CLAIMS

1. A method for identifying a gene that regulates clostridial neurotoxin activity, the method comprising:
   (a) providing a sample of human neuronal cells expressing a polypeptide that comprises a C-terminal detectable label, wherein the polypeptide is cleavable by a clostridial neurotoxin;
   (b) altering expression of a target gene of the cells;
   (c) contacting the cells with the clostridial neurotoxin;
   (d) measuring an amount of C-terminal detectable label, thereby quantifying clostridial neurotoxin activity; and
   (e) identifying the target gene as a regulator of clostridial neurotoxin activity when the quantified clostridial neurotoxin activity is different to the quantified clostridial neurotoxin activity when expression of the target gene is unaltered; or
   (f) identifying that the target gene is not a regulator of clostridial neurotoxin activity when the quantified clostridial neurotoxin activity is equivalent to the quantified clostridial neurotoxin activity when expression of the target gene is unaltered.

2. The method according to claim 1, wherein expression is altered by downregulating expression of the target gene.

~~3.~~ The method according to claim 2, wherein the target gene is identified as a positive regulator of clostridial neurotoxin activity when the quantified clostridial neurotoxin activity is less than the quantified clostridial neurotoxin activity when expression of the target gene is unaltered~~.~~

~~4.~~3. ~~The method according to claim 2 or 3~~, and/or wherein the target gene is identified as a negative regulator of clostridial neurotoxin activity when the quantified clostridial neurotoxin activity is greater than the quantified clostridial neurotoxin activity when expression of the target gene is unaltered.

~~5.~~4. The method according to any one of the preceding claims, wherein a plurality of samples of human neuronal cells are provided, and wherein the expression of a different target gene is altered in each sample of human neuronal cells.

6.5. The method according to claim 54, wherein the expression of a different target gene is altered in each sample using a human RNAi library.

7.6. The method according to any one of the preceding claims, further comprising determining whether the target gene is a direct regulator of clostridial neurotoxin activity or an indirect regulator of clostridial neurotoxin activity.

8.7. The method according to claim 76, wherein the direct regulator regulates clostridial neurotoxin activity at the level of:

(i) binding of the clostridial neurotoxin to the cell;
(ii) internalisation of the clostridial neurotoxin;
(iii) translocation of the clostridial neurotoxin L-chain out of the endosome;
(iv) catalysis; and/or
(v) persistence of the L-chain activity within the cell cytoplasm.

9.8. The method according to claim 7 6 or 87, wherein the indirect regulator regulates cellular trafficking of a clostridial neurotoxin receptor.

10.9. The method according to any one of claims 76-98, wherein the determining comprises detecting the presence or absence of a clostridial neurotoxin receptor of the cell when expression of the target gene has been altered.

11.10. The method according to any one of claims 76-109, wherein:

detecting a decreased amount of a clostridial neurotoxin receptor on the surface of the cell when expression of the target gene has been altered (preferably downregulated) when compared to an equivalent cell contacted with clostridial neurotoxin in which the expression of the target gene is unaltered indicates that the target gene indirectly regulates clostridial neurotoxin activity; or

detecting an equivalent or greater (preferably greater) amount of a clostridial neurotoxin receptor on the surface of the cell when expression of the target gene has been altered (preferably downregulated) when compared to an equivalent cell contacted with clostridial neurotoxin in which the expression of the target gene is unaltered indicates that the target gene directly regulates clostridial neurotoxin activity.

~~12. The method according to any one of claims 9-11, wherein the clostridial neurotoxin receptor is Synaptic Vesicle Glycoprotein 2A (SV2).~~

~~13.~~11. A method for identifying an agent that regulates clostridial neurotoxin activity, the method comprising:

(a) providing a sample of human neuronal cells expressing a polypeptide that comprises a C-terminal detectable label, wherein the polypeptide is cleavable by a clostridial neurotoxin;

(b) contacting the cells with the clostridial neurotoxin and an agent, wherein the contacting is sequential or simultaneous;

(c) measuring an amount of C-terminal detectable label, thereby quantifying clostridial neurotoxin activity; and

(d) identifying the agent as a regulator of clostridial neurotoxin activity when the quantified clostridial neurotoxin activity is different to the quantified clostridial neurotoxin activity in the absence of the agent; or

(e) identifying that the agent is not a regulator of clostridial neurotoxin activity when the quantified clostridial neurotoxin activity is equivalent to the quantified clostridial neurotoxin activity in the absence of the agent.

