

Supplemental Table 1. Primers used for RT-PCR analysis of inflammatory cytokines

Gene	Primer	Sequence
<i>IL-1α</i>	Forward primer	5'-CAAGATGGCCAAAGTTCGTGAC-3'
	Reverse primer	5'-GTCTCATGAAGTGAGCCATAGC-3'
<i>IL-1β</i>	Forward primer	5'-ATGGCAACTGTTCTGAACTCAACT-3'
	Reverse primer	5'-CAGGACAGGTATAGATTCTTTCCTTT-3'
<i>IL-6</i>	Forward primer	5'-AGGATACCACTCCCAACAGACCT-3'
	Reverse primer	5'-CAAGTGCATCATCGTTGTTTCATAC-3'
<i>TNF-α</i>	Forward primer	5'-TTCTGTCTACTGAACTTCGGGGTGATCGGTCC-3'
	Reverse primer	5'-GTATGAGATAGCAAATCGGCTGACGGTGTGGG-3'
<i>ICAM-1</i>	Forward primer	5'-GGCATTGTTCTCTAATGTCTCCG-3'
	Reverse primer	5'-CCGCTCAGAAGAACCACCTTGG-3'
<i>VCAM-1</i>	Forward primer	5'-TACTCCCGTCATTGAGGATATTGG-3'
	Reverse primer	5'-CTCCTTCACACACATAGACTCC-3'

Supplemental Figure 1

Apoptosis and caspase activation in human ulcerative colitis tissues. **(A)** H&E (left panel), TUNEL (red, middle panel), and active caspase 3 (red, right panel) staining of 3 matched pairs of uninvolved colonic and colitis tissues (200×). **(B)** TUNEL (brown) staining of a matched pair of uninvolved colonic and colitis tissues (400×). Arrows indicate example TUNEL positive cells. **(C)** Apoptotic index of the crypts of uninvolved colonic and colitis tissues was determined by counting TUNEL signals in 100 randomly selected crypts following TUNEL staining as in (B). Values were means \pm SD ($n = 6$ in each group). **(D)** Caspase 3 activation in a matched pair of uninvolved colonic and colitis tissues was detected by active caspase 3 (brown) immunostaining (upper panel; 400×), and active caspase 3 (green) and cytokeratin (red) double staining (lower panel; 400×). Arrows indicate example active caspase 3 positive, or active caspase 3 and cytokeratin double positive cells. **(E)** Active caspase 3 index of crypts of uninvolved colonic and colitis tissues from UC patients was determined by counting active caspase 3 signals in 20 randomly selected crypts following active caspase 3 staining as in (D). Values were means \pm SD ($n = 3$ in each group).

Supplemental Figure 2

PUMA induction and apoptosis in human ulcerative colitis tissues. **(A)** PUMA protein expression in 11 uninvolved colonic tissues (N1-N11) and 11 ulcerative colitis (UC1-UC11) tissues was analyzed by Western blotting. **(B)** A control for *PUMA* RNA *in situ* hybridization (ISH). *PUMA* expression (dark dots) was analyzed by ISH in colonic tissues from WT and *PUMA*-KO mice at 4 hr after exposure to γ -irradiation at 15 Gy (400×). Arrows indicate example *PUMA*-expressing cells. **(C)** A control for PUMA immunostaining. PUMA expression

in colonic tissues from WT and *PUMA*-KO mice was analyzed by immunostaining (200×). Arrows indicate example cells with basal PUMA expression. **(D)** PUMA (red) and TUNEL (green) double staining of an uninvolved and 2 UC tissues (400×). Arrows indicate example PUMA and TUNEL double positive cells. **(E)** Correlation between PUMA expression determined by Western blotting and apoptosis levels analyzed by TUNEL staining in colitis tissues from UC patients. Values were means \pm SD ($n = 9$ in each group).

Supplemental Figure 3

Induction of PUMA in mice following DSS or TNBS treatment. WT and *PUMA*-KO mice were treated with 5% DSS for 7 days, or 100 mg/kg of TNBS for 3 days, to induce colitis. **(A)** PUMA (red) staining of colonic tissues from the treated mice (200×). A section from untreated *PUMA*-KO mice was used as the control for staining specificity. **(B)** PUMA (red) and cytokeratin (green) double staining of colonic tissues from the treated mice (200×).

Supplemental Figure 4

Induction of PUMA in colonic epithelial cells and expression of other Bcl-2 family members in colonic mucosa of DSS- and TNBS-treated mice. WT mice were treated with 5% DSS for 7 days, or 100 mg/kg of TNBS for 3 days, to induce colitis. **(A)** Colonic epithelial cells were isolated from colonic mucosa of the treated mice as described in the Methods. The isolated epithelial cells were verified by immunostaining for EpCAM (green), an epithelial marker. **(B)** Western blot analysis of the indicated proteins in colonic epithelial cells isolated as in (A). **(C)** Western blot analysis of Bcl-2 family members in the colonic mucosa of DSS- or TNBS-treated

mice. In (B) and (C), relative expression of each sample normalized to the loading control β -actin is indicated, with that of the untreated animal arbitrarily set as 1.0.

Supplemental Figure 5

DSS-induced colonic damage in WT and *PUMA*-KO mice. (A) H&E staining of colonic tissues from WT and *PUMA*-KO mice treated with 5% DSS for 1 or 3 days (200 \times). (B) Histological scores of the treated mice were determined following H&E staining as in (A). Values were means \pm SD ($n = 3$ in each group). (C) Myeloperoxidase (MPO) activities in colonic mucosa from WT and *PUMA*-KO mice treated with 5% DSS for 7 days were measured as described in the Methods.

Supplemental Figure 6

Apoptosis in colonic tissues from WT and *PUMA*-KO mice treated with 5% DSS or 100 mg/kg of TNBS. (A) DNA fragmentation in the mice treated with DSS for 7 days was quantified by diphenylamine reaction as described in the Methods. Values were means \pm SD ($n = 3$ in each group). (B) Apoptotic index in the mice treated with DSS for 1 or 3 days was determined by counting TUNEL signals in 100 randomly selected crypts following TUNEL staining. Values were means \pm SD ($n = 3$ mice in each group). (C) TUNEL (green) and cytokeratin (red) double staining of colonic tissues from the mice treated with DSS for 3 days (400 \times). Arrows indicate example TUNEL and cytokeratin double positive cells. (D) Caspase 3 activity was measured using colonic mucosa extracts from the mice treated with DSS for 7 days. Values were means \pm SD ($n = 6$ mice in each group). (E) TUNEL (green) and cytokeratin (red) double staining of

colonic tissues from the mice treated with 100 mg/kg TNBS for 3 days (400×). Arrows indicate example TUNEL and cytokeratin double positive cells.

Supplemental Figure 7

TNBS-induced inflammation in WT and *PUMA*-KO mice. WT and *PUMA*-KO mice were treated with 100 mg/kg of TNBS for 3 days to induce colitis. **(A)** Expression of the indicated inflammatory cytokines in the treated mice was analyzed by real time RT-PCR. * $P < 0.02$ compared to the untreated control. **(B)** Myeloperoxidase (MPO) activities in colonic mucosa of the treated mice were measured as described in the Methods. Values were means \pm SD ($n = 3$ in each group).

