**Computational and Functional Analyses of HER2 Mutations Reveal Allosteric Activation Mechanisms and Altered Pharmacologic Effects**

Ishiyama et al.

**SUPPLEMENTARY INFORMATION**

**Supplementary Table S1.** List of the 35 previously determined *HER2* oncogenic mutations (29).

|  |  |
| --- | --- |
| **Oncogenic *HER2* mutationa** | **Domain involved** |
| G309A | ECD (CR1) |
| S310F | ECD (CR1) |
| S310Y | ECD (CR1) |
| C311R | ECD (CR1) |
| E321G | ECD (CR1) |
| C334S | ECD (CR1) |
| S653C | TMD |
| V659E | TMD |
| L663P | TMD |
| R678Q | JMD |
| Q709L | JMD |
| L755-T759del | KD |
| L755P | KD |
| L755S | KD |
| I767M | KD |
| D769Y | KD |
| D769H | KD |
| E770-A771insAYVM | KD |
| Y772-A775dup | KD |
| M774-A775insAYVM | KD |
| A775-G776insYVMA | KD |
| G776-V777insYVMA | KD |
| G776V | KD |
| G776delinsVC | KD |
| G776delinsLC | KD |
| V777L | KD |
| G778-P780dup | KD |
| G778-S779insG | KD |
| P780-Y781insGSP | KD |
| L841V | KD |
| V842I | KD |
| T862A | KD |
| L866M | KD |
| L869R | KD |
| R896C | KD |
| aThese mutations can be found in the OncoKB database (<https://www.oncokb.org/>).  CR, cysteine-rich; del, deletion; delins, deletion-insertion; dup, duplication; ECD, extracellular domain; ins, insertion; JMD, juxtamembrane domain; KD, kinase domain; TMD, transmembrane domain. | |

**Supplementary Table S2.** Validation of 111 *HER2* mutations by Ba/F3 cell proliferation assay allowed identification of 37 oncogenic driver mutations and 74 neutral mutations

