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Supplemental Information

Paired Related Homeobox Protein 1 Regulates Quiescence in Human Oligodendrocyte Progenitors

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Supplemental Figures

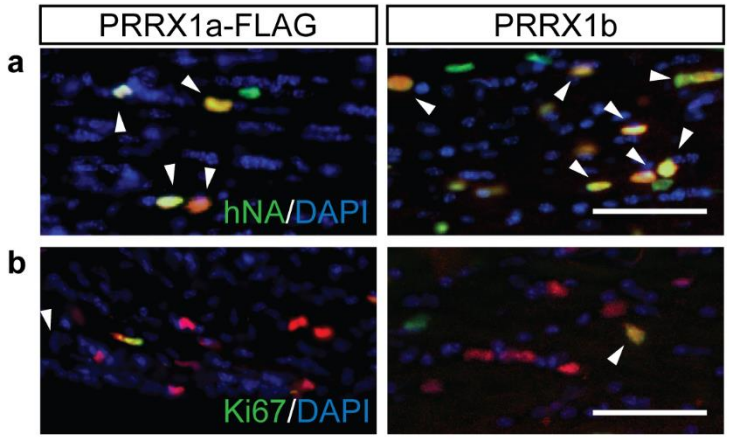

Figure S1 (related to Figure 3). PRRX1 over-expression was maintained for 12 weeks in engrafted human cells *in vivo*. Engrafted fetal human oligodendrocyte progenitors infected with PRRX1a or PRRX1b LV were examined after 12 weeks. **a,** PPRX1 expression was readily apparent in the majority of human (hNA; green) cells with antibodies recognizing the flag tag in the PRRX1a variant or specific to the PRRX1b variant (both in red). **b**, Proliferation of PRRX1 expressing engrafted cells was also confirmed by observing Ki67-PRRX1 double-positive cells. Arrowheads indicate double-positive cells. Scale: 50 µm.

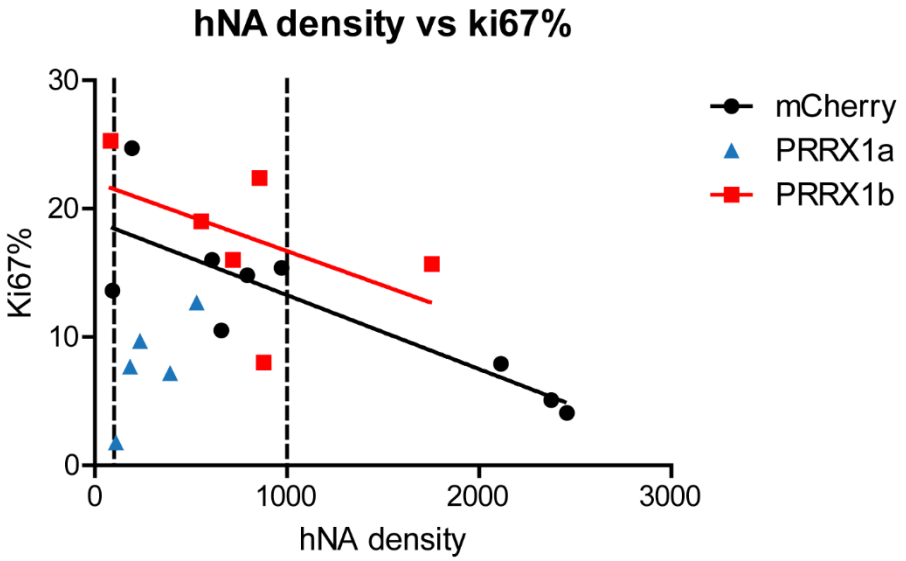

Figure S2 (related to Figure 3). Proliferation of human oligodendrocyte progenitors varies with density *in vivo*. Mice were sacrificed 12 wk following implantation of fetal human oligodendrocyte progenitors infected with a control mCherry lentivirus. Dashed lines demarcate range of densities used for Ki67 analysis. The density of implanted cells was calculated in regions within corpus callosum and plotted against the percentage of cells positive for Ki67 by immunohistochemistry ($n = 9$; $r^2 = 0.70$, $p < 0.01$, Pearson's correlation).

