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Pfizenmaier et al.

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(54) **RECOMBINANT POLYPEPTIDES OF THE MEMBERS OF THE TNF LIGAND FAMILY AND USE THEREOF**

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A61K 45/06 (2006.01)
C07K 14/705 (2006.01)
C07K 16/40 (2006.01)
G01N 33/544 (2006.01)
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CPC **C07K 14/525** (2013.01); **G01N 33/53** (2013.01); **A61K 45/06** (2013.01); **C07K 14/70575** (2013.01); **C07K 16/40** (2013.01); **G01N 33/543** (2013.01); **G01N 33/544** (2013.01); **G01N 33/6863** (2013.01); **G01N 2333/525** (2013.01); **A61K 38/00** (2013.01); **C07K 2317/622** (2013.01); **C07K 2319/00** (2013.01); **Y10S 530/811** (2013.01); **Y10S 530/812** (2013.01)
USPC **435/4**; **435/7.1**; **435/7.8**; **530/350**; **530/811**; **530/812**; **436/501**

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The invention relates to polypeptides comprising, as constituent A, at least three monomers of a member of the TNF ligand family and, as constituent B, at least two peptide linkers that link the monomers of the member of the TNF ligand family to one another. The invention also relates to the use of these polypeptides for treating diseases and for producing a medicament or a vaccine. The invention also relates to methods for producing and isolating these polypeptides, to nucleic acids that code for these polypeptides, to vectors containing these nucleic acids, to host cells transfected with these vectors, and to pharmaceutical compositions containing these inventive objects. Finally, the invention relates to methods for the extracorporeal manipulation, depletion and/or removal of components contained in body fluids, e.g. by means of apheresis.

23 Claims, 41 Drawing Sheets

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Cytotoxicity test
sTNF and scTNFs with neutralizing antibodies [AB]

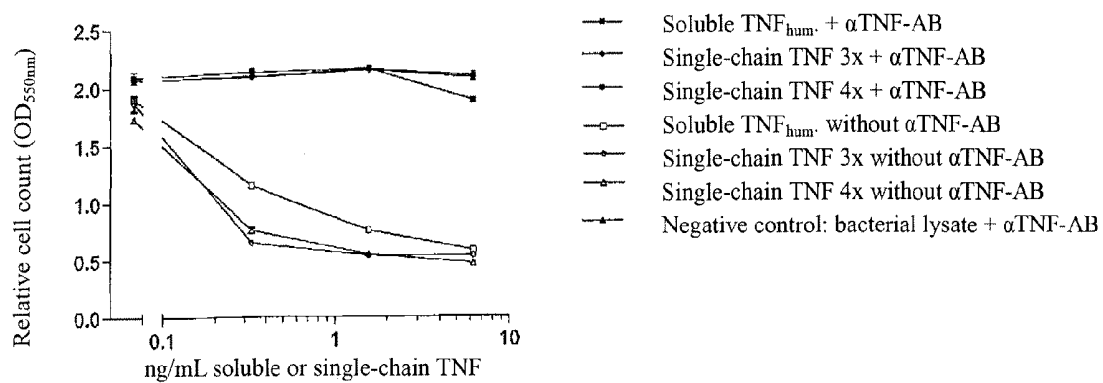


Figure 1

Cytotoxicity test
sTNF and scTNFs on MF-TNFR2

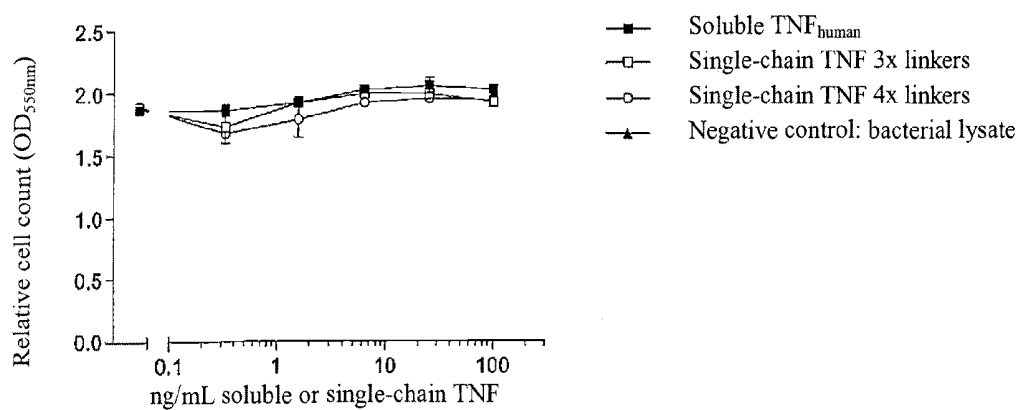


Figure 2

Cytotoxicity test
sTNF and scTNFs on MF-TNFR2 + 80M2

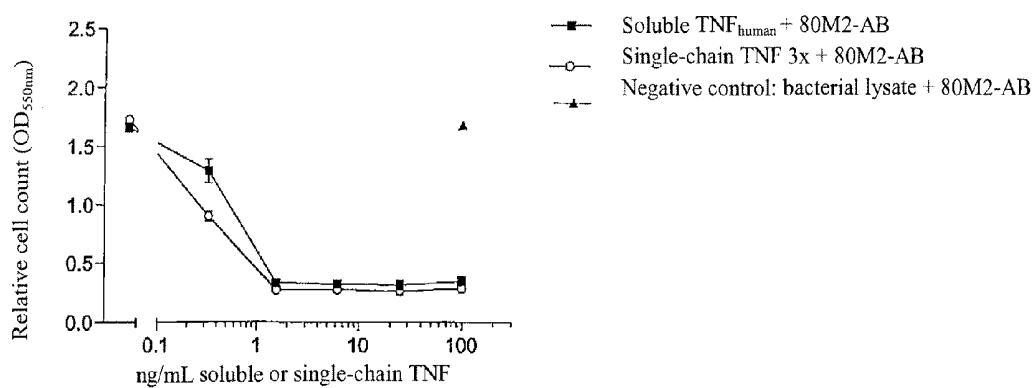
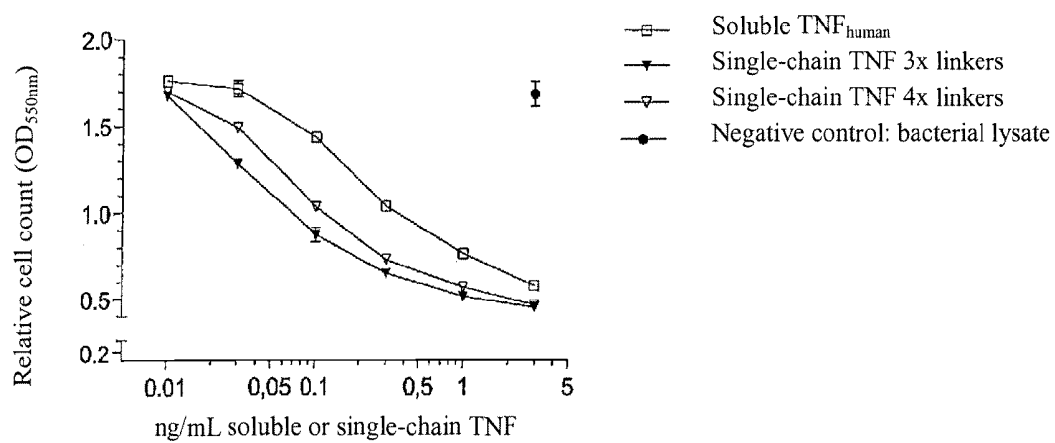
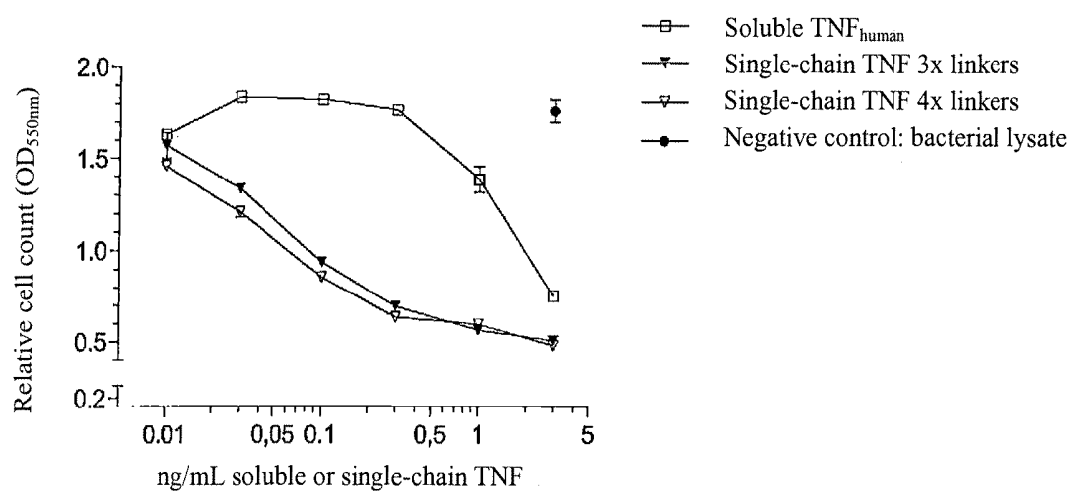
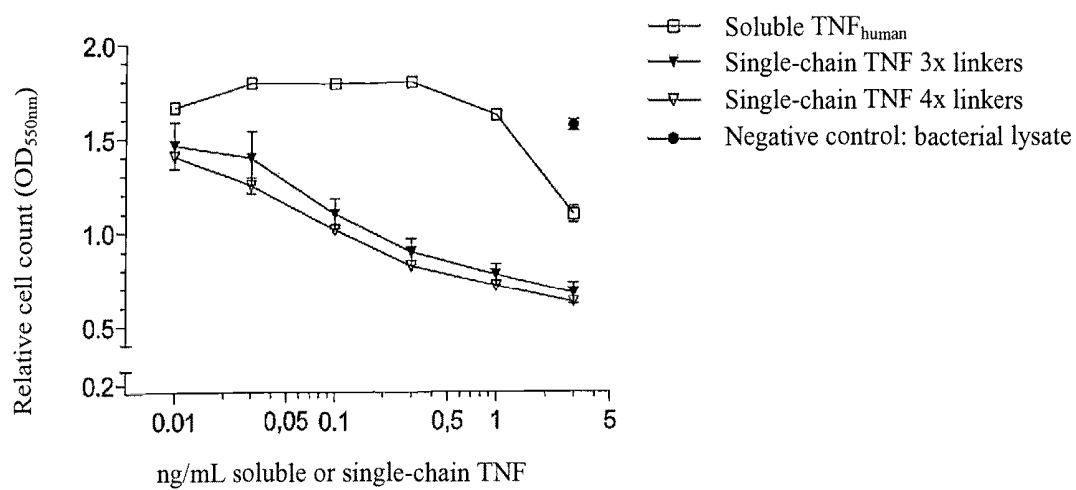
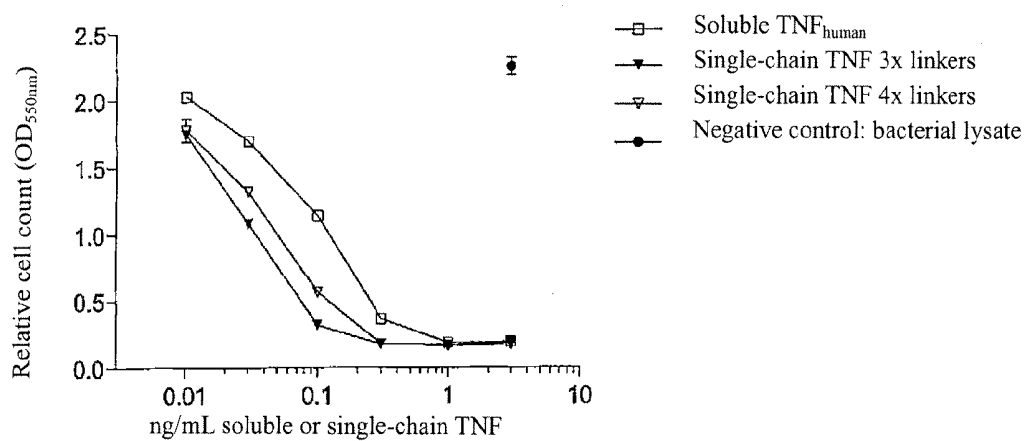


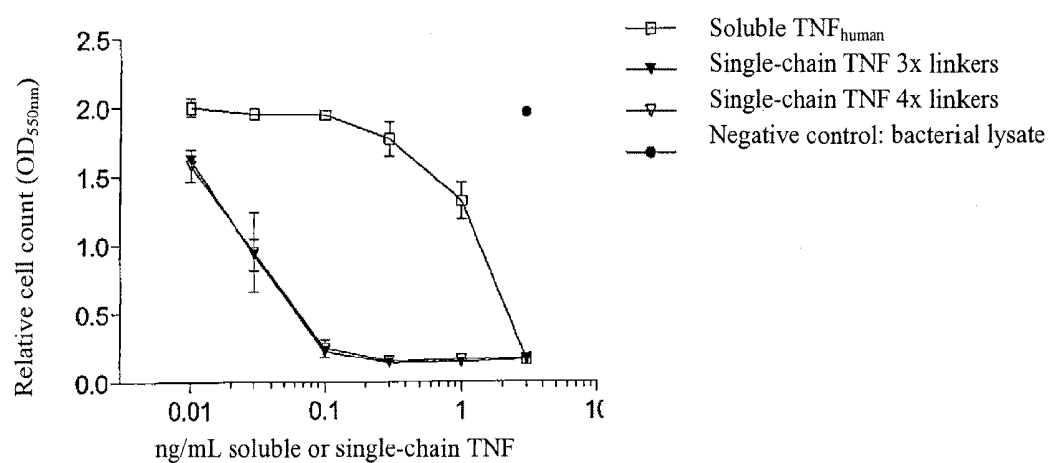
Figure 3

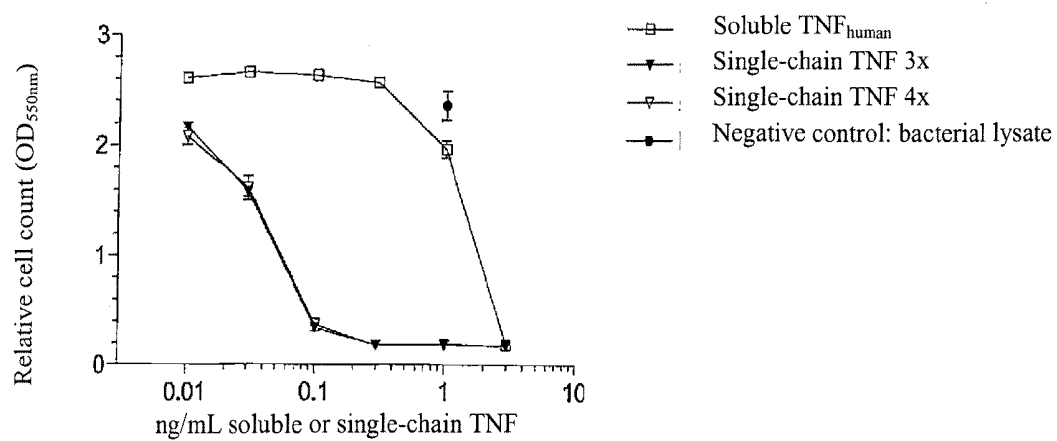
Stability test with MF – freshly titrated**Figure 4**

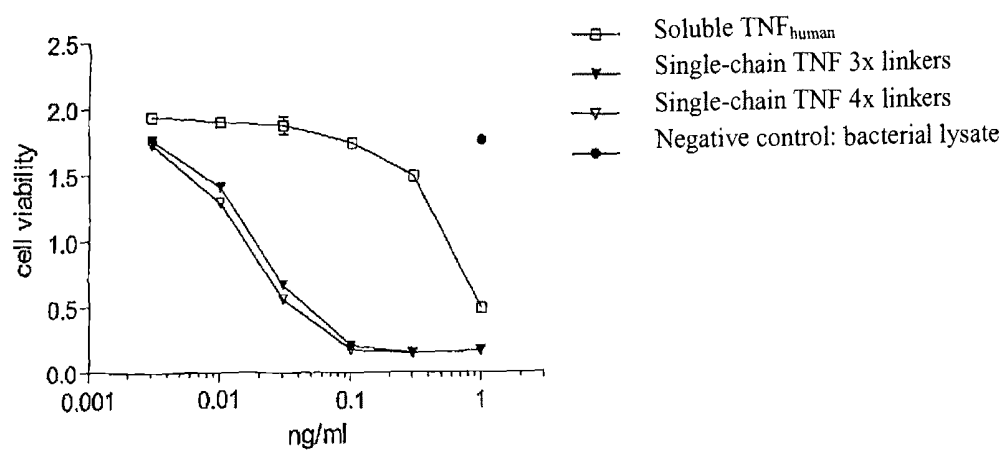
Stability test with MF – incubated for 8 days**Figure 5**

Stability test with MF – incubated for 14 days**Figure 6**

Stability test with Kym1 – freshly titrated**Figure 7**

Stability test with Kym1 – incubated for 16 days**Figure 8**

Stability test with Kym1 – incubated for 22 days**Figure 9**

Stability test with Kym1 – titrated from stock solution**Figure 10**

Stability test with human serum
Serum stability - freshly titrated

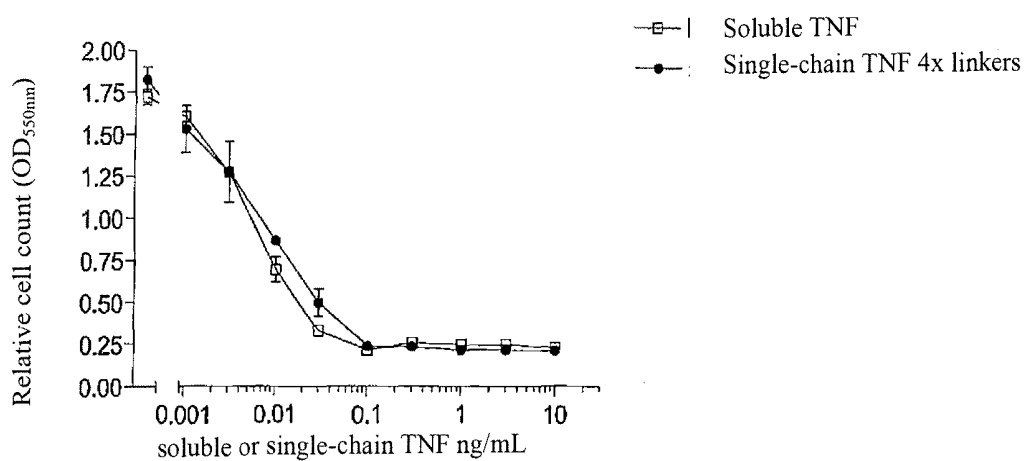


Figure 11

Stability test with human serum
Serum stability – 8 days

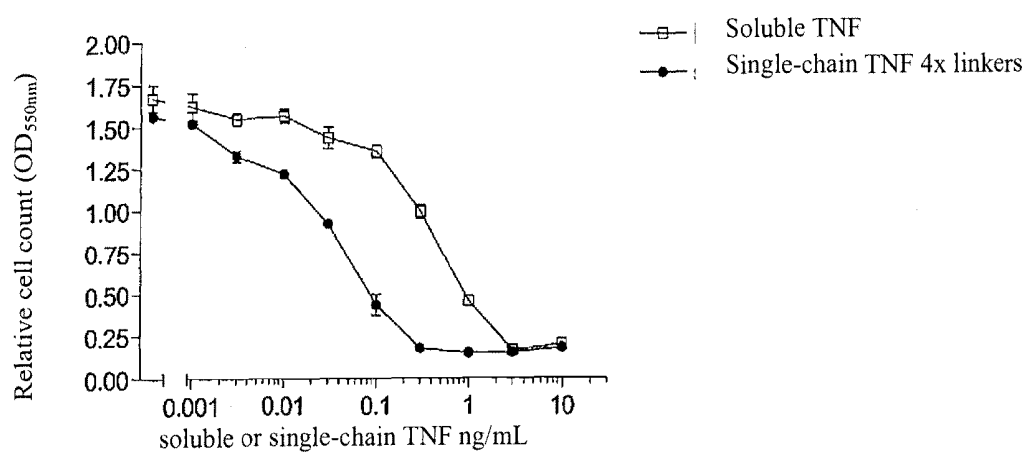


Figure 12

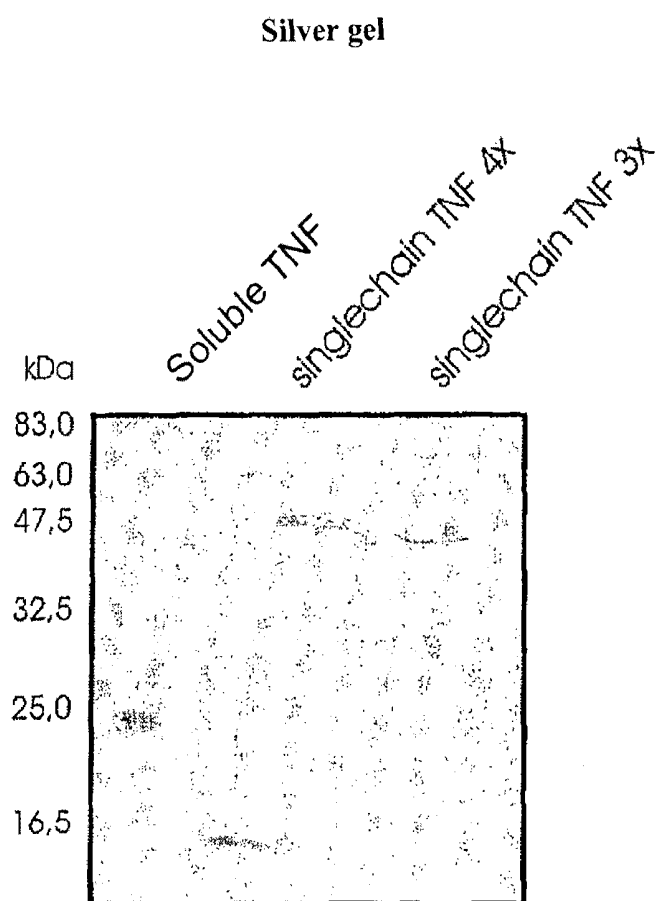


Figure 13

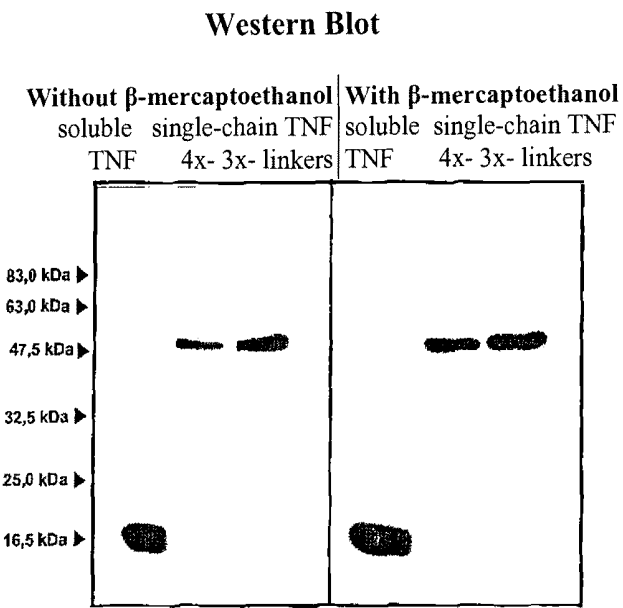
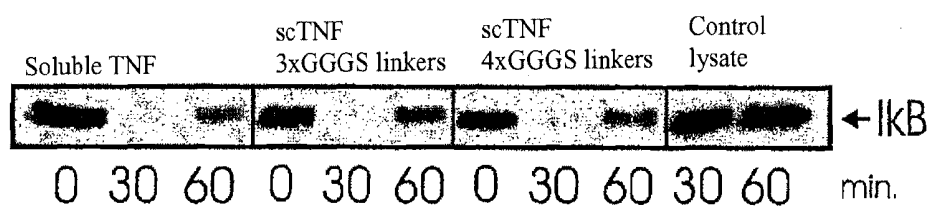
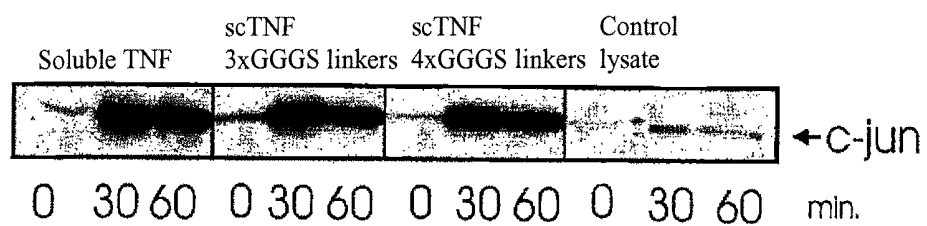
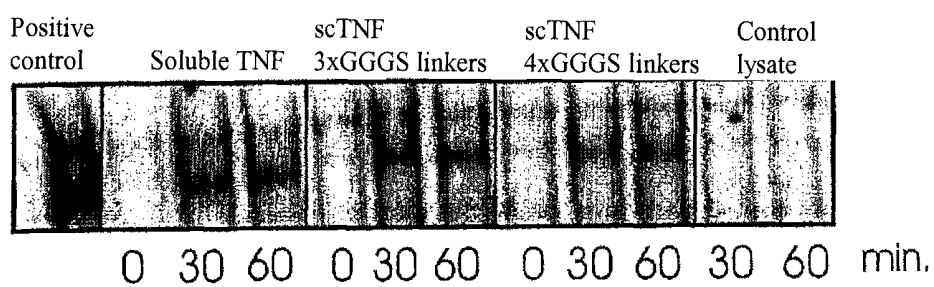


Figure 14

I κ B degradation assay**Figure 15**

JNK assay**Figure 16**

Electrophoretic Mobility Shift Assay (EMSA)**Figure 17**

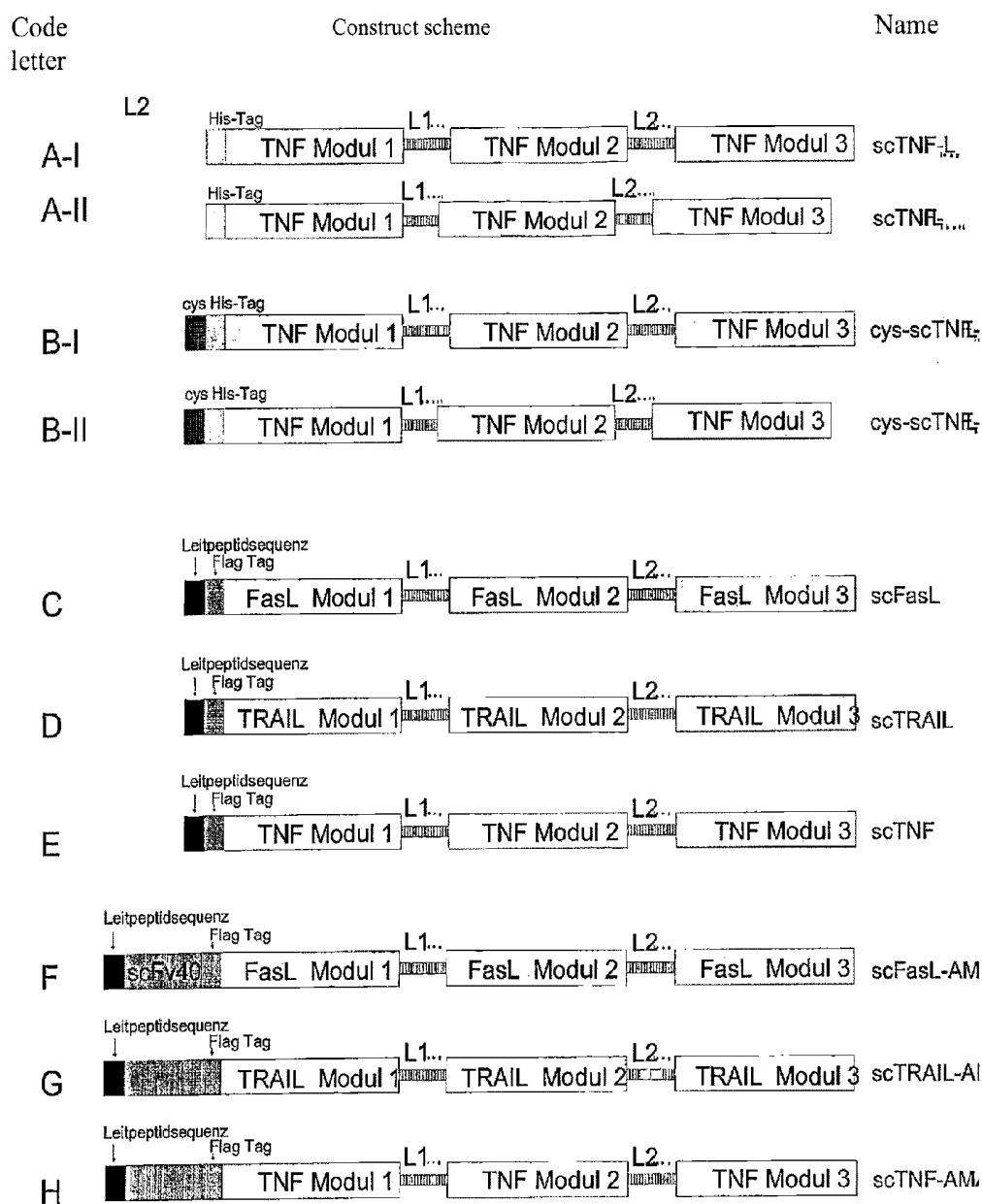


Figure 18

Key: Leitpeptidsequenz = leader peptide sequence
 Modul = module

**Nucleic acid sequence and corresponding amino acid sequence of scTNF-L_{short}
Construct A-II**

1	ATG AGA GGA TCG CAT CAC CAT CAC CAT CAC GGA TCA GCG TCG TCT	45
1	M R G S H H H H H H G S A S S	15
46	TCT TCT CGT ACC CCG TCT GAC AAA CCG GTT GCT CAC GTT GTT GCA	90
16	S S R T P S D K P V A H V V A	30
91	AAC CCG CAG GCT GAA GGT CAA CTG CAA TGG CTG AAC CGT CGT GCT	135
31	N P Q A E G Q L Q W L N R R A	45
136	AAC GCT CTG CTG GCT AAC GGT GTT GAA CTG CGT GAC AAC CAG CTG	180
46	N A L L A N G V E L R D N Q L	60
181	GTT GTT CCG TCT GAA GGC CTG TAC CTG ATC TAC TCC CAG GTT CTG	225
61	V V P S E G L Y L I Y S Q V L	75
226	TTC AAA GGC CAG GGC TGC CCG TCC ACC CAC GTT CTG CTG ACC CAC	270
76	F K G Q G C P S T H V L L T H	90
271	ACC ATC TCT CGT ATC GCT GTT TCC TAC CAG ACC AAA GTA AAC CTG	315
91	T I S R I A V S Y Q T K V N L	105
316	CTG TCT GCA ATC AAA TCT CCG TGC CAG CGT GAA ACC CCG GAA GGT	360
106	L S A I K S P C Q R E T F E G	120
361	GCT GAA GCT AAA CCG TGG TAC GAA CCG ATC TAC CTG GGT GGC GTT	405
121	A E A K P W Y E P I Y L G G V	135
406	TTT CAA CTG GAG AAA GGT GAC CGT CTG TCT GCA GAA ATT AAC CGT	450
136	F Q L E K G D R L S A E I N R	150
451	CCG GAC TAC CTG GAC TTC GCA GAA TCT GGT CAG GTT TAC TTC GGT	495
151	P D Y L D F A E S G Q V Y F G	165
496	ATC ATC GCT CTG GGT GGC GGT TCT GGT GGC GGT TCT GGT GGC GGA	540
166	I I A L G G G S G G S G G S G	180
541	TCC TCT TCT CGT ACC CCG TCT GAC AAA CCG GTT GCT CAC GTT GTT	585
181	S S S R T P S D K P V A H V V	195
586	GCA AAC CCG CAG GCT GAA GGT CAA CTG CAA TGG CTG AAC CGT CGT	630
196	A N P Q A E G Q L Q W L N R R	210
631	GCT AAC GCT CTG CTG GCT AAC GGT GTT GAA CTG CGT GAC AAC CAG	675
211	A N A L L A N G V E L R D N Q	225
676	CTG GTT GTT CCG TCT GAA GGC CTG TAC CTG ATC TAC TCC CAG GTT	720
226	L V V P S E G L Y L I Y S Q V	240
721	CTG TTC AAA GGC CAG GGC TGC CCG TCC ACC CAC GTT CTG CTG ACC	765
241	L F K G Q G C P S T H V L L T	255
766	CAC ACC ATC TCT CGT ATC GCT GTT TCC TAC CAG ACC AAA GTA AAC	810
256	H T I S R I A V S Y Q T K V N	270
811	CTG CTG TCT GCA ATC AAA TCT CCG TGC CAG CGT GAA ACC CCG GAA	855
271	L L S A I K S P C Q R E T P E	285

Figure 19A

856	GGT GCT GAA GCT AAA CCG TGG TAC GAA CCG ATC TAC CTG GGT GGC	900
286	G A E A K P W Y E P I Y L G G	300
901	GTT TTT CAA CTG GAG AAA GGT GAC CGT CTG TCT GCA GAA ATT AAC	945
301	V F Q L E K G D R L S A E I N	315
946	CGT CCG GAC TAC CTG GAC TTC GCA GAA TCT GGT CAG GTT TAC TTC	990
316	R P D Y L D F A E S G Q V Y F	330
991	GGT ATC ATC GCT CTG GGT GGC GGT TCT GGT GGC GGT TCT GGT GGC	1035
331	G I I A L G G G S G G G S G G	345
1036	GGA TCC TCT TCT CGT ACC CCG TCT GAC AAA CCG GTT GCT CAC GTT	1080
346	G S S S R T P S D K P V A H V	360
1081	GTT GCA AAC CCG CAG GCT GAA GGT CAA CTG CAA TGG CTG AAC CGT	1125
361	V A N P Q A E G Q L Q W L N R	375
1126	CGT GCT AAC GCT CTG CTG GCT AAC GGT GTT GAA CTG CGT GAC AAC	1170
376	R A N A L L A N G V E L R D N	390
1171	CAG CTG GTT GTT CCG TCT GAA GGC CTG TAC CTG ATC TAC TCC CAG	1215
391	Q L V V P S E G L Y L I Y S Q	405
1216	GTT CTG TTC AAA GGC CAG GGC TGC CCG TCC ACC CAC GTT CTG CTG	1260
406	V L F K G Q G C P S T H V L L	420
1261	ACC CAC ACC ATC TCT CGT ATC GCT GTT TCC TAC CAG ACC AAA GTA	1305
421	T H T I S R I A V S Y Q T K V	435
1306	AAC CTG CTG TCT GCA ATC AAA TCT CCG TGC CAG CGT GAA ACC CCG	1350
436	N L L S A I K S P C Q R E T P	450
1351	GAA GGT GCT GAA GCT AAA CCG TGG TAC GAA CCG ATC TAC CTG GGT	1395
451	E G A E A K P W Y E P I Y L G	465
1396	GGC GTT TTT CAA CTG GAG AAA GGT GAC CGT CTG TCT GCA GAA ATT	1440
466	G V F Q L E K G D R L S A E I	480
1441	AAC CGT CCG GAC TAC CTG GAC TTC GCA GAA TCT GGT CAG GTT TAC	1485
481	N R P D Y L D F A E S G Q V Y	495
1486	TTC GGT ATC ATC GCT CTG TGA	1506
496	F G I I A L *	501

Figure 19B

**Nucleic acid sequence and corresponding amino acid sequence of cys-scTNF-L_{short}
Construct B-II**

1	ATG GGA GAG CTC ATC GAA GGT CGC TGC GCC GGT GGA TCT GGT CAT	45
1	M G E L I E G R C A G G S G H	15
46	CAT CAT CAC CAT CAC GGC TCA GAC GGA GCG TCG TCT TCT TCT CGT	90
16	H H H H H G S D G A S S S S R	30
91	ACC CCG TCT GAC AAA CCG GTT GCT CAC GTT GTT GCA AAC CCG CAG	135
31	T P S D K P V A H V V A N P Q	45
136	GCT GAA GGT CAA CTG CAA TGG CTG AAC CGT CGT GCT AAC GCT CTG	180
46	A E G Q L Q W L N R R A N A L	60
181	CTG GCT AAC GGT GTT GAA CTG CGT GAC AAC CAG CTG GTT GTT CCG	225
61	L A N G V E L R D N Q L V V P	75
226	TCT GAA GGC CTG TAC CTG ATC TAC TCC CAG GTT CTG TTC AAA GGC	270
76	S E G L Y L I Y S Q V L F K G	90
271	CAG GGC TGC CCG TCC ACC CAC GTT CTG CTG ACC CAC ACC ATC TCT	315
91	Q G C P S T H V L L T H T I S	105
316	CGT ATC GCT GTT TCC TAC CAG ACC AAA GTA AAC CTG CTG TCT GCA	360
106	R I A V S Y Q T K V N L L S A	120
361	ATC AAA TCT CCG TGC CAG CGT GAA ACC CCG GAA GGT GCT GAA GCT	405
121	I K S P C Q R E T P E G A E A	135
406	AAA CCG TGG TAC GAA CCG ATC TAC CTG GGT GGC GTT TTT CAA CTG	450
136	K P W Y E P I Y L G G V F Q L	150
451	GAG AAA GGT GAC CGT CTG TCT GCA GAA ATT AAC CGT CCG GAC TAC	495
151	E K G D R L S A E I N R P D Y	165
496	CTG GAC TTC GCA GAA TCT GGT CAG GTT TAC TTC GGT ATC ATC GCT	540
166	L D F A E S G Q V Y F G I I A	180
541	CTG GGT GGC GGT TCT GGT GGC GGT TCT GGT GGC GGA TCC TCT TCT	585
181	L G G G S G G G S G G G S S S	195
586	CGT ACC CCG TCT GAC AAA CCG GTT GCT CAC GTT GTT GCA AAC CCG	630
196	R T P S D K P V A H V V A N P	210
631	CAG GCT GAA GGT CAA CTG CAA TGG CTG AAC CGT CGT GCT AAC GCT	675
211	Q A E G Q L Q W L N R R A N A	225
676	CTG CTG GCT AAC GGT GTT GAA CTG CGT GAC AAC CAG CTG GTT GTT	720
226	L L A N G V E L R D N Q L V V	240
721	CCG TCT GAA GGC CTG TAC CTG ATC TAC TCC CAG GTT CTG TTC AAA	765
241	P S E G L Y L I Y S Q V L F K	255
766	GGC CAG GGC TGC CCG TCC ACC CAC GTT CTG CTG ACC CAC ACC ATC	810
256	G Q G C P S T H V L L T H T I	270

