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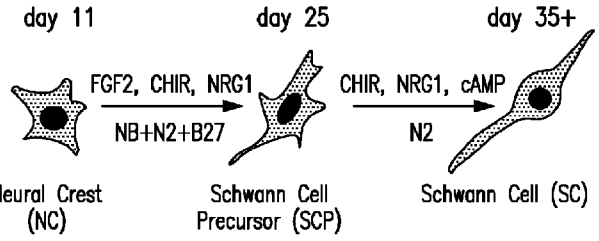

FIG. 1A

(57) Abstract: The presently disclosed subject matter relates to use of Schwann cell precursors and Schwann cells derived from stem cells (e.g., human stem cells) for drug discovery in regeneration of peripheral nervous system (PNS) and/or central nervous system (CNS), prevention and/or repair myelin damages, and/or prevention and/or treatment of Schwann cell-related disorder (e.g., peripheral neuropathy diabetic peripheral neuropathy).

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METHODS FOR DRUG DISCOVERY USING STEM CELL-DERIVED SCHWANN CELLS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application No. 62/421,816 filed November 14, 2016, and the content of which is hereby incorporated by reference in its entireties herein, and to each of which priority is claimed.

INTRODUCTION

The presently disclosed subject matter relates to use of Schwann cell precursors and Schwann cells derived from stem cells (e.g., human stem cells) for drug discovery in regeneration of peripheral nervous system (PNS) and/or central nervous system (CNS), prevention and/or repair of myelin damages, and/or prevention and/or treatment of Schwann cell-related disorder (*e.g.*, peripheral neuropathy diabetic peripheral neuropathy). It further provides for methods of treatment of Schwann cell related disorders using compounds identified by said drug discovery materials and methods.

BACKGROUND

Schwann cells (SCs) are the glia of the peripheral nervous system (PNS) and essential for PNS function. They develop from the neural crest (NC) via a Schwann cell precursor (SCP) intermediate. SCs play crucial roles in functional regulation, maintenance and repair of the PNS and exhibit a remarkable ability to promote neural repair following injury (Jessen et al., 2015; Lavdas et al., 2008). SC defects are involved in a broad range of human disorders such as Schwannomatosis, Charcot Marie Tooth Disease, Guillain Barre Syndrome and various other peripheral neuropathies including Diabetic Peripheral Neuropathy (DPN).

Diabetes Mellitus is the leading cause of peripheral neuropathy, affecting 30% (Callaghan et al., 2012) to 60% (Zochodne, 2007) of diabetic patients. It represents a major health problem causing reduced quality of life and increased morbidity and mortality (La Fontaine et al., 2014). Medical costs related to DPN in the US were estimated at $4.6 - $13.7 billion per year in 2001 and continue to increase (Gordois et al., 2003) The symptoms of DPN are diverse but include sensory dysfunction and pain as well as autonomic and ENS complications.

There are currently no effective treatments for DPN other than pursuing the primary goal of preventing further damage by carefully monitoring and adjusting glucose

levels. Symptomatic treatments include the use of antidepressants, anticonvulsants as well as opioids to cope with the neuropathic pain.

The pathogenesis of DPN likely involves several complex contributing factors that lead to cytotoxicity and degeneration in peripheral nerves (Simmons and Feldman, 2002). There is evidence that hyperglycemia, hypoxia and oxidative stress in diabetes lead to degeneration of SCs particularly in the sensory nerves (Eckersley, 2002). While the ultimate symptoms arise from dysfunction of the neurons, it is unclear whether sensory neurons or glia play a key role in the pathogenesis of DPN (Eckersley, 2002). Dissecting cell type specific mechanisms is very challenging in current animal models of DPN given the complex contribution of non-cell autonomous factors including systemic vascular abnormalities to the disease phenotype. Therefore, there remains a need for an *in vitro* disease model for DPN.

SUMMARY OF THE INVENTION

The presently disclosed subject matter relates to the discovery that Schwann cell precursors and Schwann cells derived from stem cells (e.g., human stem cells), by *in vitro* differentiation, can be used in a disease model for screening a suitable drug for regeneration of peripheral nervous system (PNS) and/or central nervous system (CNS), prevention and/or repair myelin damages, and/or preventing and/or treating a Schwann cell related disorder (*e.g.*, peripheral neuropathy, e.g., diabetic peripheral neuropathy ("DPN")). It further relates to methods of treating Schwann cell related disorders using molecules identified by said materials and methods.

The presently disclosed subject matter provides *in vitro* methods of screening a compound suitable for regeneration of peripheral nervous system (PNS), for regeneration of central nervous system (CNS), for prevention and/or repair of myelin damage, and/or for preventing and/or treating a Schwann cell related disorder. In certain embodiments, the method comprises:

(a) exposing a population of cells expressing one or more Schwann cell precursor marker to a glucose concentration of at least about 30 mM, wherein the cells are obtained from *in vitro* differentiation of stem cells;

(b) contacting the cells with a test compound after glucose exposure;

(c) measuring one or more of first sorbitol level, first glucose level, and first cell viability of the cells without treatment of the test compound;

(d) measuring one or more of second sorbitol level, second glucose level, and second cell viability of the cells treated with the test compound;

(e) comparing one or more of the followings:

(i) the second sorbitol level with the first sorbitol level,

(ii) the second glucose level with the first glucose level,

(iii) the second cell viability with the first cell viability; and

(f) identifying a test compound that is suitable for regeneration of PNS and/or CNS, for prevention and/or repair of myelin damage, and/or for preventing and/or treating of a Schwann cell related disorder where one or more of the followings is present:

(i) the second sorbitol level is lower than the first sorbitol level,

(ii) the second glucose level is lower than the first glucose level, and

(iii) the second cell viability is lower than first cell viability.

In certain embodiments, the method comprises: (a) exposing a population of cells expressing one or more Schwann cell marker to a glucose concentration of at least about 30 mM, wherein the cells are obtained from *in vitro* differentiation of stem cells;

(b) contacting the cells with a test compound after glucose exposure;

(c) measuring one or more of first sorbitol level, first glucose level, and first cell viability of the the cells without treatment of the test compound;

(d) measuring one or more of second sorbitol level, second glucose level, and second cell viability of the the cells treated with the test compound;

(e) comparing one or more of the followings:

(i) the second sorbitol level with the first sorbitol level,

(ii) the second glucose level with the first glucose level,

(iii) the second cell viability with the first cell viability; and

(f) identifying a test compound that is suitable for regeneration of PNS and/or CNS, for prevention and/or repair of myelin damage, and/or for prevention and/or treatment of a Schwann cell related disorder where one or more of the followings is present:

(i) the second sorbitol level is lower than the first sorbitol level,

(ii) the second glucose level is lower than the first glucose level, and

(iii) the second cell viability is lower than first cell viability.

In certain embodiments, the cells expressing one or more Schwann cell precursor marker are obtained from *in vitro* differentiation of stem cells by a method comprising: contacting a population of stem cells with one or more inhibitor of TGFβ/Activin-Nodal

signaling and contacting the cells with one or more Wnt activator, and further contacting the cells with one or more FGF activator for at least about 3 days.

In certain embodiments, the cells expressing one or more Schwann cell marker are obtained from *in vitro* differentiation of stem cells by a method comprising: contacting a population of stem cells with one or more inhibitor of TGFβ/Activin-Nodal signaling and contacting the cells with one or more Wnt activator, further contacting the cells with one or more FGF activator for at least about 3 days to produce the population of differentiated cells expressing one or more Schwann cell precursor marker, and subjecting the population of differentiated cells expressing one or more Schwann cell precursor marker to conditions favoring maturation of Schwann cells precursor cells into Schwann cells.

In certain embodiments, the method for differentiating the stem cells comprises contacting the cells with the one or more FGF activator for about 14 days. In certain embodiments, the method for differentiating the stem cells comprises initially contacting the cells with the one or more FGF activator no later than about 20 days from the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling. In certain embodiments, the method for differentiating the stem cells comprises initially contacting the cells with the one or more FGF activator between about 10 days and about 15 days from the initial contact of the stem cells with the one or more one or more inhibitor of TGFβ/Activin-Nodal signaling In certain embodiments, the method for differentiating the stem cells comprises initially contacting the cells with the one or more FGF activator about 11 days from the initial contact of the stem cells with the one or more one or more inhibitor of TGFβ/Activin-Nodal signaling.

In certain embodiments, the method further comprises contacting the cells with one or more SC differentiation inducer to produce a population of cells expressing one or more SC precursor marker. In certain embodiments, the method for differentiating the stem cells comprises contacting the cells with the one or more SC differentiation inducer for at least about 3 days to produce a population of differentiated cells that express one or more Schwann cell precursor marker. In certain embodiments, the method for differentiating the stem cells comprises contacting the cells with the one or more SC differentiation inducer for about 14 days. In certain embodiments, the method for differentiating the stem cells comprises initially contacting the cells with the one or more SC differentiation inducer between about 10 days and about 15 days from the initial contact of the stem cells with the one or more one or more inhibitor of

TGFβ/Activin-Nodal signaling. In certain embodiments, the method for differentiating the stem cells comprises contacting the cells with the one or more FGF activator and the one or more SC differentiation inducer concurrently.

In certain embodiments, the population of stem cells are differentiated into a population of differentiated cells that express one or more the Schwann cell precursor marker on or after about 25 days from the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling.

In certain embodiments, the method for differentiating the stem cells further comprises contacting the stem cells with one or more SMAD inhibitor. In certain embodiments, the method for differentiating the stem cells comprises contacting the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling and the one or more SMAD inhibitor concurrently.

In certain embodiments, the method for differentiating the stem cells comprises initially contacting the cells with the one or more Wnt activator no later than about 4 days from the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling. In certain embodiments, the method for differentiating the stem cells comprises initially contacting the cells with the one or more activator of Wnt signaling about 2 days from the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling. In certain embodiments, the method for differentiating the stem cells comprises initially contacting the cells with the one or more activator of Wnt signaling on the same day as the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling.

In certain embodiments, the one or more inhibitor of TGFβ/Activin-Nodal signaling is a small molecule selected from the group consisting of SB431542, derivatives thereof, and mixtures thereof. In certain embodiments, the one or more inhibitor of TGFβ/Activin-Nodal signaling is SB431542.

In certain embodiments, the one or more SMAD inhibitor is a small molecule selected from the group consisting of LDN193189, derivatives thereof, and mixtures thereof. In certain embodiments, the one or more SMAD inhibitor is a LDN193189.

In certain embodiments, the one or more Wnt activator lowers glycogen synthase kinase 3β (GSK3β) for activation of Wnt signaling. In certain embodiments, the one or more Wnt activator is a small molecule selected from the group consisting of CHIR99021,

Wnt-1, WNT3A, Wnt4, Wnt5a, derivatives thereof, and mixtures thereof. In certain embodiments, the one or more Wnt activator is CHIR99021.

In certain embodiments, the one or more SC differentiation inducer is selected from the group consisting of neuregulins, LIF, CNTF, Forskolin, TGFβ and FBS. In certain embodiments, the one or more SC differentiation inducer is NRG1.

In certain embodiments, the one or more FGF activator is selected from the group consisting of FGF1, FGF2, FGF3, FGF4, FGF7, FGF8, FGF10, FGF18, derivatives thereof, and mixtures thereof. In certain embodiments, the one or more FGF activator is FGF2.

In certain embodiments, the one or more Schwann cell precursor marker is selected from the group consisting of SOX10, GAP43, BLBP, MPZ, Dhh, P75NTR, CD49D, TFAP2, CDH19, CD44, ERBB3, POU3F1, GFAP, CALCB, GRP116, TSPYL5, ITPKA, SLC17A6, SYPL2, LOC100128252, ANGPTL7, LOC728978, ZNF502, SLC16A6, LPL, SLC30A2, and SLC10A4. In certain embodiments, the one or more Schwann cell precursor marker is selected from the genes listed in Tables 1-4. In certain embodiments, the one or more Schwann cell precursor marker is selected from the genes listed in Table 1. In certain embodiments, the one or more Schwann cell precursor marker is selected from the group consisting of CALCB, GRP116, TSPYL5, ITPKA, SLC17A6, SYPL2, LOC100128252, ANGPTL7, LOC728978, and ZNF502.

In certain embodiments, the stem cells are human stem cells. In certain embodiments, the human stem cells are selected from the group consisting of human embryonic stem cells, human induced pluripotent stem cells, human parthenogenetic stem cells, primordial germ cell-like pluripotent stem cells, epiblast stem cells, F-class pluripotent stem cells.

In certain embodiments, the conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise: contacting the cells with one or more FGF activator, and one or more Schwann cell differentiation inducer. In certain embodiments, the conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the cells with the one or more FGF activator and the one or more Schwann cell differentiation inducer for at least about 3 days. In certain embodiments, the conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the cells with the one or more FGF activator and the one or more Schwann cell differentiation inducer for about 10 days. In certain embodiments, the

conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the cells with the one or more FGF activator and the one or more Schwann cell differentiation inducer for about 35 days.

In certain embodiments, the conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise further contacting the cells with one or more SC differentiation enhancer. In certain embodiments, the conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the cells with the one or more SC differentiation enhancer for at least about 3 days. In certain embodiments, the conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the cells with the one or more SC differentiation enhancer for about 10 days. In certain embodiments, the conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the cells with the one or more SC differentiation enhancer for about 35 days. In certain embodiments, the conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the population cells with the one or more FGF activator, the one or more Schwann cell differentiation inducer, and the one or more SC differentiation enhancer concurrently.

In certain embodiments, the conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise further contacting the cells with one or more SC differentiation enhancer. In certain embodiments, the one or more SC differentiation enhancer is selected from the group consisting of neuregulins, cyclic adenosine monophosphate (cAMP), Forskolin, LIF, and CNTF. In certain embodiments, the one or more SC differentiation enhancer is cAMP.

In certain embodiments, the conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprises aggregating the cells into 3D spheroids; and contacting the 3D spheroids with the one or more FGF activator, and the one or more Schwann cell differentiation inducer. In certain embodiments, the conditions favoring maturation of Schwann cell precursor cells into Schwann cells further comprises culturing the 3D spheroids in adherent culture. In certain embodiments, the one or more Schwann cell marker is selected from the group consisting of LRRTM4, CDH1, FABP7, BDNF, UNCB5, SOSTDC1, OLIG1, PLAT, KCNJ10, SHH, NTN1, GDNF, ERBB3, GAP43, SOX10, S100, GFAP, POU3F1, PMP22, MBP, AQP4, MPZ, NGFR, NFATC4, MOG, IFNG, MAL, NTF3, TGFB1 , CD9, CD81, CD44, CD98, CD49E, CD49D, TYRP1,

ENTHD1, NT5E, HTR2B, NOV, IL8, SLC16A6, CDKN2A, PLP2, S100A6, AQP9, and CDH19. In certain embodiments, the one or more SC marker is selected from the genes listed in Tables 1-4. In certain embodiments, the one or more SC marker is selected from the genes listed in Tables 2-4. In certain embodiments, the one or more SC marker is selected from the group consisting of TYRP1, CD44, ENTHD1, NT5E, HTR2B, NOV, IL8, SLC16A6, and CDKN2A.

In certain embodiments, the glucose concentration is at least about 10 mM. In certain embodiments, the glucose concentration is about 30 mM. In certain embodiments, the measurement is performed at least about 12 hours from the initial exposure of the cells to the glucose. In certain embodiments, the measurement is performed at about 72 hours from the initial exposure of the cells to the glucose.

In certain embodiments, the Schwann cell related disorder is selected from the group consisting of peripheral neuropathy, Schwannomatosis, Charcot-Marie-Tooth Disease, and Guillain-Barre Syndrome. In certain embodiments, the Schwann cell related disorder is a peripheral neuropathy. In certain embodiments, the peripheral neuropathy disorder is a diabetic peripheral neuropathy.

In certain embodiments, the presently disclosed subject matter also provides a method of regeneration of peripheral nervous system (PNS), for regeneration of central nervous system (CNS), for prevention and/or repair of myelin damage, and/or for preventing and/or treating a Schwann cell related disorder in a subject, comprising administering an effective amount of a compound or a composition comprising thereof to the subject suffering from the Schwann cell related disorder, wherein the compound is selected from the group consisting of potassium channel blockers, norepinephrine-dopamine reuptake inhibitors, cyclopenthiazide, captopril, isradipine, condelphine, nimesulide, triamcinolone, salts thereof, solvates thereof, hydrate thereof, clathrates thereof, and prodrugs thereof, and combinations thereof.

In certain embodiments, the potassium channel blocker is a sulfonylurea compound. In certain embodiments, the sulfonylurea compound is selected from the group consisting of tolbutamide, acetohexamide, carbutamide, chlorpropamide, glycyclamide (tolhexamide), metahexamide, tolazamide, glibenclamide (glyburide), glibornuride, gliclazide, glipizide, gliquidone, glisoxepide, glyclopyramide, glimepiride, salts thereof, solvates thereof, hydrate thereof, clathrates thereof, and prodrugs thereof.

In certain embodiments, the norepinephrine-dopamine reuptake inhibitor is

selected from the group consisting of bupropion, amineptine, methylphenidate (Ritalin®, Concerta®, Metadate®, Methylin®, Rubifen®, or Stimdate®), atomoxetine, maprotiline, desoxypipradrol, dexmethylphenidate, difemetorex, diphenylprolinol, ethylphenidate, fencamfamine, fencamine, lefetamine, methylenedioxypyrovalerone, methylphenidate, nomifensine, O-2172, oxolinic acid, pipradrol, prolintane, pyrovalerone, tametraline, WY-46824, salts thereof, solvates thereof, hydrate thereof, prodrugs thereof, and clathrates thereof.

In certain embodiments, the Schwann cell related disorder is selected from the group consisting of peripheral neuropathy, Schwannomatosis, Charcot-Marie-Tooth Disease, and Guillain-Barre Syndrome. In certain embodiments, the Schwann cell related disorder is a peripheral neuropathy. In certain embodiments, the peripheral neuropathy is diabetic peripheral neuropathy.

In certain embodiments, the compound is bupropion, a salt, a solvate, a hydrate, a clathrate, or a prodrug thereof. In certain embodiments, the compound is a bupropion hydrochloride. In certain embodiments, the compound is a bupropion metabolite, or a salt, a solvate, a hydrate, a clathrate, or a prodrug thereof. In certain embodiments, the bupropion metabolite is selected from the group consisting of hydroxybupropion, threo-hydrobupropion, and erythrohydrobupropion. In certain embodiments, the potassium channel blocker is tolbutamide, a salt, a solvate, a hydrate, a clathrate, or a prodrug thereof.

In certain embodiments, the composition is a pharmaceutical composition that further comprises a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1I: Deriving SCs from hESCs. **(1A)** Schematic illustration of the protocol (day 11 - 35) for deriving Schwann cell precursors and Schwann cells. **(1B)** SOX10::GFP expression at day 11, 25 and 35 of differentiation. **(1C, 1D)** qRT-PCR for a panel of Schwann lineage markers involved in Schwann cell differentiation and myelination **(1C)** and nerve interaction and support **(1D)** Immunofluorescence of unsorted and CD49D sorted differentiated NC cells for SOX10. **(1E)** Representative immunofluorescence images of hESC-derived SCs for Schwann lineage markers at day 60. F) Quantification of markers in **(1E)**. **(1G)** Principal component analysis of CD49d purified NC, CD49d purified SCP, human primary Schwann cells, and CD98 purified hESC-derived SC at day 50 and day 100 of differentiation in comparison with CNS

precursors. **(1H)** Top 10 (normal typeface) and selected additional (bold typeface), significantly upregulated genes in day 25 SCP and day 100 SCs. **(1I)** Quantification of markers in **(1E)**. Scale bars= 100 μm in B left and middle panel and 25 μm in B right panel and E.

Figures 2A-2D: Selective vulnerability of hESC-SCs to high glucose exposure **(2A)** Schematic illustration of the experimental paradigm for modeling diabetic nerve damage in hESC-derived cell types. **(2B)** Cell death analysis of hESC-derived SCs and sensory neurons in response to exposure to different glucose concentration using LDH activity assay. **(2C)** qRT-PCR for Aldose Reductase (AR) and Sorbitol Dehydrogenase (SDH) in undifferentiated hESCs and hESC-derived Schwann cells and sensory neurons. **(2D)** Intracellular sorbitol measurements in hESC-derived SCs and sensory neurons in response to exposure to different glucose concentration.

Figures 3A-3F: Bupropion HCl protects hESC-SCs against glucotoxicity. **(3A, 3E)** Schematic illustration of high throughput drug screening for identification of compounds that enhance the viability of high glucose treated hESC-SCs. **(3B)** Primary screening data showing total number of viable hESC-SCs treated with high glucose and 1280 screened compounds. **(3C)** Dose response analysis of the selected hit compound "Bupropion HCl" for effects on cell death. **(3D)** Dose response analysis of the selected hit compound "Bupropion HCl" for effects on sorbitol levels. **(3F)** List of primary hit compounds (Z-score >3). p-values are: p-value: * $p<0.05$; ** $p<0.01$.

Figures 4A-4D: Impact of Bupropion on glucose metabolism and polyol pathway. Bupropion HCl reduces the sorbitol levels by increasing the glycolytic flux **(4A-4C)** Measurements of intracellular glucose levels **(4A)**, glucose reuptake **(4B)** and pyruvate **(4C)** in response to Bupropion HCl. **(4D)** Schematic illustration of the model of Bupropion impact on glucose metabolism in SCs. p-values are: p-value: * $p<0.05$; ** $p<0.01$.

Figures 5A-5G: Bupropion treatment prevents diabetic nerve damage in mice **(5A)** Schematic illustration of modeling diabetes and Bupropion treatment in mice. **(5B)** Blood glucose levels in normal mice and mice treated with STZ and Bupropion. **(5C)** Thermal sensitivity test measuring the latency of hind paw withdrawal in normal mice and mice treated with STZ and Bupropion. **(5D, 5E)** TUNEL staining and quantification in sciatic nerves of normal mice and mice treated with STZ and Bupropion. **(5F, 5G)** Electron transmission microscopy and quantification of damages myelin structures in sciatic nerves

of normal mice and mice treated with STZ and Bupropion. p-values are: * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p < 0.000$. Scale bars= 100 μm in D and 5 μm in F. BP, Bupropion HCL.

Figures 6A-6B: Characterization of hESC-derived SCP and SC lineages. **(6A)** Flow cytometry analysis of SOX10::GFP in hESC-derived NC (day11) and SCP (day25). **(6B)** Flow cytometry analysis of GFAP in hESC-derived SCs at different time points during *in vitro* differentiation.

Figures 7A-7C: Antibody screen identifies novel surface markers for human SCs. **(7A)** Schematic illustration of the antibody screening paradigm. **(7B)** Primary screening identifies novel surface markers for hESC-SCs. **(7C)** Immunocytochemistry and flow cytometry-based validation of surface marker expression at different stages of SC differentiation.

DETAILED DESCRIPTION OF THE INVENTION

The presently disclosed subject matter relates to use of stem cells-derived Schwann cell precursors and/or Schwann cells for drug discovery in preventing and/or treating a Schwann cell related disorder (*e.g.*, peripheral neuropathy, e.g., diabetic peripheral neuropathy). The presently disclosed subject matter also relates to use of the compounds screened from such drug discovery methods and pharmaceutical compositions of such compounds for preventing and/or treating a Schwann cell related disorder (*e.g.*, peripheral neuropathy, e.g., diabetic peripheral neuropathy).

For purposes of clarity of disclosure and not by way of limitation, the detailed description is divided into the following subsections:

1. Definitions
2. Method of Differentiating Stem Cells
3. Method of Screening Therapeutic Compounds
4. Method of Treatments
5. Kits

1. Definitions

The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them.

The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, "about" can mean within 3 or more than 3 standard deviations, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, e.g., up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, e.g., within 5-fold, or within 2-fold, of a value.

As used herein, the term "signaling" in reference to a "signal transduction protein" refers to a protein that is activated or otherwise affected by ligand binding to a membrane receptor protein or some other stimulus. Examples of signal transduction protein include, but are not limited to, a Fibroblast Growth Factor (FGF), a SMAD, a wingless (Wnt) complex protein, including beta-catnin, NOTCH, transforming growth factor beta (TGFβ), Activin, Nodal and glycogen synthase kinase 3β (GSK3P) proteins. For many cell surface receptors or internal receptor proteins, ligand-receptor interactions are not directly linked to the cell's response. The ligand activated receptor can first interact with other proteins inside the cell before the ultimate physiological effect of the ligand on the cell's behavior is produced. Often, the behavior of a chain of several interacting cell proteins is altered following receptor activation or inhibition. The entire set of cell changes induced by receptor activation is called a signal transduction mechanism or signaling pathway.

As used herein, the term "signals" refer to internal and external factors that control changes in cell structure and function. They can be chemical or physical in nature.

As used herein, the term "ligands" refers to molecules and proteins that bind to receptors, e.g., TFGβ, Activin, Nodal, bone morphogenic proteins (BMPs), etc.

"Inhibitor" as used herein, refers to a compound or molecule (*e.g.*, small molecule, peptide, peptidomimetic, natural compound, siRNA, anti-sense nucleic acid, aptamer, or antibody) that interferes with (e.g., reduces, decreases, suppresses, eliminates, or blocks) the signaling function of the molecule or pathway. An inhibitor can be any compound or molecule that changes any activity of a named protein (signaling molecule, any molecule involved with the named signaling molecule, a named associated molecule, such as a glycogen synthase kinase 3β (GSK3β)) (e.g., including, but not limited to, the signaling molecules described herein), for one example, via directly contacting SMAD signaling, contacting SMAD mRNA, causing conformational changes of SMAD, decreasing SMAD

protein levels, or interfering with SMAD interactions with signaling partners (e.g., including those described herein), and affecting the expression of SMAD target genes (e.g. those described herein). Inhibitors also include molecules that indirectly regulate SMAD biological activity by intercepting upstream signaling molecules (e.g., within the extracellular domain, examples of a signaling molecule and an effect include: Noggin which sequesters bone morphogenic proteins, inhibiting activation of ALK receptors 1,2,3, and 6, thus preventing downstream SMAD activation. Likewise, Chordin, Cerberus, Follistatin, similarly sequester extracellular activators of SMAD signaling. Bambi, a transmembrane protein, also acts as a pseudo-receptor to sequester extracellular TGFb signaling molecules. Antibodies that block activins, nodal, TGFb, and BMPs are contemplated for use to neutralize extracellular activators of SMAD signaling, and the like. Inhibitors are described in terms of competitive inhibition (binds to the active site in a manner as to exclude or reduce the binding of another known binding compound) and allosteric inhibition (binds to a protein in a manner to change the protein conformation in a manner which interferes with binding of a compound to that protein's active site) in addition to inhibition induced by binding to and affecting a molecule upstream from the named signaling molecule that in turn causes inhibition of the named molecule. An inhibitor can be a "direct inhibitor" that inhibits a signaling target or a signaling target pathway by actually contacting the signaling target.

As used herein, the term "Schwann cell precursor" refers to a cell that express one or more Schwann cell precursor marker, which includes, but not limited to, the Schwann cell precursor markers disclosed herein. Under suitable maturation conditions, Schwann cell precursors can become Schwann cells.

As used herein, the term "Schwann cell" refers to a cell that express one or more Schwann cell marker, which includes, but not limited to, the Schwann cell markers disclosed herein. The Schwann cell can be a myelinating Schwann cell or a non-myelinating Schwann cell. In certain embodiments, the Schwann cells are capable of maintaining and regenerating axons of the neurons in the peripheral nervous system (e.g., maintenance of healthy axons). In certain embodiments, the Schwann cells are capable of forming the myelin sheath. In certain embodiments, the Schwann cells are capable of forming Remak bundles.

"Activators", as used herein, refer to compounds that increase, induce, stimulate, activate, facilitate, or enhance activation the signaling function of the molecule or pathway, e.g., Wnt signaling, or FGF signaling.

As used herein, the term "derivative" refers to a chemical compound with a similar core structure.

As used herein, the term "a population of cells" or "a cell population" refers to a group of at least two cells. In non-limiting examples, a cell population can include at least about 10, at least about 100, at least about 200, at least about 300, at least about 400, at least about 500, at least about 600, at least about 700, at least about 800, at least about 900, at least about 1000 cells. The population may be a pure population comprising one cell type, such as a population of SC precursors, a population of SCs, or a population of undifferentiated stem cells. Alternatively, the population may comprise more than one cell type, for example a mixed cell population, e.g., a mixed population of SC precursors and SCs.

