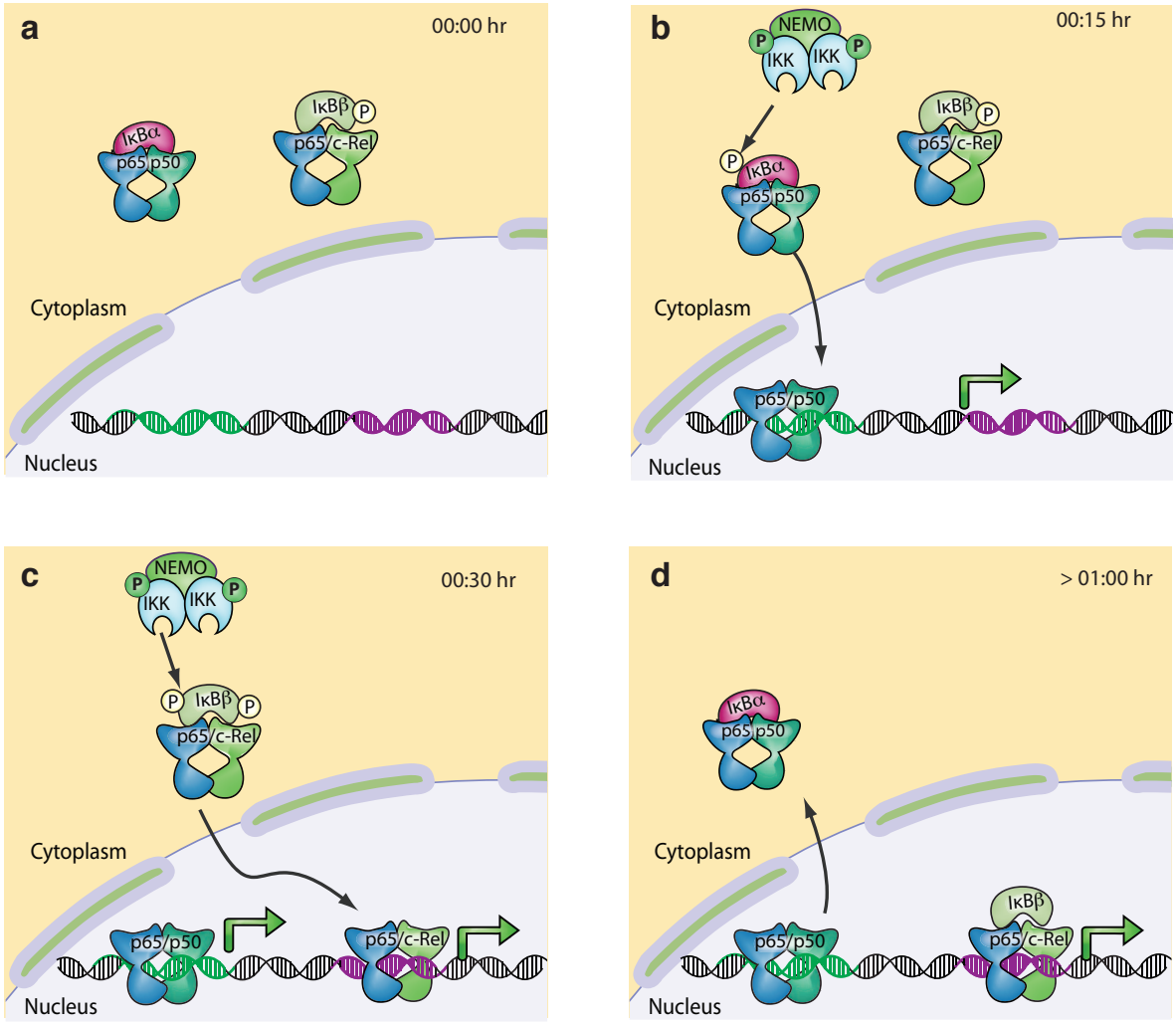
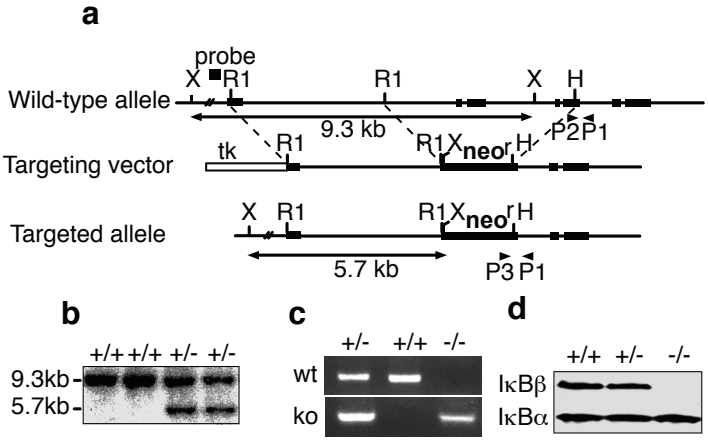
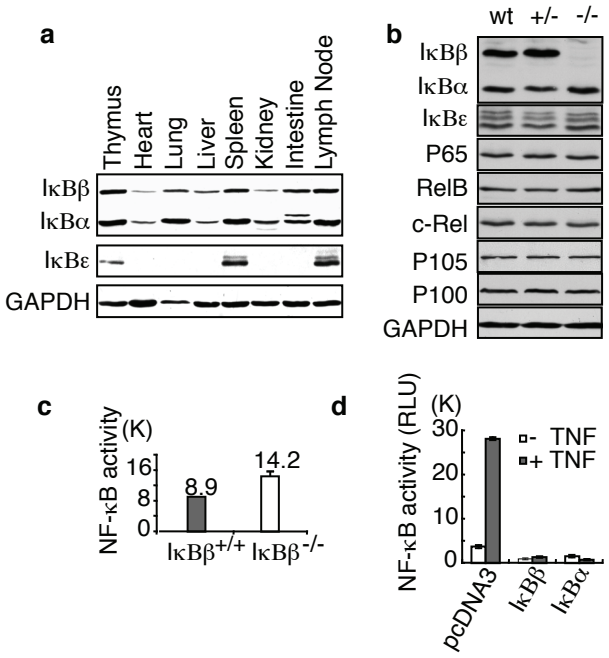


