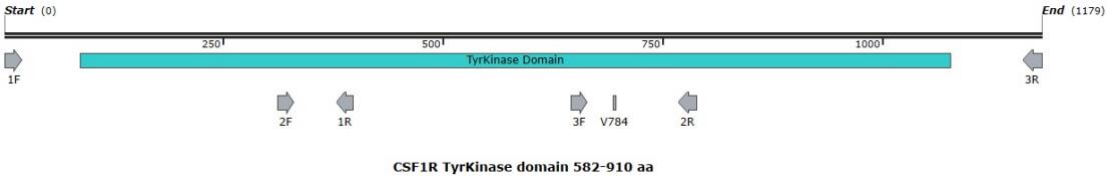
**A**




**B**

| Primer name | Sequence (5'-3') |
|---|---|
| CSF1R-TyrKinase-1F | TCGAGAGCTATGAGGGCAAC |
| CSF1R-TyrKinase-1R | GTCCCAGCATGGCCTCAG |
| CSF1R-TyrKinase-2F | AGGCCCTGTACTGGTCATCA |
| CSF1R-TyrKinase-2R | GCATTGCCCTTGACAATGTA |
| CSF1R-TyrKinase-3F | TTCCTCGCTTCCAAGAATTG |
| CSF1R-TyrKinase-3R | TCTCCTCCTCCAGCTCACTG |

**Figure S1. Sequencing strategy of the region encoding CSF1R tyrosine kinase domain.**

(A) Locations where the primers would anneal. (B) Sequences of primers.

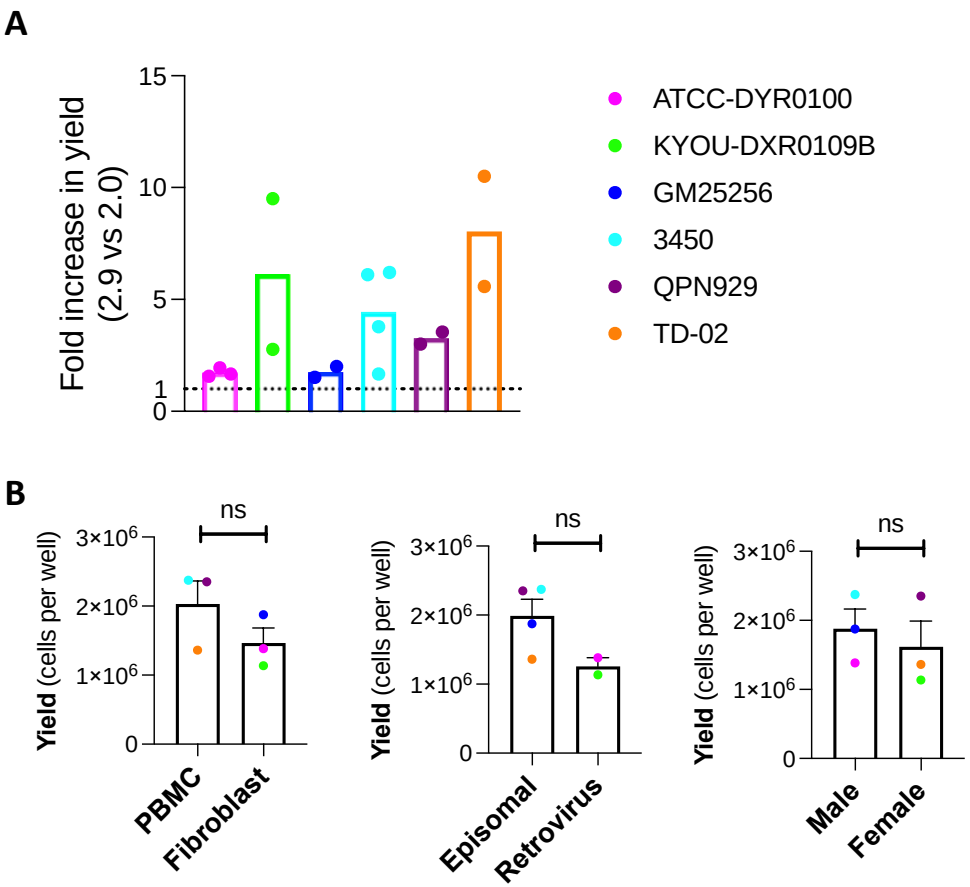
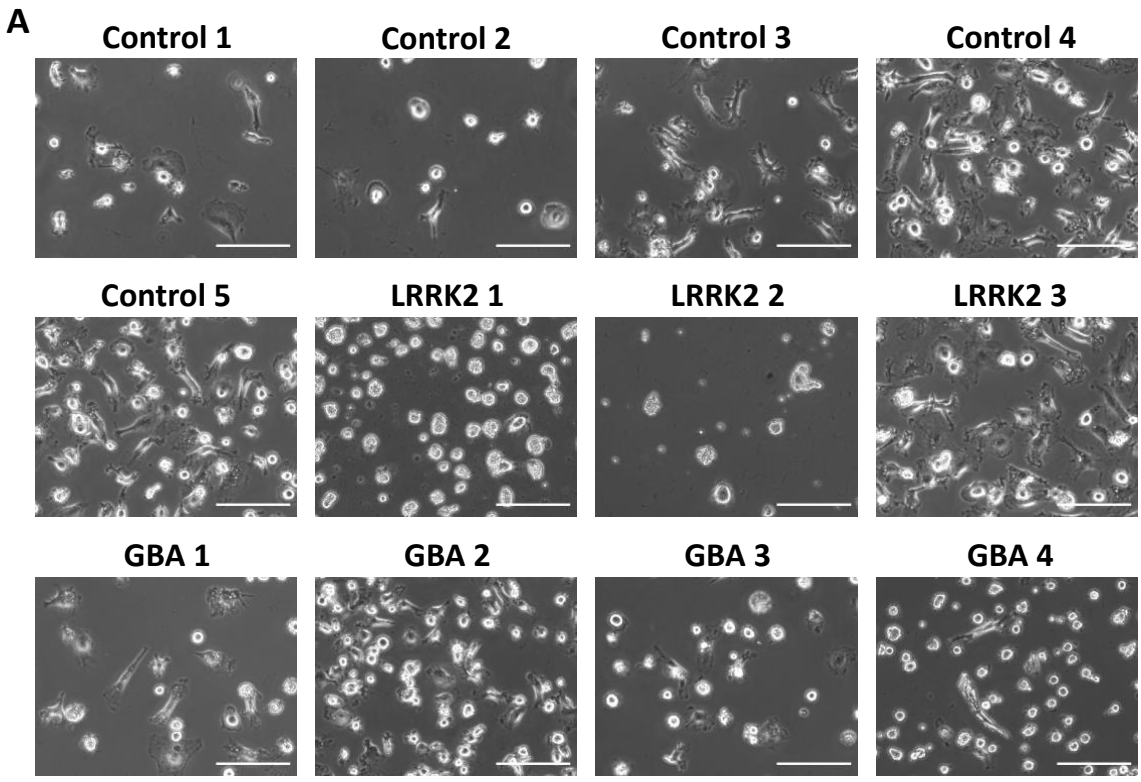



**Figure S2. Cell yield of protocols 2.0 and 2.9.** iMGL 2.0 and 2.9 were generated side-by-side from six iPSC lines. (A-B) Viable cell yield assessed by Trypan blue exclusion assay. 2-4 batches of differentiation were made per iPSC line. Average of those batches are presented in (B). T-tests were performed in (B), ns = non-significant.



