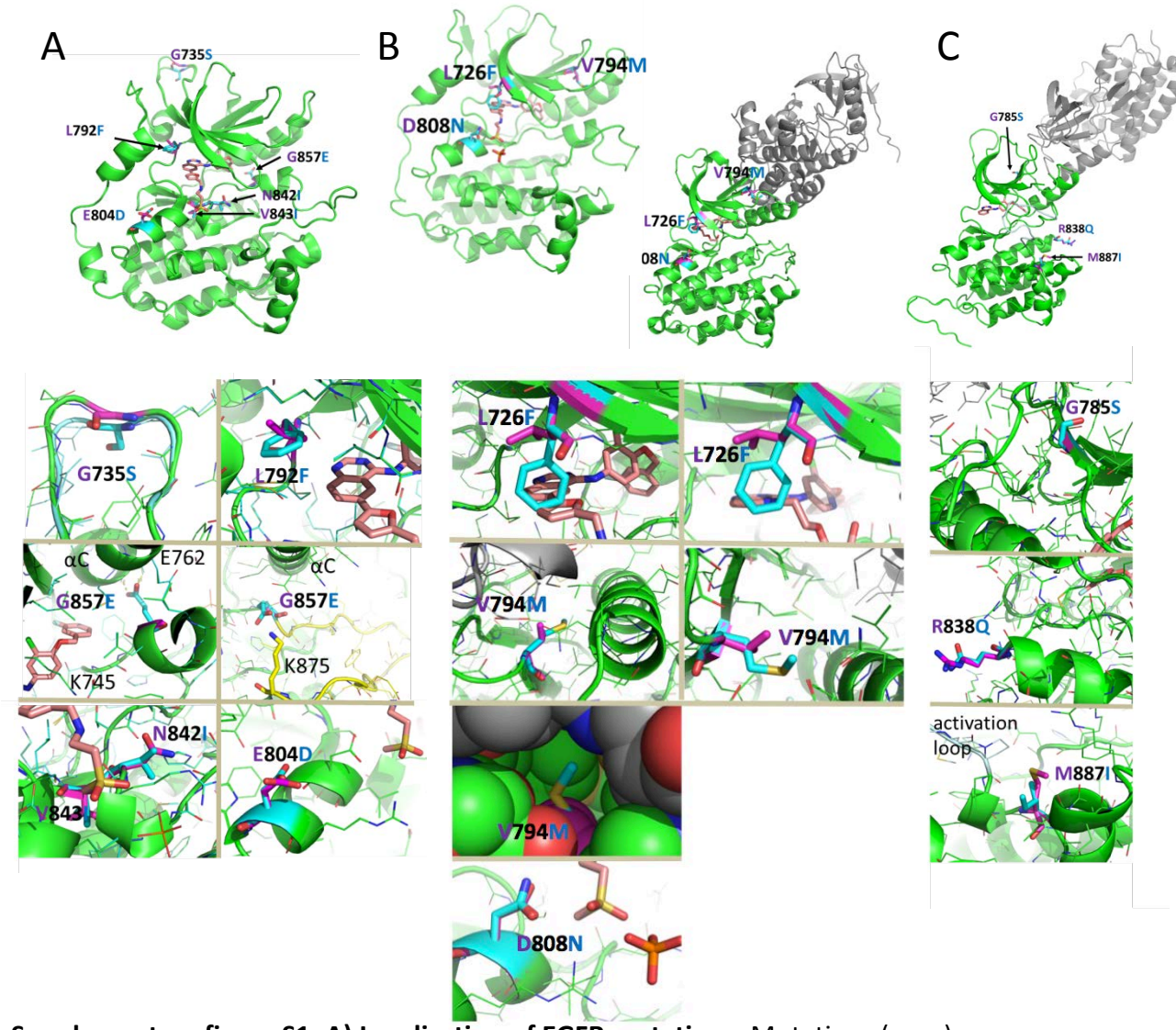
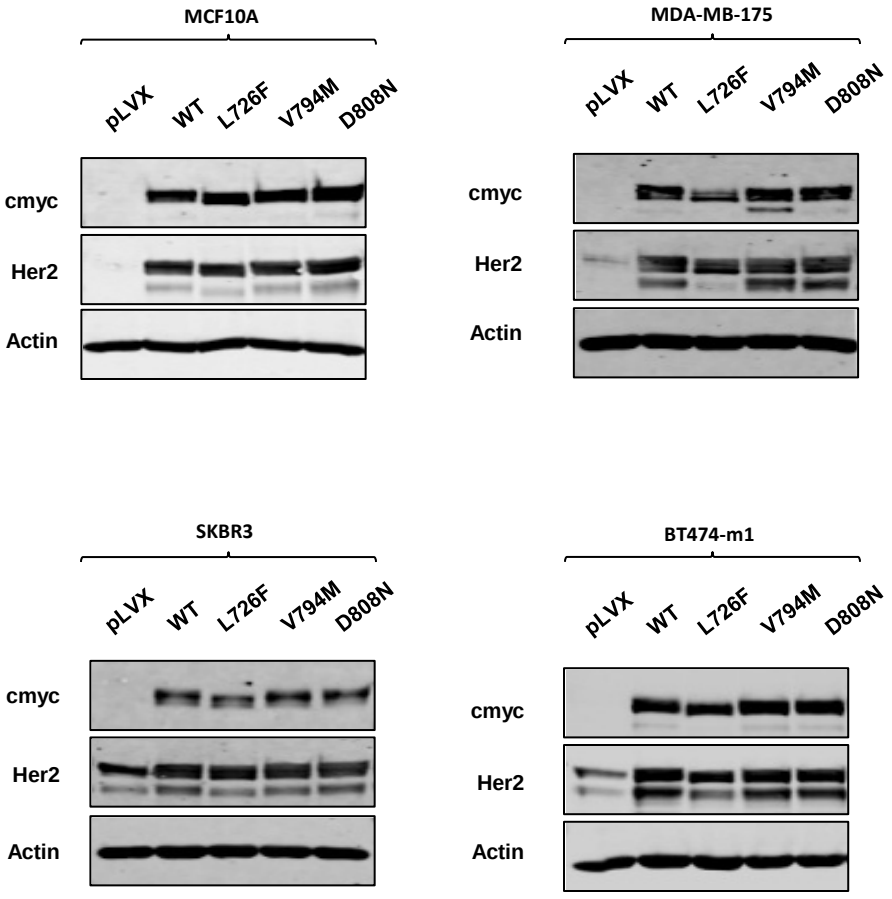