Supplemental Figure 8

DSS-induced colonic damage and apoptosis in WT and *p53*-KO mice. WT and *p53*-KO mice treated with 5% DSS for 7 days. Colonic damage and apoptosis were analyzed. **(A)** Methylene blue staining of colonic tissues from the DSS-treated mice with arrows indicating ulcers. **(B)** Colonic ulcers were counted following methylene blue staining as in (A). **(C)** H&E staining of colonic tissues from the control and DSS-treated mice (200×). **(D)** TUNEL staining of colonic tissues from the control and DSS-treated mice (200×). **(E)** Analysis of caspase 3 activation after DSS treatment by Western blotting. **(F)** Measurement of caspase 3 activities in the intestinal mucosa extracts from the DSS-treated mice. Values in (B) and (F) were means \pm SD ($n = 6$ mice in each group).

Supplemental Figure 9

TNBS-induced colonic damage and apoptosis in WT and *p53*-KO mice. WT and *p53*-KO mice treated with 100 mg/kg of TNBS for 3 days. Colonic damage and apoptosis were analyzed. (A) H&E staining of colonic tissues from the control and TNBS-treated mice (200×). (B) TUNEL staining of colonic tissues from the control and TNBS-treated mice (200×). (C) Apoptotic index was determined by counting TUNEL signals in 100 randomly selected crypts following TUNEL staining as in (B). Values were means \pm SD ($n = 6$ in each group).

Supplemental Figure 10

Bid-independent colitis induced by DSS or TNBS. WT and *Bid*-KO mice were treated with 5% DSS for 7 days, or 100 mg/kg of TNBS for 5 days, to induce colitis. (A) H&E staining of colonic tissues from the DSS-treated mice (200×). (B) Histological damage after DSS treatment was scored following H&E staining as in (A). (C) TUNEL (green) staining of colonic tissues from the DSS-treated mice (400×). Arrows indicate example TUNEL-positive cells. (D) Apoptotic index was determined by counting TUNEL signals in 100 randomly selected crypts following TUNEL staining as in (C). (E) H&E staining of colonic tissues from the TNBS-treated mice (200×). (F) Histological damage after TNBS treatment was scored following H&E staining as in (E). (G) TUNEL (green) staining of colonic tissues from the TNBS-treated mice (400×). Arrows indicate example TUNEL-positive cells. (H) Apoptotic index was determined by counting TUNEL signals in 100 randomly selected crypts following TUNEL staining as in (G). Values in (B), (D), (F) and (H) were means \pm SD ($n = 3$ mice in each group).

Supplemental Figure 11

Effects of the TNF antibody infliximib on DSS-induced colitis. WT mice were treated with 5% DSS, alone or in combination with 10 mg/kg of the TNF antibody infliximib for 7 days. **(A)** Real time RT-PCR analysis of *PUMA* mRNA expression in colonic mucosa of the treated mice. **(B)** H&E staining of colonic tissues from the treated mice (200×). **(C)** Colonic ulcers were counted following methylene blue staining. Values in (A) and (C) were means \pm SD ($n = 3$ in each group).

Supplemental Figure 12

Effects of the TNF antibody infliximib on TNBS-induced colitis. WT mice were treated with 100 mg/kg of TNBS, alone or in combination with 10 mg/kg of the TNF antibody infliximib for 3 days. **(A)** H&E staining of colonic tissues from the treated mice (200×). **(B)** TUNEL (brown) staining of colonic tissues from the treated mice (200×). **(C)** Apoptotic index was calculated by counting TUNEL signals in 100 randomly selected crypts following TUNEL staining as in (B). Values were means \pm SD ($n = 3$ in each group).

Supplemental Figure 13

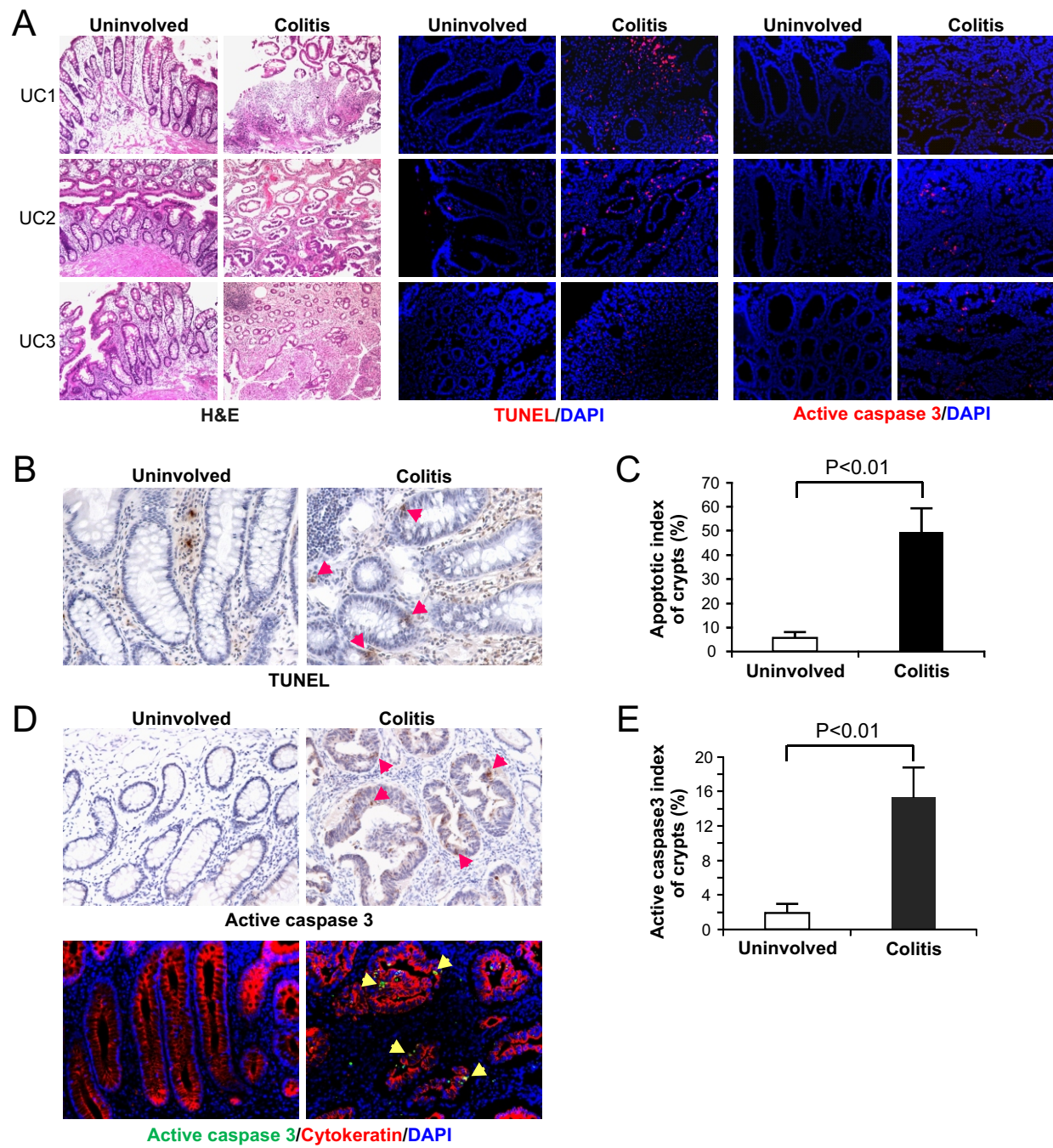
Effects of the TNF inhibitor pentoxifylline on DSS-induced colitis. WT mice were treated with 5% DSS, alone or in combination with 200 mg/kg of the TNF inhibitor pentoxifylline (PTX). **(A)** Real time RT-PCR analysis of *PUMA* mRNA expression in colonic mucosa of the mice treated for 24 hr. Values were means \pm SD ($n = 3$ in each group). **(B)** Western blot analysis of *PUMA* expression in colonic mucosa of the mice treated for 24 hr. **(C)** Disease activity index in the mice treated with DSS with or without PTX was measured at indicated time points. * $P < 0.01$ compared to DSS alone (two-way ANOVA). **(D)** Methylene blue staining of colonic tissues

from the mice treated for 7 days, with the arrow indicating an ulcer (200×). **(E)** Colonic ulcers were counted following methylene blue staining as in (D). **(F)** H&E staining of colonic tissues from the mice treated for 7 days (200×). **(G)** Histological damage in colonic tissues from the mice treated for 7 days was scored following H&E staining as in (F). **(H)** TUNEL staining (red) of colonic tissues from the mice treated for 7 days. **(I)** Apoptotic index was calculated by counting TUNEL signals in 100 randomly selected crypts. Values in (C), (E), (G) and (I) were means \pm SD ($n = 5$ in each group).

