Activating HER2 mutations in HER2 gene amplification negative breast cancer

Supplementary Figure Legends

Supplementary Figure 1. HER2 mutations differentially activate HER2 signaling in NIH3T3 fibroblasts. NIH3T3 cells were retrovirally transduced with HER2 WT or the respective mutants. Lysates were probed with the indicated antibodies. KD = kinase domain, JM = juxtamembrane region, and ECD = extracellular domain.

Supplemental Figure 2. Breast cancer-associated HER2 mutants are expressed on the cell surface. The indicated MCF10A cells were stained with anti-human HER2 antibody and analyzed by flow cytometry. Dead cells were excluded by propidium iodide staining. Cells exogenously expressing HER2 were additionally gated on GFP positivity. For the R678Q cell line, a bimodal distribution of HER2 staining was observed in all replicates.

Supplementary Figure 3. HER2 L755S is sensitive to the irreversible inhibitor, canertinib. MCF10A cells expressing HER2 WT or L755S were treated with canertinib (CI-1033) at the indicated concentrations for 4 hours, and analyzed by western blot as above.

Supplementary Figure 4. HER2 targeted drugs, lapatinib or neratinib, inhibit the spiculated and invasive-appearing morphology of MCF10A-HER2 mutants cells**.** HER2 WT or mutants were seeded on 3D Matrigel culture and allowed them to grow for 4 days. These cultures were then treated with DMSO vehicle (0.5%), lapatinib (1 μM) or neratinib (1 μM) and phase contrast images (magnification 200x) were obtained 2 days later in order to document the short term response to these drugs.

Supplementary Figure 5. HER2 mutants showed increased anchorage independent growth. MCF10A cells transduced with WT or mutant HER2 were seeded in soft agar and allowed to

grow colonies for 1 week. These cultures were subsequently treated with either DMSO vehicle (0.5%), lapatinib (0.5uM) or neratinib (0.5uM). On day 12, the colonies were stained with crystal violet and photographed using a Bio-Rad ChemiDoc XRS system.

Supplementary Figure 6. MCF7-HER2 V777L cells formed tumors more rapidly than MCF7-HER2 WT cells. 5 or 10 million MCF7 cells expressing either V777L or WT HER2 WT were injected in the flanks of nude mice. N=3 mice for V777L and N=2 for WT HER2. Tumor volume was measured weekly and day 54 measurements are shown.

Supplementary Figure 7. HER2 D769 is located near the HER2-HER3 kinase domain dimer interface. The structure of a HER2-HER3 kinase domain dimer was modeled by superimposing HER2 crystal structure 3PP0 (in green) and HER3 crystal structure 3KEX (in cyan) in PyMol. HER2 D769 side chain is indicated in red, HER2 Y772 side chain is magenta, and HER3 I919 and M923 side chains are in yellow. HER3 residue numbering is as per the 3KEX structure.