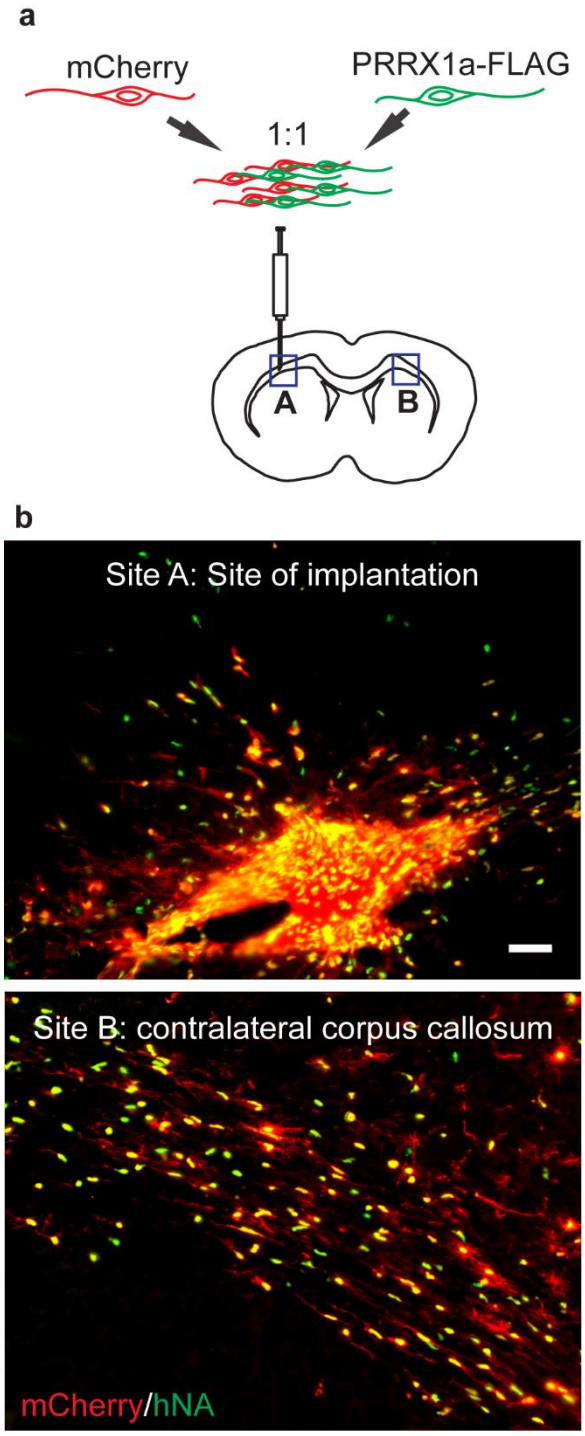

Figure S3 (related to Figure 3). Selective advantage of control hOPCs versus PRRX1-expressing quiescent hOPCs in *shiverer* forebrain. a, control mCherry-infected hOPCs were mixed 1:1 with PRRX1a-FLAG-infected hOPCs prior to unilateral engraftment into the corpus callosum of postnatal day 2-3 *shiverer/rag2* mice (n = 3). Mice were sacrificed at 4 wk to assess migration and engraftment of each population. **b**, the distribution of $mCherry^{+}hNA^{+}$ and $FLAG^{+}hNA^{+}$ cells was assessed at the site of implantation (A) and in the contralateral corpus callosum (B). The vast majority of hNA^{+} cells (green) co-expressed mCherry (red). In contrast to transplanted PRRX1a-$FLAG^{+}$ cells at 12 weeks (**Figure S1**), no $FLAG^{+}$ cells were detected at either site, indicating that PRRX1-infected hOPCs failed to successfully engraft. Scale: 50 µm.

