Experimental Procedures

Cell culture

CCD 841 CoN cells were obtained from ATCC (ATCC CRL-1790) and grown in MEM supplemented with 10% fetal bovine serum (FBS). RKO, HT-29, DLD1 and SW48 cells were provided by S. Aaronson; LoVo cells by A. Dar; and A-375 and MCF-7 cells by J. Chipuk (all of Icahn School of Medicine); and grown in DMEM (RKO, HT-29, DLD1, SW48, A-375, MCF-7) or DMEM/F-12 (LoVo), with 10% FBS.

Drugs used in cell treatments and kinase assays

Drugs used were: 3-MB-PP1 (gift of K. Shokat), THZ1 and YKL-1-116; FP, triptolide, 5-FU, 5-FUR and 5-FdUR (Sigma Aldrich), nutlin-3a (Cayman Chemical); CX5461 and ML60218 (EMD Millipore); and Z-VAD-FMK (Cell Signaling).

Antibodies

Antibodies used for immunoblotting were: PARP, cleaved Caspase 3, cleaved Caspase 7 and cleaved Caspase 8 (Cell Signaling); Cdk7, p53 and p21 (Santa Cruz); Mdm2 (Calbiochem); DR4 and DR5 (Prosci); FAS (R&D systems); and α-tubulin (Sigma).

Drug synergy, dose-response and Bliss independence analysis

CDK7$^{WT/WT}$ or *CDK7*$^{as/as}$ HCT116 cells were grown in 10 X 5 matrix in a 96-well plate and treated with drugs for 48 hr. Cell viability was measured with resazurin, and percent cell viability was determined by dividing values of drug-treated samples by DMSO controls. Predicted combination values are determined from single drug dose responses of each compound based on the formula: Fractional response of Drug 1 (Fa) + Fractional effect of Drug 2 (Fb) - (Fa*Fb). Bliss score is defined as the difference between observed and predicted values for a drug combination; scores >0 indicate synergy.

Kinase assays

After pre-incubation of Cdk7 complexes with specified drug for 20 min, kinase reactions were carried out in 20 µl reaction volume containing 10 ng Cdk7 complex (4.5 nM) in 25 mM Hepes pH 7.4, 10 mM NaCl, 10 mM $MgCl_2$, plus 1 µg purified GST-CTD for 20 min at room temperature. Phosphorylated proteins were separated by SDS-PAGE and detected by autoradiography and Typhoon FLA9500 and quantified with ImageQuant TL (GE Healthcare).

RNA interference

Cells were transfected with short interfering RNA (siRNA) oligonucleotides and Lipofectamine RNAiMAX reagent according to manufacturer's instructions. Drugs were added after 32 hr and cells were harvested after 48 hr for immunoblotting or Annexin V staining. The siRNA oligonucleotides used were DR4 (Invitrogen 10620318, TNFRSF10AHSS112945), DR5 (Invitrogen 10620318, TNFRSF10BHSS112939), FAS (sc29311) and control (sc36869).

Annexin V staining

The cells were harvested using Accutase (Cellgro 25-058-CI) to detach cells and FITC Annexin V staining was performed according to FITC-Annexin V apoptosis detection kit (BD Biosciences 556547). Cells were analyzed using flow cytometry and data analysis was performed using FlowJo 9.9.4 software.

RNAseq

HCT116 cells were treated with DMSO or 5-FU (40 µM) for 12 hr, washed twice with phosphate-buffered saline and treated for 4 hr with DMSO or 2.5 µM 3-MB-PP1. RNA

was extracted with RNeasy mini kit (Qiagen 74104). Ribo-Zero kit was used for preparation of libraries, followed by single-end sequencing.

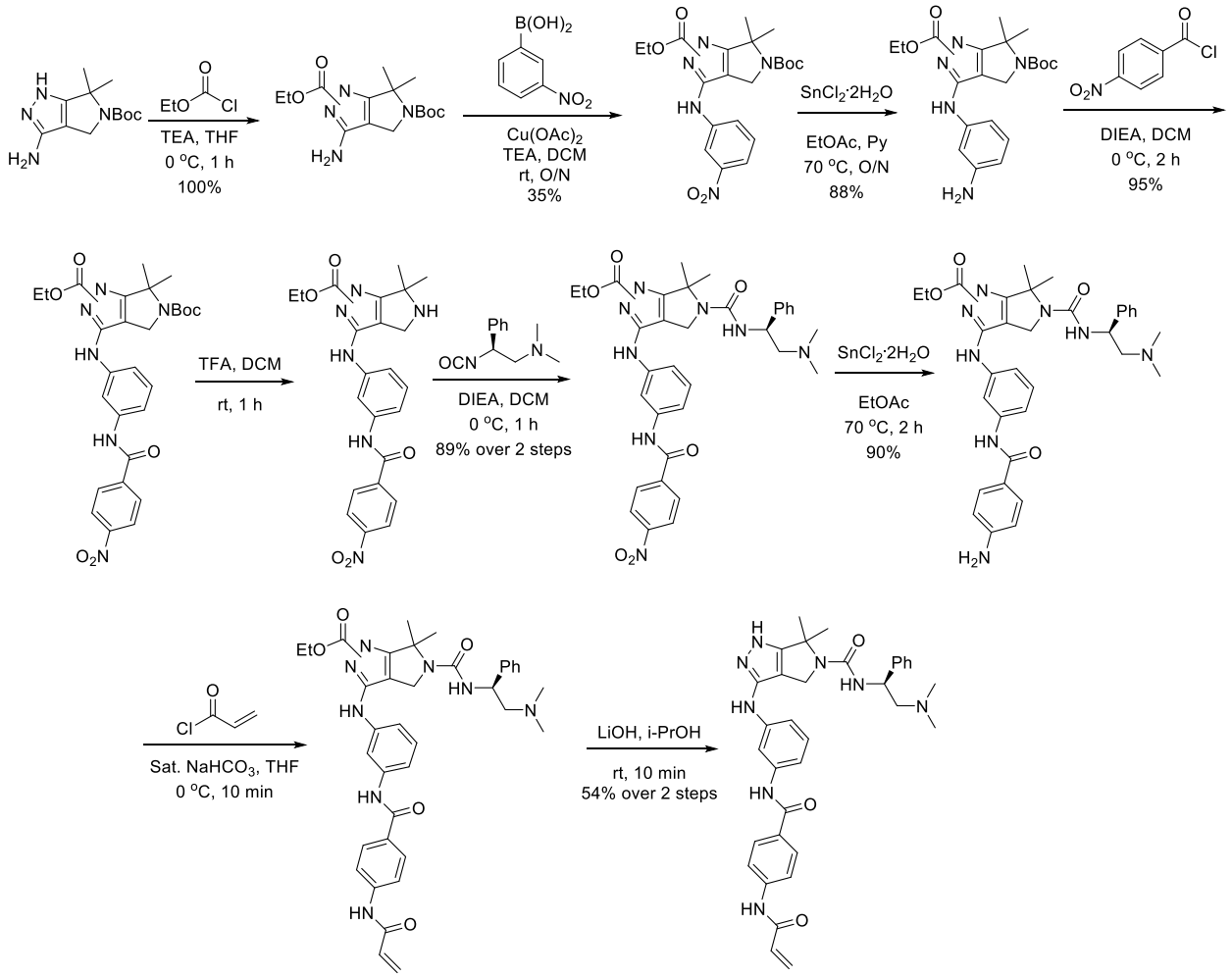
RNA extraction, cDNA synthesis and Q-PCR

