Fig. S1.

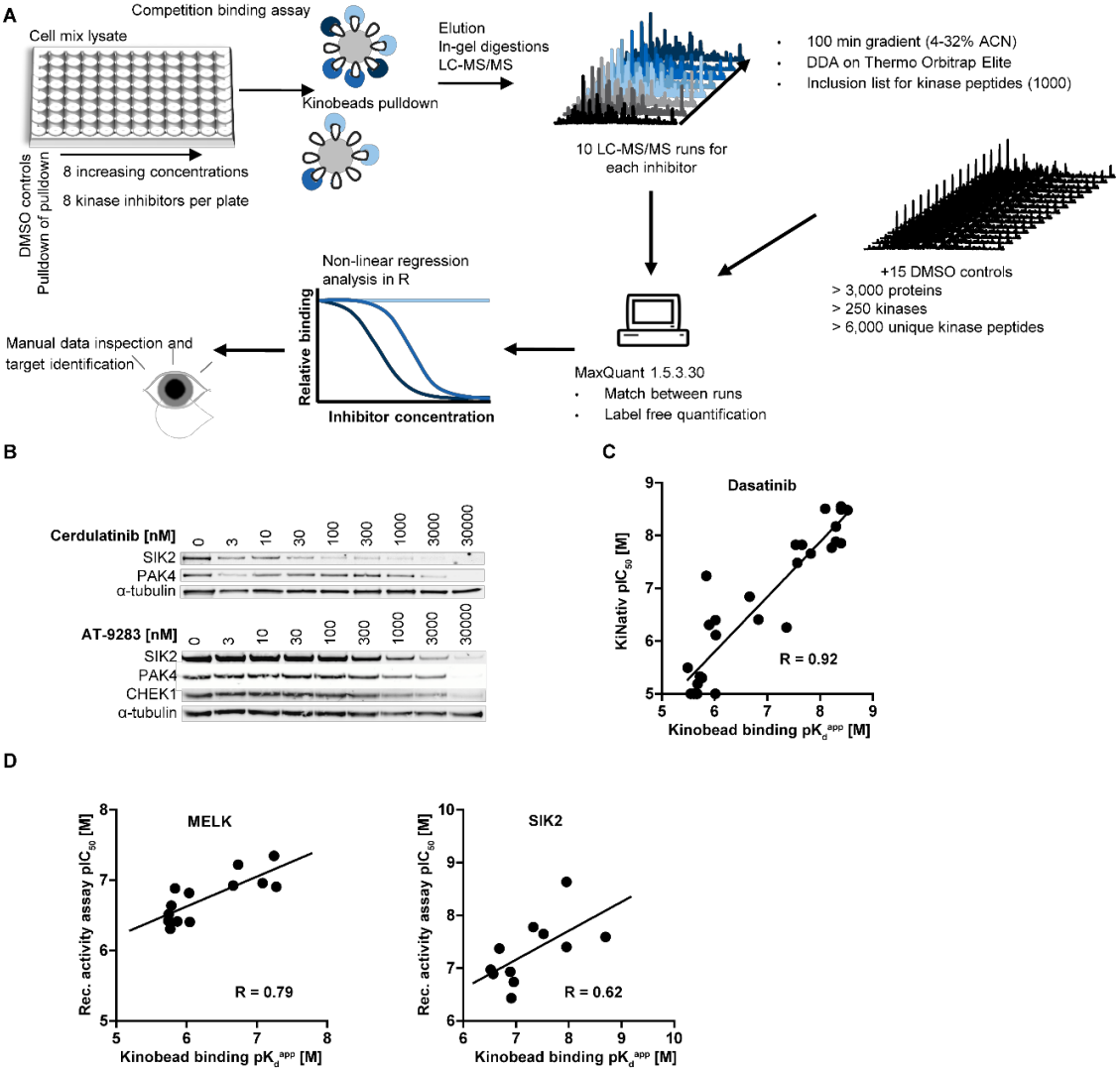

Fig. S1 | Kinobeads Drug Screen workflow and evaluation. (A) Kinobead pulldowns were performed in 96-well format. Eight doses were used for each drug plus vehicle control and a second pulldown of the vehicle control (pulldown of pulldown). This control was used to correct for protein depletion from the lysate caused by the affinity enrichment (see methods). Proteins were eluted from beads, run ~1 cm into a LDS gel and in-gel digested with trypsin. Each pulldown was analysed by liquid chromatography tandem mass spectrometry and using an inclusion list of kinase peptide m/z and retention times collected in prior experiments. MS data from the same 15 vehicle control Kinobeads pulldowns were added to the MaxQuant/Andromeda software for consistent protein identification and quantification. Dose response plots were generated and target proteins manually annotated. **(B)** Kinobead western Blot readout for selected inhibitor:protein combinations. **(C)** Dasatinib target pK_d^{app} correlate well with KiNativ binding data for the same inhibitor. **(D)** Correlation of Kinobeads binding data for clinical MELK and SIK2 inhibitors to IC_{50}-values obtained in recombinant activity assays shows reasonable agreement between the two assay formats.

