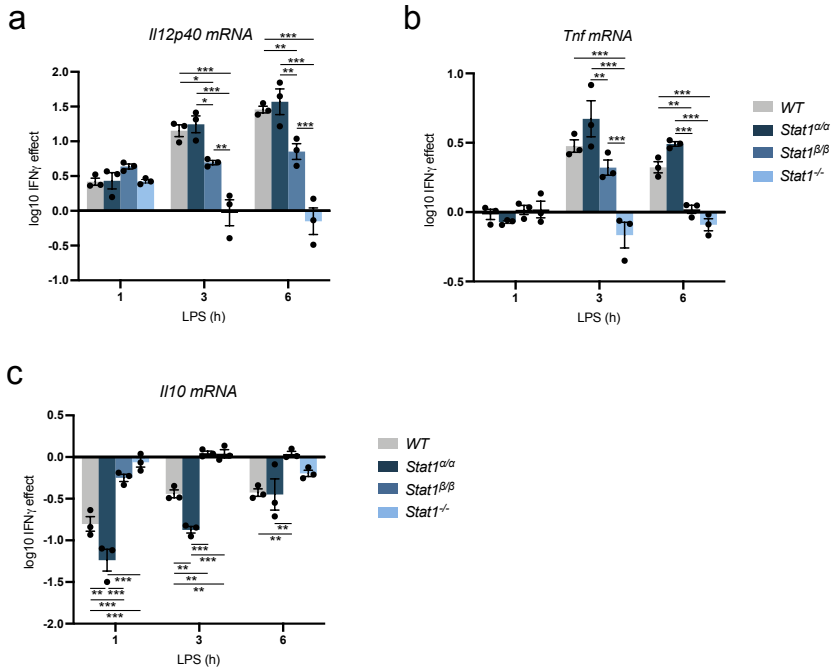
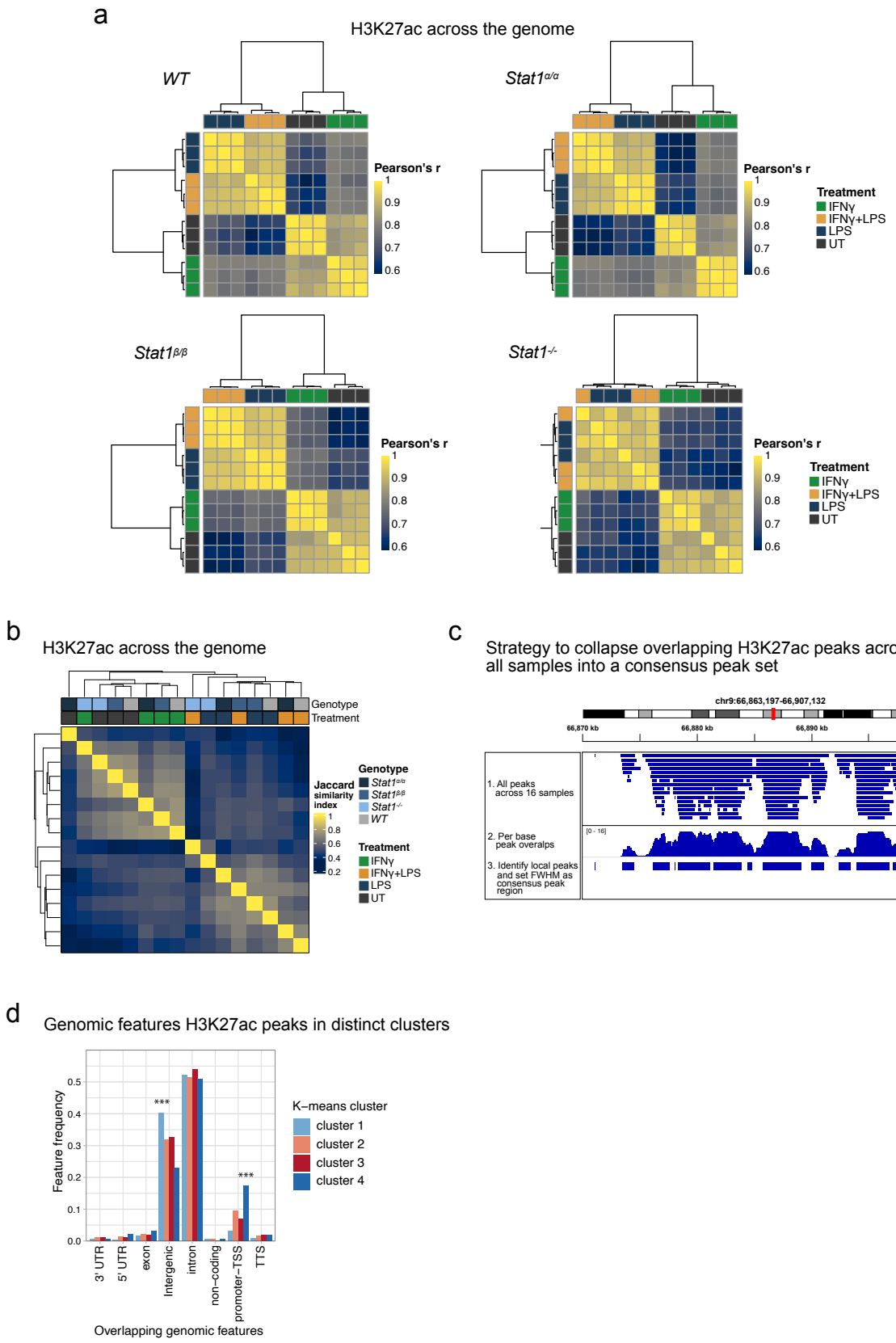
Supplementary Figures and legends

