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Supplemental information

Spatially resolved transcriptomic profiling of ovarian aging in mice

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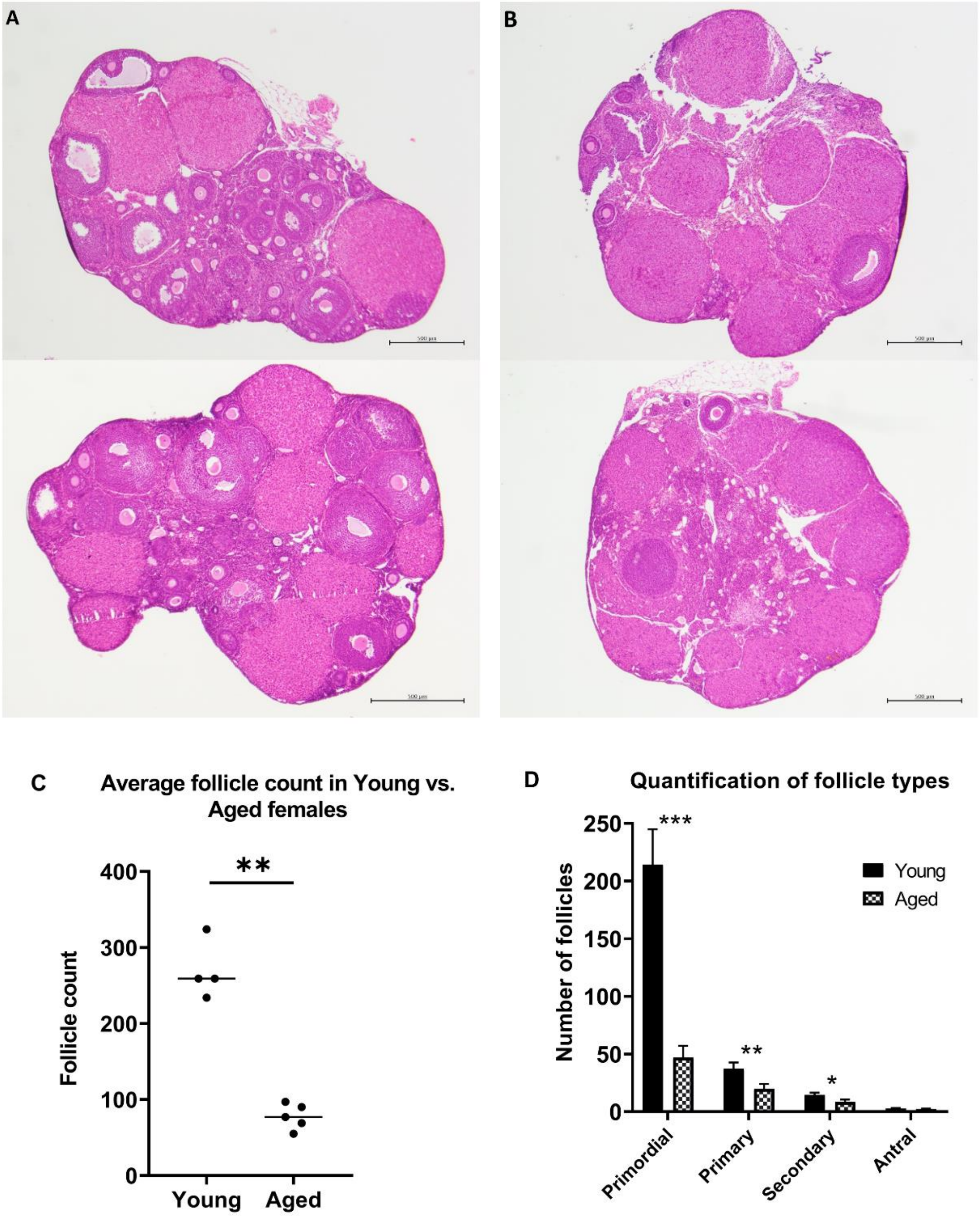

Figure S1, related to Figure 1: Female reproductive aging is associated with progressive loss of follicles in the mouse ovary. Histological staining of (A) Young and (B) Aged murine ovaries confirms diminished ovarian reserve in the Aged population and an appropriate aging model. (C) Aged ovaries exhibit 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.