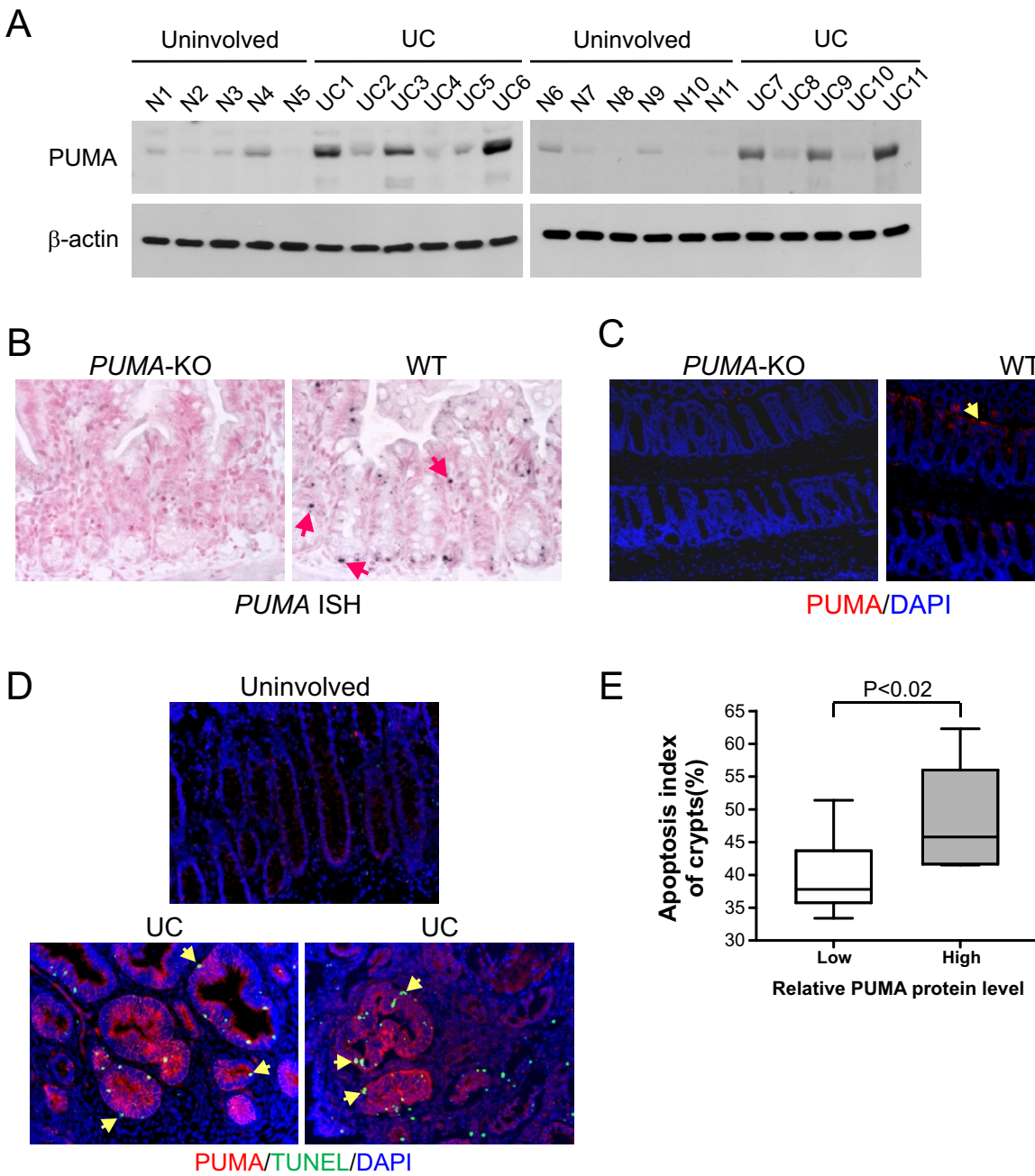
Supplemental Figure 14

Effects of the TNF inhibitor pentoxifylline on TNBS-induced colitis. WT mice were treated with 100 mg/kg of TNBS, alone or in combination with 200 mg/kg of the TNF- α inhibitor pentoxifylline (PTX). **(A)** Western blot analysis of the indicted proteins in colonic mucosa from the mice treated for 24 hr. **(B)** H&E staining of colonic tissues from the mice treated for 3 days (200×). **(C)** Histological damage in the mice treated with TNBS with or without PTX for 3 days was quantified following H&E staining as in (B). **(D)** TUNEL (brown) staining of colonic tissues from the mice treated for 3 days. Arrows indicate example TUNEL-positive cells. **(E)** Apoptotic index in the mice treated as indicated for 3 days was calculated by counting TUNEL signal in 100 randomly selected crypts. Values in (C) and (E) were means \pm SD ($n = 3$ in each group).

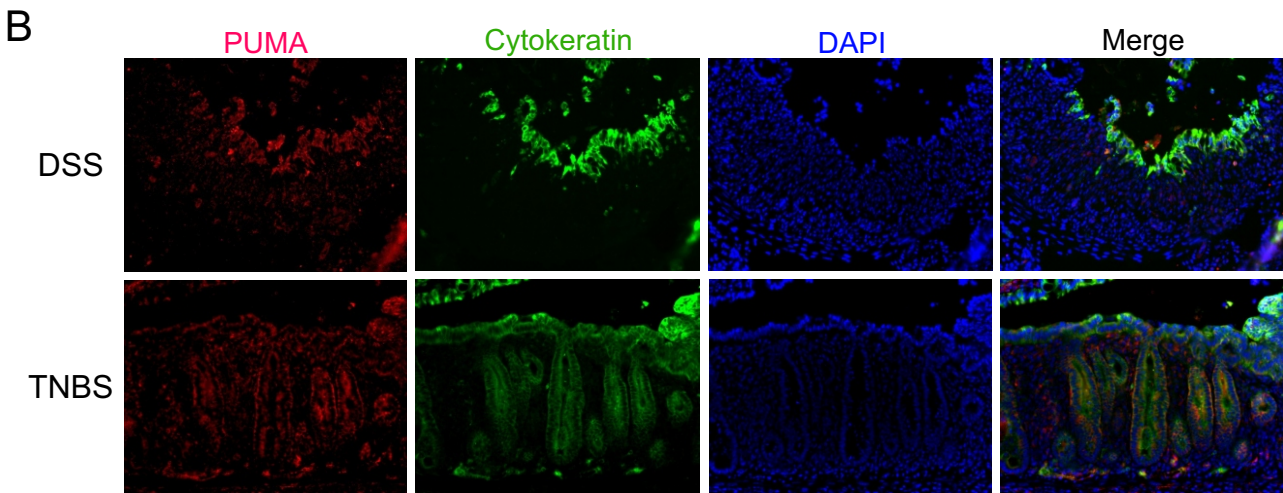
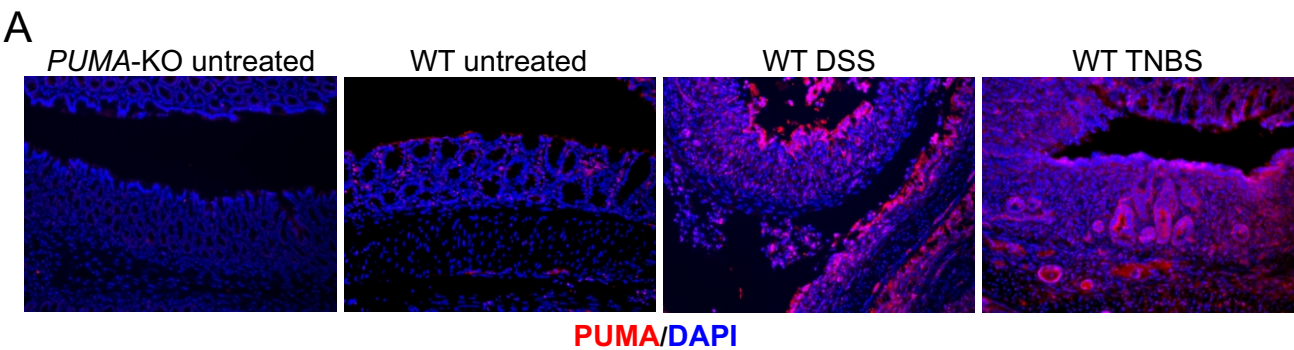
Supplemental Figure 1



