Supportive Information

Human colon cancer epithelial cells harbor active HEDGEHOG-GLI signaling that is essential for tumor growth, recurrence, metastasis and stem cell survival and expansion

Frédéric Varnat[1], Arnaud Duquet[1], Monica Malerba[1], Marie Zbinden[1], Christophe Mas[1], Pascal Gervaz[2] and Ariel Ruiz i Altaba[1]

University of Geneva Medical School
[1]Dept. Genetic Medicine and Development
1 rue Michel Servet
CH-1211 Geneva
[2]Dept of Surgery, Geneva University Hospital
24 rue Micheli-du-Crest
CH-1211 Geneva, Switzerland

Supplementary Table and Figures

Supplementary Table 1

List and medical data of all CC samples used in this study. Tumors are grouped by TNM stage.

Supplementary Figure 1

Representative images of CC frozen sections obtained fresh from the operating room stained with anti-CARCINOEMBRYONIC antigen or Pan-CYTOKERATIN antibodies (A) or with anti-βCATENIN (B) antibodies. In (A) nuclei are stained with DAPI and are shown in blue whereas specific staining is shown in red. Immunolabelings in (B) were developed with HRP-coupled antibodies and DAB. The same tumors (e.g. mCC1) often had regions of mostly nuclear (arrows) or mostly nucleocytoplasmic labeling. Other tumors had mostly membrane labeling (CC7). mCC11 had only nuclear labeling. There was no correlation between the pattern of labeling and response to HH-GLI inhibition. Scale bar = 40 µm.

Supplementary Figure 2

Heat map with numerical values corresponding to Fig 2A. Values are ratios of relative expression levels in $CD133^+$ over those in $CD133^-$ populations after normalization with housekeeping genes.

Supplementary Figure 3

A) Effects of two independent sets of siRNAs specific for each *GLI* mRNA on HT29 CC cells measuring proliferation by the BrdU index and apoptosis by the activated Caspase 3 index. Proliferation is measured by the phospho-Histone H3 (P-Histone3) labeling index (number of $P\text{-Histone3}^+$ cells over the total number of cells labeled by DAPI; top left) or by BrdU incorporation (top right). Apoptosis was measured by the activated Caspase3 index (number of activated $Caspase3^+$ cells over total number of $DAPI^+$ cells; bottom). Asterisks here and in all panels denote significative changes ($p<0.05$).

B) Histogram of the pro-proliferative effect of shPTCH1 lentivirus on transduced HT29 cells, shown as an increase in the BrdU index.

C) Rescue of the anti-proliferative effects of cyclopamine (cyc) by GLI1 as compared with tomatidine (tom) in HT29 cells transfected with GFP-expressing plasmids or

GFP- plus GLI1-expressing plasmids. The values are given as the fraction of proliferating ($BrdU^+$) cells that were also GFP^+ and equating this ratio to 1 in the control tomatidine in both conditions.

Supplementary Figure 4

A) Histograms of the effects of the concentration-dependent effects of cyclopamine (cyc) of Caco2 CC cells in vitro as compared with tomatidine (tom) as control. Numbers refer to concentrations in μM.

B) Anti-proliferative (left) and pro-apoptotic (right) effects of cyclopamine on HT29 CC cells.

C) Representative example of the anti-proliferative effects of 10μM cyclopamine (cyc) treatment vs. treatment with 10μM tomatidine (tom) in HT29 cells. Incorporated BrdU in cells that divided during the treatment period before fixation are revealed by indirect immunofluorescence (red).

