**Supplemental figure legends**

**Figure S1. HDACi promote GSK3β-mediated Mcl-1 phosphorylation and apoptosis, but not Mcl-1 degradation, in colon cancer cells. (A)** *Left*, western blotting of Mcl-1 in HCT116 cells treated with control vehicle, 40 μM regorafenib, 4 μM SAHA, or 5 μM MS-275 for 1 hr, and then with 10 μg/mL of the translation inhibitor cycloheximide (CHX) at indicated time points; *right*, Mcl-1 expression levels were quantified by the Image J program and normalized to the loading control β-actin. **(B)** *Mcl-1* mRNA expression in HCT116 cells treated with SAHA or MS-275 as in (A) at the indicated time points was analyzed by real-time RT-PCR. **(C)** Western blotting of indicated proteins in DLD1 colon cancer cells treated with SAHA or MS-275 as in (A) at indicated time points. **(D)** Western blotting of indicated proteins in RKO colon cancer cells treated with SAHA or MS-275 as in (A) at indicated time points. **(E)** HCT116 cells transfected with control scrambled or *GSK3β* siRNA were treated with 4 μM SAHA or 5 μM MS-275 for 24 hr. *Left*, western blotting of indicated proteins; p-Mcl-1: S159/T163; *right*, apoptosis was analyzed by counting condensed and fragmented nuclei after nuclear staining. **(F)** DLD1cells transfected with V5-tagged WT or 4A mutant Mcl-1 (S121A/E125A/S159A/T163A) and treated with 4 μM SAHA or 5 μM MS-275 for 24 hr. *Upper*, western blotting of transfected Mcl-1; *lower*, analysis of apoptosis as in (E). **(G)** RKO cells were transfected, treated and analyzed as in (F). In (A), (B), (E), (F) and (G), results were expressed as means ± s.d. of three independent experiments. NS, $P > 0.05$; *****, $P < 0.05$; ******, $P < 0.01$.

**Figure S2. Targeting Mcl-1 phosphorylation sites blocks apoptosis with or without involvement of Mcl-1 degradation. (A)** Schematic representation of the *Mcl-1* genomic locus and the knock-in vector highlighting the putative phosphorylation sites of Mcl-1. **(B)** Apoptosis in WT and *Mcl-1*-KI HCT116 cells transfected with control scrambled or *Mcl-1* siRNA and treated with 4

μM SAHA or 5 μM MS-275 for 24 hr was analyzed by Annexin V/Propidium Iodide (PI) staining followed by flow cytometry. **(C)** Apoptosis in WT and *Mcl-1*-KI HCT116 cells treated with indicated agents for 48 hr was analyzed by counting condensed and fragmented nuclei after nuclear staining. ABT-263, 5 μM; ABT-737, 5 μM; MS-275, 5 μM; SAHA, 4 μM; UCN-01,1 μM; sunitinib, 15 μM; sorafenib 20 μM; regorafenib, 40 μM. Results were expressed as means ± s.d. of three independent experiments.

**Figure S3. FBW7 induction determines whether Mcl-1 is degraded in drug-induced apoptosis.** **(A)** Real-time RT-PCR analysis of *FBW7* mRNA expression in HCT116 cells treated with 4 μM SAHA, 5 μM MS-275, 20 μM sorafenib, or 40 μM regorafenib for 24 hr. **(B)** Real-time RT-PCR analysis of *Mule* mRNA expression in cells treated as in (A). **(C)** Western blotting of FBW7 at the indicated time points in HCT116 cells treated with SAHA or regorafenib as in (A). **(D)** Apoptosis in WT and *FBW7*-KO HCT116 treated with SAHA or MS-275 as in (A) was analyzed by counting condensed and fragmented nuclei after nuclear staining. **(E)** Apoptosis in WT and *FBW7*-KO DLD1 treated with SAHA or MS-275 as in (A) was analyzed as in (D). **(F)** Western blotting of indicated proteins in HCT116 cells transfected with HA-tagged WT FBW7 or indicated mutants (R465C or R505C) and treated with SAHA or MS-275 as in (A). In (A), (B), (D) and (E), results were expressed as means ± s.d. of three independent experiments. NS, $P > 0.05$; *****, $P < 0.05$; ******, $P < 0.01$; *******, $P < 0.001$.

