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(54) **UNIVERSAL ANTIBODY-MEDIATED BIOSENSOR**

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(57) **ABSTRACT**

A universal antibody-mediated biosensor is provided that comprise a biosensor cell line stably expressing a novel chimeric fusion protein that can be used to detect target agents in a sample. The fusion protein has an extracellular antibody-binding domain that binds antibodies without regard to their binding specificity and a signaling domain that induces cellular activation upon antigen binding. Because the fusion protein binds to the Fc region of any antibody, it can serve as a universal pathway between extracellular signaling and intracellular activation. The biosensor can be used to detect the presence of selected antigens in a sample.

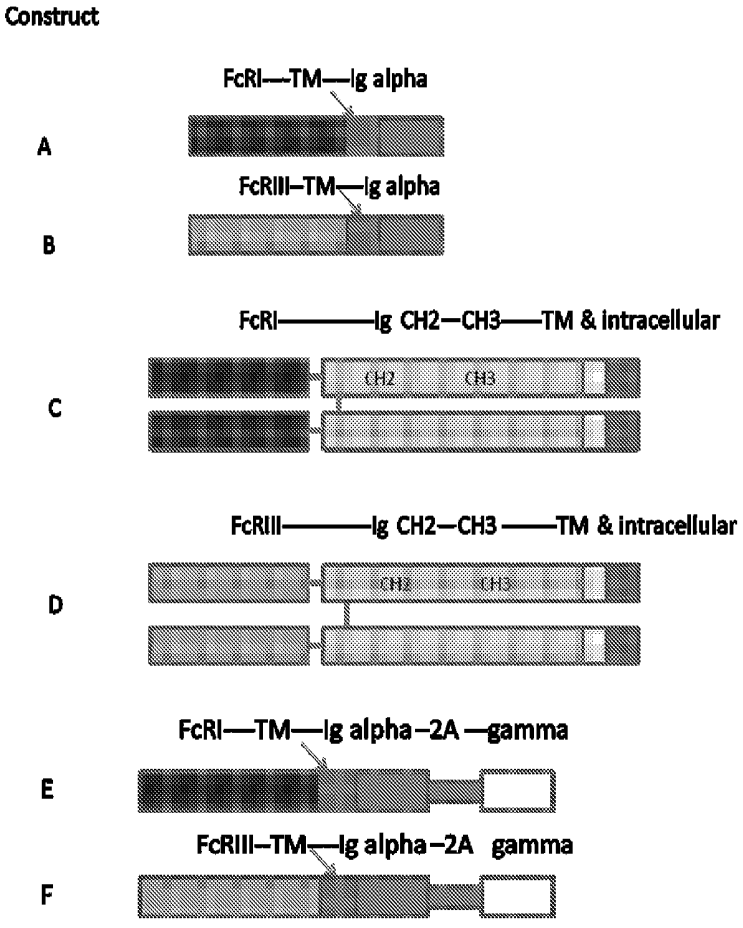

Figure 1

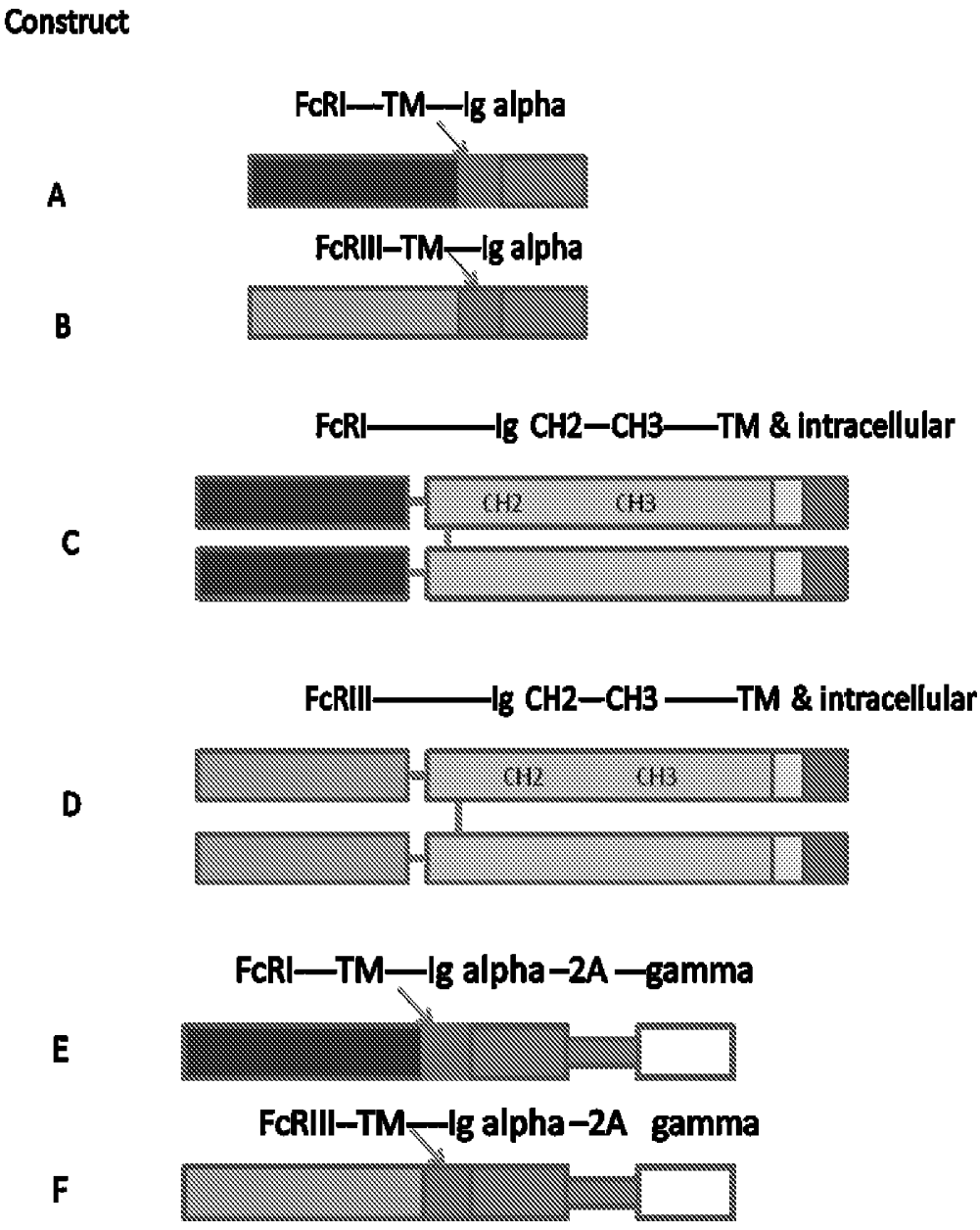
Construct
FcRI—TM—Ig alpha
A
FcRIII–TM—Ig alpha
B
FcRI————Ig CH2—CH3——TM & intracellular
CH2
CH3
C
FcRIII————Ig CH2—CH3 ——TM & intracellular
CH2
CH3
D
FcRI—TM—Ig alpha –2A —gamma
E
FcRIII–TM—Ig alpha –2A gamma
F

Figure 2

Murine FcγRI

```
ATGATTCTTACCAGCTTTGGAGATGACATGTGGCTTCTAACAACTCTGCTACTT
 M  I  L  T  S  F  G  D  D  M  W  L  L  T  T  L  L  L
TGGGTTCCAGTCGGTGGGGAAGTGGTTAATGCCACCAAGGCTGTGATCACCTTGCAGCCT
 W  V  P  V  G  G  E  V  V  N  A  T  K  A  V  I  T  L  Q  P
CCATGGGTCAGTATTTTCCAGAAGGAAAATGTCACTTTATGGTGTGAGGGGCCTCACCTG
 P  W  V  S  I  F  Q  K  E  N  V  T  L  W  C  E  G  P  H  L
CCTGGAGACAGTTCCACACAATGGTTTATCAACGGAACAGCCGTTCAGATCTCCACGCCT
 P  G  D  S  S  T  Q  W  F  I  N  G  T  A  V  Q  I  S  T  P
AGTTATAGCATCCCAGAGGCCAGTTTTCAGGACAGTGGCGAATACAGGTGTCAGATAGGT
 S  Y  S  I  P  E  A  S  F  Q  D  S  G  E  Y  R  C  Q  I  G
TCCTCAATGCCAAGTGACCCTGTGCAGTTGCAAATCCACAATGATTGGCTGCTACTCCAG
 S  S  M  P  S  D  P  V  Q  L  Q  I  H  N  D  W  L  L  L  Q
GCCTCCCGCAGAGTCCTCACAGAAGGAGAACCCCTGGCCTTGAGGTGTCACGGATGGAAG
 A  S  R  R  V  L  T  E  G  E  P  L  A  L  R  C  H  G  W  K
AATAAACTGGTGTACAATGTGGTTTTCTATAGAAATGGAAAATCCTTTCAGTTTTCTTCA
 N  K  L  V  Y  N  V  V  F  Y  R  N  G  K  S  F  Q  F  S  S
GATTCGGAGGTCGCCATTCTGAAAACCAACCTGAGTCACAGCGGCATCTACCACTGCTCA
 D  S  E  V  A  I  L  K  T  N  L  S  H  S  G  I  Y  H  C  S
GGCACGGGAAGACACCGCTACACATCTGCAGGAGTGTCCATCACGGTGAAAGAGCTGTTT
 G  T  G  R  H  R  Y  T  S  A  G  V  S  I  T  V  K  E  L  F
ACCACGCCAGTGCTGAGAGCATCCGTGTCATCTCCCTTCCCGGAGGGGAGTCTGGTCACC
 T  T  P  V  L  R  A  S  V  S  S  P  F  P  E  G  S  L  V  T
CTGAACTGTGAGACGAATTTGCTCCTGCAGAGACCCGGCTTACAGCTTCACTTCTCCTTC
 L  N  C  E  T  N  L  L  L  Q  R  P  G  L  Q  L  H  F  S  F
TACGTGGGCAGCAAGATCCTGGAGTACAGGAACACATCCTCAGAGTACCATATAGCAAGG
 Y  V  G  S  K  I  L  E  Y  R  N  T  S  S  E  Y  H  I  A  R
GCGGAAAGAGAAGATGCTGGATTCTACTGGTGTGAGGTAGCCACGGAGGACAGCAGTGTC
 A  E  R  E  D  A  G  F  Y  W  C  E  V  A  T  E  D  S  S  V
CTTAAGCGCAGCCCTGAGTTGGAGCTCCAAGTGCTTGGTCCCCAGTCATCAGCTCCTGTC
 L  K  R  S  P  E  L  E  L  Q  V  L  G  P  Q  S  S  A  P  V
TGGTTTCACATCCTGTTTTATCTGTCAGTGGGAATAATGTTTTCGTTGAACACGGTTCTC
 W  F  H  I  L  F  Y  L  S  V  G  I  M  F  S  L  N  T  V  L
TATGTGAAAATACACAGGCTGCAGAGAGAGAAGAAATACAACTTAGAAGTCCCTTTGGTT
 Y  V  K  I  H  R  L  Q  R  E  K  K  Y  N  L  E  V  P  L  V
TCTGAGCAGGGAAAGAAAGCAAATTCCTTTCAGCAAGTTAGAAGCGATGGCGTGTATGAA
 S  E  Q  G  K  K  A  N  S  F  Q  Q  V  R  S  D  G  V  Y  E
GAAGTAACAGCCACTGCGAGCCAGACCACACCAAAAGAAGCGCCCGATGGACCTCGAAGC
 E  V  T  A  T  A  S  Q  T  T  P  K  E  A  P  D  G  P  R  S
TCAGTGGGTGACTGTGGACCCGAGCAGCCTGAACCCCTTCCTCCCAGTGACAGTACTGGG
 S  V  G  D  C  G  P  E  Q  P  E  P  L  P  P  S  D  S  T  G
GCACAAACTTCCCAAAGTTGA
 A  Q  T  S  Q  S  *
```

Figure 3

Murine FcγRIII

```
ATGACTTTGGACACCCAGATGTTTCAGAATGCACACTCTGGAAGCCAATGGCTACTT
 M  T  L  D  T  Q  M  F  Q  N  A  H  S  G  S  Q  W  L  L
CCACCACTGACAATTCTGCTGCTGTTTGCTTTTGCAGACAGGCAGAGTGCAGCTCTTCCG
 P  P  L  T  I  L  L  L  F  A  F  A  D  R  Q  S  A  A  L  P
AAGGCTGTGGTGAAACTGGACCCCCCATGGATCCAGGTGCTCAAGGAAGACATGGTGACA
 K  A  V  V  K  L  D  P  P  W  I  Q  V  L  K  E  D  M  V  T
CTGATGTGCGAAGGGACCCACAACCCTGGGAACTCTTCTACTCAGTGGTTCCACAACTGG
 L  M  C  E  G  T  H  N  P  G  N  S  S  T  Q  W  F  H  N  W
AGTTCCATCCGGAGCCAGGTCCAATCCAGCTACACGTTTAAGGCCACAGTCAATGACAGT
 S  S  I  R  S  Q  V  Q  S  S  Y  T  F  K  A  T  V  N  D  S
GGAGAATATCGGTGTCAAATGGAGCAGACCCGCCTCAGCGACCCTGTAGATCTGGGAGTG
 G  E  Y  R  C  Q  M  E  Q  T  R  L  S  D  P  V  D  L  G  V
ATTTCTGACTGGCTGCTGCTCCAGACCCCTCAGCGGGTGTTTCTGGAAGGGGAAACCATC
 I  S  D  W  L  L  L  Q  T  P  Q  R  V  F  L  E  G  E  T  I
ACGCTAAGGTGCCCTAGCTGGAGGAACAAACTACTGAACAGGATCTCGTTCTTCCATAAT
 T  L  R  C  P  S  W  R  N  K  L  L  N  R  I  S  F  F  H  N
GAAAAATCCGTGAGGTATCATCACTACAAAAGTAATTTCTCTATCCCAAAAGCCAACCAC
 E  K  S  V  R  Y  H  H  Y  K  S  N  F  S  I  P  K  A  N  H
AGTCACAGTGGGGACTACTACTGCAAAGGAAGTCTAGGAAGTACACAGCACCAGTCCAAG
 S  H  S  G  D  Y  Y  C  K  G  S  L  G  S  T  Q  H  Q  S  K
CCTGTCACCATCACTGTCCAAGACCCAGCAACTACATCCTCCATCTCTCTAGTCTGGCAC
 P  V  T  I  T  V  Q  D  P  A  T  T  S  S  I  S  L  V  W  H
CACACTGCTTTCTCCCTAGTGATGTGCCTCCTGTTTGCAGTGGACACGGGCCTTTATTTC
 H  T  A  F  S  L  V  M  C  L  L  F  A  V  D  T  G  L  Y  F
TATGTACGGAGAAATCTTCAAACCCCGAGGGATTACTGGAGGAAGTCCCTGTCAATCAGA
 Y  V  R  R  N  L  Q  T  P  R  D  Y  W  R  K  S  L  S  I  R
AAGCACCAGGCTCCTCAAGACAAGTGA
 K  H  Q  A  P  Q  D  K  *
```

Figure 4

Murine Igα

```
ATGCCAGGGGGTCTAGAAGCCCTCAGAGCCCTGCCTCTCCTCCTCTTCTTGTCATACGCC
 M  P  G  G  L  E  A  L  R  A  L  P  L  L  L  F  L  S  Y  A
TGTTTGGGTCCCGGATGCCAGGCCCTGCGGGTAGAAGGGGGTCCACCATCCCTGACGGTG
 C  L  G  P  G  C  Q  A  L  R  V  E  G  G  P  P  S  L  T  V
AACTTGGGCGAGGAGGCCCGCCTCACCTGTGAAAACAATGGCAGGAACCCTAATATCACA
 N  L  G  E  E  A  R  L  T  C  E  N  N  G  R  N  P  N  I  T
TGGTGGTTCAGCCTTCAGTCTAACATCACATGGCCCCCAGTGCCACTGGGTCCTGGCCAG
 W  W  F  S  L  Q  S  N  I  T  W  P  P  V  P  L  G  P  G  Q
GGTACCACAGGCCAGCTGTTCTTCCCCGAAGTAAACAAGAACCACAGGGGCTTGTACTGG
 G  T  T  G  Q  L  F  F  P  E  V  N  K  N  H  R  G  L  Y  W
TGCCAAGTGATAGAAAACAACATATTAAAACGCTCCTGTGGTACTTACCTCCGCGTGCGC
 C  Q  V  I  E  N  N  I  L  K  R  S  C  G  T  Y  L  R  V  R
AATCCAGTCCCTAGGCCCTTCCTGGACATGGGGGAAGGTACCAAGAACCGCATCATCACA
 N  P  V  P  R  P  F  L  D  M  G  E  G  T  K  N  R  I  I  T
GCAGAAGGGATCATCTTGCTGTTCTGTGCAGTGGTGCCAGGGACGCTGCTGCTATTCAGG
 A  E  G  I  I  L  L  F  C  A  V  V  P  G  T  L  L  L  F  R
AAACGGTGGCAAAATGAGAAGTTTGGGGTGGACATGCCAGATGACTATGAAGATGAAAAT
 K  R  W  Q  N  E  K  F  G  V  D  M  P  D  D  Y  E  D  E  N
CTCTATGAGGGCCTGAACCTTGATGACTGTTCTATGTATGAGGACATCTCCAGGGGACTC
 L  Y  E  G  L  N  L  D  D  C  S  M  Y  E  D  I  S  R  G  L
CAGGGCACCTACCAGGATGTGGGCAACCTCCACATTGGAGATGCCCAGCTGGAAAAGCCA
 Q  G  T  Y  Q  D  V  G  N  L  H  I  G  D  A  Q  L  E  K  P
TGA
 *
```

