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Supplemental Information

Reprogramming Captures the Genetic and Tumorigenic Properties of Neurofibromatosis Type 1 Plexiform Neurofibromas

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FIGURE S1

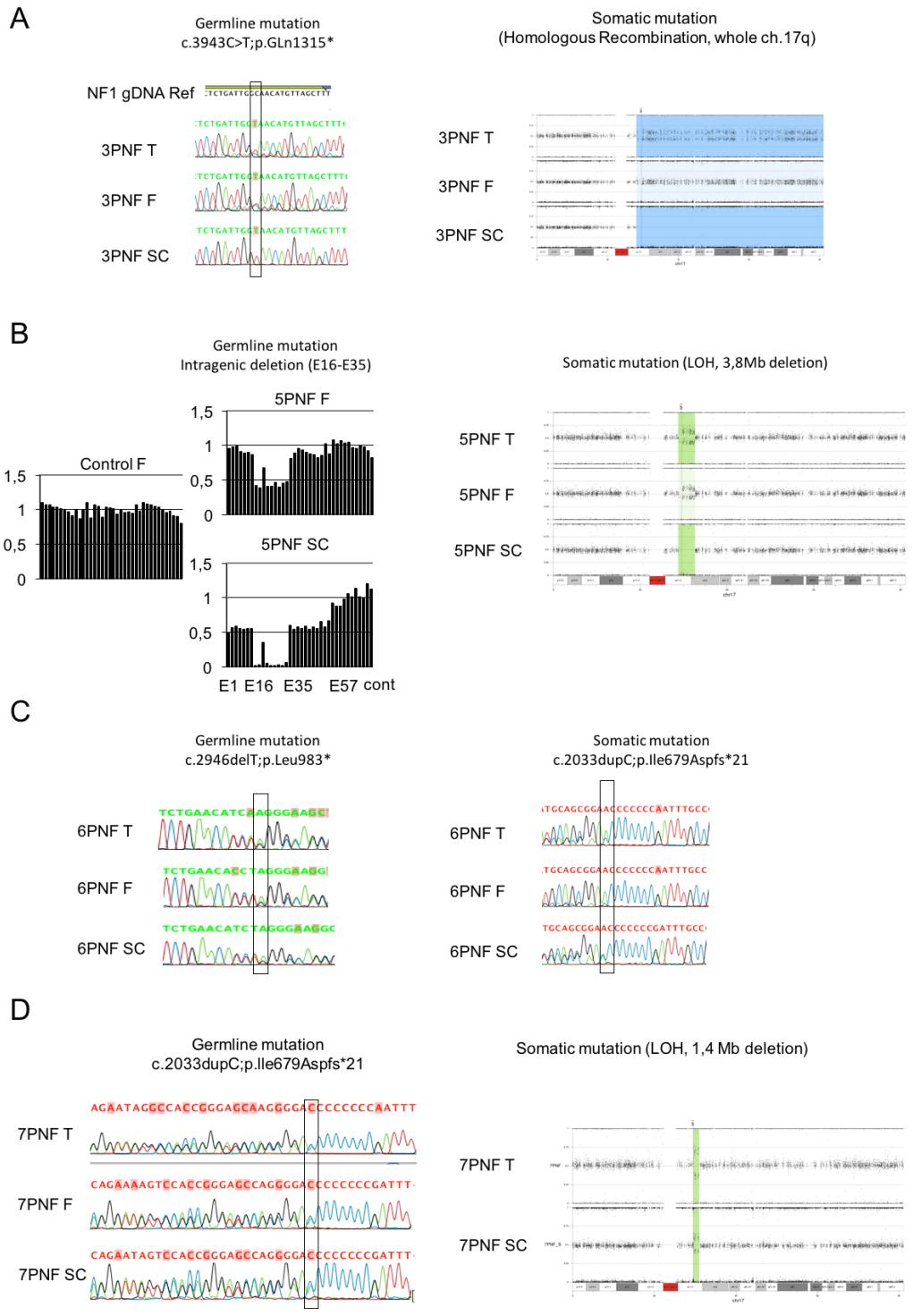
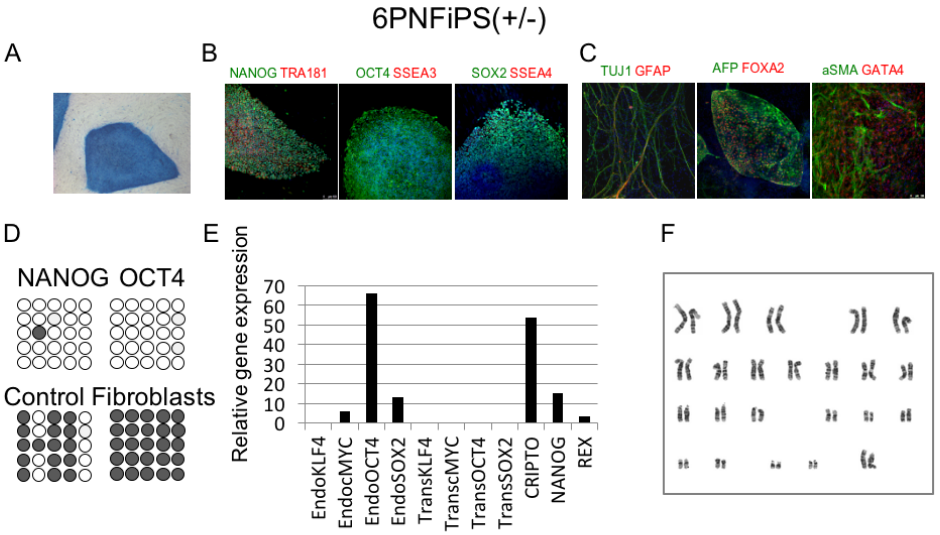
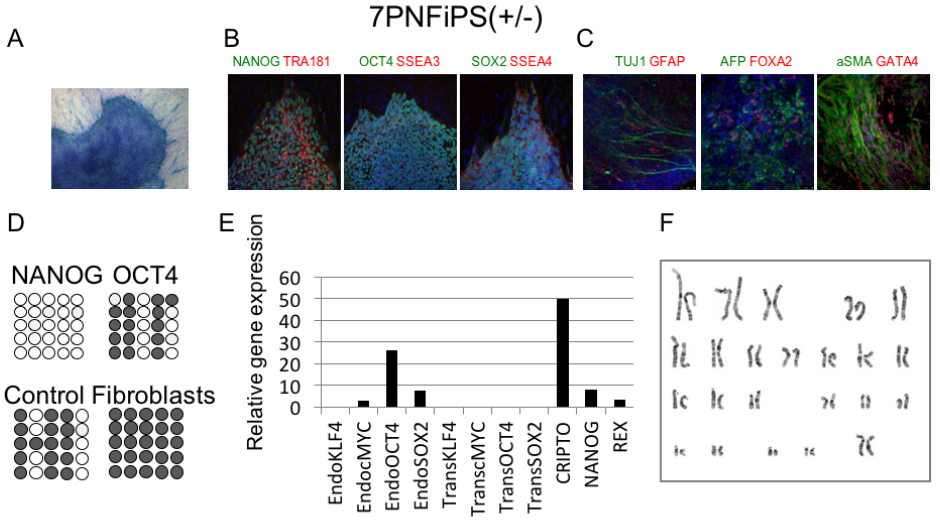