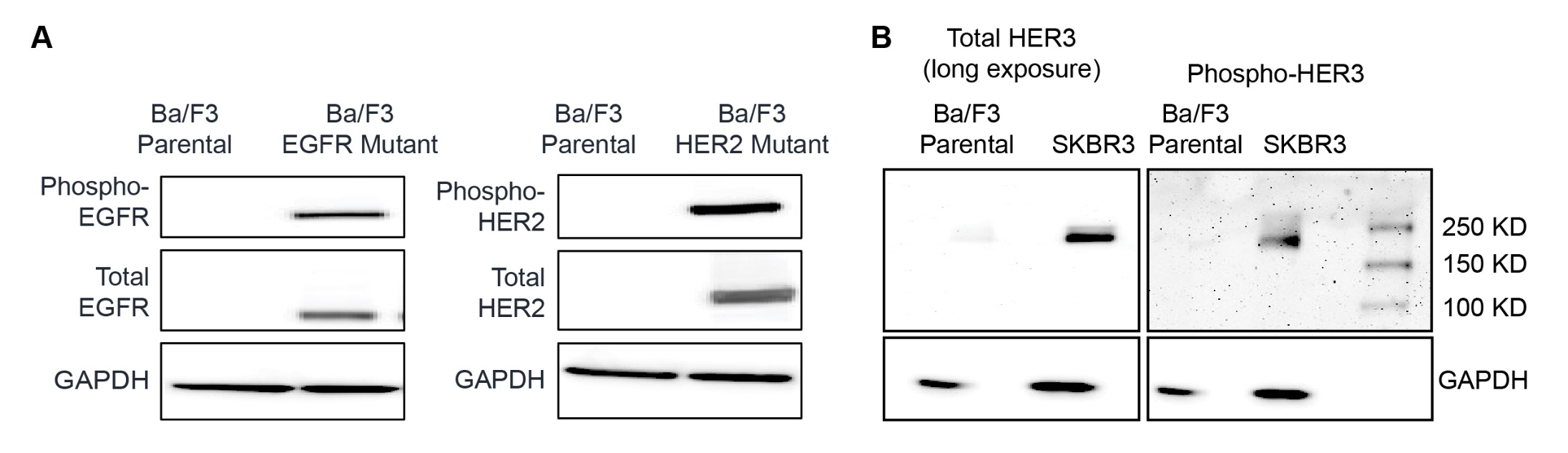
|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| ***HER2* mutation** | **Domain involved** | **Fold growtha (mean ± SEM)** | **MAP score** | **Validation results** | **VAF mean** | **VAF min** | **VAF max** |
| R34W | ECD (L1) | <1 | 0.04 | Neutral mutation | 0.28 | 0.07 | 0.48 |
| R47H | ECD (L1) | <1 | 0.31 | Neutral mutation | 0.34 | 0.15 | 0.53 |
| G58R | ECD (L1) | 3.24 ± 0.08 | 0.33 | **Oncogenic driver mutation** | 0.14 | 0.10 | 0.19 |
| T67A | ECD (L1) | <1 | 0.13 | Neutral mutation | 0.47 | 0.47 | 0.47 |
| R103Q | ECD (L1) | 7.45 ± 0.05 | 0.39 | **Oncogenic driver mutation** | 0.22 | 0.04 | 0.50 |
| P122L | ECD (L1) | 3.69 ± 0.04 | 0.28 | **Oncogenic driver mutation** | 0.35 | 0.09 | 0.49 |
| R143Q | ECD (L1) | <1 | 0.31 | Neutral mutation | 0.48 | 0.45 | 0.52 |
| G151E | ECD (L1) | <1 | 0.16 | Neutral mutation | 0.05 | 0.05 | 0.05 |
| R157Q | ECD (L1) | <1 | 0.13 | Neutral mutation | 0.29 | 0.18 | 0.49 |
| R188H | ECD (L1) | <1 | 0.14 | Neutral mutation | 0.31 | 0.11 | 0.47 |
| R217H | ECD (CR1) | <1 | 0.04 | Neutral mutation | 0.33 | 0.33 | 0.33 |
| V219I | ECD (CR1) | 3.27 ± 0.07 | 0.64 | **Oncogenic driver mutation** | 0.28 | 0.28 | 0.28 |
| G229R | ECD (CR1) | 3.07 ± 0.04 | 0.61 | **Oncogenic driver mutation** | 0.07 | 0.07 | 0.07 |
| S250F | ECD (CR1) | <1 | 0.13 | Neutral mutation | 0.15 | 0.05 | 0.25 |
| I263T | ECD (CR1) | <1 | 0.37 | Neutral mutation | 0.28 | 0.28 | 0.28 |
| T273I | ECD (CR1) | <1 | 0.11 | Neutral mutation | 0.55 | 0.55 | 0.55 |
| G292C | ECD (CR1) | 4.19 ± 0.33 | 0.72 | **Oncogenic driver mutation** | 0.10 | 0.10 | 0.10 |
| G292R | ECD (CR1) | 2.25 ± 0.08 | 0.74 | **Oncogenic driver mutation** | 0.22 | 0.10 | 0.37 |
| A293T | ECD (CR1) | 4.46 ± 0.21 | 0.13 | **Oncogenic driver mutation** | 0.28 | 0.13 | 0.44 |
| S310F | ECD (CR1) | 3.99 ± 0.03 | 0.78 | **Oncogenic driver mutation** | 0.30 | 0.02 | 0.98 |
| S310Y | ECD (CR1) | 3.52 ± 0.12 | 0.68 | **Oncogenic driver mutation** | 0.33 | 0.02 | 0.91 |
| R340Q | ECD (CR1) | <1 | 0.08 | Neutral mutation | 0.31 | 0.05 | 0.50 |
| C342Y | ECD (L2) | <1 | NAb | Neutral mutation | 0.18 | 0.18 | 0.19 |
| G346R | ECD (L2) | <1 | 0.37 | Neutral mutation | 0.12 | 0.12 | 0.12 |
| G346V | ECD (L2) | <1 | 0.31 | Neutral mutation | 0.20 | 0.20 | 0.20 |
| I370M | ECD (L2) | <1 | 0.11 | Neutral mutation | 0.56 | 0.56 | 0.56 |
| P378L | ECD (L2) | <1 | 0.11 | Neutral mutation | 0.27 | 0.17 | 0.38 |
| V424I | ECD (L2) | <1 | 0.07 | Neutral mutation | 0.51 | 0.37 | 0.72 |
| R434Q | ECD (L2) | 4.02 ± 0.30 | 0.14 | **Oncogenic driver mutation** | 0.35 | 0.20 | 0.43 |
| H470Q | ECD (L2) | <1 | 0.56 | Neutral mutation | 0.58 | 0.58 | 0.58 |
| D483N | ECD (L2) | <1 | NAb | Neutral mutation | 0.00 | 0.00 | 0.00 |
| R487Q | ECD (L2) | <1 | 0.09 | Neutral mutation | 0.54 | 0.40 | 0.84 |
| A497T | ECD (L2) | <1 | 0.13 | Neutral mutation | 0.53 | 0.26 | 0.96 |
| E507K | ECD (L2) | <1 | 0.04 | Neutral mutation | 0.18 | 0.10 | 0.29 |
| A510T | ECD (CR2) | <1 | 0.07 | Neutral mutation | 0.71 | 0.71 | 0.71 |
| A516T | ECD (CR2) | 3.34 ± 0.03 | 0.06 | **Oncogenic driver mutation** | 0.42 | 0.23 | 0.60 |
| C520F | ECD (CR2) | <1 | 0.05 | Neutral mutation | 0.30 | 0.18 | 0.41 |
