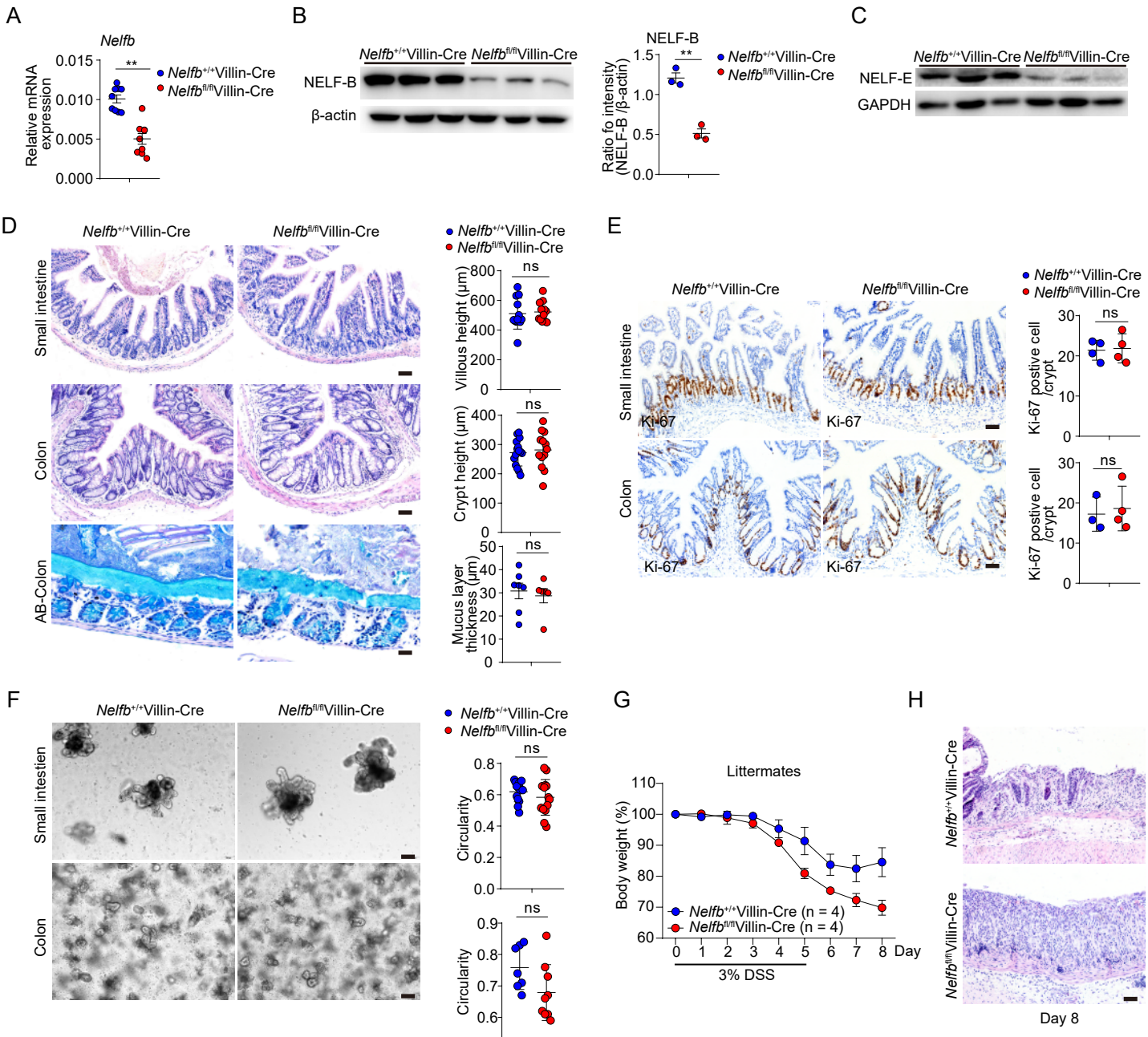

A
Nelfb
Relative mRNA expression
0.015
0.010
0.005
0.000
**
Nelfb+/+Villin-Cre
Nelfbfl/flVillin-Cre
B
Nelfb+/+Villin-Cre
Nelfbfl/flVillin-Cre
NELF-B
β-actin
NELF-B
Ratio fo intensity (NELF-B /β-actin)
1.5
1.0
0.5
0.0
**
Nelfb+/+Villin-Cre
Nelfbfl/flVillin-Cre
C
Nelfb+/+Villin-Cre
Nelfbfl/flVillin-Cre
NELF-E
GAPDH
D
Nelfb+/+Villin-Cre
Nelfbfl/flVillin-Cre
Small intestine
Colon
AB-Colon
Villous height (μm)
800
600
400
200
0
ns
Crypt height (μm)
400
300
200
100
0
ns
Mucus layer thickness (μm)
50
40
30
20
10
0
ns
E
Nelfb+/+Villin-Cre
Nelfbfl/flVillin-Cre
Small intestine
Colon
Ki-67
Ki-67 postive cell /crypt
30
20
10
0
ns
F
Nelfb+/+Villin-Cre
Nelfbfl/flVillin-Cre
Small intestien
Colon
Circularity
0.8
0.6
0.4
0.2
0.0
ns
0.9
0.8
0.7
0.6
0.5
ns
G
Littermates
Body weight (%)
110
100
90
80
70
60
Nelfb+/+Villin-Cre (n = 4)
Nelfbfl/flVillin-Cre (n = 4)
0 1 2 3 4 5 6 7 8 Day
3% DSS
H
Nelfb+/+Villin-Cre
Nelfbfl/flVillin-Cre
Day 8
I
Histological score
8
6
4
2
0
**
Nelfb+/+Villin-Cre
