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Supplemental information

The Cancer SENESCopedia:

A delineation of cancer cell senescence

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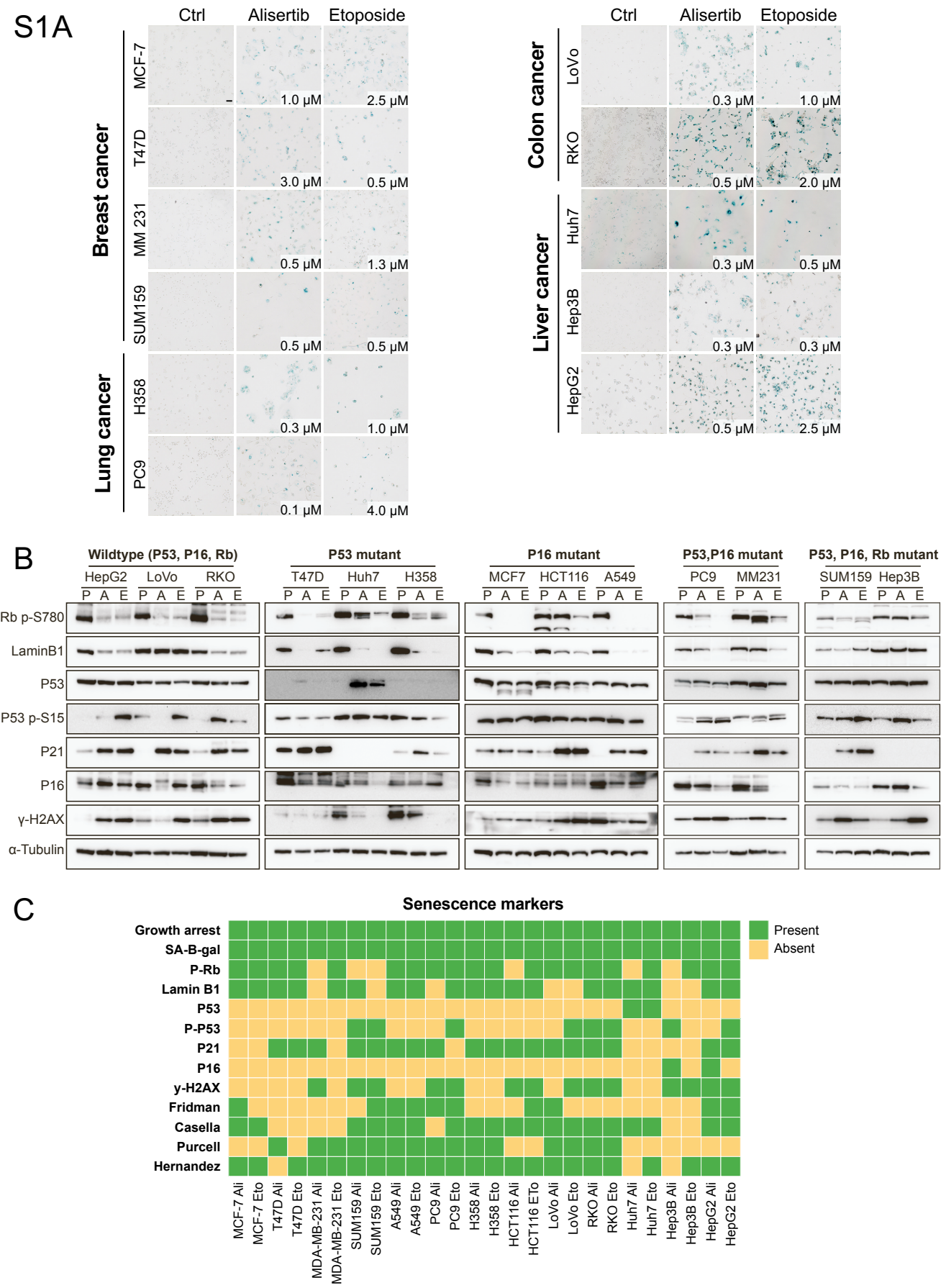

S1A
Ctrl
Alisertib
Etoposide
Breast cancer
MCF-7
T47D
MM 231
SUM159
Lung cancer
H358
PC9
Colon cancer
LoVo
RKO
Liver cancer
Huh7
Hep3B
HepG2
1.0 μM
2.5 μM
3.0 μM
0.5 μM
0.5 μM
1.3 μM
0.5 μM
0.5 μM
0.3 μM
1.0 μM
0.1 μM
4.0 μM
0.3 μM
1.0 μM
0.5 μM
2.0 μM
0.3 μM
0.5 μM
0.3 μM
0.3 μM
0.5 μM
2.5 μM
B
Wildtype (P53, P16, Rb)
HepG2
LoVo
RKO
P53 mutant
T47D
Huh7
H358
P16 mutant
MCF7
HCT116
A549
P53,P16 mutant
PC9
MM231
P53, P16, Rb mutant
SUM159
Hep3B
P A E
Rb p-S780
LaminB1
P53
P53 p-S15
P21
P16
γ-H2AX
α-Tubulin
C
Senescence markers
Growth arrest
SA-B-gal
P-Rb
Lamin B1
P53
P-P53
P21
P16
γ-H2AX
Fridman
Casella
Purcell
Hernandez
Present
Absent
MCF-7 Ali
MCF-7 Eto
T47D Ali
T47D Eto
MDA-MB-231 Ali
MDA-MB-231 Eto
SUM159 Ali
SUM159 Eto
A549 Ali
A549 Eto
PC9 Ali
PC9 Eto
H358 Ali
H358 Eto
HCT116 Ali
HCT116 ETo
LoVo Ali
LoVo Eto
RKO Ali
RKO Eto
Huh7 Ali
Huh7 Eto
Hep3B Ali
Hep3B Eto
HepG2 Ali
HepG2 Eto