As used herein, the term "stem cell" refers to a cell with the ability to divide for indefinite periods in culture and to give rise to specialized cells. A human stem cell refers to a stem cell that is from a human.

As used herein, the term "embryonic stem cell" refers to a primitive (undifferentiated) cell that is derived from preimplantation-stage embryo, capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers. A human embryonic stem cell refers to an embryonic stem cell that is from a human. As used herein, the term "human embryonic stem cell" or "hESC" refers to a type of pluripotent stem cells ("PSCs") derived from early stage human embryos, up to and including the blastocyst stage, that is capable of dividing without differentiating for a prolonged period in culture, and are known to develop into cells and tissues of the three primary germ layers.

As used herein, the term "embryonic stem cell line" refers to a population of embryonic stem cells which have been cultured under *in vitro* conditions that allow proliferation without differentiation for up to days, months to years.

As used herein, the term "totipotent" refers to an ability to give rise to all the cell types of the body plus all of the cell types that make up the extraembryonic tissues such as the placenta.

As used herein, the term "multipotent" refers to an ability to develop into more than one cell type of the body.

As used herein, the term "pluripotent" refers to an ability to develop into the three developmental germ layers of the organism including endoderm, mesoderm, and ectoderm.

As used herein, the term "induced pluripotent stem cell" or "iPSC" refers to a type of pluripotent stem cell, similar to an embryonic stem cell, formed by the introduction of certain embryonic genes (such as a OCT4, SOX2, and KLF4 transgenes) (see, for example, Takahashi and Yamanaka Cell 126, 663-676 (2006), herein incorporated by reference) into a somatic cell, for examples, CI 4, C72, and the like.

As used herein, the term "somatic cell" refers to any cell in the body other than gametes (egg or sperm); sometimes referred to as "adult" cells.

As used herein, the term "somatic (adult) stem cell" refers to a relatively rare undifferentiated cell found in many organs and differentiated tissues with a limited capacity for both self-renewal (in the laboratory) and differentiation. Such cells vary in their differentiation capacity, but it is usually limited to cell types in the organ of origin.

As used herein, the term "neuron" refers to a nerve cell, the principal functional units of the nervous system. A neuron consists of a cell body and its processes— an axon and one or more dendrites. Neurons transmit information to other neurons or cells by releasing neurotransmitters at synapses.

As used herein, the term "proliferation" refers to an increase in cell number.

As used herein, the term "undifferentiated" refers to a cell that has not yet developed into a specialized cell type.

As used herein, the term "differentiation" refers to a process whereby an unspecialized embryonic cell acquires the features of a specialized cell such as a heart, liver, or muscle cell. Differentiation is controlled by the interaction of a cell's genes with the physical and chemical conditions outside the cell, usually through signaling pathways involving proteins embedded in the cell surface.

As used herein, the term "directed differentiation" refers to a manipulation of stem cell culture conditions to induce differentiation into a particular (for example, desired) cell type, such as SC precursors.

As used herein, the term "directed differentiation" in reference to a stem cell refers to the use of small molecules, growth factor proteins, and other growth conditions to

promote the transition of a stem cell from the pluripotent state into a more mature or specialized cell fate (e.g. SC precursors, SCs, etc.).

As used herein, the term "inducing differentiation" in reference to a cell refers to changing the default cell type (genotype and/or phenotype) to a non-default cell type (genotype and/or phenotype). Thus, "inducing differentiation in a stem cell" refers to inducing the stem cell (e.g., human stem cell) to divide into progeny cells with characteristics that are different from the stem cell, such as genotype (e.g., change in gene expression as determined by genetic analysis such as a microarray) and/or phenotype (e.g., change in expression of a protein, such as SC precursor marker(s) and SC marker(s)).

As used herein, the term "cell culture" refers to a growth of cells *in vitro* in an artificial medium for research or medical treatment.

As used herein, the term "culture medium" refers to a liquid that covers cells in a culture vessel, such as a Petri plate, a multi-well plate, and the like, and contains nutrients to nourish and support the cells. Culture medium may also include growth factors added to produce desired changes in the cells.

As used herein, the term "contacting" cells with a compound (e.g., one or more inhibitor, activator, and/or inducer) refers to placing the compound in a location that will allow it to touch the cell. The contacting may be accomplished using any suitable methods. For example, contacting can be accomplished by adding the compound to a tube of cells. Contacting may also be accomplished by adding the compound to a culture medium comprising the cells. Each of the compounds (e.g., the inhibitors, activators, and inducers disclosed herein) can be added to a culture medium comprising the cells as a solution (e.g., a concentrated solution). Alternatively or additionally, the compounds (e.g., the inhibitors, activators, and inducers disclosed herein) as well as the cells can be present in a formulated cell culture medium.

As used herein, the term "*in vitro*" refers to an artificial environment and to processes or reactions that occur within an artificial environment. *In vitro* environments exemplified, but are not limited to, test tubes and cell cultures.

As used herein, the term "*in vivo*" refers to the natural environment (e.g., an animal or a cell) and to processes or reactions that occur within a natural environment, such as embryonic development, cell differentiation, neural tube formation, etc.

As used herein, the term "expressing" in relation to a gene or protein refers to making an mRNA or protein which can be observed using assays such as microarray

assays, antibody staining assays, and the like.

As used herein, the term "marker" or "cell marker" refers to gene or protein that identifies a particular cell or cell type. A marker for a cell may not be limited to one marker, markers may refer to a "pattern" of markers such that a designated group of markers may identity a cell or cell type from another cell or cell type.

As used herein, the term "derived from" or "established from" or "differentiated from" when made in reference to any cell disclosed herein refers to a cell that was obtained from (e.g., isolated, purified, etc.) a parent cell in a cell line, tissue (such as a dissociated embryo, or fluids using any manipulation, such as, without limitation, single cell isolation, cultured *in vitro*, treatment and/or mutagenesis using for example proteins, chemicals, radiation, infection with virus, transfection with DNA sequences, such as with a morphogen, etc., selection (such as by serial culture) of any cell that is contained in cultured parent cells. A derived cell can be selected from a mixed population by virtue of response to a growth factor, cytokine, selected progression of cytokine treatments, adhesiveness, lack of adhesiveness, sorting procedure, and the like.

An "individual" or "subject" herein is a vertebrate, such as a human or non-human animal, for example, a mammal. Mammals include, but are not limited to, humans, primates, farm animals, sport animals, rodents and pets. Non-limiting examples of non-human animal subjects include rodents such as mice, rats, hamsters, and guinea pigs; rabbits; dogs; cats; sheep; pigs; goats; cattle; horses; and non-human primates such as apes and monkeys.

As used herein, the term "disease" refers to any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ.

As used herein, the term "treating" or "treatment" refers to clinical intervention in an attempt to alter the disease course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Therapeutic effects of treatment include, without limitation, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastases, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. By preventing progression of a disease or disorder, a treatment can prevent deterioration due to a disorder in an affected or diagnosed subject or a subject suspected of having the disorder, but also a treatment may prevent the onset of the disorder or a

symptom of the disorder in a subject at risk for the disorder or suspected of having the disorder.

2. Method of Differentiating Stem Cells To Schwann Cells

The Schwann cell can be obtained from *in vitro* differentiation of stem cells (e.g., human stem cells). In certain embodiments, the stem cell is a human stem cell. Non-limiting examples of human stem cells include human embryonic stem cells (hESC), human pluripotent stem cell (hPSC), human induced pluripotent stem cells (hiPSC), human parthenogenetic stem cells, primordial germ cell-like pluripotent stem cells, epiblast stem cells, F-class pluripotent stem cells, somatic stem cells, cancer stem cells, or any other cell capable of lineage specific differentiation. In certain embodiments, the human stem cell is a human pluripotent stem cell. In certain embodiments, the human stem cell is a human embryonic stem cell (hESC). In certain embodiments, the human stem cell is a human induced pluripotent stem cell (hiPSC). In certain embodiments, the stem cells are non-human stem cells, including, but not limited to, mammalian stem cells, primate stem cells, or stem cells from a rodent, a mouse, a rat, a dog, a cat, a horse, a pig, a cow, a sheep, etc.

Use of dual SMAD inhibition can be used for inducing differentiation of stem cells (e.g., hPSC) to one type of neural lineage, e.g., Chambers (2009), which is incorporated by reference in its entirety. Furthermore, stem cells can be differentiated into neural crest lineage cells (e.g., nociceptors) by sequential inhibition of SMAD signaling followed by activation of Wnt signaling, e.g., Chambers (2012); Mica (2013); WO2011/149762; Fattahi (2016); and U.S. Patent Provisional application No. 62/387,468 filed December 23, 2015, all of which are incorporated by reference in their entireties.

In certain embodiments, the differentiation of stem cells to SCs include three phases: *in vitro* differentiation of stem cells to cells expressing one or more neural crest lineage marker (neural crest lineage cells), *in vitro* differentiation of neural crest lineage cells to SC precursors, and *in vitro* differentiation or maturation of SC precursors to SCs. Any suitable methods for *in vitro* differentiation of stem cells to neural crest lineage cells, including, but not limited to, those disclosed in Chambers (2012); Mica (2013); WO2011/149762; U.S. Patent Provisional application No. 62/387,468 filed December 23, 2015; and Fattahi (2016) can be used in the first phase. In certain embodiments, a population of stem cells is *in vitro* differentiated to a population of neural crest lineage

cells, which is *in vitro* differentiated to a population of SC precursors, which is further induced *in vitro* to a population of SCs.

Non-limiting examples of neural crest lineage marker include SOX10, p75, HNK1, CD49D, ERBB3, TFAP2, SNAIL and SLUG.

In certain embodiments, the neural crest lineage cells are *in vitro* differentiated from stem cells by inhibition of SMAD signaling and activation of Wnt signaling. In certain embodiments, the method comprises contacting a population of stem cells (e.g., human stem cells) with one or more inhibitor of transforming growth factor beta (TGFβ)/Activin-Nodal signaling and one or more Wnt activator.

In certain embodiments, the SC precursors are *in vitro* differentiated from neural crest lineage cells by inducing SC differentiation. In certain embodiments, the method comprises contacting a population of neural crest lineage cells (e.g., the neural crest lineage cells derived from stem cells by inhibition of SMAD signaling and activation of Wnt signaling) with one or more Wnt activator and one or more FGF activator. In certain embodiments, the method comprises contacting a population of neural crest lineage cells (e.g., the neural crest lineage cells derived from stem cells by inhibition of SMAD signaling and activation of Wnt signaling) with one or more SC differentiation inducer.

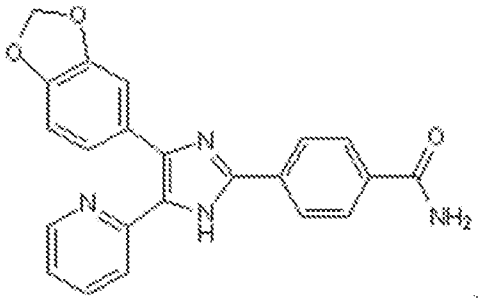
In certain embodiments, the SCs are *in vitro* differentiated from SC precursors by enhancing SC differentiation. In certain embodiments, the method comprises contacting a population of SC precursors (e.g., the SC precursors cells derived from neural crest lineage cells by inducing SC differentiation) with one or more FGF activator, one or more SC differentiation inducer. In certain embodiments, the method comprises contacting a population of SC precursors (e.g., the SC precursors cells derived from neural crest lineage cells by inducing SC differentiation) with one or more SC differentiation enhancer.

1. In vitro Differentiation of Stem Cells to Neural Crest Linage Cells

In certain embodiments, the method of *in vitro* inducing differentiation of stem cells to cells expressing one or more neural crest lineage maker comprises contacting a population of stem cells (e.g., human stem cells) with one or more inhibitor of transforming growth factor beta (TGFβ)/Activin-Nodal signaling. In certain embodiments, the inhibitor of TGFβ/Activin-Nodal signaling neutralizes the ligands including TGFβs, bone morphogenetic proteins (BMPs), Nodal, and activins, or blocking their signal pathways through blocking the receptors and downstream effectors. Non-limiting examples of inhibitors of TGFβ/Activin-Nodal signaling are disclosed in

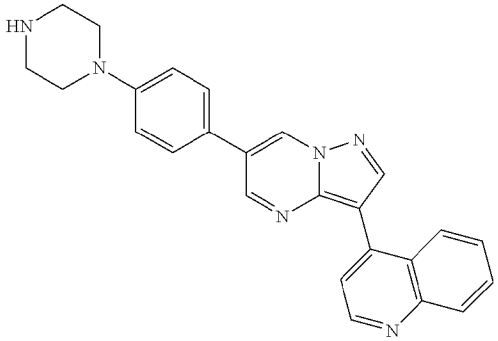
WO2011/149762, Chambers (2009), and Chambers (2012), which are incorporated by reference in their entireties. In certain embodiments, the one or more inhibitor of TGFβ/Activin-Nodal signaling is a small molecule selected from the group consisting of SB431542, derivatives thereof, and mixtures thereof. In certain embodiments, the one or more inhibitor of TGFβ/Activin-Nodal signaling is SB431542.

"SB431542" refers to a molecule with a number CAS 301836-41-9, a molecular formula of $C_{22}H_{18}N_4O_3$, and a name of 4-[4-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imidazol-2-yl]-benzamide, for example, see structure below:



In certain embodiments, the method of *in vitro* inducing differentiation of stem cells to cells expressing one or more neural crest lineage maker further comprises contacting the stem cells with one or more inhibitor of Small Mothers Against Decapentaplegic (SMAD) signaling ("SMAD inhibitor"). Non-limiting examples of SMAD inhibitors are disclosed in WO2011/149762, Chambers (2009), and Chambers (2012), which are incorporated by reference in their entireties. In certain embodiments, the one or more inhibitor of SMAD signaling is a small molecule selected from the group consisting of LDN193189, derivatives thereof, and mixtures thereof. In certain embodiments, the one or more SMAD inhibitor is LDN193189.

"LDN193189" refers to a small molecule DM-3189, IUPAC name 4-(6-(4-(piperazin-1-yl)phenyl)pyrazolo[1,5-a]pyrimidin-3-yl)quinoline, with a chemical formula of $C_{25}H_{22}N_6$ with the following formula.



LDN193189 is capable of functioning as a SMAD signaling inhibitor. LDN193189 is also highly potent small-molecule inhibitor of ALK2, ALK3, and ALK6, protein tyrosine kinases (PTK), inhibiting signaling of members of the ALK1 and ALK3 families of type I TGFβ receptors, resulting in the inhibition of the transmission of multiple biological signals, including the bone morphogenetic proteins (BMP) BMP2, BMP4, BMP6, BMP7, and Activin cytokine signals and subsequently SMAD phosphorylation of Smad1, Smad5, and Smad8 (Yu et al. (2008) Nat Med 14:1363-1369; Cuny et al. (2008) Bioorg. Med. Chem. Lett. 18: 4388-4392, herein incorporated by reference).

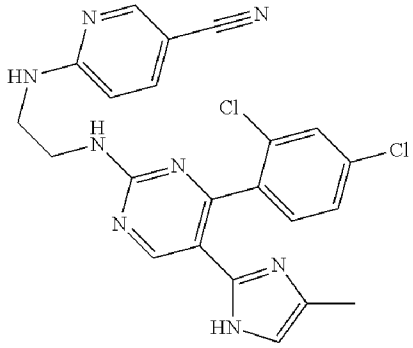
In certain embodiments, the method of *in vitro* inducing differentiation of stem cells to cells expressing one or more neural crest lineage maker comprises further comprises contacting the cells with one or more Wnt activator. As used herein, the term "WNT" or "wingless" in reference to a ligand refers to a group of secreted proteins (i.e. Intl (integration 1) in humans) capable of interacting with a WNT receptor, such as a receptor in the Frizzled and LRPDerailed/RYK receptor family. As used herein, the term "WNT" or "wingless" in reference to a signaling pathway refers to a signal pathway composed of Wnt family ligands and Wnt family receptors, such as Frizzled and LRPDerailed/RYK receptors, mediated with or without β-catenin. In certain embodiments, a WNT signaling pathway includes mediation by β-catenin, e.g., WNT / -catenin.

In certain embodiments, the one or more Wnt activator lowers glycogen synthase kinase 3β (GSK3β) for activation of Wnt signaling. Thus, the Wnt activator can be a GSK3β inhibitor. A GSK3P inhibitor is capable of activating a WNT signaling pathway, see e.g., Cadigan, et al., J Cell Sci. 2006;119:395-402; Kikuchi, et al., Cell Signaling. 2007;19:659-671, which are incorporated by reference herein in their entireties. As used herein, the term "glycogen synthase kinase 3β inhibitor" refers to a compound that inhibits a glycogen synthase kinase 3β enzyme, for example, see, Doble, et al., J Cell Sci. 2003;116:1175-1186, which is incorporated by reference herein in its entirety.

Non-limiting examples of Wnt activators or GSK3β inhibitors are disclosed in WO2011/149762, Chambers (2012), and Calder et al., J Neurosci. 2015 Aug 19;35(33):11462-81, which are incorporated by reference in their entireties. In certain embodiments, the one or more Wnt activator is a small molecule selected from the group

consisting of CHIR99021, WNT3A, Wnt-1, Wnt4, Wnt5a, derivatives thereof, and mixtures thereof. In certain embodiments, the one or more Wnt activator is CHIR99021.

"CHIR99021" (also known as "aminopyrimidine" or "3-[3-(2-Carboxyethyl)-4-methylpyrrol-2-methylidenyl]-2-indolinone") refers to IUPAC name 6-(2-(4-(2,4-dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)pyrimidin-2-ylamino) ethylamino)nicotinonitrile with the following formula.



CHIR99021 is highly selective, showing nearly thousand-fold selectivity against a panel of related and unrelated kinases, with an IC50=6.7 nM against human GSK3β and nanomolar IC50 values against rodent GSK3β homologs.

For *in vitro* differentiation of stem cells to cells expressing one or more neural crest lineage marker, the stem cells can be contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling for at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, at least about 22 days, at least about 23 days, at least about 24 days, at least about 25 days, at least about 26 days, at least about 27 days, at least about 28 days, at least about 29 days, or at least about 30 days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling for up to about 3 days, up to about 4 days, up to about 5 days, up to about 6 days, up to about 7 days, up to about 8 days, up to about 9 days, up to about 10 days, up to about 11 days, up to about 12 days, up to about 13 days, up to about 14 days, up to about 15 days, up to about 16 days, up to about 17 days, up to about 18 days, up to about 19 days, up to about 20 days, up to about 21 days, up to about 22 days, up to about 23 days, up to about 24 days, up to about 25 days, up to about 26 days, up to about 27 days, up to about 28 days, up to about 29 days, or up to about 30

days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling for between about 4 days and about 30 days, between about 4 days to about 27 days, between about 4 days and about 26 days, between about 4 days and about 25 days, between about 4 days and about 24 days, between about 4 days and about 20 days, between about 4 days and about 15 days, between about 4 days and about 10 days, between about 5 days and about 15 days, between about 5 days and about 10 days, between about 10 days and about 15 days, between about 15 days and about 20 days, between about 10 days and about 20 days, between about 20 days and about 25 days, or between about 25 days and about 30 days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling for between 10 days and about 15 days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling for about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, about 21 days, about 22 days, about 23 days, about 24 days, about 25 days, about 26 days, about 27 days, about 28 days, about 29 days, or about 30 day. In certain embodiments, the stem cells are contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling for about 10 days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling for about 11 days.

For *in vitro* differentiation of stem cells to cells expressing one or more neural crest lineage marker, the stem cells can be contacted with the one or more inhibitor of SMAD signaling for at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, at least about 22 days, at least about 23 days, at least about 24 days, at least about 25 days, at least about 26 days, at least about 27 days, at least about 28 days, at least about 29 days, or at least about 30 days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of SMAD signaling for up to about 3 days, up to about 4 days, up to about 5 days, up to about 6 days, up to about 7 days, up to about 8 days, up to about 9 days, up to about 10 days, up to about 11 days, up to about 12 days, up to about 13 days, up to about 14 days, for up to

about 15 days, up to about 16 days, up to about 17 days, up to about 18 days, up to about 19 days, up to about 20 days, up to about 21 days, up to about 22 days, up to about 23 days, up to about 24 days, up to about 25 days, up to about 26 days, up to about 27 days, up to about 28 days, up to about 29 days, or up to about 30 days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of SMAD signaling for between about 4 days and about 30 days, between about 4 days to about 27 days, between about 4 days and about 26 days, between about 4 days and about 25 days, between about 4 days and about 24 days, between about 4 days and about 20 days, between about 4 days and about 15 days, between about 4 days and about 10 days, between about 5 days and about 15 days, between about 5 days and about 10 days, between about 10 days and about 15 days, between about 15 days and about 20 days, between about 10 days and about 20 days, between about 20 days and about 25 days, or between about 25 days and about 30 days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of SMAD signaling for between 10 days and about 15 days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of SMAD signaling for about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, about 21 days, about 22 days, about 23 days, about 24 days, about 25 days, about 26 days, about 27 days, about 28 days, about 29 days, or about 30 day. In certain embodiments, the stem cells are contacted with the one or more inhibitor of SMAD signaling for about 10 days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of SMAD signaling for about 11 days.

Furthermore, the cells can be contacted with the one or more activator of Wnt signaling for at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, at least about 22 days, at least about 23 days, at least about 24 days, at least about 25 days, at least about 26 days, at least about 27 days, at least about 28 days, or at least about 29 days, at least about 30 days. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling for up to about 4 days, up to about 5 days, up to about 6 days, up to about 7 days, up to about

8 days, up to about 9 days, up to about 10 days, up to about 11 days, up to about 12 days, up to about 13 days, up to about 14 days, up to about 15 days, up to about 16 days, up to about 17 days, up to about 18 days, up to about 19 days, up to about 20 days, up to about 21 days, up to about 22 days, up to about 23 days, up to about 24 days, up to about 25 days, up to about 26 days, up to about 27 days, up to about 28 days, up to about 29 days, or up to about 30 days. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling for between about 4 days and about 30 days, between about 4 days to about 27 days, between about 4 days and about 26 days, between about 4 days and about 25 days, between about 4 days and about 24 days, between about 4 days and about 20 days, between about 4 days and about 15 days, between about 4 days and about 10 days, between about 5 days and about 15 days, between about 5 days and about 10 days, between about 10 days and about 15 days, between about 15 days and about 20 days, between about 10 days and about 20 days, between about 20 days and about 25 days, or between about 25 days and about 30 days. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling for between 5 days and about 15 days. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling for about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, about 21 days, about 22 days, about 23 days, about 24 days, about 25 days, about 26 days, about 27 days, about 28 days, about 29 days, or about 30 day. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling for about 11 days. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling for about 10 days. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling for about 9 days.

In certain embodiments, the stem cells are contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling in a concentration of from about 1 nM to about 300 nM, from about 5 nM to about 250 nM, from about 10 nM to about 200 nM, from about 10 nM to about 50 nM, from about 50 nM to about 150 nM, from about 80 nM to about 120 nM, from about 90 nM to about 110 nM, from about 50 nM to about 100 nM, from about 100 nM to about 150 nM, from about 150 nM to about 200 nM, from about 200 nM to about 250 nM, or from about 250 nM to about 300 nM. In certain embodiments, the stem cells are contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling in a

concentration of from about 80 nM to about 120 nM. In certain embodiments, the stem cells are contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling in a concentration of about 100 nM. In certain embodiments, the stem cells are contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling in any one of the above-described concentrations daily, every other day or every two days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of TGFβ/Activin-Nodal signaling in a concentration of about 100 nM daily.

In certain embodiments, the stem cells are contacted with the one or more inhibitor of SMAD signaling in a concentration of from about 1 μM to 100 μM, from about 1 μM to 20 μM, from about 1 μM to 15 μM, from about 1 μM to 10 μM, from about 1 μM to 5 μM, from about 5 μM to 10 μM, from about 5 μM to 15 μM, from about 15 μM to 20 μM, from about 20 μM to 30 μM, from about 30 μM to 40 μM, from about 40 μM to 50 μM, from about 50 μM to 60 μM, from about 60 μM to 70 μM, from about 70 μM to 80 μM, from about 80 μM to 90 μM, or from about 90 μM to 100 μM. In certain embodiments, the stem cells are contacted with the one or more inhibitor of SMAD signaling in a concentration of from about from about 5 μM to 15 μM. In certain embodiments, the stem cells are contacted with the one or more inhibitor of SMAD signaling in a concentration of about 10 μM. In certain embodiments, the stem cells are contacted with the one or more inhibitor of SMAD signaling in any one of the above-described concentrations daily, every other day or every two days. In certain embodiments, the stem cells are contacted with the one or more inhibitor of SMAD signaling in a concentration of about 10 μM daily.

In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling in a concentration of from about 1 μM to 100 μM, from about 1 μM to 20 μM, from about 1 μM to 15 μM, from about 1 μM to 10 μM, from about 1 μM to 5 μM, from about 5 μM to 10 μM, from about 5 μM to 15 μM, from about 15 μM to 20 μM, from about 20 μM to 30 μM, from about 30 μM to 40 μM, from about 40 μM to 50 μM, from about 50 μM to 60 μM, from about 60 μM to 70 μM, from about 70 μM to 80 μM, from about 80 μM to 90 μM, or from about 90 μM to 100 μM. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling in a concentration of from about 1 μM to 5 μM. In certain embodiments, the ells are contacted with the one or more activator of Wnt signaling in a concentration of about 3 μM. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling in any one of the above-described concentrations daily, every other day or every two days. In certain

embodiments, the cells are contacted with the one or more activator of Wnt signaling in a concentration of about 3 μM daily.

2. In vitro Differentiation of Neural Crest Lineage Cells to Schwann Cell Precursors

In certain embodiments, the neural crest lineage cells are differentiated into Schwann cell precursors by a method comprising contacting the cells (e.g., cells expressing one or more neural crest lineage marker, e.g., differentiated cells after contacting a population of stem cells with one or more TGFβ/Activin-Nodal signaling and optionally one or more SMAD inhibitor, and further contacting the cells with one or more Wnt activator,) with one or more Wnt activator described herein, and one or more activator of FGF signaling ("FGF activator") to produce a population of SC precursors, e.g., cells that express one or more Schwann cell precursor marker. In certain embodiments, the method comprises contacting the cells (e.g., cells expressing one or more neural crest lineage marker, e.g., differentiated cells after contacting a population of stem cells with one or more TGFβ/Activin-Nodal signaling and optionally one or more SMAD inhibitor, and further contacting the cells with one or more Wnt activator) with one or more molecule that induces Schwann cell differentiation ("SC differentiation inducer") to produce a population of SC precursors, e.g., cells that express one or more Schwann cell precursor marker.

Non-limiting examples of SC differentiation inducers include neuregulins, LIF, CNTF, Forskolin, TGFβ and FBS. In certain embodiments, the one or more SC differentiation inducer is Neuregulin 1 (NRG1).

Non-limiting examples of activators of FGF signaling include FGF1, FGF2, FGF3, FGF4, FGF7, FGF8, FGF10, FGF18, derivatives thereof, and mixtures thereof. In certain embodiments, the one or more FGF activator is FGF2.

In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more Wnt activator and one or more FGF activator, and optionally one or more SC differentiation inducer concurrently. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more Wnt activator, one or more FGF activator, and one or more SC differentiation inducer concurrently. For example, the one or more Wnt activator and one or more FGF activator, and optionally one or more SC differentiation inducer are all present in a cell culture medium comprising the cells (e.g., cells expressing one or more

neural crest lineage marker). In certain embodiments, the one or more Wnt activator, one or more FGF activator, and optionally one or more SC differentiation inducer are added together daily (or every other day or every two days) to a cell culture medium comprising the cells (e.g., cells expressing one or more neural crest lineage marker, e.g., differentiated cells after contacting a population of stem cells with one or more TGFβ/Activin-Nodal signaling and optionally one or more SMAD inhibitor, and further contacting the cells with one or more Wnt activator).

