

# **Menopause-induced $17\beta$ -estradiol and progesterone loss increases senescence markers, matrix disassembly and degeneration in mouse cartilage**

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# **Supplementary Information for “Menopause-induced 17 $\beta$ -estradiol and progesterone loss increases senescence markers, matrix disassembly and degeneration in mouse cartilage”**

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**Supplementary File 1:** Histopathology toxicity reports assessing the effects of 4-vinylcyclohexene diepoxide and sesame oil on internal organs

**Supplementary File 2:** Histopathology toxicity reports assessing the effects of sex hormone and senolytic treatment on internal organs

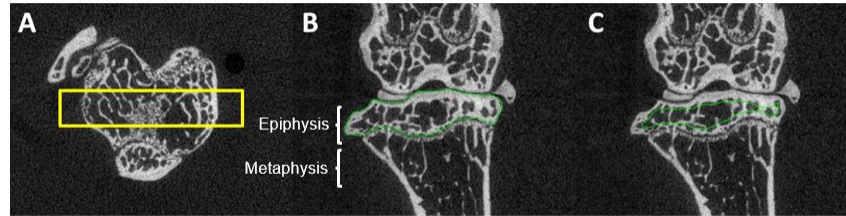
**Supplementary File 3:** Nikon Elements General Analysis 3 for CHP and Type II collagen quantification

**Supplementary File 5:** Nikon Elements General Analysis 3 for Quantification of Senescence Markers

**Supplementary File 6:** Nikon Elements General Analysis 3 Quantification of Chondrogenicity Markers

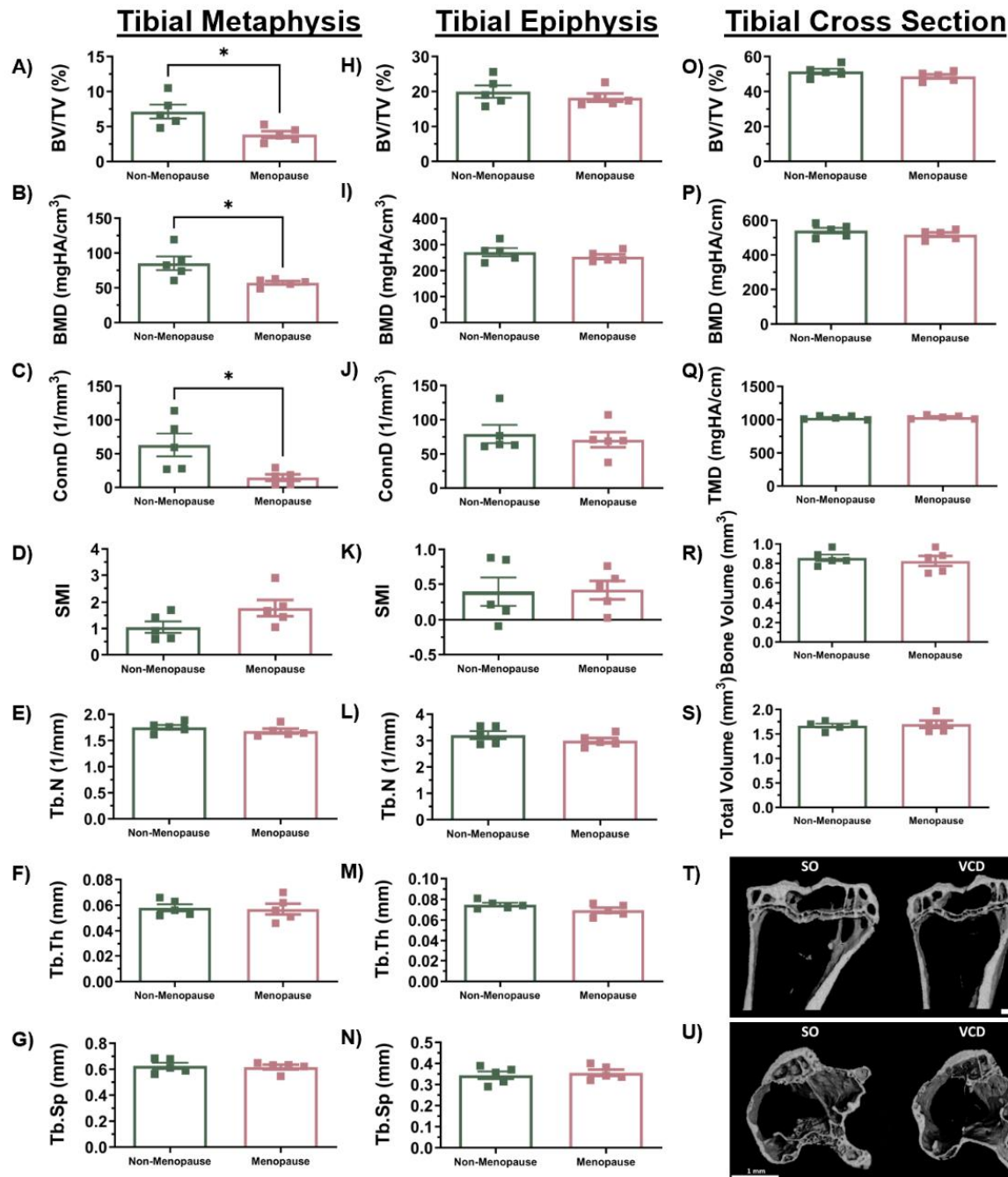
**Supplementary File 7:** ARRIVE Guidelines

**Supplementary Note**



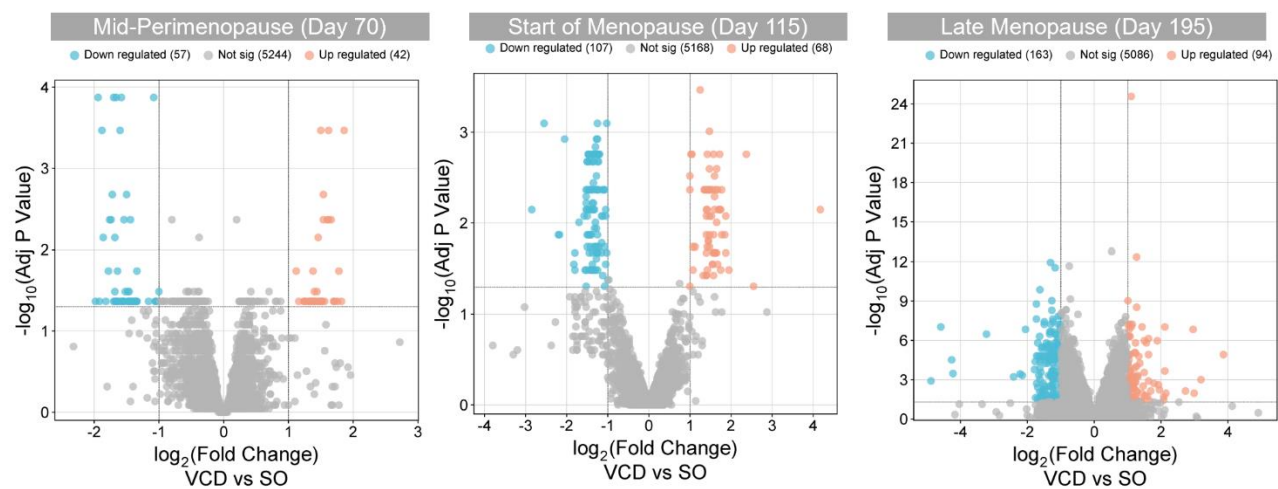
**Figure S1. Micro computed-tomography (micro-CT) scan areas shown in young samples.**

Scans were acquired in the transverse plane (A) and then axially reconstructed in the coronal plane. The whole cross-section (B) and the trabecular region (C) were manually selected (green contours) in a 1 mm (100 slice) thick region of the tibial epiphysis. The yellow box on the transverse slice (A) designates the thickness of the region that was analyzed in the coronal plane.