Figure 20A

811	TCT CGT ATC GCT GTT TCC TAC CAG ACC AAA GTA AAC CTG CTG TCT	855
271	S R I A V S Y Q T K V N L L S	285
856	GCA ATC AAA TCT CCG TGC CAG CGT GAA ACC CCG GAA GGT GCT GAA	900
286	A I K S P C Q R E T P E G A E	300
901	GCT AAA CCG TGG TAC GAA CCG ATC TAC CTG GGT GGC GTT TTT CAA	945
301	A K P W Y E P I Y L G G V F Q	315
946	CTG GAG AAA GGT GAC CGT CTG TCT GCA GAA ATT AAC CGT CCG GAC	990
316	L E K G D R L S A E I N R P D	330
991	TAC CTG GAC TTC GCA GAA TCT GGT CAG GTT TAC TTC GGT ATC ATC	1035
331	Y L D F A E S G Q V Y F G I I	345
1036	GCT CTG GGT GGC GGT TCT GGT GGC GGT TCT GGT GGC GGA TCC TCT	1080
346	A L G G G S G G G S G G G S S	360
1081	TCT CGT ACC CCG TCT GAC AAA CCG GTT GCT CAC GTT GTT GCA AAC	1125
361	S R T P S D K P V A H V V A N	375
1126	CCG CAG GCT GAA GGT CAA CTG CAA TGG CTG AAC CGT CGT GCT AAC	1170
376	P Q A E G Q L Q W L N R R A N	390
1171	GCT CTG CTG GCT AAC GGT GTT GAA CTG CGT GAC AAC CAG CTG GTT	1215
391	A L L A N G V E L R D N Q L V	405
1216	GTT CCG TCT GAA GGC CTG TAC CTG ATC TAC TCC CAG GTT CTG TTC	1260
406	V P S E G L Y L I Y S Q V L F	420
1261	AAA GGC CAG GGC TGC CCG TCC ACC CAC GTT CTG CTG ACC CAC ACC	1305
421	K G Q G C P S T H V L L T H T	435
1306	ATC TCT CGT ATC GCT GTT TCC TAC CAG ACC AAA GTA AAC CTG CTG	1350
436	I S R I A V S Y Q T K V N L L	450
1351	TCT GCA ATC AAA TCT CCG TGC CAG CGT GAA ACC CCG GAA GGT GCT	1395
451	S A I K S P C Q R E T P E G A	465
1396	GAA GCT AAA CCG TGG TAC GAA CCG ATC TAC CTG GGT GGC GTT TTT	1440
466	E A K P W Y E P I Y L G G V F	480
1441	CAA CTG GAG AAA GGT GAC CGT CTG TCT GCA GAA ATT AAC CGT CCG	1485
481	Q L E K G D R L S A E I N R P	495
1486	GAC TAC CTG GAC TTC GCA GAA TCT GGT CAG GTT TAC TTC GGT ATC	1530
496	D Y L D F A E S G Q V Y F G I	510
1531	ATC GCT CTG TGA	1542
511	I A L *	513

Figure 20B

**Nucleic acid sequence and corresponding amino acid sequence of scFasL
Construct C**

1	ATG GCT ATC ATC TAC CTC ATC CTC CTG TTC ACC GCT GTG CGG GGC	45
1	M A I I Y L I L L F T A V R G	15
46	GCG GCC GCG GAT TAC AAA GAC GAT GAC GAT AAA GAA TTC ACG CGT	90
16	A A A D Y K D D D D K E F T R	30
91	GAA AAA AAG GAG CTG AGG AAA GTG GCC CAT TTA ACA GGC AAG TCC	135
31	E K K E L R K V A H L T G K S	45
136	AAC TCA AGG TCC ATG CCT CTG GAA TGG GAA GAC ACC TAT GGA ATT	180
46	N S R S M P L E W E D T Y G I	60
181	GTC CTG CTT TCT GGA GTG AAG TAT AAG AAG GGT GGC CTT GTG ATC	225
61	V L L S G V K Y K K G G L V I	75
226	AAT GAA ACT GGG CTG TAC TTT GTA TAT TCC AAA GTA TAC TTC CGG	270
76	N E T G L Y F V Y S K V Y F R	90
271	GGT CAA TCT TGC AAC AAC CTG CCC CTG AGC CAC AAG GTC TAC ATG	315
91	G Q S C N N L P L S H K V Y M	105
316	AGG AAC TCT AAG TAT CCC CAG GAT CTG GTG ATG ATG GAG GGG AAG	360
106	R N S K Y P Q D L V M M E G K	120
361	ATG ATG AGC TAC TGC ACT ACT GGG CAG ATG TGG GCC CGC AGC AGC	405
121	M M S Y C T T G Q M W A R S S	135
406	TAC CTG GGG GCA GTG TTC AAT CTT ACC AGT GCT GAT CAT TTA TAT	450
136	Y L G A V F N L T S A D H L Y	150
451	GTC AAC GTA TCT GAG CTC TCT CTG GTC AAT TTT GAG GAA TCT CAG	495
151	V N V S E L S L V N F E E S Q	165
496	ACG TTT TTC GGC TTA TAT AAG CTC GGT GGC GGT TCT GGT GGC GGT	540
166	T F F G L Y K L G G G S G G G	180
541	TCT GGT GGC GGT TCT GGT GGC GGA TCA GAA AAA AAG GAG CTG AGG	585
181	S G G G S G G G S E K K E L R	195
586	AAA GTG GCC CAT TTA ACA GGC AAG TCC AAC TCA AGG TCC ATG CCT	630
196	K V A H L T G K S N S R S M P	210
631	CTG GAA TGG GAA GAC ACC TAT GGA ATT GTC CTG CTT TCT GGA GTG	675
211	L E W E D T Y G I V L L S G V	225
676	AAG TAT AAG AAG GGT GGC CTT GTG ATC AAT GAA ACT GGG CTG TAC	720
226	K Y K K G G L V I N E T G L Y	240
721	TTT GTA TAT TCC AAA GTA TAC TTC CGG GGT CAA TCT TGC AAC AAC	765
241	F V Y S K V Y F R G Q S C N N	255

Figure 21A

766	CTG CCC CTG AGC CAC AAG GTC TAC ATG AGG AAC TCT AAG TAT CCC	810
256	L P L S H K V Y M R N S K Y P	270
811	CAG GAT CTG GTG ATG ATG GAG GGG AAG ATG ATG AGC TAC TGC ACT	855
271	Q D L V M M E G K M M S Y C T	285
856	ACT GGG CAG ATG TGG GCC CGC AGC AGC TAC CTG GGG GCA GTG TTC	900
286	T G Q M W A R S S Y L G A V F	300
901	AAT CTT ACC AGT GCT GAT CAT TTA TAT GTC AAC GTA TCT GAG CTC	945
301	N L T S A D H L Y V N V S E L	315
946	TCT CTG GTC AAT TTT GAG GAA TCT CAG ACG TTT TTC GGC TTA TAT	990
316	S L V N F E E S Q T F F G L Y	330
991	AAG CTC GGT GGC GGT TCT GGT GGC GGT TCT GGT GGC GGT TCT GGT	1035
331	K L G G G S G G G S G G G S G	345
1036	GGC GGA TCC GAA AAA AAG GAG CTG AGG AAA GTG GCC CAT TTA ACA	1080
346	G G S E K K E L R K V A H L T	360
1081	GGC AAG TCC AAC TCA AGG TCC ATG CCT CTG GAA TGG GAA GAC ACC	1125
361	G K S N S R S M P L E W E D T	375
1126	TAT GGA ATT GTC CTG CTT TCT GGA GTG AAG TAT AAG AAG GGT GGC	1170
376	Y G I V L L S G V K Y K K G G	390
1171	CTT GTG ATC AAT GAA ACT GGG CTG TAC TTT GTA TAT TCC AAA GTA	1215
391	L V I N E T G L Y F V Y S K V	405
1216	TAC TTC CGG GGT CAA TCT TGC AAC AAC CTG CCC CTG AGC CAC AAG	1260
406	Y F R G Q S C N N L P L S H K	420
1261	GTC TAC ATG AGG AAC TCT AAG TAT CCC CAG GAT CTG GTG ATG ATG	1305
421	V Y M R N S K Y P Q D L V M M	435
1306	GAG GGG AAG ATG ATG AGC TAC TGC ACT ACT GGG CAG ATG TGG GCC	1350
436	E G K M M S Y C T T G Q M W A	450
1351	CGC AGC AGC TAC CTG GGG GCA GTG TTC AAT CTT ACC AGT GCT GAT	1395
451	R S S Y L G A V F N L T S A D	465
1396	CAT TTA TAT GTC AAC GTA TCT GAG CTC TCT CTG GTC AAT TTT GAG	1440
466	H L Y V N V S E L S L V N F E	480
1441	GAA TCT CAG ACG TTT TTC GGC TTA TAT AAG CTC TGA	1476
481	E S Q T F F G L Y K L *	491

Figure 21B

**Nucleic acid sequence and corresponding amino acid sequence of scTRAIL
Construct D**

1	ATG GCT ATC ATC TAC CTC ATC CTC CTG TTC ACC GCT GTG CGG GGC	45
1	M A I I Y L I L L F T A V R G	15
46	GCG GCC GCG GAT TAC AAA GAC GAT GAC GAT AAA GAA TTC GGA ACC	90
16	A A A D Y K D D D D K E F G T	30
91	TCT GAG GAA ACC ATT TCT ACA GTT CAA GAA AAG CAA CAA AAT ATT	135
31	S E E T I S T V Q E K Q Q N I	45
136	TCT CCC CTA GTG AGA GAA AGA GGT CCT CAG AGA GTA GCA GCT CAC	180
46	S P L V R E R G P Q R V A A H	60
181	ATA ACT GGG ACC AGA GGA AGA AGC AAC ACA TTG TCT TCT CCA AAC	225
61	I T G T R G R S N T L S S P N	75
226	TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA ATA AAC TCC TGG GAA	270
76	S K N E K A L G R K I N S W E	90
271	TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC AAC TTG CAC TTG AGG	315
91	S S R S G H S F L S N L H L R	105
316	AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG TTT TAC TAC ATC TAT	360
106	N G E L V I H E K G F Y Y I Y	120
361	TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA ATA AAA GAA AAC ACA	405
121	S Q T Y F R F Q E E I K E N T	135
406	AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT TAC AAA TAC ACA AGT	450
136	K N D K Q M V Q Y I Y K Y T S	150
451	TAT CCT GAC CCT ATA TTG TTG ATG AAA AGT GCT AGA AAT AGT TGT	495
151	Y P D P I L L M K S A R N S C	165
496	TGG TCT AAA GAT GCA GAA TAT GGA CTC TAT TCC ATC TAT CAA GGG	540
166	W S K D A E Y G L Y S I Y Q G	180
541	GGA ATA TTT GAG CTT AAG GAA AAT GAC AGA ATT TTT GTT TCT GTA	585
181	G I F E L K E N D R I F V S V	195
586	ACA AAT GAG CAC TTG ATA GAC ATG GAC CAT GAA GCC AGT TTT TTC	630
196	T N E H L I D M D H E A S F F	210
631	GGG GCC TTT TTA GTT GGC GGT GGC GGT TCT GGT GGC GGT TCT GGT	675
211	G A F L V G G G G S G G G S G	225
676	GGC GGT TCT GGT GGC GGA TCA ACC TCT GAG GAA ACC ATT TCT ACA	720
226	G G S G G S T S E E T I S T	240
721	GTT CAA GAA AAG CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA	765
241	V Q E K Q Q N I S P L V R E R	255

Figure 22A

766	GGT CCT CAG AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA	810
256	G P Q R V A A H I T G T R G R	270
811	AGC AAC ACA TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG	855
271	S N T L S S P N S K N E K A L	285
856	GGC CGC AAA ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA	900
286	G R K I N S W E S S R S G H S	300
901	TTC CTG AGC AAC TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT	945
301	F L S N L H L R N G E L V I H	315
946	GAA AAA GGG TTT TAC TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT	990
316	E K G F Y Y I Y S Q T Y F R F	330
991	CAG GAG GAA ATA AAA GAA AAC ACA AAG AAC GAC AAA CAA ATG GTC	1035
331	Q E E I K E N T K N D K Q M V	345
1036	CAA TAT ATT TAC AAA TAC ACA AGT TAT CCT GAC CCT ATA TTG TTG	1080
346	Q Y I Y K Y T S Y P D P I L L	360
1081	ATG AAA AGT GCT AGA AAT AGT TGT TGG TCT AAA GAT GCA GAA TAT	1125
361	M K S A R N S C W S K D A E Y	375
1126	GGA CTC TAT TCC ATC TAT CAA GGG GGA ATA TTT GAG CTT AAG GAA	1170
376	G L Y S I Y Q G G I F E L K E	390
1171	AAT GAC AGA ATT TTT GTT TCT GTA ACA AAT GAG CAC TTG ATA GAC	1215
391	N D R I F V S V T N E H L I D	405
1216	ATG GAC CAT GAA GCC AGT TTT TTC GGG GCC TTT TTA GTT GGC GGT	1260
406	M D H E A S F F G A F L V G G	420
1261	GGC GGT TCT GGT GGC GGT TCT GGT GGC GGT TCT GGT GGC GGA TCC	1305
421	G G S G G G S G G G S G G G S	435
1306	ACC TCT GAG GAA ACC ATT TCT ACA GTT CAA GAA AAG CAA CAA AAT	1350
436	T S E E T I S T V Q E K Q Q N	450
1351	ATT TCT CCC CTA GTG AGA GAA AGA GGT CCT CAG AGA GTA GCA GCT	1395
451	I S P L V R E R G P Q R V A A	465
1396	CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC ACA TTG TCT TCT CCA	1440
466	H I T G T R G R S N T L S S P	480
1441	AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA ATA AAC TCC TGG	1485
481	N S K N E K A L G R K I N S W	495
1486	GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC AAC TTG CAC TTG	1530
496	E S S R S G H S F L S N L H L	510
1531	AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG TTT TAC TAC ATC	1575
511	R N G E L V I H E K G F Y Y I	525
1576	TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA ATA AAA GAA AAC	1620
526	Y S Q T Y F R F Q E E I K E N	540

Figure 22B

1621	ACA	AAG	AAC	GAC	AAA	CAA	ATG	GTC	CAA	TAT	ATT	TAC	AAA	TAC	ACA	1665
541	T	K	N	D	K	Q	M	V	Q	Y	I	Y	K	Y	T	555
1666	AGT	TAT	CCT	GAC	CCT	ATA	TTG	TTG	ATG	AAA	AGT	GCT	AGA	AAT	AGT	1710
556	S	Y	P	D	P	I	L	L	M	K	S	A	R	N	S	570
1711	TGT	TGG	TCT	AAA	GAT	GCA	GAA	TAT	GGA	CTC	TAT	TCC	ATC	TAT	CAA	1755
571	C	W	S	K	D	A	E	Y	G	L	Y	S	I	Y	Q	585
1756	GGG	GGA	ATA	TTT	GAG	CTT	AAG	GAA	AAT	GAC	AGA	ATT	TTT	GTT	TCT	1800
586	G	G	I	F	E	L	K	E	N	D	R	I	F	V	S	600
1801	GTA	ACA	AAT	GAG	CAC	TTG	ATA	GAC	ATG	GAC	CAT	GAA	GCC	AGT	TTT	1845
601	V	T	N	E	H	L	I	D	M	D	H	E	A	S	F	615
1846	TTC	GGG	GCC	TTT	TTA	GTT	GGC	TGA								1866
616	F	G	A	F	L	V	G	*								622

Figure 22C

Nucleic acid sequence and corresponding amino acid sequence of scTNF**Construct E**

1	ATG GCT ATC ATC TAC CTC ATC CTC CTG TTC ACC GCT GTG CGG GGC	45
1	M A I I Y L I L L F T A V R G	15
46	GCG GCC GCG GAT TAC AAA GAC GAT GAC GAT AAA GAA TTC GGA TCA	90
16	A A A D Y K D D D D K E F G S	30
91	TCT TCT CGA ACC CCG AGT GAC AAG CCT GTA GCC CAT GTT GTA GCA	135
31	S S R T P S D K P V A H V V A	45
136	AAC CCT CAA GCT GAG GGG CAG CTC CAG TGG CTG AAC CGC CGG GCC	180
46	N P Q A E G Q L Q W L N R R A	60
181	AAT GCC CTC CTG GCC AAT GGC GTG GAG CTG AGA GAT AAC CAG CTG	225
61	N A L L A N G V E L R D N Q L	75
226	GTG GTG CCA TCA GAG GGC CTG TAC CTC ATC TAC TCC CAG GTC CTC	270
76	V V P S E G L Y L I Y S Q V L	90
271	TTC AAG GGC CAA GGC TGC CCC TCC ACC CAT GTG CTC CTC ACC CAC	315
91	F K G Q G C P S T H V L L T H	105
316	ACC ATC AGC CGC ATC GCC GTC TCC TAC CAG ACC AAG GTC AAC CTC	360
106	T I S R I A V S Y Q T K V N L	120
361	CTC TCT GCC ATC AAG AGC CCC TGC CAG AGG GAG ACC CCA GAG GGG	405
121	L S A I K S P C Q R E T P E G	135
406	GCT GAG GCC AAG CCC TGG TAT GAG CCC ATC TAT CTG GGA GGG GTC	450
136	A E A K P W Y E P I Y L G G V	150
451	TTC CAG CTG GAG AAG GGT GAC CGA CTC AGC GCT GAG ATC AAT CGG	495
151	F Q L E K G D R L S A E I N R	165
496	CCC GAC TAT CTC GAC TTT GCC GAG TCT GGG CAG GTC TAC TTT GGG	540
166	P D Y L D F A E S G Q V Y F G	180
541	ATC ATT GCC CTG GGT GGC GGT TCT GGT GGC GGT TCT GGT GGC GGT	585
181	I I A L G G G S G G G S G G G	195
586	TCT GGT GGC GGA TCA TCA TCT TCT CGA ACC CCG AGT GAC AAG CCT	630
196	S G G G S S S S R T P S D K P	210
631	GTA GCC CAT GTT GTA GCA AAC CCT CAA GCT GAG GGG CAG CTC CAG	675
211	V A H V V A N P Q A E G Q L Q	225
676	TGG CTG AAC CGC CGG GCC AAT GCC CTC CTG GCC AAT GGC GTG GAG	720
226	W L N R R A N A L L A N G V E	240
721	CTG AGA GAT AAC CAG CTG GTG GTG CCA TCA GAG GGC CTG TAC CTC	765
241	L R D N Q L V V P S E G L Y L	255

Figure 23A

766	ATC TAC TCC CAG GTC CTC TTC AAG GGC CAA GGC TGC CCC TCC ACC	810
256	I Y S Q V L F K G Q G C P S T	270
811	CAT GTG CTC CTC ACC CAC ACC ATC AGC CGC ATC GCC GTC TCC TAC	855
271	H V L L T H T I S R I A V S Y	285
856	CAG ACC AAG GTC AAC CTC CTC TCT GCC ATC AAG AGC CCC TGC CAG	900
286	Q T K V N L L S A I K S P C Q	300
901	AGG GAG ACC CCA GAG GGG GCT GAG GCC AAG CCC TGG TAT GAG CCC	945
301	R E T P E G A E A K P W Y E P	315
946	ATC TAT CTG GGA GGG GTC TTC CAG CTG GAG AAG GGT GAC CGA CTC	990
316	I Y L G G V F Q L E K G D R L	330
991	AGC GCT GAG ATC AAT CGG CCC GAC TAT CTC GAC TTT GCC GAG TCT	1035
331	S A E I N R P D Y L D F A E S	345
1036	GGG CAG GTC TAC TTT GGG ATC ATT GCC CTG GGT GGC GGT TCT GGT	1080
346	G Q V Y F G I I A L G G G S G	360
1081	GGC GGT TCT GGT GGC GGT TCT GGT GGC GGA TCA TCA TCT TCT CGA	1125
361	G G S G G G S G G G S S S S R	375
1126	ACC CCG AGT GAC AAG CCT GTA GCC CAT GTT GTA GCA AAC CCT CAA	1170
376	T P S D K P V A H V V A N P Q	390
1171	GCT GAG GGG CAG CTC CAG TGG CTG AAC CGC CGG GCC AAT GCC CTC	1215
391	A E G Q L Q W L N R R A N A L	405
1216	CTG GCC AAT GGC GTG GAG CTG AGA GAT AAC CAG CTG GTG GTG CCA	1260
406	L A N G V E L R D N Q L V V P	420
1261	TCA GAG GGC CTG TAC CTC ATC TAC TCC CAG GTC CTC TTC AAG GGC	1305
421	S E G L Y L I Y S Q V L F K G	435
1306	CAA GGC TGC CCC TCC ACC CAT GTG CTC CTC ACC CAC ACC ATC AGC	1350
436	Q G C P S T H V L L T H T I S	450
1351	CGC ATC GCC GTC TCC TAC CAG ACC AAG GTC AAC CTC CTC TCT GCC	1395
451	R I A V S Y Q T K V N L L S A	465
1396	ATC AAG AGC CCC TGC CAG AGG GAG ACC CCA GAG GGC GCT GAG GCC	1440
466	I K S P C Q R E T P E G A E A	480
1441	AAG CCC TGG TAT GAG CCC ATC TAT CTG GGA GGC GTC TTC CAG CTG	1485
481	K P W Y E P I Y L G G V F Q L	495
1486	GAG AAG GGT GAC CGA CTC AGC GCT GAG ATC AAT CGG CCC GAC TAT	1530
496	E K G D R L S A E I N R P D Y	510
1531	CTC GAC TTT GCC GAG TCT GGG CAG GTC TAC TTT GGC ATC ATT GCC	1575
511	L D F A E S G Q V Y F G I I A	525
1576	CTG TGA	1581
526	L *	526

Figure 23B

**Nucleic acid sequence and corresponding amino acid sequence of scFasL-AMAIZE
Construct F**

1	ATG	GAC	TGG	ACC	TGG	CGC	GTG	TTT	TGC	CTG	CTC	GCC	GTG	GCT	CCT	45
1	M	D	W	T	W	R	V	F	C	L	L	A	V	A	P	15
46	GGG	GCC	CAC	AGC	CAG	GTA	CAG	CTG	GTG	CAG	TCT	GGG	GGA	GGC	ATG	90
16	G	A	H	S	Q	V	Q	L	V	Q	S	G	G	G	M	30
91	GTA	GAG	CCT	GGG	GGG	TCC	CTT	AGA	CTC	TCC	TGT	GCA	GCC	TCT	GGA	135
31	V	E	P	G	G	S	L	R	L	S	C	A	A	S	G	45
136	TTC	ACT	TTC	AGT	AAT	GCC	TGG	ATG	AGC	TGG	GTC	CGC	CAG	GCT	CCA	180
46	F	T	F	S	N	A	W	M	S	W	V	R	Q	A	P	60
181	GGG	AAG	GGG	CTG	GAG	TGG	GTT	GGC	CGT	ATA	AAA	AGC	AAA	GCT	GGT	225
61	G	K	G	L	E	W	V	G	R	I	K	S	K	A	G	75
226	GGT	GGG	ACA	GCA	GAG	TAC	GCT	GCA	CCC	GTG	AAA	GGC	AGA	TTC	ACC	270
76	G	G	T	A	E	Y	A	A	P	V	K	G	R	F	T	90
271	ATC	TCA	AGA	GAT	GAT	TCA	CAA	AAC	ACG	CTG	TAT	CTG	CAA	ATG	AAC	315
91	I	S	R	D	D	S	Q	N	T	L	Y	L	Q	M	N	105
316	AGC	CTG	AAA	ACC	GAC	GAC	ACA	GCC	GTG	TAT	TAC	TGT	ACC	ACA	CAT	360
106	S	L	K	T	D	D	T	A	V	Y	Y	C	T	T	H	120
361	GTC	TAC	GGT	GCC	CCC	CGG	AAC	TGG	GGC	CAG	GGA	TCC	CTG	GTC	ACC	405
121	V	Y	G	A	P	R	N	W	G	Q	G	S	L	V	T	135
406	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	AAG	CTT	GAA	GAA	GGT	GAA	450
136	V	S	S	A	S	T	K	G	P	K	L	E	E	G	E	150
451	TTT	TCA	GAA	GCA	CGC	GTA	CAG	TCT	GTG	TTG	ACT	CAG	CCG	CCC	TCA	495
151	F	S	E	A	R	V	Q	S	V	L	T	Q	P	P	S	165
496	GTG	TCT	GCG	GCC	CCA	GGA	CAG	AAG	GTC	ACC	ATC	TCC	TGC	TCT	GGA	540
166	V	S	A	A	P	G	Q	K	V	T	I	S	C	S	G	180
541	AGC	AGC	TCC	AAC	ATT	GGA	AAT	AAT	TAT	GTC	TCC	TGG	TAC	GTT	CAA	585
181	S	S	S	N	I	G	N	N	Y	V	S	W	Y	V	Q	195
586	CTC	CCA	GGA	ACA	GCC	CCC	AAA	CTC	CTC	ATT	TAT	GAC	AAT	AAT	AAG	630
196	L	P	G	T	A	P	K	L	L	I	Y	D	N	N	K	210
631	CGA	TTC	TCA	GGA	GTT	CCT	GAC	CGA	TTC	TCT	GGC	TCC	AAG	TCT	GGC	675
211	R	F	S	G	V	P	D	R	F	S	G	S	K	S	G	225
676	ACG	TCA	GCC	ACC	CTG	GGC	ATC	ACC	GGG	CTC	CAG	ACT	GGG	GAC	GAG	720
226	T	S	A	T	L	G	I	T	G	L	Q	T	G	D	E	240
721	GCC	GAT	TAT	TAC	TGC	GGA	GCA	TGG	GAT	GGC	AGC	CTG	CGT	GAA	GCG	765
241	A	D	Y	Y	C	G	A	W	D	G	S	L	R	E	A	255

Figure 24A

766	GTA TTC GGC GGA GGG ACC AAG GTC ACC GTC CTA GGT GCG GCC GCA	810
256	V F G G G T K V T V L G A A A	270
811	GTT GAG CTC GAG GCG GCC GCG GAT TAC AAA GAC GAT GAC GAT AAA	855
271	V E L E A A A D Y K D D D D K	285
856	GAA TTC ACG CGT GAA AAA AAG GAG CTG AGG AAA GTG GCC CAT TTA	900
286	E F T R E K K E L R K V A H L	300
901	ACA GGC AAG TCC AAC TCA AGG TCC ATG CCT CTG GAA TGG GAA GAC	945
301	T G K S N S R S M P L E W E D	315
946	ACC TAT GGA ATT GTC CTG CTT TCT GGA GTG AAG TAT AAG AAG GGT	990
316	T Y G I V L L S G V K Y K K G	330
991	GGC CTT GTG ATC AAT GAA ACT GGG CTG TAC TTT GTA TAT TCC AAA	1035
331	G L V I N E T G L Y F V Y S K	345
1036	GTA TAC TTC CGG GGT CAA TCT TGC AAC AAC CTG CCC CTG AGC CAC	1080
346	V Y F R G Q S C N N L P L S H	360
1081	AAG GTC TAC ATG AGG AAC TCT AAG TAT CCC CAG GAT CTG GTG ATG	1125
361	K V Y M R N S K Y P Q D L V M	375
1126	ATG GAG GGG AAG ATG ATG AGC TAC TGC ACT ACT GGG CAG ATG TGG	1170
376	M E G K M M S Y C T T G Q M W	390
1171	GCC CGC AGC AGC TAC CTG GGG GCA GTG TTC AAT CTT ACC AGT GCT	1215
391	A R S S Y L G A V F N L T S A	405
1216	GAT CAT TTA TAT GTC AAC GTA TCT GAG CTC TCT CTG GTC AAT TTT	1260
406	D H L Y V N V S E L S L V N F	420
1261	GAG GAA TCT CAG ACG TTT TTC GGC TTA TAT AAG CTC GGT GGC GGT	1305
421	E E S Q T F F G L Y K L G G G	435
1306	TCT GGT GGC GGT TCT GGT GGC GGT TCT GGT GGC GGA TCA GAA AAA	1350
436	S G G G S G G G S G G G S E K	450
1351	AAG GAG CTG AGG AAA GTG GCC CAT TTA ACA GGC AAG TCC AAC TCA	1395
451	K E L R K V A H L T G K S N S	465
1396	AGG TCC ATG CCT CTG GAA TGG GAA GAC ACC TAT GGA ATT GTC CTG	1440
466	R S M P L E W E D T Y G I V L	480
1441	CTT TCT GGA GTG AAG TAT AAG AAG GGT GGC CTT GTG ATC AAT GAA	1485
481	L S G V K Y K K G G L V I N E	495
1486	ACT GGG CTG TAC TTT GTA TAT TCC AAA GTA TAC TTC CGG GGT CAA	1530
496	T G L Y F V Y S K V Y F R G Q	510
1531	TCT TGC AAC AAC CTG CCC CTG AGC CAC AAG GTC TAC ATG AGG AAC	1575
511	S C N N L P L S H K V Y M R N	525
1576	TCT AAG TAT CCC CAG GAT CTG GTG ATG ATG GAG GGG AAG ATG ATG	1620
526	S K Y F Q D L V M M E G K M M	540

Figure 24B

1621	AGC TAC TGC ACT ACT GGG CAG ATG TGG GCC CGC AGC AGC TAC CTG	1665
541	S Y C T T G Q M W A R S S Y L	555
1666	GGG GCA GTG TTC AAT CTT ACC AGT GCT GAT CAT TTA TAT GTC AAC	1710
556	G A V F N L T S A D H L Y V N	570
1711	GTA TCT GAG CTC TCT CTG GTC AAT TTT GAG GAA TCT CAG ACG TTT	1755
571	V S E L S L V N F E E S Q T F	585
1756	TTC GGC TTA TAT AAG CTC GGT GGC GGT TCT GGT GGC GGT TCT GGT	1800
586	F G L Y K L G G G S G G G S G	600
1801	GGC GGT TCT GGT GGC GGA TCC GAA AAA AAG GAG CTG AGG AAA GTG	1845
601	G G S G G G S E K K E L R K V	615
1846	GCC CAT TTA ACA GGC AAG TCC AAC TCA AGG TCC ATG CCT CTG GAA	1890
616	A H L T G K S N S R S M P L E	630
1891	TGG GAA GAC ACC TAT GGA ATT GTC CTG CTT TCT GGA GTG AAG TAT	1935
631	W E D T Y G I V L L S G V K Y	645
1936	AAG AAG GGT GGC CTT GTG ATC AAT GAA ACT GGG CTG TAC TTT GTA	1980
646	K K G G L V I N E T G L Y F V	660
1981	TAT TCC AAA GTA TAC TTC CGG GGT CAA TCT TGC AAC AAC CTG CCC	2025
661	Y S K V Y F R G Q S C N N L P	675
2026	CTG AGC CAC AAG GTC TAC ATG AGG AAC TCT AAG TAT CCC CAG GAT	2070
676	L S H K V Y M R N S K Y P Q D	690
2071	CTG GTG ATG ATG GAG GGG AAG ATG ATG AGC TAC TGC ACT ACT GGG	2115
691	L V M M E G K M M S Y C T T G	705
2116	CAG ATG TGG GCC CGC AGC AGC TAC CTG GGG GCA GTG TTC AAT CTT	2160
706	Q M W A R S S Y L G A V F N L	720
2161	ACC AGT GCT GAT CAT TTA TAT GTC AAC GTA TCT GAG CTC TCT CTG	2205
721	T S A D H L Y V N V S E L S L	735
2206	GTC AAT TTT GAG GAA TCT CAG ACG TTT TTC GGC TTA TAT AAG CTC	2250
736	V N F E E S Q T F F G L Y K L	750
2251	TGA	2253
751	*	751

Figure 24C

Nucleic acid sequence and corresponding amino acid sequence of scTRAIL-AMAIze
Construct G

1	ATG	GAC	TGG	ACC	TGG	CGC	GTG	TTT	TGC	CTG	CTC	GCC	GTG	GCT	CCT	45
1	M	D	W	T	W	R	V	F	C	L	L	A	V	A	P	15
46	GGG	GCC	CAC	AGC	CAG	GTA	CAG	CTG	GTG	CAG	TCT	GGG	GGA	GGC	ATG	90
16	G	A	H	S	Q	V	Q	L	V	Q	S	G	G	G	M	30
91	GTA	GAG	CCT	GGG	GGG	TCC	CTT	AGA	CTC	TCC	TGT	GCA	GCC	TCT	GGA	135
31	V	E	P	G	G	S	L	R	L	S	C	A	A	S	G	45
136	TTC	ACT	TTC	AGT	AAT	GCC	TGG	ATG	AGC	TGG	GTC	CGC	CAG	GCT	CCA	180
46	F	T	F	S	N	A	W	M	S	W	V	R	Q	A	P	60
181	GGG	AAG	GGG	CTG	GAG	TGG	GTT	GGC	CGT	ATA	AAA	AGC	AAA	GCT	GGT	225
61	G	K	G	L	R	W	V	G	R	I	K	S	K	A	G	75
226	GGT	GGG	ACA	GCA	GAG	TAC	GCT	GCA	CCC	GTG	AAA	GGC	AGA	TTC	ACC	270
76	G	G	T	A	E	Y	A	A	P	V	K	G	R	F	T	90
271	ATC	TCA	AGA	GAT	GAT	TCA	CAA	AAC	ACG	CTG	TAT	CTG	CAA	ATG	AAC	315
91	I	S	R	D	D	S	Q	N	T	L	Y	L	Q	M	N	105
316	AGC	CTG	AAA	ACC	GAC	GAC	ACA	GCC	GTG	TAT	TAC	TGT	ACC	ACA	CAT	360
106	S	L	K	T	D	D	T	A	V	Y	Y	C	T	T	H	120
361	GTC	TAC	GGT	GCC	CCC	CGG	AAC	TGG	GGC	CAG	GGA	TCC	CTG	GTC	ACC	405
121	V	Y	G	A	P	R	N	W	G	Q	G	S	L	V	T	135
406	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	AAG	CTT	GAA	GAA	GGT	GAA	450
136	V	S	S	A	S	T	K	G	P	K	L	E	E	G	E	150
451	TTT	TCA	GAA	GCA	CGC	GTA	CAG	TCT	GTG	TTG	ACT	CAG	CCG	CCC	TCA	495
151	F	S	E	A	R	V	Q	S	V	L	T	Q	P	P	S	165
496	GTG	TCT	GCG	GCC	CCA	GGA	CAG	AAG	GTC	ACC	ATC	TCC	TGC	TCT	GGA	540
166	V	S	A	A	P	G	Q	K	V	T	I	S	C	S	G	180
541	AGC	AGC	TCC	AAC	ATT	GGA	AAT	AAT	TAT	GTC	TCC	TGG	TAC	GTT	CAA	585
181	S	S	S	N	I	G	N	N	Y	V	S	W	Y	V	Q	195
586	CTC	CCA	GGA	ACA	GCC	CCC	AAA	CTC	CTC	ATT	TAT	GAC	AAT	AAT	AAG	630
196	L	P	G	T	A	P	K	L	L	I	Y	D	N	N	K	210
631	CGA	TTC	TCA	GGA	GTT	CCT	GAC	CGA	TTC	TCT	GGC	TCC	AAG	TCT	GGC	675
211	R	F	S	G	V	P	D	R	F	S	G	S	K	S	G	225
676	ACG	TCA	GCC	ACC	CTG	GGC	ATC	ACC	GGG	CTC	CAG	ACT	GGG	GAC	GAG	720
226	T	S	A	T	L	G	I	T	G	L	Q	T	G	D	E	240

Figure 25A

721	GCC GAT TAT TAC TGC GGA GCA TGG GAT GGC AGC CTG CGT GAA GCG	765
241	A D Y Y C G A W D G S L R E A	255
766	GTA TTC GGC GGA GGG ACC AAG GTC ACC GTC CTA GGT GCG GCC GCA	810
256	V F G G G T K V T V L G A A A	270
811	GTT GAG CTC GAG GCG GCC GCG GAT TAC AAA GAC GAT GAC GAT AAA	855
271	V E L E A A A D Y K D D D D K	285
856	GAA TTC GGA ACC TCT GAG GAA ACC ATT TCT ACA GTT CAA GAA AAG	900
286	E F G T S E E T I S T V Q E K	300
901	CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA GGT CCT CAG AGA	945
301	Q Q N I S P L V R E R G P Q R	315
946	GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC ACA TTG	990
316	V A A H I T G T R G R S N T L	330
991	TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA ATA	1035
331	S S P N S K N E K A L G R K I	345
1036	AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC AAC	1080
346	N S W E S S R S G H S F L S N	360
1081	TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG TTT	1125
361	L H L R N G E L V I H E K G F	375
1126	TAC TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA ATA	1170
376	Y Y I Y S Q T Y F R F Q E E I	390
1171	AAA GAA AAC ACA AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT TAC	1215
391	K E N T K N D K Q M V Q Y I Y	405
1216	AAA TAC ACA AGT TAT CCT GAC CCT ATA TTG TTG ATG AAA AGT GCT	1260
406	K Y T S Y P D P I L L M K S A	420
1261	AGA AAT AGT TGT TGG TCT AAA GAT GCA GAA TAT GGA CTC TAT TCC	1305
421	R N S C W S K D A E Y G L Y S	435
1306	ATC TAT CAA GGG GGA ATA TTT GAG CTT AAG GAA AAT GAC AGA ATT	1350
436	I Y Q G G I F E L K E N D R I	450
1351	TTT GTT TCT GTA ACA AAT GAG CAC TTG ATA GAC ATG GAC CAT GAA	1395
451	F V S V T N E H L I D M D H E	465
1396	GCC AGT TTT TTC GGG GCC TTT TTA GTT GGC GGT GGC GGT TCT GGT	1440
466	A S F F G A F L V G G G G S G	480
1441	GGC GGT TCT GGT GGC GGT TCT GGT GGC GGA TCA ACC TCT GAG GAA	1485
481	G G S G G G S G G G S T S E E	495
1486	ACC ATT TCT ACA GTT CAA GAA AAG CAA CAA AAT ATT TCT CCC CTA	1530
496	T I S T V Q E K Q Q N I S P L	510
1531	GTG AGA GAA AGA GGT CCT CAG AGA GTA GCA GCT CAC ATA ACT GGG	1575
511	V R E R G P Q R V A A H I T G	525

