Ligand-independent EGFR oligomers do not rely on the active state asymmetric kinase dimer

SUPPORTING INFORMATION

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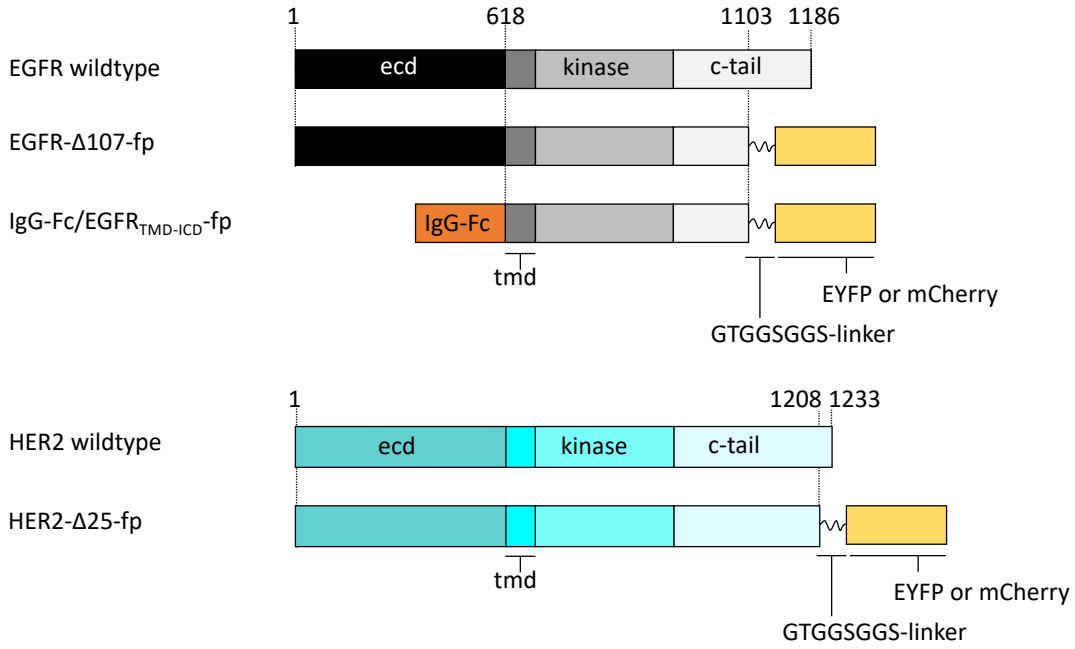
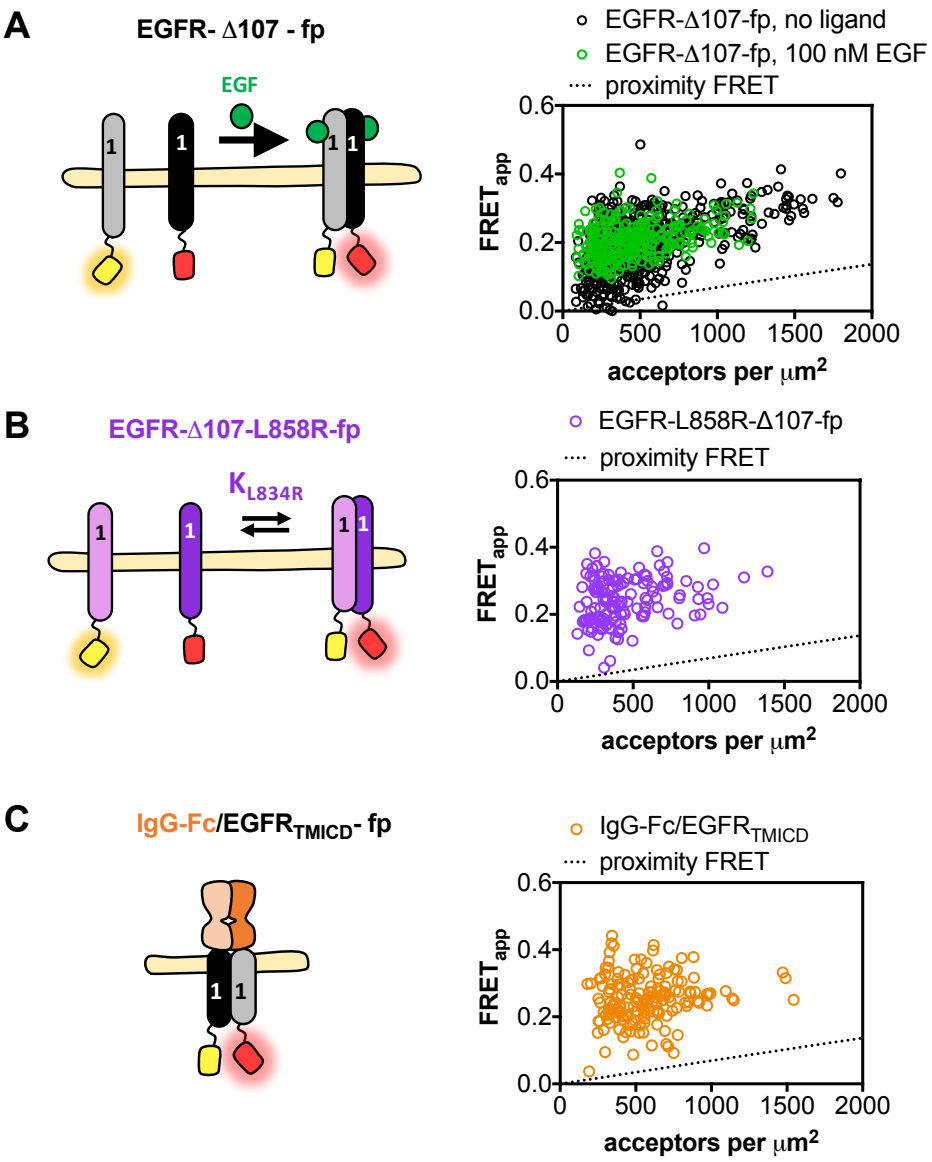
Figure S1. Schematic diagrams of EGFR/ERB1 and HER2/ERBB2 proteins used for FRET studies

