Supplemental Figure and Movie Legends

FigS1. **Characterisation of the Src-biosensor *in vitro***. A, Quantification of lifetime measurements in primary PDAC cells expressing the Src biosensor in response to 50 ng/ml EGF stimulation for 5 mins after 30 mins PP1 pre-treatment (10 μM). B, Representative lifetime maps of Src activity ± EGF 50 ng/ml. C, Western blot assessment of Src autophosphorylation using anti-phospho-Src Y416 and anti-Src and actin in response to serum starvation, PP1 pre-treatment or in response to EGF stimulation after PP1 removal. Columns, mean; bars, SE.

FigS2. **Invasion of pancreatic cancer cells upon post-invasion dasatinib treatment**. Quantification of mutant $p53^{R172H}$ PDAC cells invasion ± 2 days post-invasion treatment of 100 nM dasatinib.

FigS3. Measuring the organisation and ultrastructure of distinct ECM microenvironments in live tumors. SHG images of center versus tumor border with corresponding evaluation of collagen organization and cross-linking assessed by GLCM analysis. The homogeneity plot reflects the similarity in signal strength between pixels in the image as described previously. [37] A more homogeneous/uniformed signal strength reflects the reduced amount of cross-linked collagen present at the center of the tumor whereas border regions have a wider range of signal strengths through the increased presence of organised cross-linked collagen. Red arrow = border region, purple = SHG. $P < 0.001$ by unpaired Student's t-test. The equation for homogeneity, H, is the sum of the normalised number of times, p, that a pixel, x, and the pixel the neighbour index away, y, have the intensities i and j respectively.

FigS4. **Measuring the organisation and ultrastructure of distinct ECM manipulations in complex 3D-organotpyic matrices.** Representative maximum projection SHG images of organotypic matrices with corresponding evaluation of collagen organization and cross-linking assessed by GLCM analysis ± 10 μM Cylopamine pre-treatment. The homogeneity plot reflects the similarity in signal strength between pixels within organotypic matrix as previously described. [37] After cyclopamine treatment a more homogeneous/uniformed signal strength reflects the reduced amount of cross-linked collagen present whereas control matrices have a wider range of signal strengths through the increased presence of cross-linked collagen. Purple = SHG. Columns, mean; bars, SE. P= 0.0234 by unpaired Student's t-test. The equation for homogeneity, H, is the sum of the normalised number of times, *p*, that a pixel, x, and the pixel the neighbour index away, y, have the intensities *i* and *j* respectively.

FigS5. Summary of FLIM-FRET intravital imaging as a pre-clinical tool for the drug discovery process. Schematic demonstrating the capacity of FLIM-FRET imaging in the context of pre-clinical drug targeting regimes.

Supplementary movie M1 Mutant $p53^{R172H}$ PDACs expressing Src biosensor (green) on organotypic assay with SHG signal from ECM components (purple), interacting with fibroblasts (red) during invasion.

Supplementary movie M2 Mutant $p53^{R172H}$ PDACs expressing Src biosensor (green) invading in organotypic assay with SHG signal from ECM components (purple), z-section.

Supplementary movie M3 Mutant $p53^{R172H}$ PDACs expressing Src biosensor (green) in context of host tumor vasculature (red) and SHG from ECM components (purple).

Supplementary movies M4 Representative maximum projection SHG image of fibrillar collagen I used for GLCM analysis, control.

Supplementary movie M5 Representative maximum projection SHG image of fibrillar collagen I used for GLCM analysis, plus 10 μM cylopamine