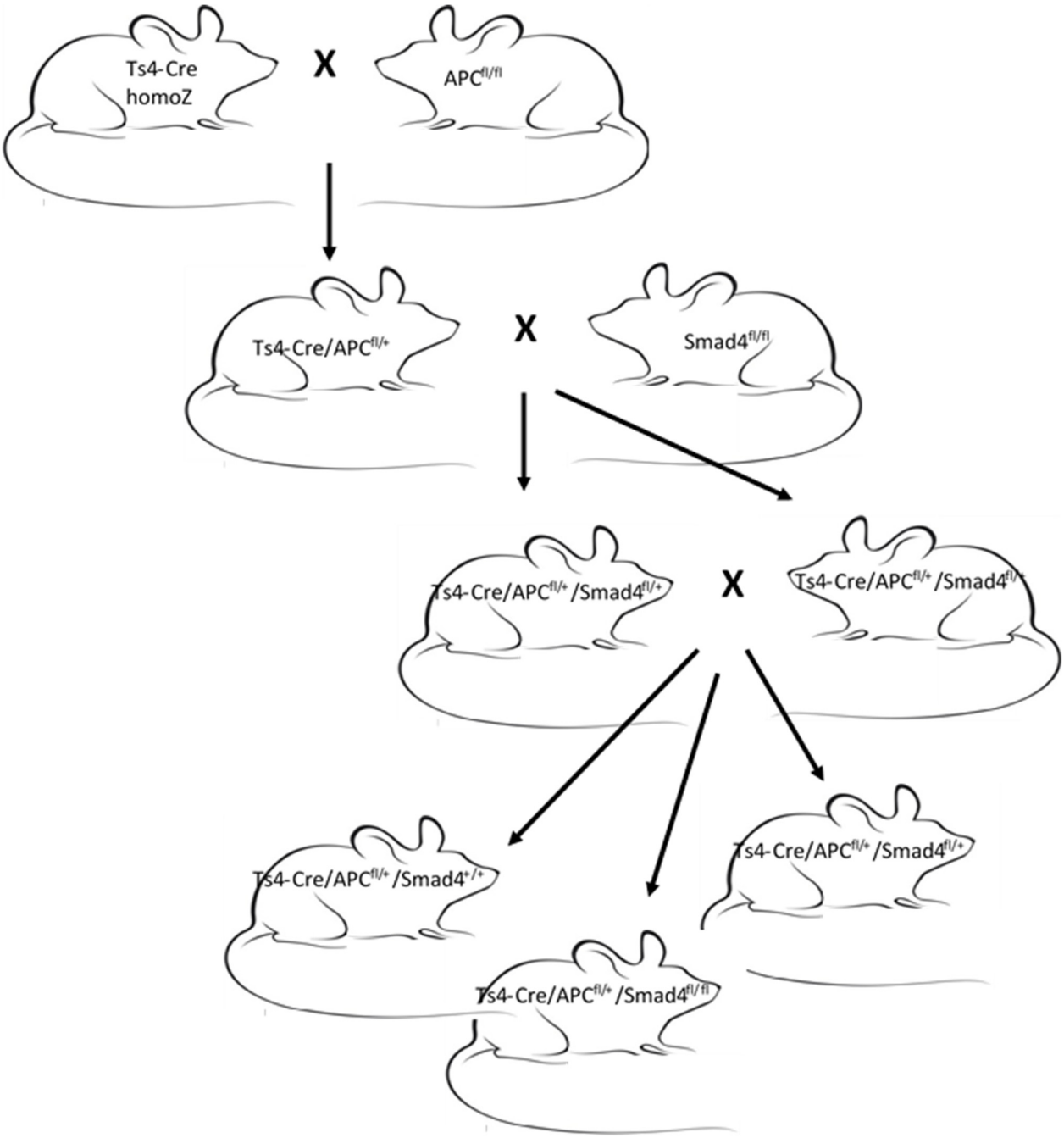


Primers:

APC$^{\Delta 468}$

Fapc up 5’- GTA TTC TCA GTC TTA GCG TTC T -3’

Fapc low 5’ - TTA ACA AGG GCA AAA GGA AAC - 3’

Ts4-Cre

Ts4Cre 5’ 5’- TGA CCG TAC ACC AAA ATT TG -3’

Ts4Cre 3’ 3’- ATT GCC CCT GTT TCA CTA TC -3’

Reaction Components:

Reaction Component	Conc	Volumes
DNA		1 ul
d H_2O		7 ul
2X GoTaq Mastermix	10x	10 ul
Primer 1	10 uM	2 ul
Primer 2	10 uM	2 ul
total		**20 ul**

Cycling Conditions:

APC

Step	Temperature	Time	Note
1	94 °C	5 min	
2	94 °C	45 sec	
3	60 °C	45 sec	-1 °C per cycle
4	72 °C	1 min	go to step 2 , 6 times
5	95 °C	30 sec	
6	54 °C	30 sec	
7	72 °C	30 sec	go to step 5 , 34 times
8	72 °C	7 min	
9	4 °C	∞	

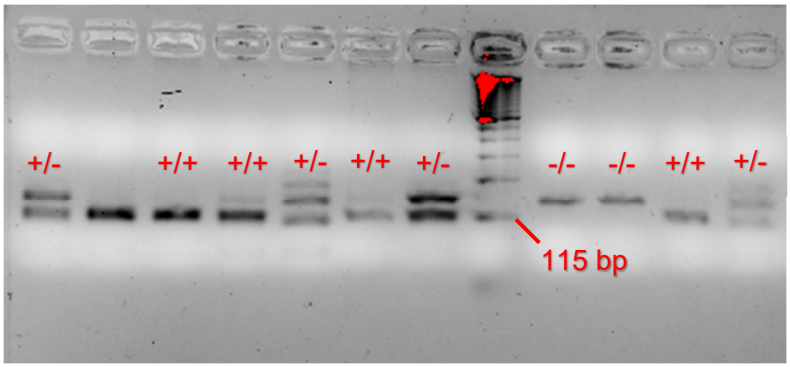
Ts4-Cre

Step	Temperature	Time	Note
1	94 °C	5 min	
2	94 °C	45 sec	
3	60 °C	45 sec	-1 °C per cycle
4	72 °C	1 min	go to step 2 , 6 times
5	95 °C	45 sec	
6	54 °C	45 sec	
7	72 °C	1 min	go to step 5 , 31 times
8	72 °C	7 min	
9	4 °C	∞	

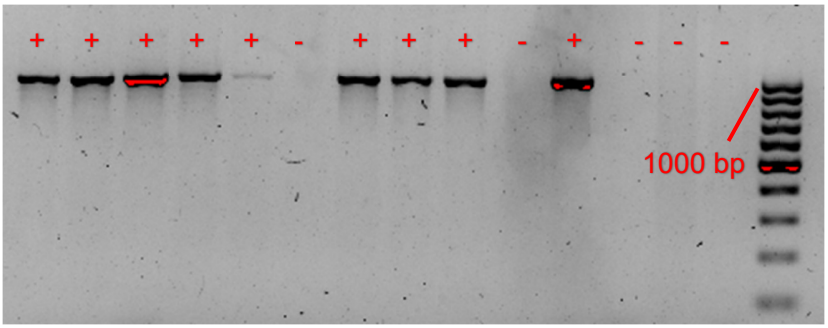
- Separated by gel electrophoresis on a **2.0%** agarose gel
- Band size:
  APC: +/+ 115bp (APC *wt*), +/- 115bp/ 135bp (het), -/- 135 (APC exons 11+12 loxp)
  Ts4-Cre 1000bp (positive; no band negative)

Sample Agarose Pictures:

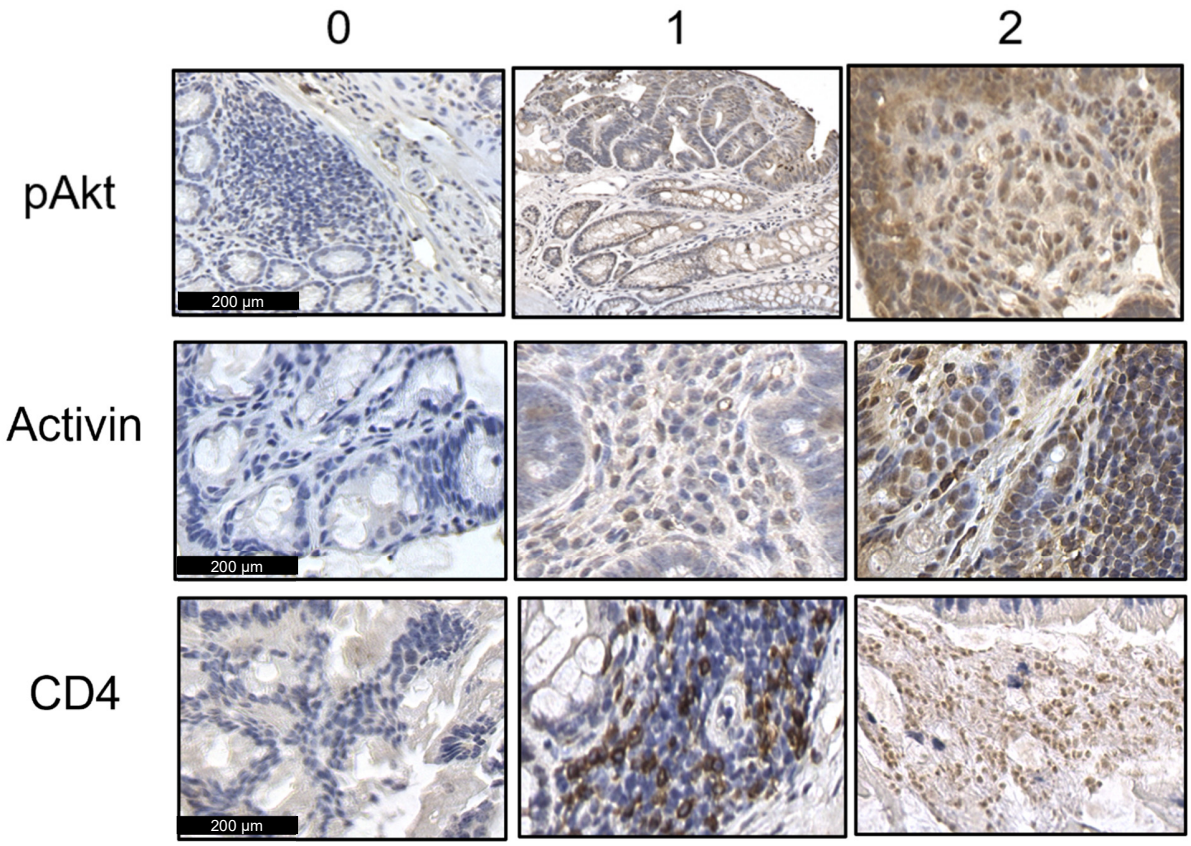
APC



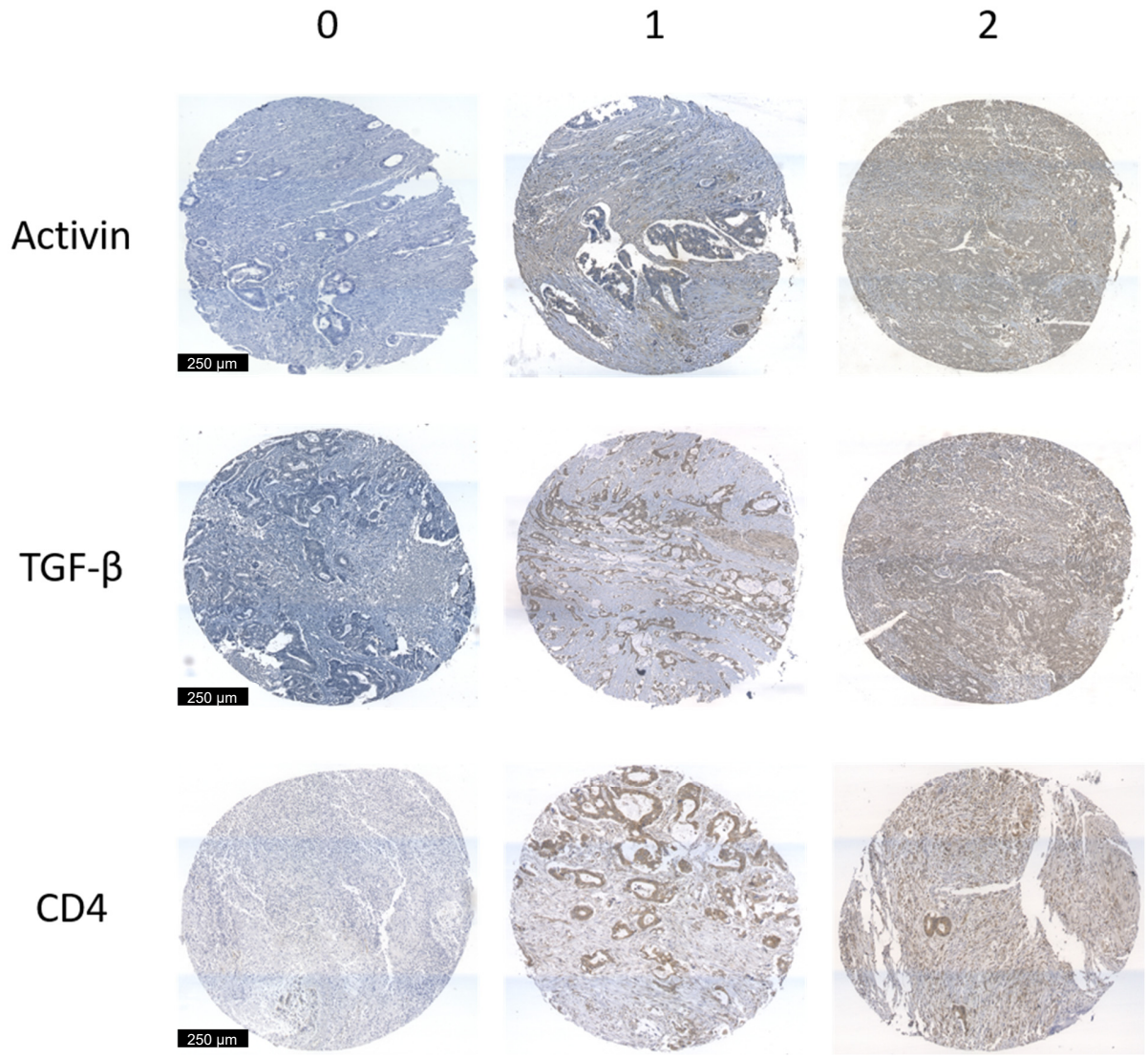
Ts4-Cre



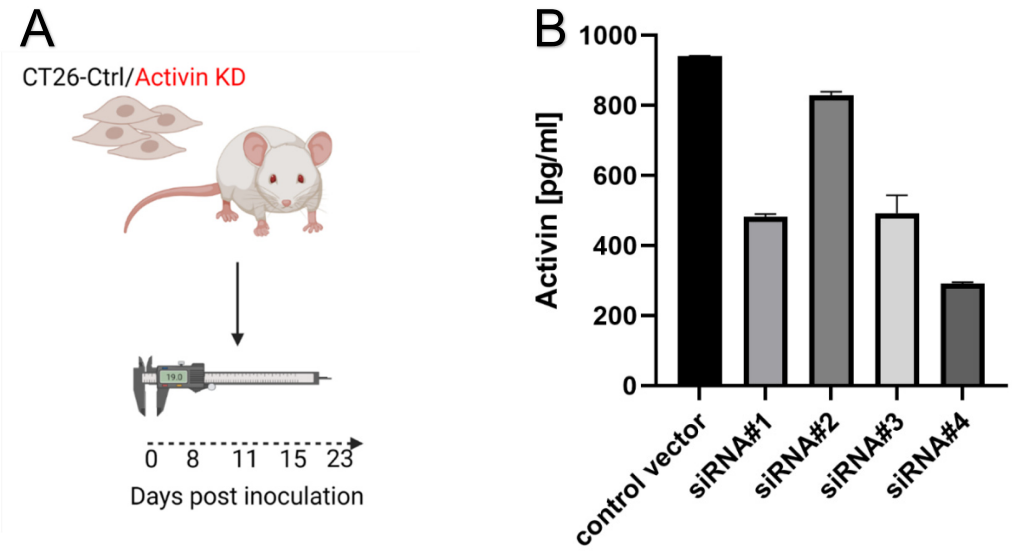
Supplemental Figure S1: Schematic of the genetic approach used to generate mice that develop polyps in the large intestine and lack the SMAD4 protein in the epithelial cells of the large intestine. PCR primer and cycle information and sample images from genotyping are also included.



Supplemental Figure S2: Representative images of low (0, left column) medium (1, middle column) and high (2, right column) scores for pAkt (top row), activin (middle row), and CD4 (bottom row) from the colon of mice in the Ts4Cre/cAPC^{flox}/Smad4^{flox} experiments.



Supplemental Figure S3: Representative images of human CRC IHC images for low (0, left column), medium (1, middle column), and high (2, right column) scores for activin (top row), TGF-β (middle row), and CD4 (bottom row).



