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Supplemental Information

Synthetic Lethal Interaction of Combined BCL-XL and MEK Inhibition Promotes Tumor Regressions in KRAS Mutant Cancer Models

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Inventory of Supplemental Information

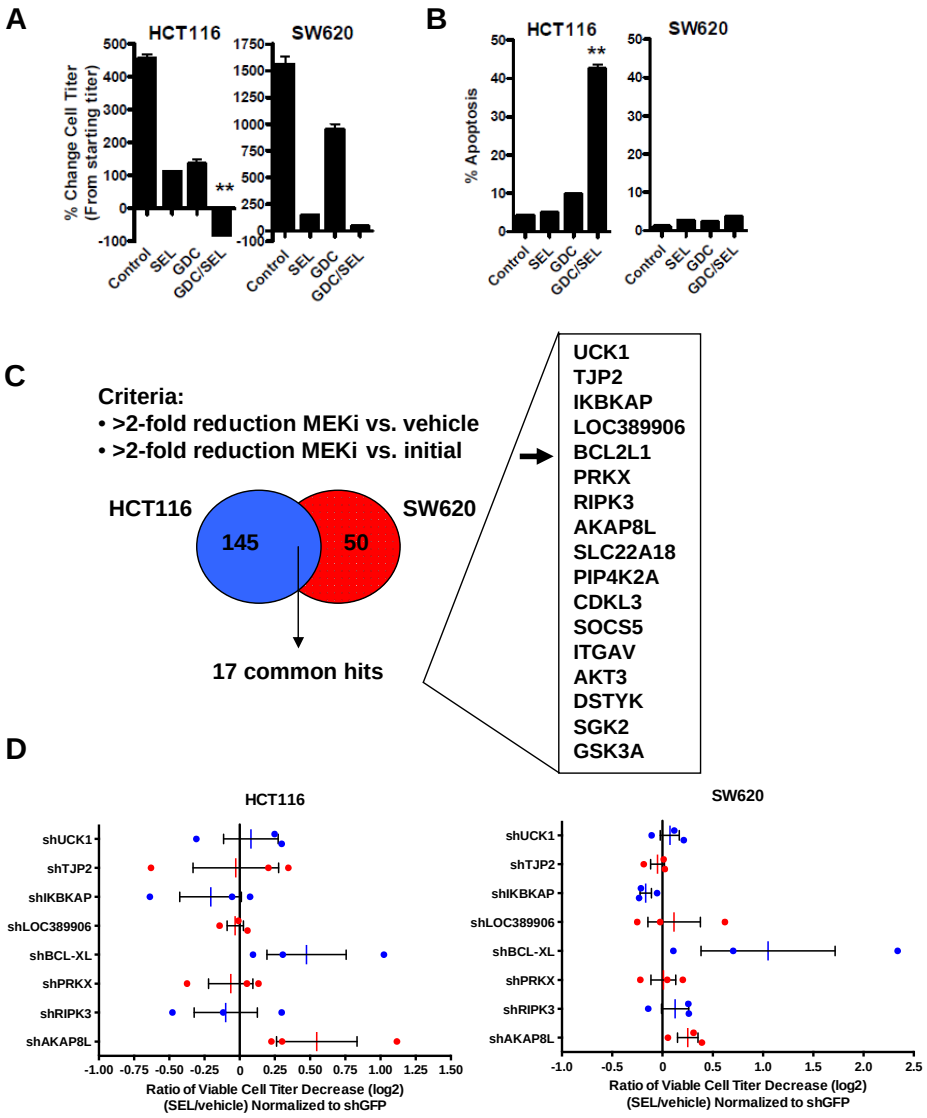

Figure S1 (related to Figure 1): Pooled shRNA-drug screen and validation studies. (A) HCT116 and SW620 cells were treated with vehicle (Control), 1μM selumetinib (SEL), 1μM of the PI3K inhibitor GDC0941 (GDC), or the combination for 72h and viable cell titer was determined by Cell TiterGlo Assay. Values represent the change in viable cell number relative to starting cell titer immediately before treatment. (B) Cells were treated as in (A) for 72 hours and the percentage of apoptotic cells was determined by Annexin V staining. Asterisks represent $p<0.001$ for the combination group vs. all other groups by one-way ANOVA with Tukey post-hoc test. (C) shRNA hits were selected using 2 criteria. A shRNA was scored as a hit if it showed: (1) a 2-fold or greater decrease in abundance in the MEK inhibitor-treated population (MEKi) vs. the vehicle-treated population, AND (2) a 2-fold or greater decrease in abundance in the MEK inhibitor-treated vs. the initial population. 145 hits were identified in HCT116 cells, and 50 hits were identified in SW620 cells. The 17 hits that were common to both cell lines are shown. *BCL-XL* (*BCL2L1*) is indicated with an arrow. (D) Validation studies for the

top 8 hits are shown. HCT116 and SW620 cells were infected with three independent shRNAs for each gene candidate. After 48 h of puromycin selection, cells were treated with vehicle or 1μM selumetinib for an additional 72 h, and cells were stained with crystal violet. Values shown represent the ratio of the fold-decrease in cell titer for each shRNA in the presence of selumetinib (relative to shGFP-infected cells treated with selumetinib) to the fold-decrease in cell titer in the presence of vehicle only (relative to shGFP-infected cells treated with vehicle only). Thus, shRNAs with values to the right of the y-axis produce a greater decrease in cell viability in the presence of selumetinib than in its absence. Each dot represents the mean value for each shRNA. Vertical hash marks represent the mean of all three shRNAs for a given gene target, and error bars represent SEM.

