Extended Data Fig. 1. IMR90 ER:RAS cells as a model of OIS. a, Quantification of immunofluorescence staining for BrdU, p16^INK4a, and SA-β-Galactosidase of IMR90 ER:RAS cells 6 or 8 days after treatment with 4-OHT or vehicle (DMSO) (n = 3). b, Representative immunofluorescence images. BrdU incorporation, which indicates proliferation, is stained green; p16^INK4a is stained red. Scale bar, 50 µm. SA-β-Galactosidase is stained red. Scale bar, 100 µm. c, Expression levels for IL8 and IL1A of senescent and control IMR90 ER:RAS cells 6 days after 4-OHT or vehicle (DMSO) (n = 4). d, DAPI staining of senescent and control IMR90 ER:RAS cells after 1 µM ABT-263 treatment for 3 days showing reduced numbers of senescent cells after ABT-263 treatment. Scale bar, 100 µM. e, Senolytic activity of the indicated drugs in the context of oncogene-induced senescence in IMR90 ER:RAS cells (n = 4). f, Quantification of immunofluorescence staining for BrdU in IMR90 ER:RAS cells expressing E6 and E7 proteins of HPV16 (n = 3). g, Senolytic activity of the indicated drugs in IMR90 ER:RAS cells expressing E6 and E7 proteins of HPV16. All error bars represent mean ± s.d; n represents independent experiments. All statistical significances were calculated using unpaired two-tailed Student’s t-tests.
Extended Data Fig. 2. Senolytic drug screen in therapy-induced senescence. a, Quantification of immunofluorescence staining for BrdU, SA-β-Galactosidase activity, p21\(^{CIP1}\) and 53BP1 in IMR90 cells treated with 50 µM etoposide (\(n = 3\)). b-c, Senolytic activity of the indicated drugs in the context of therapy-induced senescence in IMR90 (\(n = 4\), b) and oncogene-induced senescence in IMR90 ER:RAS cells (\(n = 4\), c). All error bars represent mean ± s.d; \(n\) represents independent experiments. All statistical significances were calculated using unpaired two-tailed Student’s \(t\)-tests.
Extended Data Fig. 3. The glycoside chain in CGs is dispensable for their senolytic activity. a, Dose response analysis of senolytic activity of ouabain in IMR90, control IMR90 ER:RAS cells (DMSO), senescent IMR90 ER:RAS cells (4-OHT) and IMR90 ER:RAS cells expressing E6 and E7 proteins of HPV16 (n = 3). b, Dose response analysis of senolytic activity of digitoxin in the context of oncogene-induced senescence in IMR90 ER:RAS cells (n = 3). c, Chemical structure of ouabain and its aglycone version, ouabagenin. d, Quantification of cell survival in senescent and control IMR90 ER:RAS cells after treatment with ouabagenin, the aglycone version of ouabain (n = 6). e-f, Quantification of cell survival of IMR90 ER:RAS cells undergoing OIS and the corresponding controls after treatment with the CG K-Strophanthin (e) (n = 4) or its aglycone version Strophantidin (f) (n = 5). g, Quantification of cell survival in senescent and control IMR90 ER:RAS 3 days after 1 µM ABT-263 or CG treatment (50 nM ouabain, 100 nM digoxin). Senolytic drugs were added 8 days after 4-OHT or vehicle (DMSO) (n = 4). All error bars represent mean ± s.d; n represents independent experiments. All statistical significances were calculated using unpaired two-tailed Student’s t-tests.
Extended Data Fig. 4

a

% Cell survival

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p = 0.0001

p = 0.0001

Curcumin (μM)

b

IMR90 ER:RAS

4OHT

CS +/- OVD

RNA-Seq

-7

-6

0

16h

36h

hours

c

IMR90 ER:RAS

4OHT

siRNAs

Ouabain 50 nM

Imaging

-1

0

4

6

7

9

d

Normalized cell survival

siRNA

Normalized mRNA levels

e

NOXA

mRNA levels

f

NOXA

mRNA levels

IMR90 ER:RAS + 4OHT

Vector + DMSO

Vector + Ouabain

Vector + Ouabain + shNOXA

50 nM Ouabain

1 μg/ml doxycycline
Extended Data Fig. 4. Senescent cells are more sensitive to CGs due to their altered osmotic balance. 

