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Supplemental information

A single-cell view on host immune transcriptional response to *in vivo* BCG-induced trained immunity

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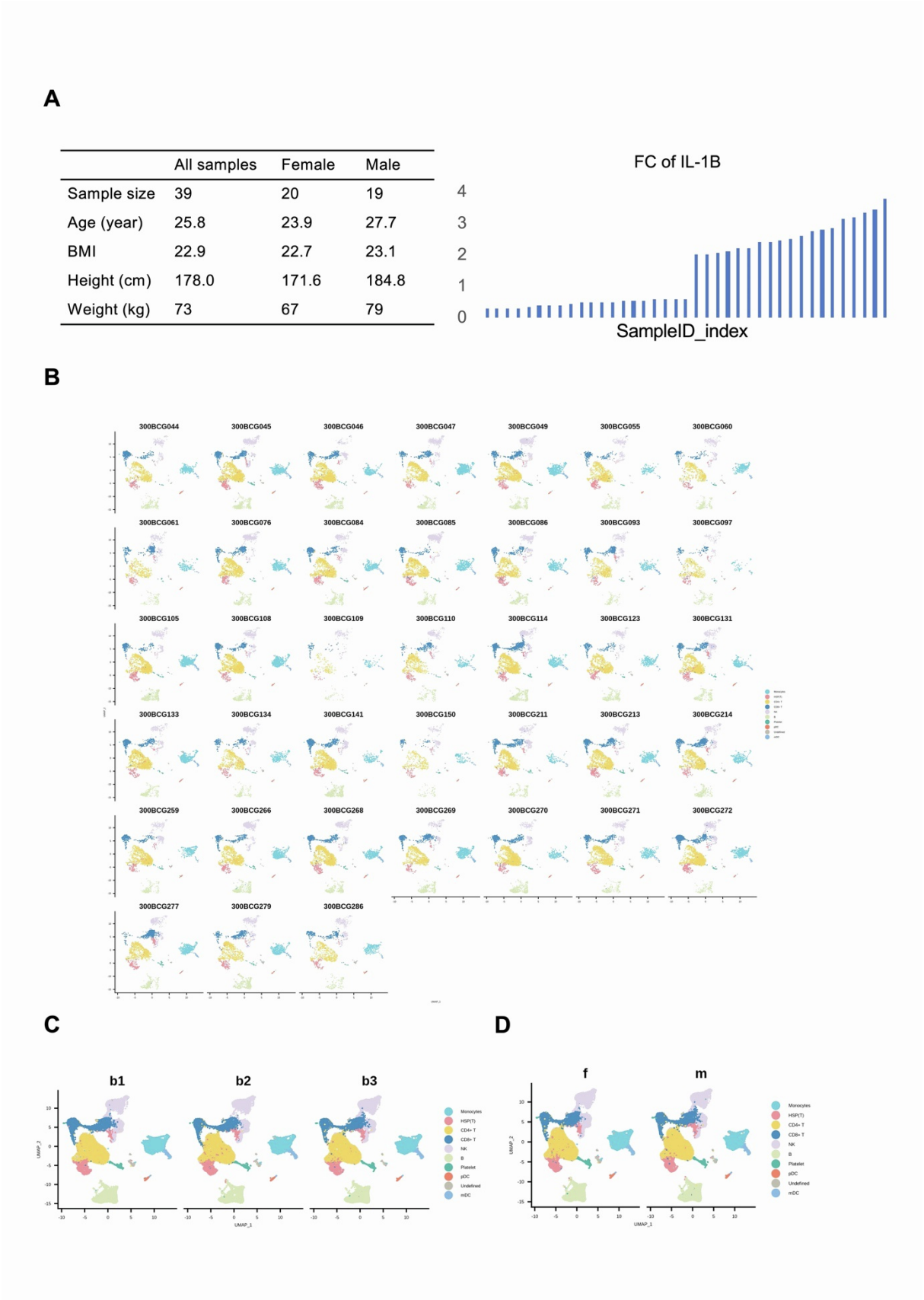

Figure S1. Overview of data quality, related to Figure 1. (**A**) Sample information. Fold Change (FC) of IL-1β in 39 individuals. Individuals with FC ≥ 2 were defined as high-responders (N = 19) and verses were defined as low-responders (N = 20). UMAP

of scRNA-seq dataset splitting by donor, batch and gender were shown in (**B-D**). All cell clusters were uniformly distributed among individuals, batches and sexes, which in turn suggested minimal effect of technical batch or donors.

