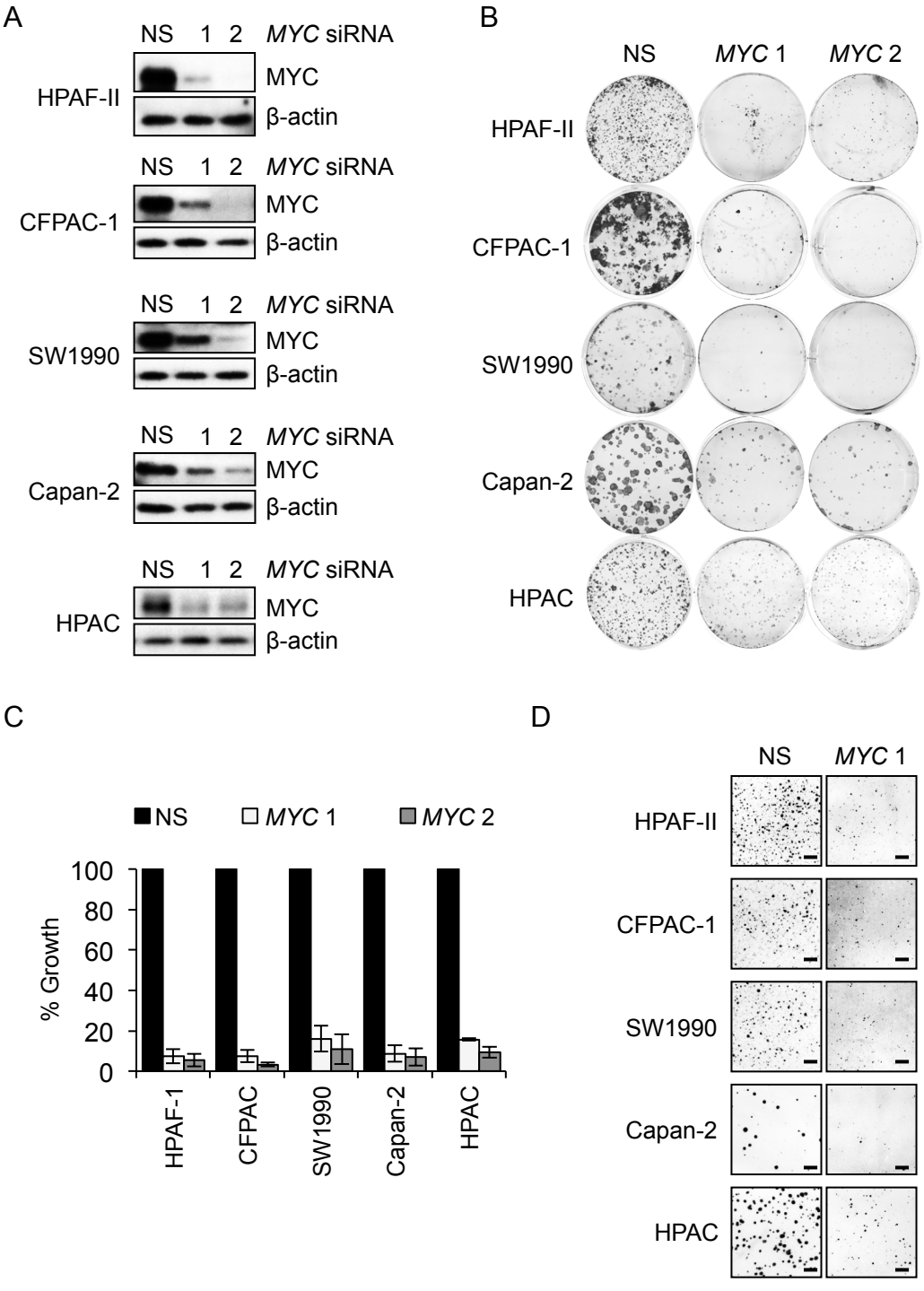
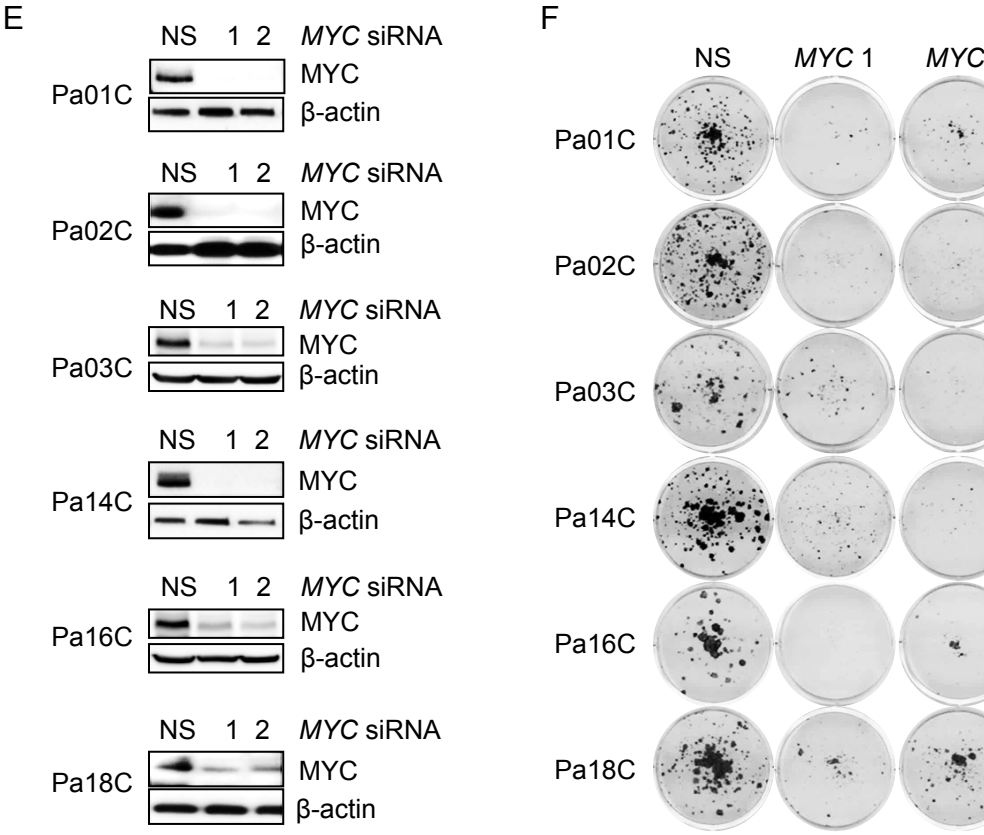
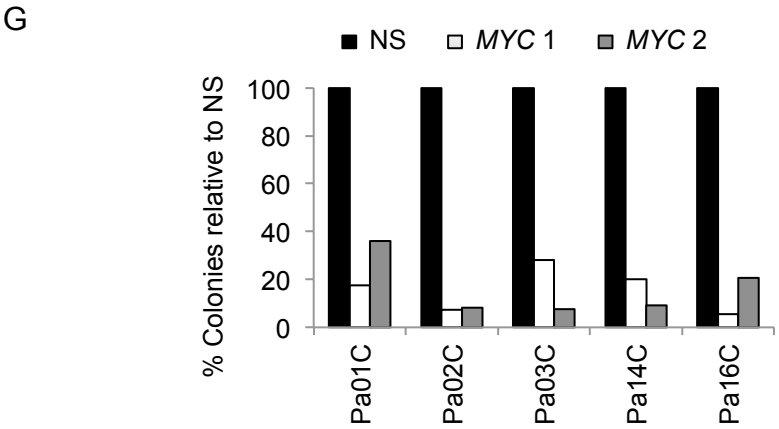

A
NS 1 2 MYC siRNA
HPAF-II
MYC
β-actin
CFPAC-1
SW1990
Capan-2
HPAC
B
NS
MYC 1
MYC 2
C
NS
MYC 1
MYC 2
% Growth
100
80
60
40
20
0
HPAF-1
CFPAC
SW1990
Capan-2
HPAC
D


E
NS 1 2 MYC siRNA
Pa01C
MYC
β-actin
Pa02C
Pa03C
Pa14C
Pa16C
Pa18C
F
NS
MYC 1
MYC 2
Pa01C
Pa02C
Pa03C
Pa14C
Pa16C
Pa18C


G
NS
MYC 1
MYC 2
% Colonies relative to NS
100
80
60
40
20
0
Pa01C
Pa02C
Pa03C
Pa14C
Pa16C

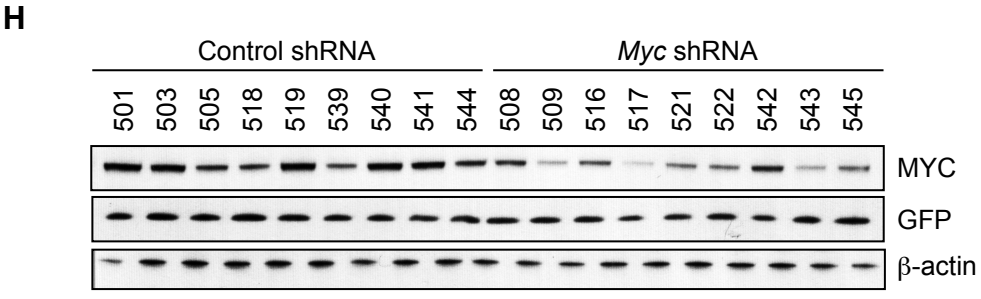

H
Control shRNA
Myc shRNA
501 503 505 518 519 539 540 541 544
508 509 516 517 521 522 542 543 545
MYC
GFP
β-actin

Figure S1. MYC is Essential for PDAC Cell Growth, Related to Figure 1

(A) *KRAS*-mutant PDAC cell lines were transfected with control nonspecific (NS) or siRNA oligos targeting two distinct *MYC* sequences to transiently suppress *MYC* expression (24 hr). Immunoblotting with anti-MYC antibody was done to monitor MYC levels and with anti-β-actin antibody to verify equivalent loading of total cellular protein.

(B) Approximately 10 days after plating, representative 6 well plates were stained with crystal violet to visualize colonies of proliferating cells.

(C) Quantitation of data in panel B. The percentage of colony formation relative to NS was determined from three biological replicates for each cell line transfected with *MYC* siRNA. Data are presented as a mean of three replicates, error bars represent standard error of the mean.

(D) Soft agar colony formation was visualized by MTT viability staining 15 days after plating the indicated cell lines transfected with *MYC* siRNA. Scale bar = 1mm.

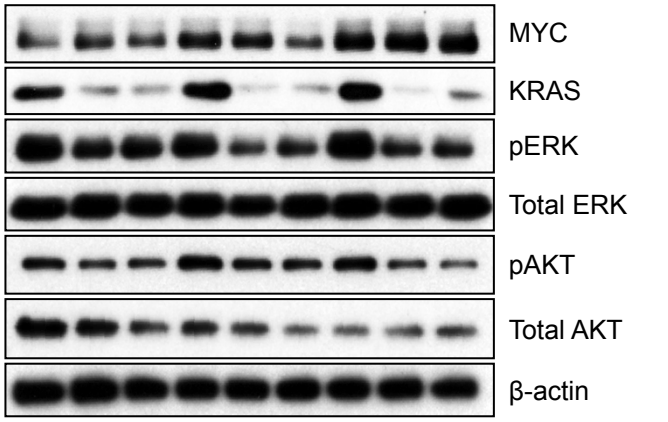
(E) *KRAS*-mutant PDX PDAC cell lines were transfected with control nonspecific (NS) or siRNA oligos targeting two distinct *MYC* sequences and immunoblotting was done as described in Figure S1A.

(F) Approximately 10 days after plating, representative 6 well plates were stained with crystal violet to visualize colonies of proliferating cells.

