Supporting Information

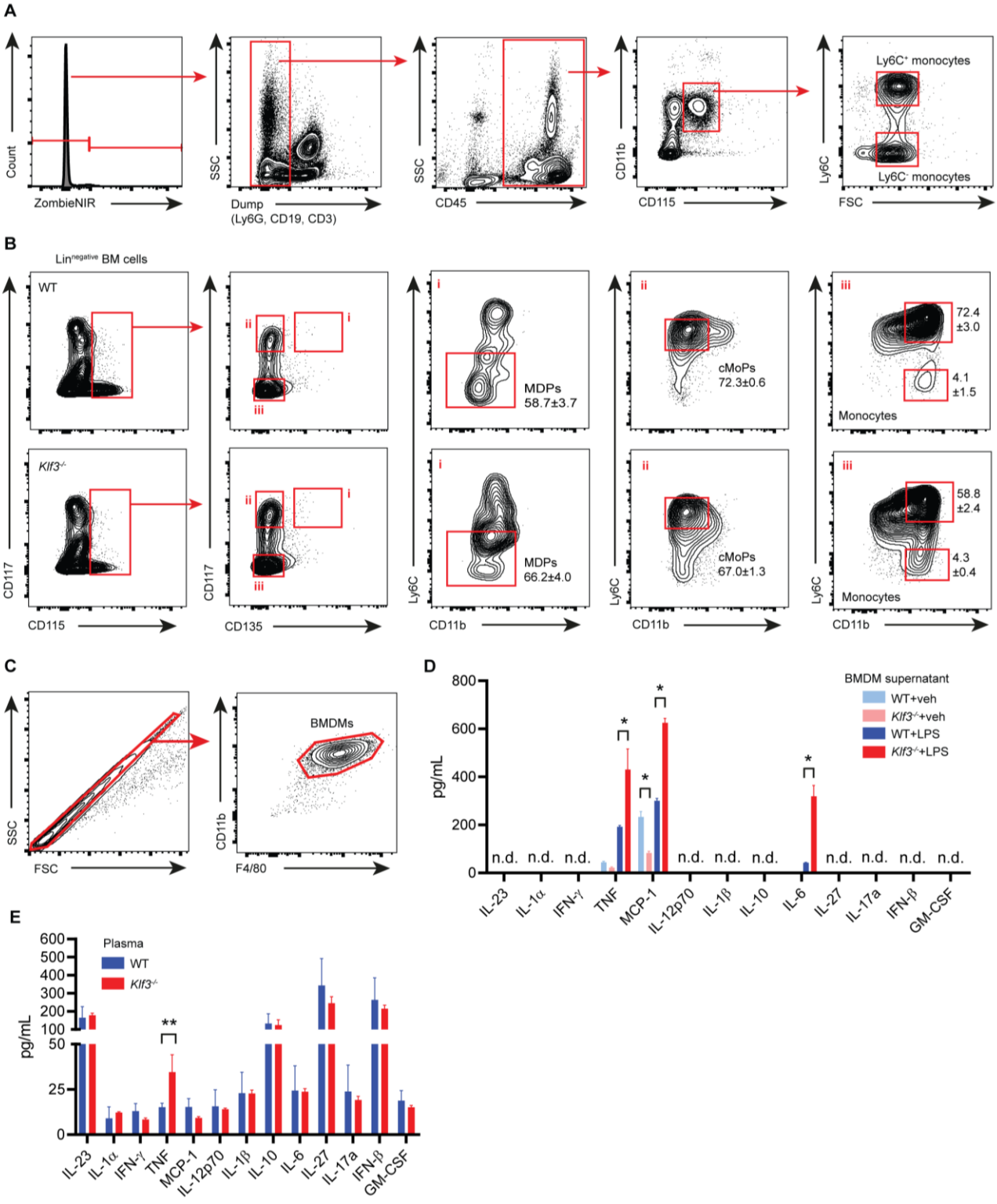

Figure S1. Related to Figure 1. Pronounced systemic inflammation in the absence of KLF3. (A) Gating strategy for identifying CD115^{+} Ly6C$^{+/-}$ monocytes, defined as live (ZombieNIR^{-}) Ly6G^{-} CD3^{-} CD19^{-} CD45^{+} CD11b^{+}

$CD115^{+}$ $Ly6C^{+/-}$. (B) Quantification of monocyte precursor populations in the bone marrow of WT and *Klf3*$^{-/-}$ mice (n=4). Lineage exclusion was performed ($Ter119^{-}$ $CD19^{-}$ $CD3^{-}$ $NK1.1^{-}$ $Ly6G^{-}$ $TCR\delta^{-}$) on live single cells, upon which monocyte precursors were defined as $CD117^{+/-}$ and $CD115^{+}$. This population was divided into three subsets based on expression of CD117 and CD135: (i) $CD117^{+}$ $CD135^{+}$ $Ly6C^{-}$ $CD11b^{-}$ macrophage-dendritic cell progenitors (MDPs), (ii) $CD117^{+}$ $CD135^{-}$ $Ly6C^{+}$ $CD11b^{-}$ common monocyte progenitors (cMoPs) and (iii) $CD117^{-}$ $CD135^{-}$ $CD11b^{+}$ $Ly6C^{+/-}$ monocytes. Plots are representative of four biological replicates and means±SEM are given as a percentage of parent population. (C) Gating strategy to confirm purity of $CD11b^{+}$ $F4/80^{+}$ BMDMs by flow cytometry. LEGENDplex kits were used to quantify inflammatory cytokines in (D) supernatants from WT and *Klf3*$^{-/-}$ BMDMs treated with vehicle (veh) or 100 ng/mL LPS for 24 h (n=3 per genotype/treatment group) and in (E) WT (n=5) and *Klf3*$^{-/-}$ (n=7) plasma. Error bars are representative of means±SEM. Non-parametric Mann-Whitney U tests were performed to assess significance between genotypes within the same condition for each cytokine tested, where **P*<0.05 and ***P*<0.01. n.d: not detected.