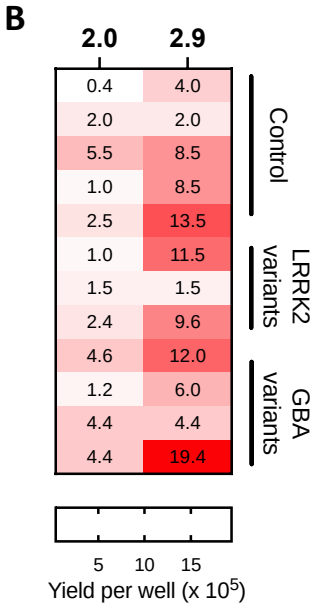




**Figure S3. Differentiation of Parkinson's disease patient-derived iPSCs into iMGL 2.9.** (A) Phase contrast images of mature iMGL. Scale bar = 150 $\mu m$. (B) Viable cell yield per well of a 6-well plate assessed by Trypan blue exclusion assay. n = 1 per cell line.

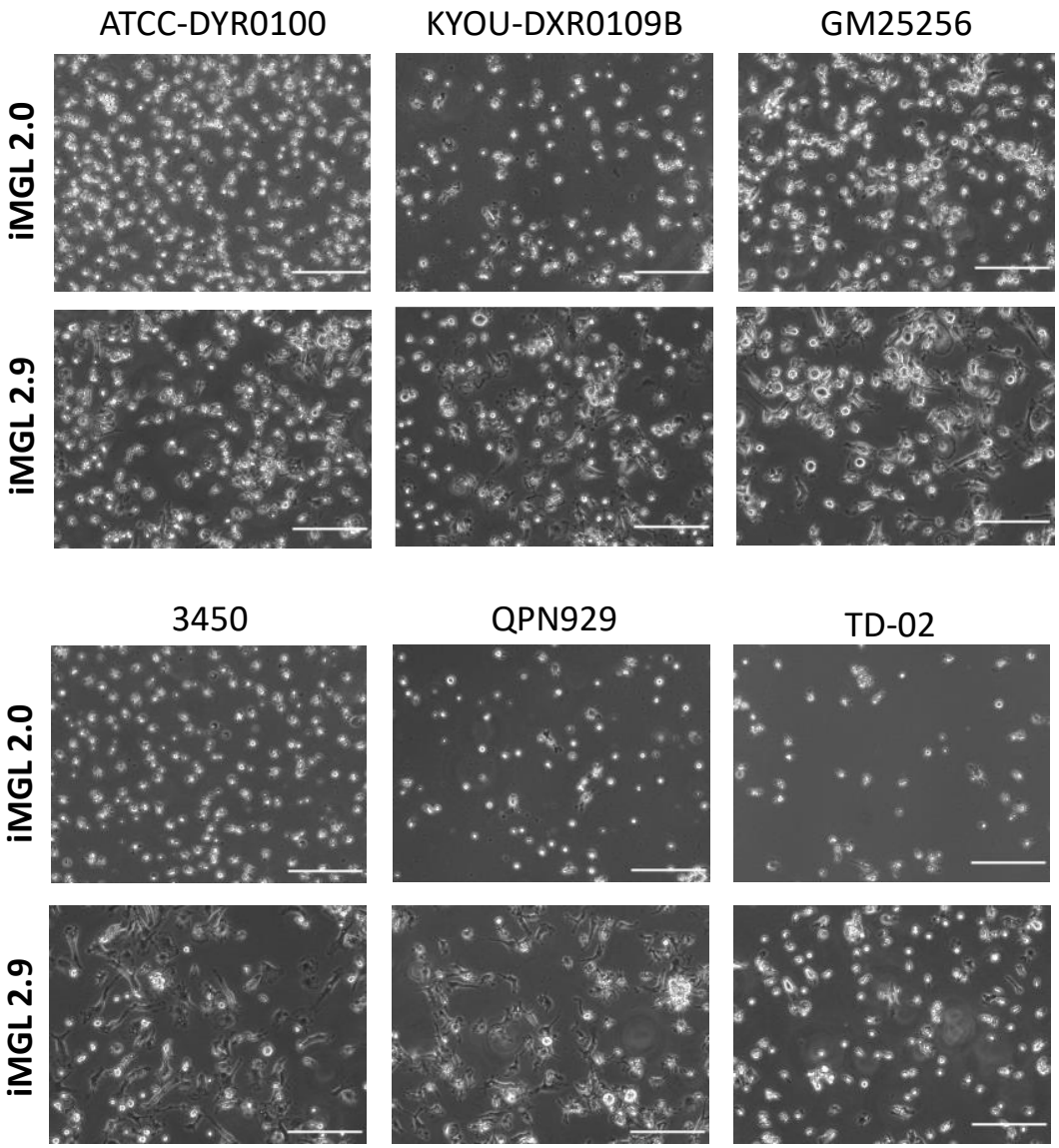



**Figure S4. Morphology of iMGL 2.0 and 2.9.** iMGL 2.0 and 2.9 were generated side-by-side from six iPSC lines. (A) Viable cell yield assessed by Trypan blue exclusion assay. n = 2-3 per iPSC line. (B) Phase contrast images. Scale bar = 150 $\mu m$.

