Supplemental Data

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Figures S1-6

Tables S1-7

Figure S1

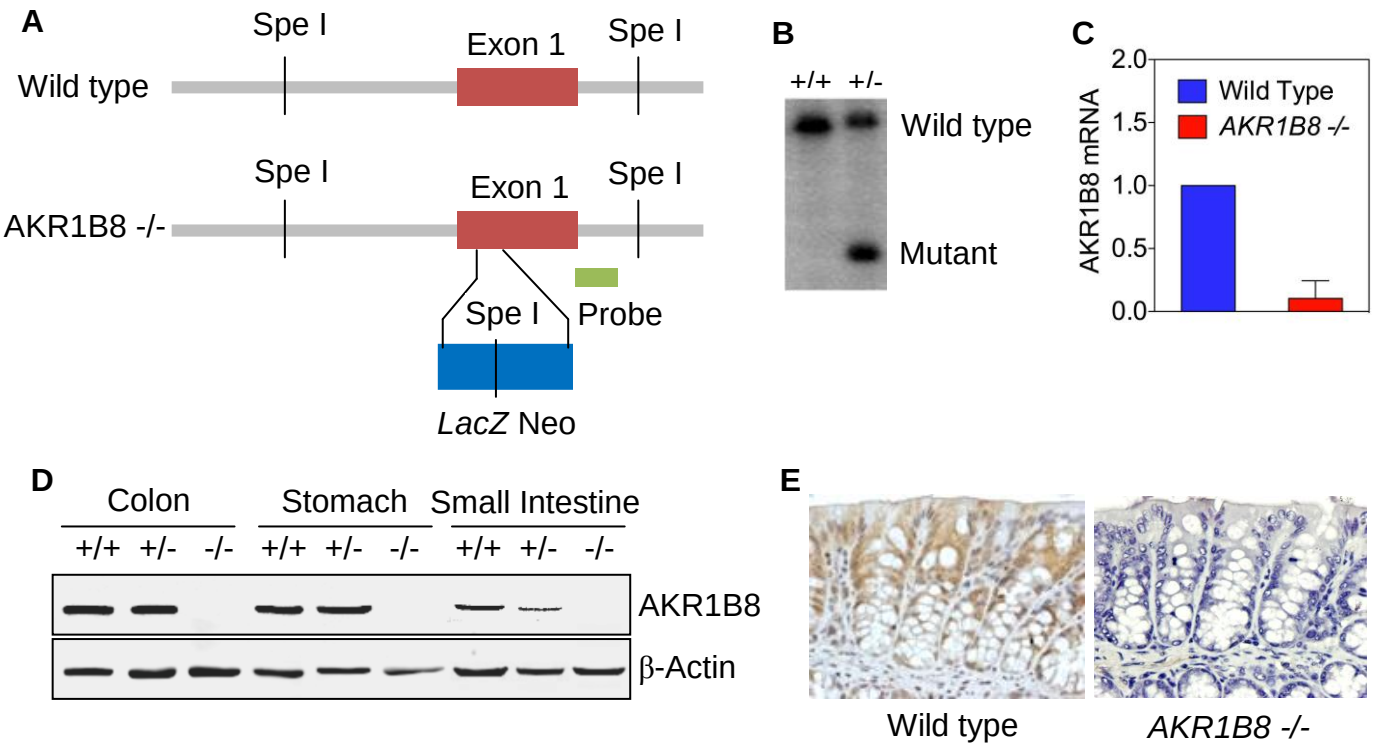


Figure S1. Targeted disruption of *AKR1B8* gene. (A) Schematic strategy for disruption of *AKR1B8* gene. Red box denotes Exon 1 of *AKR1B8* gene and blue box indicates LacZ/Neo expression cassette, a selection marker. (B) Southern blot. Mouse genomic DNA was extracted from tails, digested by SpeI, and probed by the DNA probe indicated in (A). (C) *AKR1B*8 mRNA level. Data represent mean ± SD from three mice. (D) Western blot and (E) Immunohistochemistry, indicating AKR1B8 protein levels.

