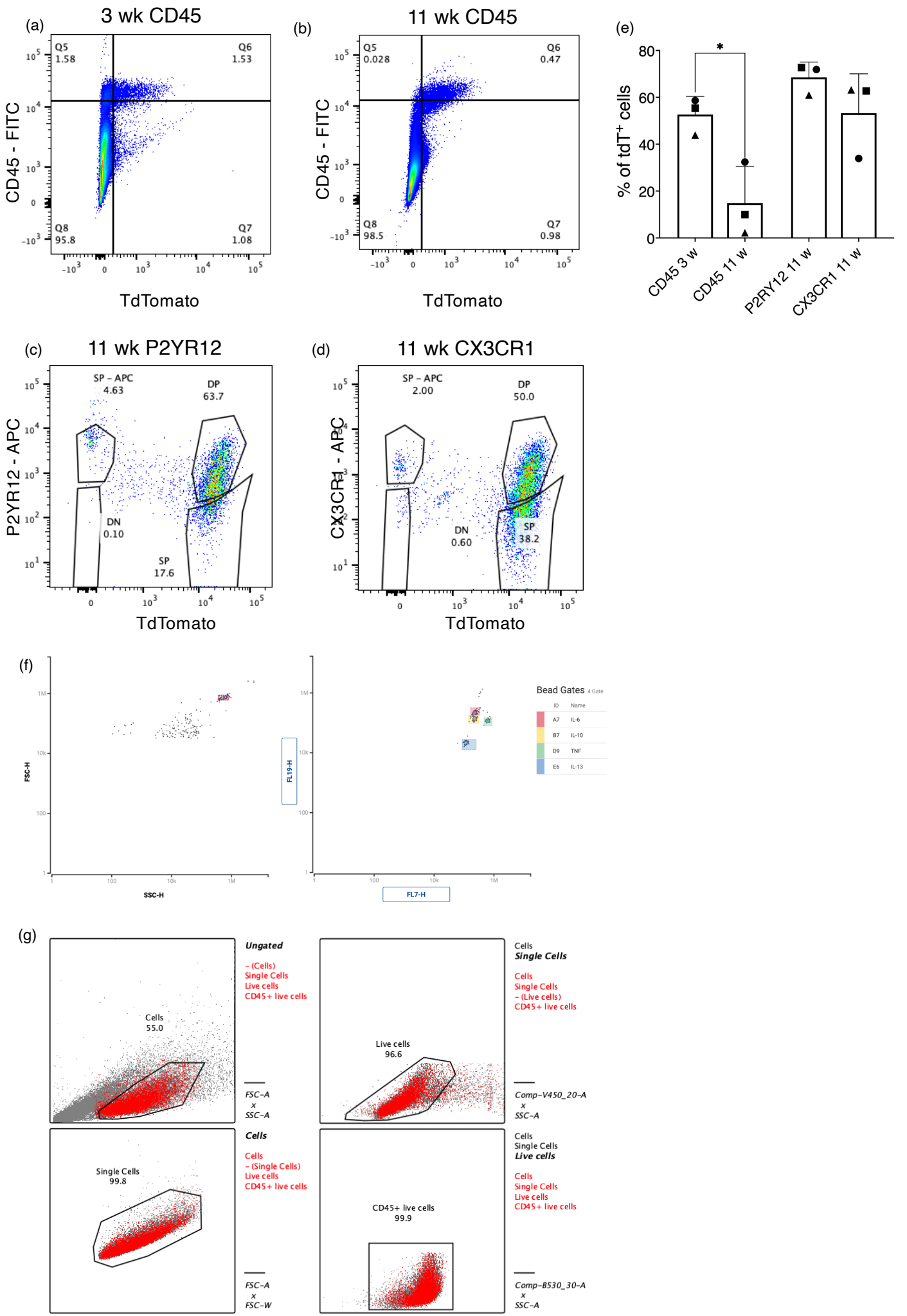


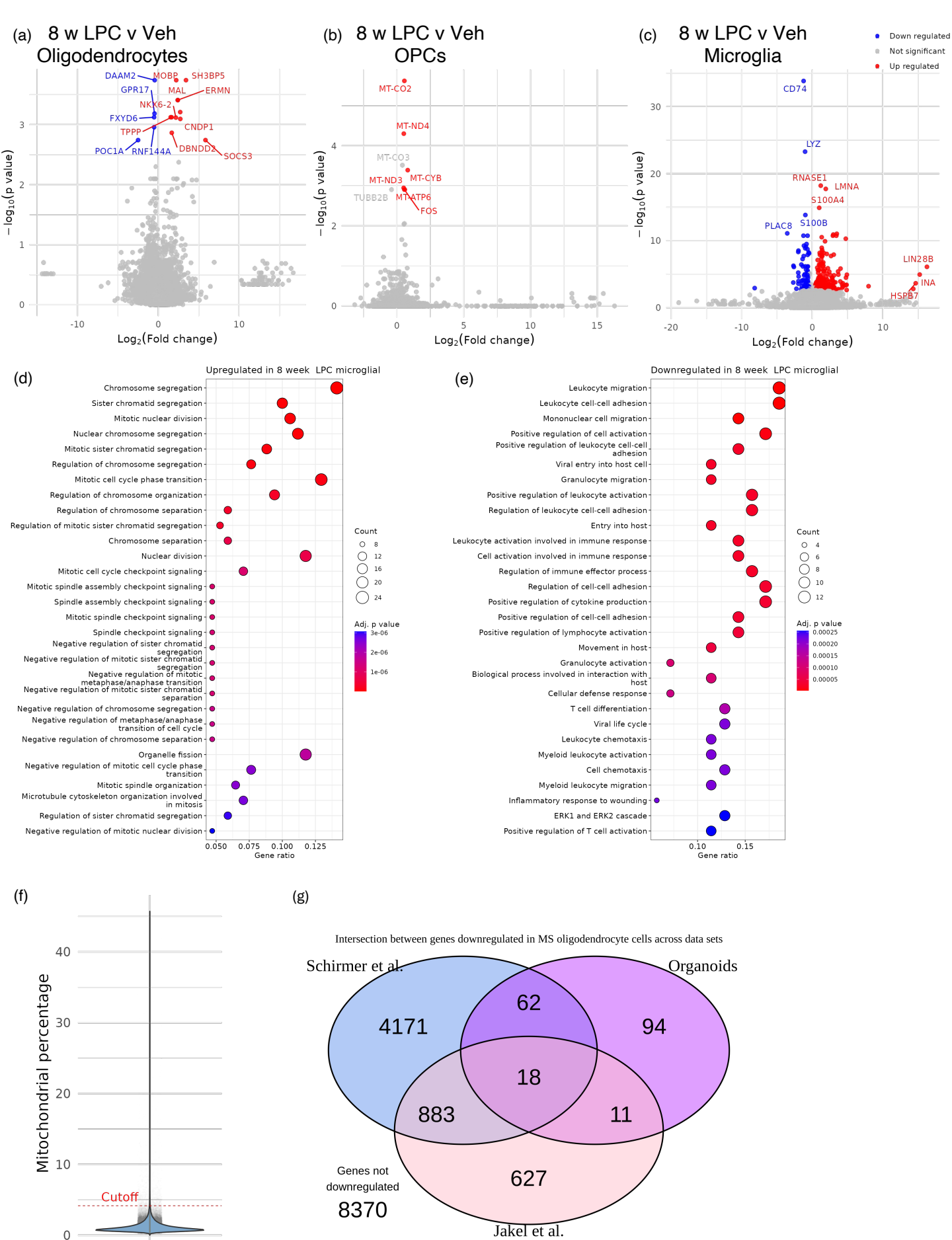
A human-induced pluripotent stem cell-derived spheroid model to investigate myelin injury and repair

