Supplementary material to:

Mee Young Chang, Courtney Smith, James B. DuHadaway, Jennifer R. Pyle, Janette Boulden, Alejandro Peralta Soler, et al. Cardiac and gastrointestinal liabilities caused by deficiency in the immune modulatory enzyme indoleamine 2,3-dioxygenase. Cancer Biol Ther 12(12): DOI: 10.4161/cbt.12.12.18142

http://www.landesbioscience.com/journals/cbt/article/18142/

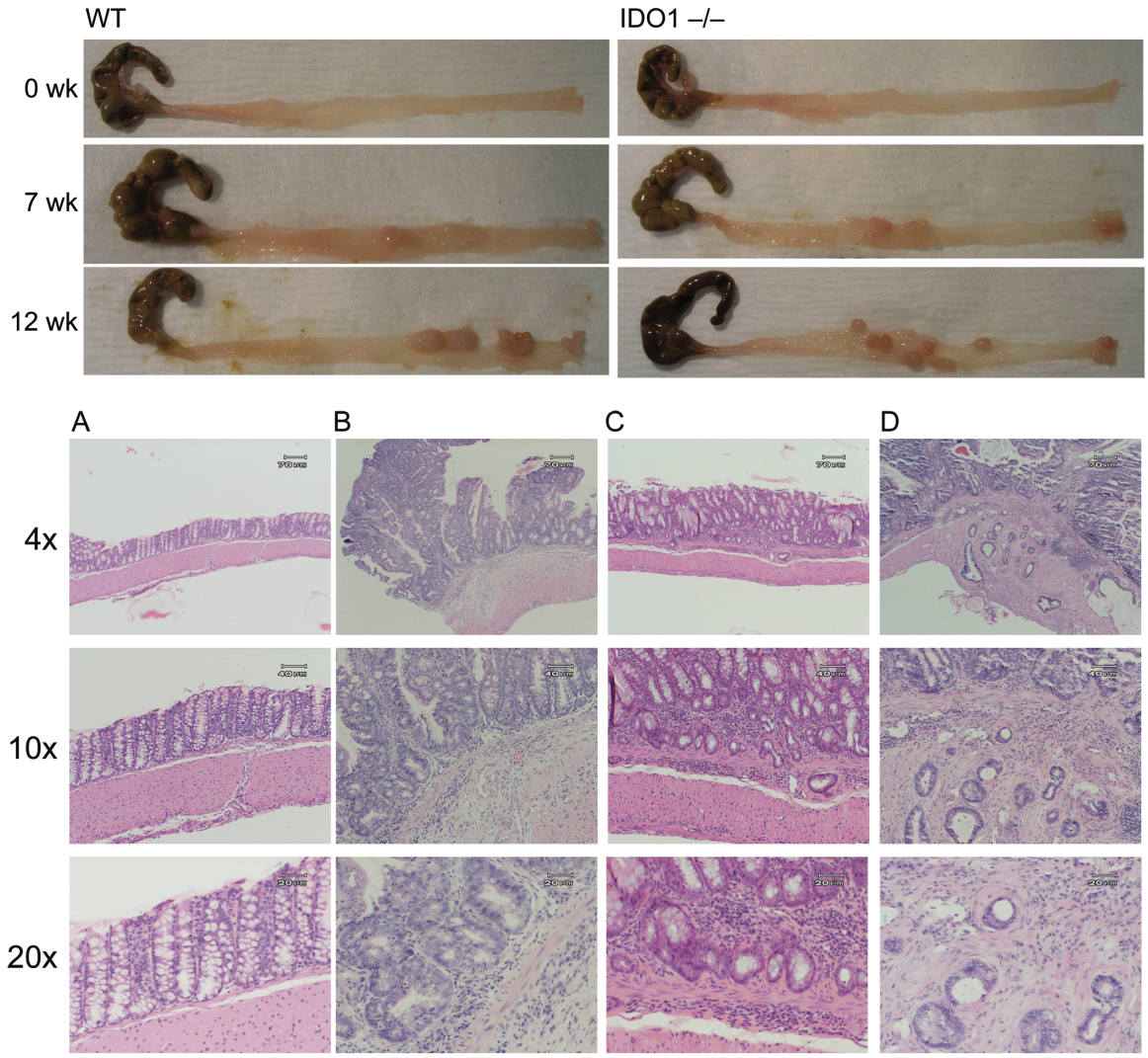

Figure S1. Dose dependence of IDO loss on colon carcinogenesis established by a high-dose DMH+DSS protocol. Mice were treated once i.p. with 30 mg/kg DMH and then 7 days 3% DSS in drinking water followed by normal drinking water to endpoints of 7, 12 or 18 wks. Colons were harvested from euthanized mice, fixed in paraffin and processed for H&E staining. Representative images of gross pathology in WT or IDO–/– mice (top panels) illustrate observations of macroscopic tumors (arrows) or prolapsed rectums (arrowheads). Representative images of histopathology in IDO–/– mice (bottom panels) illustrate observations of (A) no tumor, (B) mucosal invasion, (C) submucosal invasion and (D) muscular invasion. Original magnifications are 4x (top), 10x (middle),

20x (bottom). Tumor quantitation in this experiment is summarized in Table S2 and tumor staging in Table S3.

Table S1. IDO loss does not affect the staging of tumors developed during inflammatory colon carcinogenesis. Colon tumors generated per description in Figure 4B and Table 2 were staged histologically.

Genotype	Absence of Carcinoma	Mucosal Invasion[a]	Submucosal Invasion[b]	Muscle Invasion[c]
WT	1/8(13%)	2/8 (25%)	3/8 (38%)	2/8 (25%)
IDO–/–	0/6 (0%)	1/6 (17%)	3/6 (50%)	2/6 (33%)

[a]tumor confined to the mucosa; submucosa was not involved
[b]tumor invaded submucosa, muscle is not involved
[c]tumor invaded muscle