Supplementary Figure 1 | IκBβ exerts both typical and atypical effects on NF-κB regulation of TNFα expression. **a**, IκBβ, like IκBα functions as a typical IκB family member by sequestering NF-κB dimers in the cytoplasm. IκBβ selectively binds p65 or c-Rel containing complexes through direct binding to either p65 or c-Rel. IκBβ also binds and stabilizes the p65:c-Rel heterodimer which is otherwise not present. **b**, LPS stimulation through TLR4 and multiple intervening signaling intermediates activates the IKK complex resulting in phosphorylation of IκBα and its subsequent ubiquitination and degradation. Released dimers translocate to the nucleus where they bind κB sites in the promoters/enhancers of various genes including TNFα, IκBβ and c-Rel. **c,** IκBβ phosphorylation and degradation occurs with slower kinetic resulting in the release of NF-κB including p65:c-Rel heterodimers which bind to selective κB sites including a specific κB site (κB2) in the TNFα promoter. **d**, Newly synthesized IκBα binds and sequesters NF-κB complexes in the cytoplasm terminating transcription of most NF-κB dependent genes. Newly synthesized hypophosphorylated IκBβ binds to p65:c-Rel forming a trimeric complex that is resistant to IκBα binding and is thus capable of continued interaction with the κB site of the TNFα promoter and responsible for continued expression of TNFα.



