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Materials and Methods

iPSCs preparation: Fibroblasts were isolated from inner thigh skin punch of adult fully SLA-matched pigs (SLAdd; 5-9 months old; n=4) (*45*). Tissue explants were washed with sterile PBS + 1% penicillin/streptomycin and expanded using DMEM/F12/Glutamax (Invitrogen) +10% FBS. In about 5-9 days fibroblasts started to appear on the border of the tissue. Once expanded, fibroblasts were grown to confluence around ~70% and transduced with CytoTune-iPSc 2.0 Sendai Reprogramming Kit (ThermoFisher) in accordance with manufacturer's instructions. After transduction, cells were plated on mitotically inactivated porcine embryonic fibroblasts (PEFs), (isolated from 6-week-old embryos) with hESC medium (20% KO serum, 0.1 mM NEAA, 0.11 mM beta-mercaptoethanol, 20 ng/mL b-FGF). The iPSCs colonies were collected and passaged using manual picking into new PEFs in hESC medium containing ROCK inhibitor (Y-27632, StemCell Technologies).

Embryoid body (EB) formation: iPSCs colonies were manually picked and transferred into a 15-mL falcon tube containing 0.05% Trypsin/EDTA. Single cell suspension was generated by trituration and filtration with 40 µm cell mesh filter. Cells were re-suspended in StemDiff Neural Induction Medium (StemCell Technologies) containing 1% P/S (Gibco) and ROCK inhibitor (Y-27632, StemCell Technologies) and transferred to low attachment surface plates (Costar,). The medium was changed every 3 days. Formed EBs were kept in 3D culture for a total of 12 days.

In vitro and in vivo pluripotency (teratoma) testing: To test for pluripotency of the established iPS cell line three different assays were used: **i)** Established iPSCs-derived embryoid bodies (EBs) were continuously cultured in low-attachment plastic flasks in the presence of pig serum (0.1-1%) for 3-4 weeks. **ii)** Porcine iPSCs colonies were dissociated with accutase cell detach media (Thermo Fisher Scientific) and pipetted gently to break up large cell clumps. After spin the cells were resuspended in

MEF conditioned media containing 4 ng/ml of bFGF and plated onto prepared RGF/BME-coated chamber slides. At 3-4 days after plating cells were induced for 3–5 days using established germ layer-specific (ectoderm, endoderm or mesoderm) induction protocols (Human Pluripotent Stem Cell Functional Identification Kit, R&D Systems, Inc., cat# SC027B). After induction the EBs and induced iPSCs were fixed with 4% paraformaldehyde and stained with markers of definitive mesoderm (SMA, BRACHYURY), endoderm (AFP, SOX17) and ectoderm (OTX2,TUJ1). **iii)** A single cell suspension of iPSCs was prepared by dissociation of iPSCs colonies using accutase cell detach media (00-4555-56 – Fisher Scientific). Cells were resuspended in media/matrigel (BD Bioscience) (1:1) (at 4°C) and injected under the testes capsule of anesthetized 9-week-old male immunodeficient mice (*NOD/SCID Il2rg*$^{-/-}$; n=6), (≃1,000,000 cells in 30 µl/testes) using a 27G stainless steel needle. A palpable tumor (about 0.5 x 0.5 cm in size) was typically identified at 3-4 weeks after injection. After 5-6 weeks, the animals were sacrificed. The teratoma formation was confirmed in transverse H&E-stained sections.

NPC isolation: Embryoid bodies were collected by gravity sedimentation and transferred to Cultrex-PathClear (Trevigen) coated plates and cultured in NPC media (DMEM/F12, 0.5X B27, 0.5X N2, 1% P/S) in the presence of bFGF (10 ng/ml). Rosette-like structures that appeared after 3-4 days after plating were manually transferred with the pipette into separate plates and further expanded for several passages. To permit visualization of in vivo grafted cells, NPCs lines expressing EGFP under ubiquitin (cell type non-specific) or synapsin (neuron-specific) promoter were prepared after infection of proliferating NPCs with lentiviral vectors (HIV1-SYN-EGFP or HIV1-UBQ-EGFP; 10 M.O.I.).

Flow cytometry: Expression of intracellular NSCs-specific markers (SOX1, SOX2, PAX6 and Nestin) was determined on fixed NSCs samples using flow cytometry. Flow cytometry analysis was performed on a BD LSRFortessa (BD Biosciences) at Stem Cell Core, Sanford Consortium for Regenerative Medicine. Cells were dissociated with Accutase (Innovative Cell Technologies), washed with 1X PBS, fixed with Fixation Buffer (BD Biosciences) and permeabilized with Perm/Wash Buffer (BD Biosciences). Cells were incubated with directly conjugated antibodies (**Table S 5**) for 40-50 minutes and washed with Perm/Wash Buffer. The LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (ThermoFisher) was used to determine the viability of cells prior to the fixation and permeabilization required for intracellular antibody staining.

In vitro **iPSCs-NPCs differentiation:** For in vitro differentiation, NPCs were plated on poly-L-ornithine/laminin coated glass slides and differentiated in media containing i) cAMP (10 ng/mL, Sigma-Aldrich), BDNF (10 ng/mL, Peprotech) and GDNF (10 ng/mL, Peprotech) for 4-8 weeks, or ii) 0.1-1% porcine serum only and cultured for 20 days. To facilitate neuronal maturation some NPCs were cultured and induced on human fetal astrocyte (ScienCell) monolayer. For immunofluorescence

staining cells were fixed with 4% paraformaldehyde and stained using a standard immunofluorescence protocol (**Table S 5** for the list of primary antibodies).

In vitro **time-lapse calcium imaging in induced iPSCs-NPCs:** Two months-induced NPCs were loaded with 3 μM fluo-4AM (reconstituted in DMSO) and 0.1% Pluronic F-127 (Molecular Probes) in Hanks' Balanced Salt Solution (HBSS) for 45 min at room temperature. Excess dye was washed out with three rinses of HBSS and neurons allowed to recover for 30 min at room temperature in the dark until data acquisition. Fluorescence imaging of intracellular $Ca2^{+}$ dynamics for a period of 5 min 18 sec was performed with a Olympus BX51W1 fixed-stage upright fluorescence microscope (Olympus Corp.) equipped with a SPOT- Xplorer - XS 1.4 MP monochrome camera (Diagnostic Instruments, Inc.). The imaging system was controlled by SPOT 5.2 ADVANCED Software (Diagnostic Instruments, Inc). Neurons were imaged in time lapse with a water immersion 40x magnification, 0.8 numerical aperture (NA) LUMPlanFL N objective (Olympus). Images were captured every 1.035 sec, and under low-light level conditions. Images were collected at a slow transfer rate, which reduces background noise, and binned (2x2). Digital image processing was performed using SPOT 5.2 ADVANCED Software (Diagnostic Instruments, Inc.), image processing program ImageJ (NIH), graphics and data analysis software SigmaPlot 12.5 (Systat Software, Inc.).

Immunization of allogeneic pig with iPSCs-NPCs: Briefly, the animal (n=1) was sedated with Ketamine (5 mg/kg of BW), Xylazine (1 mg/kg) and Atropine (0.05 mg/kg) intramuscularly (IM) and anesthetized by Isoflurane via face mask. The animal received a subcutaneous injection composed of a mixture of proliferating pluripotent iPS cells (appr. $3.3x10^6$), proliferating iPSCs-NPCs (appr. $3.3x10^6$) and differentiated iPSCs-NPCs (appr. $3.3x10^6$) in PBS emulsified with CFA (Complete Freund's Adjuvant) at 1:1 in total of 2.5 ml. Differentiated iPSCs-NPCs were induced for 4 weeks with DMEM/F12 Glutamax media containing 1% P/S, 10% FBS (fetal bovine serum), B27 (1x), N2 supplement (1x), 10 ng/mL BDNF and 10 ng/mL GDNF. Cell emulsion was administered at 5 sites (bilaterally - behind the shoulder and front of the hind leg) and on the neck or hump, total of 0.5 ml per site). The whole immunization procedure was then repeated at 14 and 28 days after the first immunization. At 4 weeks after last immunization the animal was terminally anesthetized with Fatal-Plus (Vortech Pharmaceuticals, Ltd.) (0.2 ml/kg). A thoracotomy was performed, blood collected through a direct cardiac puncture and isolated serum stored at -20°C. Harvested serum was used in subsequent immunofluorescence studies as a positive control to identify the presence of circulating anti-iPSCs-NPCs antibodies using mature iPSCs-NPCs grafts in rat striatum or spinal cord.

In vivo **grafting studies**

An iPSCs-NPCs line prepared from reprogrammed skin fibroblasts harvested from animal No. 21434 was used in all in vivo grafting studies.

Intrastriatal grafting of porcine NPCs in immunodeficient rats: Immunodeficient rats (athymic rats; Crl: NIH-Foxn1^{rnu}; Charles River; n=12) were anesthetized with 2% isoflurane and received 3 bilateral intrastriatal injections of SYN-EGFP-NPCs (50,000 cells/1 µl/injection) using the following X-Y-Z coordinates (AP 1.7/04/-0.9; LAT 2.5/3.0/4.0; DEP 5.5/5.5/5.0). A Stoelting stereotaxic apparatus was used for injections and cells were delivered into the brain parenchyma using a Hamilton syringe and 32G stainless steel needle. After cell grafting animals survived for 1-10 months and were then perfusion fixed for histopathological analysis or brain freshly harvested for *ex vivo* brain slice preparation and patch clamp recording from grafted EGFP+ neurons or mRNA sequencing of porcine-specific transcripts.

Spinal grafting of iPSCs-NPCs in syngeneic fully SLA-matched pigs: Three adult fully SLA-matched pigs (*45*) previously used to harvest skin fibroblasts and generate iPSCs-NPCs were used for iPSCs-NPCs grafting. The age of animals at the time of iPSCs-NPCs grafting was 3 years 4 months (ID:21349), 3 years 2 months (ID:21434), and 3 years 10 months (ID:20975), respectively. Animals were prepared for spinal cell grafting as previously described (*14*). Pigs were pre-medicated with intramuscular azaperonum (2 mg/kg) and atropine (1 mg/kg; Biotika) and then induced with ketamine (20 mg/kg, i.v.). Animals were then intubated with a 2.5F tracheal tube and anesthesia induced and maintained with 1.5% isoflurane. Oxygen saturation was monitored throughout a procedure using a pulse oximeter (Nellcor Puritan Bennett Inc.). Anesthetized animals were then placed into prone position and prepared for spinal cell grafting. To immobilize the lumbar spinal cord, animals were mounted into a spinal immobilization apparatus and the lumbar portion of the animal was lifted 5" above the operating table to eliminate respiration-caused spinal cord pulsation. A dorsal laminectomy of L2-L5 vertebrae, corresponding to L3-L6 spinal segments, was then performed and epidural fat removed using cotton swabs. The dura was left intact. To deliver cells, the XYZ manipulator (Stoelting) was used and mounted directly to the operating table. A 30 gauge needle was then mounted into the Z-arm and connected to the digital microinjector (Stoelting) and 250 µl Hamilton syringe using PE-10 tubing. Animals then received a total of 30 injections (15 on each side; 6 µl/injection; 20,000-30,000 cells/µl; flow rate=2 µl/min) targeted into the intermediate zone (lamina VII) of L2-L5 segments. The distance between individual injections was 1-1.5 mm. All surgical interventions followed rigid aseptic procedures. All materials were subjected to autoclaving or gas sterilization. After cell grafting animals survived for 3 months. No immunosuppression was used.

