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(54) Title: METHODS AND COMPOSITIONS FOR TREATING BRAIN INJURY OR NEURODEGENERATIVE DISEASE

(57) Abstract: The present invention relates to a method for treating brain injury or neurodegenerative disease in a subject in need thereof comprising a step of administering said subject with a therapeutically effective amount of a syndecan-1 agonist. Inventors have shown a different expression pattern of Syndecan-1 between quiescent and activated neural stem cells (NSCs) and demonstrated its role in the proliferation of activated NSCs. They have shown that syndecan-1 is a marker of proliferative NSCs. Their data highlight the central role of the stem cell microenvironment in the regulation of quiescence in adult neurogenic niches. Finally, inventors unravel the role of Syndecan-1 (Sdc1) in the proliferation of activated NSCs. Interestingly, Sdc1 transcripts, highly enriched in proliferative cells, significantly decreased after BMP4 treatment. To confirm the role of SDC1 in the proliferation of activated NSCs, they performed silencing experiments using siRNA directed against Sdc1. Accordingly, inventors have found new approaches for NSC-based regenerative medicine.



METHODS AND COMPOSITIONS FOR TREATING BRAIN INJURY OR NEURODEGENERATIVE DISEASE

FIELD OF THE INVENTION:

5 The invention is in the field of neurology. More particularly, the present invention relates to methods and compositions for treating brain injury or neurodegenerative disease.

BACKGROUND OF THE INVENTION:

Adult stem cells reside within specialized microenvironments that integrate intricate signals critical for maintaining stem cell populations in an undifferentiated state, guiding cell fate decisions and modulating the regenerative potential of the niche (Papanikolaou et al., 2008). In the adult mammalian brain, neural stem cells (NSCs) continuously generate neurons throughout life in two discrete regions: the subventricular zone (SVZ) along the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus. NSCs from the adult SVZ successively give rise to transit-amplifying cells and neuroblasts that differentiate into neurons once they have reached the olfactory bulbs (Lim and Alvarez-Buylla, 2014). A key feature of NSCs is their remarkable proliferative capacity that sustains regeneration of damaged tissue through the activation of quiescent stem cells (Codega et al., 2014; Daynac et al., 2013; Doetsch et al., 1999; Llorens-Bobadilla et al., 2015; Mich et al., 2014; Morshead et al., 1994).

20 In contrast to their progeny, most adult NSCs are quiescent and a tight regulation of the balance between their quiescent and proliferative states appears essential for their long-term maintenance in neurogenic niches (Fuentealba et al., 2015; Furutachi et al., 2015). Indeed, dysregulation and/or loss of quiescence often results in premature proliferation of NSCs ultimately leading to the depletion of neural stem and progenitor cells (Kippin et al., 2005; Mira et al., 2010; Molofsky et al., 2003; Ottone et al., 2014). Deciphering the functional properties of quiescent NSCs and the associated regulatory mechanisms is thus important to develop new approaches for NSC-based regenerative medicine.

SUMMARY OF THE INVENTION:

30 The invention relates to a method for treating brain injury, aging-associated cognitive decline or neurodegenerative disease in a subject in need thereof comprising a step of administering said subject with a therapeutically effective amount of an agonist of syndecan-1. In particular, the invention is defined by claims.

DETAILED DESCRIPTION OF THE INVENTION:

Deciphering the mechanisms that regulate the quiescence of adult neural stem cells (NSCs) is crucial for the development of therapeutic strategies based on the stimulation of their endogenous regenerative potential in the damaged brain. Inventors have shown that LeXbright cells sorted from the adult mouse subventricular zone exhibit all the characteristic features of quiescent NSCs. Indeed, they constitute a subpopulation of slowly dividing cells that is able to enter the cell cycle to regenerate the irradiated niche. Comparative transcriptomic analyses showed that they express hallmarks of NSCs but display a distinct molecular signature from activated NSCs (LeX+EGFR+ cells). Particularly, numerous membrane receptors are expressed on quiescent NSCs. They further revealed a different expression pattern of Syndecan-1 between quiescent and activated NSCs and demonstrated its role in the proliferation of activated NSCs. Their data highlight the central role of the stem cell microenvironment in the regulation of quiescence in adult neurogenic niches. Studying the regulation of adult NSC proliferation in relation to the expression of SDC1 could thus provide insight into adult NSC behavior and its modulation in health and disease. Finally, inventors unravel the role of Syndecan-1 (Sdc1) in the proliferation of activated NSCs. Interestingly, Sdc1 transcripts, highly enriched in proliferative cells, significantly decreased after BMP4 treatment. To confirm the role of SDC1 in the proliferation of activated NSCs, they performed silencing experiments using siRNA directed against Sdc1. They verified the efficacy of Sdc1 silencing in neurosphere cultures both at the mRNA and protein levels 24 and 48h after electroporation. Interestingly, a decrease in the diameter of neurospheres was observed after Sdc1 silencing in comparison to a scrambled control siRNA. Subsequently, Sdc1 silencing was performed in LeX+EGFR+ cells freshly sorted from adult mice. While their clonogenic capacity was not altered, the total number of cells was reduced at day 7 suggesting a role of Sdc1 in the proliferation but not in the activation of activated NSCs. They further monitored the time required for the first cell division of LeX+EGFR+ cells after Sdc1 silencing by time-lapse videomicroscopy and found it significantly delayed by 7.1 hours. Altogether, their data hint at a role of Sdc1 in the progression of activated NSCs through the cell cycle.

Accordingly, in a first aspect, the invention relates to a method for quantifying neurogenic activity in a subject comprising following steps: i) measuring the expression level of syndecan-1 in said subject; ii) comparing the expression level measured at step i) with its predetermined reference value, and iii) concluding that neurogenic activity is stimulated in the subject when the expression level of syndecan-1 is higher than its predetermined reference

value or concluding that the neurogenic activity is not stimulated when the expression level of syndecan-1 is lower than its predetermined reference value.

In a particular embodiment, the method according to the invention is suitable for diagnosing brain injury, aging-associated cognitive decline, psychiatric disorders or neurodegenerative disease in a subject.

In another embodiment, the method according to the invention is suitable for monitoring brain injury, psychiatric disorders, aging-associated cognitive decline or neurodegenerative disease in a subject.

In a particular embodiment, the method according to the invention is suitable for monitoring the stimulation of neurogenesis or oligodendrogenesis after a therapeutic approach aiming at stimulating neurogenesis or oligodendrogenesis.

In another embodiment, the method according to the invention is suitable for a quantitative evaluation of alterations in proliferative activity in the neurogenic zones of the brain.

As used herein, the term “neurogenic activity” refers to the proliferation of neural stem progenitor cells in neurogenic niches leading to the generation of neural cells ie. neurons, oligodendrocytes and astrocytes.

In vitro methods for quantifying neurogenic activity and/or neuronal proliferation are well known in the art. Such in vitro methods for quantifying neurogenic activity are based on immunodetection of proliferating markers such as Ki67 and neural markers such as GFAP, Doublecortin etc... on histological slices.

In vivo methods (e.g. in animal models and in post mortem tissues) for quantifying neurogenic activity are well known in the art. Typically, these methods are based on incorporation of a tracer (tritiated thymidine, BrdU,...) in proliferating cells and detection of this tracer in histological slices or cell cytometry using different markers such as CD15, EGFR, Ki67 and CD24.

In another embodiment, the neurogenic activity is measured by Positron emission tomography (PET)–scan, an imaging test that uses radioactive tracers to depict the spatial distribution of metabolic or biochemical activity in the body.

In a particular embodiment, the syndecan-1 is lower than a predetermined reference value, typically after brain injury, after cancer treatment by radiotherapy and/or chemotherapy, in psychiatric disorders or neurodegenerative diseases.

In another embodiment, the neurogenic activity is higher than a predetermined reference value in some diseases such as epilepsy.

As used herein term "diagnosing" refers to classifying a disease or a symptom, determining a severity of the disease, monitoring disease progression, forecasting an outcome of a disease and/or prospects of recovery. The method according to the invention is non-invasive.

5 As used herein, the term "monitoring" refers to evaluating changes in neurogenic activity. In the context of the invention, "monitoring neurogenic activity" refers to evaluating the neurogenic activity in a subject suffering from brain injury, psychiatric disorders or neurodegenerative disease.

10 As used herein, the term "brain injury" refers to traumatic brain injury or other forms of acquired brain injuries, including without limitation: hypoxic/ischemic brain injury in the adult, perinatal hypoxia/ischemia, stroke or induced by cancer treatments including radiotherapy and/or chemotherapy. In a particular embodiment, the traumatic brain injury is a result of an acute brain injury event. In a particular embodiment, the traumatic brain injury is a result of physical brain injury event. In a particular embodiment, the traumatic brain injury is a result of brain injury caused by stroke or hypoxia. In another embodiment the traumatic brain injury is mild traumatic brain injury. In a particular embodiment, the traumatic brain injury is medium traumatic brain injury. In a particular embodiment, the traumatic brain injury is severe traumatic brain injury. In a particular embodiment, the brain injury is induced by cancer treatments including radiotherapy and/or chemotherapy.

20 As used herein, the term "psychiatric disorders" refers to a panel of behavioral or psychological symptoms that impact multiple areas of life. Typically, the psychiatric disorder is selected from the following group, but is not limited to schizophrenia, schizoaffective disorder, bipolar disorder (mania and/or depression), depression, major depression, psychotic episodes, autism, autism spectrum disorder, fragile X syndrome, and pervasive developmental disorder.

25 As used herein, the term "aging-associated cognitive decline" refers to symptoms that impact multiple areas of life. Typically, the cognitive decline is associated with a neurogenesis decline with aging and might be alleviated if neurogenesis is stimulated.

30 As used herein, the term "neurodegenerative disease" refers to a panel of diseases generated by progressive loss of structure or function of neurons. In a particular embodiment, the neurodegenerative disease is selected from the group consisting of Parkinson disease, Alzheimer disease, Huntington disease, and multiple sclerosis and amyotrophic lateral.

As used herein, the term "subject" refers to a mammal, such as a rodent, a feline, a canine, and a primate. Particularly, the subject according to the invention is a human. More

particularly, the subject according to the invention has or is susceptible to have brain injury, psychiatric disorders, aging-associated cognitive decline or neurodegenerative disease as described above.

As used herein, the term “syndecan-1” refers to a protein which in humans is encoded
5 by the SDC1 gene. This protein belongs to a family of transmembrane heparan sulfate proteoglycans. Syndecan-1 have many roles in cell-matrix interactions and mediates cell binding, cell signaling, and cytoskeletal organization.

