Supplemental Material

Supplementary Figure 1: This is a depiction of the workflow used in this experiment. First, samples were prepared by culturing fibroblasts on MirrIR slides. The cells were either treated with TGFβ1 or co-cultured with MCF7 tumorigenic breast epithelial cells or MCF10A nontumorigenic breast epithelial cells. After 0, 6, 12, and 24 h of co-culture or treatment, cells were fixed with 4% paraformaldehyde for 1 hour at 4˚C. Four samples were prepared per treatment condition and time point. Of these, half were stained for α-SMA using immunofluorescence protocols detailed in the Methods section. As expected, the positive control (TGFβ1 treated) and MCF-7 co-culture samples were positive for α-SMA expression while the negative control (the 0 h timepoint) and MCF10A co-culture samples were negative for α-SMA expression. The other samples were prepared for IR imaging. First, samples were imaged in transflection mode. Visible images were taken using the Spotlight and these were used to find confluent and subconfluent regions of each sample. Images in transflection were taken and these spectra were subsequently analyzed. For the 0 h and 24 h timepoints, samples that had previously been imaged in transflection were then imaged using ATR FT-IR. 300 µm x 300 µm regions of each sample were imaged. MNF transformation was applied to this spectral data for noise reduction before spectral analysis. Analysis revealed that a distinction could be made between nuclear and cytoplasmic pixels in ATR that could not be done with transflection data. After imaging using the ATR mode, samples were then stained for presence of α-SMA using the same immunofluorescence protocol as before. This was done as a test to specifically determine which samples were activated.