Spinal grafting of iPSCs-NPCs into allogeneic continuously or transiently-immunosuppressed pigs with chronic spinal cord injury: Induction of spinal cord injury: Adult female Gottingen-Minnesota pigs (n=6) were anesthetized and the Th9 spinal segment exposed after partial dorsal laminectomy of Th8-9 vertebra as previously described (*28*). The exposed Th9 segment was compressed (1 cm/s) with an aluminum rod (5 mm in diameter) using a computer-controlled apparatus. Compression pressure cut-off was set at 2.5 kg. After trauma, animals survived for 2.5 months before spinal iPSCs-NPC grafting. Spinal iPSCs-NPC grafting and immunosuppression: At 2.5 months after induction of spinal injury animals were re-anesthetized and a chronic jugular catheter (8G) placed into right jugular vein. The site of previous spinal cord injury was then exposed and the dura was cut open. Animals then received a total of 40 injections of iPSCs-NPCs (UBI-EGFP) (6 µl/injection; 20,000-30,000 cells/µl; flow rate=2 µl/min) targeted into the injury epicenter and above and below the injury. From the day of cell grafting animals were continuously immunosuppressed by tacrolimus (0.025 mg/kg/day) for 4 weeks using an externally mounted 11-day infusion pump (Baxter Infusor). Three animals were perfusion fixed with 4% paraformaldehyde at 4 weeks. In another 3 animals immunosuppression was stopped at 4 weeks and animals sacrificed after 2.5 additional months without any immunosuppression. Subsequently the animals were perfusion fixed with 4% paraformaldehyde for immunofluorescence analysis of spinal cord.

Preparation of *ex vivo* rat striatal slices and whole-cell patch-clamp recordings: Artificial cerebrospinal fluid (ACSF) contained (in mM) 121 NaCl, 4.2 KCl, 1.1 $CaCl_2$, 1 $MgSO_4$ (or 0.4 $MgSO_4$ and 0.3 MgCl), 29 $NaHCO_3$, 0.45 NaH_2PO_4-H_2O, 0.5 Na_2HPO_4 and 20 glucose (all chemicals from Sigma). ACSF was continuously bubbled with a mixture of CO_2 (5%) and O_2 (95%). Rats were deeply anesthetized with 5% isoflurane and immediately perfused transcardially with oxygenated cold ACSF until most blood in the brain was cleared. The cortex and hippocampus were surgically removed and coronal slices (300-µm thick) of the striatum were cut. Slicing was performed in ACSF at 4°C. After cutting, slices were brought back to 35°C for 20 min, and maintained in ACSF at 25°C before recordings. For whole-cell patch-clamp recordings, individual slices were transferred into a heated recording chamber and continuously perfused (1 ml/min) with ACSF at 25°C. For targeted whole-cell recordings of Synapsin-EGFP grafted neurons, we used a 40X water-immersion objective, differential interference contrast filters (all Olympus), an infrared digital camera (Rolera XR - Qimaging), and digidata 1440A/Multiclamp 700B and Clampex 10.3 (Molecular devices). Patch electrodes were filled with internal solutions containing 130 mM K-gluconate, 6 mM KCl, 4 mM NaCl, 10 mM Na-HEPES, 0.2 mM K-EGTA; 0.3 mM GTP, 2 mM Mg-ATP, 0.2 mM cAMP, 10 mM D-glucose, 0.15% biocytin

and 0.06% rhodamine. The pH and osmolarity of the internal solution were close to physiological conditions (pH 7.3, 290–300 mOsmol). Data were all corrected for liquid junction potentials (10 mV). Electrode capacitances were compensated on-line in cell-attached mode (~7 pF). Recordings were low-pass filtered at 2 kHz, digitized, and sampled at intervals of 50 ms (20 kHz). To control the quality and the stability of the recordings throughout the experiments, access resistance, capacitance and membrane resistance were continuously monitored on-line and recorded. The resistance of the patch pipettes was between 4 and 6 MOhm.

Swine leukocyte antigen (SLA) genotyping: SLA genotyping of three class I (*SLA-1*, *SLA-2*, *SLA-3*) and three class II (*DRB1*, *DQB1*, *DQA*) genes was performed on the iPSCs-NPCs donor and recipients using low-resolution PCR-based assays with sequence-specific typing primers as previously described (*16, 17*). Modifications were made to the typing panels to broaden the allele coverage with the increasing number of SLA alleles. SLA haplotypes and allele-level resolution were deduced based on published data (*46-48*).

Perfusion fixation, immunofluorescence staining and quantitative analysis of neuronal and glial differentiation in EGFP+ grafts: For tissue processing, rats or pigs were deeply anesthetized with 2 mg/kg pentobarbital and 0.25 mg phenytoin (0.5 mL of Beuthanasia-D, Intervet/Schering-Plough Animal Health Corp.) and transcardially perfused with heparinized saline (200 ml-rat and 2 litters-pig) followed by 4% paraformaldehyde (PFA), (250 ml-rat and 2 litters-pig) in PBS. Brain or spinal cord was then dissected from skull or vertebral column and post-fixed in 4% paraformaldehyde overnight at 4ºC. Tissue was then cryoprotected in 30% sucrose for a minimum of five days. Transverse brain or spinal cord sections were then cut on a cryostat and stored free-floating in PBS with thimerosal (0.05 wt%). Sections were stained overnight at 4°C with primary antibodies in PBS with 0.2% Triton X-100. Following washing in PBS for five minutes, sections were incubated with fluorescent-conjugated secondary donkey antibodies. All primary and secondary antibodies used for tissue or in vitro fixed cells staining are listed in **Table S 5**. Sections were then mounted on slides, dried at room temperature, and covered with Prolong anti-fade kit. Confocal images (1024 × 1024 pixels) were captured with a Fluoview FV1000 microscope (Olympus,) and ImageJ software (NIH) was used for processing.

For quantitative/qualitative analysis of grafted cell neuronal and glial differentiation sections taken from immunodeficient rats (n=4) at 7 months, syngeneic pigs (n=3) at 3 months and allogeneic pigs at 1 month (1 month of continuous immunosuppression; n=3) or 3.5 months (2.5 months after immunosuppression was terminated; n=3) after NPCs grafting were used. Four sections taken from each animal with identified EGFP+ grafts were used for staining and quantification.

In animals grafted with SYN-EGFP-tagged NPCs (immunodeficient rat and syngeneic pig) sections were stained with GFP antibody in combination with neuronal markers including: DCX, NSE and NeuN. In animals grafted with UBI-EGFP-tagged NPCs (allogeneic pig) sections were stained with GFP antibody in combination with neuronal and glial markers including: DCX, NSE, NeuN, GFAP and Olig2. The total number of double-stained grafted cells was then counted and expressed as % of the total EGFP-stained cell population.

For quantitative analysis of immune response markers (CD45 and CD8) sections taken from iPSCs-NPCs-grafted: i) syngeneic pigs at 3 months (n=3), ii) allogeneic pigs at the end of 4 weeks immunosuppression (n=3), and iii) allogeneic pigs at 2.5 months after immunosuppression was terminated (n=3), were used. The presence of positively-stained CD45 and CD8 cells was analyzed separately in the EGFP+ grafts and in surrounding host tissue. Four sections taken from each animal with identified EGFP+ grafts were used for staining and quantification. The total number of CD45 and CD8 immunoreactive cells were counted in four 1 mm^2 fields in each section. For statistical analysis the number of positively-stained cells in allogeneic animals with continuous and transient immunosuppression was compared using unpaired T-test.

RNA-Seq Preprocessing and Analysis

RNA was extracted from iPSC-NPC grafted rat striatal samples and in vitro-cultured cells using the RNeasy Mini kit (Qiagen) according to the manufacturer's protocol. Tissue samples from control pig CNS were collected and preserved in RNAlater (Thermo Fisher) until the time of processing with the rest of the samples. All extracted RNA samples were DNAase treated and RNA quality was determined using Tapestation analysis (Agilent). Stranded mRNA library preps were prepared using the TruSeq Stranded mRNA kit (Illumina) and sequencing was performed on the HiSeq4000 platform (Illumina).

RNA-Seq reads with average base quality less than 15 were filtered out and typical Illumina adapters were trimmed with BBTools (*49*). Prior to gene expression quantification the reads were further filtered by alignment to the pig genome (susScr3) with STAR (*50*) to filter out possible contaminant reads and those with more than 10% mismatches to the genome. Reads passing alignment were quantified against the Pig transcriptome using kallisto (*51*). The kallisto index was generated from a combined annotation from Ensembl and RefGene downloaded from the UCSC Genome Browser. In addition to the annotated transcripts we added many introns and genomic regions around single-exon transcripts so that kallisto was able to estimate whether or not reads were specific to the transcript or to the general region of the transcript or to a retained intron

containing the transcript. Downstream analysis of gene expression was performed in R using standard libraries as well as Rtsne for t-SNE transforms and igraph for clustering analysis.

Analysis and Preprocessing of Mixed-species RNA-Seq reads

Our species sorting pipeline breaks the raw mRNA-Seq reads up into 4 parts:

1. species 1 assigned
2. species 2 assigned
3. overall ambiguous
4. unaligned

Sequence reads were aligned to both species genomes, and the alignment with the higher score was used to assign each read to the appropriate species. The precision of this sorting was established by running single-species control samples through the read sorting pipeline and computing the ratio of confidently sorted reads (to species 1 or 2) that were sorted to the correct species. For both rat- and pig-only samples we observed consistent precision and false discovery rates (FDR) (Table S 3). Recall is calculated by considering all reads that may be potentially sorted (those aligned with alignment scores over a threshold) and computing the proportion that were correctly sorted. Recall is always less than precision because it includes ambiguously sorted reads whereas precision only reports the reads that were confidently sorted to a species. The mean precision among control rat samples was 99.6% with a FDR of 0.39%. The mean precision among control porcine NPC samples was 99.5% with a FDR of 0.47%. The mean percentage of pig-assigned reads from the grafted nude rat striatal tissue was 5.19%. This includes 9332 pig genes that were detected that passed a simple threshold of greater than 10 read counts and >1 tpm (transcripts per million mapped reads), which is a very low threshold. The provided excel spreadsheet (Porcine Gene List) lists all pig genes detected in the experiment (14,442), but many of these are below the threshold described above.

