

Supplemental material

Chen et al., <https://doi.org/10.1084/jem.20181031>

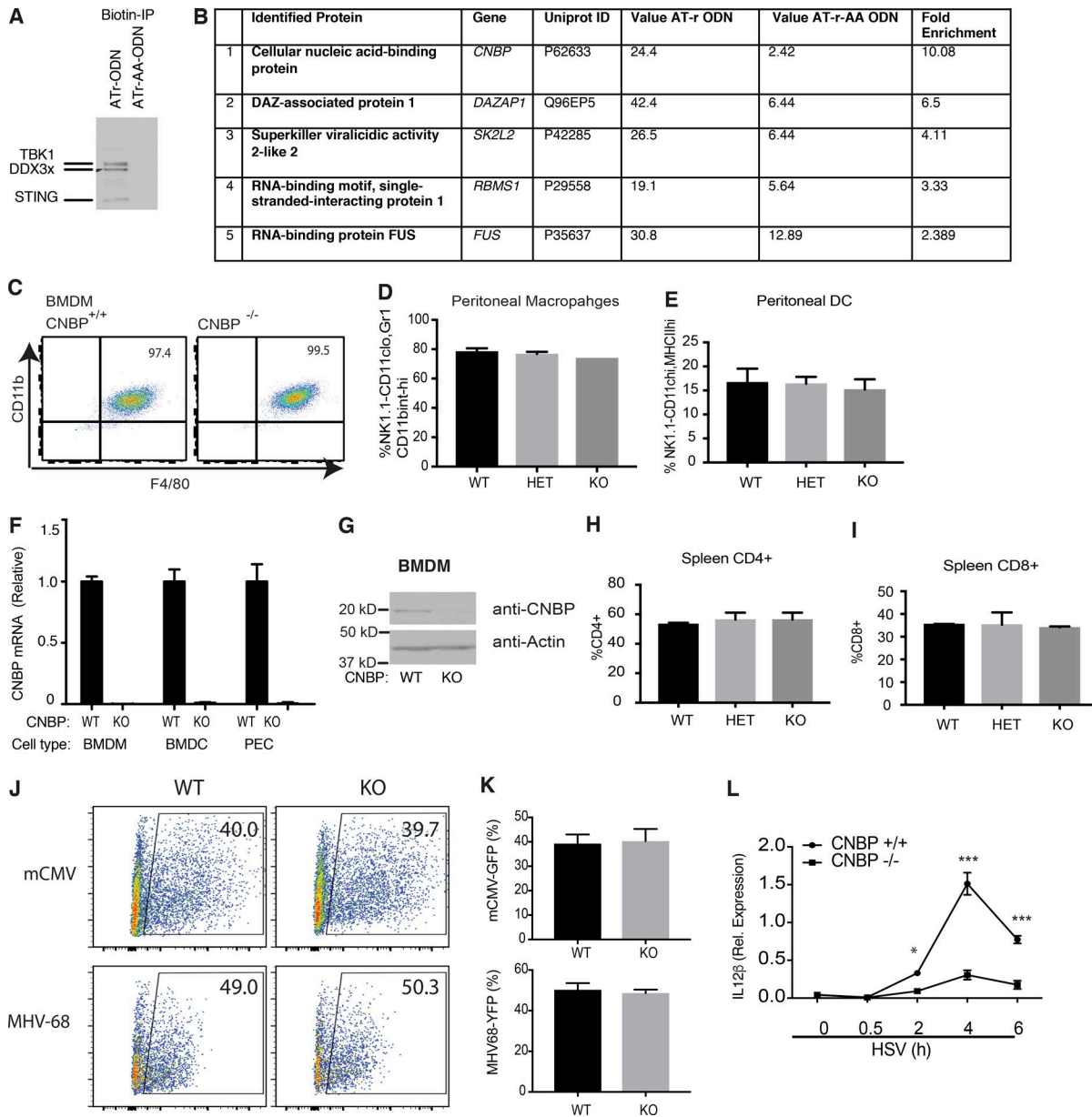


Figure S1. **Characterization of *Cnbp*-deficient mice.** (A) Immunoblotting was performed to identify known components of the DNA-sensing pathway in ATr-ODN and a nonstimulatory ODN (ATr-AA ODN) immunoprecipitation (IP) samples from macrophage cytosolic extracts. (B) LC-MS analysis of proteins identified. The top five most enriched proteins identified in ATr-ODN and nonstimulatory ATr-AA-ODN complexes. (C) CD11b/F480 levels were measured by flow cytometry on macrophage cultures differentiated in vitro from bone marrow progenitors. (D and E) Percentages of PECs (D) and peritoneal DCs (E) were analyzed by their specific lineage markers from WT (*Cnbp*^{+/+}), HET (*Cnbp*^{+/-}), and KO (*Cnbp*^{-/-}) mice. (F and G) CNBP expression levels in WT and KO cells were detected through RT-PCR (F) and Western blotting (G). (H and I) Percentages of CD4 and CD8 T cells analyzed by their specific lineage markers from WT (*Cnbp*^{+/+}), HET (*Cnbp*^{+/-}), and KO (*Cnbp*^{-/-}) mice. (J and K) The replication of reporter virus mCMV-GFP or murine gammaherpesvirus 68-YFP in CNBP WT or deficient MEFs. (L) qRT-PCR analysis of IL12 β mRNA in *Cnbp* WT or KO BMDMs mock infected or infected with HSV at different time courses. Error bars represent SD of triplicate technical replicates. All data are representative of three independent experiments with similar results. *, P < 0.05; ***, P < 0.001.