~~14.~~ The method according to claim ~~13~~11, wherein the agent is identified as a negative regulator of clostridial neurotoxin activity when the quantified clostridial neurotoxin activity is less than the quantified clostridial neurotoxin activity in the absence of the agent~~.~~

~~15.~~12. ~~The method according to claim 13 or 14~~, and/or wherein the agent is identified as a positive regulator of clostridial neurotoxin activity when the quantified clostridial neurotoxin activity is greater than the quantified clostridial neurotoxin activity in the absence of the agent.

~~16.~~ The method according to ~~any one of~~ claim~~s~~ ~~13~~11~~-15~~ or 12, further comprising determining whether the agent is a direct regulator of clostridial neurotoxin activity or an indirect regulator of clostridial neurotoxin activity, optionally ~~.~~

~~17.~~13. ~~The method according to claim 16,~~ wherein the direct regulator regulates clostridial neurotoxin activity at the level of:

(i) binding of the clostridial neurotoxin to the cell;

(ii) internalisation of the clostridial neurotoxin;

(iii) translocation of the clostridial neurotoxin L-chain out of the endosome;

(iv) catalysis; and/or

(v) persistence of the L-chain activity within the cell cytoplasm.

~~18.~~ The method according to claim ~~16 or 17~~ 13, wherein the indirect regulator regulates cellular trafficking of a clostridial neurotoxin receptor~~.~~

~~19.~~14. ~~The method according to any one of claims 16-18~~, and/or wherein the determining comprises detecting the presence or absence of a clostridial neurotoxin receptor of the cell when the cell has been targeted with the agent.

~~20.~~15. The method according to ~~any one of~~ claims 13 or ~~16~~14~~-19~~, wherein:

detecting a decreased amount of a clostridial neurotoxin receptor on the surface of the cell when the cell has been contacted with the agent when compared to an equivalent cell contacted with clostridial neurotoxin that has not been contacted with the agent indicates that the agent indirectly regulates clostridial neurotoxin activity; or

detecting an equivalent or greater (preferably greater) amount of a clostridial neurotoxin receptor on the surface of the cell when the cell has been contacted with the agent when compared to an equivalent cell contacted with clostridial neurotoxin that has not been contacted with the agent indicates that the agent directly regulates clostridial neurotoxin activity.

~~21.~~16. The method according to any one of claims 8-10 or ~~18~~14~~-20~~15, wherein the clostridial neurotoxin receptor is Synaptic Vesicle Glycoprotein 2A (SV2).

~~22. A human neuronal cell expressing a polypeptide, wherein the polypeptide is cleavable by a clostridial neurotoxin and comprises a C-terminal detectable label.~~

~~23.~~17. The method according to any one of the preceding claims ~~1-21 or the cell according to claim 22~~, wherein the human neuronal cell is a non-cancer cell.

~~24.~~18. The method ~~or cell~~ according to any one of the preceding claims, wherein the human neuronal cell is an immortalized human neural progenitor cell, or preferably wherein the human neuronal cell has been derived (e.g. differentiated) from an immortalized human neural progenitor cell.

~~25.~~19. The method ~~or cell~~ according to any one of the preceding claims, wherein the polypeptide further comprises an N-terminal detectable label, and wherein the N-terminal detectable label is different to the C-terminal detectable label.

~~26.~~20. The method ~~or cell~~ according to claim ~~25~~19, wherein one of the detectable labels is red fluorescent protein (RFP) and one of the detectable labels is selected from green fluorescent protein (GFP), cyan fluorescent protein (CFP), and yellow fluorescent protein (YFP).

