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Supplemental Information

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Supplemental Information

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Supplemental Information includes: 4 figures, Supplemental Experimental Procedures and 4 tables.

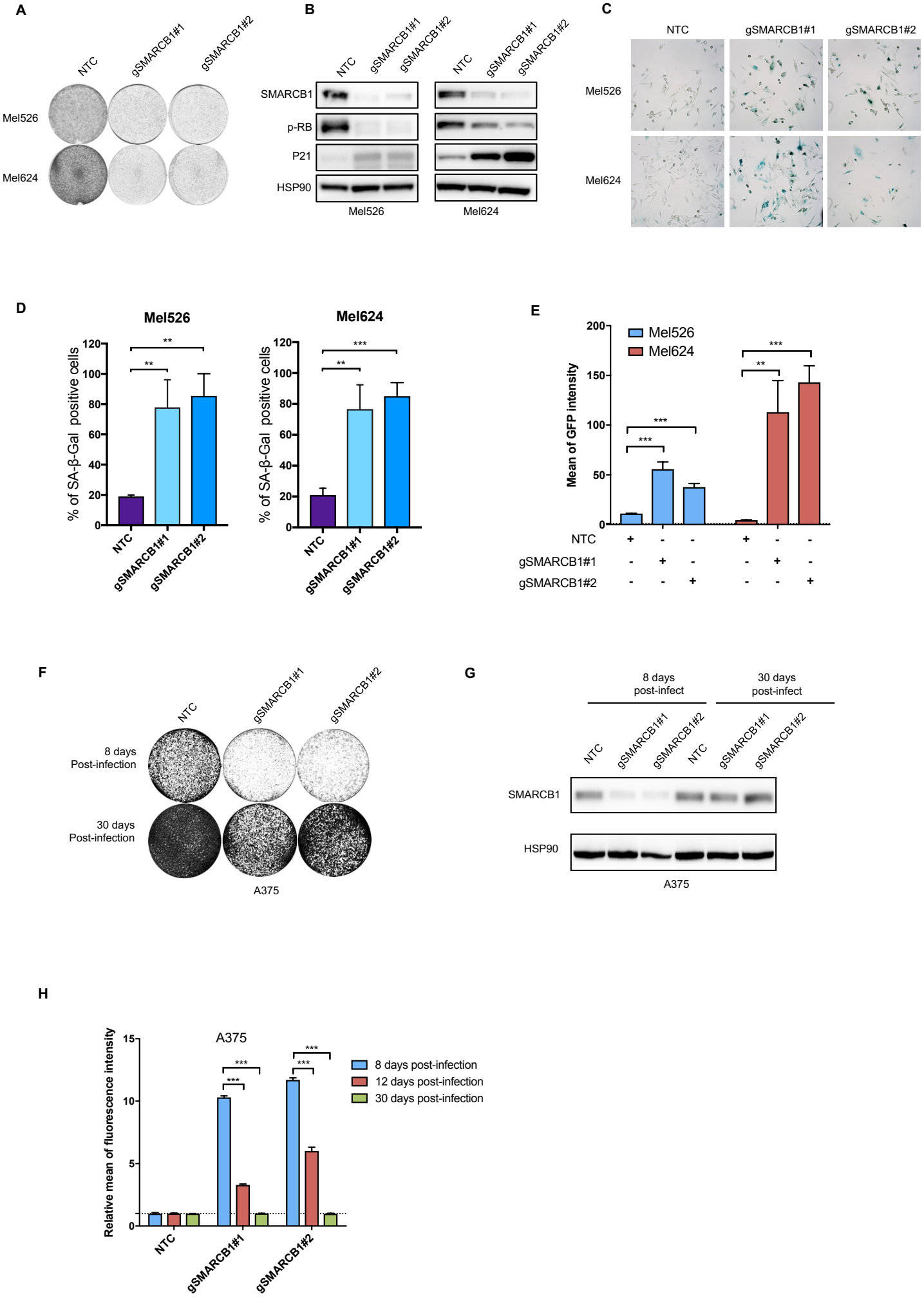
A
NTC
gSMARCB1#1
gSMARCB1#2
Mel526
Mel624
B
SMARCB1
p-RB
P21
HSP90
Mel526
Mel624
C
Mel526
Mel624
D
Mel526
Mel624
% of SA-β-Gal positive cells
E
Mel526
Mel624
Mean of GFP intensity
gSMARCB1#1
gSMARCB1#2
F
8 days
Post-infection
30 days
Post-infection
A375
G
8 days
post-infect
30 days
post-infect
SMARCB1
HSP90
A375
H
A375
Relative mean of fluorescence intensity
8 days post-infection
12 days post-infection
30 days post-infection