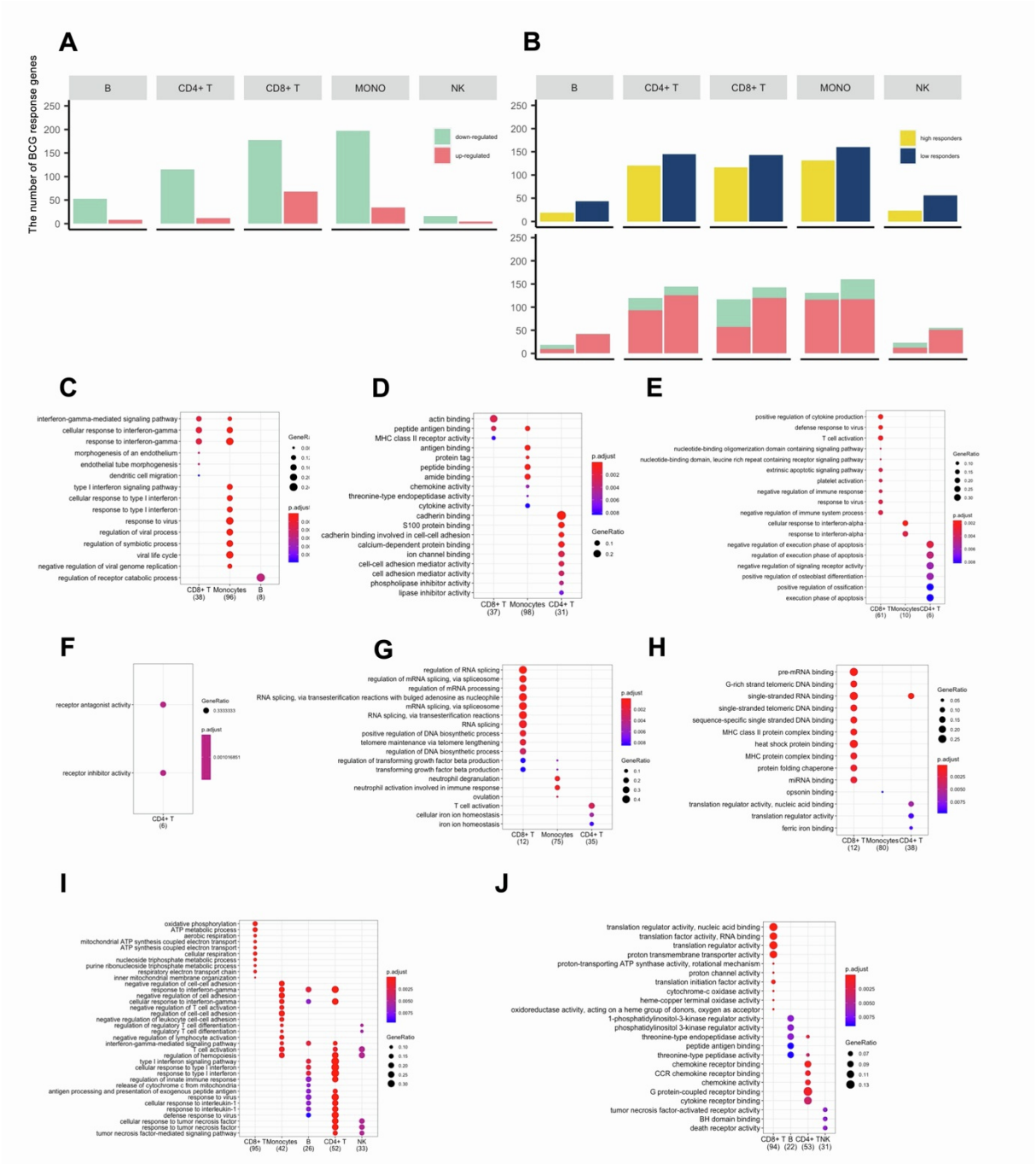

Figure S2. Comparison of TI and BCG effect and TI functional enrichment analysis, related to Figures 1 and 2. (**A**) The total number of BCG response genes in main cell types. BCG response genes were defined as the between T3m_RPMI and T0_RPMI. Up-regulated means the expression level of those genes were higher 3 months after BCG vaccination without LPS stimulation than before BCG vaccination without LPS stimulation. (**B**) The number BCG response genes in high- and low-responders, respectively. (**C-J**) Comparison was between T3m_LPS and T0_LPS. Up-regulated means the expression level of those genes were higher 3 months after BCG vaccination with LPS stimulation than before BCG vaccination with LPS stimulation. In

GO Enrichment of Biological Process (**C**) and Molecular Functions (**D**) using up-regulated trained immunity response genes in high responders. GO Enrichment of Biological Process (**E**) and Molecular Functions (**F**) using up-regulated trained immunity response genes in low responders. GO Enrichment of Biological Process (**G**) and Molecular Functions (**H**) using down-regulated trained immunity response genes in high responders. GO Enrichment of Biological Process (**I**) and Molecular Functions (**J**) using down-regulated trained immunity response genes in low responders.

