Fig. S6.

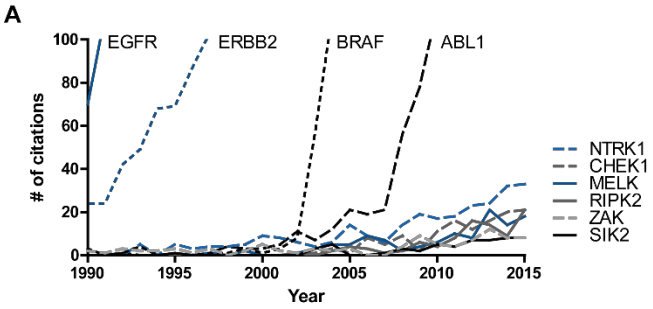

Fig. S6 | Characterization of novel off-targets. (A) Citations from PubMed (pubmed.gov, 10/2016) indicating a recent and increasing interest in particular kinases.

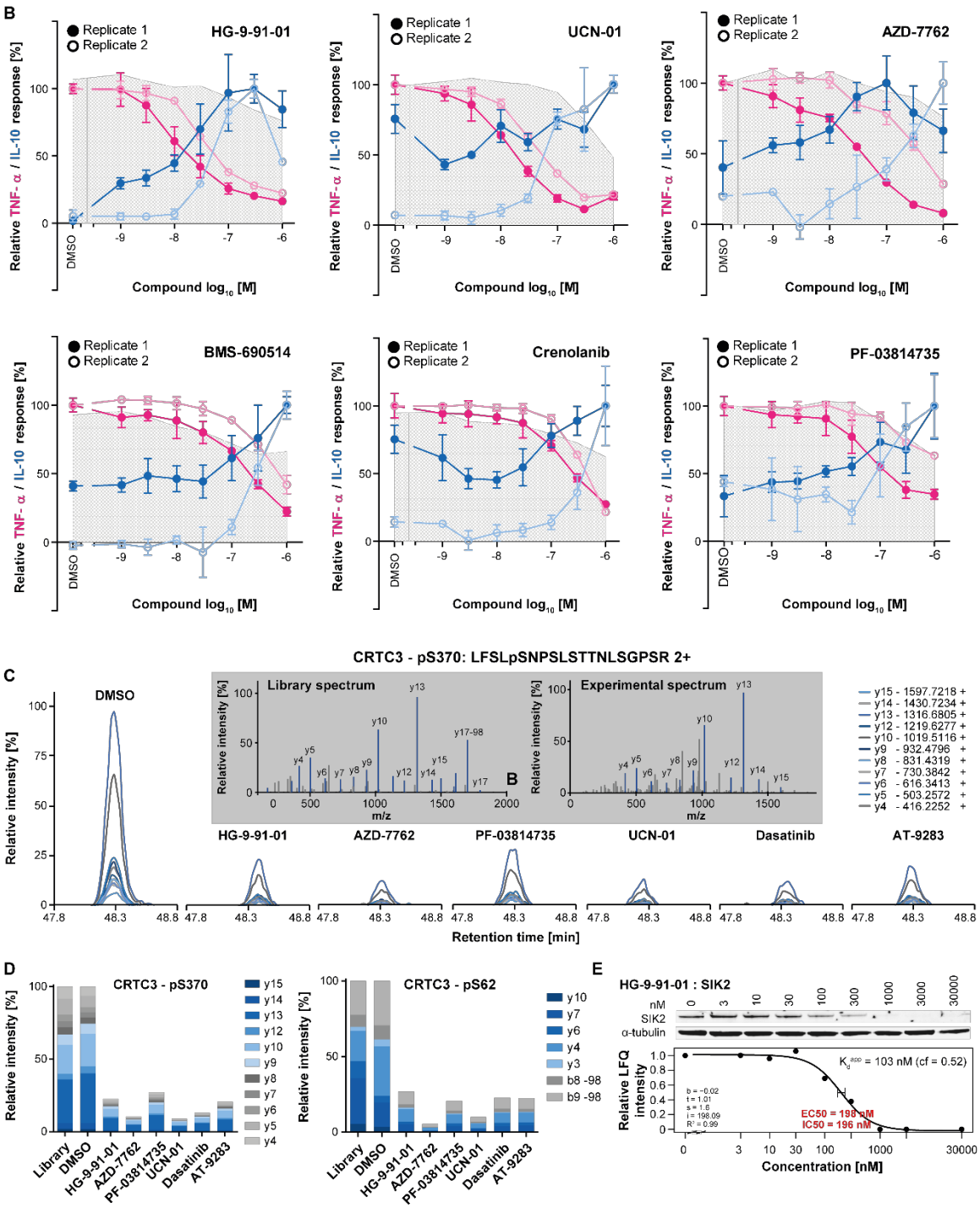

Fig. S6 continued | Characterization of novel off-targets – SIK2. (B) Mouse BMDM cells were treated with increasing concentrations of potential SIK2 inhibitors followed by cell viability (grey area), TNFα (pink) and IL-10 (blue) measurement. All shown inhibitors resulted in anti-inflammatory effects (IL-10 increase, TNFα decrease), suggesting SIK2 inhibition (error bars show standard deviation of technical triplicates). The known SIK2 inhibitor HG-9-91-01 was used as positive