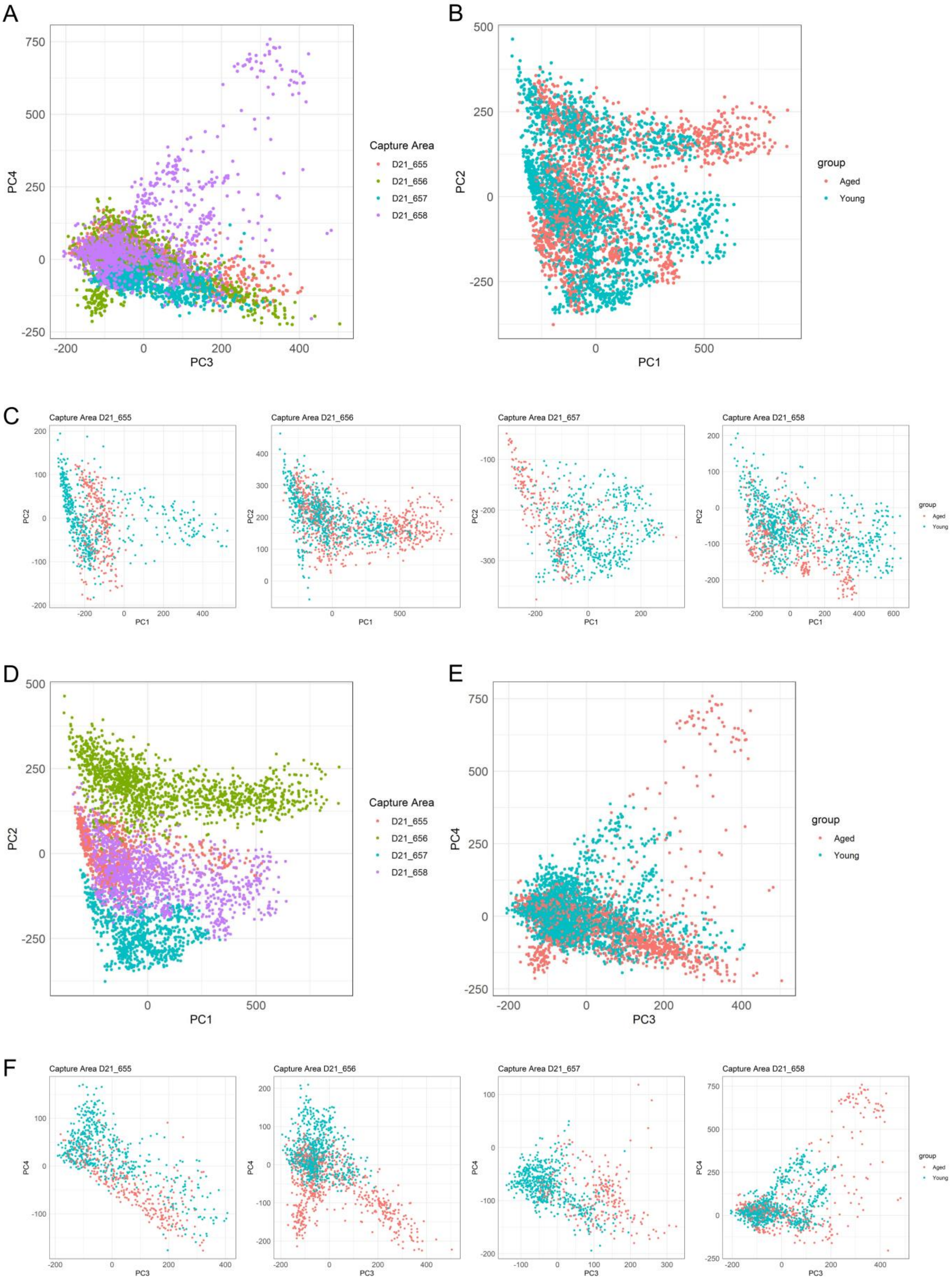
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Figure S2, related to Figure 1 and Figure 2: Principal component analysis (PCA) plots of spots. PCs 1 and 2 (A-C) and PCs 3 and 4 (D-F) are colored by the capture area (A and D) or the age group (B, C, E, and F). All spots are shown in A, B, D, and E, but spots in C and F are shown for each capture area individually.

