Table S1. Genotypes, regorafenib and sorafenib IC_{50}, and time for regorafenib-induced Mcl-1 depletion (50%) in a panel of 16 CRC cell lines.

Cell lines	*KRAS*	*BRAF*	*PIK3CA*	*p53*	*FBW7*	Regorafenib IC_{50} (μM)*	Sorafenib IC_{50} (μM)*	Time for 50% Mcl-1 reduction (hr)**
HCT116	p.G13D	WT	p.H1047R	WT	WT	7.04	20.09	1.8
DLD1	p.G13D	WT	p.E545K	p.S241F	WT	6.95	21.38	1.7
Lim1215	WT	WT	WT	WT	WT	5.1	16.9	2.2
Lim2405	WT	p.V600E	WT	WT	WT	3.45	30.97	1.2
SW480	p.G12V	WT	WT	p.R273H/p.P309S	WT	9.12	19.54	1
RKO	WT	p.V600E	p.H1047R	WT	WT	6.8	18.07	2.8
NCI-H508	WT	p.G596R	p.E545K	p.R273H	WT	12.05	29.65	3.2
DiFi	WT	WT	WT	p.K132R	WT	9.44	22.96	1.3
SW837	p.G12C	WT	WT	p.R248W	p.L403fs*34	128.83	39.72	>8
SW48	WT	WT	p.G914R	WT	p.S668fs*39	48.08	60.12	8
LoVo	p.G13D	WT	WT	WT	p.R505C	55.72	34.2	8
SW1463	p.G12C	WT	WT	p.R248Q	p.R479Q	49.55	49.55	>8
HCT-8	p.G13D	WT	p.E545K	WT	p.R658Q	76.03	85.31	>8
CCK-81	WT	WT	p.C420R	p.P278H	p.R465C	65.83	36.9	>8
SNU-C2B	p.G12D	WT	p.D725G	p.R273H	p.G579W	70.79	31.41	8
LS411N	WT	p.V600E	WT	p.Y126*	p.R505H	86.3	28.58	8

* Analyzed by MTS assay on cells treated for 72 hr. ** Analyzed by quantifying western blot bands with Image J program on cells treated with 40 μM regorafenib.

Table S2. PCR primers

Purpose	Orientation	Sequence (5'-3')
Allele-specific genomic PCR for analyzing *FBW7* mutations		
HCT116 (c.1513 C>T)	Forward	ATGCAGCATTCTAGGCTTCC
	Reverse	GCCATCATATTGAACACAGCA
Lim1215, Lim2405	Forward	ATGGGCATACTTCCACTGTGT
(c.1393 C>T)	Reverse	CCCACTTGCGGAAATAAGAAT
β-Actin	Forward	CGTCTTCCCCTCCATCGTGGG
	Reverse	AGCTCGTAGCTCTTCTCCAGG
RT-PCR		
β-Actin	Forward	GACCTGACAGACTACCTCAT
	Reverse	AGACAGCACTGTGTTGGCTA
FBW7	Forward	GTGATAGAACCCCAGTTTCA
	Reverse	CCTCAGCCAAAATTCTCCAG
***FBW7* mutant constructs**		
R465C	Forward	CATACTTCCACTGTGTGTTGTATGCATCTTC
	Reverse	GAAGATGCATACAACACACAGTGGAAGTATG
R479Q	Forward	TTGTTAGCGGTTCTCAAGATGCCACTCTTAG
	Reverse	CTAAGAGTGGCATCTTGAGAACCGCTAACAA
R505C	Forward	CATGTTGCAGCAGTCTGCTGTGTTCAATATG
	Reverse	CATATTGAACACAGCAGACTGCTGCAACATG

Supplemental Figure Legends

Figure S1. Regorafenib and sorafenib sensitivity is linked to *FBW7* mutational status in CRC cells. (A) MTS analysis of cell viability of *FBW7*-WT (black) and -mutant (red) CRC cell lines treated with sorafenib at different concentrations for 72 hr. Results represent the means ± s.d. of three independent experiments. **(B)** Comparison of sorafenib IC_{50} of *FBW7*-WT (black) and -mutant (red) CRC cell lines analyzed in (A). **(C)** Genomic DNA sequencing analysis of *FBW7* mutations in indicated CRC cell lines highlighting mutant sequences.

Figure S2. Regorafenib induces caspase activation and Mcl-1 depletion in CRC cells. (A) MTS analysis of cell viability (black) and fluorogenic analysis of caspase 3/7 activity (red) in HCT116, Lim 1215 and RKO cells treated with regorafenib as indicated for 72 hr. **(B)** Western blotting of indicated Bcl-2 family proteins in HCT116 cells treated with regorafenib at indicated concentrations for 24 hr. **(C)** Western blotting of Mcl-1 in HCT116 and DLD1 cells treated with 40 μM regorafenib continuously at indicated time points, or with regorafenib washed out at 4 hr after treatment. **(D)** Mcl-1 protein expression in untreated *FBW7*-WT (black) and -mutant (red) CRC cell lines analyzed in Fig. 1D was quantified by the Image J program and normalized to β-actin. **(E)** Western blotting of c-Myc and cyclin E in HCT116 cells treated with 40 μM regorafenib at indicated time points.

Figure S3. FBW7 is essential for regorafenib sensitivity and Mcl-1 degradation in CRC cells. (A) Regorafenib sensitivity of WT and *FBW7*-KO DLD1 cells with or without *FBW7* transfection or *Mcl-1* knockdown, which was analyzed by western blotting (left panel). **(B)** Western blotting of Mcl-1 and FBW7 in WT and *FBW7*-KO DLD1 cells treated with 5 μM regorafenib at indicated time points. **(C)** Western blotting of Mcl-1 in *FBW7*-KO HCT116 and DLD1 cells transfected with control or *FBW7* expression vector and treated with 40 μM

regorafenib at indicated time points. **(D)** Apoptosis in WT and *FBW7*-KO HCT116 and DLD1 cells transfected as in (A) and treated with 40 μM regorafenib for 48 hr was analyzed by counting apoptotic nuclei after nuclear staining. **(E)** Regorafenib sensitivity of *FBW7*-KO DLD1 cells with transfection of HA-tagged WT FBW7 or indicated mutants (R465C, R479Q and R505C), which was analyzed by western blotting (*upper* panel). **(F)** Apoptosis in *FBW7*-mutant SW837 cells with or without *FBW7* transfection or *Mcl-1* knockdown and treated with 40 μM regorafenib for 48 hr was analyzed as in (D). **(G)** Regorafenib sensitivity of *FBW7*-mutant SW48 cells with or without *FBW7* transfection or *Mcl-1* knockdown, which was analyzed by western blotting (left panel). In (A), (D), (E), (F) and (G), regorafenib sensitivity was analyzed by MTS assay on cells treated with regorafenib at indicated concentrations for 72 hr. Western blotting was performed on untreated cells at 24 hr after transfection. Results were expressed as means ± s.d. of three independent experiments. ******, P <0.01, *******, P <0.001.

