SUPPORTING INFORMATION (SI)

Obesity triggers tumoral senescence and renders poorly immunogenic malignancies amenable to senolysis

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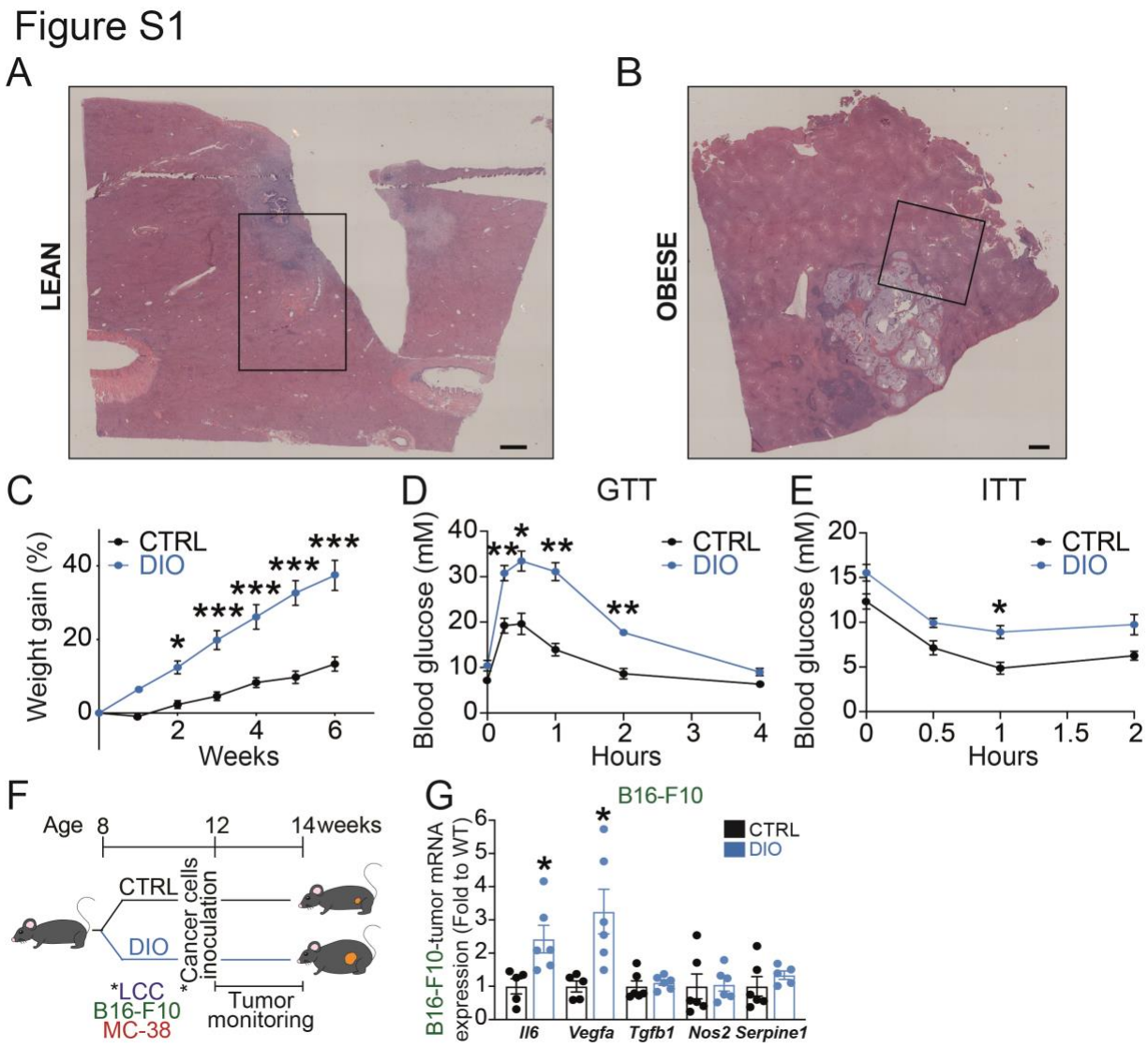
Supplementary Figures



Supplementary Figure S1. Related to Figure 1, diet-induced obesity potentiates tumor growth and triggers tumoral senescence in poorly immunogenic tumors