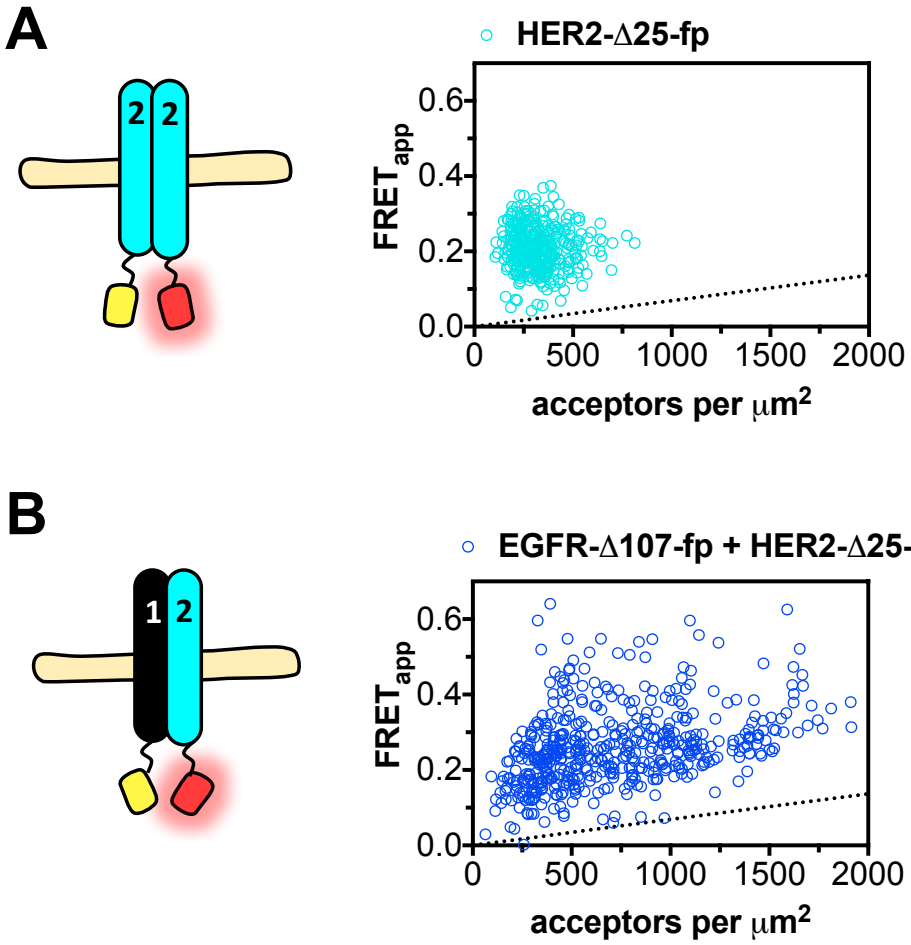


Supplementary Figure S2. Uncorrected FRET efficiency for EGFR homo-oligomerization



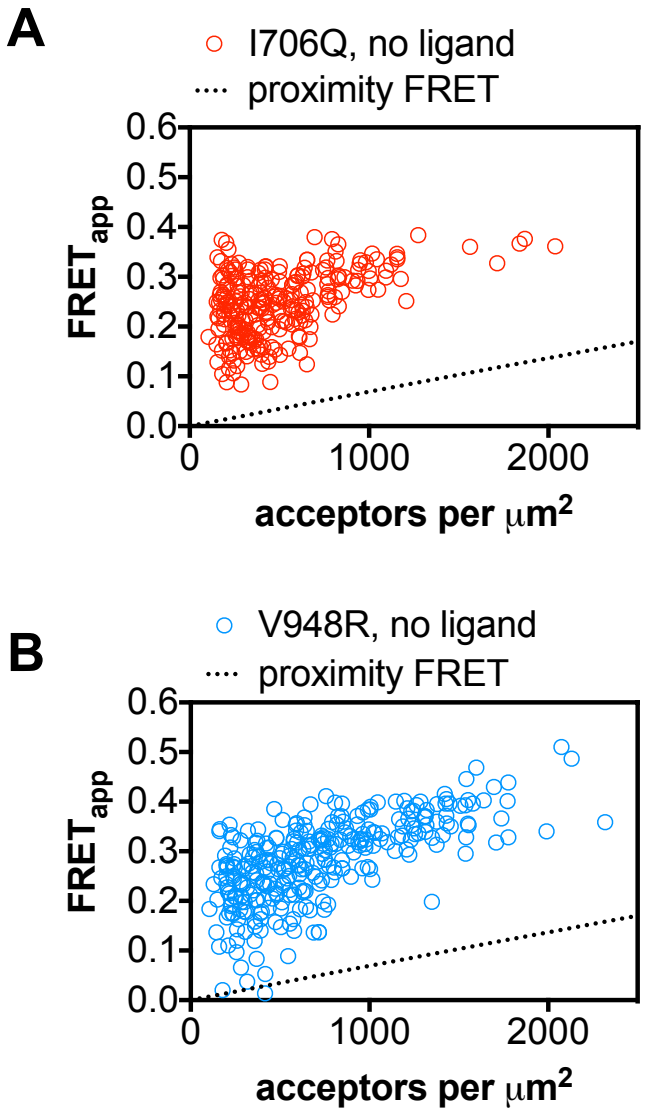
Supplementary Figure S2. Uncorrected FRET efficiency for EGFR-Δ107-fp wildtype (A), EGFR-Δ107-L858R-fp (B) and IgG-Fc/EGFR$_{TMICD}$-fp (C). The same data were used to generate these graphs and the graphs in Figure 2. Cartoons are the same as those in Figure 2. Each open circle in the graphs shows the FRET efficiency for a single vesicle. The y-axis shows the apparent FRET efficiency, the x-axis shows the number of acceptor molecules per square micron. The dotted line represents the theoretical FRET efficiency that would occur due to random proximity.