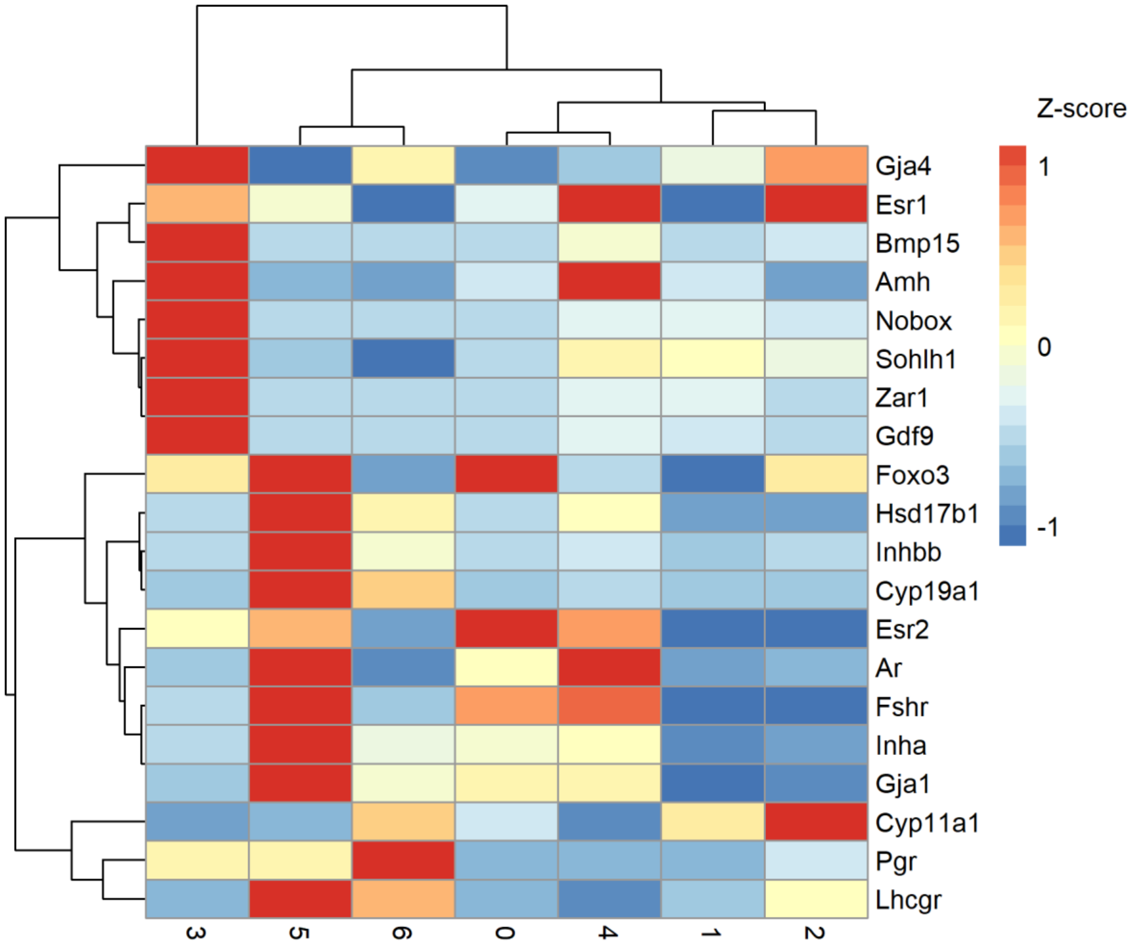

Figure S3, related to Figure 8: Gene expression of key transcripts in Follicle sub-clusters. Expression of genes well characterized in follicles of varying developmental stages used to distinguish follicle sub-clusters.