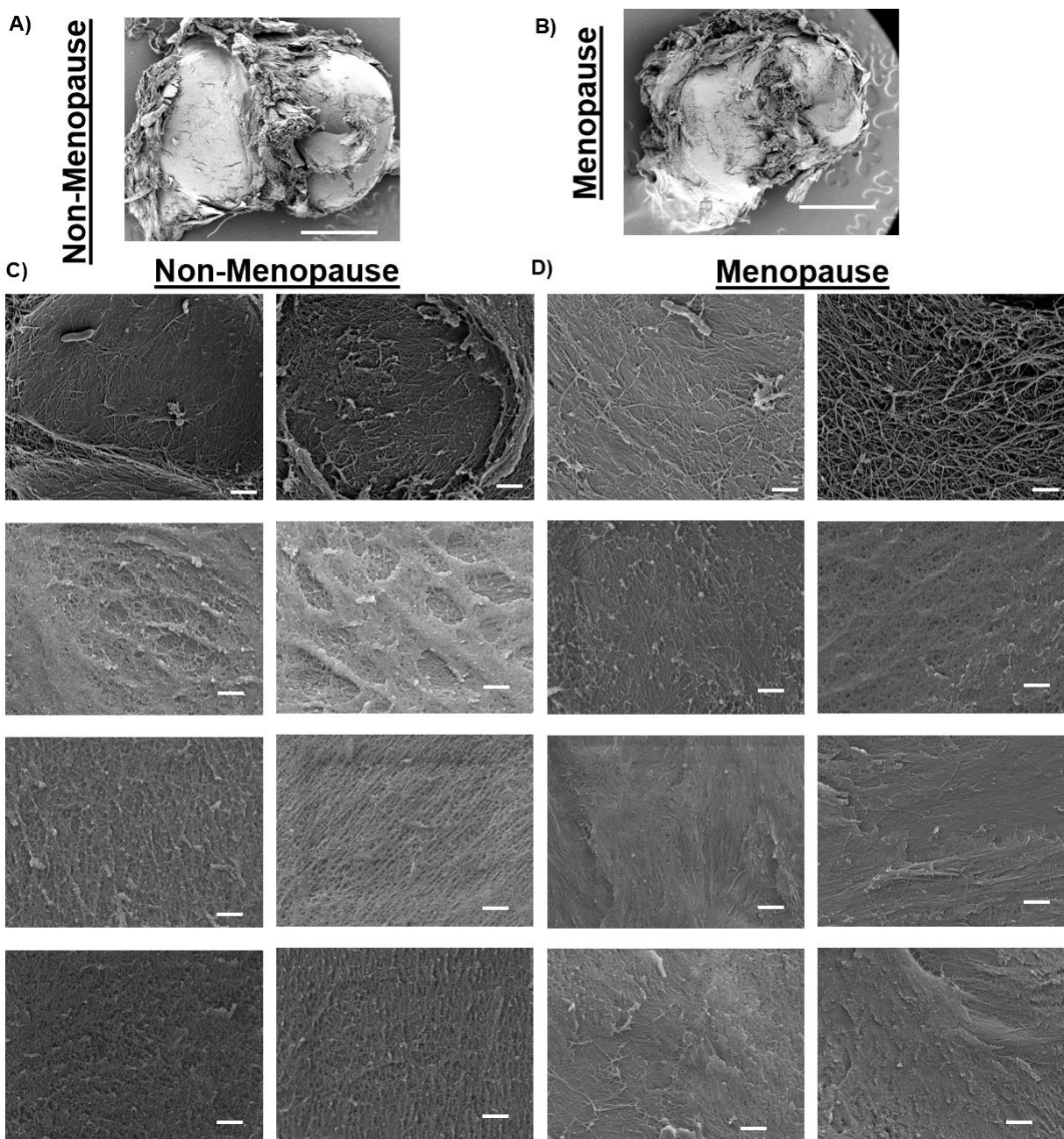


**Figure S2: Micro-CT reveals a decline in meta-physeal bone quality in the menopause group compared to the non-menopause group.**

Tibial metaphysis results: A) Bone volume (BV)/Total volume (TV). B) Bone mineral density (BMD). C) Connectivity Density (ConnD). D) Structural Model Index (SMI). E) Trabecular number (Tb.N) F) Trabecular thickness (Tb.Th). G) Trabecular space (Tb.Sp). Tibial epiphysis results: H) BV/TV. I) BMD. J) ConnD. K) SMI. L) Tb.N. M) Tb.Th. N) Tb.Sp. Tibial cross section results: O) BV/TV. P) BMD. Q) TMD. R) Bone volume. S) Total Volume. T) Representative images of coronal view. U) Representative images of transverse view. Image location on tibia is available in **Figure S1** and variable definitions are available in **Table S5**. All statistical tests for these outcome variables were Mann Whitney U tests and n=5/group. Statistical information is listed in **Table S13**. Data are presented as mean  $\pm$  standard deviations, and all statistical tests were two-sided unless otherwise noted.



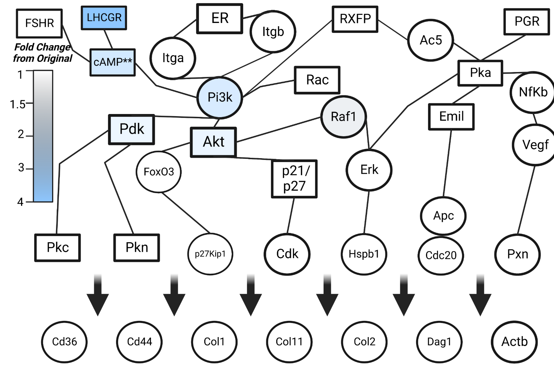
**Figure S3: Raw proteomics data by group and time point.** Y axis show  $-\log_{10}(\text{p-value})$  and x-axis shows the  $\log_2(\text{Menopause/Non-Menopause})$  protein expression. Panels are shown for menopause vs non-menopause differences at mid-perimenopause, start of menopause, and late menopause



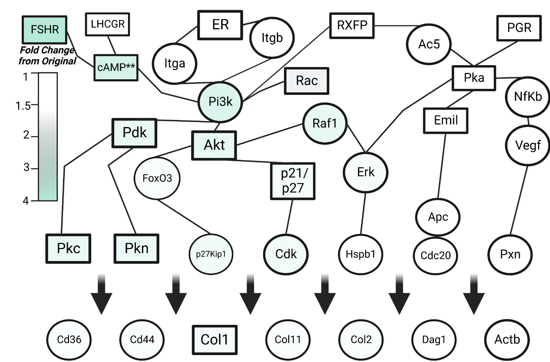
**Figure S4: Representative scanning electron microscopy (SEM) images of femoral condyles from menopause and non-menopause samples at the late menopause time point.**

A&B) 25X for menopause and non-menopause samples. Scale bar = 1 mm. C&D) 10,000X for menopause and non-menopause samples. Scale bar = 1 μm. Data were generated over three different experimental preps.

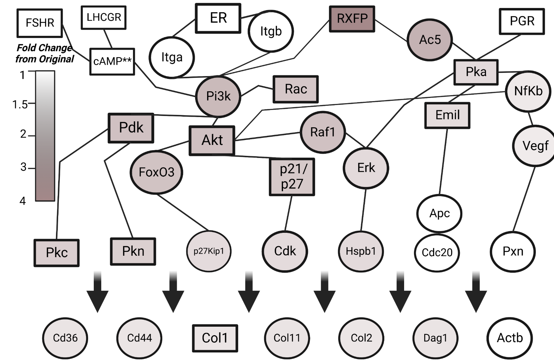
**A) Cetorelix (LH receptor Antagonist) Treatment**



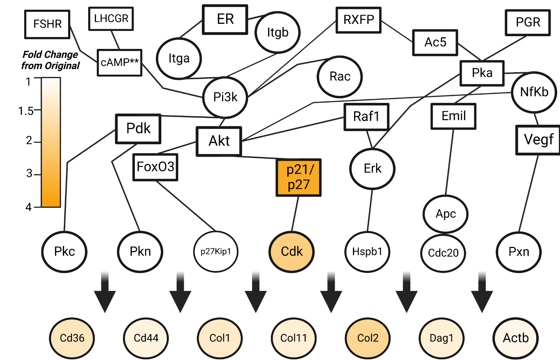
**B) Suramin (FSH receptor Antagonist) Treatment**



