Supporting Information

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SI Materials and Methods

Cell Culture and Treatments. The primary macrophages were pretreated with JQ1 (500 nM) or vehicle (DMSO) for 1 h before treatment with 100 ng/mL of LPS (Sigma-Aldrich) for 1 h.

Quantitative RT-PCR. Total RNA was isolated using TRIzol according to the manufacturer's instructions (Invitorgen) and reverse-transcribed to cDNA using iScript reagent (Bio-Rad). Quantitative RT-PCR was performed using SsoAdvanced SYBR Green reagent on the CFX384 system (BioRad).

Quantitative RT-PCR Primer Sequences. The following primer sequences were used: Ccl5-gene, forward 5′-ACCACTCC-CTGCTGCTTT-3′, reverse 5′-AGCACTTGCTGCTGGTGT-3′;

Ccl5-seRNA1, forward 5′-GGAACTGTGCTTGGGTCA-3′, reverse 5′-CTCACACTCGCACCACCT-3′; Ccl5-seRNA2, forward 5′-AAGCCAGGACAGGACAGG-3′, reverse 5′-TTTGGTGCG-CTTCTGAGT-3′; Ccl5-seRNA3, forward 5′-GGCCATCACTTG-GGATTT-3′, reverse 5′- TCTCGTGTGTGCCATGTG-3′; Ccl5-seRNA4, forward 5′-TACCCTCAATGGCAGCTTTA-3′, reverse 5′-TTGAGAGCTTGTTGGGGTAA-3′; Ccl5-seRNA5, forward 5′-GCACCCTCTAAACCTGACCT-3′, reverse 5′-T-GCTGGTCTTCAGCACACTA-3′; Irf1-gene, forward 5′-CC-TCTGTCTTTTCCCTCCAG-3′, reverse 5′-CTCTAGCCAG-GGTCTCATCC-3′; and Irf1-seRNA, forward 5′-CAGCTAT-CCCAGGTGATTGA-3′, reverse 5′-CCAAGAGGCAGAGC-ATTTT-3′.
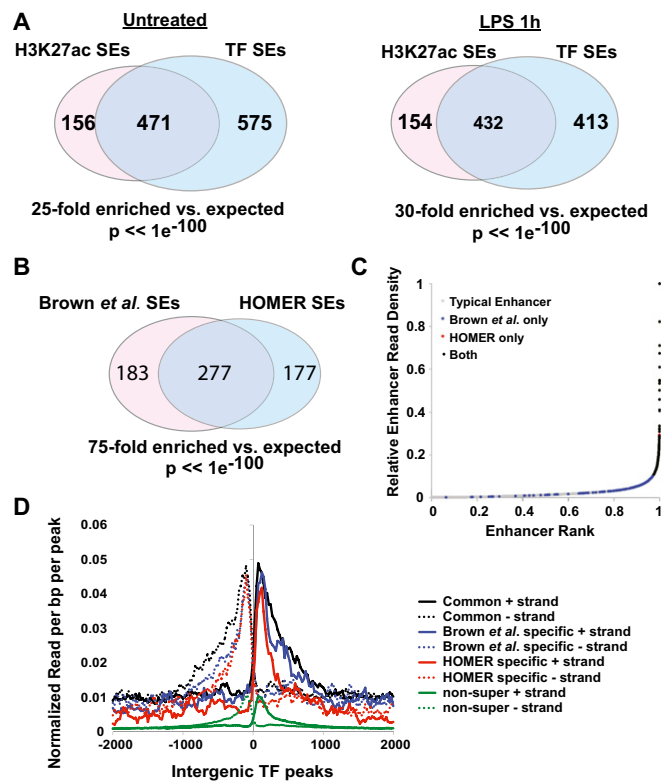
Fig. S1. Comparison of SE callings. (*A*) Venn diagram representations of SE comparisons between SEs that are called using H3K27Ac ChIP-Seq data and those that are called using key macrophage TFs used in this study in both vehicle and LPS treatment conditions. (*B* and *C*) Comparison between SEs found in Brown et al. (1) and SEs found using HOMER in the same mouse macrophage H4K12ac ChIP-Seq dataset. (*D*) Assessment of the functional quality of differential SE calls by measuring the eRNA production from intergenic TFBSs that were specific to Brown et al. or HOMER SEs.

1. Brown JD, et al. (2014) NF-κB directs dynamic super enhancer formation in inflammation and atherogenesis. *Mol Cell* 56(2):219–231.

Fig. S2. Relationship between the presence of eRNAs and SEs. Pie chart representations of the relationship between the number of eRNAs near (±50 kb from TSS) a gene and the presence of an SE (blue). The size of the pie reflects the number of genes in each class.

Fig. S3. Changes in Pol II levels in an actively down-regulated seRNA by LPS. A genome browser representation of a comparison between GRO-Seq and Pol II ChIP-Seq of actively down-regulated seRNAs and gene transcription at the Igf11 locus.

Fig. S4. Comparison of seRNA regulation by LPS and KLA. (*A*) Genome browser representations of GRO-Seq data generated with KLA stimulation in thio-glycollate-induced peritoneal macrophages (ThioMac) used in Kaikkonen et al. (1) versus LPS treatment in bone marrow-derived macrophages (BMDM) in our study. (*B*) Correlation of seRNA regulations between KLA and LPS stimulation. (*C*) Contour graph representations of the fraction of eRNAs that are either repressed or induced upon KLA stimulation within an SE.

1. Kaikkonen MU, et al. (2013) Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. *Mol Cell* 51(3):310–325.

Fig. S5. Changes in SEs upon LPS treatment in macrophages. A Venn diagram representation of the number of SEs in vehicle- or LPS-treated macrophages.

Fig. S6. The effect of JQ1 and LPS in seRNAs and gene expression in macrophages. Quantitative RT-PCR analyses of expression changes in seRNAs and their associated genes in VEH or LPS (100 ng/mL) ± JQ1 (500 nM)-treated macrophages. Each experiment was performed in biological triplicates, and error bars indicate SEM.

Table S1. The number of eRNAs near (±50 kb from TSS) a gene and the presence of an SE

No. of eRNAs	Total no. of genes	No. of genes without SEs	No. of genes with SEs	Fraction of genes with SEs
0	22,489	21,342	1,147	0.051
1	4,338	3,804	534	0.123
2 and 3	4,041	3,164	877	0.217
4 and 6	1,373	870	503	0.366
>7	172	68	104	0.604

Other Supporting Information Files

Dataset S1 (XLSX)