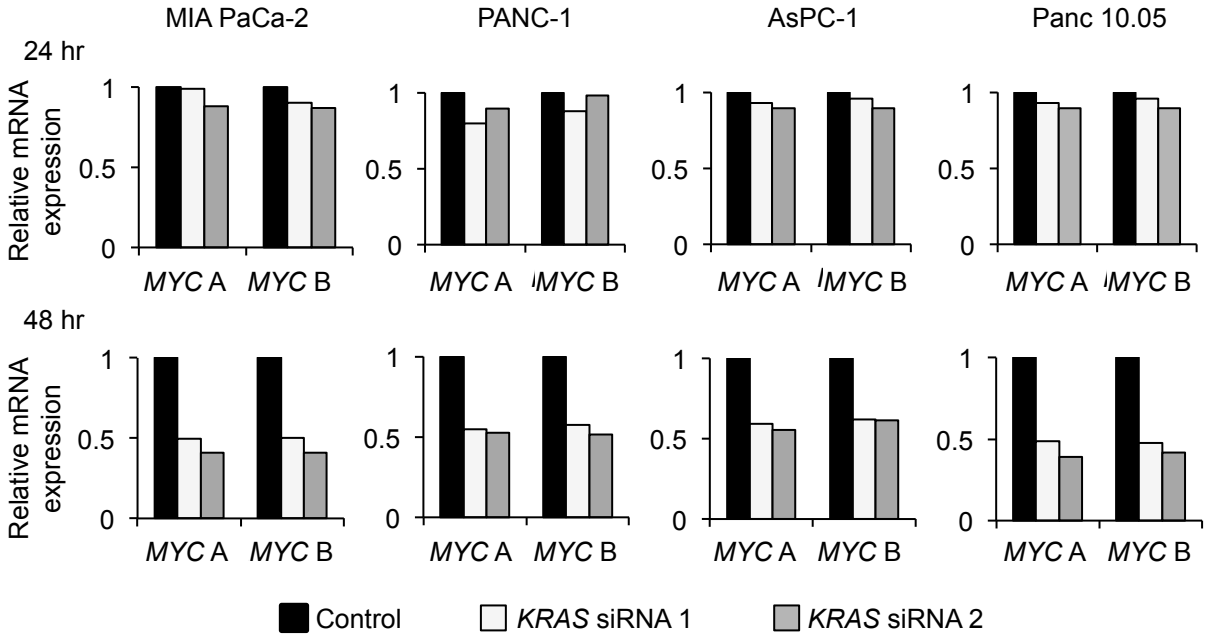
(G) Quantitation of data in panel F. Percentage of colony formation relative to NS.

(H) Immunoblotting for MYC expression of tumors from mice shown in Figure 1G that were harvested at ~40 days at time of sacrifice.

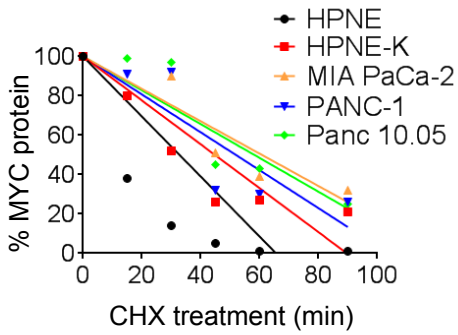
A


24
48
72
Time (hr)
NS 1 2 NS 1 2 NS 1 2
KRAS siRNA
MYC
KRAS
pERK
Total ERK
pAKT
Total AKT
β-actin

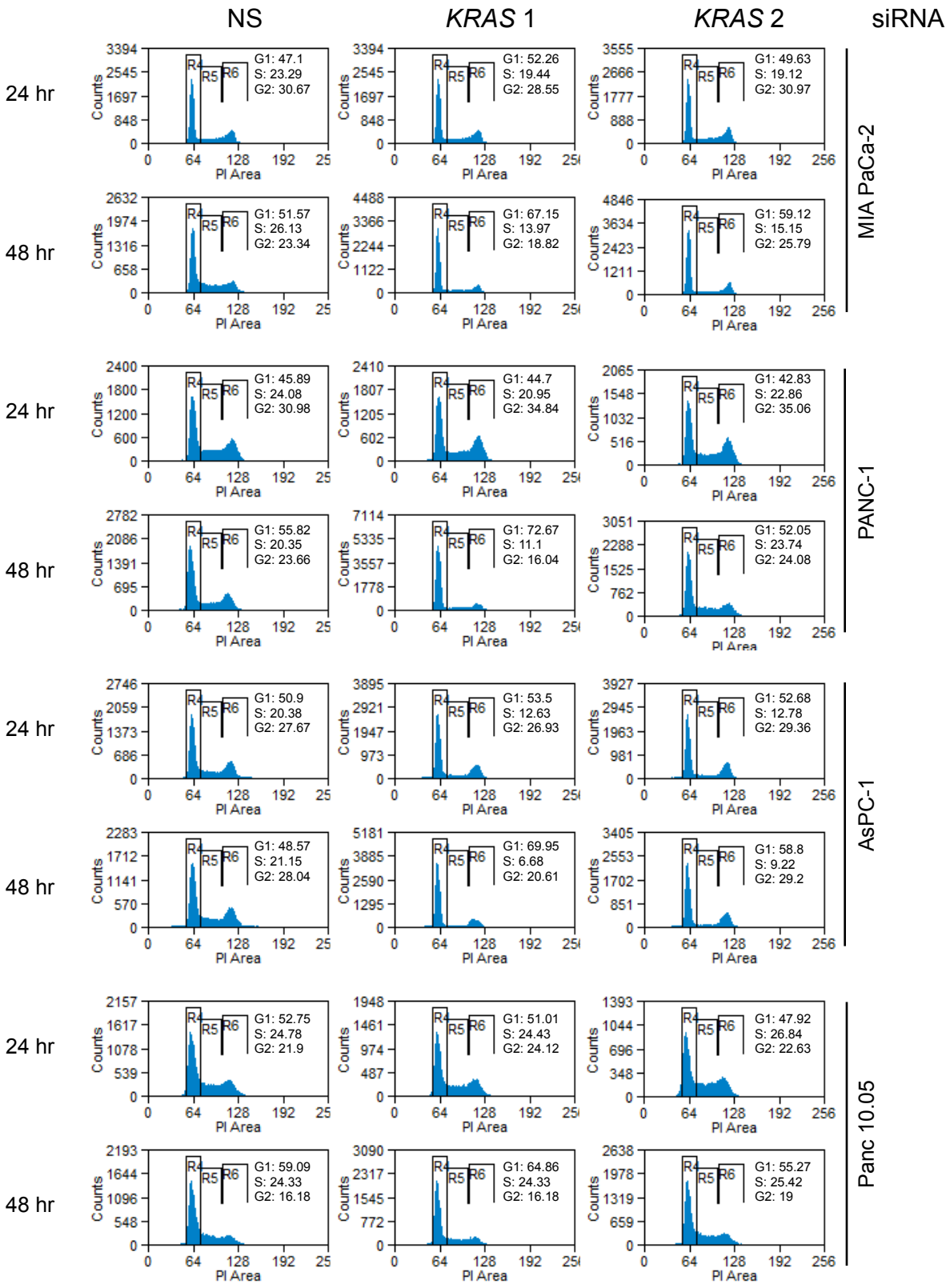
B


MIA PaCa-2
PANC-1
AsPC-1
Panc 10.05
24 hr
48 hr
Relative mRNA expression
1
0.5
0
MYC A
MYC B
Control
KRAS siRNA 1
KRAS siRNA 2

C


HPNE
HPNE-K
MIA PaCa-2
PANC-1
Panc 10.05
% MYC protein
100
80
60
40
20
0
0 20 40 60 80 100
CHX treatment (min)

D


NS
KRAS 1
KRAS 2
siRNA
24 hr
48 hr
MIA PaCa-2
PANC-1
AsPC-1
Panc 10.05
Counts
PI Area
R4
R5
R6
G1: 47.1
S: 23.29
G2: 30.67
G1: 52.26
S: 19.44
G2: 28.55
G1: 49.63
S: 19.12
G2: 30.97
G1: 51.57
S: 26.13
G2: 23.34
G1: 67.15
S: 13.97
G2: 18.82
G1: 59.12
S: 15.15
G2: 25.79
G1: 45.89
S: 24.08
G2: 30.98
G1: 44.7
S: 20.95
G2: 34.84
G1: 42.83
S: 22.86
G2: 35.06
G1: 55.82
S: 20.35
G2: 23.66
G1: 72.67
S: 11.1
G2: 16.04
G1: 52.05
S: 23.74
G2: 24.08
G1: 50.9
S: 20.38
G2: 27.67
G1: 53.5
S: 12.63
G2: 26.93
G1: 52.68
S: 12.78
G2: 29.36
G1: 48.57
S: 21.15
G2: 28.04
G1: 69.95
S: 6.68
G2: 20.61
G1: 58.8
S: 9.22
G2: 29.2
G1: 52.75
S: 24.78
G2: 21.9
G1: 51.01
S: 24.43
G2: 24.12
G1: 47.92
S: 26.84
G2: 22.63
G1: 59.09
S: 24.33
G2: 16.18
G1: 64.86
S: 24.33
G2: 16.18
G1: 55.27
S: 25.42
G2: 19

E

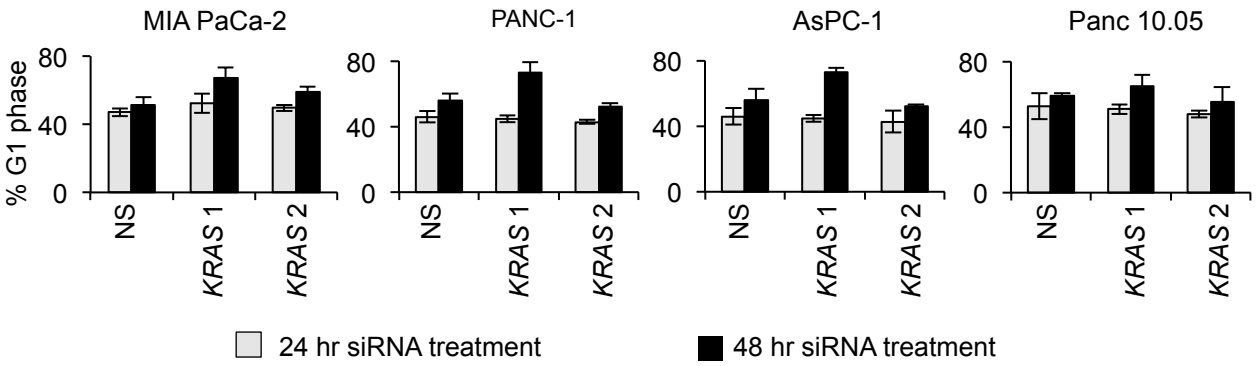


Figure S2. KRAS Regulation of MYC Protein Expression, Related to Figure 2

(A) KRAS WT BxPC-3 cells were transfected with NS or two independent *KRAS* (1 and 2) siRNAs. After the indicated times, the cultures were evaluated by immunoblotting to monitor changes in MYC and KRAS4B protein levels. Immunoblotting for phosphorylated and total ERK1/2 was done to verify loss of KRAS effector signaling, with β-actin done to verify equivalent loading of total protein.

(B) To measure *MYC* gene expression, the indicated PDAC cells were transfected with NS or *KRAS* siRNAs for 24 and 48 hr. RNA was extracted and following cDNA synthesis, real-time qPCR was performed using two sets of *MYC* specific primers (*MYC* A and *MYC* B). *MYC* mRNA expression levels were normalized to *GAPDH* mRNA levels.

(C) Linear regression graph showing quantitation of immunoblot data in panels 2F and S2C, for MYC protein remaining after initiation of CHX treatment (chase). Lower panel, MYC half-life in min ($t_{1/2}$ (min)) was calculated for each cell line using GraphPad Prism.

(D) PDAC cell lines were transfected with NS or two independent *KRAS* siRNAs and cell cycle distribution was measured by propidium iodide staining at 24 or 48 hr post-transfection.

(E) Bar-graph summary of data in panel C. Data are presented as a mean of three biological replicates, error bars represent standard error of the mean.

