Supplemental material

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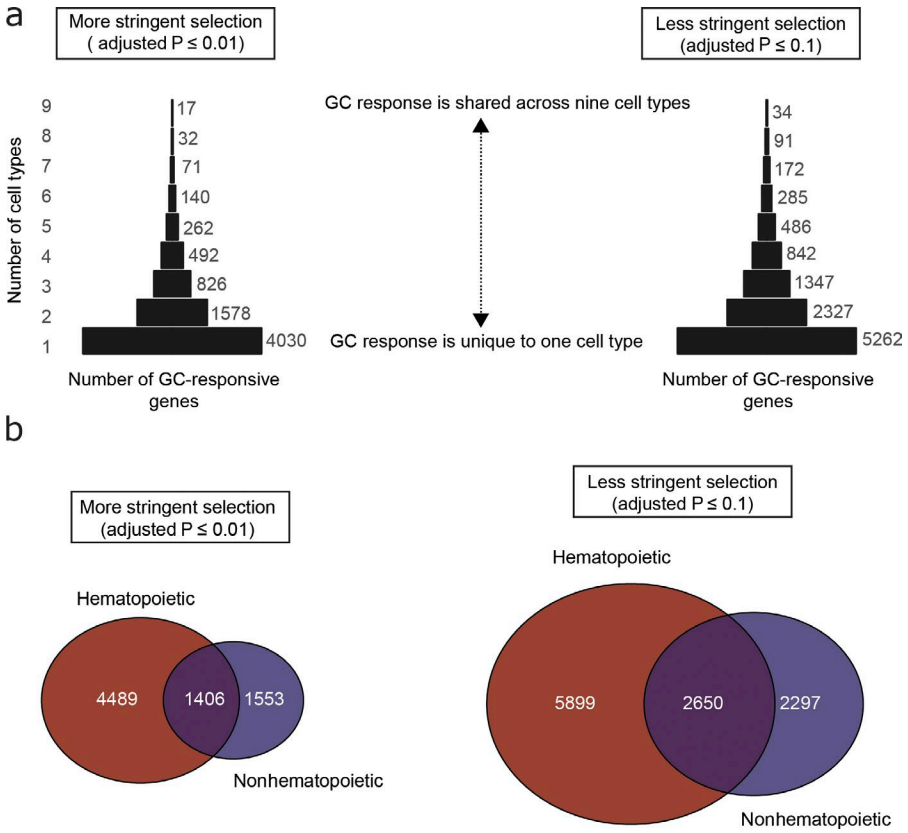

Figure S1. **Varying the threshold for differential expression does not affect the observed cell type dependence of the transcriptional response to glucocorticoid.** Four primary human hematopoietic cell types and five primary human nonhematopoietic cell types were studied. For each cell type, cells from four unrelated healthy donors were independently cultured and treated with methylprednisolone (22.7 µM) or vehicle (0.08% ethanol). Total RNA was purified 2 and 6 h after in vitro treatment. RNA-seq was performed. Differential expression was assessed by contrasting methylprednisolone-treated versus vehicle-treated cells in the four biological replicates. The statistical significance of differential expression was calculated with a Wald test, after accounting for dispersion, library size, and read count. The resulting P values for differential expression were adjusted for multiple testing by the method of Benjamini and Hochberg (1995). A glucocorticoid (GC)-responsive gene is defined as one with an adjusted P value for differential expression of ≤ 0.05. **(a)** Pyramid plots analogous to the plot in Fig. 1 b, applying a more stringent (left) or less stringent (right) cutoff value for classification of transcripts as differentially expressed in response to the glucocorticoid stimulus. **(b)** Venn diagrams analogous to the diagram in Fig. 1 c, after applying a more stringent (left) or less stringent (right) cutoff value for classification of transcripts as differentially expressed in response to the glucocorticoid stimulus.