**Figure S4. HDACi induce multiple BH-only proteins and require Bax for apoptosis induction.** **(A)-(C)** Real-time RT-PCR analysis of *PUMA* (A), *Bim* (B) and *Noxa* (C) mRNA expression in HCT116 cells treated with SAHA or MS-275 at the indicated time points. **(D)**, **(E)** *Left*, schematic diagrams of genomic locus and the targeting construct used for knocking out *Bim* (D) and *Noxa* (E) in HCT116 cells. Filled boxes represent exons. Homologous recombination resulted in a deletion

of the exon 2 of *Bim* and a part of the exon 2 of *Noxa*. The same constructs were used in the second round of gene targeting after the *Neo*, flanked by two LoxP sites, was excised from the heterozygous cells by Cre recombinase. The positions of the primers (P1 and P2) for PCR screening are indicated. *Right*, western blots of Bim (D) and Noxa (E) in two KO cell lines (KO1 and KO2) identified.

**Figure S5. HDACi-induced apoptosis is dependent on PUMA, Bim, Noxa and Bax. (A)** Apoptosis in WT, *PUMA*-KO, *Bim*-KO and *Noxa*-KO HCT116 cells treated with 4 μM SAHA or 5 μM MS-275 for 24 hr was analyzed by Annexin V/PI staining followed by flow cytometry. **(B)** MTS analysis of viability of WT (black) and *PUMA*-KO (red) DLD1 cells treated with SAHA at different concentrations for 72 hr. **(C)** Apoptosis in WT and *BAX*-knockout (*BAX*-KO) HCT116 cells treated with SAHA for 24 hr was analyzed by counting condensed and fragmented nuclei after nuclear staining. ******, $P < 0.01$. **(D)** Western blotting of cleaved (C) caspases 3 and 9 in WT and *BAX*-KO HCT116 cells treated with SAHA for 24 hr. In (A), (C) and (D), SAHA: 4 μM; MS-275: 5 μM. In (B) and (C), results were expressed as means ± s.d. of three independent experiments.

**Figure S6. Phosphorylation-dead Mcl-1 binds to PUMA, Bim and Noxa. (A)**, **(B)** WT and *Mcl-1*-KI HCT116 cells were treated with 4 μM SAHA for 24 hr. Immunoprecipitation (IP) was performed on Noxa (A), or Bim (B), followed by western blotting of indicated proteins. **(C)** WT and *Mcl-1*-KI HCT116 cells were treated with 5 μM MS-275 for 24 hr. Mcl-1 IP was performed followed by western blotting of indicated proteins. **(D)** HCT116 cells were co-transfected with V5-Mcl-1 (WT or E125A mutant) along with HA-PUMA or HA-Bim. V5 (Mcl-1) IP was performed followed by western blotting of indicated tags.

**Figure S7. HDACi-induced apoptosis can be restored by Mcl-1 inhibitors in *Mcl-1*-KI cells.** Apoptosis in WT and *Mcl-1*-KI HCT116 cells treated with 4 μM SAHA, 5 μM TW-37, 5 μM UMI-

77 alone, or the indicated combinations was by analyzed Annexin V/PI staining followed by flow cytometry.