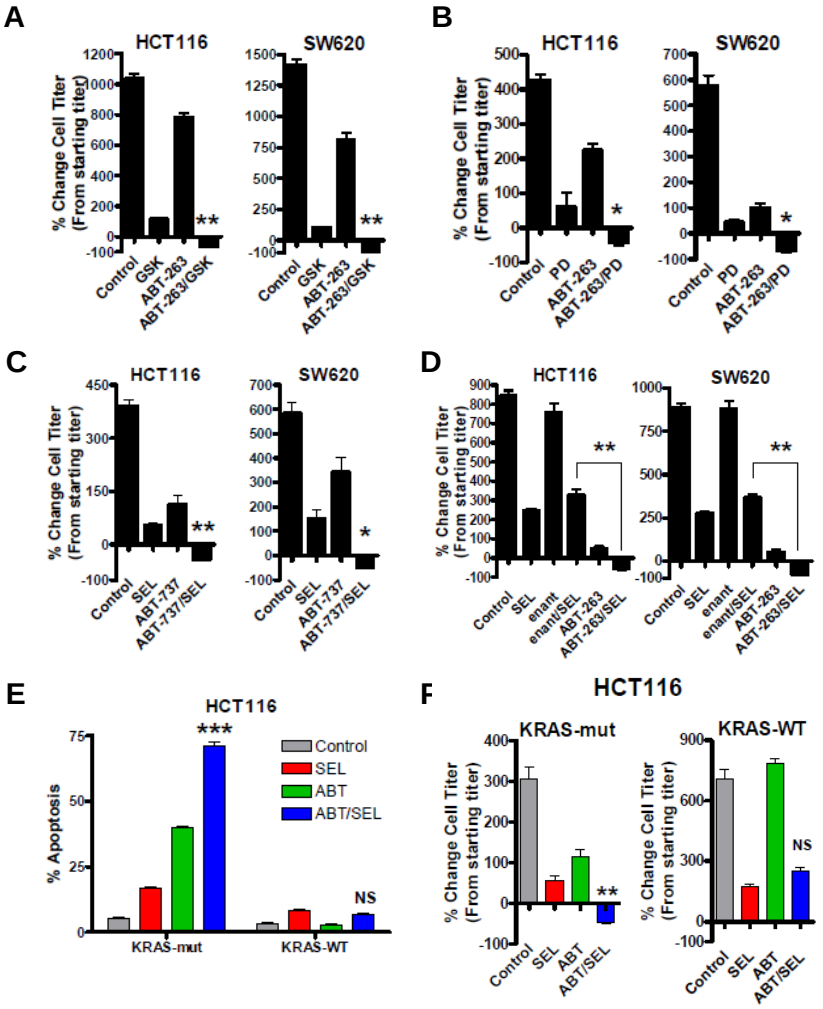

Figure S2 (related to Figure 2): Pharmacologic co-inhibition of BCL-XL and MEK. (A) HCT116 and SW620 cells were treated with vehicle (Control), 500nM of the MEK inhibitor GSK1120212 (GSK), 1μM ABT-263, or the combination for 72h and viable cell titer was determined by Cell TiterGlo Assay. Values represent the change in viable cell number relative to starting cell titer immediately before treatment. (**=p<0.01, *=p<0.05 for the combination group vs. all other groups by one-way ANOVA with Tukey post-hoc test.) (B) Cells were treated with vehicle (Control), 500nM of the MEK inhibitor PD0325901 (PD), or 1μM ABT-263, alone or in combination for 72h, as in (A). (C) Cells were treated with vehicle (Control), 1μM selumetinib, 1μM of another BH3 mimetic ABT-737, or the combination for 72h as in (A). (D) Cells were treated with vehicle (Control), 1μM selumetinib, 1μM ABT-263, or 1μM A-900526 (enant) (a less active enantiomer of ABT-263), or the combination of each with selumetinib for 72h as in (A). (E,F) HCT116 cells (KRAS-mut) and an isogenic cell line derived from HCT116 in which the mutant *KRAS* allele has been replaced by a wildtype *KRAS* allele (KRAS-WT) were treated with vehicle (Control), 1μM selumetinib, 1μM ABT-263, or the

combination for 72h. Effects of treatment on apoptosis as measured by Annexin V staining (E) or viable cell titer as measured by Cell TiterGlo (F) are shown. (NS=not significant, ***=$p<0.001$, **=$p<0.01$ by one-way ANOVAwith Tukey post-hoc test.

