SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Suppression of Notch signaling by DBZ in HT29 cells does not result in cell death *in vitro*.

Human colon cancer cell line HT29 was treated with increasing concentrations of the γ-secretase inhibitor, DBZ, for up to 72 hr. ***(A)*** The graph depicts the percentages of viable cells as determined by Trypan Blue stain. Comparison was performed by paired 2-tailed *t* test. N = 4. ***(B)*** The graph depicts the percentages of cells in the sub-G_1 fraction as determined by FACS analysis. Comparison was performed by paired 2-tailed *t* test. N = 4. ***(C)*** Western blot analysis for cleaved caspase-3 and cleaved PARP. Actin serves as a loading control. The control represents lysates obtained from cells irradiated with 12 Gy of γ-ray.

Supplementary Figure 2. The effect of Notch suppression by DBZ in HT29 cells on p27 and p57 levels.

HT29 cells were treated with increasing concentrations of the γ-secretase inhibitor, DBZ, for up to 72 hr. The levels of p27 and p57 were determined by Western blot analysis as shown. Actin was shown as a loading control.

Supplementary Figure 3. Correlation between Notch activity and rate of proliferation of HT29 cells.

(A) HT29 cells were transfected with an empty vector control or an expression construct containing NICD. Rates of proliferation were assessed by counting the number of cells daily. * $p < 0.05$; and *** $p < 0.001$ when compared to control by paired 2-tailed *t* test. N = 4. ***(B)*** HT29 cells were transfected with scrambled control siRNA or with Notch-specific siRNA. Rates of proliferation were assessed by counting the number of cells daily. *** $p < 0.001$ when compared to control by paired 2-tailed *t* test. N = 4.

Supplementary Figure 4. The mouse *Klf4* promoter contains potential HES1 binding sites.

The sequence containing 1,000 bp of the 5'-flanking region and the 5'-untranslated region of the mouse *Klf4* gene is shown (1). The initiation site of transcription is identified by an arrow and that of translation in bold letters. The TATA box is identified with a box. The five potential HES1-binding sites are identified by the italic and underlined sequences and include 3 class C, 1 reverse class C and 1 reverse N box sequences (2, 3).

Supplementary Figure 5. AB/PAS and Klf4 staining of the small intestines from control and DBZ-treated wild type mice.

Small intestines obtained from age-matched control (***A*** and ***B***) and DBZ-treated (***C*** and ***D***) wild type mice were stained with AB/PAS (***A*** and ***C***) and Klf4 (***B*** and ***D***).

Supplementary Figure 6. AB/PAS stain of the large intestines from control and DBZ-treated wild type or *Apc*$^{Min/+}$ mice.

Large intestines obtained from control ***(A)*** and DBZ-treated ***(C)*** wild type mice, and control ***(B)*** and DBZ-treated ***(D)*** *Apc*$^{Min/+}$ mice were stained with AB/PAS.

Supplementary Figure 7. Klf4 immunostaining of the large intestines from control and DBZ-treated wild type or *Apc*$^{Min/+}$ mice.

