SUPPLEMENTAL METHODS

Immunoblots

Additional primary antibodies included rabbit anti-APC (1:1000, sc-7930, Santa Cruz Biotechnology), rabbit anti-GAPDH (1:3000, 5174, Cell Signaling Technology), rabbit anti-GPX1 (1:1000, SAB5700925, Sigma-Aldrich), and rabbit anti-GPX2 (1:1000, ab137431, abcam).

Small intestine tumor area measurements

Well-oriented, H&E-stained small intestine adenomas were imaged at 10x magnification and measured in ImageJ (1) by an experimenter blinded to genotype.

Murine enteroid culture

Enteroids were established and cultured as previously described (2).

Human tumoroid culture

Human tumoroids were established and cultured as previously described (3). Known clinical characteristics are described in Table S5.

SELENOP treatments

Human tumoroids were treated with 0 or 500 ng/mL purified human SELENOP for five days prior to RNA extraction. 293 STF cells were treated with 0 or 100 ng/mL purified human SELENOP for 16 hours prior to TOPFlash assays.

Small interfering RNA (siRNA) transfections

293 STF cells were seeded in 6-well or 12-well plates (300,000 or 100,000 cells/well, respectively). Twenty-four hours later, cells were transfected with 100 nM control siRNA-A (sc37007, Santa Cruz Biotechnology) or pooled *APC* siRNAs (sequences published in (4), Dharmacon) using Lipofectamine® RNAiMAX (13778075, Invitrogen).

Proximity ligation assay

293T cells were cultured to ~10% confluency in 8-well chamber slides (PEZGS0816, Millipore), then transfected with 0.1 µg pcDNA6-N-3XFLAG-Lrp6 (123595, Addgene) and 0.1 µg pCMV6-V5-mSELENOP (5) plasmids using polyethylenimine (24314, Polysciences, Inc.). After 48 hours, cells were fixed in 3% (w/v) paraformaldehyde (158127, Sigma-Aldrich), briefly washed in PBS with 10 mM glycine

(G36050, Research Products International), and permeabilized in PBS with 0.2% (v/v) Triton™ X-100 (T8787, Sigma-Aldrich). Proximity ligation assays were then performed with mouse anti-β-catenin (1:500, 610154, BD Biosciences), rabbit anti-α-catenin (1:500, C2081, Sigma-Aldrich), mouse IgG1 (1:500, 5415, Cell Signaling Technology), rabbit IgG (1:500, 3900, Cell Signaling Technology), mouse anti-FLAG® M2 (1:500, F1804, Sigma-Aldrich), and rabbit anti-V5 (1:500, 13202, Cell Signaling Technology) antibodies and the Duolink® In Situ Red Starter Kit Mouse/Rabbit (DUO92101, Sigma-Aldrich) per the manufacturer's protocol. Slides were imaged with a Nikon Eclipse E800 upright microscope and NIS-Elements BR software.

Plasmids

pReceiver-M14-mLRP5-3XFLAG was purchased from GeneCopoeia (EX-Mm34003-M14).

SUPPLEMENTAL REFERENCES

1. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 2012;9(7):671–675.

2. Barrett C, Short S, Choksi Y, Williams C. Whole-mount Enteroid Proliferation Staining. *Bio-protocol* 2016;6(12). doi:10.21769/bioprotoc.1837

3. Short SP et al. Serine Threonine Kinase 17A maintains the epithelial state in colorectal cancer cells. *Mol Cancer Res* 2019;17(4):molcanres.0990.2018.

4. Saito-Diaz K et al. APC Inhibits Ligand-Independent Wnt Signaling by the Clathrin Endocytic Pathway. *Dev Cell* 2018;44(5):566-581.e8.

5. Kurokawa S, Bellinger FP, Hill KE, Burk RF, Berry MJ. Isoform-specific Binding of Selenoprotein P to the β-Propeller Domain of Apolipoprotein E Receptor 2 Mediates Selenium Supply. *J Biol Chem* 2014;289(13):9195–9207.

SUPPLEMENTAL TABLES

Gene Name	Assay ID
Dio1	Mm00839358_m1
Dio2	Mm00515664_m1
Dio3	Mm00548953_s1
Gapdh	Mm99999915_g1
Gpx1	Mm00656767_g1
Gpx2	Mm00850074_g1
Gpx3	Mm00492427_m1
Gpx4	Mm00515041_m1
Msrb1	Mm00489121_m1
Selenof	Mm00474111_m1
Selenoh	Mm01335355_g1
Selenoi	Mm01210813_m1
Selenok	Mm00785961_s1
Selenom	Mm00459806_m1
Selenon	Mm01188435_m1
Selenoo	Mm00662744_m1
Selenop	Mm00486048_m1
Selenos	Mm01318786_m1
Selenot	Mm01615823_m1
Selenov	Rn01475733_m1
Selenow	Mm01268252_m1
Sephs2	Mm00545980_s1
Tbp	Mm00446973_m1
Txnrd1	Mm00443675_m1
Txnrd2	Mm00496766_m1
Txnrd3	Mm00462552_m1

Table S1. TaqMan™ RT-qPCR probes.

Gene Name	**Primer Designations**	**Primer Sequences**	**Reference**
Axin2	mAxin2_RT_F mAxin2_RT_R	5' TGACTCTCCTTCCAGATCCCA 3' 5' TGCCCACACTAGGCTGACA 3'	Short et al. (2019) *Oncogene*.
AXIN2	hAXIN2_RT_F hAXIN2_RT_R	5' CAACACCAGGCGGAACGAA 3' 5' GCCCAATAAGGAGTGTAAGGACT 3'	Thompson et al. (2019) *Carcinogenesis*
Gapdh	mGapdh_RT_F mGapdh_RT_R	5' CCGCATCTTCTTGTGCA 3' 5' CGGCCAAATCCGTTCA 3'	Short et al. (2019) *Oncogene*.
GAPDH	hGAPDH_RT_F hGAPDH_RT_R	5' GGCCTCCAAGGAGTAAGACC 3' 5' AGGGGTCTACATGGCAACTG 3'	Thompson et al. (2019) *Carcinogenesis*
Lgr5	mLgr5_RT_F mLgr5_RT_R	5' CCAATGGAATAAAGACGACGGCAACA 3' 5' GGGCCTTCAGGTCTTCCTCAAAGTCA 3'	Luong-Gardiol et al. (2019) *Cancer Cell.*
LGR5	hLGR5_RT_F hLGR5_RT_R	5' GAGTTACGTCTTGCGGGAAAC 3' 5' TGGGTACGTGTCTTAGCTGATTA 3'	Liao et al. (2020) *Stem Cell Rep.*
Sox9	mSox9_RT_F mSox9_RT_R	5' GAGCCGGATCTGAAGAGGGA 3' 5' GCTTGACGTGTGGCTTGTTC 3'	Wang et al. (2020) *Cancer Cell.*
SOX9	hSOX9_RT_F hSOX9_RT_R	5' AGCGAACGCACATCAAGAC 3' 5' CTGTAGGCGATCTGTTGGGG 3'	Li et al. (2015) *PLoS One.*

Table S2. SYBR Green RT-qPCR primers.

Condition	Basal Media	Supplements	Additives
Stem cell	Advanced DMEM/F12 (12634010, Gibco) 1X B-27™ Supplement (17504044, Gibco) 1X GlutaMAX™ (35050061, Gibco) 1X N-2™ Supplement (17502048, Gibco) 1 mM HEPES (15630080, Gibco) 2% (v/v) penicillin/streptomycin (15140122, Gibco)	20% (v/v) R-spondin-conditioned media (from R-spondin-expressing cells gifted by Dr. Jeff Whitsett, The University of Cincinnati, Cincinnati, OH) 10% (v/v) Noggin-conditioned media (from Noggin-expressing cells gifted by Dr. G.R. van den Brink, Amsterdam, NL)	None
Enterocyte			2 μM IWP 2 (3533, Tocris Bioscience) 2 mM valproic acid (P4543, Sigma-Aldrich)
Paneth cell			3 μM CHIR 99021 (4423, Tocris Bioscience) 10 μM DAPT (2634, Tocris Bioscience)
Goblet cell			10 μM DAPT (2634, Tocris Bioscience) 2 μM IWP 2 (3533, Tocris Bioscience)

Table S3. Human enteroid media components.