The cells expressing one or more neural crest lineage marker can be contacted with the one or more Wnt activator, one or more FGF activator, and optionally one or more SC differentiation inducer for at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, or at least about 20 days, to produce SC precursors. In certain embodiments, the cells expressing one or more neural crest lineage marker are contacted with the one or more Wnt activator, one or more FGF activator, and optionally one or more SC differentiation inducer for at least about 10 days to produce SC precursors. In certain embodiments, the cells expressing one or more neural crest lineage marker are contacted with the one or more Wnt activator, one or more FGF activator, and optionally one or more SC differentiation inducer for up to about 15 days, up to about 16 days, up to about 17 days, up to about 18 days, up to about 19 days, up to about 20 days, up to about 21 days, up to about 22 days, up to about 23 days, up to about 24 days, up to about 25 days, up to about 26 days, up to about 27 days, up to about 28 days, up to about 29 days, or up to about 30 days, to produce SC precursors. In certain embodiments, the cells expressing one or more neural crest lineage marker are contacted with the one or more Wnt activator, one or more FGF activator, and optionally one or more SC differentiation inducer for between about 3 days and about 5 days, between about 5 days and about 10 days, between about 10 days and about 15 days, between about 15 days and about 20 days, between about 20 days and about 25 days, or between about 25 days and about 30 days, to produce SC precursors. In certain embodiments, the cells expressing one or more neural crest lineage marker are contacted with the one or more Wnt activator, one or more FGF activator, and optionally one or more SC differentiation inducer for between about 10 days and about 15 days to produce SC precursors. In certain embodiments, the cells expressing

one or more neural crest lineage marker are contacted with the one or more Wnt activator, one or more FGF activator, and optionally one or more SC differentiation inducer for about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, about 21 days, about 22 days, about 23 days, about 24 days, about 25 days, about 26 days, about 27 days, about 28 days, about 29 days, or about 30 days, to produce SC precursors. In certain embodiments, the cells expressing one or more neural crest lineage marker are contacted with the one or more Wnt activator, one or more FGF activator, and optionally one or more SC differentiation inducer for about 14 days to produce SC precursors. In certain embodiments, the cells expressing one or more neural crest lineage marker are contacted with the one or more Wnt activator, one or more FGF activator, and optionally one or more SC differentiation inducer for about 15 days to produce SC precursors.

In certain embodiments, the cells are contacted with one or more Wnt activator to produce a population of cells expressing one or more neural crest lineage marker, and the neural crest linage cell population is further contacted with the one or more Wnt activator. Thus, the cells can be contacted with the one or more Wnt activator for at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, at least about 22 days, at least about 23 days, at least about 24 days, at least about 25 days, at least about 26 days, at least about 27 days, at least about 28 days, or at least about 29 days, at least about 30 days, at least about 31 days, at least about 32 days, at least about 33 days, at least about 34 days, at least about 35 days, at least about 36 days, at least about 37 days, at least about 38 days, at least about 39 days, or at least about 40 days, in total. In certain embodiments, the cells are contacted with the one or more Wnt activator for up to about 15 days, up to about 16 days, up to about 17 days, up to about 18 days, up to about 19 days, up to about 20 days, up to about 21 days, up to about 22 days, up to about 23 days, up to about 24 days, up to about 25 days, up to about 26 days, up to about 27 days, up to about 28 days, up to about 29 days, up to about 30 days, up to about 31 days, up to about 32 days, up to about 33 days, up to t about 34 days, up to about 35 days, up to about 36 days, up to about 37 days, up to about 38 days, up to about 39 days, up to about 40 days, up to about 41 days, up to about 42 days, up to about 43 days, up to about 44 days, up to about 45 days, up to about 46 days, up to about 47 days, up to about 48 days, up to about 49 days, up to about 50 days, up to about

51 days, up to about 52 days, up to about 53 days, up to about 54 days, up to about 55 days, up to about 56 days, up to about 57 days, up to about 58 days, up to about 59 days, or up to about 60 days in total. In certain embodiments, the cells are contacted with the one or more Wnt activator for between about 14 days and about 20 days, between about 20 days and about 25 days, between about 25 days and about 30 days, between about 30 days and about 35 days, between about 35 days and about 40 days, between about 40 days and about 45 days, between about 45 days and about 50 days, between about 50 days and about 55 days, or between about 55 days and about 60 days. In certain embodiments, the cells are contacted with the one or more Wnt activator for between 20 days and about 30 days, in total. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling for between 20 days and about 25 days, in total. In certain embodiments, the cells are contacted with the one or more Wnt activator for between 25 days and about 30 days, in total. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling for about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, about 20 days, about 21 days, about 22 days, about 23 days, about 24 days, about 25 days, about 26 days, about 27 days, about 28 days, about 29 days, about 30 days, about 31 days, about 32 days, about 33 days, about 34 days, about 35 days, about 36 days, about 37 days, about 38 days, about 39 days, about 40 days, about 41 days, about 42 days, about 43 days, about 44 days, about 45 days, about 46 days, about 47 days, about 48 days, about 49 days, about 50 days, about 51 days, about 52 days, about 53 days, about 54 days, about 55 days, about 56 days, about 57 days, about 58 days, about 59 days or about 60 days, in total. In certain embodiments, the cells are contacted with the one or more Wnt activator for about 26 days in total. In certain embodiments, the cells are contacted with the one or more activator of Wnt signaling for about 25 days in total. In certain embodiments, the cells are contacted with the one or more Wnt activator for about 24 days in total. In certain embodiments, the cells are contacted with the one or more Wnt activator for about 23 days in total.

In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more activator of FGF signaling in a concentration of from about 1 nM to 100 nM, from about 1 nM to 20 nM, from about 1 nM to 15 nM, from about 1 nM to 10 nM, from about 1 nM to 5 nM, from about 5 nM to 10 nM, from about 5 nM to 15 nM, from about 15 nM to 20 nM, from about 20 nM to 30 nM, from about 30 nM to 40 nM, from about 40 nM to 50 nM, from about 50 nM to 60 nM, from

about 60 nM to 70 nM, from about 70 nM to 80 nM, from about 80 nM to 90 nM, or from about 90 nM to 100 nM, to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more activator of FGF signaling in a concentration of from about from about 5 nM to 15 nM to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more activator of FGF signaling in a concentration of about 10 nM to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more activator of FGF signaling in any one of the above-described concentrations daily , every other day or every two days to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more activator of FGF signaling in a concentration of about 10 nM daily to produce SC precursors.

In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more molecule that induces Schwann cell differentiation in a concentration of from about 1 ng/ml to 100 ng/ml, from about 1 ng/ml to 20 ng/ml, from about 1 ng/ml to 15 ng/ml, from about 1 ng/ml to 10 ng/ml, from about 1 ng/ml to 5 ng/ml, from about 5 ng/ml to 10 ng/ml, from about 5 ng/ml to 15 ng/ml, from about 15 ng/ml to 25 ng/ml, from about 15 ng/ml to 20 ng/ml, from about 20 ng/ml to 30 ng/ml, from about 30 ng/ml to 40 ng/ml, from about 40 ng/ml to 50 ng/ml, from about 50 ng/ml to 60 ng/ml, from about 60 ng/ml to 70 ng/ml, from about 70 ng/ml to 80 ng/ml, from about 80 ng/ml to 90 ng/ml, or from about 90 ng/ml to 100 ng/ml to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more molecule that induces Schwann cell differentiation in a concentration of from about from about 5 ng/ml to 15 ng/ml to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more molecule that induces Schwann cell differentiation in a concentration of about 10 ng/ml to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more molecule that induces Schwann cell differentiation in any one of the above-described concentrations daily, every other day or every two days to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more

molecule that induces Schwann cell differentiation in a concentration of about 10 ng/ml daily to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more molecule that induces Schwann cell differentiation in a concentration of about 10 ng/ml daily to produce SC precursors.

In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more activator of Wnt signaling in a concentration of from about 1 μM to 100 μM, from about 1 μM to 20 μM, from about 1 μM to 15 μM, from about 1 μM to 10 μM, from about 1 μM to 5 μM, from about 5 μM to 10 μM, from about 5 μM to 15 μM, from about 15 μM to 20 μM, from about 20 μM to 30 μM, from about 30 μM to 40 μM, from about 40 μM to 50 μM, from about 50 μM to 60 μM, from about 60 μM to 70 μM, from about 70 μM to 80 μM, from about 80 μM to 90 μM, or from about 90 μM to 100 μM, to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more activator of Wnt signaling in a concentration of from about 1 μM to 5 μM to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more activator of Wnt signaling in a concentration of about 3 μM to produce SC precursors. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more activator of Wnt signaling in any one of the above-described concentrations daily, every other day or every two days. In certain embodiments, the cells (e.g., cells expressing one or more neural crest lineage marker) are contacted with the one or more activator of Wnt signaling in a concentration of about 3 μM daily.

In certain embodiments, a cell population comprising at least about 50% cells expressing one or more neural crest lineage marker are differentiated into cells expressing one or more Schwann cell precursor marker, wherein the population of cells are contacted with one Wnt activator (e.g., CHIR99021, e.g., 3μM CHIR99021), one FGF activator (e.g., FGF2, e.g., 10nM FGF2), and one SC differentiation inducer (e.g., NRG1, e.g., 10 ng/ml NRG1) for about 15 days (e.g., about 14 days or about 15 days).

In certain embodiments, the stem cells are differentiated into cells expressing one or more Schwann cell precursor marker, wherein the cells are contacted with one inhibitor of TGFβ/Activin-Nodal signaling (e.g., SB431542, e.g., 10μM SB431542) and optionally

one SMAD inhibitor (e.g., LDN193189, e.g., 100nM LDN193189) for about 10 days (e.g., about 10 days or 11 about days); with one Wnt activator (e.g., CHIR99021, e.g., 3μM CHIR99021) for about 23 days (e.g., about 23 days or about 24 days); and with one FGF activator (e.g., FGF2, e.g., 10nM FGF2) and one SC differentiation inducer (e.g., NRG1, e.g., 10 ng/ml NRG1) for about 15 days (e.g., about 14 or 15 days).

In certain embodiments, the cells are not exposed to an activator of Sonic Hedgehog (SHH) signaling. Non-limiting examples of activators of SHH signaling include sonic hedgehog (SHH), C25II, smoothened (SMO) receptor small molecule agonists (e.g., purmorphamine), derivatives thereof, and mixtures thereof. In certain embodiments, the cells are not exposed to SHH.

3. In vitro Induction of Schwann Cell Precursors to Schwann Cells

The Schwann cell precursors can be further induced *in vitro* to Schwann cells. The differentiated SC precursors can be subjected to conditions favoring maturation of SC precursors into a population of Schwann cells. The Schwann cell can be a myelinating Schwann cell or a non-myelinating Schwann cell.

In certain embodiments, the Schwann cell precursors (SC precursors) are contacted with one or more FGF activator described herein, one or more Schwann cell differentiation inducer described herein to produce a population of SCs. In certain embodiments, the Schwann cell precursors (SC precursors) are contacted with one or more molecule that enhances Schwann cell differentiation (referred to as "SC differentiation enhancer"). Non-limiting examples of SC differentiation enhancers include neuregulins, cyclic adenosine monophosphate (cAMP), Forskolin, LIF, and CNTF. In certain embodiments, the neuregulin is NRG1. In certain embodiments, the one or more SC differentiation enhancer is cAMP. In certain embodiments, the Schwann cell precursors (SC precursors) are contacted with one FGF activator and two Schwann cell differentiation inducers to produce a population of SCs. In certain embodiments, the two Schwann cell differentiation inducers are cAMP and NRG1.

In certain embodiments, the SC precursors are contacted with the one or more FGF activator and one or more Schwann cell differentiation inducer, and optionally one or more SC differentiation enhancer in a cell culture medium to produce SCs. In certain embodiments, the cell culture medium is an NB medium supplemented with L-Glutamine (e.g., from Gibco, 25030-164), N2 (e.g., from Stem Cell Technologies, 07156), and B27 (e.g., from Life Technologies, 17504044).

In certain embodiments, the SC precursors are contacted with the one or more molecule that induces Schwann cell differentiation in a concentration of from about 1 ng/ml to 100 ng/ml, from about 1 ng/ml to 20 ng/ml, from about 1 ng/ml to 15 ng/ml, from about 1 ng/ml to 10 ng/ml, from about 1 ng/ml to 5 ng/ml, from about 5 ng/ml to 10 ng/ml, from about 5 ng/ml to 15 ng/ml, from about 15 ng/ml to 25 ng/ml, from about 15 ng/ml to 20 ng/ml, from about 20 ng/ml to 30 ng/ml, from about 30 ng/ml to 40 ng/ml, from about 40 ng/ml to 50 ng/ml, from about 50 ng/ml to 60 ng/ml, from about 60 ng/ml to 70 ng/ml, from about 70 ng/ml to 80 ng/ml, from about 80 ng/ml to 90 ng/ml, or from about 90 ng/ml to 100 ng/ml to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more molecule that induces Schwann cell differentiation in a concentration of from about 15 ng/ml to 25 ng/ml to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more molecule that induces Schwann cell differentiation in a concentration of about 20 ng/ml to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more molecule that induces Schwann cell differentiation in any one of the above-described concentrations daily, every other day or every two days to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more molecule that induces Schwann cell differentiation in a concentration of about 10 ng/ml daily to produce SCs.

In certain embodiments, the SC precursors are contacted with the one or more FGF activator, and one or more Schwann cell differentiation inducer, and optionally one or more SC differentiation enhancer for at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, at least about 8 days, at least about 9 days, at least about 10 days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, or at least about 15 days, to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more FGF activator, and one or more Schwann cell differentiation inducer, and optionally one or more SC differentiation enhancer for between about 3 days and about 40 days, between about 3 days and about 35 days, between about 3 days and about 30 days, between about 3 days and about 25 days, between about 3 days and about 20 days, between about 3 days and about 15 days, between about 10 days and about 40 days, between about 10 days and about 20 days, between about 20 days and about 40 days, between about 20 days and about 30 days, or between about 30 days and about 40 days, to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more FGF activator, and

one or more Schwann cell differentiation inducer, and optionally one or more SC differentiation enhancer for between about 3 days and about 15 days to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more FGF activator, and one or more Schwann cell differentiation inducer, and optionally one or more SC differentiation enhancer for between about 30 days and about 40 days to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more FGF activator, and one or more Schwann cell differentiation inducer, and optionally one or more SC differentiation enhancer for about 10 days to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more FGF activator, and one or more Schwann cell differentiation inducer, and optionally one or more SC differentiation enhancer for about 11 days to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more FGF activator, and one or more Schwann cell differentiation inducer, and optionally one or more SC differentiation enhancer for about 35 days to produce SCs.

In certain embodiments, the SC precursors are contacted with the one or more activator of FGF signaling in a concentration of from about 1 nM to 100 nM, from about 1 nM to 20 nM, from about 1 nM to 15 nM, from about 1 nM to 10 nM, from about 1 nM to 5 nM, from about 5 nM to 10 nM, from about 5 nM to 15 nM, from about 15 nM to 20 nM, from about 20 nM to 30 nM, from about 30 nM to 40 nM, from about 40 nM to 50 nM, from about 50 nM to 60 nM, from about 60 nM to 70 nM, from about 70 nM to 80 nM, from about 80 nM to 90 nM, or from about 90 nM to 100 nM, to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more activator of FGF signaling in a concentration of from about from about 5 nM to 15 nM to produce SC precursors. In certain embodiments, the stem cells are contacted with the one or more activator of FGF signaling in a concentration of about 10 nM to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more activator of FGF signaling in any one of the above-described concentrations daily, every other day or every two days to produce SCs. In certain embodiments, the SC precursors are contacted with the one or more activator of FGF signaling in a concentration of about 10 nM daily to produce SCs.

In certain embodiments, conditions favoring maturation from SC precursors to SCs comprise aggregating the differentiated SC precursors cells into 3D spheroids, and further contacting said 3D spheroids with the one or more FGF activator, and the one or more

Schwann cell differentiation inducer, and optionally the one or more SC differentiation enhancer. In certain embodiments, the culture medium is the suspension culture medium.

In certain embodiments, a cell population comprising at least about 50% cells expressing one or more Schwann cell precursor marker are differentiated into cells expressing one or more Schwann cell marker, wherein the population of cells are contacted with one FGF activator (e.g., FGF2, e.g., 10nM FGF2), two SC differentiation inducers (e.g., NRG1 (e.g., 10 ng/ml NRG1) and cAMP (e.g., 100mM cAMP)) for at least about 10 days.

In certain embodiments, the cells are not exposed to an activator of Sonic Hedgehog (SHH) signaling. Non-limiting examples of activators of SHH signaling include sonic hedgehog (SHH), C25II, smoothened (SMO) receptor small molecule agonists (e.g., purmorphamine), derivatives thereof, and mixtures thereof. In certain embodiments, the cells are not exposed to SHH.

4. Cell Culture Media

In certain embodiments, the above-described inhibitors, activators, inducers and enhancers are added to a cell culture medium comprising the cells, e.g., stem cells, cells expressing one or more neural crest lineage marker, cells expressing one or more SC precursor marker, cells expressing one or more SC marker, or a combination thereof. Suitable cell culture media include, but are not limited to, Knockout® Serum Replacement ("KSR") medium, N2 medium, an Essential 8® /Essential 6® ("E8/E6") medium, and a Neurobasal (NB) medium (e.g., a NB medium supplemented with N2 and B-27® Supplement). KSR medium, N2 medium, E8/E6 medium and NB medium are commercially available. In certain embodiments, a medium for *in vitro* differentiation of stem cells to cells expressing one or more neural crest lineage marker is a medium selected from the group consisting of a KSR medium, a N2 medium, and a combination thereof. In certain embodiments, a medium for *in vitro* differentiation of stem cells to cells expressing one or more neural crest lineage marker is an E8/E6 medium. In certain embodiments, a medium for *in vitro* induction of cells expressing one or more neural crest lineage marker to cells expressing one or more SC precursor marker is an NB medium. In certain embodiments, a medium for *in vitro* induction of cells expressing one or more SC precursor marker to cells expressing one or more SC marker is an NB medium.

KSR medium is a defined, serum-free formulation optimized to grow and maintain undifferentiated hESC cells in culture. The components of a KSR medium are disclosed in

WO2011/149762. In certain embodiments, a KSR medium comprises Knockout DMEM, Knockout Serum Replacement, L-Glutamine, Pen/Strep, MEM, and 13-mercaptoethanol. In certain embodiments, 1 liter of KSR medium can comprise 820 mL of Knockout DMEM, 150 mL of Knockout Serum Replacement, 10 mL of 200 mM L-Glutamine, 10 mL of Pen/Strep, 10 mL of 10 mM MEM, and 55 μM of 13-mercaptoethanol.

E8/E6 medium is a feeder-free and xeno-free medium that supports the growth and expansion of human pluripotent stem cells. E8/E6 medium has been proven to support somatic cell reprogramming. In addition, E8/E6 medium can be used as a base for the formulation of custom media for the culture of PSCs. One example E8/E6 medium is described in Chen et al., Nat Methods. 2011 May;8(5):424-9, which is incorporated by reference in its entirety. One example E8/E6 medium is disclosed in WO15/077648, which is incorporated by reference in its entirety. In certain embodiments, an E8/E6 cell culture medium comprises DMEM/F12, ascorbic acid, selenium, insulin, $NaHCO_3$, transferrin, FGF2 and TGFβ. The E8/E6 medium differs from a KSR medium in that E8/E6 medium does not include an active BMP or Wnt ingredient. Thus, in certain embodiments, when an E8/E6 medium is used to culture the stem cells, one or more SMAD inhibitor (e.g., those inhibiting BMP) is not required to be added to the E8/E6 medium.

N2 supplement is a chemically defined, animal-free, supplement used for expansion of undifferentiated neural stem and progenitor cells in culture. N2 Supplement is intended for use with DMEM/F12 medium. The components of a N2 medium are disclosed in WO2011/149762. In certain embodiments, a N2 medium comprises a DMEM/F12 medium supplemented with glucose, sodium bicarbonate, putrescine, progesterone, sodium selenite, transferrin, and insulin. In certain embodiments, 1 liter of a N2 medium comprises 985 ml dist. H_2O with DMEM/F12 powder, 1.55 g of glucose, 2.00 g of sodium bicarbonate, putrescine (100 uL aliquot of 1.61 g dissolved in 100 mL of distilled water), progesterone (20 uL aliquot of 0.032g dissolved in 100 mL 100% ethanol), sodium selenite (60 uL aliquot of 0.5 mM solution in distilled water), 100 mg of transferrin, and 25 mg of insulin in 10 mL of 5 mM NaOH.

In certain embodiments, the stem cells are initially cultured in a KSR medium, which is gradually replaced with increasing amount of a N2 medium from about 1, about 2, about 3, about 4, or about 5, about 6, about 7, or about 8 days after the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling until the

contact of the stem cells with the SC differentiation inducers and FGF activators. In certain embodiments, the stem cells are initially cultured in a KSR medium, which is gradually replaced with increasing amount of a N2 medium from day 4 to day 10 after the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling.

The cell culture medium used for culturing the presently disclosed population of stem cells not only determines the inhibitor(s), activator(s), inducer(s) and enhancer(s) to be contacted with the cells (e.g., for a KSR medium, one or more inhibitor of TGFβ/Activin-Nodal signaling and one or more SMAD inhibitor are required; and for an E8/E6 medium, only one or more inhibitor of TGFβ/Activin-Nodal signaling is required), but also determines the sequence of adding the inhibitor(s), activator(s), inducer(s) and enhancer(s) to the cell culture medium.

In certain embodiments, the initial contact of the cells with the one or more Wnt activator is no later than about 4 days (e.g., concurrently (on the same day), or between about 1 and about 4 days, e.g., about 1 day, about 2 days, about 3 days, or about 4 days) from the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling.

In certain embodiments, the cell culture medium for *in vitro* differentiation of stem cells to cell expressing one or more neural crest lineage marker is a KSR medium, and the initial contact of the cells with the one or more Wnt activator is about 2 days from the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling. In certain embodiments, the initial contact of the stem cells with the one or more SMAD inhibitor is on the same day as the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling, e.g., by initially adding the SMAD inhibitor(s) and inhibitor(s) of TGFβ/Activin-Nodal signaling to a cell culture medium comprising the stem cells on the same day.

In certain embodiments, the cell culture medium for *in vitro* differentiation of stem cells to cell expressing one or more neural crest lineage marker is an E8/E6 medium, and the initial contact of the cells with the one or more Wnt activator is on the same day as the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling, *e.g.*, by initially adding the Wnt activator(s) and inhibitor(s) of TGFβ/Activin-Nodal signaling to a cell culture medium comprising the stem cells on the same day. In certain embodiments, a BMP active agent is added to the E8/E6 medium. In

certain embodiments, the BMP active agent is withdrawn from the medium after about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, or about 10 days of culture. In certain embodiments, the BMP active agent is withdrawn from the medium after about 3 days of culturing. In certain embodiments, the BMP active agent is present in the culture medium at a concentration of from between about 0.5 and about 20 ng/mL, or between about 1 and about 15 ng/ml, or between about 2 and about 10 ng/ml, or between about 3 and about 5 ng/ ml. In certain embodiments the BMP active agent is present in the culture medium at a concentration of about 5 ng/ml. Non-limiting examples of BMP active agents include BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP10, BMP11, BMP15, derivatives thereof, and mixtures thereof.

In certain embodiments, the initial contact of the one or more FGF activator and optionally the one or more SC differentiation inducer with the cells is no later than about 20 days from the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling. In certain embodiments, the initial contact of the one or more FGF activator and optionally the one or more SC differentiation inducer with the cells is at least about 5 days from the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling. In certain embodiments, the initial contact of the one or more FGF activator and optionally the one or more SC differentiation inducer with the cells is between about 5 days and about 20 days (e.g., about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12, days, about 13 days, about 14 days, about 15 days, about 16 days, about 17 days, about 18 days, about 19 days, or about 20 days) from the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling. In certain embodiments, the initial contact of the one or more FGF activator and optionally the one or more SC differentiation inducer with the cells is about 10 days from the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling. In certain embodiments, the initial contact of the one or more FGF activator and optionally the one or more SC differentiation inducer with the cells is about 11 days from the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling.

In certain embodiments, the Wnt activator(s), FGF activator(s) and optionally SC differentiation inducer(s) are added (daily, every other day or every two days) to a NB medium supplemented with L-Glutamine (e.g., from Gibco, 25030-164), N2 (e.g., from

Stem Cell Technologies, 07156), and B27 (e.g., from Life Technologies, 17504044), to produce SC precursors.

In certain embodiments, the SC differentiation inducer(s), FGF activator(s) and optionally SC differentiation enhancer(s) are added (daily, every other day or every two days) to a NB medium supplemented with L-Glutamine (e.g., from Gibco, 25030-164), N2 (e.g., from Stem Cell Technologies, 07156), and B27 (e.g., from Life Technologies, 17504044), to produce SC precursors.

In certain embodiments, the inhibitor(s) of TGFβ/Activin-Nodal signaling, SMAD inhibitor(s), Wnt activator(s), SC differentiation inducer(s), FGF activator(s), and optionally SC differentiation enhancer(s) are added daily (or every other day or every two days) to a cell culture medium comprising the stem cells.

In certain embodiments, the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling and the one or more SMAD inhibitor is on day 0, the initial contact of the cells with the one or more Wnt activator is on day 2, the initial contact of the cells with the one or more FGF activator and optionally the one or more SC differentiation inducer is on day 11, and the initial contact of the cells with the one or more SC differentiation inducer and the one or more FGF activator, and optionally the one or more SC differentiation enhancer is on day 25. In certain embodiments, the cell culture medium for day 0 to day 10 is a KSR medium, a N2 medium, or a mixture thereof. In certain embodiments, the cell culture medium for day 0 to day 3 is a KSR medium. In certain embodiments, the cell culture medium for day 4 to day 10 is a combination of a KSR medium and a N2 medium. In certain embodiments, the cell culture medium for day 10 is a N2 medium. In certain embodiments, the cell culture medium for day 11 and after is a NB medium supplemented with L-Glutamine, N2, and B27.

In certain embodiments, the initial contact of the stem cells with the one or more inhibitor of TGFβ/Activin-Nodal signaling and the one or more Wnt activator is on day 0, the initial contact of the cells with the one or more FGF activator and optionally the one or more SC differentiation inducer is on day 11 (or every other day or every two days), and the initial contact of the cells with one or more SC differentiation inducer and the one or more FGF activator, and optionally the one or more SC differentiation enhancer is on day 25. In certain embodiments, the cell culture medium for day 0 to day 10 is an E8/E6 medium, a N2 medium, or a mixture thereof. In certain embodiments, the cell culture

medium for day 11 and after is a NB medium supplemented with L-Glutamine, N2, and B27.

In certain embodiments, the cells are contacted with one or more inhibitor of TGFβ/Activin-Nodal signaling and the one or more SMAD inhibitor for about 10 days; with the one or more Wnt activator for about 23 days; and with the one or more FGF activator for about 14 days, and optionally with the one or more SC differentiation inducer for about 14 days, to produce SC precursors. In certain embodiments, the SC precursors are contacted with one or more SC differentiation inducer, one or more FGF activator and optionally one or more SC differentiation enhancer for at least 8 days (e.g., 10 days or 35 days) to produce SCs.

In certain embodiments, the cells are contacted with one or more inhibitor of TGFβ/Activin-Nodal signaling for about 10 days; with the one or more activator of Wnt signaling for about 25 days; and with the one or more activator of FGF signaling for about 14 days, and optionally the one or more SC differentiation inducer for about 14 days, to produce SC precursors. In certain embodiments, the SC precursors are contacted with one or more SC differentiation inducer, one or more FGF activator and optionally one or more SC differentiation enhancer for at least 8 days (e.g., 10 days or 35 days) to produce SCs.

Schwann cell (SC) precursors (e.g., cells that express one or more early Schwann cell marker) can be differentiated from stem cells in less than about 35 days, in less than about 34 days, in less than about 33 days, in less than about 32 days, in less than about 31 days, in less than about 30 days, in less than about 29 days, in less than about 28 days, in less than about 27 days, in less than about 26 days, in less than about 25 days, in less than about 24 days, in less than about 23 days, in less than about 22 days, in less than about 21 days, or in less than about 20 days from initial contact with the inhibitor(s) of TGFβ/Activin-Nodal signaling. In certain embodiments, SC precursors are differentiated from the stem cells on or after about 25 days from the initial contact of the stem cells with the inhibitor(s) of TGFβ/Activin-Nodal signaling.