**C) Serelaxin (Relaxin replacement) Treatment**

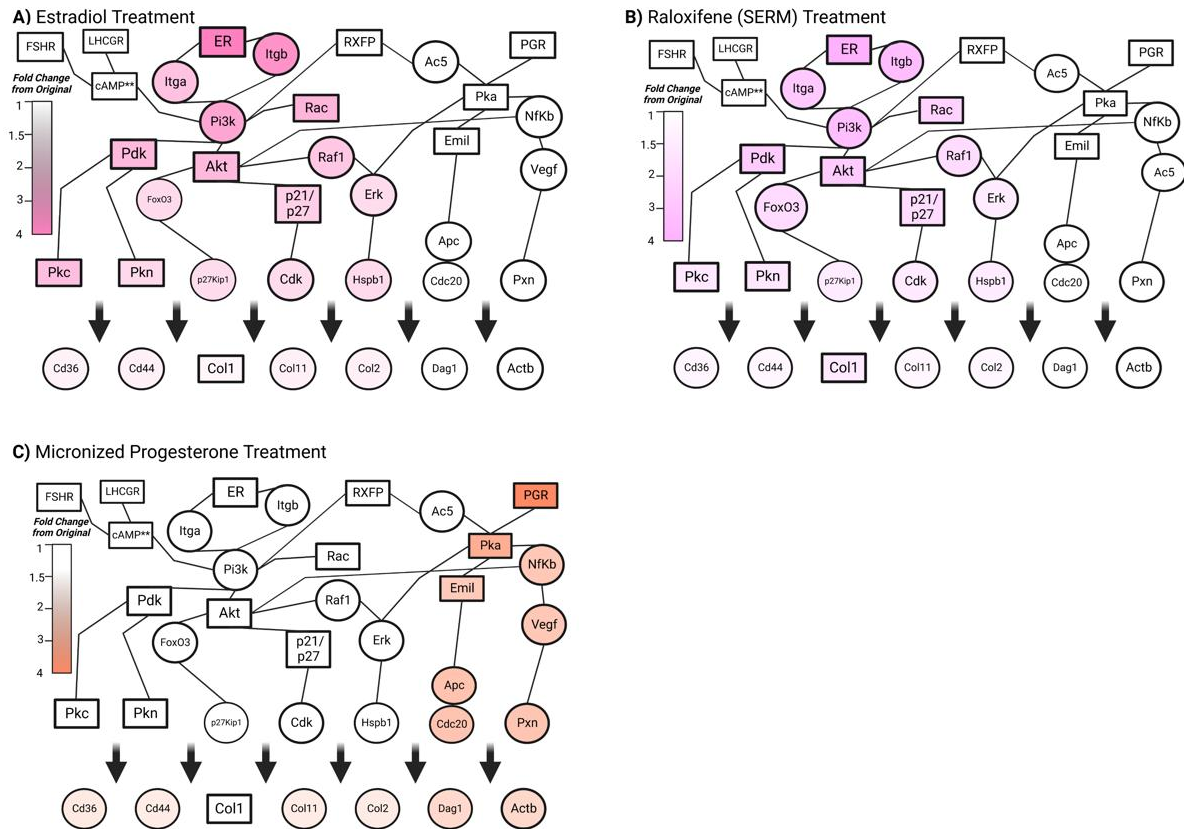


**D) SAR405838 (p53/MDM2 inhibitor) Treatment**



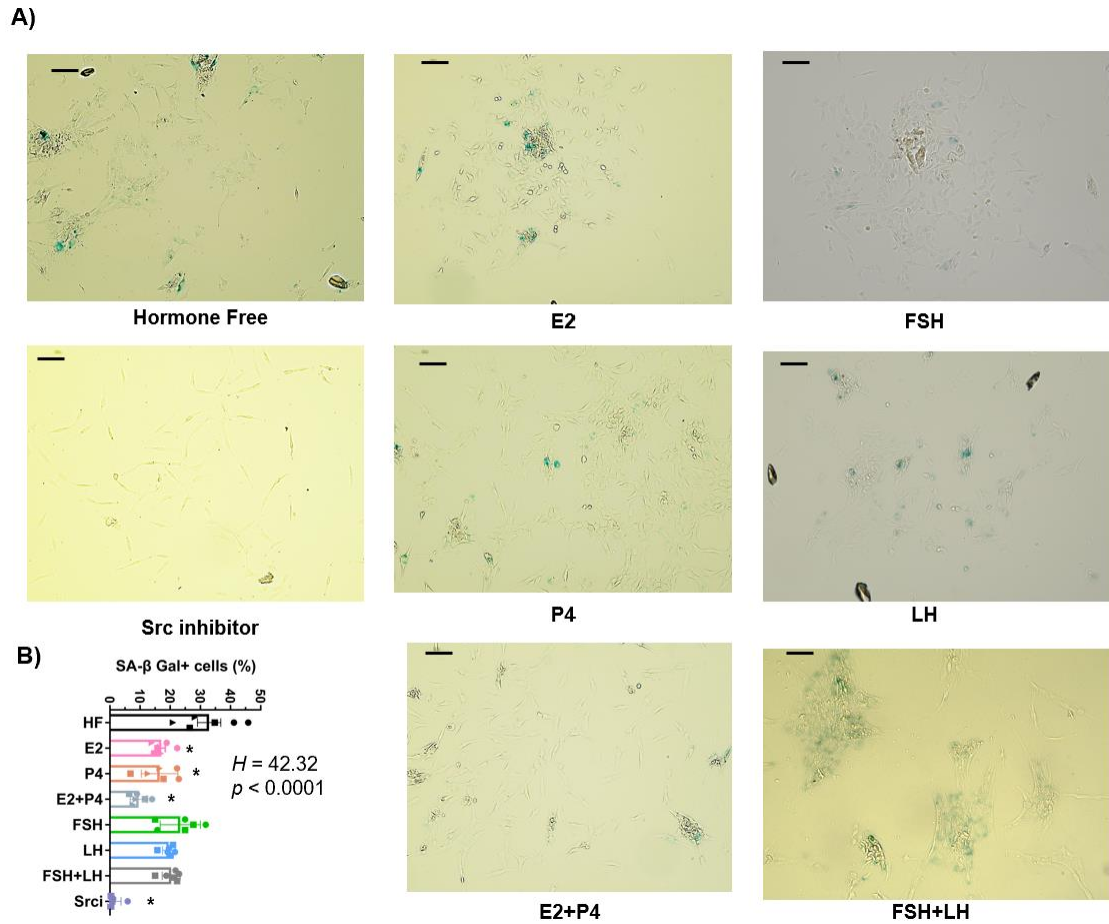
**Figure S5: Simulated effects of cetorelix, suramin, serelaxin and SAR405838 treatment on proteomic network.**

Fold change is relative to the untreated network (**Figure 4C**). A) Cetorelix treatment. B) Suramin treatment. C) Serelaxin treatment. D) SAR405838 treatment. Created in BioRender. Gilmer, G. (2023) BioRender.com/g76a916. \*\* = indicates cAMP as a summary term for other signaling.



**Figure S6: Simulated effects of 17 $\beta$ -estradiol, raloxifene and progesterone treatment on proteomic network.**

Fold change is relative to the untreated network. A) 17 $\beta$ -estradiol treatment. B) Raloxifene treatment. C) Micronized Progesterone. Created in BioRender. Gilmer, G. (2022) BioRender.com/q19a267. \*\* = indicates cAMP as a summary term for other signaling.



**Figure S7: Proportion of cells expressing senescence activated  $\beta$ -galactosidase (SA- $\beta$ -gal) in culture conditions.**

A) Representative images of SA- $\beta$ -gal. B) Quantification of proportion of SA- $\beta$ -gal cells. Kruskal Wallis test,  $n = 6/\text{group}$ . Scale bar = 200  $\mu\text{m}$ . E2 = estradiol, P4 = progesterone, Src inhibitor = Dasatinib, FSH= follicle stimulating hormone, LH = luteinizing hormone. All statistical tests were two-sided unless otherwise noted

# Histopathology toxicity reports assessing the effects of 4-vinylcyclohexene diepoxide and sesame oil on internal organs

***“Menopause-induced 17 $\beta$ -estradiol and progesterone loss increases senescence markers, matrix disassembly and degeneration in mouse cartilage”***

**Pathologist** – Lora Rigatti, VMD, DACVP, Veterinary Services, University of Pittsburgh

**Date of Report Release:** 8/15/22

**Key:**

NSL – No significant lesions

EMH – extramedullary hematopoiesis

## **Mice that received sesame oil IP injections**

Euthanized or died unexpectedly (excluded from analyses of the knee)

*Mouse A:* Final diagnosis – lung injury/disease

Lung – Multifocally, blood vessels are surrounded by mixed inflammatory cells and karyorrhectic debris.

