

SUPPLEMENTARY FIGURE LEGENDS

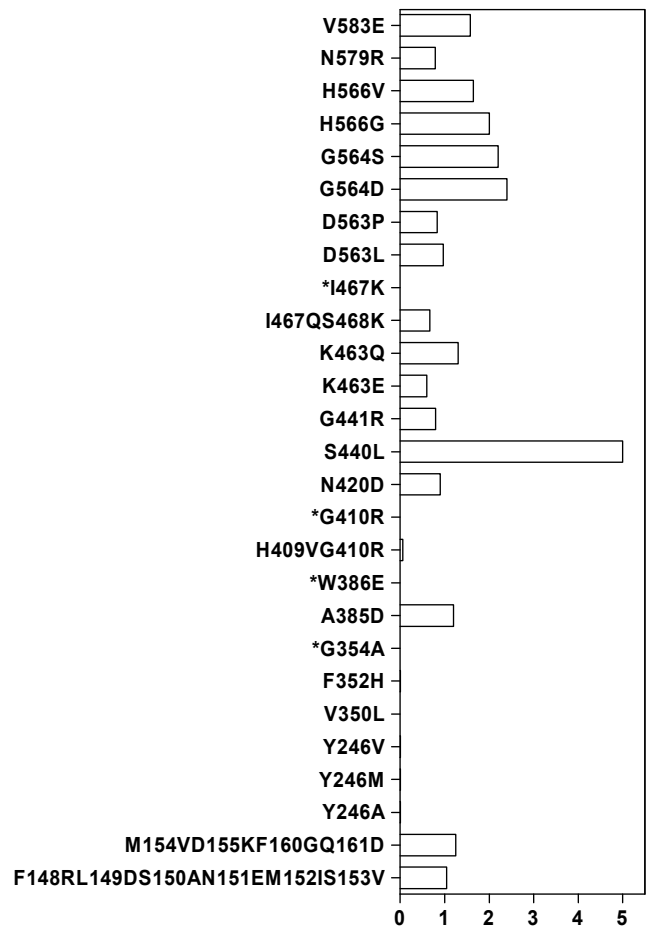
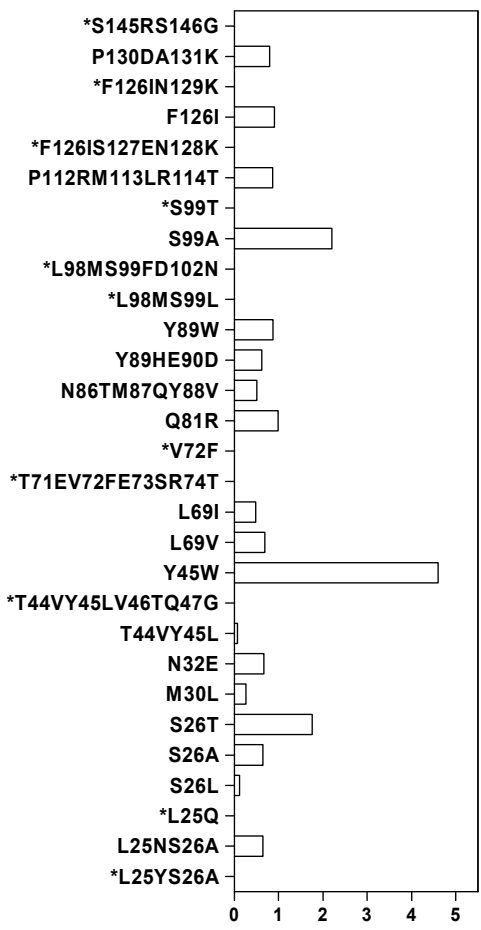
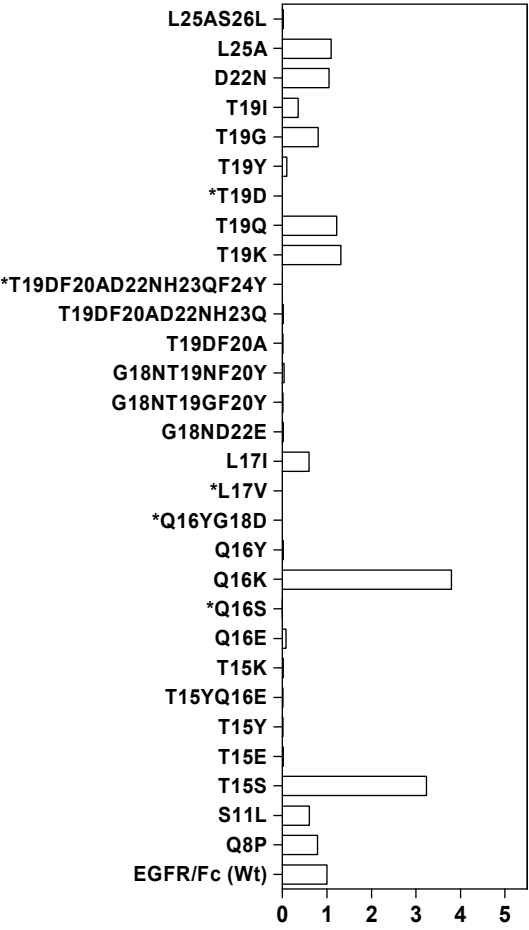
Supplementary Figure 1. Eu-EGF binding screening identifies mutants of EGFR/Fc with improved ligand binding affinity. Screening was carried out in anti-Fc-coated 96-well plates. Conditioned media of HEK293 cells transiently transfected with cDNA constructs expressing EGFR/Fc mutants were collected, and quantitated by ELISA for the presence of EGFR/Fc proteins. Twenty ng of the EGFR/Fc protein from each sample was immobilized onto the Fc-coated plates. Eu-EGF binding was carried out as described in the Methods. Eu-EGF binding of each mutant was expressed as fold of binding of the wild type (Wt) EGFR/Fc which was set as 1. Results shown are from single data point or means of duplicate measurements. Samples with * indicate no detectable secretion.

