Supporting Information

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SI Materials and Methods

Isolation of Intestinal Epithelial Cells. The terminal ileum (5 cm) was removed, flushed with HBSS/2% FBS, opened longitudinally, and cut into 0.5-cm pieces. The tissue was further washed and incubated in HBSS/2% FBS, 0.5 mM EDTA, and 1 mM DTT, at 37 °C in a shaking water bath for 45 min. The cell suspension released upon vigorous shaking was layered on a discontinuous 25%/40% Percoll gradient (Sigma) and centrifuged at 600 × *g* for 10 min. Intestinal epithelial cells (IEC) were collected from the interphase. The purity of the population was assessed by FACS analysis for the epithelial marker E-cadherin (FITC-conjugated mAb; BD) and was consistently ∼90%. The isolation of the nonepithelial compartment of the tissue was performed upon two additional 30-min incubations in 1 mM EDTA and 1 mM DTT followed by vigorous shaking for the complete depletion of IEC.

RT-PCR Analysis. RNA extraction was performed using TRIZOL reagent (Invitrogen) according to manufacturer's instructions. Twelve micrograms of total RNA was used to generate cDNA templates using M-MLV RT (Promega) and oligo-dT primers after treatment with RQ1 DNase I (Promega). Quantitative RT-PCR was performed with a Chromo4 Real-Time PCR detection system (Bio-Rad Laboratories) using Platinum SYBR Green (Invitrogen). All data were normalized to β2-microglobulin (β2m) expression. The following oligonucleotides were used: TNF forward (5′-CACGCTCTTCTGTCTACTGA-3′), TNF reverse (5′-ATCTGAGTGTGAGGGTCTGG-3′); β2m forward (5′-TTCTGGTGCTTGTCTCACTGA-3′), β2m reverse (5′-CAGTATGTTCGGCTTCCCATTC-3′); tissue inhibitor of metalloproteinases-1 (TIMP1) forward (5′-ACAAGTCCCAGAACCGCAGTG-3′), TIMP1 reverse (5′-GGACCTGATCCGTCCACAAAC-3′); matrix metalloproteinase 13 (MMP13) forward (5′-TTCTGGTCTTCTGGCACACGCTTT-3′); MMP13 reverse (5′-CCAAGCTCATGGGCAGCAACAATA-3′). Data were analyzed with RelQuant software (Bio-Rad Laboratories). Semiquantitative RT-PCR was performed with serial cDNA dilutions using the oligonucleotides: TNF forward (5′-CACGCTCTTCTGTCTACTGAACTTCG-3′); TNF reverse (5′-GGCTGGGTAGAGAATGGATGAACACC-3′); β2m forward, β2m reverse.

Bone Marrow-Derived Macrophages and ELISA. Bone marrow-derived macrophages were generated as described previously (1). TNF levels in culture supernatants were determined by ELISA (eBioscience) according to the manufacturer's instructions.

Intestinal Myofibroblast Isolation and Culture. Intestinal myofibroblast (IMF) cultures were established by enzymatic treatment of terminal ilea (5 cm). Pooled tissues from three or four individual mice of the indicated genotype were used. In brief, intestinal pieces were flushed extensively with HBSS/2% FBS and were cut longitudinally. The epithelial layer was removed by incubation in DMEM/10% FBS containing collagenase XI (300 U/mL)/dispase (0.1 mg/mL) (Sigma) for 10 min at 30 °C. The digested tissues were washed with HBSS/2% FBS and subjected to a second incubation with collagenase/dispase for 20 min at 30 °C. The mucosal samples then were washed and cultured at 37 °C in a humidified CO_2 incubator in DMEM supplemented with 10% FBS, 1% nonessential amino acids, 100 U/mL penicillin, 100 μg/mL streptomycin (Invitrogen), and 1 μg/mL amphotericin B (Sigma-Aldrich). Cells were used after three passages.

Gelatin Zymography. The gelatinolytic activity of tissue extracts from the terminal ileum [in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 μg/mL leupeptin, 2 μg/mL aprotinin, 1 mM PMSF] was assessed with gelatin zymography using 20 μg of protein as described (2). Samples were denatured before electrophoresis with incubation in sample buffer containing 4% SDS in room temperature for 10 min. The secretion of metalloproteinases by IMF was assessed with gelatin zymography in supernatants of synchronized IMF cultured in medium containing 0.5% FBS for 48 h at a density of 0.75×10^5 cells/cm^2 of well surface.

