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Supplemental information

High-throughput screening for myelination promoting compounds using human stem cell-derived oligodendrocyte progenitor cells

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Figure S1. Generation of the PT-P1-MsNL reporter cell line, related to Figure 1. **a)** schematics of the plasmids used to confirm that the secNLuc protein product separated by P2A is functional. A tdTomato-P2A sequence was cloned into the original CMV-secNluc plasmid to generate a CMV-tdTomato-P2A-secNluc plasmid. **b)** Different amount of the CMV-secNluc plasmid and CMV-tdTomato-P2A-secNluc plasmid were transfected into the HEK293 cells. **c)** Successful detection of Nanoluc activity in the culture media of the transfected cells. PCR genotyping **d)** followed by sanger sequencing of both bands **e)** was used to confirm CRISPR-Cas9 mediated successful knock-in of the sfGFP reporter sequence into PLP1 locus of PD-TT reporter line. **f)** PCR genotyping after P2A-secNluc sequence was knocked-in into the MBP locus of the PD-TT and PLPsfGFP dual reporter to make the final triple reporter PT-P1-MsNL cell line.

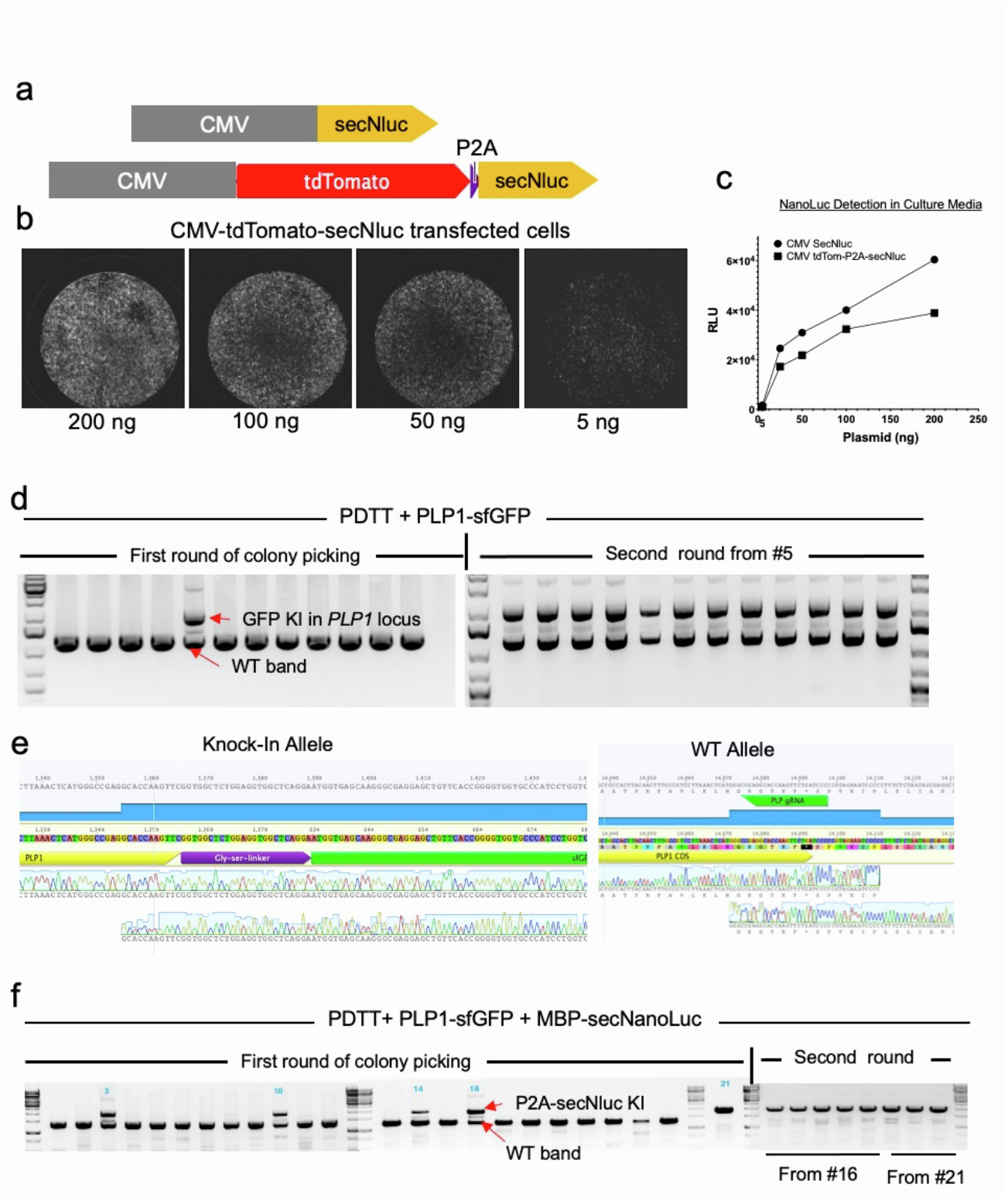


Figure S2. **Differentiation of PtT-P1-MsNL hESC reporter line to oligodendrocyte cells** related to Figure 1**.** Expression of the tdTomato and sfGFP fluorescent reporters in the differentiating oligodendrocyte cultures. First PLP1-sfGFP expressing cells appear around 60 and increase as the cells mature. Number of tdTomato expressing cells is reduced as the culture matures.