RNA was isolated from cells using RNeasy Mini kit (74104). cDNA was synthesized using Affymetrix (78070) kit , 5 µg of total RNA and a combination of random hexamers and oligo(dT) used according to the manufacturer's protocol. RT-qPCR was performed using RadiantTM Green Lo-ROX qPCR Kit (QS1020) with the primers listed below.

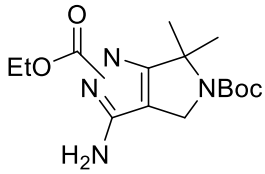
Primers used for Q-PCR

Primer	Forward	Reverse
GAPDH	GGTCATTGATGGCAACAA	AGGTGAAGGTCGGAGTCA
DR4	TTGCTTGCCTCCCATGTACAG	CAGGGACTTCTCTCTTCTTCA
DR5	GCCCCACAACAAAAGAGGTC	AGGTCATTCCAGTGAGTGCTA
FAS	GGCATCTGGACCCTCCTAC	GATAATCTAGCAACAGACGTAAG AACCA
MDM2	GGCGATTGGAGGGTAGACCT	CACATTTGCCTGGATCAGCA
p21	GAGACTCTCAGGGTCGAAA	GGATTAGGGCTTCCTCTTGG

Synthetic Scheme for YKL-1-116



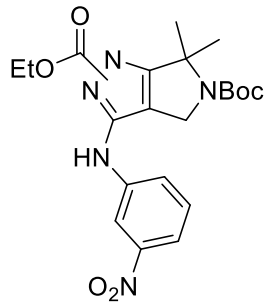
5-(*tert*-Butyl) ethyl 3-amino-6,6-dimethyl-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate



To a solution of *tert*-butyl 3-amino-6,6-dimethyl-4,6-dihydropyrrolo[3,4-c]pyrazole-5(1*H*)-carboxylate (1.0 g, 3.95 mmol) and TEA (0.6 g, 0.82 mL, 5.93 mmol) in THF (10 mL) was added ethyl chloroformate (0.43 g, 0.38 mL, 3.95 mmol) dropwise at 0 °C. The mixture was stirred at 0 °C for 1 h. The solvent was evaporated and the residue was

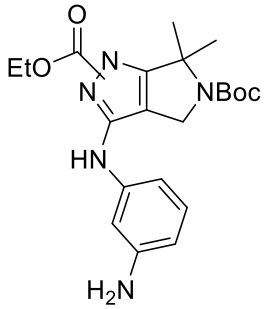
partitioned with EtOAc and sat. $NaHCO_3$. The organic layer was washed with water and brine, dried (Na_2SO_4). This was concentrated to give 5-(*tert*-butyl) ethyl 3-amino-6,6-dimethyl-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate as an off white solid (1.28 g, 100 %). LC/MS (ESI) *m*/*z* = 325.3 $(M + H)^+$.

5-(*tert*-Butyl) ethyl 6,6-dimethyl-3-((3-nitrophenyl)amino)-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate



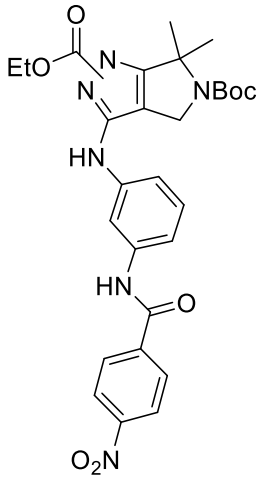
To a suspension of 5-(*tert*-butyl) ethyl 3-amino-6,6-dimethyl-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate (500 mg, 1.54 mmol), (3-nitrophenyl)boronic acid (514 mg, 3.08 mmol), and $Cu(OAc)_2$ (420 mg, 2.31 mmol) in DCM (10 mL) was added TEA (311 mg, 0.43 mL, 3.08 mmol). The mixture was stirred at room temperature overnight. Solvent was evaporated and the crude was purified by flash column chromatography on silica gel (EtOAc/hexane, 0-70%) to give 5-(*tert*-butyl) ethyl 6,6-dimethyl-3-((3-nitrophenyl)amino)-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate as a yellow solid (240 mg, 35%). LC/MS (ESI) *m*/*z* = 446.4 $(M + H)^+$.

5-(*tert*-Butyl) ethyl 3-((3-aminophenyl)amino)-6,6-dimethyl-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate



To a solution of 5-(*tert*-butyl) ethyl 6,6-dimethyl-3-((3-nitrophenyl)amino)-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate (120 mg, 0.27 mmol) in EtOAc (2 mL) and pyridine (0.2 mL) was added $SnCl_2{\cdot}2H_2O$ (305 mg, 1.35 mmol). The mixture was stirred at 70 ^{o}C overnight. The mixture was diluted with $CHCl_3$/i-PrOH (v/v 4:1) and washed with sat. $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The crude was purified by flash column chromatography on silica gel (MeOH/DCM, 0-10%) to give 5-(*tert*-butyl) ethyl 3-((3-aminophenyl)amino)-6,6-dimethyl-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate as a yellow solid (99 mg, 88%). LC/MS (ESI) *m*/*z* = 416.4 $(M + H)^{+}$.