sgRNA Name	sgRNA Designations	sgRNA Sequences
NONTARGET	NONTARGET_CRa_F_1 NONTARGET_CRa_R_1	5’ CACCGGACCTTCATTGAAGAAAAGC 3’ 5’ AAACGCTTTTCTTCAATGAAGGTCCGGTGC 3’
hSELENOP_1	hSELENOP_CRa_F_1 hSELENOP_CRa_R_1	5’ CACCGGGAAGGGCTAAGGGTAAACA 3’ 5’ AAACTGTTTACCCTTAGCCCTTCCCGGTGC 3’
hSELENOP_2	hSELENOP_CRa_F_2 hSELENOP_CRa_R_2	5’ CACCGGTTTGGGAAAGAAGGCAACT 3’ 5’ AAACAGTTGCCTTCTTTCCCAAACCGGTGC 3’
hSELENOP_3	hSELENOP_CRa_F_3 hSELENOP_CRa_R_3	5’ CACCGTTCTTTCCCAAACTATAACA 3’ 5’ AAACTGTTATAGTTTGGGAAAGAACGGTGC 3’
hSELENOP_4	hSELENOP_CRa_F_4 hSELENOP_CRa_R_4	5’ CACCGTGGGAAAGAAGGCAACTTGG 3’ 5’ AAACCCAAGTTGCCTTCTTTCCCACGGTGC 3’
mSELENOP_1	mSELENOP_CRa_F_1 mSELENOP_CRa_R_1	5’ CACCGACTTTGGACTGCACCTCAGA 3’ 5’ AAACTCTGAGGTGCAGTCCAAAGTCGGTGC 3’
mSELENOP_2	mSELENOP_CRa_F_2 mSELENOP_CRa_R_2	5’ CACCGCTGCATTTGCAAGGTCGCAG 3’ 5’ AAACCTGCGACCTTGCAAATGCAGCGGTGC 3’
mSELENOP_3	mSELENOP_CRa_F_3 mSELENOP_CRa_R_3	5’ CACCGGCTGAGGCAGTACTTACTGA 3’ 5’ AAACTCAGTAAGTACTGCCTCAGCCGGTGC 3’
mSELENOP_4	mSELENOP_CRa_F_4 mSELENOP_CRa_R_4	5’ CACCGGTTGTTTACCTCGCCCTCTG 3’ 5’ AAACCAGAGGGCGAGGTAAACAACCGGTGC 3’

Table S4. sgRNA sequences.

Construct Name	Primer Designations	Primer Sequences
pCMV6-V5-mSELENOP_tU1	mSELENOP_tU_F	5’ TACGACTAAGCAAGAATGGAGTACA GAATTAAGTG 3’
	mSELENOP_tU1_R	5’ TAAGCTGGCTTGAAGAAGAGCAACC ACTGTCACTT 3’
pCMV6-V5-mSELENOP_tU2	mSELENOP_tU_F	5’ TACGACTAAGCAAGAATGGAGTACA GAATTAAGTG 3’
	mSELENOP_tU2_R	5’ TAAGCTCTCTAAGTGACCCTGCCTG TGCTGGCCCC 3’
pCMV6-V5-mSELENOP_tU3	mSELENOP_tU_F	5’ TACGACTAAGCAAGAATGGAGTACA GAATTAAGTG 3’
	mSELENOP_tU3_R	5’ TAAGAGCTTCCTCTGGGCAAGTGAA AGGTGCAAGC 3’
pCMV6-V5-mSELENOP_tU4	mSELENOP_tU_F	5’ TACGACTAAGCAAGAATGGAGTACA GAATTAAGTG 3’
	mSELENOP_tU4_R	5’ TAAAGCAATTGCAGACCCTGACTTC TCAAATATGA 3’
pCMV6-V5-mSELENOP_Δ258-267	mSELENOP_d258-267_F	5’ TGTAAGTTGTCTAAGGAGTCCGAGG CAGCCCCCAG 3’
	mSELENOP_d258-267_R	5’ GAGCTTCCTCTGGGCAAGTGAAAGG TGCAAGCCTT 3’

pCMV6-V5-mSELENOP_Δ268-277	mSELENOP_d268-277_F	5’ CCCAGCAGCTGCTGCTGTCACTGCC GCCACCTCAT 3’
	mSELENOP_d268-277_R	5’ CAGGAGCTGGTTGATGCACCCCCTT CGACAGAGCT 3’
pCMV6-V5-mSELENOP_Δ278-287	mSELENOP_d278-287_F	5’ TTTGAGAAGTCAGGGTCTGCAATTG CTTGTCAGTG 3’
	mSELENOP_d278-287_R	5’ GGCTGCCTCGGACTCCTTAGACAAC TTACACAGGA 3’
pCMV6-V5-mSELENOP_Δ288-299	mSELENOP_d288-299_F	5’ CAGTGTGCGGAAAACCTCCCATCCT 3’
	mSELENOP_d288-299_R	5’ TATGAGGTGGCGGCAGTGACAGCAG 3’
pCMV6-V5-mSELENOP_Δ258-299	mSELENOP_d288-299_F	5’ CAGTGTGCGGAAAACCTCCCATCCT 3’
	mSELENOP_d258-267_R	5’ GAGCTTCCTCTGGGCAAGTGAAAGG TGCAAGCCTT 3’
pLX304-V5-mSELENOP	attB1-mSELENOP_F	5’ GGGGACAAGTTTGTACAAAAAAGCA GGCTTCACCATGTGGAGAAGCCTAG GGCTTGCC 3’
	attB2-mSELENOP_R	5’ GGGGACCACTTTGTACAAGAAAGCT GGGTCTTAGTTTGAATGACATTTACA CTT 3’
pLX304-V5-mSELENOP_ Δ258-299	attB1-mSELENOP_F	5’ GGGGACAAGTTTGTACAAAAAAGCA GGCTTCACCATGTGGAGAAGCCTAG GGCTTGCC 3’

	attB2-mSELENOP_R	5’ GGGGACCACTTTGTACAAGAAAGCT GGGTCTTAGTTTGAATGACATTTACA CTT 3’

Table S5. PCR primers for plasmid construction.

Line	Location	Age	Race	Sex	Stage	Dysplasia	Known Mutations	MSI/MSS	CMS
32385	Right	61	Black	Female	T3N0	HGD		MSI	1/3
35349	Sigmoid	57	White	Female	T3N0	HGD	*KRAS*^{G12D}, *TP53*^{R248W}	MSS	2/4
40299	Sigmoid	67	White	Female	T3N1b	LGD		MSS	4
82742	Right	79	Black	Male	T4aN2b	HGD		MSS	2

Table S6. Clinical characteristics of human colon tumors from which tumoroids were established. Stage and dysplasia were determined by the attending pathologist. All patients were treatment-naïve. For line 35349, mutational analysis was performed on biopsy tissue prior to resection. Lines 32385, 40299, and 82742 were not subjected to further mutational analysis. Microsatellite instability was analyzed by PCR and IHC per clinical standard of care. CMS: consensus molecular subtype, HGD: high-grade dysplasia, LGD: low-grade dysplasia, MSI: microsatellite instability, MSS: microsatellite stable.

SUPPLEMENTAL FIGURES

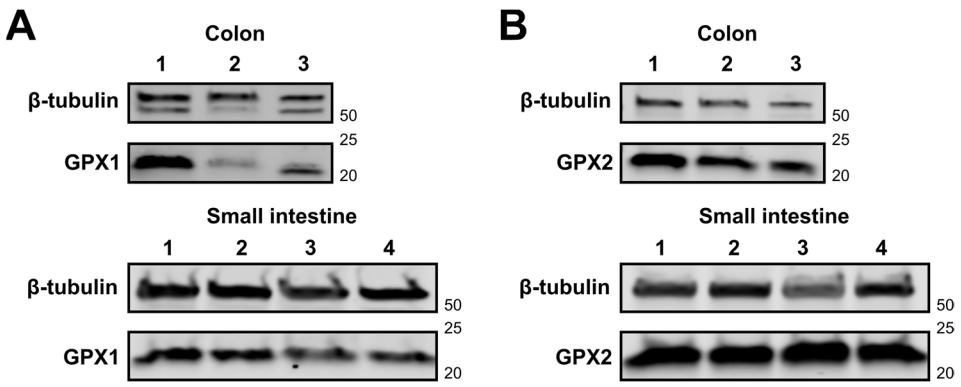


Figure S1. GPX1 and GPX2 protein expression in WT mouse colon and small intestine epithelium. Western blots for (A) GPX1, (B) GPX2, and (A, B) β-tubulin (loading control) in WT mouse colon and small intestine epithelium. n=3-4 mice.

