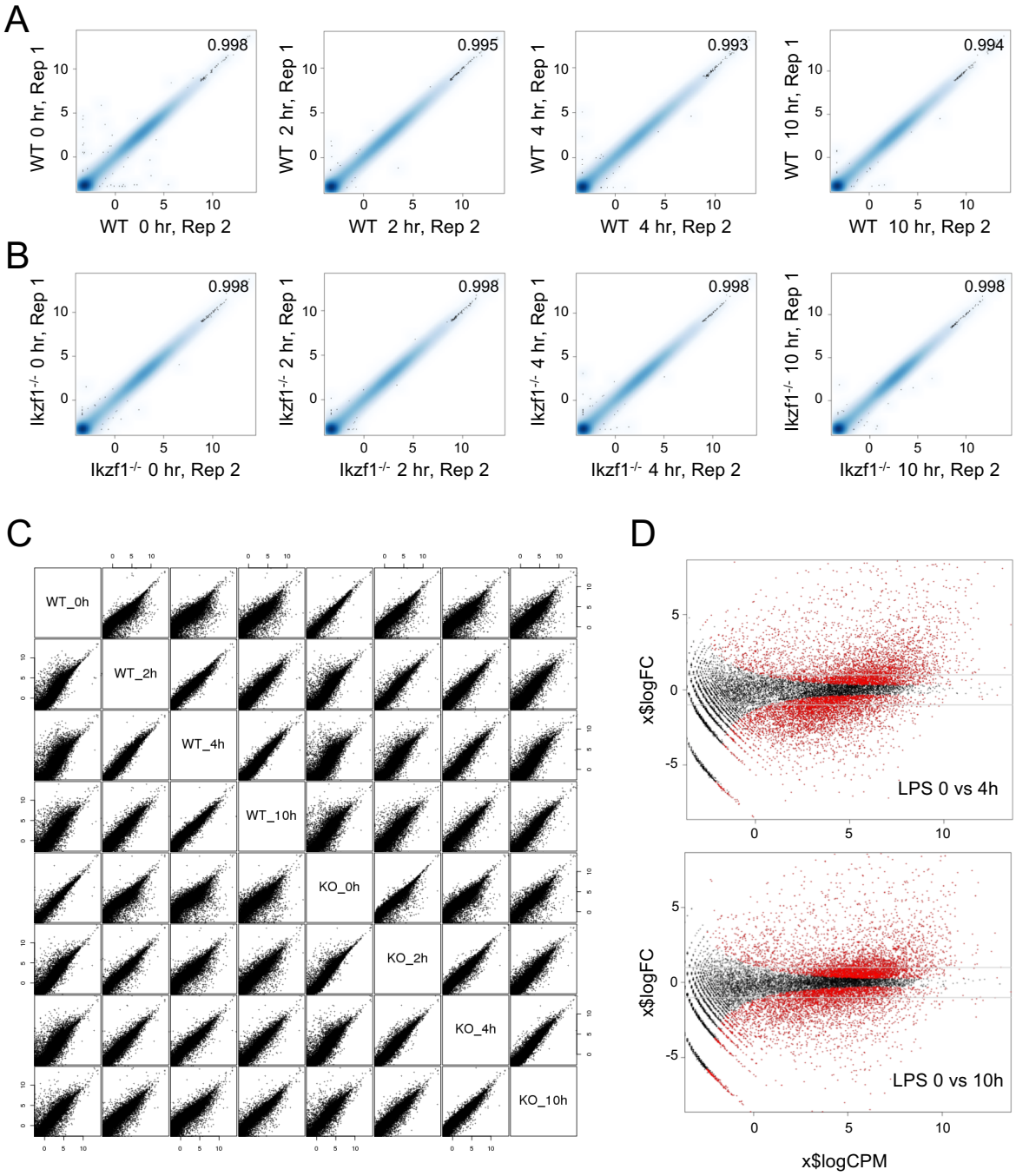


Figure S1

RNA-seq analysis in WT and Ikzf1$^{-/-}$ LPS stimulated macrophages. (A) Replicate sample correlations (Pearson) of RNA seq expression data (FPKM, log2) from WT BMDM stimulated with 10 ng/ml LPS for the indicated times. (B) Replicate sample correlations (Pearson) of RNA seq data from Ikzf1$^{-/-}$ BMDM stimulated with 10 ng/ml LPS for the indicated times. (C) Comparison matrix of scatter plots of RNA seq data averaged from replicate samples for each cell type and condition in WT and Ikzf1$^{-/-}$ (KO) BMDM. (D) edgeR analysis showing log FC (fold change over 0h LPS) versus log CPM (counts per million reads) for 4 hr and 10 hr LPS treated samples. Differentially expressed genes (FDR < 0.001) are marked in red, while gray lines denote