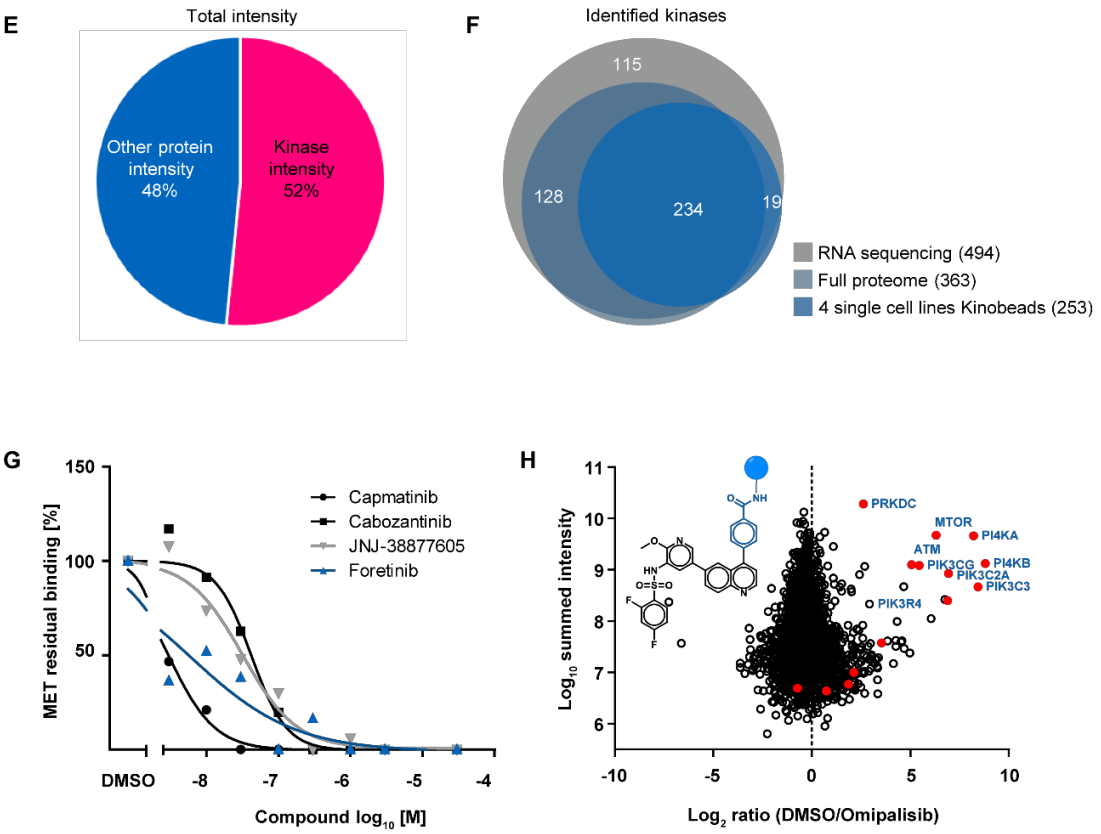

Fig. S1 continued | Kinobeads Drug Screen workflow and evaluation. (E) Intensity distribution of proteins captured on Kinobeads. From the total MS peptide intensities, 52% originated from kinase peptides illustrating good enrichment of kinases on Kinobeads. **(F)** Venn diagram of the overlap of protein kinases identified in the RNA-seq data, full proteome analysis and Kinobeads pulldowns in K-562, M-4-11, COLO 205 and SK-N-BE(2) cells. **(G)** Examples for Kinobeads experiments performed to profile MET inhibitors using a lysate mixture of the four standard cell lines supplemented with Caki-1 cell lysate (high MET expression). **(H)** Kinobeads competition data for MTOR/PI3K-family members (red) using immobilized Omipalisib (inset) and unmodified Omipalisib as the competitor.