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Supplementary Materials for

Interferon-γ signaling in human iPSC–derived neurons recapitulates neurodevelopmental disorder phenotypes

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The PDF file includes:

Figs. S1 to S7

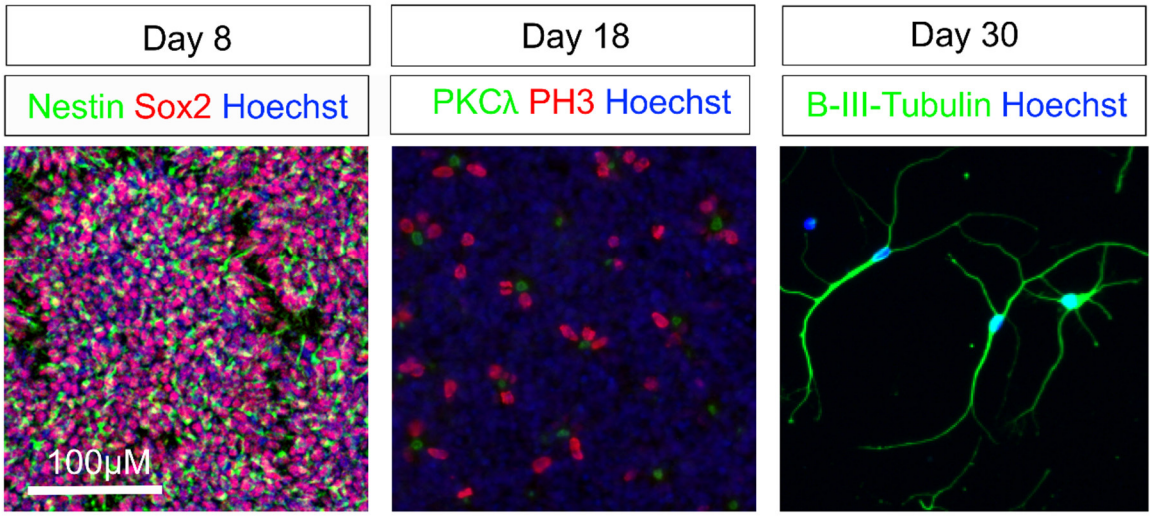
Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/34/eaay9506/DC1)