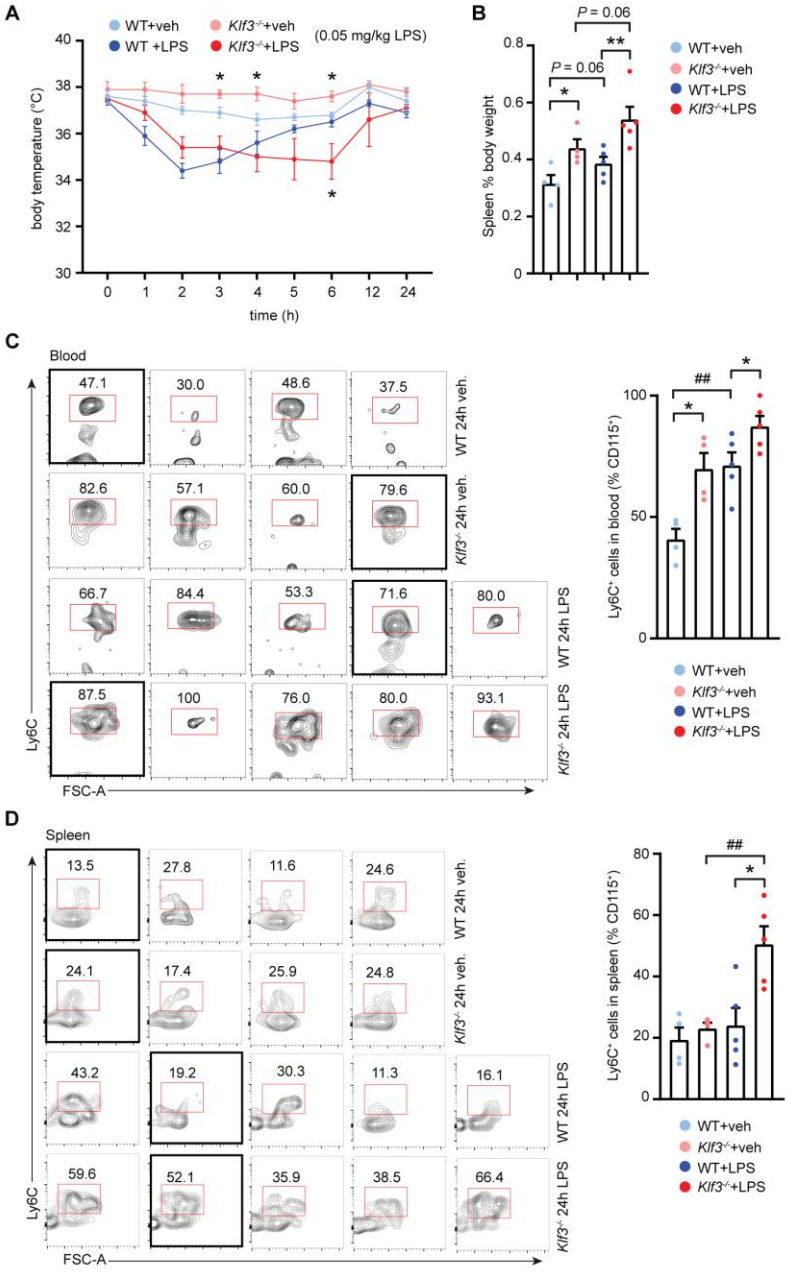

Figure S2. Related to Figure 2. Mice lacking KLF3 show a heightened and prolonged inflammatory response to endotoxin treatment. (A) WT and *Klf3*$^{-/-}$ mice were intraperitoneally administered with vehicle (veh) or 0.05 mg/kg LPS and their body temperature was measured by rectal probing over 24 h, with error bars representative of SEM (veh: n=3 per genotype and LPS: n=6 per genotype). (B) Spleens were harvested from mice treated with 0.167 mg/kg LPS, then weighed. Error bars represent the means±SEM (veh: n=4 per genotype and LPS: n=5 per genotype). Ly6C$^{+/-}$ monocyte abundance in the (C) blood and (D) spleen of WT and *Klf3*$^{-/-}$ mice given 0.167 mg/kg LPS or vehicle was measured by flow cytometry and all final gating plots shown. Abundances are shown as a percentage of the parent population, CD115^{+} monocytes, and accompanying column graphs are shown with error bars representing the means±SEM. Representative median plots, outlined in bold, were used in Figure 2G-H. For (A-D), non-parametric Mann-Whitney U tests were performed to assess statistical significance where *P<0.05,

