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Supplemental Information

**The glucocorticoid receptor
recruits the COMPASS complex to regulate
inflammatory transcription at macrophage enhancers**

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Supplemental figures, legends and tables, Greulich F. *et al.*

Supplemental Figure 1 - GR interactions with the SETD1A/COMPASS complex. (Relates to figure 1)

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Supplemental Figure 4 - Locus-specific changes in H3K4 me1, me2 and me3 at GBSs with SETD1A recruitment, and correlations with H3K27ac and mRNA expression. (Relates to figure 4)

Supplemental Figure 5 - H3K4 methylation dynamics upon SETD1A depletion. (Relates to figure 5)

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Supplemental Figure 7 - Regulation of GR targets in the absence of TLR4 signaling (Relates to figure 7)

Supplemental Table 1 – ChIP-MS data of proteins significantly enriched in α -GR IPs in macrophages (LPS+Dex). (Relates to figure 1)

Supplemental Table 2 – Differential expression of genes in macrophages, in wild type and in *Setd1a*^{Del/+} RAW264.7 cells, under various conditions (\pm LPS, \pm Dex). (Relates to figures 1, 6, 7)

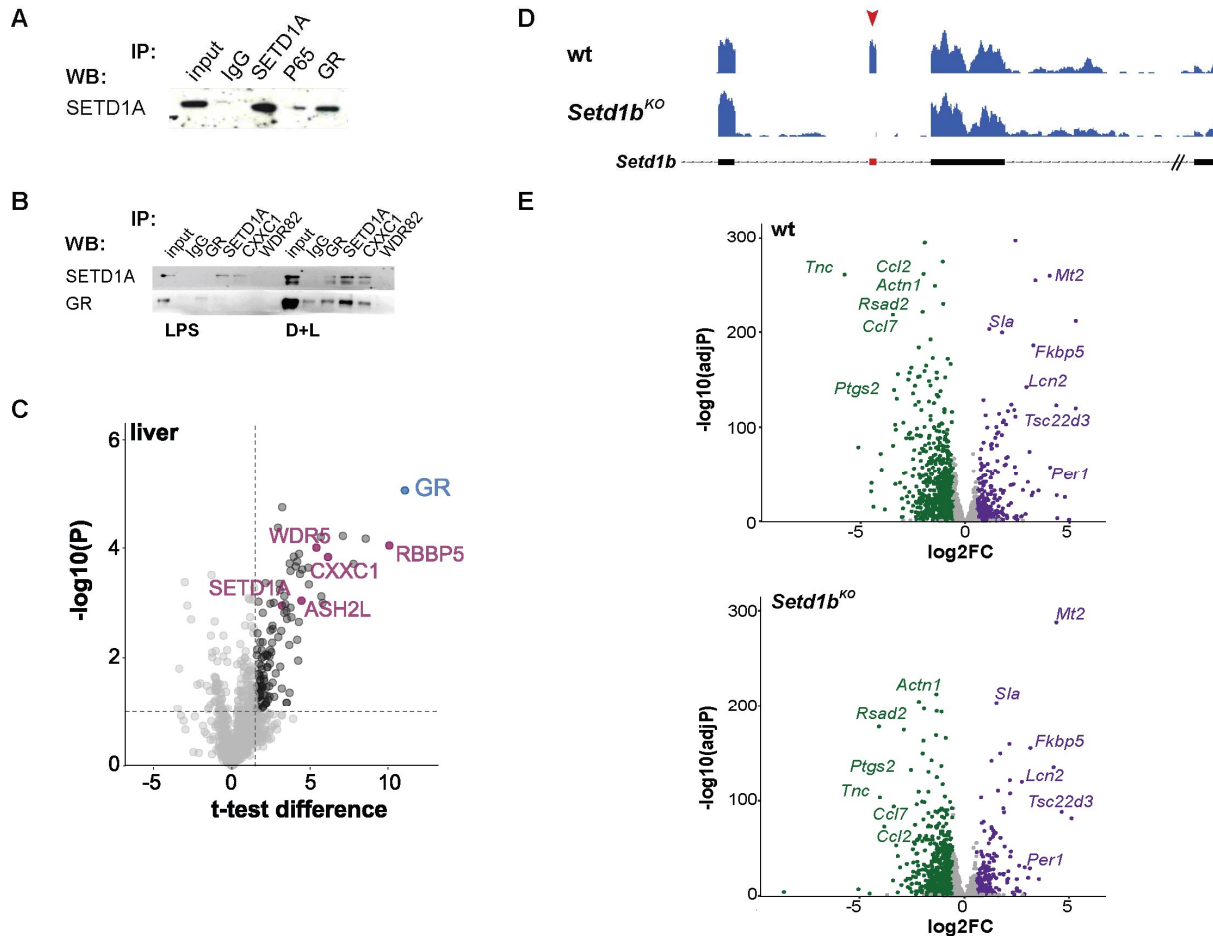
Supplemental Table 3 – GR ChIP peaks in macrophages and RAW264.7 cells (Relates to figures 3, 4, 5)