As used herein, the term “expression of syndecan-1” refers to the profile of syndecan-1 at gene, mRNA or protein level. Methods for determining or measuring the expression level of
10 syndecan-1 in tissues samples are well known in the art. The detection and quantification of a marker that is expressed by a cell is performed by flow cytometry. In some embodiments, such method comprises contacting the sample with at least one selective binding agent capable of selectively interacting with the protein of interest (i.e. syndecan-1). The selective binding agent may be polyclonal antibody or monoclonal antibody, an antibody fragment, synthetic
15 antibodies, or other protein-specific agents such as nucleic acid or peptide aptamers. For the detection of the antibody that makes the presence of the marker detectable by microscopy or an automated analysis system, the antibodies may be tagged directly with detectable labels such as enzymes, chromogens or fluorescent probes or indirectly detected with a secondary antibody conjugated with detectable labels. The binding agents such as antibodies or aptamers may be
20 labelled with a detectable molecule or substance, such as preferentially a fluorescent molecule, or a radioactive molecule or any others labels known in the art. As used herein, the terms "label" and "detectable label" refer to a molecule capable of detection, including, but not limited to, radioactive isotopes, fluorescers, chemilumescers, chromophores, enzymes, enzyme substrates, enzyme cofactors, enzyme inhibitors, chromophores, dyes, metal ions, metal sols,
25 ligands (e.g., biotin, avidin, streptavidin or haptens), intercalating dyes and the like. The term "fluorescer" refers to a substance or a portion thereof which is capable of exhibiting fluorescence in the detectable range. Labels of interest include both directly and indirectly detectable labels. Suitable labels for use in the methods described herein include any molecule that is indirectly or directly detectable by spectroscopic, photochemical, biochemical,
30 immunochemical, electrical, optical, chemical, or other means. Labels of interest include, but are not limited to, fluorescein and its derivatives; rhodamine and its derivatives; cyanine and its derivatives; coumarin and its derivatives; Cascade Blue and its derivatives; Lucifer Yellow and its derivatives; BODIPY and its derivatives; and the like. Labels of interest also include fluorophores, such as indocarbocyanine (C3), indodicarbocyanine (C5), Cy3, Cy3.5, Cy5,

Cy5.5, Cy7, Texas Red, Pacific Blue, Oregon Green 488, Alexa fluor-355, Alexa Fluor 488, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor-555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, JOE, Lissamine, Rhodamine Green, BODIPY, fluorescein isothiocyanate (FITC), carboxy-fluorescein (FAM), phycoerythrin, rhodamine, dichlororhodamine (dRhodamine), carboxy tetramethylrhodamine (TAMRA), carboxy-X- rhodamine (ROX), LIZ, VIC, NED, PET, SYBR, PicoGreen, RiboGreen, and the like. Fluorescent labels can be detected using a photodetector (e.g., in a flow cytometer) to detect emitted light. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, colorimetric labels can be detected by simply visualizing the colored label, and antigenic labels can be detected by providing an antibody (or a binding fragment thereof) that specifically binds to the antigenic label. An antibody that specifically binds to an antigenic label can be directly or indirectly detectable. For example, the antibody can be conjugated to a label moiety (e.g., a fluorophore) that provides the signal (e.g., fluorescence); the antibody can be conjugated to an enzyme (e.g., peroxidase, alkaline phosphatase, etc.) that produces a detectable product (e.g., fluorescent product) when provided with an appropriate substrate (e.g., fluorescent-tyramide, FastRed, etc.); etc. The aforementioned assays may involve the binding of the binding agents (ie. antibodies or aptamers) to a solid support. The solid surface could a microtitration plate coated with the binding partner. Alternatively, the solid surfaces may be beads, such as activated beads, magnetically responsive beads. Beads may be made of different materials, including but not limited to glass, plastic, polystyrene, and acrylic. In addition, the beads are preferably fluorescently labelled. In a preferred embodiment, fluorescent beads are those contained in TruCount(TM) tubes, available from Becton Dickinson Biosciences, (San Jose, California). According to the invention, methods of flow cytometry are preferred methods for measuring the level of the protein of interest (i.e. syndecan- 1). Flow cytometry is a well-accepted tool in research that allows a user to rapidly analyze and sort components in a sample fluid. Flow cytometers use a carrier fluid (e.g., a sheath fluid) to pass the sample components, substantially one at a time, through a zone of illumination. Each sample component is illuminated by a light source, such as a laser, and light scattered by each sample component is detected and analyzed. The sample components can be separated based on their optical and other characteristics as they exit the zone of illumination. Said methods are well known in the art. In a particular embodiment, a fluorescence activated cell sorting (FACS) is used to measure the expression level of syndecan-1. Involves using a flow cytometer capable of simultaneous excitation and detection of multiple fluorophores, such as a BD Biosciences

FACSCanto™ flow cytometer, used substantially according to the manufacturer's instructions. The cytometric systems may include a cytometric sample fluidic subsystem, as described below. In addition, the cytometric systems include a cytometer fluidically coupled to the cytometric sample fluidic subsystem. Systems of the present disclosure may include a number of additional components, such as data output devices, e.g., monitors, printers, and/or speakers, data input devices, e.g., interface ports, a mouse, a keyboard, etc., fluid handling components, power sources, etc.

The method according to the invention is suitable to perform Positron-emission tomography (PET) scan to diagnosis a brain injury, aging-associated cognitive decline, psychiatric disorders or neurodegenerative disease in a subject.

As used herein, the term "predetermined reference value" refers to a threshold value or a cut-off value. Typically, a "threshold value" or "cut-off value" can be determined experimentally, empirically, or theoretically. A threshold value can also be arbitrarily selected based upon the existing experimental and/or clinical conditions, as would be recognized by a person of ordinary skilled in the art. The threshold value has to be determined in order to obtain the optimal sensitivity and specificity according to the function of the test and the benefit/risk balance (clinical consequences of false positive and false negative). Typically, the optimal sensitivity and specificity (and so the threshold value) can be determined using a Receiver Operating Characteristic (ROC) curve based on experimental data. For example, after determining the expression level of the selected peptide in a group of reference, one can use algorithmic analysis for the statistic treatment of the expression levels determined in samples to be tested, and thus obtain a classification standard having significance for sample classification. The full name of ROC curve is receiver operator characteristic curve, which is also known as receiver operation characteristic curve. It is mainly used for clinical biochemical diagnostic tests. ROC curve is a comprehensive indicator that reflects the continuous variables of true positive rate (sensitivity) and false positive rate (1 -specificity). It reveals the relationship between sensitivity and specificity with the image composition method. A series of different cut-off values (thresholds or critical values, boundary values between normal and abnormal results of diagnostic test) are set as continuous variables to calculate a series of sensitivity and specificity values. Then sensitivity is used as the vertical coordinate and specificity is used as the horizontal coordinate to draw a curve. The higher the area under the curve (AUC), the higher the accuracy of diagnosis. On the ROC curve, the point closest to the far upper left of the coordinate diagram is a critical point having both high sensitivity and high specificity values. The AUC value of the ROC curve is between 1.0 and 0.5. When AUO0.5, the diagnostic result

gets better and better as AUC approaches 1. When AUC is between 0.5 and 0.7, the accuracy is low. When AUC is between 0.7 and 0.9, the accuracy is moderate. When AUC is higher than 0.9, the accuracy is high. This algorithmic method is preferably done with a computer. Existing software or systems in the art may be used for the drawing of the ROC curve, such as: MedCalc 9.2.0.1 medical statistical software, SPSS 9.0, ROCPOWER.SAS, DESIGNROC.FOR, MULTIREADER POWER.SAS, CREATE-ROC.SAS, GB STAT VIO.O (Dynamic Microsystems, Inc. Silver Spring, Md., USA), etc.

In a particular embodiment, the method according to the invention is suitable to determine whether a neural stem cell (NSC) has or is susceptible to have proliferative profile.

In a second aspect, the invention relates to a method for treating brain injury, aging-associated cognitive decline, psychiatric disorders or neurodegenerative disease in a subject in need thereof comprising a step of administering said subject with a therapeutically effective amount of a syndecan-1 agonist.

In a particular embodiment, the method according to the invention further comprises:
i) quantifying neurogenic activity in a subject and ii) administering said subject with a therapeutically effective amount of a syndecan-1 agonist when the neurogenic activity is lower than the predetermined reference value.

As used herein, the terms "treating" or "treatment" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of subject at risk of contracting the disease or suspected to have contracted the disease as well as subject who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse. The treatment may be administered to a subject having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment. By "therapeutic regimen" is meant the pattern of treatment of an illness, e.g., the pattern of dosing used during therapy. A therapeutic regimen may include an induction regimen and a maintenance regimen. The phrase "induction regimen" or "induction period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the initial treatment of a disease. The general goal of an induction regimen is to provide a high level of drug to a subject during the initial period of a treatment regimen. An induction regimen may employ (in part or in whole) a "loading regimen", which may include administering a greater dose of the drug than a physician would employ during a maintenance regimen, administering a drug more frequently than a physician would administer the drug during a

maintenance regimen, or both. The phrase "maintenance regimen" or "maintenance period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the maintenance of a subject during treatment of an illness, e.g., to keep the subject in remission for long periods of time (months or years). A maintenance regimen may employ continuous
5 therapy (e.g., administering a drug at regular intervals, e.g., weekly, monthly, yearly, etc.) or intermittent therapy (e.g., interrupted treatment, intermittent treatment, treatment at relapse, or treatment upon achievement of a particular predetermined criteria [e.g., pain, disease manifestation, etc.]).

As used herein, the term "syndecan-1 agonist" refers to any compound natural or not
10 that is able to bind to syndecan-1 and promotes syndecan-1 activity which consists of the proliferation of activated adult neural stem cells (NSCs) and in the neurogenesis and/or oligodendrogenesis.

In a particular embodiment, the syndecan-1 agonist is a small organic molecule. The term "small organic molecule" refers to a molecule of a size comparable to those organic
15 molecules generally used in pharmaceuticals. The term excludes biological macromolecules (e.g., proteins, nucleic acids, etc.). Preferred small organic molecules range in size up to about 5000 Da, more In particular up to 2000 Da, and most In particular up to about 1000 Da.

In a particular embodiment, the syndecan-1 agonist is an antibody or a portion thereof.

As used herein, "antibody" includes both naturally occurring and non-naturally
20 occurring antibodies. Specifically, "antibody" includes polyclonal and monoclonal antibodies, and monovalent and divalent fragments thereof. Furthermore, "antibody" includes chimeric antibodies, wholly synthetic antibodies, single chain antibodies, and fragments thereof. The antibody may be a human or nonhuman antibody. A nonhuman antibody may be humanized by recombinant methods to reduce its immunogenicity in man. In one embodiment, the agonist of
25 syndecan-1 is selected from the group consisting of chimeric antibodies, humanized antibodies or full human monoclonal antibodies. In one embodiment of the antibodies or portions thereof described herein, the antibody is a monoclonal antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a polyclonal antibody. In one embodiment of the antibodies or portions thereof described herein, the antibody is a humanized antibody. In
30 one embodiment of the antibodies or portions thereof described herein, the antibody is a chimeric antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a light chain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a heavy chain of the antibody. In one embodiment of the antibodies or portions thereof described herein,

the portion of the antibody comprises a Fab portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a F(ab')₂ portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a Fc portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a Fv portion of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises a variable domain of the antibody. In one embodiment of the antibodies or portions thereof described herein, the portion of the antibody comprises one or more CDR domains of the antibody.

Antibodies are prepared according to conventional methodology. Monoclonal antibodies may be generated using the method of Kohler and Milstein (Nature, 256:495, 1975). To prepare monoclonal antibodies useful in the invention, a mouse or other appropriate host animal is immunized at suitable intervals (e.g., twice-weekly, weekly, twice-monthly or monthly) with antigenic forms of OX1 R. The animal may be administered a final "boost" of antigen within one week of sacrifice. It is often desirable to use an immunologic adjuvant during immunization. Suitable immunologic adjuvants include Freund's complete adjuvant, Freund's incomplete adjuvant, alum, Ribi adjuvant, Hunter's Titermax, saponin adjuvants such as QS21 or Quil A, or CpG-containing immunostimulatory oligonucleotides. Other suitable adjuvants are well-known in the field. The animals may be immunized by subcutaneous, intraperitoneal, intramuscular, intravenous, intranasal or other routes. A given animal may be immunized with multiple forms of the antigen by multiple routes. Following the immunization regimen, lymphocytes are isolated from the spleen, lymph node or other organ of the animal and fused with a suitable myeloma cell line using an agent such as polyethylene glycol to form a hybridoma. Following fusion, cells are placed in media permissive for growth of hybridomas but not the fusion partners using standard methods, as described (Coding, Monoclonal Antibodies: Principles and Practice: Production and Application of Monoclonal Antibodies in Cell Biology, Biochemistry and Immunology, 3rd edition, Academic Press, New York, 1996). Following culture of the hybridomas, cell supernatants are analyzed for the presence of antibodies of the desired specificity, i.e., that selectively bind the antigen. Suitable analytical techniques include ELISA, flow cytometry, immunoprecipitation, and western blotting. Other screening techniques are well-known in the field. Preferred techniques are those that confirm binding of antibodies to conformationally intact, natively folded antigen, such as non-denaturing ELISA, flow cytometry, and immunoprecipitation. Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding

of the antibody to its epitope (see, in general, Clark, W. R. (1986) *The Experimental Foundations of Modern Immunology* Wiley & Sons, Inc., New York; Roitt, I. (1991) *Essential Immunology*, 7th Ed., Blackwell Scientific Publications, Oxford). The Fc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an F(ab')₂ fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Proceeding further, Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, as is well-known in the art, there are complementarity determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

It is now well-established in the art that the non CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody.

