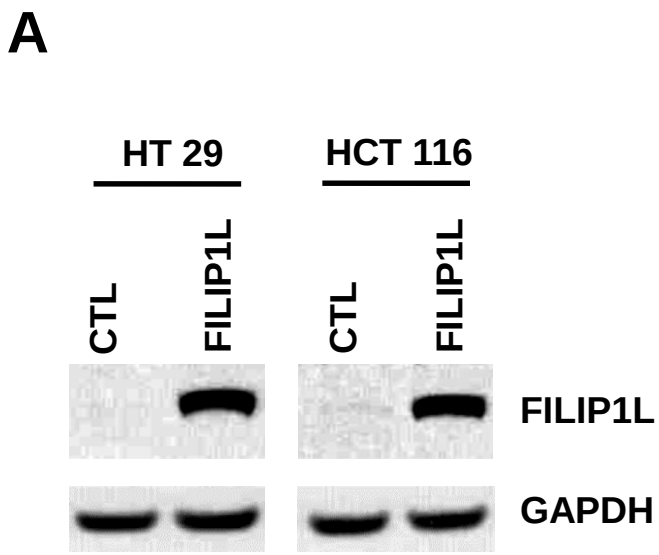
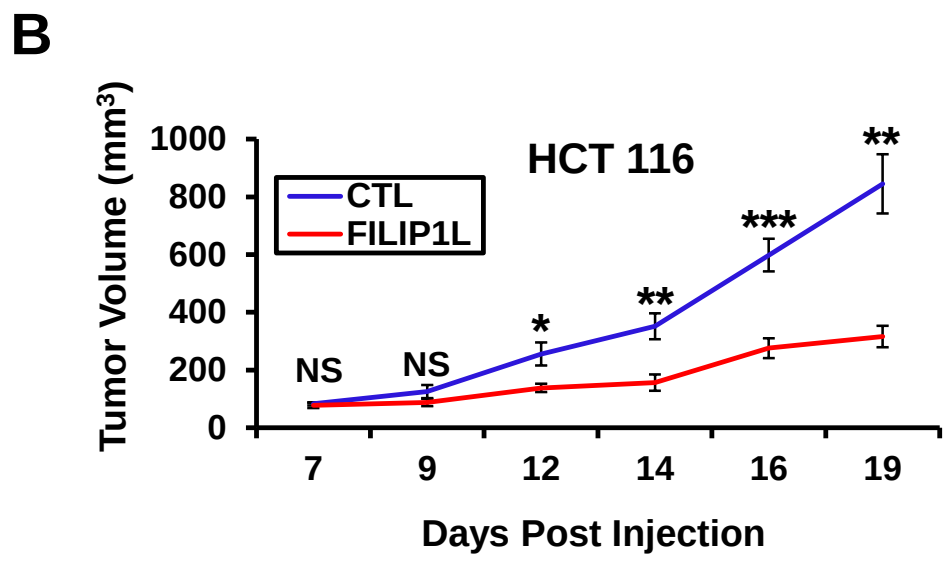
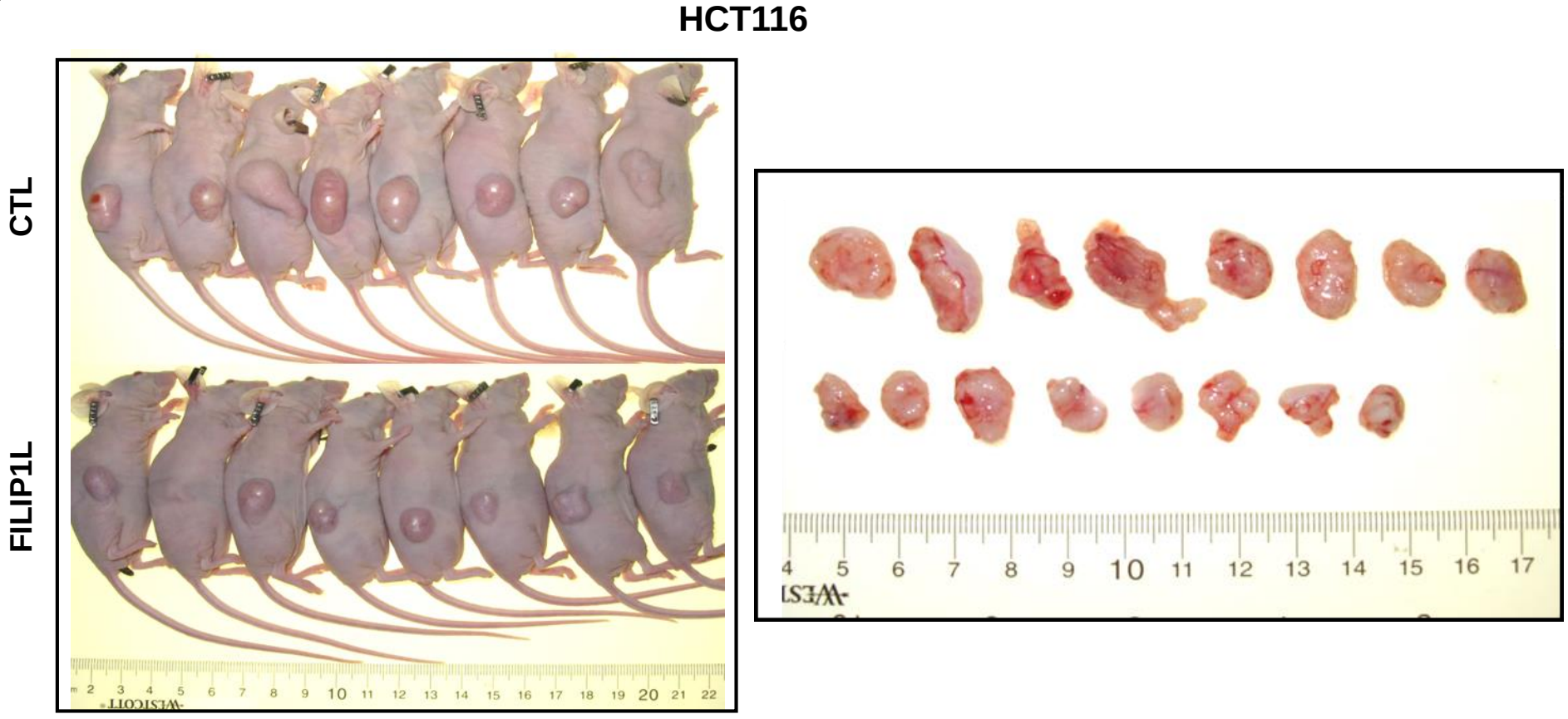
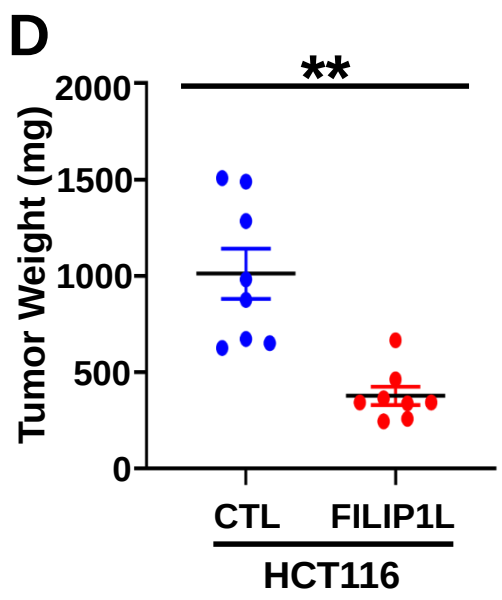


