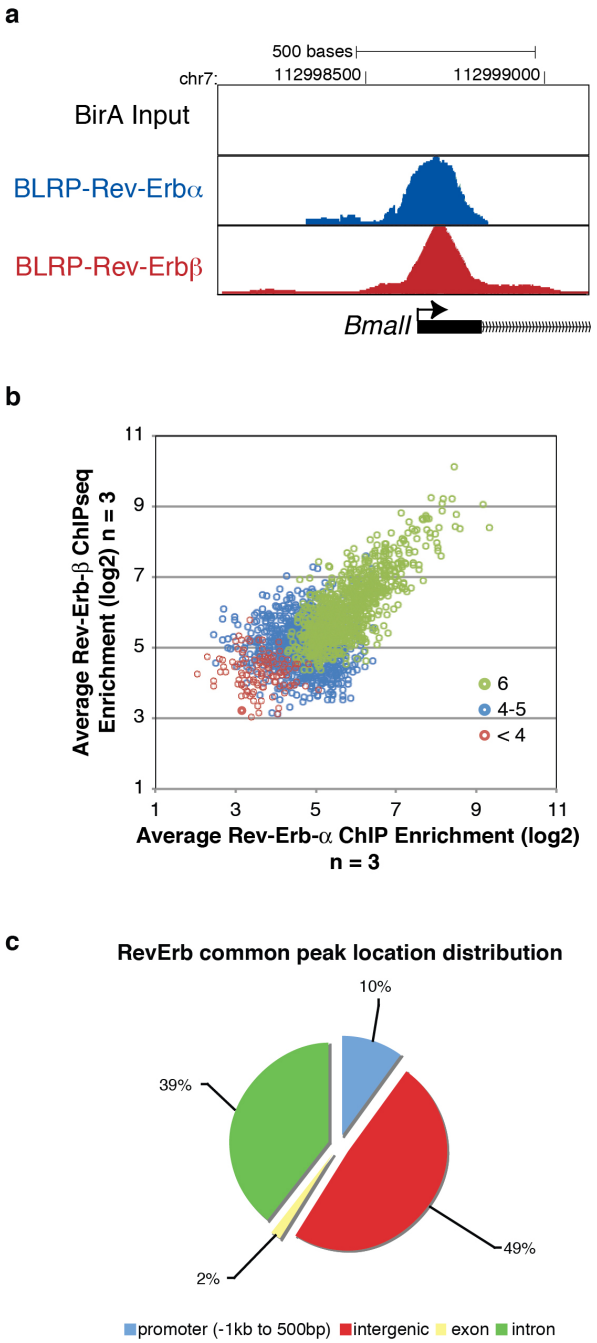
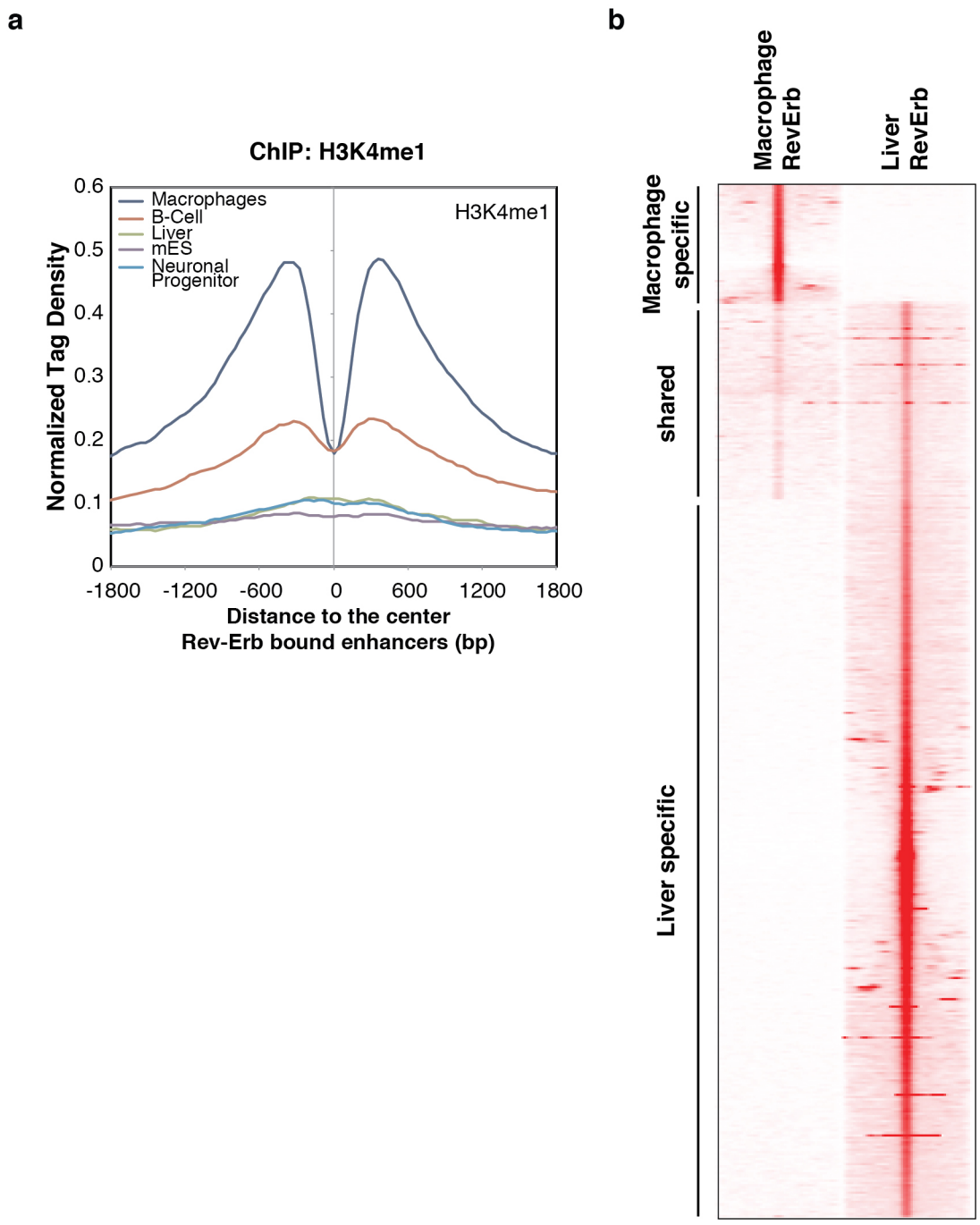
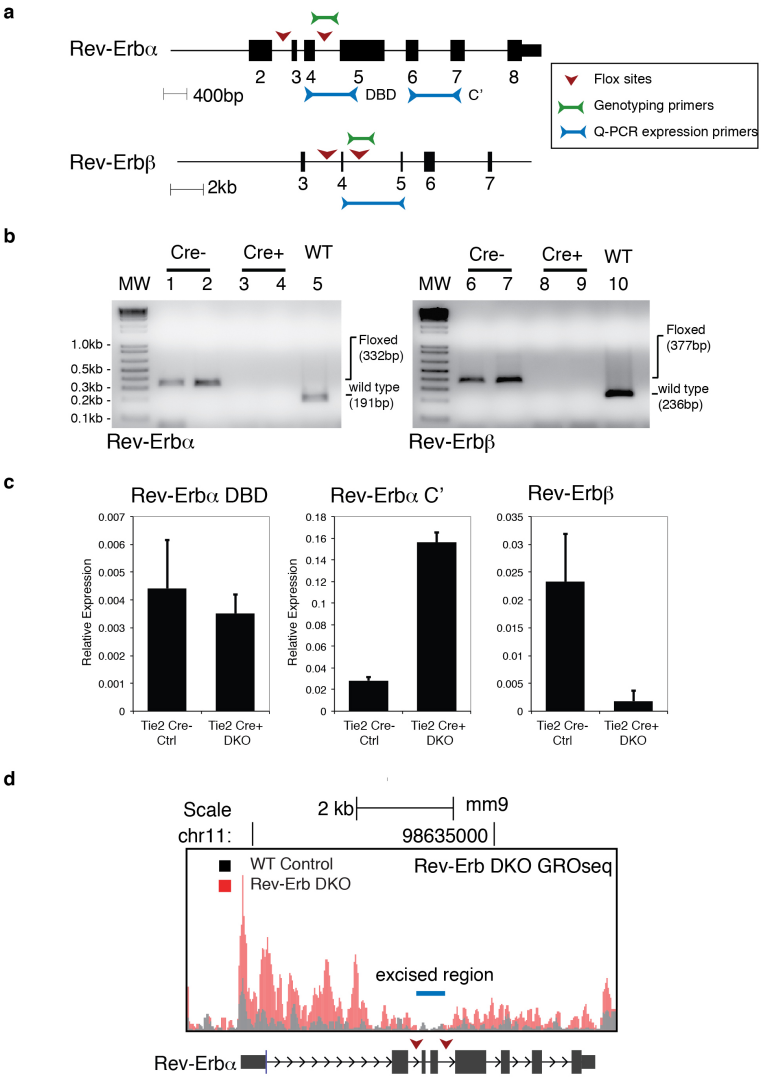
Lam, et al., Supplementary Figures