*Mouse B:* Final diagnosis - Venous thrombosis

Thrombus present within this medium sized blood vessel within the lung

*Mouse C:* Final diagnosis – Ascites

Received 10 days of sesame oil IP injections in September 2021. Control animal. Intended endpoint April 2022 as part of knee histology and cartilage microdissection.

Significant weight gain reported 12/29 with substantial distended abdomen, vet exam ddx ascites, hemoabdomen confirmed on aspirate. Euthanized 1/4/22 immediately following aspirate.

Planned Euthanasias (mice used in knee analyses)

D – 913

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – NSL

Liver – Multifocal moderate centrilobular lymphoid infiltrates

Stomach - NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat: mild multifocal chronic lymphofollicular, plasmacytic, and histiocytic infiltrates

#### E - 915

Heart – NSL

Lungs – NSL

Spleen – Peri splenic fat – moderate multifocal lymphocytic infiltrates with peripheral macrophages

Kidneys – Multifocal moderate peripelvic and perivascular lymphoid infiltrates

Liver – NSL

Stomach - NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat: mild multifocal chronic lymphofollicular, plasmacytic, and histiocytic infiltrates with extension into the parenchyma surrounding ducts

#### F - 956

Heart – Pericardiac fat – mild multifocal lymphoid infiltrates

Lungs – NSL

Spleen – Peri splenic fat – moderate multifocal lymphocytic infiltrates with peripheral macrophages \*

Kidneys – Focal chronic severe tubulointerstitial nephritis

Liver – Focal lymphohistiocytic infiltrates adjacent to gallbladder \*; Multifocal moderate centrilobular lymphoid infiltrates

Stomach - NSL

Small intestine – Mesenteric fat - multifocal moderate subacute lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Large intestine - NSL

Pancreas – Peripancreatic fat: multifocal moderate subacute lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

#### G - 968

Heart – NSL

Lungs – NSL

Spleen – Peri splenic fat – mild multifocal lymphoplasmacytic and neutrophilic infiltrates

Kidneys – NSL

Liver – NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat: multifocal moderate subacute lymphofollicular, histiocytic, and neutrophilic infiltrates

#### H - 917

Heart – NSL

Lungs – NSL

Spleen – Peri splenic fat – mild multifocal lymphoplasmacytic and neutrophilic infiltrates

Kidneys – Mild regional peripelvic and periureteral lymphofollicular infiltrates

Liver – NSL

Stomach- NSL

Small intestine – Mesenteric fat - mild multifocal lymphoplasmacytic and neutrophilic infiltrates

Pancreas – Peripancreatic fat: multifocal moderate subacute lymphoplasmacytic, histiocytic, and neutrophilic infiltrates \*

#### I - 918

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Perirenal fat - mild multifocal lymphoplasmacytic and neutrophilic infiltrates

Liver – NSL

Stomach - NSL

Small intestine – Mesenteric fat - mild multifocal lymphoplasmacytic and neutrophilic infiltrates

Pancreas – NSL

#### J - 919

Heart – NSL

Lungs – Diffuse moderate perivascular lymphoid infiltrates

Spleen – Peri splenic fat – mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – NSL

Liver – NSL

Stomach - NSL

Small intestine – Mesenteric fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Pancreas – Peri pancreatic fat – mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

#### K - 920

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – NSL

Liver – Serosal surface – mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates \*

Stomach

Small intestine – Mesenteric fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Pancreas – Peripancreatic fat – mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

V - 940

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Multifocal mild peripelvic and perivascular lymphocytic infiltrates

Liver – NSL

Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Large intestine - NSL

Pancreas – NSL

W - 941

Heart – NSL

Lungs – Severe diffuse perivascular lymphocytic infiltrates with intralesional hemosiderosis

Spleen – NSL

Kidneys – Multifocal moderate perivascular lymphocytic infiltrates with intralesional hemosiderosis

Liver – NSL

Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Large intestine - NSL

Pancreas – Peripancreatic fat -focal moderate lymphofollicular infiltrate

X - 942

Heart – NSL

Lungs – Mild multifocal perivascular lymphoid follicular infiltrates

Spleen – NSL

Kidneys – Multifocal moderate perivascular and peripelvic lymphocytic infiltrates

Liver – Mild multifocal centrilobular lymphocytic infiltrates

Small intestine – Diffuse moderate expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Large intestine - NSL

Pancreas – NSL

Y - 953

Heart – NSL

Lungs – NSL

Spleen – Peri splenic fat – moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – NSL

Liver – NSL

Small intestine – Mesenteric fat – moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Large intestine

Pancreas – Peri pancreatic fat – moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

#### Z - 954

Heart – NSL

Lungs – Severe diffuse perivascular lymphocytic infiltrates

Spleen – NSL

Kidneys – Perirenal fat – moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Liver – NSL

Small intestine – Mesenteric fat – moderate multifocal, histiocytic, and neutrophilic infiltrates

Large intestine - NSL

Pancreas – Peripancreatic fat – moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

#### AA - 955

Heart – NSL

Lungs – NSL

Spleen – Perisplenic fat – moderate multifocal lymphoplasmacytic and histiocytic infiltrates

Kidneys – Multifocal moderate perivascular and peripelvic lymphocytic infiltrates

Liver – NSL

Stomach - NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat – moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

#### AB - 904

Heart – NSL

Lungs – NSL

Spleen – Perisplenic fat – moderate multifocal lymphoplasmacytic and histiocytic infiltrates

Kidneys – Multifocal moderate perivascular and peripelvic lymphocytic infiltrates

Liver – Multifocal moderate centrilobular lymphoid infiltrates; Focal lamellated venous thrombus

Stomach - NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat – moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

AC - 907

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – NSL

Liver – NSL

Small intestine – Severe diffuse expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive); Mesenteric fat – moderate multifocal, histiocytic, and neutrophilic infiltrates

Pancreas – Mild multifocal periductal lymphoid infiltrates

AD - 902

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – NSL

Liver – Multifocal mild centrilobular lymphoid infiltrates

Small intestine – NSL

Pancreas – Peripancreatic fat – moderate multifocal lymphoplasmacytic and histiocytic infiltrates

AE - 928

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Multifocal moderate perivascular and peripelvic lymphocytic infiltrates

Liver – Multifocal moderate centrilobular lymphoid infiltrates

Small intestine - Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive); Mesenteric fat – moderate multifocal lymphocytic and histiocytic infiltrates

Pancreas – Mild multifocal periductal lymphoid infiltrates; peripancreatic fat – moderate multifocal lymphocytic and histiocytic infiltrates

AF - 944

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Focal perivascular mild lymphocytic infiltrates

Liver – Multifocal moderate centrilobular lymphoid infiltrates

Small intestine - Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive); Mesenteric fat – Severe multifocal lymphocytic and histiocytic infiltrates

Pancreas – Mild multifocal periductal lymphoid infiltrates; peripancreatic fat – Severe multifocal lymphocytic and histiocytic infiltrates

AG - 929

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Focal mild perivascular mild lymphocytic infiltrates

Liver – NSL

Small intestine – NSL

Pancreas – Mild multifocal periductal lymphoid infiltrates; peripancreatic fat – Mild multifocal lymphocytic and histiocytic infiltrates

AH -905

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – Multifocal moderate centrilobular lymphoid infiltrates

Small intestine - Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive); Mesenteric fat – Moderate multifocal lymphocytic and histiocytic infiltrates

Large intestine - NSL

Pancreas – Mild multifocal periductal lymphoid infiltrates; peripancreatic fat – Severe multifocal lymphocytic and histiocytic infiltrates

AI - 903

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Multifocal moderate perivascular and peripelvic lymphocytic infiltrates

Liver – NSL

Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Pancreas – peripancreatic fat – Mild multifocal lymphocytic and histiocytic infiltrates

AJ - 943

Heart – NSL

Lungs – NSL

Spleen – Perisplenic fat – moderate multifocal lymphoplasmacytic and histiocytic infiltrates