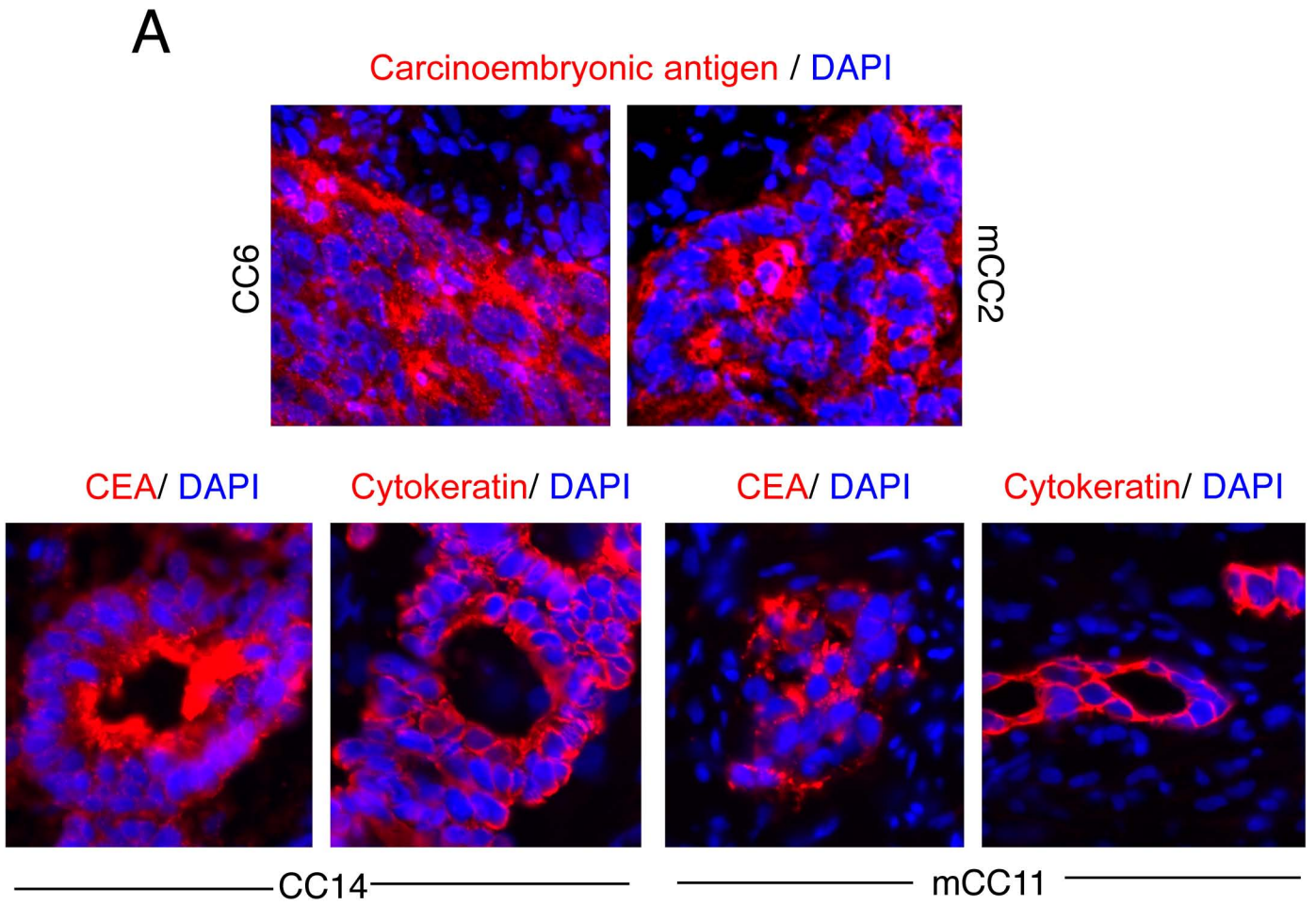
D) Representative example of increased apoptosis by siRNA against *GLI1* (siGLI1) as compared with a control siRNA (siC). Apoptotic cells express activated Caspase 3 protein shown by indirect immunofluorescence (green).

