SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Luciferase Assays- Cells were seeded on 24-well plates and grown to 70% confluency. Cells were then transfected with 100 ng of reporter constructs and 10 ng of the renilla luciferase internal control plasmid pRL-SV40 using Lipofectamine2000 transfection protocol. Twenty-four hours after transfection, cells were treated with 5 μM SAHA or 200 nM LAQ for additional 12 hours. Cells were then lysed and subjected to Dual-Luciferase assay as per the manufacturer's recommendations (Promega).

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. HDACi have no effect on $p53^{L22Q/W23S}$ transactivation. $p53^{-/-}$, $p53^{WT}$ or $p53^{L22Q/W23S}$ HCT116 cells were transiently co-transfected with the indicated firefly luciferase reporter constructs containing p21 or mdm2 promoter together with pRL-SV40 renilla luciferase vector. Cells were treated with DMSO, SAHA or LAQ and subjected to Dual-Luciferase assay. The results are represented as the mean ratio of firefly/renilla luciferase activities ± SD, n=3.

Fig. S2. Cell death and p53 expression in response to HDACi. (**A**) HCT116 wild type ($p53^{+/+}$) and $p53^{-/-}$ cells stably transfected with empty vector (Puro or Bsd), $p53^{WT}$ or $p53^{L22Q/W23S}$ were treated with either 5 μM SAHA or 200 nM LAQ for the indicated periods of time. The percentage of cell viability was determined by trypan blue dye exclusion assay. (**B**) The same cell lines treated as above for 18 hours were subjected to immunoblot analysis.

Fig. S3. Knockdown of mutant p53 reduces HDACi-induced apoptosis. (**A**) HT-29 cells infected with either scrambled control (shScr) or p53 targeting shRNA (shp53) lentivirus were treated with 5 μM SAHA or 200 nM LAQ for 36 hours and subjected to immunoblot and caspase-3 activity assay. (**B, C**) SW480 and HT-29 cells infected with shScr or shp53 lentivirus were treated with 5 μM SAHA or 200 nM LAQ for the indicated times, and cell viability was determined by trypan blue exclusion assay. (**D**) SW480 cells infected with shScr or shp53 lentivirus were treated with DMSO or 200 nM LAQ for 24 hours and subjected to Annexin V-APC/7AAD staining.

Fig. S4. SirT1 does not affect LAQ824-induced p53-dependent apoptosis. (**A, B**) H1299 $p53^{-/-}$ and $p53^{D281G}$ cells were transfected with empty pcDNA3.1 or pCDNA3.1-SirT1 expression vectors and then treated with DMSO or 200 nM LAQ for 24 hours and subjected to immunoblot and caspase-3 assays. (**C, D**) H1299 $p53^{-/-}$ and $p53^{D281G}$ cells were treated with DMSO, 1 μM EX527, 200 nM LAQ or the combination of 1 μM EX527 and 200 nM LAQ for 24 hours and subjected to western blot and AnnexinV-APC/7AAD staining.

Fig. S5. HCT116 $p53^{-/-}$ cells stably expressing TAP or TAP-$p53^{L22Q/W23S}$ were treated with 200 nM LAQ for the indicated periods of time. The percentage of cell viability was determined by trypan blue dye exclusion assay.

Fig. S6. H1299 $p53^{-/-}$ and $p53^{D281G}$ cells were infected with shScr or shKu70 lentivirus for 24 hours, treated with DMSO or 200 nM LAQ for 24 hours, and subjected to immunoblot analysis.

Fig. S7. Acetylation of p53 is required for Bax translocation but not for p53 binding to Bcl-XL. (**A**) HCT116 $p53^{-/-}$ cells stably expressing control Puro or $p53^{L22Q/W23S}$ were treated with DMSO, 5 μM SAHA or 200 nM LAQ for 18 hours and subjected to subcellular fractionation and immunoblot analysis. (**B**) HCT116 $p53^{-/-}$ cells stably transfected with empty (Puro), Myc-$p53^{L22Q/W23S}$ or Myc-$p53^{L22Q/W23S\text{-}3KR}$ were treated with DMSO, 5 μM SAHA or 200 nM LAQ for 16 hours and subjected to subcellular fractionation and immunoblot analysis. (**C, D**) HCT116 $p53^{-/-}$ cells were transiently transfected with Bcl-XL and the indicated Myc-tagged p53 expression plasmids or empty control vector for 36 hours, treated with or

without 200 nM LAQ for additional 12 hours, and subjected to immunoprecipitation in CHAPS lysis buffer with anti-Myc monoclonal antibody, followed by immunoblot analysis with the indicated antibodies.