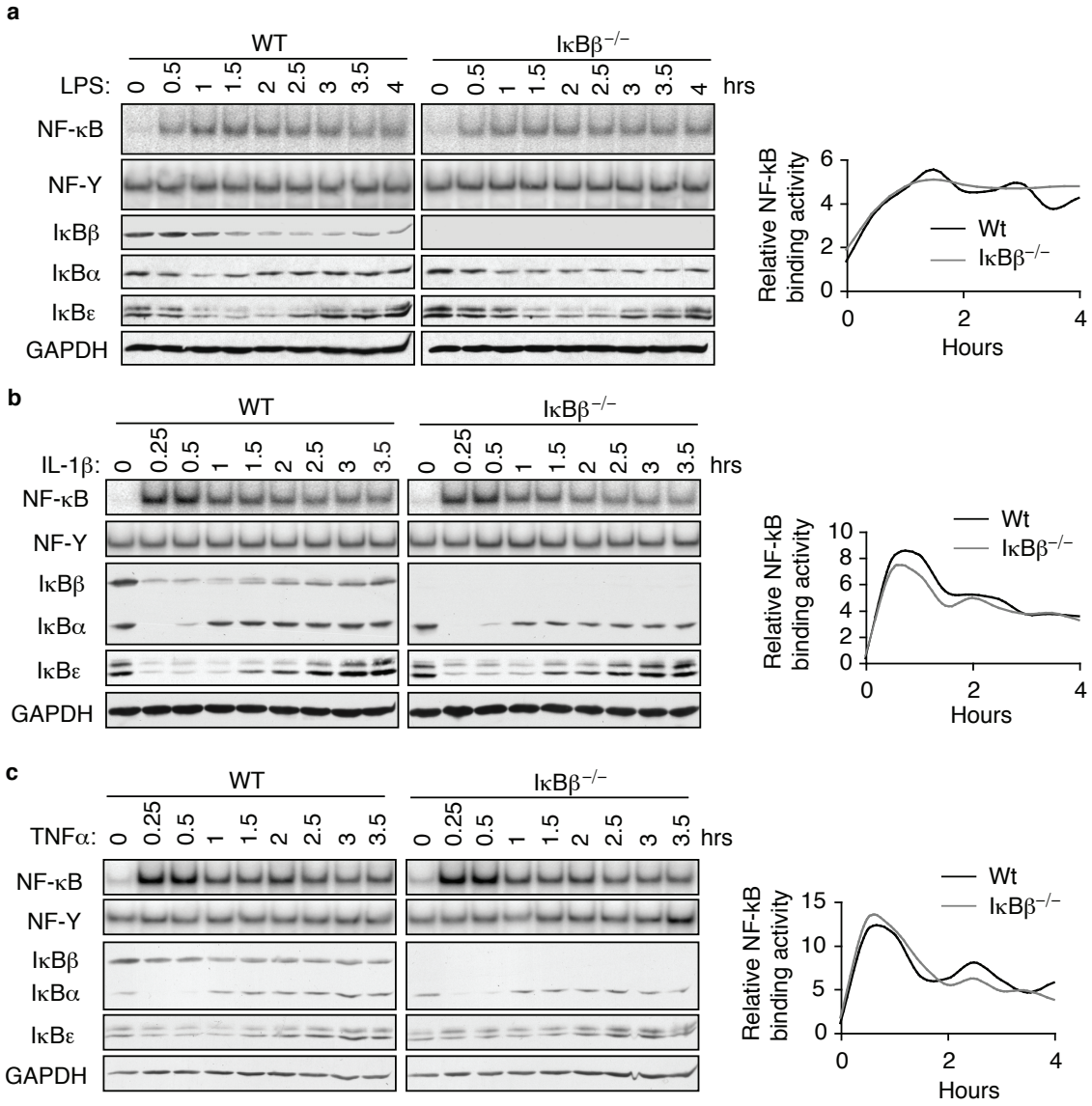
Supplementary Figure 2 | Generation of the IκBβ$^{-/-}$ mice