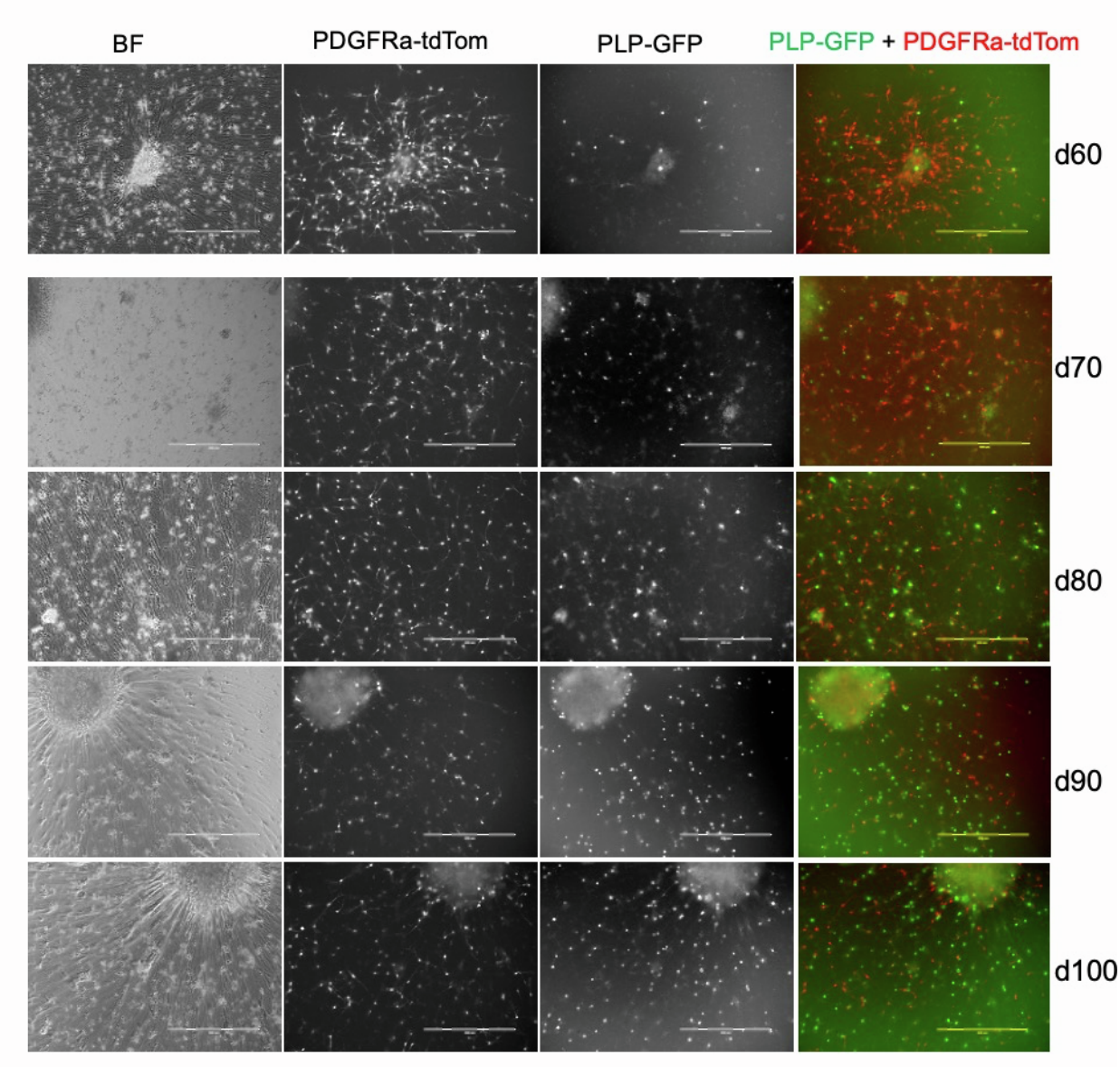


Figure S3. Flow analysis of the reporter OPCs, related to Figure 1**. a)** Gating strategy used to select live cells from the differentiating hOPC population. This BSC and FSC setup was used for all the flow analysis performed. **b)** Flow analysis showing PDGFRA-tdTomato+ and PLP1-sfGFP+ OPCs in the culture differentiated for 90 days. **c)** Significant enrichment of the tdTomato+ as well as GFP+ cells can be achieved by MACS purification using O4 antibody conjugated microbeads.

