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(54) Title: HIGH MAST2-AFFINITY POLYPEPTIDES AND USES THEREOF

(57) Abrégé/Abstract:
The invention relates to polypeptides containing a cytoplasmic domain ending with a MAST-2 binding domain, from 11 to 13 residues, the first two residues of which are S and W, and the last four residues of which are Q, T, R and L, said polypeptides presenting a high affinity for the PDZ domain of the human MAST2 protein. The invention also relates to polynucleotides, vectors, lentiviral particles, cells as well as compositions comprising the same. The invention is also directed to the use of said polypeptides, polynucleotides, vectors, lentiviral particles, cells and compositions in the treatment and/or prevention of a disease, disorder or condition, which alters the Central Nervous System (CNS) and/or the Peripheral Nervous System (PNS). The invention also concerns molecular signatures of cellular genes to determine the neurosurvival and/or neuroprotection activity of a molecule.

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(54) Title: HIGH MAST2-AFFINITY POLYPEPTIDES AND USES THEREOF

(57) Abstract: The invention relates to polypeptides containing a cytoplasmic domain ending with a MAST-2 binding domain, from 11 to 13 residues, the first two residues of which are S and W, and the last four residues of which are Q, T, R and L, said polypeptides presenting a high affinity for the PDZ domain of the human MAST2 protein. The invention also relates to polynucleotides, vectors, lentiviral particles, cells as well as compositions comprising the same. The invention is also directed to the use of said polypeptides, polynucleotides, vectors, lentiviral particles, cells and compositions in the treatment and/or prevention of a disease, disorder or condition, which alters the Central Nervous System (CNS) and/or the Peripheral Nervous System (PNS). The invention also concerns molecular signatures of cellular genes to determine the neurosurvival and/or neuroprotection activity of a molecule.

HIGH MAST2-AFFINITY POLYPEPTIDES AND USES THEREOF

FIELD OF THE INVENTION

The invention relates to polypeptides containing a cytoplasmic domain ending with a MAST-2 binding domain, the first two residues of which are S and W, and the last four residues of which are Q, T, R and L, said polypeptides presenting a high affinity for the PDZ domain of the human MAST2 protein. The invention also relates to polynucleotides, vectors, lentiviral particles, cells as well as compositions comprising the same. The invention is also directed to the use of said polypeptides, polynucleotides, vectors, lentiviral particles, cells and compositions in the treatment and/or prevention of a disease, disorder or condition, which alters the Central Nervous System (CNS) and/or the Peripheral Nervous System (PNS). The invention also concerns molecular signatures of cellular genes to determine the neurosurvival and/or neuroprotection activity of a molecule.

BACKGROUND OF THE INVENTION

During development of the nervous system, neurons extend axons over considerable distances in order to innervate their targets in an appropriate manner. This involves the stimulation in the cells of specific signaling pathways which can stimulate the activity of the growth cone.

While the developing nervous system, more particularly the developing central nervous system, is highly plastic, the adult nervous system, more particularly the adult brain, has more limited repair potential. Therefore, neurite-axon outgrowth and protection against degeneration are important factors to be considered to improve the outcome of a neurodegenerative disease, disorder or condition, such as an acute injury of the nervous system or a chronic neurodegenerative disorder. Products, which would be capable of inducing neurite outgrowth from such neuronal cells, would bring a very useful therapeutic and/or preventive and/or palliative solution to such diseases, disorders or conditions.

At the other side of the neuron developmental process, the proliferation of neuronal progenitors, which do not differentiate into matured neuronal structures, leads to nervous system neoplasm. Products, which would be capable of inducing neurite outgrowth from such progenic cells, would bring a therapeutic and/or preventive and/or palliative solution to such neoplasms.

It has been described that the pathogenicity of a rabies virus strain is inversely correlated with its ability to induce apoptosis (WO 03/048198; Ugolini 1995; Sarmento *et al.* 2005; Ugolini 2008; Jackson *et al.* 2008). Therefore, the more virulent a rabies virus strain is, the less apoptotic. The findings that virulent rabies virus strains, such as CVS strains, do not induce neuron apoptosis and explain why virulent rabies virus strains can propagate so extensively within the CNS before the appearance of signs and symptoms of the disease

More recently, Préhaud et al. (2010) reported that the C-terminal region of the cytoplasmic domain of the G protein of rabies viruses is involved in the binding of the G protein to the PDZ domain of the human microtubule associated serine threonine kinase 2 MAST2 protein. This C-terminal region bears a four amino-acid motif called PDZ-BS (PDZ binding site) which has the sequence QTRL in virulent rabies strains and the sequence ETRL in attenuated strains. Thus, the G protein of virulent rabies virus strain has been shown to bind with a high affinity to MAST-1 and MAST-2 but to not bind PTPN4, DLG2 and MPDZ. In contrast, the G protein of attenuated rabies virus strain has been shown to bind with MAST-1, MAST-2, PTPN4, DLG2 and MPDZ. This difference regarding the binding partners of the G protein of virulent and attenuated rabies virus strains seems to be correlated with the difference of virulence of these strains (Figure 2).

Thus, as demonstrated in application WO2010/116258, the nature of the amino acid residues at positions 491(H/L) and 521(Q/E) of the G protein of rabies viruses is important for the effects on neuron survival and on neurite outgrowth. The G protein of a virulent rabies virus strain presenting a H residue at position 491 and a Q residue at position 521 is non-apoptotic and favours neurite outgrowth. In contrast, the G protein of an attenuated rabies virus strain presenting a L residue at position 491 and a E residue at position 521 is apoptotic and does not promote neurite outgrowth.

Based on these results, there is a need in the art to identify and to design means having improved properties in the promotion of neurite outgrowth and in neurosurvival. The invention provides means for the regeneration and protection of neurons, which derive from the G protein of rabies virus strains, and which show unexpected properties. The invention also concerns means for the screening of molecules suitable for the regeneration and protection of neurons.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention provides a polypeptide, of at most 350 amino acids, comprising a cytoplasmic domain, wherein said cytoplasmic domain ends with a MAST-2 binding domain, wherein the size of said MAST-2 binding domain is from 11 to 13 amino acid residues, the first two residues of said MAST-2 binding domain are S and W, and the last four residues of said MAST-2 binding domain are Q, T, R and L, said MAST-2 binding domain being selected from the group consisting of:

(A) a sequence, whose size is 11 residues, consisting of $SWX_1X_2X_3X_4X_5QTRL$, wherein each of X_1, X_2, X_3, X_4 and X_5 is any amino acid residue (SEQ ID NO:19);

(B) a sequence, whose size is 11 residues, selected from the group consisting of: $SWX_1KSGGQTRL$ (SEQ ID NO:76), $SWX_1SSGGQTRL$ (SEQ ID NO:77), $SWX_1SHGGQTRL$ (SEQ ID NO:78), $SWX_1SHKGQTRL$ (SEQ ID NO:79), $SWX_1SHKSQTRL$ (SEQ ID NO:80), $SWX_1HSGGQTRL$ (SEQ ID NO:86), $SWX_1HKGGQTRL$ (SEQ ID NO:87), $SWX_1HKSGQTRL$ (SEQ ID NO:88), $SWX_1SKGGQTRL$ (SEQ ID NO:89), $SWX_1SKSGQTRL$ (SEQ ID NO:90), $SWX_1SHSGQTRL$ (SEQ ID NO:91), $SWX_1SHKGQTRL$ (SEQ ID NO:92) and $SWX_1SKGGQTRL$ (SEQ ID NO:93), wherein X_1 is any amino acid;

(C) a sequence, whose size is 12 residues, consisting of $SWX_1X_2X_3X_4X_5X_6QTRL$, wherein each of X_1, X_2, X_3, X_4, X_5 and X_6 is any amino acid residue;

(D) a sequence, whose size is 12 residues, selected from the group consisting of: $SWX_1HKSGGQTRL$ (SEQ ID NO:102), $SWX_1SKSGGQTRL$ (SEQ ID NO:103), $SWX_1SHSGGQTRL$ (SEQ ID NO:104), $SWX_1SHKGGQTRL$ (SEQ ID NO:105) and $SWX_1SHKSGQTRL$ (SEQ ID NO:106), wherein X_1 is any amino acid; and

(E) a sequence, whose size is 13 residues, consisting of $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, wherein each of X_1, X_2, X_3, X_4, X_5, X_6 and X_7, is any amino acid residue (SEQ ID NO:192), wherein said sequence does not consist of SWESHKSGGQTRL (SEQ ID NO:1),

wherein said polypeptide presents a binding affinity for the PDZ domain of the human MAST2 protein which is higher than the binding affinity of rabies virus G protein comprising the SWESHKSGGQTRL (SEQ ID NO: 1) sequence for the PDZ domain of the MAST2 protein.

The invention provides a polynucleotide encoding the polypeptide as defined herein, the sequence of which is at most 1050 nucleotides.

The invention provides a vector comprising the polynucleotide as defined herein.

The invention provides an expression lentivirus-derived vector, comprising the polynucleotide as defined herein, expression regulatory elements of said polynucleotide, a cis-acting central initiation region (cPPT) and a cis-acting termination region (CTS) both of lentiviral origin and regulatory signals of retroviral origin for reverse transcription, expression and packaging.

The invention provides a lentiviral vector pseudotyped particle comprising GAG structural proteins and a viral core made of (a) POL proteins and (b) a lentiviral vector genome comprising the polynucleotide as defined herein, expression regulatory elements of said polynucleotide, a cis-acting central initiation region (cPPT) and a cis-acting termination region (CTS) both of lentiviral origin and regulatory signals of retroviral origin for reverse transcription, expression and packaging, wherein said particle is pseudotyped with the G protein of a VSV virus or the G protein of a rabies virus.

The invention provides a cell or a cell culture transfected with the vector as defined herein or transduced by the lentiviral particle as defined herein.

The invention provides a cell transfected with the vector as defined herein or transduced by the lentiviral particle as defined herein.

The invention provides a composition comprising the polypeptide as defined herein, the polynucleotide as defined herein, the vector as defined herein, the lentiviral particle as defined herein or the cell as defined herein, and a pharmaceutically acceptable vehicle, excipient or carrier.

The invention provides a use of the polypeptide as defined herein, the polynucleotide as defined herein, the vector as defined herein, the lentiviral particle as defined herein, the cell or cell culture as defined herein or the composition as defined herein, in the treatment or and/or palliation and/or prevention of a neoplasm of the nervous system.

The invention provides a use of the polypeptide as defined herein, the polynucleotide as defined herein, the vector as defined herein, the lentiviral particle as defined herein, the cell as defined herein or the composition as defined herein, in the treatment or and/or palliation and/or prevention of a neoplasm of the nervous system.

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The invention provides a use of the polypeptide as defined herein, the polynucleotide as defined herein, the vector as defined herein, the lentiviral particle as defined herein, the cell or cell culture as defined herein or the composition as defined herein, in protecting neurons from neurotoxic agents or oxidative stress.

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The invention provides a use of the polypeptide as defined herein, the polynucleotide as defined herein, the vector as defined herein, the lentiviral particle as defined herein, the cell as defined herein or the composition as defined herein, in the treatment or and/or palliation and/or prevention of a neurogenerative disease, disorder or condition.

The invention provides a use of the polypeptide as defined herein, the polynucleotide as defined herein, the vector as defined herein, the lentiviral particle as defined herein, the cell or cell culture as defined herein or the composition as defined herein, in the treatment and/or palliation and/or prevention of a physical or ischemic injury of the nervous system, or of a disease, disorder or condition involving such an injury.

The invention provides a use of the polypeptide as defined herein, the polynucleotide as defined herein, the vector as defined herein, the lentiviral particle as defined herein, the cell as defined herein or the composition as defined herein, in the treatment and/or palliation and/or prevention of a physical or ischemic injury of the nervous system, or of a disease, disorder or condition involving such an injury.

The invention provides a use of the polypeptide as defined herein, the polynucleotide as defined herein, the vector as defined herein, the lentiviral particle as defined herein, the cell or cell culture as defined herein or the composition as defined herein, in the treatment or and/or palliation and/or prevention of a leukemia.

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The invention provides a use of the polypeptide as defined herein, the polynucleotide as defined herein, the vector as defined herein, the lentiviral particle as defined herein, the cell or cell culture as defined herein or the composition as defined herein, for the preparation of a medicament in the treatment or and/or palliation and/or prevention of a neoplasm of the nervous system.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Rabies virus (RABV) protein G processing inside the cells: upon translation, the protein G, which is a transmembrane type I glycoprotein, is synthesized at the Endoplasmic Reticulum (ER), then processed through the secretory pathway to reach the Golgi apparatus where it is glycosylated on its extracellular domain. Then, the protein is delivered to the cytoplasmic membrane where it is anchored via its transmembrane domain. The cytoplasmic domain of G protein is always in contact with the cytoplasm.

Figure 2. (A) Schematic representation of the model of action of polypeptides of the invention (Neurovita polypeptides) through the interaction with their cellular partner, the human MAST2 protein (Microtubule associated serine-threonine kinase 2) (B) Schematic representation of the involvement of the PI3K/Akt signalling pathway in neuron physiology.

Figure 3. Identification of kinases stimulated during RABV mediated neuroprotection: Kinome profiling in human post mitotic neurons NT2-N. (A) Slide of peptide microarrays covering the entire human kinome. (B) Schematic representation of the kinome profiling obtained for NT2-N cells infected with the recombinant rRABV (RABV-CVSHQ) for 45h. The dots represent the kinases which are activated upon rRABV neurosurvival infection in NT2-N cells.

Figure 4. (A) Schematic representation of G protein (first line) and Neurovita 1 polypeptide (second line); SP: Signal peptide, EC: extracellular domain, TM:

transmembrane domain, Cyto: Cytoplasmic domain, PDZ-BS: PDZ binding site. The number of amino acid residues (aa) for each domain is also indicated (B) Expression of Neurovita1 and Neurovita 1 delta PDZ-BS by lentivectors in NS cells or human neuroblastoma cells (SH-SY5Y) by Western Blot 48h post infection (p.i.) (1. Neurovita1; 2. Neurovita1 delta PDZ-BS; 3. Negative control) (C) Expression of Neurovita1 and Neurovita1 delta PDZ-BS by lentiviral vectors in NS cells by immunofluorescence 48h p.i.. In (B) and (C), detection was carried out with antibodies specific for RABV Cyto-G.

Figure 5. Neurovita 1 triggers neurite outgrowth in NS and SH-SY5Y in a PDZ-BS dependent manner (A) Neurite outgrowth assay in SH-SY-5Y human neuroblastoma cells following lentiviral vectors infection (30h, p.i.). Cells were treated with db c-AMP (10µM) (B) Neurite outgrowth assay in NS cells following lentiviral vectors infection (72h p.i.). Cells were treated with NGF (200ng/ml) (*: $p<0.05$ student's t test).

Figure 6. (A) Identification of the genetic molecular signature of Neurovita1-mediated neuroprotection; the gene expression was measured in NT2-N cells, 24h p.i., with Neurovita1-expressing lentiviral vectors, on a Human Neurogenesis and Neural Stem Cell PCR and PI3K-Akt Signaling Pathway Arrays (B) Schematic representation of the Neurovita1 genetic molecular signature obtained with the pathway-focused gene expression profiling (qRT-PCR). The cluster of genes represents the genes regulated following Neurovita1 infection but not regulated in non-infected culture or culture infected with Neurovita1 delta PDZ-BS (dots are Neurovita 1 specific genes; diamonds are connected genes).

Figure 7. Molecular signature of Neurovita 1 in absence of *MAST2*; Genetic molecular signature (24h p.i.) following infection by Neurovita1-expressing lentivector of NT2-N cells in which the MAST2 expression was knocked out by infection with Sh RNA MAST2 specific recombinant lentiviruses, 48h before infection with lentivector Neurovita1 on Human Neurogenesis and Neural Stem Cell PCR and PI3K-Akt Signaling Pathway Arrays.

Figure 8. Lentivector Neurovita 1 favours wound healing of NT2-N axons (A) Representation of the scratch assay (B) Illustration of regeneration 6 days post wounding (C) Comparison of axon regeneration after lentivector Neurovita1 or Neurovita 1 delta PDZ-BS infection.

Figure 9. Molecular signatures of Neurovita1 mediated axon regeneration; the pathway-focused gene expression profiling was established on NT2-N cell culture two days post wounding. (A) genes involved in PI3K/Akt signalling pathway (Human PI3K-AKT Signaling PCR array) (B) genes involved in cell proliferation, adhesion, differentiation, growth factors and synaptic functions (Human Neurogenesis and Neural Stem Cell PCR Array). (C) Schematic representation of the gene cluster involved in Neurovita1 mediated axon regeneration (dots are neurovita-specific genes; diamonds are related genes).

Figure 10. Structure/function analysis (A) Sequence and three-dimensional organization of the Neurovita 1 sequence. (B) Relationship between the affinity of the polypeptides of the invention (Neurovita polypeptides) for MAST2-PDZ and their neurosurvival properties.

Figure 11. (A) Schematic representation of the polypeptides of the invention; SP: Signal peptide, EC: extracellular domain, TM: transmembrane domain, Cyto: Cytoplasmic domain. The number of amino acid residues (aa) for each domain is also indicated (B) Protein sequence of 2 particular polypeptides of the invention (Neurovita2 and Neurovita3) and comparison with the sequences of Neurovita1 and Neurovita1 delta PDZ-BS polypeptides (C) Expression of Neurovita1, Neurovita1 delta PDZ-BS and Neurovita2 by lentiviral vectors (lentivectors) in NS cells, measured by western blotting 48h p.i., with antibodies specific for RABV Cyto-G (1: Neurovita2 ; 2: Neurovita1 delta PDZ-BS ; 3: Neurovita1, and 4: negative control).

Figure 12. Neurite outgrowth assay in NS cells following lentivectors infection (72h p.i.). Cells were treated with NGF (200ng/ml) (N.I. non-infected cells).

Figure 13. Neurovitas induce neurite arborisation in NS cells (A) Schematic redrawing of the arborisation of representative NS cells either infected with Neurovita2 (upper photo) or Neurovita1 (lower photo) (B) Complexity of the neurite tree measured by Sholl analysis on NS culture infected for 72h with Neurovita1 (black squares) and Neurovita2 (black circles) lentivectors.

Figure 14. Identification of the genetic molecular signature of Neurovita2. (A) Down regulation of POU4F1, DRD2, FOS and BTK, in NT2-N cells, 24h p.i., by lentivectors (B) Schematic representation of the Neurovita2 genetic molecular signature obtained with the pathway-focused gene expression profiling (qRT-PCR). The cluster of genes represents the genes regulated following Neurovita2 infection but not regulated in non-infected culture or culture infected with Neurovita1 delta PDZ-BS (dots are neurovita specific genes; diamonds are related genes).

Figure 15. Transcription of (A) the rRABV at 24h p.i. and (B) of the lentivectors at 48h p.i., in NT2-N cells following Neurovita lentiviral vectors infection. Transcription was measured by RT-QPCR.

Figure 16. Activation of innate immune genes (A) Transcription of a representative set of immunity genes (in NT2-N cells), (A) after infection with rRABV CVS HQ and rRABV CVS HΔ4 (N.I.: non-infected) or (B) after infection with Neurovita1, Neurovita1 delta PDZ-BS and Neurovita2 lentivectors (neg: negative control).

Figure 17. Axon regeneration post wounding in NT2-N cells (A) expression of tyrosine hydroxylase (TH), a marker of dopaminergic neuron in a NT2-N cell (B) TH mRNA transcription in NT2-N culture, 18S as a standard (C) Axon regeneration after lentivector infection with Neurovita 1, in presence or absence of SHRNA against MAST-2 (si MAST2); N.I.: non-infected. Results are expressed as percentages of scratched neurons which regenerate (D) Axon regeneration after infection with Neurovita 1 or Neurovita2 lentivectors; N.I.: non-infected.

Figure 18. Complexity of the neurite tree measured by Sholl analysis on NS culture, 27h post lentivirus infection (A) with a negative control in absence (circles) or presence (squares) of KCl, or (B) after infection with Neurovita2 lentivector in presence of KCl (stars).

Figure 19. Neurite outgrowth assay (A) in non-infected (N.I.) NS cells in absence or presence of LiCl and (B) in LiCl-treated NS cells, non-infected (N.I.) or after infection with Neurovita1 (NV1), Neurovita1 delta PDZ-BS (NV1Δ) or Neurovita2 (NV2) lentivectors.

Figure 20. Comparison of neurite outgrowth triggered by G full, NV1 and NV1 cyto (A) Schematic representation of RABV G full, Neurovita 1 and cytosolic form of Neurovita 1 (NV1 cyto); SP: Signal peptide, EC: extracellular domain, TM: transmembrane domain, Cyto: Cytoplasmic domain, PDZ-BS: PDZ binding site. The number of amino acid residues (aa) for each domain is also indicated. (B) Neurite outgrowth assay after infection of NS with Neurovita 1, Neurovita1 delta PDZ-BS, RABV Gfull, RABV Gfull delta PDZ-BS, Neurovita1-cyto and Neurovita1-cyto delta PDZ-BS (N.I.: non-infected).

Figure 21. Expression of Neurovita molecules from a bicistronic lentivector in NS cells (A) schematic representation of the pLenti7.3 Neurovita bicistronic lentivector (B) mRNA relative fold increase of Neurovita1 delta PDZ-BS (NV1Δ), Neurovita1 (NV1), Neurovita2 (NV2) or Neurovita3 (NV3), 18S as a standard (C) GFP expression after infection of NS cells with NV1Δ-, NV1-, NV2- or NV3-expressing lentivector by flow cytometry; results are expressed as percentages of cells

expressing GFP in the culture; Neg is non-infected cells. (D) Expression of NV1Δ, NV1, NV2, or NV3 in NS cells by Western blotting. (E) Expression of tubulin as a internal protein loading control, in the corresponding lysates.

Figure 22. Neurite outgrowth triggered by NV3 in NS cultures (A) Schematic redrawing of the arborisation of representative NS cells either non-infected (left panel) or infected with Neurovita3 (right panel); (B) Neurite outgrowth assay in NS cells following infection with NV1Δ, NV1, NV2, or NV3 lentivectors; (C) Student's t-test ($p<0.05$).

Figure 23. Tree arborisation triggered by NV3 in NS cultures (A) Complexity of the neurite tree measured by Sholl analysis on NS cells, either non-infected versus infected with Neurovita1, infected with Neurovita1 versus infected with Neurovita3 or infected with Neurovita2 versus infected with Neurovita3; (B) two way ANOVA ($p<0.05$).

Figure 24. Construction of NV3 cytosolic (NV3 cyto) lentivector (A) Schematic representation of Neurovita 3 and cytosolic form of Neurovita 3 (NV3 cyto); SP: Signal peptide, EC: extracellular domain, TM: transmembrane domain, Cyto: Cytoplasmic domain, PDZ-BS: PDZ binding site. The number of amino acid residues (aa) for each domain is also indicated; (B) schematic representation of the pLenti7.3 Neurovita bicistronic lentivector expressing NV3 or NV3-cyto ; (C) relative fold increase of NV3 and NV3cyto, 18S as a standard ; (D) GFP expression after infection with NV3 or NV3cyto-expressing lentivector by cytofluorimetry; results are expressed as percentages of GFP positive cells in the culture; neg is non-infected cells.

Figure 25. Comparison of NV3-cyto induced neurite outgrowth with those of NV1 and NV3 (A) Schematic redrawing of the arborisation of representative NS cells infected with NV1, NV3 or NV3-cyto; (B) Neurite outgrowth assay in NS cells following infection with NV1, NV2, NV3 or NV3-cyto lentivectors; (C) Student's t-test ($p<0.05$).

Figure 26. Comparison of NV3-cyto induced neurite trees with those of NV1 and NV3 (A) Complexity of the neurite tree measured by Sholl analysis on NS culture, either infected with NV1 versus infected with NV3cyto or infected with NV3 versus infected with NV3cyto; (B) two way ANOVA ($p<0.05$).

Figure 27. Neuritogenesis in culture of E16 mouse foetal cortical neurons induced by NV1 and NV1delta. Representative pictures of βIII tubulin (neuronal form) stain E16 mouse cortical neurons 3 days *in vitro* (A) non-infected or 3 days infected by NV1 (B) or NV1Δ (C) lentivectors; (D) Neurite outgrowth assay in cortical neurons following infection with NV1 or NV1Δ lentivectors.

Figure 28. Toxicity assays in new born mice injected by the intracerebral route with NV1 or NV1delta lentivectors (A) Weight determined in non-injected mice or mice injected with NV1 or NV1Δ lentivectors; (B) Expression of NV1 or NV1Δ transcripts in brain, 3 months after lentivirus infection, 18S as a standard.

Figure 29. Phenotype of mice injected with NV1 or NV1Δ lentivectors into brain. (A) NV1, day 4 post injection (pi) ; (B) mice injected with NV1, day 20 pi; (C) mice injected with NV1Δ, day 4 pi ; (D) mice injected with NV1Δ, day 20 pi ; Arrow represents a non injected (N.I.) mouse (cut tail).

Figure 30. Immunostaining of brains (striatum) of mice injected by the intracerebral route with NV1 lentivectors. (A) immunostaining of striatum with GFP fluorescence (green), Map2 staining (red) and GFAP staining (purple) (B) immunostaining of dendritic-axonal tree with GFP fluorescence (green), Map2 staining (red) and GFAP staining (purple).

Figure 31. Striatum immunostaining of mice injected with NV1Δ lentivectors into brain; GFP fluorescence (green), Map2 staining (red) and GFAP staining (purple).

DETAILED DESCRIPTION

In the present application, the inventors have unexpectedly shown that polypeptides, the sequence of which comprises the residues SW and the residues QTRL, having a high affinity for the PDZ domain of the human MAST2 protein, have particular interesting effect on the promotion of neurite outgrowth and on neurosurvival properties. Thus, the lower the constant of dissociation (K_D) of the complex formed by the polypeptides of the invention with the PDZ domain of the human MAST2 protein, the higher the neurosurvival properties of the polypeptide of the invention.

The invention is directed to a polypeptide as defined herein which presents a high affinity for the PDZ domain of the human MAST2 protein (SEQ ID NO: 6 for the full length human MAST2 protein and SEQ ID NO:7 for its PDZ domain).

In other words, the polypeptide of the invention as defined herein is designed in such a way that the constant of dissociation (K_D) of the complex that it forms with the PDZ domain of the human MAST2 protein is very low and as a consequence that its affinity for the PDZ domain of the human MAST2 protein is very high (the affinity being inverse to the K_D).

Accordingly, in a first embodiment, the polypeptide of the invention presents a binding affinity for the PDZ domain of the human MAST2 protein which is higher than the binding affinity for the PDZ domain of the MAST2 protein of a rabies virus G protein comprising the SWESHKSGGQTRL sequence (SEQ ID NO:1).

In a particular embodiment, the gain in affinity of the polypeptides of the invention as compared to a polypeptide having a MAST-2 binding domain consisting of SWESHKSGGQTRL (for example, ratio of K_D) ranges from 2.5 to 20, and in particular ranges from 5 to 20, from 5 to 15 or from 5 to 10.

In particular embodiment, the constant of dissociation (K_D) of the complex formed by the polypeptide of the invention with the PDZ domain of the human MAST2 protein is less than 1 μM, less than 0.8 μM, less than 0.5 μM, less than 0.4 μM or less than 0.3 μM. In a preferred embodiment, the constant of dissociation of the complex formed by the polypeptide of the invention with the PDZ domain of the human MAST2 protein is less than 0.2 μM, preferably less than 0.15 μM, more preferably less than 0.1 μM.

In a particular embodiment, the constant of dissociation (K_D) of the complex formed by the polypeptide of the invention with the PDZ domain of the human MAST2 protein (MAST2-PDZ) is measured by Isothermal Titration Calorimetry (ITC).

As a particular embodiment of ITC, the constant of dissociation of the complex formed by the polypeptide of the invention with the PDZ domain of the human MAST2 protein (MAST2-PDZ) is determined for a concentration of the polypeptide ranging from 250 μM to 350 μM (preferably in buffer containing 50 mM Tris-HCl, 150 mM NaCl, pH7.5) and an initial concentration of the MAST2-PDZ domain of 30 μM.

As a particular embodiment of ITC, the constant of dissociation of the complex formed by the polypeptide of the invention with the PDZ domain of the human MAST2 protein is measured as follows: the polypeptide of the invention is prepared, in a buffer containing 50 mM Tris-HCl, 150 mM NaCl , pH7.5, at initial concentrations ranging from 250 µM to 350 µM. ITC (Isothermal Titration Calorimetry) measurements are made using Microcal VP ITC200 isothermal titration calorimeter from Microcal (Northampton, MA), by titrating the MAST2-PDZ (at an initial concentration of 30 µM), at 298 K, by injection of the polypeptide of the invention as prepared above (each titration of a particular polypeptide involves 25-45 consecutive injections of aliquots of 5-7 µL at 6-min intervals). Raw data are normalized and corrected for heats of dilution of the polypeptides. Equilibrium dissociation constants are determined performing nonlinear curve fitting of the corrected data to a model with one set of sites using the Origin7.0 software (OriginLab).

The affinity of the polypeptides of the invention for the PDZ domain of the human PTPN4 protein is low, *i.e.,* the constant of dissociation (K_D) of the complex formed by the polypeptide of the invention with the PDZ domain of the human PTPN4 protein is high, in particular is more than 500 µM (for example as measured by ITC, in particular in the same conditions and with the same concentrations as for the MAST2-PDZ above). This high value of K_D (for the PDZ domain of the human PTPN4 protein) has been shown to be reached with the polypeptides of the invention in which the last four residues are Q, T, R and L.

Thus, polypeptides, having a high affinity for the PDZ domain of the human MAST2 protein and/or designed in such a way that the constant of dissociation (Kd) of the complex that it forms with the PDZ domain of the human MAST2 protein is within the above ranges, are herein described by the following structural features.

The invention accordingly relates to a polypeptide, of at most 350 amino acid residues, comprising or consisting of a cytoplasmic domain. The expression "*cytoplasmic domain*" means a protein domain ending with a MAST-2 binding domain as defined herein, and which is exposed in the cytoplasm of a cell, preferably when the polypeptide possesses a structure or sequence enabling its anchoring in the cell membrane. According to the invention, the polypeptide may comprise or not a structure or sequence enabling the anchoring of the polypeptide of the invention in the membrane. When the polypeptide does not possess the structure or sequence enabling the anchoring in the membrane, for example when the polypeptide consists of the cytoplasmic domain as defined herein, the polypeptide of the invention is cytosolic.

In a particular embodiment, the constant of dissociation (K_D) of the complex formed between the PDZ domain of the human MAST2 protein and a polypeptide of the invention which does not possess the structure or sequence enabling the anchoring in the membrane, in particular a polypeptide consisting of the cytoplasmic domain as defined herein, is less than 1µM, less than 0.5 µM, less than 0.4 µM or less than 0.3 µM, preferably less than 0.2 µM, less than 0.15 µM, and more preferably less than 0.1 µM.

In a particular embodiment, the invention relates to a polypeptide, of at most 350 amino acid residues, comprising (1) a signal peptide, (2) a domain for anchoring said polypeptide into the reticulum membrane and/or Golgi membrane (also called the anchoring domain), and (3) a domain which is exposed in the cytoplasm when the polypeptide is anchored in the membrane (also called the cytoplasmic domain). These domains are organised structurally in such a way that the signal peptide is N-terminal to the anchoring domain, which is itself N-terminal to the cytoplasmic domain. According to this embodiment, the polypeptide of the invention comprises, from N-terminal to C-terminal, (1) a signal peptide, (2) an anchoring domain, and (3) a cytoplasmic domain.

The cytoplasmic domain of the polypeptide of the invention ends with a MAST-2 binding domain, whose size is from 11 to 13 amino acid residues. By "*ends with*", it is meant that the 11 to 13 successive residues of the MAST-2 binding domain are the last C-terminal residues of the cytoplasmic domain, and in a particular embodiment the last C-terminal residues of the polypeptides of the invention.

The MAST-2 binding domain of the polypeptide of the invention consists of a sequence, whose size is from 11 to 13 residues, the first two residues of which are S and W, and the fourth last residues of which are Q, T, R and L (these 4 last amino acid residues represent the so-called PDZ-BS). The MAST-2 binding domain is defined according to one of the following groups, knowing that, whatever the group, the first two amino acid residues of the MAST-2 binding domain are S and W and the last four amino acid residues of the MAST-2 binding domain are Q, T, R and L.

(A) in a first group, the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first two residues of which are S and W, and the last four residues of which are Q, T, R and L, consisting of $SWX_1X_2X_3X_4X_5QTRL$, wherein each of X_1, X_2, X_3, X_4 and X_5 is any amino acid residue (SEQ ID NO:19).

In a particular embodiment, X_1 is E or A, more preferably E, such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first two residues of which are S, W and E, and the last four residues of which are Q, T, R and L, consisting of $SWEX_2X_3X_4X_5QTRL$ (SEQ ID NO:20) or $SWAX_2X_3X_4X_5QTRL$ (SEQ ID NO:21), wherein each of X_2, X_3, X_4 and X_5 is any amino acid residue.

In another embodiment, X_2 is S, E or V, more preferably V, such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first two residues of which are S and W and the last four residues of which are Q, T, R and L, consisting of $SWX_1VX_3X_4X_5QTRL$ (SEQ ID NO:22), $SWX_1EX_3X_4X_5QTRL$ (SEQ ID NO:23) or $SWX_1SX_3X_4X_5QTRL$ (SEQ ID NO:24), wherein each of X_1, X_3, X_4 and X_5 is any amino acid residue.

In a particular embodiment, X_3 is H, A or Y such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first two residues of which are S and W and the last four residues of which are Q, T, R and L, consisting of $SWX_1X_2HX_4X_5QTRL$ (SEQ ID NO:25), $SWX_1X_2AX_4X_5QTRL$ (SEQ ID NO:26) or $SWX_1X_2YX_4X_5QTRL$ (SEQ ID NO:27), wherein each of X_1, X_2, X_4 and X_5 is any amino acid residue.

In a particular embodiment, X_4 is G or T such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first two residues of which are S and W and the last four residues of which are Q, T, R and L, consisting of $SWX_1X_2X_3GX_5QTRL$ (SEQ ID NO:28) or $SWX_1X_2X_3TX_5QTRL$ (SEQ ID NO:29), wherein each of X_1, X_2, X_3 and X_5 is any amino acid residue.

In a particular embodiment, X_5 is G or Q such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first two residues of which are S and W and the last four residues of which are Q, T, R and L, consisting of $SWX_1X_2X_3X_4GQTRL$ (SEQ ID NO:30) or $SWX_1X_2X_3X_4QQTRL$ (SEQ ID NO:31), wherein each of X_1, X_2, X_3 and X_4 is any amino acid residue.

In a particular embodiment, X_1 is E and X_2 is S, E or V, more preferably V, such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first three residues of which are S, W and E, and the last four residues of which are Q, T, R and L, consisting of $SWEVX_3X_4X_5QTRL$ (SEQ ID NO:32), $SWESX_3X_4X_5QTRL$ (SEQ ID NO:33) or $SWEEX_3X_4X_5QTRL$ (SEQ ID NO:34), wherein each of X_3, X_4 and X_5 is any amino acid residue.

In a particular embodiment, X_1 is E and X_3 is H, A or Y, such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first three residues of which are S, W and E, and the last four residues of which are Q, T, R and L, consisting of $SWEX_2HX_4X_5QTRL$ (SEQ ID NO:35), $SWEX_2AX_4X_5QTRL$ (SEQ ID NO:36) or $SWEX_2YX_4X_5QTRL$ (SEQ ID NO:37), wherein each of X_2, X_4 and X_5 is any amino acid residue.

In a particular embodiment, X_1 is E and X_4 is G or T, such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first three residues of which are S, W and E, and the last four residues of which are Q, T, R and L, consisting of $SWEX_2X_3GX_5QTRL$ (SEQ ID NO:38) or $SWEX_2X_3TX_5QTRL$ (SEQ ID NO:39), wherein each of X_2, X_3 and X_5 is any amino acid residue.

In a particular embodiment, X_1 is E and X_5 is G or Q, such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first three residues of which are S, W and E, and the last four residues of which are Q, T, R and L, consisting of $SWEX_2X_3X_4GQTRL$ (SEQ ID NO:40) and $SWEX_2X_3X_4QQTRL$ (SEQ ID NO:41), wherein each of X_2, X_3 and X_4 is any amino acid residue.

In a particular embodiment, X_1 is E, X_2 is V and X_3 is H, A or Y, such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first four residues of which are S, W, E and V and the last four residues of which are Q, T, R and L, consisting of $SWEVHX_4X_5QTRL$ (SEQ ID NO:42), $SWEVAX_4X_5QTRL$ (SEQ ID NO:43) or $SWEVYX_4X_5QTRL$ (SEQ ID NO:44), wherein each of X_4 and X_5 is any amino acid residue.

In a particular embodiment, X_1 is E, X_2 is V and X_4 is G or T, such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first four residues of which are S, W, E and V and the last four residues of which are Q, T, R and L, consisting of SWEVX_3GX_5QTRL (SEQ ID NO:45) or SWEVX_3TX_5QTRL (SEQ ID NO:46), wherein each of X_3 and X_5 is any amino acid residue.

In a particular embodiment, X_1 is E, X_2 is V and X_5 is G or Q, such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first four residues of which are S, W, E and V and the last four residues of which are Q, T, R and L, consisting of SWEVX_3X_4GQTRL (SEQ ID NO:47) or SWEVX_3X_4QQTRL (SEQ ID NO:48), wherein each of X_3 and X_4 is any amino acid residue.

In a particular embodiment, X_1 is E, X_2 is V, X_3 is H, A or Y and X_4 is G or T, such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first four residues of which are S, W, E and V and the last four residues of which are Q, T, R and L, consisting of SWEVHGX_5QTRL (SEQ ID NO:49), SWEVHTX_5QTRL (SEQ ID NO:50), SWEVAGX_5QTRL (SEQ ID NO:51), SWEVATX_5QTRL (SEQ ID NO:52), SWEVYGX_5QTRL (SEQ ID NO:53) or SWEVYTX_5QTRL (SEQ ID NO:54), wherein X_5 is any amino acid residue.

In a particular embodiment, X_1 is E, X_2 is V, X_3 is H, A or Y and X_5 is G or Q, such that the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first four residues of which are S, W, E and V and the last four residues of which are Q, T, R and L, consisting of SWEVHX_4GQTRL (SEQ ID NO:55), SWEVHX_4QQTRL (SEQ ID NO:56), SWEVAX_4GQTRL (SEQ ID NO:57), SWEVAX_4QQTRL (SEQ ID NO:58), SWEVYX_4GQTRL (SEQ ID NO:59) or SWEVYX_4QQTRL (SEQ ID NO:60), wherein X_4 is any amino acid residue.

In a particular embodiment, the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first two residues of which are S and W, and the last four residues of which are Q, T, R and L, consisting of SW$X_1X_2X_3X_4X_5$QTRL, wherein X_1 is E or A, X_2 is S, E or V, X_3 is H, A or Y, X_4 is G or T and X_5 is G or Q (SEQ ID NO:61). In this embodiment, the MAST-2 binding domain consists of S-W-E/A-S/E/V-H/A/Y-G/T-G/Q-Q-T-R-L.

In a particular embodiment, the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first two residues of which are S and W, and the last four residues of which are Q, T, R and L, consisting of

$SWX_1X_2X_3X_4X_5QTRL$, wherein X_1 is E, X_2 is S, E or V, X_3 is H, A or Y, X_4 is G or T and X_5 is G or Q (SEQ ID NO:62). In this embodiment, the MAST-2 binding domain consists of S-W-E-S/E/V-H/A/Y-G/T-G/Q-Q-T-R-L.