a, The structure of the IκBβ gene, the targeting vector and the predicted disrupted gene. Black boxes denote the exons. Restriction enzymes: X, XbaI; R1, EcoR1; H, HindIII. P1-P3 : primers used for PCR genotyping of WT allele (P1 and P2) and targeted allele (P1 and P3). **b,** Screening for homologous recombination between the targeting vector and the endogenous IκBβ gene. ES cell genomic DNA was digested with XbaI, electrophoresed and hybridized with radiolabeled probe indicated in A. **c,** PCR genotyping of offspring from the heterozygote intercrosses. Tail DNA was obtained and PCR with primers indicated in A was carried out. **d,** *IκBβ*$^{-/-}$ mice do not express IκBβ. Western blot analysis of total cell lysate of MEF cells derived from 12.5 day embryos.



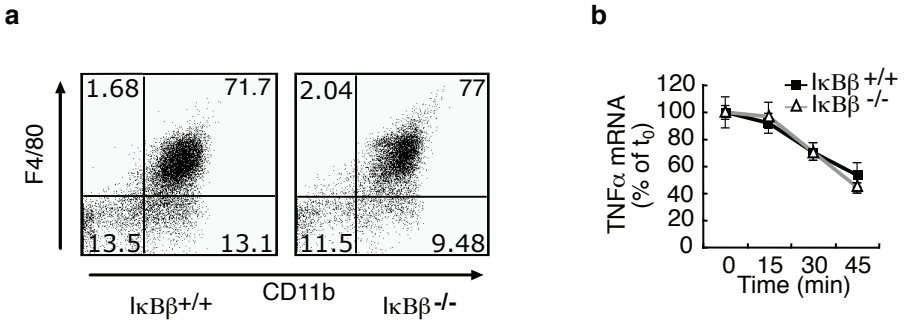
Supplementary figure 3 | NF- κB is activated normally in IκBβ$^{-/-}$ MEFs.

a, 30 ug of total cell lysate from various mouse tissues was electrophoresed and analyzed by Western blot with the indicated antibodies. **b,** 30ug of total cell lysate from MEFs was analyzed by Western blot with indicated antibodies. **c**, Basal NF- κB DNA-binding activity was analyzed by EMSA with nuclear extracts prepared from MEFs. The intensity of the gel-shift band was measured by densitometry, normalized to that of NF-Y and graphed. **d**, NF-κB luciferase assay. 293 cells were transiently transfected with pBIIx-luc together with Renilla luciferase vector and pcDNA3 vectors that express either IκBα or IκBβ. Twenty-four hours after transfection, cells were stimulated with TNFα (10ng/ml) for 4 hours, lysed and assayed for luciferase activity. Results are expressed as relative luciferase units (RLU) normalized by Renilla luciferase activity.