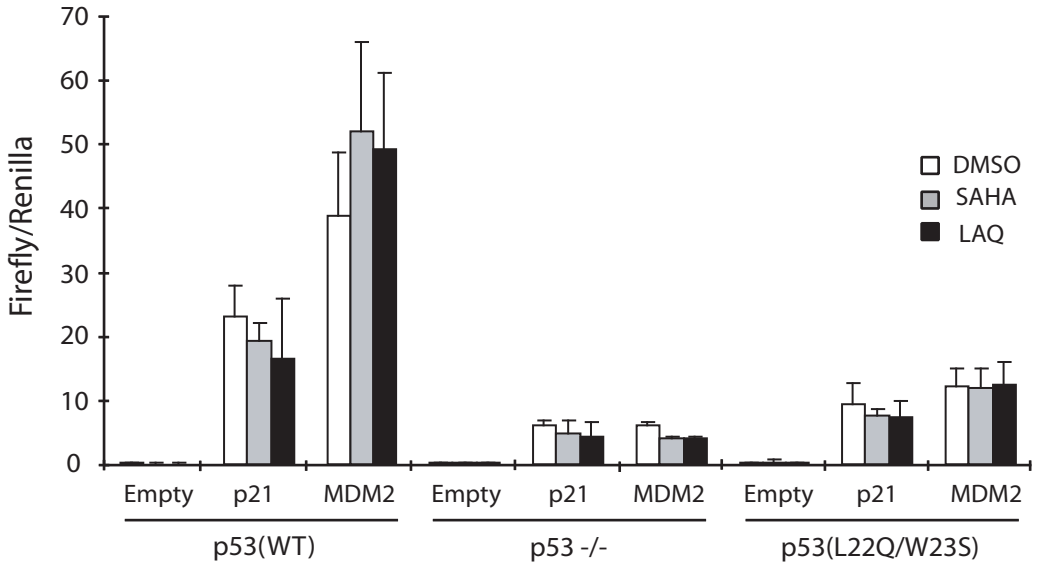

70
60
50
40
30
20
10
0
Firefly/Renilla
DMSO
SAHA
LAQ
Empty
p21
MDM2
Empty
p21
MDM2
Empty
p21
MDM2
p53(WT)
p53 -/-
p53(L22Q/W23S)