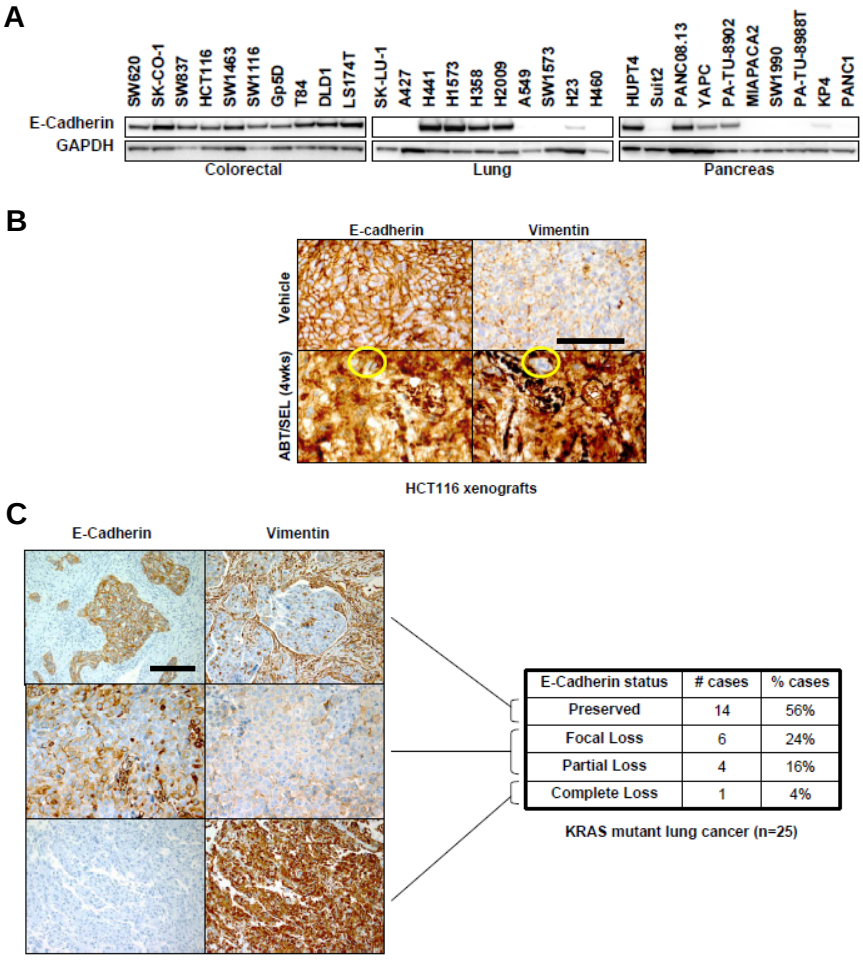

Figure S3 (related to Figure 3): E-cadherin protein expression in *KRAS*-mutant cell lines and tumors. (A) Lysates generated from the *KRAS*-mutant cell lines used in Figures 2E and 2F were assessed by western blot with an anti-E-cadherin antibody. (B) Tumors from HCT116 xenografts treated with vehicle or ABT-263/selumetinib for 28 days were assessed by immunohistochemistry for E-cadherin and vimentin. Vehicle-treated tumor shows strong membrane-restricted expression of E-cadherin and low vimentin expression, indicative of epithelial differentiation. Tumor treated with ABT-263/selumetinib shows abnormal loss of membrane expression of E-cadherin and increased vimentin staining, indicative of EMT. A small nest of cells in the combination-treated tumor that retain an epithelial phenotype (membrane expression of E-cadherin and low vimentin expression) is circled in yellow. Scale bar represents 100μm. (C) *KRAS*-mutant lung cancers from 25 patients were assessed for E-cadherin and vimentin staining. The percentage of all tumors showing preserved E-cadherin expression, focal/partial loss, or complete loss is shown. Scale bar represents 150 μm.

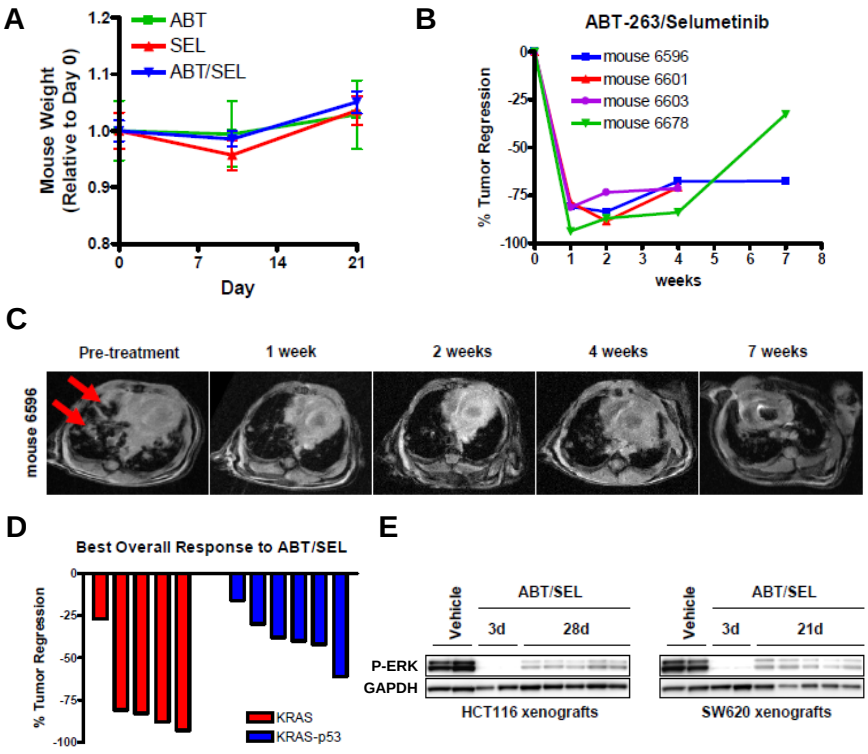

Figure S4 (related to Figure 4): Treatment of mouse models of *KRAS*-mutant cancers with ABT-263 and selumetinib. (A) Average body weights of xenograft-bearing mice treated with ABT-263 (100mg/kg daily), selumetinib (25mg/kg twice daily), or both drugs in combination are shown relative to starting body weight. Error bars represent SEM. (B) Established lung tumors induced by adenoviral-Cre inhalation in *LSL-KRAS*^{G12D} mice were treated with the combination of ABT-263 (100mg/kg daily) and selumetinib (25mg/kg twice daily) for the specified times, and the percent regression in lung tumor volume (relative to starting tumor volume) is shown for individual mice. (C) Serial MRI scans of one mouse treated for 7 weeks with ABT-263/selumetinib. Red arrows indicate dense areas of lung tumor in the pre-treatment images. (D) Established lung tumors induced by adenoviral-Cre inhalation in *LSL-KRAS*^{G12D} mice (KRAS) and *LSL-KRAS*^{G12D}*; p53*$^{lox/lox}$ mice (KRAS-p53) were treated with the combination of ABT-263 (100mg/kg daily) and selumetinib (25mg/kg twice daily). The best overall response during treatment, represented by the percent regression in lung tumor volume (relative to starting tumor volume) is shown. Each bar represents the percent regression for an individual mouse. (E) HCT116 and SW620 xenografts were treated with vehicle for 3 days or with the combination of ABT-263 (100mg/kg daily) and selumetinib (25mg/kg twice daily) for 3 days or for 21-28 days, as indicated. Tumors were harvested and P-ERK levels were assessed by western blot.

