Appendix

Figure S1: Effect of Brd4 deletion on mice survival

Figure S2: Characterization of peritoneal macrophages from Brd4 conditional KO mice

Figure S3: Effect of Brd4 deletion on LPS induced gene expression

Figure S4: GO terms of genes neighboring BRD4 and H3K27ac super-enhancers

Figure S5: Characterization of p65 in Brd4 WT and KO macrophages after LPS stimulation.

Appendix Figure S1

A Genotyping primers

Brd4 flox forward	GGACTAGAAACCTCCCAAATGTCTACAA
Brd4 flox reverse	CCTGTGTGCACTTGCTCCCGAGGAGAGA
Vav-Cre forward	ATGGTGCCCAAGAAGAAGAG
Vav-Cre reverse	CAGGTGCTGTTGGATGGTCT
LysM-Cre forward	CCCAGAAATGCCAGATTACG
LysM-Cre reverse	CTTGGGCTGCCAGAATTTCTC
Er^{T2}-Cre WT forward	AAAGTCGCTCTGAGTTGTTAT
Er^{T2}-Cre Mut reverse	CCTGATCCTGGCAATTTCG
Er^{T2}-Cre WT reverse	GGAGCGGGAGAAATGGATATG

B

Mating 1: $Brd4^{fl/+}$;Cre x $Brd4^{fl/+}$;Cre

Total=92	observed	expected
$Brd4^{fl/fl}$; Cre	3	17.24
$Brd4^{fl/+}$; Cre	41	34.5
$Brd4^{+/+}$; Cre	18	17.24
$Brd4^{fl/fl}$; +/+	11	5.76
$Brd4^{fl/+}$; +/+	14	11.5
$Brd4^{+/+}$; +/+	5	5.76

$Brd4^{fl/fl;}$Vav-Cre expected 18.75% (actual <5%)
P value=0.0025

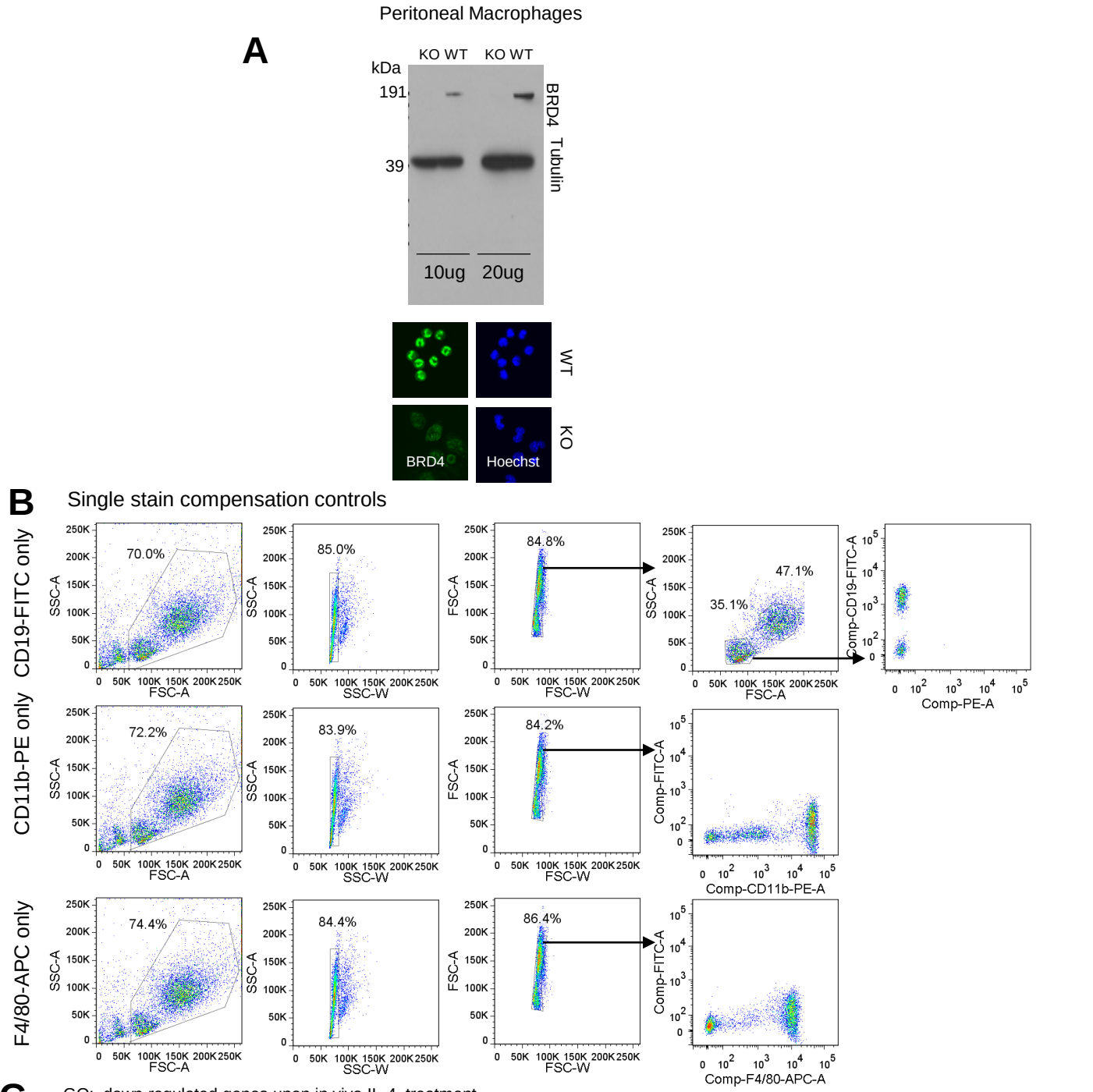
Mating 2: $Brd4^{fl/fl}$;+/+ x $Brd4^{fl/+}$;Cre

