Aspirin and Low Dose Nitric Oxide-Donating Aspirin Suppress Tumorigenesis and Increase Life Span in a Lynch Syndrome Mouse Model

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Supplementary Data

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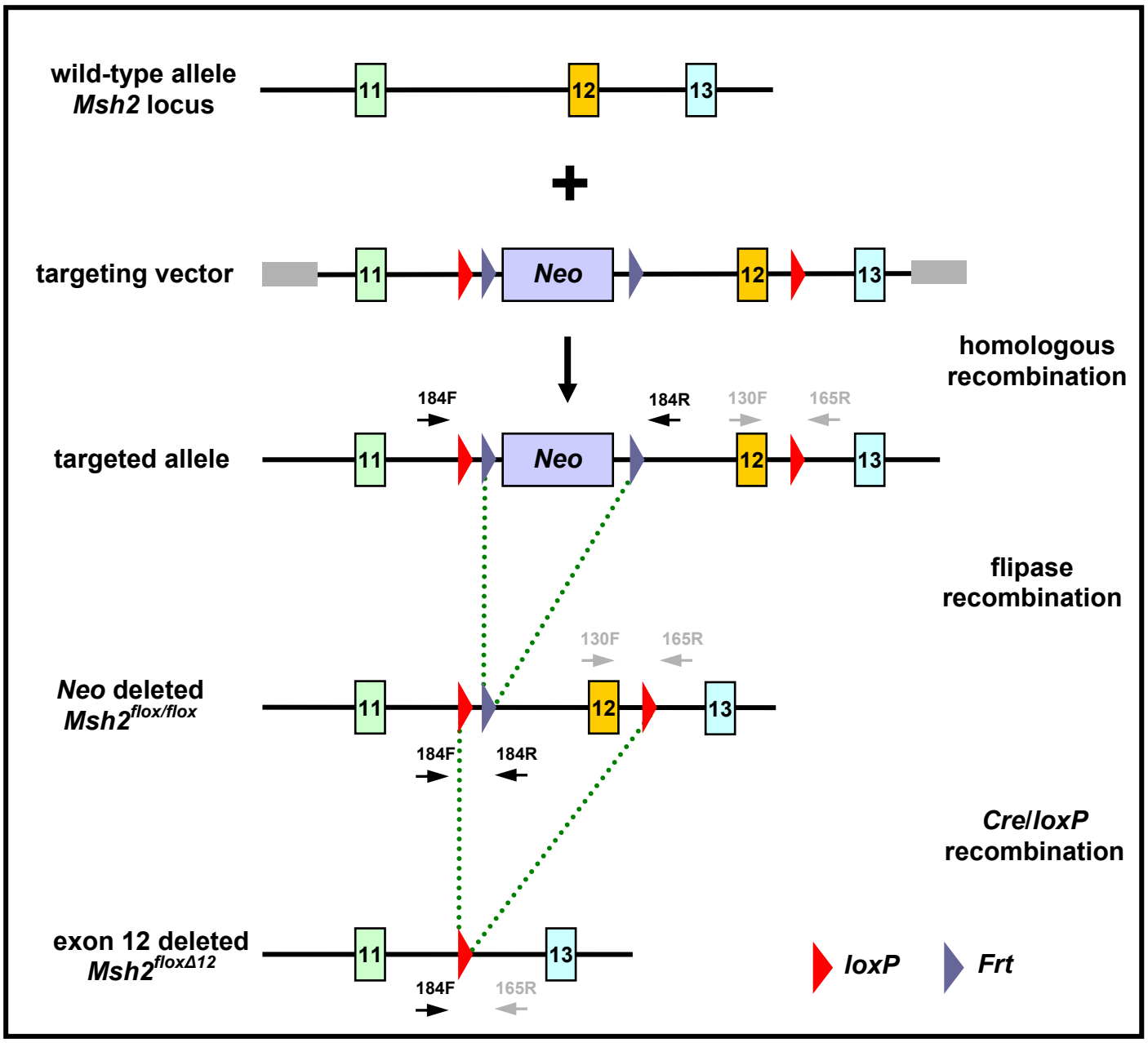
Supplementary Tables

