

SUPPLEMENTAL MATERIAL

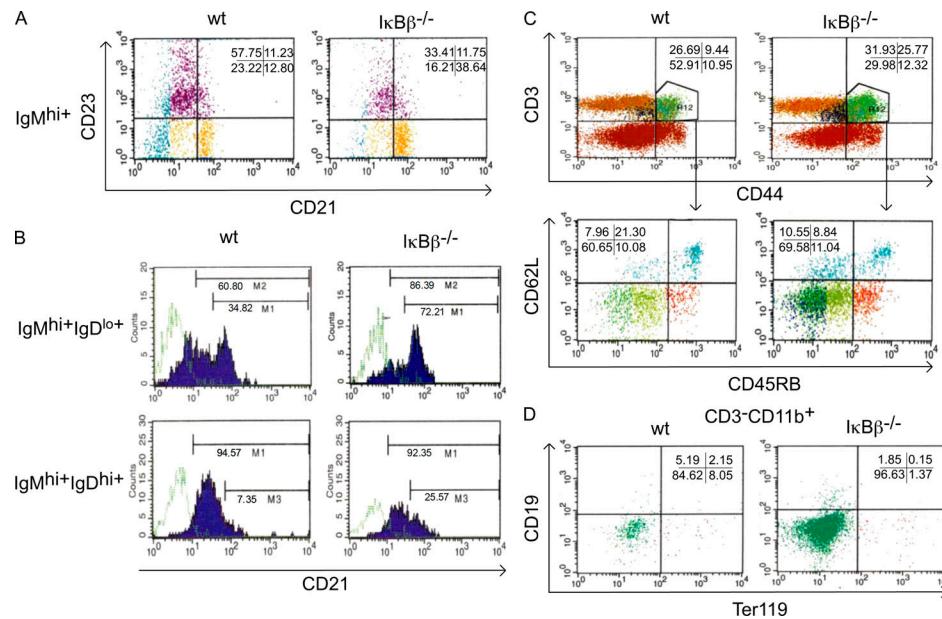
Scheibel et al., <http://www.jem.org/cgi/content/full/jem.20100864/DC1>

Figure S1. $\text{I}\kappa\text{B}\beta^{-/-}$ mice demonstrate an increase of splenic marginal zone B cells and memory T cells and an enforced differentiation of macrophages within the BM. (A) Four-color FACS analysis of isolated spleen cells from WT and $\text{I}\kappa\text{B}\beta^{-/-}$ mice and gating on IgM^{hi}-expressing cells reveals an increase in the percentage of CD23⁻CD21^{hi+} marginal zone B cells and a reduction of CD23⁺CD21^{int} naive B cells. One out of three independent experiments with similar results is shown. (B) Histogram analysis of gated IgM^{hi+Dlo+} and IgM^{hi+Dhi+} demonstrates a similar and higher expression of CD21 on marginal zone and naive B cells, respectively. One out of three independent experiments with similar results is shown. (C) A more detailed analysis of CD3⁺CD44^{hi+} T cells reveals that the obvious increase of memory T cells in the spleen of $\text{I}\kappa\text{B}\beta^{-/-}$ mice is mainly based on the rise of CD45RB⁻CD62L⁻ and CD45RB⁻CD62L⁺ cells. One out of three independent experiments with similar results is shown. (D) Analysis of CD3⁻CD11b⁺ cells within the BM of WT and $\text{I}\kappa\text{B}\beta^{-/-}$ mice discloses a strong increase of CD19⁻Ter119⁻ BM macrophages in $\text{I}\kappa\text{B}\beta^{-/-}$ mice. One out of three independent experiments with similar results is shown.

