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Supplementary Information for

Bromodomain containing 9 (BRD9) regulates macrophage inflammatory responses by potentiating glucocorticoid receptor activity

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Supplementary Information Text

Materials and Methods

BMDM isolation

BMDMs were isolated similarly as described before. Briefly, bone marrow from 6-12 weeks old male animals. After lysis with AKC lysis buffer, cells were plated in petri dishes, cultured with media containing RPMI, 20% FBS, 30% L-929 conditional medium, and 1% penicillin/streptomycin or anti-anti. Macrophages were digested with versine and plated with macrophage serum free medium.

For activation of macrophages, cells were treated with vehicle, Dex (1uM), iBRD9 (3 or 10uM), dBRD9 (250nM) or combination for overnight, followed by stimulation of LPS (100ng/ml, Sigma) for 3 hours, before harvested for RNA-seq.

Animals

All mice were housed in a 12hr light, 12 hour dark cycle. Mouse experiments were approved by IACUC at Salk Institute and IACUC at Mayo Clinic Arizona. $Brd9^{F/F}$ mice are generated by Biocytogen. LoxP sites were inserted in by CRISPR mediated homologous recombination in C57Bl6 background. LysM-Cre mice were from Jackson Laboratory (Cat. No. 004781). C57bl6 mice were from Jackson Laboratory (Cat. No. 000664) For high fat diet treatment, mice were fed with 60% fat diet paste (Bio-Serv S1850) for at least 16 weeks before serum or tissue were collected.

Immunoprecipitation

Briefly, cells are lysed using ice-cold lysis buffer (HEPES 20mM pH8, EDTA 0.2mM, NaCl 0.3 mM, NP40 0.5%, 15% glycerol) for 15 min, and after centrifuge the supernatant was collected. For Flag-tagged BRD9, immunoprecipitations were performed using Flag-magnetic beads (Sigma, M8823) for 1.5 hr at 4C. For other immunoprecipitations, primary antibodies were incubated with lysate for 2 hr followed by Protein A magnetic beads (Life Technologies, 10001D). After 3 washes with wash buffer (HEPES 10mM pH8, EDTA 0.2 mM, NaCl 0.3 mM, NP40 0.1%, 15% glycerol), the beads were boiled with NuPage LDS sample buffer and the lysate were stored at -20°C.

ChIP-seq

RAW264.7 cells were pre-treated with vehicle, Dex (1uM), or Dex+dBRD9 (250nM) for overnight and then treated with LPS (100ng/mL) for 3 hours. 20 million cells were then harvested for each ChIP assay. The experimental procedure for ChIP was as previously described(1). Briefly, after fixation, nuclei from were isolated, lysed, and sheared with a Covaris Ultrasonicator ME220 to yield DNA fragment sizes of 200–1,000 bp followed by immunoprecipitation. Antibody used for ChIP-seq: GR (Cell Signaling, Cat. No. 12041).

RNA-seq

RNA were extracted using Trizol. Samples were then processed by Novogene Co. Ltd. A total amount of 1 μg RNA per sample was used as input material for the RNA sample preparations.

CUT&RUN

RAW264.7 cells were pre-treated with vehicle, Dex (1uM), or Dex+dBRD9 (250nM) for overnight and then treated with LPS (100ng/mL) for 3 hours. 0.1 million cells were used

for each CUT&RUN. The experimental procedure for CUN&RUN was previously described. Antibody used for CUT&RUN: BRD9 (Active Motif, Cat. No. 61537).

RNA-seq Analysis

Raw data (raw reads) of FASTQ format were firstly processed through fastp. In this step, clean data (clean reads) were obtained by removing reads containing adapter and poly-N sequences and reads with low quality from raw data. At the same time, Q20, Q30 and GC content of the clean data were calculated. All the downstream analyses were based on the clean data with high quality. Paired-end clean reads were aligned to the reference genome using the Spliced Transcripts Alignment to a Reference (STAR) software. FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. Differential expression analysis between two conditions/groups (three biological replicates per condition) was performed using DESeq2 R package. DESeq2 provides statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulting P values were adjusted using the Benjamini and Hochberg's approach for controlling the False Discovery Rate (FDR). Genes with an adjusted P value < 0.05 and fold change >1.5 found by DESeq2 were assigned as differentially expressed.