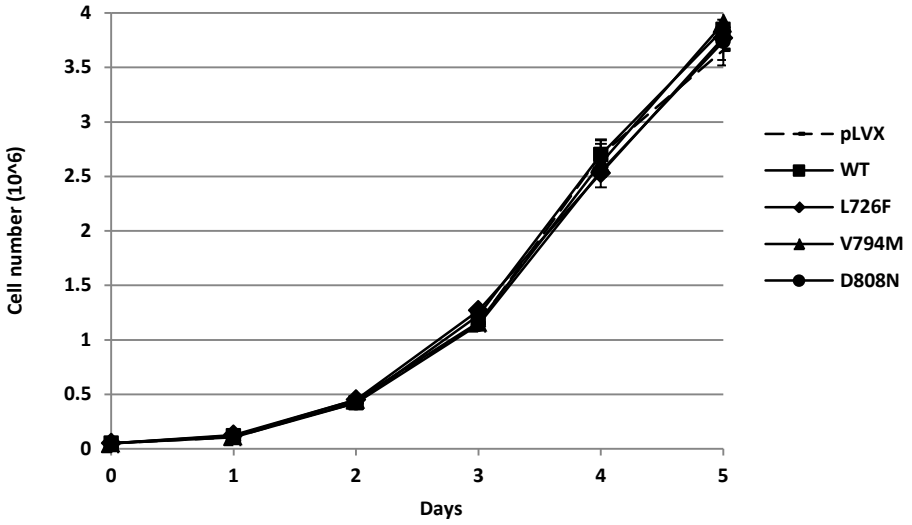