Figure S4. FBW7 is critical for sorafenib sensitivity and Mcl-1 degradation in CRC cells. **(A)** Sorafenib sensitivity of WT and *FBW7*-KO HCT116 and DLD1 cells with or without HA-FBW7 transfection or *Mcl-1* knockdown. **(B)** Western blotting of Mcl-1 and FBW7 in WT and *FBW7*-KO HCT116 and DLD1 cells treated with 5 μM sorafenib at indicated time points. **(C)** Sorafenib sensitivity of *FBW7*-KO HCT116 and DLD1 cells with transfection of HA-tagged WT *FBW7* or indicated mutants (R465C, R479Q and R505C). **(D)**Sorafenib sensitivity of *FBW7*-mutant SW837 and SW48 cells with or without HA-FBW7 transfection or *Mcl-1* knockdown. In (A), (C) and (D), sorafenib sensitivity was analyzed by MTS assay on cells treated with sorafenib at indicated concentrations for 72 hr. Results were expressed as means ± s.d. of three independent experiments.

Figure S5. Regorafenib sensitivity is not affected by *KRAS*, *BRAF*, *PIK3CA*, and *p53* mutations in CRC cells. (A)-(D) MTS analysis of cell viability of WT or parental (black) and isogenic (red) CRC cell lines with indicated genotypes of *KRAS* (A), *BRAF* (B), *PIK3CA* (C), or *p53* (D) treated with regorafenib at different concentrations for 72 hr. Results were expressed as means ± s.d. of three independent experiments.

Figure S6. Regorafenib-resistant CRC cells are defective in apoptosis and have enriched *FBW7* mutations. (A) MTS analysis of cell viability of indicated parental (black) and regorafenib-resistant (-R) (red) CRC cells treated with increasing concentrations of regorafenib for 72 hr. **(B)** Indicated parental and regorafenib-resistant CRC cells were treated with 40 μM regorafenib for 48 hr. Apoptosis was analyzed by counting condensed and fragmented nuclei after nuclear staining. **(C)** *FBW7* mutations in the indicated regorafenib-resistant CRC cell lines were analyzed by sequencing analysis of individual clones of PCR products from the genomic region of *FBW7*. The frequencies of WT and mutant clones are indicated. **(D)** *FBW7*mRNA expression in parental and regorafenib-resistant HCT116, Lim2405, and SW480 cells treated as in (B) for 24 hr was analyzed by real-time RT-PCR. Results in (A), (B) and (D) were expressed as means ± s.d. of three independent experiments.

Figure S7. Mcl-1 knockdown or inhibition restores regorafenib sensitivity in regorafenib-resistant CRC cells. (A) Western blotting of indicated proteins in parental and regorafenib-resistant HCT116, Lim2405 and SW480 cells treated with 40 μM regorafenib at indicated time points. **(B)** Parental and regorafenib-resistant CRC cells transfected with control or *Mcl-1* siRNA and treated with 40 μM regorafenib for 48 hr. *Upper*, western blot analysis of Mcl-1 knockdown; *lower*, analysis of apoptosis by counting apoptotic nuclei after nuclear staining. **(C)** Indicated parental and regorafenib-resistant CRC cells were treated with 40 μM regorafenib

alone or in combination with 1 μM of the Mcl-1 inhibitor TW-37 or the Bcl-2/Bcl-X_L inhibitor ABT-737 for 48 hr. Apoptosis was analyzed as in (B). Results in (B) and (C) were expressed as means ± s.d. of three independent experiments.

Figure S8. Regorafenib-resistant CRC cells are cross-resistant to other anticancer agents that induce Mcl-1 degradation. (A) HCT116 cells treated with 1 μM UCN-01, 1 μM 17-AAG, 1 μM YM-155, 10 μM roscovitine, 15 μM sunitinib, 10 μM crizotinib, 10 nM TRAIL, 10 μM VX680, 20 μM etoposide, 20 μM temsirolimus, or 120 μM sulindac sulfide for 24 hr. Mcl-1 expression was analyzed by western blotting. **(B)**, **(C)** Parental and regorafenib-resistant SW480 (B) and Lim2405 (C) cells were treated with indicated agents as in (A) for 48 hr. Apoptosis was analyzed by counting condensed and fragmented nuclei after nuclear staining. Results were expressed as means ± s.d. of three independent experiments.