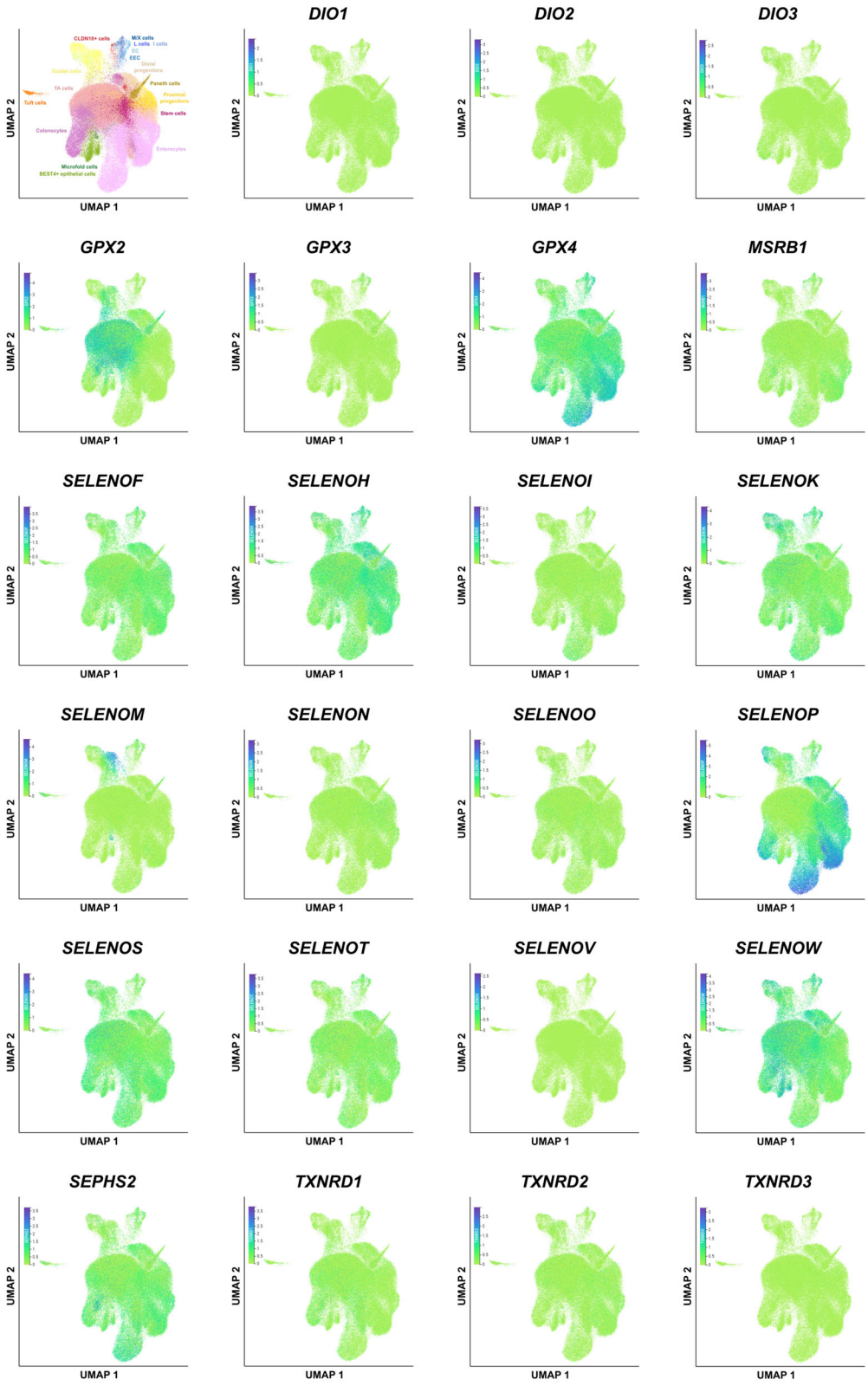


Figure S2. Selenoprotein expression in the normal human colon and small intestine. Gut Cell Atlas scRNA-seq data from human colon and small intestine epithelium queried for indicated selenoproteins. EC: enterochromaffin, EEC: enteroendocrine, TA: transit amplifying. n=6 donors.

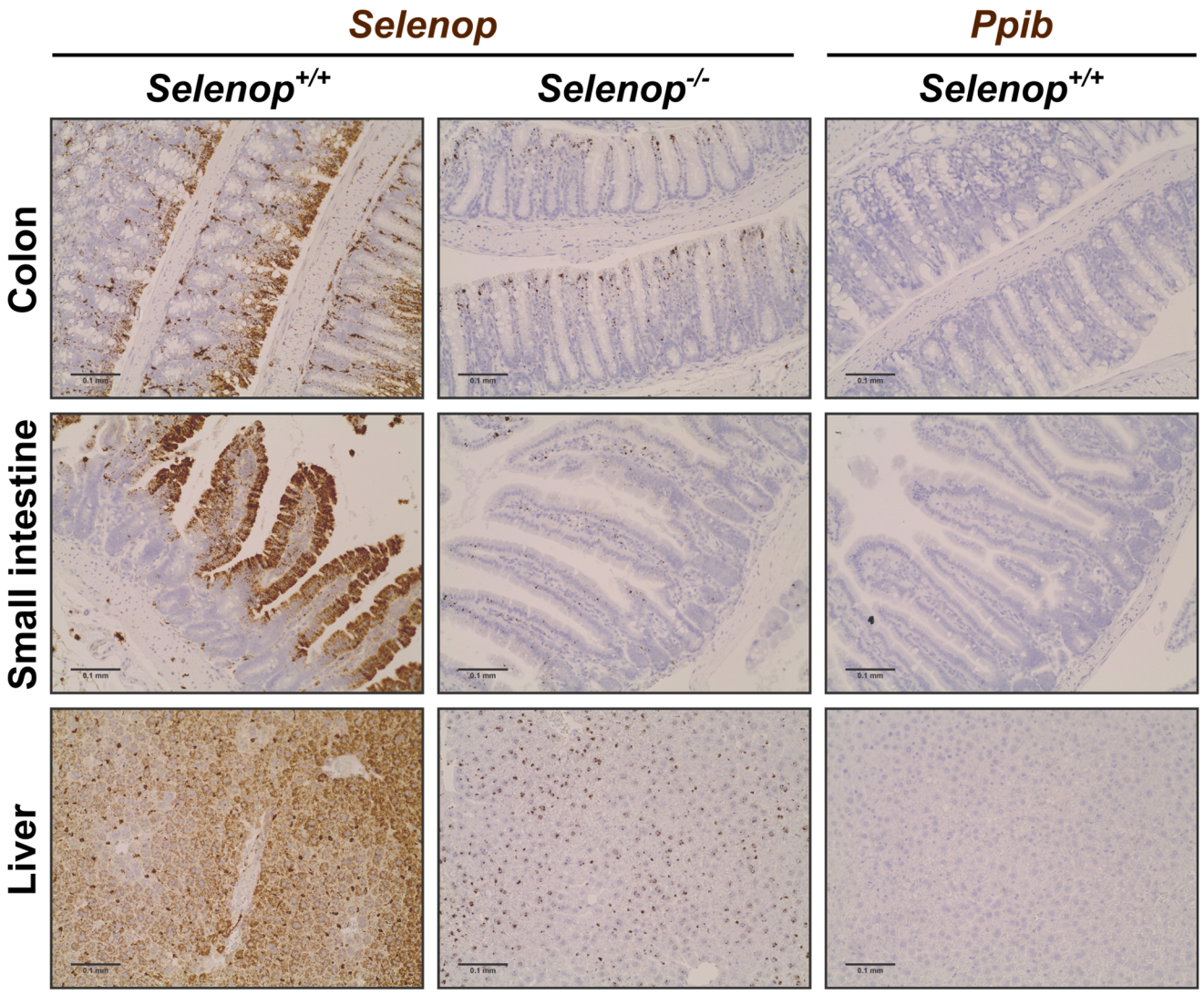


Figure S3. Validation of *Selenop* RNAscope® probe. RNAscope® of *Selenop*$^{+/+}$ and *Selenop*$^{-/-}$ colon, small intestine, and liver for *Selenop* or *Ppib* (negative control). Representative 20x images, scale bars = 100 µm.

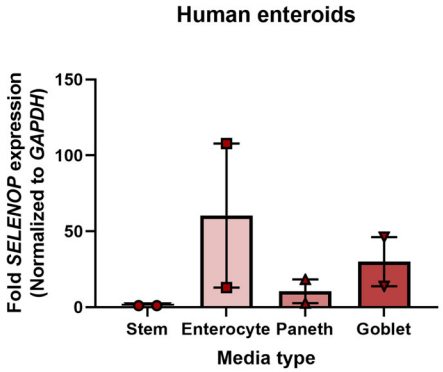


Figure S4. *SELENOP* expression in differentiated human enteroids. RT-qPCR for *SELENOP* of human enteroids subjected to directed differentiation protocols. Pooled data from n=2 independent experiments. Data are displayed as mean ± SEM.

