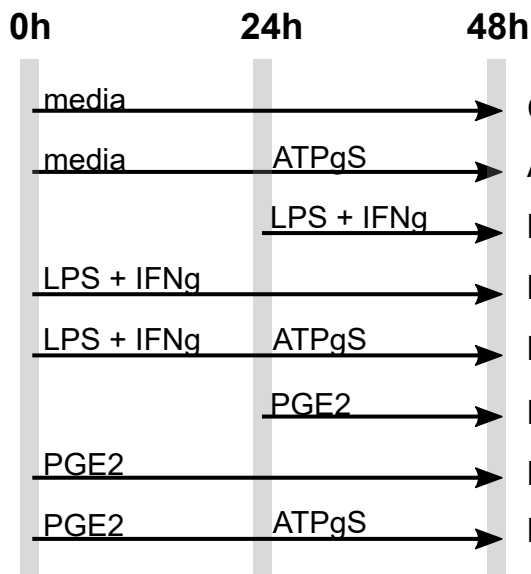


**A**

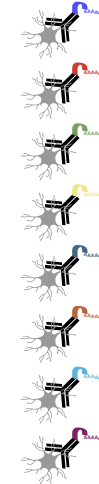


### iPSC-derived microglia

8 conditions, 4 biological replicates



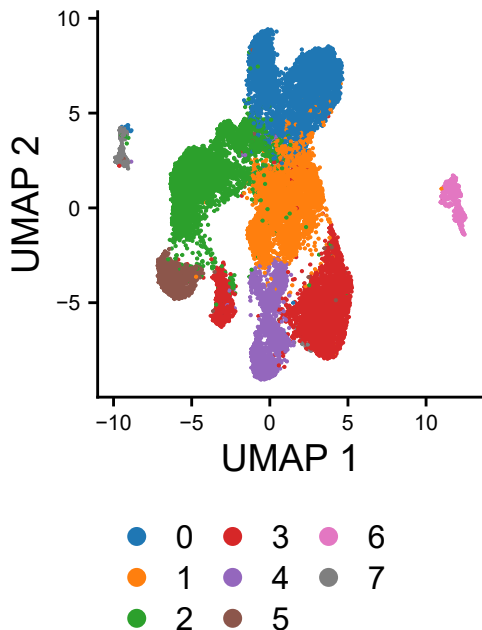
HashTagOligo labelling



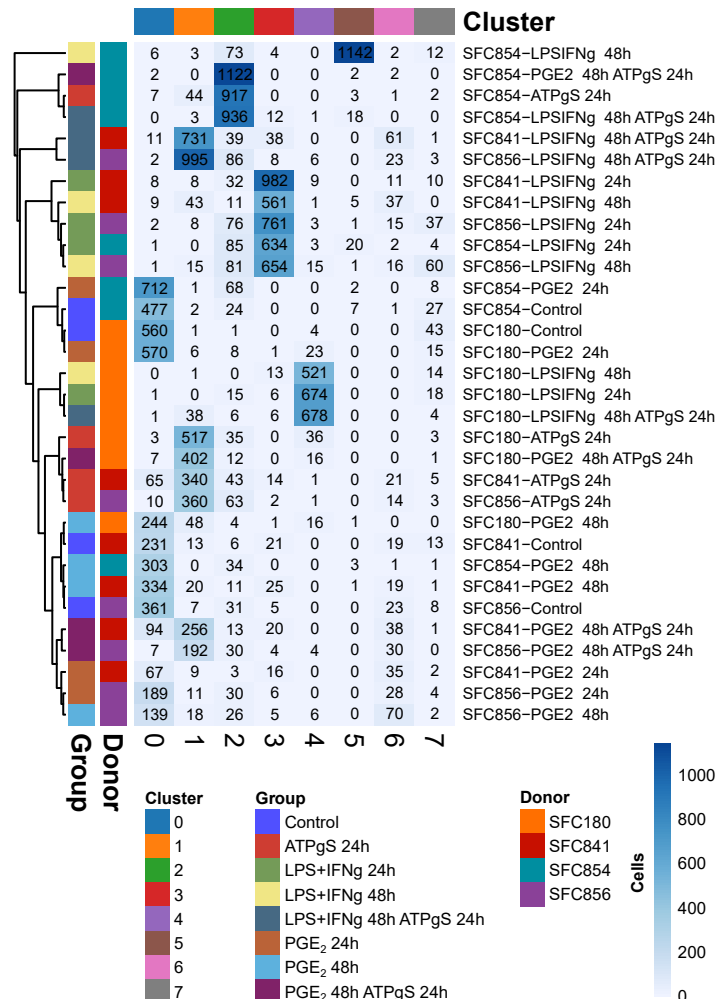
Cell pooling, 10x sequencing,  
HTO demultiplexing, downstream analysis

Concentration: ATPgS [1mM], LPS + IFNg [10ng/ml], PGE2 [500nM]