ChIP-Seq Analysis

Sequencing was performed using Illumina technology to generate paired-end reads for each sample. Sequence data quality was determined using FastQC software. Bowtie2(v2.3.3.1) (--very-sensitive) was used to map ChIP-seq reads to the mouse reference genome GRCm38. Duplicate reads were filtered out using the MarkDuplicate function from Picard tools v.2.17.0 (http://broadinstitute.github.io/picard/). RPKM-normalized bigWig files were generated with bamCoverage from deepTools v3.3.2. ChIP-Seq peaks were called using findPeaks within HOMER using default parameters for TF (-style factor). De novo and known motif analyses were carried out using the findMotifsGenome.pl module of the HOMER package with the "-size given" option.

CUT&RUN Analysis

Paired-end reads were trimmed and quality-filtered using cutadapt (V2.8). Trimmed qualified reads were aligned to mouse reference genome GRCm38 using bowtie2 (v2.3.3.1) with options: --end-to-end --very-sensitive --no-mixed --no-discordant --phred33 -I 10 -X 700. Only reads that were uniquely mapped were retained for further analysis. PCR duplicated reads were removed using "MarkDuplicates.jar" from Picard tools v.2.17.0. RPKM-normalized bigWig files were generated with bamCoverage from deepTools v3.3.2. Heatmaps were generated using computeMatrix and plotHeatmap from deepTools v3.3.2. CUT&RUN peaks were called using findPeaks within HOMER using default parameters for TF (-style factor). The identified peaks for BRD9 were further screened against 'mouse blacklisted' genomic regions, mitochondrial DNA, and pseudo-chromosomes. Known motif finding on identified peaks was done by Homer findMotifsGenome.pl using parameter '-size given'.

H&E staining

H&E staining was performed by UCSD pathology core and Mayo Clinic pathology core.

Incucyte imaging

BMDM were cultured in 96 wells. Vehicle, iBRD9, and/or LPS were added together with Cas3/7 green dye for apoptosis (cat. No. 4440). Live imaging was carried out in Incucyte S3 Live Cell Analysis system. Brightfield and GFP images were collected every four

hours for 3 days. Quantitative analysis of cas3/7 positive objects were done using Incucyte base software.

Bio-plex

Mouse serum from HFD treated BRD9 WT and BRD9 KO mice were used for Bioplex mouse 23-plex cytokine assay.

Western blotting

Cells were washed with ice-cold PBS and lysed using ice-cold lysis buffer (HEPES 20mM pH8, EDTA 0.2mM, NaCl 0.3 mM, NP40 0.5%, 15% glycerol) and cocktail protease inhibitor (Sigma-Aldrich,124469). Protein concentrations in the extracts were measured using Bradford and were made equal in different samples with the extraction reagent. Lysates were mixed with sample loading buffer and denatured at 95°C for 10 minutes. Samples were separated by SDS-PAGE, transferred onto PVDF membrane. Immunoblot analysis was performed with BRD9 antibody (Active Motif, Cat. No. 61537) and secondary anti-rabbit antibodies conjugated to horseradish peroxidase (HRP).

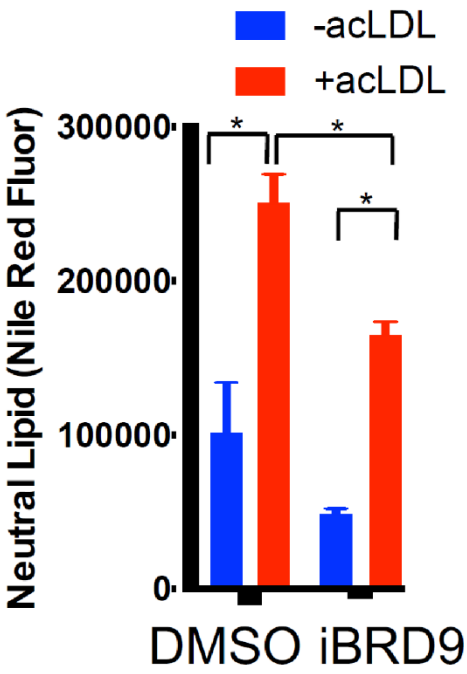


Figure S1. Inhibition of BRD9 compromises oxidized LDL induced neutral lipid accumulation in BMDMs. (n=3, *P<0.05, error bars show s.e.m.).