Total=112	observed	expected
$Brd4^{fl/fl}$; Cre	0	28
$Brd4^{fl/+}$; Cre	34	28
$Brd4^{fl/fl}$; +/+	35	28
$Brd4^{fl/+}$; +/+	43	28

$Brd4^{fl/fl;}$Vav-cre expected 25% (actual 0)
P value <0.0001

(A) Primers used for genotyping of Brd4 KO mice.
(B) Number of Brd4 KO pups generated from various $Brd4^{fl/fl}$;Vav-Cre mating

Appendix Figure S2



C GO: down regulated genes upon in vivo IL-4 treatment

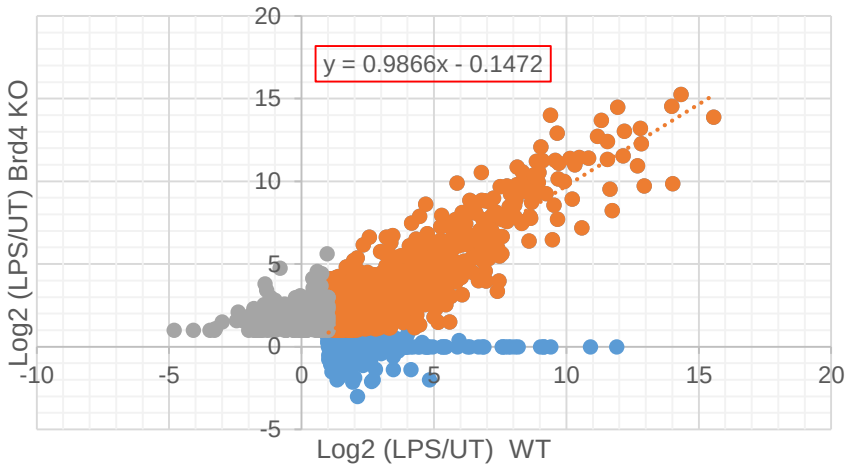
Go terms: cell cycle	overlaps	p–values
mitotic metaphase plate congression	6/59	2.61E-05
positive regulation of mitotic nuclear division	6/64	4.18E-05
attachment of mitotic spindle microtubules to kinetochore	4/201	1.86E-04
regulation of mitotic sister chromatid separation	3/201	2.38E-03
regulation of cell cycle	3/63	2.49E-02
inflammatory response	8/211	1.27E-03

