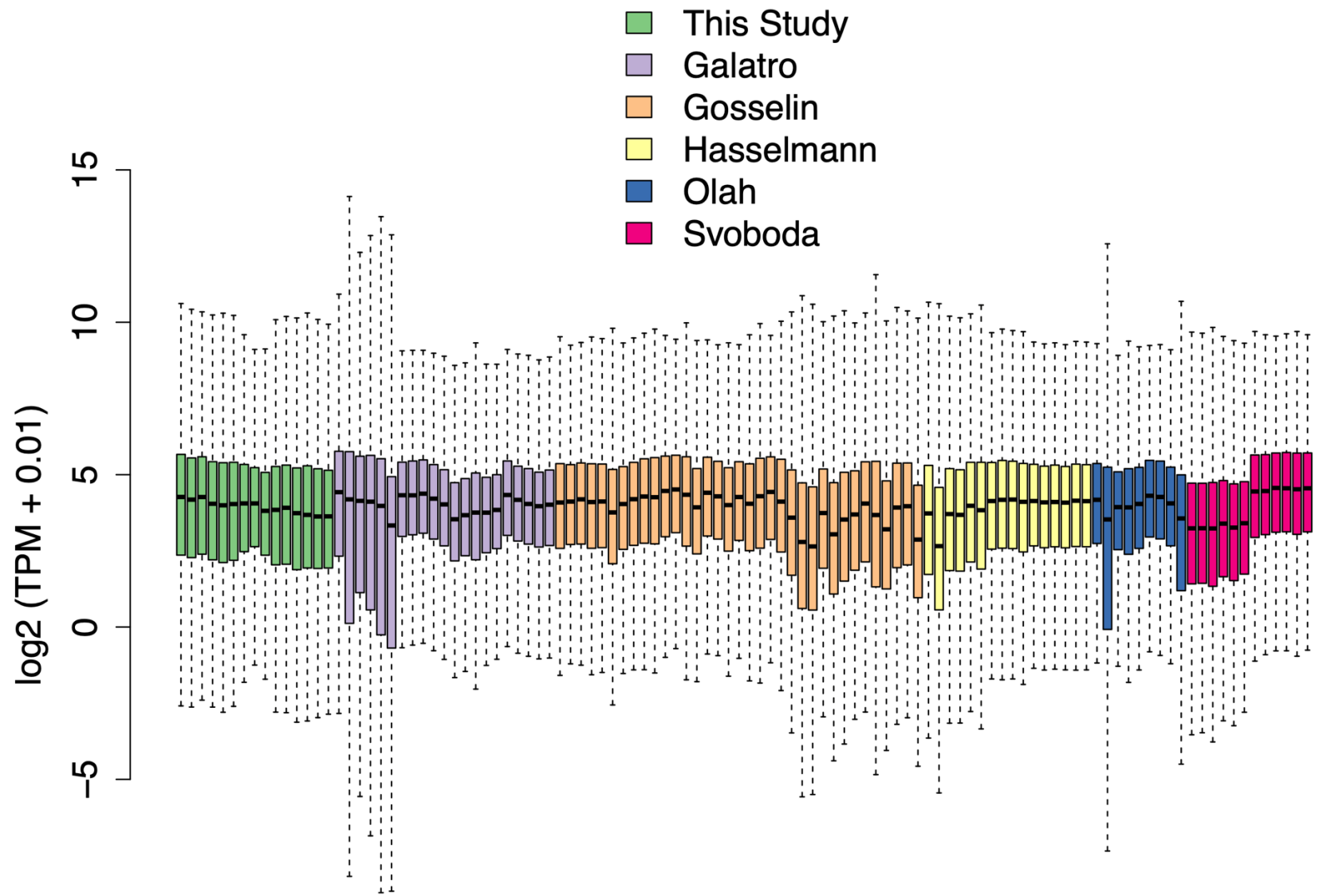
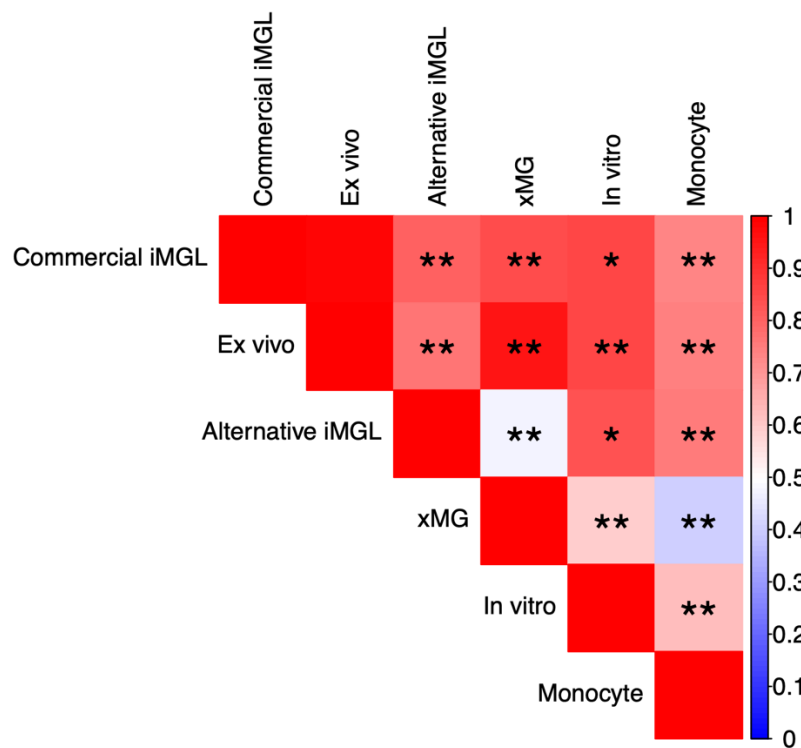
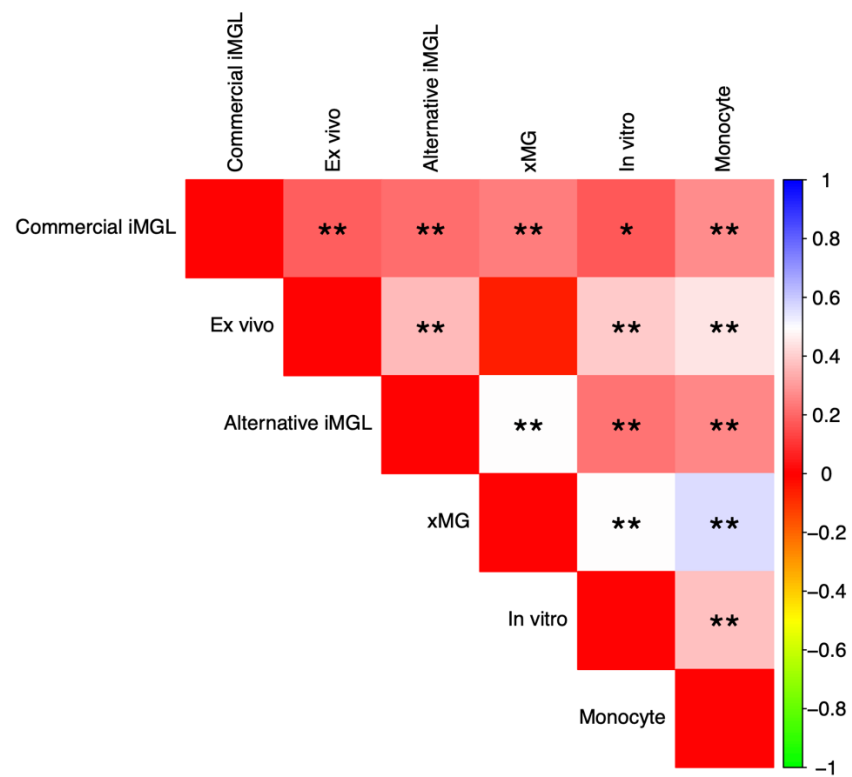


Supplementary Figure 1. Individual and composite immunofluorescence images of Commercial iMGL cells stained with *IBA1*, *P2Y12*, or *TREM2* (green) and DAPI (blue). The scale bar represents 20 μm .



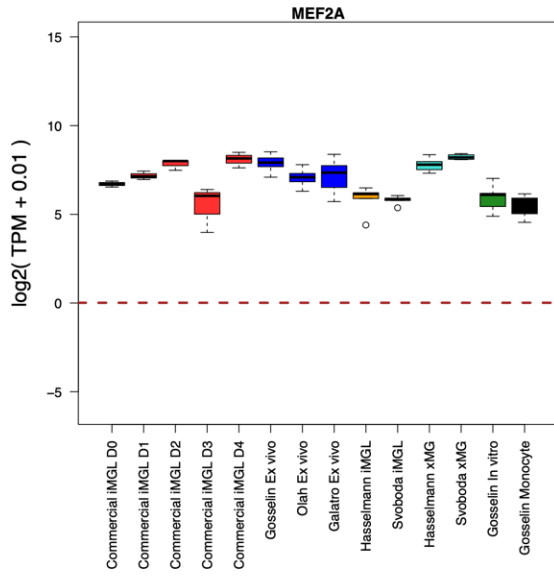
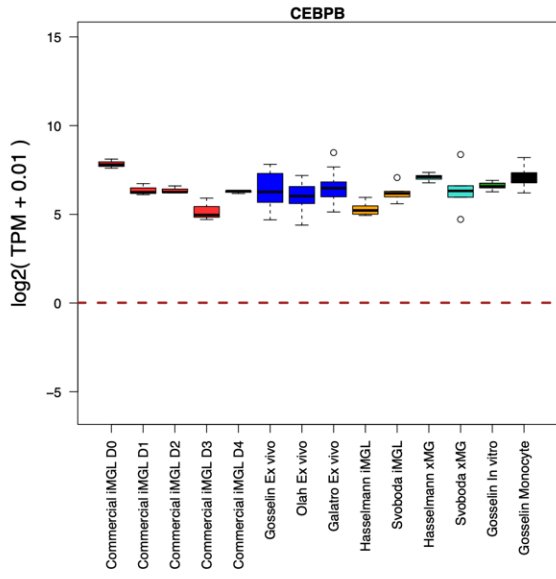
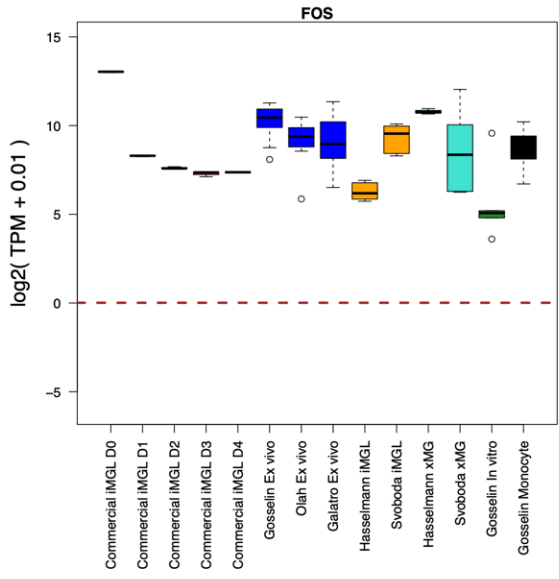
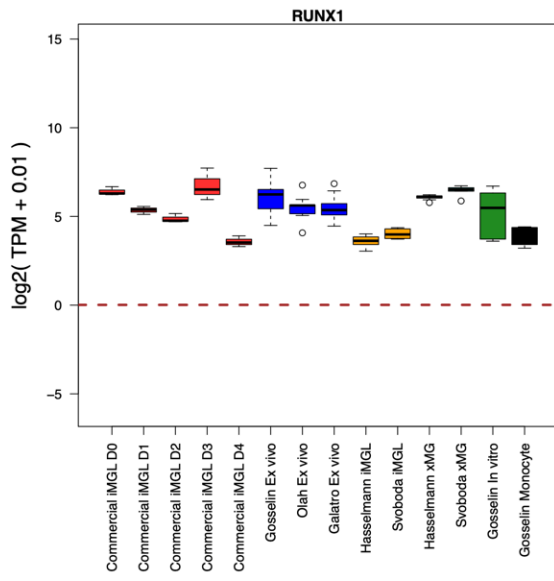
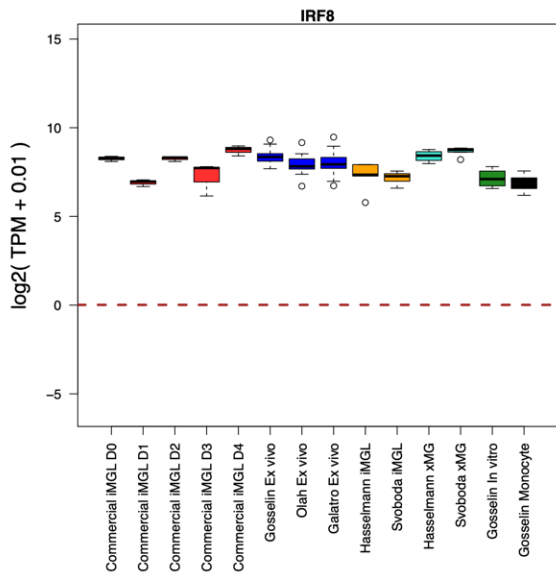
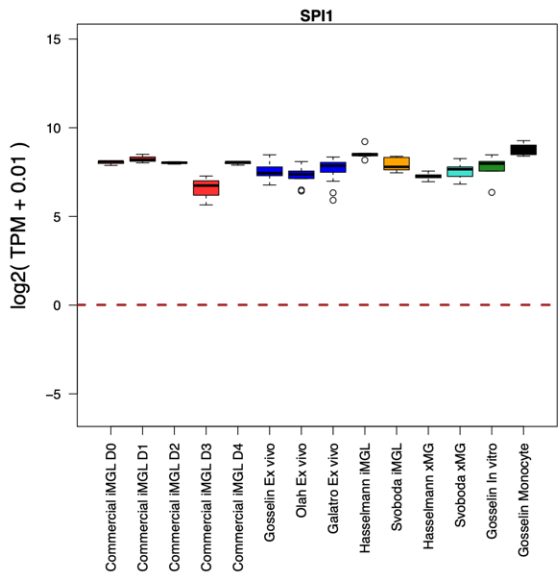
Supplementary Figure 2. Distribution of gene expression values for 12,392 expressed genes across the Commercial iMGL time course and external bulk RNA-seq datasets.

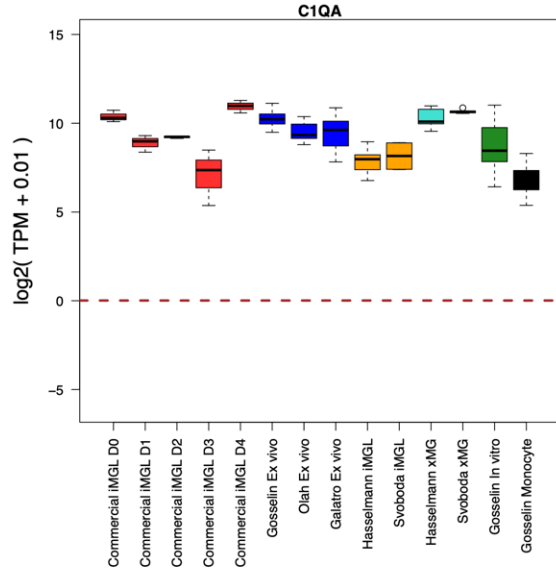
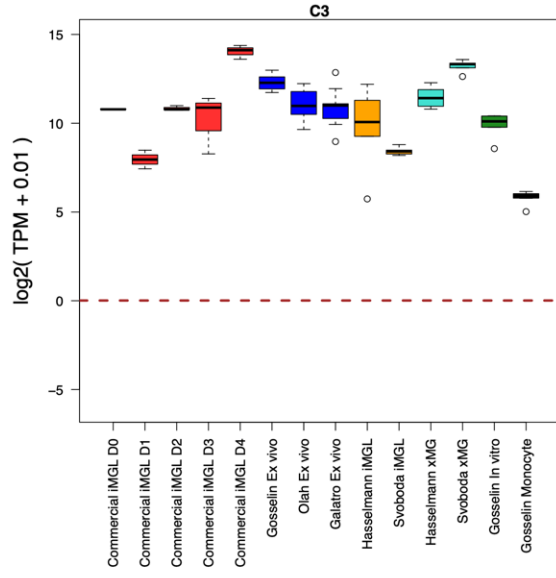
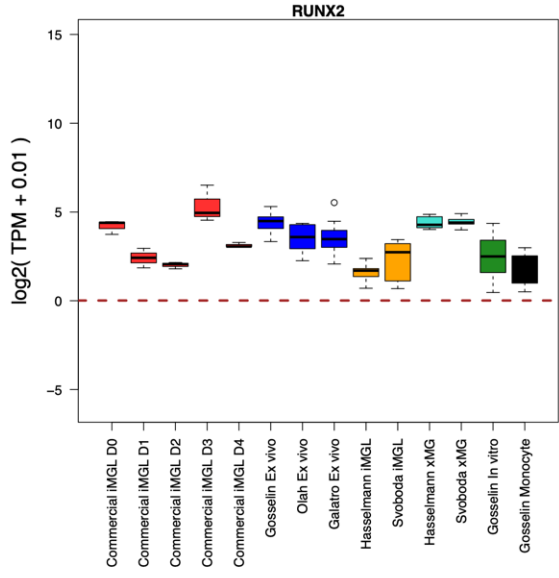
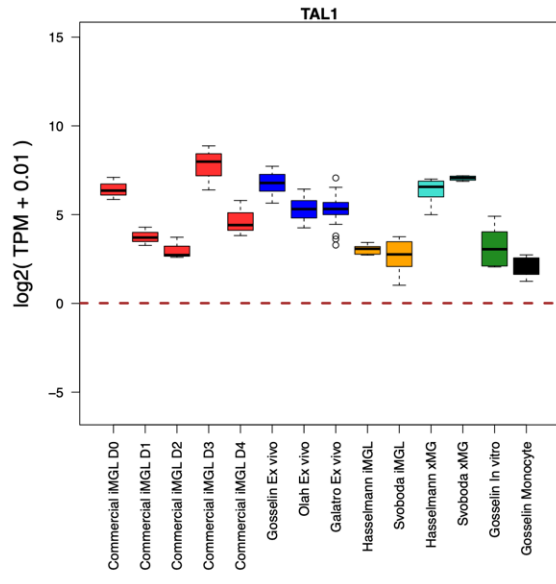
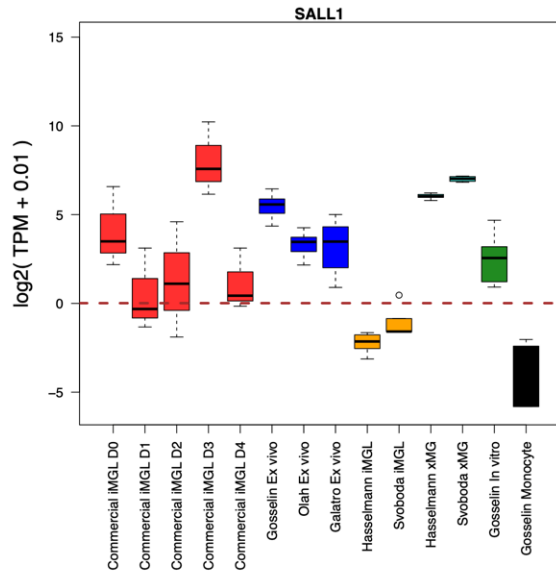
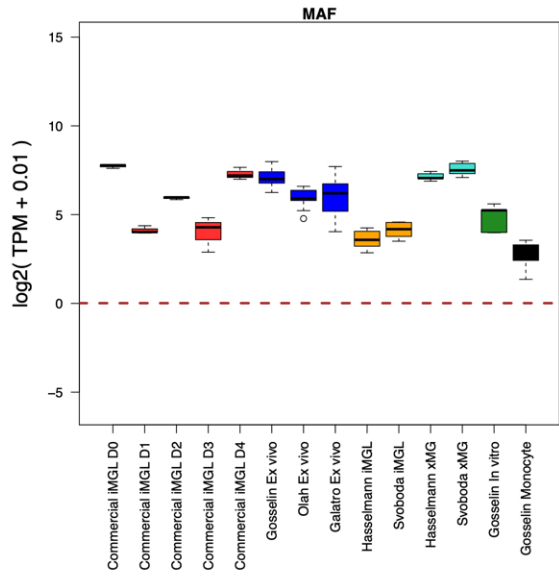
A**B**