fold change thresholds of +1 and -1. Both plots show that many genes considered differentially expressed at FDR < 0.001 have low expression changes of less than 2-fold, and thus were not included in the LPS-altered gene list (see Methods).

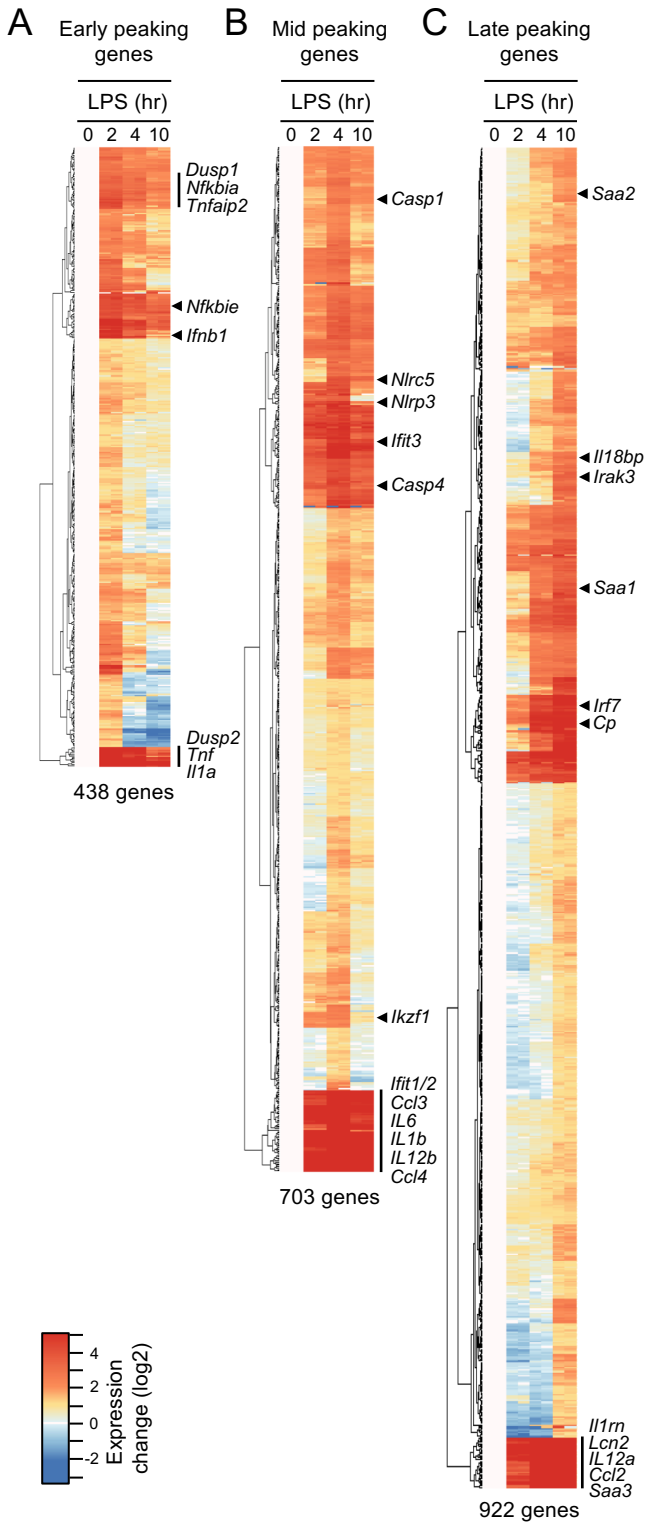


Figure S2

RNA-seq analysis of LPS-induced transcriptional dynamics in primary mouse BMDMs. BMDMs were stimulated with 10 ng/ml LPS for 2, 4 and 10 hr and transcriptional responses were measured by RNA-seq. Genes are shown that increased expression by > 2 fold FPKM from baseline in at least one time point and are categorized by the time point of peak expression into (A) early, (B) mid and (C) late response genes. Data were averaged from two independent experiments (see also Supplementary file S1 and Methods).