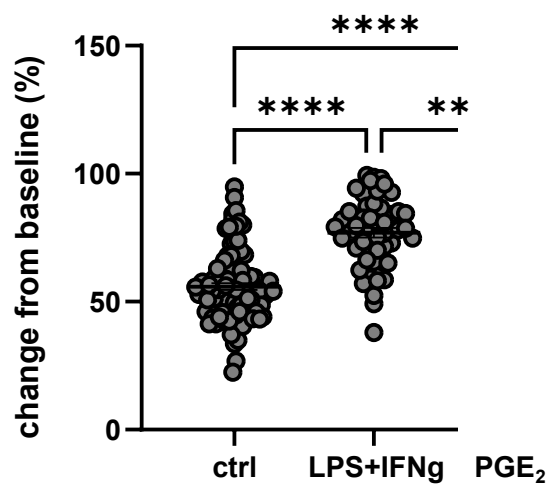
**B**



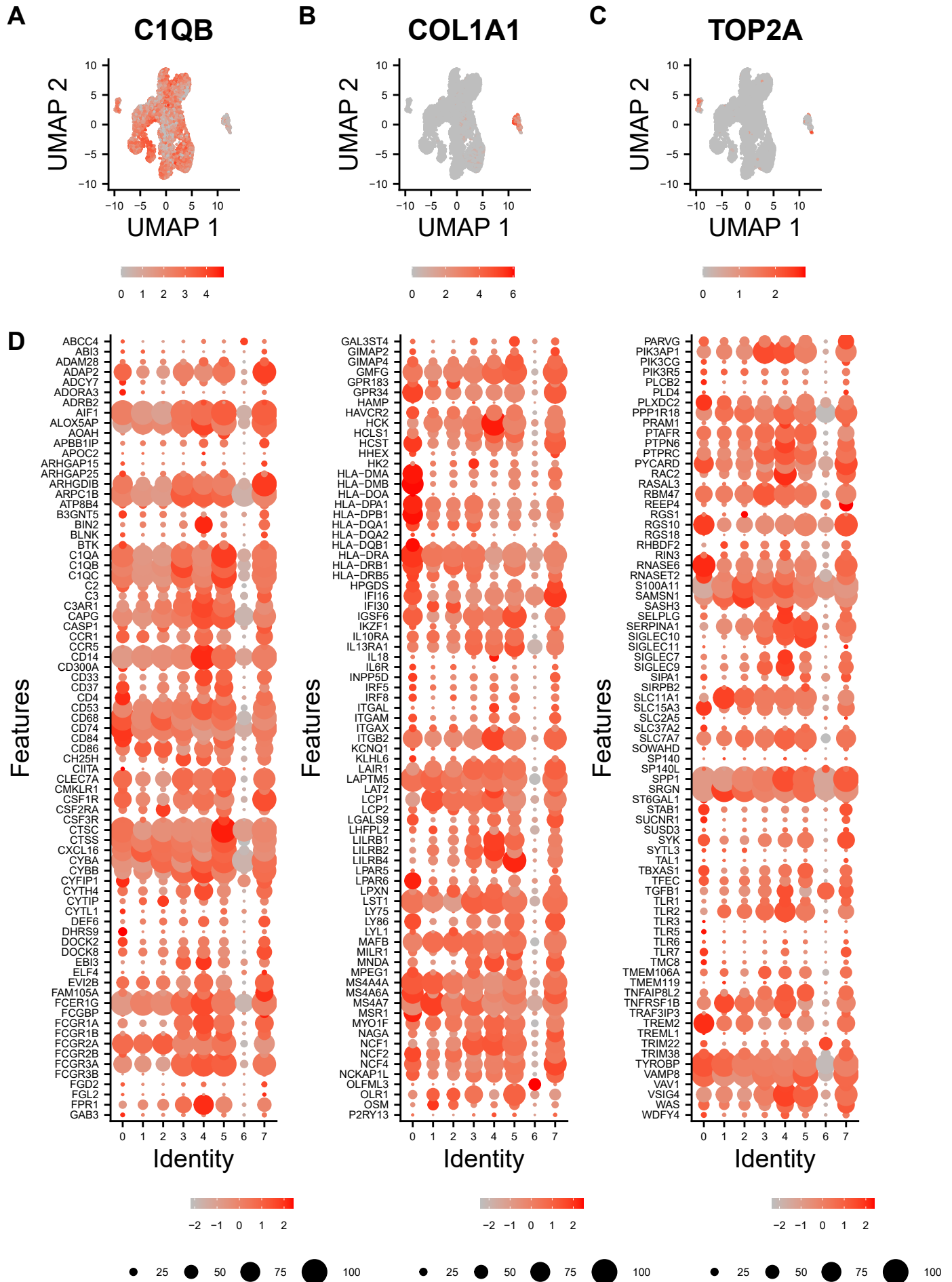
**C**



**Fig. S1. Experimental design to measure transcriptional response of iPSC-microglia and unbiased cell clustering.** **A** iPSC- microglia from 4 different donor were separated into 8 experimental groups : 1) Control iPSC-microglia maintained for 48 hours in normal media, 2) iPSC-microglia exposed to ATP $\gamma$ S [1mM] for 24 hours, 3) iPSC-microglia treated with LPS +IFN- $\gamma$  [10ng/ml] for 24 hours, 4) iPSC-microglia treated with LPS+IFN- $\gamma$  [10ng/ml] for 48 hours, 5) iPSC-microglia were exposed to LPS+IFN- $\gamma$  for 24 hours, and then to ATP $\gamma$ S for an additional 24 hours, 6) iPSC-microglia were exposed to PGE<sub>2</sub> for 24 hours. 7) iPSC-microglia exposed to PGE<sub>2</sub> for 48 hours and 8) iPSC-microglia primed with PGE<sub>2</sub> for 24 hours and then exposed to ATP $\gamma$ S for 24 hours more. We simultaneously measured the transcriptional response at the single cell level with CITE-seq for multiplexing (Stoeckius et al.), in which a particular hashtag oligo (HTO) was used to label iPSC-microglia from each condition. Library preparation and sequencing (10x) were performed, followed by in silico de-multiplexing and data analysis. **B** We detected eight unbiased cell clusters, UMAP plot coloured shows similarity of transcriptional profiles of 20,231 cells coloured by their cluster identity. **C** Heatmap shows the number of cells assigned to each of the eight unbiased clusters annotated to both the donor and the experimental group. Cluster 0 is mainly composed by control and PGE<sub>2</sub> treated cells at both 24 and 48h, cluster 1 of cells treated with ATP $\gamma$ S either alone or in combination, cluster 2 has an overrepresentation of cells from donor SFC854, clusters 3 and 4 contain most of cells treated with LPS+IFN- $\gamma$ , cluster 5 mostly cells from SFC854 treated with LPS+IFN- $\gamma$  at 48h, cluster 6 and 7 were later characterized as fibroblast-like and proliferating iPSC-microglia respectively (**Fig. S3, S4**).

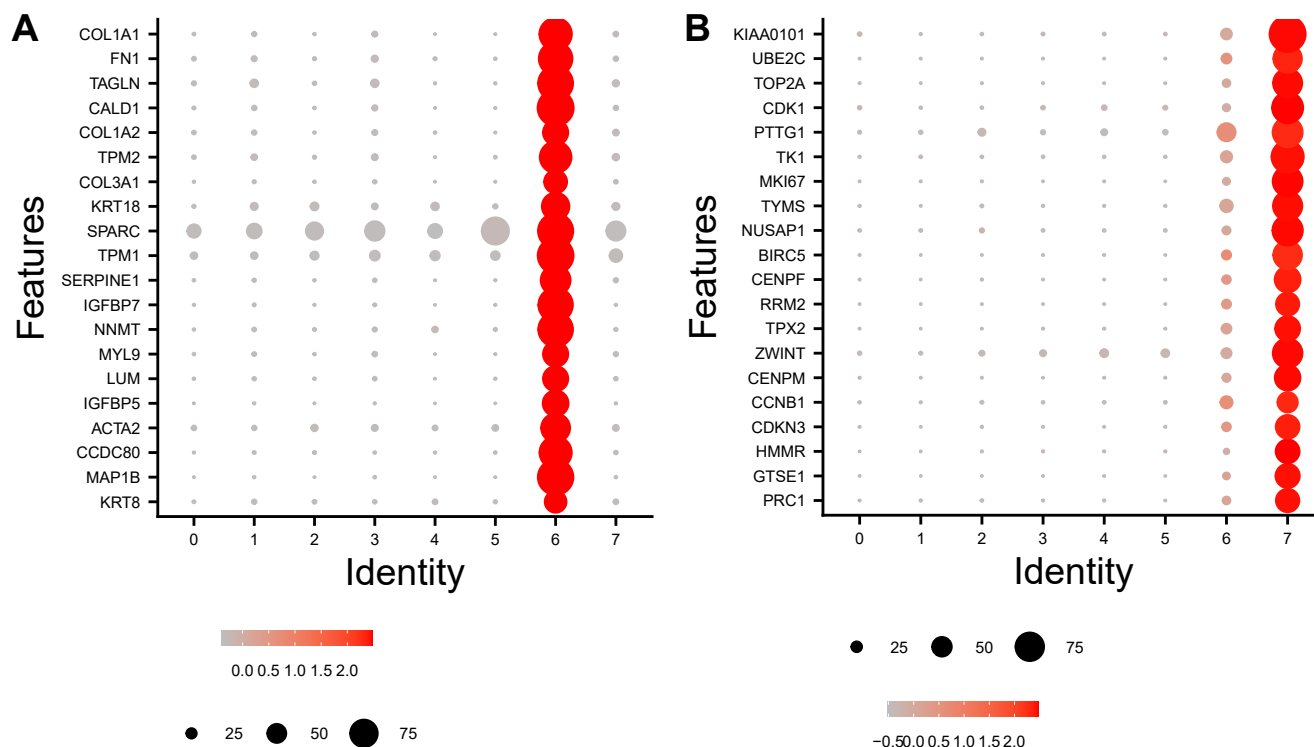


**Fig. S2.** Percentage of calcium increase, calculated from ratiometric (340/380nm) calcium imaging using Fura-2AM of iPSC-derived microglia (SFC841 line), in response to ATP $\gamma$ S at 50 $\mu$ M from baseline after pre-treatment with vehicle control or LPS (10ng/ml) + IFN- $\gamma$  (10ng/ml) or 500nM PGE<sub>2</sub> for 24 hours. One-way ANOVA; \*\*  $p < 0.01$ ; \*\*\*\*  $p < 0.0001$ .