Figure S1

Figure S1: **Effects of SMARCB1 depletion on melanoma cells. Related to Figure 2**

(A,B,C,D,E) Mel526 and Mel624 cells expressing the miR146a-eGFP reporter were infected with two independent SMARCB1 gRNAs-CAS9 (gSMARCB1) viruses and cultured for 10 days. (A) Depletion of SMARCB1 reduced cell proliferation, upregulated classic senescence markers: (B) Loss of p-RB, induction of CDKN1A ($p21^{cip1}$), (C) senescence-associated β-galactosidase activity (quantification shown in panel D) and activated miR146-eGFP senescence reporter (E) in Mel526 and Mel624. Non-targeted gRNA vector served as a control.

(F,G,H) A375 cells expressing the miR146a-eGFP reporter infected with gRNAs-CAS9 targeting SMARCB1. Non-targeted gRNA vector (NTC) served as a control. (F) The infected cells were cultured, fixed and stained after 8 and 30-day post-infection. (G) The SMARCB1 expression after 8 and 30-day post-infection with gRNAs-CAS9 targeting SMARCB1 were measured by western blot. HSP90 served as a loading control. (H) Relative GFP fluorescence was examined by flow cytometry at the indicated time point after the viral infection.

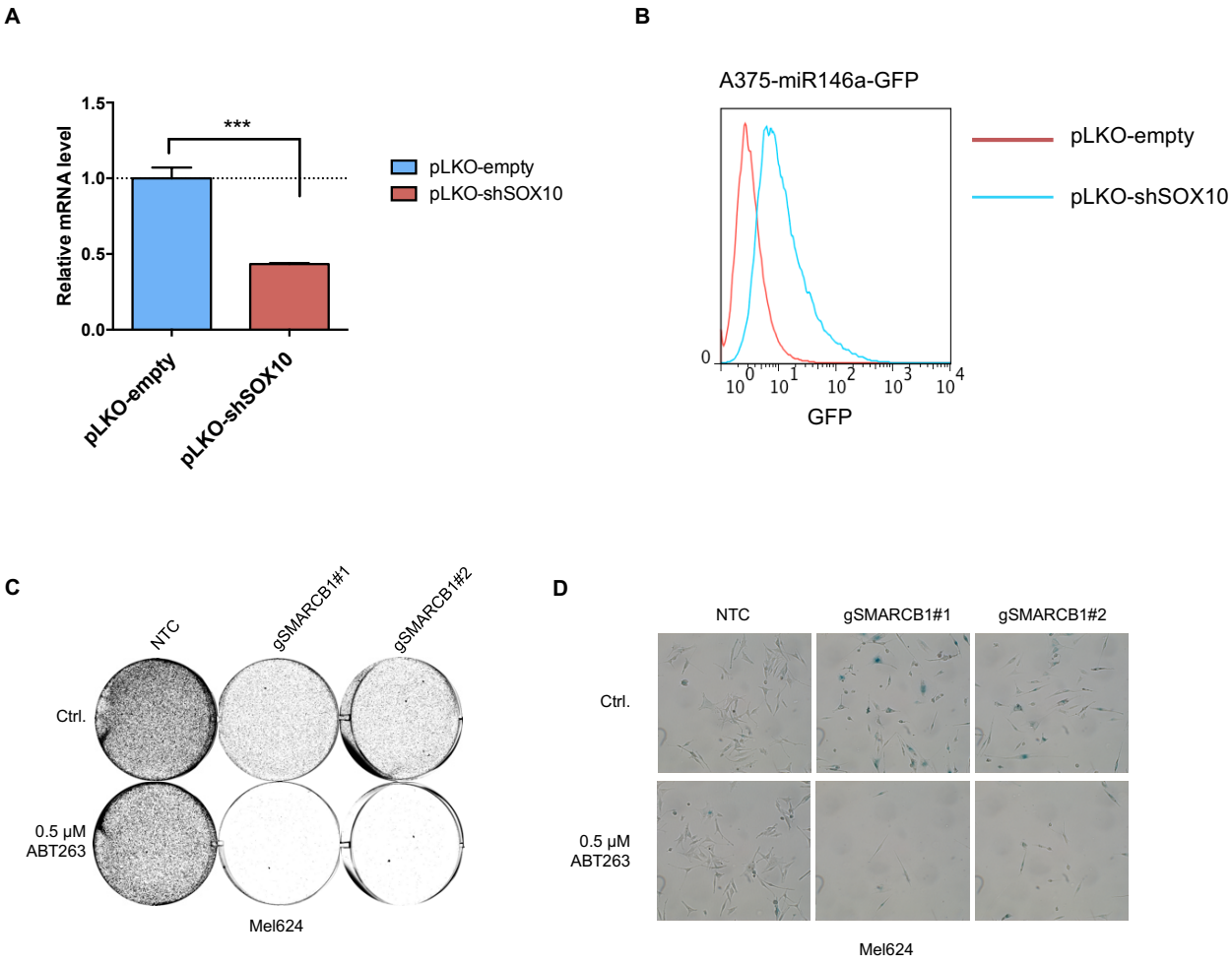

Figure S2. **Effects of SOX10 suppression and ABT263 on melanoma cells. Related to Figure 3**
(A) Real-time PCR was used to quantify the relative mRNA levels of SOX10 upon shSOX10 viral infection in A375.
