The Cancer SENESCopedia: A delineation of cancer cell senescence

Graphical abstract

Highlights

- Senescent cancer cells respond differently to senolytic ABT-263
- SASP expression in cancer is heterogeneous and influenced by cell origin
- The SENCAN classifier detects cancer cell senescence in vitro
- The Cancer SENESCopedia contains transcriptome data from 37 senescence models

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In brief
Jochems et al. define common vulnerabilities of senescent cancer cells and shared features for the unequivocal detection of cancer cell senescence. Comprehensive analysis in a cancer cell panel reveals the context dependency of cancer cell senescence and allows the establishment of a SENCAN classifier to detect cancer cell senescence.
SUMMARY

Cellular senescence is characterized as a stable proliferation arrest that can be triggered by multiple stresses. Most knowledge about senescent cells is obtained from studies in primary cells. However, senescence features may be different in cancer cells, since the pathways that are involved in senescence induction are often deregulated in cancer. We report here a comprehensive analysis of the transcriptome and senolytic responses in a panel of 13 cancer cell lines rendered senescent by two distinct compounds. We show that in cancer cells, the response to senolytic agents and the composition of the senescence-associated secretory phenotype are more influenced by the cell of origin than by the senescence trigger. Using machine learning, we establish the SENCAN gene expression classifier for the detection of senescence in cancer cell samples. The expression profiles and senescence classifier are available as an interactive online Cancer SENESCopedia.

INTRODUCTION

Cellular senescence is a stable proliferation arrest evoked by a plethora of stress-inducing factors and is characterized by alterations in cell morphology, gene expression, heterochromatin formation, and metabolic activity (Fridman and Tainsky, 2008; Hayflick, 1965; Narita et al., 2003). An important feature of senescence is the secretion of a variety of cytokines and chemokines, referred to as the senescence-associated secretory phenotype (SASP) (Coppe et al., 2008). This phenotype is heterogeneous and dynamic and depends not only on the senescence trigger, but also on the cell of origin (Basisty et al., 2020; Hernandez-Segura et al., 2017; Wiley et al., 2017).

The current understanding of senescence has derived mainly from studies in non-transformed fibroblasts and primary epithelial cells. However, both normal and cancer cells can undergo senescence after treatment with chemotherapeutic agents, a phenomenon called therapy-induced senescence (TIS) (Ewald et al., 2010). The consequence of TIS in the tumor environment is still highly controversial (Lee and Lee, 2014; Pérez-Mancera et al., 2014; Schosserer et al., 2017). On the one hand, TIS can halt tumor growth, and the SASP can create an immunogenic environment (Greten and Eggert, 2017; Haugstetter et al., 2010; Schmitt et al., 2002). On the other hand, prolonged SASP signaling can jeopardize immune clearance and promote epithelial-to-mesenchymal transition and tumorigenesis in neighboring cells (Davalos et al., 2010; Demaria et al., 2017; Freund et al., 2010). Furthermore, there is increasing evidence that senescent cells can reenter the cell cycle, promote cancer stemness, and eventually contribute to tumor relapse (Dirac and Bernards, 2003; Milanovic et al., 2018; Roberson et al., 2005; Saleh et al., 2017). This evoked interest in the removal of senescent cancer cells by so-called “senolytics”—agents that kill senescent cells (Lozano-Torres et al., 2019)—which have the potential to increase the efficacy of senescence-provoking cancer treatments and reduce potentially deleterious side effects. A potent senolytic for multiple primary senescent cell types is ABT-263 (navitoclax), a BH3 family mimetic that inhibits the anti-apoptotic proteins BCL-2, BCL-W, and BCL-xL (Chang et al., 2016; Zhu et al., 2016).
Figure 1. Senescence induction by alisertib or etoposide in a panel of 13 cancer cell lines

(A–C) A549 and HCT116 were cultured for 7 days with the indicated alisertib or etoposide concentrations using (A) colony formation assay and (B) cell proliferation curves (obtained with IncuCyte). Data represent the mean ± SEM of triplicate wells and are representative of two independent experiments. (C) Representative images of SA-β-gal staining from three independent experiments (quantification in D). Scale bar: 100 μm.

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universal markers to detect cancer cell senescence in vitro and in vivo. Besides assessing senescence-associated β-galactosidase (SA-β-gal) activity, multiple markers have to be combined to define senescence in normal cells (Gorgoulis et al., 2019). Cancer cells often harbor mutations in pathways that are involved in the onset of senescence, such as mutations in TP53 and CDKN2A, and frequently contain deficiencies in apoptotic pathways (Hanahan and Weinberg, 2011). We hypothesized that this may influence the appearance of conventional senescence markers as identified in non-cancer cells, the composition of their SASP, and their response to senolytic agents.

In this study, we analyzed a large panel of cancer cells rendered senescent by different compounds, with the aim to find common vulnerabilities to induce cell death and shared features that would allow unambiguous identification of the senescent state in the context of a cancerous phenotype.

RESULTS

Senescence induction by alisertib or etoposide in a panel of 13 cancer cell lines

To characterize cancer cell senescence, we curated a panel of 13 cell lines from four cancer types rendered senescent with two clinically relevant triggers: (1) alisertib, an aurora kinase A inhibitor that was previously identified to be an effective senescence inducer (Wang et al., 2017); and (2) etoposide, a topoisomerase II inhibitor used as a chemotherapeutic agent in a wide spectrum of human cancers (Baldwin and Osheroff, 2005).

To induce senescence, A549 and HCT116 cells were treated with increasing concentrations of alisertib and etoposide. This resulted in decreased confluency (Figure 1A) and cell proliferation arrest (Figure 1B), and at specific concentrations, cells obtained an enlarged, flattened morphology and increased SA-β-gal activity (Figure 1C). Similarly, we determined the optimal senescence-inducing concentrations in 11 additional cell lines (Figure S1), which resulted in 80%–100% SA-β-gal-positive populations (Figure 1D). Only for PC9 were 40%–60% of cells rendered positive. Next, we checked seven protein markers of senescence (retinoblastoma protein [RB] phosphorylation, laminB1, p53, p53 phosphorylation, p21, p16, and γ-H2AX) and detected at least two positive markers per condition (Figure S1B). Remarkably, even when grouped on mutation status, these markers varied strongly between cell lines.

To further study the features of senescence in this cell line panel, we collected RNA sequencing (RNA-seq) data from treated and untreated cells. We assessed the enrichment of four gene sets previously described as upregulated in non-cancer-cell senescence (Table S1): hallmark gene signatures of Fridman and Tainsky (2008), and of Casella et al. (2019) and differentially expressed genes (DEGs) from Hernandez-Segura et al. (2017) and Purcell et al. (2014). There was virtually no overlap among these gene sets (Figure 1E). We analyzed the difference in single-sample gene set enrichment analysis (ssGSEA) scores (Barbie et al., 2009) of treated samples compared to their untreated control (Figure 1F). For almost all cell lines, the ssGSEA scores were enriched in the expected direction. Only alisertib-treated Huh7 and Hep3B were enriched in the opposite direction for the Fridman and Purcell gene sets, respectively. Overall, these data support the notion that alisertib or etoposide treatment results in a senescent phenotype in the cancer cells tested, and while SA-β-gal, changed morphology, and growth arrest were universally present, the other markers were not (Figure S1C).

Senescent cancer cells respond differently to senolytic ABT-263

In the search for common vulnerabilities, we asked whether senescent cancer cells are universally sensitive to the senolytic ABT-263. We performed dose-response assays in parental and senescent cells (Figure 2A), which showed responses ranging from strong sensitization with a senolytic index (IC50SEN/IC50parental) of 220 in A549 to virtually no change in IC50 in senescent LoVo cells (Figure 2B). While the responses to ABT-263 were highly divergent for different cell lines (Figures 2C and 2D). For A549, the number of SA-β-gal-positive cells was decreased after ABT-263 treatment versus DMSO, while for LoVo, the number of SA-β-gal-positive cells was sustained after ABT-263 treatment (Figures 2E and 2F). This is consistent with the notion that senescent LoVo cells are insensitive to ABT-263. However, a substantial population of SA-β-gal-negative cells was present in SEN Eto LoVo after ABT-263 treatment, suggesting that non-senescent cells did proliferate during ABT-263 treatment. However, the reduction in SA-β-gal-positive cells (Figure S2B) correlated with the ABT-263 IC50 value of each cell line (Figure 2G), which suggests strongly that the behavior of senescent cells, rather than that of non-senescent cells, contributes to the variability in IC50 values.