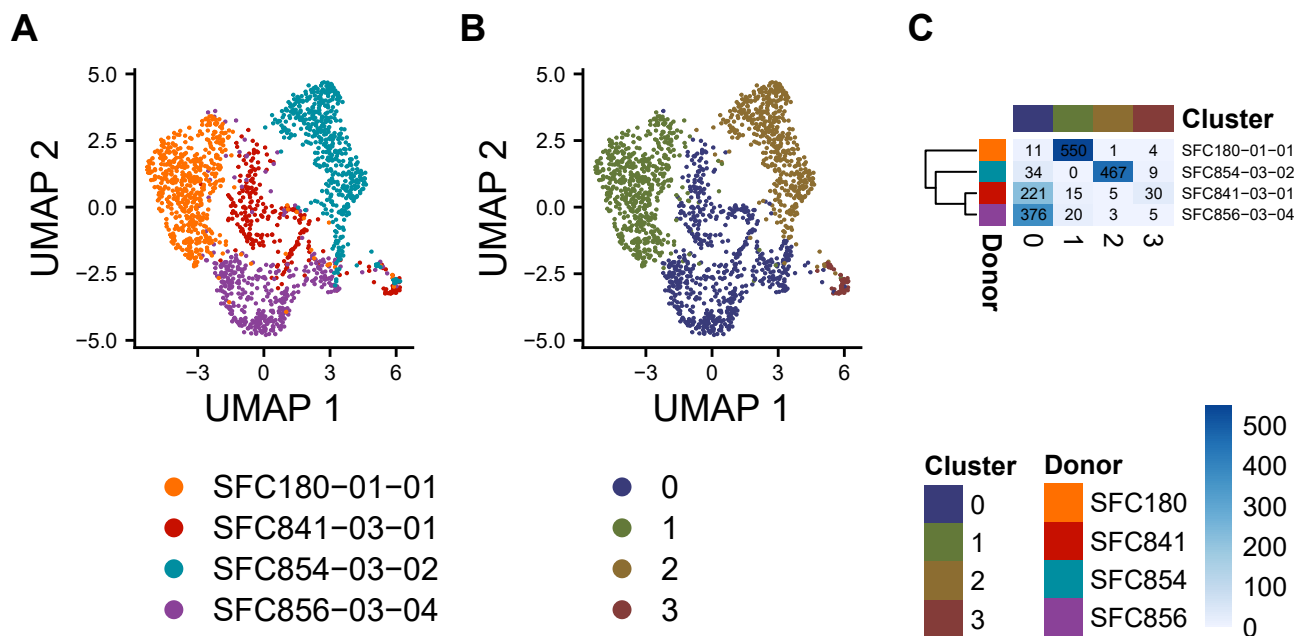




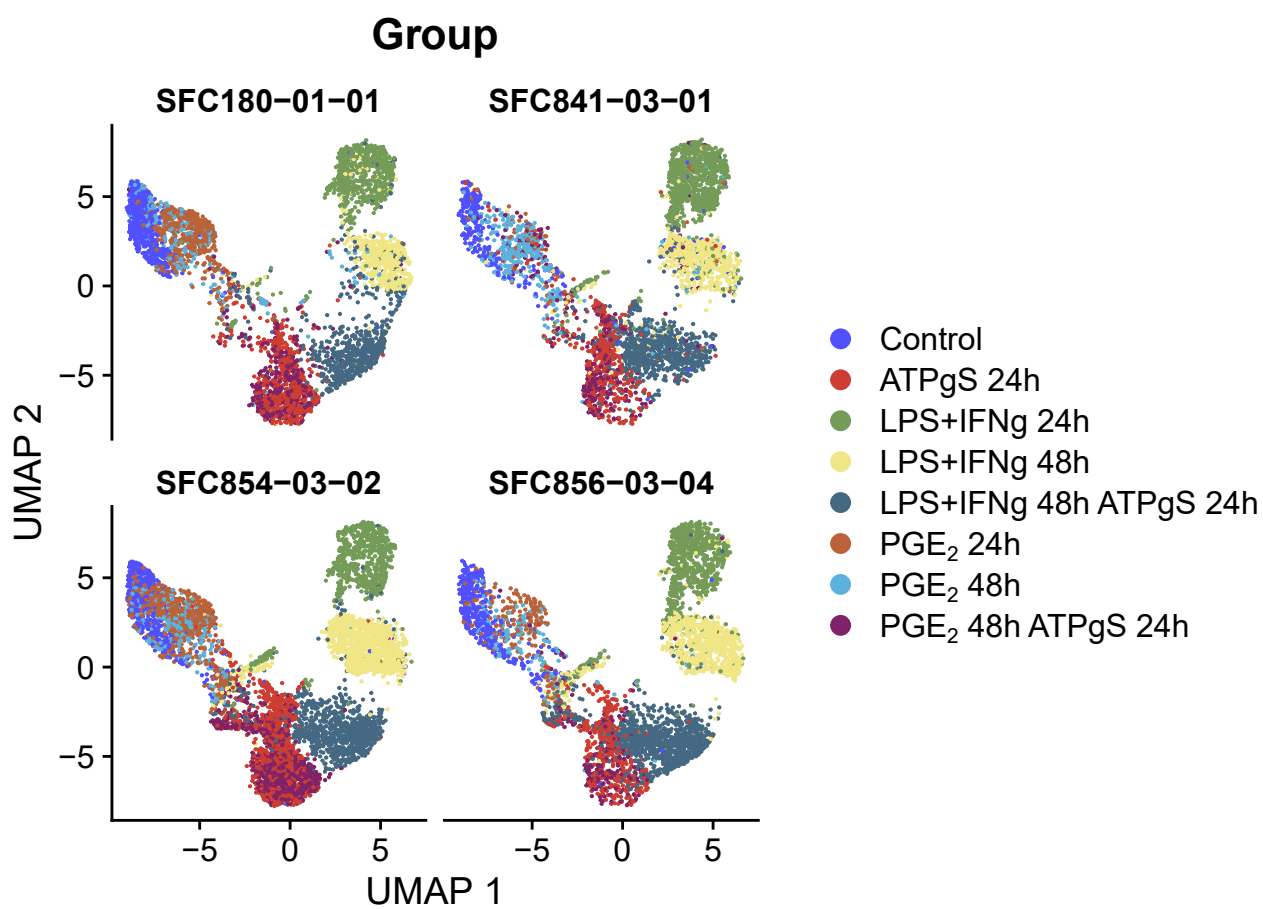
**Fig. S3. Identification of microglia-like and fibroblast like cell populations.** UMAP was based on the first 20 principal components of the top 1000 most variable genes across 20231 cells. **A** UMAP coloured by the expression of microglial marker C1QB. **B** UMAP coloured by the expression of COL1A1. **C**. UMAP coloured by the expression of TOP2A. **D** Dotplot show the expression patterns across cell clusters of core human microglia markers identified by Patir et al. (Patir et al.). Gene expression level is indicated by the colour while the proportion of cells where a gene is detected is represented by the size of the circles.



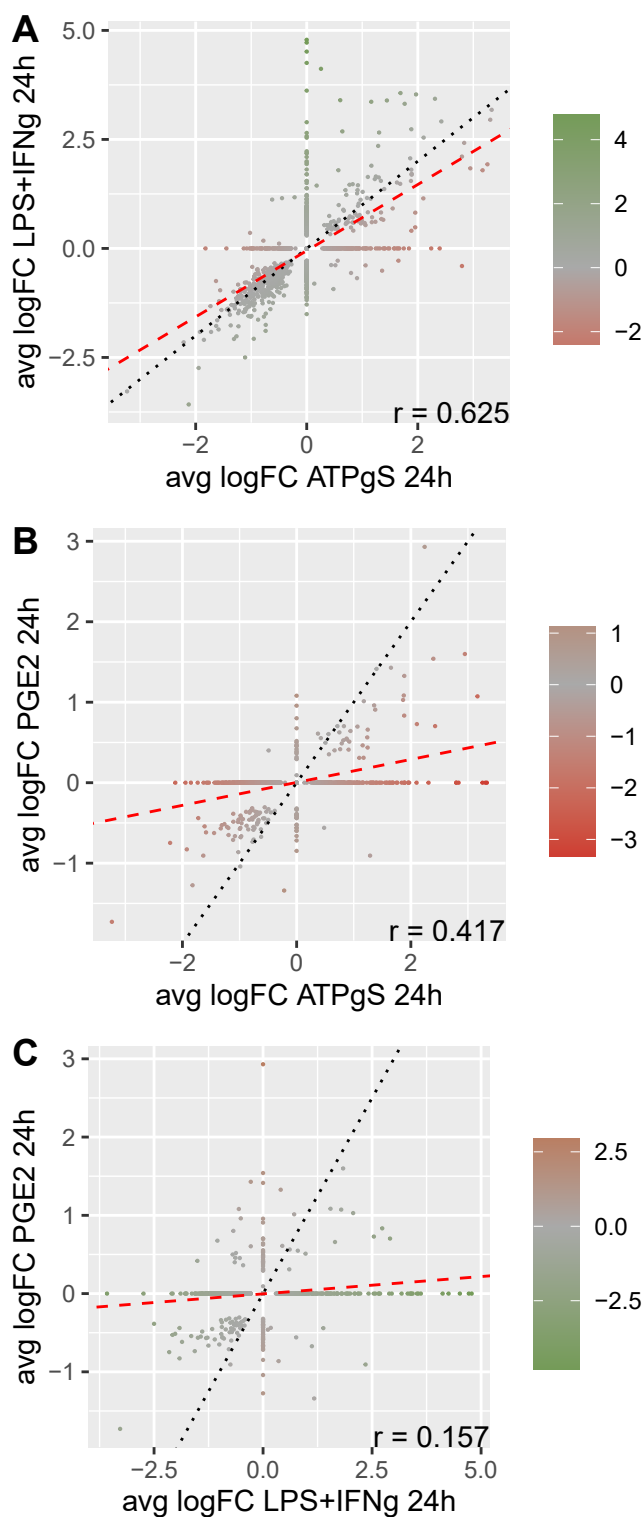
**Fig. S4. Top markers of fibroblast-like and proliferating iPSC-microglia populations.** **A** Top 20 markers of cluster 6 included genes involved in collagen fibril organization such as COL1A1 (collagen type I alpha 1 chain), COL1A2 (collagen type I alpha 2 chain), COL3A1 collagen type III alpha 1 chain and LUM (lumican) and represent a fibroblast-like population. **B** We observed genes annotated to mitotic cell cycle among the top markers of cluster 7, including TOP2A (DNA topoisomerase II alpha), TPX2 (microtubule nucleation factor), TYMS (thymidylate synthetase), UBE2C (ubiquitin conjugating enzyme E2 C), ZWINT (ZW10 interacting kinetochore protein) and RRM2 (ribonucleotide reductase regulatory subunit M2).