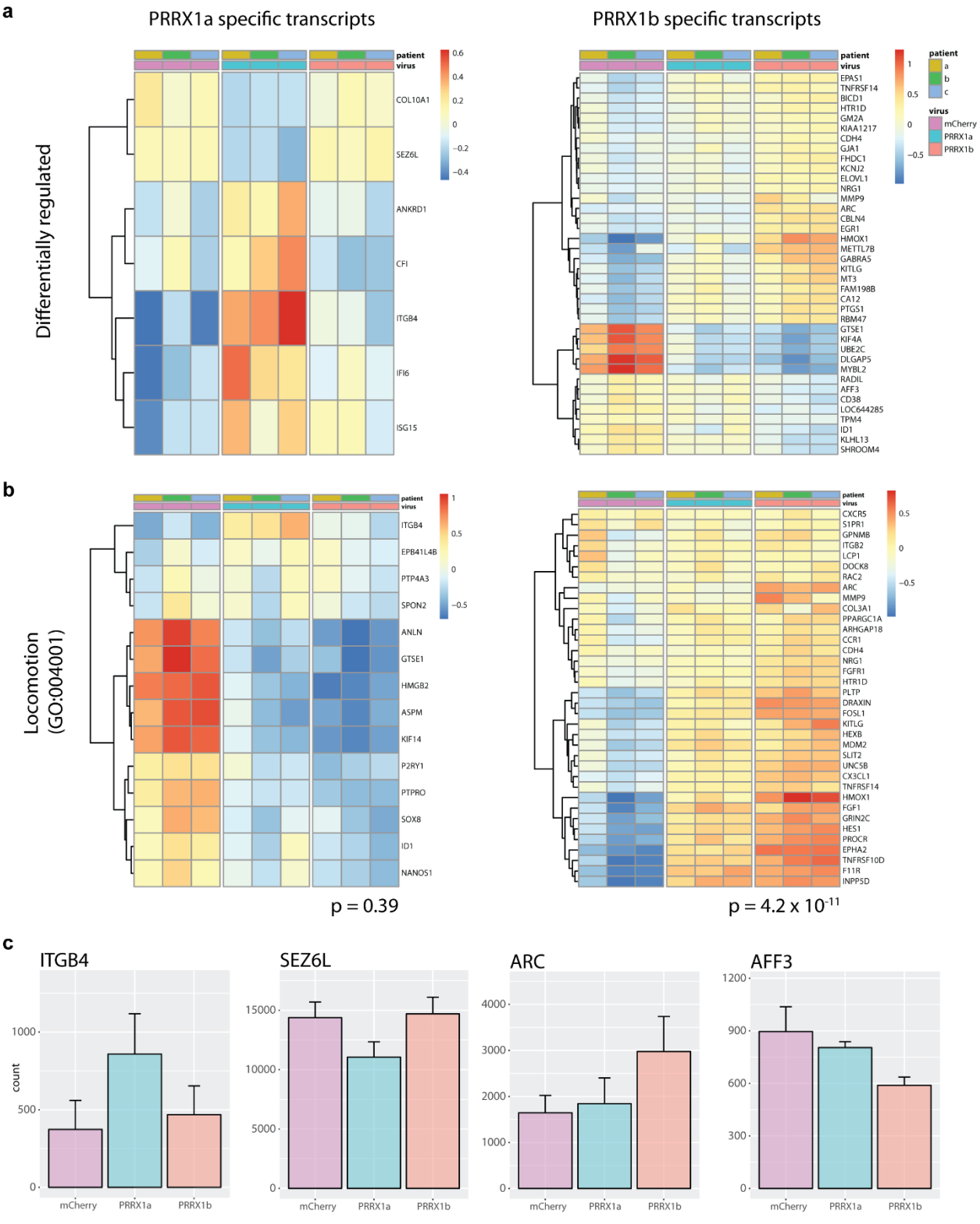
a
PRRX1a specific transcripts
PRRX1b specific transcripts
Differentially regulated
patient
virus
COL10A1
SEZ6L
ANKRD1
CFI
ITGB4
IFI6
ISG15
EPAS1
TNFRSF14
BICD1
HTR1D
GM2A
KIAA1217
CDH4
GJA1
FHDC1
KCNJ2
ELOVL1
NRG1
MMP9
ARC
CBLN4
EGR1
HMOX1
METTL7B
GABRA5
KITLG
MT3
FAM198B
CA12
PTGS1
RBM47
GTSE1
KIF4A
UBE2C
DLGAP5
MYBL2
RADIL
AFF3
CD38
LOC644285
TPM4
ID1
KLHL13
SHROOM4
a
b
c
mCherry
PRRX1a
PRRX1b
b
Locomotion
(GO:004001)
ITGB4
EPB41L4B
PTP4A3
SPON2
ANLN
GTSE1
HMGB2
ASPM
KIF14
P2RY1
PTPRO
SOX8
ID1
NANOS1
CXCR5
S1PR1
GPNMB
ITGB2
LCP1
DOCK8
RAC2
ARC
MMP9
COL3A1
PPARGC1A
ARHGAP18
CCR1
CDH4
NRG1
FGFR1
HTR1D
PLTP
DRAXIN
FOSL1
KITLG
HEXB
MDM2
SLIT2
UNC5B
CX3CL1
TNFRSF14
HMOX1
FGF1
GRIN2C
HES1
PROCR
EPHA2
TNFRSF10D
F11R
INPP5D
p = 0.39
p = 4.2 x 10-11
c
ITGB4
SEZ6L
ARC
AFF3
count
mCherry
PRRX1a
PRRX1b