Figure S1

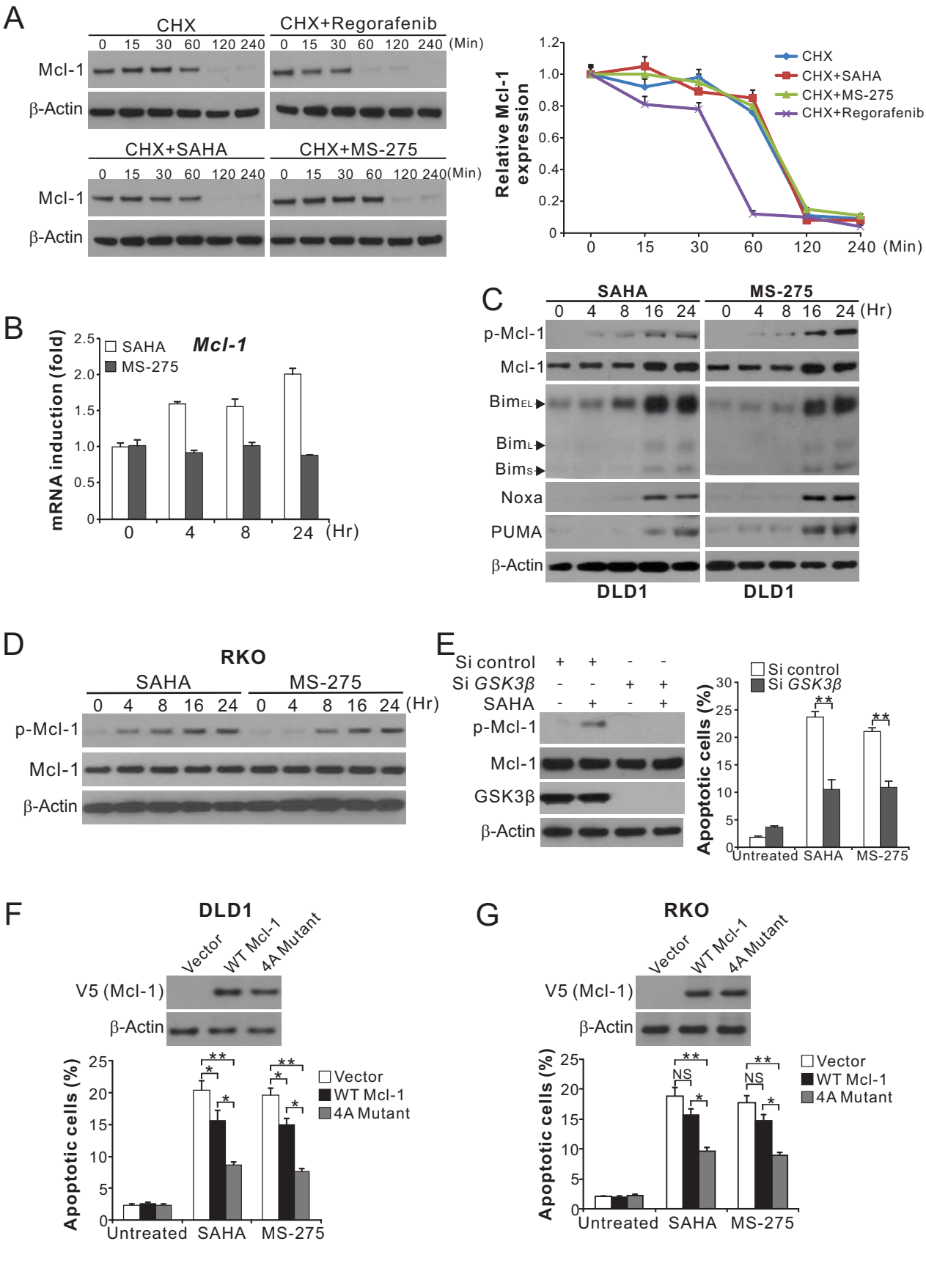

A
CHX
CHX+Regorafenib
0 15 30 60 120 240 (Min)
Mcl-1
β-Actin
CHX+SAHA
CHX+MS-275
Relative Mcl-1 expression
CHX
CHX+SAHA
CHX+MS-275
CHX+Regorafenib
B
Mcl-1
SAHA
MS-275
mRNA induction (fold)
0 4 8 24 (Hr)
C
SAHA
MS-275
p-Mcl-1
Mcl-1
BimEL
BimL
BimS
Noxa
PUMA
β-Actin
DLD1
D
RKO
SAHA
MS-275
p-Mcl-1
Mcl-1
β-Actin
E
Si control
Si GSK3β
SAHA
p-Mcl-1
Mcl-1
GSK3β
β-Actin
Apoptotic cells (%)
Untreated SAHA MS-275
F
DLD1
Vector
WT Mcl-1
4A Mutant
V5 (Mcl-1)
β-Actin
Apoptotic cells (%)
G
RKO
NS