5. Markers and Reporters

The differentiated SC precursors express one or more Schwann cell precursor marker. Non-limiting examples of Schwann cell precursor markers include SOX10, GAP43, BLBP, myelin protein zero (MPZ), Dhh, P75NTR, CD49D, TFAP2, CDH19, CD44, ERBB3, POU3F1, and glial fibrillary acidic protein (GFAP), CALCB, GRP116,

TSPYL5, ITPKA, SLC17A6, SYPL2, LOC100128252, ANGPTL7, LOC728978, ZNF502, SLC16A6, LPL, SLC30A2, SLC10A4, and the genes listed in Tables 1-4.

The SCs express one or more Schwann cell marker. Non-limiting examples of Schwann cell markers include leucine rich repeat transmembrane neuronal 4 (LRRTM4), cadherin 1 (CDH1), fatty acid binding protein 7 (FABP7), brain derived neurotrophic factor (BDNF), UNCB5, sclerostin domain containing 1 (SOSTDC1), oligodendrocyte transcription factor 1 (OLIG1), plasminogen activator (PLAT), potassium inwardly-rectifying channel subfamily J member 10 (KCNJ10), sonic hedgehog (SHH), netrin 1 (NTN1), glial cell line derived neurotrophic factor (GDNF), erb-b2 receptor tyrosine kinase 3 (ERBB3), growth associated protein 43 (GAP43), SOX10, S100, GFAP, POU3F1, PMP22, myelin basic protein (MBP), aquaporin 4 (AQP4), NGFR, NFATC4, MOG, IFNG, MAL, NTF3, TGFB1, MPZ, CD9, CD49D, CD49E, CD44, CD98, and CD81, TYRP1, ENTHD1, NT5E, HTR2B, NOV, IL8, SLC16A6, CDKN2A, PLP2, S100A6, AQP9, CDH19, and the genes listed in Tables 1-4.

The differentiated SC precursors and further matured SCs can further express one or more reporter. Non-limiting examples of reporters include fluorescent proteins (such as green fluorescent protein (GFP), blue fluorescent protein (EBFP, EBFP2, Azurite, mKalama1), cyan fluorescent protein (ECFP, Cerulean, CyPet, mTurquoise2), and yellow fluorescent protein derivatives (YFP, Citrine, Venus, YPet, EYFP)), β-galactosidase (LacZ), chloramphenicol acetyltransferase (cat), neomycin phosphotransferase (neo), enzymes (such as oxidases and peroxidases); and antigenic molecules. As used herein, the terms "reporter gene" or "reporter construct" refer to genetic constructs comprising a nucleic acid encoding a protein that is easily detectable or easily assayable, such as a colored protein, fluorescent protein such as GFP or an enzyme such as beta-galactosidase (lacZ gene).

The differentiated SC precursors and further matured SCs can be purified after differentiation, *e.g.*, in a cell culture medium. As used herein, the terms "purified," "purify," "purification," "isolated," "isolate," and "isolation" refer to the reduction in the amount of at least one contaminant from a sample. For example, a desired cell type is purified by at least about 10%, by at least about 30%, by at least about 50%, by at least about 75%, and by at least about 90%>, with a corresponding reduction in the amount of undesirable cell types. The term "purify" can refer to the removal of certain cells (e.g., undesirable cells) from a sample. The removal or selection of undesirable cells results in

an increase in the percent of desired SC precursors or SCs in the sample. In certain embodiments, the SC precursors are purified by sorting a mixed cell population into cells expressing at least one Schwann cell precursor marker. In certain embodiments, the one or more Schwann cell precursor marker is selected from the group consisting of SOX10, GAP43, BLBP, MPZ, Dhh, P75NTR, CD49D, TFAP2, CDH19, CD44, ERBB3, POU3F1, GFAP, CALCB, GRP116, TSPYL5, ITPKA, SLC17A6, SYPL2, LOC100128252, ANGPTL7, LOC728978, ZNF502, SLC16A6, LPL, SLC30A2, and SLC10A4. In certain embodiments, the one or more Schwann cell precursor marker is selected from the genes listed in Tables 1-4. In certain embodiments, the one or more Schwann cell precursor marker is selected from the genes listed in Table 1. In certain embodiments, the one or more Schwann cell precursor marker is selected from the group consisting of CALCB, GRP116, TSPYL5, ITPKA, SLC17A6, SYPL2, LOC100128252, ANGPTL7, LOC728978, and ZNF502.

The presently disclosed subject matter also provides a population of SC precursors and SCs produced by the *in vitro* methods described herein, and compositions comprising such cells.

3. Methods of Screening Therapeutic Compounds

The stem-cell-derived SC precursors or matured SCs can be an approach to determine cell-type specific mechanisms that contribute to neuropathies (e.g., peripheral neuropathy, e.g. DPN). In this approach, the individual cell types derived from hESCs can be exposed to high glucose concentrations as occurs in diabetes. The metabolic and functional consequences of these treatments can thus be evaluated in each potential contributing cell type.

The presently disclosed stem-cell-derived SC precursors or matured SCs can be used to model a Schwann cell related disorder (e.g., peripheral neuropathy, e.g., DPN) and serve as a platform to screen for candidate compounds that can overcome disease related defects in the Schwann cell related disorder. The capacity of a candidate compound to alleviate a Schwann cell related disorder (e.g., peripheral neuropathy, e.g., DPN) can be determined by assaying the candidate compound's ability to rescue a physiological or cellular defect caused by high glucose toxicity, which causes a Schwann cell related disorder (e.g., peripheral neuropathy, e.g., DPN). The inventors' identification of Bupropion in rescuing neuropathy related histological and behavioral deficits in

STZ-treated diabetic mice represents a proof-of-concept for the use of presently disclosed stem-cell-derived SC precursors or matured SCs in drug discovery. *See* Example 1.

The presently disclosed subject matter provides for *in vitro* methods of screening compounds suitable for regeneration of PNS and/or CNS, for preventing and/or treating or repairing myelin damages, and/or for preventing and/or treating Schwann cell related disorders (e.g., peripheral neuropathy, e.g., DPN). In certain embodiments, the method comprises identifying a compound that is capable of rescuing hyperglycemia-induced cytotoxicity (e.g., high glucose induced toxicity) in the presently disclosed SC precursors or matured SCs, e.g., reducing glucose level, reducing sorbitol level, and/or reducing cell viability in such cells post high glucose exposure.

In certain embodiments, the method comprise: (a) exposing a population of SC precursors and/or matured SCs obtained from *in vitro* differentiation of stem cells as disclosed herein to a glucose concentration of at least about 10mM, and (b) contacting the cell population with a test compound after glucose exposure. In certain embodiments, the method comprise (c) measuring one or more of first sorbitol level, first glucose level, and first cell viability of the cell population without treatment of said test compound, and (d) measuring one or more of second sorbitol level, second glucose level, and second cell viability of the cell population treated with said test compound. Furthermore, the method comprises (e) comparing (i) the second sorbitol level with the first sorbitol level, (ii) the second glucose level with the first glucose level, and/or (iii) the second cell viability with the first cell viability.

The method further comprises (f) identifying a test compound that is suitable for regeneration of PNS and/or CNS, for preventing and/or treating or repairing myelin damages, and/or for preventing and/or treating Schwann cell related disorders (e.g., peripheral neuropathy, e.g., DPN) where the second sorbitol level is lower than the first sorbitol level, (ii) the second glucose level is lower than the first glucose level, and/or (iii) the second cell viability is lower than the first cell viability.

In certain embodiments, the glucose concentration is at least about 10mM, e.g., between 10 mM and 100 mM. In certain embodiments, the glucose concentration is about 30mM. In certain embodiments, the measurement (including the measurement of the first and second sorbitol level, glucose level and cell viability) is performed at least about 12 hours, at least about 24 hours (1 day), about 48 hours (2 days), about 72 hours (3 days), about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, or about

10 days after the initial glucose exposure. In certain embodiments, the measurement (including the measurement of the first and second sorbitol level, glucose level and cell viability) is performed at least about 72 hours (3 days) after the initial glucose exposure.

In certain embodiments, the compounds identified by the screening method disclosed herein include, but are not limited to, potassium channel blockers, norepinephrine-dopamine reuptake inhibitors, cyclopenthiazide, captopril, isradipine, condelphine, nimesulide, and triamcinolone. In certain embodiments, the potassium channel blocker is a sulfonylurea compound. Non-limiting examples of sulfonylureas include tolbutamide, acetohexamide, carbutamide, chlorpropamide, glycyclamide (tolhexamide), metahexamide, tolazamide, glibenclamide (glyburide), glibornuride, gliclazide, glipizide, gliquidone, glisoxepide, glyclopyramide, glimepiride, salts thereof, solvates thereof, hydrate thereof, clathrates thereof, and prodrugs thereof. Non-limiting examples of norepinephrine-dopamine reuptake inhibitors include bupropion, amineptine, methylphenidate (Ritalin®, Concerta®, Metadate®, Methylin®, Rubifen®, or Stimdate®), atomoxetine, maprotiline, desoxypipradrol, dexmethylphenidate, difemetorex, diphenylprolinol, ethylphenidate, fencamfamine, fencamine, lefetamine, methylenedioxypyrovalerone, methylphenidate, nomifensine, O-2172, oxolinic acid, pipradrol, prolintane, pyrovalerone, tametraline, WY-46824, salts thereof, solvates thereof, hydrate thereof, prodrugs thereof, and clathrates thereof.

4. Method of Treatment with Therapeutic Compounds

The compounds identified in the presently disclosed method of drug discovery, or compositions comprising such compounds (e.g., pharmaceutical composition further comprising a pharmaceutically acceptable carrier and/or additional pharmacologically active ingredients), can be used therapeutically, for example and not by limitation, to promote nerve regeneration, for example in the peripheral nervous system (PNS), to preventor reduce or repair myelin damage, and/or to prevent and/or treat Schwann cell-related disorders (*e.g.*, peripheral neuropathy, such as but not limited to diabetic peripheral neuropathy).

Non-limiting examples of compounds that are suitable for the above-identified therapies include potassium channel blockers, norepinephrine-dopamine reuptake inhibitors, cyclopenthiazide, captopril, isradipine, condelphine, nimesulide, and triamcinolone. In certain embodiments, the potassium channel blocker is a sulfonylurea compound. Non-limiting examples of sulfonylureas include tolbutamide, acetohexamide,

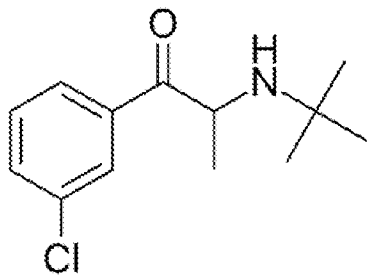
carbutamide, chlorpropamide, glycyclamide (tolhexamide), metahexamide, tolazamide, glibenclamide (glyburide), glibornuride, gliclazide, glipizide, gliquidone, glisoxepide, glyclopyramide, glimepiride, salts thereof, solvates thereof, hydrate thereof, clathrates thereof, and prodrugs thereof.

In certain embodiments, the salt is a pharmaceutically acceptable salt, e.g., a salt prepared from a pharmaceutically acceptable non-toxic inorganic or organic acid or base. Salts including pharmacologically acceptable anions, include, but are not limited to, hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, formate, acetate, propionate, succinate, camphorsulfonate, citrate, acid citrate, fumarate, gluconate, isothionate, lactate, malate, mucate, gentisate, isonicotinate, saccharate, tartrate, bitartrate, para-toluenesulfonate, glycolate, glucuronate, maleate, furoate, glutamate, ascorbate, benzoate, anthranilate, salicylate, phentylacetate, mandelate, embonate (pamoate), methanesulfonate, ethanesulfonate, pantothenate, benzenesulfonate, stearate, sulfanilate, alginate, p-toluenesulfonate, and galacturonate. In certain embodiments, the anions are hydrobromide, hydrochloride, phosphate, acid phosphate, maleate, sulfate, and acid phosphate. In certain embodiments, the anions are hydrochloride and maleate.

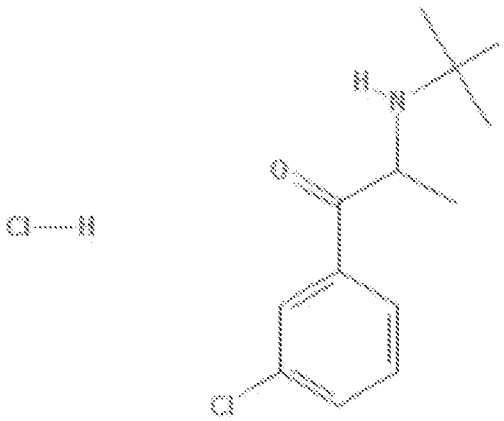
Salts including pharmacologically acceptable cations include, but are not limited to, alkali metal or alkaline earth metal salts and the calcium, magnesium, sodium or potassium salts in particular. Suitable organic bases include, but are not limited to, N,N-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumaine (N-methylglucamine), lysine, and procaine.

In certain embodiments, the compound is a Norepinephrine-Dopamine Reuptake Inhibitor (NDRI) reuptake inhibitor. Norepinephrine–dopamine reuptake inhibitors are agents that acts as reuptake inhibitors for the neurotransmitters norepinephrine and dopamine by blocking the action of the norepinephrine transporter and the dopamine transporter, respectively. Exemplary NDRIs include, but are not limited to bupropion, amineptine, methylphenidate (Ritalin®, Concerta®, Metadate®, Methylin®, Rubifen®, or Stimdate®), atomoxetine, maprotiline, desoxypipradrol, dexmethylphenidate, difemetorex, diphenylprolinol, ethylphenidate, fencamfamine, fencamine, lefetamine, methylenedioxypyrovalerone, methylphenidate, nomifensine, O-2172, oxolinic acid, pipradrol, prolintane, pyrovalerone, tametraline, WY-46824, salts thereof, solvates thereof, hydrate thereof, prodrugs thereof, and clathrates thereof.

In certain emboiments, the compound is a bupropion or a pharmaceutically acceptable salt, solvate, hydrate, a clathrate, or a prodrug thereof. "Bupropion" is a NDRI, and also has the function to interfere with potassium channel. It has been approved as antidepressant as well as for smoking cessation. The tradenames of bupropion are Wellbutrin, Elontril and Zyban. Its IUPAC name is 3-chloro-N-tert-butyl-β-keto-α-methylphenethylamine, with a chemical formula of $C_{13}H_{18}ClNO$, with the following formula:



In certain embodiments, the compound is a bupropion hydrochloride. It is the hydrochloride salt of bupropion, and is an unicyclic, aminoketone antidepressant. Its IUPAC name is 2-(tert-butylamino)-1-(3-chlorophenyl)propan-1-one;hydrochloride, with a chemical formula of $C_{13}H_{19}Cl_2NO$, with the following formula:

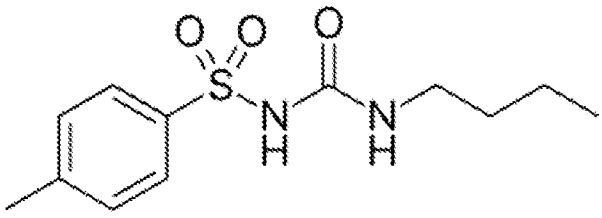


In certain embodiments, the compound is a bupropion metabolite, or a pharmaceutically acceptable salt, solvate, hydrate, a clathrate, or a prodrug thereof. Bupropion metabolites include, but are not limited to, racemic and optically pure forms of 2-(3-chlorophenyl)-2-hydroxy-3,5,5-trimethyl-morpholinol (also known as hydroxybupropion), 2-(tert-butylamino)-1-(3-chlorophenyl)-propan-1-ol (also known as dihydrobupropion), and 1-(3-chlorophenyl)-2-[(1,1-dimethylethanol)amino]-1-propanone. As used herein, the term "optically pure bupropion metabolite" includes, but is not limited

to, optically pure: (R,R)-2-(3-chlorophenyl)-2-hydroxy-3,5,5-trimethyl-morpholinol (also called (R,R)-hydroxybupropion);
(S,S)-2-(3-chlorophenyl)-2-hydroxy-3,5,5-trimethyl-morpholinol (also called (S,S)-hydroxybupropion); (R,R)-2-(tert-butylamino)-1-(3-chlorophenyl)-propan-1-ol (also called (R,R)-dihydrobupropion);
(S,R)-2-(tert-butylamino)-1-(3-chlorophenyl)-propan-1-ol (also called (S,R)-dihydrobupropion); (S,S)-2-(tert-butylamino)-1-(3-chlorophenyl)-propan-1-ol (also called (S,S)-dihydrobupropion);
(R,S)-2-(tert-butylamino)-1-(3-chlorophenyl)-propan-1-ol (also called (R,S)-dihydrobupropion);
(R)-1-(3-chlorophenyl)-2-[(1,1-dimethyl-ethanol)amino]-1-propanone; and
(S)-1-(3-chlorophenyl)-2-[(1,1-dimethylethanol)amino]-1-propanone; hydroxybupropion, threo-hydrobupropion; and erythrohydrobupropion.

In certain embodiments, the compound is a potassium channel blocker. Potassium channel blockers are agents which interfere with conduction through potassium channels, and inhibit potassium efflux through cell membranes. Blockade of potassium channels can prolong the duration of action potentials. Exemplary potassium channel blocker includes but not limited to amiodarone, dofetilide, sotalol, ibutilide, azimilide, bretylium, clofilium, E-4031, nifekalant, tedisamil, and sematilide, tetraethylammonium Cl (TEA), procaine, 4-aminopyridine (4AP), quinidine, apamin, tolbutamide, and glibenclamide.

In certain embodiments, the potassium channel blocker is a tolbutamide, or a pharmaceutically acceptable salt, solvate, hydrate, a clathrate, or a prodrug thereof. Tolbutamide is a sulphonylurea hypoglycemic agent. Its IUPAC name is 1-butyl-3-(4-methylphenyl)sulfonylurea, with a chemical formula of C12H18N2O3S, with the following formula:



In certain embodiments, the compound is a selected from a group consisting of cyclopenthiazide, captoprio, isradipine, condelphine, nimesulide, triamcinolone, and a pharmaceutically acceptable salt, solvate, hydrate, a clathrate, or a prodrug thereof.

The presently disclosed methods of treatment comprising administering the compounds or pharmaceutical composition comprising thereof via localized injection, orthotropic (OT) injection, systemic injection, intravenous injection, or parenteral administration.

The presently disclosed compounds can be conveniently provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like) and suitable mixtures thereof. Sterile injectable solutions can be prepared by incorporating the compositions of the presently disclosed subject matter, *e.g.*, a composition comprising the presently disclosed compounds in the required amount of the appropriate solvent with various amounts of the other ingredients, as desired. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (*e.g.*, methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, alum inurn monostearate and gelatin. According to the presently disclosed subject matter, however, any vehicle, diluent, or

additive used would have to be compatible with the presently disclosed compounds or a pharmaceutical composition comprising thereof.

Those skilled in the art will recognize that the components of the pharmaceutical compositions should be selected to be chemically inert and will not affect efficacy of the presently disclosed compounds. This will present no problem to those skilled in chemical and pharmaceutical principles, or problems can be readily avoided by reference to standard texts or by simple experiments (not involving undue experimentation), from this disclosure and the documents cited herein.

An "effective amount" (or "therapeutically effective amount") is an amount sufficient to affect a beneficial or desired clinical result upon treatment. An effective amount can be administered to a subject in one or more doses. In terms of treatment, an effective amount is an amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of the Schwann cell related disorder (*e.g.*, DPN), or otherwise reduce the pathological consequences of the Schwann cell related disorder (*e.g.*, DPN). The effective amount is generally determined by the physician on a case-by-case basis and is within the skill of one in the art. Several factors are typically taken into account when determining an appropriate dosage to achieve an effective amount. These factors include age, sex and weight of the subject, the condition being treated, the severity of the condition and the form and effective concentration of the cells administered.

The presently disclosed subject matter provides for a method of treating diabetic peripheral neuropathy in a subject in need of such treatment comprising administering, to the subject, an effective amount of a Norepinephrine-Dopamine Reuptake Inhibitor (NDRI) (e.g., one selected from the group consisting of bupropion, amineptine, methylphenidate (Ritalin®, Concerta®, Metadate®, Methylin®, Rubifen®, or Stimdate®), atomoxetine, maprotiline, desoxypipradrol, dexmethylphenidate, difemetorex, diphenylprolinol, ethylphenidate, fencamfamine, fencamine, lefetamine, methylenedioxypyrovalerone, methylphenidate, nomifensine, O-2172, oxolinic acid, pipradrol, prolintane, pyrovalerone, tametraline, WY-46824, salts thereof, solvates thereof, hydrate thereof, prodrugs thereof, and clathrates thereof), to reduce one or more sign or symptom of diabetic neuropathy. In certain embodiments, the NDRI is bupropion or a pharmaceutically acceptable salt, solvate, hydrate, clathrate or prodrug thereof. For example, but not by way of limitation, symptoms and/or signs of diabetic peripheral neuropathy include: numbness, tingling sensation, and/or burning sensation in one or more

extremity; reduced perception of pain and/or temperature in one or more extremity; hyperesthesia in one or more extremity; reduced reflex in one or more extremity; muscle weakness in one or more extremity; ulceration and/or infection of one or more extremity.

5. Kits

The presently disclosed subject matter provides kits for screening compounds suitable for regeneration of PNS and/or CNS, prevention and/or repair of myelin damages, and/or for treating and/or preventing a Schwann cell related disorder (e.g., peripheral neuropathy, e.g., DPN). In certain embodiments, the kit comprises an effective amount of a population of SC precursors and/or matured SCs obtained from *in vitro* differentiation from stem cells by a differentiation method disclosed herein. In certain embodiments, the kit comprises a sterile container which contains the cells; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

In certain embodiments, the kit further comprises instructions for identifying a compound that is capable of rescuing the SC precursors and/or matured SCs from high glucose induced toxicity. In certain embodiments, the instructions comprise: (a) exposing the SC precursors and/or matured SCs obtained from to a glucose concentration of at least about 10mM, and (b) contacting the cell population with a test compound after glucose exposure. In certain embodiments, the instruction comprises (c) measuring one or more of first sorbitol level, first glucose level, and first cell viability of the cells without treatment of said test compound, and (d) measuring one or more of second sorbitol level, second glucose level, and second cell viability of the cells treated with said test compound. Furthermore, the instructions comprise (e) comparing (i) the second sorbitol level with the first sorbitol level, (ii) the second glucose level with the first glucose level, and/or (iii) the second cell viability with the first cell viability.

The instructions further comprise (f) identifying a test compound that is suitable for regeneration of PNS and/or CNS, for preventing and/or treating or repairing myelin damages, and/or for preventing and/or treating Schwann cell related disorders (e.g., peripheral neuropathy, e.g., DPN), wherein the second sorbitol level is lower than the first sorbitol level, (ii) the second glucose level is lower than the first glucose level, and/or (iii) the second cell viability is lower than the first cell viability.

In certain embodiments, the glucose concentration is at least about 10mM, e.g., between 10 mM and 100 mM. In certain embodiments, the glucose concentration is about 30mM. In certain embodiments, the measurement (including the measurement of the first and second sorbitol level, glucose level and cell viability) is performed at least about 12 hours, at least about 24 hours (1 day), about 48 hours (2 days), about 72 hours (3 days), about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, or about 10 days after the initial glucose exposure. In certain embodiments, the measurement (including the measurement of the first and second sorbitol level, glucose level and cell viability) is performed at least about 72 hours (3 days) after the initial glucose exposure.

Furthermore, the presently disclosed subject matter provides kits for regeneration of PNS and/or CNS, prevention and/or repair of myelin damages, and/or for treating and/or preventing a Schwann cell related disorder (e.g., peripheral neuropathy, e.g., DPN). In certain embodiments, the kit comprises an effective amount of a compound identified by the screening method disclosed herein. In certain embodiments, the kit comprises a sterile container which contains the therapeutic composition; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

In certain embodiments, the kit comprises instructions for administering an identified compound or a composition comprising thereof to a subject, e.g., e.g., a subject suffering from a Schwann cell related disorder (e.g., peripheral neuropathy, e.g., DPN). The instructions can comprise information about the use of the compound or composition for regeneration of PNS and/or CNS, prevention and/or repair of myelin damages, and/or for treating and/or preventing a Schwann cell related disorder (e.g., peripheral neuropathy, e.g., DPN). In certain embodiments, the instructions comprise at least one of the following: description of the compounds; dosage schedule and administration for regeneration of PNS and/or CNS, prevention and/or repair of myelin damages, and/or for treating and/or preventing a Schwann cell related disorder (e.g., peripheral neuropathy, e.g., DPN) or symptoms thereof; precautions; warnings; indications; counter-indications; over dosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions can be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

EXAMPLES

The presently disclosed subject matter will be better understood by reference to the following Example, which is provided as exemplary of the presently disclosed subject matter , and not by way of limitation.

Example 1

Summary

Schwann cell precursors and Schwann Cells were derived from human stem cells (e.g., hPSC). The inventors established an hESC-based *in vitro* model of DPN that revealed the selective vulnerability of human SCs to hyperglycemia-induced cytotoxicity and activation of the polyol pathway. The inventors further used this model to perform high throughput drug screening and identified Bupropion as a compound for treatment of DPN. Bupropion can counteract the high-glucose induced cytotoxicity in SCs *in vitro* and rescues the neuropathy related histological and behavioral deficits in STZ-treated diabetic mice.

Methods and Materials

Culture of undifferentiated human Embryonic Stem cells (hESCs)

hESC line H9 (WA-09) and derivatives (SOX10::GFP; SYN::ChR2-YFP; SYN::YFP;PHOX2B:GFP ;EF1::RFP EDNRB-/-) as well as 2 independent hiPSC lines (healthy and Familial Dysautonomia, Sendai-based, OMSK (Cytotune)) were maintained on mouse embryonic fibroblasts (MEF, Global Stem, Rockville, MD) in KSR (Life Technologies, 10828-028) containing hESC medium (Chambers et. al., 2009). Cells were subjected to mycoplasma testing at monthly intervals and STR profiled to confirm cell identity at the initiation of the study.

Neural crest induction and Induction and expansion of Schwann cells from hESCs

hESCs were plated on matrigel (BD Biosciences, 354234) coated dishes (10^5 cells/cm^2) in hESC medium containing 10nM FGF2 (R&D Systems, 233-FB-001MG/CF). Differentiation was initiated in knockout serum replacement (KSR) medium (KO DMEM+15% KSR, L-glutamine (Life Technologies, 25030-081), NEAA (Life Technologies, 11140-050) containing LDN193189 (100nM, Stemgent, Cambridge, MA) and SB431542 (10μM, Tocris, Ellisville, MI). The KSR medium was gradually replaced with increasing amounts of N2 medium from day 4 through day 10 as described previously ((Chambers et. al., 2009). For Cranial NC (CNC) induction, cells are treated with 3uM CHIR99021 (Tocris Bioscience, 4423) in addition to LDN and SB from day 2 through day

11. CNS precursor control cells were generated by treatment with LDN and SB from day 0 through day 11 as previously described (Chambers et al., 2009). Throughout this Example, day 0 is the day the medium is switched from hESC medium to LDN and SB containing medium. Days of differentiation in text and figures refer to the number of days since the pluripotent stage (day 0).

At day 11, NC cells were aggregated into 3D spheroids (5 million cells/well) in Ultra Low Attachment 6-well culture plates (Fisher Scientific, 3471) and cultured in Neurobasal (NB) medium supplemented with L-Glutamine (Gibco, 25030-164), N2 (Stem Cell Technologies, 07156) and B27 (Life Technologies, 17504044) containing CHIR (3uM, Tocris Bioscience, 4423) and FGF2 (10nM, R&D Systems, 233-FB-001MG/CF) and NRG1 (10 ng/ml, R&D 378-SM-025). After 14 days of suspension culture, the spheroids are plated on Poly Ornithine/Laminin/ Fibronectin (PO/LM/FN) coated dishes (prepared as described previously) in Neurobasal (NB) medium supplemented with L-Glutamine (Gibco, 25030-164), N2 (Stem Cell Technologies, 07156) and B27 (Life Technologies, 17504044) containing NRG1 (20 ng/ml, R&D 378-SM-025), FGF2 (10nM, R&D Systems, 233-FB-001MG/CF) and cAMP (100 mM, Sigma, D0260) (Lee G et. al., 2007). The SC precursors migrate out of the plated spheroids and differentiate into SCs within 10 days. For long-term expansion, cells were cultured in Schwann cell medium (Sciencell, 1701) on PO/LM/FN coated dishes. The cells were fixed for immunostaining or harvested for gene expression analysis at Day 25, Day 35 Day 50, Day 60, and Day 100 of differentiation.