Nelfbfl/flVillin-Cre
J
Water starve Overnight
FITC-dextran 4 hours
Detect the concentration of FITC in serum
Day 0
Day 1
K
Concentration (ng/ml)
2000
1500
1000
500
0
ns
Nelfb+/+Villin-Cre
Nelfbfl/flVillin-Cre

Supplementary Figure 1. Epithelial NELF-B-deficient mice show normal crypt architecture and do not spontaneously develop colitis

(A and B) qPCR (A) and immunoblotting (B) analysis of *Nelfb* expression in colon tissues from *Nelfb*$^{+/+}$Villin-Cre and *Nelfb*$^{fl/fl}$Villin-Cre mice (n = 3-7).

(C) Immunoblotting analysis of NELF-E in colon tissues from *Nelfb*$^{+/+}$Villin-Cre and *Nelfb*$^{fl/fl}$Villin-Cre mice (n = 3).

(D) H&E and Alcian Blue (AB) staining analysis of morphologies of intestinal sections from *Nelfb*$^{+/+}$Villin-Cre and *Nelfb*$^{fl/fl}$Villin-Cre mice (n = 5-10). Scale bars, 50 μm.

(E) Immunohistochemical analysis of Ki-67 protein levels in intestinal sections of *Nelfb*$^{+/+}$Villin-Cre and *Nelfb*$^{fl/fl}$Villin-Cre mice (n = 4). Scale bars, 50 μm.

