

The chemotherapy-induced senescence-associated secretome promotes cell detachment and metastatic dissemination through metabolic reprogramming

Received: 12 August 2025

Accepted: 24 June 2026

Published online: 30 July 2026

 Check for updates

A list of authors and their affiliations appears at the end of the paper

Cellular senescence is a consequence of many chemotherapeutics that plays context-dependent roles in cancer. Senescent cells secrete an array of factors collectively known as the senescence-associated secretory phenotype (SASP). Here we show that the cisplatin-induced SASP enhances the detachment of high-grade serous ovarian cancer (HGSOC) cells in vitro and dissemination in vivo. We identify fructose as a metabolic component of the SASP that facilitates cell detachment and show that a high-fructose diet increases HGSOC dissemination in vivo. We identified complex I as the driver of SASP-mediated cell detachment and HGSOC dissemination. Mechanistically, this effect was driven by SASP-mediated inhibition of an NAD^+ –SIRT–SREBP axis, leading to decreased plasma membrane cholesterol that increased cell detachment. These findings reveal that the SASP reprograms the metabolic microenvironment, promoting metastatic dissemination in a paracrine fashion, and highlight a pro-tumorigenic metabolic effect of fructose in the SASP that may contribute to the high recurrence rate of HGSOC.

Cellular senescence is defined as a stable cell cycle arrest¹. In cancer, senescence is a common cell fate induced by chemotherapy (termed therapy-induced senescence (TIS)). TIS was initially thought to be a beneficial therapeutic response¹; however, over the past decade this paradigm has shifted, and multiple papers have reported a more nuanced and complex role for TIS in cancer. Recent evidence has shown that TIS paradoxically enhances tumorigenesis, metastasis, and chemoresistance in a paracrine fashion^{2–7}. The pro-tumorigenic role for TIS in cancer is due in part to the unique secretome of senescent cells (termed the senescence-associated secretory phenotype (SASP)). This secretome is composed of a variety of factors and is different depending on both the senescence inducer and cell type¹. The SASP is often described as a transcriptional program that alters the inflammatory phenotype of senescent cells and their tumor microenvironment (TME). However, SASP factors go beyond transcriptionally regulated inflammatory mediators, and there is evidence that metabolites such as bioactive lipids, fatty

acids, nucleobases, amino acids, and others are SASP components^{8–10}. Moreover, senescent cells reprogram their intracellular metabolism¹¹, and a few publications have pointed to depletion of certain metabolites in the SASP^{8,12}. Yet the metabolic components of the SASP remain underexplored, and it is still unknown how these metabolites influence non-senescent bystander cancer cells within the TME.

One chemotherapy well known to promote TIS is cisplatin¹³. This is of particular importance in high-grade serous ovarian cancer (HGSOC), in which platinum-based therapies such as cisplatin are standard of care¹⁴. While the majority of patients with HGSOC initially respond to platinum-based chemotherapy, disease recurrence is common, accounting for ~90% of HGSOC deaths¹⁵. The use of chemotherapy is associated with elevated metastatic dissemination of ovarian cancer^{16,17}, and patients with recurrent HGSOC all have disseminated disease¹⁸. Metastatic dissemination of HGSOC occurs most frequently through the transcoelomic route, defined as shedding of cancer cells from a

solid tumor (either the primary tumor or peritoneal tumor nodules) into the peritoneal cavity¹⁹. The first step in transcoelomic dissemination is detachment of cells from the primary tumor. The role of the cisplatin-induced SASP in detachment and dissemination remains unknown. The use of HGSOc is an ideal model to investigate these important questions in cancer senescence. In this Article, we aimed to investigate the paracrine effects of the cisplatin-induced SASP produced by HGSOc cells on metastatic dissemination.

Results

The cisplatin-induced SASP promotes HGSOc dissemination in vivo

Platinum-based therapies such as cisplatin are standard of care for patients with HGSOc, although the majority of HGSOcs initially treated with platinum will recur with widespread disseminated disease^{16,17}. Using multiple publicly available bulk RNA-seq (RNA-seq) datasets from matched pre- and post-neoadjuvant chemotherapy (NACT)-treated HGSOc tumor samples^{20,21}, we found evidence of robust senescence signatures in patients with HGSOc after platinum chemotherapy (Fig. 1a and Supplementary Tables 1–3). Similar upregulation in senescence-associated genes was also observed in three publicly available NanoString datasets of matched NACT-treated tumors (Extended Data Fig. 1a)^{16,22–25}. Together, these data show that cisplatin induces senescence. To further investigate the intratumoral senescence response to chemotherapy, we applied a senescence score derived from these signatures (see Methods for details) to publicly available single-cell RNA-seq data of matched pre- and post-NACT-treated patient tumors²⁶. As expected, multiple cell types show an increase in the senescence signature, including malignant epithelial cells and other stromal cells (Extended Data Fig. 1b). There was a heterogeneous senescence induction in malignant epithelial cell populations (Fig. 1b and Supplementary Table 4), suggesting a post-chemotherapy TME comprising both senescent malignant cells and proliferative malignant “bystander” cells (Fig. 1c). While TIS has been frequently reported, and the SASP from multiple senescent cell sources is known to affect cancer progression, the contribution of the malignant cell SASP on dissemination and tumor progression of malignant bystander cells remains unclear. Given the complexity of senescence induction in vivo, here we aimed to determine the paracrine effects of the cisplatin-induced SASP from malignant cells on HGSOc bystander cells.

Towards this goal, we first induced senescence in two different HGSOc cell lines using cisplatin (Extended Data Fig. 2a). Senescence in HGSOc cells was confirmed using multiple markers, including decreased proliferation and 5-bromo-2'-deoxyuridine (BrdU) incorporation, increased senescence-associated β -galactosidase (SA- β -Gal)

activity, and decreased lamin B1 expression (Extended Data Fig. 2b–e). We also confirmed high expression of prototypical SASP cytokines using a cytokine array (Extended Data Fig. 2f) and by RNA expression (Extended Data Fig. 2g,h). Finally, we observed increased sensitivity of cisplatin-induced senescent cells to the senolytic navitoclax (ABT-263) (Extended Data Fig. 2i). Together, these data strongly support the notion that cisplatin treatment induces a strong senescent phenotype in these cellular models. To determine how the presence of senescent cells affects HGSOc progression in vivo, we co-injected naive proliferating HGSOc cells with vehicle-treated or cisplatin-induced senescent HGSOc cells (Fig. 1d). It is important to note that we injected the same number of proliferating cells in both groups, and the senescent cells were labeled with GFP (see Methods for details). We observed an increase in tumor burden in mice co-injected with naive proliferating cells + senescent cells compared to naive proliferating cells + vehicle control cells (Fig. 1e). Moreover, we noted a metastatic tropism to the omentum, intestines, visceral fat, and peritoneum (Fig. 1f), while other sites were not markedly affected (Extended Data Fig. 3a). The increase in tumor burden was not a result of senescent cells regaining proliferative capacity in vivo (Extended Data Fig. 3b). To more directly determine the contribution of the SASP to this increase in tumor burden, we used a conditioned media approach. We treated KPCA.B HGSOc cells with vehicle or cisplatin to induce senescence, washed off the drug, and conditioned media for an additional 2 days (Extended Data Fig. 2a). We then injected conditioned media from cisplatin-induced senescent HGSOc cells (SCM) or proliferative controls (PCM) into tumor-bearing mice (Fig. 1g). SCM alone was sufficient to increase tumor burden and metastatic spread (Fig. 1h,i and Extended Data Fig. 3c). There was no marked difference in omental weights or Ki67 staining in either model (Fig. 1j,k), showing that the observed increase in tumor nodules was likely not a result of increased proliferation of cancer cells. SCM did not increase bystander HGSOc cell proliferation in vitro (Extended Data Fig. 3d). In addition, we did not observe differences in terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining in the tumors (Fig. 1l), and SCM did not affect anoikis in vitro (Extended Data Fig. 3e), showing that the SASP does not affect the viability of HGSOc cells in suspension. Together, these data show that the SASP from cisplatin-induced senescent HGSOc cells is sufficient to increase HGSOc dissemination in vivo, and this is not a result of either increased proliferation or decreased cell death.

The cisplatin-induced SASP promotes detachment of bystander HGSOc cells via NDUFA5

We found that the cisplatin-induced SASP increases the number of tumor nodules without corresponding changes in cancer cell

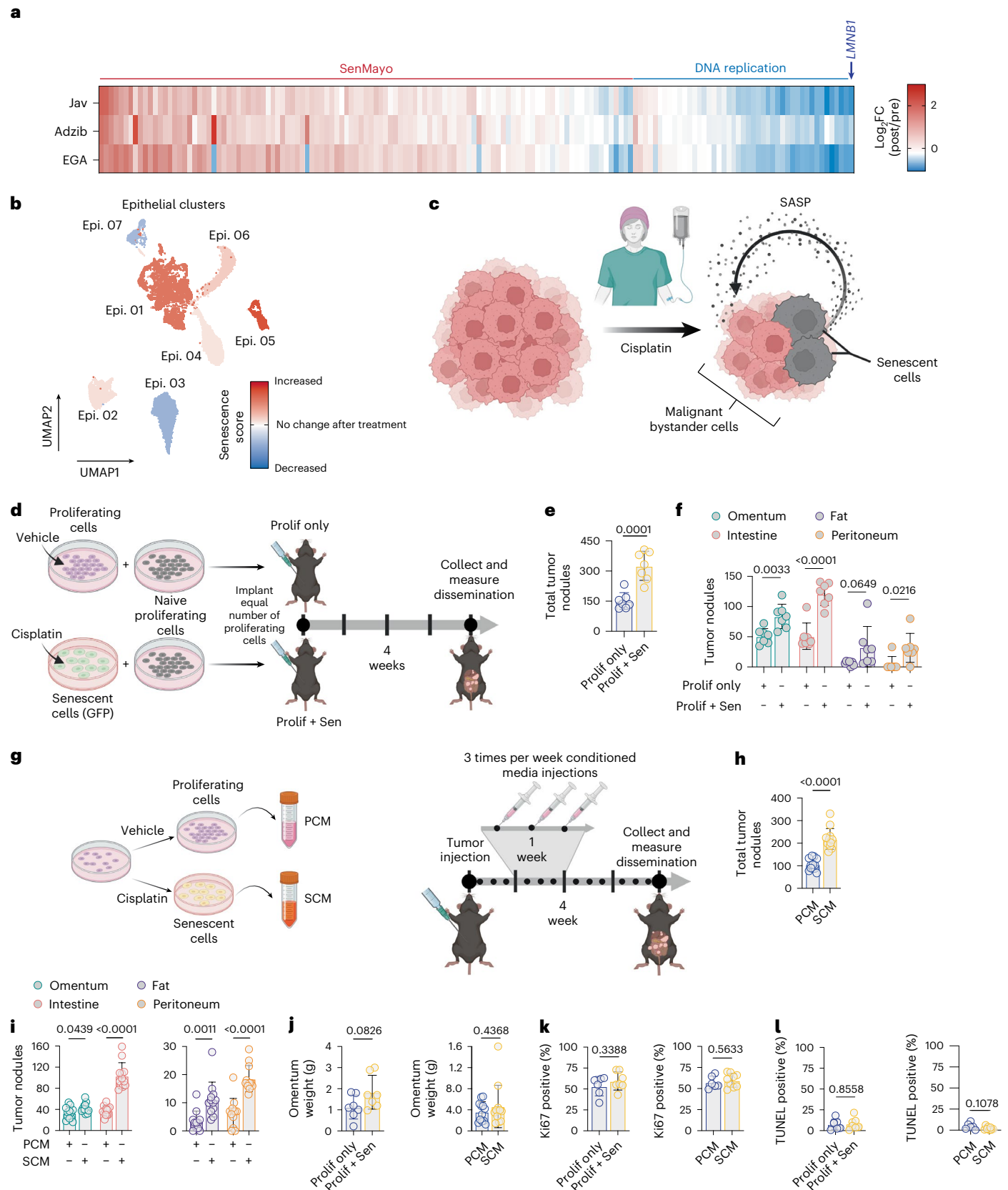
Fig. 1 | The cisplatin-induced SASP promotes dissemination of HGSOc in vivo.

a, Heat map of genes in the SenMayo signature, the DNA replication signature, and *LMNB1* in three publicly available RNA-seq datasets comparing ovarian cancer samples after neoadjuvant chemotherapy to matched, pretreatment tumor (gene sets can be found in Supplementary Table 1). **b**, Uniform manifold approximation and projection (UMAP) showing change in senescence score in epithelial clusters of ovarian cancer samples after neoadjuvant chemotherapy to matched, pre-treatment tumor. **c**, Schematic of the post-cisplatin TME in which senescent cells act on bystander cells in a paracrine manner through the SASP. **d–f**, Control or cisplatin-induced senescent cells were co-injected with naive proliferating cells. **d**, KPCA.B HGSOc cells were treated with vehicle or induced to senesce using cisplatin (see Extended Data Fig. 2 for timeline and senescence markers). Cisplatin-induced senescent cells were transduced to stably express GFP. Vehicle-treated or cisplatin-induced senescent cells were then co-injected intraperitoneally with naive proliferating KPCA.B HGSOc. The same number of proliferating cells were injected into mice in each group, and the senescent cells were injected with naive proliferating cells at a 1:1 ratio. Prolif only, vehicle-treated cells co-injected with naive proliferating cells; Prolif + Sen, cisplatin-induced senescent cells co-injected with naive proliferating cells. $n = 7$ mice per

group. **e**, End-point total intraperitoneal tumor nodules from the experiment detailed in **d**. **f**, End-point tumor nodule counts in the indicated metastatic sites from the experiment detailed in **d**. **g–i**, KPCA.B HGSOc cells were treated with vehicle or induced to senesce using cisplatin, and conditioned media was collected (see Methods for details). PCM, proliferative conditioned media, which is conditioned media collected from vehicle-treated cells; SCM, senescent conditioned media, which is conditioned media collected from cisplatin-induced senescent cells. **g**, KPCA.B HGSOc cells were injected intraperitoneally into mice and conditioned media was injected three times per week. $n = 12$ mice per group. **h**, End-point total intraperitoneal tumor nodules from the experiment detailed in **g**. **i**, End-point tumor nodule counts in the indicated metastatic sites from the experiment detailed in **g**. **j**, Omental weights from in vivo experiments detailed in **d** (left) and **g** (right). **k,l**, Quantification of Ki67 (**k**) or terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (**l**) in omental tumors detailed in **d** (left) and **g** (right). $n = 6$ (Prolif only, PCM), $n = 7$ (Prolif + Sen), or $n = 9$ (SCM) mice per group. All graphs show mean $\pm$ s.d. unless otherwise indicated. Two-tailed Student's *t*-test was used. Panels created in BioRender: **c**, Aird, K. <https://biorender.com/42ecb7u> (2026); **d,g**, Cole, A. <https://biorender.com/lecy10j> (2026).

proliferation or death (Fig. 1 and Extended Data Fig. 3), suggesting that secreted factors from malignant cells influence metastatic dissemination. During transcoelomic dissemination within the peritoneal cavity, HGSOc cells must detach from the solid tumor by loosening

their adhesion to other cells and the extracellular matrix^{19,27}. Thus, we investigated how the cisplatin-induced SASP from HGSOc cells influences detachment and adhesion of bystander HGSOc cells. To measure detachment, we performed time-lapse imaging of spheroids cultured



in PCM or SCM. Spheroids cultured in round-bottom ultra-low attachment (ULA) wells were of similar size regardless of whether they were exposed to PCM or SCM, and we did not observe differential compaction between these conditions (Extended Data Fig. 4a), showing that HGSOC cells in SCM remain able to form spheroids of the same size as those in PCM under gravitational constraint. Thus, for live cell imaging experiments, we controlled for cell number and spheroid size by forming spheroids in round-bottom ULA wells before transferring them to flat-bottom ULA wells (Fig. 2a). After transferring spheroids to flat-bottom ULA wells, we observed an increase in the total number of live cell detachment events in both Ovar8 and KPCA.B cells when bystander cells were cultured in SCM compared to PCM (Fig. 2b,c and Supplementary Video 1), indicating that cells were more loosely adherent. Staining with ethidium homodimer confirmed the detached cells were viable (Fig. 2b). Heat inactivating the SCM to denature proteins also drove detachment of live cells to the same level as regular SCM (Fig. 2c), showing that the observed increase in detachment was likely not a result of protein activity, including extracellular matrix degraders such as matrix metalloproteases. As a complementary approach, we assessed detachment using a controlled trypsinization assay (Fig. 2d), similar to a previous publication²⁸. Consistent with our live cell imaging results in spheroids (Fig. 2c), we found that bystander cells cultured in SCM had increased detachment compared to those cultured in PCM, and the increase in detachment was also retained in heat-inactivated SCM (Fig. 2e). SCM from carboplatin-induced senescent HGSOC cells, cisplatin-induced senescent melanoma cells, and gemcitabine-induced senescent pancreatic ductal adenocarcinoma cells all increased detachment of bystander cells (Extended Data Fig. 4b–d). These data indicate that the effect is not platinum- nor HGSOC cell-specific. Furthermore, SCM from normal fibroblasts undergoing replicative senescence or chemotherapy-induced senescence also increased detachment of bystander HGSOC cells (Extended Data Fig. 4e–g). Together, these data show that the SASP from multiple senescent cell types induces malignant bystander cell detachment. While most previous work has focused on the protein content of the SASP, we and others have shown that the SASP can also have differential metabolite components^{8–10,12}. Therefore, we aimed to determine whether the observed phenotype is

driven by a small molecule or metabolite by filtering the SCM to remove most proteins and large extracellular vesicles (>3 kDa), leaving small molecules such as metabolites. Bystander HGSOC cells cultured in filtered SCM also showed increased detachment to the same level as regular SCM (Fig. 2e). Together, these data provide evidence that a metabolic component of the cisplatin-induced SASP may drive the observed increased detachment of bystander HGSOC cells.

Given the observed changes in detachment in heat-inactivated and filtered SCM (Fig. 2e), we reasoned that there is a metabolic component of the SASP that likely has paracrine metabolic effects on bystander cells to decrease their adhesion. To investigate this mechanism, we conducted a CRISPR (clustered regularly interspaced short palindromic repeats) knockout screen using a metabolically focused guide RNA library. We adopted the controlled trypsinization approach to separate the attached and detached cells from SCM and PCM cultured groups. The live cell detachment from spheroids was not optimal for this purpose due to the low numbers of single cell detachment (Fig. 2a–c). Given the consistency in results between live cell imaging and the controlled trypsinization experiments (Fig. 2a–e), we used this assay for our screen. We aimed to identify genes that were negatively enriched in detached bystander cells cultured in SCM but not PCM, indicating these genes are essential for the loosely adhesive phenotype driven by the SASP (Fig. 2f). We found that oxidative phosphorylation genes were negatively enriched in the detached population of SCM-cultured bystander cells compared to adherent cells (Fig. 2g and Supplementary Tables 5 and 6). Conversely, cholesterol biosynthesis genes were positively enriched in the detached population of SCM-cultured bystander cells compared to adherent cells (Fig. 2g and Supplementary Tables 5 and 6), showing that knockout of these genes drives detachment in SCM conditions. This is in stark contrast with the gene pathways identified in PCM cultured condition (Supplementary Table 6), indicating a unique metabolic requirement for detachment driven by the cisplatin-induced SASP. The negative enrichment oxidative phosphorylation signature was mainly driven by complex I subunits (Supplementary Table 7). We confirmed that complex I genes were negatively enriched specifically in detached bystander cells cultured in SCM but not in PCM

Fig. 2 | The cisplatin-induced SASP promotes cell detachment via NDUFA5.

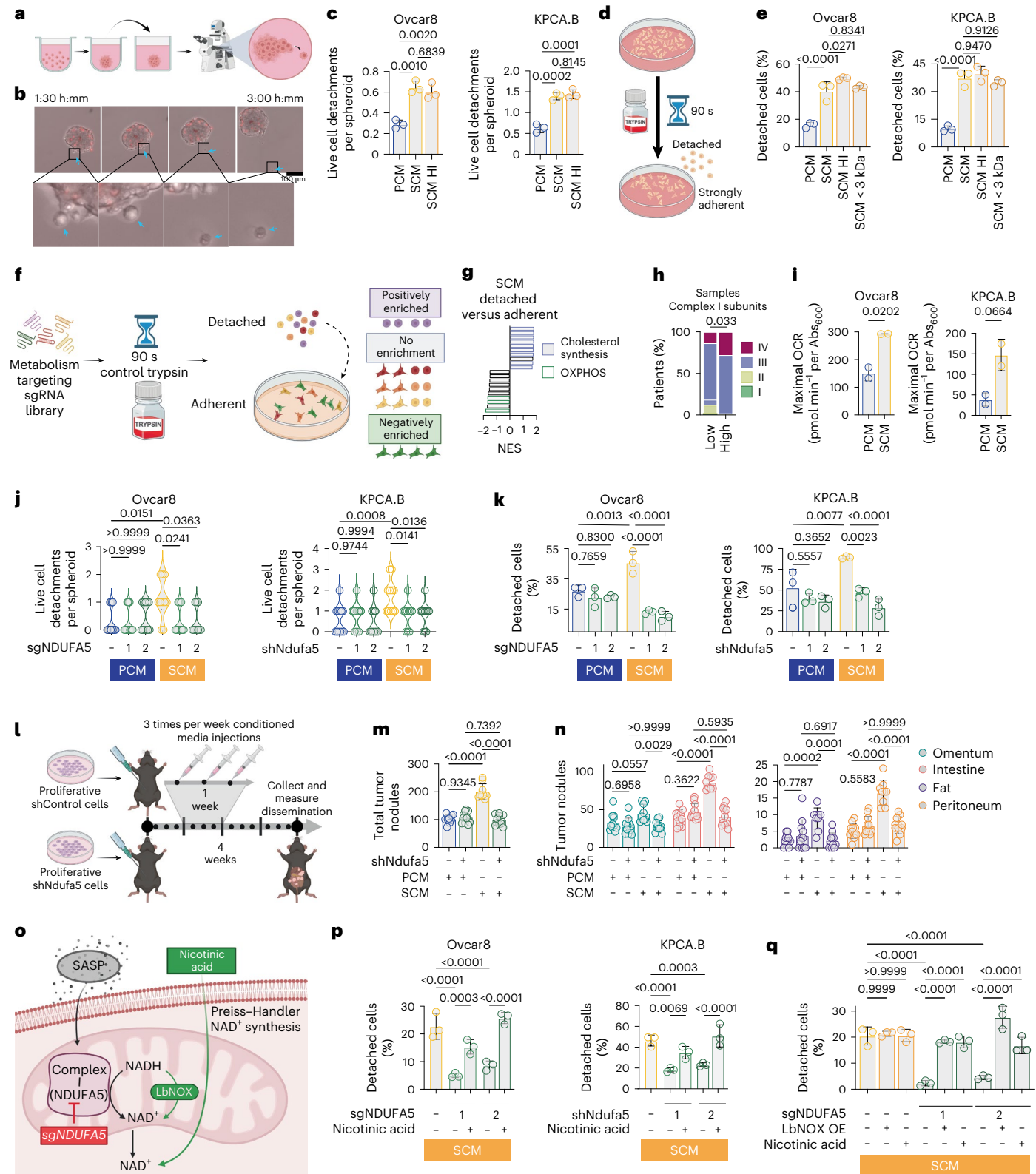
a, HGSOC cells cultured in round-bottom ULA plates for 48 h in PCM, SCM, or SCM that was heat inactivated (SCM HI) then moved to flat-bottom ULA plates. Cells were stained with ethidium homodimer and imaged for 24 h. **b**, Representative image from **a**. Blue arrows point to a live cell detaching. **c**, Quantification of imaging experiment detailed in **a**. $n = 3$ technical replicates representative of 2 independent experiments in each cell line. **d**, Schematic of controlled trypsin experiments (in **e–g**, **k**, **p**, and **q**). **e**, Bystander cells were cultured in PCM, SCM, SCM HI, or SCM passed through a 3 kDa cut-off column (SCM < 3 kDa). After 72 h, the percentage of detachment was assessed using the controlled trypsin assay ($n = 3$ technical replicates representative of 3 independent experiments in each cell line). **f**, Schematic of CRISPR knockout screen. **g**, GSEA performed on bystander cells cultured in SCM (detached versus adherent). NES, normalized enrichment score. A negative NES indicates genes whose knockout decreases detachment in SCM; a positive NES indicates genes whose knockout increases detachment in SCM. **h**, Stage of patients with HGSOC stratified by low-quartile versus high-quartile expression of complex I subunits (data from TCGA, Supplementary Table 1 for gene list). P value determined by ordered linear regression analysis. **i**, Bystander cells were cultured in PCM or SCM for 72 h, and maximal respiration was quantified ($n = 2$ technical replicates representative of 3 independent experiments in each cell line). OCR, oxygen consumption rate; Abs₆₀₀, absorbance at 600 nm. **j**, Bystander HGSOC cells with knockout of NDUFA5 (sgNDUFA5) or knockdown of Ndufa5 (shNdufa5) were cultured as detailed in the schematic in **a**. Shown are live cell detachment events per spheroid (Ovar8: sgControl PCM, $n = 14$; sgNDUFA5-1 PCM, $n = 15$; sgNDUFA5-2 PCM, $n = 11$; sgControl SCM, $n = 10$; sgNDUFA5-1 SCM, $n = 13$; sgNDUFA5-2 SCM, $n = 10$; KPCA.B: shControl PCM, $n = 12$; shNdufa5-1 PCM, $n = 9$; shNdufa5-2 PCM, $n = 10$; shControl SCM, $n = 11$; shNdufa5-1 SCM,

