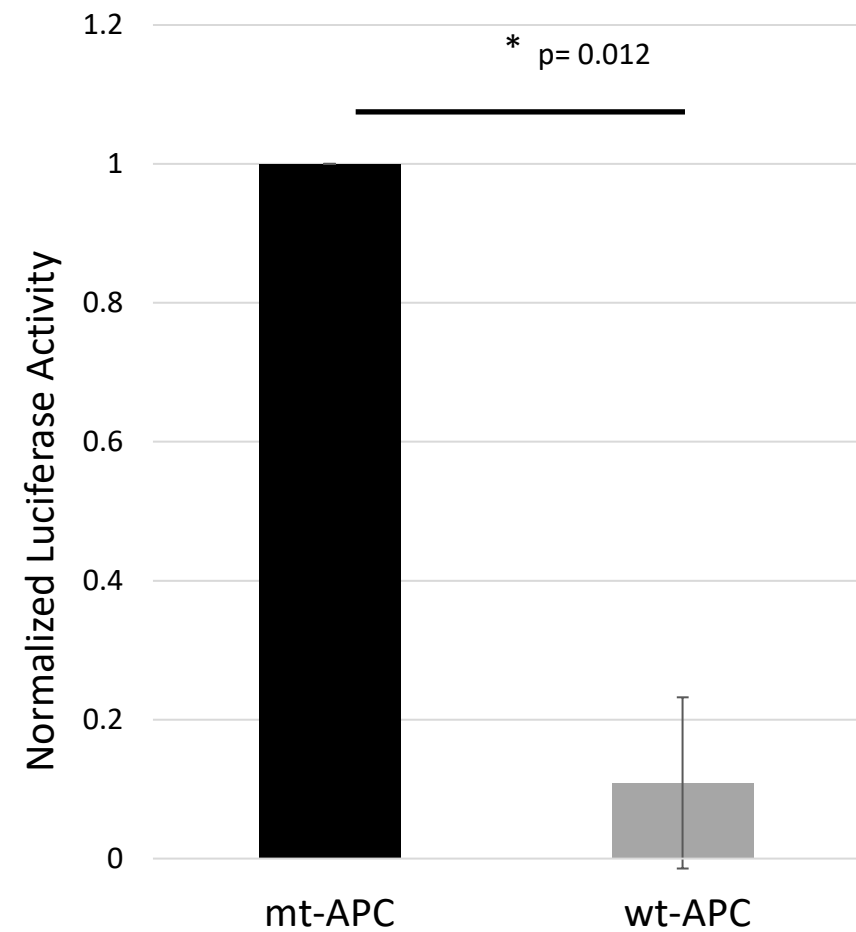


Supplementary Material

Figure S1

A.



B.

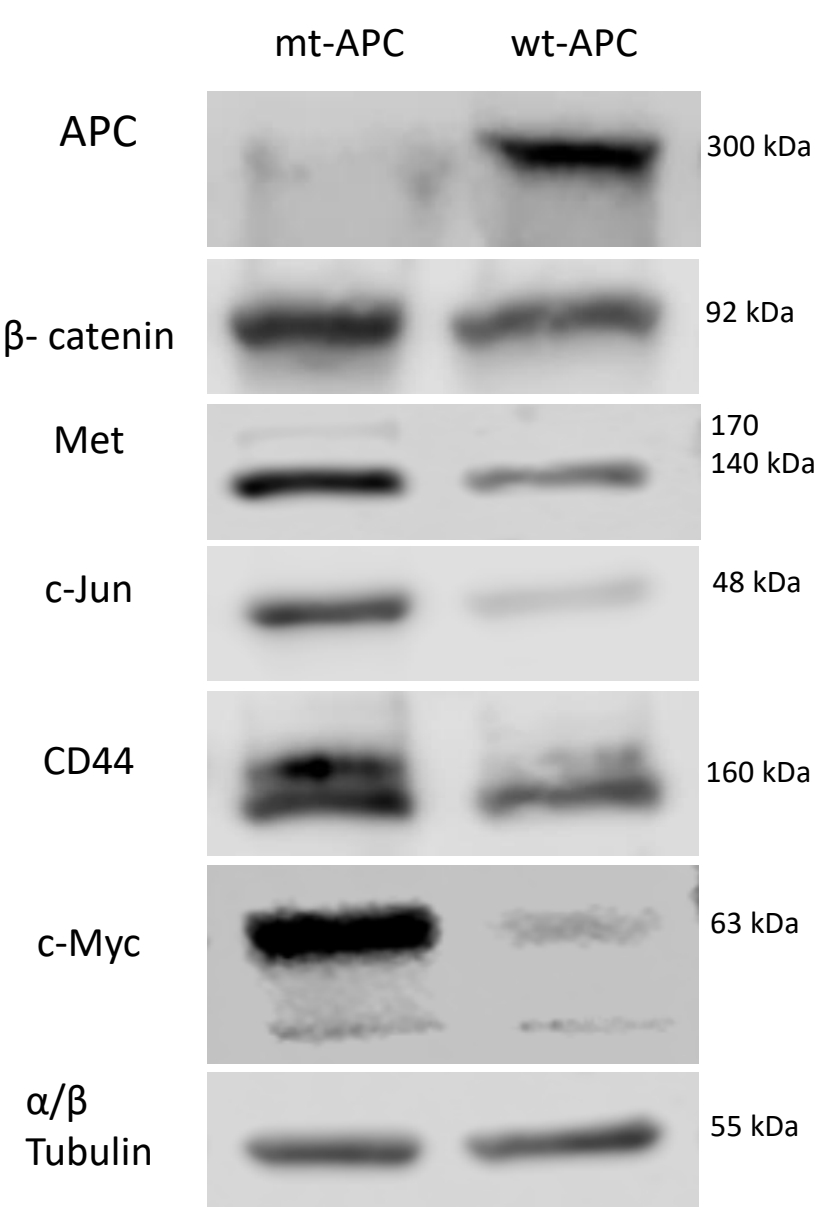
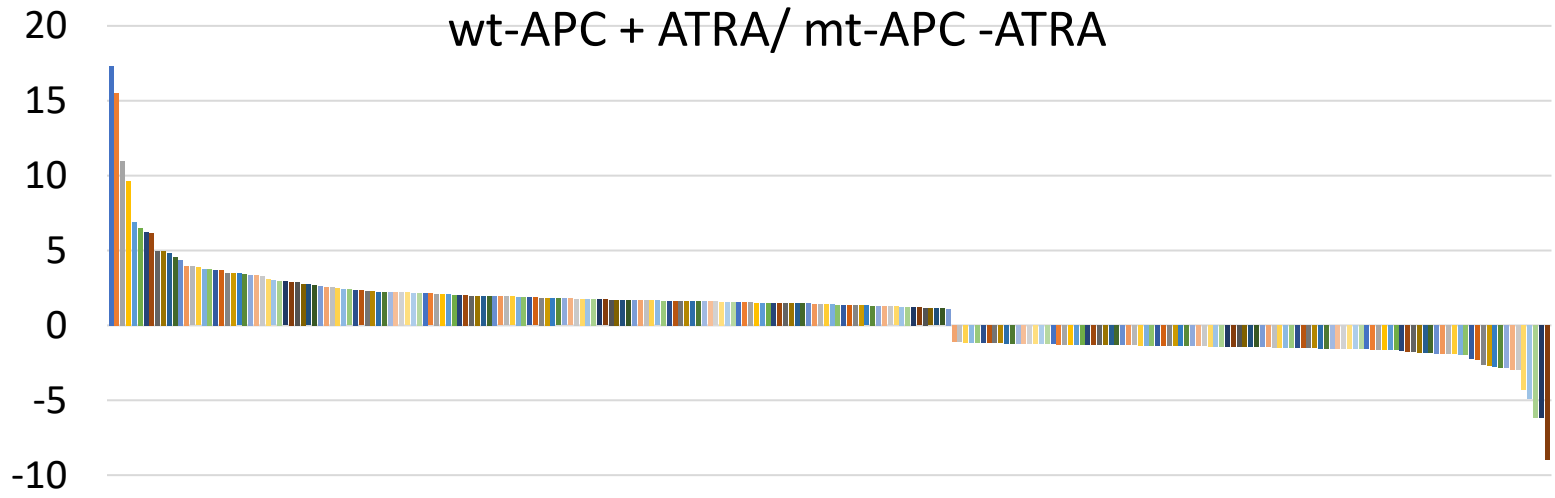


Figure S1. WNT/ β -catenin activity is reduced with expression of *wt-APC*. WNT/ β -catenin activity was measured using TCF/LEF reporter and luciferase assays. Cells were transfected with either positive or negative reporter and treated with or without zinc for 24hrs, followed by luciferase assay. **(A)** Luciferase activity was measured, and results normalized to *mt-APC*. There is a 90% decrease in WNT/ β -catenin activity when expression of *wt-APC* is induced. Student's t-test determined statistical significance as indicated. **(B)** Western blots were performed to confirm *wt-APC* expression and to assess protein expression of known WNT/TCF target proteins. Full-length, 300 kDa, APC is shown and decreased protein expression of β -catenin, MET, c-JUN, CD44, and c-MYC, are also shown.

Figure S2

A.

Fold-change of differentially expressed genes:
wt-APC + ATRA/ mt-APC -ATRA



APC	CYP26A1	DUSP5	FGF19	CDKN1A	GADD45G	KRT20	HSPA1A	IL6R	IL15
CLCF1	PLA2G4C	DUSP10	TMPRSS2	CDKN2B	LAMC2	TNFAIP3	SMAD3	PIM1	ALDH1A3
CYLD	KLF4	MAP3K14	SOCS2	PIK3CG	CSF2	BIRC3	LIF	DLL4	IL23A
ALCAM	IL1A	POLD4	PGF	PRDM1	NR4A3	SOCS1	IL11	DUSP2	CASP7
LAMB3	BAIAP3	SFN	LIG4	PLAU	WNT7B	CIC	CDH1	CALML5	KDM5C
NOS3	EPHA2	NFKBIA	CAPN2	CASP9	WNT2B	DUSP8	CDK4	STAT3	ITGA3
MMP9	ACVR1C	HSPB1	HES5	IKBK	RARA	CXXC4	SMAD4	SPP1	TNFRSF10B
IRAK2	KMT2C	PAX8	JAG1	ALDH3B1	JAK1	DKK2	SPP1	HPGD	DTX4
JAK2	ITGA2	EFNA1	KAT2B	RELA	MLLT4	HMGA1	HPGD	MAP2K1	PML
BIRC7	GNG12	CNTFR	DLL3	SGK2	KDM6A	CCND1	MAP2K1	STAT3	BDNF
CBL	ETV7	CCND3	EYA1	PLA2G2A	CASP8	PLA2G2A	STAT3	WIF1	CDC6
FLT3	MUC2	B2M	SYP	CDKN3	EP300	LEP	CDK6	ERCC3	RRAS2
IL3RA	FZD7	MED12	IDH2	COG7	CASP8	VPS33B	RAD51	RHOA	GRB2
FBXW7	MTMR14	TMUB2	BAP1	PIAS1	RAD52	PIAS1	HDAC2	VHL	GNAQ
SLC4A1AP	RAC1	ATR	BRCA1	HDAC1	MCM5	BRCA2	MCM2	PHF6	RAD50
PRKAR2A	H3F3A	ERCC2	PIK3R2	FANCC	MECOM	DNMT3A	MCM2	ERCC3	MAP3K1
HOXA9	PBRM1	STMN1	TFDP1	MGMT	MRPS5	RFC4	FANCF	ALDH7A1	BRIP1
SMC3	CDK2	MAP3K5	WNT10A	BRCA2	ENDOG	RFC4	FANCF	ALDH7A1	MAP3K1
CHEK1	HDAC3	NBN	PBX3	FANCC	MECOM	RFC4	FANCF	ALDH7A1	MAP3K1
GTF2H3	DLGAP5	MAP3K5	WNT10A	FANCC	MECOM	RFC4	FANCF	ALDH7A1	MAP3K1
PPP3CA	HELLS	STMN1	TFDP1	MGMT	MRPS5	RFC4	FANCF	ALDH7A1	MAP3K1
ZIC2	JAG2	MCM4	CDK4	CCDC77	NTHL1	FANCL	LYZ	FAS	E2F5
POLE2	TGFBR2	TP53	HOXA10	IRS1	SH6	LYZ	SKP2	RNF43	KITLG
CDC7	PIK3R1	MSH2	HOXA10	IRS1	SH6	LYZ	SKP2	RNF43	KITLG
PLA2G4A	CD44	AXIN2	DKK1	ALDH1A1	BMP4	WNT11	LGR5		

B.

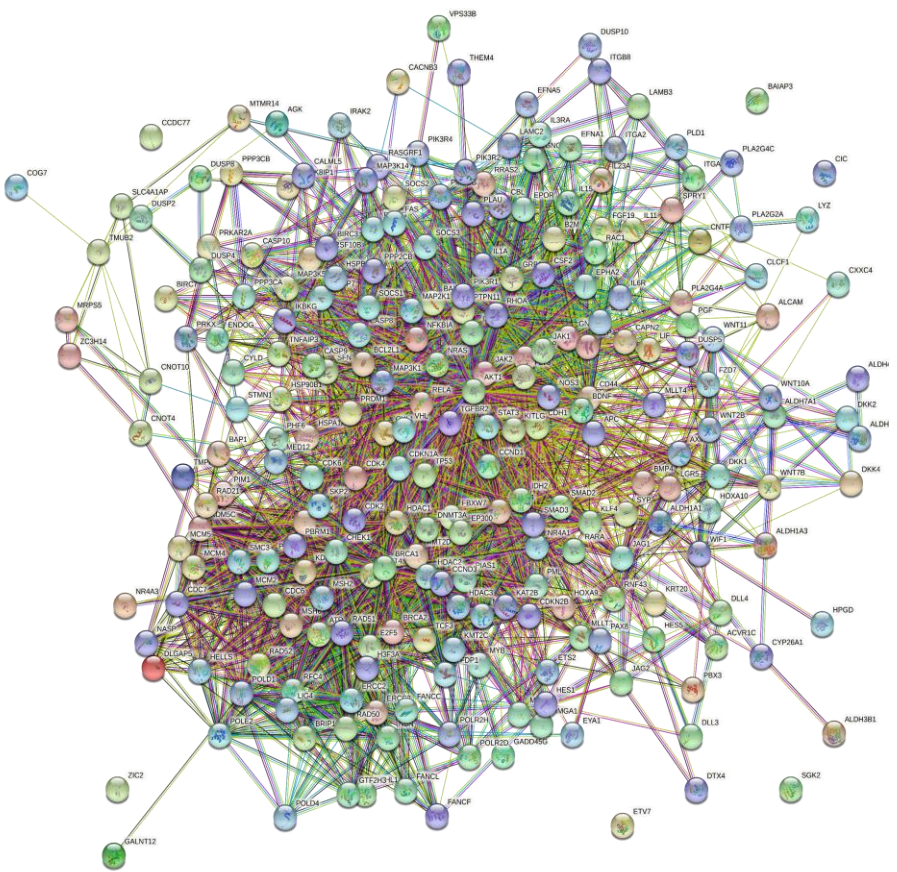


Figure S2. Inducing *wild-type APC* and treating with ATRA to drive RA signaling activates a highly regulated network of 248 proteins. HT29 cells were treated and RNA collected for NanoString profiling. Fold changes of RNA expression between treated and untreated cells were generated using nSolver software. **(A)** A list of 248- fold changes with significant *p*-values (<0.05) were selected and entered into the STRING online database. **(B)** A predicted protein network was generated, indicating a highly regulated system of protein interactions when *wild-type APC* is induced and RA signaling is activated.

