MP10485PC Merz Pharma GmbH & Co. KGaA

Claims

1. A polynucleotide encoding a fusion polypeptide comprising (i) a transcription factor domain and (ii) a cytoplasmic retention domain, separated by a linker comprising a neurotoxin cleavage site.

2. The polynucleotide of claim 1, wherein said cytoplasmic retention domain is a transmembrane protein or transmembrane spanning domain of a transmembrane protein.

3. The polynucleotide of claim 2, wherein said transmembrane protein is selected from the group consisting of: plasmalemmal neurotransmitter transporters, ion channels, G-protein coupled receptors and membrane receptors.

4. The polynucleotide of claim 1, wherein said cytoplasmic retention domain is a membrane-anchored protein or a membrane anchor domain of a membrane-anchored protein.

5. The polynucleotide of claim 4, wherein said membrane-anchored protein is selected from the group consisting of: SNAP-25, Syntaxin, synaptobrevin, synaptotagmin, vesicle associated membrane proteins (VAMPs), synaptic vesicle glycoproteins (SV2), high affinity choline transporters, Neurexins, voltage-gated calcium channels acetylcholinesterase and NOTCH.

6. The polynucleotide of claim 1, wherein said cytoplasmic retention domain is selected from the group consisting of: a globular protein and a cytoskeleton anchor protein.

7. The polynucleotide of any one of claims 1 to 6, wherein said transcription factor domain comprises a transcription factor comprising a DNA binding domain and a transactivator or silencer domain.

8. The polynucleotide of claim 7, wherein said transcription factor is selected from the group consisting of: tetracycline dependent transactivator (tet-repressor – VP 16), steroid hormone receptors, NOTCH, NFκB, p53, NFAT, MLL, E2A, HSF1, NF-IL6, STAT, R-SMAD, GAL4, and SP1.

9. A vector comprising a polynucleotide of any one of claims 1 to 8.

10. A fusion polypeptide encoded by the polynucleotide of any one of claims 1 to 8.

11. A host cell comprising the polynucleotide of any one of claims 1 to 8, the vector of claim 9 or the fusion polypeptide of claim 10.

12. The host cell of claim 11, wherein said host cell is selected from the group consisting of: primary neuronal cells, neuroblastoma cell lines, cell line N1E-115, cell line Neuro2a, cell line SH-SY5Y, cell line PC12, cell line SiMa, cell line MHH-NB-11, cell line SK-N-BE(2) and induced pluripotent stem cells.

13. The host cell of claim 11 or 12, wherein said host cell comprises a reporter gene the expression of which is modulated by the transcription factor domain upon cleavage of the fusion polypeptide.

14. A method for determining neurotoxin activity in a sample comprising the steps of:
(a) contacting a host cell comprising the fusion polypeptide of claim 10 and a reporter gene the expression of which is modulated by the transcription factor domain upon cleavage of the said fusion polypeptide with a sample suspected to comprise neurotoxin activity; and
(b) measuring the reporter gene activity, whereby neurotoxin activity in the sample is determined.

15. The method of claim 14, wherein said host cell is a host cell of any one of claims 11 to 13.

16. Use of the polynucleotide of any one of claims 1 to 8, the vector of claim 9, the fusion polypeptide of claim 10 or the host cell of claim 11 or 13 for determining neurotoxin activity in a sample.

17. A kit for determining neurotoxin activity comprising the polynucleotide of any one of claims 1 to 8, the vector of claim 9, the fusion polypeptide of claim 10 or the host cell of claim 11 or 13 and, preferably, a detection agent for detecting reporter gene activity.

Claims

1. A host cell comprising a polynucleotide encoding a fusion polypeptide comprising (i) a transcription factor domain and (ii) a cytoplasmic retention domain, separated by a linker comprising a neurotoxin cleavage site, wherein said host cell is selected from the group consisting of: primary neuronal cells, neuroblastoma cell lines, cell line N1E-115, cell line Neuro2a, cell line SH-SY5Y, cell line PC12, cell line SiMa, cell line MHH-NB-11, cell line SK-N-BE(2) and induced pluripotent stem cells.

2. The host cell of claim 1, wherein said cytoplasmic retention domain is a transmembrane protein or transmembrane spanning domain of a transmembrane protein.

3. The host cell of claim 2, wherein said transmembrane protein is selected from the group consisting of: plasmalemmal neurotransmitter transporters, ion channels, G-protein coupled receptors and membrane receptors.

4. The host cell of claim 1, wherein said cytoplasmic retention domain is a membrane-anchored protein or a membrane anchor domain of a membrane-anchored protein.

5. The host cell of claim 4, wherein said membrane-anchored protein is selected from the group consisting of: SNAP-25, Syntaxin, synaptobrevin, synaptotagmin, vesicle associated membrane proteins (VAMPs), synaptic vesicle glycoproteins (SV2), high affinity choline transporters, Neurexins, voltage-gated calcium channels acetylcholinesterase and NOTCH.

6. The host cell of claim 1, wherein said cytoplasmic retention domain is selected from the group consisting of: a globular protein and a cytoskeleton anchor protein.

7. The host cell of any one of claims 1 to 6, wherein said transcription factor domain comprises a transcription factor comprising a DNA binding domain and a transactivator or silencer domain.

8. The host cell of claim 7, wherein said transcription factor is selected from the group consisting of: tetracycline dependent transactivator (tet-repressor – VP 16), steroid

hormone receptors, NOTCH, NFκB, p53, NFAT, MLL, E2A, HSF1, NF-IL6, STAT, R-SMAD, GAL4, and SP1.

9. The host cell of any one of claims 1 to 8, wherein said polynucleotide is comprised by a vector.

10. The host cell of any of claims 1 to 9, wherein said host cell comprises a reporter gene the expression of which is modulated by the transcription factor domain upon cleavage of the fusion polypeptide.

11. A method for determining neurotoxin activity in a sample comprising the steps of:
    (a) contacting a host cell of any of claims 1 to 9 and a reporter gene the expression of which is modulated by the transcription factor domain upon cleavage of the said fusion polypeptide with a sample suspected to comprise neurotoxin activity; and
    (b) measuring the reporter gene activity, whereby neurotoxin activity in the sample is determined.

12. Use of the host cell of any of claims 1 to 10 for determining neurotoxin activity in a sample.

13. A kit for determining neurotoxin activity comprising the host cell of any of claims 1 to 10 and a detection agent for detecting reporter gene activity.