Next, we asked whether the gene expression of specific pro- or anti-apoptotic proteins could explain the divergent response to ABT-263. We tested the correlation between the expression of 16 genes involved in apoptosis (Figure S3A) and the response to ABT-263 of SEN Eto and SEN Ali. Although none of the

(D) Quantification of SA-β-gal-positive cells for cells treated for 7 days with DMSO, alisertib, or etoposide (images are shown in C and Figure S1). Bars represent the mean ± SEM. Data are obtained in biological triplicate and analyzed with a two-sided Student’s t test, *p < 0.01.
(E) Venn diagram of gene sets from hallmark gene signatures for senescence (Fridman and Casella) and DEGs in senescence (Purcell and Hernandez). Up- and downregulated genes are pooled for Fridman and Casella.
(F) RNA-seq was performed on 13 cell lines in parental and treated cells. Data were analyzed with single-sample gene set enrichment analysis (ssGSEA) for four gene sets associated with senescence. Delta ssGSEA scores represent the difference between parental and senescent samples. Values were normalized per gene set. Ali, alisertib; Eto, etoposide.
Figure 2. Senescent cancer cells respond differently to senolytic ABT-263

(A) Schematic outline of the experimental procedures used in (B)–(D). Cells were treated with senescence-inducing concentrations of alisertib or etoposide for 7 days. Senescent cells and parental cells were reseeded in 96 wells and cultured with increasing concentrations of ABT-263. After 5 days, cell viability was measured with Cell Titer Blue (CTB) assay. SEN Ali, alisertib-induced senescent; SEN Eto, etoposide-induced senescent.

(B) Normalized survival of A549 and LoVo cells treated with ABT-263. 

(C) IC50 values of ABT-263 for A549 and LoVo cells treated with ABT-263. 

(D) Correlation between senolytic index and logIC50 of ABT-263. 

(E) Representative images of A549 and LoVo cells treated with DMSO, ABT-263, and ABT-263 + B-gal activator. 

(F) Cell counts of A549 and LoVo cells treated with DMSO, ABT-263, and ABT-263 + B-gal activator. 

(G) Graph showing the IC50 values of ABT-263 for A549 and LoVo cells treated with ABT-263 + B-gal activator.

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apoptotic proteins were significantly correlated (Figure S3B), the positive correlation for BAD was potentially interesting, since this pro-apoptotic sensitizer protein has the same targets as ABT-263, and high levels of BAD could result in sensitization to this drug. However, already in the parental state, BAD ranges from high to low expression (Figure S3C), and the correlation for senescent cells is not strong enough to solely explain the sensitivity to ABT-263. For MCL1, Bik, and Bid, the difference in gene expression between ABT-263 unresponsive and responsive cells was small, or the correlation was in the opposite direction. In conclusion, the transcriptome of apoptosis-related genes could not explain the divergent response to ABT-263 of senescent cancer cells.

**Gene expression in senescent cancer cells evolves over time and is independent of senescence trigger**

The gene expression of normal senescent cells is influenced by the inducer, cell origin, and time (Hernandez-Segura et al., 2017; Wiley et al., 2017). Since this might complicate the search for a robust classifier in cancer, we asked how similar SEN Ali cells and SEN Eto cells are in transcriptome and how SEN Ali cells change over time. We used RNA-seq data from the 13 SEN Ali and SEN Eto cell lines, which were treated for 7 days (early senescent state), and included a late senescent state for 11 cell lines, where cells were treated with alisertib for 14 days (SEN Ali L). MCF-7 and RKO started to die after prolonged treatment with alisertib.

First, to assess the inducer effect, SEN Ali and SEN Eto were compared to their parental counterparts using a paired differential gene expression analysis (DGEA) across the 13 cell lines. This yielded 1,875 and 1,275 DEGs, respectively (Figures 3A and 3B). Importantly, the majority of DEGs were overlapping between SEN Ali and SEN Eto, and when compared directly to each other, no DEGs were found between the two senescent states (Figure 3C). This implies that alisertib and etoposide induce a similar senescence phenotype.

To assess the gene expression of cells at a later stage of senescence, SEN Ali and SEN Ali L were compared. DGEA identified more DEGs (2,936) between SEN Ali L and parental cells (Figure 3D) than DEGs detected between SEN Ali and parental cells (Figure 3B). SEN Ali L especially showed more genes that were downregulated. Furthermore, when SEN Ali and SEN Ali L were compared directly (Figure 3E), 69 DEGs were detected. This can be explained by gene expression evolving over time, where a large set of genes may start to change at an early time point but becomes significantly different from parental only at a later time point.

To identify genes that are differentially expressed in various senescence conditions, we compared SEN Ali, SEN Ali L, and SEN Eto samples to their parental counterparts, as outlined in Figure 3F. From 37 senescent models, 245 genes were identified as most significantly different (adjusted p value < 1 x 10^-6) with at least a 2-fold change in gene expression (Figure 3F; Table S2). GSEA of 50 hallmark gene sets (Liberzon et al., 2015) revealed that gene sets involved in proliferation (MYC proto-oncogene, bHLH transcription factor (MYC) targets, G2M checkpoint, E2F targets) were negatively enriched, as well as oxidative phosphorylation, MTORC1 signaling, DNA repair, and unfolded protein response (Figure 3G). The negative enrichment of the MTORC1 signaling and DNA repair pathway gene sets may be the result of decreased proliferation since these gene sets contain many proliferation genes. Although not statistically significant, trends for positive enrichment were seen for gene sets involved in inflammatory responses (interferon-alpha, interferon-gamma, tumor necrosis factor (TNF)-x signaling via nuclear factor (NF)-xB, interleukin (IL)-6/JAK/STAT3 signaling). Similarly, the top 20 overrepresented Gene Ontology terms included gene sets describing mitosis, RNA processing, and DNA damage responses (DDRs) (Figure 3H).

Although cellular senescence was originally characterized as a stable G1 phase cell-cycle arrest, later studies challenged this idea and confirmed the existence of a G2 arrest and G2 exit, dependent on TP53 and RB1 status (reviewed in Gire and Dulic, 2015). We asked how cell-cycle distribution changed upon senescence induction in cancer cells, often mutated in TP53, RB1, and CDKN2A. Figure S4A shows that cell-cycle gene sets have a decreased expression in senescence, consistent with the notion that senescent cells do not proliferate. Remarkably, the TP53 mutant cells show a different expression pattern than TP53 wild-type cells. For TP53 wild-type cells, the expression of G1, M/G1, and G1/S phase gene sets decreased moderately, while the other phase gene sets decreased strongly, consistent with a G1 cell-cycle arrest. On the other hand, TP53 mutant cells were not arrested in a specific cell-cycle phase. RB1 mutant cells also lacked a phase-specific cell-cycle arrest, in concordance with the observation that all RB1 mutant cells in this cell panel also have a TP53 mutation. The mutation status of other cell-cycle gene sets (CDKN1A, CDKN2A) did not appear to relate to this G1 arrest pattern. This suggests that cell-cycle arrest of senescent cancer cells is influenced by their TP53 mutation status. Indeed, it has been shown that alisertib induces a G1/4N arrest in TP53 wild-type HCT116 cells, after an initial G2/M arrest and subsequent mitotic slippage, while TP53 knockout in these cells leads to further entry into S-phase (Marxer et al., 2014).

Taken together, these data indicate that SEN Ali and SEN Eto were similar in gene expression, while the expression of SEN Ali...
Figure 3. Gene expression in senescent cancer cells changes over time and is independent of alisertib or etoposide trigger

(A–E) Volcano plots exhibit the results from edgeR paired differential expression analyses. Each dot represents a gene with its corresponding mean Log2FC (x axis) and Benjamini-Hochberg corrected p value (y axis). Black dots illustrate DEGs, using a cutoff of p < 0.01 and Log2FC > 1 or < −1.

(F) Outline of samples that were used in the DGEA. The paired samples of SEN Eto, SEN Ali, and SEN Ali later contained 13, 13, and 9 cancer cell lines, respectively. Most significant DEGs were identified using adjusted p value < 1 × 10−3 and Log2FC > 1 or < −1.