Figure S1

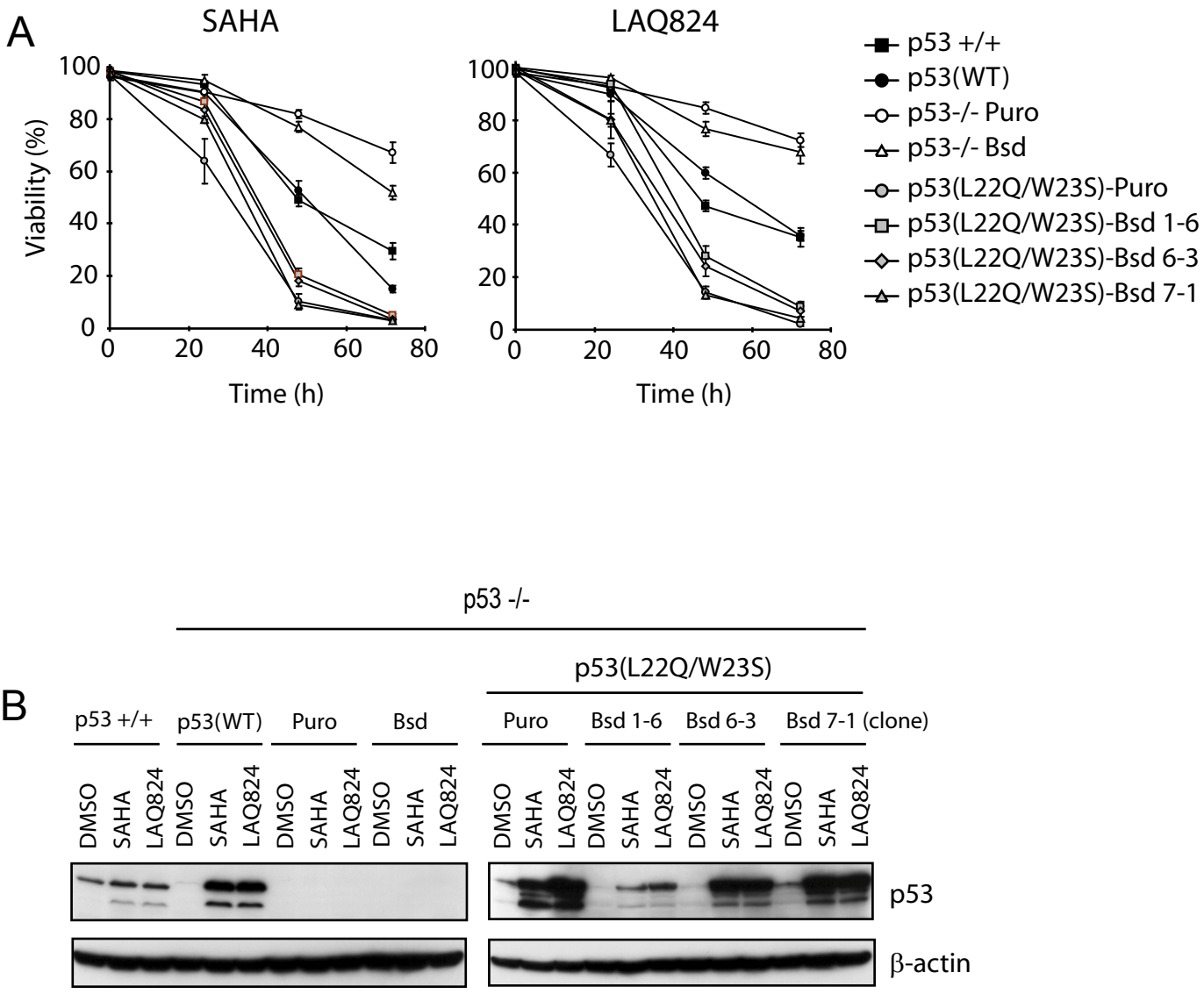

A
SAHA
LAQ824
Viability (%)
Time (h)
Time (h)
p53 +/+
p53(WT)
p53-/- Puro
p53-/- Bsd
p53(L22Q/W23S)-Puro
p53(L22Q/W23S)-Bsd 1-6
p53(L22Q/W23S)-Bsd 6-3
p53(L22Q/W23S)-Bsd 7-1
B
p53 -/-
p53(L22Q/W23S)
p53 +/+
p53(WT)
Puro
Bsd
Puro
Bsd 1-6
Bsd 6-3
Bsd 7-1 (clone)
DMSO
SAHA
LAQ824
p53
β-actin