Supplementary Figure 2. Eu-NGR1- β binding screening to identify mutants of HER3/Fc with improved ligand affinity. Screening was carried out as described in Supplementary Figure 1. Results shown are from single data point or means of duplicate measurements.

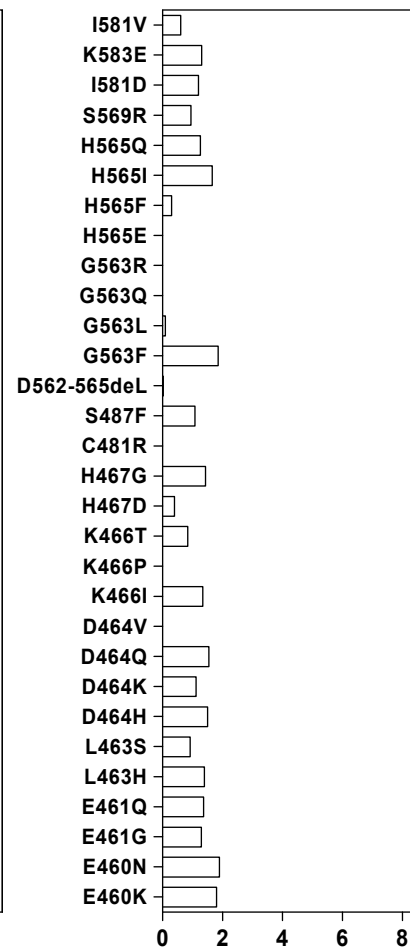
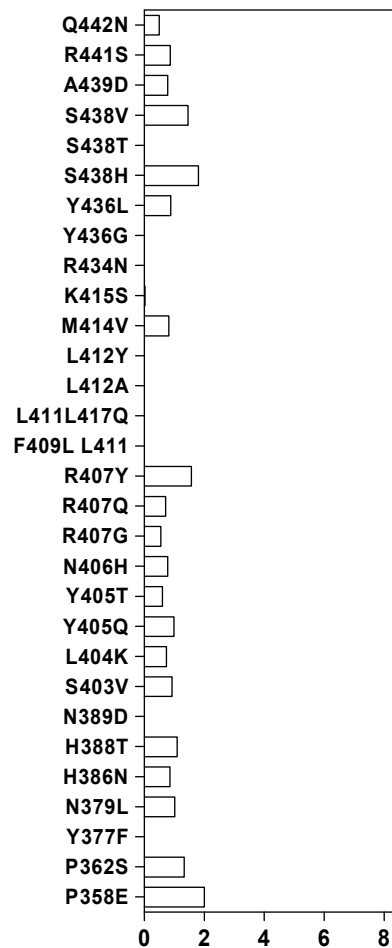
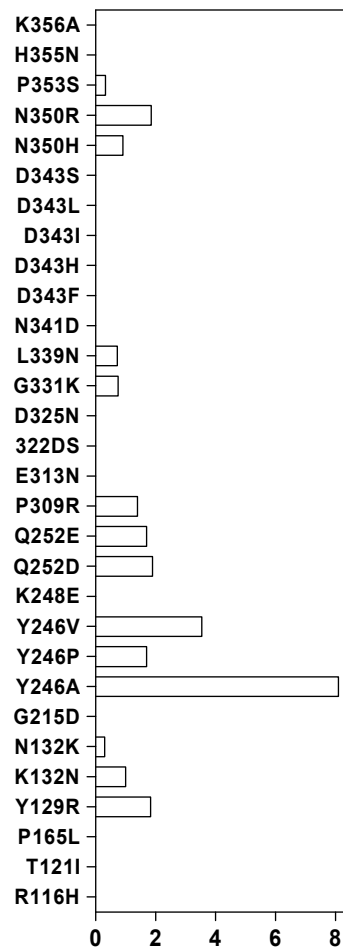
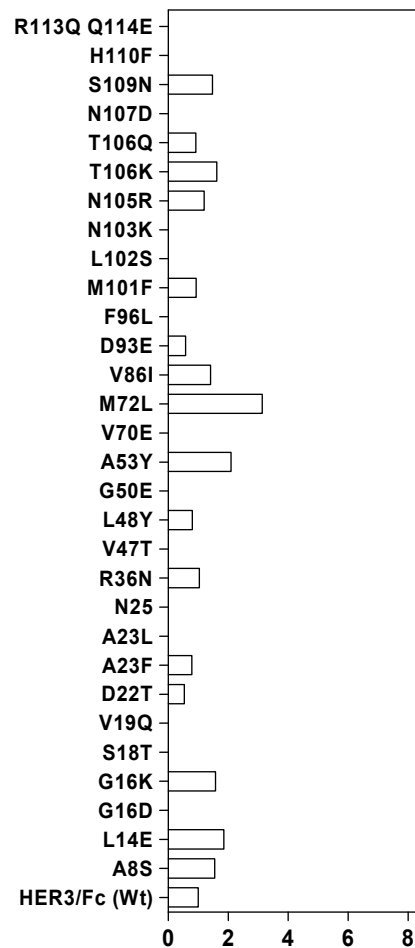
Supplementary Figure 3. Affinity-optimized HER mutants are more potent than their parent forms in inhibition of growth factor-induced EGFR and HER3 phosphorylation. Serum-starved N87 gastric cancer cells (A, B, D, E) or MCF7 breast cancer cells (C, F) were treated with 3 nM of EGF (A, D), TGF- α (B, E), or NRG1- β (C, F) in the presence of increasing amounts of the indicated homodimer (A, B, C) or heterodimer inhibitors (D, E, F). Ten minutes later, treated cells were washed with PBS and lysed. Lysates were analyzed for the presence of phosphorylated EGFR or HER3 using a quantitative phospho-ELISA assay (23). (G) Summary table of the estimated EC₅₀. Note: Not all dose-curves shown were fully titrated down to zero.

Supplementary Figure 4. Ribbon diagram of the EGFR ligand binding domain in complex with TGF- α (PDB code 1IVO-chain C) (25). (A) Shown are T15 (green) of EGFR, C32 (red) of TGF- α , and their van der Waals contact. (B) The zoom-in graph. The 3.1Å hydrogen bond formed between the T15 and C32 is measured with the Swiss-PdbViewer as described in the Methods, and is shown as a pink dashed line. The 3.1Å hydrogen bond is likely shortened to 2.7 Å in EGFR^{T15S} mutant.