This invention provides in certain embodiments compositions and methods that include humanized forms of antibodies. As used herein, "humanized" describes antibodies wherein some, most or all of the amino acids outside the CDR regions are replaced with corresponding amino acids derived from human immunoglobulin molecules. Methods of humanization include, but are not limited to, those described in U.S. Pat. Nos. 4,816,567, 5,225,539, 5,585,089, 5,693,761, 5,693,762 and 5,859,205, which are hereby incorporated by reference. The above U.S. Pat. Nos. 5,585,089 and 5,693,761, and WO 90/07861 also propose four possible criteria which may be used in designing the humanized antibodies. The first proposal was that for an

acceptor, use a framework from a particular human immunoglobulin that is unusually homologous to the donor immunoglobulin to be humanized, or use a consensus framework from many human antibodies. The second proposal was that if an amino acid in the framework of the human immunoglobulin is unusual and the donor amino acid at that position is typical for human sequences, then the donor amino acid rather than the acceptor may be selected. The third proposal was that in the positions immediately adjacent to the 3 CDRs in the humanized immunoglobulin chain, the donor amino acid rather than the acceptor amino acid may be selected. The fourth proposal was to use the donor amino acid residue at the framework positions at which the amino acid is predicted to have a side chain atom within 3 Å of the CDRs in a three dimensional model of the antibody and is predicted to be capable of interacting with the CDRs. The above methods are merely illustrative of some of the methods that one skilled in the art could employ to make humanized antibodies. One of ordinary skill in the art will be familiar with other methods for antibody humanization.

In one embodiment of the humanized forms of the antibodies, some, most or all of the amino acids outside the CDR regions have been replaced with amino acids from human immunoglobulin molecules but where some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they would not abrogate the ability of the antibody to bind a given antigen. Suitable human immunoglobulin molecules would include IgG1, IgG2, IgG3, IgG4, IgA and IgM molecules. A "humanized" antibody retains a similar antigenic specificity as the original antibody. However, using certain methods of humanization, the affinity and/or specificity of binding of the antibody may be increased using methods of "directed evolution", as described by Wu et al, /. Mol. Biol. 294: 151, 1999, the contents of which are incorporated herein by reference. Fully human monoclonal antibodies also can be prepared by immunizing mice transgenic for large portions of human immunoglobulin heavy and light chain loci. See, e.g., U.S. Pat. Nos. 5,591,669, 5,598,369, 5,545,806, 5,545,807, 6,150,584, and references cited therein, the contents of which are incorporated herein by reference. These animals have been genetically modified such that there is a functional deletion in the production of endogenous (e.g., murine) antibodies. The animals are further modified to contain all or a portion of the human germ-line immunoglobulin gene locus such that immunization of these animals will result in the production of fully human antibodies to the antigen of interest. Following immunization of these mice (e.g., XenoMouse (Abgenix), HuMAb mice (Medarex/GenPharm)), monoclonal antibodies can be prepared according to standard hybridoma technology. These monoclonal antibodies will have human

immunoglobulin amino acid sequences and therefore will not provoke human anti-mouse antibody (KAMA) responses when administered to humans.

In vitro methods also exist for producing human antibodies. These include phage display technology (U.S. Pat. Nos. 5,565,332 and 5,573,905) and in vitro stimulation of human B cells (U.S. Pat. Nos. 5,229,275 and 5,567,610). The contents of these patents are incorporated herein by reference.

Thus, as will be apparent to one of ordinary skill in the art, the present invention also provides for F(ab')₂ Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric F(ab')₂ fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies. The various antibody molecules and fragments may derive from any of the commonly known immunoglobulin classes, including but not limited to IgA, secretory IgA, IgE, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4.

In another embodiment, the antibody according to the invention is a single domain antibody. The term "single domain antibody" (sdAb) or "VHH" refers to the single heavy chain variable domain of antibodies of the type that can be found in Camelid mammals which are naturally devoid of light chains. Such VHH are also called "nanobody®". According to the invention, sdAb can particularly be llama sdAb.

In one embodiment, the syndecan-1 agonist is a peptide, peptidomimetic or polypeptide. The term "peptidomimetic" refers to a small protein-like chain designed to mimic a peptide. The term "polypeptide" refers both short peptides with a length of at least two amino acid residues and at most 10 amino acid residues, oligopeptides (11-100 amino acid residues), and longer peptides (the usual interpretation of "polypeptide", i.e. more than 100 amino acid residues in length) as well as proteins (the functional entity comprising at least one peptide, oligopeptide, or polypeptide which may be chemically modified by being glycosylated, by being lipidated, or by comprising prosthetic groups). Agonistic activity of the polypeptide is assessed from 24 hr to 7 days by any assay well known in the art. Cells are treated with or

without the polypeptide to be tested and the proliferative effect is determined 24 hr to 7 days thereafter.

In some embodiments, the polypeptide of the present invention is the functional equivalent of syndecan-1. As used herein, a "functional equivalent of syndecan-1" is a polypeptide which is capable of binding to syndecan-1, thereby promoting a syndecan-1 activity according to the invention. The term "functional equivalent" includes fragments, mutants, and muteins of syndecan-1. The term "functionally equivalent" thus includes any equivalent of syndecan-1 obtained by altering the amino acid sequence, for example by one or more amino acid deletions, substitutions or additions such that the protein analogue retains the ability to bind to syndecan-1 and promote an syndecan-1 activity according to the invention (e.g. proliferation of NSCs). Amino acid substitutions may be made, for example, by point mutation of the DNA encoding the amino acid sequence. In some embodiments, the functional equivalent is at least 80% homologous to the corresponding protein. In a preferred embodiment, the functional equivalent is at least 90% homologous as assessed by any conventional analysis algorithm such as for example, the Pileup sequence analysis software (Program Manual for the Wisconsin Package, 1996). The term "a functionally equivalent fragment" as used herein also may mean any fragment or assembly of fragments of syndecan-1 and promote the syndecan-1 activity according to the invention.

Functionally equivalent fragments may belong to the same protein family as the human syndecan-1 identified herein. By "protein family" is meant a group of proteins that share a common function and exhibit common sequence homology. Homologous proteins may be derived from non-human species. In particular, the homology between functionally equivalent protein sequences is at least 25% across the whole of amino acid sequence of the complete protein. More In particular, the homology is at least 50%, even more In particular 75% across the whole of amino acid sequence of the protein or protein fragment. More In particular, homology is greater than 80% across the whole of the sequence. More particularly, homology is greater than 90% across the whole of the sequence. More In particular, homology is greater than 95% across the whole of the sequence.

As used herein the terms "administering" or "administration" refer to the act of injecting or otherwise physically delivering a substance as it exists outside the body (e.g., an agonist of syndecan-1) into the subject, such as by mucosal, intradermal, intravenous, subcutaneous, intranasal, intramuscular delivery and/or any other method of physical delivery described herein or known in the art. When a disease, or a symptom thereof, is being treated, administration of the substance typically occurs after the onset of the disease or symptoms

thereof. When a disease or symptoms thereof, are being prevented, administration of the substance typically occurs before the onset of the disease or symptoms thereof.

By a "therapeutically effective amount" is meant a sufficient amount of an agonist of syndecan-1 for use in a method for the treatment of melanoma at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well known within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, typically from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

In a third aspect, the invention relates to a method for treating brain injury in a subject in need thereof comprising a step of administering said subject with a therapeutically effective amount of a syndecan-1 antagonist.

In a particular embodiment, the method according to the invention further comprises: i) quantifying neurogenic activity in a subject according to the invention and ii) administering said subject with a therapeutically effective amount of a syndecan-1 antagonist when the neurogenic activity is higher than the predetermined reference value.

The method according to the invention, wherein, the brain injury is epilepsy. As used herein, the term "epilepsy" refers to a long-term risk of recurrent seizures. These seizures may present in several ways depending on the part of the brain involved and the person's age.

As uses herein, the term syndecan-1 antagonist” refers to compound natural or not that is able to bind to syndecan-1 and reduces syndecan-1 activity which consists of the proliferation of activated adult neural stem cells (NSCs) and in the neurogenesis and/or oligodendrogenesis. Typically, such antagonist reduces the neurogenic activity in said subject.

5 In a particular embodiment, the antagonist is a small molecule, a peptide, an aptamer, an antibody as described above.

The syndecan-1 agonist and antagonist as described above may be combined with classical treatment of brain injury, aging-associated cognitive decline, psychiatric disorders or
10 neurodegenerative disease.

As used herein, the term “classical treatment” refers to any compound, natural or synthetic, used for the treatment of brain injury, aging-associated cognitive decline, psychiatric disorders or neurodegenerative disease.

Example of classical treatment of brain injury, aging-associated cognitive decline,
15 psychiatric disorders or neurodegenerative disease include: antipsychotic agent; vitamin E; cholinesterase inhibitors such as donepezil and rivastigmine and galantamine; memantine; diuretics, anti-seizure agent; PBT2; TNF-alpha inhibitors such as etanercept, thalidomide, lenalidomide, pomalidomide, infliximab, adalimumab, certolizumab, golumumab and bupropion.

20 As used herein, the term “anti-seizure agent” refers to chemical compounds that are effective to manage seizure. Example of anti-seizure agent include carbamazepine; benzodiazepines such as clonazepam, clobazam, clorazepate, lorazepam and diazepam; valproate; eslicarbazepine acetate; ethosuximide; gabapentin; lacosamide; methsuximide; oxcarbazepine; perampanel; phenorbital; phenytoin; pregabalin; rufinamide; tiagabine
25 hydrochloride; vigabatrin; ezogabine; felbamate; lamotrigine; levetiracetam; primidone; topiramate and zonisamide.

As used herein, the term “antipsychotic agent”, also known as neuroleptics or major tranquillizers, refers to chemical compounds that are effective to manage psychosis. Example of antipsychotic agent include butyrophenones such as benperidol, bromperidol, droperidol,
30 haloperidol, moperone, pipamerone, melperone and timiperone; diphenylbutylpiperidines such as fluspirilene, penfluridol and pimozide; phenothiazines such as acepromazine, chlorpromazine, cyamemazine, dixyrazine, fluphenazine, levomepromazine, mesoridazine, perazine, perphenazine, pipotiazine, prochlorperazine, promazine, promethazine, prothipendyl, thioproperazine, thioridazine trifluoperazine and triflupromazine; thioxanthenes such as

chlorprothixene, clopenthixol, flupentixol, thiothixene and zuclopenthixol; benzamides such as sulpiride, sultopride, veralipride, amisulpride; nemonapride, remoxipride and sultopride ; tricyclics such as asenapine, olanzapine, quetiapine, zotepine, caripramine, clocapramine, clorotepine, clotiapine, loxapine and mosapramine; molindone; benzisoaxoles such as lloperidone, lurasidone, paliperidone, paliperidone palmitate, perospirone, risperidone and ziprasidone; phenylpiperazines such as aripiprazole, aripiprazole lauxoril, brexipiparazole and cariprazine; blonanserin; pimavanserin and sertindole.

As used herein, the terms “combined treatment”, “combined therapy” or “therapy combination” refer to a treatment that uses more than one medication. The combined therapy may be dual therapy or bi-therapy.

The medications used in the combined treatment according to the invention are administered to the subject simultaneously, separately or sequentially.

As used herein, the term “administration simultaneously” refers to administration of 2 active ingredients by the same route and at the same time or at substantially the same time. The term “administration separately” refers to an administration of 2 active ingredients at the same time or at substantially the same time by different routes. The term “administration sequentially” refers to an administration of 2 active ingredients at different times, the administration route being identical or different.

In a fourth aspect, the invention relates to a method for promoting the neurogenesis and/or oligodendrogenesis, comprising the administration of an effective amount of syndecan-1 agonist.

As used herein, the term “neurogenesis” refers to the process in which nervous system cells, known as neurons, are produced by neural stem cells (NSCs) and neural progenitors. The syndecan-1 agonist as described above is suitable to activate the neurogenesis.

As used herein, the term “oligodendrogenesis” refers to a process of the proliferation, maturation of oligodendrocyte precursor cells and their migration to the site of injury to replace injured or lost oligodendrocytes. Oligodendrocyte precursor cells offer the potential for repair and recovery of injured white matter. The syndecan-1 agonist as described above is suitable to activate the oligodendrogenesis.

