Apc$^{Min/+}$ tumours and normal mouse small intestines show linear metabolite concentration and DNA cytosine hydroxymethylation gradients from pylorus to colon

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Supplementary Material

Table S1. Statistical significance of slopes of metabolite gradients between tissues.
WT = wild-type C57BL/6J, NAdj = normal tissue adjacent to *Apc*$^{Min/+}$ tumours. t-Cho, choline + phosphocholine + glycerophosphocholine; t-Cr, creatine + phosphocreatine.

Metabolite	WT vs NAdj slopes p-value	Tumour vs NAdj slopes p-value
Choline	0.4828	**0.0053**
Phosphocholine	0.1779	0.2538
Glycero-phosphocholine	0.1329	0.4096
t-Cho	0.0757	0.9489
Creatine	0.2066	**0.0382**
t-Cr	0.4194	0.0723
Glutamate	0.1963	0.1305
Glycine	0.2855	**0.0052**
Taurine	0.3301	0.4175
Leucine	0.2604	0.7754
Valine	0.6008	0.6676

Table S2. Statistical significance of Y-intercepts of metabolite gradients between tissues. WT = wild-type C57BL/6J, NAdj = normal tissue adjacent to *Apc*$^{Min/+}$ tumours. t-Cho, choline + phosphocholine + glycerophosphocholine; t-Cr, creatine + phosphocreatine.

Metabolite	Y-intercept				
	WT	NAdj	WT vs Nadj p-value	Tumour	Tumour vs Nadj p-value
Choline	1.66	1.60	0.6724	1.22	N/A
Phosphocholine	1.71	1.20	0.0956	1.57	0.22
Glycero-phosphocholine	1.94	1.58	0.9147	2.50	**0.0082**
t-Cho	5.36	4.26	0.1110	5.27	**0.0004**
Creatine	2.60	2.19	**0.0262**	2.28	N/A
t-Cr	2.61	2.34	0.1903	2.71	**<0.0001**
Glutamate	2.49	2.41	**0.0176**	4.24	**<0.0001**
Glycine	1.76	2.12	0.3397	2.38	N/A
Taurine	6.34	7.39	0.0740	7.59	0.4976
Leucine	1.09	1.25	0.7884	1.65	**0.0003**
Valine	0.73	0.83	0.4637	1.04	0.076

Table S3. Mean concentrations of metabolite in wild type C57BL/6J tissues (WT), normal tissue adjacent to *Apc*$^{Min/+}$ tumours (NAdj) and *Apc*$^{Min/+}$ Tumour tissue. t-Cho, choline + phosphocholine + glycerophosphocholine; t-Cr, creatine + phosphocreatine.

	WT V NAdj			NAdj vs Tumour		
	WT	NAdj		NAdj	Tumour	
Metabolite	Mean ± 1 s.e.m.	Mean ± 1 s.e.m.	t-test	Mean ± 1 s.e.m.	Mean ± 1 s.e.m.	t-test
Choline	0.959± 0.086	1.085± 0.093	0.3339	1.085± 0.093	1.081± 0.050	0.9649
Phosphocholine	1.564± 0.091	1.377± 0.095	0.1711	1.377± 0.095	1.532± 0.069	0.2110
Glycerophosphocholine	1.965± 0.090	1.973± 0.133	0.9581	1.973± 0.133	2.674± 0.187	0.0025
t-Cho	4.519± 0.177	4.204± 0.178	0.2196	4.204± 0.178	5.158± 0.171	0.0003
Creatine	2.025± 0.078	1.849± 0.081	0.1321	1.849± 0.081	2.309± 0.101	0.0006
t-Cr	2.168± 0.075	2.044± 0.083	0.2787	2.044± 0.083	2.781± 0.081	<0.0001
Glutamate	2.819± 0.108	2.423± 0.102	0.0120	2.423± 0.102	4.906± 0.144	<0.0001
Glycine	1.731± 0.0705	1.846± 0.126	0.3999	1.846± 0.126	2.924± 0.129	<0.0001
Taurine	6.542± 0.213	7.111± 0.216	0.0692	7.111± 0.216	6.808± 0.253	0.3630
Leucine	0.8692± 0.055	0.922± 0.075	0.5621	0.922± 0.075	1.38± 0.113	0.0009
Valine	0.5886± 0.041	0.650± 0.055	0.3637	0.650± 0.055	0.777± 0.074	0.1764

