Fig. S3.

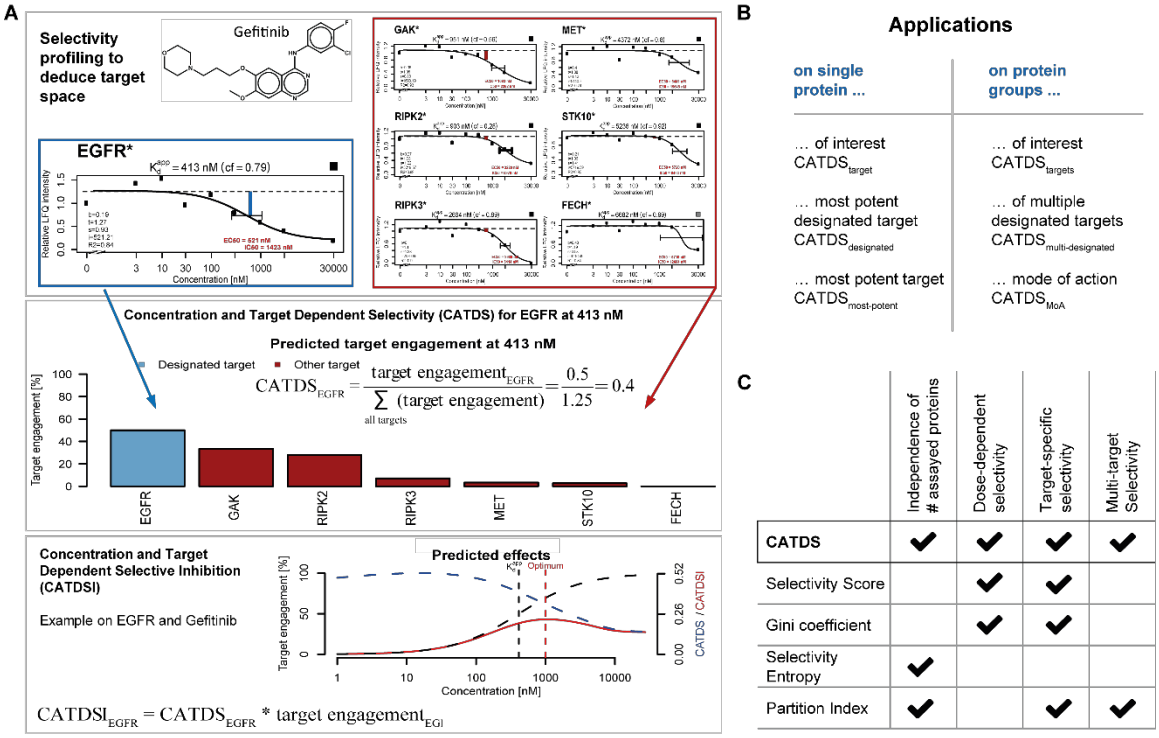

Fig. S3 | Determination of compound selectivity by the Concentration and Target Dependent Selectivity (CATDS) score. (A) An example of a CATDS calculation is provided for Gefitinib (upper panel). Kinobeads profiling determined seven targets for Gefitinib (blue box for EGFR and red box for the six other targets). The target engagement (blue vertical bar in the dose-response plot for EGFR; red vertical bar in dose response plot for other targets) is shown for all targets. The histogram in the middle panel uses the same color code. The selectivity of Gefitinib for EGFR ($CATDS_{EGFR}$) can be calculated by dividing the target engagement of EGFR (here at its K_d^{app} of 413 nM, *i.e.* 0.5, blue bar) by the sum of all target engagements (blue and red bars) at the same concentration. More generally, using the fitted curves from the Kinobeads assay, $CATDS_{EGFR}$ can be calculated at any concentration (lower panel, blue dashed line) to monitor the selectivity across the entire concentration range. A further useful metric is the Concentration and Target Dependent Selective Inhibition (CATDSI) which provides an estimate of the optimal concentration at which the highest selectivity and highest target engagement can be obtained. Thus, $CATDSI_{EGFR}$ is the $CATDS_{EGFR}$ multiplied by the target engagement of EGFR (lower panel, dashed black line). **(B)** CATDS can be flexibly used to express the selectivity of a compound for any one protein of interest ($CATDS_{target}$), one of the designated targets of a compound ($CATDS_{designated}$) or the most potent designated target of a compound ($CATDS_{most\text{-}potent}$). The score can also be used for protein groups such as multiple target proteins ($CATDS_{targets}$), multiple designated targets ($CATDS_{multi\text{-}designated}$) or multiple targets involved in a drug's mechanism of action ($CATDS_{MoA}$). **(C)** Comparison of CATDS features to four other selectivity measures. CATDS encompasses features of all the other metrics but only CATDS provides an assay-independent selectivity measure for single and multiple proteins of interest at any concentration.