Figure 25B

1576	ACC AGA GGA AGA AGC AAC ACA TTG TCT TCT CCA AAC TCC AAG AAT	1620
526	T R G R S N T L S S P N S K N	540
1621	GAA AAG GCT CTG GGC CGC AAA ATA AAC TCC TGG GAA TCA TCA AGG	1665
541	E K A L G R K I N S W E S S R	555
1666	AGT GGG CAT TCA TTC CTG AGC AAC TTG CAC TTG AGG AAT GGT GAA	1710
556	S G H S F L S N L H L R N G E	570
1711	CTG GTC ATC CAT GAA AAA GGG TTT TAC TAC ATC TAT TCC CAA ACA	1755
571	L V I H E K G F Y Y I Y S Q T	585
1756	TAC TTT CGA TTT CAG GAG GAA ATA AAA GAA AAC ACA AAG AAC GAC	1800
586	Y F R F Q E E I K E N T K N D	600
1801	AAA CAA ATG GTC CAA TAT ATT TAC AAA TAC ACA AGT TAT CCT GAC	1845
601	K Q M V Q Y I Y K Y T S Y P D	615
1846	CCT ATA TTG TTG ATG AAA AGT GCT AGA AAT AGT TGT TGG TCT AAA	1890
616	P I L L M K S A R N S C W S K	630
1891	GAT GCA GAA TAT GGA CTC TAT TCC ATC TAT CAA GGG GGA ATA TTT	1935
631	D A E Y G L Y S I Y Q G G I F	645
1936	GAG CTT AAG GAA AAT GAC AGA ATT TTT GTT TCT GTA ACA AAT GAG	1980
646	E L K E N D R I F V S V T N E	660
1981	CAC TTG ATA GAC ATG GAC CAT GAA GCC AGT TTT TTC GGG GCC TTT	2025
661	H L I D M D H E A S F F G A F	675
2026	TTA GTT GGC GGT GGC GGT TCT GGT GGC GGT TCT GGT GGC GGT TCT	2070
676	L V G G G G S G G G S G G G S	690
2071	GGT GGC GGA TCC ACC TCT GAG GAA ACC ATT TCT ACA GTT CAA GAA	2115
691	G G G S T S E E T I S T V Q E	705
2116	AAG CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA GGT CCT CAG	2160
706	K Q Q N I S P L V R E R G P Q	720
2161	AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC ACA	2205
721	R V A A H I T G T R G R S N T	735
2206	TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA	2250
736	L S S P N S K N E K A L G R K	750
2251	ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC	2295
751	I N S W E S S R S G H S F L S	765
2296	AAC TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG	2340
766	N L H L R N G E L V I H E K G	780
2341	TTT TAC TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA	2385
781	F Y Y I Y S Q T Y F R F Q E E	795
2386	ATA AAA GAA AAC ACA AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT	2430
796	I K E N T K N D K Q M V Q Y I	810

Figure 25C

2431	TAC	AAA	TAC	ACA	AGT	TAT	CCT	GAC	CCT	ATA	TTG	TTG	ATG	AAA	AGT	2475
811	Y	K	Y	T	S	Y	P	D	P	I	L	L	M	K	S	825
2476	GCT	AGA	AAT	AGT	TGT	TGG	TCT	AAA	GAT	GCA	GAA	TAT	GGA	CTC	TAT	2520
826	A	R	N	S	C	W	S	K	D	A	E	Y	G	L	Y	840
2521	TCC	ATC	TAT	CAA	GGG	GGA	ATA	TTT	GAG	CTT	AAG	GAA	AAT	GAC	AGA	2565
841	S	I	Y	Q	G	G	I	F	E	L	K	E	N	D	R	855
2566	ATT	TTT	GTT	TCT	GTA	ACA	AAT	GAG	CAC	TTG	ATA	GAC	ATG	GAC	CAT	2610
856	I	F	V	S	V	T	N	E	H	L	I	D	M	D	H	870
2611	GAA	GCC	AGT	TTT	TTC	GGG	GCC	TTT	TTA	GTT	GGC	TGA				2646
871	E	A	S	F	F	G	A	F	L	V	G	*				881

Figure 25D

Nucleic acid sequence and corresponding amino acid sequence of scTNF-AMAIze
Construct H

1	ATG	GAC	TGG	ACC	TGG	CGC	GTG	TTT	TGC	CTG	CTC	GCC	GTG	GCT	CCT	45
1	M	D	W	T	W	R	V	F	C	L	L	A	V	A	P	15
46	GGG	GCC	CAC	AGC	CAG	GTA	CAG	CTG	GTG	CAG	TCT	GGG	GGA	GGC	ATG	90
16	G	A	H	S	Q	V	Q	L	V	Q	S	G	G	G	M	30
91	GTA	GAG	CCT	GGG	GGG	TCC	CTT	AGA	CTC	TCC	TGT	GCA	GCC	TCT	GGA	135
31	V	E	P	G	G	S	L	R	L	S	C	A	A	S	G	45
136	TTC	ACT	TTC	AGT	AAT	GCC	TGG	ATG	AGC	TGG	GTC	CGC	CAG	GCT	CCA	180
46	F	T	F	S	N	A	W	M	S	W	V	R	Q	A	P	60
181	GGG	AAG	GGG	CTG	GAG	TGG	GTT	GGC	CGT	ATA	AAA	AGC	AAA	GCT	GGT	225
61	G	K	G	L	E	W	V	G	R	I	K	S	K	A	G	75
226	GGT	GGG	ACA	GCA	GAG	TAC	GCT	GCA	CCC	GTG	AAA	GGC	AGA	TTC	ACC	270
76	G	G	T	A	E	Y	A	A	P	V	K	G	R	F	T	90
271	ATC	TCA	AGA	GAT	GAT	TCA	CAA	AAC	ACG	CTG	TAT	CTG	CAA	ATG	AAC	315
91	I	S	R	D	D	S	Q	N	T	L	Y	L	Q	M	N	105
316	AGC	CTG	AAA	ACC	GAC	GAC	ACA	GCC	GTG	TAT	TAC	TGT	ACC	ACA	CAT	360
106	S	L	K	T	D	D	T	A	V	Y	Y	C	T	T	H	120
361	GTC	TAC	GGT	GCC	CCC	CGG	AAC	TGG	GGC	CAG	GGA	TCC	CTG	GTC	ACC	405
121	V	Y	G	A	P	R	N	W	G	Q	G	S	L	V	T	135
406	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	AAG	CTT	GAA	GAA	GGT	GAA	450
136	V	S	S	A	S	T	K	G	P	K	L	E	E	G	E	150
451	TTT	TCA	GAA	GCA	CGC	GTA	CAG	TCT	GTG	TTG	ACT	CAG	CCG	CCC	TCA	495
151	F	S	E	A	R	V	Q	S	V	L	T	Q	P	P	S	165
496	GTG	TCT	GCG	GCC	CCA	GGA	CAG	AAG	GTC	ACC	ATC	TCC	TGC	TCT	GGA	540
166	V	S	A	A	P	G	Q	K	V	T	I	S	C	S	G	180
541	AGC	AGC	TCC	AAC	ATT	GGA	AAT	AAT	TAT	GTC	TCC	TGG	TAC	GTT	CAA	585
181	S	S	S	N	I	G	N	N	Y	V	S	W	Y	V	Q	195
586	CTC	CCA	GGA	ACA	GCC	CCC	AAA	CTC	CTC	ATT	TAT	GAC	AAT	AAT	AAG	630
196	L	P	G	T	A	P	K	L	L	I	Y	D	N	N	K	210
631	CGA	TTC	TCA	GGA	GTT	CCT	GAC	CGA	TTC	TCT	GGC	TCC	AAG	TCT	GGC	675
211	R	F	S	G	V	P	D	R	F	S	G	S	K	S	G	225
676	ACG	TCA	GCC	ACC	CTG	GGC	ATC	ACC	GGG	CTC	CAG	ACT	GGG	GAC	GAG	720
226	T	S	A	T	L	G	I	T	G	L	Q	T	G	D	E	240
721	GCC	GAT	TAT	TAC	TGC	GGA	GCA	TGG	GAT	GGC	AGC	CTG	CGT	GAA	GCG	765
241	A	D	Y	Y	C	G	A	W	D	G	S	L	R	E	A	255

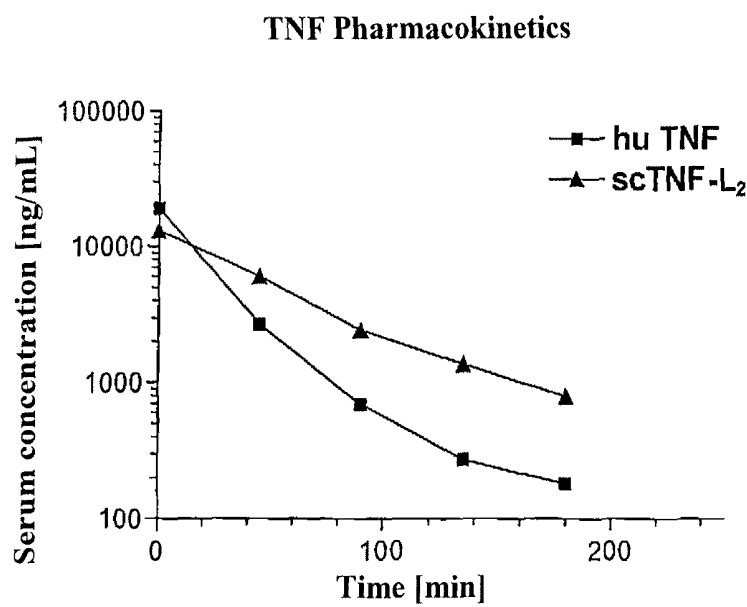
Figure 26A

766	GTA	TTC	GGC	GGA	GGG	ACC	AAG	GTC	ACC	GTC	CTA	GGT	GCG	GCC	GCA	810
256	V	F	G	G	G	T	K	V	T	V	L	G	A	A	A	270
811	GTT	GAG	CTC	GAG	GCG	GCC	GCG	GAT	TAC	AAA	GAC	GAT	GAC	GAT	AAA	855
271	V	E	L	E	A	A	A	D	Y	K	D	D	D	D	K	285
856	GAA	TTC	GGA	TCA	TCT	TCT	CGA	ACC	CCG	AGT	GAC	AAG	CCT	GTA	GCC	900
286	E	F	G	S	S	S	R	T	P	S	D	K	P	V	A	300
901	CAT	GTT	GTA	GCA	AAC	CCT	CAA	GCT	GAG	GGG	CAG	CTC	CAG	TGG	CTG	945
301	H	V	V	A	N	P	Q	A	E	G	Q	L	Q	W	L	315
946	AAC	CGC	CGG	GCC	AAT	GCC	CTC	CTG	GCC	AAT	GGC	GTG	GAG	CTG	AGA	990
316	N	R	R	A	N	A	L	L	A	N	G	V	E	L	R	330
991	GAT	AAC	CAG	CTG	GTG	GTG	CCA	TCA	GAG	GGC	CTG	TAC	CTC	ATC	TAC	1035
331	D	N	Q	L	V	V	P	S	E	G	L	Y	L	I	Y	345
1036	TCC	CAG	GTC	CTC	TTC	AAG	GGC	CAA	GGC	TGC	CCC	TCC	ACC	CAT	GTG	1080
346	S	Q	V	L	F	K	G	Q	G	C	P	S	T	H	V	360
1081	CTC	CTC	ACC	CAC	ACC	ATC	AGC	CGC	ATC	GCC	GTC	TCC	TAC	CAG	ACC	1125
361	L	L	T	H	T	I	S	R	I	A	V	S	Y	Q	T	375
1126	AAG	GTC	AAC	CTC	CTC	TCT	GCC	ATC	AAG	AGC	CCC	TGC	CAG	AGG	GAG	1170
376	K	V	N	L	L	S	A	I	K	S	P	C	Q	R	E	390
1171	ACC	CCA	GAG	GGG	GCT	GAG	GCC	AAG	CCC	TGG	TAT	GAG	CCC	ATC	TAT	1215
391	T	P	E	G	A	E	A	K	P	W	Y	E	P	I	Y	405
1216	CTG	GGA	GGG	GTC	TTC	CAG	CTG	GAG	AAG	GGT	GAC	CGA	CTC	AGC	GCT	1260
406	L	G	G	V	F	Q	L	E	K	G	D	R	L	S	A	420
1261	GAG	ATC	AAT	CGG	CCC	GAC	TAT	CTC	GAC	TTT	GCC	GAG	TCT	GGG	CAG	1305
421	E	I	N	R	P	D	Y	L	D	F	A	E	S	G	Q	435
1306	GTC	TAC	TTT	GGG	ATC	ATT	GCC	CTG	GGT	GGC	GGT	TCT	GGT	GGC	GGT	1350
436	V	Y	F	G	I	I	A	L	G	G	G	S	G	G	G	450
1351	TCT	GGT	GGC	GGT	TCT	GGT	GGC	GGA	TCA	TCA	TCT	TCT	CGA	ACC	CCG	1395
451	S	G	G	G	S	G	G	G	S	S	S	S	R	T	P	465
1396	AGT	GAC	AAG	CCT	GTA	GCC	CAT	GTT	GTA	GCA	AAC	CCT	CAA	GCT	GAG	1440
466	S	D	K	P	V	A	H	V	V	A	N	P	Q	A	E	480
1441	GGG	CAG	CTC	CAG	TGG	CTG	AAC	CGC	CGG	GCC	AAT	GCC	CTC	CTG	GCC	1485
481	G	Q	L	Q	W	L	N	R	R	A	N	A	L	L	A	495
1486	AAT	GGC	GTG	GAG	CTG	AGA	GAT	AAC	CAG	CTG	GTG	GTG	CCA	TCA	GAG	1530
496	N	G	V	E	L	R	D	N	Q	L	V	V	P	S	E	510
1531	GGC	CTG	TAC	CTC	ATC	TAC	TCC	CAG	GTC	CTC	TTC	AAG	GGC	CAA	GGC	1575
511	G	L	Y	L	I	Y	S	Q	V	L	F	K	G	Q	G	525
1576	TGC	CCC	TCC	ACC	CAT	GTG	CTC	CTC	ACC	CAC	ACC	ATC	AGC	CGC	ATC	1620
526	C	P	S	T	H	V	L	L	T	H	T	I	S	R	I	540

Figure 26B

1621	GCC	GTC	TCC	TAC	CAG	ACC	AAG	GTC	AAC	CTC	CTC	TCT	GCC	ATC	AAG	1665
541	A	V	S	Y	Q	T	K	V	N	L	L	S	A	I	K	555
1666	AGC	CCC	TGC	CAG	AGG	GAG	ACC	CCA	GAG	GGG	GCT	GAG	GCC	AAG	CCC	1710
556	S	P	C	Q	R	E	T	P	E	G	A	E	A	K	P	570
1711	TGG	TAT	GAG	CCC	ATC	TAT	CTG	GGA	GGG	GTC	TTC	CAG	CTG	GAG	AAG	1755
571	W	Y	E	P	I	Y	L	G	G	V	F	Q	L	E	K	585
1756	GGT	GAC	CGA	CTC	AGC	GCT	GAG	ATC	AAT	CGG	CCC	GAC	TAT	CTC	GAC	1800
586	G	D	R	L	S	A	E	I	N	R	P	D	Y	L	D	600
1801	TTT	GCC	GAG	TCT	GGG	CAG	GTC	TAC	TTT	GGG	ATC	ATT	GCC	CTG	GGT	1845
601	F	A	E	S	G	Q	V	Y	F	G	I	I	A	L	G	615
1846	GGC	GGT	TCT	GGT	GGC	GGT	TCT	GGT	GGC	GGT	TCT	GGT	GGC	GGA	TCA	1890
616	G	G	S	G	G	G	S	G	G	G	S	G	G	G	S	630
1891	TCA	TCT	TCT	CGA	ACC	CCG	AGT	GAC	AAG	CCT	GTA	GCC	CAT	GTT	GTA	1935
631	S	S	S	R	T	P	S	D	K	P	V	A	H	V	V	645
1936	GCA	AAC	CCT	CAA	GCT	GAG	GGG	CAG	CTC	CAG	TGG	CTG	AAC	CGC	CGG	1980
646	A	N	P	Q	A	E	G	Q	L	Q	W	L	N	R	R	660
1981	GCC	AAT	GCC	CTC	CTG	GCC	AAT	GGC	GTG	GAG	CTG	AGA	GAT	AAC	CAG	2025
661	A	N	A	L	L	A	N	G	V	E	L	R	D	N	Q	675
2026	CTG	GTG	GTG	CCA	TCA	GAG	GGC	CTG	TAC	CTC	ATC	TAC	TCC	CAG	GTC	2070
676	L	V	V	P	S	E	G	L	Y	L	I	Y	S	Q	V	690
2071	CTC	TTC	AAG	GGC	CAA	GGC	TGC	CCC	TCC	ACC	CAT	GTG	CTC	CTC	ACC	2115
691	L	F	K	G	Q	G	C	P	S	T	H	V	L	L	T	705
2116	CAC	ACC	ATC	AGC	CGC	ATC	GCC	GTC	TCC	TAC	CAG	ACC	AAG	GTC	AAC	2160
706	H	T	I	S	R	I	A	V	S	Y	Q	T	K	V	N	720
2161	CTC	CTC	TCT	GCC	ATC	AAG	AGC	CCC	TGC	CAG	AGG	GAG	ACC	CCA	GAG	2205
721	L	L	S	A	I	K	S	P	C	Q	R	E	T	P	E	735
2206	GGG	GCT	GAG	GCC	AAG	CCC	TGG	TAT	GAG	CCC	ATC	TAT	CTG	GGA	GGG	2250
736	G	A	E	A	K	P	W	Y	E	P	I	Y	L	G	G	750
2251	GTC	TTC	CAG	CTG	GAG	AAG	GGT	GAC	CGA	CTC	AGC	GCT	GAG	ATC	AAT	2295
751	V	F	Q	L	E	K	G	D	R	L	S	A	E	I	N	765
2296	CGG	CCC	GAC	TAT	CTC	GAC	TTT	GCC	GAG	TCT	GGG	CAG	GTC	TAC	TTT	2340
766	R	P	D	Y	L	D	F	A	E	S	G	Q	V	Y	F	780
2341	GGG	ATC	ATT	GCC	CTG	TGA										2358
781	G	I	I	A	L	*										785

Figure 26C

**Figure 27**

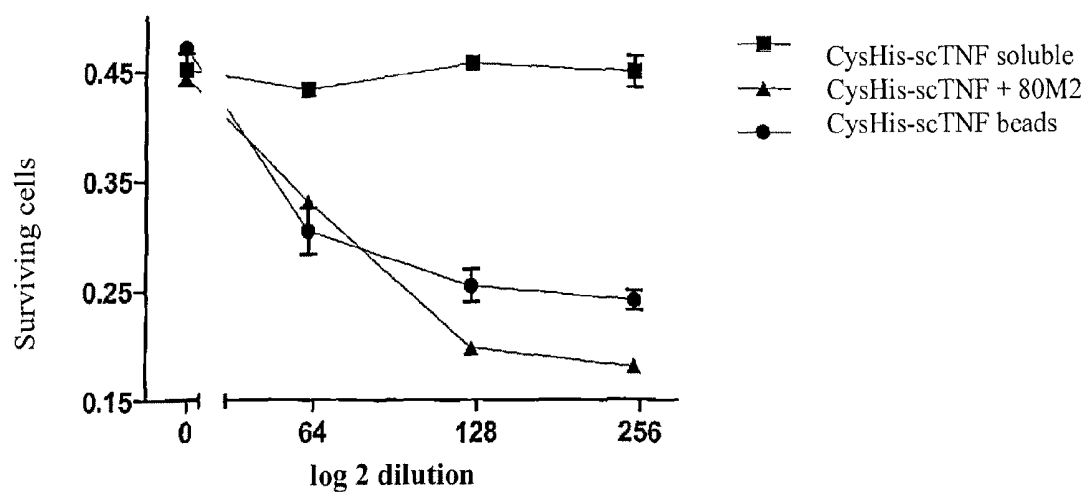


Figure 28

RECOMBINANT POLYPEPTIDES OF THE MEMBERS OF THE TNF LIGAND FAMILY AND USE THEREOF

This application is the National Phase of International Application PCT/EP2005/003158 filed Mar. 24, 2005 which designated the U.S. and that International Application was published under PCT Article 21(2) in English, and claims priority to German Application Serial No. 10 2004 014 983.6, filed Mar. 26, 2004

The present invention relates to polypeptides, which comprise at least three monomers of a member of the TNF ligand family as component A and at least two peptide linkers as component B, whereby the peptide linkers link the monomers of the member of the TNF ligand family to one another. Furthermore, the present invention relates to the use of these polypeptides for the treatment of diseases and for the preparation of a medication or vaccine. Moreover, the invention relates to methods for preparing and isolating these polypeptides and to the nucleic acids coding for the polypeptides, to vectors containing these nucleic acids, to host cells transfected with these vectors, and pharmaceutical compositions, containing these objects of the invention. Finally, the invention relates methods for the extracorporeal manipulation, depletion, and/or removal of components present in body fluids, e.g., by apheresis.

Members of the TNF ligand family are proinflammatory cytokines. Cytokines in general and members of the TNF ligand family in particular play a important role in the stimulation and coordination of the innate immune system and the humoral (antibody-mediated) immune response, the induction of apoptosis, synthesis of bone, formation of anlagen for hair growth, tooth growth, and sweat gland development, the anlage for lymph nodes, and many more (Aggarwal, B. B. (2003), *Nat. Rev. Immunol.* 3, 745-756). Defective regulation of members of the TNF ligand family, on the contrary, can lead to numerous pathological conditions. These include, for example, septic shock, autoimmune diseases, such as rheumatoid arthritis, or neurodegenerative diseases. The tumor necrosis factor (TNF) is the eponymous and arguably the most important member of this large cytokine family.

Members of the TNF ligand family exert their action in their biologically active form as homotrimers (Banner, D. W. et al., (1993) *Cell* 73, 431-445). Many trimeric structures and also aggregations of a higher order (e.g., oligomers or multimers of trimers) of proteins are encountered in nature. Examples are the cartilage matrix protein (CMP), a connective tissue protein (Beck et al. (1996), *J Mol Biol* 256, 909-923), proteins from the collagen family, such as the Clq family, which includes Clq, collagen $\alpha 1$ (X), $\alpha 2$ (VII), the hibernation protein, ACRP30, the inner ear protein, celinebrin [sic, cerebellin ?], and multimerin (Kishore and Reid, (1999), *Immunopharmacol.* 42, 15-21), and proteins of the collectin family, such as the lung surfactant protein A (SP-A) and the mannose binding protein (MBP) (Epstein et al. (1996), *Current Opinion in Immunology*, Vol 8 No. 1, 29-35).

The assembly of proteins into a trimer occurs at the surfaces of these proteins, which trimerize in solution due to interactions, such as hydrophobic interactions, hydrogen bridge formation, covalent bonds (e.g., disulfide bridges), and/or Coulomb forces, but also due to structural motifs, i.e., characteristic amino acid sequences that bring about the formation of intermolecular supersecondary structures. In the case of members of the TNF ligand family, the three monomers in the homotrimeric structure are held together non-covalently by hydrophobic bonds. In their activated form, they in turn activate their opposite members of the TNF

receptor family, which have no enzymatic activity as such. For example, TNF as a member of the TNF ligand family binds to the two membrane receptors TNFR1 and TNFR2 and mediates the trimerization of receptors or the activation of receptors already in trimer form but signal-inactive. The complex formation of receptors initiates a signal cascade, which is accompanied, inter alia, by an association of cytoplasmic adapter proteins (Wajant, H. et al (2003), *Cell Death Differ.* 10, 45-65). The trimeric structure of TNFR1 and TNFR2 forms in such a way that the receptors each bind in the interspace between two of the three TNF monomers of the TNF homotrimers (Banner et al. (1993), *supra*). It clear from this that both TNF and the other members of the TNF ligand family are biologically active only in their structure as homotrimers.

Because of their function, the members of the TNF ligand family or their membrane receptors can be used variously for the treatment of numerous diseases, such as infectious and inflammatory diseases, metabolic diseases, diseases based on defective regulation of apoptosis, neurodegenerative diseases, and many other diseases. Their use in the treatment of cancer diseases plays an especially important role, because members of the TNF ligand family are usually substances exhibiting antitumor activity. To be noted in particular in this regard are TNF itself (Eggermont, A. M. and ten Hagen, T. L. (2003), *Curr. Oncol. Rep.* 5, 79-80), TRAIL (TNF-related apoptosis-inducing ligand), also called Apo 2L (Weley et al. (1995), *Immunity* 6: 673-682; Petti et al. (1996) *J Biol Chem* 271: 12687-12689), and FasL. In vivo studies, however, showed severe systemic adverse effects of TNF and agonists of the Fas receptor and in vitro studies also indicate similar toxic effects for certain TRAIL preparations (Jo et al. (2000) *Nat Med* 6: 564-567, Ichikawa et al. (2001) *Nat Med* 7: 954-960; Ogasawara et al. (1993) *Nature* 364: 806-809). For example, agonistic antibodies to Fas, the FasL receptor, exhibited an extremely hepatotoxic effect (Ogasawara et al. (1993), *supra*). For this reason, in the case of Fas-activating ligands/agonists, clinical use has been ruled out thus far for reasons of safety. Because of the considerable importance of TNF, TRAIL, FasL, and other TNF ligand family members in this field and the adverse effects associated with their administration in the form of clinical systemic dosing, however, several approaches were pursued to minimize these adverse effects. (Eggermont, A. M. and ten Hagen, T. L. (2003), *Curr. Oncol. Rep.* 5, 79-80.)

Thus, for example, WO 02/22680 describes fusion proteins, which enable a directed and tissue-specific or cell-specific effect of cytokines by fusion of the cytokine with an antigen-binding antibody. The result achieved in this way is that the cytokines exert no effect on tissues or cells not coming into contact with these fusion proteins and that adverse effects on these tissues or cells are reduced.

DE 102 47 755 discloses an antibody-independent system, which also enables a directed and tissue-specific or cell-specific action of the cytokines. This refers to fusion proteins, in which the activation of the protein segment, contained therein, with biological function occurs via its binding to a cell-surface molecule binding domain, which represents another protein segment of the fusion protein. Apart from a reduction of adverse effects on non-target tissue, it is also possible to use this system advantageously for cell-surface molecules on the target cells, for which no antibodies or antibodies with a too low specificity are available.

Apart from the noted adverse effects, however, it is also very problematic that the active homotrimers of the members of the TNF ligand family dissociate in dilutions, and this means in physiologically meaningful concentrations as well.

This dissociation is in fact basically reversible, but the protein rapidly loses its bioactivity, because it denatures. It is assumed that this denaturation occurs via the step of unstable monomers (Smith, R. A. and Baglioni, C. (1987), *J. Biol. Chem.* 262, 6951-6954; Narhi, L. O. and Arakawa, T. (1987), *Biochem. Biophys. Res. Commun.* 147, 740-746).

Because of similar observations with TRAIL, which is especially labile, efforts were made to achieve a stability of the protein by addition of a leucine zipper as a trimerization module (Cha, S. S. et al., (1999), *Immunity* 11, 253-261). In its native state, TRAIL is stabilized by a zinc ion, which sits in the center of the trimeric ligand, and which is coordinated by cysteine residues (Hymowitz, S. G. et al. (2000), *Biochemistry* 39, 633-640). It can be a disadvantage here, however, that the addition of the leucine zipper not only achieves increased stability, but could also have a detrimental effect on other properties, e.g., structural properties such as structural changes, activity rates, or physiological properties.

There is a need, therefore, to increase the stability of active cytokines, particularly members of the TNF ligand family, without detrimentally affecting their native properties by a change in their structure, and without these substances exhibiting potent cytotoxic or other adverse effects in therapeutic applications.

No systems that achieve this are known in the related art, i.e., by which the members of the TNF ligand family are provided in an active and stable form, said members which are suitable for therapeutic application without the aforementioned disadvantages by themselves or as biologically active components of complex fusion proteins.

SUMMARY OF THE INVENTION

The object of the present invention is to provide a system by which the stability of the members of the TNF ligand family is increased.

This object is attained by the embodiments of the present invention as characterized in the claims. This object is attained in particular by the subject of claim 1, namely, by the provision of a polypeptide, which comprises at least three components A and at least two components B, whereby each component A is a monomer of a member of the TNF ligand family or a functional fragment, and/or a functional variant thereof and each component B is a peptide linker.

The present invention is based on the knowledge that naturally occurring soluble cytokine members of the TNF ligand family exhibit their full bioactivity only as homotrimers, but, on the other hand, tend to denature via dissociation of their monomers.

It is therefore imperative to prevent this dissociation of the homotrimers into monomers. This is achieved according to the invention in that at least three monomers of a member of the TNF ligand family, particularly TNF, were bound covalently to one another via their C terminals and N terminals by means of short peptide linkers to form a "single-chain" molecule ("sc" hereafter), particularly to form an scTNF. According to the invention, therefore, the entire molecule (at least three monomers of a member of the TNF ligand family with the two peptide linkers) consists of a single protein strand, so that dissociation into monomers can no longer occur. It was demonstrated according to the invention that such molecules of the invention exhibit very low loss of their bioactivity in comparison to their corresponding soluble wild-type members of the TNF ligand family. In contrast, it was not only demonstrated according to the invention that the polypeptides of the present invention have the same (qualitative) activities as their corresponding soluble wild-type mem-

ber of the TNF ligand family, but also that because of their considerably higher stability they still exhibit bioactivities at a time when the soluble wild-type member of the TNF ligand family has already lost its activity, i.e., is disassociated or denatured (reference on this point is made to the various stability tests described hereafter, which are described in the examples and in the figures).

A "soluble wild-type member of the TNF ligand family" is to be understood as a soluble extracellular segment of a membrane-associated member of the TNF ligand family. The expressions "wild type", "wt", "soluble," and "s" (for "soluble") are used hereafter synonymously with the term "soluble wild type." These can be in particular soluble wild-type TNF (as a member of the TNF ligand family), for which accordingly the synonymous terms "wild-type TNF," "wtTNF", soluble TNF, and "sTNF" are used hereafter.

A component A within the meaning of the invention is a monomer of a member of the TNF ligand family or a functional fragment or a functional variant thereof. A "monomer" is to be understood as the smallest protein unit or polypeptide unit that can be separated from an oligomeric protein without breaking the covalent bonds.

A polypeptide or a component A or a fragment or a variant thereof is functional within the meaning of the invention, provided it exhibits its biological activity or function, particularly its binding property to an interaction partner, e.g., a membrane-associated receptor, and also its trimerization property. In the case of functional fragments and the functional variants of the invention, these biological functions can in fact be changed, e.g., with respect to their specificity or selectivity, but with retention of the basic biological function.

Numerous methods for measuring the biological activity of a protein, polypeptide, or molecule are known from the related art, for example, protein assays, which use labeled substrates, substrate analyses by chromatographic methods, such as HPLC or thin-layer chromatography, spectrophotometric methods, etc. (see, e.g., Maniatis et al. (2001) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

A fragment within the meaning of the invention is to be understood as both a fragment of a monomer of a member of the TNF ligand family and a fragment of a polypeptide or protein of the present invention. These can be N-terminally, C-terminally, or intrasequentially shortened amino acid sequences of the monomer, polypeptide, or protein. In particular, the intrasequential shortenings of the polypeptide or protein may be shortenings of the sequence of one or more of the three monomers, which in turn can occur N-terminally, C-terminally, or intrasequentially.

In an especially preferred embodiment of the invention, the fragment of a monomer represents its extracellular domain, which corresponds to the entire extracellular domain of the soluble wild-type member of the TNF ligand family or a segment thereof. In particular, the fragment represents a monomer of its extracellular domain, which corresponds either to the soluble wild-type TNF (amino acids 77-233) or the entire extracellular domain (amino acids 53-233).

The preparation of such fragments of the invention is well known from the related art and can be performed by a person skilled in the art with use of standard methods (see, e.g., Maniatis et al. (2001), *Molecular Cloning: Laboratory Manual*, Cold Spring Harbor Laboratory Press). In general, the fragments of the monomers, polypeptides, or proteins can be prepared by modification of the DNA sequence, coding for the native monomer, polypeptide, or protein, followed by a transformation of this DNA sequence in a suitable host, and expression of this modified DNA sequence, provided that the

modification of the DNA does not disrupt the functional activities of the monomer, polypeptide, or protein.

The identification of a fragment of the invention can occur either by testing its functionality by measuring its biological activity, as described above, or based on a sequencing of the fragment and a subsequent comparison of the obtained sequence with the native sequence. The sequencing can occur using standard methods, which are well-known and numerous in the related art.

In particular, monomers, polypeptides, or proteins, or fragments thereof that have sequence differences relative to the corresponding native sequences are designated as variants of biologically active monomers, polypeptides, or proteins, or fragments thereof, or a component A within the meaning of the invention. These sequence deviations can be one or more insertion(s), deletion(s), and/or substitution(s) of amino acids, whereby there is a sequence homology of at least 60%, preferably 70%, more preferably 80%, also more preferably 85%, even more preferably 90%, and most preferably 97%.

To determine the percentage identity of two nucleic acid or amino acid sequences, the sequences can be aligned in order to be compared subsequently with one another. To this end, e.g., gaps can be introduced into the sequence of the first amino acid or nucleic acid sequence and the amino acids or nucleotides compared at the corresponding position of the second amino acid or nucleic acid sequence. If a position in the first amino acid sequence is occupied by the same amino acid or the same nucleotide, as is the case at a position in the second sequence, then both sequences are identical at this position. The percentage identity between two sequences is a function of the number of identical positions divided by the sequences.

The determination of the percentage identity of two sequences can be performed using a mathematical algorithm. A preferred, but not limiting example of a mathematical algorithm, which can be used for comparing two sequences, is the algorithm of Karlin et al. (1993), PNAS USA, 90:5873-5877. This type of algorithm is integrated into the NBLAST program, which can identify the sequences that have a desired identity to the sequences of the present invention. To obtain a gapped alignment, as described above, the "gapped BLAST" program can be used, as described in Altschul et al. (1997), Nucleic Acids Res, 25:3389-3402.

Biologically active, therefore functional, variants of monomers, polypeptides, or proteins, or fragments thereof within the meaning of the invention can preferably have selective receptor binding properties, whereby the variant can be optimized, e.g., with respect to their specific bioactivity or other properties, particularly their stability.

The term variants includes particularly amino acid sequences with conservative substitution compared with physiological sequences. Conservative substitutions are substitutions in which amino acids stemming from the same class are exchanged. In particular, there are amino acids with aliphatic side chains, positively or negatively charged side chains, aromatic groups in the side chains, or amino acids whose side chains may contain hydrogen bridges, for example, side chains with a hydroxy function. This means that, for example, an amino acid with a polar side chain is replaced by another amino acid with a likewise polar side chain or, for example, an amino acid characterized by a hydrophobic side chain is substituted by another amino acid with a likewise hydrophobic side chain (e.g., serine (threonine) by threonine (serine) or leucine (isoleucine) by isoleucine (leucine)). Insertions and substitutions are possible particularly at sequence positions that cause no change in the three-dimensional structure or affect the binding region. A

change in a three-dimensional structure by insertion(s) or deletion(s) can be easily checked, for example, with the use of CD spectra (circular dichroism spectra) (Urry, 1985, Absorption, Circular Dichroism and ORD of Polypeptides, in: Modern Physical Methods in Biochemistry, Neuberger et al. (eds.), Elsevier, Amsterdam).

Suitable methods for the preparation of variants, e.g., of monomers, polypeptides, or proteins, or fragments thereof with amino acid sequences that have substitutions compared with the native sequences, are disclosed, for example, in the publications U.S. Pat. No. 4,737,462, U.S. Pat. No. 4,588,585, U.S. Pat. No. 4,959,314, U.S. Pat. No. 5,116,943, U.S. Pat. No. 4,879,111, and U.S. Pat. No. 5,017,691. The preparation of variants in general is also described particularly by Maniatis et al. (2001), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press). In this case, codons can be omitted, added, or replaced. Variants can also be particularly proteins or polypeptides that are stabilized to escape physiological degradation, for example, by stabilization of the protein backbone by substitution of amide-like bonds, for example, also by the use of β -amino acids.

Variants within the meaning of the invention can also be prepared by introducing changes into the nucleic acids, coding for the variants, such as, for example, insertions, deletions, and/or substitutions in one or more nucleotides. Numerous methods for this type of changes in nucleic acid sequences are known in the related art. One of the most frequently used techniques is oligonucleotide-directed, site-specific mutagenesis (see Comack B., Current Protocols in Molecular Biology, 8.01-8.5.9, Ausubel F. et al., 1991 edition). An oligonucleotide whose sequence has a specific mutation is synthesized in this technique. This oligonucleotide is then hybridized with a template that contains the wild-type nucleic acid sequence. A single-stranded template is used preferentially in this technique. After annealing of oligonucleotide and template, a DNA-dependent DNA polymerase is used to synthesize the second strand of the oligonucleotide, which is complementary to the template-DNA strand. A heteroduplex molecule is obtained as a result, which contains a mispairing formed due to the aforementioned mutation in the oligonucleotide. The oligonucleotide sequence is then introduced into a suitable plasmid, this is introduced into a host cell, and the oligonucleotide DNA is replicated in this host cell. This technique produces nucleic acid sequences with selective changes (mutations), which can be used for preparing variants according to the invention.

Components A covered by the polypeptides of the invention can be identical or different; i.e., components A can be monomers of the same member of the TNF ligand family or different members of the TNF ligand family. For example, three different components A can be three monomers of three different members of the TNF ligand family or two monomers of the same member of the TNF ligand family and one monomer of another member of the TNF ligand family. This applies accordingly to more than three components A. For example, a polypeptide of the invention can contain 4, 5, 6, or more components A, which form a tetramer, pentamer, hexamer, etc. It is also especially preferred for the polypeptide of the invention to contain an integer multiple of a trimer, as previously described, of component A, e.g., two, three, four, or more trimers arranged one after another. Components A present in the polypeptides of the invention can be separated by linkers from one another (see below). If, as described above, two, three, four, or more trimers of components A are arranged one after another, the linkers binding the different

trimers to one another can be longer, if necessary, than the linkers binding components A in a single trimer to one another.

