

Supplementary Materials for Network analysis reveals a distinct axis of macrophage activation in response to conflicting inflammatory cues

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Supplementary Figure S1. Distinct macrophage activation network states induced by LPS+IFN γ and IL4. Each node is annotated with a bar graph that shows that node's normalized activity in response to LPS+IFN γ at 4 h (red bar) and IL4 at 4 h (blue bar).

Supplementary Figure S2. Peritoneal macrophage gene expression in response to LPS+IFN γ or IL4. A) Log10CPM-normalized read counts of the 500 genes with highest variance, sorted by hierarchical clustering (n=4 per treatment condition). B) Principal component analysis of macrophage gene expression, including all transcripts. C) Venn diagram of differentially expressed transcripts in LPS+IFN γ or IL4 treatment groups compared to control. D) Gene set enrichment analysis for transcripts with increased or decreased expression in response to LPS+IFN γ or IL4. E) Volcano plots of differential gene expression analysis of LPS+IFN γ -treated and IL4-treated macrophages, annotating selected genes from the macrophage network model.

Supplementary Figure S3. Network-wide knockdown simulations under conditions of A) LPS+IFN γ and B) IL4. In each column, the given node was knocked down by setting its ymax parameter to zero. Row shows the change in node activity in response to given knockdowns (columns). Change in activity was calculated as the difference in a node's activity with and without knockdown in response to the specified stimulus (70%) at 4 h. Network influence of a given node was quantified by summing the absolute change in all network nodes when that node was knocked down (columns). Node sensitivity was quantified by summing the absolute change in that node across all node knockdowns (rows). Influence and sensitivity calculations were used to rank nodes for Figure 3.

Supplementary Figure S4. Signaling modules distinctly regulated by combinatorial stimuli. Modules were determined by hierarchical clustering of columns in Figure 3A using the complete linkage method. After identifying 24 initial clusters, some clusters were further condensed manually based on their very similar responses and topological similarity (MyD88-dependent IL1R and TLR4; STAT6-dependent mRNA with proteins; MAPK pathways and MMP mRNAs).

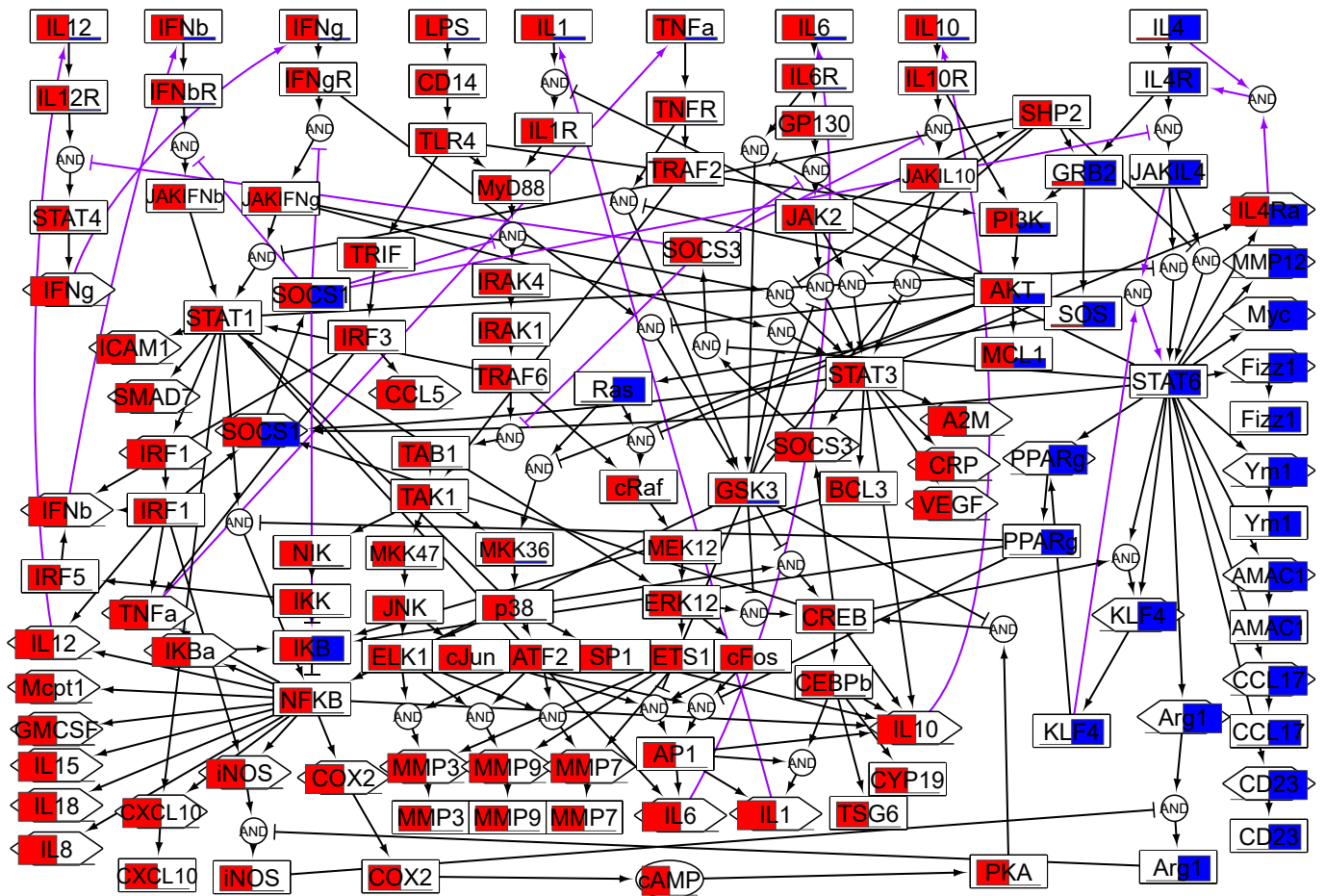
Supplementary Figure S5. Network responses to all single stimuli. A) Network-wide response to 9 single input stimuli and negative control conditions at 4 h. As in Figure 3, hierarchical clustering of stimuli (rows) was performed using the Ward method, while nodes (columns) were clustered using complete linkage. B) Principal component analysis (PCA) scores reveal relationships between phenotype clusters induced by single stimuli, color-coded based on clustering from Figure 3A. C) PCA loadings indicate the contribution of each node's activity to the PC1 and PC2 dimensions. Orange (M1-associated) and green (M2-associated) labels indicate representative signaling modules within the quadrants.

