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Supplemental information

Active elimination of intestinal cells drives oncogenic growth in organoids

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Krotenberg Garcia et al, Figure S1

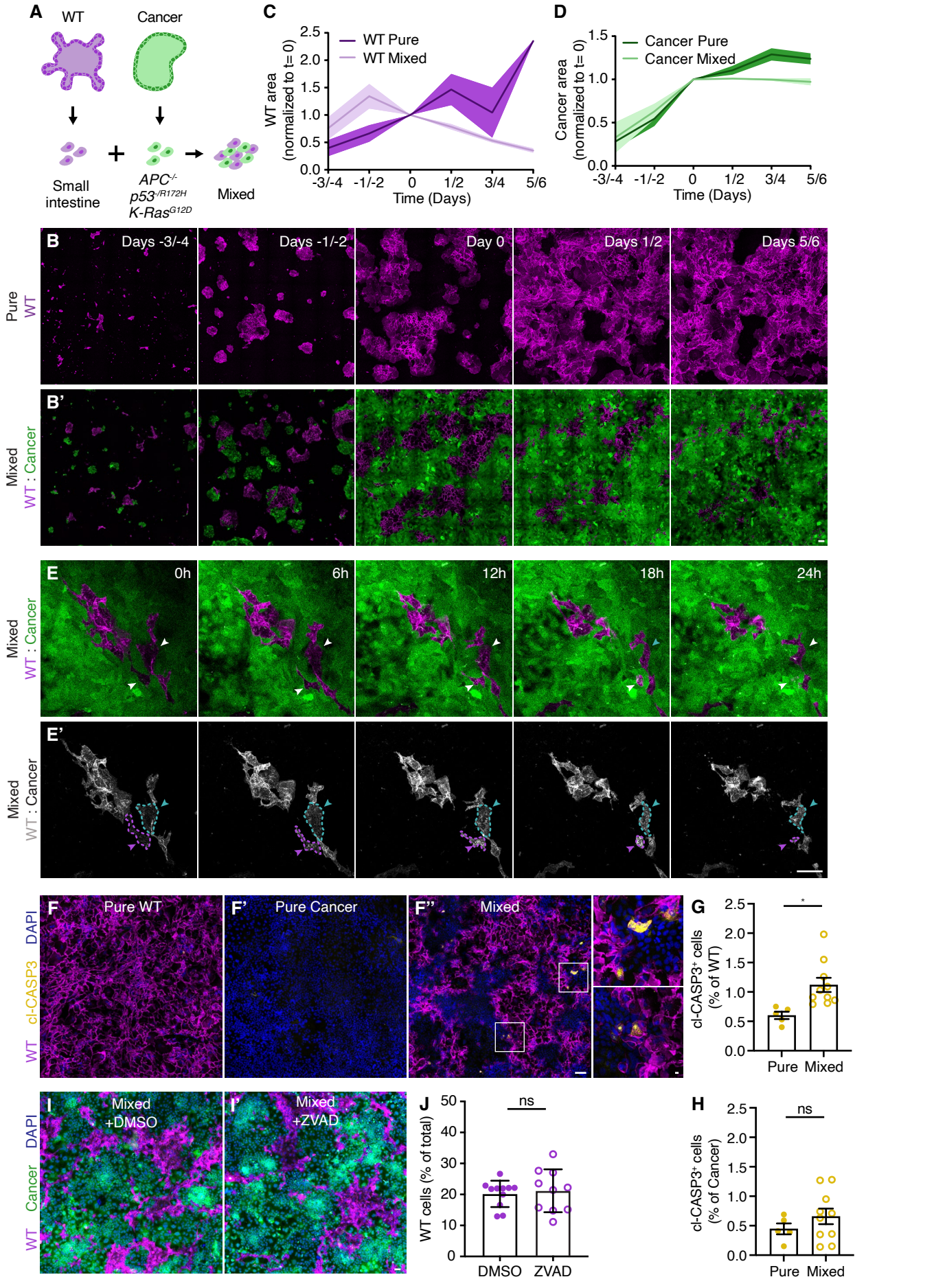

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Figure S1 - Wild-type small intestine cells are eliminated by cancer cells in enteroid monolayers. Related to Figures 1 and 2.

A) Schematic representation of a model for cell competition in murine enteroid monolayers.