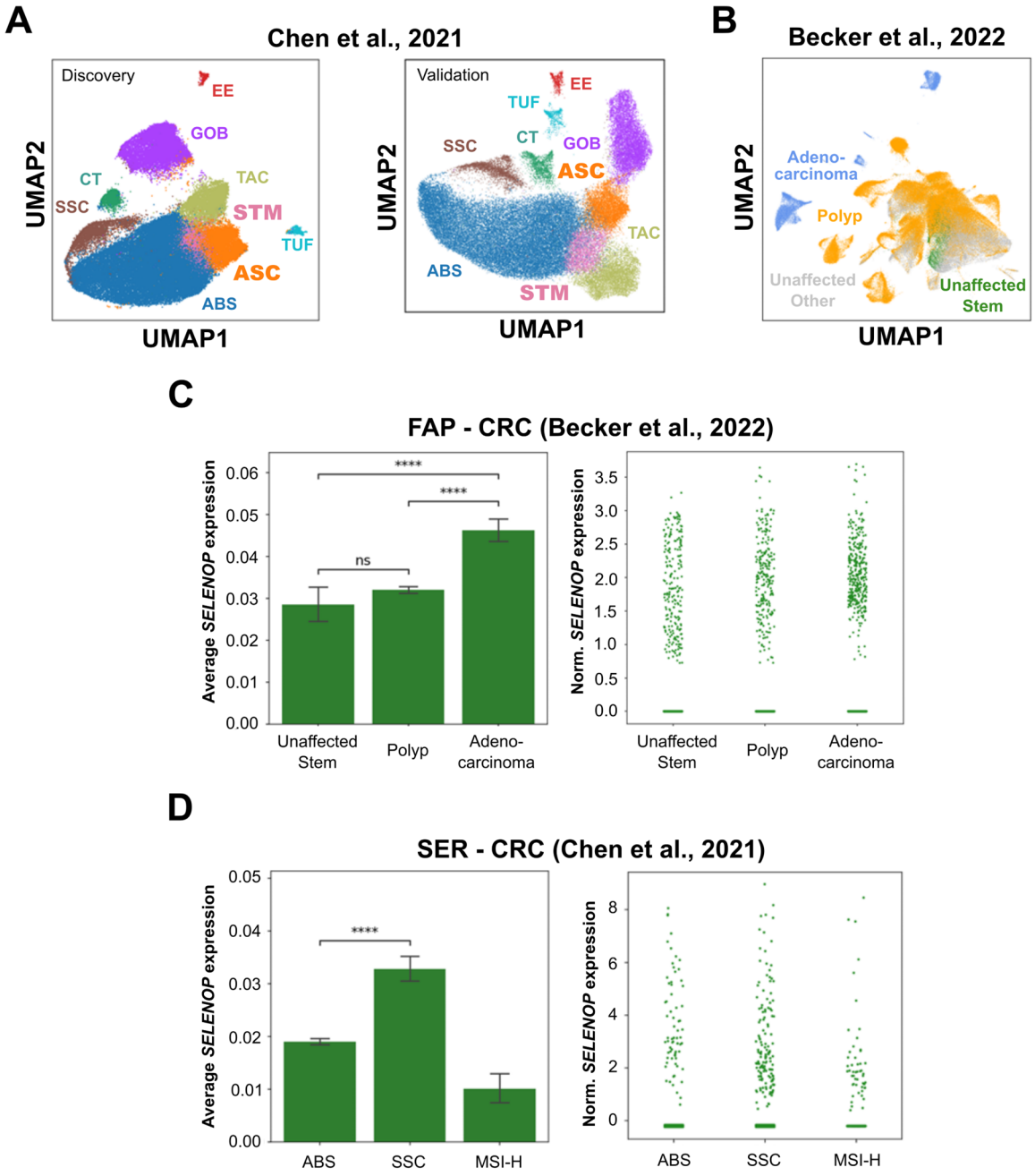


Figure S5. *SELENOP* expression throughout colorectal cancer progression. (A) scRNA-seq data from human colorectal polyps and normal colon tissue. (Left) Discovery cohort: n=35 normal samples, n=27 polyps, n=70,691 cells. (Right) Validation cohort: n=31 normal samples, n=28 polyps, n=71,374 cells. ABS: absorptive, ASC: adenoma-specific cells, CT: crypt top, EE: enteroendocrine, GOB: goblet, STM: stem, SSC: serrated-specific cells, TAC: transit amplifying cells, TUF: tuft. (B, C) snRNA-seq data from human colorectal polyps/cancers and normal colon tissue. n=23 normal samples, n=42 polyps, n=5 cancers, n=161,809 cells. (C) *SELENOP* expression by cell type. CRC: colorectal cancer, FAP: familial adenomatous polyposis. (D) scRNA-seq data from human colorectal polyps/cancers and normal colon tissue. *SELENOP* expression by cell type. ABS: absorptive, MSI-H: microsatellite instability-high, SER: serrated polyp, SSC: serrated-specific cells. n=21 normal samples, n=19 polyps, n=2 cancers. Kruskal-Wallis tests with 2-sided Mann-Whitney tests. ****p<0.0001. Data are displayed as mean ± SD.

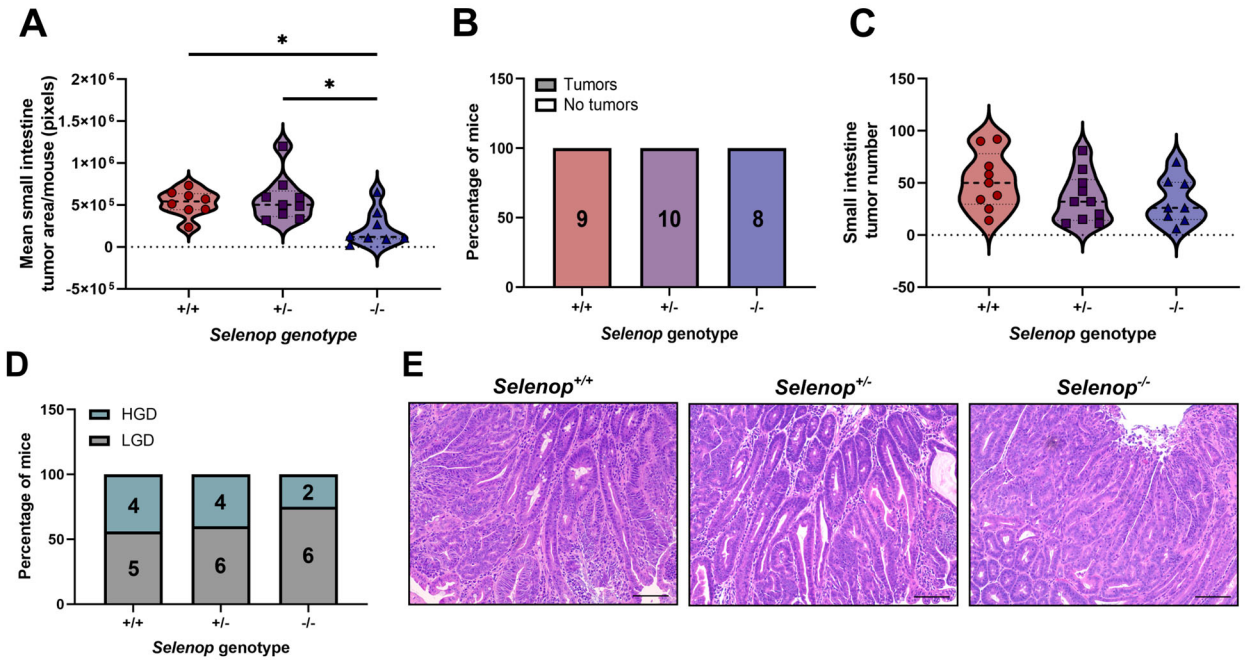


Figure S6. *Selenop* KO decreases small intestine tumor size in *Apc*-dependent tumorigenesis. (A) Small intestine tumor area, (B) small intestine tumor incidence, (C) small intestine tumor number, (D) small intestine tumor dysplasia scores (HGD: high-grade dysplasia, LGD: low-grade dysplasia), and (E) small intestine tumor histology of $Apc^{\Delta IE/+}$; $Selenop^{+/+}$ (n=9), $Selenop^{+/-}$ (n=10), and $Selenop^{-/-}$ (n=8) mice. Pooled data from n=2 independent experiments. Representative 20x images (E), scale bars = 100 μm. Kruskal-Wallis tests (A, C), Freeman-Halton tests (B, D). *p<0.05.

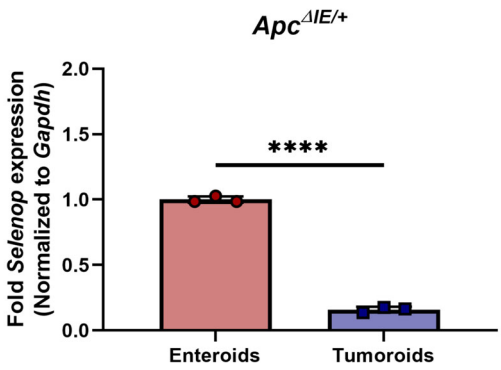


Figure S7. *Selenop* expression is reduced in tumoroids. RT-qPCR for *Selenop* of *Apc$^{\Delta IE/+}$; Selenop$^{+/+}$* enteroids and tumoroids. Pooled data from n=3 mice. 2-sided unpaired t test. ****p<0.0001. Data are displayed as mean ± SD.