Figure S3

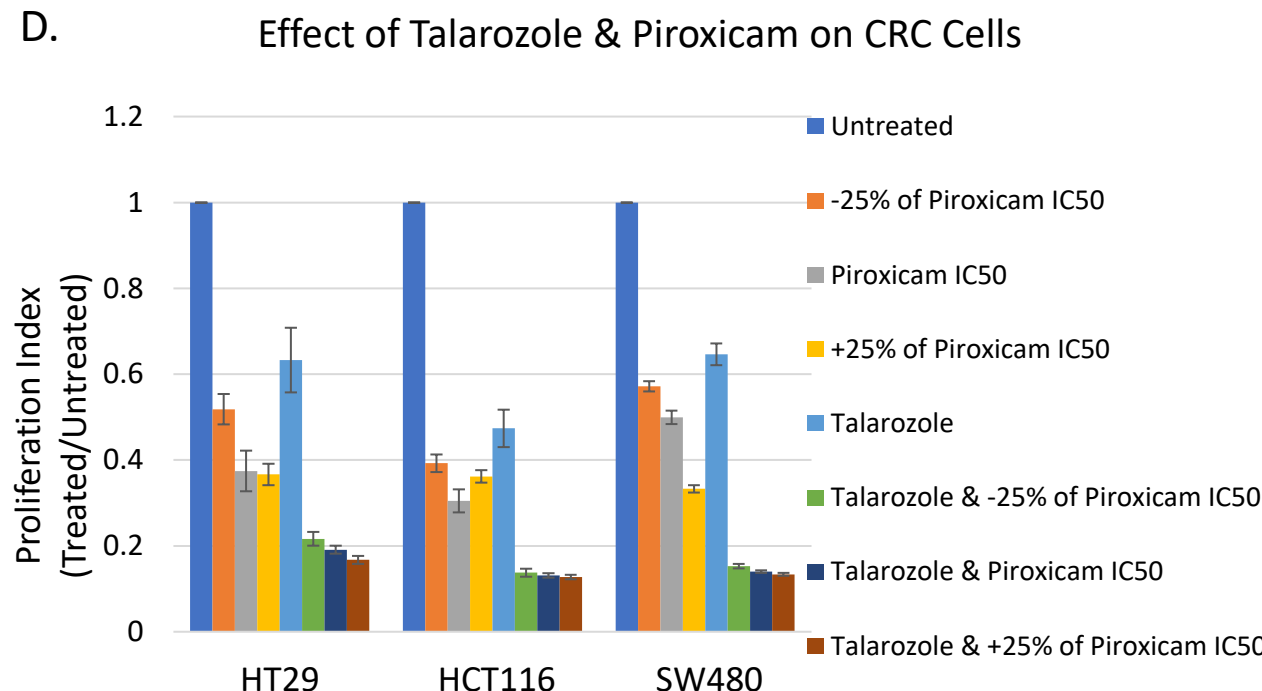
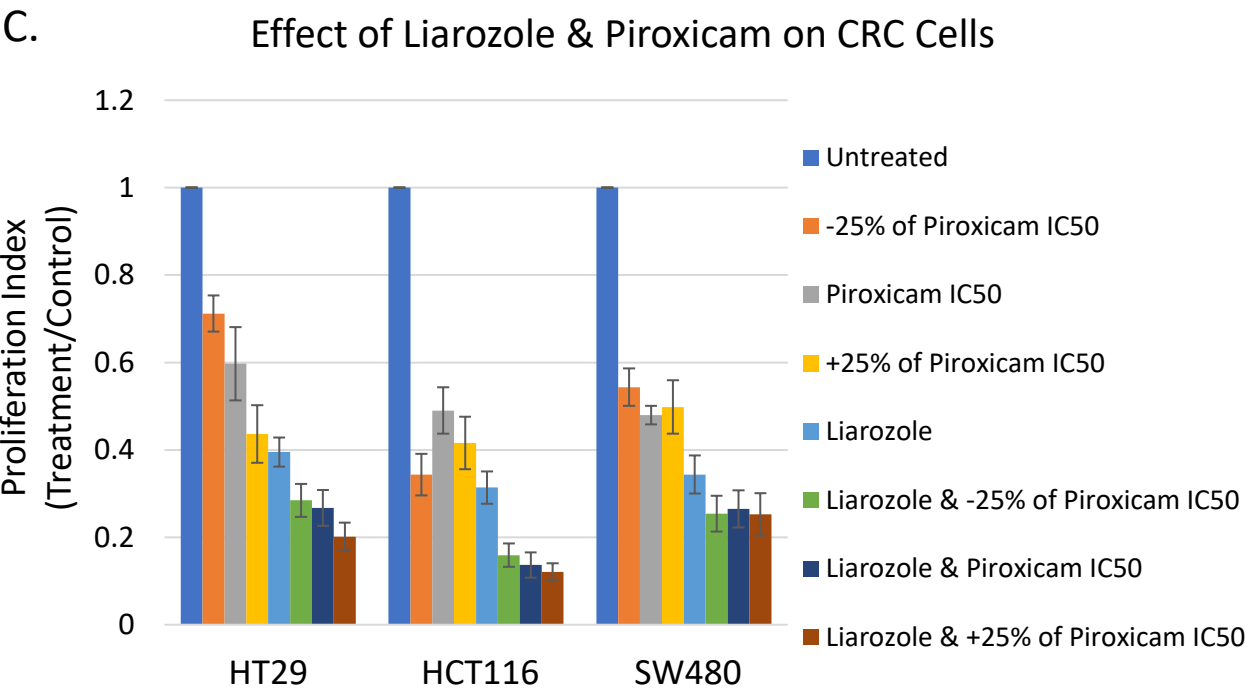
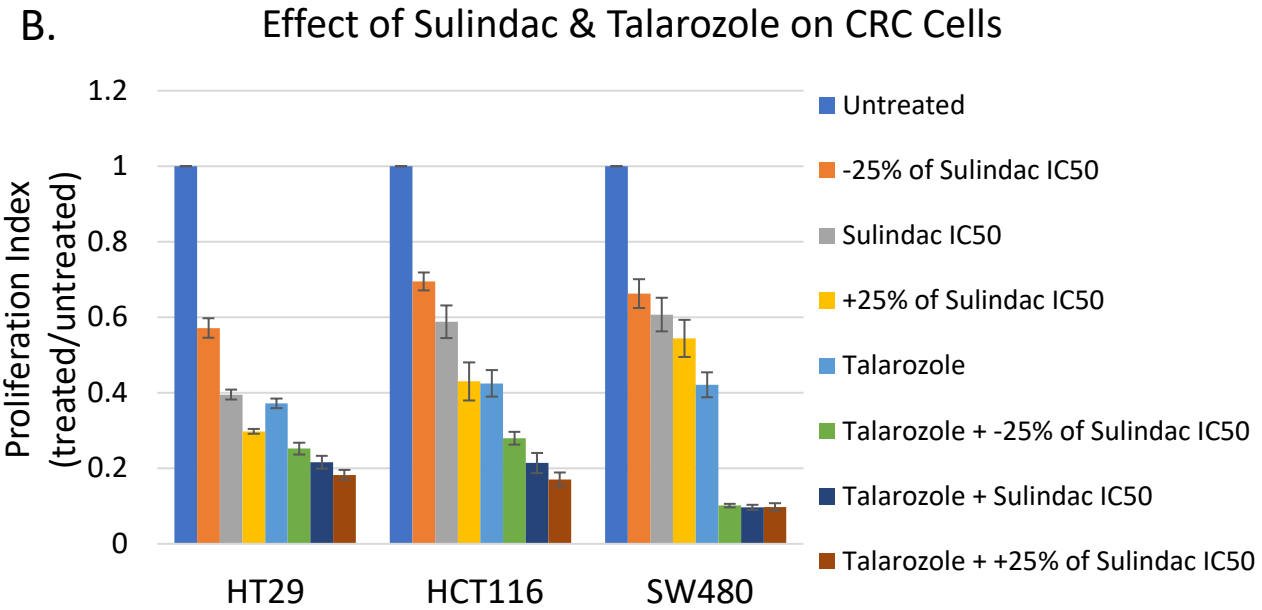
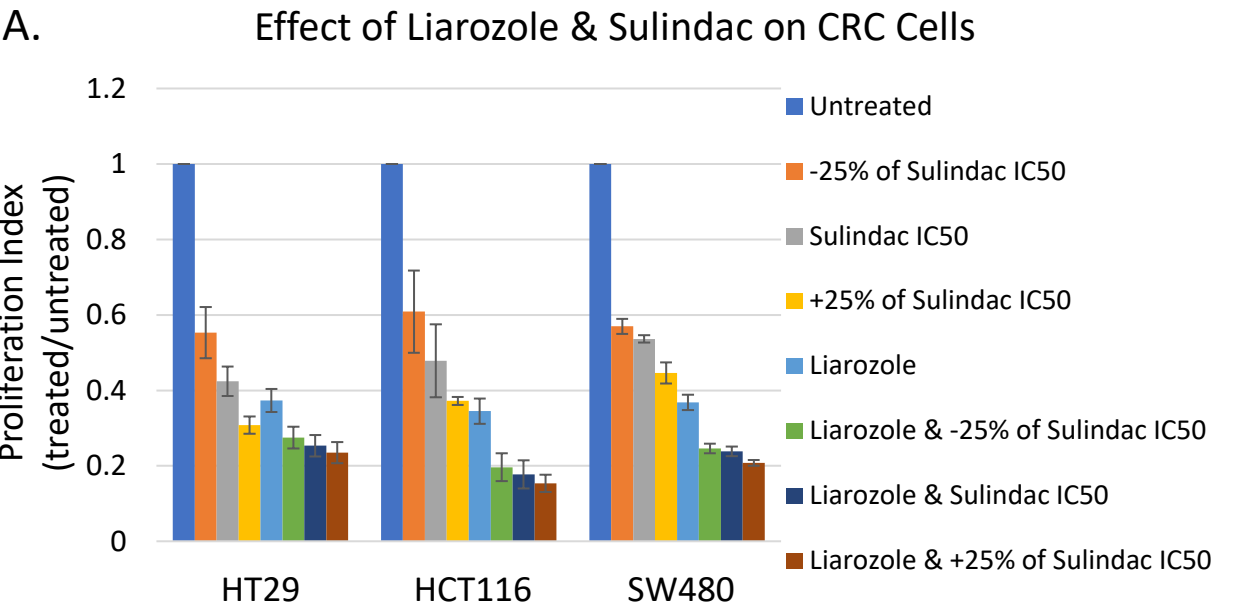
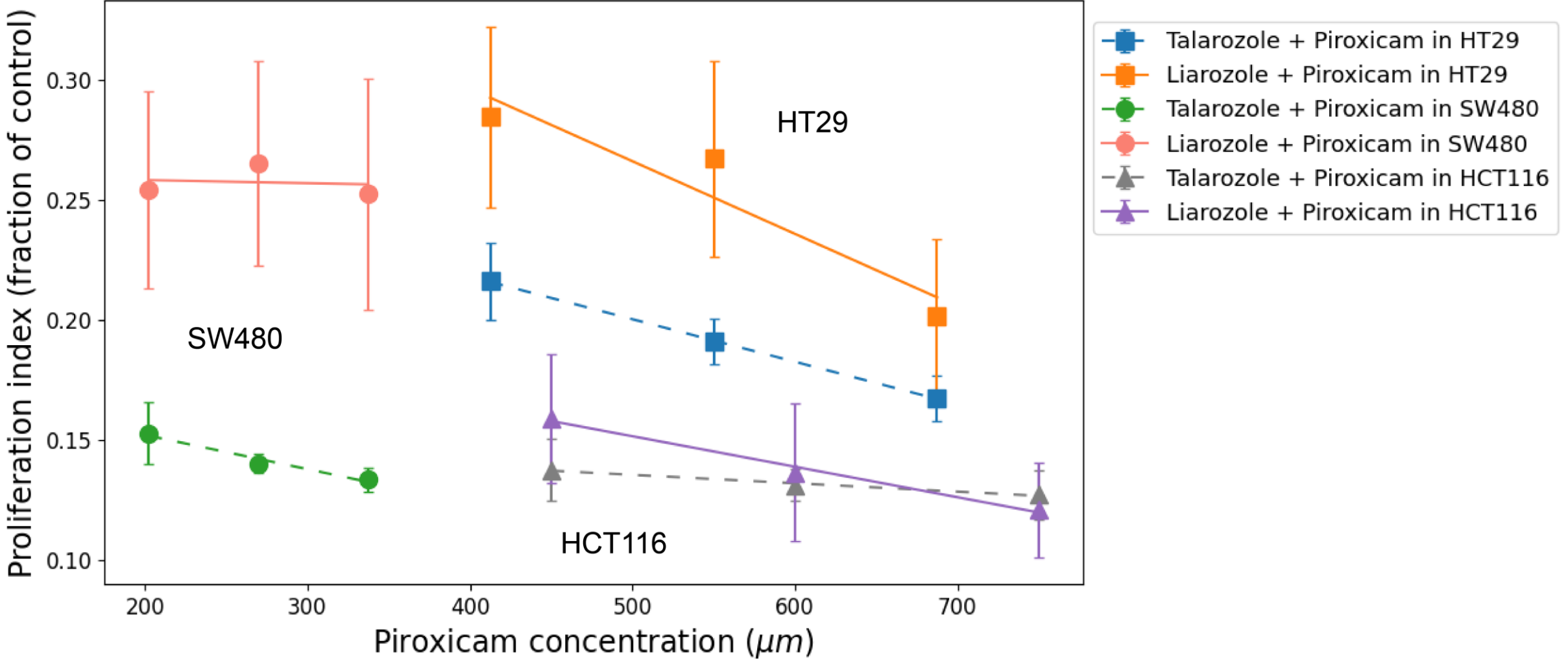