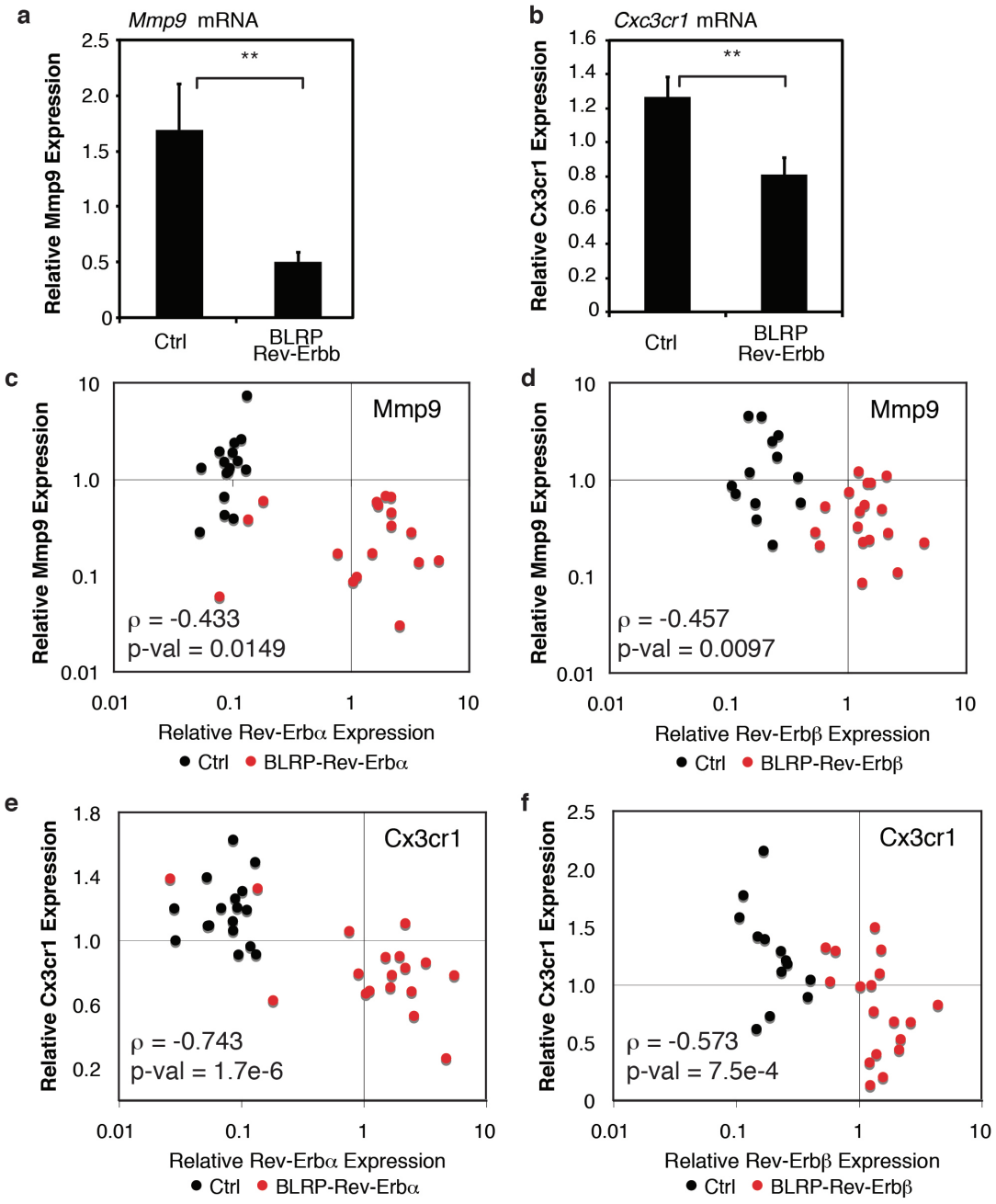
Supplementary Figure 1. ChIP-Seq indicates that Rev-Erbs predominantly bind to distal enhancers. a, Promoter of *Bmall* with ChIP-Seq track for biotin-tagged Rev-Erbα and Rev-Erbβ in RAW264.7 macrophages compared to BirA control RAW264.7 cells. b, Scatter plot comparing average ChIP-Seq tag densities for RevErbα and RevErbβ from six independent ChIP-Seq experiments. Common peaks exhibiting at least 16 tags in all six experiments are color-coded green, and peaks exhibiting at least 16 tags in 4-5 experiments are color coded in blue. c, Genomic locations of Rev-Erbα and Rev-Erbβ common peaks.



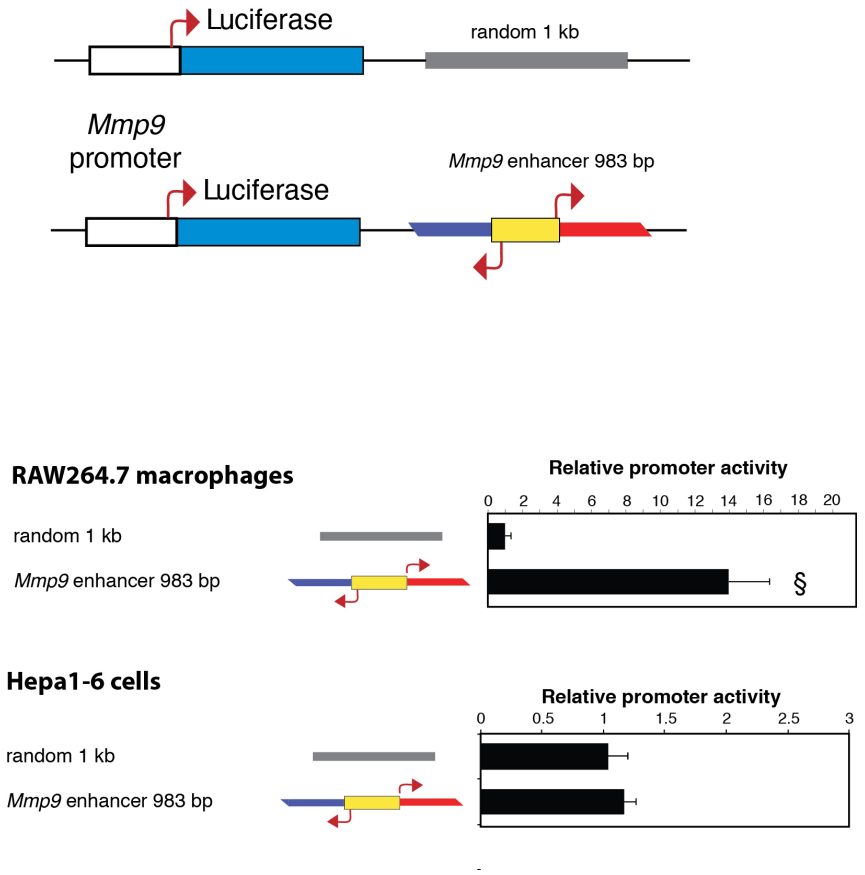
Supplementary Figure 2. Cell specificity of RevErb-regulated enhancers. a. Distribution of averaged ChIP-Seq signal of H3K4me1 at macrophage Rev-Erb bound H3K4me1^{hi} H3K4me3^{lo} (n = 1,388) gnomic locations in macrophages[1], B-cells[2], liver[3], mouse embryonic stem cells (mES), and neural progenitors[4]. b, Heat map of RevErb binding intensity at macrophage and liver genomic binding sites with at least 16 normalized tags count from Rev-Erb ChIP-Seq in at least one cell type. Respective Rev-Erb ChIP-Seq signals were plotted ±1kb from the center of each site.