The syndecan-1 agonist and antagonist as described above may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form pharmaceutical compositions. "Pharmaceutically" or

"pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The pharmaceutical compositions of the present invention for oral, sublingual, subcutaneous, intramuscular, intravenous, transdermal, local or rectal administration, the active principle, alone or in combination with another active principle, can be administered in a unit administration form, as a mixture with conventional pharmaceutical supports, to animals and human beings. Suitable unit administration forms comprise oral-route forms such as tablets, gel capsules, powders, granules and oral suspensions or solutions, sublingual and buccal administration forms, aerosols, implants, subcutaneous, transdermal, topical, intraperitoneal, intramuscular, intravenous, subdermal, transdermal, intrathecal and intranasal administration forms and rectal administration forms. Typically, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The polypeptide (or nucleic acid encoding thereof) can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium,

potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin. Sterile injectable solutions are prepared by incorporating the active polypeptides in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

Accordingly, the invention relates to a pharmaceutical composition, for use in the treatment of brain injury or neurodegenerative disease, comprising a syndecan-1 agonist.

A further object of the present invention relates to a method of screening a drug suitable for the treatment of brain injury or neurodegenerative disease comprising i) providing a test compound and ii) determining the ability of said test compound to activate and induce or reduce the activity of syndecan-1.

Any biological assay well known in the art could be suitable for determining the ability of the test compound to activate or reduce the activity of syndecan-1. In some embodiments, the assay first comprises determining the ability of the test compound to bind to syndecan-1. In some embodiments, a population of cells is then contacted and activated so as to determine the ability of the test compound to activate the activity of syndecan-1. In particular, the effect triggered by the test compound is determined relative to that of neural stem cells incubated in parallel in the presence of the test compound or in the presence of a control agent either of which is analogous to a negative control condition. The term "control substance", "control agent", or "control compound" as used herein refers a molecule that is inert or has no activity relating to an ability to modulate a biological activity or expression. It is to be understood that test compounds capable of activating the activity of syndecan-1, as determined using in vitro methods described herein, are likely to exhibit similar modulatory capacity in applications in vivo. Typically, the test compound is selected from the group consisting of peptides, peptidomimetics, small organic molecules, aptamers or nucleic acids. For example, the test compound according to the invention may be selected from a library of compounds previously synthesised, or a library of compounds for which the structure is determined in a database, or from a library of compounds that have been synthesised de novo. In some embodiments, the test compound may be selected from small organic molecules or peptides.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1: Expression of the Syndecan family members in quiescent and activated adult NSCs. Variations in the expression of Sdc1, Sdc2 and Sdc4 in freshly sorted LeX^{bright}, LeX⁺EGFR⁺ and total SVZ cells were confirmed by qRT-PCR (0: undetectable) and quantification of SDC1-, SDC2- and SDC4-positive cells reveals higher expression of SDC1

by actively proliferating LeX+EGFR⁺ cells. Data are represented as the mean $\pm$ SD from 4 independent experiments of 3 to 4 mice. * $p < 0.05$.

Figure 2: Syndecan-1 enriches in proliferating NSCs and its silencing reduces their proliferation. (A) LeXbright and LeX+EGFR⁺ cells were prospectively purified by flow cytometry from PN10 SVZ according to Sdc1 expression (Sdc1-negative: light blue; Sdc1-positive: dark blue). Primary then secondary neurospheres were determined. The effects of Sdc1 silencing in LeX+EGFR⁺ cells sorted from adult SVZ, through siRNA directed against Sdc1, were determined on the initiation of neurosphere (B), on cell proliferation (C) and on the time for the first cell division (D). Data are represented as the mean $\pm$ SD (A-B) and scattered dot plots with median $\pm$ min/max (C-D), and were obtained from independent experiments (N=3-10 for primary, N=3 for secondary and N=4 for siRNA experiments) with 3-4 pooled mice. Statistical analyses were performed using one tailed Mann Whitney (A-B-D) and Wilcoxon matched-pairs signed rank test (C). * $p < 0.05$. ND: not determined.

Figure 3: Silencing of Syndecan-1 reduces the size of neurospheres.

Neural progenitors from adult mice were electroporated with siRNA against Syndecan-1 (Sdc1) or a non-targeting/scrambled control (Ctrl). The efficacy of Sdc1 silencing was shown on Sdc-1 mRNA expression by qRT-PCR at 3 concentrations (A) and at the protein level by FACS at 100 nM (B). The size of neurospheres was measured 72 h and 7 days after siRNA electroporation. ** $p < 0.005$; p*** $p < 0.001$.

Figure 4: Neural stem cells (NSCs) are contained in the medial (MW) and the lateral walls (LW) of the ventricular-subventricular zone.

(A) A schematic coronal view of post-natal day 10 mouse forebrain showing LW and MW. (B) Relative quantities of quiescent NSCs (LeX⁺), activated NSCs (LeX⁺EGFR⁺) and TAPs (EGFR⁺) determined by flow cytometry thanks to CD24/LeX/EGF triple staining. Clonogenic capacity (C) and SDC1 expression (D) were determined on LeX⁺, LeX⁺EGFR⁺ and EGFR⁺ sorted population. SDC1 expression was confirmed by immunofluorescence on cultured NSCs from LW (E) and MW (F). Data are represented as the median $\pm$ 95% CI and each data point represents individual experiment with pooled 2-4 mice. Statistical analyses were performed using Kruskal-Wallis combined with Tukey's post-hoc test. ns: not significant. Data in D were obtained with two measurements for MW and were not compared to LW.

Figure 5: Heparanase stimulates proliferation of NSCs.

(A) Time schedule for EdU/BrdU dual incorporation and drug treatments in which the EdU-BrdU⁺ population contains NSCs that were stimulated by drugs and were not cycling before (i.e. standing in G0/G1 phase). (B) HPSE (4 ng/ml) provoked proliferation of resting

NSCs and, conversely, its inhibitor (OGT2115) blocked their proliferation. Data are represented as the mean $\pm$ SD obtained from three independent experiments. Statistical analyses were performed using one-way ANOVA and Dunnett's test for comparison with vehicle (0.02% DMSO). *: $p < 0.05$ and ns: not significant.

5

EXAMPLE:

Material & Methods

Animals and treatments

Young adult C57Bl/6J mice (2-3 months) were maintained in standard cages with access to food and water ad libitum in a colony room kept at a constant temperature (19-22°C) and humidity (40-50%) on a 12:12-hour light/dark cycle. Postnatal day 10 C57Bl/6J mice were produced in our animal facility by programmed breeding. For cell cycle analysis, we used FUCCI-Red transgenic mice (fluorescence ubiquitination-based cell cycle indicator for CDT1) (Sakaue-Sawano et al., 2008).

When indicated, mice received whole brain irradiation (4 Gy) under anesthesia using a ⁶⁰Co medical irradiator (Alcyon) as previously reported (Daynac et al., 2013).

Two month-old mice were initially injected intraperitoneally with 100 mg BrdU/kg body mass then maintained with drinking water containing BrdU (1 mg/mL, 1% glucose) for 14 consecutive days followed by a 2 and 4-week chase period until sacrifice.

Animal experiments were performed in compliance with the European Communities Council Directive of 22th September 2010 (EC/2010/63) and were approved by our institutional committee on animal welfare (authorization #12-034; CEtEA-CEA DRF IdF).

SVZ cell preparation

Adult SVZs were dissected, dissociated and labelled as previously described (Daynac, 2015). Briefly, dissected SVZs were digested with papain (1 mg/ml, Worthington) supplemented with 0.01 mg/ml DNase I (Sigma) for 10 min at 37°C. The minced tissue was then mechanically dissociated into a single-cell suspension using a P1000 micropipette in ovomucoid solution (0.7 mg/ml, Sigma). PN10 SVZs were prepared by mechanical dissociation. Papain solution was removed for the preparation of PN10 SVZ. Aggregates were removed with 20 μ m nylon filters (BD Biosciences) and cells were centrifuged at 250 g for 20 min at 4°C without brake on a 22% Percoll gradient (GE Healthcare) to remove myelin. Finally, cells were incubated for 20 min with the following antibodies: CD24 phycoerythrin [PE]-conjugated (cat#561079; 1:50 BD Biosciences), CD15/LeX fluorescein isothiocyanate [FITC]-conjugated (clone MMA, mouse IgM; 1:50 BD Biosciences) and Alexa647-conjugated EGF

ligand (1:250 Life Technologies). Rat anti-mouse Syndecan-1 PE-conjugated (cat#553714, BD Biosciences) and rat anti-mouse Syndecan-4 PE-conjugated (cat#550352, BD Biosciences) antibodies were used at 1:50 in combination with anti-CD24 PE-Cyanine7-conjugated antibody (cat#A14776, Molecular Probes). Immediately prior to FACS, Hoechst 33258 was added to a final concentration of 1 µg/mL to label dead cells. Adult SVZ cells were sorted on an INFLUX cell sorter equipped with an 86 µm nozzle at 40 psi and post-natal SVZ cells on an ARIA equipped with a 100 µm nozzle (BD Biosciences). Gates were set using Fluorescence Minus One (FMO) controls on SVZ cells.

Immunofluorescence

Sorted cells were recovered in DMEM:F12 medium supplemented with 2% B27 then plated without mitogen on poly-D-lysine- and laminin-coated 8-well glass slides (Millicell) in an incubator at 37°C 5% CO₂ for 2-4 hours and fixed in 2% paraformaldehyde. After 1 hour in blocking solution (PBS-0.1% Triton-X100–1% BSA) at RT, cells were incubated overnight at 4°C with anti-mouse CD138/SDC1 (1:100, BD Pharmingen), anti-CD362/SDC2 (1:100, AF6585, R&D System) or anti-SDC4 (1:100, NB110-41551, NovusBio) primary antibodies. After three washes in PBS, cells were incubated with an AlexaFluor donkey secondary antibody at 1:500 (Invitrogen). For BrdU detection, cells were permeabilized for 5 min at RT in 0.5% Triton X-100 in PBS. Incubation in blocking solution (PBS, 0.05% Tween 20, 4% BSA) for 1 hour was followed by a 30 min incubation at 37 °C with the anti-BrdU antibody at 1/300 (GE Healthcare) in DNase incubation buffer (0.5X PBS, 30 mM Tris-HCl pH 8, 0.3 mM MgCl₂, 0.5 mM 2-mercaptoethanol, 0.5% BSA and 10 µg/mL DNase I). After several washes, cells were incubated with an Alexa fluor 488-conjugated donkey secondary antibody at 1:500 (Invitrogen).

Cell culture

Sorted NSCs or total SVZ cell suspensions were grown at 37°C 5% CO₂ in neurosphere medium composed of DMEM/F12 (Life Technologies) supplemented with 0.6% Glucose (Sigma), 2 µg/mL heparin (STEMCELL Technologies), 1x Insulin-Selenium-Transferrin (Life Technologies), N-2 supplement (Life Technologies) and B-27 without Vit. A supplement (Life Technologies), and in the presence of 20 ng/ml EGF (Millipore) and 10 ng/ml FGF2 (Millipore).

After 7 days, neurospheres were counted under an inverted microscope. Neurospheres were centrifuged and incubated for 5 min in the presence of Accutase (Sigma) then were mechanically dissociated. Dissociated cells were plated in neurosphere medium at a density not exceeding 1.4 cells/µl in 12 or 24 well plates.

Quiescence was induced in vitro by removing growth factors and by adding 25 ng/mL hBMP4 (R&D Systems).

For RNA measurement, cells were counted on day 3 and lysed in RLT buffer (Qiagen) for RNA isolation and qRT-PCR experiments.

5 **SiRNA silencing**

Immediately after sorting or one week after initiation of neurosphere cultures, LeX+EGFR+ SVZ cells were electroporated using Neon® kit according to the manufacturer's instructions (ThermoFisher). Briefly, dissociated cells (1.2×10^3 to 12×10^3) were suspended in 20 μ L of R resuspension buffer (ThermoFisher) and split into 2 vials containing 1 μ L of
10 siRNA at 20 μ M. Cells were electroporated at 1300 V for 3 pulses during 10 ms then transferred immediately after into neurosphere medium. SiRNA were purchased from Qiagen: control nontargeting/scrambled control (cat#1027280) and a mix of four siRNA against Sdc1 (cat#1027416). Different concentrations of siRNA (10, 20 and 100 nM) were tested in a first set of experiments on neurosphere cultures initiated with total adult SVZ cells.