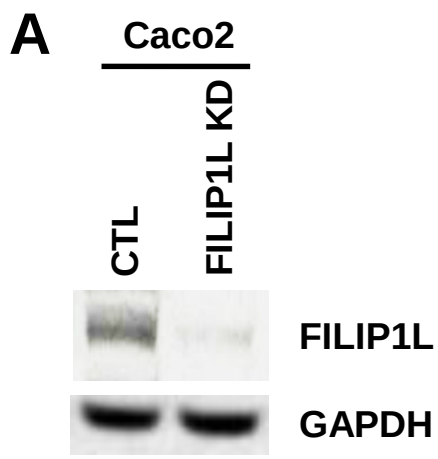


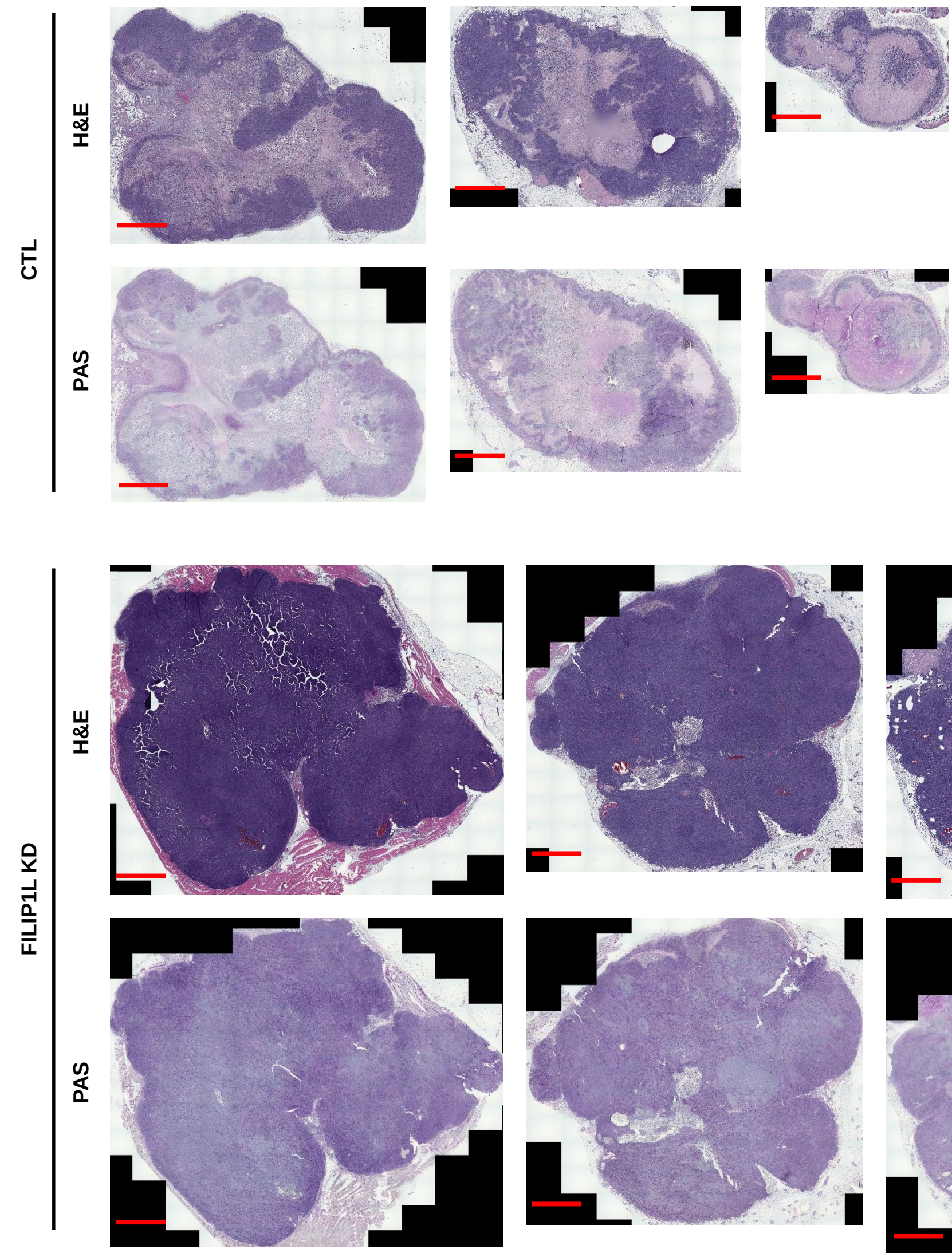




(A) FILIP1L overexpression was achieved by stable expression of lentiviral construct in low FILIP1L-expressing HT29 and HCT116 colon cancer cells. Control (CTL) clones were generated from the empty lentiviral construct. FILIP1L and GAPDH control were detected by immunoblotting. By densitometric quantification, FILIP1L protein was increased by 51-fold and 32-fold in HT29 and HCT116 clones compared to their corresponding controls, respectively. **(B-D)** HCT116 clones ($4x10^6$ cells) of either control or FILIP1L+ derivatives were subcutaneously injected into the Nude mice (8 mice per cell line). **(B)** Tumor growth was measured, every 2-3 days for a total of 19 days. The *y* axis represents tumor volume that was calculated by the formula: (length x width x height x 0.52). **(C)** Pictures of mice and xenograft tumors at the time of sacrifice (19 days) are shown. **(D)** Tumor weights from 8 mice are shown. *, ** and *** indicate $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