$n = 10$; shNdufa5-2 SCM NA, $n = 11$; replicates representative of 3 independent experiments in each cell line). **k**, Bystander HGSOC cells with knockout or knockdown of NDUFA5 were cultured in PCM or SCM. After 72 h, the percentage of detachment was assessed using the controlled trypsin assay ($n = 3$ technical replicates representative of 3 independent experiments in each cell line). **l**, Naive proliferating shNdufa5 KPCA.B HGSOC cells or controls were implanted intraperitoneally, and conditioned media was injected 3 times per week (shControl PCM, shControl SCM, and shNdufa5 SCM, $n = 9$ (shControl SCM) or $n = 11$ (shControl PCM, shNdufa5 PCM, shNdufa5 SCM) mice per group; shNdufa5 PCM, $n = 9$ mice per group). **m**, **n**, End-point total intraperitoneal tumor nodules (**m**) and the indicated metastatic sites (**n**) from the experiment detailed in **l**. **o**, Schematic of the Preiss–Handler pathway of NAD⁺ synthesis from nicotinic acid as an alternative NAD⁺ source from complex I-mediated NADH oxidation and NAD⁺ rescue strategies. **p**, Bystander HGSOC cells with knockout of NDUFA5 or knockdown of Ndufa5 were cultured in SCM with and without supplementation with nicotinic acid. After 72 h, the percentage of detachment was assessed using the controlled assay ($n = 3$ technical replicates representative of 3 independent experiments in each cell line). **q**, Ovar8 bystander cells with knockout of NDUFA5 and overexpression of LbNOX (LbNOX OE) were cultured in SCM. After 72 h, the percentage of detachment was assessed using the controlled assay ($n = 3$ technical replicates representative of 3 independent experiments). All graphs show mean $\pm$ s.d. unless otherwise indicated. In **c**, **e**, **j**, **k**, **m**, **n**, **p**, **q**, one-way analysis of variance (ANOVA) followed by Šidák's multiple comparisons test was used. In **i**, two-tailed Student's t -test was used. Panels created in BioRender: **d**, Cole, A. <https://biorender.com/38v2bpo> (2026); **f**, Cole, A. <https://biorender.com/huwfdiu> (2026); **l**, Cole, A. <https://biorender.com/53ywkqz> (2026); **o**, Cole, A. <https://biorender.com/ulplgflq> (2026).

(Extended Data Fig. 5a). A recent report showed that electron transport chain genes are enriched after patient-derived cells are cultured in ascites collected after chemotherapy²⁹. In addition, analysis of metastatic recurrence in a post-chemotherapy in vivo model identified high *Nduf412*—a complex I subunit—in malignant cells³⁰. To show the potential clinical relevance of our findings, we assessed HGSOC stage based on expression of complex I or respiration genes in which advanced

stage acts as a proxy for increased dissemination. Indeed, high expression of complex I or respiration genes corresponds to increased HGSOC stage in treatment-naïve patients (Fig. 2h and Extended Data Fig. 5b) even when adjusting for age ($P < 0.001$). Together with our data, this indicates a key role for complex I in detachment and dissemination.

Next, we aimed to validate our CRISPR screen results. Towards this goal, we used the complex I inhibitor rotenone and confirmed



the necessity of functional complex I for the increased detachment phenotype of bystander cells cultured in SCM (Extended Data Fig. 5c). Moreover, we observed an increase in both basal and maximal respiration in bystander cells cultured in SCM compared to PCM (Fig. 2i and Extended Data Fig. 5d), indicating a cisplatin-induced SASP-mediated increase in respiration in bystander HGSOC cells. For further mechanistic experiments, we chose the complex I subunit NADH: ubiquinone oxidoreductase subunit A5 (NDUFA5), which was one of the top differentially negatively enriched genes in the CRISPR screen (Extended Data Fig. 5e–g) and was required for the increase in SCM-mediated respiration in bystander cells (Extended Data Fig. 5h). We also validated the requirement of NDUFA5 knockdown for decreased detachment in bystander HGSOC cells cultured in SCM in both HGSOC models (Fig. 2j,k). Finally, we found that knockdown of *Ndufa5* abrogated the increase in dissemination in both the senescent cell co-injection model and the conditioned media treatment model in vivo (Fig. 2l–n and Extended Data Fig. 5i–k). Together, these data show that the cisplatin-induced SASP from malignant cells regulates HGSOC cell detachment and dissemination in a paracrine manner via NDUFA5.

Finally, we aimed to determine how complex I regulates the increased detachment phenotype. We did not observe an increase in reactive oxygen species (ROS) following NDUFA5 knockdown in bystander cells cultured in SCM, and scavenging ROS in these cells using N-acetyl-cysteine did not decrease detachment (Extended Data Fig. 5l–m), indicating that the SASP regulates cell adhesion via complex I activity independently of ROS generation. Complex I is also critical for oxidation of NADH to NAD⁺ (ref. 31) (Fig. 2o). Therefore, we aimed to determine whether replenishing NAD⁺ using nicotinic acid supplementation (Fig. 2o) would reverse the NDUFA5 knockdown-mediated decrease in detachment of bystander cells in the context of the SASP. Indeed, nicotinic acid supplementation of NDUFA5 knockdown bystander cells cultured in SCM decreased the NAD⁺/NADH ratio (Extended Data Fig. 5n) and restored the SASP-mediated increase in detachment (Fig. 2p). Finally, to show NADH oxidation is sufficient to drive detachment in bystander cells, we overexpressed the NADH oxidase from *Lactobacillus brevis*, LbNOX, in NDUFA5 knockout bystander cells (Fig. 2o and Extended Data Fig. 5o). LbNOX expression rescued

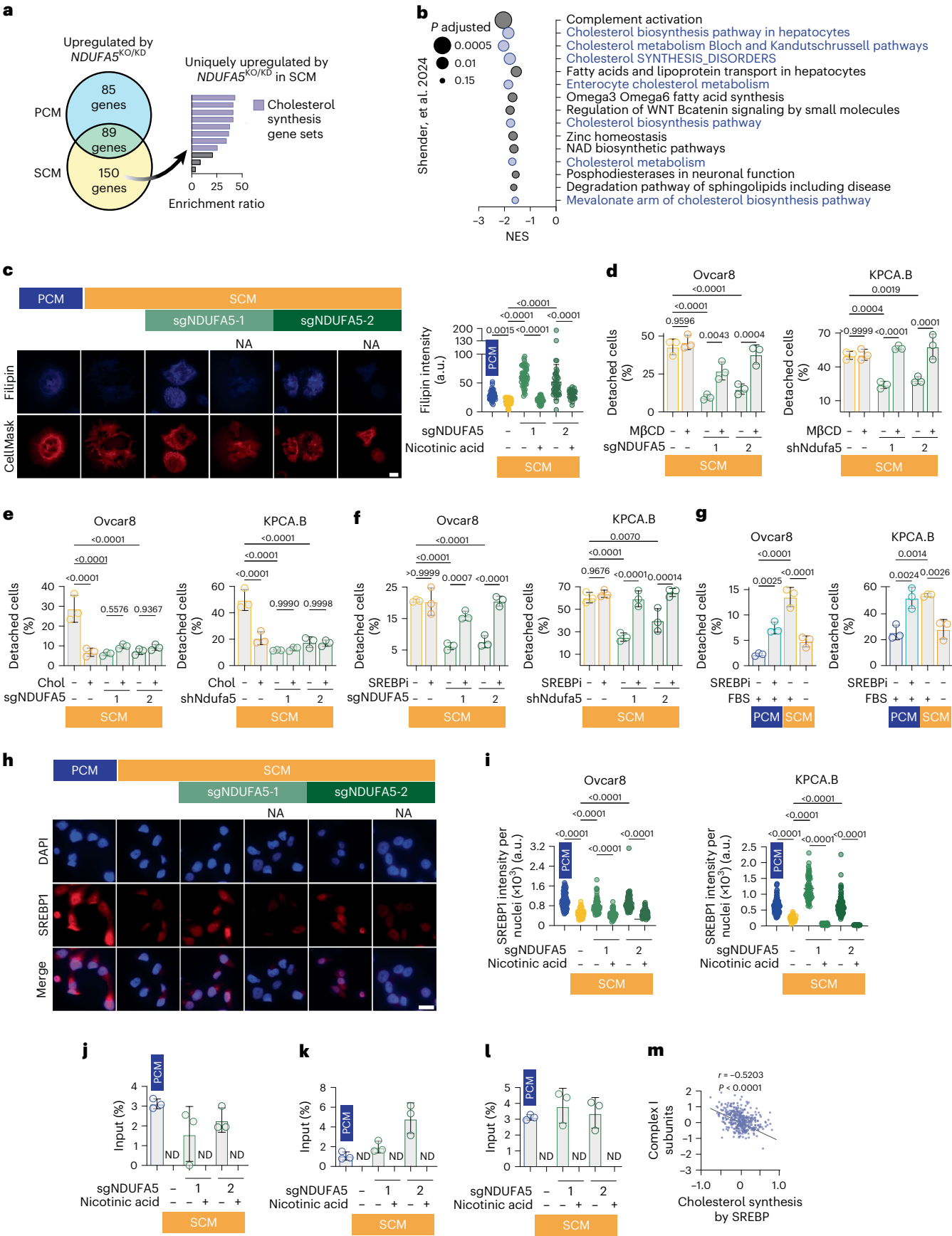
detachment in SCM-cultured NDUFA5 knockout bystanders (Fig. 2q). These data point to a critical role for complex I-mediated NAD⁺ generation in the regulation of cell attachment in the context of the SASP.

Bystander cell detachment is increased through inhibition of SREBP1-mediated plasma membrane cholesterol downstream of NDUFA5

We found that the complex I subunit NDUFA5 drives the cisplatin-induced SASP-mediated increased cell detachment in bystander HGSOC cells (Fig. 2). To understand how NDUFA5 regulates cell detachment, we performed RNA-seq on human and murine HGSOC cells with NDUFA5 knockdown (via single guide RNA or short hairpin RNA, respectively) cultured in either SCM or PCM. Pathway analysis of genes consistently upregulated by NDUFA5 knockdown in SCM, but not in PCM (Fig. 3a and Supplementary Table 8), revealed enrichment of cholesterol-related gene signatures (Supplementary Table 9). More specifically, de novo cholesterol synthesis genes were downregulated in bystander cells cultured in SCM, which was rescued by NDUFA5 knockdown (Extended Data Fig. 6a). We found a negative correlation between the senescence signature and de novo cholesterol synthesis gene signatures in NACT-treated ovarian cancer patients (Extended Data Fig. 6b and Supplementary Table 1). In addition, we used a dataset in which primary ovarian cancer cells were isolated from individual patients and cultured with autologous ascites fluid collected before or after platinum plus taxane chemotherapy²⁹. Gene sets related to cholesterol synthesis were consistently downregulated in cells cultured in ascites after chemotherapy compared to before chemotherapy (Fig. 3b). Collectively, these data support the notion that transcriptional downregulation of de novo cholesterol synthesis is a consequence of the senescent secretome. Consistent with the idea that cholesterol regulates detachment in malignant bystander cells cultured in SCM, our CRISPR knockout screen also identified multiple de novo cholesterol synthesis enzymes to be positively enriched in the detached population (Fig. 2g). In particular, *HMGCR* and *HMGCS1*, the first two enzymes of the mevalonate pathway, were both positively enriched in our CRISPR knockout screen and upregulated in SCM-cultured bystander cells following knockdown of NDUFA5 (Extended Data Fig. 6c,d). Together, these data indicate that the SCM-driven loss of detachment in bystander

Fig. 3 | SASP-induced complex I activity via NDUFA5 increases cell detachment via decreased SREBP1-driven cholesterol synthesis. **a**, Bystander cells with either knockout or knockdown of NDUFA5 were cultured in PCM or SCM for 72 h, and RNA-seq was performed. Left: Venn diagram of genes upregulated by both knockout/down of NDUFA5 (NDUFA5^{KO/KD}) bystander cells cultured in PCM or SCM. Right: overrepresentation analysis of the genes uniquely upregulated in bystander cells cultured in SCM. **b**, GSEA analysis of primary ovarian cancer cells isolated from individual patients cultured with autologous ascites fluid collected before versus after chemotherapy treatment²⁹. Wikipathways are shown. **c**, Ovcar8 bystander cells with knockout of NDUFA5 (sgNDUFA5) were cultured in PCM or SCM with or without nicotinic acid. After 72 h, plasma membrane cholesterol was assessed using filipin III staining under TIRF imaging. Left: representative images. Scale bar, 25 μ m; $\times 100$ magnification. Right: quantification (sgControl PCM, $n = 52$; sgControl SCM, $n = 46$; sgNDUFA5-1 SCM, $n = 50$; sgNDUFA5-1 SCM NA, $n = 40$; sgNDUFA5-2 SCM, $n = 44$; sgNDUFA5-2 SCM NA, $n = 32$; replicates representative of 3 independent experiments). **d**, HGSOC bystander cells with knockout or knockdown of NDUFA5 were cultured in SCM for 72 h before being treated with 10 mM M β CD for 1 h, and the percentage of detachment was assessed using the controlled trypsin assay ($n = 3$ technical replicates representative of 3 independent experiments in each cell line). **e**, HGSOC bystander cells with knockout or knockdown of NDUFA5 were cultured in SCM for 72 h before being treated with 50 μ M cholesterol for 2 h, and the percentage of detachment was assessed using the controlled trypsin assay ($n = 3$ technical replicates representative of 3 independent experiments in each cell line). **f**, HGSOC bystander cells with knockout or knockdown of NDUFA5 were cultured in SCM with 1 μ M of the SREBP inhibitor (SREBPI) fatostatin. After 72 h, the percentage of detachment was assessed using the controlled trypsin assay

($n = 3$ technical replicates representative of 3 independent experiments in each cell line). **g**, HGSOC bystander cells were cultured in PCM or SCM with 1 μ M of the SREBP inhibitor (SREBPI) fatostatin. After 48 h, cells were serum starved overnight or continued to be cultured in full media. After 72 h, the percentage of detachment was assessed using the controlled trypsin assay ($n = 3$ technical replicates representative of 3 independent experiments in each cell line). **h,i**, HGSOC bystander cells with knockout or knockdown of NDUFA5 were cultured in PCM or SCM with or without nicotinic acid supplementation. After 72 h, cells were stained with anti-SREBP1 antibody and DAPI. **h**, Representative images of SREBP1 immunofluorescent staining in Ovcar8 cells. Scale bar, 25 μ m; $\times 40$ magnification. **i**, Quantification of the average SREBP1 immunofluorescent staining intensity per cell nuclei (Ovcar8: sgControl PCM, $n = 105$; sgControl SCM, $n = 106$; sgNDUFA5-1 SCM, $n = 118$; sgNDUFA5-1 SCM NA, $n = 100$; sgNDUFA5-2 SCM, $n = 100$; sgNDUFA5-2 SCM NA, $n = 137$; KPAC.B: shControl PCM, $n = 112$; shControl SCM, $n = 61$; shNdufa5-1 SCM, $n = 62$; shNdufa5-1 SCM NA, $n = 94$; shNdufa5-2 SCM, $n = 161$; shNdufa5-2 SCM NA, $n = 76$; replicates representative of 2 independent experiments in each cell line). **j–l**, Ovcar8 bystander cells with knockout of NDUFA5 were cultured in PCM or SCM with or without nicotinic acid. After 72 h, cells were collected for ChIP using an anti-SREBP1 antibody followed by qPCR of *HMGCS1* (**j**), *HMGCR* (**k**), and *MVK* (**l**). ND, not detected ($n = 3$ technical replicates representative of 3 independent experiments). **m**, Negative correlation between the expression of genes related with complex I and cholesterol synthesis genes in tumors of HGSOC patients (data from TCGA, see Supplementary Table 1 for gene lists). Pearson's correlation, P value determined by linear regression. All graphs show mean $\pm$ s.d. unless otherwise indicated. One-way ANOVA followed by Šidák's multiple comparisons test was used.



cells is a result of transcription downregulation of de novo cholesterol synthesis downstream of NDUFA5.

Cholesterol is an important component of the plasma membrane and has been previously shown to help stabilize integrins, thereby increasing cell adhesion^{32,33}. Thus, we aimed to assess cholesterol content changes downstream of NDUFA5 using filipin III, a fluorescent cholesterol probe, and total internal reflection fluorescence (TIRF) imaging, which selectively illuminates the plasma membrane in close proximity to the coverslip. We confirmed a decrease in plasma membrane cholesterol of bystander cells cultured in SCM, which was rescued by knockdown of NDUFA5 (Fig. 3c). Supplementation with nicotinic acid increased the plasma membrane cholesterol content of NDUFA5 knockdown cells (Fig. 3c), showing this effect is through NAD⁺. We confirmed a decrease in non-esterified cholesterol by mass spectrometry (Extended Data Fig. 6e). This was not simply a result of differences in cholesterol trafficking to the plasma membrane as total cholesterol measured by widefield fluorescence filipin III was also similarly altered (Extended Data Fig. 6f,g). In addition, expression of LbNOX to regenerate NAD⁺ increased whole-cell filipin III staining (Extended Data Fig. 6h,i). To determine whether these changes in cholesterol play a functional role in cell detachment, we depleted cholesterol with either methyl- β -cyclodextrin (MBCD), which specifically depletes plasma membrane cholesterol, or statins to deplete intracellular cholesterol through blocking de novo cholesterol synthesis. Both MBCD and statins abrogated the decreased detachment in NDUFA5 knockdown bystander cells cultured in SCM (Fig. 3d and Extended Data Fig. 7a,b). Conversely, supplementation of cholesterol decreased detachment in bystander cells cultured in SCM but had no effect on NDUFA5 knockdown bystander cells cultured in SCM (Fig. 3e). Cholesterol supplementation also rescued the effects of statins, confirming that detachment was a result of their on-target effects (Extended Data Fig. 7a,b). These data show that the cisplatin-induced SASP drives differential cholesterol content in cells via NDUFA5-mediated NAD⁺, which is critical for the detachment phenotype in bystander cells.

We found that the changes in de novo cholesterol were transcriptionally driven downstream of NDUFA5 (Fig. 3a and Extended Data Fig. 6a), and decreased cholesterol drives the increased detachment phenotype of bystander cells cultured in cisplatin-induced SASP (Fig. 3c,d). Transcription of de novo cholesterol synthesis genes is well known

to be coordinated via the transcription factor sterol regulatory element-binding transcription factor (SREBP1)³⁴. Next, we sought to determine whether SREBP1 contributes to the downregulation of de novo cholesterol synthesis genes and the increase in cell detachment downstream of NDUFA5 in SCM-cultured bystander cells. Inhibition of SREBP1 increased detachment of control bystander cells cultured in PCM and NDUFA5 knockdown bystander cells cultured in SCM (Fig. 3f,g). Moreover, SREBP1 activation through serum starvation rescued detachment in SCM-cultured bystanders (Fig. 3g). Together, these data show the requirement of SREBP1 for detachment. These data are also consistent with results showing that cholesterol depletion increases detachment in NDUFA5 knockdown bystander cells cultured in SCM (Fig. 3d and Extended Data Fig. 7a,b). SREBP1 translocates to the nucleus, where it binds to target genes to regulate their transcription³⁴. We observed a decrease in both nuclear SREBP1 and SREBP1 occupancy on multiple de novo cholesterol synthesis gene promoters in bystander cells cultured in SCM that was rescued by NDUFA5 knockdown (Fig. 3h,i and Extended Data Fig. 7c). The decreased SREBP1 occupancy corresponded to decreased protein expression of HMGCR, HMGCS1, and MVK (Extended Data Fig. 7d). These data are consistent with the observed decrease in de novo cholesterol synthesis gene expression (Fig. 3a and Extended Data Fig. 6a). Nicotinic acid supplementation abrogated the NDUFA5 knockdown-mediated decrease in nuclear SREBP1 and occupancy of SREBP1 at de novo cholesterol genes (Fig. 3h–i). This reinforces a role for NAD⁺ downstream of complex I activity in regulating SREBP1 activity. In samples from patients with HGSOC, we found that expression of complex I or aerobic respiration genes inversely correlates with cholesterol synthesis genes regulated by the master transcription factor SREBP1 (Fig. 3m, Extended Data Fig. 7e, and Supplementary Table 1). Together, these data show that downregulation of SREBP1 nuclear localization and activity by SCM decreases de novo cholesterol synthesis gene transcription and adhesion downstream of NDUFA5 (Extended Data Fig. 7f).