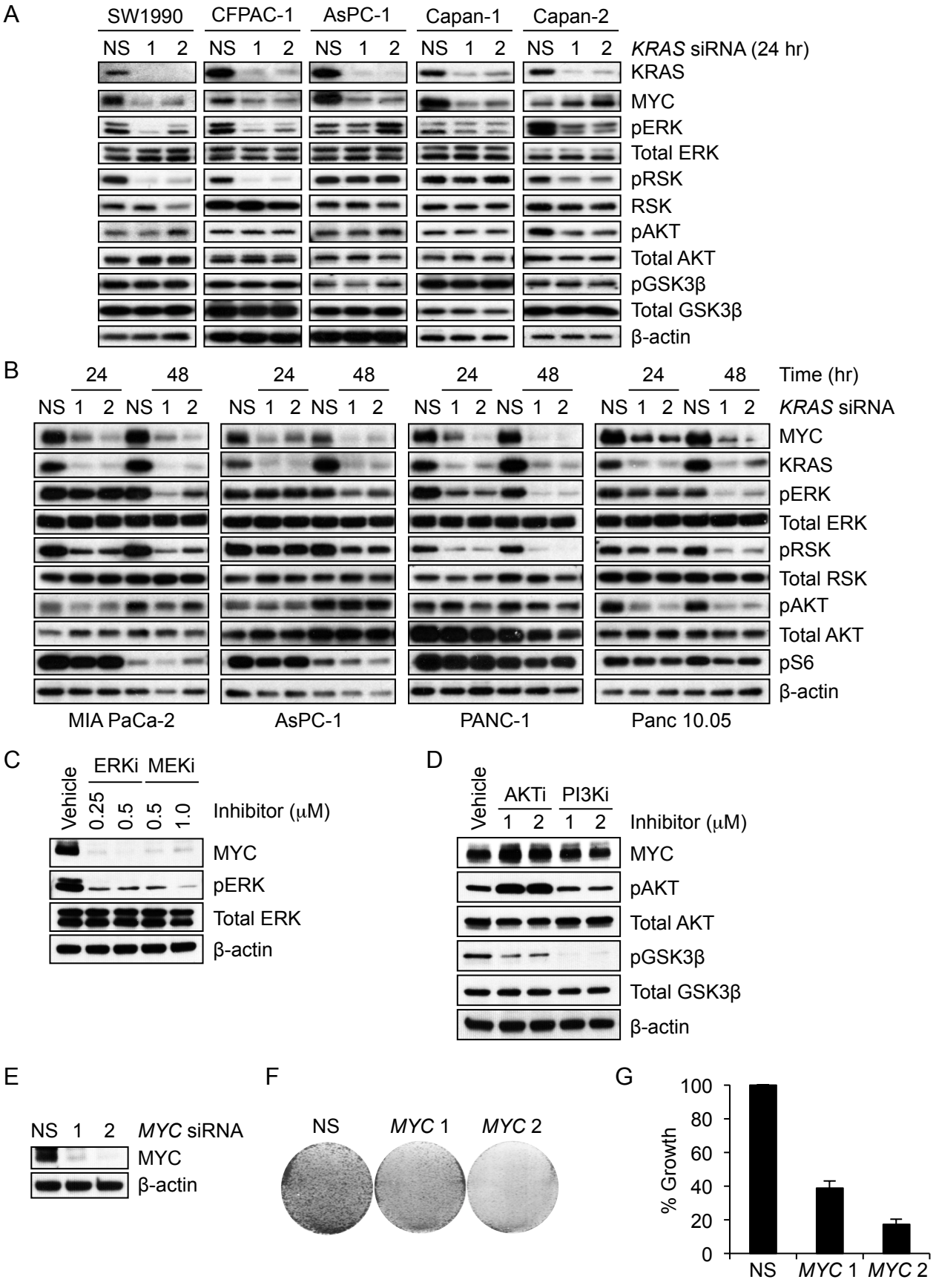

A
SW1990
CFPAC-1
AsPC-1
Capan-1
Capan-2
NS 1 2
KRAS siRNA (24 hr)
KRAS
MYC
pERK
Total ERK
pRSK
RSK
pAKT
Total AKT
pGSK3β
Total GSK3β
β-actin
B
24
48
Time (hr)
KRAS siRNA
MYC
KRAS
pERK
Total ERK
pRSK
Total RSK
pAKT
Total AKT
pS6
β-actin
MIA PaCa-2
AsPC-1
PANC-1
Panc 10.05
C
Vehicle
ERKi
MEKi
0.25
0.5
0.5
1.0
Inhibitor (μM)
MYC
pERK
Total ERK
β-actin
D
Vehicle
AKTi
PI3Ki
1 2 1 2
Inhibitor (μM)
MYC
pAKT
Total AKT
pGSK3β
Total GSK3β
β-actin
E
NS 1 2
MYC siRNA
MYC
β-actin
F
NS
MYC 1
MYC 2
G
% Growth
100
80
60
40
20
0
NS
MYC 1
MYC 2

Figure S3. KRAS Regulation of MYC Expression is Dependent on MEK-ERK1/2, but Not PI3K-AKT-GSK3 Signaling, Related to Figure 3

(A) PDAC cell lines were transfected with NS or *KRAS* siRNA (24 hr). Immunoblotting of cell lysates was done to determine total KRAS and MYC, phosphorylated ERK1/2 (pERK) and its substrate p90RSK (pRSK), and phosphorylated AKT (pAKT) and its substrate GSK3β (pGSK3β). Levels of total ERK, RSK, AKT and GSK3β were monitored to ascertain that changes in phosphorylated proteins were not due simply to changes in total proteins.

(B) PDAC cell lines were transfected with NS or *KRAS* siRNA for 24 or 48 hr. Immunoblotting of cell lysates were done to determine the levels of total KRAS or MYC and level of phosphorylated ERK1/2 (pERK), AKT (pAKT), p90RSK (pRSK), GSK-3β (pGSK-3β).

(C) BxPC3 cells were treated with ERK1/2 inhibitor SCH772984 (ERKi) or MEK inhibitor AZD6244 (MEKi) for 24 hr and cell lysates were immunoblotted with the indicated antibodies.

(D) BxPC-3 cells were treated with AKT inhibitor AZD5363 (AKTi) or PI3K inhibitor AZD8186 (PI3Ki) for 24 hr and cell lysates were immunoblotted with the indicated antibodies.

(E) BxPC-3 cells were transiently transfected (24 hr) with NS or siRNA oligos targeting two distinct *MYC* sequences (1 and 2). Immunoblotting with anti-MYC antibody was done to monitor MYC levels and with anti-β-actin antibody to verify equivalent loading of total cellular protein.

(F) Approximately 10 days after plating, representative plates from panel E were stained with crystal violet to visualize colonies of proliferating cells.

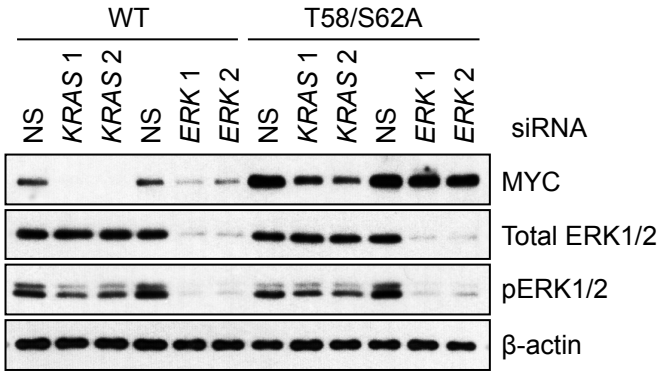
(G) Quantitation of data in panel F. The percentage of colony formation relative to NS was determined for BxPC-3 cells transfected with *MYC* siRNA. Data are presented as a mean of three biological replicates, error bars represent standard error of the mean.

Table S1. MYC and Effector Activation in PDAC tumors, Related to Figure 3

			pAKT	MYC	pERK
Spearman's rho	pAKT	r_s	1.000	0.120	-0.054
		p (2-tailed)		0.193	0.556
	MYC	r_s	0.120	1.000	0.485**
		p (2-tailed)	0.193		<0.0001
	pERK	r_s	-0.054	0.485**	1.000
		p (2-tailed)	0.556	<0.0001	

**Correlation is significant at the 0.01 level (2-tailed)

A



B

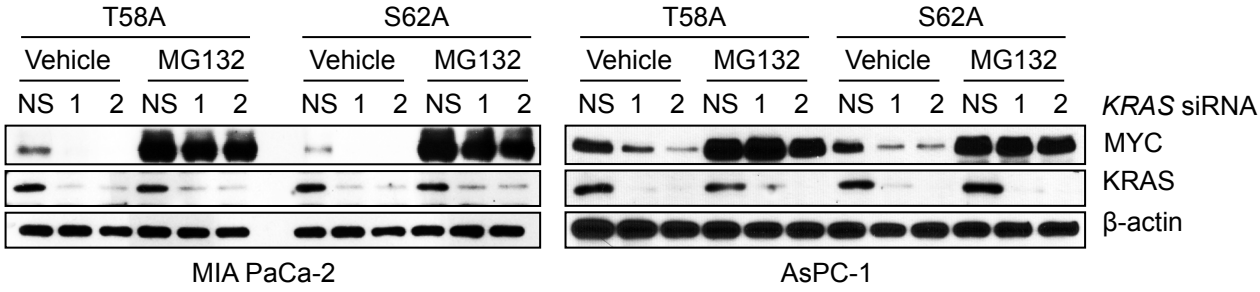


Figure S4. KRAS Regulates MYC Protein Stability Independently of MYC Residues T58 and S62, Related to Figure 4

(A) MIA PaCa-2 cells stably expressing FLAG epitope-tagged WT or phospho-deficient mutants of MYC (T58A, S62A, T58/S62A) were transiently transfected with NS or siRNA targeting KRAS or ERK1/2. After 24 hr, cell lysates were immunoblotted with the indicated antibodies.

(B) PDAC cell lines stably expressing FLAG epitope-tagged phospho-deficient MYC were transiently transfected with control NS siRNA or siRNA oligos targeting *KRAS* (1 and 2) for 20 hr. After treatment with vehicle control (DMSO) or 5 μM proteasome inhibitor MG132 for an additional 6 hr, cell lysates were immunoblotted with the indicated antibodies.