**P<0.01 between genotypes and ##P<0.01 between conditions within the same genotype. FSC-A: forward scatter (area)

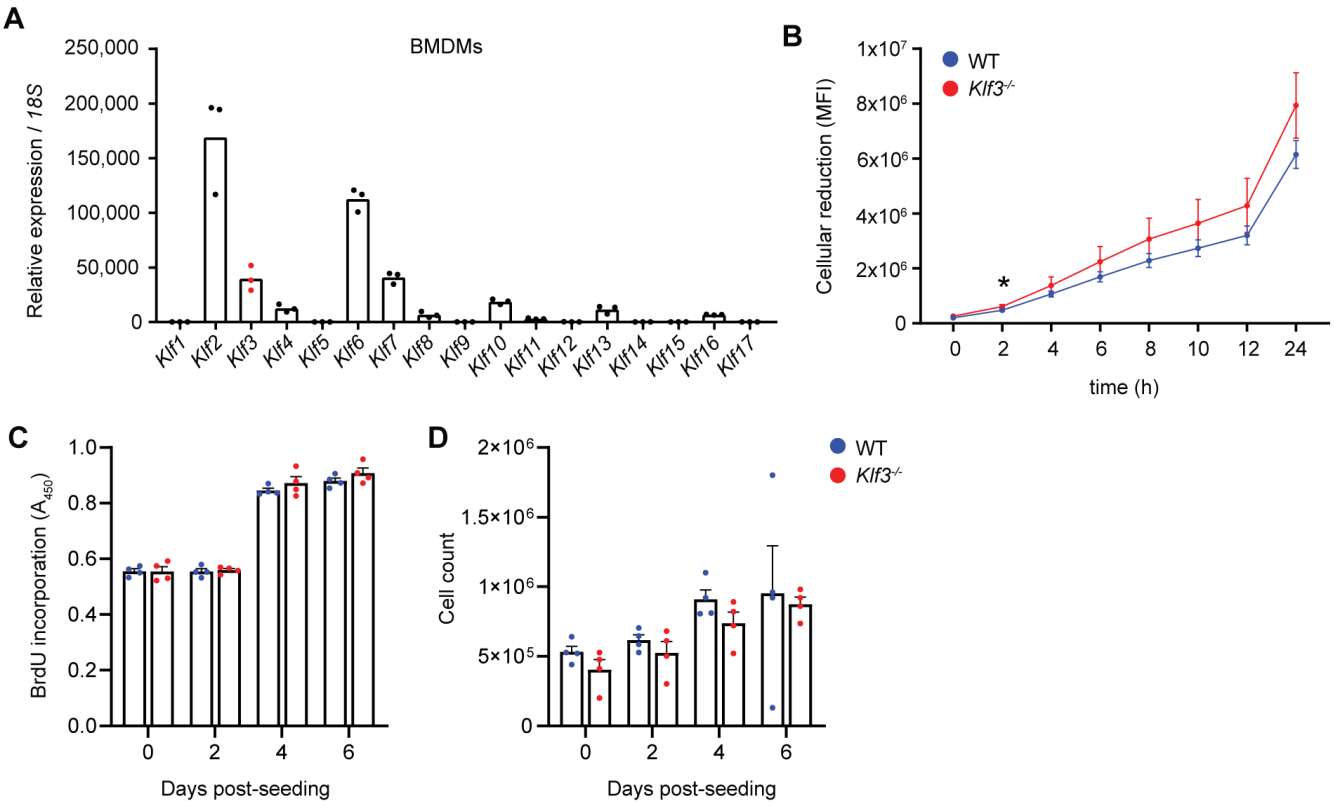

Figure S3. Related to Figure 3. Macrophages lacking KLF3 exhibit enhanced inflammatory function. (A) mRNA expression of all *Klf* family member genes was assessed in BMDMs using qPCR to determine the relative expression of *Klf3*, with values normalised to expression of *18S* rRNA levels. (B) AlamarBlue assays were conducted to assess the cellular reduction environment by measuring conversion of resazurin blue substrate in BMDMs over a 24 h period (n=8). (C) WT and *Klf3*$^{-/-}$ BMDMs were seeded then BrdU incorporation assays performed at days 0, 2, 4 and 6. Absorbance readout was obtained at 450 nm to quantify BrdU incorporation of actively replicating cells (n=4). (D) BMDM growth rate was assessed by monitoring cell counts 0, 2, 4 and 6 days post-seeding (n=4). For (B-D), error bars are representative of means±SEM of biological replicates, and non-parametric Mann-Whitney U tests were carried out where **P*<0.05.