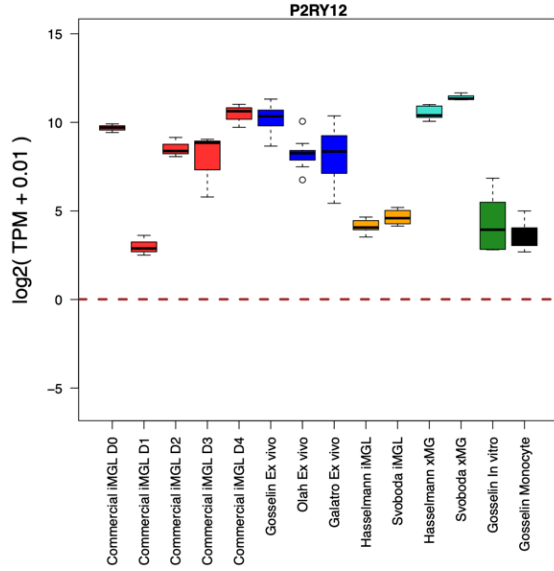
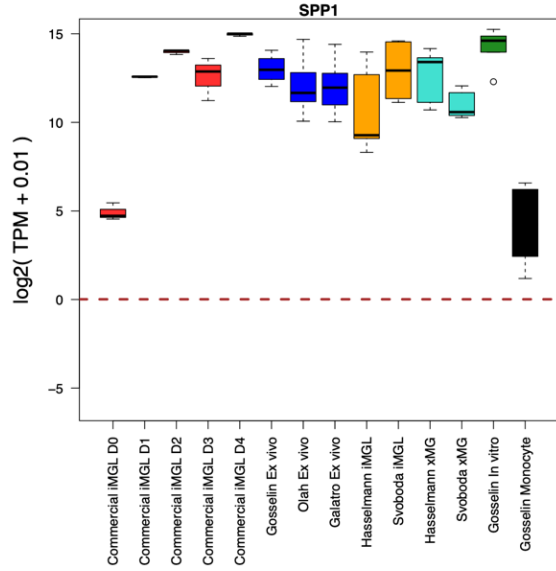
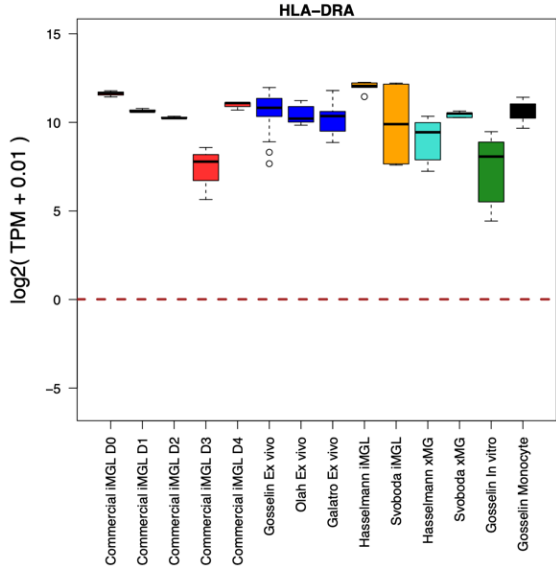
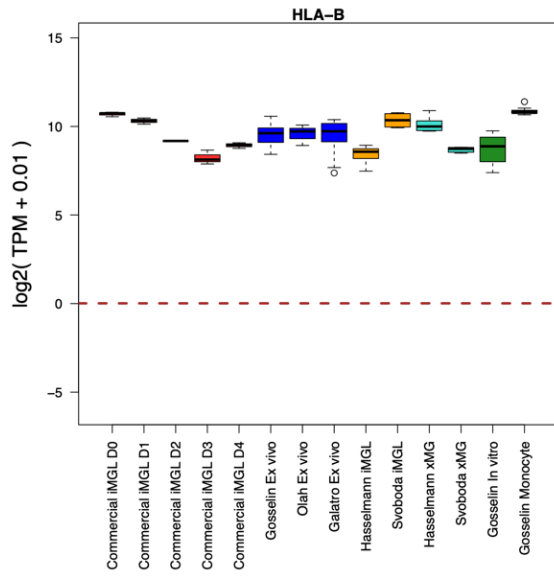
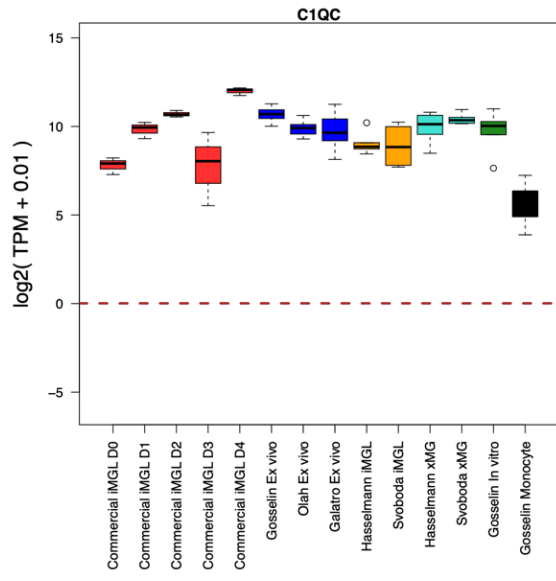
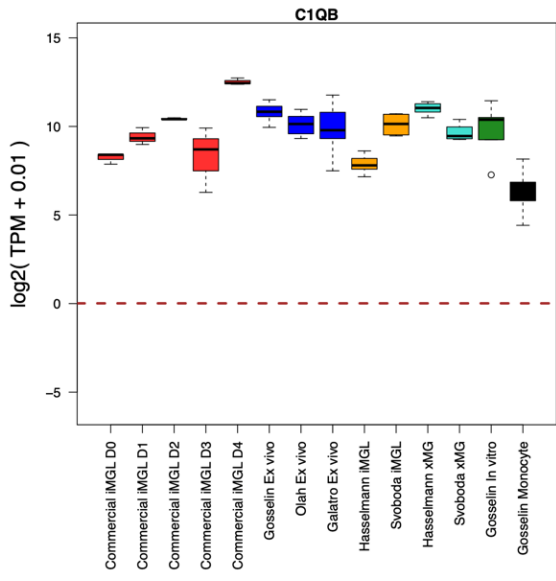
Supplementary Figure 3. Quantitative PCA comparisons

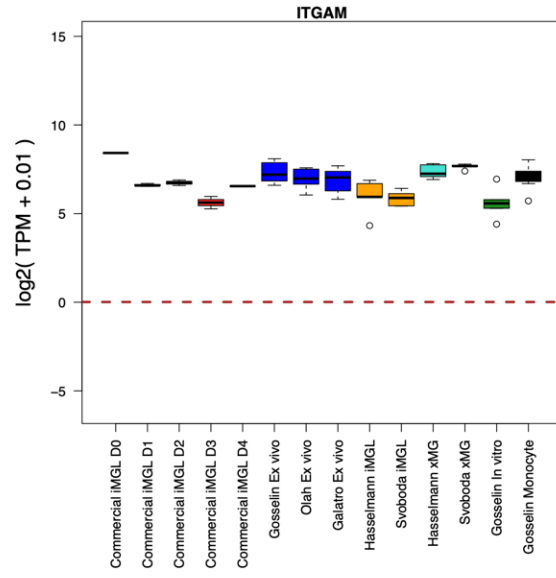
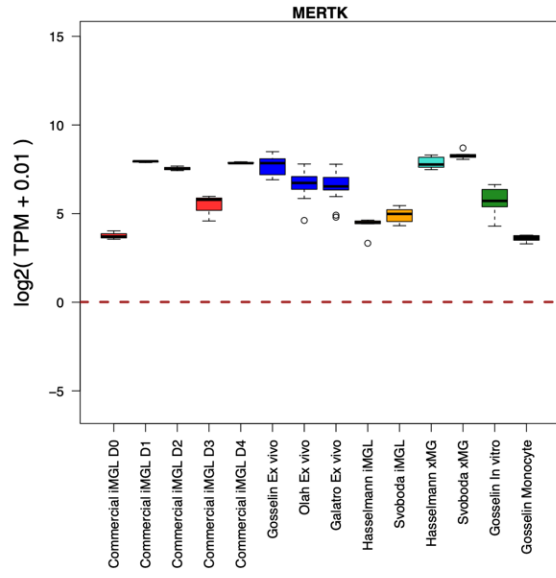
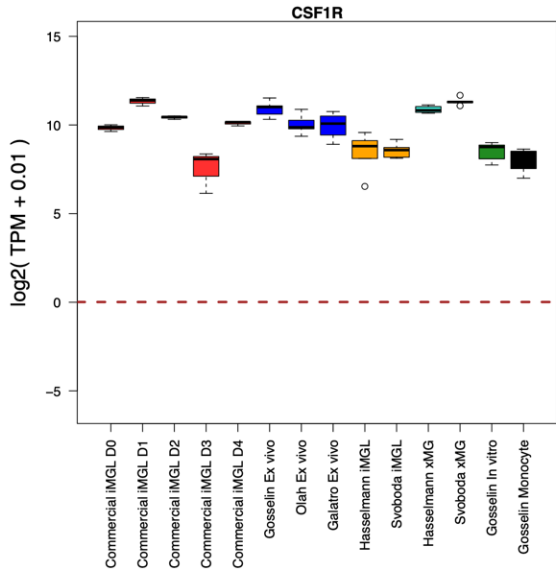
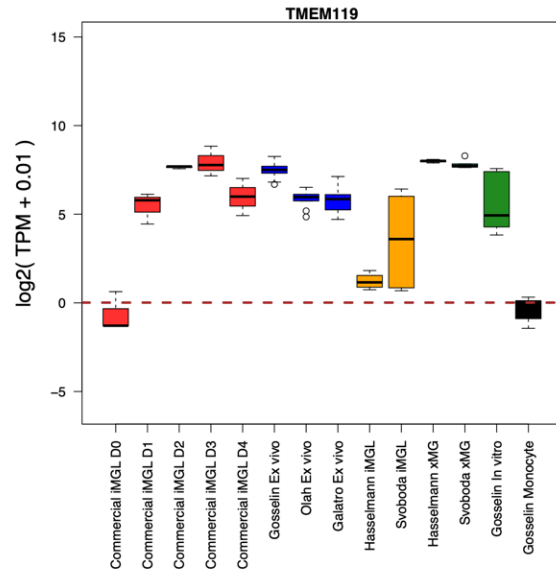
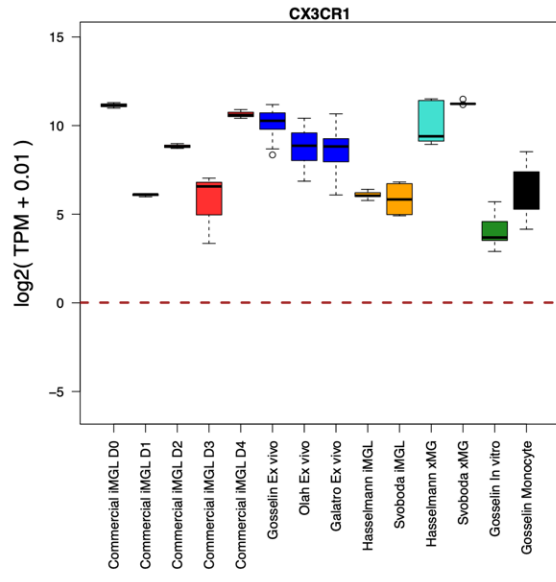
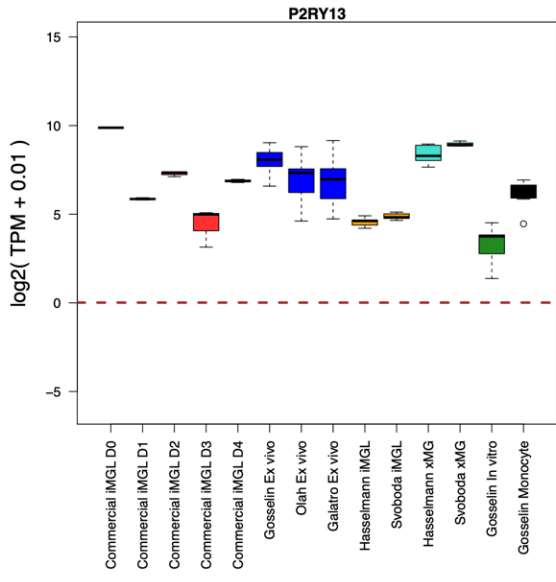
A) Pairwise Sigclust scores comparing Commercial iMGL samples and microglia comparator datasets using principal component analysis on all expressed genes. Each cell represents a comparison between two cell systems and is colored by the Sigclust score (0 to 1). * empirical p-value < 0.05, ** empirical p-value < 0.01

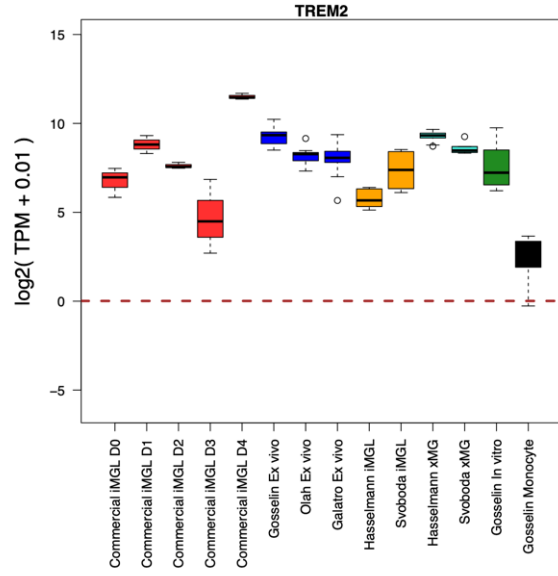
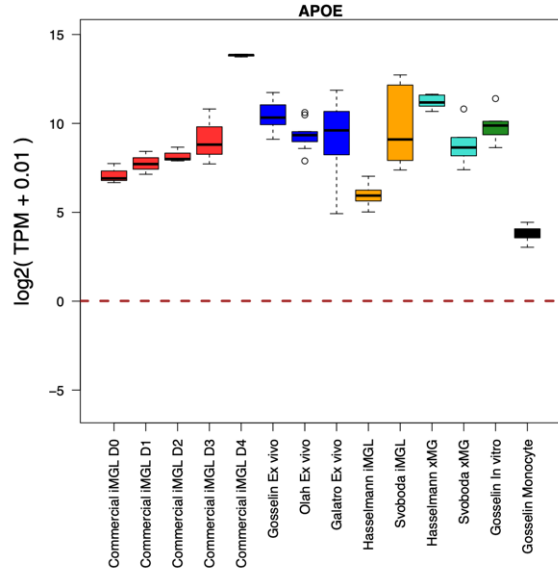
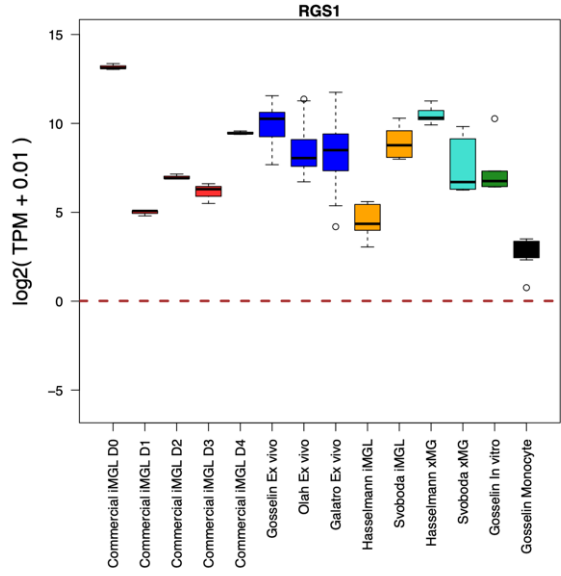
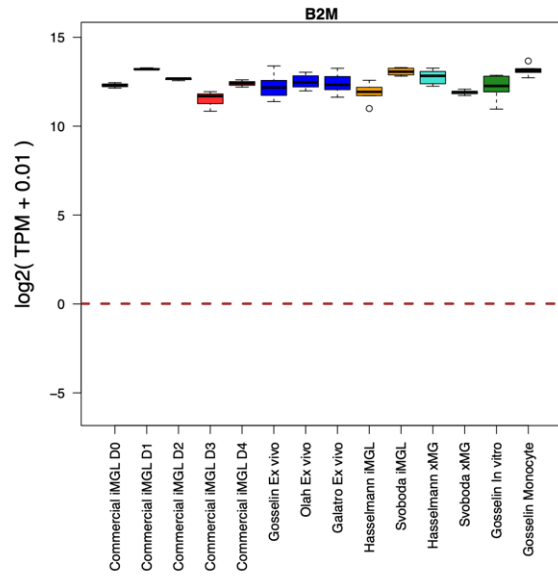
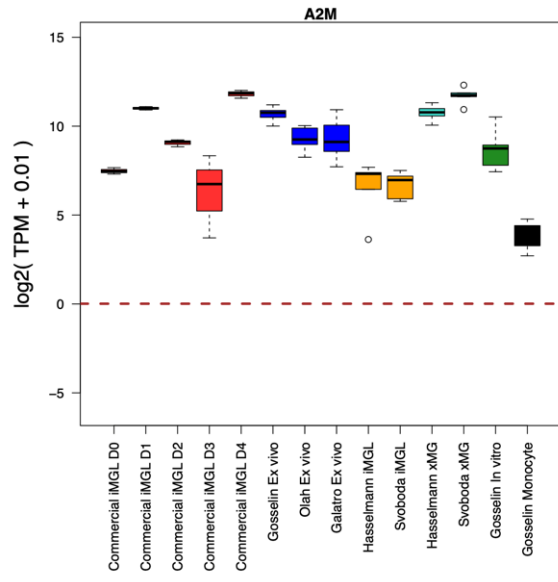
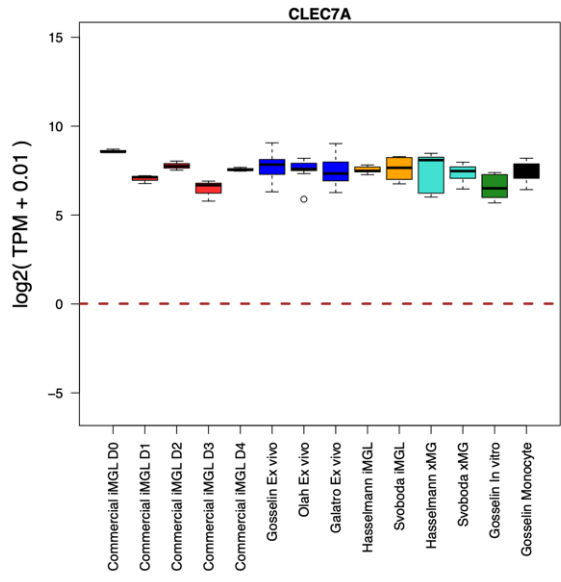
B) Pairwise average Silhouette scores comparing Commercial iMGL samples and microglia comparator datasets using principal component analysis on all expressed genes. Each cell represents a comparison between two cell systems and is colored by the average Silhouette score (-1 to 1). * empirical p-value < 0.05, ** empirical p-value < 0.01