NDUFA5 suppresses SREBP1 via increased NAD⁺-dependent SIRT activity, leading to decreased cholesterol and detachment

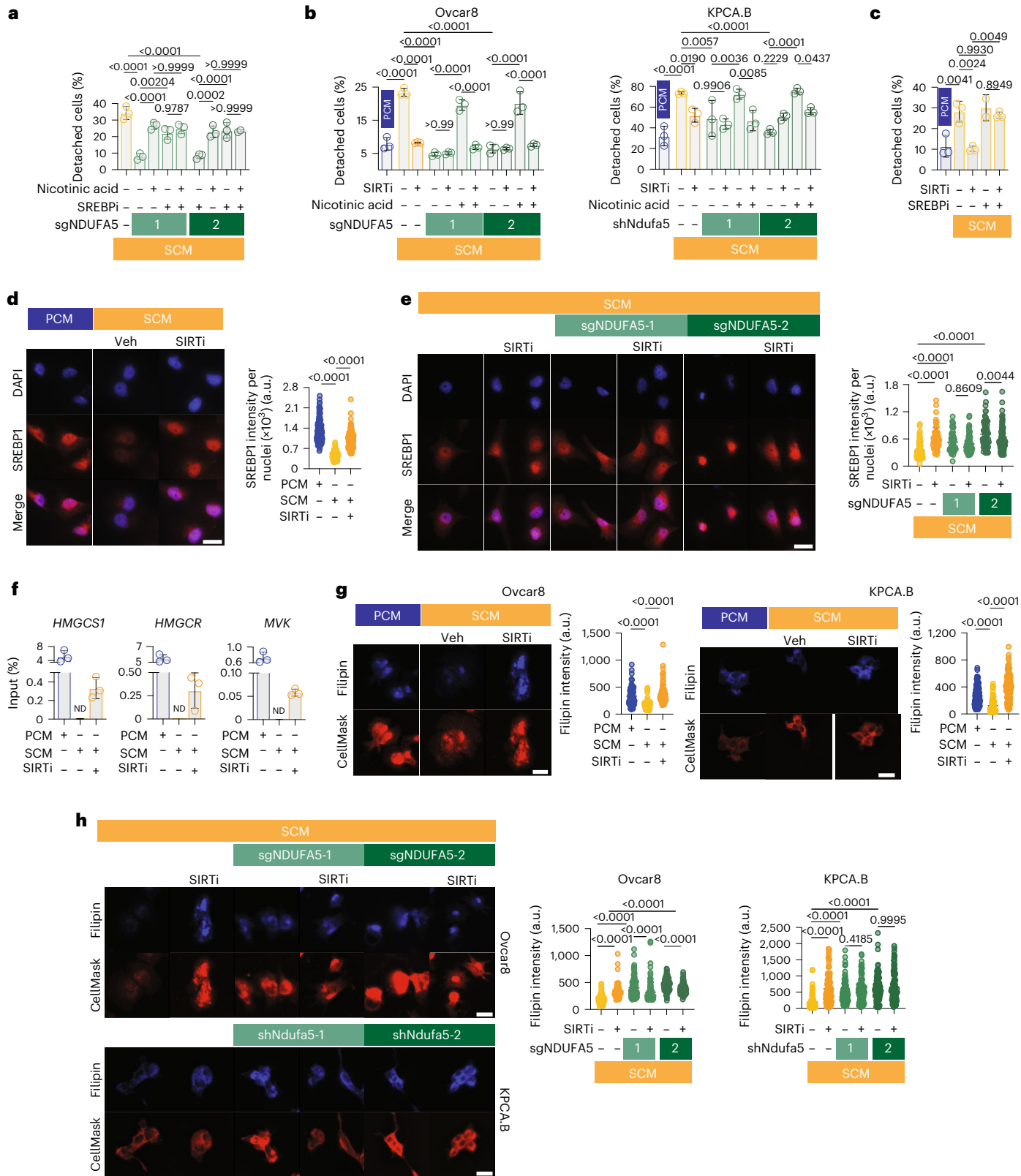
We found that SCM decreases SREBP1 activity via the complex I subunit NDUFA5, leading to increased detachment in bystander cells (Fig. 3). In addition, NAD⁺ pool replenishment with nicotinic acid reversed the decreased detachment caused by NDUFA5 knockdown and restored

Fig. 4 | SREBP1 activity is decreased by the cisplatin-induced SASP downstream of NDUFA5 through NAD⁺-dependent SIRT activity. **a**, Ovarc8 bystander cells with knockout of NDUFA5 (sgNDUFA5) were cultured in SCM alone or treated with or without the SREBP1 inhibitor (SREBPi) fatostatin and/or nicotinic acid. After 72 h, the percentage of detachment was assessed using the controlled assay ($n = 3$ technical replicates representative of 3 independent experiments). **b**, The indicated bystander cells with knockout of NDUFA5 (sgNDUFA5) or knockdown of Ndufa5 (shNdufa5) were cultured in PCM or SCM alone and treated with or without a pan-SIRT inhibitor (SIRTi) with or without nicotinic acid. After 72 h, the percentage of detachment was assessed using the controlled assay ($n = 3$ technical replicates representative of 2 independent experiments in each cell line). **c**, Ovarc8 bystander cells were cultured in PCM or SCM alone or treated with or without a pan-SIRT inhibitor and/or the SREBP1 inhibitor fatostatin. After 72 h, the percentage of detachment was assessed using the controlled assay ($n = 3$ technical replicates representative of 3 independent experiments). **d**, Ovarc8 bystander cells were cultured in PCM or SCM alone or treated with or without a pan-SIRT inhibitor. After 72 h, cells were stained with anti-SREBP1 antibody and DAPI and imaged. Left: representative images. Scale bar, 25 μ m; $\times 40$ magnification. Right: quantification of SREBP1 immunofluorescence intensity per nuclei (PCM, $n = 124$; SCM, $n = 114$; SCM + SIRTi, $n = 98$; representative of 3 independent experiments). **e**, Ovarc8 bystander cells with knockout of NDUFA5 (sgNDUFA5) were cultured in SCM alone or treated with or without a pan-SIRT inhibitor. After 72 h, cells were stained with anti-SREBP1 antibody and DAPI and imaged. Left: representative images. Scale bar, 25 μ m; $\times 40$ magnification. Right: quantification

of SREBP1 immunofluorescence intensity per nuclei (sgControl SCM, $n = 90$; sgControl SCM SIRTi, $n = 55$; sgNDUFA5-1 SCM, $n = 52$; sgNDUFA5-1 SCM SIRTi, $n = 66$; sgNDUFA5-2 SCM, $n = 66$; sgNDUFA5-2 SCM SIRTi, $n = 109$; replicates representative of 2 independent experiments). **f**, Ovarc8 bystander cells were cultured in PCM or SCM with or without a pan-SIRT inhibitor. After 72 h, cells were collected for ChIP using an anti-SREBP1 antibody followed by qPCR. Shown is occupancy of SREBP1 at the promoter of de novo cholesterol synthesis genes *HMGCS1*, *HMGCR*, and *MVK*. NS, not detected ($n = 3$ technical replicates representative of 3 independent experiments). **g**, The indicated bystander cells were cultured in PCM or SCM with or without a pan-SIRT inhibitor. After 72 h, cholesterol was assessed using filipin III fluorescent staining. Left: representative images. Scale bars, 25 μ m; $\times 20$ magnification. Right: quantification (Ovarc8 PCM, $n = 92$; Ovarc8 SCM, $n = 110$; Ovarc8 SCM SIRTi, $n = 112$; KPCA.B PCM, $n = 145$; KPCA.B SCM, $n = 103$; KPCA.B SCM SIRTi, $n = 133$; replicates representative of 2 independent experiments). **h**, The indicated bystander cells with knockout of NDUFA5 (sgNDUFA5) or knockdown of Ndufa5 (shNdufa5) were cultured in SCM alone or treated with or without a pan-SIRT inhibitor. After 72 h, cholesterol was assessed using filipin III fluorescent staining. Left: representative images. Scale bars, 25 μ m; $\times 20$ magnification. Right: quantification (Ovarc8: sgControl, $n = 111$; sgControl + SIRTi, $n = 112$; sgNDUFA5-1, $n = 103$; sgNDUFA5-1 + SIRTi, $n = 110$; sgNDUFA5-2, $n = 116$; sgNDUFA5-2 + SIRTi, $n = 110$; KPCA.B: shControl, $n = 105$; shControl + SIRTi, $n = 113$; shNdufa5-1 SCM, $n = 125$; shNdufa5-1 + SIRTi, $n = 98$; shNdufa5-2, $n = 102$; shNdufa5-2 + SIRTi, $n = 110$; replicates representative of 2 independent experiments). All graphs show mean $\pm$ s.d.; one-way ANOVA followed by Sidák's multiple comparisons test was used.

SREBP1 nuclear localization and promoter occupancy at cholesterol synthesis genes (Fig. 3h–i), altogether showing a pivotal role for NAD⁺ in this process. Replenishing NAD⁺ to SREBP-inhibited NDUF5 knockdown bystander cells cultured in SCM did not change detachment (Fig. 4a), indicating that NAD⁺ is indeed upstream of SREBP1 activity. Previous reports show that SREBPs can be deactivated by NAD⁺-dependent deacetylases known as sirtuins^{35–37} (SIRT). Thus,

we reasoned that SIRT activity driven by NAD⁺ may increase detachment downstream of NDUF5 by inhibiting SREBP1-mediated cholesterol synthesis. Indeed, inhibition of SIRTs prevented detachment in SCM-cultured bystander cells (Fig. 4b). This was not a result of a parallel pathway as inhibition of SIRTs did not affect detachment in NDUF5 knockdown cells cultured in SCM (Fig. 4b). Addition of nicotinic acid to NDUF5 knockdown cells cultured in SCM increased detachment,



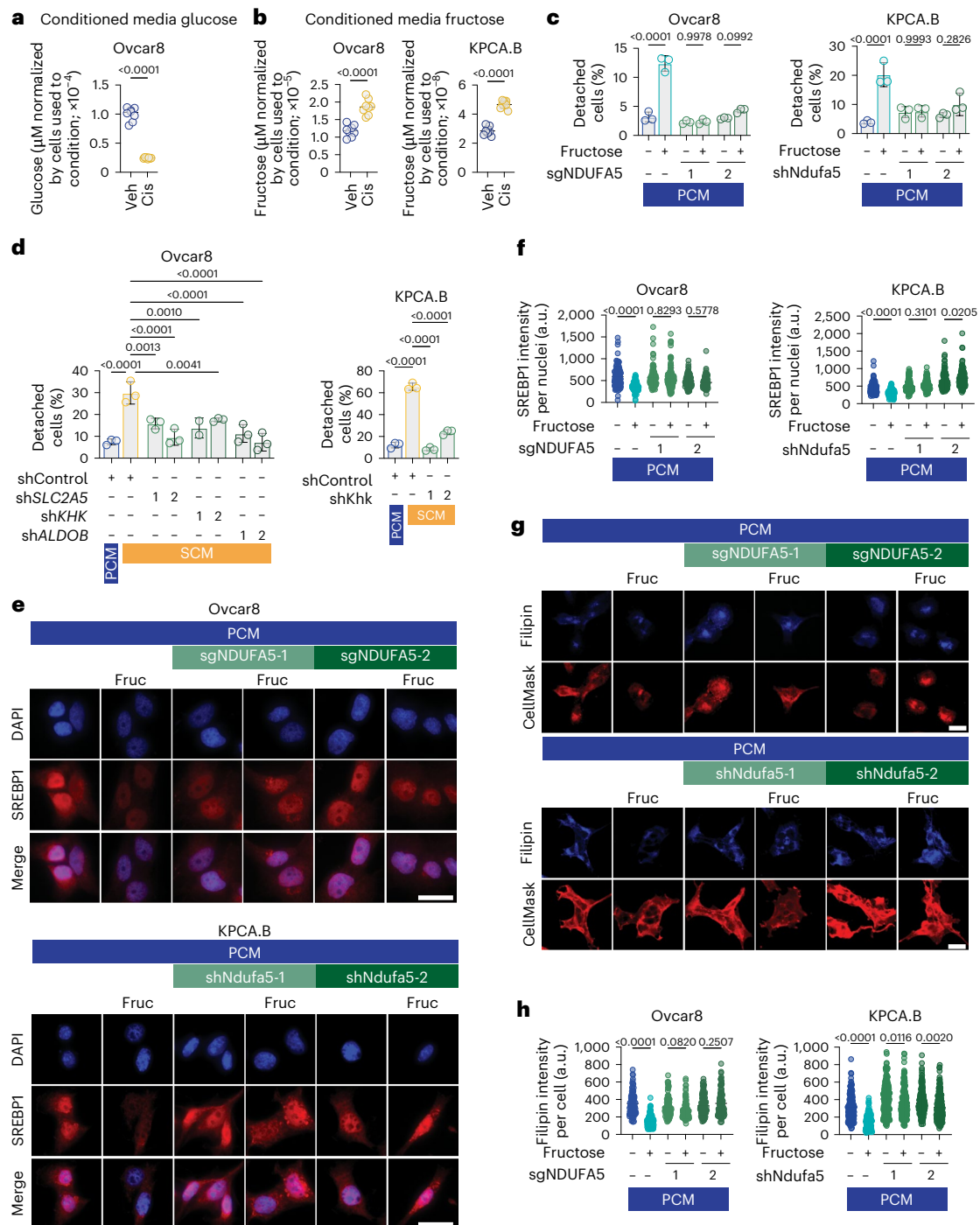
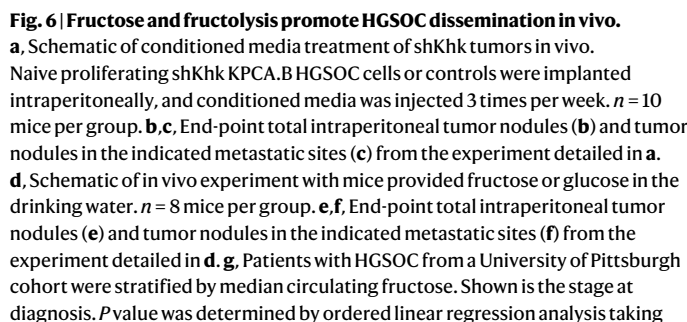


Fig. 5 | Fructose is increased in the cisplatin-induced SASP and promotes HGSOC detachment and dissemination. **a, b**, The indicated HGSOC cell lines were induced to senescence using cisplatin, and conditioned media was collected to assess glucose (**a**) or fructose (**b**) in the conditioned media by LC–MS normalized to the total number cells over the course of the conditioning timeframe ($n = 8$ per group, representative of 3 independent experiments in each cell line). **c**, The indicated bystander cells with knockout of NDUFA5 (sgNDUFA5) or knockdown of Ndufa5 (shNdufa5) were cultured in PCM alone or supplemented with fructose. After 72 h, the percentage of detachment was assessed using the controlled trypsin assay ($n = 3$ technical replicates representative of 3 independent experiments in each cell line). **d**, The indicated bystander cells with knockdown of SLC2A5 (shSLC2A5), KHK (shKHK), or knockdown of ALDOB (shALDOB) were cultured in PCM alone or supplemented with fructose. After 72 h, the percentage of detachment was assessed using the controlled trypsin assay ($n = 3$ technical replicates except for Ovarcar8 shKHK-1 SCM, $n = 2$; representative of 3 independent experiments in each cell line). **e, f**, The indicated bystander cells with knockout of NDUFA5 (sgNDUFA5) or knockdown of Ndufa5 (shNdufa5) were cultured in PCM alone or supplemented

with fructose. **e**, After 72 h, cells were stained with anti-SREBP1 antibody and DAPI. Scale bars, 25 μm ; $\times 40$ magnification. **f**, Quantification of **e**. Ovarcar8: sgControl, $n = 104$; sgControl fructose, $n = 112$; sgNDUFA5-1, $n = 103$; sgNDUFA5-1 fructose, $n = 104$; sgNDUFA5-2, $n = 150$; sgNDUFA5-2 fructose, $n = 91$; KPCA.B: shControl, $n = 101$; shControl fructose, $n = 126$; shNdufa5-1, $n = 165$; shNdufa5-1 fructose, $n = 105$; shNdufa5-2, $n = 106$; shNdufa5-2 fructose, $n = 120$; replicates representative of 2 independent experiments in each cell line. **g, h**, The indicated bystander cells with knockout of NDUFA5 (sgNDUFA5) or knockdown of Ndufa5 (shNdufa5) were cultured in PCM alone or supplemented with fructose. **g**, After 72 h, cells were stained with filipin III to assess cholesterol. Scale bars, 25 μm . $\times 40$ magnification. **h**, Quantification of **g**. Ovarcar8: sgControl, $n = 92$; sgControl fructose, $n = 107$; sgNDUFA5-1, $n = 112$; sgNDUFA5-1 fructose, $n = 119$; sgNDUFA5-2 PCM fructose, $n = 104$; KPCA.B: shControl PCM, $n = 145$; shControl fructose, $n = 111$; shNdufa5-1, $n = 207$; shNdufa5-1 fructose, $n = 184$; shNdufa5-2, $n = 265$; shNdufa5-2 fructose, $n = 265$; replicates representative of 2 independent experiments in each cell line. All graphs show mean $\pm$ s.d. In **a–c**, two-tailed Student's *t*-test was used. In **d–f** and **h**, one-way ANOVA followed by Šidák's multiple comparisons test was used.



age and body mass index into account as confounding factors. **h**, Schematic of in vivo experiments with mice provided fructose or glucose in the drinking water and implanted with control (shControl), Ndufa5 knockdown (shNdufa5), or Khk knockdown (shKhk) cells. **i,j**, End-point total tumor nodules (**i**) and tumor nodules in the indicated metastatic sites (**j**) from the experiment with knockdown of Khk detailed in **h**. shControl, $n = 10$ per group; shKhk-1 and shKhk-2, $n = 11$ per group. **k,l**, End-point total tumor nodules (**k**) and tumor nodules in the indicated metastatic sites (**l**) from the experiment with knockdown of Ndufa5 detailed in **h**. shControl, $n = 12$ per group; shNdufa5, $n = 9$ per group. All graphs show mean $\pm$ s.d.; in **e, f, k**, and **l**, two-tailed Student's *t*-test was used. In **b, c, i**, and **j**, one-way ANOVA followed by Šidák's multiple comparisons test was used. Panels **a, d** and **h** created in BioRender; Cole, A. <https://biorender.com/753ayc9> (2026).

Increased fructose in the cisplatin-induced SASP drives the paracrine increase in cell detachment

Finally, we aimed to determine the upstream signal in the SASP that regulates detachment in a paracrine fashion. We found that both heat-inactivated and <3 kD-filtered SCM increase detachment (Fig. 2e), indicating that the signal promoting detachment is a small molecule or metabolite. Senescent cells are known to increase glucose uptake^{8,12,38,39}, resulting in a glucose-depleted SASP. Indeed, we observed a significant decrease in glucose abundance in conditioned media from cisplatin-induced senescent cells versus vehicle controls (Fig. 5a and Extended Data Fig. 8a). To understand how decreased glucose in the SASP from cisplatin-induced senescent cells affects bystander cells in a paracrine fashion, we performed mass spectrometry and assessed glycolytic intermediates. Although conditioned media from cisplatin-induced senescent cells have decreased glucose (Fig. 5a), we observed an increase in glycolytic intermediates in bystander cells cultured in SCM (Extended Data Fig. 8b,c). One notable metabolite that was upregulated in bystander cells cultured in SCM is dihydroxyacetone phosphate (Extended Data Fig. 8d), which can be derived from fructolysis (Extended Data Fig. 8b). Indeed, we found that conditioned media from multiple senescent cell types and inducers had increased

fructose abundance (Fig. 5b and Extended Data Fig. 8e), consistent with previous reports showing that high glucose can induce polyol pathway activity, which converts glucose to fructose^{40–42}. To determine how the relative availability of fructose and glucose contribute to bystander cell adhesion, we cultured bystander cells in PCM supplemented with a physiological concentration of fructose or SCM supplemented with glucose. The addition of fructose to PCM cultured bystander cells was sufficient to induce detachment in controls but not NDUFA5 knockdowns (Fig. 5c), showing that fructose is upstream of NDUFA5. Conversely, the addition of glucose inhibited detachment of bystander cells cultured in SCM (Extended Data Fig. 8f), indicating that the balance between fructose and glucose drives the observed detachment. Knocking down the enzymes responsible for the first step of fructolysis, ketohexokinase (KHK) and aldolase B (ALDOB), prevented detachment in SCM-cultured bystander cells (Fig. 5d and Extended Data Fig. 8g), supporting the idea that fructolysis promotes detachment. Previous reports have shown that fructolysis can induce aerobic respiration through activating AMP-activated protein kinase (AMPK)⁴³. Indeed, we found that bystander cells cultured in SCM showed increased AMPK phosphorylation upstream of NDUFA5 (Extended Data Fig. 9a). Similarly, fructose supplementation led to increased AMPK phosphorylation in PCM cultured bystander cells, while phosphorylation is inhibited by glucose supplementation in SCM (Extended Data Fig. 9b). We also observed a modest increase in glucose-derived citrate in the presence of fructose (Extended Data Fig. 9c,d), potentially indicating increased glycolytic flux into the mitochondria to support respiration. Finally, we sought to determine whether fructose is upstream of decreased SREBP1 activity and cholesterol abundance. Addition of fructose to PCM, but not to NDUFA5 knockdown cells, was sufficient to decrease nuclear SREBP1 and cholesterol content of bystander cells (Fig. 5e–h). Conversely, the addition of glucose increased SREBP1 nuclear localization and cholesterol content of bystander cells cultured in SCM (Extended Data Fig. 9e–h). In patient samples, high expression of the fructose transporter SLC2A5 after NACT treatment corresponded with low expression of *de novo* cholesterol synthesis genes (Extended Data Fig. 9i and Supplementary Table 1). Together, these data show that fructose suppresses nuclear SREBP1, corresponding to less cholesterol and increased detachment. These data also show that restoring the balance between glucose and fructose overcomes this phenotype in SCM-cultured bystander cells.

We found that fructose is a component of the SASP and drives differential cell detachment in vitro (Fig. 5b,c). We also found that SCM increases HGSOC dissemination in vivo (Fig. 1). Thus, we interrogated whether SASP-induced fructolysis is sufficient to drive dissemination in vivo. Using the conditioned media treatment murine model, we injected control or shKhk proliferative cells intraperitoneally and treated these with conditioned media 3 times per week (Fig. 6a). Similarly to knockdown of Ndufa5 (Fig. 2l–n), knockdown of Khk prevented SASP-induced dissemination without affecting omental weights (Fig. 6b,c and Extended Data Fig. 10a). We next asked whether fructose alone is sufficient to increase dissemination in vivo, independent of senescent cells or other SASP components. Towards this goal, we provided mice with fructose in their drinking water and implanted HGSOC cells (Fig. 6d). Glucose was used to control for water sweetness. We did not observe differences in water consumption between groups (Extended Data Fig. 10b). High dietary fructose increased HGOSC dissemination in mice co-injected with senescent cells (Fig. 6e,f) without differences in omental weights (Extended Data Fig. 10c). In agreement with these findings, elevated fructose is associated with higher stage at diagnosis in patients with HGSOC, indicating more widespread disease even when adjusting for age and body mass index (Fig. 6g).