Supplemental Table 4 – Gene Ontology of various gene sets (Relates to figures 3, 6, 7)

Supplemental Table 5 – List of ChIP-Seq samples, including mapping statistics and scale factors. Scaling factors applied in this study are marked in red. (Relates to STAR methods)

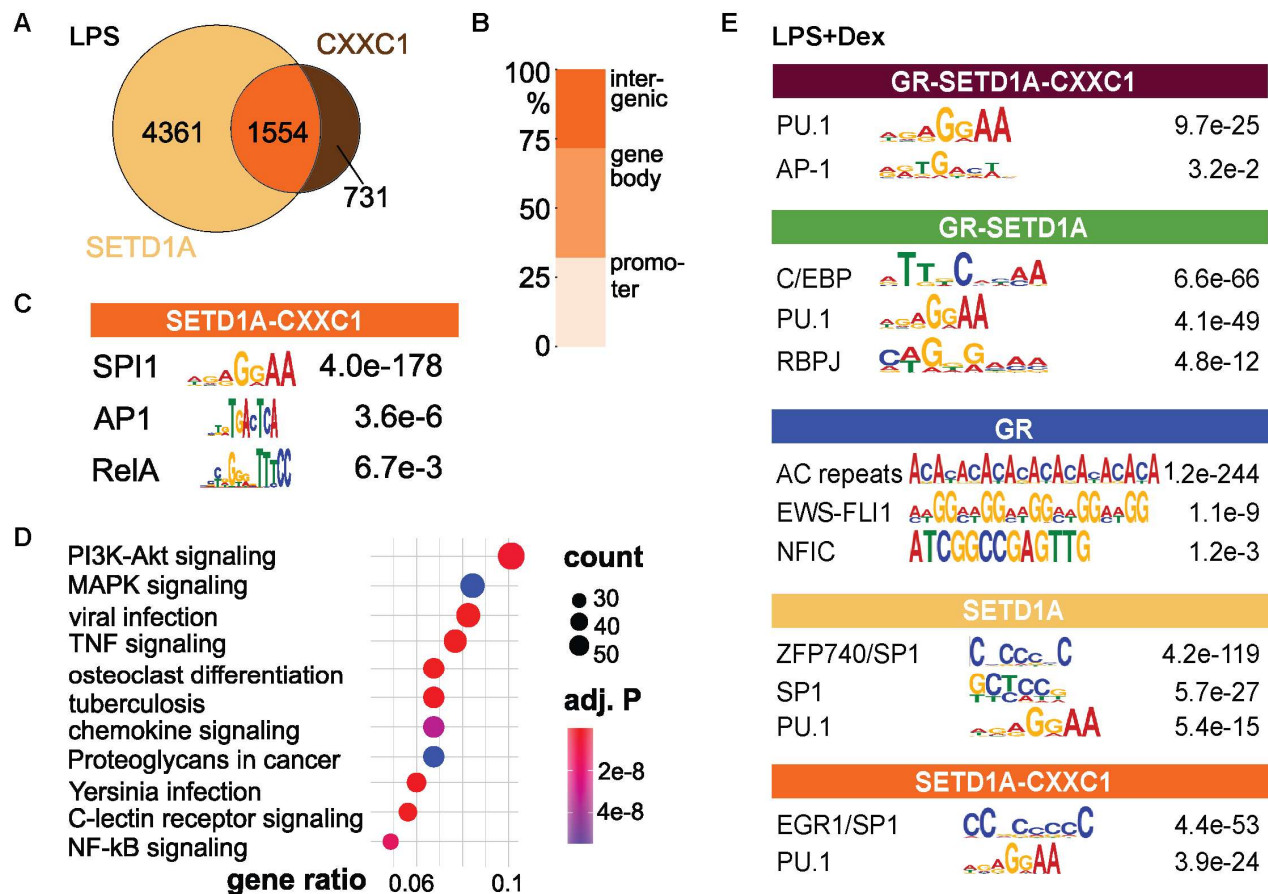
Supplemental Table 6 – List of oligos used for ChIP and mRNA qPCR as well as CRISPR cloning. (Relates to STAR methods)

