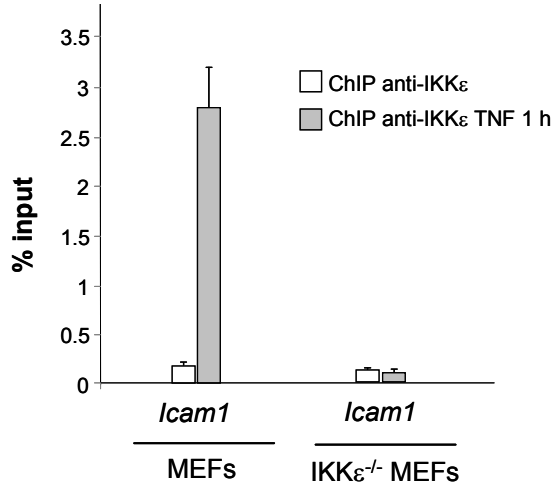
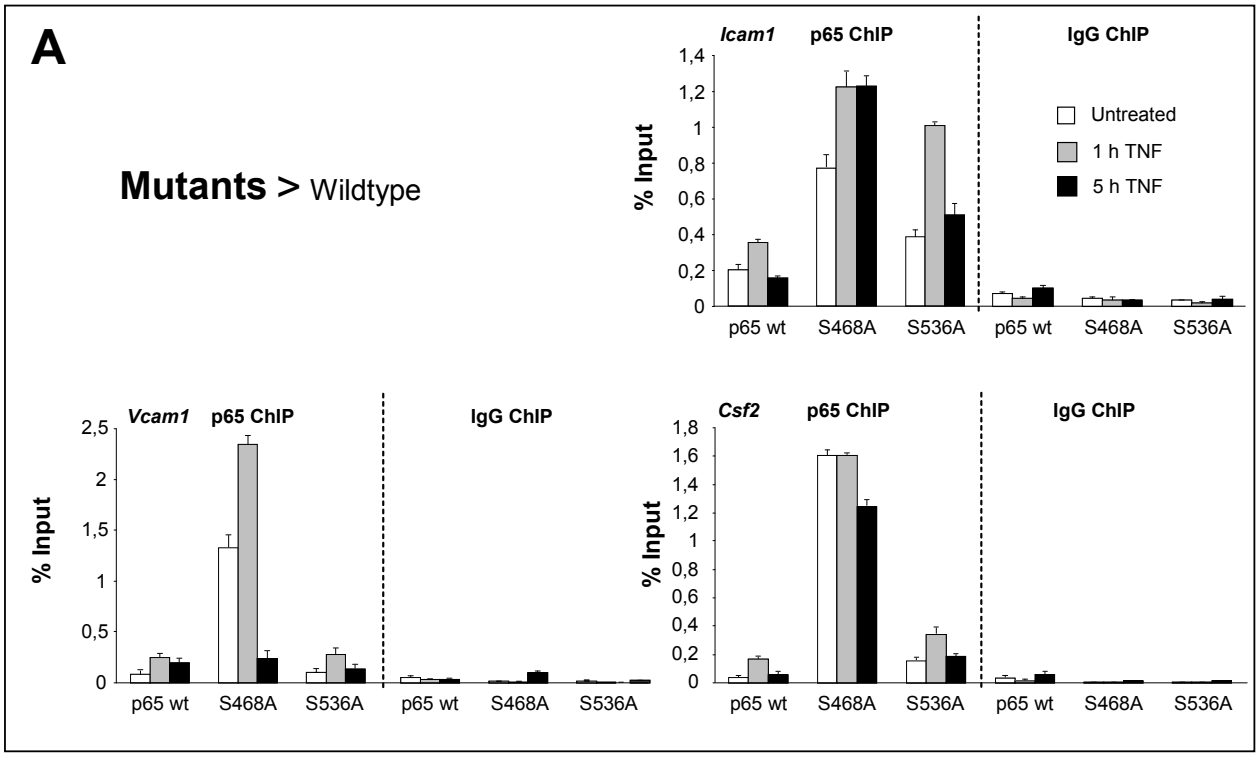