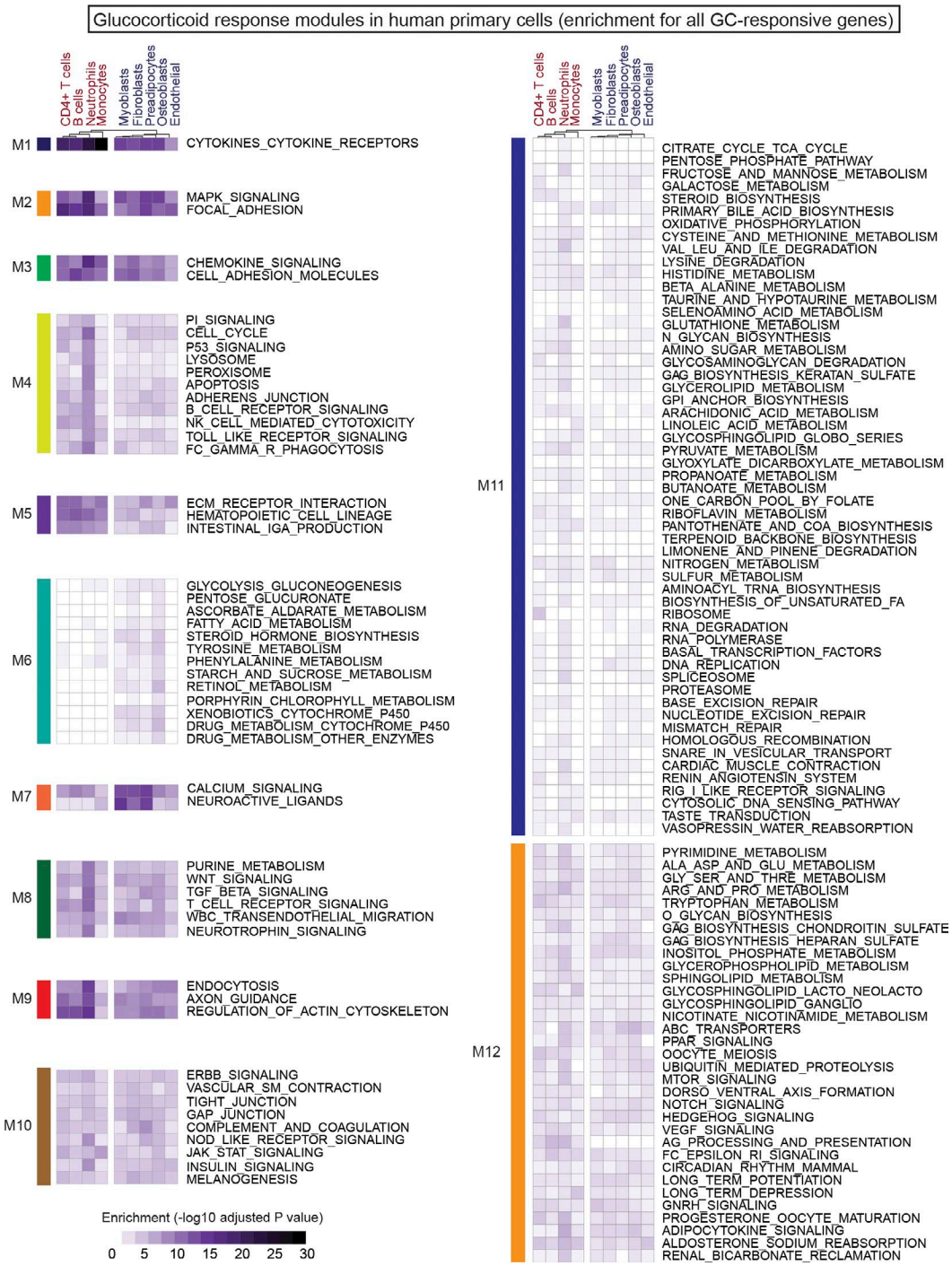

Figure S2. **A pathway-level map of the transcriptional response to glucocorticoids in nine human primary cell types.** Heat map of gene set enrichment analysis results for up-regulated or down-regulated genes. For each cell type, cells from four unrelated healthy donors were independently cultured and treated with methylprednisolone (22.7 µM) or vehicle (0.08% ethanol). Total RNA was purified 2 and 6 h after in vitro treatment. RNA-seq was performed. Differential expression was assessed by contrasting methylprednisolone-treated versus vehicle-treated cells in the four biological replicates. For each cell type, the input for the gene set enrichment analysis was a list of genes differentially expressed in response to in vitro methylprednisolone treatment for 6 h, ranked by the absolute value of the log2 fold change (methylprednisolone versus vehicle). The gene sets displayed in this plot are KEGG pathways, as defined in MSigDB v.6.2. For each pathway, the test assesses whether the genes in the pathway tend to be located near the top of the ranked list of differentially expressed genes. Enrichment P values are calculated with a Wilcoxon test, and multiple-testing correction is performed with the method of Benjamini and Hochberg (1995). Pathways that were significantly enriched for glucocorticoid (GC)-responsive genes (adjusted P value < 0.05) in at least one cell type are displayed. The values displayed are the −log10 adjusted P values for gene set enrichment. Each row represents one pathway, and each column represents one cell type. Higher values mean that a given pathway was more highly enriched for glucocorticoid-responsive genes in the respective cell type, regardless of the direction of change in gene expression. Column-wise clustering was performed by hierarchical clustering with Euclidean distances as the distance measure. Row-wise clustering was performed by k-means clustering with 100,000 starts and up to 100 iterations, partitioning the pathway enrichment results into 12 modules (M1–M12). The pathways within each module have a similar pattern of cell type specificity of the glucocorticoid response.