| G549E | ECD (CR2) | <1 | 0.55 | Neutral mutation | 0.07 | 0.07 | 0.07 |
| Q568E | ECD (CR2) | <1 | 0.13 | Neutral mutation | 0.42 | 0.42 | 0.42 |
| A588V | ECD (CR2) | 2.64 ± 0.10 | 0.21 | **Oncogenic driver mutation** | 0.35 | 0.06 | 0.64 |
| K591M | ECD (CR2) | <1 | 0.26 | Neutral mutation | 0.49 | 0.49 | 0.49 |
| F595L | ECD (CR2) | <1 | 0.15 | Neutral mutation | 0.20 | 0.13 | 0.27 |
| G603C | ECD (CR2) | 3.15 ± 0.02 | 0.27 | **Oncogenic driver mutation** | 0.11 | 0.11 | 0.11 |
| A622T | ECD (CR2) | <1 | 0.07 | Neutral mutation | 0.22 | 0.05 | 0.31 |
| P625L | ECD (CR2) | <1 | N/Aa | Neutral mutation | 0.43 | 0.43 | 0.43 |
| E645K | ECD (CR2) | <1 | 0.59 | Neutral mutation | 0.41 | 0.34 | 0.50 |
| G660D | TMD | 2.80 ± 0.05 | 0.4 | **Oncogenic driver mutation** | 0.38 | 0.08 | 0.69 |
| R678Q | JMD | 3.08 ± 0.03 | 0.76 | **Oncogenic driver mutation** | 0.27 | 0.02 | 1.00 |
| G704R | JMD | <1 | 0.1 | Neutral mutation | 0.11 | 0.11 | 0.11 |
| Q709L | JMD | 6.26 ± 0.59 | 0.57 | **Oncogenic driver mutation** | 0.25 | 0.06 | 0.47 |
| E717V | KD | 5.50 ± 0.60 | 0.23 | **Oncogenic driver mutation** | 0.32 | 0.18 | 0.45 |
| G727A | KD | <1 | 0.47 | Neutral mutation | 0.28 | 0.07 | 0.58 |
| G732A | KD | <1 | 0.44 | Neutral mutation | 0.19 | 0.19 | 0.19 |
| T733I | KD | 5.03 ± 0.04 | 0.73 | **Oncogenic driver mutation** | 0.36 | 0.04 | 0.94 |
| V734F | KD | <1 | 0.08 | Neutral mutation | 0.45 | 0.45 | 0.45 |
| A751T | KD | <1 | 0.18 | Neutral mutation | 0.29 | 0.29 | 0.29 |
| L755S | KD | 3.23 ± 0.09 | 0.87 | **Oncogenic driver mutation** | 0.31 | 0.02 | 0.96 |
| L755W | KD | <1 | 0.32 | Neutral mutation | 0.28 | 0.28 | 0.28 |
| R756M | KD | <1 | NAb | Neutral mutation | 0.00 | 0.00 | 0.00 |
| D769H | KD | 3.25 ± 0.03 | 0.89 | **Oncogenic driver mutation** | 0.40 | 0.02 | 0.98 |
| D769Y | KD | 3.11 ± 0.18 | 0.79 | **Oncogenic driver mutation** | 0.32 | 0.02 | 0.89 |
| A775-G776insSVMA | KD | 3.41 ± 0.01 | NAb | **Oncogenic driver mutation** | NAb | NAb | NAb |
| A775-G776insVVMA | KD | 3.33 ± 0.05 | NAb | **Oncogenic driver mutation** | NAb | NAb | NAb |
| A775-G776insYVMA | KD | 3.82 ± 0.05 | NAb | **Oncogenic driver mutation** | NAb | NAb | NAb |
| A775V | KD | <1 | 0.25 | Neutral mutation | 0.20 | 0.09 | 0.31 |
| G776V | KD | 2.93 ± 0.05 | 0.94 | **Oncogenic driver mutation** | 0.36 | 0.08 | 0.78 |
| G776insVC | KD | 3.63 ± 0.07 | NAb | **Oncogenic driver mutation** | NAb | NAb | NAb |
| G776-V777insAVCV | KD | <1 | NAb | Neutral mutation | NAb | NAb | NAb |
| G776-V777insAVGCV | KD | <1 | NAb | Neutral mutation | NAb | NAb | NAb |
| G776-V777insLCV | KD | <1 | NAb | Neutral mutation | NAb | NAb | NAb |
| V777L | KD | 3.07 ± 0.04 | 0.87 | **Oncogenic driver mutation** | 0.41 | 0.03 | 0.94 |
| V777-G778insC | KD | 2.57 ± 0.09 | NAb | **Oncogenic driver mutation** | NAb | NAb | NAb |
| P780-Y781insGSP | KD | 4.43 ± 0.16 | NAb | **Oncogenic driver mutation** | NAb | NAb | NAb |
| V777-G778insV | KD | 3.67 ± 0.23 | NAb | **Oncogenic driver mutation** | NAb | NAb | NAb |
| G778-S779insCPG | KD | 2.29 ± 0.02 | NAb | **Oncogenic driver mutation** | NAb | NAb | NAb |
| G778-S779insG | KD | 7.50 ± 0.77 | NAb | **Oncogenic driver mutation** | NAb | NAb | NAb |
