SUPPORTING INFORMATION

Rigidity of the extracellular part of HER2: Evidence from engineering subdomain interfaces and shared-helix DARPin-DARPin fusions

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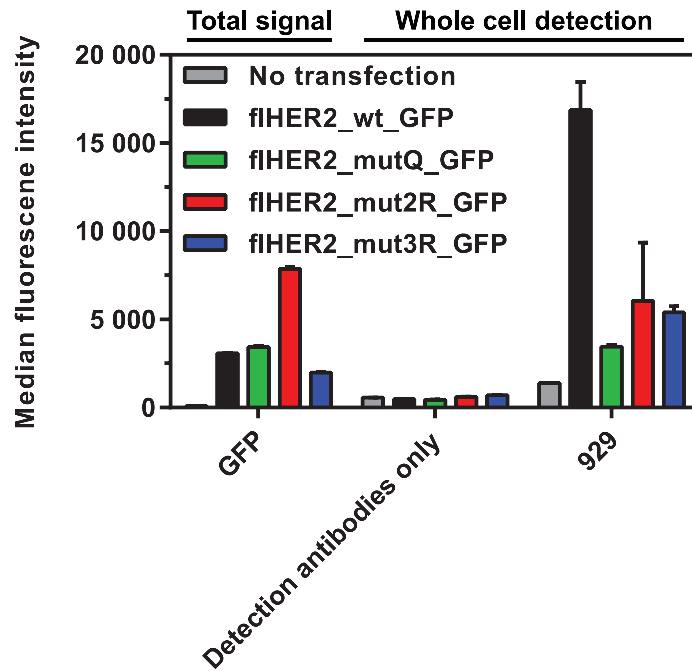

Figure S1. Mutations specifically destabilizing the open conformation of full-length HER2 (flHER2) result in little functional protein in the permeabilized whole cell. Binding of DARPin 929 to HEK293T/17 cells transiently transfected with wild-type HER2 or mutants (see Table 1 for list of mutations) after permeabilization with Tween-20 was detected in flow cytometry using anti-DARPin serum. The binding signal of DARPin 929 for the HER2 mutants (*green*, *red*, *blue* bars) is strongly reduced compared to wild-type HER2 (*black* bars). Error bars represent one standard deviation of triplicates. The GFP signal was recorded in a separate channel and is scaled for display.