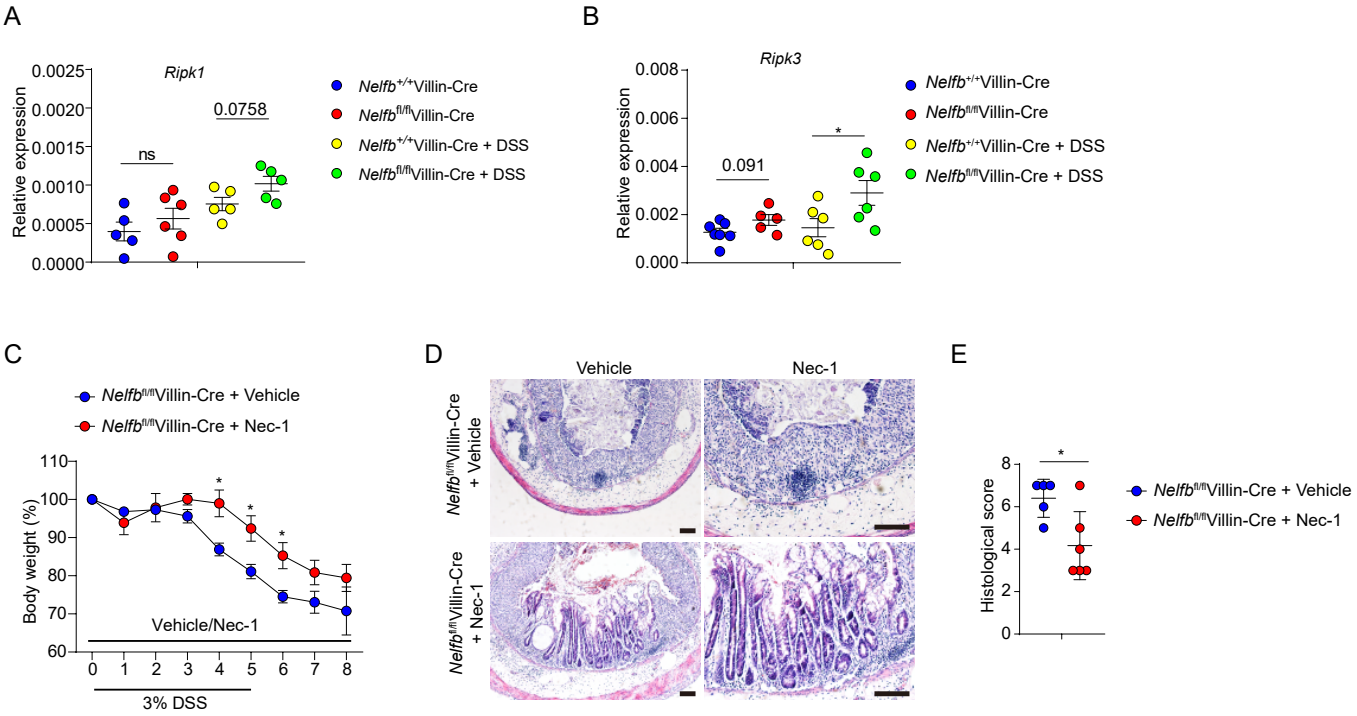
(F) Organoids of small intestine and colon from *Nelfb*$^{+/+}$Villin-Cre and *Nelfb*$^{fl/fl}$Villin-Cre mice visualized by light microscopy (n = 3). Circularities are calculated (right panel). Scale bars, 50 μm.

(G-I) Body weight (G), H&E staining (H) and histological scores (I) of *Nelfb*$^{fl/fl}$Villin-Cre mice and littermate controls (*Nelfb*$^{+/+}$Villin-Cre) after DSS treatment (n = 4). Scale bar, 50 μm.

(J) The experimental scheme for the procedure of intestinal permeability analysis.

(K) Intestinal permeability analysis of *Nelfb*$^{+/+}$Villin-Cre and *Nelfb*$^{fl/fl}$Villin-Cre mice under homeostasis condition (n = 8-9).

All data are shown as mean ± SEM. Student's *t* test was performed; $**p \leq 0.01$; ns, not significant ($p>0.05$).