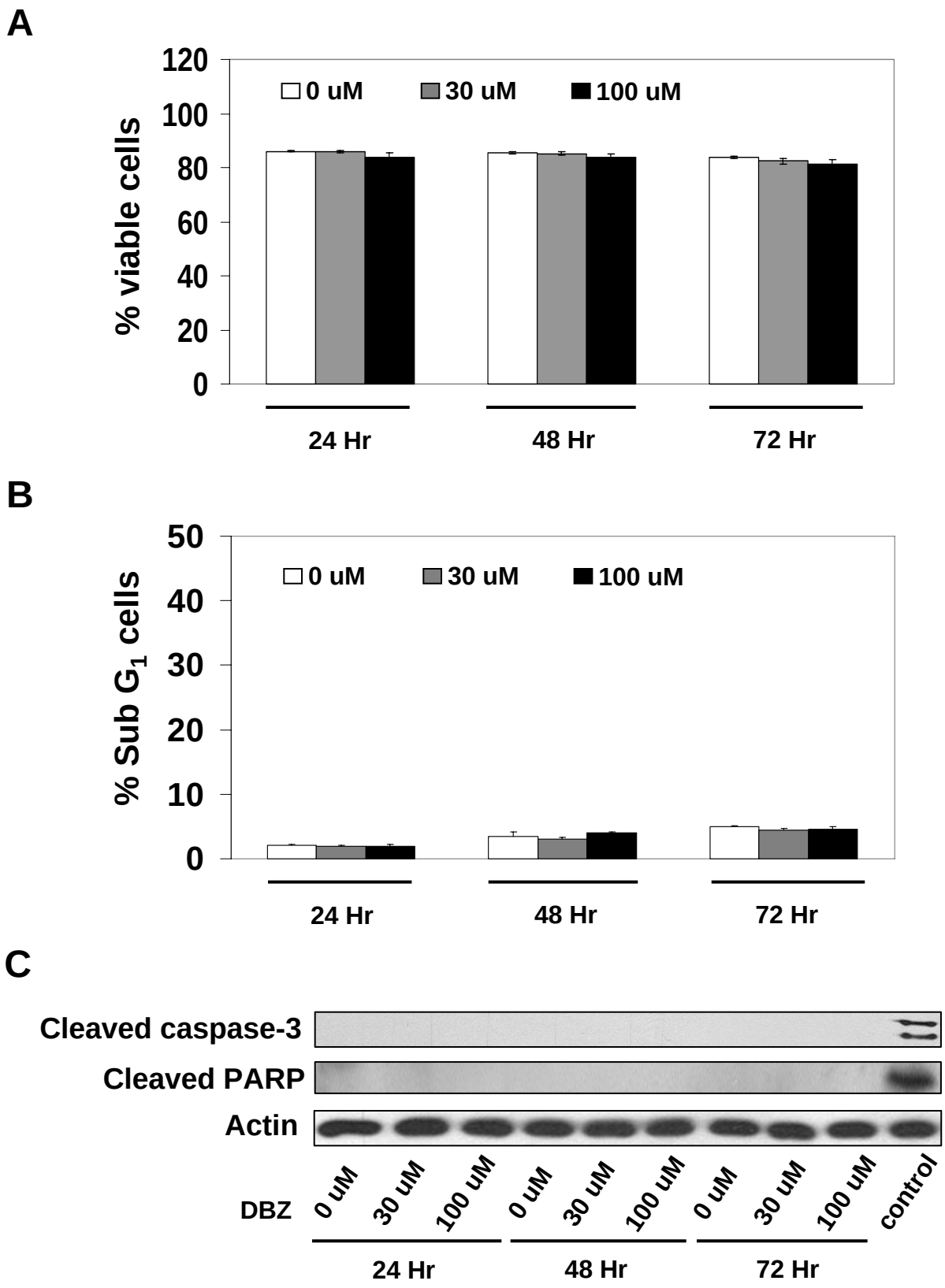
Large intestines obtained from control ***(A)*** and DBZ-treated ***(C)*** wild type mice, and control ***(B)*** and DBZ-treated ***(D)*** *Apc*$^{Min/+}$ mice were stained for Klf4.

Supplementary Figure 8. Ki67 immunostaining of the normal-appearing small intestine and adenomas from control and DBZ-treated *Apc*$^{Min/+}$ mice.

