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Supplementary Materials for

Schwann cells in the subcutaneous adipose tissue have neurogenic potential and can be used for regenerative therapies

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Other Supplementary Material for this manuscript includes the following:

Movies S1 and S2
Data files S1 and S2

SUPPLEMENTARY MATERIALS AND METHODS

Histological characterisation of the subcutaneous adipose tissue. Neural crest-derived Schwann cells in the subcutaneous adipose tissue (SAT) were visualized in double transgenic mice by crossing neural crest-specific Wnt1::Cre mice with glia-specific $Plp1^{GFP}$ mice to generate $Plp1^{GFP}$;Wnt1::Cre mice. These mice were then crossed with $ROSA26^{tdTomato}$ reporter mice to generate $Plp1^{GFP}$;Wnt1::Cre;$ROSA26^{tdTomato}$ ($Plp1^{GFP}$;Wnt1-tdT) mice. Neural crest-derived neural fibers in the SAT were visualized using the same strategy with Tau^{GFP} mice substituted for $Plp1^{GFP}$ mice to generate Tau^{GFP};Wnt1::Cre;$ROSA26^{tdTomato}$ (Tau^{GFP};Wnt1-tdT) mice. Distinctions between Schwann cells and neuron-derived structures in the SAT and nerve fibers were visualized by crossing the neuron-specific BAF53b::Cre mouse with $Plp1^{GFP}$ mice to generate $Plp1^{GFP}$;BAF53b::Cre mice which were then crossed with $ROSA26^{tdTomato}$ reporter mice to generate $Plp1^{GFP}$;BAF53b::Cre;$ROSA26^{tdTomato}$ ($Plp1^{GFP}$;BAF53b-tdT). Mice were euthanized by CO_2 affixation 6-8 weeks after birth. The SAT was collected from the entire posterior subcutaneous fat pads from the dorsal lumber to the inguinal region. For histological experiments fat pads were fixed overnight in 4% PFA overnight and washed in PBS for processing of wholemount preparations and embedding in Optimal Cutting Temperature (OCT) matrix compound (Sakura Finetek) for producing cross sections. To visualize lipids in the SAT, 20µm longitudinal sections were stained with Oil red O (Sigma-Aldrich) for 30 min, washed with distilled water and counterstained with DAPI for 2 min before being mounted and cover slipped.

Differentiation assays in SAT-NSCs. Adipogenesis assays were performed using the StemPro Adipogenesis Differentiation Kit (Life Technologies) as of manufacturers instructions. SAT-NSCs were isolated by flow cytometry from heterogenous neurospheres derived from Wnt1-tdT as described above. Wnt1-tdT^+ and Wnt1-tdT^- cells were plated at a density of 1×10^4 in tissue

culture media (16.5% FBS, 1% GlutaMAX and 1% penicillin-streptomycin in α-MEM, Life Technologies). After 4 days, the media was replaced with adipogenesis induction media or control tissue culture media which was changed every 3 days. After 12 days in adipogenesis induction media, cells were fixed in 4% PFA and stained with Oil red O to visualize adipocytes. Neural differentiation was performed using the mouse neural differentiation kit (StemCell Technologies). Spontaneous spheroids generated from the SAT of Wnt1-tdT mice or purified Wnt1-tdT neurospheres were plated on fibronectin-coated cell culture plates and cultured for 3 weeks with media changes every 3-4 days before fixation for immunohistochemical studies.

Temporal quantification of Plp1 ^{GFP} and* BAF53b-tdT *expression in nerve fiber bundle-derived spheroids.

To determine changes in the expression of Plp1^{GFP} and BAF53b-tdT in nerve fiber bundles during cell culture, the Plp1^{GFP};BAF53b-tdT mouse neuro-glial reporter system was utilized. Nerve fiber bundles from the SAT were isolated by enzymatic digestion and counter filtration as described above. The nerve fiber bundles (NFBs) were cultured in neuroproliferation medium in ultra-low attachment plates for 7 days. The mean fluorescent intensity (MFI) of Plp1^{GFP} and BAF53b-tdT expression in the NFBs was calculated by measuring the mean grey values of GFP or tdT within the region of interest (ROI) of the NFB or spheroid and subtracting the background using ImageJ software (National Institute of Health, Bethesda, MD, USA).

Schwann cell transdifferentiation experiments. To determine the ability of Schwann cells to differentiate into neurons, the Plp1^{GFP};BAF53b-tdT mouse neuro-glial reporter system was utilized. Plp1$^{GFP\ +}$, BAF53b-tdT^{+}, double positive and double negative cells were collected by flow cytometry from heterogenous SAT-derived neurospheres grown as described above. The ability of Plp1$^{GFP\ +}$ cells to generate BAF53b-tdT^{+} neurons was evaluated by seeding the purified

population of Plp1^{GFP+} cells on fibronectin-coated surfaces at a density of 1×10^3 cells for 2 weeks in neuroproliferation medium. The ability of Plp1^{GFP+} cells and double negative cells to produce BAF53b-tdT$^+$ neurons was performed by seeding equal densities of cells on Matrigel basement membrane (Corning Inc, lot #9001221) in a 1:1 dilution of the same media for 6 weeks.