Components A of the invention can stem from the same or different organisms. These can be vertebrates, particularly mammals, for example, the mouse, rat, pig, and primarily humans.

In a preferred embodiment of the invention, components A of the polypeptide of the invention are in each case a monomer of one of the members of the TNF ligand family or a functional fragment or a functional variant thereof, selected from the group consisting of FasL (GenBank Accession No. NM_000639), TRAIL (TNF-Related Apoptosis-Inducing Ligand; GenBank Accession No. NM_003810), also called Apo2L, TNF (tumor necrosis factor; GenBank Accession No. NM_000594), LT alpha (GenBank Accession No. NM_000595), lymphotoxin beta (GenBank Accession No. NM_002341), NGF (GenBank Accession No. NM_002506), CD30L (CD153; GenBank Accession No. NM_001244), CD40L (CD 154; GenBank Accession No. NM_00074), OX40L (GenBank Accession No. NM_003326), RANKL (GenBank Accession No. NM_003701), TWEAKL (GenBank Accession No. NM_003809), LTalpha, LTbeta, LIGHT (GenBank Accession No. NM_003807), CD27L (GenBank Accession No. NM_001252), 4-1BBL (GenBank Accession No. NM_003811), GITRL (GenBank Accession No. NM_005092), APRIL (GenBank Accession No. NM_172089), EDA (GenBank Accession No. NM_001399), VEGI (GenBank Accession No. NM_005118), and BAFF (GenBank Accession No. NM_006573).

In an especially preferred embodiment, component A, as defined above, of the polypeptide of the invention is a monomer of one of the members of the TNF ligand family, which is resistant to the processing enzyme TACE. TACE is a member of the ADAM protease family and represents the physiological TNF-specific processing enzyme of TNF found naturally in the cell membrane. TNF is typically cleaved from the cell membrane and released into the environment. A TACE-resistant component A of the invention preferably lacks the Ala-Val cleavage site (e.g., AA 76-77 in scTNF according to the SWISS-PROT nomenclature for human TNF, No. P1375). The cleavage site can be removed in this case according to the invention by deletion of one or both relevant amino acids Ala or Val. Alternatively or in addition, according to the invention, the recognition sequence for TACE (e.g., AA 77-88 in scTNF according to the SWISS-PROT nomenclature for human TNF, No. P1375) may be totally or partially deleted. This occurs preferably by deletion of two, three, four, or more amino acids, for example, all amino acids of the TACE recognition sequence. For scTNF, for example, the TACE-resistant sequence is in the region of amino acids 89-233 (AA 89-233) of scTNF according to the SWISS-PROT nomenclature for human TNF, No. P1375. One such deletion in component A prevents a potential cleavage of component A, e.g., scTNF, by TACE near the linkage sites of components A in the area of the linkers (component B, see below), and thereby increases the in vivo stability of the polypeptides of the invention. If necessary, the shortening of the sequence, arising due to the deletion, can be compensated by an appropriate lengthening of the linkers. The deletion of the component A sequence, described as an example for scTNF, can be used for each of the aforementioned components A. If the TACE sequence is not to be removed or to be only partially removed, care must be taken preferably that during use of additional components B or C (see below) in the polypeptides

of the invention, the TACE recognition sequence and the TACE cleavage site are not restored.

In another especially preferred embodiment, components A of the polypeptide of the invention, as defined above, are modified in such a way that the polypeptides of the invention can couple covalently to surfaces. This coupling occurs preferably to planar surfaces or to spherical particles, such as, e.g., to magnetic particles (magnetic beads), or to nanoparticles/nanoparticles, preferably as a cell-mimetic, therapeutic reagent with properties similar to membrane-bound TNF. For coupling, a coupling group, preferably the SH group of a cysteine residue, is introduced in the N-terminal region of at least one component A of the polypeptide of the invention. (Other preferred coupling groups of the invention are described further below). This can occur especially preferably by the introduction of a cysteine residue in the form of an addition and/or substitution at the desired position. The coupling group can occur at any position of the N-terminal region of component A, preferably near the N terminus. Especially preferably, the coupling group is located in a region of the first 1-15 N-terminal amino acids, and more greatly preferred in a region of the first 1-10 N-terminal amino acids. For example, the modification in a prokaryotically expressed scTNF can be introduced after an initial methionine (position 2) of the amino acid sequence or in a eukaryotically expressed scTNF directly after the cleaved leader sequence; these therefore represent the N-terminal amino acids of component A. Alternatively, in a tag used according to the invention, for example, a His tag, a Flag tag (see, e.g., FIG. 18), a coupling group is introduced according to the invention, e.g., resulting in a CysHis tag with a cysteine at amino acid position 9. Another alternative consists of the introduction of a coupling group in one or both components B (linkers) of the polypeptide of the invention.

Preferably, in a polypeptide of the invention, only the component A situated at the N terminus or the tag situated farther N-terminally is provided/modified with a coupling group as described above. In another embodiment, in the case of a polypeptide of the invention, which contains 3 components A, for example, 2 or 3 of components A modified in each case with a coupling group, so that the entire molecule (the polypeptide of the invention) can be coupled via 2 or 3 covalent bonds to a matrix suitable therefor. The coupling of the polypeptide of the invention via one or more coupling groups of all components A of a polypeptide of the invention preferably does not negatively impact the function of the individual components A, e.g., of TNF, but rather enables improvement of the immobilization, for example, of scTNF, on the surface and/or particle. As previously stated, e.g., in scTNF and also in scFasL and scTRAIL or other members of the TNF family in the sc form, each of polypeptides of the invention contains preferably the subunits necessary for the biological function (components A) in a functionally relevant arrangement. As taught by the invention, it is possible to produce surfaces or particles with, e.g., coupled TNF homotrimers or heterotrimers or -multimers, by coupling of a polypeptide of the invention to a surface/particle. The coupled TNF homotrimers or heterotrimers or -multimers are immobilized stably and bioactively on these surfaces/particles. The functionally relevant trimer state of components A is stabilized by components B by the single-chain principle of the invention; this assures that after covalent coupling to a surface/particle, dissociation of individual components A is ruled out and therefore loss of biological activity is prevented.

A preferred property of this type of polypeptide coupled to surfaces or particles according to the invention is their biomimetic activity, which corresponds to that of the natural,

membrane-associated ligands of the TNF family. For example, an immobilized scTNF of the invention achieves a high affinity for TNFR2 and thereby a high signal capacity.

Polypeptides of the invention, which contain components A modified with a coupling group, as described above, and become/are coupled to a surface and/or particle, as taught by the invention, can be used for detection, as well as for manipulation, depletion, and/or removal of binding partners of members of the TNF ligand family, as well as compounds associated therewith. Of paramount interest thereby are methods for the extracorporeal manipulation, depletion, and/or removal of components present in body fluids, e.g., by apheresis.

The following statements on the subjects of the invention relate particularly to the TNF ligand family member TNF, but they can be applied to all other members of the TNF ligand family.

The polypeptides of the present invention comprise, apart from the described components A (monomers of at least one member of the TNF ligand family), at least two components B, whereby component B has the property of a peptide linker.

Any peptide sequence expressible in a biological system is conceivable as a peptide linker within the meaning of the invention. In the polypeptide constructs of the invention, peptide linkers turned out to be, for example, a flexible compound, which, however, preferably does not negatively influence the intrinsic trimerization properties of the particular member of the TNF ligand family. Preferably, linkers are chosen with properties, particularly flexibility and length, that are capable of stabilizing the spontaneously formed homotrimers of these specific ligands (derivatives).

In a preferred embodiment of the invention, component B accordingly is a peptide linker consisting of 2 to 30, preferably between 4 and 16, and especially preferably between 4 and 12 amino acids, which preferably contain repetitive glycine-serine structures, very preferably Gly-Gly-Gly-Ser modules (GGGS modules) SEQ. ID NO: 41) in a repetitive arrangement.

Preferably, the peptide linkers of the invention are therefore peptide linkers rich in glycine (G); i.e., the amino acid sequence of a peptide linker has a high proportion of glycine, preferably from 60 to 80%, especially preferably 75%.

In especially preferred embodiments of the invention, the peptide linker (component B) comprises the amino acid sequence GGGSGGGSGGGS, also called (GGGS)₃ (SEQ. ID NO: 2) or L_{short}, or the amino acid sequence GGGSGGGSGGGSGGGS [sic], also called (GGGS)₄ (SEQ. ID NO: 3) or L_{long}. The peptide linkers according to the invention are designated, inter alia, as L1 and L2, so that designations such as L1_{short}, L2_{short}, L1_{long}, and/or L2_{long} result. It is possible to use two different linkers to stabilize a trimer molecule.

Preferably, the peptide linkers according to the invention, therefore components B, in each case link together two of the at least three components A. This linkage occurs covalently via the C terminus of a component A and the N terminus of another component A. According to the invention, the polypeptide represents a single-chain molecule (also called an sc molecule). This means that all components A and components B, which comprise the polypeptide of the invention, are located on a single polypeptide strand or protein strand.

In a preferred embodiment of the invention, components A and components B form a trimeric protein structure. Preferably, this is a homotrimeric protein structure, but the invention also comprises heterotrimeric protein structures.

The formation of this trimeric protein structure is preferably effected by component B and/or enhanced by it. Because component B, which leads to trimerization, or component B,

which enhances trimerization, of the polypeptide of the invention substantially should not form any higher aggregates, component B should typically not have any cysteine residues, which can form an intermolecular disulfide bridge. Preferably, component B in a polypeptide of the invention therefore has no cysteine residue or only such cysteine residues, which have an intramolecular disulfide bridge, therefore in the polypeptide itself of the invention, in order to avoid the formation of a covalent bond with the at least one cysteine residue of a fusion protein of another trimer under oxidizing conditions.

The sequences of native polypeptides or fragments of these native polypeptides, which are used as peptide linkers of the invention, can also be present in the form of biologically active functional variants of the same within the meaning of this invention and according to the above definition.

Components B of the invention can be identical or different naturally occurring peptide sequences. They can stem from the same or different organisms. The organisms can be vertebrates, particularly mammals, for example, the mouse, rat, pig, and primarily humans.

The polypeptide of the invention can have additional sequence segments apart from components A and B. Preferred in this regard are so-called tag sequences, for example, at least one Flag tag, therefore the amino acid sequence DYKDDDDK (SEQ. ID NO: 1), and/or, for example, at least one His tag (containing several consecutive histidines, for example, at least 5) and/or other tag or antigen sequences. An especially preferred additional sequence segment is a leader peptide sequence. Preferably, this leader peptide sequence represents a signal for the secretion of the polypeptide or protein in eukaryotic cells. This leader peptide sequence is preferably located N-terminally.

In another embodiment of the invention, a polypeptide of the invention comprises another component C, whereby this component is characterized by a specific interaction with a complementary cell surface molecule ("antigen"). The principle of action of the polypeptide constructs of the invention, such as, for example, constructs of the invention with the apoptosis inducer TRAIL or FasL as component A is particularly applicable for all members of the TNF ligand family that are active as a membrane molecule for specific receptors exclusively or to a particularly good extent. Apart from TRAIL (TNFSF10), FasL (TNFSF6), and TNF (TNFSF2), these include, for example, also the immunomodulators CD40L (TNFSF5) and CD30L (TNFSF8). Component C thereby has a "targeting" function. Accordingly, component C is an antibody fragment, preferably a single-chain antibody fragment, the so-called scFv, or an antibody derivative which recognizes a specific target molecule on the cell surface. In another embodiment, component C is not an antibody-associated protein or peptide, which, in analogy to an antibody-antigen interaction or a receptor-ligand interaction, also selectively recognizes a specific target molecule on the cell surface. In an especially preferred embodiment, the present invention comprises a polypeptide of the invention, as described above, as component A at least one member of the scTNF family, as defined above, e.g., scTNF, and as component C an antibody fragment, as described above, e.g., scFv. Smaller polypeptides of the invention are formed thereby with a "targeting domain" in each case. Such smaller polypeptides of the invention are advantageously less susceptible to, e.g., aggregation.

Preferably, component C is an antigen-binding antibody fragment or an antigen-binding antibody derivative from a mammal, particularly of murine or human origin, or a humanized antibody fragment or a humanized antibody derivative,

e.g., of mammalian origin. In the case of derivatization and/or humanizing, component C typically consists of a single-chain Fv derivative, prepared according to the related art, murine component C, humanized by CDR grafting, or component C is of completely human origin which was transformed

accordingly to an scFv derivative. In another preferred embodiment, component C is a protein ligand or peptide ligand, which as a monomer enters into a specific bond at a membrane receptor.

In another preferred embodiment, component C is a protein or peptide with specificity for cell surface molecules, which is particularly a cytokine receptor, a growth factor receptor, an integrin, or cell adhesion molecule. It is especially preferred that it is a cytokine receptor, which is selected from the group of the TNFR gene family.

Component C of a polypeptide of the invention preferably exhibits specificity for an antigen selectively or predominantly expressed in tumor tissue. This type of tumor antigen can be expressed in general in the malignant cells themselves or also in the nonmalignant part of the tumor, the stromal cells or the tumor endothelium. Such antigens of nonmalignant tissue parts of a solid tumor (carcinoma) are genetically invariant, on the one hand, but on the other, they occur in the most different tumor entities and are thereby universal tumor markers. Examples of such ligands for tumor association, against which a component C of the polypeptide of the invention can be directed, are the VEGFR or the VEGFR/VEGF complex and the integrin $\alpha_v\beta_2$ and the vitronectin isoform β F α as largely selective target structures of the tumor endothelium and the fibroblast activation protein (as a selective marker of the tumor stroma). All aforementioned examples can be effectively covered by specific scFv, which is why such scFv ("single-chain Fv") are especially suitable as component C of a polypeptide of the invention. Galectin as well is regarded hereby as a tumor marker, against which component C is directed.

Accordingly, antibody fragments in different antibodies formats, for example, scFv, particularly scFv40, are particularly preferred as component C.

In another embodiment of the present invention, the polypeptide via its component C recognizes as a specific target molecule a cell membrane-associated receptor of a member of the TNF ligand family, preferably different from the TNF ligand family member of component A. Examples, which do not represent a definitive enumeration of such possible ligands, are TNFSF1 (LT α), TNFSF2 (TNF), TNFSF3 (LT β), TNFSF4 (OX40L), TNFSF5 (CD40L), TNFSF6 (FasL), TNFSF7 (CD27L), TNFSF8 (CD30L), TNFSF9 (4-1BBL), TNFSF10 (TRAIL), TNFSF11 (RANKL), TNFSF12 (TWEAKL), TNFSF13 (APRIL), TNFSF14 (BLYS), TNFSF15 (LIGHT), TNFSF16 (VEGI), TNFSF17 (CD30L), and TNFSF18 (AITRL), and EDA, which or whose functional fragments or functional variants of the native sequence or the fragments may also be used as component A in a polypeptide construct of the invention. In particular, in this regard, all membrane-associated type II proteins (C terminus extracellular), the functional fragments or functional variants thereof, which cause a trimetric organization of their subunits as a requirement for biological activity, are also disclosed.

Another subject of the present invention is a nucleic acid, which codes for a polypeptide of the invention. This also comprises nucleic acid constructs, which contain a sequence segment coding for a polypeptide of the invention or one or more additional sequence segments. Such additional sequence segments can be, for example, sequences coding for a leader peptide, which is present as a signal for the secretion

of the polypeptide or protein according to the invention in eukaryotic cells, or codes for an scFv40, i.e., the sequence of the single-chain (scFv) antibody fragment 40, which is specific for the tumor stroma antigen FAP, or for an "Antibody Mediated Apoptosis Inducing Cytokine" (AMAIze), or for a HIS/Flag tag, a peptide sequence for the affinity purification of the expressed proteins or polypeptides. This list is provided only as an example and is not definitive.

Other sequences, which may be covered by the nucleic acid sequence, are also noncoding sequences, such as noncoding 3' and 5' sequences, including, e.g., regulatory sequences.

The nucleic acid sequences of the present invention can also be fused with nucleic acid sequences, which, for example, code for a marker sequence or code for a sequence, which codes for a polypeptide, which, for example, facilitates the isolation or purification of the polypeptide of the invention. Representative sequences, for example, include those coding for a glutathione-S transferase (GST) fusion protein, a polyhistidine (e.g., HIS 6), hemagglutinin, or HSV tag. This enumeration is by no means limiting, however.

Nucleic acids of the present invention may be DNA or RNA, particularly mRNA. The nucleic acid molecules can be double- or single-stranded. Single-stranded RNA or DNA can be either the coding (sense) or the noncoding (antisense) strand.

The nucleic acids according to the invention can be present preferably isolated. This means that the nucleic acid molecule for the nucleic acid sequence is not flanked by nucleic acid sequences, which normally flank the gene or the nucleic acid sequence (as in genomic sequences) and/or which were completely or partially purified (such as, for example, in a DNA or RNA library). For example, an isolated nucleic acid of the invention can be isolated in relation to the cellular milieu in which it occurs naturally.

In a preferred embodiment, the nucleic acid comprises one of the nucleic acid sequences presented in FIGS. 19 through 26 or consist of one of these nucleic acid sequences.

According to the invention, functional fragments or functional variants of the nucleic acids or nucleic acid constructs of the invention are also covered, to which the previous statements on the terms functional, fragment, and variant accordingly apply.

The preparation of nucleic acids, nucleic acid constructs, or functional fragments, or functional variants thereof of the invention can be carried out by means of standard methods, which are known to the person skilled in the art (see, e.g., Maniatis et al. (2001), supra). The PCR technique is used particularly in this regard. The sequence of synthesized nucleic acids according to the invention can be determined using sequencing or hybridization methods, which are also familiar to the person skilled in the art.

The invention also comprises gene products of the nucleic acids of the invention. Preferably, the gene product codes for a polypeptide of the amino acid sequences shown in FIGS. 19 through 26. A gene product of the present invention relates not only to the transcript (mRNA) but also to polypeptides or proteins, preferably in purified form. Alleles, functional fragments, or functional variants of such gene products are also included. The aforementioned definitions of these terms apply accordingly to functional fragments or functional variants of the gene products.

Another subject of the present invention is a vector, which contains the nucleic acid coding for a polypeptide of the invention. Preferably, the vectors of the invention are expression vectors, i.e., vectors that have the ability to express and/or amplify the nucleic acids in a prokaryotic and/or eukaryotic cell. The present invention relates in particular to

plasmid vectors, e.g., pBABEpuro, phages, or retroviral vectors, particularly also to all vector systems, which may be used in gene therapy, e.g., also adenoviral vector systems. Within the scope of the present invention, therefore, gene therapy methods with vectors or nucleic acids or nucleic acid constructs of the invention are also disclosed as treatment methods for the disclosed medical indications of the invention.

Vectors of the invention preferably have control sequences, which enable or enhance expression of the nucleic acid of the invention and regulate transcription. Such control sequences include, for example, polyadenylation signals, promoters, e.g., natural or synthetic promoters, enhancers to effect transcription, operator sequences to regulate transcription, silencers for tissue-specific transcription, sequences coding for suitable ribosome binding sites on mRNA, sequences stabilizing mRNA, and sequences regulating the termination of transcription and/or translation. This represents only an exemplary enumeration of possible control sequences. Other possible control sequences are well known in the related art and are described, for example, by Goeddel (1990), *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, Calif. The control sequences can be modified, e.g., by deletion, addition, or substitution of one or more nucleic acids, to potentiate their control function.

Numerous different promoters for different organisms are known in particular. For example, a preferred promoter for vectors which are used in *Bacillus subtilis* is the AprE promoter; a preferred vector used in *E. coli* is the T7/Lac promoter; a preferred promoter used in *Saccharomyces cerevisiae* is PGK1; a preferred promoter used in *Aspergillus niger* is glaA; and a preferred promoter used in *Trichoderma reesei* (reesei) is cbhl. Promoters, which are suitable for use in prokaryotic host cells, include, e.g., beta-lactamase (vector pGX2907 [ATCC39344], which contains the replicon and the beta-lactamase gene), lactose promoter systems (Chang et al. (1978), *Nature* (London, 275: 615); Goeddel et al. (1979), *Nature* (London), 281: 544), alkaline phosphatase, the tryptophan (trp) promoter system (vector pATH1 [ATCC37695]), and hybrid promoters, such as the tac promoter (isolated from the plasmid pDR540 [ATCC37282]). However, other bacterial promoters, whose nucleotide sequences are generally known, enable a skilled person to ligate these with a nucleic acid of the invention, whereby linkers or adapters may also be used, to produce the desired restriction sites. Preferably, promoters which are used in bacterial systems also contain a Shine-Dalgarno sequence, which is functionally connected to the nucleic acid.

Suitable expression vectors can consist, for example, of segments of chromosomal, nonchromosomal, and synthetic DNA. Numerous derivatives of SV40 and bacterial plasmids are known for this purpose. Examples are plasmids from *E. coli*, such as colE1, pBK, pCR1, pBR322, pMb9, pUC19, and derivatives thereof, plasmids that can be used in a wide host range, such as RP4, and phage DNA, such as numerous derivatives of the lambda phage, e.g., NM989, and other DNA phages, e.g., M13, and filamentous single-stranded DNA phages, yeast plasmids, vectors, which are suitable for use in eukaryotic cells, and vectors, which consist of a combination of plasmid and phage DNA. Numerous expression techniques for use of the expression vectors of the invention are known in the related art. Such techniques are, for example, generally described in Maniatis et al. (2001), *supra*.

Another subject of the invention relates to a host cell, which contains the nucleic acid of the invention and/or a vector of the invention.

Host cells within the meaning of the invention are cells that are capable of functioning as host and expression vehicles for a nucleic acid or vector of the invention. These can be prokaryotic and eukaryotic host cells. Prokaryotic—bacterial—host cells are, for example, *Rhodothermus marinus*, *E. coli*, *Streptomyces*, *Pseudomonas*, *Bacillus*, *Serratia marcescens*, and *Salmonella thyphimurium*. Eukaryotic host cells comprise, for example, yeasts, such as *Saccharomyces cerevisiae*, *Pichia pastoris*, insect cells, such as Sf9, or mammalian cells, such as COS and CHO. These enumerations are by no means definitive. The selection of a suitable host cell depends on several factors, e.g., in the introduction of a vector into a host cell particularly on the employed vector of the invention.

A further subject of the invention relates to the preparation of a host cell of the invention, which comprises the following steps:

- (a) Preparation of a nucleic acid of the invention or a vector of the invention,
- (b) Introduction of the nucleic acid and/or vector according to step (a) into a cell.

The preparation of a nucleic acid or a vector can occur as taught by the invention by the already described standard methods and exemplary descriptions. The introduction of the nucleic acid of the invention or the vector of the invention into the host cell can occur with use of any suitable standard method. These include, for example, transformation, electroporation, transfection with the use of, e.g., calcium chloride, lipofection, infection, transduction, etc. Various standard methods are described, for example, in Maniatis et al. (2001), *supra*.

Another subject of the present invention is a method for preparing a polypeptide of the invention, whereby this method typically comprises the following steps: (a) culturing of a host cell of the invention under suitable conditions, (b) expression of the nucleic acid or nucleic acid construct of the invention under suitable conditions, and (c) isolation of the polypeptide from the host cell and/or culture supernatant.

Culturing a host cell means making it possible for the host cell to grow in a suitable culture medium, at a suitable pH, and at suitable temperatures. Such growth conditions depend on the host cells used in the specific case and are well known to the person skilled in the art. Instructions for culturing cells can also be found in Maniatis et al. (2001), *supra*.

The expression of the polypeptide can occur here typically according to the related art in suitable expression systems, preferably as a secreted product of stable transfectants, e.g., CHO-cells or other animal cells, such as Cos7 or SF9 (insect cells), or other eukaryotic cell systems, for example, *Pichia pastoris*. Preferably, the expressed polypeptides of the invention each have leader sequences suitable for secretion in the cell system. For this reason, the vectors used for expression according to the invention also contain coding segments, which code for a functional leader sequence, e.g., as described in Brocks et al. (*Immunotechnology* 3:173-184, 1997) for mammals and insect cells or during use of, for example, pPIC-Zalpha vectors (Invitrogen, Karlsruhe, Germany) for expression and secretion in the yeast *Pichia pastoris*.

The isolation of the polypeptide of the invention from the host cell can occur with use of standard methods, such as chromatography methods, precipitation methods, etc., which are suitable for purification of polypeptides and proteins (also see Maniatis et al. (2001), *supra*).

In another embodiment of the invention, the polypeptides, but optionally also nucleic acids, nucleic acid constructs, vectors, or host cells (combined here in the category of “sub-

stances of the invention”) of the invention can also be used as a medication or for the preparation of medications for the treatment of diseases.

Provided these substances of the invention comprise a component C according to the invention (see above, an antibody fragment or another specifically binding protein/peptide), their use is appropriate particularly if the substances after binding of the polypeptide or fusion protein to a specific cell membrane-expressed target molecule exhibit the full biological effect via the appropriate receptor of the member of the TNF ligand family. By suitable selection of the specificity of the targeting component C, the activity of the substance of the invention is directed to the tissue to be treated, e.g., tumor tissue, and a therapeutic agent specifically optimized/intended for the particular indication/tumor entity can be prepared. A polypeptide of the invention is accumulated, e.g., during use as a tumor therapeutic agent, particularly for the treatment of solid tumors, but also of lymphatic tumors (benign or malignant), after in vivo administration by the targeting component C initially specifically in the tumor region by membrane markers formed by the tumor itself or the reactive tumor stroma/tumor vascular system and there presented to TNF-receptor-family-positive tumor cells or sensitive cells of the reactive tumor-supplying normal tissue, sensitive to a member of the TNF ligand family.

The use of substances of the invention, however, is always basically desirable for application in the therapeutic area, when the activation of a signal transduction chain, e.g., the signal cascades triggered by the TNF receptor family, for example, an apoptotic signal cascade, is to be triggered. Thus, the substances of the invention are used in the treatment or for the preparation of a medication for the treatment of all hyperproliferative diseases, for example, also for the targeted elimination of cells of the immune system in excessive immune responses, for example, in autoimmune diseases, such as, e.g., multiple sclerosis, rheumatoid arthritis, diabetes mellitus, and TEN, or in misdirected immune responses to foreign antigens, as can occur, e.g., in infectious diseases (bacterial (for example, caused by mycobacteria), viral, or protozoan). Also possible furthermore is the treatment of metabolic diseases or general hyperinflammatory conditions, particularly chronic inflammations, for example, also in allergies but also the treatment of rejection reactions of a patient's immune system to foreign tissue. In the aforementioned cases, the polypeptide of the invention must recognize, e.g., via a component C, a characteristic marker on the surface of the target cells, in which preferably an apoptotic signal cascade is to be triggered with the goal of cell death. In the case of treatment after transplantation of foreign tissue, therefore, for example, the body's own cells responsible for the rejection reaction in the transplantation patient's immune system serve as the target cells.

In general, the substances of the invention or medications are suitable for the treatment of cancer, particularly solid or lymphatic tumors, infectious diseases, metabolic diseases, inflammatory conditions, hyperproliferative diseases, autoimmune diseases, particularly rheumatoid/arthritis diseases, toxic epidermal necrolysis (TEN), multiple sclerosis, Hashimoto's thyroiditis, GVHD, viral hepatitis (HBV, HCV), alcohol-induced hepatitis, rejection reactions in liver transplantation, diseases based on hyperapoptotic reactions, and degenerative diseases, particularly neurodegenerative diseases.

Recombinant protein is preferably administered to the patient to be treated during use of the substances of the invention as a medication for the treatment of the aforementioned diseases. Alternatively, cells are removed from the patient for

transfection; these are transfected in vitro with the (expression) vectors of the invention, cultured, and then given to the patient as a retransplant. The transfection is preferably performed with nucleic acids, nucleic acid constructs, or (expression) vectors, which couple the expression to a regulatable promoter. The transfected autotransplant, for example, can be injected locally, depending on the specific disease and the specific target cells. Local administration is preferred, for example, in the case of tumor therapy. In this case, tumor cells are removed from the patient, transfected in vitro, and then, provided this is possible, injected directly into the tumor, for example, for the treatment of skin tumors (e.g., melanomas), and tumors of the nervous system (e.g., glioblastomas).

Other subjects of the present invention relate to the use of the substances of the invention both for the preparation of a vaccine for active or passive immunization against infectious diseases, particularly against viral infectious diseases, and also for the preparation of a vaccine for vaccination against German measles, measles, poliomyelitis, rabies, tetanus, diphtheria, BCG, malaria, yellow fever, HIV, or influenza.

Substances of the invention can also be used for in vitro diagnosis.

Another subject of the present invention relates to methods for extracorporeal (ex vivo) manipulation, depletion, and/or removal of components present in body fluids, such as, e.g., binding partners of a component A, as defined above, or cells binding thereto or associated therewith. Such extracorporeal methods comprise preferably methods such as, e.g., apheresis, particularly the basic forms of apheresis, plasmapheresis and cytappheresis.

Plasmapheresis according to the invention includes extracorporeal manipulation, depletion, and/or removal of certain soluble or suspended components in the plasma fraction of blood, and the return of the thus treated blood to the patient. To do this, peripheral blood is removed from a patient preferably by means of a pheresis machine; anticoagulant agents are optionally added to the blood, and the blood is separated into its major components—solid (red blood cells, white blood cells, and platelets) and liquid fractions (plasma). After separation into these main components, the soluble or suspended blood components, present in the thus obtained plasma fraction, can be manipulated, depleted, and/or removed in another process step, for example, with use of the polypeptides of the invention. Next, the thus treated blood plasma together with the previously separated solid blood components can be combined and reinjected into the patient. The volume loss due to the plasmapheresis can be later replaced by isotonic saline solution in the method of the invention. Plasmapheresis as taught by the invention is preferably carried out with the use of methods such as therapeutic plasma exchange (TPE), immunabsorption (IA), precipitation (HELP), differential membrane filtration, and other means. Plasma filtration columns are mentioned here as examples (see, e.g., U.S. Pat. No. 4,619,639, Asahi Medical Company, incorporated here by reference). Membrane filtration systems (MDF) with use of different particles and surfaces, for example, filters such as PlasmaFlo®OP-05(W)L and RheoFilter®AR2000 blood filters, can be used (manufactured by Asahi Medical Company, Ltd. of Japan). Alternatively, all suitable surfaces and particles can be used in the plasmapheresis methods of the invention.

In cytappheresis, as taught by the invention, in contrast to the previously described plasmapheresis, cellular components circulating in the blood and/or bound to bone marrow (red blood cells, white blood cells, stem cells, or platelets) or specific subpopulations of these cells are manipulated, depleted, and/or removed extracorporeally to achieve a clini-

cal effect. Here as well, peripheral blood is taken from a patient by means of a pheresis machine. Anticoagulant agents are optionally then added to the blood and the blood is separated into its main components. Next, in the blood fraction containing the cell components, these cell components can be manipulated, depleted, and/or removed in another process step, preferably also with use of the polypeptides of the invention. In the end, the thus treated fraction can be reinjected into the patient. Preferably, in the cytapheeresis of the invention, the separation of the blood into various fractions and optionally the separation of certain cell components in blood occur using methods such as centrifugation, differential membrane filtration, or other means. Furthermore, membrane filtration systems (MDF) with use of different particles and surfaces, for example, filters such as PlasmaFlo®OP-05(W)L and RheoFilter®AR2000 blood filters, can be used (manufactured by Asahi Medical Company, Ltd. of Japan).

In a third alternative of the invention, the aforementioned methods of plasmapheresis and cytapheeresis can be combined, for example, to manipulate, deplete, and/or remove both soluble or suspended components in the plasma fraction of blood and cellular components circulating in blood and/or bound to bone marrow (red blood cells, white blood cells, stem cells, or platelets) or specific subpopulations of these cells in order to achieve a clinical effect. Such a combination of plasmapheresis and cytapheeresis is preferably produced with use of the polypeptides of the invention.

In a preferred embodiment, the present invention relates to an (apheresis) method for extracorporeal manipulation, depletion, and/or removal of soluble, suspended, or cellular blood components comprising the following steps:

- Optionally separation of the blood into one or more fractions with solid and/or liquid components;
- Binding of soluble, suspended, or cellular blood components to a surface or particle coupled to a polypeptide of the invention; and
- Optionally separation of the bound soluble, suspended, or cellular blood components.

In a special embodiment of the apheresis method of the invention, blood can be optionally removed from a patient before the separation of the blood if necessary. Furthermore, the blood treated by the method of the invention or the thus treated blood fraction can be reinjected into the patient. To do this, it may be necessary to recombine the previously separated and possibly differently treated blood fractions with other untreated solid and/or liquid components of blood. The volume loss due to the method of the invention can be compensated by addition of appropriate fluids, e.g., by addition of an isotonic saline solution.

The step in the apheresis method of the invention for binding the soluble, suspended, or cellular blood components to a surface or particle coupled to a polypeptide of the invention can be carried out once or repeatedly as needed, in order to achieve a desired selectivity.

In the apheresis method of the invention, such surfaces and/or particles are used which were covalently coupled to the polypeptide of the invention, as defined above. To this end, the polypeptide of the invention is preferably covalently coupled to the surface and/or the particle via the coupling group(s) A present in the polypeptide.

Coupling of the polypeptides used according to the invention via the individual components A occurs preferably as described in DE 101 44 252 A1. The coupling of the polypeptides used according to the invention occurs thereby preferably to the surfaces or particles (carriers) via a bond between the first functional groups present on the carrier surface and in the polypeptide via the coupling groups present in compo-

nents A. These coupling groups are preferably complementary to the functional groups of the carrier and can enter into affinity, preferably covalent bonds with these. Preferably, the functional group of component A is positioned within component A such that it is placed a suitable distance outside the domains, responsible for the biological activity, of the polypeptide of the invention. In this way, it is possible according to the invention to immobilize TNF directionally on the carrier and with retention of its biological activity. After the immobilization, the polypeptide of the invention is fixed preferably to the carrier's surface such that the three-dimensional structure of the domain(s) necessary for biological activity has not been altered compared with the non-immobilized polypeptide of the invention, and that the domain(s) during contact with cellular reaction partners is/are freely accessible for these.

In a preferred embodiment of the invention, the functional group of the carrier surface is selected from the group consisting of the amino group, carboxy group, epoxy group, maleimido group, alkylketone group, aldehyde group, hydrazine group, hydrazide group, thiol group, and thioester group.

According to the invention, the coupling groups of components A, to be immobilized, of the polypeptide of the invention are selected from a group, which contains the same species as for the functional group of the carrier surface. A surface/particle (=carrier), which can be used in the apheresis method of the invention, therefore, has on its surface a functional group, which is covalently linked to a coupling group of the polypeptide, to be immobilized, of the invention, whereby the coupling group of the polypeptide is a group different from the functional protein of the carrier. The two coupling groups and functional groups, binding together, must thereby be complementary to one another, that is, be capable of entering into a covalent bond with one another. If, for example, an amino group is used as the functional group of the carrier according to the invention, the coupling group of component A of the polypeptide of the invention is a carboxy group. If conversely a carboxy group is used as the functional group of the carrier according to the invention, the coupling group of component A of the polypeptide of the invention is an amino group as taught by the invention. If a thiol group is selected as the functional group of the carrier according to the invention, the complementary coupling group of component A of the polypeptide of the invention is a maleimido group as taught by the invention. If conversely a maleimido group is used as the functional group of the carrier, the complementary coupling group of component A of the polypeptide of the invention is a thiol group as taught by the invention. If an alkylketone group, particularly a methylketone or aldehyde group, is used as the functional group of the carrier according to the invention, the functional complementary coupling group of component A of the polypeptide of the invention is a hydrazine or hydrazide group. If conversely according to the invention a hydrazine or hydrazide group is used as the functional group of the carrier, the functional complementary coupling group of component A of the polypeptide of the invention is an alkylketone, particularly methylketone or aldehyde group, as taught by the invention. According to the invention, the functional group on the carrier surface is preferably a maleimido group, and in the coupling group of component A of the polypeptide of the invention, a thiol group.

The immobilization thereby occurs preferably directionally. In regard to the present invention, the term "immobilized directionally" or "directional immobilization" means that a polypeptide of the invention, particularly an scTNF member, is immobilized at a defined position within the scTNF member in such a way on a carrier that the three-dimensional

structure of the domain(s) necessary for biological activity has not changed compared with the non-immobilized state and that this (these) TNF domain(s), for example, binding pockets for cellular reaction partners, during contact with cellular reaction partners is/are really accessible for these. "Immobilized directionally" also means that the coupling of the polypeptide of the invention on the carrier surface occurs in such a way that the immobilized protein during later use in a cellular and/or cell-like environment cannot be degraded or degraded only very slowly by protein-degrading enzymes. This means that an immobilized TNF molecule of the invention is aligned on the carrier surface in such a way that it offers as few sites of action as possible for proteases. In addition, the polypeptide of the invention before coupling can be made resistant to proteases by biomolecular methods, as described above, for example, for TNF members in the case of protease TACE.

The polypeptide of the invention via its coupled component(s) A retains its biological function and the ability to interact with other compounds/substances. If a polypeptide of the invention contains, for example, three components A (trimer), then typically each, two, or preferably only one of the three components A are bound covalently to the surface/particle.

For coupling of suitable surfaces and/or particles, all suitable surfaces and/or particles can be included to which the polypeptides of the invention can be coupled. In particular, culture plates or so-called microbeads, e.g., Dynabeads (DynaL Biotech GmbH), nanobeads, nanoparticles, or solid phases such as, for example, nylon wool, Sepharose, Sephadex, etc., are especially preferred. According to the invention, it is provided furthermore that the carrier systems used according to the invention are compact or hollow nanoparticles between 25 and 1000 nm in size. These consist either of organic or inorganic particle materials. The type of the material can be varied as taught by the invention according to the subsequent use, whereby the carrier of the nanoparticles consists preferably of biologically compatible and/or biologically degradable materials. In regard to the present invention, "nanoparticles" are understood to be furthermore binding matrixes, which comprise a carrier with a surface on which chemically reactive functional groups are arranged, which enter into affinity bonds, thus covalent and/or noncovalent bonds, with complementary functional groups of molecules to be bound, particularly the polypeptides of the invention, and in this way can stably fix the polypeptides of the invention on their surface. The carriers of the nanoparticles consist of chemically inert inorganic or organic materials and are less than 1 μ m in size, preferably from 25 to 500 nm.

