**How the calculations were done:**

**pERK normalization:** Raw data for each EGF concentration and timepoint were averaged (minimum of 4 each) and scaled to the maximum value. This maximum value was converted to absolute pERK values using 150nM as the amount measured in the cells and using the value of 42.5% as the maximum amount of Erk that was observed phosphorylated in MCF10A cells using targeted phosphoproteomics.

For the EGFR, , we used Forest White’s data to normalize the EGFR dose response data

Forest reported in a technique called MARQUIS how to do absolute quantification of the phosphorylation of the EGFR (Curran, T.G., Zhang, Y., Ma, D.J., Sarkaria, J.N., and White, F.M. (2015). MARQUIS: a multiplex method for absolute quantification of peptides and posttranslational modifications. Nat Comms 6, 5924–5924.).

For the different sites, the site nomenclature he uses is different than what we use. As a cheat sheet:

Y1068 = Y1092

Y1148 = Y1172

Y1173 = Y1197

For maximum phosphorylation for the different sites at ~5 minutes and 20nM EGF (120ng/ml) assuming ~250K receptors per cell:

Y1092 = 18K – 7.2% - 15nM

Y1172 = 36K – 14.4% - 29nM

Y1197 = 13K – 5.2% - 10nM

The maximum is the sum of all sites, or 54nM, which is ~27% maximum. This is consistent with the orthovanadate inhibitor studies which are capable of boosting the PY signals by about 4X. So, to calibrate our studies, we take the +mAb225 to be zero, since it blocks EGFR signaling. The remaining signal is assumed to be interfering ion current from neighboring peptides. This is then subtracted from all of the +EGF values to yield raw results. The maximal for each PY site is then set to Forest White’s maximal value at 5 min with everything else being scaled between 0->max. Total EGFR in MCF10A cells was calculated to be 201 nM based on targeted proteomics.