Supplementary Information

TLR-independent anti-inflammatory function of intestinal epithelial TRAF6 signalling prevents DSS-induced colitis in mice

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Supplementary Figure 1

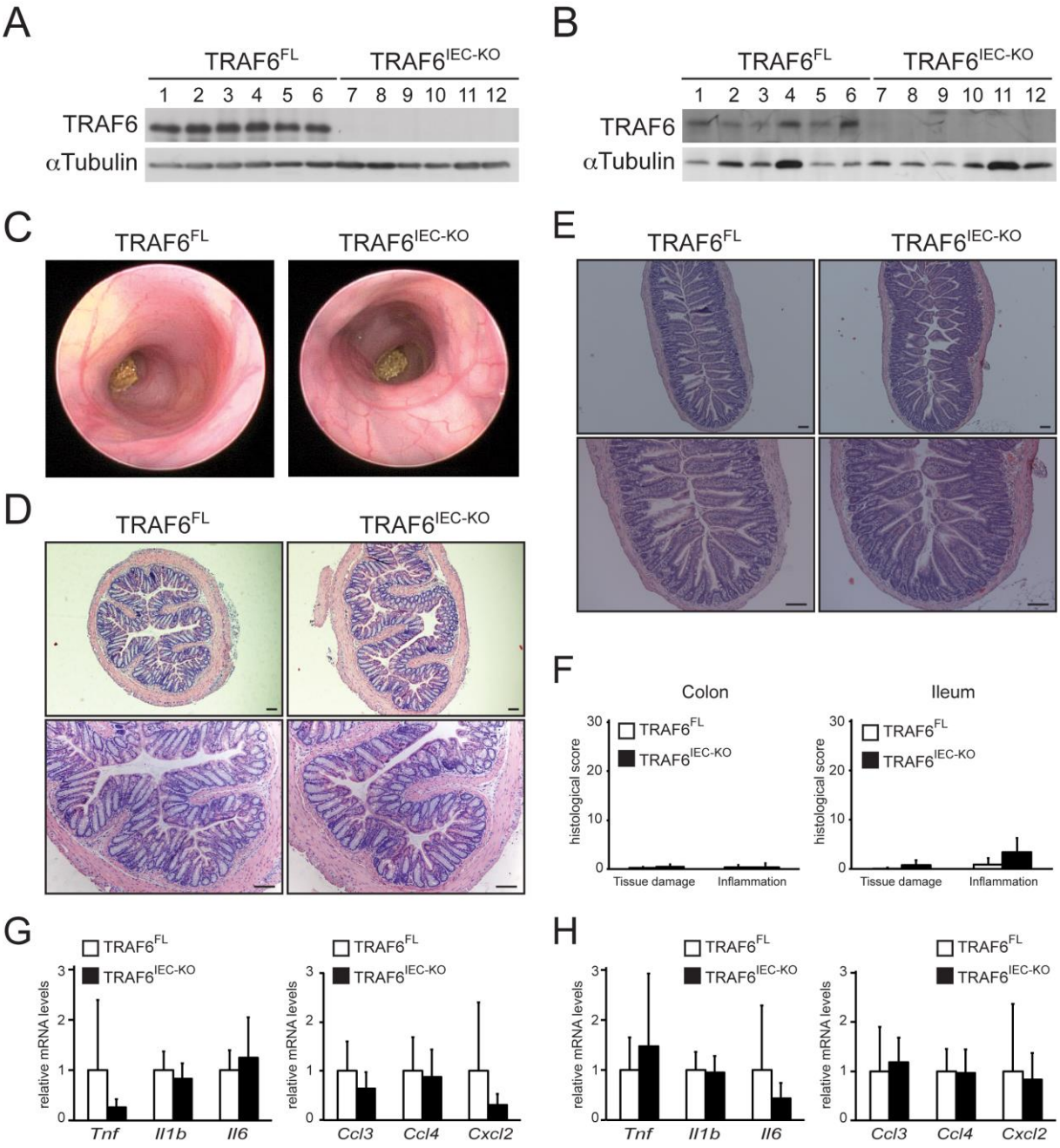

Figure S1: TRAF6^{IEC-KO} mice do not show intestinal abnormalities.

