SUPPLEMENTARY FIGURE LEGENDS

Sup. Figure 1: Validation of NFκB KO MEF cells. A) Western blot analysis of *p65*$^{-/-}$, *Ikkβ*$^{-/-}$ and *Ikkγ*$^{-/-}$ immortalized MEFs with indicated antibodies. **B)** EMSA was performed using either NFκB or AP1 consensus sequence radiolabelled probes as indicated using protein extracts from WT, *p65*$^{-/-}$, *Ikkβ*$^{-/-}$ and *Ikkγ*$^{-/-}$ immortalized MEFs treated with TNFα for the indicated time points. **C**) RNA-seq analysis of Gm16685 expression in WT, *p65*$^{-/-}$and *Ikkβ*$^{-/-}$ immortalized MEFs exposed to TNFα for indicated duration. **D)** RT-qPCR analysis of Gm16685 expression in WT, *p65*$^{-/-}$, *Ikkβ*$^{-/-}$ and *Ikkγ*$^{-/-}$immortalized MEFs exposed to TNFα for indicated duration. **E)** RT-qPCR analysis of Gm16685 expression in immortalized WT MEFs exposed to LPS (100ng/ml) for indicated time points. **F)** RT-qPCR analysis of *IL7* expression in WT, p65$^{-/-}$, IKKβ$^{-/-}$ and IKKγ$^{-/-}$ immortalized MEFs exposed to TNFα for indicated time points. Error bars indicate mean ± SD of three independent experiments. P values were calculated using Student's t-test method (**, p<0.01; ***, p<0.001).

Sup. Figure 2: Conservation and analysis of Gm16685 lncRNA. A) Multiple sequence alignment using promoter sequence of the mouse Gm16685 and sequence of 31 other species at the same region with reference to UCSC comparative genomic track. Clustal Omega was used for the alignment. **B)** Promoter motifs enrichment analysis by using Homer-v4.1.0 package. Top 10 most enriched motifs are shown. **C)** Prediction of different RBPs that can bind to Gm16685 or loc105375914 was performed using RBPmap server (http://rbpmap.technion.ac.il/). Venn diagram representing numbers of overlapping predicted RBPs that can bind both Gm16685 and loc105375914 is shown. **D)** ChIP-seq analysis of p65 (RelA) binding in mouse dendritic and macrophage cells exposed to LPS for 2 hours based on

published data (GSE36099 and GSE93736 or GSE93602). **E)** ChIP-seq analysis of RelA and Pol2 signal in human adipocyte and IMR90 fibroblasts exposed to TNFα for 1hour based on published data (GSE60462 and GSE43070). **F)** Bar plot shows the coding probability of seven NAIL (Loc105375914 for human and Gm16685 for mouse) transcript sequences (six *hNAIL* isoforms and one *mNAIL*) together with a known lncRNA sequence, TERC and actin gamma 1 (ACTG1) mRNA sequence as positive and negative control. Coding probability was calculated with CPC2 tools which indicate the closeness to the coding classification hyper-plane in the trained support vector machine (SVM) model. **G)** 293T cells were transfected with Flag, *NAIL*-Flag and PKR-Flag (Protein kinase R) vectors. Total cell lysates were resolved in SDS-PAGE gel and immunoblotted for Flag and Hsp90 proteins.

Sup. Figure 3: No difference in IL-7 expression and induction in *mNAIL*$^{\Delta NF\kappa B}$ mice. A) Gene expression profiles of mouse IL7 across different mouse tissues. All the raw FPKM values were obtained from Mouse ENCODE transcriptome data in NCBI (BioProject: PRJNA66167, Publication: PMID 25409824). **B)** Gene expression analysis was performed by RT-qPCR for mouse *IL7* in the thymus tissues of *mNAIL*^{WT} and *mNAIL*$^{\Delta NF\kappa B}$ mice (n=3). **C)** Total protein lysates isolated from the thymus tissues of *mNAIL*^{WT} and *mNAIL*$^{\Delta NF\kappa B}$ mice were analysed for mouse IL7 by western blot. Actin was used for normalization. Graph shows the quantification of IL7 western blot band intensity. **D-F)** Wild-type MEFs were transfected with si-Control, mouse IL7 siRNA#1 or mouse IL7 siRNA#2. After 48h post-transfection, cells were treated with or without TNFα and harvested for gene expression analysis or western blot. Graphs show the gene expression analysis of **D)** *IL7* and **E)** *TNFα* by RT-qPCR. Actin was used as a control. Error bars indicate mean ± SD of three independent experiments. P values were calculated using Student's t-test method (*, p<0.05; **, p<0.01; ***, p<0.001; ****,

p<0.0001; n.s., not significant). **F)** Cells lysate were analysed via western blot for the indicated proteins.