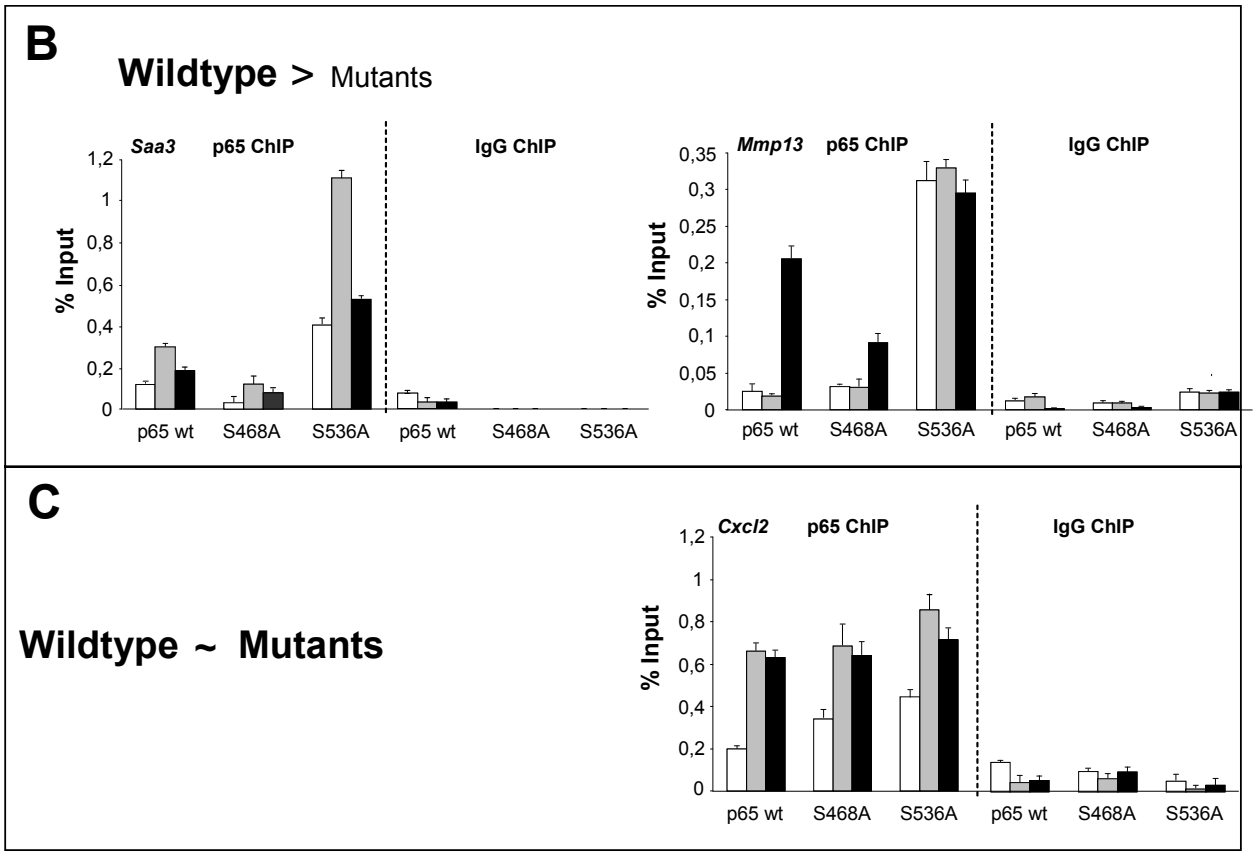
Supplementary Data