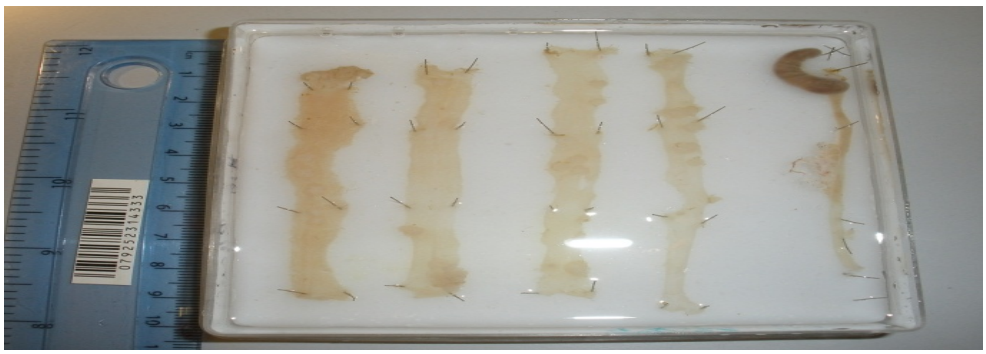
Table S4. Statistical significance of DNA cytosine hydroxymethylation (5hmC) and methylation (5mC) slopes by tissue. 5hmC = hydroxymethylation, 5mC = methylation, WT = wild-type C57BL/6J, NAdj = normal tissue adjacent to Tumour in *Apc*$^{Min/+}$.

	5hmC			5mC		
	WT	***NAdj***	***Tumour***	***WT***	***NAdj***	***Tumour***
Slope	-0.0001	0.0004	0.0002	-0.0008	-0.0014	0.0133
Is slope significantly non-zero? P-values	0.6017	0.0756	**0.0499**	0.8884	0.8224	0.0819

Table S5. Statistical significance of DNA cytosine hydroxymethylation (5hmC) and methylation (5mC) slopes between tissues. WT = wild-type C57BL/6J, NAdj = normal tissue adjacent to Tumour in *Apc*$^{Min/+}$.

	WT vs NAdj			***NAdj vs Tumour***		
	WT slope	**NAdj slope**	**p-value**	**NAdj slope**	**Tumour slope**	**p-value**
5hmC	-0.0001	0.0004	0.0869	0.0004	0.0002	0.4429
5mC	-0.0008	-0.0014	0.9485	-0.0014	0.0133	0.1373

Fig S1. Method for obtaining samples for measurements of metabolite concentrations and DNA modifications. An example of a small intestine whole-mount with the gastro-duodenal junction at the top of the left hand mount and the caecum and colon in the right hand mount. The caecum and colon (far right) were not assessed. Tissues from WT and *Apc*$^{Min/+}$ animals were dissected at measured distances from the stomach, and were then used for metabolite and DNA modification analyses.



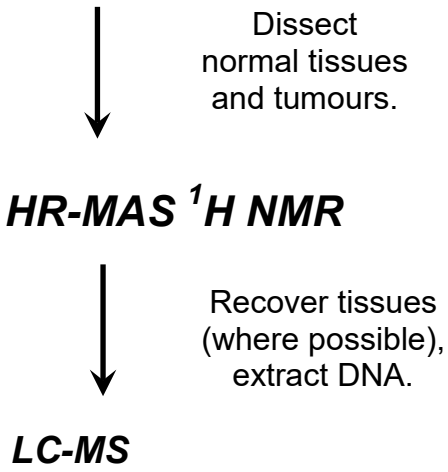


Fig S2. Plot of metabolite concentrations in WT gut tissues as a measure of tissue dissection order. Small intestine tissues were dissected proximal to distal from the pylorus (top to bottom) or distal to proximal (bottom to top) to assess whether metabolite concentrations would be dependent on time to snap freeze in liquid nitrogen. PC, phosphocholine; GPC, glycerophosphocholine.