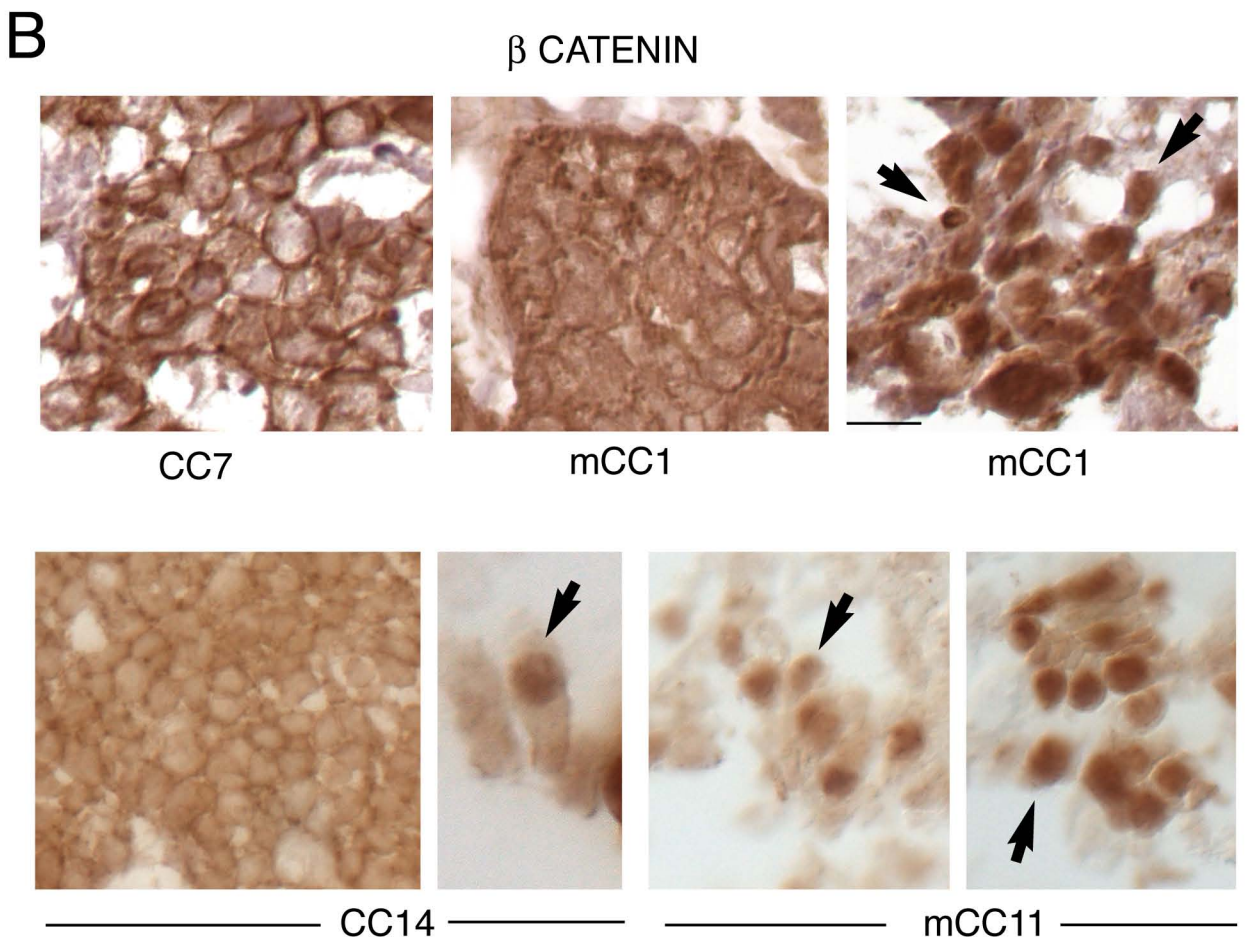
Scale bar = 150μm (C,D).