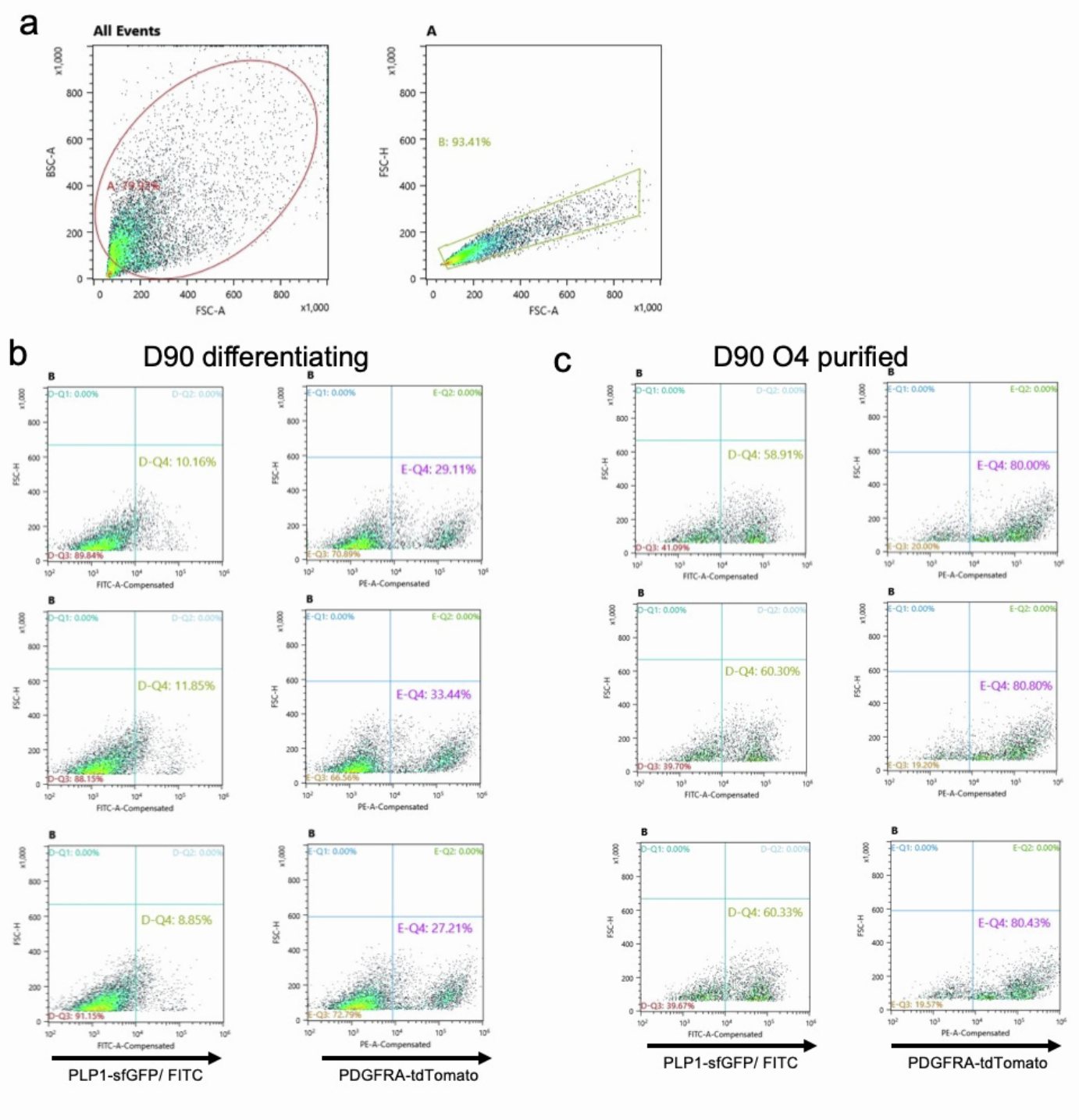


Figure S4. Optimization of the drug screening assay using reporter hESC-derived OPCs, related to Figure 2**. a)** Nluc activity was measured in different volumes cell culture media containing Nluc. **b)** Different volume of NanoLuc reagent was also tested. 20 uL of media and 2.5 uL of Nluc reagent was then used for further optimization. **c)** 1500 cells/well of a 384 well plate (7 plates) was plated to calculate variability caused by initial cell plating. **d)** The OPCs mature and produce more Nluc as a natural course of differentiation, even in control condition (i.e DMSO control). The well-to-well variability caused due to initial cell plating is negligible when compared to the RLU reading of later time-point. **e)** Dose depended effect of Tasin-1 in MBP-NLuc expression at different time-points. **f**) D10 value for 250 nM Tasin-1 presented as scatterplot to show well to well variability. For **c** and **e** data are represented as mean +/- SEM.