Supplementary Figure S1



Supplementary Fig. S1. Related to Fig. 1.
a-c The mRNA expression ratios of *Il12p40* (a), *Tnf* (b) and *Il10* (c) between IFNγ-pretreated and non-pretreated (medium) *WT*, *Stat1*$^{\alpha/\alpha}$, *Stat1*$^{\beta/\beta}$ and *Stat1*$^{-/-}$ cells stimulated with LPS for the times indicated calculated from data shown in Figure 1. Mean log10 fold-ratios ± SEM are given. For clarity, significances are only shown for the comparison between cells of the different genotypes. 2-way ANOVA with repeated measurement and Tukey HSD posthoc test; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Supplementary Figure S2

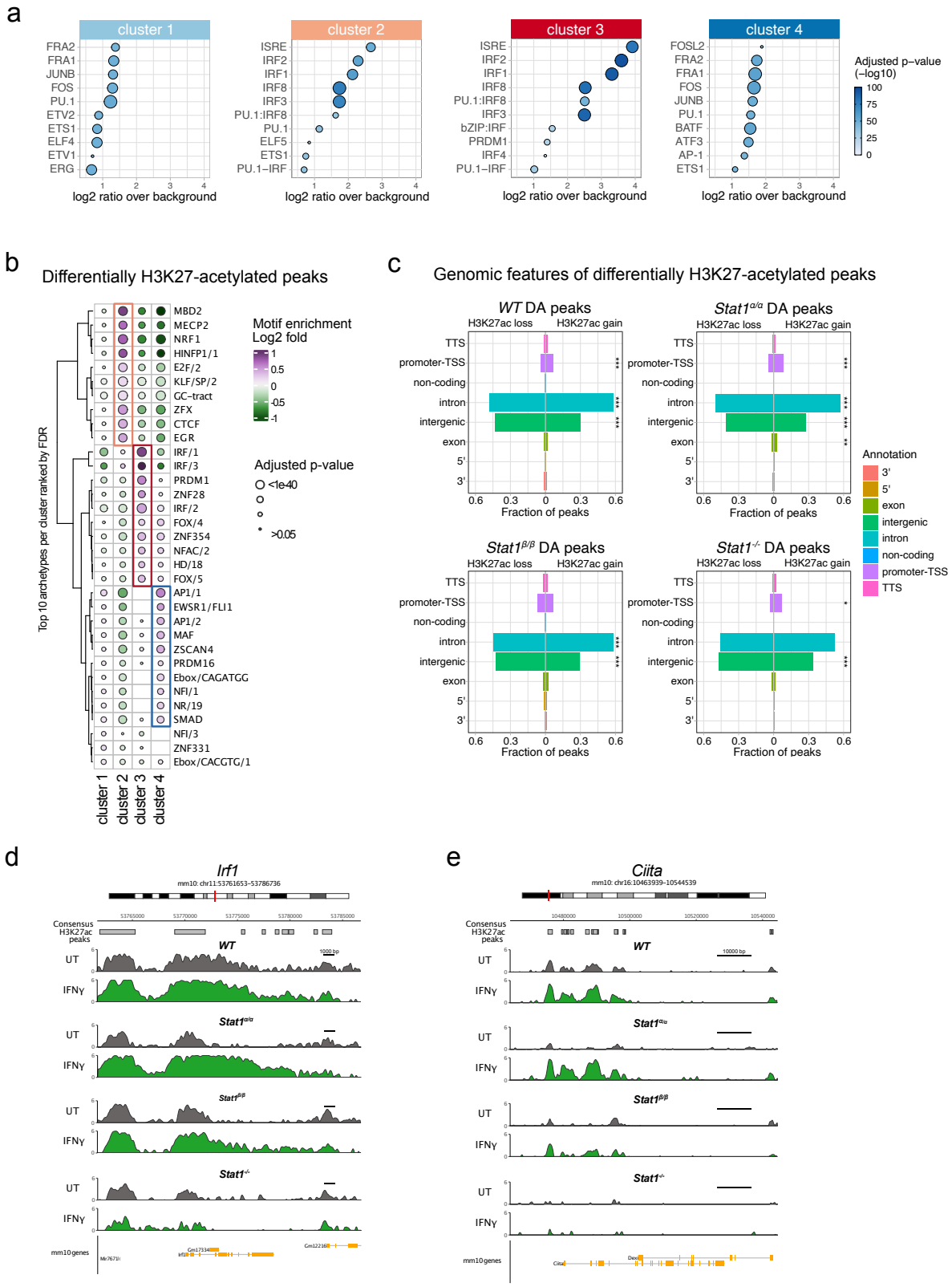


Supplementary Fig. S2. Related to Figure 2.

a Clustered heatmap depicting the Pearson's correlation value (*r*) between samples based on the binned H3K27ac normalized coverage across the genome. Samples were scaled together and heatmaps were split according to the genotype. **b** Clustered heatmap depicting the Jaccard

similarity index between samples based on the pairwise overlap of peaks called for each sample. **c** Diagram outlining the strategy developed to collapse overlapping peaks across all samples into a set of consensus peaks. FWHM: Full width at half max. **d** Genomic features of consensus H3K27ac peak locations in the distinct K-means clusters (cluster 1 to cluster 4). TSS: transcriptional start site, UTR: untranslated region, non-coding: genomic region producing a non-coding transcript. Binomial test p-values are shown (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