Figure 5

FcγRI/Igα fusion protein

```
ATGATTCTTACCAGCTTTGGAGATGACATGTGGCTTCTAACAACTCTGCTACTT
 M  I  L  T  S  F  G  D  D  M  W  L  L  T  T  L  L  L
TGGGTTCCAGTCGGTGGGGAAGTGGTTAATGCCACCAAGGCTGTGATCACCTTGCAGCCT
 W  V  P  V  G  G  E  V  V  N  A  T  K  A  V  I  T  L  Q  P
CCATGGGTCAGTATTTTCCAGAAGGAAAATGTCACTTTATGGTGTGAGGGGCCTCACCTG
 P  W  V  S  I  F  Q  K  E  N  V  T  L  W  C  E  G  P  H  L
CCTGGAGACAGTTCCACACAATGGTTTATCAACGGAACAGCCGTTCAGATCTCCACGCCT
 P  G  D  S  S  T  Q  W  F  I  N  G  T  A  V  Q  I  S  T  P
AGTTATAGCATCCCAGAGGCCAGTTTTCAGGACAGTGGCGAATACAGGTGTCAGATAGGT
 S  Y  S  I  P  E  A  S  F  Q  D  S  G  E  Y  R  C  Q  I  G
TCCTCAATGCCAAGTGACCCTGTGCAGTTGCAAATCCACAATGATTGGCTGCTACTCCAG
 S  S  M  P  S  D  P  V  Q  L  Q  I  H  N  D  W  L  L  L  Q
GCCTCCCGCAGAGTCCTCACAGAAGGAGAACCCCTGGCCTTGAGGTGTCACGGATGGAAG
 A  S  R  R  V  L  T  E  G  E  P  L  A  L  R  C  H  G  W  K
AATAAACTGGTGTACAATGTGGTTTTCTATAGAAATGGAAAATCCTTTCAGTTTTCTTCA
 N  K  L  V  Y  N  V  V  F  Y  R  N  G  K  S  F  Q  F  S  S
GATTCGGAGGTCGCCATTCTGAAAACCAACCTGAGTCACAGCGGCATCTACCACTGCTCA
 D  S  E  V  A  I  L  K  T  N  L  S  H  S  G  I  Y  H  C  S
GGCACGGGAAGACACCGCTACACATCTGCAGGAGTGTCCATCACGGTGAAAGAGCTGTTT
 G  T  G  R  H  R  Y  T  S  A  G  V  S  I  T  V  K  E  L  F
ACCACGCCAGTGCTGAGAGCATCCGTGTCATCTCCCTTCCCGGAGGGGAGTCTGGTCACC
 T  T  P  V  L  R  A  S  V  S  S  P  F  P  E  G  S  L  V  T
CTGAACTGTGAGACGAATTTGCTCCTGCAGAGACCCGGCTTACAGCTTCACTTCTCCTTC
 L  N  C  E  T  N  L  L  L  Q  R  P  G  L  Q  L  H  F  S  F
TACGTGGGCAGCAAGATCCTGGAGTACAGGAACACATCCTCAGAGTACCATATAGCAAGG
 Y  V  G  S  K  I  L  E  Y  R  N  T  S  S  E  Y  H  I  A  R
GCGGAAAGAGAAGATGCTGGATTCTACTGGTGTGAGGTAGCCACGGAGGACAGCAGTGTC
 A  E  R  E  D  A  G  F  Y  W  C  E  V  A  T  E  D  S  S  V
CTTAAGCGCAGCCCTGAGTTGGAGCTCCAAGTGCTTGGTCCCCAGTCATCAGCTCCTGTC
 L  K  R  S  P  E  L  E  L  Q  V  L  G  P  Q  S  S  A  P  V
TGGTTTCACATCCTGTTTTATCTGTCAGTGGGAATAATGTTTTCGTTGAACACGGTTCTC
 W  F  H  I  L  F  Y  L  S  V  G  I  M  F  S  L  N  T  V  L
TATGTGTTCAGGAAACGGTGGCAAAATGAGAAGTTTGGGGTGGACATGCCAGATGACTAT
 Y  V  F  R  K  R  W  Q  N  E  K  F  G  V  D  M  P  D  D  Y
GAAGATGAAAATCTCTATGAGGGCCTGAACCTTGATGACTGTTCTATGTATGAGGACATC
 E  D  E  N  L  Y  E  G  L  N  L  D  D  C  S  M  Y  E  D  I
TCCAGGGGACTCCAGGGCACCTACCAGGATGTGGGCAACCTCCACATTGGAGATGCCCAG
 S  R  G  L  Q  G  T  Y  Q  D  V  G  N  L  H  I  G  D  A  Q
CTGGAAAAGCCATGA
 L  E  K  P  *
```

Figure 6

FcγRIII/Igα fusion protein

```
ATGACTTTGGACACCCAGATGTTTCAGAATGCACACTCTGGAAGCCAATGGCTACTT
 M  T  L  D  T  Q  M  F  Q  N  A  H  S  G  S  Q  W  L  L
CCACCACTGACAATTCTGCTGCTGTTTGCTTTTGCAGACAGGCAGAGTGCAGCTCTTCCG
 P  P  L  T  I  L  L  L  F  A  F  A  D  R  Q  S  A  A  L  P
AAGGCTGTGGTGAAACTGGACCCCCCATGGATCCAGGTGCTCAAGGAAGACATGGTGACA
 K  A  V  V  K  L  D  P  P  W  I  Q  V  L  K  E  D  M  V  T
CTGATGTGCGAAGGGACCCACAACCCTGGGAACTCTTCTACTCAGTGGTTCCACAACTGG
 L  M  C  E  G  T  H  N  P  G  N  S  S  T  Q  W  F  H  N  W
AGTTCCATCCGGAGCCAGGTCCAATCCAGCTACACGTTTAAGGCCACAGTCAATGACAGT
 S  S  I  R  S  Q  V  Q  S  S  Y  T  F  K  A  T  V  N  D  S
GGAGAATATCGGTGTCAAATGGAGCAGACCCGCCTCAGCGACCCTGTAGATCTGGGAGTG
 G  E  Y  R  C  Q  M  E  Q  T  R  L  S  D  P  V  D  L  G  V
ATTTCTGACTGGCTGCTGCTCCAGACCCCTCAGCGGGTGTTTCTGGAAGGGGAAACCATC
 I  S  D  W  L  L  L  Q  T  P  Q  R  V  F  L  E  G  E  T  I
ACGCTAAGGTGCCCTAGCTGGAGGAACAAACTACTGAACAGGATCTCGTTCTTCCATAAT
 T  L  R  C  P  S  W  R  N  K  L  L  N  R  I  S  F  F  H  N
GAAAAATCCGTGAGGTATCATCACTACAAAAGTAATTTCTCTATCCCAAAAGCCAACCAC
 E  K  S  V  R  Y  H  H  Y  K  S  N  F  S  I  P  K  A  N  H
AGTCACAGTGGGGACTACTACTGCAAAGGAAGTCTAGGAAGTACACAGCACCAGTCCAAG
 S  H  S  G  D  Y  Y  C  K  G  S  L  G  S  T  Q  H  Q  S  K
CCTGTCACCATCACTGTCCAAGACCCAGCAACTACATCCTCCATCTCTCTAGTCTGGCAC
 P  V  T  I  T  V  Q  D  P  A  T  T  S  S  I  S  L  V  W  H
CACACTGCTTTCTCCCTAGTGATGTGCCTCCTGTTTGCAGTGTTCAGGAAACGGTGGCAA
 H  T  A  F  S  L  V  M  C  L  L  F  A  V  F  R  K  R  W  Q
AATGAGAAGTTTGGGGTGGACATGCCAGATGACTATGAAGATGAAAATCTCTATGAGGGC
 N  E  K  F  G  V  D  M  P  D  D  Y  E  D  E  N  L  Y  E  G
CTGAACCTTGATGACTGTTCTATGTATGAGGACATCTCCAGGGGACTCCAGGGCACCTAC
 L  N  L  D  D  C  S  M  Y  E  D  I  S  R  G  L  Q  G  T  Y
CAGGATGTGGGCAACCTCCACATTGGAGATGCCCAGCTGGAAAAGCCATGA
 Q  D  V  G  N  L  H  I  G  D  A  Q  L  E  K  P  *
```

Figure 7

Membrane Ig

```
GAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCAGCACCACCTGTGGCAGGACCGTCA
 E  R  K  C  C  V  E  C  P  P  C  P  A  P  P  V  A  G  P  S
GTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTC
 V  F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  T  P  E  V
ACGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTG
 T  C  V  V  V  D  V  S  H  E  D  P  E  V  Q  F  N  W  Y  V
GACGGCATGGAGGTGCATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACG
 D  G  M  E  V  H  N  A  K  T  K  P  R  E  E  Q  F  N  S  T
TTCCGTGTGGTCAGCGTCCTCACCGTCGTGCACCAGGACTGGCTGAACGGCAAGGAGTAC
 F  R  V  V  S  V  L  T  V  V  H  Q  D  W  L  N  G  K  E  Y
AAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAACC
 K  C  K  V  S  N  K  G  L  P  A  P  I  E  K  T  I  S  K  T
AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACC
 K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  E  E  M  T
AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTG
 K  N  Q  V  S  L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V
GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACACCTCCCATGCTGGAC
 E  W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P  M  L  D
TCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAG
 S  D  G  S  F  F  L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q
GGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAG
 G  N  V  F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K
AGCCTCTCCCTGTCTCCGGAGCTGCAACTGGAGGAGAGCTGTGCGGAGGCGCAGGACGGG
 S  L  S  L  S  P  E  L  Q  L  E  E  S  C  A  E  A  Q  D  G
GAGCTGGACGGGCTGTGGACGACCATCACCATCTTCATCACACTCTTCCTGCTAAGCGTG
 E  L  D  G  L  W  T  T  I  T  I  F  I  T  L  F  L  L  S  V
TGCTACAGTGCCACCATCACCTTCTTCAAGGTGAAGTGGATCTTCTCCTCAGTGGTGGAC
 C  Y  S  A  T  I  T  F  F  K  V  K  W  I  F  S  S  V  V  D
CTGAAGCAGACCATCGTCCCCGACTACAGGAACATGATCAGGCAGGGGGCCTAG
 L  K  Q  T  I  V  P  D  Y  R  N  M  I  R  Q  G  A  *
```

Figure 8/1

FcγRI/membrane Ig fusion protein

```
ATGATTCTTACCAGCTTTGGAGATGACATGTGGCTTCTAACAACTCTGCTACTT
 M  I  L  T  S  F  G  D  D  M  W  L  L  T  T  L  L  L
TGGGTTCCAGTCGGTGGGGAAGTGGTTAATGCCACCAAGGCTGTGATCACCTTGCAGCCT
 W  V  P  V  G  G  E  V  V  N  A  T  K  A  V  I  T  L  Q  P
CCATGGGTCAGTATTTTCCAGAAGGAAAATGTCACTTTATGGTGTGAGGGGCCTCACCTG
 P  W  V  S  I  F  Q  K  E  N  V  T  L  W  C  E  G  P  H  L
CCTGGAGACAGTTCCACACAATGGTTTATCAACGGAACAGCCGTTCAGATCTCCACGCCT
 P  G  D  S  S  T  Q  W  F  I  N  G  T  A  V  Q  I  S  T  P
AGTTATAGCATCCCAGAGGCCAGTTTTCAGGACAGTGGCGAATACAGGTGTCAGATAGGT
 S  Y  S  I  P  E  A  S  F  Q  D  S  G  E  Y  R  C  Q  I  G
TCCTCAATGCCAAGTGACCCTGTGCAGTTGCAAATCCACAATGATTGGCTGCTACTCCAG
 S  S  M  P  S  D  P  V  Q  L  Q  I  H  N  D  W  L  L  L  Q
GCCTCCCGCAGAGTCCTCACAGAAGGAGAACCCCTGGCCTTGAGGTGTCACGGATGGAAG
 A  S  R  R  V  L  T  E  G  E  P  L  A  L  R  C  H  G  W  K
AATAAACTGGTGTACAATGTGGTTTTCTATAGAAATGGAAAATCCTTTCAGTTTTCTTCA
 N  K  L  V  Y  N  V  V  F  Y  R  N  G  K  S  F  Q  F  S  S
GATTCGGAGGTCGCCATTCTGAAAACCAACCTGAGTCACAGCGGCATCTACCACTGCTCA
 D  S  E  V  A  I  L  K  T  N  L  S  H  S  G  I  Y  H  C  S
GGCACGGGAAGACACCGCTACACATCTGCAGGAGTGTCCATCACGGTGAAAGAGCTGTTT
 G  T  G  R  H  R  Y  T  S  A  G  V  S  I  T  V  K  E  L  F
ACCACGCCAGTGCTGAGAGCATCCGTGTCATCTCCCTTCCCGGAGGGGAGTCTGGTCACC
 T  T  P  V  L  R  A  S  V  S  S  P  F  P  E  G  S  L  V  T
CTGAACTGTGAGACGAATTTGCTCCTGCAGAGACCCGGCTTACAGCTTCACTTCTCCTTC
 L  N  C  E  T  N  L  L  L  Q  R  P  G  L  Q  L  H  F  S  F
TACGTGGGCAGCAAGATCCTGGAGTACAGGAACACATCCTCAGAGTACCATATAGCAAGG
 Y  V  G  S  K  I  L  E  Y  R  N  T  S  S  E  Y  H  I  A  R
GCGGAAAGAGAAGATGCTGGATTCTACTGGTGTGAGGTAGCCACGGAGGACAGCAGTGTC
 A  E  R  E  D  A  G  F  Y  W  C  E  V  A  T  E  D  S  S  V
CTTAAGCGCAGCCCTGAGTTGGAGGAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCA
 L  K  R  S  P  E  L  E  E  R  K  C  C  V  E  C  P  P  C  P
GCACCACCTGTGGCAGGACCGTCAGTCACCCTCATGATCTCCCGGACCCCTGAGGTCACG
 A  P  P  V  A  G  P  S  V  T  L  M  I  S  R  T  P  E  V  T
TTCCTCTTCCCCCCAAAACCCAAGGACGACCCCGAGGTCCAGTTCAACTGGTACGTGGAC
 F  L  F  P  P  K  P  K  D  D  P  E  V  Q  F  N  W  Y  V  D
TGCGTGGTGGTGGACGTGAGCCACGAAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTC
 C  V  V  V  D  V  S  H  E  K  P  R  E  E  Q  F  N  S  T  F
GGCATGGAGGTGCATAATGCCAAGACACACCAGGACTGGCTGAACGGCAAGGAGTACAAG
 G  M  E  V  H  N  A  K  T  H  Q  D  W  L  N  G  K  E  Y  K
CGTGTGGTCAGCGTCCTCACCGTCGTGGCCCCCATCGAGAAAACCATCTCCAAAACCAAA
 R  V  V  S  V  L  T  V  V  A  P  I  E  K  T  I  S  K  T  K
TGCAAGGTCTCCAACAAAGGCCTCCCAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG
 C  K  V  S  N  K  G  L  P  G  Q  P  R  E  P  Q  V  Y  T  L
```

Figure 8/2

CCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGC
P P S R E E M T K N Q V S L T C L V K G
TTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTAC
F Y P S D I A V E W E S N G Q P E N N Y
AAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACC
K T T P P M L D S D G S F F L Y S K L T
GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT
V D K S R W Q Q G N V F S C S V M H E A
CTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGAGCTGCAACTGGAGGAG
L H N H Y T Q K S L S L S P E L Q L E E
AGCTGTGCGGAGGCGCAGGACGGGGAGCTGGACGGGCTGTGGACGACCATCACCATCTTC
S C A E A Q D G E L D G L W T T I T I F
ATCACACTCTTCCTGCTAAGCGTGTGCTACAGTGCCACCATCACCTTCTTCAAGGTGAAG
I T L F L L S V C Y S A T I T F F K V K
TGGATCTTCTCCTCAGTGGTGGACCTGAAGCAGACCATCGTCCCCGACTACAGGAACATG
W I F S S V V D L K Q T I V P D Y R N M
ATCAGGCAGGGGGCCTAG
I R Q G A

Figure 9/1

FcγRIII/membrane Ig fusion protein

```
ATGACTTTGGACACCCAGATGTTTCAGAATGCACACTCTGGAAGCCAATGGCTACTT
 M  T  L  D  T  Q  M  F  Q  N  A  H  S  G  S  Q  W  L  L
CCACCACTGACAATTCTGCTGCTGTTTGCTTTTGCAGACAGGCAGAGTGCAGCTCTTCCG
 P  P  L  T  I  L  L  L  F  A  F  A  D  R  Q  S  A  A  L  P
AAGGCTGTGGTGAAACTGGACCCCCCATGGATCCAGGTGCTCAAGGAAGACATGGTGACA
 K  A  V  V  K  L  D  P  P  W  I  Q  V  L  K  E  D  M  V  T
CTGATGTGCGAAGGGACCCACAACCCTGGGAACTCTTCTACTCAGTGGTTCCACAACTGG
 L  M  C  E  G  T  H  N  P  G  N  S  S  T  Q  W  F  H  N  W
AGTTCCATCCGGAGCCAGGTCCAATCCAGCTACACGTTTAAGGCCACAGTCAATGACAGT
 S  S  I  R  S  Q  V  Q  S  S  Y  T  F  K  A  T  V  N  D  S
GGAGAATATCGGTGTCAAATGGAGCAGACCCGCCTCAGCGACCCTGTAGATCTGGGAGTG
 G  E  Y  R  C  Q  M  E  Q  T  R  L  S  D  P  V  D  L  G  V
ATTTCTGACTGGCTGCTGCTCCAGACCCCTCAGCGGGTGTTTCTGGAAGGGGAAACCATC
 I  S  D  W  L  L  L  Q  T  P  Q  R  V  F  L  E  G  E  T  I
ACGCTAAGGTGCCCTAGCTGGAGGAACAAACTACTGAACAGGATCTCGTTCTTCCATAAT
 T  L  R  C  P  S  W  R  N  K  L  L  N  R  I  S  F  F  H  N
GAAAAATCCGTGAGGTATCATCACTACAAAAGTAATTTCTCTATCCCAAAAGCCAACCAC
 E  K  S  V  R  Y  H  H  Y  K  S  N  F  S  I  P  K  A  N  H
AGTCACAGTGGGGACTACTACTGCAAAGGAAGTCTAGGAAGTACACAGCACCAGTCCAAG
 S  H  S  G  D  Y  Y  C  K  G  S  L  G  S  T  Q  H  Q  S  K
CCTGTCACCATCACTGTCCAAGACGAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCCA
 P  V  T  I  T  V  Q  D  E  R  K  C  C  V  E  C  P  P  C  P
GCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTC
 A  P  P  V  A  G  P  S  V  F  L  F  P  P  K  P  K  D  T  L
ATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCC
 M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H  E  D  P
GAGGTCCAGTTCAACTGGTACGTGGACGGCATGGAGGTGCATAATGCCAAGACAAAGCCA
 E  V  Q  F  N  W  Y  V  D  G  M  E  V  H  N  A  K  T  K  P
CGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCTCACCGTCGTGCACCAG
 R  E  E  Q  F  N  S  T  F  R  V  V  S  V  L  T  V  V  H  Q
GACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCAGCCCCC
 D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  G  L  P  A  P
ATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTG
 I  E  K  T  I  S  K  T  K  G  Q  P  R  E  P  Q  V  Y  T  L
CCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGC
 P  P  S  R  E  E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G
TTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTAC
 F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N  N  Y
AAGACCACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACC
 K  T  T  P  P  M  L  D  S  D  G  S  F  F  L  Y  S  K  L  T
GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT
 V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M  H  E  A
```

Figure 9/2

CTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGAGCTGCAACTGGAGGAG
L H N H Y T Q K S L S L S P E L Q L E E
AGCTGTGCGGAGGCGCAGGACGGGGAGCTGGACGGGCTGTGGACGACCATCACCATCTTC
S C A E A Q D G E L D G L W T T I T I F
ATCACACTCTTCCTGCTAAGCGTGTGCTACAGTGCCACCATCACCTTCTTCAAGGTGAAG
I T L F L L S V C Y S A T I T F F K V K
TGGATCTTCTCCTCAGTGGTGGACCTGAAGCAGACCATCGTCCCCGACTACAGGAACATG
W I F S S V V D L K Q T I V P D Y R N M
ATCAGGCAGGGGGCCTAG
I R Q G A

Figure 10

2A Sequence

```
GGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCGGAGACGGGAGGAGAACCCTGG
 G  S  G  A  T  N  F  S  L  L  K  Q  A  E  T  G  G  E  P  W
ACC
 T
```

Figure 11

Mouse FcR gamma chain

```
ATGATCTCAGCCGTGATCTTGTTCTTGCTCCTT
 M  I  S  A  V  I  L  F  L  L  L
TTGGTGGAACAAGCAGCCGCCCTGGGAGAGCCGCAGCTCTGCTATATCCTGGATGCTGTC
 L  V  E  Q  A  A  A  L  G  E  P  Q  L  C  Y  I  L  D  A  V
CTGTTTTTGTATGGTATTGTCCTTACCCTACTCTACTGTCGACTCAAGATCCAGGTCCGA
 L  F  L  Y  G  I  V  L  T  L  L  Y  C  R  L  K  I  Q  V  R
AAGGCAGCTATAGCCAGCCGTGAGAAAGCAGATGCTGTCTACACGGGCCTGAACACCCGG
 K  A  A  I  A  S  R  E  K  A  D  A  V  Y  T  G  L  N  T  R
AGCCAGGAGACATATGAGACTCTGAAGCATGAGAAACCACCCCAGTAG
 S  Q  E  T  Y  E  T  L  K  H  E  K  P  P  Q  *
```

UNIVERSAL ANTIBODY-MEDIATED BIOSENSOR

SEQUENCE LISTING

[0001] A sequence listing in electronic (ASCII text file) format is filed with this application and incorporated herein by reference. The name of the ASCII text file is "2016_0324A_ST25.txt"; the file was created on Mar. 11, 2016; the size of the file is 74 KB.