Fructose can have pleiotropic systemic effects. Thus, we finally aimed to assess whether the increased fructose-driven dissemination is due in part to cancer cell fructolysis and complex I activity. To this end, we first provided mice with fructose and then implanted control

or shKhk HGSOC cells (Fig. 6h). Khk knockdown decreased dissemination in mice with fructose supplementation without affecting omental weights (Fig. 6i,j and Extended Data Fig. 10d), indicating the necessity of malignant cell fructose utilization in driving dissemination. Next, we implanted control and shNdufa5 HGSOC cells into mice on a high-fructose diet (Fig. 6h). Ndufa5 knockdown abrogated dissemination in mice on a high-fructose diet (Fig. 6k,l) but did not affect omental weights (Extended Data Fig. 10e), which is consistent with our previous experiment using Ndufa5 knockdown cells co-injected with senescent cells (Fig. 2l–n). These data show the sufficiency of fructose to drive HGSOC dissemination through both fructolysis and complex I activity in vivo. Together with mechanistic and patient data, these studies point to a previously unrecognized role for fructose in the secretome of senescent cells in promoting cell detachment through a complex I–NAD⁺–SIRT–SREBP1–cholesterol synthesis axis and suggest that lowering dietary fructose may have beneficial clinical outcomes for patients with HGSOC (Extended Data Fig. 10f).

Discussion

The phenomenon of senescence has gained increasing attention in cancer research owing to its complex role in influencing the TME through its unique secretome. In this study, we investigated the impact of TIS cell metabolism and the malignant cell SASP on HGSOC progression to better understand how chemotherapy-induced metabolic reprogramming affects tumor progression. While the role of SASP proteins in affecting cell adhesion and detachment is well described through direct matrix remodeling or bystander cell signaling effects^{44–47}, our results show that fructose is a part of the SASP from cisplatin-induced senescent HGSOC cells, which is sufficient to increase cell detachment and drive increased metastatic dissemination in HGSOC. These studies have important implications for understanding how chemotherapy alters tumor dynamics in a paracrine fashion, show a mechanism of how senescence impacts bystander cancer cells on a molecular level, and reveal a potential role for diet in modifying HGSOC progression.

We found that the secretome from cisplatin-induced senescent HGSOC cells alone increases detachment of bystander cells and drives metastatic dissemination in a model of HGSOC, although the aggressive nature of this cell line precluded us from determining whether this affects progression-free survival. Another limitation of this study is that the in vivo models were only performed in one cell line. While previous studies have shown that senescent cells promote tumor progression and metastasis through co-injection or inducible models^{2,4,48,49}, these effects reflect the combined influence of senescent cells and their secretome. In our model, by injecting only conditioned media from senescent cancer cells, we specifically isolate the impact of the secretome in the absence of senescent cells, showing that secreted factors alone are sufficient to drive the metastatic phenotype (Fig. 1). While components of the SASP have been linked to metastasis in various cell and cancer types, many of these are pro-inflammatory mediators, and the non-protein, non-cytokine fractions of this secretome are often overlooked. In fact, there are only a handful of reports on the metabolic components of the SASP, such as elevated bioactive lipid production, nucleobases, amino acids, nitric oxide, and ROS^{8–10,50–52}. Moreover, only a small number of studies to date have indicated that specific metabolites are depleted from the SASP^{8,12}. While these reports investigated the metabolite's role in senescence induction, to our knowledge, no study to date has investigated the paracrine effects of the extracellular metabolite composition on proliferative bystander cells. Here we found that glucose is depleted in the cisplatin-induced SASP (Fig. 5a and Extended Data Fig. 8a). This is likely a result of increased glucose uptake and glycolysis of senescent cells, which has been previously reported^{8,12}. High glucose can induce polyol pathway activity, which converts glucose to fructose^{40–42}. How and why this occurs is an interesting point for future studies.

We found that high dietary fructose increases metastatic dissemination *in vivo* (Fig. 6). While glucose is the most abundant monosaccharide in the body, fructose makes up a large portion of sugar consumed. Fructose is present alone and together with glucose in sucrose in foods such as honey, fruits, vegetables, and high-fructose corn syrup. Resting plasma fructose levels are in the tens of micromolar but can increase 10-fold after consuming fructose⁵³. Although our data implicate a role of fructose acting directly on malignant bystander cells (Fig. 6), fructose metabolism can indirectly support tumor growth through the microbiome or liver lipogenesis^{54,55}, and future studies will determine the contribution of these pathways to metastatic dissemination. Given the dietary and endogenous availability and our findings on fructose driving dissemination, fructose may play an underappreciated role in modulating tumor cell behavior. This raises the possibility that restricting fructose intake could be a promising dietary intervention to assist in treatment response.

Our data reveal a critical role for respiration in reducing cell adhesion downstream of fructose, allowing cells to detach and disseminate (Fig. 2), although additional studies are needed to further interrogate the mechanism of how fructose activates complex I. There is growing evidence that mitochondrial function and efficient respiration supports tumor progression and metastasis^{56–61}. Likewise, our data support the notion that plasma membrane cholesterol depletion in cancer cells can lead to a more disseminated phenotype (Fig. 3), raising questions about the use of statins in cancer patients. Recent mechanistic investigation into statin use in cancer revealed heterogeneous responses, including increasing metastatic potential⁶². Taken together with our data, this highlights the potential context dependency in cholesterol metabolism in malignant cell phenotypes, although the interpretation of the systemic effects of long-term statin treatment can be difficult. The translation of these findings to ovarian cancer could represent a therapeutic strategy to specifically target senescent cells and mitigate the metastatic potential induced by the SASP.

It is interesting to speculate whether these changes in adhesion and detachment observed in bystander cells exposed to SCM could be therapeutically targeted. One such therapeutic route is the use of senolytics, a class of drugs designed to selectively target and eliminate senescent cells⁶³. This is a particularly attractive approach given that we observed an increase in senescence signatures in multiple cell types in addition to malignant epithelial cells (Extended Data Fig. 1b), and the SASP from non-malignant cell types is enriched in fructose (Extended Data Fig. 8e) and induces cell detachment (Extended Data Fig. 4g). Moreover, given our dietary studies (Fig. 6), it is also interesting to speculate that diet interventions could be used in concert with senolytics to attain a greater benefit. This will be an area for the field to explore further. Finally, there is a large body of literature regarding the aging-related decline in NAD⁺ and surrounding the use of supplements in aging to replenish NAD⁺ levels⁶⁴. We found that supplementing cells with nicotinic acid increased detachment *in vitro* (Fig. 2p), and previous work found that NAD⁺ promotes the pro-inflammatory SASP⁶⁵. Thus, our studies herein would indicate that nicotinic acid supplements in cancer patients, regardless of age, may be detrimental, although this needs to be experimentally investigated.

In summary, this study sheds light on a previously unexplored facet of ovarian cancer by revealing how the secretome of cisplatin-induced senescent cells contributes to HGSOc phenotypes. The SASP-induced dissemination, metabolic reprogramming leading to weakened adhesion and increased detachment, and the metabolite driving these changes provide a unique perspective on the complex interplay between senescence and cancer progression. These findings underscore the importance of understanding all components of the SASP and its multifaceted impact on the TME, offering potential therapeutic targets for mitigating ovarian cancer metastasis.

Methods

All experiments with animals were performed in accordance with institutional guidelines approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh (24024583) or The Wistar Institute (201727). Humane end points were >20% change in weight and body condition score of 2 (out of 5). None of these limits were exceeded in any of the experiments. Pretreatment blood was collected from patients with newly diagnosed epithelial ovarian cancer under University of Pittsburgh approved Institutional Review Board protocols 20050019, 19070449, and 19060197. Written, informed consent was obtained from ovarian cancer patients.

Cell lines

Ovarc8 cells were a gift from Dr. Benjamin Bitler (University of Colorado). KPCA.B cells were a gift from Dr. Robert Weinberg (MIT). IMR90 (CCL-186), YUMM5.2 (CRL-3367), and Capan-1 (HTB-79) cells were obtained from ATCC, and HEK293FT cells were obtained from Thermo Fisher (R70007). Ovarc8 cells were cultured in RPMI 1640 medium (Corning 10-040-CV) supplemented with 5% FBS (BioWest, S1620). KPCA.B cells were cultured in high-glucose DMEM (Fisher Scientific, 12430047) supplemented with 4% heat-inactivated FBS (Sigma, S1620), 2 ng ml⁻¹ hEGF (Sigma, E9644), and Insulin–Transferrin–Selenium (Fisher Scientific, 41400-045). IMR90 cells were cultured in DMEM (Corning, 10-017-CV) supplemented with 10% FBS (BioWest, S1620), L-glutamine (Corning, 25-015-CI), non-essential amino acids (Corning, 25-025-CI), sodium pyruvate (Corning, 25-000-CI), and sodium bicarbonate (Corning, 25-035-CI). YUMM5.2 cells were cultured in DMEM and Ham's F-12/50 media (Corning, 10-092-CV) supplemented with nonessential amino acids (Corning, 25-025-CI) and 10% FBS (BioWest, S1620). Capan-1 cells were cultured in IMDM (Gibco, 12440053) supplemented with 20% FBS (BioWest, S1620). HEK293FT cells were cultured in DMEM (Corning, 10-013-CV) supplemented with 10% FBS according to ATCC. All cell lines were cultured in 1% penicillin/streptomycin (Fisher Scientific, 15-140-122) at 37 °C under 5% CO₂ and atmospheric O₂ (except IMR90 cells, cultured at 2% O₂) and tested monthly for mycoplasma as described in ref. 66.

Plasmid information and cloning

The following shRNA sequences were purchased from Sigma: mouse shNdufa5-1 (TRCN0000041819) and shNdufa5-2 (TRCN0000041821), human shSLC2A5-1 (TRCN0000043005) and shSLC2A5-2 (TRCN0000043006), human shKHK-1 (TRCN0000037725) and shKHK-2 (TRCN0000037727), human shALDOB-1 (TRCN0000052508) and shALDOB-2 (TRCN0000052512), and mouse shKHK-1 (TRCN0000025511) and shKHK-2 (TRCN0000025512). shGFP control was purchased from Addgene (30323). For some experiments, KPCA.B cells were transduced with GFP (Addgene, 36083). The sgNDUFA5-1 and sgNDUFA5-2 guide sequences were cloned into the lentiviral lentiCRISPRv2 vector expressing puromycin resistance (Addgene, 52961) following standard protocols. For overexpression experiments, lentiviral plasmid Flag-LBNOX was synthesized by Twist Bioscience. The Twist Bioscience empty vector was used as the control in overexpressing experiments. Sequences can be found in Supplementary Table 10.

Lentiviral packaging and infection conditions

Lentiviral vectors were packaged as follows: 10 µg of the corresponding lentiviral vector was mixed with 7.5 µg psPAX2 (Addgene, 12260) and 7.5 µg pMD2.G (Addgene, 12259) in 1.5 ml Opti-MEM media (Gibco, 31985070) and incubated for 5 min at room temperature. In parallel, 50 µl Lipofectamine (Invitrogen, 11668019) was diluted in 1.5 ml of Opti-MEM media and incubated for 5 min at room temperature. The Lipofectamine mixture was added dropwise to the plasmid mixture and incubated for 20 min at room temperature. The Lipofectamine/DNA complexes were added dropwise to HEK293FT cells. The media was replaced 6–8 h later, and viral supernatants were collected after

72 h, which were filtered through a 0.45 μm filter (Fisherbrand, 09-720-005). Target cells were infected by incubating viral supernatant in antibiotic-free media supplemented with 8 $\mu\text{g ml}^{-1}$ polybrene (Sigma-Aldrich, H9268) for 16 h. Infected cells were selected with 1 $\mu\text{g ml}^{-1}$ puromycin (ThermoFisher, A1113802) or 3 $\mu\text{g ml}^{-1}$ blasticidin (InvivoGen, ant-bl-05).

Bioinformatic analysis of transcriptomic data from publicly available ovarian cancer patient data

Information on datasets can be found in the 'Data availability' statement.

Analysis of single cell RNA-seq. Downloaded cell type annotated data from the original study were a subject for standard Seurat v2 preprocessing. Epithelial cell clustering was performed using the first 30 principal components with default resolution. Calculation of senescence score relied on SenMayo gene set and DNA replication (GO:0044786) (DNA replication, Supplementary Table 1). Each sample score was calculated as an unweighted linear combination of log₂-scaled expression values with positive coefficient from SenMayo and negative coefficient from DNA replication gene set. Calculation of senescence scores was done on per-cell level, and significance of score differences between posttreatment and pretreatment cells within each cluster were compared using Wilcoxon test. Uniform manifold approximation and projection dimensionality reduction was used for data visualization.

Correlation with patient disease stage. Analysis of tumor stage was performed with data from The Cancer Genome Atlas (TCGA) by splitting patients into quartiles of the average z score of the genes in the indicated gene sets (Supplementary Table 1). For correlative analysis, the average z score of the genes in the indicated gene sets were plotted, and linear regression was performed.

Senescence induction and conditioned media generation

Cells were treated with senescence-inducing drugs for 48 h (see Supplementary Table 11 for doses), after which the drugs were removed, and cells were cultured in fresh media. Twenty-four hours after removal of the drugs, the media was changed and allowed to condition for 48 h. For conditioned media intended for use in vivo, no penicillin/streptomycin was added. Conditioned media was collected and filtered (Fisher Scientific, FB12566506).

Crystal violet and proliferation assays

An equal number of cells were seeded in multiwell plates and cultured for 7–10 days. Cells were fixed with 1% paraformaldehyde, stained with 0.05% crystal violet, and destained using 10% acetic acid. Absorbance (590 nm) was measured using a spectrophotometer (BioTek Epoch Microplate reader). Proliferation assays were performed using an IncuCyte S3 imaging system (Sartorius); an equal number of cells per condition were seeded in triplicates in a 96-well plate. Cells were imaged for 4–5 days with live cell imaging every 6 h. Cell quantification was performed using the IncuCyte software (Version 2023A).

SA- β -Gal assay

Cells were fixed in 2% formaldehyde/0.2% glutaraldehyde in PBS and stained (40 mM Na₂HPO₄, 150 mM NaCl, 2 mM MgCl₂, 5 mM K₃Fe(CN)₆, 5 mM K₄Fe(CN)₆, and 1 mg ml⁻¹ X-gal) overnight at 37 °C in a non-CO₂ incubator. Images were acquired at room temperature using a Nikon Eclipse Ts2 equipped with a Nikon DS-Fi3 camera with a $\times 20/0.40$ objective (Nikon LWD); >300 cells were counted per experiment.

RNA isolation, sequencing, and analysis

RNA was extracted from cells with Trizol (Ambion, 15596018) and DNase treated, cleaned, and concentrated using Zymo columns (Zymo Research, R1013). Complementary DNA libraries, next-generation

sequencing, and bioinformatics analysis were performed by Novogene. Raw and processed RNA-seq data are available on Gene Expression Omnibus (GEO, [GSE305033](#) and [GSE305002](#)).

RT-qPCR

RNA was retrotranscribed with High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, 4368814), and 20 ng of cDNA was amplified using the CFX Connect Real-time PCR system (Bio-Rad) and the PowerUp SYBR Green Master Mix (Applied Biosystems, [A25742](#)). Quantification relative to multiple reference genes was determined to ensure reproducibility using the delta-delta CT method. Primers can be found in Supplementary Table 10.

Western blotting

Cell lysates were collected in 1 $\times$ sample buffer and boiled for 10 min. An equal amount of total protein (Bradford assay (Bio-Rad, 5000006)) was resolved using SDS-PAGE gels and transferred to nitrocellulose membranes (Fisher Scientific). Membranes were blocked for 1 h at room temperature, incubated overnight at 4 °C in primary antibodies, washed 4 times in TBS-T for 5 min at room temperature, incubated with HRP-conjugated secondary antibodies for 1 h at room temperature, and washed 4 times in TBS-T for 5 min at room temperature. Proteins were visualized on film after incubation with SuperSignal West Pico PLUS Chemiluminescent Substrate (ThermoFisher).

Cytokine array

The human cytokine antibody array C1000 (RayBio, AAH-CYT-1000-2) was used. Individual spot signal densities were obtained using ImageJ software (Version 2.0.2) and normalized to cell number.

Murine ovarian cancer in vivo models

For all experiments, 6- to 8-week-old female C57BL/6J mice were purchased from Jackson Laboratories (2498079-86). Cohort numbers can be found in the figure legends. All mice were maintained in a HEPA-filtered ventilated rack. Mice were housed up to 5 mice per cage and in a 12 h light/dark cycle. For dietary studies with glucose and fructose, mice were provided 30% w/v sugar in sterile water 10 days before cell implantation and throughout the experiment. For co-injection experiments, one million KPCA.B ovarian cancer cells were injected intraperitoneally alone or together with one million cisplatin therapy-induced senescent KPCA.B cells that stably express GFP by lentiviral transduction. For conditioned media treatment experiments, mice were treated three times per week with intraperitoneal injection of 200 μl of conditioned medias. Mice were imaged by IVIS and analyzed using the Aura Spectral Imaging software (Version 5.0.1) at 5–8 days after injection and weighed weekly. To accurately analyze tumor dissemination, a synchronized end point before predefined humane end points was required. Animals were euthanized at the end of the fourth week, and tumor burden was assessed by counting the number of intraperitoneal nodules.

Immunohistochemistry

After deparaffinization and rehydration, tissues were steamed for 40 min in 1 $\times$ citrate buffer (Sigma-Aldrich, C9999) and then immersed in 3% MeOH for 20 min. Tissues were blocked before overnight incubation in primary antibodies. Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit (Abcam, 64264) was used for visualization of staining. Tissues were incubated in Mayer's Hematoxylin Solution (Sigma-Aldrich, MHS16). Tissues were dehydrated and mounted in Cytoseal (Sigma-Aldrich, 23-244257). QuPath (Version 0.7.0) was used for analysis.

Non-adherent culture and imaging

Cells were seeded 1,000 (96-well round-bottom ULA, Corning 7007), 10,000 (96-well flat-bottom ULA, Corning 3474), or 300,000 (6-well

flat-bottom ULA, Corning 3471) cells per well in a 1:1 ratio of fresh to conditioned media. To assess viability, cells were incubated with 1 mM ethidium homodimer (Sigma Aldrich, 46043) for 30 min. Time-lapse imaging to assess spheroid formation in round-bottom ULA plates was performed using IncuCyte S3 imaging system (Sartorius). Time-lapse imaging of spheroids to assess detachment was performed using a Leica Thunder Imager with an Okolab incubation chamber. Spheroids formed in round-bottom ULA culture with conditioned media were transferred to a flat-bottom ULA plate. Z-stacked images were acquired every 15 min for 12 h.

Control trypsin assay

Cells were cultured in conditioned media for 72 h with inhibitors or supplements (see Supplementary Table 11 for doses). Cells were incubated with trypsin (Corning, MT25053CI) for 90 s, and both adherent and detached cells were counted using a hemocytometer.

CRISPR dropout screen

Ovarc8 cells were infected with pooled CRISPR KO library (Addgene, 110066;) at multiplicity of infection of <0.3 to achieve ~350-fold library coverage after selection with $1 \mu\text{g ml}^{-1}$ puromycin. Cells were cultured in conditioned media for 72 h before the controlled trypsin assay was performed. The detached cell and adherent cell portions were collected separately, and DNA was isolated (Zymo Research, D4069). DNA from each condition were pooled and used for gRNA amplification, ensuring >250-fold coverage. The gRNA region was amplified using an Ex Taq DNA polymerase (Takara, RR001A). PCR amplicons were purified (Promega, A9282) and pooled. Amplicons were sequenced using Next Seq v2.5 High Output 75 cycle on an Illumina NextSeq sequencer. Data were analyzed using the MAGeCK bioinformatics pipeline to identify differentially enriched genes⁶⁷. See Supplementary Table 10 for all primer sequences.

Bioenergetic analysis of oxygen consumption rate and extracellular acidification rate

The Agilent Seahorse XFp Metabolic Analyzer (Agilent, model S7802A) was used. Cells were evenly seeded and cultured overnight in a Seahorse XFp plate (Agilent, 103022-100). The XFp sensor cartridge was hydrated (Agilent, 103022-100) at 37 °C in a humidified incubator (non-CO₂) overnight. On the day of the experiment, cell culture media was replaced with prewarmed media (Agilent, 103576-100) supplemented with 1 mM sodium pyruvate (Agilent 103578-100), 2 mM glutamine (Agilent 103579-100), and 10 mM glucose (Agilent, 103577-100). Cells were then placed into a non-CO₂ humidified incubator at 37 °C for 60 min. Three basal rate measurements (3 min measurement time each) were taken.

Immunofluorescence imaging

Cells were seeded on coverslips or in Mattek dishes coated with poly-L-ornithine (Sigma, P4957). For filipin III staining, cells were stained with CellMask deep red (ThermoFisher Scientific, C10046) and fixed with 4% paraformaldehyde. Cells were washed four times with PBS and stained with 0.2 mg ml⁻¹ filipin (Cayman Chemicals, 70440). For BrdU incorporation, cells were incubated with $1 \mu\text{mol l}^{-1}$ BrdU for 1 h. Cells were fixed with 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, and postfixed with 1% paraformaldehyde + 0.01% Tween-20. Coverslips were DNaseI treated for 10 min, and the DNaseI reaction was stopped using 20 mmol l⁻¹ EDTA. For immunofluorescence, cells were fixed and permeabilized as above. Permeabilized cells were blocked for 5 min with 3% BSA/PBS followed by incubation of corresponding primary antibody ('Reporting summary') in 3% BSA/PBS for 1 h at room temperature. Cells were washed three times with 1% Triton X-100 in PBS and incubated with secondary antibody in 3% BSA/PBS for 1 h at room temperature. Cells were counterstained with 0.15 $\mu\text{g ml}^{-1}$ DAPI and mounted (9 ml glycerol (BP229-1; Fisher Scientific), 1 ml of 1× PBS, and 10 mg p-phenylenediamine (PX0730;

EMD Chemicals); pH 8.0–9.0). Images were obtained at room temperature using a Nikon ECLIPSE Ti2 microscope equipped with a camera (ORCA-Fusion C14440) with a ×20/0.75 N.A. or ×40/0.95 N.A. dry objective (Nikon DIC N2 Plan Apo). TIRF images were obtained at room temperature using a Nikon Ti-E equipped with a camera (Photometrics Prime 95B) with an Apo TIRF ×100/1.49 N.A. oil objective. Images were acquired using NIS-Elements AR software (Version 5.30.06). For analysis, individual cells were segmented in the CellMask channel using Cellpose-SAM⁶⁸ followed by manual curation. Mean filipin intensity was quantified within segmented regions. The background was defined as the median intensity outside segmented cells and subtracted from the mean filipin signal.