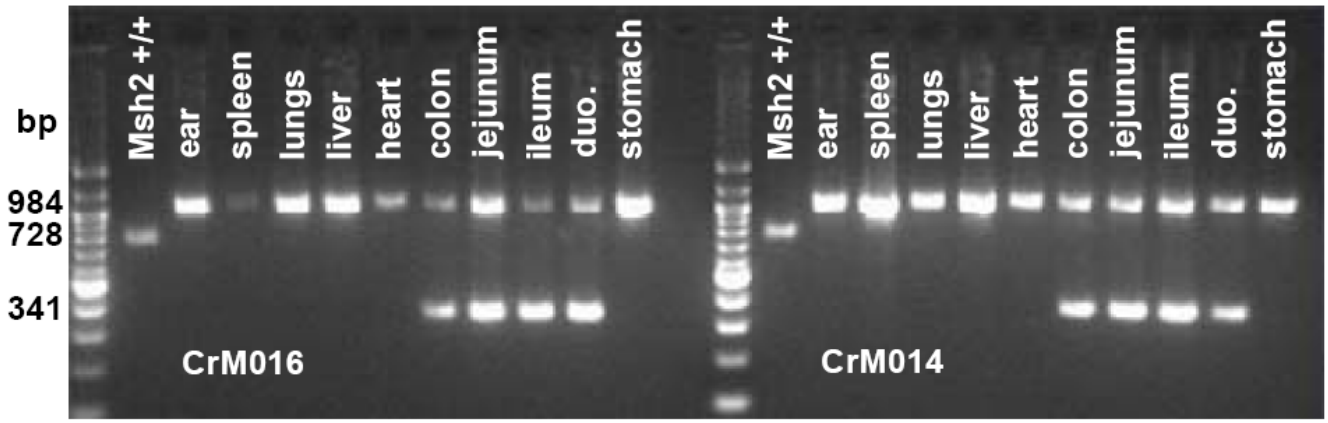
Supplementary Methods

Glutathione and thioredoxin reductase assays

Groups of *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ and *VpC*$^{+/+}$ mice (*n*=6) were treated with either 400 mg/kg ASA or 720 mg/kg NO-ASA for 120 days. Mice had been weaned and were ~25 days old when treatment was started. Untreated control groups were also included for each strain. Mice were then euthanized, their livers were removed, placed on ice and apportioned equally for separate determination of glutathione and thioredoxin reductase levels. These were assayed with kits obtained from Cayman Scientific (Glutathione Assay Kit, Catalog No. 703002; Thioredoxin Reductase Assay Kit Catalog No. 10007892). The protocols accompanying the kits were followed exactly. Final concentrations of glutathione and thioredoxin reductase were calculated according to methods for data analysis outlined in each protocol.