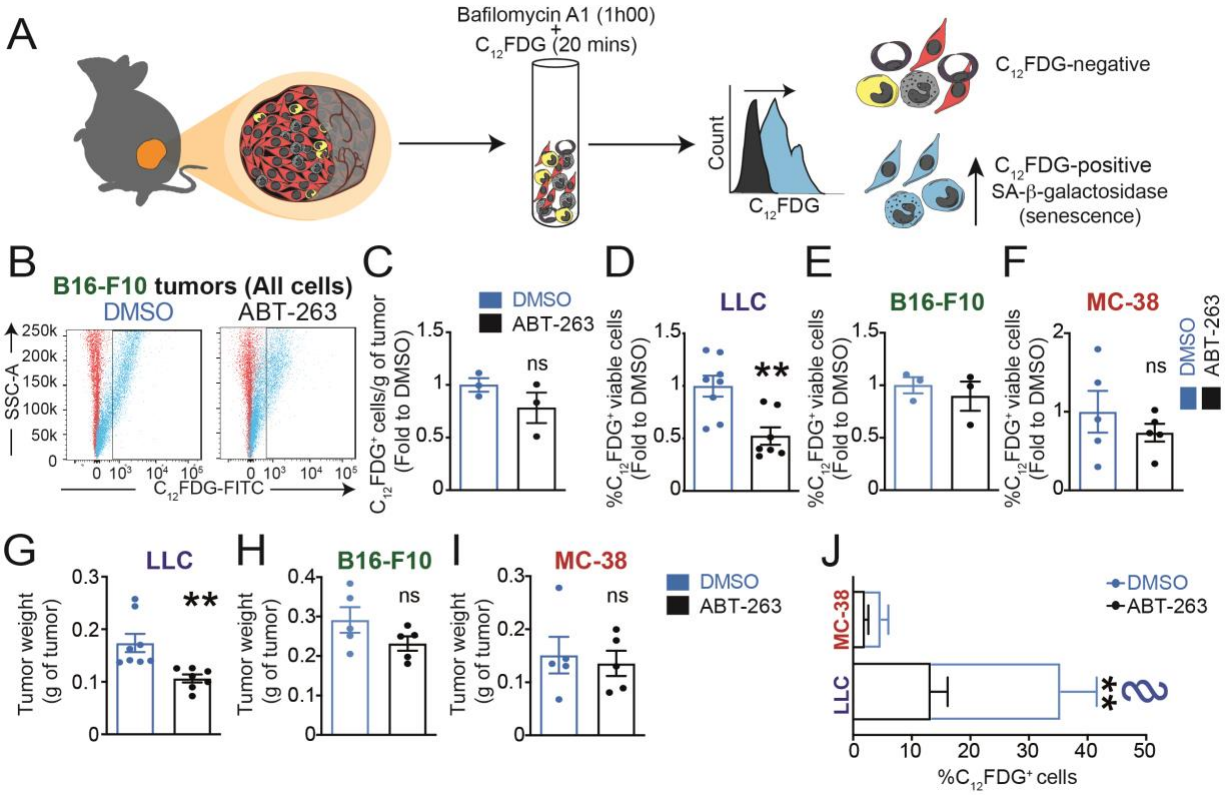
(A-B) Images shows H&E staining of human **(A)** lean and **(B)** obese samples used to represent immunofluorescence stainings in Figure 1A. Black squares represent regions from which depicted images were taken. **(C)** Weight gain curve represented as percentage of weight gain. **(D)** GTT of mice under DIO for 4 weeks and **(E)** ITT of animals under DIO for 5 weeks. **(F)** Schematic representation of the experimental paradigm combining diet-induced obesity with

LLC, B16-F10, and MC-38 cells syngeneic injections. **(G)** Bar charts represent analysis of SASP-related factors using mRNA expression of *Il6*, *Vegfa, Tgfb1, Nos2, and Serpine1* in whole B16-F10-tumor lysates.

In vivo data represent ≥2 independent experiments with $n \geq 5$ biological replicates.

Statistics: **(C-E)** Two-way ANOVA and Bonferroni post-hoc test. **(G)** Two-tailed unpaired Student's *t* test. * P <0.05, ** P <0.01, *** P <0.001; 95% CI. Error bars represent the mean ± SEM. Each *n* represents one biological replicate.