In a particular embodiment, the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first two residues of which are S and W, and the last four residues of which are Q, T, R and L, consisting of $SWX_1X_2X_3X_4X_5QTRL$, wherein X_1 is E, X_2 is V, X_3 is H, A or Y, X_4 is G or T and X_5 is G or Q (SEQ ID NO:63). In this embodiment, the MAST-2 binding domain consists of S-W-E-V-H/A/Y-G/T-G/Q-Q-T-R-L. In a more particular embodiment, the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first four residues of which are S, W, E and V and the last four residues of which are Q, T, R and L, consisting of SWEVHGGQTRL (SEQ ID NO:64), SWEVHGQQTRL (SEQ ID NO:65), SWEVHTGQTRL (SEQ ID NO:66), SWEVHTQQTRL (SEQ ID NO:67), SWEVAGGQTRL (SEQ ID NO:68), SWEVAGQQTRL (SEQ ID NO:69), SWEVATGQTRL (SEQ ID NO:70), SWEVATQQTRL (SEQ ID NO:71), SWEVYGGQTRL (SEQ ID NO:72), SWEVYGQQTRL (SEQ ID NO:73), SWEVYTGQTRL (SEQ ID NO:74) or SWEVYTQQTRL (SEQ ID NO:75).

The polypeptides fulfilling one of the definitions as described in this group are preferred, in particular when the constant of dissociation of the complex formed by a polypeptide of this group with the PDZ domain of the human MAST2 protein is less than 0.3 µM, preferably less than 0.25 µM, preferably less than 0.2 µM preferably less than 0.15 µM, more preferably less than 0.1 µM, as measured by the method defined above. In a more preferred embodiment, the polypeptides fulfilling one of the definitions described in this group have a constant of dissociation of the complex formed by the polypeptide of the invention with the PDZ domain of the human MAST2 protein which is less than 0.09 µM, less than 0.08 µM, less than 0.07 µM, less than 0.06 µM or less than 0.05 µM, as measured by the method defined above.

(B) in a second group, the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first two residues of which are S and W, and the last four residues of which are Q, T, R and L, said domain being selected from the group consisting of: $SWX_1KSGGQTRL$ (SEQ ID NO:76), $SWX_1SSGGQTRL$ (SEQ

ID NO:77), $SWX_1SHGGQTRL$ (SEQ ID NO:78), $SWX_1SHKGQTRL$ (SEQ ID NO:79), $SWX_1SHKSQTRL$ (SEQ ID NO:80), $SWX_1HSGGQTRL$ (SEQ ID NO:86), $SWX_1HKGGQTRL$ (SEQ ID NO:87), $SWX_1HKSGQTRL$ (SEQ ID NO:88), $SWX_1SKGGQTRL$ (SEQ ID NO:89), $SWX_1SKSGQTRL$ (SEQ ID NO:90), $SWX_1SHSGQTRL$ (SEQ ID NO:91), $SWX_1SHKGQTRL$ (SEQ ID NO:92) and $SWX_1SKGGQTRL$ (SEQ ID NO:93), wherein X_1 is any amino acid, preferably E or A, more preferably E. Thus, in a particular embodiment, the MAST-2 binding domain consists of the sequence, whose size is 11 residues, the first three residues of which are S, W and E, and the last four residues of which are Q, T, R and L, said domain being selected from the group consisting of: SWEKSGGQTRL (SEQ ID NO:81), SWESSGGQTRL (SEQ ID NO:82), SWESHGGQTRL (SEQ ID NO:83), SWESHKGQTRL (SEQ ID NO:84), SWESHKSQTRL (SEQ ID NO:85), SWEHSGGQTRL (SEQ ID NO:94), SWEHKGGQTRL (SEQ ID NO:95), SWEHKSGQTRL (SEQ ID NO:96), SWESKGGQTRL (SEQ ID NO:97), SWESKSGQTRL (SEQ ID NO:98), SWESHSGQTRL (SEQ ID NO:99), SWESHKGQTRL (SEQ ID NO:100) and SWESKGGQTRL (SEQ ID NO:101). In a particular embodiment, the MAST-2 binding domain of the cytoplasmic domain is SWESHGGQTRL (SEQ ID NO:83).

MAST-2 binding domain of this second group may be obtained by deletion of two amino acid residues, consecutive or not, from the SWESHKSGGQTRL sequence (SEQ ID NO:1).

(C) In a third group, the MAST-2 binding domain consists of a sequence, whose size is 12 residues, the first two residues of which are S and W, and the last four residues of which are Q, T, R and L, consisting of $SWX_1X_2X_3X_4X_5X_6QTRL$, wherein each of X_1, X_2, X_3, X_4, X_5 and X_6 is any amino acid residue (SEQ ID NO:112).

In a particular embodiment, X_1 is E, A, V or S, such that the MAST-2 binding domain consists of a sequence, whose size is 12 residues, the first two residues of which are S and W and the last four residues of which are Q, T, R and L, consisting of $SWEX_2X_3X_4X_5X_6QTRL$ (SEQ ID NO:113), $SWAX_2X_3X_4X_5X_6QTRL$ (SEQ ID NO:114), $SWVX_2X_3X_4X_5X_6QTRL$ (SEQ ID NO:115) or $SWSX_2X_3X_4X_5X_6QTRL$ (SEQ ID NO:116), wherein each of X_2, X_3, X_4, X_5 and X_6 is any amino acid residue.

In a particular embodiment, X_2 is S, V, H, A or Y, such that the MAST-2 binding domain consists of a sequence, whose size is 12 residues, the first two residues of which are S and W and the last four residues of which are Q, T, R and L, consisting of $SWX_1SX_3X_4X_5X_6QTRL$ (SEQ ID NO:117), $SWX_1VX_3X_4X_5X_6QTRL$ (SEQ ID NO:118), $SWX_1HX_3X_4X_5X_6QTRL$ (SEQ ID NO:119), $SWX_1AX_3X_4X_5X_6QTRL$ (SEQ ID NO:120) or $SWX_1YX_3X_4X_5X_6QTRL$ (SEQ ID NO:121), wherein each of X_1, X_3, X_4, X_5 and X_6 is any amino acid.

In a particular embodiment, X_3 is H, A, Y, K or Q, such that the MAST-2 binding domain consists of a sequence, whose size is 12 residues, the first two residues of which are S and W and the last four residues of which are Q, T, R and L, consisting of $SWX_1X_2HX_4X_5X_6QTRL$ (SEQ ID NO:122), $SWX_1X_2AX_4X_5X_6QTRL$ (SEQ ID NO:123), $SWX_1X_2YX_4X_5X_6QTRL$ (SEQ ID NO:124), $SWX_1X_2KX_4X_5X_6QTRL$ (SEQ ID NO:125) or $SWX_1X_2QX_4X_5X_6QTRL$ (SEQ ID NO:126), wherein each of X_1, X_2, X_4, X_5 and X_6 is any amino acid.

In a particular embodiment, X_4 is K, A, Q, S or H, such that the MAST-2 binding domain consists of a sequence, whose size is 12 residues, the first two residues of which are S and W and the last four residues of which are Q, T, R and L, consisting of $SWX_1X_2X_3KX_5X_6QTRL$ (SEQ ID NO:127), $SWX_1X_2X_3AX_5X_6QTRL$ (SEQ ID NO:128), $SWX_1X_2X_3QX_5X_6QTRL$ (SEQ ID NO:129), $SWX_1X_2X_3SX_5X_6QTRL$ (SEQ ID NO:130) or $SWX_1X_2X_3HX_5X_6QTRL$ (SEQ ID NO:131), wherein each of X_1, X_2, X_3, X_5 and X_6 is any amino acid.

In a particular embodiment, X_5 is S, H, G or T, such that the MAST-2 binding domain consists of a sequence, whose size is 12 residues, the first two residues of which are S and W and the last four residues of which are Q, T, R and L, consisting of $SWX_1X_2X_3X_4SX_6QTRL$ (SEQ ID NO:132), $SWX_1X_2X_3X_4HX_6QTRL$ (SEQ ID NO:133), $SWX_1X_2X_3X_4GX_6QTRL$ (SEQ ID NO:134) or $SWX_1X_2X_3X_4TX_6QTRL$ (SEQ ID NO:135), wherein each of X_1, X_2, X_3, X_4 and X_6 is any amino acid.

In a particular embodiment, X_6 is G, T or Q, such that the MAST-2 binding domain consists of a sequence, whose size is 12 residues, the first two residues of which are S and W and the last four residues of which are Q, T, R and L, consisting of $SWX_1X_2X_3X_4X_5GQTRL$ (SEQ ID NO:136), $SWX_1X_2X_3X_4X_5TQTRL$

(SEQ ID NO:137) or $SWX_1X_2X_3X_4X_5QQTRL$ (SEQ ID NO:138), wherein each of X_1, X_2, X_3, X_4 and X_5 is any amino acid.

In a particular embodiment, regarding the polypeptides of SEQ ID NO:112 and SEQ ID NO:122 to SEQ ID NO:138 as defined above, X_1 is E and/or X_2 is V, as disclosed in Table 1 (next page).

In a particular embodiment, the MAST-2 binding domain consists of 12 residues, its first two residues are S and W and its last four residues are Q, T, R and L, consisting of the sequence $SWX_1X_2X_3X_4X_5X_6QTRL$, wherein X_1 is E, A, V or S, X_2 is S, V, H, A or Y, X_3 is H, A, Y, K or Q, X_4 is K, A, Q, S or H, X_5 is S, H, G or T and X_6 is G, T or Q (SEQ ID NO:191). In this embodiment, the MAST-2 binding domain consists of the sequence S-W-E/A/V/S-S/V/H/A/Y-H/A/Y/K/Q-K/A/Q/S/H-S/H/G/T-G/T/Q-QTRL.

(D) In a fourth group, the MAST-2 binding domain consists of a sequence, whose size is 12 residues, the first two residues of which are S and W, and the last four residues of which are Q, T, R and L, said domain being selected from the group consisting of: $SWX_1HKSGGQTRL$ (SEQ ID NO:102), $SWX_1SKSGGQTRL$ (SEQ ID NO:103), $SWX_1SHSGGQTRL$ (SEQ ID NO:104), $SWX_1SHKGGQTRL$ (SEQ ID NO:105) and $SWX_1SHKSGQTRL$ (SEQ ID NO:106), wherein X_1 is any amino acid, preferably E or A, more preferably E. Thus, in a particular embodiment, the MAST-2 binding domain consists of the sequence, whose size is 12 residues, the first three residues of which are S, W and E, and the last four residues of which are Q, T, R and L, said domain being selected from the group consisting of: SWEHKSGGQTRL (SEQ ID NO:107), SWESKSGGQTRL (SEQ ID NO:108), SWESHSGGQTRL (SEQ ID NO:109), SWESHKGGQTRL (SEQ ID NO:110) and SWESHKSGQTRL (SEQ ID NO:111).

MAST-2 binding domain of this fourth group may be obtained by deletion of one amino acid residue from the SWESHKSGGQTRL sequence (SEQ ID NO:1).

	with X_1 is E	with X_2 is V	with X_1 is E and X_2 is V
SEQ ID NO:112	SWE$X_2X_3X_4X_5X_6$QTRL (SEQ ID NO:X113)	SWX_1V$X_3X_4X_5X_6$QTRL (SEQ ID NO:118)	SWEV$X_3X_4X_5X_6$QTRL (SEQ ID NO:190)
SEQ ID NO:122	SWEX_2H$X_4X_5X_6$QTRL (SEQ ID NO:139)	SWX_1VH$X_4X_5X_6$QTRL (SEQ ID NO:140)	SWEVH$X_4X_5X_6$QTRL (SEQ ID NO:141)
SEQ ID NO:123	SWEX_2A$X_4X_5X_6$QTRL (SEQ ID NO:142)	SWX_1VA$X_4X_5X_6$QTRL (SEQ ID NO:143)	SWEVA$X_4X_5X_6$QTRL (SEQ ID NO:144)
SEQ ID NO:124	SWEX_2Y$X_4X_5X_6$QTRL (SEQ ID NO:145)	SWX_1VY$X_4X_5X_6$QTRL (SEQ ID NO:146)	SWEVY$X_4X_5X_6$QTRL (SEQ ID NO:147)
SEQ ID NO:125	SWEX_2K$X_4X_5X_6$QTRL (SEQ ID NO:148)	SWX_1VK$X_4X_5X_6$QTRL (SEQ ID NO:149)	SWEVK$X_4X_5X_6$QTRL (SEQ ID NO:150)
SEQ ID NO:126	SWEX_2Q$X_4X_5X_6$QTRL (SEQ ID NO:151)	SWX_1VQ$X_4X_5X_6$QTRL (SEQ ID NO:152)	SWEVQ$X_4X_5X_6$QTRL (SEQ ID NO:153)
SEQ ID NO:127	SWEX_2X_3KX_5X_6QTRL (SEQ ID NO:154)	SWX_1VX_3KX_5X_6QTRL (SEQ ID NO:155)	SWEVX_3KX_5X_6QTRL (SEQ ID NO:156)
SEQ ID NO:128	SWEX_2X_3AX_5X_6QTRL (SEQ ID NO:157)	SWX_1VX_3AX_5X_6QTRL (SEQ ID NO:158)	SWEVX_3AX_5X_6QTRL (SEQ ID NO:159)
SEQ ID NO:129	SWEX_2X_3QX_5X_6QTRL (SEQ ID NO:160)	SWX_1VX_3QX_5X_6QTRL (SEQ ID NO:161)	SWEVX_3QX_5X_6QTRL (SEQ ID NO:162)
SEQ ID NO:130	SWEX_2X_3SX_5X_6QTRL (SEQ ID NO:163)	SWX_1VX_3SX_5X_6QTRL (SEQ ID NO:164)	SWEVX_3SX_5X_6QTRL (SEQ ID NO:165)
SEQ ID NO:131	SWEX_2X_3HX_5X_6QTRL (SEQ ID NO:166)	SWX_1VX_3HX_5X_6QTRL (SEQ ID NO:167)	SWEVX_3HX_5X_6QTRL (SEQ ID NO:168)
SEQ ID NO:132	SWE$X_2X_3X_4$SX_6QTRL (SEQ ID NO:169)	SWX_1VX_3X_4SX_6QTRL (SEQ ID NO:170)	SWEVX_3X_4SX_6QTRL (SEQ ID NO:171)
SEQ ID NO:133	SWE$X_2X_3X_4$HX_6QTRL (SEQ ID NO:172)	SWX_1VX_3X_4HX_6QTRL (SEQ ID NO:173)	SWEVX_3X_4HX_6QTRL (SEQ ID NO:174)
SEQ ID NO:134	SWE$X_2X_3X_4$GX_6QTRL (SEQ ID NO:175)	SWX_1VX_3X_4GX_6QTRL (SEQ ID NO:176)	SWEVX_3X_4GX_6QTRL (SEQ ID NO:177)
SEQ ID NO:135	SWE$X_2X_3X_4$TX_6QTRL (SEQ ID NO:178)	SWX_1VX_3X_4TX_6QTRL (SEQ ID NO:179)	SWEVX_3X_4TX_6QTRL (SEQ ID NO:180)
SEQ ID NO:136	SWE$X_2X_3X_4X_5$GQTRL (SEQ ID NO:181)	SWX_1V$X_3X_4X_5$GQTRL (SEQ ID NO:182)	SWEV$X_3X_4X_5$GQTRL (SEQ ID NO:183X)
SEQ ID NO:137	SWE$X_2X_3X_4X_5$TQTRL (SEQ ID NO:184)	SWX_1V$X_3X_4X_5$TQTRL (SEQ ID NO:185)	SWEV$X_3X_4X_5$TQTRL (SEQ ID NO:186)
SEQ ID NO:138	SWE$X_2X_3X_4X_5$QQTRL (SEQ ID NO:187)	SWX_1V$X_3X_4X_5$QQTRL (SEQ ID NO:188)	SWEV$X_3X_4X_5$QQTRL (SEQ ID NO:189)
wherein each of X_1, X_2, X_3, X_4, X_5 and X_6, when applicable, is any amino acid residue			

Table 1

(E) In a fifth group, the MAST-2 binding domain consists of a sequence, whose size is 13 residues, the first two residues of which are S and W, and the last four residues of which are Q, T, R and L, consisting of $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, wherein each of X_1, X_2, X_3, X_4, X_5, X_6 and X_7, is any amino acid residue (SEQ ID NO:192), wherein said MAST-2 binding domain does not consist of SWESHKSGGQTRL. In a particular embodiment, the MAST-2 binding domain, whose size is 13 residues, is neither SWESHKSGGQTRL (SEQ ID NO :1) nor SWESYKSGGQTRL (SEQ ID NO :16).

In a particular embodiment, the MAST-2 binding domain of 13 residues differs from SWESHKSGGQTRL (SEQ ID NO :1) by at least 1 substitution of amino acid residue, by at least 2 substitutions or by at least 3 substitutions, provided that the first two residues are S and W, and the fourth last residues are Q, T, R and L; in a more particular embodiment, the MAST-2 binding domain of 13 residues differing from SWESHKSGGQTRL by at least 1 substitution is not SWESYKSGGQTRL (SEQ ID NO :16).

In a particular embodiment, the MAST-2 binding domain consists of 13 residues and differs from SWESHKSGGQTRL by 1 substitution in a residue located between SW and QTRL; in particular embodiment, this is not the substitution of the histidine residue (H) in a tyrosine residue (Y).

In a particular embodiment of $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, X_1 is E or A, and each of X_2, X_3, X_4, X_5, X_6 and X_7, is any amino acid residue (SEQ ID NO:193).

In a particular embodiment of $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, X_2 is selected from polar neutral residues, negatively charged residues or hydrophobic residues (SEQ ID NO:194) and is preferably S, V or E (SEQ ID NO:195), wherein X_1, X_3, X_4, X_5, X_6 and X_7, is any amino acid residue.

In a particular embodiment of $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, X_3 is selected from positively charged residues, non polar residues with small volume and polar aromatic residues (SEQ ID NO:196), and is preferably H, A or Y (SEQ ID NO:197), wherein X_1, X_2, X_4, X_5, X_6 and X_7, is any amino acid residue.

In a particular embodiment of $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, X_4 is selected from non polar residues with small volume, polar neutral residues and positively charged residues (SEQ ID NO:198) and is preferably K, A or Q (SEQ ID NO:199), wherein X_1, X_2, X_3, X_5, X_6 and X_7, is any amino acid residue.

In a particular embodiment of $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, X_5 is selected from polar neutral residues and positively charged residues (SEQ ID NO:200), and is preferably S or H (SEQ ID NO:201), wherein X_1, X_2, X_3, X_4, X_6 and X_7, is any amino acid residue.

In a particular embodiment of $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, X_6 is selected from non polar residues with small volume, preferably flexible, and polar neutral residues (SEQ ID NO:202), and is preferably G or T (SEQ ID NO:203), wherein X_1, X_2, X_3, X_4, X_5 and X_7, is any amino acid residue.

In a particular embodiment of $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, X_7 is selected from non polar residues with small volume, preferably flexible, and polar neutral residues (SEQ ID NO:204), and is preferably G or Q (SEQ ID NO:205), wherein X_1, X_2, X_3, X_4, X_5 and X_6, is any amino acid residue.

The amino acid residues corresponding to the polar neutral residues, positively charged residues, negatively charged residues, hydrophobic residues, non polar residues with small volume and polar aromatic residues are according to the conventional literature, and confirmed in the lists below.

In a more particular embodiment, said MAST-2 binding domain consists of the sequence $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, wherein X_1 is E or A and/or X_2 is S, V or E and/or X_3 is H, A or Y and/or X_4 is K, A or Q and/or X_5 is S or H and/or X_6 is G or T and/or X_7 is G or Q, wherein X_1, X_2, X_3, X_4, X_5, X_6 and X_7 are not, together, E, S, H, K, S, G and G. In a particular embodiment, X_1, X_2, X_3, X_4, X_5, X_6 and X_7 are neither together E, S, H, K, S, G and G respectively, nor together E, S, Y, K, S, G and G respectively.

Thus, in a preferred embodiment, the sequence of the MAST-2 binding domain is S-W-E/A-S/V/E-H/A/Y-K/A/Q-S/H-G/T-G/Q-QTRL as defined in SEQ ID NO:206, provided the MAST-2 binding domain is not SWESHKSGGQTRL (SEQ ID NO :1); in a more particular embodiment, the MAST-2 binding domain of sequence S-W-E/A-S/V/E-H/A/Y-K/A/Q-S/H-G/T-G/Q-QTRL is neither SWESHKSGGQTRL (SEQ ID NO :1) nor SWESYKSGGQTRL (SEQ ID NO :16). In another preferred embodiment, the sequence of the MAST-2 binding domain is S-W-E/A-V/E-H/A-A/Q-S/H-G/T-G/Q-QTRL as defined in SEQ ID NO:207. Preferred MAST-2 binding domains consist of the sequence SWAEAQHTQQTRL (SEQ ID NO:208) or SWEVHASGGQTRL (SEQ ID NO:209)

In a particular embodiment, the MAST-2 binding domain consists of a sequence, whose size is 11 or 12 residues, the first two residues of which are S and W, and the fourth last residues of which are Q, T, R and L, selected in the groups (A), (B), (C) and (D) as detailed above.

In another embodiment, the MAST-2 binding domain consists of a sequence, whose size is 11 residues, the first two residues of which are S and W, and the fourth last residues of which are Q, T, R and L, selected in the groups (A) and (B) as detailed above.

In another embodiment, the MAST-2 binding domain consists of a sequence, whose size is 12 residues, the first two residues of which are S and W, and the fourth last residues of which are Q, T, R and L, selected in the groups (C) and (D) as detailed above.

In another embodiment, the MAST-2 binding domain consists of a sequence, whose size is 13 residues, the first two residues of which are S and W, and the fourth last residues of which are Q, T, R and L, selected in the group (E) as detailed above.

In a particular embodiment, the sequence of the cytoplasmic domain upstream of the MAST-2 binding domain of the polypeptide of the invention is either:

- a polypeptide containing 20 to 40 amino acid residues, such that the size of the entire cytoplasmic domain (sequence of the cytoplasmic domain upstream of the MAST-2 binding domain and the MAST-2 binding domain) is from 31 to 53 residues, preferably from 31 to 52 or from 31 to 51 residues. In a particular embodiment, the sequence of the cytoplasmic domain upstream of the MAST-2 binding domain consists of 25 to 45 residues. In another embodiment, the sequence of the cytoplasmic domain upstream of the MAST-2 binding domain is 31 residues, such that the entire cytoplasmic domain is from 42 to 44 residues, preferably 42 residues, 43 residues or 44 residues. Any cytoplasmic domain can be selected as long as this cytoplasmic domain enables the binding of the MAST-2 binding domain to the PDZ domain of the human MAST2 protein. Particular examples of cytoplasmic domains of G protein can be found in Schnell MJ et al. (1998) and Owens RJ et al (1993). The binding of the MAST-2 binding domain to the PDZ domain of the human MAST2 protein, and thus the affinity of the polypeptide of the invention for the PDZ domain of the human MAST2 protein, may be assayed by the method detailed above for the K_D calculation; or

- the sequence of the cytoplasmic domain upstream of the MAST-2 binding domain is a fragment of the cytoplasmic domain of a rabies virus G protein, in particular a fragment of the cytoplasmic domain of a G protein from an attenuated rabies virus strain or a fragment of the cytoplasmic domain of a G protein from a virulent rabies virus strain; in a particular embodiment, the sequence of the cytoplasmic domain upstream of the MAST-2 binding domain consists of the following sequence RRVNRSEPTQHNLRGTGREVSVTPQSGKIIS (SEQ ID NO:2) or a variant having at least 80%, at least 85% or at least 90% identity with SEQ ID NO:2, said variant retaining the ability to bind the MAST-2 binding domain of the polypeptide of the invention to the PDZ domain of the human MAST2 protein. The percentage of identity is calculated over the shortest of the two sequences *i.e.*, over the shortest of SEQ ID NO: 2 and of said variant. A variant having at least 80%, at least 85% or at least 90% identity with SEQ ID NO:2 is defined as a variant by one or more addition(s) and/or one or more deletion(s) and/or one or more substitution(s). An example of a variant of SEQ ID NO:2 is a polypeptide consisting of the sequence RRVNRSEPTQLNLRGTGREVSVTPQSGKIIS (SEQ ID NO:5). In a particular embodiment, the variant has at least 90% identity with SEQ ID NO:2 and is obtained by 1, 2 or 3 substitutions in SEQ ID NO:2, preferably by conservative substitution(s) as defined in the literature, or according to the following list:

- the group of the nonpolar (i.e., hydrophobic) amino acid residues: a first subgroup including alanine (A), glycine (G) and proline (P), and a second subgroup including leucine (L), isoleucine (I) and valine (V);

- the group of the polar neutral (uncharged) amino acid residues: a first subgroup including serine (S), threonine (T), cysteine (C) and methionine (M), and a second subgroup including asparagine (N) and glutamine (Q);

- the group of positively charged (*i.e.,* basic) residues, including arginine (R), lysine (K) and histidine (H);

- the group of negatively charged (*i.e.,* acid) residues, including aspartic acid (D) and glutamic acid (E); and

- the group of the aromatic residues, including phenylalanine (F), tryptophan (W) and tyrosine (Y).

In a particular embodiment, when the polypeptide consists of the cytoplasmic domain, the size of the polypeptide is from 31 to 53 residues, preferably from 31 to 52 or from 31 to 51 residues, or from 42 to 44 residues, preferably 42 residues, 43 residues or 44 residues.

In a particular embodiment, and whatever the sequence of the cytoplasmic domain upstream of the MAST-2 binding domain and the sequence of the MAST-2 binding domain, the anchoring domain (which may be optional as such or together with the signal peptide) of the polypeptide of the invention is either:

- a peptide, whose size is from 18 to 26 amino acids, which anchors (or has been shown to anchor) a polypeptide in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells (particularly in neuronal cells, more particularly in human neuronal cells) (Figure 1). Particular examples of anchoring domains can be found in Schroth-Diez B et al. (2000). In a particular embodiment, the size of the anchoring domain is from 20 to 24 amino acid residues. In a particular embodiment, the size of the anchoring domain is 22 amino acid residues. Any anchoring domain may be selected as long as it anchors the polypeptide of the invention in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells (particularly in neuronal cells, more particularly in human neuronal cells). In a particular embodiment, the anchoring domain is a transmembrane domain, *i.e.,* any domain able to interact with the lipid bilayer, and in particular able to anchor the polypeptide comprising it, into the lipid bilayer, especially into the cellular membrane. A transmembrane domain is a domain rich in hydrophobic residues (A, G, P, L I and/or V) and stable in a membrane, and is organized in one or several hydrophobic α-helix(es). In a particular embodiment, the transmembrane domain used in the polypeptide described herein is the transmembrane domain (as a fragment) of a known transmembrane protein. Particular examples of transmembrane domains can be found in Schroth-Diez B et al. (2000). The correct anchoring of the polypeptide of the invention may be determined by checking the affinity of the polypeptide of the invention for the PDZ domain of the human MAST2 protein, by implementing the method detailed above for the K_D calculation; or

- the transmembrane domain of a rabies virus G protein, in particular the transmembrane domain of a G protein from an attenuated rabies virus strain or the transmembrane domain of the cytoplasmic domain of a G protein from a virulent rabies virus strain; in a preferred embodiment, the transmembrane domain comprises or consists of the sequence YVLLSAGALTALMLIIFLMTCC (SEQ ID NO:4) or a variant having at least 81%, at least 86%, at least 90% or at least 95% identity with said SEQ ID NO:4, said variant retaining the capacity to anchor the polypeptide in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells; the percentage of identity is calculated over the shortest of the two sequences, *i.e.*, over the shortest of SEQ ID NO: 4 and of said transmembrane domain variant. A variant having at least 81%, at least 86%, at least 90% or at least 95% identity with SEQ ID NO:4 is defined as a variant by one or more addition(s) and/or one or more deletion(s) and/or one or more substitution(s). In a particular embodiment, a variant having at least 90% or at least 95% identity with SEQ ID NO:4 is obtained by respectively 1 or 2 substitutions in SEQ ID NO:4, preferably by conservative substitution(s) as defined in the literature, or according to the list above.

Whatever the sequence of the anchoring domain (preferably the transmembrane domain) as defined herein:

- the N-terminal extremity of said anchoring domain is, directly or indirectly, linked to the C-terminal extremity of the signal peptide as defined herein; and

- the C-terminal extremity of said anchoring domain is, directly or indirectly, preferably directly, linked to the first N-terminal amino acid residue of the cytoplasmic domain as defined herein.

In a particular embodiment, and whatever the sequence of the cytoplasmic domain upstream of the MAST-2 binding domain, the sequence of the MAST-2 binding domain and the sequence of the anchoring domain, the signal peptide (which may be optional as such or together with the anchoring domain) of the polypeptide of the invention is either:

- a peptide, whose size is from 3 to 60 residues, which targets (or has been shown to target) a polypeptide into the endoplasmic reticulum and optionally through the secretory pathway (Figure 1); in a particular embodiment, the size of the signal peptide is from 10 to 40, preferably from 15 to 30, more preferably from 15 to 25 amino acid residues. In a particular embodiment, the size of the signal peptide is 19 amino acid residues. Any signal peptide may be selected as long as it targets the polypeptide of the invention into the endoplasmic reticulum and optionally through the secretory pathway. In a particular embodiment, the signal peptide used in the polypeptide described herein is the signal peptide of a known protein, such as the CD4 and CD8 proteins, the hemagglutinin (HA) or a cytokine receptor (e.g., IL1R1, EGFR1, HER2, HER3 or HER4). The correct targeting of the polypeptide of the invention may be determined by checking the affinity of the polypeptide of the invention for the PDZ domain of the human MAST2 protein, by implementing the method detailed above for the K_D calculation; or

- the signal peptide of a rabies virus G protein, in particular the signal peptide of a G protein from an attenuated rabies virus strain or the signal peptide of the cytoplasmic domain of a G protein from a virulent rabies virus strain; in a preferred embodiment, the signal peptide of a rabies virus G protein corresponds to the 19 first amino acid residues of the G protein. In a particular embodiment, said signal peptide comprises or consists of the sequence MVPQALLFVPLLVFPLCFG (SEQ ID NO:3) or a variant having at least 68%, at least 73%, at least 89% or at least 94% identity with said SEQ ID NO:3, said variant retaining the capacity to target the polypeptide into the endoplasmic reticulum and optionally through the secretory pathway; the percentage of identity is calculated over the shortest of the two sequences, *i.e.*, over the shortest of SEQ ID NO: 3 and of said signal peptide variant. A variant having at least 68%, at least 73%, at least 89% or at least 94% identity with SEQ ID NO:3 is defined as a variant by one or more addition(s) and/or one or more deletion(s) and/or one or more substitution(s). In a particular embodiment, a variant having at least 89% or at least 94% identity with SEQ ID NO:3 is obtained by respectively 1 or 2 substitutions in SEQ ID NO:3, preferably by conservative substitution(s) as defined in the literature, or according to the list above.

Whatever the sequence of the signal peptide as defined herein, said signal peptide, when present, is the most N-terminal element of the polypeptide of the invention.

By "*direct link*", it is meant that the last C-terminal residue of a domain is linked by a peptide bond to the first N-terminal residue of the following domain.

In contrast, by "*indirect link*", it is meant that the last C-terminal residue of a domain is linked by a peptide bond to the first N-terminal residue of a peptide linker, the last C-terminal residue of which is linked to the first N-terminal residue of the following domain. In a particular embodiment, and whatever the sequence of the cytoplasmic domain upstream of the MAST-2 binding domain, the sequence of the MAST-2 binding domain, the sequence of the signal peptide and the sequence of the anchoring domain, the polypeptide of the invention optionally comprises, between the signal peptide and the anchoring domain, both defined herein, a peptide linker consisting of one to four amino acids, preferably one to four amino acids of the C-terminal end of the ectodomain of a rabies virus G protein, preferably the last two C-terminal residues of the ectodomain of a rabies virus G protein, for example amino acid residues GK.

Thus, a particular polypeptide of the invention comprises or consists of, from N-terminal to C-terminal ends:

(1) a signal peptide as defined in SEQ ID NO:3, or a variant having at least 68% identity with said SEQ ID NO:3, said variant retaining the capacity to target the polypeptide into the endoplasmic reticulum and optionally through the secretory pathway;

(2) optionally, the last two C-terminal residues of the ectodomain of a rabies virus G protein, preferably amino acid residues GK;

(3) an anchoring domain as defined in SEQ ID NO:4 or a variant having at least 81% identity with said SEQ ID NO:4, said variant retaining the capacity to anchor the polypeptide in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells; and

(4) a cytoplasmic domain comprising or consisting of (a) a peptide as defined in SEQ ID NO:2 or a variant having at least 80% identity with SEQ ID

NO:2, and (b) a MAST-2 binding domain as defined in SEQ ID NO:19 to SEQ ID NO:209, preferably chosen from the group consisting of SEQ ID NO:19 to SEQ ID NO:101, SEQ ID NO:102 to SEQ ID NO:191 and SEQ ID NO:192 to SEQ ID NO:209, more preferably as defined in SEQ ID NOS: 64-68, 71, 74 and 208-209. In a particular embodiment, the peptide as defined in SEQ ID NO:2 or the variant having at least 90% identity with SEQ ID NO:2 is located upstream (*i.e.,* N-terminal to), preferably directly linked to, the MAST-2 binding domain.

The size of the polypeptide of the invention is at most 350, at most 250, at most 200 or at most 150 amino acid residues. In a preferred embodiment, the size of the polypeptide of the invention is at most 100 amino acid residues, and is preferably from 85 to 87 amino acid residues, and more preferably is 85, 86 or 87 amino acid residues.

In a particular embodiment of the invention the polypeptide of the invention is deprived of the ectodomain of the G protein of a rabies virus, preferably with the exception of the last two amino acids of the C-terminal end of the ectodomain. More particularly, the polypeptide of the invention is not a wild type full-length G protein of a rabies virus strain, neither from a non-apoptotic strain (neurovirulent strain, such as CVS-NIV strain) nor from an apoptotic strain (attenuated strain). In another particular embodiment, the polypeptide of the invention has less than 75% identity, less than 60% identity or less than 50% identity with a wild type full-length G protein of a rabies virus strain, over the shortest of the two sequences (*i.e.*, over the shortest of the polypeptide of the invention and of a wild type full-length G protein of a rabies virus strain).

In a particular embodiment, the polypeptide of the invention consists of the sequence MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRS EPTQHNLRGTGREVSVTPQSGKIIS (SEQ ID NO:17), directly linked to a MAST-2 binding domain as defined in SEQ ID NO:19 to SEQ ID NO:209, preferably chosen from the group consisting of SEQ ID NO:19 to SEQ ID NO:101, SEQ ID NO:102 to SEQ ID NO:191 and SEQ ID NO:192 to SEQ ID NO:209, more preferably as defined in SEQ ID NOS: 64-68, 71, 74 and 208-209. In another embodiment, the polypeptide of the invention consists of the sequence

MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSEPTQLNL RGTGREVSVTPQSGKIIS (SEQ ID NO:18), directly linked to a MAST-2 binding domain as defined in SEQ ID NO:19 to SEQ ID NO:209, preferably chosen from the group consisting of SEQ ID NO:19 to SEQ ID NO:101, SEQ ID NO:102 to SEQ ID NO:191 and SEQ ID NO:192 to SEQ ID NO:209, more preferably as defined in SEQ ID NOS: 64-68, 71, 74 and 208-209.

The polypeptide of the invention does not comprise or does not consist of the sequence as defined in SEQ ID NO:9 (Neurovita 1).

Moreover, the Accession Number NCBI CAI43218 refers to the G glycoprotein consisting of the following sequence:
MVPQALLFVPLLGFSLCFGKFPIYTIPDELGPWSPIDIHHLSCPNNLVVEDEGCTN
LSEFSYMELKVGYISAIKVNGFTCTGVVTEAETYTNFVGYVTTTFKRKHFRPTPDA
CRAAYNWKMAGDPRYEESLHNPYPDYHWLRTVRTTKESLIIISPSVTDLDPYDKS
LHSRVFPGGKCSGITVSSTYCSTNHDYTIWMPENPRPRTPCDIFTNSRGKRASK
GNKTCGFVDERGLYKSLKGACRLKLCGVLGLRLMDGTWVAMQTSDETKWCPD
QLVNLHDFRSDEIEHLVVEELVKKREECLDALESIMTTKSVSFRRLSHLRKLVPGG
KAYTIFNKTLMEADAHYKSVRTWNEIIPSKGCLKVGGRCHPHVNGVFFNGIILGPD
GHVLIPEMQSSLLQQHMELLKSSVIPLMHPLADPSTVFKEGDEAEDFVEVHLPDV
YKQISGVDLGLPNWGKYVLMTAGAMIGLVLIFSLMTWCRRANRPESKQRSFGGT
GRNVSVTSQSGKVIPSWESYKSGGQTRL (SEQ ID NO : 14).

Thus, in a particular embodiment, the polypeptide of the invention does not comprise or consist of MVPQALLFVPLLGFSLCFGGKYVLMTAGAMIGLVLIFSLM TWCRRANRPESKQRSFGGTGRNVSVTSQSGKVIPSWESYKSGGQTRL (SEQ ID NO :15).

In another particular embodiment, the MAST-2 binding domain of a polypeptide of the invention is not SWESYKSGGQTRL (SEQ ID NO :16).

Particular examples of polypeptides of the invention are selected in the group consisting of (the MAST-2 binding domain is in bold):

(1) MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSE PTQHNLRGTGREVSVTPQSGKIIS**SWEVHGGQTRL** (Neurovita 2) (SEQ ID NO :210);

(2) MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSE PTQHNLRGTGREVSVTPQSGKIIS**SWEVHGQQTRL** (Neurovita 3) (SEQ ID NO :211);

(3) MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSE PTQHNLRGTGREVSVTPQSGKIIS**SWEVATQQTRL** (SEQ ID NO :212);

(4) MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSE PTQHNLRGTGREVSVTPQSGKIIS**SWEVYTGQTRL**(SEQ ID NO :213);

(5) MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSE PTQHNLRGTGREVSVTPQSGKIIS**SWEVHTGQTRL** (SEQ ID NO :214);

(6) MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSE PTQHNLRGTGREVSVTPQSGKIIS**SWEVHTQQTRL** (SEQ ID NO :215);

(7) MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSE PTQHNLRGTGREVSVTPQSGKIIS**SWEVAGGQTRL** (SEQ ID NO :216);

(8) MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSE PTQHNLRGTGREVSVTPQSGKIIS**SWAEAQHTQQTRL** (SEQ ID NO :217); and

(9) MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSE PTQHNLRGTGREVSVTPQSGKIIS**SWEVHASGGQTRL** (SEQ ID NO :218).

The term "*polypeptide*" as defined herein encompasses polypeptides, which have been modified by post-transcriptional modification and/or by synthetic chemistry, *e.g.,* by adjunction of a non-proteinous chemical group and/or by modification of the tertiary structure of the polypeptide, *e.g.*, by acetylation, acylation, hydroxylation, cyclisation, racemisation, phosphorylation, etc., as long as the resulting modified polypeptide keeps a high affinity, as defined above, for the PDZ domain of the human MAST2 protein.

The invention also relates to the MAST-2 binding domains as such, consisting from 11 to 13 amino acid residues as defined in the groups A to E above.

The invention is also directed to a polypeptide which comprises or consists of, from N-terminal to C-terminal:

(1) optionally, a signal peptide, preferably a signal peptide as defined in SEQ ID NO:3, or a variant having at least 68% identity with said SEQ ID NO:3, said variant retaining the capacity to target the polypeptide into the endoplasmic reticulum and optionally through the secretory pathway;

(2) optionally, the last two C-terminal residues of the ectodomain of a rabies virus G protein;

(3) a anchoring domain, preferably an anchoring domain as defined in SEQ ID NO:4 or a variant having at least 81% identity with said SEQ ID NO:4 retaining the capacity to anchor the polypeptide in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells; and

(4) a cytoplasmic domain comprising or consisting of (a) a cytoplasmic part upstream of the MAST-2 binding domain, preferably a peptide as defined in SEQ ID NO:2 or a variant having at least 80% identity with SEQ ID NO:2, and (b) a MAST-2 binding domain as defined above in groups A to E. In a particular embodiment, said MAST-2 binding domain is as defined in SEQ ID NO:19 to SEQ ID NO:209, preferably chosen from the group consisting of SEQ ID NO:19 to SEQ ID NO:101, SEQ ID NO:102 to SEQ ID NO:191 and SEQ ID NO:192 to SEQ ID NO:209, more preferably as defined in SEQ ID NOS: 64-68, 71, 74 and 208-209.