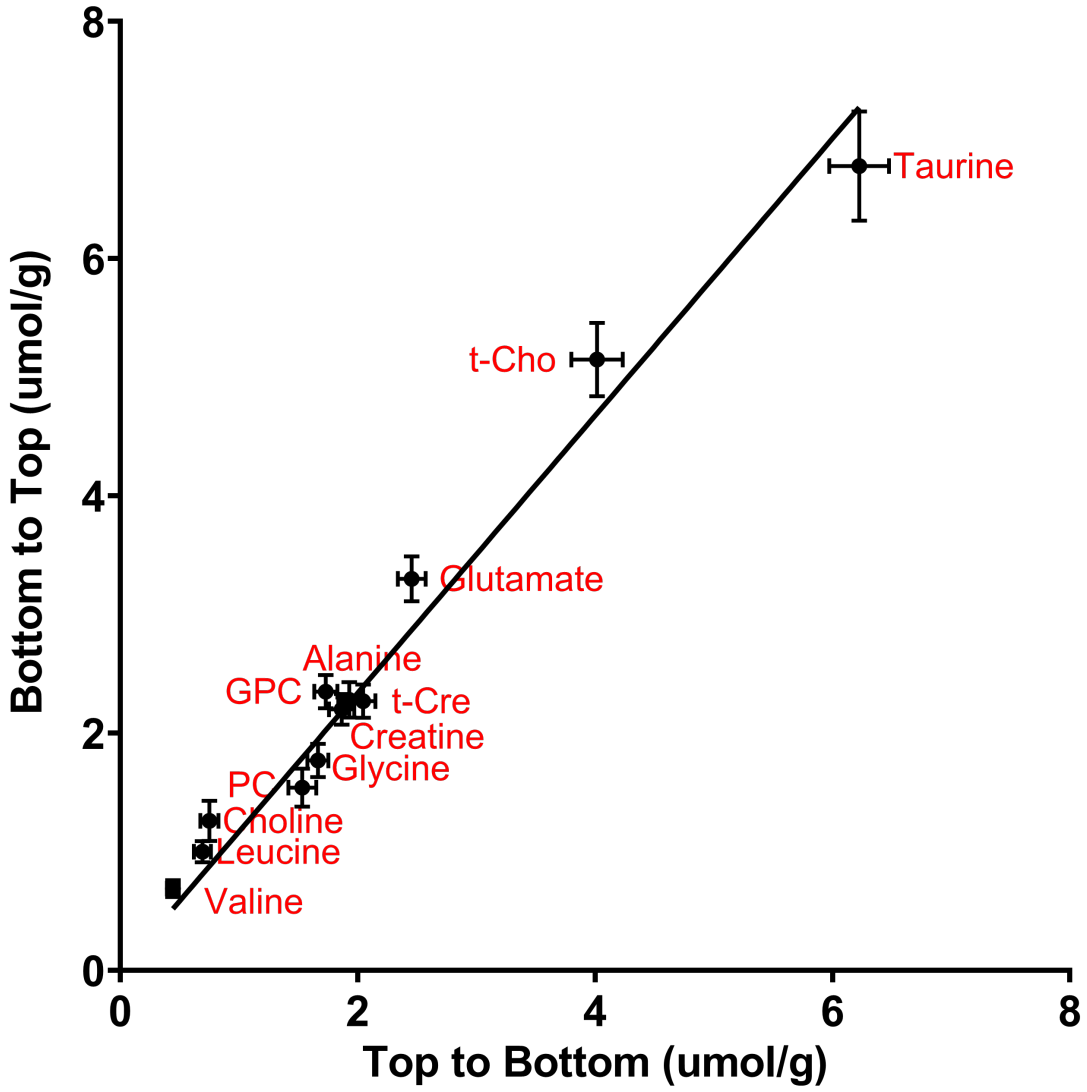


Fig S3. Plot of metabolite concentrations in normal tissue adjacent to tumours (NAdj) and Tumour tissues along the small intestine as a function of distance from pylorus. t-Cho, choline + phosphocholine + glycerophosphocholine; t-Cr, creatine + phosphocreatine.