Sample	Location	TNM	Stage	Gender	Age	Treatment
CC21	Colon L	T2N0M0	1	F	84	
CC34	Rectum	Adenoma	1	M	46	
CC2	Colon R	T4N0M0	2	M	62	
CC6	Colon R	T3N0M0	2	M	62	
CC7	Colon R	T3N0M0	2	F	85	
CC11	Colon L	T3N0M0	2	M	67	
CC16	Colon L	T3N0M0	2	M	67	
CC24	Colon R	T3N0M0	2	M	68	
CC28	Colon R	T3N0M0	2	M	87	
CC32	Colon R	T3N0M0	2	M	88	
CC33	Rectum	T3N0M0	2	F	66	
CC3	Colon L	T3N2M0	3	M	49	
CC5	Colon L	T2N1M0	3	M	83	
CC8	Colon L	T4N1M0	3	F	58	
CC13	Rectum	T4N1M0	3	M	77	
CC15	Colon L	T4N1M0	3	M	69	
CC20	Colon L	T4N1M0	3	M	75	
CC29	Colon L	T4N1M0	3	M	62	
CC30	Colon L	T3N1M0	3	M	85	
CC36	Colon L	T3N2M0	3	F	57	
CC4	Colon R	T4N2M1	4	M	53	
CC9	Colon L	T3N0M1	4	F	54	CF-AVA
CC10	Rectum	T3N2M1	4	F	74	
CC14	Colon L	T4N2M1	4	F	73	
CC18	Colon L	T3N2M1	4	M	73	
CC19	Colon R	na	4	F	81	
CC23	Colon L	T4N2M1	4	M	57	
CC25	Colon R	T4N2M1	4	F	82	
CC31	Colon R	T4N2M1	4	M	64	
mCC1	Liver Metastasis	T0N1M1	4	F	66	OCFL
mCC2	Liver Metastasis	T3N2M1	4	M	64	OCFL-AVA
mCC3	Liver Metastasis	T3N1M1	4	F	49	OCFL
mCC4	Liver Metastasis	T4N2M1	4	M	61	OCFL
mCC6	Liver Metastasis	T3N1M1	4	F	65	OCFL-AVA
mCC7	Liver Metastasis	T3N1M1	4	F	75	
mCC8	Liver Metastasis	T3N0M1	4	F	75	
mCC9	Liver Metastasis	T4N2M1	4	F	73	
mCC11	Liver Metastasis	T3N0M1	4	M	84	
mCC17	Liver Metastasis	T4N0M1	4	F	76	OCFL-AVA
mCC19	Liver Metastasis	T4N2M1	4	M	64	

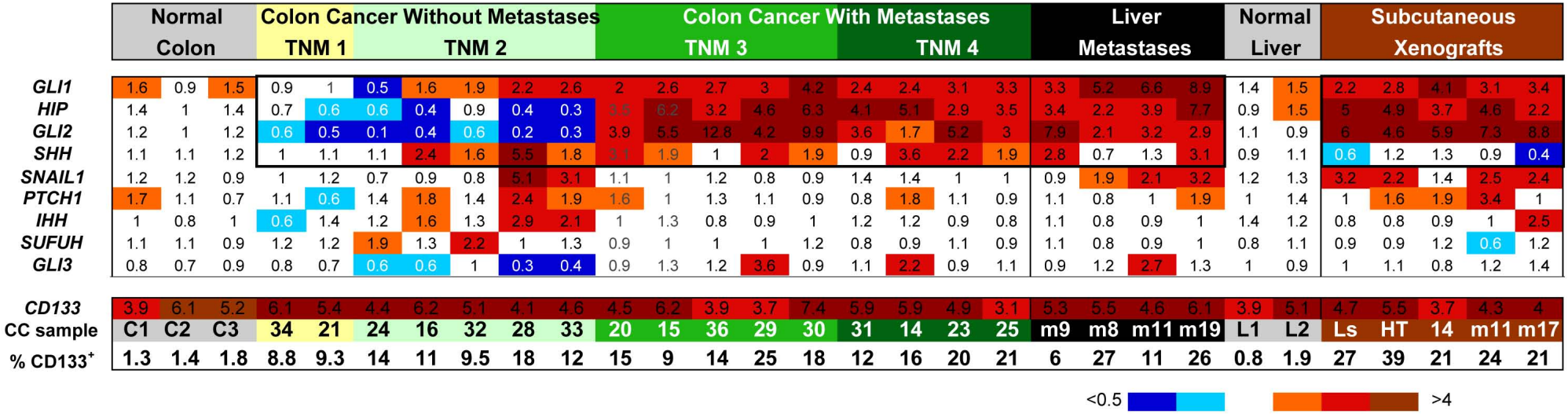
	L= left			F= female		O= Oxaliplatin
	R= right			M= male		C= Irinotecan
				deceased		F= 5-Fluorouracil
						L= Leucovirin
						AVA= Avastin

Supplementary Table 1 Varnat et al.