Figure S4 (related for Figure 5). Analysis of splice-specific effects of PRRX1 over-expression on hOPCs. To better identify transcripts regulated by PRRX1 in a splice-specific manner, we used a modified linear model in which PRRX1 transcript level was used as an additional covariate. The PRRX1a and PRRX1b-infected hOPC transcriptomes were then compared by *edgeR* analysis and pathway analysis. **a,** Differential expressed transcripts between PRRX1a and PRRX1b ($q < 0.1$) were further filtered to include only those regulated significantly vs. mCherry-infected OPCs ($q < 0.05$). 7 genes were specifically regulated by PRRX1a and 38 genes by PRRX1b. Heatmaps show relative expression of *vst* normalized counts for each gene to the median level across samples (log2 scale shown). **b**, *topGO* analysis of transcripts regulated between 1a and 1b ($q < 0.1$) revealed that locomotion (GO:004001) were among the top 5 pathways up-regulated by 1b ($p = 4.2 \times 10^{-11}$). **c**, selected genes plotted based on normalized counts (pseudocount of 0.5; mean ± SEM, n = 3). All genes were significantly regulated between PRRX1a and PRRX1b conditions.

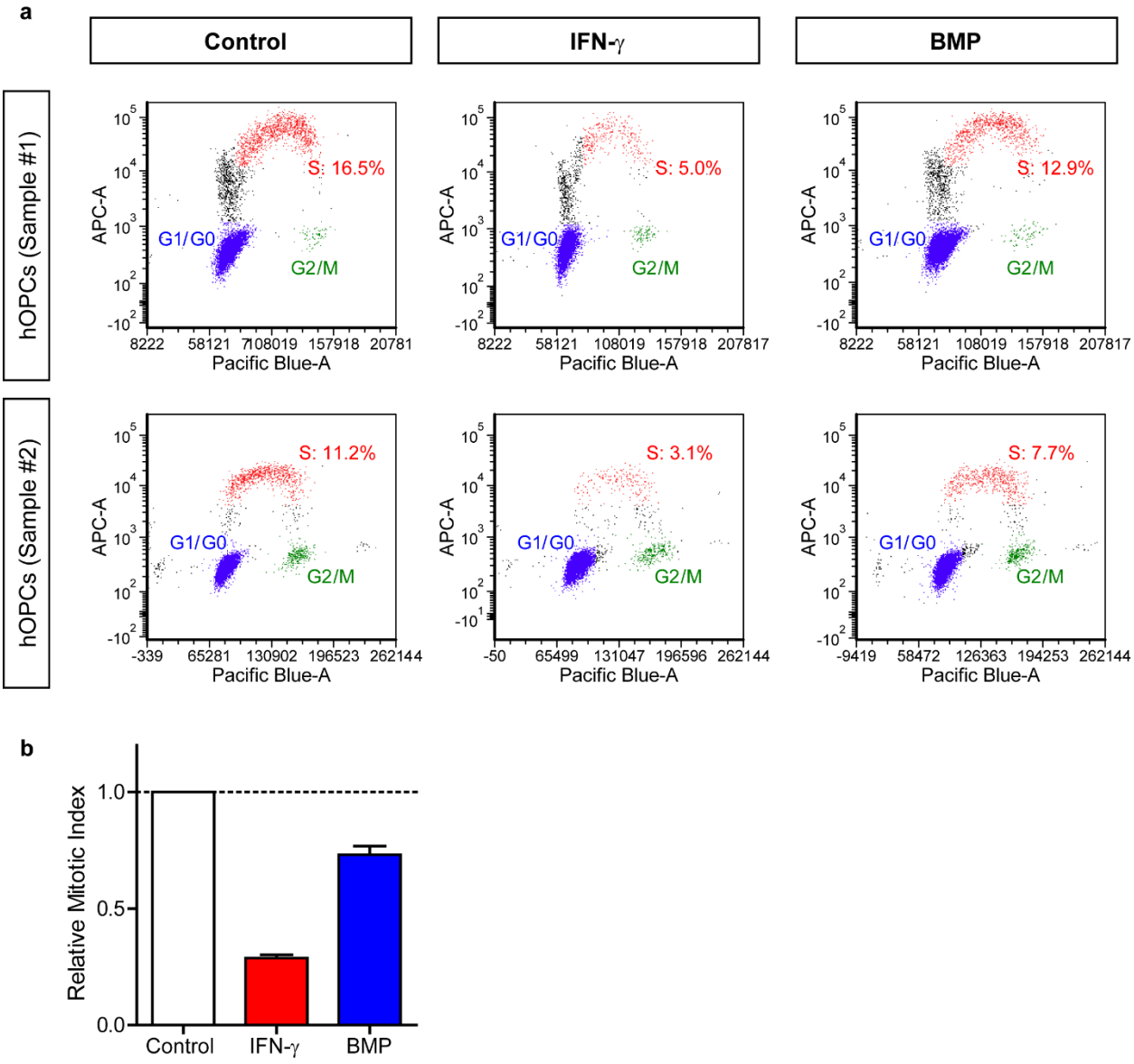

Figure S5 (related to Figure 6). IFN-γ and BMP reduce hOPC proliferation. a, hOPCs were grown in SFM without PDGF-AA (Control) or following addition of IFN-γ (10 ng/ml) or BMP-7 (50 ng/ml) for 24 hrs. Flow cytometry of S phase entry (red; 2-h EdU incorporation) and $G_{1/0}$ and $G_{2/M}$ phases (blue and green, respectively). Both IFN-γ and BMP7 treatment substantially reduced the proportion of S-phase hOPCs. The same number of events are shown in each dot plot following gating for singlet discrimination. **b**, quantification of mitotic index (defined as % S-phase relative to control cells) (mean ± SEM, n = 2 fetal human samples).