Kidneys – Multifocal moderate perivascular and peripelvic lymphocytic infiltrates  
Liver – Multifocal moderate portal and sinusoidal extramedullary hematopoiesis (EMH).  
Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).  
Pancreas – Peripancreatic fat – moderate multifocal lymphoplasmacytic and histiocytic infiltrates

AK - 926

Heart – NSL  
Lungs – NSL  
Spleen – Perisplenic fat – mild multifocal lymphoplasmacytic and histiocytic infiltrates  
Kidneys – Multifocal mild perivascular and peripelvic lymphocytic infiltrates  
Liver – Focus of mild necrosis surrounded by fibroplasia and fibrosis  
Small intestine – NSL  
Large intestine - NSL  
Pancreas – Peripancreatic fat – mild multifocal lymphoplasmacytic and histiocytic infiltrates

AL - 906

Heart – NSL  
Lungs – NSL  
Spleen – NSL  
Kidneys – Multifocal mild perivascular and peripelvic lymphocytic infiltrates  
Liver – Focal mild lymphocytic centrilobular infiltrate  
Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive)  
Large intestine - NSL  
Pancreas – Peripancreatic fat – mild multifocal lymphoplasmacytic and histiocytic infiltrates

AM - 927

Heart – NSL  
Lungs – Severe diffuse perivascular lymphocytic infiltrates  
Spleen – NSL  
Kidneys – Multifocal perivascular mild lymphocytic infiltrates  
Liver – Multifocal moderate centrilobular lymphoid infiltrates  
Small intestine – NSL  
Pancreas – Peripancreatic fat - mild multifocal lymphoplasmacytic and histiocytic infiltrates

AN - 909

Heart – NSL  
Lungs – NSL  
Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic and histiocytic infiltrates  
Kidneys – Focal perivascular mild lymphocytic infiltrates  
Liver – NSL

Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive)

Large intestine - NSL

Pancreas – Peripancreatic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BA - 963

Heart – NSL

Lungs – Mild multifocal perivascular infiltrates

Spleen – Perisplenic fat - moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Focal mild perivascular lymphocytic infiltrate

Liver – NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - moderate focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BB - 948

Heart – NSL

Lungs – Mild multifocal perivascular infiltrates

Spleen – Perisplenic fat - moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular and peripelvic mild lymphocytic infiltrates

Liver – NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - moderate focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BC - 773

Heart – NSL

Lungs – Moderate diffuse perivascular lymphocytic infiltrates; Multifocal moderate alveolar histiocytosis

Peribronchial lymph node – Severe reactive hyperplasia

Spleen – Perisplenic fat - moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular and peripelvic moderate lymphocytic infiltrates

Liver – NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BK - 772

Heart – NSL

Lungs – Mild multifocal perivascular lymphocytic infiltrates

Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – Multifocal mild centrilobular lymphocytic infiltrates

Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Large intestine - NSL

Pancreas – Peripancreatic fat – moderate focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BL - 775

Heart – NSL

Lungs – Mild multifocal perivascular lymphocytic infiltrates

Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – Multifocal mild centrilobular lymphocytic infiltrates

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat – mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BM – 650

Heart – NSL

Lungs – Mild multifocal perivascular lymphocytic infiltrates

Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular moderate lymphocytic infiltrates

Liver – Multifocal moderate centrilobular lymphocytic infiltrates

Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Large intestine - NSL

Pancreas – Peripancreatic fat – moderate focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

**Mice that received VCD IP injections**

Euthanized or died unexpectedly (excluded from analyses of the knee)

*Mouse A:* Final diagnosis – severe acute pancreatic

Pancreas - severe, regional, necrosis and acute peritonitis

*Mouse B:* Final diagnosis – Abdominal peritonitis

Abdomen - severe, acute, regionally extensive peritonitis

*Mouse C:* Final diagnosis - Mesenteric lymph node – lymphoma

Mesenteric lymph nodes identified throughout the abdomen

*Mouse D:* Final diagnosis – Abdominal peritonitis

There is multifocal necrosis and mineralization (saponification, arrows) of the perirenal fat.

*Mouse E:* Final Diagnosis – renal failure of unknown origin

Both kidneys have segmental acute necrosis of the tubules and glomeruli with segmentally thickened capillary loops

Planned Euthanasias (mice used in knee analyses)

A -901

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – NSL

Perirenal fat – Multifocal mild neutrophil and fibrin infiltrate

Liver – Multifocal moderate centrilobular lymphoid infiltrates

Stomach -Focal mild serosal mesothelial hypertrophy

Small intestine – NSL

Large intestine - NSL

Pancreas – Multifocal mild serosal lymphoplasmacytic infiltrates

B - 908

Heart – Mild mitral valve hemosiderosis

Lungs – Mild multifocal perivascular lymphoid infiltrates

Spleen – Peri splenic fat – moderate multifocal lymphocytic infiltrates with peripheral macrophages

Kidneys – Multifocal moderate peripelvic and perivascular lymphoid infiltrates

Liver – NSL

Stomach - NSL

Small intestine – NSL

Mesenteric fat – Mild, histiocytic, lymphocytic, and neutrophilic infiltrates

Large intestine - NSL

Pancreas – Mild histiocytic, lymphocytic, and neutrophilic serosal infiltrates

C - 911

Heart – NSL

Lungs – NSL

Spleen – Necrosis of peri-splenic fat with moderate acute neutrophilic infiltrates extending into the parenchyma

Kidneys - NSL

Liver – Mild multifocal subcapsular neutrophilic infiltrates; Moderate diffuse microvesicular fatty change.

Stomach – Severe diffuse serosal necrotizing neutrophilic and fibrinous infiltrates

Small intestine – Mesenteric fat: moderate multifocal necrosis with acute fibrinonecrotizing and neutrophilic infiltrates

Large intestine - Mesenteric fat: moderate multifocal necrosis with acute fibrinonecrotizing and neutrophilic infiltrates

Pancreas – Severe peripancreatic fat necrosis with acute fibrinonecrotizing and neutrophilic infiltrates that extend into the parenchyma

L - 921

Heart – NSL

Lungs – Multifocal moderate perivascular lymphoid infiltrates

Spleen – NSL

Kidneys – Perirenal fat - mild multifocal lymphoplasmacytic and neutrophilic infiltrates

Liver – Focal moderate centrilobular lymphoid infiltrates

Stomach - NSL

Small intestine – Mesenteric fat - moderate multifocal lymphoplasmacytic and neutrophilic infiltrates

Pancreas – Peripancreatic fat – moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

M - 922

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Diffuse glomerular membranous glomerular mesangial thickening by amorphous material (amyloid, presumptive); Multifocal mild chronic tubular degeneration and loss with lymphocytic infiltrates.

Liver – NSL

Small intestine – Mesenteric fat - moderate multifocal lymphoplasmacytic and neutrophilic infiltrates

Large intestine - NSL

Pancreas – Peripancreatic fat – moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

N - 930

Heart – NSL  
Lungs – NSL  
Spleen – NSL  
Kidneys – Multifocal moderate peripelvic and perivascular lymphoid infiltrates  
Liver – Focal centrilobular moderate lymphocytic infiltrates  
Stomach - NSL  
Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).  
Large intestine - NSL  
Pancreas – Peripancreatic fat – moderate multifocal lymphofollicular infiltrates

#### O - 931

Heart – NSL  
Lungs – NSL  
Spleen – NSL  
Kidneys – Focal lymphocytic mild peripelvic infiltrates  
Liver – NSL  
Stomach - NSL  
Small intestine – NSL  
Mesenteric lymph node – severe; Lymphoma, large cell  
Large intestine - NSL  
Pancreas – NSL

#### P - 934

Heart – NSL  
Lungs – NSL  
Spleen – NSL  
Kidneys – Mild multifocal lymphocytic peripelvic and perivascular infiltrates  
Liver – Focal centrilobular moderate lymphocytic infiltrates  
Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).  
Large intestine - NSL  
Pancreas – NSL

#### Q - 935

Heart – NSL  
Lungs – Multifocal mild perivascular and peripheral lung lymphoplasmacytic infiltrates  
Spleen – NSL  
Kidneys – Multifocal mild peripelvic and perivascular lymphocytic infiltrates  
Liver – NSL  
Stomach - NSL  
Small intestine – NSL  
Pancreas – NSL