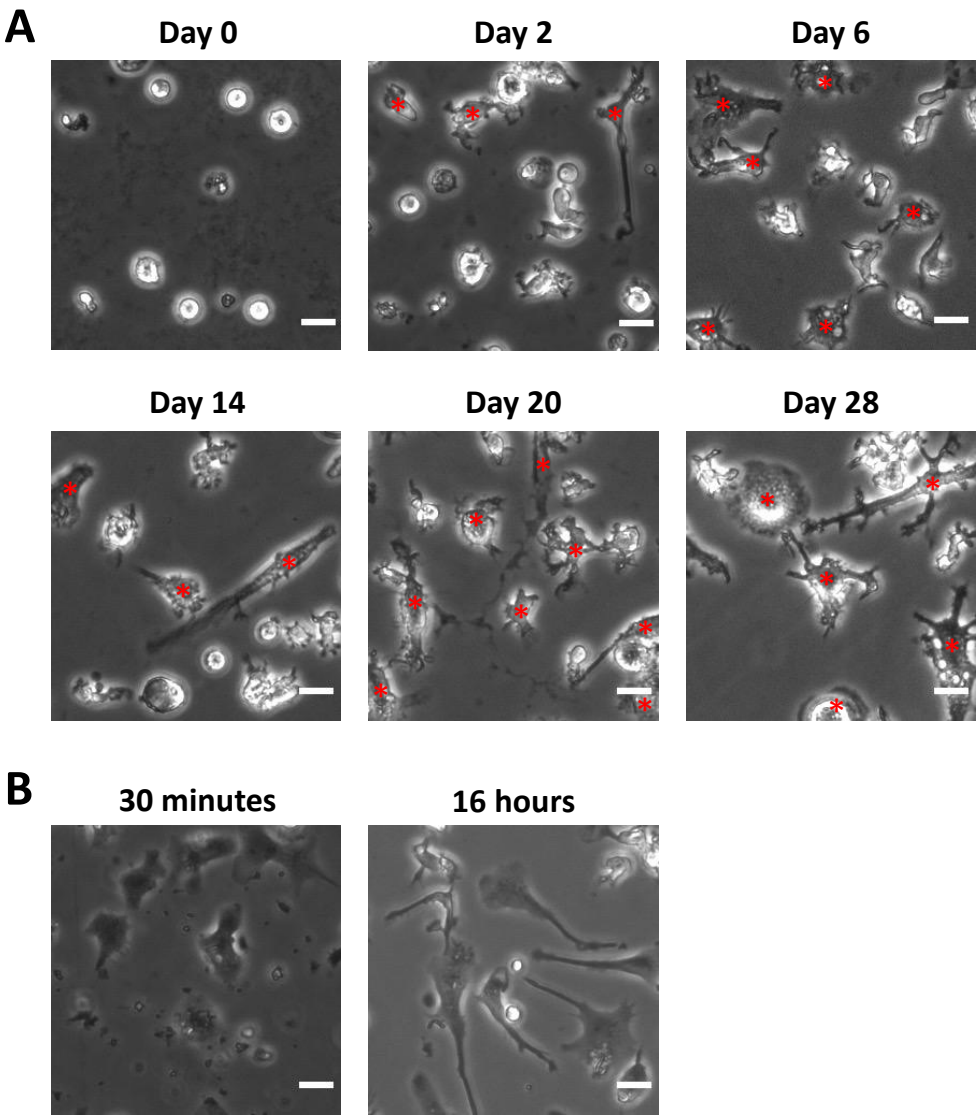



**Figure S5. Adhesive property of iMGL 2.9.** (A) Phase contrast images of iMGL 2.9 throughout microglial differentiation. Adherent cells are marked by red asterisks. Scale bar = 20 μm. (B) Phase contrast images of mature iMGL 2.9 following replating. Scale bar = 20 μm.

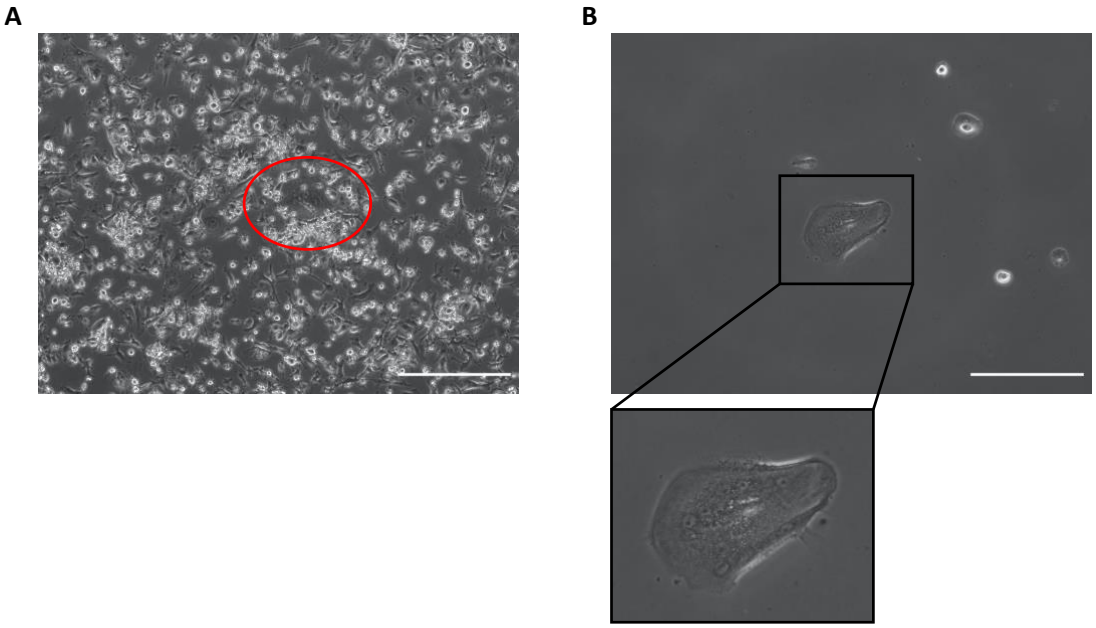



**Figure S6. Multinucleated giant cell contaminants.** Phase contrast pictures of iMGL 2.9 culture before (A) and after (B) EDTA-mediated cell harvest. Red circle in (A) depicts a multinucleated giant cell underneath mononuclear iMGL. Black square in (B) shows a zoomed-in image of a multinucleated giant cell. Scale bar = 300 μm.

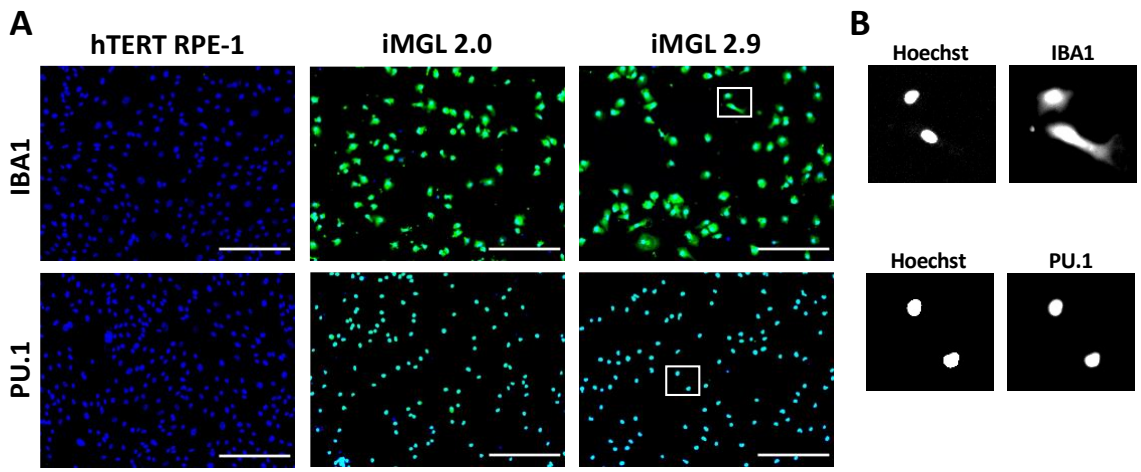



**Figure S7. IBA1 and PU.1 immunostaining.** iMGL 2.0 and 2.9 were generated side-by-side from the same iPSC line. (A) Representative images. Blue = Hoechst 33342, green = IBA1 or PU.1, scale bar = 200 μm. (B) Zoomed, unmerged images of inserts in (A).