Supplementary Figures

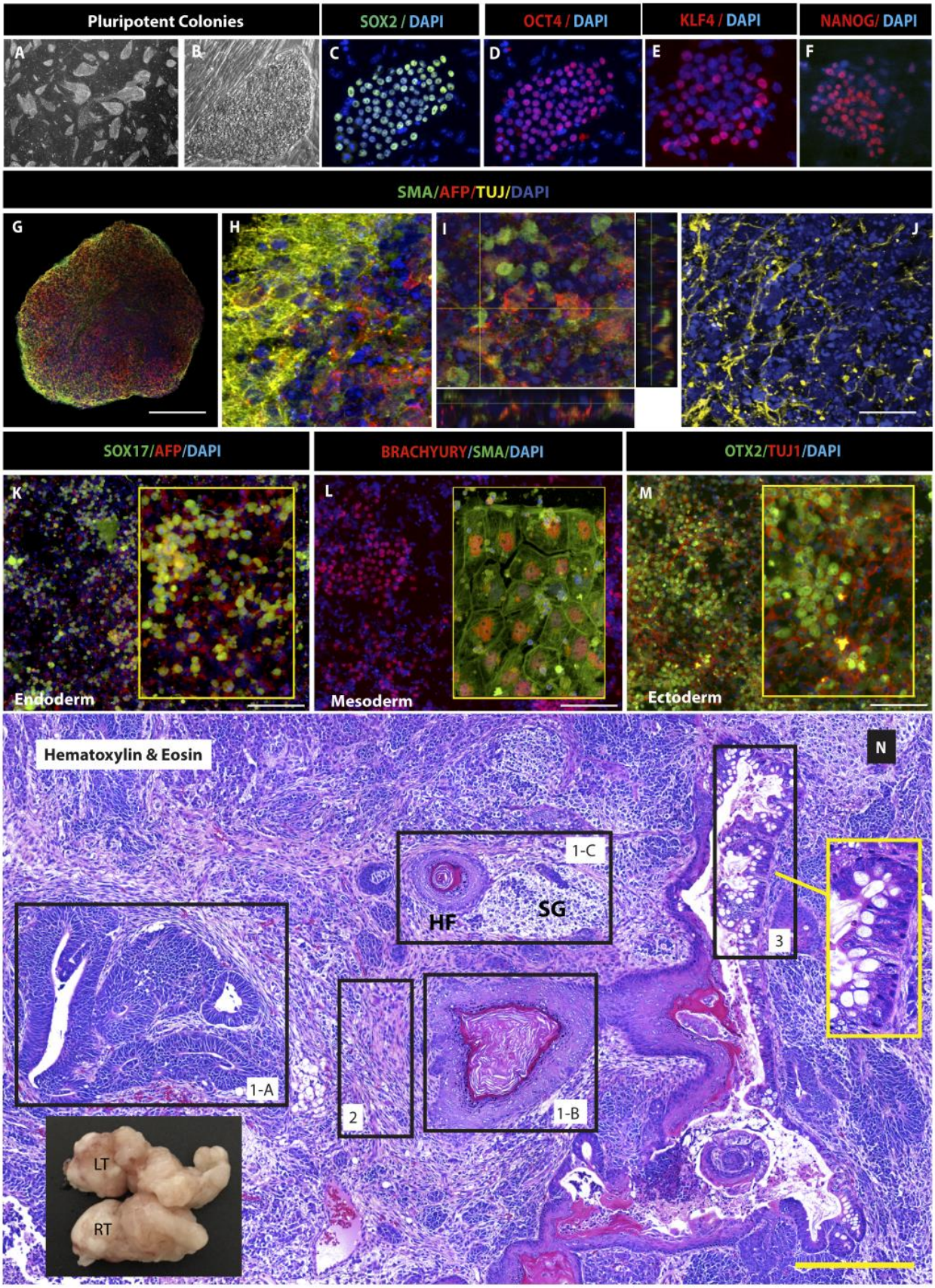
Pluripotent Colonies
SOX2 / DAPI
OCT4 / DAPI
KLF4 / DAPI
NANOG/ DAPI
A
B
C
D
E
F
SMA/AFP/TUJ/DAPI
G
H
I
J
SOX17/AFP/DAPI
BRACHYURY/SMA/DAPI
OTX2/TUJ1/DAPI
K
L
M
Endoderm
Mesoderm
Ectoderm
Hematoxylin & Eosin
N
1-C
HF
SG
3
1-A
2
1-B
LT
RT

fig. S 1A-N

Figure S 1. Porcine fibroblasts-derived iPSCs generate ectoderm, mesoderm and endoderm cell derivatives in vitro and in vivo. (A, B) Proliferating porcine iPSCs colonies re-plated from previously frozen stock (passage 18). (**C**-**F**) Immunofluorescence staining for SOX2, OCT4, KLF4 and NANOG in proliferating iPSCs colonies (passage 23). **(G-J)** Long-term in vitro-induced iPSCs-derived embryoid bodies showed expression of all three germ layers markers including SMA (mesoderm), AFP (endoderm) and TUJ1 (ectoderm). **(K-M)** Porcine iPSCs seeded as a single cell suspension in matrigel and induced for 10-14 days with endoderm, mesoderm or ectoderm-specific induction protocols show expression of markers associated with a definitive endoderm (SOX17, AFP) mesoderm (BRACHYURY, SMA) or ectoderm (OTX2, TUJ1). **(N)** Extensive bilateral teratoma formation in mouse testes at 6 weeks after intratesticular iPSCs injection (B/W background insert: LT-left testes; RT-right testes). Hematoxylin & Eosin-stained section taken from iPSCs-injected mouse testes show the presence of ectoderm (1-A-neural tube rosettes, 1-B-epiderimis with keratin and 1-C-hair follicle with sebaceous gland), mesoderm (2-smooth muscle) and endoderm (3-gut cuboidal epithelium with goblet cells) derivatives. (HF-hair follicle, SG-sebaceous gland; **Scale Bars:** G-200 µm; J-50 µm; K, L, M-100 µm; N-500 µm).

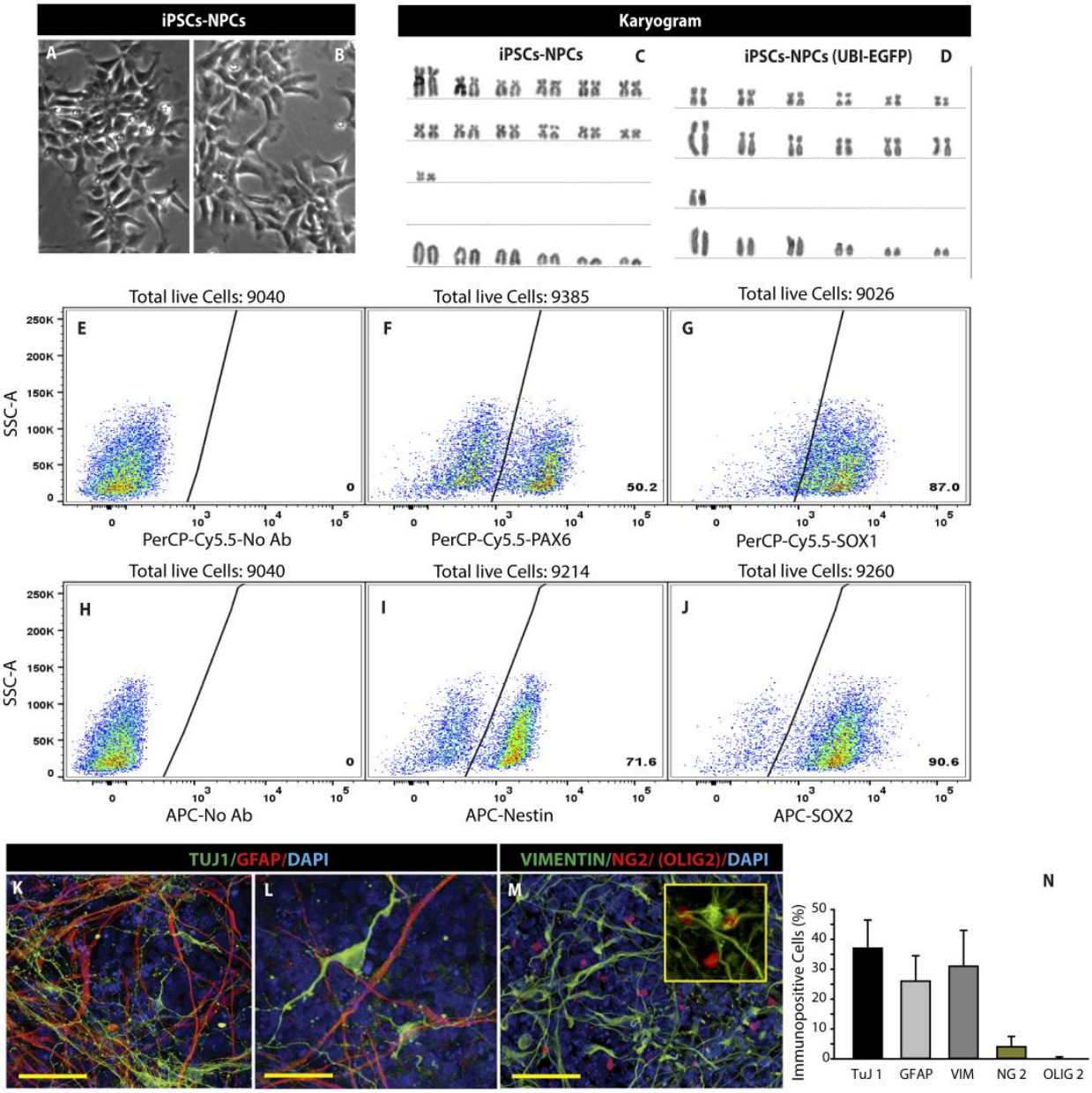
iPSCs-NPCs
A
B
Karyogram
iPSCs-NPCs
C
iPSCs-NPCs (UBI-EGFP)
D
Total live Cells: 9040
Total live Cells: 9385
Total live Cells: 9026
E
F
G
SSC-A
0
50.2
87.0
PerCP-Cy5.5-No Ab
PerCP-Cy5.5-PAX6
PerCP-Cy5.5-SOX1
Total live Cells: 9040
Total live Cells: 9214
Total live Cells: 9260
H
I
J
0
71.6
90.6
APC-No Ab
APC-Nestin
APC-SOX2
TUJ1/GFAP/DAPI
VIMENTIN/NG2/ (OLIG2)/DAPI
K
L
M
N
Immunopositive Cells (%)
TuJ 1
GFAP
VIM
NG 2
OLIG 2

fig. S 2 A-N

figure S 2. Previously frozen, in vitro expanded porcine iPSCs-NPCs show stable karyotype and generate neural derivatives (neurons, astrocytes and oligodendrocytes) after in vitro induction. (**A, B**) Proliferating iPSCs-NPCs re-plated from previously frozen stock (passage 24). Typical neural precursor morphology with multipolar organization is seen. (**C, D**) Karyotype of established naive iPSCs-NPCs (passage 15) and EGFP expressing iPSCs-NPCs prepared after infection with HIV1-UBI-EGFP lentivirus (passage 30). (**E-J**) Flow cytometry scattergrams of proliferating iPSCs-NPCs stained with PAX6, SOX1, Nestin and SOX2 antibody. (**K-N**) Qualitative and quantitative analysis of multipotent potential of iPSCs-NPCs. (**Scale Bars:** K-50 µm; L-20 µm; M-50 µm).