BACKGROUND

[0002] There is an increasing need in the fields of food safety, health care, agricultural testing, and biodefense for affordable and highly sensitive assays that rapidly and accurately identify the presence of environmental and pathogenic agents, including toxins, antigens, bacteria, and viruses, in samples of interest. To this end, a variety of biosensor products have been commercially developed and released.

[0003] A specific example of a biosensor platform currently in use is the CANARY® biosensor technology of PathSensors, Inc. This platform, based on the work of Rider et al. [1], enables reliable identification of specific airborne and liquid-based pathogens. The biological backbone of the CANARY® biosensor is comprised of a genetically-engineered B cell expressing an extracellularly bound, antigen-specific antibody that can bind its cognate antigen or pathogenic agent. In this system, when an antigen-containing sample interacts with the antibody on the extracellular surface of the biosensor, an intracellular signaling cascade is activated resulting in the release of Ca^{2+} within the B cells. In the CANARY® system, the B cells express aequorin, a Ca^{2+}-sensitive photoprotein, which results in cell luminescence in the presence of elevated intracellular Ca^{2+} levels. Thus, the luminescence can be used to indicate antigen binding.

[0004] The CANARY® system can be used to efficiently identify a number of specific antigens, including those from bacteria, viruses, and toxins. However, expansion of the antigen test repertoire is complex and costly. Different antigen- or pathogen-specific biosensors must be constructed to recognized each and every selected antigen, which requires multiple steps including production of hybridoma cell lines, cloning of nucleic acid sequences encoding the antibodies, and expressing cloned antibodies as transmembrane proteins on the surface of a B cell line genetically engineered to luminesce upon binding of the cognate antigen (e.g., a pathogen) by the antibody.

[0005] Thus, the need remains for the development of a universal biosensor that can be adapted for use in multiple testing platforms across a broad range of environmental and pathogenic agents. The present invention is directed to this and other important goals.

BRIEF SUMMARY

[0006] Provided herein are universal antibody-mediated biosensors that can be used to detect and quantify target agents in a sample, as well as methods of using the biosensors to screen samples from a selected target agent.

[0007] The biosensors of the invention generally comprise a cell line stably expressing a novel chimeric fusion protein. The fusion protein contains an antibody-binding domain (such as the extracellular domain of an Fcγ receptor (FcγR)) fused to a signaling domain (such as the intracellular activation domain of immunoglobulin-alpha (Igα)). The N-terminal, extracellular antibody-binding domain has the ability to bind to the Fc region of an antibody, while the C-terminal, intracellular signaling domain has the ability to activate cellular processes, such as Ca^{2+} release. Such activation occurs when antibodies bound to the antibody-binding domain are cross-linked by their cognate antigen.

[0008] Because the antibody-binding domain of the chimeric fusion protein binds the Fc region of an antibody, the antibody that can be bound by the fusion protein is not limited by the antigenic specificity of the antibody. Thus, the chimeric fusion protein has the ability to bind any available antibody that recognizes and binds a selected target (e.g., antigen or pathogenic agent).

[0009] The biosensor of the invention provides a rapid and economical means of testing for the presence of a wide range of different target agents using the same platform, without requiring the production of separate chimeric fusion proteins for each selected target agent. This universal biosensor can be used in conjunction with commercially available antibodies as well as antibodies produced specifically to be used with the biosensor.

Fusion Proteins

[0010] In a first embodiment, the invention is directed to chimeric fusion proteins comprising an Fcγ receptor (FcγR) antibody-binding domain, a transmembrane domain and a signaling domain. The fusion proteins have the ability to recognize and bind the Fc region of an antibody via their antibody-binding domain. The fusion proteins also have the ability to activate an intracellular signaling cascade in a cell expressing the fusion protein. In certain aspects, the intracellular signaling cascade results in the release of Ca^{2+} within the cell.

[0011] In certain aspects of this embodiment, the FcγR antibody-binding domain is the FcγRI antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3. In certain other aspects of this embodiment, the FcγR antibody-binding domain is the FcγRIII antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4. The sequence variants retain the antibody-binding activity of the antibody-binding domain upon which they are based.

[0012] In certain aspects of this embodiment, the signaling domain is the immunoglobulin alpha (Igα) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5. In certain other aspects of this embodiment, the signaling domain is the partial membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6. The sequence variants retain the signaling activity of the signaling domain upon which they are based.

[0013] In selected aspects, the fusion protein is the FcγRI/Igα fusion protein set forth in SEQ ID NO:8, the FcγRIII/Igα fusion protein set forth in SEQ ID NO:10, the FcγRI/membrane Ig fusion protein set forth in SEQ ID NO:22, or the FcγRIII/membrane Ig fusion protein set forth in SEQ ID NO:23, or a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:8, 10, 22, or 23.

[0014] The invention includes polynucleotides comprising nucleotide sequences encoding each of the fusion proteins provided in the various embodiments and aspects defined herein, as well as complementary strands thereof. The invention also includes cloning vectors comprising the polynucleotides, and host cells comprising either the polynucleotides or the expression vectors. Such host cells may be mammalian or non-mammalian cells. The invention further includes methods of producing the fusion proteins defined herein, comprising culturing the host cells under conditions promoting expression of the fusion proteins encoded by the polynucleotides and expression vectors, and recovering the fusion proteins from the cells or cell cultures.

Biosensor Cells

[0015] In a second embodiment, the invention is directed to biosensor cells stably expressing a chimeric fusion protein, wherein the chimeric fusion protein comprises an Fcγ receptor (FcγR) antibody-binding domain, a transmembrane domain and a signaling domain. The fusion proteins have the ability to recognize and bind the Fc region of an antibody via their antibody-binding domain. The fusion proteins have the ability to activate an intracellular signaling cascade in the cell expressing the fusion protein. In certain aspects, the intracellular signaling cascade results in the release of Ca^{2+} within the cell. The chimeric fusion protein is stably expressed on the surface of the cell as an integral membrane protein.

[0016] In certain aspects of this embodiment, the biosensor cell is a B cell, a T cell, a monocyte, a macrophage, a HEK293 cell, a CHO cell, P815, K562, or a Cos-1 cell, each of which stably expresses the chimeric fusion protein.

[0017] In certain aspects of this embodiment, the FcγR antibody-binding domain is the FcγRI antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3. In certain other aspects of this embodiment, the FcγR antibody-binding domain is the FcγRIII antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4. The sequence variants retain the antibody-binding activity of the antibody-binding domain upon which they are based.

[0018] In certain aspects of this embodiment, the signaling domain is the immunoglobulin alpha (Igα) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5. In certain other aspects of this embodiment, the signaling domain is the partial membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6. The sequence variants retain the signaling activity of the signaling domain upon which they are based.

Methods of Detecting an Agent

[0019] In a third embodiment, the invention is directed to methods of detecting a target agent in a sample. The method comprises (a) contacting a sample with an antibody having binding specificity for a target agent and with a biosensor cell, and (b) assaying the biosensor cell for cellular activation, wherein the biosensor cell stably expresses a chimeric fusion protein, and wherein the chimeric fusion protein comprises an Fcγ receptor (FcγR) antibody-binding domain, a transmembrane domain and a signaling domain.

[0020] The fusion proteins have the ability to recognize and bind the Fc region of an antibody via their antibody-binding domain. The fusion proteins have the ability to activate an intracellular signaling cascade in the cell expressing the fusion protein. In certain aspects, the intracellular signaling cascade results in the release of Ca^{2+} within the cell. The chimeric fusion protein is stably expressed on the surface of the cell as an integral membrane protein.

[0021] In certain aspects of this embodiment, the sample is an air sample, a liquid sample, a dry sample, vegetable sample, or a biological sample. In preferred aspects, when the sample is an air sample it is selected from the group consisting of an aerosol, an atmospheric sample, a ventilator discharge, and an engine exhaust. In preferred aspects, when the sample is a liquid sample it is selected from the group consisting of a food, a drink, a water sample, a pharmaceutical formulation, and a personal care product. In preferred aspects, when the sample is a dry sample it is selected from the group consisting of food, soil, a pharmaceutical formulation, solubilized swab samples, and a personal care product. In preferred aspects, when the sample is a vegetable sample it is selected from the group consisting of leaves, fruit, nuts, seeds, flowers, and plant tissue. In preferred aspects, when the sample is a biological sample it is selected from the group consisting of blood, serum, sweat, urine, cerebrospinal fluid, mucus, semen, stool, bronchoalveolar lavage fluid, and tissue.

[0022] In certain aspects of this embodiment, the agent is an environmental toxin, pollutant, drug, or a biologic agent. In preferred aspects, when the agent is a biologic agent it is selected from the group consisting of a bio-warfare agent, an allergen, a parasitic antigen, a fungal antigen, a viral antigen, a bacterial antigen, a cellular antigen, and an antibody.

[0023] In certain aspects of this embodiment, the biosensor cell is a B cell, a T cell, a monocyte, a macrophage, a HEK293 cell, a CHO cell, P815, K562, or a Cos-1 cell, each of which stably expresses the chimeric fusion protein.

[0024] In certain aspects of this embodiment, the cellular activation is an increase in intracellular Ca^{2+} levels.

[0025] In certain aspects of this embodiment, the FcγR antibody-binding domain is the FcγRI antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3. In certain other aspects of this embodiment, the FcγR antibody-binding domain is the FcγRIII antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4. The sequence variants retain the antibody-binding activity of the antibody-binding domain upon which they are based.

[0026] In certain aspects of this embodiment, the signaling domain is the immunoglobulin alpha (Igα) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5. In certain other aspects of this embodiment, the signaling domain is the partial membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6. The sequence variants retain the signaling activity of the signaling domain upon which they are based.

[0027] The foregoing has outlined rather broadly the features and technical advantages of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described herein, which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that any conception and specific embodiment disclosed herein may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims. The novel features which are believed to be characteristic of the invention, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that any description, figure, example, etc. is provided for the purpose of illustration and description only and is by no means intended to define the limits the invention.

BRIEF DESCRIPTION OF DRAWINGS

[0028] FIG. **1**. Cartoon representation of constructs encoding fusion proteins of the invention. Construct A encodes the FcγRI/Igα fusion protein and construct B encodes the FcγRIII/Igα fusion protein. These fusion proteins are identical except the FcγRI/Igα fusion protein has the FcγRI antibody-binding and transmembrane domains, while the FcγRIII/Igα fusion protein has the FcγRIII antibody-binding and transmembrane domain. Construct C encodes the FcγRI/membrane Ig fusion proteins and construct D encodes the FcγRIII/membrane Ig fusion proteins. These fusion proteins are identical except the FcγRI/membrane Ig fusion protein has the FcγRI antibody-binding domain, while the FcγRIII/membrane Ig fusion protein has the FcγRIII antibody-binding domain. The membrane Ig portion of these fusion proteins comprises the hinge-CH2-CH3-transmembrane-intracellular domains from a membrane-associated antibody. Constructs E and F also encode the FcγRI/Igα and FcγRIII/Igα fusion proteins, respectively, but these constructs further encode the 2A peptide and FcRγ-chain.

[0029] FIG. **2**. Sequence of murine FcγRI (SEQ ID NO:15). The extracellular, antibody-binding region is at N terminus; the shaded sequence is the predicted transmembrane region; the intracellular region is at the C terminus.

[0030] FIG. **3**. Sequence of the murine FcγRIII (SEQ ID NO:16). The extracellular, antibody-binding region at N terminus; the shaded sequence is the predicted transmembrane region; the intracellular region is at the C terminus.

[0031] FIG. **4**. Sequence of murine immunoglobulin alpha (Igα; CD79A; SEQ ID NO:17). The extracellular region at N terminus; the shaded sequence is the predicted transmembrane region; the intracellular region is at the C terminus.

[0032] FIG. **5**. Sequence of the FcγRI/Igα fusion protein (fusion protein A; SEQ ID NOs:7 and 8). The antibody-binding domain and transmembrane domain (shaded sequence) of FcγRI are fused to the Igα signaling domain (underlined) in the 5' to 3' direction.

[0033] FIG. **6**. Sequence of the FcγRIII/Igα fusion protein (fusion protein B; SEQ ID NOs:9 and 10). The antibody-binding domain and transmembrane domain (shaded sequence) of FcγRIII are fused to the Igα signaling domain (underlined) in the 5' to 3' direction.

[0034] FIG. **7**. Partial sequence of a human IgG2 membrane Ig (SEQ ID NO:18). Hinge region, followed by CH_2 domain (underlined), CH_3 domain (double underlined), transmembrane domain, and intracellular domain (underlined) in the 5' to 3' direction.

[0035] FIG. **8**. Sequence of FcγRI/membrane Ig fusion protein (fusion protein C; SEQ ID NO:22). The antibody-binding domain of FcγRI is fused to the partial human IgG2 membrane Ig domain (underlined) in the 5' to 3' direction.

[0036] FIG. **9**. Sequence of FcγRIII/membrane Ig fusion protein (fusion protein D; SEQ ID NO:23). The antibody-binding domain of FcγRIII is fused to the partial human IgG2 membrane Ig domain (underlined) in the 5' to 3' direction.

[0037] FIG. **10**. Sequence of the 2A peptide (SEQ ID NO:24).

[0038] FIG. **11**. Sequence of FcRγ-chain (SEQ ID NO:25).

DETAILED DESCRIPTION

I. Definitions

[0039] Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found, for example, in Benjamin Lewin, Genes VII, published by Oxford University Press, 2000 (ISBN 019879276X); Kendrew et al. (eds.); The Encyclopedia of Molecular Biology, published by Blackwell Publishers, 1994 (ISBN 0632021829); and Robert A. Meyers (ed.), Molecular Biology and Biotechnology: a Comprehensive Desk Reference, published by Wiley, John & Sons, Inc., 1995 (ISBN 0471186341); and other similar technical references.

[0040] As used herein, "a" or "an" may mean one or more. As used herein when used in conjunction with the word "comprising," the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more. Furthermore, unless otherwise required by context, singular terms include pluralities and plural terms include the singular.

[0041] As used herein, "about" refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term "about" generally refers to a range of numerical values (e.g., +/−5-10% of the recited value) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). In some instances, the term "about" may include numerical values that are rounded to the nearest significant figure.

II. The Present Invention

[0042] As briefly summarized above, the present invention is directed to a universal antibody-mediated biosensor comprising a cell line stably expressing a novel chimeric fusion protein on its surface. The fusion proteins can bind antibodies without regard to their antigenic-binding specificity, and cells expressing the fusion proteins on their surface can be activated upon cross-linking of the bound antibodies by their cognate antigen. Because the fusion proteins bind to the Fc region of any antibody, they can serve as a universal pathway between extracellular signaling and intracellular activation. The biosensor can be used to detect the presence

of selected antigens in a sample by contacting the sample with (i) the biosensor cells and (ii) antibodies having binding specificity for the antigen. Once added, the antibodies are bound by the chimeric fusion proteins, via binding of the Fc region of the antibody by the antibody-binding domain of the fusion proteins. Antigen recognition and binding by the antibodies leads to antibody cross-linking, which is promulgated as a signal through the fusion protein into the biosensor cell, where the intracellular signaling domain of the fusion protein triggers cellular activation. Such activation can then be assayed and, if desired, quantified. Based on the level of cellular activation, conclusions can be drawn about the presence of antigen in the sample. Very broadly speaking, when cellular activation occurs using the biosensor cells of the invention, the antigen is deemed to be present in the sample.

[0043] While the universal antibody-mediated biosensor of the invention comprises a cell line stably expressing a novel chimeric fusion protein as an integral membrane protein, the individual elements of the biosensor cells include (i) an extracellular, antibody-binding domain of the fusion protein, (ii) a transmembrane domain of the fusion protein, (iii) an intracellular signaling domain of the fusion protein, and (iv) a cell line that stably expresses the fusion protein on its surface as an integral membrane protein. These elements are discussed in the following paragraphs.

Antibody-Binding Domain

[0044] The chimeric fusion proteins of the invention comprise, at their amino termini, an extracellular, antibody-binding domain. Exemplary antibody-binding domains include, but are not limited to, the antibody-binding domain of an Fcγ receptor (FcγR), such as FcγRI or FcγRIII Because different FcγR subtypes vary in their affinity for different antibody isotypes (constant regions), biosensors of the invention can vary based on the identity of the antibody-binding domain in the fusion protein. For example, the murine FcγRI antibody-binding domain has a high-affinity for the constant regions of murine IgG2a, as well as human IgG1, IgG3 and IgG4 immunoglobulins. The antibody-binding domain of murine FcγRI binds the murine IgG2a isotype with very high affinity ($>10^8$ M^{-1}) [2]. Cross-species binding studies have demonstrated that human FcγRI can bind commercially available human mAbs, with IgG1 and IgG3 binding more strongly than IgG4 [3]. The murine FcγRIII antibody-binding domain has a lower affinity (3×10^4 to 6×10^5 M^{-1}) for the constant regions of murine IgG1, IgG2a, IgG2b, and for human IgG1, IgG2 and IgG4 immunoglobulins [3], but can also be used in the fusion proteins of the invention. Between FcγRI and FcγRIII, all mouse and human Igs (except for murine IgG3) can bind to one of these two Fc receptors. Additionally, polyclonal antibodies can bind to these FcγRs [4].

[0045] The skilled artisan will thus understand that depending on the particular agent being assayed and the particular experimental conditions, the antibody-binding domains of different Fcγ receptors will be preferable for different conditions. The present invention is thus generally directed to novel chimeric fusion proteins comprising the antibody-binding domains of the Fcγ receptors defined herein, as well as cell lines that stably express these fusion proteins.

[0046] In a first aspect, the antibody-binding domain of the Fcγ receptor used in the chimeric fusion proteins includes both the antibody-binding domain and the transmembrane domain of an Fcγ receptor. Suitable Fcγ receptor antibody-binding/transmembrane domains include, but are not limited to, the antibody-binding/transmembrane domain of mouse FcγRI set forth in SEQ ID NO:1 (where amino acids 287-319 correspond to the predicted transmembrane domain) and the antibody-binding/transmembrane domain of mouse FcγRIII set forth in SEQ ID NO:2 (where amino acids 208-233 correspond to the predicted transmembrane domain).

[0047] In a second aspect, the antibody-binding domain of the Fcγ receptor used in the chimeric fusion proteins lacks a transmembrane domain, e.g., where the transmembrane domain of the fusion protein is from an alternative source. Suitable Fcγ receptor antibody-binding domains lacking a transmembrane domain that may be used in the chimeric fusion proteins include, but are not limited to, the antibody-binding domain of mouse FcγRI set forth in SEQ ID NO:3 and the antibody-binding domain of mouse FcγRIII set forth in SEQ ID NO:4.