Supplementary Figure S3

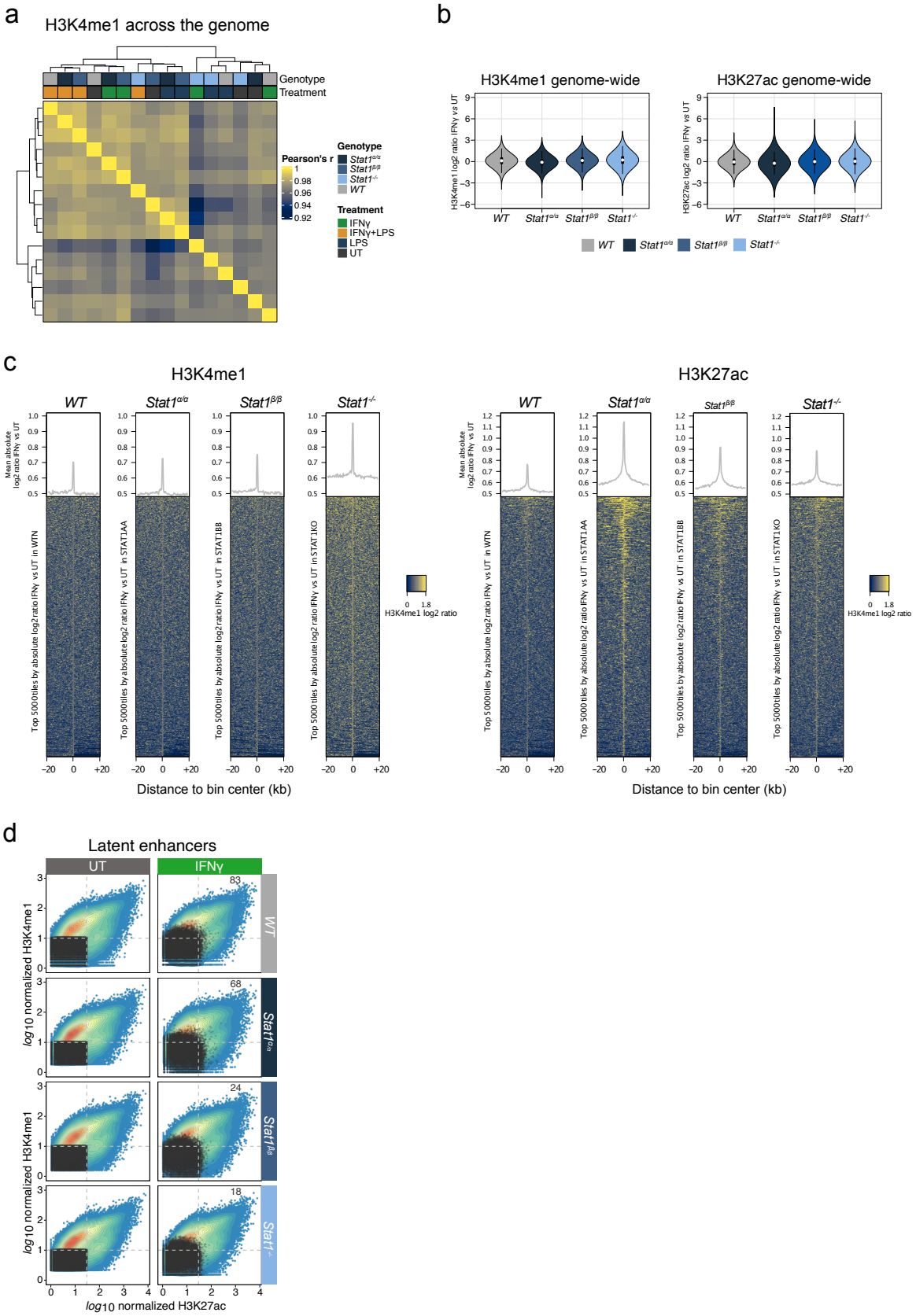


Supplementary Fig. S3. Related to Figure 2.

a The top 10 enriched transcription factor-binding motifs for genes in distinct *K*-means clusters identified using HOMER (ranked by log2 ratio over background). Points are color-code based on Benjamini-Hochberg adjusted p-value. **b** Top 10 enriched transcription factor-binding motifs archetypes in the distinct K-means clusters (cluster 1 to cluster 4) ranked by Benjamini-Hochberg adjusted p-value with log2 fold ratio > 0.5 for upregulated and downregulated