Supplementary Figure S6. Distinct macrophage activation network state induced by IFN γ , combined IFN γ +IL4, and IL4. Each node in the network is color-coded by its activation in response to IFN γ (left third), IFN γ +IL4 (middle third), and IL4 (right third) at 4 h.

Supplementary Figure S7. Network-wide knockdown simulations under conditions of A) IFN γ and B) IFN γ +IL4. In each column, the given node was knocked down by setting its y_{max} parameter to zero. Row shows the change in node activity in response to given knockdowns (columns). Change in activity was calculated as the difference in a node's activity with and without knockdown in response to the specified stimulus (70%) at 4 h. Influence (absolute sum of rows) and sensitivity (absolute sum of columns) calculations were used to rank nodes for Figure 5D.

Supplementary Figure S8. Comparison of a previously reported macrophage signaling network structure to experimental data. Rex et al. recently developed a Boolean model of bone-marrow derived macrophage (BMDM) activation in response to LPS and IL4/IL13 stimuli [32]. To evaluate the predictive performance of that network in a manner comparable to our validations performed in Figure 2C, we converted that BMDM model to our logic-based differential equation framework. mRNA expression was examined for 10 genes that overlap between the Rex et al. model, our peritoneal macrophage model, and the peritoneal macrophage RNA-seq data used for validation in Figure 2C (LPS+IFN γ or IL4 for 4h [34]). Out of the 10 genes, there are 9 semi-quantitative matches between the BMDM model predictions and experimental measurements under LPS+IFN γ stimulation, 8 matches under IL4 stimulation, and 1 additional trend match in the inconsistent genes. The overall consistency of 85% (17 in 20) is similar to our model of 91.4% (53 in 58), which has a greater overall scope and incorporates additional genes.

Supplementary Table S1. Macrophage signaling network model. This spreadsheet contains the model network structure, parameters, and supporting literature references. This spreadsheet can be opened by Netflux for network simulations.



