



**Figure S1. Extent and localization of Gli1 expression by immunofluorescence in breast cancer cell lines.** Detection of Gli1 protein by immunofluorescence in ER $\alpha$ -positive breast cancer cell lines shows that both cytoplasmic and nuclear staining are present (green staining, nuclear localization of Gli1 is demonstrated in the inset) in each cell line, with the cytoplasmic staining predominating. The nuclei are labeled with DAPI (blue). Gli1 expression was greatest, with a greater number of cells staining for Gli1, in MDA-MB-361 cells, with lower levels of expression in MCF7, T47D and BT474 cells. Therefore, the protein expression of Gli1 corresponds to the mRNA expression where the highest expression was found in MDA-MB-361 cells with lower levels in MCF7, T47D and BT474 cells (as shown in Figure 1a). Original magnification is 1000x, insets are magnified an additional 2.5x.

## **SUPPLEMENTAL METHODS**

### **Immunofluorescence**

Cells were cultured on cover-slips to sub-confluence. After fixation in 3.9% paraformaldehyde for 15 minutes, cells were blocked in PBS plus 2% horse serum and 0.1% Triton X-100 for 30 minutes. Gli-1 was then detected by a goat polyclonal antibody (N-16, Santa Cruz Biotechnology) at a dilution of 1:100, which was applied for 1 hour at room temperature. After washing with PBS, cells were then incubated with Alexa Fluor® 488 labeled donkey anti-goat secondary antibody (Invitrogen) at 1:1000 dilution for 1 hour at room temperature, followed by washing and incubation with DAPI (5 µg/ml) to label nuclei. Immunofluorescence images were acquired by a Zeiss Axioplan 2® microscope equipped with an AxioCam HRC® Camera and AxioVison® software (Carl Zeiss Inc) using identical exposure times for each image. Signals were adjusted and processed by ImageJ to generate final merged images, again using identical settings for each image.