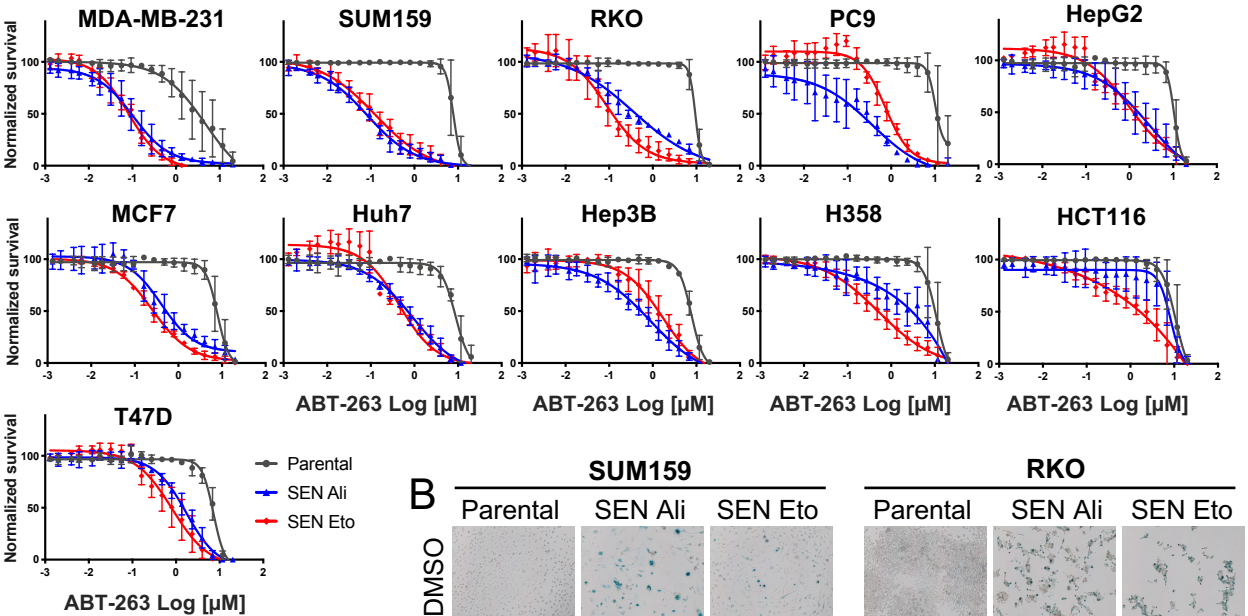
Supplementary figure 1. Senescence markers are present in cells treated with alisertib or etoposide, related to figure 1.

(A) Representative images of SA-ß-gal staining from three independent biological replicates for 11 cell lines (quantification in figure 1D). Cells were cultured for 7 days with the indicated alisertib or etoposide concentrations. Scale bar = 100 μm. MM231 = MDA-MB-231

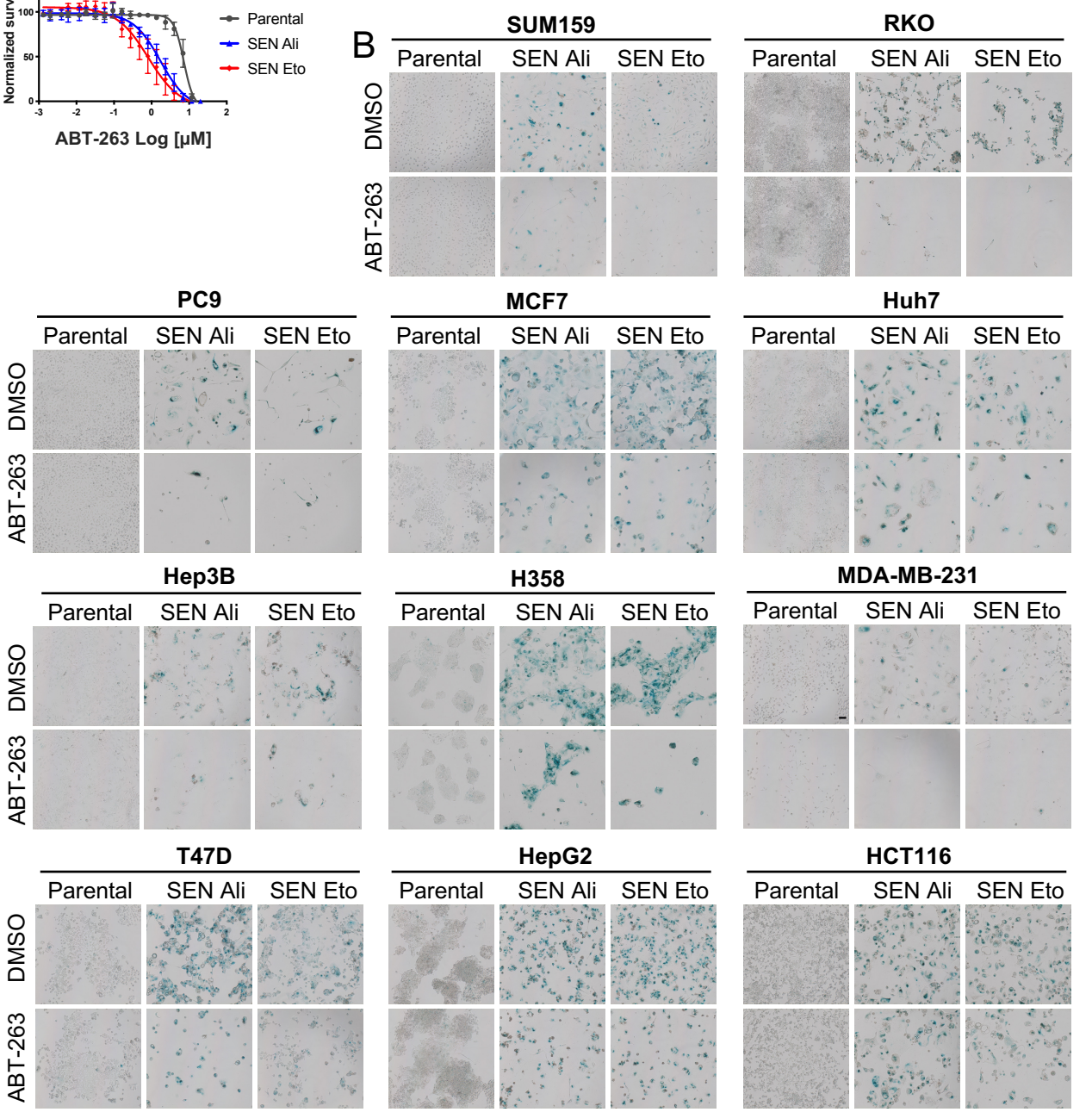
(B) Westernblot for senescence markers. Cells were lysed after a treatment for 7 days and 24h drug-washout. P = parental, E = etoposide-induced senescence, A = alisertib-induced senescence. MM231 = MDA-MB-231.