TRC clone number	Gene	Fold decrease MEK inhibitor vs. initial (log2)	Fold decrease MEK inhibitor vs. vehicle (log2)
TRCN0000007030	CPNE1	-6.691	-5.525
TRCN0000033500	BCL2L1	-4.181	-2.311
TRCN0000000379	INSR	-4.040	-1.057
TRCN0000002257	RIPK3	-3.522	-1.887
TRCN0000002378	CDKL3	-3.440	-1.244
TRCN0000000621	ERBB3	-3.415	-1.561
TRCN0000038701	SLC22A18	-3.212	-1.607
TRCN0000000480	CAMK2G	-3.051	-1.943
TRCN0000006164	TJP2	-2.872	-2.394
TRCN0000006464	RIC8B	-2.839	-1.457
TRCN0000039706	FLT3	-2.728	-1.308
TRCN0000000514	DGKG	-2.685	-2.265
TRCN0000037869	IKBKAP	-2.481	-2.216
TRCN0000001897	SOCS5	-2.358	-1.445
TRCN0000000664	MAP4K3	-2.324	-1.464
TRCN0000006038	PRKCI	-2.280	-1.610
TRCN0000001775	EPHB4	-2.103	-1.164
TRCN0000006256	PRKDC	-2.066	-1.165
TRCN0000006009	PIP4K2A	-2.036	-1.430
TRCN0000000841	GRK5	-1.995	-2.085
TRCN0000010080	ADK	-1.928	-2.253
TRCN0000002073	MCTP1	-1.854	-1.240
TRCN0000000941	SRMS	-1.816	-1.249
TRCN0000001990	MAP3K10	-1.779	-1.107
TRCN0000002278	MASTL	-1.761	-1.906
TRCN0000039744	CDK6	-1.739	-1.086
TRCN0000037759	UCK1	-1.733	-1.860
TRCN0000039629	CYLD	-1.730	-1.251
TRCN0000082471	NUCKS1	-1.704	-1.015
TRCN0000001783	PRKX	-1.580	-1.072
TRCN0000082368	LOC440354	-1.576	-2.286
TRCN0000003189	INSRR	-1.541	-1.150
TRCN0000001941	MAPK10	-1.511	-1.083
TRCN0000001844	MYLK3	-1.459	-1.079
TRCN0000082366	LOC389906	-1.458	-1.612
TRCN0000010105	LYN	-1.449	-1.127
TRCN0000037512	DSTYK	-1.344	-1.238
TRCN0000037910	AKAP7	-1.301	-2.018
TRCN0000002208	TSSK3	-1.284	-1.237
TRCN0000001612	AKT3	-1.243	-1.163
TRCN0000002113	SGK2	-1.211	-1.025
TRCN0000001010	PLD1	-1.198	-1.121
TRCN0000039672	MSH2	-1.195	-2.488
TRCN0000039764	GSK3A	-1.176	-1.280
TRCN0000003239	ITGAV	-1.153	-1.235
TRCN0000038001	AKAP8L	-1.088	-1.268

TRCN0000009999	TXK	-1.082	-1.693
TRCN0000037865	MAGI3	-1.078	-1.256
TRCN0000033306	SHH	-1.043	-1.327
TRCN0000001779	LATS1	-1.043	-1.141

Table S1 (Related to Figure 1): SW620 shRNA hits.

TRC clone number	Gene	Fold decrease MEK inhibitor vs. initial (log2)	Fold decrease MEK inhibitor vs. vehicle (log2)
TRCN0000002176	STK3	-9.282	-1.697
TRCN0000000377	GUCY2D	-5.026	-1.679
TRCN0000002403	MYO3B	-4.711	-1.492
TRCN0000001784	PRKX	-4.514	-2.386
TRCN0000010189	NME6	-4.012	-1.072
TRCN0000005986	RPA2	-3.970	-1.544
TRCN0000000832	TGFBR2	-3.954	-1.639
TRCN0000082473	LOC390641	-3.816	-2.797
TRCN0000007058	TLK1	-3.667	-1.830
TRCN0000002037	ADRBK2	-3.640	-1.235
TRCN0000039642	MYC	-3.636	-1.544
TRCN0000082563	LOC391533	-3.586	-1.315
TRCN0000038692	PDGFRL	-3.552	-3.395
TRCN0000000658	AURKA	-3.487	-1.690
TRCN0000052684	PTPMT1	-3.380	-1.856
TRCN0000006341	EXOSC10	-3.375	-1.723
TRCN0000033259	KRAS	-3.311	-1.388
TRCN0000038699	SLC22A18	-3.213	-1.258
TRCN0000006101	DLG1	-3.170	-1.900
TRCN0000003239	ITGAV	-3.162	-1.147
TRCN0000001896	SOCS5	-2.976	-2.128
TRCN0000001794	PRKCQ	-2.936	-1.552
TRCN0000052678	PPP1R14B	-2.858	-2.577
TRCN0000006437	TRIM27	-2.803	-1.977
TRCN0000082605	LOC441777	-2.692	-1.917
TRCN0000007044	STK32C	-2.661	-1.318
TRCN0000082422	LOC389069	-2.660	-1.879
TRCN0000052688	PHACTR3	-2.654	-2.735
TRCN0000010247	ETNK1	-2.644	-1.390
TRCN0000002345	MAP3K6	-2.628	-2.110
TRCN0000037409	SMG1	-2.590	-1.621
TRCN0000001386	RPS6KA1	-2.567	-1.495
TRCN0000001064	RAF1	-2.555	-1.718
TRCN0000033261	KRAS	-2.544	-1.848
TRCN0000010186	AKT3	-2.520	-2.097
TRCN0000000655	AURKA	-2.520	-3.289
TRCN0000006130	MPP2	-2.501	-1.030
TRCN0000037658	HK1	-2.424	-2.572
TRCN0000021546	MAST1	-2.420	-1.164
TRCN0000002289	NEK10	-2.343	-1.013
TRCN0000003098	FYN	-2.339	-2.078
TRCN0000009820	AKT2	-2.294	-1.783
TRCN0000039783	MTOR	-2.282	-1.222
TRCN0000000863	MERTK	-2.242	-2.312
TRCN0000082569	LOC390529	-2.226	-1.492
TRCN0000000925	RNASEL	-2.189	-2.523
TRCN0000037399	RIOK1	-2.182	-2.999