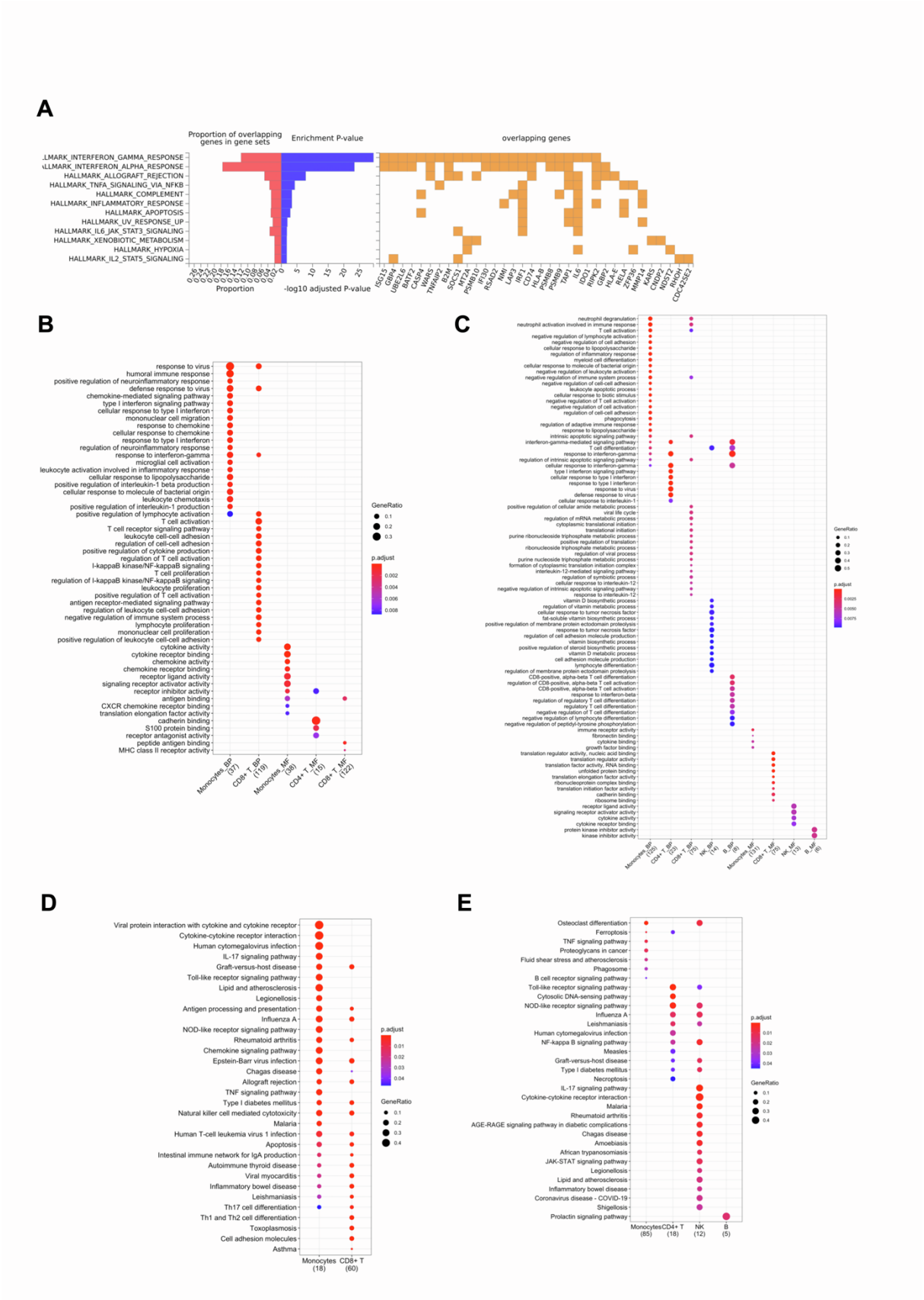

Figure S3. TIGs enrichment analysis, related to Figure2 and 3. (**A**) Enriched pathways using all genes (either significant in high-responders or low-responders) that were up-regulated in high responders but down-regulated in low responders. GO

Enrichment results of TIGs for up-regulated genes (**B**) and down-regulated (**C**) genes in each main cell type. KEGG enrichment results for up-regulated genes (**D**) and down-regulated (**E**) genes in each main cell type. T3m_LPS and T0_LPS were compared.