Immunohistochemistry and transgenic reporter visualization. Immunohistochemistry was performed on cells in vitro, cross sections and wholemount preparations as previously described (*44, 45*). Cells or tissues were fixed in 4% PFA, wholemount tissues were washed with PBS and cross sections of the SAT, colon, NFBs and neurospheres were prepared by sectioning samples embedded in OCT medium on a cryostat (Leica CM3050 S Cryostat, Leica). All samples were cut at 20µm except for human SAT cut at 50µm. Before labelling, all samples were permeabilized and blocked with 0.1% Triton X-100 for 1h containing 10% normal donkey serum. Primary antibodies included goat anti-GFAP (1:200 Abcam, ab53554), mouse anti-Tubulin β3 (TUBB3, TUJ1 clone conjugated to Alexa Fluor 488; 1:400, Biolegend), rabbit anti-p75 neurotrophin receptor (p75; 1:200; Promega, Madison, WI, #G3231), rabbit anti-GDNF (1:100, Abcam), goat anti-Notch1 (1:100, R&D systems, #967239), rabbit anti-KI67 (1:100, Abcam, ab15580), mouse anti-NSE (1:100, NSE-P1 clone, conjugated to Alexa Fluor 647, Biolegend), goat anti-VAChT (1:1000, MilliporeSigma, #ABN100), rabbit anti-Calretinin (1:200, Invitrogen, Thermo Fisher Scientific, 18-0211), rabbit anti-nNOS (1:100, Thermo Fisher Scientific, 61-7000), mouse anti-CDH19 (1:100, clone D-1 conjugated to Alexa Fluor 488, Santa Cruz Biotechnology), rabbit anti-SOX10 (1:100, clone EPR4007, Abcam), rabbit anti-NPY (1:200, Abcam #ab30914,), rabbit anti-Calcitonin Gene-Related Peptide (CGRP), (1:500, MilliporeSigma, #AB15360), rabbit anti-Enkephalin (ENK) (1:500, MilliporeSigma, #AB1975),

guinea-pig anti-Substance P (SP) (1:500, Abcam, #ab10353), sheep anti-Tyrosine Hydroxylase (TH) (1:500, MilliporeSigma, #AB1542) and mouse anti-human nuclear (1:50, clone 235-1 conjugated to Alexa Fluor 488, MilliporeSigma). Secondary antibodies included donkey anti-rabbit IgG (1:500; Alexa Fluor 546, Alexa Fluor 647, Alexa Fluor 488) and donkey anti-goat IgG (1:500, Alexa Fluor 647 and Alexa Fluor 488) (all from Thermo Fisher Scientific). Cell nuclei were stained with DAPI (Vector Labs) and images were taken using a Keyence BZX-700 All-In-One Microscopy system (Keyence America Itasca) or a Nikon A1R laser scanning confocal microscope (Nikon Instruments).

Nestin^{GFP} ***expression in neural crest derived cells.*** To determine changes in the expression of *Nestin* in Schwann cells, the Nestin^{GFP};Wnt1::Cre;ROSA26^{tdTomato} (Nestin^{GFP};Wnt1-tdT) reporter mouse was utilized. Nerve fiber bundles from the SAT containing Wnt1-tdT^{+} Schwann cells were isolated by enzymatic digestion and counter filtration as described above. The NFBs were cultured in neuroproliferation medium in ultra-low attachment plates for 10 days. The mean fluorescent intensity (MFI) of Nestin^{GFP} expression in the NFBs was calculated by measuring the mean grey values of GFP within the Wnt1-tdT^{+} region of interest (ROI) and subtracting the background using ImageJ software (National Institute of Health).

Transplantation ex vivo. Transplantation experiments performed *ex vivo* were conducted as previously described (*40*). Colons were acquired from wildtype mice, cut along the mesenteric border and dissected to obtain the muscularis externa. Tissues were cut into 1cm^2 pieces and pinned on silicone elastomer-coated tissue culture plates in tissue culture medium containing 10% FBS and 1% penicillin/streptomycin in Dulbecco's Modified Eagle Medium. Small incisions were made into the longitudinal muscle layer to lift the longitudinal muscle strips and

mouse or human neurospheres were implanted and fastened between the circular and longitudinal muscle.

***Gut-SAT-NSC co-culture experiments* in vitro.** To investigate the ability of SAT-NSCs to integrate and make physical interactions with cells of the ENS, in vitro co-culture experiments were conducted using cells from separate reporter mice. SAT-NSCs in heterogenous spheroids were cultured from the adipose of Wnt1-tdT mice as described above. Neurospheres from gut-derived cells were isolated and cultured from the muscularis propria of the intestine of $Plp1^{GFP}$ mice using the methods previously described (*46*). Cells from the gut (4×10^5 cells/well) and SAT (1×10^6 cells/well) were cultured separately in neuroproliferation medium (2000ul/well) in ultra-low attachment 6-well plates for 5 days to generate spheroids which were then transferred (1:1, 200µl/culture) to glass bottomed 8-well culture chambers (Thermo Fisher Scientific) coated in fibronectin to facilitate cell adhesion with media changes every 3-4 days.