(C) Overview of senescence markers for etoposide- and alisertib-induced senescent cells. Pospho-Rb (P-Rb) and LaminB1 are scored as present when decreased in senescence, while P53, Phospho-P53 (P-P53), P21, P16 and gamma-H2AX are present when increased upon senescence (from figure S1B). Fridman, Casella, Purcell and Hernandez are scored as absent when the Delta ssGSEA scores (from figure 1F) are between 0.2 and -0.2, higher or lower scores are scored as present. Fridman and Casella signatures score as present when both up and down signatures are present.

S2 A


MDA-MB-231
SUM159
RKO
PC9
HepG2
MCF7
Huh7
Hep3B
H358
HCT116
T47D
Normalized survival
ABT-263 Log [μM]
Parental
SEN Ali
SEN Eto

B

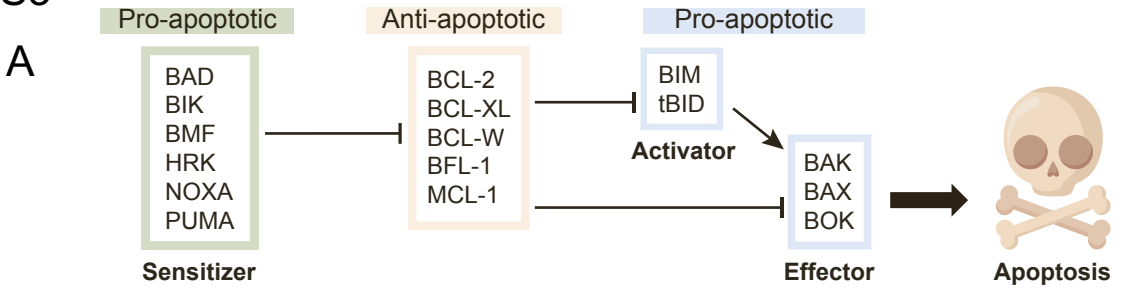

SUM159
RKO
PC9
MCF7
Huh7
Hep3B
H358
MDA-MB-231
T47D
HepG2
HCT116
Parental
SEN Ali
SEN Eto
DMSO
ABT-263

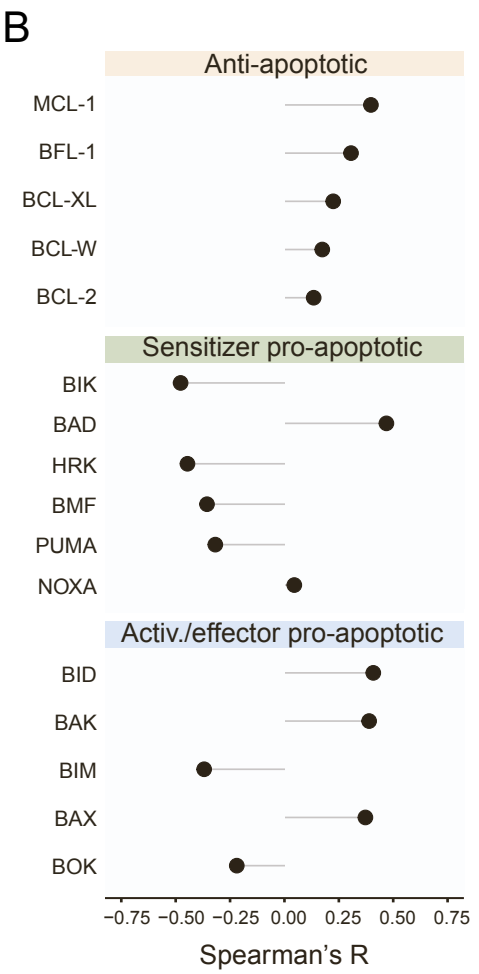
Supplementary figure 2. Drug response to ABT-263 differs between senescent cancer cells, related to figure 2.