Figure S2

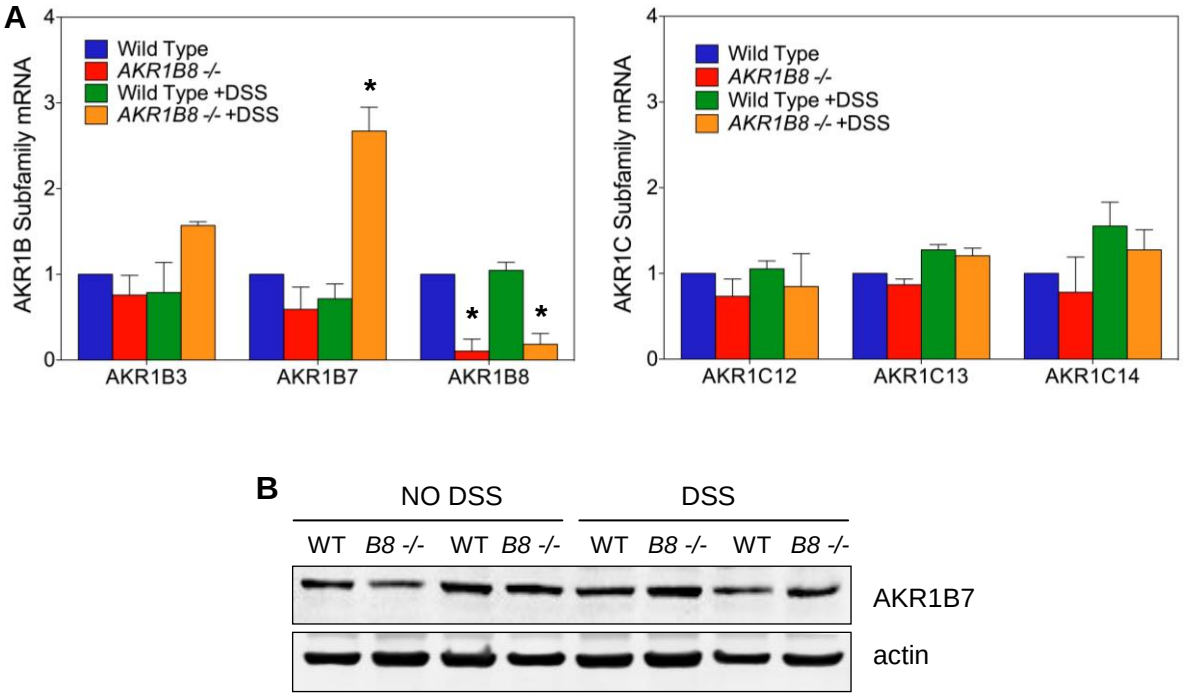
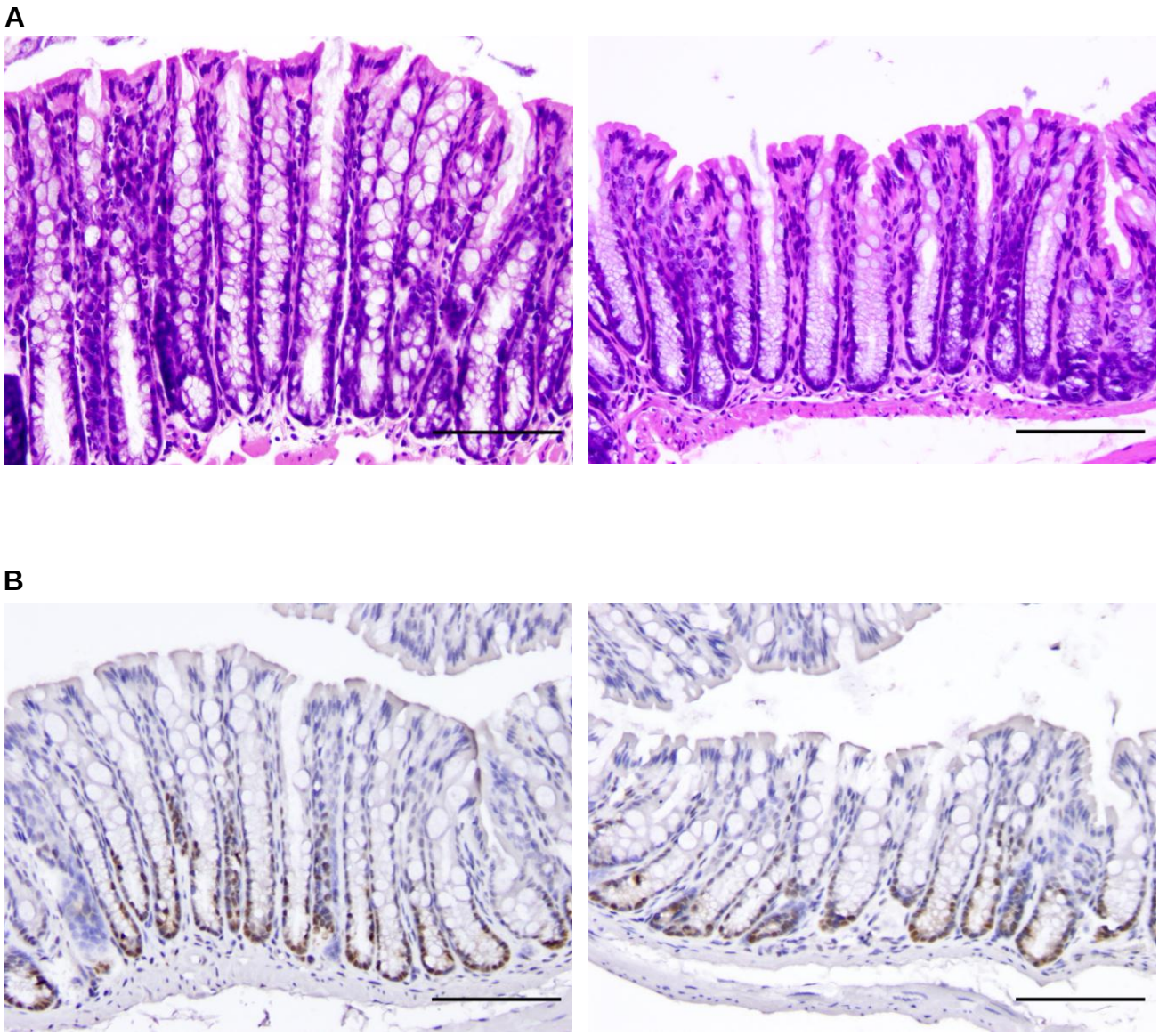


Figure S2. *AKR1B* and *AKR1C* subfamily expression. A) *AKR1B* and *AKR1C* subfamily mRNA levels detected by real-time RT-PCR. Data represent mean ± SD from three mice. * $P < 0.01$, compared to wild type control. AKR1B7 was induced by DSS- induced colitis in the *AKR1B8 -/-* mice. B) Western blot of AKR1B7. WT, wild type; B8, AKR1B8.