**Supplementary figure S1. A) Localization of EGFR mutations.** Mutations (cyan) are superimposed onto wild-type (magenta) on the model for EGFR bound to lapatinip (stick model with carbons in salmon). The initial crystallographic model (1XKK) has been completed by homology modeling. Lower panels show details of the top panel. For G857E, the left figure shows the mutation in the inactive, lapatinib-bound kinase form. The right figure shows G857E in the active kinase conformation. **B) Localization of HER2 mutations.** Top left shows the mutations projected onto HER2 in an inactive conformation. Top right shows mutations projected onto HER2 in an active conformation (with the activator kinase shown in gray). Lower panels show details of the top panels. For L726F the left panel shows the mutation projected onto the HER2 model produced from the lapatinib-bound EGFR structure (1XKK). The right panel shows the model based on the HER2 kinase in its TKI-bound active-like conformation (3PP0). For V794M, the top left panel shows the model based on activated EGFR kinase (2GS6), lower left panel shows model based on the 'receiver' molecule of EGFR (K721M) in an inactive conformation (3GOP); top left shows stick model, bottom left the corresponding van der Waals sphere model. 'activator' molecule is in gray, 'receiver' in green. Right V794M panel shows model built on HER2 in active-like conformation (open conformation, but with the αC helix in an inactive conformation; 3PP0). **C) Localization of HER4 mutations.** Lower panels show details of the top panel. For details see Supplementary Information.