A
Germline mutation
c.3943C>T;p.GLn1315*
NF1 gDNA Ref
3PNF T
3PNF F
3PNF SC
Somatic mutation
(Homologous Recombination, whole ch.17q)
3PNF T
3PNF F
3PNF SC
B
Germline mutation
Intragenic deletion (E16-E35)
Control F
5PNF F
5PNF SC
1,5
1
0,5
0
E1 E16 E35 E57 cont
Somatic mutation (LOH, 3,8Mb deletion)
5PNF T
5PNF F
5PNF SC
C
Germline mutation
c.2946delT;p.Leu983*
6PNF T
6PNF F
6PNF SC
Somatic mutation
c.2033dupC;p.Ile679Aspfs*21
6PNF T
6PNF F
6PNF SC
D
Germline mutation
c.2033dupC;p.Ile679Aspfs*21
7PNF T
7PNF F
7PNF SC
Somatic mutation (LOH, 1,4 Mb deletion)
7PNF T
7PNF SC

Figure S1. *NF1* germline and somatic mutation analysis in the four PNFs used to generate the banked iPSC lines.

A) 3PNF *NF1* mutational analysis. Left: Sanger sequencing showing the germline mutation c.3943C>T;p.GLn1315* in the *NF1* gene. The germline mutation is present in the tumor (3PNF T), in tumor fibroblasts (3PNF F) and in tumor SC (3PNF SC). Right: B-allele frequency (BAF) data (a detailed view from chromosome 17) from SNP-array analysis showing the somatic mutation of 3PNF. Somatic *NF1* inactivation is produced by mitotic recombination generating CN-LOH in 17q and the reduction to homozygosity for the constitutional *NF1* mutation. LOH is observed in 3PNF and in 100% of cells in 3PNF SC. Fibroblast culture (3PNF F) is an early passage and still exhibit a residual LOH due to the presence of tumor SCs.
B) 5PNF *NF1* mutational analysis. Left: MLPA analysis showing an intragenic deletion in the *NF1* gene, from exon 16 (E16) to exon 57 (E57). The deletion is detected in tumor fibroblasts (5PNF F) and in tumor SC (5PNF SC). Right: detailed view of BAF for chromosome 17. Somatic *NF1* inactivation is produced by a deletion generating CN-LOH in 17q and the reduction to homozygosity for the constitutional *NF1* mutation. LOH is observed in 5PNF and in 100% of cells in 5PNF SC.
C) 6PNF *NF1* mutational analysis. Left: Sanger sequencing showing the germline mutation c.2946delT;p.Leu983* present in the tumor (6PNF T), tumor fibroblasts (6PNF F) and tumor SCs (6PNF SC). Right: sanger sequencing showing the somatic mutation c.2033dupC;p.ile679Aspf*21 only present 6PNF SC and not in 6PNF F.
D) 7PNF *NF1* mutational analysis. Left: Sanger sequencing showing the germline mutation c.2033dupC;p.ile679Aspf*21 present in the tumor (7PNF T), tumor fibroblasts (7PNF F) and tumor SC (7 PNF SC). Right: detailed view of BAF for chromosome 17. Somatic *NF1* inactivation is produced by a deletion generating CN-LOH in 17q. LOH is observed in 7PNF and in 7PNF SC.