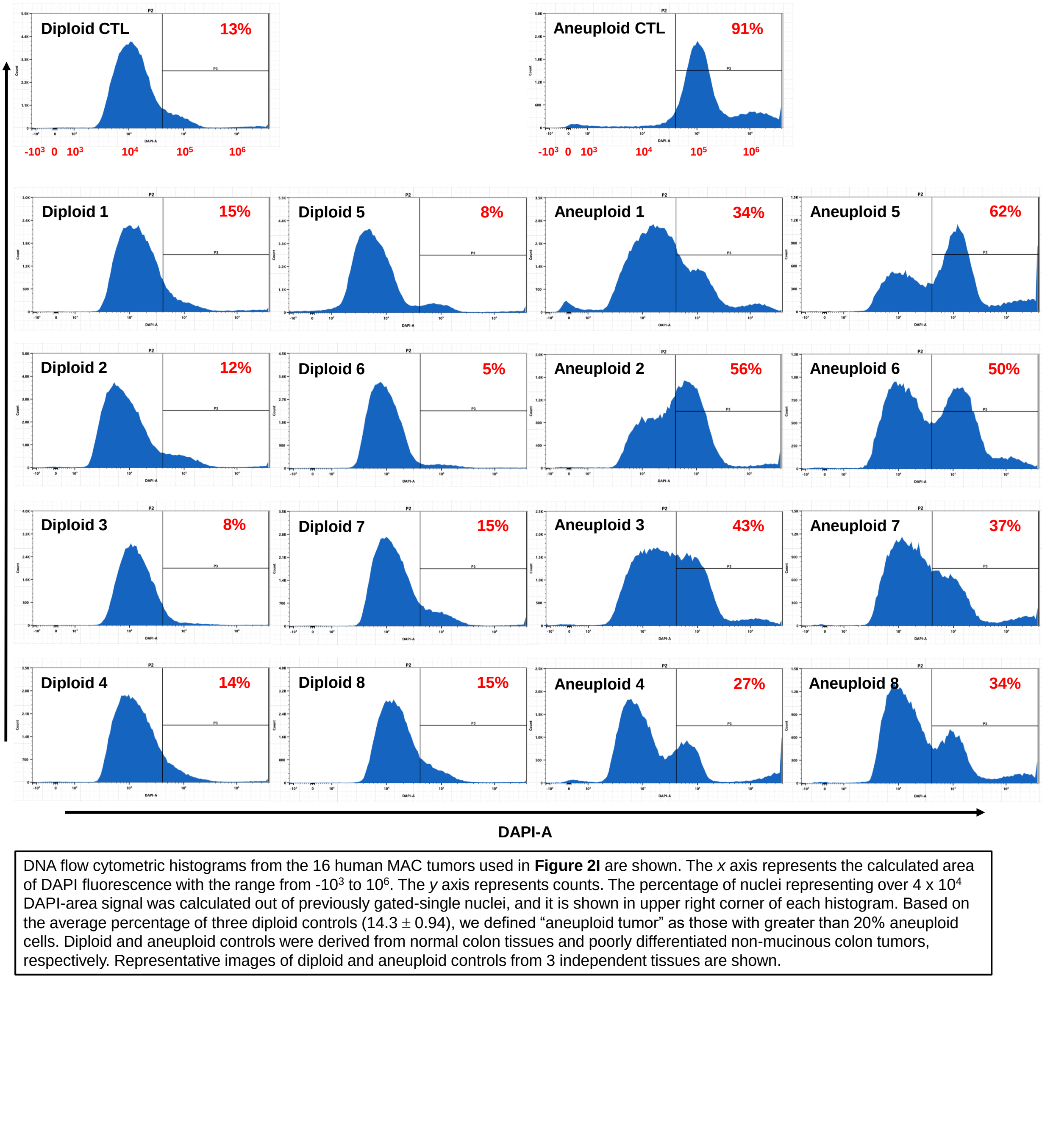
Suppl. Fig. S2





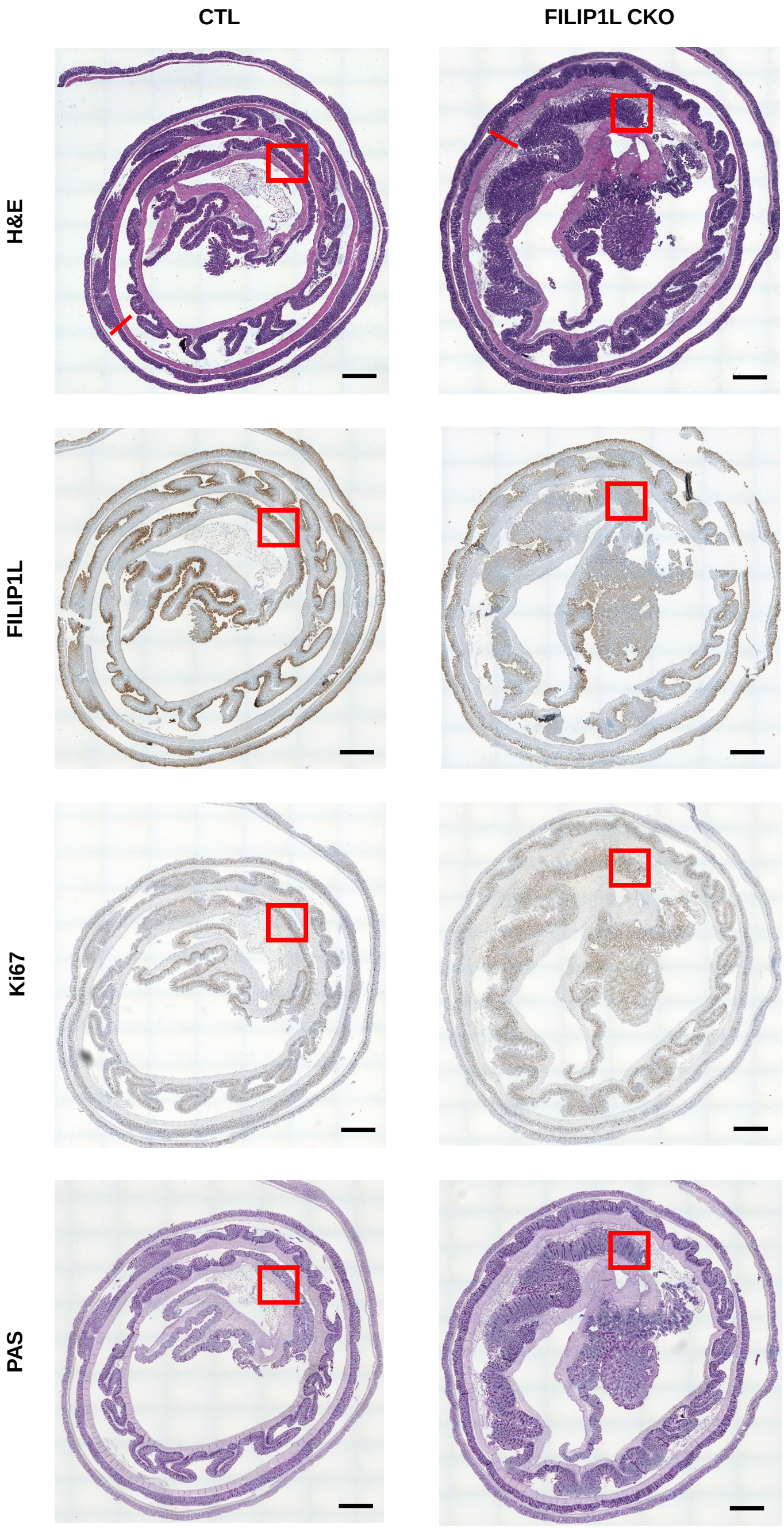
(A) FILIP1L knockdown was achieved by stable expression of Lentiviral shRNA in Caco2 colon cancer cells. Control clones were made with scrambled shRNA. FILIP1L and GAPDH control were detected by immunoblotting. **(B)** Caco2 clones ($5x10^6$) of either control or FILIP1L-knockdown derivatives were subcutaneously injected into the Nude mice (8 mice per cell line). Caco2 xenograft tumors from both groups were fixed, and stained with H&E (top panel) and PAS (bottom panel). Whole tumors were imaged by stitching methods. Representative three tumors from each group are shown. Scale bar = 2000 µm.

Suppl. Fig. S3



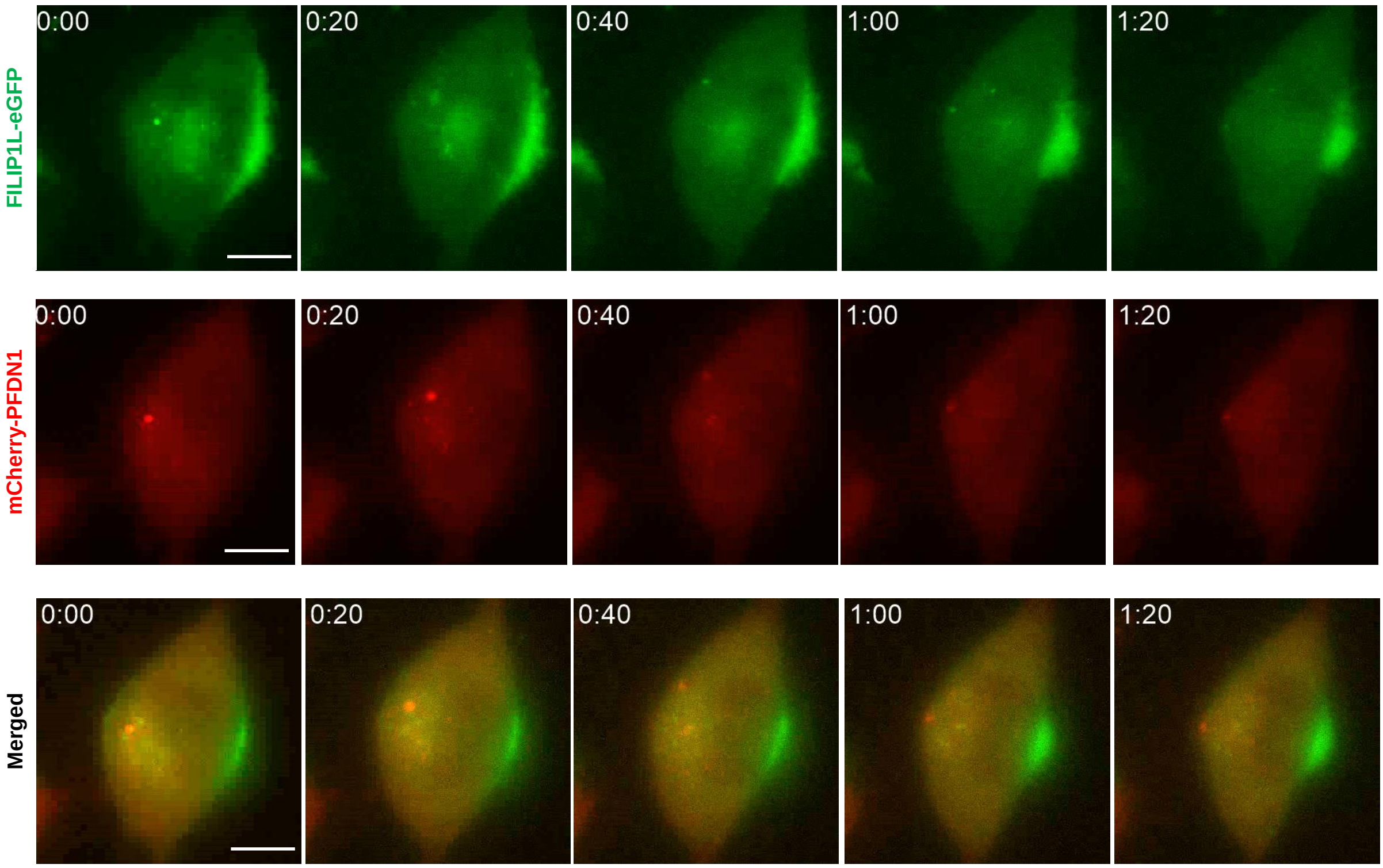
DNA flow cytometric histograms from the 16 human MAC tumors used in **Figure 2I** are shown. The *x* axis represents the calculated area of DAPI fluorescence with the range from -10^3 to 10^6. The *y* axis represents counts. The percentage of nuclei representing over 4×10^4 DAPI-area signal was calculated out of previously gated-single nuclei, and it is shown in upper right corner of each histogram. Based on the average percentage of three diploid controls (14.3 ± 0.94), we defined “aneuploid tumor” as those with greater than 20% aneuploid cells. Diploid and aneuploid controls were derived from normal colon tissues and poorly differentiated non-mucinous colon tumors, respectively. Representative images of diploid and aneuploid controls from 3 independent tissues are shown.