Signaling Domain

[0048] The chimeric fusion proteins of the invention comprise, at their carboxy termini, an intracellular signaling domain. Suitable signaling domains include those known to induce cellular activation in other contexts. For example, B cells innately transduce B cell receptor (BCR) binding of an antigen through formation of a complex with the transmembrane protein CD79. CD79 is composed of two distinct chains, immunoglobulin-alpha (Igα) and immunoglobulin-beta (Igβ), that form the heterodimer on the surface of B cells. Igα and Igβ have an extracellular domain, a single transmembrane domain, and a cytoplasmic signaling domain. It has been demonstrated that fusion proteins with the extracellular and transmembrane regions of the CD8 protein fused to either the Igα or Igβ intracellular signaling regions have signaling capacity [5]. Other studies demonstrate that protein kinases are more potent activators of the CD8/Igα fusion protein. The same study further demonstrated that Ca^{2+} signaling could be observed with the CD8/Igα fusion protein after CD8 cross-linking. Based on these studies, in one aspect the fusion proteins of the invention comprise an antibody-binding domain fused to the cytoplasmic signaling domain of Igα [6].

[0049] Thus, in a first aspect, signaling domains that may be used in the chimeric fusion proteins of the invention include, but are not limited to, the signaling domain of mouse Igα set forth in SEQ ID NO:5.

[0050] Since the affinity of binding between the fusion protein and antibodies can be quite variable, depending on the identity of the antibody-binding domain used in the fusion protein and the antibodies, it is important to have alternative signaling domains that can provide further nuances to the avidity of the fusion proteins for the antibodies. For example, the signaling domains may help with cross-linking and dimerization. It is thought that putting two antibody-binding domains in close proximity will increase the probability of maximal crosslinking. If antibody-binding domains are linked to a modified membrane-associated IgG molecule as the signaling domain, close proximity of two antibody-binding domains can be achieved. Thus, and in a second aspect, the signaling domain is a partial membrane Ig peptide comprising a hinge region followed by CH_2, CH_3, transmembrane and intracellular regions of an IgG antibody

(see fusion proteins C and D in FIG. **1**). In a specific example, such a signaling domain is set forth in SEQ ID NO:6. As this signaling domain includes a transmembrane region, it would be used in conjunction with antibody-binding domains lacking a transmembrane domain, such as the FcγRI antibody-binding domain set forth in SEQ ID NO:3 or the FcγRIII antibody-binding domain set forth in SEQ ID NO:4.

Chimeric Fusion Proteins

[0051] It will be apparent that by using different combinations of antibody-binding domains and signaling domains, the affinity of the fusion proteins for a particular antibody can be adjusted and the level of cellular activation can be controlled. Specific examples of chimeric fusion proteins included in the scope of the invention include those provided in Table 1. A representation of each of the six fusion proteins is shown in FIG. **1**.

TABLE 1

Fusion Protein	Source of Antibody-binding Domain	Source of Transmembrane Domain	Source of Signaling Domain	SEQ ID NO: for Nucleic Acid Sequence	SEQ ID NO: for Amino Acid Sequence
FcγRI/Igα	FcγRI	FcγRI	Igα	7	8
FcγRIII/Igα	FcγRIII	FcγRIII	Igα	9	10
FcγRI/membrane Ig	FcγRI	Membrane Ig	Membrane Ig	11	12
FcγRIII/membrane Ig	FcγRIII	Membrane Ig	Membrane Ig	13	14

[0052] The invention thus includes the FcγRI/Igα fusion protein set forth in SEQ ID NO:8, the FcγRIII/Igα fusion protein set forth in SEQ ID NO:10, the FcγRI/membrane Ig fusion protein set forth in SEQ ID NO:22, and the FcγRIII/membrane Ig fusion protein set forth in SEQ ID NO:23.

[0053] Because different antibody-binding domains can be paired with different signaling domains, it should be understood that the present invention also includes fusion proteins comprising the antibody-binding domain of FcγRI as set forth in SEQ ID NO:1 or 3, and fusion proteins comprising the antibody-binding domain of FcγRIII as set forth in SEQ ID NO:2 or 4. Similarly, the present invention includes fusion proteins comprising the signaling domain of Igα as set forth in SEQ ID NO:5, and fusion proteins comprising the signaling domain of membrane Ig as set forth in SEQ ID NO:6.

[0054] It will be readily understood by the skilled artisan that minor alterations can be made to the amino acid sequence of the fusion proteins of the invention without affecting the binding or signaling activity of the proteins. For example, minor alterations can be made to the antibody-binding domain of the fusion proteins while maintaining the binding activity of the fusion proteins. Similarly, minor alterations can be made to the signaling domain of the fusion proteins while maintaining the signaling activity of the fusion proteins. Further, minor alterations can be made to both the antibody-binding and signaling domains of the fusion proteins while maintaining the binding and signaling activity of the fusion proteins. Such minor alterations can be used to alter the affinity of the antibody-binding domain for antibodies as in some instances a particular binding affinity (e.g., low, medium or high) may be preferred. Similarly, such minor alterations can be used to alter the signaling activity of the signaling domain in a cell as in some instances a particular type or level of cellular activation (e.g., low, medium or high) may be preferred.

[0055] Thus, the present invention includes sequence variants of the fusion proteins disclosed herein having one or more amino acid insertions, deletions and/or substitutions, that also retain the binding and signaling activity of the fusion protein upon which they are based. In particular, the invention includes sequence variants having at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity over the entire length of the amino acid sequence set forth in SEQ ID NO:8, 10, 22, or 23.

[0056] The invention also includes sequence variants comprising an antibody-binding domain of FcγRI wherein the domain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity with SEQ ID NO:1 or 3 over the entire length of the amino acid sequence.

[0057] The invention also includes sequence variants comprising an antibody-binding domain of FcγRIII wherein the domain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity with SEQ ID NO:2 or 4 over the entire length of the amino acid sequence.

[0058] The invention further includes sequence variants comprising a signaling domain of Igα wherein the domain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity with SEQ ID NO:5 over the entire length of the amino acid sequence.

[0059] The invention further includes sequence variants comprising a signaling domain of membrane Ig wherein the domain has at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% sequence identity with SEQ ID NO:6 over the entire length of the amino acid sequence.

Polynucleotide

[0060] The invention includes polynucleotides comprising nucleotide sequences encoding each the fusion proteins provided herein, as well as complementary strands thereof. The invention also includes cloning vectors comprising the polynucleotides, and host cells comprising either the polynucleotides or the expression vectors. Such host cells may be mammalian or non-mammalian cells, including, but not limited to, *E. coli*, and insect cells. The invention further includes methods of producing the fusion proteins defined herein, comprising culturing the host cells under conditions promoting expression of the fusion proteins encoded by the polynucleotides and expression vectors, and recovering the fusion proteins from the cells or cell cultures.

Constructs Encoding the Fusion Proteins

[0061] Sequences for the murine FcγRI (SEQ ID NO:15) and FcγRIII (SEQ ID NO:16) have been cloned and confirmed. Nucleic acid constructs encoding the chimeric fusion proteins may be generated for expression of the fusion proteins by engineering sequence encoding the antibody-binding, transmembrane, and signaling domains into an expression vector. For example, antibody-binding and transmembrane domains of the FcγR receptors may be fused in frame with sequence encoding a signaling domain, for example via "SOEing" using PCR [15]. To complete the construct in the cases where the FcR-γ chain is needed (discussed below), the C-terminus of the signaling domain and the N-terminus of the FcR-γ chain would be attached by PCR to sequence encoding the 2A peptide. For construction of the FcγR-membrane Ig constructs, restriction sites at the C-terminus of the FcγR sequences may be used to link to the Ig constant regions that contain compatible restriction sites at the N-terminus.

[0062] Polynucleotide constructs encoding the fusion proteins of the invention may be transiently or stably expressed in a selected cell line. The constructs can be transfected into a selected cell line using techniques well known to the skilled artisan including, but not limited to, standard transfection kits (e.g., Fugene® or Neon™ system electroporation) or retroviral transduction methods.

[0063] Expression of the fusion protein on the cell surface can also be confirmed using standard techniques well known to the skilled artisan, including staining with fluorescently-labeled antibodies for either FcγRI or FcγRIII, and analysis using flow cytometry.

[0064] Suitable expression vectors include, but are not limited to, plasmids pcDNA 3.1+ or − (hygro), pcDNA 3.1+ or − (neomycin), pdisplay (Puro), pIRES (neomycin), pIRES Puro2, pQCXIP (puro), pQCXIN (neomycin), and pQCXIH (hygro).

[0065] Because the expression vectors can encode the fusion proteins and the FcRγ chain together in one continuous sequence, the coding sequence can be under the control of a single promoter. Alternatively, the expression vectors can encode the fusion proteins and the FcRγ chain under the control of separate promoters.

Cells

[0066] Cell lines that may be used to express the fusion proteins of the present invention, and thus serve as the biosensor cells of the invention, are limited only in that they can stably express the fusion proteins on the surface of the cell as an integral membrane protein and that activation of the signaling domain can be detected. Suitable cell lines include, but are not limited to, lymphocytes and non-lymphoid cells.

[0067] The invention thus includes cells that stably express one or more of the fusion proteins defined herein on their surface. In some instances these cells are termed "biosensor cells" herein. In particular embodiments, the invention includes biosensor cells stably expressing on their surface more or more of the FcγRI/Igα fusion protein set forth in SEQ ID NO:8, the FcγRIII/Igα fusion protein set forth in SEQ ID NO:10, the FcγRI/membrane Ig fusion protein set forth in SEQ ID NO:22, the FcγRIII/membrane Ig fusion protein as set forth in SEQ ID NO:23, and a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:8, 10, 22, or 23. The cells used to prepare the biosensor cells may be any of the cells defined herein.

Lymphocytes

[0068] Lymphocytes expressing the CD8/Igα fusion protein have been used to demonstrate that cross-linking with an anti-CD8 antibody stimulates the release of intracellular Ca^{2+} and phosphorylation of Igα in both B and T cells [5,6,10]. Mouse and human B cell lines, which normally signal using the endogenous Igα/Igβ pathway, are particularly useful in expression of the fusion proteins described herein. Suitable B cell lines that may be used in the production of the biosensor cells include, but are not limited to, Ramos, Raji, IIAI.6 and C604 cells lines. Other suitable B cell lines include A20 and LK 35.2.

[0069] Proper expression of constructs encoding any of the fusion proteins of the invention can be confirmed using fluorescently-labeled antibodies and flow cytometry. Cells may be cloned using limiting dilution, and selected based on their flow cytometry expression profiles for subsequent study.

[0070] Some B cell lines express the FcγIIb inhibitory receptor, though others, such as the Ramos and IIA1.6 B cells, do not express the protein on their cell surface [11,12]. If the inhibitory activity of the FcγIIb receptor is problematic in a particular cell line, siRNA constructs can be used to stably inhibit expression of FcγRIIb in the cells [13] or CRISPR/Cas9 technology can be used to knockout the FcγRIIb gene in these cell lines [14].

[0071] T cells expressing CD8 fused to an Igα signaling domain release Ca^{2+} after cross-linking with anti CD8 antibodies [5], which indicates that the signaling machinery in T cells can also operate through the Igα. Therefore, the fusion proteins of the invention can also be expressed in in mouse or human T cells. Suitable T cell lines that may be used in the production of the biosensor cells include, but are not limited to, Jurkat, DO-11.10 and BW5147 cell lines. Monocytes (e.g., the U937 cell line), macrophages, myoblasts (e.g., the KG! cell line), and erythroblasts (e.g., the K562 cell line) expressing the fusion proteins may also be used as biosensor cells. Since these cells do not naturally express FcγRs, there will not be any inhibition caused by the inhibitory FcγRIIb. Proper expression can also be determined using fluorescently-labeled mAbs for the FcγR using flow cytometry.

Non-Lymphoid Cells

[0072] There are a large number of established and well-characterized non-lymphoid cell lines commonly used in assays involving cell surface expression of selected proteins, such as HEK293, CHO, P815, K562, and Cos-1 cells. These cell lines are routinely used to express foreign proteins because it is easy to establish stable expression in these cells, and they have well defined growth characteristics. However, non-lymphoid cells fail to express the FcR gamma chain (FcRγ-chain) which is a secondary protein expressed in Fcγ receptor expressing cells. The FcRγ-chain is required for Fcγ receptor signaling [7]. Although non-lymphoid cells do not express the FcR-γ chain, such cells can still serve as excellent candidates for fusion protein expression and be used as biosensor cells of the invention if they are engineered to co-express the FcR-γ chain.

[0073] Non-lymphoid cells can be engineered to express the FcR-γ chain through techniques well known to the skilled artisan. One convenient technique is to include the gene encoding the FcR-γ chain on the constructs encoding the fusion proteins of the invention, where the two coding sequences are under the control of the same or separate promoters. Another convenient technique is to place expression of the fusion protein and the FcR-γ chain under the control of the same promoter. In particular, two additional elements can be added to the constructs encoding the fusion proteins. The first element is the FcR-γ chain itself (SEQ ID NO:25). As the FcR-γ chain needs to be able to adopt the correct confirmation in the cell membrane, it cannot be a part of the fusion protein. The second element addresses this problem as it is an engineered 2A peptide, a readily cleavable peptide first described in foot-and-mouth disease virus [8]. A variant of the original 2A peptide found in the porcine Teschovirus that cleaves more efficiently in a wide variety of cells tested [9] is used herein (SEQ ID NO:24). The FcR-γ chain can thus be provided to non-lymphoid cells by engineering constructs encoding the fusion proteins of the invention to include the 2A peptide sequence C-terminal of the signaling domain, following by the FcR-γ chain (see constructs E and F in FIG. **1**).

[0074] Non-lymphoid cell lines that may be used in the production of the biosensor cells of the invention include, but are not limited to, HEK293, CHO, P815, K562, and Cos-1 cell lines.

Antibodies

[0075] As will be apparent from the discussion herein, the identity of an antibody that can be used with the biosensor cells of the invention in the detection of target agents is only limited in that (i) the antibody can be bound by the fusion proteins of the invention and (ii) the antibody can bind to a target agent. Once a particular target agent is selected for detection, one can readily determine whether an antibody with binding specificity for the agent is commercially available. If it is not, an antibody with the needed binding specificity can be generated using routine methods.

[0076] As will be apparent, the antibodies can be monoclonal or polyclonal. The antibodies can be recombinant. Suitable antibodies also include fragments that retain the binding specificity of the antibody from which they are derived, such as, but are not limited to, Fab fragments, $F(ab')_2$ fragments, and single chain Fv (scFv) antibodies.

[0077] The antibodies can be conjugated to detectable labels including, but not limited to, an enzyme (e.g., peroxidase, alkaline phosphatase, glucose oxidase), a metal (e.g., gold for electron microscopy applications), a fluorescent marker (e.g., for immunofluorescence and flow cytometry applications, including CYE dyes, fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine), a fluorescence-emitting metals (e.g., ^{152}Eu), a radioactive marker (e.g., radioisotopes for diagnostic purposes, including ^{3}H, ^{131}I, ^{35}S, ^{14}C, and ^{125}I), a chemiluminescent marker (e.g., luminol, luciferin, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester), and a protein tag (e.g., biotin, phycobiliprotein, c-Myc, HA, VSV-G, HSV, FLAG, V5, or HIS).

[0078] The antibodies can also be conjugated to or coated on moieties that can be used for the isolation/separation of the antibodies from a sample after they are exposed to a target agent. Such moieties include, but are not limited to, magnetic beads, agarose beads, and polystyrene beads of various diameters.

Samples

[0079] The samples that may be screened for the presence of a target agent are similarly limited only in that they permit binding of a target agent present in the sample by an antibody. Suitable samples include, but are not limited to, air samples, liquid samples, dry samples, vegetable samples, and biological samples. Suitable air samples include, but are not limited to, an aerosol, an atmospheric sample, a ventilator discharge, and an engine exhaust. Suitable liquid samples include, but are not limited to, a food, a drink, a water sample, a pharmaceutical formulation, and a personal care product. Suitable dry samples include, but are not limited to, a food, soil, a pharmaceutical formulation, solubilized swab samples, and a personal care product. Suitable vegetable samples include, but are not limited to, leaves, fruit, nuts, seeds, flowers, and plant tissue. Suitable biological samples include, but are not limited to, blood, serum, sweat, urine, cerebrospinal fluid, mucus, semen, stool, bronchoalveolar lavage fluid, and tissue.

Agents

[0080] The biosensors of the present invention can be used to detect a wide variety of different target agents. As will be apparent to the skilled artisan, the only limitation on the target agent is that binding of the agent by an antibody must be possible. Target agents include those of biologic origin, such as, but not limited to, bio-warfare agents, allergens, parasitic antigens, fungal antigens, viral antigens, bacterial antigens, cellular antigens, and antibodies. Exemplary bio-warfare agents include, but are not limited to, ricin, anthrax spores, botulinum toxin, *Clostridium perfringens* toxin, saxitoxin, and trichothecene mycotoxins. Exemplary allergens include, but are not limited to, tree nuts, peanuts, and animal dander. Exemplary cellular antigens include, but are not limited to, antigens associated with a disease or condition in a subject, such as a human, primate or other mammal, such as, but not limited to, livestock or a companion animal, such a dog or cat. Target agents also include plant and crop agents, aquatic pathogens or disease causing agents, drugs and other chemical compounds, and molecules found in the environment such as, but not limited to, toxins and pollutants.

Detecting Cellular Activation

[0081] The biosensor cells of the invention can be used in assays to detect, and in some cases quantify, a target agent in a sample. As described above, upon binding of the agent by antibodies, and antibody binding by the fusion proteins expressed by the biosensor cells, cross-linking occurs on the surface of the cell and the signaling domain of the fusion protein transmits the binding as activation signal within the cell. As an example, when an antigen-containing sample interacts with the antibody on the extracellular surface of the biosensor, an intracellular signaling cascade is activated.

[0082] In vivo, antigen receptors (membrane-bound Ig) of B cell are non-covalently associated with a disulfide-linked transmembrane heterodimer of Igα and Igβ proteins [16]. After cross-linking of the B cell receptor upon antigen binding, several proteins are phosphorylated on tyrosine

residues by protein kinases, including Igα and Igβ [17,18]. One of the first downstream events after phosphorylation is Ca^{2+} release from intracellular stores followed by an influx of exogenous Ca^{2+} through Ca^{2+} channels in the cell membrane [19]. Such a change in intracellular calcium levels is one type of cellular activation contemplated herein that can be assayed. Changes in intracellular Ca^{2+} levels can be readily detected in cells by various chemical fluorescent compounds that can be efficiently loaded into cells.

[0083] Owing to the importance of Ca^{2+} in biology, numerous techniques for analyzing cellular Ca^{2+} activity have been established, which may be used in assaying cellular activation in the biosensor cells of the invention. A popular method is the use of fluorescent chemical Ca^{2+} indicator probes because their signal is quite large for a given change in intracellular Ca^{2+} concentration compared with other indicator types [20]. For example, cellular activation may be monitored and assayed in the biosensor cells of the invention by loading the biosensor cells with Fluo-4AM, a methyl ester of Fluo-4, which is a sensitive non-ratiometric compound used to measure Ca^{2+} concentrations inside living cells [21]. Most chemical fluorescent indicators are not membrane permeant. However, the methyl ester form of Fluo-4 can passively diffuse across the plasma membrane, and once inside the cell, intracellular esterases cleave the methyl ester group off of the probe leading to a membrane-impermeant probe. Another probe alternative for use with the cells of the invention is Fura 2, which is a UV-excited Ca^{2+} indicator that allows ratiometric Ca^{2+} measurement. Upon binding of the target agent by antibodies, a signal is transduced to the signaling domain of the biosensor cells which triggers the noted changes in Ca^{2+} levels which can, in turn, be assayed and/or quantified using a spectrometer to measure changes in cellular fluorescence.