a, Quantification of cell survival of senescent and control IMR90 ER:RAS cells after treatment with curcumin (n = 6). Statistical significance was calculated using unpaired two-tailed, Student’s t-test.  

b, Experimental design for the transcriptional profiling of senescent and control IMR90 ER:RAS cells after treatment with cardiac glycosides (CG). QVD indicates treatment with a general caspase inhibitor (Q-VD-OPh).  

c, IMR90 ER:RAS cells were transfected with 2 independent siRNAs targeting BCL-2 family genes at day 6 after senescence induction as indicated in the scheme.  

d, IMR90 ER:RAS were transfected with at least two independent siRNAs targeting BCL-2 family genes at day 6 after senescence induction (n = 3; scrambled siRNA versus three different siRNAs against NOXA, ***P < 0.001). The timeline of the experiment is shown in (c). Statistical significance was calculated using one-way ANOVA (Dunnett’s test).  

e, Expression levels of NOXA after knock down with three independent siRNAs (n = 3). Statistical significance was calculated using one-way ANOVA (Dunnett’s test).  

f, Expression levels of NOXA after knock down with four independent shRNAs (n = 3; vector versus different shRNAs against NOXA, ****P < 0.0001). Statistical significance was calculated using one-way ANOVA (Dunnett’s test). All error bars represent mean ± s.d; n represents independent experiments.
Extended Data Fig. 5. Ouabain eliminates liver preneoplastic senescent cells. 

a, Representative images of immunofluorescence staining of Nras. Mice were treated with vehicle (n = 9) or ouabain (n = 12) as explained in Fig 4a. Nras is stained in red. Scale bar, 70 µm. 

b, Immunofluorescence staining and quantification of p21Cip1 in Nras-positive senescent hepatocytes vs Nras-negative normal hepatocytes. Nras is stained in green, p21Cip1 is stained in red. White arrows indicate Nras-positive, p21Cip1-positive cells; green arrow indicates a Nras-positive, p21Cip1-negative cells (n = 10 per group). 