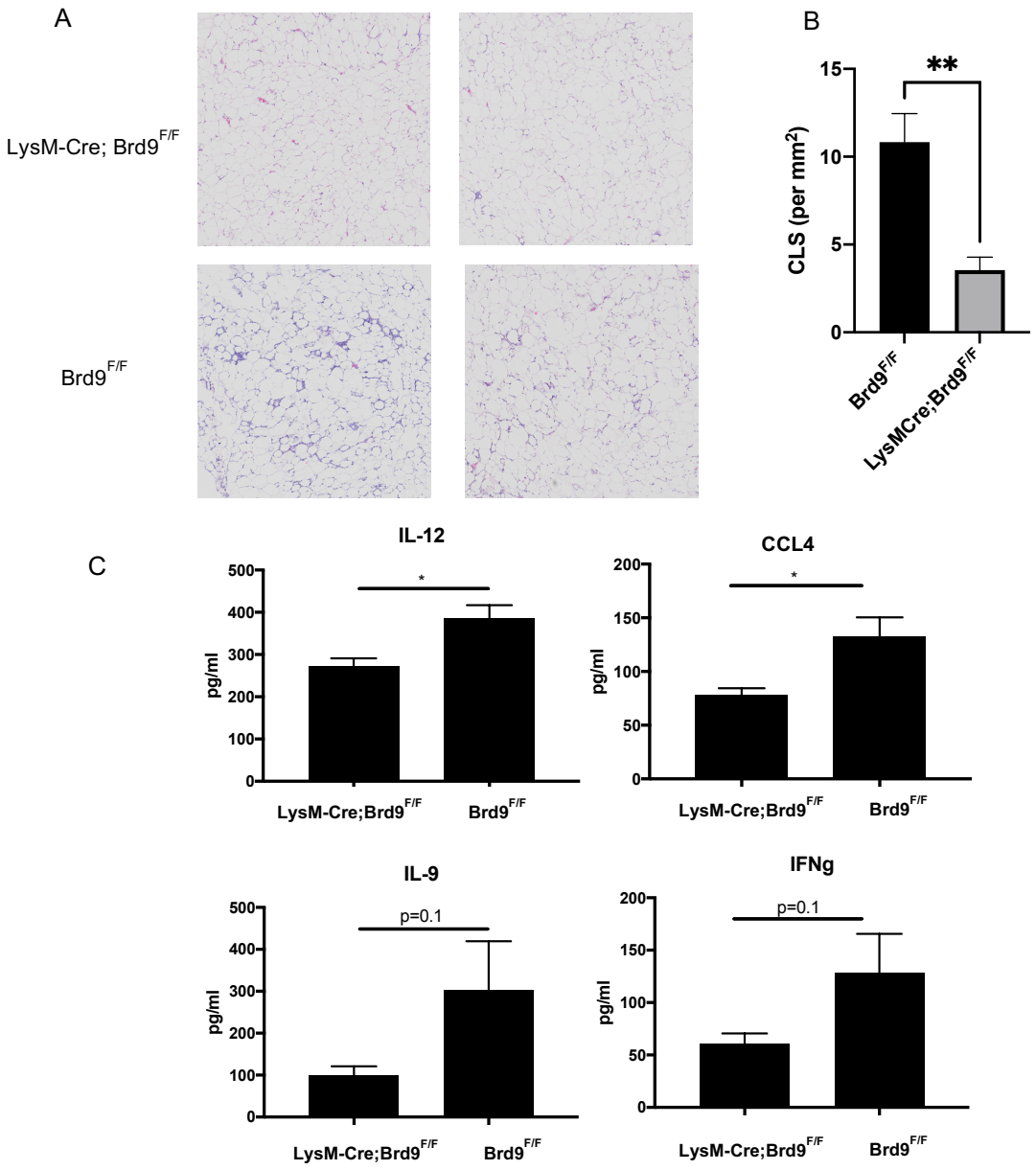


Figure S2. Inflammation is reduced in LysM-Cre;Brd9$^{F/F}$ mice treated with high fat diet.