FIGURE S2





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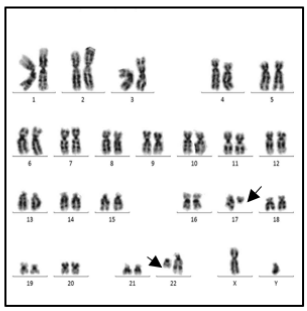
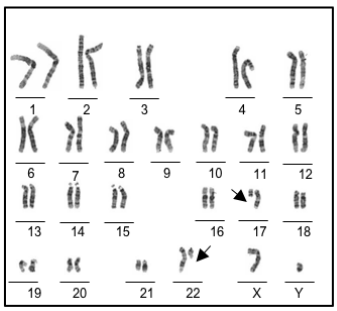


5PNF SC passage 3
46,XY,t(17;22)(q10;q13.3)



5PNFiPS (NF1-/-) passage 20
46,XY,t(17;22)(q10;q13.3)

Figure S2. iPSC Characterization of the additional banked NF1 iPSCs lines.

A) Morphology and alkaline phosphatase staining of a representative colony. Scale bar: 100µm
B) Pluripotency markers. Scale bar: 100µm
C) *In vitro* differentiation potential. Scale bar: 100µm
D) Bisulphite sequencing showing demethylation of the *NANOG and POU5F1(*OCT4*)* promoters.
E) RT-qPCR analysis characterizing the expression levels of reprogramming genes either endogenous. (iPSC) or retroviral-derived (transgenes). Expression of pluripotency markers (CRIPTO, NANOG, REX) are also shown.
F) karyotypes at passage 20.
G) Karyotype of the 5PNF-derived SCs (5PNF SC) showing the presence of the same translocation t(17;22) as in the 5PNFiPS N(-/-) cell line that causes the somatic *NF1* mutation.

FIGURE S3

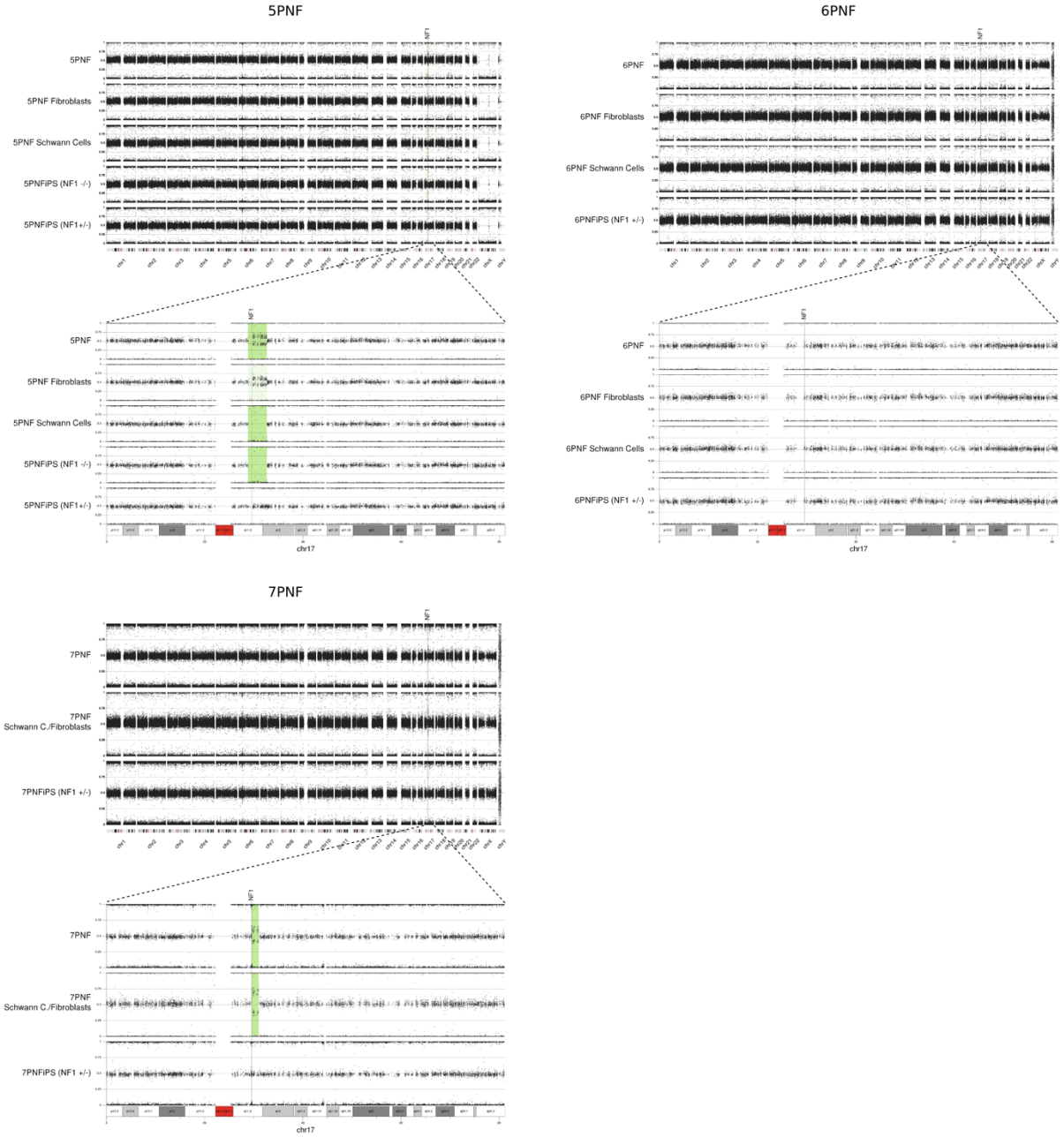


Figure S3. Genomic characterization of tumors, tumor isolated cells and corresponding iPSC lines.

B-allele frequency (BAF) data along the genome is plotted for all samples associated to each PNF. Green shaded regions denote somatic LOH due to genomic loss. Fibroblast cultures from tumor 5PNF are early passages in which LOH can still be detected due to the presence of *NF1*(-/-) Schwann cells. 7PNF Schwann cells/Fibroblasts is a heterogeneous cell culture (60% SC and 40% fibroblasts). The position of *NF1* is marked with a vertical black line.