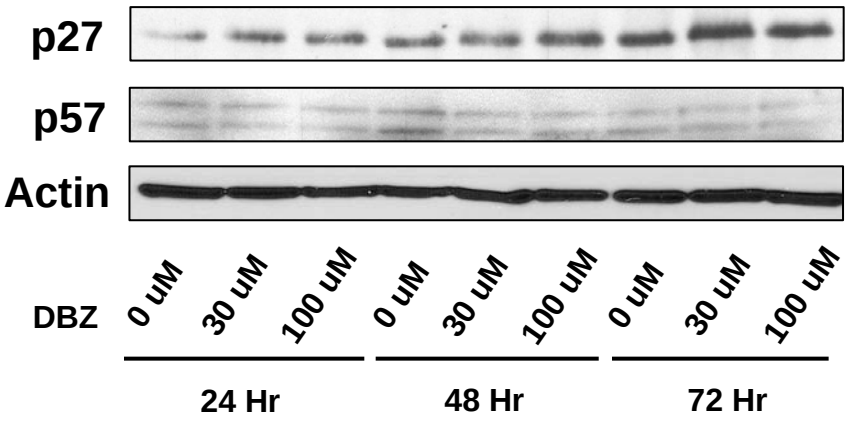
Normal-appearing small intestinal tissues (***A*** and ***C***) and small intestinal adenomas (***B*** and ***D***) obtained from age-matched control *Apc*$^{Min/+}$ mice (***A*** and ***B***) and DBZ-treated *Apc*$^{Min/+}$ mice (***C*** and ***D***) were immunostained with antibodies against Ki67.

SUPPLEMENTARY REFERENCES

1. Mahatan CS, Kaestner KH, Geiman DE, Yang VW. Characterization of the structure and regulation of the murine gene encoding gut-enriched Kruppel-like factor (Kruppel-like factor 4). Nucleic Acids Res 1999;27:4562-9.
2. Iso T, Kedes L, Hamamori Y. HES and HERP families: multiple effectors of the Notch signaling pathway. J Cell Physiol 2003;194:237-55.
3. Murata K, Hattori M, Hirai N, et al. Hes1 directly controls cell proliferation through the transcriptional repression of p27Kip1. Mol Cell Biol 2005;25:4262-71.