Calcium imaging. For calcium imaging studies, SAT-NSCs were generated from $Wnt1::Cre;Polr2a^{GCaMP5g\text{-}tdTomato}$ (Wnt1-tdT-GCaMP5) mice with constitutive expression of the *tdTomato* reporter and the genetically encoded calcium indicator, *GCaMP5*, in all neural crest-derived cells, as previously reported (*44*). Intracellular calcium ($[Ca^{2+}]_i$) transients in cells transplanted to *ex vivo* preparation were recorded using the Keyence BZX-700 All-In-One Microscopy system. Tissues were pinned in a 60 mm glass bottom dish coated with Sylgard elastomer (Dow Corning) with the mucosa layer facing upward and superfused with Krebs. SAT-NSCs derived from the SAT-NFBs of Wnt1-tdT-GCaMP5 mice were transplanted to explants of the distal colon and cultured for 8 days to allow engraftment. Ednrb KO recipient mice were utilized to assess calcium responses of transplanted cells in the aganglionic environment. Electric field stimulation (EFS) was delivered by two *parallel* silver/platinum electrodes placed on either

side of colonic preparations. EFS was applied by a CS4 constant voltage stimulator and MyoPulse software (Danish Myo Technology) at a single pulse of 50-60 V and 1ms pulse duration. Intracellular calcium concentration was measured using ImageJ by calculating the change of fluorescence intensity expressed as relative fluorescence ($\Delta F/F_0$) for selected cells [22]. Muscle contractile activity over time was measured by tracking the displacement of landmarks using Image J.

In vivo transplantation studies and disease models. To examine the engraftment, spread and differentiation of allogeneic SAT-NSCs, neurospheres were implanted to 2 month old wildtype mice to the colorectum via a perianal surgical approach or the mid-colon via laparotomy as previously described (*8, 40*). Neurospheres were generated by culturing SAT-NSC purified by flowcytometry seeded at a density of 1000 cells/cm^2 in low-attachment plates to generate spheroids between 150-200μm in diameter after 10 days for implantation. The effects of SAT-NSCs were examined in the Ednrb KO model of HSCR. Neurospheres were implanted to the aganglionic colorectum via perianal approach at postnatal day 6. To evaluate the effects of SAT-NSCs on gastroparesis the nNOS KO model was utilized. The stomach was accessed via laparotomy and neurospheres were administered to the gastric body immediately before the pyloric sphincter. In all studies, neuropheres were generated from Wnt1-tdT expressing cells purified by FACS as described above. For all cell administration procedures, three neurospheres were surgically implanted between the circular and longitudinal muscle as described (*40*).

Measurement of Contractile Force. Experiments were performed using standard organ bath techniques (*47*). Freshly excized distal colon was cut into 5-mm rings, cleaned of connective tissue and fat. The colonic rings were then mounted between two small metal hooks attached to force displacement transducers in a muscle strip myograph bath (Model 820 MS; Danish Myo

Technology) containing physiological saline (oxygenated with 95% O2 and 5% CO2) maintained at 37°C and pH 7.4. The rings were stretched to give a basal tension of 0.5-g and were equilibrated for 60min with physiological saline changed every 20 min. Force contraction was recorded and analyzed by a computer using LabChart Pro Software v8.1.16 (Power Lab). Tissues viability and integrity was checked by eliciting contraction response to 60mM KCl. Colon segments were stimulated with pulse trains of 50 - 60 V for 30 sec, with pulse duration of 0.3 ms, at a frequency of 5 Hz or with a single pulse, by using a CS4+ constant voltage stimulator with MyoPulse software (Danish Myo Technology). To confirm EFS-evoked muscle contractions were neurally-mediated, tissue preparations were incubated with 1µM tetrodotoxin (TTX) for 5 minutes to block voltage-gated sodium channels. The amplitude of contractile responses to EFS (50 V for 30 sec, with pulse duration of 0.3 ms, at a frequency of 5 Hz or with a single pulse) were assessed 5 minutes before and after TTX application to quantify the percentage of TTX-sensitive responses.

Gastric emptying assay. Solid and liquid gastric emptying assays were performed as previously described (*48*). Briefly, mice were intragastrically gavaged with 0.3 mL of barium sulfate (E-Z Paque, E-Z-EM) and 10 steel beads (diameter = 0.81-0.9 mm; Bal-tec). Radiographs were taken with a portable X-ray unit (50 kV, 1.2 mAs, ScanX14 Portable Digital Imaging Systems, ALLPRO Imaging) 90 minutes after gavage. Resulting images were analyzed using Image J. Solid gastric emptying was calculated as the percentage of total beads outside the stomach. Liquid gastric emptying was measured by calculating the integrated density (sum of pixel intensity) of liquid barium outside the stomach over the integrated density of the barium signal from the entire GI tract.