FIGURE S4

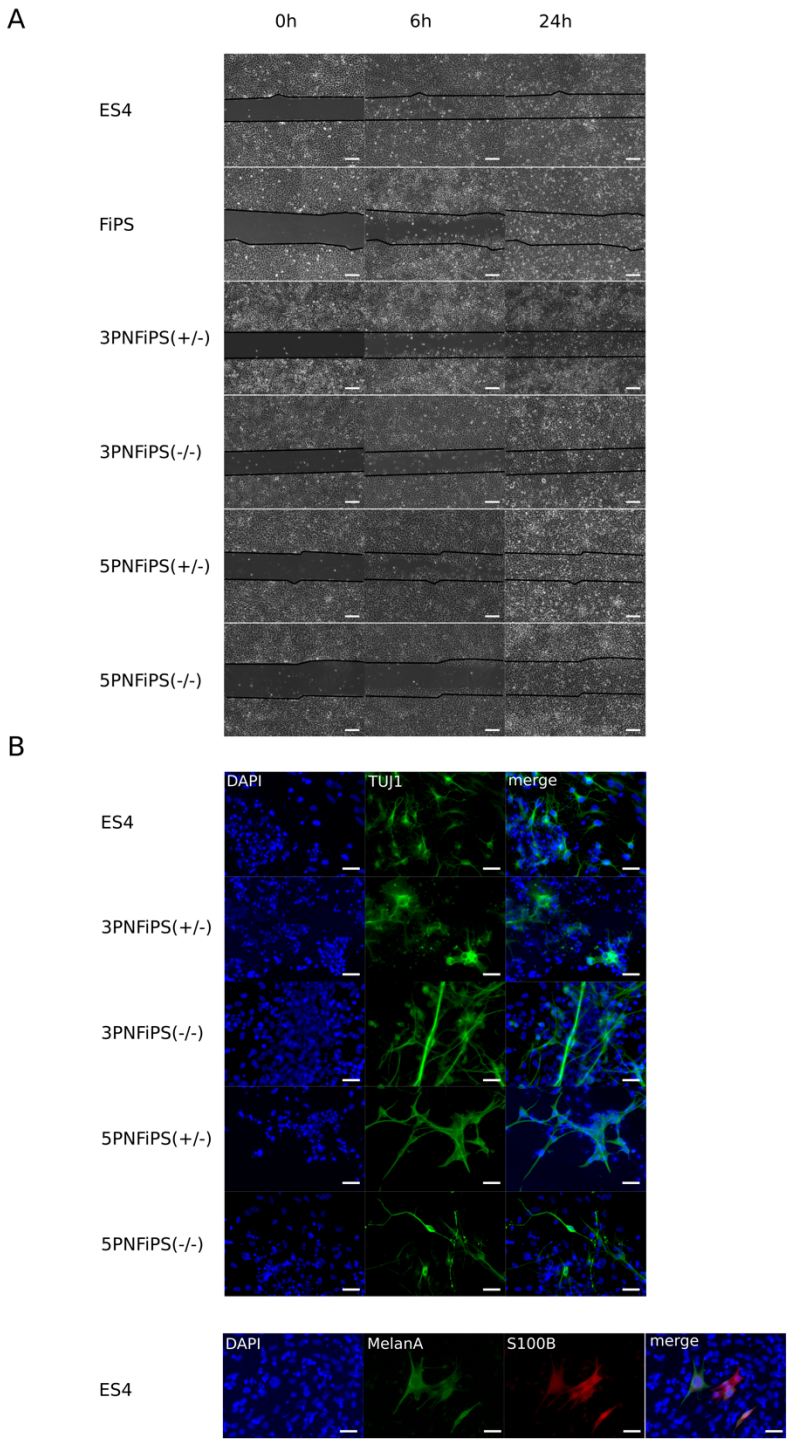


Figure S4. iPSC-derived NCSC lines have the capacity to migrate and differentiate into different NC-derivatives.

A) Scratch assay. A cell free-free gap was created using a pipette tip and migration capacity was measured by taking images of the same region at 6 and 24 hours after gap creation. Scale bar= 150µM. **B)** Differentiation capacity of generated NC towards peripheral neurons (Tuj1+) and melanocytes (MelanA+ and S100B+). Scale bar= 50µM.