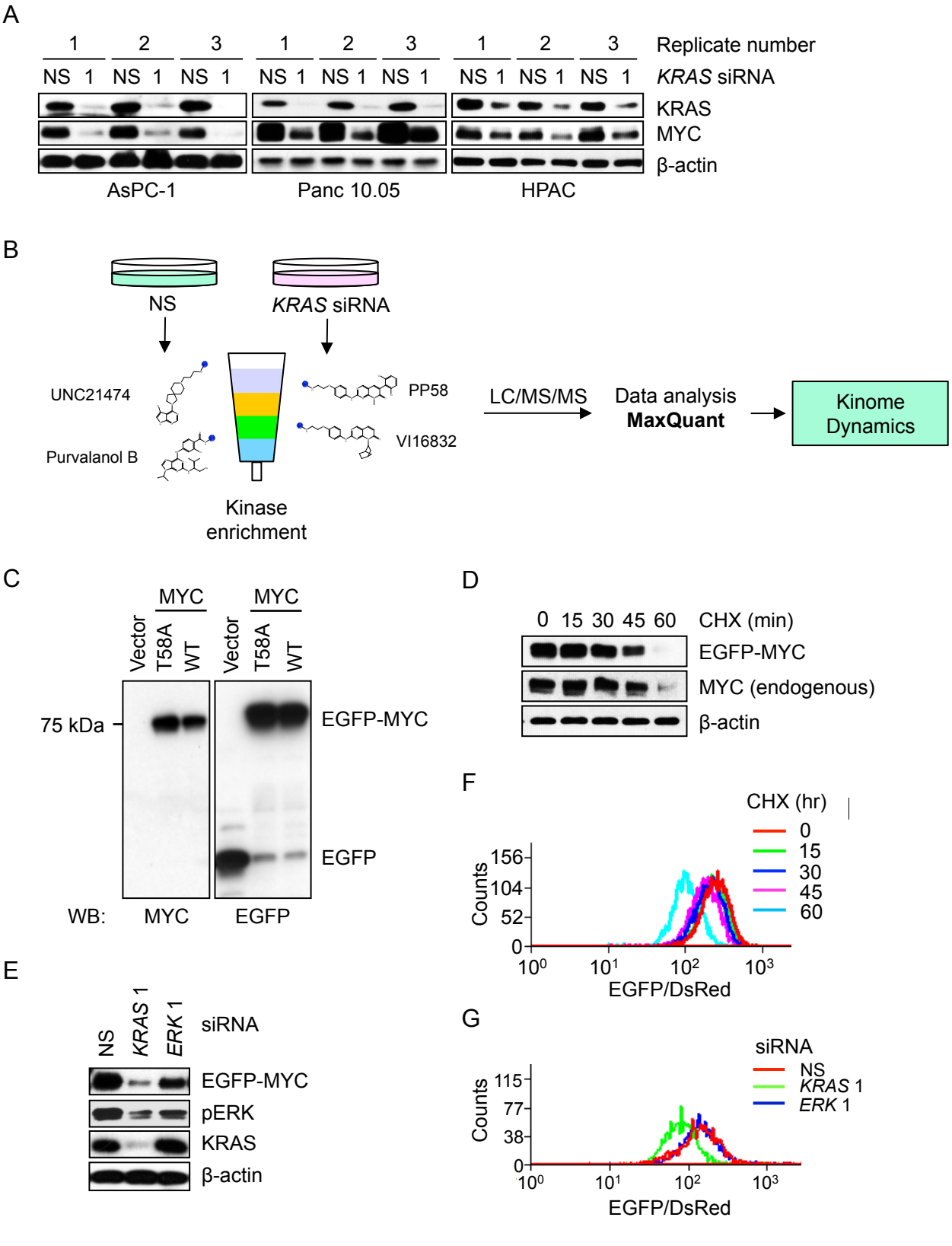

A
1 2 3 1 2 3 1 2 3
Replicate number
NS 1 NS 1 NS 1 NS 1 NS 1 NS 1 NS 1 NS 1 NS 1
KRAS siRNA
KRAS
MYC
β-actin
AsPC-1
Panc 10.05
HPAC
B
NS
KRAS siRNA
UNC21474
Purvalanol B
PP58
VI16832
Kinase enrichment
LC/MS/MS
Data analysis
MaxQuant
Kinome Dynamics
C
MYC
MYC
Vector
T58A
WT
Vector
T58A
WT
75 kDa
EGFP-MYC
EGFP
WB:
MYC
EGFP
D
0 15 30 45 60 CHX (min)
EGFP-MYC
MYC (endogenous)
β-actin
F
CHX (hr)
0
15
30
45
60
Counts
156
104
52
0
10^0
10^1
10^2
10^3
EGFP/DsRed
E
NS
KRAS 1
ERK 1
siRNA
EGFP-MYC
pERK
KRAS
β-actin
G
siRNA
NS
KRAS 1
ERK 1
Counts
115
77
38
0
10^0
10^1
10^2
10^3
EGFP/DsRed

Figure S5. Assays to Identify Protein Kinases that Regulate MYC Protein Degradation, Related to Figure 5

(A) Immunoblot of three biological replicate samples of PDAC cells used for multiplexed inhibitor beads (MIB)/mass spectroscopy (MS) analysis in Figure 5B. Lysates from cells transfected with NS or *KRAS* siRNA for 24 hr were immunoblotted to verify loss of KRAS and MYC, then subjected to MIB/MS analysis.

(B) Workflow of MIB/MS analysis to profile kinome dynamics upon KRAS knockdown in PDAC cells. Total cell lysates were prepared from PDAC cells transfected with NS or *KRAS* siRNA for 24 hr, then subjected to affinity capture of endogenous kinases, followed by quantitative mass spectrometry to measure kinome activity profiles.

(C) Cell lysates from MIA PaCa-2 cells stably expressing GPS-MYC reporter were used for immunoblotting with anti-MYC or GFP antibodies.

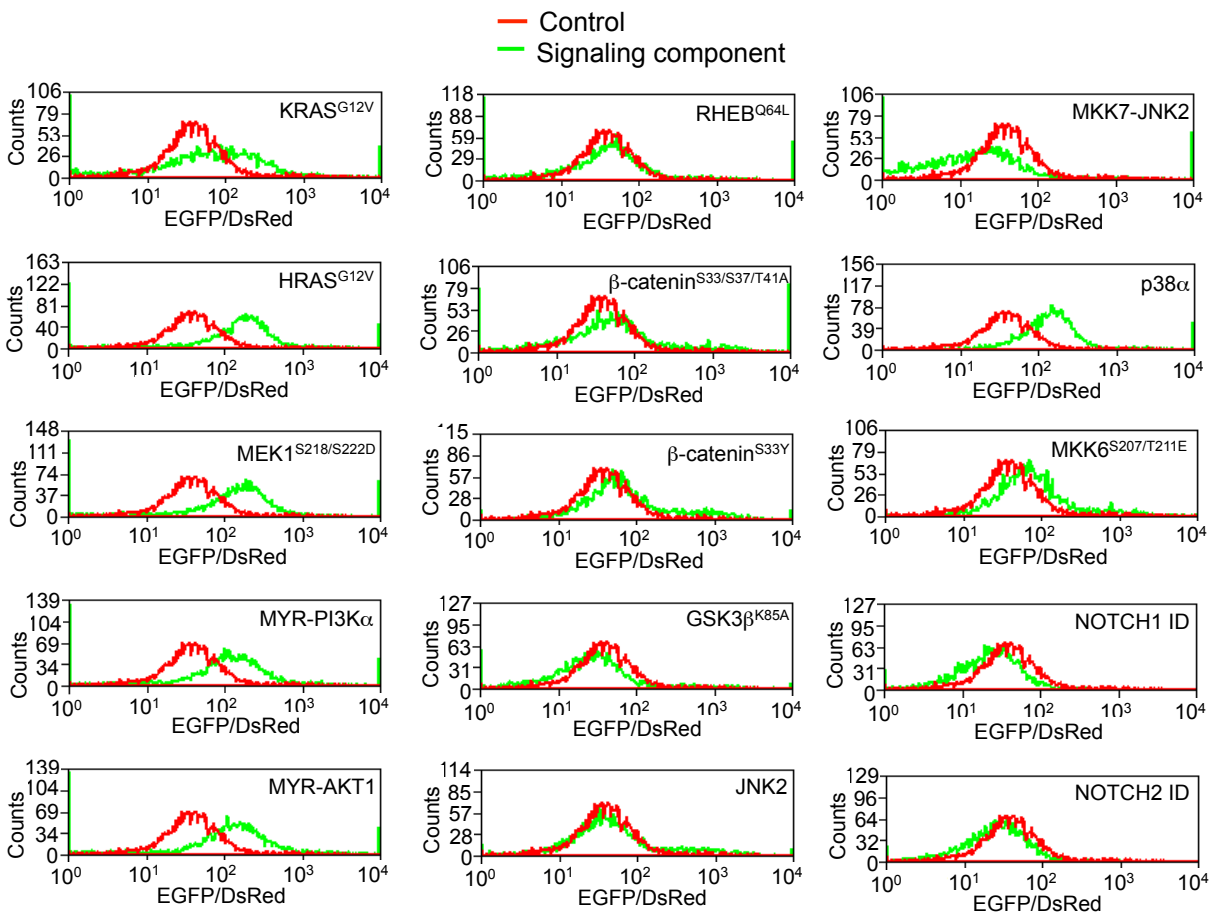
(D) MIA PaCa-2 cells stably expressing GPS-MYC were treated with CHX for the indicated times. Cell lysates were then immunoblotted with anti-MYC antibody.

(E) MIA PaCa-2 cells stably expressing GPS-MYC were transfected with NS or siRNA targeting *KRAS* or *ERK1/2* for 24 hr and cell lysates were immunoblotted with anti-MYC antibody.

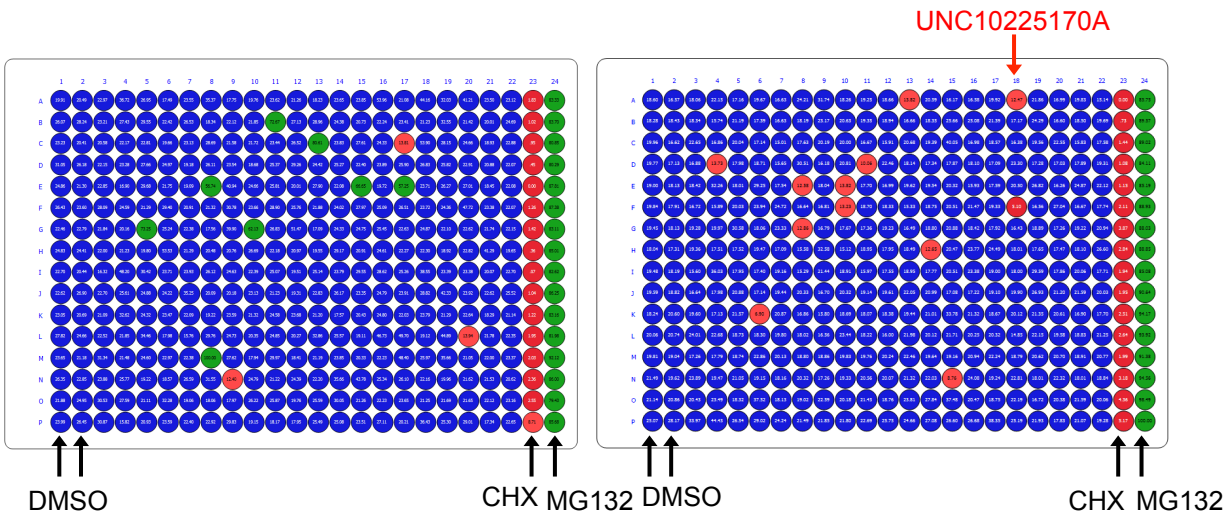
(F) EGFP/dsRed ratio histograms of MIA PaCa-2 cells stably expressing GPS-MYC, treated with CHX for the indicated times. EGFP and DsRed fluorescence was measured by FACS. Data were plotted using Summit 5.2.

(G) MIA PaCa-2 cells stably expressing GPS-MYC were transiently transfected with NS or siRNA targeting *KRAS* or *ERK1/2*. After 24 hr EGFP and DsRed fluorescence was measured by FACS to generate the EGFP/DsRed ratio histograms. Data were plotted using Summit 5.2.

A



B



Red: MYC loss (reduced EGFP/DsRed ratio)
Blue: Neutral (control EGFP/DsRed ratio)
Green: MYC stabilization (increased EGFP/DsRed ratio)

C

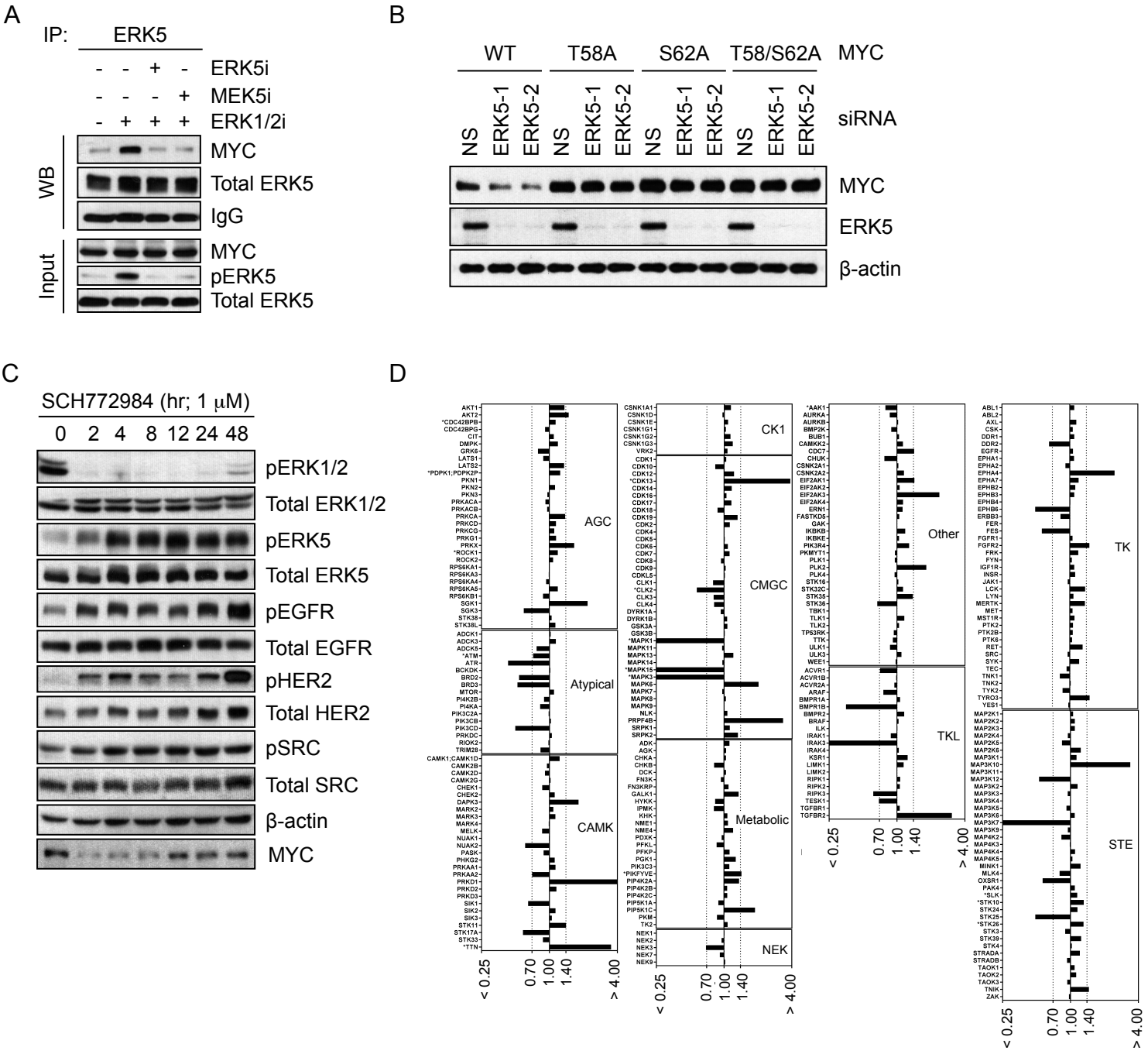
Protein	Kd (nM)
MEK5	11
MEK1	100
MEK2	270
RIPK2	440