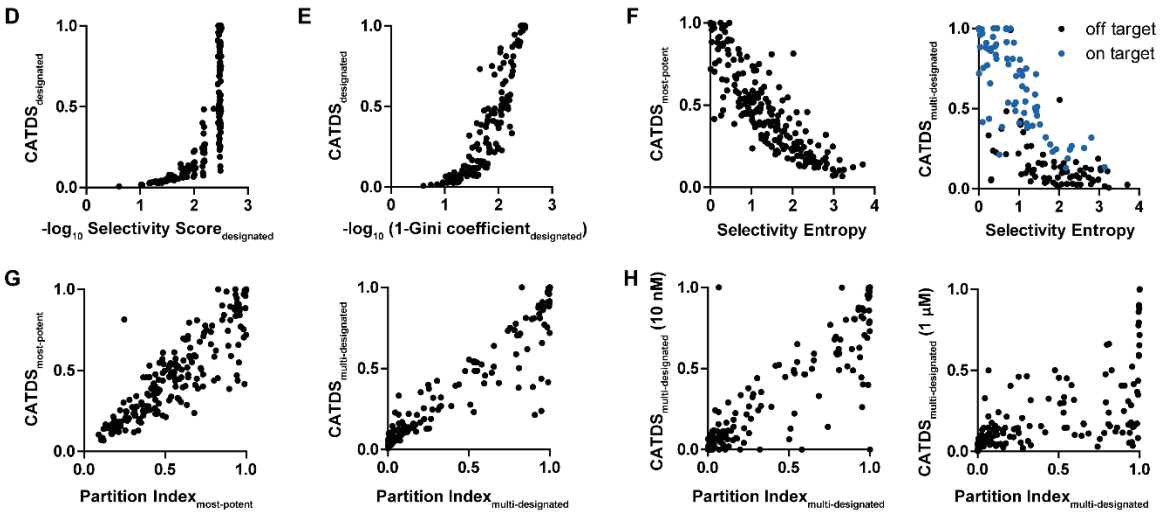

Fig. S3 continued | Determination of compound selectivity by the Concentration and Target Dependent Selectivity (CATDS) score. (D-H) Generally, there is reasonable agreement of CATDS with four other measures of selectivity. $CATDS_{designated}$ is highly comparable to **(D)** the selectivity score and **(E)** the Gini coefficient. **(F)** Selectivity entropy is comparable to $CATDS_{most\text{-}potent}$ (left panel); however, $CATDS_{multi\text{-}designated}$ indicates that the selectivity entropy is unable to differentiate compounds that are on-target (blue circles) or off-target (black circles) proteins (right panel). **(G)** There is also a high correlation between $CATDS_{most\text{-}potent}$ ($CATDS_{multi\text{-}designated}$) and the partition $index_{most\text{-}potent}$ (partition $index_{multi\text{-}designated}$), respectively. **(H)** The comparison of $CATDS_{multi\text{-}designated}$ calculated at 10 nM (left) and 1,000 nM (right) with the partition $index_{multi\text{-}designated}$ demonstrates that the partition index cannot account for the fact that selectivity is a function of drug dose.