(G and H) Results of GSEA for senescent versus control samples (presented in F). Lollipop chart shows the normalized enrichment scores (ES), where dot color indicates significance level, and dot size represents the leading edge, a measure for the number of genes that contribute to the enrichment of the gene set. (G) Top 20 enriched GSEA hallmark gene sets ranked based on normalized ES. Direction of ES indicates negative or positive enrichment in senescent samples. EMT, epithelial-to-mesenchymal transition. (H) Top 20 overrepresented GSEA Gene Ontology gene sets, ranked based on normalized ES. ES is non-directional. Sig. trans., signal transduction; Repl.-indep., replication independent; Reg., regulation; Neg., negative; Pos., positive; DDR, DNA damage response.
is dynamic over time, and genes involved in proliferation were negatively enriched in senescent cancer cells.

**SASP gene expression is heterogeneous among senescent cancer cells**

SASP factors produced by senescent cells represent a collection of secreted signaling factors that are involved in inflammatory responses (Coppé et al., 2010). It was surprising that although inflammatory responses were among the top 20 enriched hallmark gene sets, the enrichment was not significant (Figure 3G). This raised the question of which SASP factors were expressed in the individual senescence models (Figure 4A). Instead, it seems that the gene expression in the parental state predicts the change in expression upon senescence induction. For instance, MMP1, IL6, SAA1, and SAA2 are highly expressed in senescent MDA-MB-231 and SUM159 but also show higher baseline levels in the parental state. Principal-component analysis of the full transcriptome shows that cells cluster by cell line and cancer type rather than by treatment (Figure S4C). Thus, the baseline expression of parental cells seems to influence the gene expression in the senescent state and determine which SASP factors are expressed.

IL-6 (IL6) and IL-8 (CXCL8) have been described as classical SASP factors that are consistently upregulated in normal cell senescence and play a role in the maintenance and propagation of SASP expression (Coppé et al., 2008, 2010; Ortiz-Montero et al., 2017). Nonetheless, the baseline expression of IL6 and CXCL8 in cancer cells seems to be determined by the cancer tissue type of origin and, especially for CXCL8, ranges from low to high expression (Figure 4B). Upon senescence induction, expression of IL6 is mostly induced in lung and breast cancer cells with high baseline expression levels, while for CXCL8, expression increases for some cell lines but not for others. Overall, these data indicate that the SASP in senescent cancer cells is heterogeneous.

**The senescence phenotype is more influenced by cell type than senescence trigger**

Above, we showed that SEN Ali cells and SEN Eto cells had a similar transcriptome profile (Figure 3C), SASP expression (Figure 4A), and response to ABT-263 (Figures 1C and 1D). This would suggest that the senescence phenotype is less dependent on the trigger and rather dependent on the cell line model. To expand on this, we asked whether the same trend in response to ABT-263 and SASP factors is seen for two other drugs described to induce senescence: (1) doxorubicin, a DNA intercalator and topoisomerase II inhibitor (Chang et al., 1999; Gewirtz,
Figure 5. The senescence phenotype is more influenced by cell type than senescence trigger

(A) Quantification of SA-β-gal-positive cells for cells treated for 7 days with DMSO, doxorubicin, or PF-06873600 (PF) (images are shown in Figure S5A). Bars represent the mean ± SEM. Data are obtained in triplicate and analyzed with a two-sided Student’s t test. *p < 0.0001.

(B) Western blot for senescence markers for cells treated for 7 days. p, parental; D, doxorubicin.

(C) Dose-response curves for ABT-263. Values represent the mean ± SEM of three independent experiments.

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First, senescence was induced with doxorubicin or PF, as judged by a growth arrest and morphology change, an increase of SA-β-gal-positive cells (Figures 5A and SSA), and the presence of senescence protein markers (Figure 5B). The ABT-263 response of these doxorubicin- and PF-induced senescent cells (SEN Dox and SEN PF) was compared to SEN Ali and SEN Eto and was highly similar (Figure 5C). Moreover, the IC50 values of the senescent cells were significantly different between cell lines (p < 0.001, two-way ANOVA), while the senescence inducer explained only 1.84% of the total variation (p = 0.1668, two-way ANOVA). However, Tukey’s post hoc test revealed that for MCF7, the response of SEN Ali and SEN Eto was different from SEN Dox and SEN PF (Figure 5D). Although a large SA-β-gal-negative population was detected for SEN PF after ABT-263/DSMO treatment, the SA-β-gal-positive cells still showed little response to ABT-263 (Figure 5SB).

Next, we assessed whether expression of SASP genes IL6, CXCL8, IL1A, and IL1B changed upon different senescence triggers. Interestingly, the expression of IL6 and CXCL8 was induced in senescent A549 and Huh7, while HCT116 and MCF7 lacked expression, regardless of the trigger (Figures 5E and 5F). The difference in SASP expression for IL6 and CXCL8 was significantly different between cell lines (p < 0.001, two-way ANOVA), while the senescence inducer had no significant contribution (p = 0.103 and 0.08, respectively). The expression of IL1A and IL1B remained low in all samples, below the accurate detection limit of qPCR (not shown). On a cautionary note, MCF7 SEN PF did not show a decrease in LMNB1, while the other senescent MCF7 cells did, which indicates a less efficient senescence induction (Figure 5SC).

An explanation for why IL6 and CXCL8 were not expressed in senescent HCT116 and MCF7 is that these cells are intrinsically incapable of activating these genes (i.e., due to a repressive chromatin state or a lack of NF-κB activation) (Lopes-Paciencia et al., 2019). To test this, we transduced A549, HCT116, and MCF7 with a lentiviral NF-κB reporter (Wilson et al., 2013), and subsequent exposure to protein kinase C activator phorbol myristate acetate (PMA) increased NF-κB activity in A549 and HCT116, but not in MCF7. Exposure to TNF-α increased NF-κB activity in all cell lines (Figure 5G). Therefore, although NF-κB is less primed for activation in MCF7, it is still activatable. Next, we checked whether the activation of NF-κB would drive the expression of IL6 and CXCL8. Interestingly, expression was strongly increased in A549, while expression remained absent in HCT116, similar as in senescence (Figure 5H). This suggests that IL6 and CXCL8 might be silenced in HCT116, but not in A549. However, MCF7 lacked expression of IL6 but did show an increase in CXCL8 upon stimulation with PMA and TNF-α (Figure 5H). Although it is known that unraveling the variability of SASP is highly complex and still poorly understood (Cuollo et al., 2020), collectively, these results hint toward a cell line intrinsic inability to express certain cytokines. Overall, it becomes apparent that the senescence phenotype is influenced by the nature of the cell line and, to a lesser extent, by the senescence trigger.

**SENCAN classifier for cancer cell senescence**

Given the fluctuating and heterogeneous gene expression of senescent cells in general (Hernandez-Segura et al., 2017) and the apparent influence of parental cell characteristics, we studied whether a machine learning approach could be used to find a classifier that identifies senescent cancer cells based on gene expression measurements. Although the expression of genes in the existing senescence gene signatures changes consistently between senescent cells and a reference control sample, there was no threshold at which samples would be accurately classified as senescent or not using sing score (Foroutan et al., 2018) or ssGSEA (Figures 6A and S6A). Therefore, we compared several classification methods (ridge regression, elastic net, random forest, support vector machines, and Gaussian processes) through “leave-all-samples-of-one-cell-line-out” cross-validation (see Method details). Among these, elastic net (Zou and Hastie, 2005) gave the best performance, and we continued the rest of the analysis with that method. To provide the classifier with additional examples of non-senescent cells, we also included 45 untreated samples from the Cancer Cell Line Encyclopedia (Table S3). The elastic net classifier was able to classify almost all samples correctly as either senescent or not (Figure 6B). The only sample that was classified incorrectly was Hep3B after 7-day alisertib treatment. The alisertib-treated PC9 sample received a score of 0.44; however, 7-day alisertib treatment indeed only induced about 40%–60% senescent cells in this cell line (see Figure 1D).