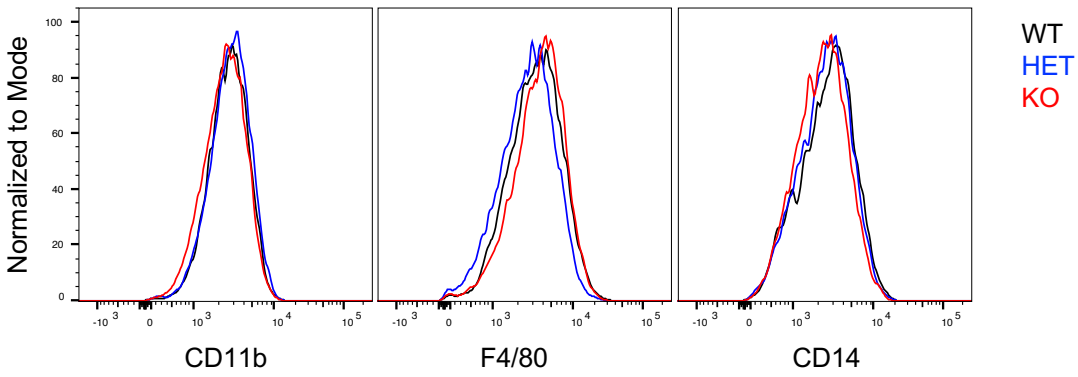
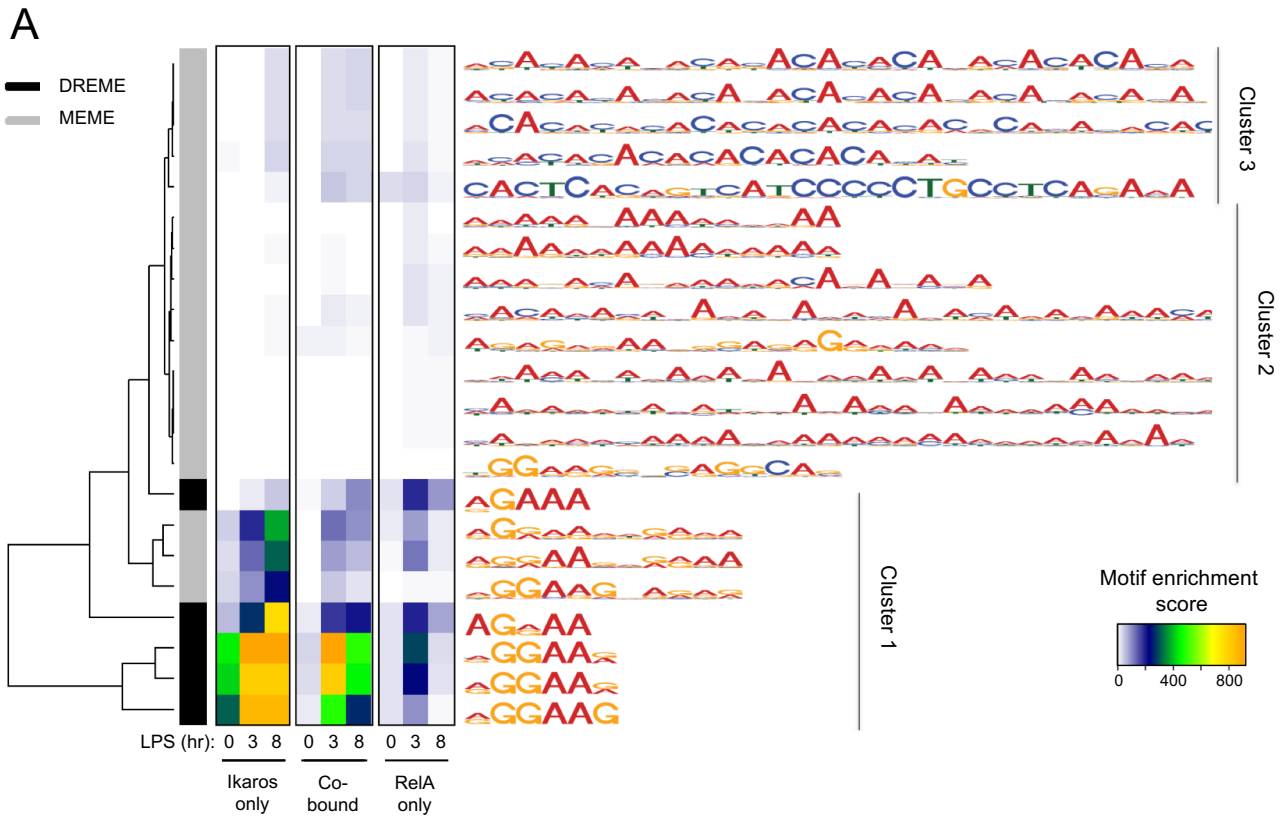