A) HE staining of inflammatory crown-like structures (CLS) in epididymal white adipose tissue from Brd9$^{F/F}$ and LysM-Cre;Brd9$^{F/F}$ mice.

B) Quantification of CLS in the epididymal white adipose tissue H&E staining sections from LysM-Cre;Brd9$^{F/F}$ and control mice (n=4, **P<0.01, error bars show s.e.m.)

C) Circulating proinflammatory markers MCP-1b (Ccl4) and IL-12 are significantly reduced in HFD-treated LysM-Cre;Brd9$^{F/F}$ mouse serum samples. (n=5, *P<0.05, error bars show s.e.m.).

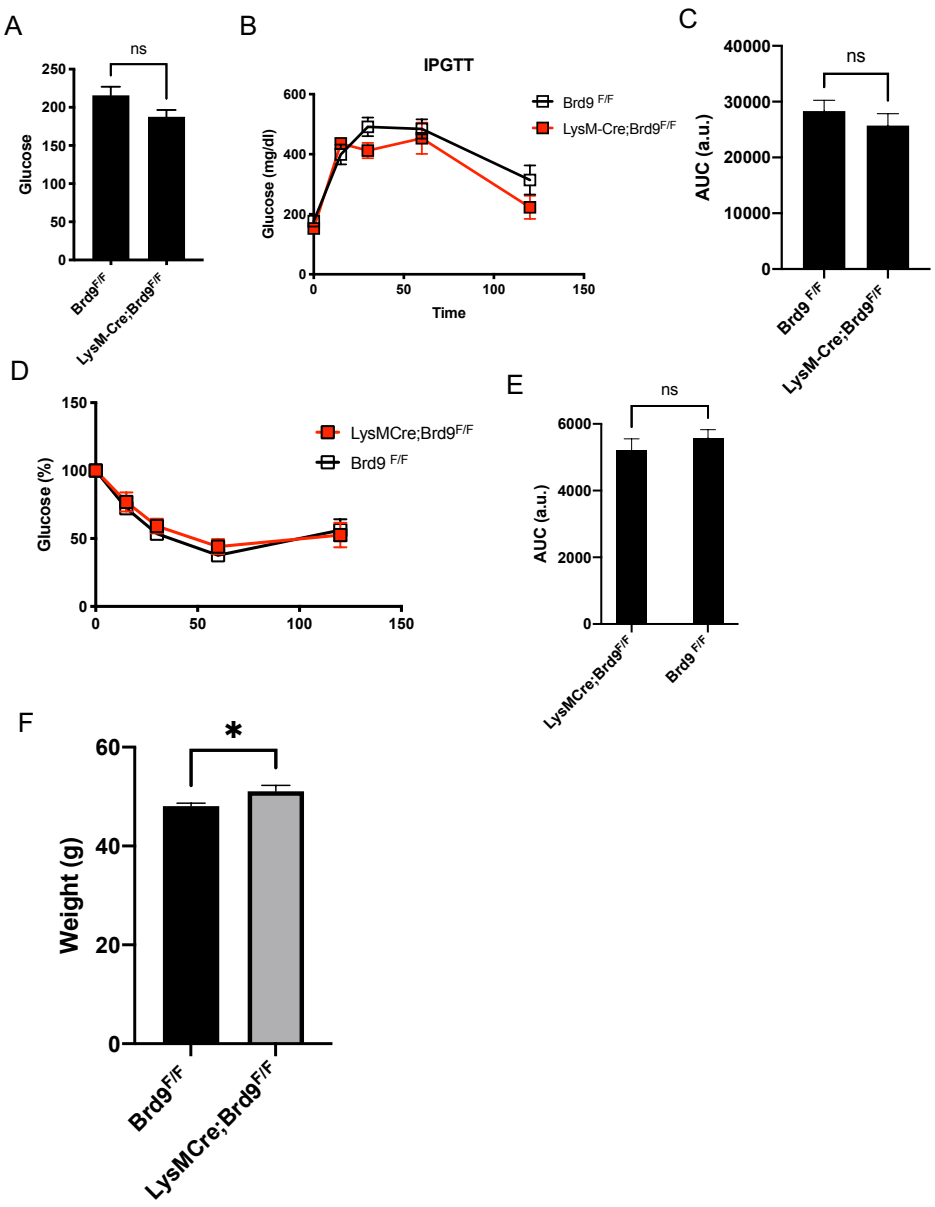


Figure S3. Metabolic phenotyping of LysM-Cre;Brd9$^{F/F}$ mice.

A) Overnight fasting glucose of high fat diet fed Brd9$^{F/F}$ and LysM-Cre;Brd9$^{F/F}$ mice (n=5).
B) Intraperitoneal glucose tolerance test (IPGTT) of high fat diet fed Brd9$^{F/F}$ and LysM-Cre;Brd9$^{F/F}$ mice (n=4-8).
C) Area under curve (AUC) of IPGTT in (B).
D) Insulin tolerance test (ITT) of high fat diet fed Brd9$^{F/F}$ and LysM-Cre;Brd9$^{F/F}$ mice (n=3-5).
E) Area under curve (AUC) of ITT in (B).
F) Weight of Brd9$^{F/F}$ and LysM-Cre;Brd9$^{F/F}$ mice after 5 months high fat diet (n=5-11, *P<0.05).