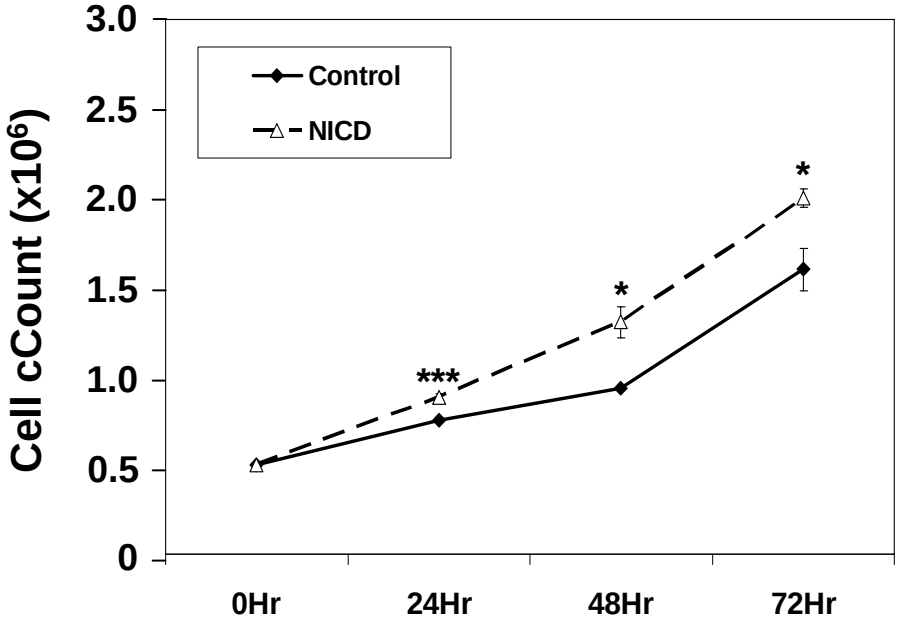


Supplementary Figure 1



Supplementary Figure 2

A



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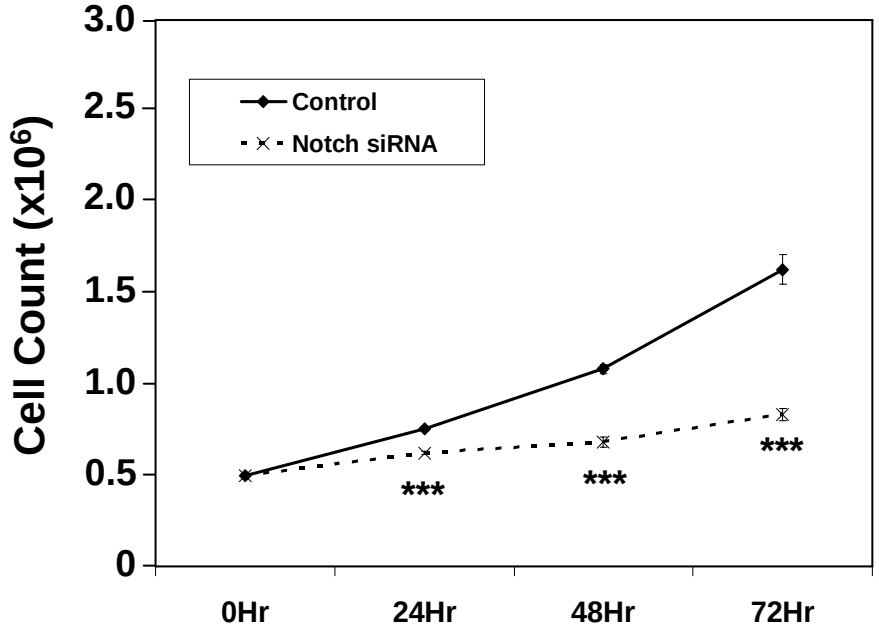