Comparisons of migration, proliferation and neural differentiation. To compare the properties of SAT-NSCs in the differing microenvironments of the colon, stomach and aganglionic intestine, single neurospheres were transplanted to *ex vivo* preparations of the muscularis from the distal colon and gastric antrum of wildtype mice, or the distal colon of aganglionic Ednrb$^{-/-}$ mice. Preparations were cultured for 7 days as described above, prior to fixation in 4% PFA, tissue processing and analysis. Migration was assessed by measuring the coverage (mm^2) of Wnt1-tdT cells using ImageJ. To quantify proliferation, the Click-iT EdU (5-ethynyl-2'-deoxyuridine) Cell Proliferation Kit for Imaging (ThermoFisher, Invitrogen) was used as of manufacturers instructions. Briefly, 10 µM EdU was added to the culture media daily. The number of DAPI and EdU positive nuclei were quantified in a total 400,000μm^2 area per sample in single slice confocal images. The average percentage of EdU positive nuclei from total nuclei was calculated for Wnt1-tdT+ transplanted cells in each sample. To assess neural differentiation, tissues were immunolabeled with TUBB3 as described above. Using ImageJ, the area occupied by Wnt1-tdT transplanted cells was acquired using binary thresholding (Huang method) to create a region of interest. The area of TUBB3 immunoreactivity was assessed using the binary threshold method (Huang method) and was measured within the Wnt1-tdT region of interest to determine the area of TUBB3 colocalization in Wnt1-tdT cells. For all experiments, analysis was performed in a 400,000μm^2 area per sample in single slice confocal images to ensure colocalization of fluorescence occurred on the same focal plane. Data were presented as the percentage of the area of TUBB3 immunoreactivity in the total Wnt1-tdT occupied area.

Quantitative PCR. Total RNA was extracted from human neurospheres using an RNeasy Mini kit (Qiagen) and RNA integrity and concentration was assessed with a nanodrop (ND-1000 spectrophotometer) following extraction. The iTaq Universal SYBR Green One-Step Kit (Bio-

Rad) was then used to reverse transcribe the total RNA and perform RT-qPCR in a Bio-Rad CFX96 real-time thermal cycler. The reaction setup listed by the manufacturer of the kit was used, with a 10 uL total reaction volume and approximately 2 ng of RNA per reaction. The primers used to amplify GAPDH were (5′→3′) GGAGCGAGATCCCTCCAAAAT (forward) and GGCTGTTGTCATACTTCTCATGG (reverse) and the primers used to amplify PLP1 were (5′→3′) TGCTGATGCCAGAATGTATGG (forward) and GCAGATGGACAGAAGGTTGGA (reverse). The thermal cycling protocol provided by the manufacturer of the kit was used. The data was then processed using the Bio-Rad CFX Manager software (version 3.1), with a constant threshold to determine crossing point (Ct) values. The Ct values for *PLP1* were normalized using *GAPDH* as an internal control for each sample. Three biological replicates were included.

Low-input RNA-seq. Gene expression profiling was performed as previously described (*44*) on Wnt1-tdT^{+} cells isolated from the SAT of mice by FACS and purified Wnt1-tdT^{+} neurospheres generated as described above. Samples were pooled equally from three 1-month old Wnt1-tdT mice (2 female and 1 male/group) and total RNA was extracted from ~50,000 cells per group following the Trizol Reagent User Guide (Thermo Fisher Scientific). For human samples, RNA was extracted from spheroids using the same method for quantitative PCR described above. RNA was processed using the SMART-Seq v4 Ultra Low Input Kit (Clontech) and Nextera XT Library Prep Kit (Illumina) as of manufacturer's instructions. Samples were sequenced using a 2x150 Paired End configuration on an Illumina HiSeq instrument, reads were aligned to mouse (mm10) or human (hg38) reference genome using *HISAT* and quantified by *FeatureCounts* via the Galaxy server (*49*). Differentially expressed genes (DEGs) between groups in human neurospheres were identified using the R package EdgeR (*50*) using default parameters with

TMM normalization, filters for lowly expressed genes (<0.5 CPM in >3 samples) and FDR values calculated using the Benjamini and Hochberg correction. DEGs between groups in mouse neurospheres were identified using the NOISeq-sim feature of the R-based package *NOIseq* using default parameters with probability scores >0.85 considered differentially expressed for further analysis (*51*). Genes set enrichment analysis was performed using the web-based tool DAVID with a $p<0.05$ threshold using the Benjamini-Hochberg correction (*52-54*). Genes reported from existing single cell RNA-Seq analysis of the SAT (*20*) were used as Schwann cell markers. The association between DEGs upregulated in SAT-NSCs and Schwann cells with embryonic properties were assessed by Chi-Squared analysis of the 'Embryonic morphogenesis' (GO:0048598) gene lists. Schwann cell, neuronal and neural crest stem cell markers were manually curated and presented as heatmaps of the LogCPM. Comparisons between the DEGs of SAT-NSCs identified from this study and enteric neural stem cells (ENSCs) were performed on previously published sets (*44*).