Normalized activity in response to:
 LPS+IFN γ ■ IL4 ■

Figure S1

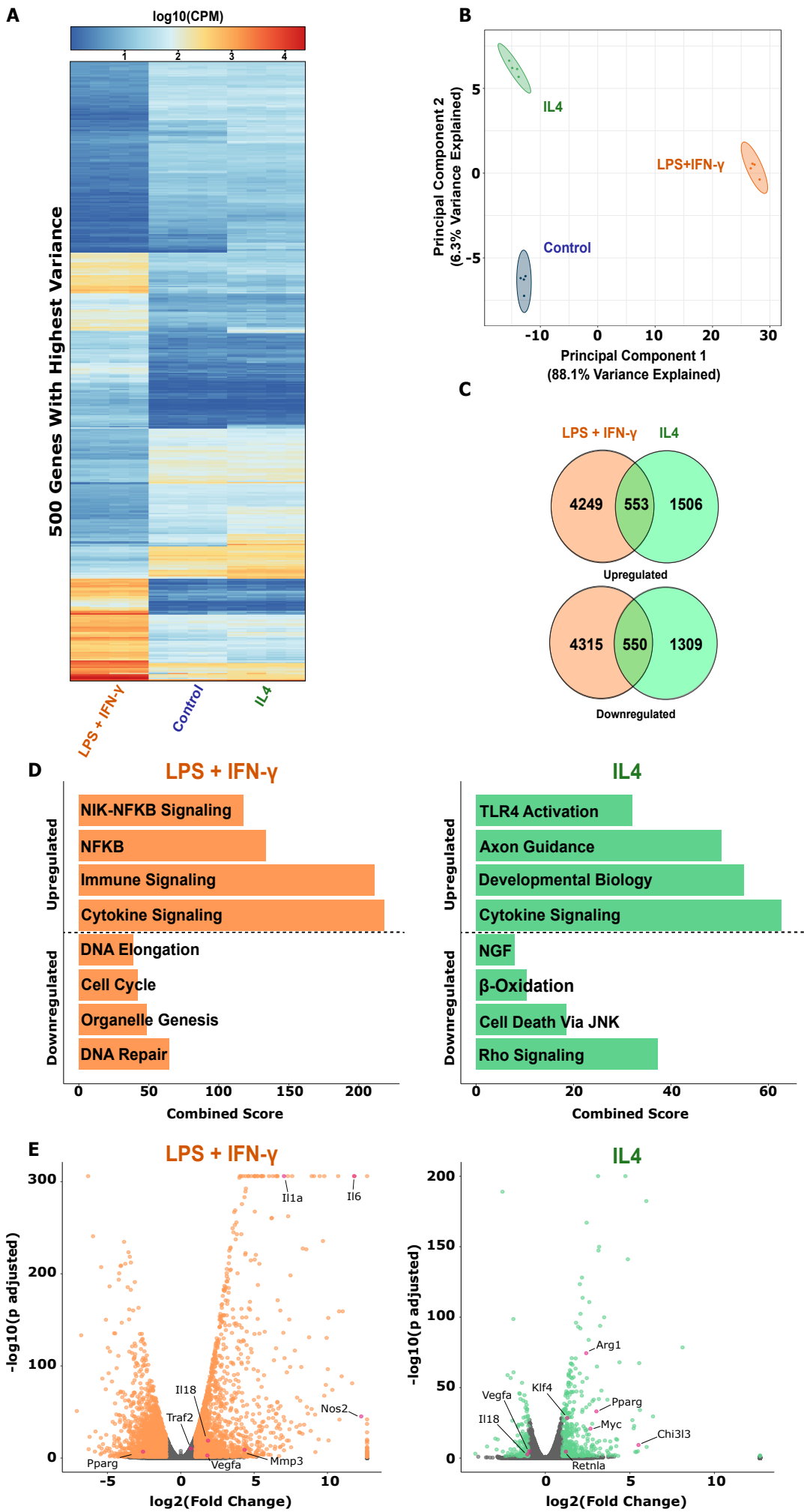