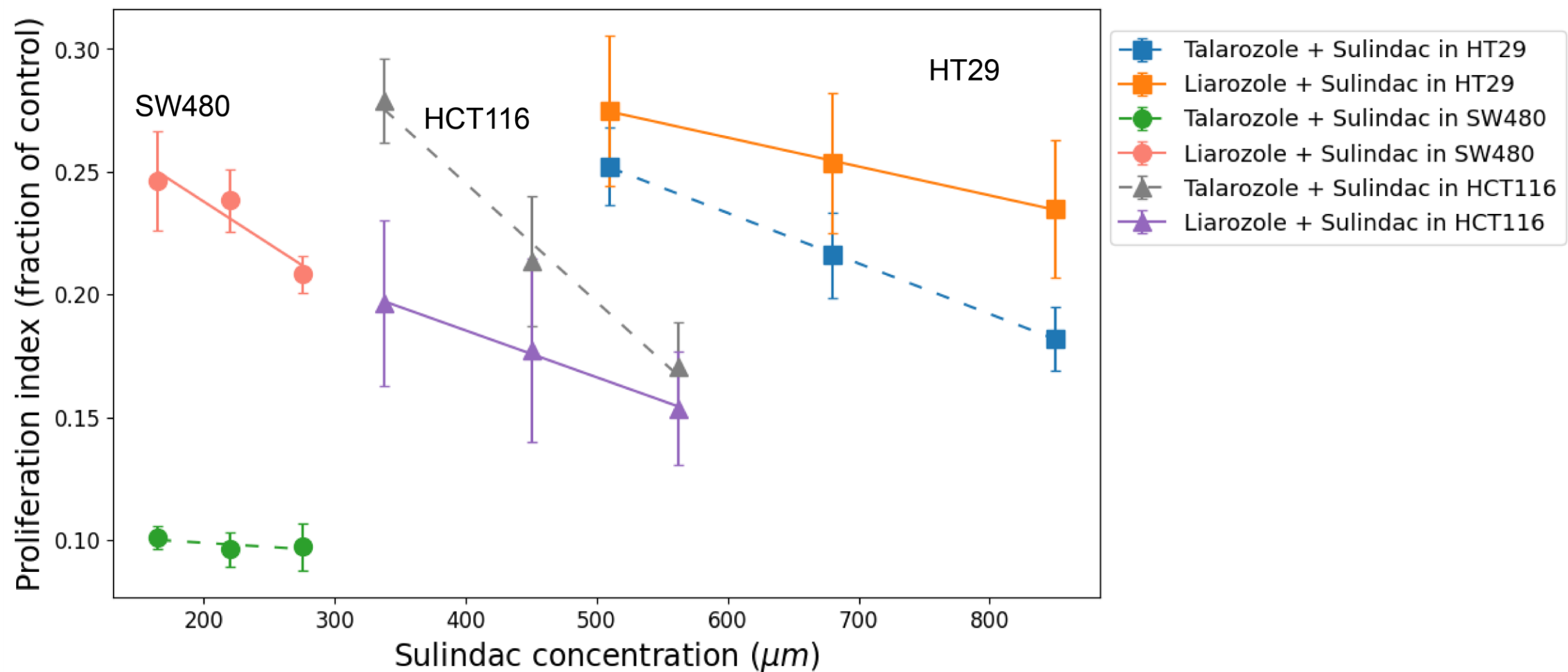
Figure S3. Extension of data showing effects of CYP26A1 and WNT inhibition on HT29, HCT116, and SW480 CRC cell lines. Cells were plated, serum-starved, and treated for 48 hrs and crystal violet assay performed, data analyzed, and plotted as shown. Cells were treated with IC50 concentrations of CYP26A1 inhibitors- Liarozole and Talarozole. WNT inhibitor concentrations are indicated as IC50 and plus (+) or minus (-) 25% of the IC50 value.

Figure S4- A



	HT29		SW480		HCT116	
	Slope ($\times 10^{-5}$)	r^2	Slope ($\times 10^{-5}$)	r^2	Slope ($\times 10^{-5}$)	r^2
Liarozole + Piroxicam	-30.23	0.8981	-1.32	0.0164	-12.69	0.9892
Talarozole + Piroxicam	-17.71	0.9997	-14.22	0.971	-3.489	0.9842

Figure S4- B



	HT29		SW480		HCT116	
	Slope ($\times 10^{-5}$)	r^2	Slope ($\times 10^{-5}$)	r^2	Slope ($\times 10^{-5}$)	r^2
Talarozole + Sulindac	-20.58	0.9996	-3.37	0.5468	-48.33	0.9842
Liarozole + Sulindac	-11.77	0.998	-34.54	0.8968	-19.07	0.997

Figure S4. Cell proliferation indexes and slopes of least-square regression plotted for combination treatments. (A) Slopes of combinations- Talarozole (22 to 30 μM) + Piroxicam and Liarozole (80 to 110 μM) + Piroxicam in HT29, SW480, and HCT116 cells. Piroxicam concentration was plotted along the X-axis and the proliferation index, generated via crystal violet staining, plotted on the Y-axis. Each mark represents a treatment combination with indicated drug concentrations and cell line ($n = 3$ replicates). Regression lines are fitted to the marks, and slopes and r^2 values indicated in table. (B) Slopes for combination treatments- Talarozole + Piroxicam and Liarozole + Piroxicam are plotted with Sulindac concentration along the X-axis and proliferation index along the Y-axis. Each mark represents a treatment combination with indicated drug concentrations and cell line ($n = 3$ replicates). Regression lines are fitted to the marks and slopes, and r^2 values are indicated in the table.

Figure S5

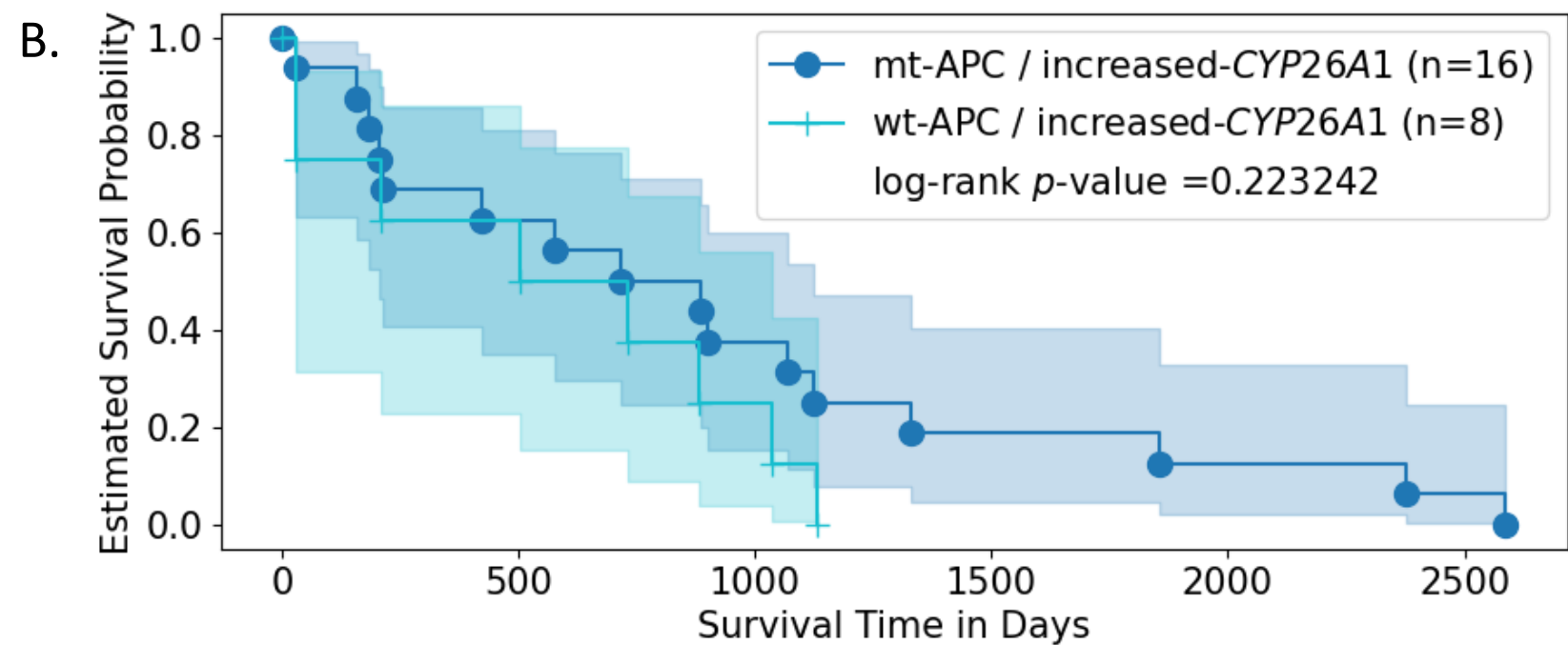
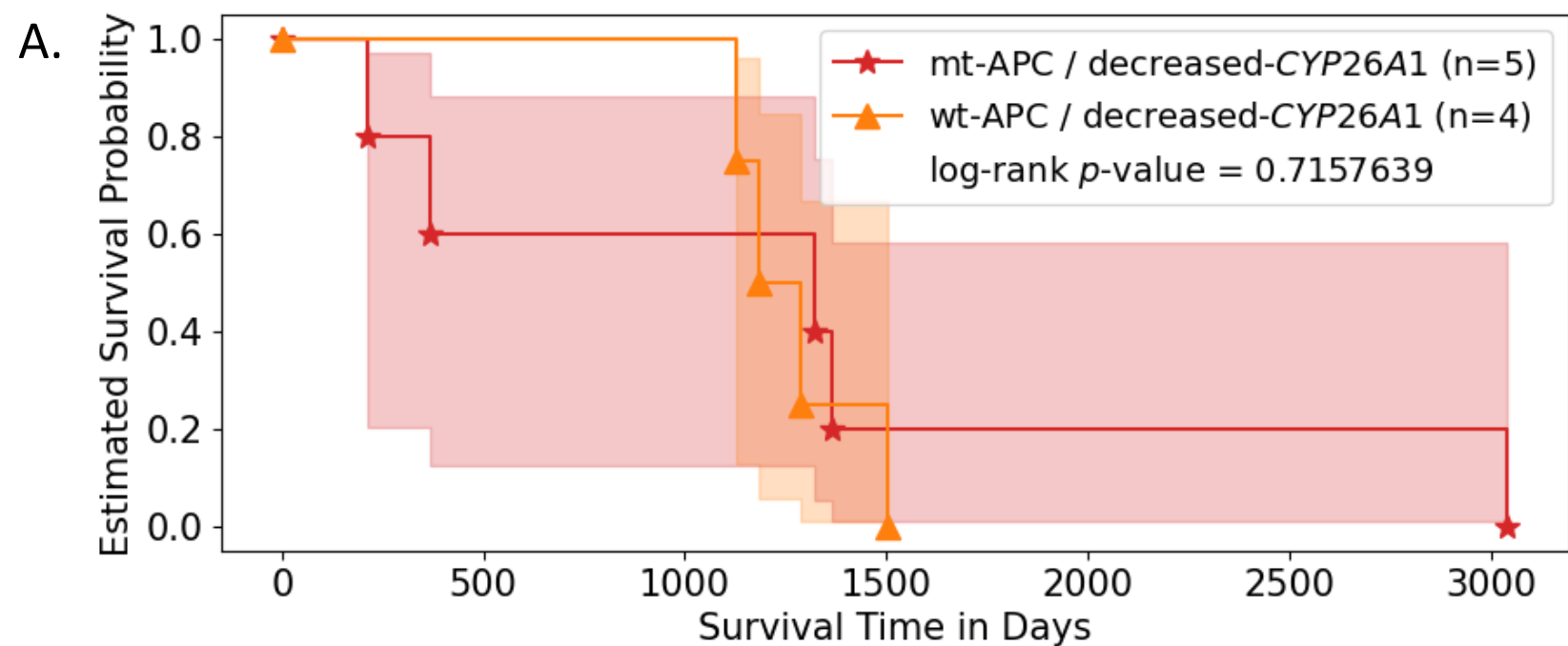


Figure S5. *APC* genotype is not a significant predictor of patient survival when patients are sub-grouped based on *CYP26A1* level. RNA-seq, mutation, and survival data of CRC patients were obtained from GDC data portal. Patients were grouped by *APC* mutation and change (either increase or decrease) of *CYP26A1* expression between CRC and normal colon samples from the same patient. Shaded area indicates the 95% confidence interval. **(A)** Decreased *CYP26A1* expression, regardless of *APC* mutation status, is not a statistically significant predictor of patient survival. **(B)** Similarly, increased *CYP26A1* expression, regardless of *APC* mutation status, is not a significant predictor of patient survival.

Table S1

Primary Antibody	Company	Catalog #	Concentration
β- catenin	Cell Signal Technologies	8480	1:1000
Met	Cell Signal Technologies	8198	1:1000
c-Jun	Cell Signal Technologies	9165	1:1000
CD44	Cell Signal Technologies	5605	1:1000
α/β-tubulin	Cell Signal Technologies	2148	1:1000
CYP26A1	Abcam	ab172474	1:1000
CHGA	NSJ Bioreagents	RQ5334	1:1000
GLP2R	Thermo Fisher Scientific	PA5-104301	1:1000
NSE	Novus Biologicals	NB-110-58870	1:2000
SSTR1	Santa Cruz Technologies	sc-25675	1:500
APC	Millipore Sigma	OP44-100ug	1:500
Secondary Antibody	Company	Catalog #	Concentration
m-IgGkBP-HRP (anti-mouse)	Santa Cruz Technologies	sc-516102	1:1000
mouse anti-rabbit IgG-HRP	Santa Cruz Technologies	sc-2357	1:1000

Table S2

A.