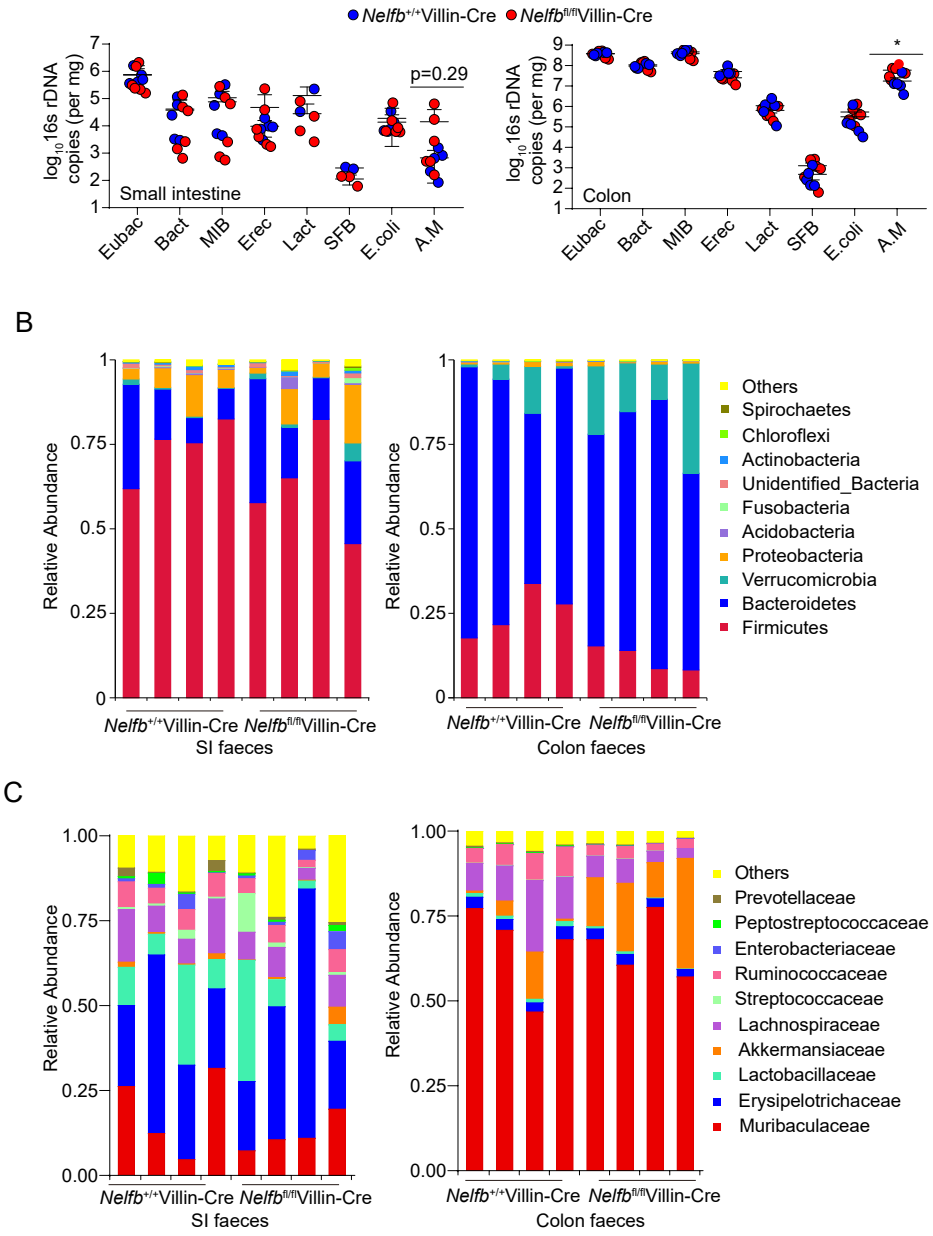
Supplementary Figure 2. Epithelial NELF inhibits IEC necroptosis

(A and B) qPCR analysis of the expression of *Ripk1* (A) and *Ripk3* (B) in *Nelfb*$^{+/+}$Villin-Cre and *Nelfb*$^{fl/fl}$Villin-Cre mice with or without DSS treatment (n = 5-7).

(C) Body weight of *Nelfb*$^{fl/fl}$Villin-Cre mice with or without necrostatin-1 stimulation after DSS treatment (n = 5-6).

(D and E) H&E staining (D) and histological scores (E) (n = 5-6) of colon sections from DSS treated mice as in (C) at day 8. Scale bars, 50 μm.

All data are shown as mean ± SEM. Student's *t* test was performed; $*p \leqslant 0.05$; ns, not significant ($p>0.05$).