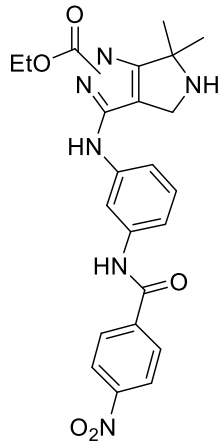
5-(*tert*-Butyl) ethyl 6,6-dimethyl-3-((3-(4-nitrobenzamido)phenyl)amino)-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate



To a solution of 5-(*tert*-butyl) ethyl 3-((3-aminophenyl)amino)-6,6-dimethyl-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate (99 mg, 0.24 mmol) and DIEA (62 mg,

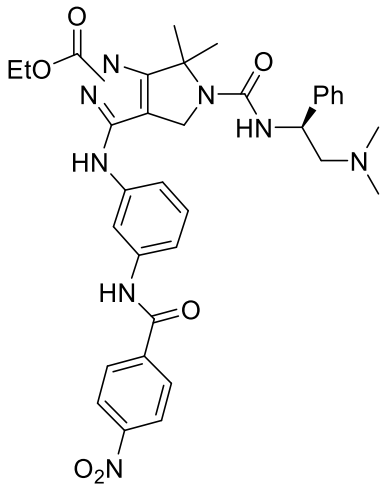
84 µL, 0.48 mmol) in DCM (2 mL) was added 4-nitrobenzoyl chloride (53 mg, 0.29 mmol) at 0 ^{o}C. The reaction was stirred at room temperature for 2 h and concentrated. The crude was purified by flash column chromatography on silica gel (MeOH/DCM, 0-5%) to give 5-(*tert*-butyl) ethyl 6,6-dimethyl-3-((3-(4-nitrobenzamido)phenyl)amino)-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate as a yellow solid (129 mg, 95%). LC/MS (ESI) *m*/*z* = 565.4 $(M + H)^{+}$.

Ethyl 6,6-dimethyl-3-((3-(4-nitrobenzamido)phenyl)amino)-5,6-dihydropyrrolo[3,4-*c*]pyrazole-1(4*H*)-carboxylate



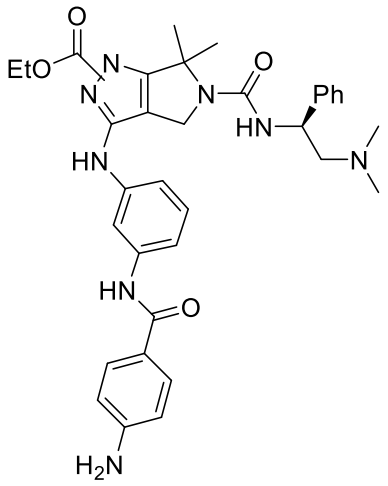
To a solution of 5-(*tert*-butyl) 1- or 2-ethyl 6,6-dimethyl-3-((3-(4-nitrobenzamido)phenyl)amino)-4,6-dihydropyrrolo[3,4-*c*]pyrazole-1,5-dicarboxylate (129 mg, 0.23 mmol) in DCM (1 mL) was added TFA (1 mL). The reaction was stirred at room temperature for 1 h and concentrated to give ethyl 6,6-dimethyl-3-((3-(4-nitrobenzamido)phenyl)amino)-5,6-dihydropyrrolo[3,4-*c*]pyrazole-1(4*H*)-carboxylate as a TFA salt, which was used directly without further purification. LC/MS (ESI) *m*/*z* = 464.4 $(M + H)^{+}$.

Ethyl (*S*)-5-((2-(dimethylamino)-1-phenylethyl)carbamoyl)-6,6-dimethyl-3-((3-(4-nitrobenzamido)phenyl)amino)-5,6-dihydropyrrolo[3,4-*c*]pyrazole-1(4*H*)-carboxylate



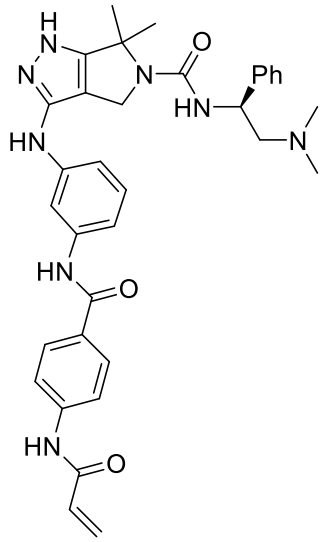
To a mixture of ethyl 6,6-dimethyl-3-((3-(4-nitrobenzamido)phenyl)amino)-5,6-dihydropyrrolo[3,4-*c*]pyrazole-1(4*H*)-carboxylate (TFA salt, 0.23 mmol) in DCM (2 mL) was added DIEA (148 mg, 0.2 mL, 1.15 mmol) at 0 ^{o}C, followed by (*S*)-2-isocyanato-*N*,*N*-dimethyl-2-phenylethan-1-amine HCl salt (62 mg, 0.27 mmol). The solution was stirred at 0 ^{o}C for 1 h and diluted with $CHCl_3$/i-PrOH (v/v 4:1) and washed with sat. $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The crude was purified by flash column chromatography on silica gel (1.75 M NH_3 in MeOH/DCM, 0-10%) to give ethyl (*S*)-5-((2-(dimethylamino)-1-phenylethyl)carbamoyl)-6,6-dimethyl-3-((3-(4-nitrobenzamido)phenyl)amino)-5,6-dihydropyrrolo[3,4-*c*]pyrazole-1(4*H*)-carboxylate as a yellow solid (134 mg, 89% over 2 steps). LC/MS (ESI) *m*/*z* = 655.4 $(M + H)^+$.

Ethyl (*S*)-3-((3-(4-aminobenzamido)phenyl)amino)-5-((2-(dimethylamino)-1-phenylethyl)carbamoyl)-6,6-dimethyl-5,6-dihydropyrrolo[3,4-*c*]pyrazole-1(4*H*)-carboxylate



To a solution of ethyl (*S*)-5-((2-(dimethylamino)-1-phenylethyl)carbamoyl)-6,6-dimethyl-3-((3-(4-nitrobenzamido)phenyl)amino)-5,6-dihydropyrrolo[3,4-*c*]pyrazole-1(4*H*)-carboxylate (134 mg, 0.20 mmol) in EtOAc (2 mL) was added $SnCl_2{\cdot}2H_2O$ (226 mg, 1.0 mmol). The mixture was stirred at 70 °C for 2 h and diluted with $CHCl_3$/i-PrOH (v/v 4:1) and washed with sat. $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The crude was purified by flash column chromatography on silica gel (1.75 M NH_3 in MeOH/DCM, 0-10%) to give ethyl (*S*)-3-((3-(4-aminobenzamido)phenyl)amino)-5-((2-(dimethylamino)-1-phenylethyl)carbamoyl)-6,6-dimethyl-5,6-dihydropyrrolo[3,4-*c*]pyrazole-1(4*H*)-carboxylate as a yellow solid (112 mg, 90%). LC/MS (ESI) *m*/*z* = 625.4 $(M + H)^+$.