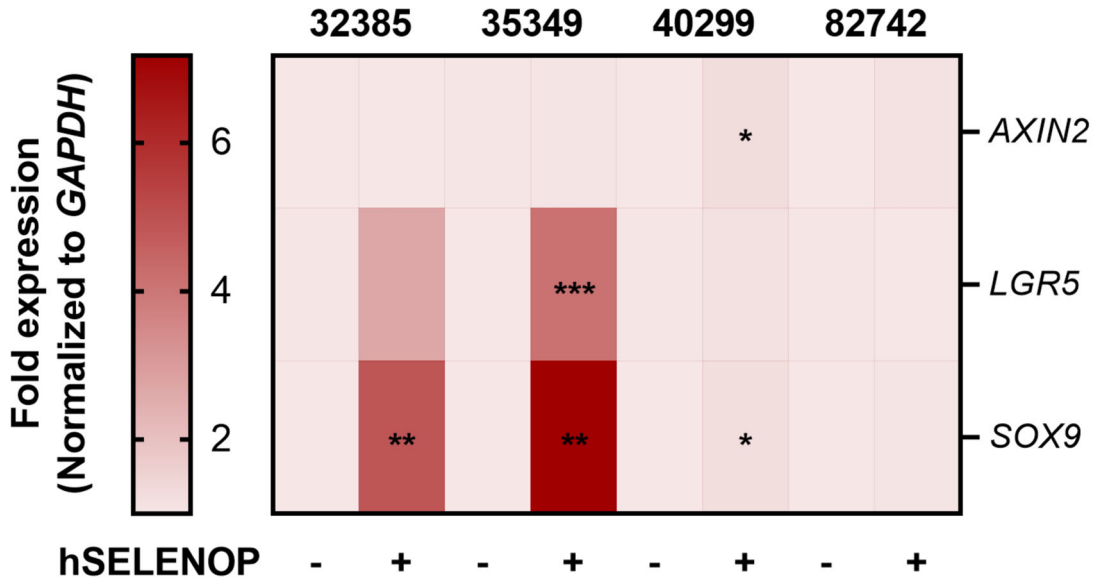


Figure S8. SELENOP increases WNT target gene expression in human tumoroids. RT-qPCR for *AXIN2*, *LGR5*, and *SOX9* of human tumoroids. Each five-digit number represents tumoroids established from one patient. Pooled data from n=3 independent experiments. 2-sided paired t tests. *p<0.05, **p<0.01, ***p<0.001. Data are displayed as mean.

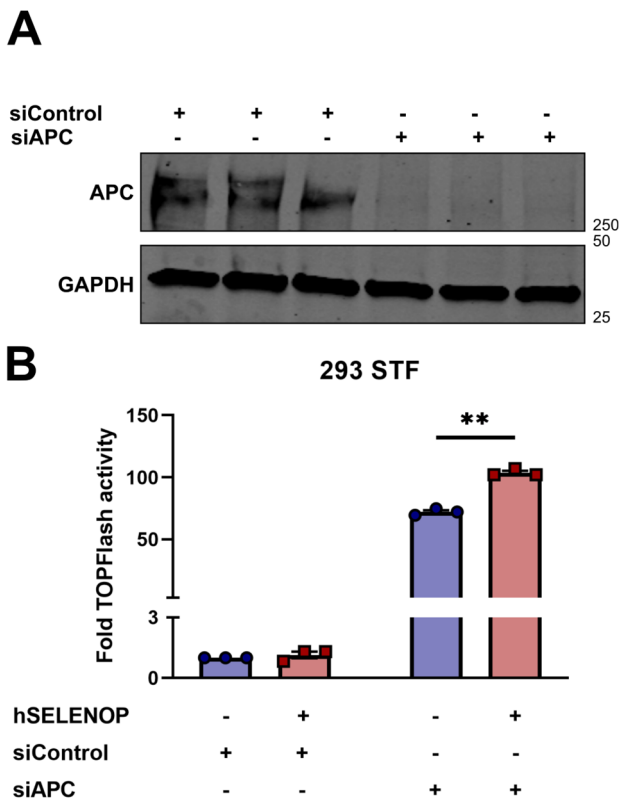


Figure S9. SELENOP acts upstream of APC. (A) Western blot for APC and GAPDH (loading control) of lysates from 293 STF cells transfected with siControl or siAPC. (B) TOPFlash activity of 293 STF cells transfected with siControl or siAPC and treated without or with hSELENOP. Pooled data from n=3 independent experiments. 2-way repeated measures ANOVA with 2-sided Sidak's multiple comparisons test. **p<0.01. Data are displayed as mean ± SEM.

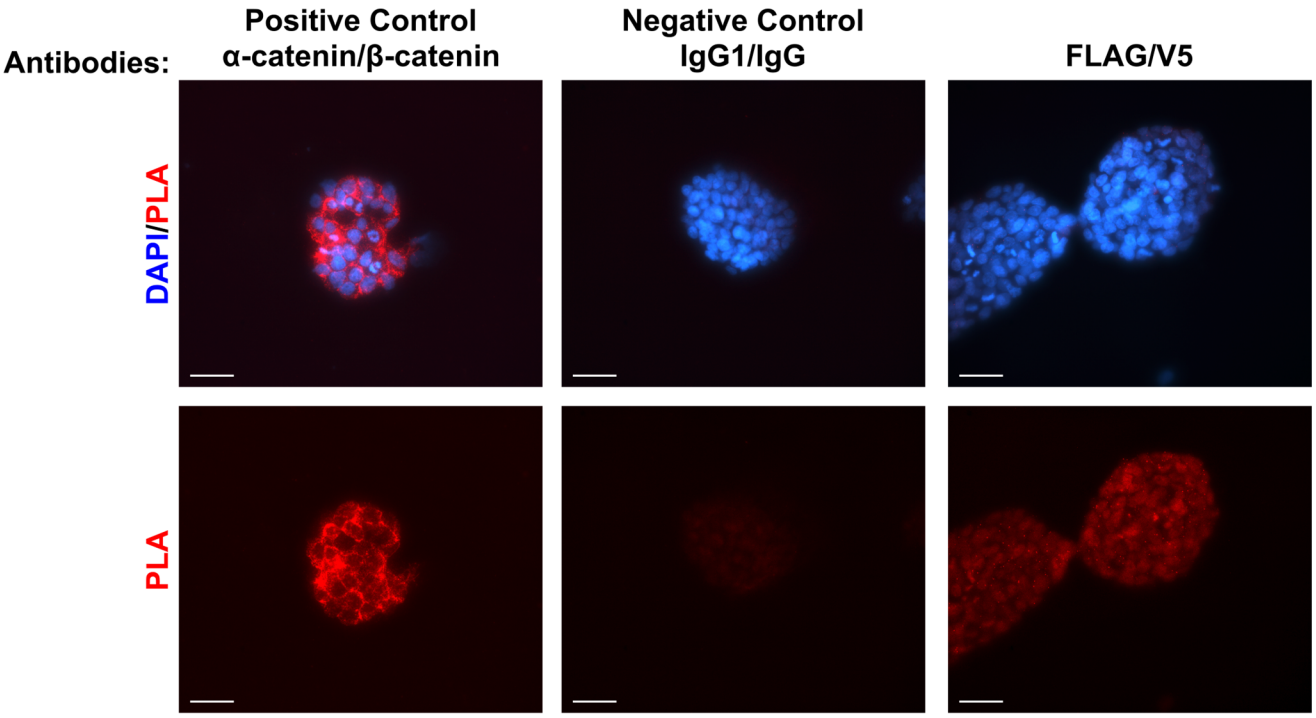


Figure S10. SELENOP interacts with LRP6. Proximity ligation assay of 293T cells co-transfected with FLAG-mLRP6 and V5-mSELENOP. Representative 40x images from n=3 independent experiments, scale bars = 50 μm.