**Fig. S5. Clustering in iPSC-microglia untreated control cells.** We performed principal component analysis using only untreated controls, UMAP and clustering analysis were based on the first 30 principal components. **A** UMAP coloured by biological replicate/donor. **B** UMAP coloured by the clusters identified in control cells only. **C** Heatmap shows the number of cells from each donor that correspond to each of cell cluster. Cluster 0 mainly correspond to cells from donor SFC854.

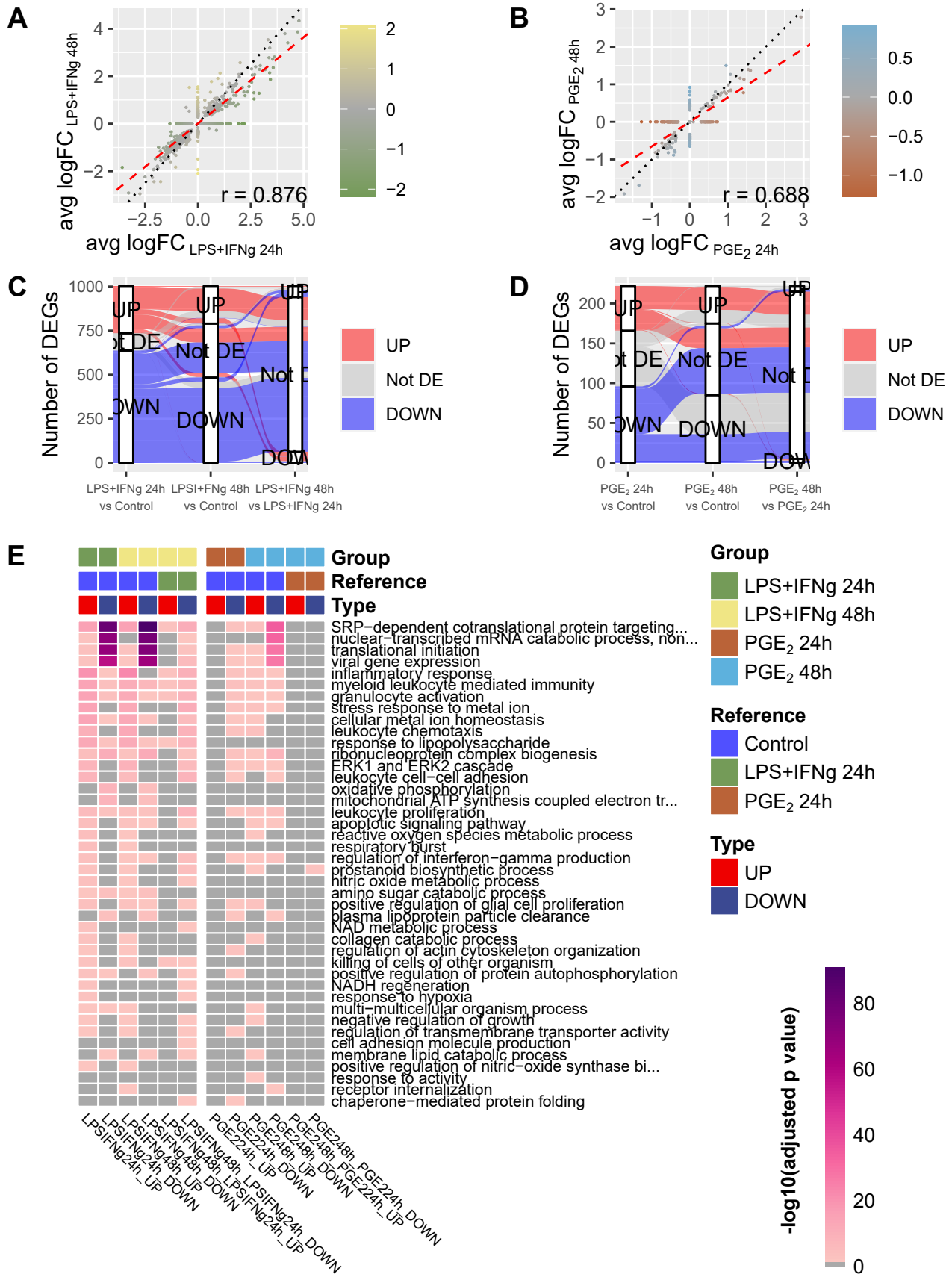


**Fig. S6. Integrated iPSC-microglia across donors. Integrated gene expression data of iPSC-microglia across donors (see Methods).** UMAP coloured by experimental condition and split by donor.