Figure S1

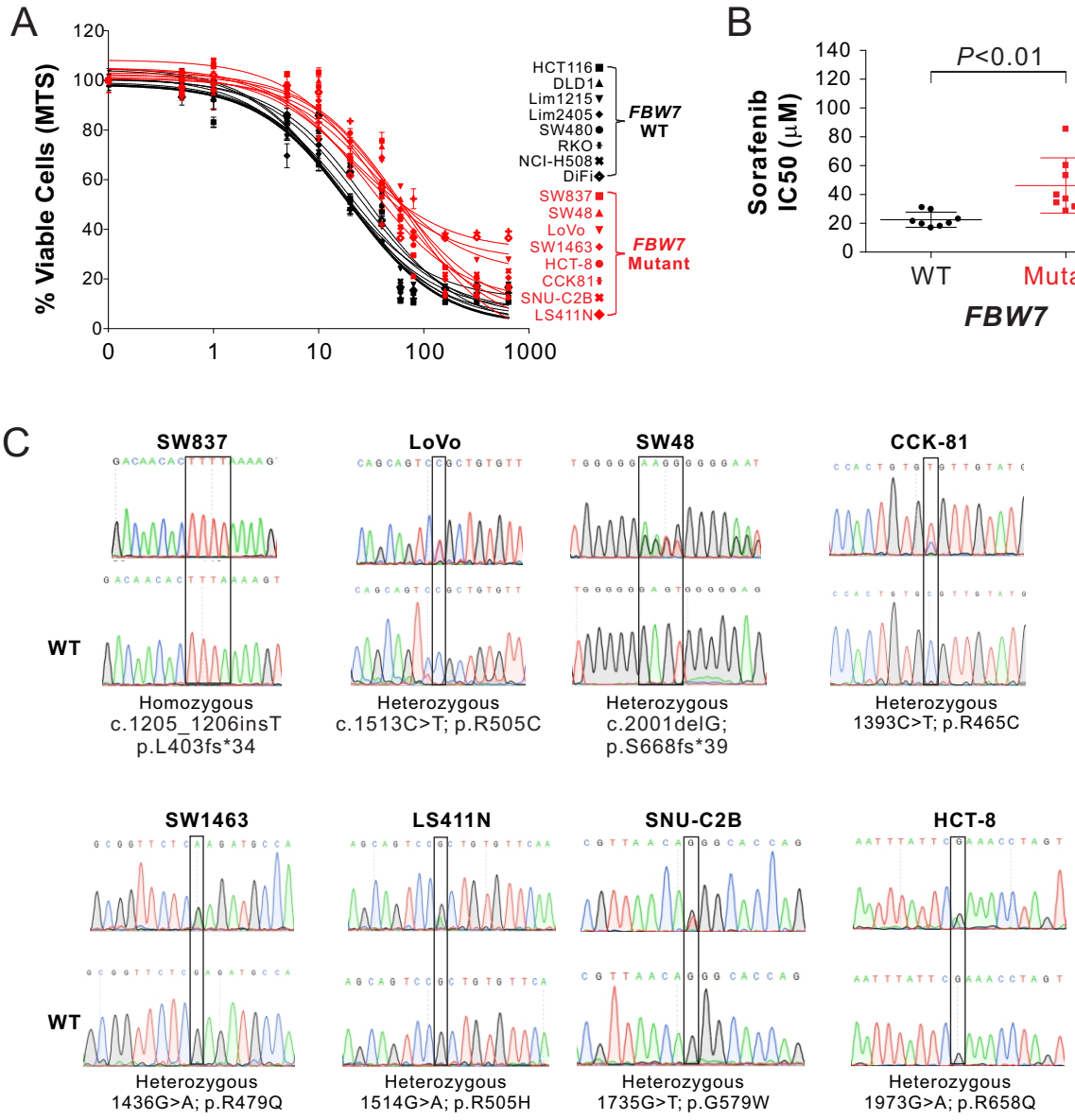

A
% Viable Cells (MTS)
120
100
80
60
40
20
0
0
1
10
100
1000
HCT116
DLD1
Lim1215
Lim2405
SW480
RKO
NCI-H508
DiFi
FBW7 WT
SW837
SW48
LoVo
SW1463
HCT-8
CCK81
SNU-C2B
LS411N
FBW7 Mutant
B
Sorafenib IC50 (μM)
P<0.01
140
120
100
80
60
40
20
0
WT
Mutant
FBW7
C
SW837
LoVo
SW48
CCK-81
WT
Homozygous
c.1205_1206insT
p.L403fs*34
Heterozygous
c.1513C>T; p.R505C
Heterozygous
c.2001delG;
p.S668fs*39
Heterozygous
1393C>T; p.R465C
SW1463
LS411N
SNU-C2B
HCT-8
WT
Heterozygous
1436G>A; p.R479Q
Heterozygous
1514G>A; p.R505H
Heterozygous
1735G>T; p.G579W
Heterozygous
1973G>A; p.R658Q