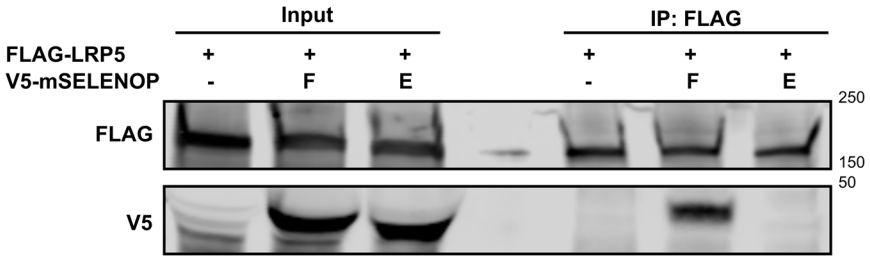
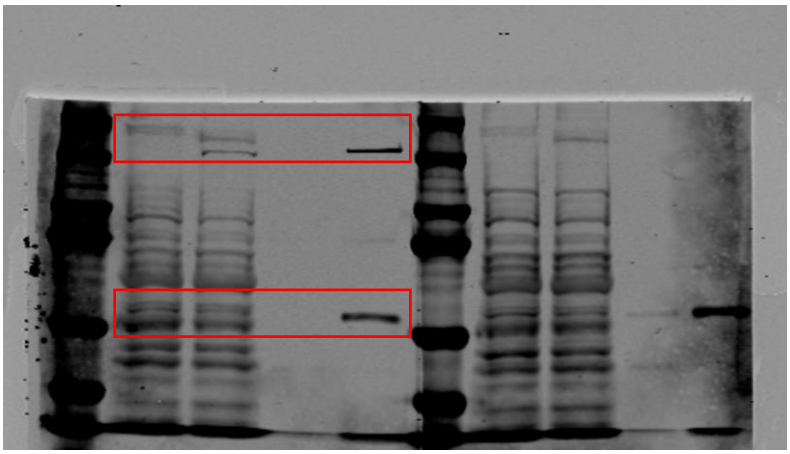


Figure S11. SELENOP$^{U258-U299}$ mediates the SELENOP:LRP5 interaction. Western blot for FLAG and V5 of FLAG IPs from 293T cells co-transfected with FLAG-mLRP5 and full-length (F) or LRP5/6-uncoupling (E) V5-mSELENOP. Representative data from n=2 independent experiments.

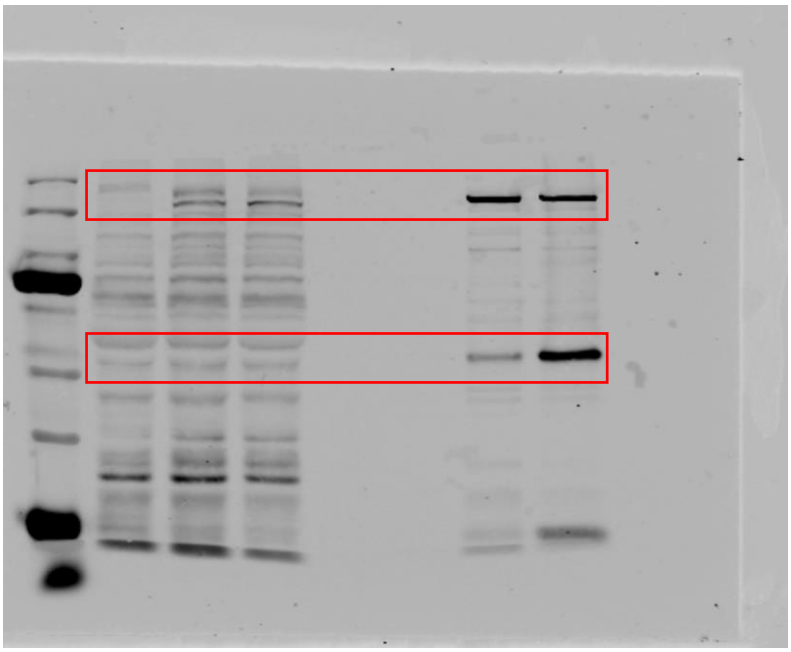
Full unedited blot for Figure 7A



Mouse anti-FLAG (F1804, Sigma-Aldrich)

Mouse anti-SELENOP (N11, VAPR)

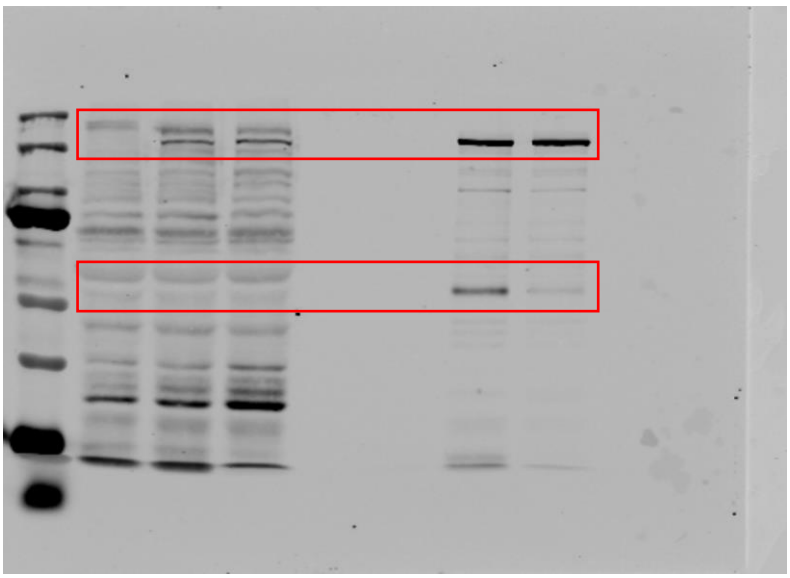
Full unedited blot for Figure 7B



Mouse anti-FLAG (F1804, Sigma-Aldrich)

Mouse anti-SELENOP (N11, VAPR)

Full unedited blot for Figure 7C



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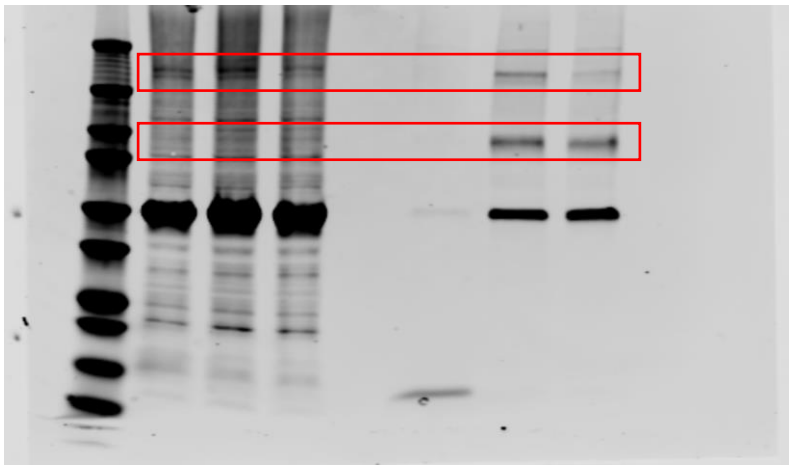
Mouse anti-SELENOP (N11, VAPR)

Full unedited blot for Figure 7D

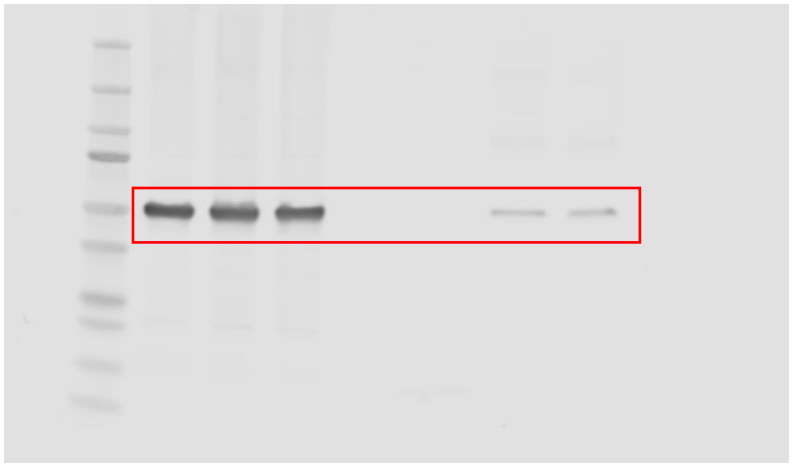


Rabbit anti-LRP6 (2560, Cell Signaling Technology)

Rabbit anti-Na$^+$/K$^+$-ATPase (3010, Cell Signaling Technology)

Rabbit anti-β-tubulin (2146, Cell Signaling Technology)

Same blot, different contrast/brightness settings on Odyssey

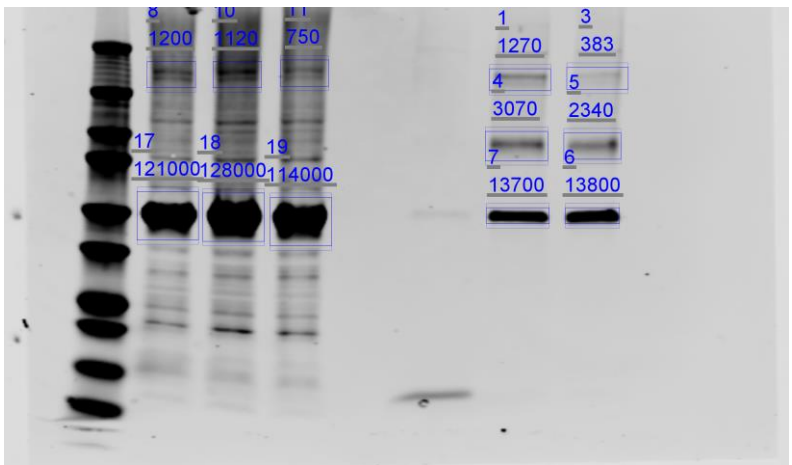


Rabbit anti-LRP6 (2560, Cell Signaling Technology)