After the optimal parameter settings were determined in cross-validation, an elastic net was trained on all samples employing the optimal settings, yielding the SENCAN classifier for classifying senescence in cancer samples. The 137 genes selected by the SENCAN classifier (Table S4) include several well-known senescence-associated genes (CDKN1A and HMGB1), as well as genes involved in mRNA processing and splicing (like SNRPA and SRSF2), interferon response genes (like IFIT2 and ISG15), histone variants (like H4C8 and H2AC20), and the telomere regulator RTEL1. Apart from CDKN1A, no other genes clearly associated with proliferation
Figure 6. Validation of SENCAN elastic net classifier for cancer cell senescence
(A) Singscores for Fridman and Casella gene expression signatures. Each dot represents a cell line. CCLE, Cancer Cell Line Encyclopedia.
(B) SENCAN scores in cross-validation; all samples of one cell line were left out, and the elastic net was trained on the remaining cell lines.
(C) SENCAN scores of external validation samples from senescent cancer cells (GEO:GSE110028; GEO:GSE129182; GEO:GSE121276; GEO:GSE102639; GEO:GSE158743).
(D) Senescence scores of 25 normal cells of Casella et al. (2019) (GEO:GSE130727).
(E) SENCAN classifier performance in cross-validation (in B) and external validation sets (in C and D).
were selected. Interestingly, the SENCAN classifier also comprised various long non-coding RNAs, novel transcripts, and antisense transcripts. Some of the novel transcripts aligned downstream and in close proximity to known protein-coding genes (such as AL512306.2, downstream of MDM4). It could be that AL512306.2 is specifically upregulated in senescence; however, the reads aligning to AL512306.2 may also be the result of transcriptional readthrough of MDM4. Transcriptional readthrough has been associated with senescence before (Muniz et al., 2017). In line with this, there were also more reads located between MDM4 and AL512306.2 in senescent samples than in the parentals (p < 0.001 by t test), while there are no transcripts annotated in that region. Altogether, it appears that the SENCAN classifier utilizes several different processes to identify senescent samples, including mRNA processing, interferon response, and a signal that may be derived from transcriptional readthrough.

To validate the SENCAN classifier, we searched the literature for RNA-seq samples of senescent cancer cells and found five datasets where senescence was induced in other cell lines and/or by means other than alisertib and etoposide (Table S5). This included data from Nojima et al. (2018), Ávila-López et al. (2021), and Kolesnichenko et al. (2019) and datasets from Wang et al. (2019) and Wang et al. (2017) derived from previous studies from our own laboratory. In these five external datasets, the SENCAN classifier was able to correctly distinguish senescent from non-senescent samples in 44 out of 49 samples (90% accuracy; Figure 6C; precision = 0.94, sensitivity = 0.8, specificity = 0.97). Three incorrectly classified samples were from Wang et al. (2019): SK-Hep1 cells treated with XL413, which were described as non-senescent due to their wild-type TP53 status but scored as senescent by the classifier (score of 0.81); and PLC and Hep3B cells treated with XL413, which were defined as senescent, but received a non-senescence score (0.10 and 0.37, respectively). The H2A.Z knockdown samples from Ávila-López et al. (2021) were also not classified as senescent, but it should be noted that although these samples were described as senescent, they contained fewer than 50% of senescent cells (Ávila-López et al., 2021). This indicates that the classifier may only classify a sample as senescent if the majority of cells are senescent.

Although our aim was to build a classifier for senescent cancer cells, we asked how the classifier would behave on normal senescent cells. Therefore, we scored samples from Casella et al. (2019), which included human aortic endothelial cell (HAEC) and human umbilical vein endothelial cell (HUVEC) cells, and IMR-90 and WI-38 fibroblast cells made senescent via various triggers (Figure 6D). Notably, the SENCAN classifier correctly classified the fibroblast and HUVEC samples but could not detect senescence in the irradiated endothelial HAEC samples. HAEC cells did not upregulate CDKN1A at the transcript level (Casella et al., 2019), so their type of senescence may indeed be different from that observed in cancer cells.

Next, to test if the classifier could distinguish senescent from quiescent cells, we attempted to obtain multiple proliferation-arrested cancer cells by serum starvation. Inducing stable quiescence only succeeded for A549, indicated by a proliferation arrest (Figure S6C), accumulation of cells in G0/G1, and increased Ki67-negative cells after serum starvation (Figure S6D). The SENCAN classifier scored both the quiescent population and the control as non-senescent (scores of 3.5 × 10⁻⁷ and 0.0016, respectively), suggesting that the SENCAN classifier is able to distinguish senescence from quiescence.

Finally, we wondered if the SENCAN classifier could be validated for detecting senescence in in vivo cancer samples. We were able to find only one publicly available dataset with RNA-seq data of senescent human cancer cells in vivo (Mainardi et al., 2018); this study used patient-derived xenografts (PDXs) of non-small-cell lung cancer in mice, which were treated with the SHP2-inhibitor SHP099. The PDX samples from which RNA-seq was available (PDX2) were not unambiguously senescent, since a minority of cancer cells stained SA-β-gal positive (Mainardi et al., 2018). The SENCAN classifier classified all of these PDX samples as non-senescent (Figure S6E). This could indicate that the classifier is not able to detect senescence in in vivo samples or that samples do not receive a high senescence score when only a minority of cells are senescent, similar to the in vitro samples of Ávila-López et al. (2021) described above. It is, therefore, unclear whether the classifier is accurate for use in vivo.

Taken together, the SENCAN classifier is able to accurately detect senescence in many cancer cells and fibroblasts (Figures 6E and S6B) in vitro, although a low senescence score does not completely rule out that cells are senescent, and a separate line of evidence is still necessary to unambiguously identify senescent cells.

**DISCUSSION**

Here, we report a Cancer SENESCopedia, which encompasses transcriptome and senolytic response data from a panel of senescent cancer cells, with the aim of finding universal weaknesses for senotherapy and ubiquitous markers of cancer cell senescence. Our data show that the senolytic response to ABT-263 is highly divergent among cell lines but remarkably similar for senescent cells induced with alisertib, etoposide, doxorubicin, and PF-06873600. Although ABT-263 has been described as a broad-spectrum senolytic and eliminated various senescent cells in vivo (Bussian et al., 2018; Chang et al., 2016; Childs et al., 2016; Demaria et al., 2017; Kim et al., 2017; Pan et al., 2017), a cell-type specificity has been reported before in senescent preadipocytes (Zhu et al., 2016) and doxorubicin-induced senescent breast cancer cells (Shahbandi et al., 2020). Since a (transient) DDR can be activated by the drugs we used to induce senescence (Montecucco et al., 2015; Niu et al., 2015; Tacar et al., 2013; Wang et al., 2020), and since the senescence transcriptome was similar between senescent cells triggered via different drugs, it might be possible that senescence is triggered via DDR and that a similar senescence phenotype is elicited in a given cell line, regardless of the DDR inducer.

Our results highlight that the therapeutic potential of senolytic approaches based on BCL-2 family inhibition in cancer treatment will be context dependent. Although the clinical use of ABT-263 is partly hampered by its on-target platelet toxicity (Kaefer et al., 2014), others have recently developed modified versions of ABT-263 that spare platelets (González-Gualda
et al., 2020; He et al., 2020; Khan et al., 2019). With these and potentially other ABT-263-based senotherapies on the horizon, it will be important to elucidate why certain senescent cells respond while others remain unresponsive. Mechanisms that are likely involved are the activity of anti-apoptotic proteins BFL-1 or MCL-1; effects of ABT-263 on the stability of MCL1 (Wang et al., 2014); or lacking activity of pro-apoptotic proteins in ABT-263-unresponsive cells. Indeed, the most unresponsive cell line, LoVo, contains biallelic frameshift mutations in BAX, and HCT116 carries a heterozygous mutation for this gene. This could explain their resistance to ABT-263. Moreover, over-expression of phorbol-12-myristate-13-acetate-induced protein 1 (NOXA), which inhibits MCL-1, conferred sensitivity in doxorubicin-induced senescent breast cancer cells (Shahbandi et al., 2020). In future experiments, we plan to investigate the pro- and anti-apoptotic signaling using BH3 profiling (Touzeau et al., 2016).

The consequences of senescent cells within a tumor microenvironment are attributed to their unique SASP mixture. Our data indicate that the expression levels of SASP genes vary strongly between various cancer types, which is in line with previous research that reported a significant heterogeneity in gene expression among senescent cells (Basisty et al., 2020; Hernandez-Segura et al., 2017), with even cell-to-cell variability (Wiley et al., 2017) and changes over time (Hernandez-Segura et al., 2017; Martinez-Zamudio et al., 2020). Our data show that even IL-6 and IL-8, often used as senescence markers, are expressed at variable levels at baseline, and senescence induction changes their expression diversely, mostly depending on the cell line and less on the trigger. Although it is still understood poorly what drives these differences, proposed mechanisms are the chromatin state of SASP genes, the TP53 mutation status, and the activity of NF-κB and CCAAT-enhancer-binding proteins (C/EBPs) isoforms (Cuollo et al., 2020). Recently, it has been shown in pre-clinical models of pancreatic cancer that a combination of pro-senescence therapy and checkpoint blockade can be an efficient combination therapy (Ruscetti et al., 2020). Given the heterogeneous nature of the SASP produced in our senescent cancer cell line panel, it remains to be determined whether this treatment strategy has broad applicability. In this context, it is important to note that two recent papers have reported that IL-8 levels in plasma, tumor, and peripheral blood mononuclear cells are associated with reduced response to immune checkpoint inhibitors and peripheral blood mononuclear cells are associated with reduced response to immune checkpoint inhibitors.