Chromatin immunoprecipitation

Cells were fixed in 1% paraformaldehyde and quenched with 2.5 M glycine. After washes, cells were lysed in chromatin immunoprecipitation (ChIP) lysis buffer (50 mM HEPES–KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, pH 8.0, 1% Triton X-100, and 0.1% deoxycholate (DOC) with 0.1 mM PMSF and EDTA-free Protease Inhibitor Cocktail (P8340; Sigma-Aldrich)). Samples were incubated on ice for 10 min and then centrifuged at 1,800g for 3 min at 4 °C. The pellet was resuspended (10 mM Tris, pH 8.0, 200 mM NaCl, 1 mM EDTA, and 0.5 mM EGTA with 0.1 mM PMSF and EDTA-free Protease Inhibitor Cocktail) and incubated at room temperature for 10 min. Samples were centrifuged at 1,800g for 5 min at 4 °C. The pellet was resuspended (10 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% DOC, and 0.5% N-lauroylsarcosine with 0.1 mM PMSF and EDTA-free Protease Inhibitor Cocktail). Cells were sonicated on ice (10 s on, 50 s off); 30 μl 10% Triton X-100 was added to each tube, and samples were centrifuged at 21,130×g for 15 min at 4 °C. Samples were precleared for 1 h at 4 °C on a rotator using protein G Dynabeads (Thermo Fisher Scientific). Chromatin was immunoprecipitated overnight on a rotator at 4 °C with antibodies conjugated to protein G Dynabeads. The following washes were performed twice for 15 min each by rotating at 4 °C: ChIP lysis buffer, ChIP lysis buffer plus 0.65 M NaCl, wash buffer (10 mM Tris–HCl, pH 8.0, 250 mM LiCl, 0.5% NP-30, 0.5% DOC, and mM EDTA, pH 8.0), and 10 mM Tris–HCl (pH 8.0) and 1 mM EDTA (pH 8.0). DNA was eluted by incubating the beads with 50 mM Tris–HCl (pH 8.0), 10 mM EDTA (pH 8.0), and 1% SDS for 30 min at 65 °C. Samples were incubated overnight at 65 °C and then digested (1 mg ml⁻¹ proteinase K at 37 °C for 5 h). DNA was purified (A9282; Promega) and analyzed by qPCR using PowerUp SYBR Green Master Mix (Applied Biosystems, A25742). Primer sets are in Supplementary Table 10.

Gene set enrichment analysis

Transcriptomic profiles of ovarian cancer cells incubated with autologous ascitic fluids from patients before and after chemotherapy was downloaded from GEO (GSE241909)²⁹. Gene set enrichment analysis (GSEA) was performed on the differential expression gene list using Metascape (Version V3.x) with the Wikipathways gene set database.

Metabolite measurement

Polar metabolites were measured by liquid chromatography-high resolution mass spectrometry adapted from previously published approaches⁶⁹. Conditioned media samples were snap frozen at –80 °C. Cell samples were quenched in 1 ml prechilled –80 °C 80:20 methanol/water (v/v) and spiked with 50 μl 1 μM isotope-labeled TCA cycle mix (Cambridge Isotope Laboratories MSK-TCA-A) prediluted in 80:20 methanol/water and 50 μl 0.02 ng μl^{-1} propionyl-L-carnitine-(N-methyl-d3) (Sigma Aldrich 52941). After vortexing for 1 min, samples were returned to –80 °C for 30 min and centrifuged at 18,000g for 10 min at 4 °C, and the supernatant was evaporated to dryness under nitrogen gas. Samples were reconstituted in 100 μl of 95:5 water:methanol (v/v), and 2 μl of the sample was injected from a 4 °C autosampler into a ZIC-PHILIC 150 × 2.1 mm 5 μm particle size column

(EMD Millipore) with a ZIC-pHILIC 20 × 2.1 guard column in a Vanquish Duo UHPLC System (Thermo Fisher Scientific) at 25 °C. Chromatography conditions were as follows: buffer A was acetonitrile; buffer B was 20 mM ammonium carbonate, 0.1% (v/v) ammonium hydroxide in water without pH adjustment, with a gradient of 0.5 min at 20% A, then a linear gradient from 20% to 80% A for 20–20.5 min, from 80% to 20% B for 20.5–28 min, and hold at 20% B at a 0.150 ml min⁻¹ flow rate. Column elute was introduced to a Q Exactive Plus with a HESI II probe operating in polarity switching mode with full scans from 70 to 1,000 *m/z* with an insource fragmentation energy of 1. Instruments were controlled via XCalibur 4.1, and data were analyzed on Tracefinder 5.1 using a 5 ppm window from the predominant ion positive or negative. The area under the curves for each analyte was normalized to the matched internal standard or the nearest surrogate internal standard. For isotope tracing, isotopologue enrichment was calculated using FluxFix (Version v0.1)⁷⁰.

Cholesterol was measured by liquid chromatography-high resolution mass spectrometry after liquid–liquid extraction. Cholesterols were extracted from cells by methyl tert-butyl ether (MTBE) extraction. Cells were pelleted and resuspended in 80% methanol/water. Samples were sonicated before transferring to a glass tube with the ratio 10:3:2.5 of MTBE/MeOH/0.9% NaCl. Samples were shaken for 30 min and centrifuged for 10 min at 1,000g at 4 °C. The organic layer was transferred to a clean tube and dried overnight under nitrogen. Samples were resuspended in 1:3 MTBE/water. ¹³C₅-cholesterol (Cambridge Isotope Laboratories) was used as an internal standard. A Vanquish UPLC system coupled to a Exploris 480 mass spectrometer with a H-ESI source was used for all cholesterol quantifications. Cholesterols were chromatographically separated using an Accucore C30 (Thermo Fisher Scientific 27826-152130) 150 mm × 2.1 mm, 2.6 μm partial size column. Solvent A was 10 mM ammonium formate in 6:4 acetonitrile/water and 0.1% formic acid. Solvent B was 10 mM ammonium formate in 88:10:2 isopropanol/acetonitrile/water. The flow rate was 0.26 ml min⁻¹. The method began at 30% B for 3 min, then increased to 43% B over 2 min and further increased to 55% B in 0.1 min. B was further increased to 65% over 10 min then further increased to 85% over 6 min. B was then increased to 100% over 2 min and maintained at 100% for 5 min. B was then decreased to 30% over 0.1 min and maintained at 30% B for 3 min. The mass spectrometer operating conditions were 3,500 V spray voltage, 400 °C ion transfer tube temperature, 200 °C vaporizer temperature, and positive ion polarity. Xcalibur was used for data acquisition and TraceFinder 5.1 was used for quantification.

Patient serum samples and clinical data

Pretreatment blood was collected from patients with newly diagnosed epithelial ovarian cancer (patient characteristics are available in Supplementary Table 12). Blood was collected in red-top tubes and centrifuged at 500g for 10 min, and serum was aliquoted and stored at –80 °C. Metabolite levels were assessed by Metabolon using the DiscoveryHD4 platform using three independent platforms: ultrahigh-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) in the negative ion mode, UPLC–MS/MS in the positive ion mode, and gas chromatography–mass spectrometry after sialylation. Compounds were identified based on chromatographic properties and mass spectra by comparing to a metabolomic library of purified standards. Clinical data were extracted from the electronic medical records by staff trained in medical record abstraction/coding (L.B.) and verified by comparison to the University of Pittsburgh Medical Center Hillman Cancer Center (UPMC/HCC) cancer registry database, which is accredited by the American College of Surgeons Committee on Cancer.

Statistics and reproducibility

GraphPad Prism (version 10.0) was used to perform statistical analysis. Point estimates with standard deviations or standard errors were reported, as indicated, and the appropriate statistical test was

performed using all observed experimental data. All statistical tests performed were two-sided, and *P* < 0.05 was considered statistically significant. Data distribution was assumed to be normal, but this was not formally tested. No statistical method was used to predetermine sample size. When necessary, outliers were identified and excluded by the Robust regression and Outlier removal (ROUT) method (*Q* = 0.1%). The investigators were not blinded to allocation during experiments and outcome assessment. In vitro experiments were not randomized. For animal studies, the number of animals assigned per condition was selected to account for the variability of the examined phenotypes based on pilot experiment and experience with the animal models. Animals were randomized into groups by random number generator before being assigned to a group for cancer cell implantation, treatment or diet. Investigators were not blinded to group allocation during experiments owing to technical limitations. Animals were excluded from analysis if they reached predetermined ethical end points defined in Institutional Animal Care and Use Committee protocols, if they did not develop tumors (measured by either IVIS imaging 7 days after implantation or omental weight compared to tumor-free omentum) or if they were determined to be statistical outliers using the ROUT method in GraphPad PRISM (version 10.0).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All source data, including western blots, are included with the paper. Raw data from the CRISPR KO screen can be found in Supplementary Table 5. RNA-seq data were deposited into the GEO database under accession number [GSE305033](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE305033) and [GSE305002](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE305002) and are available at the following URLs: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE305033> and <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE305002>. Publicly available datasets used in this study: bulk RNA-seq data: [GSE227100](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE227100) (refs. 20,21) and EGA dataset [EGAD00001006456](https://ega-archive.org/datasets/EGAD00001006456) (Fig. 1a); nanostring data: [GSE181597](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE181597) (ref. 71), [GSE201600](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE201600) (ref. 16) and [GSE319500](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE319500) (ref. 23) (Extended Data Fig. 1a); single-cell RNA-seq data: [GSE266577](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE266577) (ref. 26) (Fig. 1b and Extended Data Fig. 1b). Messenger RNA data extracted from TCGA Serous Ovarian Cancer (Nature, 2011) project were downloaded from cbioportal.org. Source data are provided with this paper.

References

- Hinterleitner, C. et al. Senescence in cancer: hallmarks, paradoxes, and therapeutic promise. *Cell* **189**, 2357–2378 (2026).
- Demaria, M. et al. Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. *Cancer Discov.* **7**, 165–176 (2017).
- Acosta, J. C. & Gil, J. Senescence: a new weapon for cancer therapy. *Trends Cell Biol.* **22**, 211–219 (2012).
- Krtolica, A., Parrinello, S., Lockett, S., Desprez, P.-Y. & Campisi, J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc. Natl Acad. Sci. USA* **98**, 12072–12077 (2001).
- Rodier, J. C. C. P. F. & Goldstein, Y. S. D. M. J. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol.* **6**, 2853–2868 (2008).
- Tsai, K. K., Chuang, E. Y.-Y., Little, J. B. & Yuan, Z.-M. Cellular mechanisms for low-dose ionizing radiation-induced perturbation of the breast tissue microenvironment. *Cancer Res.* **65**, 6734–6744 (2005).
- Alimirah, F. et al. Cellular senescence promotes skin carcinogenesis through p38MAPK and p44/42MAPK signaling. *Cancer Res.* **80**, 3606–3619 (2020).

8. James, E. L. et al. Senescent human fibroblasts show increased glycolysis and redox homeostasis with extracellular metabolomes that overlap with those of irreparable DNA damage, aging, and disease. *J. Proteome Res.* **14**, 1854–1871 (2015).
9. James, E. N. L., Bennett, M. H. & Parkinson, E. K. The induction of the fibroblast extracellular senescence metabolome is a dynamic process. *Sci. Rep.* **8**, 12148 (2018).
10. Kamal, M. et al. Senescent myoblasts exhibit an altered exometabolome that is linked to senescence-associated secretory phenotype signaling. *Am. J. Physiol. Cell Physiol.* **328**, C440–C451 (2025).
11. Wiley, C. D. & Campisi, J. The metabolic roots of senescence: mechanisms and opportunities for intervention. *Nat. Metab.* **3**, 1290–1301 (2021).
12. Aird, K. M. et al. ATM couples replication stress and metabolic reprogramming during cellular senescence. *Cell Rep.* **11**, 893–901 (2015).
13. Paffenholz, S. V. et al. Senescence induction dictates response to chemo- and immunotherapy in preclinical models of ovarian cancer. *Proc. Natl Acad. Sci. USA* **119**, e2117754119 (2022).
14. Della Pepa, C. et al. Ovarian cancer standard of care: are there real alternatives? *Chin. J. Cancer* **34**, 17–27 (2015).
15. Bowtell, D. D. et al. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nat. Rev. Cancer* **15**, 668–679 (2015).
16. Jordan, K. R. et al. The capacity of the ovarian cancer tumor microenvironment to integrate inflammation signaling conveys a shorter disease-free interval. *Clin. Cancer Res.* **26**, 6362–6373 (2020).
17. Davidson, B. et al. Proteomic analysis of malignant ovarian cancer effusions as a tool for biologic and prognostic profiling. *Clin. Cancer Res.* **12**, 791–799 (2006).
18. Amate, P. et al. Ovarian cancer: sites of recurrence. *Int. J. Gynecol. Cancer* **23**, 1590–1596 (2013).
19. Tan, D. S., Agarwal, R. & Kaye, S. B. Mechanisms of transcoelomic metastasis in ovarian cancer. *Lancet Oncol.* **7**, 925–934 (2006).
20. Adzibolosu, N. et al. Immunological modifications following chemotherapy are associated with delayed recurrence of ovarian cancer. *Front. Immunol.* **14**, 1204148 (2023).
21. Javellana, M. et al. Neoadjuvant chemotherapy induces genomic and transcriptomic changes in ovarian cancer. *Cancer Res.* **82**, 169–176 (2022).
22. James, N. E. et al. Adaptive transcriptomic and immune infiltrate responses in the tumor immune microenvironment following neoadjuvant chemotherapy in high grade serous ovarian cancer reveal novel prognostic associations and activation of pro-tumorigenic pathways. *Front. Immunol.* **13**, 2022 (2022).
23. Lodewijk, I. et al. Genomic landscape and immune-related gene expression profiling of epithelial ovarian cancer after neoadjuvant chemotherapy. *npj Precis. Oncol.* **6**, 7 (2022).
24. Van Kleunen, L. B. et al. On the predictability of progression-free survival in ovarian cancer from NanoString gene expression data. Preprint at *bioRxiv* <https://doi.org/10.64898/2026.04.22.719856> (2026).
25. Townsend, H. A. et al. Cross-assay RNA modeling reveals cancer biomarkers. Preprint at *bioRxiv* <https://doi.org/10.64898/2026.04.30.722009> (2026).
26. Launonen, I.-M. et al. Chemotherapy induces myeloid-driven spatially confined T cell exhaustion in ovarian cancer. *Cancer Cell* **42**, 2045–2063.e10 (2024).
27. Dhaliwal, D. & Shepherd, T. G. Molecular and cellular mechanisms controlling integrin-mediated cell adhesion and tumor progression in ovarian cancer metastasis: a review. *Clin. Exp. Metastasis* **39**, 291–301 (2022).
28. Moss, N. M. et al. Ovarian cancer cell detachment and multicellular aggregate formation are regulated by membrane type 1 matrix metalloproteinase: a potential role in l.p. metastatic dissemination. *Cancer Res.* **69**, 7121–7129 (2009).
29. Shender, V. O. et al. Therapy-induced secretion of spliceosomal components mediates pro-survival crosstalk between ovarian cancer cells. *Nat. Commun.* **15**, 5237 (2024).
30. Ghisoni, E. et al. Myeloid cell networks govern re-establishment of original immune landscapes in recurrent ovarian cancer. *Cancer Cell* **43**, 1568–1586.e10 (2025).
31. Wirth, C., Brandt, U., Hunte, C. & Zickermann, V. Structure and function of mitochondrial complex I. *Biochim. Biophys. Acta* **1857**, 902–914 (2016).
32. Hoque, M. et al. The cross-talk of LDL-cholesterol with cell motility: insights from the Niemann Pick Type C1 mutation and altered integrin trafficking. *Cell Adh. Migr.* **9**, 384–391 (2015).
33. Lietha, D. & Izard, T. Roles of membrane domains in integrin-mediated cell adhesion. *Int. J. Mol. Sci.* **21**, 5531 (2020).
34. Yokoyama, C. et al. SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. *Cell* **75**, 187–197 (1993).
35. Defour, A. et al. Sirtuin 1 regulates SREBP-1c expression in a LXR-dependent manner in skeletal muscle. *PLoS ONE* **7**, e43490 (2012).
36. Tao, R., Xiong, X., DePinho, R. A., Deng, C. X. & Dong, X. C. Hepatic SREBP-2 and cholesterol biosynthesis are regulated by FoxO3 and Sirt6. *J. Lipid Res.* **54**, 2745–2753 (2013).
37. Shen, S. et al. SIRT1/SREBPs-mediated regulation of lipid metabolism. *Pharmacol. Res.* **199**, 107037 (2024).
38. Kaplon, J. et al. A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. *Nature* **498**, 109–112 (2013).
39. Li, M. et al. Oncogene-induced cellular senescence elicits an anti-Warburg effect. *Proteomics* **13**, 2585–2596 (2013).
40. Yabe-Nishimura, C. Aldose reductase in glucose toxicity: a potential target for the prevention of diabetic complications. *Pharmacol. Rev.* **50**, 21–33 (1998).
41. González, R. G., Barnett, P., Aguayo, J., Cheng, H.-M., Chylack, L. Jr Direct measurement of polyol pathway activity in the ocular lens. *Diabetes* **33**, 196–199 (1984).
42. Kang, Y.-L. et al. The polyol pathway and nuclear ketohexokinase A signaling drive hyperglycemia-induced metastasis of gastric cancer. *Exp. Mol. Med.* **56**, 220–234 (2024).
43. Fang, J. H. et al. Fructose metabolism in tumor endothelial cells promotes angiogenesis by activating AMPK signaling and mitochondrial respiration. *Cancer Res.* **83**, 1249–1263 (2023).
44. Coppé, J.-P. et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLOS Biol.* **6**, e301 (2008).
45. Ohuchida, K. et al. Radiation to stromal fibroblasts increases invasiveness of pancreatic cancer cells through tumor-stromal interactions. *Cancer Res.* **64**, 3215–3222 (2004).
46. Veenstra, J. P., Bittencourt, L. F. F. & Aird, K. M. The senescence-associated secretory phenotype in ovarian cancer dissemination. *Am. J. Physiol. Cell Physiol.* **323**, C125–C132 (2022).
47. Gonzalez-Avila, G. et al. Matrix metalloproteinases participation in the metastatic process and their diagnostic and therapeutic applications in cancer. *Crit. Rev. Oncol. Hematol.* **137**, 57–83 (2019).
48. Wang, N. et al. Senescent cells promote breast cancer cells motility by secreting GM-CSF and bFGF that activate the JNK signaling pathway. *Cell Commun. Signal.* **22**, 478 (2024).
49. Liu, D. & Hornsby, P. J. Senescent human fibroblasts increase the early growth of xenograft tumors via matrix metalloproteinase secretion. *Cancer Res.* **67**, 3117–3126 (2007).

50. Huang, N. N., Wang, D. J. & Heppel, L. A. Stimulation of aged human lung fibroblasts by extracellular ATP via suppression of arachidonate metabolism. *J. Biol. Chem.* **268**, 10789–10795 (1993).
 51. Lu, S. Y. et al. Ripe areca nut extract induces G1 phase arrests and senescence-associated phenotypes in normal human oral keratinocyte. *Carcinogenesis* **27**, 1273–1284 (2006).
 52. van der Loo, B. et al. Enhanced peroxynitrite formation is associated with vascular aging. *J. Exp. Med.* **192**, 1731–1744 (2000).
 53. Ferraris, R. P., Choe, J. Y. & Patel, C. R. Intestinal absorption of fructose. *Annu. Rev. Nutr.* **38**, 41–67 (2018).
 54. Zhao, S. et al. Dietary fructose feeds hepatic lipogenesis via microbiota-derived acetate. *Nature* **579**, 586–591 (2020).
 55. Fowle-Grider, R. et al. Dietary fructose enhances tumour growth indirectly via interorgan lipid transfer. *Nature* **636**, 737–744 (2024).
 56. Bezawada, D. et al. Mitochondrial complex I promotes kidney cancer metastasis. *Nature* **633**, 923–931 (2024).
 57. Dobson, S. M. et al. Relapse-fated latent diagnosis subclones in acute B lineage leukemia are drug tolerant and possess distinct metabolic programs. *Cancer Discov.* **10**, 568–587 (2020).
 58. Farge, T. et al. Chemotherapy-resistant human acute myeloid leukemia cells are not enriched for leukemic stem cells but require oxidative metabolism. *Cancer Discov.* **7**, 716–735 (2017).
 59. Fischer, G. M. et al. Molecular profiling reveals unique immune and metabolic features of melanoma brain metastases. *Cancer Discov.* **9**, 628–645 (2019).
 60. Shelton, S. D. et al. Pathogenic mitochondrial DNA mutations inhibit melanoma metastasis. *Sci. Adv.* **10**, eadk8801 (2024).
 61. Viale, A. et al. Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature* **514**, 628–632 (2014).
 62. Dorsch, M. et al. Statins affect cancer cell plasticity with distinct consequences for tumor progression and metastasis. *Cell Rep.* **37**, 110056 (2021).
 63. Kirkland, J. L. & Tchkonja, T. Senolytic drugs: from discovery to translation. *J. Intern. Med.* **288**, 518–536 (2020).
 64. Yoshino, J., Baur, J. A. & Imai, S. I. NAD⁺ intermediates: the biology and therapeutic potential of NMN and NR. *Cell Metab.* **27**, 513–528 (2018).
 65. Nacarelli, T. et al. NAD⁺ metabolism governs the proinflammatory senescence-associated secretome. *Nat. Cell Biol.* **21**, 397–407 (2019).
 66. Uphoff, C. C. & Drexler, H. G. Detection of mycoplasma contaminations. *Methods Mol. Biol.* **290**, 13–23 (2005).
 67. Li, W. et al. MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. *Genome Biol.* **15**, 554 (2014).
 68. Pachitariu, M., Rariden, M. & Stringer, C. Cellpose-SAM: superhuman generalization for cellular segmentation. Preprint at *bioRxiv* <https://doi.org/10.1101/2025.04.28.651001> (2025).
 69. Petrova, B. et al. Redox metabolism measurement in mammalian cells and tissues by LC-MS. *Metabolites* **11**, 313 (2021).
 70. Trefely, S., Ashwell, P. & Snyder, N. W. FluxFix: automatic isotopologue normalization for metabolic tracer analysis. *BMC Bioinformatics* **17**, 485 (2016).
 71. James, N. E. et al. Adaptive transcriptomic and immune infiltrate responses in the tumor immune microenvironment following neoadjuvant chemotherapy in high grade serous ovarian cancer reveal novel prognostic associations and activation of pro-tumorigenic pathways. *Front. Immunol.* **13**, 965331 (2022).
- the CRISPR library, and J. Cassel (The Wistar Institute Cancer Center Support Grant Molecular Screening and Protein Expression Facility) for help providing shRNA constructs. We acknowledge the support of the Metabolomics and Lipidomics Core Facility at the Lewis Katz School of Medicine at Temple University. This project used the UPMC/HCC Animal Facility, Cancer Genomics Facility, Tissue and Research Pathology/Pitt Biospecimen Core, Biostatistics Core, and the Cancer Bioinformatics Services that are supported in part by award P30CA047904, and The Wistar Institute Imaging Facility, Bioinformatics Facility, Molecular Screening and Protein Expression Facility, and Animal Facility are supported in part by P30CA010815.