CYP26A1 Inhibitor IC50 Dose (μM)		
	Liarozole	Talarozole
HT29	80	30
HCT116	110	22
SW480	80	25

B.

WNT Inhibitor Doses (μM)						
	Sulindac			Piroxicam		
	-25% IC50	IC50	+25% IC50	-25% IC50	IC50	+25% IC50
HT29	510	680	850	412.5	550	687.5
HCT116	337.5	450	562.5	450	600	750
SW480	165	220	275	202.5	270	337.5

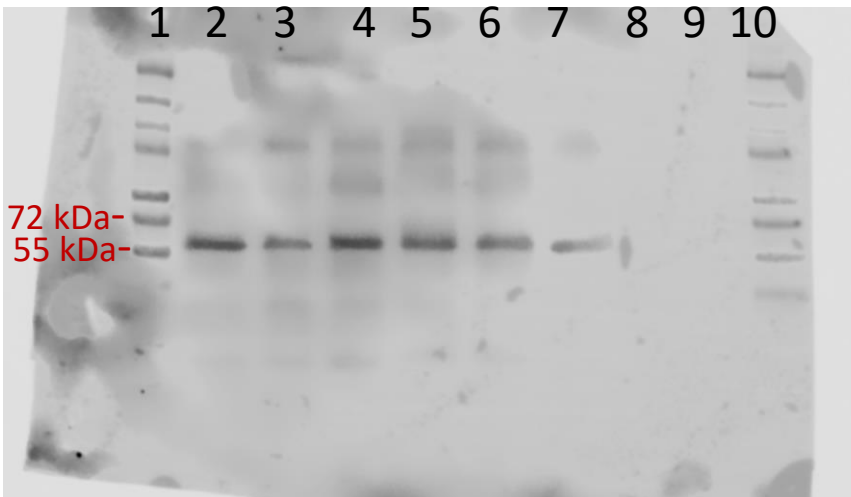
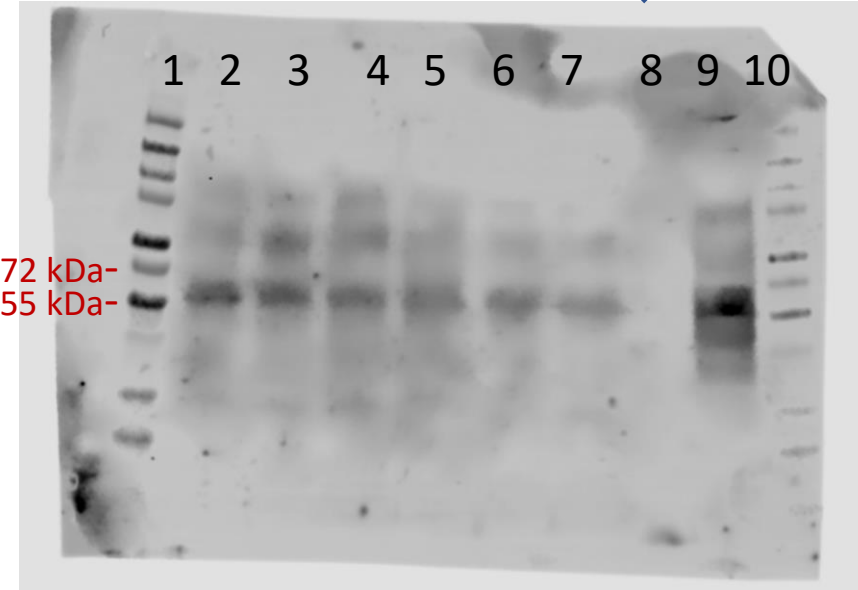
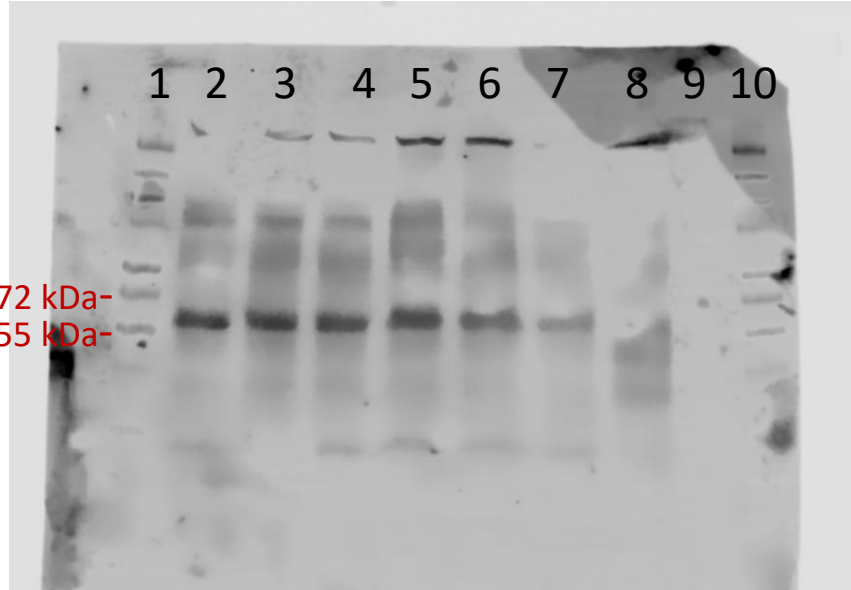
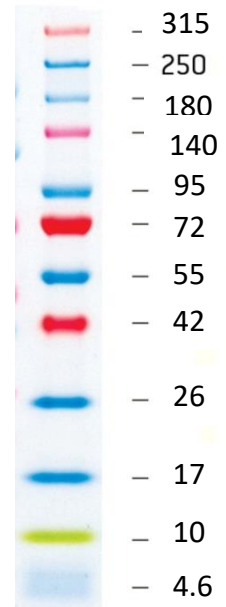
Western Blots

Figure 3

CYP26A1

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Lonza ProSieve
Quad Color
Protein Marker



1. Protein marker
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4. ATRA 20
5. DMSO
6. ATRA 10
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8. HCT116 lysate- not discussed
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10. Protein marker

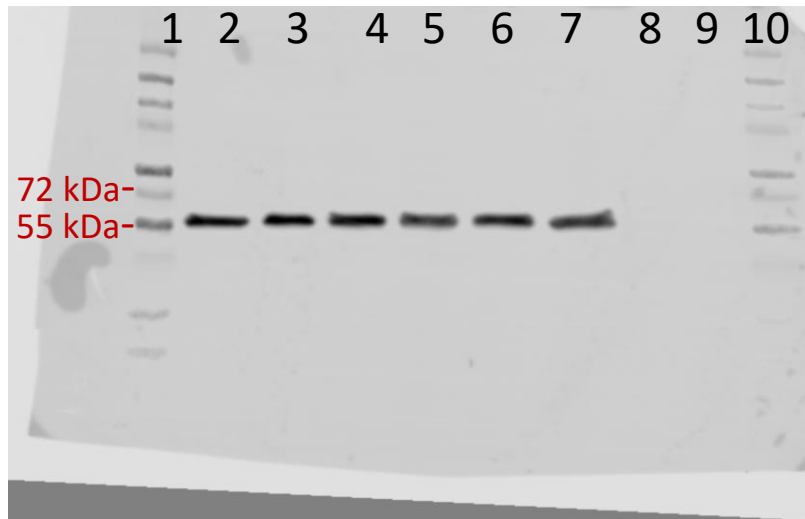
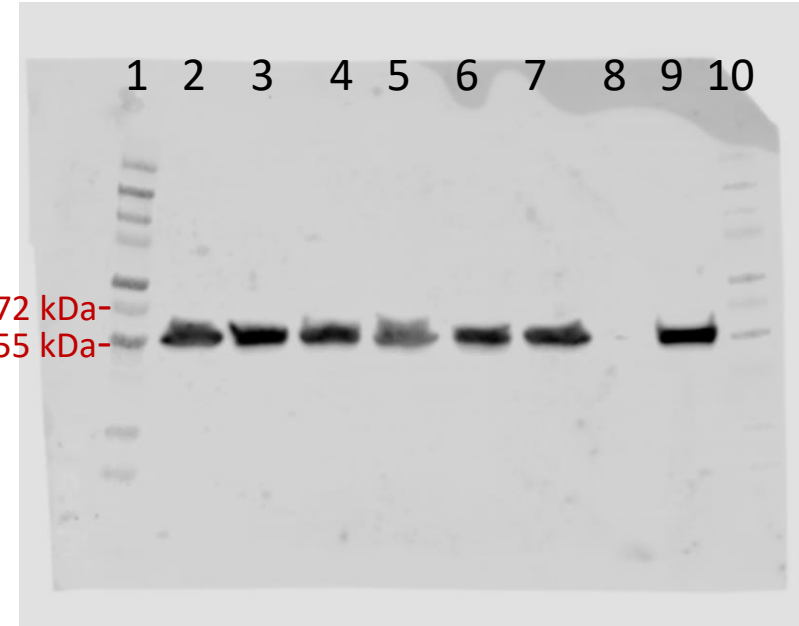
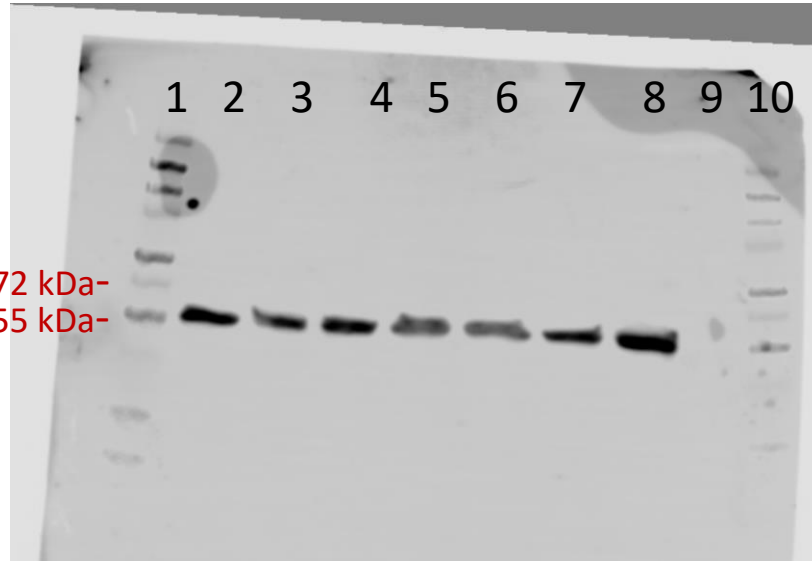
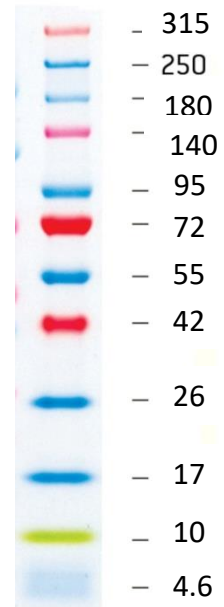
CYP26A1 Densitometry

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	0000879_02	Chemi	00002	0.0635928	0.20897	2035	7.1438E-05	Signal
	0000879_02	Chemi	00003	0.0542964	0.196611	2035	6.9933E-05	Signal
	0000879_02	Chemi	00004	0.0696345	0.198488	2035	6.3319E-05	Signal
	0000879_02	Chemi	00005	0.0432927	0.172541	2035	6.3513E-05	Signal
	0000879_02	Chemi	00006	0.0246706	0.133762	2035	5.3607E-05	Signal
gel 3	0000879_02	Chemi	00008	0.0479638	0.149997	2035	5.0139E-05	Signal
	0000879_02	Chemi	00009	0.028746	0.129758	2035	4.9637E-05	Signal
	0000879_02	Chemi	00010	0.0530092	0.168717	2035	5.6859E-05	Signal
	0000879_02	Chemi	00011	0.0469304	0.152722	2035	5.1986E-05	Signal
	0000879_02	Chemi	00012	0.0385676	0.124655	2035	4.2304E-05	Signal
	0000879_02	Chemi	00013	0.0282362	0.084056	2035	2.743E-05	Signal
gel 2	Image Name	Channel	Name	Signal	Total	Area	Bkgnd.	Type
	0000880_01	Chemi	00007	0.0227338	0.09387	1508	4.7173E-05	Signal
	0000880_01	Chemi	00008	0.0186694	0.094345	1508	5.0183E-05	Signal
	0000880_01	Chemi	00009	0.0141978	0.089194	1508	4.9732E-05	Signal
	0000880_01	Chemi	00010	0.011121	0.08638	1508	4.9907E-05	Signal
	0000880_01	Chemi	00011	0.0136076	0.078942	1508	4.3325E-05	Signal
	0000880_01	Chemi	00012	0.0098995	0.072783	1508	4.17E-05	Signal
	0000880_01	Chemi	00013	0.0325363	0.122614	1508	5.9733E-05	Signal

Figure 3

α/β -tubulin

Lonza ProSieve
Quad Color
Protein Marker



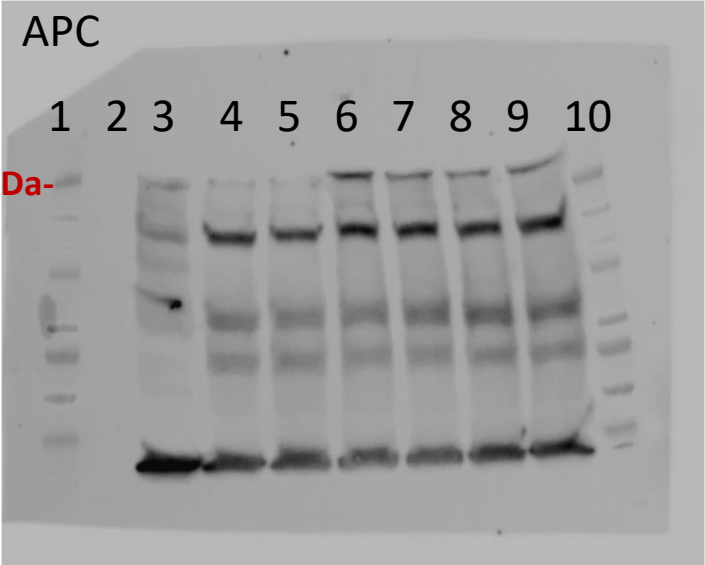
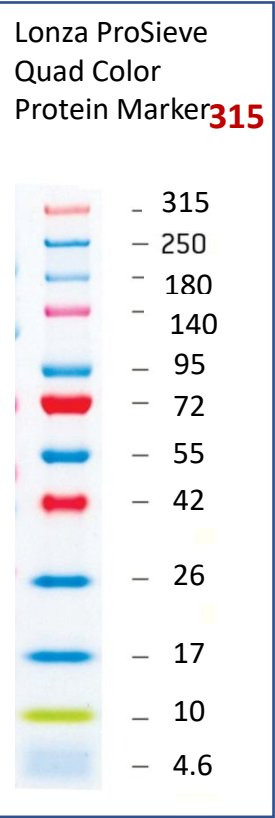
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3. ATRA 10
4. ATRA 20
5. DMSO
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8. HCT116 lysate- not discussed
9. Blank
10. Protein marker