Supplemental Figure S4: (A) Schematic displaying the approach employed for the activin knockdown (KD) *in vivo* subcutaneous tumor model. Created with BioRender.com **(B)** ELISA data collected from CT26 cells treated with control vector siRNA or one of four siRNA variants which all targeted *INHBA*. Given the greatest decrease in Activin production in response to siRNA#4, this was the vector used in the mouse experiments.

Supplemental Table S1: List of the 57 quantitative markers employed in the DSP experiments including both positive and negative controls which were used for normalization.

Immune Cell Profiling				Immune Cell Typing			
PD-1		CD45		FOXP3		FAP-alpha	
CTLA4		CD56		CD45RO		CD14	
HLA-DR		CD8		CD34		CD163	
Ki-67		CD68		CD66b			
Beta-2-macroglobulin		GZMB		Immune Cell Phenotyping			
CD11c		PD-L1		CD127		PD-L2	
CD20		PanCk		CD25		CD40	
CD3		SMA		CD80		CD44	
CD4		Fibronectin		ICOS		CD27	
MAPK Signaling Pathway				PI3K/AKT Signaling Pathway			
EGFR		Phospho-MEK1		Pan-AKT		Phospho-GSK3A/GSK3B	
pan-RAS		Phospho-p38 MAPK		MET		INPP4B	
BRAF		Phospho-p44/42 MAPK ERK 1/2		Phospho-AKT1		PLCG1	
Phospho-c-RAF		Phospho-p90 RSK		Phospho-Tuberin		Phospho-PRAS40	
Phospho-JNK				Phospho-GSK3B			
Housekeeping Proteins	Histone H3	S6	GAPDH	Negative Controls	Rb IgG	Ms IgG1	Ms IgG2a

Supplemental Table S2: Quantified data from the DSP study which is expressed as counts normalized using the approach described in the methods. Statistical differences between the groups were determined via Linear Mixed Modeling (LMM) with Benjamin-Hochberg correction test.