(B) A375 cells expressing the miR146a-GFP reporter and infected with shSOX10 virus showed induction of the GFP signal.
(C,D) Mel624 cells were infected with SMARCB1 gRNAs-CAS9 virus. After 8 days post-infection, the cells were seeded into 6-well plate and treated with 0.5 μM ABT263. (C) Colony formation assay showed that ABT263 selectively depleted cells with SMARCB1 gRNAs-CAS9. (D) Cells with increased senescence-associated β-galactosidase activity are vulnerable to ABT263.

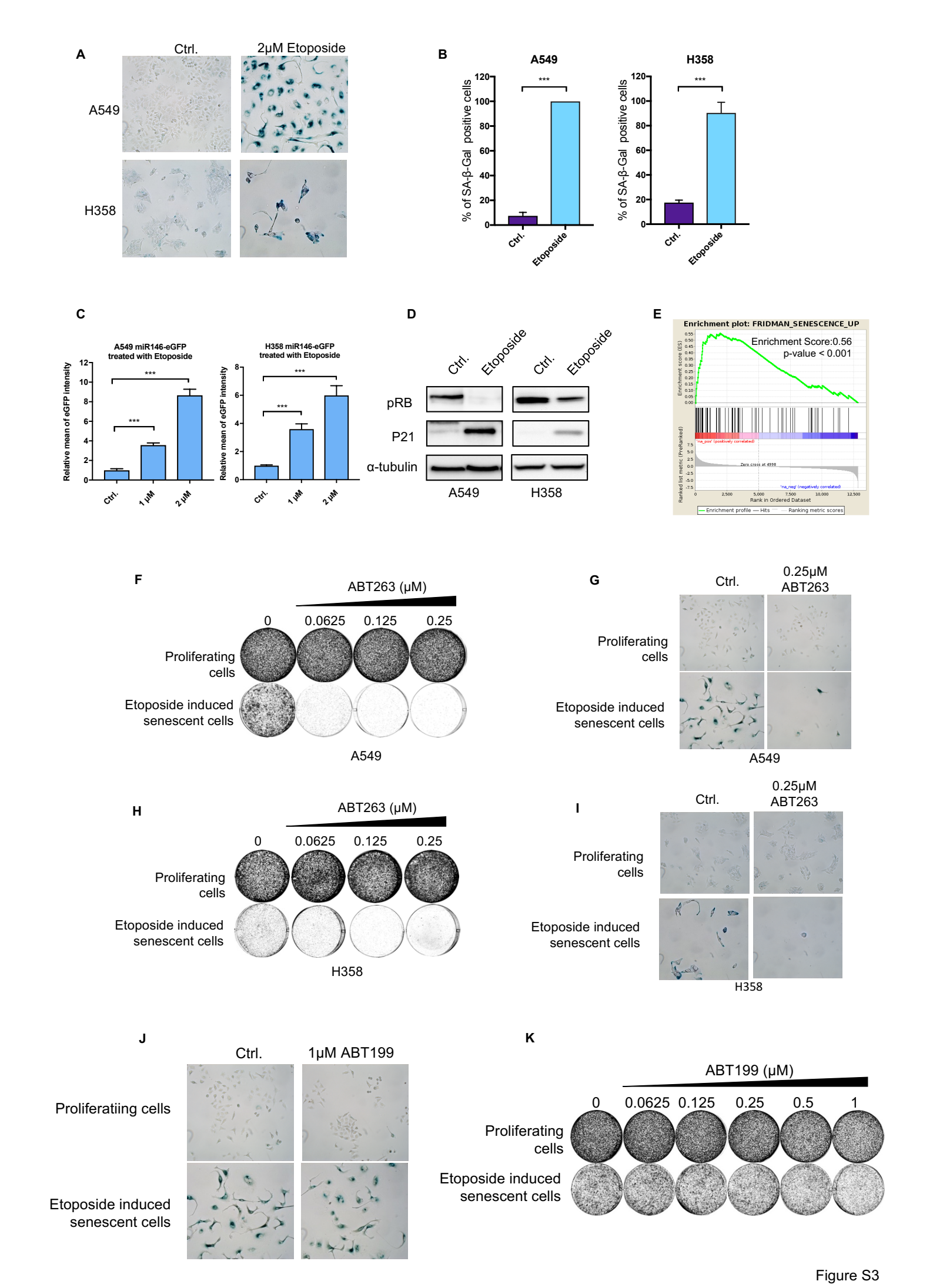
A
Ctrl.
2μM Etoposide
A549
H358
B
A549
H358
% of SA-β-Gal positive cells
***
Ctrl.
Etoposide
C
A549 miR146-eGFP treated with Etoposide
H358 miR146-eGFP treated with Etoposide
Relative mean of eGFP intensity
Ctrl.
1 μM
2 μM
D
Ctrl.
Etoposide
pRB
P21
α-tubulin
A549
H358
E
Enrichment plot: FRIDMAN_SENESCENCE_UP
Enrichment Score:0.56
p-value < 0.001
Enrichment score (ES)
Ranked list metric (PreRanked)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores
F
ABT263 (μM)
0
0.0625
0.125
0.25
Proliferating cells
Etoposide induced senescent cells
A549
G
Ctrl.
0.25μM ABT263
Proliferating cells
Etoposide induced senescent cells
A549
H
ABT263 (μM)
0
0.0625
0.125
0.25
Proliferating cells
Etoposide induced senescent cells
H358
I
Ctrl.
0.25μM ABT263
Proliferating cells
Etoposide induced senescent cells
H358
J
Ctrl.
1μM ABT199
Proliferatiing cells
Etoposide induced senescent cells
K
ABT199 (μM)
0
0.0625
0.125
0.25
0.5
1
Proliferating cells
Etoposide induced senescent cells