Supplementary Figure S3. Uncorrected FRET efficiency for HER2 homo- and hetero-oligomerization



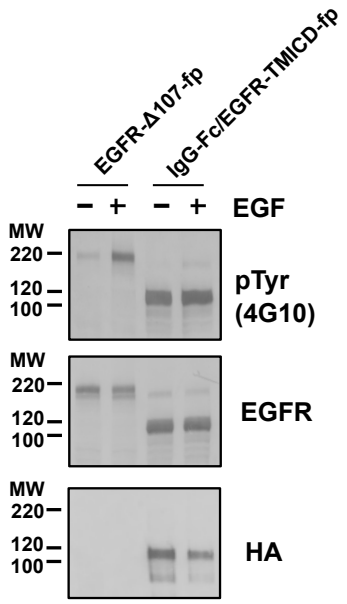
Supplementary Figure S3. Uncorrected FRET efficiency for HER2-Δ25-fp wildtype (A) and (B) HER2-Δ25-fp co-expressed with EGFR-Δ107-fp. The same data were used to generate these graphs and the graphs in Figure 3. Cartoons are the same as those in Figure 3. Each open circle in the graphs shows the FRET efficiency for a single vesicle. The y-axis shows the apparent FRET efficiency, the x-axis shows the number of acceptor molecules per square micron. The dotted line represents the theoretical FRET efficiency that would occur due to random proximity.

Supplementary Figure S4. Uncorrected FRET efficiency for the EGFR variants I706Q and V948R



Supplementary Figure S4. Uncorrected FRET efficiency for the EGFR-Δ107-fp variants I706Q (A) and V948R (B). The same data were used to generate these graphs and the graphs in Figure 5. Each open circle in the graphs shows the FRET efficiency for a single vesicle. The y-axis shows the apparent FRET efficiency, the x-axis shows the number of acceptor molecules per square micron. The dotted line represents the theoretical FRET efficiency that would occur due to random proximity.

Supplementary Figure S5. Substitution of the mouse IgG-Fc domain for the EGFR extracellular domain results in a constitutively phosphorylated chimeric receptor



Supplementary Figure S5. Western blot analysis of CHO cell lysates after transient transfection and treatment with EGF. The indicated plasmid constructs are indicated above the western blots. Antibodies are indicated to the right of each blot, molecular weight markers (kDa) inidicated to the left. The blots are representative of three independent biological experiments.

Supplementary Table S1. Previously reported fractions of preformed EGFR dimers

Cell Line	Expression Level (receptors per cell)	Oligomeric Fraction (%)	Expression method	Label	Methodology	Reference
CHO	50,000 - 200,000	0%	stable	eGFP	Number and Brightness Analysis	21
A431	did not quantify	14%	endogenous	none	FRET (lifetime decay)	19
CHO	did not quantify	20%	stable	eGFP	fluorescence intensity distribution analysis	22
CHO	600,000	30%	stable	eGFP	Number and Brightness Analysis	21
CHO	various, ranging from 41,000 to 660,000	*see below	stable, tetracycline-inducible	none	measurement of EGFR binding to I^{125}-EGF	18
BaF/3	50,000 - 100,000	55%	stable	YFP	homo-FRET	16
A431	6,000,000 - 8,000,000	68%	endogenous	none	chemical cross-linking	20
mouse fibroblast	100,000	71%	stable	none	chemical cross-linking	20
CHO	20,000 - 200,000	68 ± 8%	transient	eGFP and eRFP	fluorescence cross-correlation spectroscopy	17

*Using saturation binding data, Macdonald and Pike (Reference 18) determined the best fit value for the equilibrium dissociation constant for ligand-independent EGFR dimerization to be about 50,000 receptors per cell (i.e. ~50% dimer at 50,000 receptors per cell)