A special embodiment of the apheresis method of the invention comprises a biofunctionalized surface (planar or as particles), e.g., with the polypeptide of the invention scTNF, which was immobilized covalently and directionally on the surface in the previously described manner. This type of biofunctionalized surface, containing, for example, several immobilized scTNF molecules or variants thereof, makes possible a high affinity for the specific complementary binding partner, for example, TNFR1 and TNFR2. The receptors TNFR1 and particularly TNFR2 are found in highly elevated concentrations as processed, soluble molecules in the blood of tumor patients and in other diseases. The removal of TNFR from the blood of tumor patients demonstrably leads to clinically documented tumor regression, which is attributed to a reconstitution of the body's own defense (see, for example, Lentz M. R., (1999) *Therapeutics Apheresis*, Vol. 3, No. 1). An apheresis method with polypeptides of the invention, for example, scTNF, makes it possible that preferably both TNF

receptors can be removed simultaneously and efficiently from samples. Another advantage of this method is the above-described fact that dissociation of individual components A of the polypeptide of the invention is not possible; i.e., neither an activity loss of the functionalized surface nor contamination of the treated blood with the associated substances is expected.

The apheresis method of the invention can be carried out as a continuous or discontinuous process. As a continuous process, the method of the invention is preferably carried out directly after the taking of blood or the fractionation of the removed blood and before the return of the blood to the patient without interim storage of the blood or the obtained blood fractions. In a discontinuous process, in contrast, the removed blood or the fractions of the removed blood can be stored after each step for a specific time period.

The temperature in the apheresis method of the invention is preferably 0 to 40° C. In a continuous process, the temperature is preferably 25 to 40° C., especially preferably in a range between 36° C. and 38° C. In a discontinuous process, the temperature can be between 0° C. and 40° C.

The apheresis method of the invention as described above is used preferably for diagnosis, therapy, and/or prophylaxis in diseases associated with members of the TNF ligand family, particularly in tumor diseases, particularly solid or lymphatic tumors, particularly solid and lymphatic tumors [sic]. The restoration of the homeostasis of the immune system with its humoral and cellular components is regarded as the key to the effectiveness of the apheresis method. In another application, the apheresis system of the invention can be used for this reason also in restoring the immune homeostasis in nonmalignant diseases. These include inflammatory diseases, arthritic and rheumatic diseases, or diseases of the immune system, as well as for the treatment [sic] of infectious diseases, metabolic diseases, inflammatory conditions, hyperproliferative diseases, autoimmune diseases, particularly rheumatoid/arthritic diseases, toxic epidermal necrolysis (TEN), multiple sclerosis, Hashimoto's thyroiditis, GVHD, viral hepatitis (HBV, HCV), alcohol-induced hepatitis, rejection reactions in liver transplantation, diseases based on hyperapoptotic reactions, and degenerative diseases, particularly neurodegenerative diseases.

Tumor diseases comprise in particular colon cancer, melanomas, renal carcinomas, lymphomas, acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), gastrointestinal tumors, lung carcinomas, gliomas, thyroid tumors, breast carcinomas, prostate tumors, hepatomas, various virus-induced tumors, such as, e.g., papilloma virus-induced cancer (e.g., cervical carcinoma), adenocarcinomas, herpes virus-induced tumors (e.g., Burkitt's lymphoma, EBV-induced B cell lymphoma), hepatitis B-induced tumors (hepatocellular carcinomas), HTLV-1 and HTLV-2 induced lymphomas, acoustic neurinoma, cervical cancer, lung cancer, throat cancer, anal carcinoma, glioblastoma, lymphomas, rectal cancer, astrocytoma, brain tumors, stomach cancer, retinoblastoma, basalioma, brain metastases, medulloblastomas, vaginal cancer, pancreatic cancer, testicular cancer, melanoma, thyroid cancer, bladder cancer, Hodgkin's syndrome, meningiomas, Schneeborg's disease, bronchogenic carcinoma, pituitary tumor, mycosis fungoides, esophageal cancer, breast cancer, carcinoids, neurinoma, spinocellular carcinoma, Burkitt's lymphoma, laryngeal cancer, kidney cancer, thymoma, uterine carcinoma, bone cancer, non-Hodgkin's lymphoma, urethral cancer, CUP syndrome, head-neck tumors, oligodendroglioma, vulvar cancer, intestinal cancer, colon cancer [sic], esophageal

carcinoma, wart involvement, small bowel tumors, cranio-pharyngeomas, ovarian carcinoma, soft tissue tumors, ovarian cancer, liver cancer, pancreatic carcinoma, cervical carcinoma, endometrial carcinoma, liver metastases, penile cancer, tongue cancer, gallbladder cancer, leukemia, plas-mocytoma, uterine cancer, lid tumor, prostate cancer, etc.

Arthritic diseases comprise particularly monoarthritis, oligoarthritis, polyarthritis, acute arthritis (primarily septic, crystal-induced, reactive arthritis and acute arcoidosis), sub-acute arthritis, chronic arthritis (primarily rheumatoid arthritis, arthritis in seronegative spondylarthritis), infectious arthritis, para- or postinfectious arthritis, rheumatoid arthritis, juvenile chronic arthritis, arthritis in inflammatory connective tissue diseases and vasculitis, allergic arthritis, arthritis in conjunction with metabolic diseases and nutrition-induced disorders, arthritis in endocrine disturbances, arthritis in granulomatous diseases, arthritis in diseases of the hematopoietic system, arthritis in joint hemorrhaging due in blood coagulation disorders, neoplastic arthritis, paraneoplastic arthritis, (post-)traumatic arthritis, arthritis in diseases of the articular cartilage, arthritis in neuropathies, arthritis in pustular, abscessing, necrotizing dermatoses or dermatoses proceeding with tissue neutrophilia, arthritis in other extra-articular primary diseases, as well as allergic arthritis, chlamydia-induced arthritis, dysenteric arthritis, gonorrheal arthritis, arthritis mutilans, psoriatic arthritis, dry arthritis, syphilitic arthritis, tuberculous arthritis, uric arthritis, etc.

Another subject of the present invention is a pharmaceutical composition or a vaccine, containing the polypeptides of the invention, nucleic acid constructs of the invention, vectors of the invention, and/or host cells of the invention, as well as pharmaceutically compatible aids, additives, and/or carrier substances (e.g., also solubilizers). The pharmaceutical compositions or vaccines according to the invention are preferably used for the treatment of cancer diseases, particularly solid or lymphatic tumors, as well as for the treatment of infectious diseases, metabolic diseases, inflammatory conditions, hyperproliferative diseases, autoimmune diseases, particularly rheumatoid/arthritis diseases, toxic epidermal necrolysis (TEN), multiple sclerosis, Hashimoto's thyroiditis, GVHD, viral hepatitis (HBV, HCV), alcohol-induced hepatitis, rejection reactions in liver transplantation, diseases based on hyperapoptotic reactions, and degenerative diseases, particularly neurodegenerative diseases.

Therefore, according to the invention, a combination of substances of the invention with pharmaceutically acceptable carriers, aids, and/or additives is also disclosed. Appropriate production methods are disclosed in "Remington's Pharmaceutical Sciences" (Mack Pub. Co., Easton, Pa., 1980), which is part of the disclosure of the present invention. For parenteral administration, for example, sterile water, sterile saline solution, polyalkylene glycols, hydrogenated naphthalene, and particularly biocompatible lactide polymers, lactide/glycolide copolymers, or polyoxyethylene/polyoxypropylene copolymers may be considered as carrier materials. Pharmaceutical compositions of this type according to the invention may be used for all medical indications disclosed above. In addition, the compositions of the invention may contain fillers or substances, such as lactose, mannitol, substances for the covalent linking of polymers, such as, e.g., polyethylene glycol to inhibitors of the invention, complexing with metal ions or inclusion of materials in or on special preparations of polymer compounds, such as, e.g., polylactate, polyglycolic acid, hydrogel, or on liposomes, micro-emulsion, micelles, unilamellar or multilamellar vesicles, erythrocyte fragments, or spheroplasts. The specific embodiments of the compositions are selected depending on the

physical behavior, for example, in regard to solubility, stability, bioavailability, or degradability. Controlled or constant release of the active components of the invention in the composition includes formulations based on lipophilic depots (e.g., fatty acids, waxes, or oils). Coatings of substances of the invention or compositions containing such substances, namely, coatings with polymers, are disclosed within the scope of the present invention (e.g., poloxamers or poloxamines). Furthermore, the substances or compositions of the invention may have protective coatings, e.g., protease inhibitors or permeability enhancers.

All administration routes known in the related art are disclosed basically within the scope of the present invention for the substances of the invention or the pharmaceutical compositions of the invention or vaccines; preferably the manufacture of a medication or a vaccine occurs for the treatment of the aforementioned diseases or disorders by the parenteral, i.e., for example, subcutaneous, intramuscular, or intravenous, oral, intranasal, intra-aural, transdermal, topical (e.g., via gels, ointments, lotions, creams, etc.), intraperitoneal, intrapulmonary (e.g., AERx® inhalation technology, commercially available from Aradigm or Inhance™ pulmonary delivery system, commercially available from Inhale Therapeutics), vaginal, rectal, or intraocular administration routes. Typically, pharmaceutical compositions of the invention are solid, liquid, or aerosol-like (e.g., spray), depending on the packaging or administration.

In a preferred embodiment, the therapeutically effective amount of a polypeptide of the invention is administered as needed to a patient. The precise dose typically depends on the purpose of the basic treatment and can be determined by a person skilled in the art with use of known methods from the related art. As is known from the related art, adjustments may be necessary, for example, in regard to the metabolic breakdown of the polypeptides of the invention, in regard to systemic versus local administration, as well as to the age, body weight, general state of health, gender, nutrition, time of administration, interaction of active ingredients, or the severity of the disease. Such adjustments can be made by a person skilled in the art by means of methods known in the related art.

In a preferred embodiment, a combination of polypeptides of the invention for use as adjuvant is disclosed. An adjuvant within the meaning of this invention is particularly a composition that causes no specific immune response to an immunogen, but is capable of enhancing the immune response to this immunogen. Stated differently, administration of the adjuvant alone causes no immune response to this immunogen, whereas administration together with this immunogen produces an immune response, which is greater than the immune response after administration of the immunogen alone. An adjuvant can contain in addition pharmaceutically acceptable carriers, aids, and/or additives or be administered with them, as is disclosed here for vaccines and pharmaceutical compositions. Furthermore, an adjuvant can be administered together with the vaccines or pharmaceutical compositions disclosed above.

It can be stated in summary that the invention provides a polypeptide, which has increased stability, as a result of which the entire molecule of a member of the TNF ligand family consists of a protein strand or polypeptide strand (e.g., scTNF), so that the monomers (components A) of the members of the TNF ligand family can no longer dissociate. scTNF, as well as the polypeptides of the invention, which relate to other members of the TNF ligand family, exhibits no qualitative differences in its bioactivity to the normal soluble sTNF (also wild-type TNF or wtTNF), but is more stable by

far and therefore exhibits higher bioactivity, and thus is effective at lower concentrations. Like the normally soluble TNF (wtTNF), scTNF was able to trigger apoptosis in sensitive cells, to activate the transcription factor NF- κ B, the receptor TNFR1 but not TNFR2, and in the presence of certain antibodies (e.g., 80M2) also to activate the receptor TNFR2. This applies to polypeptides of the invention, which relate to other members of the TNF ligand family. When the polypeptides of the invention are used with a coupling group to produce biofunctionalized surfaces and the use thereof, for example, for apheresis, no bleeding of the polypeptide of the invention or a return of harmful ligands into the system is observed.

Furthermore, the polypeptide of the invention, for example, scTNF, represents an ideal starting material for the preparation of new bifunctional molecules. On the one hand, the polypeptide of the invention, for example, scTNF, or functional fragments thereof can bind covalently to cell surfaces. According to the invention, this has the advantage that only a single covalent bond must be created for stable binding of the molecule. In a normal soluble wild-type member of the TNF ligand family, for example, soluble sTNF, all three monomers of the individual homotrimer must be covalently linked to the surface in order to obtain a stable construction, because otherwise the non-covalently bound monomers dissociate off. In this embodiment of the polypeptide of the invention, use in apheresis, e.g., with scTNF as a biofunctional active substance to remove TNF receptors from body fluids is especially advantageous.

This also applies to the preparation of fusion proteins. These are especially interesting in order to concentrate a member of the TNF ligand family, for example, TNF, with use of an antibody fragment fused to TNF, for example, scFv, at a specific desired location in the body (so-called "targeting" strategies). In normal soluble TNF (wtTNF), this type of fusion protein must consist of homotrimeric TNF, in which each of the at least three monomers carries the antibody moiety, as a result of which large and unstable molecules form, which in turn dissociate into their monomers and/or tend to aggregate and thereby can be inactivated. A fusion protein derived from scTNF is linked with only one molecule of the antibody, so that the entire fusion protein in turn consists of a single, stable protein strand or polypeptide strand. The descriptions for TNF above can be applied to all members of the TNF ligand family.

It is also possible by means of the present invention, due to the covalent linkage of the monomers (components A) of the polypeptide of the invention, to introduce selectively and stably mutations in only one or also two or more of the at least three monomers (components A). Thus, e.g., point mutations are known, which force receptor selectivity; i.e., for example, in the case of TNF, binding still occurs only at one of the two TNF receptors (TNFR1 and TNFR2). It is possible according to the invention to produce for the first time, e.g., TNF mutants, which are capable of selectively binding a molecule of TNFR1 and two molecules TNFR2 simultaneously; i.e., heteromeric receptor complexes would result. In this way it is possible for the first time to prepare heteromeric, stable, single-chain members of the TNF ligand family, which selectively bind and activate only one of several possible receptors.

Of course, the explanatory statements made above as examples in regard to TNF can be applied to the other members of the TNF ligand family.

The present invention will be illustrated in greater detail by means of the following figures:

FIGS. 1-3 show the results of different biological cytotoxicity tests, in which the properties, particularly the specificity, of different scTNF variants were compared with those of

classic soluble wild-type TNF, i.e., the soluble extracellular domain of human TNF (NCBI gi:25952111, 17.09 kDa, as a monomer of the soluble form, abbreviated TNFhum or sTNF, AA 79-181).

Both transfected mouse fibroblasts and human Kym1 cells were used for these tests. Exemplary results of the analyses are presented, whereby scTNF variants were tested with peptide linkers of different length between the individual TNF modules (i.e., TNF monomers). The peptide linkers in each case consist of the threefold or fourfold amino acid repetitions GGGG (or also GlyGlyGly-Ser (SEQ. ID NO: 41) (designated as "single-chain TNF3x" or "single-chain TNF4x" or as "scTNF3x" or "scTNF4x").

DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the result of a typical cytotoxicity test of TNF. Mouse fibroblasts (MF) from TNFR1/TNFR2 double-knock-out mice, which stably express TNFR1 receptor chimeras (TNFR1-Fas) (MF TNFR1-Fas cells) were used as target cells. Such cells express a hybrid molecule, which consists extracellularly of the corresponding part of TNFR1 and thereby binds TNF, and intracellularly has the highly apoptotically acting intracellular domain of the Fas-receptor. These MF TNFR1-Fas cells can be activated by TNFR1-specific stimuli, but mediate a Fas-specific death signal. The surviving cell count was quantified after 24 hours by absorption with staining with a dye. A control bacterial lysate was used as a negative control, which was a non-scTNF expressing bacterial lysate, which was processed identically to the recombinant scTNF proteins. It can be stated as a result that both the conventional human soluble TNF (TNFhum) and the recombinant scTNF variants led to cell death. A comparable dose-dependent cytotoxic effect of TNFhum or scTNF is evident (see the bottom three curves in the diagram), whereby the specific activity of the scTNF is at least equivalent, if not even higher, than that of the wild-type TNF. As expected, the negative control shows no toxic effect.

In another test batch, the effect of neutralizing TNF-specific antibodies on the cytotoxicity of wild-type TNF (TNFhum) or scTNF was tested. Freshly prepared dilutions of soluble human TNF (TNFhum) or the above-described scTNF variants with threefold (3x) or fourfold (4x) glycine-serine peptide linkers (scTNF3x: GGGG-GGGG-GGGG (SEQ. ID NO: 2)/scTNF4x: GGGG-GGGG-GGGG-GGGG (SEQ. ID NO: 3)) were used. Concentration series with 1:3 dilutions in the presence and absence of neutralizing TNF-specific antibodies (α TNF-AB) [1 μ g/mL] were used. The above-described control bacterial lysate was used as the negative control. It is evident as the result that the cytotoxic effect of soluble human wild-type TNF or scTNF can be abolished by neutralizing TNF-specific antibodies (see the top three curves in the diagram), namely, within a broad concentration range (0.03-10 ng/mL). No differences were found between the reaction of scTNF with 3x and scTNF with 4x peptide linkers.

FIG. 2 shows the result of a cytotoxicity test in which the effect of sTNF and scTNF variants on TNFR2-receptor chimera (TNFR2-Fas) expressing cells was studied. In this test batch, mouse fibroblasts were used which stably express receptor chimeras consisting of TNFR2 and Fas (TNFR2-Fas, whereby the extracellular part stems from TNFR2 and the intracellular part from the Fas-receptor) (MF TNFR2-Fas cells). MF TNFR2-Fas cells (like the wild-type TNFR2) can only be activated by an adequate TNFR2 stimulus, for example, by membrane-associated TNF, but not by soluble TNF. Result: As expected, both the soluble human TNF (TN-

Phuman) and the scTNF variants with the 3x and 4x peptide linkers (scTNF3x, scTNF4x) had no toxic effect on the MF TNFR2-Fas cells. In other words, sTNF and the scTNF variants are equally incapable of activating TNFR2-chimera (TNFR2-Fas) expressing cells; i.e., they cause no cell death in these cells. The control bacterial lysate used as the negative control (as described above) as expected also had no toxic effect.

FIG. 3 shows the result of a cytotoxicity test in which the effect of soluble human wild-type TNF and an scTNF variant in combination with a special TNFR2-specific antibody, 80M2, on TNFR2-chimera (TNFR2-Fas) expressing cells was studied. The test batch corresponds that from FIG. 2 with the difference that the soluble wild-type TNF or the scTNF with 3x peptide linkers (scTNF3x) was given together with the antibody 80M2. The antibody 80M2 is known for conferring the special signal capacity of membrane-bound TNF on normal soluble TNF. The result shows that both the soluble wild-type TNF and scTNF in combination with the antibody 80M2 achieve a strong cytotoxic effect via the TNFR2-Fas receptor chimeras. The reason for this is that soluble TNF in combination with certain TNFR2-specific antibodies, such as, e.g., 80M2, can exhibit the activity of membrane-bound TNF. MF TNFR2-Fas cells die after incubation with soluble TNF or scTNF in the presence of such a ligand-receptor complex-stabilizing antibody (80M2-AB). In other words, in the presence of this antibody, soluble TNF and scTNF have a similar toxic effect in the MF TNFR2/FAS cells, as this otherwise can occur only with membrane-associated TNF. The control bacterial lysate used as the negative control (as described above in FIG. 1) as expected had no toxic effect.

FIGS. 4-10 show the results of different stability tests, which demonstrate that the scTNF variants of the invention due to their structure have a vastly better stability than soluble wild-type TNF.

sTNF or the scTNF variants were incubated in a serum-containing culture medium at concentrations of 3.0 to 0.01 ng/mL at 37° C. and 5% CO₂ for different time periods.

After this, the functionality of the sTNF or scTNF variants (scTNF3x, scTNF4x) was tested in the cytotoxicity tests based on MF TNFR1-Fas cells and Kym1 cells. For this cytotoxicity test, 1:3 dilutions were used starting with a 3.0 ng/mL TNF sample. A control bacterial lysate, which was employed in the cytotoxicity tests described above, was used as the negative control (non-scTNF expressing bacterial lysate, which was processed identically to the recombinant scTNF proteins).

FIG. 4 shows the bioactivity of the employed molecules BEFORE the stability test in mouse fibroblasts MF TNFR1-Fas cells. Soluble human wild-type TNF (TNFhuman) and scTNF dilutions (freshly prepared) were used and diluted out on the cells. Both the soluble control TNF and the scTNF variants after incubation with MF led to their cell death. The control bacterial lysate as expected had no toxic effect. The ED₅₀ values (half-maximal effective concentration) were about 0.2 ng/mL for the soluble human wild-type TNF and 0.1 ng/mL for the two employed different scTNF variants. The control bacterial lysate used as the negative control as expected had no toxic effect.

FIG. 5 shows the result of a stability test with mouse fibroblasts MF TNFR1-Fas cells. The wild-type TNF and scTNF dilutions, shown in FIG. 5, were hereby incubated for 8 days in a cell incubator before the test in order to be able to evaluate the stability of the samples. A clear decline in the bioactivity of the soluble wild-type TNF (TNFhuman) was measured after 8 days at 37° C., whereas the activity of both scTNF variants (scTNF3x, scTNF4x; see above for explana-

tion) remained unchanged. An ED₅₀ value of about 2 ng/mL (used freshly prepared: 0.2 ng/mL, cf. FIG. 4) was obtained for the wild-type TNF. The scTNF variants with ED₅₀ values of about 0.1 ng/mL remained as active as the freshly used samples (cf. FIG. 4). The control bacterial lysate used as the negative control as expected had no toxic effect.

FIG. 6 shows the result of a stability test with mouse fibroblasts MF TNFR1-Fas cells. The wild-type TNF and scTNF solutions were now incubated as explained in FIG. 5 for 14 days in a cell incubator. A clear further decline in the bioactivity of the wild-type TNF was measured after 14 days at 37° C., whereas the activity of the scTNF variants remained approximately the same. An ED₅₀ value of about 2.8 ng/mL (freshly prepared 0.2 ng/mL, cf. FIG. 4) was obtained for sTNF; the scTNF variants with 0.13 ng/mL remained virtually as active as the freshly used samples (0.1 ng/mL, cf. FIG. 4). The control bacterial lysate used as the negative control as expected had no toxic effect.

FIG. 7 shows the result of a stability test with the human cell line Kym1. Kym1 cells express normal human wild-type TNF receptors and also react cytotoxically to TNF. The experiment was performed similar to the stability tests with mouse fibroblasts according to FIGS. 4-6: Soluble wild-type TNF or the scTNF variants (scTNF3x, scTNF4x) were incubated in a serum-containing medium at concentrations of 3.0 to 0.01 ng/mL at 37° C. and 5% CO₂ for different time periods. Next, the functionality of the soluble sTNF or the scTNF variants was tested in cytotoxicity tests on Kym1 cells. Starting with a 3.0 ng/mL TNF samples, 1:3 dilutions were used for the test. The above-described control bacterial lysate was used as the negative control. Wild-type TNF and scTNF dilutions were freshly prepared and used. The result shows that both wild-type TNF and the scTNF variants possessed high cytotoxic activity. The ED₅₀ values are about 0.1 ng/mL for the wild-type TNF and about 0.06 ng/mL for the scTNF variants. The control bacterial lysate used as the negative control as expected had no toxic effect.

FIG. 8 shows the result of a stability test with the human cell line Kym1. The wild-type TNF and scTNF solutions were hereby incubated for 16 days in a cell incubator. Next, the Kym1 cell cytotoxicity test was performed. A clear decline in the activity of the sTNF was measured after 16 days at 37° C., whereas the toxicity, i.e., the bioactivity, of the scTNF variants remained approximately the same. An ED₅₀ value of about 1.2 ng/mL was obtained for the wild-type TNF. The scTNF variants with 0.06 ng/mL remained as active as the freshly used samples. The control bacterial lysate used as the negative control as expected had no toxic effect.

FIG. 9 shows the result of a stability test with the human cell line Kym1. sTNF and scTNF solutions were hereby incubated for 22 days in a cell incubator. A clear further decline in the activity of sTNF was measured after 22 days at 37° C. The scTNF variants continued to be stable. The control bacterial lysate used as the negative control as expected had no toxic effect.

The following Table 1 shows the results data from the stability tests according to FIGS. 7 and 9 as a comparison:

TABLE 1

Comparison of the ED ₅₀ values from FIGS. 7 + 9			
	ED ₅₀ freshly titrated (FIG. 7)	ED ₅₀ after 22 days (FIG. 9)	Loss of activity
sTNF	0.1 ng/mL	1.50 ng/mL	>90%
scTNF	0.06 ng/mL	0.07 ng/mL	Barely detectable

FIG. 10 shows the result of a stability test with the human cell line Kym1. Dilutions of 3 ng/mL were titrated out after 16 days at 37° C. In contrast to the previous FIGS. 4-9, in this test a titration curve was prepared starting with a 3 ng/mL TNF dilution stored for 16 days, whereas in the previous shown titration curves the particular dilutions were prepared and then stored for the indicated time period. It can be demonstrated that a comparison of the data from FIG. 8 (similar test with 16 days of incubation) and FIG. 10 shows no major differences. The control bacterial lysate used as the negative control as expected had no toxic effect.

FIG. 11 shows the result of a stability test in human serum. To test the stability of wild-type TNF and scTNF in human serum, in a first test batch the soluble wild-type TNF and the scTNF4x variant were diluted in 100% serum and freshly titrated out. An ED₅₀ value of 0.004 ng/mL was measured as the result for the wild-type TNF and an ED₅₀ value of 0.007 ng/mL for the scTNF variant.

FIG. 12 shows the result of a stability test in human serum. To test the stability of wild-type TNF and scTNF in human serum, in another test batch, wild-type TNF and the scTNF4x variant were stored for 8 days at 37° C. in 100% fresh serum that was not heat-inactivated, and then titrated out in Kym1 cells. An ED₅₀ value of 0.40 ng/mL was measured as the result for wild-type TNF and an ED₅₀ value of 0.03 ng/mL for the scTNF variant. The effects of the 8-day storage in 100% serum were as follows: In comparison with freshly titrated out scTNF, scTNF stored for 8 days exhibited a loss of activity by a factor of about 4.3, whereas for the soluble sTNF there was a dramatic loss of activity by a factor of 100 during storage in 100% serum. The scTNF of the invention, in comparison with sTNF, accordingly exhibited a much higher bioactivity after an 8-day incubation in serum and thereby proved to be very stable in human serum at physiological temperatures.

In the following Table 2, the data of the results from FIGS. 11 and 12 are once again presented for clarification.

TABLE 2

Comparison of the ED ₅₀ values from FIGS. 11 + 12			
	ED ₅₀ freshly titrated (FIG. 11)	ED ₅₀ after 8 days (FIG. 12)	Loss of activity
Soluble TNF	0.004 ng/mL	0.40 ng/mL	100-fold
Single-chain TNF	0.007 ng/mL	0.03 ng/mL	4.3-fold

FIG. 13 shows the result of an analysis by polyacrylamide gel electrophoresis under reducing and denaturing conditions. A silver gel of a purified wild-type TNF and the purified scTNF variants, scTNF3x and scTNF4x, is shown. The samples were each incubated with β-mercaptoethanol (final 5%) at 95° C. for 5 minutes. About 500 ng of sTNF and scTNF4x and about 150 ng of scTNF3x per lane were applied to the silver gel. The result of the silver gel of the scTNF variants separated in 15% SDS-PAGE shows that both scTNF variants under reducing conditions as well had a molecular weight of about 50 kDa, which agrees with their structure. It can also be stated that the different amounts of the applied proteins scTNF3x and scTNF4x had no effect on the result. This confirms the stability of the proteins or polypeptides of the invention under reducing and denaturing conditions. The result for sTNF, in contrast, shows that the protein breaks down into its monomers of about 17 kDa.

FIG. 14 shows the result of a stability test under reducing and denaturing conditions. In this test, a Western Blot of samples, separated in 15% SDS-PAGE, of wild-type TNF and the scTNF variants scTNF3x and scTNF4x was performed.

Two parallel batches were carried out in which the samples in one batch were each incubated with β-mercaptoethanol (final 5%) at 95° C. for 5 minutes, whereas no β-mercaptoethanol incubation occurred in the other batch. The detection after the electrophoresis run was performed with an anti-TNF antibody. It is evident as a result that the antibody specifically detected the protein of both scTNF variants in clear bands at about 50 kDa. For the wild-type TNF, the protein was specifically detected at about 17 kDa. This again confirms the stability of the scTNF3x and scTNF4x variants of the invention under reducing and denaturing conditions and also coincides as expected with the results from FIG. 13.

FIG. 15 shows the result of an IkappaB degradation assay. IkappaB (I-κB) is an inhibitor of the transcription factor nuclear factor kappa B (NF-κB), and as is generally known is caused to degrade after addition of TNF, as a result of which NF-κB is activated. To determine the degradation of I-κB, cell lysates were prepared 0, 30, and 60 minutes after stimulation with 10 ng/mL each of wild-type TNF, scTNF3x, and scTNF4x and then analyzed using the Western Blot assay with I-κB-specific antibodies. The result shows that the transient degradation of I-κB of both wild-type TNF and the two scTNF variants was induced, whereby the reaction behavior of the scTNF variants corresponds to that of the wild-type TNF. The control bacterial lysate used as the negative control (as described above, a non-scTNF expressing bacterial lysate, which was processed identically to the recombinant scTNF-proteins) as expected had no effect on the I-κB degradation.

FIG. 16 shows the result of a JNK assay. JNK (c-jun N-terminal kinase) is a stress-induced kinase, which as is known is very highly activated by TNF. After stimulation of Kym-1 cells for 0, 30, or 60 minutes with 10 ng/mL each of wild-type TNF, scTNF3x, and scTNF4x, cell lysates were prepared and the JNK activity was determined by immunoprecipitation of JNK with JNK-specific antibodies and subsequent kinase assay with GST c-Jun as the substrate. The control bacterial lysate used as the negative control (as described above) as expected did not activate JNK kinase. The result shows that both scTNF variants and wild-type TNF activated JNK, whereby the reaction behavior of the scTNF variants corresponds to that of the wild-type TNF.

FIG. 17 shows the result of an Electrophoretic Mobility Shift Assay (EMSA). Another typical test to determine the activity of TNF is the translocation of the transcription factor NF-κB into the cell nucleus after induction of the I-κB degradation. For this purpose, cell nucleus preparations of non-stimulated KYM-1 control cells (zero minutes of stimulation) or of stimulated cells (30 and 60 minutes of stimulation) were made. The stimulation of the cells occurred in each case with wild-type TNF, scTNF3x, and scTNF4x. The transcription factor NF-κB, translocated into the cell nucleus, was detected with the use of NF-κB-specific, radioactively labeled oligonucleotides. The result shows that NF-κB was translocated into the cell nucleus. The reaction behavior of the scTNF variants corresponds to that of the wild-type TNF.

FIG. 18 shows an exemplary construct scheme of the polypeptides of the invention, presented for TNF (as a member of the TNF ligand family). The designations have the following meanings:

Constructs AI/AII and BI/II have an optimized codon usage for the expression of proteins in *E. coli*, and represent molecules which are each linked with Gly-Gly-Gly-Ser-3x linker or 4x linker (SEQ. ID NOs: 2 and 3). All molecules have a histidine tag N-terminally for easier purification; the molecules BI/II in addition have a short amino acid chain with a cysteine residue for directional covalent linkage.

Constructs C to H are suitable for expression of the proteins in eukaryotic cells; they have the appropriate leader peptide sequences N-terminally.

sc= is the abbreviation for single chain,

cys= designates an N-terminal peptide linker with internal cysteine for covalent coupling,

L1_{long} or L2_{long}= designates linker 1 or 2, each with the amino acid sequence (GGGS-GGGS-GGGS-GGGS-GGGS or (GGGS)₄ (SEQ. ID NO: 3), L1_{short} or L2_{short}= designates linker 1 or 2, each with the amino acid sequence GGGS-GGGS-GGGS or (GGGS)₃ (SEQ. ID NO: 2),

Leader peptide sequence= is the amino acid sequence for the secretion of the protein from eukaryotic (host) cells.

scFv10= stands for the sequence of the single-chain (scFv) antibody fragment 40, specific for the tumor stroma antigen FAP,

AMAIZE= is the abbreviation for "Antibody-Mediated Apoptosis-Inducing Cytokines" and

His/Flag tag= stands for the peptide sequence for an affinity purification of the expressed proteins.

FIGS. 19A and 19B shows the nucleic acid sequence and the corresponding amino acid sequence of scTNF-L_{short} (construct A-II) (SEQ. ID NOs: 25 and 26)

Features of Construct A-II

His tag peptide sequence for affinity purification of the formed protein: amino acids (abbreviated as "AA" below in FIGS. 19 through 26) AA 5-10, nucleotide (abbreviated as "NT" in FIGS. 19 through 26 below) NT 13-30

Sequence of the human TNF module1 (extracellular domain, AA 79-181, of the natural human TNF molecule, sequence with optimized *E. coli* codon usage): AA 11-169, NT 31-507

Sequence of the (GGGS)₃-linker1: (SEQ. ID NO: 2) AA 170-181, NT 508-543

Sequence of the human TNF module2 (extracellular domain, AA 79-181, of the natural human TNF molecule, sequence with optimized *E. coli* codon usage): AA 182-335, NT 544-1005

Sequence of the (GGGS)₃-linker2: (SEQ. ID NO: 2) AA 336-347, NT 1006-1041

Sequence of the human TNF module3 (extracellular domain, AA 79-181, of the natural human TNF molecule, sequence with optimized *E. coli* codon usage): AA 348-501, NT 1042-1503

Stop codon: NT 1504-1506

FIGS. 20A and 20B shows the nucleic acid sequence and the corresponding amino acid sequence of cys-scTNF-L_{short} (construct B-II) (SEQ. ID NOs: 27 and 28)

Features of Construct B-II

Amino acid cysteine for covalent coupling: AA 9, NT 25-27

His tag peptide sequence for affinity purification of the formed protein: AA 15-20, NT 43-60

Sequence of the human TNF module1 (extracellular domain of the natural human TNF molecule, sequence with optimized *E. coli* codon usage): AA 21-181, NT 61-543

Sequence of the (GGGS)₃-linker1: (SEQ. ID NO: 2) AA 182-193, NT 544-579

Sequence of the human TNF module2 (extracellular domain of the natural human TNF molecule, sequence with optimized *E. coli* codon usage): AA 194-347, NT 580-1041

Sequence of the (GGGS)₃-linker2: (SEQ. ID NO: 2) AA 348-359, NT 1042-1077

Sequence of the human TNF module3 (extracellular domain of the natural human TNF molecule, sequence with optimized *E. coli* codon usage): AA 360-513, NT 1078-1539

Stop codon: NT 1540-1542

FIGS. 21A and 21B shows the nucleic acid sequence and the corresponding amino acid sequence of scFasL (construct C) (SEQ. ID NOs: 29 and 30)

Features of Construct C

Leader peptide sequence for secretion of the protein in eukaryotic cells: AA 1-15, NT 1-45

Flag tag peptide sequence for affinity purification of the formed protein: AA 19-26, NT 55-78

Sequence of the human FasL module1 (extracellular domain, AA 139-281, of the natural human FasL molecule): AA 30-173, NT, 90-519

Sequence of the (GGGS)₄-linker1: (SEQ. ID NO: 3) AA 174-189, NT 520-567

Sequence of the human FasL module2 (extracellular domain, AA 139-281, of the natural human FasL molecule): AA 190-332, NT 568-996

Sequence of the (GGGS)₄-linker2: (SEQ. ID NO: 3) AA 333-348, NT 997-1044

Sequence of the human FasL module3 (extracellular domain, AA 139-281, of the natural human FasL molecule): AA 349-491, NT 1045-1473

Stop codon: NT 1474-1476

FIGS. 22A, 22B and 22C shows the nucleic acid sequence and the corresponding amino acid sequence of scTRAIL (construct D) (SEQ. ID NOs: 31 and 32)

Features of Construct D

Leader peptide sequence for secretion of the protein in eukaryotic cells: AA 1-15, NT 1-45

Flag tag peptide sequence for affinity purification of the formed protein: AA 19-26, NT 55-78

Sequence of the human TRAIL module1 (extracellular domain, AA 95-281, of the natural human TRAIL molecule): AA 30-216, NT 88-648

Sequence of the (GGGS)₄-linker1: (SEQ. ID NO: 3) AA 217-232, NT 649-696

Sequence of the human TRAIL module2 (extracellular domain, AA 95-281, of the natural human TRAIL molecule): AA 233-419, NT 697-1257

Sequence of the (GGGS)₄-linker2: (SEQ. ID NO: 3) AA 420-435, NT 1258-1305

Sequence of the human TRAIL module3 (extracellular domain, AA 95-281, of the natural human TRAIL molecule): AA 436-622, NT 1306-1866

Stop codon: NT 1861-1863

FIGS. 23A and 23B shows the nucleic acid sequence and the corresponding amino acid sequence of scTNF (construct E) (SEQ. ID NOs: 33 and 34)

Features of Construct E

Leader peptide sequence for secretion of the protein in eukaryotic cells: AA 1-15, NT 1-45

Flag tag peptide sequence for affinity purification of the formed protein: AA 19-26, NT 55-78

Sequence of the human TNF module1 (extracellular domain of the natural human TNF molecule): AA 30-184, NT 88-552

Sequence of the (GGGS)₄-linker1: (SEQ. ID NO: 3) AA 85-200, NT 553-600

Sequence of the human TNF module2 (extracellular domain of the natural human TNF molecule): AA 201-355, NT 601-1065

Sequence of the (GGGS)₄-linker2: (SEQ. ID NO: 3) AA 356-371, NT 1066-1113

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Sequence of the human TNF module3 (extracellular domain of the natural human TNF molecule): AA 372-526, NT 1114-1581

Stop codon: NT 1799-1581

FIGS. 24A, 24B and 24C shows the nucleic acid sequence and the corresponding amino acid sequence of scFasL-AMAIZE (construct F) (SEQ. ID NOs: 35 and 36)

Features of Construct F

Leader peptide sequence for secretion of the protein in eukaryotic cells: AA 1-19, NT 1-57

Sequence of the single-chain (scFv) antibody fragment 40 specific for the tumor stroma antigen FAP: AA 20-267, NT 58-801

Flag tag peptide sequence for affinity purification of the formed protein: AA 278-285, NT 832-855

Sequence of the human FasL module1 (extracellular domain, AA 139-281, of the natural human FasL molecule): AA 290-432, NT 868-1296

Sequence of the (GGGS)₄-linker1: (SEQ. ID NO: 3) AA 433-448, NT 1297-1344

Sequence of the human FasL module2 (extracellular domain, AA 139-281, of the natural human FasL molecule): AA 449-591, NT 1345-1773

Sequence of the (GGGS)₄-linker2: (SEQ. ID NO: 3) AA 592-607, NT 1774-1821

Sequence of the human FasL module3 (extracellular domain, AA 139-281, of the natural human FasL molecule): AA 608-750, NT 1822-2250

Stop codon: NT 2251-2253

FIGS. 25A, 25B-25C and 25D shows the nucleic acid sequence and the corresponding amino acid sequence of scTRAIL-AMAIZE (construct G) (SEQ. ID NOs: 37 and 38)

Features of Construct G

Leader peptide sequence for secretion of the protein in eukaryotic cells: AA 1-19, NT 1-57

Sequence of the single-chain (scFv) antibody fragment 40 specific for the tumor stroma antigen FAP: AA 20-267, NT 58-801

Flag tag peptide sequence for affinity purification of the formed protein: AA 278-285, NT 832-855

Sequence of the human TRAIL module1 (extracellular domain, AA 95-281, of the natural human TRAIL molecule): AA 289-475, NT 865-1426

Sequence of the (GGGS)₄-linker1: (SEQ. ID NO: 3) AA 476-491, NT 1427-1476

Sequence of the human TRAIL module2 (extracellular domain, AA 95-281, of the natural human TRAIL molecule): AA 492-678, NT 1477-2034

Sequence of the (GGGS)₄-linker2: (SEQ. ID NO: 3) AA 679-694, NT 2035-208 [sic]

Sequence of the human TRAIL module3 (extracellular domain, AA 95-281, of the natural human TRAIL molecule): AA 695-, NT 2083-2643

Stop codon: NT 2644-2646

FIGS. 26A, 26B and 26C shows the nucleic acid sequence and the corresponding amino acid sequence of scTNF-AMAIZE (construct H) (SEQ. ID NOs: 39 and 40)

Features of Construct H

Leader peptide sequence for secretion of the protein in eukaryotic cells: AA 1-19, NT 1-57

Sequence of the single-chain (scFv) antibody fragment 40 specific for the tumor stroma antigen FAP: AA 20-267, NT 58-801

Flag tag peptide sequence for affinity purification of the formed protein: AA 278-285, NT 832-855

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Sequence of the human TNF module1 (extracellular domain, AA 79-181, of the natural human TNF molecule): AA 289-443, NT 865-1329

Sequence of the (GGGS)₄-linker1: (SEQ. ID NO: 3) AA 444-, NT 1330-1377

Sequence of the human TNF module2 (extracellular domain, AA 79-181, of the natural human TNF molecule): AA 460-614, NT 1378-1842

Sequence of the (GGGS)₄-linker2: (SEQ. ID NO: 3) AA 615-630, NT 1843-1890

Sequence of the human TNF module3 (extracellular domain, AA 79-181, of the natural human TNF molecule): AA 631-785, NT 1891-2353

Stop codon: NT 2356-2358

FIG. 27 shows the pharmacokinetics of human wild-type TNF and human scTNF (cf. Example 2). The data in FIG. 27 show a clear increase in the in vivo half-life of the scTNF variants. A clearly increased duration of action in vivo is expected for scTNF compared with TNF, which thereby emphasizes the value of scTNF, particularly of scTNF-L₂, as a potential therapeutic agent.