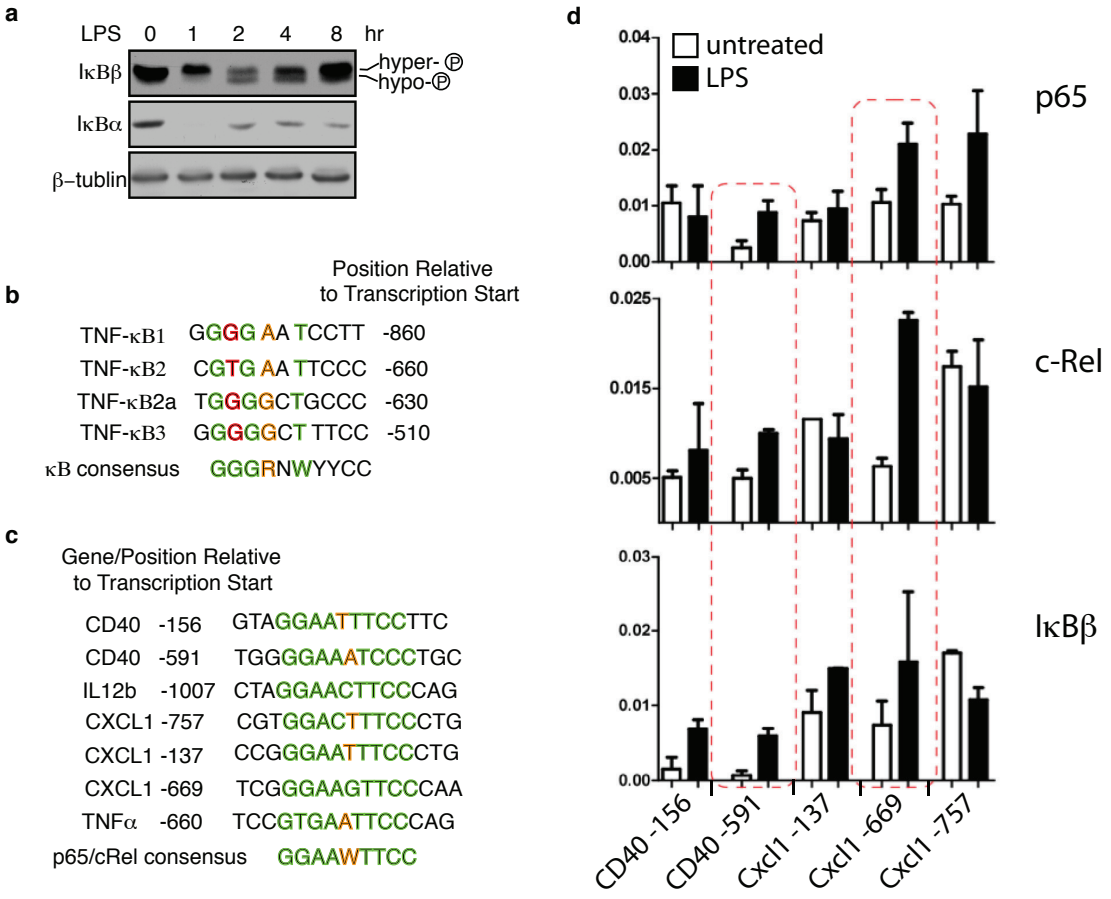
Supplementary Figure 4 | NF- κB is normally activated in *IκBβ*$^{-/-}$ MEFs.

Cells were treated with LPS (1ug/ml; **a**), IL-1β (10ng/ml; **b**) or TNF-α (10ng/ml; **c**) for the indicated times, then cytosolic and nuclear extracts were prepared. Nuclear extracts were analyzed for NF-κB DNA-binding activity by EMSA. EMSA with NF-Y DNA probe was performed as a loading control. (left panel). The intensity of the gel-shift bands were quantitated by densitometry, normalized to that of NF-Y, and graphed (right penal). Cytosolic extracts were analyzed for IκBα, IκBβ and IκBε degradation by immunoblotting with corresponding antibodies. Western blot with anti-GAPDH antibody was performed as a loading control.