In the format provided by the
authors and unedited



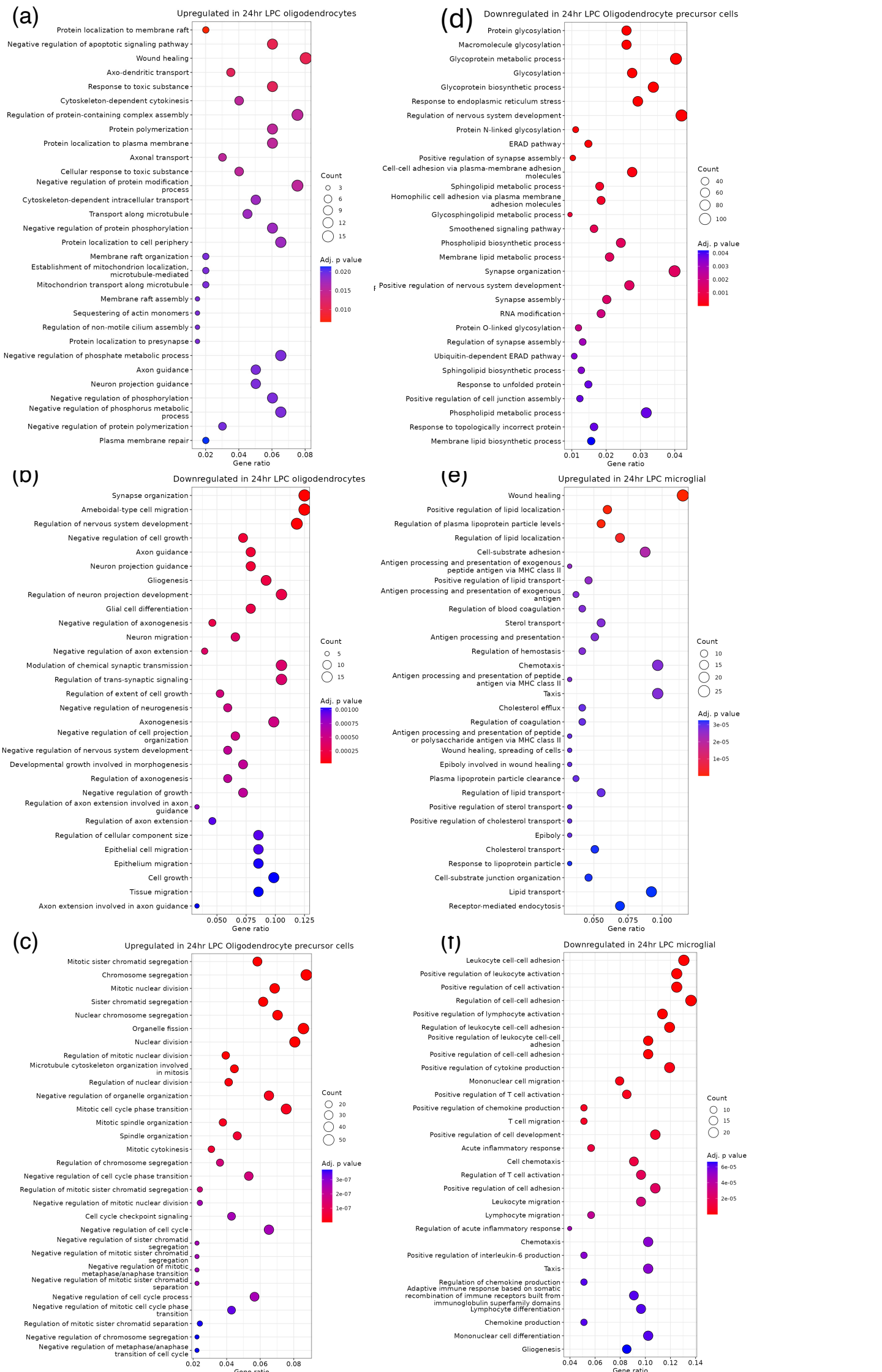
Supplemental Figure 1: FACS validation of microglia

(a) Single cell suspensions from week 3 iPSC-derived tdTomato+ macrophage-like cells were stained with CD45. Single cell suspensions from 11 week 18a spheroids containing tdtTomato+ cells were stained with immune markers: (b) CD45; (c) P2RY12; and (d) CX3CR1. (e) quantification of the proportion of tdTomato+ cells double positive for CD45, P2RY12 and CX3CR1. The CD45 3 w was a vial of frozen macrophage-like single cells; the CD45 11 w, P2RY12 and CX3CR1 were dissociated 11 w 18a spheroids. Graph is mean ± SD. Each symbol depicts averaged technical replicates from one FACS run and different symbols depict independent differentiations of spheroids from iPSC (n=3). * denotes p<0.05 (Student's t-test run between CD45 3 w and CD45 11 w). FACS plots showing gating for beads in the cytometric bead array (f) and for microglial phenotyping (g).