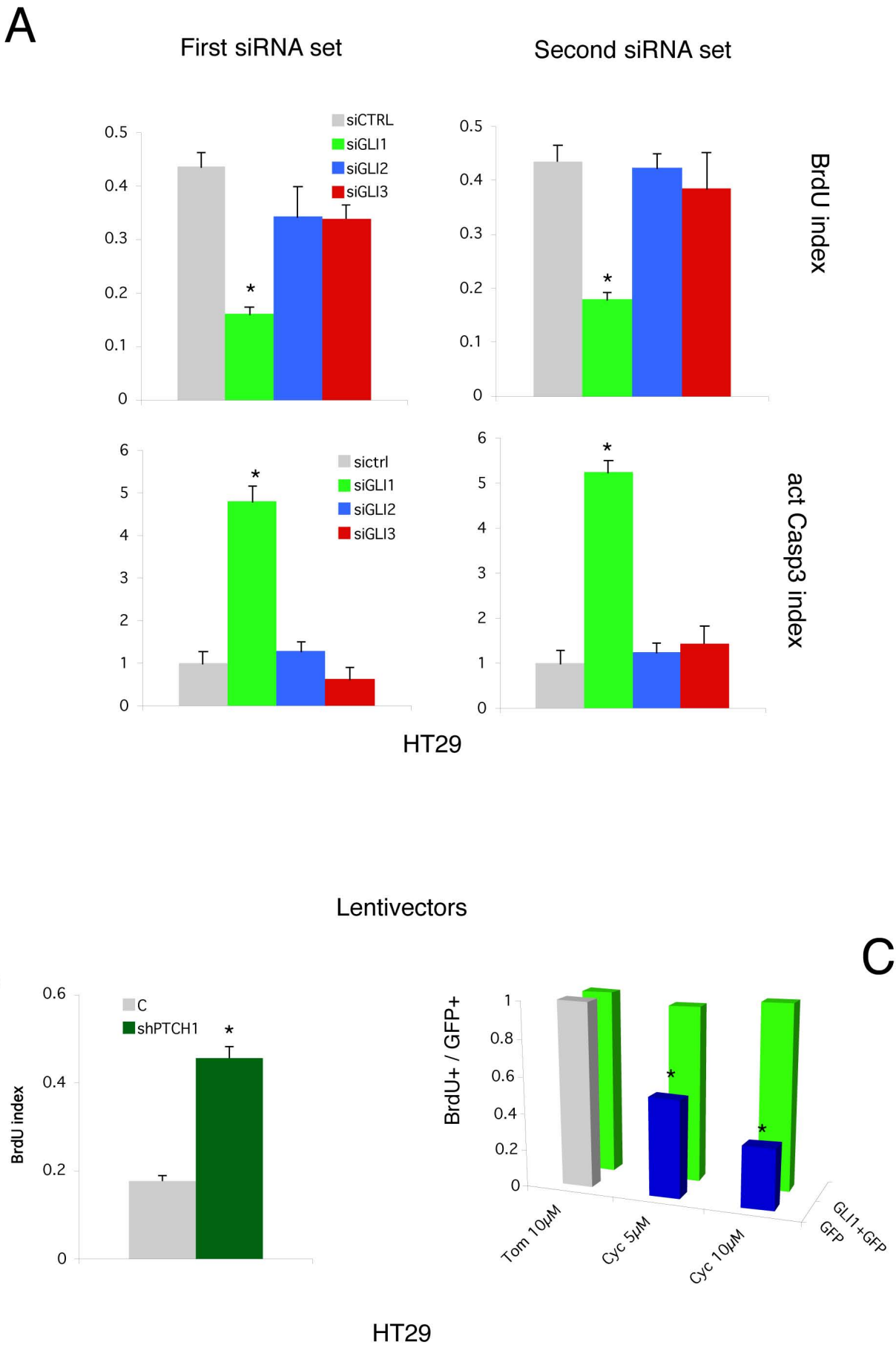




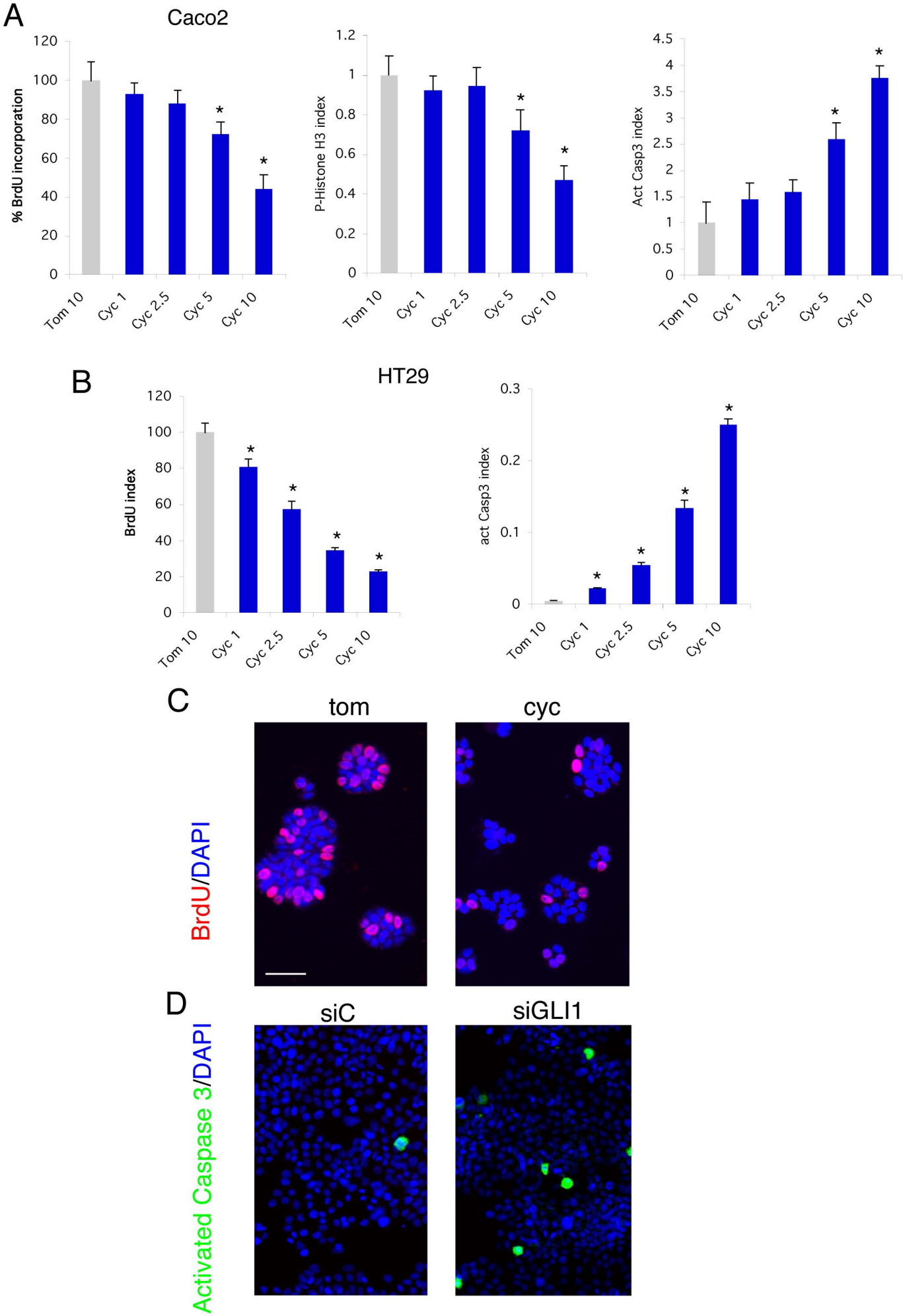
Suppl. Figure 1 Varnat et al.



Suppl Fig. 2 Varnat et al.



Supp. Figure 3. Varnat et al.



Suppl. Figure 4 Varnat et al.