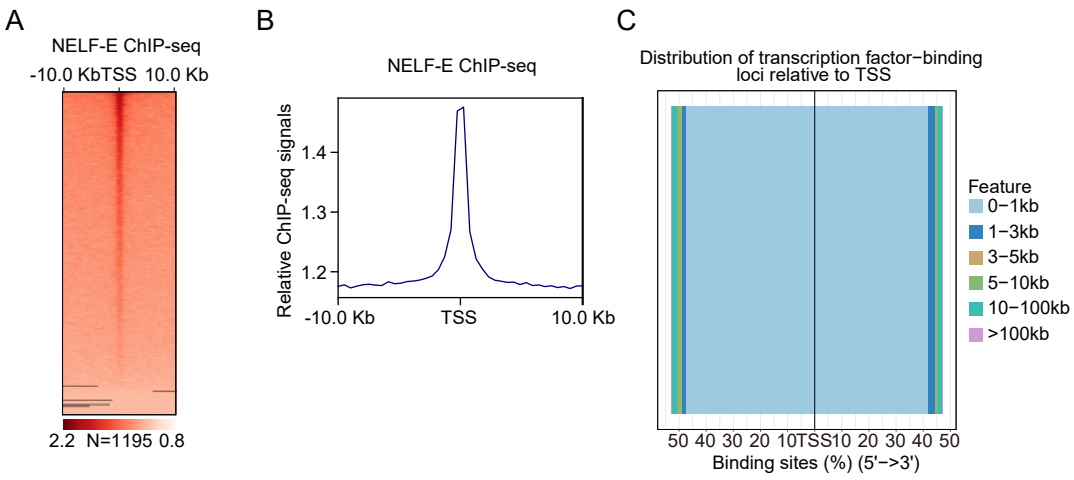


Supplementary Figure 3. Loss of epithelial NELF-B results in mild microbial changes in gut

(A) The abundance of microbiota in small intestine and colon of *Nelfb*$^{+/+}$Villin-Cre and *Nelfb*$^{fl/fl}$Villin-Cre mice was analyzed by qPCR analysis of 16S rDNA gene copy number (n = 6). Eubac, Eubacteria; Bact, *Bacteroides* spp.; MIB, mouse intestinal *Bacteroides*; Erec, *Eubaterium rectale/Clostridium coccoides*; Lact, *lactobacillus* sp.; SFB, segmented filamentous bacteria.

(B and C) 16S rDNA sequencing analysis of commensal diversity at the phylum level (B) and family level (C) in small intestine and colon of *Nelfb*$^{+/+}$Villin-Cre and *Nelfb*$^{fl/fl}$Villin-Cre littermate mice under homeostasis condition (n = 4).

All data are shown as mean ± SEM. Student's *t* test was performed; $*p \leq 0.05$; ns, not significant ($p>0.05$).