Figure S2

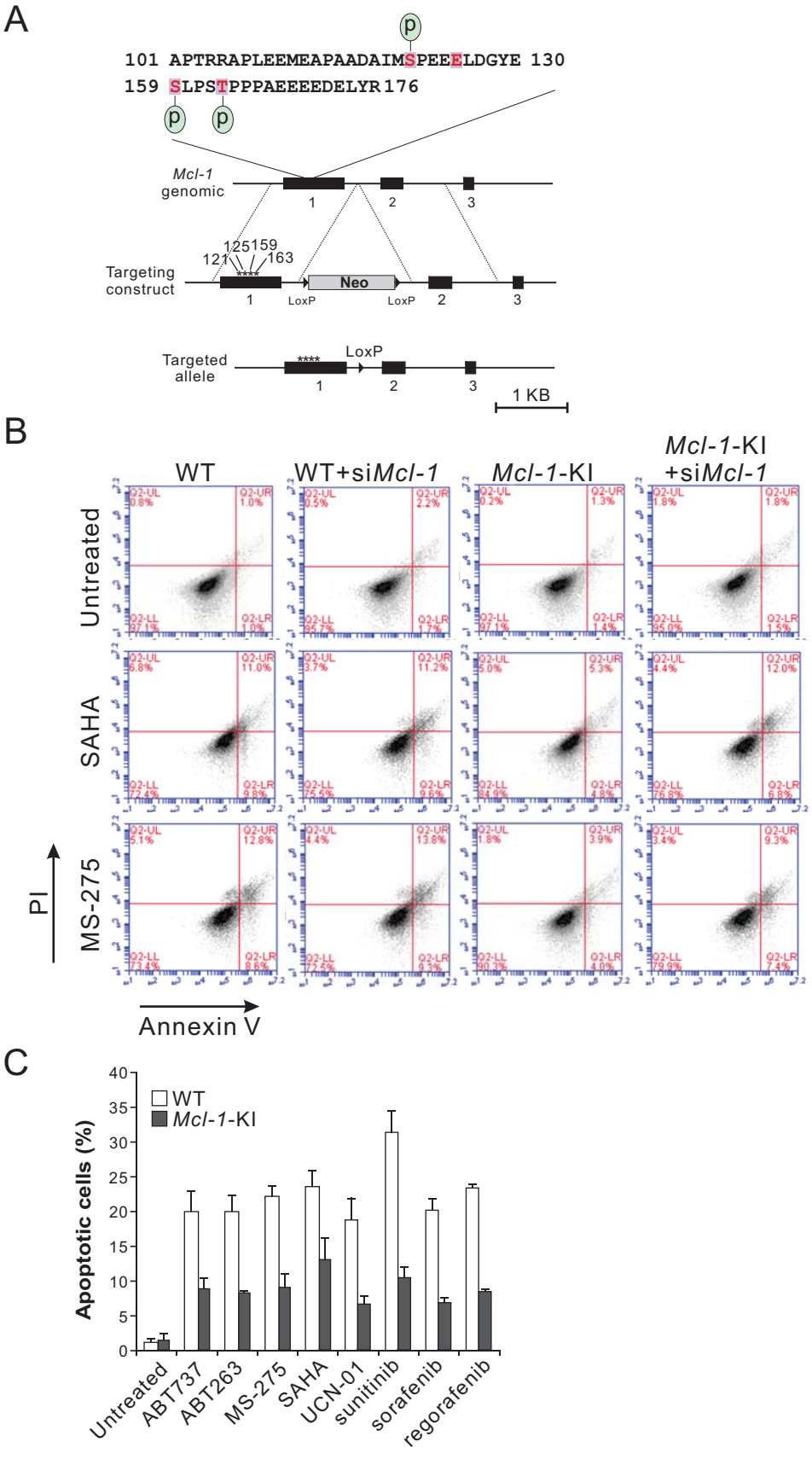
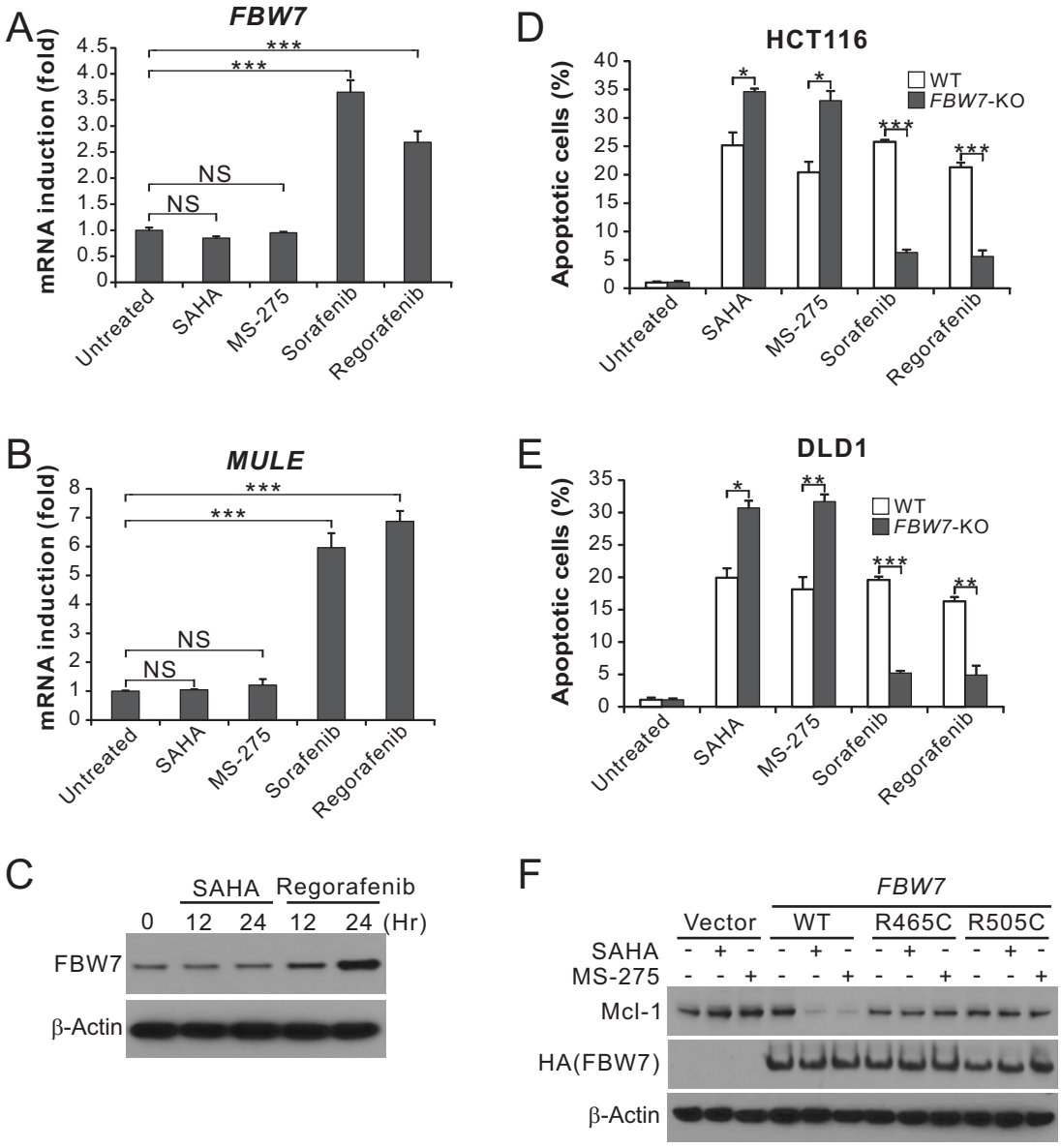