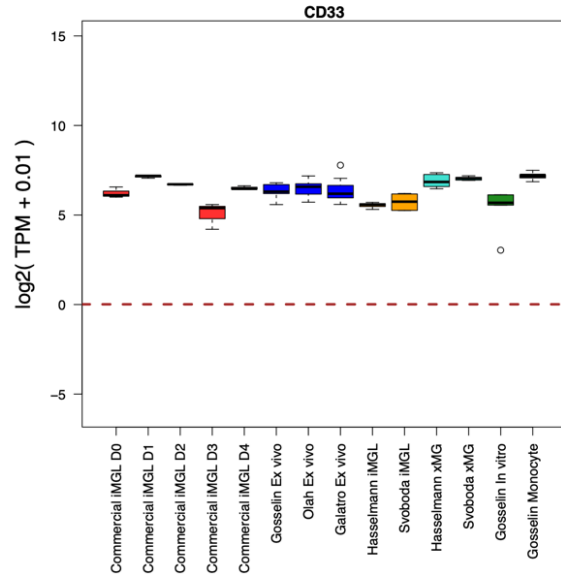
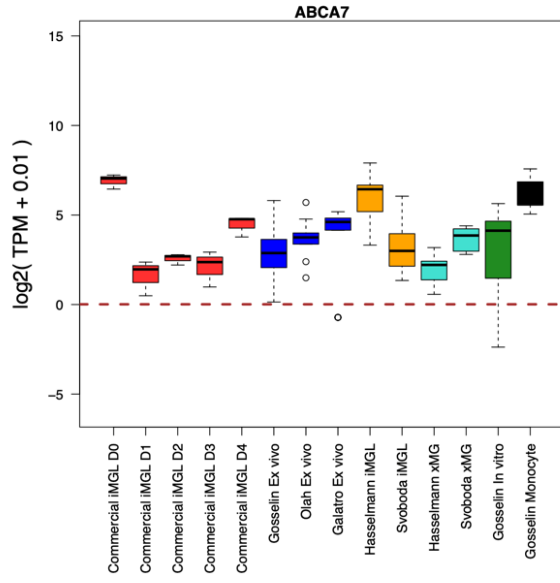
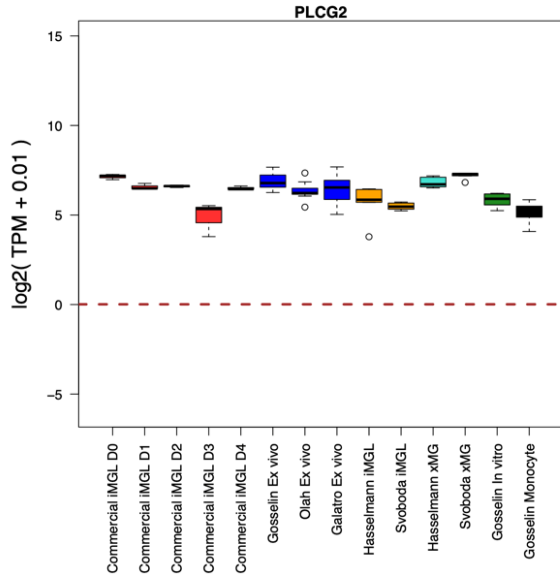
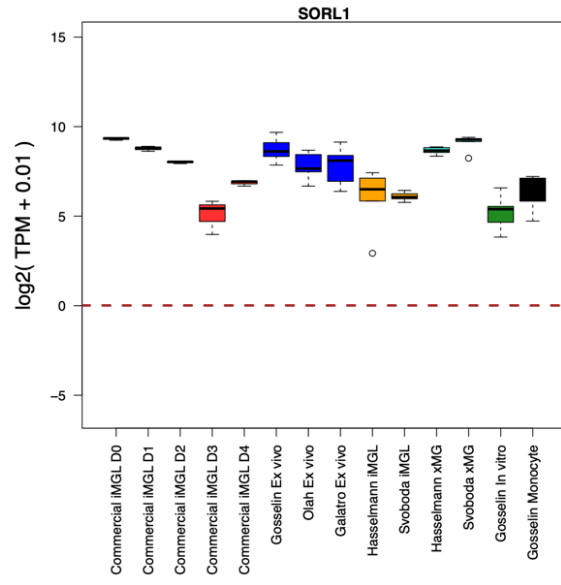
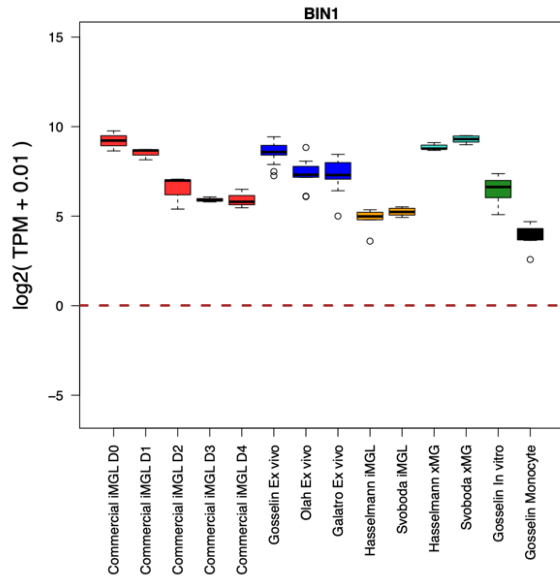
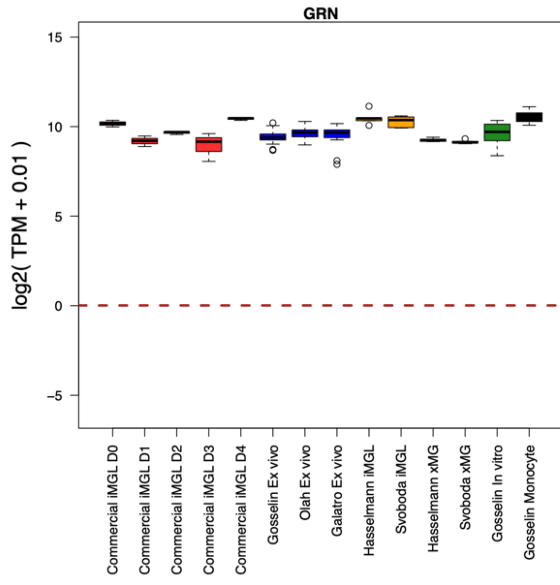


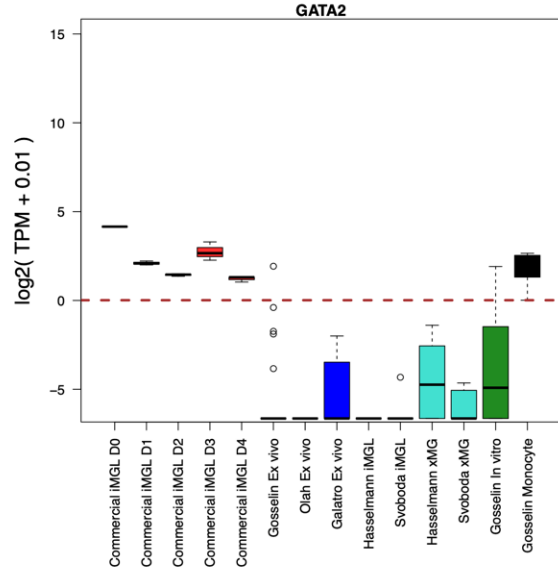
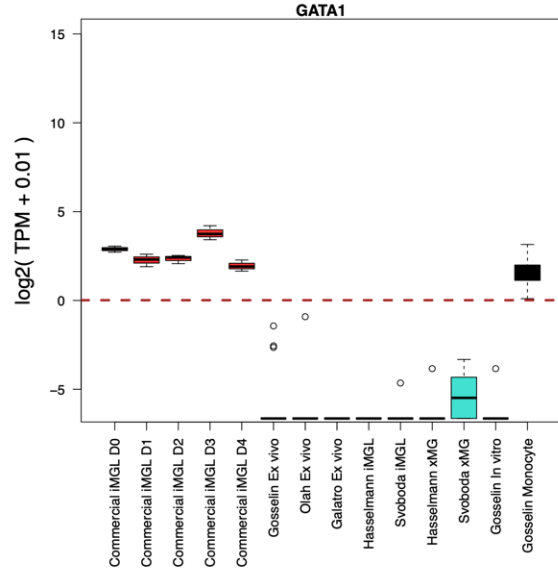
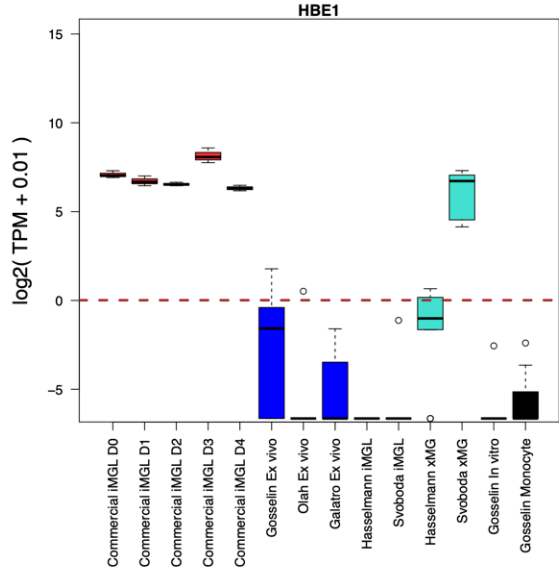
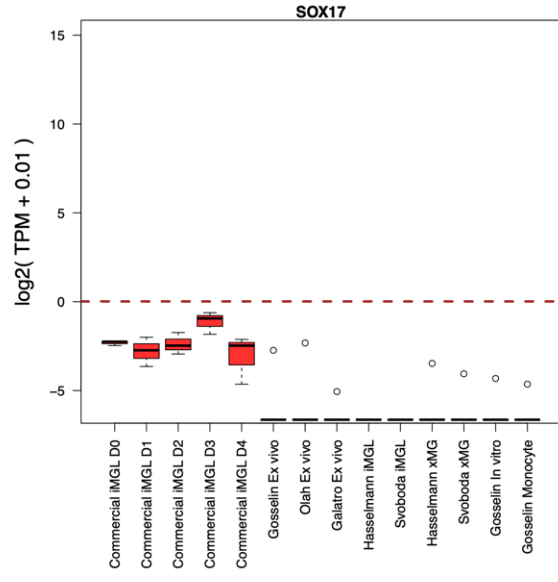
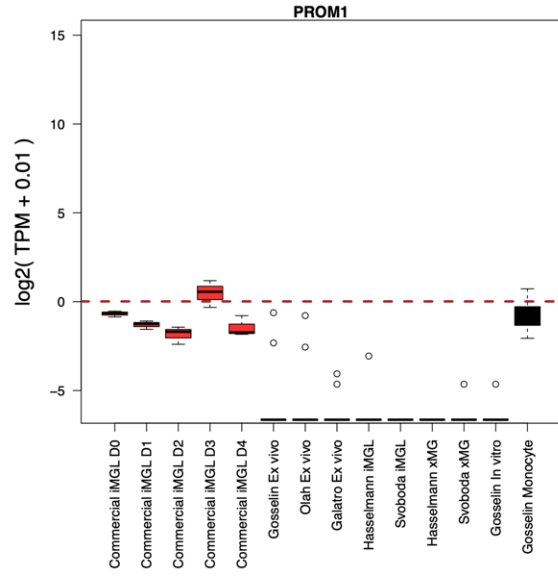
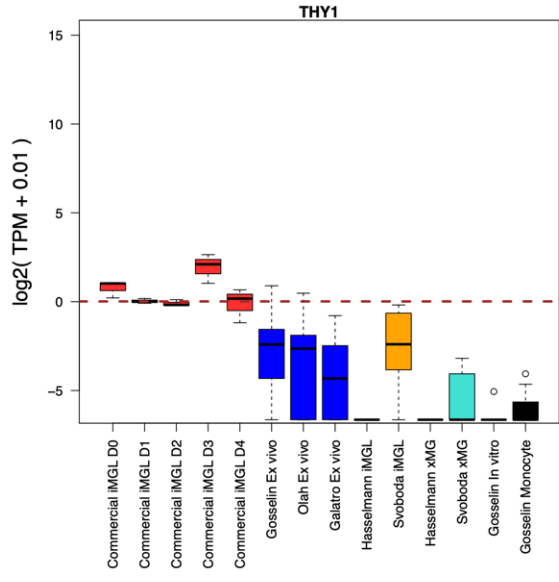


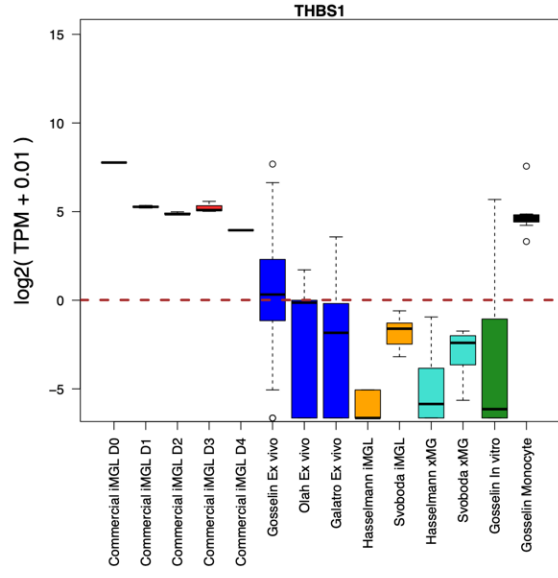
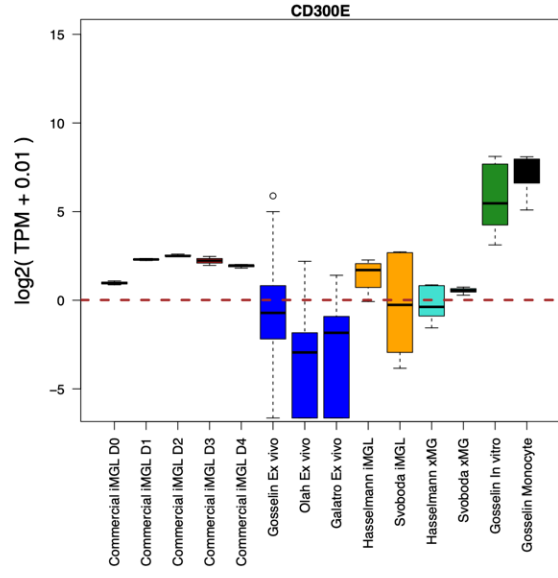
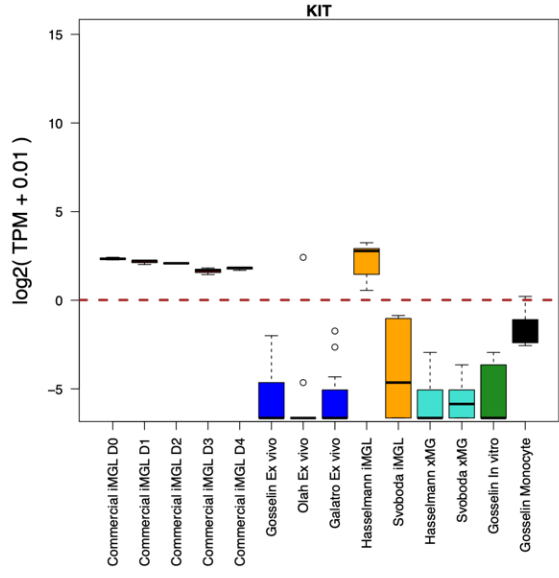
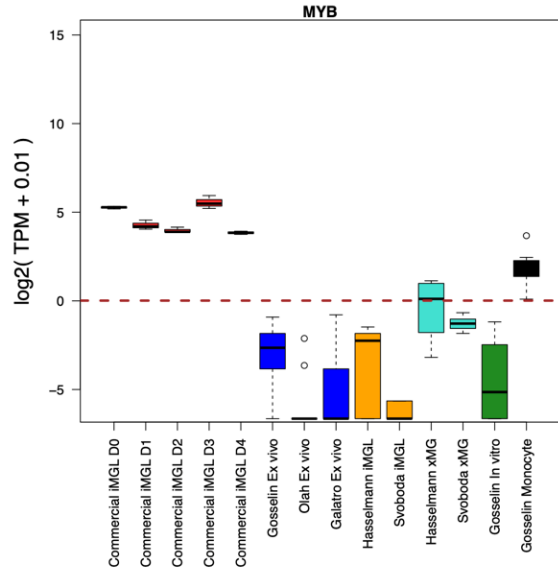
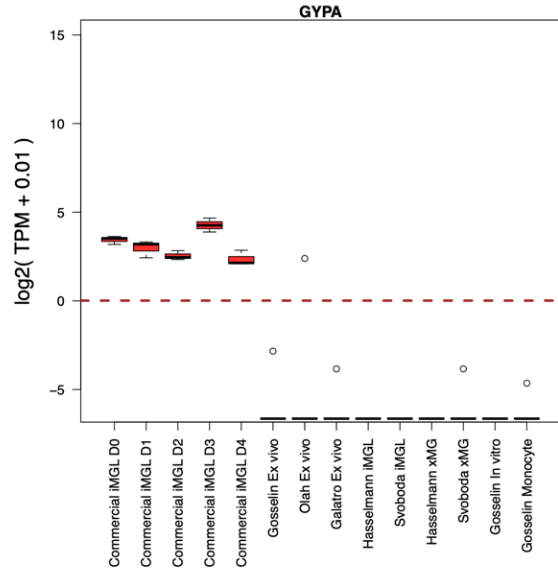
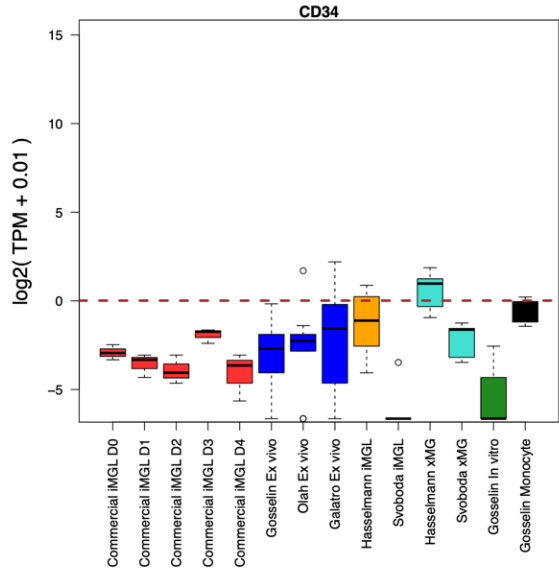