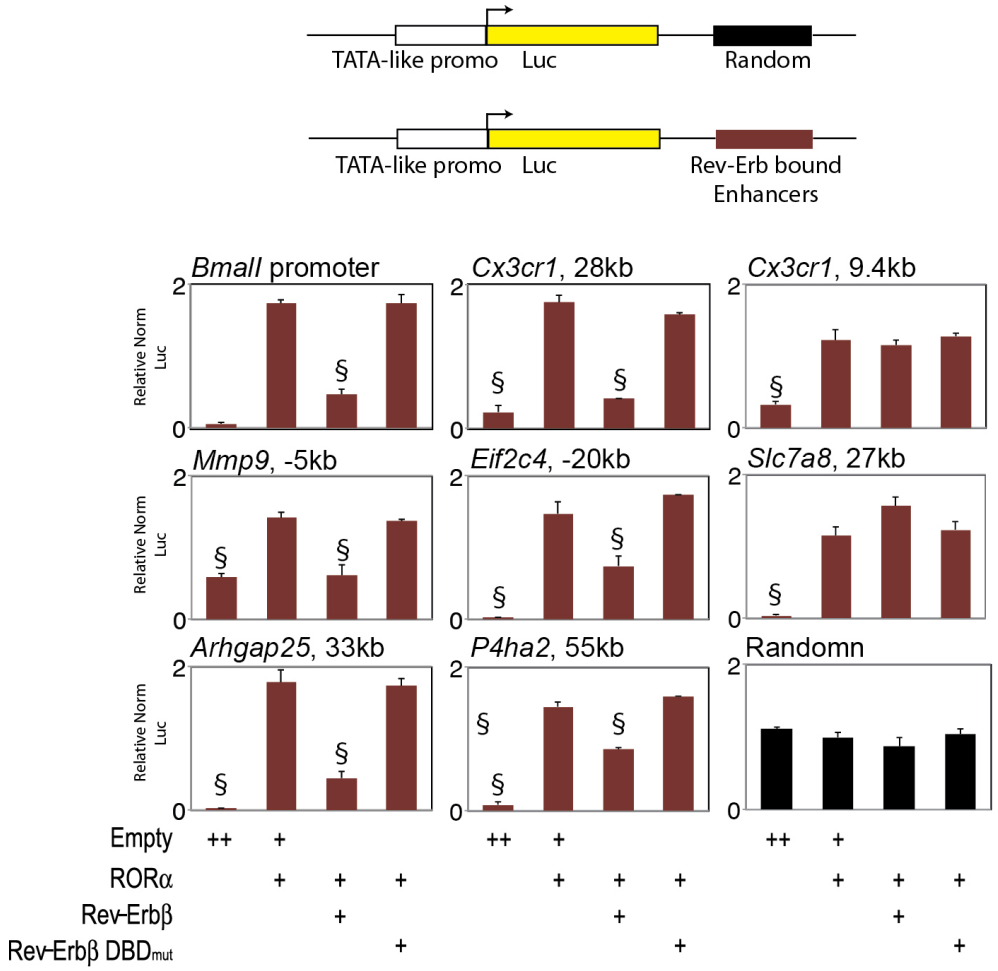
Supplementary Figure 3. Tie2-Cre mediated excision of Rev-Erbs in Rev-Erbα$^{flox/flox}$ and Rev-Erbβ$^{flox/flox}$ in macrophages. a, Illustration of floxed *Rev-Erb* genomic loci. Primer binding regions and predicted amplicons for DNA genotyping and mRNA expression are indicated. b, PCR genotyping for *Rev-Erbα* (left) and *Rev-Erbβ* (right) from DNA of BMDM with or without Tie2-Cre. Lane 5 and 10 indicated PCR results from DNA of wildtype macrophages. c, Q-PCR indicating expression of *Rev-Erbα* and *Rev-Erbβ* from macrophages with or without Tie2-Cre. Knockout of Rev-Erbβ dramatically increases *Rev-Erbα* expression. Excision for *Rev-Erbα* was estimated by comparing the derepression between the floxed region (DBD) vs. a non-excised region (*Rev-Erbα* C'). d, GRO-Seq of the Rev-Erbα genomic locus from Tie2-Cre negative (black) and Tie2-Cre positive Rev-Erb DKO macrophages (red). The excised region is indicated.