Figure S2



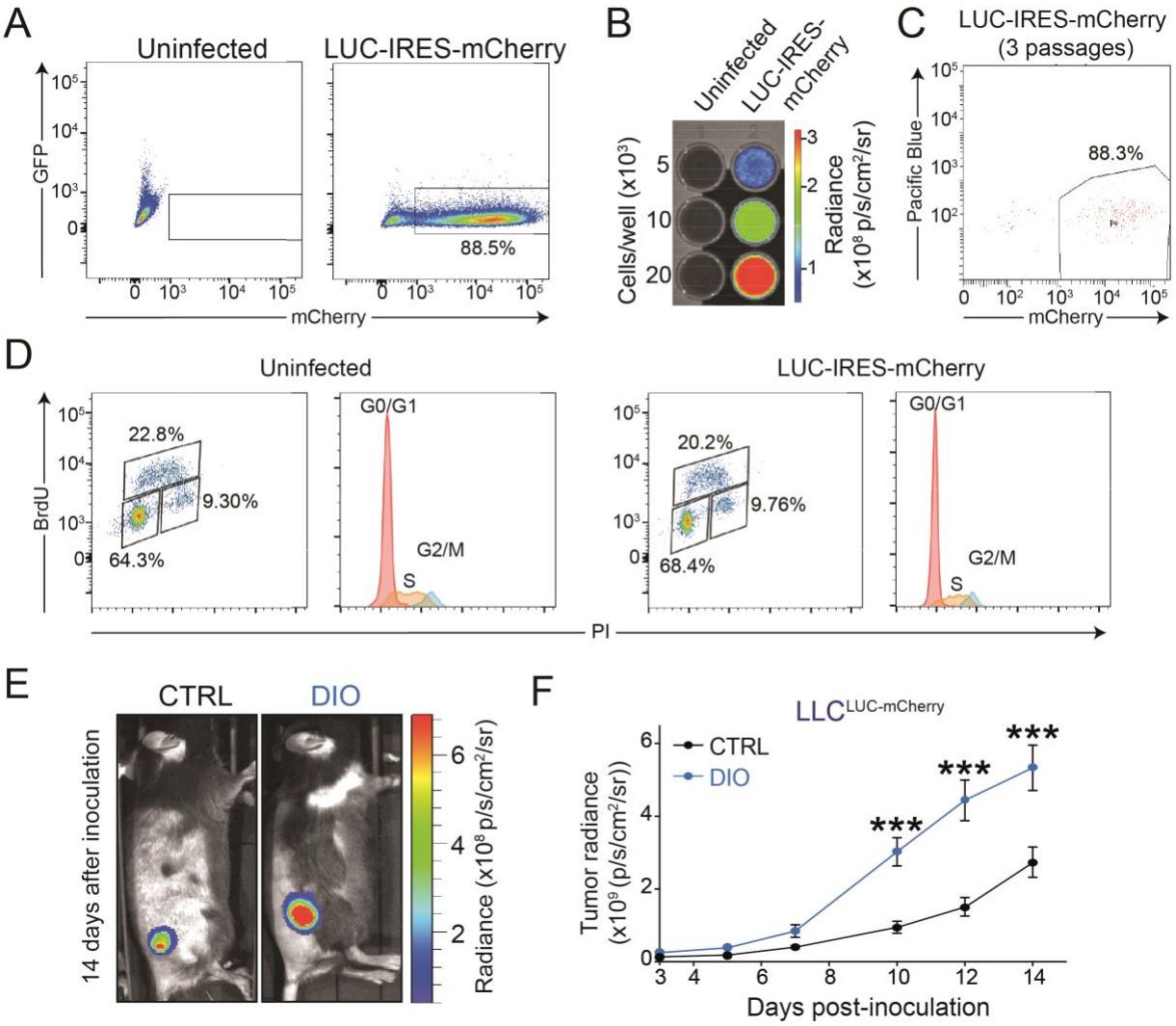
Supplementary Figure S2. Related to Figure 2, therapeutic senolysis hinders obesity-driven tumor growth

(A) Schematic representation of the experimental strategy for the detection of SA-β-Gal activity at the single cell level using C12FDG. **(B-C)** Representative **(B)** FACS histograms and **(C)** quantification of $C_{12}FDG^+$ cells in B16-F10 tumors from DMSO- or ABT-treated mice. Bar charts indicate the relative number of $C_{12}FDG^+$ cells per gram of tumor. **(D-F)** FACS quantification of $C_{12}FDG^+$ viable **(D)** LLC, **(E)** B16-F10 or **(F)** MC-38 cells in whole tumors. Bar charts indicate the relative number of $C_{12}FDG^+$ cells to total cells. **(G-I)** Bar charts indicate **(G)** LLC, **(H)** B16-F10, and **(I)** MC-38 tumors from DMSO- or ABT-treated mice. **(J)** Bar charts indicate the relative proportion of C12FDG+ cells in poorly immunogenic LLC or highly immunogenic MC-38 tumors after treatment or controls. § compares DMSO groups and * compares ABT-263 groups.

Data represent ≥2 independent experiments with ≥5 biological replicates.