[0084] Also, Ca^{2+} binding photoproteins can generate bioluminescence, which is the production of light from biological processes. Several Ca^{2+}-binding photoproteins (e.g., aequorin, obelin, mitrocomin, and clytin) have been used to measure intracellular Ca^{2+} concentration [24], each of which may be used with the biosensor cells of the invention to assay changes in cellular activation. The luminescence of these photoproteins upon Ca^{2+} binding is in the visible spectrum, which offers simplicity in terms of instrumentation or detection, and they are not affected by photobleaching.

[0085] It should be noted that while target agent binding (i.e., cellular activation) is exemplified herein based on measuring changes in Ca^{2+} levels in cells, other means can be used to assay for changes in target agent binding, including luminescence using photoproteins.

III. Examples

Example 1: Production and Expression of Constructs Encoding Fusion Proteins

[0086] Commercially available murine FcγRI and Igα cDNAs were obtained. PCR primers providing overlapping sequence of the two genes were used to sew the two sequences together, resulting in a FcγRI/Igα in frame fusion that was confirmed by sequence analysis. Alternatively, the antibody-binding and/or transmembrane domains of FcγRI are amplified with primers from cDNA encoding the receptor, and the intracellular signaling domain of Igα is similarly amplified.

[0087] Amplified fragments are gel-purified. Amplification products (e.g., FcγRI and Igα) are mixed together and denatured by boiling for 5 minutes and placed at room temperature for 30 minutes prior to amplification to create a sequence encoding the full-length fusion proteins. These sequences are gel-purified and cloned into an expression vector containing a suitable promoter (e.g., a plasmid for expressing cDNA in mammalian cells), transfected into selected cell lines using Lipofectamine LX or other suitable transfection reagent, and selected using a suitable selectable marker. Individual clones are sequenced to confirm that the proper fusion protein is being expressed. Proper surface expression of the fusion proteins is determined using labeled anti-Fc receptor antibodies (e.g., anti-CD64 antibody staining) and flow cytometry. An exemplary construct encoding the FcγRI-Igα fusion protein is one encoding the antibody-binding and transmembrane domains of FcγRI (SEQ ID NO:19) and sequence encoding the Igα signaling domain (SEQ ID NO:21) in the 5' to 3' direction.

[0088] Another exemplary construct encoding the FcγRIII-Igα fusion protein is one encoding the antibody-binding and transmembrane domains of FcγRIII (SEQ ID NO:20) and sequence encoding the Igα intracellular signaling domain (SEQ ID NO:21) in the 5' to 3' direction. A commercially obtained murine FcγRIII cDNA and Igα cDNA PCR primers providing overlaps of the two genes were used to sew the two sequences together. The product resulted in a FcγRIII/Igα in frame fusion that was confirmed by sequence analysis.

[0089] An alternative approach was used to put the FcγR receptors together with the 2A peptide and FcR-γ chain to produce the constructs shown as E and F in FIG. **1**. PCR amplification with overlap extension was used to fuse 2A sequence with the FcR-γ chain and restriction sites were placed at the ends of the 2A and FcRγ-chain cDNAs. Using PCR both the FcγRI and FcγRIII cDNAs were amplified with primers containing restriction sites on their ends that could be used to link the FcγRs to the 2A site and for subsequent cloning into an expression vector. DNA was digested with restriction endonucleases and the products eluted from a gel. The fragments were ligated and cloned into an expression vector and they were sequenced.

[0090] The following examples provide some of the instances in which the universal biosensor cells of the invention can be used in practice. These examples are only a small subset of possible ways in which the biosensor can be utilized. The biosensor can be easily adapted for single or multi-well assay formats. It should be noted that the combination of cell line, construct, and Ca^{2+} indicator can vary depending on the agent, antigen or pathogen being studied and availability of antibody isotypes, and may need to be empirically determined.

Example 2: Detection of a Plant Virus from Leaf or Root Samples

[0091] Plant pathogens, whether viral or bacterial, are of great concern as infection and resulting loss of food and fodder crops impact the economy and food security. Therefore it is important to have assays in place that can detect routine as well as emerging plant pathogens to aid in crop management and monitoring of imported crops. The testing of domestic crops at an agricultural farm is described.

[0092] Leaf or root samples are collected from a suspected plant. The samples are thoroughly ground up to release any

virus particles contained within the sample. Then magnetic beads coated with a commercially available virus-specific antibody are mixed with the sample matrix to capture the virus particles (i.e., target agents). The beads can be magnetically separated from the plant sample, thoroughly washed, and incubated with universal biosensor cells of the invention.

[0093] For example, Ramos B cells expressing either the FcγRI/Igα or FcγRIII/Igα fusion protein from a construct also encoding the FcR-γ chain (i.e., constructs E and F of FIG. **1**) may be used. Selected biosensor cells are grown to a high density (approximately 10^6 cells/mL) and the growth media is replaced with phenol red-free osmotically-balanced salt solution (i.e., HBSS, PBS). The cells are loaded for approximately 30-60 minutes in a Fluo-4 AM solution (approximately 2-9 μM) in the presence of probenecid (approximately 1-2.5 mM). Probenecid is used to minimize indicator leaking from cells. Cells are thoroughly washed to remove residual Ca^{2+} indicator. About 1-5×10^6 Fluo-4 AM-loaded cells in a small volume of HBSS with probenecid are transferred to multiple wells of a 96-well plate with dark sides. The plate containing the cells is then inserted into a fluorescence plate reader.

[0094] Several wells containing loaded cells are optically measured at 535 nm for a short period of time to establish baseline background fluorescence levels. To ensure that the cells are loaded with Fluo-4 AM, into those wells, pharmacological compounds (i.e., ATP at approximately 100-200 μM, carbachol at approximately 30-60 μM, or ionomycin at approximately 0.1-2 μM) are added to stimulate an increase in intracellular Ca^{2+} levels. Other controls, such as the use of FcγR antibodies with a cross-linking secondary antibody, are used to confirm indicator loading as well.

[0095] After confirming Fluo-4 loading, wells containing loaded cells are incubated with a commercially available virus-specific antibody (of an isotype compatible with the construct used and ideally different from the one used for the capture beads) for approximately 30-60 minutes. Then a dilution series of the virus-coated capture beads is added to the cells and changes in fluorescence is measured over a period of several minutes. Cells are also tested with both positive controls (addition of a defined virus-containing solution) and negative controls (addition of a similar solution without virus, or addition of a solution of an irrelevant antigen that does not cross-react) to ensure specificity of the signal. Increases in cellular fluorescence indicate that the selected virus is present in the sample. In some instances, the amount of change in cellular fluorescence is correlated with the amount of selected virus present in the sample, thereby permitting quantification of the amount of the virus in the sample.

Example 3: Detection of *Salmonella* from Swab Samples

[0096] *Salmonella* spp. is one of the most common food-borne pathogens and can cause serious, sometimes fatal, *salmonellosis* disease in young children, the elderly, and others with weakened immune systems. As *Salmonella* contamination arises from contact with tainted animal or human feces, a wide-range of foods can become contaminated from eggs and meats to produce and even water. Current *Salmonella* detection methods involve PCR or bacterial culture, which is time consuming and requires specialized knowledge. A simple, rapid detection assay is hence desirable for food quality monitoring to prevent outbreaks and product recalls. Testing for *Salmonella* in a chicken egg processing facility is described.

[0097] Swab samples are taken from work surfaces within the facility and exterior eggshell surfaces. The swabs are then soaked in a biocompatible solution to extract the *Salmonella* into a sample matrix that can be directly tested with the universal biosensors. In this example, C604 B cells expressing the FcγRI/membrane Ig or FcγRIII/membrane Ig fusion proteins (see constructs C and D of FIG. **1**) are used as the biosensor cells of the invention. C604 cells, being B cells, will have the endogenous Igα and Igβ to provide signaling capabilities.

[0098] The C604 cells are grown to a high density (approximately 10^6 cells/mL) and media is replaced with a phenol red-free HBSS. The cells are loaded for approximately 30-60 minutes in a Fluo-4 AM solution (approximately 1-5 μM) in the presence of probenecid (approximately 1-2.5 mM). Cells are thoroughly washed to remove residual Ca^{2+} indicator. Between 1-5×10^6 Fluo-4 loaded cells in a small volume of HBSS with probenecid are transferred to multiple wells of a 96-well plate. The plate is inserted into a fluorescence plate reader and baseline background fluorescence is established. Into a subset of cell-containing wells, anti-mouse IgM (at approximately 5-7 ng/μL) is added to stimulate a Ca^{2+} response as a positive control. Other controls are used to confirm loading such as the use of anti-FcγRI antibodies with a secondary cross-linker antibody.

[0099] Commercially available anti-*Salmonella* antibody (of an isotype compatible with FcγRI or FcγRIII) is incubated with the cells for a period of 30-60 minutes. Then a dilution series of the *Salmonella*-containing sample is added to the cells and changes in fluorescence is measured over a period of 1-2 minutes. Cells are also tested with both positive controls and negative controls to ensure specificity of the signal. Increases in cellular fluorescence indicate the presence of *Salmonella* in the sample. In some instances, the amount of change in cellular fluorescence is correlated with the amount of *Salmonella* present in the sample, thereby permitting quantification of the amount of the *Salmonella* in the sample.

Example 4: Detection of *Listeria* from Food Samples

[0100] *Listeria* (i.e., *L. monocytogenes*) is a food-borne pathogen that is the causative agent of listeriosis, a serious bacterial disease with an approximate 20% fatality rate and is most dangerous to pregnant women, infants, and those with weakened immune systems. *Listeria* can contaminate raw meats, produce, and dairy products, and prepared foods. Hence the ability to detect the bacteria and monitor for its presence is desirable in order to prevent pathogen outbreaks and product recalls. The use of the universal biosensor cells for the detection of *Listeria* in a meat processing plant that produces ready-to-eat foods (i.e., deli meats and hot dogs) is described.

[0101] As similarly described in Example 3, the work surfaces and equipment of the plant is swabbed before, during, and after meat processing to monitor for potential contamination of the products and to assess the effectiveness of decontamination procedures. Additionally, samples of processed meats may be tested. The samples are homogenized in PBS and mixed with microscopic magnetic beads

that are coated with a commercially available *Listeria*-specific antibody. The beads bind any *Listeria* present in the sample and are magnetically separated from the sample, thoroughly washed, and added to prepared universal biosensors.

[0102] COS-1 cells stably expressing either FcγRI/Igα or FcγRIII/Igα fusion proteins along with the FcR-γ chain and the bioluminescent photoprotein aequorin are used as the biosensor cells and are grown to a high density (approximately 10^6 cells/mL). The cells are incubated with approximately 2-8 μM coelenterazine (a necessary substrate of aequorin) over a period of 5-16 hours. After thorough washing to remove excess coelenterazine, cells are plated into multiple wells of a 96-well plate. Cells are then incubated with a commercially available *Listeria*-specific antibody (of an isotype compatible with the construct used and preferably a different antibody than the one used for the capture beads) for 30-60 minutes. The plate is inserted into a luminescence plate reader and a baseline background luminescence level is measured. Confirmation of successful coelenterazine loading and Ca^{2+} responsiveness is obtained by the addition of 0.15-100 μM ATP. After which, the *Listeria*-coated capture beads are added to the cells at differing dilutions and changes in luminescence signal are recorded over a period of 1-2 minutes. Increases in luminescence indicate the presence of *Listeria* in the sample. In some instances, the amount of change in luminescence is correlated with the amount of *Listeria* present in the sample, thereby permitting quantification of the amount of the *Listeria* in the sample.

Example 5: Detection of *B. anthracis* Spores from Air Samples

[0103] Anthrax is a rapid-onset and lethal disease caused by the spores of the bacterium *Bacillus anthracis*. A native soil bacterium, it can be transmitted through contact with infected meat from pasture-raised animals as well as unprocessed animal hides and wool. More recently, *B. anthracis* has been weaponized for use in biological warfare and in terrorist attacks. In this regard, reliable and rapid detection of *B. anthracis* spores is crucial. Test samples may be obtained by swabbing suspected areas or suspending suspected powders directly into PBS for analysis in solution. Alternatively, aerosol samples may be collected in a suspected area and particulates can be concentrated onto surfaces and exposed to universal biosensor cells. A suitable aerosol-sampling device (BioFlash E) is produced by PathSensors, Inc.

[0104] In this example, Jurkat cells, a human T cell line, expressing the FcγRI/Igα or FcγRIII/Igα fusion proteins and the FcRγ-chain are used as the biosensor cells. The cells are loaded with Indo-1 Ca^{2+} indicator (approximately 1-5 μM) for a period of 30-60 minutes. After thorough washing, the cells are incubated with commercially available *B. anthracis*-specific antibodies (of an isotype compatible with the construct used) and loaded into a chamber inside of the aerosol-sampling machine. Baseline background fluorescence at 405 nm is established. Confirmation of successful Ca^{2+} indicator loading is obtained by the addition of approximately 1-5 μg/mL ionomycin. Then air from the monitored area is passed through the machine and particulate matter is concentrated on an interior surface. The biosensors are then released onto the test surface to bind any *B. anthracis* spores that may be present. Changes in fluorescence signal at 405 nm are recorded over a period of 1-2 minutes. Increases in cellular fluorescence indicate the presence of anthrax in the sample. In some instances, the amount of change in cellular fluorescence is correlated with the amount of anthrax present in the sample, thereby permitting quantification of the amount of the anthrax in the sample.

[0105] While the invention has been described with reference to certain particular embodiments thereof, those skilled in the art will appreciate that various modifications may be made without departing from the spirit and scope of the invention. The scope of the appended claims is not to be limited to the specific embodiments described.

REFERENCES

[0106] All patents and publications mentioned in this specification are indicative of the level of skill of those skilled in the art to which the invention pertains. Each cited patent and publication is incorporated herein by reference in its entirety. All of the following references have been cited in this application:

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SEQUENCE LISTING

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Val Ile Thr Leu Gln Pro Pro Trp Val Ser Ile Phe Gln Lys Glu Asn
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Val Thr Leu Trp Cys Glu Gly Pro His Leu Pro Gly Asp Ser Ser Thr
    50                  55                  60

Gln Trp Phe Ile Asn Gly Thr Ala Val Gln Ile Ser Thr Pro Ser Tyr
65                  70                  75                  80

Ser Ile Pro Glu Ala Ser Phe Gln Asp Ser Gly Glu Tyr Arg Cys Gln
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Ile Gly Ser Ser Met Pro Ser Asp Pro Val Gln Leu Gln Ile His Asn
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Ser Pro Phe Pro Glu Gly Ser Leu Val Thr Leu Asn Cys Glu Thr Asn
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Leu Leu Leu Gln Arg Pro Gly Leu Gln Leu His Phe Ser Phe Tyr Val
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Gly Ser Lys Ile Leu Glu Tyr Arg Asn Thr Ser Ser Glu Tyr His Ile
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Ala Arg Ala Glu Arg Glu Asp Ala Gly Phe Tyr Trp Cys Glu Val Ala
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Thr Glu Asp Ser Ser Val Leu Lys Arg Ser Pro Glu Leu Glu Leu Gln
        275                 280                 285

Val Leu Gly Pro Gln Ser Ser Ala Pro Val Trp Phe His Ile Leu Phe
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Thr His Asn Pro Gly Asn Ser Ser Thr Gln Trp Phe His Asn Trp Ser
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Ser Ile Arg Ser Gln Val Gln Ser Ser Tyr Thr Phe Lys Ala Thr Val
                85                  90                  95

Asn Asp Ser Gly Glu Tyr Arg Cys Gln Met Glu Gln Thr Arg Leu Ser
            100                 105                 110

Asp Pro Val Asp Leu Gly Val Ile Ser Asp Trp Leu Leu Leu Gln Thr
        115                 120                 125

Pro Gln Arg Val Phe Leu Glu Gly Glu Thr Ile Thr Leu Arg Cys Pro
    130                 135                 140

Ser Trp Arg Asn Lys Leu Leu Asn Arg Ile Ser Phe Phe His Asn Glu
145                 150                 155                 160

Lys Ser Val Arg Tyr His His Tyr Lys Ser Asn Phe Ser Ile Pro Lys
                165                 170                 175

Ala Asn His Ser His Ser Gly Asp Tyr Tyr Cys Lys Gly Ser Leu Gly
            180                 185                 190

Ser Thr Gln His Gln Ser Lys Pro Val Thr Ile Thr Val Gln Asp Pro
        195                 200                 205

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Val Ile Thr Leu Gln Pro Pro Trp Val Ser Ile Phe Gln Lys Glu Asn
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Val Thr Leu Trp Cys Glu Gly Pro His Leu Pro Gly Asp Ser Ser Thr
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Gln Trp Phe Ile Asn Gly Thr Ala Val Gln Ile Ser Thr Pro Ser Tyr
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Ser Ile Pro Glu Ala Ser Phe Gln Asp Ser Gly Glu Tyr Arg Cys Gln
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Ile Gly Ser Ser Met Pro Ser Asp Pro Val Gln Leu Gln Ile His Asn
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Asp Trp Leu Leu Leu Gln Ala Ser Arg Arg Val Leu Thr Glu Gly Glu
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Pro Leu Ala Leu Arg Cys His Gly Trp Lys Asn Lys Leu Val Tyr Asn
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Val Val Phe Tyr Arg Asn Gly Lys Ser Phe Gln Phe Ser Ser Asp Ser
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Glu Val Ala Ile Leu Lys Thr Asn Leu Ser His Ser Gly Ile Tyr His
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Cys Ser Gly Thr Gly Arg His Arg Tyr Thr Ser Ala Gly Val Ser Ile
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Thr Val Lys Glu Leu Phe Thr Thr Pro Val Leu Arg Ala Ser Val Ser
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Ser Pro Phe Pro Glu Gly Ser Leu Val Thr Leu Asn Cys Glu Thr Asn
210 215 220

Leu Leu Leu Gln Arg Pro Gly Leu Gln Leu His Phe Ser Phe Tyr Val
225 230 235 240

Gly Ser Lys Ile Leu Glu Tyr Arg Asn Thr Ser Ser Glu Tyr His Ile
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Thr His Asn Pro Gly Asn Ser Ser Thr Gln Trp Phe His Asn Trp Ser
65                  70                  75                  80

Ser Ile Arg Ser Gln Val Gln Ser Ser Tyr Thr Phe Lys Ala Thr Val
                85                  90                  95

Asn Asp Ser Gly Glu Tyr Arg Cys Gln Met Glu Gln Thr Arg Leu Ser
            100                 105                 110

Asp Pro Val Asp Leu Gly Val Ile Ser Asp Trp Leu Leu Leu Gln Thr
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Pro Gln Arg Val Phe Leu Glu Gly Glu Thr Ile Thr Leu Arg Cys Pro
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Ser Trp Arg Asn Lys Leu Leu Asn Arg Ile Ser Phe Phe His Asn Glu
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Lys Ser Val Arg Tyr His His Tyr Lys Ser Asn Phe Ser Ile Pro Lys
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<213> ORGANISM: Mus musculus

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        35                  40                  45