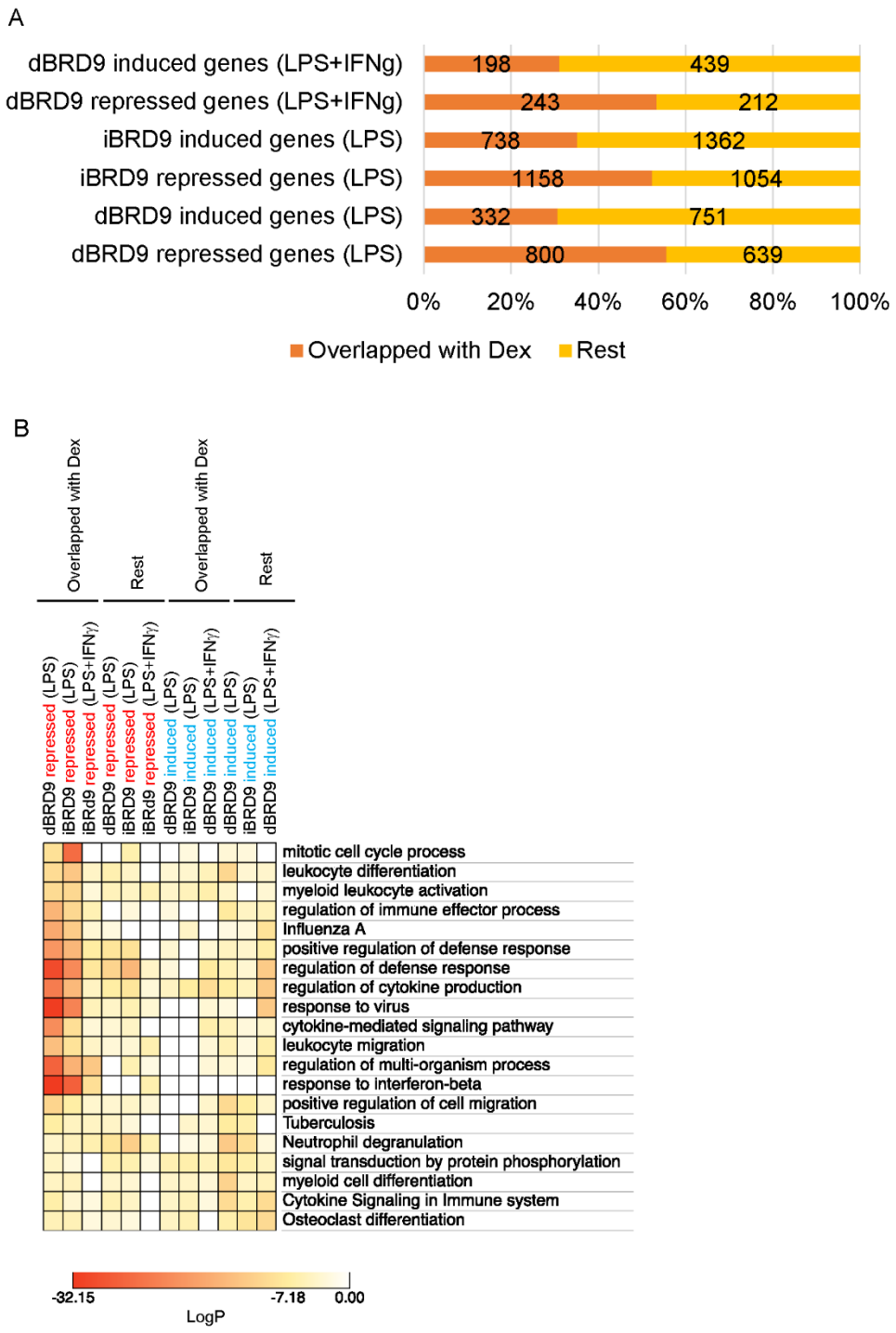


Figure S4. Overlap of dBRD9/iBRD9-regulated and dexamethasone-regulated genes suggests the co-regulation by BRD9 and GR.

A) Overlap of dBRD9 and iBRD9 regulated and dexamethasone (dex) regulated genes.

B) Gene ontology analysis shows that class A, containing genes repressed by both iBRD9/dBRD9 or dex, are the most related to inflammatory process.