TRCN0000003150	MAPKAP1	-2.122	-1.459
TRCN0000037841	UGP2	-2.106	-2.129
TRCN0000006165	TJP2	-2.103	-1.668
TRCN0000037809	IP6K3	-2.103	-3.549
TRCN0000001571	MAPK6	-2.094	-1.266
TRCN0000010263	CNP	-2.085	-1.440
TRCN0000037419	ULK3	-2.085	-1.430
TRCN0000037763	UCK1	-2.079	-2.395
TRCN0000003141	CDK19	-2.074	-1.202
TRCN0000000819	EPHB1	-2.070	-1.663
TRCN0000002257	RIPK3	-2.068	-1.245
TRCN0000038701	SLC22A18	-2.053	-1.169
TRCN0000037794	PIK3C3	-2.036	-1.064
TRCN0000037519	TP53RK	-2.025	-1.397
TRCN0000007065	KSR2	-2.000	-1.092
TRCN0000001068	RAF1	-1.990	-1.622
TRCN0000082496	LOC402434	-1.974	-1.043
TRCN0000006447	GZMB	-1.971	-1.159
TRCN0000052590	PRKCSH	-1.932	-1.327
TRCN0000003153	MAPKAP1	-1.919	-1.082
TRCN0000001001	MAP3K12	-1.897	-1.990
TRCN0000001612	AKT3	-1.867	-1.410
TRCN0000007121	MCTP2	-1.866	-1.214
TRCN0000037946	AKAP8	-1.853	-2.159
TRCN0000037871	IKBKAP	-1.844	-1.423
TRCN0000010021	ITK	-1.838	-1.147
TRCN0000021535	LOC400588	-1.832	-1.214
TRCN0000007094	GMIP	-1.830	-1.457
TRCN0000021423	TWF2	-1.824	-1.143
TRCN0000038683	GSK3A	-1.817	-1.140
TRCN0000000994	MAP3K5	-1.778	-1.539
TRCN0000010168	NME4	-1.752	-1.498
TRCN0000039674	IGF1R	-1.713	-1.523
TRCN0000082366	LOC389906	-1.710	-1.933
TRCN0000021401	NRBP2	-1.687	-1.974
TRCN0000082487	C15orf42	-1.650	-1.439
TRCN0000002138	PLCB2	-1.648	-1.241
TRCN0000010230	EIF2AK1	-1.642	-1.246
TRCN0000037606	PI4K2A	-1.637	-1.473
TRCN0000001734	MAST2	-1.635	-1.079
TRCN0000007108	PAK7	-1.627	-1.366
TRCN0000002355	PLCD1	-1.623	-1.488
TRCN0000000758	CLK1	-1.619	-1.922
TRCN0000037554	AK4	-1.613	-1.959
TRCN0000000501	CHEK1	-1.612	-1.253
TRCN0000039843	SDHD	-1.554	-1.177
TRCN0000039607	PIK3CA	-1.551	-1.473
TRCN0000006139	FXN	-1.541	-1.180
TRCN0000006009	PIP4K2A	-1.516	-1.360
TRCN0000082493	LOC402434	-1.514	-1.464

TRCN0000033501	BCL2L1	-1.477	-1.219
TRCN0000006292	BRAF	-1.477	-1.463
TRCN0000002038	TNK2	-1.476	-1.087
TRCN0000021427	BRD4	-1.465	-2.619
TRCN0000001419	DDR2	-1.461	-1.131
TRCN0000010282	SGK3	-1.446	-1.111
TRCN0000037887	LAMTOR3	-1.430	-3.056
TRCN0000082583	LOC441992	-1.429	-1.280
TRCN0000001587	OXSR1	-1.426	-1.082
TRCN0000000759	PLK3	-1.419	-1.581
TRCN0000002337	PIK3C2G	-1.406	-1.404
TRCN0000033315	DHH	-1.405	-1.579
TRCN0000038728	SUZ12	-1.394	-1.170
TRCN0000021543	KIAA0226	-1.393	-1.089
TRCN0000003230	TTBK2	-1.363	-1.625
TRCN0000002233	MAP4K2	-1.353	-2.567
TRCN0000037512	DSTYK	-1.337	-1.309
TRCN0000039849	EXT2	-1.321	-1.031
TRCN0000006289	BRAF	-1.301	-1.785
TRCN0000000511	MAPK14	-1.295	-1.906
TRCN0000002376	CDKL3	-1.284	-1.461
TRCN0000006014	PIP4K2B	-1.283	-1.181
TRCN0000052581	CALM2	-1.282	-1.141
TRCN0000002112	SGK2	-1.262	-1.551
TRCN0000000727	CDKL2	-1.252	-1.481
TRCN0000039826	FUS	-1.250	-1.370
TRCN0000021421	TWF2	-1.219	-1.013
TRCN0000039947	CHEK2	-1.218	-1.128
TRCN0000037517	TNIK	-1.208	-1.164
TRCN0000000406	RET	-1.208	-1.055
TRCN0000082410	61E3.4	-1.156	-1.123
TRCN0000001067	RAF1	-1.156	-1.772
TRCN0000001375	MAPK4	-1.155	-1.098
TRCN0000037544	CKS2	-1.120	-1.015
TRCN0000039913	MAP2K4	-1.118	-1.365
TRCN0000082414	LOC442075	-1.111	-2.516
TRCN0000021399	NRBP2	-1.072	-1.314
TRCN0000010239	RET	-1.070	-1.210
TRCN0000001367	GRK6	-1.069	-1.483
TRCN0000038002	AKAP8L	-1.068	-1.688
TRCN0000001594	FGR	-1.060	-2.389
TRCN0000003240	ITGAV	-1.047	-1.506
TRCN0000039718	FOXO4	-1.042	-1.495
TRCN0000002327	PRKCG	-1.024	-1.652
TRCN0000002293	PDIK1L	-1.023	-1.001
TRCN0000002368	CDK14	-1.010	-1.425
TRCN0000037914	AKAP6	-1.007	-1.608
TRCN0000002305	MAP3K3	-1.000	-1.095

Table S2 (Related to Figure 1): HCT116 shRNA hits.