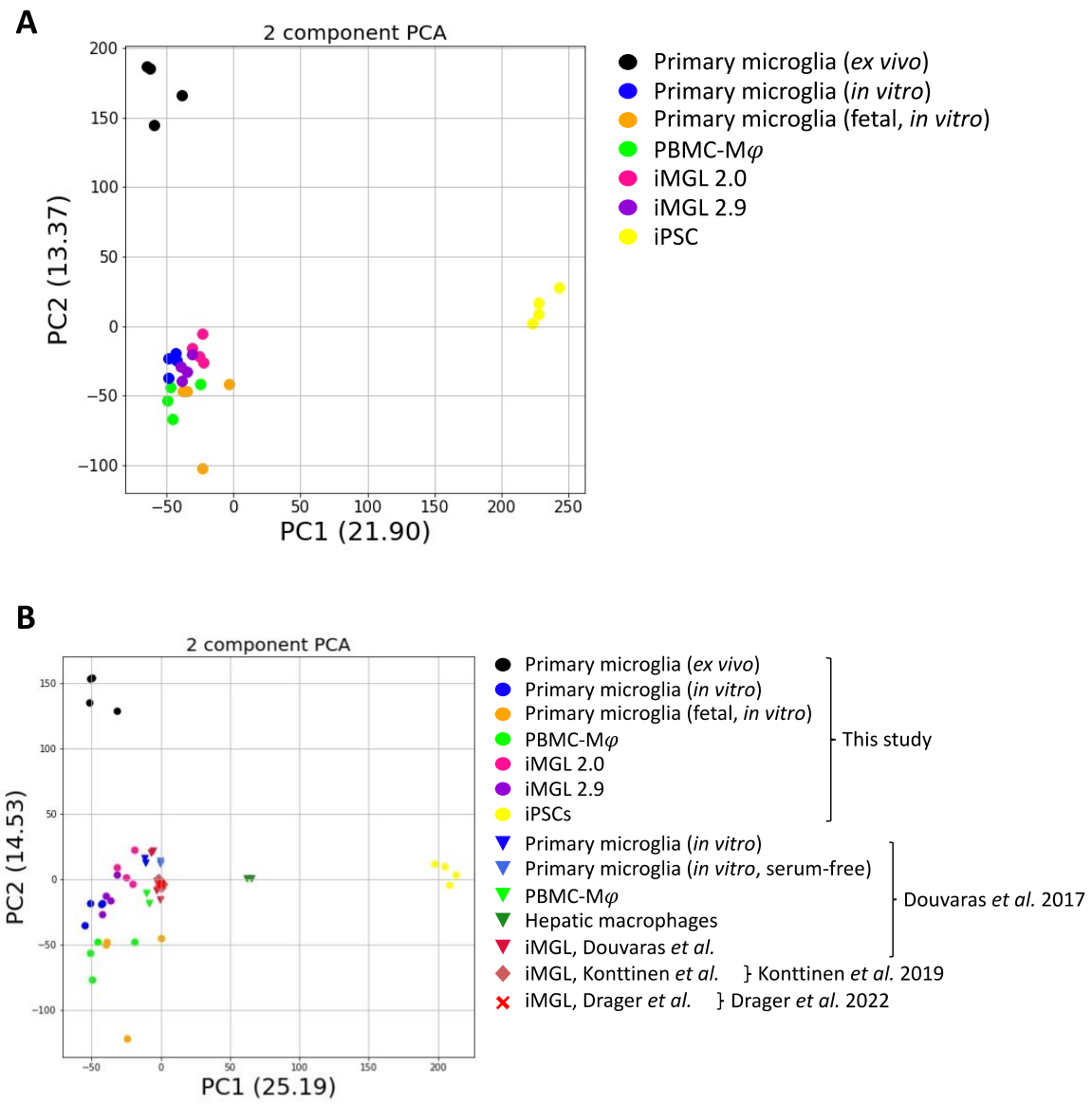



**Figure S8. Transcriptome of iMGL 2.0 and 2.9 compared to primary microglia and iPSCs.** (A-B) PCA plots of RNA-sequencing data are presented. iMGL 2.0 and 2.9 were differentiated side-by-side from the same four iPSC lines.