FACS Analysis of Freshly Isolated IMF. A modified version of a method originally described for human tissues (3) has been used. Pooled terminal ilea from three individual mice of the indicated genotype were washed and incubated in HBSS/2% FBS, 1 mM EDTA, and 1 mM DTT, at 37 °C in a shaking water bath for 15 min for the depletion of the IEC layer. Then the tissue was washed, cut into small pieces, and incubated in DMEM/10% FBS containing 300 U/mL Collagenase IA (Sigma), 0.1 mg/mL dispase, and 50 U/mL DNase-V (Sigma) in a shaking water bath for 3 h at 37 °C. The resulting cell suspension was filtered through a 40-μm cell strainer (BD Falcon). To detect IMF and measure intercellular adhesion molecule-1 (ICAM1) expression levels with FACS analysis, surface staining was performed with mAbs for the mesenchymal marker CD90.2 (Biolegend), for the endothelial marker CD31 (BD), and for ICAM1 (BD), followed by intracellular staining for the smooth muscle/myofibroblast marker αSMA (Sigma) using the BD Cytofix/Cytoperm kit.

Isolation and FACS Analysis of Lamina Propria Cells. Isolation of lamina propria cells was performed as described previously (4). Surface staining was performed with mAbs against CD11b (BD), Gr-1 (BioLegend), T-cell receptor β (TCRβ) (eBioscience), CD4 (eBioscience), and CD8β (BD). Intracellular cytokine staining was performed as described below.

Intracellular Cytokine Staining of Lamina Propria Cells and Splenocytes. Cells were incubated for 6 h with 50 ng/mL phorbol 12-myristate 13-acetate (PMA) (Sigma), 500 ng/mL ionomycin (Sigma), and GolgiStop protein transport inhibitor containing monensin (BD). Intracellular staining was performed with mAbs against TNF (eBioscience), IL-17 (eBioscience), and IFN-γ (eBioscience). Flow cytometric analysis was performed as previously described (4).

TUNEL Assay and E-cadherin Immunostaining. TUNEL assay was performed in paraffin sections of the terminal ileum with the DeadEnd Fluorimetric TUNEL System (Promega) according to the manufacturer's instructions. E-cadherin immunostaining was performed in paraffin sections upon antigen retrieval by boiling in citrate buffer for 30 min and staining with FITC-conjugated anti–E-cadherin mAb (BD) at a 1:200 dilution. Sections were stained with DAPI, mounted with GelMount (Biomeda), and analyzed with a fluorescent microscope (Nikon, Eclipse E800) equipped with a Nikon Dxm1200F camera and ACT1.1 software.

Quantitation of TNF-Induced IEC Apoptosis. For quantitation of TNF-induced apoptosis, a modified version of a method originally described elsewhere (5) was used. Starved mice fed with water containing sugar were injected i.v. with TNF. Forty-five minutes after injection, the small intestine was flushed with 10 mL PBS containing 2% FBS, and the solution collected was filtered two

times through a nylon-mesh filter to remove debris. The collected cells were washed and counted with a cell counter (Beckman Coulter), stained with anti–E-cadherin (BD) and anti-CD45 (BD) mAbs, and fixed in PBS containing 1% paraformaldehyde. Then the cells were incubated in PBS containing 0.2% Triton X-100, 5 μg/mL propidium iodide, and 0.02 μg/μL RNaseA for 40 min at 4 °C and analyzed with flow cytometry. To obtain a diploid (live) IEC population as a control, an untreated mouse was used for IEC isolation upon incubation of the terminal ileum in HBSS/2% FBS, 0.5 mM EDTA, 1 mM DTT, at 37 °C in a shaking water bath for 15min.

1. Kontoyiannis D, Pasparakis M, Pizarro TT, Cominelli F, Kollias G (1999) Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: Implications for joint and gut-associated immunopathologies. *Immunity* 10:387–398.
2. Armaka M, et al. (2008) Mesenchymal cell targeting by TNF as a common pathogenic principle in chronic inflammatory joint and intestinal diseases. *J Exp Med* 205:331–337.
3. Saada JI, et al. (2006) Subepithelial myofibroblasts are novel nonprofessional APCs in the human colonic mucosa. *J Immunol* 177:5968–5979.
4. Apostolaki M, et al. (2008) Role of beta7 integrin and the chemokine/chemokine receptor pair CCL25/CCR9 in modeled TNF-dependent Crohn's disease. *Gastroenterology* 134:2025–2035.
5. Piguet PF, Vesin C, Guo J, Donati Y, Barazzone C (1998) TNF-induced enterocyte apoptosis in mice is mediated by the TNF receptor 1 and does not require p53. *Eur J Immunol* 28:3499–3505.