Cell Line	Tumor type	KRAS	PIK3CA	p53
SW620	Colorectal	G12V	wt	MUT
SK-CO-1	Colorectal	G12V	wt	wt
SW837	Colorectal	G12C	wt	MUT
HCT116	Colorectal	G13D	H1047R	wt
SW1463	Colorectal	G12C	Wt	MUT
SW1116	Colorectal	G12A	Wt	MUT
Gp5D	Colorectal	G12D	H1047L	wt
T84	Colorectal	G13D	E542K	MUT
DLD1	Colorectal	G13D	E545K	MUT
LS174T	Colorectal	G13D	H1047R	wt
SK-LU-1	Lung	G12D	wt	MUT
A427	Lung	G12D	wt	wt
H441	Lung	G12V	wt	MUT
H1573	Lung	G12A	wt	MUT
H358	Lung	G12C	wt	wt
H2009	Lung	G12A	wt	MUT
A549	Lung	G12S	wt	wt
SW1573	Lung	G12C	K111E	wt
H23	Lung	G12C	wt	MUT
H460	Lung	Q61H	E545K	wt
HUPT4	Pancreas	G12V	wt	MUT
Suit2	Pancreas	G12D	wt	MUT
PANC08.13	Pancreas	G12D	wt	wt
YAPC	Pancreas	G12V	wt	MUT
PA-TU-8902	Pancreas	G12V	wt	MUT
MIAPACA2	Pancreas	G12C	wt	MUT
SW1990	Pancreas	G12D	wt	wt
PA-TU-8988T	Pancreas	G12V	wt	MUT
KP4	Pancreas	G12D	wt	wt
PANC1	Pancreas	G12D	wt	MUT

Table S3 (Related to Figure 2): Mutational profile of cell lines used. Cell lines and tumor type are shown. Specific mutations in KRAS and PIK3CA are displayed for each cell line. The status of p53 for each cell line is indicated as wild-type (wt) or mutated (MUT).

Gene Set Name [# Genes]	Description	# Genes in Overlap	p value
CHARAFE_BREAST_CANCER_LUMINAL_VS_MESENSENCHYMAL_UP [456]	Genes up-regulated in luminal-like breast cancer cell lines compared to the mesenchymal-like ones.	20	0 e^{0}
ONDER_CDH1_TARGETS_2_DN [473]	Genes down-regulated in HMLE cells (immortalized nontransformed mammary epithelium) after E-cadhedrin (CDH1) [Gene ID=999] knockdown by RNAi.	23	0 e^{0}
WU_CELL_MIGRATION [186]	Genes associated with migration rate of 40 human bladder cancer cells.	14	0 e^{0}
COLDREN_GEFITINIB_RESISTANCE_DN [228]	Genes down-regulated in NSCLC (non-small cell lung carcinoma) cell lines resistant to gefitinib [PubChem=123631] compared to the sensitive ones.	21	0 e^{0}
CHARAFE_BREAST_CANCER_BASAL_VS_MESENCHNCHYMAL_UP [123]	Genes up-regulated in basal-like breast cancer cell lines as compared to the mesenchymal-like ones.	13	0 e^{0}
JAEGER_METASTASIS_DN [264]	Genes down-regulated in metastases from malignant melanoma compared to the primary tumors.	13	1.48 e^{-13}
MCBRYAN_PUBERTAL_BREAST_4_5WK_UP [279]	Genes up-regulated during pubertal mammary gland development between week 4 and 5.	13	3 e^{-13}
WAMUNYOKOLI_OVARIAN_CANCER_LMP_UP [268]	Genes up-regulated in mucinous ovarian carcinoma tumors of low malignant potential (LMP) compared to normal ovarian surface epithelium tissue.	9	3.14 e^{-8}
DELYS_THYROID_CANCER_UP [400]	Genes up-regulated in papillary thyroid carcinoma (PTC) compared to normal tissue.	9	9.27 e^{-7}
AIGNER_ZEB1_TARGETS [29]	Genes up-regulated in MDA-MB-231 cells (breast cancer) after knockdown of ZEB1 [Gene ID=6935] by RNAi.	4	9.63 e^{-7}

Table S4 (Related to Figure 3): Gene Set Enrichment Analysis (GSEA) of genes correlating with sensitivity to ABT-263/AZD6244. GSEA was performed using the genes identified in Figure 3A. Top 10 enriched gene sets are shown, ranked by p-value. Gene sets related to epithelial vs. mesenchymal differentiation are shown in red.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell Lines and Reagents

All cell lines were grown in DMEM/F12 (GIBCO) with 10% FBS and assayed in DMEM/F12 with 5% FBS and were obtained from the Massachusetts General Hospital Center for Molecular Therapeutics, which performs routine cell line authentication testing by SNP and STR analysis. Isogenic HCT116 cells lacking mutant *KRAS* were kindly provided by Kevin Haigis (Massachussets General Hospital, Boston, MA). Chemical inhibitors from the following sources were dissolved in DMSO for in vitro studies: ABT-263 (Active Biochem), selumetinib (Otava Chemicals), ABT-737 (Selleck Chemicals), PD0325901 (Selleck Chemicals), GSK1120212 (kindly provided by GlaxoSmithKline), A-900526, a less active enantiomer of ABT-263 (kindly provided by Abbott Laboratories; Tse et al., 2008).