15 **Live cell imaging**

Freshly sorted LeX+EGFR+ cells (2.10^3) from adult FUCCI-Red mice were electroporated with siRNA (Ctrl or Sdc1) at a final concentration of 50 nM. Brightfield and fluorescent images for Cdt1-red were captured through a Plan Apo VC 320 DIC objective (NA: 0.75) on a Nikon A1R confocal laser scanning microscope system attached to an inverted
20 ECLIPSE Ti (Nikon, Corp., Tokyo, Japan) thermostated at 37°C under 5% CO₂/20% O₂ atmosphere as previously reported (Daynac et al., 2014). Recording was started 4 h after electroporation. Proliferating cells (n = 20-30 cells) were individually followed and the time of the first cell division was determined in 4 independent experiments.

RNA isolation, microarrays and qRT-PCR

25 NSCs were sorted into tubes containing RLT lysis buffer and total RNAs were isolated with the RNeasy Micro Kit with DNase treatment (Qiagen). For microarray experiments, RNA transcripts were converted into cDNAs and amplified using the Ovation Pico WTA System (NuGEN). cDNA were fragmented and biotinylated; then, labeled cRNA were hybridized to Affymetrix MOE430 2.0 arrays according to the manufacturer's protocol at PartnerShip (Evry,
30 France). The data were normalized with the MAS5 algorithm and quality controlled with the Expression Console software (Affymetrix).

For qRT-PCR experiments, total RNAs were reverse-transcribed into cDNA using the Reverse Transcription High Capacity Master Mix (Applied Biosystems) with specific primers

(Sigma-Aldrich). q-PCR was performed on an ABI PRISM 7900 Sequence Detector System using SYBR Green for RT-PCR. Expression levels were normalized to GAPDH.

Microarray analysis

Data were normalized with GC-robust multi-array analysis (GC-RMA) using log₂ transformed expression levels in Genespring GX12 (Agilent Technologies). For comparative analysis of datasets, differentially expressed probes were filtered by an average expression greater than 50 in at least 1 population, at least 2-fold change and a Student's t-test p-value < 0.05 (control LeXbright vs irradiated LeXbright) or corrected p-value < 0.05 (LeXbright vs LeX+EGFR+). An overrepresentation analysis of GO biological processes (p < 0.05) with Bonferroni correction and cellular component ontology analysis were carried out with PANTHER software (<http://www.pantherdb.org>). Statistically enriched GO terms were then hand-curated into thematic categories. Heat maps were generated with Gene-E (<https://software.broadinstitute.org/GENE-E/>).

Accession number

All data are deposited in NCBI GEO under accession number GSE99777.

Statistical analyses

The data are expressed as the mean $\pm$ SD. Non-parametric Mann-Whitney test was conducted to compare qRT-PCR data using GraphPad PRISM software (GraphPad, San Diego, CA). Significance was set at p < 0.05.

Results

LeXbright cells exhibit properties of slowly dividing NSCs

We recently developed a cell sorting strategy based on the exclusion of CD24-positive cells and on the detection of the surface markers LeX and EGFR to simultaneously isolate quiescent NSCs (CD24-EGFR-LeXbright, hereafter LeXbright cells), activated NSCs (CD24-LeX+EGFR+, hereafter LeX+EGFR+ cells) and transit-amplifying cells (CD24-EGFR+, hereafter EGFR+ cells) from the adult SVZ (data not shown) (Daynac et al., 2013; Daynac, 2015). In contrast to the other neurogenic SVZ cell populations, we previously showed that the vast majority of LeXbright cells are not proliferating (Daynac et al., 2013). To explore their cell cycle in more detail, we used Fluorescence Ubiquitination Cell Cycle Indicator (FUCCI)-Red mice (Sakaue-Sawano et al., 2008), which allow the visualization of cells in G1 with the presence of a G1 specific red-Cdt1 reporter (FUCCI-Redpositive cells) while it is absent in cells during the S-G2/M phases (FUCCI-Rednegative cells). Besides, FUCCI-Redbright cells have been shown to have exited the cell cycle (G0) (Daynac et al., 2014; Roccio et al., 2013). While most activated LeX+EGFR+ cells progressed through S-G2/M phases (40.3 $\pm$ 4.0% FUCCI-

Rednegative), LeXbright cells were for the most part distributed in G0 ($66.5 \pm 6.5\%$ FUCCI-Redbright) or in G1 ($31.7 \pm 7.3\%$ FUCCI-Redpositive) in accordance with their quiescent state (data not shown).

To further characterize the cell cycle dynamics of LeXbright cells, we administrated BrdU to mice for two weeks and assessed their ability to retain BrdU labeling for extended chase periods (data not shown). Immediately after BrdU treatment, the great majority of rapidly dividing LeX+EGFR+ cells had incorporated BrdU ($89.7 \pm 5.3\%$) while they had almost all lost the BrdU labeling after 2 and 4 weeks of chase (data not shown). By contrast, only $6.8 \pm 0.7\%$ of LeXbright cells had incorporated BrdU after 2 weeks reflecting their much slower rate of division. Moreover, $64 \pm 24\%$ of LeXbright cells retained the BrdU labelling after 4 weeks of chase (data not shown). These data confirmed that LeXbright cells correspond to a subpopulation of slowly dividing NSCs in vivo.

LeXbright cells enter oxidative metabolism after irradiation

We have previously shown that the vast majority of slowly dividing LeXbright cells that survived to radiation exposure entered the cell cycle to regenerate the irradiated niche (Daynac et al., 2013), recapitulating what is observed after antimitotic treatment with Ara-C (Doetsch et al., 1999).

Here, we performed a transcriptomic analysis of LeXbright cells sorted from 2-month-old control mice and 48 hours after mice were irradiated using whole-genome Affymetrix MOE430 2.0 arrays.

The obtained datasets are visualized as sets of coordinates using principal component analysis (PCA). PCA is an unsupervised pattern recognition and visualization tool used to reduce the dimensionality of datasets derived from transcriptomic arrays, making it possible to visually assess similarities and differences between cell populations (Ringner, 2008). This PCA illustrates the transcriptomic shift of qNSCs induced by irradiation concomitantly to the entry in the cell cycle of a subset of these cells we reported before (Daynac et al., 2013). The comparative gene expression profile of LeXbright cells revealed an altered expression of 927 probes. The resulting set of genes enriched in control LeXbright cells included 439 genes, whereas 409 genes were upregulated in irradiated LeXbright cells. As expected, gene ontology (GO) term analysis revealed that genes upregulated after irradiation in LeXbright cells were mainly associated with the cell cycle and DNA/RNA processes. Moreover, many of these genes were linked to translation and ribosomal activity. Interestingly, Gene Set Enrichment Analysis (GSEA) (Subramanian et al., 2005) also showed enrichment in genes associated with the TCA cycle and respiratory electron transport. Therefore, the cell cycle entry of LeXbright cells after

radiation was accompanied with a shift toward an oxidative metabolism that was consistent with that observed in stem cells during proliferation and differentiation (Huang et al., 2012).

Distinct molecular signatures of quiescent and activated NSCs

In order to gain insights into the mechanisms regulating stem cell quiescence, we performed a microarray analysis of LeXbright and LeX+EGFR⁺ cells, i.e. quiescent and activated NSCs, sorted from 2-month-old mouse SVZ. We compared their global mRNA expression patterns to those obtained from previous studies either characterizing NSCs (Codega et al., 2014) or differentiated cells (Cahoy et al., 2008) using PCA (data not shown). Direct comparison of LeXbright and LeX+EGFR⁺ transcriptome profiles revealed that they were clustered away from differentiated cells (astrocytes, oligodendrocytes and neurons) (data not shown). Moreover, the clear separation of LeXbright cells from LeX+EGFR⁺ cells confirmed their distinct cellular identity (data not shown). Importantly, LeXbright and LeX+EGFR⁺ cells were closely clustered to GFAP::GFP+CD133⁺ and GFAP::GFP+CD133+EGFR⁺ cells, previously shown to correspond respectively to quiescent and proliferating NSCs in the adult SVZ (Codega et al., 2014), providing additional validation of our cell sorting approach (data not shown). Besides, transcriptional hallmarks of NSCs such as Slc1a3/Glast, Prominin1/CD133, Nr2e1/Tlx, Hes5 and Sox2 were found substantially expressed in both LeXbright and LeX+EGFR⁺ cells. It is noteworthy that our cell sorting technique does not require transgene expression to identify the stem cell population and is thus easily transferable to any other mouse model.

To further define genes enriched in each cellular state, the transcriptomes of LeXbright and LeX+EGFR⁺ cells were compared. Probes were filtered by an average expression greater than 50 in at least 1 population, a differential expression of at least 2-fold and a Student's t-test corrected p-value < 0.05. As shown on the volcano plot, the comparative gene expression profile of LeXbright and LeX+EGFR⁺ cells revealed an altered expression of 1278 probes. The resulting set of LeXbright-enriched genes included 433 genes (548 probe sets), whereas 563 genes were upregulated in LeX+EGFR⁺ cells (730 probe sets).

GO term analysis was then performed using a statistical overrepresentation test to delineate the molecular features of quiescent and activated NSCs. In accordance with their proliferating state, the transcriptome of LeX+EGFR⁺ cells was enriched in genes linked to the cell cycle, DNA repair, DNA/RNA metabolism, transcription and translation. Strikingly, cellular component analysis also revealed a drastically different cellular location of the differentially expressed gene products. As expected due to their transcriptionally active state, 15.3% of the genes enriched in LeX+EGFR⁺ cells encoded proteins associated with the

nucleus, as opposed to only 2.3% of those enriched in LeXbright cells. In contrast, the vast majority of the genes enriched in LeXbright cells were related to GO categories linked to lipid metabolic process, transport, response to stimulus, cell localization, cell communication and cell adhesion (data not shown). Importantly, most genes enriched in LeXbright cells encoded proteins associated with the membrane (data not shown) emphasizing the key role played by the microenvironment in the regulation of the quiescent state in the adult SVZ (Chaker et al., 2016).

Transcription factors enriched in quiescent and activated NSCs

In order to identify putative transcriptional regulators of the quiescent and proliferative states of adult NSCs, we focused on transcription factors (TFs) and co-factors either enriched in LeXbright or LeX+EGFR⁺ cells. Analysis of our dataset using public databases (Zhang et al., 2012) revealed a total of 75 differentially expressed TFs – 14 of which were upregulated in LeXbright cells and the remaining 61 in LeX+EGFR⁺ cells (data not shown).

Among the TFs upregulated in LeXbright cells were Sox9 and Id2, which have been previously associated with quiescent NSCs (Llorens-Bobadilla et al., 2015). Interestingly, Klf9, a member of the family of Kruppel-like TFs found upregulated in quiescent muscle satellite stem cells (Pallafacchina et al., 2010), was also enriched in LeXbright cells.

Among the TFs and co-factors that were the most enriched in LeX+EGFR⁺ cells, several were linked to the cell cycle (E2f1, E2f2, Rbl1, Ccne1, Trp53 and Tfdp1). Of particular interest, LeX+EGFR⁺ cells expressed high levels of 3 members of the high-mobility group box (HMGB) protein family - Hmgb1/2/3 (data not shown). Besides the broad role of HMGs in the control of transcription as well as replication, recent studies have linked HMGBs to the control of the proliferation and maintenance of embryonic and adult NSCs (Abraham et al., 2013). Additionally, transcripts for Ascl1 were 200 times higher in LeX+EGFR⁺ cells compared to LeXbright cells (data not shown) in accordance to recent studies that have reported its key role in the proliferation of NSCs and in the exit of stem cells from quiescence in both the adult hippocampus and the SVZ (Urban et al., 2016). Finally, the proliferating state was also associated with the expression of the immediate early gene Fos and SoxC factors (Sox4 and Sox11) which have been linked to stem cell activation (Adepoju et al., 2014; Foronda et al., 2014).

Quiescent LeXbright cells integrate signals from the microenvironment

We found that most genes enriched in LeXbright cells were linked to the cell membrane (data not shown).

Various adhesion molecules, such as neural cell adhesion molecule 1 and 2 (Ncam1, Ncam2), and cadherins/protocadherins (Cdh10, Cdh20, Pcdh7, Pcdh9, Pcdh10, Pcdhb19) were found overexpressed in LeXbright cells (data not shown). Adhesion molecules have been shown to play a key role in the NSC niche by maintaining stem cell niche architecture and homeostasis (Marthiens et al., 2010). Indeed disruption of Vcam1 was previously shown to lead to a massive activation of quiescent NSCs and consequent depletion of the NSC population (Kokovay et al., 2012). In addition, the proliferative status of NSC is dynamically modulated by the cleavage of Cdh2 (or N-cadherin) on NSCs in the adult SVZ (Porlan et al., 2014). Noticeably, both Vcam1 and Cdh2 were found upregulated in LeXbright cells (data not shown).