Figure S2

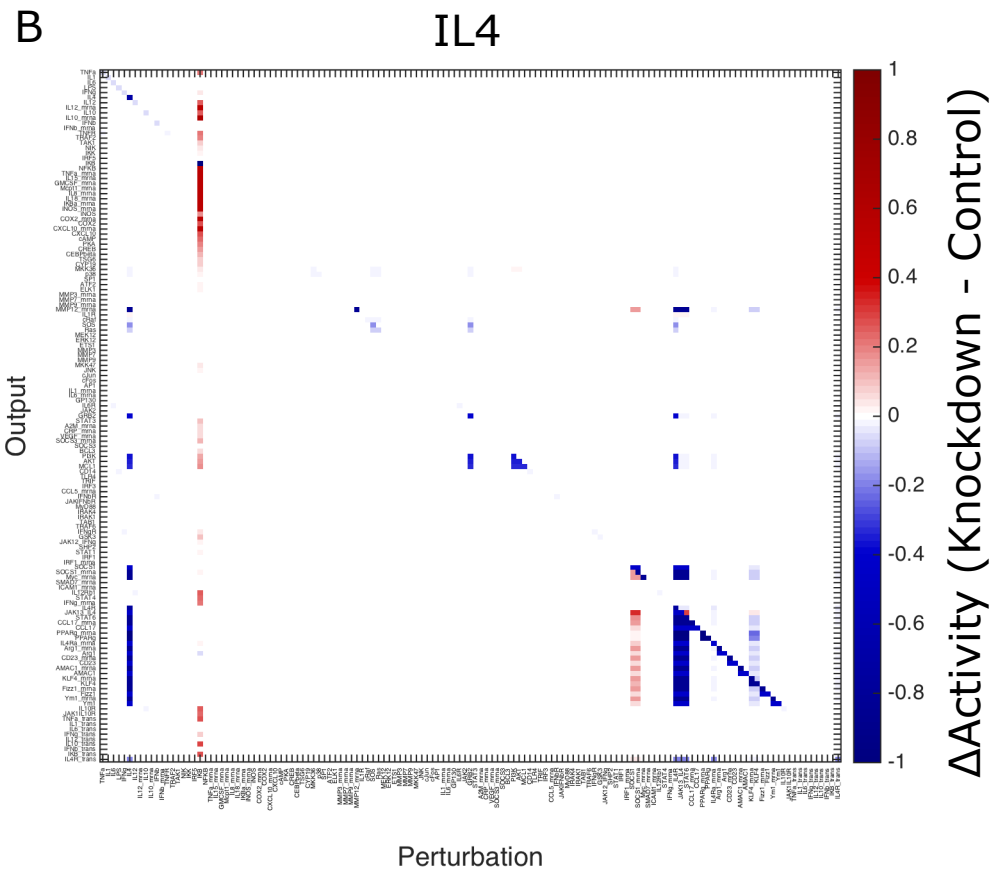
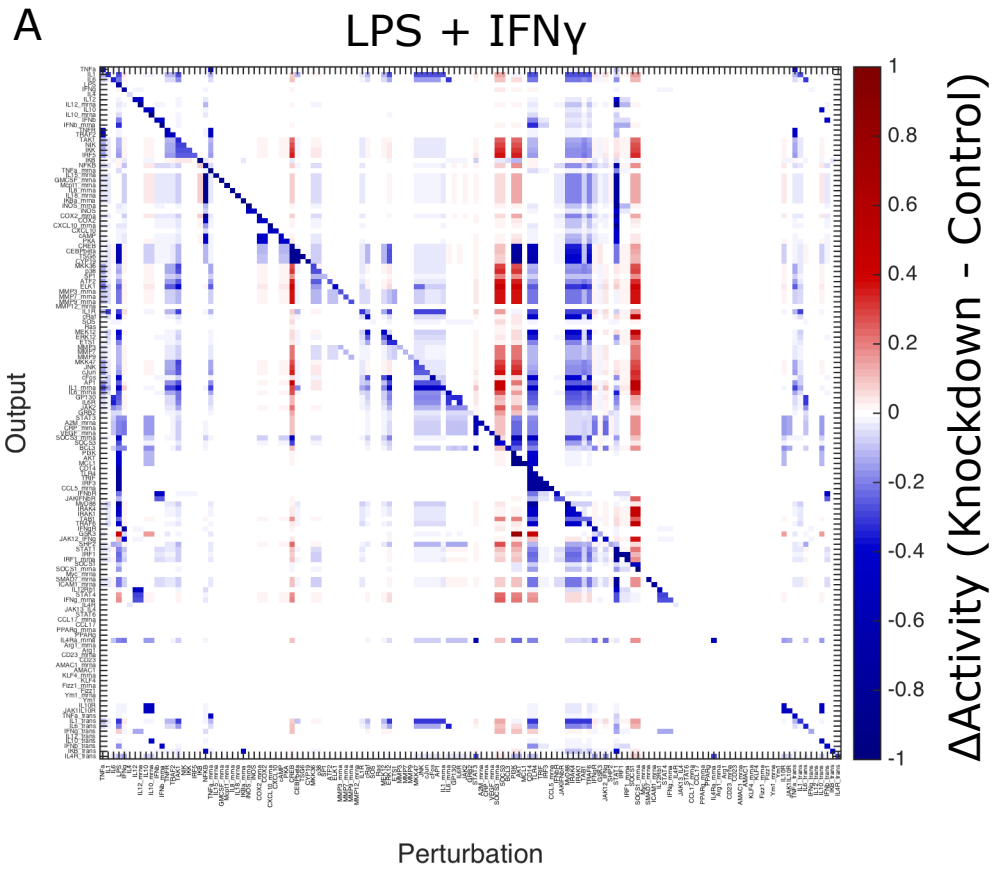