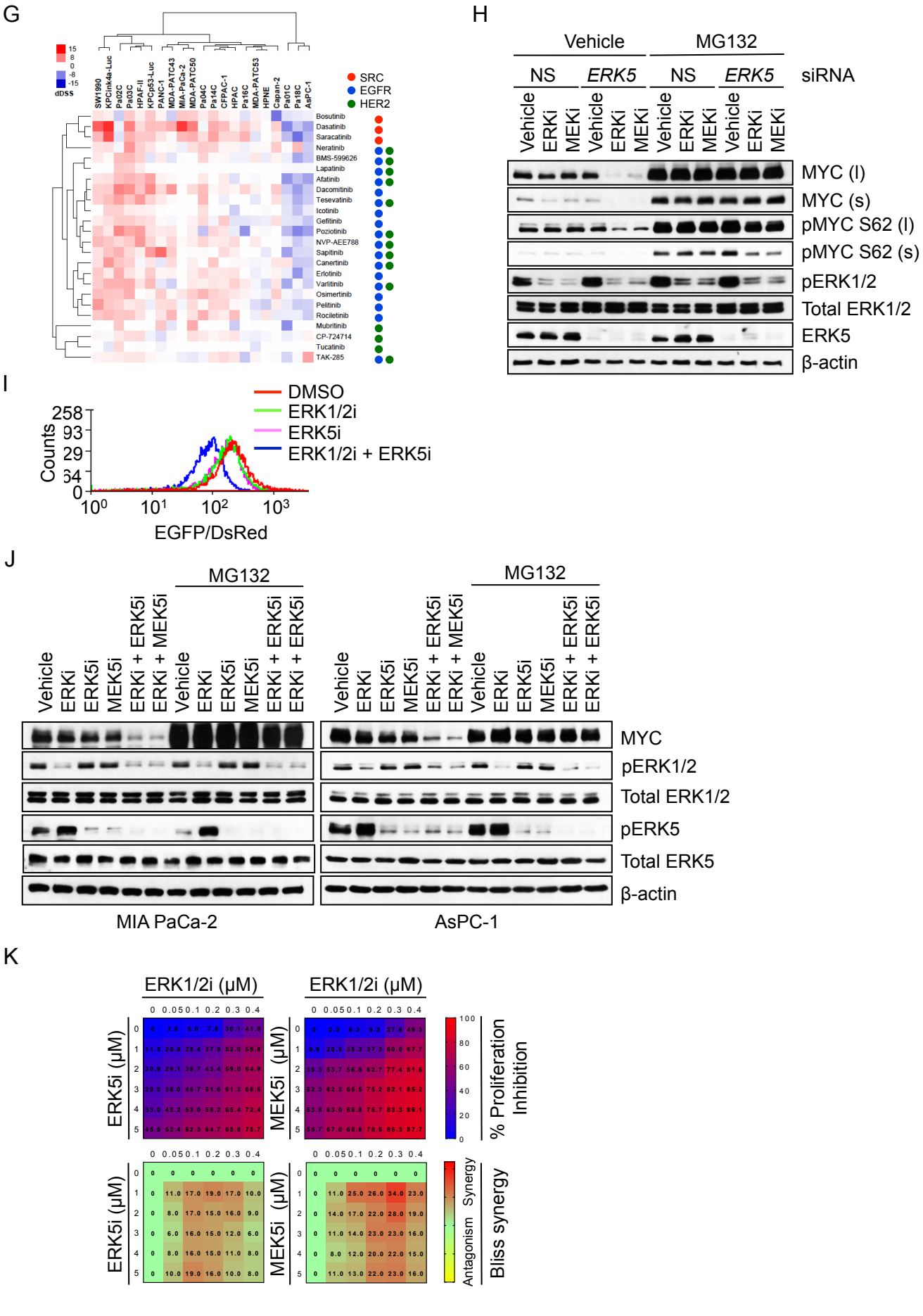
Figure S6. Identification of Regulators of MYC Protein Stability, Related to Figure 6

(A) MIA PaCa-2 cells stably expressing GPS-MYC were infected with control (red) or lentivirus expression vectors encoding the indicated protein (green). EGFP and DsRed fluorescence was measured by FACS to generate the EGFP/DsRed ratio histograms. Data were plotted using Summit 5.2.

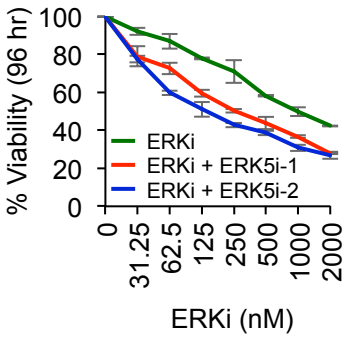
(B) EGFP/DsRed ratio heat maps of 384-well plates from a high-throughput flow-cytometry-based screen with the Published Kinase Inhibitor Set (PKIS) of 843 compounds. MIA PaCa-2 cells stably expressing GPS-MYC were plated in 384-well plates using robotic liquid handling, next day washed and treated with PKIS for 4 hr and analyzed with Intellicyte iQue high throughput flow cytometer. Shown are heat maps of two plates treated with two different sets of PKIS. Treatment with vehicle (DMSO), MG132 (increased GPS-MYC expression) and CHX (decreased GPS-MYC expression) treatments served as internal controls.

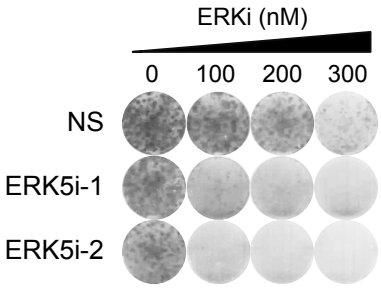
(C) UNC10225170A activity against the top kinases from a total of 392 wild-type human kinases evaluated. Compiled from Supplementary Table 3 (Drewry et al., 2017).


A
IP: ERK5
- - + - ERK5i
- - - + MEK5i
- + + + ERK1/2i
WB
MYC
Total ERK5
IgG
Input
MYC
pERK5
Total ERK5
B
WT
T58A
S62A
T58/S62A
MYC
NS
ERK5-1
ERK5-2
siRNA
MYC
ERK5
β-actin
C
SCH772984 (hr; 1 μM)
0 2 4 8 12 24 48
pERK1/2
Total ERK1/2
pERK5
Total ERK5
pEGFR
Total EGFR
pHER2
Total HER2
pSRC
Total SRC
β-actin
MYC
D
AGC
Atypical
CAMK
CK1
CMGC
Metabolic
NEK
Other
TKL
TK
STE
< 0.25
0.70
1.00
1.40
> 4.00
E
1 2
Replicate #
- + - + - +
ERK1/2i (1 hr)
MYC
pERK
Toal ERK1/2
β-actin
F
ERK1/2i
vehicle
Vehicle
Poziotinib
Erlotinib
Saracatinib
LY2874455
pERK5
Total ERK5
pERK1/2
Total ERK1/2

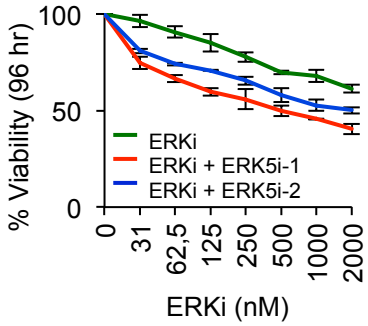

G
dDSS
SW1990
KPCinK4a-Luc
Pa02C
Pa03C
HPAF-II
KPCp53-Luc
PANC-1
MDA-PATC43
MIA-PaCa-2
MDA-PATC50
Pa04C
Pa14C
CFPAC-1
HPAC
Pa16C
MDA-PATC53
HPNE
Capan-2
Pa01C
Pa18C
AsPC-1
SRC
EGFR
HER2
Bosutinib
Dasatinib
Saracatinib
Neratinib
BMS-599626
Lapatinib
Afatinib
Dacomitinib
Tesevatinib
Icotinib
Gefitinib
Poziotinib
NVP-AEE788
Sapitinib
Canertinib
Erlotinib
Varlitinib
Osimertinib
Pelitinib
Rociletinib
Mubritinib
CP-724714
Tucatinib
TAK-285
H
Vehicle
MG132
NS
ERK5
siRNA
Vehicle
ERKi
MEKi
MYC (l)
MYC (s)
pMYC S62 (l)
pMYC S62 (s)
pERK1/2
Total ERK1/2
ERK5
β-actin
I
DMSO
ERK1/2i
ERK5i
ERK1/2i + ERK5i
Counts
EGFP/DsRed
J
MG132
Vehicle
ERKi
ERK5i
MEK5i
ERKi + ERK5i
ERKi + MEK5i
MYC
pERK1/2
Total ERK1/2
pERK5
Total ERK5
β-actin
MIA PaCa-2
AsPC-1
K
ERK1/2i (μM)
ERK5i (μM)
MEK5i (μM)
% Proliferation Inhibition
Bliss synergy
Synergy
Antagonism

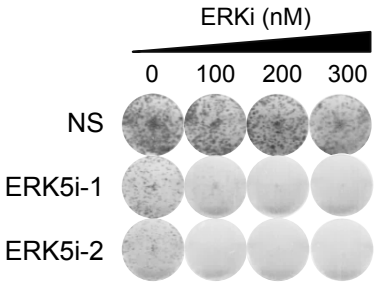
L



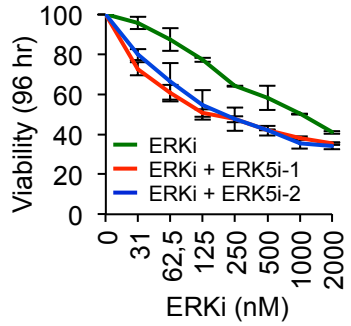


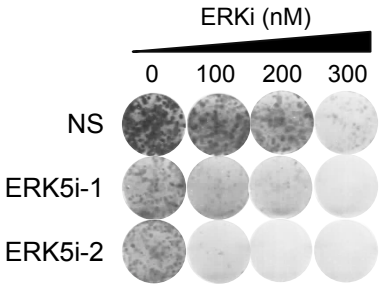
MIA PaCa-2	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i-1	0.1
ERKi + ERK5i-2	0.08