Figure S2

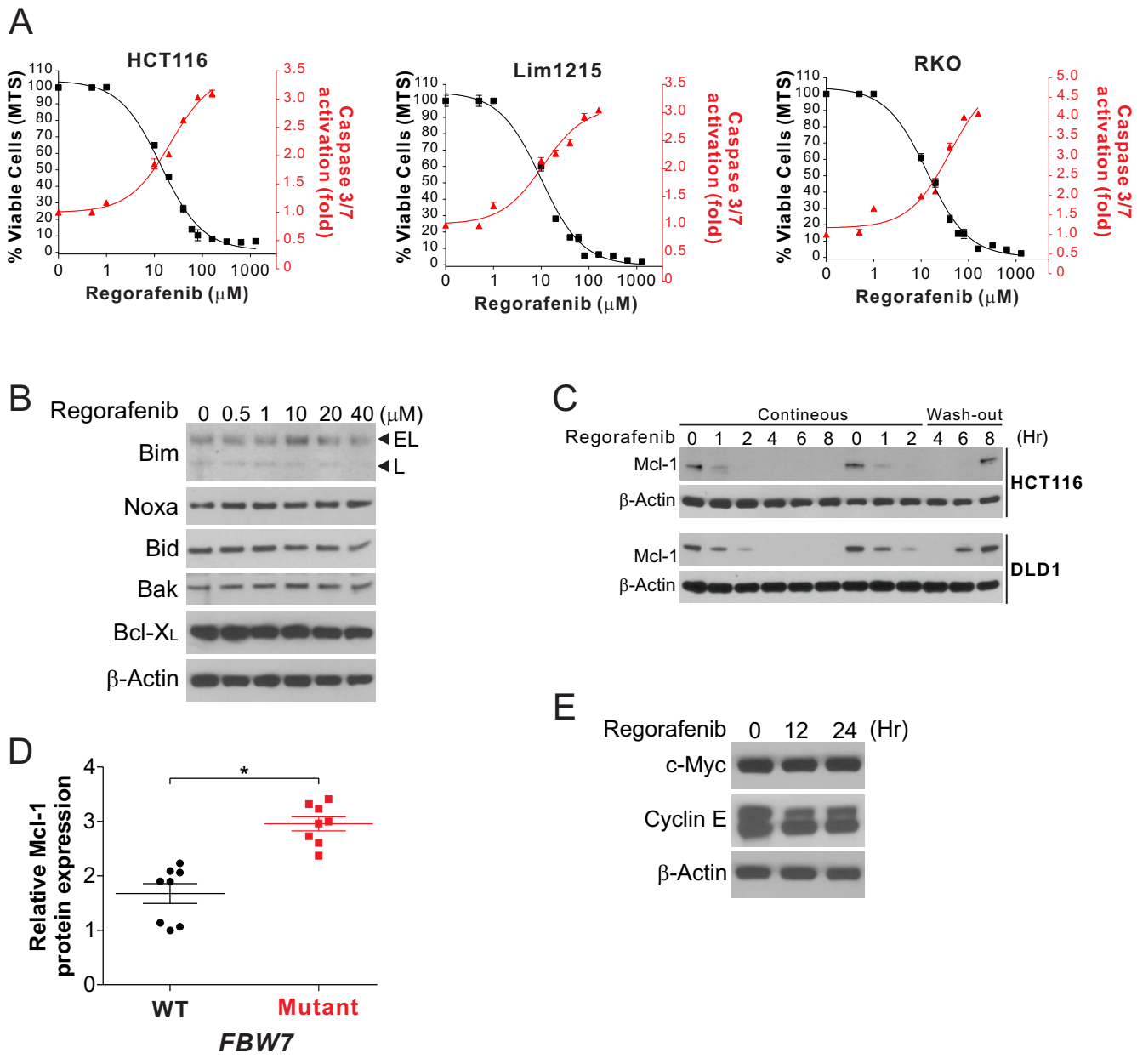

A
HCT116
Lim1215
RKO
% Viable Cells (MTS)
Caspase 3/7 activation (fold)
Regorafenib (μM)
B
Regorafenib 0 0.5 1 10 20 40 (μM)
Bim
EL
L
Noxa
Bid
Bak
Bcl-XL
β-Actin
C
Contineous
Wash-out
Regorafenib 0 1 2 4 6 8 0 1 2 4 6 8 (Hr)
Mcl-1
β-Actin
HCT116
DLD1
D
Relative Mcl-1 protein expression
WT
Mutant
FBW7
E
Regorafenib 0 12 24 (Hr)
c-Myc
Cyclin E
β-Actin