Figure S3

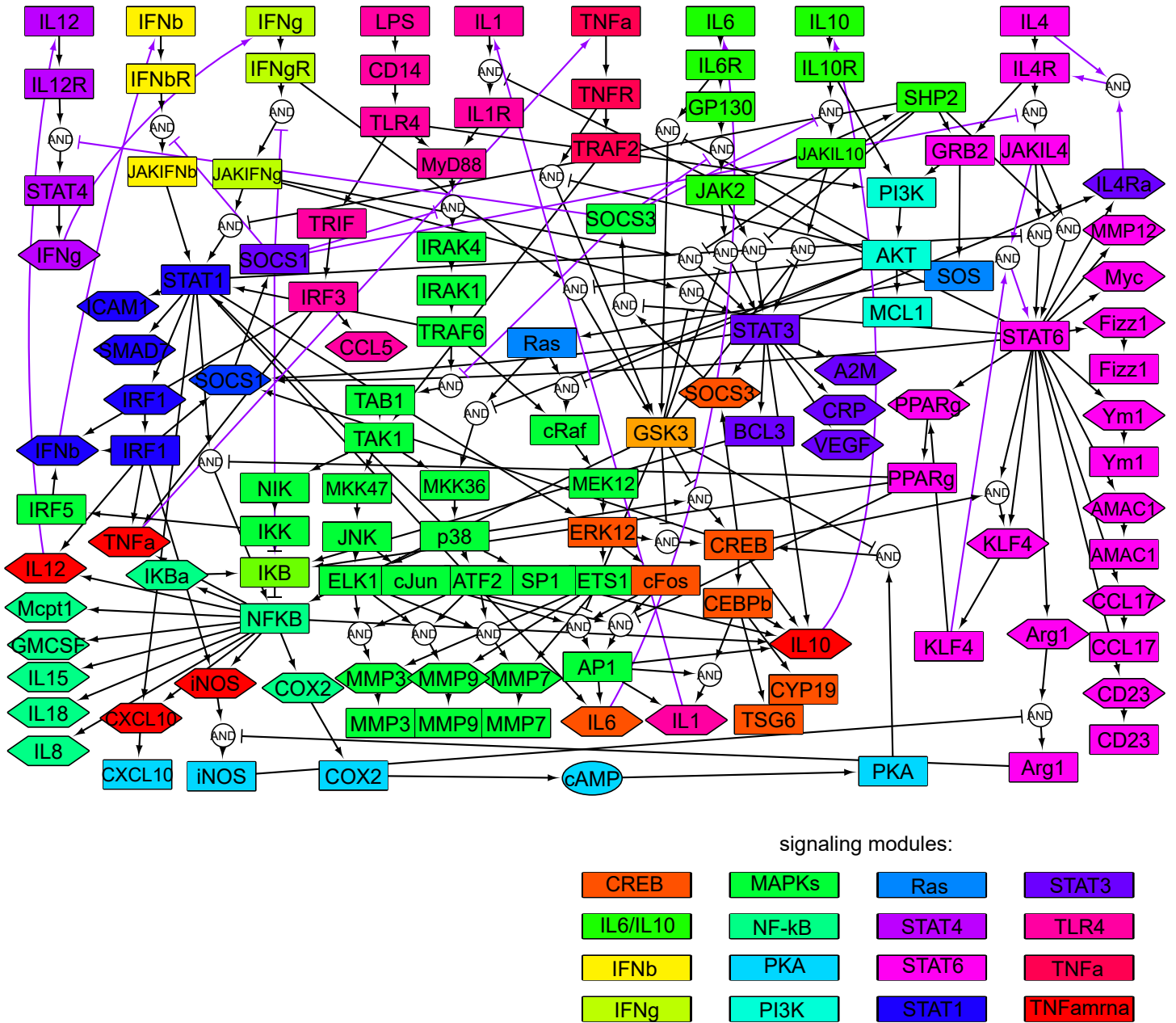


Figure S4

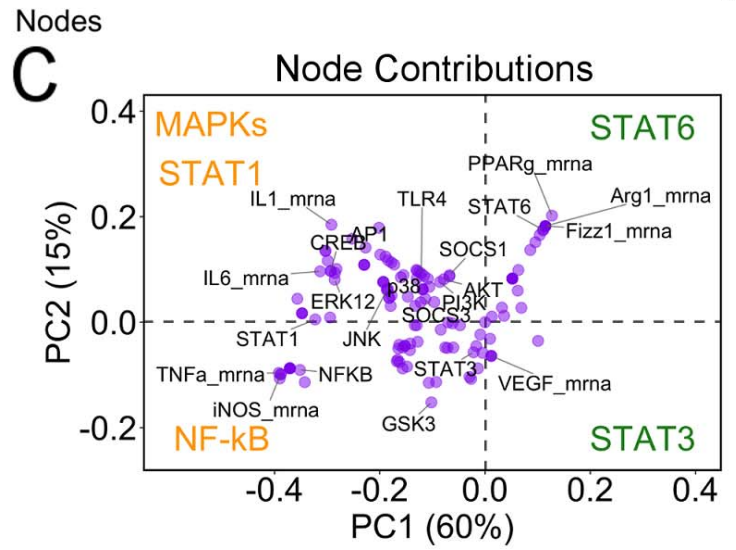
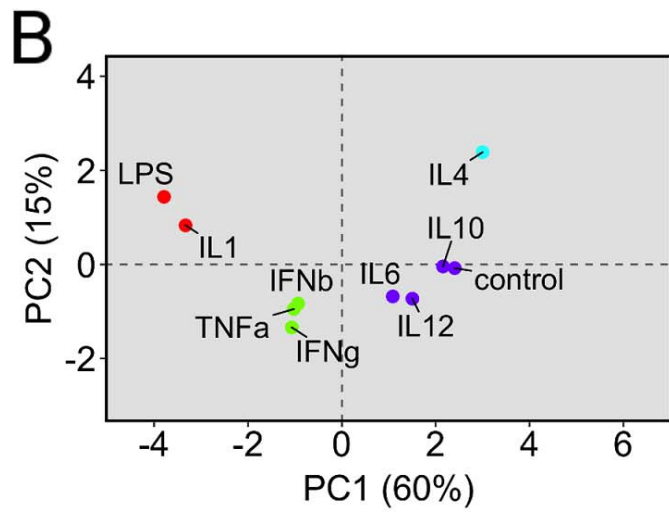
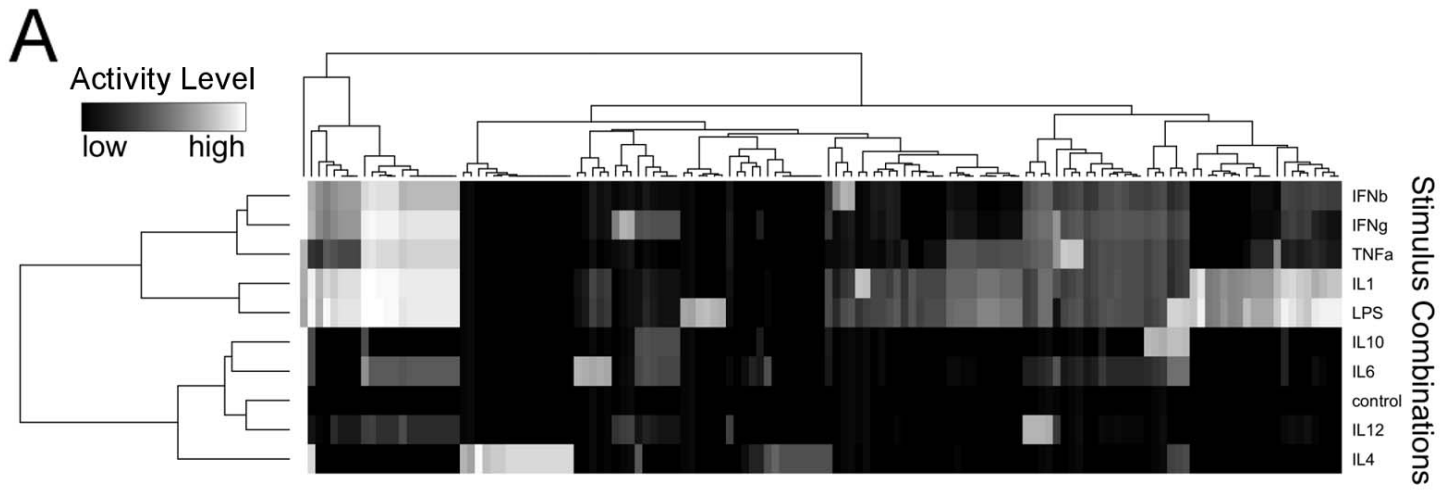