B-D) Representative pictures of sequential imaging of enteroid monolayers in pure (B) and mixed (B') conditions and quantification of the surface covered by wild-type (C) or cancer (D) populations over time normalized to Day 0 (Mean ±SEM). Day 0 is the moment a full monolayer is formed in mixed conditions.

E) Representative images of time-lapse series of a competing enteroid monolayer, arrow heads in (E') indicate examples of wild-type cells that are shrinking (cyan) and being eliminated (magenta).

F-H) Representative confocal images of pure wild-type (F) pure cancer (F') and mixed (F'') enteroid monolayers. Apoptotic cells are marked by cl-CASP3 (yellow). The insets display a 5.75x magnification of the area in the white box. G-H) Quantification of the cl-CASP3+ cells relative to the total wild-type (G) and cancer (H) cell population, each dot represents one imaged well (Mean ±SEM, unpaired t-test, two-tailed, p=0.0128 (G), p=0.3092, n=5 & 10 wells).

I-J) Representative confocal images of control (I) and Z-VAD-FMK (I') treated enteroid monolayers. J) Quantification of the number of wild-type cells relative to the cell population, each dot represents one imaged well (Mean ±SEM, unpaired t-test, two-tailed, p=0.6926, n=11 & 10 wells).

Scale bars = 100µm, excluding magnifications in (F) where scale bar = 10µm.