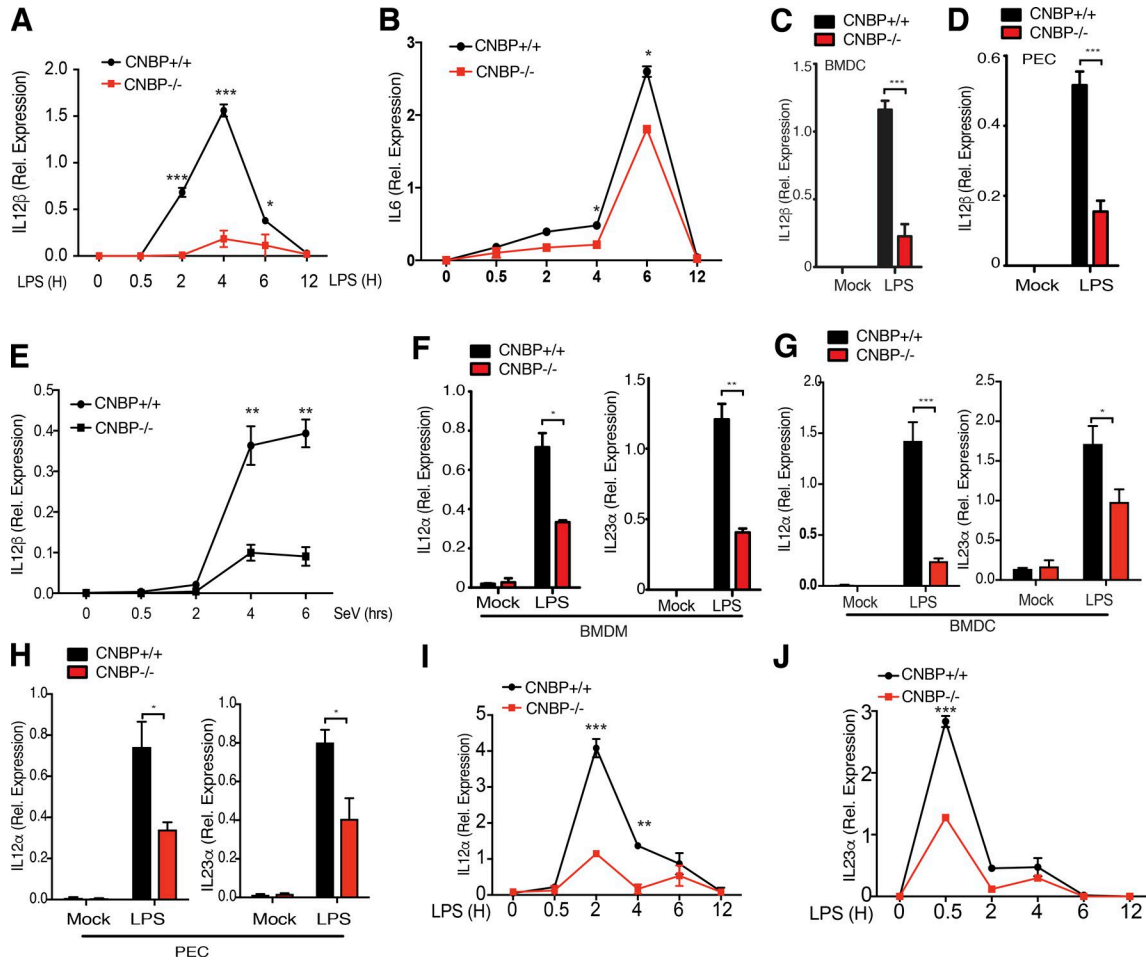


Figure S2. **Cytokine analysis in CNBP-KO cells after stimulation with various ligands at different time points.** (A and B) qRT-PCR analysis of IL-12β (A) or IL6 (B) mRNA in Cnbp WT or KO BMDMs left unstimulated or stimulated with LPS at different time courses. (C and D) qRT-PCR analysis of IL-12β in Cnbp WT or KO BMDC (C) or PECs (D) left unstimulated or stimulated with LPS. (E) qRT-PCR analysis of IL-12β mRNA in Cnbp WT or KO BMDMs mock infected or infected with SeV at different time courses. (F-H) qRT-PCR analysis of IL-12α and IL-23α mRNA in Cnbp WT or KO BMDMs (F), BMDCs (G), or PECs (H) left unstimulated or stimulated with LPS. (I and J) qRT-PCR analysis of IL-12α (I) or IL-23α (J) mRNA in Cnbp WT or KO BMDMs left unstimulated or stimulated with LPS at different time courses. Error bars represent SD of triplicate technical replicates. All data are representative of three independent experiments with similar results. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

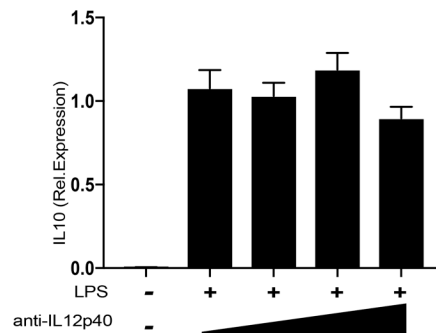


Figure S3. **IL-10 induction was not affected by IL-12.** Primary macrophages were incubated with anti-IL-12 neutralizing antibody before LPS stimulation and IL-10 mRNA expression levels were determined by qPCR. Error bars represent SD of triplicate technical replicates. All data are representative of three independent experiments with similar results.