Figure S3



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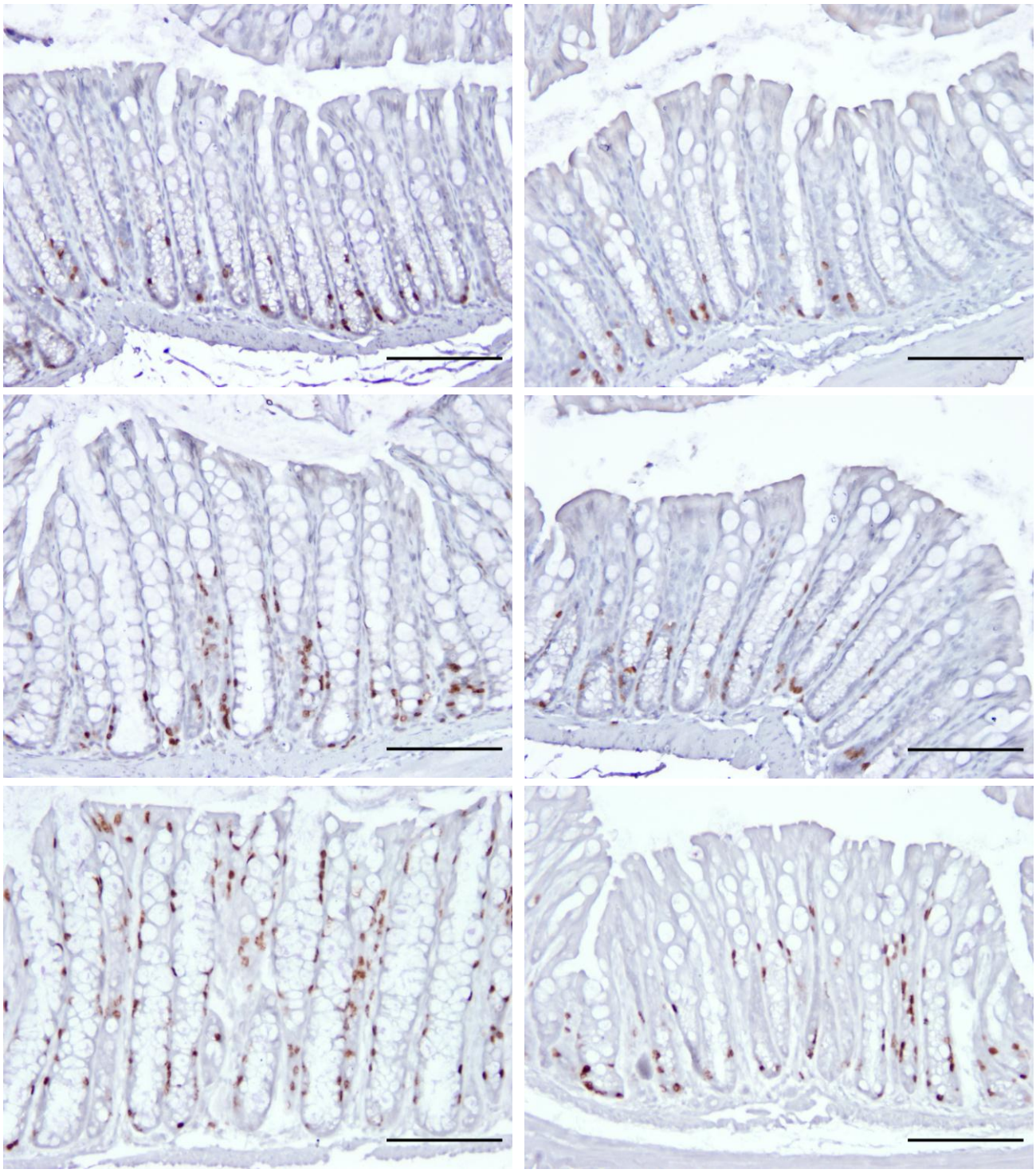