Thus, as a particular embodiment, the invention relates to a polypeptide, of at most 350 amino acids, comprising, from N-terminal to C-terminal, a domain for anchoring said polypeptide into the reticulum membrane and/or Golgi membrane (*i.e.,* the anchoring domain), and a domain exposed cytoplasmically (*i.e.,* the cytoplasmic domain) when the polypeptide is anchored in the membrane, wherein said cytoplasmic domain ends with a MAST-2 binding domain as defined in the groups A to E above, whose size is from 11 to 13 amino acid residues. This polypeptide corresponds to the polypeptide as defined above but deprived of their signal peptide, following the cleavage of this signal peptide once the polypeptide as defined above anchors into the membrane. Thus, the invention also concerns a polypeptide which comprises or consists of, from N-terminal to C-terminal:

(1) optionally, the last two C-terminal residues of the ectodomain of a rabies virus G protein;

(2) a anchoring domain, preferably an anchoring domain as defined in SEQ ID NO:4 or a variant having at least 81% identity with said SEQ ID NO:4 retaining the capacity to anchor the polypeptide in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells; and

(3) a cytoplasmic domain comprising or consisting of (a) a cytoplasmic part upstream of the MAST-2 binding domain, preferably a peptide as defined in SEQ ID NO:2 or a variant having at least 80% identity with SEQ ID NO:2, and (b) a MAST-2 binding domain as defined above in groups A to E. In a particular embodiment, said MAST-2 binding domain is as defined in SEQ ID NO:19 to SEQ ID NO:209, preferably chosen from the group consisting of SEQ ID NO:19 to SEQ ID NO:101, SEQ ID NO:102 to SEQ ID NO:191 and SEQ ID NO:192 to SEQ ID NO:209, more preferably as defined in SEQ ID NOS: 64-68, 71, 74 and 208-209.

As a particular embodiment, this polypeptide consists of the following sequence:

- residues 20 to 74 of SEQ ID NOs: 17 or 18, directly linked to a MAST-2 binding domain as defined above in groups A to E, such as the ones as defined in SEQ ID NO:19 to SEQ ID NO:209, preferably chosen from the group consisting of SEQ ID NO:19 to SEQ ID NO:101, SEQ ID NO:102 to SEQ ID NO:191 and SEQ ID NO:192 to SEQ ID NO:209, more preferably as defined in SEQ ID NOS: 64-68, 71, 74 and 208-209; or

- residues 20 to 85 of one of the sequences SEQ ID NOs:210 to 216 or residues 20 to 87 of SEQ ID NO:217 or 218.

The invention is also directed to any polynucleotide (or nucleic acid) encoding a polypeptide of the invention as defined herein, in accordance with the universal genetic code, taking due account of its degeneracy. In a particular embodiment, the polynucleotide of the invention is DNA, RNA either as a positive strand or negative strand (when for example found in a viral particle) or as cDNA (when for example expressed in a cell transfected by a viral particle). The size of the polynucleotide of the invention is at most 1050, at most 750, at most 600 or at most 450 base pairs (bp). In a preferred embodiment, the size of the polynucleotide of the invention is at most 300 bp and is preferably from 255 to 261 bp, and more preferably is 255, 258 or 261 bp.

These are examples of polynucleotides encoding the different domains of the polypeptides of the invention described herein:

- the signal peptide is for example encoded by a polynucleotide located from nucleotides 1 to 57 of SEQ ID NO:219 below;

- the 2 last amino acid residues of the ectodomain are for example encoded by a polynucleotide located from nucleotides 58 to 63 of SEQ ID NO:219 below;

- the transmembrane domain is for example encoded by a polynucleotide located from nucleotides 64 to 129 of SEQ ID NO:219 below; and

- the cytoplasmic part upstream of the MAST-2 binding domain is for example encoded by a polynucleotide located from nucleotides 130 to 222 of SEQ ID NO:219 below.

According to the size of the MAST-2 binding domain, the polynucleotide encoding the MAST-2 binding domain is located either from nucleotides 223 to 255 of a SEQ ID chosen from the group consisting of SEQ ID NOs:219 to 225, or from nucleotides 223 to 261 of SEQ ID NO: 226 or 227.

In a particular embodiment, the polynucleotides of the invention comprise, at their N-terminal part, a polynucleotide encoding a signal peptide.

Particular polynucleotides consist of the following sequences:

(1) polynucleotide encoding Neurovita2 (as defined in SEQ ID NO:210):
ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGT
TTTGGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGT
TGATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACG
CAACACAATCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGC
GGGAAGATCATATCTTCATGGGAAGTACACGGGGGTCAGACCAGACTGTGA
(SEQ ID NO :219);

(2) polynucleotide encoding Neurovita3 (as defined in SEQ ID NO:211):
ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGT
TTTGGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGT
TGATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACG
CAACACAATCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGC

GGGAAGATCATATCTTCATGGGAAGTACACGGGCAGCAGACCAGACTGTGA (Neurovita3) (SEQ ID NO :220);

(3) polynucleotide encoding the polypeptide as defined in SEQ ID NO:212: ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGT TTTGGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGT TGATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACG CAACACAATCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGC GGGAAGATCATATCTTCATGGGAAGTAGCCACGCAGCAGACCAGACTGTGA (SEQ ID NO :221);

(4) polynucleotide encoding the polypeptide as defined in SEQ ID NO:213: ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGT TTTGGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGT TGATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACG CAACACAATCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGC GGGAAGATCATATCTTCATGGGAAGTATACACGGGGCAGACCAGACTGTGA (SEQ ID nO:222);

(5) polynucleotide encoding the polypeptide as defined in SEQ ID NO:214: ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGT TTTGGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGT TGATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACG CAACACAATCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGC GGGAAGATCATATCTTCATGGGAAGTACACACGGGGCAGACCAGACTGTGA (SEQ ID NO :223);

(6) polynucleotide encoding the polypeptide as defined in SEQ ID NO:215: ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGT TTTGGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGT TGATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACG CAACACAATCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGC GGGAAGATCATATCTTCATGGGAAGTACACACGCAGCAGACCAGACTGTGA (SEQ ID NO :224);

(7) polynucleotide encoding the polypeptide as defined in SEQ ID NO:216: ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGT TTTGGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGT TGATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACG CAACACAATCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGC GGGAAGATCATATCTTCATGGGAAGTAGCCGGGGGGCAGACCAGACTGTGA (SEQ ID NO :225);

(8) polynucleotide encoding the polypeptide as defined in SEQ ID NO:217: ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGT TTTGGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGT TGATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACG CAACACAATCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGC GGGAAGATCATATCTTCATGGGCCGAAGCCCAGCACACGCAGCAGACCAGA CTGTGA (SEQ ID NO :226);

(9) polynucleotide encoding the polypeptide as defined in SEQ ID NO:218: ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGT TTTGGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGT TGATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACG CAACACAATCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGC GGGAAGATCATATCTTCATGGGAAGTACACGCCTCTGGGGGGCAGACCAGA CTGTGA (SEQ ID NO :227).

The polynucleotide encoding a polypeptide of the invention does not comprise or consist of the sequence as defined in SEQ ID NO:8 (Neurovita1).

The invention also relates to a nucleic acid vector (such as a plasmid) comprising a polynucleotide as defined herein, *i.e.,* a polynucleotide encoding a polypeptide of the invention. In a particular embodiment, the vector is an expression vector, *i.e.,* a vector which comprises, besides the elements explicitly mentioned, all the elements necessary to drive the expression of the polynucleotide of the invention (expression regulatory elements), and particularly transcription regulatory elements. "*Transcription regulatory element*" defines any DNA regions involved in the regulation of transcription of the polynucleotide and encompasses a promoter, such as CMV or EF1α, enhancer or cis-acting regulatory elements. These elements, and particularly the promoter, are chosen depending upon the nature of the cells to be transfected with the nucleic acid vector. The determination of the suitable promoter, according to the expression level sought or to the transfected cell, makes part of the knowledge of the person skilled in the art. It is noteworthy that, when the nucleic vector contains several polynucleotides (one of which is a polynucleotide of the invention), the transcription regulatory element(s) may be unique for all the polynucleotides or shared by some of them or in contrast each polynucleotide may be associated with one or more particular transcription regulatory element(s). In the latter case, the several transcription regulatory elements may be similar or different.

Within the present invention, the expression regulatory elements inserted into the nucleic acid vector of the invention are preferably adapted for an expression of the polynucleotide of the invention in neuronal cells, in particular in human neuronal cells, such as the human neuroblastoma cell line SH-SY5Y. These promoters include, but are not limited to, the following promoters: neuron specific enolase (NSE), synapsin-1 (SYN), platelet-derived growth factor (PDGF), tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) (Boulaire et al.; (2009).

The invention also concerns an expression lentivirus-derived vector, in particular a plasmid, comprising, in addition to the polynucleotide of the invention (*i.e.,* a polynucleotide encoding a polypeptide of the invention), regulatory signals for transcription and expression of said polynucleotide (expression regulatory elements), a cis-acting central initiation region (cPPT) and a cis-acting termination region (CTS) both of lentiviral origin and regulatory signals of retroviral origin for reverse transcription, expression and packaging. This vector is the transfer vector when used in a transcomplementation system (vector/packaging system) (see below).

In a particular embodiment, the expression lentivirus-derived vector can be prepared from the genome of a lentivirus or retrovirus, and only contains, apart from the polynucleotide or the nucleic acid construct of the invention, the sequences of the lentiviral or retroviral genome which are non-coding regions of said genome, necessary to provide recognition signals for DNA or RNA synthesis and processing. Hence, an expression lentivirus-derived vector may be a replacement vector in which all the viral coding sequences, between the 2 long terminal repeats (LTRs) of a lentivirus or retrovirus genome, have been replaced by the polynucleotide of the invention, regulatory signals for transcription and expression of said polynucleotide (expression regulatory elements), a cis-acting central initiation region (cPPT) and a cis-acting termination region (CTS) both of lentiviral origin and regulatory signals of retroviral origin for reverse transcription, expression and packaging. In a particular embodiment, the expression lentivirus-derived vector is obtained from a HIV genome, in particular from a HIV-1 genome, in which all the viral coding sequences, between the 2 long terminal repeats (LTRs) have been replaced by the polynucleotide of the invention, regulatory signals for transcription and expression of said polynucleotide (expression regulatory elements), a cis-acting central initiation region (cPPT) and a cis-acting termination region (CTS) both of lentiviral origin and regulatory signals of retroviral origin for reverse transcription, expression and packaging, to give an expression HIV-derived vector, in particular an expression HIV-1-derived vector.

The invention also relates to the lentiviral vector genome *i.e.,* the genetic material contained in the lentiviral vector particle, following the formation of the particles in the transcomplementation system, as well as any nucleic acid intermediates between the expression lentivirus-derived vector and the genetic material contained in the lentiviral vector particle, said lentiviral genome or nucleic acid intermediates comprising the polynucleotide of the invention, regulatory signals for transcription and expression of said polynucleotide (expression regulatory elements), a cis-acting central initiation region (cPPT) and a cis-acting termination region (CTS) both of lentiviral origin and regulatory signals of retroviral origin for reverse transcription, expression and packaging.

Thus, the invention also encompasses any appropriate nucleic acid, *i.e.,* DNA or RNA, either double or single stranded, including in the form containing the DNA flap as a triplex sequence, depending upon the stage of cycle of the particles, including the expression lentivirus-derived - used for cotransfection of the host cells with the encapsidation plasmid and the envelope plasmid - for expression of the particles, or the RNA genome of the particles when formed, or including the various forms of the nucleic acid of this genome in the transduced cells of the host to whom particles are administered, including the vector pre-integration complex.

Thus, the expression lentivirus-derived vector, the lentiviral vector genome or any nucleic acid intermediates of the invention, comprise regulatory signals for transcription and expression of non lentiviral origin, such as a promoter and/or an enhancer, preferably promoter adapted for an expression of the polynucleotide of the invention in neuronal cells, in particular in human neuronal cells as described above. Examples of promoters are CMV also referred to as CMVie (CMV immediate early), EF1α promoter, PGK.... In a particular embodiment, the polynucleotide of the invention is under the control of regulatory signals for transcription and expression.

The expression lentivirus-derived vector, the lentiviral vector genome or any nucleic acid intermediates of the invention also comprises a cis-acting central initiation region (cPPT) and a cis-acting termination region (CTS) both of lentiviral origin. These two regions are known as DNA Flap or DNA triplex. The DNA flap suitable for the invention may be obtained from a lentivirus or from a retrovirus-like organism such as retrotransposon, or may be prepared synthetically (chemical synthesis) or by amplification of the DNA flap from any lentivirus genome such as by Polymerase chain reaction (PCR). The DNA flap may be obtained from a lentivirus and in particular a HIV retrovirus, or from the CAEV (Caprine Arthritis Encephalitis Virus) virus, the EIAV (Equine Infectious Anaemia Virus) virus, the VISNA virus, the SIV (Simian Immunodeficiency Virus) virus or the FIV (Feline Immunodeficiency Virus) virus. In a more preferred embodiment, the DNA flap is obtained from an HIV retrovirus, for example HIV-1 or HIV-2 virus including any isolate of these two types. Preferred DNA flap comprises or consists in the sequences as defined in SEQ ID NOs: 228 to 234. It is noteworthy that the DNA flap is used as a DNA fragment isolated from its natural (lentiviral genome) nucleotide context *i.e.,* out of the context of the *pol* gene in which it is naturally contained in the lentivirus genome. Therefore, the DNA flap is used, in the present invention, deleted from the unnecessary 5' and 3' parts of the *pol* gene and is recombined with sequences of different origin.

According to a particular embodiment, a DNA flap has a nucleotide sequence of about 90 to about 140 nucleotides. In HIV-1, the DNA flap is a stable

99-nucleotide-long plus strand overlap. When used in the genome vector of the lentiviral vector of the invention, it may be inserted as a longer sequence, especially when it is prepared as a PCR fragment. A particular appropriate polynucleotide comprising the structure providing the DNA flap is a 178-base pair polymerase chain reaction (PCR) fragment encompassing the cPPT and CTS regions of the HIV-1 DNA.

In a particular embodiment, the cPTT and CTS regions are inserted, in a functional orientation, into the vector or lentiviral genome, in order to adopt a triplex conformation during reverse transcription.

In a particular embodiment, the DNA flap is inserted immediately upstream of the polynucleotide of the invention or immediately upstream from the promoter controlling the expression of the polynucleotide of the invention, advantageously to have a central or nearly central position in the vector genome.

The expression lentivirus-derived vector, the lentiviral vector genome or any nucleic acid intermediates of the invention also comprises regulatory signals of retroviral origin for reverse transcription, expression and packaging. Examples of such elements are at least one (preferably two) long terminal repeats (LTR), such as a LTR5' and a LTR3' and a psi sequence involved in the lentiviral genome encapsidation. In a particular embodiment of the invention, the LTR, preferably the LTR3', is deleted for the promoter and the enhancer of the U3 region; this modification has been shown to increase substantially the transcription of the transgene inserted in the lentiviral genome (WO01/27304).

The expression lentivirus-derived vector, the lentiviral vector genome or any nucleic acid intermediates of the invention may also optionally comprise at least one the following elements:

- elements selected among a splice donor site (SD), a splice acceptor site (SA) and/or a Rev-responsive element (RRE); and/or

- several unique restriction sites for cloning the polynucleotide of the invention; and/or

- a sequence of DNA at which replication is initiated , origin of replication (ori), whose sequence is dependent on the nature of cells where the lentiviral genome has to be expressed. Said ori may be from mammalian origin, most preferably of human origin, preferably adapted for replication in human neuronal cells. It is an advantageous embodiment of the invention to have an ori inserted into the lentiviral genome or the expression lentivirus-derived vector of the invention when the lentiviral genome does not integrate into the cell host genome; thus, the presence of an ori ensures that at least one lentiviral genome is present in each cell, even after cell division; and/or

- at least one scaffold attachment region (SAR) and/or a matrix attachment region (MAR). Indeed, these AT-rich sequences enable to anchor the lentiviral genome to the matrix of the cell chromosome, thus regulating the transcription of the polynucleotide of the invention.

In particular embodiments of the invention, either independently of or in combination with the embodiments discussed throughout the specification, the expression lentivirus-derived vector or the lentiviral vector genome is devoid of functional *gag*, *pol* and/or *env* lentiviral genes. By "*functional*" it is meant a gene that is correctly transcribed, and/or correctly expressed. Thus, the expression lentivirus-derived vector or the lentiviral vector genome of the invention in this embodiment contains at least one of, preferably all, the *gag*, *pol* and *env* genes that is either not transcribed or incompletely transcribed; the expression "*incompletely transcribed*" refers to the alteration in the transcripts gag, gag-pro or gag-pro-pol, one of these or several of these being not transcribed. In a particular embodiment the expression lentivirus-derived vector or the lentiviral vector genome is devoid of *gag*, *pol* and/or *env* lentiviral genes. In a particular embodiment, the expression lentivirus-derived vector or the lentiviral vector genome is also devoid of the coding sequences for *Vif*-, *Vpr*-, *Vpu*- and *Nef*-accessory genes (for HIV-1 lentiviral vectors), or of their complete or functional genes.

The lentiviral vector of the invention is non replicative *i.e.,* the expression lentivirus-derived vector or the lentiviral vector genome are not able to form new particles budding from the infected host cell. This may be achieved by the absence in the expression lentivirus-derived vector or in the lentiviral vector genome of the *gag*, *pol* or *env* genes, as indicated in the above paragraph; this can also be achieved by deleting other viral coding sequence(s) and/or cis-acting genetic elements needed for particles formation. The absence of formation of particles should be distinguished from the replication of the expression lentivirus-derived vector or the lentiviral vector genome. Indeed, as described before, the expression lentivirus-derived vector or the lentiviral vector genome may contain an origin of replication ensuring the replication of the expression lentivirus-derived vector or the lentiviral vector genome without ensuring the formation of particles.

The invention also concerns a lentiviral vector pseudotyped particle comprising GAG structural proteins and a viral core made of (a) POL proteins and (b) a lentiviral vector genome comprising the polynucleotide of the invention, expression regulatory elements of said polynucleotide, a cis-acting central initiation region (cPPT) and a cis-acting termination region (CTS) both of lentiviral origin and regulatory signals of retroviral origin for reverse transcription, expression and packaging, wherein said particle is pseudotyped with the G protein of a VSV virus or the G protein of a rabies virus.

The expression "*lentiviral vector pseudotyped particle*" encompasses a lentiviral particle that comprises both proteins and genetic material, preferably encapsidated into these proteins. Particles are made of viral envelope proteins (encoded by an *env* gene) as well as structural proteins (encoded by a *gag* gene). Inside the particles, a viral core (or capsid) formed of three enzymes (encoded by a *pol* gene), *i.e.*, the reverse transcriptase, the integrase and the protease, and genetic material (the lentiviral genome). The features of the expression regulatory elements of said polynucleotide, a cis-acting central initiation region (cPPT) and a cis-acting termination region (CTS) both of lentiviral origin and regulatory signals of retroviral origin for reverse transcription, expression and packaging contained in the lentiviral genome are as defined above for the expression lentivirus-derived vector. Indeed, the lentiviral genome contained in the lentiviral particle is a transcript of the nucleic acid contained in the expression lentivirus-derived vector.

The envelope protein of the lentiviral vector of the invention may be pseudotyped with the envelope protein of the lentivirus used to prepare the lentiviral vector, or alternatively with a heterogeneous envelope protein that is chosen with respect to the cells to be targeted into the host.

In a particular embodiment, said lentiviral particle is pseudotyped with a VSV-G protein. The VSV-G protein originates from the serotype Indiana, New Jersey, Piry, Chandipura, Isfahan, Cocal or the combination of at least two of these serotypes. In a particular embodiment, the VSV-G protein originating from a VSV is modified with respect to its native form, especially to improve pseudotyping.

In another embodiment, said lentiviral particle is pseudotyped with the G protein of a rabies virus. In a particular embodiment, the G protein originates from an attenuated strain such as the ERA-NIV (ERA) strain. In a particular embodiment, the G protein originates from a virulent strain such as the CVS-NIV (CVS) strain, the CVS-Gif-sur-Yvette strain (Préhaud *et al.* 1988), the CVS-11 strain, the N2C strain or the CVS-24 strain. In a particular embodiment, the G protein originates from the CVS24 B2c strain (Morimoto et al. 1998; Mentis et al. 2006). A lentiviral particle, comprising in its lentiviral genome a polynucleotide encoding for a polypeptide of the invention, pseudotyped with the G protein of a rabies virus, is a preferred product of the invention.

The original ERA and CVS strains of rabies virus (RABV) are available from the ATCC under deposit number vr332 and vr959, respectively (Prehaud C et al,, 2003, and WO2010/116258).

The sequence of the G protein of the CVS-NIV strain is available under accession number AF406694 and is as defined in SEQ ID NO:12. The G protein of the CVS-NIV strain is available from the recombinant *E. coli* strain deposited, under number I-2758, on the 30^{th} of November, 2001 at the CNCM (Institut Pasteur, 25 rue du Docteur Roux, 75724 PARIS Cedex 15 - France) under the terms of the Budapest Treaty. This recombinant *E. coli* comprises a plasmid (plasmid pRev-TRE-G-CVS; WO 03/048198), which inducibly expresses the G protein of the CVS-NIV strain.

The sequence of the G protein of the ERA-NIV strain is available under accession number AF406693 and is as defined in SEQ ID NO:13. The G protein of the ERA strain is available from the recombinant *E. coli* strain deposited, under number I-2760, on the 30^{th} of November, 2001 at the CNCM under the terms of the Budapest Treaty. This recombinant *E. coli* comprises a plasmid (plasmid pRev-TRE-G-ERA; WO 03/048198), which inducibly expresses the G protein of the ERA strain.

Appropriate conditions for the cultivation of the recombinant *E. coli* strain containing the plasmid CNCM I-2758 or the plasmid CNCM I-2760 comprise the incubation of said recombinant *E. coli* strain at 37°C on a standard LB-TYM growth medium (in the presence of ampicillin).

The nucleotide and protein sequences of the G protein of the CVS24 B2c strain are as defined in SEQ ID NO:287 and SEQ ID NO:288, respectively.

In a particular embodiment, the integrase protein contained in the lentiviral vector pseudotyped particle is defective. The integrase protein is one of the proteins encoded by the *pol* gene. By "*defective*", it is meant that the integrase, of lentiviral origin, is devoid of the capacity of integration of the lentiviral genome into the genome of the host cells *i.e.,* an integrase protein mutated to specifically alter its integrase activity. Accordingly the integrase capacity of the protein is altered whereas the correct expression of the GAG, PRO and POL proteins and/or the formation of the capsid and hence of the vector particles, as well as other steps of the viral cycle, preceding or subsequent to the integration step, such as the reverse transcription, the nucleus import, stay intact. An integrase is said defective when the integration that it should enable is altered in such a way that an integration step takes place less than 1 over 1000, preferably less than 1 over 10000, when compared to a lentiviral vector containing a corresponding wild-type integrase.

In a particular embodiment of the invention, the property of the integrase of being defective, results from a mutation of class 1, preferably amino acid substitutions (one-amino acid substitution) or short deletions giving rise to a protein fulfilling the requirements of the preceding paragraph. The mutation is carried out within the *pol* gene. Examples of mutations altering HIV-1 and enabling to obtain a non-functional integrase for integration (integration-incompetent integrase) are the following: H12N, H12C, H16C, H16V, S81 R, D41A, K42A, H51A, Q53C, D55V, D64E, D64V, E69A, K71A, E85A, E87A, D116N, D116I, D116A, N120G, N120I, N120E, E152G, E152A, D35E, K156E, K156A, E157A, K159E, K159A, K160A, R166A, D167A, E170A, H171A, K173A, K186Q, K186T, K188T, E198A, R199C, R199T, R199A, D202A, K211A, Q214L, Q216L, Q221 L, W235F, W235E, K236S, K236A, K246A, G247W, D253A, R262A, R263A and K264H. Another proposed substitution is the replacement of the amino acids residues RRK (positions 262 to 264) by the amino acids residues AAH. In a particular embodiment, the following substitutions are preferred: H12N, H12C, H16C, H16V, S81 R, D41A, K42A, H51A, Q53C, D55V, D64E, D64V, E69A, K71A, E85A, E87A, D116I, D116A, N120G, N120I, N120E, E152G, E152A, D35E, K156E, K156A, E157A, K159E, K159A, K160A, R166A, D167A, E170A, H171A, K173A, K186Q, K186T, K188T, E198A, R199C, R199T, R199A, D202A, K211A, Q214L, Q216L, Q221 L, W235F, W235E, K236S, K236A, K246A, G247W, D253A, R262A, R263A and K264H. Other mutations are disclosed in Wanisch and Yáñez-Muñoz (2009). A particularly proper mutation is the D64V mutation.

Whatever the elements contained in the lentiviral vector genome, the nature of the envelope protein of the particle and the defective feature or not of the integrase protein, the lentiviral vector pseudotyped particle is preferably obtained by a transcomplementation system (vector/packaging system). Thus, a permissive cell (such as 293T cells) is *in vitro* transfected with a transfer vector which is a expression lentivirus-derived vector as defined herein and with at least one other plasmid providing, *in trans*, the *gag*, *pol* and *env* sequences encoding the polypeptides GAG, POL and the envelope protein(s), or for a portion of these polypeptides sufficient to enable formation of lentiviral particles. The transfer vector generates, as a transcript, the lentiviral genome, whereas the *gag*, *pol* and *env* provide respectively the GAG structural proteins, the POL protein for the viral core (preferably with a defective integrase) and the pseudotyped ENV proteins (preferably a G protein from VSV or a G protein from a rabies virus).

As an example, permissive cells are transfected with a first plasmid which is the expression lentivirus-derived vector of the invention (transfer vector), a second plasmid (envelope expression plasmid or pseudotyping env plasmid) comprising a gene encoding an envelope protein(s) (such as VSV-G or the protein G of a rabies virus), and a third plasmid (encapsidation plasmid or packaging construct) expressing the GAG and POL proteins.

The invention is also directed to a cell (preferably isolated) or a cell culture transfected with a vector of the invention or transduced by a lentiviral particle of the invention. Thus, the cell or cell culture of the invention comprises or expresses at least one polypeptide of the invention, and/or comprises at least one polynucleotide of the invention and/or at least one vector of the invention.

Said cell can be a eukaryotic cell [or a cell culture made of eukaryotic cells], preferably a mammal cell, for example a human cell or a non-human cell, most preferably a human cell. Preferably, said cell is not a human embryonic cell or a human germinal cell.

In a particular embodiment, said cell is a neuronal cell, preferably a human neuronal cell. In a particular embodiment, said cell are human pre-mitotic neurons, immature human neurons, such as neuroblastoma cells, Ntera 2D1 (ATCC CRL-1973), SK-N-SH (ATCC HTB11), SH-SY-5Y (ATCC CRL-2266), U373MG (human astrocytoma cell line; Babault N et al, 2011) (Prehaud C. et al, 2010, 2005 and 2003 ; Lafon M. et al, 2008 and 2006 ; Megret F. et al. 2007).

These are particular cells or cell culture that may be transfected or transduced according to the present specification:

- the SH-SY5Y cell culture, a human neuroblastoma cell line, which is available from the American Type Culture Collection (ATCC; 10801 University Blvd.; Manassas, Virginia 20110-2209; U.S.A.) under deposit number CRL-2266. These cells, which are a sub clone of the human neuroblastoma cell line SK-N-SH (ATCC, HTB11), may differentiate when they are treated with the cell permeable db-cAMP. These differentiated cells have shown high plasticity, outgrowth and retraction (Loh SHY et al. 2008);

- pure post-mitotic human neurons (NT2-N), which are obtained from the embryonic carcinoma cell line NTera 2cl.-D1 (ATCC CRL-1973), as described in the art, *e.g.,* in Préhaud et al. 2005. Tera cells N2D1 can differentiate into pure cultures of human post-mitotic neurons (NT2-N) after induction of differentiation by all-trans retinoic acid (ATRA), then treatment with inhibitors of mitosis and purification arranged by trypsinization (Andrews PW, 1998). NT2-N cells have all the specific markers of differentiated human neurons (Guillemain I. 2000). They can establish *in vitro* synaptic contacts between them and the functional contacts with astrocytes in co-culture, as well as functional synapses.

- rat pheochromocytoma cells (NS cells, Cellomics USA), which are a subclone of PC12 cells, differentiated with NGF. These differentiated cells present a strong and organized neurite network and have been validated for high throughput screening. NS cells extend neurites, become electrically excitable, become more responsive to exogenously applied acetylcholine, have increased numbers of calcium channels, and increase the synthesis of several neurotransmitters. NS cells grown in the presence of NGF resemble sympathetic neurons and are a model of noradrenergic neurons.

- the SK-N-SH human neuroblastoma cell line (ATCC HTB11), which is a prototype of adrenergic immature neurons (Von Reitzentstein, 2001). These cells can be differentiated further by treatment with ATRA (Gaitonde et al. 2001; Wainwright et al. 2001).

Alternatively, said cell can be a prokaryotic cell [or a cell culture made of prokaryotic cells], preferably a bacterium, for example *E. coli*.

In a particular embodiment, the cell contains, integrated in its genome, the polynucleotide of the invention (expressing the polypeptide of the invention), especially when the cell or cell culture has been previously transduced by a lentiviral particle of the invention. Alternatively, the polynucleotide of the invention is not integrated in the genome of the cells, even when it has been previously transduced by a lentiviral particle of the invention, as a result of a defective integrase. In this latter case, the polynucleotide of the invention advantageously comprises an origin of replication.

The invention also concerns a composition comprising a polypeptide of the invention, a polynucleotide of the invention, a vector of the invention, an expression lentivirus-derived vector of the invention, a lentiviral vector pseudotyped particle of the invention or a cell of the invention, and optionally a pharmaceutically acceptable vehicle, excipient or carrier. The composition comprises the polypeptide, the polynucleotide, the vector, the expression lentivirus-derived vector, the lentiviral vector pseudotyped particle or the cell of the invention, as active principle, said composition being suitable for administration into a host, preferably a human host.

A preferred composition comprises a lentiviral vector particle pseudotyped with a rabies G protein of the invention. Indeed, the lentiviral vector particle pseudotyped with a rabies G protein of the invention combines at least the two advantageous features:

(1) the polypeptide of the invention, via the features of the MAST-2 binding domain of the cytoplasmic domain as defined above, has a high affinity for the PDZ domain of the human MAST2 protein. Thus, the use of the polypeptide of the invention improves the effects observed on the induction and/or the stimulation of the neurite outgrowth and/or on the neurosurvival, as compared to the polypeptides of the prior art; and

(2) the pseudotyping of the particle with the G protein of a rabies virus enables to specifically target the neuronal cells, by retrograde transport from the muscle (site of injection of the composition), and thus to avoid the unnecessary transduction of other cell types.

A "*pharmaceutically acceptable carrier*" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any conventional type. A "*pharmaceutically acceptable carrier*" is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation; suitable carriers include, but are not limited to, phosphate buffered saline solutions, distilled water, emulsions such as an oil/water emulsions, various types of wetting agents sterile solutions and the like, dextrose, glycerol, saline, ethanol, and combinations thereof. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like. Carriers for parenteral administration include aqueous solutions of dextrose, mannitol, mannose, sorbitol, saline, pure water, ethanol, glycerol, propyleneglycol, peanut oil, sesame oil, polyoxyethylene-polyoxypropylene block polymers, and the like.

A "*pharmaceutically acceptable excipient*" refers to a substance that is used as a carrier or for the manufacturing of the administrable form of polypeptide(s) of the invention. Suitable excipients include fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize, wheat, rice, or potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Thus, for oral use of the polypeptide(s) of the invention, a solid excipient can be used, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Examples of excipients for coating of dragee or tablet are concentrated sugar solutions which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

The invention also relates to a polypeptide of the invention, a polynucleotide of the invention, a vector of the invention, an expression lentivirus-derived vector of the invention, a lentiviral vector pseudotyped particle of the invention, a cell of the invention or a composition of the invention (disclosed hereinafter as the products of the invention), for use as a medicament or a drug.

Regarding the use as a medicament, as well as the uses and treatments detailed below, a lentiviral vector particle pseudotyped with a rabies virus G protein of the invention or a composition comprising lentiviral vector particle(s) pseudotyped with a rabies virus G proteins of the invention, are preferred.

Thus, the products of the invention are used for inducing and/or stimulating neurite outgrowth, more particularly in the treatment and/or palliation and/or prevention of a disease, disorder or condition involving an insufficient or impaired neuritogenesis, more particularly an insufficient or impaired neurite outgrowth.

In accordance with the invention, the products of the invention, is intended as an effector of neurite outgrowth (and/or of axon and/or dendrite development), *e.g.*, for neuron differentiation from neuron progenitors or neoplastic neurons, and/or for neuron regeneration of impaired neurons (both effects being obtained through stimulation of neurite outgrowth). In a particular embodiment, the products of the invention are for use to induce and/or to stimulate neuritogenesis, more particularly neurite outgrowth, still more particularly human neurite outgrowth. In another particular embodiment, the products of the invention are for use to induce and/or to stimulate neuritogenesis, more particularly neurite outgrowth from pre-mitotic neurons, neoplastic neurons, neuron progenitors, as well as from impaired neurons.

The products of the invention are for use as a neuroregenerative (generation of new functional neurons, glia, axons, myelin, and/or synapses) and/or neuroprotective agent (protection of neurons from apoptosis or degeneration).

The products of the invention are for use to stimulate and/or to induce neurite sprouting and/or axon growth and/or dendritic tree extension.

The products of the invention are for use to stimulate and/or to induce synaptogenesis and/or neurotransmission. Indeed, the polypeptide of the invention stimulates the activity of the growth cone. Furthermore, it prevents growth cone from collapsing upon contact with a growth collapsing agent, such as LPA or oxidative stress.

The products of the invention are for use to stimulate neuronal development and/or neuronal regeneration and/or axon growth and/or dendrite development and/or dendritic tree extension and/or neuronal plasticity and/or synaptogenesis and/or neurotransmission.

The products of the invention are for use to prevent and/or to inhibit and/or to block any kind of neurotoxicity which would lead to neurite retraction and/or growth cone collapse.

The products of the invention are for use to stimulate and/or to induce neurite outgrowth and/or growth cone activity after said neurite and/or cone has been in contact with a neurotoxic agent.

The products of the invention are for use to prevent and/or to inhibit and/or to block growth cone collapse and/or neurite retraction and/or axodendritic damage or lesion and/or disruption of synaptic integrity and/or loss of neuron connectivity and/or damage to nerve endings and/or neurotransmission impairment.

The products of the invention are for use to induce and/or stimulate neurite outgrowth, which is notably useful

- in inducing neuron differentiation, for example in the treatment and/or palliation and/or prevention of a neoplasm of the nervous system, as well as

- in regenerating impaired neurons, more particularly impaired neurites, for example

- in the treatment and/or palliation and/or prevention of a neurodegenerative disease, disorder or condition, in the treatment and/or palliation and/or prevention of microbial infections of the neurons, or in protecting neurons from neurotoxic agents or oxidative stress.

Therefore, the invention relates to products of the invention, for use in the treatment and/or palliation and/or prevention of any disease, disorder or condition which involves an insufficient or impaired neuritogenesis, more particularly an insufficient or impaired neurite outgrowth or an insufficient dendrites arborisation.

Said disease, disorder or condition is alternatively or complementarily defined as any disease, disorder or condition involving an unbalanced neuron cell cycle, wherein said neuron cell cycle is unbalanced:

- either by excessive or undesired presence of pre-mitotic neurons (more particularly, by insufficient neuron differentiation and/or by excessive or undesired re-entry of post-mitotic neurons into the neuron cell cycle, as is the case when a neoplasm develops in the nervous system), or

- by excessive or undesired neuron degeneration, more particularly excessive or undesired neurite degeneration (as is the case for a neurodegenerative disease, disorder or condition, and for certain microbial infection of the neurons).

The products of the invention are for use in the treatment and/or palliation and/or prevention of a disease, disorder or condition, which alters the Central Nervous System (CNS) and/or the Peripheral Nervous System (PNS), for example as a neurorestorative therapy and/or prevention and/or palliation. The expression "*Central Nervous System*" or "*CNS*" is herein intended as meaning the brain and (in case of a vertebrate animal) the spinal cord. The peripheral nervous system (PNS) is the vast network of spinal and cranial nerves linking the body to the brain and spinal cord. The PNS is subdivided into the autonomic nervous system (sympathetic NS and parasympathetic NS) and the somatic nervous system. The PNS consists of sensory neurons running from stimulus receptors to the CNS and motor neurons running from the CNS to the muscle and glands.

According to an embodiment of the invention, said disease, disorder or condition is or involves a microbial infection of the nervous system, such as a bacterial and/or viral infection, more particularly a viral infection. Preferably, said microbial infection is a microbial infection that induces neuron apoptosis, such as poliomyelitis (Blondel *et al.*, 2005). As an example of viral infection is poliovirus infection or West Nile virus infection.

According to another embodiment of the invention, said disease, disorder or condition is or involves a non-viral disease, disorder or condition, more preferably a non-bacterial and non-viral disease, disorder or condition, still more preferably a non-microbial disease, disorder or condition.

According to an embodiment of the invention, said disease or disorder is or involves a neurodegenerative disease or disorder (for example, a chronic neurodegenerative disease or disorder), such as non-viral encephalopathy, Alzheimer's disease, Parkinson's disease, ALS, Huntington disease, multiple sclerosis (MS) or rare genetic disease. Preferably, said neurodegenerative disease or disorder is a non-viral disease or disorder, more preferably a non-bacterial and non-viral disease or disorder, still more preferably a non-microbial disorder.

According to an embodiment of the invention, said condition is or involves a neurodegenerative condition, such as aging. Preferably, said neurodegenerative condition is a non-viral condition, more preferably a non-bacterial and non-viral condition, still more preferably a non-microbial condition.

According to an embodiment of the invention, said disease, disorder or condition is or involves a physical or ischemic injury of the nervous system, such as seizure, stroke, trauma, epilepsy. Preferably, said physical or ischemic injury is a non-viral disease, disorder or condition, more preferably a non-bacterial and non-viral disease, disorder or condition, still more preferably a non-microbial disease, disorder or condition.

According to an embodiment of the invention, said disease, disorder or condition involves the presence of a chemical neurotoxic agent and/or of an oxidative stress. Preferably, said disease, disorder or condition is a non-viral disease, disorder or condition, more preferably a non-bacterial and non-viral disease, disorder or condition, still more preferably a non-microbial disease, disorder or condition.

According to an embodiment of the invention, said disease is a neoplasm, more particularly a neoplasm which comprises neoplastic neurons. The term "*neoplasm*" is herein more particularly intended as a malignant neoplasm, more particularly a cancer, still more particularly a tumor or a leukaemia, even still more particularly a tumor.

Any administration mode that the skilled person may find appropriate is encompassed by the present invention. Depending on how the product of the invention is formulated, it can be administered by parenteral or enteral (*e.g.*, oral) administration, preferably by parenteral administration, more preferably by parenteral injection.

The invention also concerns the use of a polynucleotide of the invention, a vector of the invention, an expression lentivirus-derived vector of the invention, a lentiviral vector pseudotyped particle of the invention, a cell or a composition of the invention, for the manufacture of a medicament or a drug for the treatment and/or palliation and/or prevention of any disease, disorder or condition as defined above.

The invention also relates to a method of treatment of a subject, more particularly of a human being, in need thereof, which comprises administering to said subject or human being at least a polynucleotide of the invention, a vector of the invention, an expression lentivirus-derived vector of the invention, a lentiviral vector pseudotyped particle of the invention, a cell of the invention or a composition of the invention. This method of treatment is intended for the treatment and/or palliation and/or prevention of any disease, disorder or condition as defined above.

