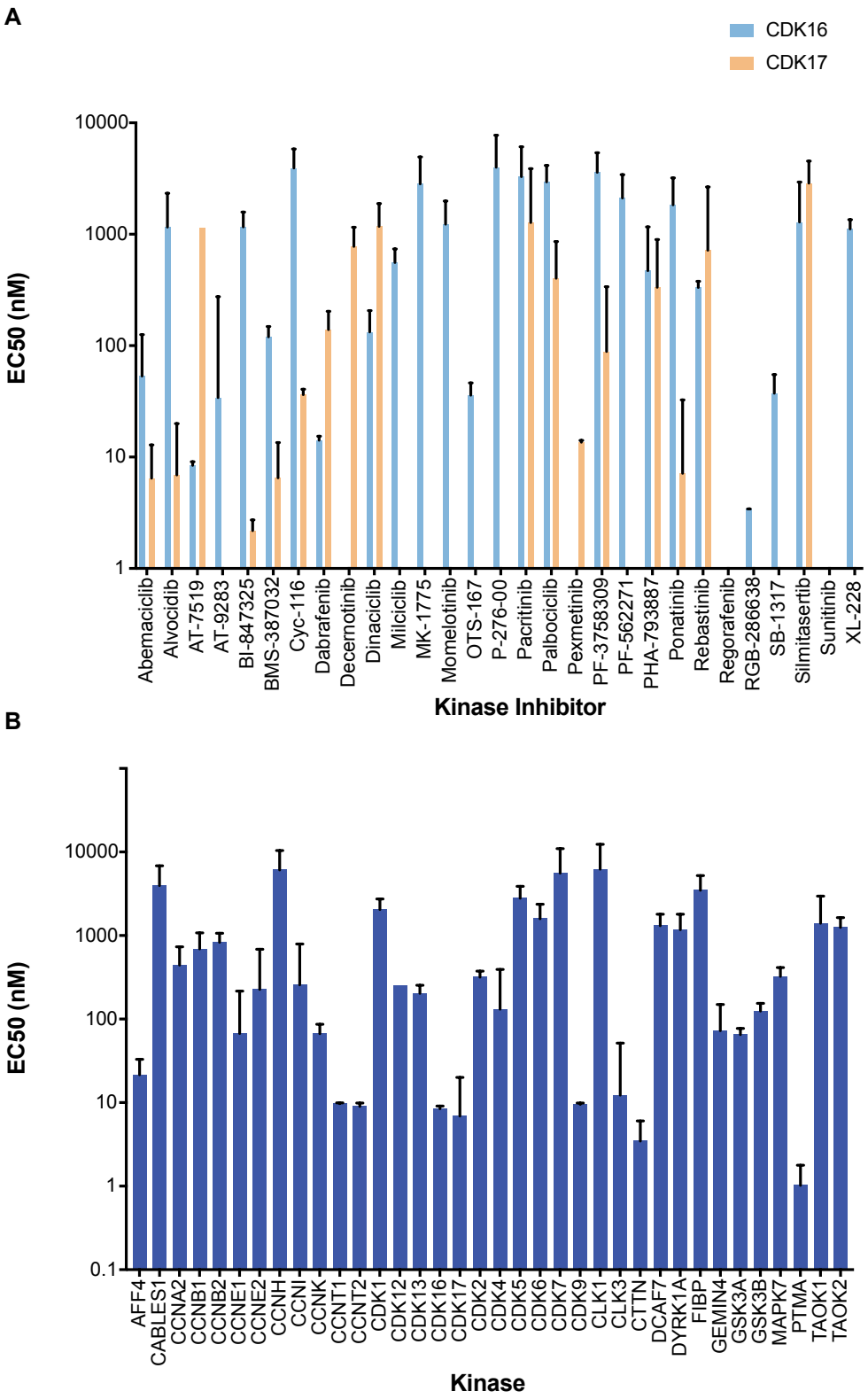

Figure S1. Target characterization of clinical kinase inhibitors. *Related to Figure 2.* (A) Clinical kinase drugs that inhibit CDK16 and/or CDK17 in cells, analyzed from published data.(Klaeger et al., 2017) (B) Kinase targets of AT7519, assessed by kinobeads, analyzed from published data.(Klaeger et al., 2017)