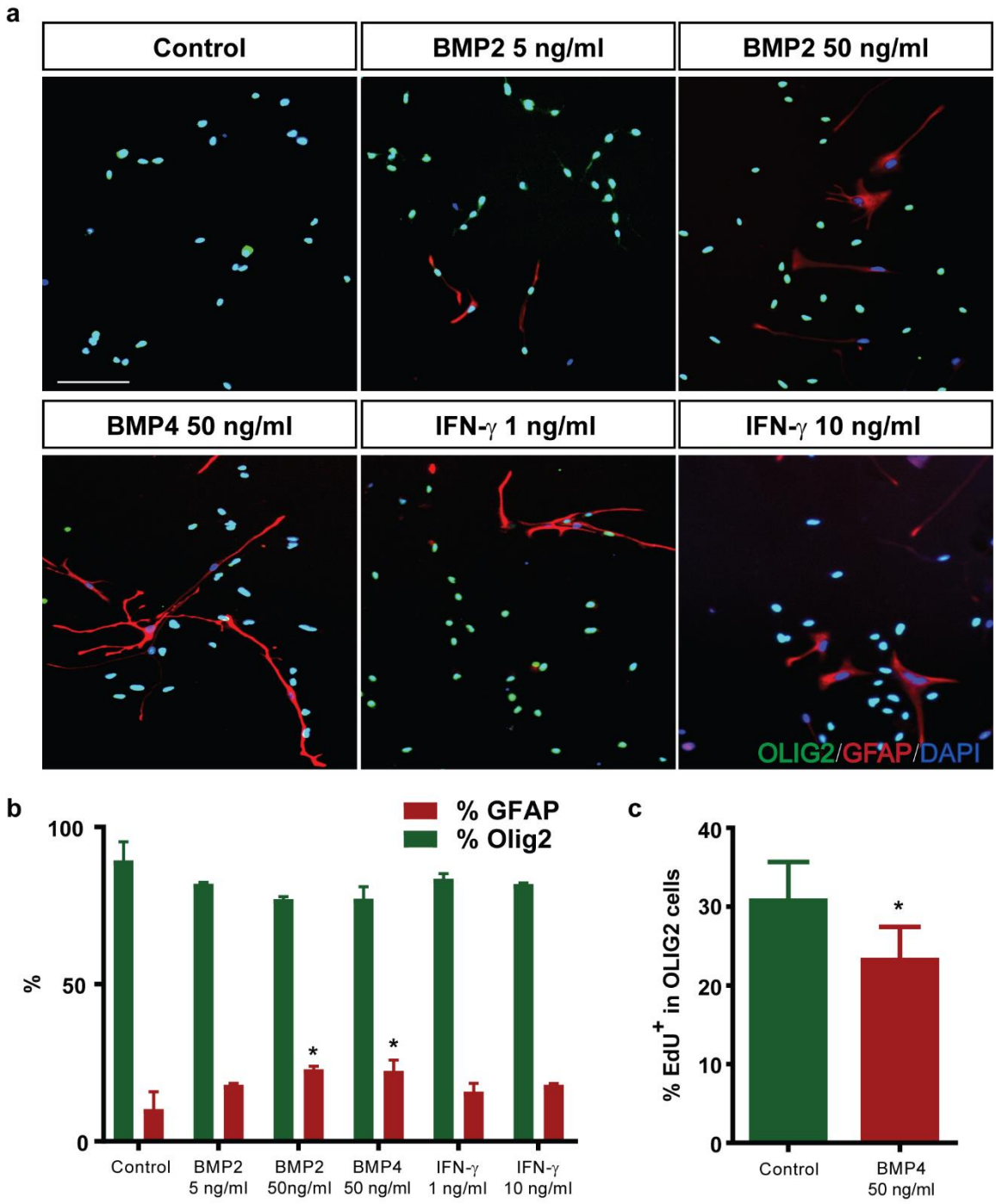

Figure S6 (related to Figure 6). Effect of BMP2/4 and IFN-γ on hOPC lineage and astrocyte differentiation. a, hOPC were treated with BMP2 (5-50 ng/ml), BMP4 (50ng/ml), or IFN-□ (1-10 ng/ml) for 24 hours. The effect of cytokine treatment on oligodendrocyte lineage commitment (OLIG2, green) and astrocyte fate (GFAP, red) was assessed by immunocytochemistry. **b**, Quantification of OLIG2+ and GFAP+ cells among DAPI+ cells (mean ± SEM, n = 3 fetal human samples, *p < 0.05, one-way ANOVA, Dunnett's multiple comparisons post-test vs. control). **c**, Proliferation of $OLIG2^{+}$ cells was assessed following terminal 8-hr EdU incorporation at 24 hours of BMP treatment. BMP4 (50 ng/ml) treatment caused a significant decrease in OLIG2+ cell proliferation relative to controls (paired t-test, $p < 0.05$, n = 3 fetal human samples). Scale: 50 µm.