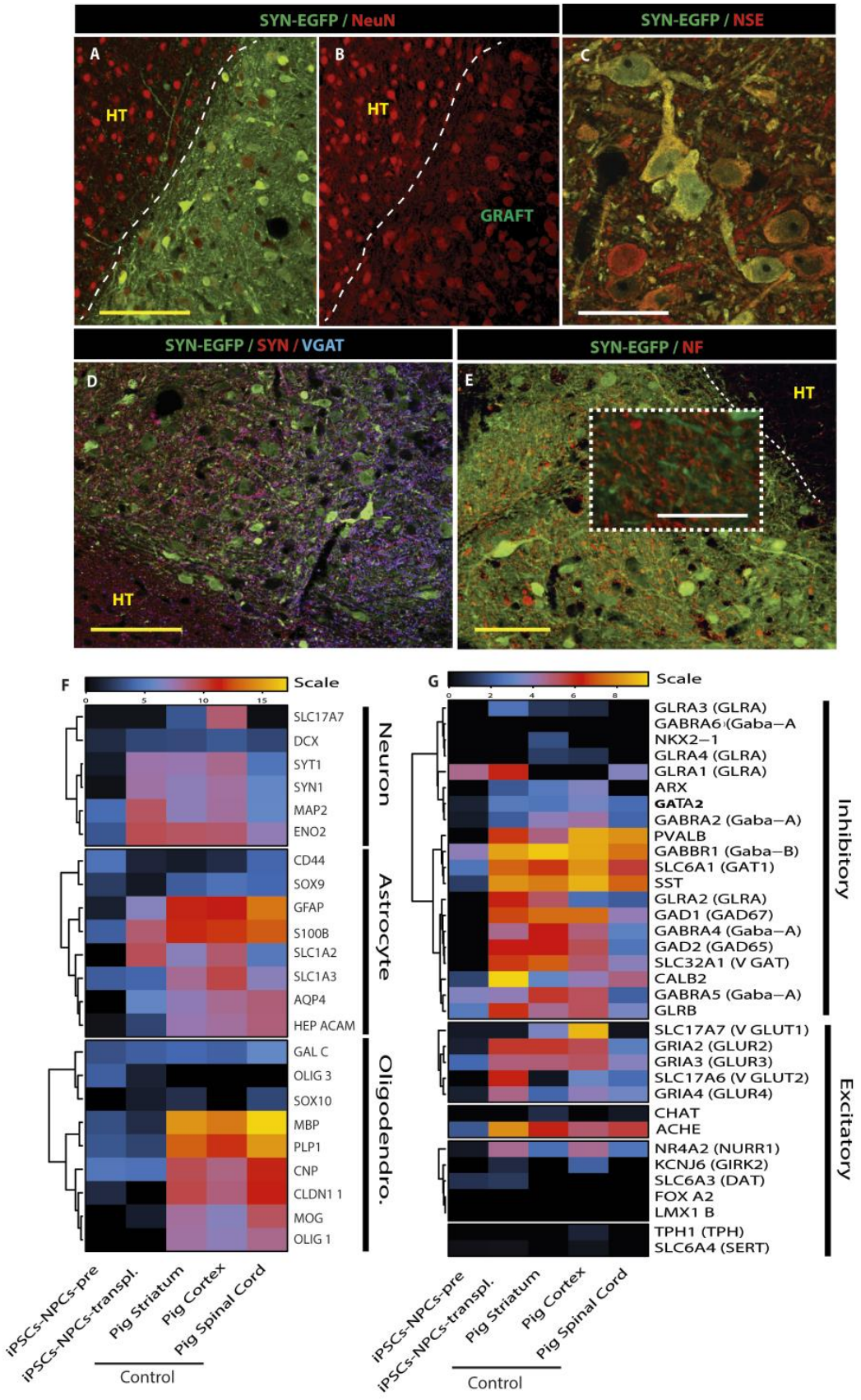
SYN-EGFP / NeuN
SYN-EGFP / NSE
A
B
C
HT
HT
GRAFT
SYN-EGFP / SYN / VGAT
SYN-EGFP / NF
D
E
HT
HT
F
Scale
0
5
10
15
SLC17A7
DCX
SYT1
SYN1
MAP2
ENO2
Neuron
CD44
SOX9
GFAP
S100B
SLC1A2
SLC1A3
AQP4
HEP ACAM
Astrocyte
GAL C
OLIG 3
SOX10
MBP
PLP1
CNP
CLDN1 1
MOG
OLIG 1
Oligodendro.
iPSCs-NPCs-pre
iPSCs-NPCs-transpl.
Pig Striatum
Pig Cortex
Pig Spinal Cord
Control
G
Scale
0
2
4
6
8
GLRA3 (GLRA)
GABRA6 (Gaba–A
NKX2–1
GLRA4 (GLRA)
GLRA1 (GLRA)
ARX
GATA2
GABRA2 (Gaba–A)
PVALB
GABBR1 (Gaba–B)
SLC6A1 (GAT1)
SST
GLRA2 (GLRA)
GAD1 (GAD67)
GABRA4 (Gaba–A)
GAD2 (GAD65)
SLC32A1 (V GAT)
CALB2
GABRA5 (Gaba–A)
GLRB
Inhibitory
SLC17A7 (V GLUT1)
GRIA2 (GLUR2)
GRIA3 (GLUR3)
SLC17A6 (V GLUT2)
GRIA4 (GLUR4)
CHAT
ACHE
NR4A2 (NURR1)
KCNJ6 (GIRK2)
SLC6A3 (DAT)
FOX A2
LMX1 B
TPH1 (TPH)
SLC6A4 (SERT)
Excitatory
iPSCs-NPCs-pre
iPSCs-NPCs-transpl.
Pig Striatum
Pig Cortex
Pig Spinal Cord
Control

fig. S 3 A-G

figure S 3. Long-term grafted (7-10 months) porcine iPSCs-NPCs in rat striata show protein- and mRNA-defined signature which is consistent with mature porcine CNS tissue. (A, B) SYN-EGFP+ grafts containing high density NeuN+ neurons. **(C)** A majority of SYN-EGFP+ grafted neurons show porcine-specific NSE expression. **(D)** High density of double SYN/VGAT-stained puncta in SYN-EGFP+ grafts. **(E)** High density of NF+ neurites in the core of SYN-EGFP+ graft. (**F**) Heat maps comparing the gene expression levels of a subset of genes commonly used to mark neurons, astrocytes, and oligodendrocytes between pre-implantation iPSCs-NPCs and grafted iPSCs-NPCs at 10 months post-transplantation into the striata of an immunodeficient rat. **(G)** Heat map of relative gene expression of a subset of genes associated with either inhibitory (top) or excitatory (bottom) neuronal transmission. (**HT-**host tissue**, Scale Bars:** A-100 µm; C-20 µm; D-100 µm; E-50 µm).

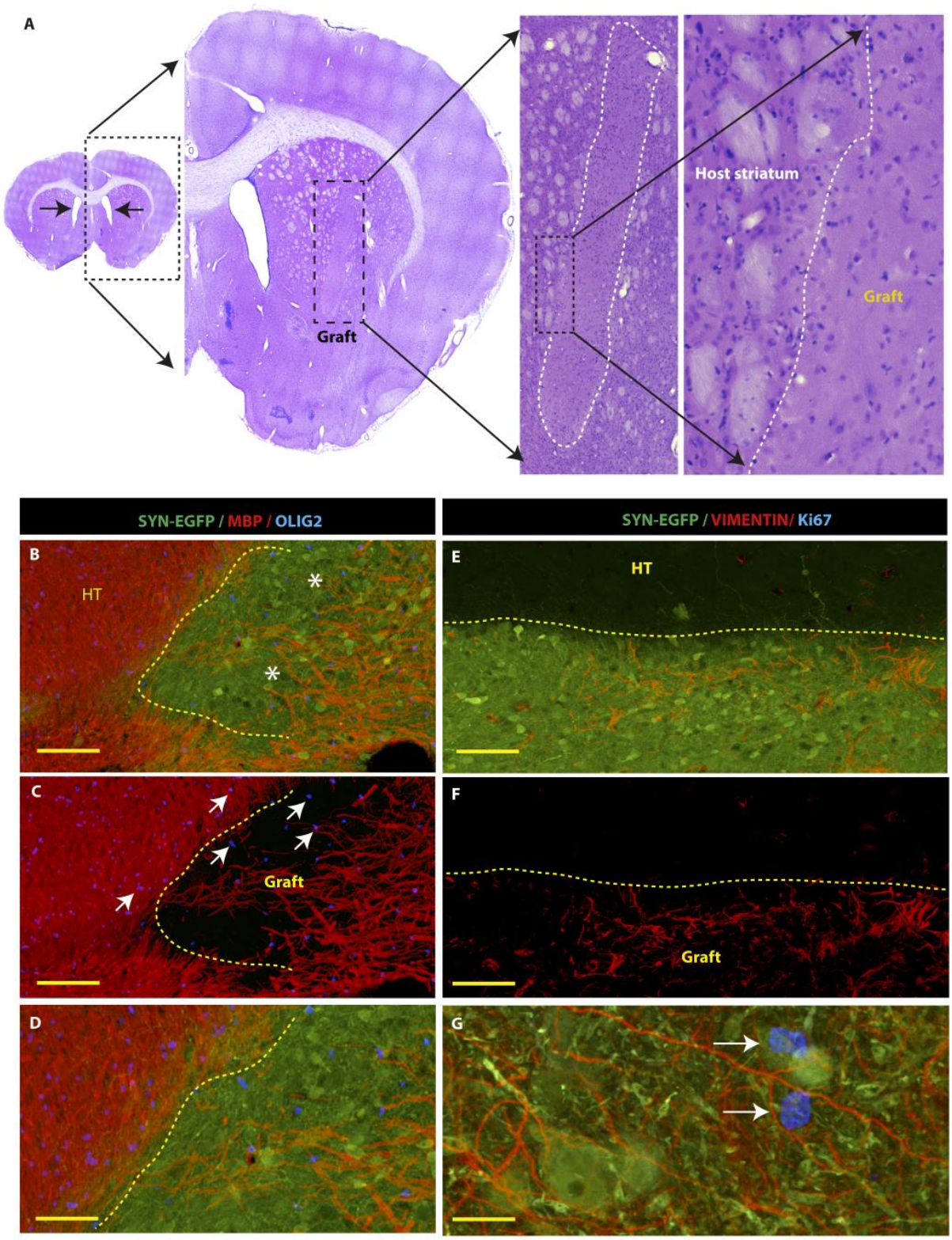
Porcine iPSCs-NPCs in rat striatum: 7 months post-grafting.
A
Graft
Host striatum
Graft
SYN-EGFP / MBP / OLIG2
SYN-EGFP / VIMENTIN/ Ki67
B
HT
E
HT
C
Graft
F
Graft
D
G

fig. S 4 A-G

figure S 4. Porcine iPSCs-NPCs grafted into rat striata show no tumor formation and incomplete myelinization at 7 months postgrafting. (**A**) H&E staining of coronal brain section shows well incorporated iPSCs-NPCs graft. No signs of tumor formation or ventricular compression are seen (black arrows). **(B-D)** Staining with myelin basic protein (MBP) and oligodendrocyte marker (Olig2) showed incomplete myelination of EGFP+ grafts. Olig2+ cells are regularly distributed in the EGFP+ graft and in the host tissue (C-white arrows). (**E-G**) Staining with VIMENTIN and Ki67 antibody show a continuing proliferation of glial (VIMENTIN+) cells within EGFP+ graft. (HT-host tissue; **Scale Bars:** B, C-100 µm; D-50 µm; E, F-200 µm; G-40 µm).