Figure S3

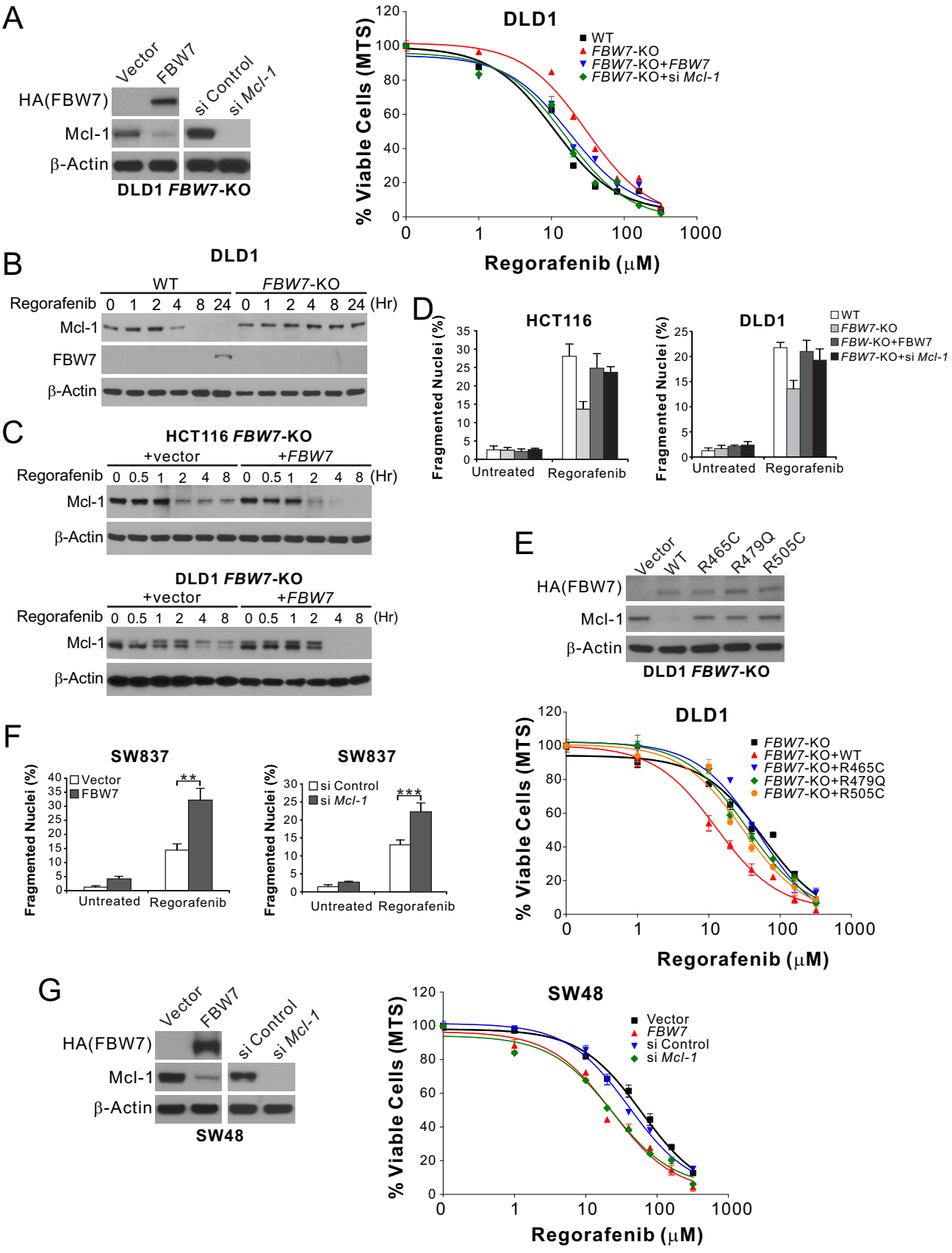

A
Vector
FBW7
si Control
si Mcl-1
HA(FBW7)
Mcl-1
β-Actin
DLD1 FBW7-KO
DLD1
WT
FBW7-KO
FBW7-KO+FBW7
FBW7-KO+si Mcl-1
% Viable Cells (MTS)
Regorafenib (μM)
B
DLD1
WT
FBW7-KO
Regorafenib 0 1 2 4 8 24 0 1 2 4 8 24 (Hr)
Mcl-1
FBW7
β-Actin
C
HCT116 FBW7-KO
+vector
+FBW7
Regorafenib 0 0.5 1 2 4 8 0 0.5 1 2 4 8 (Hr)
Mcl-1
β-Actin
DLD1 FBW7-KO
+vector
+FBW7
Regorafenib 0 0.5 1 2 4 8 0 0.5 1 2 4 8 (Hr)
Mcl-1
β-Actin
D
HCT116
DLD1
Fragmented Nuclei (%)
Untreated
Regorafenib
WT
FBW7-KO
FBW-KO+FBW7
FBW7-KO+si Mcl-1
E
Vector
WT
R465C
R479Q
R505C
HA(FBW7)
Mcl-1
β-Actin
DLD1 FBW7-KO
DLD1
FBW7-KO
FBW7-KO+WT
FBW7-KO+R465C
FBW7-KO+R479Q
FBW7-KO+R505C
% Viable Cells (MTS)
Regorafenib (μM)
F
SW837
Vector
FBW7
Fragmented Nuclei (%)
Untreated
Regorafenib
SW837
si Control
si Mcl-1
Fragmented Nuclei (%)
Untreated
Regorafenib
G
Vector
FBW7
si Control
si Mcl-1
HA(FBW7)
Mcl-1
β-Actin
SW48
SW48
Vector
FBW7
si Control
si Mcl-1
% Viable Cells (MTS)
Regorafenib (μM)

Figure S4

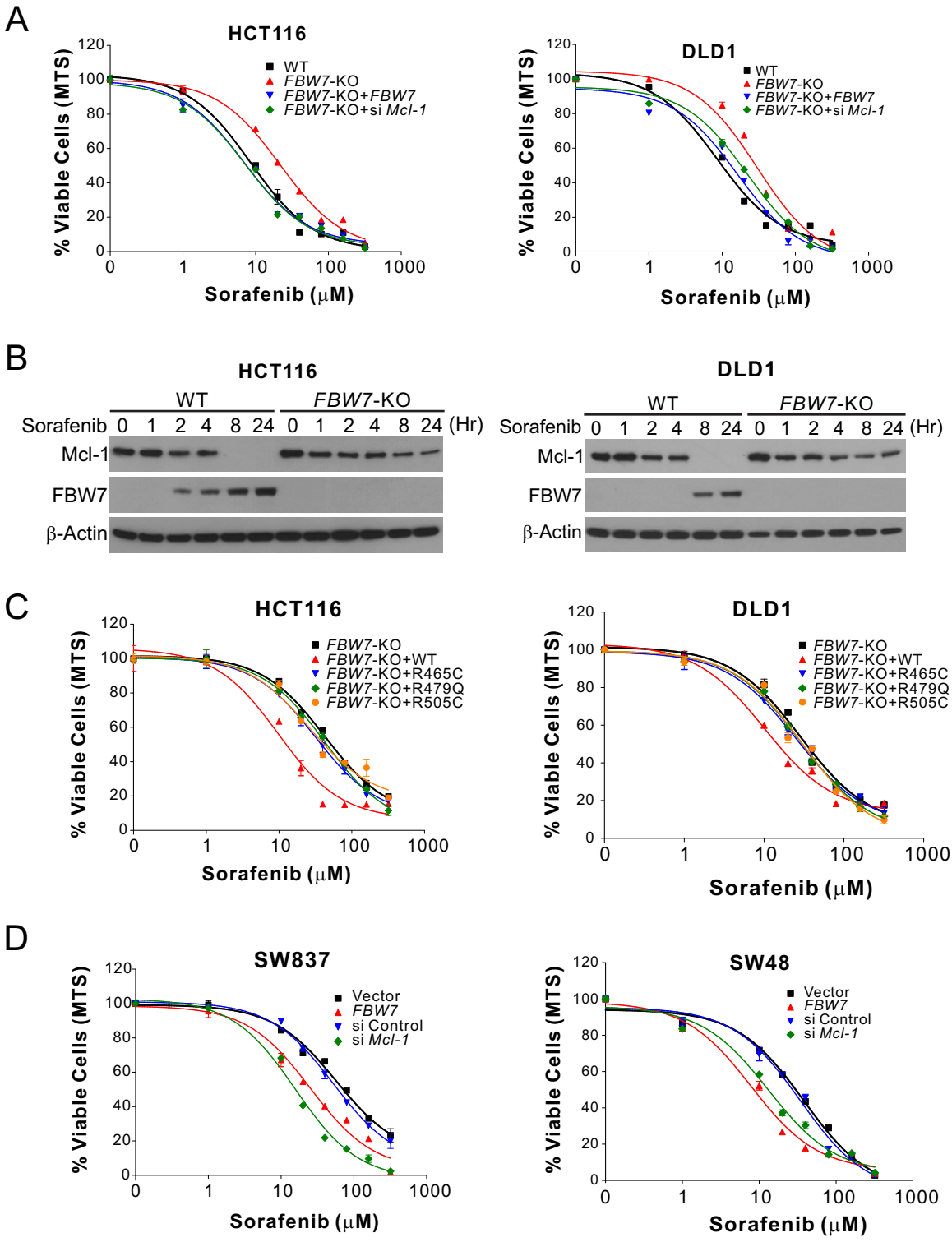

A
HCT116
% Viable Cells (MTS)
Sorafenib (μM)
WT
FBW7-KO
FBW7-KO+FBW7
FBW7-KO+si Mcl-1
DLD1
B
HCT116
DLD1
WT
FBW7-KO
Sorafenib 0 1 2 4 8 24 0 1 2 4 8 24 (Hr)
Mcl-1
FBW7
β-Actin
C
HCT116
DLD1
FBW7-KO
FBW7-KO+WT
FBW7-KO+R465C
FBW7-KO+R479Q
FBW7-KO+R505C
D
SW837
SW48
Vector
FBW7
si Control
si Mcl-1