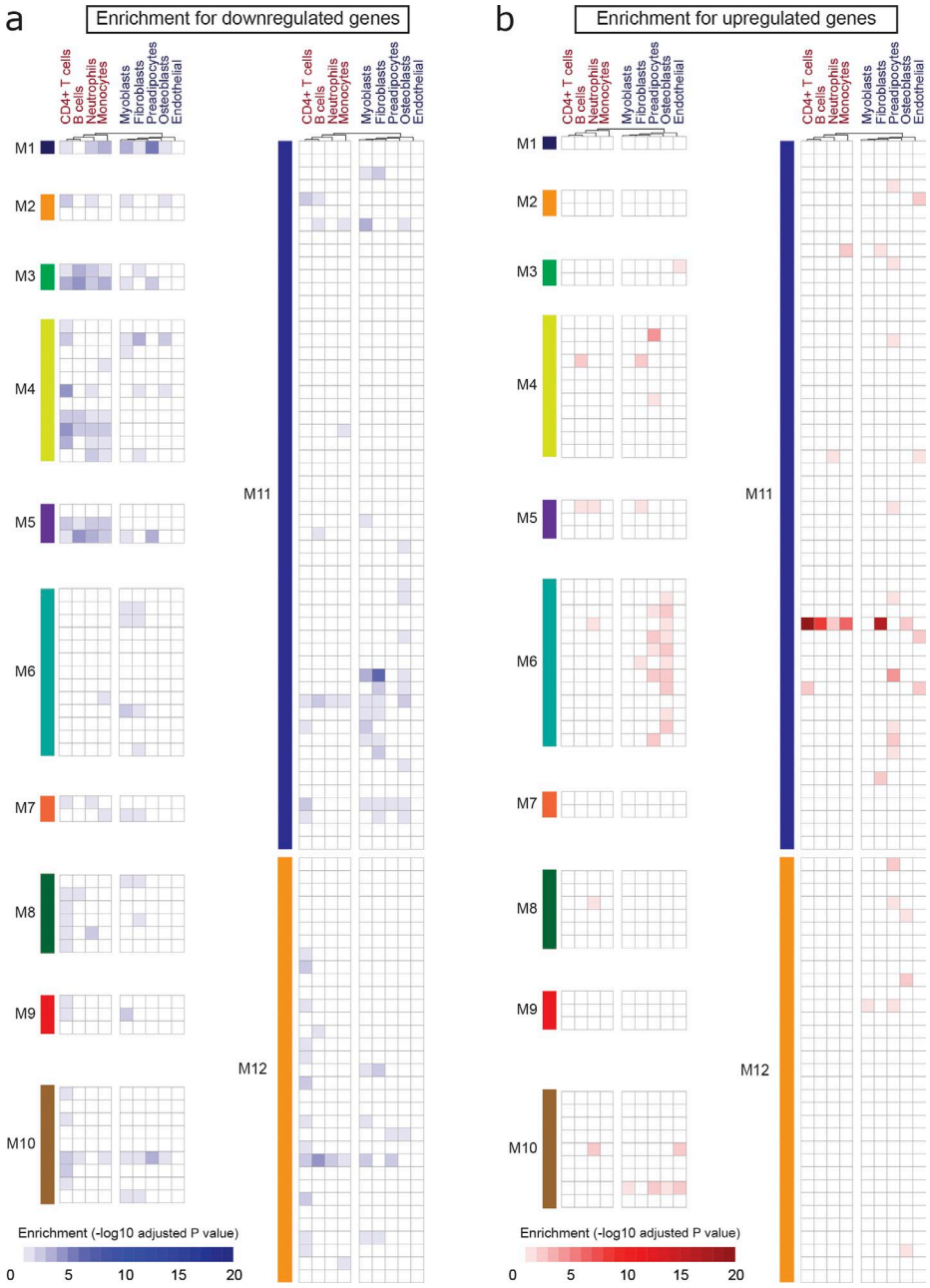

Figure S3. **Enrichment for down-regulated or up-regulated genes among pathways affected by the glucocorticoid stimulus. (a and b)** Heat maps of gene set enrichment analysis results with independent analyses for genes down-regulated (a) or up-regulated (b) in response to in vitro glucocorticoid treatment. Each row represents one gene set (one KEGG pathway), and each column represents one cell type. The pathway modules and the order in which pathways and cell types are displayed are maintained from Fig. S2, to facilitate comparison. For each cell type, cells from four unrelated healthy donors were independently cultured and treated with methylprednisolone (22.7 µM) or vehicle (0.08% ethanol). Total RNA was purified 2 and 6 h after in vitro treatment. RNA-seq was performed. Differential expression was assessed by contrasting methylprednisolone-treated versus vehicle-treated cells in the four biological replicates. For each cell type, the input for the gene set enrichment analyses was a list of genes differentially expressed in response to in vitro methylprednisolone treatment for 6 h, ranked by the value of the log2 fold change (methylprednisolone versus vehicle). Genes at the top of the list are up-regulated, and genes at the bottom of the list are down-regulated in response to glucocorticoid. **(a)** Pathway enrichment for genes down-regulated in response to in vitro glucocorticoid treatment. For each pathway, the test assessed whether the genes in the pathway tend to be located near the bottom of the ranked list of differentially expressed genes (down-regulated in response to glucocorticoid). Enrichment P values are calculated with a Wilcoxon test, and multiple-testing correction is performed with the method of Benjamini and Hochberg (1995). The values displayed are the −log10 adjusted P values for gene set enrichment. Higher values mean that a given pathway was more highly enriched for genes down-regulated by the glucocorticoid in the respective cell type. **(b)** Pathway enrichment for genes up-regulated in response to in vitro glucocorticoid treatment. For each pathway, the test assessed whether the genes in the pathway tend to be located near the top of the ranked list of differentially expressed genes (up-regulated in response to glucocorticoid). Enrichment P values are calculated with a Wilcoxon test, and multiple-testing correction is performed with the method of Benjamini and Hochberg (1995). The values displayed are the −log10 adjusted P values for gene set enrichment. Higher values mean that a given pathway was more highly enriched for genes up-regulated by the glucocorticoid in the respective cell type.

