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Supplemental Information

UCP2 Deficiency Increases Colon Tumorigenesis by Promoting Lipid Synthesis and Depleting NADPH for Antioxidant Defenses

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Figure S1

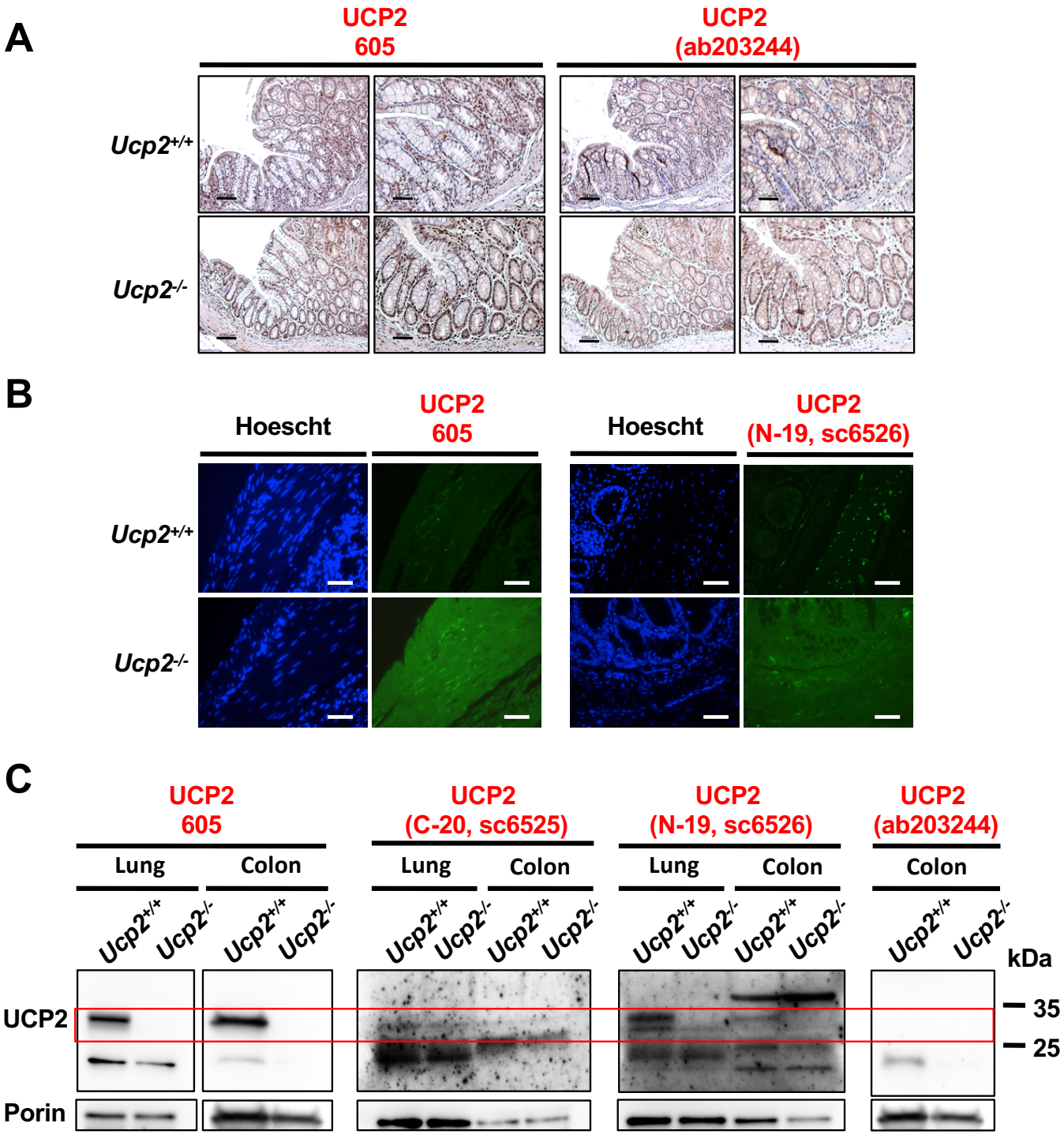

Figure S1, related to Figure 1. UCP2 detection by immunohistochemistry, immunofluorescence and western blot analysis.