Suppl. Fig. S4



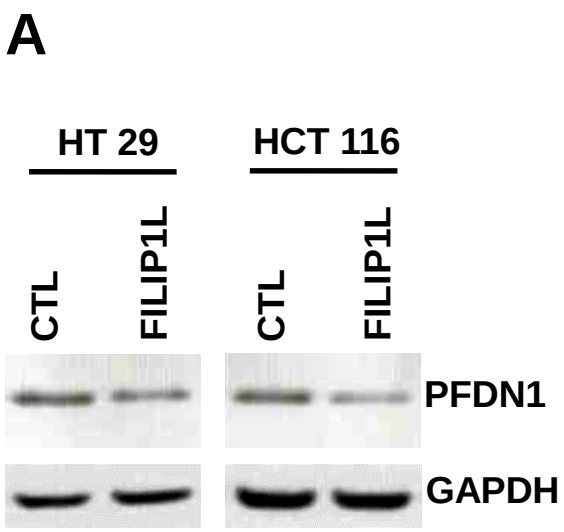
Swiss-rolled whole colons from Filip1l$^{fl/fl}$ (CTL) and Filip1l$^{fl/fl}$; Cdx2-CreER^{T2} (CKO) mice were fixed and stained with H&E and PAS. They were also immunohistochemically stained for FILIP1L and Ki67. Proximal colon (center) to rectum (outer end) of stitched images are shown. Boxed areas indicate the matching proximal colon regions that were captured as representative images in **Figure 3D-G**. The lines in H&E images indicate the junction between proximal and mid colon. Scale bar = 1000 µm.