Figure S3

Surface phenotype of Ikaros deficient, heterozygous, and WT BMDM. BMDM differentiated from Ikaros +/+, Ikaros +/- and Ikaros -/- littermate mice were stained for the macrophage surface markers CD11b, F4/80 and CD14 and analyzed by flow cytometry. Data are representative of two independent experiments.



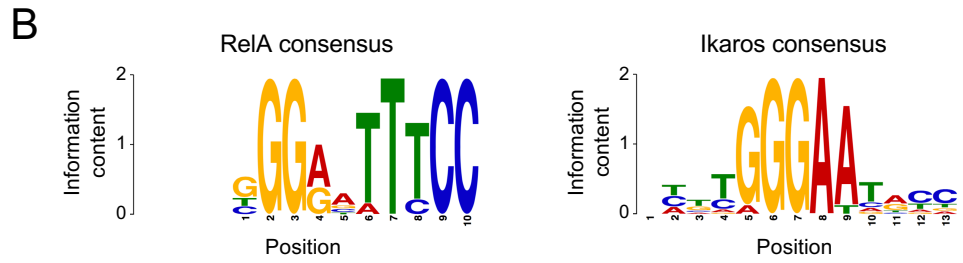


Figure S4

Analysis of Ikaros and RelA bound ChIP sites. (A) Motif enrichments identified by the MEME and DREME algorithms at sites either uniquely bound or co-bound by Ikaros and RelA. (B) Consensus sites for RelA and Ikaros.