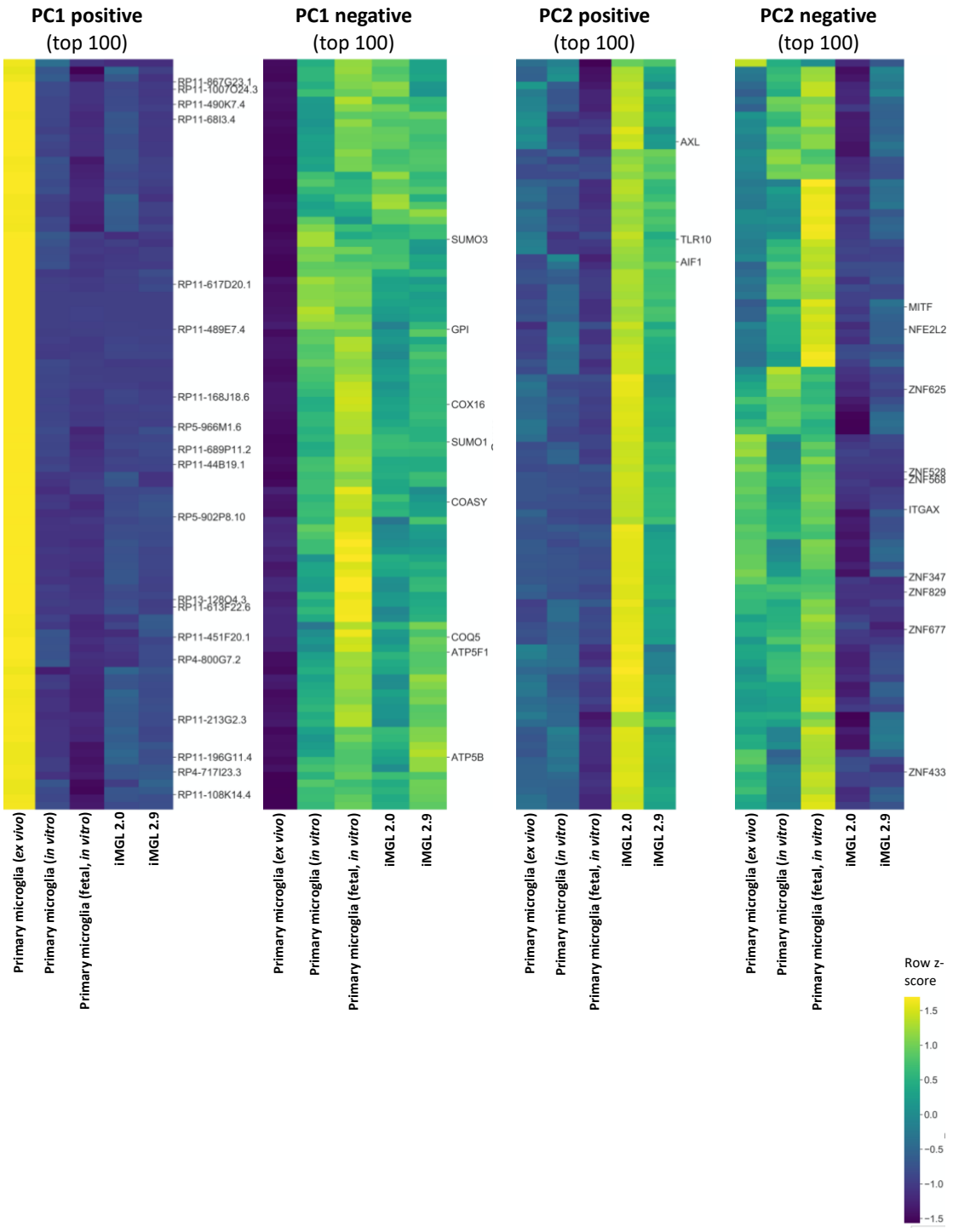



**Figure S9. Top genes driving PC1 and PC2 variances.** iMGL 2.0 and 2.9 were generated side-by-side from the same iPSC lines. n = 4 donors for primary cells, 4 lines for iMGL**.**

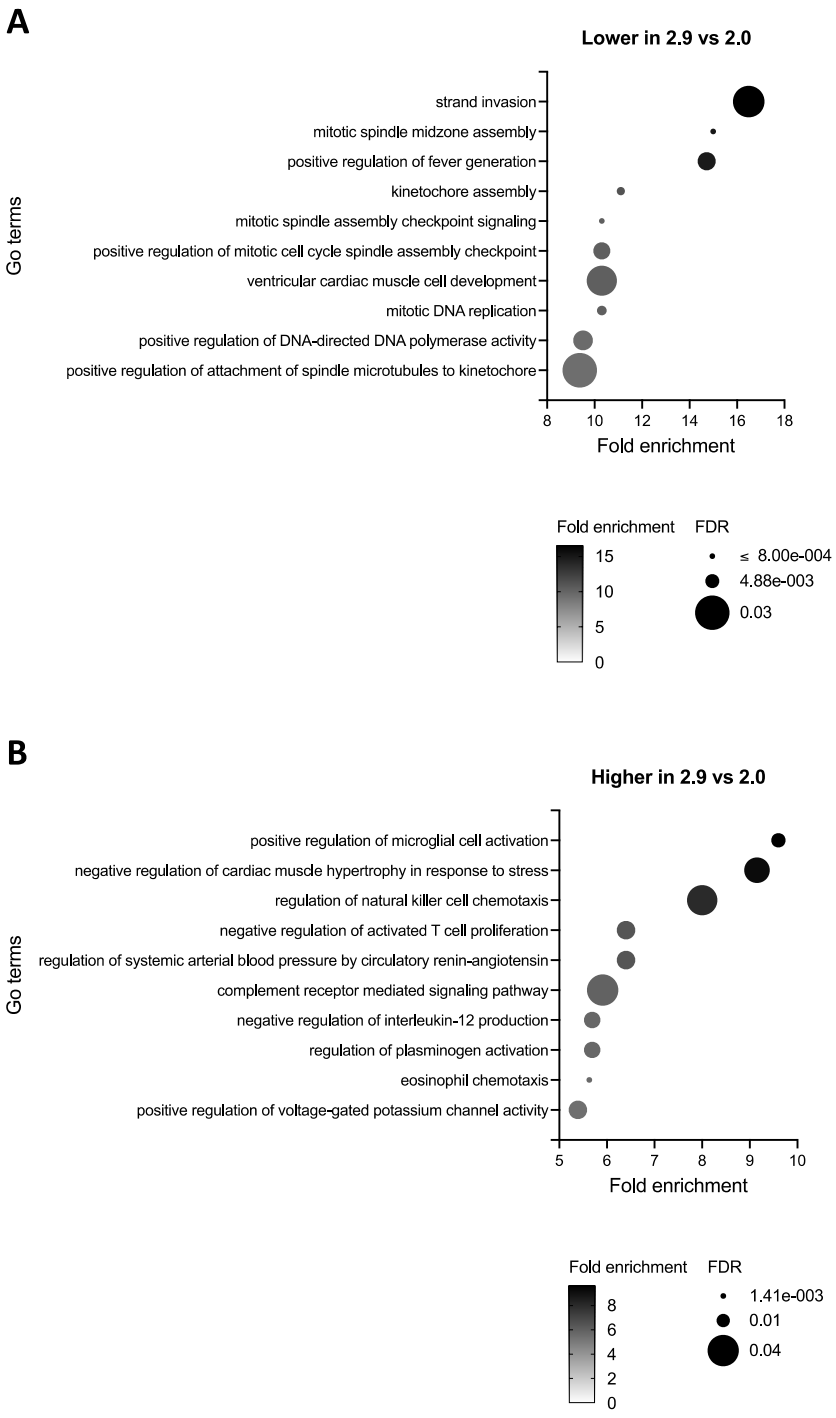



**Figure S10. GO term analysis of DEGs identified between iMGL 2.9 and 2.0.** iMGL 2.0 and 2.9 were generated side-by-side from the same four iPSC lines. FDR = false discovery rate.