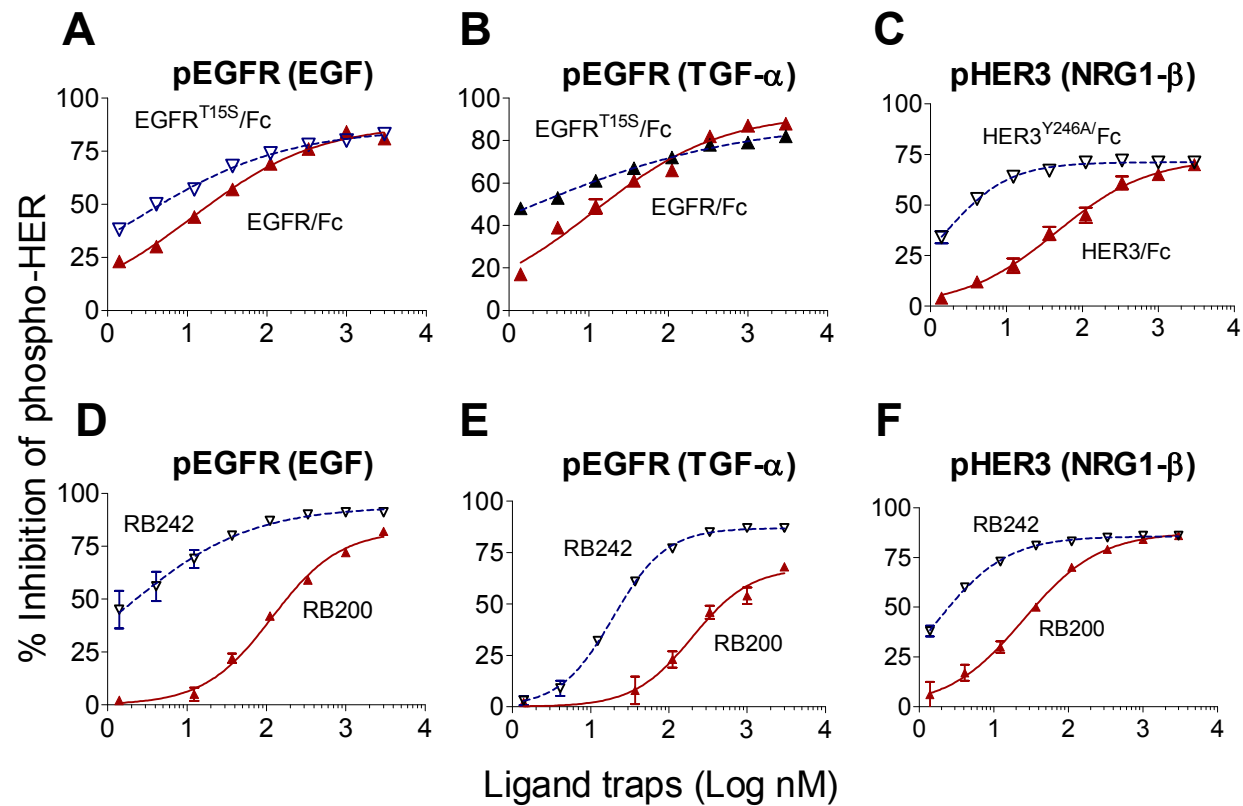
P Jin et al - Supplementary Figure 1



P Jin et al, Supplementary Figure 2



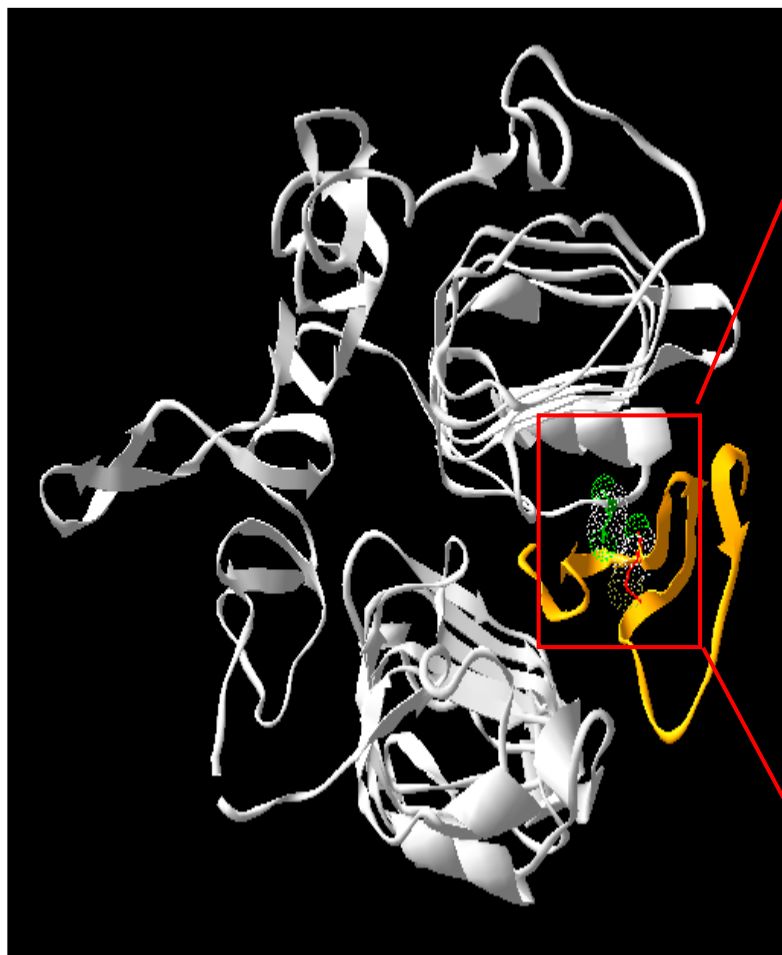
P Jin et al, Supplementary Figure 3



G

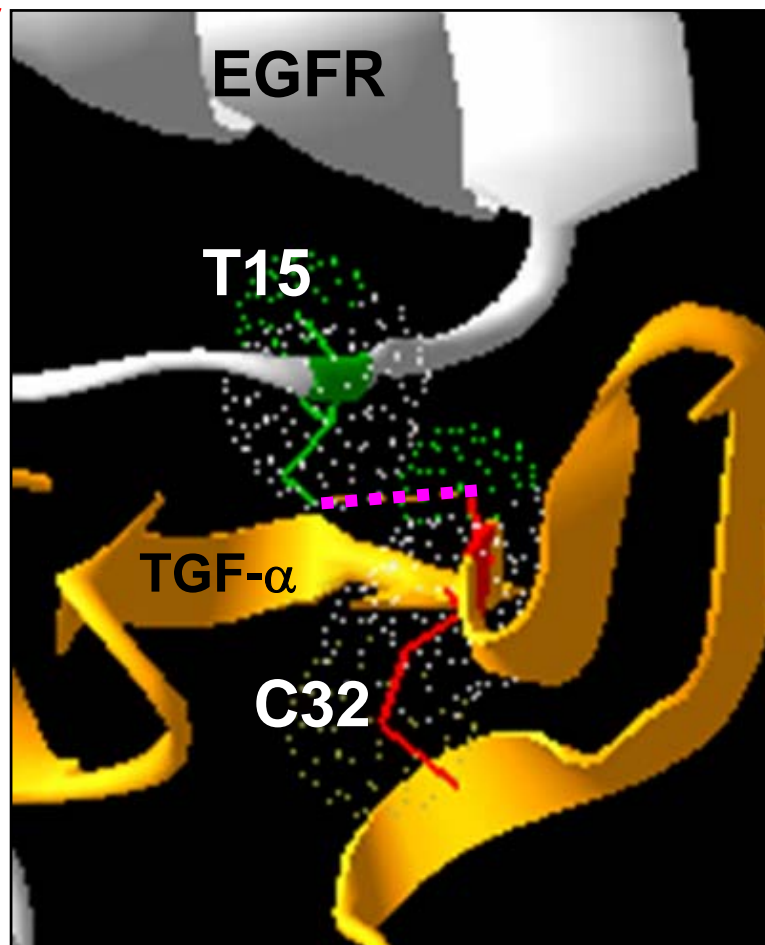
	Approximate EC ₅₀ (nM)		
	EGF	TGF- α	NRG1- β
EGFR/Fc	11.6	11.4	NT
EGFR ^{T15S} /Fc	2.2	1.0	NT
HER3/Fc	NT	NT	45.5
HER3 ^{Y246A} /Fc	NT	NT	1.5
RB200	117.3	199.0	25.1
RB242	1.8	19.4	1.7

A



EGFR in complex with TGF- α

B



Zoom in