Krotenberg Garcia et al, Figure S2

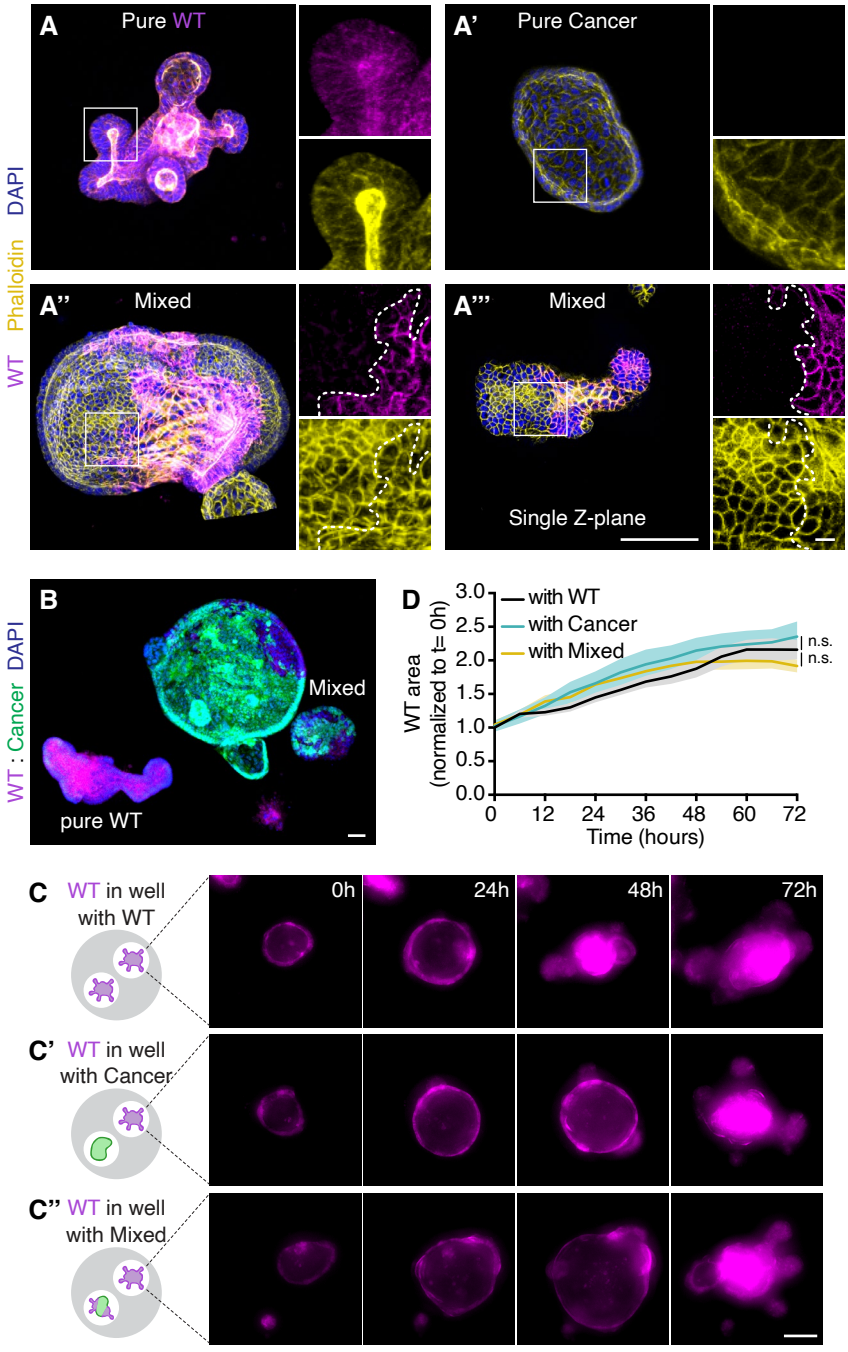
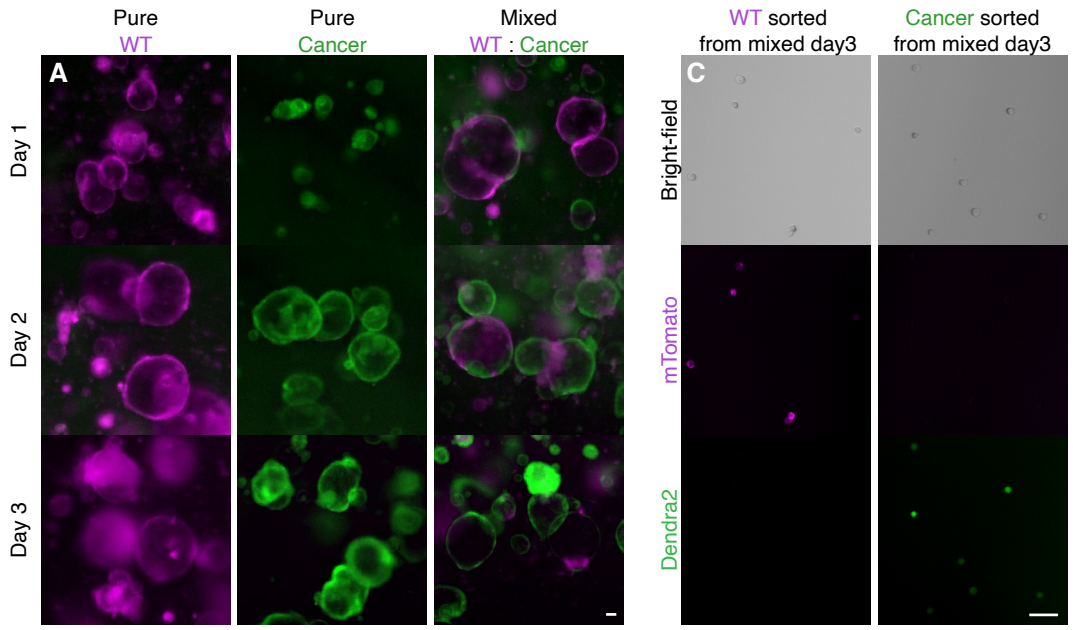
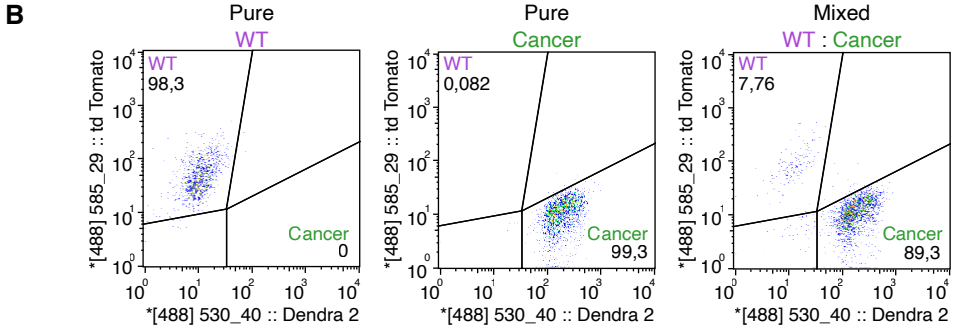
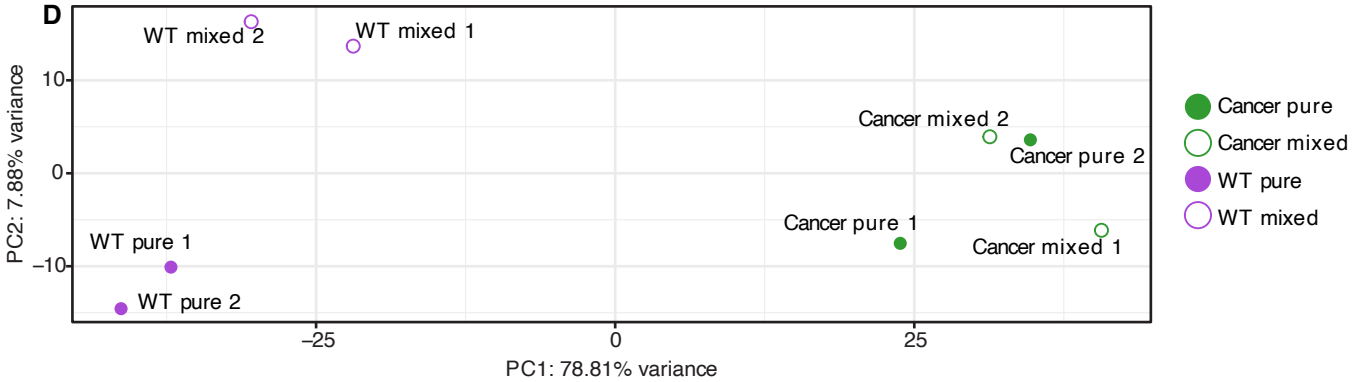