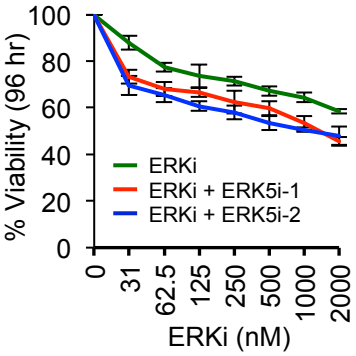


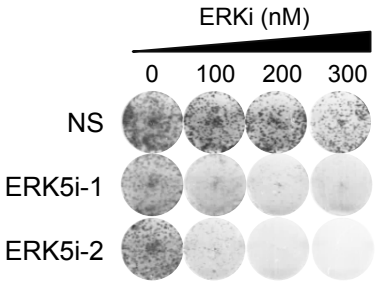
AsPC-1	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i-1	0.5
ERKi + ERK5i-2	0.7





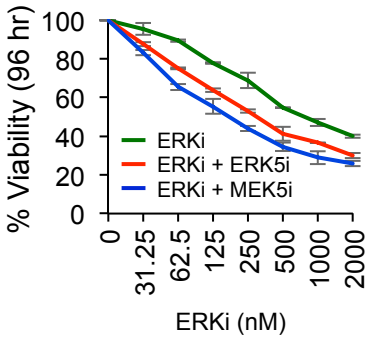
Panc 10.05	
Treatment	GI_{50} (μM)
ERKi	0.8
ERKi + ERK5i-1	0.09
ERKi + ERK5i-2	0.089

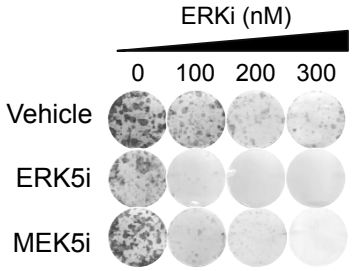




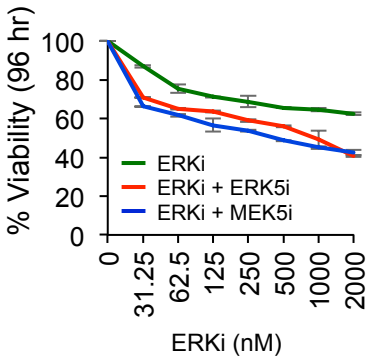
SW1990	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i-1	1
ERKi + ERK5i-2	0.21

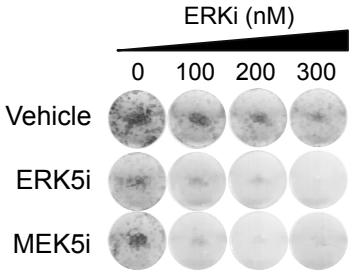
M



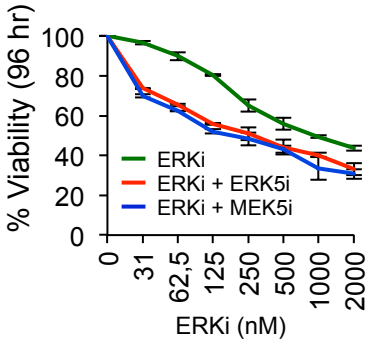


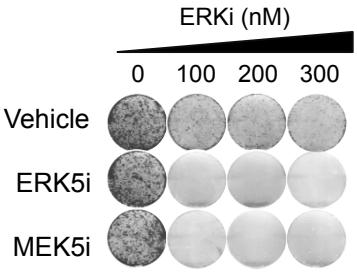
MIA PaCa-2	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	0.47
ERKi + MEK5i	0.21



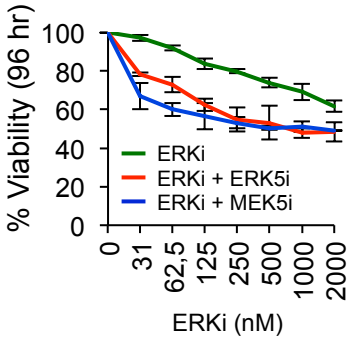


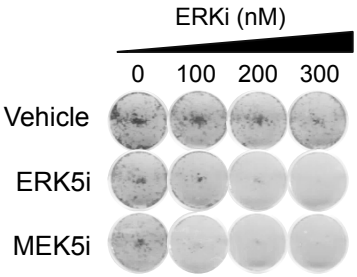
AsPC-1	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	>2
ERKi + MEK5i	0.2





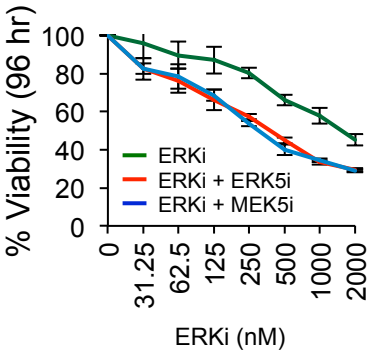
Panc 10.05	
Treatment	GI_{50} (μM)
ERKi	0.8
ERKi + ERK5i	0.28
ERKi + MEK5i	0.23

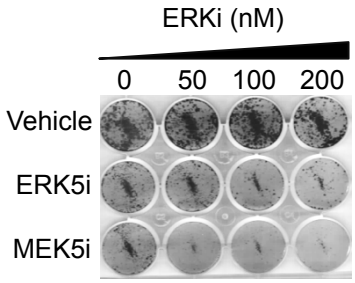




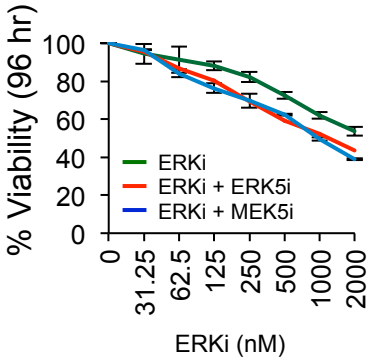
SW1990	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	0.08
ERKi + MEK5i	>2

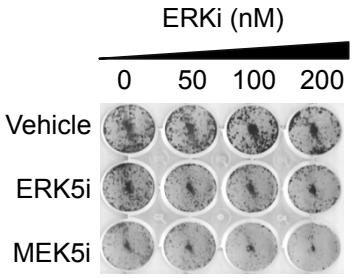
N



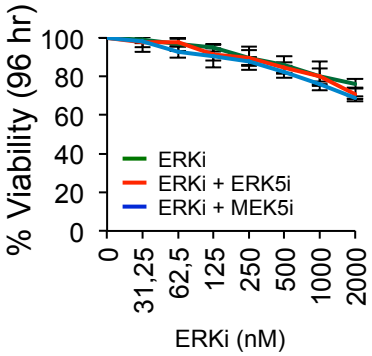


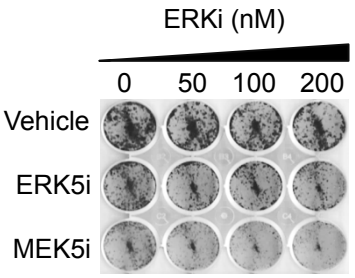
MIA PaCa-2 (Empty vector)	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	0.12
ERKi + MEK5i	0.082



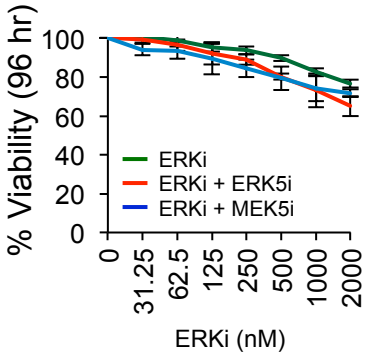


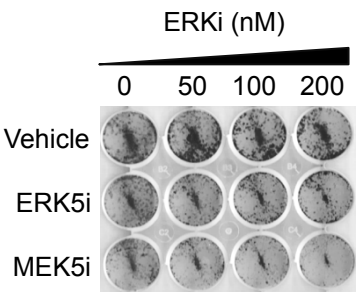
MIA PaCa-2 (MYC WT)	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	>2
ERKi + MEK5i	0.09



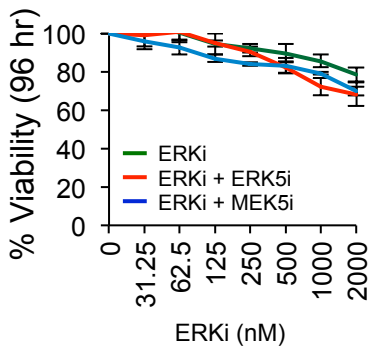


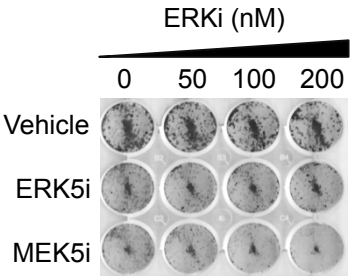
MIA PaCa-2 (MYC S62A)	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	>2
ERKi + MEK5i	>2





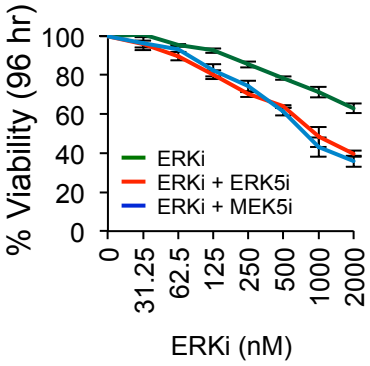
MIA PaCa-2 (MYC T58A)	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	>2
ERKi + MEK5i	>2

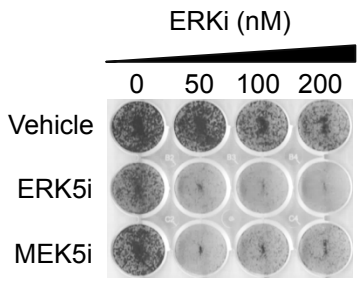




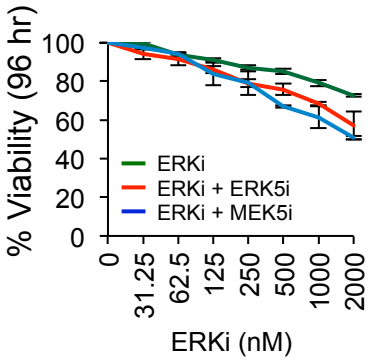
MIA PaCa-2 (MYC T58/S62A)	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	1.17
ERKi + MEK5i	0.13

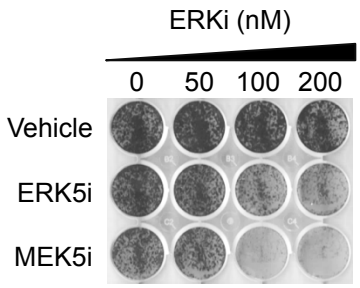
O



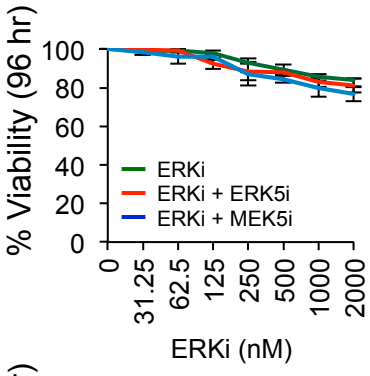


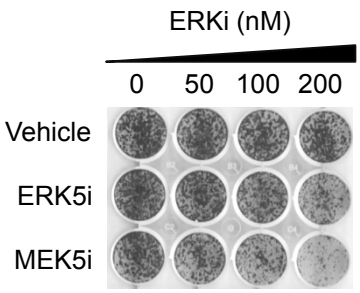
AsPC-1 (Empty vector)	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	0.46
ERKi + MEK5i	0.38



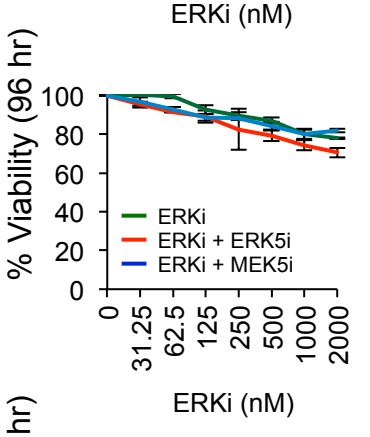


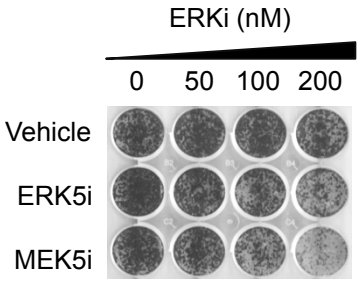
AsPC-1 (MYC WT)	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	>2
ERKi + MEK5i	0.70