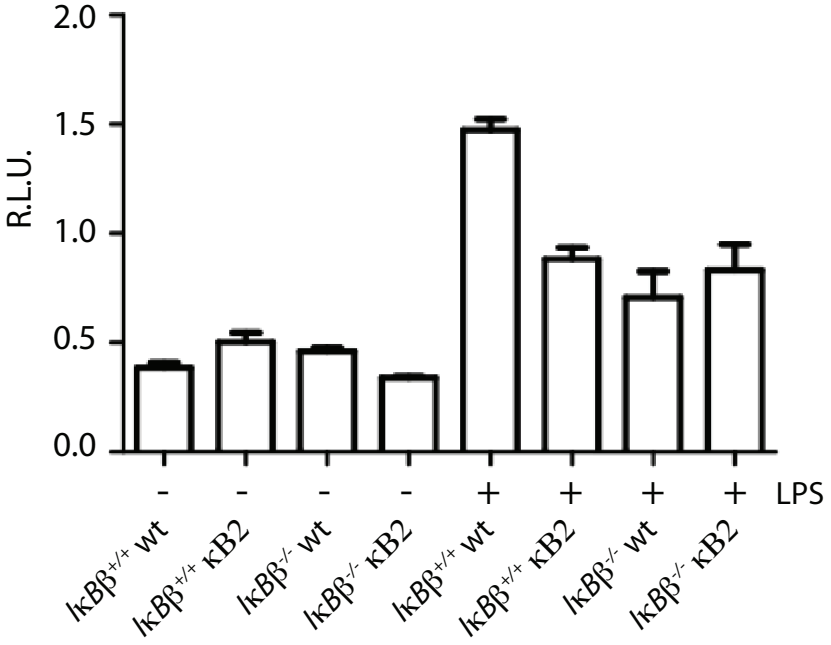
Supplementary Figure 5 | Normal phenotype of and TNFα mRNA stability in thioglycolate-elicited macrophages (TEMP) from IκBβ$^{-/-}$ mice.

a, Characterization of TEMPs. 6- to 8-week-old littermate mice were injected intraperitoneally with 2 ml of 3% thioglycollate broth and three days later, the mice were sacrificed and their peritoneal cavities were washed with 10 ml cold PBS. Cell pellets were washed once with DMEM supplemented with 10% FBS and cultured at the concentration of 5X10^5 cells/ml. 2 hours later the dishes were washed with medium to remove nonadherent cells. TEPMs were stained for CD11b and F4/80 and analyzed by flow cytometry. **b**, WT and IκBβ$^{-/-}$ TEMPs generated as in (a) were stimulated with LPS for 3 hours, after which the transcriptional inhibitor DRB was added (t_0). The degradation curves represent RNA level measured by qRT-PCR normalized to β-actin mRNA level and expressed as a percentage of the level at t_0.



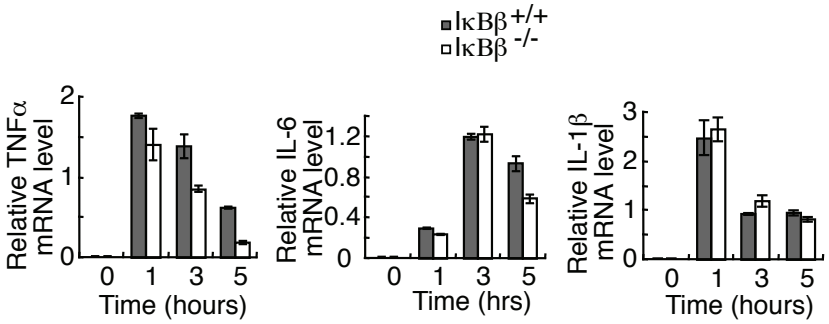
Supplementary Figure 6 | Diversity in NF-κB binding sites in the TNFα promoter

a, Two phosphorylation forms of IκBβ. Raw264.7 cells were treated with LPS (1 mg/ml) for the indicated times. Total cell lysates were prepared and subjected to immunoblotting with the indicated antibodies. **b,** The four NF-κB sites on TNFα promoter. R, N, W and Y denote purine, any nucleotide, A or T, and pyrimidine bases. **c,** Potential p65:c-Rel binding NF-κB sites on the promoter of genes whose expression pattern is similar to TNFα in microarray analysis. Sites were chosen based on the following criteria: fit 9bp κB consensus site preferred by p65 and c-Rel; extra G at 5' end of the consensus sequence is less preferred; five or six A/T base-pairs at the center of binding site is preferred (Huang, D. B., Phelps CB, Fusco AJ, Ghosh G. JOURNAL OF MOLECULAR BIOLOGY, 346, p147-160, 2005) **d,** Raw 264.7 cells were left unstimulated (open bars) or were stimulated with 100ng/ml LPS for 2 hours (closed bars), and were then fixed with 1% formaldehyde for 15 min at room temperature, washed twice with ice-cold PBS, and re-suspended in ChIP lysis buffer. The lysates were subsequently sonicated to shear the genomic DNA into fragments of approximately 200 bp. ChIP analysis was performed on the supernatants with anti-IκBβ, anti-cRel and anti-p65 antibodies, and precipitated DNA were purified and subjected to qPCR with primers flanking each of the indicated sites of respective promoters.