Figure S3

Figure S3. **Etoposide can be used as an efficient senescence inducer. Related to Figure 4.**

(A,B,C,D) A549 and H358 cells expressing the miR-146-eGFP reporter were treated with 2μM etoposide for 7 days. (A,B) Etoposide treatment induced senescence-associated β-galactosidase activity (quantification shown in panel B), (C) activated reporter and increased GFP fluorescence signal measured by flow cytometry. (D) Etoposide treatment induces classic senescence markers: Loss of phosphorylated-RB and induction of P53 and CDKN1A ($p21^{cip1}$).

(E) RNA sequencing was performed on etoposide treated cells, and followed by GSEA analysis of etoposide treated cells versus untreated control for the 'FRIDMAN_SENESCENCE_UP' geneset.

(F, G) The etoposide-induced senescent A549 cells were seeded into 6 well plates and treated with ABT263. Parental A549 were used as a control. (F) Colony formation assay demonstrated that etoposide-induced senescent cells were selectively sensitive to ABT263 compared to proliferating cells. (G) Etoposide pre-treated cells stained strong positively with senescence-associated β-galactosidase activity, and these cells can be significantly depleted with ABT263 within 96 hours

(H, I) The etoposide-induced senescent H358 cells were seeded into 6 well plate and treated with ABT263. Parental H358 were used as a control. (H) Colony formation assay demonstrated that etoposide-induced senescent cells were selectively sensitive to ABT263 compared to proliferating cells. (I) Etoposide pre-treated cells stained strong positively with senescence-associated β-galactosidase activity, and these cells can be significantly depleted with ABT263 in 96 hours.

(J, K) The etoposide-induced senescent A549 cells were seeded into 6 well plate and treated with ABT199. Parental A549 were used as a control. (J) Etoposide pre-treated cells were stained for senescence-associated β-galactosidase activity, and these cells were not sensitive to ABT199. (K) Colony formation assay demonstrated that etoposide-induced senescent cells were not responsive to ABT199 compared to proliferated cells.