FIGURE S5

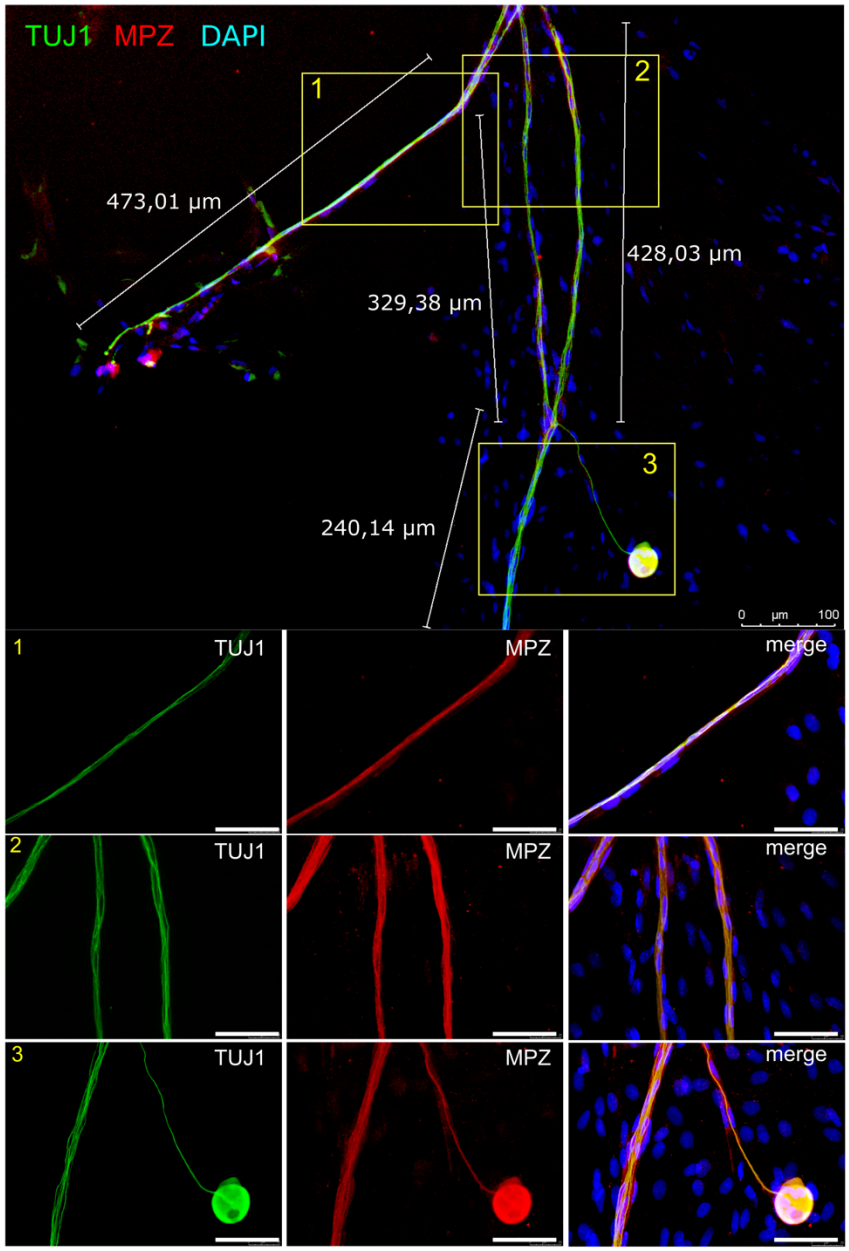


FIGURE S5. Myelin quantification of FiPS-differentiated SC.

The myelination capacity of FiPS-differentiated SCs was assessed by co-culturing cells at 7 days of differentiation with rat DRG neurons for 30 days. SC specification and myelination was measured by immunostaining for TUJ1 (green) andMPZ (red). The length of myelinated axons was measured using LEICA LASAF software and are marked by a white line. Scale bar: 50µm.

TABLE S1. Reprogramming information

TUMOR INFORMATION	REPROGRAMMING INFORMATION						
Tumor ID	Method	Reprogrammed cell	Num. of clones	NF1 mutation			iPSC LINE BANKING NAME
				Germline	Somatic		
					yes	No	
3PNF	Rv	PNF skin fibroblasts*	7	7	7*		
		PNF Schwann cells	10	10	10		
	Sv	PNF skin fibroblasts*	9	9		2	3PNFiPS(+/-) 3PNF_FiPSsv_PM
					7*		
		PNF Schwann cells	12	12	12		3PNFiPS(-/-) 3PNF_SiPSsv_MM
5PNF	Rv	PNF endoneurial fibroblasts	0				
		PNF Schwann cells	2	2	2		5PNFiPS(-/-)_Rv
	Sv	Digested PNF	12	12		1	5PNFiPS(+/-) 5PNF_TDiPSsv_PM
					11		5PNFiPS(-/-) 5PNF_TDiPSsv_MM
6PNF	Rv	PNF endoneurial fibroblasts	1	1	0	1	6PNFiPS(+/-)_Rv
		PNF Schwann cells	10	10	0	10	6PNFiPS(+/-) 6PNF_SiPSrv_PM
	Sv	Digested PNF	10	10	0	10	
7PNF	Rv	PNF endoneurial fibroblasts	1	1	0	1	
		Mix population T(40% PNF Schwann cell, 60% endoneurial fibroblasts)	12	12	0	12	7PNFiPS(+/-) 7PNF_TDiPSrv_PM
13PNF	Sv	PNF endoneurial fibroblasts	11	11	0	11	13PNFiPS(+/-)
		PNF Schwann cells	40	40	0	40	13PNFiPS(+/-)
		Digested PNF	27	27	0	27	

*The skin used was covering the PNF. When skin was separated, part of the tumor was still left.

TABLE S2. Sample authentication.

AmpFISTR Identifiler loci	3PNF	5PNF	6PNF	7PNF
CSF1PO	11,14	10	10,12	11,13
D2S1338	17,24	17,19	17,23	24
D3S1358	15,16	15,16	16,18	15,17
D5S818	10,11	12,13	11,13	10,12
D7S820	10	10,13	11	8,12
D8S1179	10,14	8,13	10,12	13,14
D13S317	8,13	8,12	11,14	8,13
D16S539	9,12	12,13	9,13	11,12
D18S51	13,15	15,16	11,13	12,15
D19S433	12,14.2	14,15	13,14	13,15
D21S11	30,32.2	29	29,30.2	29,30
FGA	21,24	21,23	21,27	20,25
THO1	8,9.3	9,3	7,9.3	6,9.3
TPOX	8	10,11	9,11	10,11

TABLE S3. List of somatic mutations.
The list includes all somatic mutations identified in each sample, meeting the following criteria: exonic or present in canonical splice sites, passing manual validation and excluding synonymous mutations. The list includes only non-*NF1* mutations.
See attached Supplemental_Table_S3.xls

TABLE S4. Primers for RT-qPCR.