Author contributions

A.R.C.: conceptualization, investigation, methodology, visualization, writing—original draft, visualization, writing—review and editing, and funding acquisition. R.B.: investigation, methodology, and writing—review and editing. A.U.: investigation, methodology, and writing—review and editing. E.L.: investigation. A.T.: investigation. H.W.: investigation. K.M.K.: investigation and methodology. A. Chatoff: investigation and methodology. A.A.H.: investigation and methodology. M.M.M.: investigation and methodology. A.A.: investigation, methodology, and writing—review and editing. B.Y.: investigation and writing—review and editing. N.K.T.: investigation and writing—review and editing. E.N.: investigation. J.D.: investigation. M.N.: investigation. A.T.E.: investigation and methodology. S.W.: investigation and methodology. D.L.: investigation and methodology. C.T.W.: investigation and methodology. F.K.: investigation and methodology. F.L.: investigation. E.E.: resources. L.B.: resources. H.A.T.: investigation and methodology. R.D.D.: methodology and supervision. A. Clauset: methodology and supervision. D.E.J.: investigation and methodology. M.S.L.: investigation and methodology. H.A.: methodology. D.D.: writing—review and editing. L.G.C.: methodology and supervision. G.T.: supervision. S.O.: methodology and writing—review and editing. A.K.: investigation and methodology. Q.L.: investigation and methodology. A.P.G.: supervision and writing—review and editing. A.U.G.: methodology, supervision, and writing—review and editing. F.J.S.: methodology, supervision, and writing—review and editing. F.M.: resources and supervision. S.C.W.: investigation, methodology, and supervision. I.K.Z.: investigation, methodology, and supervision. B.G.B.: resources, supervision, and writing—review and editing. W.S.: investigation, methodology, and supervision. N.H.: investigation, methodology, supervision, and writing—review and editing. N.W.S.: investigation, methodology, supervision, and writing—review and editing. K.M.A.: conceptualization, visualization, writing—original draft, writing—review and editing, supervision, project administration, and funding acquisition.

Funding

A.R.C. discloses support for this research from the National Institutes of Health (T32GM133332) to support his dissertation research. R.B. discloses support for this research from the National Institutes of Health (P50CA272218) to support in vivo ovarian cancer studies (Figs. 1 and 6) and the Melanoma Research Foundation to support melanoma studies. A.U. discloses support for this research from the Ovarian Cancer Research Alliance (MIG-2023-2-1018) and the HERA Ovarian Cancer Foundation to support in vitro and in vivo ovarian cancer studies. K.M.K. discloses support for this research from the Chan Zuckerberg Initiative DAF, an advised fund of Silicon Valley Community Foundation (2023-329680) to support image analysis (Figs. 3–5). A.A. discloses support for this research from the HERA Ovarian Cancer Foundation to support in vitro and in vivo ovarian cancer studies. N.K.T. discloses support for this research from the HERA Ovarian Cancer Foundation and the Congressionally Directed Medical Research Program (OC230324) to support in vitro

Acknowledgements

We thank U. Chandran and J. Wang (UPMC/HCC Bioinformatics Core) for help with bioinformatic analysis of the CRISPR screen, M. Lyons (UPMC/HCC Cancer Genomics Core) for help with sequencing of

and in vivo ovarian cancer studies. L.G.C. discloses support for this research from the National Institutes of Health (U01AG077923) and the Congressionally Directed Medical Research Program (OC210139) for conceptual support related to aging and the clinical course of ovarian cancer. F.M. discloses support for this research from the National Institutes of Health (R21CA267050 and R21CA291905) and the Janet Burroughs Ovarian Cancer Foundation to assess circulating fructose (Fig. 6g) and the Congressionally Directed Medical Research Program (W81XWH2110338) to support collection of samples. N.H. discloses support for this research from the National Institutes of Health (R01CA242021) to assess ovarian cancer spheroids (Extended Data Figs. 3 and 4). N.W.S. discloses support for this research from the National Institutes of Health (R01CA259111) to support pilot studies for metabolite assessment of ovarian cancer samples (pilot experiments for Extended Data Figs. 6 and 8) and for M.M.M. in the Snyder lab, and R01CA298386 to support metabolite assessment of ovarian cancer samples (Fig. 5 and Extended Data Fig. 8) and A.A.H. and A. Chatoff in the Snyder lab. K.M.A. discloses support for this research from the National Institutes of Health (R37CA240625) to study senescence in melanoma models in Extended Data Figs. 4 and 8 and support R.B. and N.K.T. in the Aird lab; R01CA259111 to support 3D ovarian cancer spheroid studies (Extended Data Fig. 3), senescence induction in ovarian cancer models (Extended Data Fig. 1), and pilot studies for metabolite assessment of ovarian cancer samples (pilot experiments for Extended Data Figs. 6 and 8) and A.U. in the Aird lab; and R01CA298386 to support the assessment of KPCA.B cells in vivo (pilot studies for Figs. 1 and 6), immunohistochemistry of ovarian cancer tumors (Fig. 1), and metabolite assessment of ovarian cancer samples (Fig. 5 and Extended Data Fig. 8) and A.U. and M.N. in the Aird lab), the American Cancer Society (RSG-19-113-01-CCG) to support CHIP and RNA-seq studies, the Congressionally Directed Medical Research Program (HT9425-23-1-0436) to support studies related to the ovarian cancer SASP (all figures), and institutional funds from both the UPMC/HCC and The Wistar Institute. E.L., A.T., H.W., A. Chatoff, A.A.H., M.M.M., B.Y., E.N., J.D., M.N., A.T.E., S.W., D.L., C.T.W., F.K., F.L., E.E., L.B., H.A.T., R.D.D., A. Clauset, D.E.J., M.S.L., H.A., D.D., G.T., S.O., A.K., Q.L., A.P.G., A.U.G., F.J.S., S.C.W., I.K.Z., B.G.B., and W.S. declare no relevant funding. Authors with foreign affiliations (D.D.) received no funding, salary support, resources, materials, or any other form of support from the National Institutes of Health awards or other federal funds listed above and did not participate in any activities supported by these awards.

Competing interests

The authors declare no competing interests.

Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s43587-026-01172-5>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s43587-026-01172-5>.

Correspondence and requests for materials should be addressed to Katherine M. Aird.

Peer review information *Nature Aging* thanks the anonymous reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

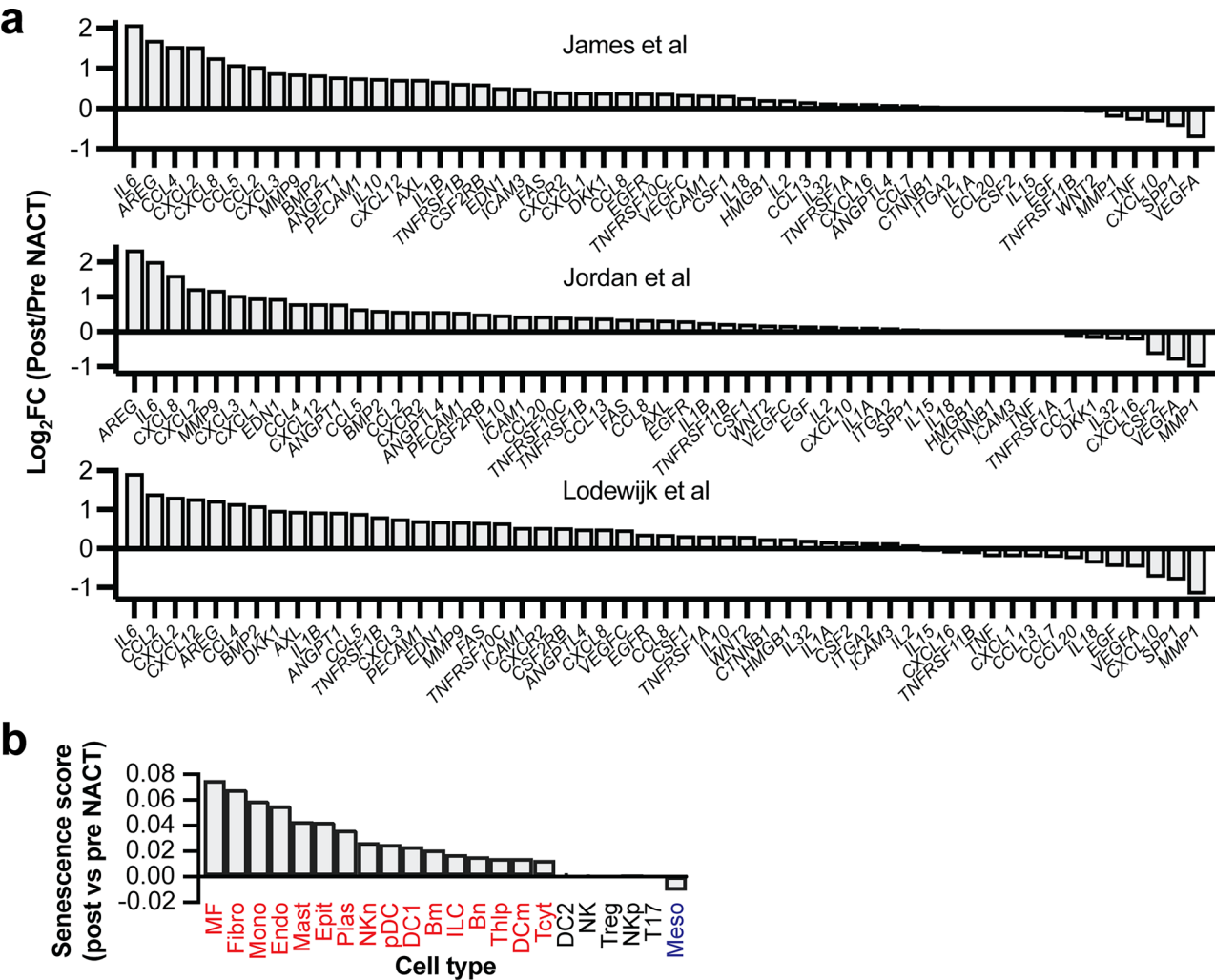
Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2026

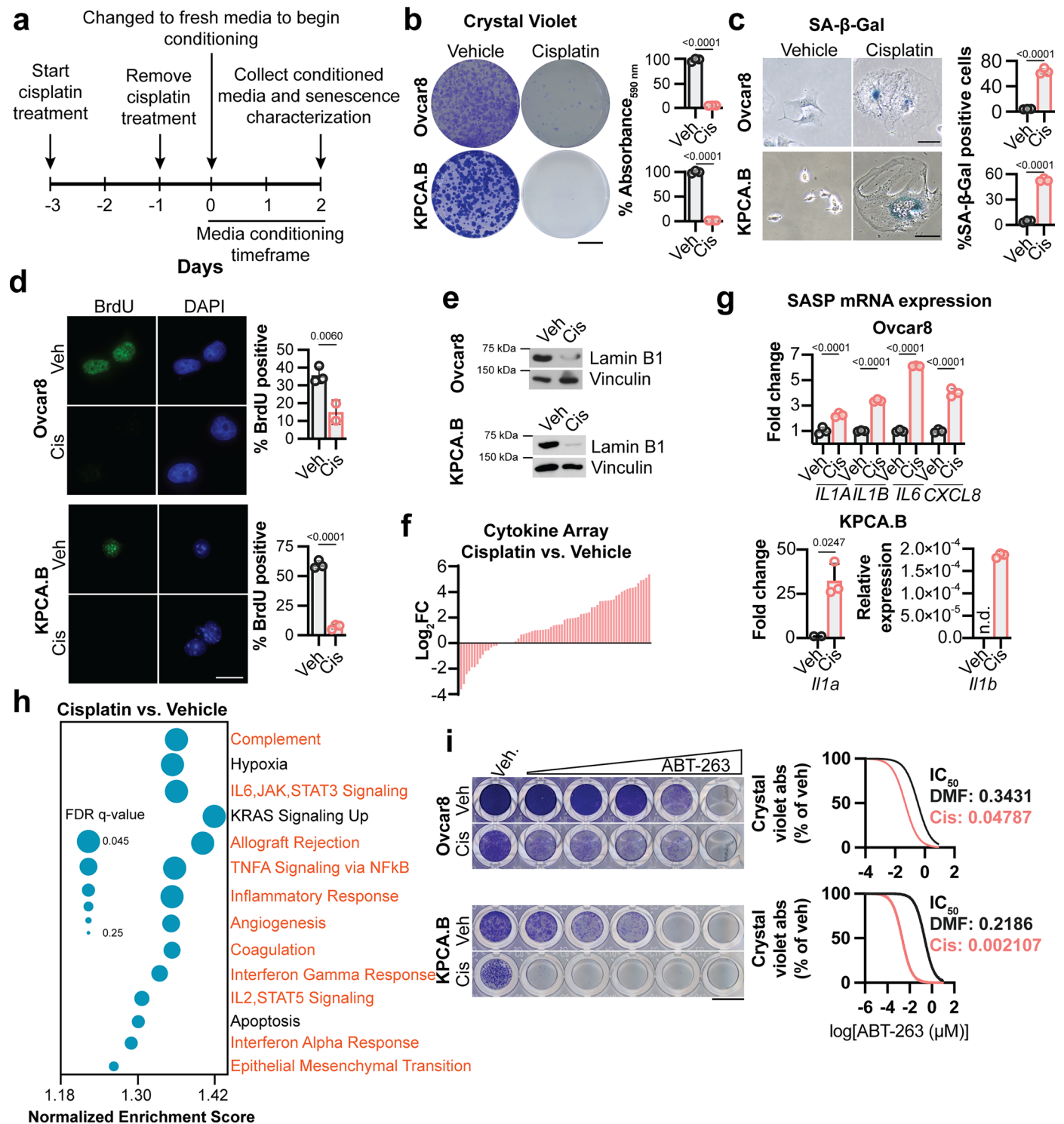
Aidan R. Cole^{1,2}, **Raquel Buj**^{1,2}, **Apoorva Uboveja**^{1,2}, **Evan Levasseur**², **Alexander Tom**¹, **Hui Wang**², **Katarzyna M. Kedziora**³, **Adam Chatoff**⁴, **Andrea Andress Huacachino**⁴, **Mariola M. Marcinkiewicz**⁴, **Amandine Amalric**^{1,2}, **Baixue Yang**^{1,2,23}, **Naveen Kumar Tangudu**², **Emily Noonan**⁴, **Jeff Danielson**², **Miho Naruse**^{1,2}, **Amal Taher Elhaw**⁵, **Sierra White**⁵, **Danyang Li**⁶, **Callen T. Wallace**³, **Frederick Keeney**¹, **Felicia Lazure**⁷, **Esther Elishaev**⁸, **Lauren Borho**⁹, **Hope A. Townsend**^{10,11,12}, **Robin D. Dowell**^{10,11,12}, **Aaron Clauset**^{12,13}, **Dorota E. Jazwinska**¹⁴, **Matthew S. Laird**¹⁴, **Huda Atiya**¹⁵, **Denarda Dangaj**¹⁶, **Lan G. Coffman**¹⁵, **George Tseng**⁶, **Steffi Oesterreich**², **Andrew Kossenkov**¹⁷, **Qin Liu**¹, **Ana P. Gomes**⁷, **Aditi U. Gurkar**¹⁸, **Francisco J. Schopfer**^{19,20}, **Francesmary Modugno**⁸, **Simon C. Watkins**³, **Ioannis K. Zervantonakis**¹⁴, **Benjamin G. Bitler**²¹, **Wayne Stallaert**²², **Nadine Hempel**⁵, **Nathaniel W. Snyder**⁴ & **Katherine M. Aird**^{1,2} ✉

¹Molecular and Cellular Oncogenesis Program, Ellen and Ronald Caplan Cancer Center, The Wistar Institute, Philadelphia, PA, USA. ²Department of Pharmacology & Chemical Biology and UPMC Hillman Cancer Center, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ³Department of Cell Biology, Center for Biologic Imaging, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ⁴Aging + Cardiovascular Discovery Center, Department of Cardiovascular Sciences, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA. ⁵Department of Medicine and UPMC Hillman Cancer Center, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ⁶Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA, USA. ⁷Department of Molecular Oncology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL, USA. ⁸Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ⁹Department of Obstetrics, Gynecology and Reproductive Sciences and Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ¹⁰BioFrontiers Institute, University of Colorado Boulder, Boulder, CO, USA. ¹¹Department of Molecular, Cellular and Developmental Biology, University of Colorado Boulder, Boulder, CO, USA. ¹²Department of

Computer Science, University of Colorado Boulder, Boulder, CO, USA. ¹³Santa Fe Institute, Santa Fe, NM, USA. ¹⁴Department of Bioengineering and UPMC Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA, USA. ¹⁵Division of Malignant Hematology & Medical Oncology, Department of Medicine, UPMC Hillman Cancer Center, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ¹⁶Ludwig Institute for Cancer Research, University of Lausanne (UNIL), Lausanne, Switzerland. ¹⁷Genome Regulation and Cell Signaling Program, Ellen and Ronald Caplan Cancer Center, The Wistar Institute, Philadelphia, PA, USA. ¹⁸Aging Institute of UPMC and the Division of Geriatric Medicine, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ¹⁹Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ²⁰Pittsburgh Heart, Lung, Blood and Vascular Medicine Institute (VMI); Pittsburgh Liver Research Center (PLRC); and Center for Immunometabolism, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ²¹Department of Obstetrics and Gynecology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA. ²²Department of Computational and Systems Biology and UPMC Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA, USA. ²³Present address: Tsinghua University School of Medicine, Beijing, People's Republic of China. ✉e-mail: kaird@wistar.org



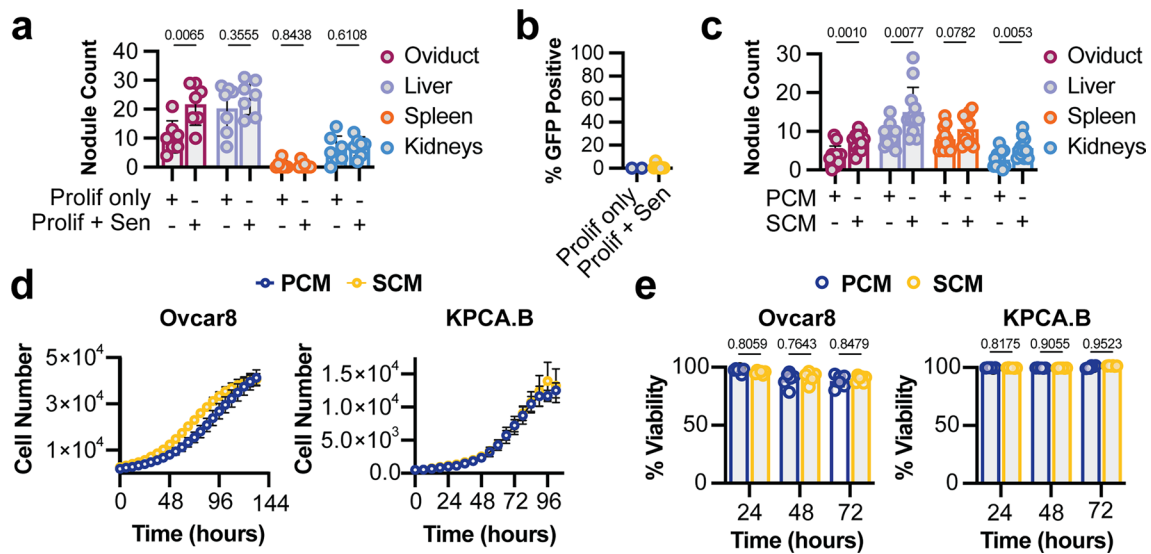
Extended Data Fig. 1 | Upregulation of senescence-associated genes in patients receiving neoadjuvant chemotherapy. (a) Log₂ fold change of senescence associated genes (from SenMayo geneset) present in NanoString analysis of patient tumors after neoadjuvant chemotherapy compared to matched pre-treatment tumor. (b) Senescence score in different cellular compartments after neoadjuvant chemotherapy.



Extended Data Fig. 2 | Confirmation of cisplatin-induced senescence in HGSOc models.

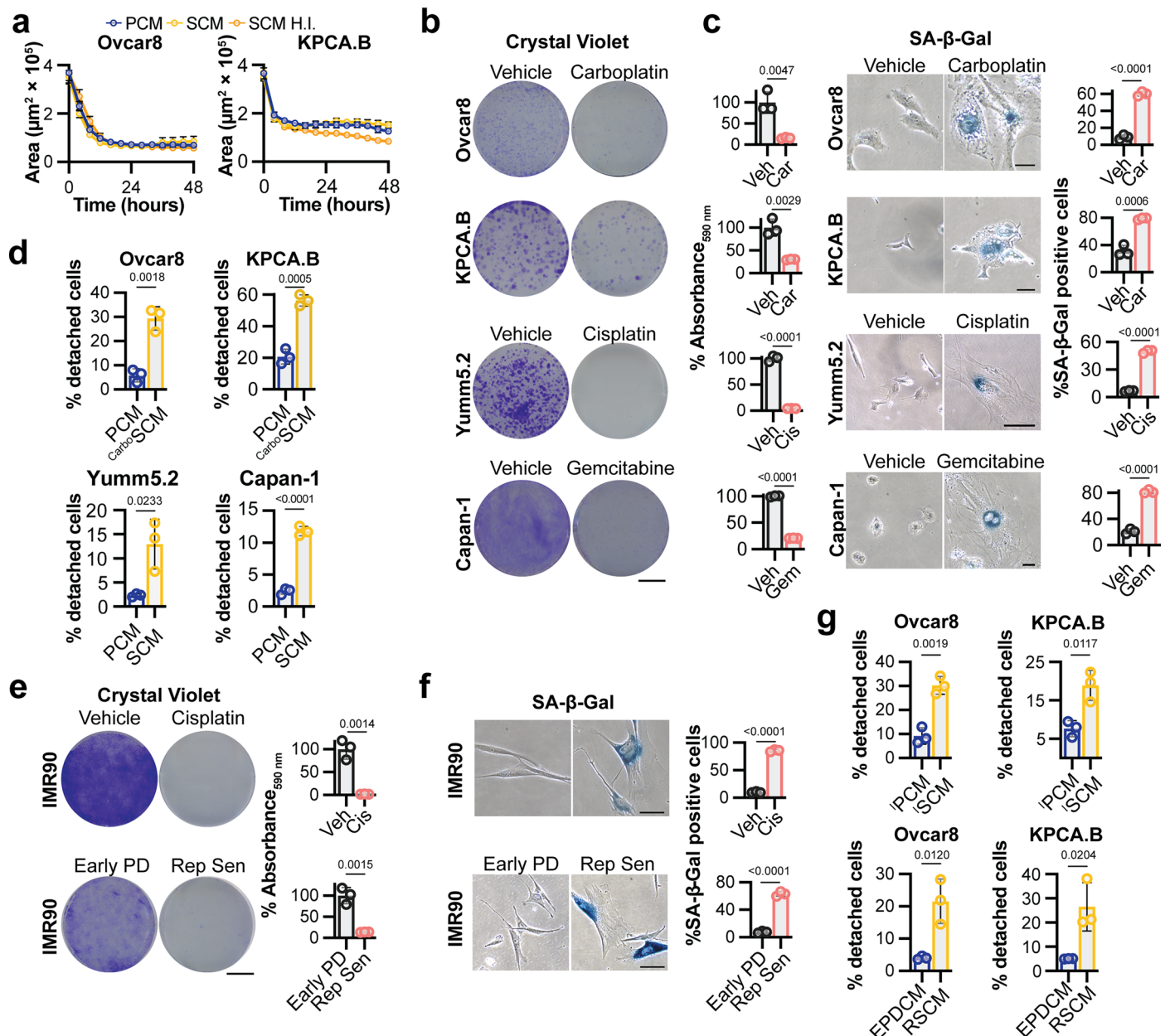
(a) Timeline of senescence induction experiments indicating when conditioned media was harvested and senescence markers were characterized. (b-e) The indicated cells were treated with cisplatin as in (a). (b) Cells were stained with crystal violet after 7-10 days in culture (left: representative images, scale bar = 10 mm; right: quantification). (n = 3 technical replicates representative from 3 independent experiments in each cell line). (c) Senescence-associated-beta-galactosidase (SA-β-Gal) activity (left: representative images, scale bar = 25 μm, 20X magnification; right: quantification, n = 3 technical replicates representative from 3 independent experiments in each cell line). (d) BrdU incorporation in the indicated cells (left: representative images, scale bar = 10 μm, 40X magnification; right: quantification, n = 3 technical replicates representative from 2 independent experiments in each cell line).