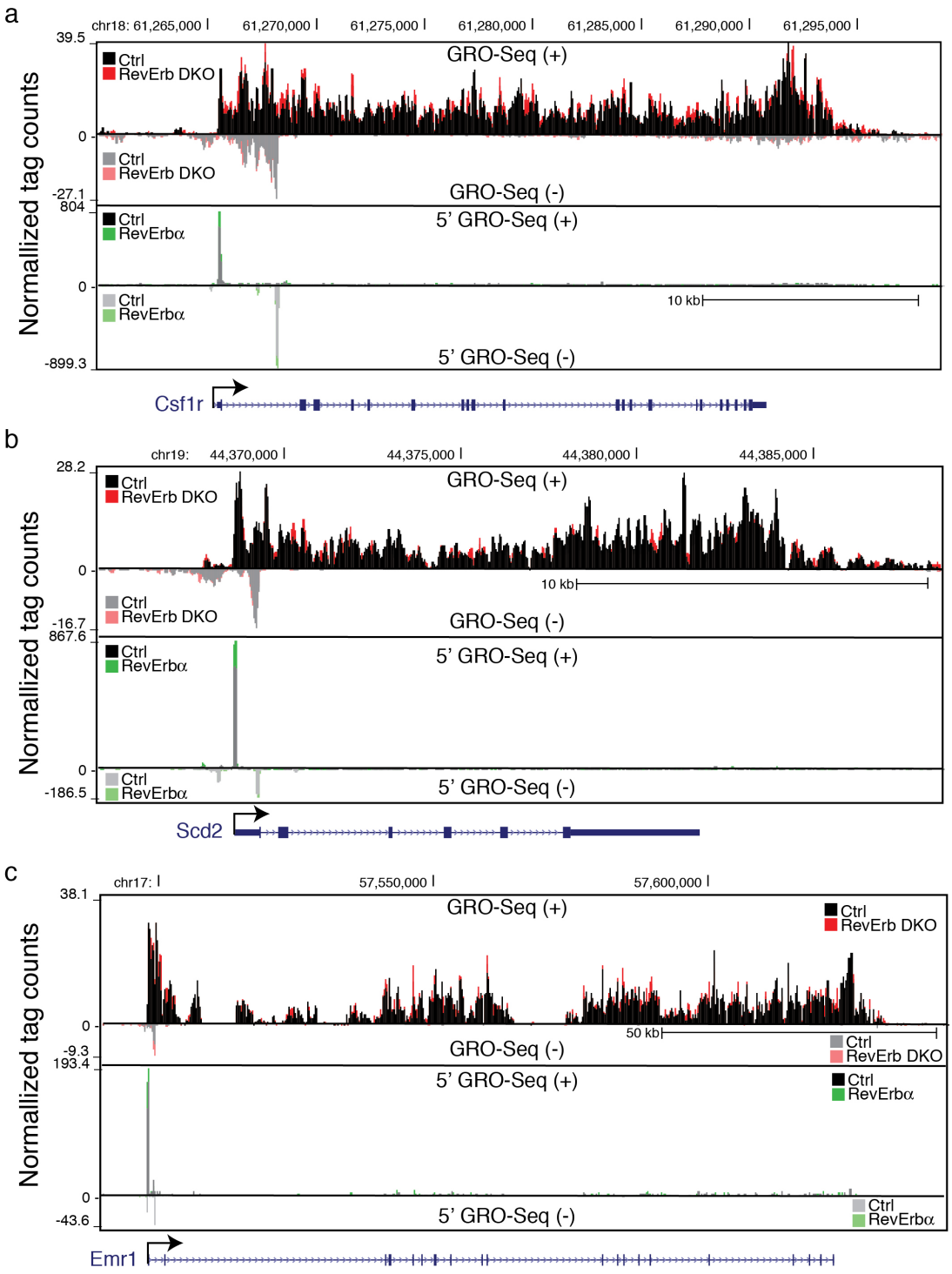
Supplementary Figure 4. Repression of *Mmp9* and *Cx3cr1* are inversely correlated to the expression levels of exogenous Rev-Erb. a,b, Q-PCR analysis of *Mmp9* and *Cx3cr1* mRNA in RAW264.7 macrophages engineered to stably express BLRP-Rev-Erbβ (N_{ctrl} = 13, N_{beta} = 18 independent lines). Data represent mean ± s.e.m. Statistical significance was determined by two tail Student's t-test. P-value **, $P < 0.01$ versus control. c-f, Q-PCR analysis of *Mmp9* (a-b) and *Cx3cr1* (c-d) mRNA expression in independent RAW264.7 macrophages cell lines expressing Rev-Erbα (a, c, n = 17), Rev-Erbβ (b, d, n = 18) (red) or empty expression vector (black, n = 16 for Rev-Erbα experiment, n = 13 for Rev-Erbβ experiment). Expressions of Rev-Erbs were plotted on x-axis; *Mmp9* or *Cx3cr1*, y-axis. Statistic significances for correlation were determined by Spearman rank correlation test.



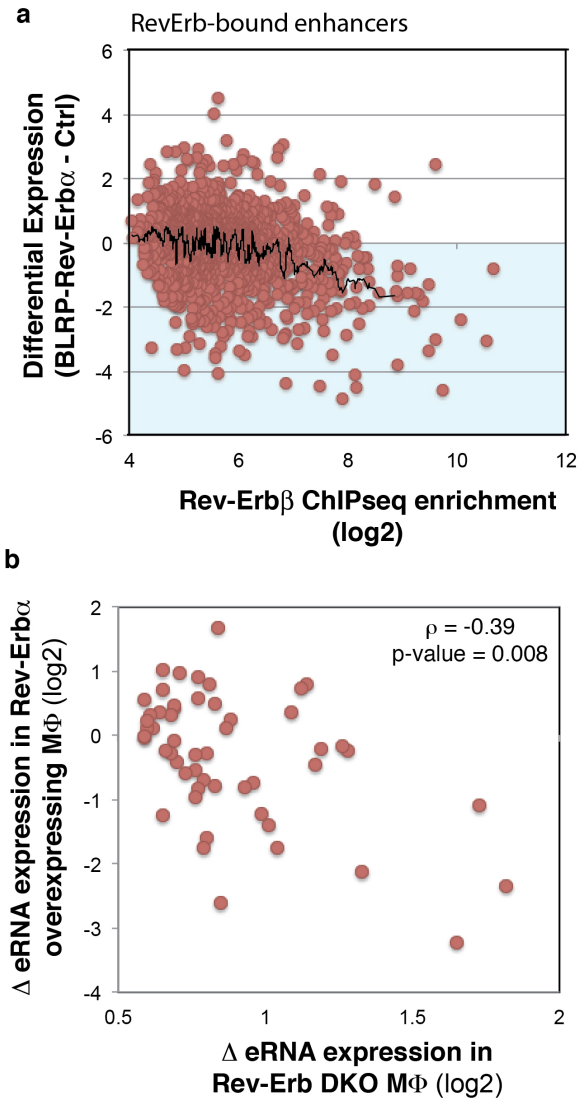
Supplementary Figure 5. Cell-specific function of the *Mmp9* -5kb enhancer. Top, Enhancer reporter assay schematic with Mmp9 -5kb enhancer element or a random 1kb genomic region cloned downstream of the *Mmp9* promoter-luciferase reporter. Luciferase activity of reporters was determined following transfection into RAW264.7 macrophages or Hepa1-6 cells. Data represent mean $\pm$ s.d. from 3 independent experiments. Statistical significance was determined by student's t-test P-value §, $P < 0.005$ versus.



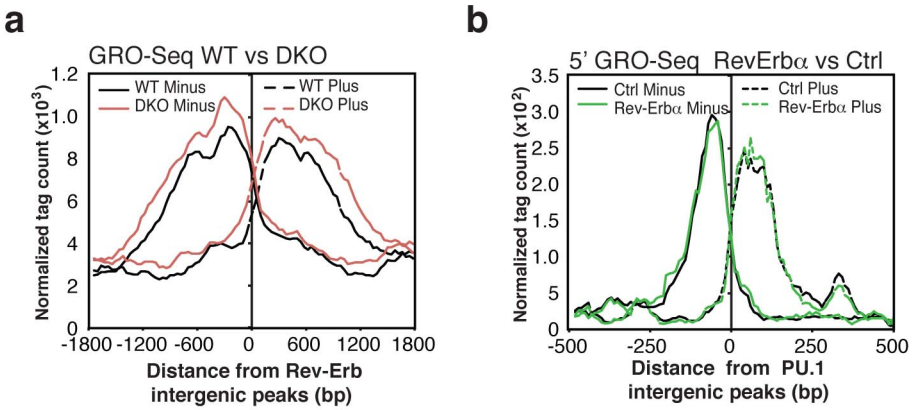
Supplementary Figure 6. Rev-Erb bound enhancer elements are sufficient for Rev-Erb mediated repression in enhancer reporter assay.
Enhancer reporter assay schematic with Rev-Erb bound enhancer element cloned downstream of the luciferase reporter. Enhancer reporters were co-transfected with expression vectors for RORα, Rev-Erbβ, or Rev-Erbβ with mutation in the DNA binding domain (DBDmut) as indicated. Luciferase activity for reporters containing indicated genomic regions are shown. *BmalI* promoter was used as positive control. A 1kb genomic region without enhancer-like element (black) was used as negative control. Data represent mean ± s.d. from at least 3 independent experiments. Statistical significance was determined by one-way ANOVA followed by Tukey HSD test. P-value §, $P < 0.005$ versus reactions with RORα transfection only and RORα co-transfected with Rev-Erbβ DBDmut.