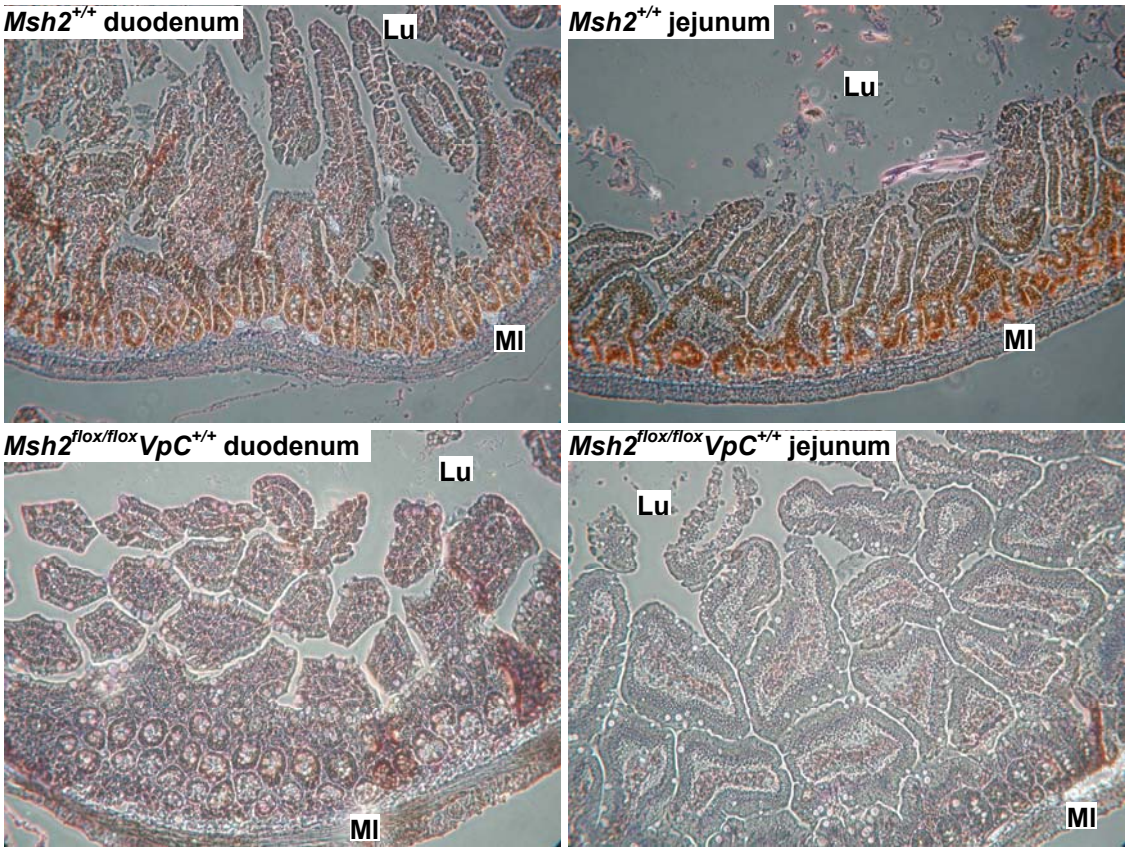


Supplementary Figure S1. Targeting strategy used to generate *Msh2*$^{flox/flox}$ mice. The above figure outlines the procedures that were adopted to generate conditional *Msh2* knockout mice that had exon 12 flanked by two *loxP* sites. 184F, 184R, 130F and 165 indicate the approximate location of primers that were used in subsequent genotyping reactions to confirm that the correct targeting events had occurred. *Msh2*$^{flox/flox}$ mice were then crossed with mice carrying a *Cre* transgene, B6.SJL-Tg(Vil-cre)997Gum/J. Expression of Cre recombinase in the intestine from the villin gene *(Vil1)* promoter, (VpC=**V**illin **p**romoter **C**re recombinase), resulted in tissue-specific ablation of *Msh2* in this cellular compartment, thus creating the *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ strain of mice.

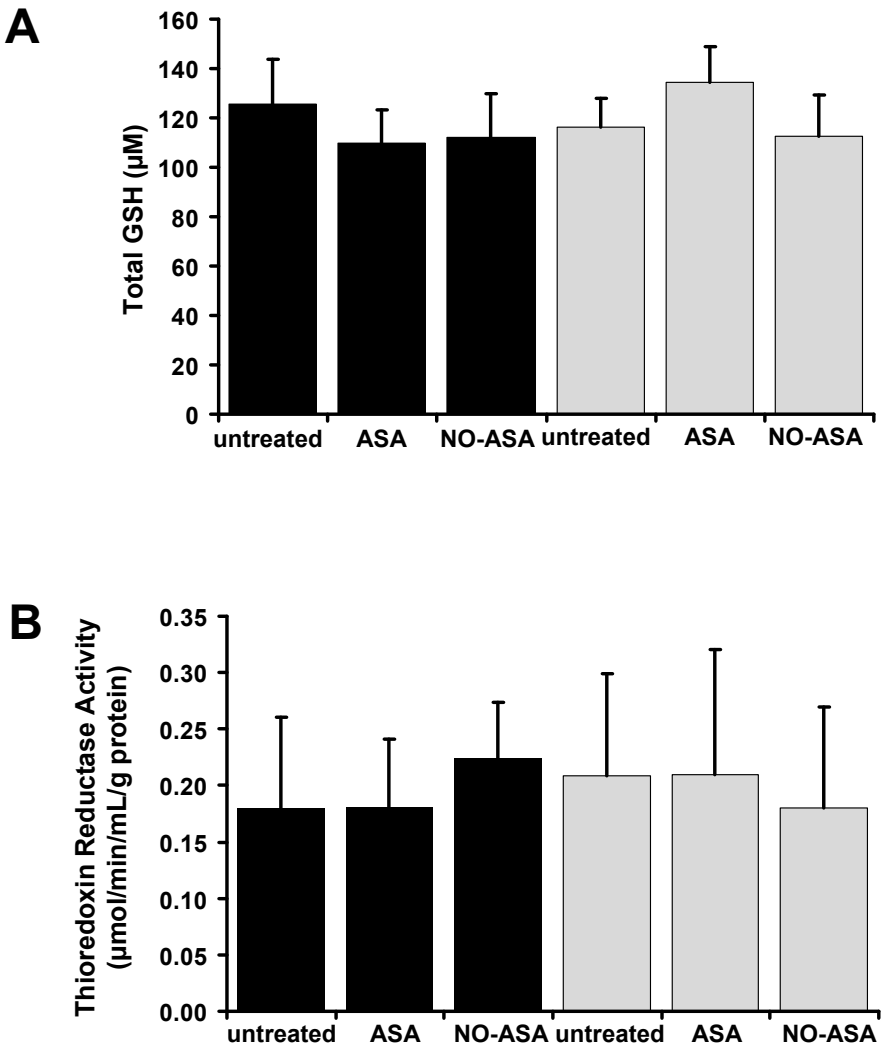


728 bp (genomic sequence) *Msh2*$^{+/+}$ allele primers 130F/165R
984 bp (exon 12 + *loxP* intact) *Msh2*$^{flox/flox}$ allele primers 130F/165R
341 bp (exon 12 deleted) *Msh2*^{floxΔ12} allele primers 184F/165R

Supplementary Figure S2. Cre-mediated deletion of *Msh2* exon 12 occurs only in the intestinal tissues of *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice. DNA was isolated from various tissues of two *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice, CrM016 and CrM014, and analyzed at the molecular level by PCR with primers 130F, 165R and 184F. A 984 bp product, corresponding to the intact floxed *Msh2* allele was amplified from all tissues examined. The 341 bp product, corresponding to the *Msh2*^{floxΔ12} allele, was only amplified from intestinal tissues, including the colon. Samples were not micro-dissected to obtain more homogenous populations of epithelial cells, which is why both PCR products were amplified from intestinal tissues. The 728 bp product corresponds to the wild-type, unfloxed genomic sequence around *Msh2* exon 12. It was amplified from DNA from a wild-type control C57Bl/6J mouse.