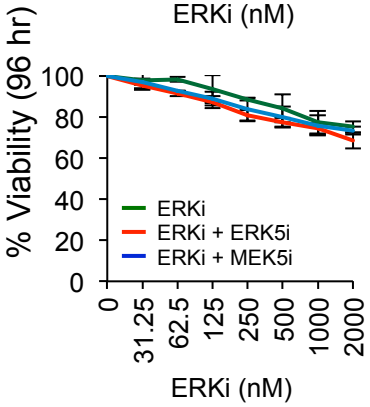


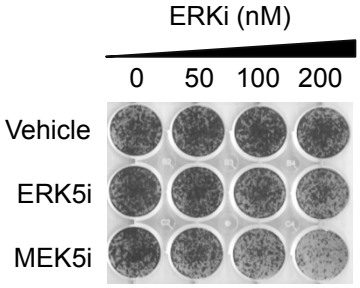
AsPC-1 (MYC S62A)	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	>2
ERKi + MEK5i	>2





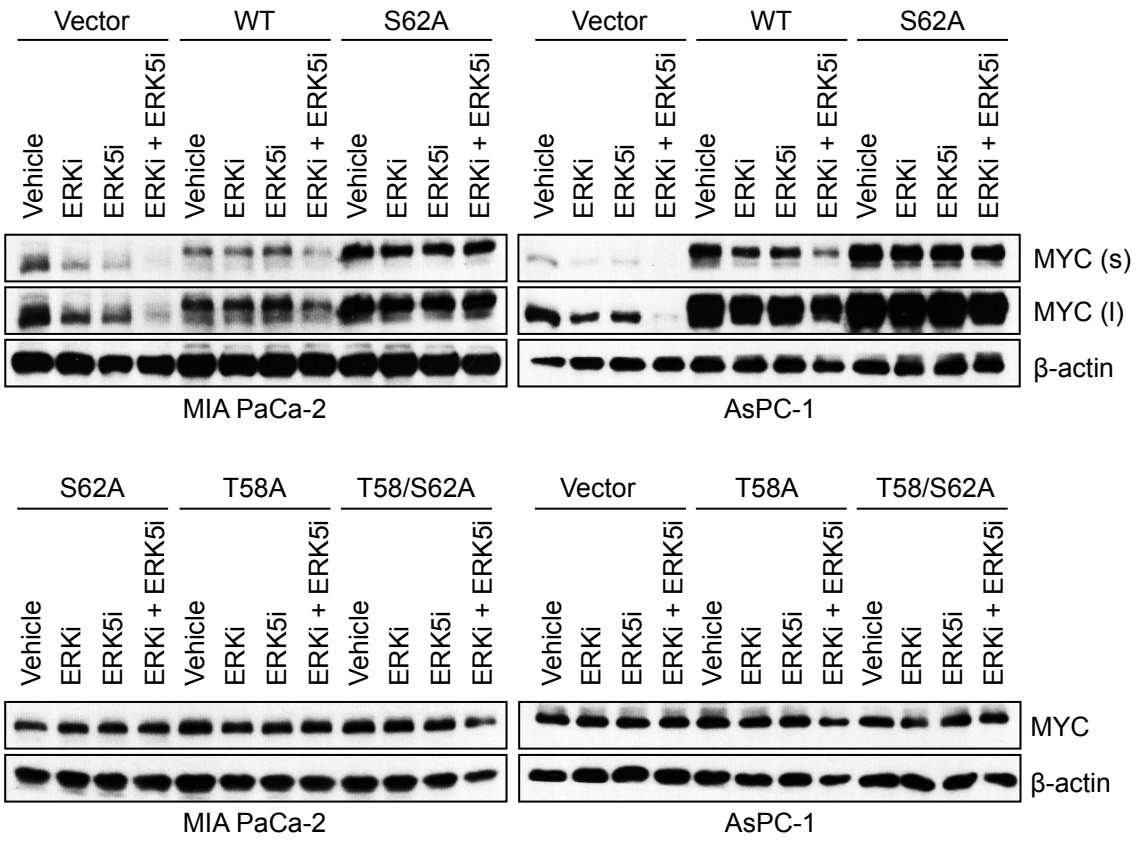
AsPC-1 (MYC T58A)	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	>2
ERKi + MEK5i	>2



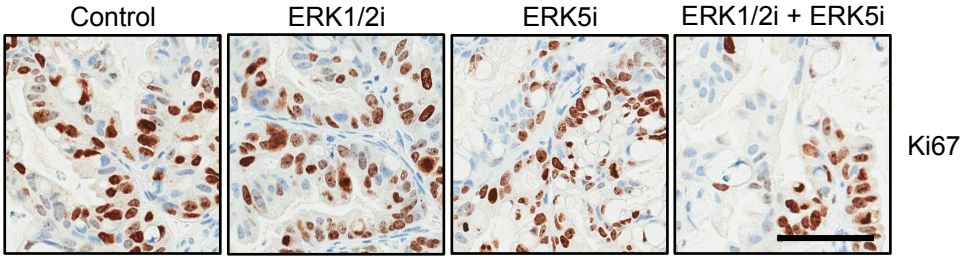


ASPC-1 (MYC S62A/T58A)	
Treatment	GI_{50} (μM)
ERKi	>2
ERKi + ERK5i	>2
ERKi + MEK5i	>2

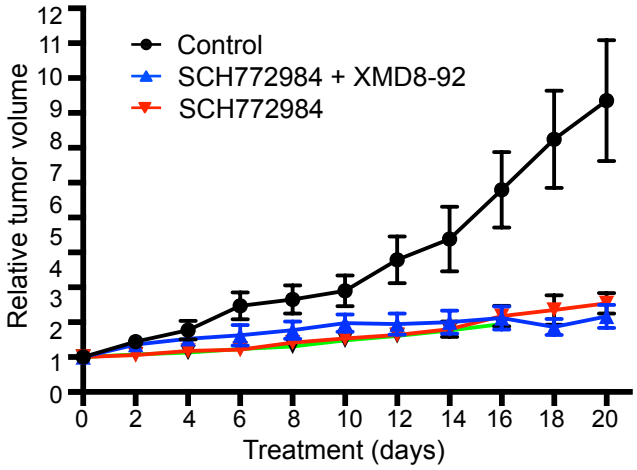
P

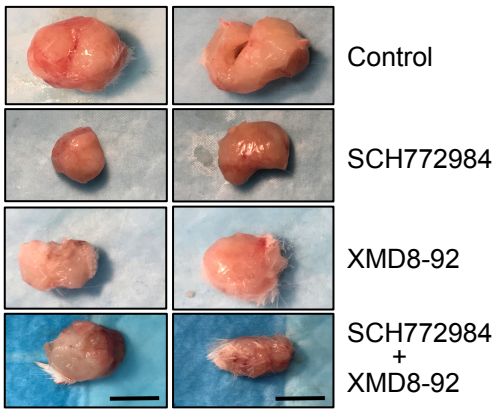

Vector
WT
S62A
Vehicle
ERKi
ERK5i
ERKi + ERK5i
MYC (s)
MYC (l)
β-actin
MIA PaCa-2
AsPC-1
T58A
T58/S62A
MYC

