

## SUPPLEMENTAL FIGURE LEGENDS

**Supplemental Figure 1: Pan-ErbB inhibitor CI-1033 inhibits HER2 phosphorylation and cell growth of both wild-type and T798M mutant cells.** *Top panel.* Cells were treated for 3 h with increasing concentrations of CI-1033 as indicated. Protein extracts were then prepared and subjected to immunoblot analyses using the indicated antibodies. *Bottom panel.* Cells seeded in triplicates in 6-well plates were treated with increasing concentrations of CI-1033 for 7 days. Cells were trypsinised and counted using a hemocytometer on days 1, 4 and 7. Each bar represents the mean number of cells  $\pm$  SEM.

**Supplemental Figure 2: Expression of T798M HER2 results in increased HER3 phosphorylation and HER3-p85 association.** Protein extracts were prepared from BT474<sup>GFP</sup> and BT474<sup>T798M</sup> cells and subjected to immunoprecipitation with a p85 antibody. Pulldowns were separated by SDS-PAGE followed by immunoblot analyses with the indicated antibodies. The bottom three panels represent immunoblot analyses of whole cell lysates prior to precipitation with the p85 antibody.

**Supplemental Figure 3: Dilutional cloning of T798M shows mutant expression in 3% of alleles.** *Left panel.* Exon 19-20 was amplified from BT474<sup>T798M</sup> cells and cloned into pCR Blunt vector followed by direct sequencing of pooled and individual clones as described in Methods. *Right panel.* Representative traces of direct sequencing of HER2<sup>WT</sup> and HER2<sup>T798M</sup>. The T798M mutation was observed in 3.1% of the sequenced clones.

**Supplemental Figure 4: T798M mutant expression in as low as 5% of cells is sufficient to confer resistance.** *A.* BT474<sup>GFP</sup> and BT474<sup>T798M</sup> cells ( $5 \times 10^3$ /well) were plated on 3D Matrigel alone or together mixed in the indicated ratios and treated with DMSO or 1  $\mu$ M lapatinib for 14 days. Fresh media with inhibitor was replenished every 3 days. At the end of treatment, images were captured using a Leica DM IRB inverted microscope equipped with a Nikon DXM1200C camera. An example of GFP-fluorescent cells is indicated by the arrowhead. *B.* Cells ( $5 \times 10^4$ /well) were seeded in 6-well plates in triplicate. Cells were trypsinized and counted using a hemocytometer on days 3, 6 and 10. Each data point on the plot represents the mean of three wells  $\pm$  SEM.

**Supplemental Figure 5: HER3 expression is required for growth of T798M mutant-expressing cells.** *A.* Cells ( $5 \times 10^4$ /well) were seeded in 24-well plates in triplicate. Reverse transfection was performed with SmartPool siRNA against HER3 using Lipofectamine RNAi Max as described in Methods. Seven days post-transfection, the cells were trypsinised and counted using a Coulter counter. Scrambled siRNA was used as control. Bars represent mean of 3 wells  $\pm$  SEM. *B.* Cells were treated for 7 days with 5  $\mu$ M of the locked nucleic acid (LNA) ErbB3 antisense oligonucleotide EZN-3920. EZN-4455 (5 $\mu$ M) was used as a control. Protein extracts were prepared at the end of treatment and subjected to immunoblot analyses with the indicated antibodies.

**Supplemental Figure 6: Inhibition of P-Akt in xenografts treated with trastuzumab  $\pm$  cetuximab.** *A.* Xenografts from mice treated with vehicle, trastuzumab, cetuximab, or the combination (Figure 5I) were fixed in formalin. Paraffin-embedded tumor sections were stained with a S473 P-Akt antibody. Representative images from 3 tumors in each group are shown at 100X magnification. *B.* Intensity of cytoplasmic staining was

determined on a 0 to 3+ scale by an expert pathologist (MVE) blinded to the treatment group. The distribution of cytoplasmic scores is shown for each treatment group. **C.** H-scores were calculated from cytoplasmic intensity as described in Methods. The mean  $\pm$  SEM H-score for each treatment group is shown (n=3 tumors per treatment group; p=0.1204 by ANOVA).

**Supplemental Figure 7: Inhibition of P-Akt in xenografts treated with trastuzumab  $\pm$  lapatinib.** **A.** Xenografts from mice treated with vehicle, trastuzumab, lapatinib, or the combination (Figure 6D) were fixed in formalin. Paraffin-embedded tumor sections were stained with a S473 P-Akt antibody. Representative images from 3 tumors in each group are shown at 100X magnification. **B.** Intensity of cytoplasmic staining was determined on a 0 to 3+ scale by an expert pathologist (MVE) blinded to the treatment group. The distribution of cytoplasmic scores is shown for each treatment group. **C.** H-scores were calculated from cytoplasmic intensity as described in Methods. The mean  $\pm$  SEM H-score for each treatment group is shown (n=4-5 tumors per treatment group; p=0.0669 by ANOVA).