A
101 APTRRAPLEEMEAPAADAIMSPEEELDGYE 130
159 SLPSTPPPAEEEEDELYR 176
Mcl-1 genomic
1
2
3
125159
121
163
Targeting construct
Neo
LoxP
LoxP
Targeted allele
LoxP
1 KB
B
WT
WT+siMcl-1
Mcl-1-KI
Mcl-1-KI +siMcl-1
Untreated
SAHA
MS-275
PI
Annexin V
C
Apoptotic cells (%)
WT
Mcl-1-KI
Untreated
ABT737
ABT263
MS-275
SAHA
UCN-01
sunitinib
sorafenib
regorafenib

Figure S3


A
FBW7
mRNA induction (fold)
***
***
NS
NS
Untreated
SAHA
MS-275
Sorafenib
Regorafenib
B
MULE
mRNA induction (fold)
***
***
NS
NS
Untreated
SAHA
MS-275
Sorafenib
Regorafenib
C
SAHA
Regorafenib
0
12
24
12
24 (Hr)
FBW7
β-Actin
D
HCT116
Apoptotic cells (%)
WT
FBW7-KO
Untreated
SAHA
MS-275
Sorafenib
Regorafenib
E
DLD1
Apoptotic cells (%)
WT
FBW7-KO
Untreated
SAHA
MS-275
Sorafenib
Regorafenib
F
FBW7
Vector
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R465C
R505C
SAHA
MS-275
Mcl-1
HA(FBW7)
β-Actin