Supplemental figures and legends



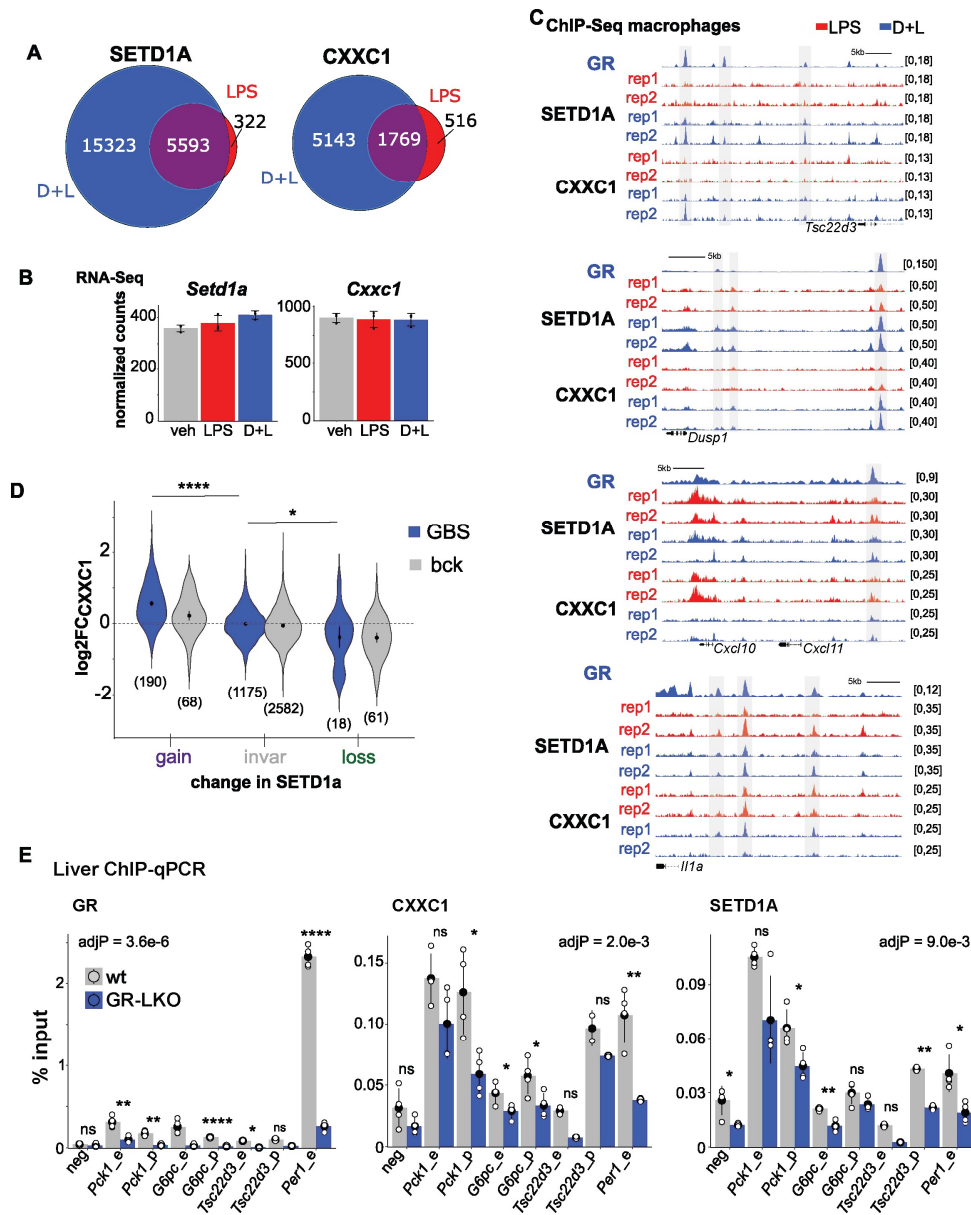
Supplemental Figure 1. GR interactions with the SETD1A/COMPASS complex.

This figure relates to figure 1. **(A)** Co-IP of SETD1A with GR and p65 in RAW264.7 cells. WB - Western blot, IP - immunoprecipitation **(B)** Co-IPs of SETD1A, CXXC1 and GR in nuclear extracts from RAW264.7 cells treated either with LPS only or with Dex+LPS (D+L). **(C)** ChIP-MS for GR in murine liver. COMPASS proteins present in the hepatic interactome are colored in purple (Hemmer et al., 2019) **(D)** Genome browser tracks of *Setd1b* mRNA in wild type and *Setd1b* KO macrophages after Dex and LPS treatment. Gray shadow indicates the deletion of exon 5 (orange) in *Setd1b* conditional cells as described in Bledau 2014. **(E)** Volcano plots of RNA-Seq results from wild type and *Setd1b* knockout BMDMs. Displayed is the negative log₁₀-transformed Benjamini-Hochberg adjusted p-value (-log₁₀(adjP)) over the log₂FoldChange (log₂FC). Green – reduced expression. Purple – increased expression. Selected classical GR target genes are labeled.