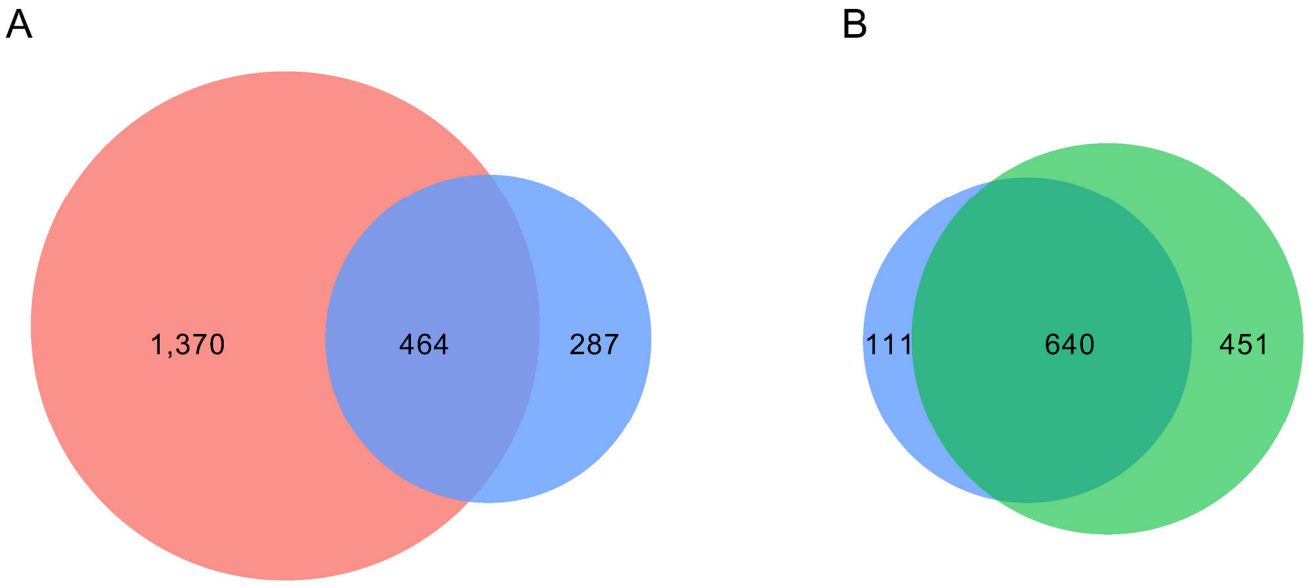
Tables S1 to S6

SUPPLEMENTARY MATERIAL

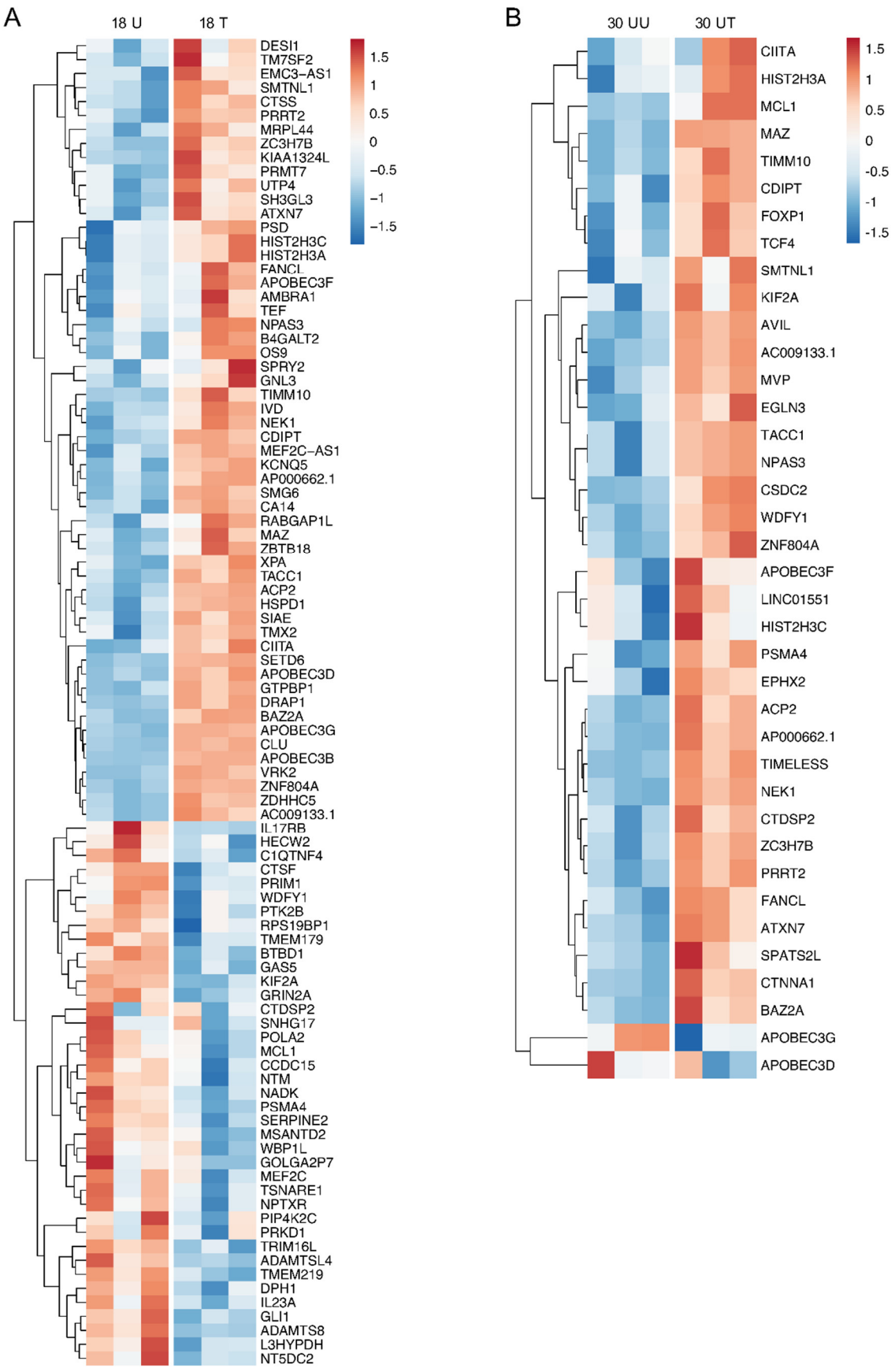
Supplementary Figures



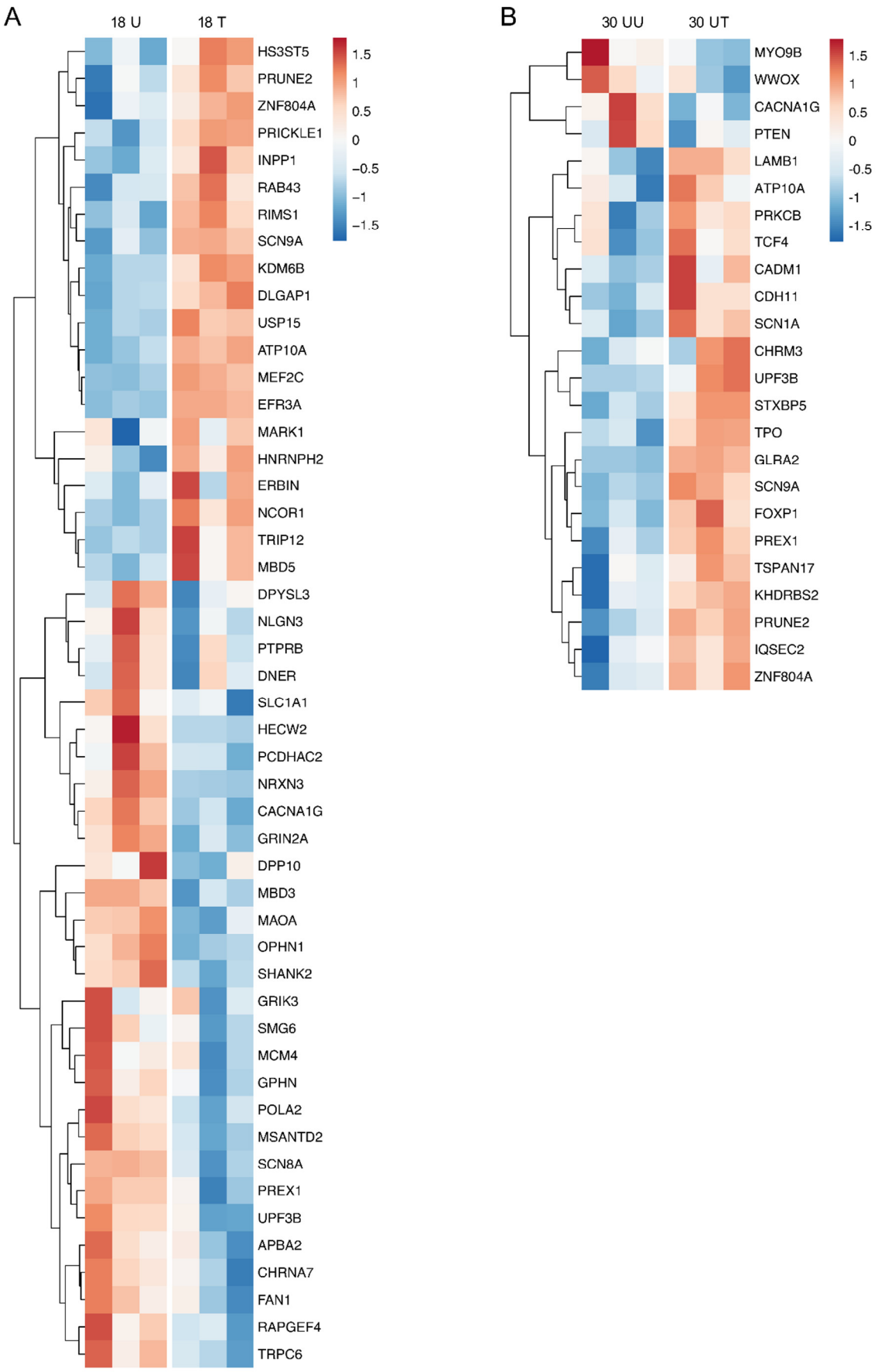
Supplementary Figure 1: Characterisation of hiPSC neuronal differentiation. Representative confocal images of neural progenitor cells (NPCs), neural rosettes (also composed of NPCs) and neuronal markers at D8, D18 and D30 during hiPSC neuronal differentiation.