Protein of Interest	Activin (+) Tumor n = 10	Activin (-) Tumor n = 8	Activin (+) Stroma n = 18	Activin (-) Stroma n = 20
αSMA	96.57 ± 28.61	87.03 ± 16.99	469.06 ± 123.14	646.45 ± 125.132
PanCK	8.89 ± 2.26	5.44 ± 2.07	3.06 ± 1.01	2.21 ± 0.56
CD45	2.35 ± 0.41	3.24 ± 0.73	6.67 ± 1.36	8.46 ± 1.28
CD4	1.17 ± 0.38	0.67 ± 0.16	2.16 ± 0.39	2.02 ± 0.21
CD8	1.04 ± 0.17	0.87 ± 0.18	2.63 ± 0.41	2.00 ± 0.32
CTLA-4	14.49 ± 5.33	7.56 ± 5.44	19.21 ± 5.56	3.66 ± 0.82
FOXP3	0.87 ± 0.28	0.21 ± 0.07	0.77 ± 0.20	0.27 ± 0.05
CD25	0.82 ± 0.27	0.27 ± 0.07	0.95 ± 0.20	0.36 ± 0.08
PD-1	1.97 ± 0.67	0.47 ± 0.13	1.47 ± 0.30	0.99 ± 0.16
HLA-DR	2.47 ± 0.97	2.70 ± 1.31	4.71 ± 1.33	7.17 ± 2.15
CD80	1.07 ± 0.35	0.16 ± 0.06	1.01 ± 0.28	0.32 ± 0.07
CD40	0.91 ± 0.26	0.28 ± 0.15	1.00 ± 0.31	0.46 ± 0.14
PLCG1	0.96 ± 0.23	0.40 ± 0.09	0.87 ± 0.20	0.41 ± 0.06
Phospho-PRAS40	1.47 ± 0.61	0.25 ± 0.07	1.17 ± 0.38	0.34 ± 0.06
Phospho-Tuberin	0.97 ± 0.28	0.22 ± 0.06	0.79 ± 0.19	0.34 ± 0.07

Supplemental Table S3: P-values for each significantly differentially expressed protein found in the DSP analysis. Statistical differences between the groups were determined via Linear Mixed Modeling (LMM) with Benjamin-Hochberg correction test.

Protein of Interest	Activin (+) Tumor vs. Activin (-) Tumor	Activin (+) Stroma vs. Activin (-) Stroma	Activin (+) Tumor vs. Activin (+) Stroma	Activin (-) Tumor vs. Activin (-) Stroma
αSMA	*n.s.* p = 0.881	** p = 0.0046	**** p = 0.00008	**** p = 5.997 x 10^{-7}
PanCK	* p = 0.0186	*n.s.* p = 0.316	**** p = 0.000036	* p = 0.036
CD45	*n.s.* p = 0.182	* p = 0.0283	*** p = 0.00012	*** p = 0.00012
CD4	*n.s.* p = 0.164	*n.s.* p = 0.596	* p = 0.0242	*** p = 0.00023
CD8	*n.s.* p = 0.511	*n.s.* p = 0.304	* p = 0.0138	* p = 0.0227
CTLA-4	** p = 0.0033	*** p = 0.0003	*n.s.* p = 0.651	*n.s.* p = 0.417
FOXP3	** p = 0.00498	* p = 0.0151	*n.s.* p = 0.775	*n.s.* p = 0.375
CD25	* p =0.0286	** p = 0.0093	*n.s.* p = 0.824	*n.s.* p = 0.836
PD-1	* p = 0.0104	*n.s.* p = 0.504	*n.s.* p = 0.668	*n.s.* p = 0.0628
HLA-DR	*n.s.* p = 0.462	** p = 0.00604	*n.s.* p = 0.0552	*** p = 0.00041
CD80	*** p = 0.00032	** p = 0.0027	*n.s.* p = 0.899	*n.s.* p = 0.111
CD40	* p = 0.0131	* p = 0.0231	*n.s.* p = 0.725	*n.s.* p = 0.122
PLCG1	* p = 0.0245	* p = 0.0218	*n.s.* p = 0.576	*n.s.* p = 0.820
Phospho-PRAS40	** p = 0.0045	* p =0.023	*n.s.* p = 0.538	*n.s.* p = 0.357
Phospho-Tuberin	** p = 0.0013	** p = 0.00598	*n.s.* p = 0.663	*n.s.* p = 0.272