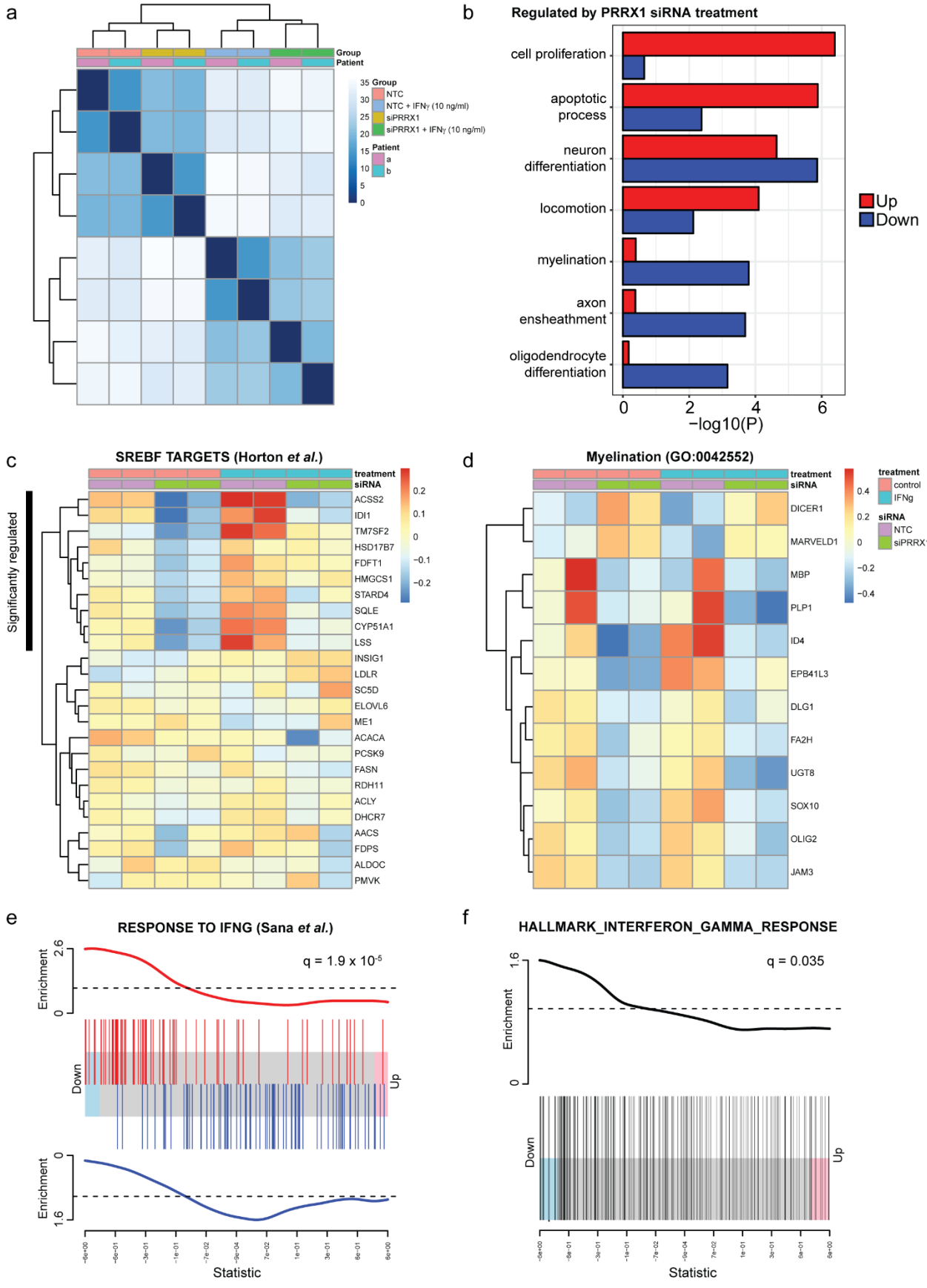
a
Group
Patient
35 Group
NTC
NTC + IFNγ (10 ng/ml)
siPRRX1
siPRRX1 + IFNγ (10 ng/ml)
Patient
a
b
b
Regulated by PRRX1 siRNA treatment
cell proliferation
apoptotic process
neuron differentiation
locomotion
myelination
axon ensheathment
oligodendrocyte differentiation
Up
Down
−log10(P)
c
SREBF TARGETS (Horton et al.)
Significantly regulated
treatment
siRNA
ACSS2
IDI1
TM7SF2
HSD17B7
FDFT1
HMGCS1
STARD4
SQLE
CYP51A1
LSS
INSIG1
LDLR
SC5D
ELOVL6
ME1
ACACA
PCSK9
FASN
RDH11
ACLY
DHCR7
AACS
FDPS
ALDOC
PMVK
d
Myelination (GO:0042552)
treatment
siRNA
DICER1
MARVELD1
MBP
PLP1
ID4
EPB41L3
DLG1
FA2H
UGT8
SOX10
OLIG2
JAM3
control
IFNg
NTC
siPRRX1
e
RESPONSE TO IFNG (Sana et al.)
q = 1.9 x 10-5
Enrichment
Down
Up
Statistic
f
HALLMARK_INTERFERON_GAMMA_RESPONSE
q = 0.035
Enrichment
Down
Up
Statistic

