Supplemental Materials

Molecular Biology of the Cell

Peckys et al.

Phenotypic characterization of HCC1954 cells

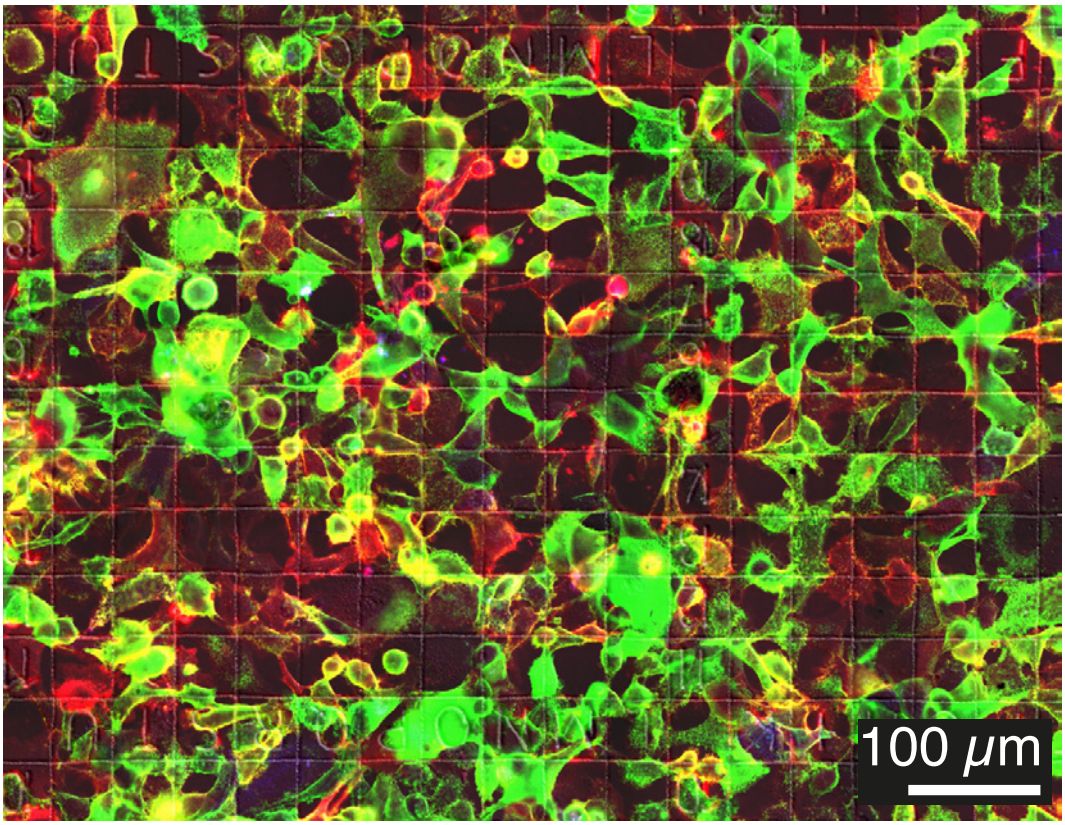

FIGURE S1: Fluorescence microscopy image of adherent HCC1954 HER2 breast cancer cells. QD labels indicating membrane-bound HER2 appear in red, CD44 protein is shown in green, and CD24 in blue. Compared to the fluorescence image of SKBR3 cells shown in Figure 1 of the main text, the signal from the green fluorescence channel is shown with reduced intensity and the red QD fluorescence signal is enhanced, in order to assure a good visibility of each signal. It can be seen that almost all HCC1954 cells belong to the $CD44^{+}/CD24^{-}$ phenotype.