His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Met Glu
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Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
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Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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Gln Trp Phe Ile Asn Gly Thr Ala Val Gln Ile Ser Thr Pro Ser Tyr
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Ser Ile Pro Glu Ala Ser Phe Gln Asp Ser Gly Glu Tyr Arg Cys Gln
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Ile Gly Ser Ser Met Pro Ser Asp Pro Val Gln Leu Gln Ile His Asn
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    290                 295                 300

Tyr Leu Ser Val Gly Ile Met Phe Ser Leu Asn Thr Val Leu Tyr Val
305                 310                 315                 320

Phe Arg Lys Arg Trp Gln Asn Glu Lys Phe Gly Val Asp Met Pro Asp
                325                 330                 335

Asp Tyr Glu Asp Glu Asn Leu Tyr Glu Gly Leu Asn Leu Asp Asp Cys
            340                 345                 350

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<211> LENGTH: 888
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric fusion protein construct comprising Fc-gammaRIII and Ig alpha

<400> SEQUENCE: 9

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atgactttgg acacccagat gtttcagaat gcacactctg gaagccaatg gctacttcca     60

ccactgacaa ttctgctgct gtttgctttt gcagacaggc agagtgcagc tcttccgaag    120

gctgtggtga aactggaccc cccatggatc caggtgctca aggaagacat ggtgacactg    180

atgtgcgaag ggacccacaa ccctgggaac tcttctactc agtggttcca caactggagt    240

tccatccgga gccaggtcca atccagctac acgtttaagg ccacagtcaa tgacagtgga    300

gaatatcggt gtcaaatgga gcagacccgc ctcagcgacc ctgtagatct gggagtgatt    360

tctgactggc tgctgctcca gacccctcag cgggtgtttc tggaagggga aaccatcacg    420

ctaaggtgcc ctagctggag gaacaaacta ctgaacagga tctcgttctt ccataatgaa    480

aaatccgtga ggtatcatca ctacaaaagt aatttctcta tcccaaaagc caaccacagt    540

cacagtgggg actactactg caaaggaagt ctaggaagta cacagcacca gtccaagcct    600

gtcaccatca ctgtccaaga cccagcaact acatcctcca tctctctagt ctggcaccac    660

actgctttct ccctagtgat gtgcctcctg tttgcagtgt tcaggaaacg gtggcaaaat    720

gagaagtttg gggtggacat gccagatgac tatgaagatg aaaatctcta tgagggcctg    780

aaccttgatg actgttctat gtatgaggac atctccaggg gactccaggg cacctaccag    840

gatgtgggca acctccacat tggagatgcc cagctggaaa agccatga                888
```

<210> SEQ ID NO 10
<211> LENGTH: 295
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric fusion protein construct comprising Fc-gammaRIII and Ig alpha

<400> SEQUENCE: 10

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```
Met Thr Leu Asp Thr Gln Met Phe Gln Asn Ala His Ser Gly Ser Gln
1               5                  10                  15

Trp Leu Leu Pro Pro Leu Thr Ile Leu Leu Leu Phe Ala Phe Ala Asp
            20                  25                  30

Arg Gln Ser Ala Ala Leu Pro Lys Ala Val Val Lys Leu Asp Pro Pro
        35                  40                  45

Trp Ile Gln Val Leu Lys Glu Asp Met Val Thr Leu Met Cys Glu Gly
    50                  55                  60

Thr His Asn Pro Gly Asn Ser Ser Thr Gln Trp Phe His Asn Trp Ser
65                  70                  75                  80

Ser Ile Arg Ser Gln Val Gln Ser Ser Tyr Thr Phe Lys Ala Thr Val
                85                  90                  95

Asn Asp Ser Gly Glu Tyr Arg Cys Gln Met Glu Gln Thr Arg Leu Ser
            100                 105                 110

Asp Pro Val Asp Leu Gly Val Ile Ser Asp Trp Leu Leu Leu Gln Thr
        115                 120                 125

Pro Gln Arg Val Phe Leu Glu Gly Glu Thr Ile Thr Leu Arg Cys Pro
    130                 135                 140

Ser Trp Arg Asn Lys Leu Leu Asn Arg Ile Ser Phe Phe His Asn Glu
145                 150                 155                 160

Lys Ser Val Arg Tyr His His Tyr Lys Ser Asn Phe Ser Ile Pro Lys
                165                 170                 175

Ala Asn His Ser His Ser Gly Asp Tyr Tyr Cys Lys Gly Ser Leu Gly
            180                 185                 190

Ser Thr Gln His Gln Ser Lys Pro Val Thr Ile Thr Val Gln Asp Pro
        195                 200                 205

Ala Thr Thr Ser Ser Ile Ser Leu Val Trp His His Thr Ala Phe Ser
    210                 215                 220

Leu Val Met Cys Leu Leu Phe Ala Val Phe Arg Lys Arg Trp Gln Asn
225                 230                 235                 240

Glu Lys Phe Gly Val Asp Met Pro Asp Asp Tyr Glu Asp Glu Asn Leu
                245                 250                 255

Tyr Glu Gly Leu Asn Leu Asp Asp Cys Ser Met Tyr Glu Asp Ile Ser
            260                 265                 270

Arg Gly Leu Gln Gly Thr Tyr Gln Asp Val Gly Asn Leu His Ile Gly
        275                 280                 285

Asp Ala Gln Leu Glu Lys Pro
    290                 295


<210> SEQ ID NO 11
<211> LENGTH: 1752
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric fusion protein construct comprising
      Fc-gammaRI and membrane Ig

<400> SEQUENCE: 11

atgattctta ccagctttgg agatgacatg tggcttctaa caactctgct actttgggtt     60

ccagtcggtg gggaagtggt taatgccacc aaggctgtga tcaccttgca gcctccatgg    120

gtcagtattt tccagaagga aaatgtcact ttatggtgtg aggggcctca cctgcctgga    180

gacagttcca cacaatggtt tatcaacgga acagccgttc agatctccac gcctagttat    240
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agcatcccag aggccagttt tcaggacagt ggcgaataca ggtgtcagat aggttcctca 300

atgccaagtg accctgtgca gttgcaaatc cacaatgatt ggctgctact ccaggcctcc 360

cgcagagtcc tcacagaagg agaacccctg gccttgaggt gtcacggatg gaagaataaa 420

ctggtgtaca atgtggtttt ctatagaaat ggaaaatcct ttcagttttc ttcagattcg 480

gaggtcgcca ttctgaaaac caacctgagt cacagcggca tctaccactg ctcaggcacg 540

ggaagacacc gctacacatc tgcaggagtg tccatcacgg tgaaagagct gtttaccacg 600

ccagtgctga gagcatccgt gtcatctccc ttcccggagg ggagtctggt caccctgaac 660

tgtgagacga atttgctcct gcagagaccc ggcttacagc ttcacttctc cttctacgtg 720

ggcagcaaga tcctggagta caggaacaca tcctcagagt accatatagc aagggcggaa 780

agagaagatg ctggattcta ctggtgtgag gtagccacgg aggacagcag tgtccttaag 840

cgcagccctg agttggagga gcgcaaatgt tgtgtcgagt gcccaccgtg cccagcacca 900

cctgtggcag gaccgtcagt cttcctcttc cccccaaaac ccaaggacac cctcatgatc 960

tcccggaccc ctgaggtcac gtgcgtggtg gtggacgtga gccacgaaga ccccgaggtc 1020

cagttcaact ggtacgtgga cggcatggag gtgcataatg ccaagacaaa gccacgggag 1080

gagcagttca acagcacgtt ccgtgtggtc agcgtcctca ccgtcgtgca ccaggactgg 1140

ctgaacggca aggagtacaa gtgcaaggtc tccaacaaag gcctcccagc ccccatcgag 1200

aaaaccatct ccaaaaccaa agggcagccc cgagaaccac aggtgtacac cctgccccca 1260

tcccgggagg agatgaccaa gaaccaggtc agcctgacct gcctggtcaa aggcttctac 1320

cccagcgaca tcgccgtgga gtgggagagc aatgggcagc cggagaacaa ctacaagacc 1380

acacctccca tgctggactc cgacggctcc ttcttcctct acagcaagct caccgtggac 1440

aagagcaggt ggcagcaggg gaacgtcttc tcatgctccg tgatgcatga ggctctgcac 1500

aaccactaca cacagaagag cctctccctg tctccggagc tgcaactgga ggagagctgt 1560

gcggaggcgc aggacgggga gctggacggg ctgtggacga ccatcaccat cttcatcaca 1620

ctcttcctgc taagcgtgtg ctacagtgcc accatcacct tcttcaaggt gaagtggatc 1680

ttctcctcag tggtggacct gaagcagacc atcgtccccg actacaggaa catgatcagg 1740

caggggcct ag 1752

<210> SEQ ID NO 12
<211> LENGTH: 583
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric fusion protein construct comprising
Fc-gammaRI and membrane Ig

<400> SEQUENCE: 12

Met Ile Leu Thr Ser Phe Gly Asp Asp Met Trp Leu Leu Thr Thr Leu
1 5 10 15

Leu Leu Trp Val Pro Val Gly Gly Glu Val Val Asn Ala Thr Lys Ala
20 25 30

Val Ile Thr Leu Gln Pro Pro Trp Val Ser Ile Phe Gln Lys Glu Asn
35 40 45

Val Thr Leu Trp Cys Glu Gly Pro His Leu Pro Gly Asp Ser Ser Thr
50 55 60

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Gln Trp Phe Ile Asn Gly Thr Ala Val Gln Ile Ser Thr Pro Ser Tyr
65                  70                  75                  80

Ser Ile Pro Glu Ala Ser Phe Gln Asp Ser Gly Glu Tyr Arg Cys Gln
                85                  90                  95

Ile Gly Ser Ser Met Pro Ser Asp Pro Val Gln Leu Gln Ile His Asn
            100                 105                 110

Asp Trp Leu Leu Leu Gln Ala Ser Arg Arg Val Leu Thr Glu Gly Glu
        115                 120                 125

Pro Leu Ala Leu Arg Cys His Gly Trp Lys Asn Lys Leu Val Tyr Asn
    130                 135                 140

Val Val Phe Tyr Arg Asn Gly Lys Ser Phe Gln Phe Ser Ser Asp Ser
145                 150                 155                 160

Glu Val Ala Ile Leu Lys Thr Asn Leu Ser His Ser Gly Ile Tyr His
                165                 170                 175

Cys Ser Gly Thr Gly Arg His Arg Tyr Thr Ser Ala Gly Val Ser Ile
            180                 185                 190

Thr Val Lys Glu Leu Phe Thr Thr Pro Val Leu Arg Ala Ser Val Ser
        195                 200                 205

Ser Pro Phe Pro Glu Gly Ser Leu Val Thr Leu Asn Cys Glu Thr Asn
    210                 215                 220

Leu Leu Leu Gln Arg Pro Gly Leu Gln Leu His Phe Ser Phe Tyr Val
225                 230                 235                 240

Gly Ser Lys Ile Leu Glu Tyr Arg Asn Thr Ser Ser Glu Tyr His Ile
                245                 250                 255

Ala Arg Ala Glu Arg Glu Asp Ala Gly Phe Tyr Trp Cys Glu Val Ala
            260                 265                 270

Thr Glu Asp Ser Ser Val Leu Lys Arg Ser Pro Glu Leu Glu Glu Arg
        275                 280                 285

Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
    290                 295                 300

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
305                 310                 315                 320

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
                325                 330                 335

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Met Glu Val His
            340                 345                 350

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
        355                 360                 365

Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
    370                 375                 380

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
385                 390                 395                 400

Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
                405                 410                 415

Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
            420                 425                 430

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
        435                 440                 445

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
    450                 455                 460

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
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465                 470                 475                 480

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
                485                 490                 495

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
            500                 505                 510

Glu Leu Gln Leu Glu Glu Ser Cys Ala Glu Ala Gln Asp Gly Glu Leu
        515                 520                 525

Asp Gly Leu Trp Thr Thr Ile Thr Ile Phe Ile Thr Leu Phe Leu Leu
    530                 535                 540

Ser Val Cys Tyr Ser Ala Thr Ile Thr Phe Phe Lys Val Lys Trp Ile
545                 550                 555                 560

Phe Ser Ser Val Val Asp Leu Lys Gln Thr Ile Val Pro Asp Tyr Arg
                565                 570                 575

Asn Met Ile Arg Gln Gly Ala
            580
```

<210> SEQ ID NO 13
<211> LENGTH: 1515
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric fusion protein construct comprising
Fc-gammaRIII and membrane Ig