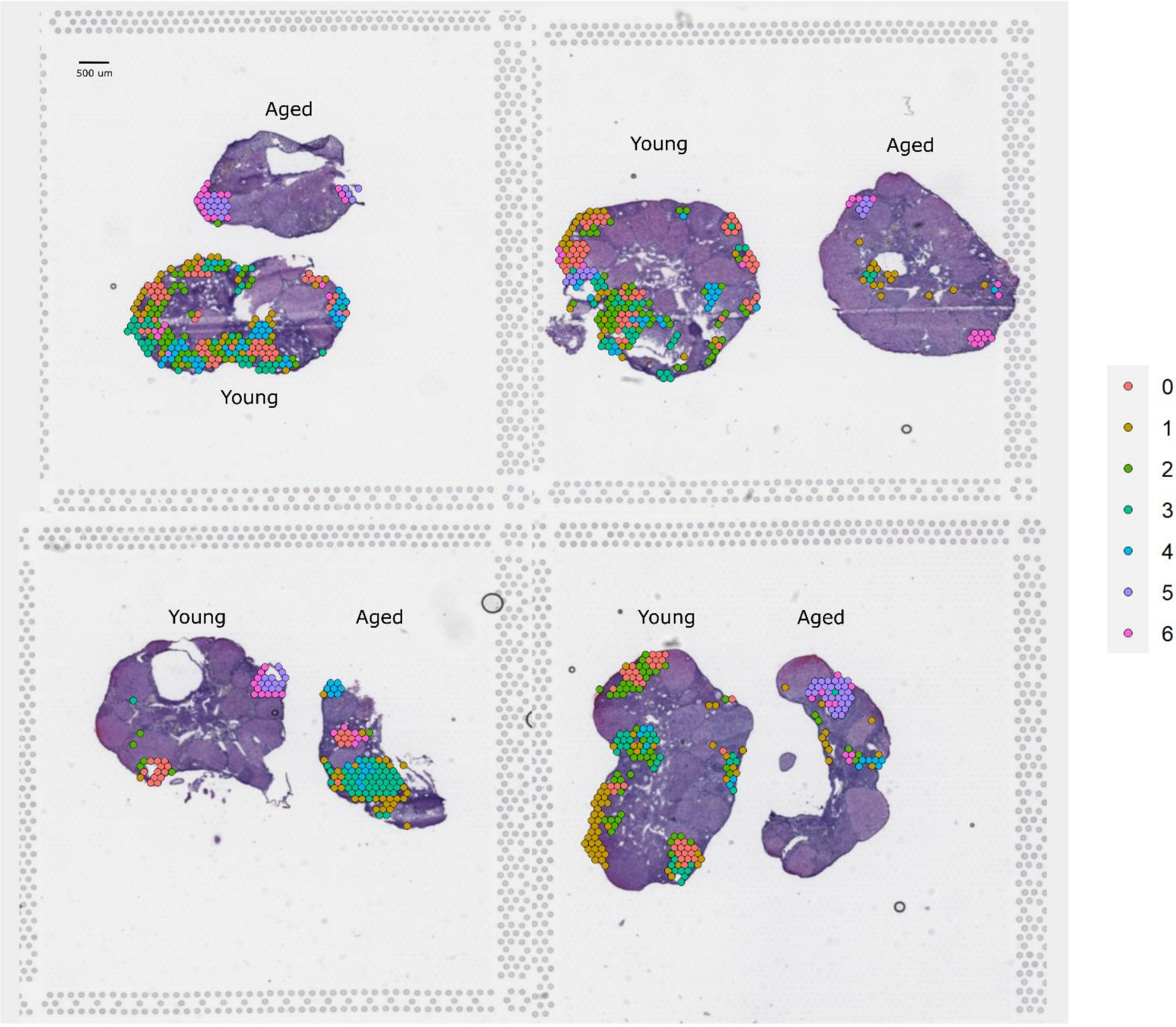

Figure S4, related to Figure 3: Spatial mapping of Follicle sub-clusters. Follicle sub-clusters aligned with tissue sections to validate cluster identities and distinguish follicles at varying stages of development. Scale bar is 500µm.