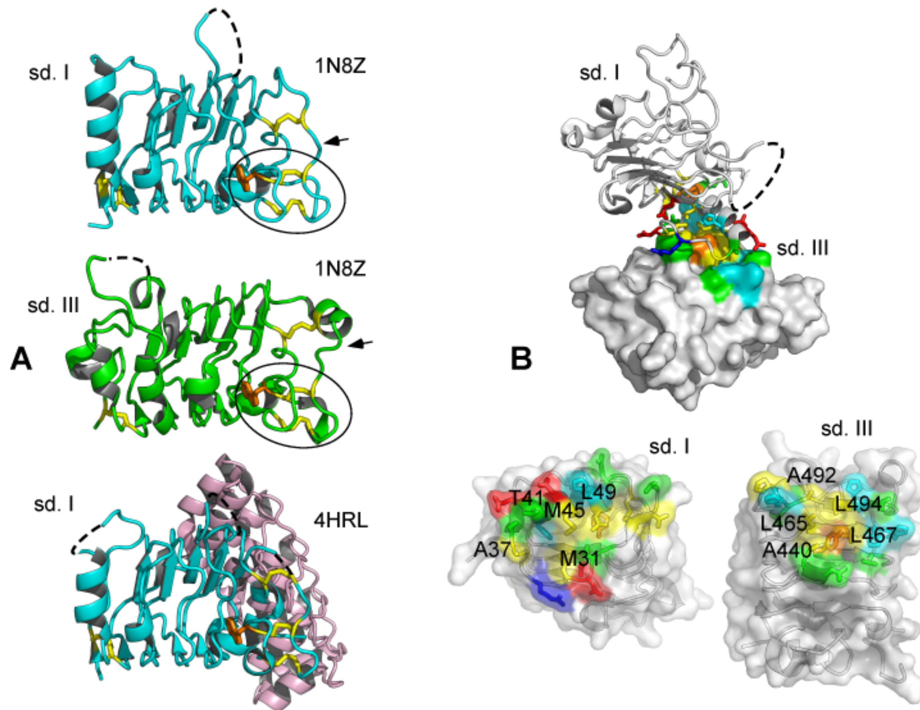

Figure S2: (A) Boundaries between HER2 subdomains (sd.) I and II, III and IV. Based on sequence features, the boundary between subdomain I and cystein-rich subdomain II in HER2 was historically placed after Cys 192, the boundary between subdomain III and cysteine-rich subdomain IV after Cys 504 (arrow). Our structural analysis had shown, however, that residues 193-212 and 505-528 are essential for proper folding of the two domains, as Trp 205 and Trp521 are firmly embedded in the respective domains. Thus, the subdomain boundaries have to be placed after Cys 212 and after Cys 528. If these domain boundaries are used, the isolated domains fold properly, as demonstrated by the structures of HER2_I in complex with DARPin 929 (PDB ID 4HRL[1]) and with scFv chA21 (PDB ID 3H3B[2]). (B) Interface between HER2 subdomains I and III: Interface residues were color-coded according to the following scheme: aromatic (FYW), *orange*; aliphatic and cysteine-containing (AILPV, M, C), *yellow*; uncharged hydrophilic (STNQ), *green*; acidic (DE), *red*; basic (H) *cyan*; (KR) *dark blue*. Mutations were introduced to disrupt the hydrophobic

interaction across the interface, and labeled positions were mutated in at least one of the three constructs (Table 1).

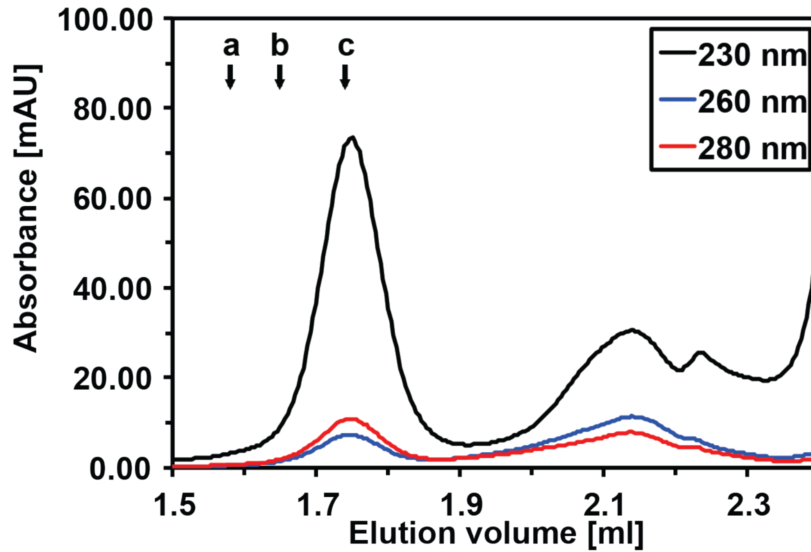

Figure S3: Size exclusion chromatography of purified HER2_I (expected molecular weight: 22.9 kDa): Immobilized metal affinity chromatography (IMAC)-purified HER2_I was applied at a concentration of 10 µM to a Superdex 75 column, recording absorbance at 230, 260 and 280 nm. Peak maxima of protein standards (*a*, catalase monomer, 58 kDa; *b*, ovalbumin, 42 kDa; *c*, chymotrypsinogen A, 25 kDa) are indicated by arrows. HER2_I protein eluted as a single, monomeric peak at an elution volume of 1.75 ml, corresponding to an apparent molecular weight of 24 kDa. The non-proteinaceous peak eluting at 2.15 ml is caused by low molecular weight DNA contaminants, co-purified during IMAC.

SI references

1. Jost C, Schilling J, Tamaskovic R, Schwill M, Honegger A, Plückthun A (2013) Structural basis for eliciting a cytotoxic effect in HER2-overexpressing cancer cells via binding to the extracellular domain of HER2. Structure 21:1979-1991.
2. Zhou H, Zha Z, Liu Y, Zhang H, Zhu J, Hu S, Shen G, Cheng L, Niu L, Greene MI, Teng M, Liu J (2011) Structural insights into the down-regulation of overexpressed p185her2/neu protein of transformed cells by the antibody chA21. J Biol Chem 286:31676-31683.