<400> SEQUENCE: 13

```
atgactttgg acacccagat gtttcagaat gcacactctg gaagccaatg gctacttcca     60

ccactgacaa ttctgctgct gtttgctttt gcagacaggc agagtgcagc tcttccgaag    120

gctgtggtga aactggaccc cccatggatc caggtgctca aggaagacat ggtgacactg    180

atgtgcgaag ggacccacaa ccctgggaac tcttctactc agtggttcca caactggagt    240

tccatccgga gccaggtcca atccagctac acgtttaagg ccacagtcaa tgacagtgga    300

gaatatcggt gtcaaatgga gcagacccgc ctcagcgacc ctgtagatct gggagtgatt    360

tctgactggc tgctgctcca gacccctcag cgggtgtttc tggaagggga aaccatcacg    420

ctaaggtgcc ctagctggag gaacaaacta ctgaacagga tctcgttctt ccataatgaa    480

aaatccgtga ggtatcatca ctacaaaagt aatttctcta tcccaaaagc caaccacagt    540

cacagtgggg actactactg caaaggaagt ctaggaagta cacagcacca gtccaagcct    600

gtcaccatca ctgtccaaga cgagcgcaaa tgttgtgtcg agtgcccacc gtgcccagca    660

ccacctgtgg caggaccgtc agtcttcctc ttcccccaa aacccaagga caccctcatg    720

atctcccgga cccctgaggt cacgtgcgtg gtggtggacg tgagccacga agaccccgag    780

gtccagttca actggtacgt ggacggcatg gaggtgcata atgccaagac aaagccacgg    840

gaggagcagt tcaacagcac gttccgtgtg gtcagcgtcc tcaccgtcgt gcaccaggac    900

tggctgaacg gcaaggagta caagtgcaag gtctccaaca aaggcctccc agcccccatc    960

gagaaaacca tctccaaaac caaagggcag ccccgagaac cacaggtgta caccctgccc   1020

ccatcccggg aggagatgac caagaaccag gtcagcctga cctgcctggt caaaggcttc   1080

taccccagcg acatcgccgt ggagtgggag agcaatgggc agccggagaa caactacaag   1140

accacacctc ccatgctgga ctccgacggc tccttcttcc tctacagcaa gctcaccgtg   1200

gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggctctg   1260
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cacaaccact acacacagaa gagcctctcc ctgtctccgg agctgcaact ggaggagagc 1320

tgtgcggagg cgcaggacgg ggagctggac gggctgtgga cgaccatcac catcttcatc 1380

acactcttcc tgctaagcgt gtgctacagt gccaccatca ccttcttcaa ggtgaagtgg 1440

atcttctcct cagtggtgga cctgaagcag accatcgtcc ccgactacag gaacatgatc 1500

aggcaggggg cctag 1515

<210> SEQ ID NO 14
<211> LENGTH: 504
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric fusion protein construct comprising
Fc-gammaRIII and membrane Ig

<400> SEQUENCE: 14

Met Thr Leu Asp Thr Gln Met Phe Gln Asn Ala His Ser Gly Ser Gln
1 5 10 15

Trp Leu Leu Pro Pro Leu Thr Ile Leu Leu Leu Phe Ala Phe Ala Asp
20 25 30

Arg Gln Ser Ala Ala Leu Pro Lys Ala Val Val Lys Leu Asp Pro Pro
35 40 45

Trp Ile Gln Val Leu Lys Glu Asp Met Val Thr Leu Met Cys Glu Gly
50 55 60

Thr His Asn Pro Gly Asn Ser Ser Thr Gln Trp Phe His Asn Trp Ser
65 70 75 80

Ser Ile Arg Ser Gln Val Gln Ser Ser Tyr Thr Phe Lys Ala Thr Val
85 90 95

Asn Asp Ser Gly Glu Tyr Arg Cys Gln Met Glu Gln Thr Arg Leu Ser
100 105 110

Asp Pro Val Asp Leu Gly Val Ile Ser Asp Trp Leu Leu Leu Gln Thr
115 120 125

Pro Gln Arg Val Phe Leu Glu Gly Glu Thr Ile Thr Leu Arg Cys Pro
130 135 140

Ser Trp Arg Asn Lys Leu Leu Asn Arg Ile Ser Phe Phe His Asn Glu
145 150 155 160

Lys Ser Val Arg Tyr His His Tyr Lys Ser Asn Phe Ser Ile Pro Lys
165 170 175

Ala Asn His Ser His Ser Gly Asp Tyr Tyr Cys Lys Gly Ser Leu Gly
180 185 190

Ser Thr Gln His Gln Ser Lys Pro Val Thr Ile Thr Val Gln Asp Glu
195 200 205

Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
210 215 220

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
225 230 235 240

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
245 250 255

Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Met Glu Val
260 265 270

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
275 280 285

Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly

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    290                 295                 300

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
305                 310                 315                 320

Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
                325                 330                 335

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
            340                 345                 350

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
        355                 360                 365

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
    370                 375                 380

Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
385                 390                 395                 400

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                405                 410                 415

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
            420                 425                 430

Pro Glu Leu Gln Leu Glu Glu Ser Cys Ala Glu Ala Gln Asp Gly Glu
        435                 440                 445

Leu Asp Gly Leu Trp Thr Thr Ile Thr Ile Phe Ile Thr Leu Phe Leu
    450                 455                 460

Leu Ser Val Cys Tyr Ser Ala Thr Ile Thr Phe Phe Lys Val Lys Trp
465                 470                 475                 480

Ile Phe Ser Ser Val Val Asp Leu Lys Gln Thr Ile Val Pro Asp Tyr
                485                 490                 495

Arg Asn Met Ile Arg Gln Gly Ala
            500


<210> SEQ ID NO 15
<211> LENGTH: 1215
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (1)..(1212)

<400> SEQUENCE: 15

atg att ctt acc agc ttt gga gat gac atg tgg ctt cta aca act ctg       48
Met Ile Leu Thr Ser Phe Gly Asp Asp Met Trp Leu Leu Thr Thr Leu
1               5                   10                  15

cta ctt tgg gtt cca gtc ggt ggg gaa gtg gtt aat gcc acc aag gct       96
Leu Leu Trp Val Pro Val Gly Gly Glu Val Val Asn Ala Thr Lys Ala
            20                  25                  30

gtg atc acc ttg cag cct cca tgg gtc agt att ttc cag aag gaa aat      144
Val Ile Thr Leu Gln Pro Pro Trp Val Ser Ile Phe Gln Lys Glu Asn
        35                  40                  45

gtc act tta tgg tgt gag ggg cct cac ctg cct gga gac agt tcc aca      192
Val Thr Leu Trp Cys Glu Gly Pro His Leu Pro Gly Asp Ser Ser Thr
    50                  55                  60

caa tgg ttt atc aac gga aca gcc gtt cag atc tcc acg cct agt tat      240
Gln Trp Phe Ile Asn Gly Thr Ala Val Gln Ile Ser Thr Pro Ser Tyr
65                  70                  75                  80

agc atc cca gag gcc agt ttt cag gac agt ggc gaa tac agg tgt cag      288
Ser Ile Pro Glu Ala Ser Phe Gln Asp Ser Gly Glu Tyr Arg Cys Gln
                85                  90                  95
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ata ggt tcc tca atg cca agt gac cct gtg cag ttg caa atc cac aat      336
Ile Gly Ser Ser Met Pro Ser Asp Pro Val Gln Leu Gln Ile His Asn
            100                 105                 110

gat tgg ctg cta ctc cag gcc tcc cgc aga gtc ctc aca gaa gga gaa      384
Asp Trp Leu Leu Leu Gln Ala Ser Arg Arg Val Leu Thr Glu Gly Glu
        115                 120                 125

ccc ctg gcc ttg agg tgt cac gga tgg aag aat aaa ctg gtg tac aat      432
Pro Leu Ala Leu Arg Cys His Gly Trp Lys Asn Lys Leu Val Tyr Asn
    130                 135                 140

gtg gtt ttc tat aga aat gga aaa tcc ttt cag ttt tct tca gat tcg      480
Val Val Phe Tyr Arg Asn Gly Lys Ser Phe Gln Phe Ser Ser Asp Ser
145                 150                 155                 160

gag gtc gcc att ctg aaa acc aac ctg agt cac agc ggc atc tac cac      528
Glu Val Ala Ile Leu Lys Thr Asn Leu Ser His Ser Gly Ile Tyr His
                165                 170                 175

tgc tca ggc acg gga aga cac cgc tac aca tct gca gga gtg tcc atc      576
Cys Ser Gly Thr Gly Arg His Arg Tyr Thr Ser Ala Gly Val Ser Ile
            180                 185                 190

acg gtg aaa gag ctg ttt acc acg cca gtg ctg aga gca tcc gtg tca      624
Thr Val Lys Glu Leu Phe Thr Thr Pro Val Leu Arg Ala Ser Val Ser
        195                 200                 205

tct ccc ttc ccg gag ggg agt ctg gtc acc ctg aac tgt gag acg aat      672
Ser Pro Phe Pro Glu Gly Ser Leu Val Thr Leu Asn Cys Glu Thr Asn
    210                 215                 220

ttg ctc ctg cag aga ccc ggc tta cag ctt cac ttc tcc ttc tac gtg      720
Leu Leu Leu Gln Arg Pro Gly Leu Gln Leu His Phe Ser Phe Tyr Val
225                 230                 235                 240

ggc agc aag atc ctg gag tac agg aac aca tcc tca gag tac cat ata      768
Gly Ser Lys Ile Leu Glu Tyr Arg Asn Thr Ser Ser Glu Tyr His Ile
                245                 250                 255

gca agg gcg gaa aga gaa gat gct gga ttc tac tgg tgt gag gta gcc      816
Ala Arg Ala Glu Arg Glu Asp Ala Gly Phe Tyr Trp Cys Glu Val Ala
            260                 265                 270

acg gag gac agc agt gtc ctt aag cgc agc cct gag ttg gag ctc caa      864
Thr Glu Asp Ser Ser Val Leu Lys Arg Ser Pro Glu Leu Glu Leu Gln
        275                 280                 285

gtg ctt ggt ccc cag tca tca gct cct gtc tgg ttt cac atc ctg ttt      912
Val Leu Gly Pro Gln Ser Ser Ala Pro Val Trp Phe His Ile Leu Phe
    290                 295                 300

tat ctg tca gtg gga ata atg ttt tcg ttg aac acg gtt ctc tat gtg      960
Tyr Leu Ser Val Gly Ile Met Phe Ser Leu Asn Thr Val Leu Tyr Val
305                 310                 315                 320

aaa ata cac agg ctg cag aga gag aag aaa tac aac tta gaa gtc cct     1008
Lys Ile His Arg Leu Gln Arg Glu Lys Lys Tyr Asn Leu Glu Val Pro
                325                 330                 335

ttg gtt tct gag cag gga aag aaa gca aat tcc ttt cag caa gtt aga     1056
Leu Val Ser Glu Gln Gly Lys Lys Ala Asn Ser Phe Gln Gln Val Arg
            340                 345                 350

agc gat ggc gtg tat gaa gaa gta aca gcc act gcg agc cag acc aca     1104
Ser Asp Gly Val Tyr Glu Glu Val Thr Ala Thr Ala Ser Gln Thr Thr
        355                 360                 365

cca aaa gaa gcg ccc gat gga cct cga agc tca gtg ggt gac tgt gga     1152
Pro Lys Glu Ala Pro Asp Gly Pro Arg Ser Ser Val Gly Asp Cys Gly
    370                 375                 380

ccc gag cag cct gaa ccc ctt cct ccc agt gac agt act ggg gca caa     1200
Pro Glu Gln Pro Glu Pro Leu Pro Pro Ser Asp Ser Thr Gly Ala Gln
385                 390                 395                 400
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act tcc caa agt tga                                                    1215
Thr Ser Gln Ser


<210> SEQ ID NO 16
<211> LENGTH: 804
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (1)..(801)

<400> SEQUENCE: 16

atg act ttg gac acc cag atg ttt cag aat gca cac tct gga agc caa       48
Met Thr Leu Asp Thr Gln Met Phe Gln Asn Ala His Ser Gly Ser Gln
1               5                   10                  15

tgg cta ctt cca cca ctg aca att ctg ctg ctg ttt gct ttt gca gac       96
Trp Leu Leu Pro Pro Leu Thr Ile Leu Leu Leu Phe Ala Phe Ala Asp
            20                  25                  30

agg cag agt gca gct ctt ccg aag gct gtg gtg aaa ctg gac ccc cca      144
Arg Gln Ser Ala Ala Leu Pro Lys Ala Val Val Lys Leu Asp Pro Pro
        35                  40                  45

tgg atc cag gtg ctc aag gaa gac atg gtg aca ctg atg tgc gaa ggg      192
Trp Ile Gln Val Leu Lys Glu Asp Met Val Thr Leu Met Cys Glu Gly
    50                  55                  60

acc cac aac cct ggg aac tct tct act cag tgg ttc cac aac tgg agt      240
Thr His Asn Pro Gly Asn Ser Ser Thr Gln Trp Phe His Asn Trp Ser
65                  70                  75                  80

tcc atc cgg agc cag gtc caa tcc agc tac acg ttt aag gcc aca gtc      288
Ser Ile Arg Ser Gln Val Gln Ser Ser Tyr Thr Phe Lys Ala Thr Val
                85                  90                  95

aat gac agt gga gaa tat cgg tgt caa atg gag cag acc cgc ctc agc      336
Asn Asp Ser Gly Glu Tyr Arg Cys Gln Met Glu Gln Thr Arg Leu Ser
            100                 105                 110

gac cct gta gat ctg gga gtg att tct gac tgg ctg ctg ctc cag acc      384
Asp Pro Val Asp Leu Gly Val Ile Ser Asp Trp Leu Leu Leu Gln Thr
        115                 120                 125

cct cag cgg gtg ttt ctg gaa ggg gaa acc atc acg cta agg tgc cct      432
Pro Gln Arg Val Phe Leu Glu Gly Glu Thr Ile Thr Leu Arg Cys Pro
    130                 135                 140

agc tgg agg aac aaa cta ctg aac agg atc tcg ttc ttc cat aat gaa      480
Ser Trp Arg Asn Lys Leu Leu Asn Arg Ile Ser Phe Phe His Asn Glu
145                 150                 155                 160

aaa tcc gtg agg tat cat cac tac aaa agt aat ttc tct atc cca aaa      528
Lys Ser Val Arg Tyr His His Tyr Lys Ser Asn Phe Ser Ile Pro Lys
                165                 170                 175

gcc aac cac agt cac agt ggg gac tac tac tgc aaa gga agt cta gga      576
Ala Asn His Ser His Ser Gly Asp Tyr Tyr Cys Lys Gly Ser Leu Gly
            180                 185                 190

agt aca cag cac cag tcc aag cct gtc acc atc act gtc caa gac cca      624
Ser Thr Gln His Gln Ser Lys Pro Val Thr Ile Thr Val Gln Asp Pro
        195                 200                 205

gca act aca tcc tcc atc tct cta gtc tgg cac cac act gct ttc tcc      672
Ala Thr Thr Ser Ser Ile Ser Leu Val Trp His His Thr Ala Phe Ser
    210                 215                 220

cta gtg atg tgc ctc ctg ttt gca gtg gac acg ggc ctt tat ttc tat      720
Leu Val Met Cys Leu Leu Phe Ala Val Asp Thr Gly Leu Tyr Phe Tyr
225                 230                 235                 240

gta cgg aga aat ctt caa acc ccg agg gat tac tgg agg aag tcc ctg      768
Val Arg Arg Asn Leu Gln Thr Pro Arg Asp Tyr Trp Arg Lys Ser Leu
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               245                 250                 255

tca atc aga aag cac cag gct cct caa gac aag tga                    804
Ser Ile Arg Lys His Gln Ala Pro Gln Asp Lys
            260                 265


<210> SEQ ID NO 17
<211> LENGTH: 663
<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (1)..(660)

<400> SEQUENCE: 17

atg cca ggg ggt cta gaa gcc ctc aga gcc ctg cct ctc ctc ctc ttc      48
Met Pro Gly Gly Leu Glu Ala Leu Arg Ala Leu Pro Leu Leu Leu Phe
1               5                   10                  15

ttg tca tac gcc tgt ttg ggt ccc gga tgc cag gcc ctg cgg gta gaa      96
Leu Ser Tyr Ala Cys Leu Gly Pro Gly Cys Gln Ala Leu Arg Val Glu
            20                  25                  30

ggg ggt cca cca tcc ctg acg gtg aac ttg ggc gag gag gcc cgc ctc     144
Gly Gly Pro Pro Ser Leu Thr Val Asn Leu Gly Glu Glu Ala Arg Leu
        35                  40                  45

acc tgt gaa aac aat ggc agg aac cct aat atc aca tgg tgg ttc agc     192
Thr Cys Glu Asn Asn Gly Arg Asn Pro Asn Ile Thr Trp Trp Phe Ser
    50                  55                  60

ctt cag tct aac atc aca tgg ccc cca gtg cca ctg ggt cct ggc cag     240
Leu Gln Ser Asn Ile Thr Trp Pro Pro Val Pro Leu Gly Pro Gly Gln
65                  70                  75                  80

ggt acc aca ggc cag ctg ttc ttc ccc gaa gta aac aag aac cac agg     288
Gly Thr Thr Gly Gln Leu Phe Phe Pro Glu Val Asn Lys Asn His Arg
                85                  90                  95

ggc ttg tac tgg tgc caa gtg ata gaa aac aac ata tta aaa cgc tcc     336
Gly Leu Tyr Trp Cys Gln Val Ile Glu Asn Asn Ile Leu Lys Arg Ser
            100                 105                 110

tgt ggt act tac ctc cgc gtg cgc aat cca gtc cct agg ccc ttc ctg     384
Cys Gly Thr Tyr Leu Arg Val Arg Asn Pro Val Pro Arg Pro Phe Leu
        115                 120                 125

gac atg ggg gaa ggt acc aag aac cgc atc atc aca gca gaa ggg atc     432
Asp Met Gly Glu Gly Thr Lys Asn Arg Ile Ile Thr Ala Glu Gly Ile
    130                 135                 140

atc ttg ctg ttc tgt gca gtg gtg cca ggg acg ctg ctg cta ttc agg     480
Ile Leu Leu Phe Cys Ala Val Val Pro Gly Thr Leu Leu Leu Phe Arg
145                 150                 155                 160

aaa cgg tgg caa aat gag aag ttt ggg gtg gac atg cca gat gac tat     528
Lys Arg Trp Gln Asn Glu Lys Phe Gly Val Asp Met Pro Asp Asp Tyr
                165                 170                 175

gaa gat gaa aat ctc tat gag ggc ctg aac ctt gat gac tgt tct atg     576
Glu Asp Glu Asn Leu Tyr Glu Gly Leu Asn Leu Asp Asp Cys Ser Met
            180                 185                 190

tat gag gac atc tcc agg gga ctc cag ggc acc tac cag gat gtg ggc     624
Tyr Glu Asp Ile Ser Arg Gly Leu Gln Gly Thr Tyr Gln Asp Val Gly
        195                 200                 205

aac ctc cac att gga gat gcc cag ctg gaa aag cca tga                 663
Asn Leu His Ile Gly Asp Ala Gln Leu Glu Lys Pro
    210                 215                 220


<210> SEQ ID NO 18
<211> LENGTH: 894
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (1)..(891)

<400> SEQUENCE: 18

```
gag cgc aaa tgt tgt gtc gag tgc cca ccg tgc cca gca cca cct gtg       48
Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val
1               5                   10                  15

gca gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc       96
Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            20                  25                  30

atg atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg agc      144
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        35                  40                  45

cac gaa gac ccc gag gtc cag ttc aac tgg tac gtg gac ggc atg gag      192
His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Met Glu
    50                  55                  60

gtg cat aat gcc aag aca aag cca cgg gag gag cag ttc aac agc acg      240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
65                  70                  75                  80

ttc cgt gtg gtc agc gtc ctc acc gtc gtg cac cag gac tgg ctg aac      288
Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn
                85                  90                  95

ggc aag gag tac aag tgc aag gtc tcc aac aaa ggc ctc cca gcc ccc      336
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro
            100                 105                 110

atc gag aaa acc atc tcc aaa acc aaa ggg cag ccc cga gaa cca cag      384
Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln
        115                 120                 125

gtg tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc      432
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
    130                 135                 140

agc ctg acc tgc ctg gtc aaa ggc ttc tac ccc agc gac atc gcc gtg      480
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
145                 150                 155                 160

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc aca cct      528
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                165                 170                 175

ccc atg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc      576
Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
            180                 185                 190

gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg      624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        195                 200                 205

atg cat gag gct ctg cac aac cac tac aca cag aag agc ctc tcc ctg      672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    210                 215                 220

tct ccg gag ctg caa ctg gag gag agc tgt gcg gag gcg cag gac ggg      720
Ser Pro Glu Leu Gln Leu Glu Glu Ser Cys Ala Glu Ala Gln Asp Gly
225                 230                 235                 240

gag ctg gac ggg ctg tgg acg acc atc acc atc ttc atc aca ctc ttc      768
Glu Leu Asp Gly Leu Trp Thr Thr Ile Thr Ile Phe Ile Thr Leu Phe
                245                 250                 255

ctg cta agc gtg tgc tac agt gcc acc atc acc ttc ttc aag gtg aag      816
Leu Leu Ser Val Cys Tyr Ser Ala Thr Ile Thr Phe Phe Lys Val Lys
            260                 265                 270
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tgg atc ttc tcc tca gtg gtg gac ctg aag cag acc atc gtc ccc gac      864
Trp Ile Phe Ser Ser Val Val Asp Leu Lys Gln Thr Ile Val Pro Asp
        275                 280                 285