Figure S5

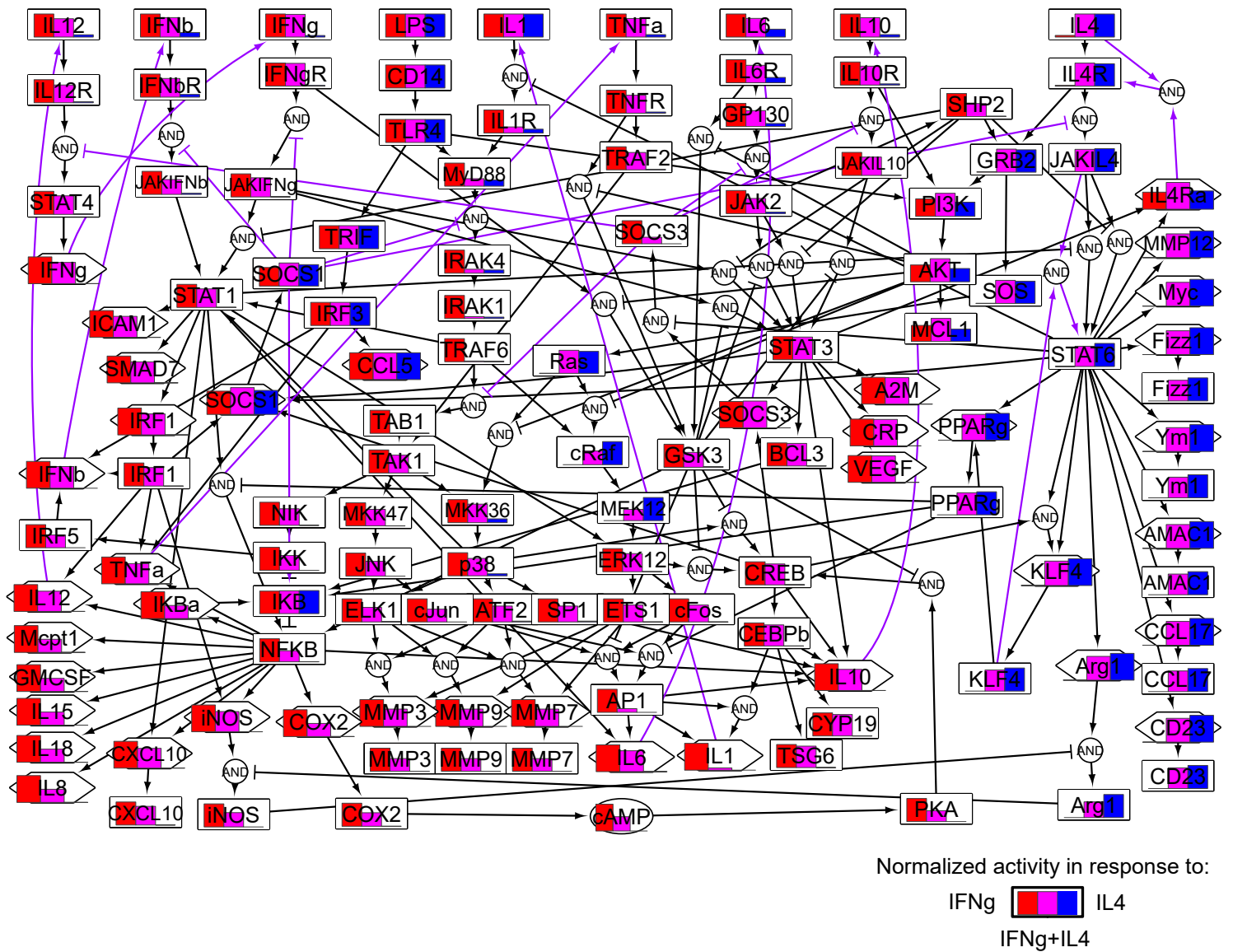


Figure S5