α/β tubulin Densitometry

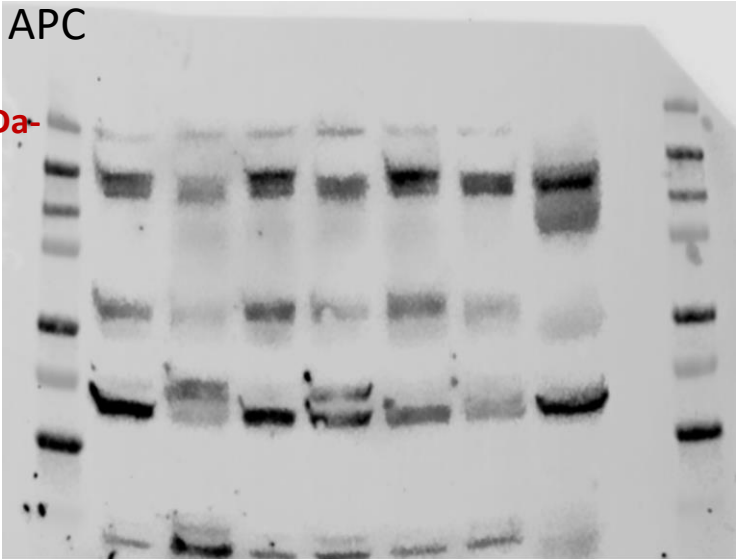
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	0000904_02	Chemi	00002	2.8248708	3.290296	2170	0.00021448	Signal
	0000904_02	Chemi	00003	3.6436223	3.970928	2170	0.00015083	Signal
	0000904_02	Chemi	00004	3.1781306	3.568618	2170	0.00017995	Signal
	0000904_02	Chemi	00005	2.7641	3.29008	2170	0.00024239	Signal
	0000904_02	Chemi	00006	3.2635473	3.586648	2170	0.00014889	Signal
	0000904_02	Chemi	00007	5.6133482	6.476317	2170	0.00039768	Signal
gel 3	0000904_02	Chemi	00008	2.8590233	3.16025	2170	0.00013881	Signal
	0000904_02	Chemi	00009	2.6162436	2.841784	2170	0.00010394	Signal
	0000904_02	Chemi	00010	3.02755	3.239487	2170	9.7667E-05	Signal
	0000904_02	Chemi	00011	2.6370391	2.836209	2170	9.1783E-05	Signal
	0000904_02	Chemi	00012	2.8342912	3.049364	2170	9.9112E-05	Signal
	0000904_02	Chemi	00013	2.8589504	3.258442	2170	0.0001841	Signal
gel 2	Image Name	Channel	Name	Signal	Total	Area	Bkgnd.	Type
	0000905_01	Chemi	00002	1.819918	2.216599	2016	0.00019677	Signal
	0000905_01	Chemi	00003	1.9979991	2.472499	2016	0.00023537	Signal
	0000905_01	Chemi	00004	1.9158432	2.196268	2016	0.0001391	Signal
	0000905_01	Chemi	00005	1.3559578	1.766118	2016	0.00020345	Signal
	0000905_01	Chemi	00006	1.7369985	1.980431	2016	0.00012075	Signal
	0000905_01	Chemi	00007	1.9097523	2.238864	2016	0.00016325	Signal
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Figure 5

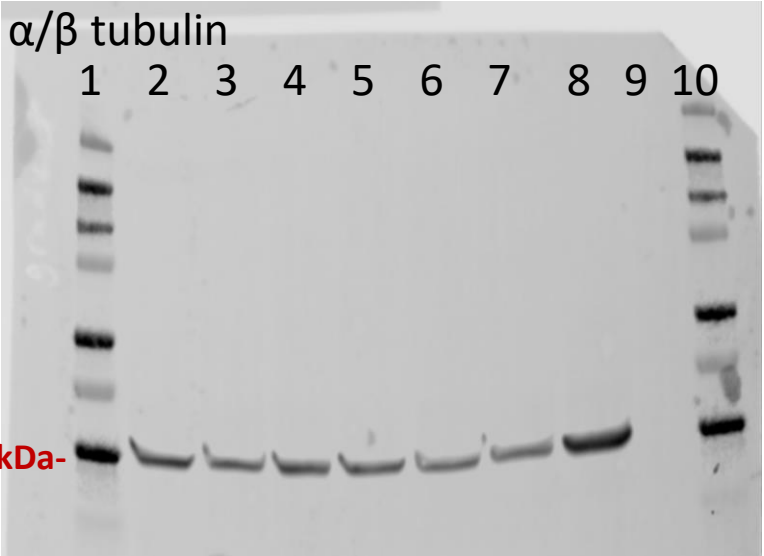
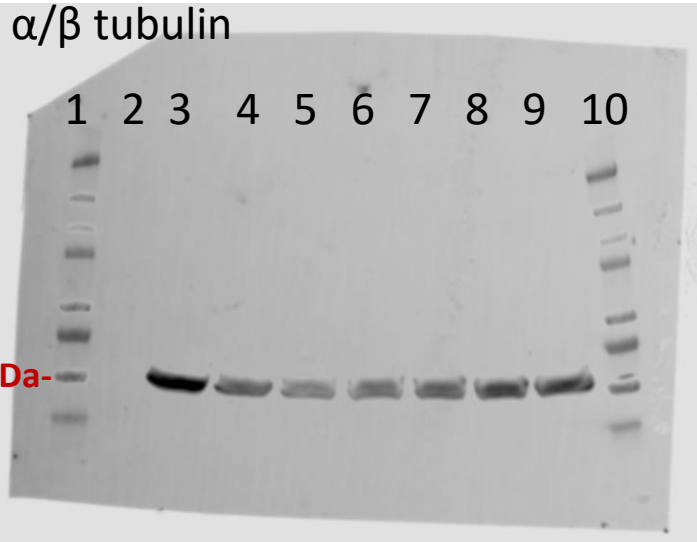
APC- not quantified



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 8. 80
 9. 100
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μM E64

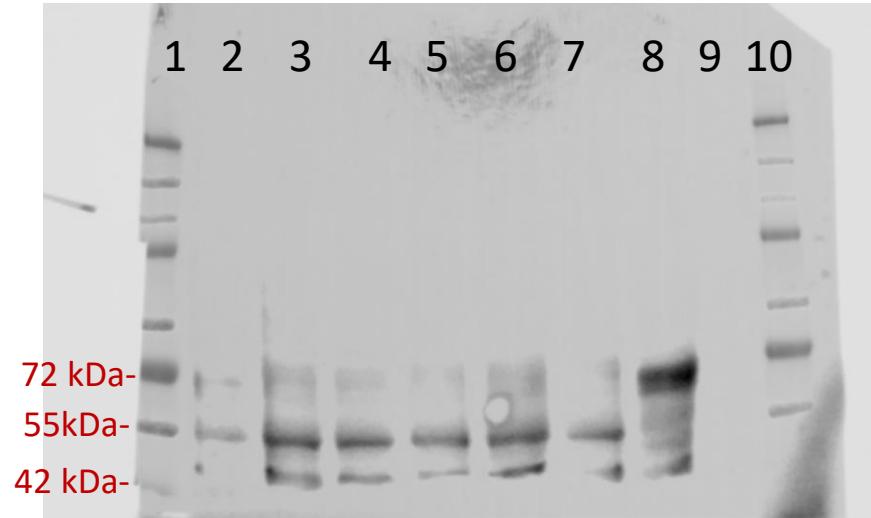
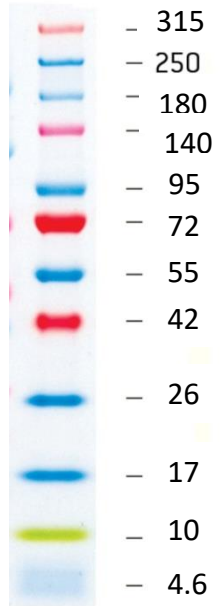


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n=3

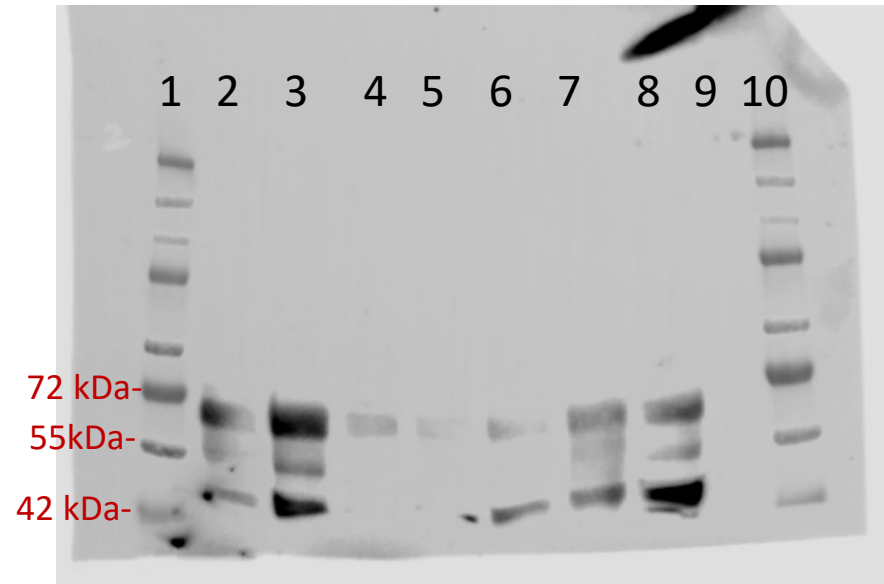


CHGA

Lonza ProSieve
Quad Color
Protein Marker



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n=2 (lanes 4-5)
n=3 (lanes 6-7)

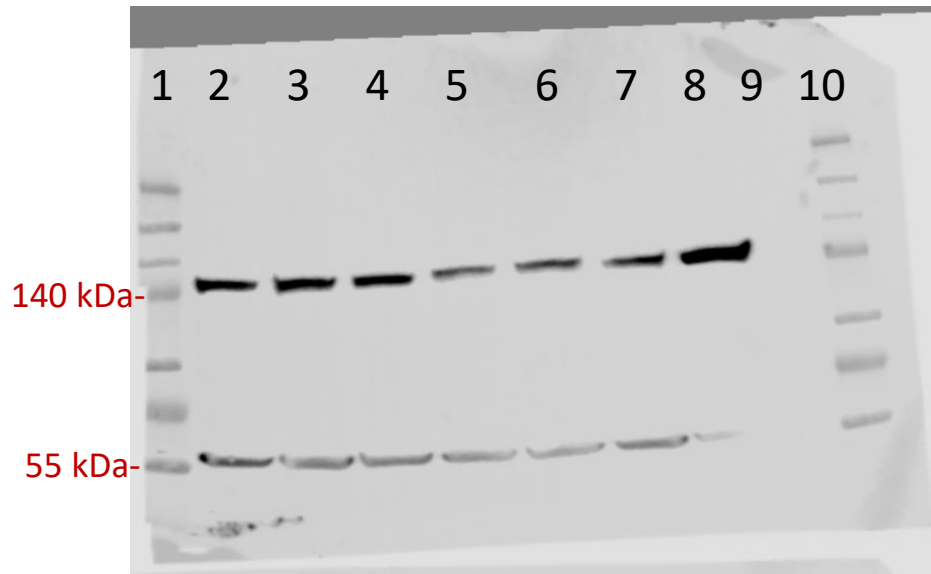
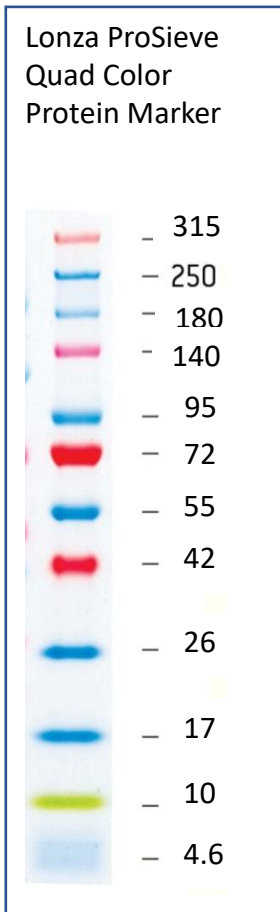