| L785F | KD | <1 | 0.13 | Neutral mutation | 0.42 | 0.28 | 0.55 |
| D808E | KD | <1 | 0.1 | Neutral mutation | 0.78 | 0.67 | 0.89 |
| L823P | KD | <1 | 0.2 | Neutral mutation | 0.07 | 0.07 | 0.07 |
| G832E | KD | <1 | 0.07 | Neutral mutation | 0.14 | 0.14 | 0.14 |
| G832V | KD | <1 | 0.26 | Neutral mutation | 0.27 | 0.27 | 0.27 |
| V842I | KD | 3.89 ± 0.05 | 0.89 | **Oncogenic driver mutation** | 0.30 | 0.02 | 0.98 |
| T862A | KD | 2.55 ± 0.06 | 0.79 | **Oncogenic driver mutation** | 0.32 | 0.05 | 0.86 |
| G865E | KD | <1 | 0.19 | Neutral mutation | 0.13 | 0.13 | 0.13 |
| G865R | KD | <1 | 0.11 | Neutral mutation | 0.19 | 0.04 | 0.48 |
| L869R | KD | 2.90 ± 0.07 | 0.69 | **Oncogenic driver mutation** | 0.40 | 0.10 | 0.81 |
| Y877C | KD | <1 | 0.39 | Neutral mutation | 0.22 | 0.22 | 0.22 |
| Q902H | KD | <1 | 0.13 | Neutral mutation | 0.12 | 0.12 | 0.12 |
| S903I | KD | <1 | 0.19 | Neutral mutation | 0.08 | 0.08 | 0.08 |
| W906C | KD | <1 | 0.03 | Neutral mutation | 0.25 | 0.25 | 0.25 |
| W906G | KD | <1 | NAb | Neutral mutation | 0.00 | 0.00 | 0.00 |
| P922H | KD | <1 | 0.05 | Neutral mutation | 0.41 | 0.41 | 0.41 |
| D950Y | KD | <1 | 0.11 | Neutral mutation | 0.04 | 0.04 | 0.04 |
| S977T | KD | <1 | 0.06 | Neutral mutation | 0.63 | 0.63 | 0.63 |
| G1015E | CTD | <1 | NAb | Neutral mutation | 0.00 | 0.00 | 0.00 |
| E1022K | CTD | <1 | 0.22 | Neutral mutation | 0.08 | 0.08 | 0.08 |
| P1037L | CTD | <1 | 0.01 | Neutral mutation | 0.43 | 0.36 | 0.49 |
| A1039T | CTD | <1 | 0.01 | Neutral mutation | 0.15 | 0.06 | 0.23 |
| R1048C | CTD | <1 | 0.01 | Neutral mutation | 0.32 | 0.14 | 0.46 |
| R1053G | CTD | <1 | 0.01 | Neutral mutation | 0.46 | 0.44 | 0.48 |
| G1056S | CTD | <1 | 0 | Neutral mutation | 0.50 | 0.15 | 0.82 |
| K1096N | CTD | <1 | 0.01 | Neutral mutation | 0.25 | 0.04 | 0.45 |
| P1102L | CTD | <1 | 0.01 | Neutral mutation | 0.52 | 0.43 | 0.67 |
| V1128I | CTD | <1 | 0.03 | Neutral mutation | 0.36 | 0.11 | 0.85 |
| R1146W | CTD | <1 | 0.01 | Neutral mutation | 0.30 | 0.18 | 0.36 |
| L1157R | CTD | <1 | 0.01 | Neutral mutation | 0.49 | 0.29 | 0.75 |
| R1161Q | CTD | <1 | NAb | Neutral mutation | 0.00 | 0.00 | 0.00 |
| K1177E | CTD | <1 | 0.02 | Neutral mutation | 0.22 | 0.22 | 0.22 |
| K1182Q | CTD | <1 | 0.01 | Neutral mutation | 0.44 | 0.36 | 0.50 |
| Q1200H | CTD | <1 | 0.01 | Neutral mutation | 0.14 | 0.14 | 0.14 |
| R1230Q | CTD | 5.31 ± 0.32 | 0 | **Oncogenic driver mutation** | 0.30 | 0.14 | 0.47 |
| aProliferation of Ba/F3 cells transformed with HER2 variants and cultured in the absence of IL-3 was measured over a 72-hour period. Fold growth calculated as reading at 72 hours divided by reading at time plating. bMissing MAP scores are due to a number of possible reasons: indels were excluded from the calculations; some missense mutations were either not found in the mutation database used for MAP analysis or excluded from MAP scoring due to missing features.  CR, cysteine-rich; CTD, C-terminal domain; del, deletion; delins, deletion-insertion; dup, duplication; ECD, extracellular domain; IL, interleukin; ins, insertion; JMD, juxtamembrane domain; KD, kinase domain; TMD, transmembrane domain; VAF, variant allelic fraction. | | | | | | | |