Go terms: Immune related	overlaps	p-values
positive regulation of cell proliferation	17/442	3.27E-04
inflammatory response	11/211	3.13E-04
positive regulation of GTPase activity	10/160	1.36E-04
extracellular matrix organization	9/143	2.78E-04
negative regulation of cell migration	8/126	5.69E-04
cellular response to hypoxia	5/58	1.63E-03

(A) Brd4 KO peritoneal macrophages were tested for deletion by western blotting and immunocytochemistry. (B) Single stain compensation setup. Peritoneal cells were stained with one of each single stain (CD19-FITC, CD11b-PE and F4/80-APC) and an unstained sample. Compensation was calculated by BD FACSDiva software.
(C) GO analysis of genes downregulated in KO macrophages from IL-4C treated mice. Results obtained from the Enrichr program. See Figure 2E.

Appendix Figure S3

A LPS induced global gene expression in WT and Brd4 KO macrophages



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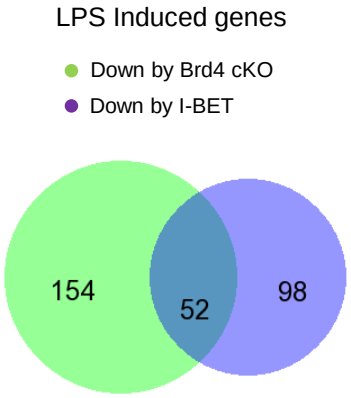


GO term	Down by Brd4 cKO only	Down by I-BET but not by Brd4 cKO	Down by cKO & I-BET
Immune response	*Oas1g, Fcgr2b, Ccl24, Cxcl1, H2-Q6, H2-Q7, H2-Q8, H2-T23, H2-T24, Nfil3, Procr, Slpi, Tnfsf8*	*Oas2, Oas3, Oasl2, Ccl12, Cxcl9, Irf8, Il19, Il23a, P2ry14, Tnfsf4*	*H2-M2, Tnfsf10, Tnfsf15*
Innate immune response	*Fgr, Samhd1, Bst1, Gsdmd, Iigp1, Irf7, Marco, Nod1, Slpi, Flt1, Ccl24, Cx3cl1, Pdgfrb*	*Ifnb1, Klrk1, Lck, Ptx3, Tlr6, Tnfsf4,*	*Oas1a,DEXH,Fcgr1, Apobec3,Cfb,Isg20, Lcn2,Slamf1,*
Cell adhesion	*Epha4, Col18a1, Flt3, Has1, Itgal, Klra2, Siglece, Tnc,*		
Inflammatory response	*S100a8, S100a9, Ccl24, Cxcl3, Gsdmd, Mmp25, Ptgir, Tlr1*	*Bcl6, Ccl12, Cxcl9, Fpr2, Il19, Il23a, Il27, Nos2, Tlr6, Tnfsf4*	*Mefv, Fpr1, Il6, Ptgs2, Smpdl3b, Tlr3*

(A) Regression plot to identify changes in global gene expression. Genes that were induced by LPS in both WT and KO (orange dots). Grey and blue dots were excluded because their values were not consistent in different experiments. (B) Comparison of Brd4KO and BET inhibitor effects. Venn diagram shows number of LPS induced genes downregulated by Brd4 deletion (from $Brd4^{fl/fl}$ ER^{T2}-Cre) or downregulated by a BET inhibitor (I-BET) reported in (Nicodeme, 2010) (left). The table shows representative examples of genes downregulated by Brd4KO only or I-BET only or by both.