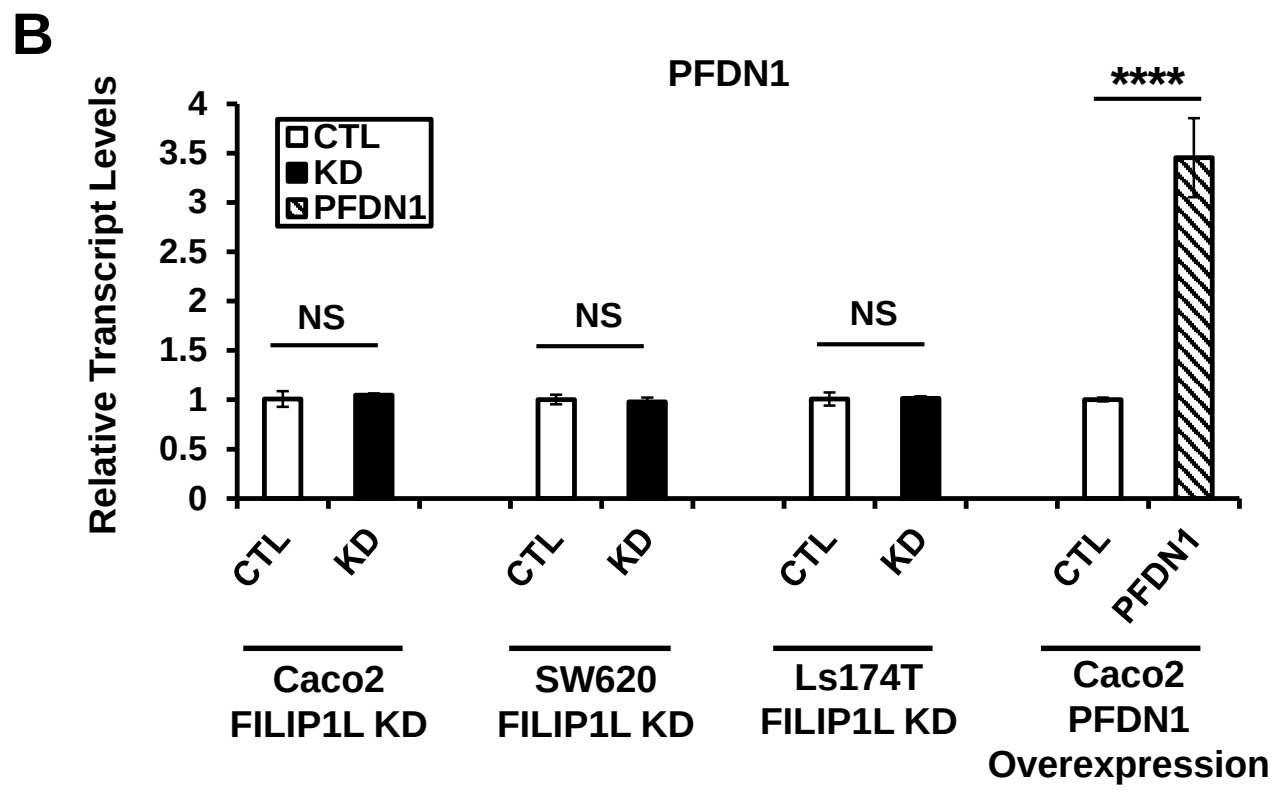
Suppl. Fig. S5

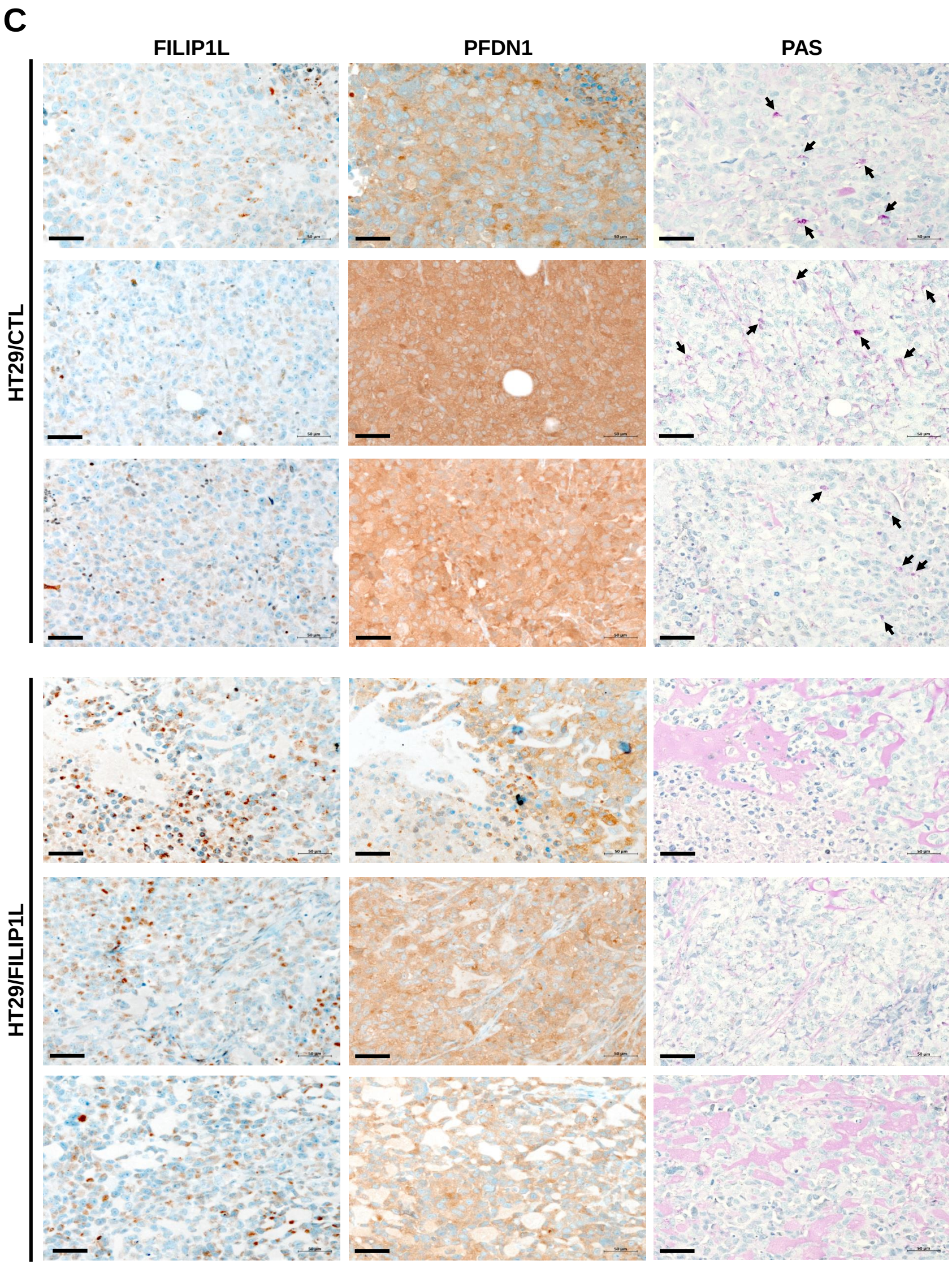


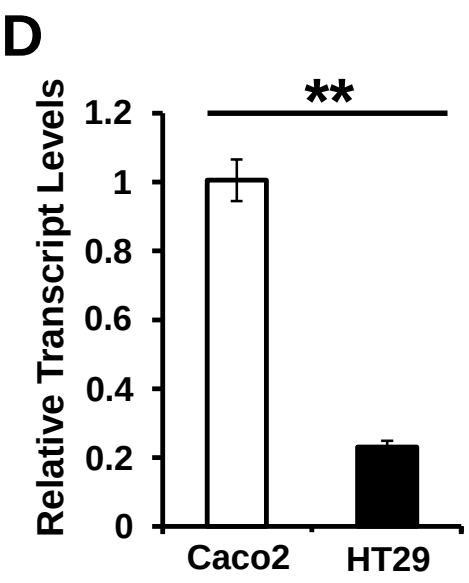
Time-lapse imaging of HEK293 cells transfected with FILIP1L-eGFP and mCherry-PFDN1. Representative tiled images are shown. Time is designated in the upper left (hr:min). Scale bar = 10 µm.