Figure S7 (related to Figure 6). PRRX1 siRNA reduces cholesterol biosynthesis and reverses IFN-γ induced gene expression. Human hOPCs were treated with IFN-γ (10 ng/ml) at 8 hrs post transfection and RNA extracted 24hrs later. **a**, Heatmap demonstrating clustering of individual RNA samples by sample-to-sample distances. The effects of IFN-γ and PRRX1 siRNA treatment on hOPC transcriptome are clearly distinguishable. **b**, Gene Ontology-based over-representation analysis of genes regulated by PRRX1 siRNA in hOPCs. **c-d,** Pathway analysis by gene set enrichment and topGO revealed PRRX1 target gene regulation of cholesterol biosynthesis genes (**c**, *camera* q = 0.0059) and myelination-associated transcripts (**d**, *topGO* $p = 1.6 \times 10^{-4}$). **e-f**, Gene set enrichment analysis examining the effect of PRRX1 siRNA on the IFN-γ induced transcriptional response demonstrated that PRRX1 siRNA was able to attenuate the induction of IFN-related transcripts. Red bars indicate the position of up-regulated genes and blue bars down-regulated genes in each gene set. The red/blue traces *above/below* show relative enrichment of up and down-regulated genes respectively (q-value shown).

Supplemental Tables

Gene / Target sequence	Sequence (5' to 3')
PRRX1a	Fwd: CGCAGGAATGAGAGAGCCAT Rev: AACATCTTGGGAGGGACGAG
PRRX1b	Fwd: ACGCTTCCCTCCTCAAATCC Rev: AGTAGCCATGGCGCTGTACG
CCNA1	Fwd: TTCCCGCAATCATGTACCCTG Rev: TGTAGCCAGCACAACTCCACT
CCNB1	Fwd: CGCCTGAGCCTATTTTGGTTG Rev: AGTGACTTCCCGACCCAGTA
CCNE2	Fwd: TCTTCACTGCAAGCACCATC Rev: ACCTCATTATTCATTGCTTCCAA
SGOL1	Fwd: GACCCCAATAGTGATGACAGC Rev: GAAATGATTCTCCTTGTCCTGG
RRM2	Fwd: CGGAGCCGAAAACTAAAGCAG Rev: TCTCCTCGGGTTTCAGGGAT
ANLN	Fwd: GCTGCGTAGCTTACAGACTTAGC Rev: AAGGCGTTTAAAGGTGATAGGTG
IRF1	Fwd: CAACATGCCCATCACTCGGA Rev: TGCTTTGTATCGGCCTGTGT

Table S1 (related to Figures 5 & 6). qPCR primers and Taqman assays used in this study.