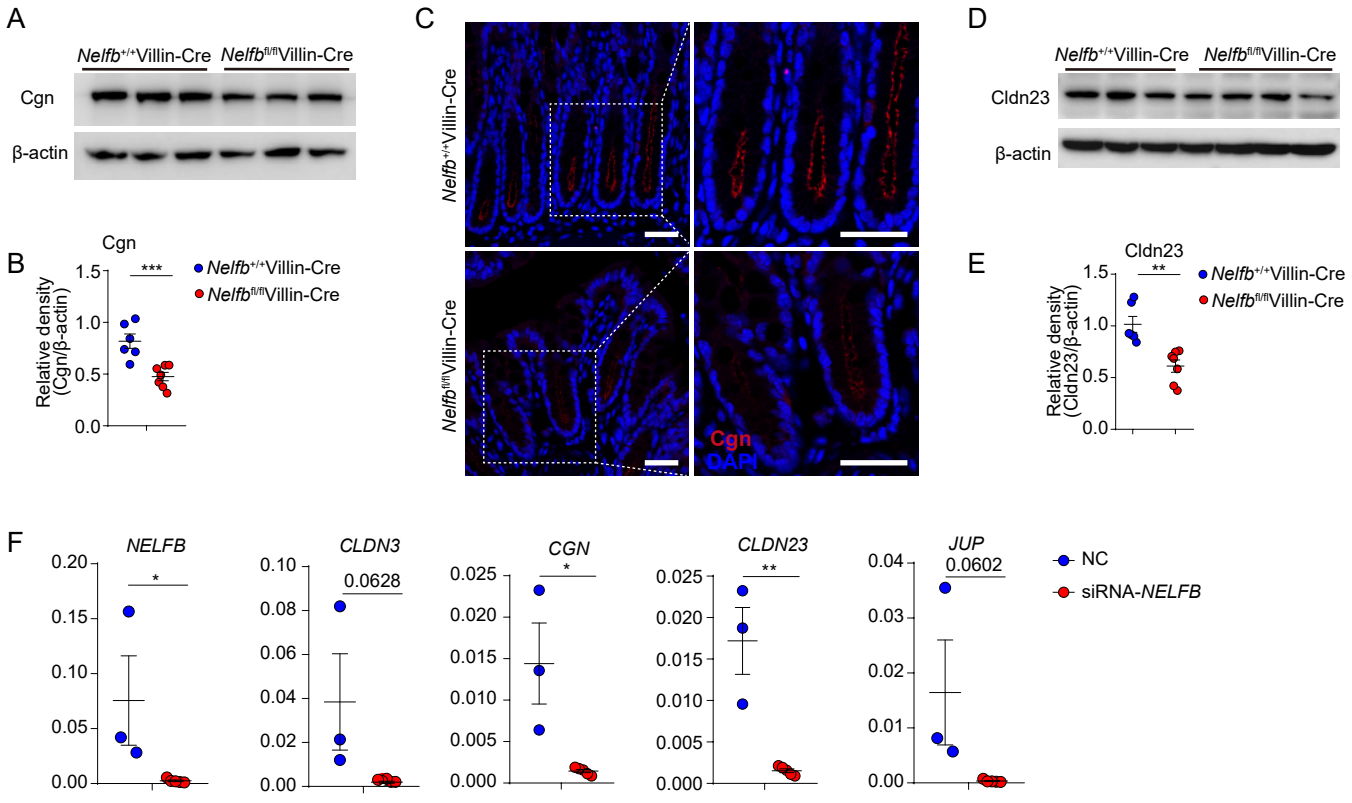


Supplementary Figure 4. ChIP-seq analysis results for NELF-B marks in colonic IECs

(A) Heat map of NELF-E ChIP-seq signals around TSS regions for colonic IEC-expressed genes in *Nelfb*$^{+/+}$Villin-Cre mice at steady state. Each row indicates one gene sorted by the peak heights in *Nelfb*$^{+/+}$Villin-Cre mice. The signals were normalized to input signals. The number of peaks in the whole genome is shown at the bottom.

(B) Relative NELF-E ChIP-seq signals around TSS regions as (A) shown.

(C) Distribution of NELF-E ChIP-seq peaks around transcription factor−binding loci relative to TSS.