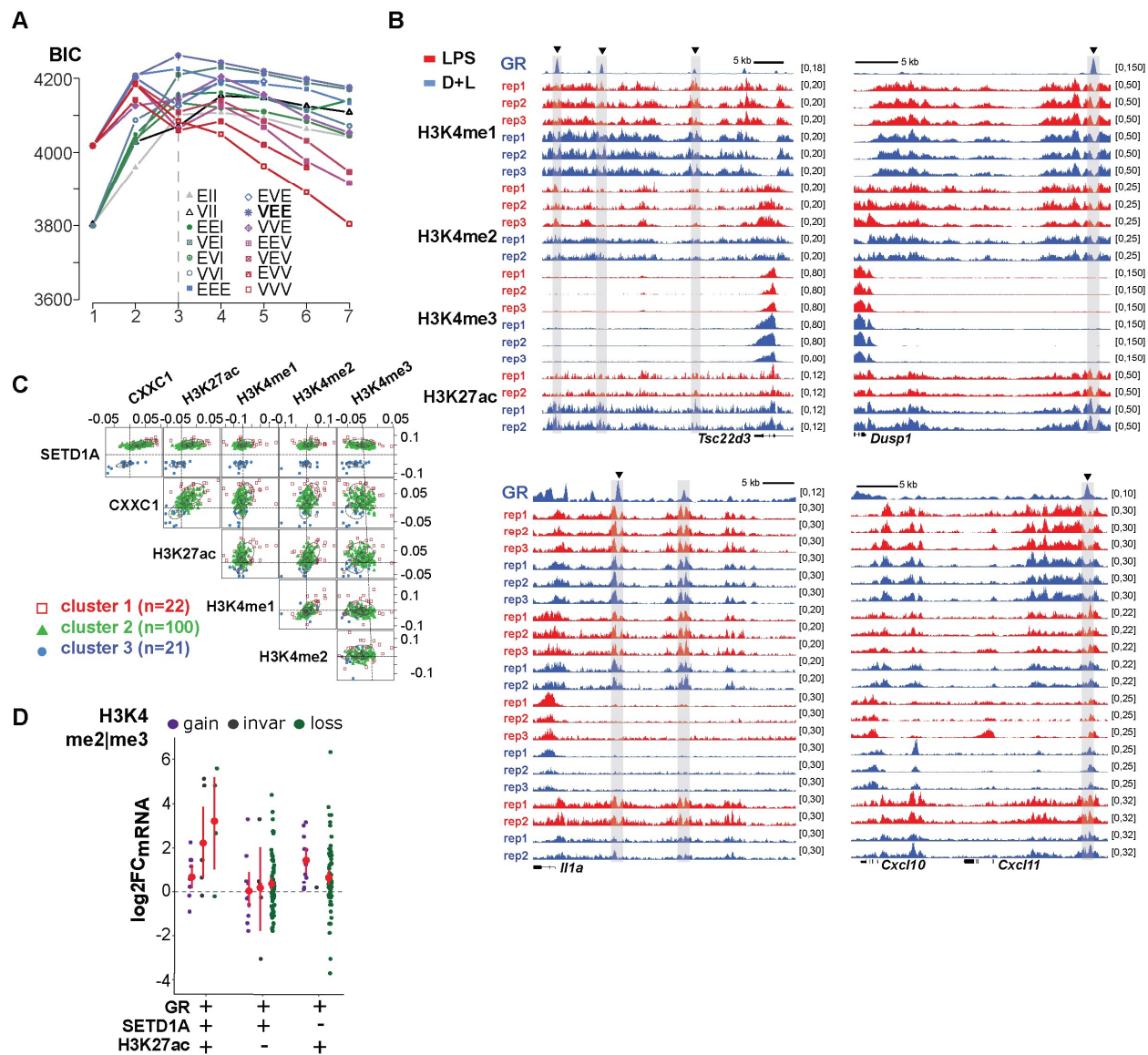
Supplemental Figure 2. SETD1A/COMPASS cistromes in primary macrophages.

This figure relates to figure 2. **(A)** Overlap of SETD1A and CXXC1 binding sites in LPS treated BMDMs. Numbers are called peaks. **(B)** Genomic feature distribution (%) of SETD1A-CXXC1 co-occupied regions in LPS treated BMDMs. **(C)** MEME motif enrichment for the SETD1A-CXXC1 overlap. Consensus sequence and E-values are displayed **(D)** KEGG pathway enrichment for the SETD1A/CXXC1 peak overlap in LPS- stimulated BMDMs (1554 peaks mapped to the nearest TSSs). **(E)** MEME motif enrichment for indicated peak subsets, as defined in **Fig. 2A**. The union of GR, SETD1A and CXXC1 peaks was used as background. Displayed are the consensus sequences and E-values. Motifs already displayed in Fig. 2A are not shown here.