Sup. Figure 4: **No difference in stem cells and precursor cells between *mNAIL*^{WT} and *mNAIL*$^{\Delta NF\kappa B}$ mice. A-E)** Bone marrow cells were isolated from *mNAIL*^{WT} and *mNAIL*$^{\Delta NF\kappa B}$ mice (n=7) and treated with or without DSS at day 8. Cells were stained for Lineage marker cocktail, CD34, IL7ra, c-KIT, Sca-1 and CD16/32 cell surface markers. **A)** Cells were analysed by FACS and firstly gated as LIN- cells. LIN- cells were further gated as KL, KSL and K-low S-low cells based on the c-KIT and Sca-1 expression. KL cells were further gated into MEP, CMP and GMP according to CD16/32 and CD34 expression. K-low and S-low cells were gated for CLP population based on the IL7ra expression. Representative FACS data was shown for *mNAIL*^{WT} and *mNAIL*$^{\Delta NF\kappa B}$ mice treated with or without DSS. Quantification of **B)** GMP, **C)** CMP, **D)** MEP and **E)** CLP cells. Error bars indicate mean ± SD of 3 UT and 7 DSS treated samples. P values were calculated using Student's t-test method (n.s., not significant).

Sup. Figure 5: Genome editing process did not cause non-specific global transcriptome changes. **A)** RNA-seq was performed with colon tissues obtained at Day 8 from *mNAIL*^{WT} and *mNAIL*$^{\Delta NF\kappa B}$ mice treated with or without DSS. Venn diagram representing numbers of overlapping differentially expressed genes between different experimental groups is shown. **B)** GO analysis was performed from the genes that are differentially expressed between *mNAIL*^{WT} and *mNAIL*$^{\Delta NF\kappa B}$ mice treated with DSS.

Sup. Figure 6: *mNAIL*$^{\Delta NF\kappa B}$ mice are protected from DSS induced colitis. A, B) Colon sections of the DSS treated *mNAIL*^{WT} and *mNAIL*$^{\Delta NF\kappa B}$ mice (n=3) at day 8 were stained with *mNAIL* specific FISH probe (Quasar570), F4/80 (AF647) and p-p38 (AF488) antibodies. Tissue slides were analysed for the indicated molecules by LSM800 confocal microscopy.

Nuclei were counterstained with DAPI. Representative images for co-staining of **A)** *mNAIL,* F4/80 and p-p38 is shown**.** Quantification of **B)** F4/80, *mNAIL* and p-p38 positive cells is shown as bar graphs. Error bars indicate mean ± SD of three independent fields examined per mouse (n=3 per group). *, $p < 0.05$; ****, $p < 0.0001$. p-values were calculated by two-tailed student's t-test method based on three independent experiments. **C)** Representative H & E staining and **D)** quantification of histology scores of colon sections from DSS treated $mNAIL^{WT}$ and $mNAIL^{\Delta NF\kappa B}$ mice at day 8. Error bars indicate mean ± SD of three independent experiments (n=9 for UT, n=15 for DSS treated samples)

Sup. Figure 7: *NAIL* alters phosphorylation of p65 and p38 but not upstream molecules. A-B) Graphs show the gene expression analysis of **A)** *mNAIL* and **B)** *TNFα* by RT-qPCR in intestinal epithelial cells isolated from colon tissues of $mNAIL^{WT}$ and $mNAIL^{\Delta NF\kappa B}$ mice treated with and without DSS for 8 days. Actin was used as a control. Error bars indicate mean ± SD of 6 mice. P values were calculated using Student's t-test method (**, $p<0.01$; ***, $p<0.001$). n.s., not significant. **C)** $mNAIL^{WT}$ MEFs were transfected with si-*Control* or si-*NAIL* and after 48h post-transfection, cells were stimulated with TNFα for indicated time points. Cell lysates were analyzed by western blot for the indicated proteins. **D-E)** Bone marrow cells isolated from $mNAIL^{WT}$ and $mNAIL^{\Delta NF\kappa B}$ mice (n=4) were differentiated into bone marrow derived macrophages (BMDM) for 7 days. BMDM cells were treated with or without LPS (200ng/ml) for 4 hours. p65 immunoprecipitation was performed. Total and co-immunoprecipitated proteins were resolved on SDS-PAGE and immunoblotted for indicated proteins. **F)** Graph shows quantification of co-precipitated Wip1 from **D-E.** Error bars indicate mean ± SD of four replicates. P values were calculated using Student's t-test method (**, $p<0.01$).

Sup. Figure 8: Wip1 is a key determinant of *NAIL* action. A-C) $mNAIL^{WT}$ and $mNAIL^{\Delta NF\kappa B}$ MEFs were transfected with control siRNA or Wip1 siRNA. After 48h post-transfection, cells

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were treated with or without TNFα and harvested for gene expression analysis or western blot. Graphs show the gene expression analysis of **A)** *IL1β* and **B)** *TNFα* by RT-qPCR. Actin was used as a control. **C)** siRNA transfected and TNFα stimulated MEFs cell lysates were analysed via western blot for the indicated proteins. **D-F)** *mNAIL*^{WT} and *mNAIL*$^{\Delta NF\kappa B}$ BMDMs were transfected with control siRNA or Wip1 siRNA. After 48h post-transfection, cells were treated with or without LPS and harvested for gene expression analysis or western blot. Graphs show the gene expression analysis of **D)** *IL1β* and **E)** *TNFα* by RT-qPCR. Actin was used as a control. Error bars indicate mean ± SD of three independent experiments. P values were calculated using Student's t-test method (***, p<0.001). **F)** siRNA transfected and LPS stimulated BMDM cell lysates were analysed via western blot for the indicated proteins.