Single-cell quantification of membrane bound HER2

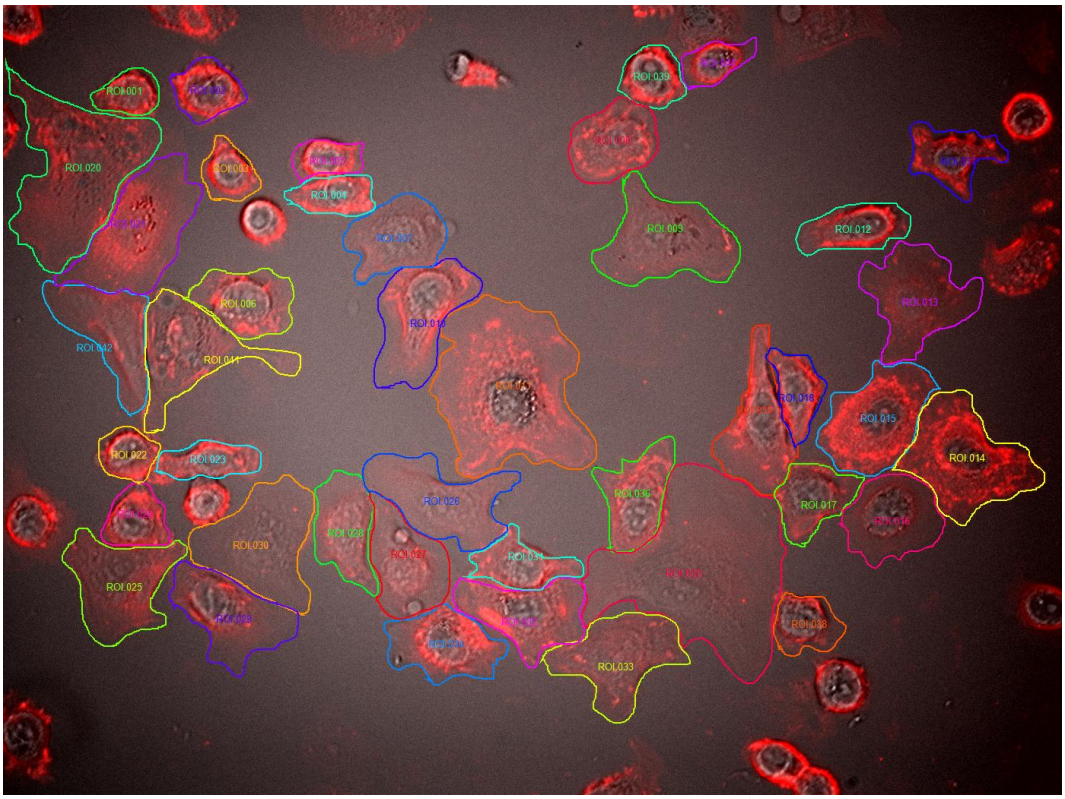

FIGURE S2: Measuring fluorescence intensity in single cells. The image shows the overlay of direct interference contrast (DIC) contrast and QD-fluorescence. The outline of each cell was manually marked and the total fluorescence intensity within the individual cells measured from the cumulative intensity of all selected pixels within the outline, using the microscope software.

Trastuzumab-induced endocytosis of HER2

To examine a possible drug-induced endocytosis of HER2 the cellular uptake (endocytosis) of HER2 in SKBR3 cells induced by incubation with trastuzumab (10 μg/ml) was studied using light microscopy. Instead of adding the HER2 label after trastuzumab incubation, a fluorescent Affibody label was applied at the onset of the experiment. To minimize the impact of the label on the uptake process, a smaller label was used than the QD, namely an anti-HER2 Affibody conjugated with fluorescein (Affibody AB, Bromma, Sweden). Cells in dishes were labeled with 400 nM HER2-AFF-Fluo, for 20 min at 37° C, then exposed to the drug or to cell culture medium without FBS, for 1 h at 37° C, washed and fixed (as described in the methods section). CD44/CD24 labeling was omitted. A representative group of cells, including ruffled and flat cells, before exposure to trastuzumab is shown in Figure S3. The ruffled bulk cells display HER2 in ruffles and at the outlines of cells (Figure S3E), while the flat cells show a homogeneous distribution of the protein (Figure S3F). Endocytosis of the fluorescent label in ruffled cells is visible from a reduction of the fluorescence from the ruffles and the appearance of fluorescent vesicles (round bright shapes) (Figure S3H). In contrast, the flat cell showed only very few or no fluorescent vesicles after 1 h of trastuzumab incubation (Figure S3I).

