Supplemental Figure Legends

Supplemental Figure 1. Relationship between GFP intensity and cell number. MCF-7eGFP cells were plated ranging from 5,000 to 100,000 cells per well of a 96-well plate and GFP fluorescence intensity was measured after 24 hours on a plate reader. A linear curve fit was used to evaluate the use of GFP intensity as a measure of cell number.

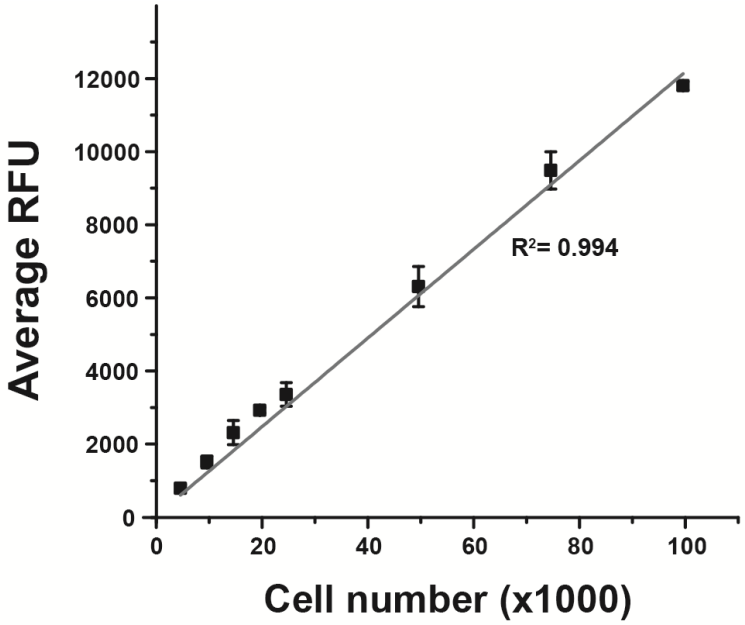
Supplemental Figure 2. Evaluation of measurements of fluorescence intensity by image analysis. (A) GFP intensity is linear with cell number. MCF-7eGFP cells were plated in single microchannels ranging from 500 to 2,000 cells per channel and images were collected by microscopy after 24 hours of culture. ImageJ was used to quantify the fluorescence intensity of GFP. A linear curve fit was used to evaluate the relationship between GFP intensity and cell number. (B) Feature intensity differences are quantifiable. InSpeck microscope calibration beads (6μm) with defined relative fluorescence intensities were seeded at density of 1,000 beads per μL in single microchannels, and images were collected by microscopy. ImageJ was used to quantify the fluorescence intensity of the population of beads, as described in Figure 3B. A linear curve fit was used to determine the linear range at which changes in intensity can be detected.

Supplemental Figure 3. ERα protein expression in ERα-positive breast cancer cell line, T47D. Co-cultures of T47D cells and HS-5 cells were performed twice for 24 hour time periods in 6-well plates. 10nM 17β-estradiol (E2) or vehicle (EtOH) were added for the last 8 hours of co-culture as a control. Total cell lysates were used for Western blots using Li-Cor Odyssey system (representative blot, left panel) for quantification (right panel). The doublet seen is consistent for all ERα-positive cell lines, and is representative of phosphorylation of the receptor.