control. **(C)** PRM assay for phosphorylation of CRTC3 S370 – a substrate site of SIK2 – showing reduced phosphorylation for all SIK2 inhibitors compared to DMSO. **(D)** Quantitative readout of PRM transitions for either pS370 or pS62 after inhibitor treatment. **(E)** Western blot and mass spectrometry readout of Kinobeads binding of SIK2 by HG-9-91-01.

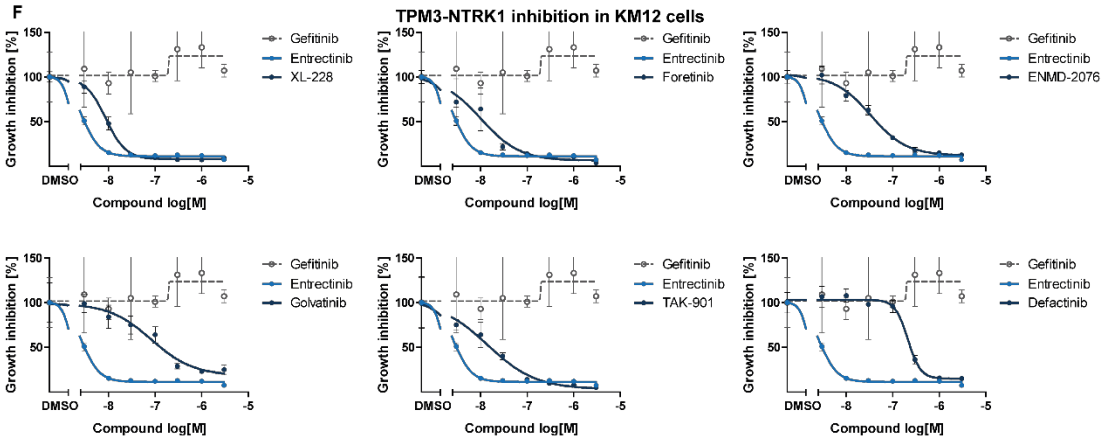

Fig. S6 continued | Characterization of novel off-targets – NTRK1. (F) Potential NTRK1 inhibitors but not Gefitinib reduced the viability of TPM3-NTRK1 dependent KM12 cells in a dose-dependent fashion (error bars show standard deviation of technical triplicates). The designated NTRK1 inhibitor Entrectinib was used as positive control.