Western blot on IECs isolated from the small intestine (A) or colon (B) showing efficient deletion of TRAF6 in TRAF6^{IEC-KO} mice. Each lane represents a different mouse. (C) Representative endoscopic, (D) histological colon and (E) histological ileum pictures of TRAF6^{FL} and TRAF6^{IEC-KO} mice. (F) Histological tissue damage and inflammation scoring of colon and ileum from TRAF6^{FL} and TRAF6^{IEC-KO} mice. (G-H) Cytokine and chemokine mRNA levels in colon (G) or small intestine (H) of naïve TRAF6^{FL} and TRAF6^{IEC-KO} mice. All statistical analyses were performed with unpaired two-sided Student's *t*-tests with unequal variance.

Supplementary Figure 2

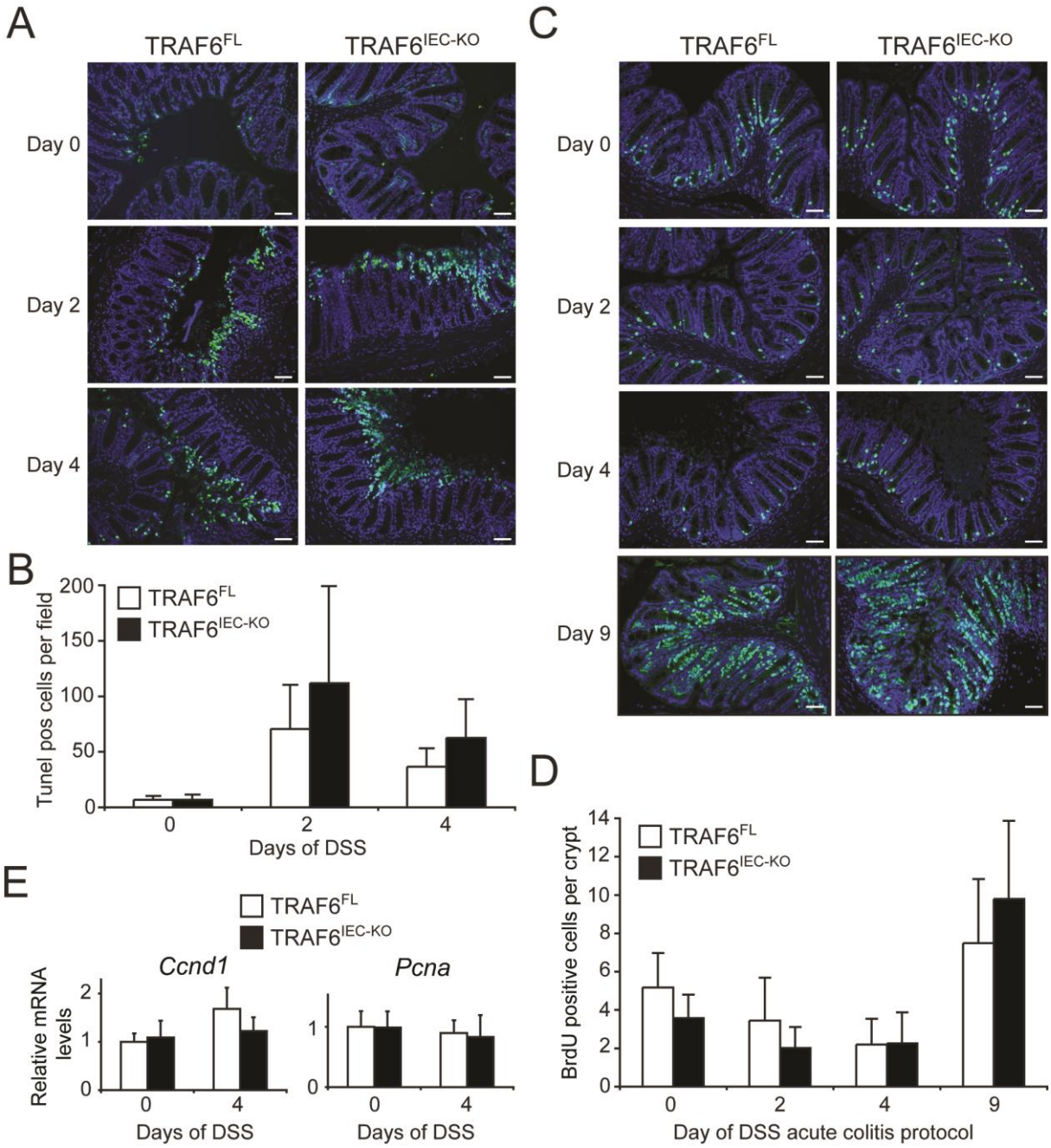

Figure S2: TRAF6^{IEC-KO} mice display normal epithelial cell death and proliferation responses upon DSS treatment.

(A) Representative TUNEL stainings and (B) mean number of TUNEL positive cells per 200x magnification field +/- SEM of TRAF6^{FL} and TRAF6^{IEC-KO} mice that were either untreated (day 0) or treated with 2% DSS for 2 or 4 days (*n*=5-8 per group). Bars, 100 μm. At least 3 fields of 3 different colon cross-sections were counted per mouse. (C) Representative BrdU stainings and (D) mean number of BrdU positive cells per crypt +/- SEM of TRAF6^{FL} and TRAF6^{IEC-KO} mice that were either untreated (day 0) or at day 2, 4 or 9 of the acute DSS colitis protocol (*n*=5-8 per group). Bars, 100 μm. BrdU positive cells in well-oriented and

longitudinally sectioned crypts in at least 3 fields of 3 different sections were counted per mouse. (E) Relative mRNA expression of proliferation marker genes *Ccdn1* and *Pcna* in isolated IECs from $TRAF6^{FL}$ and $TRAF6^{IEC\text{-}KO}$ mice that were either untreated (day 0) or treated with 2% DSS for 4 days (*n*=4-10 per group). All statistical analyses were performed with unpaired two-sided Student's *t*-tests with unequal variance.