Supplementary Figures

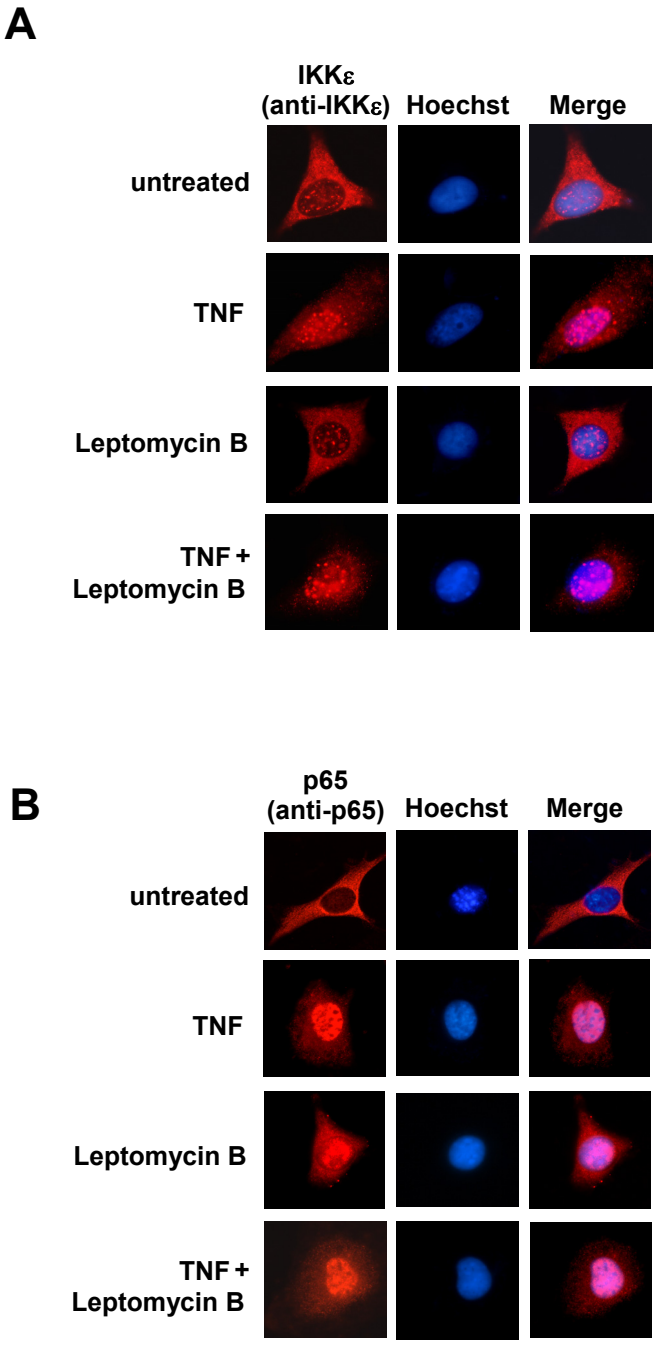


Supplementary Fig. 1. Wildtype MEFs and *Ikbke*-deficient MEFs were stimulated with TNF for 1 hr, followed by ChIP analysis using the indicated specific and anti-IgG control antibodies. IKKε association with the *Icam1* gene promoter region was detected by real-time PCR using specific primers, error bars show standard deviations.

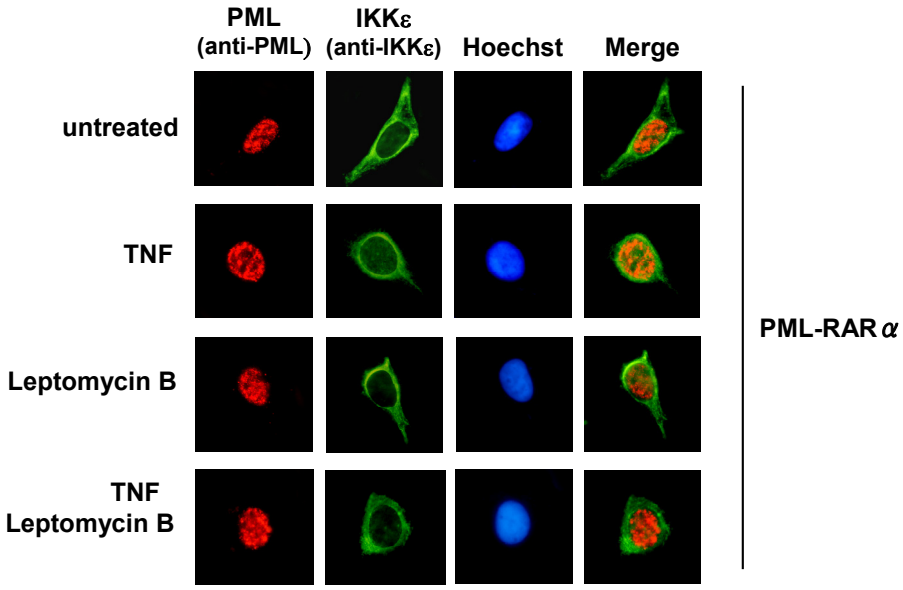




Supplementary Fig. 2. Analysis of phosphorylation-dependent p65 promoter occupancy. $p65^{-/-}$ MEFs stably reconstituted to express p65 and the indicated mutants were stimulated with TNF for 1 and 5 hr. ChIP assays were performed using anti-p65 or anti-IgG control antibodies. Binding of p65 to the indicated promoters was quantified by real-time PCR using specific primers. Experiments were performed in triplicates, error bars display standard deviations. The analyzed promoters are arranged in three groups (A-C) according to the regulation of the target genes by p65 phosphorylation. The amounts of p65-associated DNA are presented as the percentage recovered out of the total input DNA (percent input).