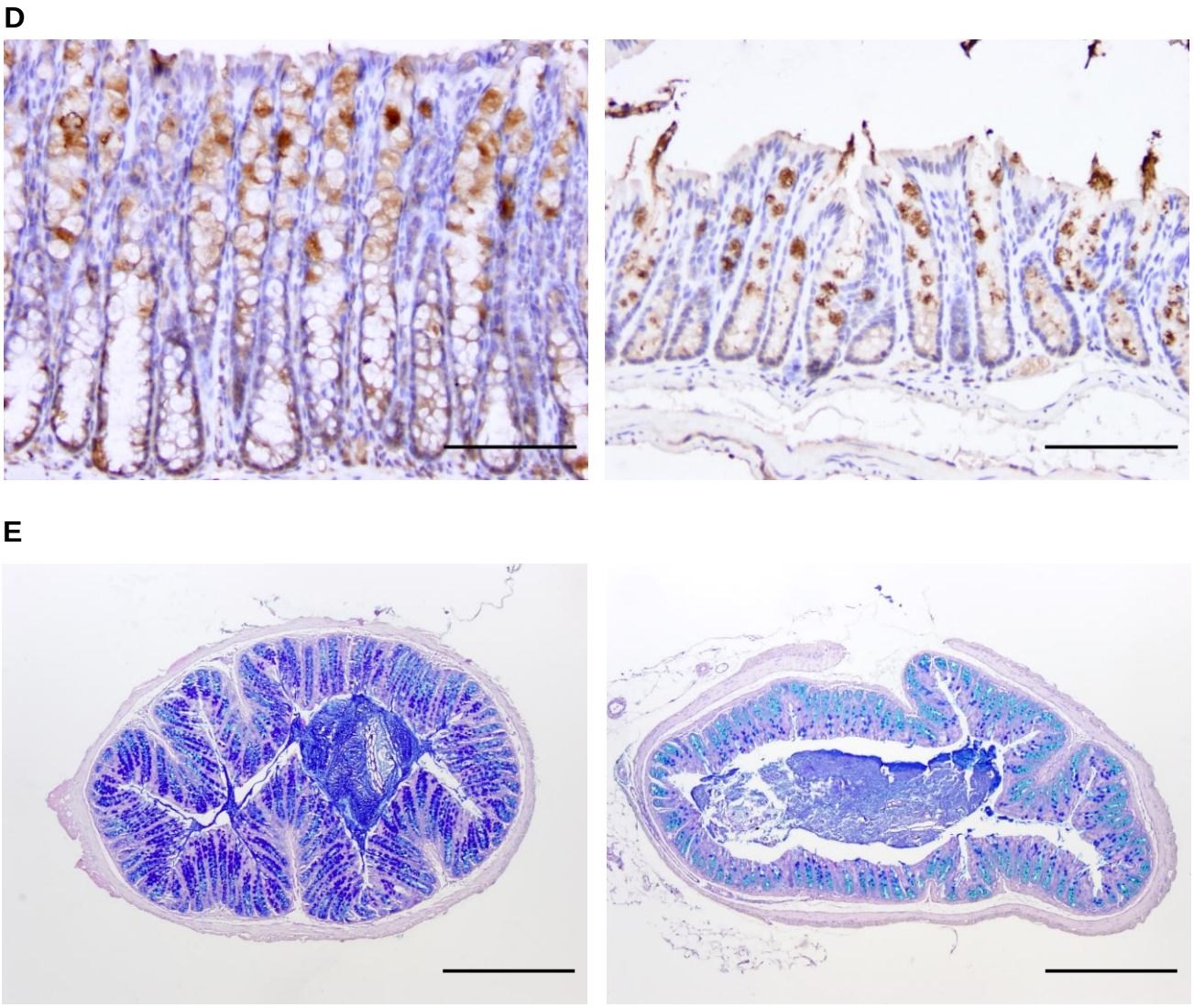


Figure S3. Deficiency of proliferation and self-renewal of colonic crypt cells in *AKR1B8* -/- mice. A) H & E histology; B) PCNA expression; C) BrdU labeling at 1, 24, and 48 hours; D) ITF expression; and E) Alcian blue and Periodic acid Schiff staining (PAS/AB). Scale bars indicate 50μm in PAS/AB, and 100μm in others.

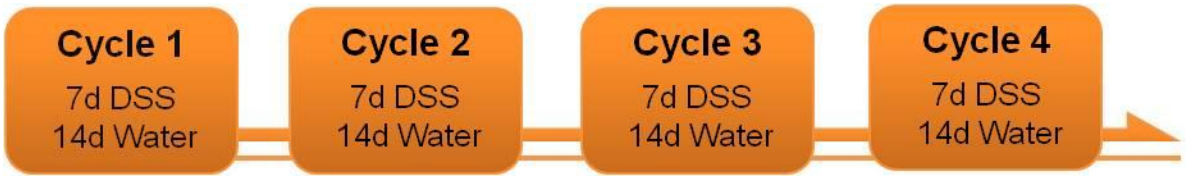
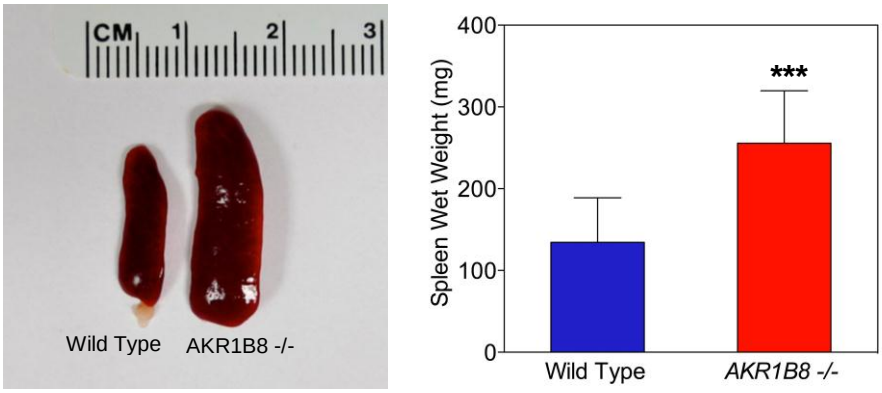


Figure S4. DSS treatment schedules. *AKR1B8 -/-* and wild type mice were fed with 2% dextran sulfate sodium (DSS) for 7 days, followed by 14 days DSS-free water, which is counted as one cycle. Animals experienced one to four cycles depending on experimental needs.