~~27.~~21. The method ~~or cell~~ according to any one of the preceding claims, wherein the polypeptide comprises an N-terminal RFP and a C-terminal GFP.

~~28.~~22. The method ~~or cell~~ according to any one of the preceding claims, wherein the polypeptide:

(a) is encoded by a nucleotide sequence comprising:

(i) a nucleotide sequence having at least 70%, 80%, or 90% sequence identity to SEQ ID NO: 3;

(ii) a nucleotide sequence having at least 70%, 80%, or 90% sequence identity to SEQ ID NO: 9; and/or

(iii) a nucleotide sequence having at least 70%, 80%, or 90% sequence identity to SEQ ID NO: 11; or

(b) is encoded by a nucleotide sequence having at least 70%, 80%, or 90% sequence identity to SEQ ID NO: 1; or

(c) comprises a polypeptide sequence comprising:

(i) a polypeptide sequence having at least 70%, 80%, or 90% sequence identity to SEQ ID NO: 4;

~~(1)~~(ii) a polypeptide sequence having at least 70%, 80%, or 90% sequence~~;~~ identity to SEQ ID NO: 10; and/or

~~(2)~~(iii) a polypeptide sequence having at least 70%, 80%, or 90% sequence identity to SEQ ID NO: 12; or

~~(d)~~ comprises a polypeptide sequence having at least 70%, 80%, or 90% sequence identity to SEQ ID NO: 2.

~~29. The method or cell according to any one of the preceding claims, wherein the polypeptide:~~

~~(a) is encoded by a nucleotide sequence comprising:~~

~~(i)a nucleotide sequence having at least 80% sequence identity to SEQ ID NO: 3;~~
~~(ii)a nucleotide sequence having at least 80% sequence identity to SEQ ID NO: 9; and/or~~
~~(iii)a nucleotide sequence having at least 80% sequence identity to SEQ ID NO: 11; or~~
~~(b) is encoded by a nucleotide sequence having at least 80% sequence identity to SEQ ID NO: 1; or~~
~~(c) comprises a polypeptide sequence comprising:~~
~~(i)a polypeptide sequence having at least 80% sequence identity to SEQ ID NO: 4;~~
~~(ii)a polypeptide sequence having at least 80% sequence identity to SEQ ID NO: 10; and/or~~
~~(iii)a polypeptide sequence having at least 80% sequence identity to SEQ ID NO: 12; or~~
~~(d) comprises a polypeptide sequence having at least 80% sequence identity to SEQ ID NO: 2.~~

~~30. The method or cell according to any one of the preceding claims, wherein the polypeptide:~~
~~(a) is encoded by a nucleotide sequence comprising:~~
~~(i)a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 3;~~
~~(ii)a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 9; and/or~~
~~(iii)a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 11; or~~
~~(b) is encoded by a nucleotide sequence having at least 90% sequence identity to SEQ ID NO: 1; or~~
~~(c) comprises a polypeptide sequence comprising:~~
~~(i)a polypeptide sequence having at least 90% sequence identity to SEQ ID NO: 4;~~
~~(ii)a polypeptide sequence having at least 90% sequence identity to SEQ ID NO: 10; and/or~~
~~(iii)a polypeptide sequence having at least 90% sequence identity to SEQ ID NO: 12; or~~
~~(d) comprises a polypeptide sequence having at least 90% sequence identity to SEQ ID NO: 2.~~

~~31.~~23. The method ~~or cell~~ according to any one of the preceding claims, wherein the polypeptide:

(a) is encoded by a nucleotide sequence comprising:

(i) SEQ ID NO: 3;

(ii) SEQ ID NO: 9; and

(iii) SEQ ID NO: 11; or

(b) is encoded by a nucleotide sequence comprising SEQ ID NO: 1; or

(c) comprises:

(i) SEQ ID NO: 4;

(ii) SEQ ID NO: 10; and

(iii) SEQ ID NO: 12; or

(d) comprises or consists of SEQ ID NO: 2.