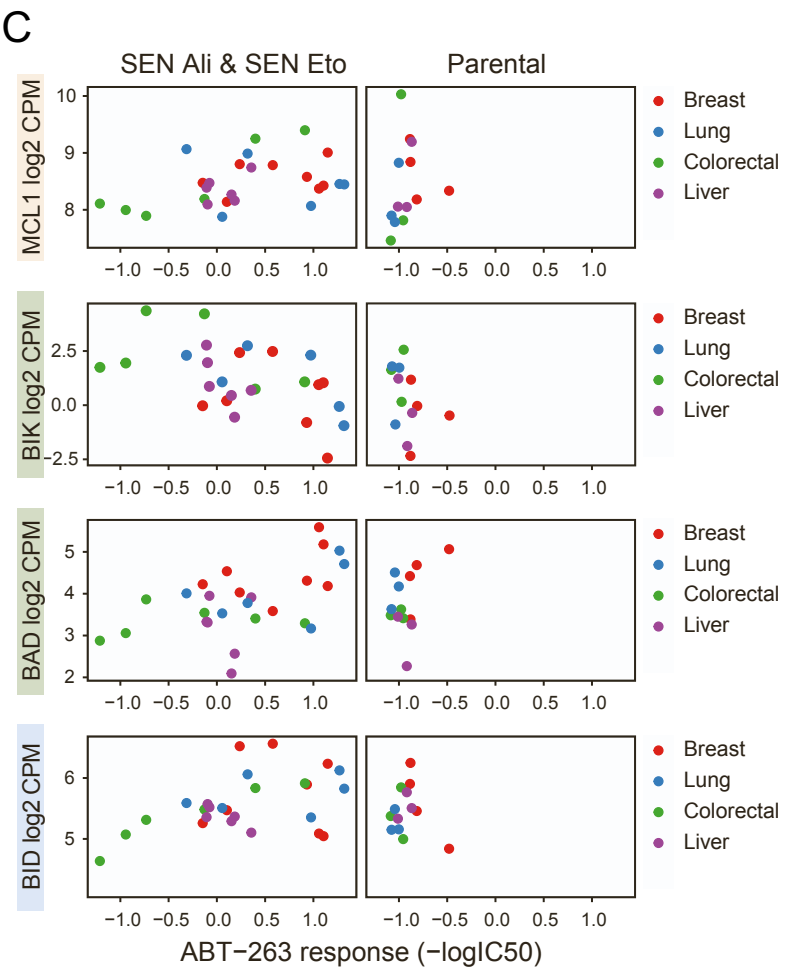
(A) Dose-response curves for increasing concentrations of ABT-263 for 13 cell lines. Cells were treated with senescence-inducing concentrations of alisertib or etoposide for seven days. Senescent cells and parental cells were reseeded in 96-wells and cultured with increasing concentrations of ABT-263. After five days, cell viability was measured with Cell Titer Blue (CTB) assay. SEN Ali = alisertib-induced senescent, SEN Eto = etoposide-induced senescent. Values represent the mean ±SEM of three independent biological experiments.

(B) Representative images of SA-ß-gal staining from three technical replicates for 13 cell lines. Cells were seeded into a 6-well and treated with alisertib or etoposide for 7 days, and medium was subsequently replaced by medium supplemented with DMSO or 1μM ABT-263. Cells were cultured for another 5 days before SA-ß-gal staining was performed. Scale bar = 100 μm.

S3


A
Pro-apoptotic
Anti-apoptotic
Pro-apoptotic
BAD
BIK
BMF
HRK
NOXA
PUMA
Sensitizer
BCL-2
BCL-XL
BCL-W
BFL-1
MCL-1
BIM
tBID
Activator
BAK
BAX
BOK
Effector
Apoptosis


B
Anti-apoptotic
MCL-1
BFL-1
BCL-XL
BCL-W
BCL-2
Sensitizer pro-apoptotic
BIK
BAD
HRK
BMF
PUMA
NOXA
Activ./effector pro-apoptotic
BID
BAK
BIM
BAX
BOK
−0.75 −0.50 −0.25 0.00 0.25 0.50 0.75
Spearman's R