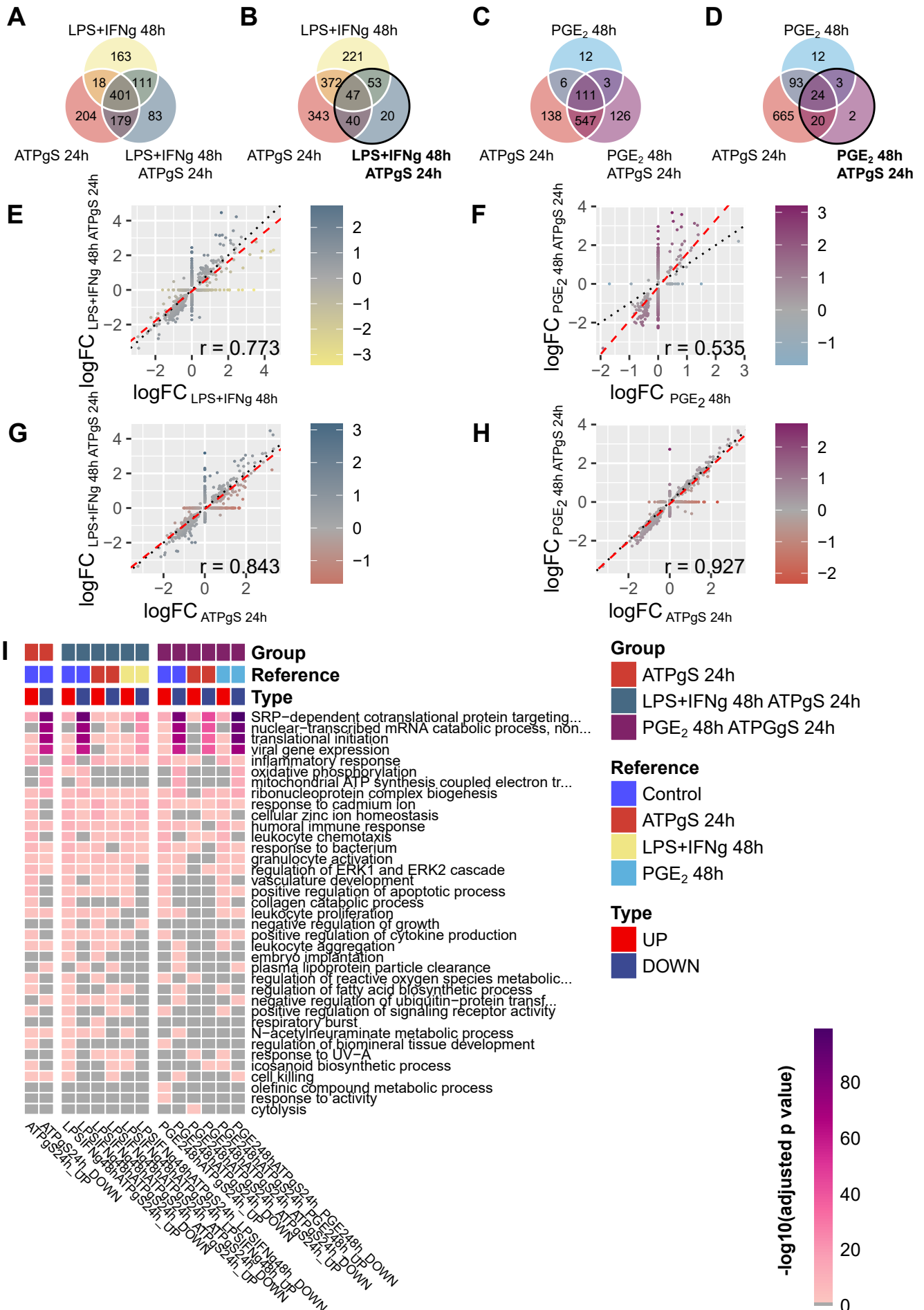


**Fig. S7. Highest correlation between ATP $\gamma$ S and LPS+IFN- $\gamma$  gene expression changes at 24h.**

Comparison between the log transformed fold changes (logFC) at 24h. **A** Gene expression changes in response to ATP $\gamma$ S and to LPS+IFN- $\gamma$  at 24h. **B** Gene expression changes in response to ATP $\gamma$ S and to PGE2 at 24h. **C** Gene expression changes in response to LPS+IFN- $\gamma$  and to PGE2 at 24h. Pearson correlation coefficient between the average logFC is indicated in the bottom right corner. Black dotted line marks equal fold changes, while red dashed line indicates the linear regression between the average log Fold Changes. Colour scheme based on the difference of the absolute fold changes and correspond to colours in Fig. 1A.



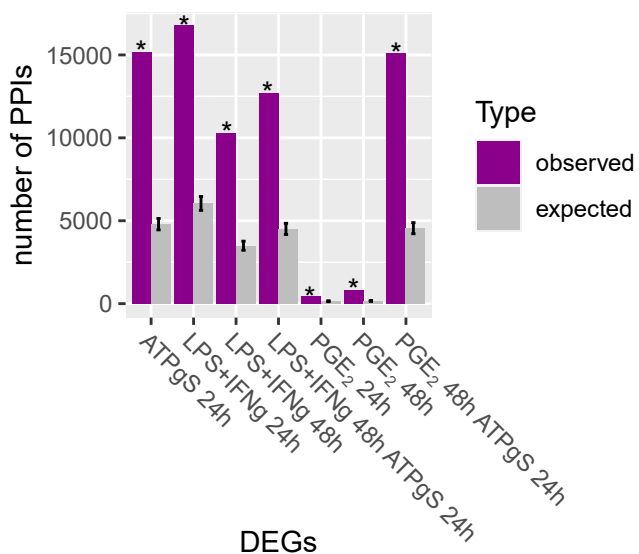
**Fig. S8. Comparison of gene expression changes at 24 and 48 hours.** **A** Comparison between the average log transformed fold changes observed at 24 and 48h after the exposure to LPS+IFN- $\gamma$ . **B** Comparison between the average log transformed fold changes observed at 24 and 48h after the exposure to PGE<sub>2</sub>. Colour scheme related to Figure 1A reflecting the condition where we observed the strongest change in expression, based on the difference of the absolute fold changes. Black dotted line marks equal fold changes, while red dashed line indicates the linear regression between the average log Fold Changes. Pearson correlation is indicated in the top left corner. **C** Sankey diagram shows DEGs in response to LPS+IFN- $\gamma$  at 24 and 48h compared to control and at 48 compared to 24h. Colour indicate if genes were up or down regulated at 24h compared to control. **D.** Sankey diagram shows DEGs in response to PGE<sub>2</sub> at 24 and 48h compared to control and at 48h compared to 24h. Similarly, colour indicate if DEGs were up or down regulated at 24h compared to control. **C** Enriched biological processes among the genes differentially expressed through time in response to either LPS+IFN- $\gamma$  or PGE<sub>2</sub>. iPSC-microglia treated with either LPS+IFN- $\gamma$  or PGE<sub>2</sub> at 24h were compared against untreated control cells, while those exposed for 48h were compared to both the control and 24h treatment. Gene Ontology enrichment analysis of biological processes was performed separately for up and down regulated genes. Heatmap shows the transformed adjusted p value for each enriched biological process in shades of pink (if adjusted p value < 0.05, otherwise is coloured in grey).



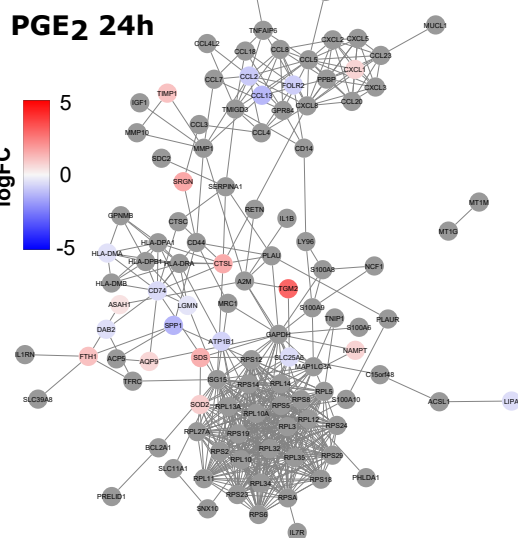


**Fig. S9. Combined treatments with ATP $\gamma$ S.** Venn diagrams show the overlap between differentially expressed genes (combined p value < 0.05): **A** Overlapping DEGs in response to LPS+IFN- $\gamma$  48h ATP $\gamma$ S 24h and each individual condition, in all comparisons the reference group was untreated Control cells. **B** The combined effect of LPS+IFN- $\gamma$  and ATP $\gamma$ S was relatively small (20 genes) once we condition to the response to ATP $\gamma$ S. Bold and black circle indicate that the reference group for this comparison was ATP $\gamma$ S 24h. **C** Overlapping DEGs in response to the combined PGE2 48h ATP $\gamma$ S 24h. **D** Similarly, only 2 genes were uniquely differentially expressed when exposed to both PGE2 and ATP $\gamma$ S that were not detected on either of these conditions alone once we condition the response to ATP $\gamma$ S. Gene expression changes given as the average log Fold Change. **E** The combined treatment with LPS+IFN- $\gamma$  and ATP $\gamma$ S compared to only LPS+IFN- $\gamma$ . **F** The combined and individual treatment with PGE2. **G** The combined treatment with LPS+IFN- $\gamma$  and ATP $\gamma$ S compared to ATP $\gamma$ S only. **H** The combined treatment with PGE2 and ATP $\gamma$ S compared to ATP $\gamma$ S alone. Colour scheme related to Figure 1A reflecting the condition where we observed the strongest change in expression, based on the difference of the absolute fold changes. Black dotted line marks equal fold changes, while red dashed line indicates the linear regression between the average log Fold Changes. Pearson correlation is indicated in the bottom right corner. **I** Enriched biological processes among the genes differentially expressed in response to ATP $\gamma$ S. Sets of differentially expressed genes include those in response to ATP $\gamma$ S alone (ATP $\gamma$ S 24h) and in combination with either LPS+IFN- $\gamma$  treatment or PGE2. Heatmap shows the transformed adjusted p value for each enriched biological process in shades of pink (if adjusted p value < 0.05, otherwise is coloured in grey).