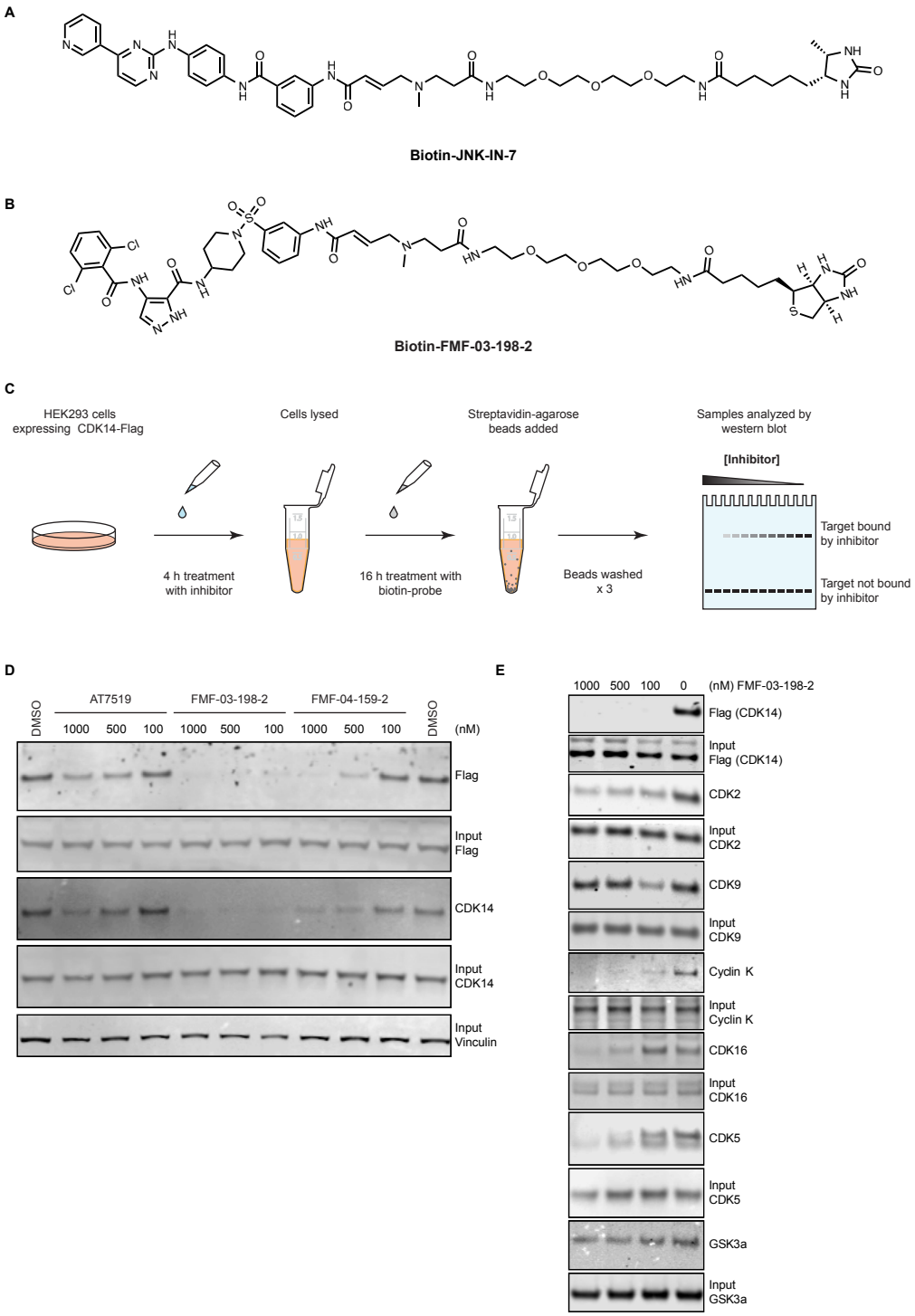

Figure S2. Pull down reagents, schematic, and experimental validation using biotin compounds, performed in the course of FMF compound characterization. *Related to Figure 1, Table 1, Figure 2, Figure 3 and STAR Methods* (A) Chemical structure of desthiobiotin-PEG3-3-amidopropanamide-JNK-IN-7, dubbed Biotin-JNK-IN-7, used as a pull-down reagent. (B) Chemical structure of Biotin-FMF-03-198-2, used as a pull-down reagent. (C) Schematic depicting pull-down assay protocol. (D) Representative western blot data reported in Table 1 for cellular pulldown using Biotin-JNK-IN-7. (E) Pre-treatment of cells with FMF-03-198-2 results in dose-dependent competition of pull down of several CDKs and CMGC kinases using Biotin-FMF-03-198-2.

Table S2. Dependence of cell proliferation on TAIRE kinases in HCT116 cells using RNAi. *Related to Figure 3.* Data from Novartis Project DRIVE.(McDonald et al., 2017) CDK1 data shown for reference. A significant dependency is defined by an ATARIS score of -3 or less.

Kinase	ATARIS score
CDK14	-0.36
CDK15	-0.625
CDK16	-0.605
CDK17	-1.871
CDK18	-1.129
CDK1	-3.143

Table S3. IC_{50} results for NanoBRET (Promega) CDK14 assay. *Related to Figure 3.* IC_{50} comparing direct compound treatment for 6 h to compound treatment for 4 h following by washout for 2 h.

Compound	IC50 (nM)
FMF-04-159-2	39.6 ± 2.8
FMF-04-159-2 washout	56.3 ± 6.0
FMF-04-159-R	563 ± 145
FMF-04-159-R washout	3417 ± 1154
AT7519	77.7 ± 5.9
AT7519 washout	532 ± 160