YKL-01-116. (*S*)-3-((3-(4-acrylamidobenzamido)phenyl)amino)-*N*-(2-(dimethylamino)-1-phenylethyl)-6,6-dimethyl-4,6-dihydropyrrolo[3,4-*c*]pyrazole-5(1*H*)-carboxamide



To a mixture of ethyl (*S*)-3-((3-(4-aminobenzamido)phenyl)amino)-5-((2-(dimethylamino)-1-phenylethyl)carbamoyl)-6,6-dimethyl-5,6-dihydropyrrolo[3,4-*c*]pyrazole-1(4*H*)-carboxylate (20 mg, 0.032 mmol) in THF (1 mL) and sat. $NaHCO_3$ (1 mL) was added acryloyl chloride (5.8 mg, 0.064 mmol) dropwise at 0 ^oC. The mixture was stirred at 0 ^oC for 10 min and diluted with $CHCl_3$/i-PrOH (v/v 4:1) and washed with sat. $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The crude ethyl (*S*)-3-((3-(4-acrylamidobenzamido)phenyl)amino)-5-((2-(dimethylamino)-1-phenylethyl)carbamoyl)-6,6-dimethyl-5,6-dihydropyrrolo[3,4-*c*]pyrazole-1(4*H*)-carboxylate was dissolved in i-PrOH (1 mL) and LiOH (1 M, 1 mL) was added. After stirred at room temperature for 10 min, the mixture was diluted with $CHCl_3$/i-PrOH (v/v 4:1) and washed with sat. $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated. The crude was purified by reverse phase preparative HPLC (MeOH/H_2O, 0-100%) to give (*S*)-3-((3-(4-acrylamidobenzamido)phenyl)amino)-*N*-(2-(dimethylamino)-1-phenylethyl)-6,6-dimethyl-4,6-dihydropyrrolo[3,4-*c*]pyrazole-5(1*H*)-carboxamide (**YKL-01-116**) as a white solid (10.5 mg, 54%). ^1H NMR: 600 MHz (DMSO-d_6) δ 10.44 (d, J = 4.2 Hz, 1H), 10.04 (s, 1H), 8.33 (s, 1H), 7.96-7.93 (m, 2H), 7.82-7.80 (m, 2H), 7.34-7.32 (m, 2H), 7.26-7.23 (m, 2H), 7.20-7.10 (m, 3H), 6.52-6.46 (m, 1H), 6.35-6.30 (m, 1H), 6.09 (s, 1H), 5.84-5.81 (m,

1H), 4.89-4.83 (m, 1H), 4.35 (d, *J* = 19.8 Hz, 2H), 2.64-2.57 (m, 1H), 2.45-2.38 (m, 1H), 2.18 (s, 6H), 1.65 (d, *J* = 4.2 Hz, 3H), 1.58 (d, *J* = 4.8 Hz, 3H); MS *m/z*: 607.4 [M+1].

Evaluation of YKL-1-116 inhibitory specificity

To measure efficiency of Cdk7 target engagement by YKL-1-116, 7.5 x10^7 Jurkat cells were treated with DMSO, YKL-1-116, or the moderately selective, reversible Cdk7 inhibitor BS-181 (Ali et al., 2009) for 6 hr before extract preparation. Lysates were incubated with DMSO or biotin-conjugated YKL-1-116 at 4°C overnight, and interacting proteins were precipitated with streptavidin beads and analyzed by immunoblotting for recovery of Cdk7. To assess Cdk7-selectivity, we performed KiNativ™ kinome profiling. Cells were treated with DMSO or YKL-1-116 (1 μM) for 4 hr. PBS-washed cell pellets were flash-frozen and subjected to KiNativ™ kinome profiling at ActivX Biosciences, Inc., using a desthiobiotin-ATP probe. Peptide sequences shown in Supplemental Table 1 belong to the indicated kinase(s) and were detected by mass spectrometry (MS) under DMSO control conditions following enrichment for biotinylated proteins by streptavidin pull-down and subsequent proteolysis. Only kinases accessible to desthiobiotin-ATP probe binding are labeled in this assay. Results in the presence of drug are normalized to paired DMSO controls and numbers represent the percentage of MS signal lost (compared to DMSO control) for sequences of an indicated kinase, so that values approaching 100% indicate that the test compound effectively out-competed the desthiobiotin ATP probe for binding to the kinase, leading to decreased labeling and recovery of peptides representing this kinase.

