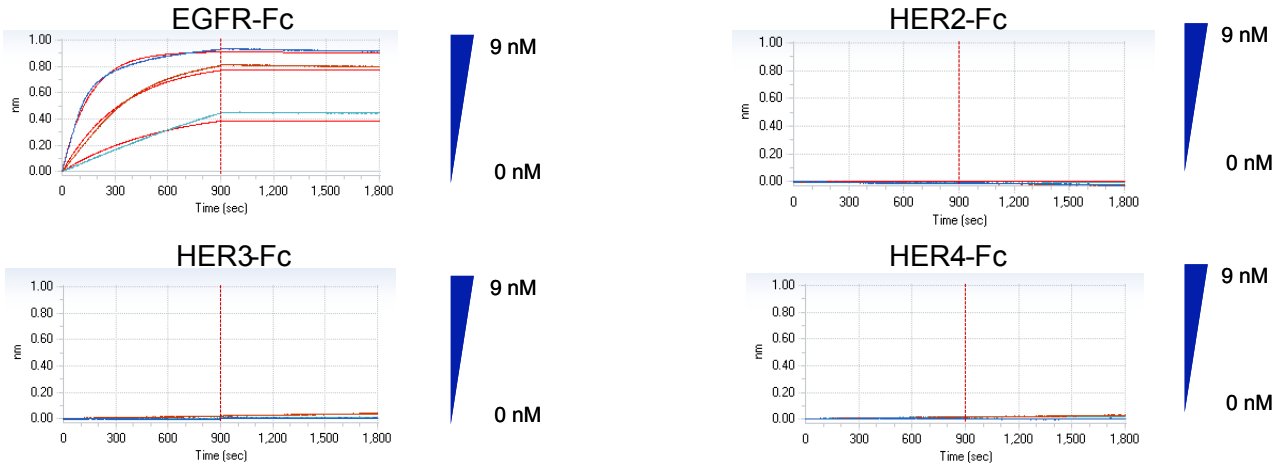
Dong J et al

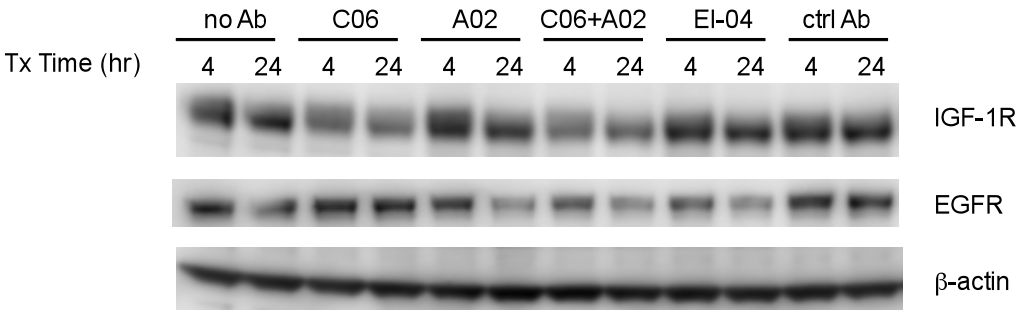
Supplementary Figure S1



EI-04 binds specifically to human EGFR (HER1) but not the other HER family members. EGFR/ErbB1/HER1-Fc, ErbB2/HER2-Fc), ErbB3/HER3-Fc and ErbB4/HER4-Fc (R&D Systems), were all diluted to concentrations of 1, 3 and 9 nM in OB buffer (PBS, pH 7.4, 0.01% (w/v) NaN3, 1 mg/ml BSA, 0.02 % (v/v) Tween 20). Biotinylated EI-04 diluted in OB buffer to 0.2 μg/ml, was captured on Streptavidin-coated SA tips. EI-04-coated tips were washed and then transferred to wells containing the aforementioned HER-Fc solutions, where association with EI-04 was measured for 15 minutes. Following this step, tips were transferred to OB-containing wells, where HER-Fc dissociation from EI-04 was measured, again for 15 minutes. Sensorgrams were processed and analyzed using Octet Red Analysis software (ForteBio). Raw data are shown in shades of blue, while the fit to the data is shown in red.