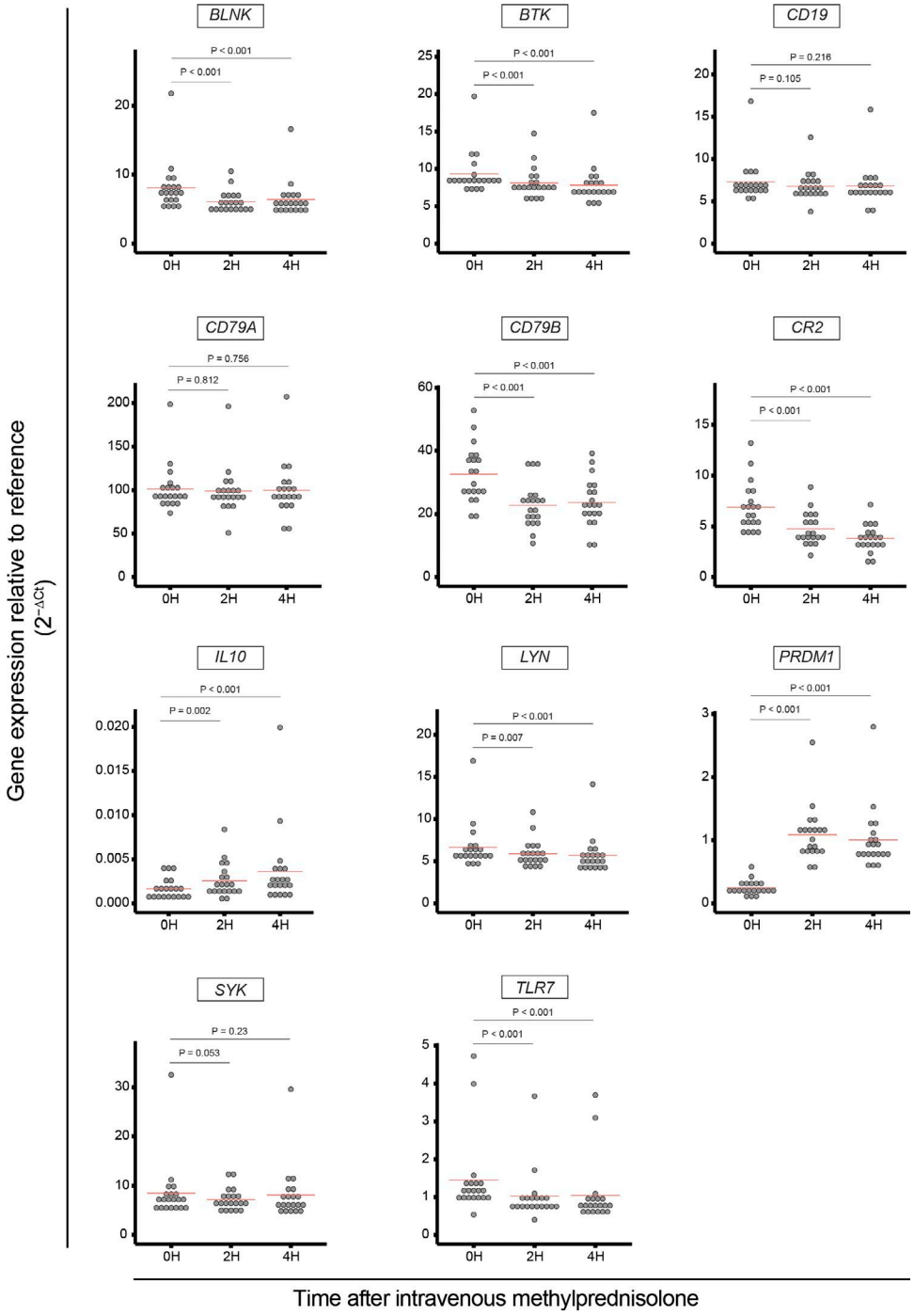

Figure S4. **Gene expression over time in key BCR signaling genes before and after in vivo treatment with methylprednisolone.** 20 unrelated healthy donors were treated with a single intravenous dose of methylprednisolone (250 mg). Circulating B cells were purified before (baseline), 2 h after, and 4 h after medication administration. Gene expression was measured by real-time PCR. For each gene, results are presented as gene expression relative to the reference gene *TBP* ($2^{-\Delta Ct}$). Each dot represents one biological replicate (one donor). Horizontal red bars display the mean. Statistical testing results are from paired (signed-rank) Wilcoxon tests, where paired values are the $2^{-\Delta Ct}$ of glucocorticoid-treated and baseline cells from the same subject. The data presented here are intended to complement the plots in Figs. 4 c, 5 c, and 7, where the same data are presented in the standard $2^{-\Delta\Delta Ct}$ format. That format is intuitive and facilitates understanding as it represents the fold change in expression at each time point relative to baseline. Because this experiment used a paired study design, statistical testing involved a paired analysis of the expression values from each subject before and after glucocorticoid administration. Therefore, in addition to the fold change data in Figs. 4 c, 5 c, and 7, the authors believe it is important to display the expression data at each time point that was used as input for statistical testing.