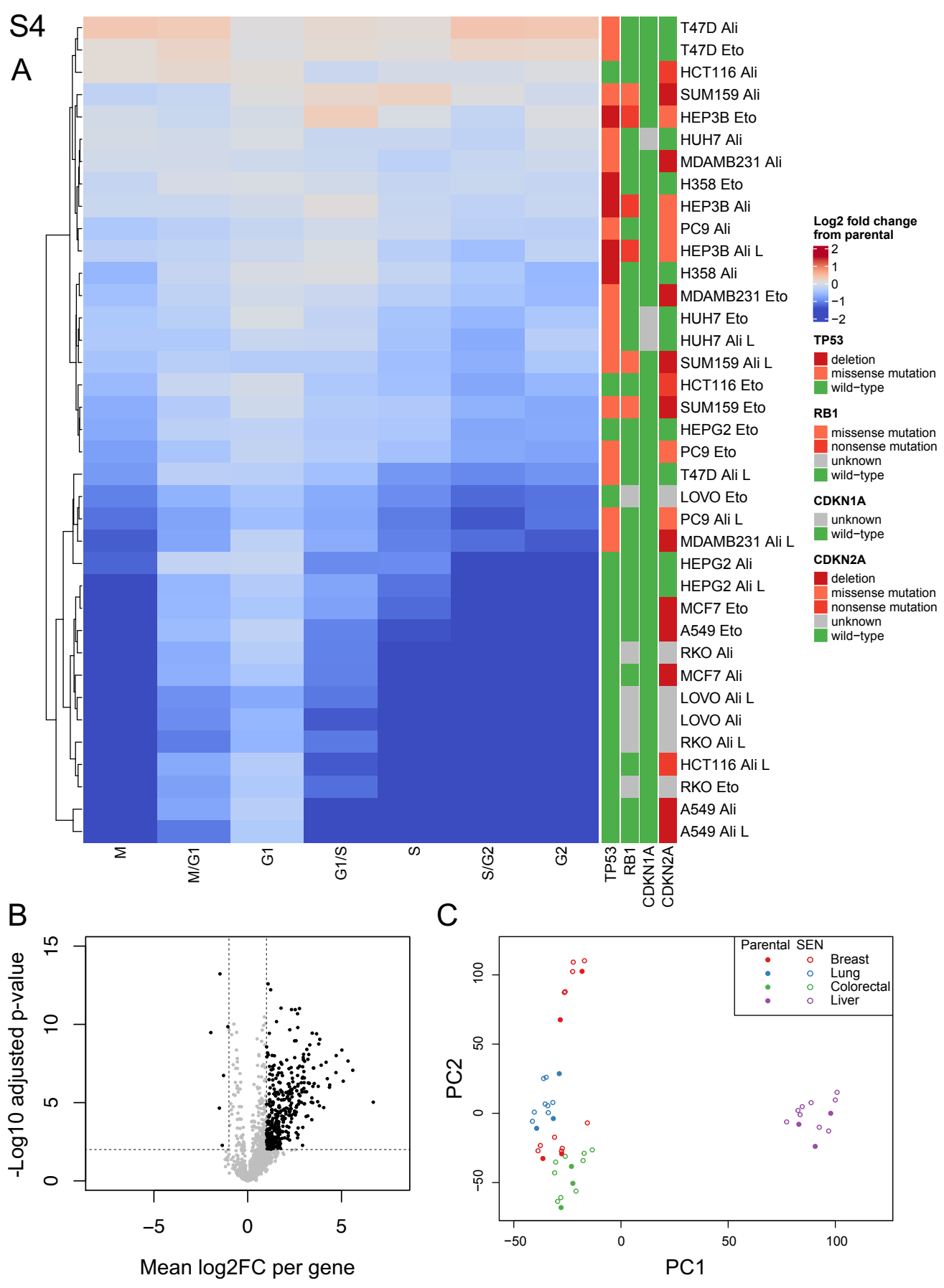

C
SEN Ali & SEN Eto
Parental
MCL1 log2 CPM
BIK log2 CPM
BAD log2 CPM
BID log2 CPM
Breast
Lung
Colorectal
Liver
ABT−263 response (−logIC50)

Supplementary figure 3. Gene expression of BAD correlates with response to ABT-263, related to figure 2.

(A) Schematic outline of the actions of BCL-2 family proteins in apoptosis. tBID = truncated BID.

(B) Lollipop chart shows the results from the Spearman rank correlation analysis in which the gene expression of 16 apoptotic proteins was compared to the ABT-263 response (-logIC50) in etoposide- and alisertib-induced senescent cells. All proteins scored higher than Benjamini-Hochberg (FDR) corrected *p*-value 0.05. Activ. = activator.

(C) Scatter plots depict ABT-263 response (-logIC50) versus gene expression of BCL-2 family members that are significantly correlated based on Spearman rank correlation analysis (in B). In the left plots, each dot represents a cell line that was treated with alisertib or etoposide. On the right plots, each dot represents a cell line in parental state. CPM = counts per million. SEN Ali = alisertib-induced senescent cells. SEN Eto = etoposide-induced senescent cells.


S4
A
T47D Ali
T47D Eto
HCT116 Ali
SUM159 Ali
HEP3B Eto
HUH7 Ali
MDAMB231 Ali
H358 Eto
HEP3B Ali
PC9 Ali
HEP3B Ali L
H358 Ali
MDAMB231 Eto
HUH7 Eto
HUH7 Ali L
SUM159 Ali L
HCT116 Eto
SUM159 Eto
HEPG2 Eto
PC9 Eto
T47D Ali L
LOVO Eto
PC9 Ali L
MDAMB231 Ali L
HEPG2 Ali
HEPG2 Ali L
MCF7 Eto
A549 Eto
RKO Ali
MCF7 Ali
LOVO Ali L
LOVO Ali
RKO Ali L
HCT116 Ali L
RKO Eto
A549 Ali
A549 Ali L
M
M/G1
G1
G1/S
S
S/G2
G2
TP53
RB1
CDKN1A
CDKN2A
Log2 fold change from parental
2
1
0
−1
−2
TP53
deletion
missense mutation
wild−type
RB1
missense mutation
nonsense mutation
unknown
wild−type
CDKN1A
unknown
wild−type
CDKN2A
deletion
missense mutation
nonsense mutation
unknown
wild−type
B
-Log10 adjusted p-value
0
5
10
15
−5
0
5
Mean log2FC per gene
C
Parental SEN
Breast
Lung
Colorectal
Liver
PC2
−50
0
50
100
PC1
−50
0
50
100