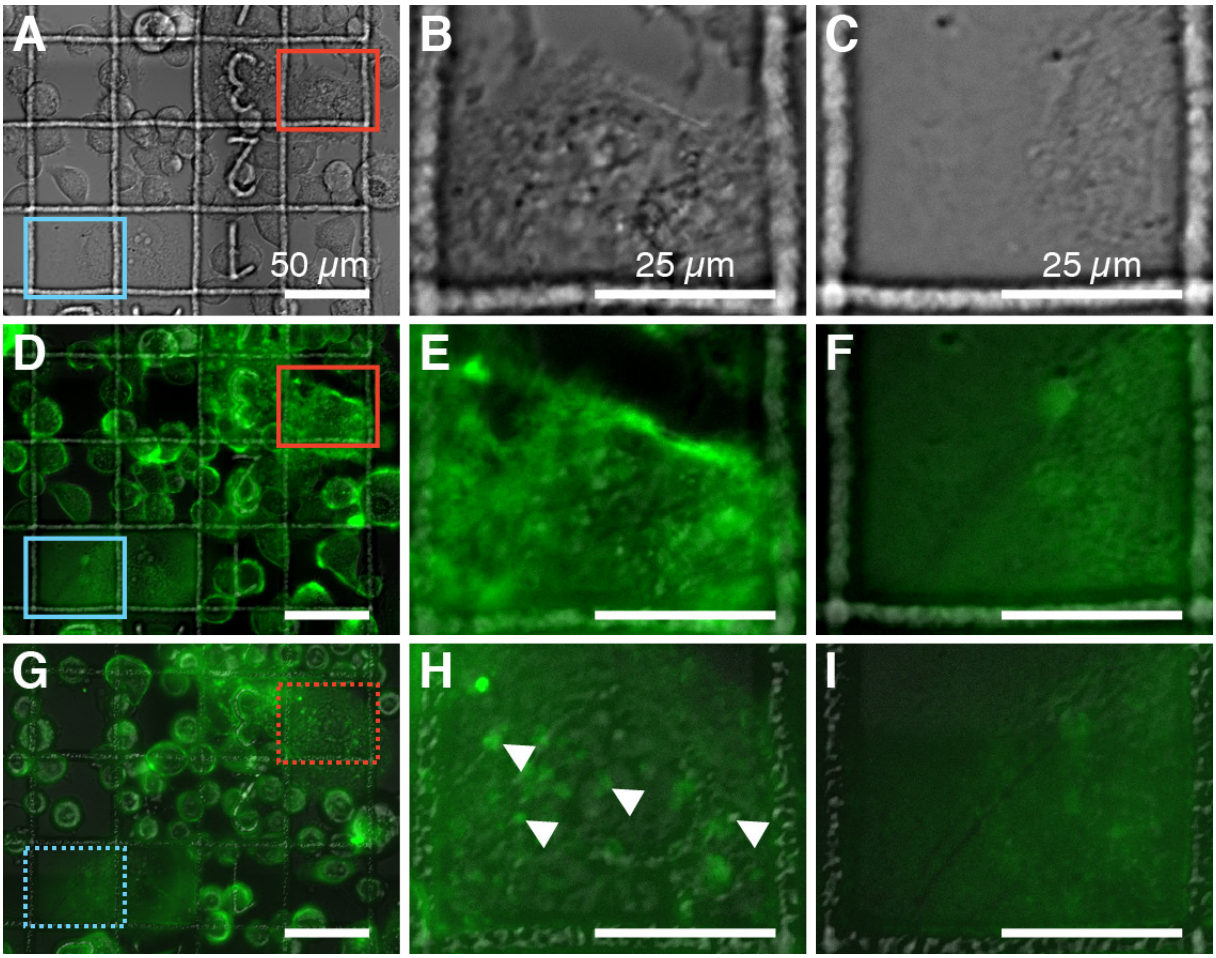

FIGURE S3: Trastuzumab-Induced Endocytosis of Affibody-Labeled HER2. Live cell DIC (A - C) and corresponding fluorescence images (D – I) acquired from Affibody-fluorescein labeled HER2 in SKBR3 cells shown before (A – F) and after 1 h incubation with trastuzumab (G – I). The red, boxed area in *A* is positioned over a ruffled cell; the image detail is shown enlarged in B, to demonstrate the ruffled structure of the surface. The blue, boxed area in a captures an area of a big, flat cell, which is shown enlarged in C. (D) Fluorescence image of the cells. All cells show fluorescence at the plasma membrane. HER2 enrichment is found in ruffles and at the cell border. (E) Details of D corresponding to region shown in B. (F) Details of D corresponding to C. The flat cell shows a homogenous HER2 distribution. (G) The same group of cells as in A and D but shown after 1 h incubation with trastuzumab. (H) The ruffled cell (now marked with a red, dashed box) shows accumulation of HER2-fluorescence in small vesicles, examples are indicated with arrowheads. (I) The flat cell (blue, dashed box) still shows the same diffuse and membrane-bound fluorescence pattern, indicating absence of HER2 endocytosis.

