Supplemental information

Spatially resolved transcriptomic profiling
of ovarian aging in mice

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Figure S1, related to Figure 1: Female reproductive aging is associated with progressive loss of follicles in the mouse ovary. Histological staining of (A) Young and (B) Aged murine ovaries confirms diminished ovarian reserve in the Aged population and an appropriate aging model. (C) Aged ovaries exhibit significantly decreased follicle counts (**p<0.01) compared to the Young population. (D) Quantification of follicle types in Young versus Aged ovaries (**p<0.001, *p<0.01, *p<0.05). Scale bar is 500μm.
Figure S2, related to Figure 1 and Figure 2: Principal component analysis (PCA) plots of spots. PCs 1 and 2 (A-C) and PCs 3 and 4 (D-F) are colored by the capture area (A and D) or the age group (B, C, E, and F). All spots are shown in A, B, D, and E, but spots in C and F are shown for each capture area individually.
Figure S3, related to Figure 8: Gene expression of key transcripts in Follicle sub-clusters. Expression of genes well characterized in follicles of varying developmental stages used to distinguish follicle sub-clusters.
Figure S4, related to Figure 3: Spatial mapping of Follicle sub-clusters. Follicle sub-clusters aligned with tissue sections to validate cluster identities and distinguish follicles at varying stages of development. Scale bar is 500μm.
Figure S5, related to Figure 8: Spatial mapping of Oocyte sub-clusters. Oocyte sub-clusters aligned with tissue sections to validate cluster identities and distinguish oocytes at varying stages of development. Scale bar is 500\(\mu\)m.
Figure S6, related to Figure 1: Spatial transcriptomics tissue optimization. Each capture area contains one Young and one Aged ovary tissue section. Fluorescently labeled cDNA footprints generated after various lengths of tissue permeabilization; times shown in bottom left corner of each capture area. The upper left capture area shows a positive RNA control with no tissue (Pos) and the bottom right capture area shows a negative control with tissue not exposed to permeabilization reagents (Neg). The optimal permeabilization time for mouse ovaries was determined to be 18 minutes based on brightness of cDNA footprint.
Figure S7, related to Figure 1: Gene Expression slide imaging. Each capture area contains one Young and one Aged ovary tissue section (n=4). Sections stained by hematoxylin and eosin (left side) versus the detected tissue in Space Ranger by mRNA capture (right side) shows excellent coverage, mRNA capture, and tissue recognition by Space Ranger software. Scale bar is 500μm.
| Cluster/Sub-Cluster | Total Spots | Total Young Spots | Young Proportion | Total Aged Spots | Aged Proportion | p-value  | OR  | FDR |
|---------------------|-------------|------------------|-----------------|-----------------|----------------|----------|-----|-----|
| **Figure 1A**       |             |                  |                 |                 |                |          |     |     |
| Cluster Corpus Luteum-R | 1138 | 680 | 0.265 | 458 | 0.282 | 0.2395709 | 0.919 | 0.3359992 |
| Cluster Corpus Luteum-P | 525 | 859 | 0.335 | 539 | 0.332 | 0.8422357 | 0.319 | 0.4571919 |
| Stromal Cluster | 1398 | 639 | 0.249 | 236 | 0.145 | 2.72E-16 | 1.951 | 1.90E-15 |
| Follicle Cluster | 875 | 245 | 0.095 | 280 | 0.172 | 5.02E-13 | 0.507 | 1.76E-12 |
| Epithelium Cluster | 168 | 104 | 0.041 | 64 | 0.039 | 0.8720064 | 0.130 | 0.8720064 |
| Epithelium Cluster | 25 | 29 | 0.011 | 26 | 0.017 | 0.1314329 | 0.652 | 0.1300755 |