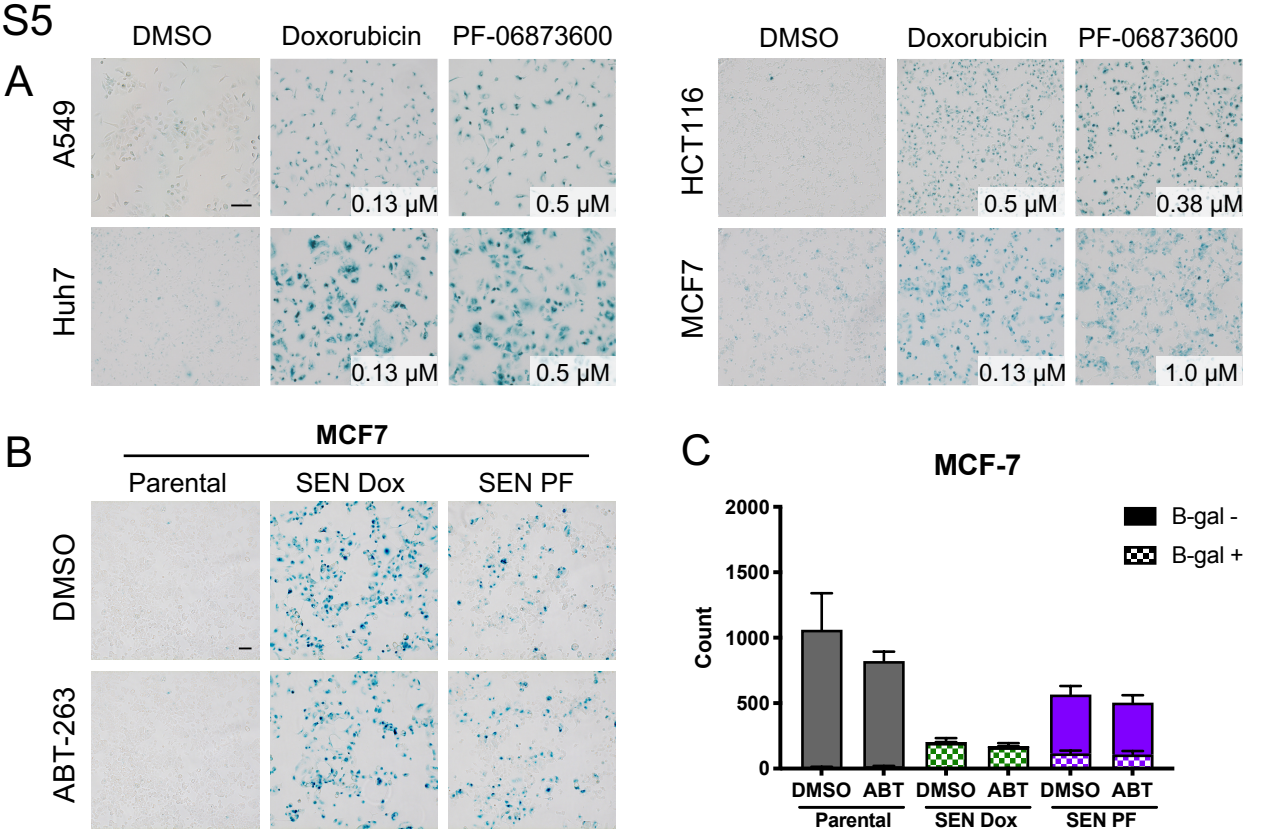
Supplementary figure 4. Gene expression pattern of senescent cancer cells is dictated by the parental state, related to figure 4.

(A) Relative estimation of cell cycle phases, calculated by the difference in mean log expression of marker genes for each phase between treated and untreated samples.

(B) Volcano plot exhibit the results from edgeR paired differential expression analyses, showing only the genes which have a secreted protein product. Each dot represents a gene with its corresponding mean log2 foldchange (x-axis) and multiple testing corrected p-value (-log10, y-axis). Black dots illustrate differentially expressed genes. Log2FC = log2 foldchange.

(C) Principal component analysis for parental and senescent cells (SEN) covering the full transcriptome.

S5


A
DMSO
Doxorubicin
PF-06873600
A549
Huh7
HCT116
MCF7
0.13 μM
0.5 μM
0.38 μM
1.0 μM
B
MCF7
Parental
SEN Dox
SEN PF
DMSO
ABT-263
C
MCF-7
Count
2000
1500
1000
500
0
B-gal -
B-gal +
DMSO
ABT
Parental
SEN Dox
SEN PF
D
LMNB1
Normalized expression
4
3
2
1
0
Parental
SEN Ali
SEN Eto
SEN Dox
SEN PF
A549
HCT116
MCF7
Huh7