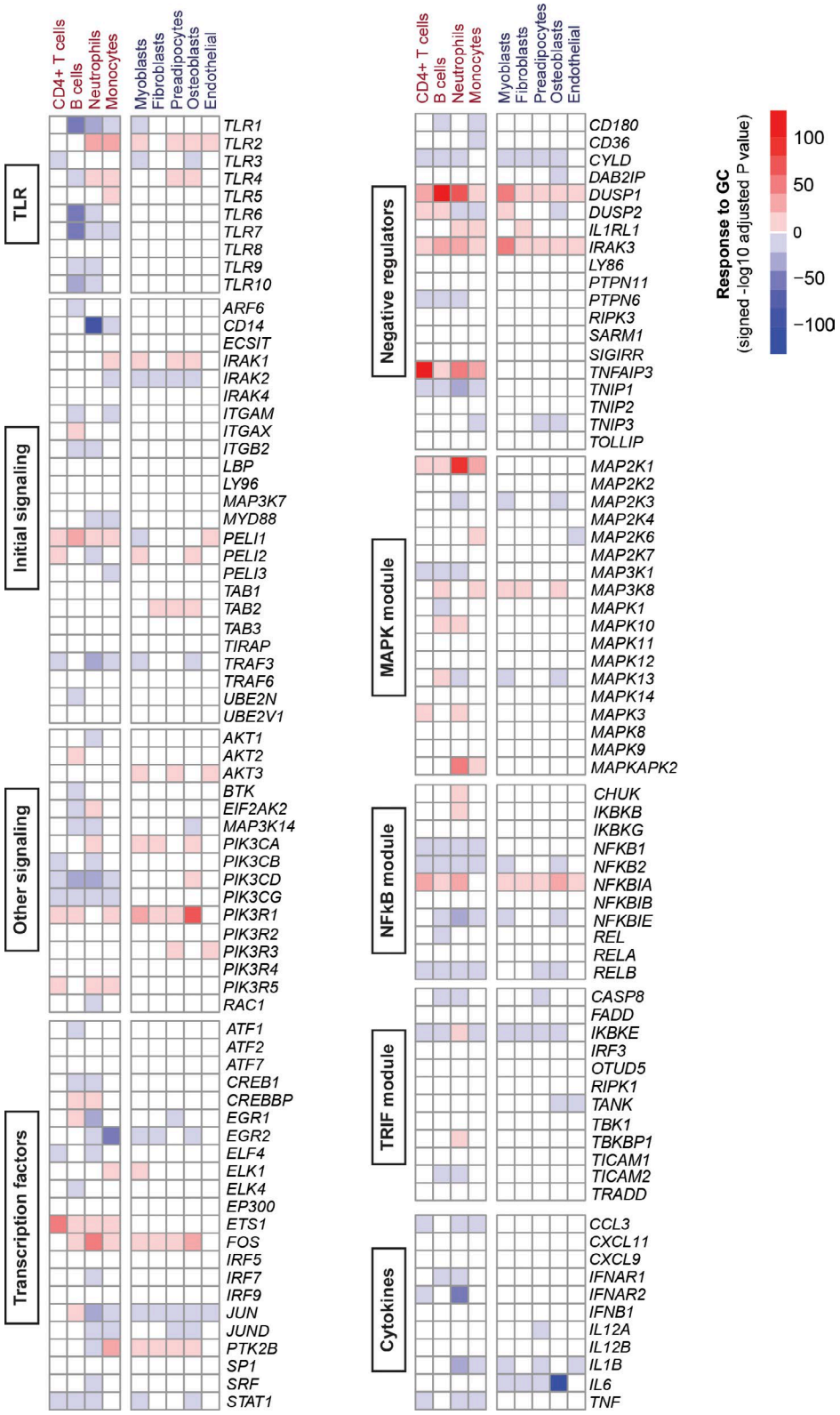

Figure S5. **Transcriptional effects of glucocorticoids on TLR signaling genes.** For each cell type, cells from four unrelated healthy donors were independently cultured and treated with methylprednisolone (22.7 µM) or vehicle (0.08% ethanol). Total RNA was purified 2 and 6 h after in vitro treatment. RNA-seq was performed. Differential expression was assessed by contrasting methylprednisolone-treated versus vehicle-treated cells in the four biological replicates. The statistical significance of differential expression was calculated with a Wald test, after accounting for dispersion, library size, and read count. The resulting P values for differential expression were adjusted for multiple testing by the method of Benjamini and Hochberg (1995). The values displayed are the signed −log10 adjusted P values for differential expression. Higher positive values mean stronger evidence of up-regulation, and lower negative values mean stronger evidence of down-regulation, after 6 h of in vitro exposure to methylprednisolone.

References

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J. R. Stat. Soc. Series B Stat. Methodol.* 57:289–300.