Statistics: (C-J) Two-tailed unpaired Student's *t* test. *P <0.05, **P <0.01, ***P <0.001. Error bars represent the mean ± SEM. Each *n* represents one biological replicate

Figure S3



Supplementary Figure S3. Related to Figure 3, senolysis targets SA-βgal⁺ cancer cells in obese mice to limit tumor growth

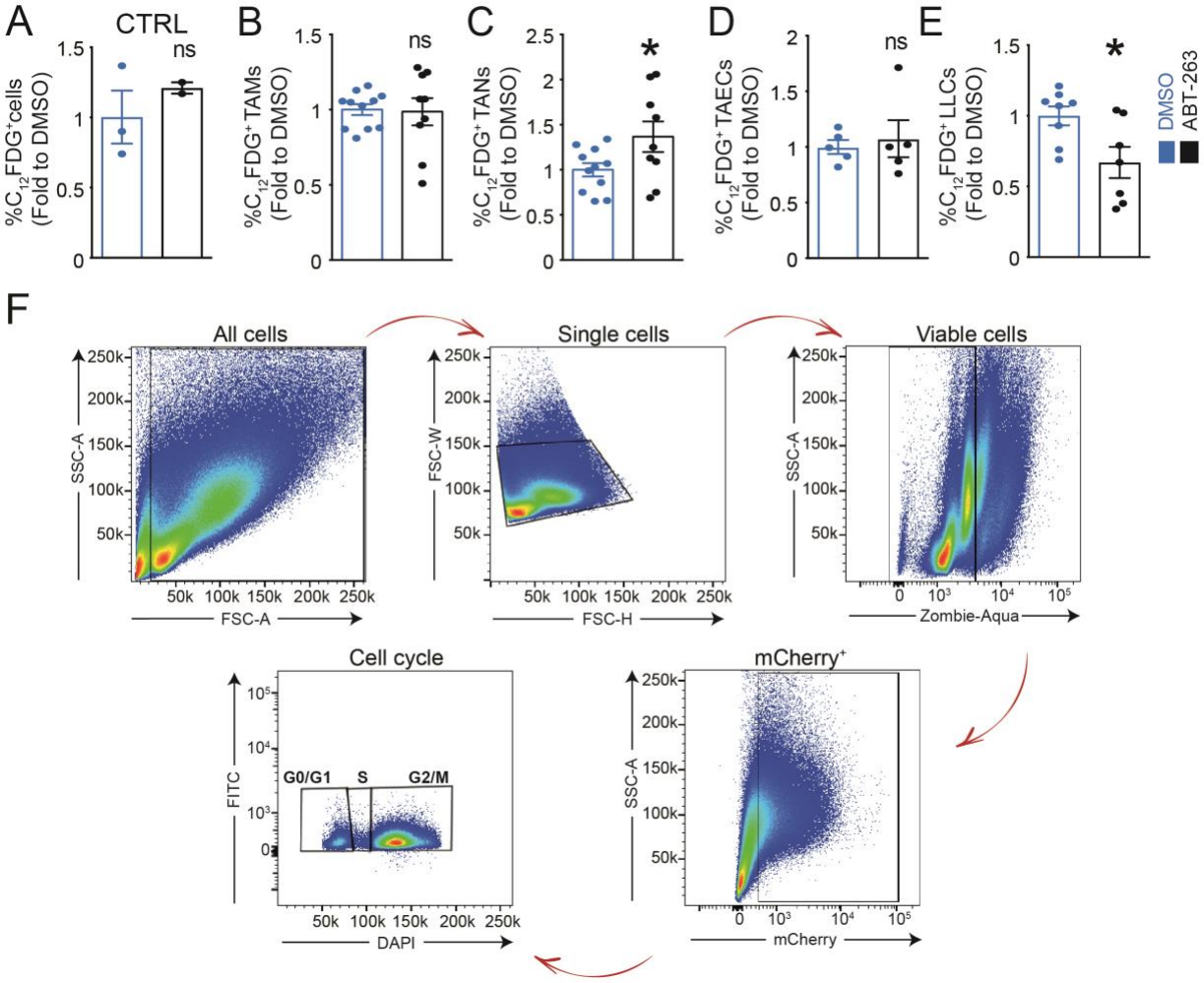
(A) Illustrative histogram showing the population of mCherry⁺ cells following LLC cells transduction with the MSCV-LUC-IRES-mCherry plasmid. **(B)** Luciferase activity of mCherry⁺ LLC cells (LUC-IRES-mCherry cells) is dependent on the cell concentration *in vitro*. **(C)** Illustrative histogram showing the population of LUC-IRES-mCherry LLC cells *in vitro* following transduction. Percentage indicates proportion of mCherry⁺ LLC cells on total LLC cells **(D)** Illustrative histograms depicting the cell cycle state of uninfected LLC cells

compared to LUC-IRES-mCherry LLC cells using PI and BrdU. Percentages indicate proportions of LLC cells in each cell cycle phase to total LLC cells. **(E)** Representative images of tumors *in vivo* from mice under DIO or CTRL diet at 14-days post-inoculation of $LLC^{LUC\text{-}mCherry}$ cells obtained with *IVIS*. Images overlay the luminescent signals and are represented as radiance ($p/s/cm^2/sr$). Imaging thresholds were optimized for minimizing inter-batch variation (min: 3.85e7; max:6.90e8). **(F)** Curve represents the tumor growth of $LLC^{LUC\text{-}mCherry}$ cells in mice under DIO or CTRL diet monitored with *IVIS*.