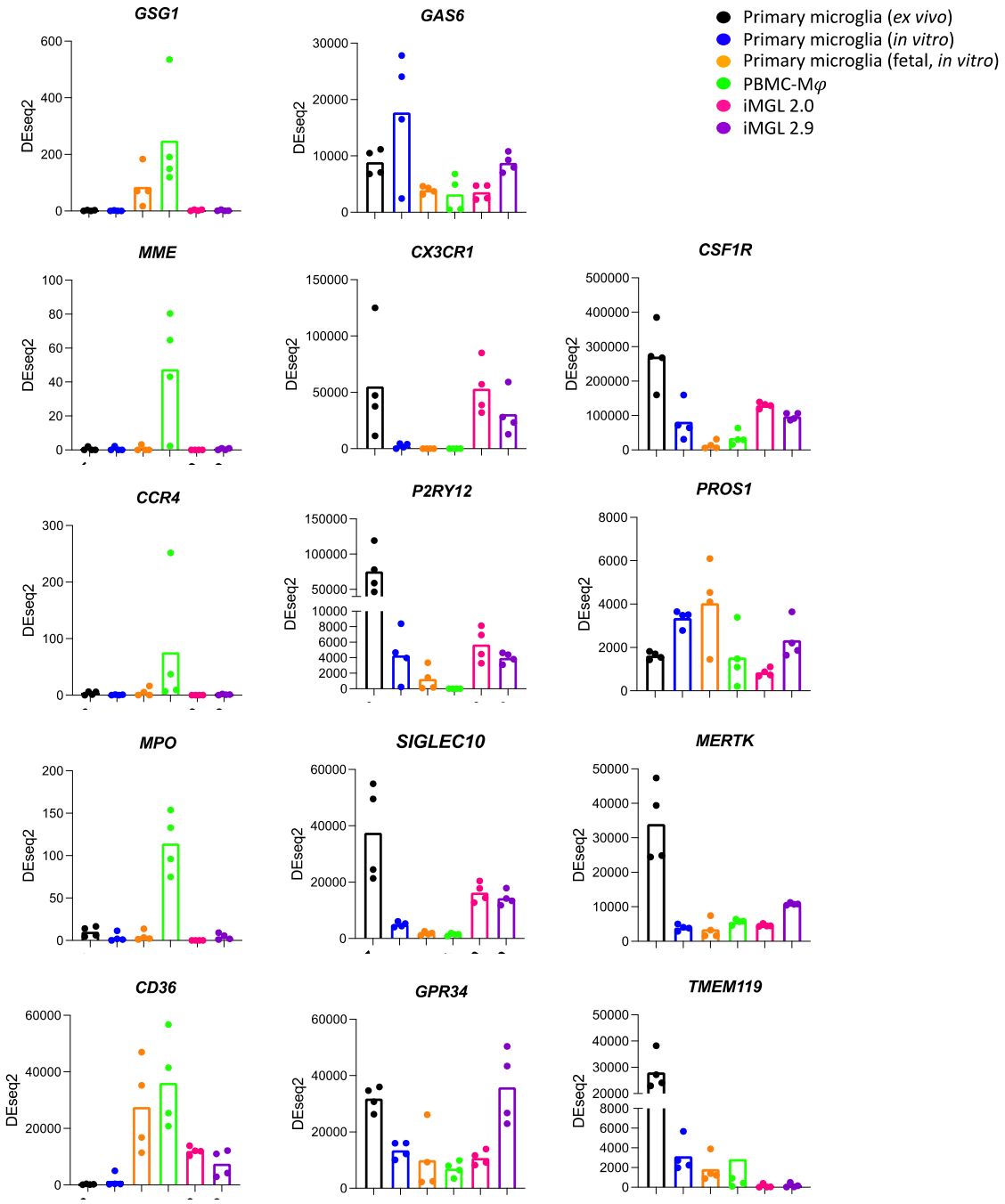



**Figure S11. RNAseq analysis of iMGL 2.9 transcriptome.** iMGL 2.0 and 2.9 were generated side-by-side from the same iPSC lines. Expression of select macrophage and microglia markers are presented. n = 4 donors for primary cells, 4 lines for iMGL.

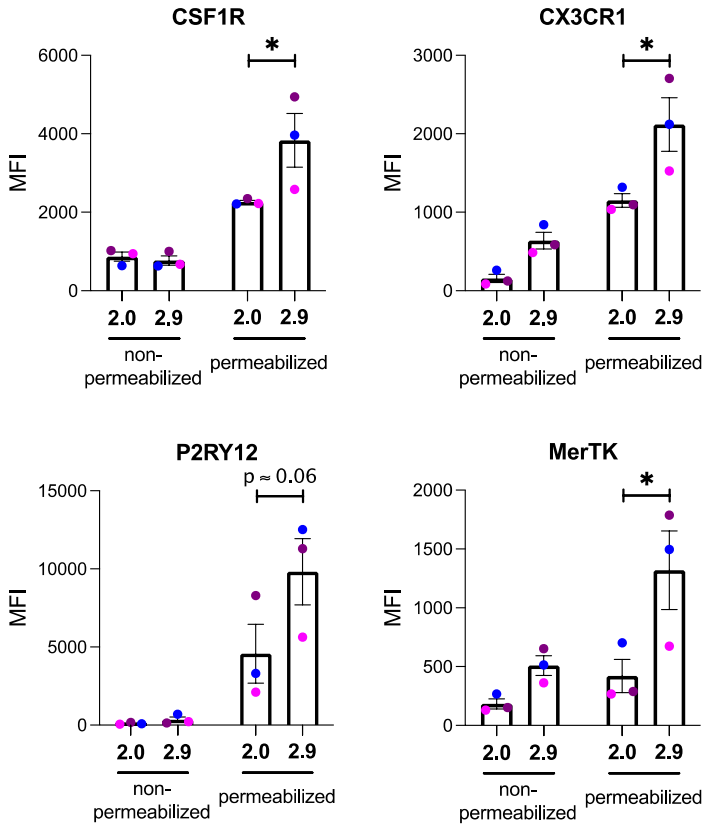



**Figure S12. Flow cytometry assessment of microglia markers in iMGL 2.0 and 2.9.** iMGL 2.0 and 2.9 were generated side-by-side from the same iPSC lines. Two-way ANOVA were performed, followed by Sidak's post hoc test. n = 3 lines, * $p < 0.05$. MFI = median fluorescence intensity.