Suppl. Fig. S6-1

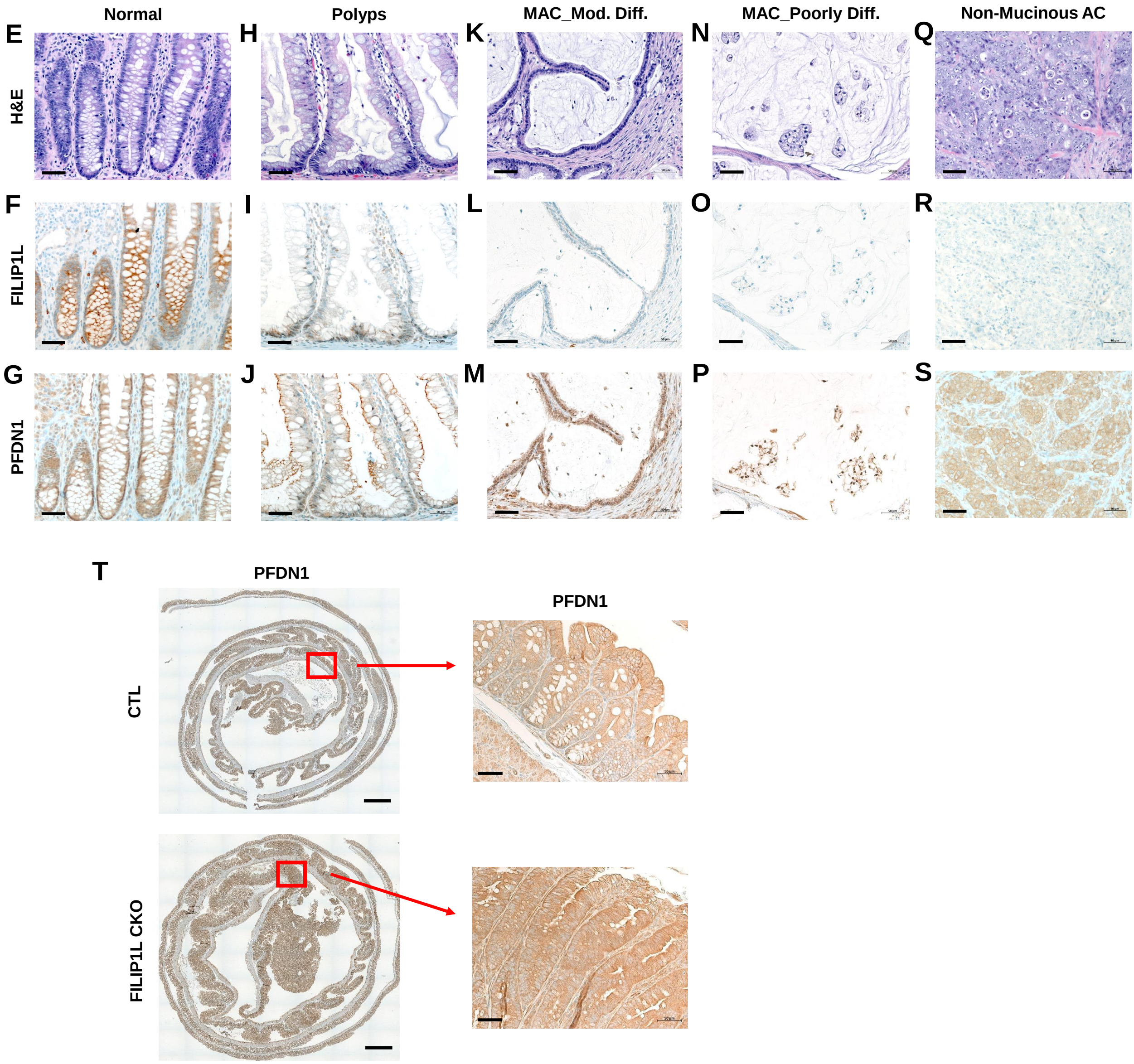




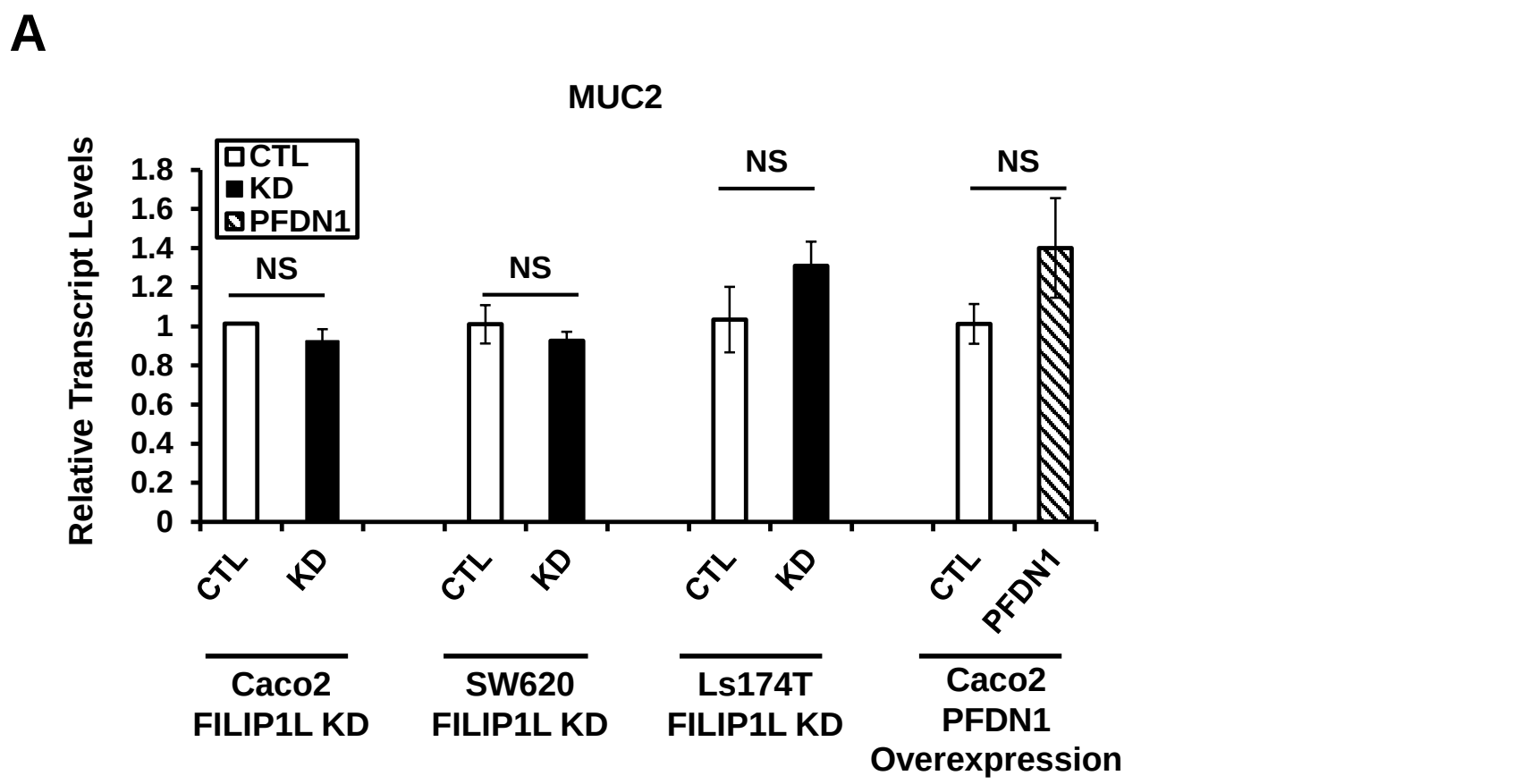


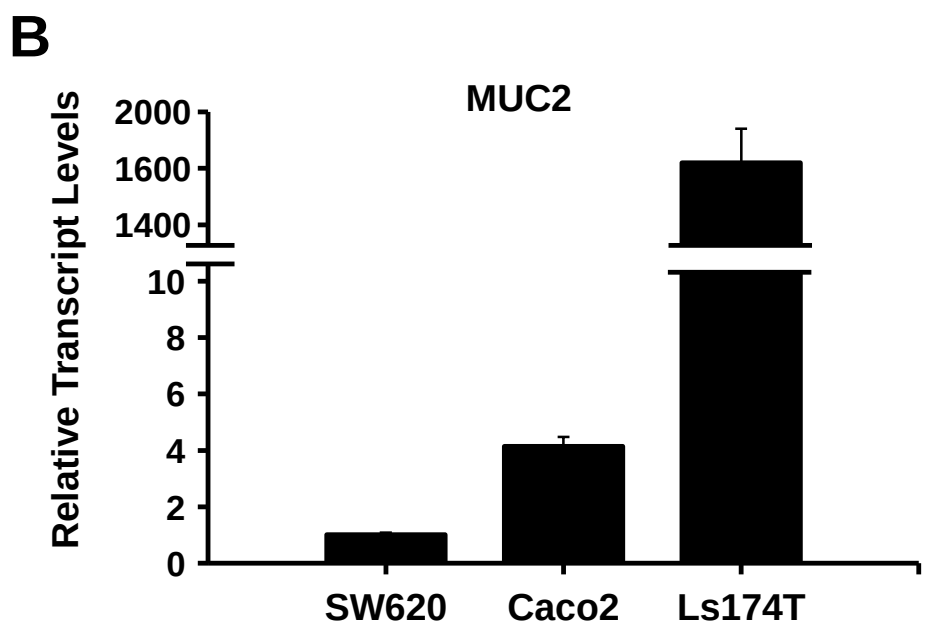


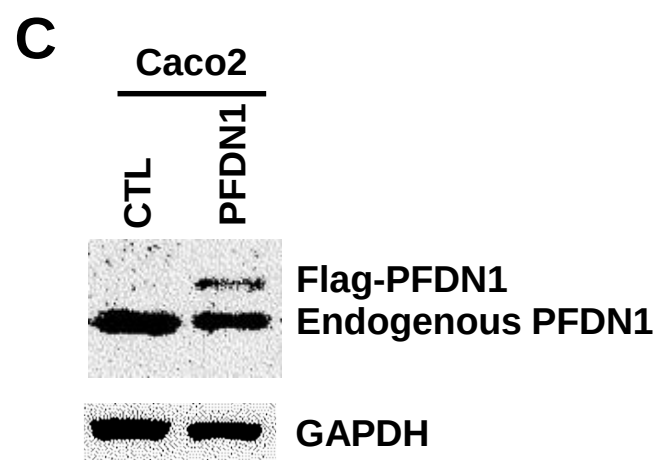
Suppl. Fig. S6-2



(A) FILIP1L overexpression was achieved by stable expression of lentiviral construct in low FILIP1L-expressing HT29 and HCT116 colon cancer cells. Control (CTL) clones were generated from the empty lentiviral construct. PFDN1 and GAPDH control were detected by immunoblotting. **(B)** mRNA levels of PFDN1 in clones from either control or FILIP1L knockdown (Caco2, SW620 and Ls174T clones) as well as those from either control or PFDN1 over-expression (Caco2 clones) were measured by qRT-PCR. The *y* axis represents fold change over their respective control clones, where each value was standardized with the housekeeping gene GAPDH. **** indicate $P<0.0001$. **(C)** HT29 xenograft tumors from either control or FILIP1L+ derivatives (shown in panel **A**) were fixed and immunohistochemically stained for FILIP1L and PFDN1. They were also stained with PAS. The exact same regions of each tumor were imaged. Note that tumors from FILIP1L+ derivatives displayed considerably less compact cells, and light pink regions in PAS-stained cells indicate acellular areas that were depleted of mucin. True mucin stains indicated by magenta color were only shown in control tumors (indicated by black arrows). Scale bar = 50 µm. **(D)** MUC2 mRNA levels in Caco2 and HT29 cells were measured by qRT-PCR. The *y* axis represents fold change over Caco2 cells, where each value was standardized with the housekeeping gene GAPDH. Note that HT29 cells express 5-fold less MUC2 mRNA compared to Caco2 cells, which explains considerably less PAS stain in panel **C** compared to that in **Figure 1G**. **(E-S)** H&E staining **(E, H, K, N, Q)**, FILIP1L **(F, I, L, O, R)** and PFDN1 **(G, J, M, P, S)** expression was analyzed in specimens from non-tumor adjacent colon tissues (NATs, n=16), serrated polyps (n=9), well/moderately differentiated mucinous adenocarcinoma (n=4), poorly differentiated mucinous adenocarcinoma (n=12) and poorly differentiated non-mucinous colorectal adenocarcinoma (n=7). Scale bar = 50 µm. **(T)** Swiss-rolled whole colons from $Filip1l^{fl/fl}$ (CTL) and $Filip1l^{fl/fl}$; Cdx2-$CreER^{T2}$ (CKO) mice were fixed and immunohistochemically stained for PFDN1. Proximal colon (center) to rectum (outer end) of stitched images are shown. Scale bar = 1000 µm. Magnified images (scale bar = 50 µm) from the boxed areas of matching proximal colon regions are also shown.