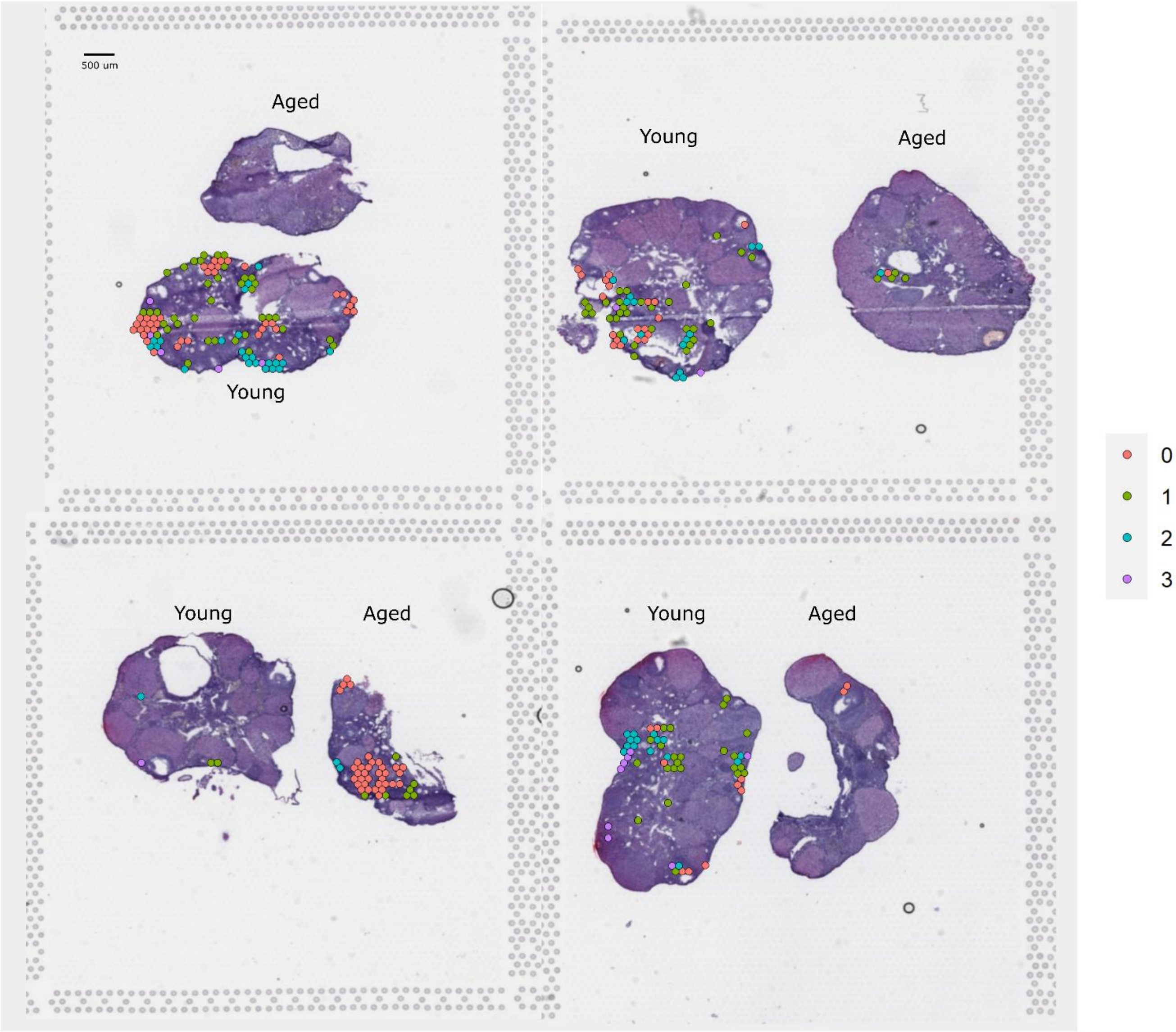

Figure S5, related to Figure 8: Spatial mapping of Oocyte sub-clusters. Oocyte sub-clusters aligned with tissue sections to validate cluster identities and distinguish oocytes at varying stages of development. Scale bar is 500µm.