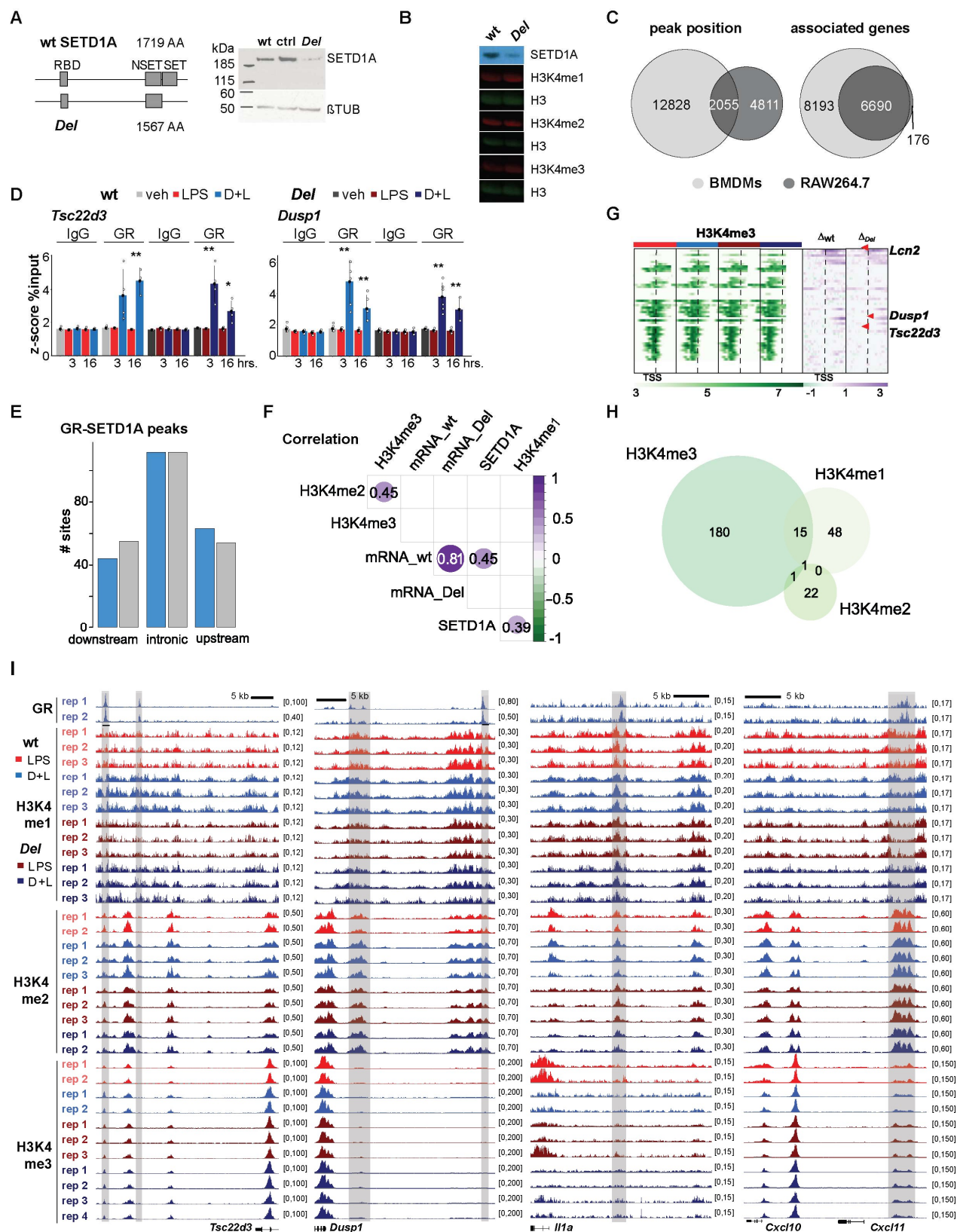
Supplemental Figure 3. GR recruits SETD1A/COMPASS to its enhancers in murine macrophages and livers.