Western Blot Analysis, Immunoprecipitation, and Antibodies

Western blotting was performed using standard methods. After treatment with indicated drugs, cells were washed with cold PBS and lysed in the following lysis buffer: 20 mM Tris pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 10% glycerol, 1 mM EDTA, 1 mM EGTA, 5 mM sodium pyrophosphate, 50 mM NaF, 10 nM β–glycerophosphate, 1 mM sodium vanadate, 0.5 mM DTT, 4 μg/mL leupeptin, 4 μg/mL pepstatin, 4 μg/mL aprotinin, 1 mM phenylmethylsulfonyl fluoride. Lysates were centrifuged at 16,000 x *g* for 5 min at 4°C. Protein concentrations were determined by BCA assay (Thermo Scientific). Proteins were resolved by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Hybond-P, Amersham). Immunoblotting was performed per antibody manufacturer's specifications.All antibodies were purchased from Cell Signaling, except for GAPDH (Millipore), E-cadherin (BD Bioscience), and MCL-1 and Zeb1 (Santa Cruz Biotechnology). For immunoprecipitation experiments, cells were lysed using the same lysis buffer as above and immunoprecitpiated with anti-BIM antibody (Cell Signaling #2819) and Protein A sepharose (GE Healthcare) after incubation overnight at 4 ^{o}C. The immunoprecipitate, the supernatant, and a sample of the initial whole cell lysate for each condition were then analyzed by western blot.

Determination of Cell Titer

Cells were seeded at 2,000 cells per well in parallel 96-well plates. After overnight incubation, one plate was frozen immediately to represent the starting cell titer, and the other plate was treated in for 72h (six wells per condition) and then frozen. Plates were thawed simultaneously, and cell titer was determined using Cell Titer Glo assay (Promega) according to the manufacturer's protocol. Change in cell titer for each treatment condition was calculated relative to starting cell titer.

Annexin V Apoptosis Assays

Cells were seeded at ~30-40% confluence in 6cm plates. After overnight incubation, media was aspirated and replaced with media with or without various concentrations of indicated drugs. After 72h, media was collected. Cells were washed with PBS and trypsinized. PBS wash and trypsinized cells were added to the collected media in a single tube. Cells were pelleted, washed once with PBS and resuspended in Annexin binding buffer (BD Biosciences) at ~1 x 10^6 cells/mL. Cells were stained with propidium iodide (BD Biosciences) and Annexin V Cy5 (Biovision) according to the manufacturer's protocol and assayed on a LSRII flow cytometer (BD Biosciences).

Immunohistochemistry

IHC on formalin-fixed paraffin-embedded tissue was performed for P-ERK as previously described (Engelman et al., 2008). IHC for Ki67 was performed using Ki67 antibody (Novocastra/Leica NCL-Ki67p at 1:1000 dilution in PBS/3% BSA) and developed using Dako Envision+ system-HRP (DAB). IHC for Cleaved Caspase-3 was performed using the Apoptosis Marker: SignalStain Cleaved Caspase-3 (Asp175) IHC Detection Kit (Cell Signaling #8210) according to the manufacturer's protocol. E-cadherin and vimentin was performed by the Clinical Pathology Laboratory at the Massachusetts General Hospital.

Pooled shRNA screen and analysis

Lentivital pooled shRNA library was constructed using a subset of the RNA Consortium (TRC) shRNA library targeting "druggable" genes, such as kinases and regulators of cell proliferation and survival using previously described techniques, available at www.broad.mit.edu/rnai/trc (Moffat et al., 2006; Luo et al., 2008; Barbie et al., 2009). 3 x 10^6 target cells were infected with the pooled lentiviral library at a multiplicity of infection of 0.3, and the morning after infection, cells were selected in 2μg/mL puromycin for 48 h to eliminate uninfected cells. Cells were split into three aliquots of ~1.5 x 10^6 cells. One aliquot was immediately frozen to represent the initial population. The remaining two aliquots were seeded into two separate 15 cm plates. The following day, media with 5% FBS containing DMSO vehicle or 1μM selumetinib was added, and cells were cultured for 7 days. Fresh media and drug was added after day 3. At all times, sufficient cell numbers were used so that an average of at least 200 cells per shRNA were maintained. After 7 days, cells were trypsinized, pelleted, and frozen. Genomic DNA was isolated from the initial cell aliquot and the vehicle and selumetinib-treated aliquots using a QIAamp DNA Blood Midi Kit (QIAGEN) according to the manufacturer's protocol.

Quantification of shRNA abundance in genomic DNA samples was performed by the Partners Center for Personalized Genetic Medicine (www.hpcgg.org), using established protocols summarized below. Briefly, shRNA inserts were PCR-amplified from 1.5μg of each genomic DNA sample in a 75μL PCR reaction containing Amplification Buffer (Fidelity Systems), 0.5mM each dNTPs (Roche), 1.66 U/μLTopoTaq enzyme (Fidelity Systems). Forward and reverse primers flanking the shRNA insert region and containing the P5 and P7 flowcell adapters, respectively, were used at a final concentration of 200nM. PCR was performed under the following conditions: 98^{o}C fro 30s; then 30 cycles of 98^{o}C for 10s, 65^{o}C for 30s, 72^{o}C for 30s; followed by 72^{o}C for 5 min. PCR reactions were purified using Ampure beads (Beckman Coulter) and eluted in 25μL water. Product solutions were adjusted to10nM and sequenced on the Illumina GAII to generate single-end 26 base-pair reads. Bases 2-21 of the sense shRNA sequence were used to align to the reference sequence of the complete TRC shRNA library.

The abundance of each shRNA sequence was quantified in terms of number of individual shRNA sequence reads per one-million total reads. The ratio of the abundance of each shRNA in MEK inhibitor-treated samples vs. both the vehicle-treated and initial samples was calculated. For each cell line, a given shRNA was considered a "hit" if that shRNA showed a decrease in abundance of at least 2-fold relative to **both** the vehicle-treated and initial samples. These criteria were chosen to select shRNAs that not only illustrated enhanced effect in the presence of MEK inhibitor, but also that caused the most profound suppression of cell number in the presence of MEK inhibitor relative to the initial population.