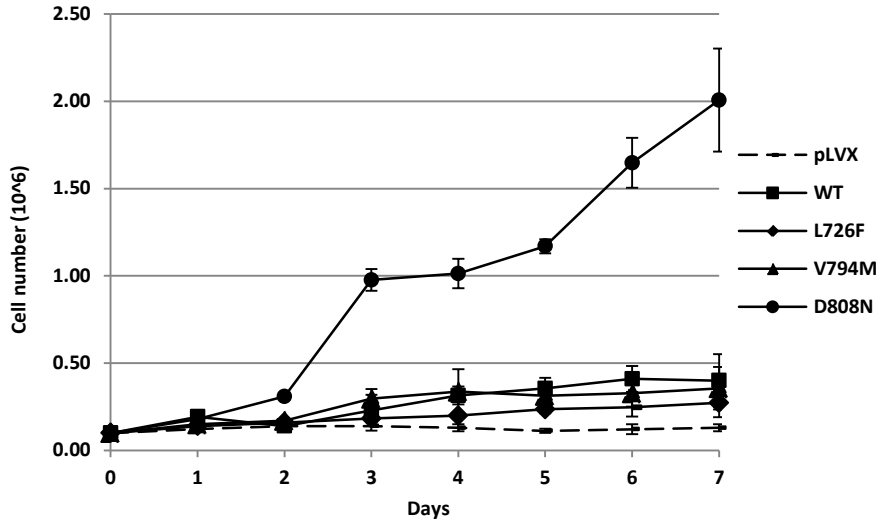



**Supplementary figure S2.** Expression levels of exogenous HER2 wt and mutants in each cell line are comparable.

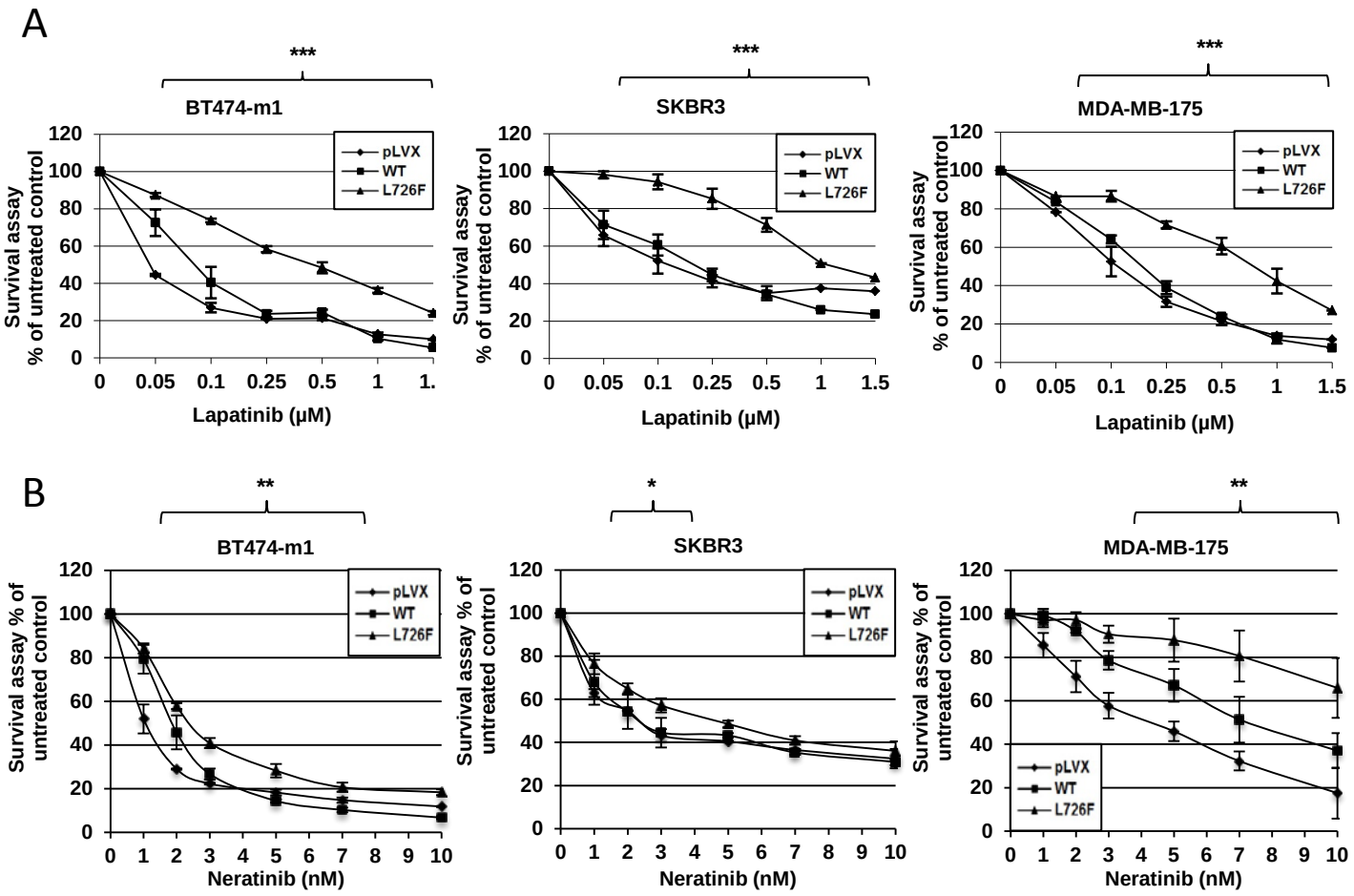
A