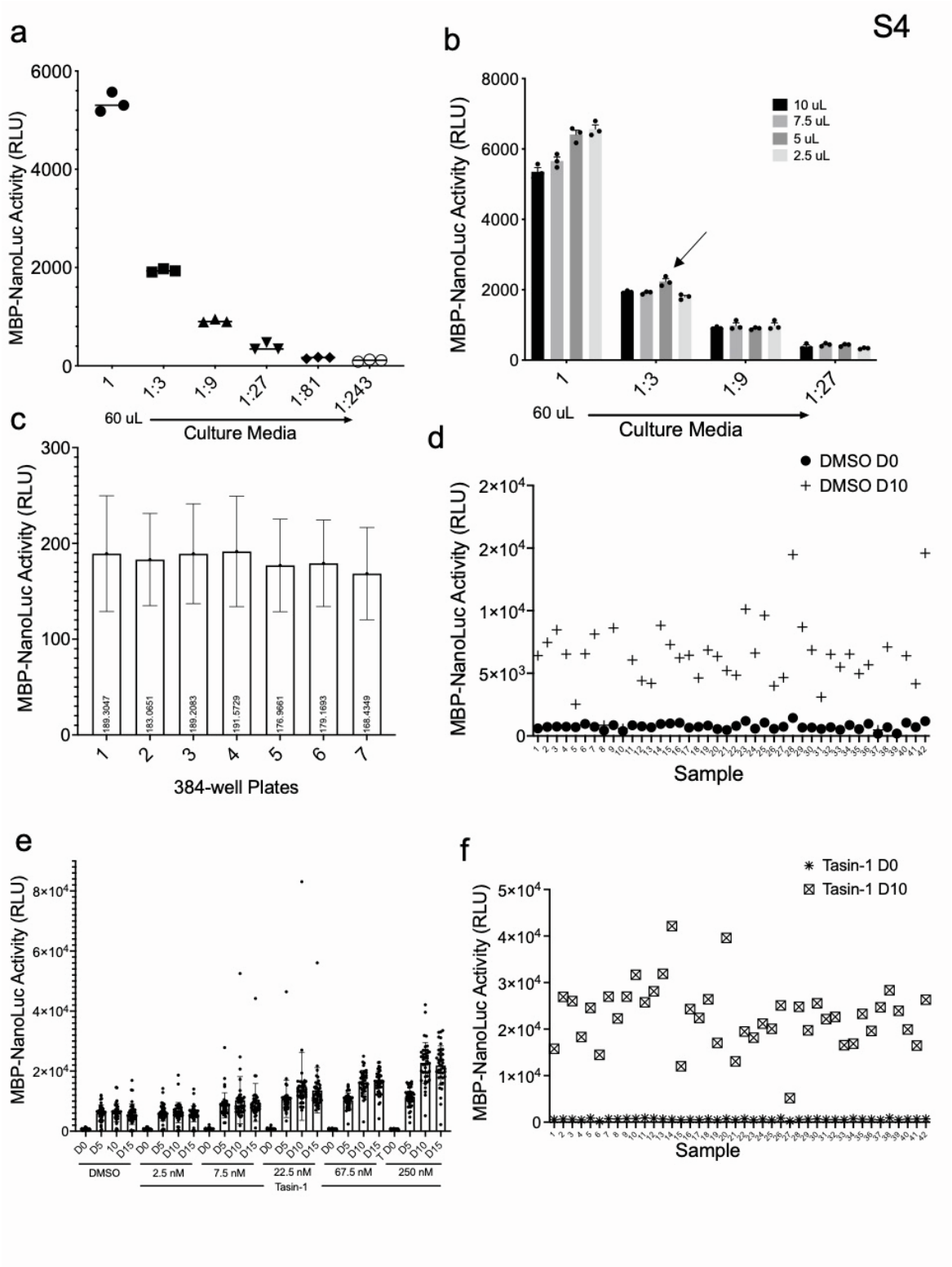


Figure S5. Workflow of the drug screening setup, related to Figures 2 and 3. A simplified version of the workflow established in our laboratory for the drug screening to identify pro-myelinating compounds using human hPSC-derived OPCs.

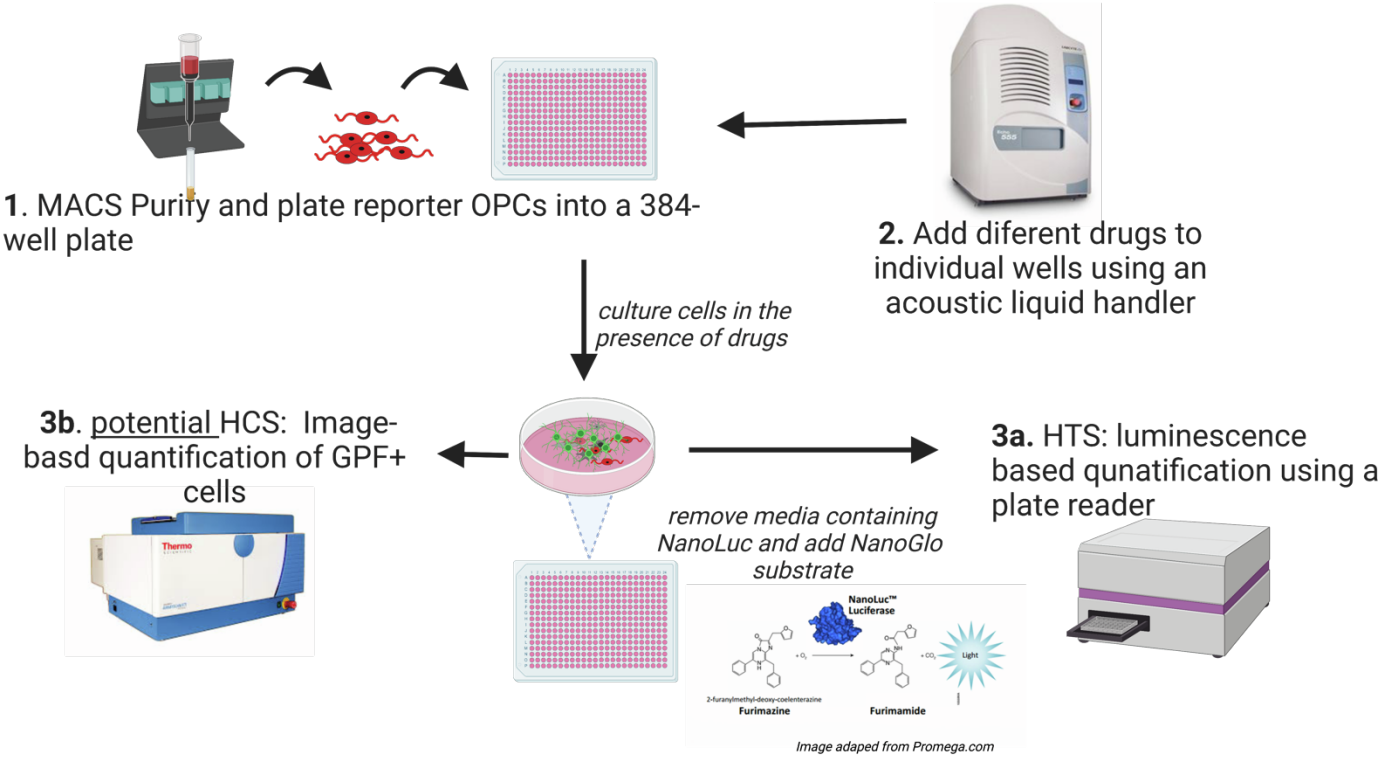
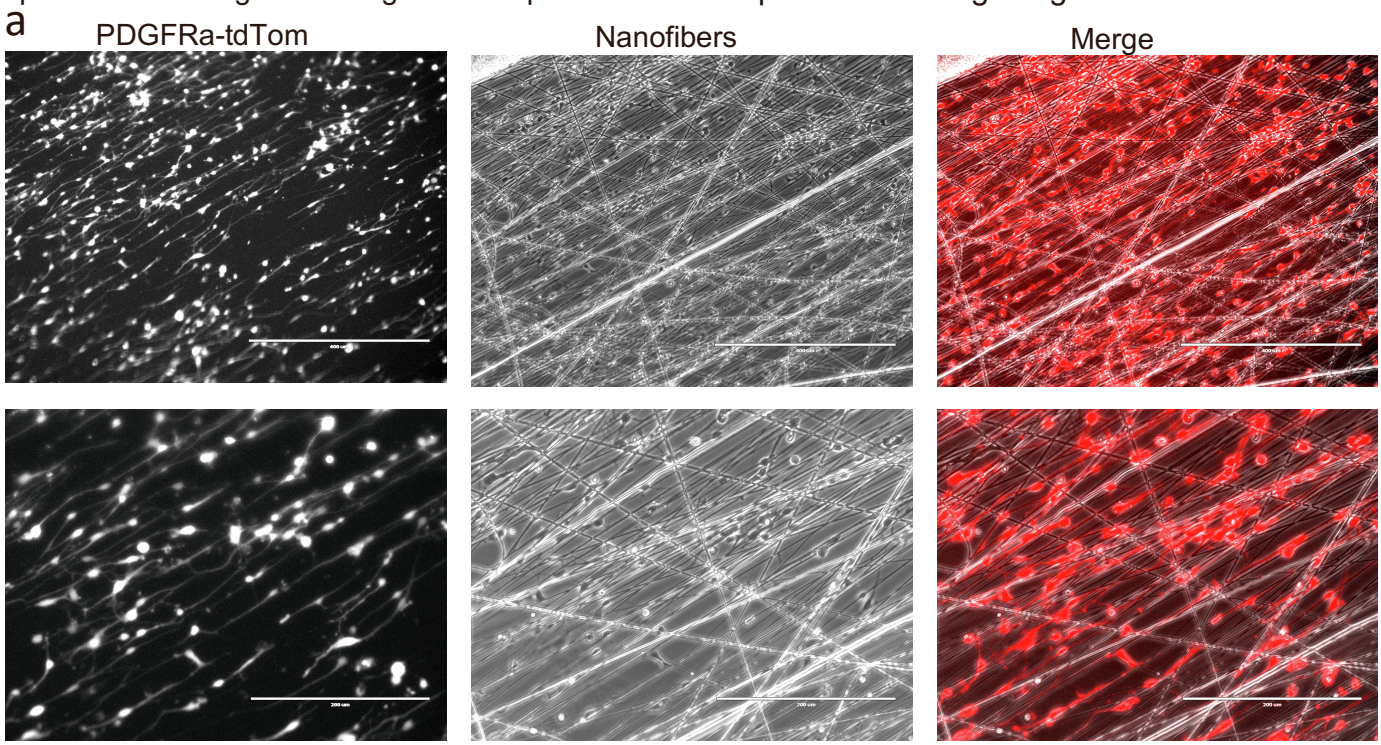
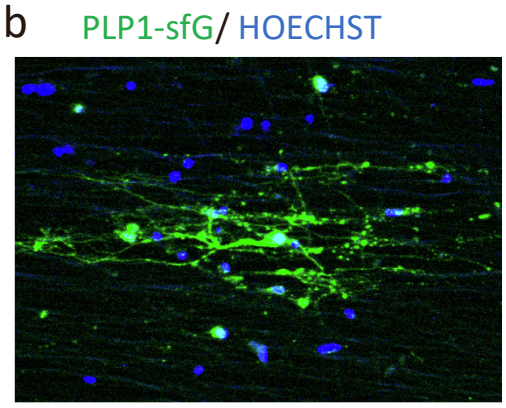
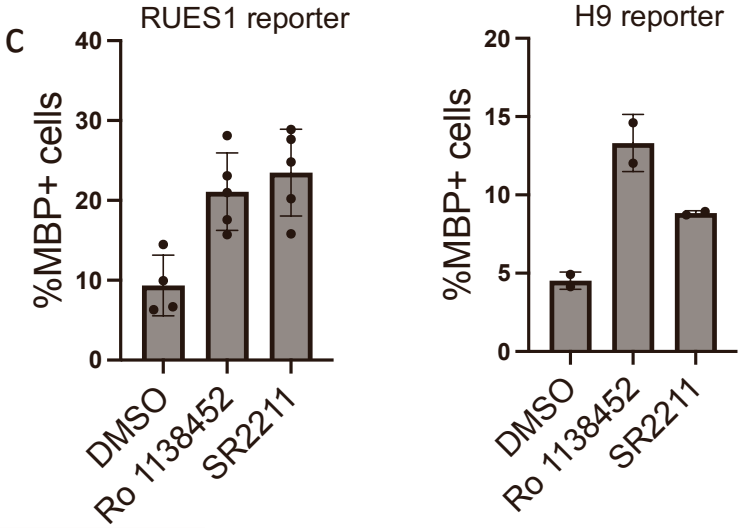