Figure S2 - Short-range communication is essential for cell competition. Related to Figure 2.
A) Representative 3D-reconstructed confocal images of pure WT (A), pure cancer (A') and mixed (A'') organoids, and a single Z-plane of A'' (A'''). The actin cytoskeleton is stained with Phalloidin (yellow), nuclei with DAPI (blue) and borders between wild-type and cancer cells are indicated by dashed lines. The insets display a 2.5x magnification of the area in the white box.
B) Representative confocal image of a mixed culture containing a pure WT organoid, nuclei are visualized with DAPI (blue).
C-D) Representative images from live-imaging of pure WT organoids co-cultured with pure WT (C), pure cancer (C') or mixed (C'') organoids and quantification of the area covered by wild-type cells within indicated organoids (D) normalized to the start of the time-lapse (Mean ±SEM, 2-way ANOVA, multiple comparisons, n=18 organoids for each condition, 'WT in WT' vs. 'WT in cancer' p= 0.5453, 'WT in WT' vs. 'WT in Mix' p= 0.9689).
Scale bars = 100µm, excluding magnifications in (A) where scale bar = 10µm.

Krotenberg Garcia et al, Figure S3







E

GeneSet	mFetal
Enrichment Score (ES)	0.4131525
Normalized Enrichment Score (NES)	2.0948782
Nominal p-value	0.000
FDR q-value	0.000
FWER p-Value	0.000

F

GeneSet	mRepair
Enrichment Score (ES)	0.43847787
Normalized Enrichment Score (NES)	1.8192958
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FDR q-value	5.030181E-4
FWER p-Value	0.001

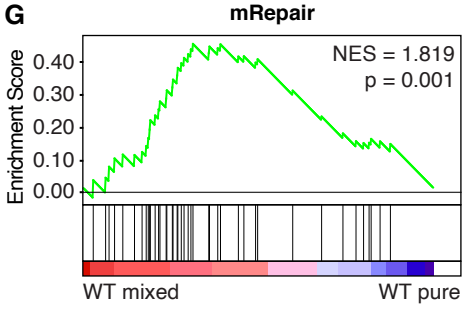

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Figure S3 - Cell competition induces a fetal-like state in WT cells. Related to Figure 4.
A-C) Flow cytometry sorting of wild-type and cancer cells from pure and mixed cultures. A) Representative images of pure and mixed cultures 1, 2 and 3 days after plating. B-C) Analysis of cells after sorting, graphs in B show an analysis of 10.000 cells, numbers in the corners display the percentage of sorted cells. Representative images of sorted cells are shown in (C) Scale bars = 50µm. D-G) Gene expression analysis of wild-type and cancer cells in pure and mixed conditions. D) displays a principal component analysis of all sample. D) Parameters of a gene Set Enrichment Analysis showing enrichment of a fetal signature (Yui et al., 2018) in mixed wild-type cells. F-G) Parameters and graph of a gene Set Enrichment Analysis showing enrichment of a repair signature (Yui et al., 2018) in mixed wild-type cells.