Gene	Sequence(5′-3′)		UPL
POU5F1	*Forward*	cttcgcaagccctcatttc	60
	Reverse	gagaaggcgaaatccgaag	
POU3F1	*Forward*	ttctcaagtgccccaagc	78
	Reverse	ccggttgcagaaccagac	
NGFR	*Forward*	ccttccacgctgtctcca	60
	Reverse	cctaggcaagcatcccatc	
SOX10	*Forward*	gacacggttttccacttccta	25
	Reverse	gtcctcgcaaagagtccaac	
TFAP2A	*Forward*	ggtgaaccccaacgaagtc	73
	Reverse	accgtgaccttgtacttcgag	
S100B	*Forward*	ggaaggggtgagacaagga	73
	Reverse	ggtggaaaacgtcgatgag	
CDH19	*Forward*	tgtaccagaggaaatgaatacgac	78
	Reverse	catatatgtcacctgttctttcatca	
ITGA4	*Forward*	atgcaggatcggaaagaatc	78
	Reverse	ccacaaggttctccattaggg	
PLP1	*Forward*	cttcaacacctggaccacct	60
	Reverse	ccatgggagaacaccataca	
GAP43	*Forward*	gctccaagcctgatgagc	12
	Reverse	gctctgtggcagcatcac	
EGR2	*Forward*	gctgctacccagaaggcata	60
	Reverse	ggatgaggctgtggttgaa	
PMP22	*Forward*	ctgtcgatcatcttcagcattc	29
	Reverse	agcactcatcacgcacagac	
MPZ	*Forward*	ttcccatctcctgcatcc	55
	Reverse	ctgggccacctggtagag	
EndoKLF4	*Forward*	agcctaaatgatggtgcttggt	68
	Reverse	ttgaaaactttggcttccttgtt	
EndoMYC	*Forward*	cgggcgggcactttg	55
	Reverse	ggagagtcgcgtccttgct	
EndoOCT4	*Forward*	gggtttttgggattaagttcttca	63
	Reverse	gcccccacccttttgtgtt	
EndoSOX2	*Forward*	caaaaatggccatgcaggtt	63
	Reverse	agttgggatcgaacaaaagctatt	
TransKLF4	*Forward*	tggactacaaggacgacgatga	60
	Reverse	cgtcgctgacagccatga	
TransMYC	*Forward*	tggactacaaggacgacgatga	77
	Reverse	gttcctgttggtgaagctaacgt	
TransOCT4	*Forward*	tggactacaaggacgacgatga	58
	Reverse	caggtgtcccgccatga	
TransSOX2	*Forward*	gctcgaggttaacgaattcatgt	57
	Reverse	gcccggcggcttca	
CRIPTO	*Forward*	cggaactgtgagcacgatgt	66
	Reverse	gggcagccaggtgtcatg	
NANOG	*Forward*	acaactggccgaagaatagca	63
	Reverse	ggttcccagtcgggttcac	
REX	*Forward*	cctgcaggcggaaatagaac	61
	Reverse	gcacacatagccatcacataagg	

TABLE S5. Antibody list.

Antibody	Supplier	Reference	Dilution
Rabbit anti-NF1	Bethyl laboratories	A300-140A	1:1000 (WB)
Mouse IgG anti-OCT3/4	Santa Cruz Biotechnology	Sc-5279	1:60
Rabbit IgG anti-SOX2	Pierce Antibodies	PA1-16968	1:100
Goat IgG anti-NANOG	R&D Systems	AF1997	1:25
Rat IgM anti-SSEA3	Hybridoma Bank	MC-631	1:3
Mouse IgG anti-SSEA4	Hybridoma Bank	MC-813-70	1:3
Mouse IgM anti TRA-1-81	Millipore	MAB4381	1:200
Rabbit IgG anti-alpha-1-fetoprotein	Dako	A0008	1:400
Goat IgG anti-FOXA2	R&D Systems	AF2400	1:50
Rabbit IgG anti-GATA4	Santa Cruz Biotechnology	sc-9053	1:50
Mouse IgG anti SMA	Sigma	A5228	1:400
Mouse IgM anti-ASA	Sigma	A2172	1:400
Mouse IgG anti-TUJ1	Bio Legend	MMS-435P	1:500
Rabbit IgG anti GFAP	Dako	Z0334	1:500
Rabbit IgG anti NF200	Sigma	N4142	1:100
Mouse IgG anti-Nerve growth factor (p75) receptor (ME20.4)	Advanced targeting System	AB-N07	1:100 (IF) 1:1000 (FACS)
Rabbit IgG anti-S100B	Dako	Z0311	1:1000
Mouse IgG anti-AP2	Thermo Scientific	MA1-872	1:50
Rabbit IgG anti-Sox10	Abcam	ac108408	1:50
Mouse IgG anti-MelanA	Ventana	790-2990	1:100
Rabbit IgG anti-MPZ	Abcam	Ab31851	1:500
Rabbit IgG anti-PLP	Abcam	ab28486	1:100
Rabbit IgG anti-GAP43	Novus Biologicals	NB300-143SS	1:500
Mouse IgG anti-Ki67	Santa Cruz Biotechnology	sc-23900	1:50
Mouse IgG anti-HNK1	SIGMA	C6680	1:1000 (FACS)