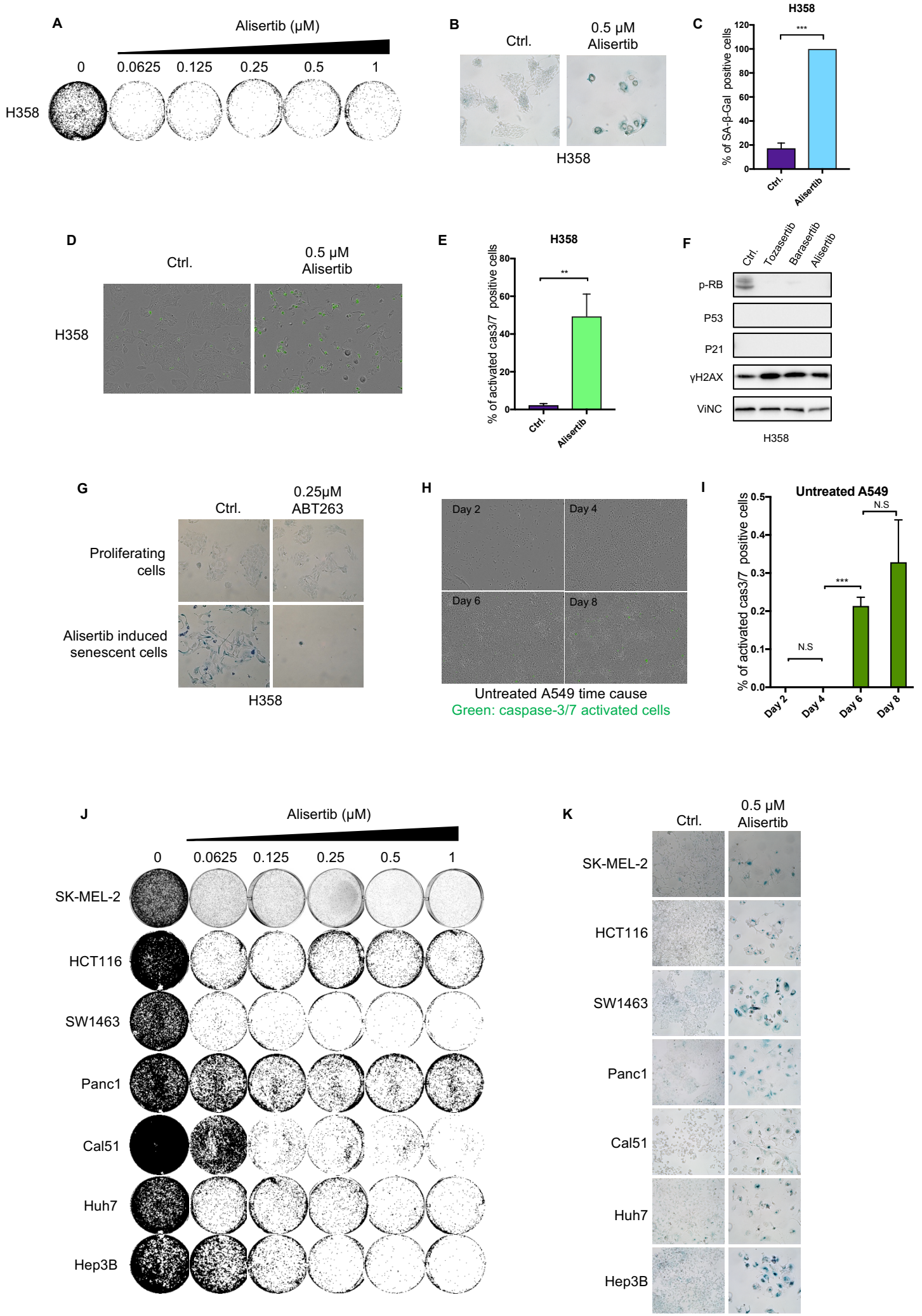
A
Alisertib (μM)
0
0.0625
0.125
0.25
0.5
1
H358
B
Ctrl.
0.5 μM Alisertib
H358
C
H358
% of SA-β-Gal positive cells
Ctrl.
Alisertib
D
Ctrl.
0.5 μM Alisertib
H358
E
H358
% of activated cas3/7 positive cells
Ctrl.
Alisertib
F
Ctrl.
Tozasertib
Barasertib
Alisertib
p-RB
P53
P21
γH2AX
ViNC
H358
G
Ctrl.
0.25μM ABT263
Proliferating cells
Alisertib induced senescent cells
H358
H
Day 2
Day 4
Day 6
Day 8
Untreated A549 time cause
Green: caspase-3/7 activated cells
I
Untreated A549
% of activated cas3/7 positive cells
N.S
N.S
Day 2
Day 4
Day 6
Day 8
J
Alisertib (μM)
0
0.0625
0.125
0.25
0.5
1
SK-MEL-2
HCT116
SW1463
Panc1
Cal51
Huh7
Hep3B
K
Ctrl.
0.5 μM Alisertib
SK-MEL-2
HCT116
SW1463
Panc1
Cal51
Huh7
Hep3B

