**Materials and Methods**

**Proteomic Analysis**

**Tissue Lysis and Homogenization**

Ovarian tissues from reproductively-young (n=10) and reproductively-old (n=10) mice were prepared for proteomic analysis. Samples were homogenized in 600 µL lysis buffer containing 8 M urea, 2% sodium dodecyl sulfate (SDS), 1 µM trichostatin A (TSA), 3 mM nicotinamide adenine dinucleotide (NAD), 75 mM sodium chloride, and 1X protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA) in 200 mM triethylammonium bicarbonate (TEAB) by adding them to 2.0 mL safe-lock tubes (VWR International, Radnor, PA) containing stainless steel beads and subjected to three intervals of high-speed shaking (25 Hz, 1 min) using a Qiagen TissueLyser II (Qiagen, Hilden, Germany). Tissue homogenates were centrifuged at 15,700 x g for 10 min at 4°C, and the supernatant was collected for label-free quantitative proteomics experiments. Protein concentration was determined using Bicinchoninic Acid (BCA) assay (Thermo Fisher Scientific, Waltham, MA).

**Protein Digestion and Desalting**

Aliquots of 200 µg protein lysates for each sample were brought to the same overall volume of 100 µL with water, reduced using 20 mM dithiothreitol in 50 mM TEAB at 50°C for 10 min, cooled to room temperature (RT) and held at RT for 10 min, and alkylated using 40 mM iodoacetamide in 50 mM TEAB at RT in the dark for 30 min. Samples were acidified with 12% phosphoric acid to obtain a final concentration of 1.2% phosphoric acid. S-Trap buffer consisting of *90% methanol in 100 mM TEAB at pH ~7.1,* was added and samples were loaded onto the S-Trap micro spin columns. The entire sample volume was spun through the S-Trap micro spin columns at 4,000 x g and RT, binding the proteins to the micro spin columns. Subsequently, S-Trap micro spin columns were washed twice with S-Trap buffer at 4,000 x g and RT and placed into clean elution tubes. Samples were incubated for one-hour at 47 oC with sequencing grade trypsin (Promega, San Luis Obispo, CA) dissolved in 50 mM TEAB at a 1:25 (w/w) enzyme:protein ratio, and then digested overnight at 37 oC.

Peptides were sequentially eluted from micro S-Trap spin columns with 50 mM TEAB, 0.5% formic acid (FA) in water, and 50% acetonitrile (ACN) in 0.5% FA. After centrifugal evaporation, samples were resuspended in 0.2% FA in water and desalted with Oasis 10-mg Sorbent Cartridges (Waters, Milford, MA). The desalted elutions were then subjected to an additional round of centrifugal evaporation and re-suspended in 0.1% FA in water at a final concentration of 1 µg/µL. Eight microliters of each sample was diluted with 2% ACN in 0.1% FA to obtain a concentration of 400 ng/µL. One microliter of indexed Retention Time Standard (iRT, Biognosys, Schlieren, Switzerland) was added to each sample, thus bringing up the total volume to 20 µL.1

**Mass Spectrometric Analysis**

Reverse-phase HPLC-MS/MS analyses were performed on a Dionex UltiMate 3000 system coupled online to an Orbitrap Exploris 480 mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The solvent system consisted of 2% ACN, 0.1% FA in water (solvent A) and 80% ACN, 0.1% FA in ACN (solvent B). Digested peptides (400 ng) were loaded onto an Acclaim PepMap 100 C18 trap column (0.1 x 20 mm, 5 µm particle size; Thermo Fisher Scientific) over 5 min at 5 µL/min with 100% solvent A. Peptides (400 ng) were eluted on an Acclaim PepMap 100 C18 analytical column (75 µm x 50 cm, 3 µm particle size; Thermo Fisher Scientific) at 300 nL/min using the following gradient: linear from 2.5% to 24.5% of solvent B in 125 min, linear from 24.5% to 39.2% of solvent B in 40 min, up to 98% of solvent B in 1 min, and back to 2.5% of solvent B in 1 min. The column was re-equilibrated for 30 min with 2.5% of solvent B, and the total gradient length was 210 min. Each sample was acquired in **data-independent acquisition (DIA)** mode.2-4 Full MS spectra were collected at 120,000 resolution (Automatic Gain Control (AGC) target: 3e6 ions, maximum injection time: 60 ms, 350-1,650 *m/z*), and MS2 spectra at 30,000 resolution (AGC target: 3e6 ions, maximum injection time: Auto, Normalized Collision Energy (NCE): 30, fixed first mass 200 *m/z*). The isolation scheme consisted of 26 variable windows covering the 350-1,650 *m/z* range with an overlap of 1 *m/z*.3

**DIA Data Processing and Statistical Analysis**

DIA data was processed in Spectronaut (version 14.10.201222.47784) using directDIA. Data extraction parameters were set as dynamic and non-linear iRT calibration with precision iRT was selected. Data was searched against the *Mus musculus* reference proteome with 58,430 entries (UniProtKB-TrEMBL), accessed on 01/31/2018. Trypsin/P was set as the digestion enzyme and two missed cleavages were allowed. Cysteine carbamidomethylation was set as a fixed modification while methionine oxidation and protein N-terminus acetylation were set as dynamic modifications. Identification was performed using 1% precursor and protein q-value. Quantification was based on the peak areas of extracted ion chromatograms (XICs) of 3 – 6 MS2 fragment ions, specifically b- and y-ions, with local normalization and q-value sparse data filtering applied (Supplemental table Sx). In addition, iRT profiling was selected. Differential protein expression analysis comparing either 1) Young to Old Native or 2) Young to Old ECM-enriched was performed using a paired t-test, and p-values were corrected for multiple testing, using the Storey method.5 Specifically, group wise testing corrections were applied to obtain q-values. Protein groups with at least two unique peptides, q-value < 0.01, and absolute Log2(fold-change) > 0.58 are significantly-altered (Figure x, Supplemental Table Sx).

**Statistical Processing:**

Partial least square-discriminant analysis (PLS-DA) of the proteomics data was performed using the package mixOmics6 in R (version 4.0.2; RStudio, version 1.3.1093). Volcano plots for differential analysis were generated using R (RStudio, version 1.3.1093).

**Pathway Analysis**

An over-representation analysis (ORA) was performed using Consensus Path DB-mouse (Release MM11, 14.10.2021), developed by the bioinformatics group at the Max Planck Institute for Molecular Genetics (Berlin, Germany).7, 8 The following comparisons were used to evaluate which gene ontology terms, including biological processes, molecular functions, and cellular components, were significantly enriched: 1) Young vs. Old Native and 2) Young vs. Old ECM-enriched. Gene ontology terms identified from the ORA were subjected to the following filters: q-value < 0.05, term category = b (biological processes), and term level = 4. Dot plots were generated using the ggplot2 package9 in R (version 4.0.5; RStudio, version 1.4.1106) to visualize significantly-enriched biological processes from each comparison (Supplemental Figure Sx).

**Data Availability**

Raw data and complete MS data sets have been uploaded to the Mass Spectrometry Interactive Virtual Environment (MassIVE) repository, developed by the Center for Computational Mass Spectrometry at the University of California San Diego, and can be downloaded using the following link: xxx (MassIVE ID number: xxx; ProteomeXchange ID: xxx).