Microarray analysis

Available expression profiles for *KRAS*-mutant cell lines were obtained from the Cancer Cell Line Encyclopedia web site www.broadinstitute.org/ccle using the GENE-E tool and the CCLE_Expression_Entrez_2012-04-06.res data file for 18988 probe IDs (Barretina et al., 2012). Cell lines were divided into two groups based on percent apoptosis induced by ABT-263/selumetinib with 25% as the threshold for sensitivity. This threshold was chosen since 90% of cell lines showed <25% apoptosis induction in the presence of selumetinib alone. The prediction analysis for microarrays module for R (PAMR) was used to create a list of differentially expressed genes (Tibshirani et al., 2002). A threshold of 3 was used as this gave the lowest cross-validation error rate and had a false discovery rate of <0.2. Average linkage hierarchical clustering was performed on this list using Cluster/Treeview to generate a heatmap (Eisen et al., 1998).

Gene Set Enrichment Analysis (GSEA) was performed on the gene list identified through the PAMR algorithm as above using GSEA software available at www.broadinstitute.org/gsea (Mootha et al., 2003; Subramanian et al., 2005).

Lentiviral shRNA experiments

shRNA constructs in the pLKO.1 lentiviral vector containing the following targeting sequences were used:

shGFP: 5'-GCAAGCTGACCCTGAAGTTCAT-3'

shBCL-XL#1: 5'- GCTCACTCTTCAGTCGGAAAT -3'

shBCL-XL#2:	5’-GTGGAACTCTATGGGAACAAT-3’
shBCL-XL#3	5’-GTTTAGTGATGTGGAAGAGAA-3’
shUCK1#1:	5’- GCCTTGAAAGGACAGTACAAT-3’
shUCK1#2:	5’- CCTCTGGCAAACGGTCACATT-3’
shUCK1#3:	5’- CGTGTGTGAGAAGATCATGGA-3’
shTJP2#1:	5’- CGAGTGGTAGACACACTGTAT-3’
shTJP2#2:	5’- GCGATCAACTTAGGGACAATA- 3’
shTJP2#3:	5’- CGTCATCAGTATTCTGATTAT-3’
shIKBKAP#1:	5’- CGGTTCTAGGTCCCAATTCTA-3’
shIKBKAP#2:	5’- GCCAGATATTTAAGTACCTTT-3’
shIKBKAP#3:	5’- GCTGTGCTCTTGCTGTTAGAA-3’
shLOC309906#1:	5’- ACCGTGGGAAAGCAACTAGAA-3’
shLOC309906#2:	5’- CAATGGGAAAGGAAGTCGCTT-3’
shLOC309906#3:	5’- CGTTTCTAAGTCCGTTGATGA-3’
shPRKX#1:	5’- CAAGGCGATTAGGAAACATGA-3’
shPRKX#2:	5’- GCGATTAGGAAACATGAAGAA-3’
shPRKX#3:	5’- CCTACTGTGATGTCTTGGTTT-3’
shRIPK3#1:	5’- CTGAGAGACAAGGCATGAACT-3’
shRIPK3#2:	5’- CACAGGGTTGGTATAATCATA-3’
shRIPK3#3:	5’- GCACTCTCGTAATGATGTCAT-3’
shAKAP8L#1:	5’- CCACCAACTATGGGTATGGTA -3’
shAKAP8L#2:	5’- CCGCAGTATTCTCAACAACAA -3’
shAKAP8L#3:	5’- CGACTTCCGAACCAAGAAGAA-3’

Lentiviral particles were generated and target cells were infected as described previously (Moffat et al, 2006). The day prior to infection, HCT116 or SW620 cells were seeded in 6-well plates at 1-2 x 10^5 cells per well for western blot experiments and at 0.5-1 x 10^5 cells per well for cell viability assays. The morning after infection, cells were treated with 2 μg/mL puromycin for 48 h to eliminate uninfected cells. Media without puromycin containing the indicated concentrations of drug was then added for 24 h for western blot analysis or for 72 h for cell viability assays. For cell viability assays, crystal violet staining and quantification was performed as follows. Cells were fixed with

gluteraldehyde at room temperature for 10 minutes and washed with distilled water. Cells were stained with 0.1% crystal violet in water for 30 minutes, washed with water, dried, and photographed. For quantification, 10% acetic acid was added for 5 minutes and the absorbance of the resulting solution was measured at 590nm.

Mouse Treatment Studies

Lung tumors were induced in *LSL-KRAS*^{G12D} mice or in *LSL-KRAS*^{G12D}*; p53*$^{lox/lox}$ mice by inhalation of adenoviral Cre recombinase, and were monitored and measured by serial MRI scans, as previously described (Engelman et al., 2008; Chen et al., Nature 2012). ABT-263 and selumetinib for in vivo studies were obtained from Active Biochem. ABT-263 was formulated in 60% Phosal 50 PG (Phospholipoid GmBH), 30% PEG-400, and 10% ethanol and dosed at 100mg/kg daily by oral gavage. Selumetinib was formulated in 0.5% methylcellulose and 0.4% polysorbate and dosed at 25mg/kg twice daily by oral gavage. For pharmacodynamic studies, tumor tissue was harvested and formalin-fixed 3h after the morning doses of drug on the specified day of treatment. Animal care and treatment was performed in accordance with institutional guidelines.

Xenograft Tumor Mutational Analysis

HCT116 and SW620 xenograft tumors harvested following 28 days or 21 days of treatment, respectively, with ABT-263 and selumetinib were subjected to mutational analysis and compared to control tumors harvested pre-treatment to identify mutations acquired during treatment. Hot-spot mutations were analyzed using a multiplexed genotyping assay (Dias-Santagata et al., 2010) covering the following genes: *AKT1, APC, BRAF, CTNNB1, EGFR, ERBB2, IDH1, KIT, KRAS, MEK1, NOTCH1, NRAS, PIK3CA, PTEN, TP53*.

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