This work underscores the context dependency of the senescence cancer phenotype and compares the characteristics of senescent cancer cells in a sizeable number of senescence models. Our data also emphasize the need for a systematic approach to study the pleiotropic nature of senescence. Besides already-existing (relational) databases, like SeneQuest (Gorgoulis et al., 2019), Human Ageing Genomic Resources (HAGR) (de Magalhaes et al., 2005), and SASP Atlas (Basisty et al., 2020), multi-parametric testing will be pivotal to map senotherapy responses, SASP compositions, and the various effects of senescent tumor cells. We share an interactive online Cancer SENSE-Copedia interface (available at https://ccb.nki.nl/publications/cancer-senescence/), which allows interrogation of the gene expression pattern of senescent cancer cells and provides the SENCAN classifier as a tool to classify whether cancer cells are senescent using transcriptome data as input. This will help to unify the validation and detection of cancer cell senescence in the path toward understanding and eliminating senescent cells in each cancerous context.

**STAR★METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
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  - Western blot
  - NF-KB activation experiments
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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2021.109441.

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AUTHOR CONTRIBUTIONS

Conceptualization, F.J., B.T., and R.B.; formal analysis, F.J. and B.T.; investigation, F.J., B.T., G.D.C., R.J., Z.P., K.G., L.W., A.S., C.W., H.J., and R.L.d.O.; writing - original draft, F.J., B.T., and R.B.; writing – review & editing; G.D.C., R.J., Z.P., K.G., L.W., A.S., C.W., H.J., R.L.d.O.; visualization, F.J. and B.T.; supervision, R.B. and L.F.A.W.; funding acquisition, R.B.

DECLARATION OF INTERESTS

R.B. is the founder of the company Oncosence (https://www.oncosence.com), which aims to develop senescence-inducing and senolytic compounds to treat cancer. L.F.A.W. received research funding from Genmab BV.

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Phospho-Rb (Ser780) | Cell Signaling Technology | RRID: AB_10950972 |
| Lamin B1            | Cell Signaling Technology | RRID: AB_2650517  |
| P53                 | Cell Signaling Technology | RRID: AB_331743   |
| Phospho-p53 (Ser15) | Cell Signaling Technology | RRID: AB_331741   |
| P21                 | Abcam  | RRID: AB_2890611 |
| P16                 | Abcam  | RRID: AB_10858268  |
| Phospho-Histone H2A.X (Ser139) | Sigma-Aldrich | RRID: AB_309864 |
| Alpha-Tubulin       | Sigma-Aldrich | RRID: AB_477593 |
| Goat anti-Rabbit IgG (H L)- HRP Conjugated | Bio-Rad | RRID: AB_11125142 |
| Goat anti-mouse IgG (H L)- HRP Conjugated | Bio-Rad | RRID: AB_11125547 |
| **Bacterial and virus strains** |        |            |
| Lentiviral NF-kB reporter | Wilson et al., 2013 | RRID: Addgene_49343  |
| **Chemicals, peptides, and recombinant proteins** |        |            |
| Alisertib (MLM8237) | MedKoo Biosciences | Cat:#201931 |
| Etoposide (VP16)   | MedKoo Biosciences | Cat:#100330 |
| Navitoclax (ABT-263) | MedKoo Biosciences | Cat:#201970 |
| Doxorubicin hydrochloride | Sigma-Aldrich | Cat:#D1515 |
| PF-06873600        | MedChemExpress | Cat:#HY-114177 |
| **Critical commercial assays** |        |            |
| Senescence Cells Histochemical Staining Kit | Sigma-Aldrich | Cat:CS0030 |
| RNeasy mini kit    | QIAGEN  | Cat:#74106 |
| TruSeq Stranded mRNA samples preparation kit | illumina inc. | Part:#15031047 |
| Luciferase Assay System | Promega | Cat:#E1500 |
| **Deposited data** |        |            |
| RNA sequencing data of etoposide- and alisertib- induced senescent cells | This paper | https://www.ebi.ac.uk/arrayexpress Accesion: E-MTAB-9970 |
| SENCAN classifier  | This paper | https://ccb.nki.nl/publications/cancer-senescence/ |
| SENESCopedia webtool with processed RNA sequencing data of etoposide- and alisertib-induced senescent cells | This paper | https://ccb.nki.nl/publications/cancer-senescence/ |
| **Experimental models: Cell lines** |        |            |
| MCF-7               | ATCC    | RRID:CVCL_0031 |
| T47D                | ATCC    | RRID:CVCL_0553 |
| MDA-MB-231          | ATCC    | RRID:CVCL_0062 |
| SUM159              | Metello Innocenti (NKI, Amsterdam) | RRID:CVCL_5423 |
| A549                | ATCC    | RRID:CVCL_0023 |
| PC9                 | ATCC    | RRID:CVCL_B260 |
| H358                | ATCC    | RRID:CVCL_1559 |
| HCT116              | ATCC    | RRID:CVCL_0291 |

(Continued on next page)
## REAGENT or RESOURCE SOURCE IDENTIFIER

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| LoVo                | ATCC   | RRID:CVCL_0399 |
| RKO                 | ATCC   | RRID:CVCL_0504 |
| Huh7                | Erasmus university (Rotterdam, Netherlands) | RRID:CVCL_0336 |
| Hep3B               | Erasmus university (Rotterdam, Netherlands) | RRID:CVCL_0326 |
| HepG2               | Erasmus university (Rotterdam, Netherlands) | RRID:CVCL_0027 |

## Oligonucleotides

| Oligonucleotides | Resource | Identifier |
|------------------|----------|------------|
| GAPDH_Fw         | Integrated DNA Technologies | AATCCCATCACCATCTTCCA |
| GAPDH_Rv         | Integrated DNA Technologies | TGGACCTCCACGAGTACTCA |
| IL6_Fw           | Integrated DNA Technologies | ACTCACCTCTTCAAGAAGATTG |
| IL6_Rv           | Integrated DNA Technologies | CCATCTTTGGAAGGTCAGGTA |
| IL8_Fw           | Integrated DNA Technologies | TTCTGATTTCTGCAGCTCTG |
| IL8_Rv           | Integrated DNA Technologies | AAATTTGGTTGGAAGGAAGT |
| LMNB1_Fw         | Integrated DNA Technologies | TTCTCGAAGCTTGATCTGGG |
| LMNB1_Rev        | Integrated DNA Technologies | GATCGAGCTGGGCAAGTG |

## Software and algorithms

| Software and algorithms | Resource | Identifier |
|-------------------------|----------|------------|
| Graphpad Prism (v8)     | GraphPad Software, San Diego, CA, USA | https://www.graphpad.com |
| R statistical programming language (v3.6.0) | R Foundation for Statistical Computing, Vienna, Australia | https://www.r-project.org |
| edgeR (v3.26.8)         | McCarthy et al., 2012 | http://bioinf.wehi.edu.au/edgeR |
| Flowjo (v10.7.1)        | Becton, Dickson and Company | https://www.flowjo.com |
| GSVA R package          | Hänelmann et al., 2013 | https://github.com/rcastelo/GSVA |
| Flexgsea R package      | Bismeijer and Kim, 2019 | https://github.com/NKI-CCB/flexgsea-r |
| REVIGO                  | Supek et al., 2011 | http://revigo.irb.hr |
| MSigDB Hallmark and Gene Ontology (GO) biological process sets | Liberzon et al., 2015 | https://www.gsea-msigdb.org/gsea/index.jsp |
| CLASTR                  | Robin et al., 2020 | https://web.expasy.org/ceoliosaurus-str-search/ |
| Glmnet R package        | Friedman et al., 2010 | https://www.jstatsoft.org/article/view/v033i01 |

## Other

| Other | Resource | Identifier |
|-------|----------|------------|
| Cell cycle distribution gene set | Mizuno et al., 2009 | PMID: 19331659 |
| Senescence signature Fridman | Fridman and Tainsky, 2008 | https://www.gsea-msigdb.org/gsea/index.jsp PMID: 18711403 |
| Senescence signature Casella | Casella et al., 2019 | PMID: 31251810 |
| Geneset Hernandez | Hernandez-Segura et al., 2017 | PMID: 28844647 |
| Geneset Purcell | Purcell et al., 2014 | PMID: 25483067 |
| External validation data-set senescent cancer cells 1 | Wang et al., 2017 | GEO: GSE102639 |
| External validation data-set senescent cancer cells 2 | Wang et al., 2019 | GEO: GSE121276 |
| External validation data-set senescent cancer cells 3 | Nojima et al., 2018 | GEO: GSE110028 |
| External validation data-set senescent cancer cells 4 | Ávila-López et al., 2021 | GEO: GSE129182 |

(Continued on next page)
**RESOURCE AVAILABILITY**

**Lead contact**
Requests for further information and reagents may be addressed to the corresponding author: René Bernards (r.bernards@nk.nl).