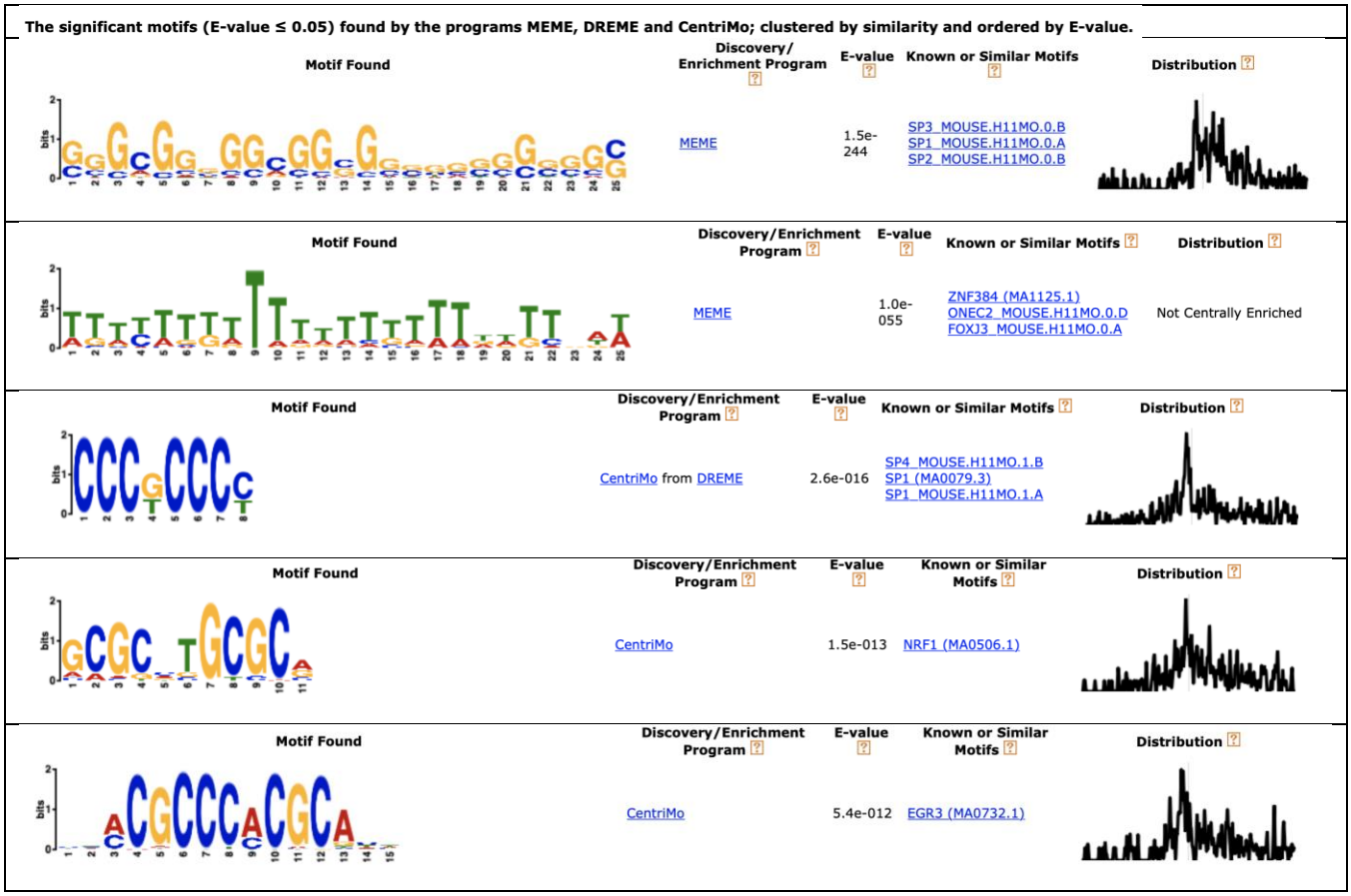

Figure S4. Related to Figure 4. The promoter regions of differentially expressed genes between WT and *Klf3*$^{-/-}$ BMDMs treated with LPS for 8 h are enriched for Sp/KLF family binding sites. *De novo* motif discovery and known motif enrichment analysis using MEME-ChIP on differentially expressed genes from microarray analysis comparing WT and *Klf3*$^{-/-}$ BMDMs treated with LPS for 8 h. The top 5 most highly enriched motifs and the transcription factors that are known to bind to these or similar motifs from the MEME-ChIP analysis are shown. The first and third most highly enriched motifs match known motifs for the Sp/KLF transcription factor family, which includes KLF3.