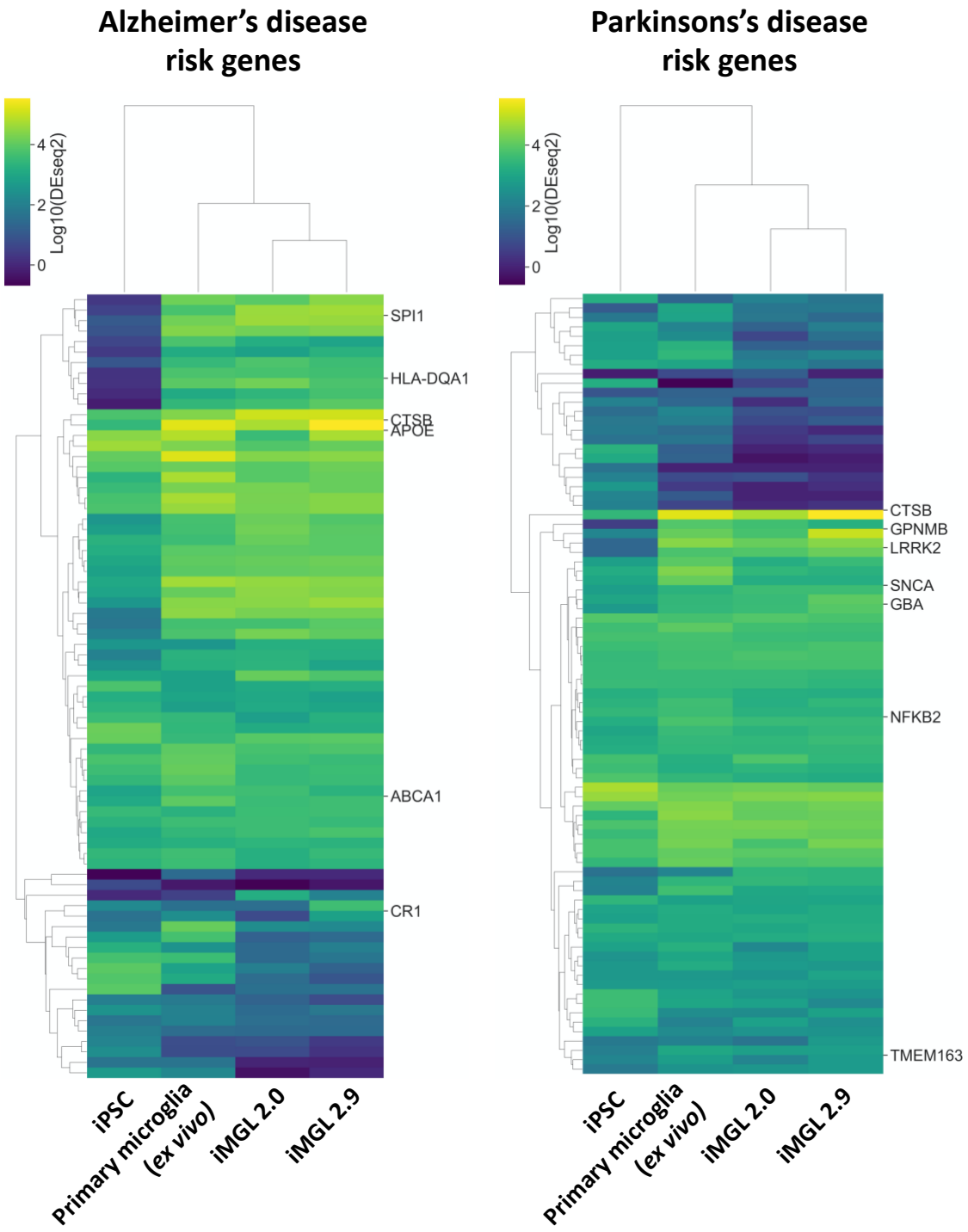



**Figure S13. Expression of disease-associated risk genes in iMGL 2.0 and 2.9.** iMGL 2.0 and 2.9 were generated side-by-side from the same iPSC lines. n = 4 donors for primary microglia, 4 lines for iMGL and iPSCs.

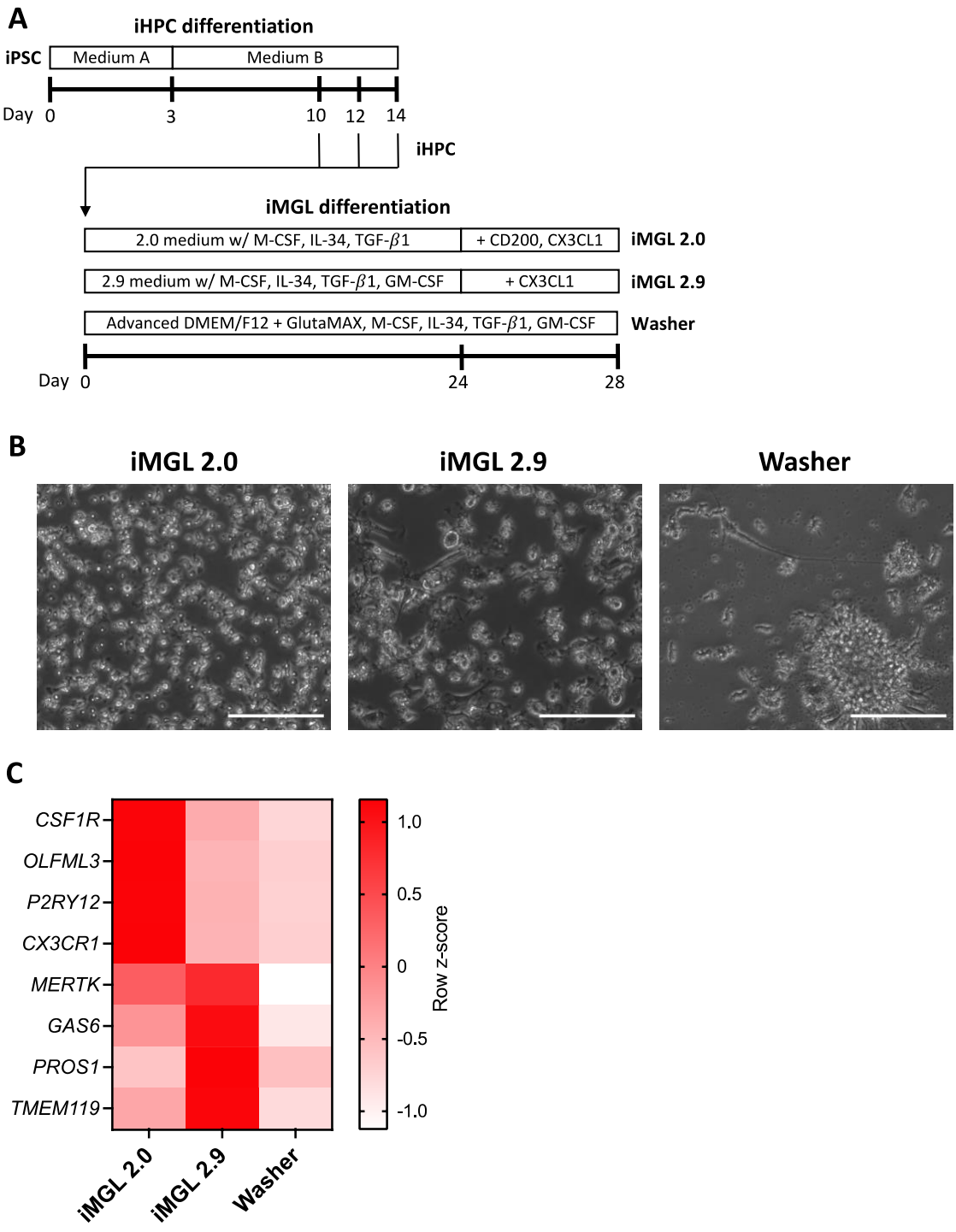



**Figure S14. iMGL derived using Washer *et al.*'s microglial differentiation medium.** (A) Schematic of the experimental design. iHPCs were subjected to microglial differentiation using the 2.0, the 2.9 or Washer *et al.*'s differentiation media, side-by-side

from the same iPSC lines. Scale bar = 150 $\mu m$. (B) Phase contrast images taken at day 28. (C) qRT-PCR assessment of microglia marker genes. n = 2 lines.