SUPPLEMENTARY FIGURES

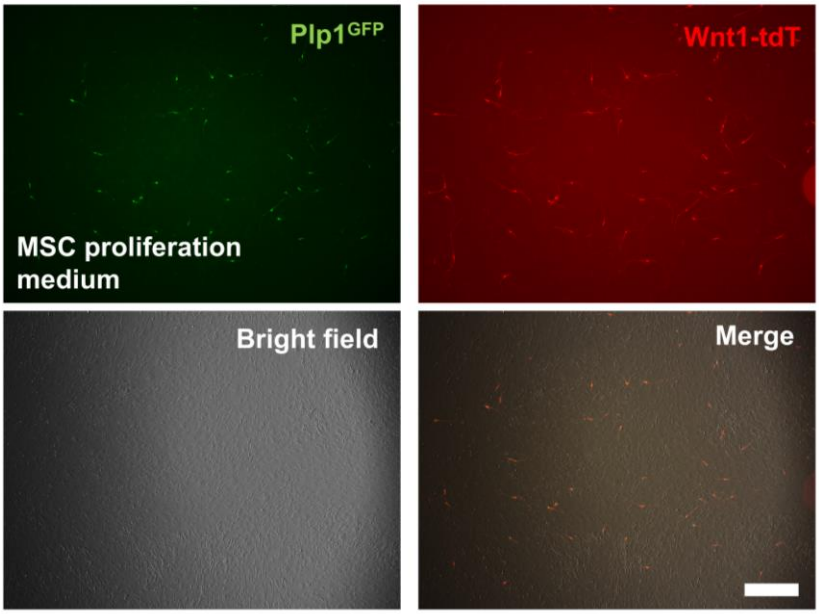


Fig. S1. Schwann cells contaminate cultures of mesenchymal stem cells. Representative images of neural crest-derived (Wnt1-tdT) Schwann cells ($Plp1^{GFP}$) in cultures using protocols for mesenchymal stem cell (MSC) isolation from mice. Cells were isolated from digested SAT, filtered for cells <40µm and were cultured on plastic in monolayer conditions in MSC proliferation medium containing 16.5% FBS, 1% Glutamax and 1% penicillin-streptomycin in α-MEM basal media. Scale bar = 500µm.

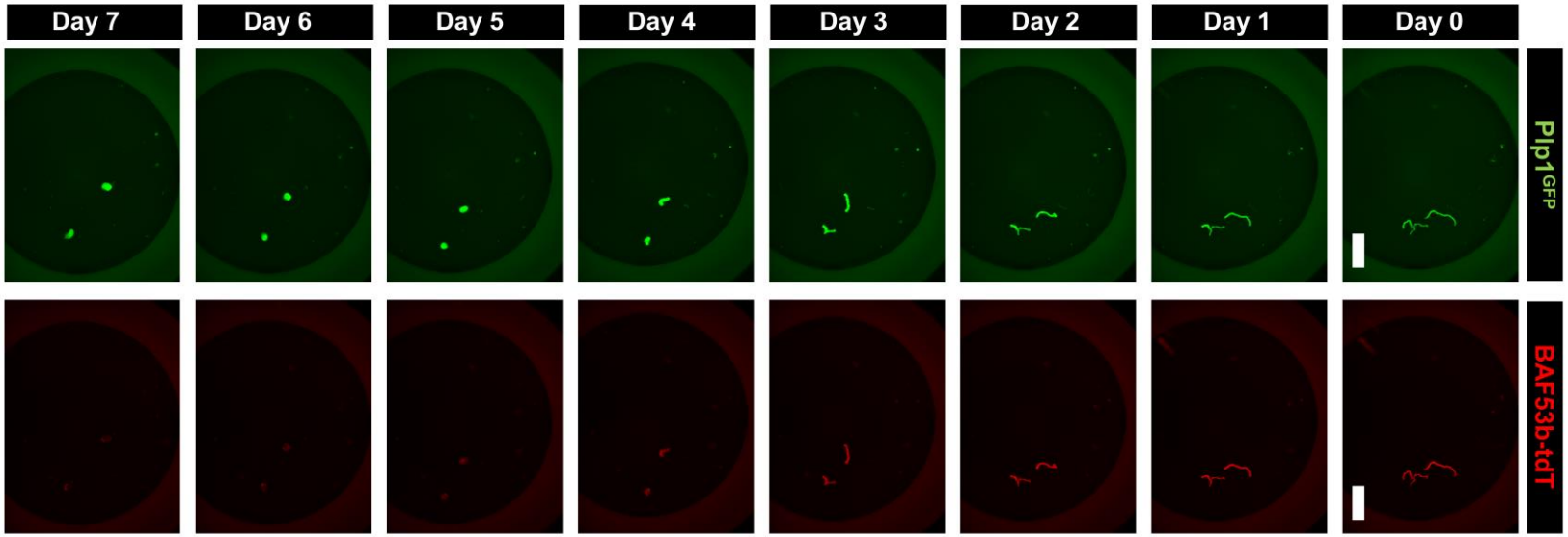
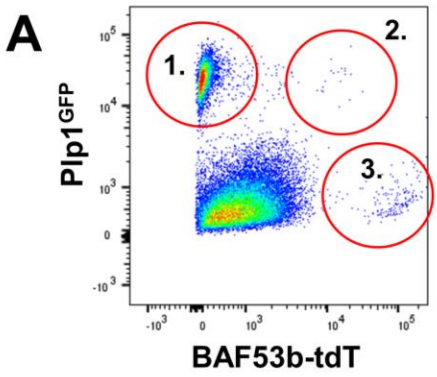