H3K27ac peaks. **c** Relative frequency of genomic features of differentially active consensus H3K27ac peak locations in *WT*, *Stat1*$^{\alpha/\alpha}$, *Stat1*$^{\beta/\beta}$ and *Stat1*$^{-/-}$ cells. Binomial test p-values are shown as asterisks (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). TSS: transcriptional start site, UTR: untranslated region, non-coding: genomic region producing a non-coding transcript. **d, e** Normalized H3K27ac density tracks over H3K27ac consensus peaks around the *Irf1* (d) and *Ciita* (e) genes.

Supplementary Figure S4

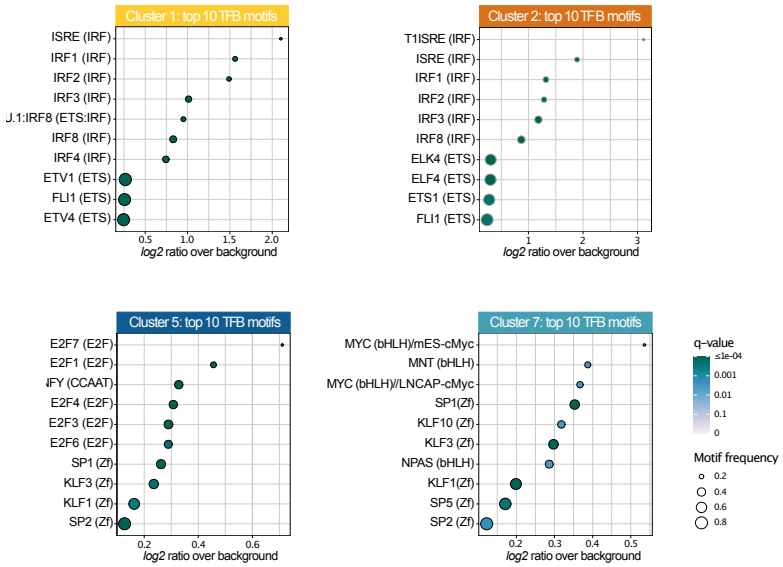


Supplementary Fig. S4. Related to Figure 3.