**Supplementary Table S3.** Clinical data, *HER2* mutations, and other co-affected genes

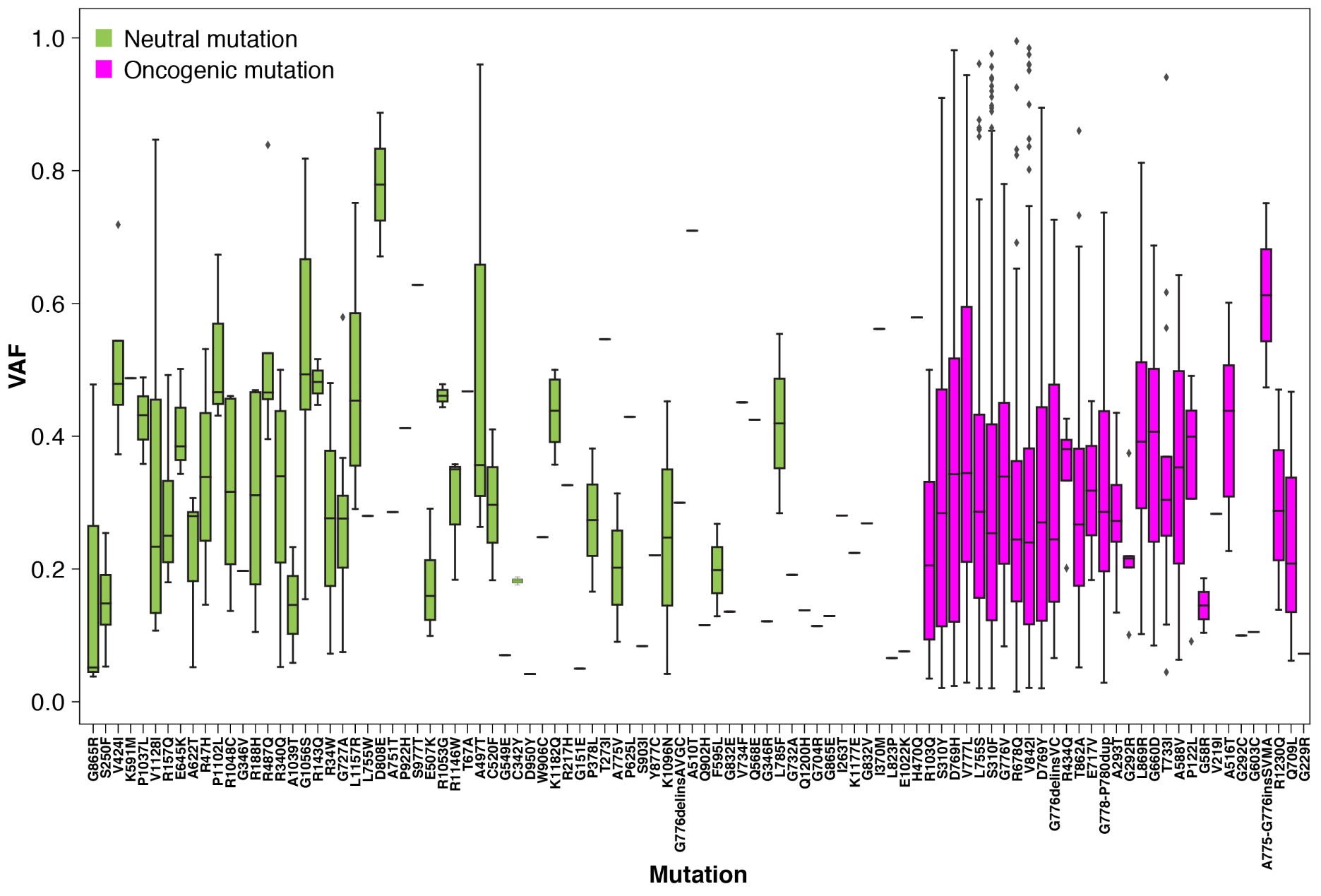
[Table provided as an excel file]