In vivo data represent ≥2 independent experiments with $n \geq 5$ biological replicates.

Statistics: **(F)** Two-way ANOVA $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Error bars represent the mean ± SEM. Each *n* represents one biological replicate.

Figure S4



Supplementary Figure S4. Related to Figure 4, ABT-263 eliminates growth-arrested cancer cells in poorly immunogenic tumors during DIO

(A) FACS quantification of all cells in Ctrl mice inoculated with LLC cells. Bar charts indicate the relative percentage of $C_{12}FDG^+$ cells on DMSO. **(B-E)** FACS quantification of $C_{12}FDG^+$ **(B)** TAMs, **(C)** TANs, **(D)** TAECs, and **(E)** LLCs cells in whole tumors. Bar charts indicate the relative number of $C_{12}FDG^+$ cells to total cells. **(F)** Gating strategy used to analyze the cell cycle status of viable LLC cells in tumors.

FACS data represent more than 1 independent experiment with ≥2 biological replicates. (A-E) Two-tailed unpaired Student's *t* test. *P <0.05, **P <0.01, ***P <0.001. Error bars represent the mean ± SEM. Each *n* represents one biological replicate.

ARRIVE guidelines for animal research

Essential 10

1. Study design

1a. **Groups compared**: Our study uses the combination of two experimental models: A diet-induced obesity model (DIO) and a syngeneic tumor inoculation model. We fed 8-week-old C57Bl/6 WT mice with either a HFD (DIO) or a nutritionally matched RD (CTRL) for 6 weeks. After 4 weeks of diet, we inoculated LLC, B16-F10 and MC-38 cells via syngeneic injections and monitored tumor growth every second day for 2 weeks. We also orally administered ABT-263 to the animals for 5 consecutive days (from D7 to D11 post tumor inoculation). The dosage was of 75mg/kg/day and the control is DMSO (vehicle). For each in vivo experiments, tumors and other relevant tissues were dissected 14 days after tumor inoculations. Diagrams representing our experimental paradigms can be found in **Supplementary Figure S1, S2 and Figure 2**.

1b. **Experimental unit**: Each experimental unit (n) is typically an animal, or a tumor from an animal. Regarding the single cell analysis, the experimental unit is all the sorted cells from one tumor. When the number of cells of a sorted cell type in one tumor is low, we pooled multiple tumors together. The experimental unit is then the pooled cells from 2 or more tumors. The specific nature of the experimental unit is always specified in the figure legends.

2. Sample size

2a. **The exact number of experimental units**: The exact number of animals used might differ from the total experimental units showed in the figures following the respect of our inclusion/exclusion criteria (See next section). **Supplementary Table S5** recapitulates all experimental units and animal numbers for each *in vivo* experiments of our study.

2b. **Rationale behind sample size**: The sample size of each experiment was based on previous experiments and existing literature references. 4 to 17 biological replicates per group were used for *in vivo* studies, as indicated in **Supplementary Table S5**.

3. Inclusion and exclusion criteria

3a. **Criteria**

Tumor ulceration: An experimental unit or an animal is excluded from the experiment when the tumor shows signs of an important ulceration. In addition to causing unnecessary pain to the animal, tumor ulceration affects the integrity of the tumor micro-environment and is therefore considered as an exclusion criterion.

Mouse reaches limit points: All animals used in our study were treated in agreement with the Animal Care Committee of the Maisonneuve-Rosemont Hospital Research Center and following the guidelines established by the Canadian Council on Animal Care. All animals that would reach the limit points of our animal protocol (e.g. tumor ulceration, >20% weight loss, tumor volume that is exceeding 1.5cm^3) would be euthanized and excluded from our study.

3b. All excluded units have not been considered in the statistical analysis and are presented in **Supplementary Table S5**.

3c. The exact values of n for all experiments of our study are shown in **Supplementary Table S5** and in the **Figure section**.

4. Randomization

4a. **Randomization method**: Animals were randomized using the online GraphPad Prism random number generator. However, for the sake of space at our animal facility, mice were kept in the least cage possible. Thus, small modifications to the randomization were done with respect to the space constraints. Also, ABT-263 treated mice (vs DMSO vehicle) were determined before tumor inoculation by another investigator to avoid biases.

4b. **Strategy to minimize potential confounders**: All tumor inoculations and tumor monitoring measurements were done by the same person. Tumor inoculations were performed blindly. All animal cages were always kept on the same lanes on the experimental rack in the animal facility, but cages were moved among that lane at every tumor monitoring day (every second day). All tumor monitoring handling and animal dissections were performed in the morning by the same investigators (7h00-10h00).