Analyzed n1 and n3

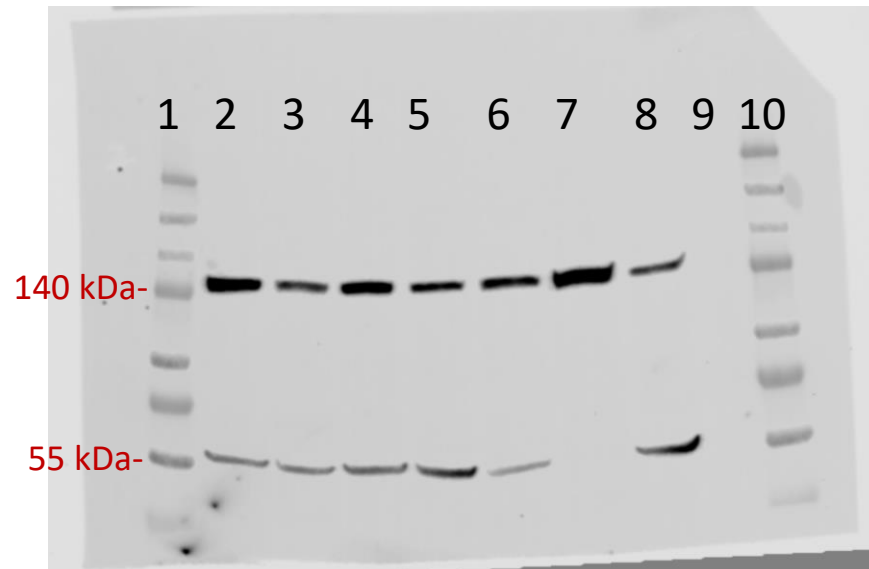
CHGA Densitometry

	CHGA	n=2 was too faint to compare.														
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n=3	no zinc	Chemi	00001	0.055831	0.134429	1800	4.37E-05	Signal	0.74216	1	top	1	3.529656			
	middle	Chemi	00002	0.014418	0.08745	1800	4.06E-05	Signal	0.191663	1	middle	1	4.165847			
	bottom	Chemi	00003	0.086348	0.175677	1800	4.96E-05	Signal	1.147832	1	bottom*	1	6.503912	1.222415	0.864378	
	plus zinc	Chemi	00004	0.13446	0.252919	1800	6.58E-05	Signal	2.544741	3.428829						
	middle	Chemi	00005	0.0502	0.155646	1800	5.86E-05	Signal	0.950072	4.957003						
	bottom	Chemi	00006	0.342035	0.505902	1800	9.1E-05	Signal	6.47324	5.639534						
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n=1	0000585_02	Chemi	no z top	0.130288	0.311586	2394	7.57E-05	Signal	1.253706	1						
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	0000585_02	Chemi	no z middle	0.036226	0.206204	2394	7.1E-05	Signal	0.355272	1						
	0000585_02	Chemi	z middle	0.084889	0.271037	2394	7.78E-05	Signal	1.198932	3.37469						
	0000585_02	Chemi	no z bottom	0.035362	0.203078	2394	7.01E-05	Signal	0.346795	1						
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GLP2R



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 7. +zinc
 8. HCT116 lysate, not discussed
 9. Blank
 10. Protein marker
- n=1
- n=2
- n=3



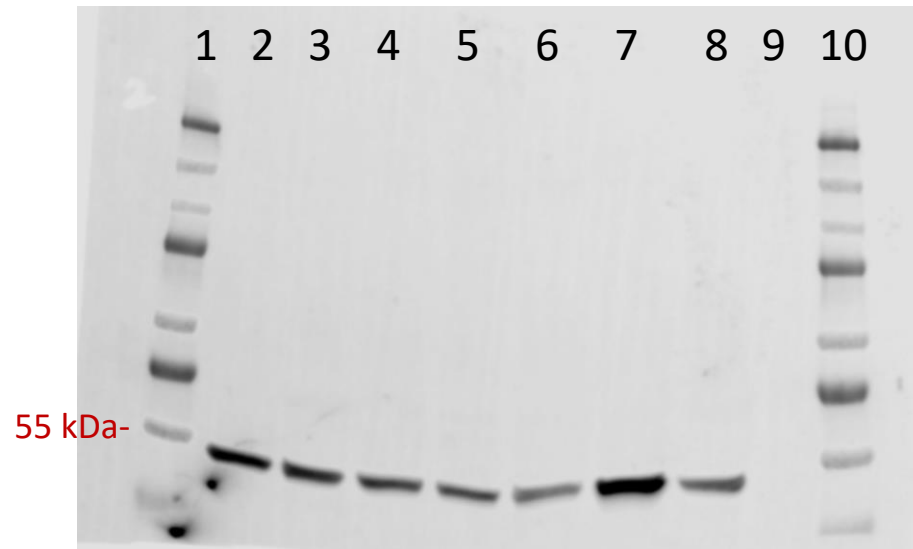
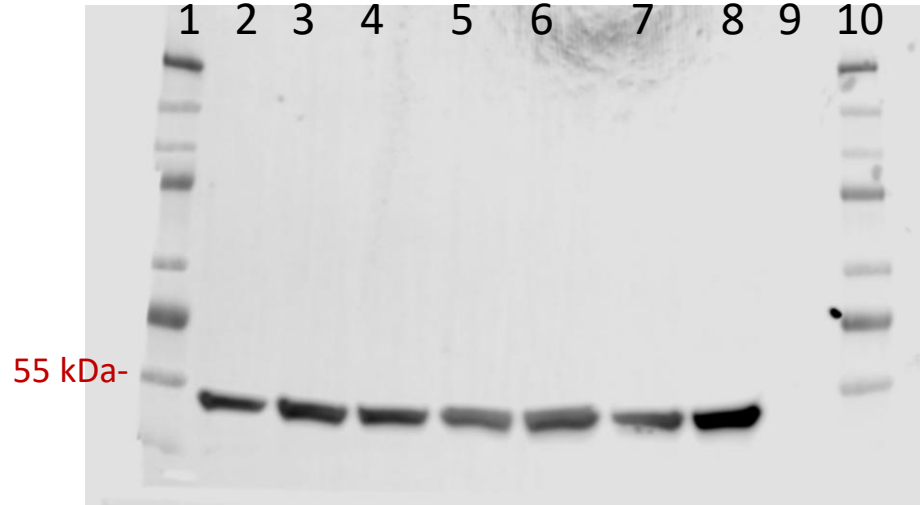
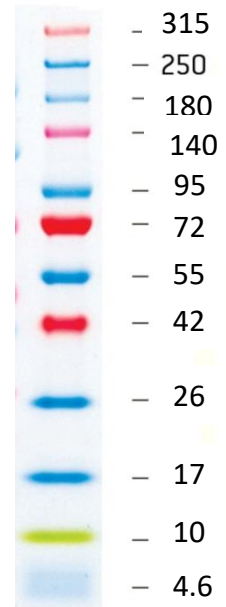
Analyzed 60 kDa band

GLP2R Densitometry

	GLP2R												
	Image Name	Channel	Name	Signal	Total	Area	Bkgnd.	Type	norm to tub	norm to control	average	st dev	st err
n=1	0000595_02	Chemi	00001	0.031794	0.040883	1728	5.26E-06	Signal	0.311805	1	2.159973	1.481436	0.855307
	0000595_02	Chemi	00002	0.026924	0.036439	1728	5.51E-06	Signal	0.380267	1.219567			
n=2	0000595_02	Chemi	00003	0.039411	0.046879	1728	4.32E-06	Signal	0.448849	1			
	0000595_02	Chemi	00004	0.048722	0.059078	1728	5.99E-06	Signal	0.625106	1.392686			
n=3	0000595_02	Chemi	00005	0.022542	0.028511	1728	3.45E-06	Signal	0.299656	1			
	0000595_02	Chemi	00006	0.061238	0.069057	1728	4.52E-06	Signal	1.158968	3.867665			

Enolase/ NSE

Lonza ProSieve
Quad Color
Protein Marker



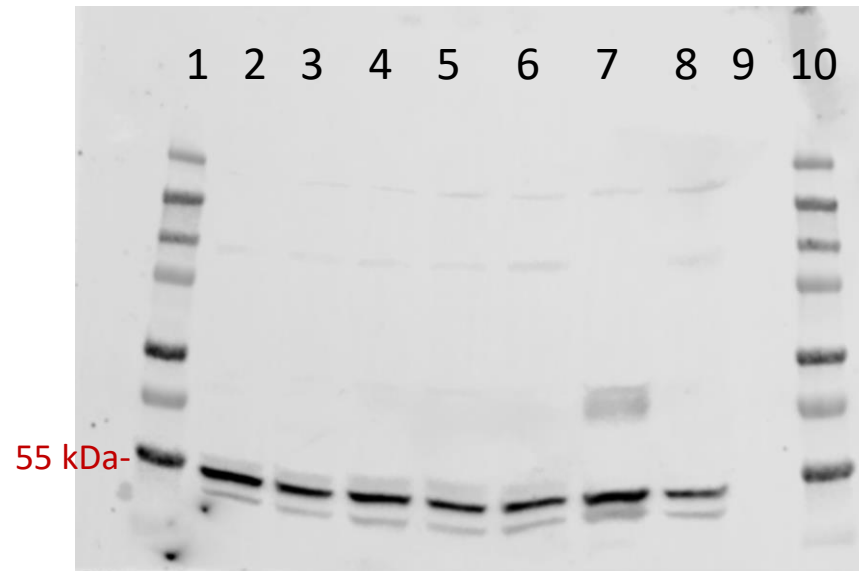
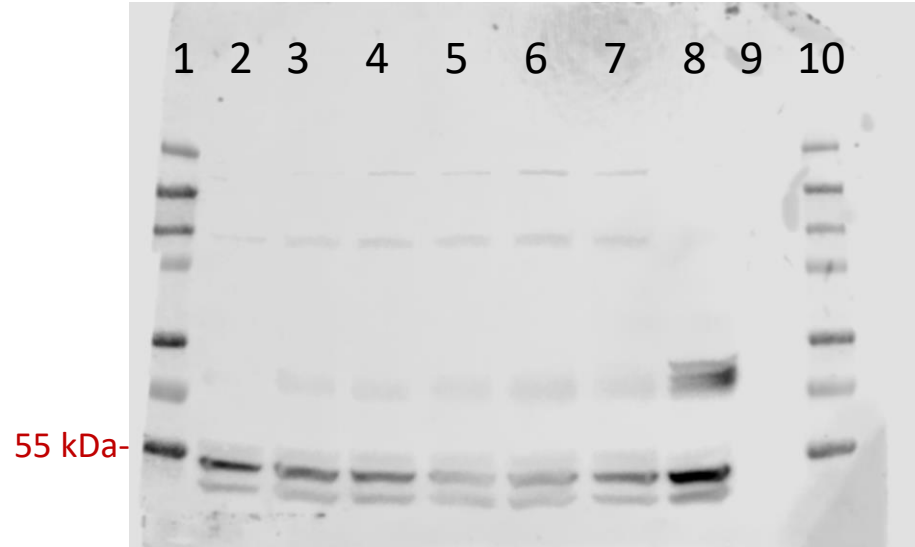
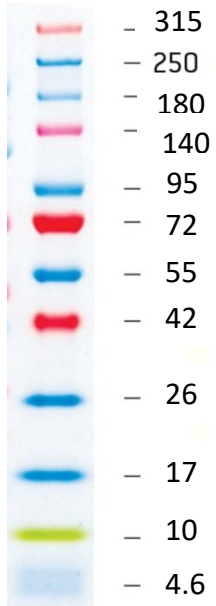
1. Protein marker
 2. -zinc
 3. +zinc
 4. -zinc
 5. +zinc
 6. -zinc
 7. +zinc
 8. HCT116 lysate, not discussed
 9. Blank
 10. Protein marker
- Groupings:
- n=1: 2. -zinc, 3. +zinc
 - n=2: 4. -zinc, 5. +zinc
 - n=3: 6. -zinc, 7. +zinc

NSE Densitometry

	Enolase/ NSE												
	Image Name	Channel	Name	Signal	Total	Area	Bkgnd.	Type	norm to tub	norm to control	average	st dev	st err
n=1	0000601_02	Chemi	00001	9.498009	10.29861	1920	0.000417	Signal	93.14737	1	1.308985	0.408328	0.235748
	0000601_02	Chemi	00002	7.670272	8.603663	1920	0.000486	Signal	108.3312	1.163009			
n=2	0000601_02	Chemi	00003	6.562607	7.560053	1920	0.00052	Signal	74.74105	1			
	0000601_02	Chemi	00004	5.788858	6.508143	1920	0.000375	Signal	74.27075	0.993708			
n=3	0000601_02	Chemi	00005	4.791397	5.919724	1920	0.000588	Signal	63.69249	1			
	0000601_02	Chemi	00007	5.957571	6.565863	1920	0.000317	Signal	112.7509	1.770239			

SSTR1

Lonza ProSieve
Quad Color
Protein Marker



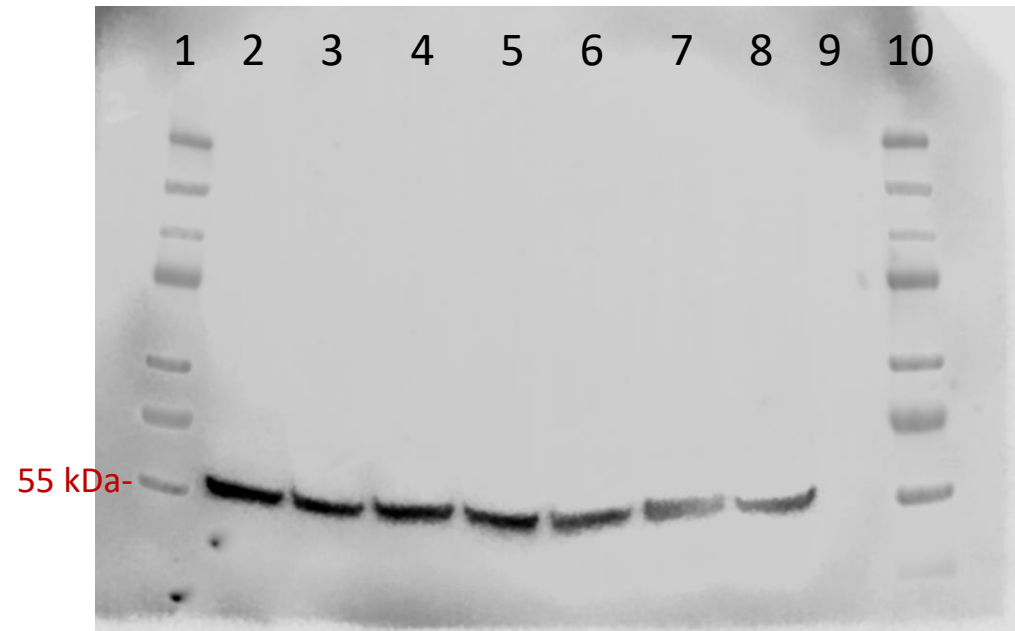
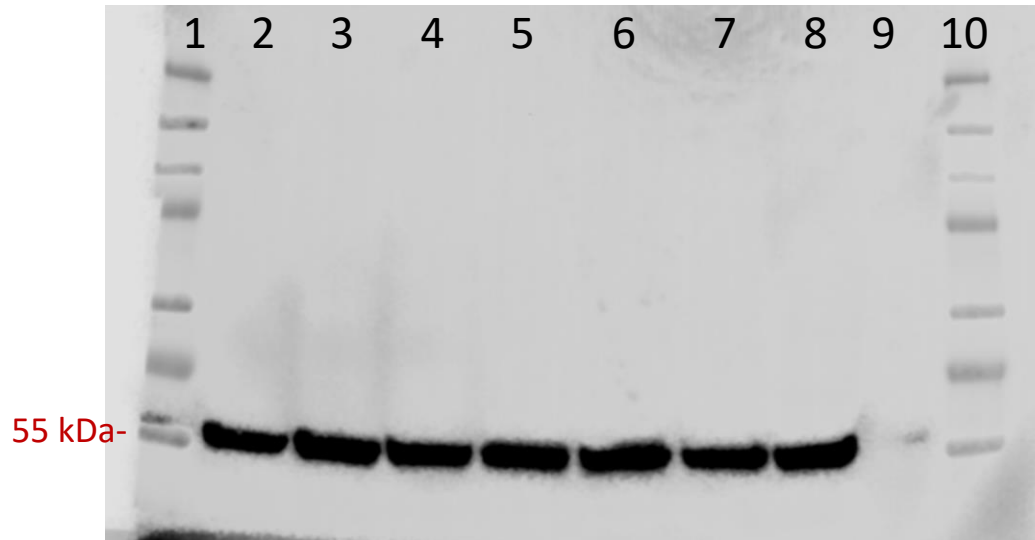
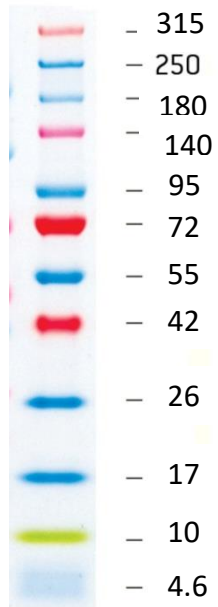
1. Protein marker
 2. -zinc
 3. +zinc
 4. -zinc
 5. +zinc
 6. -zinc
 7. +zinc
 8. HCT116 lysate, not discussed
 9. Blank
 10. Protein marker
- Blue brackets indicate groupings: n=1 for lanes 2-3, n=2 for lanes 4-5, and n=3 for lanes 6-7.