Appendix Figure S4

A

GO: SE nearest genes: UT

GO term	overlaps	p-values
Neutrophil deregulation	24/487	3.24E-08
Toll like receptor 4 signalling pathway	7/195	5.77E-06
Positive regulation of leukocyte migration	8/151	8.86E-04
proteosomal protein catabolic process	6/195	1.23E-04
Cellular response to interferon alpha	4/193	7.87E-04
peptidyl-tyrosine phosphorylation	10/327	1.16E-02
Negative regulation of IL12 production	3/210	9.10E-04
Inflammatory response	8/211	6.96E-03

GO: SE nearest genes: LPS

GO term	overlaps	p-value
LPS mediated signaling pathway	15/69	3.42E-14
Tnf mediated signalling pathway	20/272	2.02E-09
Inflammatory response	18/211	1.26E-09
Positive regulation of NF-kB activity	15/189	8.07E-08
Chemokine mediated signalling pathway	13/139	8.83E-08
Type I interferon signalling pathway	13/148	1.85E-07
Regulation of import of NF-kB to nucleus	12/170	5.53E-06
Negative regulation of apoptotic process	20/467	1.16E-05

B H3K27ac WT & H3K27ac KO overlap: 194 genes

GO term	overlaps	p-values
inflammatory response	17/211	2.69E-11
lipopolysaccharide-mediated signaling pathway	10/196	1.21E-09
I-kappaB phosphorylation	16/346	2.87E-07
negative regulation of cell proliferation	16/399	1.88E-06
positive regulation of transcription from RNA pol II promoter	27/117	2.70E-05
positive regulation of transcription in G2/M transition of mitotic cell cycle	20/721	2.51E-05

(A) GO terms for genes neighboring BRD4 SEs, based on Enrichr program (GO biological process 2017b) (B) GO terms for genes neighbored by H3K27ac SEs in WT and KO macrophages.