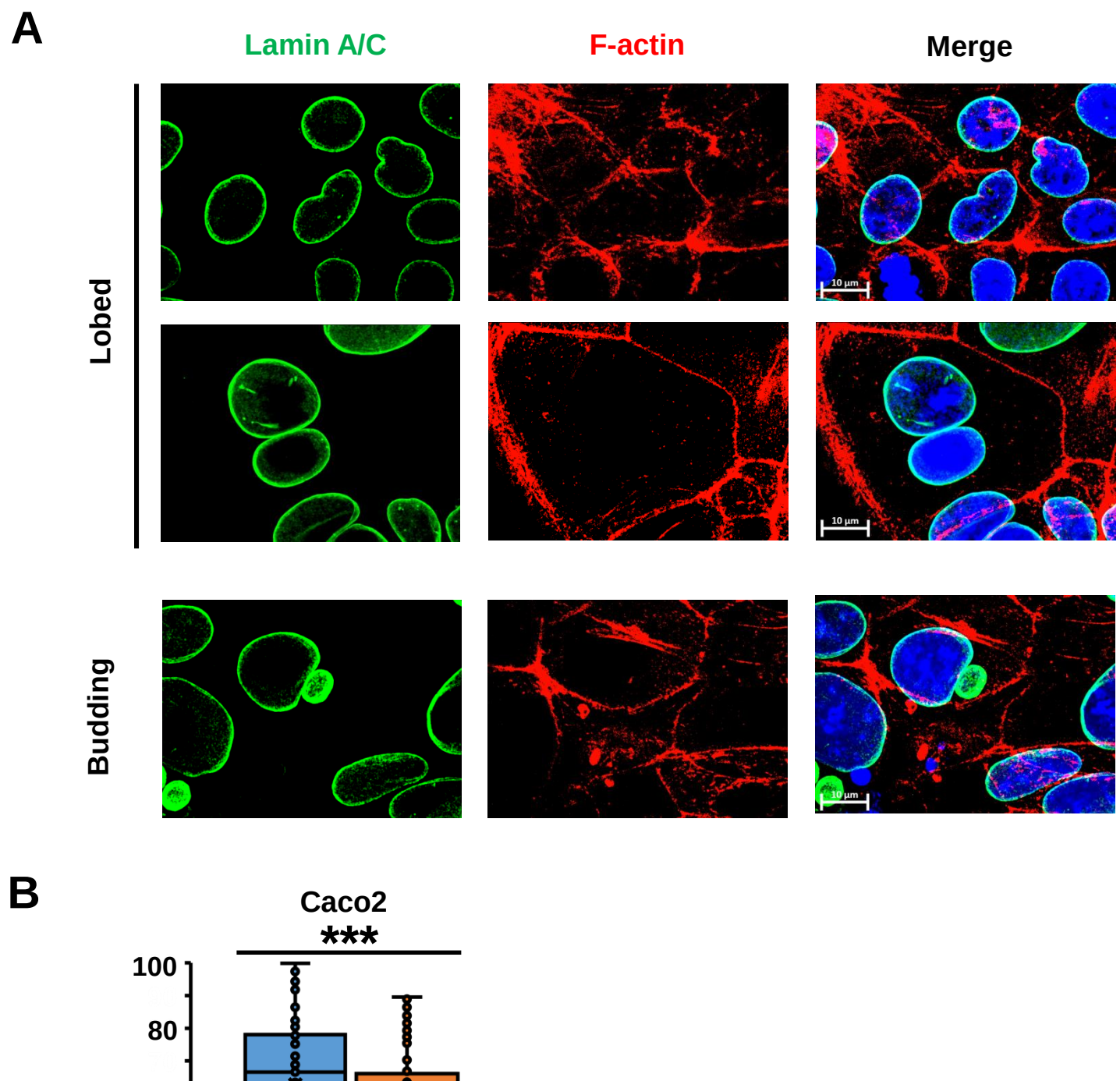


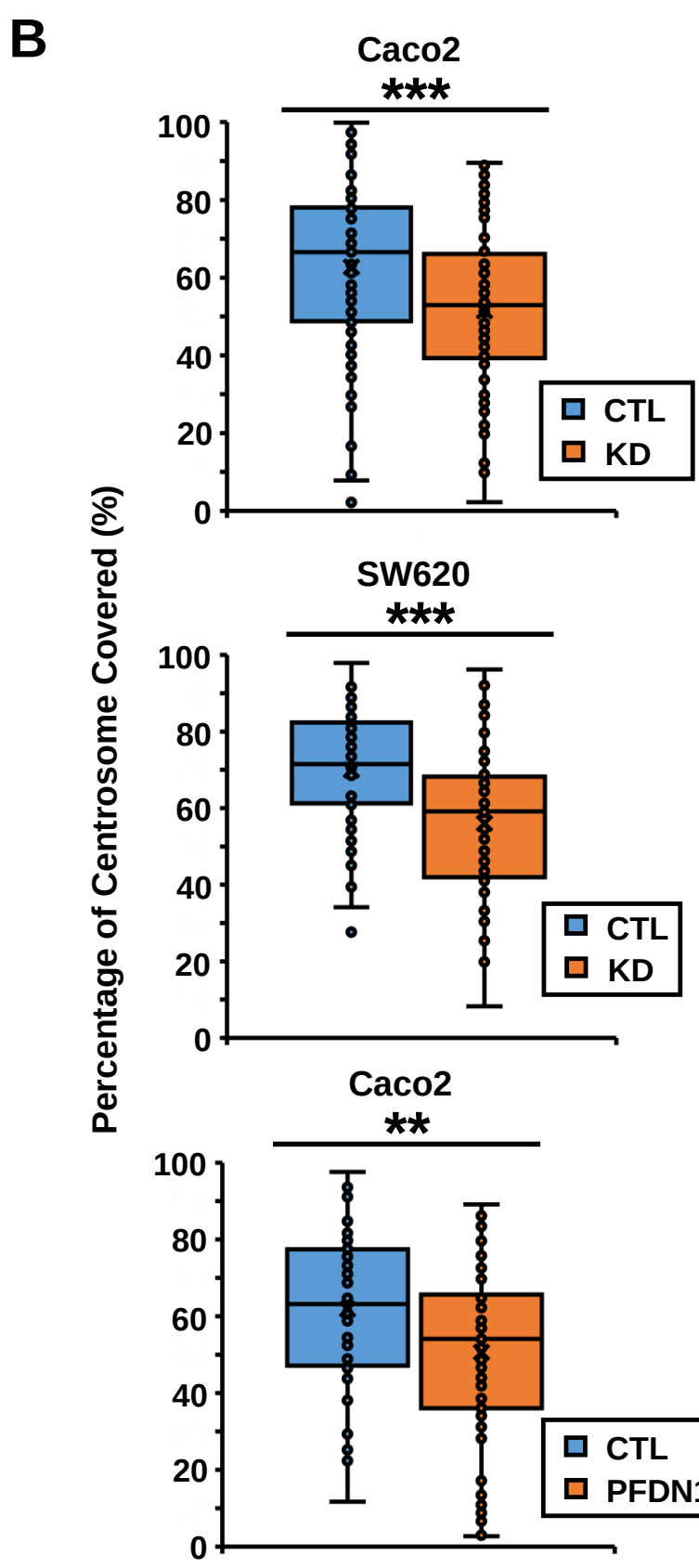




(A) mRNA levels of MUC2 in clones from either control or FILIP1L knockdown (Caco2, SW620 and Ls174T clones) as well as those from either control or PFDN1 over-expression (Caco2 clones) were measured by qRT-PCR. The *y* axis represents fold change over their respective control clones, where each value was standardized with the housekeeping gene GAPDH. **(B)** MUC2 mRNA levels in Caco2, SW620 and Ls174T cells were measured by qRT-PCR. The *y* axis represents fold change over SW620 cells, where each value was standardized with the housekeeping gene GAPDH. **(C)** PFDN1 over-expression was achieved by stable expression of Flag-tagged lentiviral construct in Caco2 colon cancer cells. Control (CTL) clones were generated from the empty lentiviral construct. PFDN1 and GAPDH control were detected by immunoblotting.

Suppl. Fig. S8





(A) FILIP1L knockdown clones from Caco2 cells were stained for lamin A/C (green), F-actin (red) and DAPI (blue). Representative images (either lobed or budding) for the criteria of multi-nuclei quantification are shown. Scale bar = 10 µm. **(B)** Clones from either control or FILIP1L knockdown (Caco2 and SW620 clones) as well as those from either control or PFDN1 over-expression (Caco2 clones) were stained for pericentrin (green), PFDN1 (red) and DAPI (blue), and the area of centrosome occupied by PFDN1 in metaphase-cells was quantified. The *y* axis represents the percentage of centrosome coverage in each metaphase-cells. 42-92 cells were counted. ** and *** indicate $P<0.01$ and $P<0.001$, respectively.

Supplementary Information

Target protein for antibody	Vendor	Catalogue#	Application
Flag tag	Sigma	A2220	IP
HA tag	Sigma	A2095	IP
Ubiquitin	Cytoskeleton	UBA01	IP
HA tag	BioLegend	901501	IB
Flag tag	Sigma	F1804	IB, IF
FILIP1L	Atlas	HPA043133	IB, IF
PFDN1	Thermo	PA5-52304	IB, IF
GAPDH	Sigma	MAB374	IB
α Tubulin	Santa Cruz	sc-23948	IF
γ Tubulin	Santa Cruz	sc-7396	IF
Centriolin	Santa Cruz	sc-365521	IF
Pericentrin	Abcam	ab28144	IF
Pericentrin	Abcam	ab4448	IF
ZO-1	Thermo	61-7300	IF
F-actin (Alexa Fluor™ 488 Phalloidin)	Thermo	A12379	IF
F-actin (Alexa Fluor™ 568 Phalloidin)	Thermo	A12380	IF
Lamin A/C	Cell Signaling	4777	IF
MUC2	Santa Cruz	sc-7314	IF
FILIP1L	Aviva	ARP52360_P050	IHC
PFDN1	Atlas	HPA006499	IHC
Ki-67	Cell Signaling	9449	IHC
Periodic Acid Schiff (PAS) Stain Kit	Newcomer Supply	9162B	

IP: Immunoprecipitation, IB: Immunoblot, IF: Immunofluorescence, IHC: Immunohistochemistry

SYBR Primer list

Gene (official symbol)	Forward primer	Reverse primer
PFDN1	CAGAACGAAAAAGCATGCAC	CTTCGTGCCATCAGCATCT
MUC2	CACCTGTGCCCTGGAAGGC	CGGTCACGTGGGGCAGGTTC
GAPDH	TGCACCACCAACTGCTTAG	AGAGGCAGGGATGATGTTC
mFilip1l	CAGACTACAAGAGCCTCATTCC	AGCACAGACTCCTCCTCATTA
mGAPDH	ATCACTGCCACCCAGAAGACT	CATGCCAGTGAGCTTCCCGTT
mAtoh1	GGGGTTGTAGTGGACGAGC	CGTTGTTGAAGGACGGGATAAC
mSpdef	AAGGCAGCATCAGGAGCAATG	CTGTCAATGACGGGACACTG
mNeurog3	CCAAGAGCGAGTTGGCACT	CGGGCCATAGAAGCTGTGG
mMuc2	ATGCCCACCTCCTCAAAGAC	GTAGTTTCCGTTGGAACAGTGAA
mAgr2	CCTGCTTCTTGTGGCCATTT	GGGTCTGTTGCTTGTCTTGG
mActb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT

Quantification procedures for crypt length measurement

Steps 1-4 and 6 were performed in EVOS Analysis Software (ThermoFisher).
Steps 5, 7 and 8 were performed in Microsoft Excel Software.