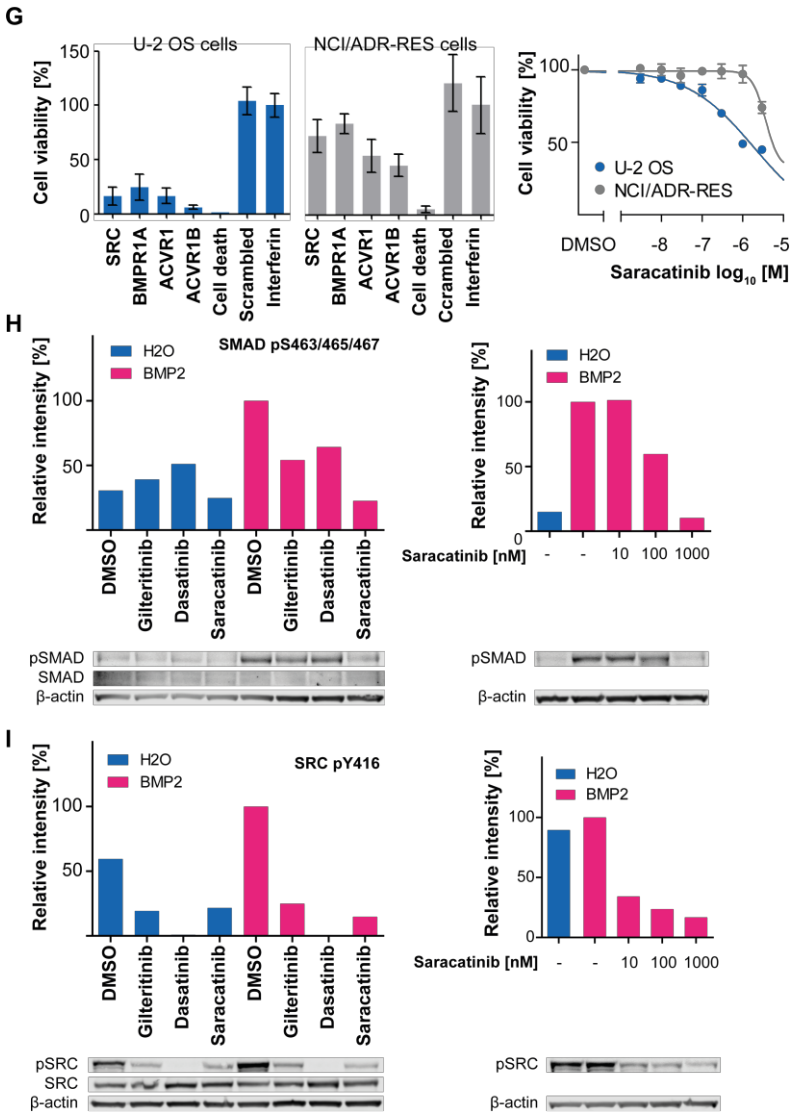

Fig. S6 continued | Characterization of novel off-targets - Saracatinib. (G) siRNA knock-down experiments in U-2 OS cells (osteosarcoma) and NCI/ADR-RES cells (ovarian cancer; left panel) suggested that Saracatinib efficacy in U-2 OS cells (right panel) is owing to a concerted inhibition of SRC and BMP receptor signaling in osteosarcoma. Transfection reagent and scrambled siRNAs were used as negative control and cell death inducing siRNA as positive control (error bars depict standard deviation of technical triplicates). **(H)** Western blot readout for phospho-SMAD in U-2 OS cells treated with inhibitor in the presence or absence of BMP2. Saracatinib treatment results in decreased phosphorylation of SMAD downstream of BMP receptors and **(I)** decreased autophosphorylation of SRC Y419 showing target/pathway engagement of the drug.