(e) Western blot of Lamin B1. Vinculin was used as a loading control. Representative from 3 independent experiments in each cell line. (f) Waterfall plots of cytokine secretion in conditioned media from Ovcar8 cells treated with cisplatin (1 independent experiment). (g) Relative expression of SASP genes by qPCR (n = 3 technical replicates representative from 3 independent experiments in each cell line). n.d. = not detected. (h) GSEA analysis enriched pathways of RNA-Seq data from Ovcar8 cells treated with cisplatin. Pathways highlighted in orange are related pathways associated with the SASP. (i) Cisplatin-induced senescent cells were subsequently treated with increasing doses of the senolytic ABT-263 for 4 days post senescence induction. Cells were stained with crystal violet (left: representative images, scale bar = 6 mm; right: dose response curves). n = 3 technical replicates representative from 2 independent experiments in each cell line. All graphs show mean ± SD unless otherwise indicated. Two-tailed student's t-test.



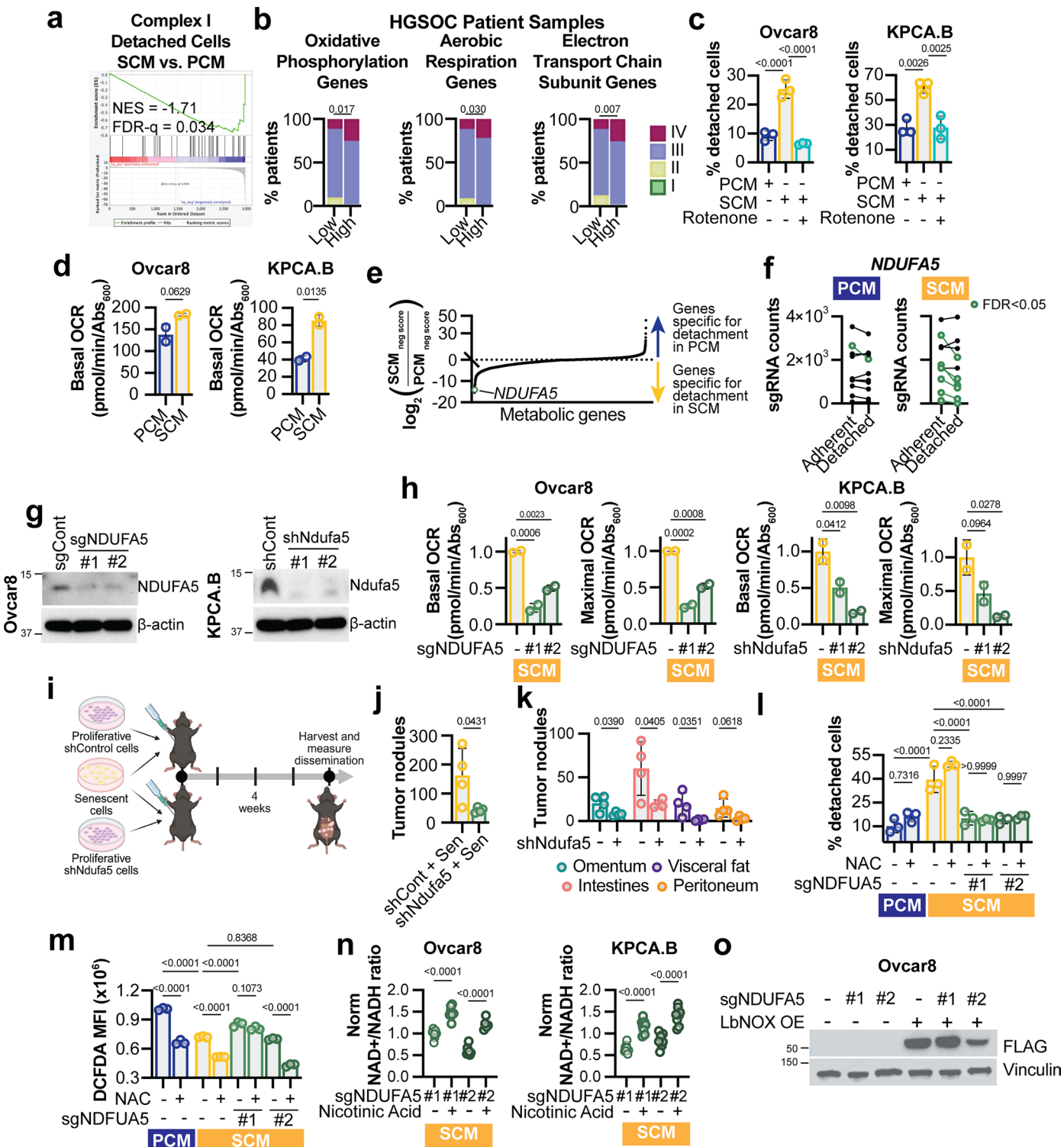
Extended Data Fig. 3 | The cisplatin-induced SASP does not affect proliferation or anoikis of bystander HGSOc cells. (a) KPCA.B HGSOc cells were treated with vehicle or induced to senesce using cisplatin. Cisplatin-induced senescent cells stably express GFP. Vehicle treated or cisplatin-induced senescent cells were then co-injected intraperitoneally with naïve proliferating KPCA.B HGSOc cells. The same number of proliferating cells were injected into mice in each group, and the senescent cells were injected with naïve proliferating cells at a 1:1 ratio. Prolif only = vehicle treated cells co-injected with naïve proliferating cells. Prolif + Sen = cisplatin-induced senescent cells co-injected with naïve proliferating cells. Shown are endpoint tumor nodules in the indicated sites. $n = 7$ mice/group. (b) % total cells labeled with GFP recovered from disaggregated Prolif + Sen omental tumors from (a). Two Prolif only tumors were used as negative controls. (c) KPCA.B HGSOc cells were treated with vehicle or induced to senesce using cisplatin, and conditioned media was harvested. PCM = proliferative conditioned

media, conditioned media harvested from vehicle treated cells. SCM = senescent conditioned media, conditioned media harvested from cisplatin-induced senescent cells. KPCA.B HGSOc cells were injected intraperitoneally into mice, and conditioned media was injected 3X per week. Shown are endpoint tumor nodules in the indicated sites. $n = 12$ mice/group. (d) Proliferation curves of the indicated bystander cells cultured in PCM or SCM. Ovar8: $n = 3$; KPCA.B: $n = 6$; technical replicates representative from 3 independent experiments in each cell line. (e) The indicated bystander cells in ultra-low attachment plates were cultured in PCM or SCM, and viability was assessed using fluorescent imaging of calcein AM (live cell probe) and ethidium homodimer (dead cell probe). ($n = 6$ technical replicates representative from 3 independent experiments in each cell line). All graphs show mean $\pm$ SD unless otherwise indicated. (a, c) Two-tailed Student's t -test; (e) One-way ANOVA followed by Šidák's multiple comparisons test.



Extended Data Fig. 4 | The SASP from other senescence models also increased bystander cell detachment. (a) Spheroid compaction of HGSOc bystander cells cultured in proliferative conditioned media (PCM), senescent conditioned media (SCM), or heat inactivated SCM (SCM H.I.) in ultra-low attachment round bottom plates (n = 6 technical replicates representative from 3 independent experiments in each cell line). (b-c) Cells treated with cisplatin, carboplatin, or gemcitabine following the timeline in Extended Data Fig. 2a. (b) Cells were stained with crystal violet (left: representative images, scale bar = 10 mm.; right: quantification, n = 3 technical replicates representative from 3 independent experiments in each cell line). (c) Senescence-associated-beta-galactosidase (SA-β-Gal) activity (left: representative images, scale bar = 25 μm, 20X magnification; right: quantification, n = 3 technical replicates representative from 2 independent experiments in each cell line). (d) Bystander cells were cultured in PCM or SCM (CarboSCM = media from carboplatin-induced senescent cells). After 72 h, % detachment was assessed by the controlled trypsin assay (n = 3 technical

replicates representative from 2 independent experiments in each cell line). (e-f) Normal IMR90 fibroblasts treated with cisplatin to induce senescence following the timeline in Extended Data Fig. 2a or passaged to replicative senescence (Rep Sen; controls = Early PD). (e) Cells were stained with crystal violet (left: representative images, scale bar = 10 mm.; right: quantification, n = 3 technical replicates representative from 3 independent experiments in each cell line). (f) SA-β-Gal activity (left: representative images, scale bar = 25 μm, 20X magnification; right: quantification, n = 3 technical replicates representative from 3 independent experiments in each cell line). (g) Bystander HGSOc cells were cultured in PCM or SCM for cisplatin induced IMR90 fibroblasts (PCM or SCM) or from early PD conditioned media (EPDCM) or replicative senescent (RSCM) IMR90 fibroblasts. After 72 h, % detachment was assessed by the controlled trypsin assay (n = 3 technical replicates representative from 3 independent experiments in each cell line). All graphs show mean ± SD unless otherwise indicated. Two-tailed student's t-test.

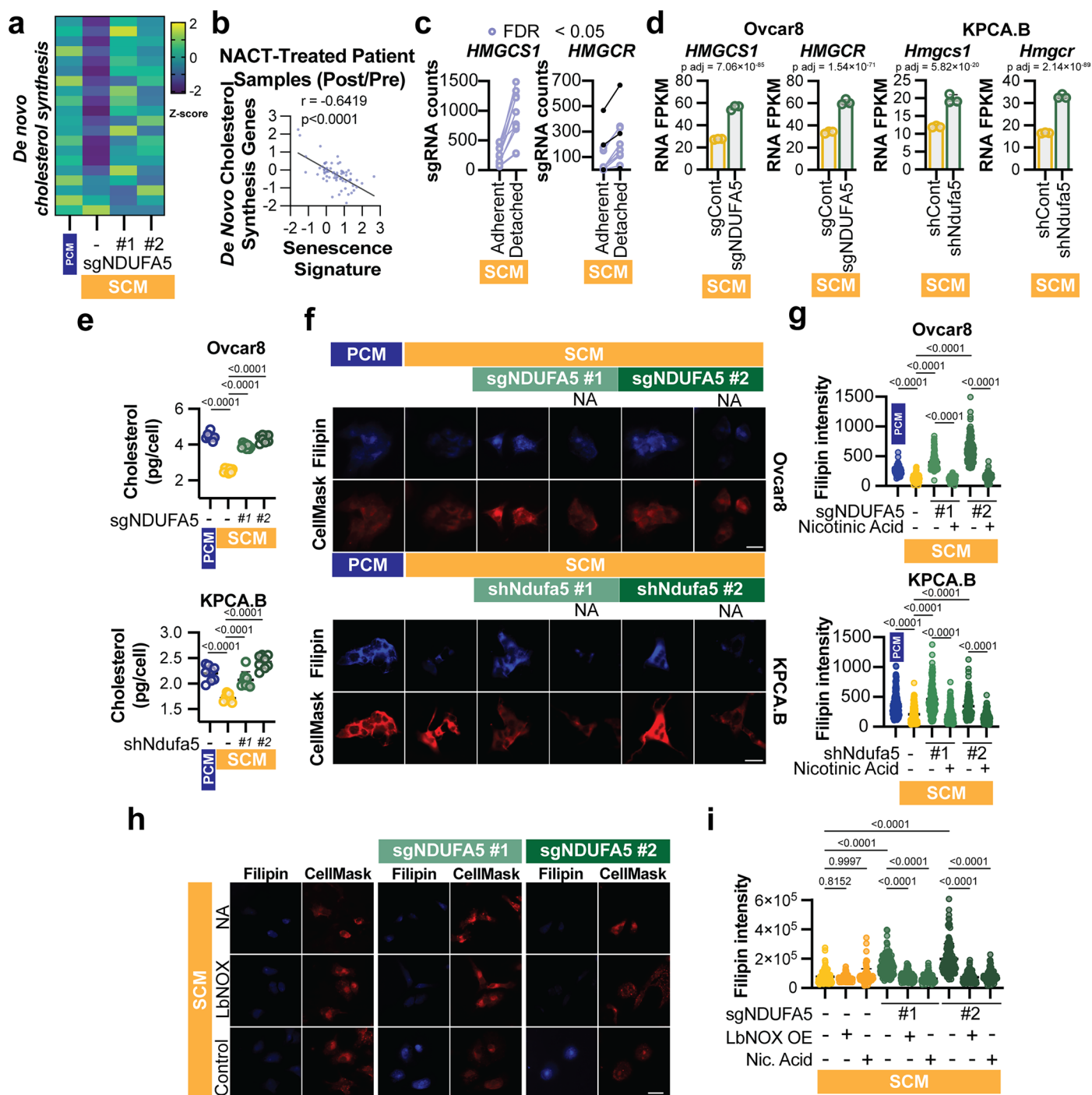


Extended Data Fig. 5 | See next page for caption.

Extended Data Fig. 5 | Validation of the CRISPR KO screen in multiple models.

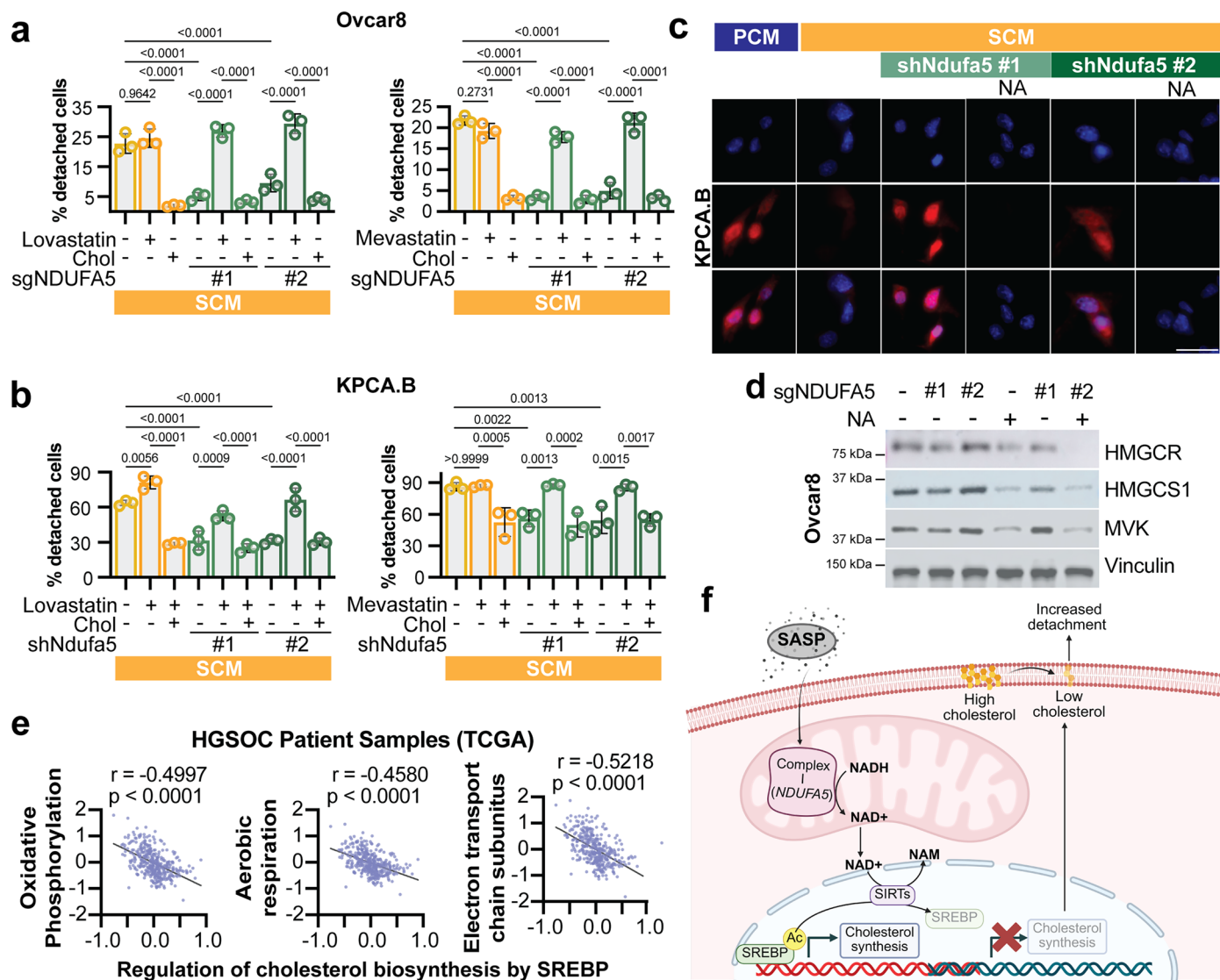
(a) Gene set enrichment analysis of the complex I submit gene set in detached cells cultured in SCM vs. PCM from the CRISPR KO screen. (b) HGSOC patients from TCGA stratified by quartiles based on sum of indicated genes expression (Supplementary Table 1 for gene lists). P value was determined by ordered linear regression analysis adjusting for age. (c) % detachment was assessed by the controlled trypsin assay after culture in PCM or SCM +/- rotenone (n = 3 technical replicates representative from 3 independent experiments in each cell line). (d) Basal respiration of cells cultured in PCM or SCM (n = 2 technical replicates representative from 3 independent experiments in each cell line). (e) Ranked genes from the CRISPR knockout screen. (f) Individual *NDUFA5* sgRNAs from the CRISPR knockout screen. Green: FDR < 0.05. (g) Western blot of cells transduced with sg/shRNA targeting *NDUFA5*. Representative from 2 independent experiments in each cell line. (h) Basal/maximal respiration of cells cultured in SCM (n = 2 technical replicates representative from 3 independent experiments in each cell line). (i) Schematic of senescent cells and proliferative shNdufa5 cell co-injection *in vivo*. n = 4 mice/group. (j-k) Total intraperitoneal tumor nodules (j) and tumor nodule counts from the indicated sites (k) from

(i). shCont + Sen = proliferating shControl cells co-injected with senescent cells. shNdufa5 + Sen = proliferating shNdufa5 cells co-injected with senescent cells. (l-m) *NDUFA5* knockout Ovarcar8 bystander cells cultured in PCM or SCM +/- N-acetyl-cysteine (NAC) supplementation. (l) % detachment was assessed by controlled trypsin assay (n = 3 technical replicates representative from 2 independent experiments). (m) ROS was assessed by DCFDA (n = 3 technical replicates representative from 2 independent experiments). (n) NAD⁺ /NADH was assessed by mass spectroscopy (n = 8 for Ovarcar8 sgNDUFA5 #1, sgNDUFA5 #1 NA, sgNDUFA5 #2, KPCA.B shNdufa5 #1, shNdufa5 #2, shNdufa5 #2 NA; n = 6 for Ovarcar8 sgNDUFA5 #2 NA; n = 9 for KPCA.B shNdufa5 #1 NA; technical replicates representative from 2 independent experiments in each cell line). (o) Western blot of control or *NDUFA5* knockout cells transduced with empty vector or vector expressing Flag-tagged LbNOX (LbNOX OE). Representative from 3 independent experiments. All graphs show mean ± SD unless otherwise indicated. (d, j, k) Two-tailed student's t-test. (c, h, j, l-n) One-way ANOVA followed by Šidák's multiple comparisons test. Panel i created in BioRender; Cole, A. <https://biorender.com/llaueed> (2026).



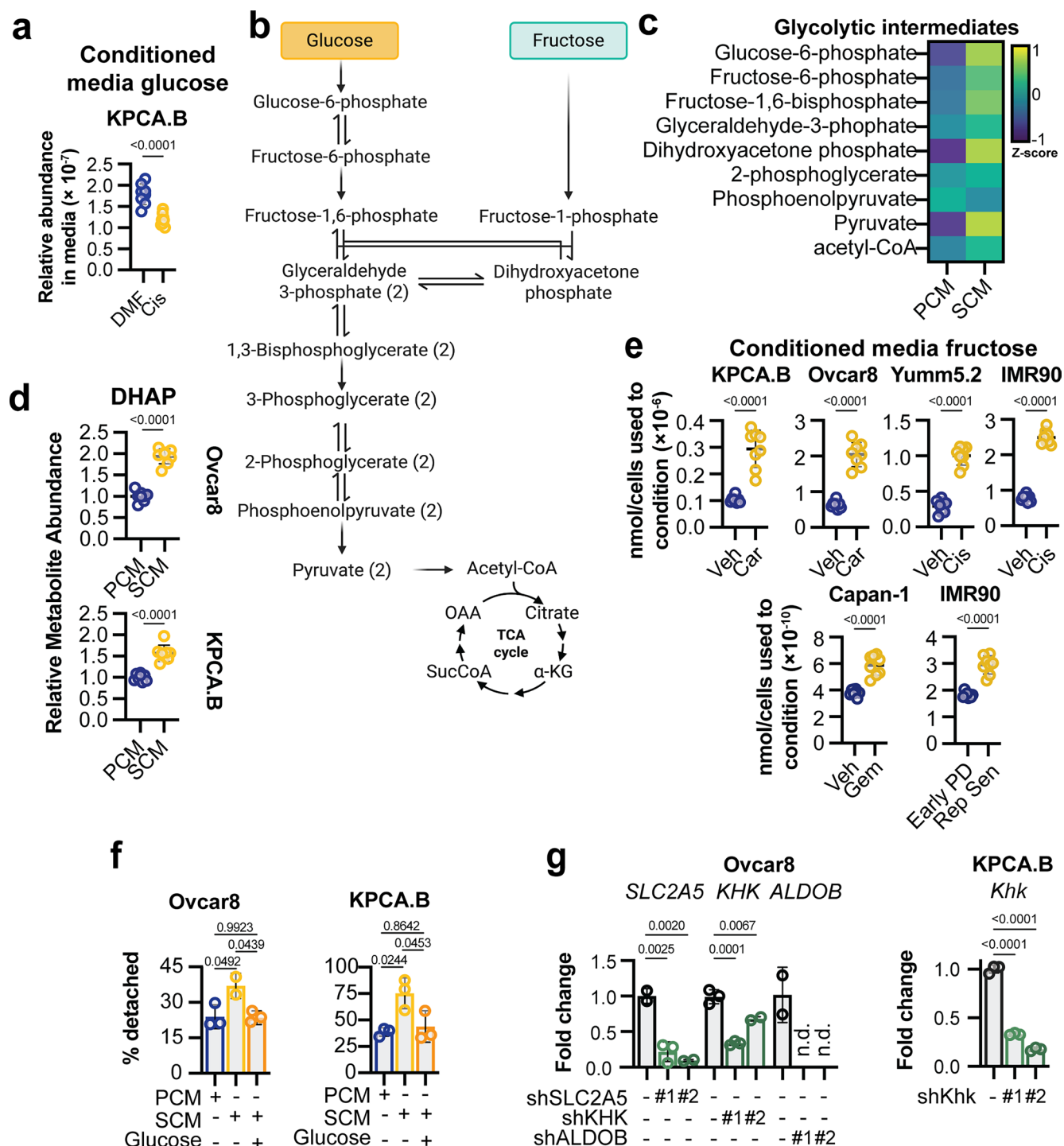
Extended Data Fig. 6 | Bystander HGSOC cells cultured in SCM have decreased cholesterol synthesis genes that is reversed by NDUF5 knockdown or knockout. (a) Heatmap of *de novo* cholesterol gene expression in the indicated Ovar8 bystander cells. (b) Correlation between senescent and *de novo* cholesterol gene expression in neoadjuvant treated ovarian cancer patient (post vs. pre). Pearson's correlation, p-value determined by linear regression. (c) HMGCS1/HMGCR sgRNAs in the controlled trypsin CRISPR knockout screen. Purple: FDR < 0.05. (d) mRNA of *de novo* cholesterol synthesis genes in the indicated bystander cells. (n = 3 technical replicates representative from 1 independent RNA-Seq experiment in each cell line). (e) Non-esterified cholesterol in the indicated bystander cells was assessed by mass spectroscopy (n = 8 Ovar8 sgControl SCM, sgNDUFA5 #1, sgNDUFA5 #2, KPCA.B shControl PCM, shControl Ndufa5#1, shNdufa5 #2; n = 7 for Ovar8 sgControl PCM, KPCA.B sgControl SCM; technical replicates representative from 2 independent experiments in each cell line). (f) Cholesterol was assessed in the indicated bystander cells by filipin III staining. NA = nicotinic acid. Representative

images from 3 experiments in each cell line. Scale bar = 25 μ m. 20X magnification. (g) Quantification of (f). sgControl PCM: n = 110; sgControl SCM: n = 112; sgControl SCM: n = 107; sgNDUFA5 #2 SCM: n = 109; sgNDUFA5 #2 SCM NA: n = 123; shControl PCM: n = 144; shControl SCM: n = 102; shNdufa5 #1 SCM: n = 144; shNdufa5 #1 SCM NA: n = 136; shNdufa5 #2 SCM: n = 107; shNdufa5 #2 SCM NA: n = 167; technical replicates representative from 3 independent experiments in each cell line). (h) Cholesterol was assessed in the indicated bystander cells by filipin III staining. Representative images from 3 experiments in each cell line. Scale bar = 25 μ m. 20X magnification. (i) Quantification of (h). sgControl: n = 100; sgControl LbNOX: n = 118; sgControl NA: n = 100; sgNDUFA5 #1: n = 147; sgNDUFA5 #1 LbNOX: n = 180; sgNDUFA5 #1 NA: n = 105; sgNDUFA5 #2: n = 131; sgNDUFA5 #2 LbNOX: n = 99; sgNDUFA5 #2 NA: n = 111; technical replicates representative from 3 independent experiments. All graphs show mean $\pm$ SD unless otherwise indicated. One-way ANOVA followed by Šidák's multiple comparisons test.