(A) UCP2 detection by immunohistochemistry analysis using colon tissue from *Ucp2*$^{+/+}$ and *Ucp2*$^{-/-}$ AOM/DSS-treated mice. Our homemade UCP2-605 antibody and the commercial UCP2 antibody ab203244 were tested. For each antibody: low magnification (left), scale bar 100 µm, and high magnification (right), scale bar 50 µm.

(B) UCP2 detection by immunofluorescence (green) using colon samples. Our homemade UCP2-605 antibody and the commercial UCP2 antibody sc6526 were tested. Nuclei were stained with Hoechst 3342 (blue). Scale bar = 100 µm.

(C) Western blot analysis of UCP2 protein levels using mitochondria protein lysates from colon and lung samples from *Ucp2*$^{+/+}$ and *Ucp2*$^{-/-}$ mice. Our homemade UCP2-605 antibody and the commercial UCP2 antibodies sc6525, sc6526 and ab203244 were used. Porin was used as a loading control.

Figure S2

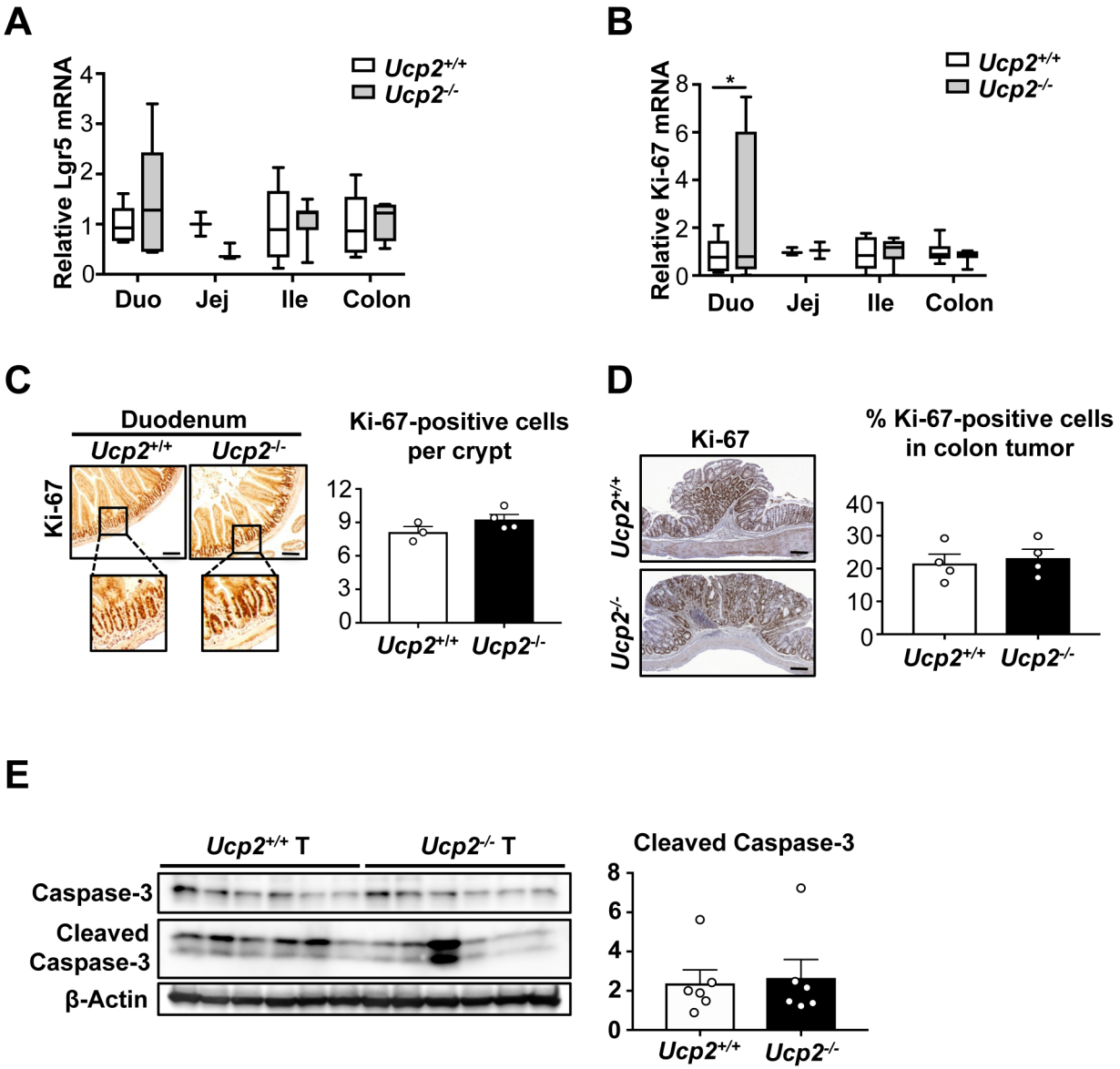

Figure S2 , related to Figure 2. UCP2 invalidation does not affect the rate of cell proliferation and cell death.
(A) Lgr5 mRNA levels relative to *Ucp2*$^{+/+}$ samples. Data represent mean ± SEM for duodenum (Duo, n = 6), jejunum (n = 3), ileum (Ile, n = 7) and colon (n = 7).
(B) Ki-67 mRNA levels relative to *Ucp2*$^{+/+}$ samples. Data represent mean ± SEM for duodenum (Duo, n = 7), jejunum (n = 3), ileum (Ile, n = 7) and colon (n = 7).
(C) Representative duodenum sections stained with Ki-67. Data represent mean ± SEM (n = 3-4). Scale bar = 100 µm.
(D) Representative colonic sections, showing tumoral and non-tumoral regions, stained with Ki-67. Data represent mean ± SEM (n = 4). Scale bar = 200 µm.
(E) Immunoblot of caspase-3 and its cleaved form. Data represent mean ± SEM (n = 6).