FIG. 28 shows CysHis-scTNF coupled covalently to particles (silica). This covalently coupled CysHis-scTNF is bio-active and possesses the special activity of membrane-bound TNF; i.e., it activates TNFR2. FIG. 28 shows that cells from mouse fibroblasts, which are transfected with the construct TNFR2-Fas and were treated with serial dilutions of the indicated reagents, are totally resistant to soluble wtTNF. After covalent coupling of reduced CysHis-scTNF to silica micro-particles (beads) according to established protocols (DPA 2001, No. DE 10144252), these cause a strong cytotoxic response (circle), like a positive control consisting of CysHis-scTNF and a TNFR2 cross-linking antibody, mAk 80M2 (triangle). Cys-His scTNF, which is not coupled, as expected shows no activity on TNFR2-positive cells (squares).

The present invention will be illustrated below by the examples:

DETAILED DESCRIPTION OF THE INVENTION

Examples

Example 1

Preparation of Different Polypeptide Constructs of the Invention

All clonings were performed according to standard protocols. The conditions for these are given below.

Standard PCR:

60 ng of template, 0.5 μ L, of 100 μ M primer, 1 μ L of 10 mM dNTPs, 5 μ L of 10 \times buffer, and 2 U of Taq polymerase were amplified in a reaction volume of 50 μ L using the following PCR program. Denaturation: 94 $^{\circ}$ C. 3 minutes; 15 cycles: denaturation 94 $^{\circ}$ C. 30 seconds, annealing 55 $^{\circ}$ C. 30 seconds, elongation 72 $^{\circ}$ C. 90 seconds; final elongation: 72 $^{\circ}$ C. 7 minutes.

Digestion of PCR Products:

The PCR product was purified over an agarose gel and eluted and then digested with the appropriate restriction enzymes (see specific instructions) in a 40- μ L reaction batch at an optimal cleavage temperature (indicated by the manufacturer) for 2 hours.

Digestion of Vectors:

1 μ g of vector was digested with 5 U of the appropriate restriction enzymes in a 20- μ L reaction volume for 2 hours at an optimal cleavage temperature (this depends on employed

enzyme and is indicated by the manufacturer). To dephosphorylate the vectors, 10 U of alkaline phosphatase was added for 1 hour to the reaction digest.

Fill-In Reaction:

The batch from a vector digestion was combined with 33 μ M dNTPs and 1 U/1 μ g of DNA Klenow fragment of DNA polymerase I and incubated for 15 minutes at 25° C. This reaction was stopped with 10 mM EDTA for 20 minutes at 75° C.

Ligation:

Vector and insert were ligated at a molar ratio of 1:5 together with 400 U of ligase in a 10- μ L volume overnight at 16° C.

I. Preparation of scTNF_{human} (scTNF) with 4x or 3x Linkers:

Two scTNF variants were prepared, which differ in the length of the peptide linker between the individual modules. Primers with a 4x or 3x peptide-linker sequence were used: linker_{long} with the (GGGS)₄: (SEQ. ID NO: 3) sequence or linker_{short} with the (GGGS)₃: (SEQ. ID NO: 2) sequence. The produced constructs accordingly contained either only two 4x linkers (L1 or L2 with (GGGS)₄: (SEQ. ID NO: 3)—designated as “long”) or only two 3x linkers (L1 or L2 with (GGGS)₃:—designated as “short”).

1. A standard PCR was run with primers V and I or II (for linker_{long}) and the pQE-9 vector with a TNF module (pQE9-HisTNF) as the template. The vector bears a His tag sequence for later affinity purification of the produced protein.

2. The obtained PCR product I was then digested with 20 U of the appropriate restriction enzymes StuI and HindIII at 37° C. The same digestion and a dephosphorylation reaction were performed with the pQE9-HisTNF vector, and the PCR product I was inserted into the pQE9-HisTNF vector by ligation. The result of this step was a His tag-TNF module1 with a linker1_{short or long} or the following construct in the pQE9 vector:

EcoRI-His tag-TNF module1-linker1_{short or long}-BamHI

3. PCR product II was produced by another PCR with primers III and I or II (for linker_{long}) and the pQE9-HisTNF vector as the template. PCR product II was then cut each time with 20 U of the restriction enzymes EcoRI and HindIII and inserted into the pQE9-HisTNF vector, which was cut with the same enzymes and dephosphorylated. The result of this cloning was a pQE9 vector, which was as follows:

TNF module2-linker2_{short or long}-BamHI

This construct has no sequence for a His tag before the TNF sequence.

4. The cloned PCR product II from step 3 was first cut from the vector with the restriction enzyme Hind III and then partially with BamHI (1 U/ μ g of DNA). The pQE9-His tag TNF module1-linker1_{short or long} vector was also cut sequentially with the restriction enzymes BamHI and HindIII, dephosphorylated, and the PCR product II ligated into this vector. The result was a pQE9 vector with following construct:

His tag-TNF module1-linker1_{short or long} TNF module2-linker2_{short or long}.

5. Another PCR was performed under standard conditions with the pQE9-HisTNF vector as the template and primers III and IV, and the obtained PCR product III was sequentially digested with restriction enzymes BamHI (40 U) and HindIII (40 U). This fragment was then ligated into a pBluescript SKII vector, which was also cut with the restriction enzymes BamHI and HindIII. The result of this cloning was a pBluescript SKII vector, which contains the TNF module 3 without linkers.

6. The pQE9 vector with the His tag-TNF module1-linker1_{short or long} TNF module2-linker2_{short or long} construct was cut with the restriction enzyme EcoRI; this vector was then treated with the Klenow fragment of DNA polymerase I from *E. coli*, to carry out a fill in. After this step, a partial restriction digestion was carried out with the enzyme BamHI (1 U/ μ g DNA).

7. Parallel to Step 6, the pBluescript SKII vector, which contains the TNF module 3, was cut with the restriction enzyme XbaI; this vector was then treated with the Klenow fragment of DNA polymerase I from *E. coli*, to carry out a fill in. After this step, a second restriction digestion was carried out with the enzyme BamHI under standard conditions with additional dephosphorylation.

8. The fragment obtained by the restriction digestion from Step 6 was then ligated into the linear vector generated in Step 7. The constructs were in reverse order as follows:

HindIII-TNF module3-linker2_{short or long}-TNF module2-linker2_{short or long}-TNF module1-His tag-EcoRI

9. The reverse TNF construct from Step 8 was cut from the pBluescript SKII vector with the restriction enzymes EcoRI and HindIII and ligated into the pQE9-HisTNF vector, which was treated with the same enzymes and dephosphorylated. As a result, the following construct with the complete scTNF in correct orientation formed:

EcoRI-His Tag-TNF module1-linker1_{short or long}-TNF module2-linker2_{short or long}-TNF module3-HindIII

10. To prepare an scTNF with an N-terminal cysteine, the oligos cys-scTNF VI and VII were annealed (20 μ L each of 100 μ M oligo VI or VII were heated together for 5 minutes at 95° C. and slowly allowed to cool to room temperature), and oligo1 was formed in this way. The construct from Step 9 was digested with the restriction enzymes EcoRI and BbsI and the oligo1, which has the same cut sites, was ligated into the vector. Alternatively, the cysteine was inserted via PCR mutagenesis. The result of this cloning was the following construct:

EcoRI-Cysteine-His Tag-TNF module1-linker1_{short or long}-TNF module2-linker2_{short or long}-TNF module3-HindIII

All constructs were verified by sequencing.

The expression was carried out in the *E. coli* strain XL-1 blue. The purification of the expressed scTNF variants occurred with use of chromatographic methods (His tag affinity and anion exchange chromatography).

The sequences of the employed primers are given below:

Peptide linker sequences at the protein level (SEQ. ID NOs: 2 and 3)

3x GGGS-linker (short) = (GGGS)₃:
GGGS GGGS GGGS

4x GGGS-linker (long) = (GGGS)₄:
GGGS GGGS GGGS GGGS

Peptide linker sequences at the nucleotide level (SEQ. ID NOs: 4 and 5)

3x GGGS-linker (short):
5'GGT GGC GGT TCT GGT GGC GGT TCT GGT GGC GGA
TCC3'

4x GGGS-linker (long):
5'GGT GGC GGT TCT GGT GGC GGT TCT GGT GGC GGT TCT
GGT GGC GGA TCC3'

Primers for the Clonings

scTNF Primer I (SEQ. ID NO: 6)
 5'-TCG ATT AAG CTT CCC GGG GGA TCC GCC ACC AGA ACC
 GCC ACC AGA ACC GCC ACC CAG AGC GAT GAT ACC GAA
 GTA AAC CTG ACC-3'

scTNF Primer II (SEQ. ID NO: 7)
 5'-ATC GAT TAA GCT TCC CGG GGG ATC CGC CAC CAG AAC
 CGC CAC CAG AAC CGC CAC CAG AAC CGC CAC CCA GAG
 CGA TGA TAC CGA AGT AAA CCT GAC C-3'

scTNF Primer III (SEQ. ID NO: 8)
 5'-CCC CGA ATT CGG ATC CTC TTC TCG TAC CCC GTC TGA
 CAA ACC G-3'

scTNF Primer IV (SEQ. ID NO: 9)
 5'-GGG GGG GAA GCT TAT CGA TAG TTA GAT ATC ATC ACA
 GAG CGA TGA TAC CGA AG-3'

scTNF Primer V (SEQ. ID NO: 10)
 5'-CCT GTA CCT GAT CTA CTC CCA GGT TCT GTT CAA AGG
 CCA GG-3'

Oligo for CysHis Insertion:

cys-scTNF Primer VI (SEQ. ID NO: 11)
 AAT TCA TTA AAG AGG AGA AAT TAA CTA TGG GAG AGC
 TCA TCG AAG GTC GCT GCG CCG GTG GAT CTG GTC ATC
 ATC ATC ACC ATC ACG GCT CAG ACG G

cys-sscTNF Primer VII (SEQ. ID NO: 12)
 CGC TCC GTC TGA GCC GTG ATG GTG ATG ATG ACC
 AGA TCC ACC GGC GCA GCG ACC TTC GAT GAG CTC TCC
 CAT AGT TAA TTT CTC CTC TTT AAT G

II. Preparation of scFasL in pcDNA3 and AMAIZE Constructs:

The standard conditions given in Example 1 were used for the following clonings.

A. Generation of the HA Signal in pcDNA3

1. Digestion of the pcDNA3 vector with KpnI and NotI.
2. Preparation of the HA oligo with the KpnI-HA-signal-NotI sequence by annealing of the primers HA-IF and HA-IIR for the leader peptide sequence:
3. Ligation of the HA oligo (contains KpnI and NotI cut sites) into the pcDNA3 vector

B. Preparation of scFasL in the pcDNA3(+) Vector

1. PCR with primers FasL#1F and FasL#2R on the template FasL-AMAIZE vector. The preparation of this type of constructs is described in German Patent Application DE 10045591.3, which is herewith incorporated in its entirety in the disclosure of the present invention. The product of this PCR 1 was a NotI-Flag tag-FasL module1-linker1-BamHI-XbaI construct.
2. Using the NotI and XbaI restriction cut sites, this construct was cloned in the pcDNA3-HA sequence vector with the same enzymes, so that the following construct forms:
 HA sequence-Flag tag-FasL module1-linker1-BamHI-XbaI
3. The following PCR product 2 was generated with the use of another PCR on the template FasL-AMAIZE vector and the primers FasL#3 and FasL#1:
 blunt end-FasL module2-linker2-BamHI-XbaI
4. In the next step, the pcDNA3 vector from Step 2 was digested with BamHI; this cut site was filled with the Klenow enzyme and then cut with XbaI, so that a "blunt end" and a "sticky end" formed. PCR product 2 was then

cloned in this thus modified vector, with the formation of the following construct:

HA sequence-Flag tag-FasL module1-linker1-FasL module2-linker2-BamHI-XbaI

5. For the third module, another PCR was performed with the template FasL-AMAIZE vector and the primers FasL#4 and FasL#5. The formed PCR product was then digested with the restriction enzymes BamHI and XbaI and ligated into the vector, cut with the same enzymes, from Step 4. The result of this cloning was the following construct in the pcDNA3 vector:

HA sequence-NotI-Flag tag-FasL module1-linker1-FasL module2-linker2-FasL module3-stop-XbaI.

- To prepare the scFasL- or scTNF-AMAIZE constructs, the corresponding scFasL or scTNF was digested with the restriction enzymes NotI or EcoRI and XbaI and the inserts were inserted as cassettes into the corresponding AMAIZE vectors (see German Patent Application DE 10045591.3), whereby these vectors were also cut with the enzymes NotI or EcoRI and XbaI. The following constructs were prepared in this way:

Leading peptide-scFv40-Flag tag-FasL module1-linker1-FasL module2-linker2-FasL module3

Leading peptide-scFv40-Flag tag-TNF module1-linker1-TNF module2-linker2-TNF module3

- The sequences of the employed primers are given below: (SEQ. ID NOs: 13-19)

FasL#1R:
 5'ATCGATTTCCTAGACCCGGGGATCGCCACCAGAACCCGCCACCAGAACC
 GCCACCAGAACCCGCCACCAGCTTATATAAGCCGAAAAACGCTGAGATT
 C3'

FasL#2F:
 5'GGGGTAGCGGCCGCTGTCGACGATTACAAAGAC3'

FasL#3F:
 5'AGAAAAAAGGAGCTGAGGAAAGTGG3'

FasL#4F:
 5'GGGGCGGATCCGAAAAAAGGAGCTGAGGAAAGTGG3'

FasL#5R:
 5'GGGGCCTCTAGAATCGATGGTCAGAGCTTATATAAGCCGAAAAACGTC
 TG3'

HA-IF
 5'CGCCAT GGCTATCATC TACCTCATCC TCCTGTTTCAC
 CGTGTGCGG GGAGC3'

HA-IIR
 5'GGC CGC TGC CCC GCA CAG CGG TGA ACA GGA GGA TGA
 GGT AGA TGA TAG CCA TGG CGG TAC3'

III. scTRAIL Cloning and Preparation of scTRAIL AMAIZE Constructs

The standard conditions given in Example 1 were used for the following clonings.

1. PCR with primers TRAIL#1 and TRAIL#2 on the template pcDNA3-sc40-TRAIL (see German Patent Application DE 10045591.3). PCR product 1 was cut with EcoRI and XbaI and ligated into the pcDNA3-scFasL vector digested with the same restriction enzymes. This digestion deleted the FasL sequence, whereby the HA and Flag tag sequence was retained and the following construct was now formed:
 HA sequence-Flag tag-TRAIL module1-linker1-BamHI-XbaI
2. Using the primers TRAIL#1R and TRAIL#2F, PCR product 2 was generated with the template TRAIL-AMAIZE (see German Patent Application DE 10045591.3). This was cut only with XbaI, a blunt end and a sticky end forming. The construct from Step 1 was digested with BamHI and then treated with the Klenow enzyme, so that the ends were filled. After this, an XbaI digestion was performed and the

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PCR product 2 was cloned in this vector. The result was the following construct: HA sequence-Flag tag-TRAIL module1-linker1-TRAIL module2-linker2-BamHI-XbaI

3. For the cloning of TRAIL module 3, a PCR was carried out with the primers TRAIL#4 and TRAIL#5 on the template TRAIL-AMAIze, the product was then digested with BamHI and XbaI, and cloned in the construct from Step 2—also digested with BamHI and XbaI, as a result of which the following construct formed:

HA sequence-Flag tag-TRAIL module1-linker1-TRAIL module2-linker2-TRAIL module3 Stop-XbaI

For the preparation of the scTRAIL-AMAIze constructs, the specific scTRAIL vectors were digested with the restriction enzymes NotI or EcoRI and XbaI and the inserts were inserted as cassettes into the corresponding AMAIze vectors (see German Patent Application DE 10045591.3), whereby these vectors were also cut with the enzymes NotI or EcoRI and XbaI. The following constructs were prepared in this way:

HA svFv40-Flag tag-TRAIL module1-linker1-TRAIL module2-linker2-TRAIL module3

The sequences of the employed primers are given below: Primers for the scTRAIL cloning (SEQ. ID NOs: 20-24)

TRAIL#1R:

5' ATCGATTCTAGACCCGGGGATCCGCCACCAGAACGCCACCAGAACCGCCACCAGAACCGCCCACTAAAGGCCCGAAAAAATGGCTT-CATGGTC3'

TRAIL#2F:

5' GGGGTAGAAATTCGAACCTCTGAGGAAACCATTTCTACAGTTCAAG3'

TRAIL#3F:

5' AACCTCTGAGGAAACCATTTCTACAG3'

TRAIL#4F:

5' GGGGCGGATCCACCTCTGAGGAAACCATTTCTACAG3'

TRAIL#5R:

5' GGGGCTCTAGAAATCGATGGTCAGCCAACTAAAGGCCCGAAAAAATGGTC3'

Example 2

Pharmacokinetics of Human Wild-Type TNF and Human scTNF

Six-week-old Balb/c mice were injected i.v. with 12 µg of TNF or scTNF (3 mice in each case). Blood was taken every 45 minutes and collected, and the concentration of TNF in the serum was determined using a human TNF-specific ELISA kit. The data in FIG. 27 show a clear increase in the in vivo half-life of the scTNF variants. It is therefore expected for scTNF that it has a clearly increased duration of biological action in vivo, which thereby emphasizes the value of scTNF as a potential therapeutic agent.

Example 3

CysHis-scTNF Coupled Covalently to Particles (Silica)

Mouse fibroblasts, transfected with the construct TNFR2-Fas, were treated with serial dilutions of the indicated reagents. These cells are completely resistant to soluble wtTNF. After covalent coupling of reduced CysHis-scTNF to silica microparticles (beads) according to established protocols (DPA 2001, No. DE 10144252), these cause a strong cytotoxic response (circle), like a positive control consisting

38

of CysHis-scTNF and a TNFR2 cross-linking antibody, mAk 80M2 (triangle). CysHis-scTNF, which is not coupled, as expected shows no activity on TNFR2 positive cells (squares). Covalently coupled CysHis-scTNF is bioactive and possesses the special activity of membrane-bound TNF; i.e., it activates TNFR2.

Example 4

Comparison of Standard Recombinant Human (Rh)TNF and scTNF in In Vivo Tumor Necrosis Models and In Vitro L929 Cytotoxicity Activity

Mice: C3H/HeJ (female), 17-19 g, from Charles River

Tumor cells: CFS-1 methylcholanthrene-induced fibrosarcoma cell line derived from C3H/HeN mouse (reference: Hafner M., P. Orosz, A. Krüger, and D. N. Männel. 1996 TNF promotes metastasis by impairing natural killer cell activity. *Internat. J. Cancer* 66:388-392).

Tumor necrosis experiment: The mice received 1.6×10^7 CFS-1 cells in 50 µL of medium (RPMI, 10% FCS) intradermally in the back; the tumors were allowed to grow for 12 days until they reached a size of about 5-6 mm in diameter, before the intraperitoneal injection of TNF (10 µg per mouse) in 200 µL of PBS or PBS alone as control. The tumor size was measured daily and examined grossly. The mice were sacrificed on Day 6 after the treatment and the tumors were removed for histology. The tumors were excised, fixed overnight in 4% PBS-buffered formalin, and embedded in paraffin. Equatorial vertical sections (4 µm) were stained with hematoxylin and eosin, and examined microscopically for necrosis (as described, e.g., in: Lucas R. et al., 2001, *Int J Cancer*, 91:543-549).

TNF: rhTNF specific activity 6.6×10^6 U/mg (48-hour L929 test without Act D) scTNF

In vitro experiment, LD₅₀ activity in the L929 cytotoxicity assay with Act D for:

rhTNF=391 pg/mL

scTNF=39 pg/mL

(tested with the same TNF samples, which were used for in vivo experiments) scTNF in this in vitro experiment shows a 10-fold increased activity.

In Vivo Tumor Necrosis Experiment:

Group	n	d0	d4	Necrosis		
				Tumor diameter	microscopic**	
				gross*	<5%	>10%
PBS	6	5.2 ± 1	7.4 ± 0.5	1	2	0
rhTNF	7	5.2 ± 0.9	6.6 ± 1.7	3	6	1
scTNF	7	5.9 ± 0.5	7.3 ± 0.3	5	0	7

*= grossly clearly discernible superficial necrosis

**= on microscopic examination, central hemorrhagic necrosis, <5% or >10% of the tumor tissue

CONCLUSION

After 4 days of treatment with single doses, there was no difference in the tumor size (for an overview on TNF as a tumor therapeutic agent, see, e.g., Eggermont et al, *Lancet Oncol.* 4, 429 (2003)).

rhTNF induced small hemorrhagic necrosis (<5% of the tumor area), visible grossly in only 3/7 of the animals. scTNF induced larger hemorrhagic necroses (>10% of the tumor area) in all tumors (7/7), 5/7 of which can be seen grossly.

scTNF>>rhTNF in relation to tumor cytotoxicity in vitro and induction of necrosis.

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<223> OTHER INFORMATION: Description of Artificial Sequence: Primer
TRAIL#1R

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<220> FEATURE:
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tct cgt acc ccg tct gac aaa ccg gtt gct cac gtt gtt gca aac ccg          96
Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro
      20      25      30

cag gct gaa ggt caa ctg caa tgg ctg aac cgt cgt gct aac gct ctg          144
Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
      35      40      45

ctg gct aac ggt gtt gaa ctg cgt gac aac cag ctg gtt gtt ccg tct          192
Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser
      50      55      60

gaa ggc ctg tac ctg atc tac tcc cag gtt ctg ttc aaa ggc cag ggc          240
Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
      65      70      75      80

tgc ccg tcc acc cac gtt ctg ctg acc cac acc atc tct cgt atc gct          288
Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala
      85      90      95

gtt tcc tac cag acc aaa gta aac ctg ctg tct gca atc aaa tct ccg          336
Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro
      100      105      110

tgc cag cgt gaa acc ccg gaa ggt gct gaa gct aaa ccg tgg tac gaa          384
Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu
      115      120      125

ccg atc tac ctg ggt ggc gtt ttt caa ctg gag aaa ggt gac cgt ctg          432
Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu
      130      135      140

tct gca gaa att aac cgt ccg gac tac ctg gac ttc gca gaa tct ggt          480
Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly
      145      150      155      160

cag gtt tac ttc ggt atc atc gct ctg ggt ggc ggt tct ggt ggc ggt          528
Gln Val Tyr Phe Gly Ile Ile Ala Leu Gly Gly Gly Ser Gly Gly Gly
      165      170      175

tct ggt ggc gga tcc tct tct cgt acc ccg tct gac aaa ccg gtt gct          576
Ser Gly Gly Gly Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala
      180      185      190

cac gtt gtt gca aac ccg cag gct gaa ggt caa ctg caa tgg ctg aac          624
His Val Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn
      195      200      205

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ctg ttc aaa ggc cag ggc tgc ccg tcc acc cac gtt ctg ctg acc cac Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His 245 250 255	768
acc atc tct cgt atc gct gtt tcc tac cag acc aaa gta aac ctg ctg Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu 260 265 270	816
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gag aaa ggt gac cgt ctg tct gca gaa att aac cgt ccg gac tac ctg Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu 305 310 315 320	960
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ggt gaa ctg cgt gac aac cag ctg gtt gtt ccg tct gaa ggc ctg tac Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr 385 390 395 400	1200
ctg atc tac tcc cag gtt ctg ttc aaa ggc cag ggc tgc ccg tcc acc Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr 405 410 415	1248
cac gtt ctg ctg acc cac acc atc tct cgt atc gct gtt tcc tac cag His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln 420 425 430	1296
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acc ccg gaa ggt gct gaa gct aaa ccg tgg tac gaa ccg atc tac ctg Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu 450 455 460	1392
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aac cgt ccg gac tac ctg gac ttc gca gaa tct ggt cag gtt tac ttc Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe 485 490 495	1488
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Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
          35          40          45

Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser
          50          55          60

Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
65          70          75          80

Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala
          85          90          95

Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro
          100         105         110

Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu
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Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu
          130         135         140

Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly
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Gln Val Tyr Phe Gly Ile Ile Ala Leu Gly Gly Gly Ser Gly Gly Gly
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Ser Gly Gly Gly Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala
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His Val Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn
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Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn
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Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val
225         230         235         240

Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His
          245         250         255

Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu
          260         265         270

Ser Ala Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu
          275         280         285

Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu
          290         295         300

Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu
305         310         315         320

Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu Gly
          325         330         335

Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Ser Ser Arg Thr Pro
          340         345         350

Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro Gln Ala Glu Gly
          355         360         365

Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly
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Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr
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His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln
 420 425 430

Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln Arg Glu
 435 440 445

Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu
 450 455 460

Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile
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His His His His Gly Ser Asp Gly Ala Ser Ser Ser Ser Arg Thr Pro	
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tct gac aaa ccg gtt gct cac gtt gtt gca aac ccg cag gct gaa ggt	144
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gtt gaa ctg cgt gac aac cag ctg gtt gtt ccg tct gaa ggc ctg tac	240
Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr	
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ctg atc tac tcc cag gtt ctg ttc aaa ggc cag ggc tgc ccg tcc acc	288
Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr	
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His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln	
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acc aaa gta aac ctg ctg tct gca atc aaa tct ccg tgc cag cgt gaa	384
Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln Arg Glu	
115 120 125	
acc ccg gaa ggt gct gaa gct aaa ccg tgg tac gaa ccg atc tac ctg	432
Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu	
130 135 140	
ggt ggc gtt ttt caa ctg gag aaa ggt gac cgt ctg tct gca gaa att	480
Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile	
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tcc tct tct cgt acc ccg tct gac aaa ccg gtt gct cac gtt gtt gca Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala 195 200 205	624
aac ccg cag gct gaa ggt caa ctg caa tgg ctg aac cgt cgt gct aac Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn 210 215 220	672
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ccg tct gaa ggc ctg tac ctg atc tac tcc cag gtt ctg ttc aaa ggc Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly 245 250 255	768
cag ggc tgc ccg tcc acc cac gtt ctg ctg acc cac acc atc tct cgt Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg 260 265 270	816
atc gct gtt tcc tac cag acc aaa gta aac ctg ctg tct gca atc aaa Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys 275 280 285	864
tct ccg tgc cag cgt gaa acc ccg gaa ggt gct gaa gct aaa ccg tgg Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp 290 295 300	912
tac gaa ccg atc tac ctg ggt ggc gtt ttt caa ctg gag aaa ggt gac Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp 305 310 315 320	960
cgt ctg tct gca gaa att aac cgt ccg gac tac ctg gac ttc gca gaa Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu 325 330 335	1008
tct ggt cag gtt tac ttc ggt atc atc gct ctg ggt ggc ggt tct ggt Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu Gly Gly Gly Ser Gly 340 345 350	1056
ggc ggt tct ggt ggc gga tcc tct tct cgt acc ccg tct gac aaa ccg Gly Gly Ser Gly Gly Gly Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro 355 360 365	1104
ggt gct cac gtt gtt gca aac ccg cag gct gaa ggt caa ctg caa tgg Val Ala His Val Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp 370 375 380	1152
ctg aac cgt cgt gct aac gct ctg ctg gct aac ggt gtt gaa ctg cgt Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg 385 390 395 400	1200
gac aac cag ctg gtt gtt ccg tct gaa ggc ctg tac ctg atc tac tcc Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser 405 410 415	1248
cag gtt ctg ttc aaa ggc cag ggc tgc ccg tcc acc cac gtt ctg ctg Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu 420 425 430	1296
acc cac acc atc tct cgt atc gct gtt tcc tac cag acc aaa gta aac Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn 435 440 445	1344
ctg ctg tct gca atc aaa tct ccg tgc cag cgt gaa acc ccg gaa ggt Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly 450 455 460	1392
gct gaa gct aaa ccg tgg tac gaa ccg atc tac ctg ggt ggc gtt ttt Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe 465 470 475 480	1440

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caa ctg gag aaa ggt gac cgt ctg tct gca gaa att aac cgt ccg gac	1488
Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp	
485 490 495	
tac ctg gac ttc gca gaa tct ggt cag gtt tac ttc ggt atc atc gct	1536
Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala	
500 505 510	
ctg tga	1542
Leu	

<210> SEQ ID NO 28
 <211> LENGTH: 513
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: cys-scTNF-L short peptide

<400> SEQUENCE: 28

Met Gly Glu Leu Ile Glu Gly Arg Cys Ala Gly Gly Ser Gly His His	
1 5 10 15	
His His His His Gly Ser Asp Gly Ala Ser Ser Ser Ser Arg Thr Pro	
20 25 30	
Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro Gln Ala Glu Gly	
35 40 45	
Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly	
50 55 60	
Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr	
65 70 75 80	
Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr	
85 90 95	
His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln	
100 105 110	
Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln Arg Glu	
115 120 125	
Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu	
130 135 140	
Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile	
145 150 155 160	
Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe	
165 170 175	
Gly Ile Ile Ala Leu Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly	
180 185 190	
Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala	
195 200 205	
Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn	
210 215 220	
Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val	
225 230 235 240	
Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly	
245 250 255	
Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg	
260 265 270	
Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys	
275 280 285	
Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp	
290 295 300	

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Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp
 305 310 315 320
 Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu
 325 330 335
 Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu Gly Gly Gly Ser Gly
 340 345 350
 Gly Gly Ser Gly Gly Gly Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro
 355 360 365
 Val Ala His Val Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp
 370 375 380
 Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg
 385 390 395 400
 Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser
 405 410 415
 Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu
 420 425 430
 Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn
 435 440 445
 Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly
 450 455 460
 Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe
 465 470 475 480
 Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp
 485 490 495
 Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala
 500 505 510
 Leu

<210> SEQ ID NO 29
 <211> LENGTH: 1476
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: scFasL
 sequence
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1473)

<400> SEQUENCE: 29

atg gct atc atc tac ctc atc ctc ctg ttc acc gct gtg cgg ggc gcg	48
Met Ala Ile Ile Tyr Leu Ile Leu Leu Phe Thr Ala Val Arg Gly Ala	
1 5 10 15	
gcc gcg gat tac aaa gac gat gac gat aaa gaa ttc acg cgt gaa aaa	96
Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys Glu Phe Thr Arg Glu Lys	
20 25 30	
aag gag ctg agg aaa gtg gcc cat tta aca ggc aag tcc aac tca agg	144
Lys Glu Leu Arg Lys Val Ala His Leu Thr Gly Lys Ser Asn Ser Arg	
35 40 45	
tcc atg cct ctg gaa tgg gaa gac acc tat gga att gtc ctg ctt tct	192
Ser Met Pro Leu Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser	
50 55 60	
gga gtg aag tat aag aag ggt ggc ctt gtg atc aat gaa act ggg ctg	240
Gly Val Lys Tyr Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu	
65 70 75 80	
tac ttt gta tat tcc aaa gta tac ttc cgg ggt caa tct tgc aac aac	288
Tyr Phe Val Tyr Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys Asn Asn	
85 90 95	

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ctg ccc ctg agc cac aag gtc tac atg agg aac tct aag tat ccc cag Leu Pro Leu Ser His Lys Val Tyr Met Arg Asn Ser Lys Tyr Pro Gln 100 105 110	336
gat ctg gtg atg atg gag ggg aag atg atg agc tac tgc act act ggg Asp Leu Val Met Met Glu Gly Lys Met Met Ser Tyr Cys Thr Thr Gly 115 120 125	384
cag atg tgg gcc cgc agc agc tac ctg ggg gca gtg ttc aat ctt acc Gln Met Trp Ala Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn Leu Thr 130 135 140	432
agt gct gat cat tta tat gtc aac gta tct gag ctc tct ctg gtc aat Ser Ala Asp His Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val Asn 145 150 155 160	480
ttt gag gaa tct cag acg ttt ttc ggc tta tat aag ctc ggt ggc ggt Phe Glu Glu Ser Gln Thr Phe Phe Gly Leu Tyr Lys Leu Gly Gly Gly 165 170 175	528
tct ggt ggc ggt tct ggt ggc ggt tct ggt ggc gga tca gaa aaa aag Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Glu Lys Lys 180 185 190	576
gag ctg agg aaa gtg gcc cat tta aca ggc aag tcc aac tca agg tcc Glu Leu Arg Lys Val Ala His Leu Thr Gly Lys Ser Asn Ser Arg Ser 195 200 205	624
atg cct ctg gaa tgg gaa gac acc tat gga att gtc ctg ctt tct gga Met Pro Leu Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser Gly 210 215 220	672
gtg aag tat aag aag ggt ggc ctt gtg atc aat gaa act ggg ctg tac Val Lys Tyr Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu Tyr 225 230 235 240	720
ttt gta tat tcc aaa gta tac ttc cgg ggt caa tct tgc aac aac ctg Phe Val Tyr Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys Asn Asn Leu 245 250 255	768
ccc ctg agc cac aag gtc tac atg agg aac tct aag tat ccc cag gat Pro Leu Ser His Lys Val Tyr Met Arg Asn Ser Lys Tyr Pro Gln Asp 260 265 270	816
ctg gtg atg atg gag ggg aag atg atg agc tac tgc act act ggg cag Leu Val Met Met Glu Gly Lys Met Met Ser Tyr Cys Thr Thr Gly Gln 275 280 285	864
atg tgg gcc cgc agc agc tac ctg ggg gca gtg ttc aat ctt acc agt Met Trp Ala Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn Leu Thr Ser 290 295 300	912
gct gat cat tta tat gtc aac gta tct gag ctc tct ctg gtc aat ttt Ala Asp His Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val Asn Phe 305 310 315 320	960
gag gaa tct cag acg ttt ttc ggc tta tat aag ctc ggt ggc ggt tct Glu Glu Ser Gln Thr Phe Phe Gly Leu Tyr Lys Leu Gly Gly Gly Ser 325 330 335	1008
ggg ggc ggt tct ggt ggc ggt tct ggt ggc gga tcc gaa aaa aag gag Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Glu Lys Lys Glu 340 345 350	1056
ctg agg aaa gtg gcc cat tta aca ggc aag tcc aac tca agg tcc atg Leu Arg Lys Val Ala His Leu Thr Gly Lys Ser Asn Ser Arg Ser Met 355 360 365	1104
cct ctg gaa tgg gaa gac acc tat gga att gtc ctg ctt tct gga gtg Pro Leu Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser Gly Val 370 375 380	1152
aag tat aag aag ggt ggc ctt gtg atc aat gaa act ggg ctg tac ttt Lys Tyr Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu Tyr Phe 385 390 395 400	1200
gta tat tcc aaa gta tac ttc cgg ggt caa tct tgc aac aac ctg ccc Val Tyr Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys Asn Asn Leu Pro	1248