Supplementary Figure 3

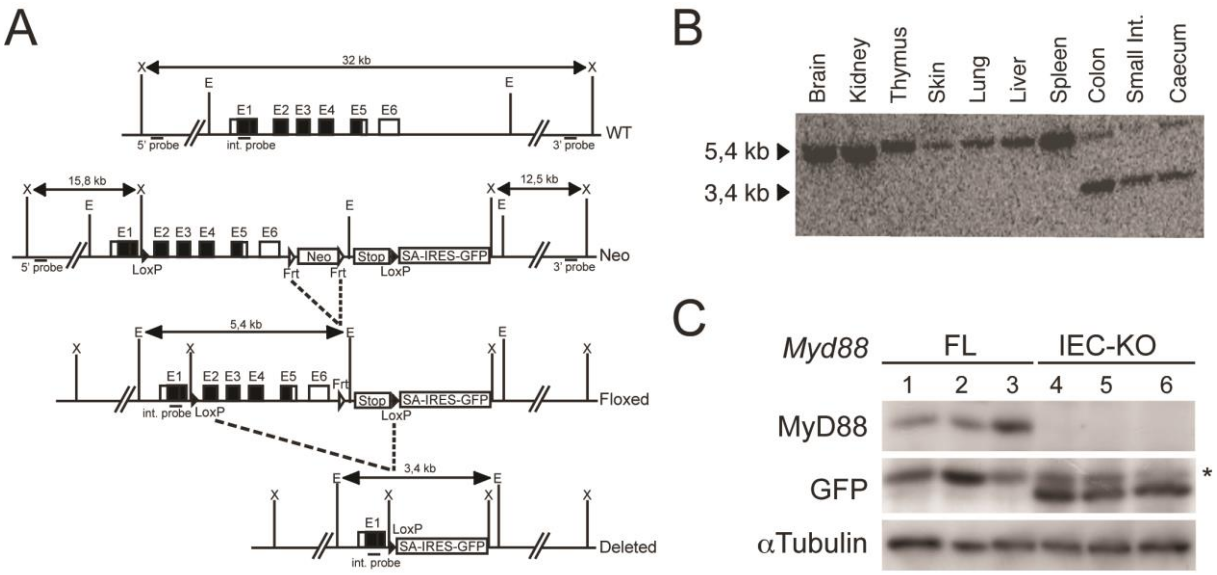

Figure S3: Generation of MyD88^{IEC-KO} mice.

(A) Schematic showing the targeting strategy used for the generation of MyD88^{FL} mice. Exons 2-6 of the *Myd88* gene were flanked by loxP sites and a cassette consisting of an adenoviral splice acceptor site fused to an IRES and a GFP gene was inserted downstream of the second loxP site. In this way, deletion of the loxP-flanked sequences generates a null *Myd88* allele, and at the same time allows expression of the GFP reporter gene under the control of the endogenous promoter. Non-coding and coding exons are depicted as white and black boxes, respectively; E, EcoRI; X, XhoI (B) Southern blot on genomic DNA from a MyD88^{IEC-KO} mouse showing selective deletion of the floxed *Myd88* allele in intestinal tissues, as indicated by the band at 3,4 kb upon EcoRI digest and hybridization with the internal probe. (C) Western blot on colon IECs showing efficient deletion of MyD88 and concomitant expression of GFP in MyD88^{IEC-KO} mice. Each lane represents a different mouse, * indicates a non-specific band.