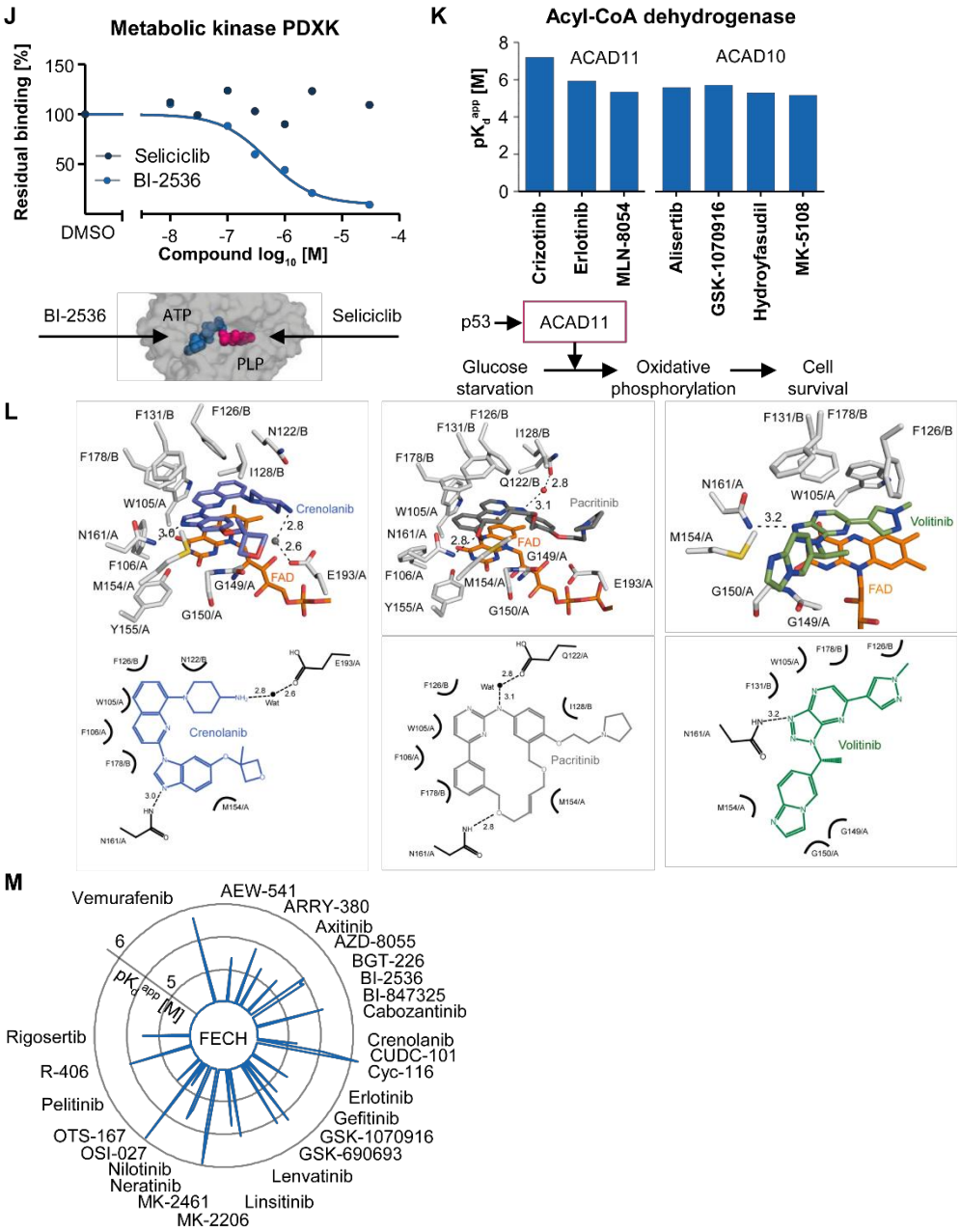

Fig. S6 continued | Characterization of novel off-targets – beyond protein kinases. (J) Metabolic kinase PDXK as off-target of kinase inhibitors. Seliciclib is known to bind to the pyridoxal binding site (PLP) but does not score in the Kinobeads assay. Conversely, BI-2536 showed binding inhibition in Kinobeads and therefore likely interacts with the ATP-binding pocket of PDXK. **(K)** Acyl-CoA dehydrogenases ACAD10 and ACAD11 as off-targets of kinase inhibitors. Inhibition of ACAD11 may block the metabolic switch of cancer cells from glucose to fatty acid metabolism under conditions of glucose starvation. **(L)** Co-crystal structures of NQO2 and Crenolanib (left two panels), Pacritinib (middle two panels) and Volitinib (right two panels). All molecules interact with FAD by π-stacking and residues in the active site. **(M)** Radar plot of pK_d^{app} values for FECH revealing interaction with a total of 26 compounds.