Krotenberg Garcia et al, Figure S4

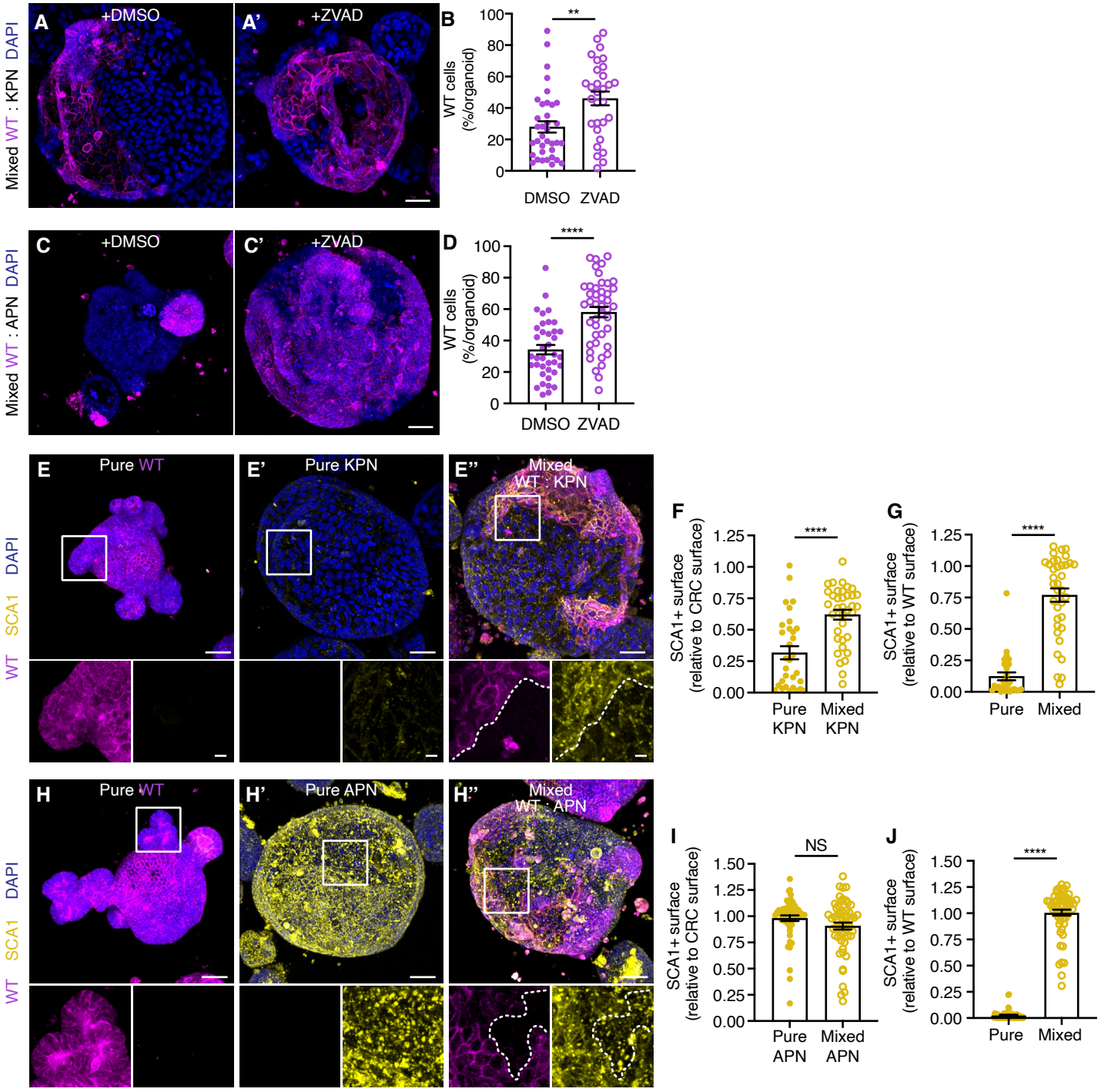

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Figure S4 - Multiple types of intestinal cancer compete with WT cells. Related to Figures 2 and 4.

A-B) Representative 3D-reconstructed confocal image of control (A) and apoptosis inhibited (A') mixed organoids formed by KPN cancer and wild-type intestinal cells, nuclei are stained with DAPI (blue), and quantification of the percentage of wild-type cells contributing to mixed organoids (B), each dot represents one organoid (Mean ±SEM, unpaired t-test, two-tailed, $p=0.019$, n=36 & 30 organoids).

