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Sinai SCENT TMC - 5x5 Project Mouse Harvesting

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Cellular Senescence Net…

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Protocol status: Working
We use this protocol and it's working

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Abstract

We propose a total of 10 C57BL/6 mice (5 males and 5 females) to be studied for each study group or cellular senescence inducer intervention.

Materials

See the steps

Experimental Design

1 Males and females are grouped and housed in standard cages, provided LabDiet 5K0G and acidified water ad libitum. Euthanasia is performed by cervical dislocation (CMQ93-26).

- **Control:** Tissues collected at 32 weeks.
- **Doxorubicin:** IP injection (5 mg/kg) at 26 weeks on days 0 and 10, with tissue collection at 32 weeks.
- **Palbociclib:** Daily oral gavage (150 mg/kg) for 10 days starting at 26 weeks, with tissue collection at 32 weeks.
- **High-Fat Diet:** From 24 weeks, animals receive a 45% fat diet until tissue collection at 32 weeks.
- **Aging Controls:** Tissues collected at 24 months.

Lung Tissue Collection

2 **Materials:** 26G needle, 1 mL syringe, balance, surgical board, 70% EtOH, scissors, 2 forceps, suture thread, 19G cannula, E-tube (1 per sample), normal formalin, tools for lung inflation, histology container with 10 mL of normal formalin (1 per sample)

3 **Preparation:** After euthanasia, apply 70% ethanol to the throat to wet the fur.

4 **Incision:** Lift the skin and make a vertical cut on the throat to expose the salivary glands. Use forceps to expose the trachea.

5 **Tracheal Access:** Make a horizontal incision between the second and third tracheal rings. Insert a 19G cannula and secure it with a suture thread.

6 *Optional: Go to "Bronchoalveolar Lavage Collection"*

7 *Optional: Go to "Blood Collection and Serum Separation"*

8 **Lung Isolation:** Carefully remove the lung with the trachea and cannula intact.

9 **Tissue Processing:**

- **Right Lobe:** Isolate, freeze in liquid nitrogen, and store at -80°C.
- **Left Lobe:** Fix in 10% formalin overnight, transfer to 70% ethanol, and prepare for histology.

Bronchoalveolar Lavage (BAL) Collection

10 **Materials:** 26G-syringe, 70% ethanol spray, surgery board with needles/pins, 2 forceps, 1 scissors, 4.0-suture string, 19G-cannula (brown color), PBS, 1 mL syringe, e-tube, notebook/paper, pens

11 Slowly inject 0.8 mL of ice-cold PBS into the lungs (speed: 0.1 mL/sec), pause for 2 – 3 sec, and then slowly retrieve the BAL. Record the collected volume of BAL and transfer BAL to theE-tube (label: BAL cells) on ice. Repeat Steps with another 0.8 mL of ice-cold PBS. Save the BAL in one tube/sample.

12 Save the collected BAL on the ice during harvest and then continue for the “BALF and BAL cell separation”
procedure

BAL Fluid (BALF) and BAL cell separation

13 **Materials:** 1 mL pipette, tips, e-tubes, marker pen, dry ice, waste bin, e-tube rack, timer, PBS (keep on ice), ice bucket, RBC lysis buffer

14 Centrifuge BAL sample: 3,000 rpm, 10 min, 4°C

15 Transfer the supernatant (=BALF) to a new E-tube (label: BALF) → dry ice or liquid nitrogen → -80'C

16 CRITICAL: Cell pellet + 1 mL room temperature RBC lysis buffer → immediately vortex for 3 seconds →
Incubation: 5 min, ice. Incubation time is critical.

17 Centrifuge: 3,000 rpm, 10 min, 4°C

18 Discard supernatant using a pipette. Do not disturb the pellet.

19 If you want to count the WBC, proceed to step 5. Or you can save BAL cell → dry ice or liquid nitrogen →
-80'C

20 Optional: Cell pellet + 500 uL of ice-cold PBS. Make the single-cell suspension by pipetting.
CRITICAL Un-even cell suspension affects cell number as well as the quality of the Cytospin

Blood Collection and Serum Separation

21 **Materials:** 26G-syringe, surgery board with needles/pins, 1 forceps, 1 scissors, 23G-syringe, e-tube

22 Open the abdominal cavity. Remove all abdominal organs to reveal the inferior vena cava, which is located in the middle of the back of the body.

23 Insert a 26G syringe into the inferior vena cava and collect the blood slowly.

24 Transfer the collected blood to the e-tube. CRITICAL do not drop the blood. gently transfer the blood from
the syringe to E-tube unless RBC is destroyed and affects the colorimetric analysis.

25 Leave the tube at room temperature for 30 min. Do not close the lid

26 Close the lid and centrifuge: 1,000 g, 10 min, 4°C

27 Collect the supernatant (=serum) carefully and transfer it to a new e-tube. Saved it at -80'C