~~32. A nucleotide sequence encoding a polypeptide, wherein the polypeptide is cleavable by a clostridial neurotoxin and comprises an N-terminal RFP and a C-terminal GFP, wherein the nucleotide sequence comprises:~~

~~(a) a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 3;~~

~~(b) a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 9; and~~

~~(c) a nucleotide sequence having at least 70% sequence identity to SEQ ID NO: 11.~~

~~33. The nucleotide sequence according to claim 32, wherein the nucleotide sequence has at least 70% sequence identity to SEQ ID NO: 1.~~

~~34. The nucleotide sequence according to claim 32 or 33, wherein the nucleotide sequence has at least 80% sequence identity to SEQ ID NO: 1.~~

~~35. The nucleotide sequence according to any one of claims 32-34, wherein the nucleotide sequence has at least 90% sequence identity to SEQ ID NO: 1.~~

~~36. The nucleotide sequence according to any one of claims 32-35, wherein the nucleotide sequence comprises SEQ ID NO: 1.~~

~~37. The method or cell according to any one of claims 1-31 or the nucleotide sequence according to any one of claims 32-36, wherein the nucleotide sequence consists of SEQ ID NO: 1.~~

~~38. A vector comprising the nucleotide sequence according to any one of claims 32-37.~~

~~39. A polypeptide that is cleavable by a clostridial neurotoxin and comprises an N-terminal RFP and a C-terminal GFP, wherein the polypeptide comprises:~~

~~(a) a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 4;~~
~~(b) a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 10; and~~
~~(c) a polypeptide sequence having at least 70% sequence identity to SEQ ID NO: 12.~~

~~40. The polypeptide according to claim 39, wherein the polypeptide sequence has at least 70% sequence identity to SEQ ID NO: 2.~~

~~41. The polypeptide according to claim 39 or 40, wherein the polypeptide sequence has at least 80% sequence identity to SEQ ID NO: 2.~~

~~42. The polypeptide according to any one of claims 39-41, wherein the polypeptide sequence has at least 90% sequence identity to SEQ ID NO: 2.~~

~~43. The polypeptide according to any one of claims 39-42, wherein the polypeptide sequence comprises SEQ ID NO: 2.~~

~~44. The method or cell according to any one of claims 1-31 or 37 or the polypeptide according to any one of claims 39-43, wherein the polypeptide sequence consists of SEQ ID NO: 2.~~

~~45.~~24. The method according to any one of the preceding claims ~~1-31, 37 or 44~~, wherein the method comprises contacting the cells with a buffer comprising glial cell-derived neurotrophic factor (GDNF), cell-permeable cyclic adenosine monophosphate (cAMP), $CaCl_2$ and KCl.

~~46.~~25. The method according to claim ~~45~~24, wherein the GDNF is present in the buffer at a concentration of 1-100 ng/ml, cAMP is present in the buffer at a concentration of 0.1-5 mM, $CaCl_2$ is present in the buffer at a concentration of 0.1-7 mM and/or KCl is present in the buffer at a concentration of 1-100 mM.

~~47. A kit comprising:~~
~~(a) the cell according to any one of claims 22-31, 37 or 44; or~~
~~(b) the nucleotide sequence according to any one of claims 32-37; or~~
~~(c) the vector according to claim 38; and~~
~~(d) optionally instructions for use of the same.~~

48. The kit according to claim 47, further comprising a buffer, said buffer comprising GDNF, cell-permeable cyclic adenosine monophosphate (cAMP), $CaCl_2$ and KCl.

49. A composition comprising:

(a) a clostridial neurotoxin; and

(b) a buffer, the buffer comprising GDNF, cell-permeable cyclic adenosine monophosphate (cAMP), $CaCl_2$ and KCl.

50. The kit according to claim 48 or the composition according to claim 49, wherein the GDNF is present in the buffer at a concentration of 1-100 ng/ml, cAMP is present in the buffer at a concentration of 0.1-5 mM, $CaCl_2$ is present in the buffer at a concentration of 0.1-7 mM and KCl is present in the buffer at a concentration of 1-100 mM.