Figure S3

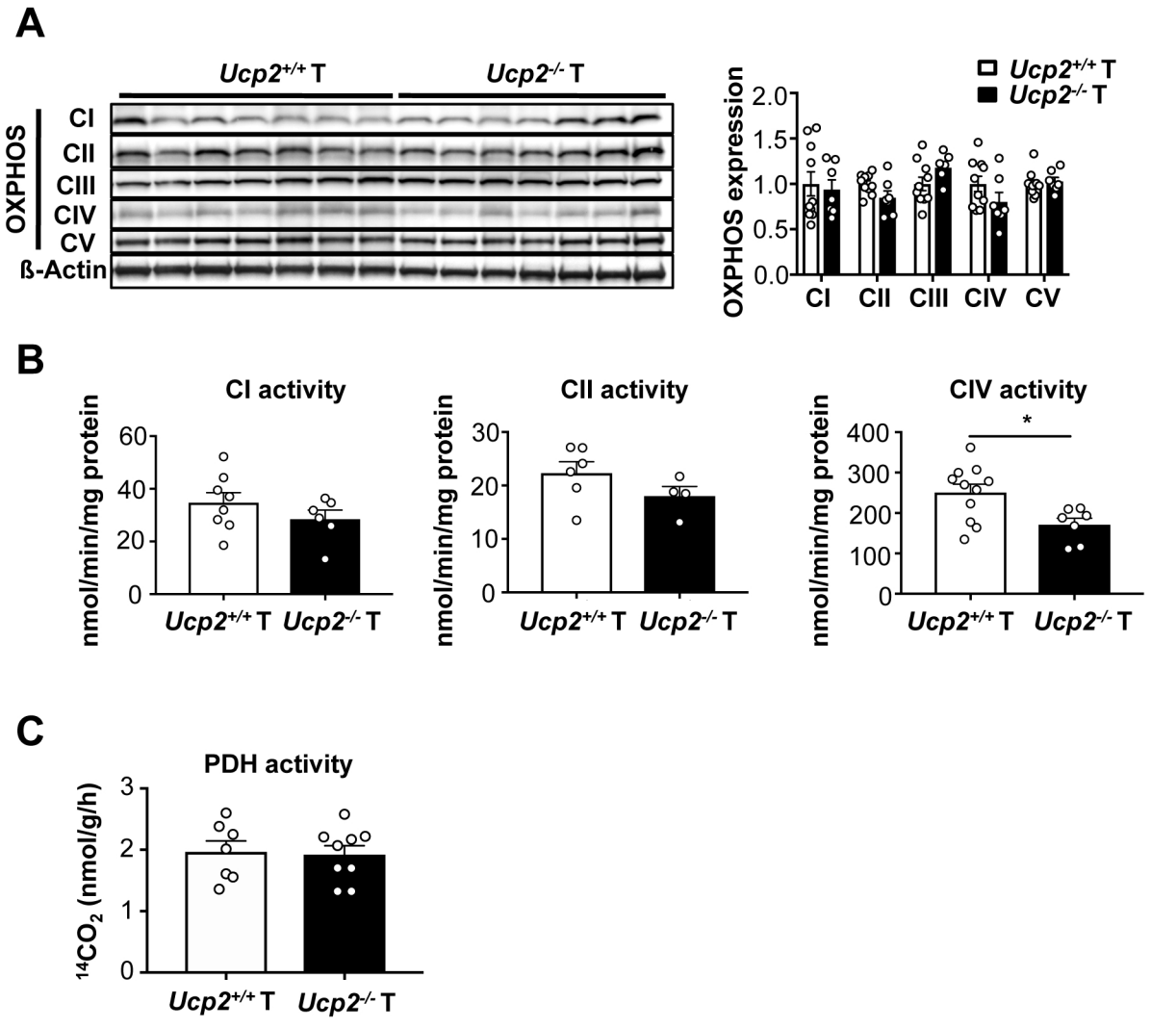

Figure S3, related to Figure 5. Complex IV activity is reduced in *Ucp2*$^{-/-}$ tumors.

(A) Representative immunoblot analysis of OXPHOS complexes (CI to CV) using whole cell extracts (*Ucp2*$^{+/+}$ T n = 10, *Ucp2*$^{-/-}$ T n = 7). Data indicate mean ± SEM.

(B) Enzyme activities of OXPHOS complexes CI, CII and CIV normalized to protein content. Data indicate mean ± SEM.

(C) PDH activity measured as $^{14}CO_2$ release from [1-^{14}C]-pyruvate (*Ucp2*$^{+/+}$ T n = 7, *Ucp2*$^{-/-}$ T n = 9). Data indicate mean ± SEM.