SSTR1 Densitometry

	SSTR1													
	Image Name	Channel	Name	Signal	Total	Area	Bkgnd.	Type	norm to tub	norm to control	average	st dev	st err	
n=1	0000608_0 2	Chemi	00001	0.484174	0.735849	1440	0.000175	Signal	4.748314	1	1.012268	0.210822	0.121718	
	0000608_0 2	Chemi	00002	0.344003	0.499221	1440	0.000108	Signal	4.858538	1.023213				
n=2	0000608_0 2	Chemi	00003	0.400521	0.579465	1440	0.000124	Signal	4.561509	1				
	0000608_0 2	Chemi	00004	0.283073	0.448719	1440	0.000115	Signal	3.631809	0.796186				
n=3	0000608_0 2	Chemi	00005	0.306848	0.482729	1440	0.000122	Signal	4.078959	1				
	0000608_0 2	Chemi	00006	0.262381	0.351485	1440	6.19E-05	Signal	4.965741	1.217404				

α/β tubulin

Lonza ProSieve
Quad Color
Protein Marker



1. Protein marker
 2. -zinc
 3. +zinc
 4. -zinc
 5. +zinc
 6. -zinc
 7. +zinc
 8. HCT116 lysate, not discussed
 9. Blank
 10. Protein marker
- Groupings:
- n=1: 2. -zinc, 3. +zinc
 - n=2: 4. -zinc, 5. +zinc
 - n=3: 6. -zinc, 7. +zinc

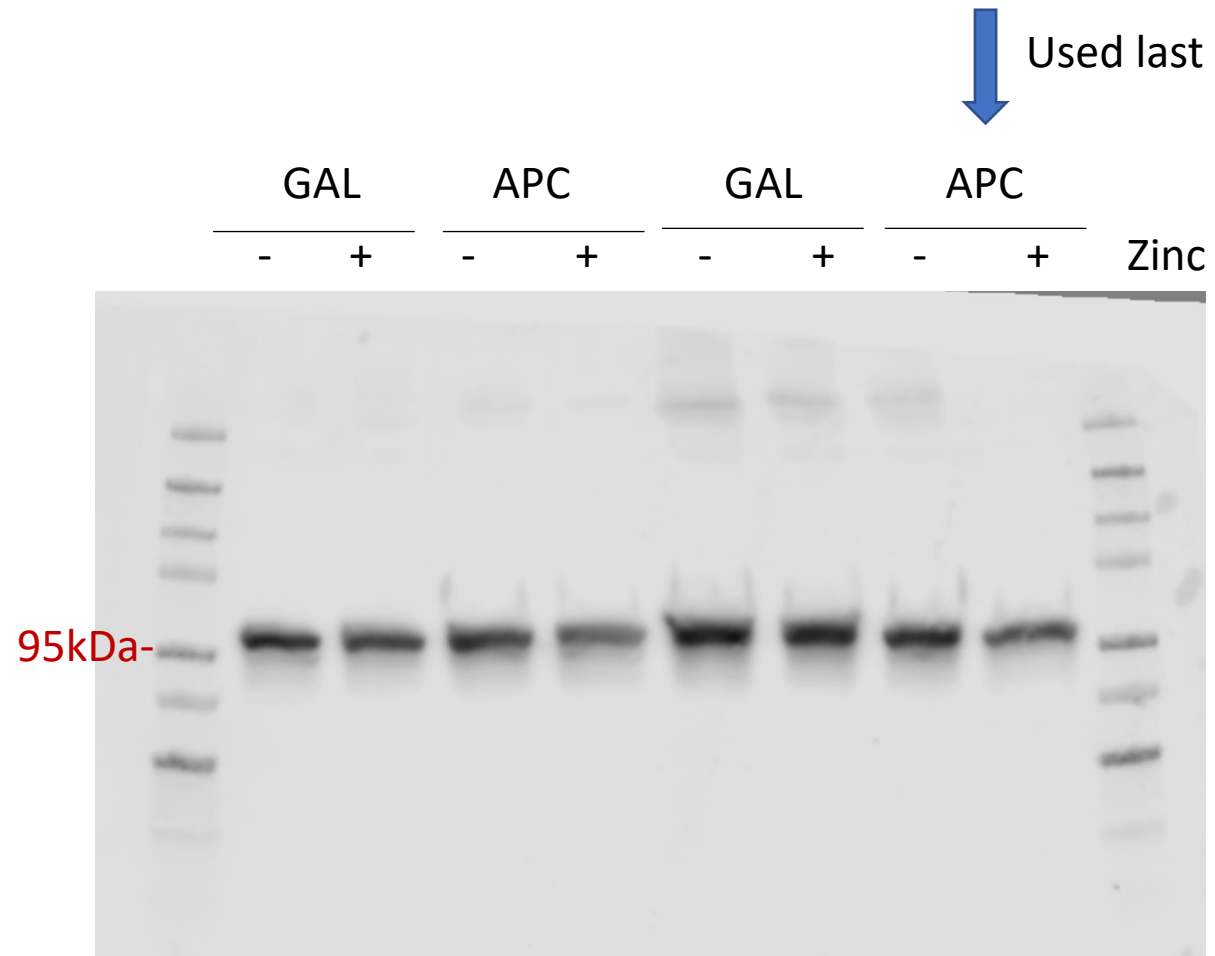
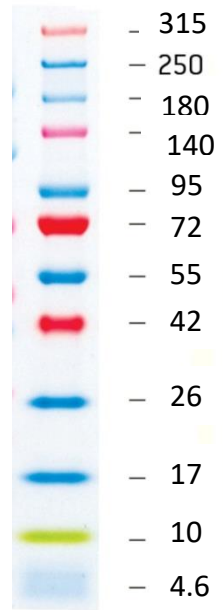
α/β tubulin Densitometry

	tubulin							
	Image Name	Channel	Name	Signal	Total	Area	Bkgnd.	Type
n=1	0000604_02	Chemi	00001	0.101968	0.169665	2145	3.16E-05	Signal
	0000604_02	Chemi	00002	0.070804	0.129999	2145	2.76E-05	Signal
n=2	0000604_02	Chemi	00003	0.087805	0.137475	2145	2.32E-05	Signal
	0000604_02	Chemi	00004	0.077943	0.122627	2145	2.08E-05	Signal
n=3	0000604_02	Chemi	00005	0.075227	0.100542	2145	1.18E-05	Signal
	0000604_02	Chemi	00007	0.052838	0.066135	2145	6.2E-06	Signal
hct116	0000604_02	Chemi	00006	0.059316	0.074914	2145	7.27E-06	Signal