Day 7
Day 6
Day 5
Day 4
Day 3
Day 2
Day 1
Day 0
Plp1GFP
BAF53b-tdT

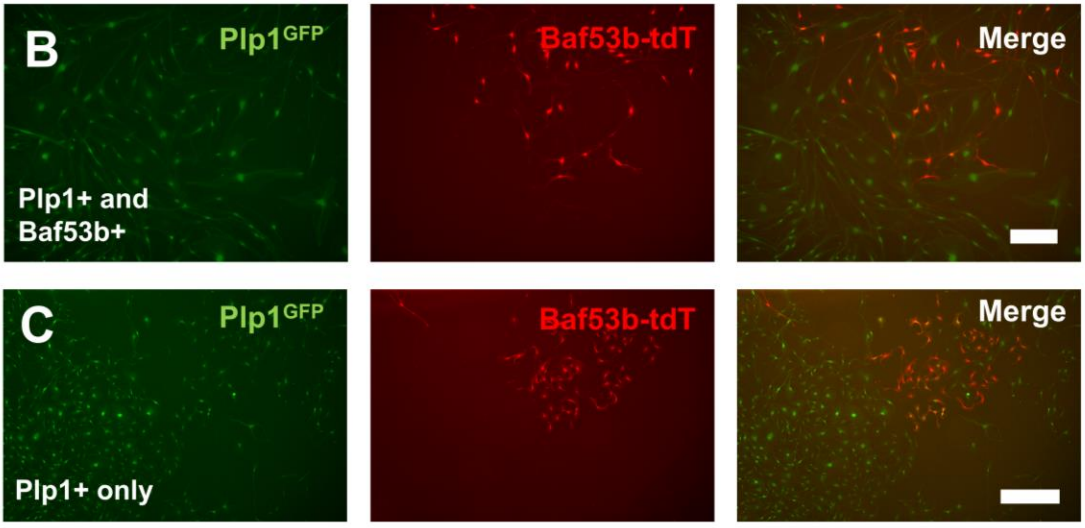
Fig. S2. Temporal expression of Plp1^{GFP} and BAF53b-tdT during the transition of nerve fiber bundles to neurospheres. Nerve fiber bundles isolated from the subcutaneous adipose tissue of Plp1^{GFP};BAF53b-tdT mice cultured in neuroproliferation medium to generate neurospheres. Representative images of transgenic Plp1^{GFP} (left panels) and BAF53b-tdT (right panels) expression in the same sample over 7 days. Scale bars = 500µm.



1) Plp1^{GFP}+ (glial)

2) Plp1^{GFP}+ Baf53b-tdT+ (transitioning)

3) Baf53b-tdT+ (neuronal)