C-D) Representative 3D-reconstructed confocal image of control (C) and apoptosis inhibited (C') mixed organoids formed by APN cancer and wild-type intestinal cells, nuclei are stained with DAPI (blue), and quantification of the percentage of wild-type cells contributing to mixed organoids (D), each dot represents one organoid (Mean ±SEM, unpaired t-test, two-tailed, $p<0.0001$, n=38 & 44 organoids).

E-G) Representative 3D-reconstructed confocal images of pure WT (E), pure KPN cancer (E'), mixed KPN (E'') organoids and quantification of the SCA1+ surface relative to the total KPN cancer (F) or wild-type (G) surface area. The organoids were stained for SCA1 (yellow), nuclei are visualized with DAPI (blue). The insets display a 2.5x magnification of the area in the white box. Each dot in (F) and (G) represent one organoid (Mean ±SEM, Non-parametric, ANOVA, multiple comparisons: $p<0.0001$, n=31 & 36 organoids (F); $p<0.0001$, n=28 & 36 organoids (G)).

H-J) Representative 3D-reconstructed confocal images of pure WT (H), pure APN cancer (H'), mixed APN (H'') organoids and quantification of the SCA1+ surface relative to the total APN cancer (I) or wild-type (J) surface area. The organoids were stained for SCA1 (yellow), nuclei are visualized with DAPI (blue). The insets display a 2.5x magnification of the area in the white box. Each dot in (I) and (J) represent one organoid (Mean ±SEM, Non-parametric, ANOVA, multiple comparisons: $p=0.0939$, n=54 & 58 organoids (I); $p<0.0001$, n=50 & 58 organoids (J)).