To examine the uptake of HER2 in vesicles, the cells were first labeled with HER2 AFF-fluorescein, then exposed to the drug for 1 h, and fixed. Chemical fixation allowed the recording of high-resolution fluorescence images revealing the presence of vesicle, presumably early endosomes (Figure S4A). Vesicles were mostly absent from the control cells (Figure S4B).

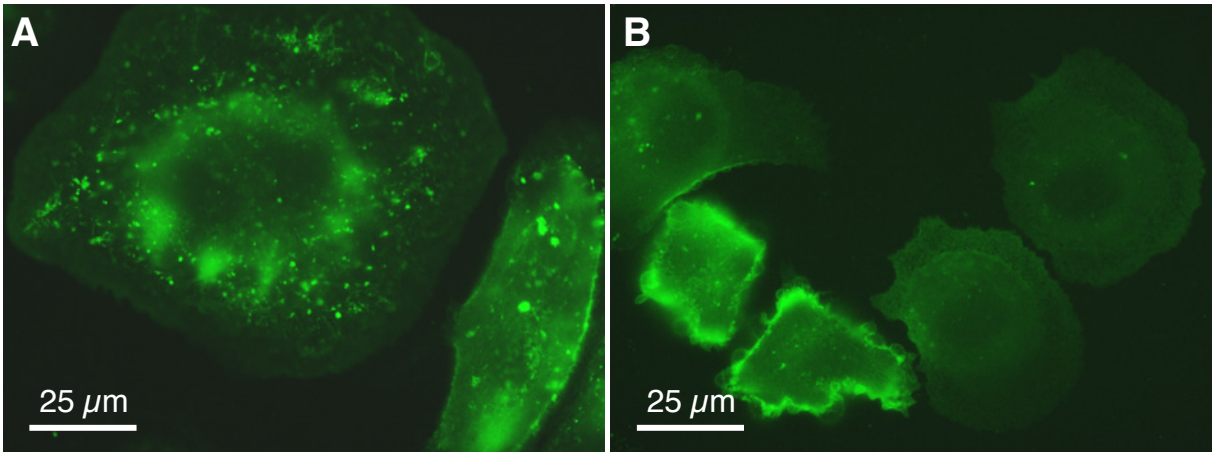

FIGURE S4: Trastuzumab-induced endocytosis of Affibody-fluorescein labeled HER2. (A) Image of fixed cells after 1 h drug exposure acquired with 63 × oil objective. The cells showed HER2-fluorescence localized at their membranes, and a fraction of labeled HER2 was found in vesicles visible as round bright shapes. (B) Control cells after 1 h incubation with cell culture medium (without drug) retained the Affibody-labeled HER2 predominantly at their membrane, where it often concentrated in ruffles at the cell periphery. These cells had only very few small vesicles with fluorescence-labeled HER2. Note that brightness and contrast were independently adjusted between the panels.