FACS and Immunofluorescence (IF) analysis

For IF, the cells were fixed with 4% paraformaldehyde (PFA, Affymetrix-USB, 19943) for 20 minutes, then blocked and permeabilized using 1% Bovine Serum Albumin (BSA, Thermo Scientific, 23209) and 0.3% triton X-100 (Sigma, T8787). The cells were then incubated in primary antibody solutions overnight at 4°C (Celsius) and stained with fluorophore conjugated secondary antibodies at RT for 1 hour, the stained cells were then incubated with DAPI (1 ng/ml, Sigma, D9542-5MG) and washed several times before imaging. For Flow Cytometry analysis, the cells are dissociated with Accutase (Innovative Cell Technologies, AT104) and fixed and permeabilized using BD Cytofix/Cytoperm (BD Bioscience, 554722) solution, then washed, blocked and permeabilized using BD Perm/Wash buffer (BD Bioscience, 554723) according to the manufacturer's instructions. The cells are then stained with primary (overnight at 4) and secondary (30 min at room

temperature) antibodies and analyzed using a flow Cytometer (FlowJo software). A list of primary antibodies and dilutions is provided in **Table 5**.

Surface marker screening

Screening for specific surface antigens was performed using BD Lyoplate library® (BD, 560747) on hESC-SCs at day 80 of differentiation. Cells were plated in 96 well plates (10,000 cells/well) and stained with primary and secondary antibodies according to manufacturer's instructions. The stained wells were fixed for total plate imaging and quantification. The percentage of double positive cells out of total GFAP was quantified for each antibody. Top hits (>60% double positive) were validated further using flow cytometry.

Gene expression analysis

For RNA sequencing, total RNA was extracted using RNeasy RNA purification kit (Qiagen, 74106). For qRT-PCR assay, total RNA samples were reverse transcribed to cDNA using Superscript II Reverse Transcriptase (Life Technologies, 18064-014). qRT-PCR reactions were set up using QuantiTect SYBR Green PCR mix (Qiagen, 204148). Each data point represents three independent biological replicates. RNA-seq reads were mapped to the human reference genome (hg19) using TopHat v2.0. TopHat was run with default parameters with exception to the coverage search. Alignments were then quantified using HTSeq and differential gene expression was calculated using DESeq normalized to the cranial neural crest sample.

Viability ssay

To monitor the viability of SCs, cells were assayed for LDH activity using CytoTox 96 cytotoxicity assay kit (Promega, G1780). Briefly, the cells were plated in 96 well plates at 30,000 cells/cm^2. The supernatant and the cell lysate is harvested 24 hours later and assayed for LDH activity using a plate reader (490 nm absorbance). Viability was calculated by dividing the LDH signal of the lysate by total LDH signal (from lysate plus supernatant). The cells were cultured in Schwann cell medium (Sciencell, 1701) on PO/LM/FN coated dishes during the assay.

Metabolite measurements

High glucose, low glucose and drug treated SCs and sensory neurons were subjected to biochemical assays for Sorbitol (abcam, ab118968), glucose (abcam, ab65333), Pyruvate (abcam, ab65342) and 2DG uptake (abcam, ab136955) measurements

according to manufacturer's instructions. Data was normalizing according to cell numbers and averaged over 3-6 biological replicates.

High content screening assay for drugs reversing glucose-mediated SC cytotoxicity

The chemical compound screening was performed using the Prestwick Chemical Library®. RFP-labeled hESC-SCs were plated in 384 well plates (1,000/well) and treated with 30 mM glucose immediately before addition of the compounds. The compounds were added at 1μM concentration. 72 hours later, the plates were treated with DAPI for ten minutes, washed twice and fixed for total plate imaging. The number of viable cells was quantified for each well by counting the number of DAPI negative, RFP positive cells. The percentage of dead cells were calculated for each well by dividing the number of DAPI positive cells by total RFP positive cells.

For validation of the selected hit compound (Bupropion HCL, Sigma, B102), the cells were treated with various concentrations of the compound for dose response analysis. The highest non-toxic dose (0.7μM-based on optimal sorbitol reduction and viability) was used for follow-up experiments.

Drug treatment of diabetic mice

All procedures were performed following NIH guidelines, and were approved by the local Institutional Animal Care and Use Committee (IACUC). 3-8 weeks old male C57BL6 mice were treated with one dose IP injection of streptozotocin (180 mg/kg, sigma, 85882) to induce pancreatic beta cell death. Blood glucose levels was measured using a standard glucometer (Freestyle Lite) in weekly intervals starting at one week post-treatment, by drawing a drop of blood from tail tip. BP treatment was initiated one week post-streptozotocin treatment. BP was mixed with standard chow at 1.63 mg/gram of chow to be administered orally at ~300 mg/kg daily. The dose was calculated on the basis of average daily food intake (5.5 gram/day) and initial body weight (30 grams).

Mouse thermal sensitivity test

Thermal nociception was assessed using the hot plate test. The hot plate (Ugo Basile 35100) consisted of a metal surface (55 °C) with a transparent plexiglass cylinder to contain the mouse. The subject is placed upon a constant temperature hot plate and the latency necessary to demonstrate discomfort, assessed by either licking/shaking the hind paw or jumping, is determined. Typical baseline latencies are 5-10 seconds, with maximal latencies of 30 seconds. Any animal that does not

demonstrate discomfort behavior will be removed after 30 seconds, the maximal latency, to avoid tissue damage.

Statistical analysis

Data are presented as mean ± SEM and were derived from at least 3 independent experiments. Data on replicates (n) is given in figure legends. Statistical analysis was performed using the Student t-test (comparing 2 groups) or ANOVA with Dunnett test (comparing multiple groups against control). Distribution of the raw data approximated normal distribution (Kolmogorov Smirnov normality test) for data with sufficient number of replicates to test for normality. Survival analysis was performed using log rank (Mantel-Cox) test. Z-scores for primary hits were calculated as $Z= (x-\mu)/\sigma$. X is the migration score value and is 3 for all hit compounds. μ is the mean migration score value and σ is the standard deviation for all compounds and DMSO controls

Results

Derivation and prospective isolation of SC lineages from hESCs

To dissect the cell type specific mechanisms of sensory nerve damage in DPN human sensory neurons and SCs were generated from hPSCs. Methods for induction of sensory neurons from hESCs are described in Cai et al., 2016; Chambers et al., 2012, but the derivation of SCs has remained elusive. Therefore, as a first step towards establishing an hESC model for DPN, an effective strategy to differentiate hESCs into SCs was established.

More recent studies reported on the derivation of SC-like cells from hPSCs but did not show expression of key lineages markers such as SOX10 and failed to demonstrate functional myelination (Liu et al., 2012; Ziegler et al., 2011). During embryonic development, SCs were thought to arise from $SOX10^+$ NC cells in a stepwise process. Based on studies in the mouse and chick embryo, NC first gives rise to SC precursors that associate with neuronal bundles in the developing nerves. The associated neurons express NRG1 and promote survival and further differentiation of SC precursors by activating their ERBB3 receptors (Newbern and Birchmeier, 2010). By E13.5 of mouse development, SC precursors give rise to immature SCs that up regulate lineage markers such as GFAP, S100 and POU3F1 while maintaining the expression of SOX10. Terminal differentiation of SCs into myelinating and non-myelinating fates continues until after birth (Jessen et al., 2015).

Initial hESC-based NC differentiation protocols relied on the delamination of putative NC cells from neuroepithelial lineages combined with the prospective isolation of p75+ and/or HNK1+ NC precursors (Bajpai et al., 2010; Lee et al., 2007). While those protocols yield various NC-derived lineages, the levels of SOX10 expression are generally low. In contrast, more directed NC induction protocols based on timed exposure to activators of WNT signaling show robust induction of SOX10 in the majority of cells by day 11 of differentiation (Fattahi et al., 2016; Menendez et al., 2011; Mica et al., 2013). . Upon further culture, those hESC-derived NC cells can be directed into SOX10+ melanocytes (Mica et al., 2013) but also give rise to SOX10- mesenchymal and neuronal precursors (Mica et al., 2013). Since SOX10 expression is a key marker retained in SC lineages throughout the development, it was first focused to establish conditions to maintain $SOX10^+$ precursors in culture before instructing them towards a glial fate. The percentage of SOX10+ cells in 2D or 3D NC cultures in the presence of modulators of EGF, FGF, WNT, Notch, TGFβ, BMP, NRG and Endothelin 3 signaling was determined. Combination of a 3D aggregation step and activation of WNT signaling by CHIR99021 in addition to FGF2 and NRG1 treatment resulted in maintenance of SOX10 expression (**Figure 6A**) and the induction of S100 and other early SC markers by day 25 (**Figures 1A-1C**). At this stage, treatment of day 25 cultures precursors with FGF2, NRG1 and cAMP for 10 additional days promotes a robust induction of several SC markers such as GFAP, POU3F1, PMP22, MBP, AQP4, MPZ and upregulation of genes involved in neuronal interaction and support including GDNF, ERBB3, and GAP43 among others (**Figures 1A-1D**). Longer-term culture resulted in the enrichment of GFAP+ cells yielding almost homogeneous populations of SCs by day 60-90 (**Figure 6B**) based on the expression of S100, MBP and GFAP (**Figures 1E, 1F and 1I**). These hESC-derived populations can proliferate for several additional weeks while maintaining a high percentage of S100, MBP and GFAP expressing SCs (**Figures 1E, 1F, and 1I**).

To enable the prospective isolation of hESC-SCs during the differentiation, a library of 242 antibodies for surface antigens that specifically mark GFAP+ SCs were screened (**Figure 7A**). It was determined that CD44, CD49e, CD81 and CD98 label the GFAP+ cell population (**Figure 7B**). F urther validations revealed that CD98 was the only marker specifically expressed in day 60 SCs but not in day 11 NC or day 25 SCP cells (**Figure 7C**) which expressed CD49D, a marker previously shown to label early SOX10+ NC lineages (Fattahi et al., 2016). RNA sequencing of purified cells demonstrated that

day 25 hESC-derived SCPs were closely related to early NC cells while day 50 and, in particular, day 100 SCs showed a gene expression pattern closely matching primary adult human SCs (**Figure 1G**). The gene expression data also yielded novel candidate SCP and SC markers by comparing day 25 and 100 cells with day 11 NC (**Figure 1H**). A list of top 200 enriched transcripts for each lineage is provided in **Tables 1-4.**

hESC-SCs are selectively vulnerable to glucotoxicity

The above findings established an efficient protocol to derive functional SCs. To identify the specific cell type mediating DPN, the effect of diabetes on hESC-derived SCs as well as sensory neurons was investigated by evaluating their response to high glucose exposure as occurs in diabetes (**Figure 2A**). To assess cell type specific vulnerability, cultures of hESC-derived sensory neurons and SCs were treated with a range of glucose concentrations. Sensory neurons showed no toxicity at glucose levels up to 45 mM. hPSC-derived SCs however were sensitive to moderately increased glucose levels (**Figures 2B, 2E**).

Elevated levels of glucose in diabetes may activate the polyol pathway in certain cell types (Oates, 2002). The polypol pathway metabolizes the excessive glucose into sorbitol and subsequently into fructose via two enzymatic steps catalyzed by aldose reductase (AR) and by sorbitol dehydrogenase (SDH), respectively. The polyol pathway and sorbitol mediated osmotic and oxidative stress might be a potential mediator of tissue damage by high glucose in studies on glucose induced lens cataracts in the rat (Van Heyningen, 1959). Sorbitol accumulation is also implicated in diabetes induced peripheral nerve damage in animal models (Mizisin, 2014; Oates, 2002). Given the toxic effects of glucose on hESC-SCs, whether they show a buildup of sorbitol in response to high glucose exposure was assessed, which could suggest a potential mechanism for their selective vulnerability. In agreement with previous reports (Maekawa et al., 2001; Mizisin and Powell, 1993), an increased AR to SDH ratio in hESC-derived SCs versus sensory neurons was observed (**Figure 2C**). This was further supported by the observation that SCs but not sensory neurons exhibited increased levels of sorbitol when exposed to high glucose (**Figure 2D**).

High content screening identifies drugs that counteract glucotoxicity in hESC-SCs

These data demonstrated the vulnerability of SCs to high glucose, reducing which presented novel therapeutic opportunities for DPN. The ability to recapitulate this cellular damage in hESC-SCs, offered a platform for high throughput screening (HTS) for drugs

than can counteract this toxicity. Therefore, a HTS system that measured viability of hPSC-derived SCs in the presence of 30mM glucose was established (**Figures 3A and 3E**). The inventors screened the Prestwick library containing 1,280 small molecules of approved drugs (FDA, EMA or other regulatory agencies). The most potent compound in enhancing the viability of high glucose treated hESC-SCs was an antidepressant, bupropion (BP), marketed as Wellbutrin® (**Figure 3B**). Validation studies showed a dose-response for BP in rescuing SC viability and in decreasing sorbitol levels with an IC50 of 263 nM (**Figures 3C, 3D**), and a maximum effect at 0.7 μM, the dose used for all further studies. BP levels above 2 μM induced a gradual dose-dependent toxicity. We next asked whether BP acts by modulating intracellular glucose levels. Given the impact of BP on the levels of sorbitol, the inventor investigated whether BP treatment changes the levels of glucose in SCs. BP reduced the intracellular glucose levels in hESC-Scs without affecting the glucose uptake (**Figures 4A, 4B**). It was therefore hypothesized that BP lowers glucose levels by increasing glycolytic flux and shifting the balance away from sorbitol production. This hypothesis was supported by the increased levels of pyruvate which is the glycolytic end product in the hESC-SCs that were treated with BP (**Figure 4C).** Taken together, these studies demonstrate that exposure to high glucose in hESC-SCs leads to elevation of intracellular glucose and sorbitol which can be normalized by treatment with BP (**Figure 4D**).

Bupropion rescues the disease phenotypes in a mouse model of DPN

Given the remarkable ability of BP in rescuing the viability of high glucose treated hESC-SCs *in vitro*, the therapeutic effects of BP treatment in a mouse model of DPN was studied. Wild type C57BL6 mice were treated with the pancreatic beta cell specific toxin streptozotocin (STZ) which leads to beta cell death, impaired insulin production and hyperglycemia, as murine model of type 1 diabetes (Wu and Huan, 2008). This method is widely used to model type 1 diabetes in rodents (Akbarzadeh et al., 2007; Wu and Huan, 2001). In DPN patients and animal models, sensory nerve damage oftentimes leads to loss of sensation in the extremities. The impact of BP treatment on DPN phenotypes in STZ-treated mice was evaluated using a thermal sensation test as a functional readout and by histological analysis of the sciatic nerve to assess structural damage (**Figure 5A**). STZ-treated mice showed a significant increase in the levels of blood glucose independent of BP treatment as compared to non-diabetic control animals (**Figure 5B),** indicating that BP treatment does not affect glucose levels. Diabetic mice that were not treated with BP

showed a delayed response to thermal stimulation at seven and eight weeks post STZ treatment. BP treated diabetic mice showed a significantly improved response time comparable to that of normal, non-diabetic animals (**Figure 5C**). Histological analysis revealed an increase in the percentage of TUNEL+ apoptotic cells in the sciatic nerves of STZ mice. BP treated animals showed significantly fewer apoptotic cells than animals in the untreated group (**Figures 5D, 5E**). Next, the impact of STZ and BP treatment on peripheral myelin was evaluated using transmission electron microscopy. A large percentage of profoundly damages myelin were observed in the sciatic nerves of STZ treated animals, however this percentage was significantly reduced in STZ mice that were treated with BP (**Figures 5F-5G**). These studies demonstrate robust therapeutic effects of BP in the STZ-model of DPN.

Discussion

SCs play important roles in peripheral nerve development, function and repair. However, their development and function are poorly understood in humans due to limitations in obtaining them in workable numbers from primary tissue. Others previously reported Schwann-like cells from hPSCs after long-term maintenance of P75+/HNK1+ NC precursors (Lee et al., 2007), however, the these studies have the limitations of low induction efficiency, and months of *in vitro* culture, protracted differentiation, limited SC maturation and lack of myelination data (Lee et al., 2007; Liu et al., 2012; Ziegler et al., 2011). Attempts to derive human SCs by others also resulted in low yield, limited phenotypic characterization and lack of *in vitro* or *in vivo* myelination (Li et al., 2015; Mica et al., 2013). A highly efficient approach for generation of well characterized and pure population of human SCs was established, which sets the stage for future in depth developmental studies and translational applications such as disease modeling, and cell therapy.

An important feature of our hESC-based platform is the scalability and purity of the resulting SCs and the ability to culture cells for extended periods without losing SC properties. In contrast, primary SCs tend to rapidly lose their properties upon extended culture which results in the increasing contamination of Schwann cell cultures with fibroblast-like cells. Important developmental questions that are now accessible using this novel differentiation technology include the mechanisms controlling the transition from a multipotent NC stem cell to committed SCs and the study of human SC plasticity given recent data in the mouse suggesting that both melanocytes and parasympathetic neurons

can be derived from early SC-lineages (Adameyko et al., 2009; Espinosa-Medina et al., 2014). The identification of CD98 as a surface marker for the prospective isolation of committed SCs represents a powerful tool for such studies. The modeling of PNS pathologies could be of particular interest. A surprising feature of the cultured hESC-derived Schwann cell is their gene expression pattern that not only confirms Schwann cell identity but suggests that pluripotent-derived cells match the expression pattern of adult Schwann cells. This is in contrast to most other *in vitro* derived hPSC-lineages such as neurons expressing fetal stage markers (Studer et al., 2015).

The ability to generate myelinating SCs enable the modeling of genetic myelination disorders such as Charcot Marie Tooth (CMT) Disease. Current efforts to model CMT have been limited to defining early molecular events in SC precursors without capturing the actual disease phenotype. CMT is the most common genetic disorder of the PNS, however, the majority of peripheral neuropathies are acquired with diabetes being the most frequent cause (15 – 60% of all diabetic patients affected(Martyn and Hughes, 1997)).

The inventors present an hESC-based DPN model that revealed a selective vulnerability of SCs to diabetes-associated hyperglycemia. While most of the data were obtained in response to high glucose exposure, the inventors observed decreases in SC viability even at very moderate levels of increased glucose. The ability of BP to counteract glucose-mediated Schwann cell toxicity correlated with a decrease in intracellular glucose and sucrose levels but an increase in pyruvate levels suggesting an increase in oxygen consumption. Interestingly, BP appears to be the only antidepressive drug commonly associated with moderate weight loss in patients rather than a weight gain and thus BP mediated changes in glucose metabolism may be related to those systemic effects. The *in vivo* studies demonstrated that BP treatment can rescue DPN-related behavioral deficits and nerve damage. BP has shown some benefit in the treatment of patients suffering from neuropathic pain (Semenchuk et al., 2001) raising the question whether those effects of the drug for alternative indications could also be mediated by its effect on SC vulnerability. In addition to BP, the inventors identified several additional compounds capable or rescuing Schwann cell vulnerability. It will be interesting to determine whether those compounds act via a common or distinct mechanism and whether they show *in vivo* activity in STZ mice comparable to BP.

The study can lead to advancements in modeling other acquired peripheral neuropathies. Iatrogenic neuropathies are commonly observed in cancer patients following treatment with chemotherapy drugs such as cisplatin (Quasthoff and Hartung; Thompson et al., 1984). The combination of *in vitro* cytotoxicity tests using hPSC-derived lineages and HTS could serve as a platform for identification of potential drugs that reverse the cell-type specific side effects of these chemotherapy agents.

In conclusion, this study presents an effective framework to access human SC lineages for exploring their biology in health and disease and developing novel therapies for DPN. Directed differentiation of hPSCs represent an effective approach for large scale derivation of human SCs with broad implications in basic and translational research. This framework offers new possibilities for in-depth studies of the role of glia in the PNS biology and disease and contributes to the development of new therapeutics for peripheral neuropathies in future. The work further implicates SC defects in the pathogenesis of DPN and presents BP as an FDA-approved drug that can treat DPN-related damage *in vitro* and *in vivo*.

Table 1. Top 200 upregulated genes in hESC-derived Schwann cell precursors (day 25) versus hESC-derived NC (day 11).

Gene ID	**log2FoldChange**
CALCB	13.12
GPR116	9.29
TSPYL5	8.44
ITPKA	8.33
SLC17A6	8.28
SYPL2	8.12
LOC100128252	7.73
ANGPTL7	7.47
LOC728978	7.31
ZNF502	7.22
ZNF229	7.17
XLOC_003498	7.12
STK32A	7.08
LOC100507341	7.01
EEF1A2	6.98
TRIM54	6.95
SEZ6L	6.77
SLC16A6	6.66
C20orf26	6.64
LPL	6.58
STMN4	6.57

CNGA3	6.55
QPCT	6.54
C12orf69	6.42
CACNG5	6.33
BAAT	6.30
AGBL4	6.28
COL12A1	6.22
SPOCK2	6.21
XLOC_000972	6.20
ABCB1	6.18
ANGPTL1	6.14
CHRNA1	6.07
DHRS2	6.06
MFAP4	6.01
ARHGDIG	5.97
XLOC_003411	5.97
ABLIM3	5.97
LINC00152	5.97
HLA-DOB	5.95
P2RX3	5.93
PLA2G4C	5.87
CAV1	5.86
CD44	5.85
FAM26E	5.82
SRPX2	5.81
LUM	5.80
CRHBP	5.74
HOXD9	5.73
ADAMTS8	5.71
SLC30A2	5.66
C7orf29	5.65
DSCAM	5.65
PHOX2A	5.63
KCNK9	5.62
GAL	5.59
SST	5.58
DMGDH	5.53
KCNH5	5.52
TRIM67	5.52
GPR64	5.50
GPR115	5.49
PTPRN	5.48
NKX6-2	5.48
PNPLA4	5.48
NOV	5.47
ABCG1	5.42
NELL1	5.39
SPARCL1	5.37

LOC375010	5.37
APLNR	5.35
DCN	5.35
SLC10A4	5.35
NCAN	5.33
PLEK2	5.32
HSPB7	5.32
CLCA2	5.30
FAIM2	5.29
CALB1	5.28
SLC6A15	5.26
LOC100132891	5.24
SCG2	5.23
NFIB	5.22
RUNDC3B	5.21
XLOC_010607	5.21
C5AR1	5.21
MICAL2	5.20
SGIP1	5.17
GNG3	5.14
LOC541471	5.14
KCNA2	5.14
FOXF2	5.11
IFI44L	5.10
HPCAL4	5.10
LOC100507043	5.09
TNFAIP6	5.09
TMEM132D	5.07
KLHDC7B	5.04
GMPR	5.03
CMKLR1	5.02
PPP1R27	5.01
REEP1	5.01
PALM3	5.00
PTPN5	4.99
GPRIN3	4.99
MGP	4.97
ATP8A2	4.97
SERPINB2	4.95
TCN1	4.94
IFI44	4.94
CLVS2	4.94
DGKI	4.93
FAM20C	4.91
TPH2	4.91
TGFA	4.90
ACTG2	4.89
ULBP2	4.89

RMRP	4.89
XLOC_011087	4.88
NPFFR2	4.88
GDAP1L1	4.86
INHBA	4.85
CHSY3	4.85
PPYR1	4.84
CD163L1	4.83
MIR7-3HG	4.82
ZNF542	4.79
CDH13	4.78
TM4SF1	4.77
TYRP1	4.77
SYT9	4.77
CACNG7	4.76
PDLIM3	4.76
FAM135B	4.74
NETO1	4.74
CD207	4.73
TNC	4.73
TNFRSF8	4.73
XLOC_013083	4.73
SYT5	4.73
PMP2	4.72
PTPRH	4.72
ZFP28	4.71
LHFPL4	4.70
TTBK1	4.69
HOXB7	4.68
HCST	4.67
SERPINB7	4.67
LOC653513	4.67
MSC	4.66
SYNGR3	4.65
POPDC3	4.65
PENK	4.62
CFI	4.59
C3AR1	4.59
SERPINE1	4.58
NT5E	4.57
C4orf32	4.57
TMEM59L	4.56
RIPPLY2	4.54
STEAP3	4.54
SLC1A2	4.54
HECW1	4.54
IL8	4.54
FAM65B	4.53

TLR4	4.53
ADAMTS5	4.52
CDKN2B	4.51
LGI2	4.51
KCNMA1	4.50
ANKRD1	4.50
XLOC_009257	4.50
MXRA5	4.49
HIGD1B	4.49
ALX4	4.47
RUNX3	4.46
ETV4	4.46
HOXD8	4.45
FLNC	4.45
HRK	4.45
HRH3	4.45
LOC338651	4.45
CAV2	4.44
HCST	4.67
SERPINB7	4.67
LOC653513	4.67
MSC	4.66
SYNGR3	4.65
POPDC3	4.65
PENK	4.62
CFI	4.59
C3AR1	4.59
SERPINE1	4.58
NT5E	4.57
C4orf32	4.57
TMEM59L	4.56
RIPPLY2	4.54
STEAP3	4.54
SLC1A2	4.54
HECW1	4.54
IL8	4.54
FAM65B	4.53
TLR4	4.53
ADAMTS5	4.52
CDKN2B	4.51
LGI2	4.51
KCNMA1	4.50
ANKRD1	4.50
XLOC_009257	4.50
MXRA5	4.49
HIGD1B	4.49
ALX4	4.47
RUNX3	4.46

ETV4	4.46
HOXD8	4.45
FLNC	4.45
HRK	4.45
HRH3	4.45
LOC338651	4.45
CAV2	4.44

Table 2. Top 200 upregulated genes in hESC-derived Schwann cells (day 50) versus hESC-derived NC (day 11)

Gene ID	**log2FoldChange**
STT3B	11.85
CTAGE5	11.30
KBTBD6	10.63
B3GALTL	10.46
PAX9	10.07
APOO	9.45
XLOC_011326	9.15
HSPE1	8.84
TRIM3	8.73
RAP2B	8.53
TRAPPC9	8.49
TXNDC15	8.35
THBS2	8.34
GMPPB	8.32
PLP2	8.20
NCS1	8.09
ABL1	7.92
FMNL2	7.77
NDUFA12	7.39
XLOC_009725	7.34
PTRH2	7.24
CNBD1	7.24
XLOC_000576	7.21
ZNF224	7.19
PDXDC2P	7.11
GSTM3	7.07
CENPM	7.06
GCLM	7.04
NCAPH	6.98
C15orf37	6.98
JAK1	6.97
STARD3	6.87
TRIB3	6.80
DOPEY2	6.77
APAF1	6.72

NCOA4	6.71
PSMB6	6.68
COX20	6.64
PIK3CB	6.63
HAX1	6.58
KITLG	6.58
CNTD1	6.54
ETNK2	6.47
LRRC57	6.47
CDK2	6.43
GOLGA7	6.42
CCDC90B	6.38
GSTP1	6.31
PPP1R8	6.28
C7orf50	6.28
POLR2L	6.25
ITGB1	6.24
TYRP1	6.18
DNAJC3	6.14
THY1	6.14
GOSR2	6.12
FAM123B	6.12
HIGD1A	6.10
ELMOD3	6.10
NME5	6.04
TUSC2	6.02
C11orf10	5.93
SIPA1	5.93
JUP	5.92
NCKAP5	5.90
THYN1	5.90
RUNX1	5.81
FLJ46906	5.80
XLOC_004725	5.77
MGC57346	5.74
RTP4	5.72
PLD3	5.70
NYAP1	5.69
TLN2	5.65
XLOC_009577	5.62
FBLN5	5.57
LRFN5	5.56
CDH7	5.56
XLOC_003471	5.54
BRWD3	5.53
RAX2	5.49
MRPS16	5.38
CUL4A	5.36