We then looked for receptors that were differentially expressed between LeXbright and LeX+EGFR⁺ cells in an attempt to identify additional markers of quiescent and activated NSCs as well as signalling pathways involved in the regulation of NSC behaviour (data not shown). In the adult SVZ, gamma-aminobutyric acid (GABA) produced by neuroblasts decreased the proliferation of adult NSCs (Liu et al., 2005). Moreover, inhibition of GABA signaling through the GABAA receptors led to an entry in proliferation of LeXbright cells (Daynac et al., 2013). Interestingly, transcripts for several GABAA receptor chains ($\alpha 4$, $\beta 1$, $\gamma 1$ and $\gamma 3$) were expressed in LeXbright cells with the $\gamma 1$ chain (Gabrg1) being almost 40 times higher in comparison to LeX+EGFR⁺ cells (data not shown). Moreover, Lrig1, a pan ErbB inhibitor that has been used as a marker of quiescent stem cells in the epidermis as well as in the intestine and was shown to negatively regulate proliferation (Jensen and Watt, 2006; Powell et al., 2012), was also enriched in LeXbright cells. Another receptor well described in neurogenesis is Ptch1, a member of the patched gene family and main receptor for sonic hedgehog (Shh), overexpressed in LeXbright cells (Ahn and Joyner, 2005; Balordi and Fishell, 2007; Ferent et al., 2014). We have recently shown that the activation of the SHH pathway through deletion of the Patched receptor in NSCs resulted in an increase of the pool of quiescent NSCs (Daynac et al., 2016b).

This receptors could therefore be used as markers of quiescent NSCs and/or could act as putative regulators of the balance between quiescence and proliferation.

Syndecan-1 is a marker of proliferating NSCs

Syndecan family members were among the receptors that were found differentially expressed between LeXbright and LeX+EGFR⁺ cells. The pattern of expression of three out of the four members of the syndecan family was particularly interesting as they were either enriched in LeXbright cells (Sdc2 and Sdc4) or strongly upregulated in LeX+EGFR⁺ cells (Sdc1), hinting at the possibility of their use as markers to discriminate quiescent NSCs from proliferating NSCs (Fig. 1). However, SDC2 and SDC4 were found expressed on the vast

majority of quiescent and activated NSCs at the protein level (Fig. 1) suggesting that they undergo complex post-transcriptional/post-translational regulation in these cells. By contrast, the specificity of SDC1 expression in actively dividing LeX+EGFR+ cells was confirmed at the protein level (Fig. 1).

To further determine if Sdc1 was linked to the proliferative status of NSCs, we modeled NSC quiescence in culture with BMP4 as previously described (Martynoga et al., 2013; Mathieu et al., 2008) (data not shown). We confirmed that addition of BMP4 to proliferating SVZ cells drastically reduced the formation of neurospheres and that the total number of cells was decreased (data not shown). Interestingly, Sdc1 transcripts, highly enriched in proliferative cells, significantly decreased after BMP4 treatment (data not shown).

We then sought to evaluate whether SDC1 could be used as a prospective marker of proliferating NSCs using flow cytometry. Irrespective of the enzymatic cocktail used for cell dissociation, cell-membrane bound SDC1 was shed rendering its labeling impossible on adult NSCs (data not shown). As an enzymatic dissociation is required to obtain single cell suspensions of NSCs from adult SVZ, we examined SDC1 expression on NSCs prepared by mechanical dissociation of postnatal day 10 (PN10) SVZ. Importantly, PN10 LeXbright, LeX+EGFR+ cells, as well as EGFR+ had similar FACS profiles to those of adult SVZ although some differences in their frequencies were observed (data not shown). Similarly to what was observed in adult NSCs, SDC4 was expressed on quiescent and proliferating NSCs at PN10 while SDC1 was present on most LeX+EGFR+ cells and absent from the vast majority of LeXbright cells (data not shown).

LeX+EGFR+ as well as LeXbright cells were then sorted according to SDC1 expression and a colony-forming neurosphere assay was performed to assess the clonogenic capacity of the different NSC subpopulations. In accordance with what was previously reported for LeXbright cells isolated from adult SVZ (Daynac et al., 2013), PN10 LeXbright cells very rarely formed neurospheres, regardless of the expression of SDC1 (Fig. 2A). Importantly, a 1.6-fold increase in the number of primary neurospheres was observed for LeX+EGFR+SDC1+ cells as compared with their SDC1-negative counterparts (Fig. 2A). This increase was also found when we performed secondary neurosphere formation, hinting at a higher long-term self-renewal potential of LeX+EGFR+SDC1+ cells (Fig. 2A).

To confirm the role of SDC1 in the proliferation of activated NSCs, we performed silencing experiments using siRNA directed against Sdc1. We verified the efficacy of Sdc1 silencing in neurosphere cultures both at the mRNA and protein levels 24 and 48h after electroporation (Fig. 3A). Interestingly, a decrease in the diameter of neurospheres was

observed after Sdc1 silencing in comparison to a scrambled control siRNA (Fig. 3B). Subsequently, Sdc1 silencing was performed in LeX+EGFR+ cells freshly sorted from adult mice. While their clonogenic capacity was not altered, the total number of cells was reduced at day 7 (Fig. 2B-C) suggesting a role of Sdc1 in the proliferation but not in the activation of activated NSCs. We further monitored the time required for the first cell division of LeX+EGFR+ cells after Sdc1 silencing by time-lapse videomicroscopy and found it significantly delayed by 7.1 hours (Fig. 2D). Altogether, our data hint at a role of Sdc1 in the progression of activated NSCs through the cell cycle.

Activated neural stem cells express syndecan-1 in different regions of the ventricular-subventricular zone of the postnatal mouse brain.

To further investigate whether syndecan-1 (SDC1) is involved in proliferation of Neural Stem Cells (NSCs) from different brain regions, the medial (MW) and the lateral walls (LW) of the V-SVZ were dissected from postnatal day-10 mouse forebrains (Fig 4A). Relative contents of quiescent NSCs (LeX⁺), activated NSCs (LeX⁺EGFR⁺) and TAPs (EGFR⁺) were determined by flow cytometry thanks to CD24/LeX/EGF triple staining (Daynac et coll. 2013) and revealed no difference between LW and MW (Fig. 4B). In addition, LeX⁺EGFR⁺ and EGFR⁺ sorted populations had the same clonogenic capacity, regardless LW or MW, whereas LeX⁺ cells rarely initiated neurospheres (Fig. 4C). SDC1 analysis by flow cytometry revealed its presence on the majority of proliferating LeX⁺EGFR⁺ and EGFR⁺ cells, disregarding MW or LW origins, whereas it was absent from most of LeX⁺ quiescent cells (Fig. 4D). The SDC1 expression was also confirmed on almost all cultured NSCs from LW (Fig. 4E) and MW by immunofluorescence (Fig. 4F).

Heparanase stimulates proliferation of NSCs.

Heparanase (HPSE) is known to cleave Heparan sulfate chains of SDC1 increasing its shedding and up-regulating growth factor expression signaling (Ramani et al, 2013). Therefore, we tested whether recombinant HPSE (Bio-Techne) or its inhibitor (OGT2115; Bio-Techne) could influence the proliferation of NSCs. LeX⁺EGFR⁺ and EGFR⁺ from MW and LW were pooled and cultured as neurospheres for 6 days then seeded on laminin-coated wells. A dual EdU/BrdU incorporation was performed allowing the identification of the EdU⁺BrdU⁺ population that contains NSCs that were stimulated by the treatment and were not cycling before (i.e. standing in G0/G1 phase) (Fig. 5A). The results showed that HPSE (4 ng/ml) provoked the proliferation of resting NSCs and, conversely, its inhibitor (5μM OGT2115) blocked their proliferation (Fig. 5B). On the other hand, activated NSCs (EdU⁺BrdU⁺) that

already progressed through the cell cycle were neither affected by HPSE nor by OGT2115 (Fig. 5B). Therefore, shedding/recycling of SDC1 might be an approach to induce NSC proliferation.

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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CLAIMS:

1. A method for quantifying neurogenic activity in a subject comprising following steps:
i) measuring the expression level of syndecan-1 in said subject; ii) comparing the
5 expression level measured at step i) with its predetermined reference value, and iii)
concluding that neurogenic activity is stimulated in the subject when the expression
level of syndecan-1 is higher than its predetermined reference value or concluding that
the neurogenic activity is not stimulated when the expression level of syndecan-1 is
lower than its predetermined reference value.
- 10 2. The method according to claim 1 is suitable for diagnosing brain injury, aging-
associated cognitive decline, psychiatric disorders or neurodegenerative disease in a
subject.
3. The method according to claim 1 is suitable for monitoring brain injury, aging-
associated cognitive decline, psychiatric disorders or neurodegenerative disease in a
15 subject.
4. A method for treating brain injury, aging-associated cognitive decline, psychiatric
disorders or neurodegenerative disease in a subject in need thereof comprising a step of
administering said subject with a therapeutically effective amount of a syndecan-1
agonist.
- 20 5. The method according to claim 4 further comprises: i) quantifying neurogenic activity
in a subject according to claim 1 and ii) administering said subject with a therapeutically
effective amount of a syndecan-1 agonist when the neurogenic activity is lower than the
predetermined reference value.
6. The method according to claim 1 to 4, wherein the neurodegenerative disease is selected
25 from the group consisting of Parkinson disease, Alzheimer disease, Huntington disease,
multiple sclerosis.
7. The method according to claim 4, wherein, the syndecan-1 is a small molecule.
8. The method according to claim 4, wherein, the syndecan-1 agonist is a peptide.

9. A method for treating brain injury in a subject in need thereof comprising a step of administering said subject with a therapeutically effective amount of a syndecan-1 antagonist.
- 5 10. The method according to claim 9 further comprises: i) quantifying neurogenic activity in a subject according to the invention and ii) administering said subject with a therapeutically effective amount of a syndecan-1 antagonist when the neurogenic activity is higher than the predetermined reference value.
11. The method according to claim 10, wherein, the brain injury is epilepsy.
- 10 12. A pharmaceutical composition, for use in the treatment of brain injury, aging-associated cognitive decline, psychiatric disorders or neurodegenerative disease, comprising an agonist of syndecan-1.
13. A method for promoting the neurogenesis and/or oligodendrogenesis, comprising the administration of an effective amount of a syndecan-1 agonist.