Figure S2

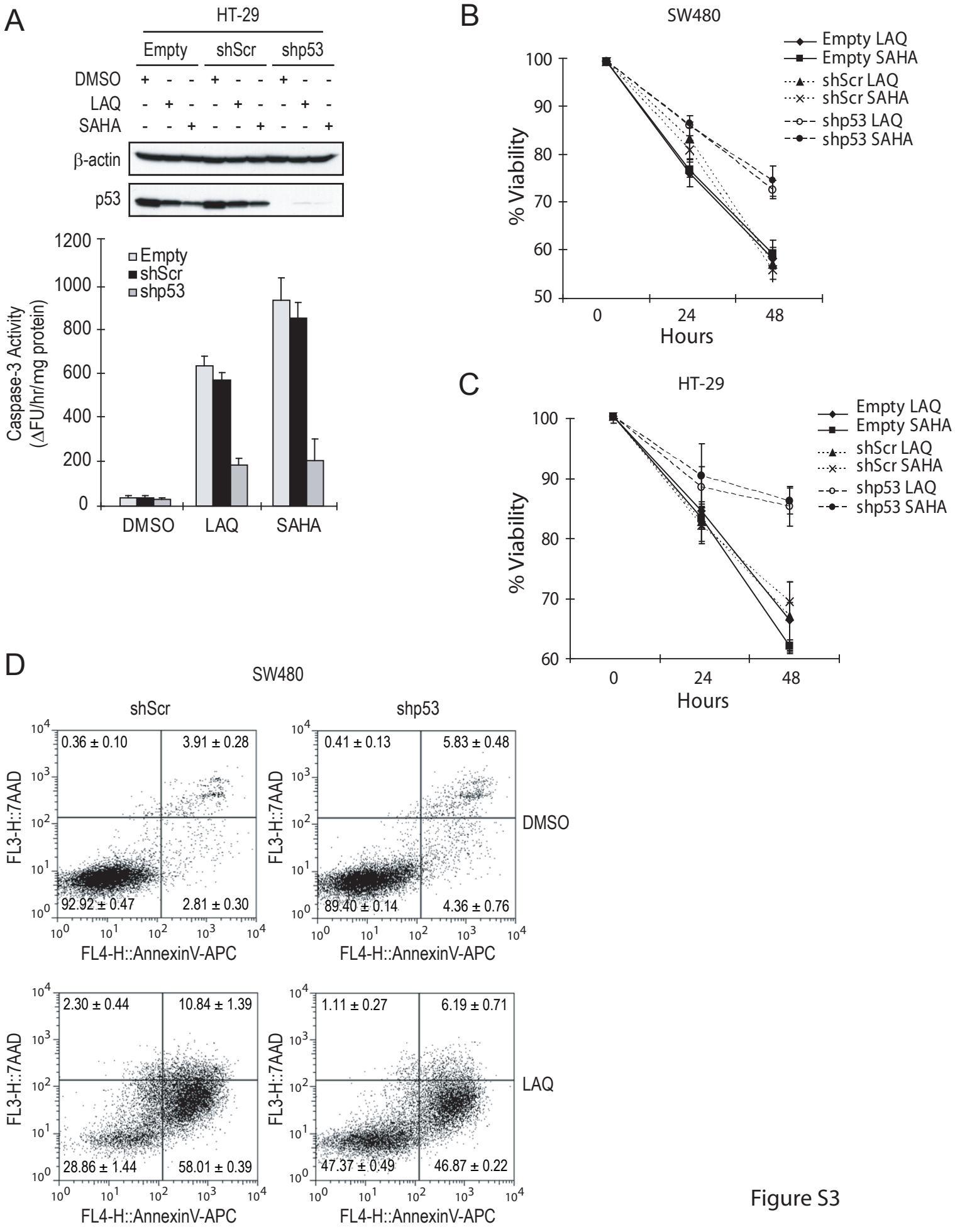

A
HT-29
Empty
shScr
shp53
DMSO
LAQ
SAHA
β-actin
p53
Caspase-3 Activity (ΔFU/hr/mg protein)
Empty
shScr
shp53
DMSO
LAQ
SAHA
B
SW480
% Viability
Hours
Empty LAQ
Empty SAHA
shScr LAQ
shScr SAHA
shp53 LAQ
shp53 SAHA
C
HT-29
% Viability
Hours
D
SW480
shScr
shp53
FL3-H::7AAD
FL4-H::AnnexinV-APC
0.36 ± 0.10
3.91 ± 0.28
92.92 ± 0.47
2.81 ± 0.30
0.41 ± 0.13
5.83 ± 0.48
89.40 ± 0.14
4.36 ± 0.76
DMSO
2.30 ± 0.44
10.84 ± 1.39
28.86 ± 1.44
58.01 ± 0.39
1.11 ± 0.27
6.19 ± 0.71
47.37 ± 0.49
46.87 ± 0.22
LAQ