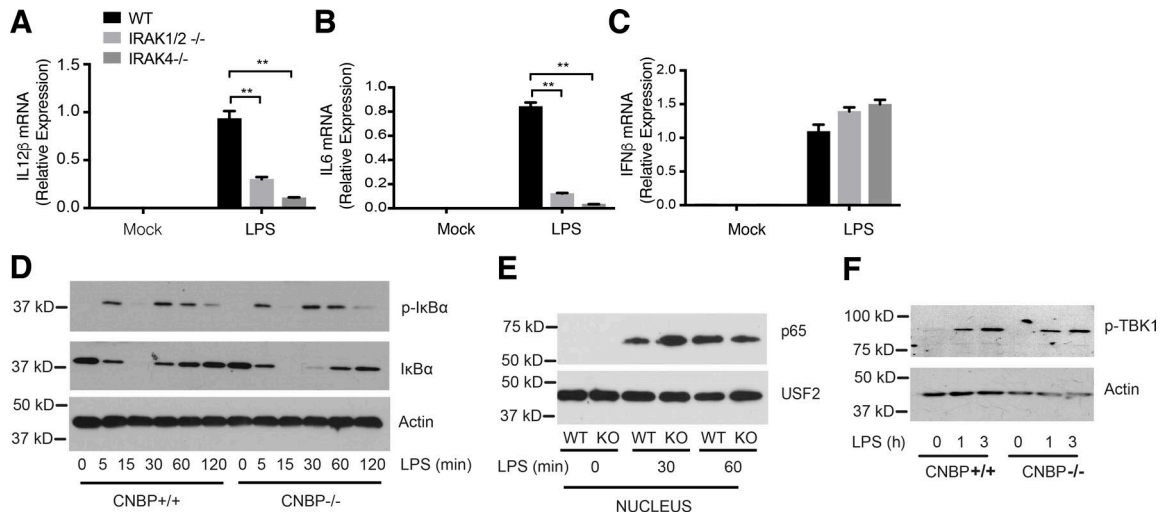


Figure S4. **Analysis of the NF- κ B signaling and cytokine inductions in IRAK1/2 $^{-/-}$ or IRAK4 $^{-/-}$ BMDMs.** (A–C) qRT-PCR analysis of IL12 β (A), IL6 (B), and IFN β (C) in IRAK1/2 $^{-/-}$ or IRAK4 $^{-/-}$ BMDMs left unstimulated or stimulated with LPS. (D) Immunoblot analysis of phospho-I κ B α or total I κ B α in whole cell lysates of Cnbp WT or KO BMDMs stimulated for different times with LPS. (E) Nuclear extracts were analyzed for p65 by Western blotting in Cnbp WT and KO BMDMs treated with LPS. (F) Immunoblot analysis of p-TBK1 in whole cell lysates of Cnbp WT or KO BMDMs stimulated for various times with LPS. Error bars represent SD of triplicate technical replicates. All data are representative of three independent experiments with similar results. **, P < 0.01.