The products of the invention are not immunogenic agents or adjuvants, or at the very least are not used as immunogenic agents or adjuvants and are not used under conditions which would enable the polypeptide of the invention to act as an immunogenic agent or adjuvant. The products of the invention do not raise a detectable humoral immune response after administration.

The invention also concerns a method to determine the neurosurvival and/or neuroprotection activity of a given molecule in a cell, comprising:

(a) adding a molecule to be assayed in contact with a cell or cell culture;

(b) measuring the expression of a set of genes consisting of or comprising the five cellular genes ROBO1, POU4F1, PTN, PARD6B and PAFAH1B1, in a cell or cell culture of step a); and

(c) normalizing the expression of each of the genes measured in step b) on the expression of the same genes measured in a cell of the same cell type, which has not been in contact with the said molecule,

wherein a statistically significant modulation of the expression of the genes of said set reveals that said molecule may have a neurosurvival and/or neuroprotection activity.

In a particular embodiment, step b) further comprises the measurement of the expression of at least one additional gene selected from the group consisting of the twelve cellular genes PIK3CG, BMP2, DRD1, PAX5, S100A6, DRD2, HDAC7, HEY2, INHBA, SHH, BTK and FOS. In that case, each additional gene is normalized in step c) on the expression of the same additional gene measured in a cell of the same cell type, which has not been contacted with said molecule.

Thus, in a particular embodiment, the method to determine the neurosurvival and/or neuroprotection activity of a molecule in a cell, comprises:

(a) adding a molecule to be assayed in contact with a cell or cell culture;

(b) measuring the expression of a set of genes consisting of or comprising the five cellular genes ROBO1, POU4F1, PTN, PARD6B and PAFAH1B1, and optionally at least one additional gene selected from the group consisting of the twelve cellular genes PIK3CG, BMP2, DRD1, PAX5, S100A6, DRD2, HDAC7, HEY2, INHBA, SHH, BTK and FOS, in a cell or cell culture of step a); and

(c) normalizing the expression of each of the genes measured in step b) on the expression of the same genes measured in a cell of the same cell type, which has not been in contact with said molecule,

wherein a statistically significant modulation of the expression of the genes of said set reveals that said molecule may have a neurosurvival and/or neuroprotection activity.

The nucleotide sequences of the ROBO1, POU4F1, PTN, PARD6B, PAFAH1B1, PIK3CG, BMP2, DRD1, PAX5, S100A6, DRD2, HDAC7, HEY2, INHBA, SHH, BTK and FOS genes are as defined in SEQ ID NO:236 to SEQ ID NO:252 respectively.

This method (or process) comprises, in a first step, adding a molecule to be assayed in contact with a cell or cell culture.

By "*adding a molecule to be assayed in contact with a cell or cell culture*", it is meant that the molecule must be able to interact with the PDZ domain of the human MAST2 protein, and therefore must be expressed in the cytoplasm of this cell or cell culture, in particular in a cell or cell culture expressing the human MAST-2 protein. Thus, the first step consists of expressing the molecule to be assayed in the cytoplasm of the cell.

Thus, any method known from the person skilled in the art may used to transfect or transform cells, or make cell permeable to the molecule in particular according to the nature of the molecule.

As an illustration of said expression into the cytoplasm, whatever the nature of the molecule to be assayed, the molecule may be transported into the cytoplasm of a cell, using liposomes, by contacting said cell with a liposome containing the molecule to be assayed, or by electroporation or by nanoparticles delivery.

As a particular embodiment, and when the molecule is a protein or a polypeptide, the expression can result from the transfection of this cell by a nucleic acid, a plasmid or a vector containing the nucleic acid sequence encoding this protein or polypeptide. In this embodiment, the first step of the method consists in transfecting said cell with a nucleic acid, any plasmid or a vector containing the nucleic acid sequence encoding this protein or polypeptide. In a particular embodiment, the molecule is a polypeptide of the invention as defined herein. In this embodiment, the first step of the method consists in transfecting said cell with a polynucleotide or a vector as defined in the specification. Known methods encompass chemical-based transfection, such as calcium phosphate, cationic liposomes (DOTMA and DOPE, Lipofectamine and UptiFectin), cationic polymers (DEAE-dextran, polyethylenimine, Fugene, LT-1, GeneJuice and JetPEI), and non chemical methods, such as electroporation, sono-poration, optical transfection, gene electrotransfer or impalefection.

Alternatively, a cell or cell culture may also be transduced by a viral particle, which comprises in its viral genome, the nucleic acid sequence encoding the protein or polypeptide to be assayed. As a particular embodiment, the particles as defined in the present specification may be used to transduce cells or cell culture.

To determine the neurosurvival and/or neuroprotection activity of a molecule, the method is implemented into neuronal cell, in particular expressing the MAST-2 protein, preferably a human neuronal cell. In a particular embodiment, said cell are human pre-mitotic neurons, immature human neurons, such as neuroblastoma cells. The method is preferably implemented on the SH-SY5Y cells, the NT2-N cells, the NS cells or the NS-SK-N-SH cells, as defined above.

The second step comprises measuring, in a cell or cell culture; which has been in contact with the molecule to be assayed, the expression of a set of genes consisting of or comprising the five cellular genes ROBO1, POU4F1, PTN, PARD6B and PAFAH1B1. This step may further comprise the measurement of the expression of at least one additional gene, in particular 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 genes, selected from the group consisting of the twelve cellular genes PIK3CG, BMP2, DRD1, PAX5, S100A6, DRD2, HDAC7, HEY2, INHBA, SHH, BTK and FOS.

By "*measuring*", it is meant assaying, in particular detecting, the product or several products resulting from the expression of a cellular gene, this product being in the form of a nucleic acid, especially RNA, mRNA, cDNA, polypeptide, protein or any other formats. In a particular embodiment, the measurement of the gene expression comprises detecting a set of nucleotide targets, each nucleotide target corresponding to the expression product of a gene encompassed in the set.

The expression "*nucleotide target*" means a nucleic acid molecule whose expression must be measured, preferably quantitatively measured. By "*expression measured*", it is meant that the expression product(s), in particular the transcription product(s) of a gene, are measured. By "*quantitative*" it is meant that the method is used to determine the quantity or the number of copies of the expression products, in particular the transcription products or nucleotide targets. This must be opposed to the qualitative measurement, whose aim is to determine the presence or absence of said expression product(s) only.

A nucleotide target is in particular a RNA, and most particularly a total RNA. In a preferred embodiment, the nucleotide target is mRNA or transcripts.

According to the methods used to measure the gene expression level, the mRNA may be used to obtain cDNA or cRNA, which is then detected and possibly measured.

The expression products or the nucleotide targets are preferably prepared from a cell culture, in particular after isolation or even purification. When the nucleotide targets are mRNA, a further step comprising or consisting in the retro-transcription of said mRNA into cDNA (complementary DNA) may also be performed prior to the step of detecting expression. Optionally, the cDNA may also be transcribed *in vitro* to provide cRNA.

During the step of preparation, and before assaying the expression, the expression product(s) or the nucleotide target(s) may be labelled, with isotopic (such as radioactive) or non isotopic (such as fluorescent, coloured, luminescent, affinity, enzymatic, magnetic, thermal or electrical) markers or labels.

It is noteworthy that steps carried out for assaying the gene expression must not alter the qualitative or the quantitative expression (number of copies) of the expression product(s) or of the nucleotide target(s), or must not interfere with the subsequent step comprising assaying the qualitative or the quantitative expression of said expression product(s) or nucleotide target(s).

The step of profiling gene expression comprises determining the expression of a set of genes. Such a set is defined as a group of genes that must be assayed for one test, and especially performed at the same time, on the same cell culture.

A set of gene consists of or comprises the five cellular genes ROBO1, POU4F1, PTN, PARD6B and PAFAH1B1. The set of genes may further comprise at least one additional gene selected from the group consisting of the twelve cellular genes PIK3CG, BMP2, DRD1, PAX5, S100A6, DRD2, HDAC7, HEY2, INHBA, SHH, BTK and FOS.

Moreover, in addition to these genes, step b) may encompass the measurement of the expression of other cellular genes, and in particular the measurement of the expression of at least one cellular gene(s) selected from the group consisting of genes involved in the PI3K/Akt signalling pathway or genes involved in cell proliferation, cell adhesion, cell differentiation, growth factors and synaptic functions.

In a particular embodiment, the set of genes used in step b) includes from 5 to 17 genes, in particular (1) exactly the five cellular genes ROBO1, POU4F1, PTN, PARD6B and PAFAH1B1, or (2) at least the five cellular genes ROBO1, POU4F1, PTN, PARD6B and PAFAH1B1, and from 1 to 12 genes, in particular 1 to 10 or 1 to 5 genes, selected from the group consisting of the twelve cellular genes PIK3CG, BMP2, DRD1, PAX5, S100A6, DRD2, HDAC7, HEY2, INHBA, SHH, BTK and FOS. Thus, in a particular embodiment, the set of genes consists of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 genes.

In step c) of the method, the expression of each gene of the set, as measured in step b) is normalized, *i.e.,* that for each gene, the expression measured in step b) is compared to the expression of the same gene as measured in a cell of the same cell type, in particular of the same cell culture or cell line, which has not been in contact with the molecule to be assayed. Thus, the following ratio is calculated:

$$\frac{\text{expression of a gene, measured in a cell in contact with the molecule to be assayed}}{\text{expression of the same gene as measured in a cell not in contact with said molecule.}}$$

This ratio enables to determine the relative expression of each gene, *i.e.,* whether the expression of each gene is increased or decreased as a result of the contact with the molecule to be assayed. By "*increase*" or "*decrease*", it is meant that the expression of a gene is statistically higher or statistically lower in a cell contacted with the molecule to be assayed as compared to a cell of the same cell type not contacted with this molecule. An expression is considered statistically different when the p-value (p) as calculated by the Student t test is < 0.05.

Carrying out the method as described herein, a molecule, and in particular a polypeptide of the invention is considered as having a neurosurvival phenotype (*i.e.,* neuroprotection, and/or neurogenesis and/or neuroregeneration and/or arborisation and/or neurorestoration), when the expression of the genes contained in the set as defined herein is modified in a statistically significant manner.

As an example, a molecule, and in particular a polypeptide of the invention is considered as having a neurosurvival phenotype (*i.e.,* neuroprotection, and/or neurogenesis and/or neuroregeneration and/or arborisation and/or neurorestoration), when the respective ROBO1, POU4F1, PTN, PARD6B and PAFAH1B1genes are statistically under-expressed, in particular by a fold of at least 1.5, when compared to the expression of the same genes in a cell (culture) which has not been in contact with said molecule (negative control); the under-expression may be calculated by implementing the experiment described below in point A.5 (results in point B.10), in which the negative control is a mock-infected cell culture.

The invention is also directed to a kit, suitable to carry out the method as defined herein, comprising

a. a plurality of pairs of primers specific for a set of genes as defined herein, in particular a set of genes consisting of or comprising the five cellular genes ROBO1, POU4F1, PTN, PARD6B and PAFAH1B1, and optionally at least one additional gene selected from the group consisting of the twelve cellular genes PIK3CG, BMP2, DRD1, PAX5, S100A6, DRD2, HDAC7, HEY2, INHBA, SHH, BTK and FOS; and

b. optionally reagents necessary for the amplification of the nucleotide targets of these cellular genes by said primers, and optionally reagents for detecting the amplification products.

As defined herein, a pair of primers consists of a forward polynucleotide and a backward polynucleotide, each primer having the capacity to match its nucleotide target and to amplify, when appropriate conditions and reagents are brought, a nucleotide sequence framed by their complementary sequence, in the sequence of their nucleotide target.

The pairs of primers present in the kits of the invention are specific for a gene *i.e.,* each pair of primers amplifies the nucleotide targets of one and only one gene among the set. Therefore, it is excluded that a pair of primers specific for a gene amplifies, in a exponential or even in a linear way, the nucleotide targets of another gene and/or other nucleic acids contained in sample. In this way, the sequence of a primer (whose pair is specific for a gene) is selected to be not found in a sequence found in another gene, is not complementary to a sequence found in this another gene and/or is not able to hybridize in amplification conditions as defined in the present application with the sequence of the nucleotide targets of this another gene.

In a particular embodiment, the forward and/or backward primer(s) may be labelled, either by isotopic (such as radioactive) or non isotopic (such as fluorescent, biotin, fluorochrome) methods. The label of the primer(s) leads to the labelling of the amplicon (product of amplification), since the primers are incorporated in the final product.

The design of a pair of primers is well known in the art and in particular may be carried out by reference to Sambrook et al. (Molecular Cloning, A laboratory Manual, Third Edition; chapter 8 and in particular pages 8.13 to 8.16). Various softwares are available to design pairs of primers, such as Oligo™ or Primer3.

Therefore, each primer of the pair (forward and backward) has, independently from each other, the following features:

- their size is from 10 and 50 bp, preferably 15 to 30 bp; and
- they have the capacity to hybridize with the sequence of the nucleotide targets of a gene.

In a particular embodiment, when the pairs of primers are used in a simultaneous amplification reaction carried out on the sample, the various primers have the capacity to hybridize with their respective nucleotide targets at the same temperature and in the same conditions.

Conventional conditions for PCR amplification are well known in the art and in particular in Sambrook et al. An example of common conditions for amplification by PCR is dNTP (200 mM), $MgCl_2$ (0.5 - 3 mM) and primers (100-200 nM).

In a particular embodiment, the sequence of the primer is 100% identical to one of the strands of the sequence of the nucleotide target to which it must hybridize with, *i.e.* is 100% complementary to the sequence of the nucleotide target to which it must hybridize. In another embodiment, the identity or complementarity is not 100%, but the similarity is at least 80%, at least 85%, at least 90% or at least 95% with its complementary sequence in the nucleotide target. In a particular embodiment, the primer differs from its counterpart in the sequence of the sequence of the nucleotide target by 1, 2, 3, 4 or 5 mutation(s) (deletion, insertion and/or substitution), preferably by 1, 2, 3, 4 or 5 nucleotide substitutions. In a particular embodiment, the mutations are not located in the last 5 nucleotides of the 3' end of the primer.

In a particular embodiment, the primer, which is not 100% identical or complementary, keeps the capacity to hybridize with the sequence of the nucleotide target, similarly to the primer that is 100% identical or 100% complementary with the sequence of the nucleotide target (in the hybridization conditions defined herein). In order to be specific, at least one of the primers (having at least 80% similarity as defined above) of the pair specific for a gene can not hybridize with the sequence found in the nucleotide targets of another gene of the set and of another gene of the sample.

Examples of primers that may be used to measure the expression of the cellular genes listed herein are disclosed in Table 2.

Gene	Forward primer (SEQ ID)	Backward primer (SEQ ID)
ROBO1	GTGTGGTGTGTGGCTTCA (253)	GTATACAGTCTCATGCC (254)
POU4F1	CCCTCCCTGAGCACAAG (255)	GTGGGCAGGCAGGCCC (256)
PTN	GGCAAGAAACAGGAGAAGA (257)	GTTTGCTGATGTCCTTT (258)
PARD6B	CATATAGTCATTAGTATG (259)	CTGGGAGAATATCCACG (260)
PAFAH1B1	CGGCAAGCTTCTGGCTTC (261)	GCATTCAAAGCCCTG (262)
PIK3CG	CGAGATCTACGACAAGTACC (263)	CCGGTGCGTGGCCTTCCAGT (264)
BMP2	CCACCATGAAGAATCTTTG (265)	ATTAAAGAAGAATCTCCGG (266)
DRD1	GTGTCAGAGCCCCTGATGTG (267)	GTCCCGTCCATGGCAGAG (268)
PAX5	CGTCAGTTCCATCAACAGG (269)	GGAAGCTGGGACTGGTTG (270)
S100A6	CACCGACCGCTATAAGG (271)	GCCAAATGCGACGCGAGCG (272)
DRD2	CATTGTCACCCTGCTGGTC (273)	GGTGTTGACTCGCTTGC (274)
HDAC7	GTAGTAGCAGCACGCCCG (275)	AGGATGGGATTGGGGC (276)
HEY2	GCAGCCCTGCTCCAGCCCA (277)	CTGAAGTTGTGGAGAGG (278)
INHBA	GGGGGAGAGGAGTGAACTG (279)	GAAGACATGCCAGGTGC (280)
SHH	GCTGGCCCGCCTGGCGGTGG (281)	GCAGTGGATATGTGCCTTGG (282)
BTK	GAATATTTTATCTTGGAGGA (283)	AGCCTTCCTGCCCATTTTT (284)
FOS	GAGGAGGCCTTCACCCTGCC (285)	TGCTCTTGACAGGTTCCACT (286)

Table 2

In the application, the term "*comprising*", which is synonymous with "*including*" or "*containing*", is open-ended, and does not exclude additional, unrecited element(s), ingredient(s) or method step(s), whereas the term "*consisting of*" is a closed term, which excludes any additional element, step, or ingredient which is not explicitly recited.

The term "*essentially consisting of*" is a partially open term, which does not exclude additional, unrecited element(s), step(s), or ingredient(s), as long as these additional element(s), step(s) or ingredient(s) do not materially affect the basic and novel properties of the invention.

The term "*comprising*" (or "*comprise(s)*") hence includes the term "*consisting of*" ("*consist(s) of*"), as well as the term "*essentially consisting of*" ("*essentially consist(s) of*"). Accordingly, the term "*comprising*" (or "*comprise(s)*") is, in the present application, meant as more particularly encompassing the term "*consisting of*" ("*consist(s) of*"), and the term "*essentially consisting of*" ("*essentially consist(s) of*").

The following examples are offered by way of illustration, and not by way of limitation.

EXAMPLES

A. Material and methods

A.1. Isothermal Titration Calorimetry (ITC) calculation

ITC measurements were made using VP-ITC VP-ITC200 calorimeters (MicroCal). MAST2-PDZ was titrated at 298 K by injections of the polypeptides (25-45 consecutive aliquots of 5-7 µL at 6-min intervals). Raw data were normalized and corrected for heats of dilution of polypeptides. Equilibrium dissociation constants (K_D) were determined performing nonlinear curve fitting of the corrected data to a model with one set of sites using the Origin7.0 software (OriginLab). All samples were prepared in a buffer containing 50 mM Tris-HCl, 150 mM NaCl , pH7.5. Data were recorded with MAST2-PDZ at an initial concentration of 30 µM and with peptide at initial concentrations ranging from 250 µM to 350 µM.

A.2. Cell culture

Human neuroblastoma cells, SH-SY-5Y and human NT2-N cell culture, were described respectively in Prehaud C. et al (2010) and Prehaud C. et al ——

(2005), and are detailed in the above-specification. The NS cells are grown in RPMI medium as described by manufacturer's instructions (http://www.cellomics.com/content/menu/Neuroscreen-1_Cells/).

A.3. Lentivirus production

The nucleotide sequences of Neurovita 1 and Neurovita1 delta PDZ-BS were cloned from the plasmid G-[SP-(2aa)-TM-Cyto] described in WO2010/116258 application. The nucleotide sequences of Neurovita1 and Neurovita1 delta PDZ-BS (disclosed in WO2010/116258) and of Neurovita2 were obtained by PCR and cloned in the pLenti6.3/V5-TOPO® by using the TA cloning kit (K5315-20, Invitrogen, France). Lentiviruses were obtained in 293T cells, transfected with vectors encoding the Neurovita1, Neurovita1 delta PDZ-BS or the Neurovita2 polypeptides, by the standard procedures as described in Vitry S. et al (2009). HIV particles quantity was assayed by using the HIV p24 ELISA kit (Perkin Elmer, NEK050). Infectivity of recombinant lentivectors on NS, SH-SY-5Y and NT2-N cells were systematically monitored by qRT-PCR.

A.4. Kinome profiling

Kinome profiling was undertaken by Pepscan presto (Netherlands) on PepChip Kinase arrays according to the manufacturer instructions (http://www.pepscan.com/presto/products-services/pepchip/#kinase-profiling). Briefly, NT2-N cells were either mock-infected (control) or infected with the neurosurvival rRABV CVS HQ (Prehaud C. et al, 2010) for 45h before harvesting. The rRABV CVS HQ (CVS-NIV strain) has been deposited at the CNCM on the 1st of April, 2009 under deposit number I-4140. 10000 mm^2 of NT2-N cells were treated in duplicates for each condition. Cell lysates were prepared in anti-proteases-containing MPER buffer (Pierce, Thermofisher, France) and supernatants were deep frozen in liquid nitrogen before using. The PepChip kinase arrays covered the entire human kinome (Manning G. Et al, 2002). Data were subjected to Kolmogorov-Smirnov statistical analysis with a cut-off of p=0.001 before validation. A threshold means ratio >1.96SE was chosen (high stringency). Dots represent the organized kinase cluster as defined by Gene Network Central pro (Qiagen, Germany)

A.5. Pathway-focused profiling

Gene expression was monitored by using the following pathway-focused profiling PCR arrays from QIAGEN (Germany) according to the manufacturer's instructions (http://www.sabiosciences.com/RTPCR.php): the Human PI3K-AKT Signaling PCR Array (ref.: PAHS-058) and the Human Neurogenesis and Neural Stem Cell PCR Array (ref.: PAHS-404). Briefly 15.7 mm^2 of NT2-N cells were infected with 900ng of p24 lentivectors. Total RNA was isolated by using the RNEasy purification kit (QIAGEN, including the DNAse 1 treatment) and subjected to cDNA synthesis (SABioscience, USA) and qPCR (ABI Fast 7500 real time PCR apparatus). Experiments were realized in duplicates and quality control was assayed for each PCR plate. Data were analyzed with the QIAGEN web interface (http://www.sabiosciences.com/pcrarraydataanalysis.php).

Fold regulations were calculated accordingly to the comparative method. In the comparative or ΔΔCt method of qPCR data analysis, the Ct values obtained from two different experimental RNA samples were directly normalized to a housekeeping gene and then compared. This method assumes that the amplification efficiencies of the gene of interest and the housekeeping genes are close to 100% (meaning a standard or calibration curve slope of -3.32). First, the difference between the Ct values (ΔCt) of the gene of interest and the housekeeping gene was calculated for each experimental sample. Then, the difference in the ΔCt values (ΔΔCt), between the experimental and control samples (mock-infected cells) was calculated. The fold-change in expression of the gene of interest between the two samples is then equal to 2^(-ΔΔCt). The fold difference (fold change) is calculated by the equation 2(-ΔΔC(t). For the fold regulation, any fold regulation (or fold change) less than 1 (meaning that the gene is down regulated) was negatively inversed, changing the fractional number into a whole number [for example, for a gene having a fold change value of 0.31, the fold regulation given is -3.2 fold, meaning that this particular gene is down regulated by 3.2 fold].

Genes clustering was realized by using Gene Network Central pro (Qiagen, Germany).

<u>A.6. Neurite outgrowth</u>

Neurite outgrowth assays have been extensively described in Prehaud C. et al, 2010. SH-SY5Y human neuroblastoma cells are seeded on 24-well plates (Cell Bind plastic ware, Corning, USA) at a density of 40,000 cells per well in non differentiating medium [DMEMF12 (Invitrogen, U.K.) with 20% Fetal Bovine Serum plus 1%Pen:Strep and 1% Glutamine], and cultured overnight at 37°C. 24h post seeding non differentiation medium is replaced with differentiating medium [Neurobasal medium (Invitrogen, U.K.) supplemented with B27 supplement (Invitrogen, U.K.), 1% P/S, 1% Glutamine and 1mM db-cAMP (dibutyril c-AMP is membrane permeable, Sigma)], and the cells are incubated for 6h. Then, cells are infected with 30 ng of p24 lentivector in differentiating medium. After 1h of incubation, cells are washed once with differentiating medium, and after adding differentiating medium they are incubated for 24h at 37°C. Thirty hours post differentiation, the cells are fixed with 3% paraformaldehyde in phosphate buffered saline (PBS) for 20 min at room temperature (RT) followed by treatment for 5mn with 0.1% Triton-X-100 and 50% normal goat serum (NGS) in PBS for 1h at RT. Neuronal specific anti βIII tubulin Ab (Promega, France) and anti-RABV nucleocapsid Ab are used to stain the neurite processes and to reveal RABV infection respectively. Alternatively, cells are also stained with crystal violet which preserves the neurites processes.

NS cells were monitored for 72h and treated with 200ng/ml of NGF at time=0 (one hit only). Neurite outgrowth (NO) was basically undertaken as described above for SH-SY5Y with the exception that the NO was monitored 72h post infection and NS cells were always grown in their feeding medium (Cellomics, USA).

In both cases, SH-SY5Y human neuroblastoma cells and NS cells are imaged using a Leica DM 5000B UV microscope equipped with a DC 300FX camera (x40 or x20 objectives) and analyzed using ImageJ 1.38X Software (Wayne Rasband, NIH, USA, http://rsb.info.nih.gov/ij/) and its plug-in NeuronJ (Meijering *et al.* 2004; http://www.imagescience.org/meijering/software/neuronj/). The average neurite length per neuron is determined from triplicate experiments.

A.7. Scratch assay (axon regeneration)

For scratch-induced assays, 200mm^2 of NT2-N cells (n=8), infected with 30ng of p24 lentivectors, were seeded on poly-D-Lysin-laminin coated cell+(Sarstedt, Germany) 12 wells plastic ware, and were grown for two days in order to recover completely after trypsinisation. The medium was changed 2h before scratching. Individual wounds were made with an injection needle (26GX1/2", 12-4.5). At least 10 scratching were made on each individual well. Cells were fixed with PFA (4%) 6 days post wounding and stained with crystal violet solution. Cells are imaged using a Leica DM 5000B microscope equipped with a DC 300FX camera (x20 objective) and analysed using ImageJ 1.38X Software (Wayne Rasband, NIH, USA, http://rsb.info.nih.gov/ij/) and its plug-in NeuronJ. The average percentage of neuron in regeneration is determined from 8 experiments.

A.8. Arborisation

Sholl analysis which is a mean of measuring dendritic arborisation was assayed according to Sahay A. et al and Lioy DT. et al Nature 2011. Neurite complexity was analysed from 8-bit images by using the ImageJ Sholl Analysis plug-in (http://www-biology.ucsd.edu/labs/ghosh/software/). Images were taken 72h post infection (p.i.) with lentivectors.

A.9. Silencing of MAST2

MAST2 gene expression was silenced with the specific set of shRNA based lentiviruses developed by the RNAI Consortium (TRC, MIT and Harvard, sold by Thermoscientific-ABgene, RHS4533-NM_015112). Recombinant lentiviruses were produced as described above. 15.7 mm^2 of NT2-N cells were infected with 900ng of p24 of each lentivector. The efficiency of silencing was assessed by qRT-PCR, 2 days post infection with the specific primer set QT00042574 (Qiagen, Germany). Then, cells were used immediately after for experiments

A.10. Neuritogenesis in mouse fœtal cortical neurons

E16 swiss mouse cortical neurons were prepared according to Vitry et al (2009). 10^4 cortical neurons were plated on 96 well dark sided cell bind plates

(#3340, Corning, USA) and infected with 10ng p24/well of lentivectors (NV1 eGFP or NV1Δ eGFP) 2 hours after seeding. Medium was changed 12 hours after infection. Three days post infection medium was removed carefully and neurons were fixed with 4% PFA for 20mn at room temperature. Plates were washed three times with PBS and then cells were permeabilized with 0.3 % Triton X100 for 10mn at room temperature. βIII neuronal tubulin immunofluorescence was carried out according to Loh Shy et al. (2008). Neurite outgrowth was monitored by high throughput screening on a cellomics (USA) CellInsight reader by using the neuronal profiling bioapplication (n= 10 wells, 20 fields/well, 250 neurons per well). Student t test was carried out on GraphPad Prism 6 (USA).

A.11. Mice experiments with lentivectors NV1 eGFP and NV1Δ eGFP

Groups of 10 swiss mice (3 days old) were injected directly into brain with 100ng p24 of each lentivector (vehicle was 1% BSA containing PBS) or vehicle alone (1 mouse) as described in Vitry et al (2003). Mice development and phenotype were recorded over a four-month period. Weight was monitored for 20 days post injection. 4 months after injection, animals were euthanized and brains were isolated for immunochemistry and real time PCR as described by Vitry et al (2003). Neurovita expression was monitored with e-GFP expression. Immunostaining for Map2 antigen was used to detect the dendrites. Immunostaining for GFAP (Glial fibrillary acidic protein) was used to monitor astrogliosis. The anti-map2 antibody was from SIGMA, US (M1406); the anti-GFAP antibody was from Dako (Z0334); the anti-GFP antibody was from Rockland (600-106-215).

B. Results

B.1. Kinome profiling in NT2-N cells during RABV-mediated neuroprotection

Incubation of the PepChip Kinomics array (covering the entire human kinome, *i.e.,* more than 518 kinases) with cell lysates derived from NT2-N cells infected (45h) with the neurosurvival rRABV CVS HQ reveals that only 17 kinases are stimulated, in high stringency conditions (Table 3). All these kinases are linked together, as shown in Figure 3B.

Kinase	Name	Fold regulation of activation	Kinase Group
AKT1*	protein kinase B	6.69693	AGC
PIK3CG*	phosphoinositide-3-kinase, catalytic, gamma polypeptide	3.81055	Other
CSNK2A1*	casein kinase 2, alpha 1	3.30198	Other
SRC	proto-oncogene tyrosine-protein kinase	2.52567	TKL
PDLIM5	PDZ and LIM domain 5	2.47084	TKL
MAP4K1= MEKKK1	mitogen-activated protein kinase kinase kinase kinase 1	2.26839	STE
ROCK1	Rho-associated, coiled-coil containing protein kinase 1	2.14479	AGC
GRK6	G protein-coupled receptor kinase 6	2.10186	AGC
GRK5	G protein-coupled receptor kinase 5	2.05385	AGC
PDK1*	pyruvate dehydrogenase kinase, isozyme 1	1.82555	AGC
CDK1	cyclin-dependent kinase 1	1.82134	GMC
ACVR1B	activin A receptor, type IB	1.65481	TKL
RPS6KA2	ribosomal protein S6 kinase, 90kDa	1.65099	AGC
MAPK14* = p38 alpha	mitogen-activated protein kinase 14	1.61328	CMGC
ATM*	ataxia telangiectasia mutate	0.61628	Atypical
PRKCA*	protein kinase C, alpha	0.35932	PKC
MAPK8* JNK, JNK1, JNK1A2, JNK21B1/2, PRKM8, SAPK1	mitogen-activated protein kinase 8	0.06722	CMGC
Kolmogorov-Smirnov statistical analysis; Cut-off p=0.001 Threshold means ratios >1.96 SE *: involved in the Pi3K-AKT signalling pathway			

Table 3

<u>B.2. Neurite outgrowth in SH-SY5Y cells and in NS cells transduced with Neurovita1-expressing lentivectors</u>

The following constructs were designed: Neurovita1 polypeptide and Neurovita1 delta PDZ-BS polypeptide (Figure 4A). Their nucleotide and protein sequences are as follows:

<u>Neurovita 1</u>

- polynucleotide:

ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGTTTTGGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGTTGATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACGCAACACAATCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGCGGGAAGATCATATCTTCATGGGAATCACACAAGAGTGGGGGTCAGACCAGACTGTGA (SEQ ID NO:8).

- polypeptide:

MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSEPTQHNLRGTGREVSVTPQSGKIISSWESHKSGGQTRL (SEQ ID NO:9).

<u>Neurovita1 delta PDZ-BS</u> <u>(without the PDZ-BS domain)</u>

- polynucleotide:

ATGGTTCCTCAGGCTCTCCTGTTTGTACCCCTTCTGGTTTTTCCATTGTGTTTTGGGGGGAAGTATGTATTACTGAGTGCAGGGGCCCTGACTGCCTTGATGTTGATAATTTTCCTGATGACATGTTGTAGAAGAGTCAATCGATCAGAACCTACGCAACACAATCTCAGAGGGACAGGGAGGGAGGTGTCAGTCACTCCCCAAAGCGGGAAGATCATATCTTCATGGGAATCACACAAGAGTGGGGGTTGA (SEQ ID NO:10).

- polypeptide:

MVPQALLFVPLLVFPLCFGGKYVLLSAGALTALMLIIFLMTCCRRVNRSEPTQHNLRGTGREVSVTPQSGKIISSWESHKSGG (SEQ ID NO:11).

The Western Blot experiments show that Neurovita1 and Neurovita 1 delta PDZ-BS are expressed in both cell lines (Figure 4B). Moreover, Figure 4C confirms that Neurovita1 and Neurovita 1 delta PDZ-BS exhibit a typical immunofluorescence pattern expected for Rhabdovirus glycoprotein.

Neurite outgrowth assay in SH-SY5Y cells shows that the Neurovita1 polypeptide exhibits a strong neurite outgrowth phenotype, which is PDZ-BS mediated (compare the polypeptide with and without the PDZ-BS domain, in Figure 5A). Similarly, neurite outgrowth assay in NS cells shows that the Neurovita1 polypeptide not only exhibits a strong neurite outgrowth phenotype, which is PDZ-BS mediated but also increases the neurites network in the infected culture (Figure 5B).

<u>B.3. Molecular signature of Neurovita1-mediated protection</u>

Pathway-focused gene expression profiling (Human Neurogenesis and Neural Stem Cell Array and PI3K/Akt signalling pathway) of NT2-N cells transfected with Neurovita1-expressing lentivector reveals a genetic molecular signature as represented in Figures 6A and 6B. This signature is characterized by the following fold regulation: SHH gene (-1.82), ROBO1 gene (-1.69), PTN gene (-1.83), POU4F1 gene (-1.50), PARD6B gene (-1.62), PAFAH1B1 gene (-1.57), INHBA gene (-1.92), HEY2 gene (-3.49), HDAC7 gene (-1.60), DRD2 gene (-1.64), S100A6 gene (+3.38), PAX5 gene (+1.97), DRD1 gene (+1.59), BMP2 gene (+1.52) and PIK3CG gene (+1.79).

This gene expression profiling was compared with the one obtained in NT2-N cells knocked out for the human MAST2 protein (76% of MAST-2 silencing), and also transfected with Neurovita1-expressing lentivector. Thus, the cluster of genes identified in Figure 7B represents the genes regulated in Neurovita1 infection but differently regulated in NT2-N cells wherein the MAST2 expression was knocked down. The genetic molecular signature is characterized by the following fold regulation: SHH gene (-2.52), ROBO1 gene (+1.00), PTN gene (+1.01), POU4F1 gene (+1.21), PARD6B gene (+1.46), PAFAH1B1 gene (+1.03), INHBA gene (-1.31), HEY2 gene (-2.27), HDAC7 gene (-1.17), DRD2 gene (-1.15), S100A6 gene (-1.44), PAX5 gene (-1.01), DRD1 gene (+2.31), BMP2 gene (+1.23) and

PIK3CG gene (-2.24). Of note is the inverted regulation of the genes ROBO1, PTN, POU4F1, PARD6B, PAFAH1B1, S100A6, PAX5 and PIK3CG when MAST2 expression is silenced, leading to the conclusion that MAST-2 controls the gene survival pattern mediated by Neurovita.

B.4. Axon regeneration

Scratch assay performed on NT2-N cells shows that only Neurovita1-infected NT2-N cells can regenerate their axons post-scratching. Neither non infected nor Neurovita1 delta PDZ-BS-infected cells can do it ($p<0.0001$) (Figure 8).

B.5. Molecular signature of Neurovita1-mediated axon regeneration

Figure 9 represents the pathway-focused gene expression profiling (on Human Neurogenesis and Neural Stem Cell and Human PI3K-AKT Signaling PCR Arrays) implementing Neurovita1-infected NT2-N cells tested after scratching (point B.4. above) as compared to non-infected cells after scratching. The genetic molecular signature is characterized by the following fold regulation:

Figure 9A: Human PI3K-AKT Signaling PCR Array

EIF2AK2 (+1.57), FASLG (+2.13), FOXO3 (+1.5), GSK3B (+1.53), HRAS (+1.54), IRAK1 (+1.51), MAPK8 (+1.53), MTCP1 (+1.76), PDK1 (+2.71), PIK3CG (+3.78), RHEB (+1.84), RPS6KA1 (+1.99), TCL1A (+2.10), APC (-1.82), BTK (-1.61), GRB10 (-1.78), RPS6KB1 (-1.68) and TLR4 (-1.63); and

Figure 9B: Human Neurogenesis and Neural Stem Cell Array

ALK (+2.49), GDNF (+1.5), NPTX1 (+1.56), NRG1 (+1.52), PAX5 (+1.65), S100A6 (+1.99), ASCL1 (-1.56), BDNF (-1.78), BMP15 (-2.27), BMP4 (-1.58), EGF (-2.10), INHBA (-1.89), NDP (-1.68), NEUROD1 (-3.92), NOTCH2 (-1.57), POU3F3 (-1.55) and ROBO1 (-1.98).

These molecular signatures show a strong regulation of genes involved in PI3K/Akt signalling pathway (Figure 9A) and of genes involved in cell proliferation, adhesion and differentiation, growth factors and synaptic functions (Figure 9B), and demonstrate that these genes are highly connected (Figure 9C).

B.6. Design of polypeptides with optimized sequences (dissociation constant and thermodynamic parameters)

From our high resolution NMR structures of MAST2-PDZ in complex with endogenous and viral ligands (Protein Data Bank codes 2KQF & 2KYL), an unexpected large surface of interaction between the domain and the polypeptides was characterized. It was demonstrated that the polypeptides interact similarly with the PDZ target not only through the very last four amino acids of canonical C-terminal regions, but also with another anchoring region at the N-terminal end of the assayed peptides. Even if the C-terminal residues of the peptides contribute mainly to the binding strength with MAST2-PDZ, the presence of an additional N-terminal interaction implying a specific position of the peptide and an original feature of the PDZ domain clearly reinforces the specificity and affinity of the interaction. This large and original surface of interaction is an opportunity to optimize peptide affinity and specificity for MAST2-PDZ.

Our detailed thermodynamical and structural (at an atomic level) descriptions of MAST2-PDZ/peptide complexes (Figure 10) were used to design optimized sequences. We proposed a model of relationship between the affinity of polypeptides for MAST2-PDZ (Neurovita) and their neurosurvival properties, in which the highest the affinity of polypeptides for the MAST2-PDZ domain (*i.e.,* the lowest the K_D), the highest the neurosurvival properties of these polypeptides (Figure 10B).

In order to retain the high specificity driven by the N-terminal and C-terminal binding sites of the peptide and selected by the endogenous and viral ligands for the interaction with MAST2-PDZ, the terminal sequences were left unchanged. The central region of the peptide was modified in order to increase the affinity of sequences for MAST2-PDZ, by designing polypeptides whose MAST2-binding domain is from 11 to 13 amino acid residues. The general structure of these polypeptides is represented in Figure 11A.

The polypeptides as described in the list below have been designed, and a complete thermodynamical description for each MAST2-PDZ/polypeptide complex (with the estimation of enthalpic and entropic parameters taking into account the flexibility and the polar contacts contributions to the binding strength) has been carried out (Table 4):

- a polypeptide ending with the MAST-2 binding domain as defined in SEQ ID NO:235;
- a polypeptide as defined in SEQ ID NO:9, ending with the MAST-2 binding domain as defined in SEQ ID NO:1;
- a polypeptide as defined in SEQ ID NO:215, ending with the MAST-2 binding domain as defined in SEQ ID NO:67;
- a polypeptide as defined in SEQ ID NO:210, ending with the MAST-2 binding domain as defined in SEQ ID NO:64;
- a polypeptide as defined in SEQ ID NO:218, ending with the MAST-2 binding domain as defined in SEQ ID NO:209;
- a polypeptide as defined in SEQ ID NO:217, ending with the MAST-2 binding domain as defined in SEQ ID NO:208;
- a polypeptide as defined in SEQ ID NO:213, ending with the MAST-2 binding domain as defined in SEQ ID NO:74;
- a polypeptide as defined in SEQ ID NO:211, ending with the MAST-2 binding domain as defined in SEQ ID NO:65;
- a polypeptide as defined in SEQ ID NO:214, ending with the MAST-2 binding domain as defined in SEQ ID NO:66;
- a polypeptide as defined in SEQ ID NO:216, ending with the MAST-2 binding domain as defined in SEQ ID NO:68; and
- a polypeptide as defined in SEQ ID NO:212, ending with the MAST-2 binding domain as defined in SEQ ID NO:71.