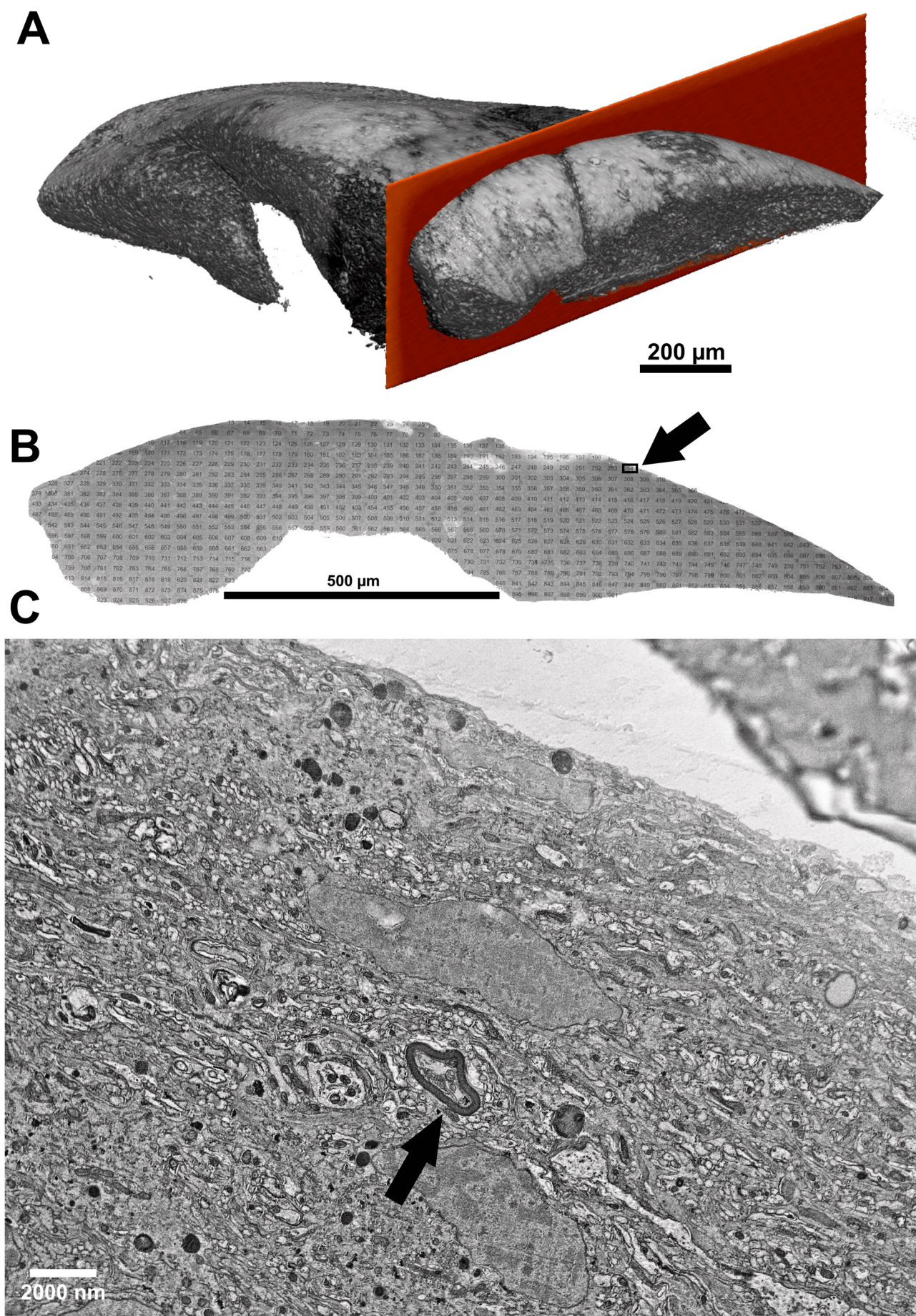


Supplemental Figure 2: Single cell RNA sequencing enrichment analysis on 8 w LPC/Veh spheroids

Volcano plot of genes ($|\log_2\text{FC}| > 0.43$, $\text{FDR} < 0.0025$) for the 8 w oligodendrocytes (a), OPCs (b) and microglia (c). The top 30 (ordered by p-value, $\text{FDR} < 0.05$) gene ontology enrichment terms from overrepresentation analysis for upregulated (c) and downregulated (d) genes in 8 w microglia. (f) Violin plot of the MT-percent across cells for quality control. (g) Venn diagram of the intersection of the downregulated genes between our LPC 24 h oligodendrocyte data, Jäkel et al. data (oligodendrocytes from active lesions) [19] and Schirmer et al. data (oligodendrocytes from active lesions) [20] shows a significant association between datasets (Fischer's exact test $p < 0.05$).