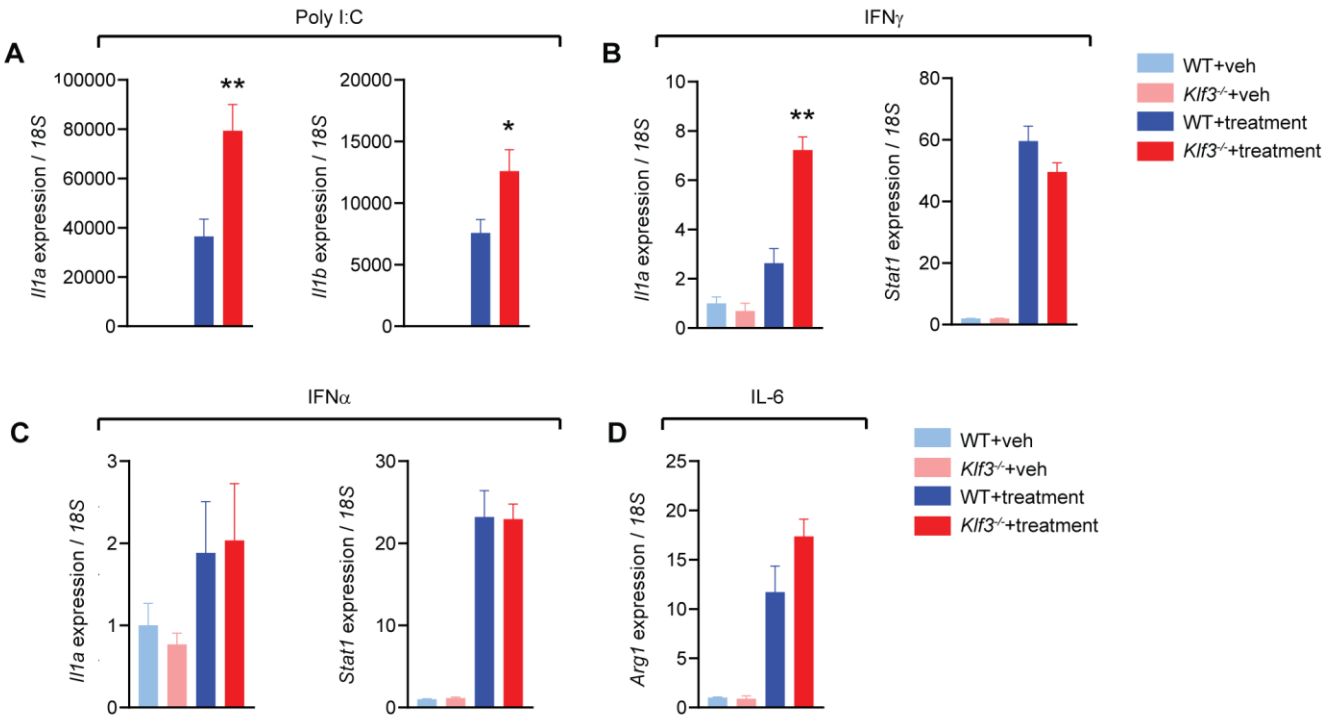

Figure S5. Related to Figure 4. Stimulation of macrophages with ligands which trigger NF-κB-dependent or -independent inflammatory pathways suggests that the effect of KLF3 deficiency on gene expression is specific to the NF-κB pathway. WT and *Klf3*$^{-/-}$ BMDMs were treated for 8 h with vehicle or 10 μg/mL poly I:C (A), 10 ng/mL IFNγ (B), 10 ng/mL IFNα (C) and 10 ng/mL IL-6 (D). Gene expression was normalised to *18S* rRNA levels and the WT vehicle set to 1. Error bars are representative of the means±SEM of four biological replicates for each experiment. Non-parametric Mann Whitney U tests were carried out where **P*<0.05 and ***P*<0.01.

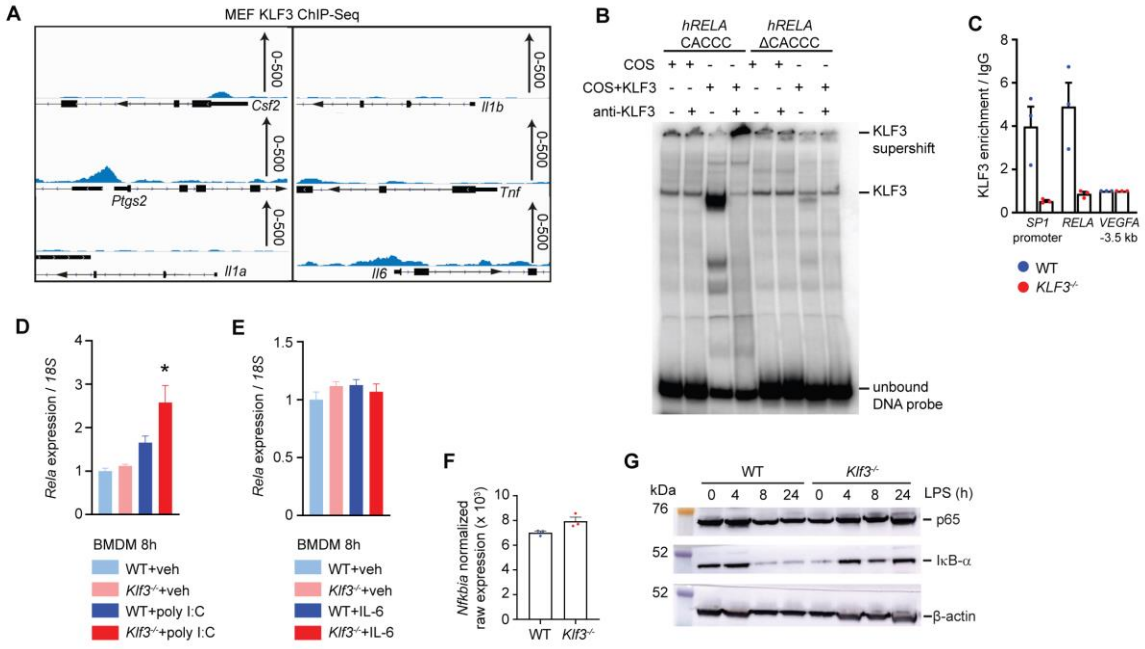

Figure S6. Related to Figure 5. KLF3 suppresses activation of NF-κB genes. (A) KLF3-V5 ChIP-Seq in murine embryonic fibroblasts showing KLF3 enrichment at promoter regions cytokine genes deregulated in *Klf3*$^{-/-}$ BMDMs (Accession No. GSE44748) (35). (B) EMSA was used to detect KLF3 binding to a radiolabelled DNA probe comprised of the wild type (CACCC) or mutated (ΔCACCC) human *RELA* promoter consensus sequence. Nuclear extracts from COS-7 cells over-expressing pMT3-Klf3 and untransfected COS-7 cells were used as positive and negative controls respectively. Polyclonal anti-KLF3 antibody (α-KLF3) raised in rabbit was added to confirm the identity of bound protein as KLF3, labelled as a supershift band. Unbound radiolabelled DNA probe can be seen as an intense dark band at the bottom of the gel. (C) *In vivo* KLF3 binding at the human *RELA* promoter in WT and *KLF3*$^{-/-}$ K562 cells was performed using ChIP-qPCR (n=3 individual clonal populations per genotype). The *SP1* promoter serves as a positive control region, where KLF3 is known to bind, and *VEGFA* is a negative control locus. Error bars represent means±SEM. WT and *Klf3*$^{-/-}$ BMDMs were treated for 8 h with vehicle (veh), 10 μg/mL poly I:C (D) or 10 ng/mL IL-6 (E) and expression of *Rela* normalized to *18S* levels. The WT veh treatment was set to 1. For (D-E), error bars represent means±SEM and non-parametric Mann-Whitney U tests were used to assess significance between genotypes under each condition where *P<0.05. (F) Normalized raw expression of *Nfkbia* from microarrays in WT and *Klf3*$^{-/-}$ BMDMs treated with 100 ng/mL LPS for 8 h (n=3). Error bars represent the means±SEM. (G) Total cellular levels of the NF-κB p65 subunit (RELA) and IκB-α were measured in WT and *Klf3*$^{-/-}$ BMDMs following stimulation with 100 ng/mL LPS for 0-24 h. 20 μg of whole cell extract was loaded per lane alongside a Rainbow Molecular Weight Marker (GE Healthcare). Membranes were probed with a monoclonal anti-p65 antibody (raised in rabbit, 65 kDa), monoclonal anti-IκB-α (raised in rabbit; ab32518) and anti-β-actin antibody (raised in mouse; A1978).