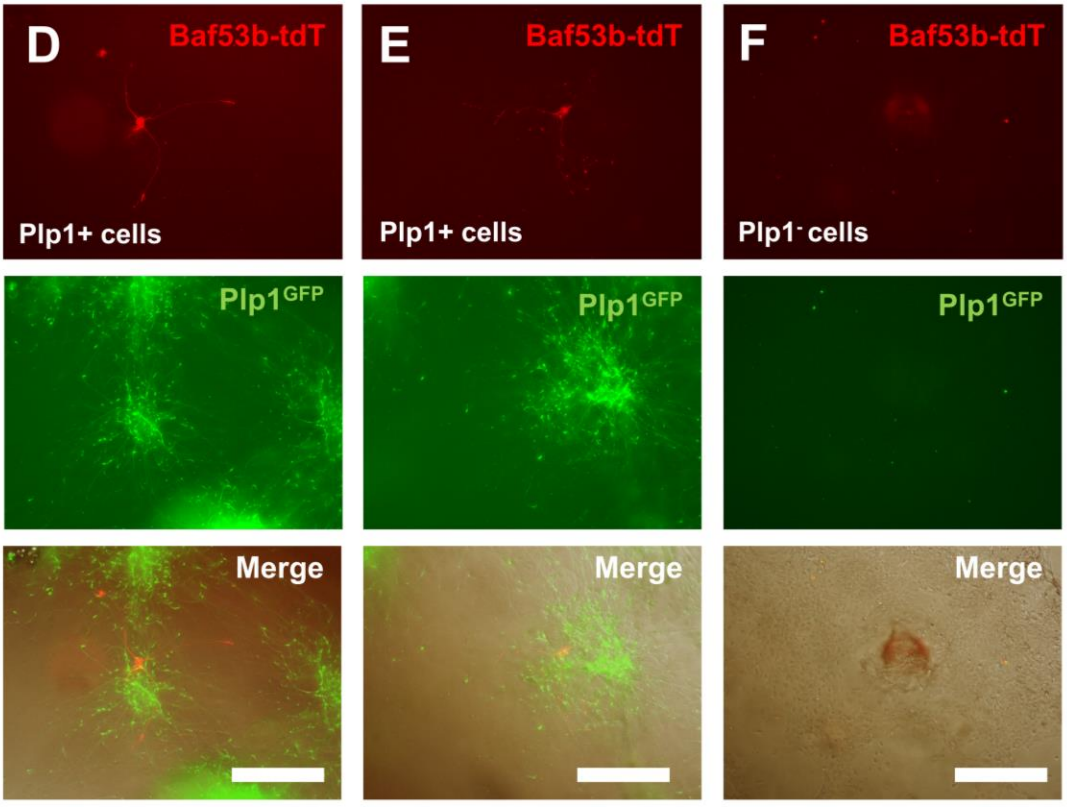


Fig. S3. Plp1^{GFP} expressing SAT-NSCs differentiate to neurons in vitro. A) Representative flow cytometry plot of cells isolated from the SAT and cultured in free-floating conditions in neuroproliferation medium from Plp1^{GFP};BAF53b-tdT transgenic mice. B) Validation by microscopy of transgene expression profiles in Plp1^{GFP+}, BAF53b-tdT^{+} and double positive cells from SAT-derived spheroids isolated by flowcytometry and cultured on fibronectin. Scale bar = 200µm. C) Identification of Plp1^{GFP+}, BAF53b-tdT^{+} and double positive cells in cultures of only Plp1^{GFP+} cells purified by flow cytometry indicating Schwann-like cells gives rise to BAF53b-tdT^{+} neurons in vitro. Scale bar = 500µm. D-E) Representative images of Plp1^{GFP+} cell differentiation into multipolar BAF53b-tdT^{+} Plp1^{GFP-} neurons in 3D culture conditions. Scale bar = 500µm. F) No evidence of BAF53b-tdT^{+} neuron differentiation was observed in cultures of Plp1^{GFP-} BAF53b-tdT^{-} cells in the same culture conditions. Scale bar = 500µm.

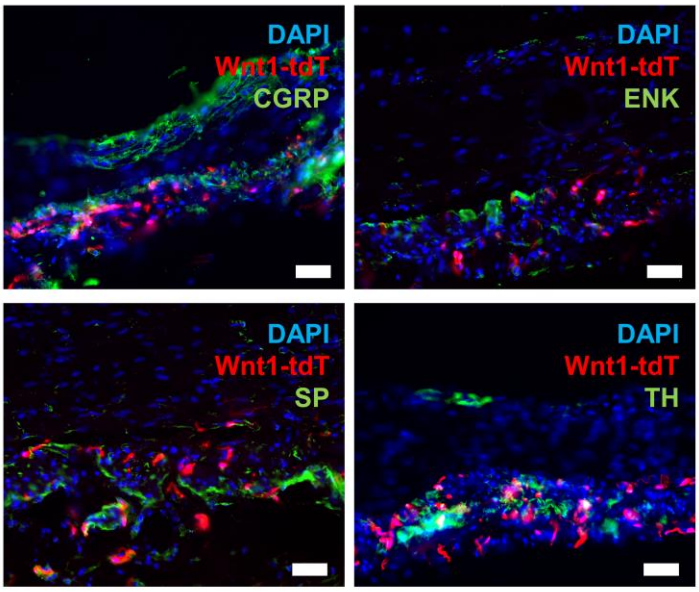


Fig. S4. Immunohistochemical labelling of SAT-NSCs for neurochemical subtype markers after transplantation to the colon. Cross sections of the colon containing Wnt1-tdT positive SAT-NSCs were labelled for Calcitonin Gene-Related Peptide (CGRP), Enkephalin (ENK), Substance P (SP) and Tyrosine Hydroxylase (TH) with minimal overlap observed with Wnt1-tdT (Scale bar = 50µm).