Figure S5

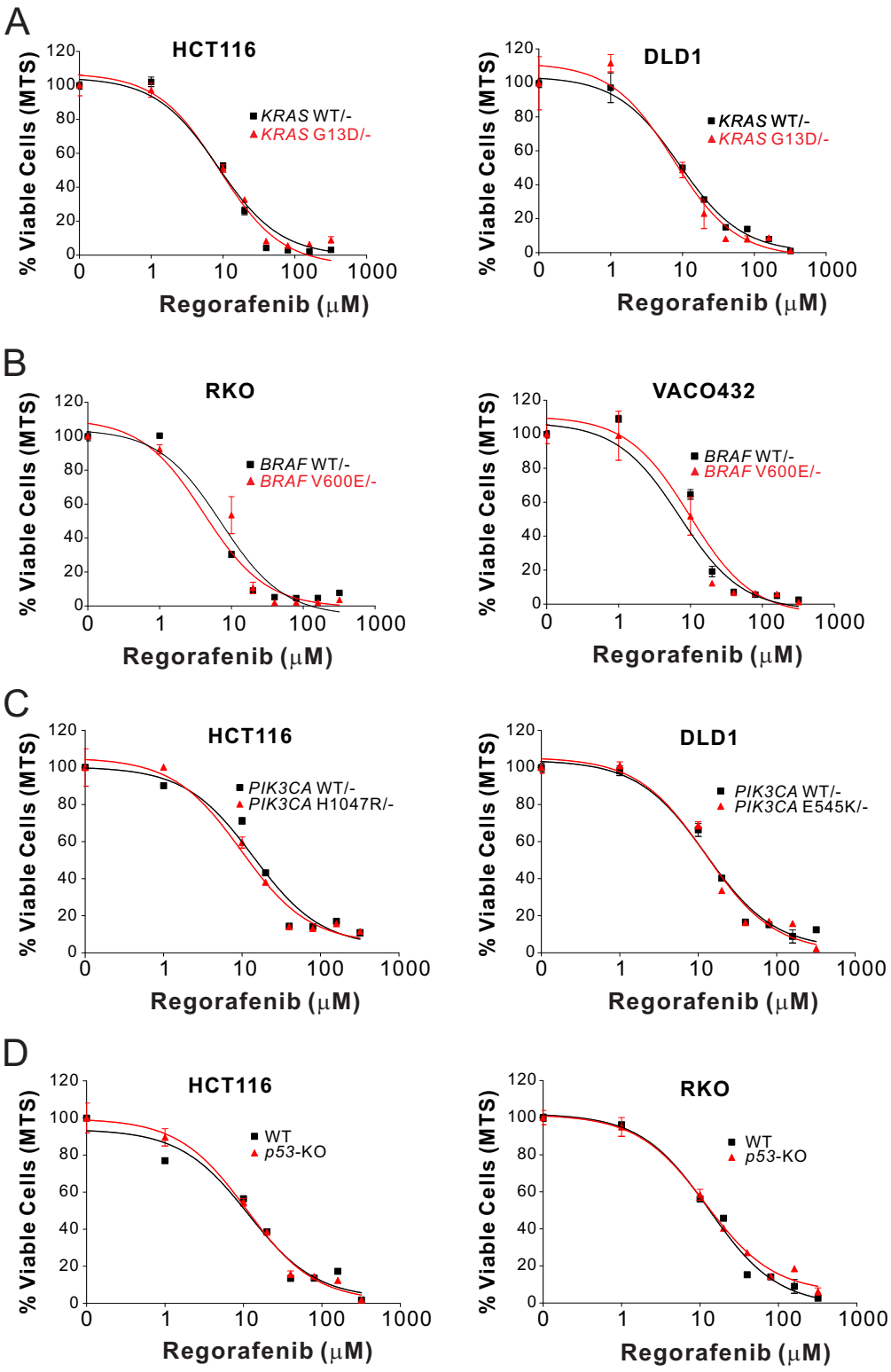

A
HCT116
KRAS WT/-
KRAS G13D/-
DLD1
KRAS WT/-
KRAS G13D/-
B
RKO
BRAF WT/-
BRAF V600E/-
VACO432
BRAF WT/-
BRAF V600E/-
C
HCT116
PIK3CA WT/-
PIK3CA H1047R/-
DLD1
PIK3CA WT/-
PIK3CA E545K/-
D
HCT116
WT
p53-KO
RKO
WT
p53-KO
% Viable Cells (MTS)
Regorafenib (μM)