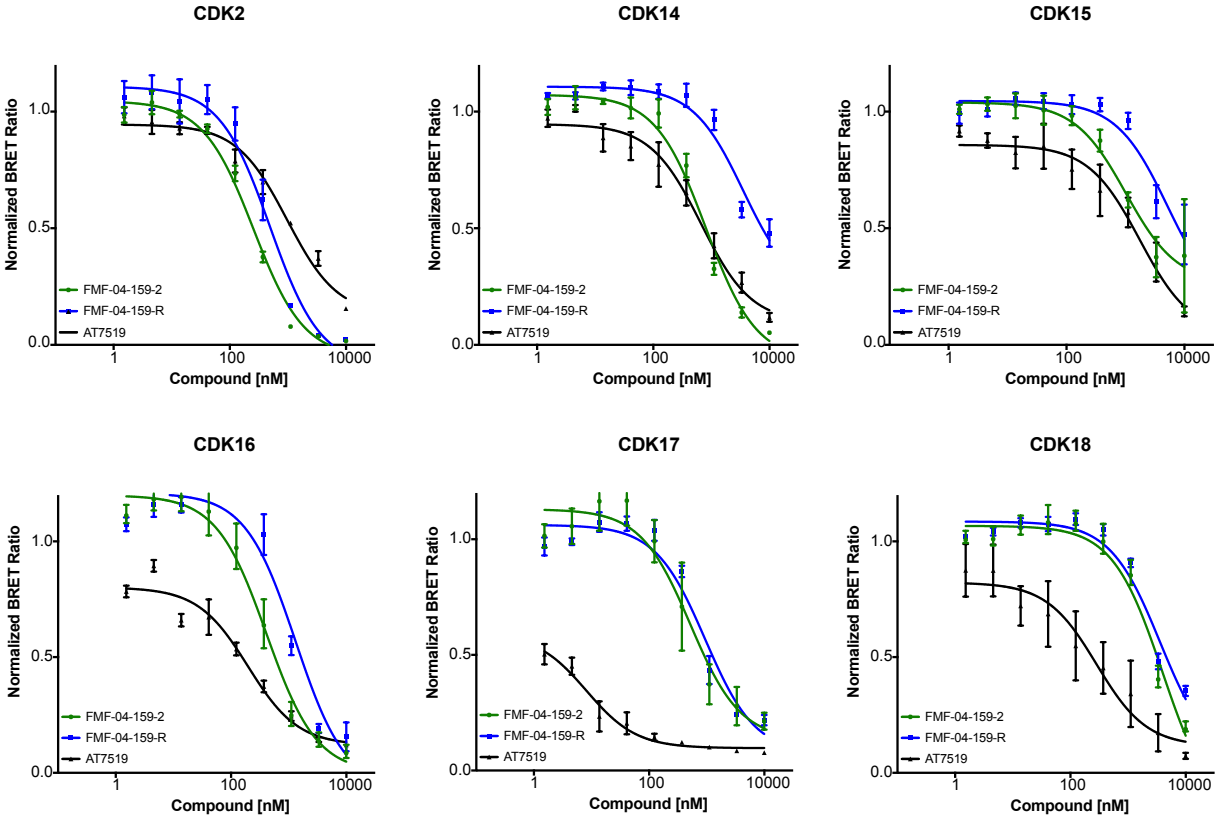

Figure S3: NanoBRET (Promega) live-cell target engagement. *Related to Figure 3 and Table S3.* NanoBRET results in HCT116 cells of the indicated CDK targets for FMF-04-159-2, FMF-04-159-R and AT7519 2 h treatment.

Table S4: NanoBRET (Promega) live-cell target engagement IC50 values. *Related to Figure 3 and Figure S3.*

Compound	CDK2 IC50 (nM)	CDK14 IC50 (nM)	CDK15 IC50 (nM)	CDK16 IC50 (nM)	CDK17 IC50 (nM)	CDK18 IC50 (nM)
FMF-04-159-2	256± 26	803 ± 111	1014 ± 320	413 ± 67	521 ± 151	3977 ± 951
FMF-04-159-R	493 ± 81	3540 ± 988	5165 ± 2097	1315 ± 326	942 ± 210	3607 ± 918
AT7519	969 ± 160	661 ± 124	1748 ± 500	197 ± 41	8.2 ± 2.1	285 ± 110