| Epithelium Cluster | 30 | 10 | 0.004 | 20 | 0.012 | 0.0022555 | 0.314 | 0.003865 |
| **Figure 7A - Corpus Luteum-R** |             |                  |                 |                 |                |          |     |     |
| Cluster Corpus Luteum-R | 269 | 262 | 0.385 | 7 | 0.015 | 4.00E-59 | 40.295 | 3.60E-58 |
| Cluster Corpus Luteum-P | 225 | 100 | 0.147 | 121 | 0.264 | 1.33E-08 | 0.481 | 1.33E-08 |
| Stromal Cluster | 193 | 69 | 0.101 | 124 | 0.271 | 1.73E-13 | 0.305 | 5.20E-13 |
| Follicle Cluster | 158 | 118 | 0.174 | 40 | 0.087 | 3.53E-05 | 2.193 | 3.98E-05 |
| Epithelium Cluster | 164 | 45 | 0.056 | 73 | 0.340 | 2.92E-03 | 0.009 | 2.58E-02 |
| Epithelium Cluster | 58 | 6 | 0.009 | 52 | 0.114 | 1.61E-15 | 0.070 | 5.24E-15 |
| Epithelium Cluster | 55 | 55 | 0.081 | 0 | 0.000 | 3.83E-13 | Inf | 6.89E-13 |
| Epithelium Cluster | 35 | 1 | 0.001 | 34 | 0.074 | 3.74E-13 | 0.018 | 6.89E-13 |
| Epithelium Cluster | 25 | 25 | 0.035 | 1 | 0.002 | 4.69E-05 | 16.697 | 4.69E-05 |
| **Figure 7B - Corpus Luteum-P** |             |                  |                 |                 |                |          |     |     |
| Cluster Corpus Luteum-R | 165 | 88 | 0.359 | 77 | 0.275 | 0.047682 | 1.477 | 0.095364 |
| Cluster Corpus Luteum-P | 158 | 81 | 0.331 | 77 | 0.275 | 0.182161 | 1.361 | 0.206236 |
| Stromal Cluster | 148 | 76 | 0.310 | 72 | 0.257 | 0.206236 | 1.298 | 0.206236 |
| Follicle Cluster | 54 | 0 | 0.000 | 54 | 0.193 | 2.54E-16 | 0.000 | 1.02E-15 |
| **Figure 7C - Stroma** |             |                  |                 |                 |                |          |     |     |
| Cluster Stroma | 288 | 111 | 0.129 | 177 | 0.328 | 1.03E-18 | 0.304 | 2.75E-18 |
| Cluster Stroma | 262 | 253 | 0.295 | 9 | 0.017 | 2.25E-48 | 24.549 | 1.81E-47 |
| Cluster Stroma | 225 | 124 | 0.144 | 101 | 0.187 | 0.03629 | 0.732 | 0.041476 |
| Cluster Stroma | 189 | 44 | 0.051 | 145 | 0.269 | 2.49E-30 | 0.147 | 9.95E-30 |
| Cluster Stroma | 147 | 133 | 0.155 | 14 | 0.026 | 7.51E-16 | 0.663 | 3.03E-16 |
| Cluster Stroma | 140 | 77 | 0.090 | 60 | 0.117 | 0.1003 | 0.744 | 0.85E-17 |
| Cluster Stroma | 88 | 72 | 0.084 | 16 | 0.030 | 3.59E-05 | 2.988 | 5.75E-05 |
| Cluster Stroma | 7 | 59 | 0.052 | 14 | 0.026 | 0.019507 | 2.072 | 0.026009 |
| **Figure 7D - Follicle** |             |                  |                 |                 |                |          |     |     |
| Cluster Follicle | 168 | 160 | 0.250 | 8 | 0.034 | 6.75E-16 | 9.506 | 3.55E-15 |
| Cluster Follicle | 166 | 114 | 0.178 | 52 | 0.220 | 0.173922 | 0.769 | 0.202909 |
| Cluster Follicle | 159 | 152 | 0.203 | 7 | 0.030 | 1.67E-15 | 10.195 | 3.90E-15 |
| Cluster Follicle | 147 | 99 | 0.165 | 48 | 0.263 | 0.10279 | 0.718 | 1.43E-04 |
| Cluster Follicle | 140 | 70 | 0.130 | 24 | 0.137 | 0.806317 | 0.744 | 0.85E-17 |
| Cluster Follicle | 75 | 23 | 0.036 | 52 | 0.230 | 1.02E-15 | 0.133 | 3.55E-15 |
| Cluster Follicle | 66 | 21 | 0.033 | 45 | 0.191 | 3.51E-13 | 0.145 | 6.04E-13 |
| **Figure 8G - Oocyte** |             |                  |                 |                 |                |          |     |     |
| Cluster Oocyte | 104 | 67 | 0.295 | 37 | 0.725 | 1.91E-08 | 0.160 | 7.64E-08 |
| Cluster Oocyte | 103 | 92 | 0.405 | 11 | 0.216 | 0.015444 | 2.471 | 0.020592 |
| Cluster Oocyte | 55 | 52 | 0.229 | 3 | 0.059 | 0.00564 | 4.735 | 0.011279 |
| Cluster Oocyte | 3 | 16 | 0.070 | 0 | 0.000 | 0.049406 | Inf | 0.049406 |
**Table S2, related to Figure 2B: Top 10 Marker Genes per Cluster**

| Cluster | Gene | P-value | Function |
|---------|------|---------|----------|