Figure S6

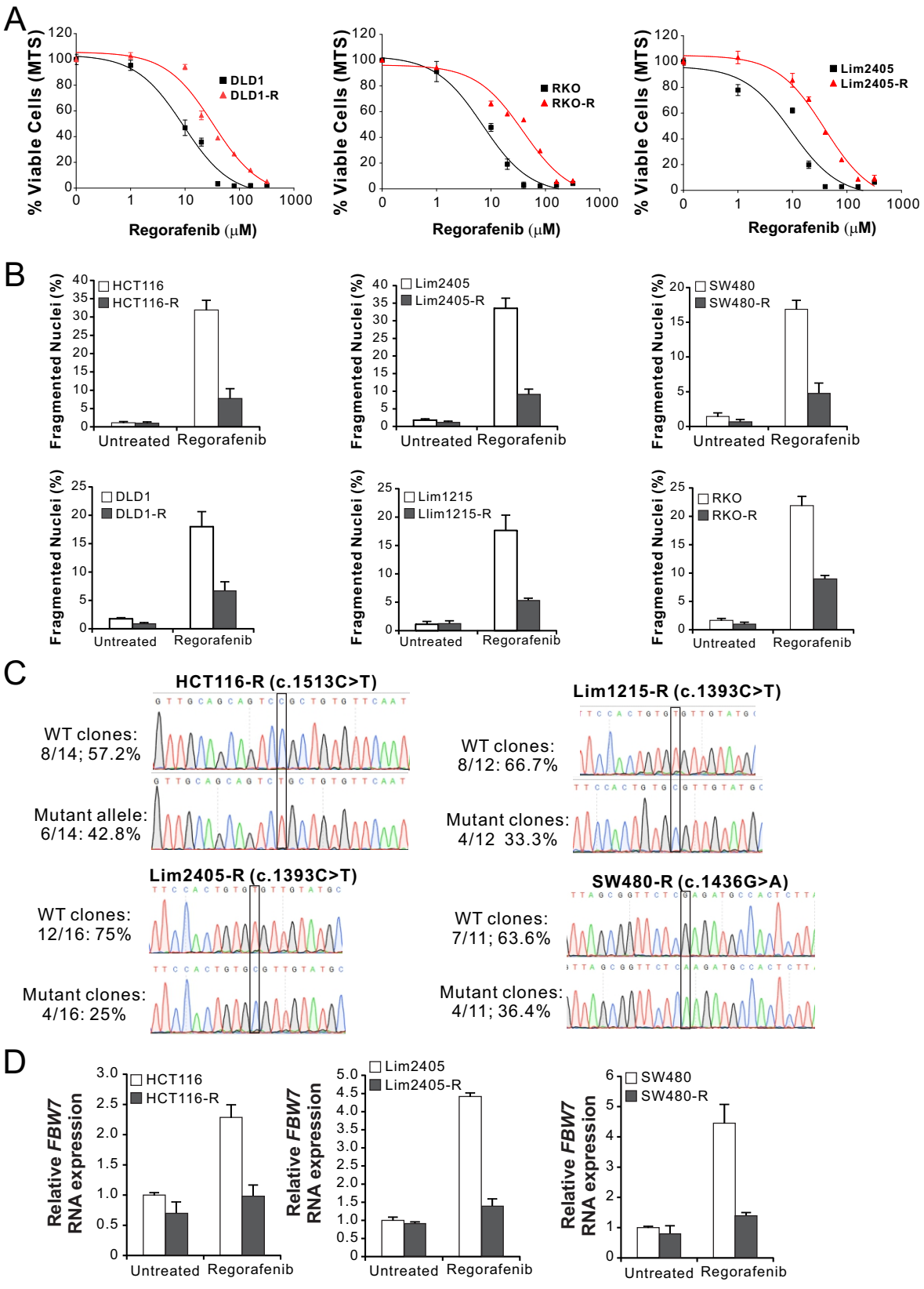

A
% Viable Cells (MTS)
Regorafenib (μM)
DLD1
DLD1-R
RKO
RKO-R
Lim2405
Lim2405-R
B
Fragmented Nuclei (%)
HCT116
HCT116-R
Lim2405
Lim2405-R
SW480
SW480-R
DLD1
DLD1-R
Lim1215
Llim1215-R
RKO
RKO-R
Untreated
Regorafenib
C
HCT116-R (c.1513C>T)
WT clones: 8/14; 57.2%
Mutant allele: 6/14: 42.8%
Lim1215-R (c.1393C>T)
WT clones: 8/12: 66.7%
Mutant clones: 4/12 33.3%
Lim2405-R (c.1393C>T)
WT clones: 12/16: 75%
Mutant clones: 4/16: 25%
SW480-R (c.1436G>A)
WT clones: 7/11; 63.6%
Mutant clones: 4/11; 36.4%
D
Relative FBW7 RNA expression
HCT116
HCT116-R
Lim2405
Lim2405-R
SW480
SW480-R
Untreated
Regorafenib