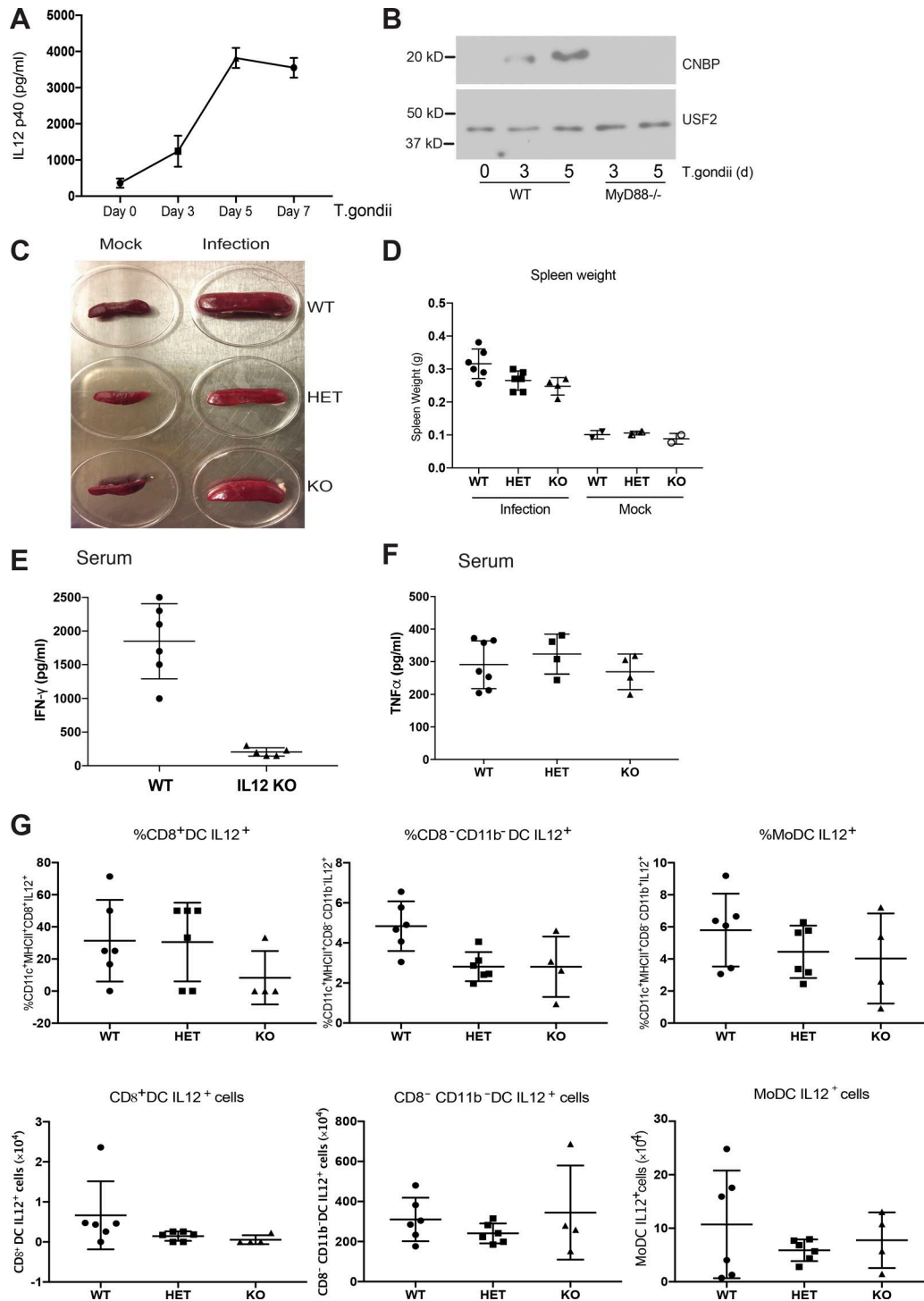


Figure S5. **Analysis of CNBP-deficient mice after *T. gondii* infection.** (A) ELISA quantification of IL-12p40 levels in the serum after *T. gondii* infection at different time points after infection. (B) Nuclear extracts were analyzed for Cnbp by Western blotting in spleen cells from WT and MyD88^{-/-} mice after *T. gondii* infection at different time after infection. (C and D) Gross appearance (C) and weight (D) of the spleen in the indicated mice. (E) ELISA quantification of IL12p40 levels in the serum after *T. gondii* infection in WT and IL12^{-/-} (KO) mice. (F) ELISA quantification of TNF-α levels in the serum after *T. gondii* infection in WT, CNBP^{+/-} (HET), and CNBP^{-/-} (KO) mice. (G) IL12 production in different DC subsets from WT, CNBP^{+/-} (HET), and CNBP^{-/-} (KO) mice 7 d after *T. gondii* infection. Each symbol represents an individual mouse; small horizontal lines indicate the mean. All data are representative of at least two to three independent experiments with similar results.

Table S1. sgRNA, CHIP, and qPCR primers

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
qPCR primers		
IL12b	GGAAGCACGGCAGCAGAATA	AACTTGAGGGAGAAGTAGGAATGG
IL12a	ACGAGAGTTGCCTGGCTACTAG	CCTCATAGATGCTACCAAGGCAC