Figure S5

A



B

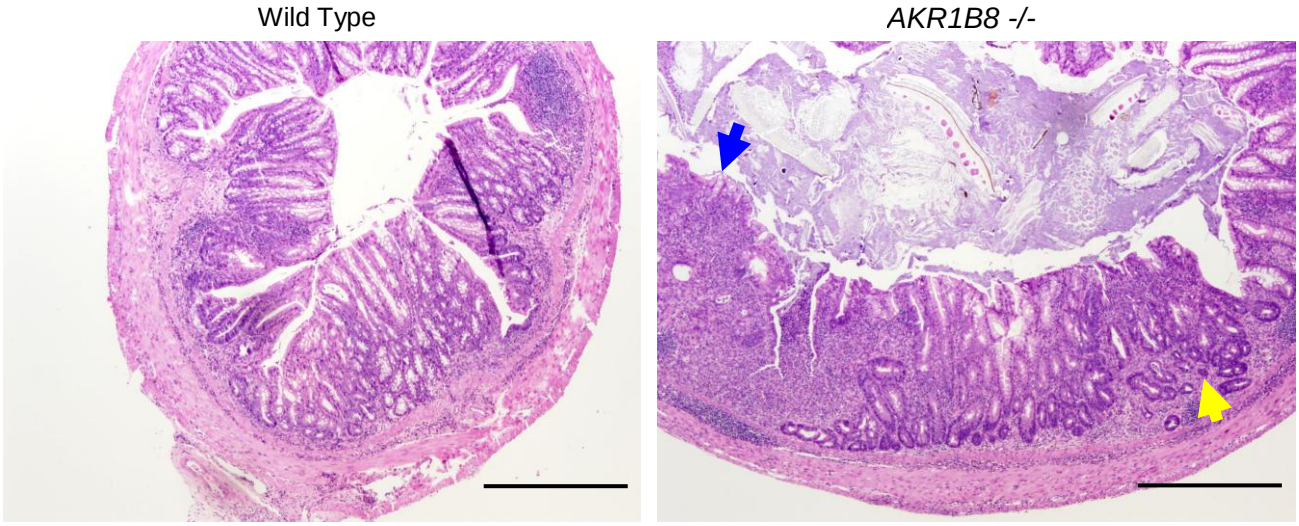
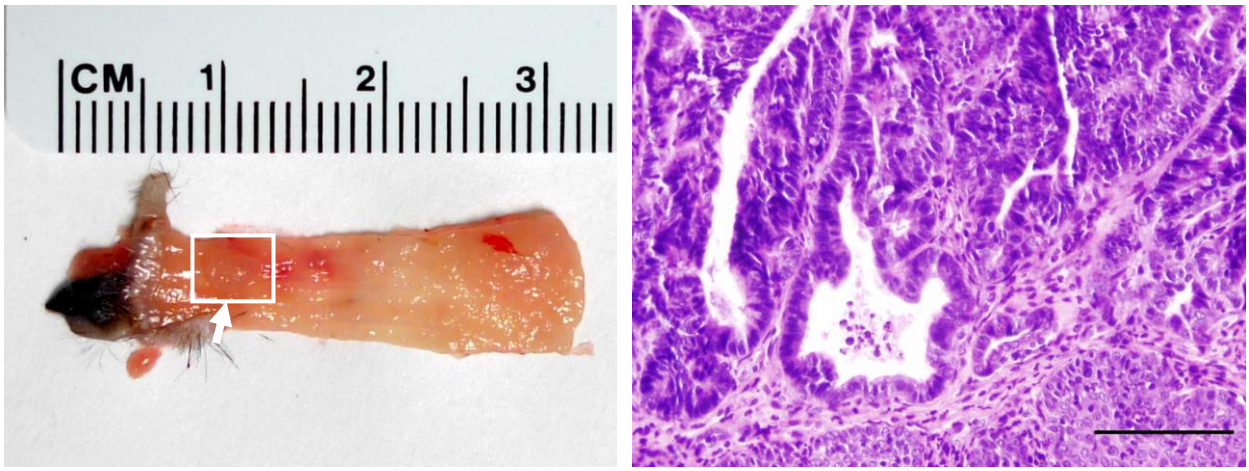


Figure S5. Spleen size and histological inflammation of *AKR1B8 -/-* mice treated with 2% DSS. A) Spleen size. Spleen in *AKR1B8 -/-* mice was significantly enlarged by >2 fold over that in wild type. Data denote mean ± SD, n= 15, *** $P< 0.0001$. B) Histological inflammatory lesions in another representative mouse, showing ulcer (blue arrow). Dysplasia occurred in *AKR1B8 -/-* mice (white arrow). *AKR1B8 -/-* mouse colon was swelling and enlarged.