Figure S3

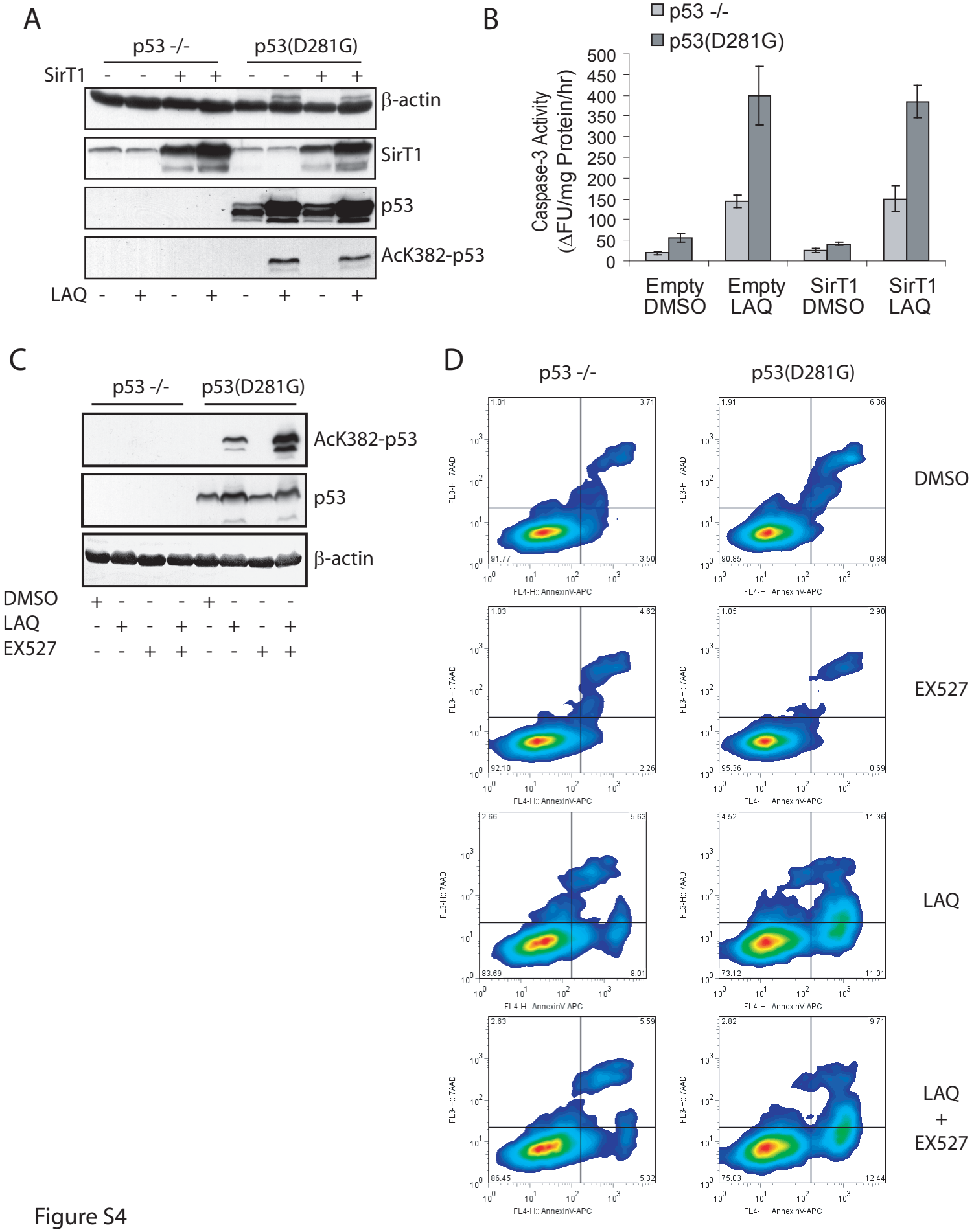

A
p53 -/-
p53(D281G)
SirT1
β-actin
SirT1
p53
AcK382-p53
LAQ
B
p53 -/-
p53(D281G)
Caspase-3 Activity (ΔFU/mg Protein/hr)
Empty DMSO
Empty LAQ
SirT1 DMSO
SirT1 LAQ
C
p53 -/-
p53(D281G)
AcK382-p53
p53
β-actin
DMSO
LAQ
EX527
D
p53 -/-
p53(D281G)
DMSO
EX527
LAQ
LAQ + EX527
FL3-H:: 7AAD
FL4-H:: AnnexinV-APC