Figure S6*. In vitro* myelination culture, related to Figures 2, 4, and 5. **a)** purified OPCs when plated on a plate containing 950 nM of electrospun nanofibers align their processes within 2 days of plating the cells. **b)** Within 3 weeks they mature into OLs and appear to myelinate the fibers. **c)** Quantification of percent MBP+ cells upon treatment with the two hit molecules for 10 days. Data are represented as mean +/- SEM. **d)** Representative images showing how the quantification was performed using imageJ.







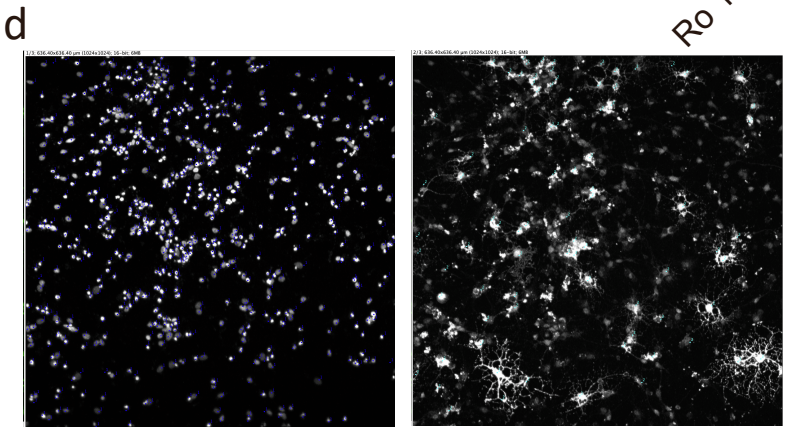


Figure S7. Flow analysis on the purified reporter OPCs treated with small molecules, related to Figure 4. Flow analysis of **a)** DMSO, **b)** Ro1138452, or **c)** SR2211 drug treated OPCs. **d)** Representative image showing the back scatter and forward scatter gating used to select the cells used for flow analysis.