EPHA5	5.36
SPTBN2	5.34
SMYD5	5.31
CDKN2AIP	5.30
ZNF829	5.28
OLFM2	5.27
PNMA6C	5.24
DNAJB11	5.23
NIPAL2	5.20
ZNF622	5.19
STRADA	5.18
CEP57L1	5.17
SHISA6	5.16
CKAP2	5.15
IGFBP7	5.14
GRSF1	5.14
GRWD1	5.13
CD101	5.13
PLIN2	5.08
LOC100129361	5.04
PRKG1	5.04
SERF2	5.04
RUNX3	5.04
FAM91A1	5.02
ALDH3B1	5.01
CCDC96	5.00
NNMT	4.98
C11orf71	4.98
ZNF804A	4.98
DNAJA1	4.94
CHCHD1	4.93
SRPX	4.91
XLOC_007995	4.89
C11orf61	4.87
TNFAIP8	4.86
CSPG4	4.86
ALX3	4.86
SSR4P1	4.85
CES4A	4.84
IFI44	4.84
PLCD3	4.84
XLOC_009509	4.83
PPP2R5E	4.82
C19orf53	4.81
SPARCL1	4.79
UBE3B	4.78
HPGD	4.77
ADM2	4.74

TLR1	4.73
NYNRIN	4.73
PHF8	4.73
IL2RB	4.72
TEX9	4.72
IGFBP1	4.71
PLAC8L1	4.69
DHX34	4.68
TOPBP1	4.67
BCAP31	4.67
RHBDF2	4.66
TP53BP2	4.65
DIRAS2	4.63
DNMT1	4.63
TMEM9B	4.63
OSGIN1	4.63
SWI5	4.63
CILP	4.61
GLTPD2	4.60
LSMD1	4.58
SAMD11	4.58
BCAR1	4.58
ENTHD1	4.57
PTTG1IP	4.54
PAFAH1B1	4.53
SERPINB1	4.50
BPI	4.49
GNL3	4.48
APOE	4.48
DR1	4.47
TUBGCP3	4.47
C11orf82	4.47
ANTXR1	4.45
DLG5	4.44
PLK1S1	4.41
EGLN2	4.40
GTF2A1	4.40
COL6A2	4.40
CAPZA1	4.39
PRR24	4.38
SMUG1	4.36
ZNF626	4.36
MAGED2	4.36
EHBP1	4.35
LAMA5	4.35
XLOC_008024	4.34
RPS10	4.33
THTPA	4.33

PHF2	4.32
CCDC71L	4.31
KLHL18	4.30
FAM49A	4.29
TIMP4	4.29
ANAPC10	4.28
C19orf29-AS1	4.28
SKIV2L2	4.27
C15orf52	4.26
ATP6AP2	4.25
FASTKD5	4.25
WDR45	4.24
AP2S1	4.24
HS2ST1	4.23
G6PC3	4.21
ANKRD44	4.17
GIT2	4.16
MIR22HG	4.16
SH3TC2	4.15
ALPK1	4.15
POLE	4.14

Table 3. Top 200 upregulated genes in hESC-derived Schwann cells (day 100) versus hESC-derived NC (day 11)

Gene ID	**Log2FoldChange**
TYRP1	15.79
CD44	13.21
ENTHD1	11.71
NT5E	11.69
HTR2B	11.48
NOV	10.78
IL8	10.49
SLC16A6	10.35
CDKN2A	9.92
GPNMB	9.90
HSPB7	9.46
EMP1	9.29
RIT2	9.29
PAEP	9.16
TYR	8.99
SYNC	8.98
XLOC_008700	8.83
NLRC5	8.71
FAIM3	8.68
RGS20	8.64
CBR3	8.63

TMEM173	8.63
GJA3	8.59
SAMD9	8.33
EVI2B	8.30
FBXO32	8.26
TSPYL5	8.25
TLR4	8.09
SERPINE1	7.90
HOXD-AS1	7.88
CITED1	7.87
KCNA5	7.81
ATP10A	7.75
OCA2	7.75
IRF4	7.73
MMP8	7.71
GAL	7.70
CD109	7.68
LGI3	7.57
LGALS3	7.53
TRIM63	7.51
XLOC_003498	7.46
LOC285000	7.45
KLHL38	7.39
HOXB2	7.35
PTHLH	7.30
MBP	7.29
CARD16	7.27
TFF3	7.23
IL13RA2	7.22
LINC00152	7.21
ISM1	7.21
MLPH	7.16
ECM1	7.12
CHSY3	7.11
CXCL1	7.08
KLF2	7.08
ASB11	7.07
KRTAP19-1	7.02
C10orf90	7.01
ITGA3	6.99
LOC646329	6.98
THBD	6.96
FLJ43663	6.95
HR	6.92
C1orf127	6.89
NFIX	6.88
LY96	6.85
LOC100128252	6.85

TRIM47	6.79
XLOC_002736	6.77
COL8A1	6.76
RUNX3	6.74
ZNF229	6.72
C15orf52	6.71
CABLES1	6.69
FOSL1	6.67
RASGRP3	6.64
TBX18	6.63
SPON2	6.58
THBS2	6.58
LOC541471	6.53
AHRR	6.52
SGCD	6.50
ZNF502	6.47
CSPG4	6.45
BARX2	6.44
MYC	6.44
SLC7A4	6.43
MLIP	6.43
VGF	6.42
DHRS2	6.41
HOXD3	6.41
SYPL2	6.39
SGK1	6.39
MLANA	6.39
DUSP10	6.35
ITGB3	6.35
KCNJ13	6.32
ST8SIA6	6.32
MME	6.32
PLXNC1	6.32
SUSD5	6.26
DLX1	6.26
MMP1	6.22
ANO4	6.21
C19orf71	6.20
STK32A	6.19
CAV1	6.16
PSMB8	6.12
PLP2	6.12
BCL2A1	6.11
HOXD4	6.11
LOC100507463	6.10
TFPI2	6.07
NFIB	6.06
TNFRSF14	6.05

ANKRD1	6.03
IFI16	6.01
DDIT4L	6.01
KCNQ5	6.01
NR4A3	6.00
IFIT2	6.00
XLOC_013026	6.00
SH2D4B	5.99
XLOC_001215	5.98
FAM129A	5.96
GREM1	5.96
HSPA6	5.92
TM4SF1	5.92
HOXB7	5.92
MET	5.91
MFSD12	5.90
IL6R	5.89
RUNX1	5.86
CATSPER1	5.83
FAM20C	5.83
GMPR	5.82
GOLGA7B	5.80
PHLDA2	5.80
MIR612	5.77
GALNTL6	5.77
MGAT5B	5.76
HSF4	5.75
SLC1A4	5.74
CD97	5.74
SLC24A5	5.74
XLOC_004803	5.74
LOC375010	5.73
COL12A1	5.68
PNPLA4	5.66
LOC100133445	5.66
TSPAN10	5.64
OSGIN1	5.63
GIPC3	5.62
CPNE7	5.59
OAS3	5.59
GRIN2B	5.59
CD300LB	5.59
KDR	5.58
UPP1	5.58
S100A6	5.58
SH3TC2	5.55
WFDC1	5.54
AQP9	5.53

XLOC_001738	5.52
XLOC_007040	5.51
LYL1	5.51
SLC6A15	5.49
SYK	5.49
C7orf29	5.46
XLOC_009274	5.45
RIPK3	5.45
S100A4	5.41
NFATC2	5.40
CEBPE	5.39
GEM	5.37
MYOT	5.37
ABCG2	5.37
XLOC_007085	5.35
ERG	5.33
ARID5B	5.32
TRPV2	5.31
LPL	5.31
XLOC_008985	5.31
SERPINB2	5.31
IFI35	5.27
MIR221	5.27
S100A2	5.27
BMPR1B	5.25
SP100	5.24
LOC400643	5.24
PDGFB	5.24
XLOC_001228	5.20
HRK	5.20
BHLHE41	5.19
LDHAL6B	5.18
GPR65	5.17
XLOC_006252	5.16
LGALS1	5.13
XLOC_008985	5.31
LGALS1	5.13

Table 4. Top 200 upregulated genes in human primary Schwann cells versus hESC-derived NC (day 11)

Gene ID	***log2FoldChange***
KBTBD6	12.13
FAHD2B	11.98
CTAGE5	11.64
C19orf45	11.30
XLOC_003345	10.85

WDR90	10.83
B3GALTL	10.55
PAX9	9.77
GMPPB	9.65
HSPE1	9.64
XLOC_008617	9.53
TRIM3	9.32
SPINT2	9.08
PLP2	9.05
ADAMTS20	8.91
CD8A	8.84
COX20	8.84
UNC5CL	8.75
DIRAS2	8.45
THBS2	8.41
RIMS3	8.20
ZNF273	8.20
GCLM	8.20
CLDN3	8.16
CCDC167	8.15
RAP2A	8.10
NCS1	8.10
TXNDC15	8.08
DSN1	8.01
ZNF224	7.97
NYAP1	7.96
SIPA1	7.90
XLOC_009279	7.88
JAK1	7.83
NDUFA12	7.73
XLOC_009509	7.70
EFNB3	7.69
SHISA6	7.66
XLOC_000734	7.45
APAF1	7.45
HAX1	7.42
PTRH2	7.41
TLN2	7.40
KLF12	7.38
STT3B	7.33
MESP2	7.31
RASGRP3	7.25
ZNF559	7.25
PRR11	7.24
FAM123B	7.22
MBD1	7.18
CNTN2	7.18
LRFN5	7.16

WBP4	7.12
CLCN5	7.08
ABL1	7.05
ORC6	7.04
CCL27	7.02
KAT7	6.96
KITLG	6.95
MIR4746	6.92
ARHGEF38	6.92
CCDC90B	6.90
MIR3176	6.89
PPP1R8	6.88
MCMBP	6.84
FAM199X	6.81
TRIB3	6.78
GNL3	6.77
BRWD3	6.77
IRX2	6.76
SPTBN2	6.75
CTTNBP2	6.74
KIAA1609	6.74
ZC3H12B	6.72
TEX9	6.70
THYN1	6.68
DCC	6.66
UG0898H09	6.64
STARD3	6.61
ZNF804A	6.61
C11orf71	6.60
ITGB1	6.57
FLNB	6.54
NCOA4	6.53
INMT	6.51
CBY3	6.51
TAGLN3	6.50
ST6GALNAC3	6.48
POLR2L	6.47
XLOC_007995	6.43
MAGEE1	6.40
LOC400604	6.39
GOSR2	6.39
LOC285889	6.39
MGC57346	6.37
TIMP4	6.27
CNKSR1	6.26
GOLGA7	6.26
GLTPD2	6.24
XLOC_009224	6.23

PSMB6	6.22
RTDR1	6.18
MTL5	6.17
IGLON5	6.17
HIGD1A	6.16
PBK	6.15
SPATA5L1	6.14
XLOC_014081	6.14
CUL4A	6.13
MCM5	6.10
SMYD5	6.10
LIN28A	6.06
ANTXR2	6.04
LRRC57	6.03
RUNX1	6.02
XLOC_006828	6.01
FAM66C	5.99
RAX2	5.99
ERMN	5.98
RLBP1	5.95
TP73	5.93
PPOX	5.88
BRD9	5.88
TTC40	5.87
BCL7A	5.83
PAEP	5.83
SWI5	5.81
SPTBN4	5.80
DNAJC3	5.79
MACROD2	5.79
FBLN5	5.78
ALX3	5.78
LAMA5	5.77
RBM28	5.77
GRWD1	5.74
XLOC_012069	5.71
LOC339874	5.70
AGAP8	5.70
NIPAL2	5.70
NNMT	5.69
EPHA5	5.67
CNTNAP3B	5.67
XLOC_010236	5.64
EHBP1	5.64
PLAC8L1	5.64
GPR162	5.63
MYEF2	5.63
CAPZA1	5.60

DIRC3	5.60
RHBDF2	5.59
ZNF610	5.59
XLOC_011568	5.58
GRHL3	5.58
SETDB2	5.56
UBE3B	5.55
CD97	5.54
XLOC_012905	5.54
XLOC_011507	5.54
MOB3B	5.53
NRARP	5.53
SRPX	5.51
LOC100506314	5.51
ZNF497	5.50
CALM2	5.49
XLOC_003249	5.49
PCSK9	5.48
CSPG4	5.48
XLOC_013922	5.47
C12orf76	5.45
CTAGE10P	5.45
ZNF622	5.44
XLOC_012288	5.44
LSMD1	5.41
XLOC_003726	5.39
ATRNL1	5.37
XLOC_007094	5.37
LOC100287036	5.34
ELMOD3	5.34
PLCD3	5.34
THY1	5.33
KCNH8	5.33
XLOC_003433	5.33
XLOC_011075	5.33
MYEF2	5.63
C15orf52	5.33
ROBO2	5.31
SDK1	5.31
TSNARE1	5.31
MTRR	5.30
SDR9C7	5.29
GSTM3	5.29
ZNF829	5.29
IFI44	5.28
IRAK3	5.28
CHCHD1	5.27
LOC100506801	5.27

COG1	5.25
TMEM200C	5.25
TBRG1	5.24
PPFIA1	5.23

Table 5. List of primary antibodies and working dilutions

Antibody	**Source**	**Dilution**
CD49D	Biolegend	1:800
TUJ1	Covance	1:1000
CHAT	Millipore	1:1000
GFAP	Dako	1:1000
S100B	Dako	1:200
MBP	Millipore	1:200
MAG	Millipore	1:200
NFH	Encore	1:1000
PanNa	Joel Black	1:1000
Kv1.2	Joel Black	1:200
CASPR	Joel Black	1:1000
SC101	STEM Cell Tech	1:1000
Antibody	Source	Dilution

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What is claimed is:

1. An *in vitro* method of screening a compound that is suitable for regeneration of peripheral nervous system (PNS), for regeneration of central nervous system (CNS), for prevention and/or repair of myelin damage, and/or for preventing and/or treating a Schwann cell related disorder, comprising:

(a) exposing a population of cells expressing one or more Schwann cell precursor marker to a glucose concentration of at least about 30 mM, wherein said cells are obtained from *in vitro* differentiation of stem cells,

(b) contacting said cells with a test compound after glucose exposure,

(c) measuring one or more of first sorbitol level, first glucose level, and first cell viability of said cells without treatment of said test compound;

(d) measuring one or more of second sorbitol level, second glucose level, and second cell viability of said cells treated with said test compound;

(e) comparing one or more of the followings:

(i) said second sorbitol level with said first sorbitol level,

(ii) said second glucose level with said first glucose level,

(iii) said second cell viability with said first cell viability; and

(f) identifying a test compound that is suitable for regeneration of PNS and/or CNS, for prevention and/or repair of myelin damage, and/or for preventing and/or treating of a Schwann cell related disorder where one or more of the followings is present:

(i) said second sorbitol level is lower than said first sorbitol level,

(ii) said second glucose level is lower than said first glucose level, and

(iii) said second cell viability is lower than first cell viability.

2. An *in vitro* method of screening a compound that is suitable for regeneration of peripheral nervous system (PNS), for regeneration of central nervous system (CNS), for prevention and/or repair of myelin damage, and/or for preventing and/or treating a Schwann cell related disorder, comprising:

(a) exposing a population of cells expressing one or more Schwann cell marker to a glucose concentration of at least about 30 mM, wherein said cells are obtained from *in vitro* differentiation of stem cells,

(b) contacting said cells with a test compound after glucose exposure,

(c) measuring one or more of first sorbitol level, first glucose level, and first cell viability of said cells without treatment of said test compound;

(d) measuring one or more of second sorbitol level, second glucose level, and second cell viability of said cells treated with said test compound;

(e) comparing one or more of the followings:

(i) said second sorbitol level with said first sorbitol level,

(ii) said second glucose level with said first glucose level,

(iii) said second cell viability with said first cell viability; and

(f) identifying a test compound that is suitable for regeneration of PNS and/or CNS, for prevention and/or repair of myelin damage, and/or for prevention and/or treatment of a Schwann cell related disorder where one or more of the followings is present:

(i) said second sorbitol level is lower than said first sorbitol level,

(ii) said second glucose level is lower than said first glucose level, and

(iii) said second cell viability is lower than first cell viability.

3. The method of claim 2, wherein said cells expressing one or more Schwann cell precursor marker are obtained from *in vitro* differentiation of stem cells by a method comprising: contacting a population of stem cells with one or more inhibitor of TGFβ/Activin-Nodal signaling and contacting said cells with one or more Wnt activator, and further contacting said cells with one or more FGF activator for at least about 3 days.

4. The method of claim 3, wherein said cells expressing one or more Schwann cell marker are obtained from *in vitro* differentiation of stem cells by a method comprising:

contacting a population of stem cells with one or more inhibitor of TGFβ/Activin-Nodal signaling and contacting said cells with one or more Wnt activator,

further contacting said cells with one or more FGF activator for at least about 3 days to produce the population of differentiated cells expressing one or more Schwann cell precursor marker, and

subjecting said population of differentiated cells expressing one or more Schwann cell precursor marker to conditions favoring maturation of Schwann cells precursor cells into Schwann cells.

5. The method of claim 3 or 4, wherein said method for differentiating the stem cells comprises contacting said cells with said one or more FGF activator for about 14 days.

6. The method of any one of claims 3-5, wherein said method for differentiating the stem cells comprises initially contacting said cells with said one or more FGF activator no

later than about 20 days from the initial contact of said stem cells with said one or more inhibitor of TGFβ/Activin-Nodal signaling.

7. The method of claim 6, wherein said method for differentiating the stem cells comprises initially contacting said cells with said one or more FGF activator between about 10 days and about 15 days from the initial contact of said stem cells with said one or more one or more inhibitor of TGFβ/Activin-Nodal signaling.

8. The method of claim 7, wherein said method for differentiating the stem cells comprises initially contacting said cells with said one or more FGF activator about 11 days from the initial contact of said stem cells with said one or more one or more inhibitor of TGFβ/Activin-Nodal signaling.

9. The method of any one of claims 3-8, further comprising contacting said cells with one or more SC differentiation inducer to produce a population of cells expressing one or more SC precursor marker.

10. The method of claim 9, wherein said method for differentiating the stem cells comprises contacting said cells with said one or more SC differentiation inducer for at least about 3 days to produce a population of differentiated cells that express one or more Schwann cell precursor marker.

11. The method of claim 10, wherein said method for differentiating the stem cells comprises contacting said cells with said one or more SC differentiation inducer for about 14 days.

12. The method of any one of claims 9-11, wherein said method for differentiating the stem cells comprises initially contacting said cells with said one or more SC differentiation inducer between about 10 days and about 15 days from the initial contact of said stem cells with said one or more one or more inhibitor of TGFβ/Activin-Nodal signaling.

13. The method of any one of claims 9-12, wherein said method for differentiating the stem cells comprises contacting said cells with said one or more FGF activator and said one or more SC differentiation inducer concurrently.

14. The method of any one of claims 3-13, wherein said population of stem cells are differentiated into a population of differentiated cells that express one or more said Schwann cell precursor marker on or after about 25 days from the initial contact of said stem cells with said one or more inhibitor of TGFβ/Activin-Nodal signaling.

15. The method of any one of claims 3-14, wherein said method for differentiating the stem cells further comprises contacting said stem cells with one or more SMAD inhibitor.

16. The method of claim 15, wherein said method for differentiating the stem cells comprises contacting said stem cells with said one or more inhibitor of TGFβ/Activin-Nodal signaling and said one or more SMAD inhibitor concurrently.

17. The method of any one of claims 3-16 , wherein said method for differentiating the stem cells comprises initially contacting said cells with said one or more Wnt activator no later than about 4 days from the initial contact of said stem cells with said one or more inhibitor of TGFβ/Activin-Nodal signaling.

18. The method of claim 17, wherein said method for differentiating the stem cells comprises initially contacting said cells with said one or more activator of Wnt signaling about 2 days from the initial contact of said stem cells with said one or more inhibitor of TGFβ/Activin-Nodal signaling.

19. The method of claim 17, wherein said method for differentiating the stem cells comprises initially contacting said cells with said one or more activator of Wnt signaling on the same day as the initial contact of said stem cells with said one or more inhibitor of TGFβ/Activin-Nodal signaling.

20. The method of any one of claims 3-19, wherein said one or more inhibitor of TGFβ/Activin-Nodal signaling is a small molecule selected from the group consisting of SB431542, derivatives thereof, and mixtures thereof.

21. The method of claim 20, wherein said one or more inhibitor of TGFβ/Activin-Nodal signaling is SB431542.

22. The method of any one of claims 15-21, wherein said one or more SMAD inhibitor is a small molecule selected from the group consisting of LDN193189, derivatives thereof, and mixtures thereof.

23. The method of claim 22, wherein said one or more SMAD inhibitor is a LDN193189.

24. The method of any one of claims 3-23, wherein said one or more Wnt activator lowers glycogen synthase kinase 3β (GSK3β) for activation of Wnt signaling.

25. The method of claim 24, wherein said one or more Wnt activator is a small molecule selected from the group consisting of CHIR99021, Wnt-1, WNT3A, Wnt4, Wnt5a, derivatives thereof, and mixtures thereof.

26. The method of claim 25, wherein said one or more Wnt activator is CHIR99021.

27. The method of any one of claims 9-26, wherein said one or more SC differentiation inducer is selected from the group consisting of neuregulins, LIF, CNTF, Forskolin, TGFβ and FBS.

28. The method of claim 27, wherein said one or more SC differentiation inducer is NRG1.

29. The method of any one of claims 4-28, wherein said one or more FGF activator is selected from the group consisting of FGF1, FGF2, FGF3, FGF4, FGF7, FGF8, FGF10, FGF18, derivatives thereof, and mixtures thereof.

30. The method of claim 29, wherein said one or more FGF activator is FGF2.

31. The method of any one of claims 1, 3-30, wherein said one or more Schwann cell precursor marker is selected from the group consisting of SOX10, GAP43, BLBP, MPZ, Dhh, P75NTR, CD49D, TFAP2, CDH19, CD44, ERBB3, POU3F1, GFAP, CALCB, GRP116, TSPYL5, ITPKA, SLC17A6, SYPL2, LOC100128252, ANGPTL7, LOC728978, ZNF502, SLC16A6, LPL, SLC30A2, and SLC10A4.

32. The method of any one of claims 3-31, wherein said stem cells are human stem cells.

33. The method of claim 32, wherein said human stem cells are selected from the group consisting of human embryonic stem cells, human induced pluripotent stem cells, human parthenogenetic stem cells, primordial germ cell-like pluripotent stem cells, epiblast stem cells, F-class pluripotent stem cells.

34. The method of claim 4, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise: contacting said cells with one or more FGF activator, and one or more Schwann cell differentiation inducer.

35. The method of claim 34, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting said cells with the one or more FGF activator and the one or more Schwann cell differentiation inducer for at least about 3 days.

36. The method of claim 35, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the cells with the one or more FGF activator and the one or more Schwann cell differentiation inducer for about 10 days.

37. The method of claim 35, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the cells with the one or more FGF activator and the one or more Schwann cell differentiation inducer for about 35 days.

38. The method of any one of claims 33-37, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise further contacting the cells with one or more SC differentiation enhancer.

39. The method of claim 38, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the cells with the one or more SC differentiation enhancer for at least about 3 days.

40. The method of claim 39, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the cells with the one or more SC differentiation enhancer for about 10 days.

41. The method of claim 39, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise contacting the cells with the one or more SC differentiation enhancer for about 35 days.

42. The method of any one of claims 38-41, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprises contacting the population cells with the one or more FGF activator, the one or more Schwann cell differentiation inducer, and the one or more SC differentiation enhancer concurrently.

43. The method of any one of claims 33-42, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise: further contacting said cells with one or more SC differentiation enhancer.

44. The method of any one of claims 38-43, wherein said one or more SC differentiation enhancer is selected from the group consisting of neuregulins, cyclic adenosine monophosphate (cAMP), Forskolin, LIF, and CNTF.

45. The method of claim 44, wherein said one or more SC differentiation enhancer is cAMP.

46. The method of any one of claims 3-45, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells comprise aggregating said cells into 3D spheroids; and contacting said 3D spheroids with said one or more FGF activator, and said one or more Schwann cell differentiation inducer.

47. The method of claim 46, wherein said conditions favoring maturation of Schwann cell precursor cells into Schwann cells further comprise culturing said 3D spheroids in adherent culture.

48. The method of any one of claims 4, and 34-37, wherein said one or more Schwann cell marker is selected from the group consisting LRRTM4, CDH1, FABP7, BDNF,

UNCB5, SOSTDC1, OLIG1, PLAT, KCNJ10, SHH, NTN1, GDNF, ERBB3, GAP43, SOX10, S100, GFAP, POU3F1, PMP22, MBP, AQP4, MPZ, NGFR, NFATC4, MOG, IFNG, MAL, NTF3, TGFB1 , CD9, CD81, CD44, CD98, CD49E, CD49D, TYRP1, ENTHD1, NT5E, HTR2B, NOV, IL8, SLC16A6, CDKN2A, PLP2, S100A6, AQP9, and CDH19.

49. The method of any one of claims 1-48, wherein said glucose concentration is at least about 10 mM.

50. The method of claim 49, wherein said glucose concentration is about 30 mM.

51. The method of any one of claims 1-50, wherein said measurement is performed at least about 12 hours from the initial exposure of said cells to said glucose.

52. The method of claim 51, wherein said measurement is performed at about 72 hours from the initial exposure of said cells to said glucose.

53. The method of any one of claims 1-52, wherein the Schwann cell related disorder is selected from the group consisting of peripheral neuropathy, Schwannomatosis, Charcot-Marie-Tooth Disease, and Guillain-Barre Syndrome.

54. The method of claim 53, wherein the Schwann cell related disorder is a peripheral neuropathy.

55. The method of claim 54, wherein the peripheral neuropathy disorder is a diabetic peripheral neuropathy.

56. A method of regeneration of peripheral nervous system (PNS), for regeneration of central nervous system (CNS), for prevention and/or repair of myelin damage, and/or for preventing and/or treating a Schwann cell related disorder in a subject, comprising administering an effective amount of a compound or a composition comprising thereof to the subject suffering from the Schwann cell related disorder, wherein the compound is selected from the group consisting of potassium channel blockers, norepinephrine-dopamine reuptake inhibitors, cyclopenthiazide, captopril, isradipine, condelphine, nimesulide, triamcinolone, salts thereof, solvates thereof, hydrate thereof, clathrates thereof, and prodrugs thereof, and combinations thereof.

57. The method of claim 56, wherein said potassium channel blocker is a sulfonylurea compound.

58. The method of claim 57, wherein said sulfonylurea compound is selected from the group consisting of tolbutamide, acetohexamide, carbutamide, chlorpropamide, glycyclamide (tolhexamide), metahexamide, tolazamide, glibenclamide (glyburide),

glibornuride, gliclazide, glipizide, gliquidone, glisoxepide, glyclopyramide, glimepiride, salts thereof, solvates thereof, hydrate thereof, clathrates thereof, and prodrugs thereof.

59. The method of any one of claims 56-58, wherein said norepinephrine-dopamine reuptake inhibitor is selected from the group consisting of bupropion, amineptine, methylphenidate (Ritalin®, Concerta®, Metadate®, Methylin®, Rubifen®, or Stimdate®), atomoxetine, maprotiline, desoxypipradrol, dexmethylphenidate, difemetorex, diphenylprolinol, ethylphenidate, fencamfamine, fencamine, lefetamine, methylenedioxypyrovalerone, methylphenidate, nomifensine, O-2172, oxolinic acid, pipradrol, prolintane, pyrovalerone, tametraline, WY-46824, salts thereof, solvates thereof, hydrate thereof, prodrugs thereof, and clathrates thereof.

60. The method of any one of claims 56-59, wherein the Schwann cell related disorder is selected from the group consisting of peripheral neuropathy, Schwannomatosis, Charcot-Marie-Tooth Disease, and Guillain-Barre Syndrome.

61. The method of claim 60, the Schwann cell related disorder is a peripheral neuropathy.

62. The method of claim 61, the peripheral neuropathy is diabetic peripheral neuropathy.

63. The method of any one of claims 56-62, the compound is bupropion, a salt, a solvate, a hydrate, a clathrate, or a prodrug thereof.

64. The method of any one of claims 56-62, the compound is a bupropion hydrochloride.

65. The method of any one of claims 56-62, the compound is a bupropion metabolite, a salt, a solvate, a hydrate, a clathrate, or a prodrug thereof.