Supplementary Figure S3. Msh2 protein is not expressed in the intestinal epithelial cells of *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice. Tissues were prepared from wild-type (*Msh2*$^{+/+}$)*VpC*$^{+/+}$ and *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice, washed in PBS and fixed in formalin. They were embedded in paraffin and subjected to immunohistochemistry with an affinity purified rabbit antibody against Msh2 (Bethyl Laboratories, catalogue number IHC-00082) at a 1:250 dilution. Msh2 protein was visualized with horseradish peroxidase, indicated by a brown nuclear stain, and counter-stained with hematoxylin (blue). Msh2 is clearly visible in the crypts, and to a lesser extent the villi, of the duodenum and jejunum of *Msh2*$^{+/+}$ mice (upper panels). It is not detectable in the corresponding regions of *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice due to localized Cre-mediated ablation of *Msh2* expression (deletion of exon 12) in these tissues. (Ml = muscularis; Lu = lumen)



Supplementary Figure S4. Continuous treatment with NO-ASA does not significantly depress the glutathione or thioredoxin systems. A, Total glutathione levels in both *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ and *VpC*$^{+/+}$ mice livers were not significantly lowered by prolonged dietary exposure to NO-ASA. B, Similarly, levels of thioredoxin reductase were not significantly perturbed by long-term treatment with either NO-ASA or ASA. (Black bars = *VpC*$^{+/+}$; grey bars = *Msh2*$^{flox/flox}$*VpC*$^{+/+}$; *n*=6 per group). Error bars show standard deviation around the mean. Differences between groups were compared by a *t*-test; none were statistically significant.

Supplementary Table S1. Histopathologic classification of tumors from *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice

Table S1 A. Untreated mice, plain food

Block	Location	Diagnosis
CrM049	small bowel	invasive, poorly differentiated adenocarcinoma
CrM065 (A)	small bowel	invasive, poorly differentiated adenocarcinoma arising in high grade dysplastic adenoma
CrM065 (B)	small bowel	invasive, poorly differentiated adenocarcinoma
CrM118	small bowel	invasive, poorly differentiated adenocarcinoma
CrM121	small bowel	high grade dysplastic adenoma
CrM143 (A)	small bowel	invasive, poorly differentiated adenocarcinoma
CrM143 (B)	small bowel	high grade dysplastic adenoma
CrM144	small bowel	invasive, poorly differentiated adenocarcinoma
CrM145	small bowel	invasive, poorly differentiated adenocarcinoma
CrM176	small bowel	aberrant crypt foci
CrM177	small bowel	invasive, very poorly differentiated adenocarcinoma
CrM194	small bowel	invasive, very poorly differentiated adenocarcinoma
CrM202	small bowel	invasive, poorly differentiated adenocarcinoma

Table S1 B. Mice treated with 400 mg/kg ASA

Block	Location	Diagnosis
CrM139	small bowel	invasive moderately differentiated adenocarcinoma and adenoma
CrM157	small bowel	invasive, poorly differentiated adenocarcinoma
CrM158	small bowel	invasive, poorly differentiated adenocarcinoma
CrM162 (A)	small bowel	invasive moderately differentiated adenocarcinoma
CrM162 (B)	small bowel	invasive, poorly differentiated adenocarcinoma
CrM165	small bowel	benign mucosa
CrM178	small bowel	invasive, poorly differentiated adenocarcinoma
CrM180	small bowel	high grade dysplastic adenoma with focal superficial invasion; peritumoral lymphoid infiltrate
CrM186	small bowel	moderately dysplastic adenoma
CrM190	small bowel	invasive, poorly differentiated adenocarcinoma
CrM193	small bowel	invasive, poorly differentiated adenocarcinoma
CrM198	small bowel	invasive, very poorly differentiated adenocarcinoma

Supplementary Table S1. (cont.)