**Materials availability**
This study did not generate new unique reagents.

**Data and code availability**
- Raw RNA sequencing data of HepG2, Huh7, Hep3B, MCF-7, T47D, MDA-MB-231, SUM159, HCT116, LoVo, RKO, A549, H358, PC9, in parental, alisertib-induced senescent and etoposide-induced senescent state are available in ArrayExpress. The accession number is listed in the Key Resources Table. The SENCAN classifier established in this study is functional at the online Cancer SENESCopedia website, which is listed in the Key Resources Table.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

Liver cancer cell lines HepG2 (RRID:CVCL_0027), Huh7 (RRID:CVCL_0336), Hep3B (RRID:CVCL_0326), triple-negative breast cancer (TNBC) cell line MDA-MB-231 (RRID:CVCL_0062) were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS, Serana), 1% penicillin/streptomycin (P/S, GIBCO) and 2mM L-glutamine (GIBCO). Colon cancer cell lines HCT116 (RRID:CVCL_0291), RKO (RRID:CVCL_0399) and lung cancer cell lines A549 (RRID:CVCL_0023), PC9 (RRID:CVCL_B260) and H358 (RRID:CVCL_1559) were cultured in RPMI medium (GIBCO) supplemented with 10% FBS, 1% P/S and 2mM L-glutamine (GIBCO). ER+ breast cancer cell lines MCF-7 (RRID:CVCL_0031) and T47D (RRID:CVCL_0553) were grown in RPMI (GIBCO) supplemented with 10% FBS, 1% P/S and 10 µg/ml insulin (Sigma-Aldrich). TNBC cell line SUM159 (RRID:CVCL_5423) was maintained in DMEM/F12 (GIBCO) medium supplemented with 10% FBS, 1% P/S and 5 µg/ml insulin and 1ug/ml hydrocortisone (Sigma-Aldrich). HepG2 (RRID:CVCL_0027), Huh7 (RRID:CVCL_0336), and Hep3B (RRID:CVCL_0326) were provided by Erasmus University (Rotterdam, Netherlands), SUM159 was provided by Metello Innocenti (NKI, Amsterdam). Other cell lines were purchased from ATCC. All cell lines were regularly tested for mycoplasma contamination and their identity was validated via short tandem repeat analysis (by Eurofins Genomics). The STR profiles were analyzed with CLASTR (Robin et al., 2020). Cell lines derived from female: MCF7, T47D, MDA-MB-231, SUM159, RKO, Huh7, Hep3B, PC9. Cell lines derived from male: HCT116, LoVo, HepG2, A549, H358. Based on STR profiling, HCT116, LoVo, and HepG2 have lost their Y chromosome.

**METHOD DETAILS**

**Colony formation assay**
Cells were grown for 7 days in 6-well until 80%-90% confluency, 15k cells were seeded for A549 and 20k cells for HCT116. Alisertib (Medkoo Bioscience) and etoposide (Medkoo Bioscience) drugs were refreshed every 3-4 days. Colonies were fixed with 4% paraformaldehyde (v/v) and stained with 0.5% crystal violet (w/v) for visualization.

**IncuCyte assay**
A549 cells (20k/well) and HCT116 cells (40k/well) were seeded in 96-well and cultured for 7 days. Confluency was measured by IncuCyte © every 4 h.
Senescence-associated β-galactosidase activity (SA-β-Gal) staining

Cells were stained for SA-β-Gal activity with the Senescence Cells Histochemical Staining Kit (cat#: CS0030) from Sigma-Aldrich. The staining was performed according to manufacturer’s instructions. To assess senescence-induction, SA-β-gal positive cells were determined from three independent experiments, and at least 100 cells were counted per condition.

For quantification of SA-β-gal cells after ABT-263 treatment, 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 1 μM ABT-263. Cells were cultured for another 5 days before SA-β-gal staining was performed. The total number of cells were counted from 3 images from the same experiment. Images were taken at 100x magnification, 5 images per well.

Senescence-induction

Cells were seeded in 150 mm dishes at the following cell densities and treated with the following alisertib (Ali), etoposide (Eto), doxorubicin (Dox), or PF-06873600 (PF) concentrations: MCF7, 2.5x10^6 cells, 1 μM Ali, 2.5 μM Eto, 0.13 μM Dox, 1 μM PF; T47D, 2.5x10^6 cells, 3 μM Ali, 0.5 μM Eto; MDA-MB-231, 2.0x10^6 cells, 0.5 μM Ali, 1.25 μM Eto; SUM159, 7.5x10^5 cells, 0.5 μM Ali, 0.5 μM Eto; HCT116, 2.5x10^6 cells, 0.5 μM Ali, 3 μM Eto, 0.38 μM PF; LoVo, 3.0x10^5 cells, 0.25 μM Ali, 1 μM Eto; RKO, 2.0x10^6 cells, 0.5 μM Ali, 2.0 μM Eto; Huh7, 5.0x10^5 cells, 0.25 μM Ali, 0.5 μM Eto, 0.13 μM Dox, 0.5 μM PF; Hep3B, 1.0x10^6 cells, 0.25 μM Ali, 0.321 μM Eto; HepG2, 1.0x10^6 cells, 0.5 μM Ali, 2.5 μM Eto. A549, 5.0x10^5 cells, 0.5 μM Ali, 1.25 μM Eto, 0.13 μM Dox, 0.5 μM PF; NCI-H358, 1.0x10^6 cells, 0.25 μM Ali, 1 μM Eto; PC9, 1.0x10^5 cells, 0.125 μM Ali, 4 μM Eto. 24h after seeding, cells were treated and drug was refreshed every 3 to 4 days. After 7 days of treatment, the cells reached about 70%–90% confluency and were senescent. For downstream applications, senescent cells from one 150 mm dish were reseeded into a full 96-well plate (96 x 96-wells) or a full 6-well plate (6 x 6-wells).

Dose-response assay

After senescence-induction of 7 days (see senescence-induction method), cells were reseeded in 96-well and treated with a range of concentrations of ABT-263 using the HP D300 Digital Dispenser. Cell viability was assessed in a CellTiter-Blue assay with resazurin sodium salt (sigma-Aldrich, #R7017) and measured with EnVision 2104 Multilabel Reader (PerkinElmer). Half maximal inhibitory concentration (IC_{50}) was estimated using the three-parameter logistic curve

$$y = a + \frac{(1-a)}{1 + 10^{(b\cdot(x-x_{50})-\log_{10}(\frac{1}{2\cdot\pi}-1))}}$$

where y is the viability measurement normalized to the positive and negative controls, x is the drug concentration, a is the maximal inhibition, b is the steepness and IC_{50} is the log_{10}-transformed half maximal inhibitory concentration. This equation was fitted to the viability measurements with robust non-linear regression (M-estimator with iterative reweighted least-squares). Phenylarsine oxide (Sigma-Aldrich, cat#:P3075) was used as a positive control and DMSO as a negative control. The senolycy index for ABT-263 represents the IC_{50} value for parental cells divided by the mean IC_{50} value of etoposide- and alisertib-induced senescent cells. Assays were run in technical triplicates, in three independent experiments.