Figure S4

Figure S4. **Alisertib induces senescence in additional cancer models. Related to Figure 4.**

(A) Colony formation assay using H358 cells in the presence of alisertib demonstrating that treatment of alisertib reduces cell proliferation. (B,C) H358 cells were treated with 0.5μM alisertib for 7 days and stained for senescence-associated β-galactosidase activity (quantification shown in panel C). (D, E) Alisertib treatment also induced apoptosis in H358 at day 6. The quantification is shown in panel E. Data were represented as Mean ± SD. (F) Three independent aurora kinase inhibitors treatments induced classic senescence markers in H358: Loss of phosphorylated-RB, upregulation of P53 and CDKN1A ($p21^{cip1}$). Induction of γH2AX was also observed. VINC served as a loading control.

(G) The Alisertib-induced senescent H358 cells were seeded into 6 well plate and treated with ABT263. Parental H358 were used as a control. Alisertib pre-treated H358 cells were stained for senescence-associated β-galactosidase activity, and these cells can be significantly depleted with ABT263 in 96 hours.

(H, I) The control for experiment in Figure 4H, A549 cells were seeded in 96 well plate and incubated with caspase-3/7 green apoptosis assay reagent. Images were taken by Incucyte at different time points. Green fluorescent staining indicated caspase-3/7 dependent apoptosis. The quantification was shown in panel I. Data were represented as Mean ± SD.

(J, K) Various cancer models were treated with alisertib for 7 days. (J) Alisertib treatment reduced cell proliferations. (K) 0.5μM of alisertib induced senescence-associated β-galactosidase activity in these cancer cell lines.

Supplemental tables titles and legends

Table S1. **Genes and gRNAs of the epigenome CRISPR library and the hits in the senescence screen in A375 cells. Related to Figure 1I-J.**

List of Customized epigenome CRISPR library containing 5230 gRNAs targeting 446 epigenetic modifiers. The library also contain the gRNAs of non-targeting controls and essential (straight-lethal) genes to determine screen quality. This table also includes list of significantly enriched gRNAs in the GFP+ cells, which are considered as the robust senescence regulators. The hits ranking were generated by rank algorithm as part of the MAGeCK software.

Table S2. **RNAseq data. Related to Figure 1G and 4G.**

RNA sequencing results of (1) A375 cells and SMARCB1 depleted counterparts. (2) A549 cells and aurora kinase inhibitor, etoposide treated counterparts. 'FRIDMAN_SENESCENCE_UP' geneset was used to determine the enrichment of senescence-related genes.

Table S3. **Normalized reading of the compound screen in A549. Related to Figure 4B.**

The measurement of CellTiterBlue readout and for the Luciferase readout. The negative controls (DMSO) was created and used to normalized to determine the fold changes of both readout upon different drug responses, and the mean value of the biological replicates was tested for significance. Etoposide was considered as a positive control in the screen.

Table S4. **Top hits in the compound screen in A549. Related to Figure 4C.**

Among 941 unique compounds in the combined drug library, there were 27 unique aurora kinase inhibitors. 47 compounds were considered as hits. Among these 47 drug hits were 18 independent aurora kinase inhibitors.