R – 936

Heart – NSL

Lungs - NSL

Spleen – NSL

Kidneys – NSL

Liver – NSL

Stomach - NSL

Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Pancreas – Peripancreatic fat – mild multifocal lymphofollicular infiltrates

S - 937

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Multifocal mild peripelvic and perivascular lymphocytic infiltrates

Liver – NSL

Small intestine – NSL

Large intestine- NSL

Pancreas – Mild multifocal periductular atrophy and lymphocytic infiltrates; Peripancreatic fat – mild multifocal lymphofollicular infiltrates

T - 938

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Multifocal mild peripelvic and perivascular lymphocytic infiltrates

Liver – NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Mild multifocal periductular atrophy and lymphocytic infiltrates; Peripancreatic fat – mild multifocal lymphofollicular infiltrates

U - 939

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Multifocal mild peripelvic and perivascular lymphocytic infiltrates

Liver – Multifocal centrilobular moderate lymphocytic infiltrates

Small intestine – NSL

Large intestine - NSL

Pancreas – Mild multifocal periductular atrophy and lymphocytic infiltrates; Peripancreatic fat – mild multifocal lymphofollicular infiltrates

AO - 957

Heart – NSL

Lungs – NSL

Spleen – Perisplenic fat - moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

AP - 960

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – NSL

AQ - 970

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – NSL

Liver – NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

AR - 965

Heart – NSL

Lungs – NSL

Spleen – Perisplenic fat - moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

AS -964

Heart – NSL

Lungs – NSL

Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – Multifocal mild centrilobular lymphocytic infiltrates

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

AT - 910

Heart – NSL

Lungs – NSL

Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – Multifocal mild centrilobular lymphocytic infiltrates

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

AU -912

Heart – NSL

Lungs – NSL

Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – NSL

Liver – NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

AV -916

Heart – NSL  
Lungs – NSL  
Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates  
Kidneys – NSL  
Liver – NSL  
Small intestine – NSL  
Large intestine - NSL  
Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

#### AW - 914

Heart – NSL  
Lungs – Focal moderate adenoma  
Spleen – NSL  
Kidneys – Multifocal perivascular mild lymphocytic infiltrates  
Liver – NSL  
Small intestine – NSL  
Large intestine - NSL  
Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

#### AX - 962

Heart –  
Lungs – Moderate diffuse perivascular lymphocytic infiltrates  
Peribronchial lymph node – Severe reactive hyperplasia  
Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates  
Kidneys – Multifocal perivascular and peripelvic mild lymphocytic infiltrates  
Liver – NSL  
Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).  
Large intestine - NSL  
Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

#### AY - 959

Heart – NSL  
Lungs – NSL  
Spleen – Perisplenic fat - moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates  
Kidneys – Multifocal perivascular and peripelvic mild lymphocytic infiltrates  
Liver – NSL

Small intestine – Diffuse moderate expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Large intestine - NSL

Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

AZ - 958

Heart – NSL

Lungs – Focal mild adenoma

Spleen – Perisplenic fat - moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Focal mild perivascular lymphocytic infiltrate

Liver – NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BD - 947

Heart – NSL

Lungs – NSL

Spleen – Perisplenic fat - moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular and peripelvic moderate lymphocytic infiltrates

Liver – NSL

Small intestine – Diffuse moderate expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Large intestine- NSL

Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BE - 946

Heart – NSL

Lungs – Moderate diffuse perivascular lymphocytic infiltrates

Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – NSL

Liver – NSL

Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Large intestine - NSL

Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BF - 945

Heart – NSL

Lungs – NSL

Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – Multifocal mild centrilobular lymphocytic infiltrates

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - moderate focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BG - 774

Heart – NSL

Lungs – Mild diffuse perivascular lymphocytic infiltrates

Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates \*

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – Multifocal mild centrilobular lymphocytic infiltrates

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat - moderate focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BH - 932

Heart – NSL

Lungs – Mild diffuse perivascular lymphocytic infiltrates

Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – Multifocal mild centrilobular lymphocytic infiltrates

Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Large intestine - NSL

Pancreas – Peripancreatic fat - mild focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BI - 771

Heart – NSL

Lungs – Moderate multioccal perivascular lymphocytic infiltrates

Spleen – Perisplenic fat - moderate multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – NSL

Liver – NSL

Small intestine – Diffuse severe expansion of villous lamina propria by eosinophilic amorphous material (amyloid, presumptive).

Large intestine - NSL

Pancreas – Peripancreatic fat – moderate focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

BJ - 933

Heart – NSL

Lungs – Mild multifocal perivascular lymphocytic infiltrates

Spleen – Perisplenic fat - mild multifocal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

Kidneys – Multifocal perivascular mild lymphocytic infiltrates

Liver – Multifocal mild centrilobular lymphocytic infiltrates

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat – moderate focal lymphoplasmacytic, histiocytic, and neutrophilic infiltrates

# Histopathology toxicity reports assessing the effects of sex hormone and senolytic treatment on internal organs

***“Menopause-induced 17 $\beta$ -estradiol and progesterone loss increases senescence markers, matrix disassembly and degeneration in mouse cartilage”***

**Pathologist** – Samantha St Jean, DVM, MSc, DACVP & Julie Feldstein, MD, ABP, FCAP, HistoWiz Pathology Services

**Date of Report Release:** 11/27/2023

## **Key:**

NSL – No significant lesions

EMH – extramedullary hematopoiesis

All mice in these studies received 4-vinycyclohexen diepoxide IP injections for 10 days as described in the manuscript, and all mice presented here were planned euthanasias and used in the osteoarthritis outcomes presented in the paper.

## **Mice that received control treatment**

100

Heart – NSL

Lungs – Mild, focal peribronchiolar mononuclear infiltrate; Adjacent fat: Mild, multifocal mononuclear infiltrates

Spleen – NSL

Kidneys – NSL

Liver – NSL

Stomach - Mild to moderate, multifocal mononuclear infiltrates

Small intestine – NSL

Large intestine - NSL

Pancreas – NSL

150

Heart – NSL

Lungs – Moderate, multifocal perivascular mononuclear infiltrates; Adjacent fat: Mild, multifocal mononuclear infiltrates

Spleen – NSL

Kidneys – Mild, multifocal mononuclear interstitial infiltrates with mild tubular epithelial cells basophilia and tubular dilation

Liver – Mild, multifocal centrilobular to random mononuclear infiltrates with single cell hepatocyte apoptosis  
Stomach - NSL  
Small intestine – NSL  
Large intestine - NSL  
Pancreas – NSL

144

Heart – NSL  
Lungs – Moderate, multifocal perivascular mononuclear infiltrates; Adjacent fat: Mild, multifocal mononuclear infiltrates  
Spleen – NSL  
Kidneys – NSL  
Liver – Mild, multifocal centrilobular to random mononuclear infiltrates with single cell hepatocyte apoptosis  
Stomach - Mild to moderate, multifocal mononuclear infiltrates  
Small intestine – NSL  
Large intestine - NSL  
Pancreas – NSL

218

Heart – NSL  
Lungs – NSL  
Spleen – NSL  
Kidneys – Moderate, multifocal mononuclear interstitial infiltrates with mild tubular epithelial cells basophilia and tubular dilation  
Liver – NSL  
Stomach - Mild to moderate, multifocal mononuclear infiltrates  
Small intestine – NSL  
Large intestine - NSL  
Pancreas – NSL

#### **Mice that received E2 treatment**

85

Heart – NSL  
Lungs – NSL  
Spleen – NSL  
Kidneys – Mild, multifocal mineralization with minimal mononuclear interstitial infiltrates and tubular epithelial cell basophilia  
Liver – NSL  
Stomach - Moderate, multifocal muscularis mineralization with minimal glandular dilation

Small intestine – Lymphoma  
Large intestine - Lymphoma  
Pancreas – Mild, multifocal acinar cell vacuolation, suspect

136

Heart – Mild, multifocal myocardial mineralization  
Lungs – Moderate, multifocal perivascular mononuclear infiltrates; Adjacent fat: Mild, multifocal mononuclear infiltrates  
Spleen – Mild, multifocal mineralization  
Kidneys – Moderate, multifocal mononuclear interstitial infiltrates with mild tubular epithelial karyomegaly and basophilia, capsular mineralization, and tubular dilation  
Liver – Mild, multifocal mineralization with minimal, multifocal centrilobular mononuclear infiltrates and extramedullary hematopoiesis  
Stomach - Moderate, multifocal serosal and muscularis mineralization  
Small intestine – NSL  
Large intestine - NSL  
Pancreas – NSL