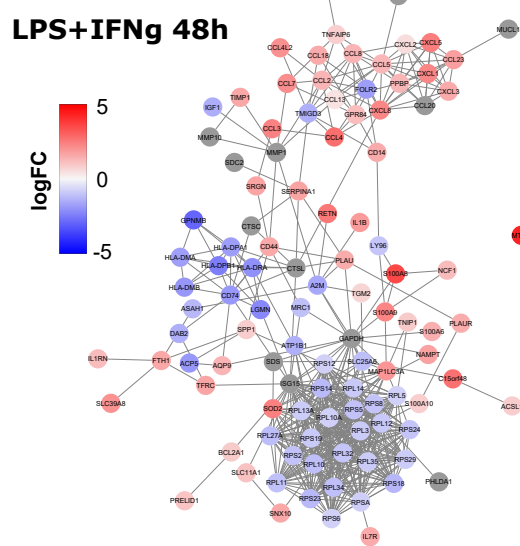
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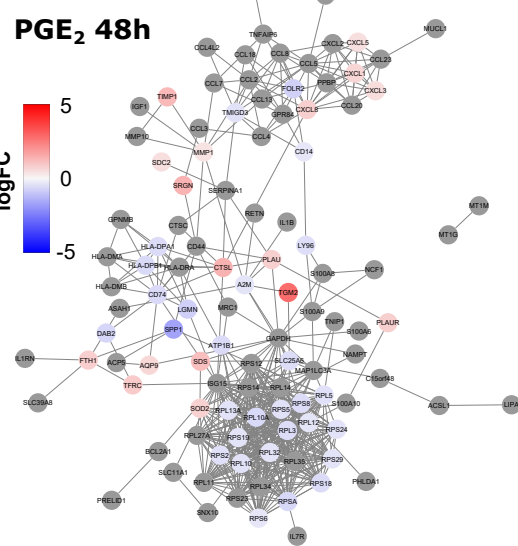
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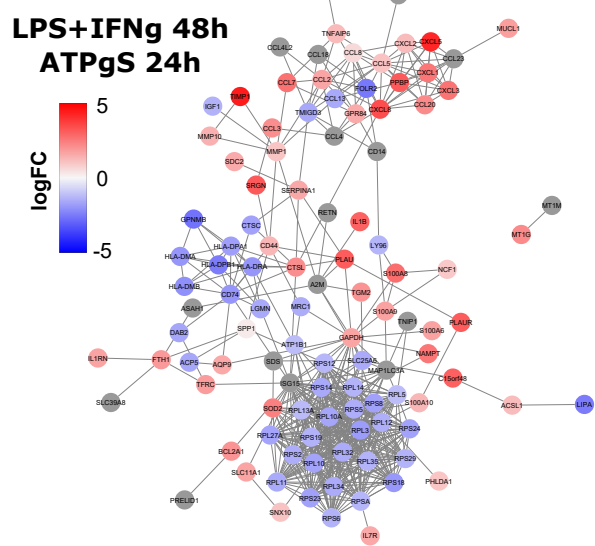
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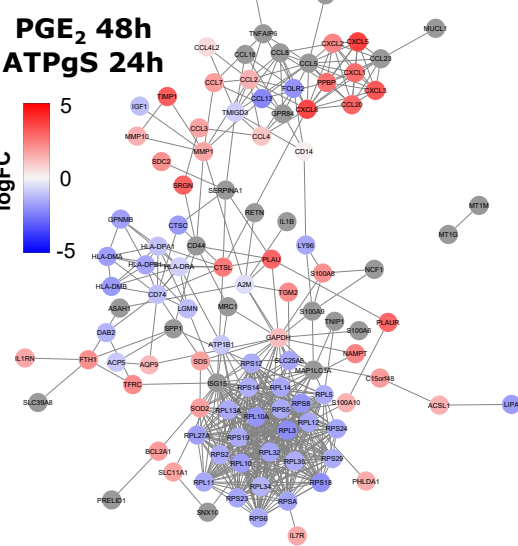
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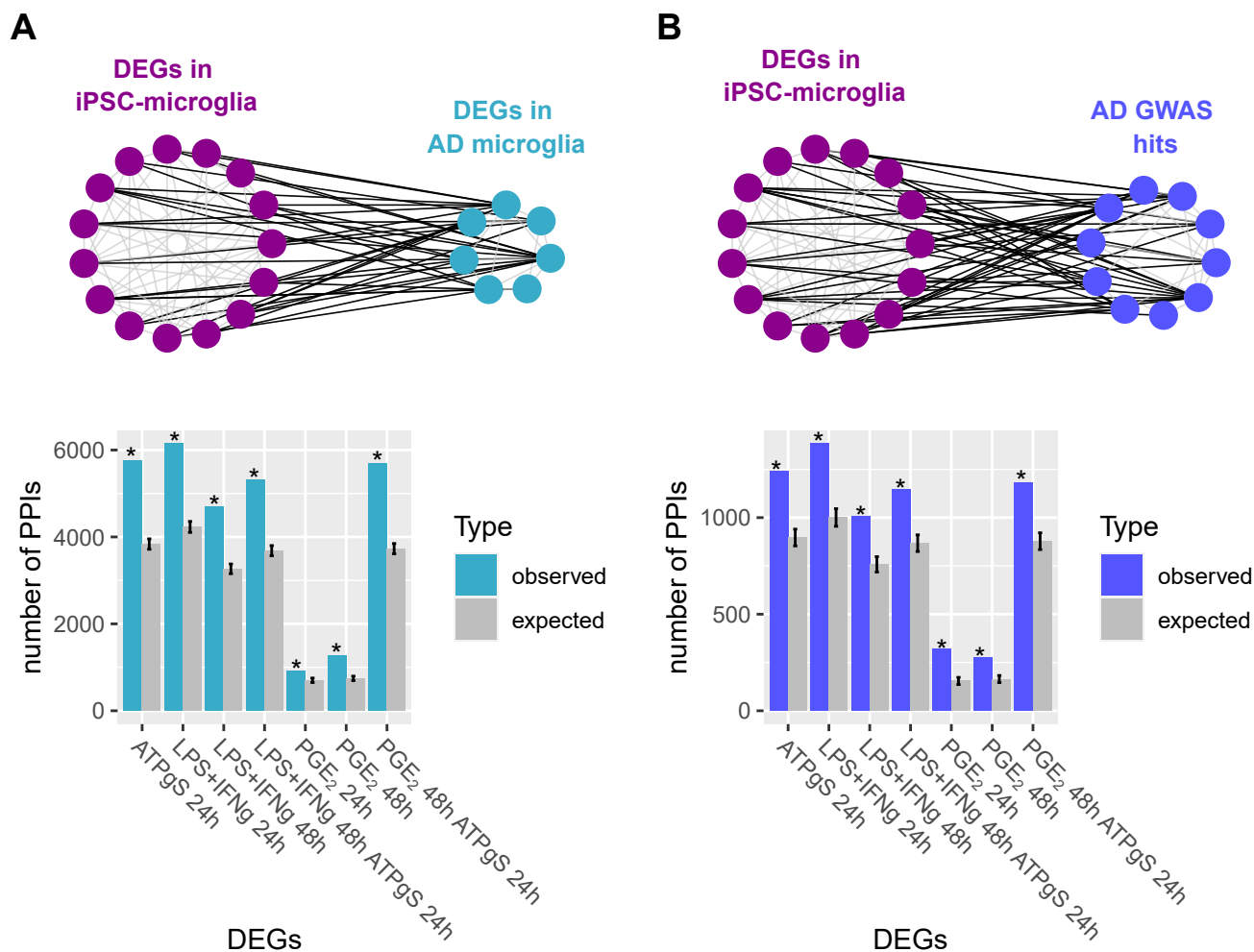
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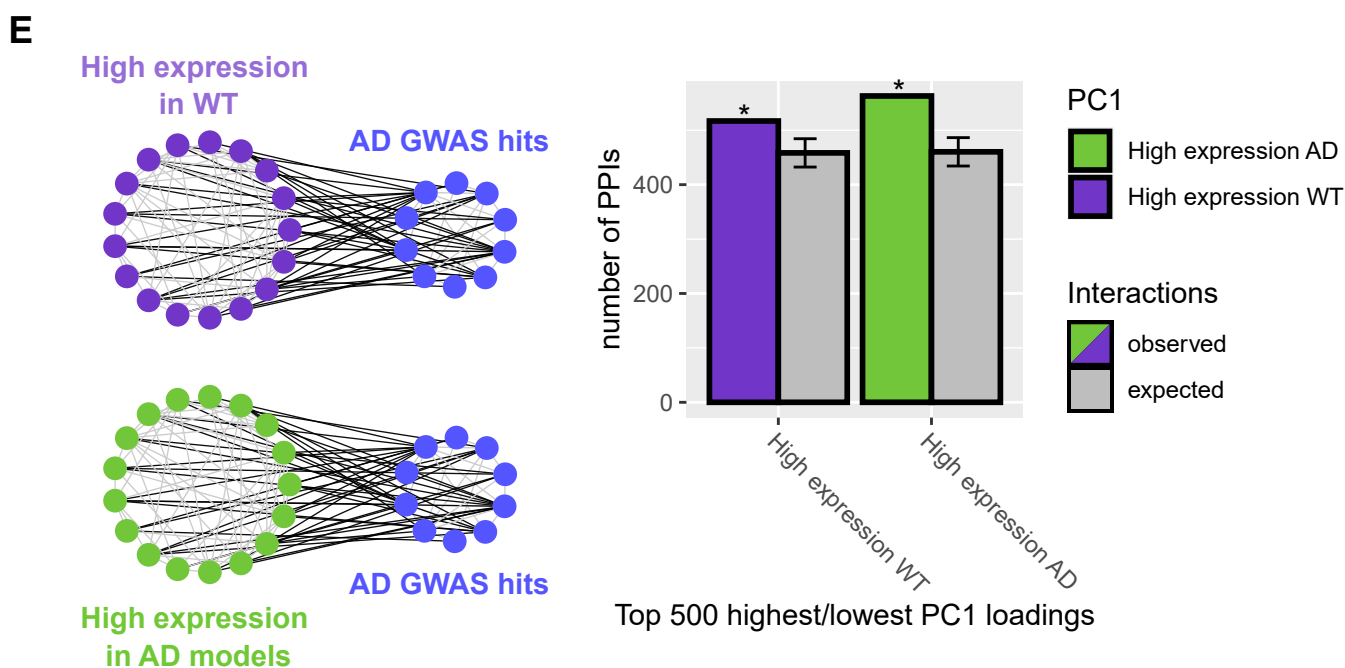
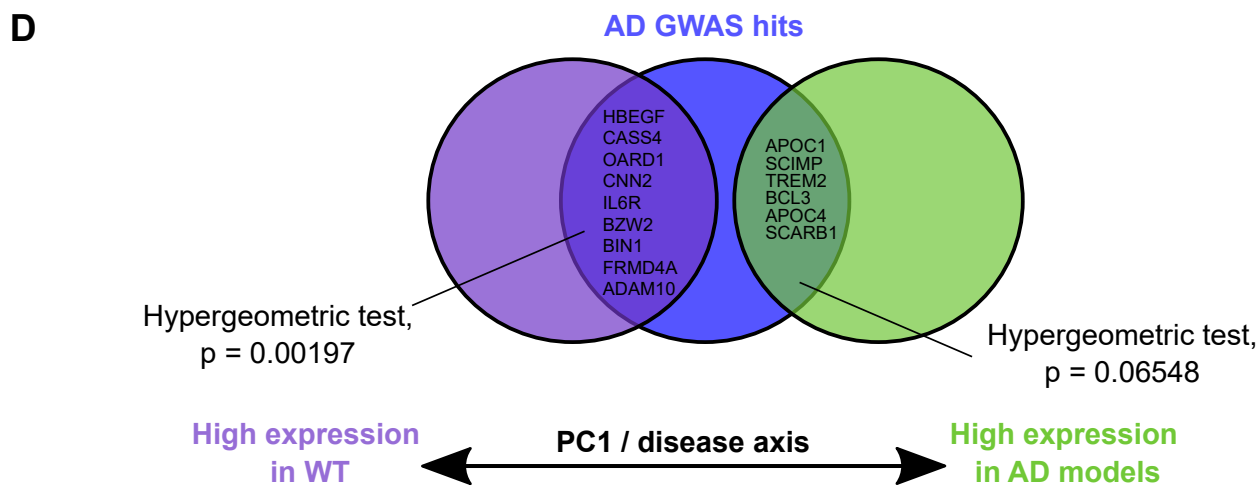
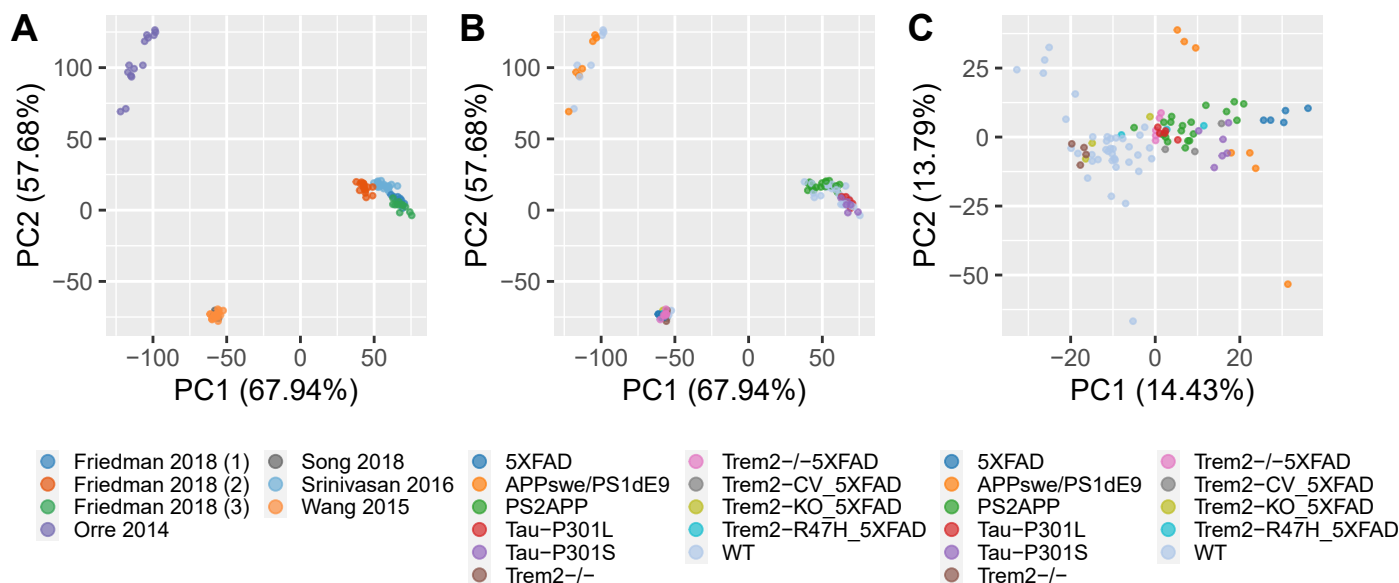
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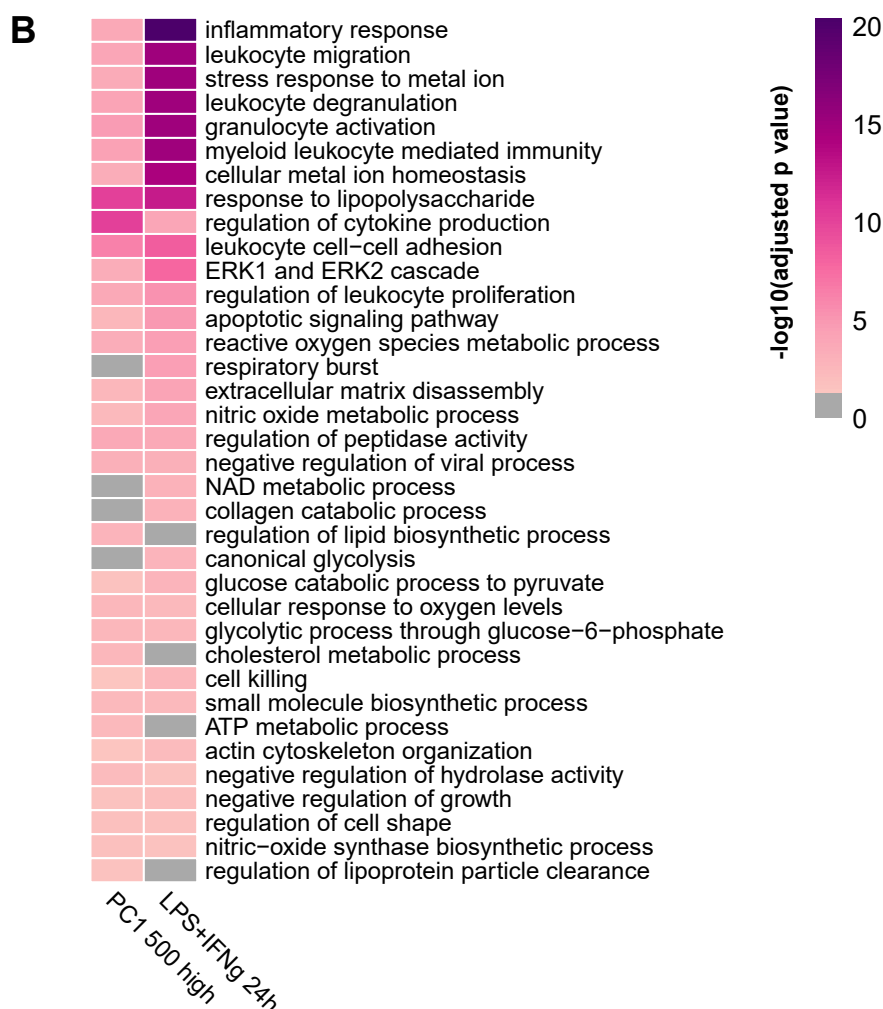
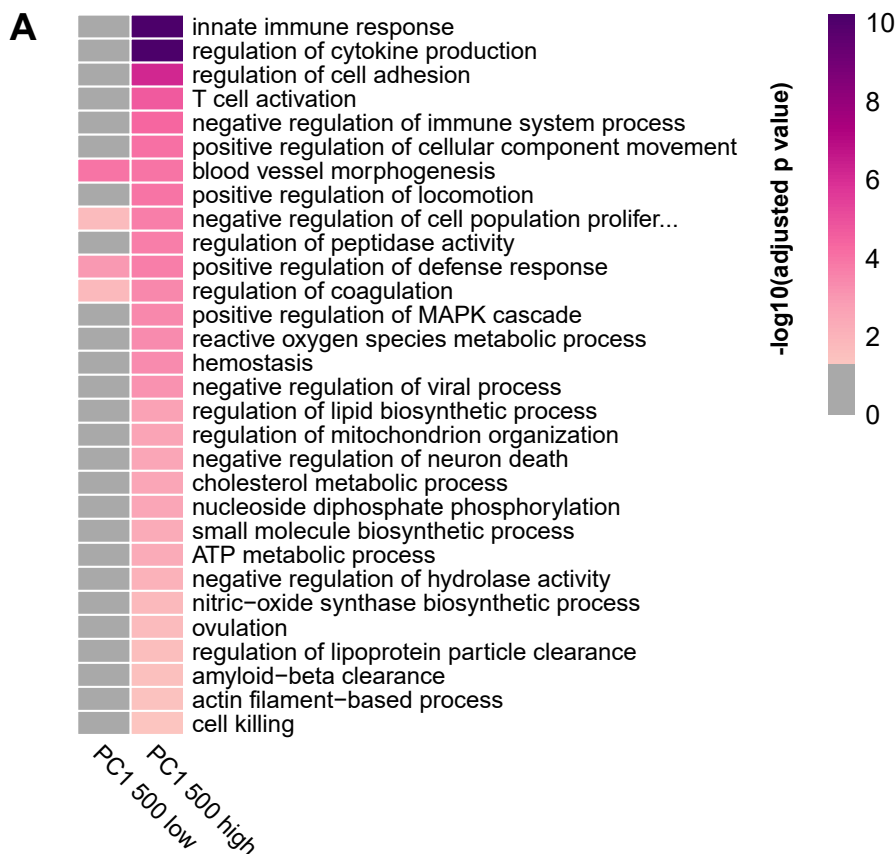
**Fig. S10. Protein-protein interaction network of the genes with the strongest changes in expression in iPSC-microglia in response to the different treatments.** DEGs in response to each of the different treatments in iPSC-microglia show more protein-protein interactions than expected by chance. **A** Bars shows the number of observed and expected protein-protein interaction between the gene products of DEGs in iPSC-microglia after 24 and 48 h treatment with ATP $\gamma$ S, LPS+IFN- $\gamma$  and PGE2, as well as the combined treatments with ATP $\gamma$ S. Error bars show the S.E.M. based on 10,000 randomizations, \* indicates an estimated p value < 0.0001. PPIs network of the corresponding DEGs in iPSC microglia with the strongest changes in expression (absolute logFC  $\geq$  1.5, combined p value < 0.05). Nodes indicate genes, edges known protein-protein interactions, and colour fold changes in response **B** LPS +IFN- $\gamma$  48h, **C** LPS+IFN- $\gamma$  48h ATP $\gamma$ S at 24h, **D** PGE2 24h, **E** PGE2 48h and **F** PGE2 at 48h ATP $\gamma$ S 24h.