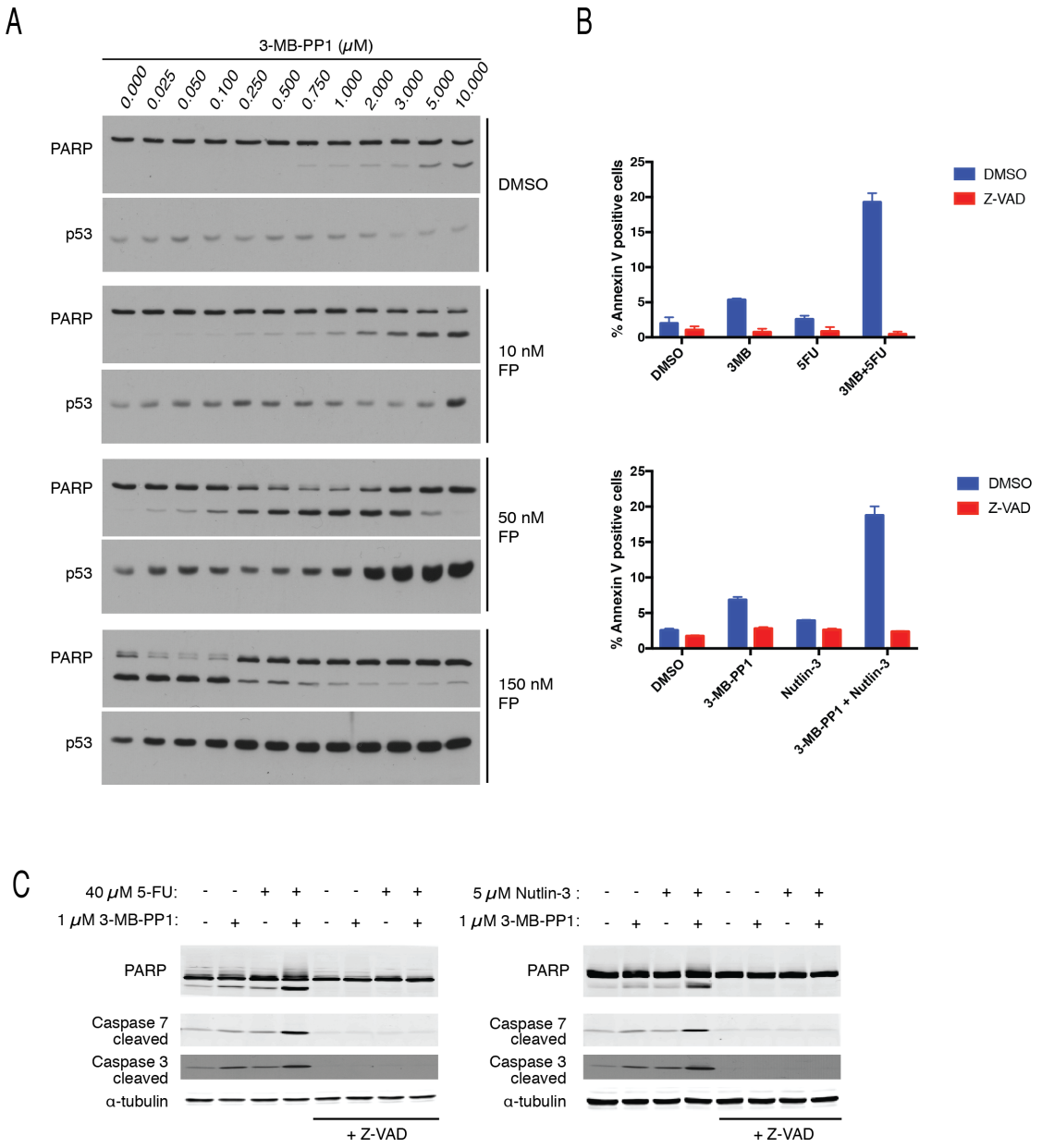


Figure S1 (related to Figure 1) Synthetic lethality of Cdk7 inhibition with p53-activating agents

(A) *CDK7*$^{as/as}$ cells were treated with indicated doses of 3-MB-PP1, without FP (DMSO) or with addition of 10, 50 or 150 nM flavopiridol (FP), as indicated, for 14 hr prior to extract preparation and immunoblot detection of PARP and p53.

(B) Addition of Z-VAD (50 μM) prevents synthetic lethality of 3-MB-PP1 combined with either 5-FU (top) or nutlin-3 (bottom), detected by annexin V staining.

(C) *CDK7*$^{as/as}$ cells were treated with indicated combinations of 3-MB-PP1 with either 5-FU (left) or nutlin-3 (right), without (left four lanes in each panel) or with Z-VAD (50 μM) (right four lanes) prior to extract preparation and immunoblot detection of PARP, cleaved caspase 7 and α-tubulin.

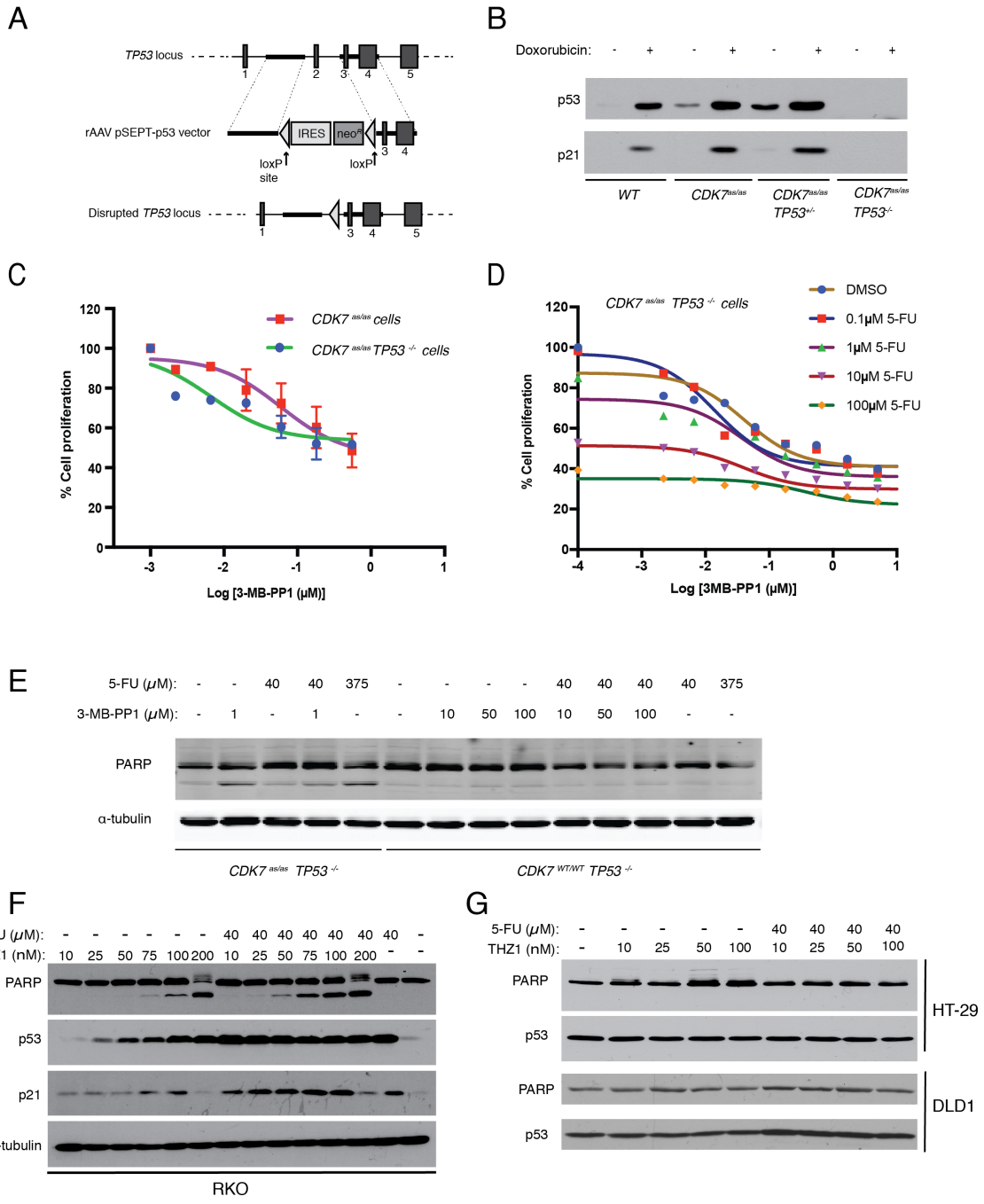


Figure S2 (related to Figure 2) Synthetic lethality of Cdk7 inhibition in colon cancer-derived cells is dependent on p53 status

(A) Construction of *CDK7*$^{as/as}$ *TP53*$^{-/-}$ cell line with recombinant adeno-associated virus (rAAV) vector pSEPT-p53.