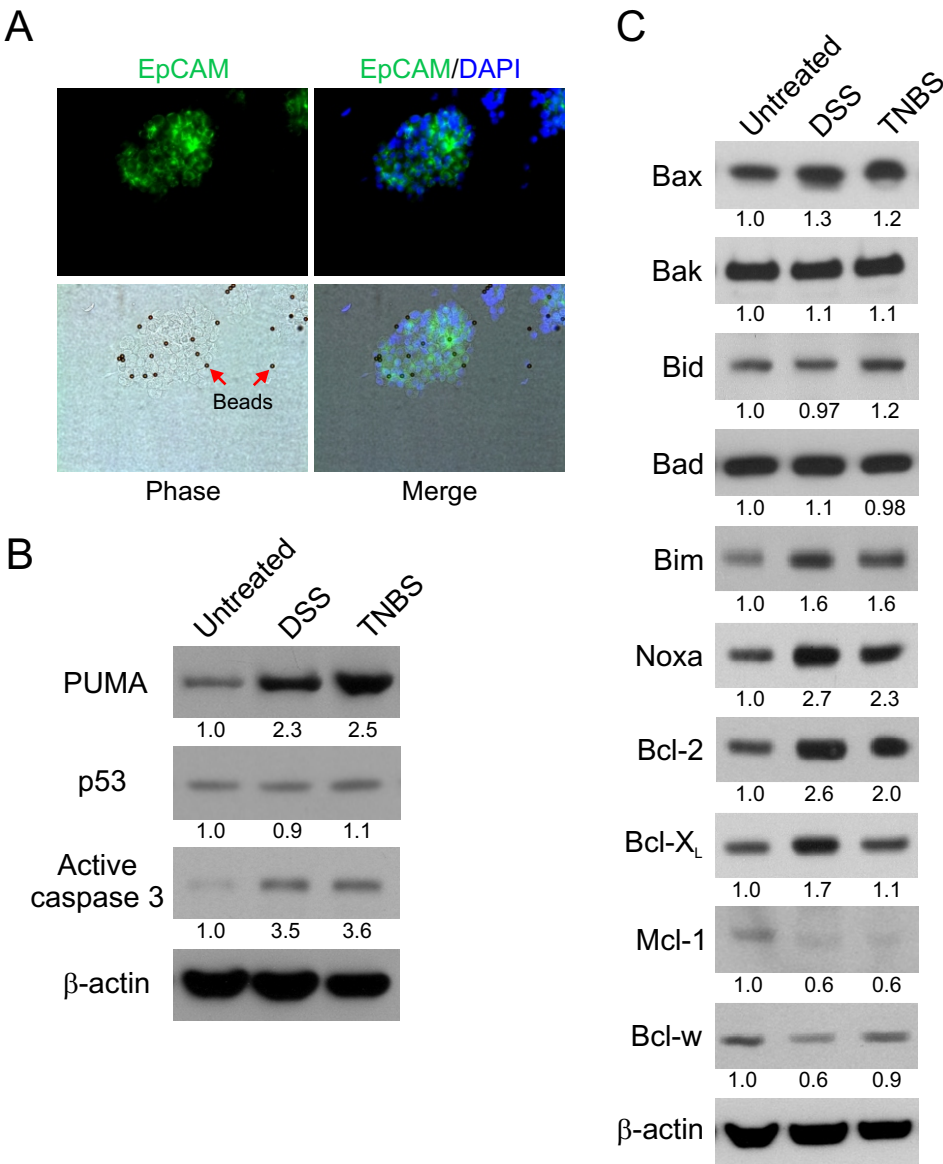
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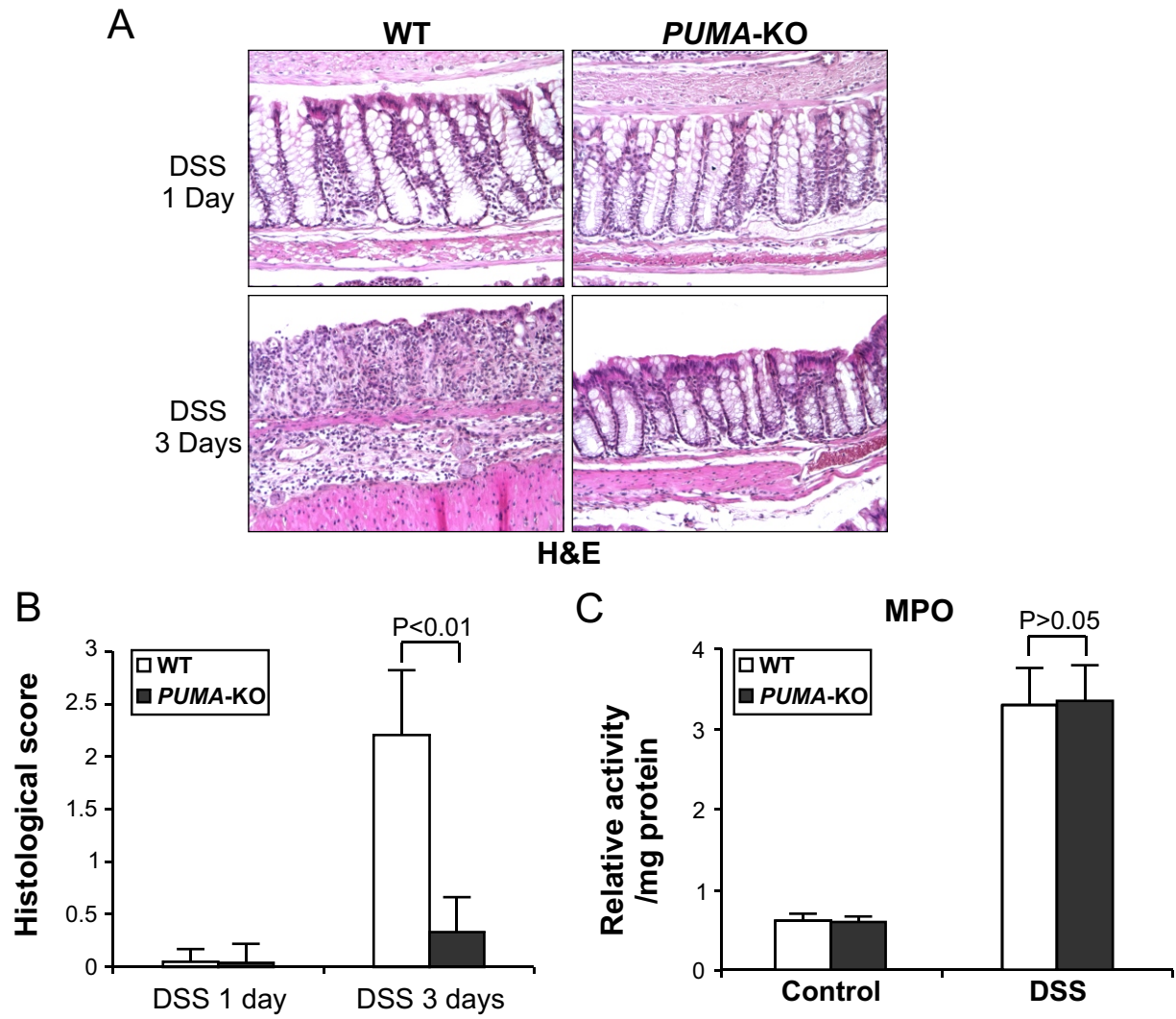
Supplemental Figure 3