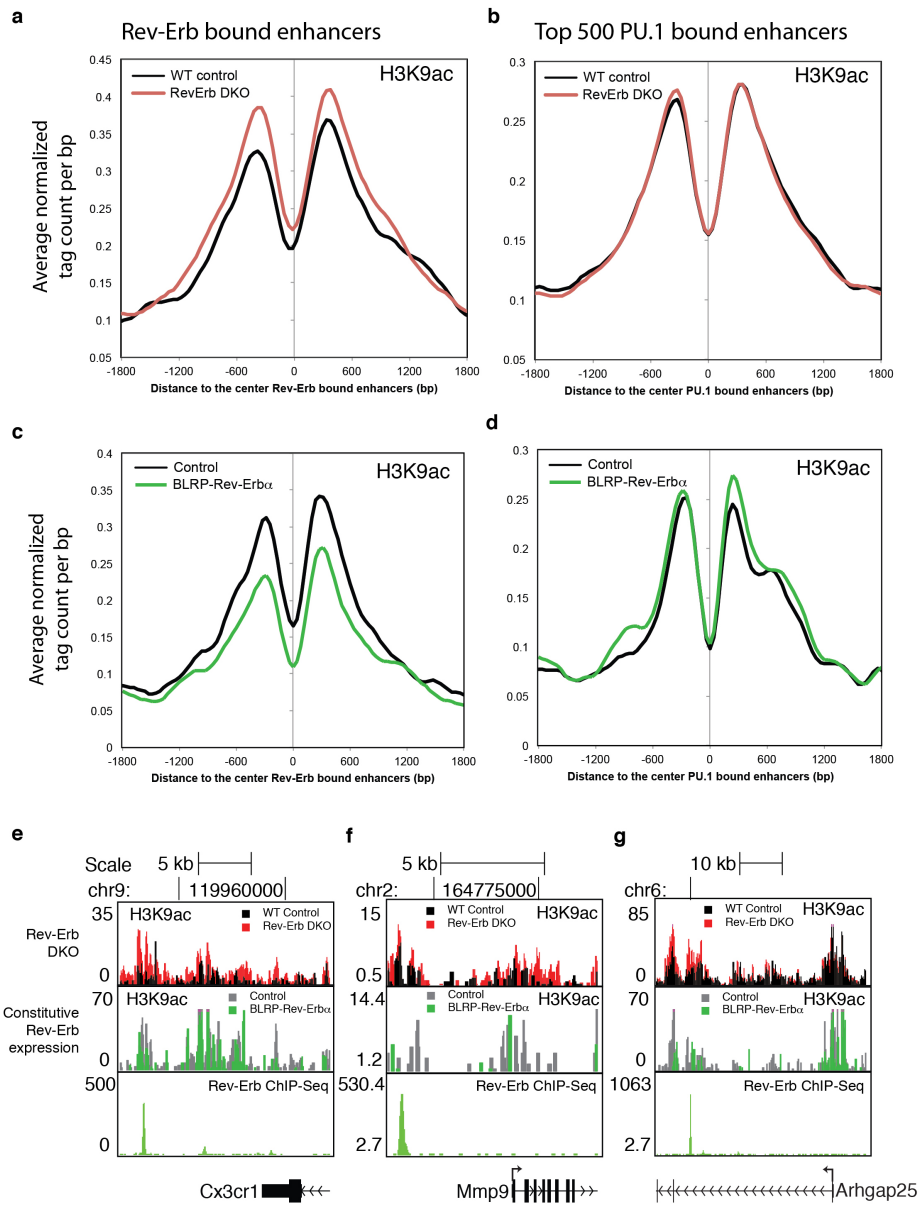
Supplementary Figure 7. Validation of 5'GRO-Seq assay. Genome browser images comparing 5'GRO-Seq and GRO-Seq signal in BirA control RAW264.7 cells (Ctrl) and BLRP-Rev-Erbα expressing RAW264.7 cells (Rev-Erbα) are illustrated for a, *Csf1r*, b, *Scd2* and c, *Emr1* (F4/80).



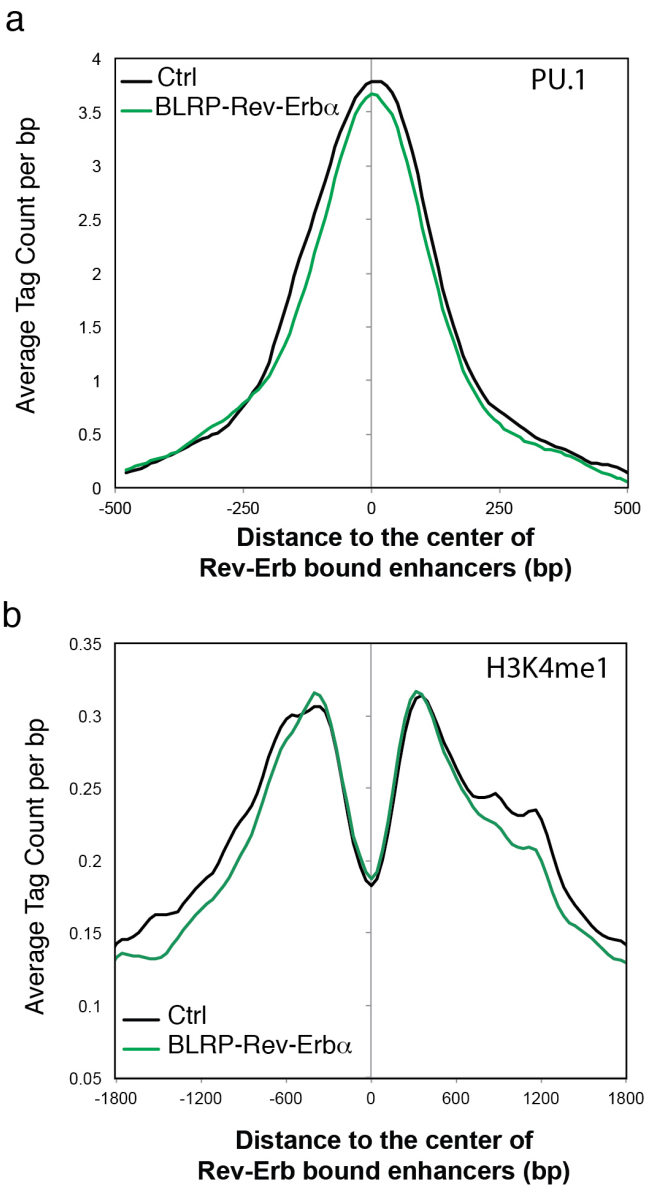
Supplementary Figure 8. RevErb binding and eRNA expression. Relationship of local eRNA expression as defined by 5'GRO-Seq signal to RevErbβ ChIP-Seq enrichment. The change in 5'GRO-Seq signal comparing BirA control to RevErbβ overexpression at a RevErb binding site is plotted as a function of RevErb binding strength at that site (log2 normalized tag count). b, Rev-Erb bound enhancers with eRNA de-repression in Rev-Erb deficient macrophages have inversely correlated eRNA repression upon constitutive expression of Rev-Erbα. Fifty-three Rev-Erb bound enhancers with de-repressed GRO-Seq signal in Rev-Erb DKO macrophages (x-axis) are plotted against changes of 5'GRO-Seq signal upon overexpression of Rev-Erbα in RAW264.7 macrophages (y-axis). Changes in eRNA were determined by the $\log_2$ difference of expression between DKO and wild type macrophages, or between Rev-Erbα overexpressing and control macrophages. Statistical significance for correlation was determined by Spearmen rank correlation test.