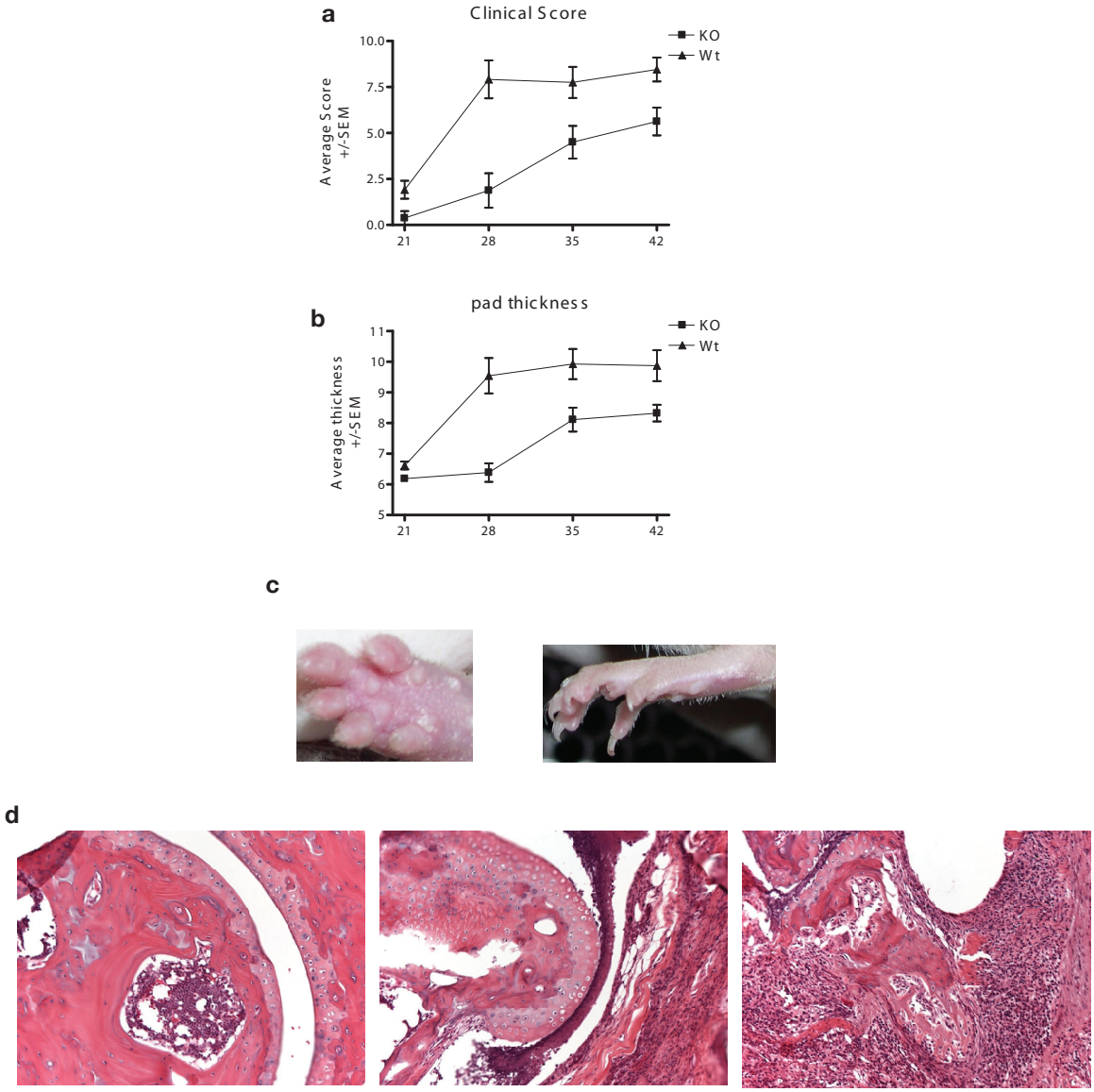
Supplementary Figure 7 | TNFα κB2 is required for optimal activation of TNFα transcription in IκBβ$^{+/+}$ but not in IκBβ$^{-/-}$ macrophages.