66. The method of claim 65, the bupropion metabolite is selected from the group consisting of hydroxybupropion, threo-hydrobupropion, and erythrohydrobupropion.

67. The method of any one of claims 56-66, the potassium channel blocker is tolbutamide, a salt, a solvate, a hydrate, a clathrate, or a prodrug thereof.

68. The method of any one of claims 56-67, the composition is a pharmaceutical composition that further comprises a pharmaceutically acceptable carrier.

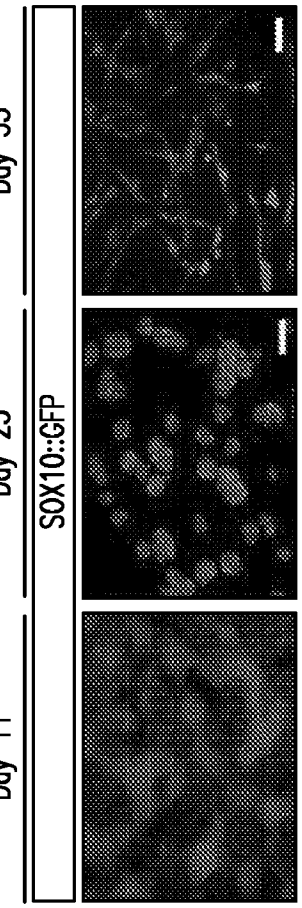

FIG. 1B

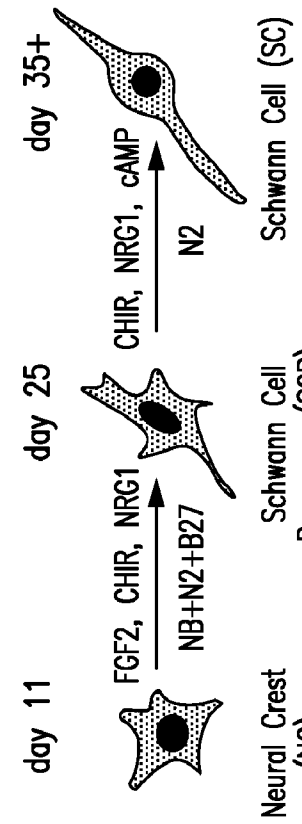

FIG. 1A

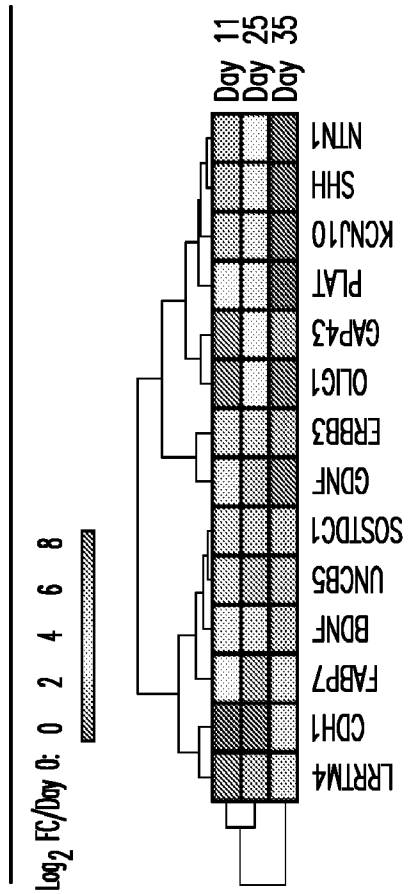

FIG. 1D

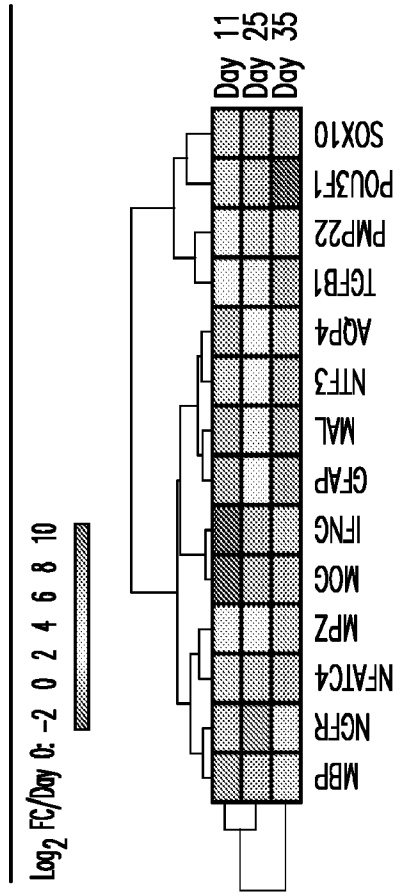

FIG. 1C

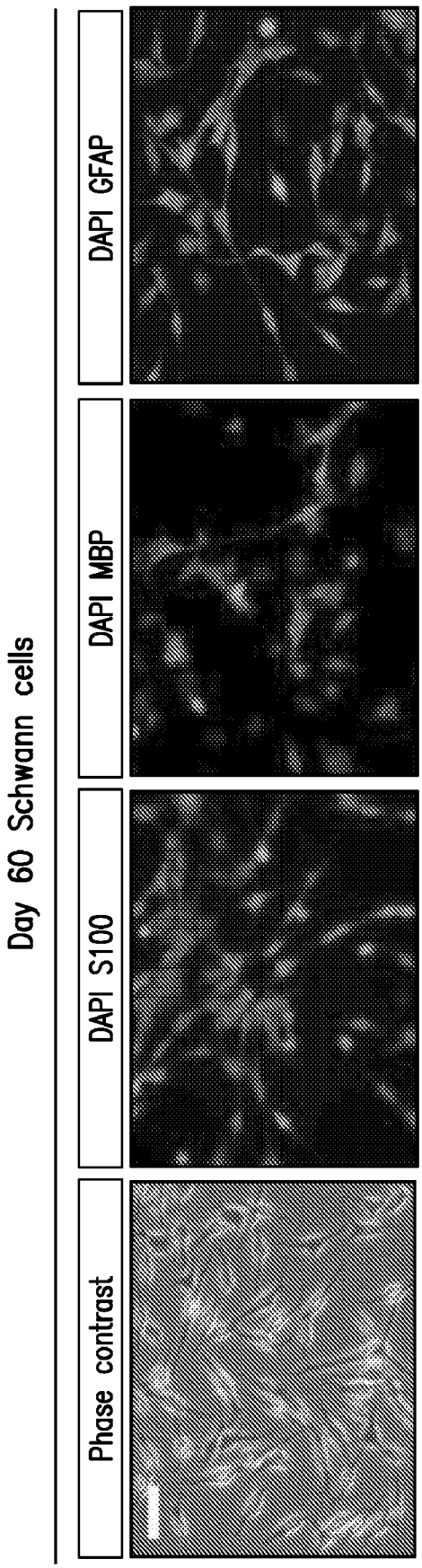
Day 60 Schwann cells
Phase contrast
DAPI S100
DAPI MBP
DAPI GFAP

FIG. 1E

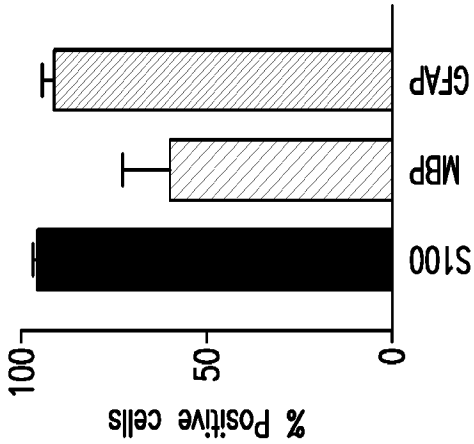
% Positive cells
100
50
0
S100
MBP
GFAP

FIG. 1F

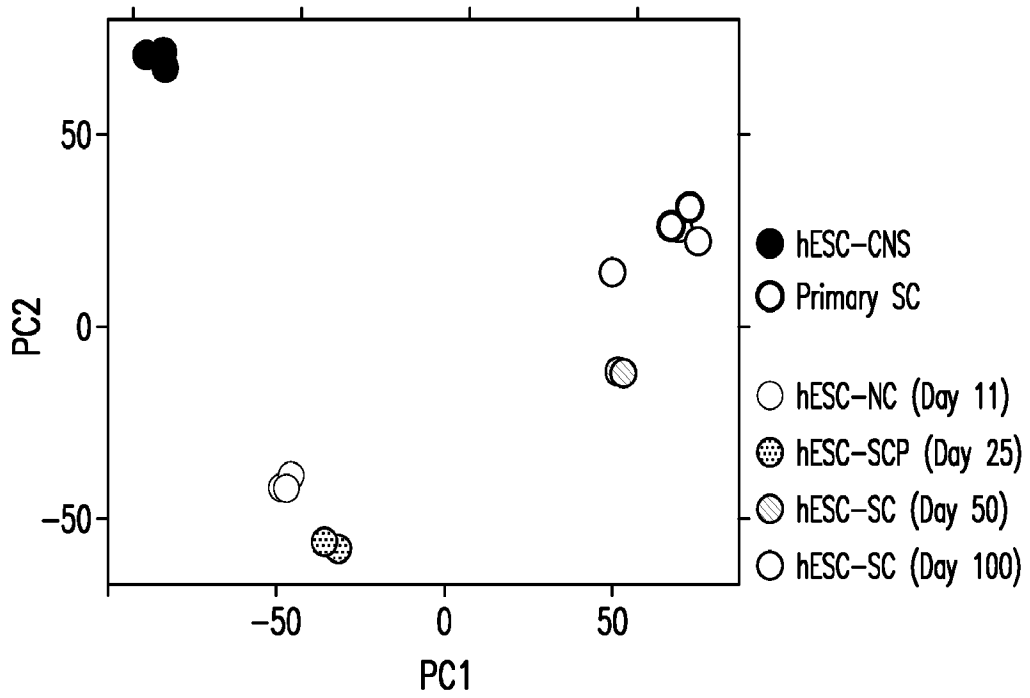
50
0
−50
PC2
−50
0
50
PC1
hESC−CNS
Primary SC
hESC−NC (Day 11)
hESC−SCP (Day 25)
hESC−SC (Day 50)
hESC−SC (Day 100)

FIG. 1G

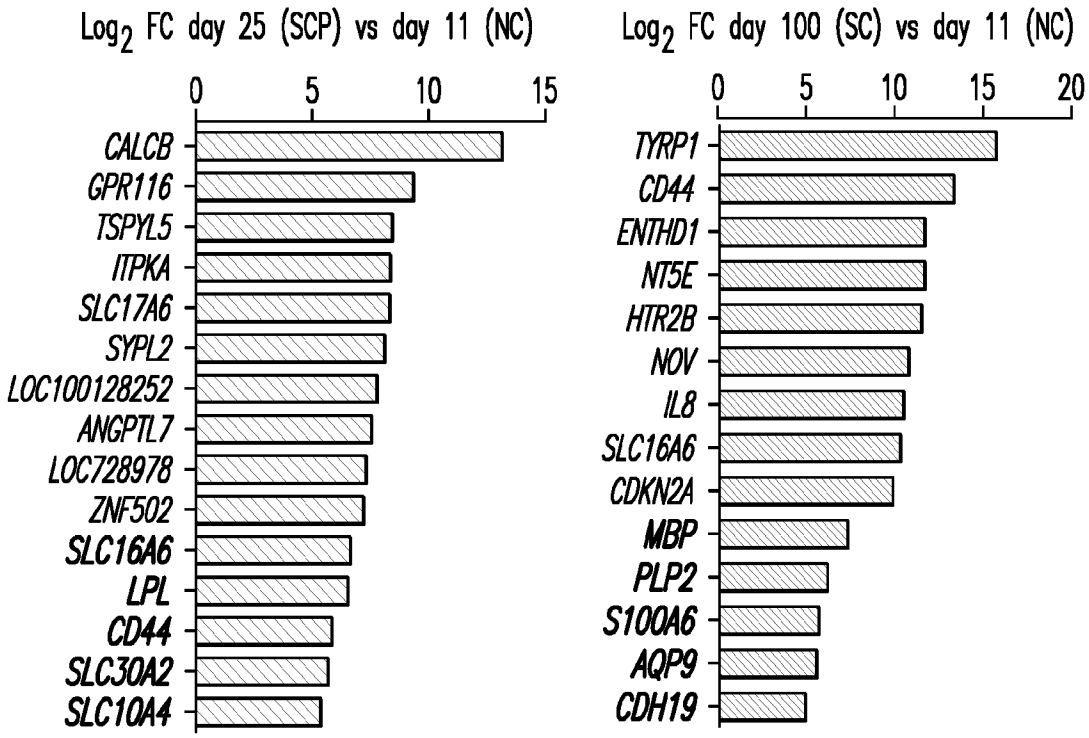
Log2 FC day 25 (SCP) vs day 11 (NC)
0
5
10
15
CALCB
GPR116
TSPYL5
ITPKA
SLC17A6
SYPL2
LOC100128252
ANGPTL7
LOC728978
ZNF502
SLC16A6
LPL
CD44
SLC30A2
SLC10A4
Log2 FC day 100 (SC) vs day 11 (NC)
0
5
10
15
20
TYRP1
CD44
ENTHD1
NT5E
HTR2B
NOV
IL8
SLC16A6
CDKN2A
MBP
PLP2
S100A6
AQP9
CDH19

FIG. 1H

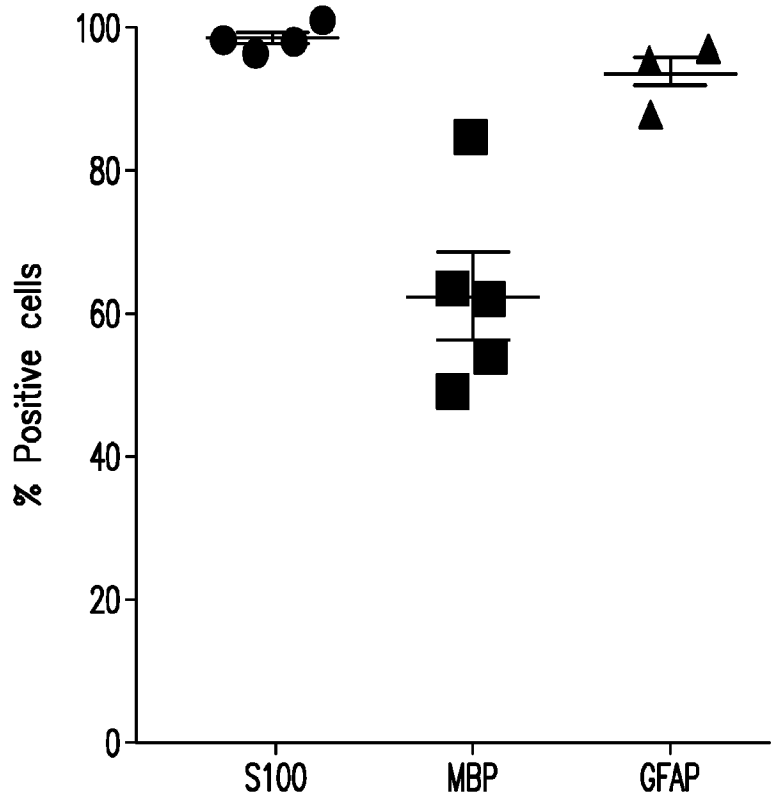

FIG. 1I

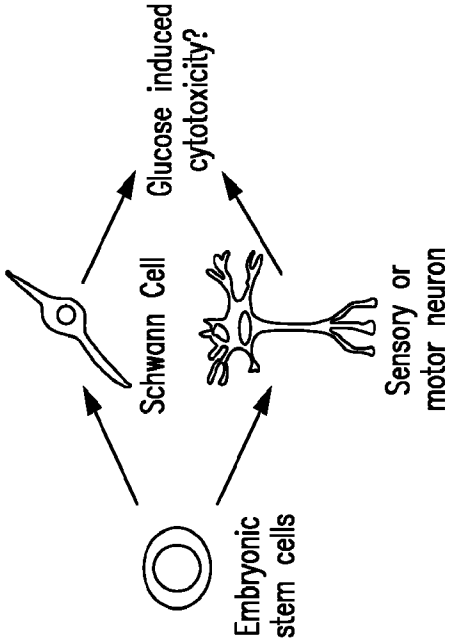

FIG. 2A

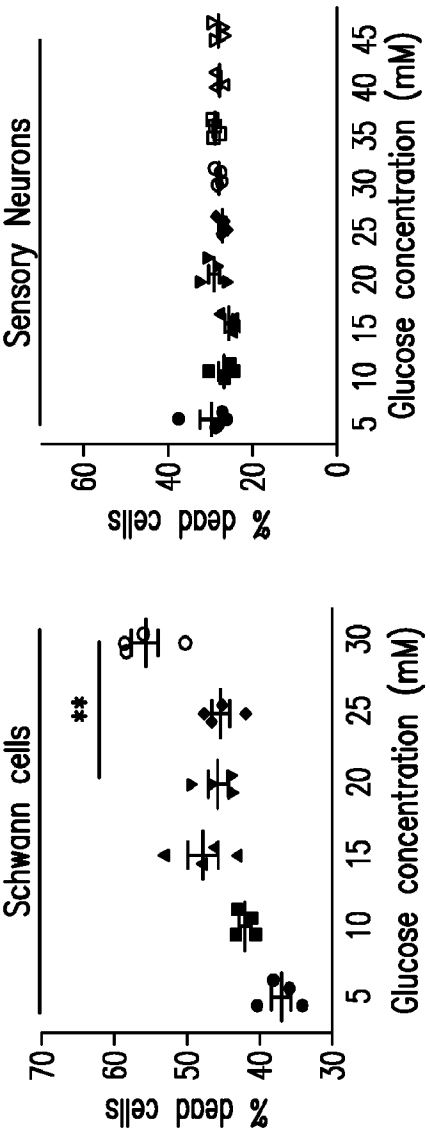

FIG. 2B

FIG. 2C

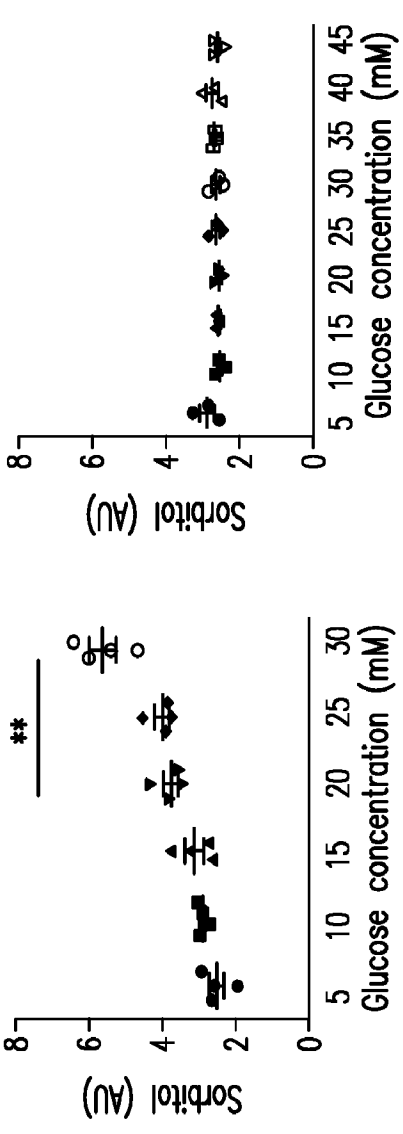

FIG. 2D

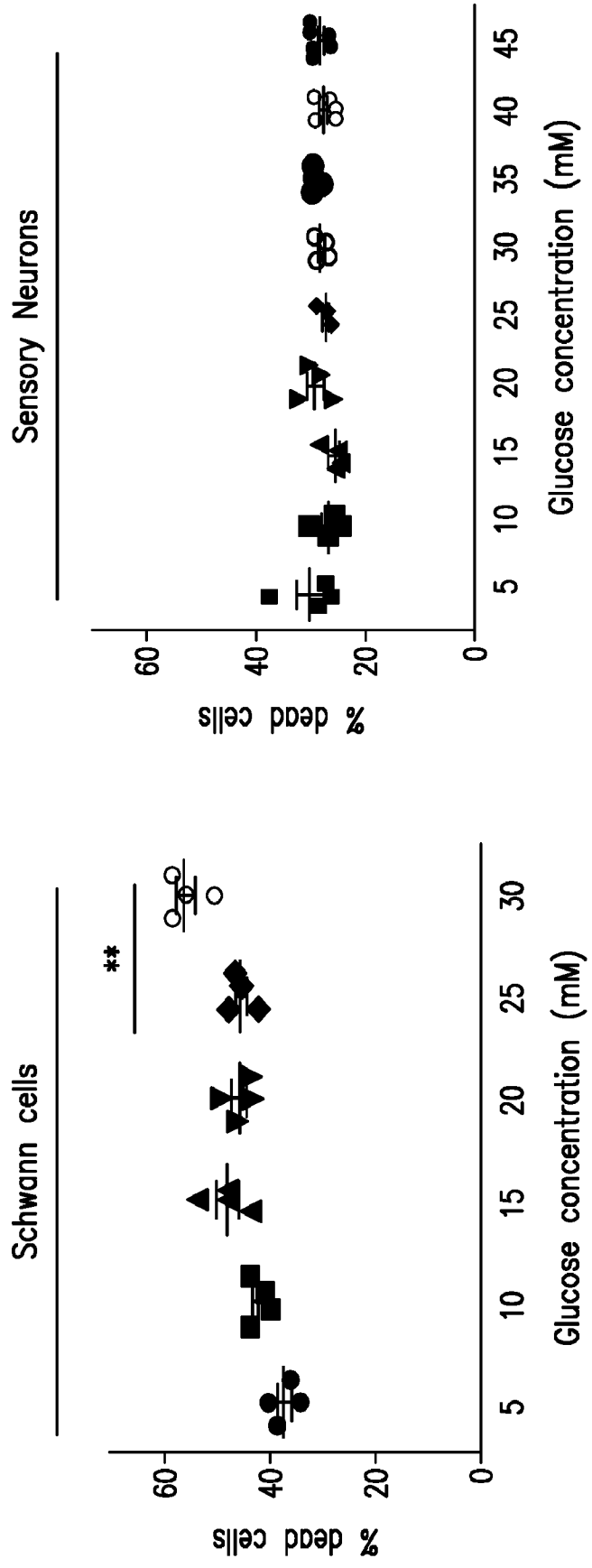
Sensory Neurons
% dead cells
0
20
40
60
5
10
15
20
25
30
35
40
45
Glucose concentration (mM)
Schwann cells
**
% dead cells
0
20
40
60
5
10
15
20
25
30
Glucose concentration (mM)

FIG. 2E

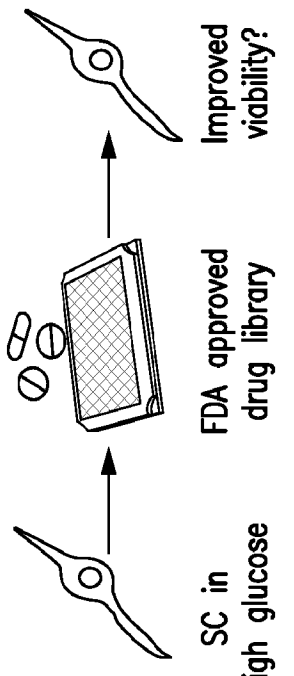

FIG. 3A

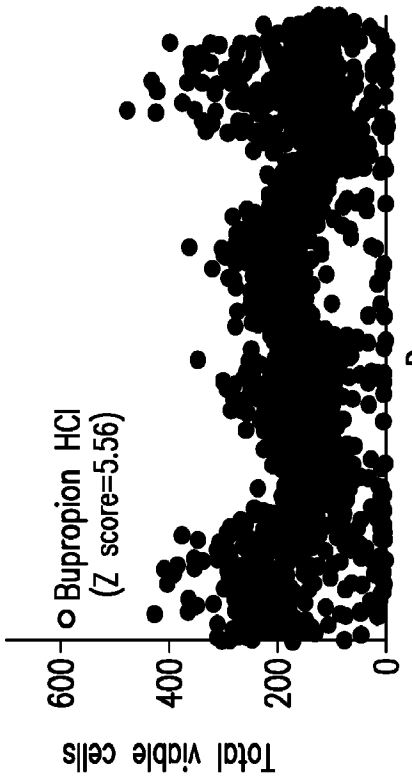

FIG. 3B

FIG. 3C

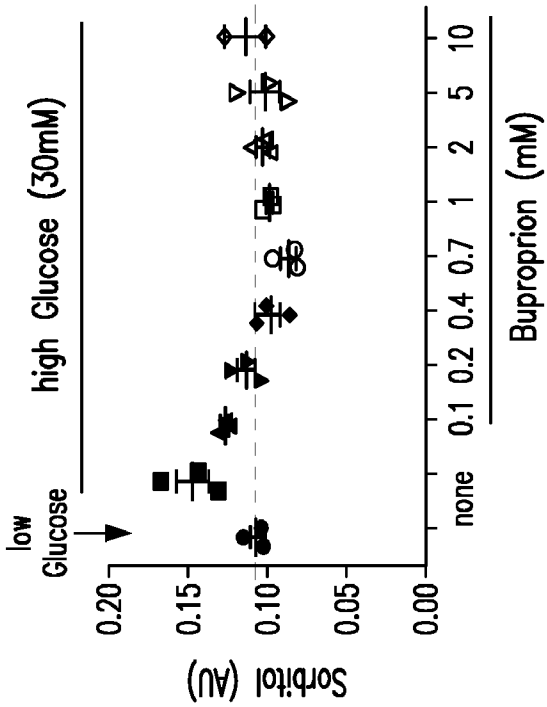

FIG. 3D

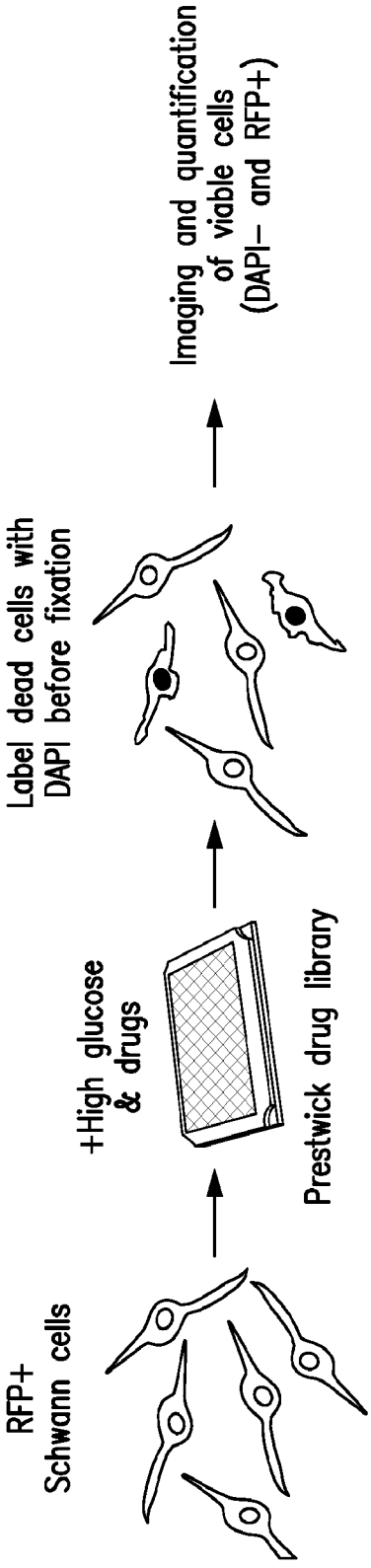
RFP+
Schwann cells
+High glucose
& drugs
Prestwick drug library
Label dead cells with
DAPI before fixation
Imaging and quantification
of viable cells
(DAPI– and RFP+)

FIG. 3E

Compound	Total viable cells	Z-score
Bupropion Hydrochloride	583	5.561673904
Cyclopenthiazide	478	4.165937472
Captopril	428	3.501301075
Isradipine	425	3.461422891
Condelphine	423	3.434837435
Nimesulide	409	3.248739244
Tolbutamide	405	3.195568333
Triamcinolone	393	3.036055597