5. Blinding

Tumor inoculations were performed blindly. One investigator put the animals to sleep, while the other injected the cancer cells subcutaneously without knowing about the animal diet, genetic background or treatment (ABT-263 or DMSO). During tumor monitoring, the principal investigator knew which group was assessed in which order. Concerning the senolytic experiment, animals receiving the treatment were determined prior to tumor inoculation in a randomized fashion and the investigator treating mice by gavage became aware of the dedicated group of each mouse at the moment of the first treatment. The outcome analysis was always confirmed by at least a second independent investigator, and data analysis were confirmed or performed by a different investigator (third party) to avoid bias.

6. Outcome measures

6a. **Outcome measure assessed:** The outcome measures are always specified in figure legends to allow total transparency. Here is the outcome measures justifications for the *in vivo* experiments. All *ex vivo* outcome measures are also clearly specified in the Figure legends section.

- Tumor monitoring units: volumetric measurements (length and width) and radiance measures (p/s/cm^2/sr) were employed to evaluate tumor volume at each time point. We used two complementary assessments to overcome possible biases and/or artefacts, such as skin color, tumor depth, cell types heterogeneity, tissue fibrosis, etc. Bioluminescent signal from cancer cells were showed as radiance to illustrate the photon emission intensity from an animal per second, as opposed to a total count of photon incident on a detector.
- Weight gain units: We are showing percentage of weight gain through time. This outcome measure considers the initial weight of each animal on the average weight gain of each group.

6b. **Primary outcome measure:** This work does not primarily focus on a therapy efficiency or the impact of a given treatment on a phenotype. Our hypothesis was not only verified via therapeutical *in vivo* experiments. Several outcome measures were considered and explicitly described in the Methods and Figure legends sections.

7. Statistical methods

7a. **Statistical methods details:** All the statistical methods that were used are mentioned and explained in the appropriate section of the Figure legends. Every graph illustrates the mean value ± the standard error of mean (Mean ± SEM) and each symbol on the graphs represent one experimental unit. The choice of the statistical analysis took into consideration the number of groups and the number of variables that are compared within one single analysis. When necessary, our data was normalized on a housekeeping gene and on the control group (RD-WT). Further information regarding data normalization is mentioned in the Figure legends section.

7b. **Methods used to assess whether the data met the assumption of the statistical approach:** We did not use any test for normality or performed any data transformation. All included experimental units (see **Supplementary Table S5**) were plotted on graphs and represented as a symbol to show biological variability among groups and parametric analysis were performed to assess whether difference observed were biologically relevant and significant.

8. Experimental animals

8a & b. **Animals appropriate details:** Details about experimental animals and cell lines that were either used or produced for this article are exhaustively described in the Methods section. In brief, the cancer cell lines cultured and inoculated were the Lewis Lung Carcinoma cell line LLC1 (referred in this paper as LLC), the B16-F10 melanoma cell line and the MC-38 colorectal cell line. We generated an LLC transgene expressing the mCherry and the Luciferase protein reporters, which allowed us to follow LLC cells both *in vivo* and *ex vivo* using their fluorescent and bioluminescent properties. C57BL/6J WT mice were purchased from the Jackson Laboratory and bred in house. 8-week-old male mice were put under a HFD (DIO) or a control nutritionally matched RD (CTRL) for 4 weeks prior to tumor inoculation.

9. Experimental procedures

9a. **What was done, how it was done and what was used?** Experimental paradigms are exhaustively described in the Methods section and schematics representing the experimental timeline are available in the paper figures.

9b. **When and how often?** All strains were kept in 12h-12h light & dark cycles with free access to water and food. All the *in vivo* tumor monitoring experiments and animal sacrifices/terminal surgical interventions were performed in the morning (7am-10am). All weight measurements were done once a week in the afternoon (1pm-4pm).

9c. **Where?** All animals' procedures were performed by the same investigators in the same dedicated space within the animal facility. All the procedures were done at the same site for all the animals involved in this study.

9d. **Why?** Rationale for the different procedures is described in the Methods section.

10. Results

10a. **Summary descriptive statistics for each experimental group:** See **Supplementary Table S5** and section 7 for a summary descriptive. The Figure legends section also presents an exhaustive summary of all experimental units and statistics used for each experiment.

10b. **Effect size with a confidence interval:** All analysis performed, with a statistically significant result or not, are reported in the Figure section along with the experimental units that were used for each one of them so that the reader can appreciate the attention we put on biological relevance (See also **Supplementary Table S5**). Moreover, every experimental unit is shown and represented as a symbol on the graphs. In sum, although we did not strictly perform effect size calculations, we always took into consideration the biological relevance of an experimental change by considering fold change between groups and confidence intervals.