(B) Genotypes of gene-targeted HCT116 cell derivatives confirmed by immunoblotting, showing no expression of p53 or induction of p21 by doxorubicin in *TP53*$^{-/-}$ lines.

(C) Dose response curves of HCT116 *CDK7*$^{as/as}$ *and CDK7*$^{as/as}$ *TP53*$^{-/-}$ cells with % reduction in metabolic activity as measured by resazurin staining, relative to DMSO-treated cells (values indicate mean of two biological replicates).

(D) Dose response curves of *CDK7*$^{as/as}$ *TP53*$^{-/-}$ cells for 5-FU and 3-MB-PP1 combination

with % reduction in metabolic activity as measured by resazurin staining, relative to DMSO-treated cells (values indicate mean of two biological replicates).
(E) *CDK7*$^{as/as}$ *TP53*$^{-/-}$ or *TP53*$^{-/-}$ HCT116 cells were treated with indicated drugs at doses indicated for 14 hr prior to lysis and immunoblot detection of PARP, p53 and α-tubulin.
(F) Colon cancer-derived RKO cells, which have wild-type p53 function, were treated with indicated concentrations of 5-FU and/or THZ1 before extract preparation and immunbolot detection of PARP, p53, p21 and α-tubulin.
(G) Colon cancer-derived HT-29 or DLD1 cells, which have inactivating mutations in *TP53*, were treated with indicated concentrations of 5-FU and/or THZ1 before extract preparation and immunoblot detection of PARP and p53. (Note elevated level of basal p53 expression due to defective p53 function.)

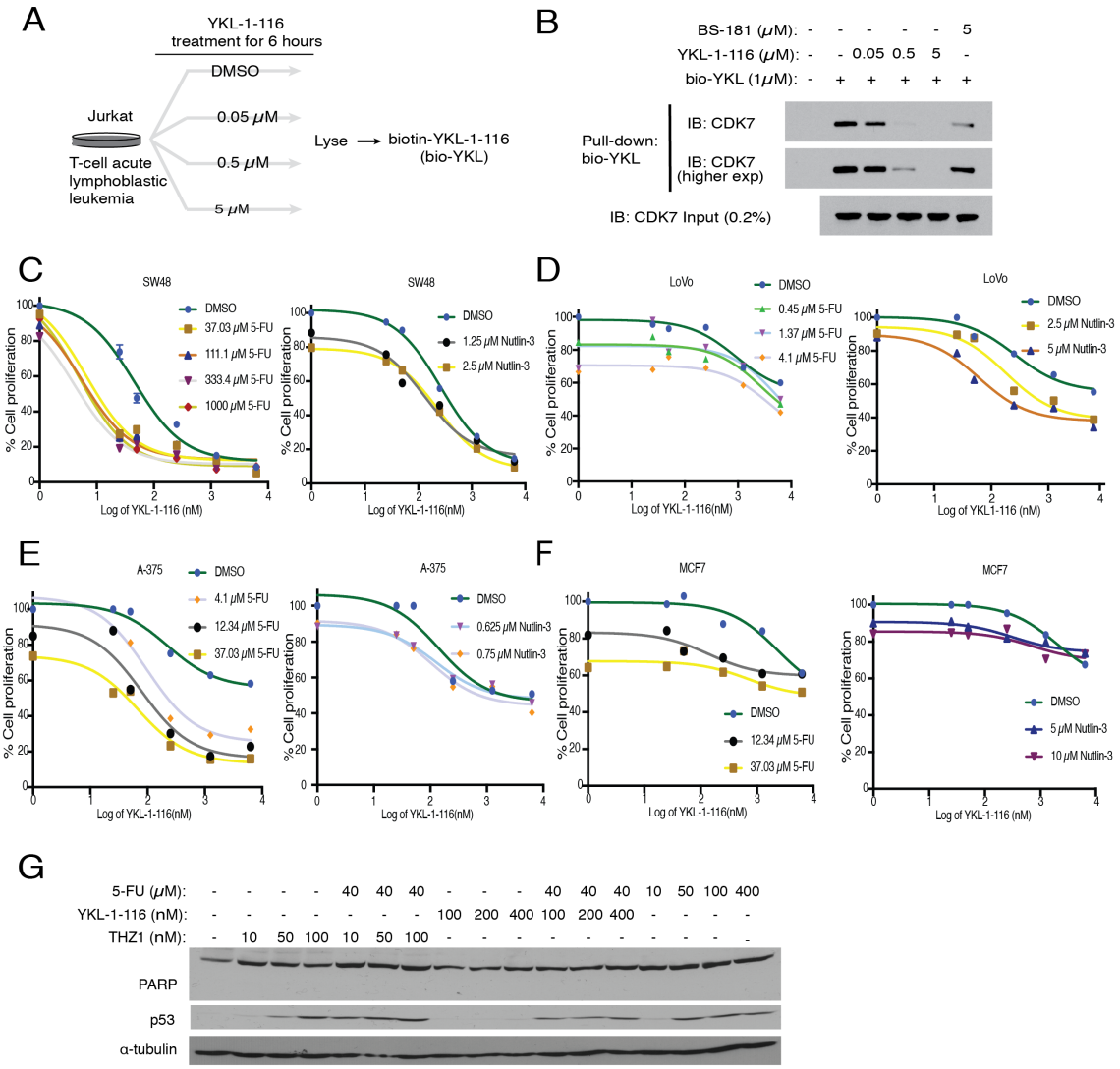


Figure S3 (related to Figure 3) YKL-1-116, a selective, covalent inhibitor of Cdk7
(A) Schematic diagram of assay to measure Cdk7-binding by YKL-1-116 in human cells (Jurkat T-ALL cells).
(B) Cells were exposed to indicated concentrations of YKL-1-116 or the reversible CDK inhibitor BS-181. Whole-cell extracts were prepared, subjected to pulldown with biotin-YKL-1-116 ("bio-YKL") conjugated to streptavidin beads, and analyzed by immunoblot for recovered Cdk7.
(C-F) Dose response curves of *TP53*$^{+}$ cancer-derived cells with YKL-1-116 and 5-FU (left panel) and YKL-1-116 and nutlin-3 (right panel), % reduction in metabolic activity as measured by resazurin staining, relative to DMSO-treated cells (data indicate mean of two biological replicates). The cell lines analyzed were: colon cancer-derived SW48 (C) and LoVo (D), melanoma-derived A-375 (E), and breast cancer-derived MCF7 (F)
(F) CCD 841 CoN *TP53*$^{+}$ normal colorectal epithelial cells were treated with indicated drugs, at indicated doses, for 14 hr prior to lysis and immunoblot detection of PARP, p53 and α-tubulin.