FIG. 3F

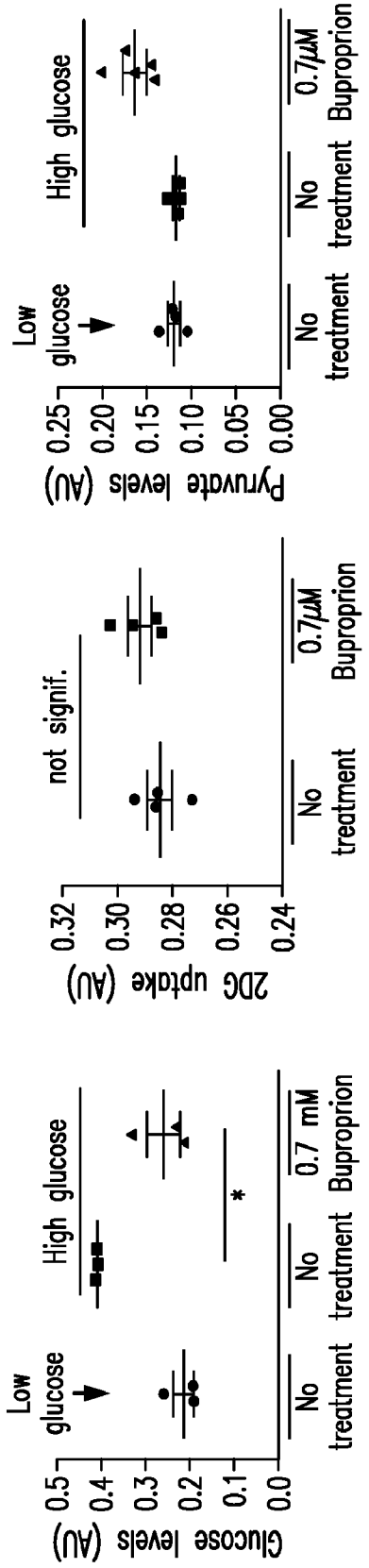

FIG. 4A

FIG. 4B

FIG. 4C

FIG. 4D

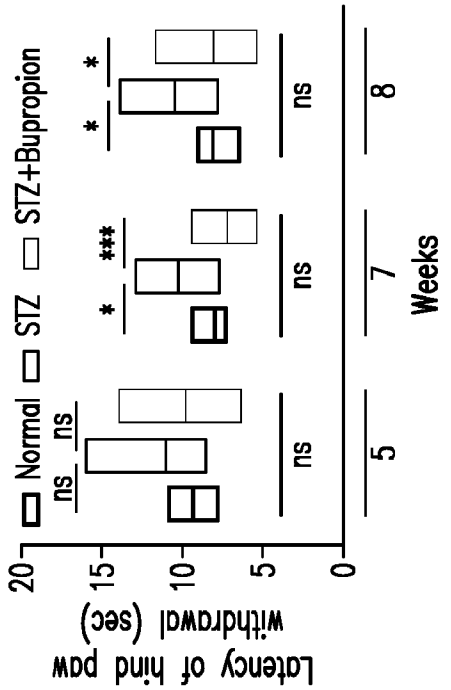

FIG. 5C

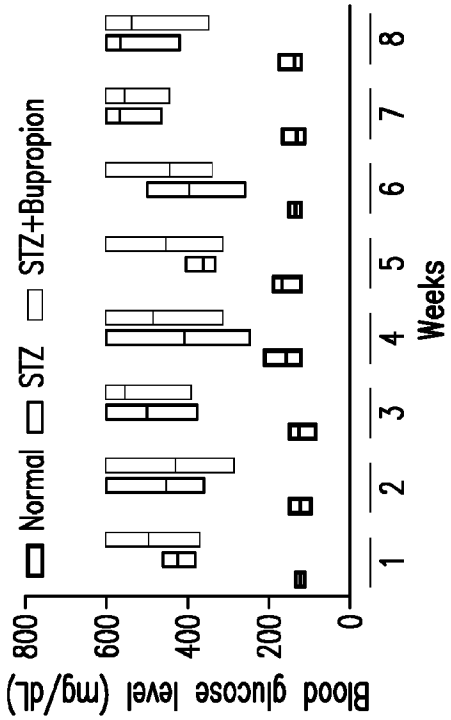

FIG. 5B

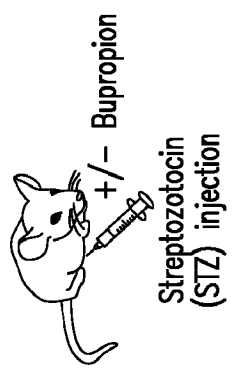

FIG. 5A

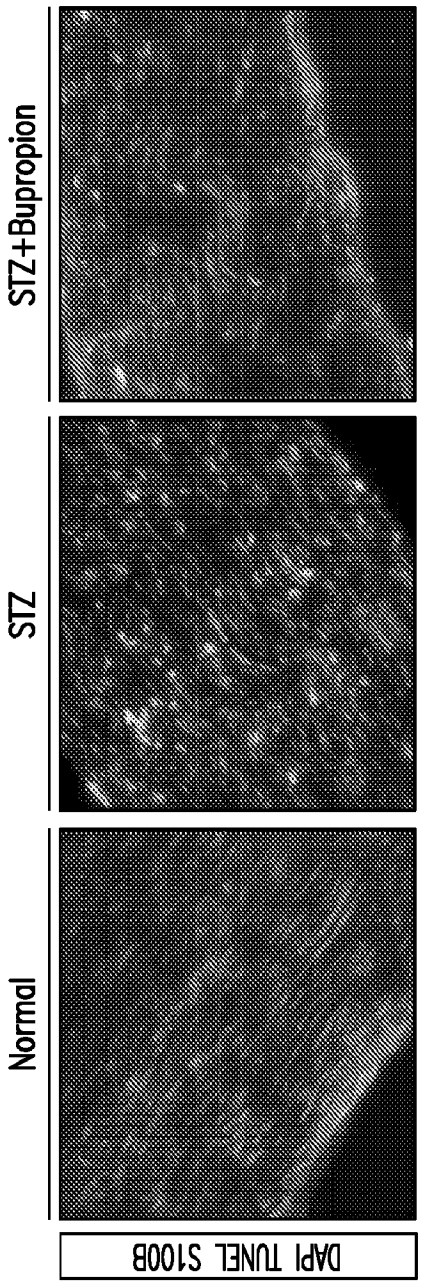

FIG. 5D

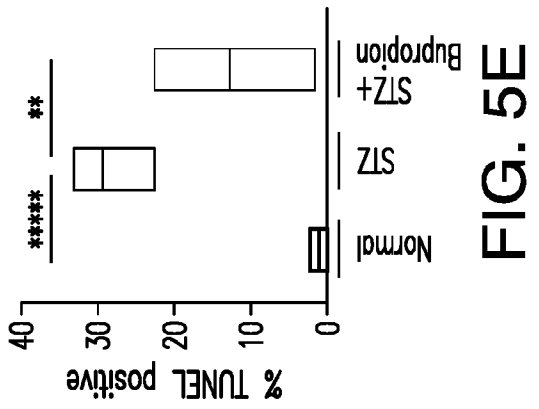

FIG. 5E

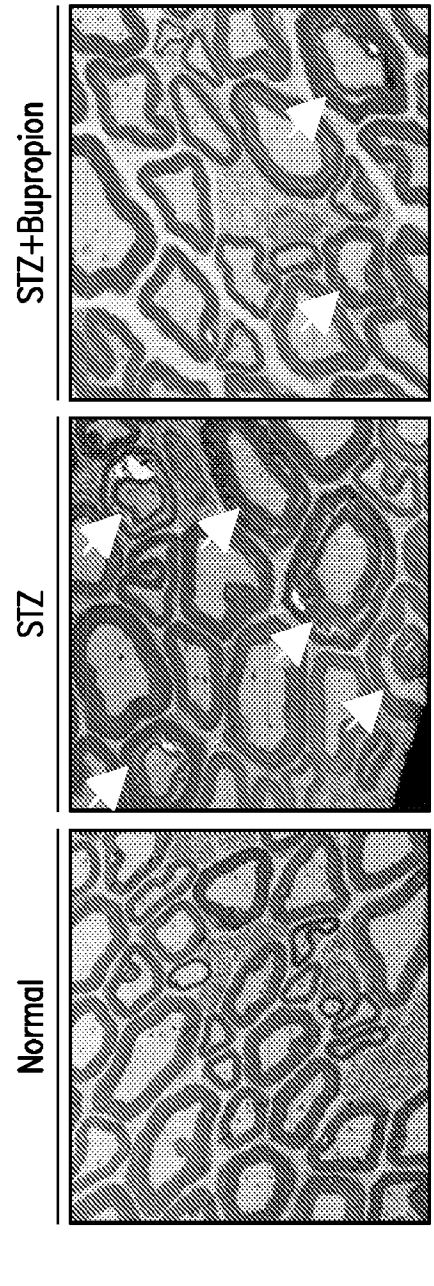

FIG. 5F

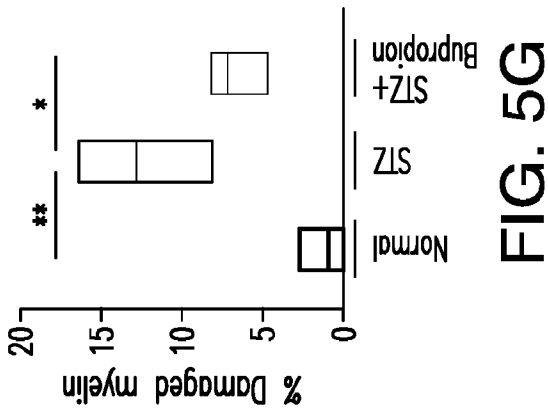

FIG. 5G

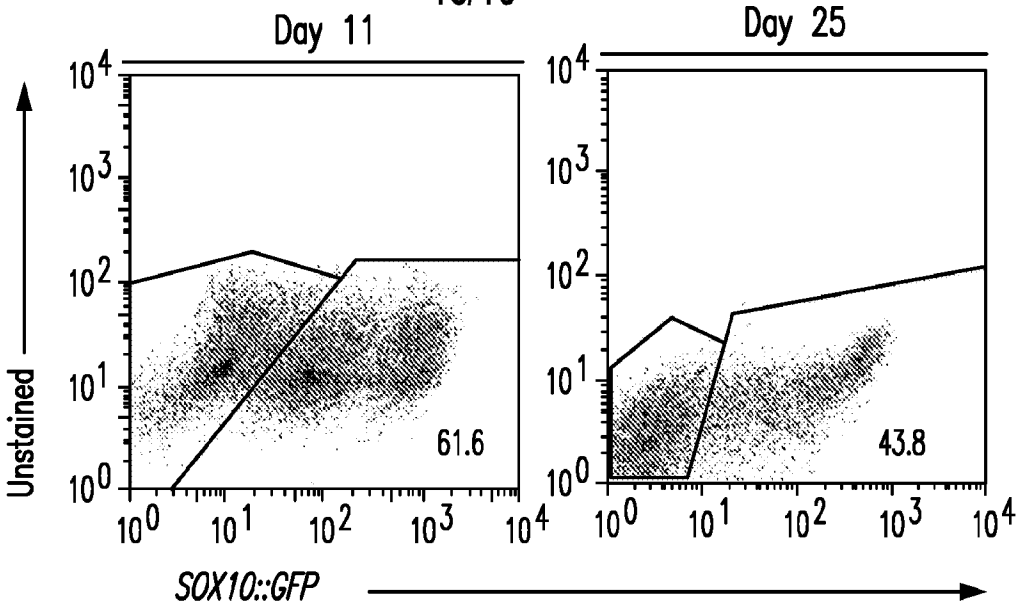
Day 11
Day 25
Unstained
SOX10::GFP
61.6
43.8

FIG. 6A

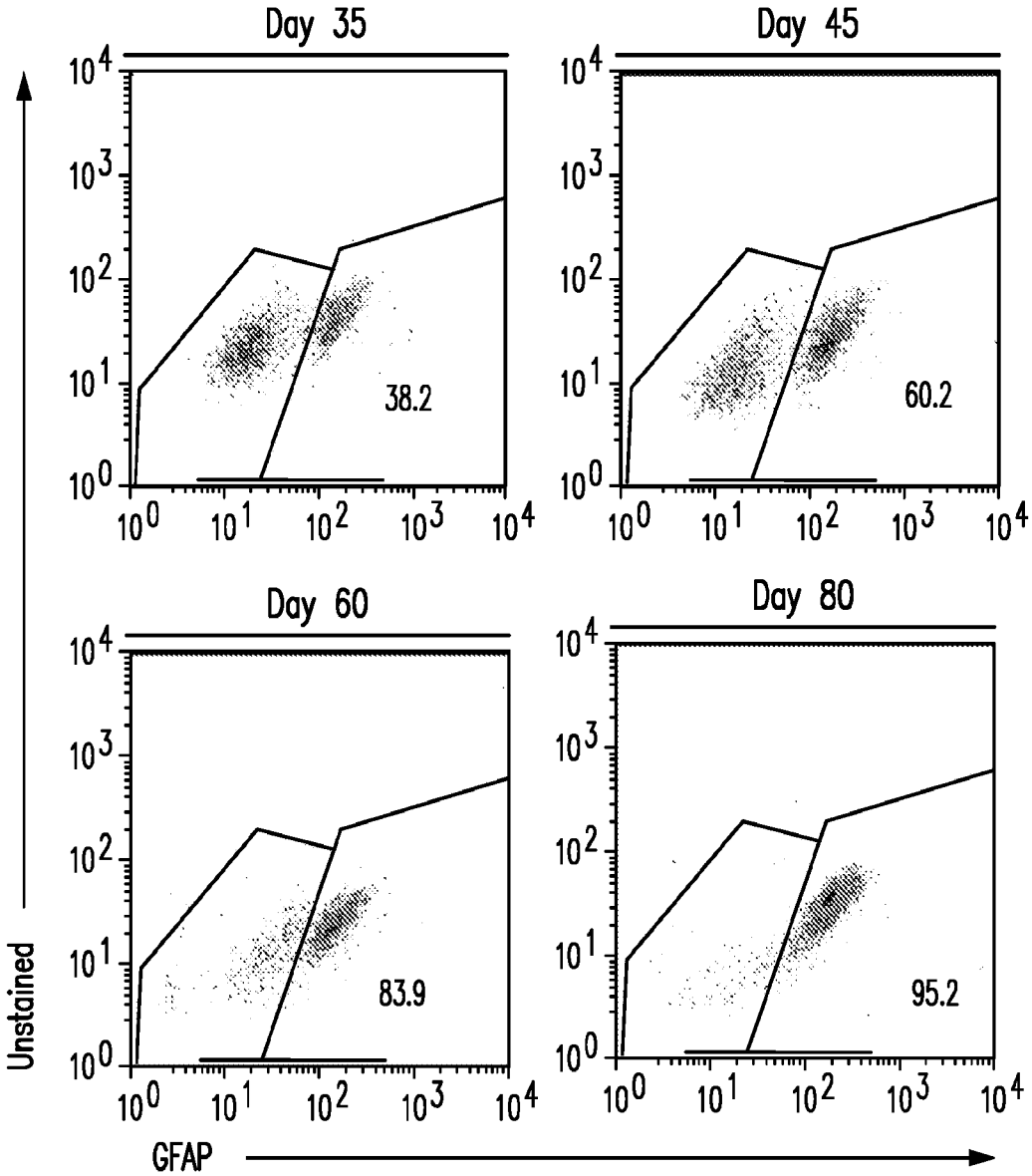
Day 35
Day 45
Day 60
Day 80
Unstained
GFAP
38.2
60.2
83.9
95.2

FIG. 6B

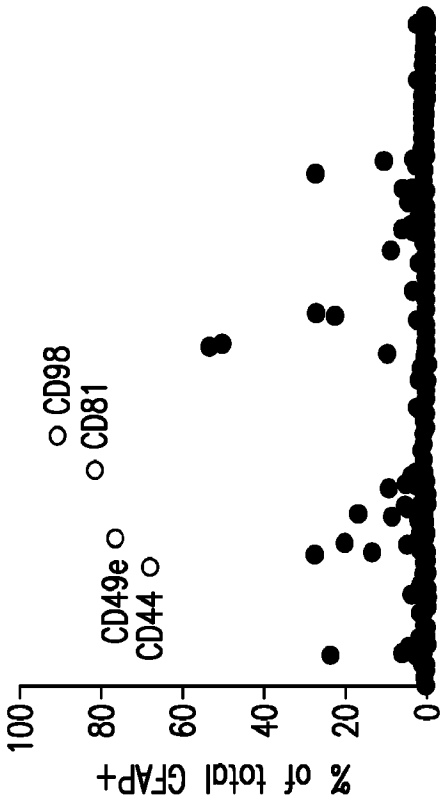
% of total GFAP+
100
80
60
40
20
0
CD49e
CD44
CD98
CD81
Antibodies

FIG. 7B

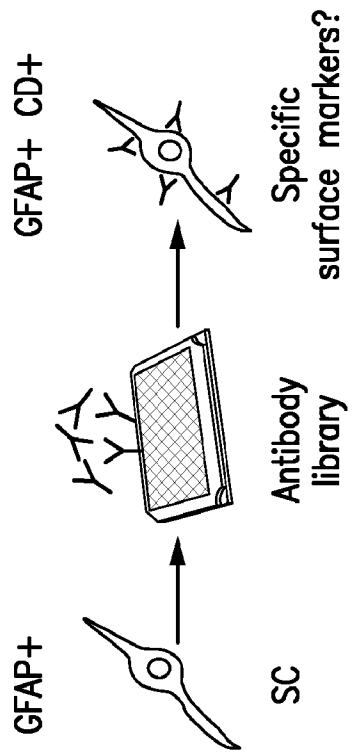
GFAP+
SC
Antibody library
GFAP+ CD+
Specific surface markers?

FIG. 7A

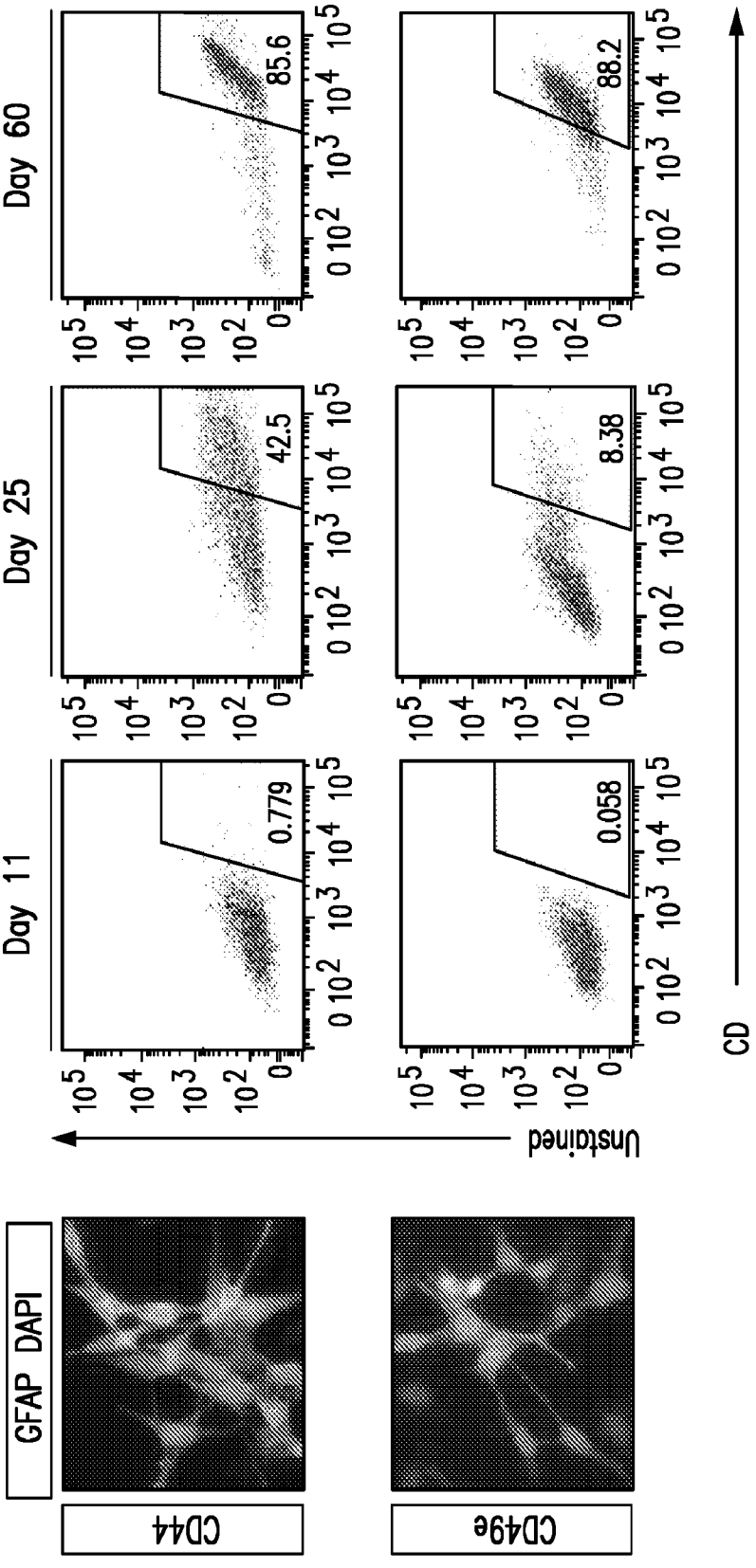
Day 11
Day 25
Day 60
Unstained
CD
0.779
42.5
85.6
0.058
8.38
88.2
GFAP DAPI
CD44
CD49e

FIG. 7C

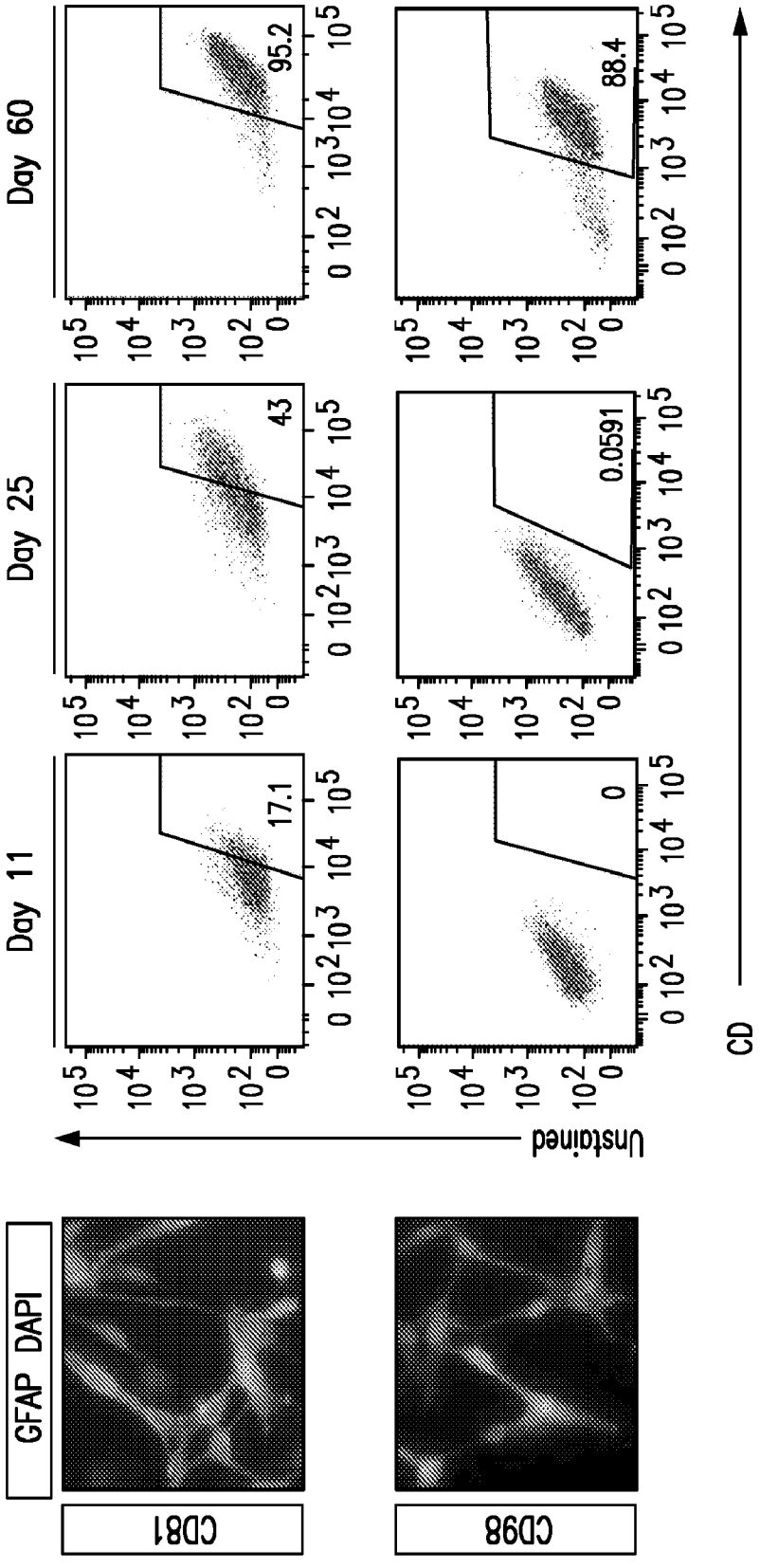

FIG. 7C continued

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/061549

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 35/12; A61K 35/30; A61K 35/32; A61K 35/34; A61K 35/35; A61P 19/00 (2018.01)

CPC - A61K 35/12; A61K 35/30; C12N 5/0622; C12N 5/0623; C12N 5/0653; C12N 5/0655; C12N 5/0661; C12N 2500/90; C12N 2501/15; C12N 2501/155; C12N 2501/727; C12N 2501/999; C12N 2506/1307; G01N 33/5008; G01N 33/5058 (2018.01)

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)

See Search History document

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

USPC - 424/85.6; 424/133.1; 435/6.12; 435/7.1; 435/29; 435/366; 514/225.8; 514/304; 514/428; 514/653 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2000/19993 A2 (BROWNLEE) 13 April 2000 (13.04.2000) entire document	1-5, 34-37, 56-59
Y	LEE et al. "Isolation and Directed Differentiation of Neural Crest Stem Cells Derived from Human Embryonic Stem Cells," Nature Biotechnology, 25 November 2007 (25.11.2007), Vol. 25, No. 12, Pgs. 1468-1475. entire document	1-5, 34-37
Y	US 2013/0183674 A1 (STUDER et al) 18 July 2013 (18.07.2013) entire document	3-5, 34-37
Y	US 2014/0105861 A1 (MARCH et al) 17 April 2014 (17.04.2014) entire document	56-59
Y	US 2014/0038949 A1 (SCHULTZ et al) 06 February 2014 (06.02.2014) entire document	59
A	DELANEY et al. "Insulin-Like Growth Factor-I and Over-Expression of Bcl-xL Prevent Glucose Mediated Apoptosis in Schwann Cells," Journal of Neuropathology & Experimental Neurology, 01 February 2001(01.02.2001), Vol. 60, Iss. 2, Pgs. 147-160. entire document	1-5, 34-37, 56-59
A	DENHAM et al. "Multipotent Caudal Neural Progenitors Derivedfrom Human Pluripotent Stem Cells That GiveRise to Lineages of the Central and Peripheral Nervous System," Stem Cells, 01 June 2015 (01.06.2015), Vol. 33, No. 6, Pgs. 1759-1770. entire document	1-5, 34-37, 56-59
A	WO 2015/011031 A1 (F. HOFFMANN-LA ROCHE AG et al) 29 January 2015 (29.01.2015) entire document	1-5, 34-37, 56-59

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 January 2018	07 FEB 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/061549

C (Continuation). *DOCUMENTS CONSIDERED TO BE RELEVANT*

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NAT, R. "Chapter 15: From Human Pluripotent Stem Cells to Peripheral Neurons," In Pluripotent Stem Cells-From the Bench to the Clinic, Ed. Minoru Tomizawa, 20 July 2016 (20.07.2016), Pgs. 307-329. entire document	1-5, 34-37, 56-59

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/061549

Box No. II ***Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)***

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-33, 38-55, 60-68
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.