SUPPLEMENTARY TABLES

Supplementary Table S1. Clinical data of tumors analyzed in the study

PATIENT	BMI	SEX	AGE	RUBBIA BRANDT	BLAZER	# LESIONS	TUMOR (cm)
LEAN 1	18.9	M	65	4	minor	1	3.2
LEAN 2	15.7	M	56	3	major	1	2.5
LEAN 3*	18.2	F	64	3	major	2	0.6
OBESE 1	39.7	M	62	no data	no data	1	5.0
OBESE 2*	45.6	F	68	4	minor	1	1.5
OBESE 3	35.1	M	67	4	minor	3	2.5

Supplementary Table S2. Primary antibodies

ANTIBODY	METHOD	CLONE	SPECIE	DILUTION	SOURCE	CAT#
BrdU (APC)	FACS	Bu20a	Mouse	Titration by lot	Biolegend	339807
β-ACTIN	WB	N/A	Mouse	1:5000	Cell Signalling	3700S
BCL-2	WB	N/A	Rabbit	1:1000	Abcam	ab182858
BCL-w	WB	N/A	Goat	1:1000	R&D Systems	AF8241
BCL-xL	WB	N/A	Rabbit	1:1000	Cell Signalling	2764S
C_{12}FDG-FITC	FACS	N/A	N/A	2mM	Invitrogen	D2893
CD11B (BV711)	FACS	M1/70	Rat	Titration by lot	Biolegend	101242
CD11C (BV786)	FACS	N418	Armenian Hasmter	Titration by lot	Biolegend	117335
Caspase3	WB	N/A	Rabbit	1:1000	Cell Signaling	9662S
CD16/32 (Fc Block)	FACS	93	Rat	1μL/10^6 cells	Biolegend	101330

CD31 (APC)	FACS	MEC 13.3	Rat	Titration by lot	BD Pharmingen	551262
CD45.2 (AlexaFluor700)	FACS	104	Mouse	Titration by lot	Biolegend	109822
CD64 (APC)	FACS	X54-5/7.1	Mouse	Titration by lot	Biolgend	139305
F4/80 (PE/Cy7)	FACS	BM8	Rat	Titration by lot	Biolegend	123114
F4/80	IF	BM8	Rat	1:300	Invitrogen	14-4801-82
$IgG2_{aK}$ (APC)	FACS	N/A	Rat	Titration by lot	BD Pharmingen	553932
Ly-6G (APC/Cy7)	FACS	1A8	Rat	Titration by lot	Biolegend	127624
$p16^{INK4A}$	WB	N/A	Rabbit	1:500	Abcam	ab211542
$p16^{INK4A}$	IF	N/A	Rat	1:100	Abcam	ab241543
$p21^{CIP1}$	WB	N/A	Rabbit	1:2000	Abcam	ab109199
PAI-1	IF	N/A	Mouse	1:100	Santa Cruz	sc-5297
PAI-1	WB	N/A	Mouse	1:500	Santa Cruz	sc-5297

Supplementary Table S3. Primer sequences

Gene	Primer sequence	
mActb	Forward	GACGGCCAGGTCATCACTATTG
	Reverse	CCACAGGATTCCATACCCAAGA
mIl6	Forward	CTTCCATCCAGTTGCCTTC
	Reverse	ATTTCCACGATTTCCCAGAG
mNos2	Forward	CGGCAAACATGACTTCAGGC
	Reverse	GCACATCAAAGCGGCCATAG
mSerpine1	Forward	TGACGTCGTGGAACTGC
	Reverse	GAAAGACTTGTGAAGTCGGC
mTgfb1	Forward	ACGCCTGAGTGGCTGTCTTTTGAC
	Reverse	GGGCTGATCCCGTTGATTTCCACG
mVegfa	Forward	GCCCTGAGTCAAGAGGACAG
	Reverse	CTCCTAGGCCCCTCAGAAGT

Supplementary Table S4. Key resources related to the Methods

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
Gamma-retrovirus	Neault M *et al.*, Cell Reports, 2016	N/A
Chemicals, Peptides, and Recombinant Proteins		
ABT-263	Selleckchem.com	Cat# S1001
Bafilomycin A1	Sigma Aldrich	Cat# B1793
BrdU	Abcam	Cat# ab142567
C_{12}FDG (FITC)	Invitrogen	Cat# D2893
Collagenase IV	Worthington	Cat# LS004188
DAPI	Sigma Aldrich	Cat# D9542
D-Luciferine potassium salt	Perkin Elmer	Cat# 122799
DNaseI	Sigma Aldrich	Cat# D4527
Hexadimethrine bromide	Sigma Aldrich	Cat#107689
HumulinR (Insulin)	Lilly	Cat# DIN00586714
Liberase TL	Sigma Aldrich	Cat#540120001
Lipofectamine® 2000	Invitrogen	Cat# 11-668-019
Phosphatase inhibitor	Sigma Aldrich	Cat# P5726
2-Propanol	Sigma Aldrich	Cat# 650447
Propidium Iodide	Sigma Aldrich	Cat# P4864-10mL
Protease inhibitor	Sigma Aldrich	Cat# P8340
RBC Lysis Buffer	Invitrogen	Cat# 00-4333057
UltraComp eBeads	Invitrogen	Cat# 01-2222-42
X-Gal	BioShop	Cat# XGA00.1