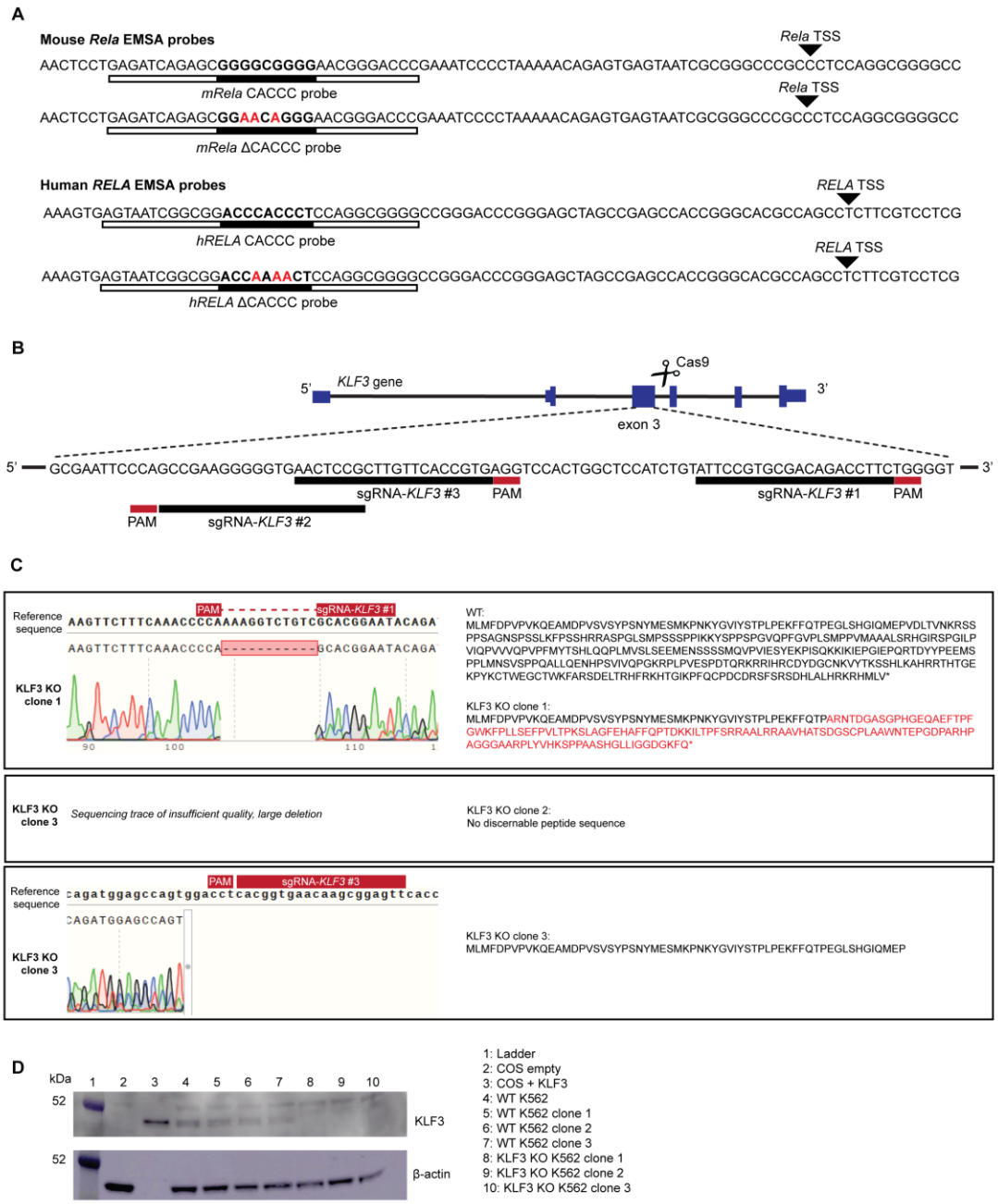

Figure S7. Related to Figure 5. KLF3 suppresses activation of NF-κB genes. (A) EMSA probes designed to encompass the KLF3 binding site (CACCC box; bold) in the mouse and human *Rela/RELA* promoters, and mutated probes (mutated base pairs in red) to show loss of KLF3 binding. (B) The CRISPR/Cas9 *KLF3* knockout strategy, utilising three separate guide RNAs (sgRNA) to recruit Cas9 to cut at exon 3. (C) Sanger sequencing traces for *KLF3*$^{-/-}$ K562 clones compared to reference sequence, showing disruptions to the *KLF3* gene and peptide sequence. (D) Western blot showing the presence or absence of KLF3 (~48 kDa) in WT (lanes 5-7) and *KLF3*$^{-/-}$ (lanes 8-10) K562 nuclear extracts. Extracts from untransfected COS-7 cells (lane 2), COS-7 cells over-expressing pMT3-Klf3 (lane 3) and WT K562 cells (lane 4) were used as control lanes, and for testing the specificity of the polyclonal anti-KLF3 antibody (Thermo Fisher). Nitrocellulose membranes were probed for β-actin using an anti-β-actin antibody (Sigma), and size determined with reference to a Rainbow Molecular Weight Marker (GE Healthcare; lane 1). TSS: transcription start site. PAM: proto-spacer adjacent motif. kDa: kilodaltons. COS: COS-7 cells.