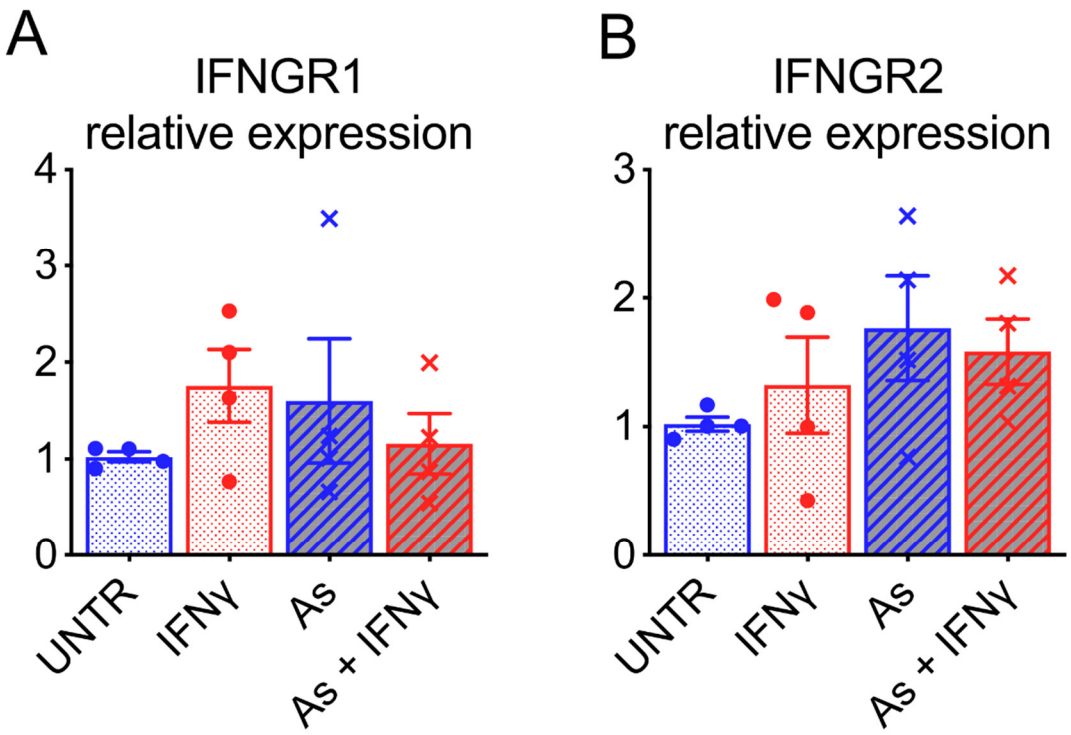
Supplementary Figure 2: Area proportional Venn diagrams of DEG overlap. Visualisation of overlapping DEGs from **(A)** 18U vs 18T (red) and 30UU vs 30UT (blue) and **(B)** 30UU vs 30UT (blue) and 30UU vs 30TT (green) conditions.