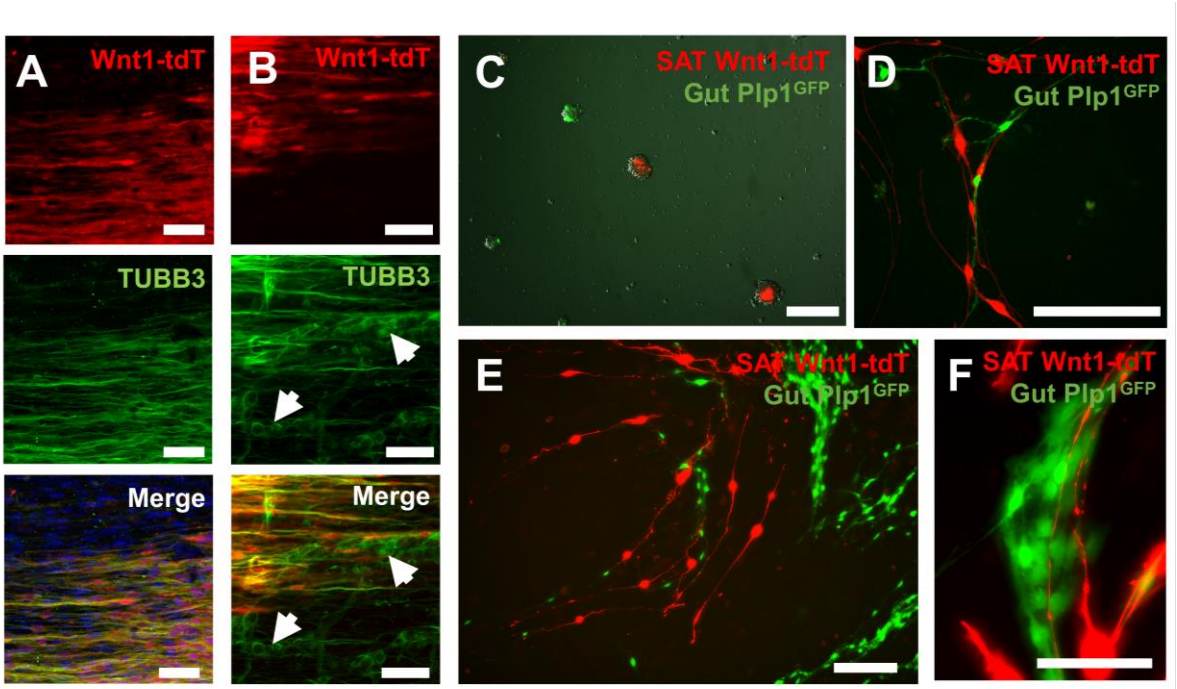


Fig. S5. SAT-NSCs integrate with the ENS *ex vivo* and in vitro. Representative images of transplanted cells located alone (A) and in proximity to endogenous myenteric ganglia (B). White arrows indicate endogenous TUBB3^{+} cells. Recipient muscularis propria tissues visualized for Wnt1-tdT^{+} cells (A-B, top panels), immunohistochemical labelling of TUBB3 (A-B, middle panels) and merged images (A-B, bottom panels). Scale bar = 50µm. C-F) Representative images of co-cultured heterogenous neurospheres isolated from the SAT of Wnt1-tdT mice (SAT-NSCs) and the colonic muscularis propria of Plp1^{GFP} mice (enteric glial cells) on fibronectin. SAT-NSCs and enteric glia migrated out of spheroids (C, Scale bar = 500µm) and began forming connections between each other after one week (D, Scale bar = 200µm). By 3 weeks, SAT-NSCs exhibited multipolar morphologies (E, Scale bar = 100µm) with nerve fiber projections penetrating through enteric ganglia-like structures (F, Scale bar = 100µm).

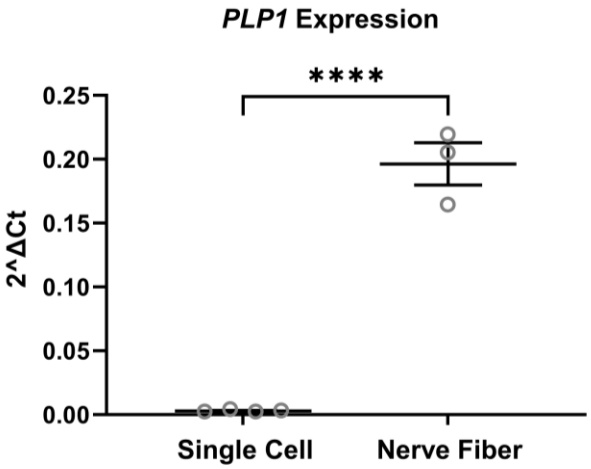


Fig. S6. Quantitative PCR of *PLP1* in human subcutaneous adipose tissue-derived spheroids after culture in neuroproliferation medium. Spheroids were generated from single cell suspensions of the digested subcutaneous adipose tissue (Single Cell) or cultured from intact nerve fiber bundles isolated from subcutaneous adipose tissue (Nerve Fiber). Unpaired t-test, ****p<0.0001. Single Cell-derived, n = 4 independent cultures; Nerve Fiber-derived, n = 3 independent cultures.

Table S1: Mice strains used for breeding in this study

Mouse	**Procurement**	**Usage**
Wnt1::Cre	Tg(Wnt1-Cre)11Rth - JAX Stock #003829	Neural crest tracer Cre (*46, 55*)
ROSA26^{tdTomato}	ROSA26R-tdTomato (R26R-tdT) reporter - JAX Stock #007914	tdT fluorescent reporter for Cre (*46, 55*)
Plp1^{GFP}	Kindly gifted by Wendy Macklin PhD, U Colorado	GFP fluorescent reporter for *Plp1* (glial) (*43*)
Tau^{GFP}	Mapttm1(EGFP)Klt/J - JAX Stock #004779	GFP fluorescent reporter for *Tau* (Neuronal) (*46*)
BAF53b::Cre	Tg(Actl6b-Cre)4092Jiwu/J - JAX Stock #027826	Mature neuron specific Cre (*56*)
Nestin^{GFP}	Kindly gifted by Jorg Dietrich MD PhD, MGH	Fluorescent reporter for *Nestin* (Stem cells) (*42*)
Polr2a$^{GCaMP5g\text{-}tdTomato}$	Polr2aTn(pb-CAG-GCaMP5g,-tdTomato)Tvrd (GCaMP5-tdT) reporter - JAX Stock #024477	Genetically encoded calcium indicator and tdT fluorescent reporter for Cre (*57, 58*)
Ednrb$^{-/-}$ (KO)	Ednrbtm1Ywa/J - JAX Stock #003295	Colonic aganglionosis (Hirschsprung disease model) (*40*)
nNOS$^{-/-}$ (KO)	B6.129S4-Nos1tm1Plh/J - JAX Stock #002986	Delayed gastric emptying (Gastroparesis model) (*59*)

Movie S1. Plp1-GFP BAF53b-tdT nerve fiber bundle transition to neurosphere

Movie S2. Intracellular calcium responses to EFS in SAT-NSCs transplanted to the aganglionic colon.

Data File S1 - Data for Figs 1-7

Data File S2 – Additional data for Fig 7