Table S1 C. Mice treated with 720 mg/kg NO-ASA

Block	Location	Diagnosis
CrM076	small bowel	invasive, poorly differentiated adenocarcinoma; superficial high grade adenoma
CrM086	small bowel	adenoma with high grade dysplasia/intramucosal carcinoma; superficial high grade adenoma, transition to benign mucosa
CrM088	small bowel	invasive adenocarcinoma (x2); superficial adenomas with high grade dysplasia (x2)
CrM089 (A)	small bowel	multiple foci of superficial adenoma with moderate to high grade dysplasia
CrM089 (B)	small bowel	multiple foci of superficial adenoma with moderate to high grade dysplasia
CrM093	small bowel	no neoplasm
CrM096 (A)	small bowel	invasive, poorly differentiated adenocarcinoma
CrM096 (B)	small bowel	invasive, poorly differentiated adenocarcinoma
CrM096 (C)	small bowel	adenoma with high grade dysplasia/intramucosal carcinoma
CrM097 (A)	small bowel	intramucosal carcinoma high grade, possibly invasive; several foci of superficial high grade adenoma
CrM097 (B)	small bowel	several foci of adenoma with moderate to high grade dysplasia; invasive, poorly differentiated adenocarcinoma
CrM097 (C)	small bowel	several foci of superficial high grade adenoma; possible intramucosal carcinoma

Table S1 D. Mice treated with 1500 mg/kg NO-ASA

Block	Location	Diagnosis
CrM216 (A)	small bowel	invasive, poorly differentiated adenocarcinoma
CrM216 (B)	small bowel	adenoma with moderate dysplasia
CrM217	small bowel	high grade dysplastic adenoma
CrM224	small bowel	invasive, poorly differentiated adenocarcinoma
CrM228	small bowel	invasive, poorly differentiated adenocarcinoma
CrM229	small bowel	invasive, poorly differentiated adenocarcinoma
CrM232	small bowel	high grade dysplastic adenoma
CrM234	small bowel	high grade dysplastic adenoma
CrM237	small bowel	high grade dysplastic adenoma, focally invasive
CrM241	small bowel	invasive, poorly differentiated adenocarcinoma
CrM245	small bowel	high grade dysplastic adenoma
CrM246	small bowel	high grade dysplastic adenoma
CrM252	small bowel	superficially invasive, poorly differentiated adenocarcinoma

Supplementary Table S2. MSI analysis of ear, normal (N) and tumor (T) intestinal tissues

S2 A. *VpC*$^{+/+}$ mice, 720 mg/kg NO-ASA

Tissue N=16	DNA	MSM 01	MSM 02	MSM 03	MSM 04	MSM 05	[A]Com.MSI
093	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Je	0	0	0	0	0	
094	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Je	0	0	0	0	0	
100	Ear	0	0	0	0	0	
	Je	0	0	0	0	0	n/a
	il	0	0	0	0	0	
104	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Je	0	0	0	0	0	
106	Ear	0	0	0	0	0	
	Je	0	0	0	0	0	n/a
	Co	0	0	0	0	0	
111	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Je	0	0	0	0	0	
112	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Je	0	0	0	0	0	
113	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Je	0	0	0	0	0	
114	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Je	0	0	0	0	0	
115	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Je	0	0	0	0	0	
116	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Co	0	0	0	0	0	
117	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Co	0	0	0	0	0	
121	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Je	0	0	0	0	0	
122	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Je	0	0	0	0	0	
123	Ear	0	0	0	0	0	
	Du	0	0	0	0	0	n/a
	Je	0	0	0	0	0	
141	Ear	0	0	0	0	0	
	N=Je	0	0	0	0	0	MSS
	T=Je	0	0	0	0	0	

[A] Comparative MSI is for (N v. T) intestinal tissues only.