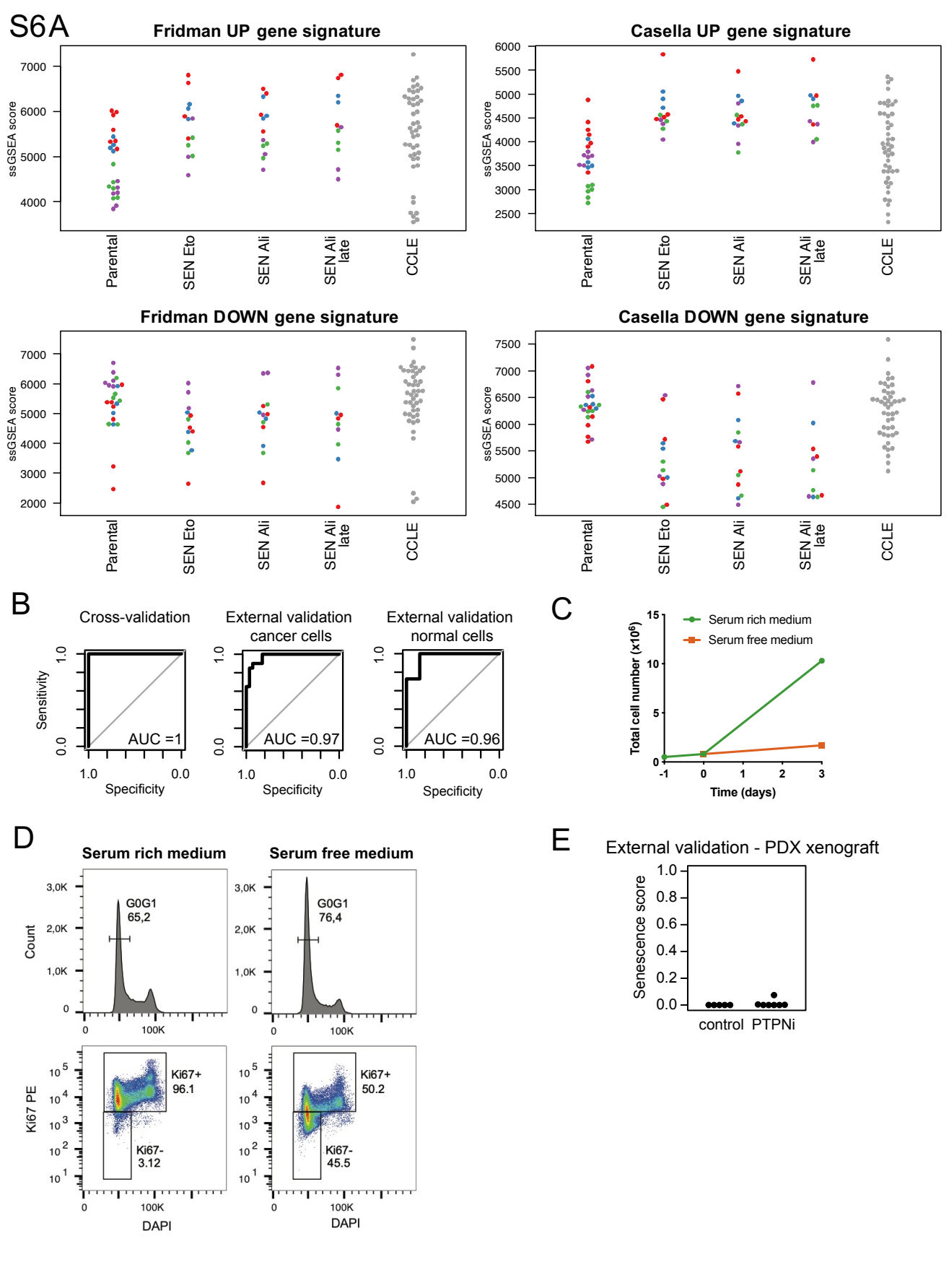
Supplementary figure 5. The senescence phenotype is more influenced by cell type than senescence trigger, related to figure 5.

(A) Representative images of SA-ß-gal staining from three biological replicates. Cells were treated for 7 days. Scale bar =100 µm.

(B) Representative images of SA-ß-gal staining from three technical replicates. Cells were seeded into a 6-well and treated with alisertib or etoposide for 7 days, and medium was subsequently replaced by medium supplemented with DMSO or 1µM ABT-263. Cells were cultured for another 5 days before SA-ß-gal staining was performed. Scale bar = 100 µm

(C) Quantification of SA-ß-gal + and SA-ß-gal – cells for MCF7. Calculations were performed on images shown in B.

(D) Normalized mRNA expression determined by qPCR. Expression is relative to GAPDH and normalized to T47D parental.


S6A
Fridman UP gene signature
Casella UP gene signature
Fridman DOWN gene signature
Casella DOWN gene signature
ssGSEA score
Parental
SEN Eto
SEN Ali
SEN Ali late
CCLE
B
Cross-validation
External validation cancer cells
External validation normal cells
Sensitivity
Specificity
AUC =1
AUC =0.97
AUC =0.96
C
Serum rich medium
Serum free medium
Total cell number (x10^6)
Time (days)
D
Serum rich medium
Serum free medium
Count
G0G1 65,2
G0G1 76,4
Ki67 PE
Ki67+ 96.1
Ki67- 3.12
Ki67+ 50.2
Ki67- 45.5
DAPI
E
External validation - PDX xenograft
Senescence score
control
PTPNi

Supplementary figure S6. Signature performance and quiescence induction, related to figure 6.

(A) ssGSEA scores for Fridman and Casella gene expression signatures.

(B) Receiver Operating Characteristic (ROC) curve of the SENCAN classifier for cross-validation, and external validation in cancer and normal cells.

(C) Cell counts of A549 cultured in serum rich and serum starved medium for 3 days. 500 k A549 cells were seeded at day -1. Medium was changed after one day to serum free or serum rich medium.

(D) Cell cycle analysis of cells stained with DAPI and anti-KI67 PE-conjugated antibody.

(E) SENCAN senescence scores for *in vivo* validation samples from PDX xenograft models treated with SHP2-inhibitor SHP099 (GSE109270).

Supplementary tables

Table S1. Genes in senescence gene sets, related to figure 1 and STAR methods

Casella_UP	Casella_DOWN	Fridman_UP	Fridman_DOWN	Purcell	Hernandez
TMEM159	MCUB	CRYAB	ALDH1A1	OASL	FAM214B
CHPF2	FBL	IGFBP7	BMI1	APOL1	P4HA2
SLC9A7	NIBAN1	SERPINE1	CCN4	IFI27	CCND1
PLOD1	ANP32B	CDKN1A	CCNB1	COL17A1	DYNLT3
FAM234B	PARP1	MMP1	CDC25B	CD82	SCOC
DHRS7	LBR	ISG15	COL3A1	IL32	TAF13
SRPX	SSRP1	IGFBP6	E2F4	APOL3	TOLLIP
SRPX2	TMSB15A	MDM2	EGR1	C3	GBE1
TNFSF13B	CBS	IGFBP5	ID1	CTSS	CHMP5
PDLIM1	CDCA7L	IGFBP1	LAMA1	RSPO3	UFM1
ELMOD1	H1-4	OPTN	LDB2	HERC5	DGKA
CCND3	CBX2	FN1	MARCKS	PLAU	PLXNA3
TMEM30A	PTMA	CCND1		BIRC3	DDA1
STAT1	ITPRIPL1	PEA15		CLDN1	TMEM87B
RND3	AC074135.1	GSN		CD163L1	ZBTB7A
TMEM59		SMPD1		MYD88	RAI14
SARAF		NRG1		IL1B	TSPAN13
SLCO2B1		RRAS		TNFAIP3	ZNHIT1
ARRDC4		IGFBP4		BATF2	ACADVL
PAM		GUK1		GNG11	SLC10A3
WDR78		MAP1LC3B		PMAIP1	POFUT2
WDR63		RAB5B		MLKL	B4GALT7
NCSTN		SPARC		GCA	BCL2L2
SLC16A14		TNFAIP2		DUSP6	NOL3
GPR155		RAB13		ICAM1	KLC1
CLDN1		THBS1		ATF3	PLK3
JCAD		CD44		LGALS9	SLC16A3
BLCAP		IFI16		GMPR	ADPGK
FILIP1L		STAT1		ISG20	
TAP1		TNFAIP3		CXCL2	
TNFRSF10C		CREG1		TNFRSF18	
SAMD9L		CLTB			
SMCO3		S100A11			
POFUT2		CITED2			
KIAA1671		RAC1			
LRP10		HSPA2			
DIO2		VIM			
MAP4K3-DT		RABGGTA			