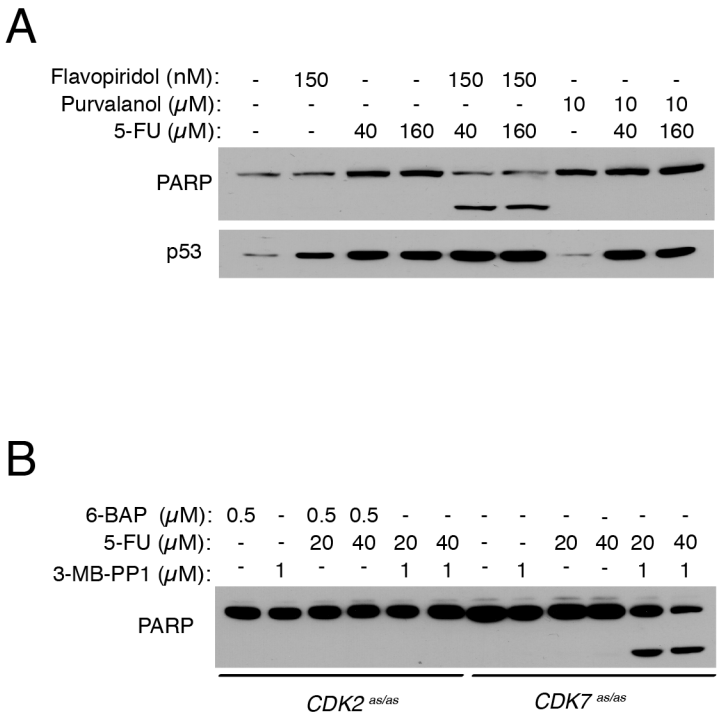


Figure S4 (related to Figure 4) Synthetic lethality with 5-FU due to inhibition of transcriptional, not cell-cycle, CDKs

(A) *CDK7*$^{WT/WT}$ HCT116 cells were treated with indicated doses of 5-FU and either 150 nM flavopiridol or 10 μM purvalanol A prior to extract preparation and immunoblot detection of PARP and p53.

(B) *CDK2*$^{as/as}$ or *CDK7*$^{as/as}$ HCT116 cells, as indicated, were treated with indicated doses of 5-FU and either 0.5 μM 6-benzylaminopurine (6-BAP)—an adenine analog that rescues the cyclin-binding defect of CDK2^{as} without inhibiting its kinase activity—or 1 μM 3-MB-PP1—which inhibits CDK2^{as} and CDK7^{as}—prior to extract preparation and immunoblot detection of PARP.

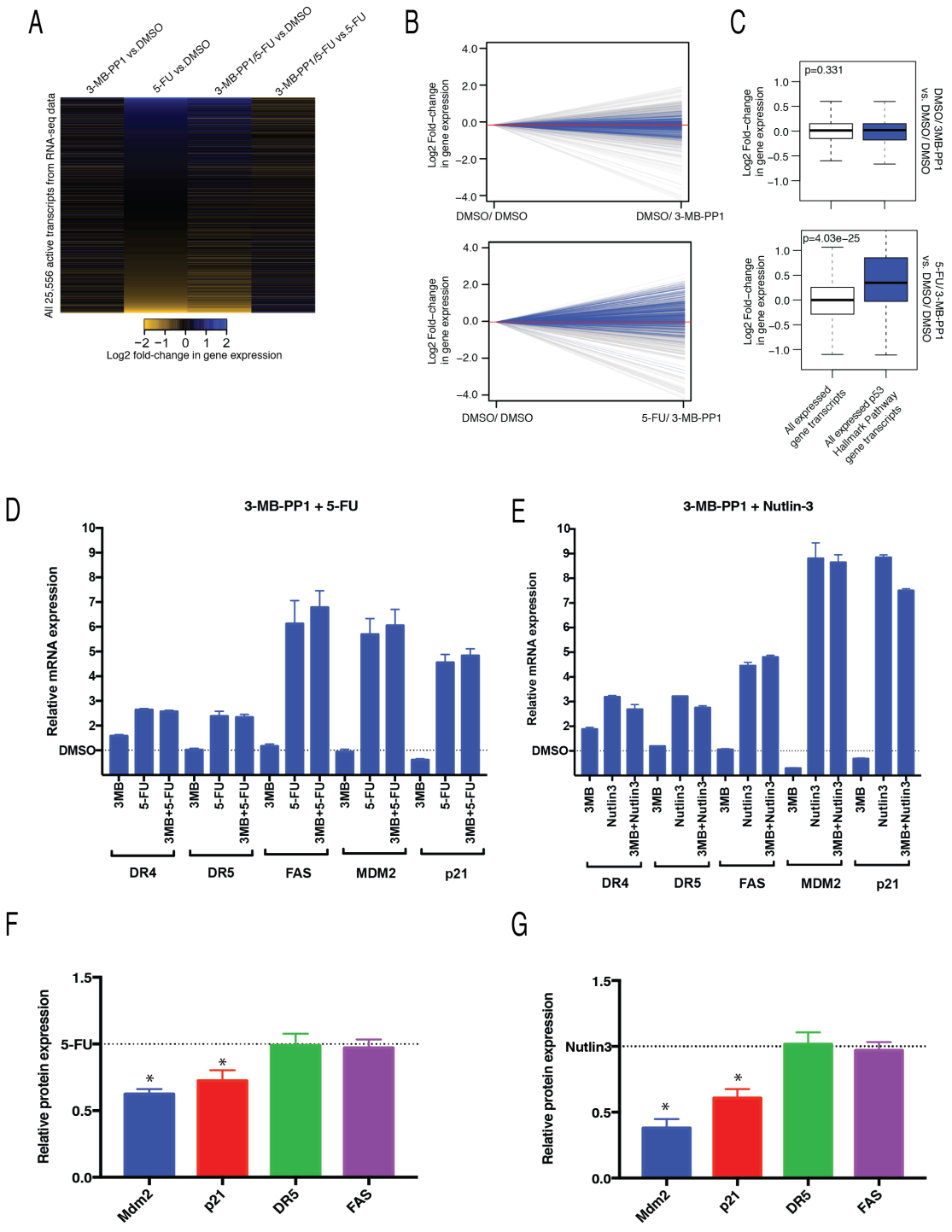


Figure S5 (related to Figure 6) Cdk7 inhibition modulates the transcriptional response to p53 activation

(A) HCT116 cells were treated as indicated in Figure 6A. Heatmaps display the Log2 fold change in gene expression vs. DMSO for the 25,556 transcripts expressed in DMSO. The three rightmost columns are identical to Figure 6B and are included for comparison.