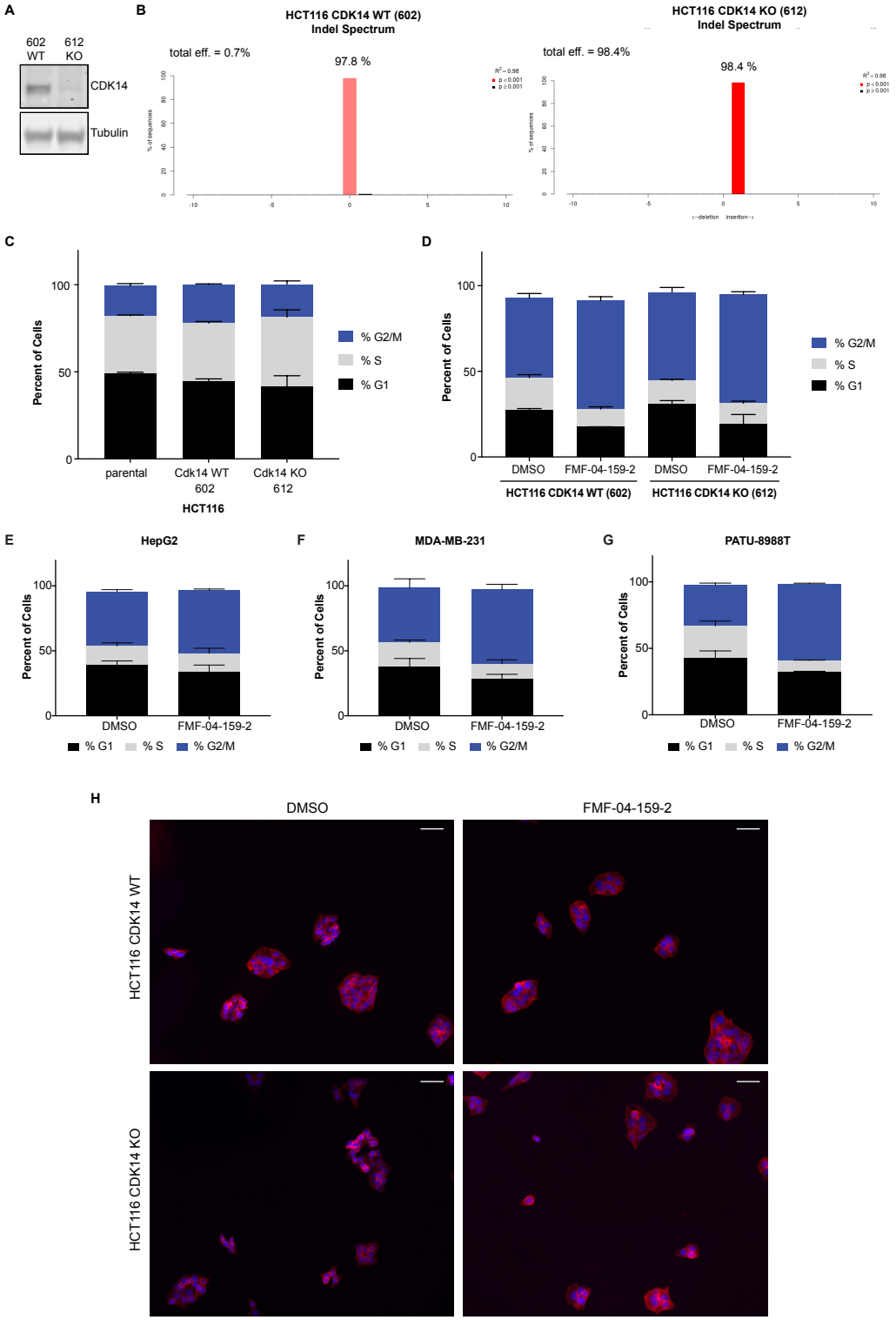

Figure S4. Cell cycle and phenotypic characterization of FMF-04-159-2. *Related to Figure 4.* (A) Western blot analysis of HCT116 CDK14 knockout (KO) cell line (612) and CDK14 wild-type (WT) control. (B) Indel spectrum for both cell lines based on genomic DNA PCR, generated using the TIDE online software.(Brinkman et al., 2014) (C) FACS PI analysis of parental HCT116 cells compared to HCT116

CDK14 KO and WT clonal cell lines. (D) FACS PI analysis of HCT116 CDK14 KO and WT cells, treated with DMSO or FMF-04-159-2 for 24 h. (E) FACS PI cell cycle analysis following 24 h treatment with DMSO or FMF-04-159-2 of HepG2 (F) of MDA-MD-231 and (G) of PATU-8988T cells. (H) HCT116 cells with CRISPR-mediated CDK14 knockout (KO) or wild-type (WT), treated with DMSO or FMF-04-159-2 for 24 h then stained with Phalloidin-488 and DAPI. Scale bar represents 50 microns. Images are representative of n=3 biological replicates.