Du=duodenum; Je=jejunum; il=ileum; Co=colon

n/a - not applicable

S2 B. *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice, plain food

Tissue N=18	DNA	MSM 01	MSM 02	MSM 03	MSM 04	MSM 05	[A]Com.MSI
020	Ear	0	0	0	0	0	
	N1=Je	0	0	-2	-2	-1	MSS
	T1=Je	0	0	-2	-2	-1	
027	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	0	MSI-H
	T=Je	0	0	0, -2	-2, -4	-1, -2	
028	Ear	0	0	0	0	0	
	N1=Je	0	0	-2	-2	0	MSS
	T1=Je	0	0	-2	-2	0	
028	Kidney	0	0	0	0	0	
	N2=Je	0	0	-2	-2	0	MSI-L
	T2=Je	0	0	-2	-2	-1	
028	Spleen	0	0	0	0	0	
	N3=Je	0	0	-2	-2	-1	MSS
	T3=Je	0	0	-2	-2	-1	
033	Ear	0	0	0	0	0	
	Du	0	0	-2	0	-1	MSI-L
	Je	0	0	-2	-2	-1	
044	Ear	0	0	0	0	0	
	N1=Je	0	0	-2	0	0	MSI-H
	T1=Je	-2	0	-2	-2, -4	-2	
057	Ear	0	0	0	0	0	
	N1=Du	0	0	-2	-2	-1	MSI-L
	T1=Du	0	0	-2	-2	-1, -2	
057	Liver	0	0	0	0	0	
	N2=Je	0	0	-2	-2	-1	MSS
	T2=Je	0	0	-2	-2	-1	
049	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	-1, -2	MSI-L
	T=Je	-2	0	-2	-2	-1, -2	
065	Ear	0	0	0	0	0	
	N1=Du	0, -2	0	-2	-2	-1	MSI-H
	T1=Du	-2	0	-4	-4	-1	
065	Ear	0	0	0	0	0	
	N2=Je	0	0	-2	-4	-1	MSI-L
	T2=Je	0	0	-2	-2	-1	
118	Ear	0	0	0	0	0	
	N=Je	0	0	-2	0	0	MSI-L
	T=Je	0	0	-2	0	-1, -2	
136	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	0, -1	MSS
	T=Je	0	0	-2	-2	0, -1	
144	Ear	0	0	0	0	0	
	N1=Du	0	0	0, -2	0	0	MSI-H
	T1=Du	0	0	-2	-2	0, -1	
177	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	-1	MSI-H
	T=Je	-2	0	-2, -4	-4, -6	-2	
194	Ear	0	0	0	0	0	
	N1=Je	0	0	-2	-2	0	MSI-

	T1=Je	-2	0	-2, -4	-4	-1	H
202	Ear	0	0	0	0	0	
	N1=Je	0	0	-2	-2	0, -1	MSI-L
	T1=Je	0	0	-2	-2	-1	

Supplementary Table S2. (cont.)

S2 C. *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice, 400 mg/kg ASA

Tissue N=18	DNA	MSM 01	MSM 02	MSM 03	MSM 04	MSM 05	[A]Com.MSI
139	Ear	0	0	0	0	0	
	N1=Je	-2	0	-2	-2	-1	MSS
	T1=Je	-2	0	-2	-2	-1	
139	Ear	0	0	0	0	0	
	N2=il	0, -2	0	-2	-2	-1	MSI-L
	T2=il	0	0	-2	-2	-1	
154	Liver	0	0	0	0	0	
	Du	0	0	-2	-2	0, -1	MSI-L
	Co	0	0	-2	-2	-1	
157	Ear	0	0	0	0	0	
	N=Du	0	0	-2	-2	-1	MSS
	T=Du	0	0	-2	-2	-1	
158	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	0, -1	MSS
	T=Je	0	0	-2	-2	0, -1	
162	Ear	0	0	0	0	0	
	N=Je	0	0	0	-2	0	MSI-H
	T=Je	0	0	0, -2	-4	-1	
164	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	-1	MSS
	T=Je	0	0	-2	-2	-1	
165	Ear	0	0	0	0	0	
	Du	0	0	0, -2	-2	-1	MSI-L
	Co	0	0	-2	-2	-1	
168	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	0	MSI-H
	T=Je	-2	0	-2	-2	-1	
178	Ear	0	0	0	0	0	
	N=Du	0	0	-2	-2	-1	MSI-L
	T=Du	0	0	-2	-2	-1, -2	
180	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	-1	MSI-L
	T=Je	-2	0	-2	-2	-1	
186	Ear	0	0	0	0	0	
	N1=Du	0	0	-2	-2	-1	MSI-H
	T1=Du	-2	0	-2	-4	-1	
190	Ear	0	0	0	0	0	
	N1=Du	0	0	0	-2	0	MSI-L
	T1=Du	0	0	0	-2	-1	
190	Ear	0	0	0	0	0	
	N2=Je	0, -2	0	-2	-2	-1	MSI-H
	T2=Je	0, -4	0	-2	-2, -4	-1	
193	Ear	0	0	0	0	0	
	N=Je	0	0	0	-2	0, -1	MSI-L
	T=Je	0	0	0	-2	-2	
197	Ear	0	0	0	0	0	
	N=Je	0	0	0	-2	-1	MSI-L
	T=Je	0	0	-2	-2	-1	
198	Ear	0	0	0	0	0	
	N=Du	0	0	0, -2	-2	0	MSI-
	T=Du	0	0	-2	-2	0	L
200	Ear	0	0	0	0	0	
	N=Je	0	0	0	-2	0	MSI-L
	T=Je	0	0	0	-2	0	