Figure S4

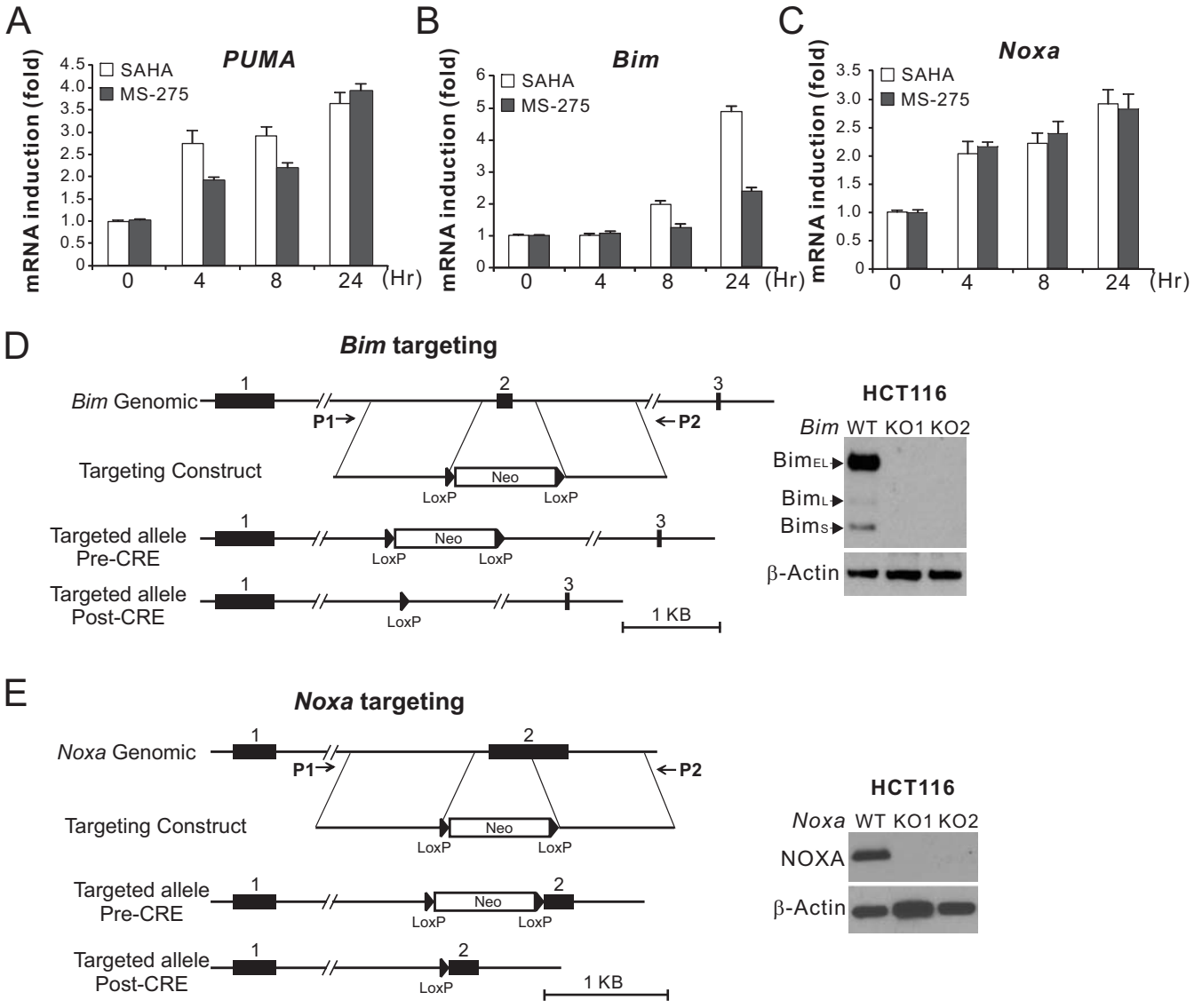

A
PUMA
mRNA induction (fold)
SAHA
MS-275
0 4 8 24 (Hr)
B
Bim
C
Noxa
D
Bim targeting
Bim Genomic
P1
P2
Targeting Construct
Neo
LoxP
Targeted allele Pre-CRE
Targeted allele Post-CRE
1 KB
HCT116
Bim WT KO1 KO2
BimEL
BimL
BimS
β-Actin
E
Noxa targeting
Noxa Genomic
NOXA