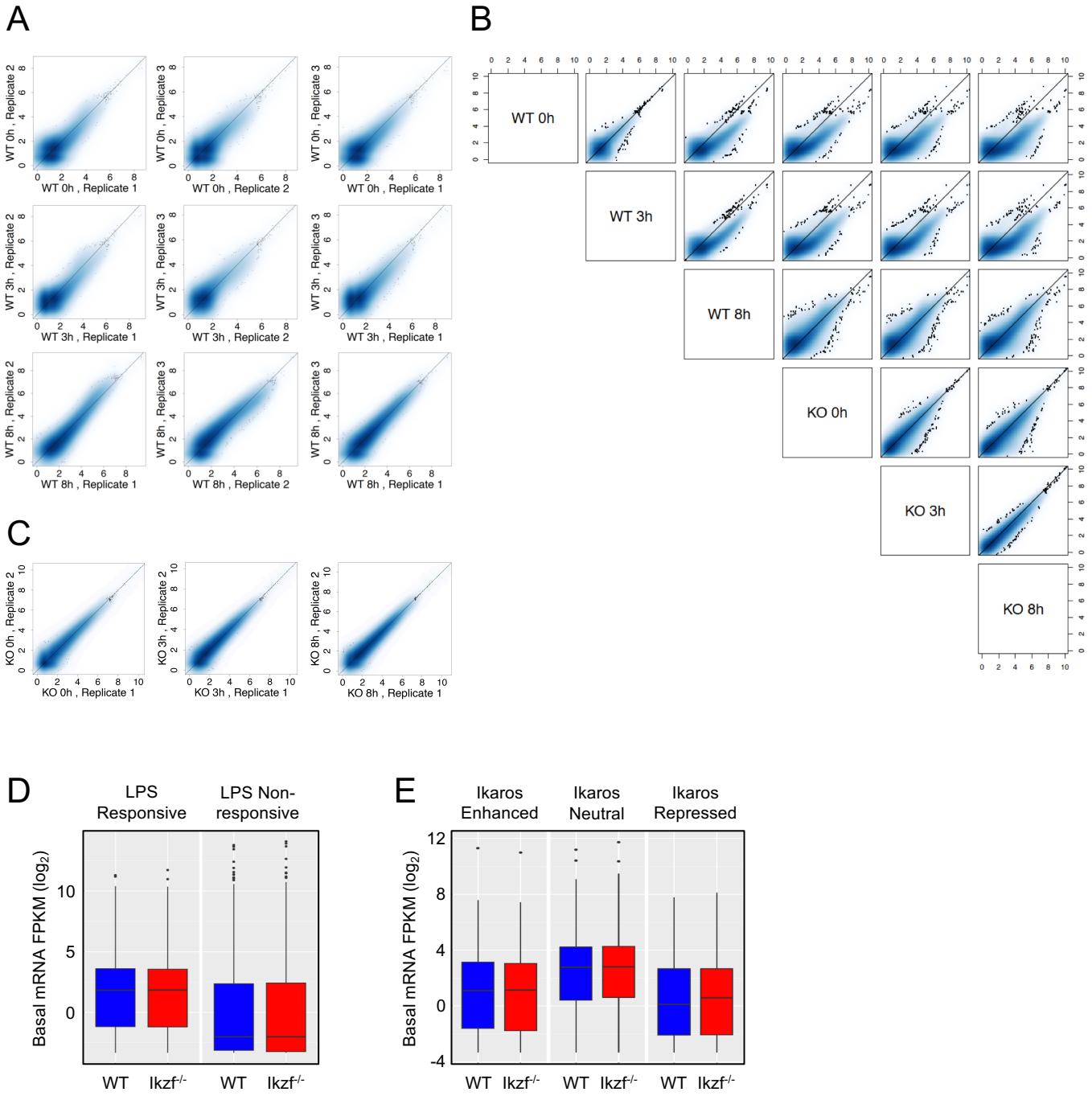


Figure S5

Comparison of WT and Ikzf1$^{-/-}$ LPS stimulated macrophages by DNase-seq. (A) Replicate sample correlations of DNase-seq density from WT BMDM stimulated with 10 ng/ml LPS for the indicated times. Three biological replicates were prepared from independent batches of BMDM: two replicates were analyzed initially, and subsequently one additional set was generated from WT littermates of the *Ikzf1*$^{-/-}$ samples used in (C). (B) Comparison matrix of scatter plots of DNase-seq density, averaged from replicate samples, for each treatment condition in WT and *Ikzf1*$^{-/-}$ BMDM. (C) Replicate sample correlations of DNase-seq density from *Ikzf1*$^{-/-}$ BMDM stimulated with 10 ng/ml LPS for the indicated times. (D,E) Comparison of basal gene expression levels in WT and Ikzf1-/- macrophages at (D) LPS responsive and non-responsive genes and (E) Ikaros enhanced, Ikaros neutral and Ikaros repressed LPS-responsive genes. Dnase-seq data were averaged from 3 (WT) or 2 (Ikzf1$^{-/-}$) independent experiments. Biological replicates were prepared from independent batches of BMDM. See also Supplementary file S3.