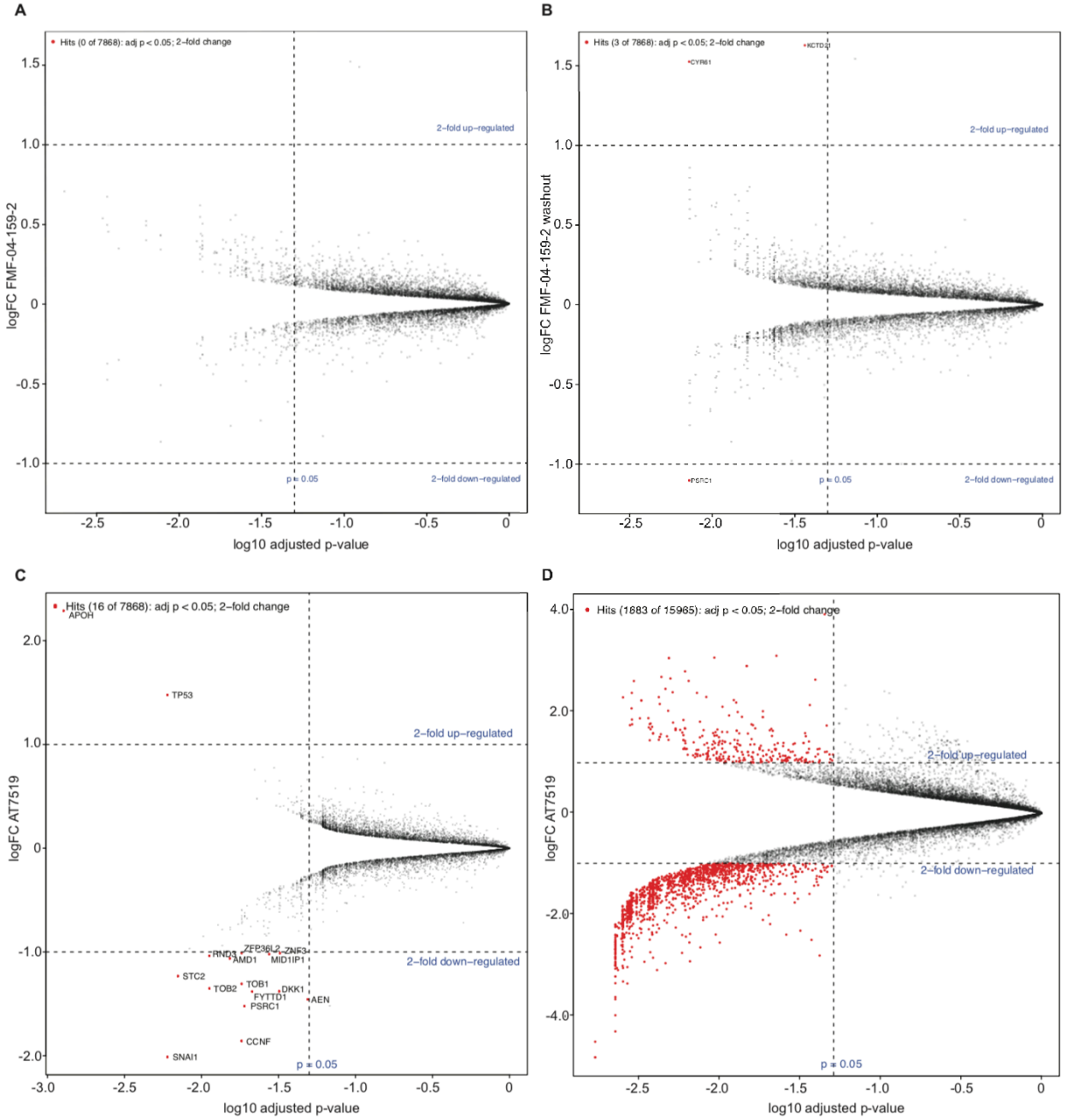

Figure S5. Proteomics and phospho-proteomics analysis for additional compounds and conditions. *Related to Figure 5.* (A) Proteome-wide effect of FMF-04-159-2 4 h treatment of HCT116 cells. (B) Proteome-wide effect of FMF-04-159-2 4 h treatment of HCT116 cells followed by 2 h compound washout. (C) Proteome-wide effect of AT7519 4 h treatment of HCT116 cells. (D) Phospho-proteomics for AT7519 4 h treatment of HCT116 cells.

