Supplementary Material.

Plasmids. PAGFP-Farn2Palm was amplified by PCR using the primers PAGFP BamHI/NheI 5'-GTACCGGGATCCACCGCTAGCC-3' and PAGFP-FarnPalmXbaI 5'GTACTCTCAGATCAGGAGAGCACACACTTGCAGCTCATGCAGCCGGGGC CACTCTCATCAGGAGGGTTCAGCTTCTTGTACAGCTCGTCCATGC-3' containing the coding sequence for farnesylation and palmitoylation signal from H-Ras

Cells. A431 Human epithelial carcinoma cells were cultured in DMEM (Invitrogen, UK) supplemented with 10 % FBS and 2 mM L-glutamine (Invitrogen, UK). Cells were transfected using the Amaxa Nucleofector transfection system as described by manufacturers protocol (Amaxa, Germany). Cells were selected using 0.6 mg/ml G418 and single cell clones were isolated using a standard dilution cloning procedure.

Photo-activation and photo-bleaching *in vitro*. For *in vitro* / *in vivo* comparisons 1.5 x 10^6 cells were plated onto glass-bottomed 30 mm tissue culture dishes (Iwaki Cell Biology, Sterilin Ltd, United Kingdom) and left to adhere overnight. Culture media was replaced prior to imaging. Photo-bleaching and -activation experiments were performed using an Olympus FV1000 confocal microscope with SIM scanner. Cells were maintained at 37 ºC in a temperature controlled chamber and imaging performed using the following settings: pixel dwell time 4 µs/px, pixel resolution 512 x 512, 5 % 488 nm laser power. Prior to photo-activation, the membrane probe could be faintly localized to the cell periphery using 488 nm excitation or weak illumination at 405 nm, which was sufficient to target photo-activation at cell edges. Effective photo-bleaching was achieved using 50 % 405 nm laser power, 4 µs/pixel dwell time, and a 200 ms bleach time. Images were captured every 10 s for 50 frames. Photo-activation

was achieved using 5 % 405 nm laser power, 4 µs/pixel dwell time, and a 1 frame activation pulse. Images were captured every 5 s for 75 frames. Up to 40 cells were imaged for each probe (see **Supplementary Table 1**).

Photo-activation and photo-bleaching *in vivo*. Following trypsination, 1 x 10^6 cells were resuspended in 100 µl HBSS (Invitrogen, Paisley, UK) and subcutaneously injected into the rear flank of a nude mouse. Tumors were allowed to develop until 0.5 – 1 cm in diameter. To permit imaging mice were anaesthetised using an anaesthetic combination of 1:1 hypnorm - H_2O + 1:1 hypnovel - H_2O. Following induction of anaesthesia the subcutaneous tumor was surgically exposed and the mouse restrained on a 37 ºC heated stage. Mice were sacrificed after the experiment within 4hr of imaging. Photo-bleaching and -activation experiments were performed using an Olympus FV1000 confocal microscope with SIM scanner using the following image acquisition settings: pixel dwell time 4 µs/pixel, pixel resolution 512 x 512, and 5 % 488 nm laser power. Effective photo-bleaching of GFP-E-cadherin was achieved using 70 % 405 nm laser power, 4 µs/pixel dwell time, and a 200 ms bleach time. Images were captured every 10 s for 75 frames. Photo-activation of PAGFP-Farn2Palm was achieved using 5 % 405 nm laser power, 4 µs/pixel dwell time, and a 1 frame activation pulse. Images were captured every 5 s for 75 frames. At least three animals were imaged for each probe, and care was taken to minimize pixel saturation in the raw data. The level of photo-bleaching which did not cause photo-damage was estimated to be 40 % *in vivo*, and therefore limited to this value *in vivo.* Cell-cell junctions could be imaged up to a depth of 70 µm *in vivo*, however, targeted photo-activation and photo-bleaching were not possible at this depth due to light scattering by the specimen. To facilitate comparative analysis of the results, all experiments were

therefore performed at ~20 µm sample depth, which is on the order of sample thickness *in vitro*.

Drug treatment *in vitro* and *in vivo.* Dasatinib (a kind gift from Bristol Myers Squibb, Princeton, NJ) was administered by oral gavage in 80 mmol/L citrate buffer [30 mg/kg] or 200 nM *in vitro* and subsequently imaged within 4 h of administration.

Data Analysis. Fluorescent intensity measurements derived from the region of interest used to bleach / activate were averaged in Excel and used to plot recovery / decay curves. Average measurements for each time-point were exported into SigmaPlot (Systat Inc, London, UK) for exponential curve fitting. Data were fit using the following exponential functions: photo-bleaching: $y = y_0 + a \bullet (1 - e^{-bt})$; photo-activation: $y = y_0 + a \bullet e^{-bt}$. The half-time of recovery was calculated using the formula ln2 / b, where b was obtained from the exponential curve fit. The immobile fraction was calculated as follows using values derived from the curve fit: photo-bleaching: immobile fraction = $100 \bullet (1 - a / (1 - y_0))$; photo-activation: immobile fraction = $100 \bullet a / (a + y_0 - pre)$, where pre is the pre-bleach intensity. Standard error values for y_0, a, and b were given by SigmaPlot and propagated through the equations for half-time of recovery and immobile fraction listed above.

Supplementary Table 1. The number of cells targeted, half-times of recovery and immobile fractions for GFP-E-cadherin and PAGFP-Farn2Palm derived *in vitro* and *in vivo*.

Supplementary Figure 1. Movies of GFP-E-cadherin photo-bleaching *in vitro* and *in vivo* respectively.

Supplementary Figure 2. Western blots showing that E-cadherin levels do not change in response to dasatinib treatment. However auto-phosphorylation of Src on tyrosine 416, used as a marker of Src activity[10], is reduced in response to drug treatment both *in vitro* and *in vivo*.

Supplementary Movie 1, 2. Photo-bleaching of migratory and stationary cells respectively. Images were acquired every 5 seconds. Bar = 20 μm.

Supplementary Movie 3, 4. Photo-bleaching *in vitro* and *in vivo* respectively. Images were acquired every 10 seconds. Bar = 20 μm.

Supplementary Movie 5, 6. PAGFP-Farn2Palm photo-activation *in vitro* and *in vivo* respectively. Images were acquired every 5 seconds. Bar = 10 μm.