a Clustered heatmap depicting the Pearson's correlation value (*r*) between samples based on the H3K4me1 coverage across the genome. **b** Violin plots showing the genome-wide log2 ratio of H3K4me1 (left) and H3K27ac (right) enrichment between IFNγ and untreated *WT*, *Stat1*$^{\alpha/\alpha}$,

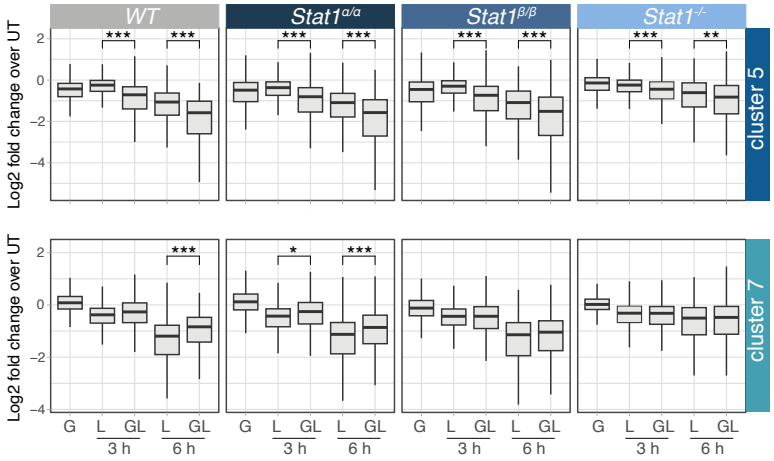
Stat1$^{\beta/\beta}$ and *Stat1*$^{-/-}$ cells. **c** Heatmaps and summary density plot (top) depicting the log2 ratio of H3K4me1 (left) and H3K27ac (right) between IFNγ-treated and untreated *WT*, *Stat1*$^{\alpha/\alpha}$, *Stat1*$^{\beta/\beta}$ and *Stat1*$^{-/-}$ cells for the top 5000 genomic bins (5kb bin size) ranked by absolute log2 ratio. IgG subtracted and RPGC normalized signal ± 20kb from the bin center in along 500 bp windows is shown. **d** Scatter plots of H3K4me1 *versus* H3K27ac with the number of latent enhancers indicated in the respective quadrant for *WT*, *Stat1*$^{\alpha/\alpha}$, *Stat1*$^{\beta/\beta}$ and *Stat1*$^{-/-}$ BMDM.