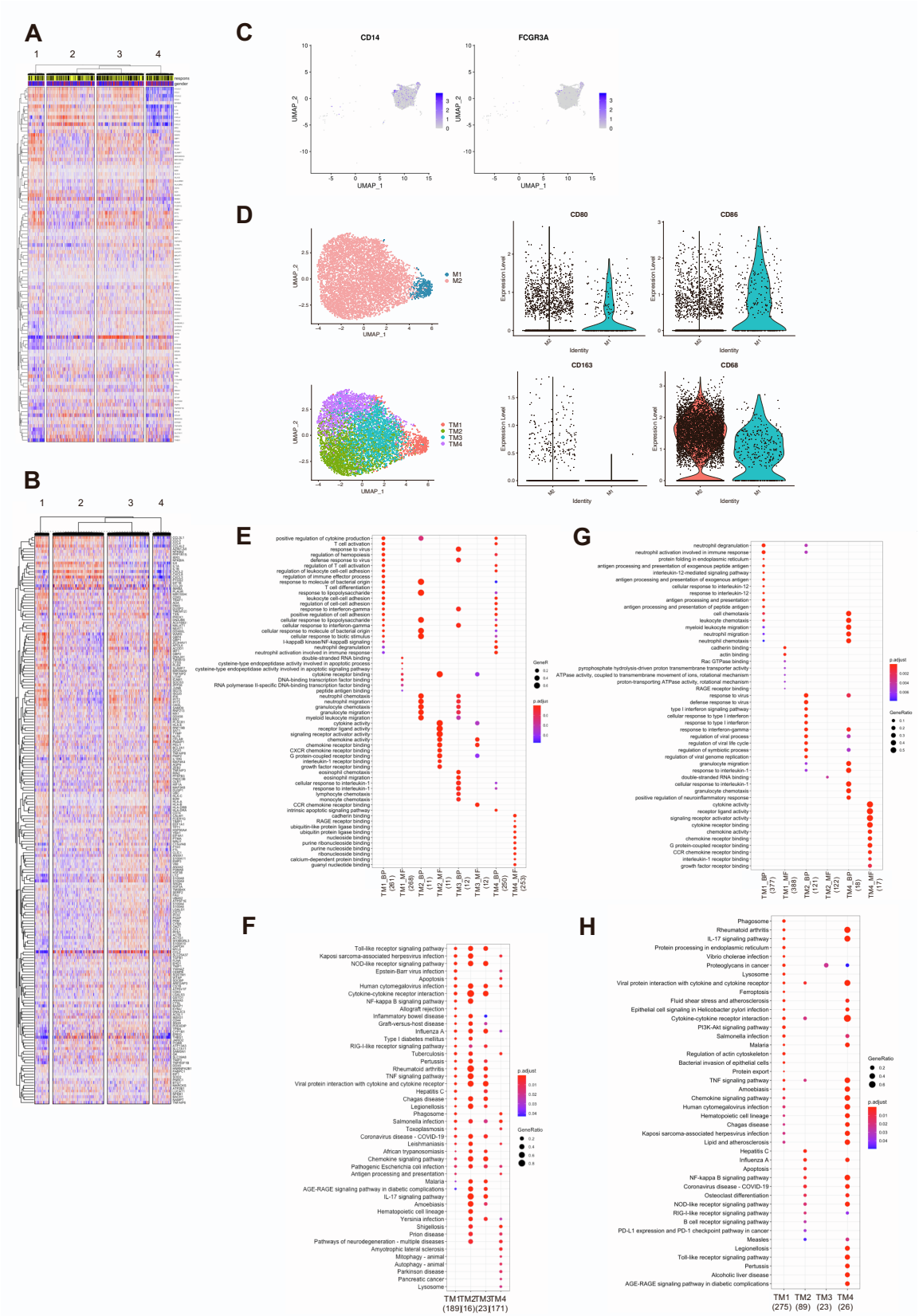

Figure S4. Validation of clustering robustness and functional analysis, related to Figure 3 and 4. (**A**) Unsupervised clustering using top 100 most variable genes. (**B**) Unsupervised clustering using top 300 most variable genes. (**C**) FeaturePlot of the

expression levels of markers for classical monocytes (*CD14*) and non-classical monocytes (*FCGR3A*) after being trained (T3m_LPS)**.** (**D**) FeaturePlot of the expression levels of markers for M1 (*CD80*, *CD86*) and M2 (*CD163*, *CD68*) after being trained (T3m_LPS)**.** GO (**E**) and KEGG (**F**) enrichment analysis for up-regulated TIGs. GO (**G**) and KEGG (**H**) enrichment of down-regulated TIGs, related to Figure 3 and 4.