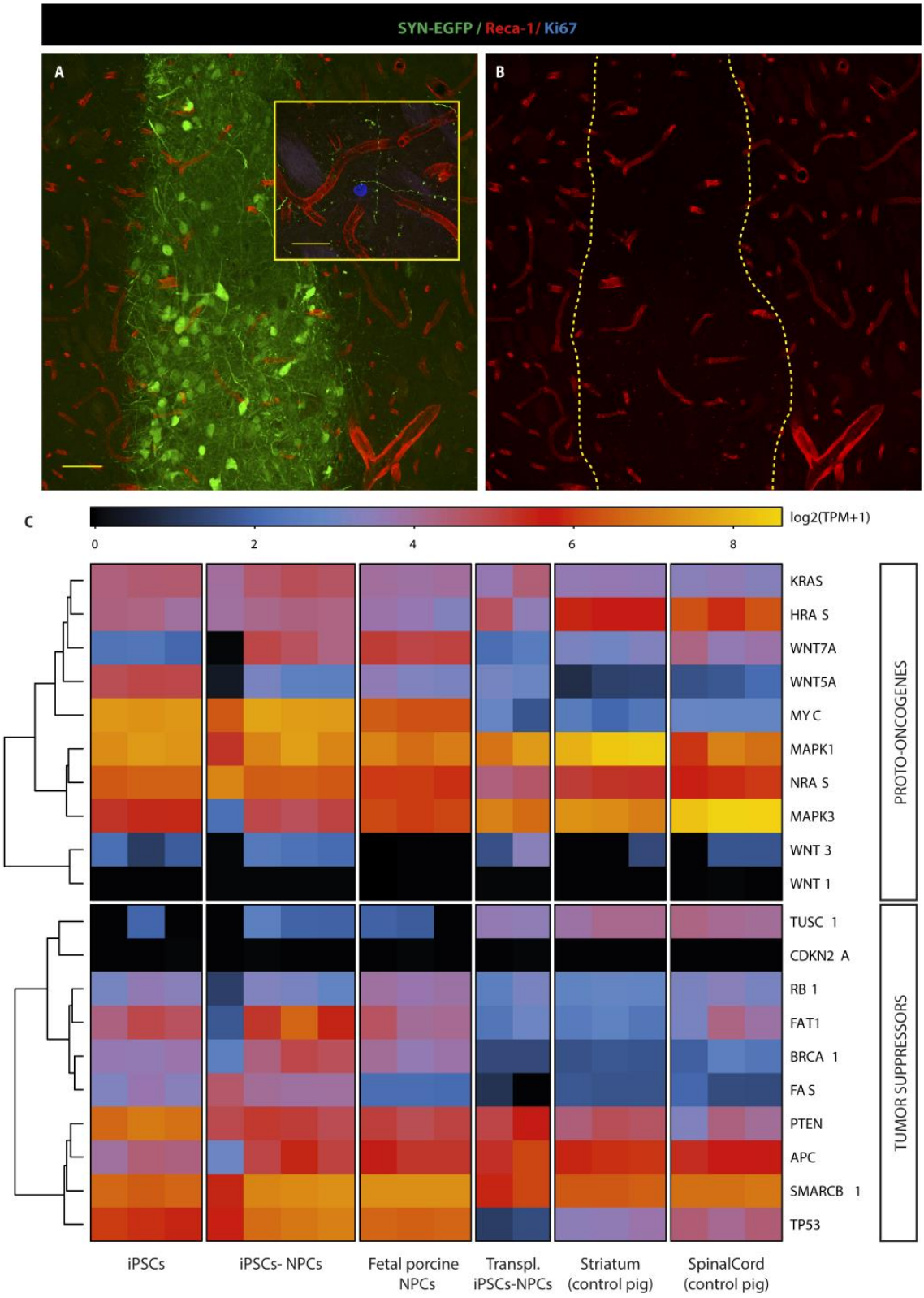
SYN-EGFP / Reca-1/ Ki67
A
B
C
log2(TPM+1)
0
2
4
6
8
KRAS
HRA S
WNT7A
WNT5A
MY C
MAPK1
NRA S
MAPK3
WNT 3
WNT 1
PROTO-ONCOGENES
TUSC 1
CDKN2 A
RB 1
FAT1
BRCA 1
FA S
PTEN
APC
SMARCB 1
TP53
TUMOR SUPPRESSORS
iPSCs
iPSCs- NPCs
Fetal porcine NPCs
Transpl. iPSCs-NPCs
Striatum (control pig)
SpinalCord (control pig)

fig. S 5 A-C

figure S 5. Porcine iPSCs-NPCs–EGFP+ grafts in rat striata show normal vascularization and no changes in tumor suppressors or proto-oncogenes at 7-10 months post-grafting. (**A, B**) Triple staining with GFP, Reca-1 and Ki67 antibody of coronal brain sections taken from rat striata and containing EGFP+ graft at 7 months after grafting. **(C)** Heat map generated from mRNA sequencing illustrating relative gene expression levels of a subset of 10 proto-oncogenes and tumor suppressors genes. (**Scale Bars:** A-100 µm).

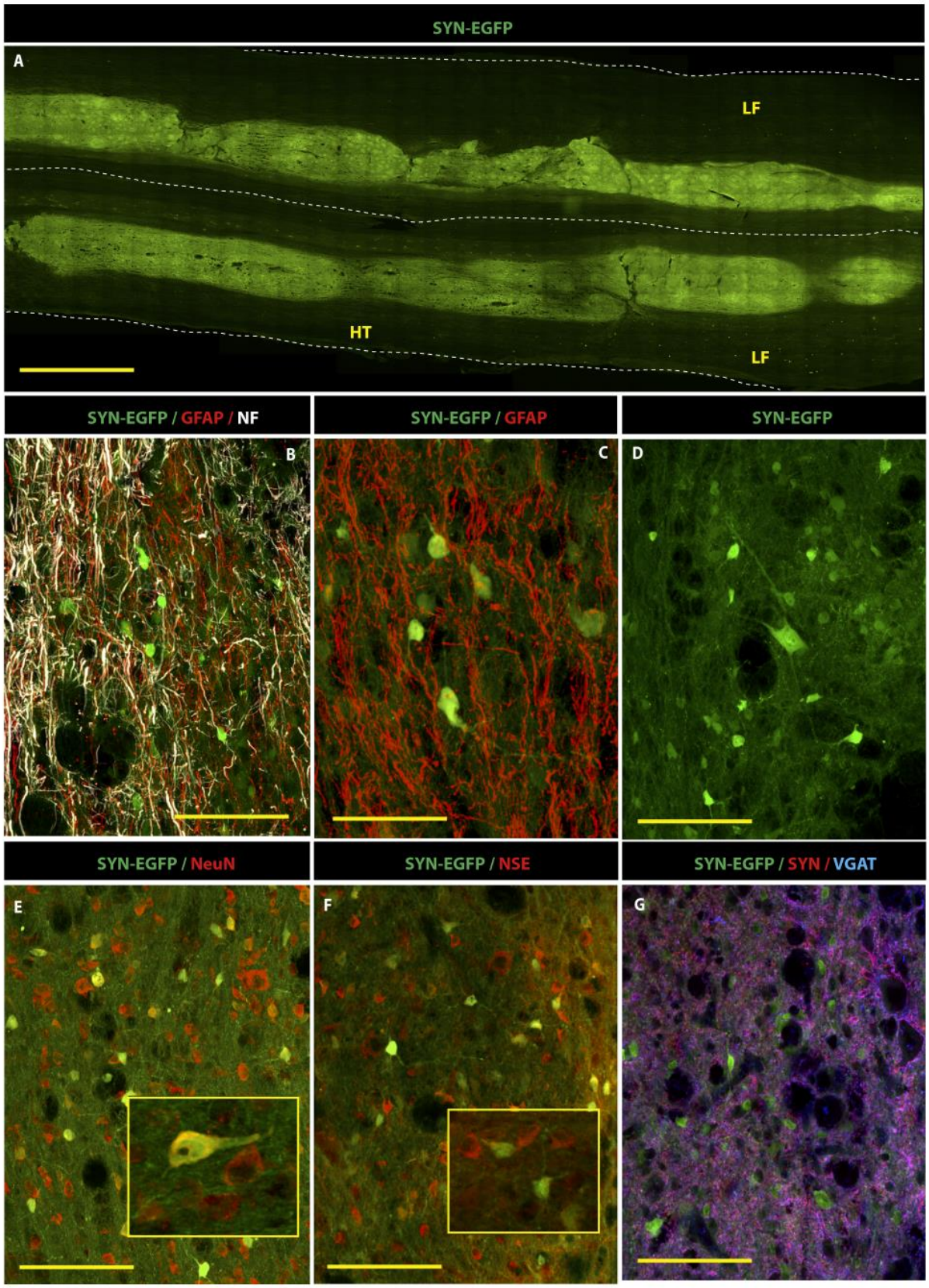
SYN-EGFP
A
LF
HT
LF
SYN-EGFP / GFAP / NF
B
SYN-EGFP / GFAP
C
SYN-EGFP
D
SYN-EGFP / NeuN
E
SYN-EGFP / NSE
F
SYN-EGFP / SYN / VGAT
G

fig. S 6 A-G

figure S 6. iPSCs-NPCs grafted into syngeneic pig spinal cord in the absence of immunosuppression show long-term survival and neuronal and glial differentiation at 3 months after transplantation. (A) Extensive bilaterally-localized SYN-EGFP+ grafts in horizontally cut spinal cord. **(B, C)** A high density of GFAP and neurofilament (NF)+ processes in SYN-EGFP+ grafts. **(D-F)** SYN-EGFP+ grafts show the presence of double-stained EGFP+/NeuN+ and EGFP+/NSE+ neurons. **(G)** High density of double SYN/VGAT-stained puncta in SYN-EGFP+ grafts. (**LF**-lateral funiculus, **HT**-host tissue, **Scale Bars:** A-2500 µm; B, D, E, F, G-125 µm; C-60 µm).