Table S2. Tumor quantitation in the high dose DMH+DSS protocol. Colon tumors were initiated in WT and IDO–/– mice with a single i.p. injection of 30 mg/kg DMH followed by 7 days treatment with 3% DSS in drinking water. All animals then received regular drinking water through the course of the experiment. Mice were euthanized at an endpoint of 18 weeks and colons were dissected for processing and histological evaluation.

Genotype	Wks after DMH	Absence of carcinoma	Early Carcinoma	Carcinoma	Multiple Carcinoma
WT	7	0/7 (0%)	1/7 (14%)	5/7 (71%)	1/7 (14%)
IDO -/-	7	2/6 (33%)	0/6 (0%)	0/6 (0%)	4/6 (67%)
WT	12	2/12 (17%)	0/12 (0%)	0/12 (0%)	10/12 (83%)
IDO -/-	12	2/14 (14%)	0/14 (0%)	0/14 (0%)	12/14 (86%)
WT	18	0/8 (0%)	0/8 (0%)	0/8 (0%)	8/8 (100%)
IDO -/-	18	0/11 (0%)	0/11 (0%)	0/11 (0%)	11/11 (100%)

At 7 wk 100% WT mice and 67% IDO–/– mice displayed tumors, but multiplicity was greater in IDO–/– mice with 100% of the animals exhibiting multiple carcinomas compared to only 14% of the WT animals. However, by 12 and 18 wk there was no longer any statistical difference in the tumor incidence.

Table S3. Tumor stages in the high dose DMH+DSS protocol. Colon tumors generated as described in Figure S1 and and Table S2 were graded histologically.

Genotype	Wks after DMH	Absence of Carcinoma	Mucosal Invasion[a]	Submucosal Invasion[b]	Muscle Invasion[c]
WT	7	0/7 (0%)	2/7 (28%)	3/7 (43%)	2/7 (29%)
IDO -/-	7	2/6 (33%)	0/6 (0%)	3/6 (50%)	1/6 (17%)
WT	12	2/12 (17%)	2/12 (17%)	7/12 (50%)	2/12 (17%)
IDO -/-	12	2/14 (14%)	2/14 (14%)	6/14 (43%)	4/14 (29%)
WT	18	0/8 (0%)	0/8 (0%)	4/8 (50%)	4/8 (50%)
IDO -/-	18	0/11 (0%)	0/11 (0%)	6/11 (55%)	5/11 (45%)

[a]tumor confined to the mucosa; submucosa was not involved
[b]tumor invaded submucosa, muscle is not involved
[c]tumor invaded muscle

Using the high-dose colon carcinogenesis regimen there appeared to be no significant difference in tumor grade observed in tumors scored at any endpoint. By 18 weeks, all WT and IDO–/– mice developed multiple carcinomas and 50% of them were muscle invasive tumors.