Supplemental Experimental Procedures

STAR Methods

Resource Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
HSP 90alpha/beta (H-114) Rabbit polyclonal antibody	Santa Cruz Biotechnology	Cat#: sc-7947 RRID: AB_2121235
p21 (C-19) Rabbit polyclonal antibody	Santa Cruz Biotechnology	Cat#: sc-397 RRID: AB_632126
Phospho-Rb (Ser780) Rabbit polyclonal antibody	Cell Signaling Technology	Cat#: 39033 RRID:AB_330015
Phospho-MEK1/2 (Ser217/221) (41G9) Rabbit monoclonal antibody	Cell Signaling Technology	Cat#: 9154S RRID: AB_2138017
MEK1/2 (L38C12) Mouse monoclonal antibody	Cell Signaling Technology	Cat#: 4694S RRID: AB_10695868
EGF Receptor Mouse monoclonal antibody	BD Biosciences	Cat#: 610017 RRID: AB_2096701
P27, Kip-1 Mitotic Inhibitor, aa. 1-197 Mouse monoclonal antibody	BD Biosciences	Cat#: 610242 RRID: AB_397637
SMARCB1 Rabbit polyclonal antibody	Atlas Antibodies	Cat#: HPA018248 RRID: AB_1234535
SOX10 Rabbit monoclonal antibody	Abcam	Cat#: Ab155279 RRID:AB_1234535
c-Jun (H-79) Rabbit polyclonal antibody	Santa Cruz Biotechnology	Cat#: sc-1694 RRID:AB_ 631263
p-c-Jun (KM-1) Mouse monoclonal antibody	Santa Cruz Biotechnology	Cat#: sc-822 RRID:AB_ 627262
phospho-Rsk1 (Thr359/Ser363) Rabbit monoclonal antibody	Millipore	Cat#: 04-419 RRID: AB_11213444
RSK1 (D6D5) Rabbit monoclonal antibody	Cell Signaling Technology	Cat#: 8408S RRID: AB_10828594
P53 (DO-1) Mouse monoclonal antibody	Santa Cruz Biotechnology	Cat#: sc-126 RRID: AB_628082
γH2AX Mouse monoclonal antibody	Millipore	Cat # 05-636 RRID: AB_309864
Chemicals, Peptides, and Recombinant Proteins		
Navitoclax (ABT-263)	Selleck Chemicals	Cat#: S1001
Venetoclax (ABT199)	Medkoo Bioscience	Cat#: 205807
Etoposide	LC laboratories	Cat#: E-4488
Vemurafenib	Selleck Chemicals	Cat#: S1267
GPCR Compound Library	Selleck Chemicals	Cat#: L2200
Kinase Inhibitor Library	Selleck Chemicals	Cat#: L1200
Epigenetics Compound Library	Selleck Chemicals	Cat#: L1900
Tozasertib	Selleck Chemicals	Cat#: S1048
Barasertib	Selleck Chemicals	Cat#: S1147

Alisertib	Selleck Chemicals	Cat#: L1133
NCI approved oncology drug set	Provided by NCI	
Paraquat	Sigma-Aldrich	Cat#: 36541
Doxorubicin	Selleck Chemicals	Cat#: S1208
Critical Commercial Assays		
Senescence Cells Histochemical Staining Kit	Sigma-Aldrich	Cat#: CS0030-1KT
FastStart Universal SYBR Green Master (Rox)	Roche	Cat#: 04913850001
Quick-RNA™ MiniPrep	Zymo Research	Cat#: R1055
Maxima First Strand cDNA Synthesis Kit for RT-qPCR	Thermo Fisher	Cat#: K1641
IncuCyte® Caspase-3/7 Green Apoptosis Assay Reagent	Essen Bioscience	Cat#: 4440
Deposited Data		
FACS-assisted CRIPSR screen raw data	This paper	GEO: 102639
RNA-Seq of A375 SMARCB1 Knock-out	This paper	GEO: 102639
Experimental Models: Cell Lines		
A375, H358, A549, HCT116, SW1463, SK-MEL-2, Panc1	ATCC	NA
Cal51	K. Jastrzebski (NKI, Amsterdam, The Netherlands)	NA
Huh7 and Hep3B	S. Huang ((NKI, Amsterdam, The Netherlands)	NA
Mel888, Mel624 and Mel526	D. Peeper (NKI, Amsterdam, The Netherlands)	NA
Plasmids and Recombinant DNA		
miR146-GFP reporter	*Kang et al., 2015*	NA
miR146-Gaussia-Dura luciferase reporter	This paper	
DNA fragment sequence Gaussia-Dura luciferase	Snapgene	Cat#: 533
DNA fragment Gaussia-Dura luciferase gBlock	IDT	NA
pLenti-CRISPR V2.1	*Evers et al. 2016*	NA
pLKO 0.1 (TRC)	Sigma-Aldrich	SHCLNG
pLX304-GFP	SSCB Broad ORF lentiviral expression collection	NA
pLX304-SOX10	SSCB Broad ORF lentiviral expression collection	NA
Sequence-Based Reagents		
gRNA sequences (without PAM)		
gSMARCB1#1 CCTGTTAAAAGCCTCGGAAG	This paper	NA
gSMARCB1#2 GTTCTACATGATCGGCTCCG	This paper	NA
Non-target control (NTC): CTACTAAAAGAGAGGGATC	This paper	NA
shRNA target sequences		
shSMARCB1#1 CCGGCACACATTCCATTTGTT	Sigma-Aldrich	TRCN0000039583
shSMARCB1#2 GCCTCGGAAGTGGAAGAGATT	Sigma-Aldrich	TRCN0000039586
shSMARCB1#3 GAAGTTGATGACGCCTGAGAT	Sigma-Aldrich	TRCN0000039587
shSOX10 CCTCATTCTTTGTCTGAGAAA	Sigma-Aldrich	TRCN0000018984