**Fig. S11. Linking DEGs in iPSC-microglia to AD through protein-protein interactions. A** Proteins from DEGs in stimulated iPSC-microglia show more protein-protein interactions (PPIs) to those of the DEG in microglia of AD patients. At the top the schematic representation of the protein-protein interactions between the gene products of DEGs in iPSC-microglia and those DEG in human post-mortem AD microglia. At the bottom, barplot indicates the observed and expected number of PPIs between each set of DEGs in iPSC-microglia and the protein products of the DEG in microglia from AD patients. **B** Top. Schematic representation of the protein- protein interactions (edges) between the corresponding proteins of DEGs in challenged iPSC microglia and those of genes associated to AD through GWAS. Bottom barplot indicates the number of observed and expected PPIs between DEG in iPSC-microglia and the protein products of the genes mapped to AD loci from GWAS. Error bars represent the S.E.M. based on 10,000 randomizations, \* indicates an estimated p value < 0.0001.



**Fig. S12. Genes that drive PC1 / mouse model disease axis overlap and are connected to AD GWAS through protein-protein interactions.** Principal component analysis (PCA) based on the gene expression data from multiple datasets from microglia AD mouse models before batch correction widely segregated samples by study and after by correction broadly segregated WT from transgenic mouse. **A** First two principal components before batch correction coloured by the study. **B** First two principal components before batch correction coloured by genotype. **C** First two principal components after batch correction coloured by genotype. **D** Overlap between the top 500 genes with the lowest and highest loadings along the disease axis (or highest expression in WT or AD models) and those genes associated to AD through GWAS. **E** Number of observed and expected protein-protein interactions between the top 500 driving the disease axis (either with highest or lowest loadings along PC1) and the AD GWAS hits compared to random sets of genes. Error bars represent the S.E.M. based on 10,000 randomizations, \* indicates an estimated p value < 0.0001.



**Fig. S13. Functional characterization of genes along the disease axis and convergence in functional pathways with the genes up regulated in response to LPS+IFN- $\gamma$  in iPSC-microglia at 24h. A** Gene Ontology enrichment analysis among the top 500 genes with the highest and lowest loadings along the disease axis (PC1 from mouse meta-analysis). GO terms were reduced to a non-redundant set based on the semantic similarity in mouse. **B** We observed a strong pathway convergence from those genes driving the disease axis (top 500 highest PC1 loadings) and among the DEGs upregulated in response to LPS+IFN- $\gamma$  at 24h. Heatmap shows the minus log transformed p value for the enriched biological processes among each set of genes (shades of pink if adjusted p value < 0.05, grey otherwise).





**Fig. S14. Post-mortem microglia from AD show a minor shift along disease axis based on genetic mouse models of AD. A** Single cell gene expression from human post-mortem microglia from the prefrontal cortex was aggregated from 24 controls (low  $\beta$  amyloid pathology) and AD cases (high  $\beta$  amyloid) reduce to one to one human to mouse orthologs and projected into the first principal component from the mouse meta-analysis. **B** Single cell gene expression from human post-mortem microglia from the entorhinal cortex was aggregated from 6 AD cases and 6 controls, reduced to one to one ortholog genes from human to mice and projected into the disease axis from the meta-analysis of mouse disease models of AD.