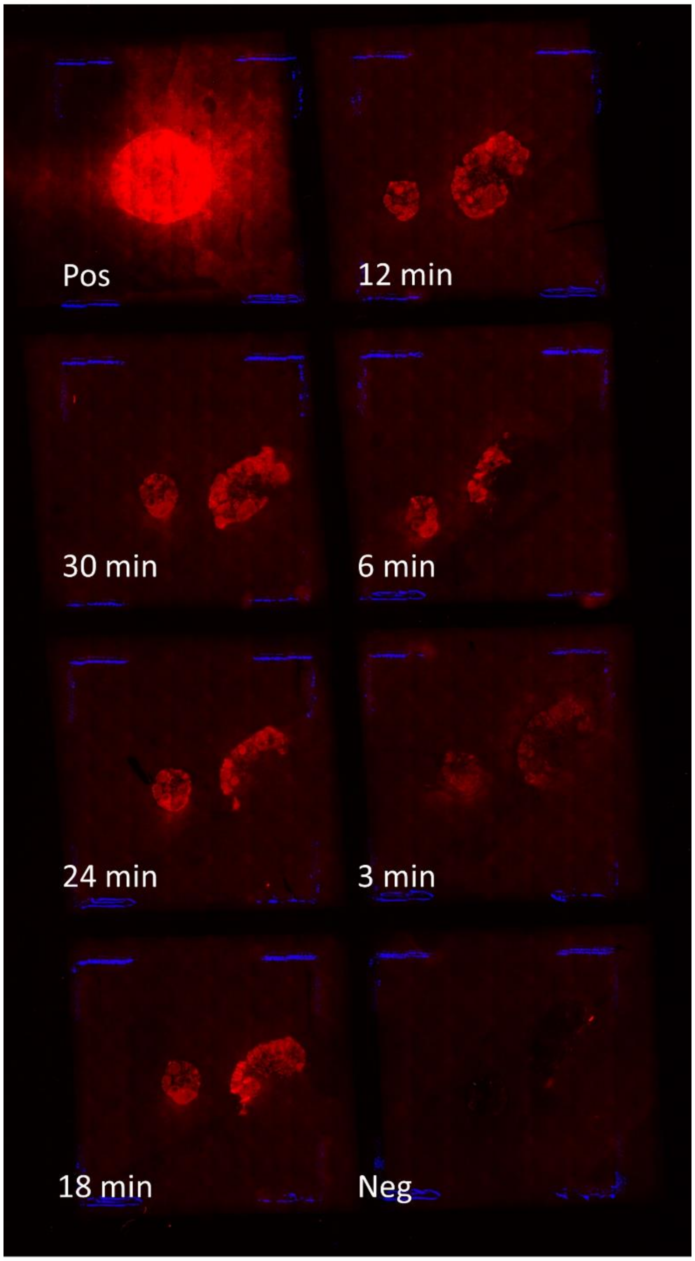

Figure S6, related to Figure 1: Spatial transcriptomics tissue optimization. Each capture area contains one Young and one Aged ovary tissue section. Fluorescently labeled cDNA footprints generated after various lengths of tissue permeabilization; times shown in bottom left corner of each capture area. 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.