S2 D. *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice, 72 mg/kg NO-ASA

Tissue N=13	DNA	MSM 01	MSM 02	MSM 03	MSM 04	MSM 05	[A]Com.MSI
366	Ear	0	0	0	0	0	
	N=Je	0,-2	0	-2	-2	0	MSI-H
	T=Je	-2	0	-4	-2	-1	
367	Ear	0	0	0	0	0	
	N=Je	0	0	-2	0	0	MSI-H
	T=Je	0	0	-4	0	-2	
369	Ear	0	0	0	0	0	
	N1=Je	0	0	-2	-2	-1	MSI-H
	T1=Je	-2	0	-2	-4	-1	
369	Ear	0	0	0	0	0	
	N2=Du	0	0	-2	-2	0	MSI-L
	T2=Du	0	0	-2	-2	-1	
408	Ear	0	0	0	0	0	
	N=Du	0	0	0	0	0	MSI-L
	T=Du	0	0	0	-2	0	
409	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	0	MSI-L
	T=Je	0	0	-4	-2	0	
410	Ear	0	0	0	0	0	
	N=Je	-2	0	-4	-4	-1	MSI-H
	T=Je	-4	0	-6	-4	-2	
412	Ear	0	0	0	0, -2	0	
	N=Je	0	0	-2	-2	0	MSI-L
	T=Je	0	0	-2	-2	-1	
419	Ear	0	0	0	0	0	
	N=Du	-2	0	-4	-2	-1	MSI-L
	T=Du	-2	0	-4	-4	-1	
421	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	0	MSI-H
	T=Je	-2	0	-2	-6	-2	
444	Ear	0	0	0	0	0	
	N=Du	0	0	-2	-2	-1	MSS
	T=Du	0	0	-2	-2	-1	
445	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	0	MSI-H
	T=Je	-4	0	-2	-2	-3	
473	Ear	0	0	0	0	0	
	N=Du	0	0	-2	-2	0	MSS
	T=Du	0	0	-2	-2	0	

[A] Comparative MSI is for (N v. T) intestinal tissues only.

Du=duodenum; Je=jejunum; il=ileum; Co=colon

Supplementary Table S2. (cont.)

S2 E. *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice, 720 mg/kg NO-ASA

Tissue N=16	DNA	MSM 01	MSM 02	MSM 03	MSM 04	MSM 05	[A]Com.MSI
076	Ear	0	0	0	0	0	
	N=Du	0,-2	0	0	-2	0	MSI-H
	T=Du	-2	0	-2	-2	-1	
078	Ear	0	0	0	0	0	
	N=Je	-2	0	0	0	-1	MSI-H
	T=Je	-4	0	0	-2	-1	
081	Ear	0	0	0	0	0	
	Je	-2	0	0, -2	0	-1	MSI-H
	Co	-2	0	-2	-2	-1	
085	Ear	0	0	0	0	0	
	N=Je	-2	0	0, -2	-2	-1	MSS
	T=Je	-2	0	0, -2	-2	-1	
086	Ear	0	0	0	0	0	
	N=Du	0	0	-4	-2	0	MSI-H
	T=Du	-2	0	-4	-4	-1	
088	Ear	0	0	0	0	0	
	N=Du	0	0	-2	-2	0, -1	MSI-H
	T=Du	-2	0	-4	-2	-1	
091	Ear	0	0	0	0	0	
	N=Du	0	0	-2	0	0	MSI-H
	T=Du	0, -2	0	-2	-2	-1	
093	Ear	0	0	0	0	0	
	N=Je	0	0	0	0, -2	0	MSI-H
	T=Je	-2	0	0, -2	-2	-1	
096	Ear	0	0	0	0	0	
	N=Du	0	0	-2	-2	-1	MSI-H
	T=Du	-2	0	-4	-2, -4	-2	
097	Ear	0	0	0	0	0	
	N=Du	-2	0	-2	-2	-1	MSI-L
	T=Du	-2	0	-4	-2	-1	
103	Ear	0	0	0	0	0	
	N1=Du	-2	0	-2	-2	-1	MSI-H
	T1=Du	-4	+2	-2, -4	-4	-2	
106	Spleen	0	0	0	0	0	
	N=Du	0	0	-2	-2	0	MSI-H
	T=Du	0	0	-2, -4	-2	-1	
107	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	-1	MSI-L
	T=Je	-2	0	-2	-2	-1	
108	Ear	0	0	0	0	0	
	N=Du	0	0	0	0, -2	-1	MSI-H
	T=Du	-2	0	-2	-2	-1	
109	Ear	0	0	0	0	0	
	N1=Du	0, -2	0	-2	-2	-1	MSI-H
	T1=Du	-2	0	-4	-4	-1	
114	Ear	0	0	0	0	nd	
	N=Du	-2	0	-2	+1	nd	MSI-L
	T=Du	-4	0	-2	+1	nd	

S2 F. *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice, 1500 mg/kg NO-ASA