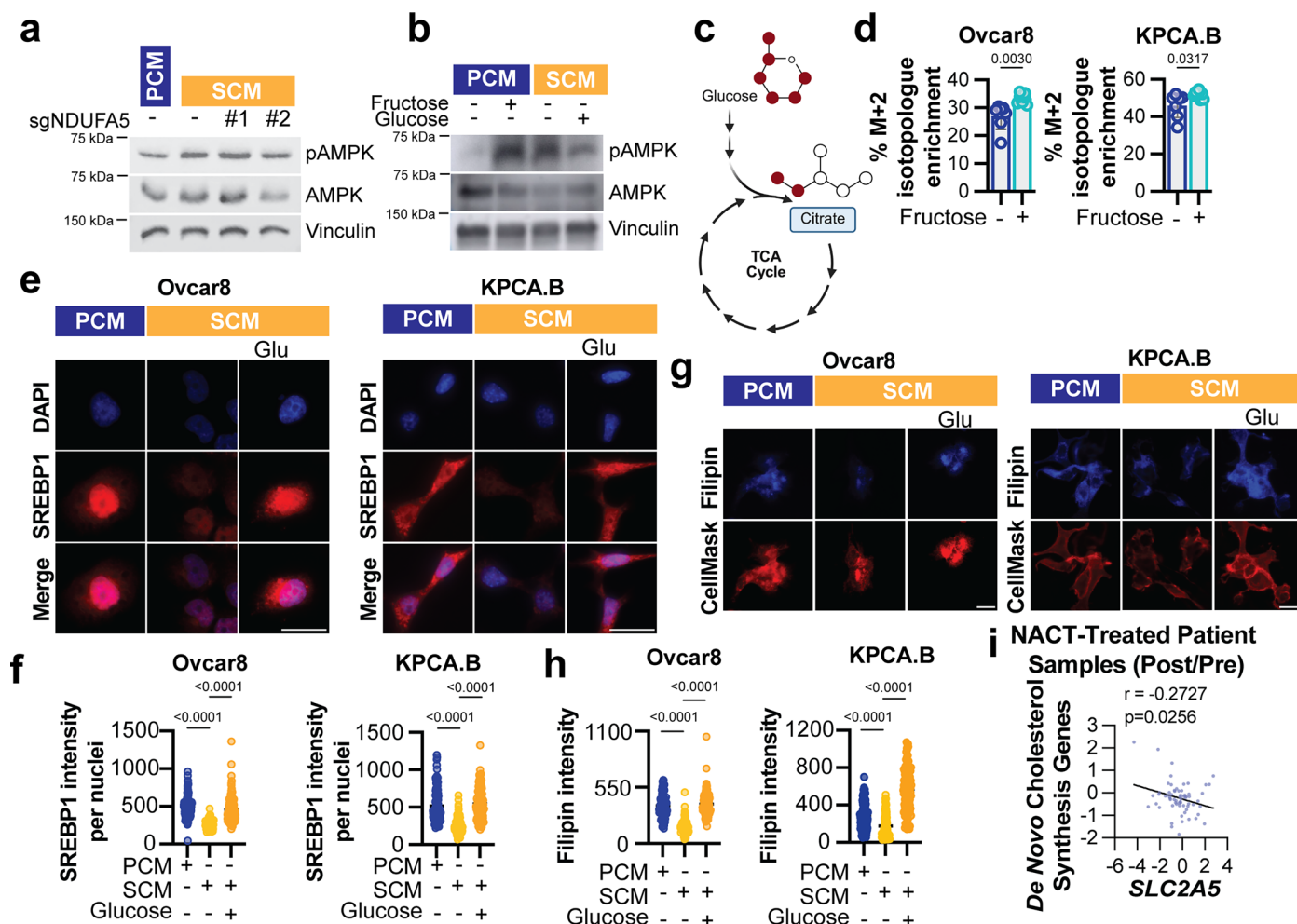
Extended Data Fig. 7 | Detachment in SCM is increased by inhibition of *de novo* cholesterol synthesis using statins. (a) Ovar8 bystander cells with knockout of NDUF5 (sgNDUF5) with or without lovastatin or mevastatin. After 72 h, cells were either incubated with vehicle or cholesterol for 2 h, and % detachment was assessed by the controlled trypsin assay ($n = 3$ technical replicates representative from 2 independent experiments). (b) KPCA.B bystander cells with knockdown of Ndufa5 (shNdufa5) were cultured in PCM or SCM with or without lovastatin or mevastatin. After 72 h, cells were either incubated with vehicle or cholesterol for 2 h, and % detachment was assessed using the controlled trypsin assay ($n = 3$ technical replicates representative from 2 independent experiments). (c) KPCA.B bystander cells with knockdown of Ndufa5 (shNdufa5) were cultured in PCM or SCM with or without nicotinic acid (NA). After 72 h, cells were stained with anti-SREBP1 antibody and DAPI and imaged. Shown are representative images from 3 experiments. Scale bar = 25 μ m. 20X magnification. Quantification can

be found in Fig. 3i. (d) The indicated bystander cells with knockout of NDUF5 (sgNDUF5) or knockdown of Ndufa5 (shNdufa5) were cultured in PCM or SCM with or without nicotinic acid. After 72 h, protein was harvested and western blots run probing for the indicated *de novo* cholesterol synthesis genes. Vinculin was used as a loading control. (e) Negative correlation between the expression of genes related with oxidative phosphorylation and cholesterol synthesis genes in TCGA HGSOC patient tumors (see Supplementary Table 1 for gene lists). Pearson's correlation, p value determined by linear regression. (f) Schematic showing regulation of cholesterol synthesis downstream of SASP-mediated complex I activity through the NAD⁺-SIRT-SREBP axis. Graphs show mean $\pm$ SD. All graphs show mean $\pm$ SD unless otherwise indicated. One-way ANOVA followed by Šidák's multiple comparisons test. Panel f created in BioRender; Cole, A. <https://biorender.com/589zte8> (2026).



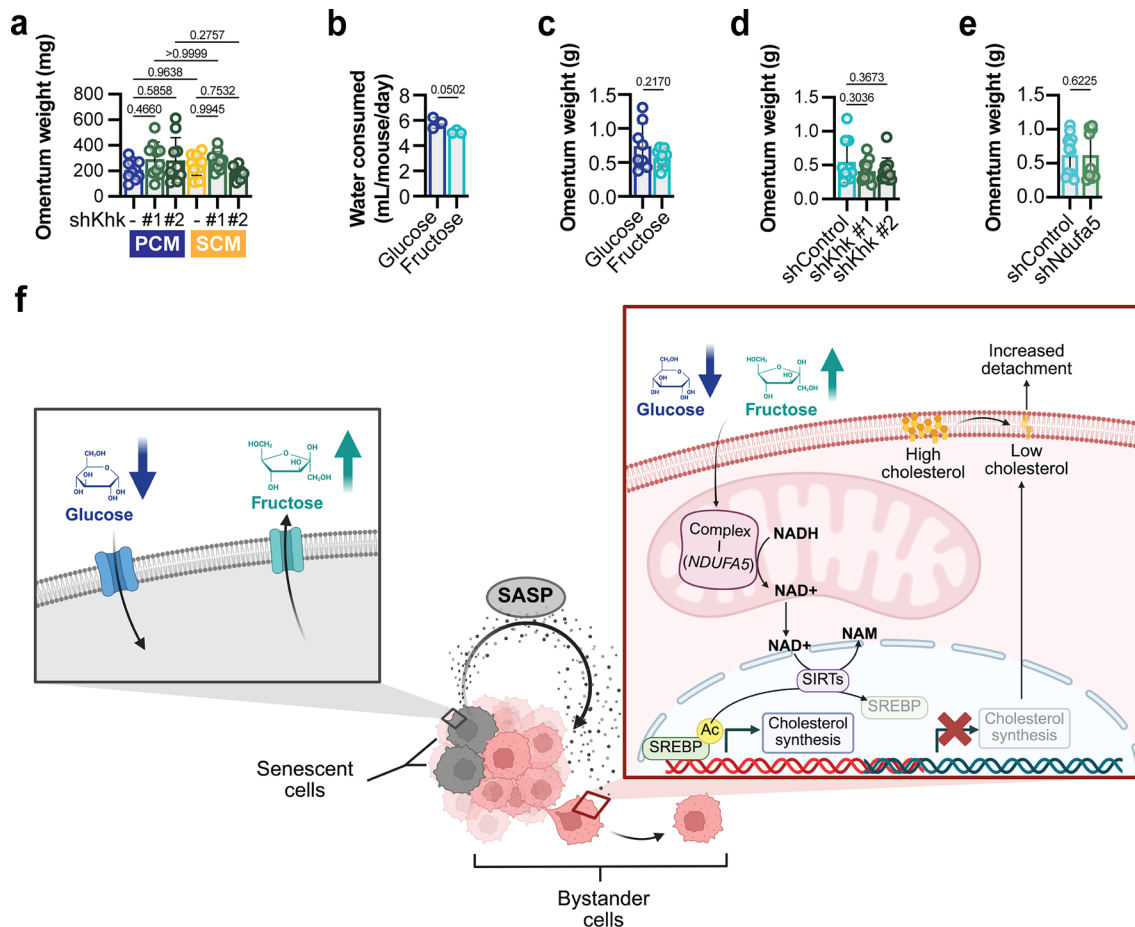
Extended Data Fig. 8 | Glycolytic intermediates and DHAP are increased in bystander cells cultured in SCM, while fructose is increased in the media of multiple senescent models. (a) Quantification of glucose in the conditioned media of senescent cells by LC-MS normalized to the total number cells over the course of the conditioning timeframe ($n = 8$ /group, representative from 3 independent experiments). (b) Schematic of the fructolytic and glycolytic pathways. (c) Ovar8 bystander cells were cultured in PCM or SCM. After 72 h, cells were harvested and glycolytic intermediates were assessed using LC-MS. Shown is a heatmap of z-scores ($n = 8$ technical replicates representative from 1 independent experiment). (d) The indicated bystander cells were cultured in PCM or SCM. After 72 h, cells were harvested and relative DHAP abundance normalized to AUC was assessed by LC-MS ($n = 8$ technical replicates representative from 1 independent experiment in each cell line). (e) The indicated cell lines were induced to senescence using carboplatin (Car),

cisplatin (Cis), gemcitabine (Gem), or replication (Rep Sen) and conditioned media was harvested. Quantification of fructose in the conditioned media of senescent cell by LC-MS normalized to the total number cells over the course of the conditioning timeframe ($n = 8$ technical replicates representative from 2 independent experiments in each cell line). (f) The indicated bystander cells were cultured in PCM or SCM alone or supplemented with glucose. After 72 h, % detachment was assessed using the controlled trypsin assay ($n = 3$ technical replicates representative from 3 independent experiments in each cell line). (g) The indicated cells were transduced with shRNA controls or shRNA targeting SLC2A5, KHK, or ALDOB, and mRNA expression was assessed by RT-qPCR ($n = 3$ technical replicates representative from 2 independent experiments in each cell line). All graphs show mean $\pm$ SD unless otherwise indicated. (a, d, e) Two-tailed student's test. (f-g) One-way ANOVA followed by Šidák's multiple comparisons test. Panel b created in BioRender; Cole, A. <https://biorender.com/likytne> (2026).



Extended Data Fig. 9 | Fructose decreases while glucose increases nuclear SREBP1 and cholesterol. (a–b) Western blot of the indicated bystander cells. Representative from 2 independent experiments. (c) Schematic of $^{13}\text{C}_6$ -glucose tracing. (d) HGSOC cells were incubated with $^{13}\text{C}_6$ -glucose alone or together with fructose for 2 h. Citrate M + 2 isotopologue enrichment was assessed by mass spectrometry (Control: n = 7; Fructose: n = 8; technical replicates representative from 1 independent experiment in each cell line). Two-tailed student's test. (e–f) Bystander cells were cultured in PCM/SCM +/- glucose. (e) Cells were stained with anti-SREBP1 antibody and DAPI. Representative images from 2 independent experiments. Scale bar = 25 μm . 40X magnification. (f) Quantification of (e). Ovarcar8 PCM: n = 112; Ovarcar8 SCM: n = 103; Ovarcar8 SCM + glucose: n = 119; KPCA.B PCM: n = 132; KPCA.B SCM: n = 103; KPCA.B SCM + glucose: n = 119; technical replicates representative from 2 independent experiments in each

cell line. (g–h) Bystander cells were cultured in PCM/SCM +/- glucose. (g) Cells stained with filipin III. Representative images from 2 independent experiments. Scale bar = 25 μm . 20X magnification. (h) Quantification of (g). Ovarcar8 PCM: n = 91; Ovarcar8 SCM: n = 110; Ovarcar8 SCM + glucose: n = 99; KPCA.B PCM: n = 145; KPCA.B SCM: n = 103; KPCA.B SCM + glucose: n = 134; technical replicates representative from 2 independent experiments in each cell line. (i) Correlation between *SLC2A5* and *de novo* cholesterol gene expression in neoadjuvant treated ovarian cancer patient (post vs. pre). Pearson's correlation, p value determined by linear regression. All graphs show mean $\pm$ SD unless otherwise indicated. One-way ANOVA followed by Šidák's multiple comparisons test unless otherwise indicated. Panel c created in BioRender; Cole, A. <https://biorender.com/ehdzqti> (2026).



Extended Data Fig. 10 | No change in omental weights in tumor-bearing mice.

(a) Omental weights from mice at endpoint. $n = 10$ mice/group (b) Average water intake of mice provided glucose or fructose in their drinking water. $n = 3$ cages/group (c-e) Omental weights from mice at endpoint. $n = 8$ /group glucose, fructose in (c); $n = 10$ /group (shControl in (d)); $n = 11$ /group (shKHK #1 and shKHK #2 in (d)); $n = 12$ /group (shControl in (e)), $n = 9$ /group (shNdufa5 in (e)). (f) Final

proposed model. High fructose in the SASP leads to metabolic reprogramming in bystander cells through the complex I-NAD⁺-SIRT-SREBP1 axis leading to low plasma membrane cholesterol and increased detachment. All graphs show mean $\pm$ SD. (a, d) One-way ANOVA followed by Šidák's multiple comparisons test; (c, e) Two-tailed student's test. Panel f created in BioRender; Cole, A. <https://biorender.com/Szuc0pm> (2026).

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	NIS Elements Imaging software for Immunofluorescence, live cell timelapse, and TIRF image capturing, Leica LAS-X Imaging software for live cell timelapse image capturing.
Data analysis	Image J version 1.54f for microscopic image analysis. QuPath was used for analysis of IHC. Incucyte software was using for cell quantification. Rstudio was used to analyze the CRISPR screen. Microsoft Excel (Excel 2016 microsoft office), GraphPad Prism v10 (GraphPad software)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All source data, including western blots, are included with the manuscript. Raw data from the CRISPR KO screen can be found in Supplementary Table 5.

RNA-Seq data were deposited into the Gene Expression Omnibus database under accession number GSE305033 and GSE305002 and are available at the following URLs: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE305033> and <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE305002>

Publicly available datasets used in this study:

Bulk RNA-Seq data: GSE227100 and EGA dataset EGAD00001006456 (Fig 1a)

Nanostring data: GSE181597, GSE201600, and GSE319500 (Extended Data Fig. 1a)

scRNA-Seq data: GSE266577 (Fig. 1b and Extended Data Fig. 1b)

The Cancer Genome Atlas (TCGA) Serous Ovarian Cancer (Nature, 2011) project was downloaded from cbiportal.org.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	All data reported from patients are from ovarian cancer patients and are therefore only female.
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	For Pitt cohort: Human samples were from ovarian cancer patients treated at the University of Pittsburgh. BMI and age were considered in the analysis. For TCGA data: Transcriptomic data downloaded from cBioportal (Nature, 2011). https://doi.org/10.1038/nature10166
Recruitment	For Pitt cohort: De-identified samples were collected under University of Pittsburgh IRB approved protocols (20050019, 19070449, and 19060197). For TCGA data: See Nature, 2011 https://doi.org/10.1038/nature10166
Ethics oversight	For Pitt cohort: The collection of patient samples was approved by the University of Pittsburgh IRB (protocols: 20050019, 19070449, and 19060197). Written, informed consent was obtained from ovarian cancer patients. For TCGA data: See Nature, 2011 https://doi.org/10.1038/nature10166

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes were chosen on the basis of extensive experience with the assays we have performed. For metabolomics data, an n = 8 was chosen based on prior experience and variability in the data. For detachment assays, n = 3 was chosen based on prior experience. For imaging experiments, >100 cells or a maximum number in imaged fields were analyzed. The data in figures are representative of one independent experiment in each cell line.
Data exclusions	Outliers were removed using PRISM outlier analysis (ROUT method). For in vivo studies, mice without tumor development, measured by IVIS imaging seven days post-implantation or by endpoint omental weight compared to tumor free omentum, were excluded. Further outliers were determined using ROUT method in GraphPad PRISM.
Replication	Experiments were repeated in multiple cell lines with at least 2 replicates, and all experiments were reproducible.
Randomization	All in vivo experiments and tests were randomly assigned, but no randomization was performed for cell culture experiments.
Blinding	Immunofluorescence analysis was blinded when possible. Investigators were not blinded during the other experiments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

The following antibodies were obtained from the indicated suppliers: Primary antibodies: rabbit anti-Lamin B1 (#ab16048, RRID AB_443298, WB 1:1000) from Abcam, rabbit anti-NDUFA5 (#16640-1-AP, RRID AB_2251270, WB 1:1000), rabbit anti-SREBF1 (#14088-1-AP, RRID AB_2255217, IF 1:100, ChIP 1 ug) from Proteintech, rabbit anti-SREBP1 (#ab28481, RRID AB_778069, IF 1:150) from Abcam, rabbit anti-HMGCR (#PA5-37367, RRID AB_2554032, WB 1:1000) from Invitrogen, rabbit anti-HMGCS1 (#17643-1-AP, RRID AB_2248359, WB 1:1000) from Proteintech, rabbit anti-MVK (#12228-1-AP, RRID AB_2147565, WB 1:1000) from Proteintech, rabbit anti-AMPKa (#2532, RRID AB_330331, WB 1:1000) from Cell Signaling Technology, rabbit anti-phosphoAMPKa (Thr172) (40H9) (#2535, WB 1:1000) from Cell Signaling Technology, and rabbit anti-DYDDDDK Tag (D6W5B) (#14793, RRID AB_2572291, WB 1:500, rat anti-BrdU (#ab6326, RRID: AB_305426, Clone BU1/75 (ICR1), IF 1:500) from Abcam

Validation

All antibodies used in this study were purchased from commercial vendors who had validated specificity in human cells/mouse tissues for the specific assays (Western Blot, Chromatin Immunoprecipitation and Immunofluorescence). NDUFA5 antibody was validated by knockdown studies.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Human ovarian cancer Ovar8 cells were a gift from Dr. Benjamin Bitler (University of Colorado). KPCA.B cells were a kind gift from Dr. Robert Weinberg (Whitehead Institute of Biomedical Research, Cambridge, MA). YUMM5.2 cells were obtained from ATCC (ATCC, cat# CRL-3367, RRID: CVCL_JK43). Capan-1 cells were obtained from ATCC (ATCC cat# HTB-79). Normal diploid IMR90 human fibroblasts were obtained from ATCC (ATCC, cat# CCL-186). HEK293FT cells were obtained from Thermo Fisher (R70007).

Authentication

Ovar8 cells were STR fingerprinted. The other cell lines were not authenticated.

Mycoplasma contamination

All the cells were tested monthly for Mycoplasma and cells used in this study were mycoplasma negative.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified cell lines were used in this study.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Eight-week-old female mice were purchased from Jackson Laboratories (C57Bl6/J mice: cat#000664). All mice were maintained in a HEPA-filtered ventilated rack system either at the Animal Facility of the Assembly Building of The Hillman Cancer Center at the University of Pittsburgh School of Medicine or the Animal Facility of The Wistar Institute. Mice were housed up to 5 mice per cage and in a 12-hour light/dark cycle. All experiments with animals were performed in accordance with institutional guidelines approved by the Institutional Animal Care and Use Committee (IACUC) at either the University of Pittsburgh School of Medicine or the Wistar Institute.

Wild animals

No wild animals were used in the study.

Reporting on sex

Only female mice were used in this study.

Field-collected samples

No field collected samples were used in the study.

Ethics oversight

All experiments with animals were performed in accordance with institutional guidelines approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pittsburgh School of Medicine or The Wistar Institute.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

No plants were used in the study.

Novel plant genotypes

No plants were used in the study.

Authentication

No plants were used in the study.