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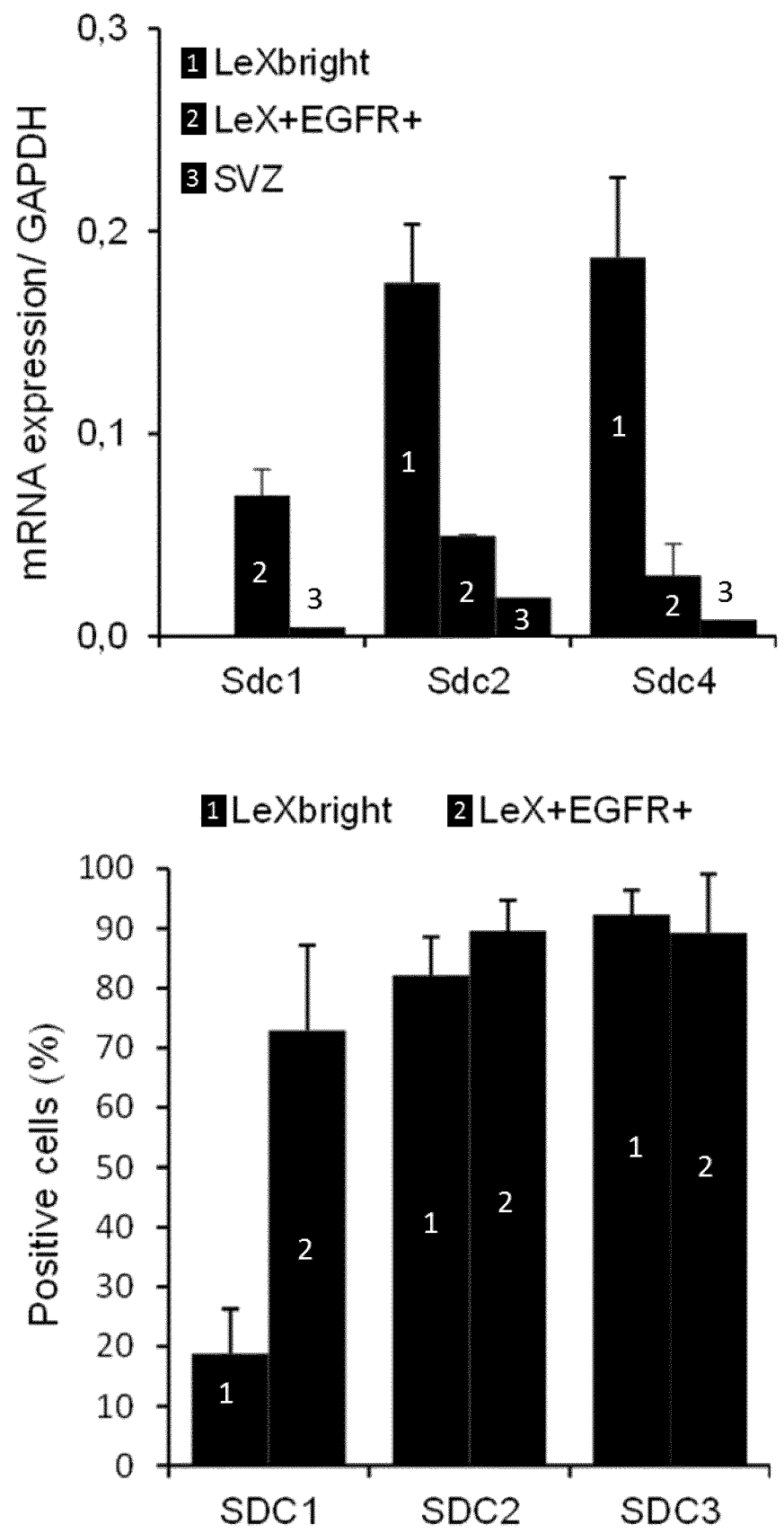


Figure 1

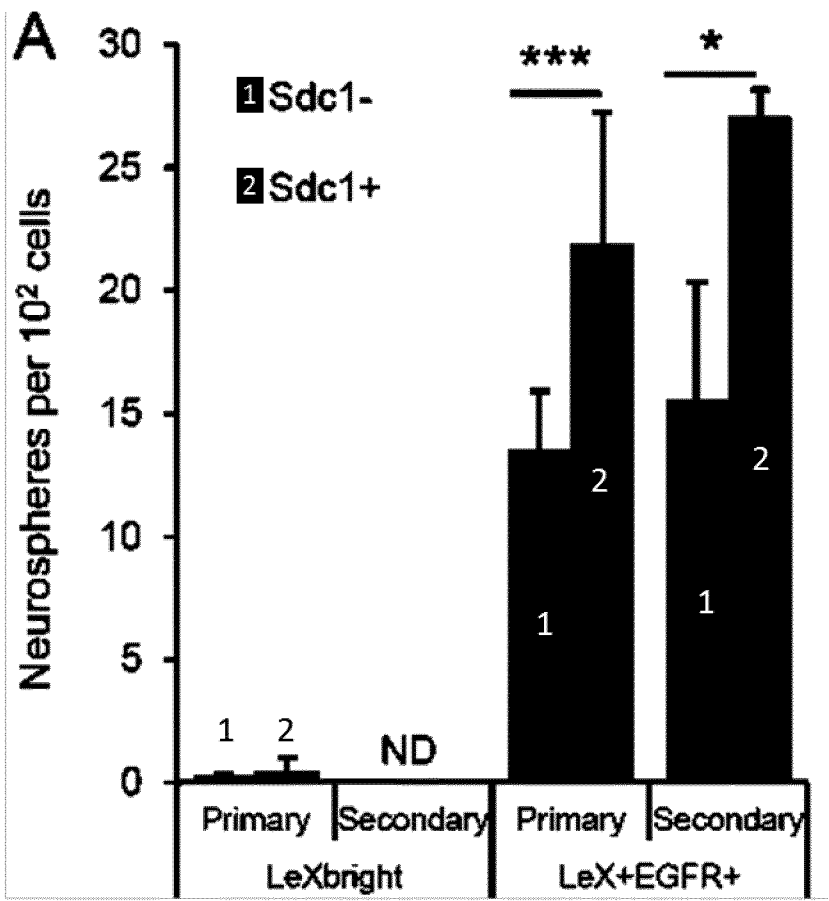


Figure 2A

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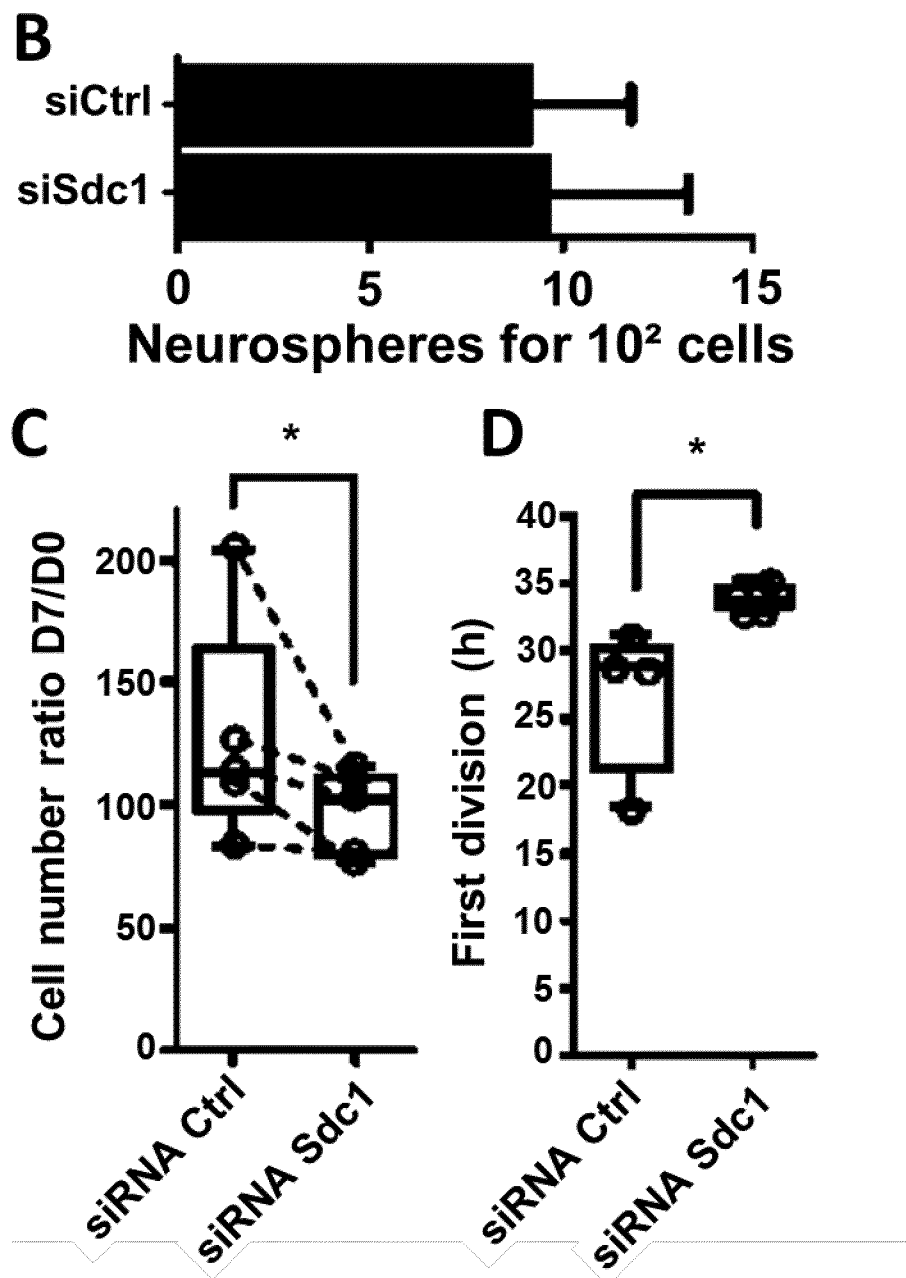