Table S1. Mammalian expression plasmids

Plasmid name	Source	Application
pSpCas9(BB)-2A-GFP (PX458)	Feng Zhang (Addgene plasmid 48138)	CRISPR/Cas9 genome editing
pMT3-*Klf3*	Jose Perdomo, University of Sydney	*Klf3* over-expression in COS-7 cells

Table S2. Oligonucleotides for CRISPR/Cas9 genome editing

Oligo name	Sequence
hKLF3 ex3 sgRNA#1 Fwd	CACCGATTCCGTGCGACAGACCTTC
hKLF3 ex3 sgRNA#1 Rev	AAACGAAGGTCTGTCGCACGGAATC
hKLF3 ex3 sgRNA#2 Fwd	CACCGAACTCCGCTTGTTCACCGTG
hKLF3 ex3 sgRNA#2 Rev	AAACCACGGTGAACAAGCGGAGTTC
hKLF3 ex3 sgRNA#3 Fwd	CACCGCGGAGTTCACCCCCTTCGGC
hKLF3 ex3 sgRNA#3 Rev	AAACGCCGAAGGGGGTGAACTCCGC
hKLF3 ex3 PCR Fwd/Sequencing	CTTATTTGGCTGTTGACACG
hKLF3 ex3 PCR Rev	GGCAGATGAGTATTTCCTTTG

Table S3. EMSA probes

Probe name	Sequence
CACCC sense	TAGAGCCACACCCTGGTAAG
CACCC antisense	CTTACCAGGGTGTGGCTCTA
mRela CACCC sense	GAGATCAGAGCGGGGCGGGGAACGGGACCC
mRela CACCC antisense	GGGTCCCGTTCCCCGCCCCGCTCTGATCTC
mRela ΔCACCC sense	GAGATCAGAGCGGAACAGGGAACGGGACCC
mRela ΔCACCC antisense	GGGTCCCGTTCCCTGTTCCGCTCTGATCTC
hRELA CACCC sense	AGTAATCGGCGGACCCACCCTCCAGGCGGGG
hRELA CACCC antisense	CCCCGCCTGGAGGGTGGGTCCGCCGATTACT
hRELA ΔCACCC sense	AGTAATCGGCGGACCAAAACTCCAGGCGGGG
hRELA ΔCACCC antisense	CCCCGCCTGGAGTTTTGGTCCGCCGATTACT

Table S4. Quantitative real-time PCR primers

Oligo name	Sequence
18S rRNA Fwd	CACGGCCGGTACAGTGAAAC
18S rRNA Rev	AGAGGAGCGAGCGACCAA
mKlf1 Fwd	AGACTGTCTTACCCTCCATCAGTACA
mKlf1 Rev	CCGCCACCACTTGAGGAA
mKlf2 Fwd	ACCAAGAGCTCGCACCTAAA
mKlf2 Rev	TCCTTCCCAGTTGCAATGAT
mKlf3 exon 4/5 Fwd	GAAATGTCACCCCCTTTAATGAAC
mKlf3 exon 4/5 Rev	CACGATGACGGAAGGATGGT
mKlf3 exon 1b Fwd	AGAAATAATTGATGAGAGGCACAGATT
mKlf3 exon 1b Rev	ATGTCTGGCCTCACTCTTCCA
mKlf4 Fwd	GTGGCAAAACCTATACCAAGAGTTC
mKlf4 Rev	GGTAGTGCCTGGTCAGTTCATC
mKlf5 Fwd	CCGGAGACGATCTGAAACAC
mKlf5 Rev	GGAGCTGAGGGGTCAGATACTT
mKlf6 Fwd	CACATCAGCGCACTCACACA
mKlf6 Rev	CAAAACGCCACTCACAACCTT
mKlf7 Fwd	TCCCACTTAAAGGCCCACC
mKlf7 Rev	TGTGTGTTTCCTGTAGTGCC
mKlf8 Fwd	TGGTTCGATGCAGGTATTCA
mKlf8 Rev	GGGGCATGTTGAAATCACTC
mKlf9 Fwd	ATACAGGTGAACGGCCCTTT
mKlf9 Rev	TCACTCCTCATGAAGCGCTT
mKlf10 Fwd	GCAGCCAACCATGCTCAAC
mKlf10 Rev	CCCCTCTCTGGGCTTTTCAG
mKlf11 Fwd	ACTCTGTGTATAACTCCTCCTCA
mKlf11 Rev	CAGTGACCATGCATCCTTTG
mKlf12 Fwd	CGCCCTTGAGAACAGAATGC
mKlf12 Rev	GGGTAGTTGTGGACGTTTGGA