c, SCID/beige mice were treated with saline or Digoxin (1mg/kg) on two consecutive days, 5 days after hydrodynamic transduction of Nras-GFP. Mice were culled 6 hours after the second treatment. Representative images of immunofluorescence staining of GFP and cleaved caspase-3 and quantification of intensity levels in 1/2 independent experiments (n = 200 cells). Scale bar, 50 µm. Statistical significance was calculated using unpaired two-tailed Student's t-test. Data represent mean ± s.d; n represents number of mice.
Extended Data Fig. 6. Ouabain eliminates preneoplastic senescent cells. a, Representative images of immunofluorescence staining of β-catenin (green) and synaptophysin (red) in tumoral pituitaries from 18.5dpc Hesx1\textsuperscript{Cre+};Ctnnb1\textsuperscript{lox(ex3)+} mice that were cultured in the presence of either ABT-737 (2.5 μM), ouabain (250 nM and 500 nM) or vehicle (DMSO) (n = 10 per group). Scale bar, 50 μm. b, Quantitative analysis of the immunofluorescence in (a) demonstrates that ABT-737 and ouabain significantly reduce the number of β-catenin-positive cells. Statistical significance was calculated using Kruskal-Wallis and Dunn’s multiple comparisons test. c, Representative images of immunofluorescence staining of ACTH (adrenocorticotrophic hormone; magenta). Scale bar: 50 μm. d, qRT-PCR analysis revealing that the senescent marker Cdkn1a (encoding for p21\textsuperscript{Cip1}) and the SASP components Il1b and Il6 are reduced in neoplastic pituitaries treated with 100 nM ouabain and 100 nM digoxin relative to vehicle controls (n = 3 per group). Statistical significance was calculated using unpaired two-tailed Student’s t-test; data represent mean ± s.d; n represents number of mice.
Extended Data Fig. 7. Anti-cancer effect of cardiac glycosides across different human cancer cell lines. a-b, Quantification of cell survival by trypan blue staining of Huh7 cells (a) and HLF cells (b) after treatment with the indicated drug combinations \((n = 3)\). Timeline of the experiment is shown in Supplementary Fig 4a. Statistical significance was calculated using unpaired two-tailed Student's \(t\)-test. c, Quantification of cell survival of senescent (alisertib, palbociclib) and control (DMSO) SK-Mel-5 melanoma cells \((n = 4)\). Statistical significance was calculated using two-way ANOVA (Dunnett's test). d, Quantification of cell survival of senescent (doxorubicin, palbociclib) and control (DMSO) MCF-7 or MCF-7 breast cancer cells infected with a shRNA against \(TP53\) \((n = 4)\). Statistical significance was calculated using two-way ANOVA (Dunnett's test). e-f, mRNA expression levels of \(TP53\) in MCF-7 cells (e) and HCT-116 cells \((n = 3)\). Statistical significance was calculated using unpaired two-tailed, Student's \(t\)-test. Data represent mean ± s.d; \(n\) represents independent experiments; ns, not significant.
Extended Data Fig. 8. Ouabain treatment reverses age-associated changes in old mice. a, Ouabain levels in plasma were assessed by ELISA 24 hours after finishing a 4-day course of daily 1mg/kg ouabain i.p. injections. (n = 6). b, Phosphate and amylase levels of young (n = 7) and old mice, either treated with vehicle (n = 6) or ouabain (n = 9), were determined in whole-blood samples at the endpoint of the experiment. Statistical significance was calculated using unpaired two-tailed Student’s t-test. c, Grip strength assessment in old mice treated with vehicle (n = 7) or ouabain (n = 9) 10 weeks after the start of the experiment, referred to the basal test. Statistical significance was calculated using unpaired two-tailed Student’s t-test. d, Expression levels of p16\textsuperscript{Ink4a} in heart and kidney were determined by qRT-PCR following treatment with vehicle (n = 6) or ouabain (n = 7). mRNA expression levels in young mice (n = 7) were used as reference. Statistical significance was calculated using unpaired two-tailed Student’s t-test. e-f, GSEA signature for chemokines, oncogene-induced senescence (e) and ageing (f). g, Quantitative analysis (left) and representative IHC pictures (right) of p21\textsuperscript{Cip1} positive hepatocytes in the liver of young (n = 6) and old mice treated with ouabain (n = 8) or vehicle (saline) (n = 6). Scale bar, 100 µm. Data represent mean ± s.e.m. Statistical significance was calculated using one-way ANOVA with Tukey’s post hoc comparison. h, Quantitative analysis (left) and representative IHC pictures (right) of LINE-1 ORF in the liver of young (n = 6) and old mice treated with ouabain (n = 8) or vehicle (saline) (n = 7). Statistical significance was calculated using one-way ANOVA with Tukey’s post hoc comparison. Scale bar, 50 µm. Data represent mean ± s.d; n represents number of mice.
Extended Data Fig. 9. Ouabain treatment resets immune infiltration in old mice. a, xCell analysis of the transcriptome data predicts changes in immune infiltration in the liver of old mice that could be reverted with ouabain. RNA-Seq data from the livers of young (n = 6) and old mice, either treated with vehicle (n = 6) or ouabain (n = 6) was used. Statistical significance was calculated using unpaired two-tailed Student's t-test. b, Blood analysis at the end of the experiment show that ouabain treatment does not change immune composition. Blood from young (n = 8) and old mice, either treated with vehicle (n = 6) or ouabain (n = 8) was used. Statistical significance was calculated using unpaired two-tailed Student's t-test. c-d, Representative IHC images (c) and quantification (d) of the indicated immune cell markers in the liver of young (n = 6) and old mice, either treated with vehicle (n = 6) or ouabain (n = 8). Scale bar: 100µm. Statistical significance was calculated using one-way ANOVA with Tukey’s post hoc comparison. Data represent mean ± s.e.m.; n represents number of mice; ns, not significant.