Extended Methodology

iPSC characterization

Alkaline phosphatase activity was demonstrated using the Alkaline Phosphatase Blue Membrane Substrate Solution (Sigma). Briefly, iPSC were grown on top of mitotically inactivated human foreskin fibroblasts (HFF) during one week. Cells were fixed during 2 min in 3.7% paraformaldehyde and exposed to the substrate solution. After 20 min incubation in the dark, blue staining was evident in iPS colonies. Detection of pluripotency-associated markers (nuclear: OCT4, SOX2 and NANOG; cytoplasmic: SSEA3, SSEA4 and Tra-1-81) was performed on iPSC cultured on HFF for 8 days and fixed with 4% paraformaldehyde (PFA). Then, samples were processed for immunocytochemistry. *In vitro* differentiation ability to the three germ layers was carried out through embryoid body (EB) formation. For endoderm, EBs were plated on 0.1% gelatin (Millipore) coated coverslips and cultured 3 weeks in KODMEM (Gibco) supplemented with 20% fetal bovine serum (Hyclone), 1x penicillin/streptomycin (Gibco), 1x Glutamax (Gibco), 0.05 mM 2-mercaptoethanol (Gibco), non essential aminoacids (Lonza). For mesoderm induction the same medium was used as before mentioned with the addition of 0.5 mM L-ascorbic acid (Sigma). Ectoderm differentiation was done culturing the EBs in suspension in N2B27 medium (Neurobasal:DMEM:F12 50:50 v/v, 1x N2 supplement, 1x B27 supplement, 1x Glutamax) supplemented with b-FGF as described (Sánchez-Danés, 2012). After 10 days in culture, EBs were plated on Matrigel (Corning) coated coverslips and cultured for additional three weeks in N2B27 medium without b-FGF. Differentiated cells were fixed with 4% PFA. Immunocytochemistry was performed by standard methods as previously reported (Martí, 2013). Primary antibodies used are listed in table S5. Secondary antibodies were of the Alexa Fluor series from Jackson Immuno Research and used between 1:250 and 1:500 dilution. Cell nuclei were counterstained with 0.5 µg/ml DAPI (Invitrogen). Images were acquired with an SP5 Leica confocal microscope. For karyotyping, iPSC were cultured on matrigel in the absence of HFF and treated with colcemide (Gibco) at a final concentration of 0.1 µg/mL and processed as described (Campos, 2009).

In the case of retroviral reprogrammed cell lines qPCR was performed to confirm the silencing of the transgenes. Sendai virus reprogrammed iPSC lines were subjected to qualitative PCR to check that they were vector-free at passage 10. The genetic expression of endogenous pluripotency-associated genes (*OCT4*, *NANOG*, *CRIPTO* and *Rex1*) were confirmed by qPCR. Primers employed are listed in Table S4.

For DNA methylation analysis, genomic DNA was extracted from cell pellets using QIAamp DNA Mini Kit (Qiagen 51304). DNA methylation analysis was performed with Methylamp DNA Modification kit (Epigentek P-1001-1) according to manufacturer's specifications. Oct4 and Nanog promoters were amplified by PCR using primers previously described in Freberg et al (2007), amplified in DH5a cells, purified and sequenced. Severe combined immunodeficient (SCID) beige mice (Charles River Laboratories) were used to generate teratomas from two iPSC line, 5PNFiPS(+/-) and 5PNFiPS(-/-). Animal assays were conducted following experimental procedures previously approved by the Institutional Ethics Committee on Experimental Animals, in full compliance with Spanish and European laws and regulations. Teratomas were stained with hematoxylin eosin and also the detection of the three germ layers was done by immunocytochemistry. Antibodies used are included in Table S5.

DNA extraction

Genomic DNA from tumors was extracted using the Gentra Puregene Kit (Qiagen, Chatsworth, CA) following manufacturer's instructions, after tissue homogenization using Tissue Lyser (Qiagen). Genomic DNA from primary cells and iPSCs was extracted using Promega Maxwell 16 system following manufacturer's instructions.

NF1 genetic analysis

NF1 germline and somatic mutations were detected by *NF1* cDNA Sanger sequencing, by gDNA sequencing using the I2HCP NGS custom panel (Castellanos *et al*, 2017) and MLPA from cultured PNF-derived Schwann cells treated with 250 µg/ml puromycin (Sigma) or PNFs DNA following Genetic Diagnostics for Hereditary Cancer Unit protocols. Germline mutations were confirmed by DNA Sanger sequencing from cultured PNF-derived fibroblast cells. Loss of heterozigosity of NF1 locus was detected by Microsatellite multiplex PCR analysis (MMPA) of chromosome 17 (Garcia-Linares *et al*, 2012). Reference sequence used was GeneBank: NG_009018_1, NM_000267_3, NP_000258.1. For intragenic deletions we used GeneBank: NM_001042492.2.

SNP-array analysis

SNP-array analysis was performed on selected samples using Illumina HumanOmniExpress v1 BeadChips (730,525 SNPs). Raw data was processed with Illumina Genome Studio v2011.1 with the Genotyping module v1.9.4 to extract B Allele frequency (BAF) and log R ratio (LRR) and then analyzed with the R package ASCAT (Van Loo *et al*, 2010) to obtain loss of heterozygosity (LOH) and allele specific copy

number (CN) profiles. All samples were analyzed independently and treated as unpaired samples, using the germline genotype prediction functionality from ASCAT.