Figure 2B-D

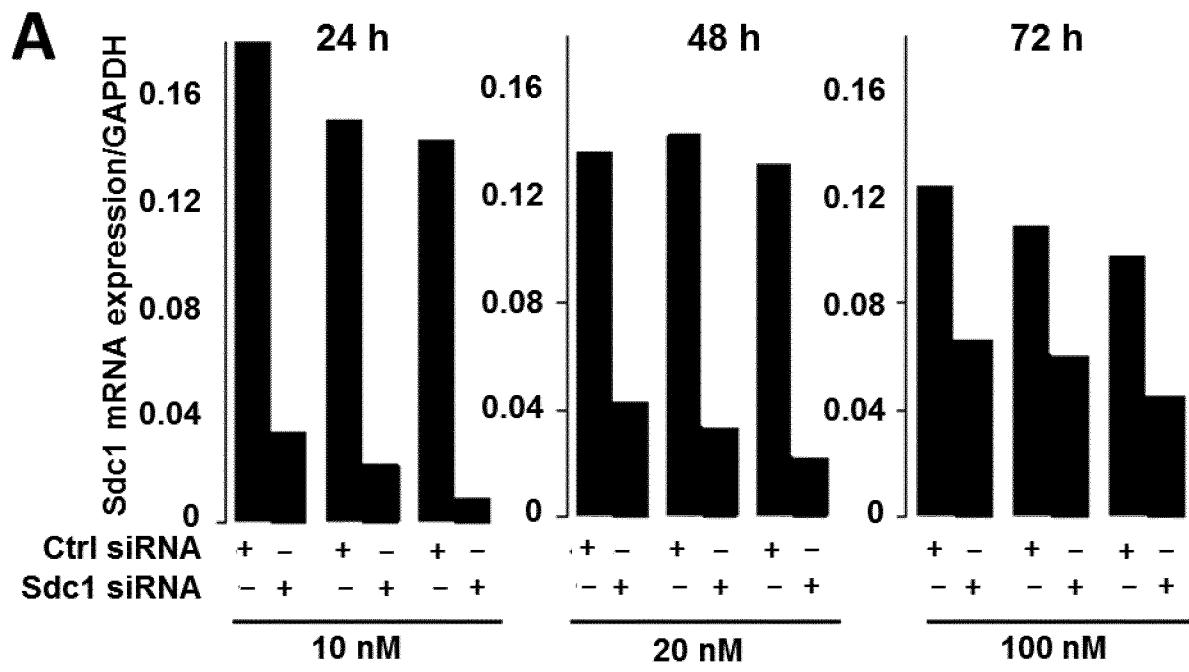


Figure 3A

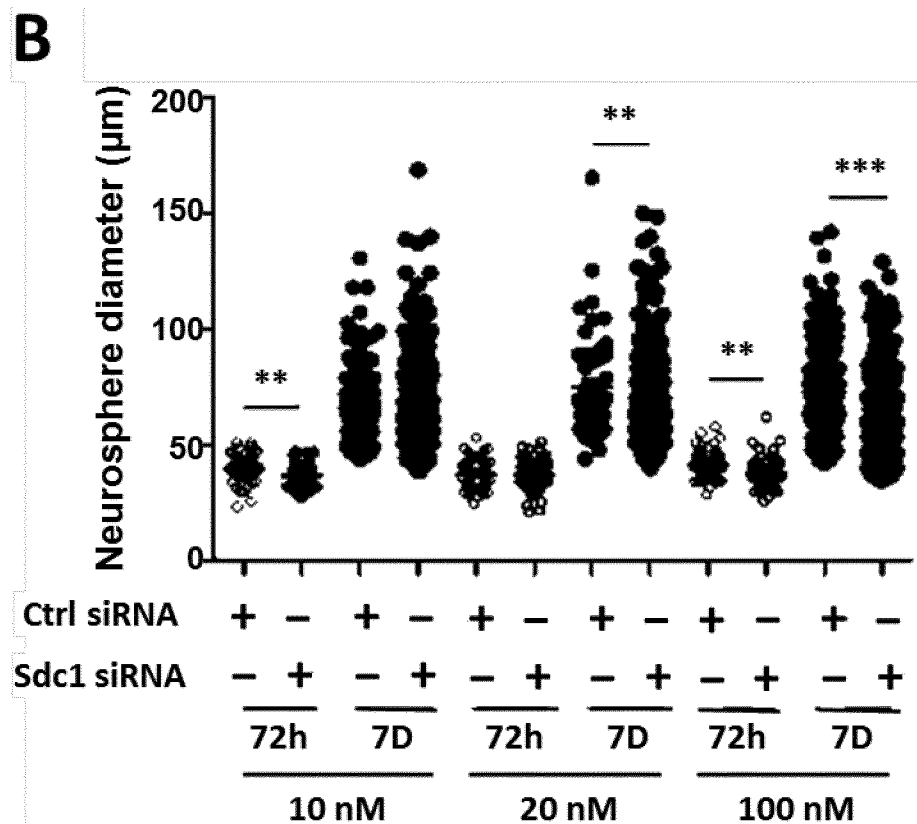


Figure 3B

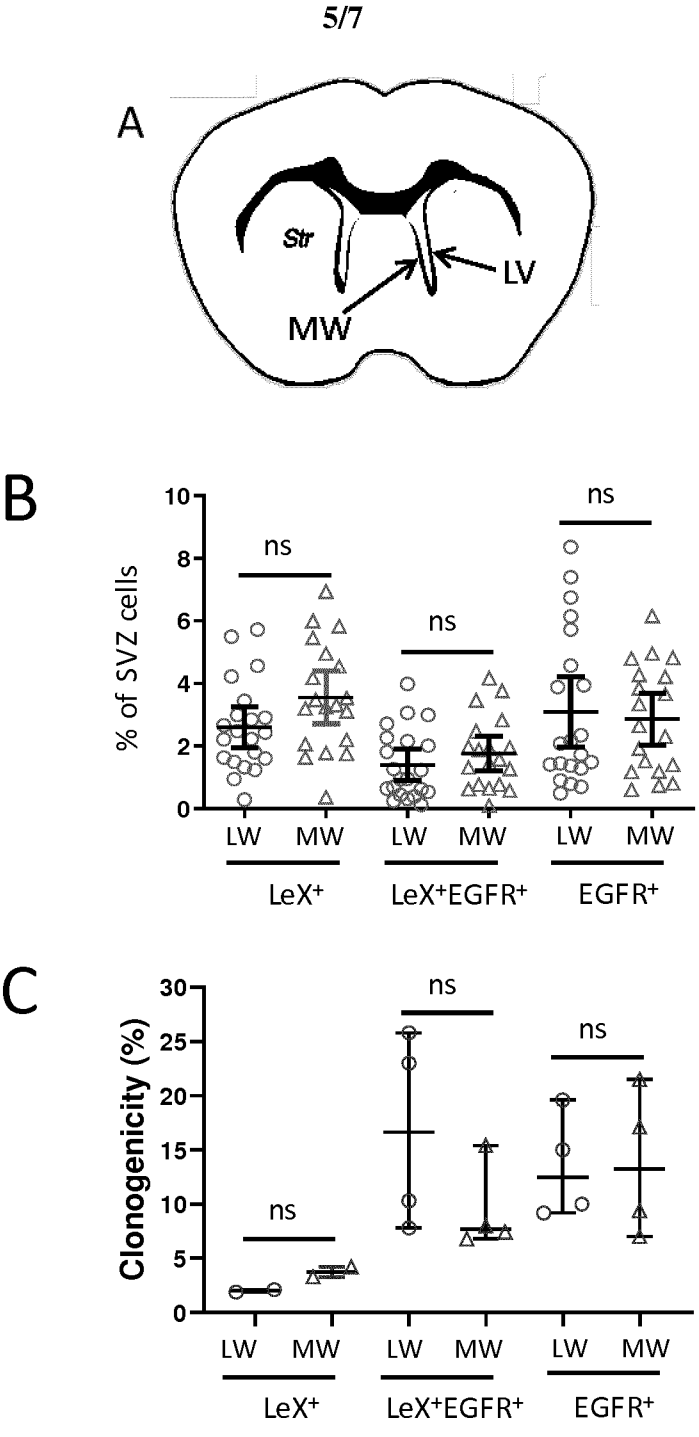


Figure 4A-C

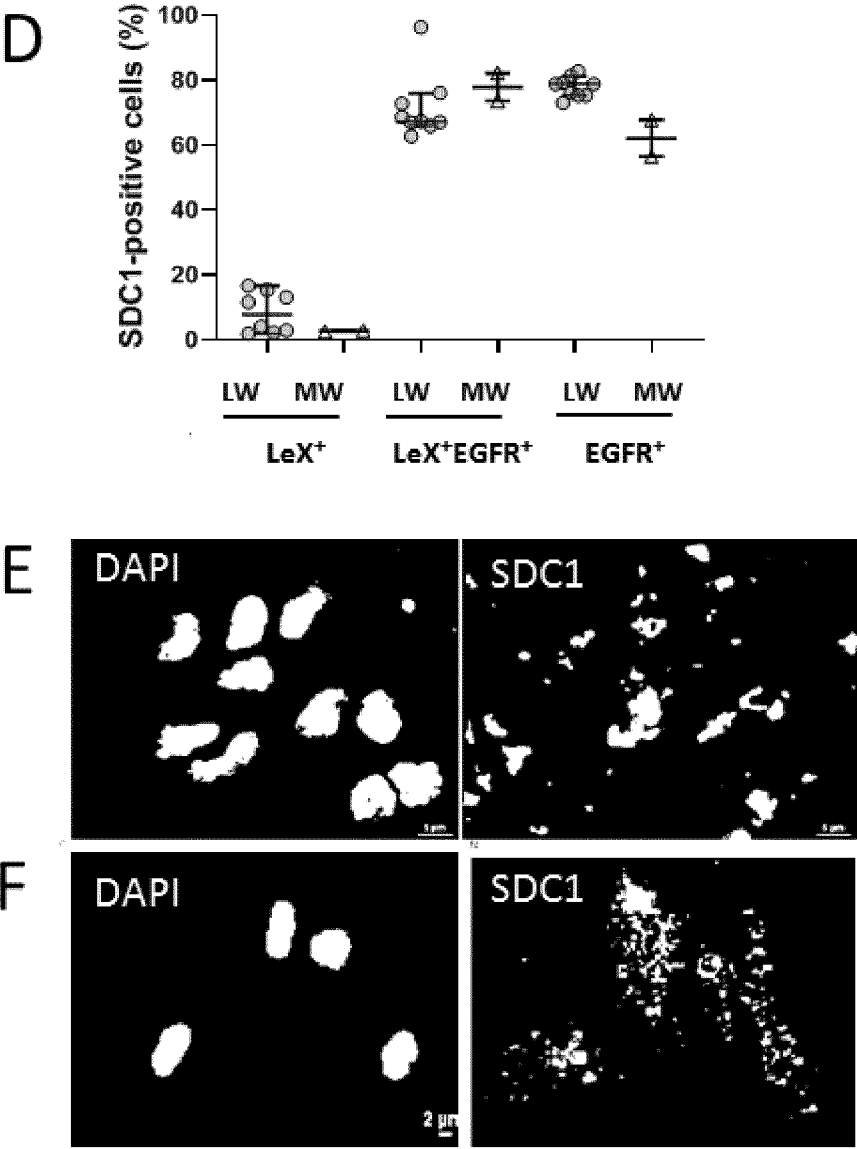


Figure 4D-F

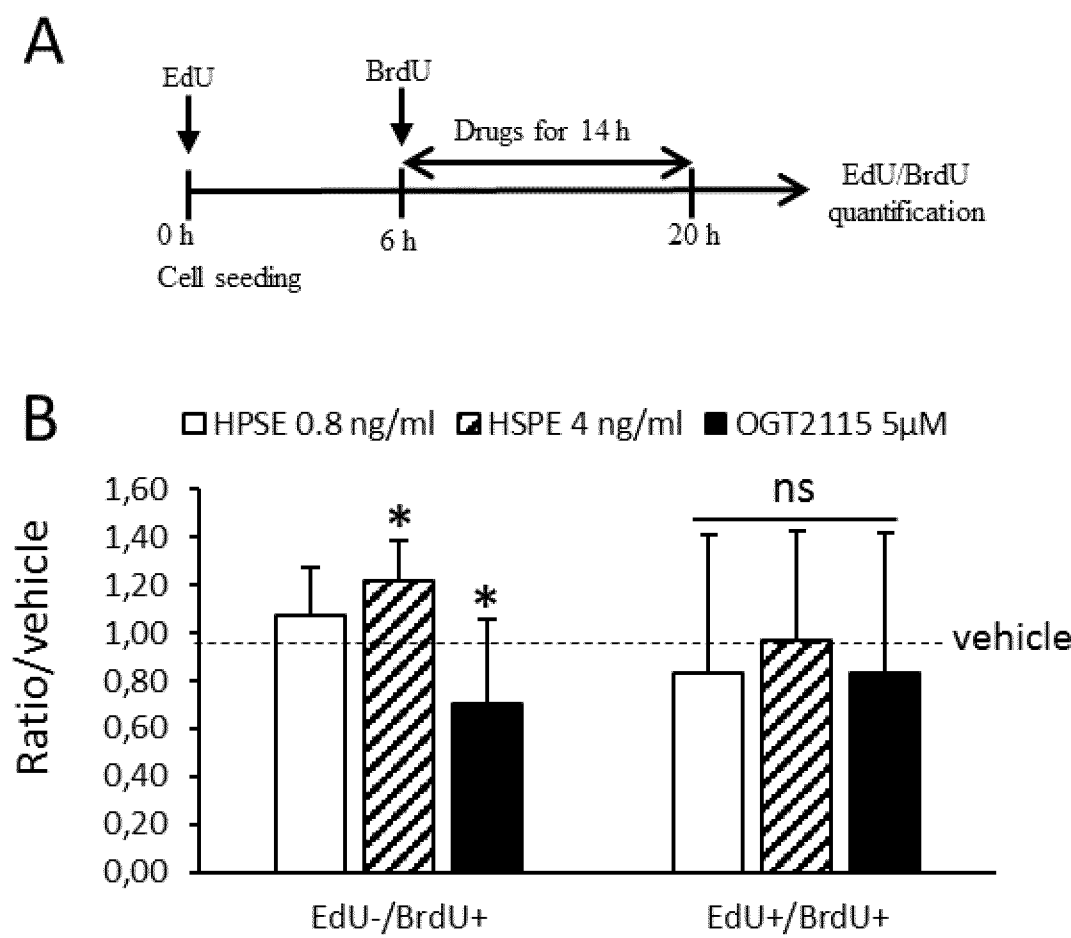


Figure 5A-B

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/067819

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K45/00 A61K39/00 A61P25/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2009/105624 A2 (MASSACHUSETTS INST TECHNOLOGY [US]; EDELMAN ELAZER R [US]; BAKER AARON) 27 August 2009 (2009-08-27) claims 1, 3, 6 -----	1-13
A	WO 01/98539 A2 (HITACHI CHEMICAL CO LTD [JP]; HITACHI CHEMICAL RES CT INC [US]; HITACH) 27 December 2001 (2001-12-27) claim 1 -----	1-13
A	WO 2012/074888 A2 (ALERE SAN DIEGO INC [US]; ARNOLD WILLIAM D [US]; JOST CHRISTELLE [US];) 7 June 2012 (2012-06-07) paragraph [0010] ----- -/-	1-13



Further documents are listed in the continuation of Box C.



See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

1 October 2019

Date of mailing of the international search report

11/10/2019

Name and mailing address of the ISA/

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Authorized officer

Cattell, James

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/067819

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCHOENFELD KURT ET AL: "Indatuximab ravtansine (BT062) combination treatment in multiple myeloma: pre-clinical studies", JOURNAL OF HEMATOLOGY & ONCOLOGY, BIOMED CENTRAL LTD, LONDON UK, vol. 10, no. 1, 1 January 2017 (2017-01-01), page 13, XP009509316, ISSN: 1756-8722, DOI: 10.1186/S13045-016-0380-0 [retrieved on 2017-01-11] abstract -----	9
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Information on patent family members

International application No

PCT/EP2019/067819

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