Quantitative real-time PCR (qPCR)

Total RNA was extracted using ISOLATE II RNA Mini kit (Bioline, cat#BIO-52073). RNA was retrotranscribed using SensiFAST cDNA Synthesis kit (Bioline, cat#BIO-65054). qPCR reactions were performed with SensiFAST SYBR Lo-ROX mix (Bioline, cat#BIO94020) and the following primers: GAPDH_Fw: AATCCCATCACCATCTTCCA, GAPDH_Rv: TGGACTCCACGACGTACTCA, IL6_Fw: ACTCA

TGGGGTGGAAAGGTT, LMNB1_Fw: TTCTCGAAGCTTGATCTGGG, LMNB1_Rev: GATCGAGCTGGGCAAGTG. All procedures were executed according to the manufacturer’s instructions. Briefly, qPCR assays were run in 96-well on the 7500 Fast Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific) with the following thermal profile: 1 cycle at 95°C for 10 s, 60°C for 30 s, and 72°C for 15 s. Melting curve analysis was performed ramping from 60°C to 90°C and rising by 0.5°C every 2 s. Normalized mRNA expression was calculated using the 2^{-ΔΔCT} method. GAPDH was used as the housekeeping gene, and A549 parental sample served as control in Figures 5E, 5F, and 5H, while parental T47D parental served as control sample in Figure S5E. Assays were run in technical duplicates, in three independent experiments.

Western blot

Cells were seeded in 150 mm culture dishes and were treated with alisertib, etoposide for 7 days (See senescence-induction method), and 24h drug-washout. Then, cells were washed with cold PBS and lysed with RIPA buffer [25 mM Tris-Cl (pH7.5), 1% NP40, 1% sodium deoxycholate, 0.1% SDS, and 150 mM NaCl], supplemented with Halt Protease & Phosphatase Single-Use Inhibitor cocktail (Thermo Scientific, cat:#78442). After centrifugation for 30 min at 14,000 rpm at 4°C, supernatant was collected. Protein concentrations were determined using Pierce BCA assay (Thermo Scientific, cat#: 23225), according to manufacturer’s instructions. Samples were denatured with Bolt Sample Reducing Agent (Thermo Fisher, cat#:B0009) followed by 5 min incubation at 95°C.
After that, samples were resolved on a 5%–15% Bis-Tris gel (Thermo Fisher) and run (SDS-PAGE) for 30 min at 200V. Proteins were transferred to a polyvinylidene fluoride (PVDF) membrane at 340 mA for 120 min. Blocking, incubation of primary and secondary antibody was performed under conditions recommended by the manufacturer. Primary antibodies detected Phospho-S780 Rb (RRID: AB_10950972), Lamin B1 (RRID:AB_2650517), PS3 (RRID:AB_331743), Phospho-S15 P53 (RRID: AB_331741), P21 (RRID:AB_2890611), P16 (RRID:AB_10858268), Phospho-Histone (gamma) H2A.X (Ser139) (RRID:AB_309864), Alpha-Tubulin (RRID: AB_477593). Membranes were incubated with a 1:10000 dilution of secondary HRP conjugated anti-mouse (RRID: AB_11125547) or anti-rabbit (RRID: AB_11125142) antibody for 1 hour in blocking solution. After washing, a chemiluminescence substrate (ECL, Bio-Rad) was added to visualize the protein signal on the ChemiDoc (Bio-Rad).

**NF-KB activation experiments**

To generate NF-κB reporter cell lines, cells were transduced with a lentiviral construct containing four tandem copies of the NF-kB consensus sequence upstream a minimal promoter controlling the firefly luciferase reporter gene (Wilson et al., 2013) (addgene #49343). Transduced cells have been FACS sorted by using the constitutive expression of the GFP reporter gene present in the construct.

For the NF-κB stimulation experiments, reporter cells were plated in 96-wells for overnight stimulation with PMA 100 ng/ml or TNF 10 ng/ml. NF-κB activation level has been assessed by measuring luciferase signal with the Luciferase Assay System (Promega, cat#:E1500), according to manufacturer’s protocol. Luciferase signal is shown as average of three biological replicates.

**RNA-sequencing**

Cells were seeded in 150-mm cell culture dishes and were treated with alisertib or etoposide for 7 days (See senescence-induction method). Senescent cells were cultured for 24 h without drug before total RNA was extracted with RNeasy mini kit (QIAGEN, cat #E1500), according to manufacturer’s instructions. Quality and quantity of total RNA was assessed by the 2100 Bioanalyzer using a Nano chip (Agilent, Santa Clara, CA). Total RNA samples having RIN > 8 were subjected to library generation.

Strand-specific libraries were generated using the TruSeq Stranded mRNA samples preparation kit (Illumine Inc., San Diego, RS-122-2101/2) according to manufacturer’s instructions (Illumina, part #15031047 Rev.E). Briefly, polyadenylated RNA from intact total RNA was purified using oligo-dT beads. Following purification, the RNA was fragmented, random primed and reverse transcribed using SuperScript II Reverse Transcriptase (Invitrogen, part # 18064-014) with the addition of Actinomycin D. Second strand synthesis was performed using Polymerase I and RNaseH with replacement of dTTP for dUTP. The generated cDNA fragments were 3’-end adenylated and ligated to Illumina Paired-end sequencing adapters and subsequently amplified by 12 cycles of PCR. The libraries were analyzed on a 2100 Bioanalyzer using a 7500 chip (Agilent, Santa Clara, CA), diluted and pooled equimolar into a multiplex sequencing pool. The libraries were sequenced with single-end 65bp reads on a HiSeq 2500 using V4 chemistry (Illumina inc., San Diego).

Transcript levels were quantified using kallisto (v0.46) and summed to gene level. As reference transcriptome, Gencode v34 was used, where we filtered out transcripts from genes annotated as unexpressed pseudogenes as well as transcripts with a transcript support level of 5, unless the transcript has a consensus coding sequence identifier. The gene expression levels were normalized between samples with edgeR (v3.26.8) using trimmed mean of M-values with singleton pairing. For differential gene expression analysis the estimated kallisto counts were used directly, and for other downstream analysis, log2-transformed counts per millions were used. Transformed gene expression values are available in Table S6.

**Gene set enrichment analysis**

For calculating single-sample gene set scores, we used single-sample gene set enrichment analysis (ssGSEA) (Barbie et al., 2009), as implemented in the GSVA R package (Hänzelmann et al., 2013). Delta ssGSEA scores represent the difference between treated samples and their parental counterparts. The scores were adjusted for gene set size and normalized per gene set. Senescent gene sets are summarized in Table S1.

For differential gene set enrichment analysis, we used the flexgsea R package (Bismeijer and Kim, 2019) with the default weighted KS-like statistic (Subramanian et al., 2005). Significance was estimated by sample permutation. For gene sets without direction, absolute enrichment scores were used. As gene sets, we used the MSigDB Hallmark and Gene Ontology (GO) biological process sets (Liberzon et al., 2015). For the GO terms, REVIGO (Supek et al., 2011) was used on the enrichment scores with the “small” setting to obtain a summarized list.

**Differential gene expression analysis**

Differentially expressed genes were identified with edgeR using generalized linear models (GLMs) (McCarthy et al., 2012) and quasi-likelihood F-tests. First, the dispersion was estimated from the samples which were sequenced in duplicate, with a GLM with a factor for every condition. Using these dispersion estimates, a GLM was fitted with a factor for each cell line, and either a factor per treatment (for the treatment-specific DGEA), or a factor for senescence status (for the DGEA of all treatments together). P-values were adjusted for multiple testing by false discovery rate correction.
Cell cycle analysis

To estimate the change in cell cycle distribution from the gene expression values, we calculated the average expression of genes specific for each cell cycle phase in the untreated cells relative to the untreated cells. For this, we used the marker genes from Mizuno et al. (2009). First, we calculated the average expression for each cell cycle phase as follows, for every sample:

\[ y_{ij} = \frac{1}{N_i} \sum_{k=1}^{N_i} x_{ij,k}, \]

where \( i \) indicates a geneset of a specific cell cycle phase, \( j \) indicates a sample and \( k \) indices the genes in geneset \( i \); \( x \) is the log\(_2\) counts per million, and \( N_i \) is the amount of genes in geneset \( i \), and \( y_{ij} \) is the resulting score. The scores shown in the heatmap in Figure S4 were calculated by taking the difference in the score \( y \) for a sample and the corresponding untreated cell.

Senescence classifier

We used our cell line panel and CCLE samples as input to the classifier. We first selected CCLE samples corresponding to the same cell lines used in our panel and performed a DGEA to identify genes which were significantly different between our study and the CCLE samples – these genes reflect genes which are variable between sites and were excluded from the classifier (1,448 genes). We then selected 45 other CCLE samples (3 cell lines each for the 15 main cancer types in CCLE which were not included in our panel, Table S3). In the classifier, these CCLE samples were given a weight such that the total weight of untreated and senescent samples was equal. Log\(_2\) counts per million for each gene were mean-centered and normalized by dividing by the weighted standard deviation over all samples. We further excluded genes for which the 95\(^{th}\) percentile was below 1 count per million and filtered out all mitochondrial-encoded genes as the mitochondrial content is likely to differ significantly between sample preparations, leaving 17,405 genes constituting of leaving out all samples from one cell line and training on the remaining set (resulting in two cell lines being left out in the inner loop). Several other classification methods were tried, but were found to give worse performance.