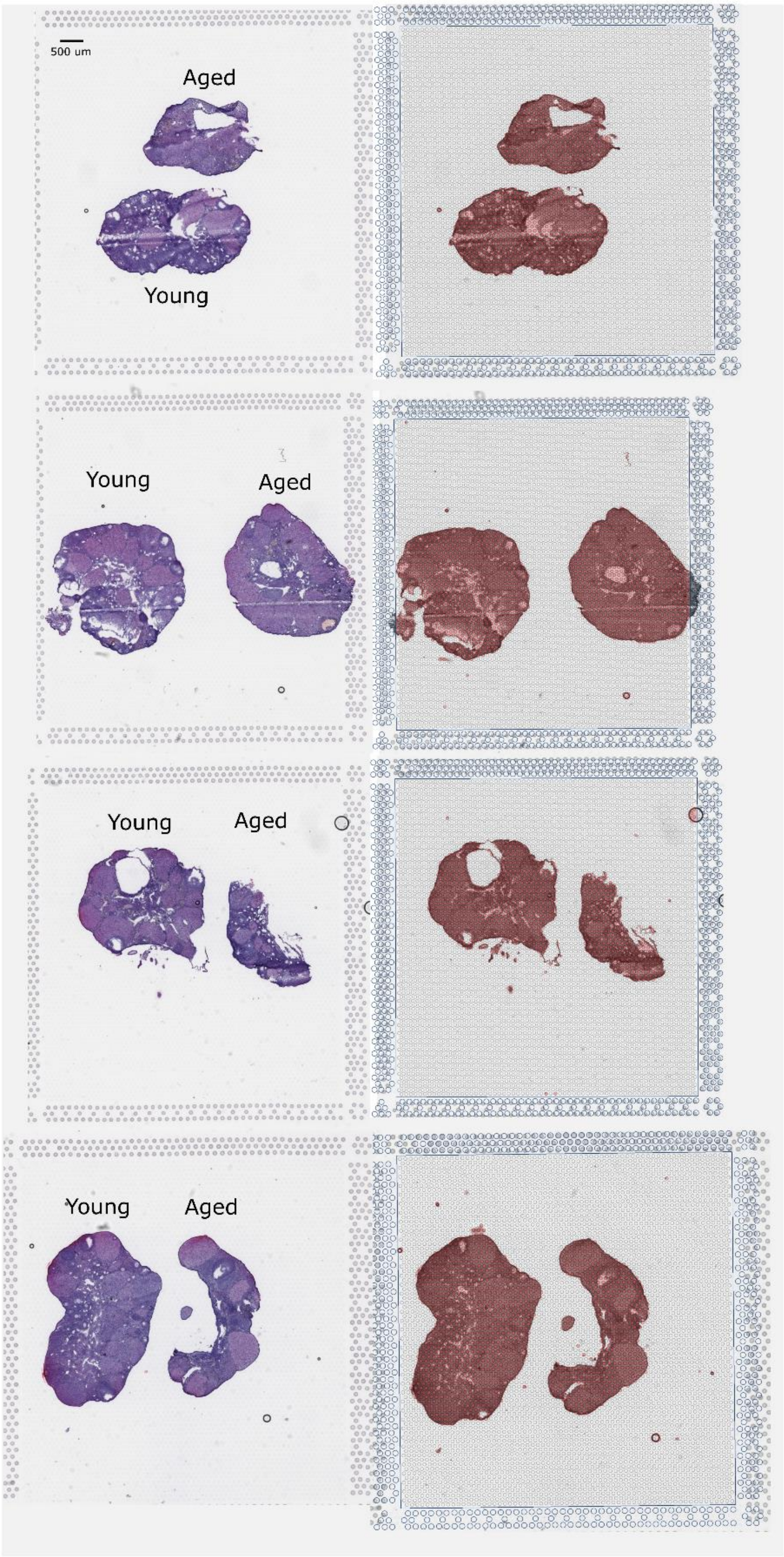

Figure S7, related to Figure 1: Gene Expression slide imaging. Each capture area contains one Young and one Aged ovary tissue section (n=4). Sections stained by hematoxylin and eosin (left side) versus the detected tissue in Space Ranger by mRNA capture (right side) shows excellent coverage, mRNA capture, and tissue recognition by Space Ranger software. Scale bar is 500μm.

Table S1, related to Figure 1A, Figure 3A, Figure 7A-D, Figure 8G: Spots Analyzed in UMAP Plots

