

Supplemental Figures Legends

Supplemental Figure S1: Characterization of the KPCX mouse model of pancreatic cancer

A, Recombination efficiency of the *Rosa^{Confetti}* Cre-reporter system. The table depicts the percentage of total Dapi⁺ cells expressing any one of 4 Confetti lineage labels. Data are presented as mean percentage \pm SEM. Data are pooled from n=4 CX mice with >16,000 Dapi⁺ pancreatic cells counted per animal. The confetti allele was activated in CX on day 0 through oral gavage of the mother with TAM.

B, Fluorescent images of cultured RFP⁺ tumor cells derived from KPCX mice. Cells imaged at passage 1 and 5 of culture (passages separated by 3 weeks) exhibit no evidence of Cre-independent color switching. Images are representative of n > 5000 observed cells in 5 replicates.

C, Age distribution (weeks) of age at detection of pancreatic tumors in *Pdx1-Cre; Kras^{G12D}; p53^{fl/+}; Rosa^{YFP}* (“KPCY”) and KPCX mice. The mean age of tumor detection was 18.35 weeks in KPCY mice (■, n=35) and 16.12 weeks in KPCX mice (▼, n=29). ns, non-significant.

D, H&E images demonstrating histological similarities between KPCY- and KPCX-derived tumors. The KPCY image is taken from a paraffin section while the KPCX image is from a fixed frozen section.

Scale bars 50 μ m for **A** and **B** and 100 μ m for **D**.

Supplemental Figure S2: Polyclonal cell clusters in peritoneal and diaphragm metastases are spaced widely apart

A, Fluorescent image of a peritoneal micro-metastasis. Insert shows a magnified view of the lesion.

B, Combined brightfield and fluorescent stereomicroscope image of two well-separated macroscopic metastases in a large section of peritoneal tissue.

C, Brightfield (left panel) and fluorescent (middle and right panel) stereomicroscope image of two well-separated macroscopic metastases in a large section of diaphragmatic tissue.

Scale bars 500 μm for wide view in **A** (25 μm for insert), 1 mm for **B**. In **C**, the scale bars in the left and middle panels are 1mm, and in the inserts they are 500 μm (i) and 100 μm (ii).

Supplemental Figure S3: Derivation of 458d cells for assessing the origins of polyclonal metastases

A, A RFP/YFP diaphragmatic metastasis was isolated and sorted into single RFP (458d_R) and YFP (458d_Y) cells and cultured for <4-6 passages. Cells were injected either intraperitoneally (IP) for studies of peritoneal metastasis or retro-orbitally (RO) for studies of lung metastasis as a mixture of single 458d_R and 458d_Y cells or as RFP/YFP clusters.

B, Fluorescent Image showing multi-color tumor cell clusters created by gentle mixing of equal numbers of 458d_R and 458d_Y cells for 8-12 h.

Scale bar 25 μm (**B**).

Supplemental Figure S4: Distribution of lung and liver metastases according to size

A and **B**, Representative images from monochromatic lung (**A**) and liver (**B**) metastases.

Individual metastatic lesions were binned based on the number of cells per lesion: Single (1 cell), Nano (2-10 cells), Micro (11-100 cells), and Macro (>100 cells).

C, Percentage of total metastatic lesion in each size category pooled from n=6 (lung) and n=5 (liver) mice. Total number of lesions counted in lung and liver were 1512 and 763 respectively.

Scale bars 50 μ m for **A** and **B**.

Supplemental Figure S5: Changes in clonal representation with growth of liver metastases

A, Fluorescent images of bi-chromatic liver metastases showing a change in the ratio of lineage labeled populations during metastatic growth.

B, Curve showing the trend depicted in (A). The ratio of the number of cells in the minor fraction relative to the total number of fluorescent cells in a metastatic lesion (y-axis) is plotted as a function of the total fluorescent cell number in the lesion (x-axis). Each (●) represents a single liver metastasis. Data were taken from 33 individual liver metastases pooled from n=5 mice (lesions with identical ratios and size are represented as a single data point). Trend is significant with $p < 0.0001$ by Wald Chi-square test.

Scale bars 25 μ m.

Supplemental Figure S6: Lung metastatic burden following single and cluster injections

A, Stereomicroscope brightfield (top panel) and fluorescent (bottom panel) images of lung metastasis 3 weeks following injection of equal number of cells (20,000) as either single cells (left panel) or as clusters (right panel).

B, Graph showing the trend depicted in (A). Data are taken from n=4 mice per group and presented as mean \pm SEM. * $p < 0.001$ by Student's t test.

Scale bars 1 mm.