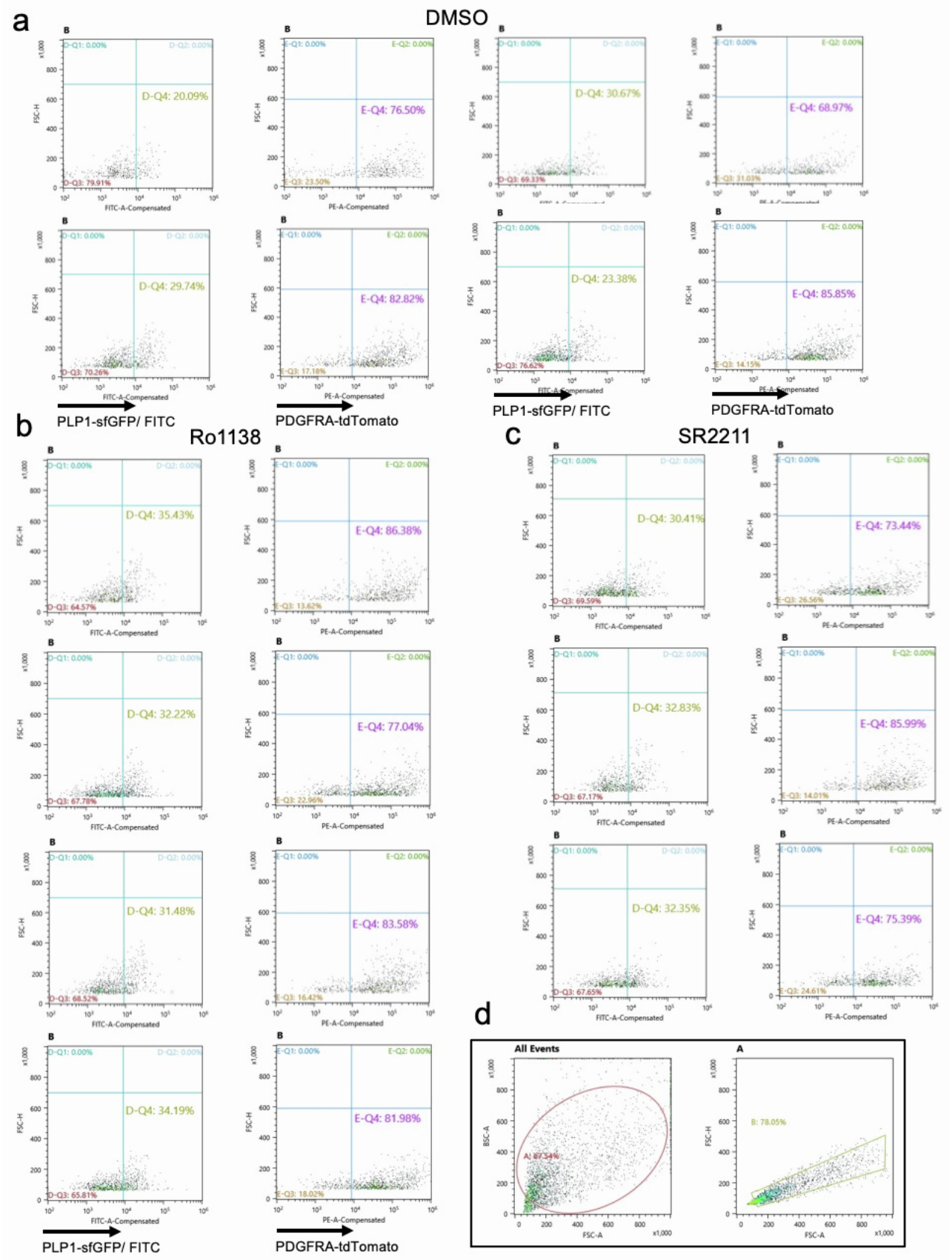


Figure S8. Validation of the RPD- OPC cells, related to Figure 5**.** Additional immunostaining images corresponding to figure 5c demonstrating that the MACS purified tdTomato+ cells express the OPC markers SOX10, OLIG2, NKX2.2 and NG2.