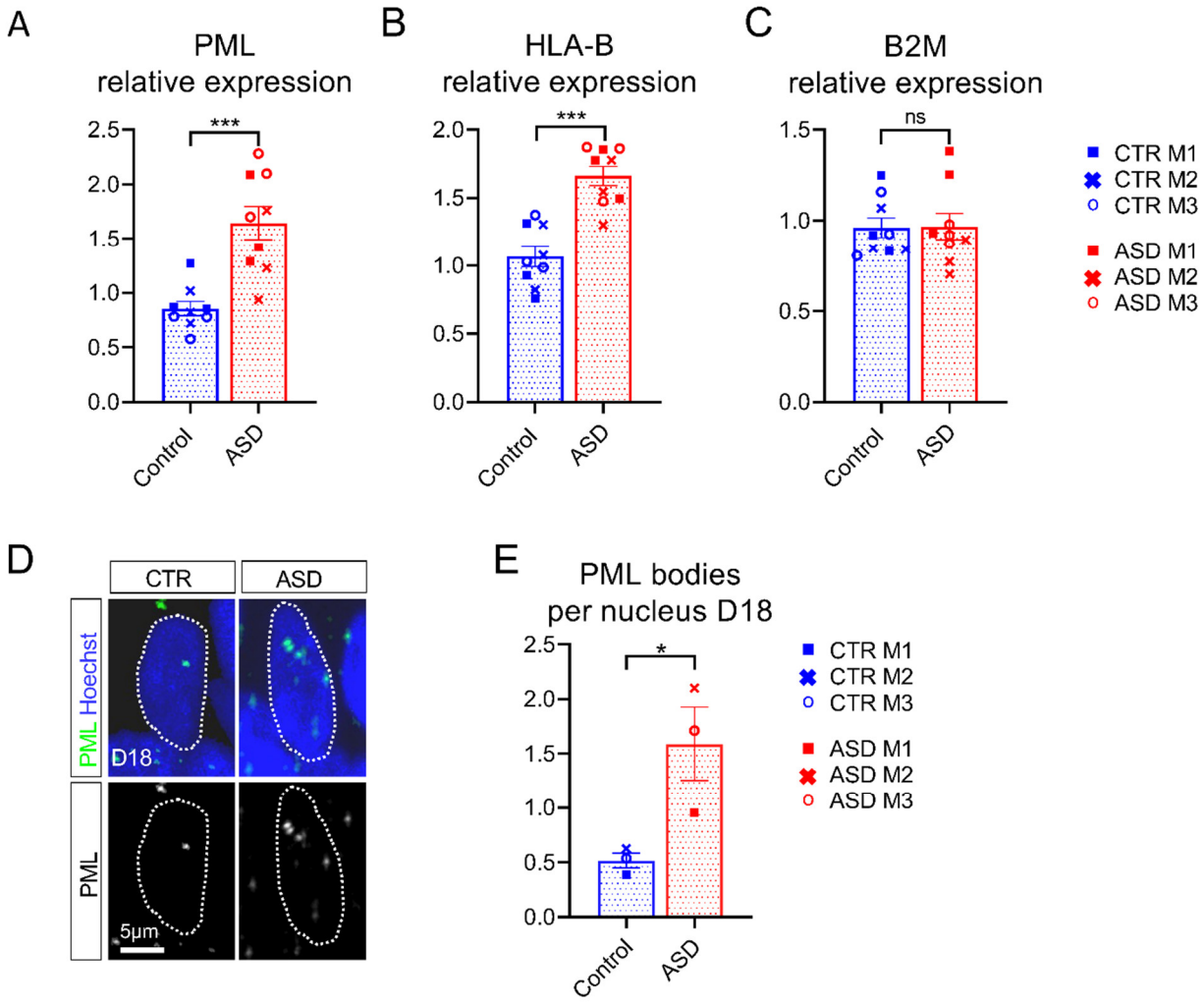
Supplementary Figure 3: Relative expression of IFNγ-responding SZ risk genes. Heatmaps illustrating the SZ risk genes from the PsychENCODE gene set that were differentially expressed in response to IFNγ in NPCs (A) and neurons (B). Expression is scaled by row.



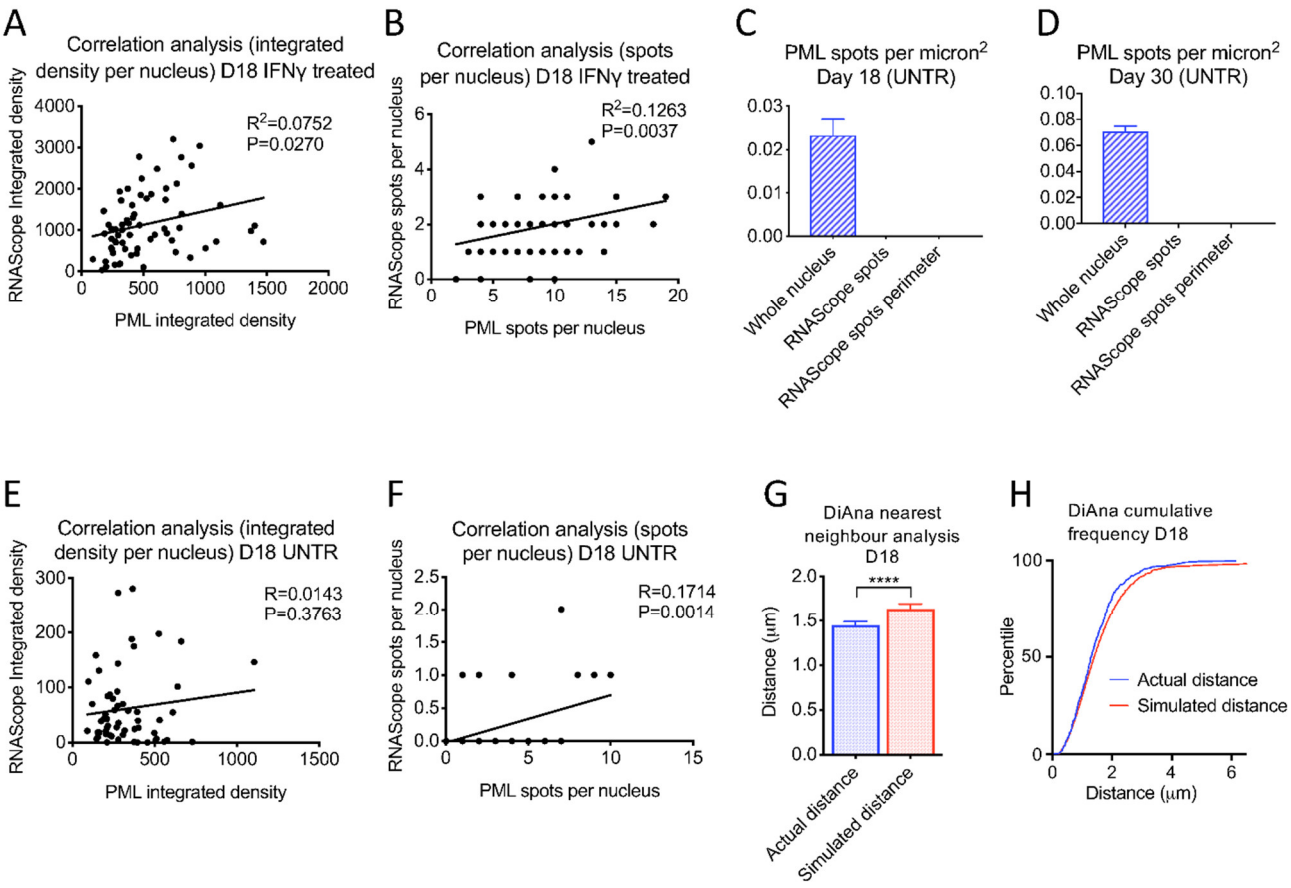
Supplementary Figure 4: Relative expression of IFNγ-responding ASD risk genes. Heatmaps illustrating the ASD risk genes from the SFARI database, category 1-4, that were differentially expressed in response to IFNγ in NPCs (A) and neurons (B). Expression is scaled by row.