Figure S5

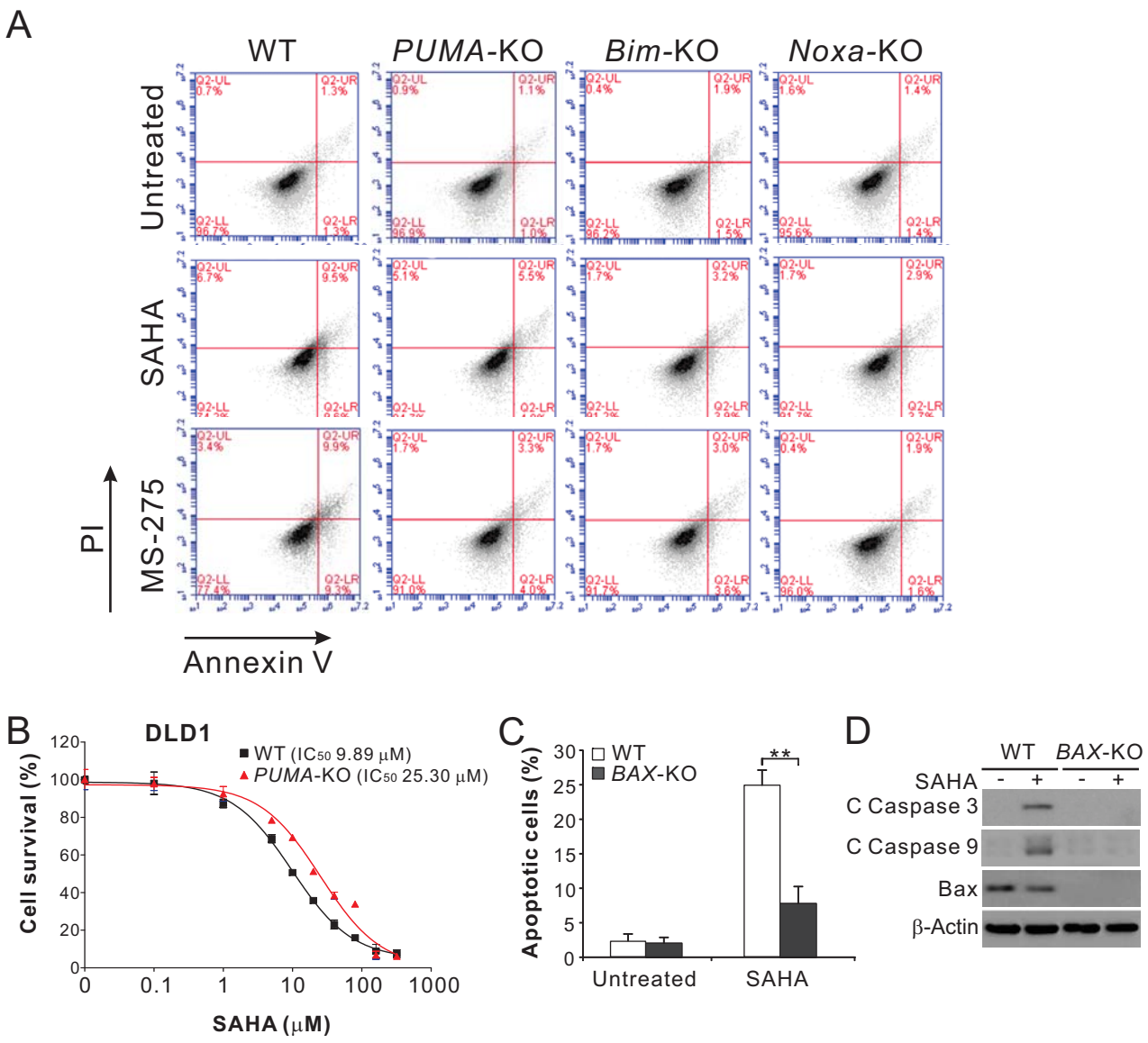

A
WT
PUMA-KO
Bim-KO
Noxa-KO
Untreated
SAHA
MS-275
PI
Annexin V
B
DLD1
WT (IC50 9.89 μM)
PUMA-KO (IC50 25.30 μM)
Cell survival (%)
SAHA (μM)
C
WT
BAX-KO
Apoptotic cells (%)
**
Untreated
SAHA
D
WT
BAX-KO
SAHA
C Caspase 3
C Caspase 9
Bax
β-Actin