Supplemental Figure 4

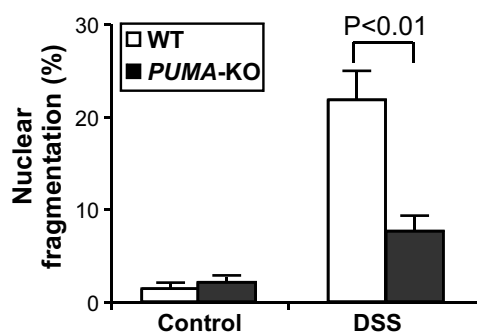


Supplemental Figure 5

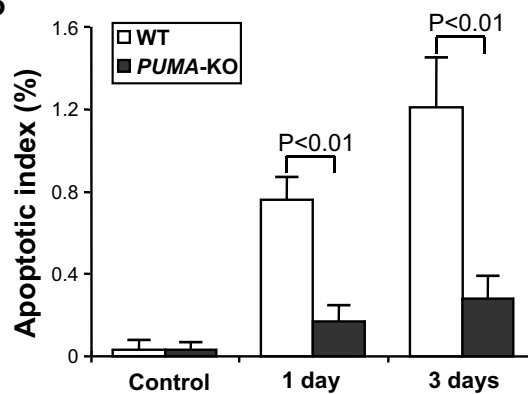


Supplemental Figure 6

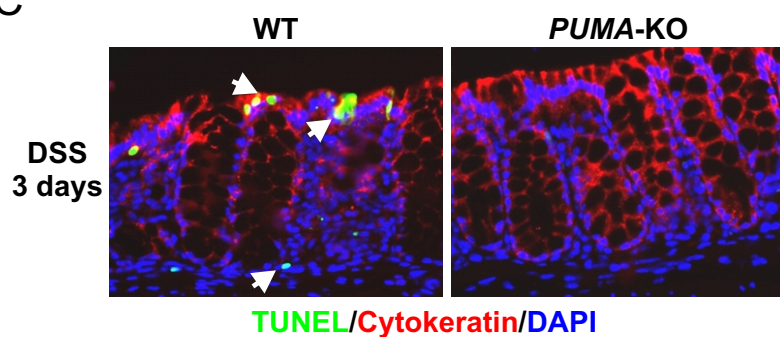
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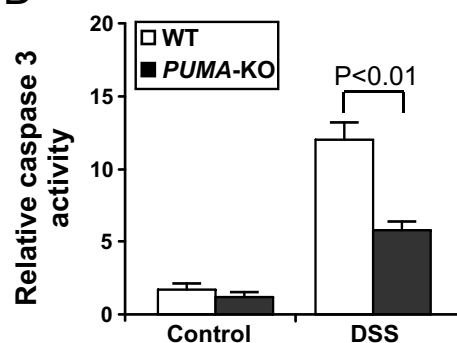
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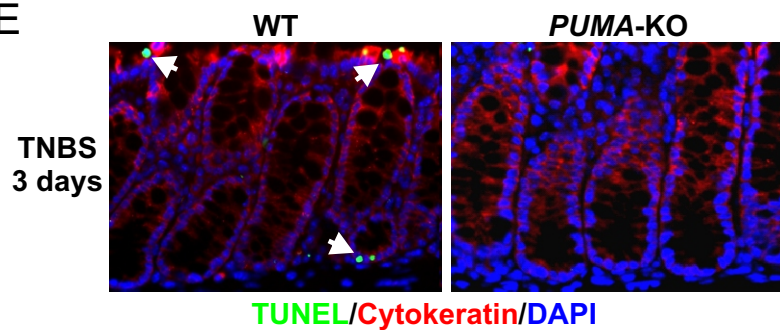