mKlf13 Fwd	CGAGAAAGTTTACGGGAAATCTTC
mKlf13 Rev	CAGGCGAAAGGCCTCTCA
mKlf14 Fwd	TCCATGGACCGGTTCCAT
mKlf14 Rev	AGAGCCACAGACAGCGGTTAG
mKlf15 Fwd	TACACCAAGAGCAGCCACCT
mKlf15 Rev	AACTCATCTGAGCGGGAAAA
mKlf16 Fwd	TCACACCTGCGGACTCACA
mKlf16 Rev	CAGAACGGGCGAACTTCTTG
mKlf17 Fwd	GCAGGACAATAAGGAACAGGC
mKlf17 Rev	AAGTATTGAGTGGCTGGTGG
mTnf Fwd	GCCACCACGCTCTTCTGTCT
mTnf Rev	GCCATAGAACTGATGA
mIl6 Fwd	AAAGCCAGAGTCCTTCAGAGAGATAC
mIl6 Rev	CTGTTAGGAGAGCATTGGAAATTG
mCsf2 Fwd	TTGGAAGCATGTAGAGGCCA
mCsf2 Rev	CGGGTCTGCACACATGTTAG
mIl1a Fwd	ACAGTTCTGCCATTGACCATC
mIl1a Rev	CCTTGAAGGTGAAGTTGGACA
mIl1b Fwd	TGTAATGAAAGACGGCACACC
mIl1b Rev	TCTTCTTTGGGTATTGCTTGG
mPtgs2 Fwd	GAACAACATCCCCTTCCTGC
mPtgs2 Rev	AGAGGTTGGAGAAGGCTTCC
mRela Fwd	GCGAATCCAGACCAACAATAAC
mRela Rev	CTGTCACCTGGAAGCAGAG
mArg1 Fwd	CTCCAAGCCAAAGTCCTTAGAG
mArg1 Rev	AGGAGCTGTCATTAGGGACATC
mStat1 Fwd	TCCCGTACAGATGTCCATGA
mStat1 Rev	ACTTTACTGTCCAGCTCCTTCT
mLgals3 positive control Fwd	TGGAAAAACACCCGTGCCTCTGA
mLgals3 positive control Rev	CAGTGCCTACGCCCAGATGACTC

mRela promoter Fwd	TGAGATCAGAGCGGGGCG
mRela promoter Rev	GGCCCGACTACAAGCTCC
mKlf8 -4.5 kb negative control Fwd	GGTTTCTGAGACCTAACACTTCACACT
mKlf8 -4.5 kb negative control Rev	CCATTTAGTCATCCAGCGAACAA
hSP1 promoter Fwd	ACCTCTCCGCCCACTAGGA
hSP1 promoter Rev	CAACGGCCAACCAGAATCC
hVEGFA -3.5 kb Fwd	ACTGGGTCTTGCTGTTTTCC
hVEGFA -3.5 kb Rev	TTCAGGCTGTGAACCTTGG
hRELA promoter Fwd	TTTAGCTGGAGAGGGACGAG
hRELA promoter Rev	CCTGACAGTGCATCAAGAGC

Table S5. Antibodies

Antibody name	**Raised in**	**Supplier/Product#**	**Application**
Mouse monoclonal β-actin clone AC-15	Mouse	Sigma A1978	Western blotting (1:20,000)
Mouse/human monoclonal anti-NF-κB/p65 clone C22B4	Rabbit	Cell Signalling Technology #4764	Western blotting (1:1,000)
Mouse monoclonal anti-IκBα clone E130	Rabbit	Abcam ab32518	Western blotting (1:500)
ECL™ anti-mouse IgG		GE Healthcare NA931	Western blotting (1:10,000-20,000)
ECL™ anti-rabbit IgG		GE Healthcare NA934	Western blotting (1:10,000)
Mouse/human polyclonal anti-KLF3	Goat	Thermo Fisher Pierce PA5-18030	Western blotting (1:1,000) ChIP (15 μg per IP)
Normal goat IgG		Santa Cruz SC-2028	ChIP (15 μg per IP)
Mouse polyclonal anti-KLF3	Rabbit	Homemade	EMSA
Anti-mouse CD16/32 Fc block clone 2.4G2	Rat	BD Pharmingen 553141	Flow cytometry (1:25)
FITC anti-mouse CD11b clone M1/70	Rat	BD Pharmingen 557396	Flow cytometry (1:100)
BUV395 anti-mouse CD115 clone T38-320	Rat	BD Optibuild 743642	Flow cytometry (1:50)

Biotin anti-mouse CD115 clone AFS98	Rat	eBioscience 13-1152-82	Flow cytometry (1:20)
BV711 Streptavidin		BD Horizon 563262	Flow cytometry (1:100)
APC anti-mouse CD19 clone 1D3	Rat	eBioscience 17-0193-80	Flow cytometry (1:20)
APC anti-mouse CD3 clone 145-2C11	Rat	BD Pharmingen 553066	Flow cytometry (1:20)
APC anti-mouse Ly6G clone 1A8	Rat	BD Pharmingen 560599	Flow cytometry (1:20)
BV421 anti-mouse Ly6C clone AL-21	Rat	BD Horizon 562727	Flow cytometry (1:33)
PE anti-mouse CD45 clone 30-F11	Rat	BD Pharmingen 553081	Flow cytometry (1:100)
PE/Cy5 anti-mouse F4/80 clone BM8	Rat	eBioscience 15-4801-80	Flow cytometry (1:100)
APC anti-mouse CD135 clone A2F10.1	Rat	BD Biosciences 560718	Flow cytometry (1:100)
PE anti-mouse CD117 clone 2B8	Rat	BD Pharmingen 561075	Flow cytometry (1:100)
Biotin anti-mouse Ter119 clone Ter-119	Rat	BD Biosciences 553672	Flow cytometry (1:200)
Biotin anti-mouse CD19 clone 1D3	Rat	BD Pharmingen 553784	Flow cytometry (1:200)
Biotin anti-mouse CD3 clone 145-2C11	Armenian hamster	eBioscience 13-0031-82	Flow cytometry (1:200)
Biotin anti-mouse NK1.1 clone PK136	Mouse	eBioscience 13-5941-82	Flow cytometry (1:200)
Biotin anti-mouse Ly6G clone	Rat	BD Pharmingen 553124	Flow cytometry (1:200)
Biotin anti-mouse TCRγδ clone GL3	Armenian hamster	eBioscience 13-5711-82	Flow cytometry (1:200)
PE-CF594 Streptavidin		BD Biosciences 562318	Flow cytometry (1:300)