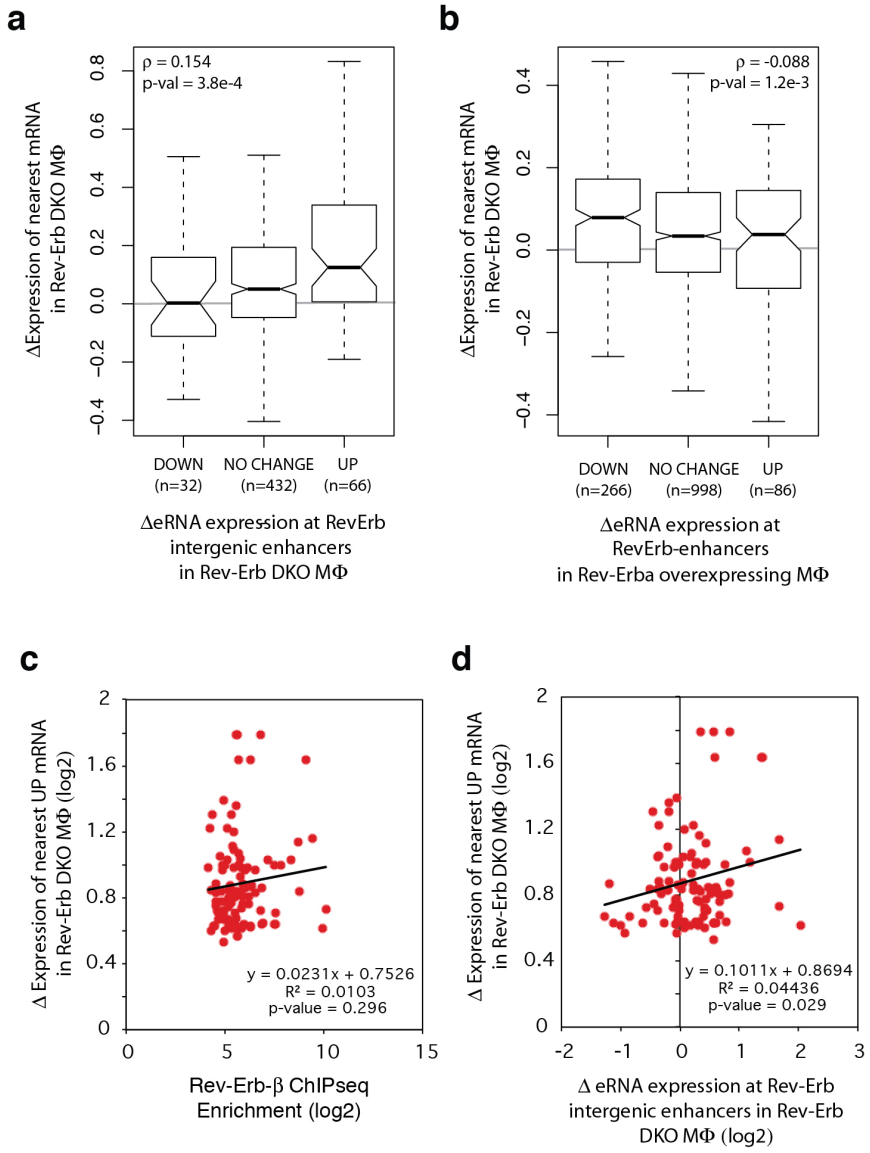
Supplementary Figure 9. Effect of RevErb loss and gain of function on eRNA expression is specific for Rev-Erb bound enhancers. **a**, Distribution of averaged GRO-Seq eRNA signal from Rev-Erb DKO (red) and control (black) BMDM at the top 100 Rev-Erb bound intergenic sites. **b**, Distribution of average 5'GRO-Seq signal from Rev-Erbα overexpressing (green) and control RAW264.7 macrophages (black) flanking the top 100 PU.1-bound intergenic sites.



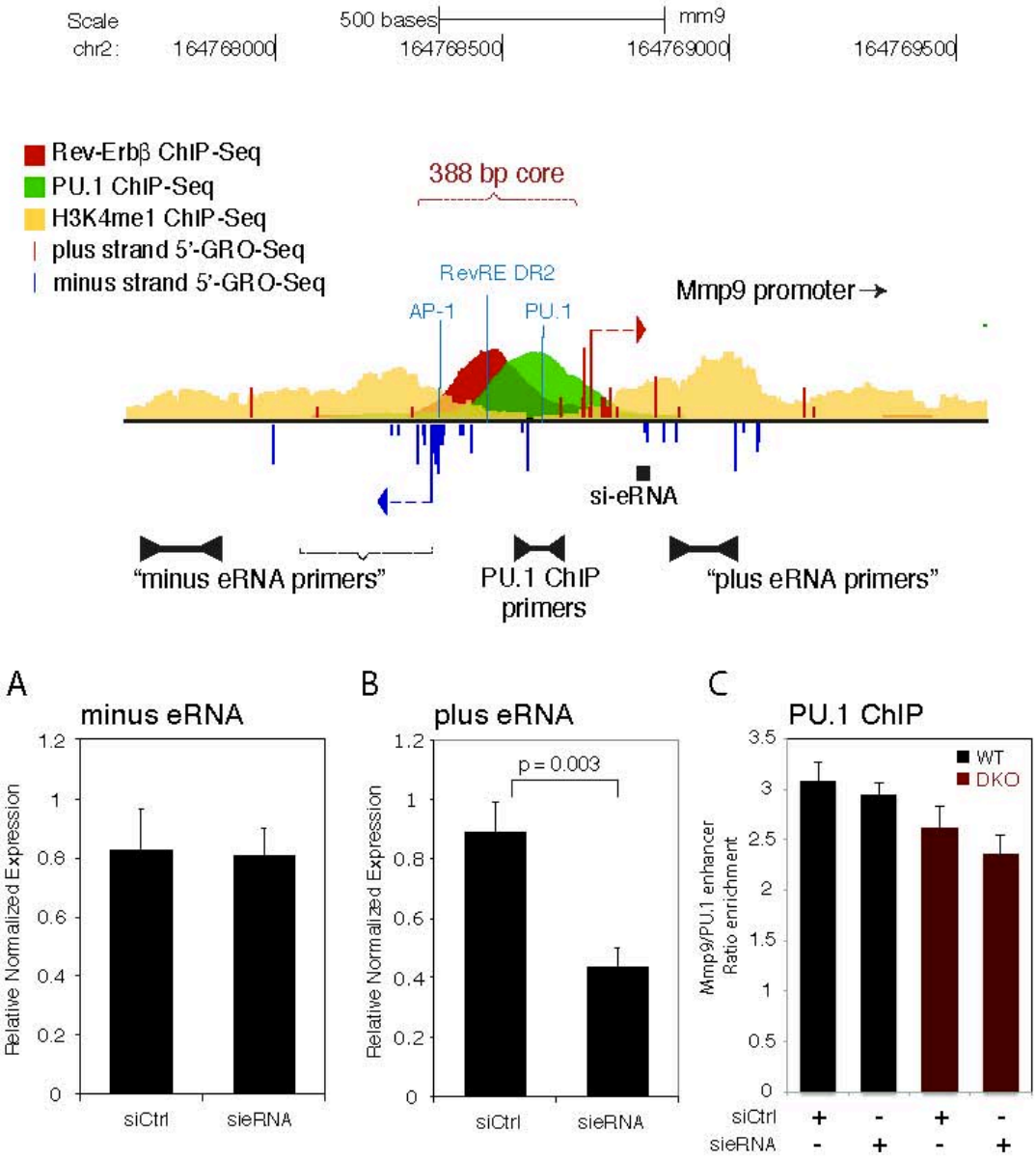
Supplementary Figure 10. Rev-Erb alters acetylation of H3K9 at Rev-Erb bounded enhancers. a-b, Average distribution of H3K9ac ChIP-Seq signal flanking (a) 53 Rev-Erb bound enhancers which have de-repressed GRO-Seq signal or (b) the top 500 enriched PU.1 bound enhancers for WT (black) and Rev-Erb DKO (red) macrophages. c-d, Average distribution of H3K9ac ChIP-Seq signal in control (black) and Rev-Erbα overexpressing (green) RAW264.7 macrophages flanking (c) 266 Rev-Erb bound enhancer with repressed 5'GRO-Seq signal or (d) the top 500 enriched PU.1 bound enhancers. e-g, Changes of H3K9ac ChIP-Seq signal at Rev-Erb bound genomic loci for (e) *Cx3cr1*, (f) *Mmp9* and (g) *Arhgap25*. Panels indicate H3K9ac ChIP-Seq in Rev-Erb DKO BMDM (top) and Rev-Erbα overexpressing RAW264.7 macrophages (middle). The bottom panels indicate ChIP-Seq signal for Rev-Erbβ.