Supplementary Figure 3

-1,000 bp

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TTCTCATCTTCAAAATGAAGAGTGCGAGTGCGTAGCTTGTAAAGAGCTTT
GAGATCC*CTGGTG*AAAGGTATCTCCGCGCCTCGGTCCCTCTCCGCGTTCC Reverse N box
CACTCCCCCACCCCCAATGTAAATCTGGAACGCCTTCCTTGTTCCTTCCA
TTATCAAGATGACTACGTGCCCAATCCCGGCCGCTCTCTTTCATAGCAGG
CGCGGAACCCCTTCTCCTAGCTTCTGAGATTCCTAGCGCCCCCCCCCAAT
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AGGCCAGGCCCTGCGCCGACACGGCCCGGCGTCTGCGTGCGTATGCGAGA
GAGGGCGGGCTGGATGAGT*CACGCG*GATAATCGCGCTCTCGAGTGCGCAG Class C
ACGACAGGACAAGCGCGTACGCGAGCAGGGGTAGGGGCGCGCCGCCTCTT
CCCCCCCACACACACACACCCCCAACCTCCTGTCCCCCGCCAGGGTTCCC
GTGGCAGGACTTTCGAGCCCAGGGAACCGACCGTGGCCGCCCAGAGGACC
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CCGTCGCCGCCACCGCCGCCGGCGTCAGCACGGCTCCAGCCCGCCAGCTG
CCTGGCTGGCGTCACGGCCTGGCCCCGCCCCGCCCGCGCCCCTCCCTCGC
CCCCGCCCCC*CACGTG*CGCGGAGTTTGTTTATTTAGCTACCATGGCAACGC Class C
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CCCCCCACCCAGCAACCCG*CCCGTG*ACCCGCGCCCATGGCCGCGCGCACC Reverse Class C
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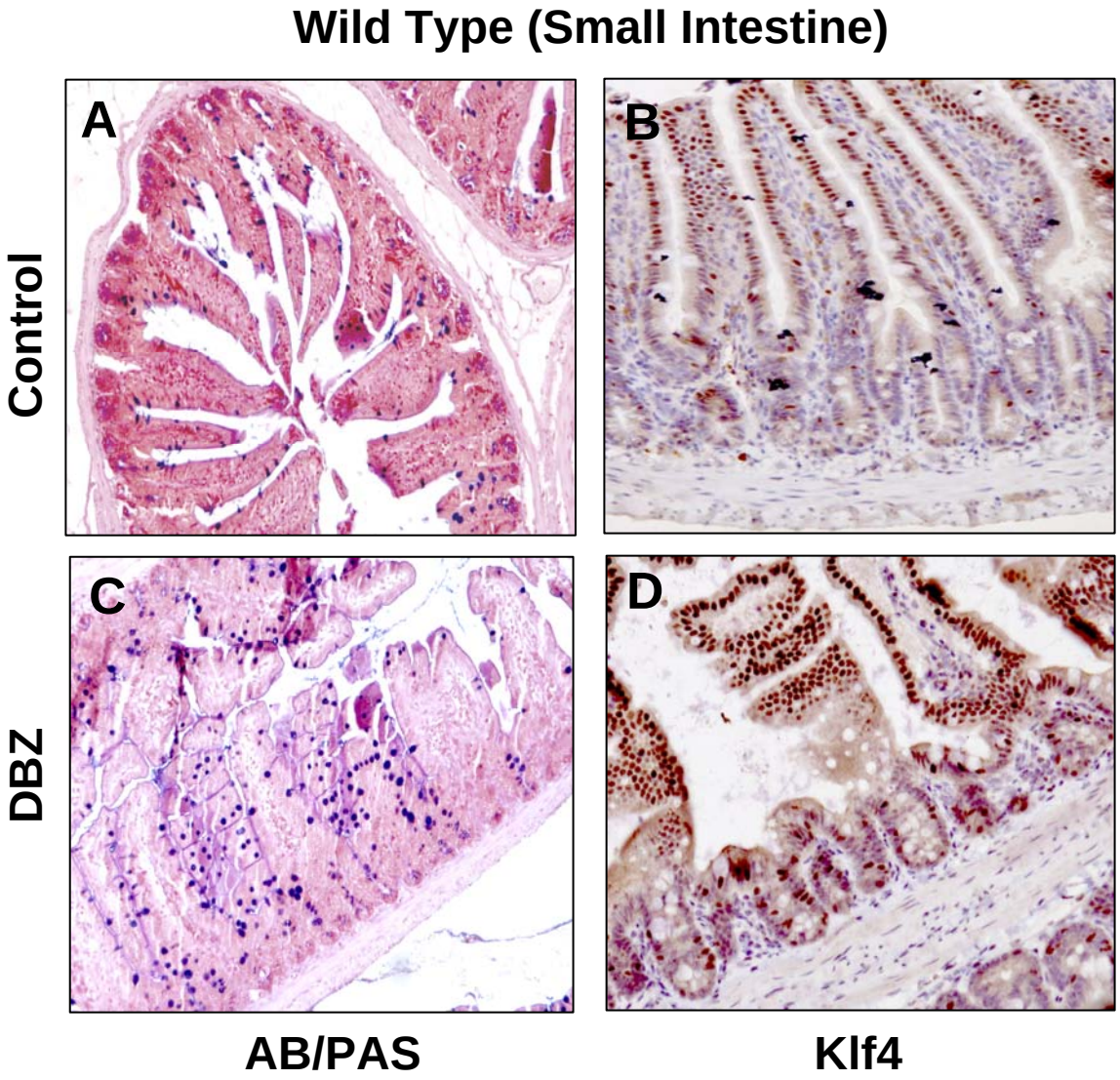
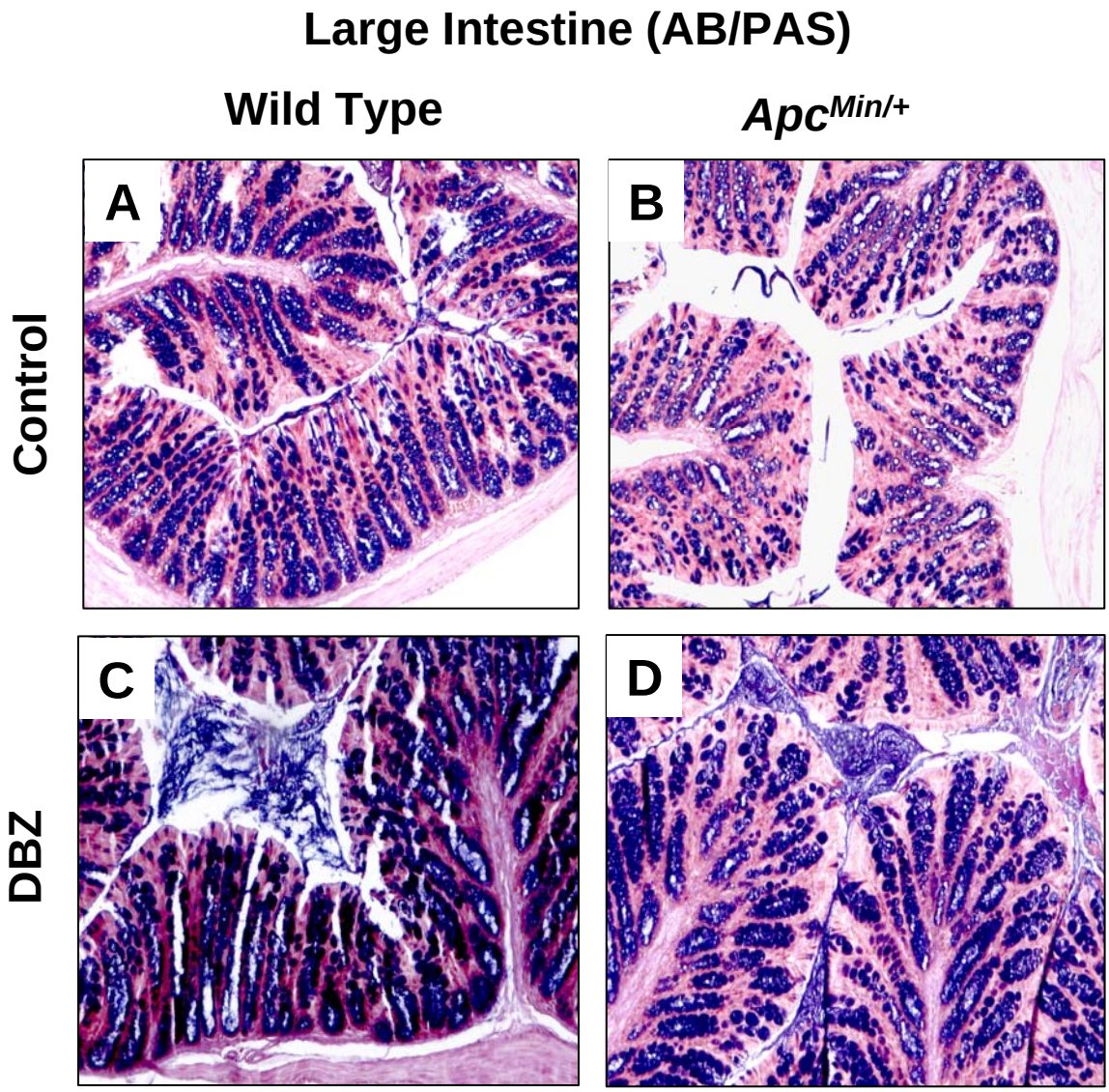
Supplementary Figure 4