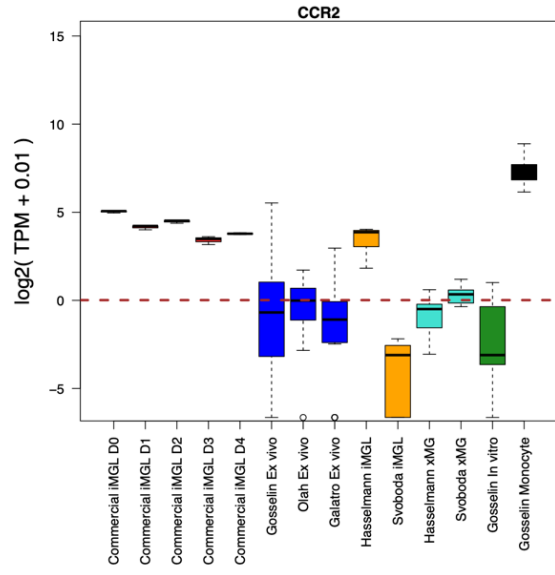
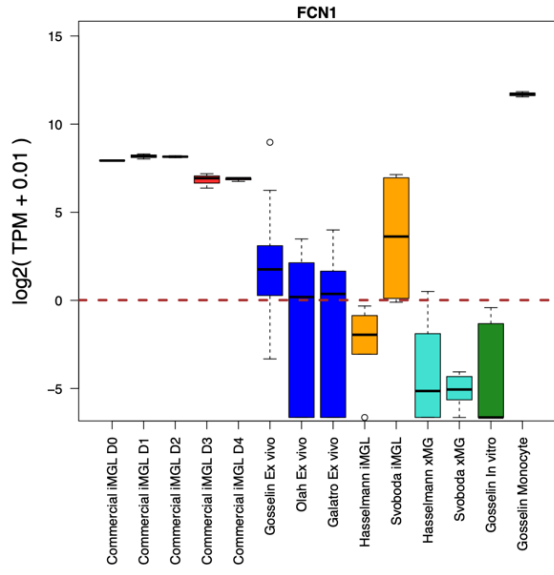
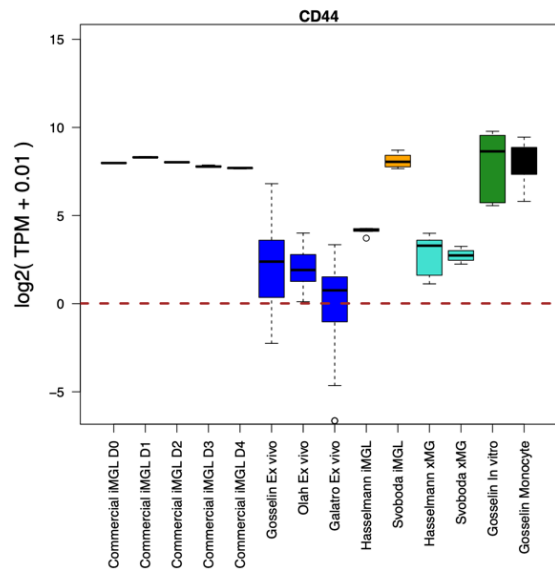
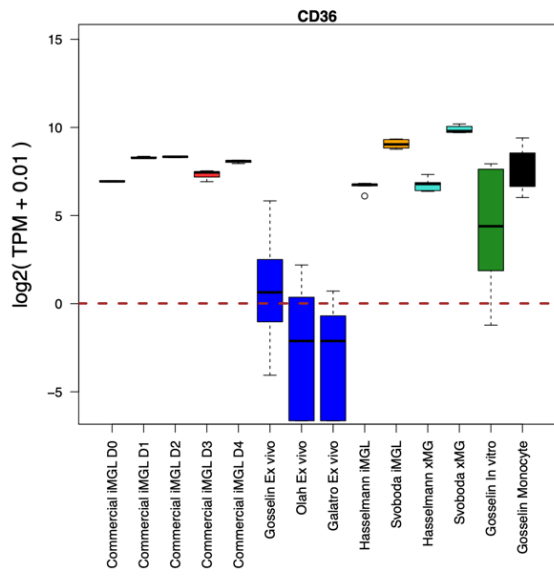












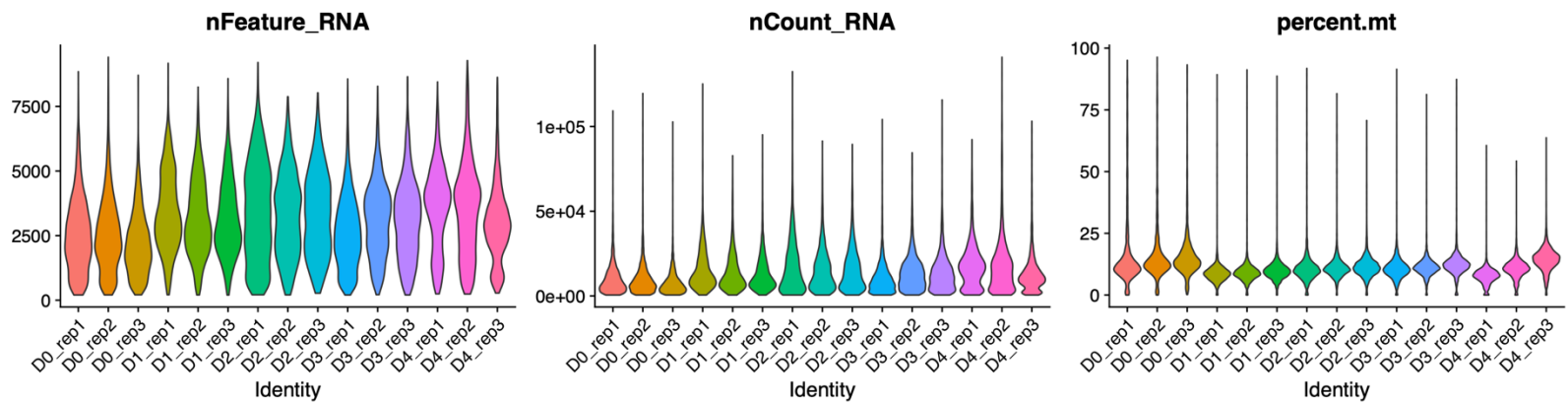
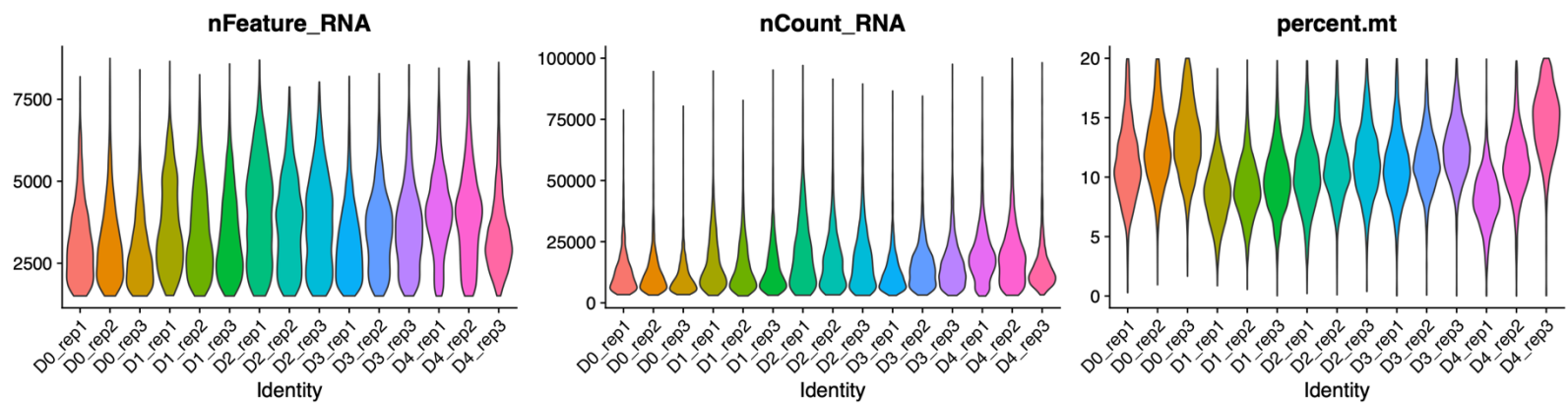
Supplementary Figure 4. Expression of key microglia related genes across the Commercial iMGL samples and microglia comparator datasets.

Microglia TFs: SPI1, IRF8, RUNX1, FOS, CEBPB, MEF2A, MAF, SALL1, TAL1, RUNX2

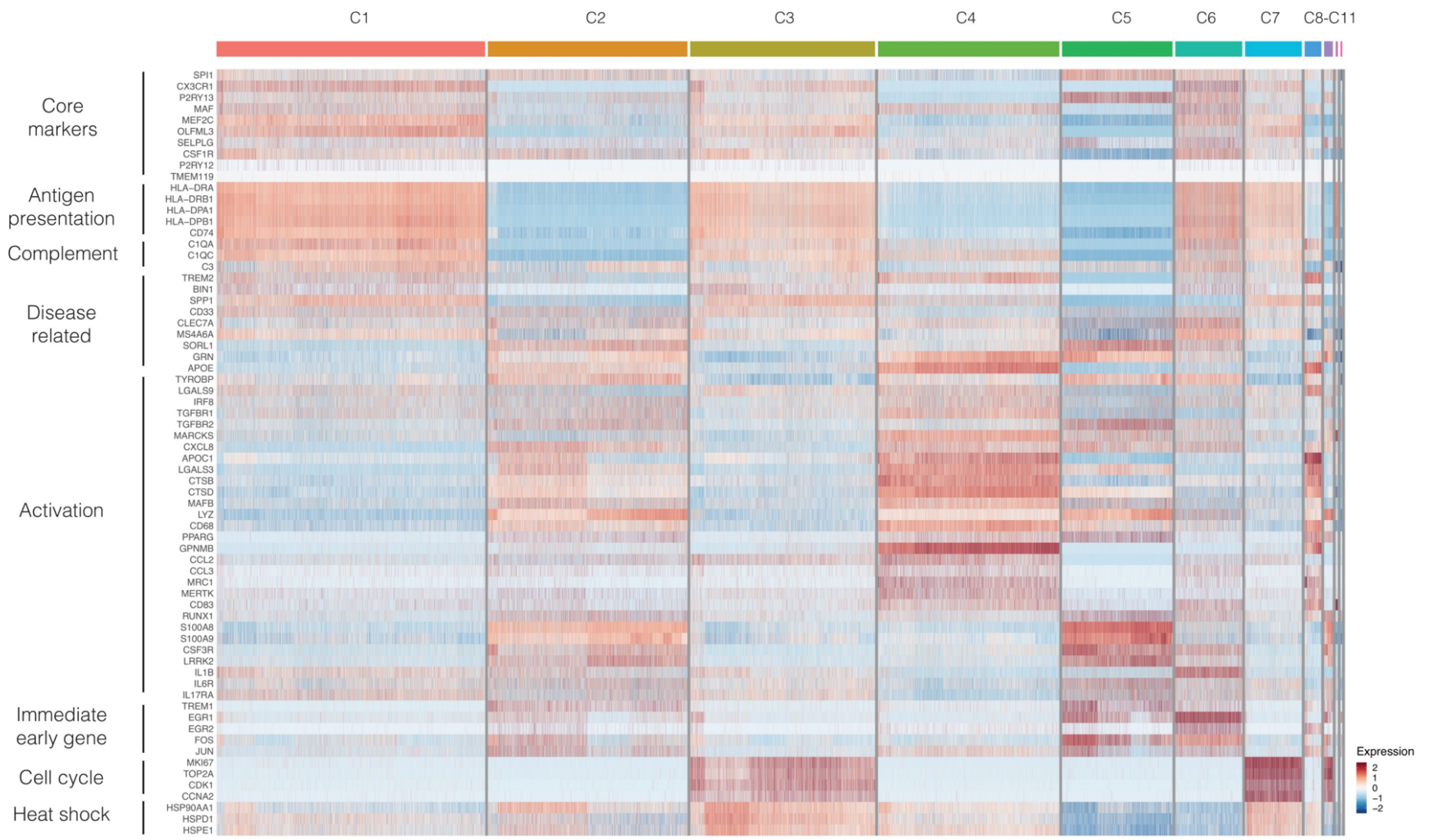
Microglia core marker genes: C3, C1QA, C1QB, C1QC, HLA-B, HLA-DRA, SPP1, P2RY12, P2RY13, CX3CR1, TMEM119, CSF1R, MERTK, ITGAM, CLEC7A, A2M, B2M, RGS1

Disease genes: APOE, TREM2, GRN, BIN1, SORL1, PLCG2, ABCA7, CD33

Myeloid progenitor and monocyte marker genes: THY1, PROM1, SOX17, HBE1, GATA1, GATA2, CD34, GYPA, MYB, KIT, CD300E, THBS1, CD36, CD44, FCN1, CCR2

A**B**

Supplementary Figure 5. Quality control (QC) metrics for Commercial iMGL time course single cell RNA-seq dataset. The number of genes (nFeature_RNA), reads (nCount_RNA), and percentage of mitochondrial reads (percent.mt) for cells in each sample **A**) before and **B**) after applying QC filters.



Core markers

Antigen presentation
Complement

Disease related

Activation

Immediate early gene

Cell cycle

Heat shock

SPH1
CX3CR1
P2RY13
MAF
MEF2C
OLFML3
SELP1G
CSF1R
P2RY12
TMEM119
HLA-DRA
HLA-DRB1
HLA-DPA1
HLA-DPB1
CD74
C1QA
C1QC
C3
TREM2
BIN1
SPP1
CD33
CLEC7A
MS4A6A
SORL1
GRN
APOE
TYROBP
LGALS9
IRF8
TGFBRI1
TGFBRI2
MARCKS
CXCL8
APOC1
LGALS3
CTSB
CTSD
MAFB
LYZ
CD68
PPARG
GPNMB
CCL2
CCL3
MRC1
MERTK
CD83
RUNX1
S100A8
S100A9
CSF3R
LRRK2
IL1B
IL5R
IL17RA
TREM1
EGR1
EGR2
FOS
JUN
MKI67
TOP2A
CDK1
CCNA2
HSP90AA1
HSPD1
HSPET1