Polypeptide	Sequence of the MAST-2 binding domain	SEQ ID	MAST2-PDZ					PTPN4-PDZ
			Kd (µM) *(dissociation constant)*	erreur	ΔH *(enthalpy)*	TΔS *(entropy)*	n *(stoechiometry)*	Kd (µM)
ATT13	**SWESHKSGGETRL**	235	0.57	+/- 0.052	-	-	-	160
VIR13 (Neurovita1)	**SWESHKSGGQTRL**	1	1.26	+/- 0.11	-9929	-1878.39	0.9996	560
439	**SWEVHTQQTRL**	67	0.21	+/- 0.002	-8454	646.66	1.022	
441 (Neurovita2)	**SWEVHGGQTRL**	64	0.12	+/- 0.001	-10230	-808.176	1.069	544
442	**SWEVHASGGQTRL**	209	0.49	+/- 0.001	-10340	-1737.34	1.037	-
443	**SWAEAQHTQQTRL**	208	0.4	+/- 0.003	-9088	-360.878	1.007	-
453	**SWEVYTGQTRL**	74	0.238	-	-6511	2521.08	0.846	-
454 (Neurovita 3)	**SWEVHGQQTRL**	65	0.0629	-	-8715	1108.56	1.01	-
455	**SWEVHTGQTRL**	66	0.13	-	-9434	-42.316	0.906	-
460	**SWEVAGGQTRL**	68	0.188	-	-7484	1686.68	0.916	-
461	**SWEVATQQTRL**	71	0.126	-	-7688	1722.44	0.806	-

Table 4

Measure of the dissociation constant (K_D) and thermodynamics parameters (ΔH and $T\Delta S$) by ITC of MAST2-PDZ shows a significant gain of affinity of the optimized polypeptides of the invention for the MAST2-PDZ domain (Table 4). Thus, all the assayed polypeptides have a K_D lower than 0.5 µM, *i.e.,* a gain of affinity of at least 2.5 as compared to Neurovita1. The K_D of the assayed polypeptides varies from 0.0629 to 0.49 µM, *i.e.* a gain of affinity as compared to Neurovita1 ranging from 2.5 to 20. A particularly interesting polypeptide is the polypeptide 454 (Neurovita3), whose MAST2-binding domain consists of SWEVHGQQTRL, which has a K_D of 0.0629 µM.

Consequently, by modifying the sequence of the central flexible linker of the Neurovita sequence, taking into account the entropy/enthalpy compensation of the complexes, the affinity of the polypeptides of the invention for MAST2-PDZ was drastically enhanced.

As an example, the Neurovita2 polypeptide (whose MAST2-binding domain consists of SWEVHGGQTRL) shows a 10 fold gain in affinity for the MAST2-PDZ domain as compared to Neurovita1 (0.12 µM versus 1.26 µM). The comparison of the sequence of the cytoplasmic domain of Neurovita2 with the one of Neurovita1 and Neurovita1 delta PDZ-BS is described in Figure 11B.

Expression of the Neurovita2 polypeptide in NS cells infected by a lentivector (in which the Neurovita2 sequence was cloned) was similar to the one obtained with infection by lentiviral vectors expressing Neurovita1 and Neurovita1 delta PDZ-BS polypeptides (Figure 11C).

<u>B.7. Neurite outgrowth in NS cells infected with Neurovita2-expressing lentivectors</u>

Neurite outgrowth assay in NS cells infected with Neurovita2-expressing lentivectors shows that Neurovita2, like Neurovita1, exhibits a strong neurite outgrowth phenotype which is PDZ-BS mediated ($p<0.0001$) (Figure 12).

B.8. Arborisation in NS cells transduced with Neurovita2-expressing lentivectors

Arborisation in NS cells transduced with Neurovita2-expressing lentivectors demonstrates that the Neurovita2-mediated neurite outgrowth in NS cells is characterized by a stronger complexity of the neurite tree which is specific of sympathetic neurons fully functional, as compared to neurovita1. Thus, Neurovita 2 increases the strength and complexity of the neurite tree which is a trait of functionality and survival (Figure 13).

B.9. Molecular signature of Neurovita2-mediated neuroprotection

The expression of four cellular genes, previously identified, have been assayed in NT2-N cells, 24h p.i. Figure 14 shows the following fold down-regulation: BTK (-1.64), FOS (-1.82), DRD2 (-2.35) and POU4F1 (-1.53).

B.10. Genetic molecular signatures of Neurovita 1 and Neurovita2 molecules

Table 5 presents a summary of the fold regulation obtained with the Neurovita 1 or Neurovita2 infection of NT2-N cells.

A Black square indicates a gene which is regulated in the scratch assay; a white square indicates a gene for which regulation is inverted when MAST2 is silenced; a hatched square indicates a gene regulated in Neurovita2 infection (threshold $x<-1.5$, or $x>+1.5$).

The core of neurosurvival gene signature is then ROBO1, POU4F1, PTN, PARD6B and PAFAH1B1 which are genes regulated in the same way in both Neurovita1 and Neurovita2 infections and which regulation is inverted when MAST2 is silenced. Of note ROBO1 is also downregulated in the scratch assay.

The other genes are used to characterize more specifically either Neurovita1 (*i.e.,* PIK3CG, BMP2, DRD1, PAX5, S100A6, HDAC7, HEY2, INHBA, SHH) or Neurovita2 (*i.e.,* DRD2, BTK, FOS).

These genes and their function are listed in Table 7 below.

Genes	Neurovita 1	Neurovita2
PIK3CG	1.79	-1.14
BMP2	1.52	-1.09
DRD1	1.59	-1.19
PAX5	1.97	-1.37
S100A6	3.38	-1.05
DRD2	-1.64	-2.35
HDAC7	-1.6	-1.05
HEY2	-3.49	-1.03
INHBA	-1.92	1.3
PAFAH1B1	-1.57	-1.04
PARD6B	-1.62	-1.34
POU4F1	-1.5	-1.53
PTN	-1.83	-1.29
ROBO1	-1.69	-1.07
SHH	-1.82	-1.10
BTK	-1.02	-1.64
FOS	-1.41	-1.82

Table 5

Genes regulated for Neurovita1 axon regeneration
Genes disregulated when MAST2 is silenced
Genes regulated in Neurovita2

>1.5
<-1.5
-1.5<x>1.5

Table 6 is a heat map representation of the Neurovita1 genetic molecular signature (a) and Neurovita 2 genetic molecular signature (b).

a)

PIK3CG	S100A6	INHBA	PTN
BMP2	DRD2	PAFAH1B1	ROBO1
DRD1	HDAC7	PARD6B	SHH
PAX5	HEY2	POU4F1	BTK
FOS			

Table 6

b)

PIK3CG	S100A6	INHBA	PTN
BMP2	DRD2	PAFAH1B1	ROBO1
DRD1	HDAC7	PARD6B	SHH
PAX5	HEY2	POU4F1	BTK
FOS			

Table 7

Genes	Known functions
Neurovita 1	
PIK3CG	Familly member of the PI3/Akt signaling pathway (regulated by PTEN)
PAX5	Regulator cell differentiation
S100A6	Regulator cell cycle and cell proliferation
PAFAH1B1	Regulator cell motility and cell migration
PARD6B	Regulator cell cycle and cell proliferation
POU4F1	Transcription factor, repression early neurogenic genes, control terminal differentiation
PTN	Cytokine, regulator cell cycle
ROBO1	Regulator cell adhesion
INHBA	Negative regulator of cell cycle
Neurovita2	
BTK	Regulator neurite outgrowth
FOS	Positive regulator of Apoptosis
DRD2	Inhibitor of Wnt signaling pathway (Wnt is a major signaling pathway involved in neuronal growth, survival and branching)
POU4F1	Transcription factor, repression early neurogenic genes, control terminal differentiation

B.11. Transcription of rRABV and lentivectors in NT2-N cells

NT2-N cells were either infected with recombinant rabies viruses (rRABV CVS HQ ,or rRABV CVS HΔ4) or the lentivectors as described above. rRABV CVS HQ virus expresses a full length G protein ending with a MAST-2 binding domain as defined in SEQ ID NO:1; rRABV CVS HΔ4 virus expresses a full length G protein ending with a MAST-2 binding domain consisting of SEQ ID NO:1 in which the Q, T, R and L residues have been deleted.

Total RNA were extracted 24 h p.i. for rRABV infections and 48h for lentivectors infections. The specific transcription of the recombinant viruses (rRABV, lentiviruses) was assayed by RT-QPCR. The graph showed that NT2-N cells are efficiently infected with both types of viruses.

B.12. Transcription of a representative set of immunity genes in NT2-N cells

The transcription was assayed by RT-qPCR in the cultures harvested above (Figure15).

The relative fold induction of a set of immunity genes were assayed in NT2-N cells infected with either the CVS-HQ strain or the CVS HΔ4 strain. Comparison of Figure 16A and 16B shows that the induction of immunity gene cluster and neurosurvival phenotype are dissociated.

Moreover, the relative fold induction of a set of immunity genes were assayed in NT2-N cells infected with lentiviral vectors expressing the Neurovita1, Neurovita1 delta PDZ-BS or Neurovita2. Figure 16B shows that none of the Neurovita polypeptides alone is able to trigger immune gene response in human post mitotic neurons.

B.13. Axon regeneration

Figure 8 has shown that Neurovita1-infected NT2-N cells can regenerate their axons post-scratching. The inventors have assayed the involvement of MAST-2 in the regeneration mechanism.

As demonstrated in Figure 17C, the silencing of the *MAST2* expression (NV1 / siMAST2) dramatically decreases the axon regeneration post-scratching, meaning that the promotion of axon regeneration by lentivector NV1 in human post mitotic dopaminergic Neurons (NT2-N) is dependent upon the expression of *MAST2*.

Figure 17D shows that, like Lentivector Neurovita1 (NV1), Lentivector Neurovita2 (NV2) promotes axon regeneration in human post mitotic dopaminergic Neurons (NT2-N).

B.14. Protection against excessive arborisation by Neurovita2

As shown in Figure 18A, addition of KCl in non-infected NS cells (black squares) stimulates excessive outgrowth and arborisation. This observation is explained by the fact that KCl mimicks the depolarizing effects of persistant neuronal activity (neuron firing).

Infection with Neurovita2 lentivector in KCl-treated NS cells reduces the outgrowth and arborisation of NS cells as compared to the same treated, but non-infected, cells (stars, Figure 18B). These results demonstrate that Neurovita2, not only stimulates the pathways involved in neuritogenesis, but also protects against excessive arborisation by controlling these pathways and by avoiding their runaway. Interestingly, this modulating effect of Neurovita 2 is also a sign for its non toxicity.

B.15. Protection against LiCl toxicity by Neurovita 1 and Neurovita2

As shown in Figure 19A, addition of LiCl in non-infected NS cells inhibits neuritogenesis.

Neurovita 1 or Neurovita2 lentivector, but not Neurovita 1 Δ PDZ-BS lentivector, exhibits a neurite outgrowth phenotype which is PDZ-BS mediated in LiCl-treated NS cells (Figure 19B). These results demonstrate that Neurovita 1 and Neurovita2 protects against the toxic effect of LiCl.

B.16. Neurite outgrowth in NS cells infected with RABV G and Neurovita1- derived polypeptides, delivered by lentivectors

6 types of polypeptides have been assayed (Figure 20A): the RABV G full protein, the RABV G protein deleted for the PDZ-BS domain, the neurovita1 polypeptide, the neurovita1 polypeptide deleted for the PDZ-BS domain, the cytosolic form of the neurovita1 polypeptide and the cytosolic form of the neurovita1 polypeptide deleted for the PDZ-BS domain. Figure 20 shows that, in neurite outgrowth assay, the neurovita1 polypeptide is the optimized form.

B.17. Expression of Neurovita polypeptides from a bicistronic lentivector

Various Neurovita polypeptides (NV1, NV1Δ, NV2 and NV3) have been expressed via a bicistronic lentivector in NS cells. All these Neurovita polypeptides have been correctly expressed (Western Blot). NV1 and NV2 mRNAs are found at approximately the same level, whereas NV3 mRNAs are found at a higher level (Figure 21).

The experiments also show that the GFP protein is correctly and sufficiently expressed in cells from this bicistronic lentivector

B.18. Neurite outgrowth and arborisation in NS cells transduced with Neurovita3-expressing lentivectors

Arborisation experiments in NS cells transduced with Neurovita3-expressing lentivectors demonstrates that Neurovita3 promotes a strong complexity of the neurite tree as compared to a negative control (Figure 22A). As reported previously in Table 4, Neurovita 3 has an affinity for MAST2 that is 20 times higher than the one of NV1.

Moreover, neurite outgrowth assay in NS cells infected with Neurovita3-expressing lentivectors shows that Neurovita3 exhibits a strong neurite outgrowth phenotype, more importantly than Neurovita1 ($p<0.00001$) and Neurovita 2 ($p<0.0003$) (Figures 22 B and C).

A comparison of the number of crossings (arborisation) between NV1, NV2 and NV3 has demonstrated that NV3 promotes neurite tree arborisation in NS cells more efficiently than NV1 ($p<0.0001$) and than NV2 ($p<0.0007$) (Figure 23).

B.19. Experiments with a cytosolic form of Neurovita3

The Neurovita3 (NV3) polypeptide and its cytosolic form (NV3 cyto) (Figure 24A) has been assayed for expression, for neurite outgrowth and for arborisation in NS cells. NV3 polypeptide has a high affinity for MAST2 (20x higher than the one of NV1), is processed by the ER and Golgi, and possesses a transmembrane domain (TM) domain allowing its anchorage into the cytoplasmic membrane. The NV3cyto polypeptide has the same affinity for MAST-2 than NV3, but is a cytosolic molecule.

Transcription analysis, via the bicistronic lentivector (Figure 24B) shows that NV3cyto is correctly expressed, but at a lower level than NV3 (Figure 24C). The expression of GFP from this biscistronic lentivector is also correct (Figure 24D).

Arborisation experiments in NS cells transduced with NV3- and NV3 cyto-expressing lentivectors demonstrates that NV3cyto does not promote a neurite tree as complex as NV3 does (Figure 25A).

Moreover, neurite outgrowth assay in NS cells infected with NV3- and NV3 cyto-expressing lentivectors confirms that NV3cyto promotes neurite outgrowth in

NS, but not as efficiently as NV3 does. However, NV3-cyto is as good as NV1 (anchored form) to promote neurite outgrowth in NS cells (Figures 25B and 25C).

Interestingly, the absence of SP and TM domains in NV3cyto (absence which is known to reduce the neurite outgrowth promotion of Neurovita polypeptides; see NV1cyto in Figure 20B) is counterbalanced by the high affinity of NV3 cyto (and NV3) for MAST2.

This conclusion is confirmed in neurite tree arborisation experiments, wherein NV3-Cyto is as good as NV1 to promote neuritic tree arborisation in NS cells (Figure 26A, top panel).

Altogether, these experiments demonstrate that the neurite outgrowth promotion and neurite tree arborisation promotion of Neurovita polypeptides, such as the polypeptides of the invention, are dependent upon two factors: (1) the affinity of this polypeptide for MAST2, and (2) the anchoring of this polypeptide in the cytoplasmic membrane. Thus, a polypeptide, anchored into the cytoplasmic membrane and having a MAST-2 affinity comparable to the one of Neurovita1 (1.26 µM) or higher, is efficient to promote neurite outgrowth and neurite tree arborisation. Moreover, a cytosolic polypeptide having a MAST-2 affinity higher than the one of Neurovita1, and preferably comparable to the one of Neurovita3, is still efficient to promote neurite outgrowth and neurite tree arborisation, despite the absence of anchoring in the membrane.

<u>B.20. Neuritogenesis in E16 mouse foetal cortical neurons</u>

Neurite outgrowth experiments demonstrate that NV1 stimulates neuritogenesis in E16 mouse foetal cortical neurons (Figure 27), in agreement with the results obtained in SH-SY-5Y and NS cells.

<u>B.21. Absence of toxicity of NV1 or NV1Δ lentivectors for newborn mice infected with lentivectors by intracerebral route</u>

As reported in Figure 28A, there is no difference of weight between non-infected mice or mice infected with NV1 or NV1Δ lentivectors. Moreover, no obvious phenotypic difference between those different mice could be detected, at day 4 or 20 post injection (Figure 29).

Immunofluorescence histochemistry brains analysis indicated that NV1 (eGFP) and NV1Δ (eGFP) are expressed in the neurons of the striatum (Figures 30 and 31, panels marked with the neuronal marker Map2). Both for NV1 (eGFP) and NV1Δ (eGFP) infection, astrogliosis is very mild (Figures 30 and 31, panels marked with GFAP).

In addition, NV1 infected neurons exhibited neuritogenesis and extended differentiation of the dendritic-axonal tree (Figure 30B), suggesting that NV lentivector allows neuritogenesis and neurite tree development not only *in vitro* but also *in vivo*.These *in vivo* results confirm, one the hand, the *in vivo* efficiency of the Neurovita polypeptides, such as NV1, and on the other hand, the safety of these Neurovita polypeptides on animal development, and in particular on brain development.

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CLAIMS

1. Polypeptide, of at most 350 amino acids, comprising a cytoplasmic domain, wherein said cytoplasmic domain ends with a MAST-2 binding domain, wherein the size of said MAST-2 binding domain is from 11 to 13 amino acid residues, the first two residues of said MAST-2 binding domain are S and W, and the last four residues of said MAST-2 binding domain are Q, T, R and L, said MAST-2 binding domain being selected from the group consisting of:

(A) a sequence, whose size is 11 residues, consisting of $SWX_1X_2X_3X_4X_5QTRL$, wherein each of X_1, X_2, X_3, X_4 and X_5 is any amino acid residue (SEQ ID NO:19);

(B) a sequence, whose size is 11 residues, selected from the group consisting of: $SWX_1KSGGQTRL$ (SEQ ID NO:76), $SWX_1SSGGQTRL$ (SEQ ID NO:77), $SWX_1SHGGQTRL$ (SEQ ID NO:78), $SWX_1SHKGQTRL$ (SEQ ID NO:79), $SWX_1SHKSQTRL$ (SEQ ID NO:80), $SWX_1HSGGQTRL$ (SEQ ID NO:86), $SWX_1HKGGQTRL$ (SEQ ID NO:87), $SWX_1HKSGQTRL$ (SEQ ID NO:88), $SWX_1SKGGQTRL$ (SEQ ID NO:89), $SWX_1SKSGQTRL$ (SEQ ID NO:90), $SWX_1SHSGQTRL$ (SEQ ID NO:91), $SWX_1SHKGQTRL$ (SEQ ID NO:92) and $SWX_1SKGGQTRL$ (SEQ ID NO:93), wherein X_1 is any amino acid;

(C) a sequence, whose size is 12 residues, consisting of $SWX_1X_2X_3X_4X_5X_6QTRL$, wherein each of X_1, X_2, X_3, X_4, X_5 and X_6 is any amino acid residue;

(D) a sequence, whose size is 12 residues, selected from the group consisting of: $SWX_1HKSGGQTRL$ (SEQ ID NO:102), $SWX_1SKSGGQTRL$ (SEQ ID NO:103), $SWX_1SHSGGQTRL$ (SEQ ID NO:104), $SWX_1SHKGGQTRL$ (SEQ ID NO:105) and $SWX_1SHKSGQTRL$ (SEQ ID NO:106), wherein X_1 is any amino acid; and

(E) a sequence, whose size is 13 residues, consisting of $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, wherein each of X_1, X_2, X_3, X_4, X_5, X_6 and X_7, is any amino acid residue (SEQ ID NO:192), wherein said sequence does not consist of SWESHKSGGQTRL (SEQ ID NO:1),

wherein said polypeptide presents a binding affinity for the PDZ domain of the human MAST2 protein which is higher than the binding affinity of rabies virus G protein comprising the SWESHKSGGQTRL (SEQ ID NO: 1) sequence for the PDZ domain of the MAST2 protein.

2. The polypeptide according to claim 1, consisting of a cytoplasmic domain, wherein said cytoplasmic domain ends with a MAST-2 binding domain, wherein the size of said MAST-2 binding domain is from 11 to 13 amino acid residues, the first two residues of said MAST-2 binding domain are S and W, and the last four residues of said MAST-2 binding domain are Q, T, R and L, said MAST-2 binding domain being selected from the group consisting of:

(A) a sequence, whose size is 11 residues, consisting of $SWX_1X_2X_3X_4X_5QTRL$, wherein each of X_1, X_2, X_3, X_4 and X_5 is any amino acid residue (SEQ ID NO:19);

(B) a sequence, whose size is 11 residues, selected from the group consisting of: $SWX_1KSGGQTRL$ (SEQ ID NO:76), $SWX_1SSGGQTRL$ (SEQ ID NO:77), $SWX_1SHGGQTRL$ (SEQ ID NO:78), $SWX_1SHKGQTRL$ (SEQ ID NO:79), $SWX_1SHKSQTRL$ (SEQ ID NO:80), $SWX_1HSGGQTRL$ (SEQ ID NO:86), $SWX_1HKGGQTRL$ (SEQ ID NO:87), $SWX_1HKSGQTRL$ (SEQ ID NO:88), $SWX_1SKGGQTRL$ (SEQ ID NO:89), $SWX_1SKSGQTRL$ (SEQ ID NO:90), $SWX_1SHSGQTRL$ (SEQ ID NO:91), $SWX_1SHKGQTRL$ (SEQ ID NO:92) and $SWX_1SKGGQTRL$ (SEQ ID NO:93), wherein X_1 is any amino acid;

(C) a sequence, whose size is 12 residues, consisting of $SWX_1X_2X_3X_4X_5X_6QTRL$, wherein each of X_1, X_2, X_3, X_4, X_5 and X_6 is any amino acid residue;

(D) a sequence, whose size is 12 residues, selected from the group consisting of: $SWX_1HKSGGQTRL$ (SEQ ID NO:102), $SWX_1SKSGGQTRL$ (SEQ ID NO:103), $SWX_1SHSGGQTRL$ (SEQ ID NO:104), $SWX_1SHKGGQTRL$ (SEQ ID NO:105) and $SWX_1SHKSGQTRL$ (SEQ ID NO:106), wherein X_1 is any amino acid; and

(E) a sequence, whose size is 13 residues, consisting of $SWX_1X_2X_3X_4X_5X_6X_7QTRL$, wherein each of X_1, X_2, X_3, X_4, X_5, X_6 and X_7, is any amino acid residue (SEQ ID NO:192), wherein said sequence does not consist of SWESHKSGGQTRL (SEQ ID NO:1),

wherein said polypeptide presents a binding affinity for the PDZ domain of the human MAST2 protein which is higher than the binding affinity of rabies virus G protein comprising the SWESHKSGGQTRL (SEQ ID NO: 1) sequence for the PDZ domain of the MAST2 protein.

3. The polypeptide according to claim 1 or 2, wherein X_1 is E or A and/or X_2 is S, E or V.

4. The polypeptide according to any one of claims 1 to 3, wherein the sequence is

(A) any one of SEQ ID NOs: 20, 21 or 22 to 24;

(B) any one of SEQ ID NOs: 81 to 85 or 94 to 101;

(C) SEQ ID NO: 112; or

(D) any one of SEQ ID NOs: 107 to 111.

5. The polypeptide according to any one of claims 1 to 3, wherein said MAST-2 binding domain consists of the 11-residue sequence $SWX_1X_2X_3X_4X_5QTRL$ (SEQ ID NO: 19), wherein X_1 is E or A and/or X_2 is S, E or V and/or X_3 is H, A or Y and/or X_4 is G or T and/or X_5 is G or Q.

6. The polypeptide according to claim 5, which has the sequence S-W-E/A-S/E/V-H/A/Y-G/T-G/Q-Q-T-R-L as defined in SEQ ID NO:61.

7. The polypeptide according to any one of claims 1 to 3, wherein said MAST-2 binding domain consists of the 11-residue sequence S-W-E-V-H/A/Y-G/T-G/Q-Q-T-R-L as defined in SEQ ID NO:63.

8. The polypeptide according to claim 7, wherein said MAST-2 binding domain is selected from the group consisting of SWEVHGGQTRL (SEQ ID NO:64), SWEVHGQQTRL (SEQ ID NO:65), SWEVHTGQTRL (SEQ ID NO:66), SWEVHTQQTRL (SEQ ID NO:67), SWEVAGGQTRL (SEQ ID NO:68), SWEVAGQQTRL (SEQ ID NO:69), SWEVATGQTRL (SEQ ID NO:70), SWEVATQQTRL (SEQ ID NO:71) SWEVYGGQTRL (SEQ ID NO:72), SWEVYGQQTRL (SEQ ID NO:73), SWEVYTGQTRL (SEQ ID NO:74) and SWEVYTQQTRL (SEQ ID NO:75).

9. The polypeptide according to claim 1, wherein said MAST-2 binding domain consists of the 12-residue sequence SW$X_1X_2X_3X_4X_5X_6$QTRL (SEQ ID NO: 112), wherein X_1 is E, A, V or S, X_2 is S, V, H, A or Y, X_3 is H, A, Y, K or Q, X_4 is K, A, Q, S or H, X_5 is S, H, G or T and X_6 is G, T or Q, as defined in SEQ ID NO:191.

10. The polypeptide according to claim 1, wherein said MAST-2 binding domain consists of the 13-residue sequence SW$X_1X_2X_3X_4X_5X_6X_7$QTRL (SEQ ID NO: 192), wherein X_1 is E or A and/or X_2 is S, V or E and/or X_3 is H, A or Y and/or X_4 is K, A or Q and/or X_5 is S or H and/or X_6 is G or T and/or X_7 is G or Q.

11. The polypeptide according to claim 10, wherein said MAST-2 binding domain has the sequence S-W-E/A-S/V/E-H/A/Y-K/A/Q-S/H-G/T-G/Q-QTRL as defined in SEQ ID NO:206.

12. The polypeptide according to claim 10, wherein said MAST-2 binding domain consists of SWAEAQHTQQTRL (SEQ ID NO:208) or SWEVHASGGQTRL (SEQ ID NO:209).

13. The polypeptide according to any one of claims 1 to 12, wherein the sequence of the cytoplasmic domain upstream of the MAST-2 binding domain is either:

- a polypeptide containing 20 to 40 amino acid residues; or
- a fragment of the cytoplasmic domain of a rabies virus G protein consisting of the sequence as defined in SEQ ID NO:2, or
- a variant having at least 80% identity with SEQ ID NO:2.

14. The polypeptide according to claim 13, wherein the variant having at least 80% identity with SEQ ID NO: 2 comprises RRVNRSEPTQLNLRGTGREVSVTPQSGKIIS (SEQ ID NO:5).

15. The polypeptide according to any one of claims 1 to 14, further comprising a signal peptide and an anchoring domain for anchoring said polypeptide into the reticulum membrane and/or Golgi membrane, wherein said polypeptide comprises, from N-terminal to C-terminal, (1) said signal peptide, (2) said anchoring domain, and (3) said cytoplasmic domain.

16. The polypeptide according to claim 15, wherein the anchoring domain is either:

- a peptide, whose size is from 18 to 26 residues, which anchors the polypeptide in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells; or
- the transmembrane domain of a rabies virus G protein consisting of the sequence as defined in SEQ ID NO:4 or a variant having at least 81% identity with said SEQ ID NO:4 retaining the capacity to anchor the polypeptide in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells.

17. The polypeptide according to claim 16, wherein the peptide is a transmembrane domain.

18. The polypeptide according to any one of claims 15 to 17, wherein the signal peptide is either:

- a peptide, whose size is from 3 to 60 residues, which targets the polypeptide into the endoplasmic reticulum; or
- a signal peptide of a rabies virus G protein consisting of the sequence as defined in SEQ ID NO:3 or a variant having at least 68% identity with said SEQ ID NO:3 retaining the capacity to target the polypeptide into the endoplasmic reticulum.

19. The polypeptide according to claim 18, wherein the signal peptide targets the polypeptide into the endoplasmic reticulum through the secretory pathway.

20. The polypeptide according to any one of claims 15 to 19, which comprises, between the signal peptide and the anchoring domain, a linker consisting of 1 to 4 amino acid residues.

21. The polypeptide according to claim 20, wherein the linker consists of the amino acid residues GK.

22. The polypeptide according to any one of claims 1 to 21 which comprises, from N-terminal to C-terminal:

(1) a signal peptide as defined in SEQ ID NO:3, or a variant having at least 68% identity with said SEQ ID NO:3 retaining the capacity to target the polypeptide into the endoplasmic reticulum;

(2) an anchoring domain as defined in SEQ ID NO:4 or a variant having at least 81% identity with said SEQ ID NO:4 retaining the capacity to anchor the polypeptide in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells; and

(3) a cytoplasmic domain comprising (a) a peptide as defined in SEQ ID NO:2 or a variant having at least 80% identity with SEQ ID NO:2, and (b) a MAST-2 binding domain as defined in any one of SEQ ID NO:19 to SEQ ID NO:209.

23. The polypeptide according to claim 22, which comprises, from N-terminal to C-terminal:

(1) a signal peptide as defined in SEQ ID NO:3, or a variant having at least 68% identity with said SEQ ID NO:3 retaining the capacity to target the polypeptide into the endoplasmic reticulum and through the secretory pathway;

(2) the amino acid residues GK.;

(3) an anchoring domain as defined in SEQ ID NO:4 or a variant having at least 81% identity with said SEQ ID NO:4 retaining the capacity to anchor the polypeptide in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells; and

(4) a cytoplasmic domain comprising (a) a peptide as defined in SEQ ID NO:2 or a variant having at least 80% identity with SEQ ID NO:2, and (b) a MAST-2 binding domain as defined in any one of SEQ ID NOS: 64-68, 71, 74 and 208-209.

24. The polypeptide according to claim 22 which consists of, from N-terminal to C-terminal:

(1) a signal peptide as defined in SEQ ID NO:3, or a variant having at least 68% identity with said SEQ ID NO:3 retaining the capacity to target the polypeptide into the endoplasmic reticulum;

(2) an anchoring domain as defined in SEQ ID NO:4 or a variant having at least 81% identity with said SEQ ID NO:4 retaining the capacity to anchor the polypeptide in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells; and

(3) a cytoplasmic domain comprising (a) a peptide as defined in SEQ ID NO:2 or a variant having at least 80% identity with SEQ ID NO:2, and (b) a MAST-2 binding domain as defined in any one of SEQ ID NO:19 to SEQ ID NO:209.

25. The polypeptide according to claim 23 which consists of

(1) a signal peptide as defined in SEQ ID NO:3, or a variant having at least 68% identity with said SEQ ID NO:3 retaining the capacity to target the polypeptide into the endoplasmic reticulum and through the secretory pathway;

(2) the amino acid residues GK.;

(3) an anchoring domain as defined in SEQ ID NO:4 or a variant having at least 81% identity with said SEQ ID NO:4 retaining the capacity to anchor the polypeptide in the membrane of the endoplasmic reticulum and/or the membrane of the Golgi apparatus in cells; and

(4) a cytoplasmic domain comprising (a) a peptide as defined in SEQ ID NO:2 or a variant having at least 80% identity with SEQ ID NO:2, and (b) a MAST-2 binding domain as defined in any one of SEQ ID NOS: 64-68, 71, 74 and 208-209.

26. The polypeptide according to any one of claims 1 to 25, for which the constant of dissociation (K_D) of a complex formed between this polypeptide and the PDZ domain of the human MAST2 protein is less than 0.5 µM, measured by Isothermal Titration Calorimetry (ITC).

27. The polypeptide according to claim 26, for which the constant of dissociation (K_D) of the complex formed between this polypeptide and the PDZ domain of the human MAST2 protein is less than 0.2 μM, measured by Isothermal Titration Calorimetry (ITC).

28. The polypeptide according to claim 26, for which the constant of dissociation (K_D) of the complex formed between this polypeptide and the PDZ domain of the human MAST2 protein is less than 0.1 μM, measured by Isothermal Titration Calorimetry (ITC).

29. The polypeptide according to any one of claims 1 to 28, the sequence of which is selected from the group consisting of SEQ ID NO:210, SEQ ID NO: 211, amino-acids 44-85 of SEQ ID NO: 211, SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 216, SEQ ID NO: 217, and SEQ ID NO:218.

30. A polynucleotide encoding the polypeptide as defined in any one of claims 1 to 29, the sequence of which is at most 1050 nucleotides.

31. The polynucleotide according to claim 30, selected from the group consisting of: SEQ ID NO:219, SEQ ID NO:220, nucleotides 130-258 of SEQ ID NO:220, SEQ ID NO:221, SEQ ID NO:222, SEQ ID NO:223, SEQ ID NO:224, SEQ ID NO:225, SEQ ID NO:226, and SEQ ID NO:227.

32. A vector comprising the polynucleotide as defined in claim 30 or 31.

33. The vector according to claim 32, which is an expression vector wherein said polynucleotide is under the control of transcription regulatory element(s).

34. The vector according to claim 33, wherein the transcription regulatory element is the CMV promoter.

35. An expression lentivirus-derived vector, comprising the polynucleotide as defined in claim 30 or 31, expression regulatory elements of said polynucleotide, a cis-acting central initiation region (cPPT) and a cis-acting termination region (CTS) both of lentiviral origin and regulatory signals of retroviral origin for reverse transcription, expression and packaging.

36. The expression lentivirus-derived vector of claim 35, which is a plasmid.

37. A lentiviral vector pseudotyped particle comprising GAG structural proteins and a viral core made of (a) POL proteins and (b) a lentiviral vector genome comprising the polynucleotide as defined in claim 30 or 31, expression regulatory elements of said polynucleotide, a cis-acting central initiation region (cPPT) and a cis-acting termination region (CTS) both of lentiviral origin and regulatory signals of retroviral origin for reverse transcription, expression and packaging, wherein said particle is pseudotyped with the G protein of a VSV virus or the G protein of a rabies virus.

38. The lentiviral particle according to claim 37, wherein the integrase protein of the POL protein is defective.

39. A cell transfected with the vector as defined in claim 35 or 36 or transduced by the lentiviral particle as defined in claim 37 or 38.

40. A composition comprising the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38 or the cell as defined in claim 39, and a pharmaceutically acceptable vehicle, excipient or carrier.

41. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, in the treatment or and/or palliation and/or prevention of a neoplasm of the nervous system.

42. The use according to claim 41, wherein the neoplasm of the nervous system is a cancer tumor.

43. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, in the treatment or and/or palliation and/or prevention of a microbial infection of the neurons.

44. The use according to claim 43 wherein the microbial infection induces neuron apoptosis.

45. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, in protecting neurons from neurotoxic agents or oxidative stress.

46. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, in the treatment or and/or palliation and/or prevention of a neurogenerative disease, disorder or condition.

47. The use according to claim 46, wherein the neurogenerative disease, disorder or condition is selected amongst: non-viral encephalopathy, Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis ALS, Huntington disease, multiple sclerosis (MS) and a rare genetic disease.

48. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, in the treatment and/or palliation and/or prevention of a physical or ischemic injury of the nervous system, or of a disease, disorder or condition involving such an injury.

49. The use according to claim 48, in the treatment and/or palliation and/or prevention of a seizure, stroke, trauma, or epilepsy.

50. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, in the treatment or and/or palliation and/or prevention of a leukemia.

51. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for the preparation of a medicament in the treatment or and/or palliation and/or prevention of a neoplasm of the nervous system.

52. The use according to claim 51, wherein the neoplasm of the nervous system is a cancer tumor.

53. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for the preparation of a medicament in the treatment or and/or palliation and/or prevention of a microbial infection of the neurons.

54. The use according to claim 53 wherein the microbial infection induces neuron apoptosis.

55. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for the preparation of a medicament for protecting neurons from neurotoxic agents or oxidative stress.

56. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for the preparation of a medicament in the treatment or and/or palliation and/or prevention of a neurogenerative disease, disorder or condition.

57. The use according to claim 56, wherein the neurogenerative disease, disorder or condition is selected amongst: non-viral encephalopathy, Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis ALS, Huntington disease, multiple sclerosis (MS) and a rare genetic disease.

58. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for the preparation of a medicament in the treatment and/or palliation and/or prevention of a physical or ischemic injury of the nervous system, or of a disease, disorder or condition involving such an injury.

59. The use according to claim 58, in the treatment and/or palliation and/or prevention of a seizure, stroke, trauma, or epilepsy.

60. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for the preparation of a medicament in the treatment or and/or palliation and/or prevention of a leukemia.

61. The polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for use in the treatment or and/or palliation and/or prevention of a neoplasm of the nervous system.

62. The polypeptide, the polynucleotide, the vector, the lentiviral particle, the cell or the composition for use according to claim 61, wherein the neoplasm of the nervous system is a cancer tumor.

63. The polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for use in the treatment or and/or palliation and/or prevention of a microbial infection of the neurons.

64. The polypeptide, the polynucleotide, the vector, the lentiviral particle, the cell or the composition for use according to claim 63, wherein the microbial infection induces neuron apoptosis.

65. The polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for use for protecting neurons from neurotoxic agents or oxidative stress.

66. Use of the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for use in the treatment or and/or palliation and/or prevention of a neurogenerative disease, disorder or condition.

67. The polypeptide, the polynucleotide, the vector, the lentiviral particle, the cell or the composition for use according to claim 66, wherein the neurogenerative disease, disorder or condition is selected amongst: non-viral encephalopathy, Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis ALS, Huntington disease, multiple sclerosis (MS) and a rare genetic disease.

68. The polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for use in the treatment and/or palliation and/or prevention of a physical or ischemic injury of the nervous system, or of a disease, disorder or condition involving such an injury.

69. The polypeptide, the polynucleotide, the vector, the lentiviral particle, the cell or the composition for use according to claim 68, in the treatment and/or palliation and/or prevention of a seizure, stroke, trauma, or epilepsy.

70. The polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40, for use in the treatment or and/or palliation and/or prevention of a leukemia.

71. Kit comprising the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40 and instructions for the treatment or and/or palliation and/or prevention of a neoplasm of the nervous system.

72. Kit comprising the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40 and instructions for the treatment or and/or palliation and/or prevention of a microbial infection of the neurons.

73. Kit comprising the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40 and instructions for protecting neurons from neurotoxic agents or oxidative stress.

74. Kit comprising the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40 and instructions for the treatment or and/or palliation and/or prevention of a neurogenerative disease, disorder or condition.

75. Kit comprising the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40 and instructions for the treatment or and/or palliation and/or prevention of a physical or ischemic injury of the nervous system, or of a disease, disorder or condition involving such an injury.

76. Kit comprising the polypeptide as defined in any one of claims 1 to 29, the polynucleotide as defined in claim 30 or 31, the vector as defined in any one of claims 32 to 36, the lentiviral particle as defined in claim 37 or 38, the cell as defined in claim 39 or the composition as defined in claim 40 and instructions for the treatment or and/or palliation and/or prevention of a leukemia.