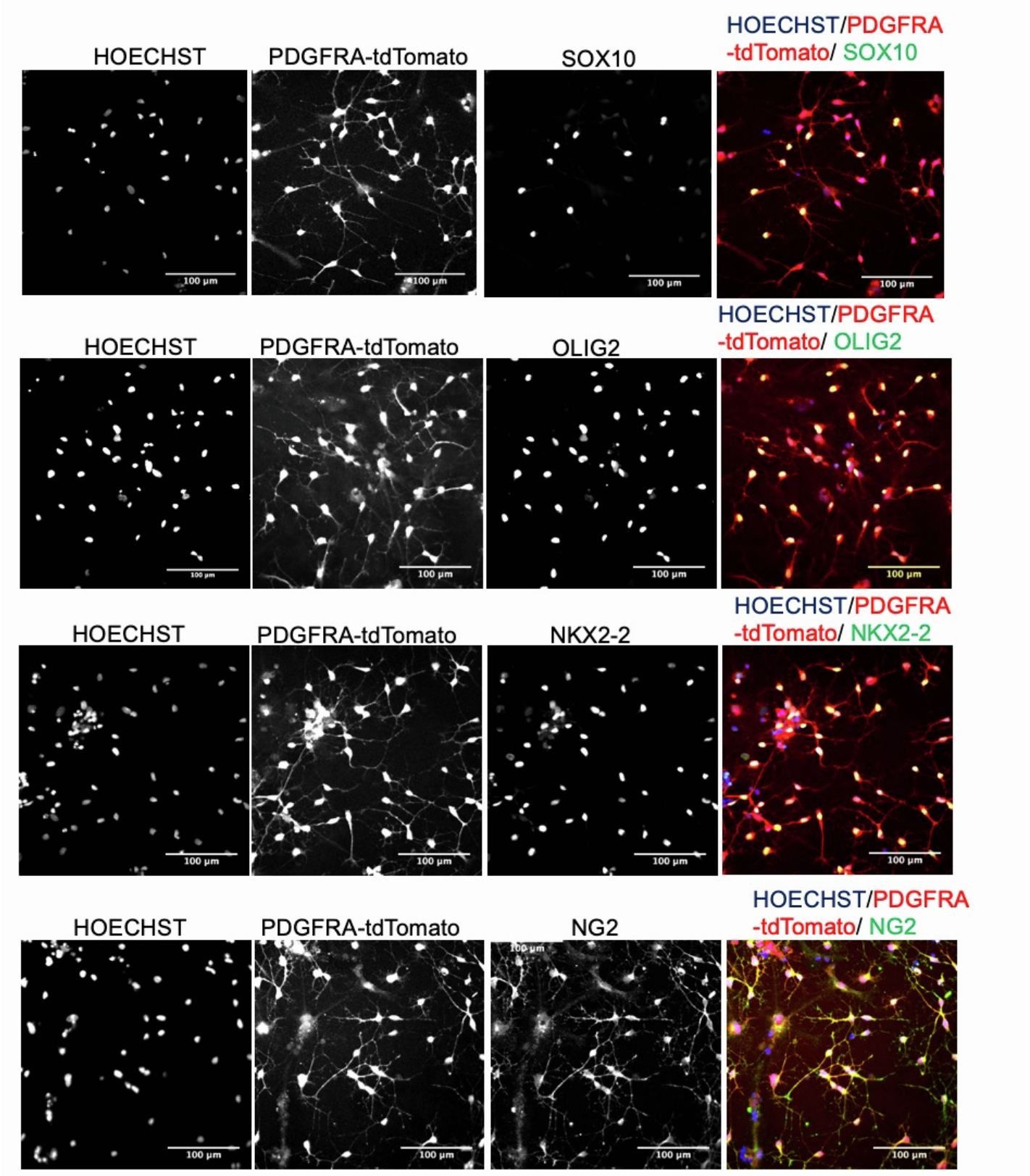


Figure S9. The identified hit molecules promote maturation of rodent OPCs, related to Figure 6. The purified P7-8 mice OPCs were treated with either Ro1138412, SR2211, T3 as positive control or DMSO. Cells were immunostaining with antibody for MBP. **a)** Representative images showing how images were captured for analysis. Nineteen random fields per well of a 96 well plate was captured for analysis. **b-c)** The experiment was repeated three times. For each experiment, MBP+ area and OLIG2+ cell number was measured and the MBP+ area compared relative to OLIG2+ was calculated. Result from one of the three experiment is presented in Figure 6. Other two experiments are presented here as **b** and **c**. Data are represented as mean +/- SEM.

