Material and Methods

Animal models

As mentioned previously [1], wild-type (WT) mice used in the study were between 9 and 10 weeks old at the beginning of the experimental protocol and were maintained on a 12-h dark-light cycle and allowed free access to pelleted, nonpurified diet and tap water. The Animal Care Committee of the Emory University, Atlanta approved all procedures performed on animals and was in accordance with the Guide for the Care and Use of Laboratory Animals, published by the U.S. Public Health Service.

Induction of colitis associated cancer and inhibition of Notch1 by pharmacological inhibitor in animals

Age- and sex-matched C57B6 WT and MMP9$^{-/-}$ mice (n=20/ group) were injected i.p. with 7.6 mg/kg AOM (Sigma, St. Louis, MO) on day 0. On day 7, one group each [1 2] of WT and MMP9$^{-/-}$ mice was exposed to 3% (w/v) DSS (MP Biomedicals, Solon, OH), given through drinking water *ad libitum* for 7 days. On day 14, their water was changed back to regular drinking water. On day 28, their drinking water was again changed to 3% DSS for a second 7 day cycle of DSS exposure. After this week, these mice were returned to regular drinking water and sacrificed on day 56. Their colons were opened longitudinally. Body weight, stool consistency, and stool occult blood of all the mice during the DSS treatment and recovery phase were monitored. Inhibition of Notch1 signaling during each cycle of DSS was achieved by five consecutive i.p. injections of 10 µmol/kg DAPT [3 4] ((Sigma), a γ-secretase inhibitor), while controls received vehicle alone. DAPT, solubilized in DMSO, was suspended in phosphate-buffered saline (PBS) containing 0.5% (w/v) hydroxypropylmethylcellulose (Methocel E4M) (Dow Chemicals, Midland, MI) and 0.01% (v/v) Tween 80 [4]. Mice were sacrificed on day 56 and colonoscopy (Xenon Nova 475, STORZ, Tuttlingen, Germany) was performed. Colons of the mice were cut open, and the

number and size of the polyps were determined under the Zeiss microscope (Olympus, Center Valley, PA).

Protein extraction and Western blot analysis

For Western blot analysis, mucosal strippings were obtained after sacrificing the mice. Antibodies used were: MMP9 (1:1,000; Abcam), p53 (1:250; BD Transduction Laboratories), Bax-1 (1:500; Biolegend), caspase-3 (1:1,000; Cell Signaling), Notch1 intracellular domain (NICD; 1:500; Millipore), and $p21^{WAF1/Cip1}$ (1:1,000; BD Biosciences). For p53 and $p21^{WAF1/Cip1}$, the goat anti-mouse secondary antibody (1:4,000; Bio-Rad) was used. For MMP9, Bax-1, caspase-3, and NICD, the goat anti-rabbit (1:2,500; Bio-Rad) was used as secondary antibody.

Isolation of MEFs and cell culture.

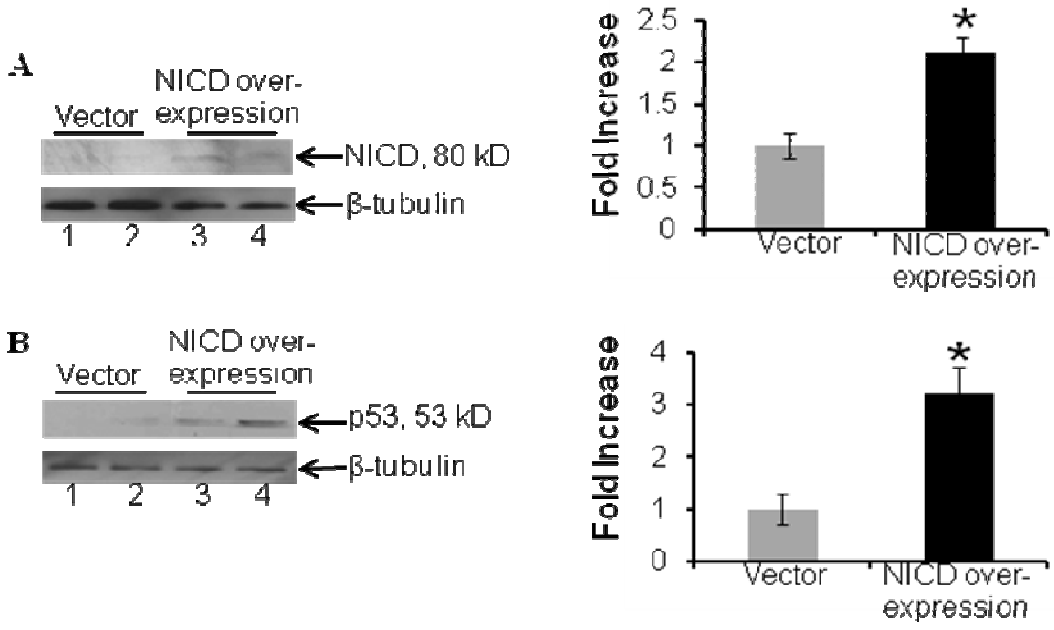
WT or $MMP9^{-/-}$ mouse embryonic fibroblasts (MEFs) were derived from day 13.5 embryos using the 3T3 protocol as described previously [5] [6]. Briefly, MEFs were maintained in Dulbecco's modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin at 37ºC in atmosphere containing 5% CO_2. HCT116 cells were also used as *in vitro* model, as they express wild type p53. $MMP9^{-/-}$ MEFs were transiently transfected with pEGFP plasmid without NICD construct as well as with NICD construct (a generous gift from Prof. Kopan, St. Louis) [7]. Cell lysates were collected after 72 hours and were processed for western blot. HCT116 cells were transfected for 72 hours with a pEGFP plasmid with and without the MMP9 gene. Transfected clones were selected under an antibiotic (Geneticin, GIBCO, Grand Island, NY) and fluorescent cells were isolated using flow cytometry (FACS), then used for the detection of MMP9, NICD, p53, $p21^{WAF1/Cip1}$ and Bax-1 protein expression. Stably transfected HCT116 cells were maintained in McCoy's 5A medium supplemented with

10% FBS, 1% penicillin-streptomycin and 800µg/ml Geneticin at 37ºC in atmosphere containing 5% CO_2.

Endoscopic assessment of colitis

Direct visualization of DSS-induced colonic mucosal damage *in vivo* was performed using the Coloview (Karl Storz Veterinary Endoscopy, Tuttlingen, Germany). Mice were supplied with food and water until the endoscopy was performed. Mice were anesthetized with 1.5 to 2% isoflurane and 3 cm of the colon proximal to the anus was visualized after inflation of the colon with air.

Supplementary Figure 1



NICD overexpression results in activation of p53 among MMP-9$^{-/-}$ MEFs. MEFs isolated from MMP9$^{-/-}$ mice were plated on 6 well plates and were transiently transfected with a pEGFP plasmid, without the NICD gene as well as with a plasmid with NICD gene (see Method). Cell lysates were collected and immunoblotted for A: NICD, B: p53. β-tubulin was used as the loading control. Western blots were quantitated by scanning densitometry and graphed adjacently. Each lane shows protein (17µg/lane). Values are representative of two experiments, mean ± SE; *p<0.05.

References

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