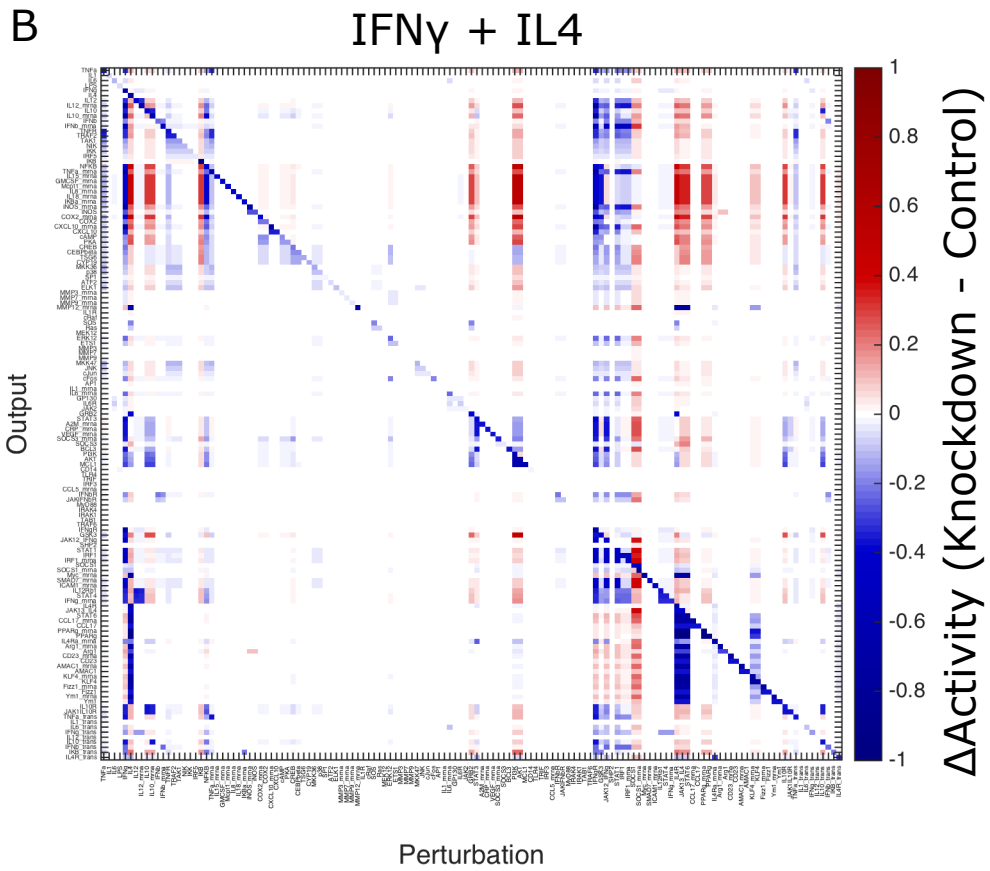
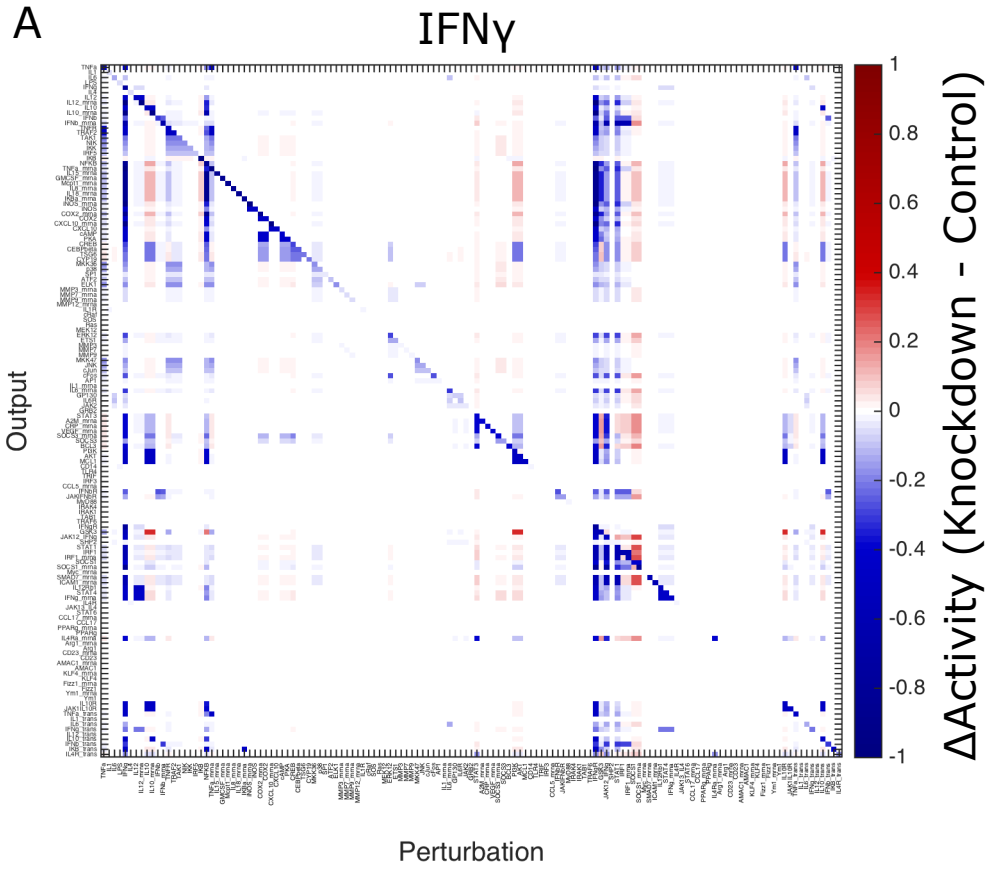