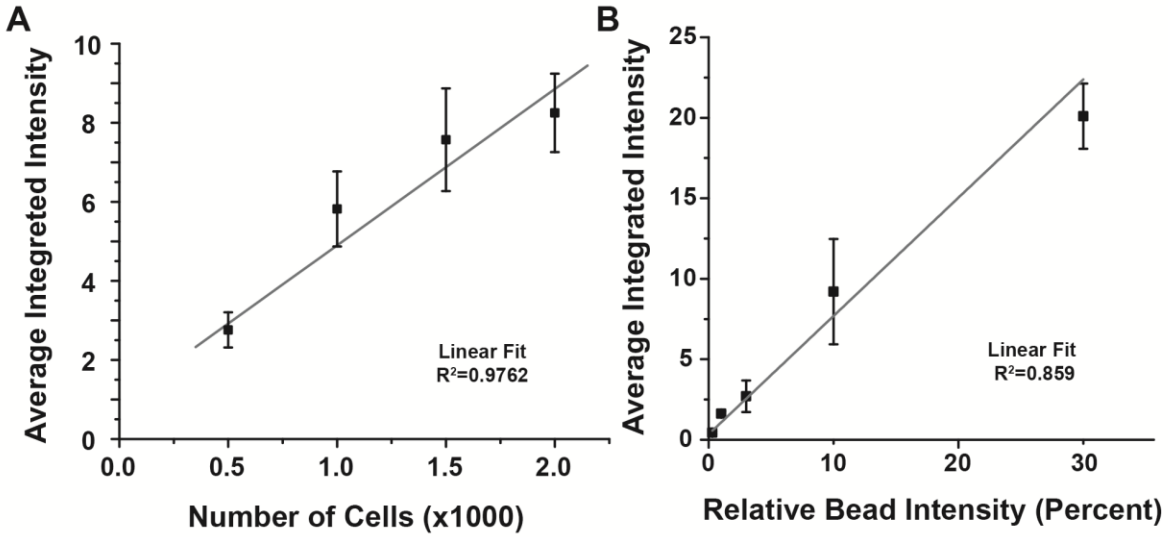
Supplemental Figure 4. Decrease in ERα protein in HS-5 co-culture is not inhibited by inhibitors of ligand-dependent activation of ERα. (A) Co-culture of MCF7eGFP cells with HS-5

cells for 24 hours in single microchannels in the presence of 100nM E2, or 100nM Tamoxifen (OHT). ERα was quantified as in Figure 4B. (B) Co-culture of MCF7eGFP cells with HS-5 cells in 6-well plates for 24 hours in the presence of the aromatase inhibitor, Anastrozole (Ana). Doses of Ana used were 10nM, 100nM, 1μM, 10μM, where the highest dose exceeds typical doses used in the literature by 10-fold. Total cell lysates were used for Western blots using Li-Cor Odyssey system, and quantification is shown. ERα protein levels were normalized to GFP levels to control for MCF-7 cell number, and all values are shown relative to mono-cultured, untreated control. Quantification from one representative blot is shown.

Supplemental Figure 1

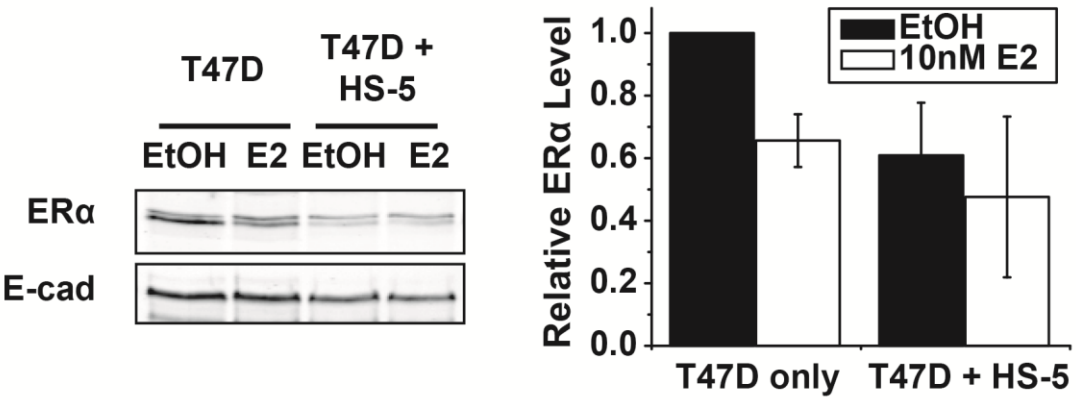


Supplementary Figure 2



Electronic Supplementary Material (ESI) for Integrative Biology

Supplementary Figure 3



Supplementary Figure 4