LINC02154		AOPEP			
TM4SF1-AS1		IRF5			
PTCHD4		TSPYL5			
H2AJ		MAP2K3			
PURPL		RBL2			
		HPS5			
		IGFBP3			
		RGL2			
		RAB31			
		TES			
		SOD1			
		TP53			
		HBS1L			
		ING1			
		TGFB1I1			
		IGFBP2			
		CCN2			
		F3			
		IRF7			
		HTATIP2			
		CDKN2D			
		EIF2S2			
		SMURF2			
		IGSF3			
		CYP1B1			
		TFAP2A			
		ALDH1A3			
		RHOB			
		CDKN1C			
		CDKN2B			
		CDKN2A			

Table S3. Validation samples from the Cancer Cell Line Encyclopedia (CCLE), related to figure 6

SRA sample id	CCLE name	Cell line	Tissue type
SRR8615692	U2OS BONE	U-2 OS	bone
SRR8616101	SAOS2 BONE	Saos-2	bone
SRR8616211	HOS BONE	HOS	bone
SRR8615746	SF268 CENTRAL NERVOUS SYSTEM	SF268	central nervous system
SRR8615748	SF539 CENTRAL NERVOUS SYSTEM	SF539	central nervous system

SRR8615745	SF295 CENTRAL NERVOUS SYSTEM	SF-295	central nervous system
SRR8615493	ISHIKAWAHERAKLIO02ER ENDOMETRIUM	Ishikawa (Heraklio) 02 ER-	endometrium
SRR8615275	HEC1A ENDOMETRIUM	HEC-1-A	endometrium
SRR8615907	KLE ENDOMETRIUM	KLE	endometrium
SRR8616198	BV173	BV173	haematopoietic and lymphoid tissue
SRR8615717	K562 HAEMATOPOIETIC AND LYMPHOID TISSUE	K-562	haematopoietic and lymphoid tissue
SRR8616133	HL60 HAEMATOPOIETIC AND LYMPHOID TISSUE	HL-60	haematopoietic and lymphoid tissue
SRR8616015	A498 KIDNEY	A-498	kidney
SRR8615272	CAKI1 KIDNEY	Caki-1	kidney
SRR8615333	UO31 KIDNEY	UO-31	kidney
SRR8615226	OE19 OESOPHAGUS	OE19	oesophagus
SRR8615228	OE33 OESOPHAGUS	OE33	oesophagus
SRR8615484	JHESOAD1 OESOPHAGUS	JH-EsoAd1	oesophagus
SRR8615505	SKOV3 OVARY	SK-OV-3	ovary
SRR8615844	IGROV1 OVARY	IGROV1	ovary
SRR8615870	OVCAR4 OVARY	OVCAR-4	ovary
SRR8615377	PANC1005	Panc10.05	pancreas
SRR8615519	MIAPACA2	MiaPaCa2	pancreas
SRR8615685	PANC1	Panc1	pancreas
SRR8615641	PC3 PROSTATE	PC-3	prostate
SRR8615547	LNCAPCLONEFGC PROSTATE	LNCaP clone FGC	prostate
SRR8615300	DU145 PROSTATE	DU 145	prostate
SRR8616020	A375	A375	skin
SRR8615414	HS294T	Hs294T	skin
SRR8616054	SKMEL28	Sk-Mel-28	skin
SRR8615722	RH30	RH-30	soft tissue
SRR8615595	RD SOFT TISSUE	RD	soft tissue
SRR8615861	SKUT1 SOFT TISSUE	SK-UT-1	soft tissue
SRR8615362	KATOIII STOMACH	KATO III	stomach
SRR8615495	NCIN87 STOMACH	NCI-N87	stomach
SRR8615934	SNU16 STOMACH	SNU-16	stomach
SRR8615869	FTC133 THYROID	FTC-133	thyroid
SRR8615773	BCPAP THYROID	B-CPAP	thyroid
SRR8615644	8505C THYROID	8505C	thyroid
SRR8616100	SCC15 UPPER AERODIGESTIVE TRACT	SCC-15	upper aerodigestive tract
SRR8616097	SCC25 UPPER AERODIGESTIVE TRACT	SCC-25	upper aerodigestive tract
SRR8615267	CAL27 UPPER AERODIGESTIVE TRACT	CAL 27	upper aerodigestive tract
SRR8616045	HT1197 URINARY TRACT	HT-1197	urinary tract
SRR8615858	T24 URINARY TRACT	T24	urinary tract
SRR8615826	TCCSUP URINARY TRACT	TCCSUP	urinary tract