Figure S6

A



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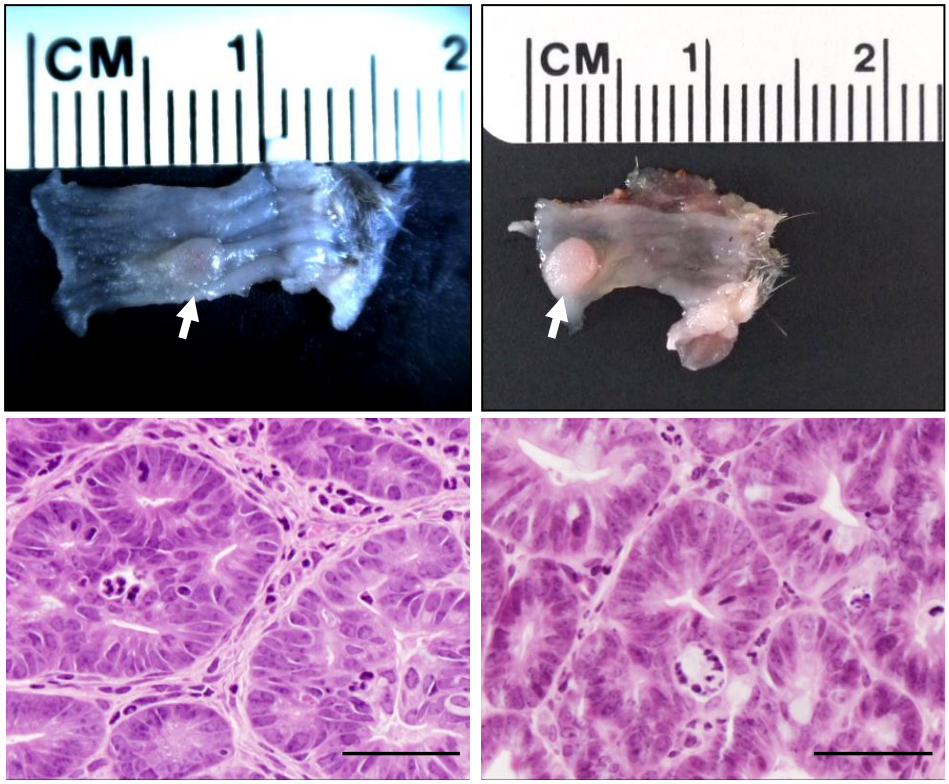

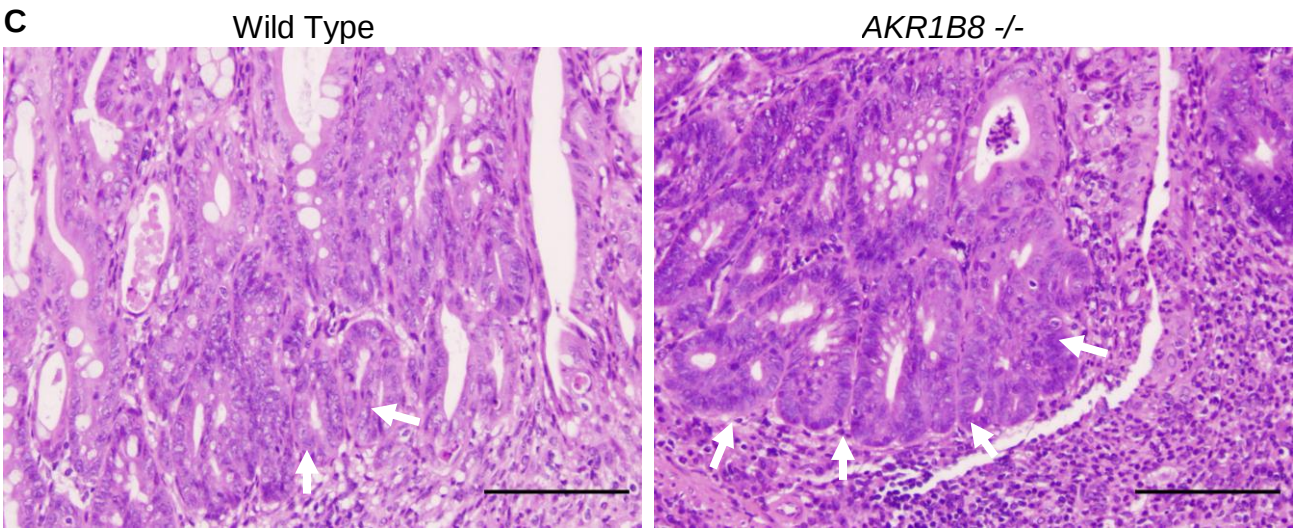

Figure S6. Colitis-associated tumorigenesis in *AKR1B8 -/-* mice. A) A tumor mass (arrow) from an *AKR1B8 -/-* mouse that received 2 cycles of DSS treatment. *Right:* H & E histology. B) Two more representative palpable tumors (arrows) from *AKR1B8 -/-* mice received 4 cycles of DSS treatment. *Below:* H & E histology. C) Micro-dysplasias (white arrows). Dysplastic lesions in *AKR1B8 -/-* mouse were at higher grade than in wild type. Scale bars indicate 200μm in (B), and 100μm in the others.

Supplemental Table S1. Coverage summary of Exome sequencing.