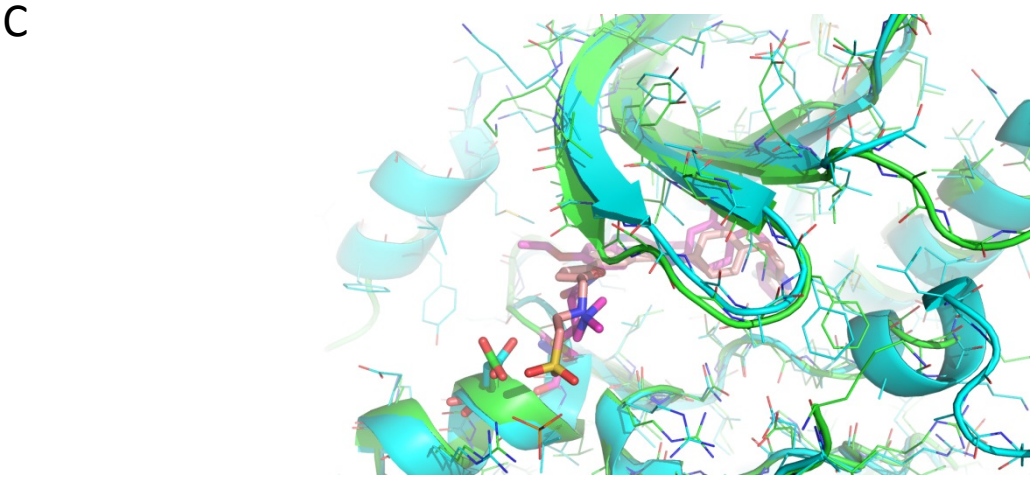



B

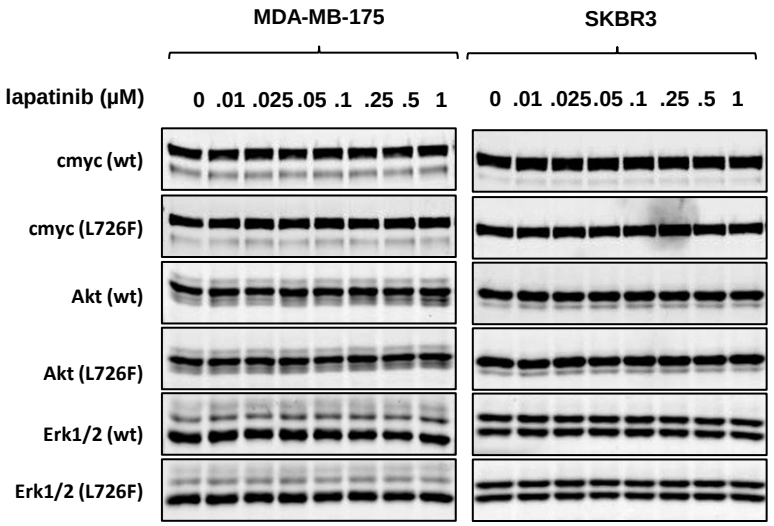



**Supplementary figure S3.** Proliferation of MCF10A cells in complete growth medium (A) and in serum-starved conditions (B).