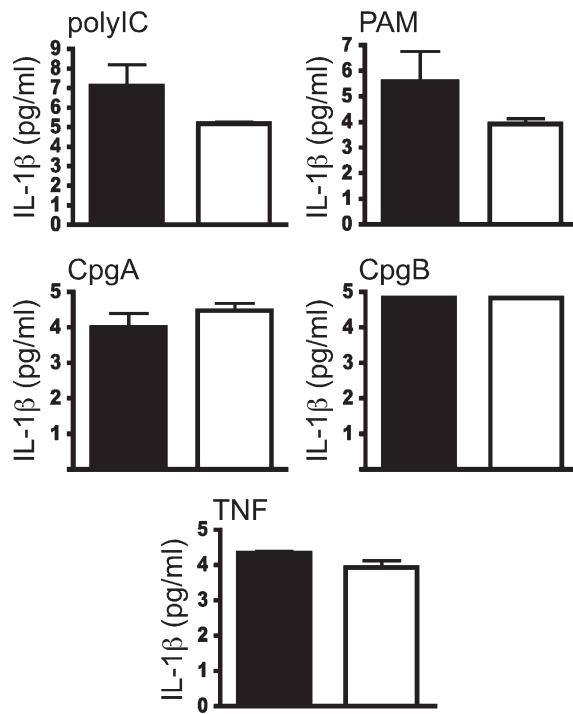


Figure S2. IL-1 β secretion in response to NF- κ B activators in I κ B β $^{-/-}$ BMDMs. WT and I κ B β $^{-/-}$ BMDMs were stimulated with polyI:C (62.5 μ g/ml), PAM3CSK4 (1 μ g/ml), CpgA (1 μ M), CpgB (1 μ M), and TNF (100 ng/ml) as indicated. 24 h after the treatment, IL-1 β secretion was determined by ELISA. Data were obtained from two independent experiments performed in triplicate, and the results are presented as mean and SEM.

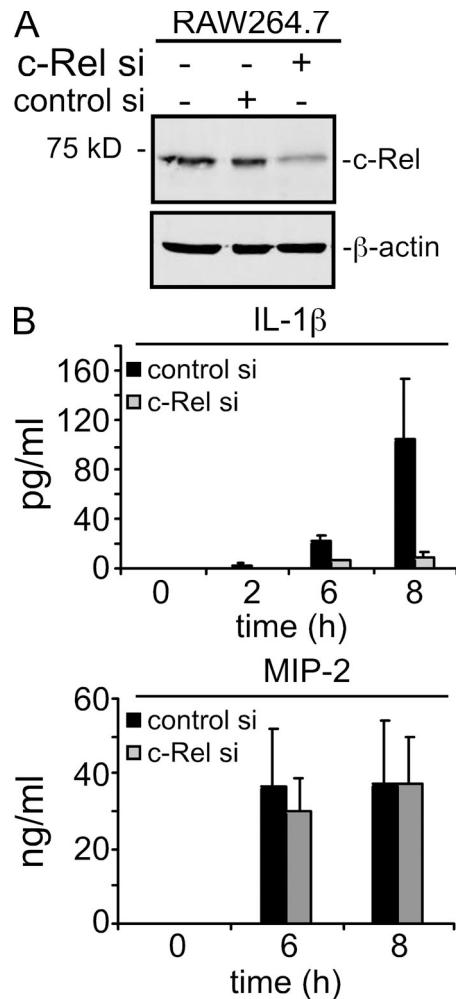


Figure S3. c-Rel knock down results a significant reduction in IL-1 β cytokine expression and secretion. (A) Silencing of c-Rel expression in RAW264.7 macrophages using siRNA. RAW264.7 macrophages were transfected with control or c-Rel-specific siRNAs or were left as an untransfected control. After 48 h, Western blot detected c-Rel expression. β -actin was used as loading control. Knockdown of c-Rel was controlled in at least three independent experiments. (B) Measurement of IL-1 β and MIP-2 cytokine secretion in control and c-Rel siRNA-transfected RAW264.7 cells. 48 h after transfection, cells were treated with LPS (100 ng/ml) for the indicated time points and cytokine secretion was measured by ELISA. Data were obtained from three independent experiments performed in triplicate, and the results are presented as mean and SEM.