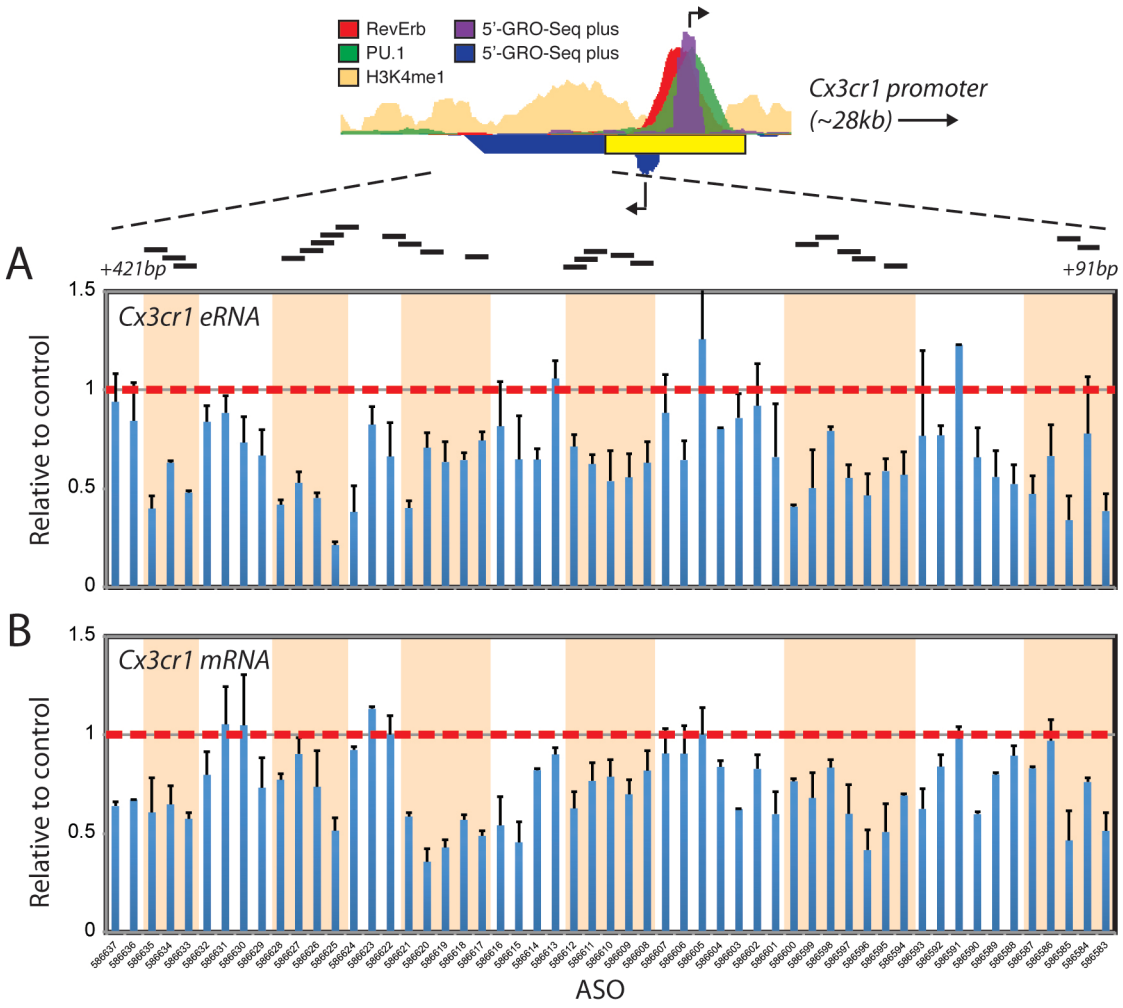
Supplementary Figure 11. Overexpression of Rev-Erbα does not change PU.1 or H3K4me1 enrichment at Rev-Erb bound enhancers with repressed eRNA expression.
a-b, Average distribution of (a) PU.1 and (b) H3K4me1 ChIP-Seq signal flanking the 266 Rev-Erb bound enhancers with repressed 5'GRO-Seq signal upon overexpression of Rev-Erbα. ChIP-Seq signal for control are indicated in black; for Rev-Erbα overexpressing RAW264.7 macrophages, in green.



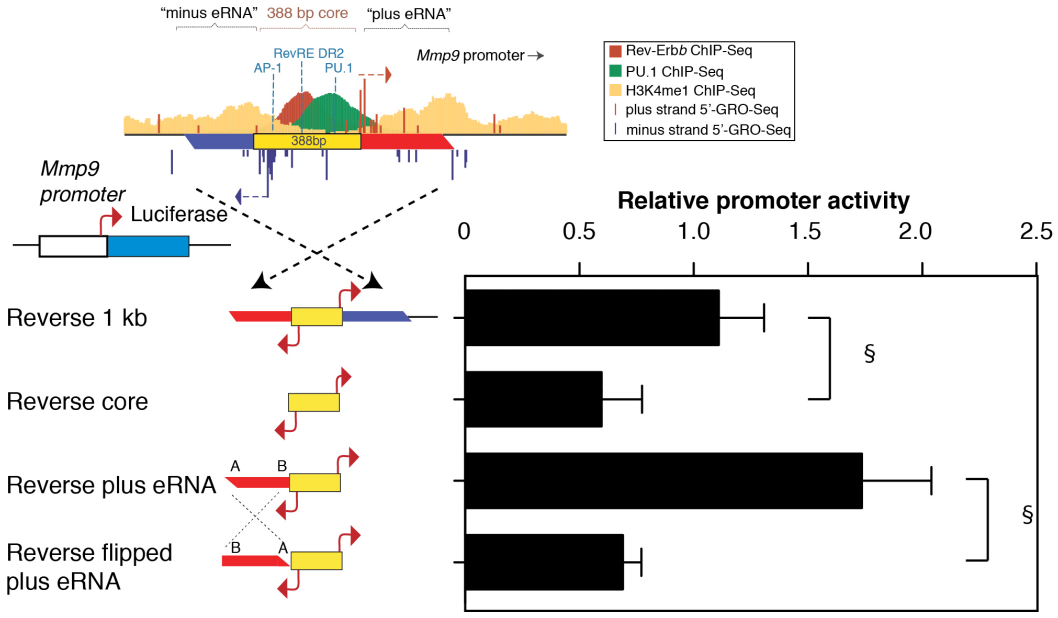
Supplementary Figure 12. Differential eRNA expression is correlated to differential expression of the protein coding genes nearest to Rev-Erb bound enhancers.
a-b, Box-and-whiskers plot for differential expression of the protein-coding genes between Rev-Erb DKO and control BMDM to the nearest Rev-Erb bound enhancers that have decreased, unaffected, or increased eRNA expression in (a) Rev-Erb DKO BMDM or (b) Rev-Erbα overexpressing RAW264.7 macrophages. The edges of the box represent first and third quartiles, and the whiskers represent 1.5X of the interquartile range. c-d, Correlation plots of up-regulated protein coding genes in Rev-Erb DKO macrophages to (c) Rev-Erb binding enrichment or (d) eRNA expression at Rev-Erb intergenic enhancers. Each point represents a Rev-Erb intergenic enhancer located within 1Mb of a nearest Rev-Erb regulated gene. Changes in protein coding genes or eRNA expression are defined by log2 difference of GRO-Seq signal between DKO and wild type macrophages. Statistical significances for correlation were determined using (a,b) Spearman rank correlation or (c,d) regression correlation.