**Supplementary Table S4.** Raw data generated to develop Figure 5A

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **HER2 WT** | | | **HER2** **Δ16** | | | **HER2 p95-M611** | | | **HER2 S310F** | | |
| **Lapatinib, µM** | **Rep1** | **Rep2** | **Rep3** | **Rep1** | **Rep2** | **Rep3** | **Rep1** | **Rep2** | **Rep3** | **Rep1** | **Rep2** | **Rep3** |
| 1.0000 | -0.412 | -0.441 | -0.452 | -0.178 | -0.194 | -0.232 | -0.209 | -0.217 | -0.240 | -0.231 | -0.272 | -0.256 |
| 0.3333 | -0.300 | -0.330 | -0.327 | 0.070 | 0.060 | -0.013 | -0.193 | -0.201 | -0.227 | 0.529 | 0.527 | 0.564 |
| 0.1111 | -0.163 | -0.212 | -0.249 | 0.771 | 0.821 | 0.710 | -0.177 | -0.183 | -0.194 | 0.803 | 0.863 | 0.813 |
| 0.0370 | 0.031 | -0.014 | -0.017 | 1.075 | 1.061 | 1.002 | 0.414 | 0.373 | 0.532 | 0.899 | 1.020 | 0.906 |
| 0.0123 | 0.533 | 0.593 | 0.483 | 1.056 | 1.094 | 1.025 | 0.907 | 1.081 | 1.219 | 1.049 | 1.082 | 0.990 |
| 0.0041 | 0.700 | 0.799 | 0.642 | 1.087 | 1.022 | 1.006 | 0.980 | 1.123 | 1.195 | 1.097 | 1.101 | 0.997 |
| 0.0014 | 0.963 | 1.020 | 0.951 | 1.100 | 1.043 | 0.965 | 1.007 | 1.089 | 1.116 | 1.034 | 1.093 | 1.032 |

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**Supplementary Figure S1.** Western blot to confirm lack of endogenous EGFR, HER2 and HER3 protein expression in Ba/F3 cells. Relative mutant EGFR/HER2 expressions in transformed Ba/F3 cells are shown in Panel **A**. HER3 expression levels (total and phosphorylated HER3) in SKBR3 cells are shown as a control in Panel **B**.