This figure relates to figure 3. **(A)** Venn diagram illustrating the SETD1A and CXXC1 peak union overlap upon Dex+LPS (D+L) and LPS treatment in BMDMs. **(B)** *Setd1a* and *Cxxc1* expression in vehicle (veh), LPS or Dex+LPS (D+L) treated BMDMs, as measured by RNA-Seq. Mean DESeq2-normalized counts from three replicates are shown. Single dots are individual data points. Error bars reflect the standard deviation. **(C)** Example genome browser tracks of ChIP-Seq for GR, SETD1A and CXXC1 in macrophages treated either with LPS (red) or with Dex+LPS (blue). Grey boxes highlight GR-bound enhancers. **(D)** Log2FoldChange (FC) of CXXC1 occupancy at GR-bound sites (blue) or non-GR-bound (grey) intergenic regions (+/-1kb from any gene) summarized as Violin plot. Loci are categorized by gained ($p < 0.1$, $FC > 1.5$), lost ($p < 0.1$, $FC < -1.5$) or invariant ($p > 0.1$, $-1.5 < FC < 1.5$) SETD1A binding upon Dex+LPS treatment compared to LPS alone. Dunn's test shows the significance of CXXC1 dynamics between different SETD1A categories. * $p < 0.05$; **** $p < 0.0001$. Numbers in parentheses are subset sizes. **(E)** ChIP qPCR for GR, CXXC1 and SETD1A in murine livers from wild type (wt) and hepatocyte-specific GR knockout (GR-LKO) mice (pairwise Wilcoxon-Mann-Whitney test, adjP – Benjamini-Hochberg adjusted p-value). Pair-wise comparisons of individual enhancer by two-sided Student's t-test ($n = 4$). ns – non-significant, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.



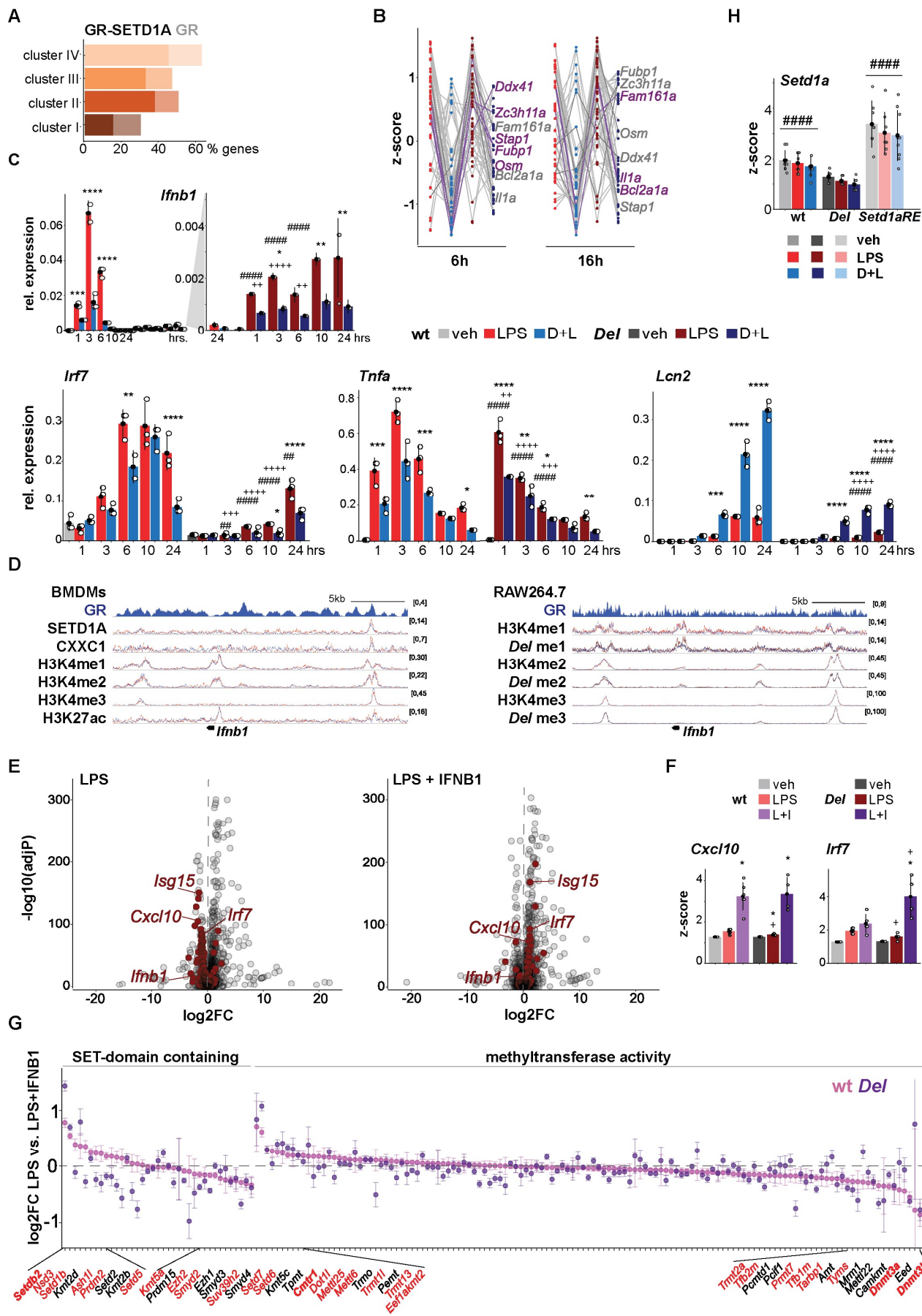
Supplemental Figure 4. Locus-specific changes in H3K4 me1, me2 and me3 at GBSs with SETD1A recruitment, and correlations with H3K27ac and mRNA expression.