Dong J et al

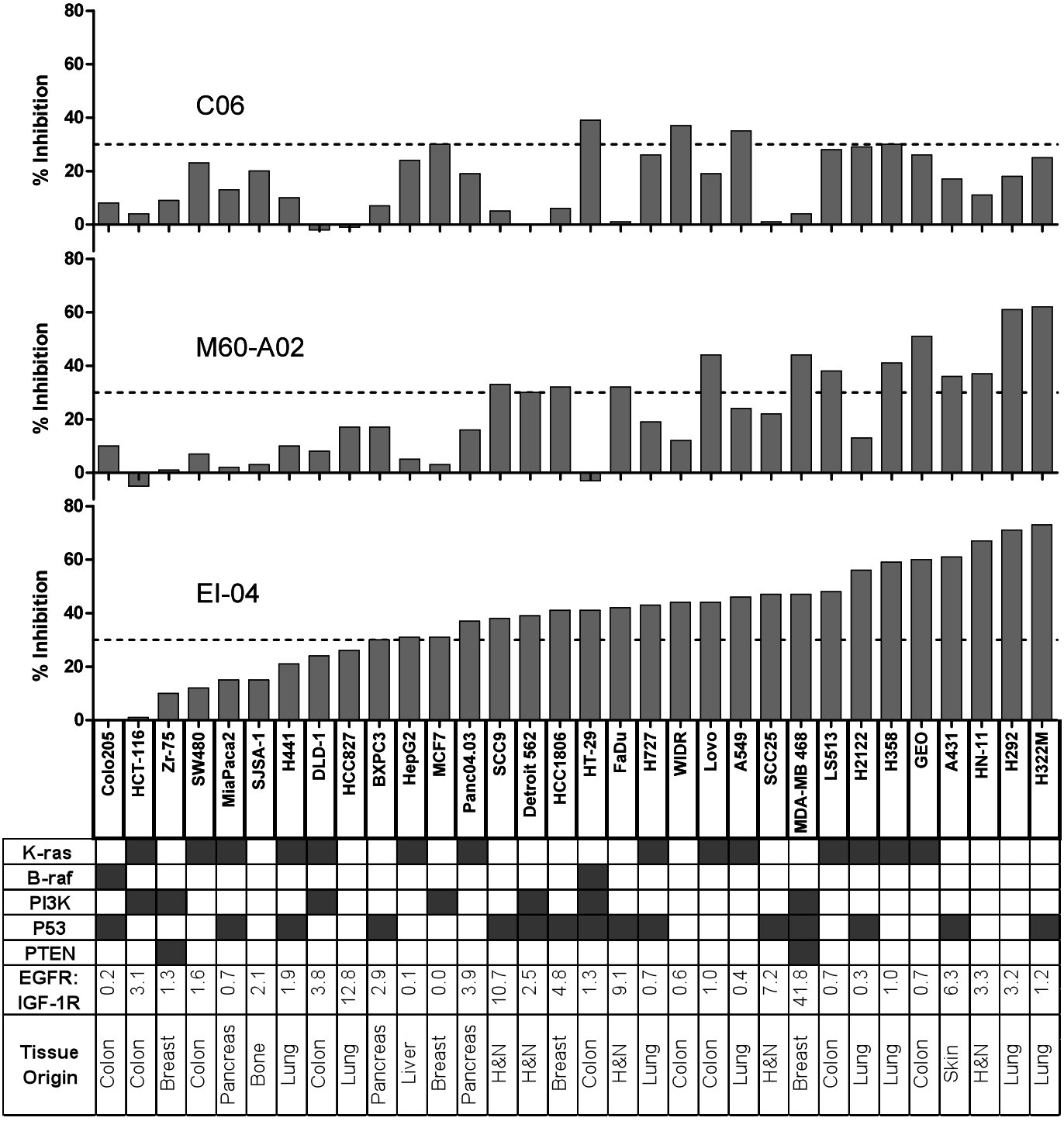
Supplementary Figure S2



EGFR and IGF-1R downregulation by EI-04 and mAb controls in GEO cells. Cells grown in culture medium supplemented with 10% FBS were treated with the indicated antibodies against EGFR and IGF-1R or a control IgG (ctrl Ab) for 4 or 24 hrs. Total EGFR, total IGF-1R and b-actin in cell lysates were analyzed by western blot analysis.

Dong J et al

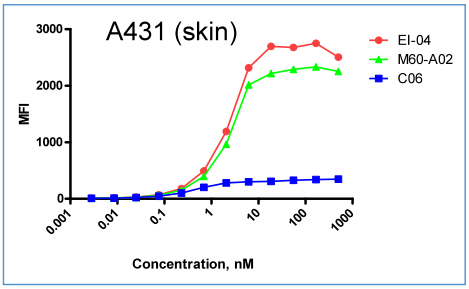
Supplementary Figure S3

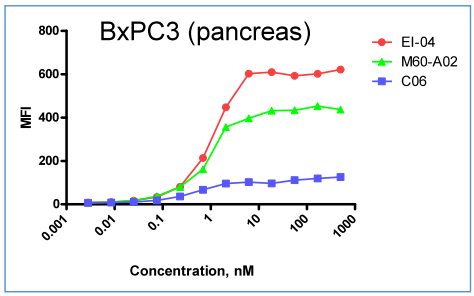


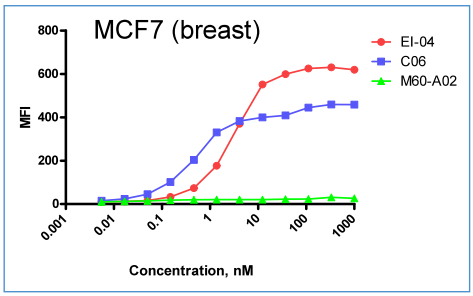
Superior inhibitory activity of EI-04 observed across a panel of tumor cell lines. Tumor cells grown in culture medium supplemented with 10% FBS were treated with 300 nM of C06, M60-A02 or EI-04 for three days prior to cell viability determination. Percent growth inhibition was calculated relative to no antibody treatment control. Mutation status information was obtained from the Sanger database or in-house sequence analysis. The relative expression levels of EGFR and IGF-1R were determined by FACS analysis.

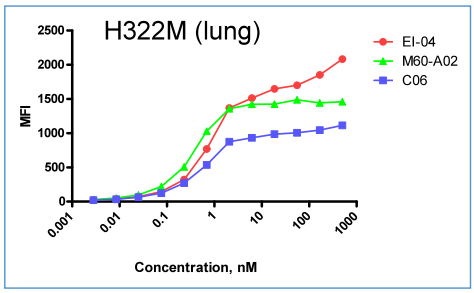
Dong J et al

Supplementary Figure S4









Flow cytometric measurement of the direct binding activities of anti-EGFR mAb M60-A02, anti-IGF-1R mAb C06 and BsAb EI-04 to 4 tumor cell lines expressing varying levels of EGFR and IGF-1R. Cells were stained with serially diluted antibodies followed by a PE-labelled secondary antibody. Mean fluorescence intensity was plotted as a function of antibody concentrations.