Supplementary Figure S5



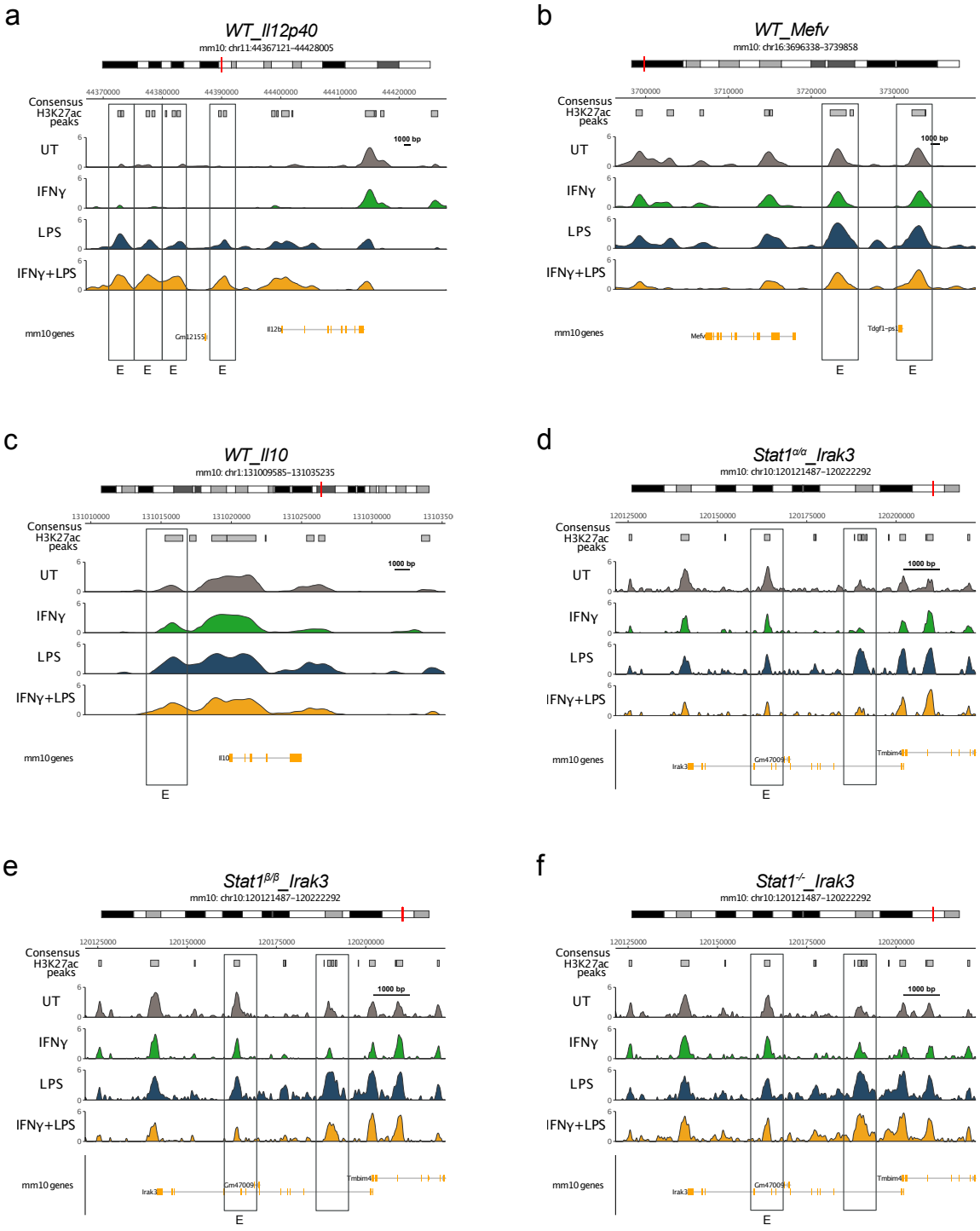
Supplementary Fig. S5. Related to Figure 4.
The top 10 enriched transcription factor-binding motifs for genes in distinct *K*-means clusters identified using HOMER ranked by log2 fold ratio over background. Points are color-coded based on Benjamini-Hochberg adjusted p-value.

Supplementary Figure S6



Supplementary Fig. S6. Related to Figure 5.
Box plots showing log2 fold changes in gene expression of the different treatments over untreated *WT*, *Stat1*$^{\alpha/\alpha}$, *Stat1*$^{\beta/\beta}$ and *Stat1*$^{-/-}$ cells in cluster 5 (top) and cluster 7 (bottom). For reasons of clarity, statistical significances are only given for the differences in the log2 fold change over untreated between LPS alone (L) and after pre-treatment with IFNγ (GL) across the genotypes. See Additional file 5: Table S4 for the Tukey HSD results of all comparisons. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