This figure relates to figure 4. **(A)** Bayesian Information Criterion (BIC) plot for classification of intergenic GR-bound enhancers with differential SETD1A occupancy ($|FC| > 1.5$, $p < 0.1$) in Fig. S4B. The best model used for enhancer classification is indicated by the dashed line and bold text. **(B)** Representative ChIP-Seq genome browser tracks for LPS- and Dex+LPS- stimulated BMDMs. Individual replicates are shown, corresponding to the mean representation in the main figure. **(C)** Scatter plots of scaled SETD1A, CXXC1, H3K27ac and H3K4me1/me2/me3 log2FoldChanges (FC) at GBSs with significantly changed SETD1A occupancy ($p < 0.1$, $-1.5 > FC > 1.5$) upon Dex and LPS treatment. Colors reflect clusters identified by "mclust". The dashed line represents axis centers and circles mark the uncertainty border **(D)** Scatter plot showing log2FC in mRNA expression of indicated groups, separated according to their change in H3K4me2 or me3. Red dots indicate the mean and the red bars the 95% confidence intervals. + gain; - invariant or lost.



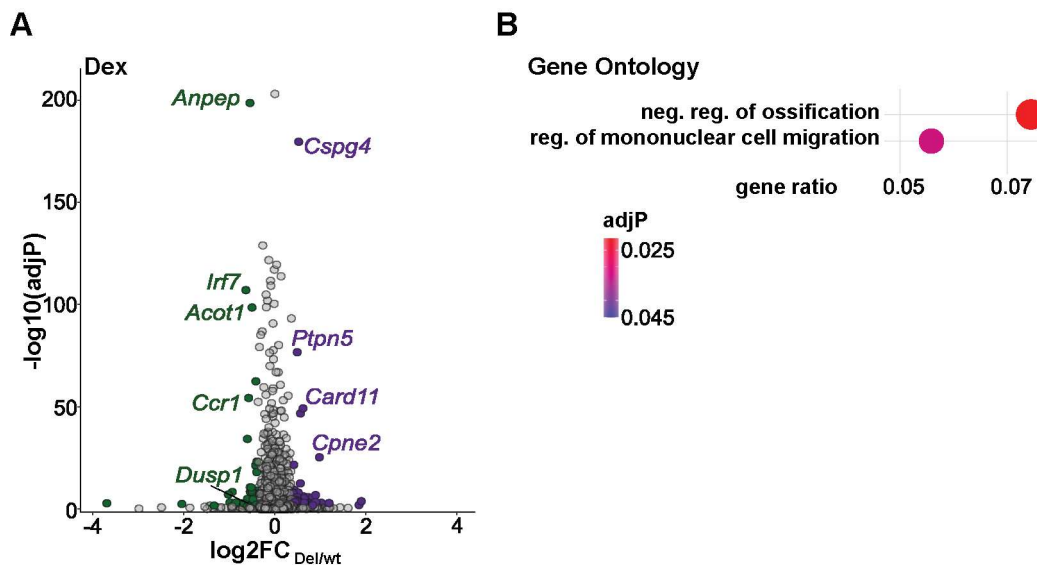
Supplemental Figure 5. H3K4 methylation dynamics upon SETD1A depletion.

This figure relates to figure 5. **(A)** *Setd1a*^{Del/+} (*Del*) RAW264.7 cells, carrying a deletion of the SET domain, show reduced SETD1A expression by Western Blot. Loading control: beta tubulin (β TUB); RBD: RNA-binding domain. **(B)** Western blot for SETD1A, H3K4me1, H3K4me2, H3K4me3 and total H3 in wild type (wt) and *Setd1a*^{Del/+} (*Del*) RAW264.7 cells. **(C)** Venn diagram comparing GR ChIP-Seq peaks and associated genes in RAW264.7 cells (dark) and primary macrophages (lighter shade) after Dex+LPS treatment. **(D)** ChIP qPCR against IgG and GR for the *Tsc22d3* and *Dusp1* enhancers (indicated by the black line in I) after vehicle (veh), LPS or Dex+LPS (D+L) treatment. Dex treatment time as indicated. (n=4, Kruskal-Wallis with post-hoc Dunn's test. P values are Benjamini-Hochberg adjusted. * p<0.05; ** p<0.01. Bar plots display the mean z-score of % input and error bars show the standard deviation. **(E)** Genomic localization of GR-SETD1A overlapping sites shown in figure 5. **(F)** Correlation plot of Dex-induced log2FCs for SETD1A, H3K4me1/me2/me3 and mRNA in wild type RAW264.7, as well as mRNA changes in *Setd1a*^{Del/+} (*Del*) cells for the GR subset with significantly gained SETD1A occupancy (p<0.1, FC>1.5-fold). **(G)** Spike-in normalized plus differential heatmaps (Δ compares Dex+LPS over LPS) for H3K4me3 ChIP-Seq signals at \pm 2kb around TSSs in wild type (wt) and *Setd1a*^{Del/+} (*Del*) RAW264.7 cells treated with LPS or Dex+LPS. The mean of 2-4 replicates is displayed. TSSs are sorted by descending order of H3K4me3 fold-changes in response to Dex in wild type cells. Arrows point at loci of interest. **(H)** Venn Diagram of the overlap of GR-SETD1A co-occupied intergenic regions with >1.5-fold difference in H3K4 me1, me2 or me3 (p<0.05) in Dex+LPS treated wild type RAW264.7 cells when compared to LPS-treated cells. **(I)** Example genome browser tracks of normalized ChIP-Seq signal for GR and H3K4me1/me2/me3 in RAW264.7 cells. Gray boxes mark GR peaks. Black lines indicate the position of the qPCR primers.