Supplementary Fig. 3. (A) HeLa cells were incubated for 4 hr with leptomycin B (10 ng/mL) or left untreated prior to TNF stimulation for 30 min. Subcellular localization of endogenous IKKε was visualized using an anti-IKKε antibody (red). (B) The experiment was done as in (A) with the exception that localization of the endogenous p65 protein was analyzed.



Supplementary Fig. 4. (A) HeLa cells were transfected to express the PML-RARα fusion protein. 36 hr after the transfection, cells were incubated for 4 hr with Leptomycin B or left untreated prior to TNF stimulation for 30 min. Subcellular localization of PML and endogenous IKKε was visualized using an anti-PML (red) and anti-IKKε antibody (green).

Supplementary Materials and methods

Real-time PCR for the quantification of gene expression

The following primers were used to quantify the murine NF-κB target genes:

CSF2_FW 5'-TCCTGGGCATTGTGGTCT-3'
CSF2_RV 5'-CGGGTGACAGTGATGGGT-3'
VCAM-1_FW 5'-AGTTGGGGATTCGGTTGTTCT-3'
VCAM-1_RV 5'-CCCCTCATTCCTTACCACCC-3'
CXCL2_FW 5'-AGTGAACTGCGCTGTCAATG-3'
CXCL2_RV 5'-CTTCAGGGTCAAGGCAAACT-3'
ICAM-1_FW 5'-GGAGACGCAGAGGACCTTAAC-3'
ICAM-1_RV 5'-CGCTCAGAAGAACCACCTTC-3'
SAA3_FW 5'-CTGTTCAGAAGTTCACGGGAC-3'
SAA3_RV 5'-AGCAGGTCGGAAGTGGTT-3'
MMP13_FW 5'-ACCTCCACAGTTGACAGGCT-3'
MMP13_RV 5'-AGGCACTCCACATCTTGGTTT-3'
MMP3_FW 5'-ACCTATTCCTGGTTGCTG-3'
MMP3_RV 5'-GCCTTGGCTGAGTGGTAG-3'

COX-2_FW 5′-TCTCCAACCTCTCCTACTAC-3′
COX-2_RV 5′-GCACGTAGTCTTCGATCACT-3′
IL6_FW 5′-TGGATGCTACCAAACTGGAT-3′
IL6_RV 5′-GGACTCTGGCTTTGTCTTTC-3′
IP10_FW 5′-AATCATCCCTGCGAGCCTAT-3′
IP10_RV 5′-TTTGGCTAAACGCTTTCATT-3′
β-ACTIN_FW 5'-GAGATTACTGCTCTGGCTCCTA-3'
β-ACTIN_RV 5'-TCATCGTACTCCTGCTTGCT-3'.

ChIP and re-ChIP assays

The following primers were used to quantify the genomic DNA fragments containing a NF-κB binding site.

CSF2_FW 5'-CCTGGCTTCTATACCC-3'
CSF2_RV 5'-CTCACAAGTCCACCTCA-3'
VCAM-1_FW 5'-TCAGCCCAGAAAGCAGC-3'
VCAM-1_RV 5'-AAAGTGTTCAGCCTCCA-3'
CXCL2_FW 5'- AGGGCAGGGCAGTAGAATGA -3'
CXCL2_RV 5'- TGTGGCTGGAGTCTGGAGTG -3'
ICAM-1_FW 5'-AGGGGACTAGGCAGTAGTCAATCAG-3'
ICAM-1_RV 5'-GAACGAGGGCTTCGGTATTT-3'
SAA3_FW 5'-ATTATGGGTAAGTGGG-3'
SAA3_RV 5'-GCTGAGGAGATGTGGC-3'
MMP13_FW 5'-GCAGACATTTTCCTTA-3'
MMP13_RV 5'-ATGTTTGTGACTTGGA-3'