tac agg aac atg atc agg cag ggg gcc tag                              894
Tyr Arg Asn Met Ile Arg Gln Gly Ala
    290                 295
```

<210> SEQ ID NO 19
<211> LENGTH: 960
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 19

atgattctta ccagctttgg agatgacatg tggcttctaa caactctgct actttgggtt 60

ccagtcggtg gggaagtggt taatgccacc aaggctgtga tcaccttgca gcctccatgg 120

gtcagtattt tccagaagga aaatgtcact ttatggtgtg aggggcctca cctgcctgga 180

gacagttcca cacaatggtt tatcaacgga acagccgttc agatctccac gcctagttat 240

agcatcccag aggccagttt tcaggacagt ggcgaataca ggtgtcagat aggttcctca 300

atgccaagtg accctgtgca gttgcaaatc cacaatgatt ggctgctact ccaggcctcc 360

cgcagagtcc tcacagaagg agaacccctg gccttgaggt gtcacggatg gaagaataaa 420

ctggtgtaca atgtggtttt ctatagaaat ggaaaatcct ttcagttttc ttcagattcg 480

gaggtcgcca ttctgaaaac caacctgagt cacagcggca tctaccactg ctcaggcacg 540

ggaagacacc gctacacatc tgcaggagtg tccatcacgg tgaaagagct gtttaccacg 600

ccagtgctga gagcatccgt gtcatctccc ttcccggagg ggagtctggt caccctgaac 660

tgtgagacga atttgctcct gcagagaccc ggcttacagc ttcacttctc cttctacgtg 720

ggcagcaaga tcctggagta caggaacaca tcctcagagt accatatagc aagggcggaa 780

agagaagatg ctggattcta ctggtgtgag gtagccacgg aggacagcag tgtccttaag 840

cgcagccctg agttggagct ccaagtgctt ggtccccagt catcagctcc tgtctggttt 900

cacatcctgt tttatctgtc agtgggaata atgttttcgt tgaacacggt tctctatgtg 960

<210> SEQ ID NO 20
<211> LENGTH: 699
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 20

atgactttgg acacccagat gtttcagaat gcacactctg gaagccaatg gctacttcca 60

ccactgacaa ttctgctgct gtttgctttt gcagacaggc agagtgcagc tcttccgaag 120

gctgtggtga aactggaccc cccatggatc caggtgctca aggaagacat ggtgacactg 180

atgtgcgaag ggacccacaa ccctgggaac tcttctactc agtggttcca caactggagt 240

tccatccgga gccaggtcca atccagctac acgtttaagg ccacagtcaa tgacagtgga 300

gaatatcggt gtcaaatgga gcagacccgc ctcagcgacc ctgtagatct gggagtgatt 360

tctgactggc tgctgctcca gacccctcag cgggtgtttc tggaagggga aaccatcacg 420

ctaaggtgcc ctagctggag gaacaaacta ctgaacagga tctcgttctt ccataatgaa 480

aaatccgtga ggtatcatca ctacaaaagt aatttctcta tcccaaaagc caaccacagt 540

cacagtgggg actactactg caaaggaagt ctaggaagta cacagcacca gtccaagcct 600

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gtcaccatca ctgtccaaga cccagcaact acatcctcca tctctctagt ctggcaccac 660

actgctttct ccctagtgat gtgcctcctg tttgcagtg 699

<210> SEQ ID NO 21
<211> LENGTH: 189
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 21

ttcaggaaac ggtggcaaaa tgagaagttt ggggtggaca tgccagatga ctatgaagat 60

gaaaatctct atgagggcct gaaccttgat gactgttcta tgtatgagga catctccagg 120

ggactccagg gcacctacca ggatgtgggc aacctccaca ttggagatgc ccagctggaa 180

aagccatga 189

<210> SEQ ID NO 22
<211> LENGTH: 1752
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric fusion protein construct comprising
Fc-gammaRI and membrane Ig
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (1)..(1749)

<400> SEQUENCE: 22

atg att ctt acc agc ttt gga gat gac atg tgg ctt cta aca act ctg 48
Met Ile Leu Thr Ser Phe Gly Asp Asp Met Trp Leu Leu Thr Thr Leu
1 5 10 15

cta ctt tgg gtt cca gtc ggt ggg gaa gtg gtt aat gcc acc aag gct 96
Leu Leu Trp Val Pro Val Gly Gly Glu Val Val Asn Ala Thr Lys Ala
20 25 30

gtg atc acc ttg cag cct cca tgg gtc agt att ttc cag aag gaa aat 144
Val Ile Thr Leu Gln Pro Pro Trp Val Ser Ile Phe Gln Lys Glu Asn
35 40 45

gtc act tta tgg tgt gag ggg cct cac ctg cct gga gac agt tcc aca 192
Val Thr Leu Trp Cys Glu Gly Pro His Leu Pro Gly Asp Ser Ser Thr
50 55 60

caa tgg ttt atc aac gga aca gcc gtt cag atc tcc acg cct agt tat 240
Gln Trp Phe Ile Asn Gly Thr Ala Val Gln Ile Ser Thr Pro Ser Tyr
65 70 75 80

agc atc cca gag gcc agt ttt cag gac agt ggc gaa tac agg tgt cag 288
Ser Ile Pro Glu Ala Ser Phe Gln Asp Ser Gly Glu Tyr Arg Cys Gln
85 90 95

ata ggt tcc tca atg cca agt gac cct gtg cag ttg caa atc cac aat 336
Ile Gly Ser Ser Met Pro Ser Asp Pro Val Gln Leu Gln Ile His Asn
100 105 110

gat tgg ctg cta ctc cag gcc tcc cgc aga gtc ctc aca gaa gga gaa 384
Asp Trp Leu Leu Leu Gln Ala Ser Arg Arg Val Leu Thr Glu Gly Glu
115 120 125

ccc ctg gcc ttg agg tgt cac gga tgg aag aat aaa ctg gtg tac aat 432
Pro Leu Ala Leu Arg Cys His Gly Trp Lys Asn Lys Leu Val Tyr Asn
130 135 140

gtg gtt ttc tat aga aat gga aaa tcc ttt cag ttt tct tca gat tcg 480
Val Val Phe Tyr Arg Asn Gly Lys Ser Phe Gln Phe Ser Ser Asp Ser
145 150 155 160

gag gtc gcc att ctg aaa acc aac ctg agt cac agc ggc atc tac cac 528
Glu Val Ala Ile Leu Lys Thr Asn Leu Ser His Ser Gly Ile Tyr His

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                165                 170                 175

tgc tca ggc acg gga aga cac cgc tac aca tct gca gga gtg tcc atc      576
Cys Ser Gly Thr Gly Arg His Arg Tyr Thr Ser Ala Gly Val Ser Ile
            180                 185                 190

acg gtg aaa gag ctg ttt acc acg cca gtg ctg aga gca tcc gtg tca      624
Thr Val Lys Glu Leu Phe Thr Thr Pro Val Leu Arg Ala Ser Val Ser
        195                 200                 205

tct ccc ttc ccg gag ggg agt ctg gtc acc ctg aac tgt gag acg aat      672
Ser Pro Phe Pro Glu Gly Ser Leu Val Thr Leu Asn Cys Glu Thr Asn
    210                 215                 220

ttg ctc ctg cag aga ccc ggc tta cag ctt cac ttc tcc ttc tac gtg      720
Leu Leu Leu Gln Arg Pro Gly Leu Gln Leu His Phe Ser Phe Tyr Val
225                 230                 235                 240

ggc agc aag atc ctg gag tac agg aac aca tcc tca gag tac cat ata      768
Gly Ser Lys Ile Leu Glu Tyr Arg Asn Thr Ser Ser Glu Tyr His Ile
                245                 250                 255

gca agg gcg gaa aga gaa gat gct gga ttc tac tgg tgt gag gta gcc      816
Ala Arg Ala Glu Arg Glu Asp Ala Gly Phe Tyr Trp Cys Glu Val Ala
            260                 265                 270

acg gag gac agc agt gtc ctt aag cgc agc cct gag ttg gag gag cgc      864
Thr Glu Asp Ser Ser Val Leu Lys Arg Ser Pro Glu Leu Glu Glu Arg
        275                 280                 285

aaa tgt tgt gtc gag tgc cca ccg tgc cca gca cca cct gtg gca gga      912
Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly
    290                 295                 300

ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc      960
Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
305                 310                 315                 320

tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg agc cac gaa     1008
Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
                325                 330                 335

gac ccc gag gtc cag ttc aac tgg tac gtg gac ggc atg gag gtg cat     1056
Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Met Glu Val His
            340                 345                 350

aat gcc aag aca aag cca cgg gag gag cag ttc aac agc acg ttc cgt     1104
Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg
        355                 360                 365

gtg gtc agc gtc ctc acc gtc gtg cac cag gac tgg ctg aac ggc aag     1152
Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly Lys
    370                 375                 380

gag tac aag tgc aag gtc tcc aac aaa ggc ctc cca gcc ccc atc gag     1200
Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu
385                 390                 395                 400

aaa acc atc tcc aaa acc aaa ggg cag ccc cga gaa cca cag gtg tac     1248
Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
                405                 410                 415

acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg     1296
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu
            420                 425                 430

acc tgc ctg gtc aaa ggc ttc tac ccc agc gac atc gcc gtg gag tgg     1344
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
        435                 440                 445

gag agc aat ggg cag ccg gag aac aac tac aag acc aca cct ccc atg     1392
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met
    450                 455                 460

ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac     1440
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
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465                 470                 475                 480

aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat     1488
Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
                485                 490                 495

gag gct ctg cac aac cac tac aca cag aag agc ctc tcc ctg tct ccg     1536
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
            500                 505                 510

gag ctg caa ctg gag gag agc tgt gcg gag gcg cag gac ggg gag ctg     1584
Glu Leu Gln Leu Glu Glu Ser Cys Ala Glu Ala Gln Asp Gly Glu Leu
        515                 520                 525

gac ggg ctg tgg acg acc atc acc atc ttc atc aca ctc ttc ctg cta     1632
Asp Gly Leu Trp Thr Thr Ile Thr Ile Phe Ile Thr Leu Phe Leu Leu
    530                 535                 540

agc gtg tgc tac agt gcc acc atc acc ttc ttc aag gtg aag tgg atc     1680
Ser Val Cys Tyr Ser Ala Thr Ile Thr Phe Phe Lys Val Lys Trp Ile
545                 550                 555                 560

ttc tcc tca gtg gtg gac ctg aag cag acc atc gtc ccc gac tac agg     1728
Phe Ser Ser Val Val Asp Leu Lys Gln Thr Ile Val Pro Asp Tyr Arg
                565                 570                 575

aac atg atc agg cag ggg gcc tag                                    1752
Asn Met Ile Arg Gln Gly Ala
            580


<210> SEQ ID NO 23
<211> LENGTH: 1515
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chimeric fusion protein construct comprising
      Fc-gammaRIII and membrane Ig
<220> FEATURE:
<221> NAME/KEY: exon
<222> LOCATION: (1)..(1512)

<400> SEQUENCE: 23

atg act ttg gac acc cag atg ttt cag aat gca cac tct gga agc caa       48
Met Thr Leu Asp Thr Gln Met Phe Gln Asn Ala His Ser Gly Ser Gln
1               5                   10                  15

tgg cta ctt cca cca ctg aca att ctg ctg ctg ttt gct ttt gca gac       96
Trp Leu Leu Pro Pro Leu Thr Ile Leu Leu Leu Phe Ala Phe Ala Asp
            20                  25                  30

agg cag agt gca gct ctt ccg aag gct gtg gtg aaa ctg gac ccc cca      144
Arg Gln Ser Ala Ala Leu Pro Lys Ala Val Val Lys Leu Asp Pro Pro
        35                  40                  45

tgg atc cag gtg ctc aag gaa gac atg gtg aca ctg atg tgc gaa ggg      192
Trp Ile Gln Val Leu Lys Glu Asp Met Val Thr Leu Met Cys Glu Gly
    50                  55                  60

acc cac aac cct ggg aac tct tct act cag tgg ttc cac aac tgg agt      240
Thr His Asn Pro Gly Asn Ser Ser Thr Gln Trp Phe His Asn Trp Ser
65                  70                  75                  80

tcc atc cgg agc cag gtc caa tcc agc tac acg ttt aag gcc aca gtc      288
Ser Ile Arg Ser Gln Val Gln Ser Ser Tyr Thr Phe Lys Ala Thr Val
                85                  90                  95

aat gac agt gga gaa tat cgg tgt caa atg gag cag acc cgc ctc agc      336
Asn Asp Ser Gly Glu Tyr Arg Cys Gln Met Glu Gln Thr Arg Leu Ser
            100                 105                 110

gac cct gta gat ctg gga gtg att tct gac tgg ctg ctg ctc cag acc      384
Asp Pro Val Asp Leu Gly Val Ile Ser Asp Trp Leu Leu Leu Gln Thr
        115                 120                 125
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cct cag cgg gtg ttt ctg gaa ggg gaa acc atc acg cta agg tgc cct      432
Pro Gln Arg Val Phe Leu Glu Gly Glu Thr Ile Thr Leu Arg Cys Pro
    130                 135                 140

agc tgg agg aac aaa cta ctg aac agg atc tcg ttc ttc cat aat gaa      480
Ser Trp Arg Asn Lys Leu Leu Asn Arg Ile Ser Phe Phe His Asn Glu
145                 150                 155                 160

aaa tcc gtg agg tat cat cac tac aaa agt aat ttc tct atc cca aaa      528
Lys Ser Val Arg Tyr His His Tyr Lys Ser Asn Phe Ser Ile Pro Lys
                165                 170                 175

gcc aac cac agt cac agt ggg gac tac tac tgc aaa gga agt cta gga      576
Ala Asn His Ser His Ser Gly Asp Tyr Tyr Cys Lys Gly Ser Leu Gly
            180                 185                 190

agt aca cag cac cag tcc aag cct gtc acc atc act gtc caa gac gag      624
Ser Thr Gln His Gln Ser Lys Pro Val Thr Ile Thr Val Gln Asp Glu
        195                 200                 205

cgc aaa tgt tgt gtc gag tgc cca ccg tgc cca gca cca cct gtg gca      672
Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala
    210                 215                 220

gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg      720
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
225                 230                 235                 240

atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg agc cac      768
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
                245                 250                 255

gaa gac ccc gag gtc cag ttc aac tgg tac gtg gac ggc atg gag gtg      816
Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Met Glu Val
            260                 265                 270

cat aat gcc aag aca aag cca cgg gag gag cag ttc aac agc acg ttc      864
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe
        275                 280                 285

cgt gtg gtc agc gtc ctc acc gtc gtg cac cag gac tgg ctg aac ggc      912
Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp Leu Asn Gly
    290                 295                 300

aag gag tac aag tgc aag gtc tcc aac aaa ggc ctc cca gcc ccc atc      960
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ala Pro Ile
305                 310                 315                 320

gag aaa acc atc tcc aaa acc aaa ggg cag ccc cga gaa cca cag gtg     1008
Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val
                325                 330                 335

tac acc ctg ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc     1056
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
            340                 345                 350

ctg acc tgc ctg gtc aaa ggc ttc tac ccc agc gac atc gcc gtg gag     1104
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
        355                 360                 365

tgg gag agc aat ggg cag ccg gag aac aac tac aag acc aca cct ccc     1152
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
    370                 375                 380

atg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg     1200
Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
385                 390                 395                 400

gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg     1248
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
                405                 410                 415

cat gag gct ctg cac aac cac tac aca cag aag agc ctc tcc ctg tct     1296
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
            420                 425                 430
```

-continued

```
ccg gag ctg caa ctg gag gag agc tgt gcg gag gcg cag gac ggg gag     1344
Pro Glu Leu Gln Leu Glu Glu Ser Cys Ala Glu Ala Gln Asp Gly Glu
        435                 440                 445

ctg gac ggg ctg tgg acg acc atc acc atc ttc atc aca ctc ttc ctg     1392
Leu Asp Gly Leu Trp Thr Thr Ile Thr Ile Phe Ile Thr Leu Phe Leu
    450                 455                 460

cta agc gtg tgc tac agt gcc acc atc acc ttc ttc aag gtg aag tgg     1440
Leu Ser Val Cys Tyr Ser Ala Thr Ile Thr Phe Phe Lys Val Lys Trp
465                 470                 475                 480

atc ttc tcc tca gtg gtg gac ctg aag cag acc atc gtc ccc gac tac     1488
Ile Phe Ser Ser Val Val Asp Leu Lys Gln Thr Ile Val Pro Asp Tyr
                485                 490                 495

agg aac atg atc agg cag ggg gcc tag                                 1515
Arg Asn Met Ile Arg Gln Gly Ala
            500


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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Glu Thr Gly
1               5                   10                  15

gga gaa ccc tgg acc                                                  63
Gly Glu Pro Trp Thr
            20


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<212> TYPE: DNA
<213> ORGANISM: Mus musculus
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1               5                   10                  15

gcc gcc ctg gga gag ccg cag ctc tgc tat atc ctg gat gct gtc ctg       96
Ala Ala Leu Gly Glu Pro Gln Leu Cys Tyr Ile Leu Asp Ala Val Leu
            20                  25                  30

ttt ttg tat ggt att gtc ctt acc cta ctc tac tgt cga ctc aag atc      144
Phe Leu Tyr Gly Ile Val Leu Thr Leu Leu Tyr Cys Arg Leu Lys Ile
        35                  40                  45

cag gtc cga aag gca gct ata gcc agc cgt gag aaa gca gat gct gtc      192
Gln Val Arg Lys Ala Ala Ile Ala Ser Arg Glu Lys Ala Asp Ala Val
    50                  55                  60

tac acg ggc ctg aac acc cgg agc cag gag aca tat gag act ctg aag      240
Tyr Thr Gly Leu Asn Thr Arg Ser Gln Glu Thr Tyr Glu Thr Leu Lys
65                  70                  75                  80

cat gag aaa cca ccc cag tag                                         261
His Glu Lys Pro Pro Gln
                85
```

What is claimed is:

1. A method of detecting a target agent in a sample, comprising

(a) contacting a sample with an antibody having binding specificity for a target agent and with a biosensor cell, and

(b) assaying the biosensor cell for cellular activation,

wherein the biosensor cell stably expresses a chimeric fusion protein, and wherein the chimeric fusion protein comprises an Fcγ receptor (FcγR) antibody-binding domain and a signaling domain.

2. The method of claim **1**, wherein the sample is an air sample, a liquid sample, a vegetable sample, or a dry sample.

3. The method of claim **1**, wherein the sample is a biological sample selected from the group consisting of blood, serum, sweat, urine, cerebrospinal fluid, mucus, semen, stool, bronchoalveolar lavage fluid, and tissue.

4. The method of claim **1**, wherein the agent is an environmental toxin, pollutant, or drug.

5. The method of claim **1**, wherein the agent is a biologic agent selected from the group consisting of a bio-warfare agent, an allergen, a parasitic antigen, a fungal antigen, a viral antigen, a bacterial antigen, a cellular antigen, and an antibody.

6. The method of claim **1**, wherein the biosensor cell is a B cell, a T cell, a monocyte, a macrophage, a HEK293 cell, a CHO cell, P815, K562, or a Cos-1 cell that stably expresses the chimeric fusion protein.

7. The method of claim **1**, wherein cellular activation is an increase in intracellular Ca^{2+} levels.

8. The method of any one of claims **1-7**, wherein the FcγR antibody-binding domain is the FcγRI antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3.

9. The method of any one of claims **1-7**, wherein the FcγR antibody-binding domain is the FcγRIII antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4.

10. The method of claim **8**, wherein the signaling domain is the immunoglobulin alpha (Igα) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5.

11. The method of claim **9**, wherein the signaling domain is the immunoglobulin alpha (Igα) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5.

12. The method of claim **8**, wherein the signaling domain is the membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6.

13. The method of claim **9**, wherein the signaling domain is the membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6.

14. A biosensor cell stably expressing a chimeric fusion protein, wherein the chimeric fusion protein comprises an Fcγ receptor (FcγR) antibody-binding domain and a signaling domain.

15. The biosensor cell of claim **14**, wherein the biosensor cell is a B cell, a T cell, a monocyte, a macrophage, a HEK293 cell, a CHO cell, P815, K562, or a Cos-1 cell that stably expresses the chimeric fusion protein.

16. The biosensor cell of claim **14**, wherein the FcγR antibody-binding domain is the FcγRI antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3.

17. The biosensor cell of claim **14**, wherein the FcγR antibody-binding domain is the FcγRIII antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4.

18. The biosensor cell of any one of claims **14-17**, wherein the signaling domain is the immunoglobulin alpha (Igα) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5.

19. The biosensor cell of any one of claims **14-17**, wherein the signaling domain is the membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6.

20. A chimeric fusion protein comprising an Fcγ receptor (FcγR) antibody-binding domain and a signaling domain.

21. The chimeric fusion protein of claim **20**, wherein the FcγR antibody-binding domain is the FcγRI antibody-binding domain set forth in SEQ ID NO:1 or 3, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:1 or 3.

22. The chimeric fusion protein of claim **20**, wherein the FcγR antibody-binding domain is the FcγRIII antibody-binding domain set forth in SEQ ID NO:2 or 4, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:2 or 4.

23. The chimeric fusion protein of any one of claims **20-22**, wherein the signaling domain is the immunoglobulin alpha (Igα) signaling domain set forth in SEQ ID NO:5, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:5.

24. The chimeric fusion protein of any one of claims **20-22**, wherein the signaling domain is the membrane Ig set forth in SEQ ID NO:6, or a sequence variant thereof having at least 95% sequence identity over the entire length of SEQ ID NO:6.

25. The chimeric fusion protein of claim **20**, wherein the fusion protein is the FcγRI/Igα fusion protein set forth in SEQ ID NO:8 or a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:8.

26. The chimeric fusion protein of claim **20**, wherein the fusion protein is the FcγRIII/Igα fusion protein set forth in SEQ ID NO:10 or a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:10.

27. The chimeric fusion protein of claim **20**, wherein the fusion protein is the FcγRI/membrane Ig fusion protein set forth in SEQ ID NO:22 or a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:22.

28. The chimeric fusion protein of claim **20**, wherein the fusion protein is the FcγRIII/membrane Ig fusion protein set forth in SEQ ID NO:23 or a sequence variant having at least 95% sequence identity over the entire length of SEQ ID NO:23.

29. A polynucleotide sequence encoding a chimeric fusion protein of any one of claims **20-28**, or a complementary strand thereof.

30. A cloning vector comprising a polynucleotide sequence of claim **29**.

31. A cell comprising a polynucleotide sequence of claim **29**.

32. A cell comprising a cloning vector of claim **30**.

33. A method of producing a chimeric fusion protein comprising culturing a cell of claim **31** under conditions promoting expression of the fusion protein, and recovering the fusion protein from the cell or cell culture.

34. A method of producing a chimeric fusion protein comprising culturing a cell of claim **32** under conditions promoting expression of the fusion protein, and recovering the fusion protein from the cell or cell culture.

* * * * *

专利名称(译)	通用抗体介导的生物传感器		
公开(公告)号	US20180057562A1	公开(公告)日	2018-03-01
申请号	US15/557603	申请日	2016-03-11
[标]申请(专利权)人(译)	舒尔茨丹		
申请(专利权)人(译)	SCHULZE，DAN		
当前申请(专利权)人(译)	马里兰州巴尔的摩大学		
[标]发明人	SCHULZE DAN		
发明人	SCHULZE, DAN		
IPC分类号	C07K14/735 C07K16/12 G01N33/50 C12Q1/02 G01N33/53		
CPC分类号	C07K19/00 C07K2319/03 C07K14/70535 C07K16/12 G01N33/5038 G01N33/5041 C12Q1/02 G01N33/53 C07K16/00 C07K2319/00 G01N33/6854 G01N2333/70535		
优先权	62/132729 2015-03-13 US		
外部链接	Espacenet USPTO		

摘要(译)

提供了通用抗体介导的生物传感器，其包含稳定表达新型嵌合融合蛋白的生物传感器细胞系，所述嵌合融合蛋白可用于检测样品中的靶因子。融合蛋白具有结合抗体的细胞外抗体结合结构域而不考虑它们的结合特异性和在抗原结合时诱导细胞活化的信号结构域。因为融合蛋白结合任何抗体的Fc区，它可以作为细胞外信号传导和细胞内激活之间的通用途径。生物传感器可用于检测样品中所选抗原的存在。