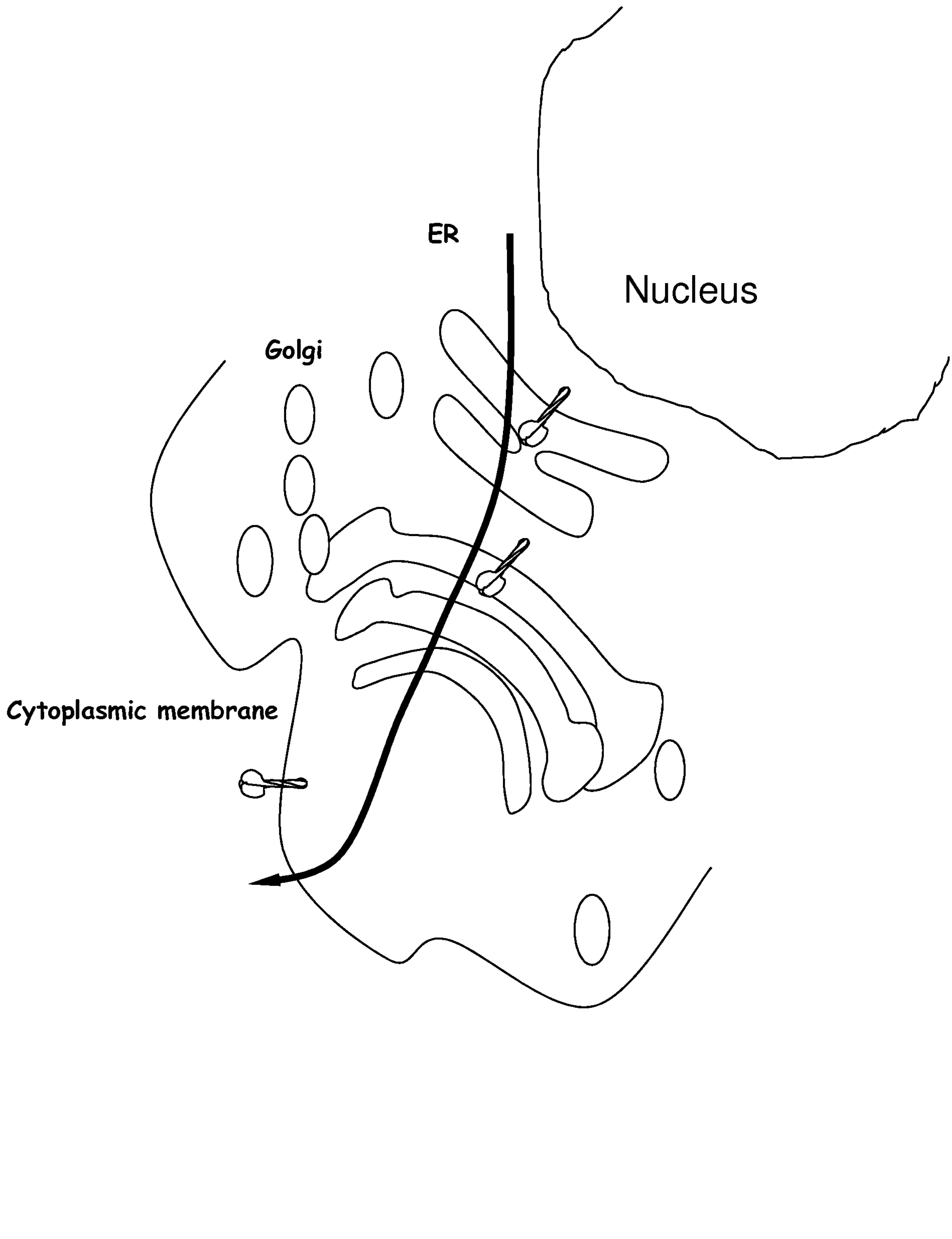


FIG. 1

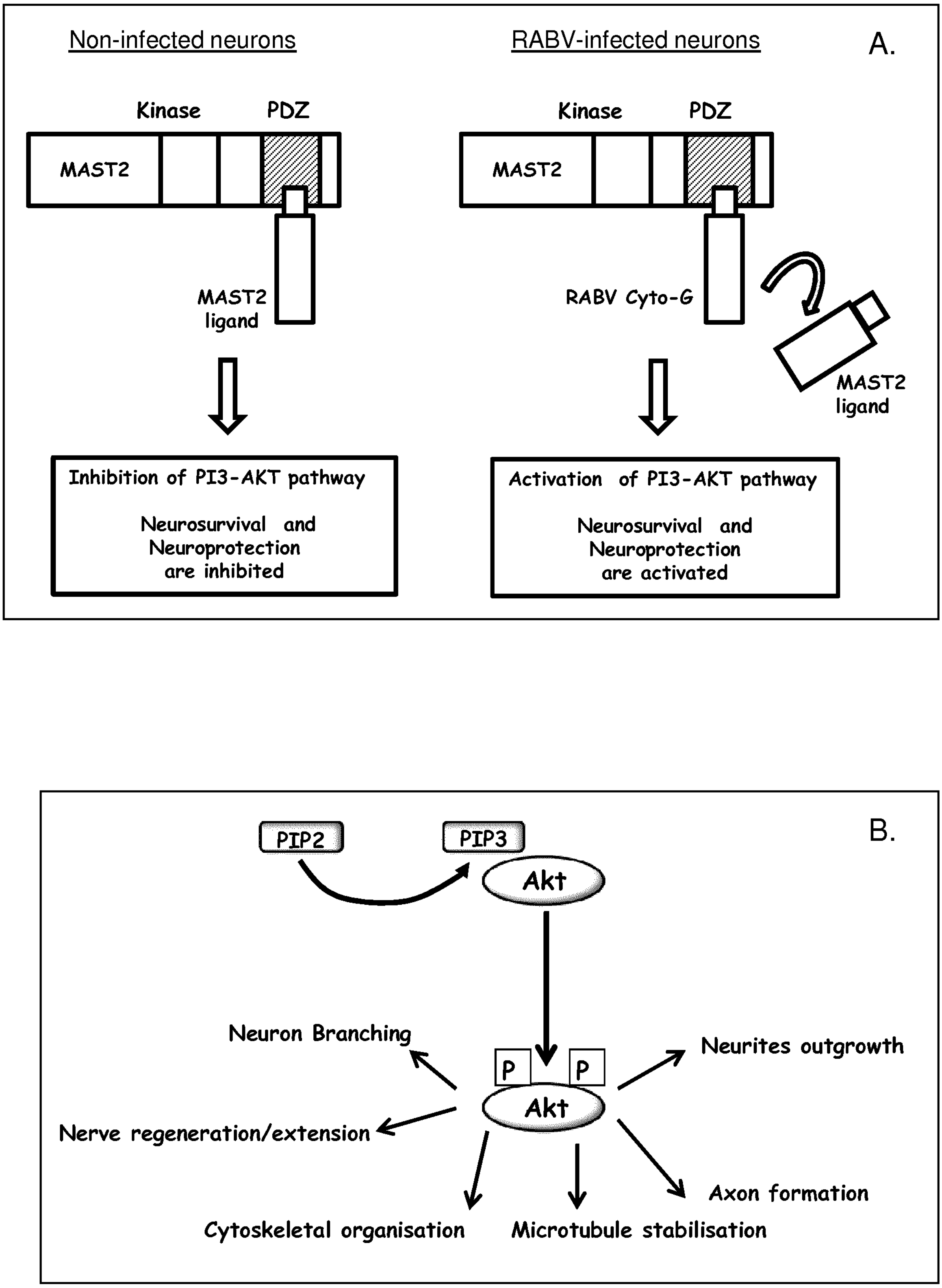
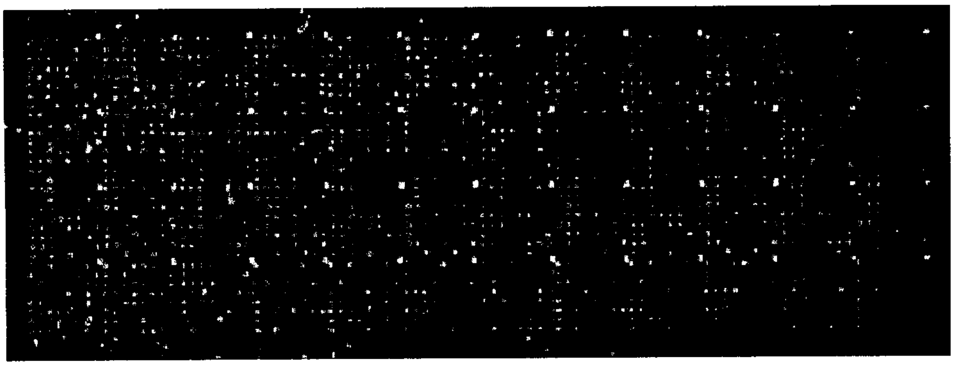

Non-infected neurons
RABV-infected neurons
A.
Kinase
PDZ
MAST2
MAST2
ligand
Kinase
PDZ
MAST2
RABV Cyto-G
MAST2
ligand
Inhibition of PI3-AKT pathway
Neurosurvival and
Neuroprotection
are inhibited
Activation of PI3-AKT pathway
Neurosurvival and
Neuroprotection
are activated
B.
PIP2
PIP3
Akt
Neuron Branching
P
P
Akt
Neurites outgrowth
Nerve regeneration/extension
Axon formation
Cytoskeletal organisation
Microtubule stabilisation

FIG. 2

A.



B.

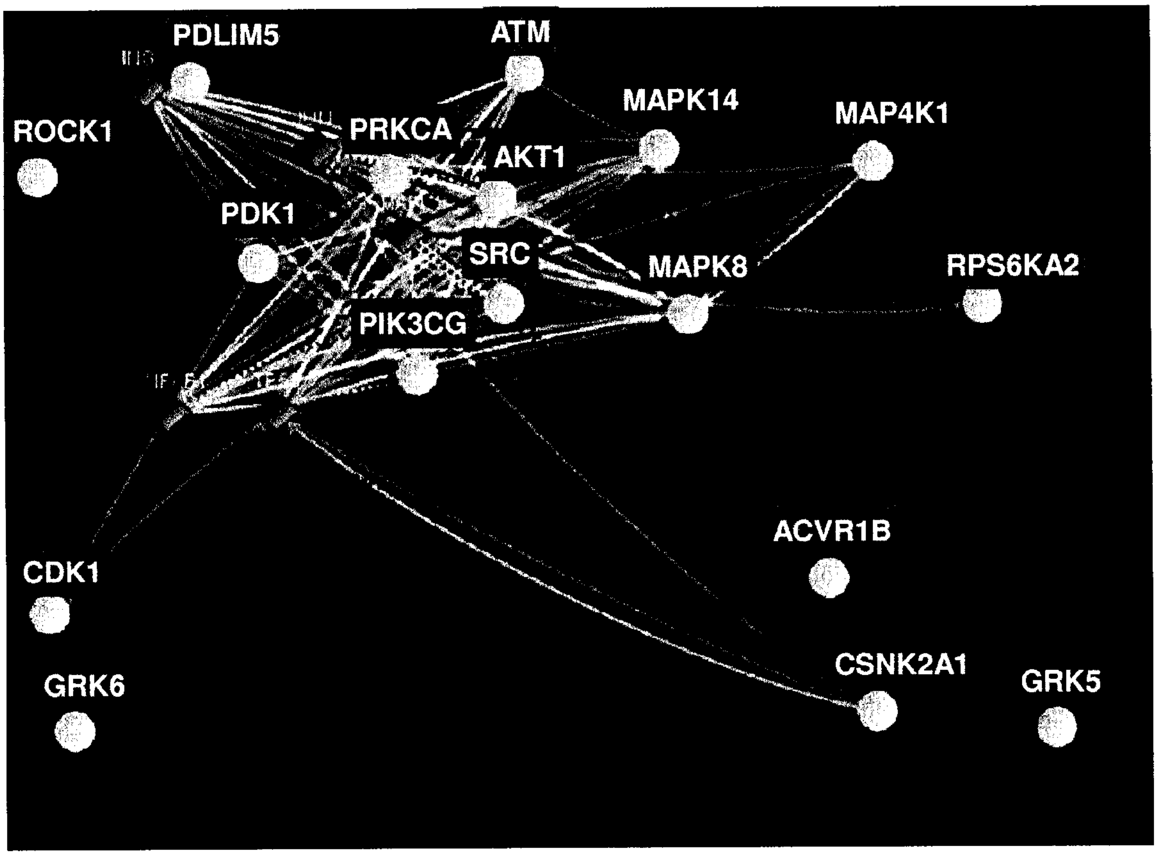

PDLIM5
ATM
MAPK14
MAP4K1
ROCK1
PRKCA
AKT1
PDK1
SRC
MAPK8
RPS6KA2
PIK3CG
ACVR1B
CDK1
CSNK2A1
GRK5
GRK6

FIG. 3

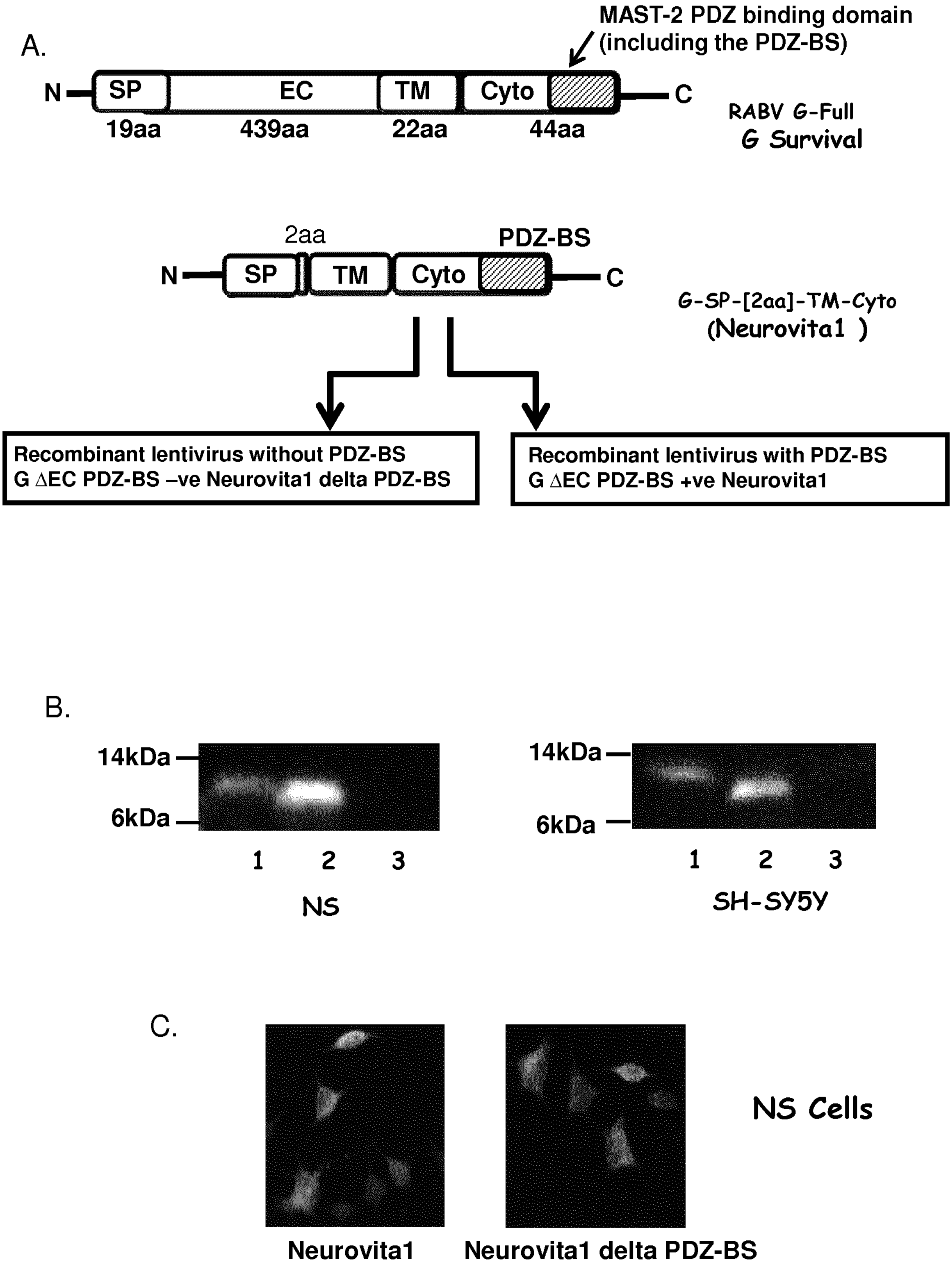

A.
MAST-2 PDZ binding domain
(including the PDZ-BS)
N
SP
EC
TM
Cyto
C
19aa
439aa
22aa
44aa
RABV G-Full
G Survival
2aa
PDZ-BS
N
SP
TM
Cyto
C
G-SP-[2aa]-TM-Cyto
(Neurovita1)
Recombinant lentivirus without PDZ-BS
G ΔEC PDZ-BS –ve Neurovita1 delta PDZ-BS
Recombinant lentivirus with PDZ-BS
G ΔEC PDZ-BS +ve Neurovita1
B.
14kDa
6kDa
1 2 3
NS
14kDa
6kDa
1 2 3
SH-SY5Y
C.
NS Cells
Neurovita1
Neurovita1 delta PDZ-BS

FIG. 4

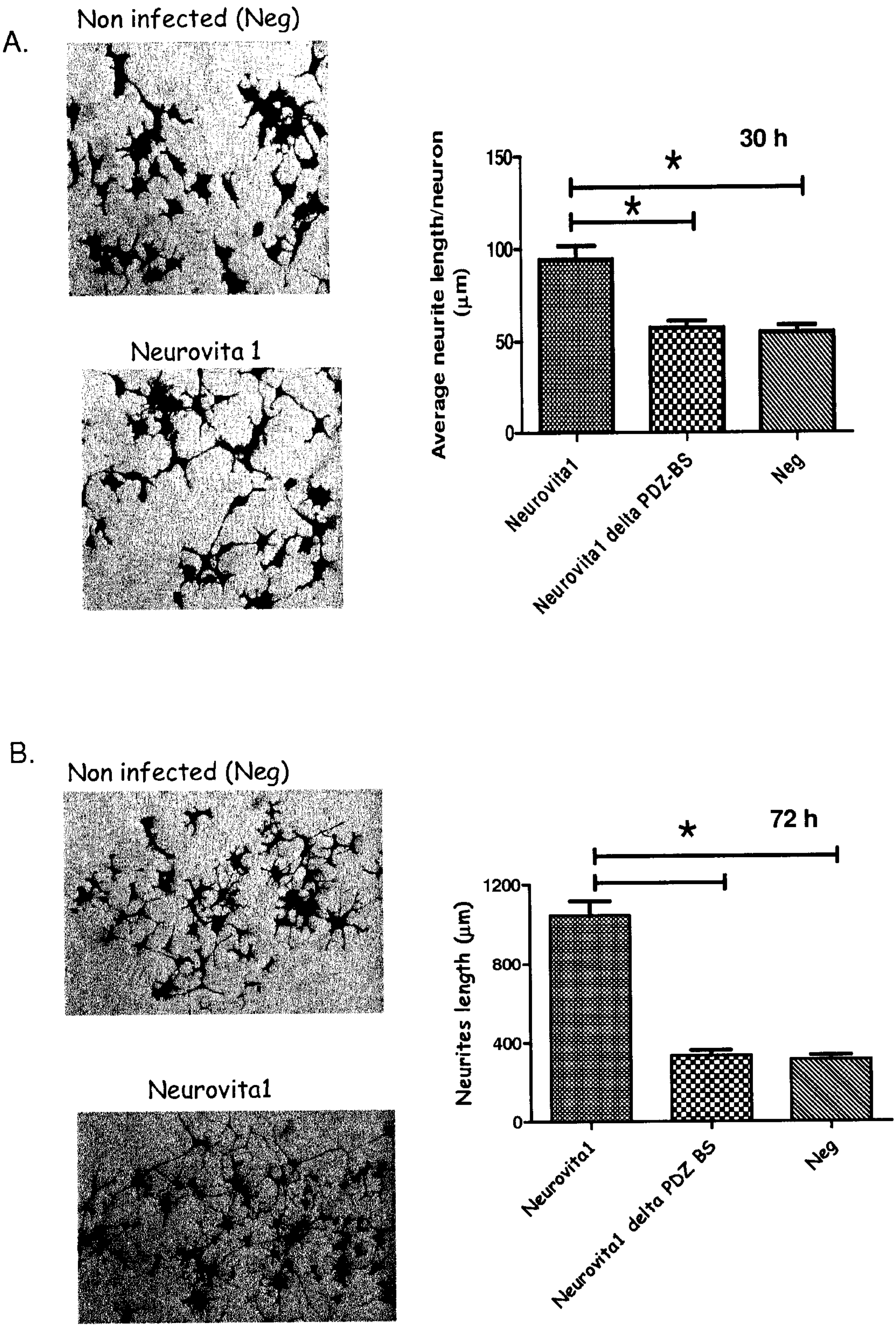

A.
Non infected (Neg)
Neurovita 1
30 h
Average neurite length/neuron (µm)
150
100
50
0
*
*
Neurovita1
Neurovita1 delta PDZ-BS
Neg
B.
Non infected (Neg)
Neurovita1
72 h
*
Neurites length (µm)
1200
800
400
0
Neurovita1
Neurovita1 delta PDZ BS
Neg

FIG. 5

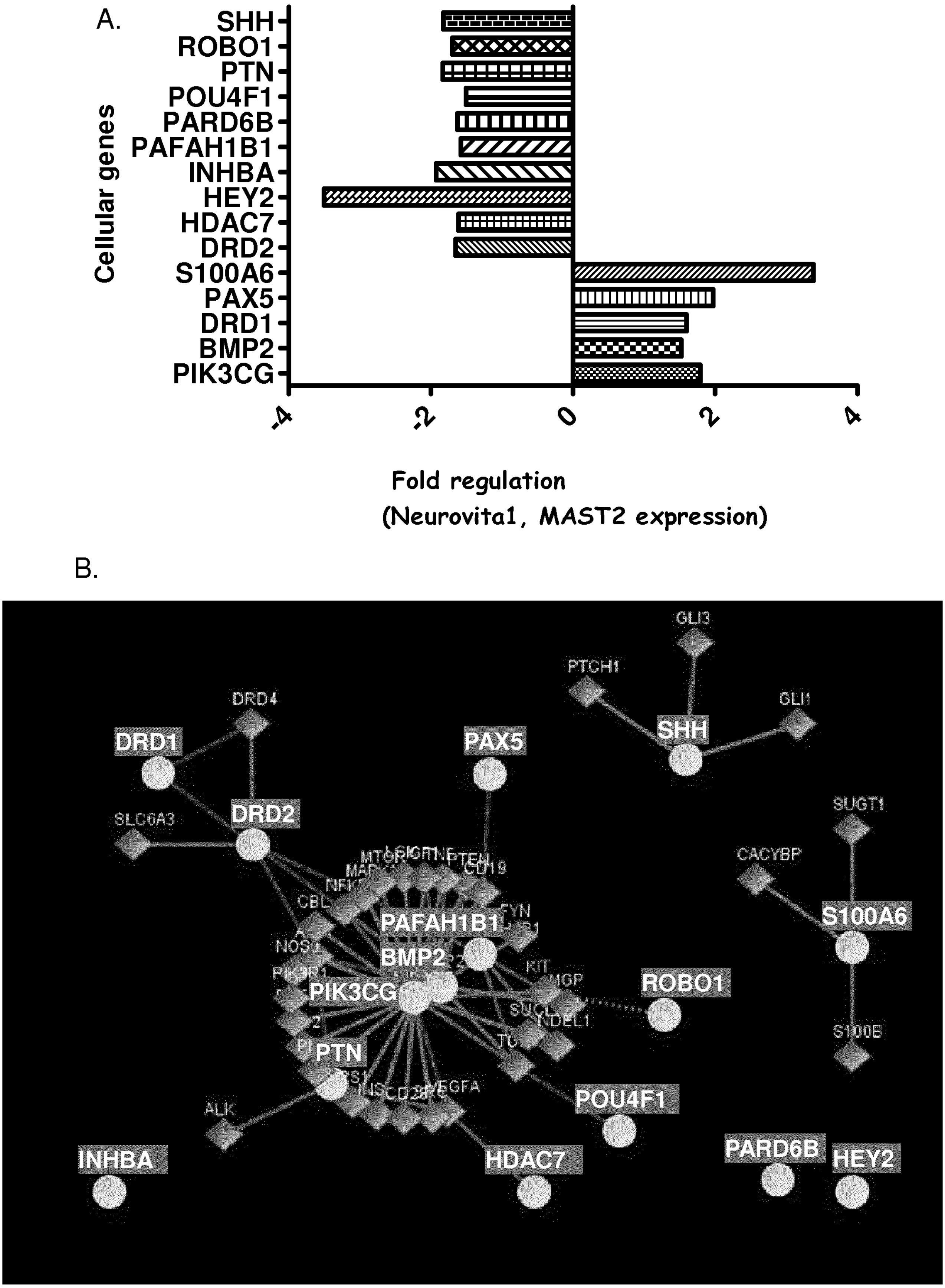

A.
SHH
ROBO1
PTN
POU4F1
PARD6B
PAFAH1B1
INHBA
HEY2
HDAC7
DRD2
S100A6
PAX5
DRD1
BMP2
PIK3CG
Cellular genes
-4
-2
0
2
4
Fold regulation
(Neurovita1, MAST2 expression)
B.
GLI3
PTCH1
GLI1
SHH
DRD4
DRD1
PAX5
SLC6A3
DRD2
SUGT1
CACYBP
S100A6
CD19
CBL
FYN
PAFAH1B1
BMP2
KIT
MGP
ROBO1
PIK3CG
NDEL1
S100B
PTN
ALK
VEGFA
POU4F1
INHBA
HDAC7
PARD6B
HEY2

FIG. 6

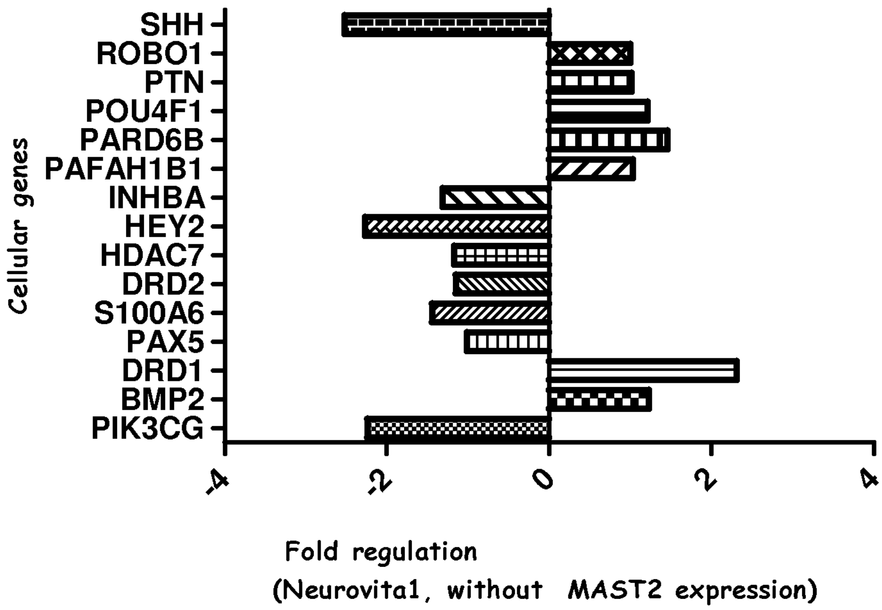

SHH
ROBO1
PTN
POU4F1
PARD6B
PAFAH1B1
INHBA
HEY2
HDAC7
DRD2
S100A6
PAX5
DRD1
BMP2
PIK3CG
Cellular genes
-4
-2
0
2
4
Fold regulation
(Neurovita1, without MAST2 expression)

FIG. 7

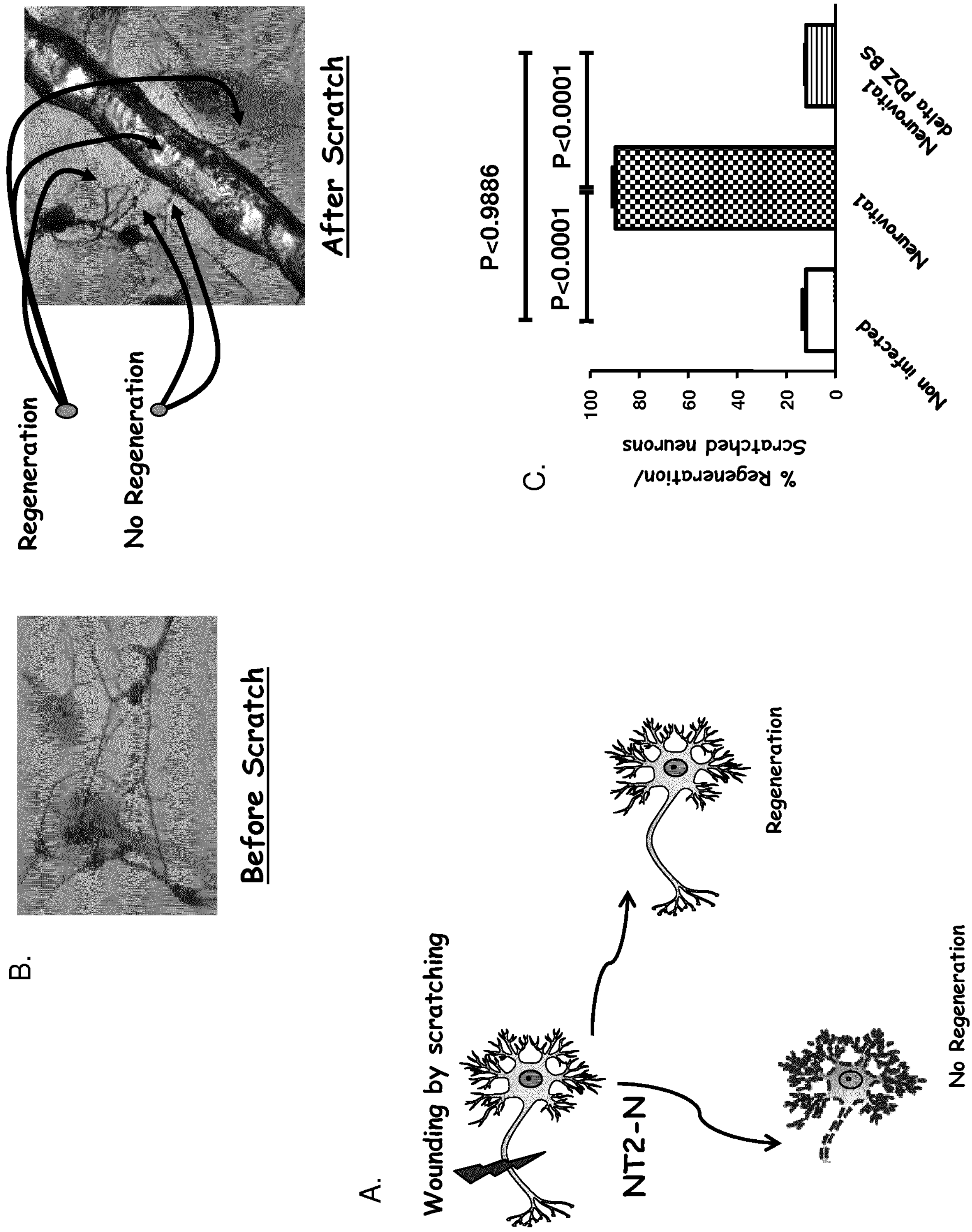

A.
Wounding by scratching
NT2-N
Regeneration
No Regeneration
B.
Before Scratch
After Scratch
Regeneration
No Regeneration
C.
% Regeneration/
Scratched neurons
0
20
40
60
80
100
Non infected
Neurovita1
Neurovita1
delta PDZ BS
P<0.9886
P<0.0001
P<0.0001

FIG. 8

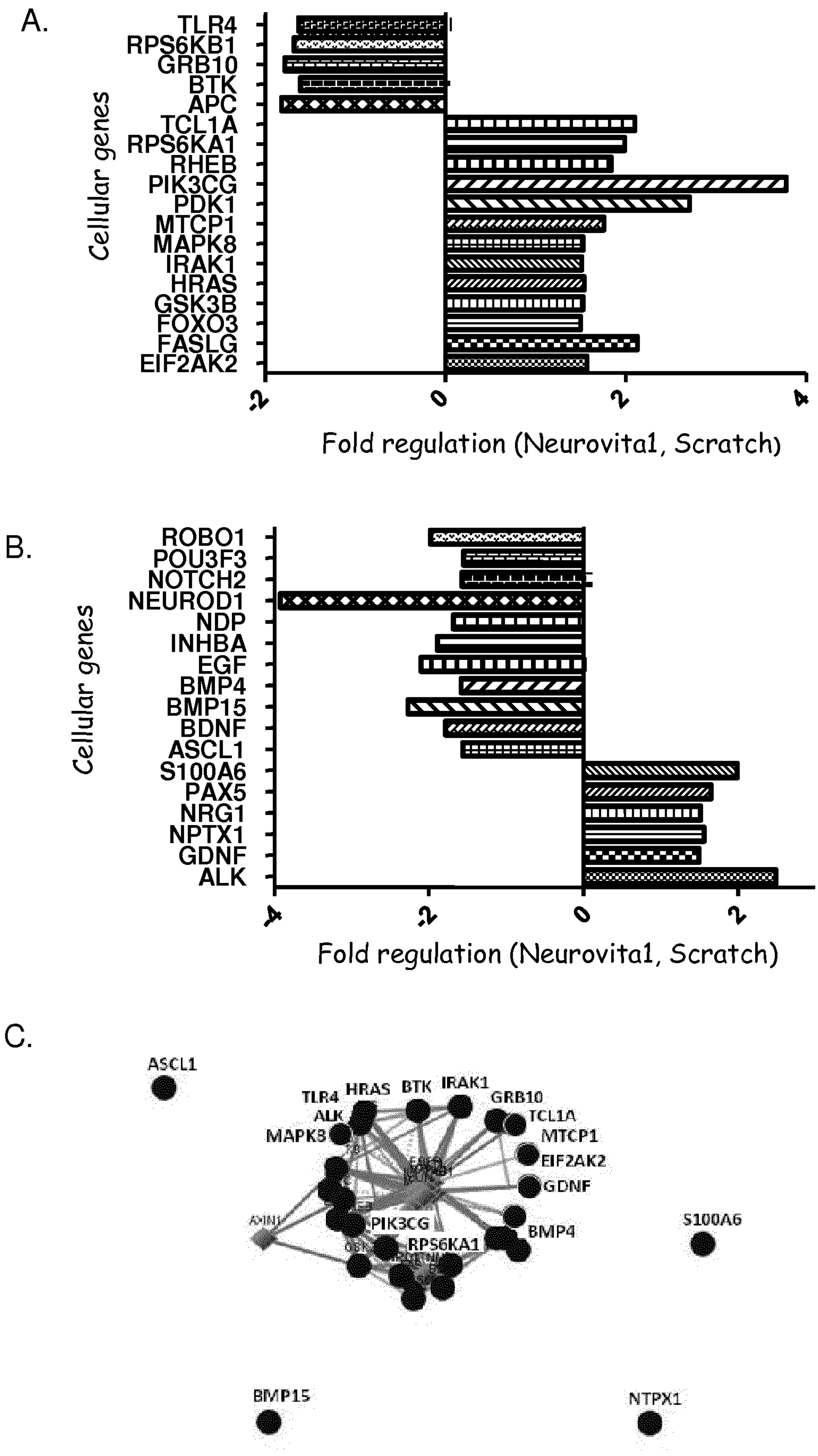

A.
TLR4
RPS6KB1
GRB10
BTK
APC
TCL1A
RPS6KA1
RHEB
PIK3CG
PDK1
MTCP1
MAPK8
IRAK1
HRAS
GSK3B
FOXO3
FASLG
EIF2AK2
Cellular genes
-2
0
2
4
Fold regulation (Neurovita1, Scratch)
B.
ROBO1
POU3F3
NOTCH2
NEUROD1
NDP
INHBA
EGF
BMP4
BMP15
BDNF
ASCL1
S100A6
PAX5
NRG1
NPTX1
GDNF
ALK
Cellular genes
-4
-2
0
2
Fold regulation (Neurovita1, Scratch)
C.
ASCL1
TLR4
HRAS
BTK
IRAK1
GRB10
ALK
TCL1A
MAPK8
MTCP1
EIF2AK2
GDNF
PIK3CG
BMP4
S100A6
RPS6KA1
BMP15
NTPX1

FIG. 9

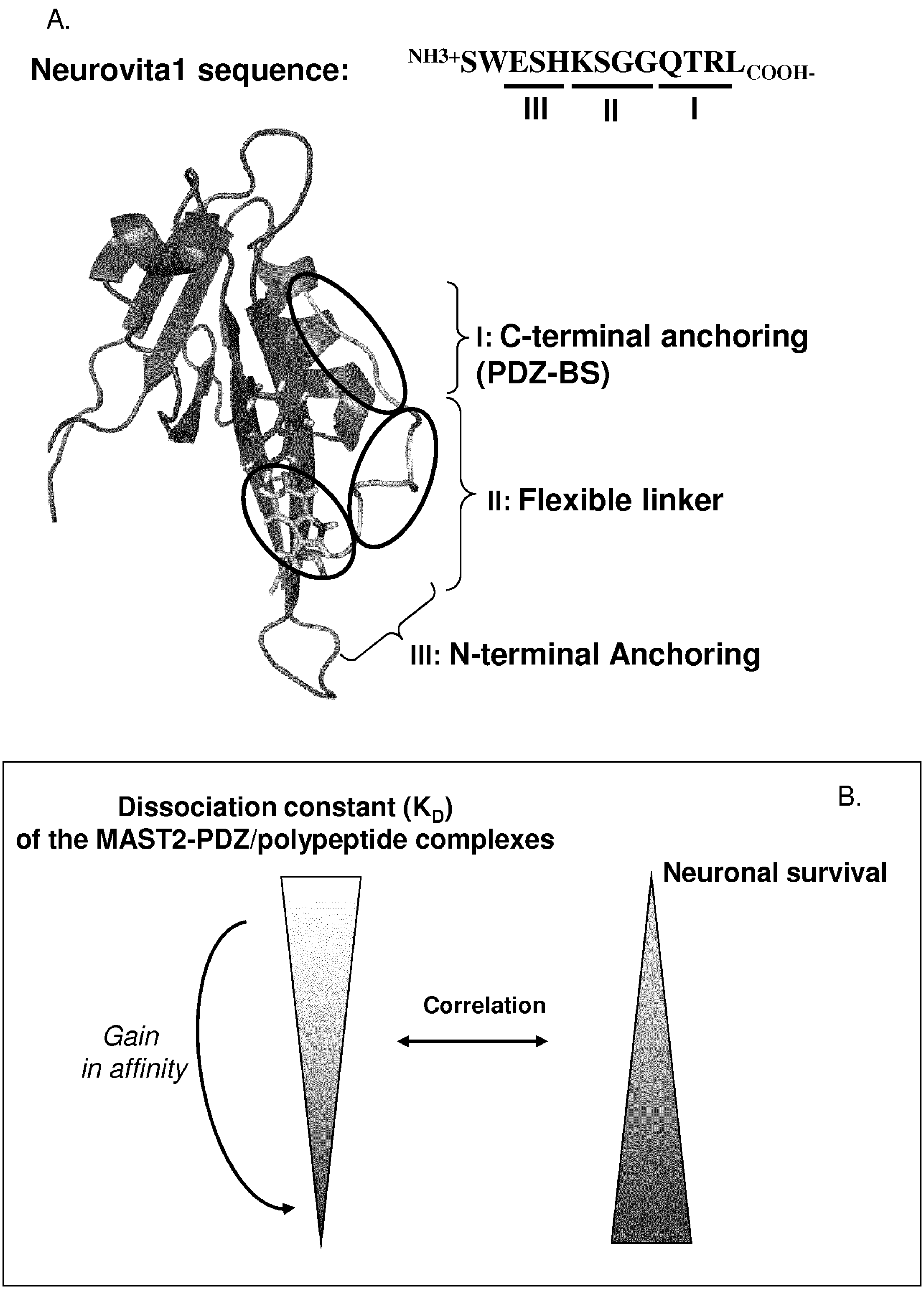

A.
Neurovita1 sequence:
NH3+SWESHKSGGQTRLCOOH-
III
II
I
I: C-terminal anchoring (PDZ-BS)
II: Flexible linker
III: N-terminal Anchoring
Dissociation constant (KD) of the MAST2-PDZ/polypeptide complexes
B.
Neuronal survival
Correlation
Gain in affinity