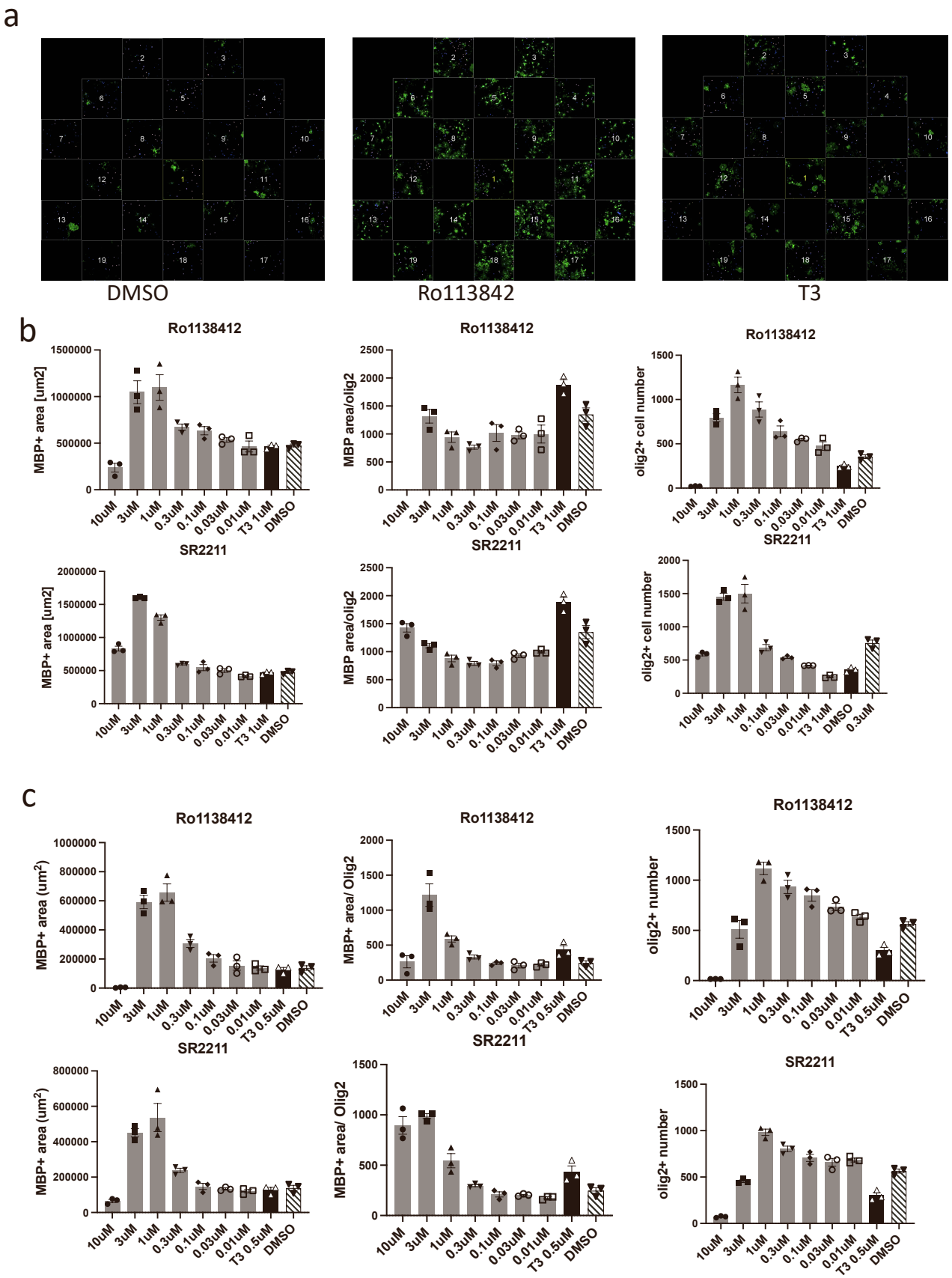


Figure S10. Single cell transcriptomic analysis of the small molecule treated OPCs, related to Figures 7 and 8**. a)** Quality control metrics applied to the scRNAseq data of the DMSO and small molecules treated OPCs. **b)** Average expression of oligodendrocyte markers genes (*MAG* and *MOG*) in the OL cluster. **c)** Proportion of different cell-types in the DMSO vs small molecule treated samples. Data are represented as mean +/- SEM.

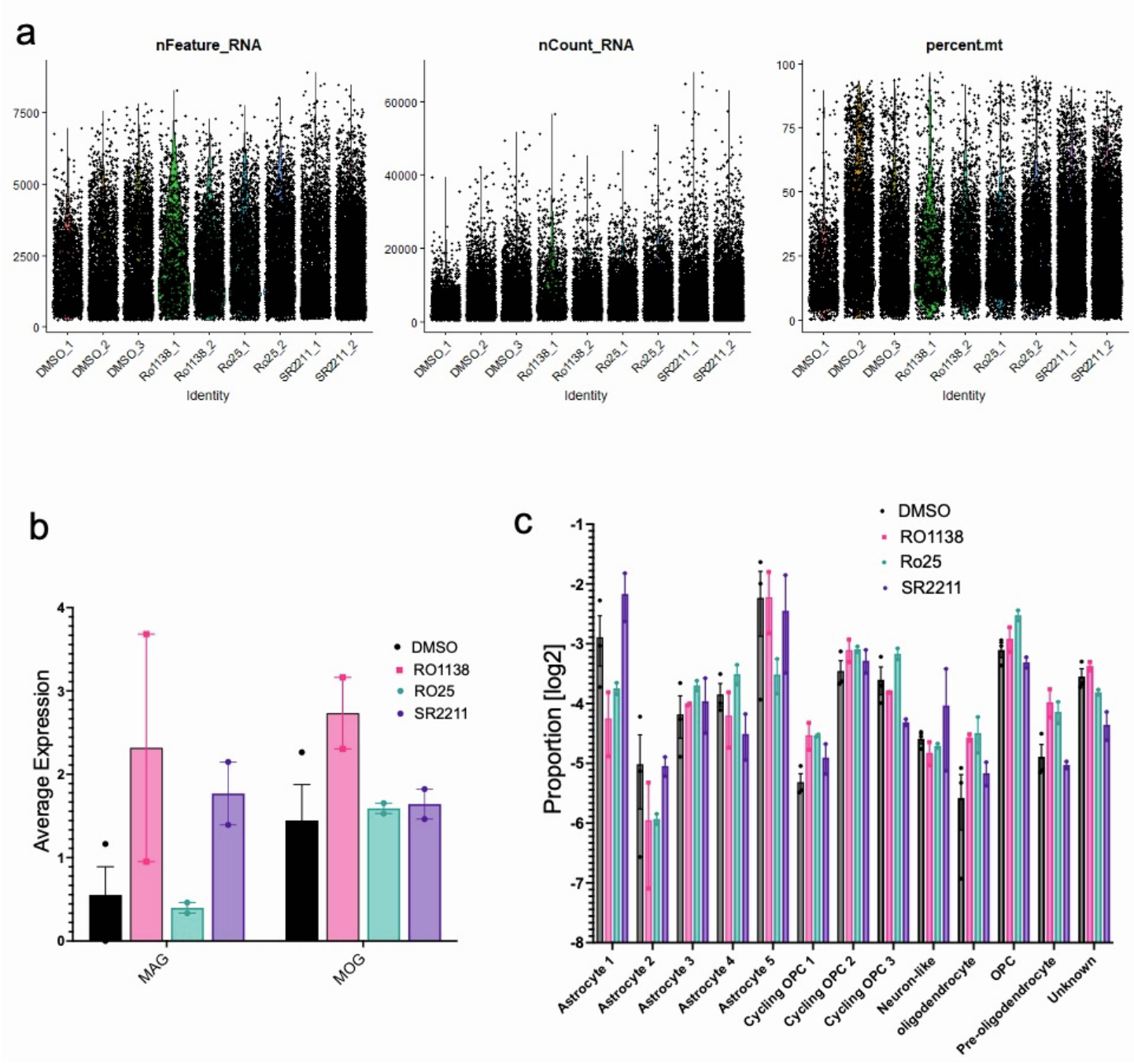


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Figure S11. UMAP clustering of the single cell data, related to Figures 7 and 8**. a)** A table illustrating the number of cells used for the analysis for each sample after quality control. **b)** cell-type label probability per cell transferred from the reference. **c)** Distribution of various genetic markers among the different clusters presented as an enrichment heatmap. Blue color represents enrichment. **d)** Pseudotime analysis show the distribution of cell by maturity within the UMAP cluster, and the trajectory analysis illustrates potential path through which the cells mature from cycling progenitor to oligodendrocytes and astrocytes.

a

sample	Cell number
DMSO1	1705
DMSO2	1452
DMSO3	2244
SR2211-1	3507
SR2211-2	3937
Ro1138-1	800
Ro1138-2	1503
Ro25-1	1553
Ro25-2	2012