Figure S7

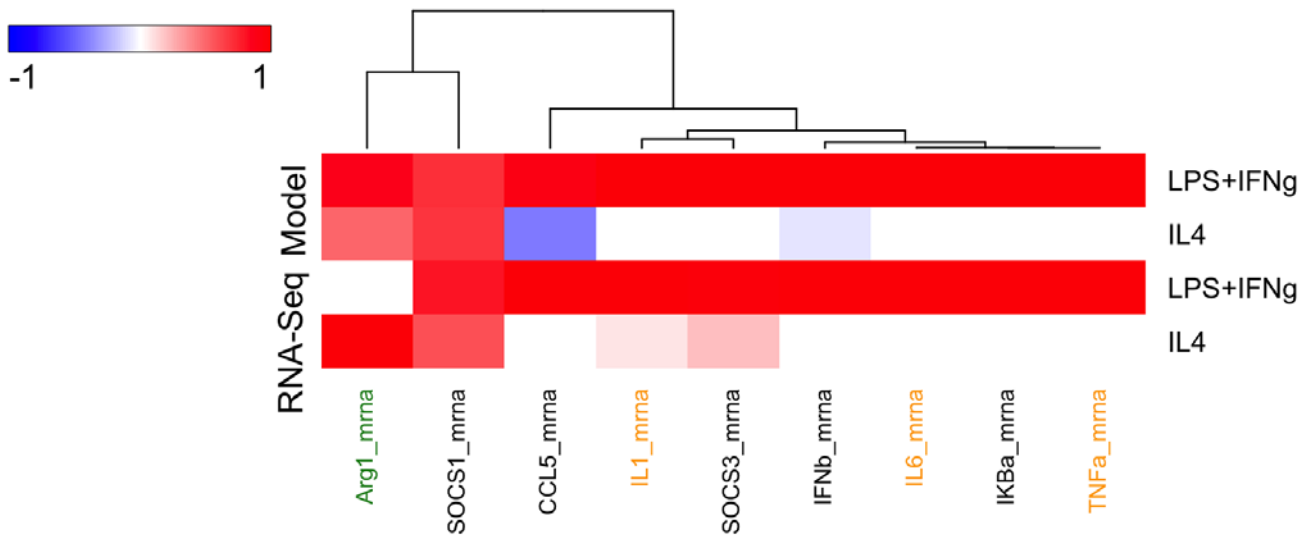


Figure S8