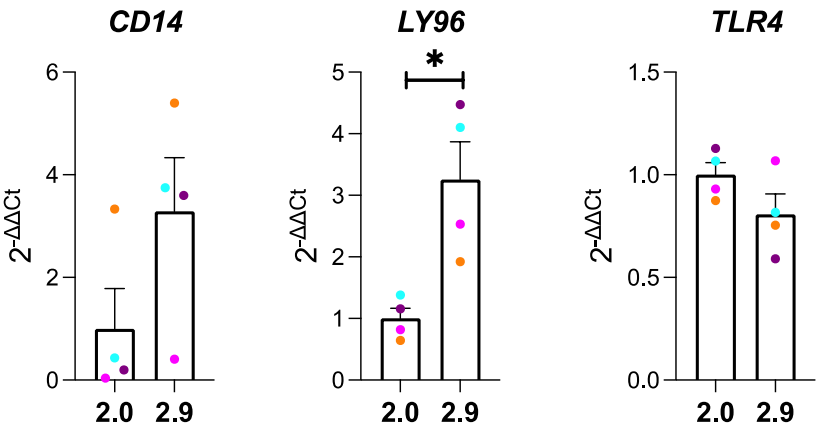



**Figure S15. qRT-PCR assessment of *CD14*, *LY96* and *TLR4* expression in iMGL 2.0 and 2.9.** iMGL 2.0 and 2.9 were differentiated side-by-side from the same iPSC lines. T-tests were performed. n = 4 lines, * $p < 0.05$.

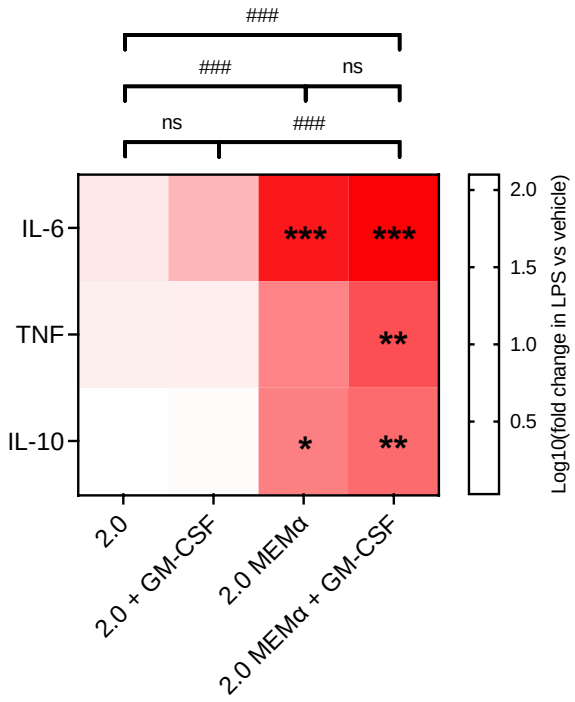



**Figure S16. Media-dependent inflammatory response of iMGL to LPS.** iMGL were differentiated using the indicated media formulation and then treated with vehicle or LPS (100 ng/mL) for 24 hours. Cytokine secretion in cell supernatants was assessed. A two-way ANOVA was performed, followed by Dunnett's post hoc test (asterisks) and Tukey's post hoc test (hash signs). n = 3 lines, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs iMGL 2.0, ns = non-significant, ### $p < 0.001$.

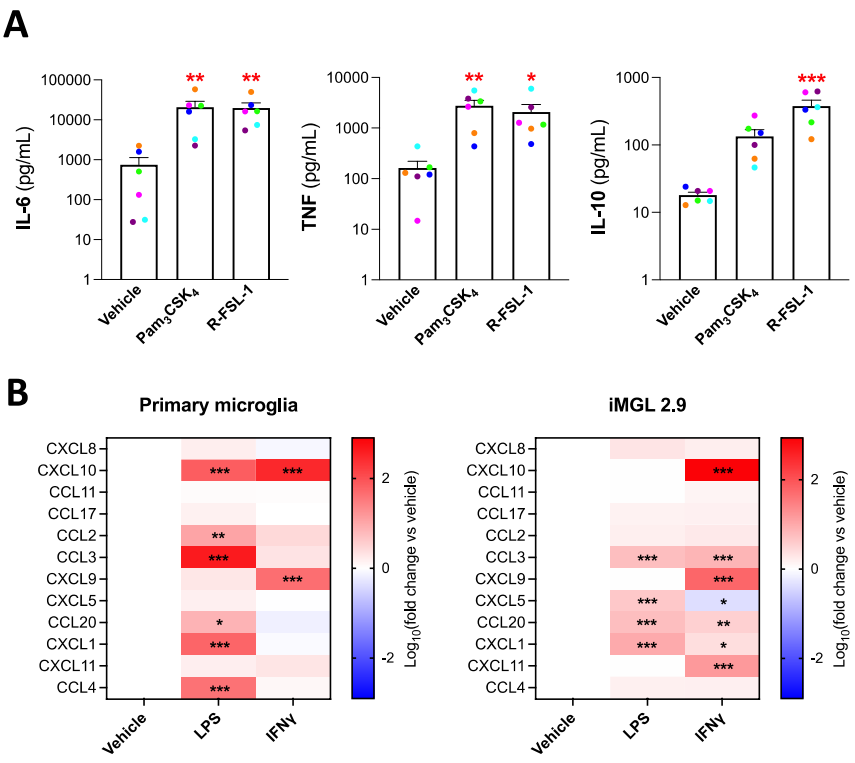



**Figure S17. Inflammatory response of iMGL 2.9 to a variety of stimuli.** (A) IL-6, TNF and IL-10 concentrations in supernatants from iMGL 2.9 treated with vehicle, $Pam_3CSK_4$ (100 ng/mL) or R-FSL-1 (250 ng/mL) for 24 hours. One-way ANOVA were performed, followed by Dunnett's post hoc tests. n = 6 lines, * $p < 0.05$, ** $p < 0.01$ vs vehicle. (B) Heatmap representing chemokine secretion from primary microglia or iMGL 2.9 following a 24-hour treatment with LPS (100 ng/mL) or IFNγ (10 ng/mL). Two-way ANOVA were performed, followed by Dunnett's post hoc tests. n = 2 donors for primary microglia and 3 lines for iMGL. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs vehicle.