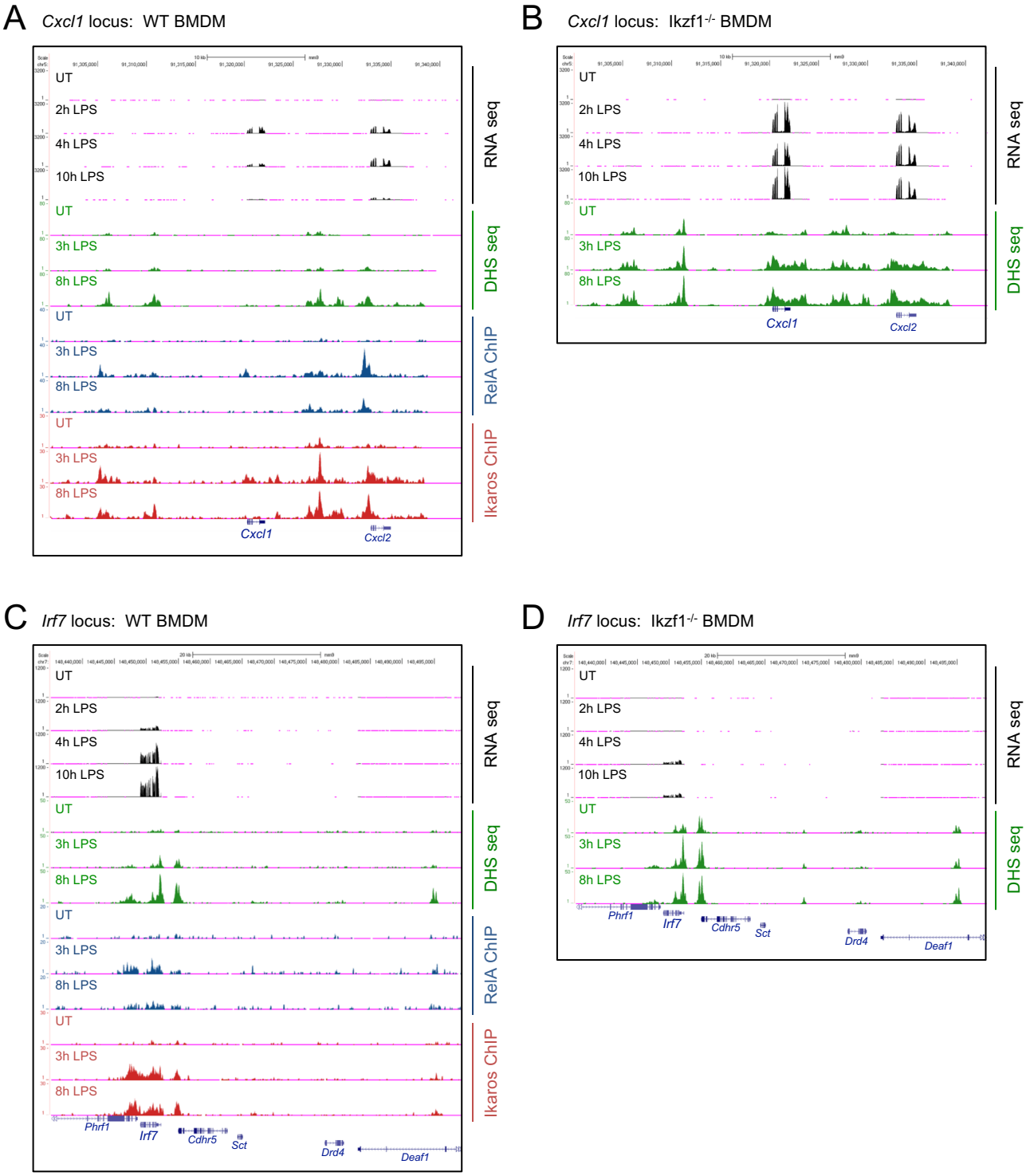


Figure S6

Genome browser images of LPS-induced gene expression, chromatin accessibility and RelA/Ikaros binding in WT and Ikzf1$^{-/-}$ macrophages. UCSC genome browser images of RNA seq reads, DNase-seq reads and RelA and Ikaros bound ChIP-seq hotspots at the indicated LPS treatment times in WT macrophages (A, C) and RNA + DNase seq reads in Ikzf1$^{-/-}$ macrophages (B, D) at the (A, B) *Cxcl1* and (C, D) *Irf7* gene loci.