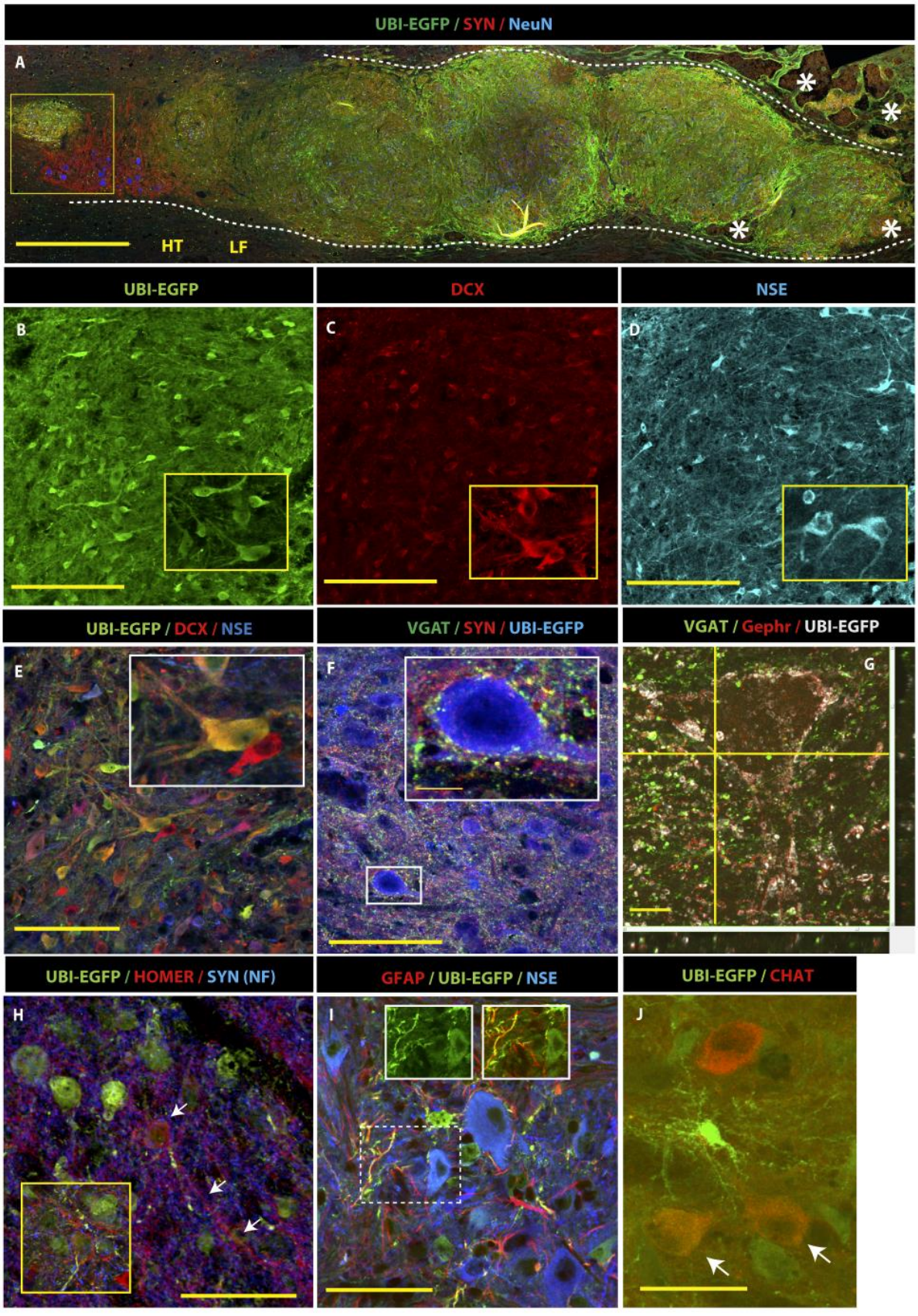
UBI-EGFP / SYN / NeuN
A
HT
LF
UBI-EGFP
DCX
NSE
B
C
D
UBI-EGFP / DCX / NSE
VGAT / SYN / UBI-EGFP
VGAT / Gephr / UBI-EGFP
E
F
G
UBI-EGFP / HOMER / SYN (NF)
GFAP / UBI-EGFP / NSE
UBI-EGFP / CHAT
H
I
J

fig. S 7 A-J

figure S 7. Porcine iPSCs-NPCs grafted spinally in allogeneic, transiently-immunosuppressed (1 month immunosuppression) pigs with previous spinal traumatic injury show neuronal and glial differentiation at 3.5 months after grafting. (A) Extensive UBI-EGFP+ graft in areas of previous traumatic injury (white dotted area). **(B-E)** Double staining with DCX and NSE antibody showed the presence of a mixed population of grafted EGFP+ neurons expressing DCX and/or NSE. **(F)** High density of double SYN/VGAT-stained puncta in UBI-EGFP+ grafts. (**G**) Staining with GFP/VGAT and postsynaptic inhibitory marker gephyrin (Gephr) antibody show double-stained EGFP/VGAT+ terminals in opposition to membrane-bound Gephr puncta on EGFP-negative neuron. (**H**) A high density Homer + puncta on neuronal soma or axons of grafted EGFP+ neuron (white arrows). **(I)** Numerous EGFP+/GFAP+ astrocyte processes in the close vicinity of grafted EGFP+/NSE+ neurons. **(J)** Some EGFP+ grafted neurons showed CHAT expression (white arrows). (**LF**-lateral funiculus, **HT**-host tissue, **Scale Bars:** A-2000 µm; B, C, D, E-100 µm; F-60 µm; G-10 µm; H-20 µm; I-60 µm; J-40 µm).

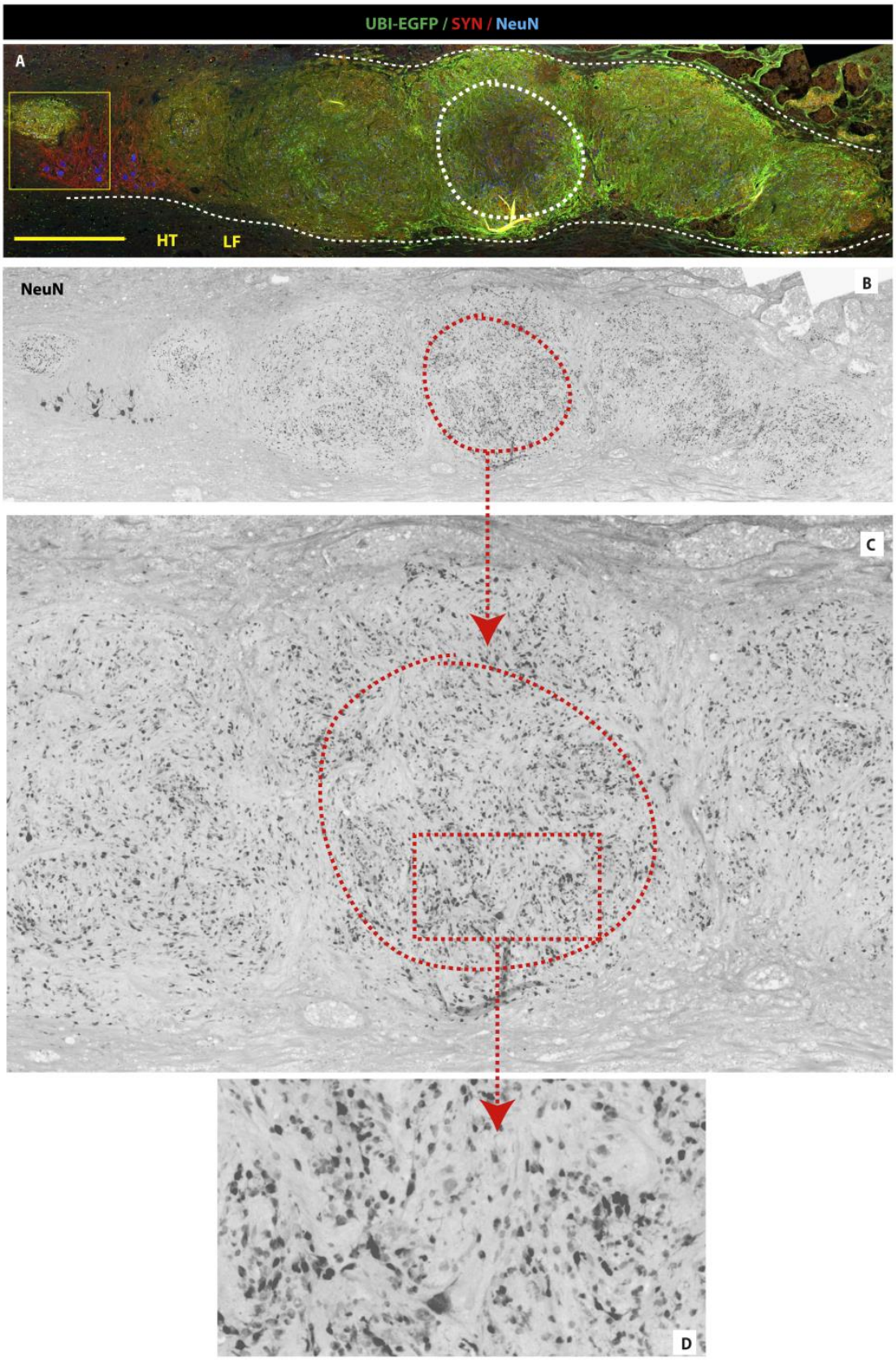

fig. S 8 A-D

figure S 8. Spinally-grafted iPSCs-NPCs in allogeneic, spinally-injured pig with transient immunosuppression (1 month) show extensive neuronal (NeuN) differentiation at 3.5 months after grafting. (**A-D**) A high density of NeuN+ neurons in the whole grafted region. (**Scale Bar:** A-2000 µm).