Tissue N=14	DNA	MSM 01 137	MSM 02 143	MSM 03 154	MSM 04 152	MSM 05 140	[A]Com.MSI (N v
228	Ear	0	0	0	0	0	
	N=Du	0	0	-2	-2	-1	MSI-L
	T=Du	0	0	-2	-2	-1, -2	
229	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	-1	MSI-H
	T=Je	0	0	-2, -4	-2	-1, -2	
231	Ear	0	0	0	0	0	
	N=Du	0	0	-2	-2	-1	MSI-H
	T=Du	-2	0	-4	-2, -4	-2	
232	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	-1	MSI-L
	T=Je	0	0	-2	-2	-2	
237	Liver	0	0	0	0	0	
	N=Je	0	0	-2	-2	-1, -2	MSI-L
	T=Je	0	0	-2	-2	-2	
238	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	-1	MSI-L
	T=Je	0	0	-2	-2	-1, -2	
241	Ear	0	0	0	0	0	
	N1=Du	0	0	-2	-2	-1	MSI-H
	T1=Du	-2	0	-2	-2	-2	
241	Ear	0	0	0	0	0	
	N2=Je	0	0	-2	-2	-1	MSI-L
	T2=Je	0	0	-2	-2	-1, -2	
246	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	-1	MSS
	T=Je	0	0	-2	-2	-1	
253	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	-1	MSI-L
	T=Je	0	0	-2	-2	-2	
249	Ear	0	0	0	0	0	
	N=Je	0	0	-2, -4	-2	-1	MSI-L
	T=Je	0	0	-4	-2	-1	
252	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	0	MSI-H
	T=Je	-2	0	-4	-4	-1	
257	Ear	0	0	0	0	0	
	N=Du	0	0	-2, -4	-2	0, -1	MSI-H
	T=Du	-2	0	-4	-2	-1	
271	Ear	0	0	0	0	0	
	N=Je	0	0	-2	-2	0	MSS
	T=Je	0	0	-2	-2	0	

[A] Comparative MSI is for (N v. T) intestinal tissues only.

Du=duodenum; Je=jejunum; il=ileum; Co=colon

Supplementary Table S2. Five microsatellite loci (MSM01-MSM05) were evaluated for matched sets of ear (E), intestinal tumor (T) and adjacent normal (N) intestinal tissues from *Msh2*$^{flox/flox}$*VpC*$^{+/+}$ mice in each treatment group. Overall, the *VpC*$^{+/+}$ control groups did not develop tumors, so usually tissue samples were taken from two different (normal) locations along the intestinal tract. The MSS ear tissues provide constitutional microsatellite profiles. They represent the standard allele sizes of these markers, indicated by 0 in each column to signify 0 bp deviation from the established marker size. MSM01, 02, 03 and 04 are dinucleotide markers. Differences in the lengths of microsatellite markers between normal and tumor samples and their cognate ear samples were expressed in units of 2 (bp). MSM05 is a mononucleotide marker; any differences in allele lengths were expressed in units of 1 (bp). If two major alleles were observed, both were recorded. Comparative MSI (N vs. T) was calculated by simply assessing any changes between only the normal and tumor samples at the different markers. The relative degree of MSI in the tumors was subsequently scored as follows: MSS, no changes in microsatellite status; low microsatellite instability (MSI-L), MSI in 1 of 5 microsatellite markers; high microsatellite instability (MSI-H), MSI in at least 2 of 5 markers. Note that normal and tumor samples may be MSI-H if compared to the MSS ear but in the context of comparative MSI they may be scored as MSS, MSI-L or MSI-H. All tissues examined for *VpC*$^{+/+}$ mice treated with 720 mg/kg *ortho* NO-ASA were MSS, including any of the rare intestinal tumors that did develop. The criteria for comparative MSI were not applicable to these samples.

Supplementary Table S3. Primer sequences for microsatellite instability analysis

primer sets	amplicon size	primer name	primer sequence	accession number
MSM01 (TG_{27})	137 bp	MSM 01F	GGA TCA CTC GAT GTA CGG CTA CTC	AC098712
		MSM 01R	CCA GGC AGG CAA AGC ATT TAT	
MSM02 (TA_{27})	143 bp	MSM 02F	CAC CCC TTG CTA CCA CTA AGA AA	AC122340
		MSM 02R	CTC ATT GGA GTT TGA CCC ATC A	
MSM03 (GA_{29})	154 bp	MSM 03F	CAG GAG GTC AAG GTC ATC CTA AG	AC083948
		MSM 03R	CCA CCA TGG TAG GAG CTT GCT A	
MSM04 (CT_{25}/CA_{27})	152 bp	MSM 04F	GGA GAT TCT GCT GTT TCA AAC AAG	AC079442
		MSM 04R	TTC CTA TAC ATG GGT GGA GTA GGA	
MSM05 (A_{33})	140 bp	MSM 05F	TAC AGA GGA TTG TCC TCT TGG AG	AC096777
		MSM 05R	GCT GCT TCA CTT GGA CAT TGG CT	