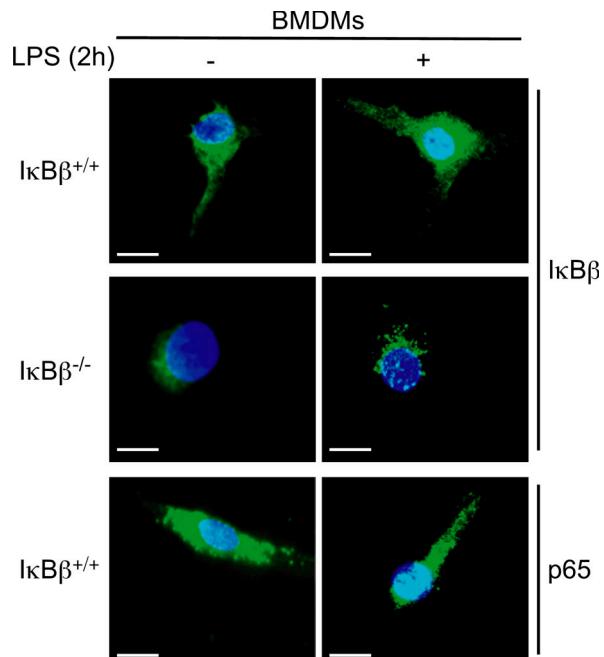


Figure S4. LPS induces the nuclear translocation of IκBβ in BMDMs. Immunohistochemistry demonstrating the nuclear accumulation of IκBβ. WT (first and last row) and IκBβ^{-/-} (middle row) BMDMs were stimulated with LPS (100 ng/ml) for 2 h and stained with IκBβ- and RelA/p65-specific primary antibodies, followed by FITC-labeled secondary antibodies (green) as indicated. Nuclei were counterstained with DAPI (blue). Overlay, pale blue. Bars 5 μm. One out of three independent experiments with similar results is shown.

Table S1. $\text{I}\kappa\text{B}\beta$ -dependent LPS-induced genes in BMDM

Title	Gene symbol	Gene Id	Fold induction	Fold induction
			WT	KO
chemokine (C-X-C motif) ligand 1	Cxcl1	14825	292.2	87.6
interleukin 1 beta	Il1b	16176	41.5	4.3
suppressor of cytokine signaling 3	Socs3	12702	33.3	16.5
tumor necrosis factor, alpha-induced protein 3	Tnfaip3	21929	31.5	10.7
interleukin 1 alpha	Il1a	16175	24.4	13.1
oxidized low density lipoprotein (lectin-like) receptor 1	Olr1	108078	21.0	7.2
tumor necrosis factor (ligand) superfamily, member 9	Tnfsf9	21950	19.3	7.0
Notch gene homolog 4 (<i>Drosophila</i>)	Notch4	18132	12.5	0.7
interleukin 12b	Il12b	16160	11.1	3.5
phosphodiesterase 4B, cAMP specific	Pde4b	18578	10.6	6.1
ras homolog gene family, member E	Rnd3	74194	10.2	4.1
CDC42 effector protein (Rho GTPase binding) 2	Cdc42ep2	104252	9.6	4.2
inhibitor of DNA binding 3	Id3	15903	9.6	3.4
Jun dimerization protein 2	Jdp2	81703	9.1	3.9
gap junction membrane channel protein alpha 1	Gja1	14609	8.7	2.5
serine (or cysteine) proteinase inhibitor, clade E, member1	Serpine1	18787	7.9	2.4
monocyte to macrophage differentiation-associated	Mmd	67468	7.9	1.7
G protein-coupled receptor 84	Gpr84	80910	7.8	3.4
calcitonin receptor-like	Calcr1	54598	7.5	2.9
tumor necrosis factor receptor superfamily, member 5	cd40	21939	7.3	2.2
regulator of calcineurin 1	Rcan1	54720	7.1	2.2
G protein-coupled receptor 85	Gpr85	64450	7.1	2.5
early growth response 2	Egr2	13654	7.0	1.8
selenium binding protein 1	Selenbp1	20341	6.5	2.7
DnaJ (Hsp40) homolog, subfamily C, member 2	Dnajc2	22791	6.4	2.0
pellino 1	Peli1	67245	6.4	2.7
jagged 1	Jag1	16449	6.1	1.9
proviral integration site 1	Pim1	18712	6.0	3.1
syndecan 4	Sdc4	20971	6.0	1.6
integrin alpha 5 (fibronectin receptor alpha)	Itga5	16402	5.9	1.4
tumor necrosis factor, alpha-induced protein 2	Tnfaip2	21928	5.8	3.2
phospholipid scramblase 1	Plscr1	22038	5.6	1.3
potassium voltage-gated channel, shaker-related, subfamily, member 3	Kcnq3	16491	5.5	2.1
tripartite motif protein 13	Trim13	66597	5.3	3.1
Mediterranean fever	Mefv	54483	5.2	2.2
tumor necrosis factor receptor superfamily, member 1b	Tnfrsf1b	21938	5.0	1.5