(B) Per-transcript line plots showing the Log2 fold-change in expression following

DMSO/ 3-MB-PP1 treatment in comparison to DMSO/ DMSO treatment (top panel) and following 5-FU/ 3-MB-PP1 in comparison to DMSO/ DMSO treatment (bottom panel). Gray lines indicate the top 10% of most highly expressed transcripts in DMSO. Blue lines indicate “p53 Hallmark Pathway Gene” transcripts. Red line indicates no change in gene expression.
(C) Box plots showing the distribution of Log2 fold-changes in expression for the indicated transcript sets following DMSO/ 3-MB-PP1 treatment in comparison to DMSO/ DMSO treatment (top panel) and following 5-FU/ 3-MB-PP1 in comparison to DMSO/ DMSO treatment (bottom panel).
(D) RT-qPCR analysis of selected p53 target gene expression in *CDK7*$^{WT/WT}$ cells after simultaneous exposure to 5-FU and/or 3-MB-PP1, relative to levels in DMSO-treated cells. Error bars indicate range of values obtained in two biological replicates.
(E) RT-qPCR analysis of selected p53 target gene expression in *CDK7*$^{WT/WT}$ cells after simultaneous treatment with nutlin-3 and/or 3-MB-PP1, relative to levels in DMSO-treated cells. Error bars indicate range of values obtained in two biological replicates.
(F) Quantification of protein expression after sequential treatment with 5-FU (12 hr) and 3-MB-PP1 (4 hr), relative to treatment with 5-FU alone (defined as 1.0, dashed horizontal line), measured by densitometry of immunoblots with α-tubulin as normalization control (representative blot shown in figure 6I). Cells were harvested for protein extraction at 12 hr (8 hr after removal of 3-MB-PP1). Data represents mean (± S.E.M.) of 3 biological replicates (* represents $p < 0.05$).
(G) Quantification of protein expression after simultaneous treatment with nutlin-3 and 3-MB-PP1 (12 hr), relative to treatment with nutlin-3 alone (defined as 1.0, dashed horizontal line), measured by densitometry of immunoblots with α-tubulin as normalization control (representative blot shown in figure 6J). Cells were harvested for protein extraction at 12 hr. Data represents mean (± S.E.M.) of 3 biological replicates (* represents $p < 0.05$).

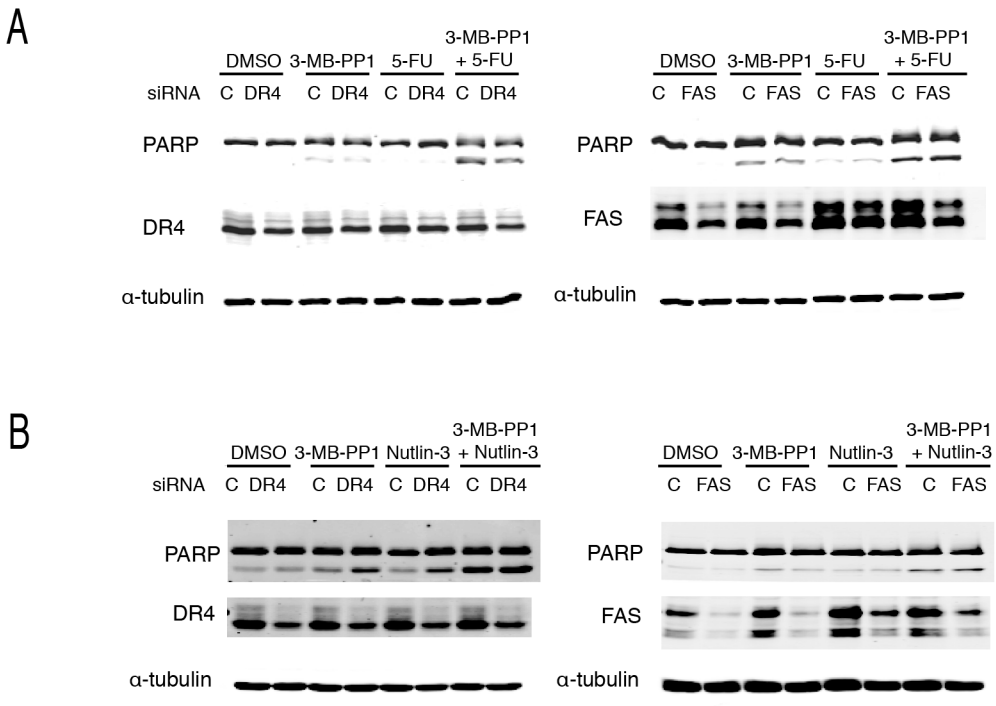


Figure S6 (related to Figure 7) Death-receptor specificity of synthetic lethality due to Cdk7 inhibition combined with p53 activation

(A) *CDK7*$^{as/as}$ cells were transfected with siRNA targeting DR4 or FAS or control siRNA ("C") and treated with indicated drugs (doses: 40 μM 5-FU, 1 μM 3-MB-PP1) prior to extract preparation and immunoblot detection of PARP, DR4 or FAS, and α-tubulin.
(B) *CDK7*$^{as/as}$ cells were transfected as in (A) and treated with indicated drugs (doses: 5 μM nutlin-3, 1 μM 3-MB-PP1) prior to extract preparation and immunoblot detection of PARP, DR4 or FAS, and α-tubulin.

Kinase Tested	IC50 (nM)
MELK	102
CDK9/cyclin K	> 10E+03
CDK2/cyclin A	1140
CDK2/cyclin A	1380
CHEK2 (CHK2)	7.37
FGR	5.15
HIPK4	178
PRKCQ (PKC theta)	4.88
RET	63.5
SRC	3.89
CDK7/cyclin H/MNAT1	7.6

Supplemental Table 2 In vitro IC50 for YKL-1-116 potency in binding to selected CDKs
The LanthaScreen Eu Kinase Binding assay (Invitrogen) was performed with indicated CDKs and their associated cyclins in the presence of different concentration of YKL-1-116. The IC50 values indicate the affinity of YKL-1-116 toward the ATP binding pocket of CDK.