Gene expression qPCR primer sequences		
GAPDH Forward: 5'-AAGGTGAAGGTCGGAGTCAA-3'	Invitrogen	NA
GAPDH Reverse: 5'-AATGAAGGGGTCATTGATGG-3'	Invitrogen	NA
SMARCB1 Forward: 5'-AACGTCAGCGGGTTCAAAT-3'	Invitrogen	NA
SMARCB1 Reverse: 5'-GCCTTCACCTGGAACATGAA-3'	Invitrogen	NA
EGFR Forward: 5'-TCCTCTGGAGGCTGAGAAAA-3'	Invitrogen	NA
EGFR Reverse: 5'-GGGCTCTGGAGGAAAAGAAA-3'	Invitrogen	NA
c-Jun Forward: 5'-GTCCTTCTTCTCTTGCGTGG-3'	Invitrogen	NA
c-Jun Reverse: 5'-GGAGACAAGTGGCAGAGTCC-3'	Invitrogen	NA
SOX10 Forward: 5'-CTTTCTTGTGCTGCATACGG-3'	Invitrogen	NA
SOX10 Reverse: 5'- AGCTCAGCAAGACGCTGG -3'	Invitrogen	NA
Software and Algorithms		
GraphPad Prism 7.0	GraphPad	NA
FlowJo version 7.6.5	FlowJo, LLC	NA
DESeq2	*Evers et al. 2016*	NA
MAGeCK software version 0.5	*Evers et al. 2016*	NA
Senescence miR146-drug screen Algorithms	This paper	NA
'FRIDMAN_SENESCENCE_UP' geneset	GSEA	Senescence gene signature gene set

miR146-Dura Gaussia luciferase reporter cloning

To create the miR146-Gaussia reporter system, the *Gaussia*-Dura luciferase was fused to the miR-146a promotor. Accordingly, eGFP was replaced by commercial *Gaussia*-Dura from gBlock IDT via *Xho*I/*Apa*I restriction cloning in pHage-promiR-146a-eGFP. The reporter plasmid and gene fragments were digested with corresponding enzymes (NEB), according to manufacturer's instructions. Digestion products were separated on an agarose gel, following standard procedures. Correct fragments were excised and purified with a gel purification kit (28 9034-70; GE healthcare) following manufacturer's instructions. Purified fragments were ligated using T4 DNA ligase (NEB), standard procedures were followed, except for an increased incubation time of 1h at RT. Endura cells (Lucigen) were transformed with the ligated constructs via electroporation, as recommended by the supplier. Vector DNA was isolated with PureLink Quick Plasmid Miniprep Kit (K210011; ThermoFisher scientific), as indicated by the manufacturer. The plasmid DNA was subjected to Sanger sequencing with BigDye Terminator v3.1 (Thermo Fisher Scientific) at the Genomics Core Facility (NKI, Amsterdam).

CONTACT FOR REAGENT AND RESOURCE SHARING

Requests for further information and reagents may be addressed to the corresponding author: Rene Bernards (r.bernards@nki.nl).

Supplementary References

Kang, C., Xu, Q., Martin, T.D., Li, M.Z., Demaria, M., Aron, L., Lu, T., Yankner, B.A., Campisi, J., and Elledge, S.J. (2015). The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4. Science *349*, aaa5612.

Evers, B., Jastrzebski, K., Heijmans, J.P., Grernrum, W., Beijersbergen, R.L., and Bernards, R. (2016). CRISPR knockout screening outperforms shRNA and CRISPRi in identifying essential genes. Nat Biotechnol *34*, 631-633.