| **CL-R** |      |         |          |
| Lgmn   | 0    | Protease required for lysosomal protein degradation; regulates cell proliferation via EGFR degradation |
| Gyp4f18 | 0    | Monooxygenase important for synthesis of cholesterol and steroids; inflammation regulation |
| S100a4 | 1.11E-291 | Motility, angiogenesis, cell differentiation, apoptosis and autophagy, cytokine production and inflammation |
| A001b | 7.75E-288 | Translocates drugs and phospholipids across the membrane |
| Hgcc | 5.54E-210 | Modulates cell cycle-specific kinases; induced by ps3 in response to DNA damage; overexpression suppresses cell cycle progression |
| Lep3 | 2.33E-196 | Protects cells and enzymes from oxidative damage |
| Mgs1 | 1.16E-146 | Protects endoplasmic reticulum and outer mitochondrial membrane from oxidative stress |
| Sectlm1b | 2.46E-57 | Immune system processes; cytokine activity |
| Mt | 2.58E-46 | Heavy metal-binding; degrades various components of the ECM (collagen) |
| mt-Co2 | 3.43E-11 | Mitochondrial electron transport chain; oxidative phosphorylation |
| **Stroma** |      |         |          |
| Ap | 6.15E-135 | Potent regulator of blood pressure by acting directly on vascular smooth muscle as a potent vasoconstrictor |
| Mgarp | 3.46E-80 | Mitochondria trafficking via microtubules; role in stereoidogenesis via maintenance of mitochondrial abundance and morphology |
| Igf2 | 2.24E-12 | Antigen binding; innate immune response |
| Ig hg2c | 9.79E-30 | Antigen binding; innate immune response |
| Ig hg2b | 2.14E-36 | Antigen binding; immunoglobulin receptor binding; innate immune response |
| Igm | 1.3E-83 | Binds oocyte zona pellucida |
| Apoe | 4.83E-14 | Key regulator of active cholesterol uptake in ovarian follicle cells for steroligenesis |
| Lyz2 | 1.04E-08 | Lysozyme with primarily bactericidal function |
| Cpe | 1.05E-93 | Directs prohormones to the regulated secretory pathway |
| Chs | 3.46E-14 | Intracellular protein breakdown; amyloid precursor protein (APP) processing and degradation |
| **Follicle** |      |         |          |
| Inha | 0 | Inhibin; inhibits the secretion of follistatin (FSH) by the pituitary gland; oocyte development and maturation |
| Rps4x | 1.84E-133 | Ribosomal protein |
| Rps2x | 3.04E-121 | Ribosomal protein |
| Hps3a | 1.6E-114 | Ribosomal protein |
| Ldhb | 2.93E-110 | Oxidoreductase involved in synthesizing lactate from pyruvate |
| Hifdb | 1.15E-12 | Inhibits the secretion of FSH by the pituitary gland; oocyte development and maturation |
| Hps17 | 1.77E-18 | Ribosomal protein |
| Pdcd6 | 2.96E-09 | Oocyte cytoskeletal organization; cytoplasmic lattice (CPL) component in oocytes |
| Inha | 2.42E-31 | Inhibin; inhibits the secretion of FSH by the pituitary gland; oocyte development and maturation |
| Grea1 | 1.91E-21 | Estrogen-stimulated cell proliferation |
| **CL-P** |      |         |          |
| Hsx1b7 | 0 | Steroid-hormone metabolism and cholesterol biosynthesis; catalyzes reduction of estrogens and androgens, regulating their biological potency |
| Hmgcs1 | 0 | Cholesterol synthesis; catalyzes the condensation of acetyl-CoA to mevalonate |
| Hspa9b1 | 4.11E-04 | Molecular chaperone that functions in the processing and transport of secreted proteins |
| Timp1 | 5.66E-03 | Inhibits metalloproteinases, such as collagenases, by binding to their catalytic zinc cofactor |
| Csa | 2.87E-41 | Extracellular chaperone that prevents aggregation of non-native proteins; inhibits apoptosis when associated with the mitochondrial membrane; following stress, promotes apoptosis |