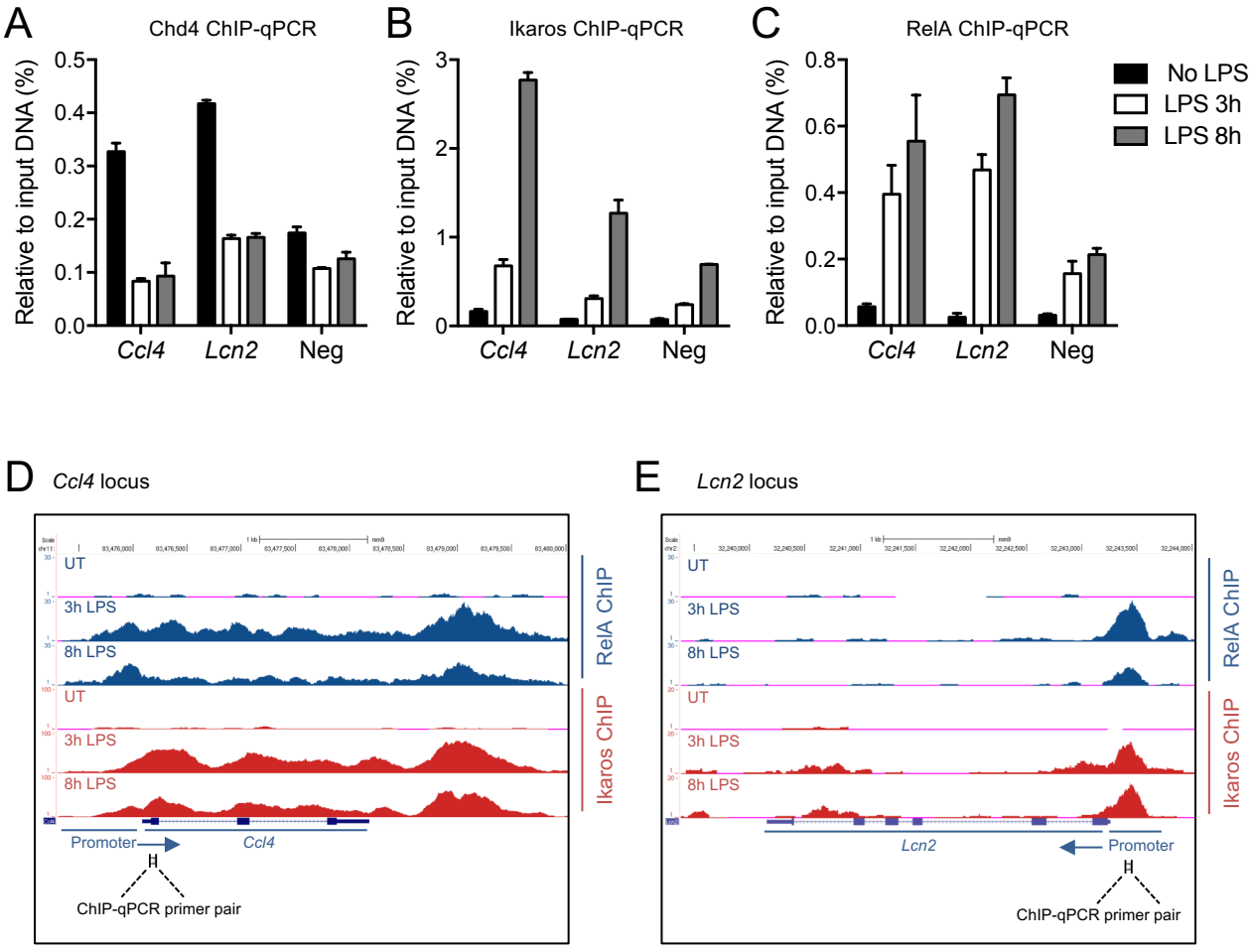


Figure S7

Binding of RelA, Ikaros and Chd4 at the Ccl4 and Lcn2 gene loci in response to LPS. ChIP-qPCR of (A) Chd4, (B) Ikaros and (C) RelA binding at *Ccl4* and *Lcn2* loci in LPS treated WT BMDM. Data were averaged from replicate experiments (mean + s.d.). Neg: negative control PCR primer set. (D, E) UCSC genome browser images are shown to indicate location of the ChIP-qPCR primers at the (D) *Ccl4* and (E) *Lcn2* gene loci.