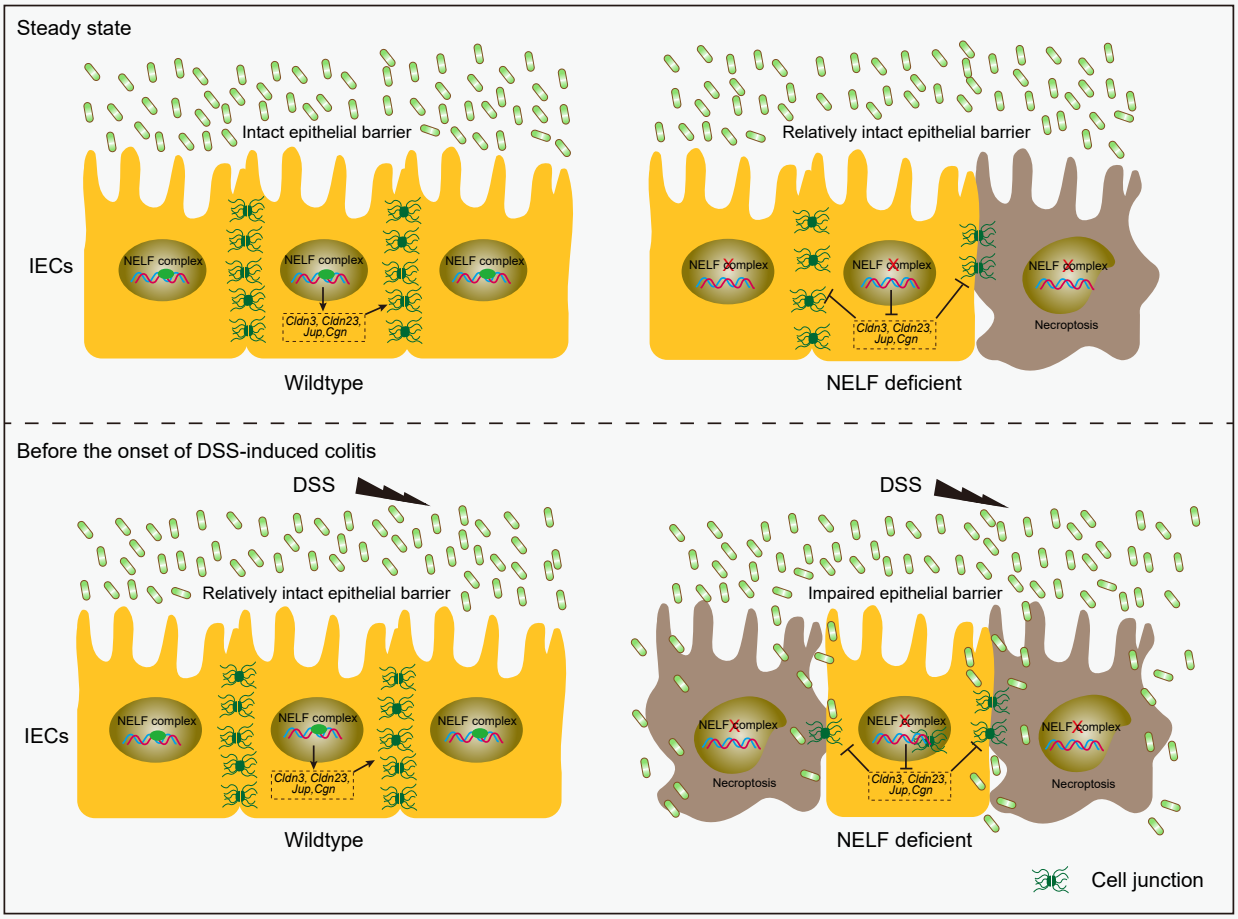
Supplementary Figure 5. Epithelial NELF promotes the expression of a subset of cell junction related genes

(A-C) Immunoblotting analysis (A), quantification (B) and immunofluorescent (C) analysis of Cgn expression in *Nelfb*$^{+/+}$Villin-Cre and *Nelfb*$^{fl/fl}$Villin-Cre mice (n = 6-7).

(D and E) Immunoblotting analysis (D) and quantification (E) analysis of Cldn23 expression in *Nelfb*$^{+/+}$Villin-Cre and *Nelfb*$^{fl/fl}$Villin-Cre mice (n = 6-7).

(F) qPCR analysis of cell junction-related genes expression in LS174T cells after siRNA against *NELFB*.

All data are shown as mean ± SEM. Student's t test was performed; $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$; ns, not significant ($p>0.05$).



Supplementary Figure 6. Epithelial NELF maintains intestinal barrier function to repress colitis

A working model for epithelial NELF maintaining intestinal barrier function. Under steady state, genetic deletion of NELF complex in IECs results in reduction of genes associated with junctional integrity (such as *Cldn3*and *Cldn23*) and increased IEC necroptosis without significantly affecting epithelial barrier integrity in mice. However, upon DSS treatment, loss of epithelial NELF complex leads to reduction of genes associated with junctional integrity, impaired epithelial barrier characterized by increased permeability, bacterial invasion, and epithelial necroptosis before the onset of colitis, and consequently increased susceptibility to intestinal inflammation.