Xylenes	Sigma Aldrich	Cat# 214736
Zombie aqua	Biolegend	Cat#423102
Critical Commercial Assays		
Caspase-Glo® 3/7 Assay System	Promega	Cat#G8090
Cytofix/Cytoperm kit	Biosciences	Cat# 554714
Mouse & rabbit HRP/DAB (ABC) detection IHC kit	Abcam	ab64264
QuantiPro BCA Assay Kit	Sigma Aldrich	Cat# QBPCA-1KT
Deposited Data		
Experimental Models: Cell Lines		
B16-F10	ATCC	CRL-6475
HEK 293T	ATCC	CRL-3216
Lewis Lung Carcinoma (LLC)	ATCC	CRL-1642
Lewis Lung Carcinoma ($LLC^{LUC\text{-}mCherry}$)	This paper	N/A
MC-38	ATCC	
Experimental Models: Organisms/Strains		
Mouse: C57BL/6J (referred as WT)	Jackson Laboratories	No.000664
Recombinant DNA		
Plasmid: MSCV-LUC-IRES-mCherry	MI-Luciferase-IRES-mCherry was a gift from Xiaping Sun (Addgene plasmid; http://n2t.net/addgene:75020)	Cat# 75020; RRID: Addgene_75020

Plasmid: pCL-ECO	pCL-ECO was a gift from Inder Verma (Addgene plasmid # 12371 ; http://n2t.net/addgene:12371)	Cat#12371; RRID: Addgene_12371
Software and Algorithms		
Fiji (ImageJ)	https://imagej.net/Fiji	V1.0
FlowJo	https://www.flowjo.com/	V10.2
GraphPad Prism	https://www.graphpad.com/scientific-software/prism/	V8.4.3
Living Image	Perkin Elmer (https://www.perkinelmer.com/fr/lab-products-and-services/resources/in-vivo-imaging-software-downloads.html)	V4.7.3
Other		
AlphaTrak2 blood glucose test strips	Zoetis	Cat# 71681-01
Mouse diet: High Fat Diet 60% lipids	Research Diet	Cat#D12492
Mouse diet: Regular Diet 10% lipids	Research Diet	Cat#D12450J

Supplementary Table S5. Sample size justification for *in vivo* experiments

Figure	Experiment	Cancer cell line	Group/ Condition	N	Total mouse number (Δ)	Exclusion/inclusion criteria
1C	Tumor monitoring *in vivo*	LLC	CTRL-WT	6	6	1. Tumor ulceration 2. Mouse reaches limit points (CCPA)
			DIO-WT	7	7	
1D	Tumor monitoring *in vivo*	B16-F10	CTRL-WT	6	6	
			DIO-WT	5	5	
1E	Tumor monitoring *in vivo*	MC38	CTRL-WT	6	6	
			DIO-WT	6	6	
S1C	Weight gain monitoring	N/A	CTRL-WT	10	10	1. Mouse reaches limit points (CCPA)
			DIO-WT	11	11	
S1D-E	GTT/ITT	N/A	CTRL-WT	5	5	1. Mouse reaches limit points (CCPA)
			DIO-WT	5	5	
2B	Tumor monitoring *in vivo*	LLC	DIO-WT-DMSO	9	9	1. Tumor ulceration 2. Mouse reaches limit points (CCPA)
			DIO-WT-ABT263	9	9	
2C	Tumor monitoring *in vivo*	B16-F10	DIO-WT-DMSO	5	5	1. Tumor ulceration 2. Mouse reaches limit points (CCPA)
			DIO-WT-ABT263	5	5	
2D	Tumor monitoring *in vivo*	MC-38	DIO-WT-DMSO	5	5	1. Tumor ulceration 2. Mouse reaches limit points (CCPA)
			DIO-WT-ABT263	5	5	
3D-E	Tumor monitoring *in vivo*	$LLC^{LUC\text{-}mCherry}$	CTRL-WT-DMSO	6	6	1. Tumor ulceration 2. Mouse reaches limit points (CCPA)
			CTRL-WT-ABT263	5	5	
			DIO-WT-DMSO	4	4	

			DIO-WT-ABT263	5	5	
S3E-F	Tumor monitoring *in vivo*	$LLC^{LUC-mCherry}$	CTRL-WT	5	6(1)	1. Tumor ulceration 2. Mouse reaches limit points (CCPA)
			DIO-WT	7	7	

*Numbers in red represent the number of excluded animals when applicable