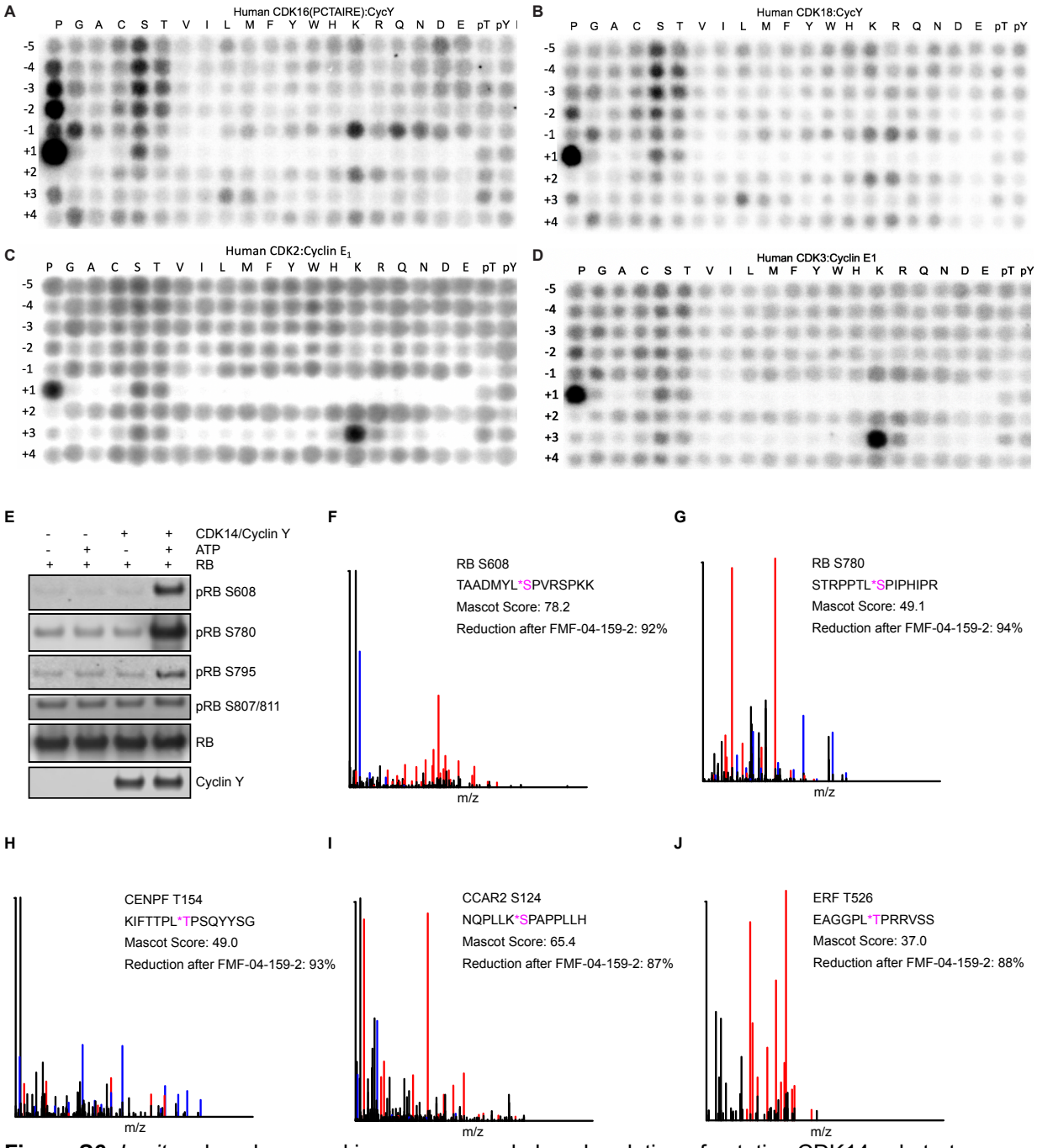

Figure S6: *In vitro* phospho-array kinase assay and phosphorylation of putative CDK14 substrates. *Related to Figure 5*. In vitro kinase assay results for phosphorylation of peptide library array by (A) CDK16/Cyclin Y (B) CDK18/Cyclin Y (C) CDK2/Cyclin E1 and (D) CDK3/Cyclin E1. (E) *In vitro* kinase assay using CDK14/Cyclin Y with recombinant RB protein, followed by western blotting with phospho-specific RB antibodies. (F-I) MS/MS spectra of five indicated synthetic peptides phosphorylated in vitro by CDK14. Phosphorylated residue is indicated in pink. Singly charged, doubly charged, and phosphate neutral loss fragment ions are highlighted (y-type ions: red, b-type ions: blue). Included are Mascot search scores and the percentage of phospho-peptide precursor signal lost after CDK14 kinase was incubated with 1 μM FMF-4-129-2 for 1 h prior to in vitro phosphorylation reactions.