Coverage Summary	Wild Type		*AKR1B8 -/-*		Tumors	
Reads mapped (%)	34,726,317 (95.87)	30,643,916 (96.01)	37,536,791 (95.55)	34,026,043 (95.58)	37,781,378 (95.49)	32,203,249 (95.66)
Mapped confidently	32,112,619 (88.65)	28,095,115 (88.02)	34,842,474 (88.69)	31,287,103 (87.89)	34,475,395 (87.13)	29,696,048 (88.21)
Mapped repetitively	2,613,698 (7.22)	2,548,801 (7.99)	2,694,317 (6.86)	2,738,940 (7.69)	3,305,983 (8.36)	2,507,201 (7.45)
Reads not mapped (%)	1,496,567 (4.13)	1,274,818 (3.99)	1,750,207 (4.45)	1,572,689 (4.42)	1,785,596 (4.51)	1,460,649 (4.34)
No mapping	591,030 (1.63)	501,817 (1.57)	833,889 (2.12)	743,808 (2.09)	831,032 (2.10)	672,032 (2.00)
Low quality	842,400 (2.33)	727,953 (2.28)	865,717 (2.20)	772,845 (2.17)	854,899 (2.16)	730,565 (2.17)
Ribosomal RNA	4,797 (0.01)	4,901 (0.02)	4,540 (0.01)	4,775 (0.01)	4,195 (0.01)	3,938 (0.01)
Primer	1,721 (0.00)	299 (0.00)	856 (0.00)	548 (0.00)	783 (0.00)	611 (0.00)
Control (phiX-174)	56,529 (0.16)	39,742 (0.12)	45,127 (0.11)	50,638 (0.14)	94,560 (0.24)	53,339 (0.16)
Poly-A	46 (0.00)	47 (0.00)	34 (0.00)	40 (0.00)	65 (0.00)	79 (0.00)
Poly-C	2 (0.00)	3 (0.00)	3 (0.00)	2 (0.00)	3 (0.00)	5 (0.00)
Poly-G	0 (0.00)	2 (0.00)	1 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Poly-T	42 (0.00)	54 (0.00)	40 (0.00)	33 (0.00)	59 (0.00)	80 (0.00)
Total reads (%)	36,222,884 (100.00)	31,918,734 (100.00)	39,286,998 (100.00)	35,598,732 (100.00)	39,566,974 (100.00)	33,663,898 (100.00)
Total bases (%)	3,622,288,400 (100.00)	3,191,873,400 (100.00)	3,928,699,800 (100.00)	3,559,873,200 (100.00)	3,956,697,400 (100.00)	3,366,389,800 (100.00)

Supplemental Table S2. Variations detected throughout the mouse Exome sequencing. Data were broken down by variant types and zygosity. *, $P < 0.05$ and **, $P < 0.01$ compared to wild type. SNP, single nucleotide polymorphisms; MNP, multi-nucleotide polymorphisms.

Variant Types and Zygosity		Wild Type (n=2)	*AKR1B8* -/- (n=2)	Tumors (n=2)
SNPs	Homozygous	1470 ± 107	3480 ± 304*	4246 ± 70**
	Heterozygous	9330 ± 317	9698 ± 258	9491 ± 202
	Subtotal	**10800 ± 421**	**13178 ± 93****	**13736 ± 474***
MNPs	Homozygous	68 ± 1	102 ± 5**	127 ± 8**
	Heterozygous	698 ± 59	705 ± 47	760 ± 48
	Subtotal	**766 ± 57**	**806 ± 52**	**887 ± 57**
Insertions	Homozygous	94 ± 7	146 ± 19	175 ± 3**
	Heterozygous	237 ± 11	318 ± 7**	355 ± 23*
	Subtotal	**331 ± 4**	**464 ± 26****	**530 ± 25****
Deletions	Homozygous	142 ± 18	217 ± 28	245 ± 6**
	Heterozygous	1496 ± 30	1382 ± 25	1387 ± 99
	Subtotal	**1638 ± 12**	**1599 ± 53**	**1632 ± 52**
Total variant loci		**13533 ± 494**	**16046 ± 224***	**16785 ± 660***

Supplemental Table S3. Oncogenes and tumor suppressor genes mutated in *AKR1B8 -/-* colitis and tumors only, but not in wild type colitis. Data were produced by Exome sequencing (Otogenetics, GA), n=2 mice each group.

Gene Name	Zygosity	Transcript Name	Mutant Type	Mutant Numbers	Original AA	Mutant AA	Function	Ref
Abtb1	Het	NM_030251	Non-Synonym	1	Y	H	Tumor suppressor	(50)
Chd5	Het	NM_001081376	Non-Synonym	1	R	L	Tumor suppressor	(51)
Chl1	Hom	NM_007697	Non-Synonym	3	S	A	Tumor suppressor	(52)
Chn2	Hom	NM_023543	Non-Synonym	1	M	V	Tumor suppressor	(53)
Dfna5	Hom	NM_018769	Non-Synonym	1	L	P	Tumor suppressor	(54)
Dlx5	Hom	NM_010056	Frameshift	4			Oncogene	(55)
Dok1	Hom	NM_010070	Non-Synonym	1	V	A	Tumor suppressor	(56)
Fam176a	Hom	NM_145570	Non-Synonym	1	P	S	Tumor suppressor	(57)
Fbxo5	Het	NM_025995	Non-Synonym	2	F	C	Tumor suppressor	(58)
Fgd5	Hom	NM_172731	Non-Synonym	1	Q	R	Oncogene	(59)
Fhl3	Het	NM_010213	Frameshift	1			Tumor suppressor	(60)
	Het		Non-Synonym	3	C	Y		
Herc5	Hom	NM_025992	Non-Synonym	19	S	G	E3 protein ligase; Oncogene?	(61)
	Hom		Stop Gain	1	Q	*		
Hoxa10	Hom	NM_008263	Non-Synonym	1	V	G	Transcription factor, tumor suppressor?	(62)
Hoxa13	Hom	NM_008264	Non-Synonym	1	R	A	Transcription factor, oncogene?	(63)
Hoxa5	Hom	NM_010453	Non-Synonym	1	A	V	Transcription factor, tumor suppressor?	(64)
L3mbtl4	Het	NM_177278	Non-Synonym	1	E	G	Transcription factor, tumor suppressor	(65)
Lrig1	Het	NM_008377	Non-Synonym	1	A	T	Tumor suppressor	(66)
Mkrn1	Hom	NM_018810	Non-Synonym	1	N	Y	E3 ubiquitin ligase, oncogene?	(67)
Mxd1	Hom	NM_010751	Non-Synonym	1	S	G	Tumor suppressor	(68)
NSPC1	Hom	NM_197992	Non-Synonym	1	R	C	Transcription factor, oncogenic	(69)
Pdzk1	Het	NM_001146001	Non-Synonym	2	N	D	Oncogenic	(70)
PP2Cm	Hom	NM_175523	Non-Synonym	2	S	L	Cell death/survival	(71)
Rtkn	Hom	NM_001136227	Non-Synonym	3	A	V	A scaffold protein, oncogenic?	(72)
Samd9l	Hom	NM_010156	Non-Synonym	2	I	V	oncogene	(73)
Smo	Hom	NM_176996	Non-Synonym	2	D	N	Tumor suppressor	(74)
ECRG2	Het	NM_001001803	Non-Synonym	1	C	S	Tumor suppressor	(75)
AMSH	Hom	NM_024239	Non-Synonym	1	V	I	Cytokine signaling, oncogenic	(76)
Sulf1	Het	NM_172294	Non-Synonym	1	D	N	Tumor suppressor	(77)
Subtotal: 28 genes								