145

Heart – Mild, multifocal epicardial and endocardial mineralization  
Lungs – Moderate, multifocal perivascular mononuclear infiltrates; Adjacent fat: Moderate, multifocal mononuclear infiltrates  
Spleen – NSL  
Kidneys – Mild, multifocal mononuclear interstitial infiltrates  
Liver – NSL  
Stomach - NSL  
Small intestine – NSL  
Large intestine - Mild, multifocal muscularis mineralization  
Pancreas – NSL

219

Heart – NSL  
Lungs – NSL  
Spleen – NSL  
Kidneys – NSL  
Liver – Mild, multifocal extramedullary hematopoiesis with intrahistiocytic pigment  
Stomach - Mild to moderate, multifocal mononuclear infiltrates  
Small intestine – Lymphoma  
Large intestine - Lymphoma  
Pancreas – NSL

## Mice that received P4 treatment

99

Heart – NSL  
Lungs – NSL  
Spleen – NSL  
Kidneys – NSL  
Liver – NSL  
Stomach - NSL  
Small intestine – Marked, locally extensive lamina propria amyloid deposits; Mild, focal lymphoid follicular hyperplasia  
Large intestine - NSL  
Pancreas – NSL

106

Heart – NSL  
Lungs – NSL  
Spleen – NSL  
Kidneys – NSL  
Liver – Mild, multifocal centrilobular to random mononuclear infiltrates; Adjacent fat: Moderate, mononuclear inflammation with multinucleated giant cells containing pigmented material  
Stomach - NSL  
Small intestine – Lymphoma; Moderate, multifocal lamina propria amyloid deposits  
Large intestine - NSL  
Pancreas – NSL

132

Heart – NSL  
Lungs – Mild to moderate, multifocal perivascular mononuclear infiltrates; Adjacent fat: Minimal, multifocal mononuclear infiltrates  
Spleen – NSL  
Kidneys – NSL  
Liver – NSL  
Stomach - NSL  
Small intestine – Mild, focal lymphoid follicular hyperplasia  
Large intestine - NSL  
Pancreas – NSL

147

Heart – NSL  
Lungs – Mild, multifocal perivascular mononuclear infiltrates; Adjacent fat: Mild, multifocal mononuclear infiltrates  
Spleen – NSL  
Kidneys – NSL  
Liver – NSL

Stomach - NSL

Small intestine – Moderate, multifocal lamina propria amyloid deposits; Minimal, multifocal lymphoid follicular hyperplasia; Adjacent fat: Mild, multifocal mononuclear infiltrates with intrahistiocytic pigment

Large intestine - NSL

Pancreas – NSL

### **Mice that received E2+P4 treatment**

79

Heart – NSL

Lungs – Mild to moderate, multifocal perivascular mononuclear infiltrates; Adjacent fat: Minimal, multifocal mononuclear infiltrates

Spleen – NSL

Kidneys – NSL

Liver – NSL

Stomach - NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – NSL

108

Heart – NSL

Lungs – Mild, multifocal perivascular mononuclear infiltrates; Adjacent fat: Minimal, multifocal mononuclear infiltrates

Spleen – NSL

Kidneys – NSL

Liver – NSL

Stomach - NSL

Small intestine – NSL

Large intestine - NSL

Pancreas – NSL

197

Heart – NSL

Lungs – Mild, multifocal perivascular mononuclear infiltrates; Adjacent fat: Mild, multifocal mononuclear infiltrates

Spleen – NSL

Kidneys – NSL

Liver – NSL

Stomach - NSL  
Small intestine – NSL  
Large intestine - NSL  
Pancreas – Peripancreatic fat: Mild, multifocal mononuclear infiltrates

227

Heart – NSL  
Lungs – Mild to moderate perivascular mononuclear infiltrates; Adjacent fat: Minimal, multifocal mononuclear infiltrates  
Spleen – NSL  
Kidneys – NSL  
Liver – Mild, multifocal extramedullary hematopoiesis  
Stomach - NSL  
Small intestine – NSL  
Large intestine - Adjacent fat: Mild, multifocal mononuclear infiltrates with intrahistiocytic pigment  
Pancreas – Peripancreatic fat and periductular: Mild to moderate, multifocal mononuclear infiltrates

#### **Mice that received Src inhibitor treatment**

223

Heart – NSL  
Lungs – Moderate to marked, multifocal mononuclear infiltrates  
Spleen – NSL  
Kidneys – Moderate, multifocal interstitial and pelvic mononuclear infiltrates with mild tubular epithelial cell degeneration, simplification, and basophilia, Bowman's space dilation, tubular dilation, rare hyaline casts, and multifocal interstitial adipocyte accumulation  
Liver – NSL  
Stomach - NSL  
Small intestine – Mild, focal lymphocytic muscular infiltrate with minimal multifocal lamina propria amyloid deposits  
Large intestine - NSL  
Pancreas – NSL

225

Heart – NSL  
Lungs – Mild, focal subserosal alveolar foamy histiocytic infiltrate; Mild to moderate, multifocal, perivascular mononuclear infiltrates; Adjacent fat: Mild, multifocal mononuclear infiltrates  
Spleen – NSL

Kidneys – NSL

Liver – Hepatocellular adenoma; Mild, multifocal random mononuclear infiltrates with intrahistiocytic pigment and oval cell hyperplasia

Stomach - Mild, multifocal mononuclear infiltrates with intrahistiocytic pigment

Small intestine – NSL

Large intestine - Adjacent fat: Mild, multifocal mononuclear infiltrates with intrahistiocytic pigment

Pancreas – Peripancreatic fat and periductular: Moderate, multifocal mononuclear infiltrates with intrahistiocytic pigment

478

Heart – NSL

Lungs – NSL

Spleen – NSL

Kidneys – Mild, focal ossification; Mild, multifocal mononuclear interstitial infiltrates with minimal tubular epithelial simplification and basophilia and tubular dilation

Liver – NSL

Stomach - Mild, multifocal mononuclear infiltrates with intrahistiocytic pigment

Small intestine – NSL

Large intestine - NSL

Pancreas – Peripancreatic fat and periductular: Mild, multifocal mononuclear infiltrates

479

Heart – NSL

Lungs – Mild, multifocal perivascular mononuclear infiltrates; Adjacent fat: Minimal, focal mononuclear infiltrate

Spleen – NSL

Kidneys – Moderate, multifocal mononuclear interstitial and pelvic infiltrates with mild tubular epithelial cell degeneration, simplification, and basophilia, Bowman's space dilation, and tubular dilation

Liver – NSL

Stomach - Mild, multifocal mononuclear infiltrates with intrahistiocytic pigment

Small intestine – NSL

Large intestine - Moderate, multifocal lymphoid follicular hyperplasia

Pancreas – Peripancreatic fat: Mild, multifocal mononuclear infiltrates

### **Mice that received p53/MDM2 inhibitor treatment**

70

Heart – NSL

Lungs – Mild, multifocal perivascular mononuclear infiltrates  
Spleen – NSL  
Kidneys – Moderate, multifocal mononuclear interstitial and pelvic infiltrates with mild tubular epithelial cell degeneration, basophilia, and simplification, Bowman's space dilation, tubular dilation, and multifocal interstitial adipocyte accumulation  
Liver – Mild, multifocal centrilobular mononuclear infiltrates with single cell hepatocyte apoptosis  
Stomach - NSL  
Small intestine – NSL  
Large intestine - NSL  
Pancreas – NSL

71

Heart – NSL  
Lungs – NSL  
Spleen – NSL  
Kidneys – NSL  
Liver – NSL  
Stomach - NSL  
Small intestine – NSL  
Large intestine - NSL  
Pancreas – Peripancreatic fat and periductular: Mild, multifocal mononuclear infiltrates

324

Heart – NSL  
Lungs – Mild, multifocal perivascular mononuclear infiltrates; Adjacent fat: Mild, focal mononuclear infiltrate  
Spleen – NSL  
Kidneys – Mild, multifocal tubular dilation with minimal tubular epithelial cell simplification  
Liver – NSL  
Stomach - NSL  
Small intestine – NSL  
Large intestine - NSL  
Pancreas – Peripancreatic fat and periductular: Mild, multifocal mononuclear infiltrates