Figure S6

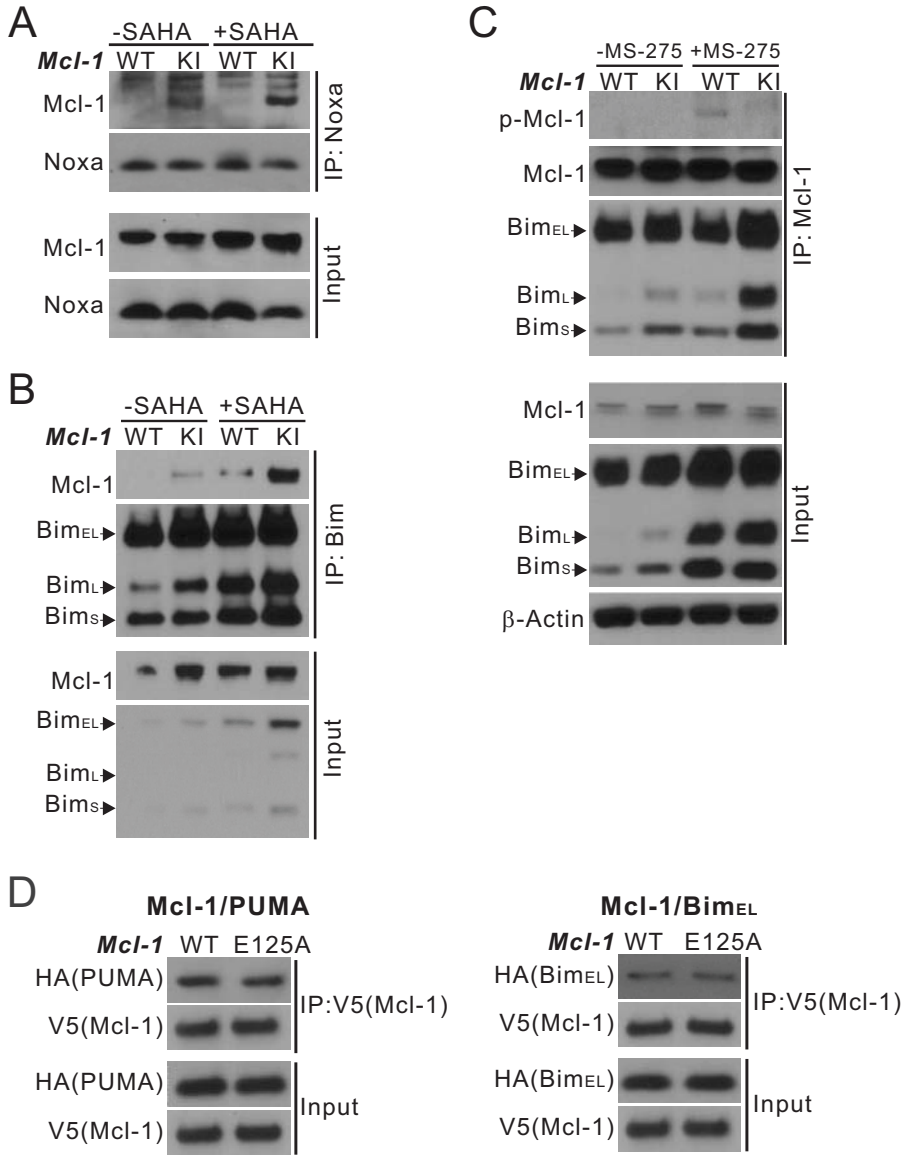
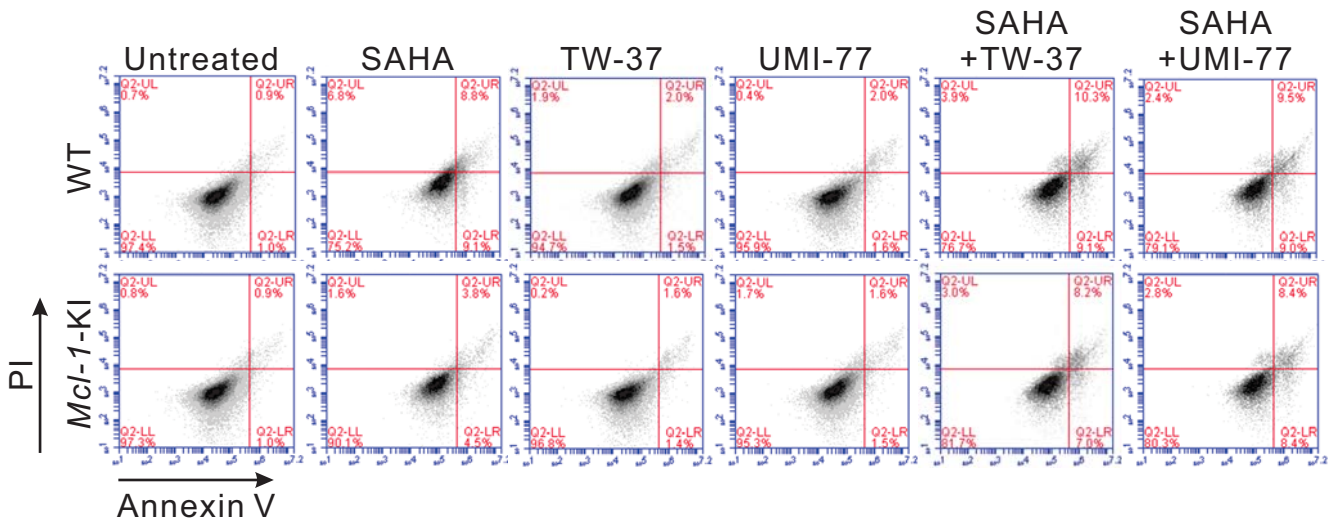

A
-SAHA
+SAHA
Mcl-1
WT
KI
WT
KI
Mcl-1
Noxa
IP: Noxa
Mcl-1
Noxa
Input
B
-SAHA
+SAHA
Mcl-1
WT
KI
WT
KI
Mcl-1
BimEL
BimL
BimS
IP: Bim
Mcl-1
BimEL
BimL
BimS
Input
C
-MS-275
+MS-275
Mcl-1
WT
KI
WT
KI
p-Mcl-1
Mcl-1
BimEL
BimL
BimS
IP: Mcl-1
Mcl-1
BimEL
BimL
BimS
β-Actin
Input
D
Mcl-1/PUMA
Mcl-1
WT
E125A
HA(PUMA)
V5(Mcl-1)
IP:V5(Mcl-1)
HA(PUMA)
V5(Mcl-1)
Input
Mcl-1/BimEL
Mcl-1
WT
E125A
HA(BimEL)
V5(Mcl-1)
IP:V5(Mcl-1)
HA(BimEL)
V5(Mcl-1)
Input

Figure S7


Untreated
SAHA
TW-37
UMI-77
SAHA +TW-37
SAHA +UMI-77
WT
Mcl-1-KI
PI
Annexin V