C1

C2

C3

C4

C5

C6

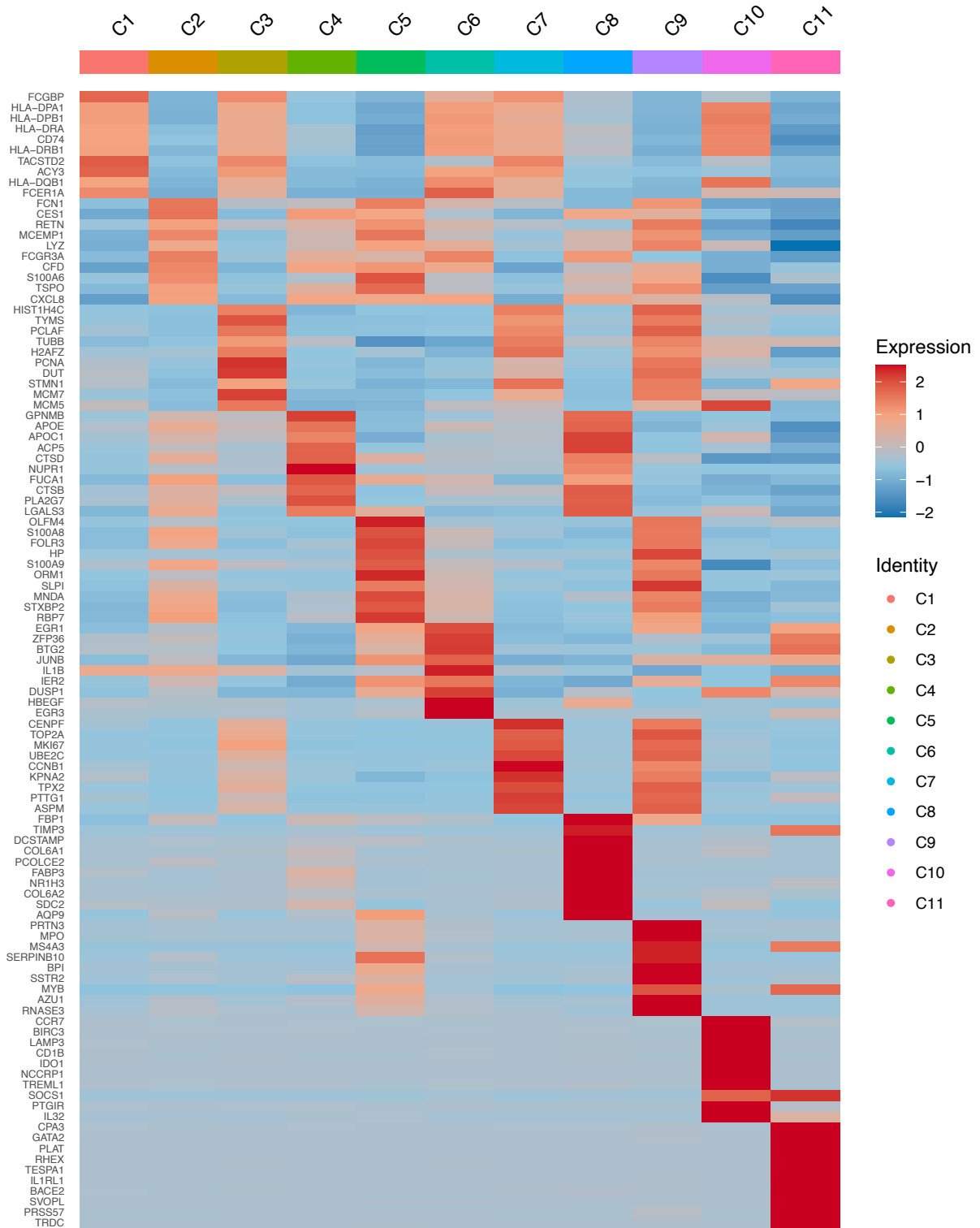
C7

C8-C11

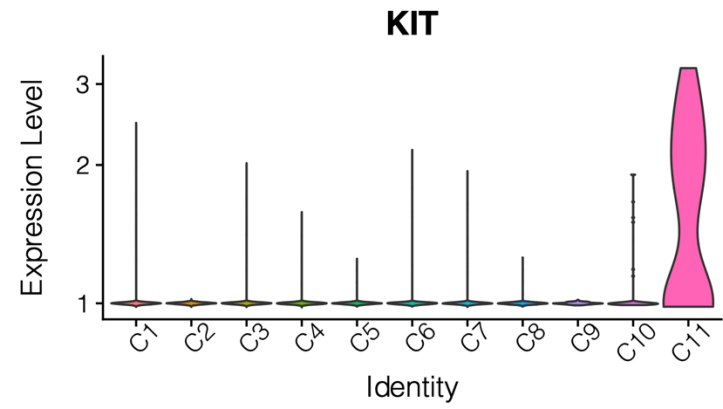
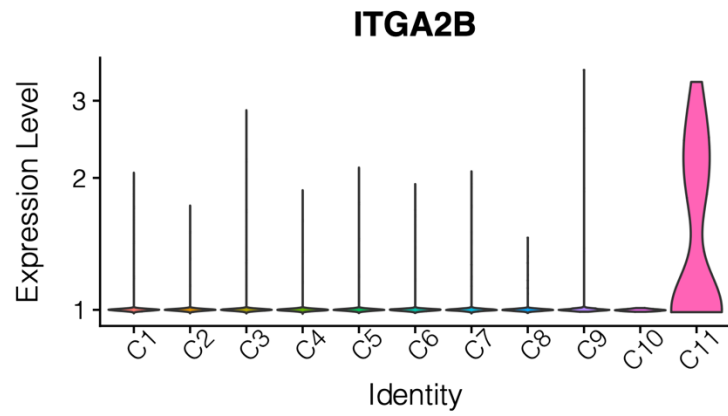
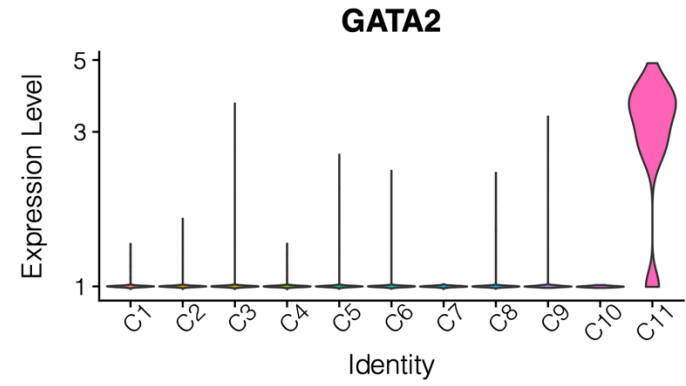
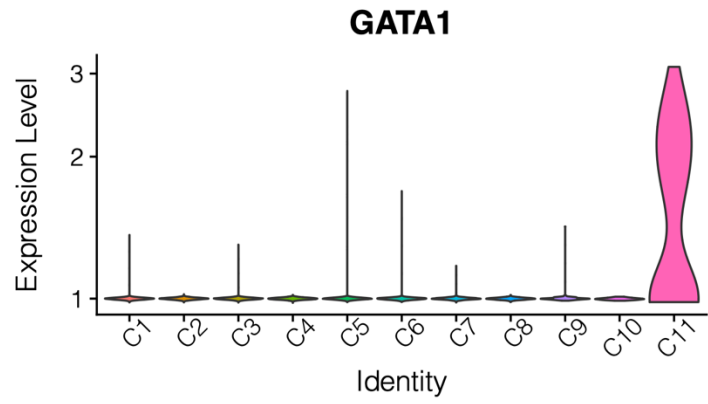
Expression
2
1
0
-1
-2

Supplementary Figure 6. Gene expression heatmap of microglia functional marker genes in the time course single cell RNA-seq dataset.

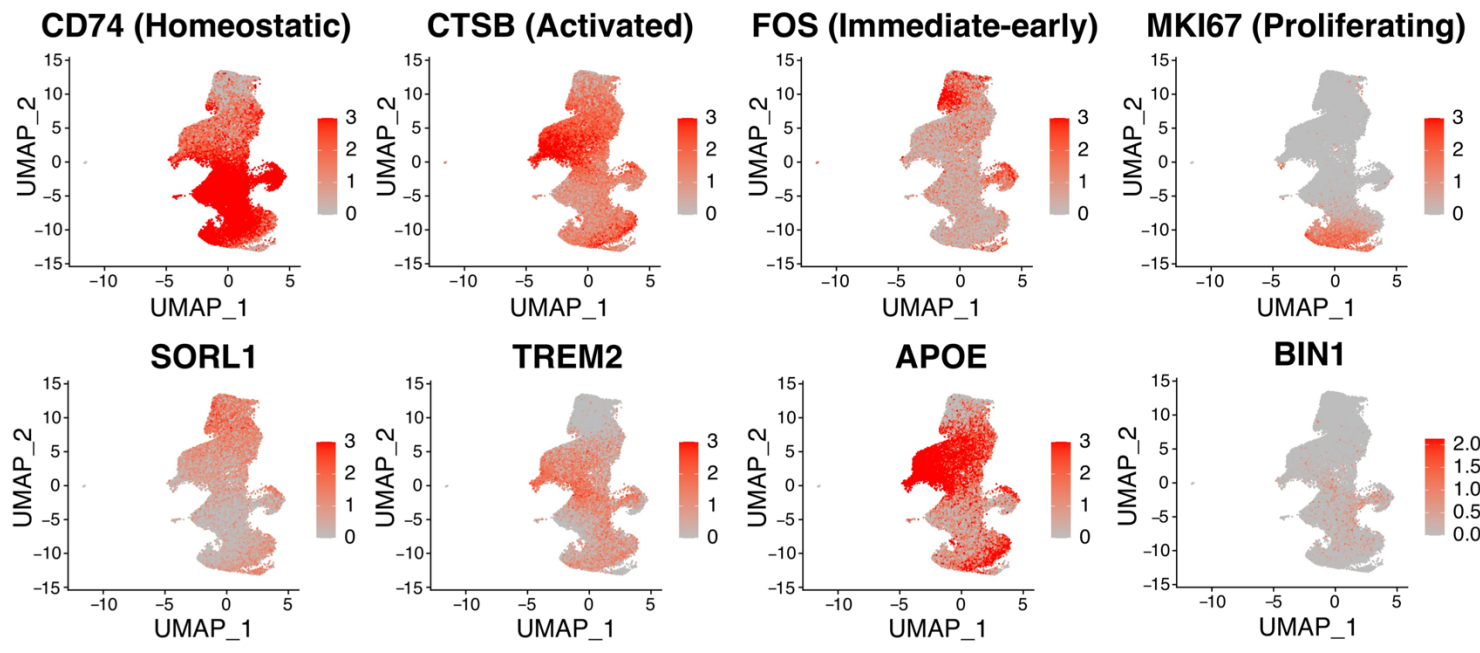
Supplementary Figure 7. Identification of preferentially expressed marker genes and their top gene ontology enrichments for each cellular cluster in the time course single cell RNA-seq dataset.



Supplementary Figure 8. Heatmap of average gene expression per cluster of the top 10 marker genes for each cluster in the time course single cell RNA-seq dataset.



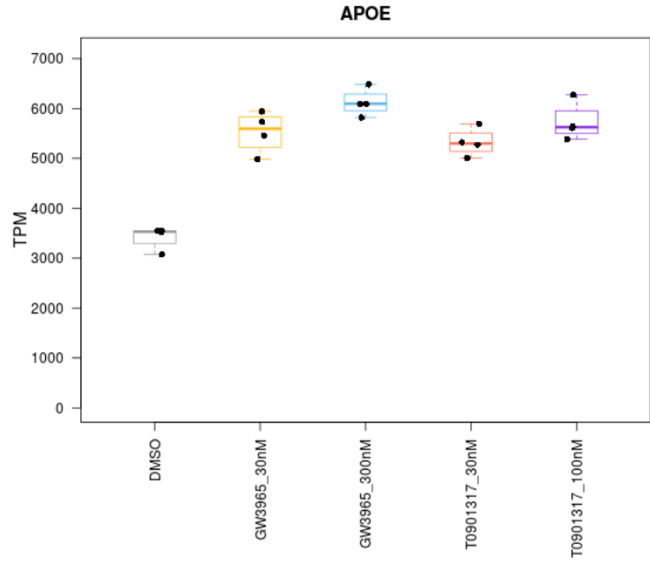
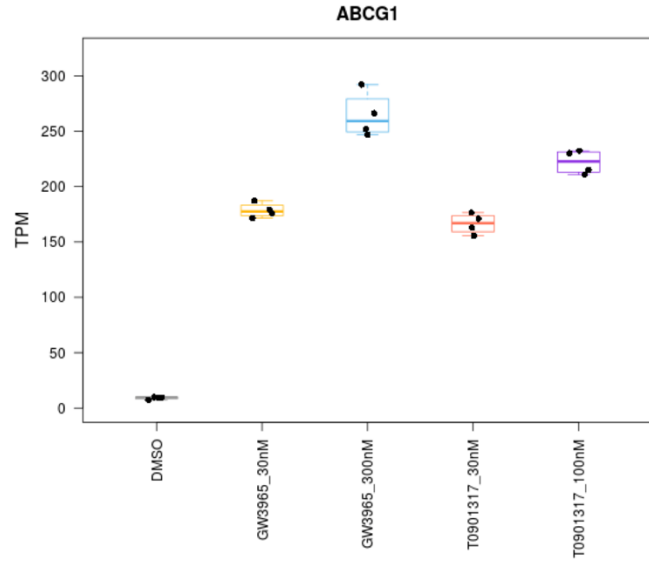
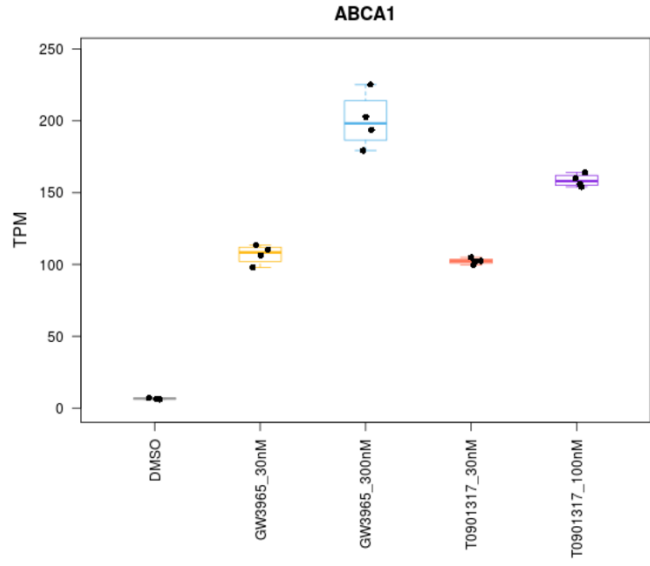
Supplementary Figure 9. Expression of progenitor (GATA1, GATA2, KIT) and monocyte (ITGA2B) marker genes across the 11 single cell clusters.



Supplementary Figure 10. Relative expression of key homeostatic (CD74), activated (CTSB), immediate-early (FOS), proliferating (MKI67), and AD-associated (SORL1, TREM2, APOE, BIN1) genes across the individual cells. Expression values were scaled using the FeaturePlot method in Seurat.

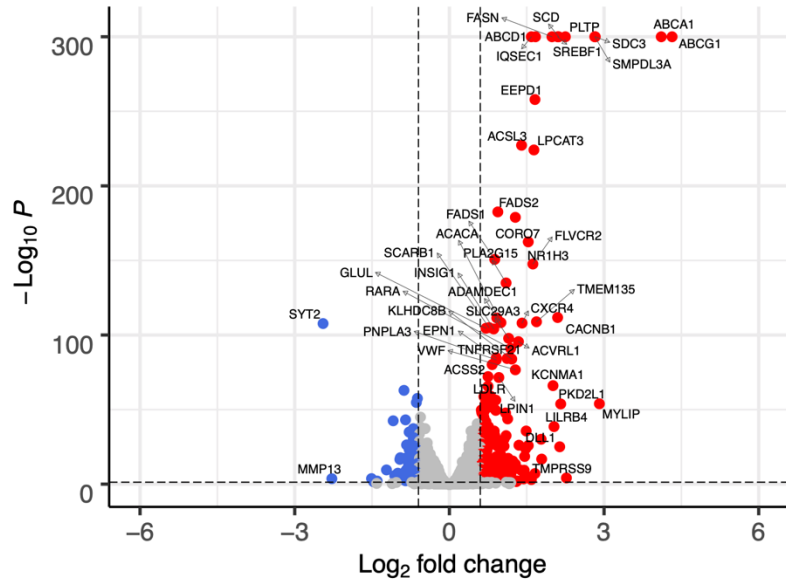
<u>Ex vivo subcluster</u>	<u>Functional annotation</u>
Olah 1	Homeostatic
Olah 2	Homeostatic
Olah 3	Activated, distressed
Olah 4	Activated, interferon response
Olah 5	Activated, anti-inflammatory
Olah 6	Activated, anti-inflammatory
Olah 7	Homeostatic, antigen presentation
Olah 8	Metabolic
Olah 9	Proliferative
Olah 10	Monocytes
Olah 11	T cells
Olah 12	B cells
Olah 13	GFAP+ cells
Olah 14	Red blood cells
Sankowski 1	Activated
Sankowski 2	Homeostatic, antigen presentation
Sankowski 3	Homeostatic
Sankowski 5	Activated
Sankowski 6	Metabolic
Sankowski 7	Metabolic
Sankowski 8	Activated
Sankowski 9	Activated

Supplementary Figure 11. Functional annotations of ex vivo cell clusters curated from their descriptions in the Olah and Sankowski publications.

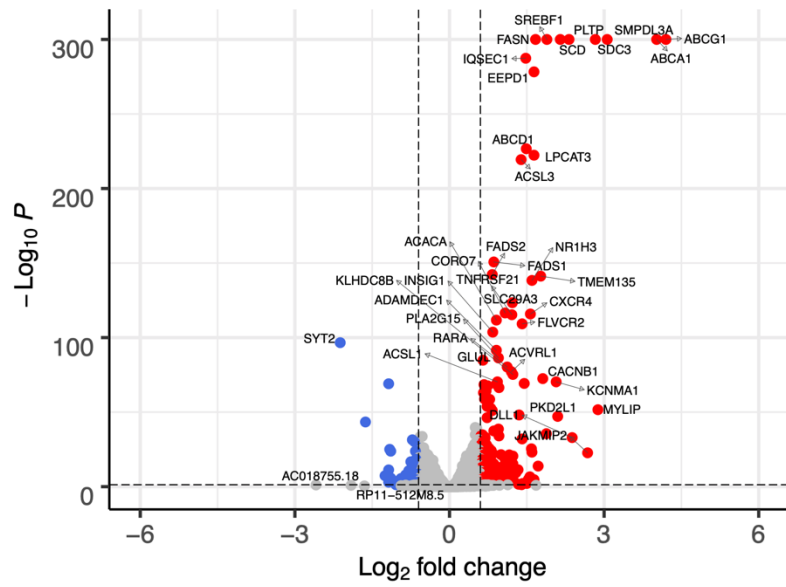


Supplementary Figure 12. Expression of known LXR pathway response genes (ABCA1, ABCG1, APOE) in the LXR pathway agonist treatment bulk RNA-seq dataset.

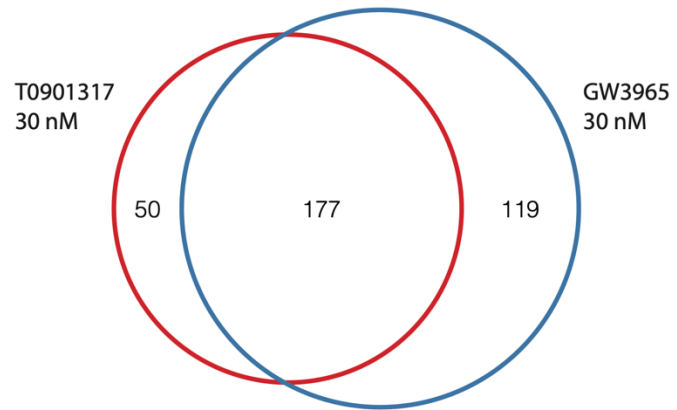
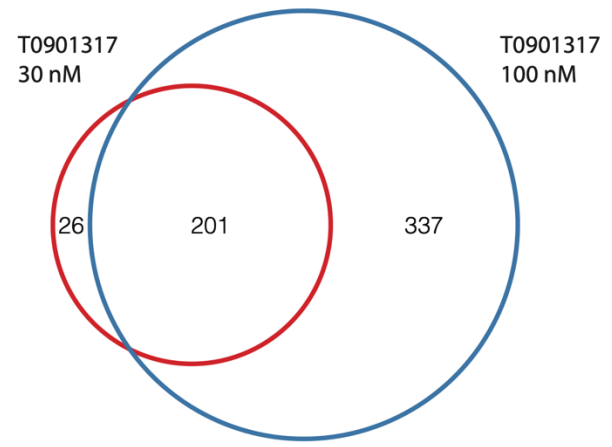
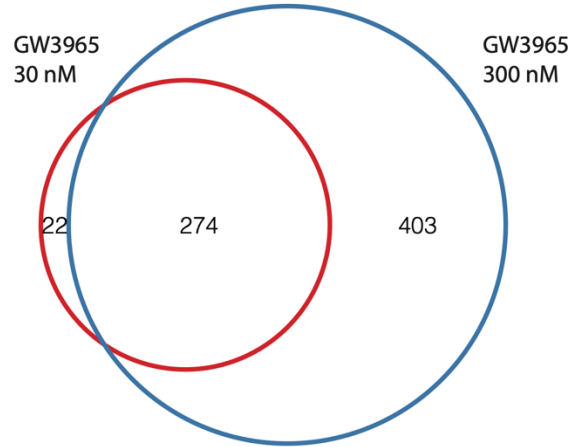
GW3965 30nM:



T0901317 30nM:



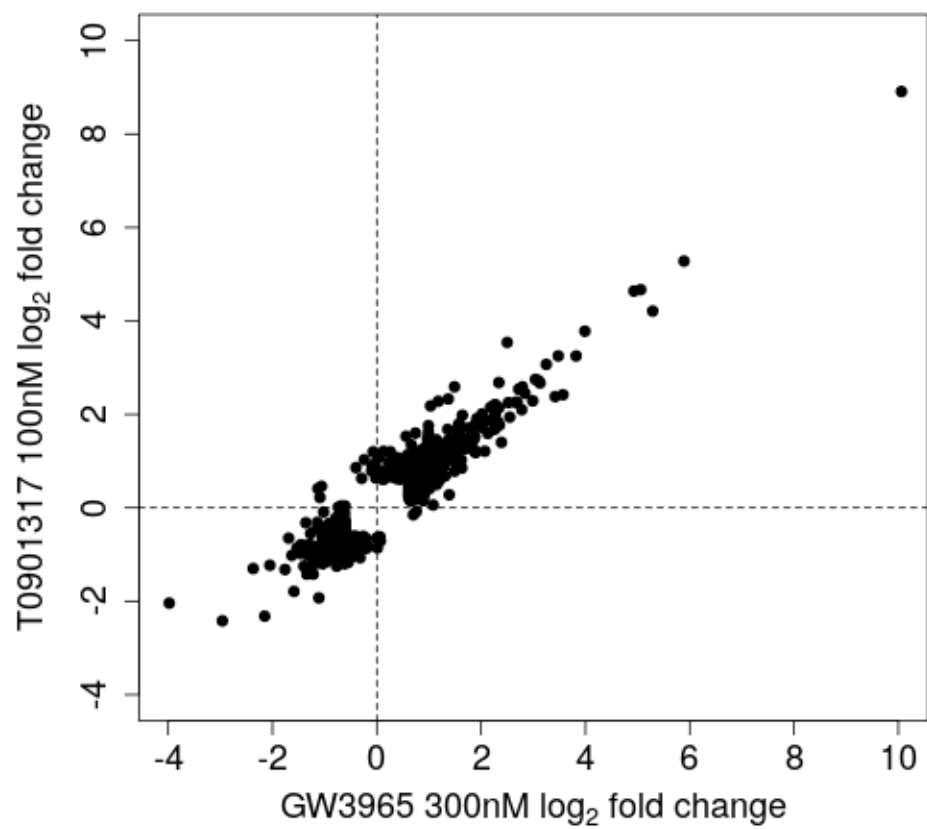
Supplementary Figure 13. Volcano plots showing relationship between expression fold change and significance for differential gene expression between GW3965 30nM and T0901317 30nM with DMSO treated cells. Genes with significant upregulation and downregulation are colored red and blue, respectively.