Supplemental Table S4. Disease Activity Index

Score	Weight loss (%)	Stool consistency	Occult/gross blood in stools
0	≤ 1	Normal stool (well-formed pellets)	Normal (no blood in stools)
1	1 <≤ 5		
2	5 <≤ 10	Loose stools (pasty and semi-formed, not adhered to the anus)	Positive occult blood in stools
3	10 <≤ 20		
4	20 <	Diarrhea (liquid, adhered to the anus)	Gross bleeding in stools

Supplemental Table S5. Histopathological Inflammation Score

Score	Epithelial Damage (E)	Infiltration (I)
0	Normal morphology	No infiltrate
1	Loss of goblet cells	Infiltrate around crypt basis
2	Loss of goblet cells in large areas	Infiltrate reaching to muscularis mucosa
3	Loss of crypts	Extensive infiltration reaching the muscularis mucosa, thickening of the mucosa with abundant oedema
4	Loss of crypts in large area	Infiltration of the submucosa

Supplemental Table S6. Definition of histopathology.

Histopathology	
Regenerative mucosa	Regenerative mucosa is usually due to damage (ulceration). In the background, there are some changes indicating the prior damages, such as thrombus formation/ hyaline thrombus in small vessels, necrosis, ulceration, granulation tissue, fibrin, hemosiderin/hemorrhage, edema in lamina propria, and inflammatory exudate (early) or stromal fibrosis (late).
Hyperplasia without dysplasia	Elongated glands/crypts usually with serrated lumen, mixed goblet cells and absorptive cells, bland cytology (no atypia) with brush borders and basal nuclei, the basement membrane is usually thickened, cells at the base of crypt are a little bit immature including nuclear elongation, crowding and increased mitotic rate, but this is not present at the top of crypt (maturation).
Mild dysplasia	Pseudostratification of nuclei that reach only half of the cell, apical mucin is present, nuclei are elongated and hyperchromatic. Mitotic activity present but atypical mitosis and loss of polarity are absent or minimal.
Moderate dysplasia	Changes are between mild and high grade dysplasia.
Severe dysplasia	Cytological changes: nuclei are rounded, hyperchromatic or vesicular with prominent nucleoli; nuclei are stratified and reach luminal surface. Architectural changes: back to back glands, cribriform, mitotic and atypical mitotic figures are prominent with reduced mucin.
Adenoma	Dome-shaped elevated lesions with mild dysplasia.

Supplemental Table S7. Gene specific primers.

Gene Description	Primers	Sequence (5' → 3')
AKR1B3	Forward primer	CAA CAG GAA CTG GAG GGT GTG
	Reverse primer	GAA CAG GTG CAA GCC ACT TG
AKR1B7	Forward primer	GAT CCC CAA GTC TGT GAC AC
	Reverse primer	CAG TCC TTG CAT CCA ACA G
AKR1B8	Forward primer	CAT CCT TAC CTC ACC CAG GA
	Reverse primer	GAT CAC CAC CAC GTT CCT CT
AKR1C12	Forward primer	GAC ACA TGG GAG AGG TTG GAG
	Reverse primer	GTC CTG GCT TGT TGA GGA TTC
AKR1C13	Forward primer	CCT TGT TGA CCA CCC AGA G
	Reverse primer	CTG TCC ACA CAC AGG GAC AC
AKR1C14	Forward primer	GGA GGC CAT GGA AAA GTG TA
	Reverse primer	GAT GGC ATT CTA CCT GGT TGC
IL-1β	Forward primer	GCA ACT GTT CCT GAA CTC AAC
	Reverse primer	ATC TTT TGG GGT CCG TCA ACT
IL-6	Forward primer	TAG TCC TTC CTA CCC CAA TTT CC
	Reverse primer	TTG GTC CTT AGC CAC TCC TTC
IFNγ	Forward primer	GCG TCA TTG AAT CAC ACC TG
	Reverse primer	GAC CTG TGG GTT GTT GAC C
IKKβ	Forward primer	CTG AAG ATC GCC TGT AGC AAA
	Reverse primer	TCC ATC TGT AAC CAG CTC CAG

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