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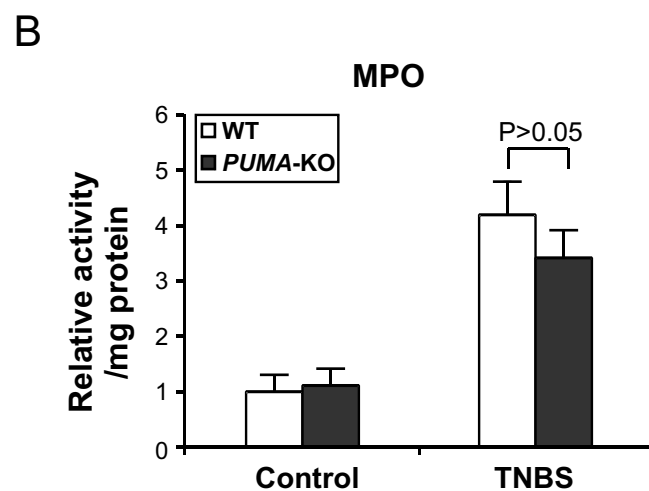
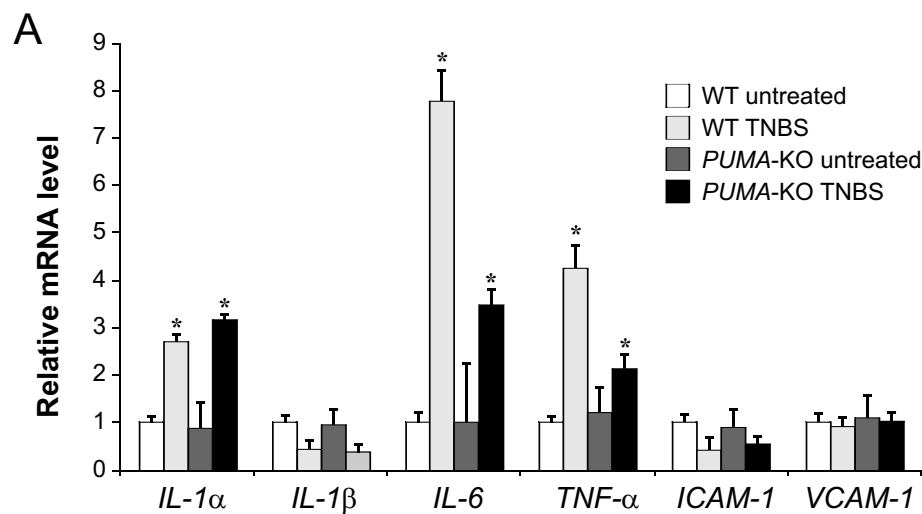
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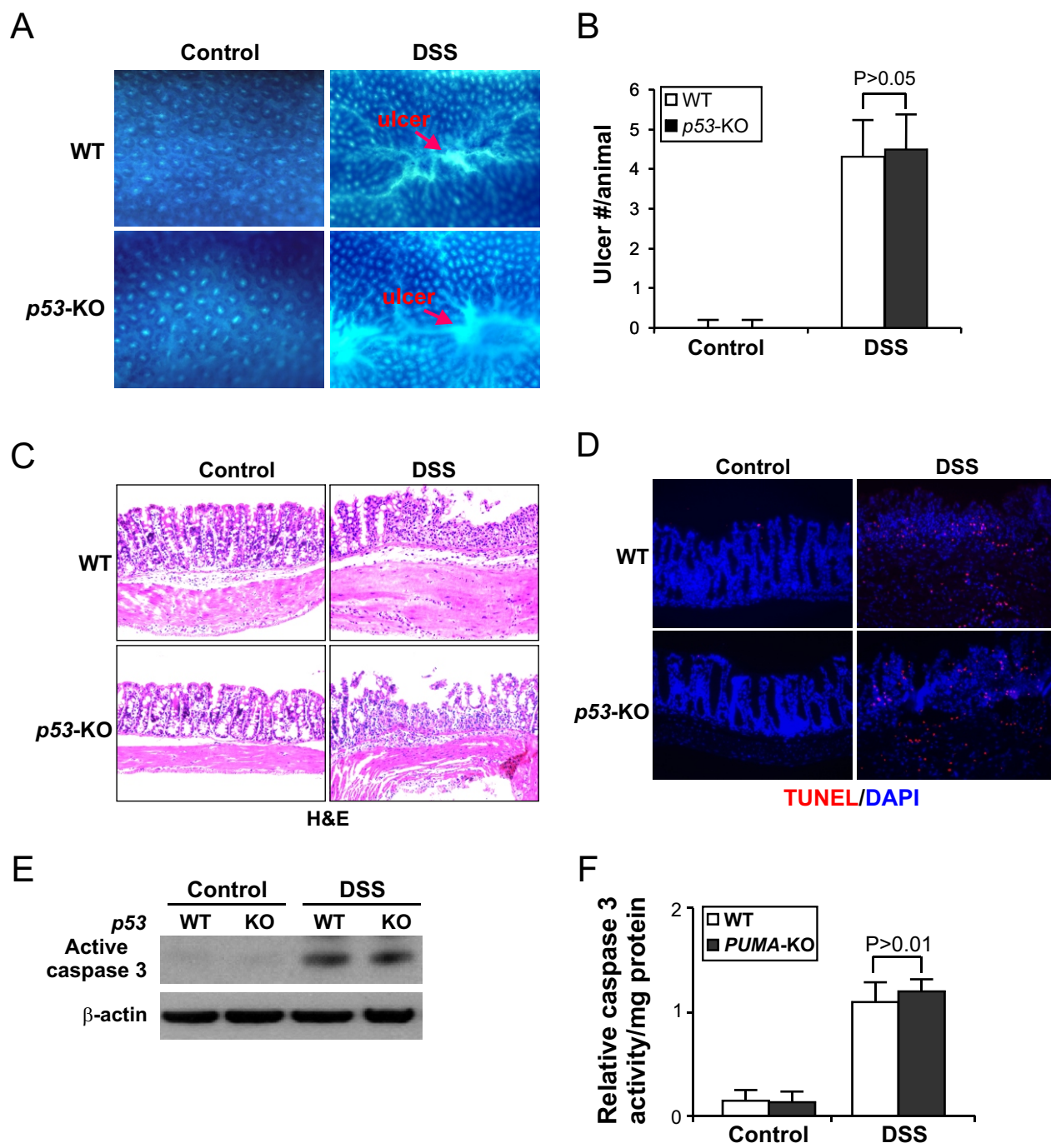
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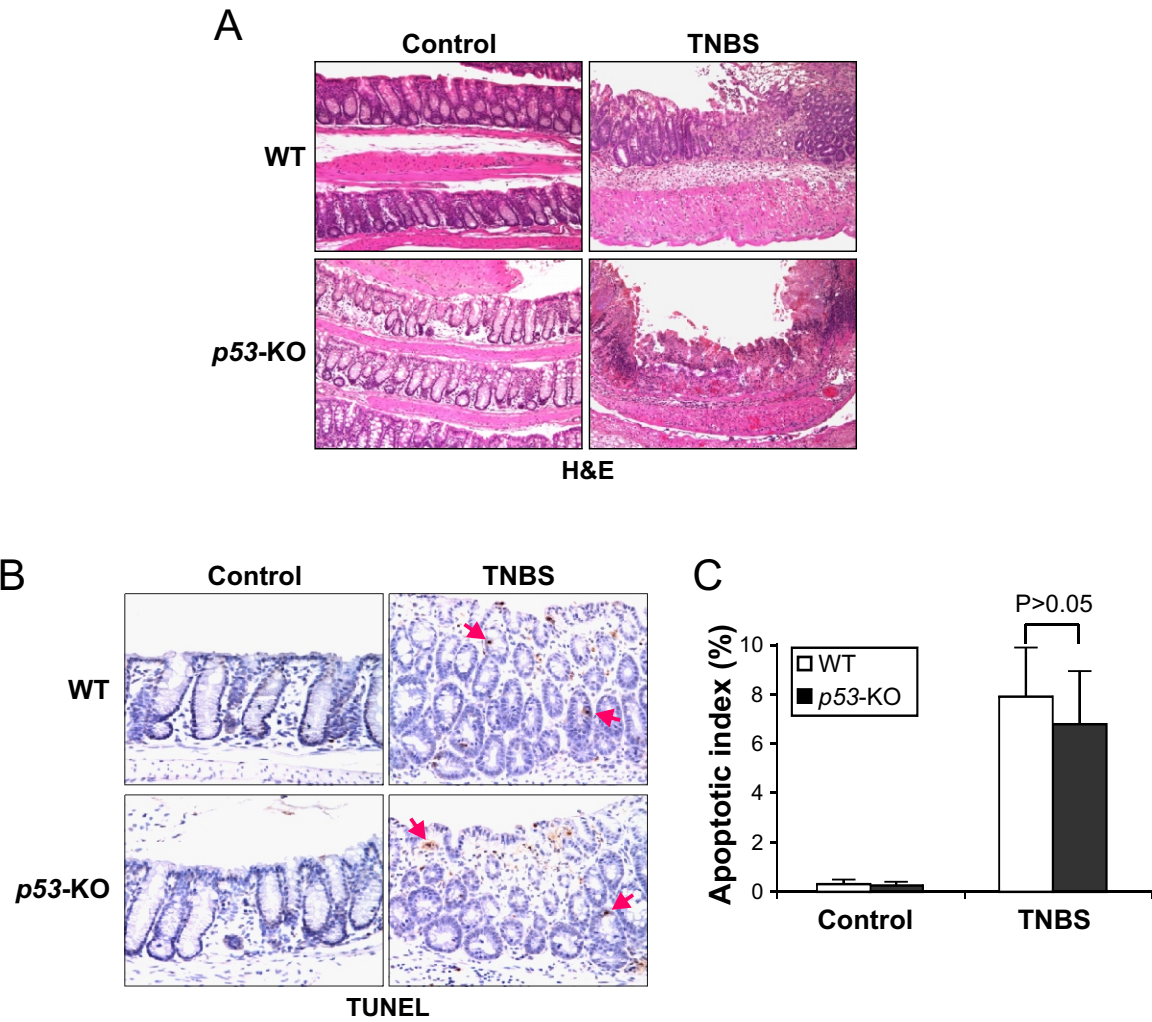
Supplemental Figure 7