**Supplementary Figure S2:** Distribution of VAF for oncogenic and neutral *HER2* mutations.

**A picture containing chart

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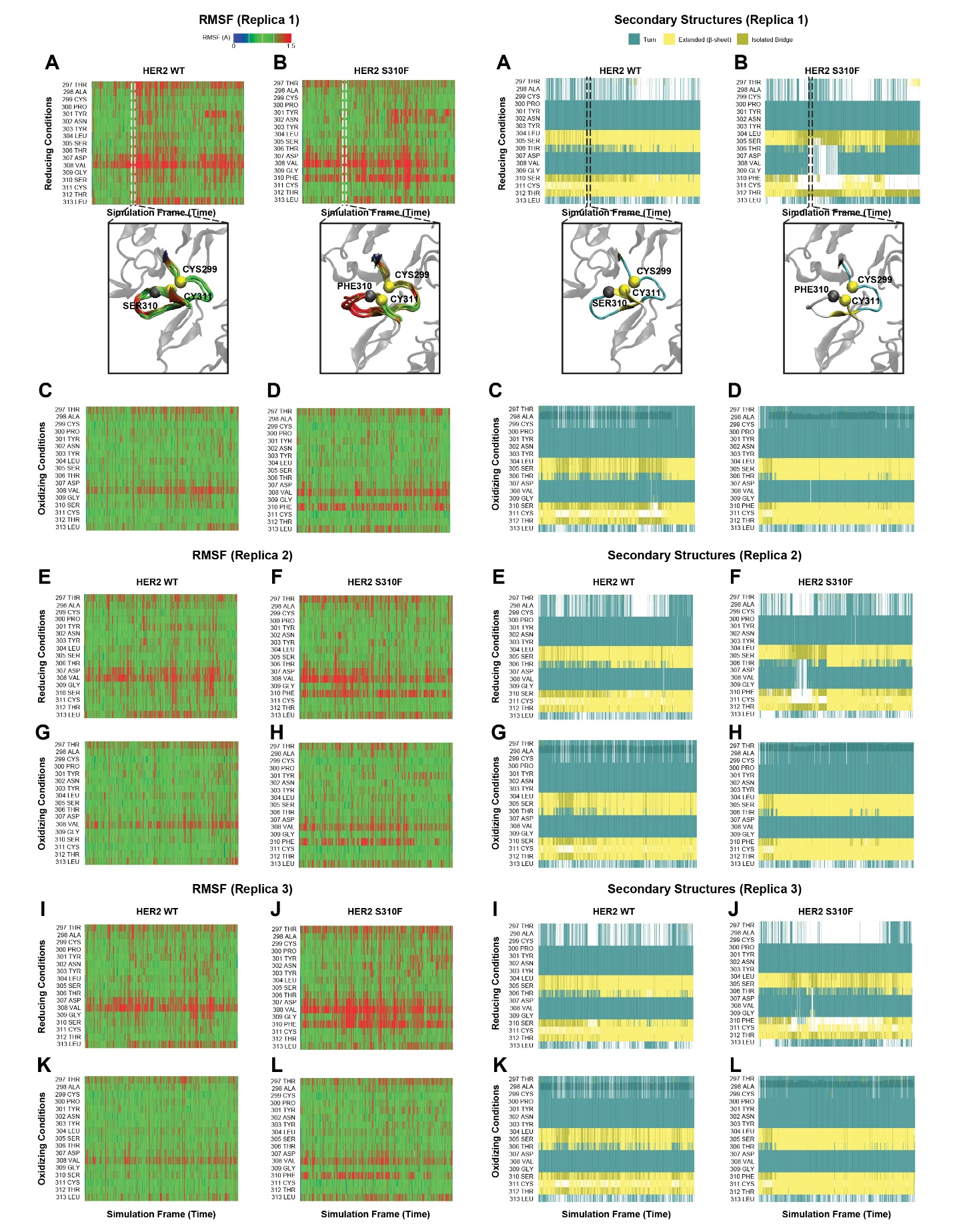
**Supplementary Figure S3:** Prevalence of validated driver mutations in **A,** solid tumor, **B,** breast, **C,** lung, **D,** bladder, and **E,** colorectal cancer.

Diagram

Description automatically generated

**Supplementary Figure S4.** MD simulations of S310F substitution

The effects of the S310F substitution is simulated by examining four different MD simulations using HER2 ECD structure (PDB IDs: 6J71) (see Methods). The four initial structures as MD starting conformation are: (1) HER2 WT structure (with Ser at position 310) with disulfide bond between Cys299-Cys311 (HER2 WT oxidized, captured in **Panel A**); (2) HER2 WT structure with no disulfide bond formed between Cys299-Cys311 (HER2 WT reduced, captured in   
**Panel B**); (3) S310F-mutated structure (with Phe at position 310) with disulfide bond between Cys299-Cys311 (HER2 S310F oxidized, captured in **Panel A**); (4) S310F-mutated structure with no disulfide bond formed between Cys299-Cys311 (HER2 S310F reduced, captured in **Panel B**).



**Supplementary Figure S5.** TOP: Root mean square fluctuations (RMSF; left) and secondary structure changes (right) are shown on representative structures from WT (**A**) and mutant (**B**) simulations (Replica 1). The fluctuations and secondary structure annotations for residues between 297-313 are highlighted and the rest of the protein structures shown in transparent grey cartoon. Note that RMSF values for each residue from 5 windows (frames 183-187) around frame 185 are projected on the structure.