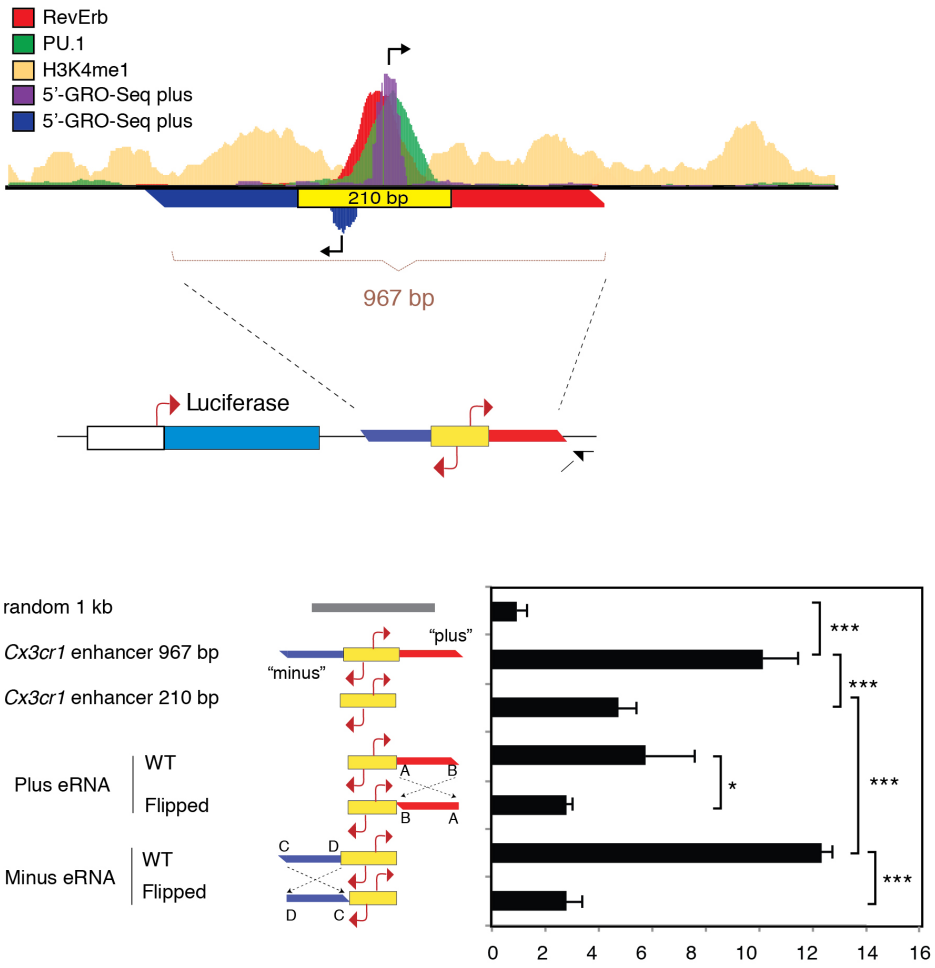
Supplementary Figure 13. Lack of effect of siRNA against *Mmp9* -5 kb enhancer plus strand eRNA on expression of the minus strand eRNA or PU.1 recruitment. The top figure illustrates the siRNA targeting *Mmp9* -5kb eRNA (sieRNA) and the qPCR primers. Thioglycollate-elicited macrophages were transfected with control or sieRNA. Total RNA was collected 48-hour post-transfection for measurement of the (a) minus and (b) plus strand eRNAs using QT-PCR (n = 6-7 independent samples). c. PU.1 ChIP recruitment at Mmp9 enhancer in WT (black) or DKO (red) macrophages transfected with control or sieRNA. Statistical significance was tested by two-tails student's t-test.



Supplementary Figure 14. ASO screen for targeting *Cx3cr1* minus strand eRNA. Top figure shows the schematic of *Cx3cr1* 28kb enhancers with indicated ChIP-seq or 5'GRO-Seq signals. Relative positions of ASOs are indicated with black lines. RAW macrophages were transfected with tiling ASOs from 91-411bp downstream of the enhancer transcription start sites (TSS). The changes in (A) Cx3cr1 eRNA and (B) mRNA are as indicated relative to the average of 3 independent ASO. Regions with higher propensity to ASO knockdown are highlighted. qPCR for each reaction was performed in technical duplicates with error bar representing SD.



Supplementary Figure 15. Functional analysis of the Mmp9 enhancer in the reverse orientation. The top part of the figure illustrates that experimental design for testing the *Mmp9* enhancer in the inverse orientation. 983 bp of the *Mmp9* enhancer was cloned downstream of the luciferase reporter gene driven by the *Mmp9* promoter in the opposite orientation from that used in experiments presented in Figure 4. The yellow box represents the 388 bp core mediating transcription factor binding as indicated by the ChIP-Seq tracks. Transcription factor motifs are indicated in blue. 'Plus eRNA' refers to the eRNA directed by the enhancer portion proximal to the *Mmp9* promoter at the endogenous locus; 'minus eRNA', distal. Directional arrows represent eRNA transcription start sites defined by 5'GRO-Seq. Enhancer reporter constructs driven by *Mmp9* promoter containing the indicated DNA fragments were transfected into RAW264.7 macrophages. Bars represent mean normalized values from 5 independent experiments $\pm$ s.d, (§ $P < 0.005$ versus all other indicated conditions).



Supplementary Figure 16. Functional analysis of the *Cx3cr1* 28 kb enhancer. Top, Experimental design for testing eRNA coding sequence in the *Cx3cr1* enhancer. 967 bp of the *Cx3cr1* enhancer was cloned downstream of the luciferase reporter gene driven by the *Cx3cr1* promoter. The yellow box represents the 210 bp core mediating transcription factor binding as indicated by the ChIP-Seq tracks. 'Plus eRNA' refers to the eRNA directed by the enhancer portion proximal to the *Cx3cr1* promoter at the endogenous locus; 'minus eRNA', distal. Directional arrows represent eRNA transcription start sites defined by 5'GRO-Seq. Bottom, Luciferase activities of enhancer reporter constructs driven by the *Cx3cr1* promoter containing the indicated DNA fragments transfected in RAW264.7 macrophages. Dashed lines represent inversions of the plus or minus DNA sequence relative to the core enhancer element (yellow). Bars represent mean values from one of four experiments ± s.d, (*** $P < 0.005$, versus indicated conditions).

References for Supplementary Figures

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[2] Lin, Y.C. *et al.*, A global network of transcription factors, involving E2A, EBF1 and Foxo1, that orchestrates B cell fate. Nature Immunology 11 (7), 635 (2010).

[3] Creyghton, M.P. *et al.*, Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc Natl Acad Sci USA 107 (50), 21931-21936 (2010).

[4] Meissner, A. *et al.*, Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature 454 (7205), 766-770 (2008).