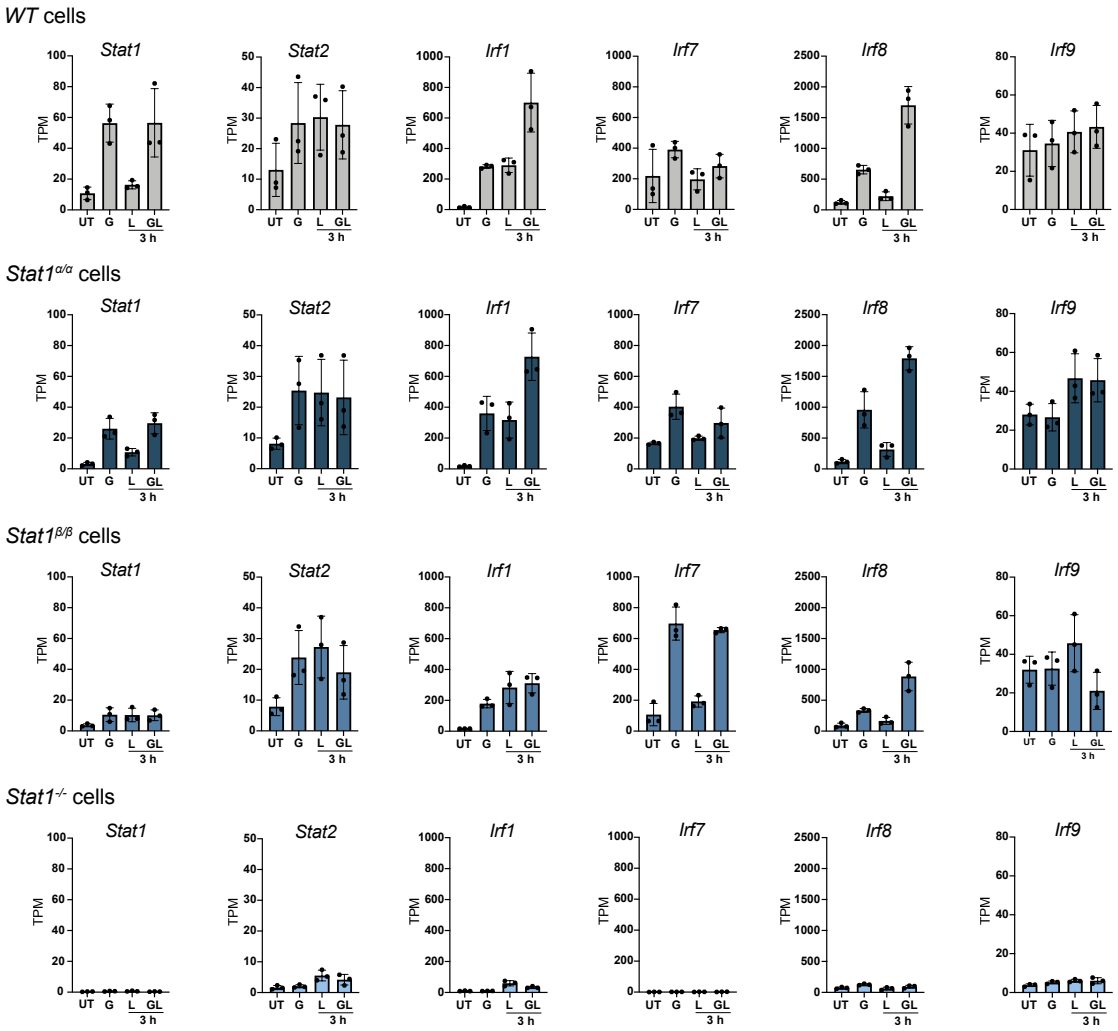
Supplementary Figure S7



Supplementary Fig. S7. Related to Figure 6.

a-f Normalized H3K27ac density tracks for H3K27ac around the *Il12b* (a), *Mefv* (b) and *Il10* (c) genes in *WT* cells and the *Irak3* gene in *Stat1*$^{\alpha/\alpha}$ (d) *Stat1*$^{\beta/\beta}$ (e) and *Stat1*$^{-/-}$ (f) cells. Mean values of 3 independent experiments are shown. H3K27ac peaks are indicated by a box below the genes and enhancers identified by Lara-Astiaso et al. [52] are indicated by an E.

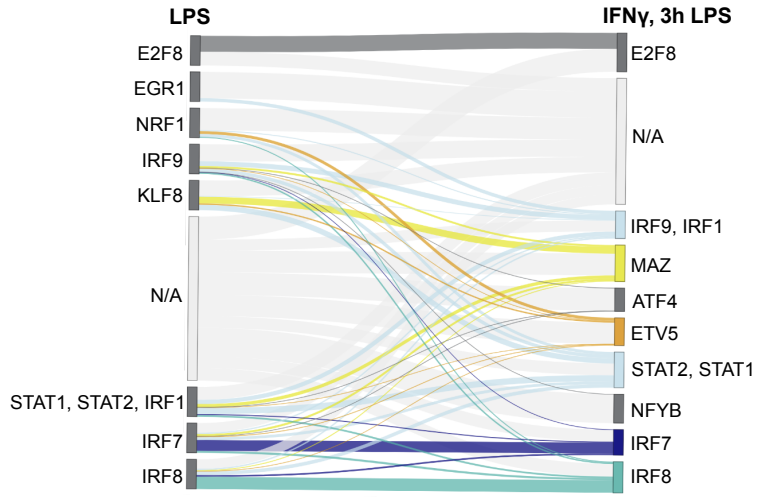
Supplementary Figure S8



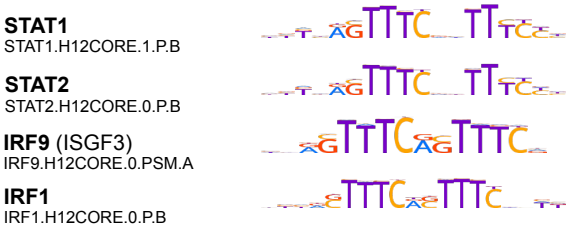
Supplementary Fig. S8. Expression of STAT1, STAT2 and IRF family members.
RNA-seq data (TPM) for *Stat1*, *Stat2*, *Irf1*, *Irf7*, *Irf8* and *Irf9* in *WT*, *Stat1*$^{\alpha/\alpha}$, *Stat1*$^{\beta/\beta}$ and *Stat1*$^{-/-}$ cells.