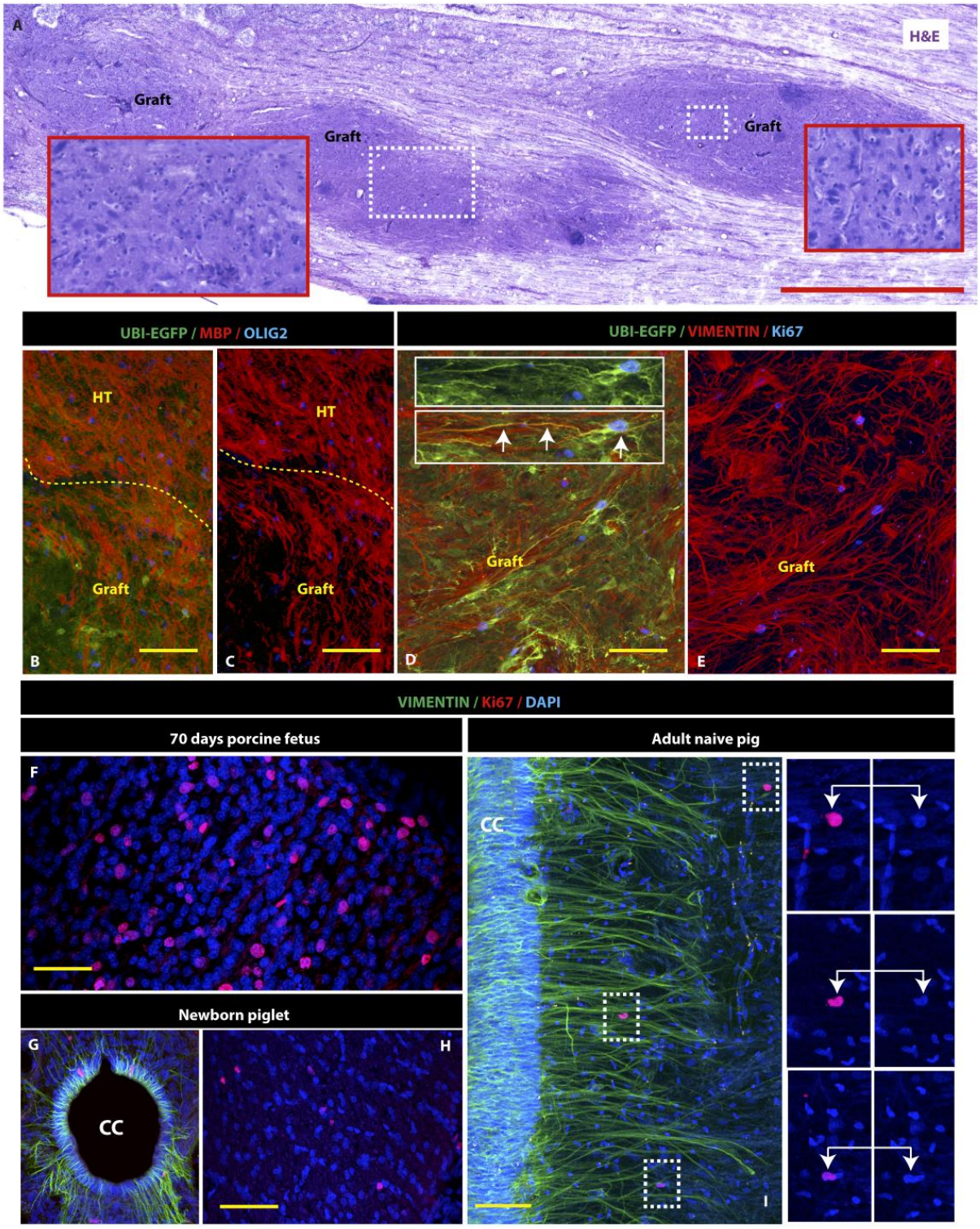
A
H&E
Graft
Graft
Graft
UBI-EGFP / MBP / OLIG2
HT
Graft
B
HT
Graft
C
UBI-EGFP / VIMENTIN / Ki67
Graft
D
Graft
E
VIMENTIN / Ki67 / DAPI
70 days porcine fetus
F
Adult naive pig
CC
I
Newborn piglet
G
CC
H

fig.9 S A-I

figure S 9. Porcine iPSCs-NPCs grafted spinally in allogeneic pig with previous spinal injury don't form tumors and show incomplete myelination at 3.5 months postgrafting. (**A**) H&E staining of horizontal spinal cord section show three well incorporated NPCs grafts with no signs of tumor. (**B-E**) Staining with MBP, OLIG2, VIMENTIN and Ki67 antibody in areas occupied by EGFP+ graft. (**F-I**) Ki67+ cells in spinal cord of 70-day-old porcine fetus, newborn piglet and adult (18 months) pig. (HT-host tissue; CC-central canal; **Scale Bars:** A-1000 µm; B, C-40 µm; D, E-100 µm; F-50 µm; H-100 µm; I-150 µm).

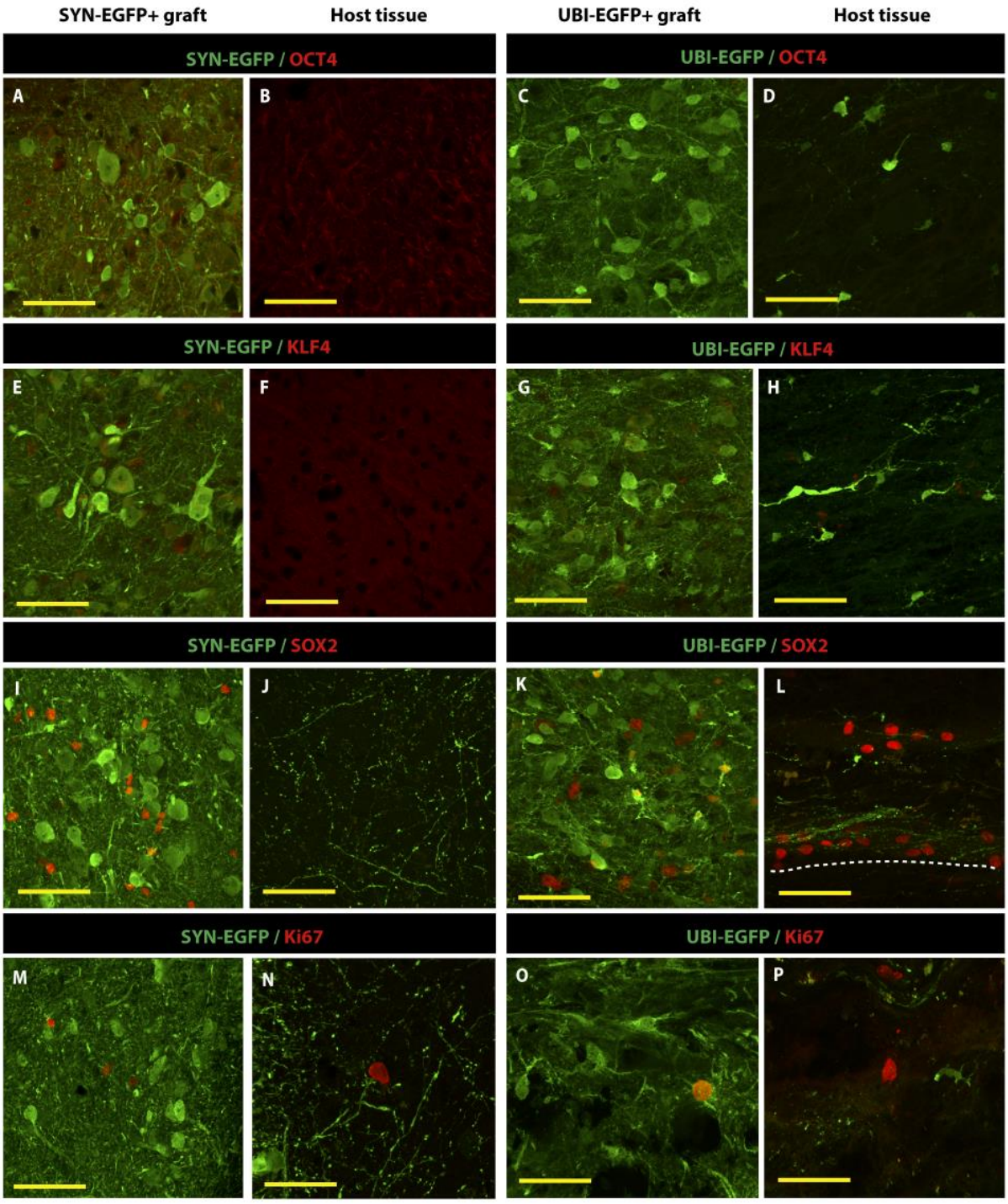
iPSCs-NPCs-SYN-EGFP-grafted rat striatum
(7 months postgrafting)
iPSCs-NPCs-UBI-EGFP-grafted allogeneic
injured spinal cord (3.5 months postgrafting)
SYN-EGFP+ graft
Host tissue
UBI-EGFP+ graft
Host tissue
SYN-EGFP / OCT4
UBI-EGFP / OCT4
A
B
C
D
SYN-EGFP / KLF4
UBI-EGFP / KLF4
E
F
G
H
SYN-EGFP / SOX2
UBI-EGFP / SOX2
I
J
K
L
SYN-EGFP / Ki67
UBI-EGFP / Ki67
M
N
O
P

fig. S 10 A-P

figure S 10. Reprogramming factors (*OCT4, KLF4*) are silenced in mature iPSCs-NPCs grafts in rat striata or spinal cord in allogeneic pig with previous spinal traumatic injury. (A-L) OCT4, KLF4 and SOX2 protein staining of rat striatal and pig allogeneic spinal cord sections at 7 or 3.5 months after iPSCs-NPCs-EGFP grafting. **(M-P)** Ki67 staining in SYN-EGFP or UBI-EGFP+ grafts. (L; white dotted line-the ependymal cells of the central canal of the host; **Scale Bars:** A-M-60 µm; N, O, P-90 µm).

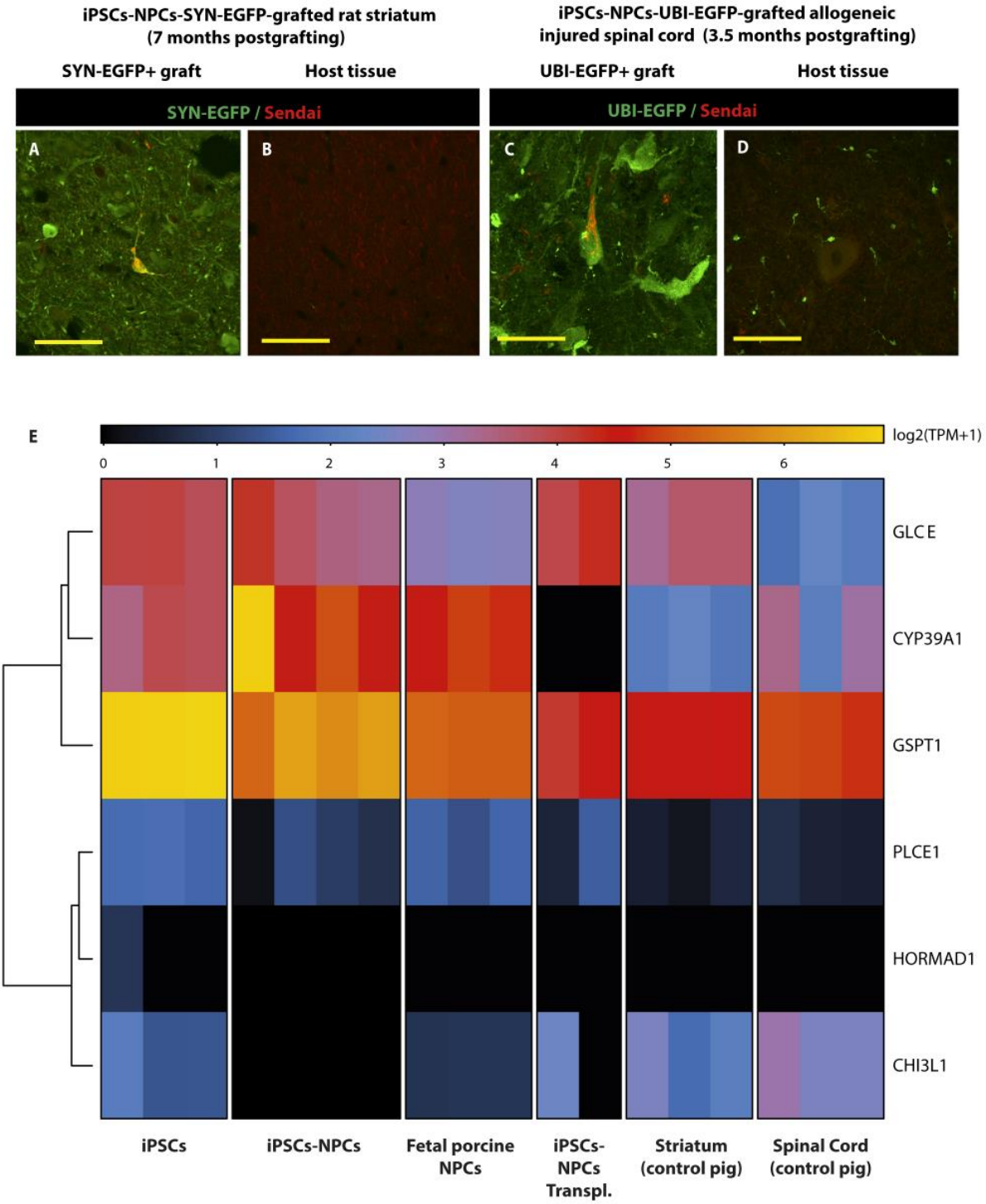
iPSCs-NPCs-SYN-EGFP-grafted rat striatum
(7 months postgrafting)
SYN-EGFP+ graft
Host tissue
SYN-EGFP / Sendai
A
B
iPSCs-NPCs-UBI-EGFP-grafted allogeneic
injured spinal cord (3.5 months postgrafting)
UBI-EGFP+ graft
Host tissue
UBI-EGFP / Sendai
C
D
E
log2(TPM+1)
0
1
2
3
4
5
6
GLCE
CYP39A1
GSPT1
PLCE1
HORMAD1
CHI3L1
iPSCs
iPSCs-NPCs
Fetal porcine NPCs
iPSCs-NPCs Transpl.
Striatum (control pig)
Spinal Cord (control pig)