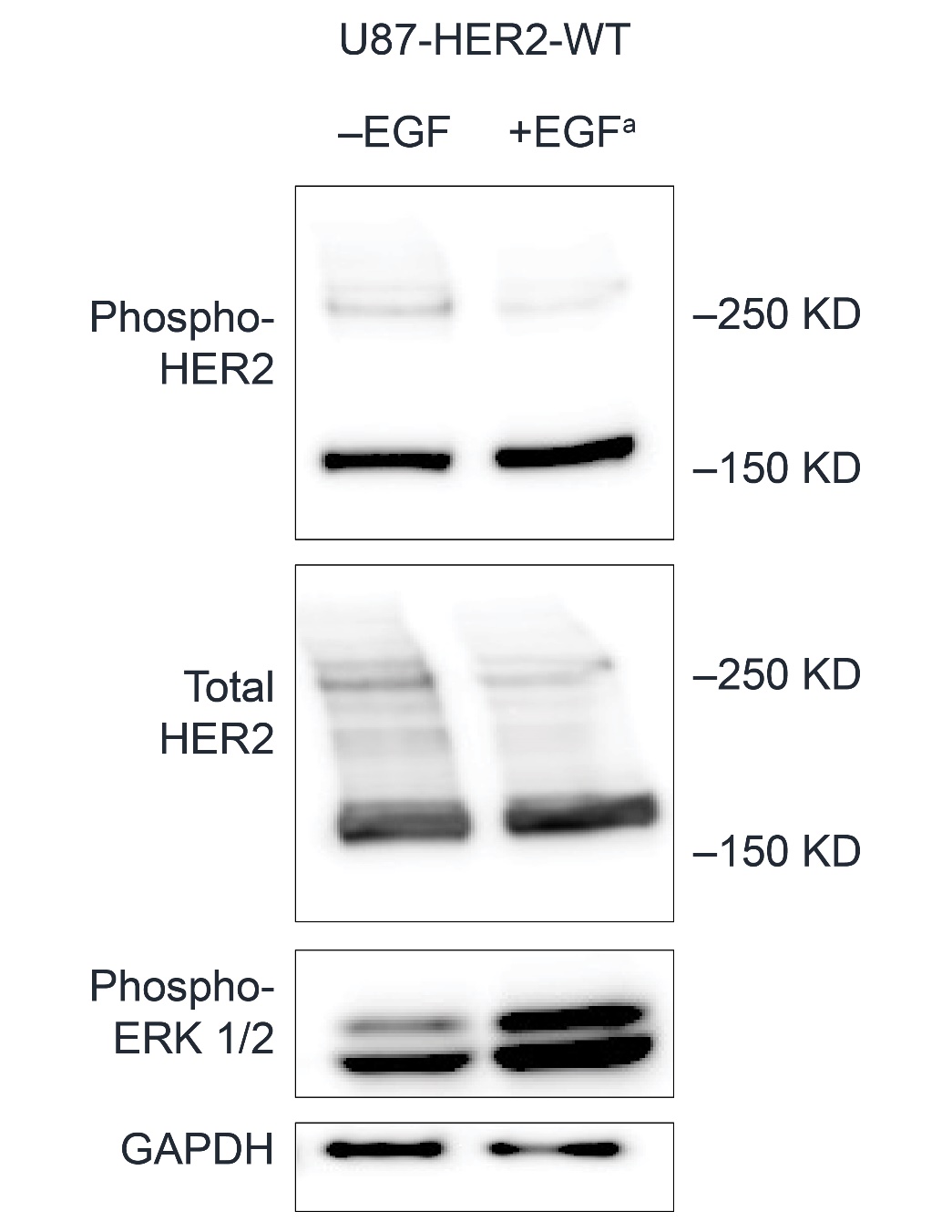
BOTTOM: RMSF (left) and secondary structure changes (right) during WT (**A, C, E, G, I, K**) and mutant (**B, D, F, H, J, L**) simulations in reducing and oxidizing conditions. In each replica simulation of HER2 S310F-reduced (**B, F, J**), we have observed substantially larger fluctuations around residues 308-310 upon substitution of Ser310 with Phe, compared with the residue fluctuations from the HER2 WT-oxidized, the HER2 WT-reduced, and HER2 S310F-oxidized simulations. It was also observed that the distance between Thr306 and Phe310 becomes larger in the HER2 S310F-reduced simulation (Fig. 4B). The larger fluctuations and distance between Thr306 and Phe310 residues may result in distortion of β-sheet spanning residues 310-312. This data from MD simulations suggests that S310F mutation may destabilize β-sheet–spanning residues 310-312 by destabilizing/weakening residue-residue interactions. As a consequence of the destabilization due to S310F mutation, free Cys311 may reach another Cys on its adjacent chain to form a disulfide bond for HER2 ECD dimerization.

Chart

Description automatically generated

**Supplementary Figure S6.** Perturbation-response scanning (PRS) profile of HER2 ECD structure.

The effectiveness and sensitivity plots **(A, C**) describe the propensity of residues to propagate structural changes (as effectors, **A**), and to be sensitive to such effects (as sensors, **C**), respectively. The dotted black lines on the figures mark the cutoff (0.5) for defining the strongest effectors and sensors. The high-medium-low values of the effectors (**B**) and sensors (**D**) are indicated on the 3D structure of HER2 ECD using the red-green-blue spectrum. The highest effectors (>0.5) are shown with red circles and located at the interfaces of CR1, L1 and L2 domains of ECD structure (**B**).



**Supplementary Figure S7.** Western blot for WT HER2 expressed in U87MG cells. WT HER2 does not exist as a covalent dimer.

aU87MG cells were stimulated with 100 ng/mL of EGF for 5 minutes.