

## SUPPLEMENTAL DATA

Supplement 1:

Details about references, targeted epitopes and cross-reactivity of the therapeutic mAbs and of the anti-HER antibodies used for western blotting, FACS and TR-FRET assays.

| Target        | Antibody    | Type                 | Provided by    | Use          | Epitope                    | Reactivity           |
|---------------|-------------|----------------------|----------------|--------------|----------------------------|----------------------|
| <b>EGFR</b>   | #2232       | Rabbit polyclonal Ab | Cell Signaling | western-blot | residues surrounding Y1068 | Human, Mouse, Rabbit |
|               | m225        | Mouse IgG1           | ATCC           | FACS         | ligand binding site        | Human                |
|               | m425        | Mouse IgG1           | Merck, AG      | TR-FRET      | domain III                 | Human                |
|               | cetuximab   | Chimeric IgG1        | Merck, AG      | treatment    | ligand binding site        | Human                |
| <b>p-EGFR</b> | #2236S      | Mouse Ab             | Cell Signaling | western-blot | Y1068                      | Human                |
| <b>HER2</b>   | #2242       | Rabbit polyclonal Ab | Cell Signaling | western-blot | residues surrounding Y1222 | Human                |
|               | FSP77       | Mouse IgG1           | NE Hynes       | FACS         | extracellular domain       | Human                |
|               | FRP5        | Mouse IgG1           | NE Hynes       | TR-FRET      | extracellular domain       | Human                |
|               | trastuzumab | Humanized IgG1       | Roche          | treatment    | domain IV                  | Human                |
|               | pertuzumab  | Humanized IgG1       | Roche          | treatment    | domain II                  | Human                |
| <b>p-HER2</b> | #06-229     | Rabbit polyclonal Ab | Upstate        | western-blot | Y1248                      | Human, Rat           |

Supplemental 2:

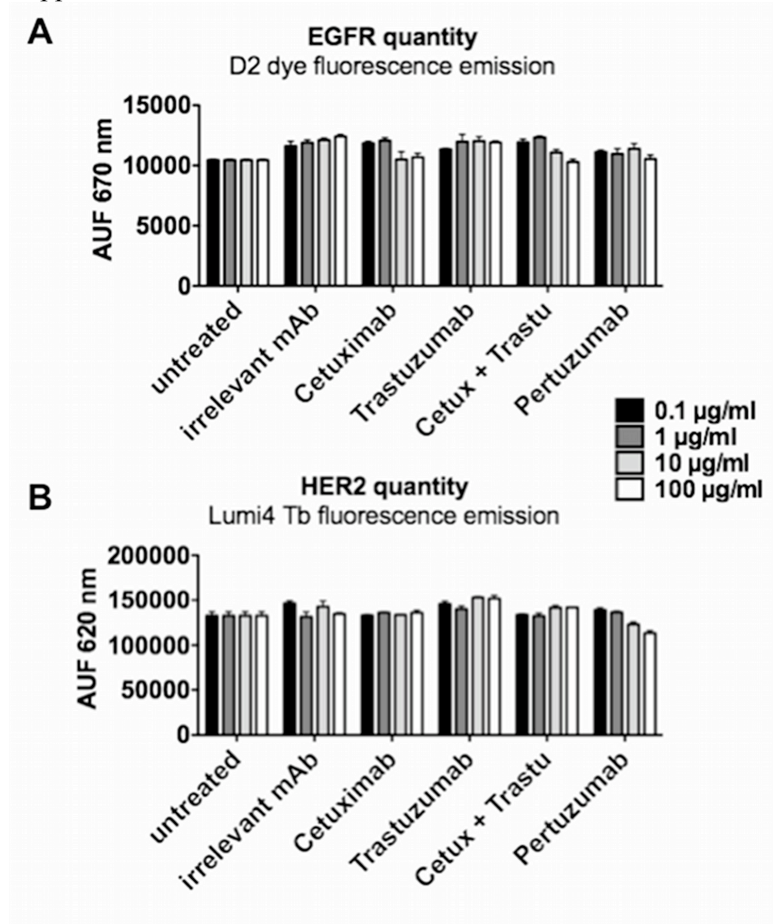


Fig. S2. Effect of therapeutic anti-EGFR or -HER2 mAbs on EGFR and HER2 expression levels in SKOV-3 cells.  $10^5$  cells/well were treated with increasing concentrations (from 0.1 to 100 µg/ml) of Px (irrelevant antibody), Cetuximab, Trastuzumab, Cetuximab + Trastuzumab (ratio 1:1) and Pertuzumab. Cells were then fixed with formalin for 2 min and EGFR/HER2 heterodimers were quantified using the antibody-based TR-FRET assay with anti-EGFR (d2-labeled m425) and anti-HER2 (Lumi4 Tb-labeled FRP5) antibodies. After extensive washes, different measurements were performed. *A*) The EGFR expression level was quantified by measurement of fluorescence emission of D2 dye coupled with an anti-EGFR (m425). *B*) The HER2 expression level was quantified by measurement of time resolved fluorescence emission of Lumi4 Tb coupled with an anti-HER2 (FRP5). Data are the mean  $\pm$  SEM of three independent experiments performed in triplicate.