Scale bars = 50µm, excluding magnifications in (E and H) where scale bar = 10µm

Krotenberg Garcia et al, Figure S5

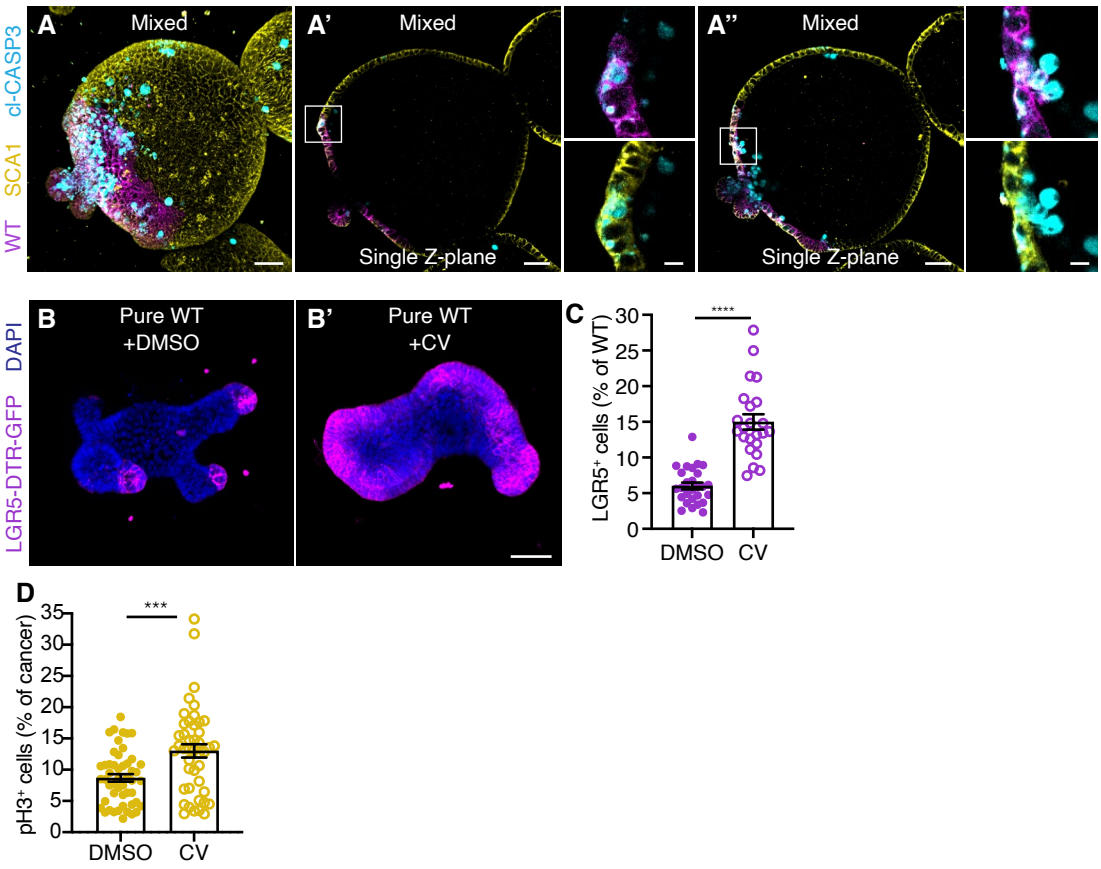

Figure S5 - Increased stemness prevents cell competition. Related to Figures 2 and 5.
A) Representative 3D-reconstructed (A) and single Z-plane (A' and A") confocal images of a mixed organoid. The organoids were stained for cl-CASP3 (cyan) and SCA1 (yellow). The insets display a 3.5x magnification of the area in the white box. Organoid is from the same dataset used in panel 2E.
B) Representative 3D-reconstructed confocal images of control (B) and CV treated (B') pure WT organoids. LGR5+ Intestinal stem cells (magenta) and nuclei (blue) are visualized.
(C) Graph displays the number of LGR5+ cells relative to total number of wild-type cells, each dot represents one organoid (Mean ±SEM, unpaired T-test, two-tailed, $p<0.0001$, n=27 & 23 organoids). Displayed DMSO control organoids are from the same dataset used in panel 5B.
D) Graph displays the number of pH3+ cells in pure organoids relative to the total number of cancer cells, each dot represents one organoid (Mean ±SEM, one-way ANOVA, multiple comparisons, $p=0.0005$, n=50 & 44 organoids).
Scale bars = 50µm

Krotenberg Garcia et al, Figure S6

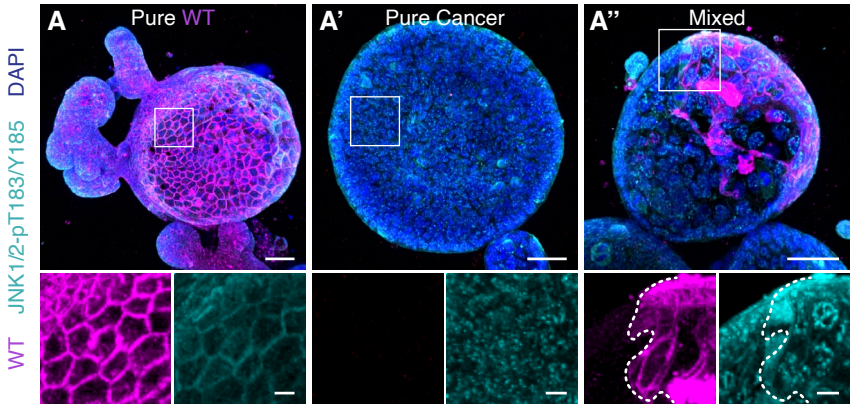

Figure S6 - JNK signaling drives cell competition. Related to Figure 6.
Representative 3D-reconstructed confocal images of pure WT (A), pure cancer (A'), mixed (A'') organoids, stained for activated JNK1/2-pT183/Y185 (cyan), nuclei are visualized with DAPI (blue). The insets display a 2.5x-3.5x magnification of the area in the white box.