Figure S4

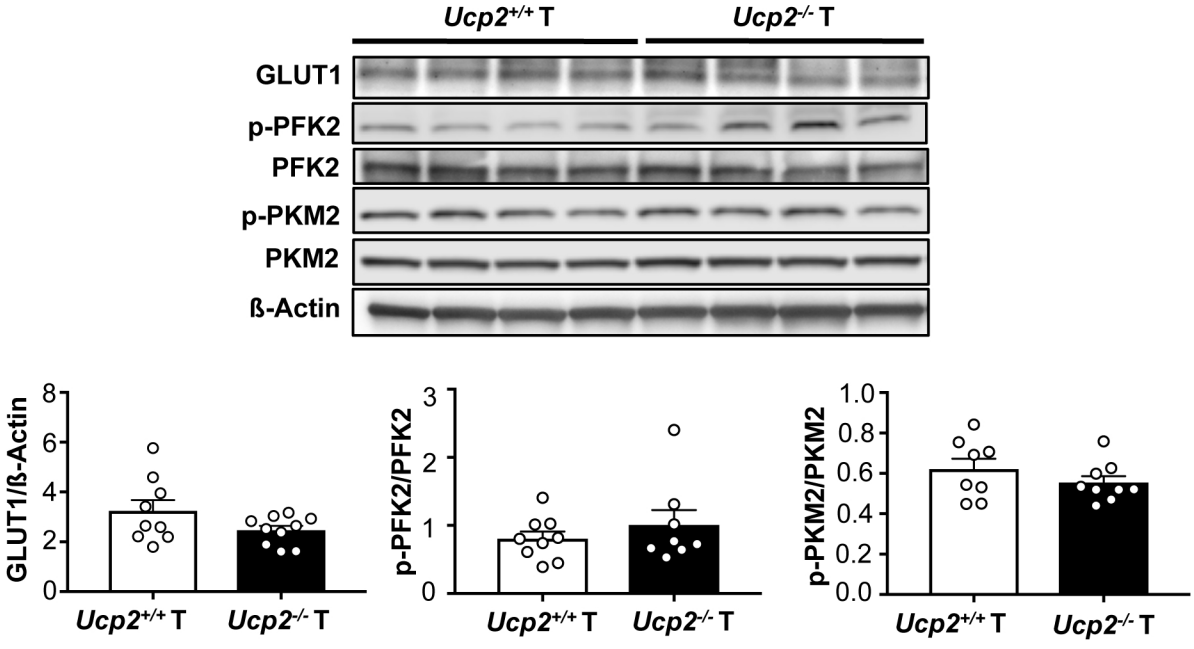

Figure S4, related to Figure 5. Effect of UCP2 invalidation on the expression of enzymes from glycolysis. Representative immunoblot of GLUT1, p-PFK2, PFK, p-PKM2 and PKM2 using whole cell extracts from tumors (T) with ß-Actin as a loading control for quantification. Data indicate mean ± SEM (n = 7-10).

Table S1, related to Figure 1. UCP2 expression in multiple microarray colorectal cancer data sets available from Oncomine (http://www.oncomine.com).

Data set	Normal type	Colorectal cancer subtype	Samples (N)	p value	Changes
GAEDKE	Rectum	Rectal adenocarcinoma	65 vs. 65	0.0817	=
GASPAR	Intestinal mucosa	Colorectal adenoma	22 vs. 47	0.0012	↑
GRAUDENS	Colon	Colorectal carcinoma	12 vs. 18	0.3038	=
HONG	Colon	Colorectal carcinoma	12 vs. 70	0.0662	=
KAISER	Colon	Colon adenocarcinoma	5 vs. 41	0.1171	=
	Colon	Colon mucinous adenocarcinoma	5 vs. 13	0.2508	=
	Colon	Rectal adenocarcinoma	5 vs. 8	0.3692	=
	Colon	Rectal mucinous adenocarcinoma	5 vs. 4	0.7165	=
	Colon	Rectosigmoid adenocarcinoma	5 vs. 10	0.0010	↑
KI	Colorectal mucosa	Colorectal	28 vs. 53	0.6584	=
