SUPPLEMENTAL METHODS

Exposures

High Fat Diet: 42% Fat diet (HFD) was ordered from Harlan Laboratories (TD.88137). At 8-10 weeks of age, mouse chow was changed to HFD vs. normal diet (4% fat) after initial baseline imaging. New diet was refilled once a week. Twenty male and female SKH1-E p16\textsuperscript{+/LUC} mice were analyzed per dietary cohort (42% vs. 4%) for 84 weeks.

Hepatic Steatosis Evaluation: Livers were incubated in 4% Osmium overnight, washed with diH\textsubscript{2}O, and then fixed with 10% Formalin. Tissues were then imbedded, sectioned and stained with Hematoxilyn and Eosin using the Immunohistochemistry core facility.

Data Analysis: Linear regression analysis was completed to determine the relationship between p16\textsuperscript{LUC} signal and weight in HFC cohort at 52 weeks of exposure.

Arsenic: Sodium arsenite (As) was ordered from Sigma (71287-250G) and dissolved in sterile water at 50 ppm. At 10-12 weeks of age, mouse water was changed to 50 ppm vs. 0 ppm arsenic after initial baseline imaging. New water was refilled once a week. Twenty male and female SKH1-E p16\textsuperscript{+/LUC} mice were analyzed per arsenic cohort (50 ppm vs. 0 ppm) for 48 weeks. Arsenic accumulation in liver was measured using mass spectrometry analysis.

Pancreatic Islet Measurements: Randomized 10x histological images were taken of the pancreas in each cohort. Each islet was then carefully traced and the area was measured using imageJ analysis.

Cigarette Smoke: Cigarettes were ordered from the University of Kentucky (3R4F)—through the Reference Cigarette Program at College of Agriculture in Lexington, Kentucky. Beginning at age 10-12 weeks after baseline imaging, mice were exposed to one hour of cigarette smoke/day, five days/week. For exposure, mice were put into an inExpose exposure system providing both mainstream and side stream smoke exposure. Control animals (no smoking
exposure) were handled in an identical manner except were not exposed to tobacco smoke. Ten male SKH1-E p16+/LUC mice were analyzed per tobacco cohort (smoking vs. non-smoking). Animals were exposed to tobacco smoke for 24 weeks, but were imaged for an additional 32 weeks after exposure.

**UV light:** A UVB lamp from UVP was used for these studies, with an emission spectrum of 290-350 nm light, and with a peak emission at 312 nm. At 8-10 weeks of age, mice were exposed to 353 J/m³ of UVB light three times per week for 24 weeks. UV exposure began after initial baseline imaging. Fifteen male SKH1-E p16+/LUC mice were analyzed per UVB cohort (exposed vs. unexposed) for 16 more weeks after exposure. UV exposed mice were sacrificed for morbidity or tumor burden in accordance with established laboratory protocols.

**Phospho-Histone H2A.X Immunohistochemistry:** Skin tissues were incubated overnight with 1/500 of Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Cell Signaling Cat # 9718) according to Cell Signaling standard immunohistochemistry instructions.

**Data analysis:** All H2AX cells in the epithelial layer of the exposed skin were divided by the total number of epithelial cells in the microscopic field. Fisher’s exact test was used to determine statistical significance.

**Senescence-Associated ß-galactosidase (SA-ß-gal) activity:** Skin was assayed using Cellular Senescence Assay Kit (KAA002; Millipore, USA) according to manufacturer’s instructions.

**Imaging**

**In vivo Luminescence Imaging:** To serially monitor luciferase induction, mice were imaged as previously described (18) using a Xenogen IVIS LUMINA/KINETIC Imaging System. Imaging was completed under anesthesia, immediately after D-luciferin injection (10 mg/ml in sterile PBS). Sequential imaging was used to determine optimal luminescence. A sequence of 2 min, 2 min, 2 min, 2 min, 30 sec, and 1 min were used. The 4th two minute image (8
minutes after initial injection) was used for all reported imaging measurements. For arsenic, high fat diet, and cigarette smoke exposure images were taken of the ventral side of the mouse. For UV light, the dorsal (exposed) and ventral (unexposed) sides of each mouse were taken. Living Image 3.1 Software (Caliper Life Sciences) was used to analyze the images at the same image exposure time point (8 min) over the life span of the mouse. Representative images are shown with exposed and unexposed animals for each cohort, as well as appropriate imaging scales to account for imaging settings. For data analyses, the raw average radiance values (photon/sec/cm$^2$/steradian) from the original images were used. A wide angle lens (FOV-24) was used to capture images of 3 or more mice.