494

Heart – NSL  
Lungs – Mild, multifocal perivascular mononuclear infiltrates  
Spleen – NSL  
Kidneys – Mild, multifocal mononuclear interstitial infiltrates with minimal tubular epithelial cell degeneration, simplification, and basophilia, Bowman's space dilation, and tubular dilation  
Liver – Mild, multifocal extramedullary hematopoiesis with single cell hepatocyte apoptosis  
Stomach - Mild, multifocal mononuclear infiltrates  
Small intestine – Lymphoma; Mild, multifocal lamina propria amyloid deposits  
Large intestine - Lymphoma

Pancreas – NSL

**Supplementary File 3: Nikon Elements General Analysis 3 for CHP and Type II collagen quantification**

<https://www.dropbox.com/scl/fi/25wor5hdvu1772qbh3pq8/CHP-and-Type-II-collagen-quantification-Feb28-2024.ga3?rlkey=8v83q7kueuj1bik7lugf0csvm&dl=0>

**Supplementary File 5: Nikon Elements General Analysis 3 for Quantification of Senescence Markers**

[https://www.dropbox.com/scl/fi/c0m5ckuowdo71qfxfid7/SF3\\_p16\\_ki67\\_cellCounts.ga3?rlkey=7g8mrl1jdm2zzbdbq1lqq5fy&e=1&dl=0](https://www.dropbox.com/scl/fi/c0m5ckuowdo71qfxfid7/SF3_p16_ki67_cellCounts.ga3?rlkey=7g8mrl1jdm2zzbdbq1lqq5fy&e=1&dl=0)

**Supplementary File 6: Nikon Elements General Analysis 3 Quantification of Chondrogenicity Markers**

[https://www.dropbox.com/scl/fi/u6xfjfqea3ra46v0ldmh/SF4\\_CellArea\\_TypelICollagenArea\\_Sox9-Cells.ga3?rlkey=lb1ncprc2d9kkgrryb5cjbpu&e=1&dl=0](https://www.dropbox.com/scl/fi/u6xfjfqea3ra46v0ldmh/SF4_CellArea_TypelICollagenArea_Sox9-Cells.ga3?rlkey=lb1ncprc2d9kkgrryb5cjbpu&e=1&dl=0)



# The ARRIVE guidelines 2.0: author checklist

## The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

| Item                                    | Recommendation                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Section/line number, or reason for not reporting |
|-----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|
| <b>Study design</b>                     | 1 For each experiment, provide brief details of study design including: <ul style="list-style-type: none"> <li>a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.</li> <li>b. The experimental unit (e.g. a single animal, litter, or cage of animals).</li> </ul>                                                                                                                                                                                                 |                                                  |
| <b>Sample size</b>                      | 2 a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.<br>b. Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done.                                                                                                                                                                                                                                              |                                                  |
| <b>Inclusion and exclusion criteria</b> | 3 a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established <i>a priori</i> . If no criteria were set, state this explicitly.<br>b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.<br>c. For each analysis, report the exact value of <i>n</i> in each experimental group. |                                                  |
| <b>Randomisation</b>                    | 4 a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.<br>b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.                                                                                                                                                |                                                  |
| <b>Blinding</b>                         | 5 Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).                                                                                                                                                                                                                                                                                                                                          |                                                  |
| <b>Outcome measures</b>                 | 6 a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).<br>b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.                                                                                                                                                                                                                                                                            |                                                  |
| <b>Statistical methods</b>              | 7 a. Provide details of the statistical methods used for each analysis, including software used.<br>b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.                                                                                                                                                                                                                                                                            |                                                  |
| <b>Experimental animals</b>             | 8 a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.<br>b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.                                                                                                                                                                                                       |                                                  |
| <b>Experimental procedures</b>          | 9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: <ul style="list-style-type: none"> <li>a. What was done, how it was done and what was used.</li> <li>b. When and how often.</li> <li>c. Where (including detail of any acclimatisation periods).</li> <li>d. Why (provide rationale for procedures).</li> </ul>                                                                                                                                  |                                                  |
| <b>Results</b>                          | 10 For each experiment conducted, including independent replications, report: <ul style="list-style-type: none"> <li>a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).</li> <li>b. If applicable, the effect size with a confidence interval.</li> </ul>                                                                                                                                                                              |                                                  |

# The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

| Item                                           |    | Recommendation                                                                                                                                                                                                                                                                                                                                                                                                                 | Section/line number, or reason for not reporting |
|------------------------------------------------|----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|
| <b>Abstract</b>                                | 11 | Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.                                                                                                                                                                                                                                                                                |                                                  |
| <b>Background</b>                              | 12 | <ul style="list-style-type: none"> <li>a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.</li> <li>b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.</li> </ul>                                                                                |                                                  |
| <b>Objectives</b>                              | 13 | Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.                                                                                                                                                                                                                                                                                                          |                                                  |
| <b>Ethical statement</b>                       | 14 | Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.                                                                                                                                                                       |                                                  |
| <b>Housing and husbandry</b>                   | 15 | Provide details of housing and husbandry conditions, including any environmental enrichment.                                                                                                                                                                                                                                                                                                                                   |                                                  |
| <b>Animal care and monitoring</b>              | 16 | <ul style="list-style-type: none"> <li>a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.</li> <li>b. Report any expected or unexpected adverse events.</li> <li>c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.</li> </ul> |                                                  |
| <b>Interpretation/ scientific implications</b> | 17 | <ul style="list-style-type: none"> <li>a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.</li> <li>b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.</li> </ul>                                                              |                                                  |
| <b>Generalisability/ translation</b>           | 18 | Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).                                                                                                                                                                                                                                    |                                                  |
| <b>Protocol registration</b>                   | 19 | Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.                                                                                                                                                                                                                      |                                                  |
| <b>Data access</b>                             | 20 | Provide a statement describing if and where study data are available.                                                                                                                                                                                                                                                                                                                                                          |                                                  |
| <b>Declaration of interests</b>                | 21 | <ul style="list-style-type: none"> <li>a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.</li> <li>b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.</li> </ul>                                                                                             |                                                  |

## Supplementary Note

While female humans have a menstrual cycle (defined as shedding of the uterine lining as a result of changing sex hormone circulation), most female mouse species have an estrous cycle (defined as cyclical changes in sex hormones that do not result in shedding of the uterine lining). In mice, the estrous cycle is divided into four phases (proestrus, estrus, metestrus, diestrus,) and the cycle repeats every 4-5 days unless disrupted via pregnancy or anestrus (i.e., sexual quiescence most often observed post-partum). Estrous cycling begins in mice ~26 days after birth, at which point the vagina opens and cornifies via an apoptosis driven mechanism. The start of estrous cycling drives the development of secondary characteristics and can be used as a predictor of puberty and an increase in circulating estradiol levels.

Proestrus is the preparatory stage for the mouse to come into heat. The proestrus phase can be detected on vaginal cytology as an abundance of nucleated epithelial cells and on gross inspection of the vagina as an enlargement of the vaginal opening with the tissues appearing moist and reddish-pink. The estrus phase is analogous to the ovulation window in humans, and this is the time in the cycle where mice can become pregnant. The estrus cycle is detected by visualizing an abundance of cornified epithelial cells via cytology, and on gross exam, the vagina appears similar to the proestrus phase, with the tissues being less pink. Metestrus is characterized by a decline in corpus luteum function in the absence of conception. On vaginal cytology, metestrus is characterized by a mix of nucleated and cornified epithelial cells and a few leukocytes. On gross exam during metestrus, the vagina appears pale and dry. Lastly, diestrus is a period of rest during the estrous cycle, where leukocytes dominate vaginal cytology and the vaginal opening closes.

<https://fertilityresearchandpractice.biomedcentral.com/articles/10.1186/s40738-020-00074-3>

<https://books.google.com/books?hl=en&lr=&id=8pQxAQAAMAAJ&oi=fnd&pg=PA295&ots=U2r54 Exie&sig=wh-hL 6SrxAtnArnCAYC1DnZYNy#v=onepage&q&f=false>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3325956/#:~:text=In%20the%20mouse%2C%20the%20estrous,pregnancy%2C%20pseudopregnancy%2C%20or%20anestrus>

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