Supplementary Figure 5



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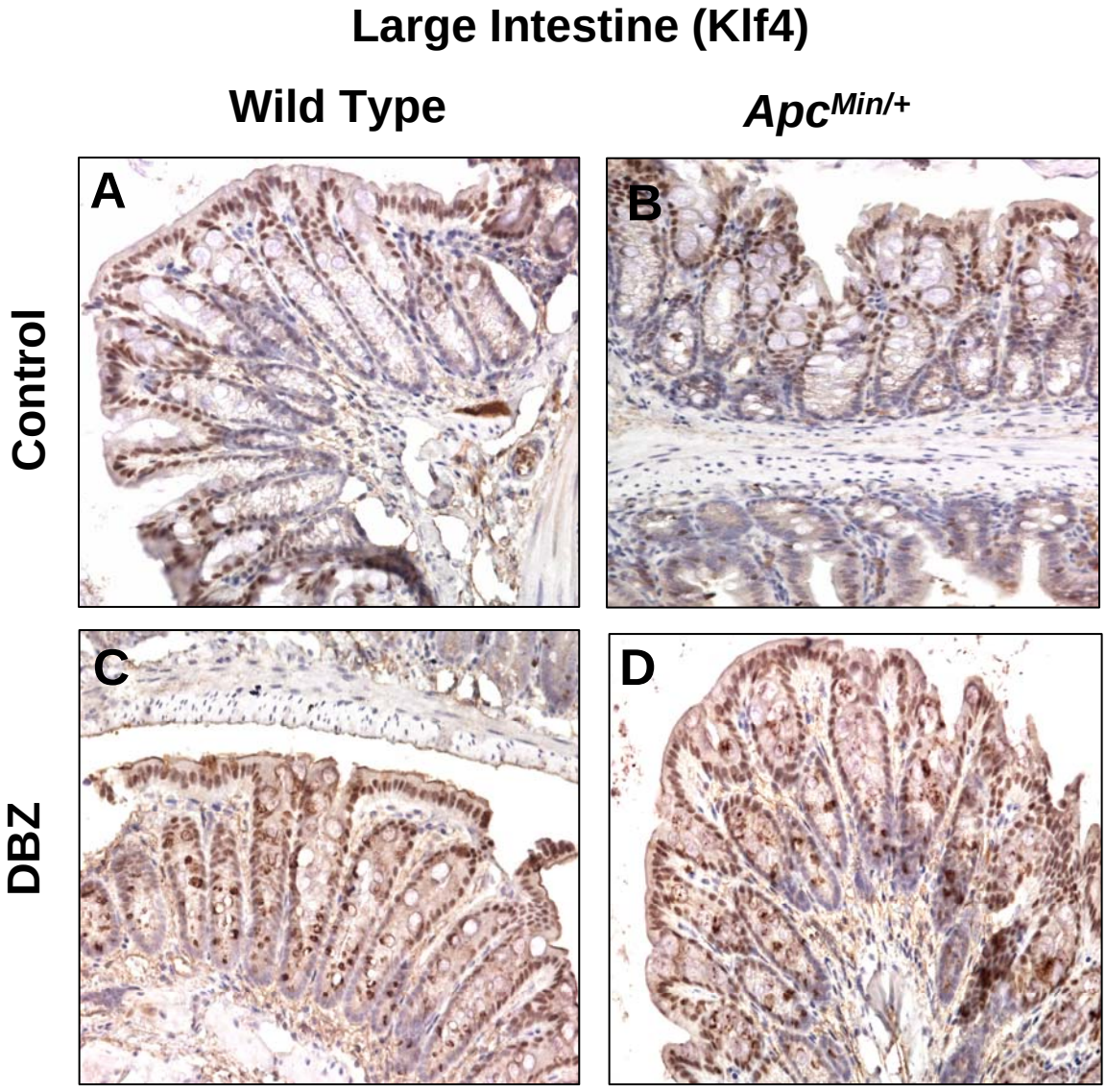