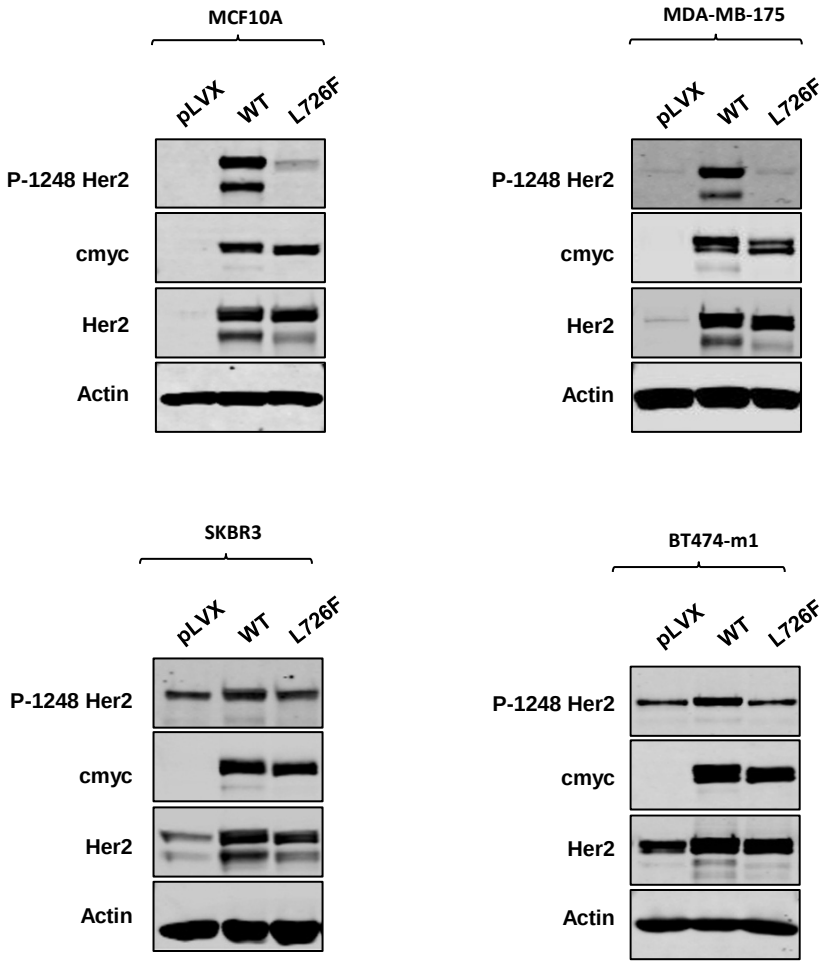


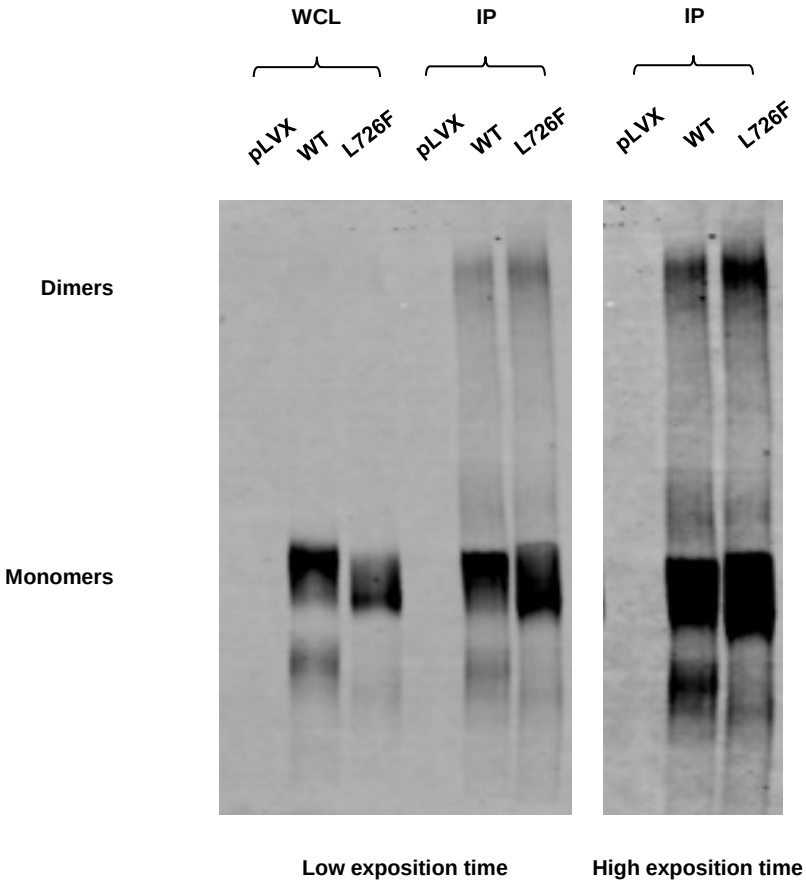

**Supplementary figure S4. A) Response to lapatinib.** Cell survival assay using HER2-positive breast cancer cells expressing equal amounts of exogenous HER2 wt or L726F and incubated with lapatinib. $IC_{50}$ calculated using GraphPad Prism 6 : BT474-m1/WT=0.09 µM and BT474-m1/L726F=0.41 µM ; SKBR3/WT=0.19 µM and SKBR3/L726F=1.03 µM ; MDA-MB-175/WT=0.16 µM and MDA-MB-175/L726F=0.68 µM. **B) Response to neratinib.** Cell survival assay using HER2-positive breast cancer cells expressing equal amounts of exogenous HER2 wt or L726F and incubated with neratinib. **C) Lapatinib and neratinib bind similarly to the kinase active site.** Comparison of the experimental structures of EGFR bound to lapatinib (kinase: cyan; compound: pale orange; PDB 1XKK) and to neratinib (kinase: green; compound: magenta; PDB 2JIV).