Figure S4

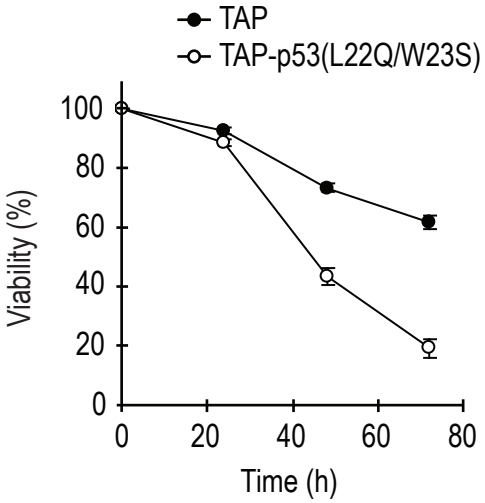

TAP
TAP-p53(L22Q/W23S)
Viability (%)
100
80
60
40
20
0
0
20
40
60
80
Time (h)

Figure S5

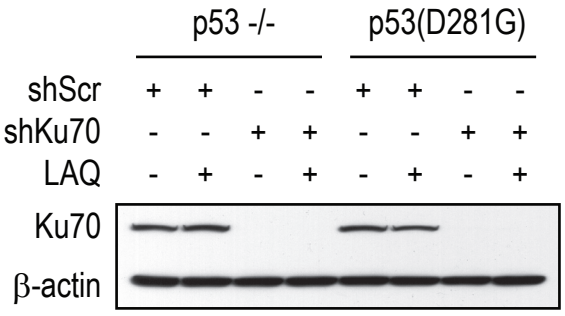
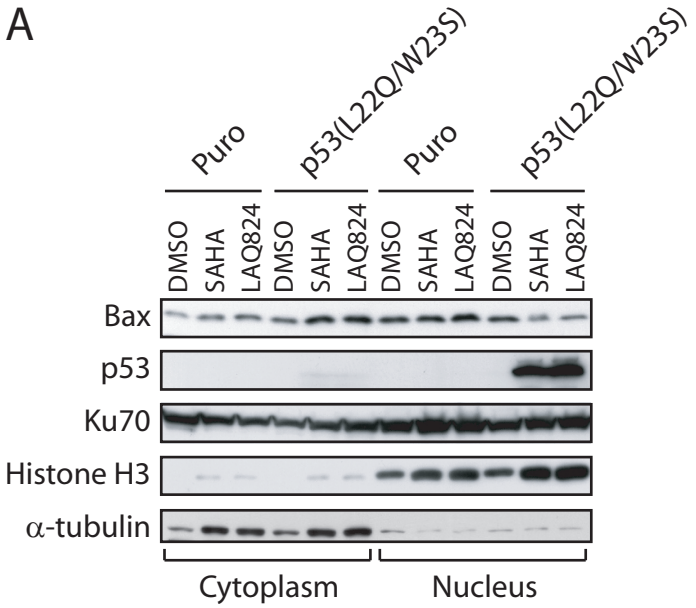
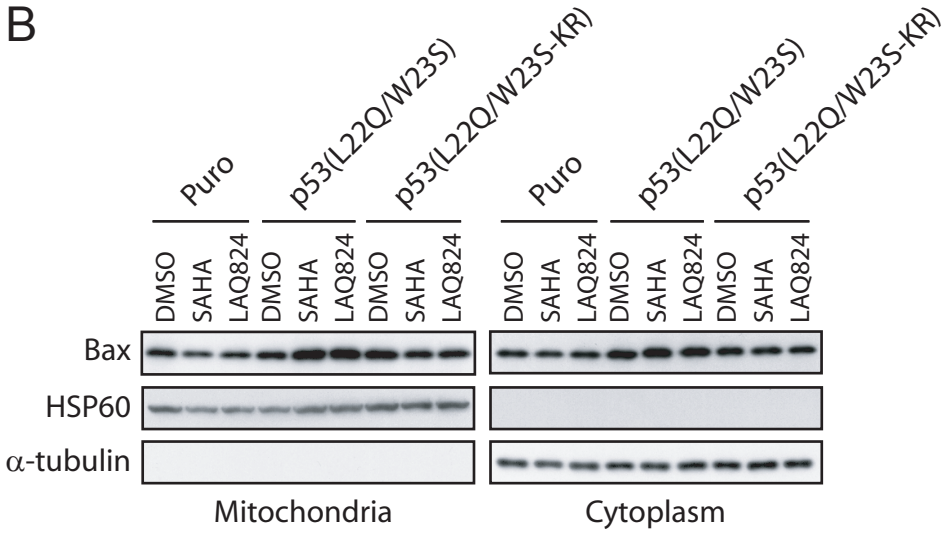
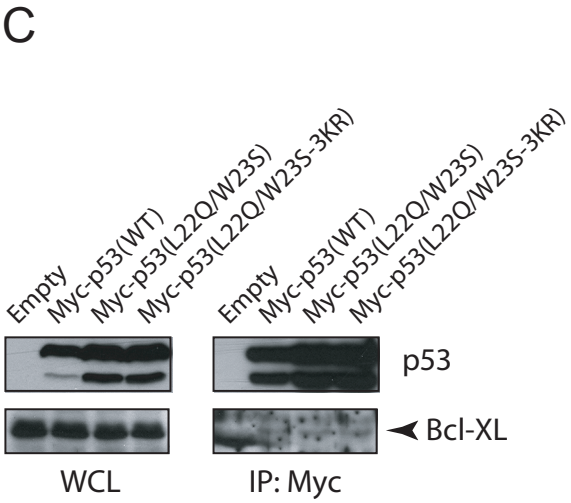