Supplementary Figure 4

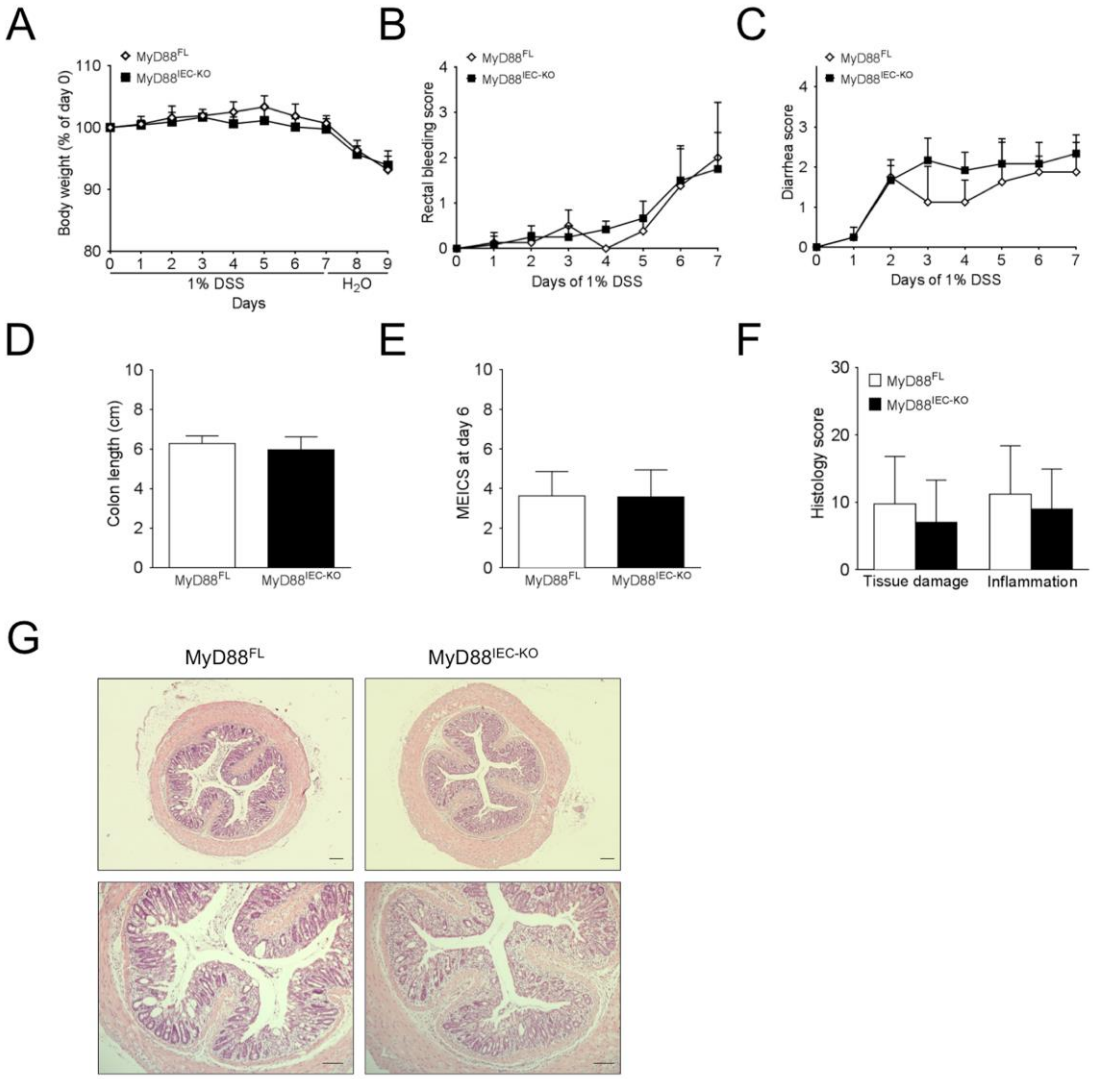

Figure S4: MyD88^{IEC-KO} mice are not more susceptible to DSS-induced acute colitis than control mice.

(A) Body weight change, (B) rectal bleeding score, and (C) diarrhoea score of age- and sex-matched MyD88^{FL} (*n*=4) and MyD88^{IEC-KO} (*n*=6) mice that were administered 1% DSS for 7 days followed by 2 days of normal drinking water. (D) Colon length of MyD88^{FL} and MyD88^{IEC-KO} mice at day 9 of the DSS colitis protocol. (E) Mean endoscopic index of colitis severity (MEICS) of MyD88^{FL} and MyD88^{IEC-KO} mice after 6 days of DSS treatment. (F) Histological tissue damage and inflammation scoring and (G) representative H&E stained colon cross-sections of MyD88^{FL} and MyD88^{IEC-KO} mice at day 9 of the DSS colitis protocol. Bars, 100 μm. All data shown are representative of 3 independent experiments. All statistical analyses were performed with unpaired two-sided Student's *t*-tests with unequal variance.