IκBβ$^{+/+}$ and IκBβ$^{-/-}$bone marrow derived macrophages were transfected with mouse TNFα luciferase reporters as indicated and phRL-TK (Promega) as an internal control. 24 hours later, the transfected cells were stimulated with LPS for 4 hours and luciferase activity were plotted as reporter (firefly)/control (renilla).



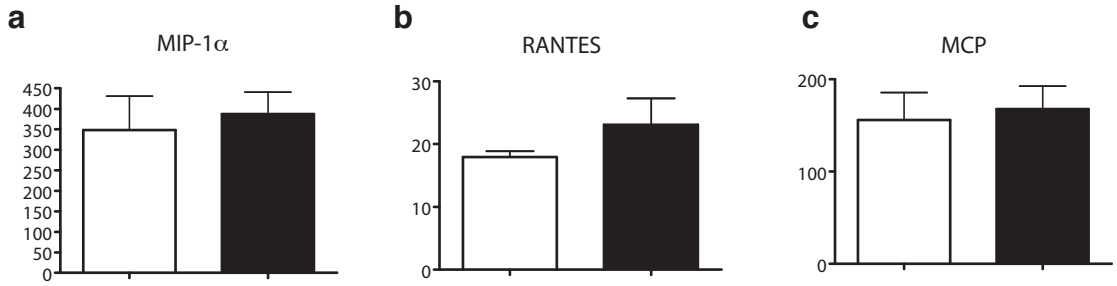
Supplementary Figure 8 | Relative mRNA expression level of TNFα, IL-6 and IL-1β gene.

BMDM from both wild type (*IκBβ*$^{+/+}$) and *IκBβ*$^{-/-}$ were stimulated with LPS (1mg/ml) for the indicated times. Relative mRNA abundance was determined by qRT-PCR (normalized using β-actin mRNA levels).



Supplementary Figure 9 | IκBβ knock out mice exhibited delayed onset and attenuated bone destruction in an experimental model of CIA.

Severity of arthritis, assessed by clinical scoring (a) or paw thickness measurement (b), in wild type DBA or IκBβ knock out mice (backcrossed for eight generations to the DBA background). **c**, Representative examples of the paw swelling in mice from wild type DBA (left) or IκBβ knock out (right) mice at day 28. **d**, Representative histological analysis of H&E-stained (upper) sections of joints from wild type DBA or IκBβ knock out mice. Left panel, largely intact joint of IκBβ knock out mouse (Clinical score 0). Middle panel, joint of IκBβ knock out mouse (Clinical score 2), exhibited serious synovial tissue proliferation and inflammatory infiltration, however, bone and cartilage destruction was minimal. Right panel: joint of a wild type DBA mouse (Clinical score 4), exhibited extensive inflammatory cell infiltration and severe destruction of bone/joint structure.



Supplementary Figure 10 | IκBβ knock out mice exhibited normal production of MIP-α, RANTES and MCP in an experimental model of CIA.

Serum MIP-α (a), RANTES (b), MCP (c) levels in wild type DBA (filled bars) or IκBβ knock out (open bars) mice.