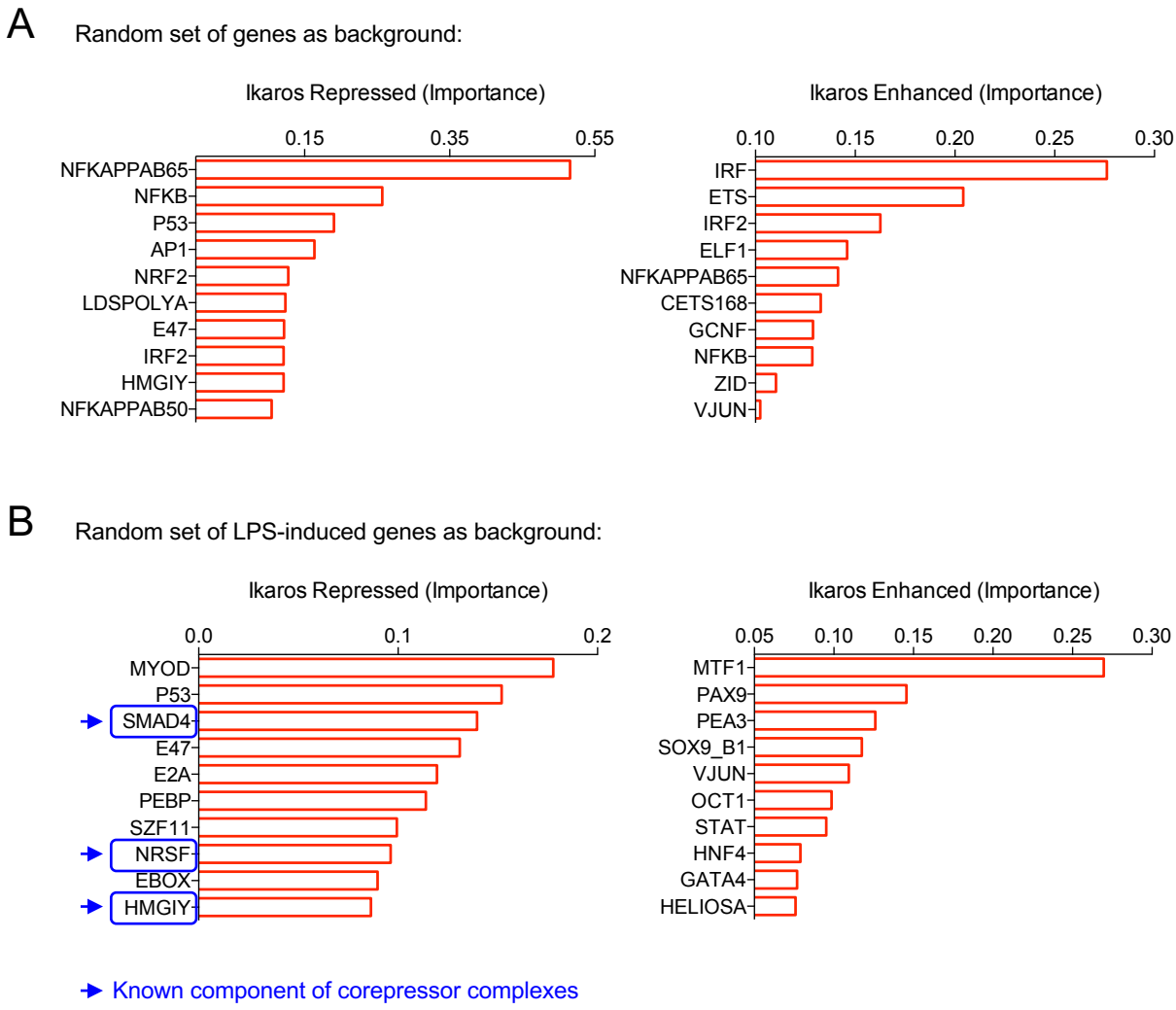


Figure S8

Identification of corepressor-associated transcription factor motifs among Ikaros repressed genes in LPS activated macrophages. The Ikaros Repressed and Ikaros Enhanced genes sets (Fig. 1) were analyzed using DiRE (see Methods) to identify enriched regulatory motifs. Enrichment analyses were conducted using a background gene set randomly selected from either (A) the entire genome (B) the LPS induced set identified in this study (Fig. S2). The Importance parameter for enriched transcription factors is defined as the product of TF occurrence (fraction of candidate enhancers containing binding motif) and weight score (binding motif prevalence compared to background gene set).