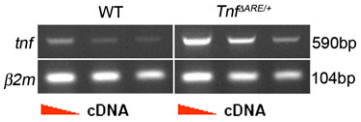

Fig. S1. IEC from *Tnf*$^{\Delta ARE/+}$ mice overexpress TNF. Semiquantitative RT-PCR for TNF expression in primary unstimulated, isolated IEC from WT and *Tnf*$^{\Delta ARE/+}$ 4-wk-old mice. Data are representative of WT (n = 7) and *Tnf*$^{\Delta ARE/+}$ (n = 6) mice analyzed individually in two separate experiments.

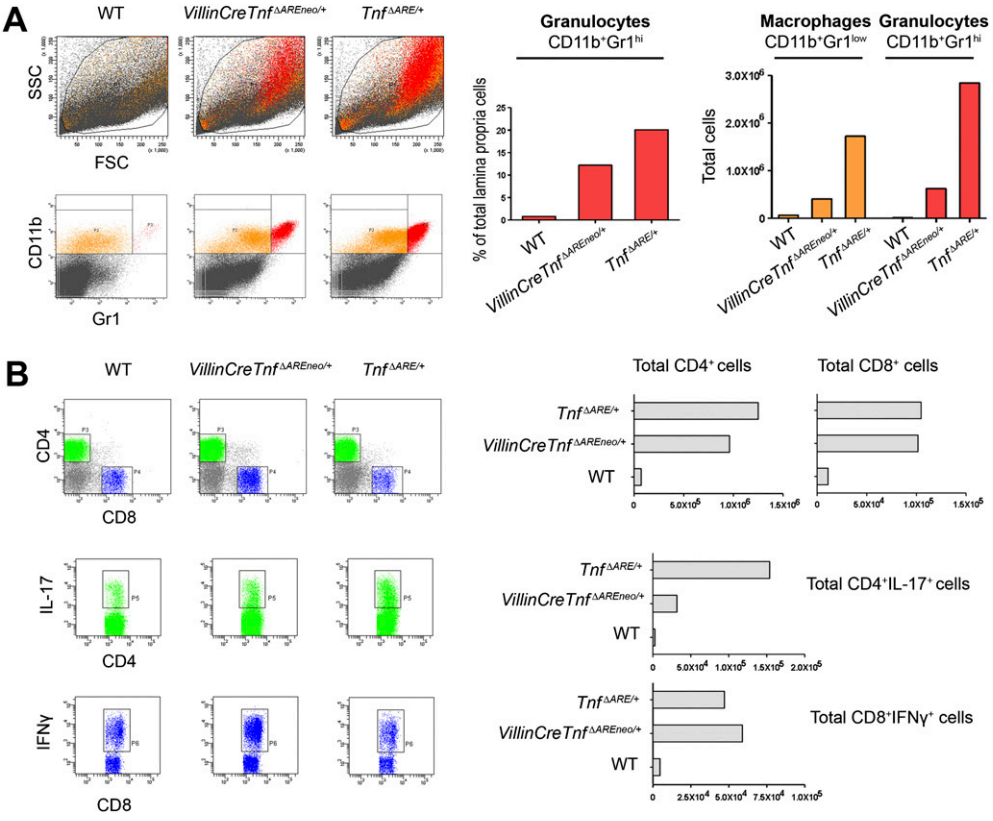

Fig. S2. FACS analysis of the lamina propria cell populations in the ileum of diseased *VillinCreTnf*$^{\Delta AREneo/+}$ mice. Lamina propria cells were isolated from pooled tissues of littermate 4-mo-old WT or *VillinCreTnf*$^{\Delta AREneo/+}$ mice (n = 4 or 5). Lamina propria cells from fully diseased 3-mo-old *Tnf*$^{\Delta ARE/+}$ mice (n = 3) were used as a positive control. (*A*) Quantitation of macrophage (CD11b^{+}Gr1^{low}) and granulocyte (CD11b^{+}Gr1^{hi}) populations. The absolute cell numbers are calculated per mouse. FSC, forward scatter; SSC, side scatter. (*B*) Quantitation of TCRβ^{+}CD4^{+} and TCRβ^{+}CD8^{+} cell populations and intracellular cytokine staining to determine the number of IL-17–producing TCRβ^{+}CD4^{+} cells and IFNγ-producing TCRβ^{+}CD8^{+} cells. The absolute cell numbers are calculated per mouse. Results are representative of two independent experiments.