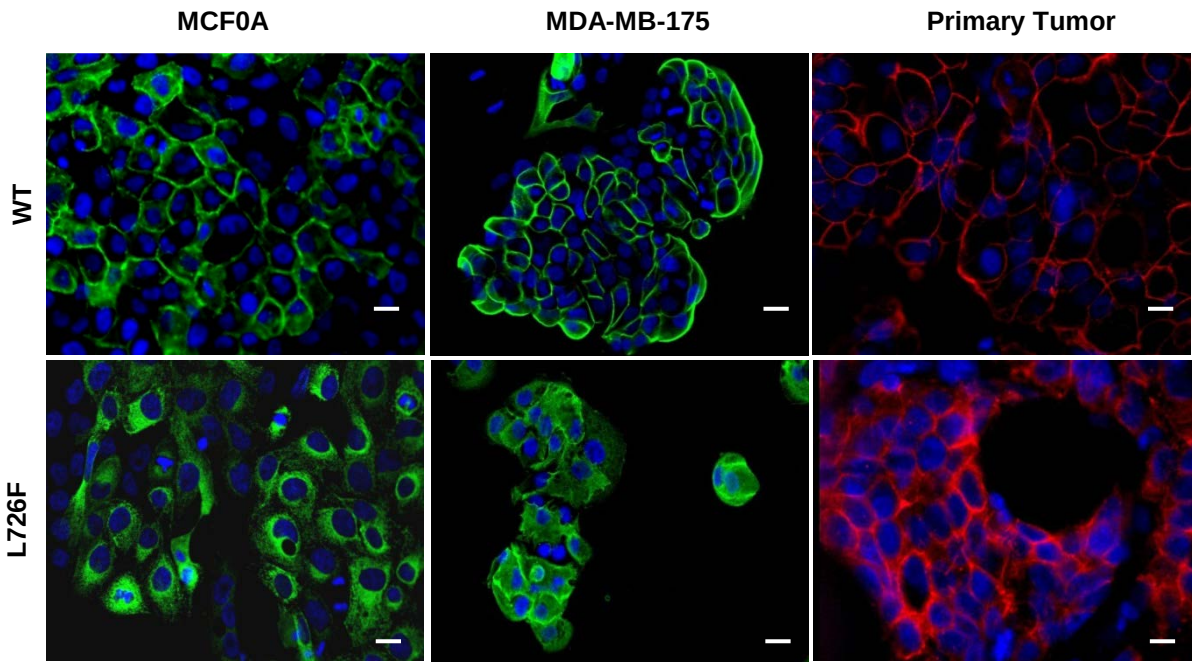

**Supplementary figure S5:** Protein levels from figure 5C.




**Supplementary figure S6:** Underphosphorylation of HER2 L726F was consistent in all cell lines.



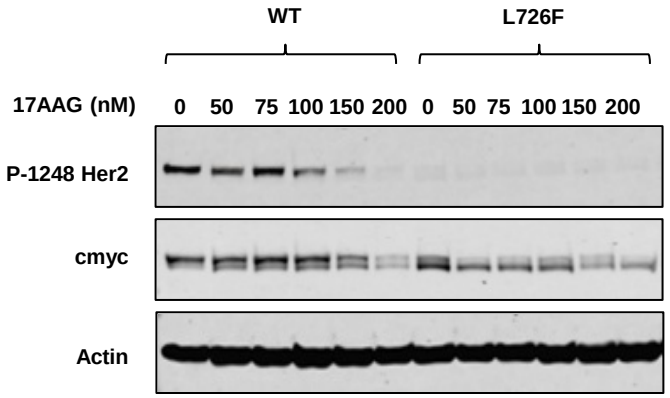

**Supplementary figure S7:** The underphosphorylated state of HER2 L726F is not due to an impaired homodimerization of HER2.




**Supplementary figure S8:** HER2 localization is impaired in both breast cancer cells and tissues.

| Mutation | MT peak/WT peak estimate (%) |
|---|---|
| EGFR. L792F | ~50% |
| EGFR. V843I | ~15% |
| EGFR. G857E | ~40% |
| EGFR. E804D | ~15% |
| EGFR. G735S | ~30% |
| EGFR. N842I | ~10% |
| HER2. L726F | ~95% |
| HER2. V794M | 100% |
| HER2. D808N | ~30% |
| HER4. M887I | ~20% |
| HER4. R838Q | ~25% |
| HER4. G785S | ~15% |

**Supplementary figure S9:** Estimated mutant to wild-type peak ratios for all the variants detected .




**Supplementary figure S10:** HER2 WT and L726F expression after 48 hours incubation with 17AAG in MCF10A.