FIG. 10

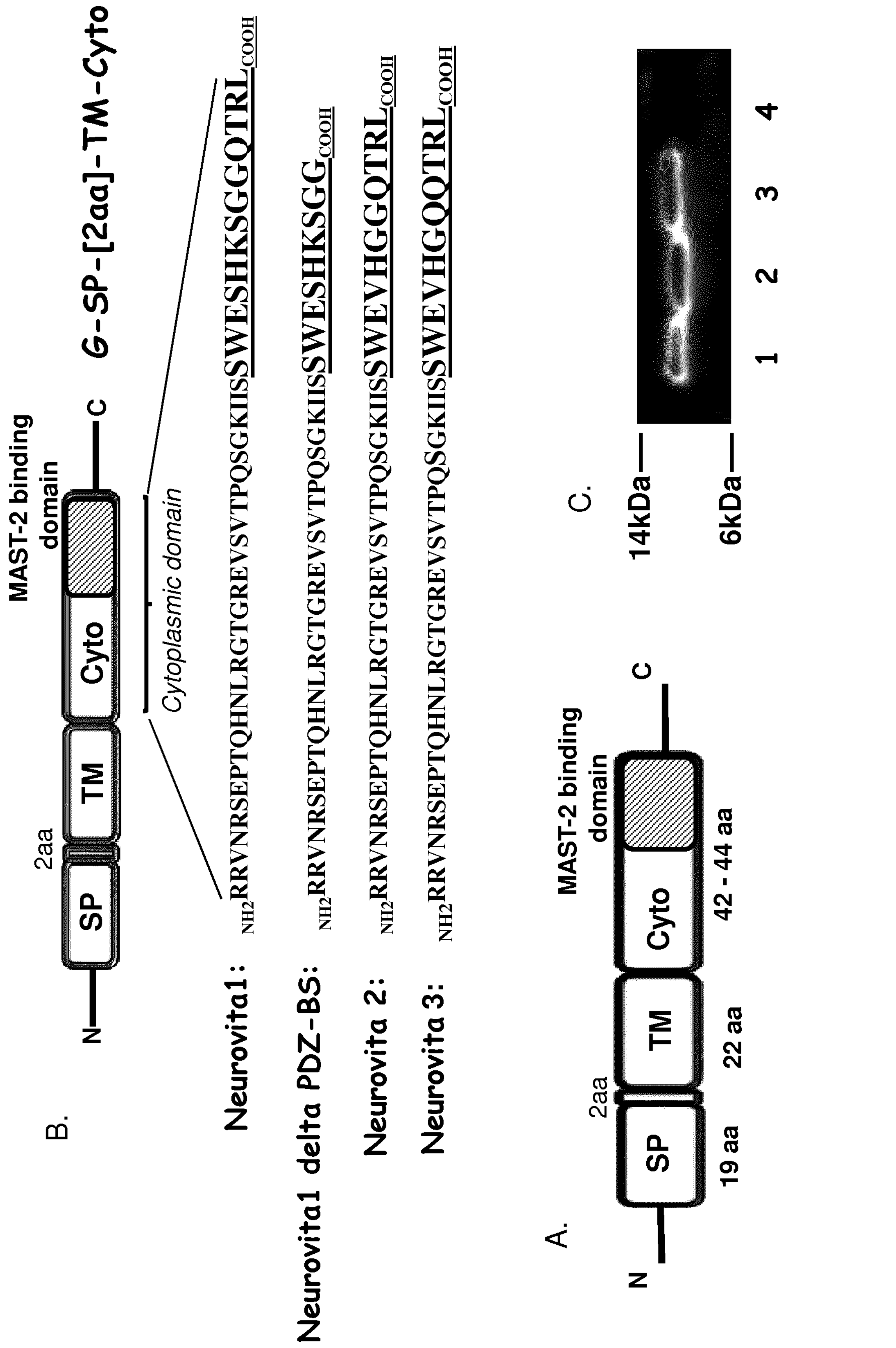

B.
N
SP
2aa
TM
Cyto
MAST-2 binding domain
C
G-SP-[2aa]-TM-Cyto
Cytoplasmic domain
Neurovita1: NH2 RRVNRSEPTQHNLRGTGREVSVTPQSGKIISSWESHKSGGQTRL COOH
Neurovita1 delta PDZ-BS: NH2 RRVNRSEPTQHNLRGTGREVSVTPQSGKIISSWESHKSGG COOH
Neurovita 2: NH2 RRVNRSEPTQHNLRGTGREVSVTPQSGKIISSWEVHGGQTRL COOH
Neurovita 3: NH2 RRVNRSEPTQHNLRGTGREVSVTPQSGKIISSWEVHGQQTRL COOH
A.
N
SP
19 aa
2aa
TM
22 aa
Cyto
42 - 44 aa
MAST-2 binding domain
C
C.
14kDa
6kDa
1
2
3
4

FIG. 11

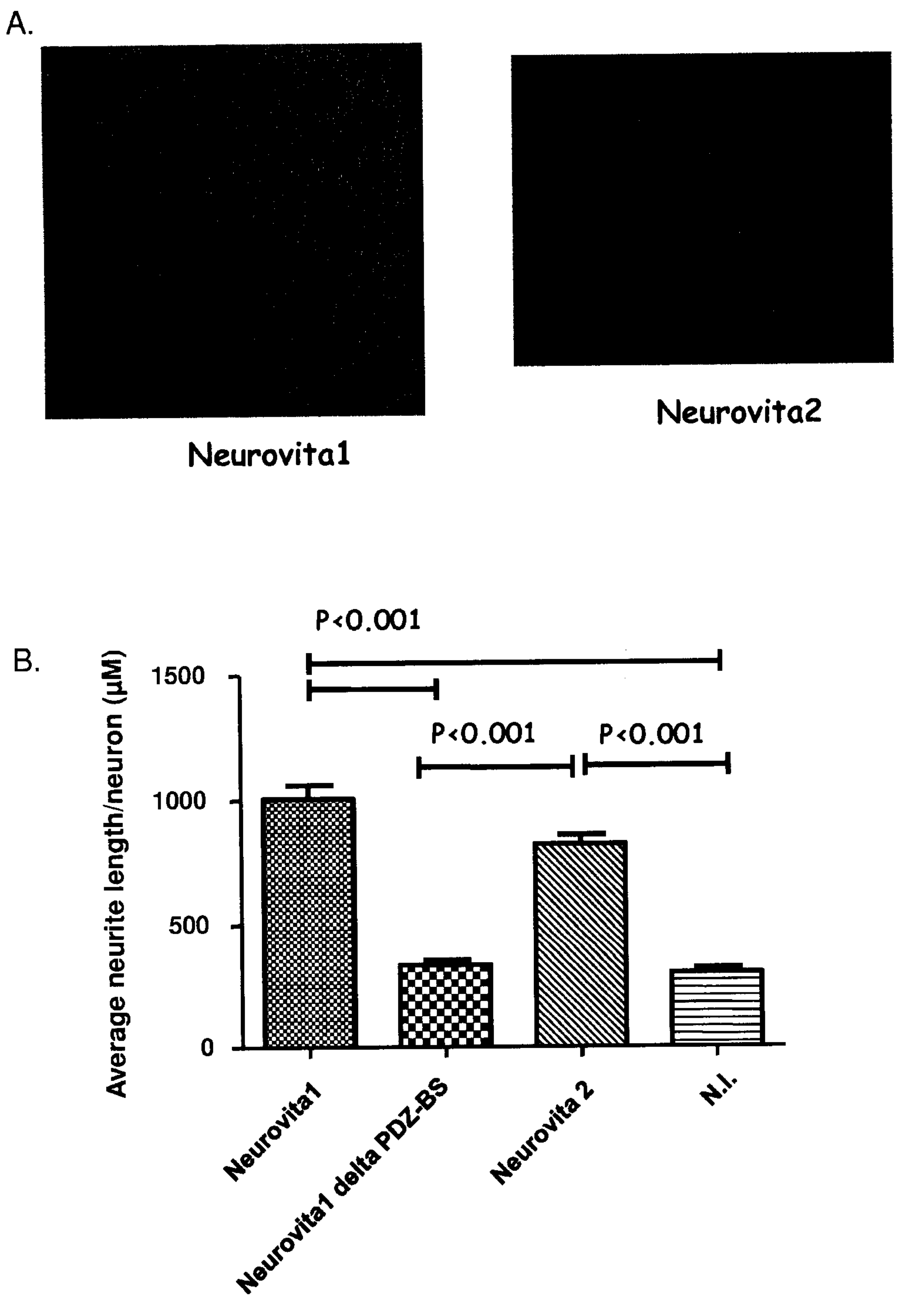

A.
Neurovita1
Neurovita2
B.
P<0.001
P<0.001
P<0.001
Average neurite length/neuron (µM)
1500
1000
500
0
Neurovita1
Neurovita1 delta PDZ-BS
Neurovita 2
N.I.

FIG. 12

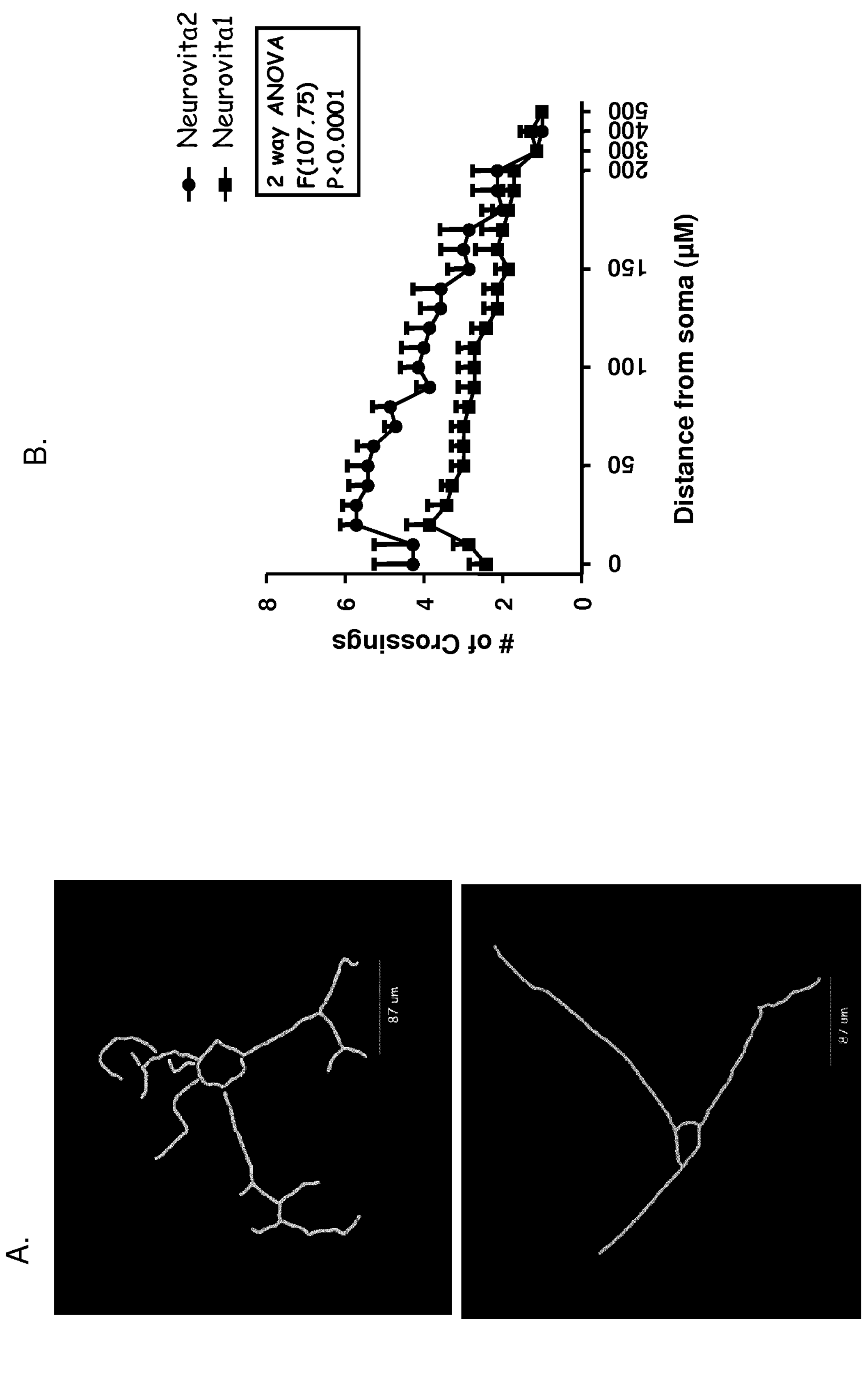
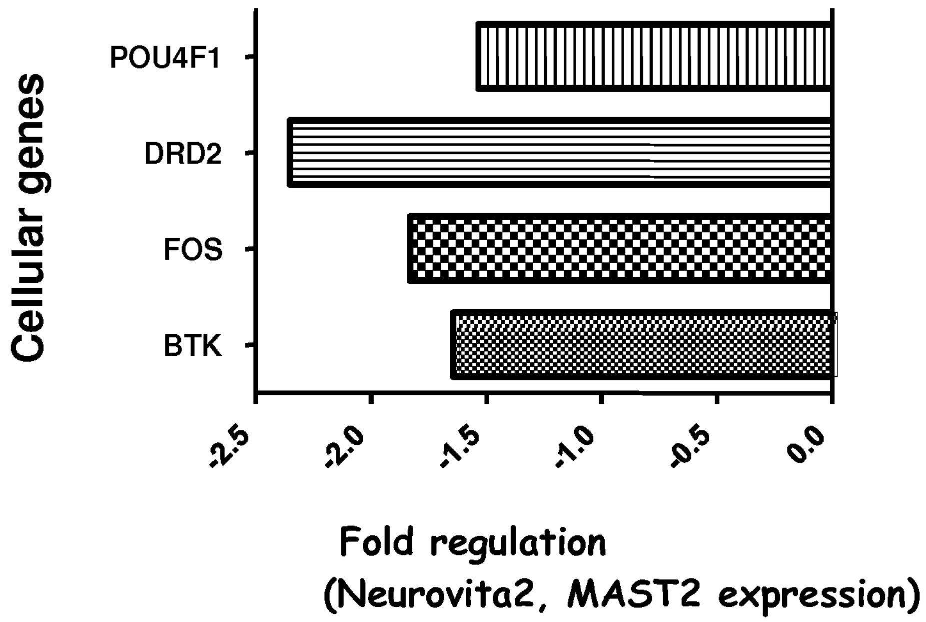

A.
87 um
87 um
72 h
B.
Neurovita2
Neurovita1
2 way ANOVA
F(107.75)
P<0.0001
# of Crossings
0
2
4
6
8
0
50
100
150
200
300
400
500
Distance from soma (μM)

FIG. 13

A.


Cellular genes
POU4F1
DRD2
FOS
BTK
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
Fold regulation
(Neurovita2, MAST2 expression)

B.

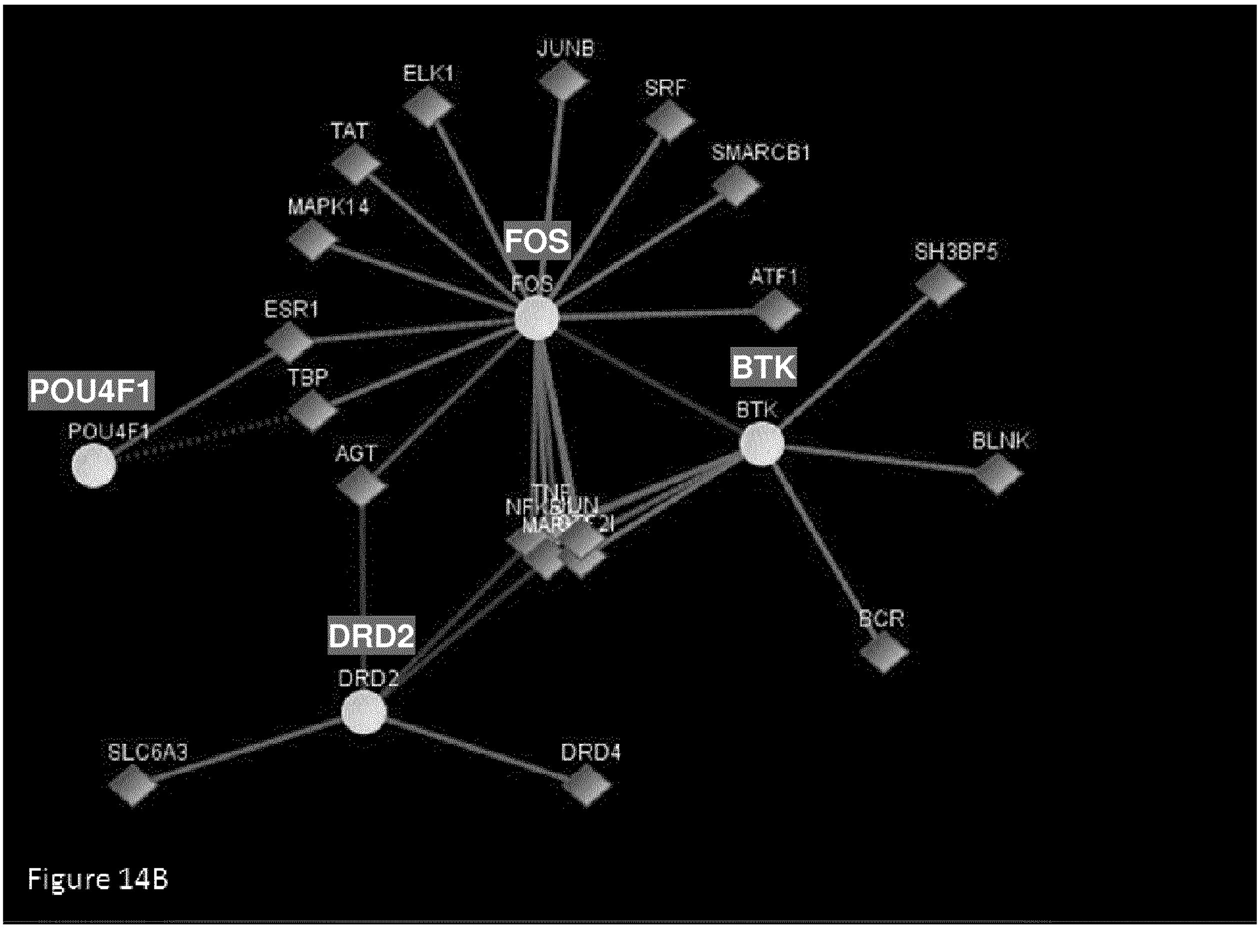

JUNB
ELK1
SRF
TAT
SMARCB1
MAPK14
FOS
SH3BP5
ATF1
ESR1
BTK
POU4F1
TBP
BLNK
AGT
BCR
DRD2
SLC6A3
DRD4
Figure 14B

FIG. 14

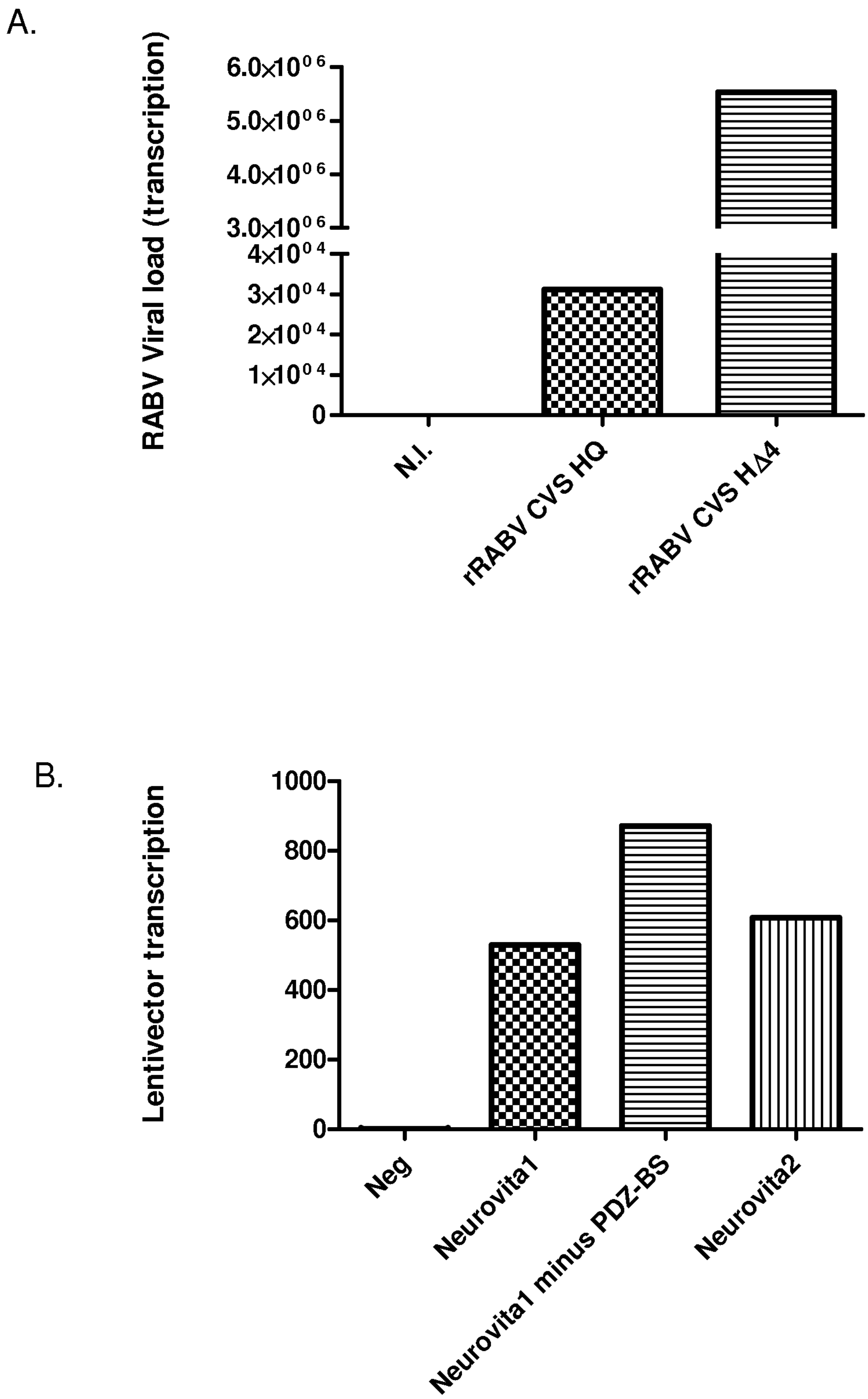
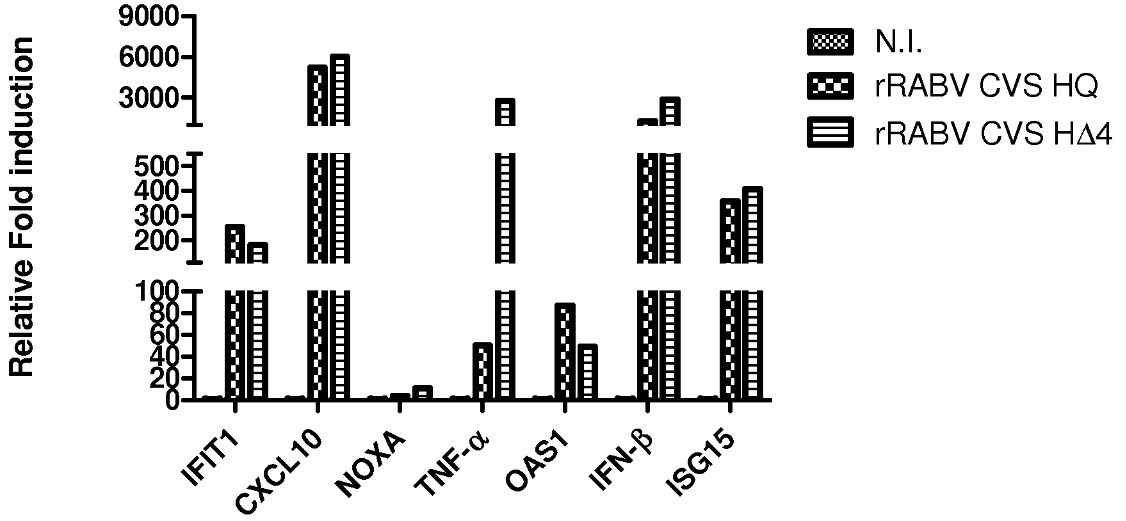

A.
RABV Viral load (transcription)
6.0×10⁰⁶
5.0×10⁰⁶
4.0×10⁰⁶
3.0×10⁰⁶
4×10⁰⁴
3×10⁰⁴
2×10⁰⁴
1×10⁰⁴
0
N.I.
rRABV CVS HQ
rRABV CVS HΔ4
B.
Lentivector transcription
1000
800
600
400
200
0
Neg
Neurovita1
Neurovita1 minus PDZ-BS
Neurovita2

FIG. 15

A.


9000
6000
3000
500
400
300
200
100
80
60
40
20
0
Relative Fold induction
N.I.
rRABV CVS HQ
rRABV CVS HΔ4
IFIT1
CXCL10
NOXA
TNF-α
OAS1
IFN-β
ISG15

B.

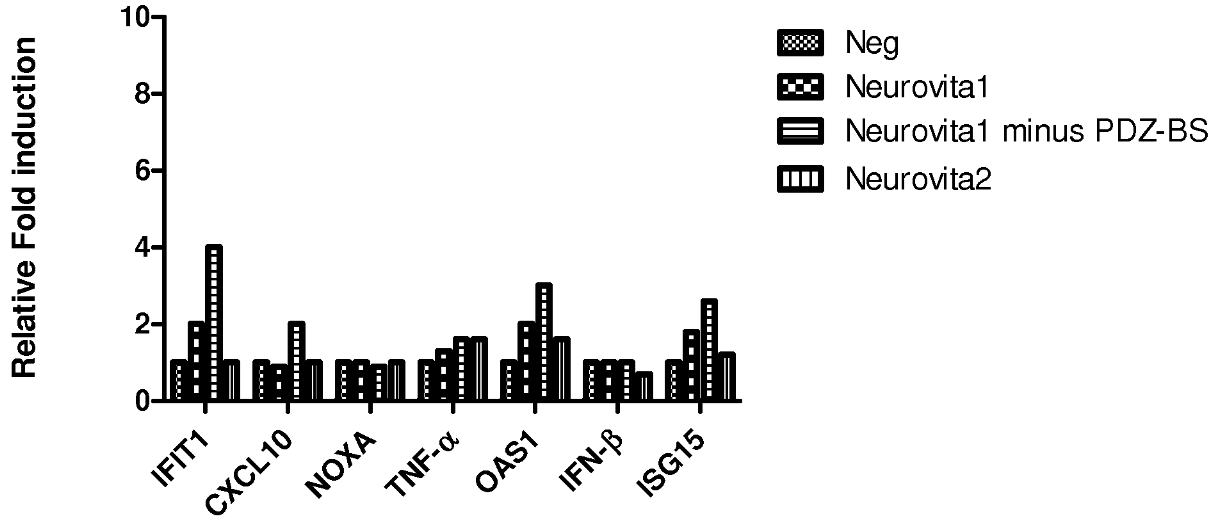

10
8
6
4
2
0
Relative Fold induction
Neg
Neurovita1
Neurovita1 minus PDZ-BS
Neurovita2
IFIT1
CXCL10
NOXA
TNF-α
OAS1
IFN-β
ISG15

FIG. 16

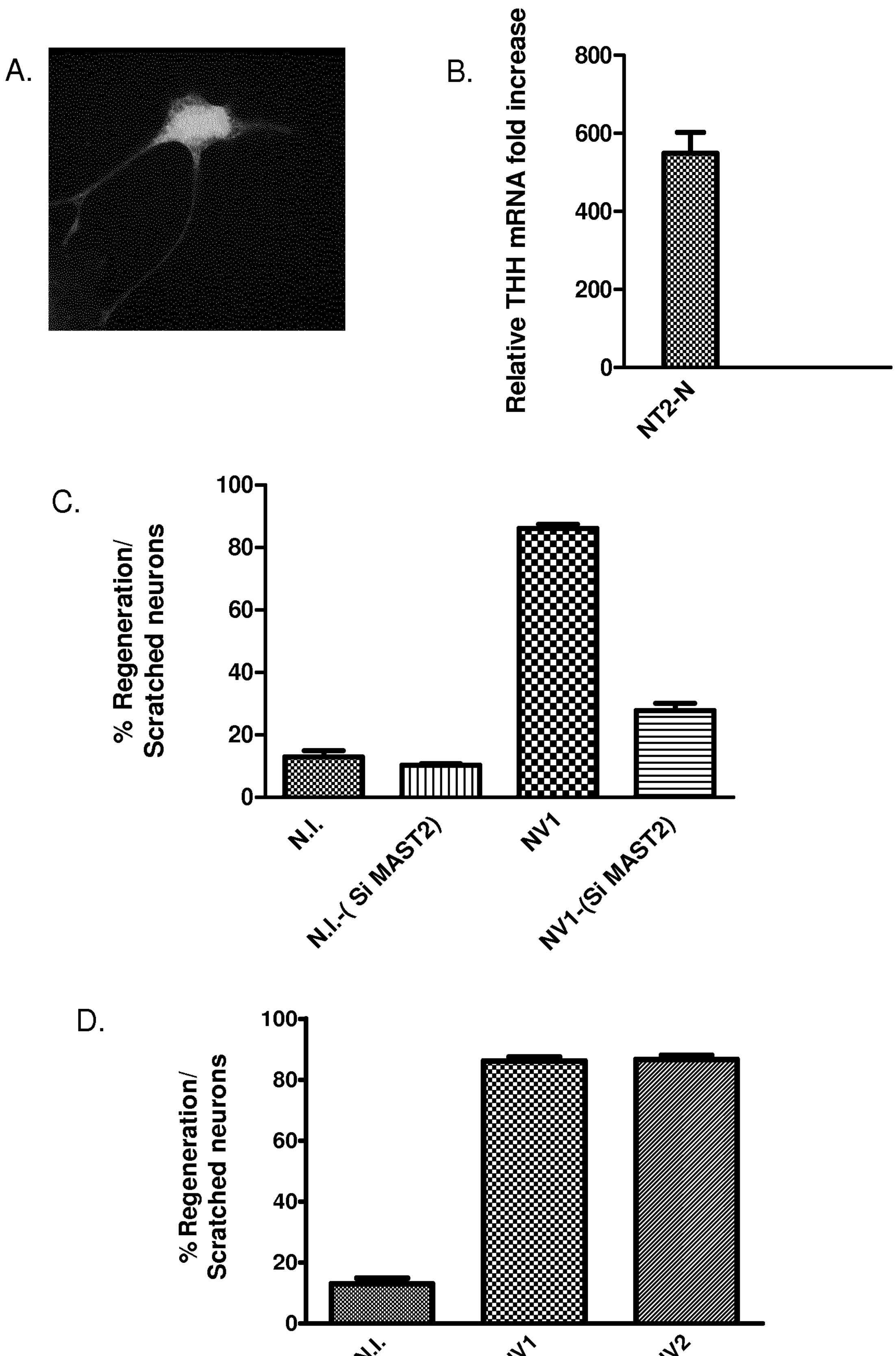

A.
B.
Relative THH mRNA fold increase
800
600
400
200
0
NT2-N
C.
% Regeneration/
Scratched neurons
100
80
60
40
20
0
N.I.
N.I.-(Si MAST2)
NV1
NV1-(Si MAST2)
D.
% Regeneration/
Scratched neurons
100
80
60
40
20
0
N.I.
NV1
NV2

FIG. 17

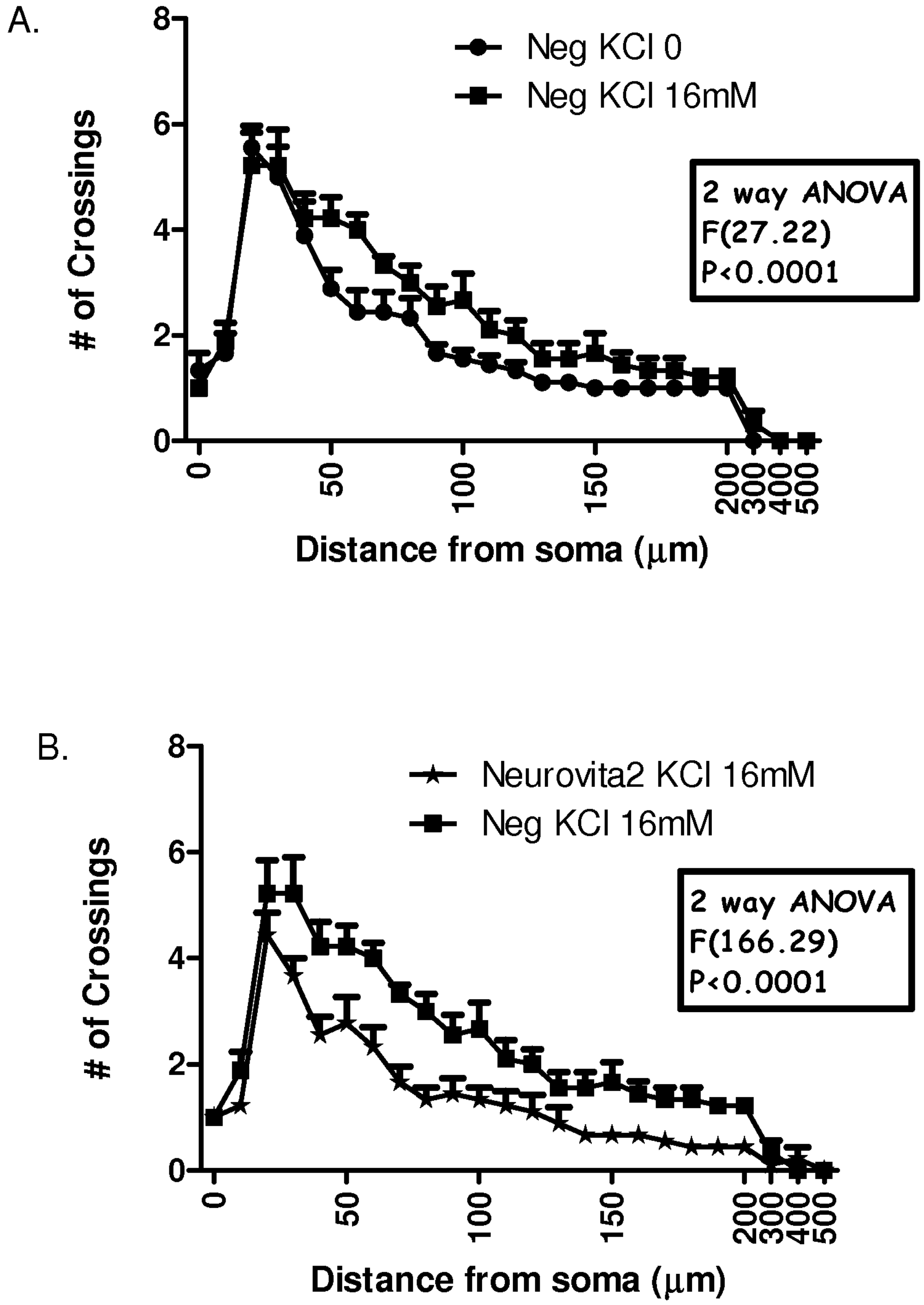

A.
8
6
4
2
0
# of Crossings
Neg KCl 0
Neg KCl 16mM
2 way ANOVA
F(27.22)
P<0.0001
0
50
100
150
200
300
400
500
Distance from soma (μm)
B.
8
6
4
2
0
# of Crossings
Neurovita2 KCl 16mM
Neg KCl 16mM
2 way ANOVA
F(166.29)
P<0.0001
0
50
100
150
200
300
400
500
Distance from soma (μm)

FIG. 18

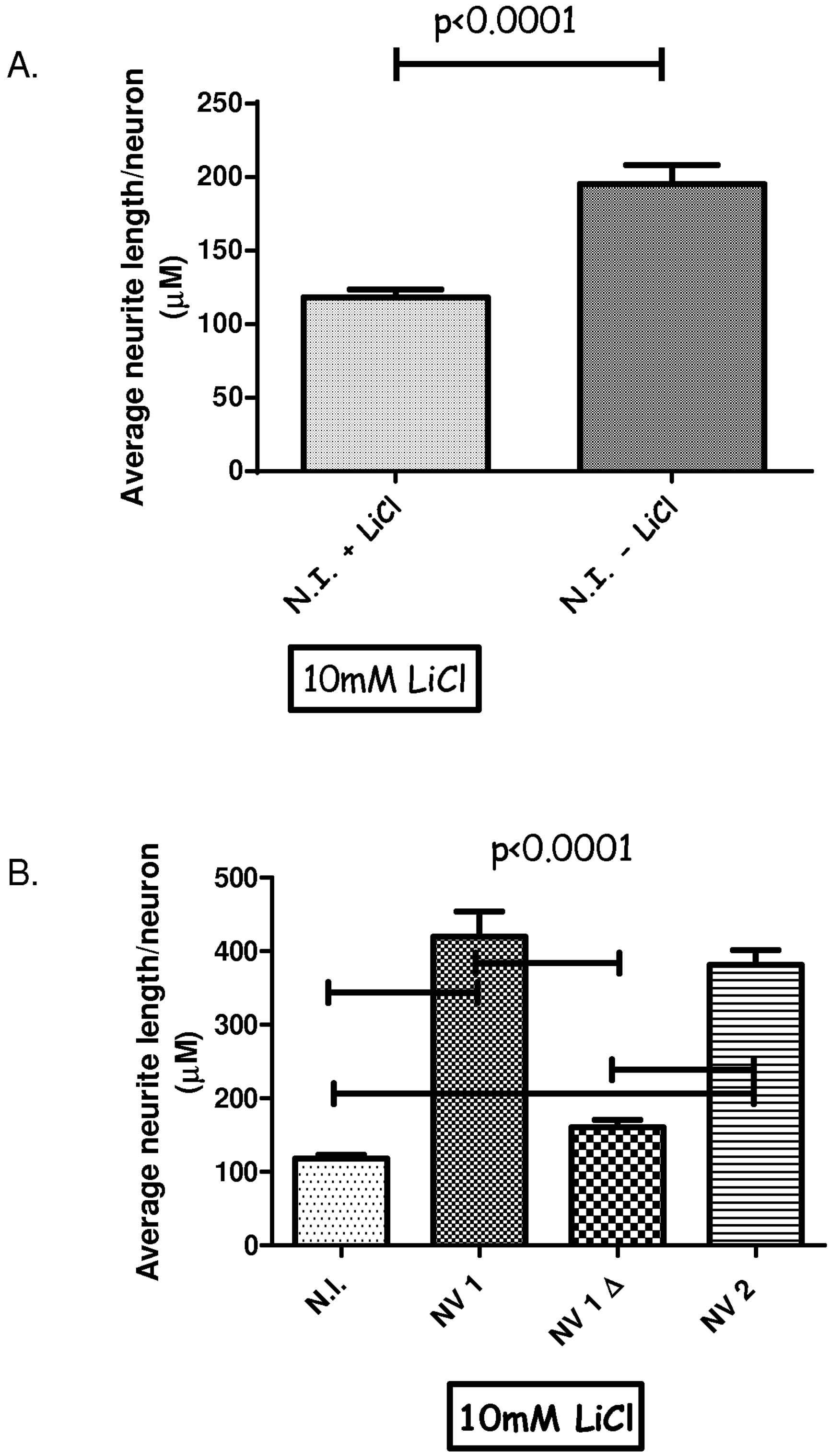

A.
p<0.0001
Average neurite length/neuron (μM)
250
200
150
100
50
0
N.I. + LiCl
N.I. - LiCl
10mM LiCl
B.
p<0.0001
Average neurite length/neuron (μM)
500
400
300
200
100
0
N.I.
NV 1
NV 1 Δ
NV 2
10mM LiCl

FIG. 19

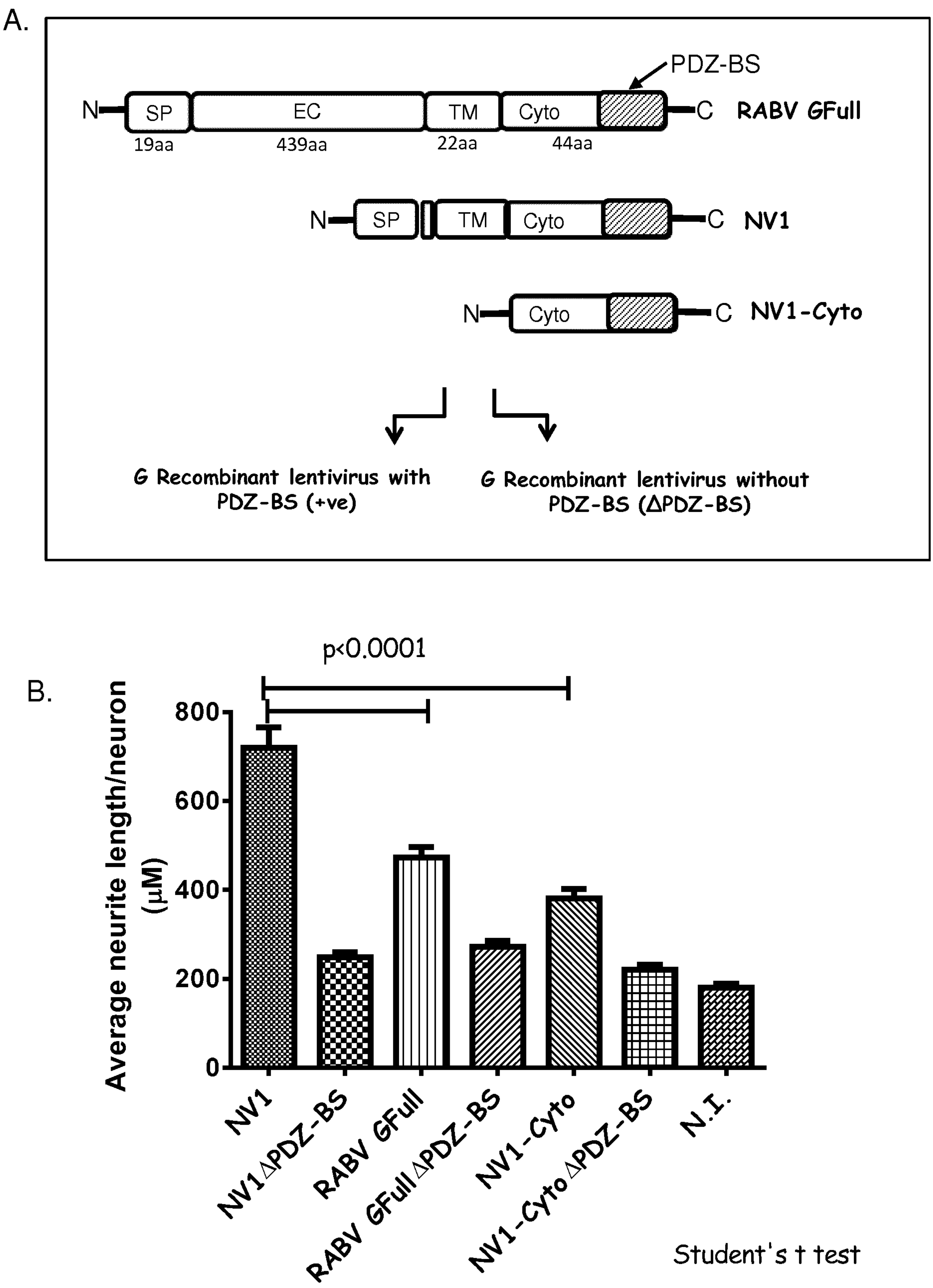
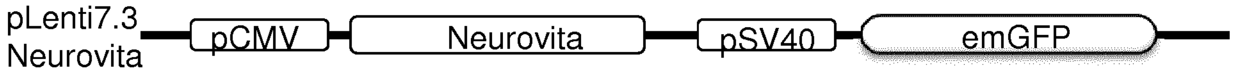
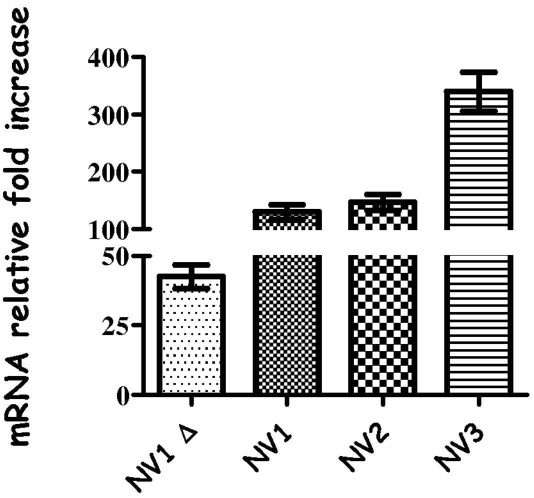

A.
PDZ-BS
N SP EC TM Cyto C RABV GFull
19aa 439aa 22aa 44aa
N SP TM Cyto C NV1
N Cyto C NV1-Cyto
G Recombinant lentivirus with PDZ-BS (+ve)
G Recombinant lentivirus without PDZ-BS (ΔPDZ-BS)
B.
p<0.0001
Average neurite length/neuron (μM)
800
600
400
200
0
NV1
NV1ΔPDZ-BS
RABV GFull
RABV GFullΔPDZ-BS
NV1-Cyto
NV1-CytoΔPDZ-BS
N.I.
Student's t test

FIG. 20

A.