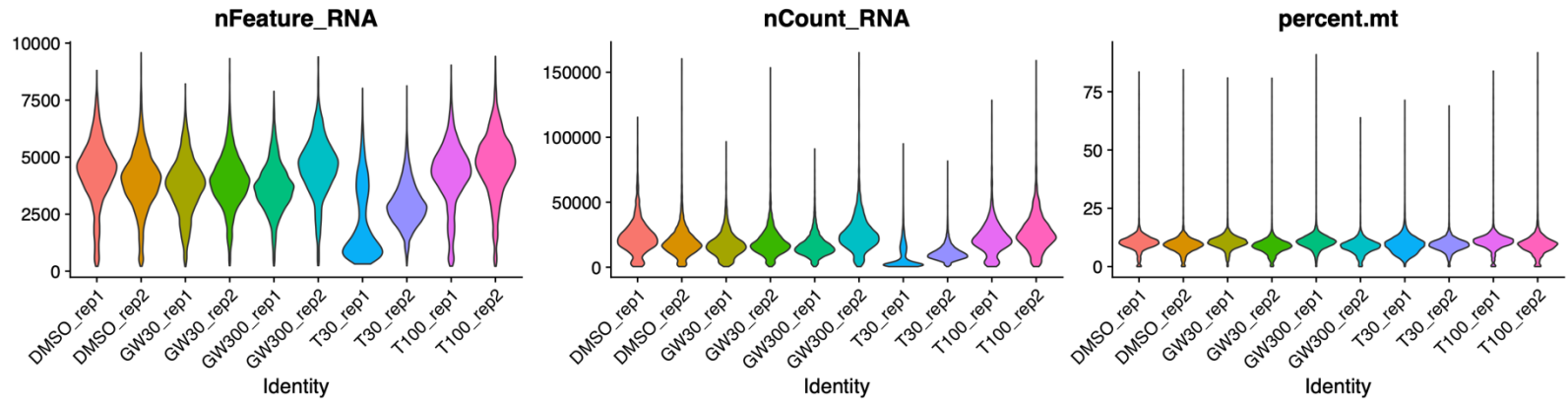
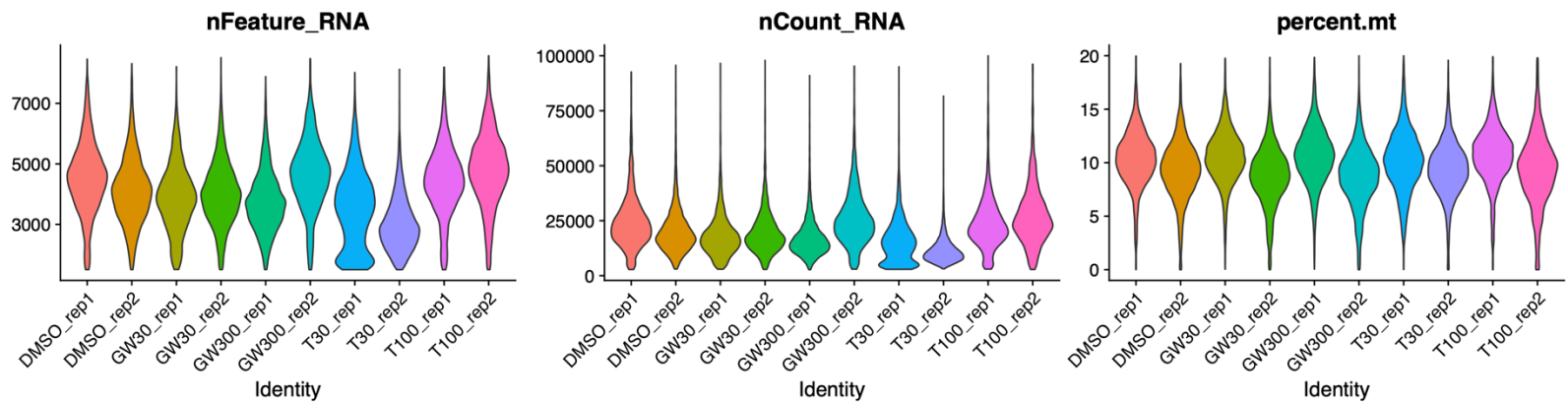
Supplementary Figure 14. Overlap in DEGs between GW3965 30nM and 300nM treatments.

Overlap in DEGs between T0901317 30nM and 100nM treatments. Overlap in DEGs between

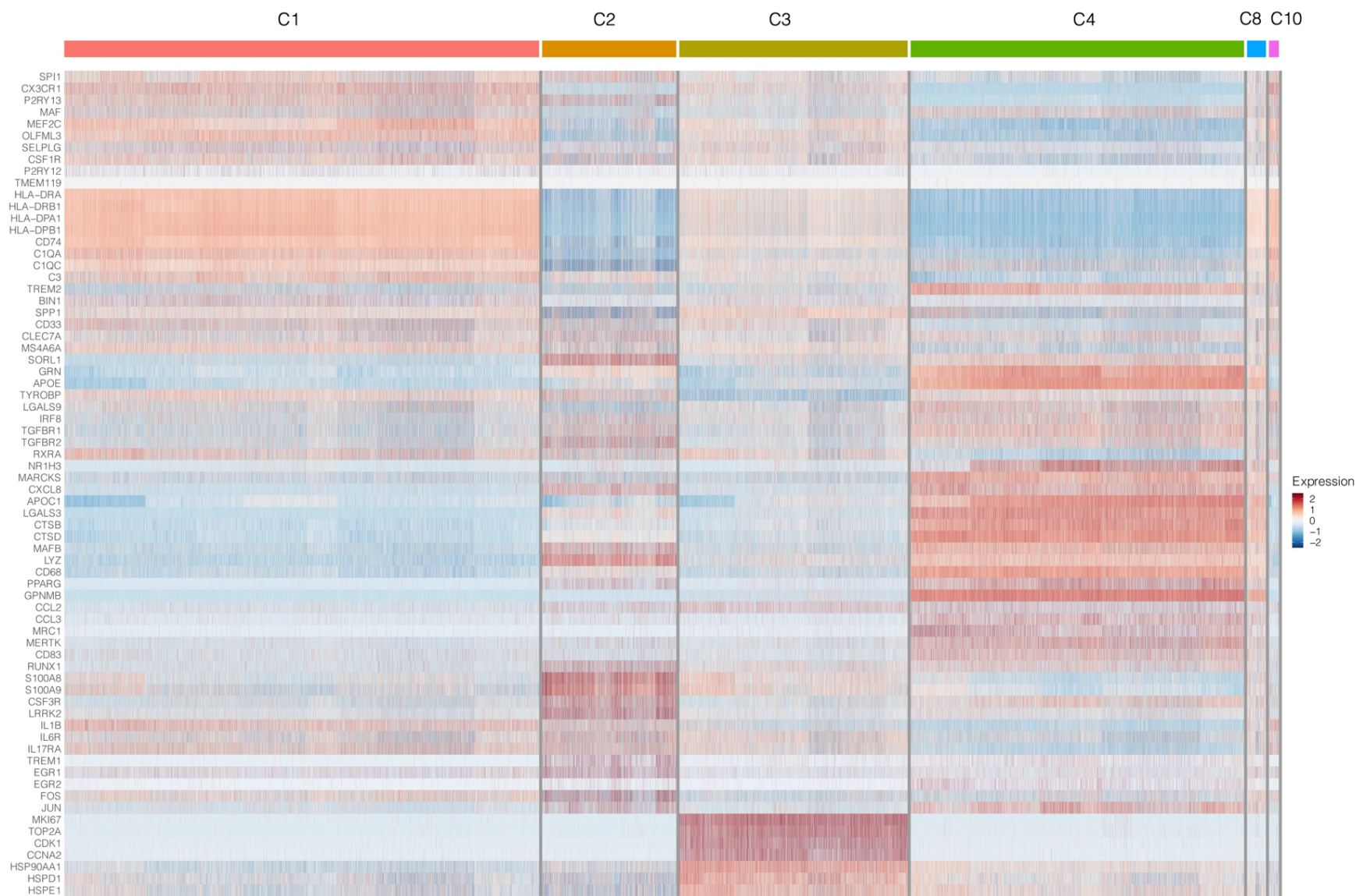
GW3965 30nM and T0901317 30nM treatments.



Supplementary Figure 15. Comparison of DEG fold changes between GW3965 300nM and T0901317 100nM treatments. The union of DEGs between the two treatments were used in this comparison.

A**B**

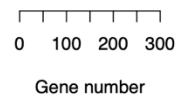
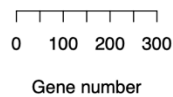
Supplementary Figure 16. QC metrics for LXR pathway agonist treatment single cell RNA-seq dataset. The number of genes (nFeature_RNA), reads (nCount_RNA), and percentage of mitochondrial reads (percent.mt) for cells for cells in each sample **A**) before and **B**) after applying QC filters.



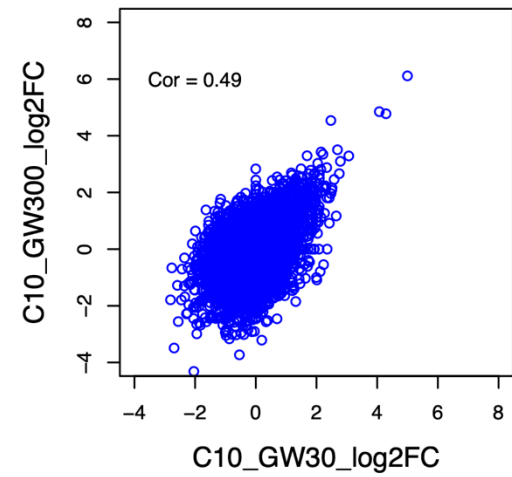
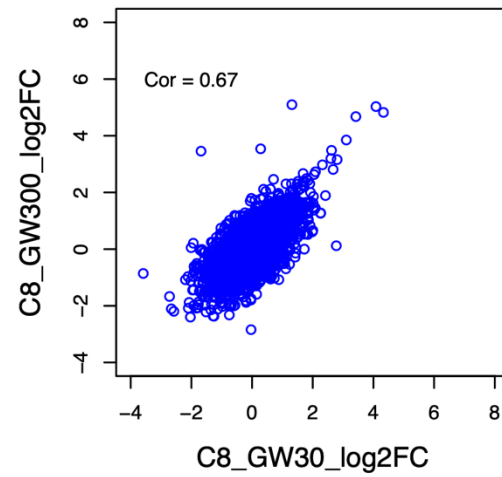
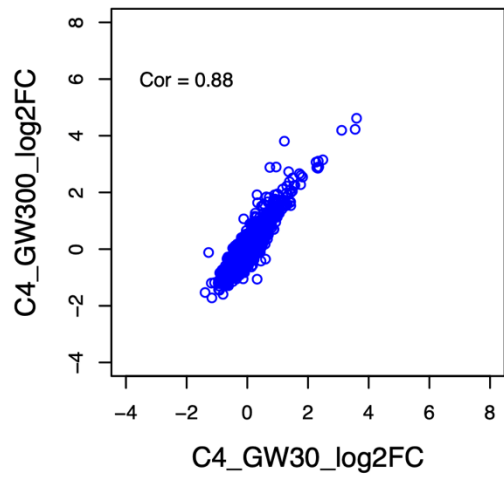
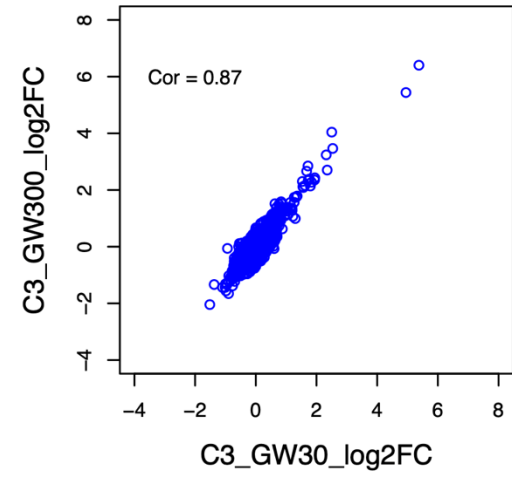
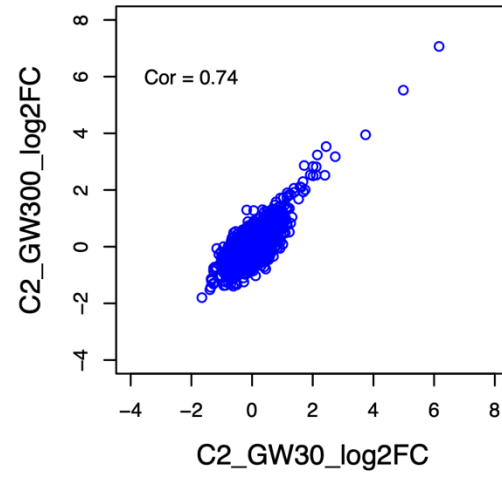
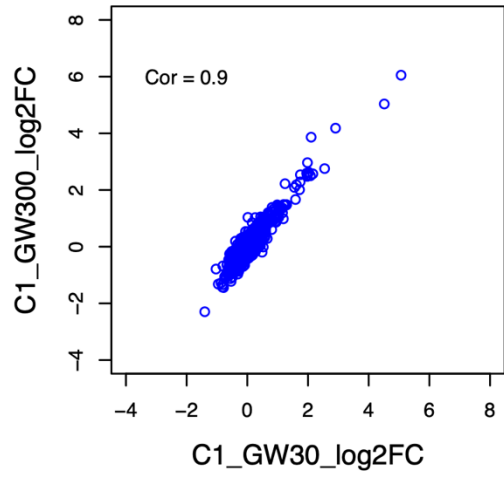
Supplementary Figure 17. Gene expression heatmap of microglia functional marker genes in the LXR pathway agonist treatment single cell RNA-seq dataset.