Supplementary Table 1: Primers used in this study

Primer sequences for regular qPCR		
Gene	**Forward Primer**	**Reverse Primer**
Nelfb	TGAGGCTTCTCCTCCACTT	GGTCTAACTGCTCCAACTTCTC
Cldn3	CACCCACCAAGATCCTCTATTC	TTCATCGACTGCTGGTAGTG
Cldn23	TCTGTGTTGAGCTTGTTGAGC	AGGTATCTCGGGAACAGGACA
Jup	CTGTTTGACTCCCACCACCT	CGCATGCCCCTTACTTCCTC
Cgn	ACAAAAGACCCTCCTTATGGCTT	AGAGGCAAACCATCCCCAT
Retnlb	TCAGTCGTCAAGAGCCTAAGA	CATAGCCACAAGCACATCCA
Ang4	ACTCTGGCTCAGAATGAAAGGT	GACATCTTTGCAAGGCGAGG
Itln1	GCTGAAGAGAACCTGGACAC	GGTCTGGTAGATGACACCATTC
Spink4	ACATGGCTGAGCTTCCAAA	AGCATTGGCCATCCTTCAT
Mptx1	ACAGCATCTTCTCCTACAACAC	GACTCCCAGTTCACACAGATAC
Tgm3	GTCTCCCCACACCATCTCTGT	CGTTTTGGATCTGTAAAGCACTCA
Slc37a2	GGAATGGTGCTAAGTGGCCT	ATGAATGACAGGCCCCAGTG
Fabp6	GTGAAGATGGAGGGTGGCAA	CGCTCATAGGTCACATCCCC
Cyp3a44	TTGGTCCTGCTGGCAATCAT	GCCCAGGAATCCCCTGTTTC
Gm20605	CGAGCTTTTTGGGGACCTGA	CAACATCCTGCGACTGGTGA
Ripk1	CCACTTCAGCACCGAACTCC	CTTCTCCAGCAGGTCACTGG
Ripk3	GCCTTCCTCTCAGTCCACAC	ACGCACCAGTAGGCCATAAC
Human NELFB	GTATTCGATGAGCTGCGGGA	TTTCTCCGGAACCTTGGGC
Human CLDN3	GTCTAAGGGACAGACGCAGG	AAGTATTGGCGGTCACCCAG
Human CGN	CCCGGCTAGGGACTCCTC	CTGGCTCTGTGATGAAGCGA
Human CLDN23	ACTCCGACCTCTAGACGCTT	CAAGTGTCCGGGTTCCAACT
Human JUP	GCCTCGTCGATACTACCTGC	GGTGTATGTCTGCTGCCACT
Eubacteria (Universal)	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGGCTGCTGGC
Bacteroides (Bact)	GGTTCTGAGAGGAGGTCCC	CTGCCTCCCGTAGGAGT
Mouse Intestinal *Bacteroides* (MIB)	CCAGCAGCCGCGGTAATA	CGCATTCCGCATACTTCTC
Lactobacillus/Enterococcus Group (Lact)	AGCAGTAGGGAATCTTCC	CACCGCTACACATGGA
Eubacterium rectale/ Clostridium coccoides group (Erec)	ACTCCTACGGGAGGCAGC	GCTTCTTAGTCAGGTACCGTCA
Segmented filamentous bacteria (SFB)	GACGCTGAGGCATGAGAGCA	GACGGCACGGATTGTTATTC
E. coli	CATGCCGCGTGTATGAAGAA	CGGGTAACGTCAATGAGCAAA
A. muciniphila	CAGCACGTGAAGGTGGGGAC	CCTTGCGGTTGGCTTCAGAT
Primer sequences for ChIP assays		
Gene	**Forward Primer**	**Reverse Primer**
Cldn3 TSS	CCTCCTCCTCTAGGCACCAA	GGCTTTGGAGACTGGCTTCT
Cldn23 TSS	CGAAACCAGCTCCGAGTCC	CCACCCGGTAGGGTTTGG
Cgn TSS	TAGCTTAGCGCCAGAGCATC	GATGAAGGGCAGAAAGGGCT
Jup TSS	CAGCCTGACTATCCCATCGAG	GAGCGCATAAACAGAGGCGG
Jun TSS	CGTCTGTCTGTCTGTCTGCC	GCTCAGGCTGGATAAGGACTC
Hbb TSS	CAGGGAGAAATATGCTTGTCATCA	GTGAGCAGATTGGCCCTTACC