Rabbit anti-Na$^+$/K$^+$-ATPase (3010, Cell Signaling Technology)

Rabbit anti-β-tubulin (2146, Cell Signaling Technology)

Same blot, quantified on Odyssey

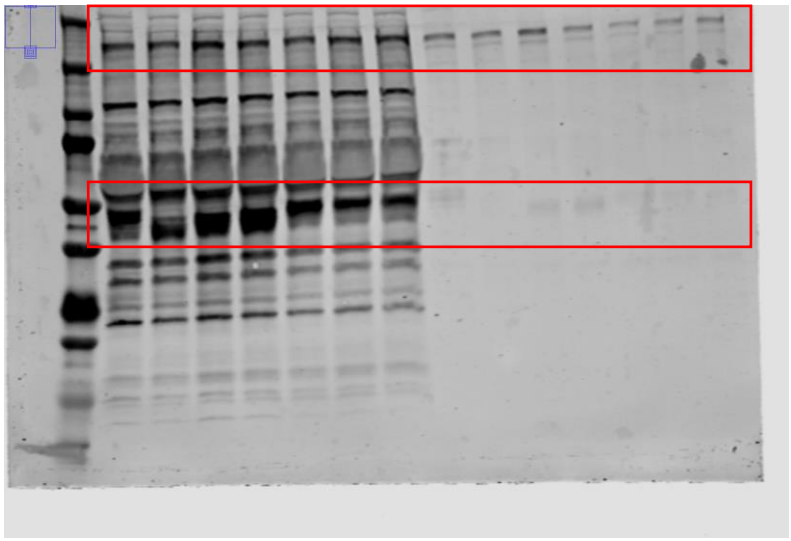


Rabbit anti-LRP6 (2560, Cell Signaling Technology)

Rabbit anti-Na$^+$/K$^+$-ATPase (3010, Cell Signaling Technology)

Rabbit anti-β-tubulin (2146, Cell Signaling Technology)

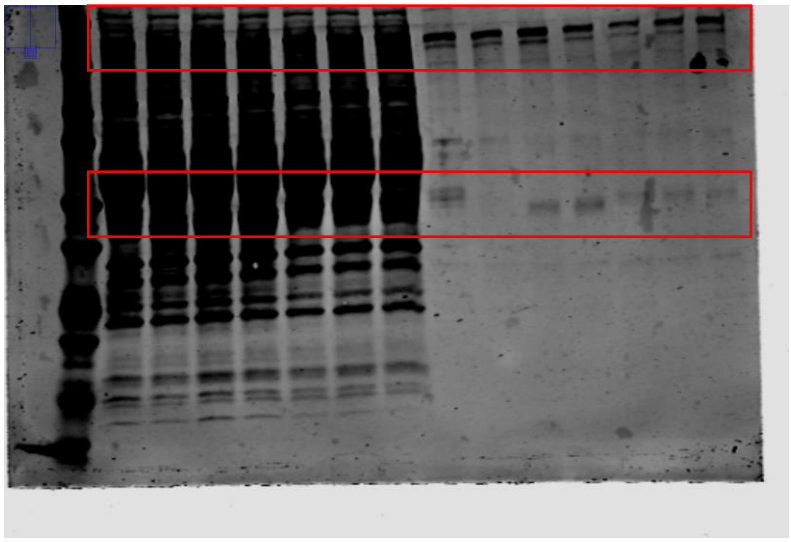
Full unedited blot for Figure 8B



Rabbit anti-LRP6 (3395, Cell Signaling Technology)

Rabbit anti-SELENOP (Proteintech Group)

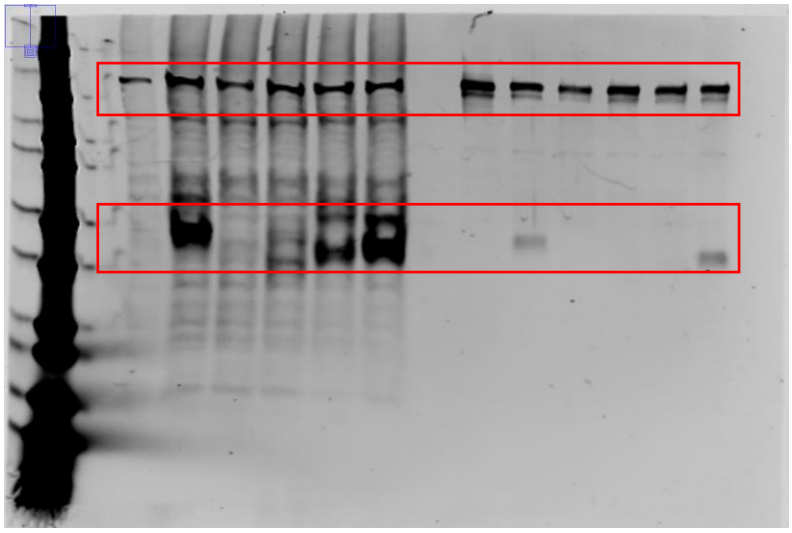
Same blot, different contrast/brightness settings on Odyssey



Rabbit anti-LRP6 (3395, Cell Signaling Technology)

Rabbit anti-SELENOP (Proteintech Group)

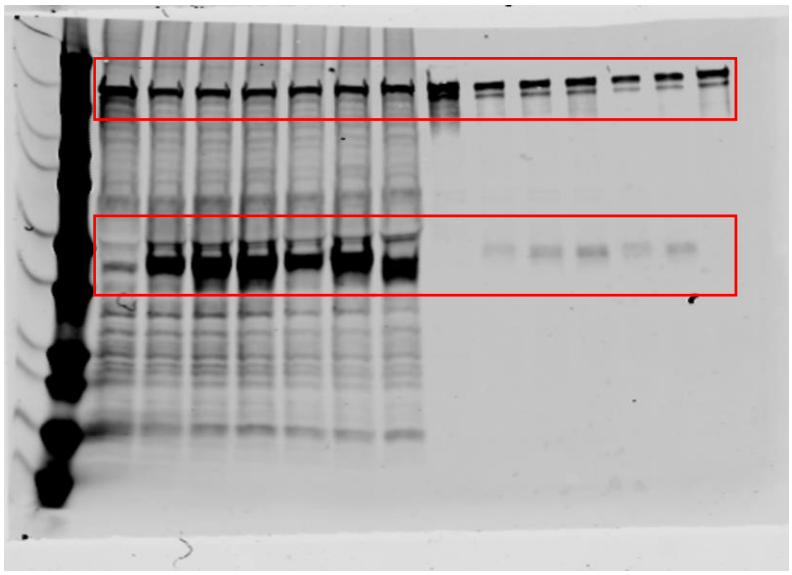
Full unedited blot for Figure 8D



Rabbit anti-LRP6 (3395, Cell Signaling Technology)

Rabbit anti-V5 (13202, Cell Signaling Technology)

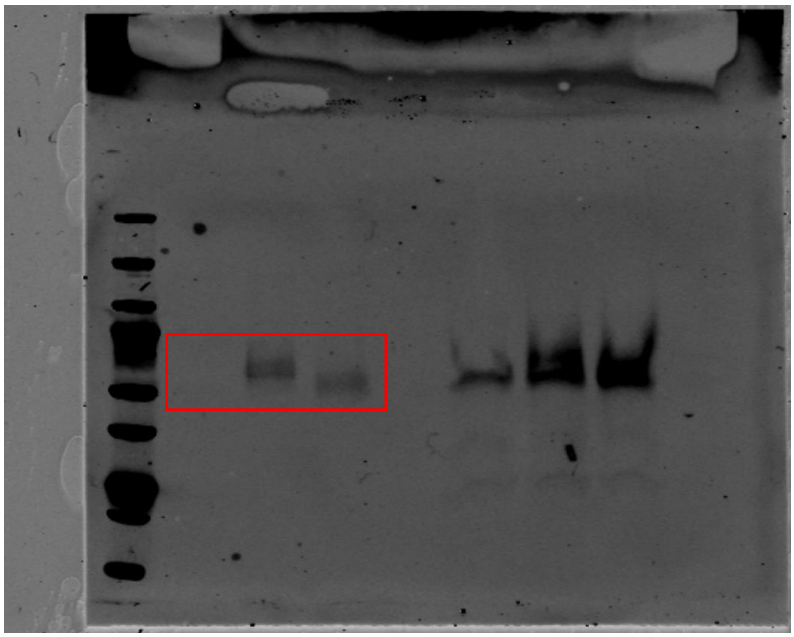
Full unedited blot for Figure 9B



Rabbit anti-LRP6 (3395, Cell Signaling Technology)