Disappearance of Membrane Ruffles after Trastuzumab Binding

For studying drug effects on membrane topographies and HER2 membrane distribution, cells in dishes were labeled with HER2-AFF-B (as described in the methods), then exposed to the drug or to cell culture medium without FBS, for 1 h at 37° C, washed, fixed and QD-labeled (as described above for cells in dishes). In these experiments CD44/CD24 labeling was omitted. Figure S6 shows that incubation with the drug resulted in the disappearance of the membrane ruffles compared to the control.

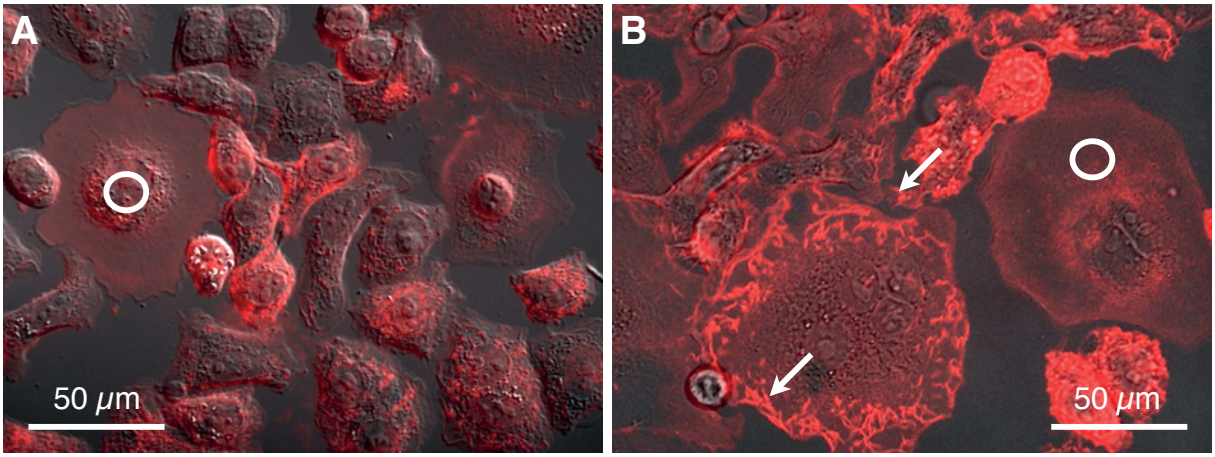

FIGURE S5: Trastuzumab-induced disappearance of membrane ruffles. DIC and red fluorescence overlay images showing SKBR3 cell membrane topography and distribution of QD-Affibody labeled membrane-bound HER2 acquired with 40 × objective. (A) Examples of cells after exposure to trastuzumab for 1 h. The majority of cells showed rough membrane topographies but without the distinct elongated ruffles. HER2 exhibited a patchy distribution pattern. One flat cell (marked with circle) showed a homogenous membrane HER2 distribution. (B) Control experiment without drug. Most cells exhibited membrane ruffles (for example at the arrows). Ruffles showed higher HER2 intensities than the surrounding membrane. A rare flat cell (circle) lacked ruffles and exhibited a homogenous membrane distribution of HER2. Note that brightness and contrast of the fluorescence images in A and B were adjusted to obtain similar fluorescence appearance, because the drug-treated cells exhibited a lower fluorescence signal than the control.