Q

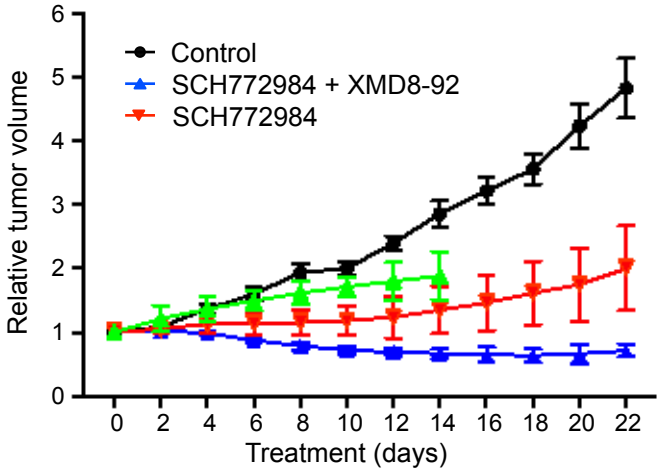

Control
ERK1/2i
ERK5i
ERK1/2i + ERK5i
Ki67

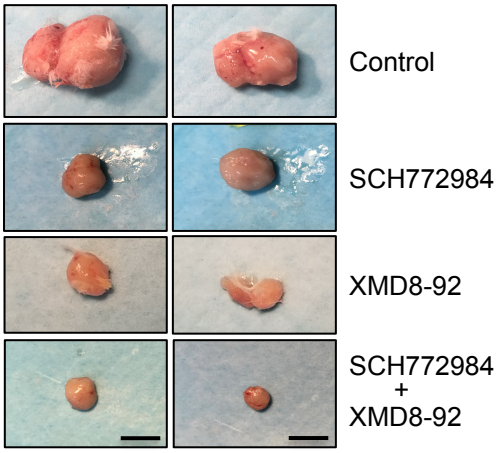
R


Relative tumor volume
Treatment (days)
Control
SCH772984 + XMD8-92
SCH772984

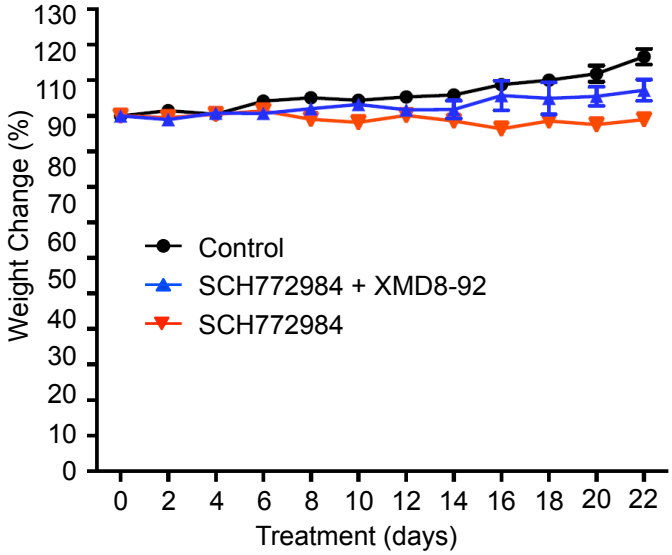

Control
SCH772984
XMD8-92
SCH772984
+
XMD8-92

S


Relative tumor volume
Treatment (days)
Control
SCH772984 + XMD8-92
SCH772984


Control
SCH772984
XMD8-92
SCH772984
+
XMD8-92

T


Weight Change (%)
Treatment (days)
Control
SCH772984 + XMD8-92
SCH772984

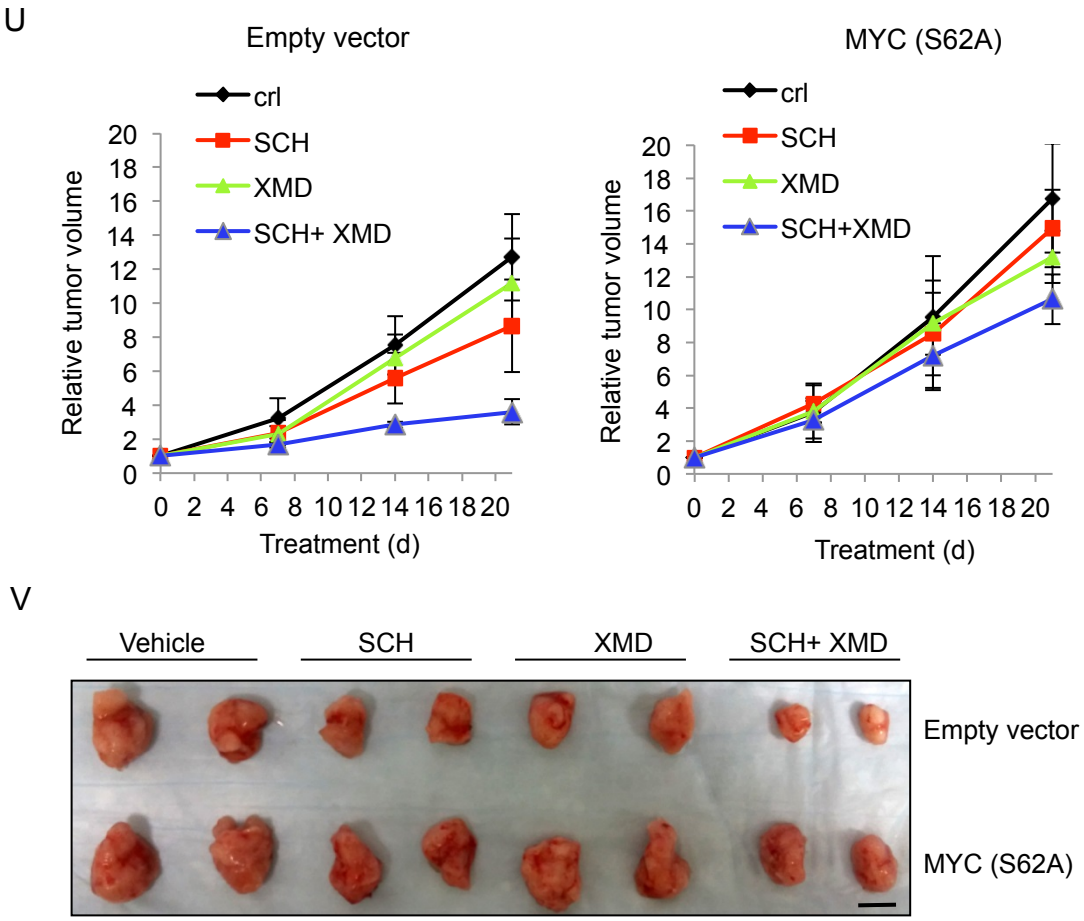


Figure S7. EGFR/SRC-Dependent ERK5 Compensatory Mechanism Regulates MYC Protein Stability, Related to Figure 7.

(A) MIA PaCa-2 cells were treated with the ERK1/2i SCH772984 (100 nM), together with vehicle (DMSO), ERK5i XMD8-92 (2 μM) or MEK5i BIX02188 (4 μM) for 10 hr. Six hr prior to lysis and immunoprecipitation, cells were treated with MG132. ERK5 complexes were isolated and analyzed as described in Figure 7A.

(B) MIA PaCa-2 cells stably expressing the indicated WT or phospho-deficient MYC-FLAG proteins were transfected with *ERK5* siRNAs for 48 hr and protein levels were detected by immunoblotting.

(C) AsPC-1 cells were treated with SCH772984 (ERK1/2i) for the indicated times. Cell lysates were immunoblotted for total or phospho-specific antibodies (pEGFR, Y1068; pHER2 Y1248; pSRC, Y416).

(D) Panc10.05 cells treated for 1 hr with 1 μM SCH772984 and then subjected to affinity capture of endogenous kinases, followed by quantitative mass spectrometry. The kinase abundance ratios (ERKi/DMSO control), as determined by LFQ quantitative analysis, were averaged from three independent experiments (left panel). A ratio ≤ 0.7 denotes decreased MIB binding while a ratio ≥ 1.4 denotes increased MIB binding.

(E) Immunoblotting with the indicated antibodies was done to assess ERK1/2 inhibition and MYC protein loss for the samples used in figure S7D.

(F) MIA PaCa-2 cells were treated for 12 hr with 200 nM ERK1/2i SCH772984 in the presence or absence of 500 nM inhibitors: pan-EGFRi poziotinib, EGFRi erlotinib, SRCi saracatinib or pan-FGFRi LY2874455. Immunoblotting of cell lysates was done with indicated antibodies to determine activation of pERK5 and pERK1/2.
(G) Human or mouse PDAC cell lines were exposed to dose-dependent drug sensitivity testing against 525 oncology-related compounds in the presence or absence of SCH772984 (100 nM). Cell viability was measured using CellTiter-Glo and drug responses were calculated as drug sensitivity scores (DSS). The deltaDSS values (DSS in the presence of ERK/MEK inhibitor – DSS in the absence of overlaid inhibitor) for each condition are plotted in the heatmap, where red signifies potential synergies and blue indicates negative interactions between the two drugs. Drugs where the deltaDSS remained between 5 and 5 for all four conditions were excluded from the heatmap.
(H) AsPC-1 cells were transiently transfected with NS or *ERK5* siRNA for 30 hr, then treated with vehicle (DMSO), SCH772984 (100 nM) or trametinib (2 nM) for an additional 8 hr. Where indicated, MG132 treatment was for 6 hr. Cell lysates were immunoblotted with the indicated antibodies. MYC phosphorylated at residue S62 (pMYC S62) was detected with phospho-specific antibody. Short (s) and long (l) exposures are shown for phosphorylated and total MYC proteins.
(I) MIA PaCa-2 cells stably expressing the GPS-MYC reporter were treated with either SCH772984 (100 nM) or XMD8-92 (2 μM), or both, for 8 hr. EGFP and DsRed fluorescence was measured by FACS and EGFP/DsRed fluorescence ratio was plotted using Summit 5.2.
(J) PDAC cells were treated with SCH772984 (100 nM) or ERK5 inhibitors XMD8-92 (2 μM) and BIX02189 (4 μM) alone or in combination for 8 hr. Where indicated, MG132 treatment was for 6 hr. Blot analyses of total cell lysates were done with indicated antibodies.
(K) The indicated cell lines were treated with increasing doses of SCH772984 in combination with increasing doses of either XMD8-92 (ERK5i) or BIX02189 (MEK5i) for 96 hr, and proliferation was monitored by the CellTiter-Blue Cell Viability Assay. Bliss scores were calculated using Combenefit and heatmaps were generated using Graphpad Prism 7.
(L) PDAC cells were transfected with NS or *ERK5* siRNA, plated in 96 or 6 well plates and treated with SCH772985 as indicated. Quantitation of cell viability in 96-well plates, 96 hr after SCH772985 treatments (left panel). Data are presented as the mean of three replicates, error bars represent standard error of the mean. Clonogenic survival visualized by crystal violet staining, 10 days after treatment, 12-well plates (middle panel). GI_{50} values were calculated using Graphpad Prism 7 (right panel).
(M) PDAC cell lines were treated with the indicated concentrations of SCH772984, together with vehicle (DMSO), XMD8-92 (2 μM), or BIX02189 (4 μM) for 96 hr. Quantitation of cell viability, 96 hr after treatments (left panel). Data are presented as the mean of three replicates, error bars represent standard error of the mean. Ten days after treatment, clonogenic growth was visualized by staining with crystal violet in 12-well plates (middle panel). GI_{50} values were calculated using Graphpad Prism 7 (right panel).
(N) MIA PaCa-2 cells stably expressing empty vector, FLAG epitope-tagged WT or phospho-deficient MYC mutants were treated with the indicated concentrations of SCH772984, together with vehicle (DMSO), XMD8-92 (2 μM), or BIX02189 (4 μM) for 96 hr. Quantitation of cell viability, 96 hr after treatments (left panel). Data are presented as the mean of three replicates, error bars represent standard error of the mean Ten days after treatment, clonogenic growth was visualized by staining with crystal violet in 12-well plates (middle panel). GI_{50} values were calculated using Graphpad Prism 7.

(O) AsPC-1 cells stably expressing empty vector, FLAG epitope-tagged WT or phospho-deficient MYC mutants were treated with the indicated concentrations of SCH772984, together with vehicle (DMSO), XMD8-92 (2 μM), or BIX02189 (4 μM) for 96 hr. Quantitation of cell viability 96 hr after treatments (left panel). Data are presented as the mean of three replicates, error bars represent standard error of the mean. Ten days after treatment, clonogenic growth was visualized by staining with crystal violet in 12-well plates (middle panel). GI50 values were calculated using Graphpad Prism 7.
(P) MIA PaCa-2 and AsPC-1 cells stably expressing empty vector, FLAG epitope-tagged WT or phospho-deficient MYC mutants were treated with SCH772984 (100 nM) or ERK5 inhibitor XMD8-92 alone or in combination for 8 hr. Blot analyses of total cell lysates were done with indicated antibodies.
(Q) Ki67 immunohistochemical staining of representative EMC1222 tumors from Figure 7F. Scale bar= 100 μm.
(R-S) Immunocompromised (NSG) mice with the implanted *KRAS*-mutant PDX tumors AZ1013 (top) and AZ97 (bottom) were treated with SCH772984 (ERKi) alone or together with ERK5i XMD 8-92 for the indicated days (left panels). The relative tumor volume and standard error of the mean were graphed. Images of representative tumors are shown in the right panels. AZ97 PDX tumors have the following mutations: *KRAS*G12V, *TP53* p.R248Q, *SMAD4* p.Y131* and *CDKN2A*. AZ1013 PDX tumors have the following mutations: *KRAS* G12D, *TP53* R248W, *NF1* I1186N, *KMT2C* R4541*, *SMAD4* and *CDKN2A* deletions. Scale bar = 1 cm.
(T) Graph summarizing mouse weight change over days of drug treatment. The relative tumor volume and standard error of the mean were graphed.
(U) NOD *scid* mice inoculated subcutaneously with AsPC-1 cells stably infected with empty vector or encoding MYC S62A were treated with SCH772984 (ERKi) alone or together with XMD 8-92 (ERK5i) for the indicated days. The relative tumor volume and standard error of the mean were graphed.
(V) Images of representative tumors from Figure S7U. Scale bar =1 cm.

Table S2. Oligonucleotides, Related to Star Methods

NS siRNA oligo: 5'-XXXXXXXXXX-3'	N/A	Thermo Fisher Sci. Cat# 4390843
MYC siRNA oligo 1: 5'-XXXXXXXXXX-3'	AGACCUUCAUCAAAAACAUtt	Thermo Fisher Sci. Cat# 4392420 ID# s9129
MYC siRNA oligo 2: 5'-XXXXXXXXXX-3'	GAGCUAAAACGGAGCUUUUtt	Thermo Fisher Sci. Cat# 4392420 ID# s9130
Myc shRNA: 5'-XXXXXXXXXX-3'	GAUGAUGACCGAGUUACUU	ID# V3IMMMCG_12968867
NS shRNA:	N/A	Thermo Fisher Sci. Cat# VSC6584
KRAS siRNA oligo 1	CUAUGGUCCUAGUAGGAAAtt	Thermo Fisher Sci. Cat# 4390824 ID# s7939
KRAS siRNA oligo 2	GCCUUGACGAUACAGCUAAtt	Thermo Fisher Sci. Cat# 4390824 ID# s7940
MYC RNA primer A:	1214087 B2	ID# Hs00153408_m1
MYC RNA primer B:	1133740 A6	ID# Hs00153408_m1
GAPDH		
ERK1 siRNA oligo 1:	GGACCGGAUGUUAACCUUUtt	Thermo Fisher Sci. Cat# 4457298 ID# s11141
ERK1 siRNA oligo 2	UGAUGGAGACUGACCUGUAtt	Thermo Fisher Sci. Cat# 4392420 ID# s230179
ERK2 siRNA oligo 1	CAGGGUUCCUGACAGAAUAtt	Thermo Fisher Sci. Cat# 4390824 ID# s11137
ERK2 siRNA oligo 2:	CAACCAUCGAGCAAAUGAAtt	Thermo Fisher Sci. Cat# 4390824 ID# s11138
FBXW7 siRNA oligo 1:	GCAUAUGAUUUUAUGGUAAtt	Thermo Fisher Sci. Cat# 4392420 ID# s30663
FBXW7 siRNA oligo 2:	GGGUUGUUAGUGGAGCAUAtt	Thermo Fisher Sci. Cat# 4392420 ID# s30664
FBW7 RNA primer	1326276 B4	ID# Hs00217794_m1
ERK5 siRNA oligo 1:	GCUGAACAUCAGUACUUCAtt	Thermo Fisher Sci. Cat# 4390824 ID# s11149
ERK5 siRNA oligo 2:	GAGGAAUUCAUAAACCAGUtt	Thermo Fisher Sci. Cat# 4392420 ID# s11150
ERK5 siRNA oligo 3:	CCAUUGAUCUGACCCUGCAtt	Thermo Fisher Sci. Cat# 4392420 ID# s11151