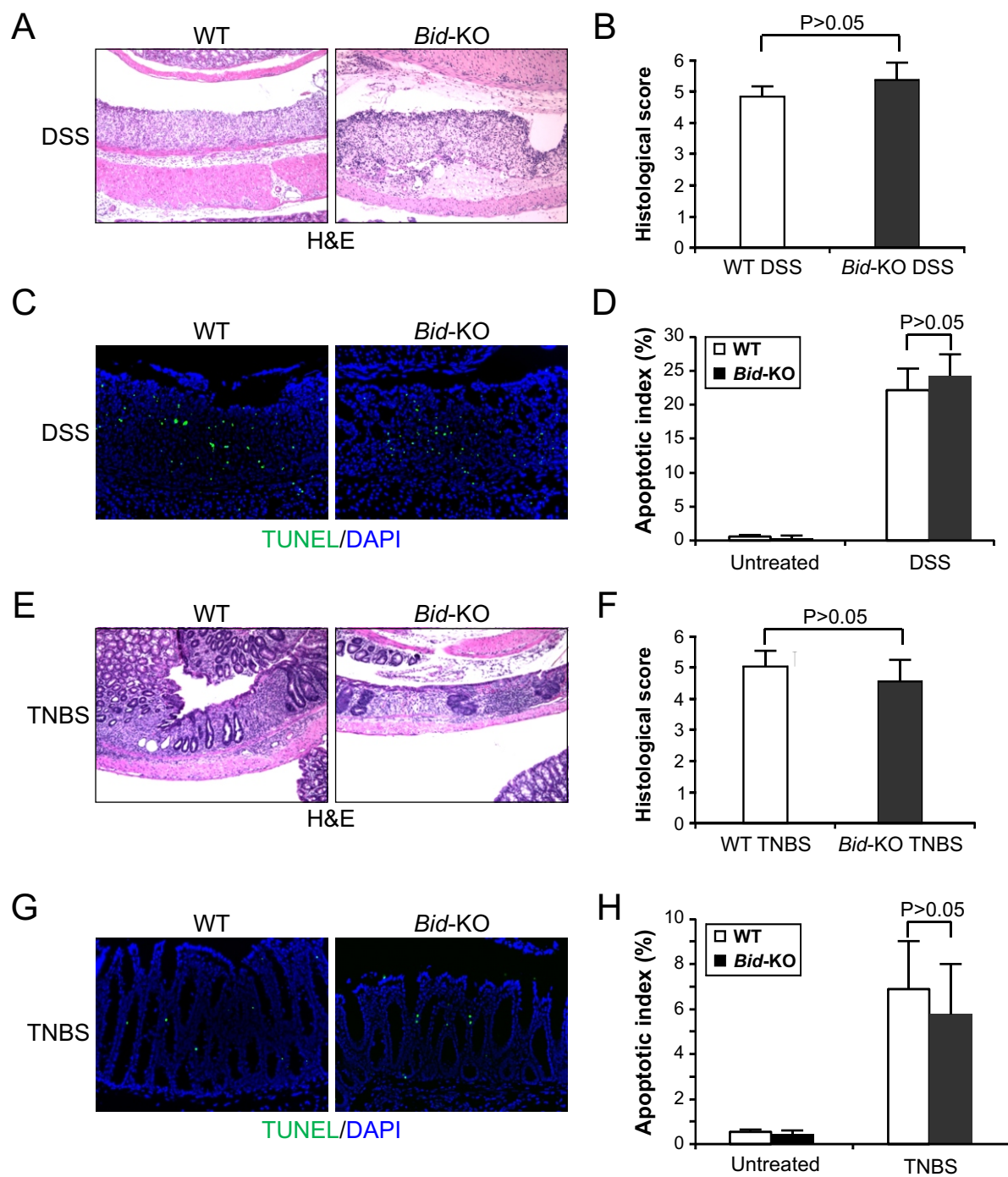
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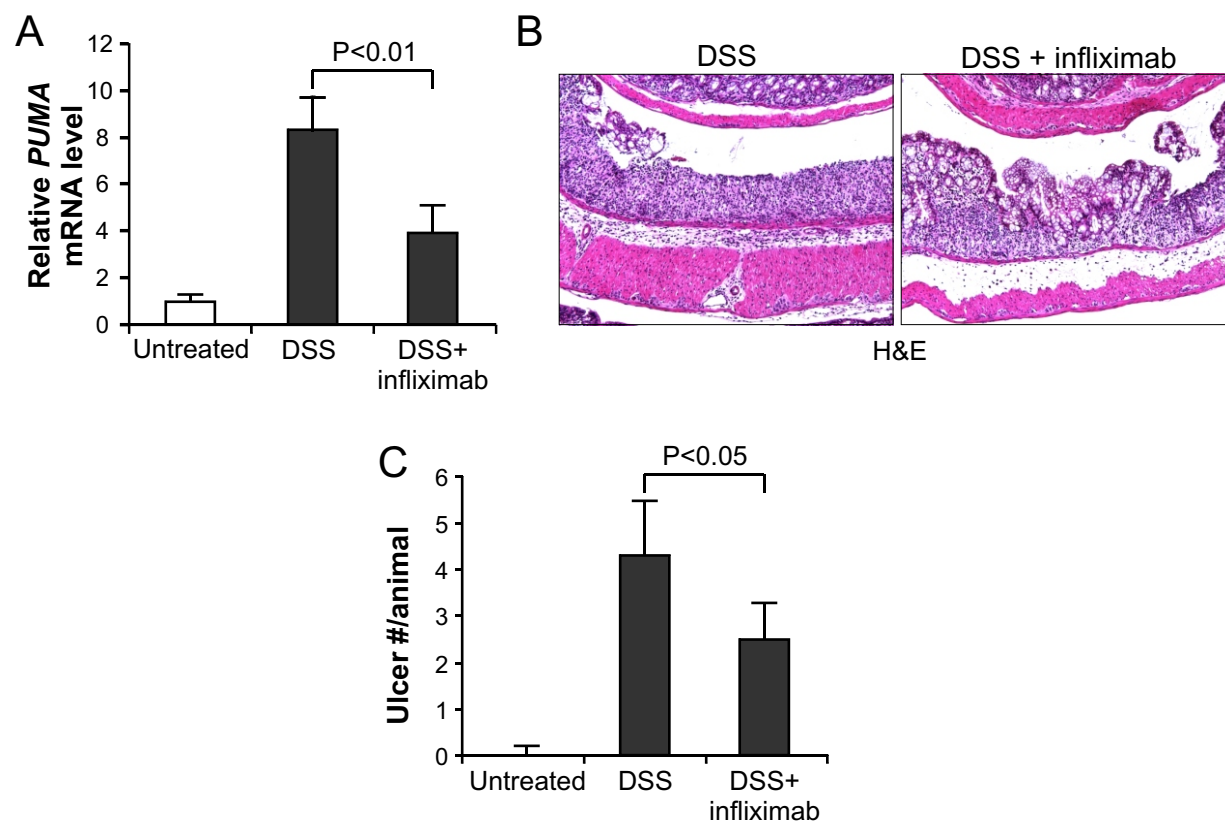
Supplemental Figure 9