Exome sequencing

Exome was captured using Agilent SureSelect Human All Exon V5 kit (Agilent, Santa Clara, CA, US) according to the manufacturer's instructions and sequenced in a HiSeq instrument (Illumina) producing 100-base long paired-end reads. Reads were aligned to the hs37d5 reference genome using BWA MEM (Li H 2013) (bwa-0.7.13). After that, duplicates were marked using Picard (v2.0.1) and the Genome Analysis Toolkit (GATK) (McKenna *et al*, 2010) (v.3.4.46) was used for local realignment around indels. GATK's Mutect2 (Cibulskis *et al*, 2013) was used to detect somatic variants specific to primary tumors, SC and iPSC with respect to their associated fibroblasts. Variants were annotated using annovar (Wang *et al*, 2010)(v20160201), filtered using custom R scripts and further validated by manual inspection.

Data visualization

Genomic plots were created with the R/Bioconductor package karyoploteR (Gel & Serra, 2017) and additional custom R scripts. Graphs were created with Graphpad Prism 7.0.

RT-qPCR analysis

Total RNA was extracted from cultured cells using the 16 LEV simplyRNA Purification Kit, from Maxwell technology following manufacturer's instructions. RNA (0,5 µg) was reverse-transcribed using the Superscript III reverse transcriptase enzyme (Life technologies) according to manufacturer's instructions. Quantitative polymerase chain reaction (qPCR) was performed with Roche Universal Probe Library (UPL) technology and analyzed using the Light-Cycler® 480 Real-Time PCR System (Roche Diagnostics). Gene expression was normalized to two selected reference genes (*EP300* and *TBP*) and expressed as Normalized Relative Expression (NRE). Primer sequences used are listed in Table S4. A Microsoft Excel spreadsheet was used to analyze qPCR data for relative expression calculations (Terribas *et al*, 2013).

Western Blotting

Cells were washed with chilled PBS twice and lysed with RIPA buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1mM EDTA, 0.5% Igepal CA-630) supplemented with 3mM DTT (Roche), 1mM PMSF (Fluka), 1mM sodium orthovanadate (Sigma), 5mM NaF (Honeywell), 10 ug/ml leupeptin (Sigma), 5ug/ml aprotinin (Sigma) and 1xPhosSTOP (Roche). Lysates were boiled with 1X Laemmli buffer and 90 µg of protein was subjected to SDS-PAGE and transfered onto PVDF membranes (18 hours 90mA at 4ºC). Membranes were blocked with Odyssey Blocking Buffer (PBS)(LI-COR) and incubated with rabbit anti-NF1 Antibody (Bethyl laboratories) at 4ºC overnight; and with mouse anti-αtubulin (Sigma-Aldrich) 1 h at room temperature. Membranes were then incubated with IRDye 680LT and IRDye 800CW secondary antibodies (1:25,000 and 1:15,000, respectively; LI-COR) for 1 h at room temperature and scanned using the Odyssey Infrared Imaging System (LI-COR).

Immunocytochemistry and flow cytometry

For immunofluorescence, cells were fixed in 4% para-formaldehyde in PBS for 15min at RT, permeabilized with 0.1%Triton-X 100 in PBS for 10 min at RT, blocked in 10% FBS in PBS for 15 min at RT and stained with the primary antibodies (Table S4) overnight at 4ºC. Secondary antibodies were Alexa Fluor 488- and Alexa Fluor 568- (Invitrogen). Nuclei were stained with DAPI and images captured using LEICA DMIL6000 and LASAF software. Confocal images from spheres were captured using AxioObserver Z1 Confocal LSM 710, and ZEN Black 2012 software. For flow cytometry assays, cells were dissociated with accutase, resuspended in 0.1% BSA in PBS, incubated for 30 min on ice with unconjugated primary antibody p75 and detected with Alexa Fluor 568-conjugated secondary antibodies, following incubation for 30 min on ice with unconjugated primary antibody Hnk1 and detected with Alexa Fluor 488-conjugated secondary antibodies Cells were analyzed by flow cytometry using BD LSR Fortessa SORP and BD FACSDiva 6.2 software.

Proliferation Assay (Click-iT Edu assay)

Two hundred thousand iPSCs (ES4, FiPS and iPSC) were plated on matrigel-coated 6-well plates, and feeded daily with mTESR medium. After 72h cells were treated with 20 µM EdU for 2 hours, fixed, permeabilized and click labeled with Alexa Fluor 488 azide using Click-iT Plus EdU Flow Cytometry Assay Kits (Thermo Fisher) according to the manufacturer protocol. Cells were also stained with propidium iodide to detect DNA content. Data was collected and analyzed using an BD LSR Fortessa SORP and BD FACSDiva 6.2 software.

Scratch assay

$0.5x10^6$ NC cells were plated onto matrigel–coated 6-well dishes. When cells reached confluence a scratch area was created using a sterile tip. Medium was replaced and migration was measured after 6 and 24 hours. To obtain data in cell migration 9 fields covering the scratch were imaged with a 10x lens at 0, 6 and 24 hours after the scratch. The 9 images were joined using tilescan tool from the LASAF software (Leica).

In vitro Myelination assay

In vitro myelination assay was performed as described in Kim H-S et al (Kim *et al*, 2017) co-culturing Rat Dorsal Root Ganglion (DRG) neurons (Innoprot, Spain) with SCP (7 days differentiation)-FiPS, for 30 days.

Statistical Analyses

Statistical analysis was carried out using GraphPad Prism software v7. For multiple group comparisons, a two-tailed unpaired t test was performed. The number of biological replicates (n) for each experiment and average ± SEM are indicated when applicable, and statistical significance is indicated by *p < 0.05, **p < 0.01, and ***p < 0.001.

References

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