1. Open previously acquired raw stitched image of Swiss-rolled whole colon in EVOS Analysis Software. The tiff file should contain calibration data required for measurement.
2. Under the Measurements and Annotations tool, select the 'draw line' icon.
3. Starting at the center of the sample (proximal colon), select crypts with an open longitudinal axis for analysis.
4. Draw a line from the basal side of the bottom most crypt cell to the apical side of the surface epithelium.
    a. If the walls of the crypt are uneven, select the midpoint as the point of measurement.
5. Record the measurement in a table.
6. Repeat steps 4 and 5, spiraling outward toward the rectal end of the colon.
7. Note the transition from the proximal to middle colon in the table.
8. Run a t-test on comparative groups.

Quantification procedures for Ki67 area

Steps 1-2 were performed in ZEN 3.1 software (Zeiss).

Steps 3-8 were performed in CellProfiler 2.1.1 software.

Steps 9-10 were performed in Microsoft Excel software.

1. Acquire Z-stack images of desired tissue (IF stained for Ki67 and DAPI) from randomly selected representative areas.
2. Create a maximum intensity projection TIFF image from all Z-stacks.
3. Split the RGB TIFF image into two grayscale images.
4. Threshold the DAPI (blue) grayscale image to include DAPI positive stained areas.
5. Using the binary image from step 4, create an image mask on the Ki67 (green) grayscale image.
6. Threshold the masked Ki67 grayscale image to include positive Ki67 stained areas within the positive DAPI stained area.
7. Measure the image area occupied by positive Ki67 staining from step 6.
8. Measure the image area occupied by positive DAPI staining from step 4.
9. Divide the area from step 7 by the area from step 8 and multiply by 100 for the percentage of DAPI stained areas covered by positive Ki67 staining.
10. Repeat for all images, display the data on a box and whisker plot, and perform a t-test.

Quantification procedures for time-lapse imaging

Steps 1-4 and step 12 were performed in EVOS FL Auto 2 software (ThermoFisher).
Steps 5-11 and steps 13-15 were performed in Microsoft Excel Software.
Steps 16-18 were performed in Celleste software (ThermoFisher, Version 4.1.1).

1. Acquisition parameters determined by prior experiments were set at a laser intensity of 0.65, exposure time of 60 msec and gain of 1.4 for fluorescent channels. Phase contrast was set at intensity of 0.06, exposure time of 6.3 msec and gain of 1.
2. Acquire fluorescent and phase contrast images of 40 random fields on EVOS FL Auto 2 microscope with EVOS Onstage Incubator with conditions of 37°C, 5% CO_2, 20% O_2 and 80%

humidity. Z stack distance was set at 1.7 μm, with 5 minute acquisition intervals, experiment durations of 4 hours.

3. In the EVOS FL Auto 2 software, open the scan using the review function.
4. Select the first field, play the time lapse and locate mitotic cells.
5. In a table, note the field number, location within the field and z-stack of best resolution for each channel.
6. Note the frame number for NEBD.
    a. Defined by loss of nuclear envelope, rounding of the cell and/or condensing of the nucleus.
7. Note the frame number for anaphase.
    a. Defined by separation of the chromatids and/or membrane invagination.
8. Note the frame number for completion of cytokinesis by phase contrast.
    b. Defined by membrane fission of two daughter cells.
9. Note cells that undergo prolonged cytokinesis/time to membrane fission.
    a. Defined as greater than the average time to membrane fission of experimental group.
10. Note cells of high resolution and signal as candidates for representative images.
11. In a separate column, subtract the anaphase and membrane fission columns from the NEBD column. The difference in frame numbers multiplied by the interval will give the time to mitosis and time to membrane fission in minutes.
12. Repeat for all acquired fields.
13. Run a t-test on comparative groups for both endpoints.
14. To generate images, review the table for cells previously identified as candidates.
15. Create separate folders for these image files organized by experiment date, field number, z-slice and channel. Copy the raw image files from scan folder into these folders.
16. Load image files, adjust image parameters and merge channels to create a composite image sequence.
17. Save individual frames to generate a tiled sequence of fluorescent signal, and fluorescent plus phase signal
18. Save entire frame range composite as sequence to generate a video file.

Quantification procedures for mucin 2 intensity in 3D cell clusters

Steps 1-2 were performed in ZEN 3.1 software (Zeiss).
Steps 3-9 were performed in CellProfiler 2.1.1 software.
Steps 10-11 were performed in Microsoft Excel software.

1. Acquire Z-stack images of desired cells (IF stained for F-actin, Mucin 2, and DAPI) displaying 3D growth. The stack distance should be 2.0 um.
2. Create a maximum intensity projection TIFF image using all Z-stacks.
3. Split the RGB TIFF image into three grayscale images.
4. Add the DAPI grayscale and Phalloidin grayscale images together. Summing these images ensures there is one large object identified per cell cluster rather than multiple small ones that complicate analysis.
5. Identify cell clusters as primary objects using the combined Phalloidin/DAPI grayscale image to yield one object per cell cluster.
6. Expand these objects slightly to include a small area around the cell cluster for intensity analysis.
7. Using the expanded cell cluster object from step 6, create an image mask on the Mucin 2 grayscale image.
8. Calculate the sum of pixel intensity in the masked Mucin 2 grayscale image.

9. Measure the area occupied by the pre-expanded cell cluster.
10. Divide the total pixel intensity from step 8 by the cell cluster area from step 9 to calculate the average pixel intensity per cell cluster.
11. Repeat for all images, display the data on a box and whisker plot, and perform a t-test.

Quantification procedures for multi-nuclei

Steps 1-2 were performed in ZEN 3.1 software (Zeiss).
Steps 3-8 and step 11 were performed in ImageJ software as well as Zen 3.1 software.
Steps 9-10 and steps 12-13 were performed in Microsoft Excel software.

1. Acquire Z-stack images of desired cells (IF stained for Lamin, ZO-1/F-actin, and DAPI) displaying 2D growth. The stack distance should be 0.55 um.
2. Create a maximum intensity projection TIFF image using all Z-stacks.
3. Open the TIFF image. Open the ZVI image to scan Z-stacks manually when nuclei are stacked on top of one another.
4. Count all normal nuclei.
5. Count all lobular nuclei.
    a. Cells that have an irregular nucleus. Lamin staining is useful in determining a smooth nuclear boundary versus an irregular one.
6. Count all fused nuclei.
    a. Cells that have multiple distinct nuclei within one ZO-1/F-actin boundary.
7. Count all mega nuclei.
    a. Cells that have a nucleus more than double the average size.
8. Count all budding nuclei.
    a. Cells that have one or more small budding nucleus.
9. Sum all of the counts per image.
10. Record all of the counts in a table.
11. Count the nuclei that overlap per image.
12. Record the number of overlapping cells.
13. Repeat for all images and perform a t-test.

Quantification procedures for PFDN1 occupancy in centrosomes

Steps 1-3 were performed in ZEN 3.1 software (Zeiss).

Steps 4-10 were performed in CellProfiler 2.1.1 software.

Steps 11-12 were performed in Microsoft Excel software.

1. Acquire Z-stack images of desired cells (IF stained for Pericentrin, PFDN1, and DAPI) with at least one metaphase cell per image. The stack distance should be 0.55 um.
2. Create a maximum intensity projection TIFF image from the range of Z-stacks that contain centrosomes for a chosen metaphase cell.
3. Crop the TIFF image down to contain just the metaphase cell.
4. Split the RGB TIFF image into three grayscale images.
5. Clean the Pericentrin grayscale image to reduce noise and smooth objects.
6. Identify centrosomes as primary objects using the cleaned Pericentrin grayscale image.
7. Using the centrosome objects from step 6, create an image mask on the PFDN1 grayscale image.

8. Threshold the masked PFDN1 grayscale image to clean background and ensure noise is removed.
9. Measure the image area occupied by positive PFDN1 staining within the centrosome mask.
10. Measure the image area occupied by centrosome objects.
11. Divide the area from step 9 by the area from step 10 and multiply by 100 for the percentage of centrosome covered by PFDN1.
12. Repeat for all images, display the data on a histogram with bin width set to 10% (ten bins of 10%) and perform a t-test.