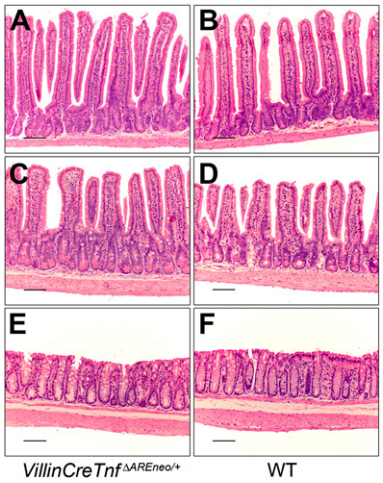

Fig. S3. *VillinCreTnf*$^{\Delta ARE neo/+}$ mice do not show signs of intestinal pathology in the duodenum, jejunum, or colon at age 8 mo. Histological examination of the duodenum (*A* and *B*), jejunum (*C* and *D*), and colon (*E* and *F*) of *VillinCreTnf*$^{\Delta ARE neo/+}$ mice and WT littermates at age 8 mo. Paraffin sections were stained with H&E. (Scale bars: 100 μm.)

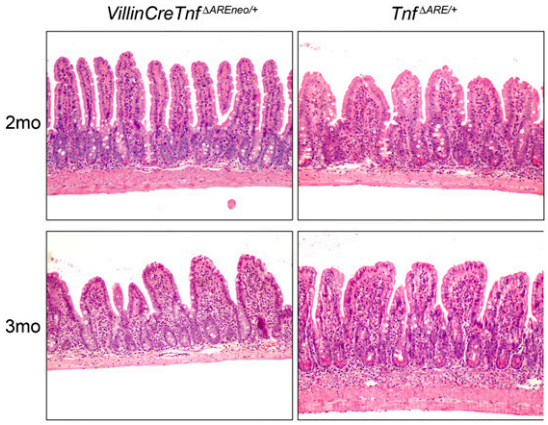

Fig. S4. Comparative histological examination of the ileum of *VillinCreTnf*$^{\Delta ARE neo/+}$ and *Tnf*$^{\Delta ARE/+}$ mice at age 2 and 3 mo. Paraffin sections were stained with H&E. The data presented are representative of mice analyzed in parallel and maintained on the same genetic background.

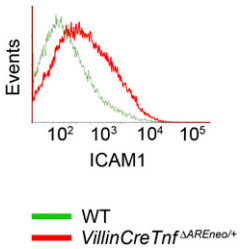

Fig. S5. FACS analysis showing increased ICAM1 expression on cultured IMF isolated from 2-mo-old *VillinCreTnf*$^{\Delta ARE neo/+}$ and littermate WT control mice. Data are representative of two independent experiments. Events represent gated IMF (CD90.2^{+} cells).

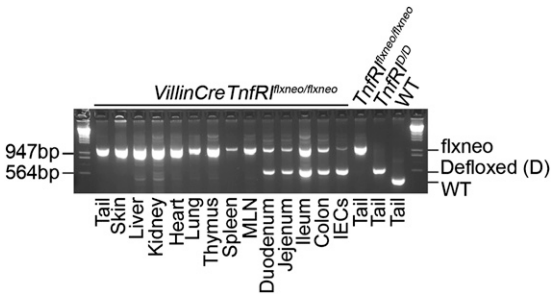

Fig. S6. IEC-specific reactivation of the *TnfRI* allele in *VillinCreTnfRI*$^{flxneo/flxneo}$ mice. PCR analysis for the detection of the activated *TnfRI* allele in tissues and in primary isolated IEC from *VillinCreTnfRI*$^{flxneo/flxneo}$ mice. MLN, mesenteric lymph node.

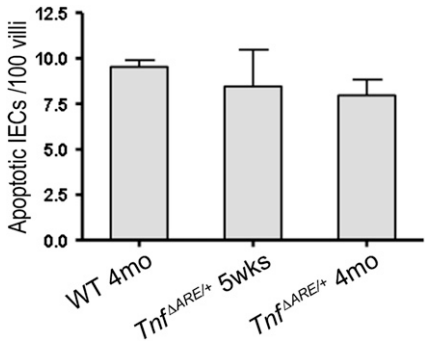

Fig. S7. Apoptosis of IEC in *Tnf*$^{\Delta ARE/+}$ mice. Number of apoptotic IEC in a portion (~5 cm long) of the terminal ileum of 4-mo-old WT and 5-wk-old and 4-mo-old *Tnf*$^{\Delta ARE/+}$ mice (n = 3 per group) as measured with TUNEL assay. Data shown include combined results from two independent experiments.