Supplementary Figure 5

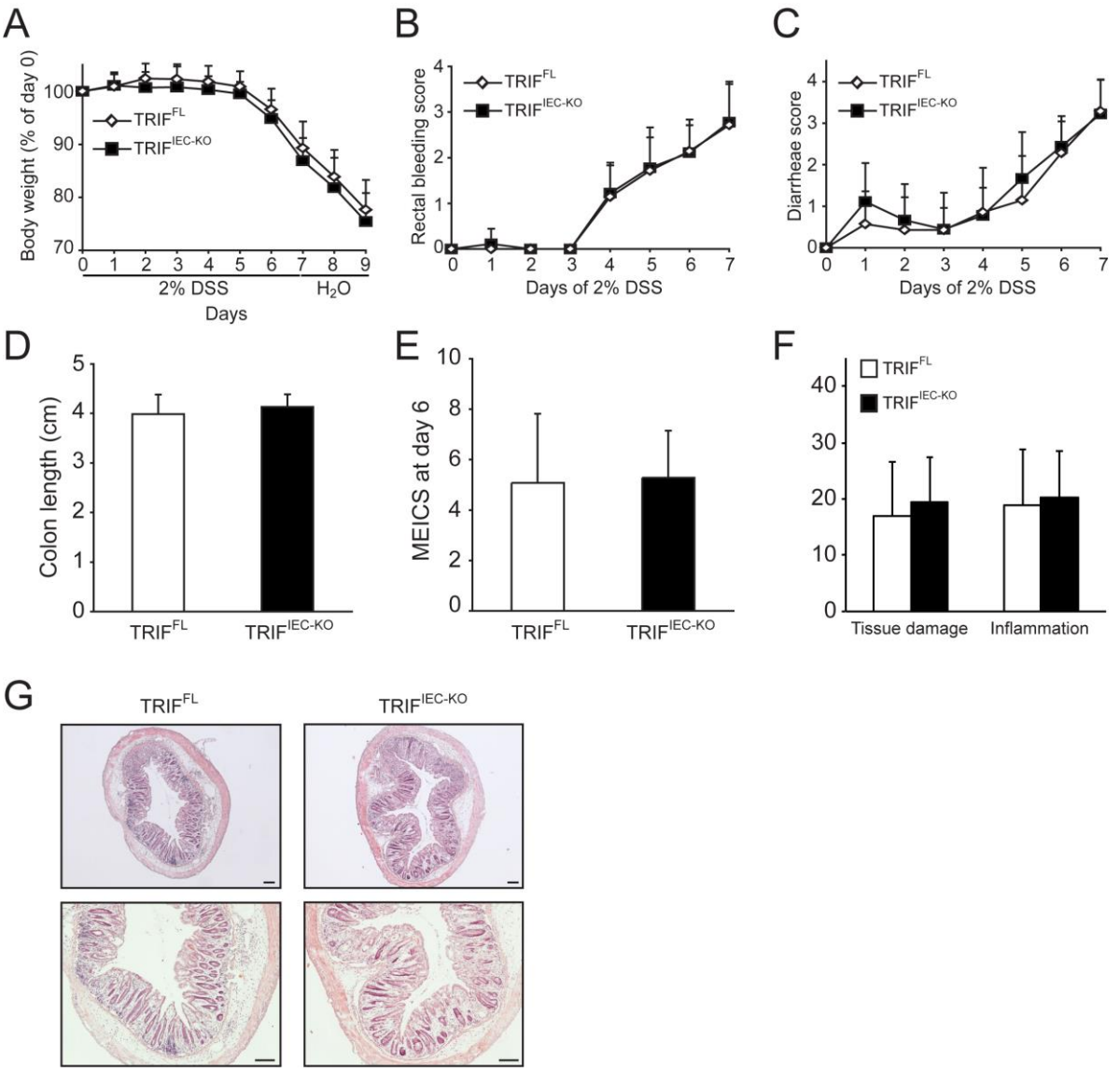

Figure S5: TRIF^{IEC-KO} mice are not more susceptible to DSS-induced acute colitis than control mice.

(A) Body weight change, (B) rectal bleeding score, and (C) diarrhoea score of age- and sex-matched TRIF^{FL} (*n*=7) and TRIF^{IEC-KO} (*n*=9) mice that were administered 2% DSS for 7 days followed by 2 days of normal drinking water. (D) Colon length of TRIF^{FL} and TRIF^{IEC-KO} mice at day 9 of the DSS colitis protocol. (E) Representative endoscopic pictures and mean endoscopic index of colitis severity (MEICS) of TRIF^{FL} and TRIF^{IEC-KO} mice after 6 days of DSS treatment. (F) Histological tissue damage and inflammation scoring and (G) representative H&E stained colon cross-sections of indicated mice at day 9 of the DSS colitis protocol. Bars, 100 μm. All data shown are representative of 3 independent experiments. All statistical analyses were performed with unpaired two-sided Student's *t*-tests with unequal variance.