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405	410	415	
ctg agc cac aag gtc tac atg agg aac tct aag tat ccc cag gat ctg			1296
Leu Ser His Lys Val Tyr Met Arg Asn Ser Lys Tyr Pro Gln Asp Leu			
420	425	430	
gtg atg atg gag ggg aag atg atg agc tac tgc act act ggg cag atg			1344
Val Met Met Glu Gly Lys Met Met Ser Tyr Cys Thr Thr Gly Gln Met			
435	440	445	
tgg gcc cgc agc agc tac ctg ggg gca gtg ttc aat ctt acc agt gct			1392
Trp Ala Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn Leu Thr Ser Ala			
450	455	460	
gat cat tta tat gtc aac gta tct gag ctc tct ctg gtc aat ttt gag			1440
Asp His Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val Asn Phe Glu			
465	470	475	480
gaa tct cag acg ttt ttc ggc tta tat aag ctc tga			1476
Glu Ser Gln Thr Phe Phe Gly Leu Tyr Lys Leu			
485	490		
<210> SEQ ID NO 30			
<211> LENGTH: 491			
<212> TYPE: PRT			
<213> ORGANISM: Artificial Sequence			
<220> FEATURE:			
<223> OTHER INFORMATION: Description of Artificial Sequence: scFasL peptide sequence			
<400> SEQUENCE: 30			
Met Ala Ile Ile Tyr Leu Ile Leu Leu Phe Thr Ala Val Arg Gly Ala			
1	5	10	15
Ala Ala Asp Tyr Lys Asp Asp Asp Lys Glu Phe Thr Arg Glu Lys			
	20	25	30
Lys Glu Leu Arg Lys Val Ala His Leu Thr Gly Lys Ser Asn Ser Arg			
	35	40	45
Ser Met Pro Leu Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser			
	50	55	60
Gly Val Lys Tyr Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu			
65	70	75	80
Tyr Phe Val Tyr Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys Asn Asn			
	85	90	95
Leu Pro Leu Ser His Lys Val Tyr Met Arg Asn Ser Lys Tyr Pro Gln			
	100	105	110
Asp Leu Val Met Met Glu Gly Lys Met Met Ser Tyr Cys Thr Thr Gly			
	115	120	125
Gln Met Trp Ala Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn Leu Thr			
	130	135	140
Ser Ala Asp His Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val Asn			
145	150	155	160
Phe Glu Glu Ser Gln Thr Phe Phe Gly Leu Tyr Lys Leu Gly Gly Gly			
	165	170	175
Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Glu Lys Lys			
	180	185	190
Glu Leu Arg Lys Val Ala His Leu Thr Gly Lys Ser Asn Ser Arg Ser			
	195	200	205
Met Pro Leu Glu Trp Glu Asp Thr Tyr Gly Ile Val Leu Leu Ser Gly			
	210	215	220
Val Lys Tyr Lys Lys Gly Gly Leu Val Ile Asn Glu Thr Gly Leu Tyr			
225	230	235	240
Phe Val Tyr Ser Lys Val Tyr Phe Arg Gly Gln Ser Cys Asn Asn Leu			

				245						250						255			
Pro	Leu	Ser	His	Lys	Val	Tyr	Met	Arg	Asn	Ser	Lys	Tyr	Pro	Gln	Asp				
			260					265					270						
Leu	Val	Met	Met	Glu	Gly	Lys	Met	Met	Ser	Tyr	Cys	Thr	Thr	Gly	Gln				
		275					280					285							
Met	Trp	Ala	Arg	Ser	Ser	Tyr	Leu	Gly	Ala	Val	Phe	Asn	Leu	Thr	Ser				
	290					295					300								
Ala	Asp	His	Leu	Tyr	Val	Asn	Val	Ser	Glu	Leu	Ser	Leu	Val	Asn	Phe				
305				310					315					320					
Glu	Glu	Ser	Gln	Thr	Phe	Phe	Gly	Leu	Tyr	Lys	Leu	Gly	Gly	Gly	Ser				
			325					330					335						
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Glu	Lys	Lys	Glu				
			340				345					350							
Leu	Arg	Lys	Val	Ala	His	Leu	Thr	Gly	Lys	Ser	Asn	Ser	Arg	Ser	Met				
	355					360					365								
Pro	Leu	Glu	Trp	Glu	Asp	Thr	Tyr	Gly	Ile	Val	Leu	Leu	Ser	Gly	Val				
	370				375					380									
Lys	Tyr	Lys	Lys	Gly	Gly	Leu	Val	Ile	Asn	Glu	Thr	Gly	Leu	Tyr	Phe				
385				390					395				400						
Val	Tyr	Ser	Lys	Val	Tyr	Phe	Arg	Gly	Gln	Ser	Cys	Asn	Asn	Leu	Pro				
			405					410					415						
Leu	Ser	His	Lys	Val	Tyr	Met	Arg	Asn	Ser	Lys	Tyr	Pro	Gln	Asp	Leu				
		420				425					430								
Val	Met	Met	Glu	Gly	Lys	Met	Met	Ser	Tyr	Cys	Thr	Thr	Gly	Gln	Met				
	435					440					445								
Trp	Ala	Arg	Ser	Ser	Tyr	Leu	Gly	Ala	Val	Phe	Asn	Leu	Thr	Ser	Ala				
	450				455				460										
Asp	His	Leu	Tyr	Val	Asn	Val	Ser	Glu	Leu	Ser	Leu	Val	Asn	Phe	Glu				
465				470				475					480						
Glu	Ser	Gln	Thr	Phe	Phe	Gly	Leu	Tyr	Lys	Leu									
		485				490													

<210> SEQ ID NO 31
 <211> LENGTH: 1869
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence: scTRAIL sequence
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1866)

<400> SEQUENCE: 31

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atg gct atc atc tac ctc atc ctc ctg ttc acc gct gtg cgG ggc gcg      48
Met Ala Ile ile Tyr Leu Ile Leu Leu Phe Thr Ala Val Arg Gly Ala
1          5              10             15

gcc gcg gat tac aaa gac gat gac gat aaa gaa ttc gga acc tct gag      96
Ala Ala Asp Tyr Lys Asp Asp Asp Lys Glu Phe Gly Thr Ser Glu
                20           25            30

gaa acc att tct aca gtt caa gaa aag caa caa aat att tct ccc cta     144
Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro Leu
        35              40              45

gtg aga gaa aga ggt cct cag aga gta gca gct cac ata act ggg acc     192
Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr
        50              55              60

aga gga aga agc aac aca ttg tct tct cca aac tcc aag aat gaa aag     240
Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys
    
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65	70	75	80	
gct ctg ggc cgc aaa ata aac tcc tgg gaa tca tca agg agt ggg cat				288
Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His				
	85	90	95	
tca ttc ctg agc aac ttg cac ttg agg aat ggt gaa ctg gtc atc cat				336
Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile His				
	100	105	110	
gaa aaa ggg ttt tac tac atc tat tcc caa aca tac ttt cga ttt cag				384
Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln				
	115	120	125	
gag gaa ata aaa gaa aac aca aag aac gac aaa caa atg gtc caa tat				432
Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr				
	130	135	140	
att tac aaa tac aca agt tat cct gac cct ata ttg ttg atg aaa agt				480
Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys Ser				
	145	150	155	160
gct aga aat agt tgt tgg tct aaa gat gca gaa tat gga ctc tat tcc				528
Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser				
	165	170	175	
atc tat caa ggg gga ata ttt gag ctt aag gaa aat gac aga att ttt				576
Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile Phe				
	180	185	190	
gtt tct gta aca aat gag cac ttg ata gac atg gac cat gaa gcc agt				624
Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala Ser				
	195	200	205	
ttt ttc ggg gcc ttt tta gtt ggc ggt ggc ggt tct ggt ggc ggt tct				672
Phe Phe Gly Ala Phe Leu Val Gly Gly Gly Gly Ser Gly Gly Gly Ser				
	210	215	220	
ggg ggc ggt tct ggt ggc gga tca acc tct gag gaa acc att tct aca				720
Gly Gly Gly Ser Gly Gly Gly Ser Thr Ser Glu Glu Thr Ile Ser Thr				
	225	230	235	240
gtt caa gaa aag caa caa aat att tct ccc cta gtg aga gaa aga ggt				768
Val Gln Glu Lys Gln Gln Asn Ile Ser Pro Leu Val Arg Glu Arg Gly				
	245	250	255	
cct cag aga gta gca gct cac ata act ggg acc aga gga aga agc aac				816
Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg Gly Arg Ser Asn				
	260	265	270	
aca ttg tct tct cca aac tcc aag aat gaa aag gct ctg ggc cgc aaa				864
Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala Leu Gly Arg Lys				
	275	280	285	
ata aac tcc tgg gaa tca tca agg agt ggg cat tca ttc ctg agc aac				912
Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser Phe Leu Ser Asn				
	290	295	300	
ttg cac ttg agg aat ggt gaa ctg gtc atc cat gaa aaa ggg ttt tac				960
Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu Lys Gly Phe Tyr				
	305	310	315	320
tac atc tat tcc caa aca tac ttt cga ttt cag gag gaa ata aaa gaa				1008
Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Glu Ile Lys Glu				
	325	330	335	
aac aca aag aac gac aaa caa atg gtc caa tat att tac aaa tac aca				1056
Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile Tyr Lys Tyr Thr				
	340	345	350	
agt tat cct gac cct ata ttg ttg atg aaa agt gct aga aat agt tgt				1104
Ser Tyr Pro Asp Pro Ile Leu Met Lys Ser Ala Arg Asn Ser Cys				
	355	360	365	
tgg tct aaa gat gca gaa tat gga ctc tat tcc atc tat caa ggg gga				1152
Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly				
	370	375	380	
ata ttt gag ctt aag gaa aat gac aga att ttt gtt tct gta aca aat				1200

Val	Arg	Glu	Arg	Gly	Pro	Gln	Arg	Val	Ala	Ala	His	Ile	Thr	Gly	Thr		
50					55					60							
Arg	Gly	Arg	Ser	Asn	Thr	Leu	Ser	Ser	Pro	Asn	Ser	Lys	Asn	Glu	Lys		
65					70					75					80		
Ala	Leu	Gly	Arg	Lys	Ile	Asn	Ser	Trp	Glu	Ser	Ser	Arg	Ser	Gly	His		
85					90					95							
Ser	Phe	Leu	Ser	Asn	Leu	His	Leu	Arg	Asn	Gly	Glu	Leu	Val	Ile	His		
100					105					110							
Glu	Lys	Gly	Phe	Tyr	Tyr	Ile	Tyr	Ser	Gln	Thr	Tyr	Phe	Arg	Phe	Gln		
115					120					125							
Glu	Glu	Ile	Lys	Glu	Asn	Thr	Lys	Asn	Asp	Lys	Gln	Met	Val	Gln	Tyr		
130					135					140							
Ile	Tyr	Lys	Tyr	Thr	Ser	Tyr	Pro	Asp	Pro	Ile	Leu	Leu	Met	Lys	Ser		
145					150					155					160		
Ala	Arg	Asn	Ser	Cys	Trp	Ser	Lys	Asp	Ala	Glu	Tyr	Gly	Leu	Tyr	Ser		
165					170					175							
Ile	Tyr	Gln	Gly	Gly	Ile	Phe	Glu	Leu	Lys	Glu	Asn	Asp	Arg	Ile	Phe		
180					185					190							
Val	Ser	Val	Thr	Asn	Glu	His	Leu	Ile	Asp	Met	Asp	His	Glu	Ala	Ser		
195					200					205							
Phe	Phe	Gly	Ala	Phe	Leu	Val	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser		
210					215					220							
Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Thr	Ser	Glu	Glu	Thr	Ile	Ser	Thr		
225					230					235					240		
Val	Gln	Glu	Lys	Gln	Gln	Asn	Ile	Ser	Pro	Leu	Val	Arg	Glu	Arg	Gly		
245					250					255							
Pro	Gln	Arg	Val	Ala	Ala	His	Ile	Thr	Gly	Thr	Arg	Gly	Arg	Ser	Asn		
260					265					270							
Thr	Leu	Ser	Ser	Pro	Asn	Ser	Lys	Asn	Glu	Lys	Ala	Leu	Gly	Arg	Lys		
275					280					285							
Ile	Asn	Ser	Trp	Glu	Ser	Ser	Arg	Ser	Gly	His	Ser	Phe	Leu	Ser	Asn		
290					295					300							
Leu	His	Leu	Arg	Asn	Gly	Glu	Leu	Val	Ile	His	Glu	Lys	Gly	Phe	Tyr		
305					310					315					320		
Tyr	Ile	Tyr	Ser	Gln	Thr	Tyr	Phe	Arg	Phe	Gln	Glu	Glu	Ile	Lys	Glu		
325					330					335							
Asn	Thr	Lys	Asn	Asp	Lys	Gln	Met	Val	Gln	Tyr	Ile	Tyr	Lys	Tyr	Thr		
340					345					350							
Ser	Tyr	Pro	Asp	Pro	Ile	Leu	Leu	Met	Lys	Ser	Ala	Arg	Asn	Ser	Cys		
355					360					365							
Trp	Ser	Lys	Asp	Ala	Glu	Tyr	Gly	Leu	Tyr	Ser	Ile	Tyr	Gln	Gly	Gly		
370					375					380							
Ile	Phe	Glu	Leu	Lys	Glu	Asn	Asp	Arg	Ile	Phe	Val	Ser	Val	Thr	Asn		
385					390					395					400		
Glu	His	Leu	Ile	Asp	Met	Asp	His	Glu	Ala	Ser	Phe	Phe	Gly	Ala	Phe		
405					410					415							
Leu	Val	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly		
420					425					430							
Gly	Gly	Ser	Thr	Ser	Glu	Glu	Thr	Ile	Ser	Thr	Val	Gln	Glu	Lys	Gln		
435					440					445							
Gln	Asn	Ile	Ser	Pro	Leu	Val	Arg	Glu	Arg	Gly	Pro	Gln	Arg	Val	Ala		
450					455					460							
Ala	His	Ile	Thr	Gly	Thr	Arg	Gly	Arg	Ser	Asn	Thr	Leu	Ser	Ser	Pro		

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465	470	475	480	
Asn Ser Lys Asn Glu Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu				
	485	490	495	
Ser Ser Arg Ser Gly His Ser Phe Leu Ser Asn Leu His Leu Arg Asn				
	500	505	510	
Gly Glu Leu Val Ile His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln				
	515	520	525	
Thr Tyr Phe Arg Phe Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp				
	530	535	540	
Lys Gln Met Val Gln Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro				
	545	550	555	560
Ile Leu Leu Met Lys Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala				
	565	570	575	
Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys				
	580	585	590	
Glu Asn Asp Arg Ile Phe Val Ser Val Thr Asn Glu His Leu Ile Asp				
	595	600	605	
Met Asp His Glu Ala Ser Phe Phe Gly Ala Phe Leu Val Gly				
	610	615	620	
 <210> SEQ ID NO 33				
<211> LENGTH: 1581				
<212> TYPE: DNA				
<213> ORGANISM: Artificial Sequence				
<220> FEATURE:				
<223> OTHER INFORMATION: Description of Artificial Sequence: scTNF				
sequence				
<220> FEATURE:				
<221> NAME/KEY: CDS				
<222> LOCATION: (1)..(1578)				
 <400> SEQUENCE: 33				
atg gct atc atc tac ctc atc ctc ctg ttc acc gct gtg cgg ggc gcg				48
Met Ala Ile Ile Tyr Leu Ile Leu Leu Phe Thr Ala Val Arg Gly Ala				
1 5 10 15				
gcc gcg gat tac aaa gac gat gac gat aaa gaa ttc gga tca tct tct				96
Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys Glu Phe Gly Ser Ser Ser				
20 25 30				
cga acc ccg agt gac aag cct gta gcc cat gtt gta gca aac cct caa				144
Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro Gln				
35 40 45				
gct gag ggg cag ctc cag tgg ctg aac cgc cgg gcc aat gcc ctc ctg				192
Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu				
50 55 60				
gcc aat gcc gtg gag ctg aga gat aac cag ctg gtg gtg cca tca gag				240
Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu				
65 70 75 80				
ggc ctg tac ctc atc tac tcc cag gtc ctc ttc aag ggc caa ggc tgc				288
Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys				
85 90 95				
ccc tcc acc cat gtg ctc ctc acc cac acc atc agc cgc atc gcc gtc				336
Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val				
100 105 110				
tcc tac cag acc aag gtc aac ctc ctc tct gcc atc aag agc ccc tgc				384
Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys				
115 120 125				
cag agg gag acc cca gag ggg gct gag gcc aag ccc tgg tat gag ccc				432
Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro				
130 135 140				

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atc tat ctg gga ggg gtc ttc cag ctg gag aag ggt gac cga ctc agc Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser 145 150 155 160	480
gct gag atc aat cgg ccc gac tat ctc gac ttt gcc gag tct ggg cag Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln 165 170 175	528
gtc tac ttt ggg atc att gcc ctg ggt ggc ggt tct ggt ggc ggt tct Val Tyr Phe Gly Ile Ile Ala Leu Gly Gly Gly Ser Gly Gly Gly Ser 180 185 190	576
ggt ggc ggt tct ggt ggc gga tca tca tct tct cga acc ccg agt gac Gly Gly Ser Gly Gly Gly Ser Ser Ser Ser Arg Thr Pro Ser Asp 195 200 205	624
aag cct gta gcc cat gtt gta gca aac cct caa gct gag ggg cag ctc Lys Pro Val Ala His Val Val Ala Asn Pro Gln Ala Glu Gly Gln Leu 210 215 220	672
cag tgg ctg aac cgc cgg gcc aat gcc ctc ctg gcc aat ggc gtg gag Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu 225 230 235 240	720
ctg aga gat aac cag ctg gtg gtg cca tca gag ggc ctg tac ctc atc Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile 245 250 255	768
tac tcc cag gtc ctc ttc aag ggc caa ggc tgc ccc tcc acc cat gtg Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val 260 265 270	816
ctc ctc acc cac acc atc agc cgc atc gcc gtc tcc tac cag acc aag Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys 275 280 285	864
gtc aac ctc ctc tct gcc atc aag agc ccc tgc cag agg gag acc cca Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro 290 295 300	912
gag ggg gct gag gcc aag ccc tgg tat gag ccc atc tat ctg gga ggg Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly 305 310 315 320	960
gtc ttc cag ctg gag aag ggt gac cga ctc agc gct gag atc aat cgg Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg 325 330 335	1008
ccc gac tat ctc gac ttt gcc gag tct ggg cag gtc tac ttt ggg atc Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile 340 345 350	1056
att gcc ctg ggt ggc ggt tct ggt ggc ggt tct ggt ggc ggt tct ggt Ile Ala Leu Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly 355 360 365	1104
ggc gga tca tca tct tct cga acc ccg agt gac aag cct gta gcc cat Gly Gly Ser Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His 370 375 380	1152
gtt gta gca aac cct caa gct gag ggg cag ctc cag tgg ctg aac cgc Val Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg 385 390 395 400	1200
cgg gcc aat gcc ctc ctg gcc aat ggc gtg gag ctg aga gat aac cag Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln 405 410 415	1248
ctg gtg gtg cca tca gag ggc ctg tac ctc atc tac tcc cag gtc ctc Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu 420 425 430	1296
ttc aag ggc caa ggc tgc ccc tcc acc cat gtg ctc ctc acc cac acc Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr 435 440 445	1344
atc agc cgc atc gcc gtc tcc tac cag acc aag gtc aac ctc ctc tct Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser 450 455 460	1392

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```

gcc atc aag agc ccc tgc cag agg gag acc cca gag ggg gct gag gcc    1440
Ala Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala
465                      470                      475                      480

```

```

aag ccc tgg tat gag ccc atc tat ctg gga ggg gtc ttc cag ctg gag    1488
Lys Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu
                      485                      490                      495

```

```

aag ggt gac cga ctc agc gct gag atc aat cgg ccc gac tat ctc gac    1536
Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp
                      500                      505                      510

```

```

ttt gcc gag tct ggg cag gtc tac ttt ggg atc att gcc ctg tga    1581
Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
                      515                      520                      525

```

<210> SEQ ID NO 34

<211> LENGTH: 526

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: scTNF
peptide sequence

<400> SEQUENCE: 34

```

Met Ala Ile Ile Tyr Leu Ile Leu Leu Phe Thr Ala Val Arg Gly Ala
1           5           10           15

```

```

Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys Glu Phe Gly Ser Ser Ser
                20           25           30

```

```

Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro Gln
35           40           45

```

```

Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu
50           55           60

```

```

Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu
65           70           75           80

```

```

Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys
85           90           95

```

```

Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val
100          105          110

```

```

Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys
115          120          125

```

```

Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro
130          135          140

```

```

Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser
145          150          155          160

```

```

Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln
165          170          175

```

```

Val Tyr Phe Gly Ile Ile Ala Leu Gly Gly Gly Ser Gly Gly Gly Ser
180          185          190

```

```

Gly Gly Gly Ser Gly Gly Gly Ser Ser Ser Ser Arg Thr Pro Ser Asp
195          200          205

```

```

Lys Pro Val Ala His Val Val Ala Asn Pro Gln Ala Glu Gly Gln Leu
210          215          220

```

```

Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu
225          230          235          240

```

```

Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile
245          250          255

```