**Data Analysis:** For each exposure, mice were imaged at least every other month for up to one year, although some animals were imaged more frequently during the initial stages of each exposure period. Each mouse was circled to measure average radiance values (p/s/cm$^2$/sr) over the region of interest. The area for each mouse was held constant for each experiment. A blank area on the image was also circled to allow for subtraction of background noise. To omit background noise from each individual mouse image, the background circle was subtracted from the mouse circle. To graph the luciferase induction over time, all murine luciferase signals in a specific cohort were averaged at time zero and normalized to a value of one. All subsequent mouse images were then normalized relative to the average time zero luminescence.
**Supplemental Figure 1.** Physiologically effective doses of high fat diet do not affect the survival rates of mice, and quantitative realtime PCR validates p16\(^{Ink4a}\) expression as measured by TBLI. (A) The body mass of mice fed a high fat (HFD; 42% fat) or normal (ND; 4% fat) diet during the 52-week period of exposure (orange bar) (HFD, n=24; ND, n=15). *p<0.05, **p<0.01. (B) Representative histopathological images of hemotoxylin and eosin (top) and osmium tetroxide (bottom) stained liver tissue from HFD mice ND mice at 84 weeks of exposure. Hepatocytic lipid droplets are stained black and indicate severe steatosis in the high fat chow liver (right). (C) Kaplan-Meier curve of mortality in the HFD and ND cohorts (p=0.8078) (D) Graph measuring individual weight vs luciferase induction (\(r^2=0.024\)). (E) Quantitative realtime PCR analysis of p16\(^{Ink4a}\) expression in livers and spleens of each cohort at the after 84 weeks of exposure. Orange bar on x-axis indicates duration of exposure. Error bars indicate standard error of the mean.
Supplemental Figure 2. Physiologically effective doses of arsenic do not affect the survival rates of mice, and quantitative realtime PCR validates p16\(^{\text{INK4a}}\) expression as measured by TBLI. (A) Level of arsenic (ng/g) found by mass spectrometry in the liver of mice treated with 50 ppm or 0 ppm arsenic for 48 weeks. (B) Representative hematoxylin and eosin stained sections of the pancreas 48 weeks following exposure to 0 ppm or 50 ppm arsenic. (C) Area of individual islets in the pancreas of mice exposed to 0 ppm or 50 ppm arsenic. (D) Kaplan-Meier curve of mortality in the 50 ppm and 0 ppm cohorts (p=0.5164). (E) Quantitative realtime PCR of p16\(^{\text{INK4a}}\) in the liver and spleen in 50 ppm and 0 ppm mice after 52 weeks of exposure. Orange bar on x-axis indicates duration of exposure. Error bars indicate standard error of the mean. ***p<0.0001
Supplemental Figure 3. Cigarette smoke, at the concentrations and durations of exposure in these experiments, does not affect the survival rates of mice, and quantitative realtime PCR validates p16^{Ink4a} expression as measured by TBLI. (A) Kaplan-Meier curve of mortality in the CS and AA cohorts (p=0.4361). (B) Quantitative realtime PCR of p16^{Ink4a} expression in the livers, spleens, lungs and lymph nodes of CS and AA mice after 52 weeks of exposure. Orange bar on x-axis indicates duration of exposure. Error bars indicate standard error of the mean. ***p<0.0001
Supplemental Figure 4. **UV light leads to a senescent phenotype in skin.**

(A) Quantitative analysis of H2AX staining in the back skin of UV exposed and non-exposed cohorts. (B) SA β-galactosidase staining of skin from the backs and abdomens of UV exposed and unexposed mice. Unexposed back skin from young mice (8 weeks old) was used to determine background staining (bottom). **p<0.01