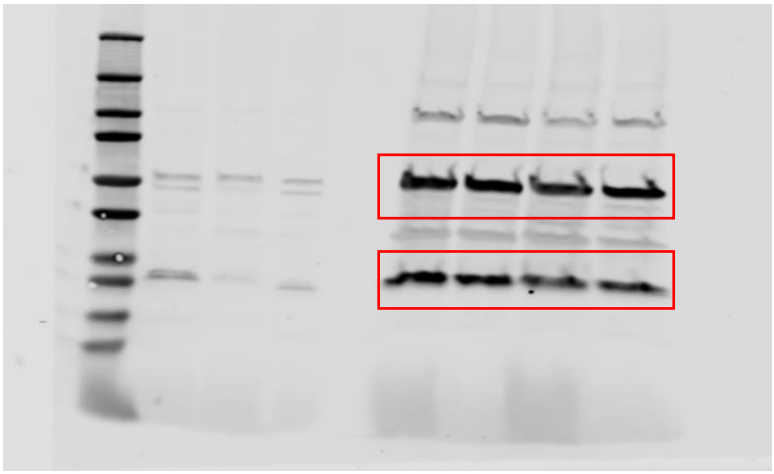
Rabbit anti-V5 (13202, Cell Signaling Technology)

Full unedited blot for Figure 9C



Mouse anti-V5 (ab27671, abcam)

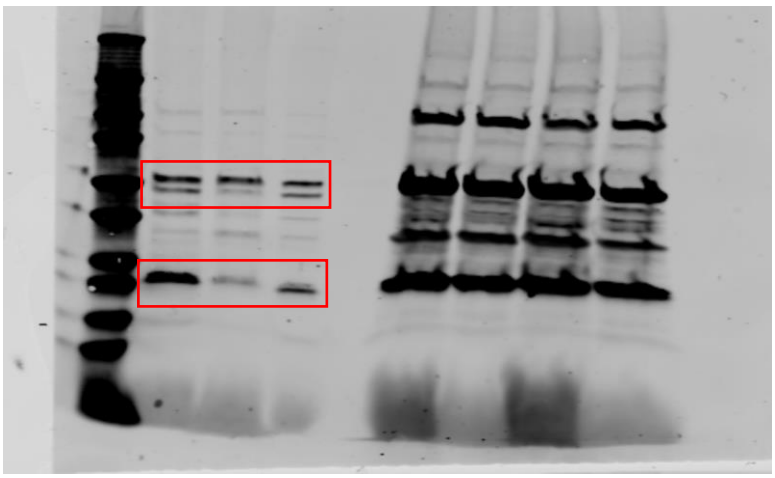
Full unedited blot for Figure S1A



Rabbit anti-β-tubulin (2146, Cell Signaling Technology)

Rabbit anti-GPX1 (SAB2700534, Sigma-Aldrich)

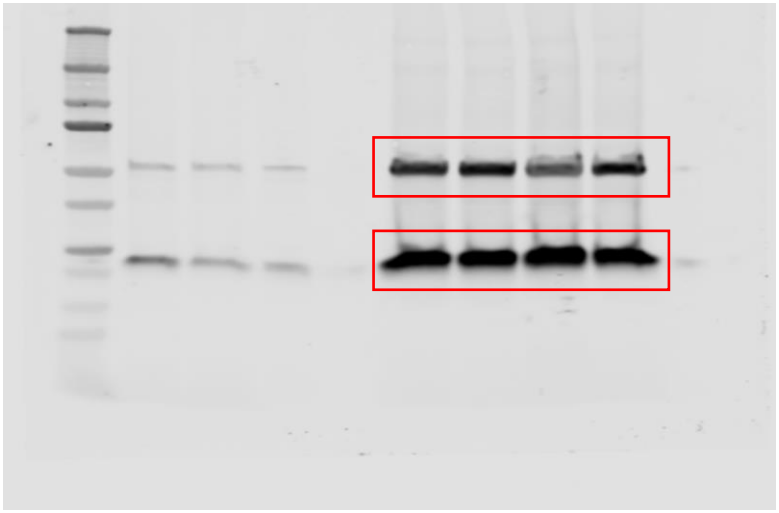
Same blot, different contrast/brightness settings on Odyssey



Rabbit anti-β-tubulin (2146, Cell Signaling Technology)

Rabbit anti-GPX1 (SAB2700534, Sigma-Aldrich)

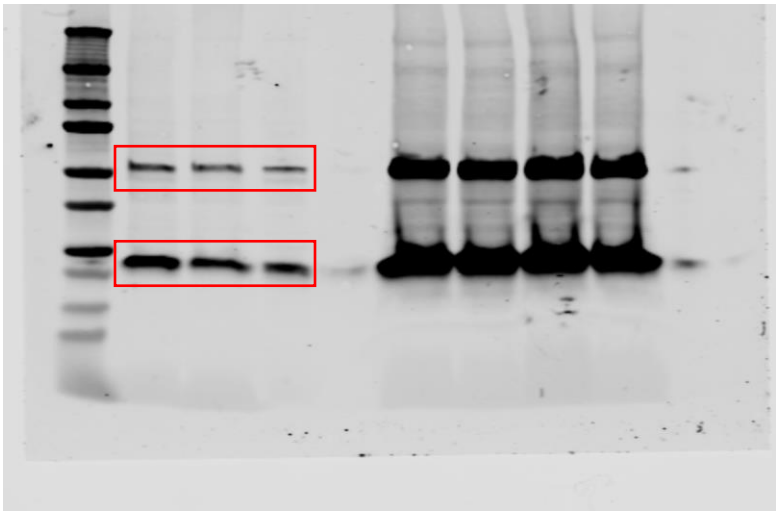
Full unedited blot for Figure S1B



Rabbit anti-β-tubulin (2146, Cell Signaling Technology)

Rabbit anti-GPX2 (ab137431, abcam)

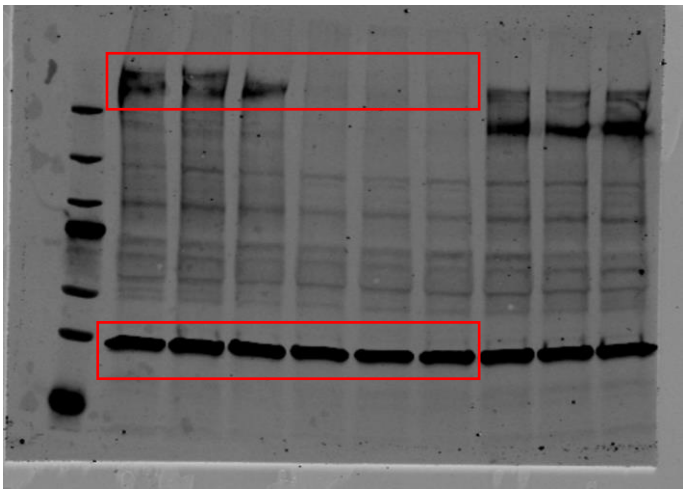
Same blot, different contrast/brightness settings on Odyssey



Rabbit anti-β-tubulin (2146, Cell Signaling Technology)

Rabbit anti-GPX2 (ab137431, abcam)

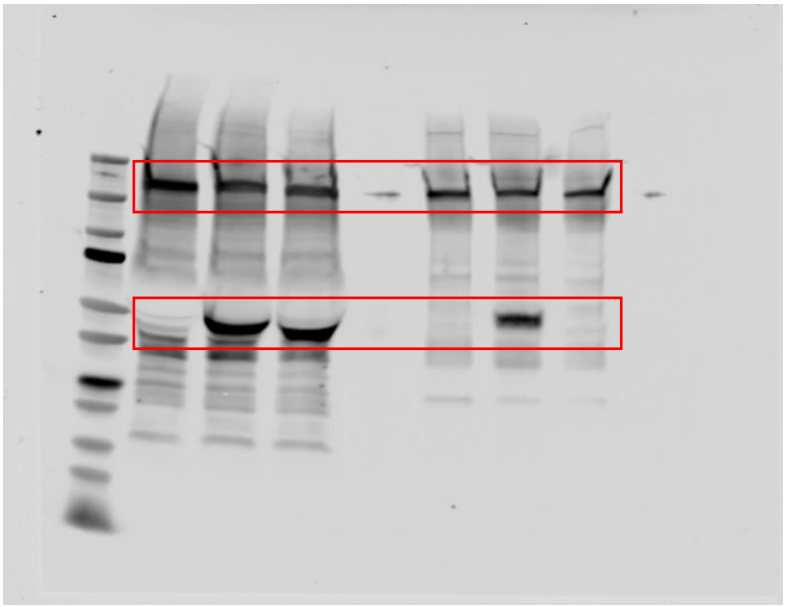
Full unedited blot for Figure S9A



Rabbit anti-APC (sc-7930, Santa Cruz Biotechnology)

Rabbit anti-GAPDH (5174, Cell Signaling Technology)

Full unedited blot for Figure S11



Mouse anti-FLAG (F1804, Sigma-Aldrich)

Rabbit anti-V5 (13202, Cell Signaling Technology)