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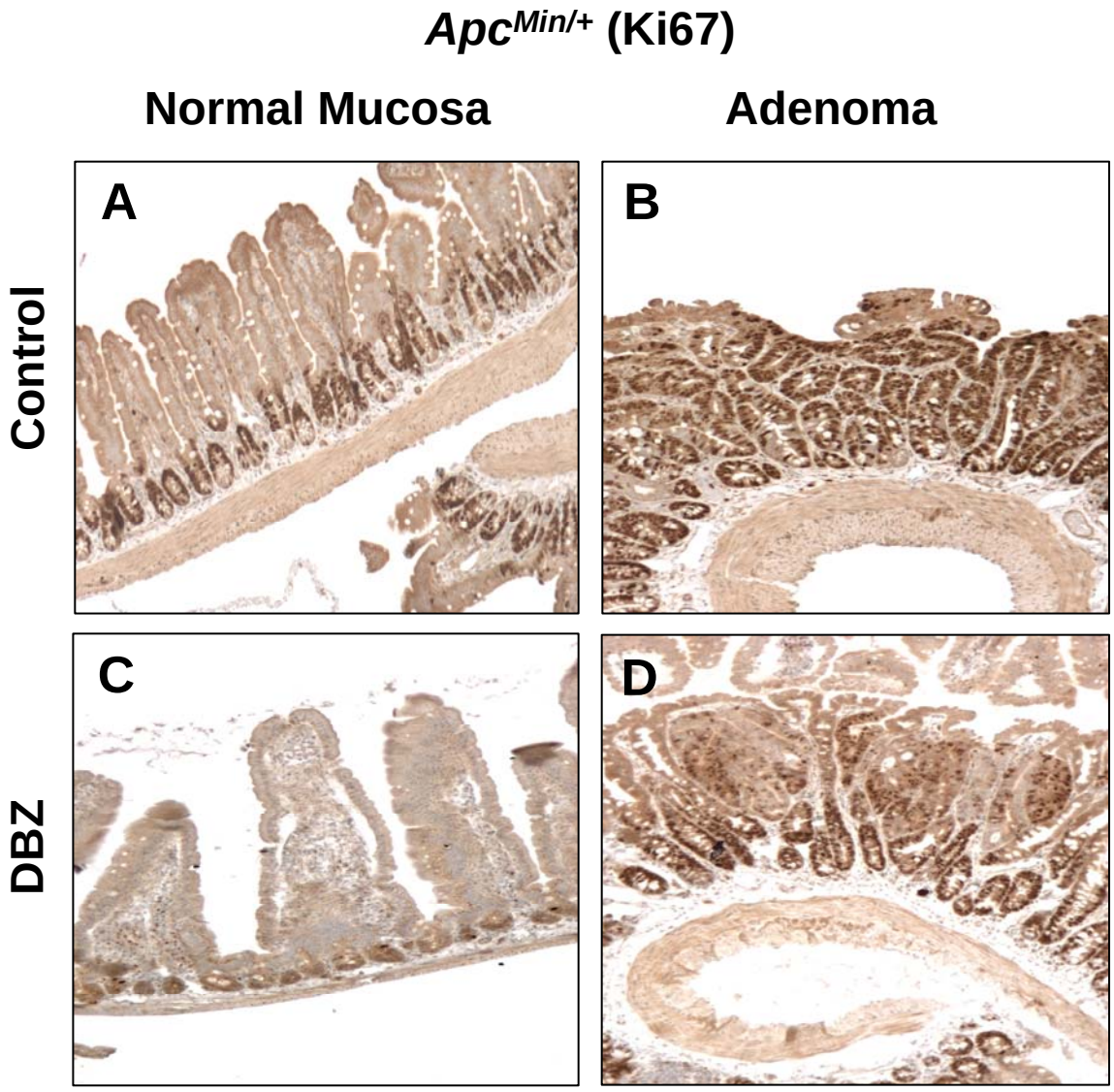

Supplementary Figure 8