fig. S 11 A-E

figure S 11. Long-term grafted iPSCs-NPCs in rat striata or spinal cord of allogeneic pig show only occasional presence of Sendai virus-associated protein in grafted cells and show no change in expression of immunogenic genes. (A-D) Staining of rat striatal and pig allogeneic spinal cord sections at 7 or 3.5 months after iPSCs-NPCs-EGFP grafting with anti Sendai-associated protein antibody (Sendai). (**E**) Heat map generated from mRNA sequencing illustrating relative gene expression levels of a subset of 6 immunogenic genes. (**Scale Bars:** A, B-70 µm; C-40 µm; D-120 µm).

Table S 1. Quantitative analysis of neuronal and glial differentiation in EGFP grafts.

	Postgrafting survival	**EGFP/NeuN**	**EGFP/NSE**	**EGFP/DCX**	**EGFP/GFAP**	**EGFP/Olig2**
Rat striatum	7 months	88.9 ± 1.2	89.8 ± 2.2	5.6 ± 0.3	ND	ND
Syngeneic Pig-Spinal cord	3 months	82.0 ± 1.3	55.3 ± 2.8	31.8 ± 1.0	ND	ND
Allogeneic Pig-Spinal cord	3.5 months	77.7 ± 4.2	82.7 ± 6.6	12.1 ± 1.4	14.9 ± 2.5	12.2 ± 5.3

Data are expressed as % of double-stained EGFP/DCX, EGFP/hNSE, EGFP/NeuN, EGFP/GFAP, and EGFP/Olig2 relative to total number of EGFP+ cells.

Table S 2. Electrophysiological properties of three transplanted iPSCs-NPCs-EGFP neurons into the striatum of a rat at 8 months post-grafting.

	mean	**SEM**
Voltage-gated sodium current peak (pA)	-5745	95
Evoked action potential firing rate above -10mV (Hz)	18	1.3
Action potential peak (mV)	35	1.8
Action potentail amplitude (mV)	114	2.3
Action potential threshold (mV)	-54	1.4
Resting membrane potential, measure at 0pA (mV)	-78	0.8

Table S 3. mRNA sequencing species sorting quantification.

CONTROL SAMPLES

									Rat Control			Pig Control		
Sample ID	RN Sorted	SUS Sorted	Ambiguous	Aligned	Unaligned	Pct. Rat	Pct. Pig	Pct. Ambig.	Precision	Recall	FDR	Precision	Recall	FDR
Nude_left_striatum2_S1_L001_R1_001	2,850,964	7,844	37,458	2,896,266	103,734	98.44%	0.27%	1.29%	0.9973	0.9844	0.0027			
Nude_left_striatum3_S2_L001_R1_001	2,809,267	12,512	91,895	2,913,674	86,326	96.42%	0.43%	3.15%	0.9956	0.9642	0.0044			
Nude_right_striatum1_S3_L001_R1_001	2,731,581	11,895	160,654	2,904,130	95,870	94.06%	0.41%	5.53%	0.9957	0.9406	0.0043			
Nude_right_striatum2_S4_L001_R1_001	2,832,853	10,153	36,175	2,879,181	120,819	98.39%	0.35%	1.26%	0.9964	0.9839	0.0036			
Nude_right_striatum3_S5_L001_R1_001	2,754,475	11,539	148,389	2,914,403	85,597	94.51%	0.40%	5.09%	0.9958	0.9451	0.0042			
SeNPC_1_S14_L001_R1_001	12,393	2,635,195	79,730	2,727,318	272,682	0.45%	96.62%	2.92%				0.9953	0.9662	0.0047
SeNPC_2_S15_L001_R1_001	11,509	2,655,950	47,491	2,714,950	285,050	0.42%	97.83%	1.75%				0.9957	0.9783	0.0043
SeNPC_4_S16_L001_R1_001	12,545	2,664,193	55,569	2,732,307	267,693	0.46%	97.51%	2.03%				0.9953	0.9751	0.0047
SeNPC_5_S33_L002_R1_001	14,145	2,679,793	62,902	2,756,840	243,160	0.51%	97.21%	2.28%				0.9947	0.9721	0.0053
								Mean:	0.9961	0.9636	0.0039	0.9953	0.9729	0.0047

iPSCs-NPCs IMPLANTED RAT STRIATA SAMPLES

Sample ID	RN Sorted	SUS Sorted	Ambiguous	Aligned	Unaligned	Pct. Rat	Pct. Pig	Pct. Ambig.
Nude_left_striatum_implanted_S6_L001_R1_001	2,719,723	139,384	38,213	2,897,320	102,680	93.87%	4.81%	1.32%
Nude_right_striatum_implanted_S7_L001_R1_001	2,689,896	160,722	39,869	2,890,487	109,513	93.06%	5.56%	1.38%
					Mean:	93.47%	5.19%	1.35%

Table S 4A. SLA haplotypes of donor iPSCs-NPCs and allogeneic graft recipients.

iPSCs-NPCs allogeneic graft recipients	**Low-resolution (Lr) SLA haplotype**
N162	Lr-39.23 / 85.26
P41	Lr-2.2 / 10.11
P44	Lr-10.11 / 85.26
Donor iPS-NPCs	Lr-4.4 / 4.4

Table S 4B. Expected high-resolution (Hp) SLA typing and haplotype**.**

	SLA Haplotype				
	Donor	**Recipient**			
Locus	**Hp-4.4**	**Hp-2.2**	**Hp-10.11**	**Hp-39.23**	**Hp-85.26**
SLA-1	04:01	02:01, 07:01	05:01	Blank	07:05
SLA-3	04:01	Null	08:01	05:XX*	07:XX*
SLA-2	04:01 / 04:02	02:01	03:02	10:XX*	16:XX*
DRA	01:01	01:01	02:02	02:01	NT
DRB1	02:01	02:01	09:01	10:01	11:02 / 11:07 / 11:08
DQA	02:02	02:01	03:XX*	01:01	02:XX*
DQB1	04:01	02:01	04:02	06:01	04:01 / 04:03

* High-resolution typing not available

Blank: Allele not identified by assays used

NT: Not typed

Null: Gene not expressed

Table S 5. Antibodies used for Flow cytometry, and immunofluorescence staining.

Antibodies used for Flow cytometry		
Catalog #	***Name***	***Company***
560341	NESTIN - Alexa Fluor® 647	BD Biosciences
561549	SOX1 - PerCP-Cy™5.5	BD Biosciences
562388	PAX6 - PerCP-Cy™5.5	BD Biosciences
560302	SOX2 - Alexa Fluor® 647	BD Biosciences

Primary Antibodies used for Indirect immunofluorescence and Histology		
Catalog #	***Name***	***Company***
4903	NANONG (D73G4) XP®	Cell Signaling Technology
4038	KLF4	Cell Signaling Technology
3579	SOX2 (D6D9) XP®	Cell Signaling Technology
2840	OCT4A (C30A3)	Cell Signaling Technology
5605	c-MYC (D84C12)	Cell Signaling Technology
PD029	Anti-Sendai Virus	MBL International Corp
ABN78	NeuN	EMD Millipore
MAB377	NeuN	EMD Millipore
AB114P	CHAT	Millipore AB114P
A-2052	GABA	Sigma-Aldrich
VP-S285	Synaptophysin	Vector
131002	VGAT	Synaptic Systems
AB951	Neuron Specific Enolase	Chemicon
sc-8066	Doublecortin (DCX)	Santa Cruz Biotechnology
C9205	GFAP (Cy3-labeled)	Sigma-Aldrich
MAB2018	Sox2	R&D Systems

M0762	Neurofilament	Dako Corporation
AB9610	Olig2	EMD Millipore
SF-1099	PAX6 Hu-Cy5 SmartFlare Probe 1	EMD Millipore
MMS-435P	TUJ	Covance
147011	Gephyrin	Synaptic Systems
019-19741	Iba-1	Wako
AV5733	Vimentin	Millipore
MCA1222GA	CD45	Bio-Rad, ABD Serotec
MCA1222F	CD45	Bio-Rad, ABD Serotec
MCA48R	CD8	Bio-Rad, ABD Serotec
Ab5864	MBP	Millipore

Table S 5-Continuation. Antibodies used for Flow cytometry, and immunofluorescence staining.

Primary Antibodies used for Indirect immunofluorescence and Histology		
Catalog #	***Name***	***Company***
6667	Ki67	Abcam
160003(SY)	Homer-1	Synaptic Systems
Ab5320	NG2	Millipore
Ab9774	Reca-1	Abcam
ab34000	MHC-II	Abcam
S193	Synapsin	Sigma
AF1924	SOX17	R&D Systems
AM31985PU-S	AFP	Acris
MAB20851	Brachyury	R&D Systems
ab21027	SMA (anti-alpha smooth muscle Actin)	Abcam
AF1979	OTX2	R&D Systems
TUJ	Tubulin β-3	Aves Labs

Fluorescent-conjugated secondary donkey antibodies		
Dilution	***Name***	***Company***
1:500	Alexa® Fluor 488	Jackson Immuno Research
1:500	Alexa® Fluor 647	Jackson Immuno Research
1:500	Alexa® Fluor 555	Thermo Fisher Scientific