Appendix Figure S5

A

Transcription factor interaction

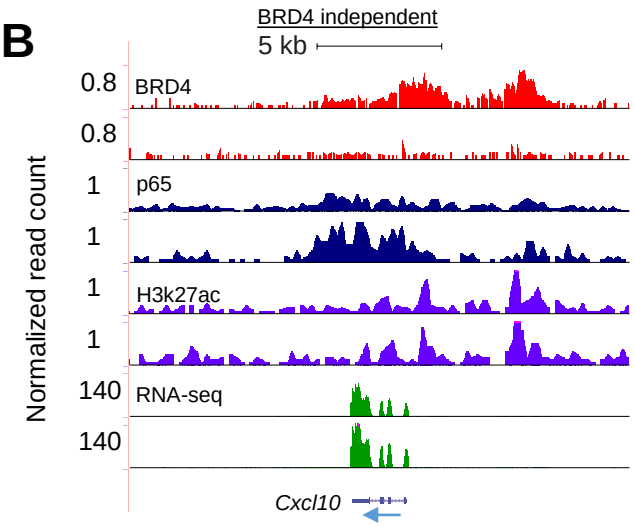
BRD4 independent

TF's	P-value
NFKB1	9.58E-14
STAT3	8.84E-09
STAT6	2.57E-06
NOD2	1.38E-06
STAT1	2.24E-05
IRF3	2.87E-05

BRD4 dependent

TF's	P-value
STAT3	4.16E-02
STAT1	5.17E-02
IRF3	4.19E-02
USF2	2.69E-02
SMAD3	9.20E-02
IRF8	4.24E-02

B



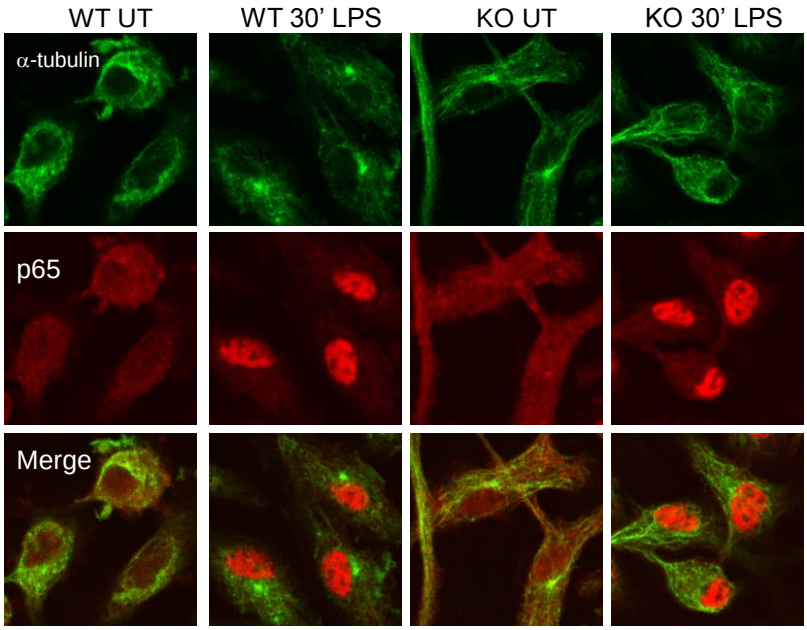
C GO: nearest genes to p65 enhancers- WT

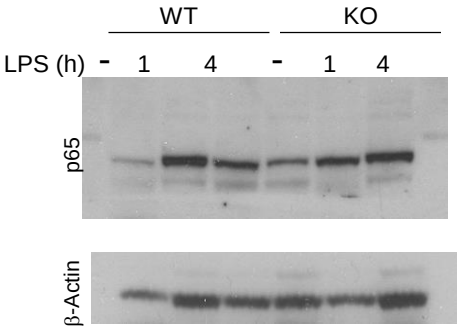
GO terms	overlaps	p-value
lipopolysaccharide-mediated signaling pathway	5/69	2.53E-07
positive regulation of natural killer cell chemotaxis	3/15	3.32E-06
chemokine (C-C motif) ligand 5 signaling pathway	4/49	2.72E-06
inflammatory response	5/211	6.07E-05
neutrophil chemotaxis	3/55	1.81E-04
TNF-mediated signaling pathway	5/272	2.00E-04
type I interferon signaling pathway	3/148	3.21E-03
positive regulation of GTPase activity	3/160	4.00E-03

GO: nearest genes to p65 enhancers- KO

GO terms	overlaps	p-values
inflammatory response	14/211	8.02E-12
lipopolysaccharide-mediated signaling pathway	10/69	3.59E-12
tumor necrosis factor-mediated signaling pathway	12/272	2.75E-08
chemokine-mediated signaling pathway	8/139	8.72E-07
neutrophil chemotaxis	6/55	5.04E-07
positive regulation of natural killer cell chemotaxis	4/15	1.05E-06
cellular response to interferon-gamma	6/82	5.41E-06
I-kappaB phosphorylation	10/346	1.82E-05

D





(A) Transcription factor interaction prediction by Enrichr program. Ranking of possible TFs for BRD4 independent and BRD4 dependent genes. Note the top ranking for NFKB1 for BRD4 independent genes. (B) Gene tracks of BRD4, p65 and H3K27ac ChIP-seq peaks and RNA-seq profiles on Cxcl10 (BRD4 independent gene). (C) GO terms for genes near the p65 binding in WT (left) and KO (right) macrophages, assessed by Enrichr program. (D) WT and KO macrophages were grown on coverslips, treated with or without LPS for 30 min, fixed, permeabilized and stained with antibody for p65 (diluted 1:100) and a-tubulin (diluted 1:500) and visualized by confocal microscopy (left). No discernible differences were noted in p65 staining between WT and KO macrophages. P65 protein expression in WT and Brd4 KO macrophages was detected by immunoblot analysis (right). Thirty μg of whole cell extracts was used. β-actin was tested as a loading control.