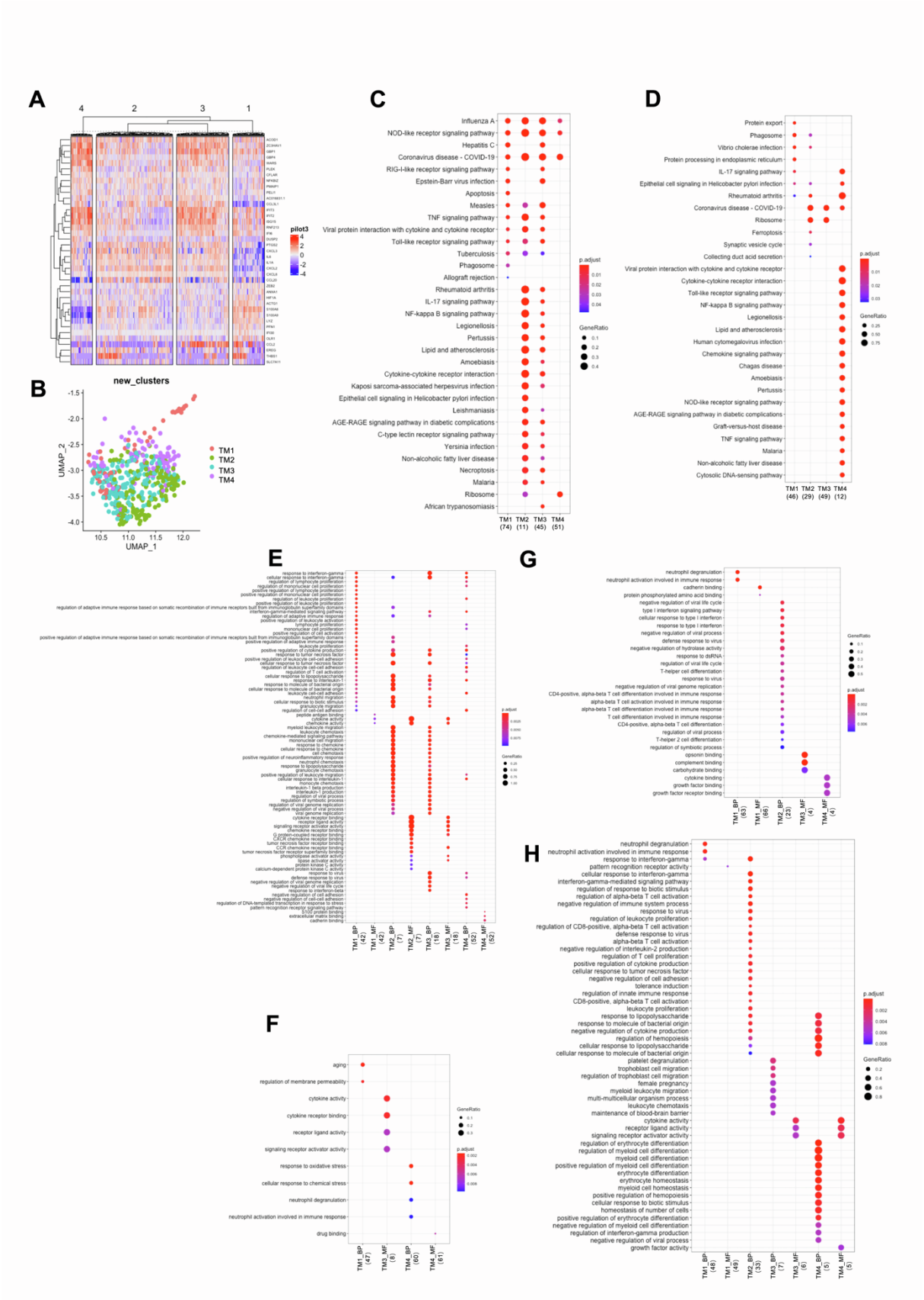

Figure S5. Validation using an independent pilot cohort and functional analysis, related to Figure 3 and 4. (**A**) Heatmap of un-supervised clustering using markers identified in 300BCG scRNA-seq dataset. (**B**) UMAP of identified sub-populations.

KEGG enrichment using up-regulated TIGs (**C**) and down-regulated TIGs (**D**). GO Enrichment for up- (**E**) and down-regulated (**F**) TIGs in high-responders. GO Enrichment for up- (**G**) and down-regulated (**H**) TIGs in low-responders.