Supplementary Figure 5: Impact of IFNγ treatment on expression of IFNγ-realted genes. qPCR analysis of **(A)** *IFNGR1* and **(B)** *IFNGR2* relative expression in D18 'UNTR', 'IFNγ', 'As' and 'As + IFNγ' conditions. *n* = 4 biological replicates from 3 control cell lines. No significant differences observed. One-way ANOVA with Tukey's multiple comparison test.



Supplementary Figure 6: NPCs generated from ASD-hiPSCs display elevated levels of PML and HLA-B expression. (A-C) QPCR analysis of *PML*, *HLA-B* and *B2M* in day 18 NPCs. For all genes, *n* = 9 biological replicates from 3 control or 3 ASD hiPSC lines. *PML* and *HLA-B* but not *B2M*, showed increased expression in NPCs from ASD hiPSCs. Unpaired t test. **(D)** Confocal images of PML nuclear bodies in D18 NPCs derived from control and ASD hiPSCs. **(E)** Quantification of PML nuclear bodies at D18 in NPCs derived from *n* = 3 control and *n* = 3 ASD hiPSC lines. CTR: 136 cells analysed; ASD: 280 cells analysed. Unpaired t test. Bar chart results are presented as means +/- SEM. **P* < 0.05; ****P* < 0.001; ns: not significant.



Supplementary Figure 7: *HLA-B* RNAScope and PML correlation and spatial analysis in D18 UNTR NPCs. (A and B) Correlation analysis of *HLA-B* RNAScope and PML spots per nucleus and integrated density per nucleus in D18 IFNγ-treated cells. *n* = 65 cells from 3 control cell lines. Linear regression analysis. **(C and D)** Quantification of PML spots per micron^2 in whole nuclei, *HLA-B* RNAScope spots and *HLA-B* RNAScope spot perimeters in D18 NPCs **(C)** and D30 neurons **(D)**, untreated condition. D18 UNTR: *n* = 59 cells; D30 UNTR: *n* = 56 cells; 3 control cell lines. **(E and F)** Correlation analysis of *HLA-B* RNAScope and PML integrated density and spots per nucleus in D18 untreated cells. *n* = 57 cells from 3 control cell lines. Linear regression analysis. **(G and H)** DiAna distance analysis of centre-centre distances from *HLA-B* RNAScope spots to PML spots in real images and following random shuffle of PML and RNAScope spots, using nuclei as a bounding box. n = 405 measurements; 3 control cell lines. Wilcoxon matched-pairs test. Results are presented as means +/- SEM. ****$P < 0.0001$.