Supplemental Figure 6. *Setd1a* is required for GR-mediated inflammatory gene regulation.

This figure relates to figure 6. (A) Percent of Dex- and *Setd1a*-dependent genes associated with a GR peak, clustered as in Fig. 6A. (B) Slope graph showing the gene expression patterns from cluster III (Fig. 6A). Each gene is shown by one line and one dot per treatment. Purple: genes with lost Dex-dependent repression in *Del* RAW264.7 cells after 6h or 16h treatment. (C) Time series qRT-PCR. (n=3, ANOVA with post-hoc pairwise t-test, Benjamini-Hochberg adjusted; * treatment effect Dex+LPS over LPS; # genotype effect compared to wild type cells treated with LPS; + genotype effect compared to wild type cells (Dex+LPS treatment)). (D) Genome browser tracks with ChIP-Seq data for the *Ifnb1* locus from primary macrophages and RAW264.7 cells treated with LPS or Dex+LPS, as described. (E) Volcano plots of transcript changes in *Setd1a*^{Del/+} versus wild type RAW264.7 cells after LPS and LPS+IFN β 1 stimulation. The negative log-transformed Benjamini-Hochberg adjusted p-value (adjP) is plotted over the log2FC (fold change). Brown dots represent genes from cluster I (Fig. 6A). Four selected genes are labeled. (F) qRT-PCR of wild type and *Del* cells stimulated with vehicle (veh), LPS or LPS plus IFN β 1 (I+L). (n=6, Kruskal-Wallis with post-hoc pairwise Wilcoxon-Mann-Whitney test, Benjamini-Hochberg adjusted; * treatment effect; + genotype effect compared to wild type cells). (G) Log2FC of methyltransferase mRNA expression in LPS+IFN β 1 and LPS treated wild type (pink) and *Setd1a*^{Del/+} (purple) cells. Dots represent the mean with the standard deviation as error bars. The top up- and downregulated genes are shown in red when significantly regulated (Benjamini-Hochberg adjusted p-value <0.05) and in bold when regulated >1.5fold in wild type cells. (H) *Setd1a* expression (qRT-PCR) in vehicle (veh), LPS or Dex plus LPS (D+L) treated RAW264.7 cells. (n=3, Kruskal-Wallis with post-hoc pairwise Wilcoxon-Mann-Whitney test, Benjamini-Hochberg adjusted; # genotype effect compared to *Del* cells). * # + p<0.05; ** ## ++ p<0.01; *** ### +++ p<0.001; **** #### ++ p<0.0001; ***** +++++ p<0.0001.



Supplemental Figure 7. Regulation of GR targets in the absence of TLR4 signaling

This figure relates to figure 7. (A) Volcano plot of mRNA profiles in Dex-treated (compared to vehicle) wild type and *Setd1a*^{Del/+} RAW264.7 cells. $-\log_{10}$ Benjamini-Hochberg adjusted p value (Wald test) for the genotype dependence of the gene expression model (full model: $\sim\text{genotype}+\text{treatment}+\text{treatment:genotype}$ versus reduced model: $\sim\text{treatment}$) is shown over the difference in log2FC between *Setd1a*^{Del/+} and wild type cells. Purple: genes with lost gene repression (adjP<0.05, FC>1.3); green: genes with impaired activation (adjP<0.05, FC>1.3). Selected transcripts are labelled. (B) Gene Ontology enrichment for biological processes, for genes >1.3-fold differentially regulated in *Setd1a*^{Del/+} RAW264.7 cells in response to Dex (adjP<0.05). Colors are Benjamini-Hochberg adjusted p-values.