SABATES-BELLVER	Colon mucosa	Colorectal adenoma	32 vs. 32	0.0007	↑
	Colon mucosa	Rectal adenoma	32 vs. 7	0.1769	=
SKRZPCZAK	Colon	Colorectal adenocarcinoma	24 vs. 45	0.0112	↑
	Colon	Colorectal carcinoma	24 vs. 36	0.7489	=
SKRZPCZAK 2	Colon	Colon adenoma	10 vs. 5	0.0011	↑
	Colon	Colon carcinoma	10 vs. 5	0.7276	=
TCGA	Colon	Colon adenocarcinoma	19 vs. 102	0.0023	↓
	Colon	Colon mucinous adenocarcinoma	19 vs. 20	0.4327	=
	Colon	Rectal adenocarcinoma	19 vs. 60	0.0010	↓
	Colon	Rectal mucinous adenocarcinoma	19 vs. 6	0.0565	=
ZOU	Colon	Colon carcinoma	8 vs. 9	0.3845	=

Table S3, related to STAR Methods. Sequences of primers used in this study.

Gene		Sequence (5'-3')
mouse Ki-67	forward reverse	AGGATGGAAGCAAGCCAACA GGCCCTTGGCATACACAAAA
mouse Lgr5	forward reverse	CGAGCCTTACAGAGCCTGATACC TTGCCGTCGTCTTTATTCCATTGG
mouse UCP2	forward reverse	TCGAAGCCTACAAGACCATTGCAC ACCAGCTCAGCACAGTTGACAATG
human UCP2	forward reverse	CTACAGCCAGCGCCCAGTA TCAGTACGCACCATGGTCAGA
mouse cyclophilin	forward reverse	ATGGCACTGGCGGCAGGTCC TTGCCATTCCTGGACCCAAA
human cyclophilin	forward reverse	GTCAACCCCACCGTGTTCTT CTGCTGTCTTTGGGACCTTGT