Figure S7

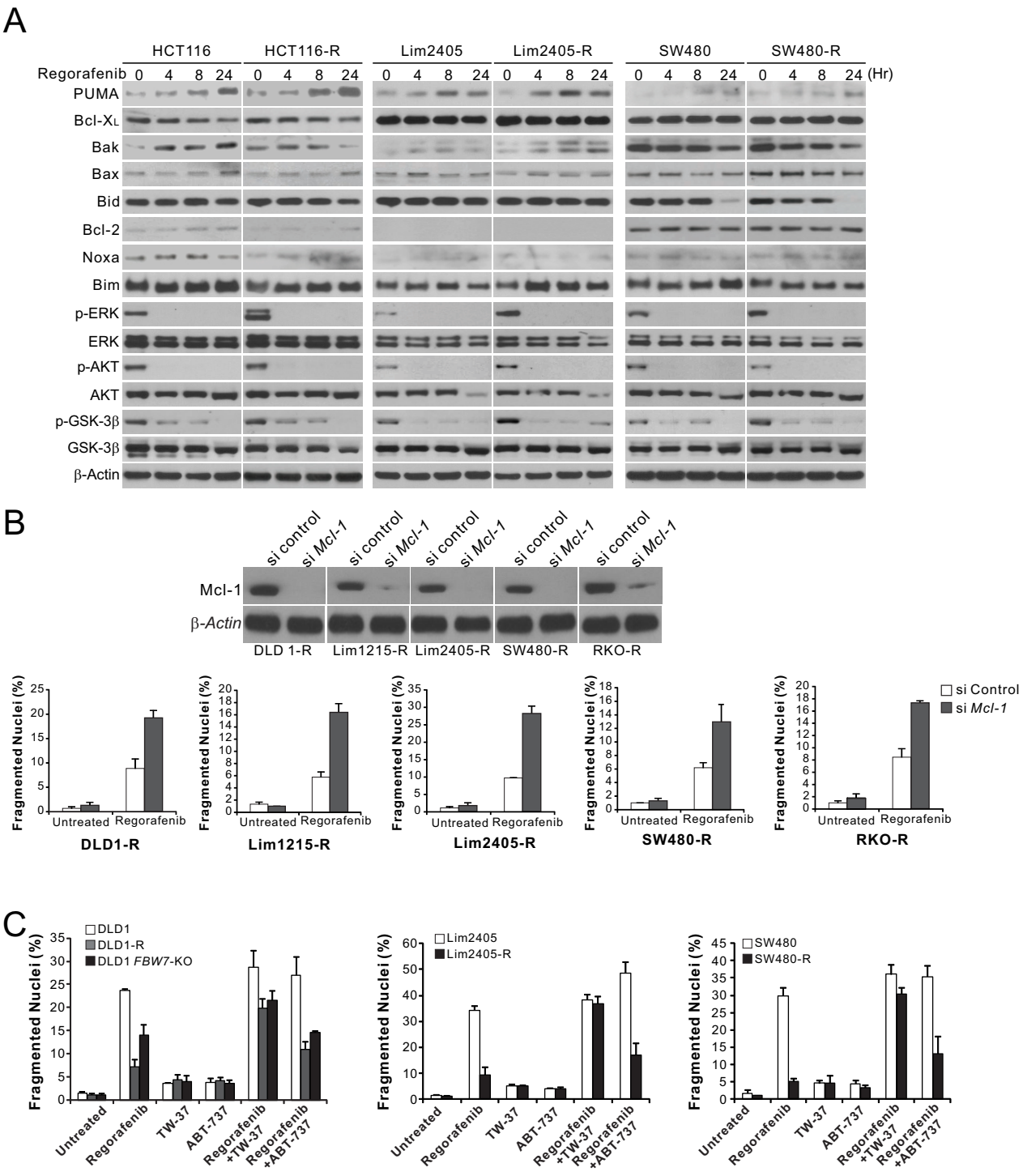
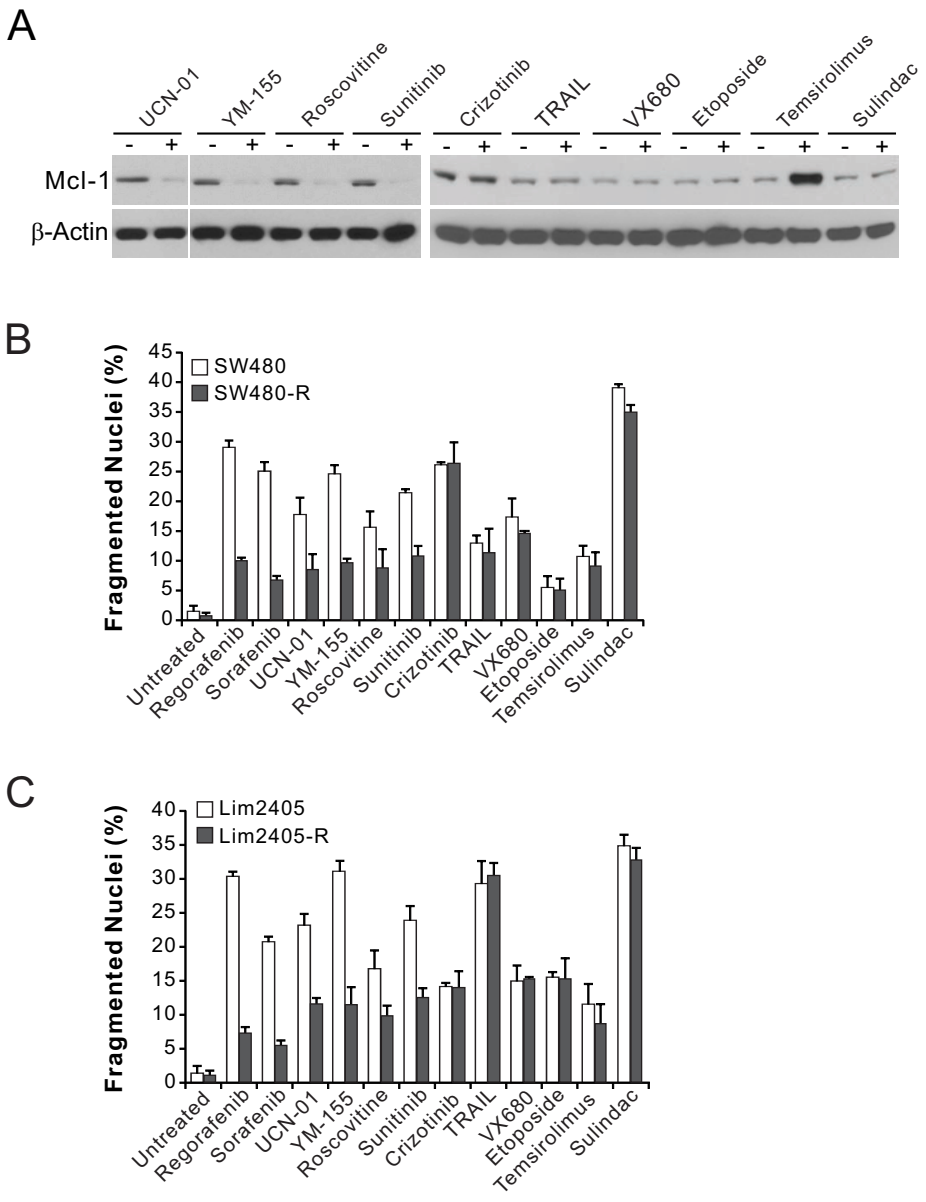

A
HCT116
HCT116-R
Lim2405
Lim2405-R
SW480
SW480-R
Regorafenib 0 4 8 24 0 4 8 24 0 4 8 24 0 4 8 24 0 4 8 24 0 4 8 24 (Hr)
PUMA
Bcl-XL
Bak
Bax
Bid
Bcl-2
Noxa
Bim
p-ERK
ERK
p-AKT
AKT
p-GSK-3β
GSK-3β
β-Actin
B
si control
si Mcl-1
Mcl-1
β-Actin
DLD 1-R
Lim1215-R
Lim2405-R
SW480-R
RKO-R
Fragmented Nuclei (%)
Untreated
Regorafenib
DLD1-R
Lim1215-R
Lim2405-R
SW480-R
RKO-R
si Control
si Mcl-1
C
DLD1
DLD1-R
DLD1 FBW7-KO
Lim2405
Lim2405-R
SW480
SW480-R
Fragmented Nuclei (%)
Untreated
Regorafenib
TW-37
ABT-737
Regorafenib +TW-37
Regorafenib +ABT-737

Figure S8


A
UCN-01
YM-155
Roscovitine
Sunitinib
Crizotinib
TRAIL
VX680
Etoposide
Temsirolimus
Sulindac
- + - + - + - + - + - + - + - + - + - +
Mcl-1
β-Actin
B
Fragmented Nuclei (%)
SW480
SW480-R
0 5 10 15 20 25 30 35 40 45
Untreated
Regorafenib
Sorafenib
UCN-01
YM-155
Roscovitine
Sunitinib
Crizotinib
TRAIL
VX680
Etoposide
Temsirolimus
Sulindac
C
Fragmented Nuclei (%)
Lim2405
Lim2405-R
0 5 10 15 20 25 30 35 40
Untreated
Regorafenib
Sorafenib
UCN-01
YM-155
Roscovitine
Sunitinib
Crizotinib
TRAIL
VX680
Etoposide
Temsirolimus
Sulindac