| Poc1e | 1.06E-02 | Binds C-terminal pro-peptide of type I procollagen and enhances procollagen C-proteinase activity |
| Fdn1 | 4.77E-05 | Stores iron in a soluble, non-toxic readily available form; important for iron homeostasis |
| mt-Co1 | 3.62E-02 | Mitochondrial electron transport chain; oxidative phosphorylation |
| Spp1 | 3.15E-08 | Important for cell-matrix interactions; inflammatory cytokine activity |
| Star | 3.65E-09 | Steroid hormone synthesis; cholesterol transport and metabolism |
| **Epi1** |      |         |          |
| Lgals2 | 3.30E-157 | Binds beta-galactoside; physiological function unknown |
| Gpd2 | 4.23E-146 | Hydrolyzes GTP to GDP; major product is GDP |
| Ucp2 | 3.14E-14 | Negative regulation of glucose import |
| Ly9e | 6.00E-106 | Regulation of T-lymphocytes proliferation, differentiation, and activation |
| Igfbp5 | 6.59E-105 | Prolongs the half-life of insulin-like growth factors (IGFs); implicated in aging |
| Cgap | 3.00E-01 | Acts in sarcoplasmic transport and absorption |
| Laptm4b | 7.20E-70 | Required for lysosomal function; blocks EGFR-stimulated EGFR intramitochondial sorting and degradation; negative regulator of TGB1 production in regulatory T-cells |
| Krt7 | 2.75E-02 | Keratin; constitutes Type II intermediate filaments of the intracytoplasmic cytoskeleton |
| Lgsa1 | 1.6E-46 | Cell-cell and cell-matrix interactions; apoptosis, cell proliferation and cell differentiation |
| Pima | 1.71E-17 | Immune function; apoptosis |
| **Epi2** |      |         |          |
| Dynlnb2 | 8.01E-79 | Involved in linking dynein to cargos and adapter proteins; cytoplasmic dynein 1 acts as a motor for intracellular retrograde motility of vesicles and organelles along microtubules |
| Cdc153 | 1.06E-76 | Collected-domain containing protein |
| Tipp3 | 1.06E-74 | Regulator of microtubule dynamic that has microtubule bundling activity |
| Fam3B | 1.07E-66 | Cytoskeleton component |
| Ovq3x | 6.95E-52 | Binds oocyte zona pellucida in vivo; may play a role in the fertilization process |
| M03 | 3.16E-50 | Binds heavy metals |
| Eor1 | 3.65E-04 | Transcription elongation factor implicated in maintenance of chromatin structure in actively transcribed regions |
| Hsp90a1 | 4.42E-04 | Molecular chaperone; promotes maturation, structural maintenance and proper regulation of specific target proteins involved in cell cycle control and signal transduction; mitochondrial import; inflammatory response |
| Tmem212 | 2.56E-73 | Transmembrane protein |
| **Epi3** |      |         |          |
| Des | 2.47E-42 | Muscle-specific type III intermediate filament essential for proper muscular structure and function; sarcomeric microtubule-anchoring protein |
| Cm1n | 1.82E-34 | Thin filament-associated protein implicated in the regulation and modulation of smooth muscle contraction |
| Pdmd3 | 1.44E-29 | Organization of actin filament arrays within muscle cells |
| Act2 | 1.22E-26 | Gamma actins exist in most cell types as components of the cytoskeleton and as mediators of internal cell motility |
| Cita2a | 4.76E-27 | Function unknown; expressed in activated T-cells |
| Casp1 | 2.24E-21 | Neuronal development |
| Myo9 | 2.33E-20 | Myosin regulatory subunit important in regulation of smooth muscle and non-muscle cell contractile activity via phosphorylation |
| Tnsh | 7.49E-20 | Actin cross-linking protein |
| Acta2 | 1.47E-14 | Alpha actins are found in muscle tissues and are a major constituent of the contractile apparatus |
| C21ta | 5.32E-04 | Inhibits hemopoiesis and stimulates chemotaxis; chemotactic in vitro for thromocytes and activated T-cells |