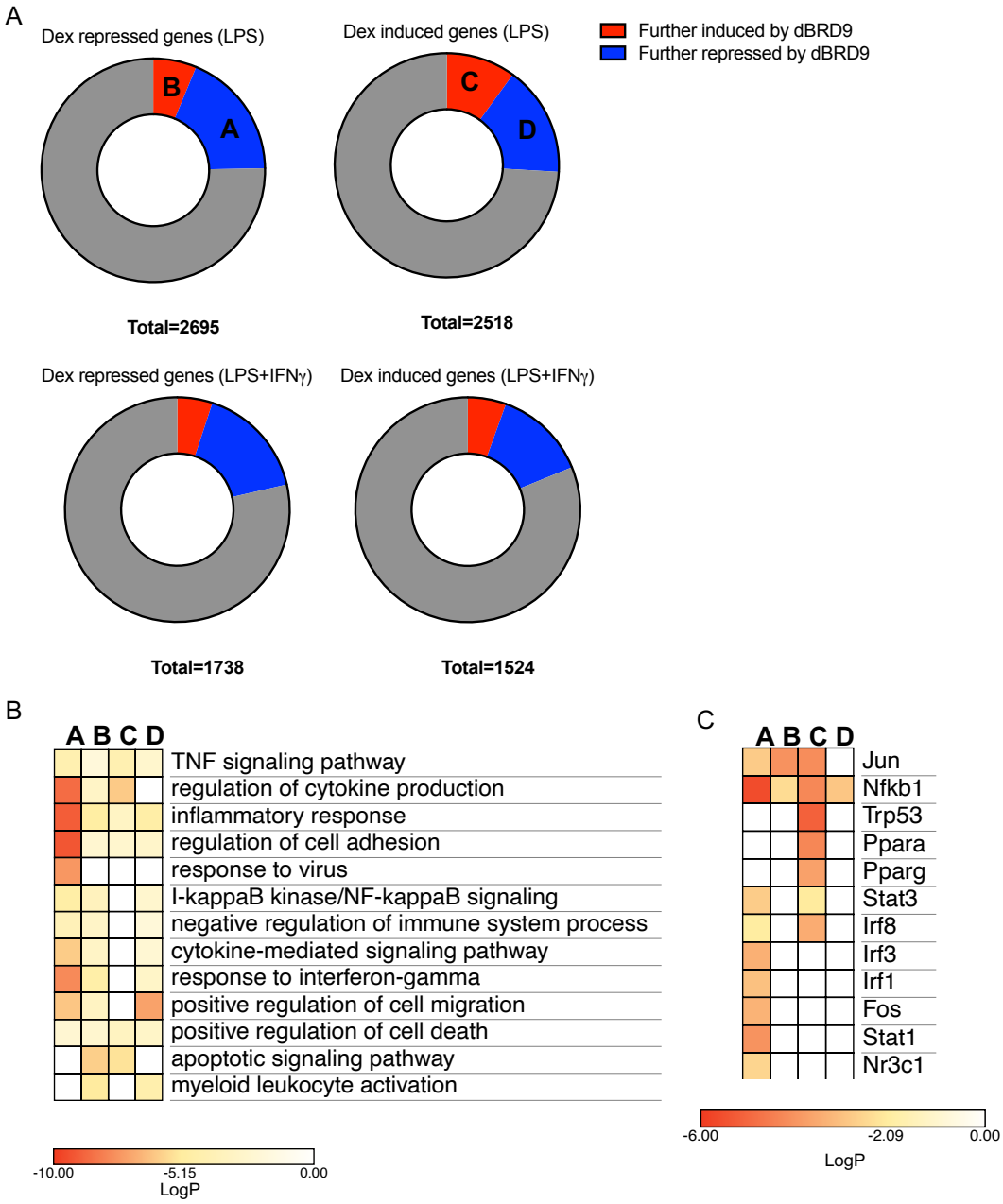


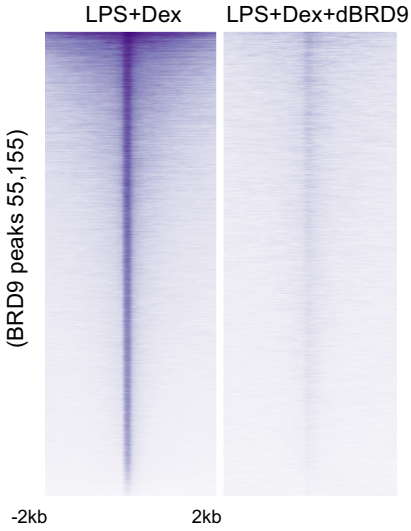
Figure S5. dBRD9 and Dex synergistically repress genes related to the inflammatory process.

A) Pie charts show the fraction of Dex-induced or Dex-repressed genes that can be further regulated by addition of dBRD9.

B) Gene ontology of categories in A). Class A genes, which are synergistically repressed by both Dex and dBRD9, are mostly related to inflammatory responses.

C) TRRUST prediction of putative upstream regulators of all categories of genes in A).

A



B

Motif	TF	P value
	JUN-AP1	1 e-2119
	CTCF	1 e-2497
	IRF8	1 e-270
	PU.1	1 e-1257
	KLF3	1 e-437
	GRE (IR3)	1 e-7

C

Group	Motif	TF	P value
Class I		GRE	1e-44
		Jun-AP1	1e-17
		AP-1	1e-16
		PU.1	1e-7
Class II		PGR	1e-131
		ARE	1e-113
		CEBP	1e-30
		ETS1	1e-28
Class III		GATA3	1e-9
		GRE	1e-5
		Etv2	1e-3
		IRF2	1e-3

Figure S6. CUT&RUN profile of BRD9.

A) Left: Heatmap of all BRD9 peaks in LPS+Dex. Right: binding intensity of BRD9 at the same location in LPS+Dex+dBRD9.
B) Motif analysis of all motifs enriched in the BRD9 peaks in LPS+Dex.
C) Enriched motifs in individual categories of GR peaks listed in Figure 4A.

References

1. G. D. Barish *et al.*, Bcl-6 and NF-kappaB cistromes mediate opposing regulation of the innate immune response. *Genes Dev* **24**, 2760-2765 (2010).