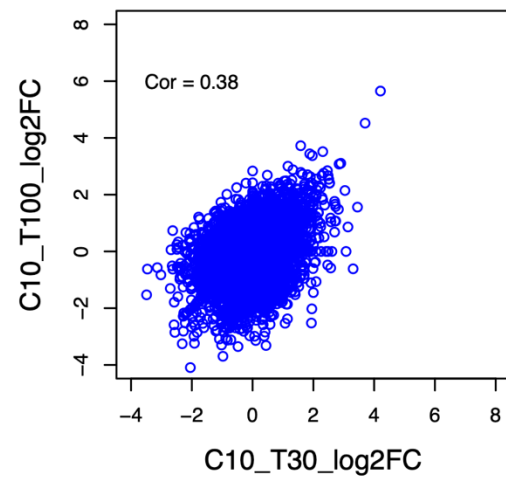
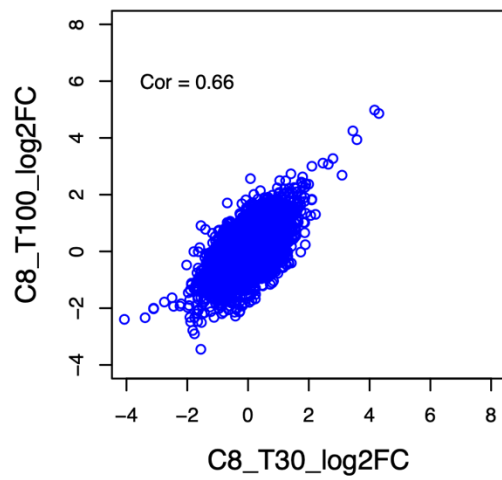
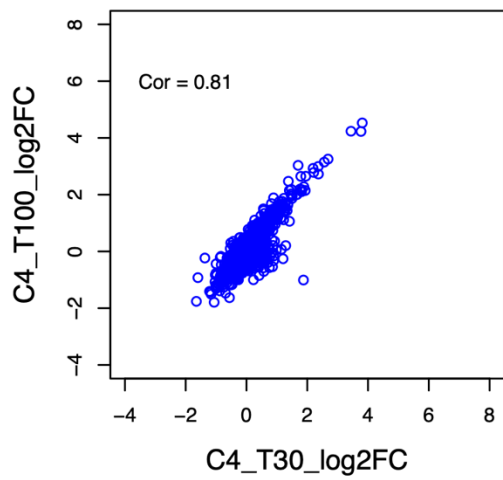
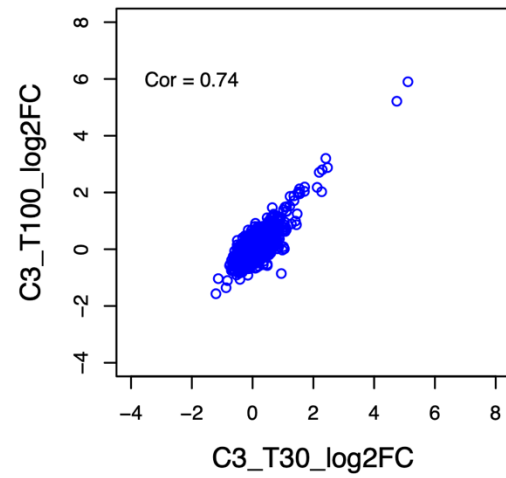
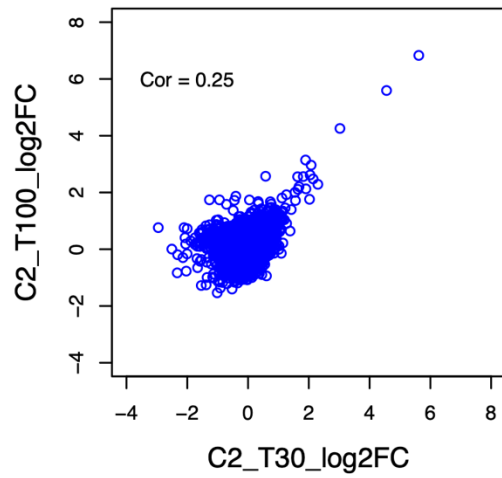
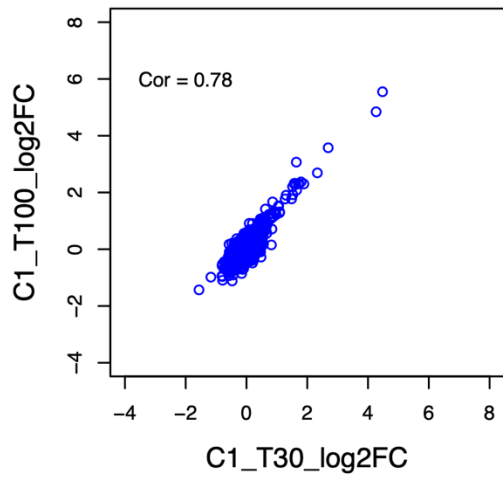
Upregulated

Downregulated

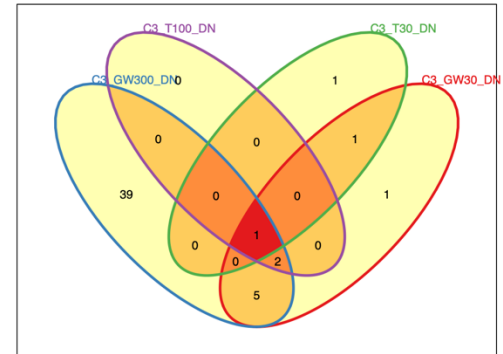
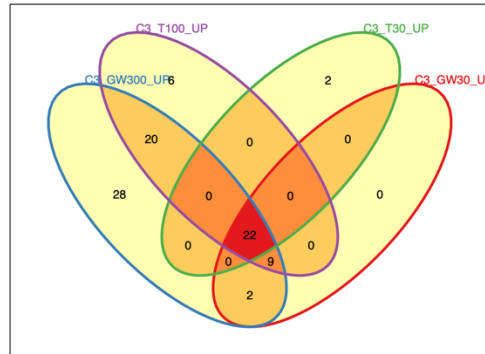
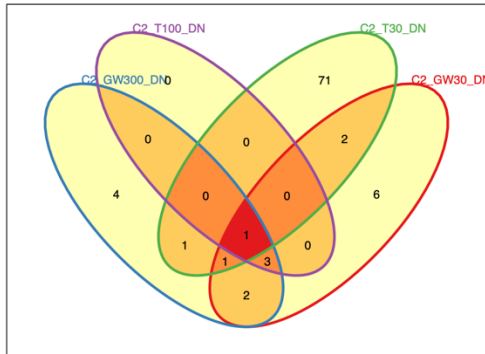
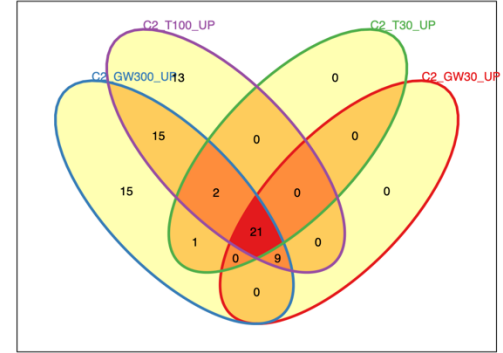
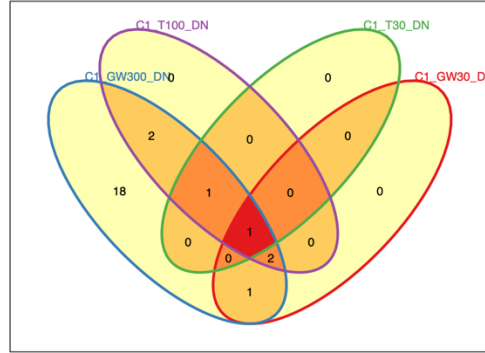
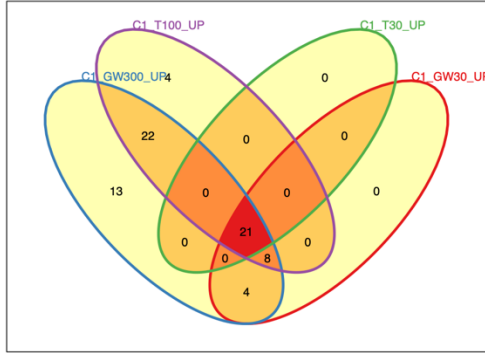


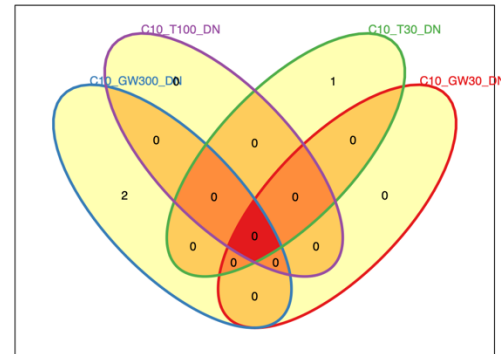
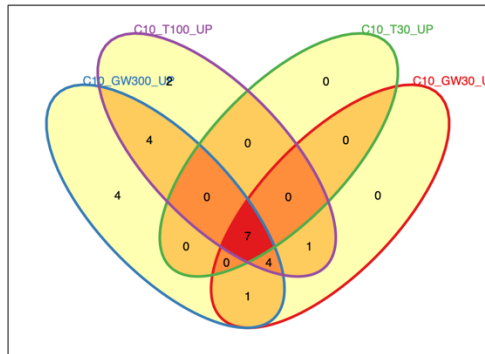
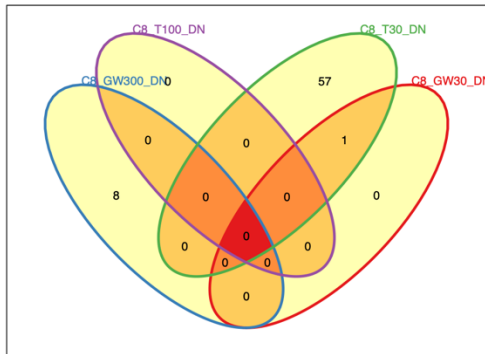
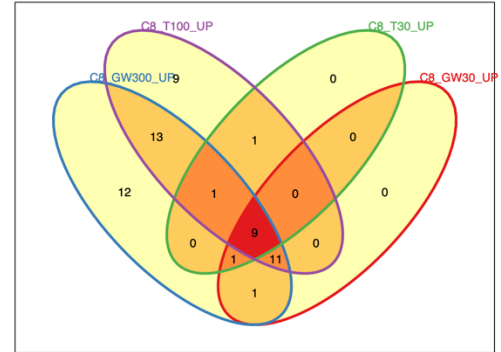
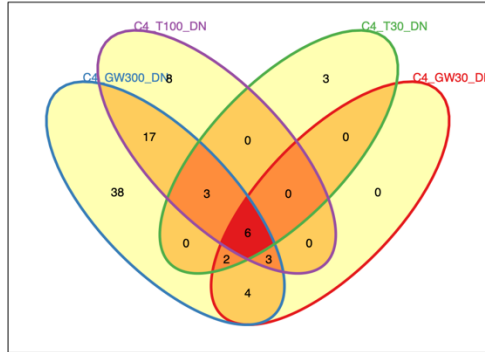
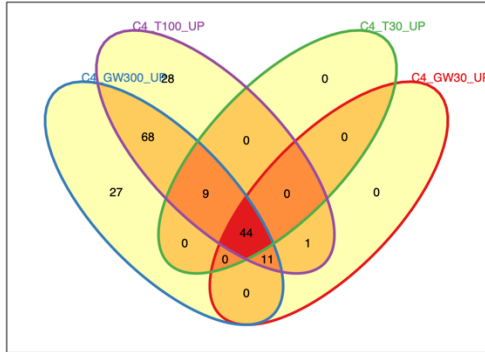
Supplementary Figure 18. Number of DEGs identified in each single cell cluster for each treatment condition against DMSO. GW30: GW3965 30 nM, GW300: GW3965 300 nM, T30: T0901317 30 nM, T100: T0901317 100 nM.





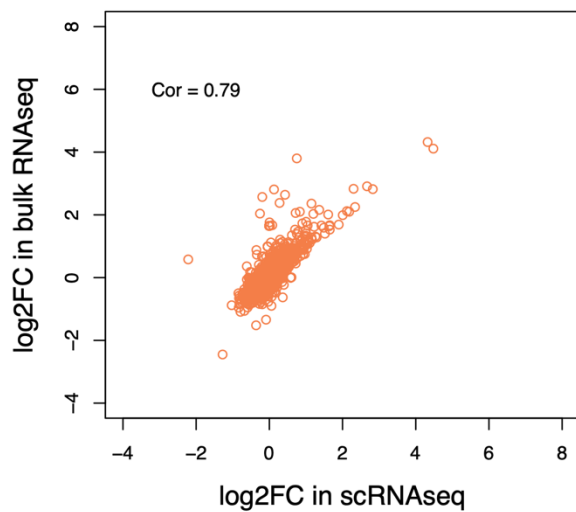
Supplementary Figure 19. Correlation of gene fold changes between the low and high dose of each treatment within the same single cell cluster. Pearson's correlations are shown. GW30: GW3965 30 nM, GW300: GW3965 300 nM, T30: T0901317 30 nM, T100: T0901317 100 nM.



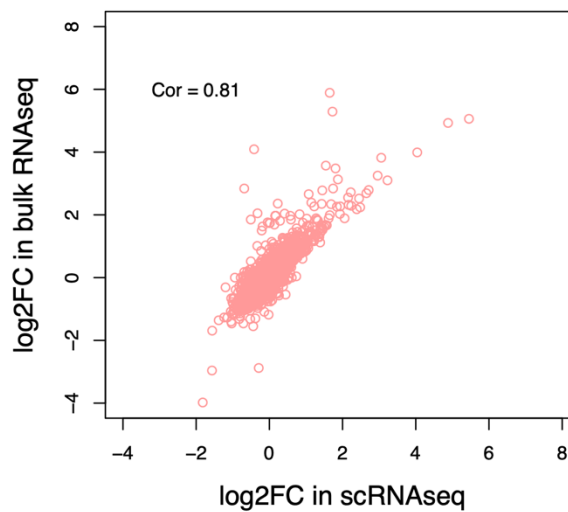


Supplementary Figure 20. Overlap in DEGs identified in each treatment within the same single cell cluster. UP: upregulated in the treatment compared to DMSO, DN: downregulated in the treatment compared to DMSO. GW30: GW3965 30 nM, GW300: GW3965 300 nM, T30: T0901317 30 nM, T100: T0901317 100 nM.

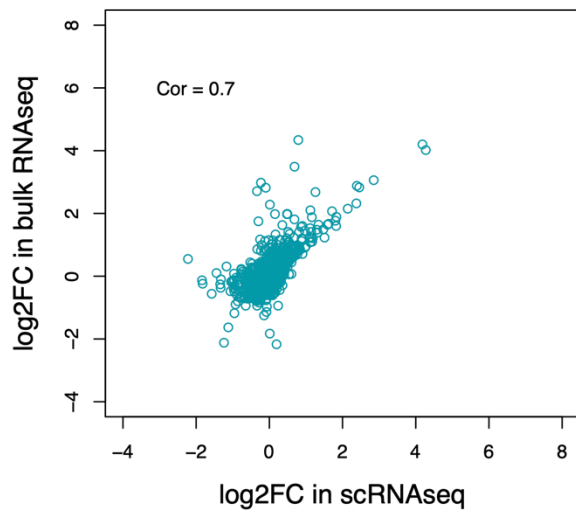
GW3965 30nM vs DMSO



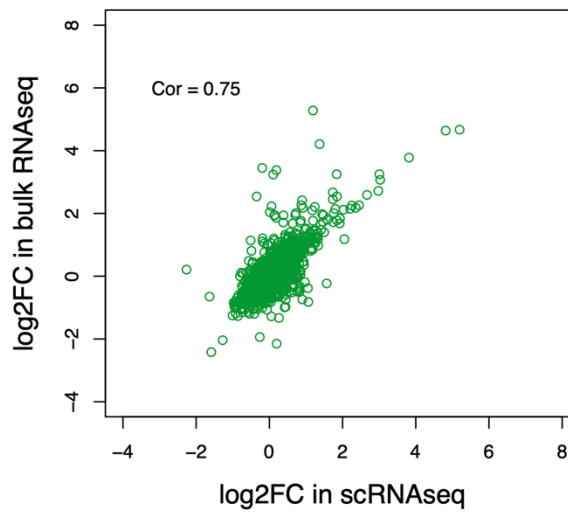
GW3965 300nM vs DMSO



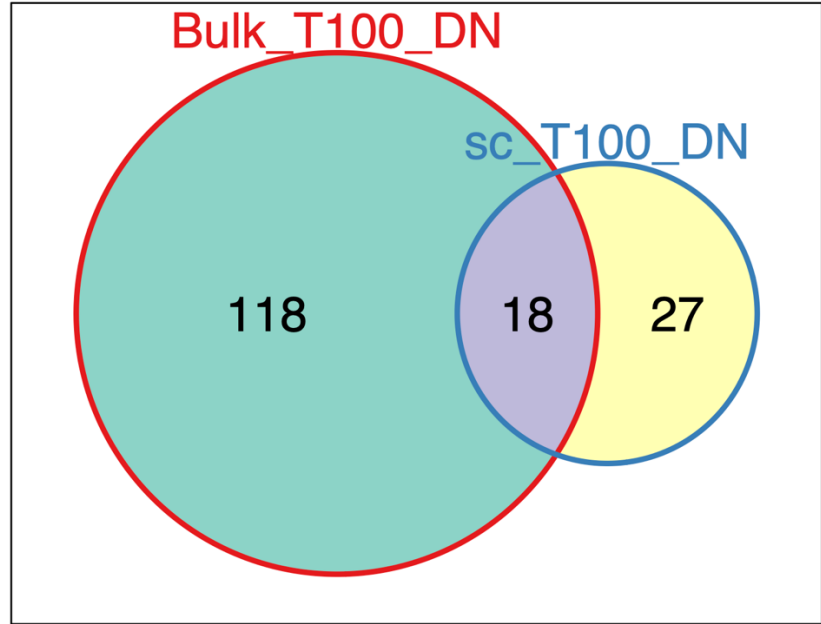
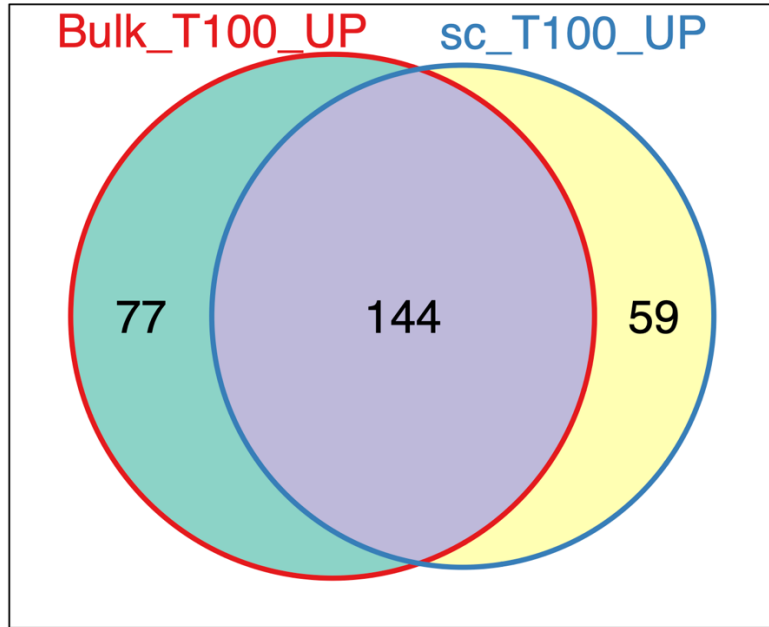
T0901317 30nM vs DMSO



T0901317 100nM vs DMSO

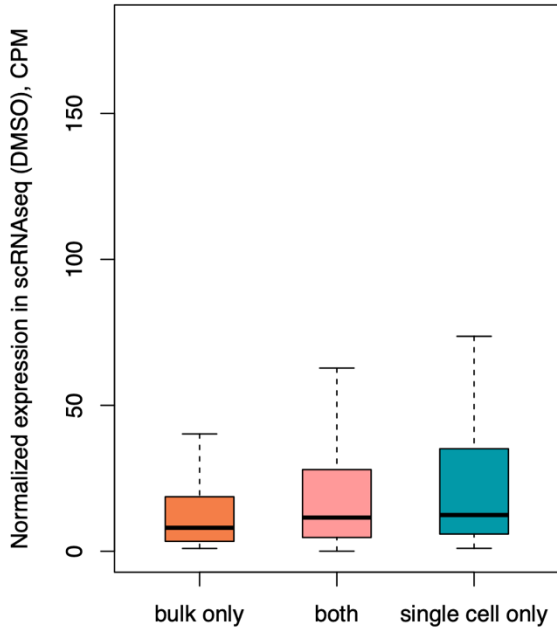


Supplementary Figure 21. Correlation of gene fold changes between bulk and single cell RNA-seq for each treatment. Single cell fold changes were calculated using the mean expression across all single cells associated with a treatment compared to the mean expression across all single cells associated with DMSO. Pearson's correlations are shown.