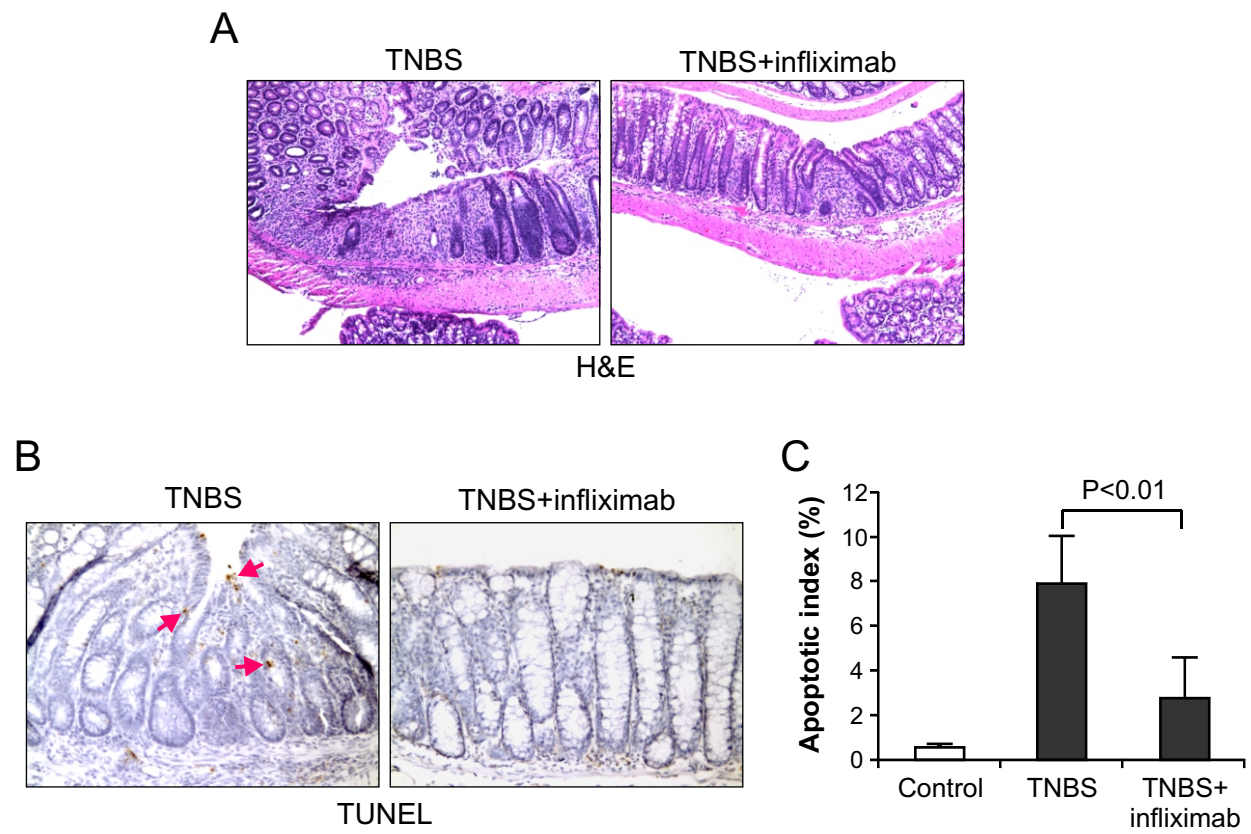
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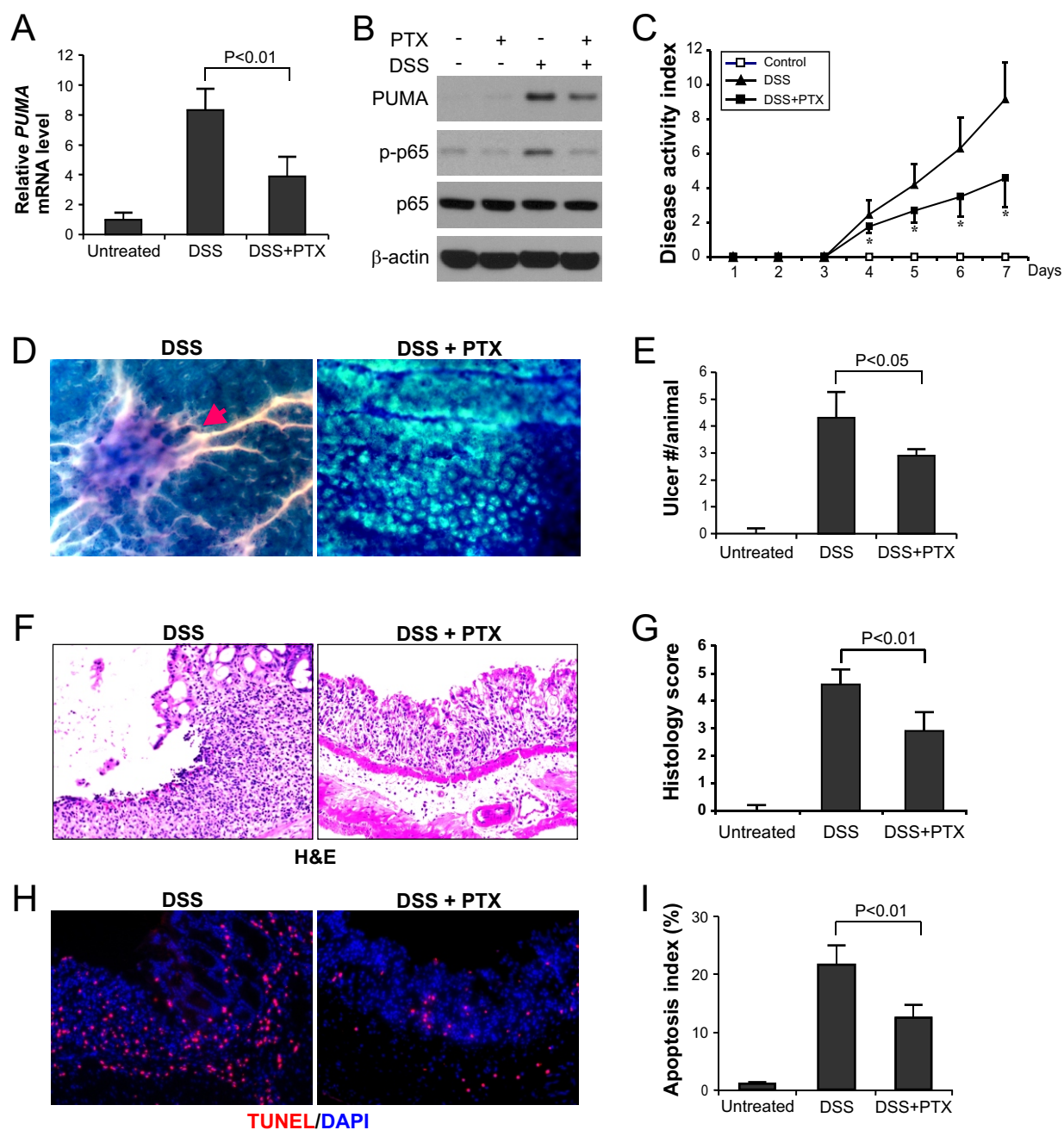
Supplemental Figure 11



Supplemental Figure 12



Supplemental Figure 13



Supplemental Figure 14