Supplemental Figure 3: Single cell RNA sequencing on 24 h LPC/Veh spheroids
The top 30 (ordered by p-value, FDR<0.05) gene ontology enrichment terms through overrepresentation analysis for upregulated (a) genes and downregulated (b) genes in 24 h LPC oligodendrocytes compared to Veh. The top 30 (ordered by p-value, FDR<0.05) gene ontology enrichment terms through overrepresentation analysis for upregulated (c) genes and downregulated (d) genes in 24 h LPC OPCs compared to Veh. The top 30 (ordered by p-value, FDR<0.05) gene ontology enrichment terms through overrepresentation analysis for upregulated (e) genes and downregulated (f) genes in 24 h LPC microglia compared to Veh.



Supplemental Figure 4: FIB-SIM targeting of myelinated axons

Myelinated axon targeting. A – 3D-rendering of a micro-CT dataset, the edge, which was chosen for cross section, is marked with a plane; B – SEM image of the edge with numbered tiles, each tile is 6144×4096 pixels. The tile containing a myelinated axon (C and black arrow showing position of myelinated axon). Scale bars: A – 200 μm , B – 500 μm , C – 2 μm .

Supplemental Table 1: Summary of the number of iPSC-organoid differentiations and cell numbers quantified in Veh and LPC organoids at different treatment timepoints			
Time	Number of differentiations	Number of cells	
		Veh	LPC
5 h	3	2523	843
24 h	3	539	1081
7 d	4	4529	3114
14 d	3	4296	3523

Supplemental Table 2: Summary of differences in microglial protein expression between LPC and Veh treated iPSC-derived organoids.				
A IBA1				
Time	Difference log(mean AI) ± SE (Veh - LPC)	p-value	Cohen's d ± SE	Biological significance
5 h	-0.0654 ± 0.0100	< 0.0001	-0.3005 ± 0.0459	Medium
24 h	-0.0186 ± 0.0119	0.1155	-0.0856 ± 0.0544	-
7 d	0.0002 ± 0.0059	0.9765	0.0008 ± 0.0271	-
14 d	0.0735 ± 0.0050	< 0.0001	0.3377 ± 0.0230	Medium
B L-ferritin				
Time	Difference log(mean AI) ± SE (Veh - LPC)	p-value	Cohen's d ± SE	Biological significance
5 h	0.0386 ± 0.0098	0.0001	0.1820 ± 0.0460	Low
24 h	-0.0360 ± 0.0116	0.0019	-0.1700 ± 0.0545	Low
7 d	0.0949 ± 0.0058	< 0.0001	0.4470 ± 0.0272	Medium
14 d	0.0841 ± 0.0049	< 0.0001	0.3960 ± 0.0230	Medium
C HLA-DR				
Time	Difference log(mean AI) ± SE (Veh - LPC)	p-value	Cohen's d ± SE	Biological significance
5 h	-0.0429 ± 0.0132	0.0011	-0.1500 ± 0.0459	Low
24 h	-0.3335 ± 0.0156	< 0.0001	-1.163 ± 0.0544	High
7 d	-0.2611 ± 0.0078	< 0.0001	-0.9110 ± 0.0271	High
14 d	0.1213 ± 0.0066	< 0.0001	0.4230 ± 0.0230	Medium
D CD68				
Time	Difference log(mean AI) ± SE (Veh - LPC)	p-value	Cohen's d ± SE	Biological significance
5 h	0.0285 ± 0.0122	0.0200	0.1070 ± 0.0459	Low
24 h	-0.1240 ± 0.0145	< 0.0001	-0.4650 ± 0.0544	Medium
7 d	0.0784 ± 0.0072	< 0.0001	0.2940 ± 0.0271	Low
14 d	0.0997 ± 0.0062	< 0.0001	0.3730 ± 0.0230	Medium
Abbreviations: AI, average intensity; d, days; h, hours; SE, standard error				