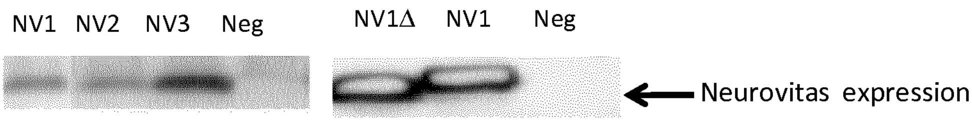
B.



C.

	% of GFP expression
Neg	0
NV1Δ	15,56
NV1	48,98
NV2	33,66
NV3	59,7

D.



E.

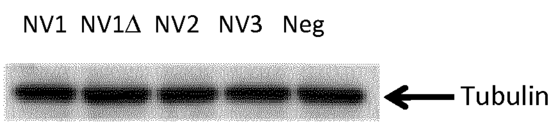


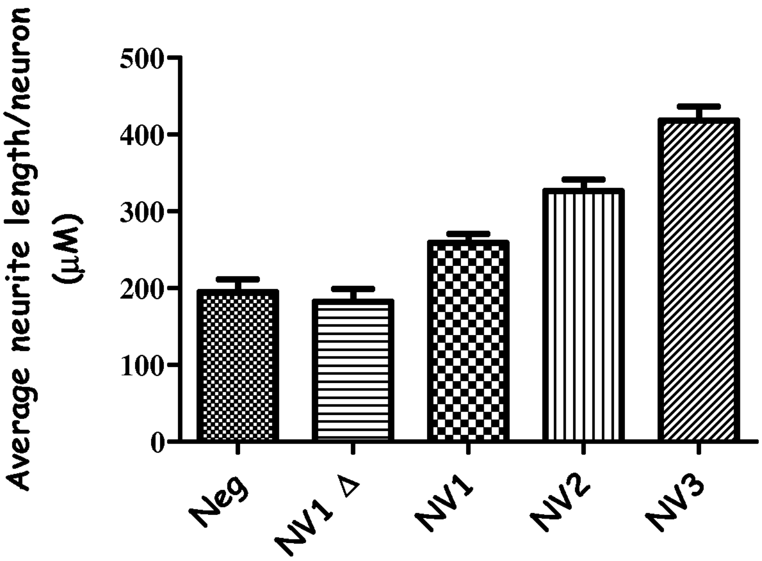
FIG. 21

A.

Non infected NS cells (Neg)

NV3 infected NS cells

B.

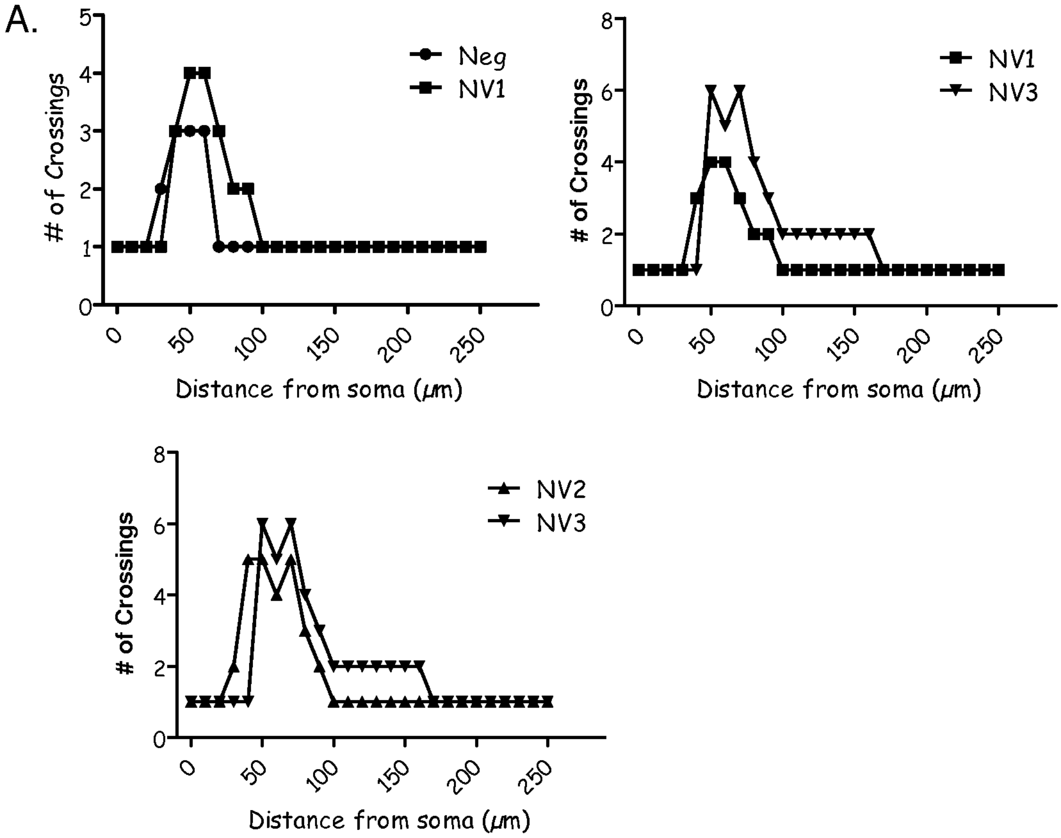


C.

Neg					
NV1Δ	0,6076				
NV1	0,047	0,0010			
NV2	< 0,0001	< 0,0001	0,0010		
NV3	< 0,0001	< 0,0001	< 0,0001	0,0003	
	Neg	NV1Δ	NV1	NV2	NV3

FIG. 22

A.

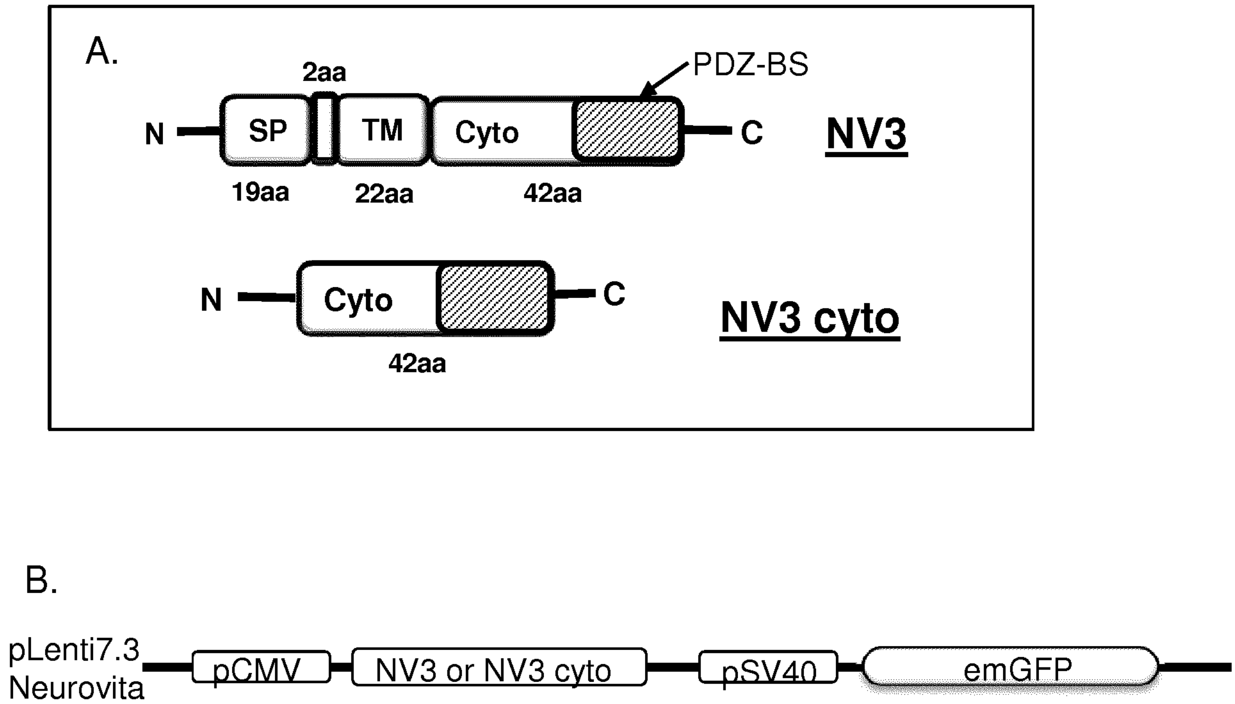


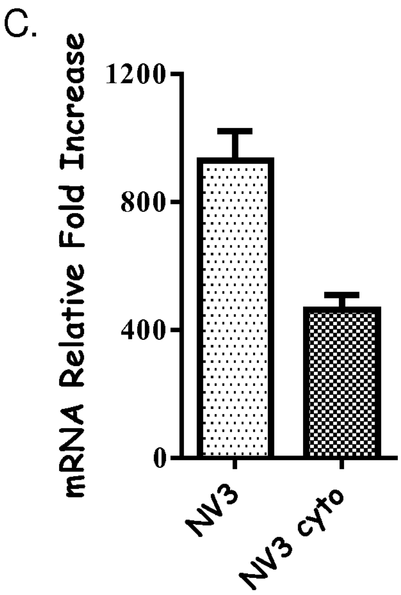
B.

	Neg	NV1
NV2	0,0178	0,0053

	Neg	NV1	NV2
NV3	< 0,0001	< 0,0001	0,0007

FIG. 23



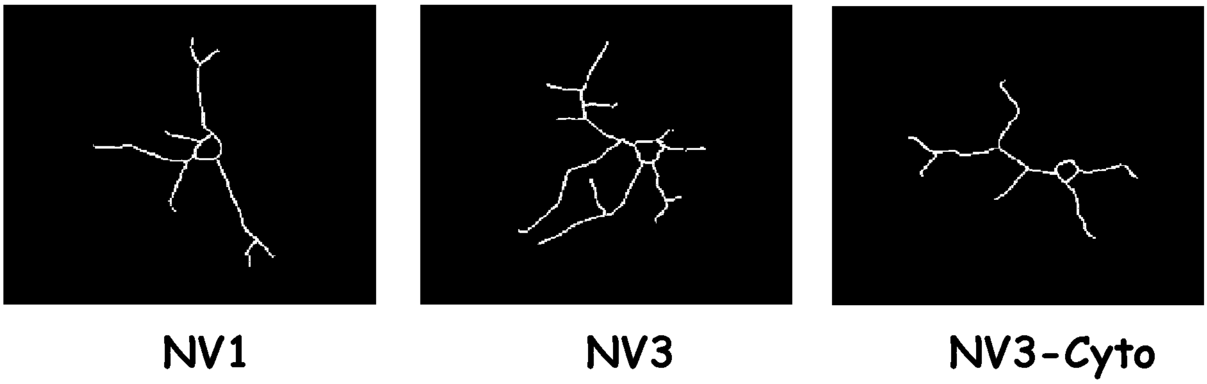


D.

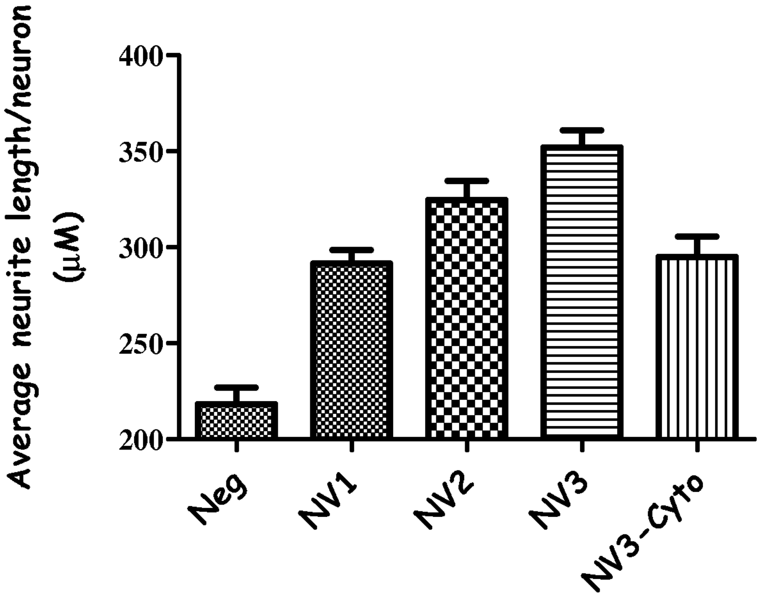
	Neg	NV3	NV3 cyto
% of GFP expression	0	60.41	36.71

FIG. 24

A.



B.

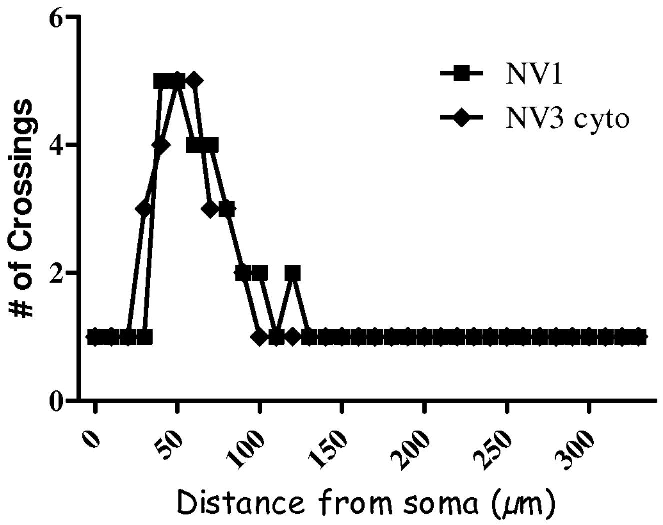


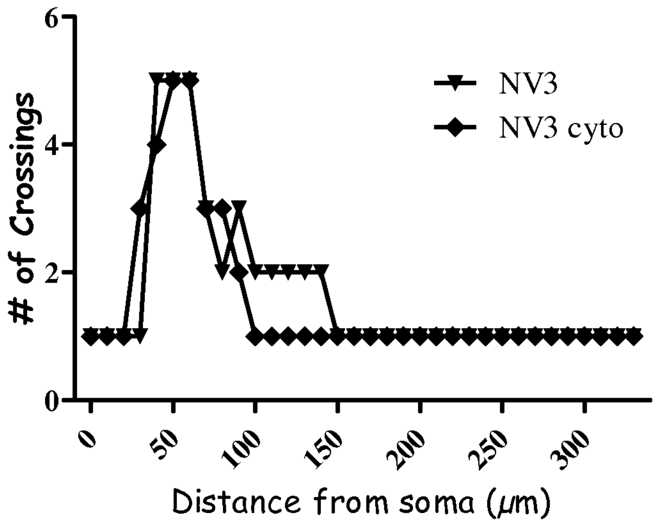
C.

	Neg	NV1	NV2	NV3
NV3 cyto	< 0,0001	0,7784	0,0457	< 0,0001

FIG. 25

A.





B.

	Neg	NV1	NV2	NV3
NV3 cyto	< 0,0001	0,088	0,006	< 0,0001

FIG. 26

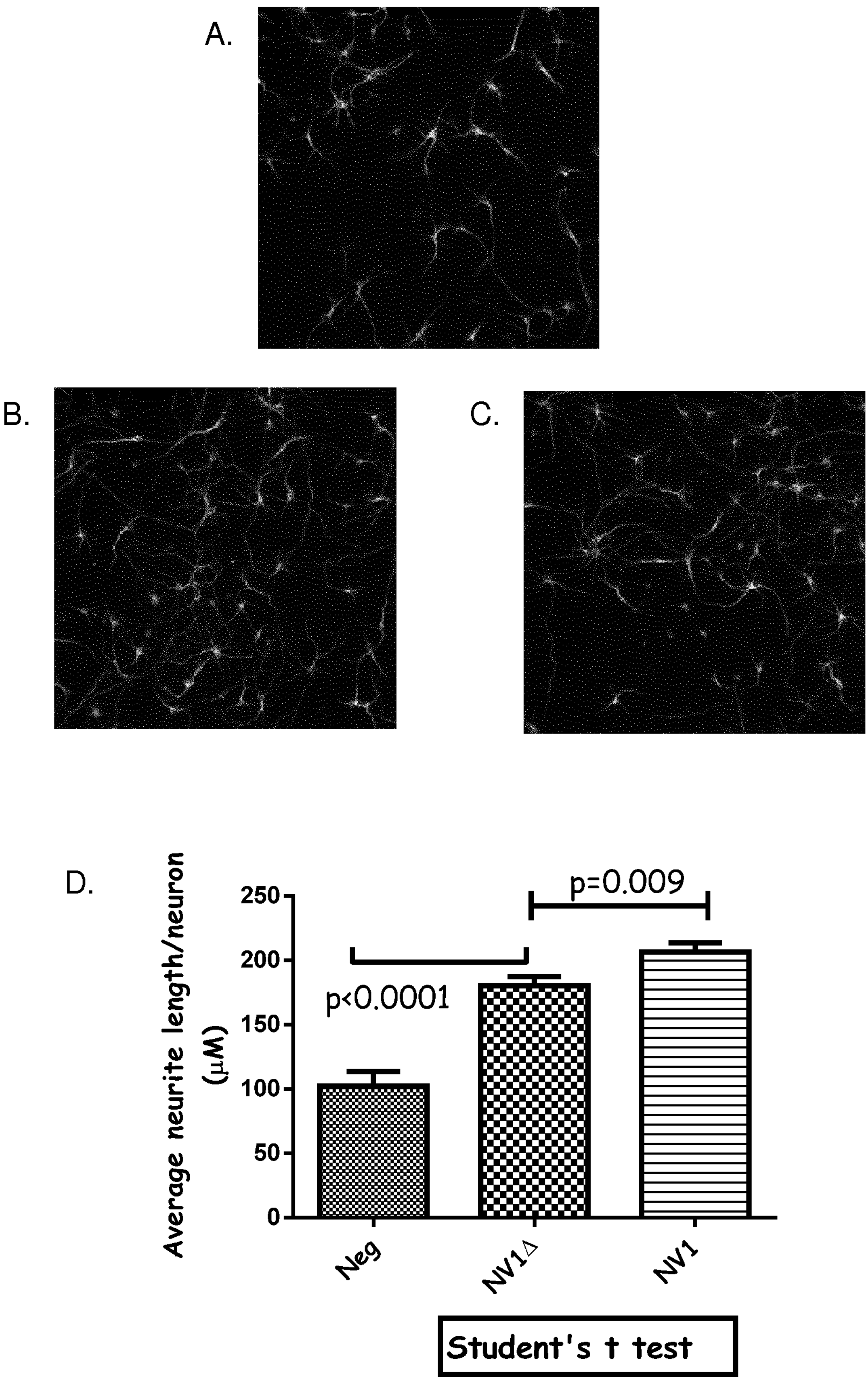

A.
B.
C.
D.
Average neurite length/neuron (μM)
250
200
150
100
50
0
p=0.009
p<0.0001
Neg
NV1Δ
NV1
Student's t test

FIG. 27

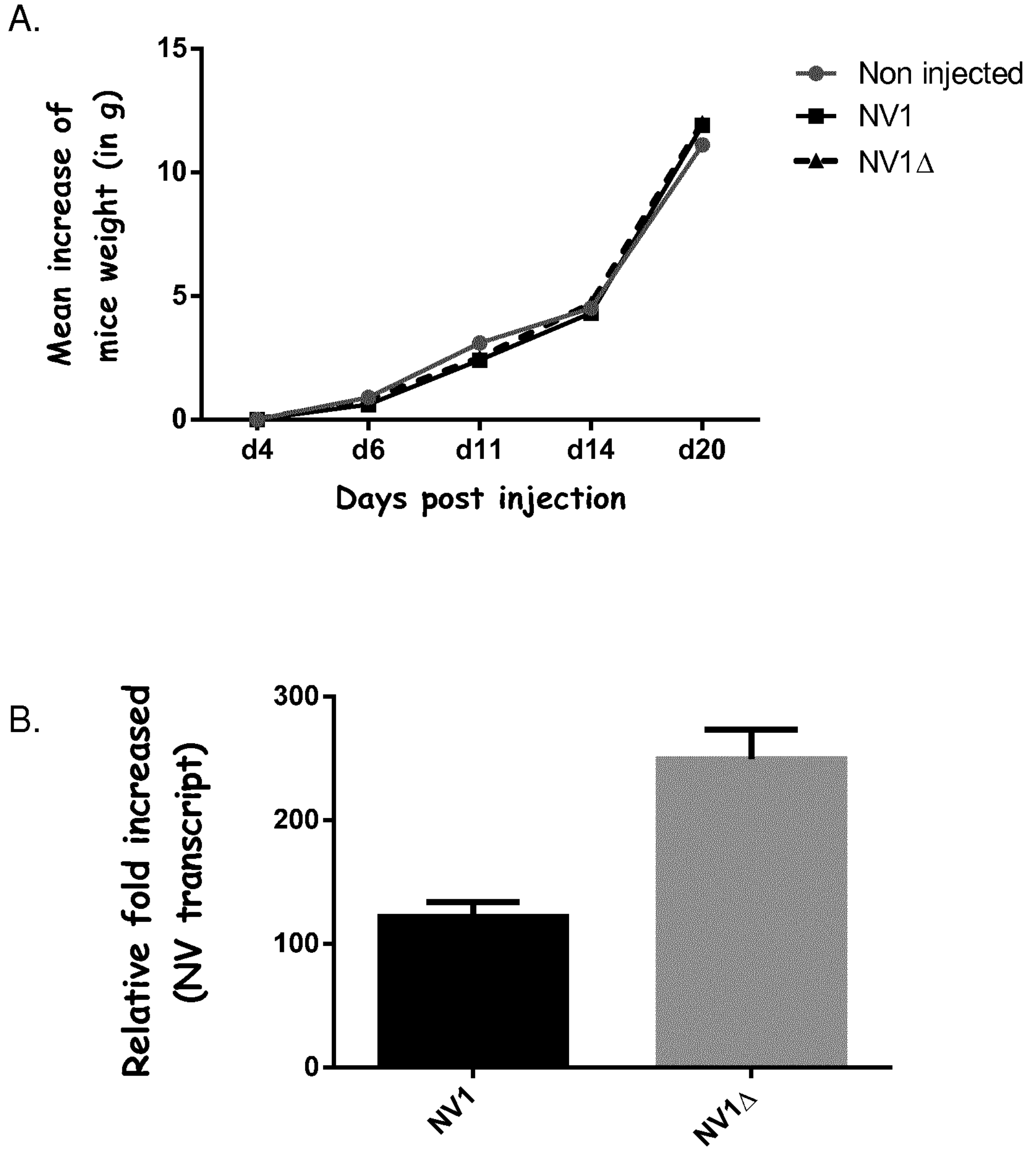

A.
15
10
5
0
Mean increase of mice weight (in g)
d4
d6
d11
d14
d20
Days post injection
Non injected
NV1
NV1Δ
B.
300
200
100
0
Relative fold increased (NV transcript)
NV1
NV1Δ

FIG. 28

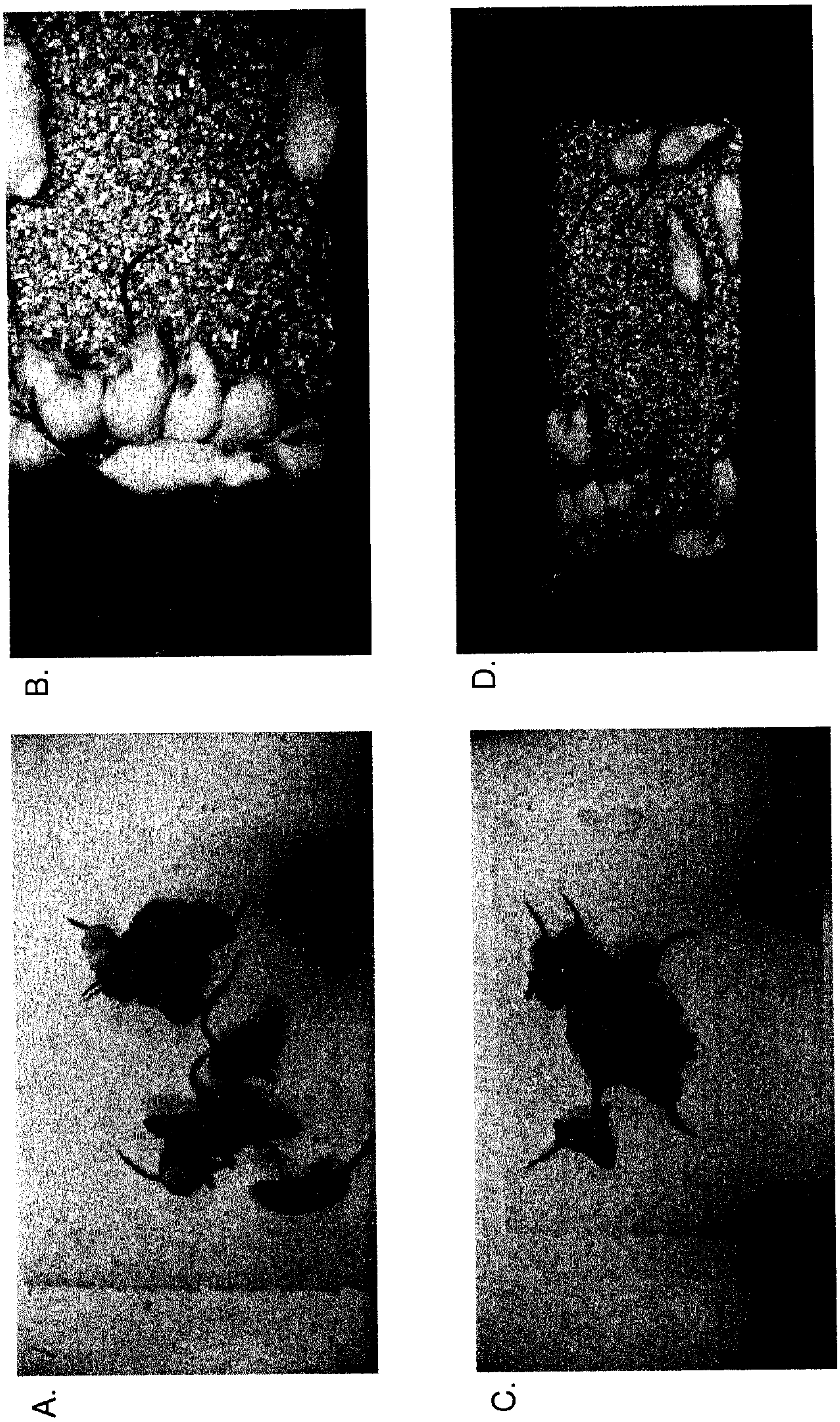


FIG. 29

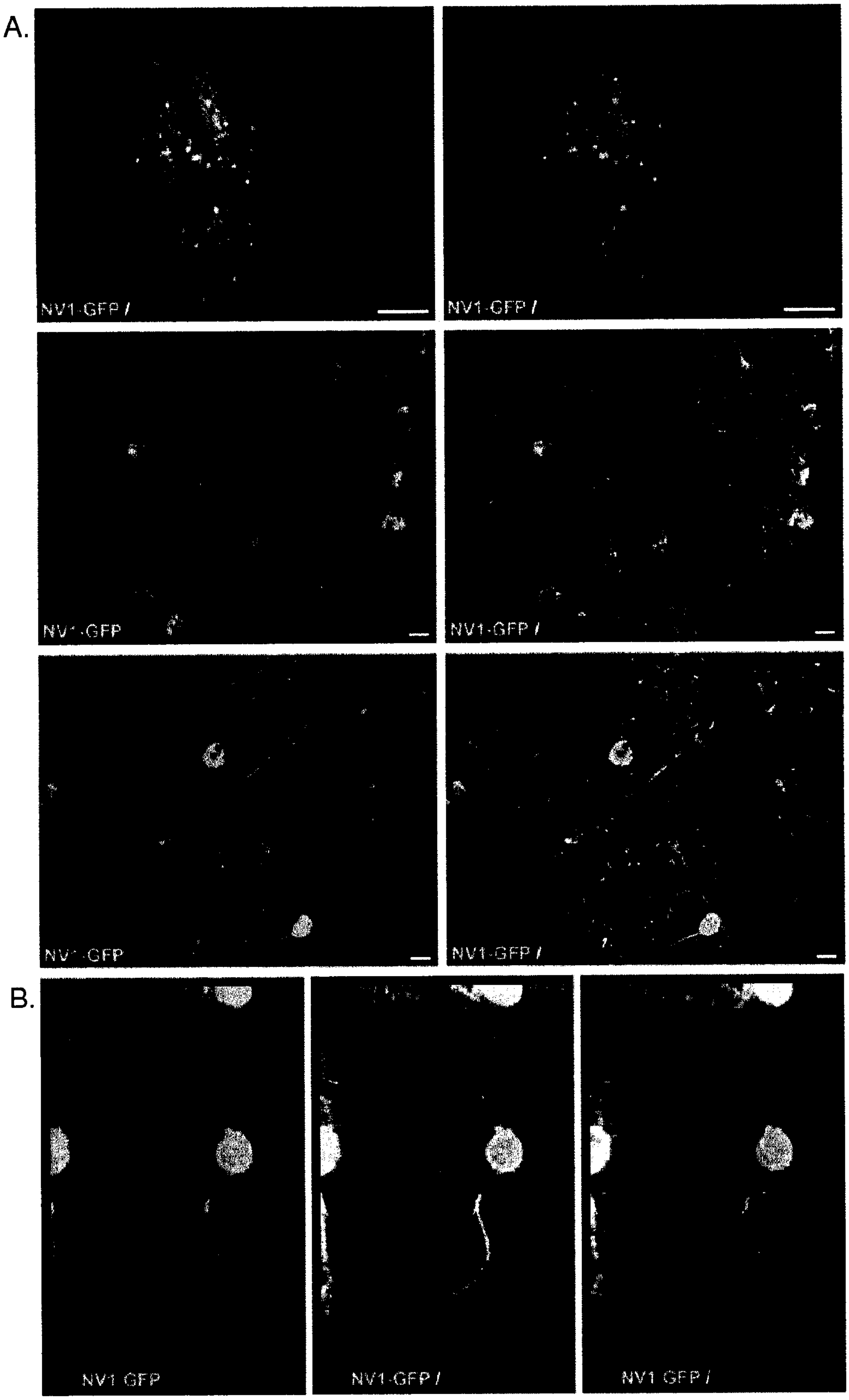

A.
NV1-GFP /
NV1-GFP /
NV1-GFP
NV1-GFP /
NV1-GFP
NV1-GFP /
B.
NV1 GFP
NV1-GFP /
NV1 GFP /

FIG. 30

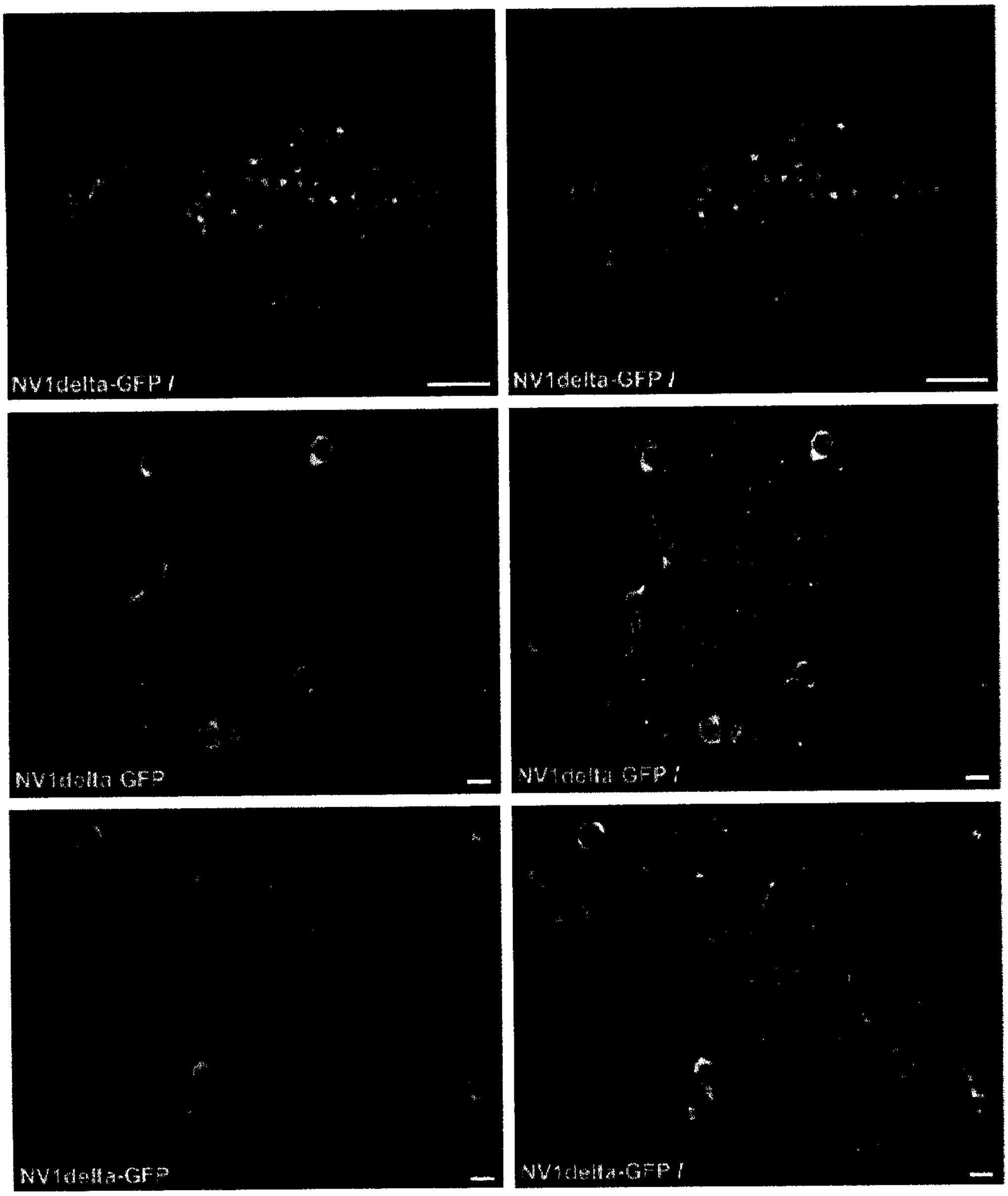

NV1delta-GFP /
NV1delta-GFP /
NV1delta GFP
NV1delta GFP /
NV1delta-GFP
NV1delta-GFP /

FIG. 31