Supplementary Figure S9

a

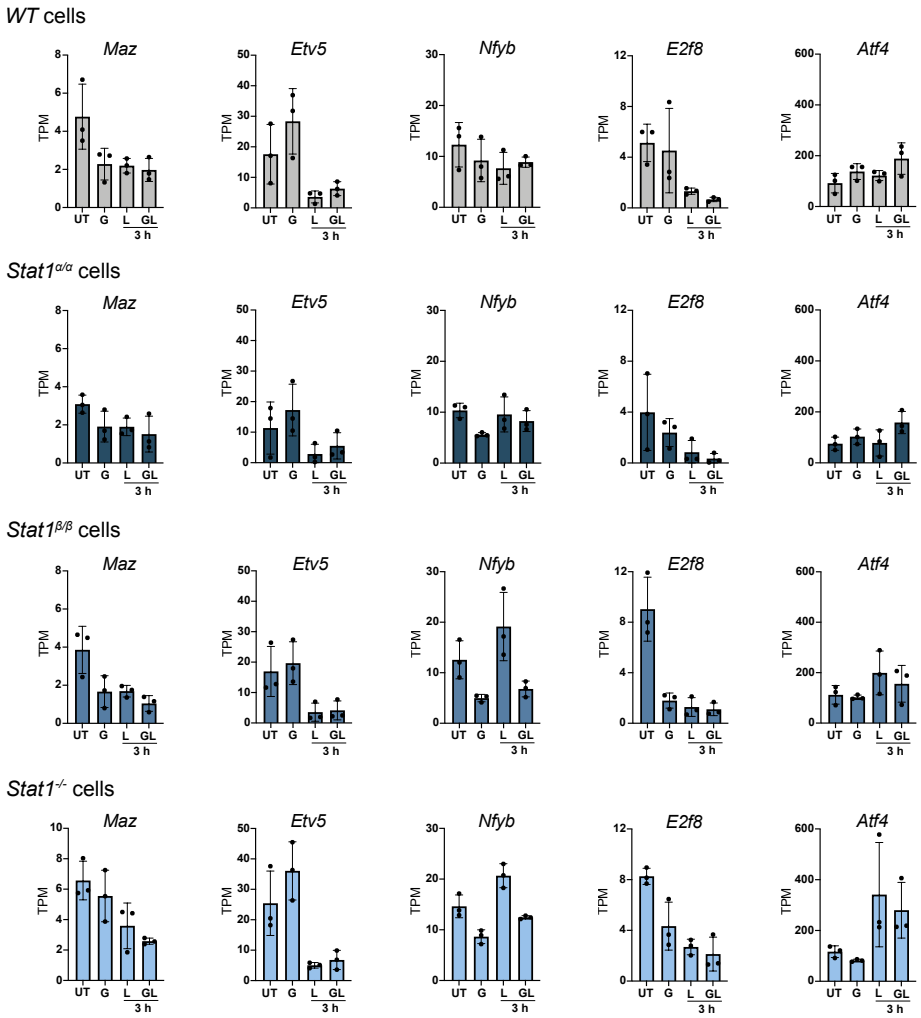


b



Supplementary Fig. S9. Related to Figure 7.
a Sankey diagram showing the changes in TF-communities between LPS treatment and IFNγ+LPS treatment. Treatment-specific target genes in communities are indicated in grey (not annotated, N/A, in the respective other treatment), target genes that are found in the same or another community in both treatments are coloured according to their community assignment in the IFNγ+LPS treatment. **b** Binding motifs annotated for STAT1, STAT2, IRF1 and IRF9 in the HOCOMOCO (version 12) database.

Supplementary Figure S10



Supplementary Fig. S10. Expression of selected TFs identified in the TF-network analysis shown in Figure 7c.

RNA-seq data (TPM) of key transcription factors identified with the regulatory network analysis in *WT*, *Stat1*$^{\alpha/\alpha}$, *Stat1*$^{\beta/\beta}$ and *Stat1*$^{-/-}$ cells.