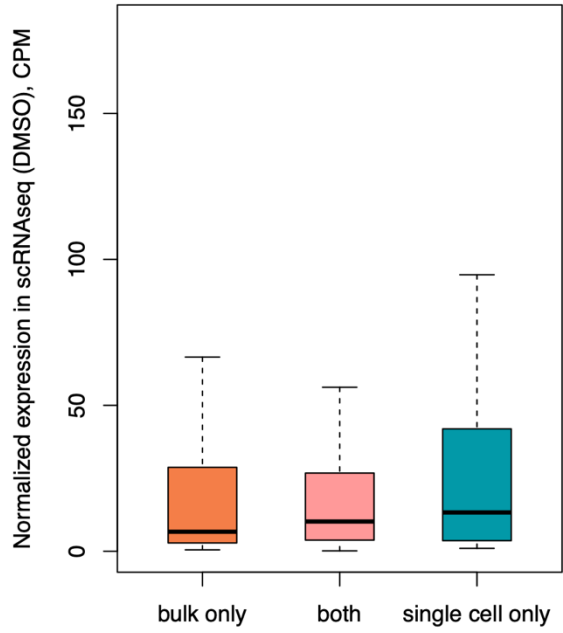


Supplementary Figure 22. Overlap in upregulated (UP) and downregulated (DN) DEGs between bulk RNA-seq and the union of single cell RNA-seq DEGs in clusters C1, C2, C3, C4, C8, and C10 with T0901317 100nM treatment. Bulk DEGs were filtered to genes tested in at least one single cell contrast.

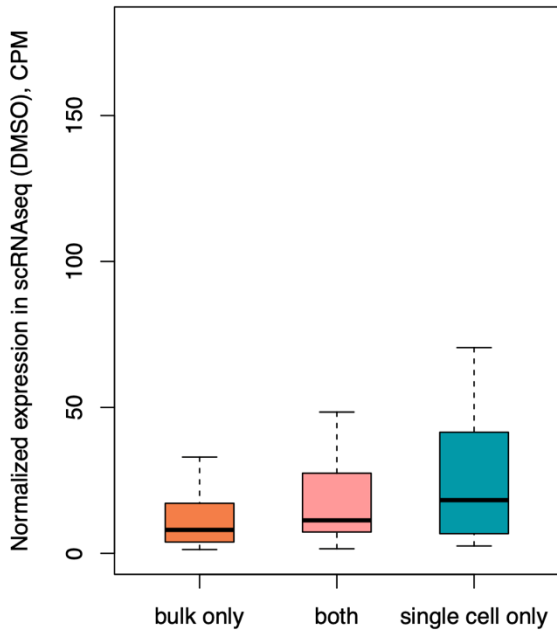
GW3965 300nM upregulated genes



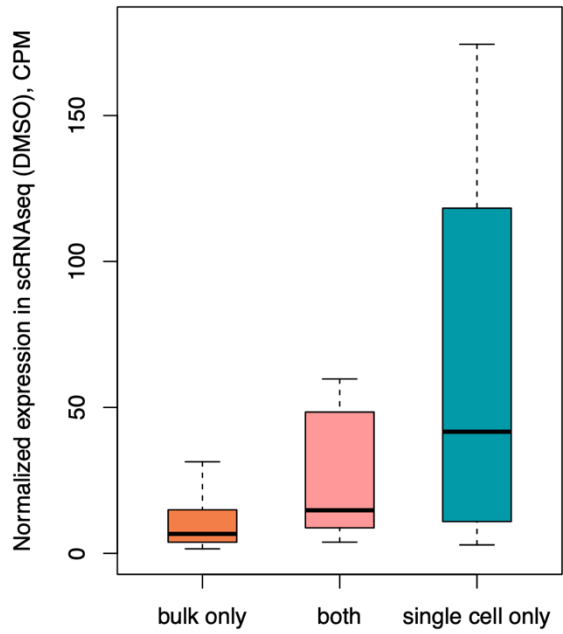
T0901317 100nM upregulated genes



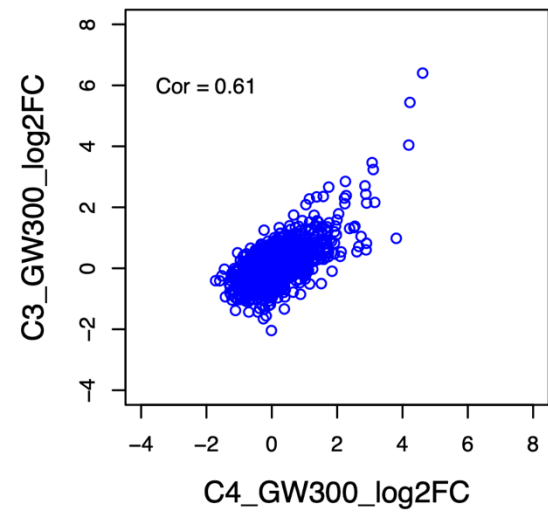
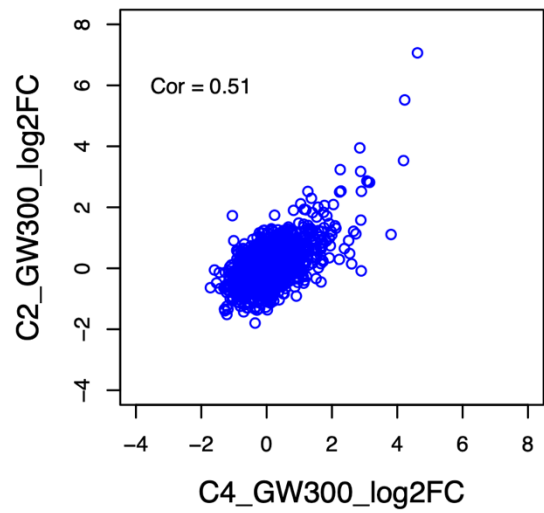
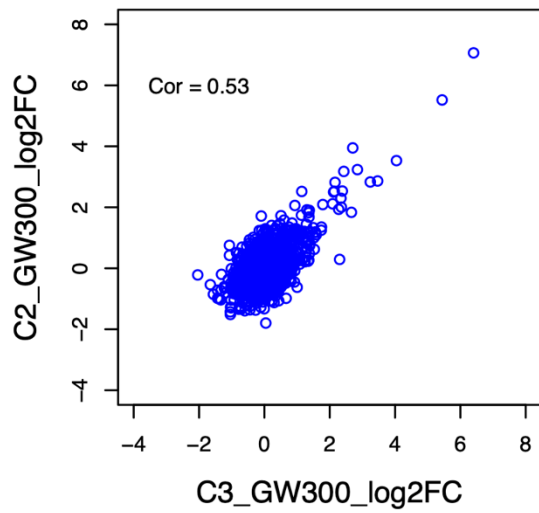
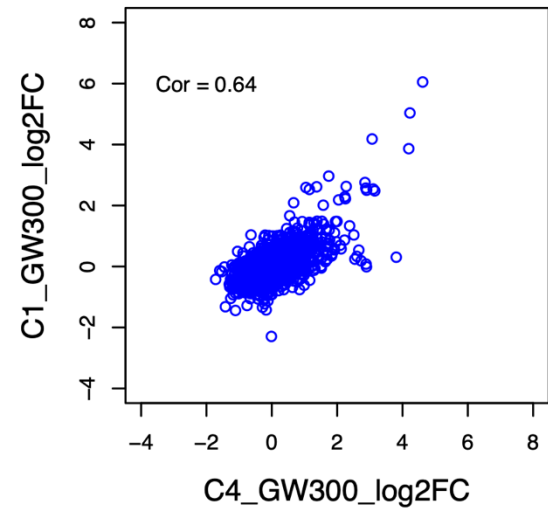
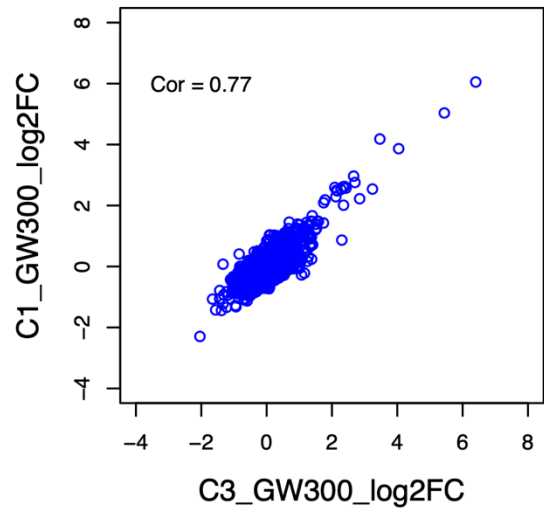
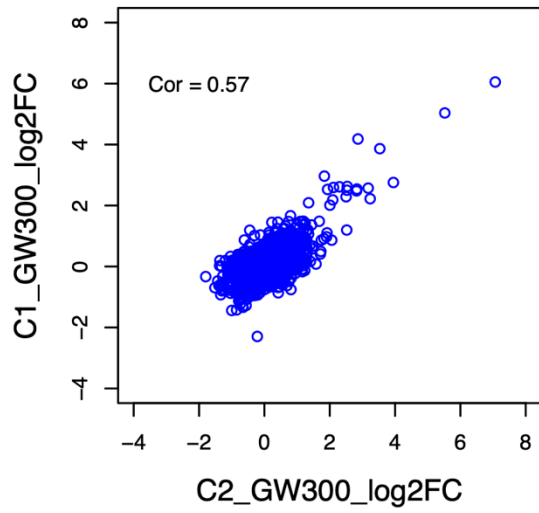
GW3965 300nM downregulated genes

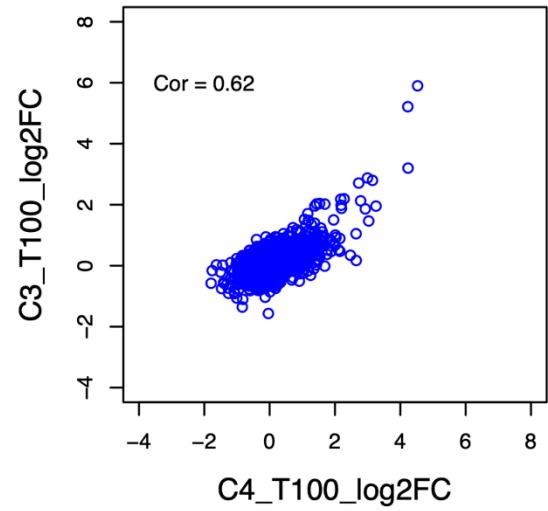
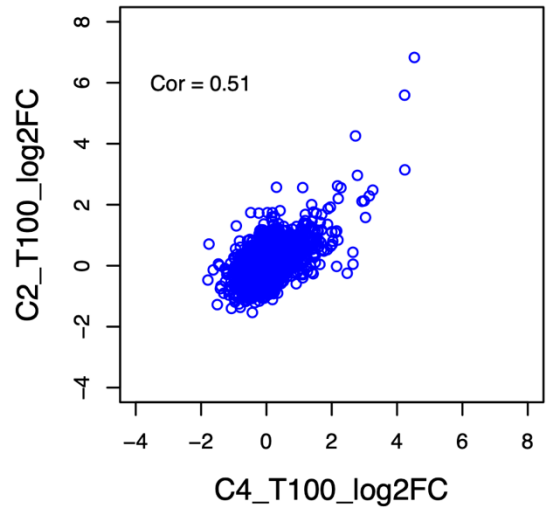
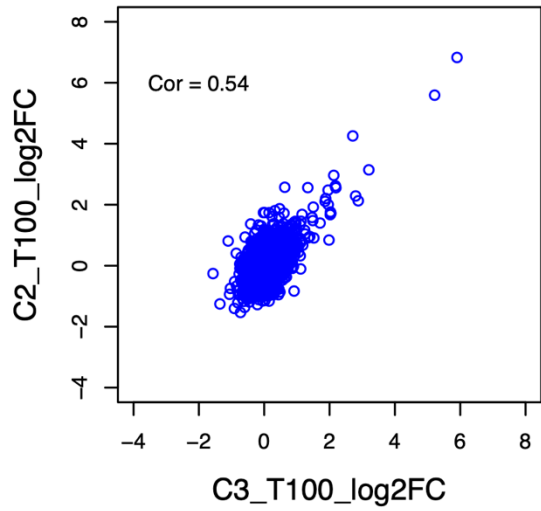
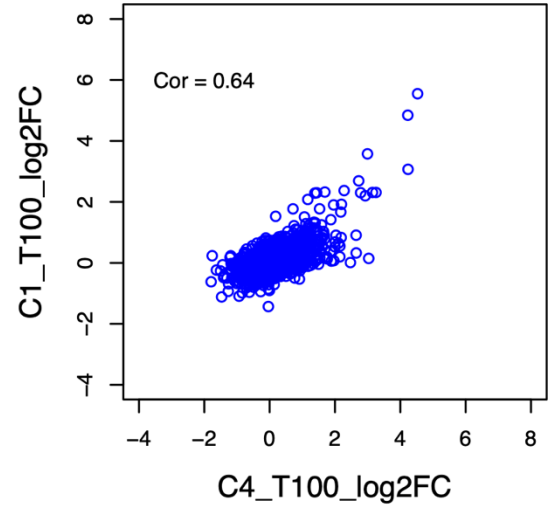
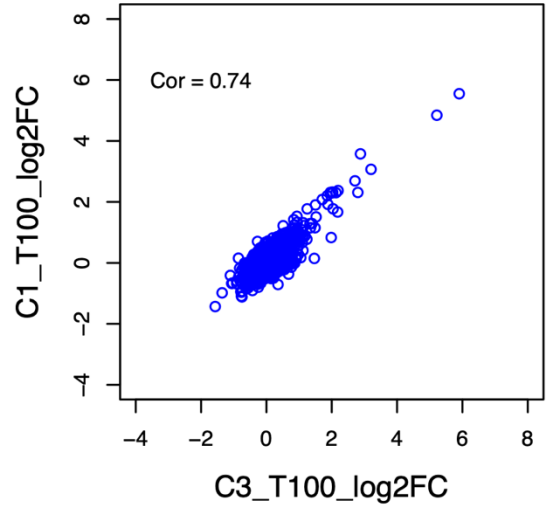
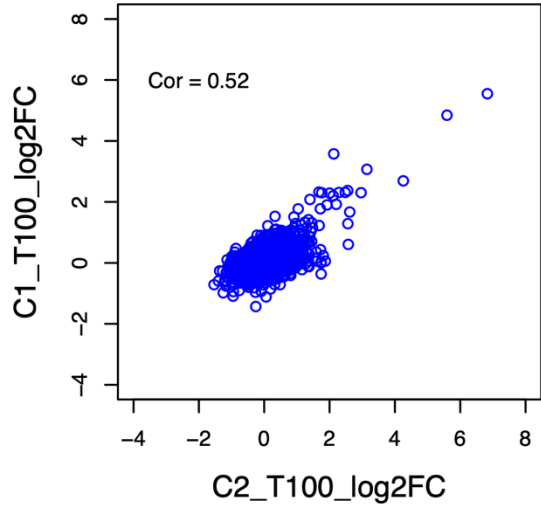


T0901317 100nM downregulated genes

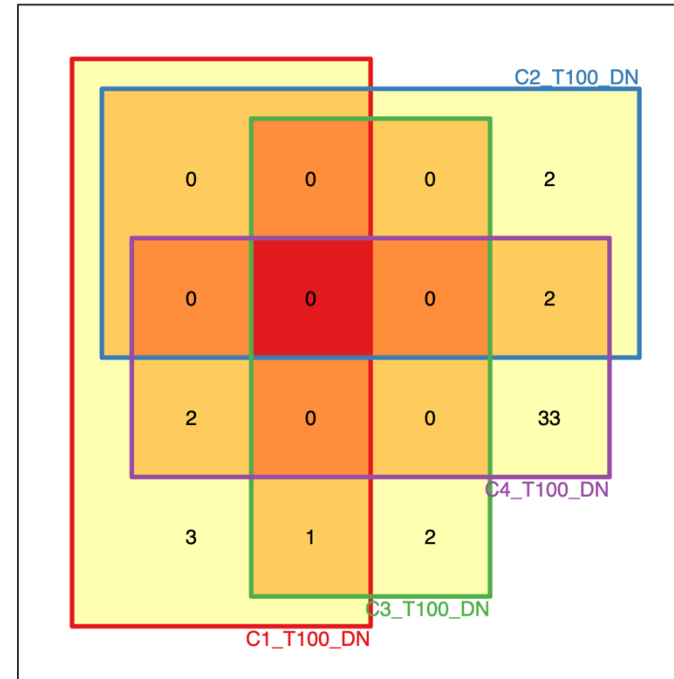
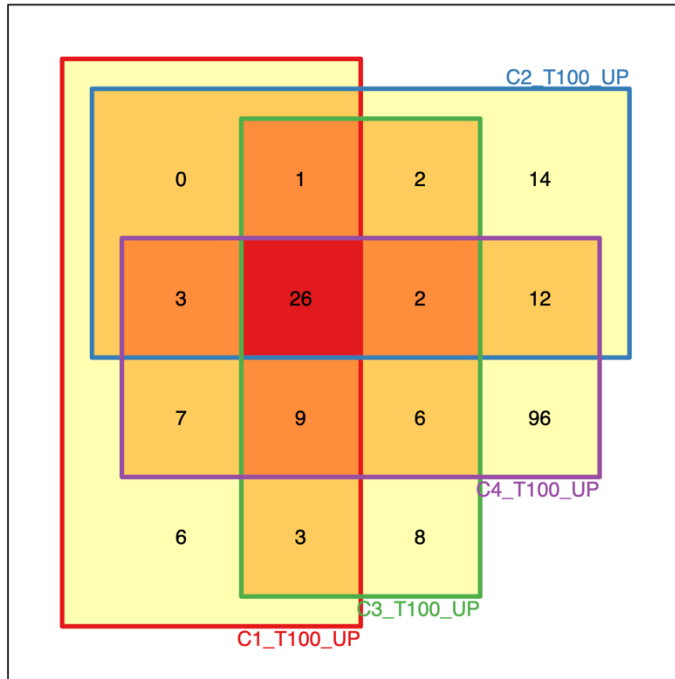


Supplementary Figure 23. Comparison of single cell expression levels in counts per million (CPM) for DEGs identified by bulk RNA-seq only, single cell RNA-seq only, or both methods for GW3965 300nM and T0901317 100nM treatments. Bulk DEGs were filtered to genes tested in at least one single cell contrast. For each gene, its single cell expression level was calculated as the mean expression across single cells in the DMSO samples.





Supplementary Figure 24. Pairwise correlation of gene fold changes for the high doses of GW3965 and T0901317 across single cell clusters C1, C2, C3, and C4. Pearson's correlations are shown. GW300: GW3965 300 nM, T100: T0901317 100 nM.



Supplementary Figure 25. Overlap in upregulated (UP) and downregulated (DN) DEGs for single cell clusters C1, C2, C3, and C4 with T0901317 100nM treatment (T100).