Krotenberg Garcia et al, Figure S7

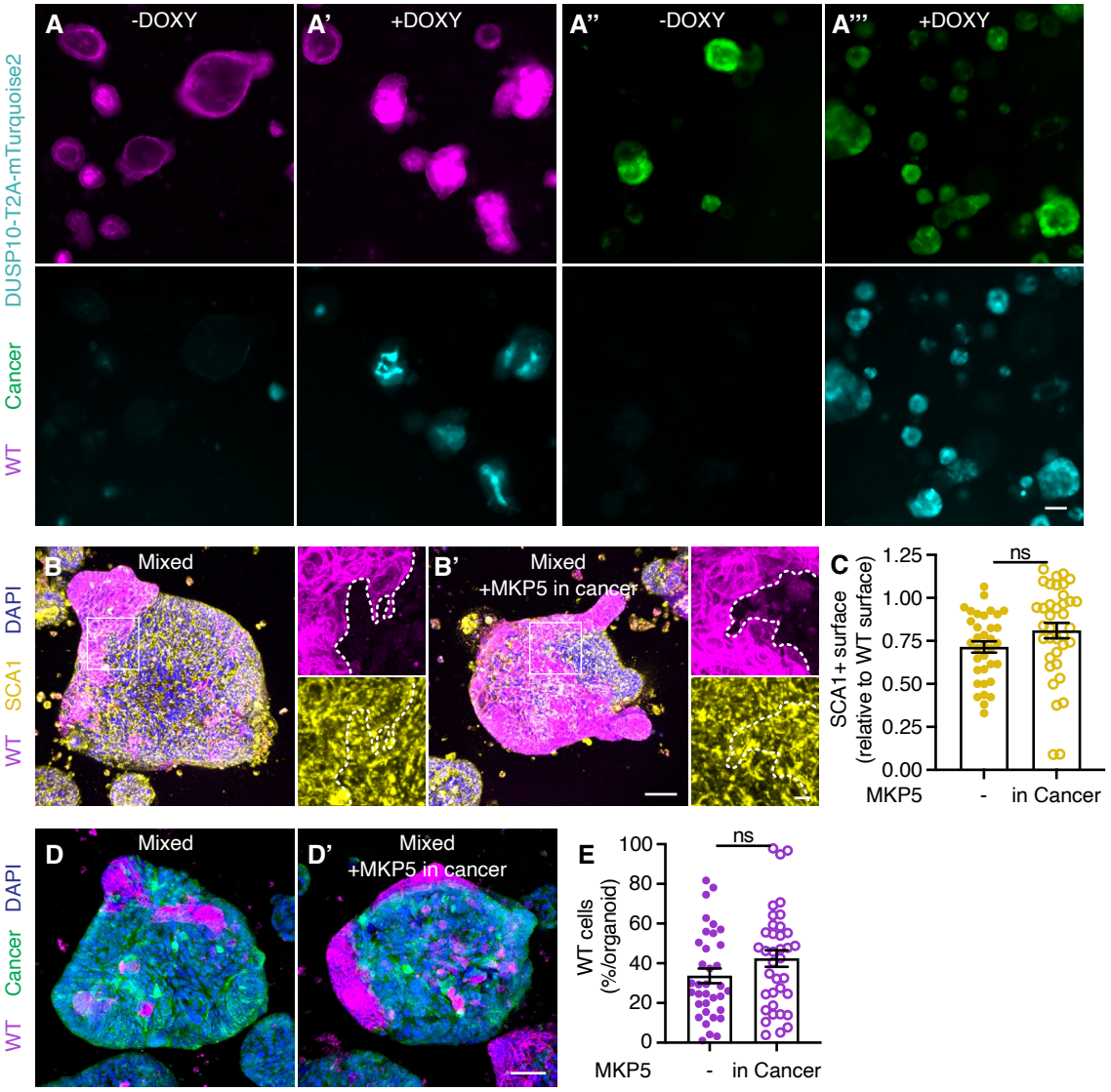

Figure S7 - JNK activity in wild-type cells is required for cell competition. Related to Figure 7.
A) Representative images of control (A) and doxycycline treated (A') organoids formed by TET-inducible MKP5 wild-type cells and control (A'') and doxycycline treated (A''') organoids formed by TET-inducible MKP5 cancer cells, co-expression of mTurquoise2 (Cyan) is shown.
B-C) Representative 3D-reconstructed confocal images of control (B) and doxycycline treated (B') mixed organoids formed by TET-inducible MKP5 cancer and control wild-type cells. The organoids were stained for SCA1 (yellow), nuclei are visualized with DAPI (blue). The insets display a 2.5x magnification of the area in the white box. C) Quantification of the SCA1+ surface relative to the total wild-type surface area, each dot represents one organoid (Mean ±SEM, ANOVA, multiple comparisons, p=0.2337, n=35 & 37 organoids).
D-E) Representative 3D-reconstructed confocal image of control (D) and doxycycline treated (E') mixed organoids formed by TET-inducible MKP5 cancer and control wild-type cells, nuclei are stained with DAPI (blue), and quantification of the percentage of wild-type cells contributing to mixed organoids (E), each dot represents one organoid (Mean ±SEM, ANOVA, multiple comparisons, p=0.2892, n=35 & 37 organoids).
Scale bars = 50µm, excluding magnifications in (B) where scale bar = 10µm