Figure S1

β -catenin

Lonza ProSieve
Quad Color
Protein Marker



GAL= HT29-galactosidase cells
APC= HT29-wt-APC cells

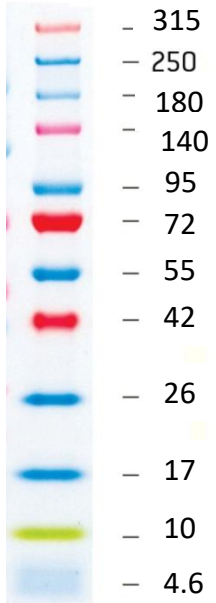
2 different passages shown

Met

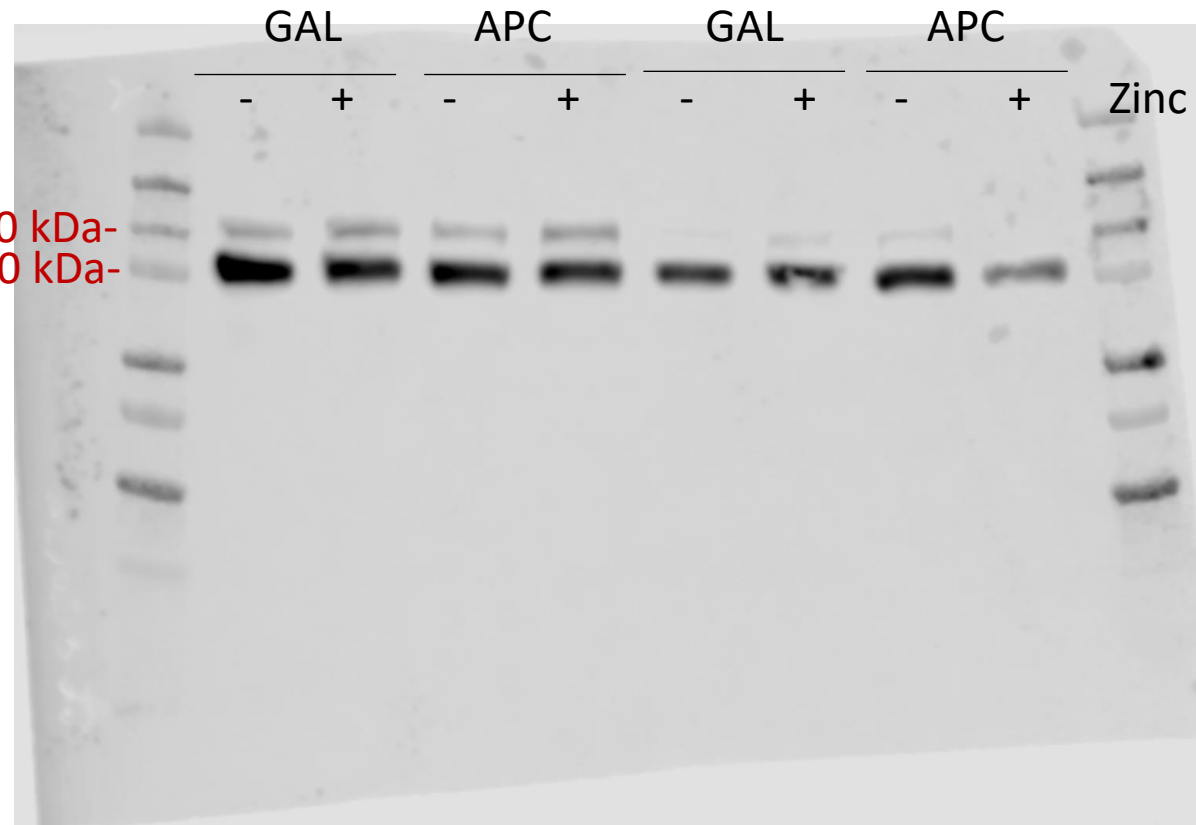


Used last 2 lanes for figure

Lonza ProSieve
Quad Color
Protein Marker



180 kDa-
140 kDa-



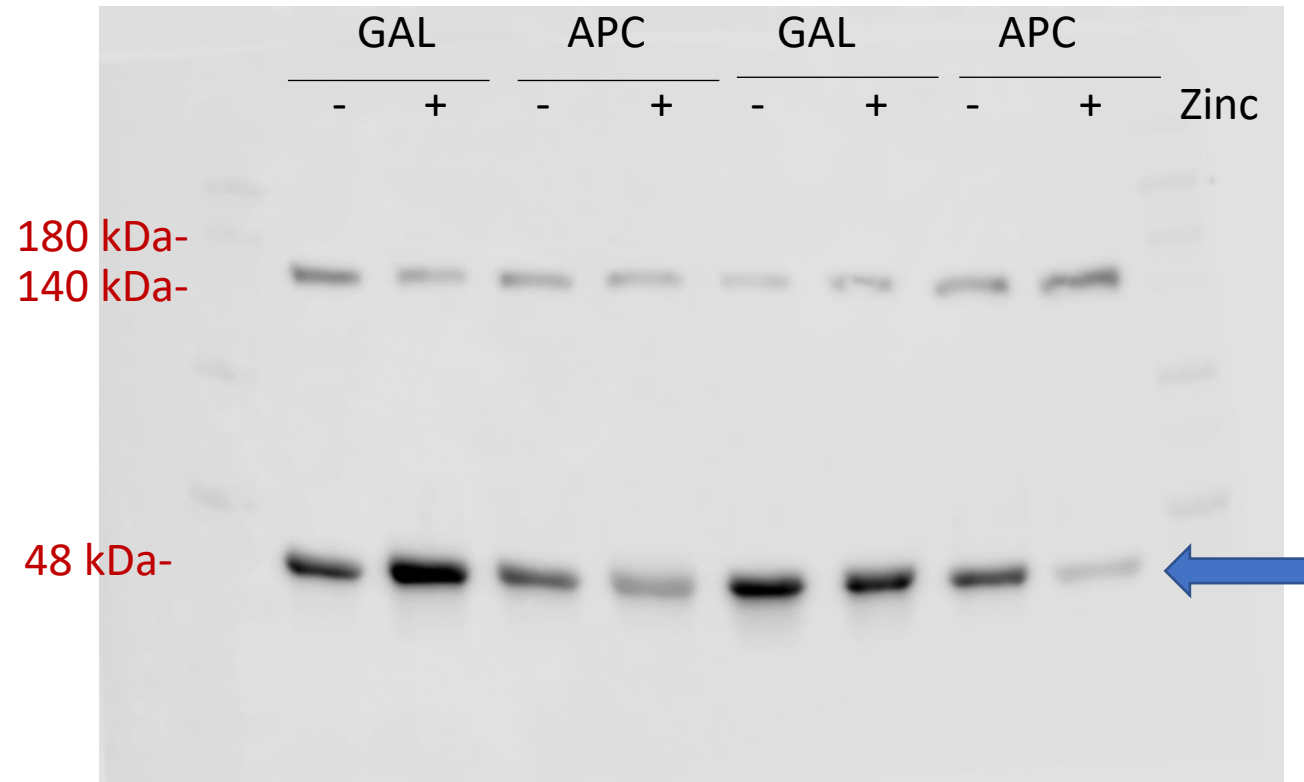
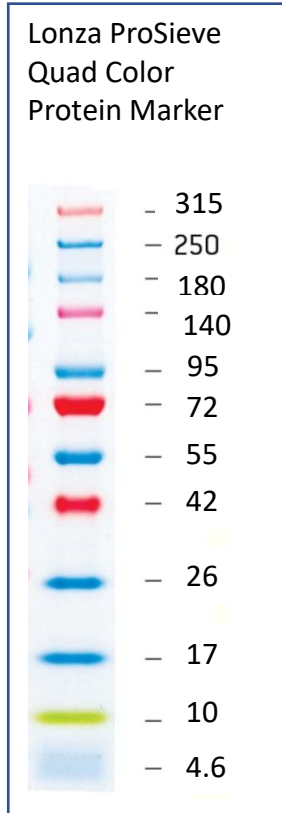
GAL= HT29-galactosidase cells
APC= HT29-wt-APC cells

2 different passages shown

c-Jun



Used last 2 lanes for figure



GAL= HT29-galactosidase cells
APC= HT29-wt-APC cells

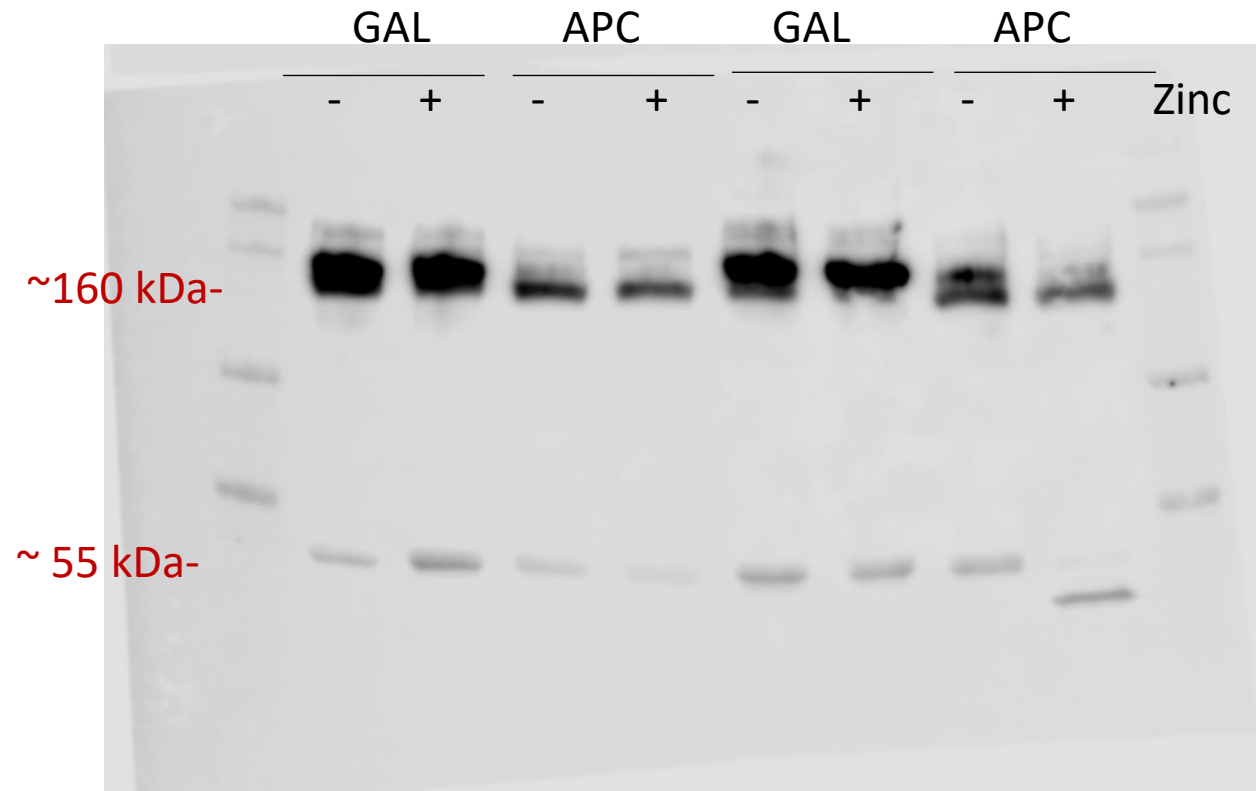
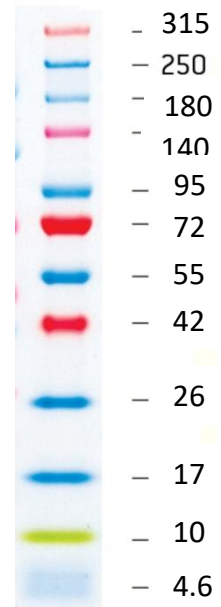
2 different passages shown

CD44



Used last 2 lanes for figure

Lonza ProSieve
Quad Color
Protein Marker

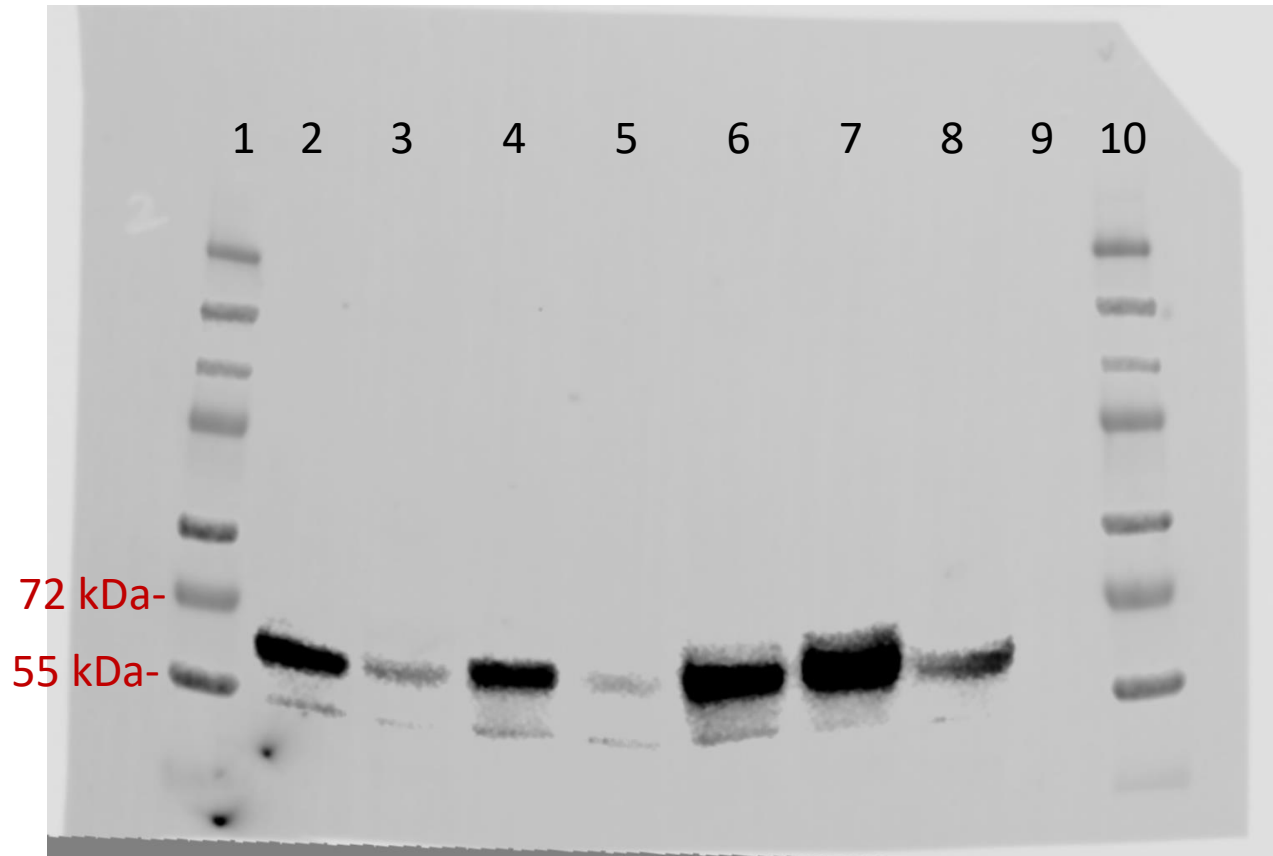
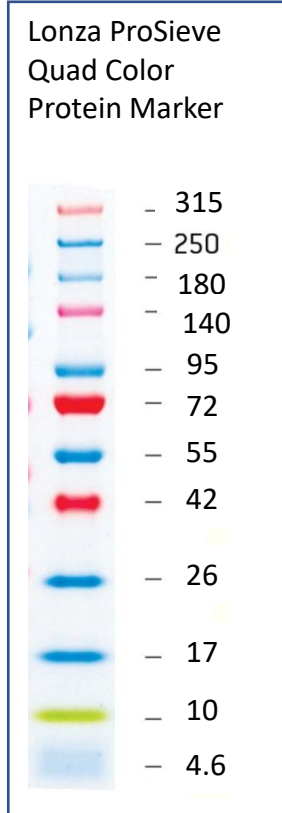


GAL= HT29-galactosidase cells
APC= HT29-wt-APC cells

2 different passages shown

c-Myc

From series in Figure 5
Used n=2 for supplemental figure



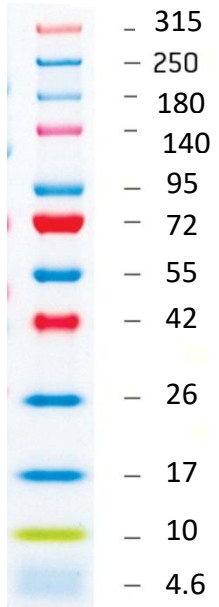
1. Protein marker
 2. -zinc
 3. +zinc
 4. -zinc
 5. +zinc
 6. -zinc
 7. +zinc
 8. HCT116 lysate, not discussed
 9. Blank
 10. Protein marker
- n=1
- n=2
- n=3

α/β - tubulin

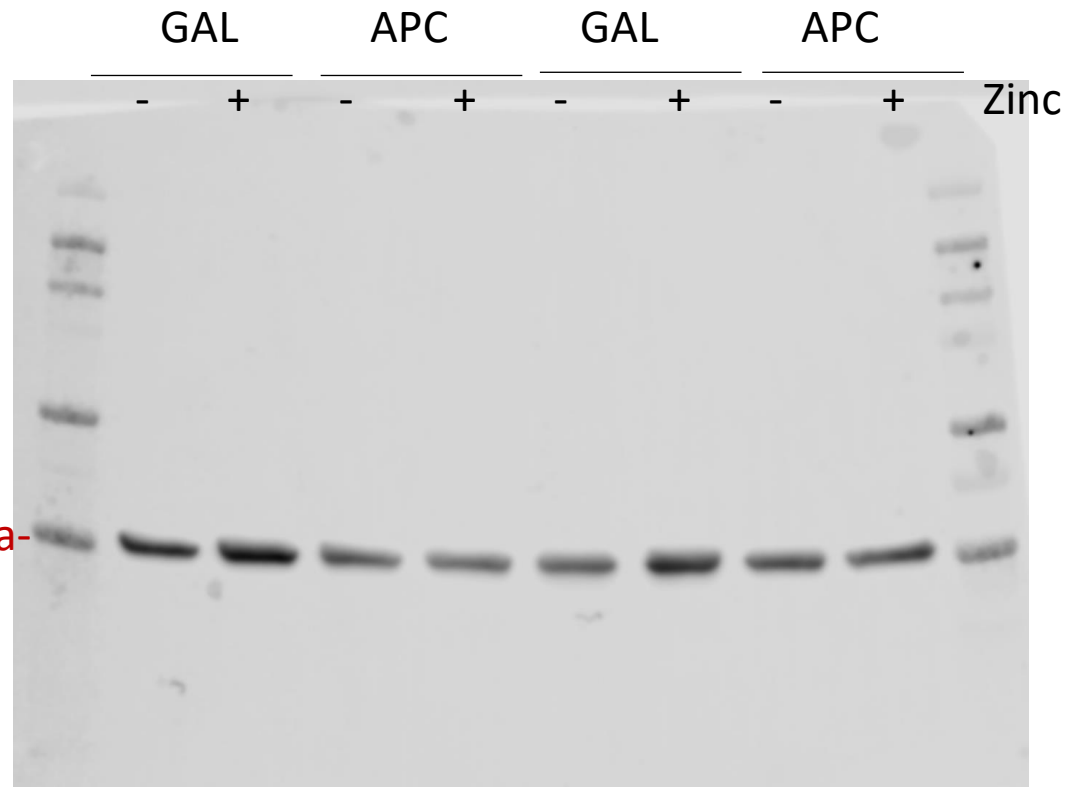
Used last 2 lanes for figure



Lonza ProSieve
Quad Color
Protein Marker



55 kDa-



GAL= HT29-galactosidase cells
APC= HT29-wt-APC cells

2 different passages shown