IL23a	CATGCTAGCCTGGAACGCACAT	ACTGGCTGTTGTCCTTGAGTCC
IL10	CGGGAAGACAATAACTGCACCC	CGGTTAGCAGTATGTTGTCCAGC
IL6	TACCACCTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
TNF- α	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAAGTATGATGAGAGGGAG
IFN- β	ATAAGCAGCTCCAGCTCCA	CTGTCTGCTGGTGGAGTCA
CNBP	ATCTGCTACCGCTGTGGTGAGT	GCCACCTCTACCGCAGTTATAG
CCL4	TTCCTGCTGTTTCTCTTACACCT	CTGTCTGCCTCTTTGGTCAG
CXCL9	GGAGTTCGAGGAACCTAGTG	GGGATTGTAGTGGATCGTGC
IL15	GTAGGTCTCCCTAAAACAGAGGC	TCCAGGAGAAAGCAGTTTATTGC
iNOS2	GTTCTCAGCCAAACAATAACA	GTGGACGGGTGATGTCAC
IRF7	CAGCGAGTGTGTTTGGAGAC	AAGTTCGTACACCTTATGCGG
IL4i1	GGATGAGAAGACAGGCTGGATAG	GCAGCTTACATTATGCACCTCC
Med21	AGTGTGGTCTCCTGCCTCTTT	CTGTTCGTGCAATCAGTGCTGC
GAPDH	TGGCAAAGTGGAGATTGTTGCC	AAGATGGTATGGGCTTCCCG
CHIP primers		
IL12b pro -600	CATAAGAGACGCCCTCAAA	TGTTTGGCTCCACGTACC
IL12b pro -500	TGGAGCCAAACAGGAGGTAAT	GTGTTACAGGCCCAAGAATAAA
IL12b pro -400	GGGCCTGTAACACCTACTTATTT	CGGCAATGGCTAACCTCTC
IL12b pro -300	GCCGCCTCTATTCACCTTAG	GACGTCGAAATCCAGGTTA
IL12b pro -200	TCGACGCTATATTCCTCTGTAT	CTAGTCTCAATTGCAACACTGAAA
IL12b pro -100	TTCAGTGTGCAATTGAGACTA	TGTTCTTCTGCTGCCTTG
sgRNAs		
hCNBP sgRNA1	CACCGCCGTGTGCAGACCCGCGTG	AAACCACGGGGTCTGCACACGGC
hCNBP sgRNA2	CACCGCGTCCGAGTCTCCGCCGCTG	AAACCACGGCGGAGACTCGGACGC