```

Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val
260          265          270

```

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Leu Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys
 275 280 285
 Val Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro
 290 295 300
 Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly
 305 310 315 320
 Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg
 325 330 335
 Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile
 340 345 350
 Ile Ala Leu Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly
 355 360 365
 Gly Gly Ser Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His
 370 375 380
 Val Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg
 385 390 395 400
 Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln
 405 410 415
 Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu
 420 425 430
 Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr
 435 440 445
 Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser
 450 455 460
 Ala Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala
 465 470 475 480
 Lys Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu
 485 490 495
 Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp
 500 505 510
 Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
 515 520 525

<210> SEQ ID NO 35
 <211> LENGTH: 2253
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:
 scFasL-AMAIZE sequence
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(2250)
 <400> SEQUENCE: 35

atg gac tgg acc tgg cgc gtg ttt tgc ctg ctc gcc gtg gct cct ggg	48
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly	
1 5 10 15	
gcc cac agc cag gta cag ctg gtg cag tct ggg gga ggc atg gta gag	96
Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Gly Gly Met Val Glu	
20 25 30	
cct ggg ggg tcc ctt aga ctc tcc tgt gca gcc tct gga ttc act ttc	144
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe	
35 40 45	
agt aat gcc tgg atg agc tgg gtc cgc cag gct cca ggg aag ggg ctg	192
Ser Asn Ala Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu	
50 55 60	
gag tgg gtt ggc cgt ata aaa agc aaa gct ggt ggt ggg aca gca gag	240

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Glu 65	Trp	Val	Gly	Arg	Ile 70	Lys	Ser	Lys	Ala	Gly 75	Gly	Gly	Thr	Ala	Glu 80	
tac	gct	gca	ccc	gtg	aaa	ggc	aga	ttc	acc	atc	tca	aga	gat	gat	tca	288
Tyr	Ala	Ala	Pro	Val	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asp	Ser	
			85						90					95		
caa	aac	acg	ctg	tat	ctg	caa	atg	aac	agc	ctg	aaa	acc	gac	gac	aca	336
Gln	Asn	Thr	Leu	Tyr	Leu	Gln	Met	Asn	Ser	Leu	Lys	Thr	Asp	Asp	Thr	
			100					105					110			
gcc	gtg	tat	tac	tgt	acc	aca	cat	gtc	tac	ggc	gcc	ccc	cgg	aac	tgg	384
Ala	Val	Tyr	Tyr	Cys	Thr	Thr	His	Val	Tyr	Gly	Ala	Pro	Arg	Asn	Trp	
		115					120					125				
ggc	cag	gga	tcc	ctg	gtc	acc	gtc	tcc	tca	gcc	tcc	acc	aag	ggc	cca	432
Gly	Gln	Gly	Ser	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	
	130						135					140				
aag	ctt	gaa	gaa	ggc	gaa	ttt	tca	gaa	gca	cgc	gta	cag	tct	gtg	ttg	480
Lys	Leu	Glu	Glu	Gly	Glu	Phe	Ser	Glu	Ala	Arg	Val	Gln	Ser	Val	Leu	
	145					150				155					160	
act	cag	ccg	ccc	tca	gtg	tct	cgc	gcc	cca	gga	cag	aag	gtc	acc	atc	528
Thr	Gln	Pro	Pro	Ser	Val	Ser	Ala	Ala	Pro	Gly	Gln	Lys	Val	Thr	Ile	
			165						170					175		
tcc	tgc	tct	gga	agc	agc	tcc	aac	att	gga	aat	aat	tat	gtc	tcc	tgg	576
Ser	Cys	Ser	Gly	Ser	Ser	Ser	Asn	Ile	Gly	Asn	Asn	Tyr	Val	Ser	Trp	
			180					185					190			
tac	gtt	caa	ctc	cca	gga	aca	gcc	ccc	aaa	ctc	ctc	att	tat	gac	aat	624
Tyr	Val	Gln	Leu	Pro	Gly	Thr	Ala	Pro	Lys	Leu	Leu	Ile	Tyr	Asp	Asn	
		195					200					205				
aat	aag	cga	ttc	tca	gga	gtt	cct	gac	cga	ttc	tct	ggc	tcc	aag	tct	672
Asn	Lys	Arg	Phe	Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Lys	Ser	
	210						215					220				
ggc	acg	tca	gcc	acc	ctg	ggc	atc	acc	ggg	ctc	cag	act	ggg	gac	gag	720
Gly	Thr	Ser	Ala	Thr	Leu	Gly	Ile	Thr	Gly	Leu	Gln	Thr	Gly	Asp	Glu	
	225					230				235				240		
gcc	gat	tat	tac	tgc	gga	gca	tgg	gat	ggc	agc	ctg	cgt	gaa	gcg	gta	768
Ala	Asp	Tyr	Tyr	Cys	Gly	Ala	Trp	Asp	Gly	Ser	Leu	Arg	Glu	Ala	Val	
			245						250					255		
ttc	ggc	gga	ggg	acc	aag	gtc	acc	gtc	cta	ggc	gcg	gcc	gca	gtt	gag	816
Phe	Gly	Gly	Gly	Thr	Lys	Val	Thr	Val	Leu	Gly	Ala	Ala	Ala	Val	Glu	
			260						265				270			
ctc	gag	gcg	gcc	gcg	gat	tac	aaa	gac	gat	gac	gat	aaa	gaa	ttc	acg	864
Leu	Glu	Ala	Ala	Ala	Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Glu	Phe	Thr	
			275					280					285			
cgt	gaa	aaa	aag	gag	ctg	agg	aaa	gtg	gcc	cat	tta	aca	ggc	aag	tcc	912
Arg	Glu	Lys	Lys	Glu	Leu	Arg	Lys	Val	Ala	His	Leu	Thr	Gly	Lys	Ser	
	290						295				300					
aac	tca	agg	tcc	atg	cct	ctg	gaa	tgg	gaa	gac	acc	tat	gga	att	gtc	960
Asn	Ser	Arg	Ser	Met	Pro	Leu	Glu	Trp	Glu	Asp	Thr	Tyr	Gly	Ile	Val	
	305					310				315				320		
ctg	ctt	tct	gga	gtg	aag	tat	aag	aag	ggc	ctt	gtg	atc	aat	gaa		1008
Leu	Leu	Ser	Gly	Val	Lys	Tyr	Lys	Lys	Gly	Gly	Leu	Val	Ile	Asn	Glu	
			325						330					335		
act	ggg	ctg	tac	ttt	gta	tat	tcc	aaa	gta	tac	ttc	cgg	ggc	caa	tct	1056
Thr	Gly	Leu	Tyr	Phe	Val	Tyr	Ser	Lys	Val	Tyr	Phe	Arg	Gly	Gln	Ser	
			340						345					350		
tgc	aac	aac	ctg	ccc	ctg	agc	cac	aag	gtc	tac	atg	agg	aac	tct	aag	1104
Cys	Asn	Asn	Leu	Pro	Leu	Ser	His	Lys	Val	Tyr	Met	Arg	Asn	Ser	Lys	
			355						360				365			
tat	ccc	cag	gat	ctg	gtg	atg	atg	gag	ggg	aag	atg	atg	agc	tac	tgc	1152
Tyr	Pro	Gln	Asp	Leu	Val	Met	Met	Glu	Gly	Lys	Met	Met	Ser	Tyr	Cys	
			370					375					380			

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act	act	ggg	cag	atg	tgg	gcc	cgc	agc	agc	tac	ctg	ggg	gca	gtg	ttc	1200
Thr	Thr	Gly	Gln	Met	Trp	Ala	Arg	Ser	Ser	Tyr	Leu	Gly	Ala	Val	Phe	
385					390					395					400	
aat	ctt	acc	agt	gct	gat	cat	tta	tat	gtc	aac	gta	tct	gag	ctc	tct	1248
Asn	Leu	Thr	Ser	Ala	Asp	His	Leu	Tyr	Val	Asn	Val	Ser	Glu	Leu	Ser	
			405					410					415			
ctg	gtc	aat	ttt	gag	gaa	tct	cag	acg	ttt	ttc	ggc	tta	tat	aag	ctc	1296
Leu	Val	Asn	Phe	Glu	Glu	Ser	Gln	Thr	Phe	Phe	Gly	Leu	Tyr	Lys	Leu	
			420					425					430			
ggg	ggc	ggg	tct	ggg	ggg	tct	ggg	ggc	ggg	tct	ggg	ggc	gga	tca		1344
Gly	Gly		Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	
		435				440					445					
gaa	aaa	aag	gag	ctg	agg	aaa	gtg	gcc	cat	tta	aca	ggc	aag	tcc	aac	1392
Glu	Lys	Lys	Glu	Leu	Arg	Lys	Val	Ala	His	Leu	Thr	Gly	Lys	Ser	Asn	
	450					455					460					
tca	agg	tcc	atg	cct	ctg	gaa	tgg	gaa	gac	acc	tat	gga	att	gtc	ctg	1440
Ser	Arg	Ser	Met	Pro	Leu	Glu	Trp	Glu	Asp	Thr	Tyr	Gly	Ile	Val	Leu	
465					470					475					480	
ctt	tct	gga	gtg	aag	tat	aag	aag	ggg	ggc	ctt	gtg	atc	aat	gaa	act	1488
Leu	Ser	Gly	Val	Lys	Tyr	Lys	Lys	Gly	Gly	Leu	Val	Ile	Asn	Glu	Thr	
			485					490						495		
ggg	ctg	tac	ttt	gta	tat	tcc	aaa	gta	tac	ttc	cgg	ggg	caa	tct	tgc	1536
Gly	Leu	Tyr	Phe	Val	Tyr	Ser	Lys	Val	Tyr	Phe	Arg	Gly	Gln	Ser	Cys	
			500					505					510			
aac	aac	ctg	ccc	ctg	agc	cac	aag	gtc	tac	atg	agg	aac	tct	aag	tat	1584
Asn	Asn	Leu	Pro	Leu	Ser	His	Lys	Val	Tyr	Met	Arg	Asn	Ser	Lys	Tyr	
		515					520					525				
ccc	cag	gat	ctg	gtg	atg	atg	gag	ggg	aag	atg	atg	agc	tac	tgc	act	1632
Pro	Gln	Asp	Leu	Val	Met	Met	Glu	Gly	Lys	Met	Met	Ser	Tyr	Cys	Thr	
	530					535					540					
act	ggg	cag	atg	tgg	gcc	cgc	agc	agc	tac	ctg	ggg	gca	gtg	ttc	aat	1680
Thr	Gly	Gln	Met	Trp	Ala	Arg	Ser	Ser	Tyr	Leu	Gly	Ala	Val	Phe	Asn	
545					550					555					560	
ctt	acc	agt	gct	gat	cat	tta	tat	gtc	aac	gta	tct	gag	ctc	tct	ctg	1728
Leu	Thr	Ser	Ala	Asp	His	Leu	Tyr	Val	Asn	Val	Ser	Glu	Leu	Ser	Leu	
			565					570						575		
gtc	aat	ttt	gag	gaa	tct	cag	acg	ttt	ttc	ggc	tta	tat	aag	ctc	ggg	1776
Val	Asn	Phe	Glu	Glu	Ser	Gln	Thr	Phe	Phe	Gly	Leu	Tyr	Lys	Leu	Gly	
		580						585					590			
ggc	ggg	tct	ggg	ggc	ggg	tct	ggg	ggc	ggg	tct	ggg	ggc	gga	tcc	gaa	1824
Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Glu	
		595				600					605					
aaa	aag	gag	ctg	agg	aaa	gtg	gcc	cat	tta	aca	ggc	aag	tcc	aac	tca	1872
Lys	Lys	Glu	Leu	Arg	Lys	Val	Ala	His	Leu	Thr	Gly	Lys	Ser	Asn	Ser	
	610					615					620					
agg	tcc	atg	cct	ctg	gaa	tgg	gaa	gac	acc	tat	gga	att	gtc	ctg	ctt	1920
Arg	Ser	Met	Pro	Leu	Glu	Trp	Glu	Asp	Thr	Tyr	Gly	Ile	Val	Leu	Leu	
625					630					635					640	
tct	gga	gtg	aag	tat	aag	aag	ggg	ggc	ctt	gtg	atc	aat	gaa	act	ggg	1968
Ser	Gly	Val	Lys	Tyr	Lys	Lys	Gly	Gly	Leu	Val	Ile	Asn	Glu	Thr	Gly	
			645					650						655		
ctg	tac	ttt	gta	tat	tcc	aaa	gta	tac	ttc	cgg	ggg	caa	tct	tgc	aac	2016
Leu	Tyr	Phe	Val	Tyr	Ser	Lys	Val	Tyr	Phe	Arg	Gly	Gln	Ser	Cys	Asn	
			660					665					670			
aac	ctg	ccc	ctg	agc	cac	aag	gtc	tac	atg	agg	aac	tct	aag	tat	ccc	2064
Asn	Leu	Pro	Leu	Ser	His	Lys	Val	Tyr	Met	Arg	Asn	Ser	Lys	Tyr	Pro	
		675					680				685					
cag	gat	ctg	gtg	atg	atg	gag	ggg	aag	atg	atg	agc	tac	tgc	act	act	2112
Gln	Asp	Leu	Val	Met	Met	Glu	Gly	Lys	Met	Met	Ser	Tyr	Cys	Thr	Thr	
	690					695					700					

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ggg cag atg tgg gcc cgc agc agc tac ctg ggg gca gtg ttc aat ctt      2160
Gly Gln Met Trp Ala Arg Ser Ser Tyr Leu Gly Ala Val Phe Asn Leu
705                      710                      715                      720

acc agt gct gat cat tta tat gtc aac gta tct gag ctc tct ctg gtc      2208
Thr Ser Ala Asp His Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val
                      725                      730                      735

aat ttt gag gaa tct cag acg ttt ttc ggc tta tat aag ctc tga      2253
Asn Phe Glu Ser Gln Thr Phe Phe Gly Leu Tyr Lys Leu
                      740                      745                      750

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<210> SEQ ID NO 36
<211> LENGTH: 750
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
      scFasL-AMAIZE peptide sequence

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<400> SEQUENCE: 36

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Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
1                      5                      10                      15

Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Gly Gly Met Val Glu
                      20                      25                      30

Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
                      35                      40                      45

Ser Asn Ala Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50                      55                      60

Glu Trp Val Gly Arg Ile Lys Ser Lys Ala Gly Gly Gly Thr Ala Glu
65                      70                      75                      80

Tyr Ala Ala Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
                      85                      90                      95

Gln Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Asp Asp Thr
100                      105                      110

Ala Val Tyr Tyr Cys Thr Thr His Val Tyr Gly Ala Pro Arg Asn Trp
115                      120                      125

Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
130                      135                      140

Lys Leu Glu Glu Gly Glu Phe Ser Glu Ala Arg Val Gln Ser Val Leu
145                      150                      155                      160

Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln Lys Val Thr Ile
165                      170                      175

Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Tyr Val Ser Trp
180                      185                      190

Tyr Val Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Asp Asn
195                      200                      205

Asn Lys Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser
210                      215                      220

Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln Thr Gly Asp Glu
225                      230                      235                      240

Ala Asp Tyr Tyr Cys Gly Ala Trp Asp Gly Ser Leu Arg Glu Ala Val
245                      250                      255

Phe Gly Gly Gly Thr Lys Val Thr Val Leu Gly Ala Ala Ala Val Glu
260                      265                      270

Leu Glu Ala Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys Glu Phe Thr
275                      280                      285

Arg Glu Lys Lys Glu Leu Arg Lys Val Ala His Leu Thr Gly Lys Ser

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290					295					300					
Asn 305	Ser	Arg	Ser	Met	Pro 310	Leu	Glu	Trp	Glu	Asp 315	Thr	Tyr	Gly	Ile	Val 320
Leu	Leu	Ser	Gly	Val 325	Lys	Tyr	Lys	Lys	Gly 330	Gly	Leu	Val	Ile	Asn 335	Glu
Thr	Gly	Leu	Tyr	Phe 340	Val	Tyr	Ser	Lys	Val 345	Tyr	Phe	Arg	Gly 350	Gln	Ser
Cys	Asn 355	Asn	Leu	Pro	Leu	Ser	His 360	Lys	Val	Tyr	Met	Arg 365	Asn	Ser	Lys
Tyr	Pro 370	Gln	Asp	Leu	Val	Met 375	Met	Glu	Gly	Lys	Met 380	Met	Ser	Tyr	Cys
Thr 385	Thr	Gly	Gln	Met	Trp 390	Ala	Arg	Ser	Ser	Tyr 395	Leu	Gly	Ala	Val	Phe 400
Asn	Leu	Thr	Ser	Ala 405	Asp	His	Leu	Tyr	Val 410	Asn	Val	Ser	Glu	Leu 415	Ser
Leu	Val	Asn	Phe	Glu 420	Glu	Ser	Gln	Thr	Phe 425	Phe	Gly	Leu	Tyr 430	Lys	Leu
Gly	Gly	Gly	Ser	Gly 435	Gly	Gly	Ser	Gly	Gly 440	Gly	Ser	Gly 445	Gly	Gly	Ser
Glu	Lys 450	Lys	Glu	Leu	Arg	Lys 455	Val	Ala	His	Leu	Thr	Gly 460	Lys	Ser	Asn
Ser 465	Arg	Ser	Met	Pro	Leu	Glu 470	Trp	Glu	Asp 475	Thr	Tyr	Gly	Ile	Val	Leu 480
Leu	Ser	Gly	Val	Lys 485	Tyr	Lys	Lys	Gly	Gly 490	Leu	Val	Ile	Asn	Glu 495	Thr
Gly	Leu	Tyr	Phe 500	Val	Tyr	Ser	Lys	Val 505	Tyr	Phe	Arg	Gly 510	Gln	Ser	Cys
Asn	Asn 515	Leu	Pro	Leu	Ser	His	Lys 520	Val	Tyr	Met	Arg 525	Asn	Ser	Lys	Tyr
Pro 530	Gln	Asp	Leu	Val	Met 535	Met	Glu	Gly	Lys	Met 540	Met	Ser	Tyr	Cys	Thr
Thr 545	Gly	Gln	Met	Trp 550	Ala	Arg	Ser	Ser	Tyr 555	Leu	Gly	Ala	Val	Phe	Asn 560
Leu	Thr	Ser	Ala 565	Asp	His	Leu	Tyr	Val 570	Asn	Val	Ser	Glu	Leu 575	Ser	Leu
Val	Asn	Phe	Glu 580	Glu	Ser	Gln	Thr	Phe 585	Phe	Gly	Leu	Tyr	Lys 590	Leu	Gly
Gly	Gly	Ser	Gly 595	Gly	Gly	Ser	Gly 600	Gly	Gly	Ser	Gly 605	Gly	Gly	Ser	Glu
Lys 610	Lys	Glu	Leu	Arg	Lys 615	Val	Ala	His	Leu	Thr 620	Gly	Lys	Ser	Asn	Ser
Arg 625	Ser	Met	Pro	Leu	Glu 630	Trp	Glu	Asp	Thr 635	Tyr	Gly	Ile	Val	Leu	Leu 640
Ser	Gly	Val	Lys 645	Tyr	Lys	Lys	Gly	Gly 650	Leu	Val	Ile	Asn	Glu 655	Thr	Gly
Leu	Tyr	Phe	Val 660	Tyr	Ser	Lys	Val	Tyr 665	Phe	Arg	Gly	Gln	Ser 670	Cys	Asn
Asn	Leu 675	Pro	Leu	Ser	His	Lys 680	Val	Tyr	Met 685	Arg	Asn	Ser	Lys	Tyr	Pro
Gln 690	Asp	Leu	Val	Met	Met 695	Glu	Gly	Lys	Met 700	Met	Ser	Tyr	Cys	Thr	Thr
Gly 705	Gln	Met	Trp 710	Ala	Arg	Ser	Ser	Tyr	Leu 715	Gly	Ala	Val	Phe	Asn	Leu 720

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Thr Ser Ala Asp His Leu Tyr Val Asn Val Ser Glu Leu Ser Leu Val
 725 730 735

Asn Phe Glu Glu Ser Gln Thr Phe Phe Gly Leu Tyr Lys Leu
 740 745 750

<210> SEQ ID NO 37
 <211> LENGTH: 2646
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Description of Artificial Sequence:
 scTRAIL-AMAIZE sequence
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(2643)

<400> SEQUENCE: 37

atg gac tgg acc tgg cgc gtg ttt tgc ctg ctc gcc gtg gct cct ggg	48
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly	
1 5 10 15	
gcc cac agc cag gta cag ctg gtg cag tct ggg gga ggc atg gta gag	96
Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Gly Gly Met Val Glu	
20 25 30	
cct ggg ggg tcc ctt aga ctc tcc tgt gca gcc tct gga ttc act ttc	144
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe	
35 40 45	
agt aat gcc tgg atg agc tgg gtc cgc cag gct cca ggg aag ggg ctg	192
Ser Asn Ala Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu	
50 55 60	
gag tgg gtt ggc cgt ata aaa agc aaa gct ggt ggt ggg aca gca gag	240
Glu Trp Val Gly Arg Ile Lys Ser Lys Ala Gly Gly Gly Thr Ala Glu	
65 70 75 80	
tac gct gca ccc gtg aaa ggc aga ttc acc atc tca aga gat gat tca	288
Tyr Ala Ala Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser	
85 90 95	
caa aac acg ctg tat ctg caa atg aac agc ctg aaa acc gac gac aca	336
Gln Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Asp Asp Thr	
100 105 110	
gcc gtg tat tac tgt acc aca cat gtc tac ggt gcc ccc cgg aac tgg	384
Ala Val Tyr Tyr Cys Thr Thr His Val Tyr Gly Ala Pro Arg Asn Trp	
115 120 125	
ggc cag gga tcc ctg gtc acc gtc tcc tca gcc tcc acc aag ggc cca	432
Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro	
130 135 140	
aag ctt gaa gaa ggt gaa ttt tca gaa gca cgc gta cag tct gtg ttg	480
Lys Leu Glu Glu Gly Glu Phe Ser Glu Ala Arg Val Gln Ser Val Leu	
145 150 155 160	
act cag ccg ccc tca gtg tct gcg gcc cca gga cag aag gtc acc atc	528
Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln Lys Val Thr Ile	
165 170 175	
tcc tgc tct gga agc agc tcc aac att gga aat aat tat gtc tcc tgg	576
Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Tyr Val Ser Trp	
180 185 190	
tac gtt caa ctc cca gga aca gcc ccc aaa ctc ctc att tat gac aat	624
Tyr Val Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Asp Asn	
195 200 205	
aat aag cga ttc tca gga gtt cct gac cga ttc tct ggc tcc aag tct	672
Asn Lys Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser	
210 215 220	
ggc acg tca gcc acc ctg ggc atc acc ggg ctc cag act ggg gac gag	720
Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln Thr Gly Asp Glu	

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225	230	235	240	
gcc gat tat tac tgc gga gca tgg gat ggc agc ctg cgt gaa gcg gta Ala Asp Tyr Tyr Cys Gly Ala Trp Asp Gly Ser Leu Arg Glu Ala Val 245 250 255				768
ttc ggc gga ggg acc aag gtc acc gtc cta ggt gcg gcc gca gtt gag Phe Gly Gly Gly Thr Lys Val Thr Val Leu Gly Ala Ala Ala Val Glu 260 265 270				816
ctc gag gcg gcc gcg gat tac aaa gac gat gac gat aaa gaa ttc gga Leu Glu Ala Ala Ala Asp Tyr Lys Asp Asp Asp Lys Glu Phe Gly 275 280 285				864
acc tct gag gaa acc att tct aca gtt caa gaa aag caa caa aat att Thr Ser Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile 290 295 300				912
tct ccc cta gtg aga gaa aga ggt cct cag aga gta gca gct cac ata Ser Pro Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile 305 310 315 320				960
act ggg acc aga gga aga agc aac aca ttg tct tct cca aac tcc aag Thr Gly Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys 325 330 335				1008
aat gaa aag gct ctg ggc cgc aaa ata aac tcc tgg gaa tca tca agg Asn Glu Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg 340 345 350				1056
agt ggg cat tca ttc ctg agc aac ttg cac ttg agg aat ggt gaa ctg Ser Gly His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu 355 360 365				1104
gtc atc cat gaa aaa ggg ttt tac tac atc tat tcc caa aca tac ttt Val Ile His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe 370 375 380				1152
cga ttt cag gag gaa ata aaa gaa aac aca aag aac gac aaa caa atg Arg Phe Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met 385 390 395 400				1200
gtc caa tat att tac aaa tac aca agt tat cct gac cct ata ttg ttg Val Gln Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu 405 410 415				1248
atg aaa agt gct aga aat agt tgt tgg tct aaa gat gca gaa tat gga Met Lys Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly 420 425 430				1296
ctc tat tcc atc tat caa ggg gga ata ttt gag ctt aag gaa aat gac Leu Tyr Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp 435 440 445				1344
aga att ttt gtt tct gta aca aat gag cac ttg ata gac atg gac cat Arg Ile Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His 450 455 460				1392
gaa gcc agt ttt ttc ggg gcc ttt tta gtt ggc ggt ggc ggt tct ggt Glu Ala Ser Phe Phe Gly Ala Phe Leu Val Gly Gly Gly Gly Ser Gly 465 470 475 480				1440
ggc ggt tct ggt ggc ggt tct ggt ggc gga tca acc tct gag gaa acc Gly Gly Ser Gly Gly Ser Gly Gly Ser Thr Ser Glu Glu Thr 485 490 495				1488
att tct aca gtt caa gaa aag caa caa aat att tct ccc cta gtg aga Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro Leu Val Arg 500 505 510				1536
gaa aga ggt cct cag aga gta gca gct cac ata act ggg acc aga gga Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg Gly 515 520 525				1584
aga agc aac aca ttg tct tct cca aac tcc aag aat gaa aag gct ctg Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala Leu 530 535 540				1632
ggc cgc aaa ata aac tcc tgg gaa tca tca agg agt ggg cat tca ttc				1680

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Gly	Arg	Lys	Ile	Asn	Ser	Trp	Glu	Ser	Ser	Arg	Ser	Gly	His	Ser	Phe	
545					550					555					560	
ctg	agc	aac	ttg	cac	ttg	agg	aat	ggg	gaa	ctg	gtc	atc	cat	gaa	aaa	1728
Leu	Ser	Asn	Leu	His	Leu	Arg	Asn	Gly	Glu	Leu	Val	Ile	His	Glu	Lys	
			565					570						575		
ggg	ttt	tac	tac	atc	tat	tcc	caa	aca	tac	ttt	cga	ttt	cag	gag	gaa	1776
Gly	Phe	Tyr	Tyr	Ile	Tyr	Ser	Gln	Thr	Tyr	Phe	Arg	Phe	Gln	Glu	Glu	
		580					585					590				
ata	aaa	gaa	aac	aca	aag	aac	gac	aaa	caa	atg	gtc	caa	tat	att	tac	1824
Ile	Lys	Glu	Asn	Thr	Lys	Asn	Asp	Lys	Gln	Met	Val	Gln	Tyr	Ile	Tyr	
		595					600					605				
aaa	tac	aca	agt	tat	cct	gac	cct	ata	ttg	ttg	atg	aaa	agt	gct	aga	1872
Lys	Tyr	Thr	Ser	Tyr	Pro	Asp	Pro	Ile	Leu	Leu	Met	Lys	Ser	Ala	Arg	
	610					615					620					
aat	agt	tgt	tggt	tct	aaa	gat	gca	gaa	tat	gga	ctc	tat	tcc	atc	tat	1920
Asn	Ser	Cys	Trp	Ser	Lys	Asp	Ala	Glu	Tyr	Gly	Leu	Tyr	Ser	Ile	Tyr	
	625				630					635				640		
caa	ggg	gga	ata	ttt	gag	ctt	aag	gaa	aat	gac	aga	att	ttt	gtt	tct	1968
Gln	Gly	Gly	Ile	Phe	Glu	Leu	Lys	Glu	Asn	Asp	Arg	Ile	Phe	Val	Ser	
			645					650						655		
gta	aca	aat	gag	cac	ttg	ata	gac	atg	gac	cat	gaa	gcc	agt	ttt	ttc	2016
Val	Thr	Asn	Glu	His	Leu	Ile	Asp	Met	Asp	His	Glu	Ala	Ser	Phe	Phe	
		660					665					670				
ggg	gcc	ttt	tta	gtt	ggc	ggg	ggg	tct	ggg	ggg	ggg	tct	ggg	ggc		2064
Gly	Ala	Phe	Leu	Val	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	
	675					680					685					
ggg	tct	ggg	ggc	gga	tcc	acc	tct	gag	gaa	acc	att	tct	aca	gtt	caa	2112
Gly	Ser	Gly	Gly	Gly	Ser	Thr	Ser	Glu	Glu	Thr	Ile	Ser	Thr	Val	Gln	
	690					695				700						
gaa	aag	caa	caa	aat	att	tct	ccc	cta	gtg	aga	gaa	aga	ggg	cct	cag	2160
Glu	Lys	Gln	Gln	Asn	Ile	Ser	Pro	Leu	Val	Arg	Glu	Arg	Gly	Pro	Gln	
	705				710					715				720		
aga	gta	gca	gct	cac	ata	act	ggg	acc	aga	gga	aga	agc	aac	aca	ttg	2208
Arg	Val	Ala	Ala	His	Ile	Thr	Gly	Thr	Arg	Gly	Arg	Ser	Asn	Thr	Leu	
		725					730							735		
tct	tct	cca	aac	tcc	aag	aat	gaa	aag	gct	ctg	ggc	cgc	aaa	ata	aac	2256
Ser	Ser	Pro	Asn	Ser	Lys	Asn	Glu	Lys	Ala	Leu	Gly	Arg	Lys	Ile	Asn	
		740					745						750			
tcc	tggt	gaa	tca	tca	agg	agt	ggg	cat	tca	ttc	ctg	agc	aac	ttg	cac	2304
Ser	Trp	Glu	Ser	Ser	Arg	Ser	Gly	His	Ser	Phe	Leu	Ser	Asn	Leu	His	
		755				760						765				
ttg	agg	aat	ggg	gaa	ctg	gtc	atc	cat	gaa	aaa	ggg	ttt	tac	tac	atc	2352
Leu	Arg	Asn	Gly	Glu	Leu	Val	Ile	His	Glu	Lys	Gly	Phe	Tyr	Tyr	Ile	
	770					775					780					
tat	tcc	caa	aca	tac	ttt	cga	ttt	cag	gag	gaa	ata	aaa	gaa	aac	aca	2400
Tyr	Ser	Gln	Thr	Tyr	Phe	Arg	Phe	Gln	Glu	Glu	Ile	Lys	Glu	Asn	Thr	
			785			790				795				800		
aag	aac	gac	aaa	caa	atg	gtc	caa	tat	att	tac	aaa	tac	aca	agt	tat	2448
Lys	Asn	Asp	Lys	Gln	Met	Val	Gln	Tyr	Ile	Tyr	Lys	Tyr	Thr	Ser	Tyr	
			805					810					815			
cct	gac	cct	ata	ttg	ttg	atg	aaa	agt	gct	aga	aat	agt	tgt	tggt	tct	2496
Pro	Asp	Pro	Ile	Leu	Leu	Met	Lys	Ser	Ala	Arg	Asn	Ser	Cys	Trp	Ser	
			820					825					830			
aaa	gat	gca	gaa	tat	gga	ctc	tat	tcc	atc	tat	caa	ggg	gga	ata	ttt	2544
Lys	Asp	Ala	Glu	Tyr	Gly	Leu	Tyr	Ser	Ile	Tyr	Gln	Gly	Gly	Ile	Phe	
		835				840						845				
gag	ctt	aag	gaa	aat	gac	aga	att	ttt	gtt	tct	gta	aca	aat	gag	cac	2592
Glu	Leu	Lys	Glu	Asn	Asp	Arg	Ile	Phe	Val	Ser	Val	Thr	Asn	Glu	His	
	850					855					860					

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ttg ata gac atg gac cat gaa gcc agt ttt ttc ggg gcc ttt tta gtt      2640
Leu Ile Asp Met Asp His Glu Ala Ser Phe Phe Gly Ala Phe Leu Val
865                      870                      875                      880

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ggc tga      2646
Gly

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<210> SEQ ID NO 38
<211> LENGTH: 881
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
      scTRAIL-AMAIZe peptide sequence

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<400> SEQUENCE: 38

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Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala Val Ala Pro Gly
1          5          10          15
Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Gly Gly Met Val Glu
20          25          30
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
35          40          45
Ser Asn Ala Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50          55          60
Glu Trp Val Gly Arg Ile Lys Ser Lys Ala Gly Gly Gly Thr Ala Glu
65          70          75          80
Tyr Ala Ala Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
85          90          95
Gln Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Asp Asp Thr
100         105         110
Ala Val Tyr Tyr Cys Thr Thr His Val Tyr Gly Ala Pro Arg Asn Trp
115         120         125
Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
130         135         140
Lys Leu Glu Glu Gly Glu Phe Ser Glu Ala Arg Val Gln Ser Val Leu
145         150         155         160
Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln Lys Val Thr Ile
165         170         175
Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Tyr Val Ser Trp
180         185         190
Tyr Val Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Asp Asn
195         200         205
Asn Lys Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser
210         215         220
Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln Thr Gly Asp Glu
225         230         235         240
Ala Asp Tyr Tyr Cys Gly Ala Trp Asp Gly Ser Leu Arg Glu Ala Val
245         250         255
Phe Gly Gly Gly Thr Lys Val Thr Val Leu Gly Ala Ala Ala Val Glu
260         265         270
Leu Glu Ala Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys Glu Phe Gly
275         280         285
Thr Ser Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile
290         295         300
Ser Pro Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile
305         310         315         320
Thr Gly Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys

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325								330				335			
Asn	Glu	Lys	Ala	Leu	Gly	Arg	Lys	Ile	Asn	Ser	Trp	Glu	Ser	Ser	Arg
			340					345					350		
Ser	Gly	His	Ser	Phe	Leu	Ser	Asn	Leu	His	Leu	Arg	Asn	Gly	Glu	Leu
			355					360					365		
Val	Ile	His	Glu	Lys	Gly	Phe	Tyr	Tyr	Ile	Tyr	Ser	Gln	Thr	Tyr	Phe
			370					375					380		
Arg	Phe	Gln	Glu	Glu	Ile	Lys	Glu	Asn	Thr	Lys	Asn	Asp	Lys	Gln	Met
385						390					395		400		
Val	Gln	Tyr	Ile	Tyr	Lys	Tyr	Thr	Ser	Tyr	Pro	Asp	Pro	Ile	Leu	Leu
			405					410						415	
Met	Lys	Ser	Ala	Arg	Asn	Ser	Cys	Trp	Ser	Lys	Asp	Ala	Glu	Tyr	Gly
			420					425					430		
Leu	Tyr	Ser	Ile	Tyr	Gln	Gly	Gly	Ile	Phe	Glu	Leu	Lys	Glu	Asn	Asp
			435					440					445		
Arg	Ile	Phe	Val	Ser	Val	Thr	Asn	Glu	His	Leu	Ile	Asp	Met	Asp	His
			450					455					460		
Glu	Ala	Ser	Phe	Phe	Gly	Ala	Phe	Leu	Val	Gly	Gly	Gly	Gly	Ser	Gly
465						470					475		480		
Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Thr	Ser	Glu	Glu	Thr
			485					490						495	
Ile	Ser	Thr	Val	Gln	Glu	Lys	Gln	Gln	Asn	Ile	Ser	Pro	Leu	Val	Arg
			500					505						510	
Glu	Arg	Gly	Pro	Gln	Arg	Val	Ala	Ala	His	Ile	Thr	Gly	Thr	Arg	Gly
			515					520					525		
Arg	Ser	Asn	Thr	Leu	Ser	Ser	Pro	Asn	Ser	Lys	Asn	Glu	Lys	Ala	Leu
			530					535					540		
Gly	Arg	Lys	Ile	Asn	Ser	Trp	Glu	Ser	Ser	Arg	Ser	Gly	His	Ser	Phe
545						550					555		560		
Leu	Ser	Asn	Leu	His	Leu	Arg	Asn	Gly	Glu	Leu	Val	Ile	His	Glu	Lys
			565					570						575	
Gly	Phe	Tyr	Tyr	Ile	Tyr	Ser	Gln	Thr	Tyr	Phe	Arg	Phe	Gln	Glu	Glu
			580					585						590	
Ile	Lys	Glu	Asn	Thr	Lys	Asn	Asp	Lys	Gln	Met	Val	Gln	Tyr	Ile	Tyr
			595					600					605		
Lys	Tyr	Thr	Ser	Tyr	Pro	Asp	Pro	Ile	Leu	Leu	Met	Lys	Ser	Ala	Arg
			610					615					620		
Asn	Ser	Cys	Trp	Ser	Lys	Asp	Ala	Glu	Tyr	Gly	Leu	Tyr	Ser	Ile	Tyr
625						630					635		640		
Gln	Gly	Gly	Ile	Phe	Glu	Leu	Lys	Glu	Asn	Asp	Arg	Ile	Phe	Val	Ser
			645					650						655	
Val	Thr	Asn	Glu	His	Leu	Ile	Asp	Met	Asp	His	Glu	Ala	Ser	Phe	Phe
			660					665						670	
Gly	Ala	Phe	Leu	Val	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly
			675					680					685		
Gly	Ser	Gly	Gly	Gly	Ser	Thr	Ser	Glu	Glu	Thr	Ile	Ser	Thr	Val	Gln
			690					695					700		
Glu	Lys	Gln	Gln	Asn	Ile	Ser	Pro	Leu	Val	Arg	Glu	Arg	Gly	Pro	Gln
705						710					715		720		
Arg	Val	Ala	Ala	His	Ile	Thr	Gly	Thr	Arg	Gly	Arg	Ser	Asn	Thr	Leu
			725					730						735	
Ser	Ser	Pro	Asn	Ser	Lys	Asn	Glu	Lys	Ala	Leu	Gly	Arg	Lys	Ile	Asn
			740					745					750		

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Ser Trp Glu Ser Ser Arg Ser Gly His Ser Phe Leu Ser Asn Leu His
755 760 765

Leu Arg Asn Gly Glu Leu Val Ile His Glu Lys Gly Phe Tyr Tyr Ile
770 775 780

Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Glu Ile Lys Glu Asn Thr
785 790 795 800

Lys Asn Asp Lys Gln Met Val Gln Tyr Ile Tyr Lys Tyr Thr Ser Tyr
805 810 815

Pro Asp Pro Ile Leu Leu Met Lys Ser Ala Arg Asn Ser Cys Trp Ser
820 825 830

Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly Ile Phe
835 840 845

Glu Leu Lys Glu Asn Asp Arg Ile Phe Val Ser Val Thr Asn Glu His
850 855 860

Leu Ile Asp Met Asp His Glu Ala Ser Phe Phe Gly Ala Phe Leu Val
865 870 875 880

Gly

<210> SEQ ID NO 39

<211> LENGTH: 2358

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence:

sctNF-AMAIze sequence

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(2355)

<400> SEQUENCE: 39

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gcc cac agc cag gta cag ctg gtg cag tct ggg gga ggc atg gta gag	96
Ala His Ser Gln Val Gln Leu Val Gln Ser Gly Gly Gly Met Val Glu	
20 25 30	
cct ggg ggg tcc ctt aga ctc tcc tgt gca gcc tct gga ttc act ttc	144
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe	
35 40 45	
agt aat gcc tgg atg agc tgg gtc cgc cag gct cca ggg aag ggg ctg	192
Ser Asn Ala Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu	
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gag tgg gtt ggc cgt ata aaa agc aaa gct ggt ggt ggg aca gca gag	240
Glu Trp Val Gly Arg Ile Lys Ser Lys Ala Gly Gly Gly Thr Ala Glu	
65 70 75 80	
tac gct gca ccc gtg aaa ggc aga ttc acc atc tca aga gat gat tca	288
Tyr Ala Ala Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser	
85 90 95	
caa aac acg ctg tat ctg caa atg aac agc ctg aaa acc gac gac aca	336
Gln Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Asp Asp Thr	
100 105 110	
gcc gtg tat tac tgt acc aca cat gtc tac ggt gcc ccc cgg aac tgg	384
Ala Val Tyr Tyr Cys Thr Thr His Val Tyr Gly Ala Pro Arg Asn Trp	
115 120 125	
ggc cag gga tcc ctg gtc acc gtc tcc tca gcc tcc acc aag ggc cca	432
Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro	
130 135 140	
aag ctt gaa gaa ggt gaa ttt tca gaa gca cgc gta cag tct gtg ttg	480
Lys Leu Glu Glu Gly Glu Phe Ser Glu Ala Arg Val Gln Ser Val Leu	

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145	150	155	160	
act cag ccg ccc tca gtg tct gcg gcc cca gga cag aag gtc acc atc				528
Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln Lys Val Thr Ile				
	165	170	175	
tcc tgc tct gga agc agc tcc aac att gga aat aat tat gtc tcc tgg				576
Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Tyr Val Ser Trp				
	180	185	190	
tac gtt caa ctc cca gga aca gcc ccc aaa ctc ctc att tat gac aat				624
Tyr Val Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Asp Asn				
	195	200	205	
aat aag cga ttc tca gga gtt cct gac cga ttc tct ggc tcc aag tct				672
Asn Lys Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser				
	210	215	220	
ggc acg tca gcc acc ctg ggc atc acc ggg ctc cag act ggg gac gag				720
Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln Thr Gly Asp Glu				
	225	230	235	240
gcc gat tat tac tgc gga gca tgg gat ggc agc ctg cgt gaa gcg gta				768
Ala Asp Tyr Tyr Cys Gly Ala Trp Asp Gly Ser Leu Arg Glu Ala Val				
	245	250	255	
ttc ggc gga ggg acc aag gtc acc gtc cta ggt gcg gcc gca gtt gag				816
Phe Gly Gly Gly Thr Lys Val Thr Val Leu Gly Ala Ala Ala Val Glu				
	260	265	270	
ctc gag gcg gcc gcg gat tac aaa gac gat gac gat aaa gaa ttc gga				864
Leu Glu Ala Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys Glu Phe Gly				
	275	280	285	
tca tct tct cga acc ccg agt gac aag cct gta gcc cat gtt gta gca				912
Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala				
	290	295	300	
aac cct caa gct gag ggg cag ctc cag tgg ctg aac cgc cgg gcc aat				960
Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn				
	305	310	315	320
gcc ctc ctg gcc aat ggc gtg gag ctg aga gat aac cag ctg gtg gtg				1008
Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val				
	325	330	335	
cca tca gag ggc ctg tac ctc atc tac tcc cag gtc ctc ttc aag ggc				1056
Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly				
	340	345	350	
caa ggc tgc ccc tcc acc cat gtg ctc ctc acc cac acc atc agc cgc				1104
Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg				
	355	360	365	
atc gcc gtc tcc tac cag acc aag gtc aac ctc ctc tct gcc atc aag				1152
Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys				
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agc ccc tgc cag agg gag acc cca gag ggg gct gag gcc aag ccc tgg				1200
Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp				
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tat gag ccc atc tat ctg gga ggg gtc ttc cag ctg gag aag ggt gac				1248
Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp				
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cga ctc agc gct gag atc aat cgg ccc gac tat ctc gac ttt gcc gag				1296
Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu				
	420	425	430	
tct ggg cag gtc tac ttt ggg atc att gcc ctg ggt ggc ggt tct ggt				1344
Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu Gly Gly Gly Ser Gly				
	435	440	445	
ggc ggt tct ggt ggc ggt tct ggt ggc gga tca tca tct tct cga acc				1392
Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Ser Ser Ser Arg Thr				
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ccg agt gac aag cct gta gcc cat gtt gta gca aac cct caa gct gag				1440

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Pro 465	Ser	Asp	Lys	Pro	Val	Ala	His	Val	Val	Ala	Asn	Pro	Gln	Ala	Glu	
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ggg	cag	ctc	cag	tgg	ctg	aac	cgc	cgg	gcc	aat	gcc	ctc	ctg	gcc	aat	1488
Gly	Gln	Leu	Gln	Trp	Leu	Asn	Arg	Arg	Ala	Asn	Ala	Leu	Leu	Ala	Asn	
				485					490					495		
ggc	gtg	gag	ctg	aga	gat	aac	cag	ctg	gtg	gtg	cca	tca	gag	ggc	ctg	1536
Gly	Val	Glu	Leu	Arg	Asp	Asn	Gln	Leu	Val	Val	Pro	Ser	Glu	Gly	Leu	
			500					505					510			
tac	ctc	atc	tac	tcc	cag	gtc	ctc	ttc	aag	ggc	caa	ggc	tgc	ccc	tcc	1584
Tyr	Leu	Ile	Tyr	Ser	Gln	Val	Leu	Phe	Lys	Gly	Gln	Gly	Cys	Pro	Ser	
		515					520					525				
acc	cat	gtg	ctc	ctc	acc	cac	acc	atc	agc	cgc	atc	gcc	gtc	tcc	tac	1632
Thr	His	Val	Leu	Leu	Thr	His	Thr	Ile	Ser	Arg	Ile	Ala	Val	Ser	Tyr	
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Gln	Thr	Lys	Val	Asn	Leu	Leu	Ser	Ala	Ile	Lys	Ser	Pro	Cys	Gln	Arg	
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gag	acc	cca	gag	ggg	gct	gag	gcc	aag	ccc	tgg	tat	gag	ccc	atc	tat	1728
Glu	Thr	Pro	Glu	Gly	Ala	Glu	Ala	Lys	Pro	Trp	Tyr	Glu	Pro	Ile	Tyr	
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Leu	Gly	Gly	Val	Phe	Gln	Leu	Glu	Lys	Gly	Asp	Arg	Leu	Ser	Ala	Glu	
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Ile	Asn	Arg	Pro	Asp	Tyr	Leu	Asp	Phe	Ala	Glu	Ser	Gly	Gln	Val	Tyr	
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Phe	Gly	Ile	Ile	Ala	Leu	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	
	610					615					620					
ggg	tct	ggg	ggc	gga	tca	tca	tct	tct	cga	acc	cgg	agt	gac	aag	cct	1920
Gly	Ser	Gly	Gly	Gly	Ser	Ser	Ser	Ser	Arg	Thr	Pro	Ser	Asp	Lys	Pro	
				630						635				640		
gta	gcc	cat	gtt	gta	gca	aac	cct	caa	gct	gag	ggg	cag	ctc	cag	tgg	1968
Val	Ala	His	Val	Val	Ala	Asn	Pro	Gln	Ala	Glu	Gly	Gln	Leu	Gln	Trp	
				645					650					655		
ctg	aac	cgc	cgg	gcc	aat	gcc	ctc	ctg	gcc	aat	ggc	gtg	gag	ctg	aga	2016
Leu	Asn	Arg	Arg	Ala	Asn	Ala	Leu	Leu	Ala	Asn	Gly	Val	Glu	Leu	Arg	
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		675					680					685				
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Gln	Val	Leu	Phe	Lys	Gly	Gln	Gly	Cys	Pro	Ser	Thr	His	Val	Leu	Leu	
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acc	cac	acc	atc	agc	cgc	atc	gcc	gtc	tcc	tac	cag	acc	aag	gtc	aac	2160
Thr	His	Thr	Ile	Ser	Arg	Ile	Ala	Val	Ser	Tyr	Gln	Thr	Lys	Val	Asn	
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ctc	ctc	tct	gcc	atc	aag	agc	ccc	tgc	cag	agg	gag	acc	cca	gag	ggg	2208
Leu	Leu	Ser	Ala	Ile	Lys	Ser	Pro	Cys	Gln	Arg	Glu	Thr	Pro	Glu	Gly	
			725					730						735		
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Ala	Glu	Ala	Lys	Pro	Trp	Tyr	Glu	Pro	Ile	Tyr	Leu	Gly	Gly	Val	Phe	
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cag	ctg	gag	aag	ggg	gac	cga	ctc	agc	gct	gag	atc	aat	cgg	ccc	gac	2304
Gln	Leu	Glu	Lys	Gly	Asp	Arg	Leu	Ser	Ala	Glu	Ile	Asn	Arg	Pro	Asp	
			755				760					765				
tat	ctc	gac	ttt	gcc	gag	tct	ggg	cag	gtc	tac	ttt	ggg	atc	att	gcc	2352
Tyr	Leu	Asp	Phe	Ala	Glu	Ser	Gly	Gln	Val	Tyr	Phe	Gly	Ile	Ile	Ala	
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ctg tga 2358
 Leu
 785

<210> SEQ ID NO 40
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 <213> ORGANISM: Artificial Sequence
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 <223> OTHER INFORMATION: Description of Artificial Sequence: TNF-AMAIZE
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 Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
 35 40 45
 Ser Asn Ala Trp Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
 50 55 60
 Glu Trp Val Gly Arg Ile Lys Ser Lys Ala Gly Gly Gly Thr Ala Glu
 65 70 75 80
 Tyr Ala Ala Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser
 85 90 95
 Gln Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Asp Asp Thr
 100 105 110
 Ala Val Tyr Tyr Cys Thr Thr His Val Tyr Gly Ala Pro Arg Asn Trp
 115 120 125
 Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 130 135 140
 Lys Leu Glu Glu Gly Glu Phe Ser Glu Ala Arg Val Gln Ser Val Leu
 145 150 155 160
 Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln Lys Val Thr Ile
 165 170 175
 Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Tyr Val Ser Trp
 180 185 190
 Tyr Val Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Asp Asn
 195 200 205
 Asn Lys Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Lys Ser
 210 215 220
 Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln Thr Gly Asp Glu
 225 230 235 240
 Ala Asp Tyr Tyr Cys Gly Ala Trp Asp Gly Ser Leu Arg Glu Ala Val
 245 250 255
 Phe Gly Gly Gly Thr Lys Val Thr Val Leu Gly Ala Ala Ala Val Glu
 260 265 270
 Leu Glu Ala Ala Ala Asp Tyr Lys Asp Asp Asp Asp Lys Glu Phe Gly
 275 280 285
 Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala
 290 295 300
 Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn
 305 310 315 320
 Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val
 325 330 335
 Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly

340										345										350									
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355							360							365															
Ile	Ala	Val	Ser	Tyr	Gln	Thr	Lys	Val	Asn	Leu	Leu	Ser	Ala	Ile	Lys														
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385							390							395							400								
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405							410							415															
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Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Ser	Ser	Ser	Ser	Arg	Thr													
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Pro	Ser	Asp	Lys	Pro	Val	Ala	His	Val	Val	Ala	Asn	Pro	Gln	Ala	Glu														
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485							490							495															
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500							505							510															
Tyr	Leu	Ile	Tyr	Ser	Gln	Val	Leu	Phe	Lys	Gly	Gln	Gly	Cys	Pro	Ser														
515							520							525															
Thr	His	Val	Leu	Leu	Thr	His	Thr	Ile	Ser	Arg	Ile	Ala	Val	Ser	Tyr														
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Gln	Thr	Lys	Val	Asn	Leu	Leu	Ser	Ala	Ile	Lys	Ser	Pro	Cys	Gln	Arg														
545							550							555							560								
Glu	Thr	Pro	Glu	Gly	Ala	Glu	Ala	Lys	Pro	Trp	Tyr	Glu	Pro	Ile	Tyr														
565							570							575															
Leu	Gly	Gly	Val	Phe	Gln	Leu	Glu	Lys	Gly	Asp	Arg	Leu	Ser	Ala	Glu														
580							585							590															
Ile	Asn	Arg	Pro	Asp	Tyr	Leu	Asp	Phe	Ala	Glu	Ser	Gly	Gln	Val	Tyr														
595							600							605															
Phe	Gly	Ile	Ile	Ala	Leu	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	Gly														
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Val	Ala	His	Val	Val	Ala	Asn	Pro	Gln	Ala	Glu	Gly	Gln	Leu	Gln	Trp														
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Leu	Asn	Arg	Arg	Ala	Asn	Ala	Leu	Leu	Ala	Asn	Gly	Val	Glu	Leu	Arg														
660							665							670															
Asp	Asn	Gln	Leu	Val	Val	Pro	Ser	Glu	Gly	Leu	Tyr	Leu	Ile	Tyr	Ser														
675							680							685															
Gln	Val	Leu	Phe	Lys	Gly	Gln	Gly	Cys	Pro	Ser	Thr	His	Val	Leu	Leu														
690							695							700															
Thr	His	Thr	Ile	Ser	Arg	Ile	Ala	Val	Ser	Tyr	Gln	Thr	Lys	Val	Asn														
705							710							715							720								
Leu	Leu	Ser	Ala	Ile	Lys	Ser	Pro	Cys	Gln	Arg	Glu	Thr	Pro	Glu	Gly														
725							730							735															
Ala	Glu	Ala	Lys	Pro	Trp	Tyr	Glu	Pro	Ile	Tyr	Leu	Gly	Gly	Val	Phe														
740							745							750															
Gln	Leu	Glu	Lys	Gly	Asp	Arg	Leu	Ser	Ala	Glu																			

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Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala
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Leu
 785

<210> SEQ ID NO 41
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 <223> OTHER INFORMATION: Description of Artificial Sequence: Peptide
 linker sequence
 <400> SEQUENCE: 41
 Gly Gly Gly Ser
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20

The invention claimed is:

1. A method for extracorporeal depletion or removal of tumor necrosis factor receptor (TNFR) from blood or blood fractions comprising the following steps:

- a) Optional separation of the blood into one or more blood fractions with solid or liquid components;
- b) Binding of the blood or the blood fractions expressing a TNFR to a surface or particle coupled to a polypeptide wherein the polypeptide comprises at least three components A and at least two components B, wherein each component A comprises a tumor necrosis factor (TNF) monomer or a functional fragment or a functional variant thereof, and each component B is a peptide linker under conditions allowing binding of TNFR in the blood or the blood fractions to the surface or the particle; and
- c) Separating the bound TNFR from the blood or the blood fractions, thereby depleting or removing TNFR from the blood or the blood fractions.

2. The method according to claim 1, wherein before step a) or b) blood is taken from a patient.

3. The method according to claim 1, wherein after a step b) or c), the blood or blood fraction is injected or reinjected into a patient.

4. The method according to claim 1, wherein components A are identical or different.

5. The method according to claim 1, wherein the blood or the blood fraction is from the same organism or different organisms.

6. The method according to claim 1, wherein components B each link together two of the at least three components A.

7. The method according to claim 1, wherein at least one of components B has the amino acid sequence (GGGS)₃ (SEQ ID NO:2) or (GGGS)₄ (SEQ ID NO:3).

8. The method according to claim 1, wherein components A and components B form a trimeric protein structure.

9. The method according to claim 8, wherein components A and components B form a homotrimeric protein structure.

10. The method according to claim 8, wherein components A and components B form a heterotrimeric protein structure.

11. The method according to claim 1, wherein components B are identical or different.

12. The method according to claim 1, wherein components B are from the same organism or different organisms.

13. The method according to claim 1, wherein the polypeptide has an N-terminal tag sequence.

14. The method according to claim 13, wherein the N-terminal tag sequence is a His tag sequence or a Flag tag sequence.

15. The method according to claim 1, wherein the polypeptide has an N-terminal leader peptide sequence.

16. The method according to claim 1, wherein the polypeptide further comprises component C, wherein component C comprises an antibody fragment or a different protein or peptide, which selectively recognizes a specific target molecule on a cell surface.

17. The method according to claim 16, wherein component C is an antibody fragment from a mammal.

18. The method according to claim 17, wherein the mammal is human.

19. The method according to claim 17, wherein the antibody fragment is a humanized antibody fragment.

20. The method according to claim 16, wherein the antibody fragment comprises scFv.

21. The method according to claim 1, wherein at least one component A comprises a soluble tumor necrosis factor (TNF) monomer.

22. The method according to claim 1, wherein each component A comprises a soluble tumor necrosis factor (TNF) monomer.

23. The method according to claim 1, wherein the polypeptide is coupled to the surface or particle by a cysteine residue in the first 1-15 N-terminal amino acids of the polypeptide.

* * * * *

专利名称(译)	TNF配体家族成员的重组多肽及其用途		
公开(公告)号	US8927205	公开(公告)日	2015-01-06
申请号	US10/594189	申请日	2005-03-24
[标]申请(专利权)人(译)	UNIVERISTAT STUTTGART		
申请(专利权)人(译)	UNIVERISTAT STUTTGART		
当前申请(专利权)人(译)	斯图加特大学的		
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摘要(译)

本发明涉及多肽，其包含作为组分A的至少三种TNF配体家族成员的单体，和作为组分B的至少两种肽接头，其将TNF配体家族成员的单体彼此连接。本发明还涉及这些多肽用于治疗疾病和生产药物或疫苗的用途。本发明还涉及产生和分离这些多肽的方法，编码这些多肽的核酸，含有这些核酸的载体，用这些载体转染的宿主细胞，以及含有这些本发明目的的药物组合物。最后，本发明涉及用于体外操作，消耗和/或去除体液中所含成分的方法，例如，体液。通过单采血液成分术。

Cytotoxicity test
sTNF and scTNFs with neutralizing antibodies [AB]

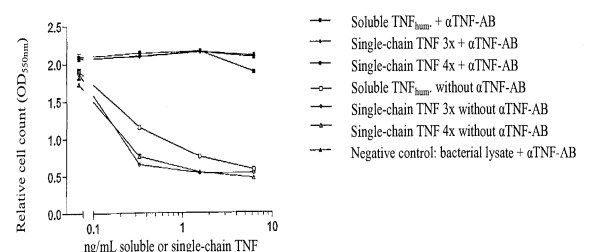


Figure 1