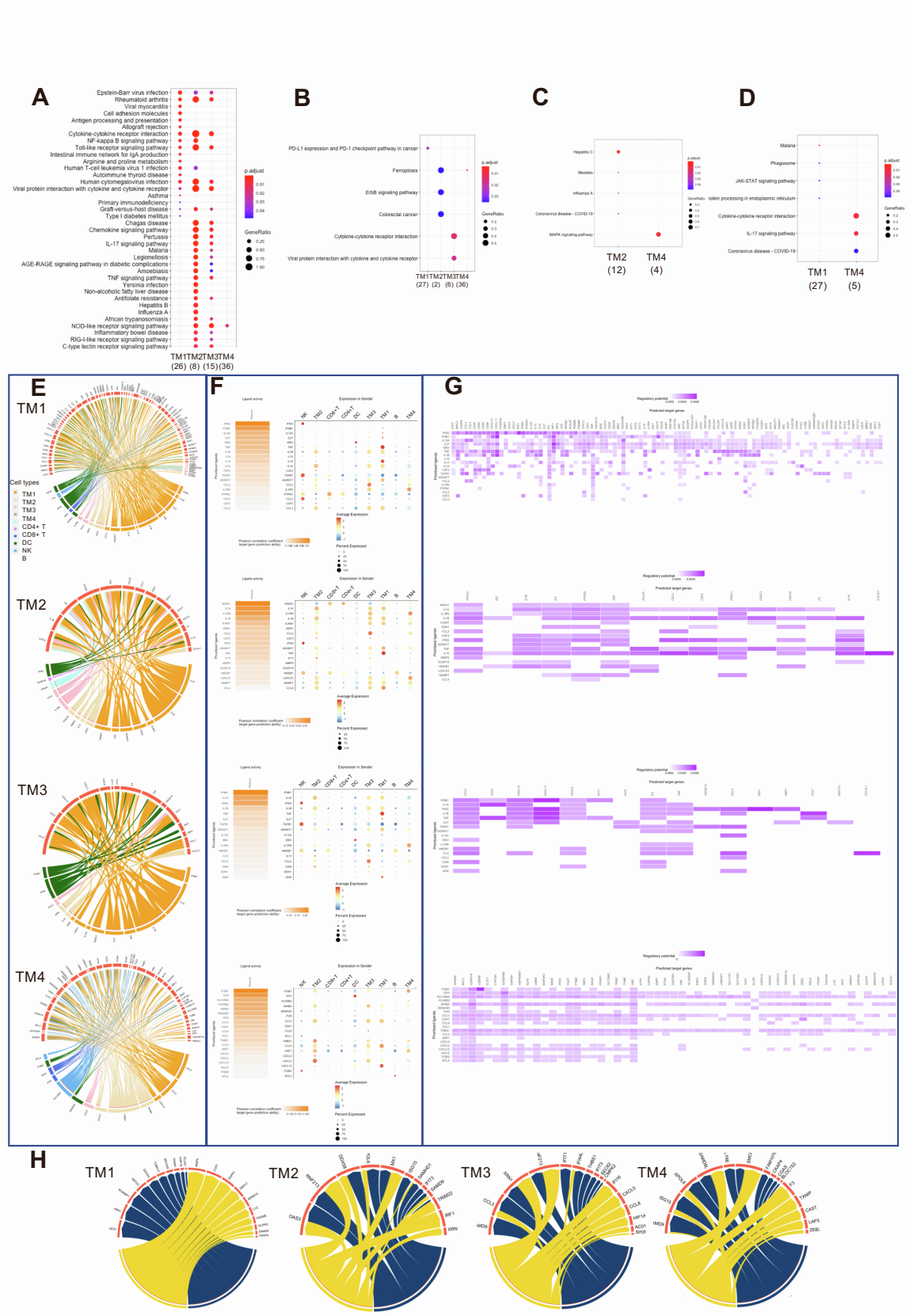

Figure S6. Functional analysis, cell-cell interaction and reconstruction of *STAT1* regulated network, related to Figure 4 and 5. KEGG Enrichment for up-regulated TIGs in high- (**A**) and low- (**B**) responders. KEGG Enrichment for down-regulated TIGs

in high- (**C**) and low- (**D**) responders. (**E-G**) Cell-cell interaction between TM1-TM4 (receiver) and other cell types (sender), respectively. The circos plot (**E**) and heatmap (**G**) are ligands-targets connection, and dotplot shows the expression level of ligands in each cell type. (**H**) Predicted targets of *STAT1* in each monocyte sub-population in high- and low- responders, respectively.

Table S4. The number of TIGs that were located in TI QTLs areas (p-value < 1×10^{-4}) in each monocyte sub-population, related to Table 1.

	TIGs (logFC > 0)	IL1b (TIGs/snps)
TM1	350	3(21)
TM2	19	0
TM3	35	0
TM4	309	9(59)