Figure S6


A
Puro
p53(L22Q/W23S)
Puro
p53(L22Q/W23S)
DMSO
SAHA
LAQ824
Bax
p53
Ku70
Histone H3
α-tubulin
Cytoplasm
Nucleus


B
Puro
p53(L22Q/W23S)
p53(L22Q/W23S-KR)
DMSO
SAHA
LAQ824
Bax
HSP60
α-tubulin
Mitochondria
Cytoplasm


C
Empty
Myc-p53(WT)
Myc-p53(L22Q/W23S)
Myc-p53(L22Q/W23S-3KR)
p53
Bcl-XL
WCL
IP: Myc

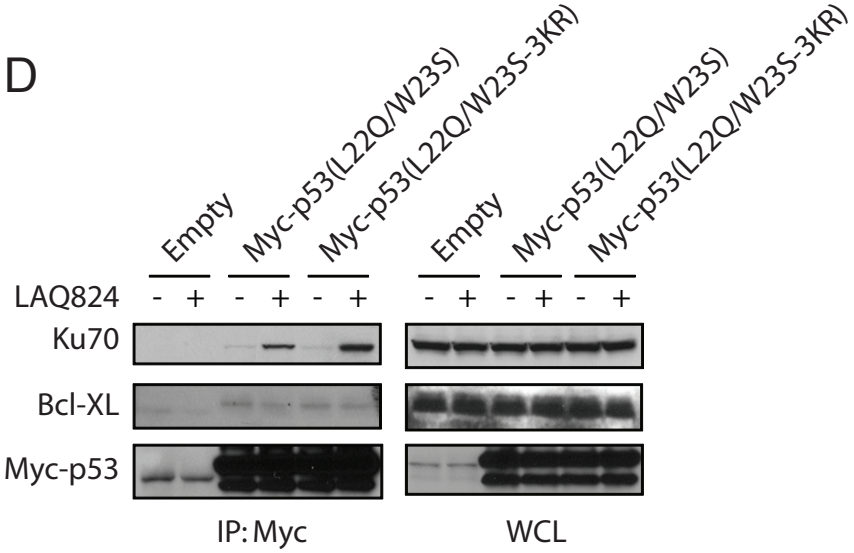

D
Empty
Myc-p53(L22Q/W23S)
Myc-p53(L22Q/W23S-3KR)
LAQ824
Ku70
Bcl-XL
Myc-p53
IP: Myc
WCL

Figure S7