After this cross-validation, an elastic net was fitted on all samples with single-loop CV to optimize \( \lambda \). Gene expression of external samples was re-quantified from the raw reads using the same procedure described above in the RNA-sequencing section, with the same reference transcriptome. Estimated read counts were adjusted to our gene expression data using edgeR with trimmed mean of M-values with singleton pairing against the reference sample in our dataset. To ensure that samples with a very high sequencing depth have comparable expression values, log\(_2\) counts per million values below the minimum value observed in our dataset were clamped to that minimum (−2.99). The gene expression values were centered and scaled with the mean and standard deviation of our dataset. A script for calculating senescence scores with this classifier is available at https://ccb.nki.nl/publications/cancer-senescence/.

For the validation analysis on normal senescent cells from Casella et al. (2019) included only the main set of 25 samples and excluded their second set of 12 samples, as the latter samples had a strong 3’UTR bias in the sequencing reads, indicative of RNA degradation, given that ribosome-depletion was used. Importantly, these 12 samples were also excluded from part of the analysis by Casella et al. (2019).

Quiescence-induction

A549 cells (0.5x10\(^6\)) were plated in 150-mm dishes in complete media (RPMI 10% FBS). After 24h, cells were starved in serum free media for 72h. Cells were collected, counted and fixed in 70% ethanol and incubated at −20C for 2h.

For cell cycle analysis, 10\(^6\) cells were stained with anti-Ki67 PE-conjugated antibody (Clone B56, BD Pharmigen) for 30 min at RT. Finally, cells were re-suspended in PBS containing DAPI (5 ug/ml) and incubated at 4°C for 2h before acquisition. Samples were acquired with LSR Fortessa (BD) and analyzed using FlowJo software (v10).

For RNaseq analysis, after 72h of starvation, cells were washed with cold PBS and collected in RLT buffer (QIAGEN) for RNA extraction.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Statistical details of experiments can be found in the figure legends and results. How significance was defined can be found in the legends and results. Data was analyzed using Graphpad Prism v8.4.3 and R statistical computing language (v3.6.0).

**ADDITIONAL RESOURCES**

SENESCopedia webtool with processed RNA sequencing data of senescent and parental cells, and the SENCAN classifier: ‘https://ccb.nki.nl/publications/cancer-senescence/.’
Supplemental information

The Cancer SENESCopedia:
A delineation of cancer cell senescence

Fleur Jochems, Bram Thijssen, Giulia De Conti, Robin Jansen, Ziva Pogacar, Kelvin Groot, Liqin Wang, Arnout Schepers, Cun Wang, Haojie Jin, Roderick L. Beijersbergen, Rodrigo Leite de Oliveira, Lodewyk F.A. Wessels, and René Bernards
### S1A

|                | Ctrl | Alisertib | Etoposide |
|----------------|------|-----------|-----------|
| *S1A*          |      |           |           |
| **Breast cancer** |      |           |           |
| MCF-7          |      |           |           |
| T47D          |      |           |           |
| MM 231       |      |           |           |
| SUM159       |      |           |           |
| **Lung cancer** |      |           |           |
| PC9          |      |           |           |
| H358       |      |           |           |

### B

|                | P53 Wildtype (P53, P16, Rb) | P53 mutant | P16 mutant | P53, P16 mutant | P53, P16, Rb mutant |
|----------------|-----------------------------|------------|------------|-----------------|---------------------|
| HepG2         | P   | A   | E       | P   | A   | E       | P   | A   | E       | P   | A   | E       | P   | A   | E       | P   | A   | E       |
| LoVo          | P   | A   | E       | P   | A   | E       | P   | A   | E       | P   | A   | E       | P   | A   | E       | P   | A   | E       |
| RKO           | P   | A   | E       | P   | A   | E       | P   | A   | E       | P   | A   | E       | P   | A   | E       | P   | A   | E       |

### 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          |         |        |
| T47D          |         |        |
| MM 231       |         |        |
| SUM159       |         |        |
| Hep3B        |         |        |
| HepG2        |         |        |
| Hep3B        |         |        |

### Notes

- Alisertib: 0.1 µM, 0.3 µM, 0.5 µM, 1.0 µM, 2.5 µM, 3.0 µM, 4.0 µM
- Etoposide: 0.1 µM, 0.3 µM, 0.5 µM, 1.0 µM, 2.5 µM, 3.0 µM, 4.0 µM
Supplementary figure 1. Senescence markers are present in cells treated with alisertib or etoposide, related to figure 1.

(A) Representative images of SA-β-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) Western blot 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. Phospho-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.
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-β-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-β-gal staining was performed. Scale bar = 100 μm.
**Pro-apoptotic**
- BAD
- BIK
- BMF
- HRK
- NOXA
- PUMA

**Anti-apoptotic**
- BCL-2
- BCL-XL
- BCL-W
- BFL-1
- MCL-1

**Activator**
- BAK
- BAX
- BOK

**Effector**
- BID

**Sensitizer**
- SEN Ali & SEN Eto

**Activator**
- tBID

**Effector**
- Apoptosis

**BCL-W**
- BCL-XL
- BFL-1
- MCL-1

**BAD**
- HRK
- BMF
- PUMA
- NOXA

**Sensitizer pro-apoptotic**
- BIK
- BAD
- HRK
- BMF
- PUMA
- NOXA

**Activator effector pro-apoptotic**
- BID
- BAK
- BIM
- BAX
- BOK

**Spearman’s R**

**MCL1 log2 CPM**

**BIK log2 CPM**

**BAD log2 CPM**

**ABT-263 response (−logIC50)**

**SEN Ali & SEN Eto**

**Parental**

**Breast**
- Red

**Lung**
- Blue

**Colorectal**
- Green

**Liver**
- Purple
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.
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.
A549 HCT116 MCF7 Huh7

Parental SEN Ali SEN Eto SEN Dox SEN PF

DMSO Doxorubicin PF-06873600

A549

HCT116

MCF7

Huh7

DMSO Doxorubicin PF-06873600

MCF7

DMSO ABT-263

B-gal - B-gal +

LMNB1

Normalized expression

Parental SEN Dox SEN PF

DMSO ABT-263

LMNB1

Normalized expression

Parental SEN Ali SEN Eto SEN Dox SEN PF

DMSO ABT-263

DMSO ABT-263

DMSO ABT-263

DMSO ABT-263

DMSO ABT-263
Supplementary figure 5. The senescence phenotype is more influenced by cell type than senescence trigger, related to figure 5.

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

(B) Representative images of SA-β-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-β-gal staining was performed. Scale bar = 100 μm

(C) Quantification of SA-β-gal + and SA-β-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.
Fridman UP gene signature

Fridman DOWN gene signature

Casella UP gene signature

Casella DOWN gene signature

B. Cross-validation

- AUC = 1
- AUC = 0.97
- AUC = 0.96

C. Total cell number (x10^6)

- Serum rich medium
- Serum free medium

D. Serum rich medium

- G0G1
- 65.2

- KI67+
- 96.1

D. Serum free medium

- G0G1
- 76.4

- KI67+
- 50.2

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).
### 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   | CCN1      |
| PLOD1      | ANP32B      | CDKN1A     | CCNB1        | COL17A1 | DYNLT3    |
| FAM234B    | PARP1       | MMP1       | CDC25B       | CD82    | SCOC      |
| DHR57      | 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        | RSP03   | 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      | RA1I4        |         |           |
| SARAF      | NRG1        | IL1B       | TSPAN13      |         |           |
| SLCO2B1    | RRAS        | TNAIP3     | 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     |             |             |         |           |
| POFTU2     | CITED2      |             |             |         |           |
| K1AA1671   | RAC1        |             |             |         |           |
| LRP10      | HSPA2       |             |             |         |           |
| DIO2       | VIM         |             |             |         |           |
| MAP4K3-DT  | RABGGA      |             |             |         |           |
| 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 |

Table S3. Validation samples from the Cancer Cell Line Encyclopedia (CCLE), related to figure 6.
| Accession  | Description                                      | Symbol | Tissue Type                                      |
|------------|--------------------------------------------------|--------|-------------------------------------------------|
| 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 | LNCAPCLOSENFGC 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                                  |