Cluster/Sub-Cluster	Total Spots	Total Young Spots	Young Proportion	Total Aged Spots	Aged Proportion	p-value	OR	FDR
Figure 1A								
0	349	338	0.132	11	0.007	2.64E-60	22.251	6.33E-59
1	324	143	0.056	181	0.111	1.01E-10	0.471	2.20E-10
2	289	273	0.106	16	0.010	1.27E-41	11.970	1.52E-40
3	258	105	0.041	153	0.094	8.94E-12	0.411	2.15E-11
4	247	60	0.023	187	0.115	8.16E-34	0.184	6.53E-33
5	234	207	0.081	27	0.017	2.68E-21	5.192	9.17E-21
6	214	52	0.020	162	0.100	3.07E-29	0.187	1.84E-28
7	204	178	0.069	26	0.016	7.70E-17	4.583	2.31E-16
8	186	138	0.054	48	0.030	0.000155	1.867	0.000286
9	176	168	0.065	8	0.005	3.29E-27	14.156	1.58E-26
10	172	91	0.035	81	0.050	0.025103	0.701	0.032279
11	168	104	0.041	64	0.039	0.872006	1.030	0.90992
12	158	83	0.032	75	0.046	0.024544	0.691	0.032279
13	155	95	0.037	60	0.037	1	1.003	1
14	149	81	0.032	68	0.042	0.086845	0.746	0.104214
15	145	94	0.037	51	0.031	0.386667	1.174	0.421819
16	138	41	0.016	97	0.060	4.35E-14	0.256	1.16E-13
17	137	71	0.028	66	0.041	0.025555	0.672	0.032279
18	131	47	0.018	84	0.052	2.85E-09	0.342	5.69E-09
19	124	92	0.036	32	0.020	0.00263	1.851	0.004209
20	88	66	0.026	22	0.014	0.007675	1.923	0.011512
21	58	0	0.000	58	0.036	7.25E-25	0.000	2.90E-24
22	57	29	0.011	28	0.017	0.131433	0.652	0.150209
23	30	10	0.004	20	0.012	0.002255	0.314	0.003865
Figure 3A								
Corpus Luteum-R	1138	680	0.265	458	0.282	0.2395709	0.919	0.3353992
Corpus Luteum-P	525	859	0.335	539	0.332	0.8402217	1.014	0.8720064
Stroma	1398	639	0.249	236	0.145	2.72E-16	1.951	1.90E-15
Follicle	875	245	0.095	280	0.172	5.02E-13	0.507	1.76E-12
Epithelium 1	168	104	0.041	64	0.039	0.8720064	1.030	0.8720064
Epithelium 2	57	29	0.011	28	0.017	0.1314329	0.652	0.2300075
Epithelium 3	30	10	0.004	20	0.012	0.0022546	0.314	0.0052608
Figure 7A - Corpus Luteum-R								
0	269	262	0.385	7	0.015	4.00E-59	40.295	3.60E-58
1	221	100	0.147	121	0.264	1.33E-06	0.481	1.71E-06
2	193	69	0.101	124	0.271	1.73E-13	0.305	5.20E-13
3	158	118	0.174	40	0.087	3.53E-05	2.193	3.98E-05
4	124	45	0.066	79	0.172	3.29E-08	0.340	4.94E-08
5	58	6	0.009	52	0.114	1.16E-15	0.070	5.24E-15
6	55	55	0.081	0	0.000	3.83E-13	Inf	6.89E-13
7	35	1	0.001	34	0.074	3.74E-13	0.018	6.89E-13
8	25	24	0.035	1	0.002	4.69E-05	16.697	4.69E-05
Figure 7B - Corpus Luteum-P								
0	165	88	0.359	77	0.275	0.047682	1.477	0.095364
1	158	81	0.331	77	0.275	0.182161	1.301	0.206238
2	148	76	0.310	72	0.257	0.206238	1.298	0.206238
3	54	0	0.000	54	0.193	2.54E-16	0.000	1.02E-15
Figure 7C - Stroma								
0	288	111	0.129	177	0.328	1.03E-18	0.304	2.75E-18
1	262	253	0.295	9	0.017	2.26E-48	24.549	1.81E-47
2	225	124	0.144	101	0.187	0.036292	0.732	0.041476
3	189	44	0.051	145	0.269	2.49E-30	0.147	9.95E-30
4	147	133	0.155	14	0.026	1.51E-16	6.863	3.03E-16
5	140	77	0.090	63	0.117	0.10038	0.744	0.10038
6	88	72	0.084	16	0.030	3.59E-05	2.988	5.75E-05
7	59	45	0.052	14	0.026	0.019507	2.072	0.026009
Figure 7D - Follicle								
0	168	160	0.250	8	0.034	6.75E-16	9.506	3.55E-15
1	166	114	0.178	52	0.220	0.173922	0.769	0.202909
2	159	152	0.238	7	0.030	1.67E-15	10.195	3.90E-15
3	147	99	0.155	48	0.203	0.102789	0.718	0.143904
4	94	70	0.110	24	0.102	0.806317	1.087	0.806317
5	75	23	0.036	52	0.220	1.02E-15	0.133	3.55E-15
6	66	21	0.033	45	0.191	3.45E-13	0.145	6.04E-13
Figure 8G - Oocyte								
0	104	67	0.295	37	0.725	1.91E-08	0.160	7.64E-08
1	103	92	0.405	11	0.216	0.015444	2.471	0.020592
2	55	52	0.229	3	0.059	0.00564	4.735	0.011279
3	16	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
	Cyp4f18	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
	Abcb1b	7.75E-288	Translocates drugs and phospholipids across the membrane
	Rgcc	5.54E-210	Modulates cell cycle-specific kinases; induced by p53 in response to DNA damage; overexpression suppresses cell cycle progression
	Gpx3	2.13E-159	Protects cells and enzymes from oxidative damage
	Mgst1	1.66E-148	Protects endoplasmic reticulum and outer mitochondrial membrane from oxidative stress
	Sectm1b	2.46E-57	Immune system processes; cytokine activity
	Mt1	2.58E-46	Heavy metal-binding; degrades various components of the ECM (collagen)
	mt-Co2	9.43E-11	Mitochondrial electron transport chain; oxidative phosphorylation
Stroma			
	Agt	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 steroidogenesis via maintenance of mitochondrial abundance and morphology
	Igkc	2.54E-12	Antigen binding; innate immune response
	Ighg2c	9.79E-30	Antigen binding; innate immune response
	Ighg2b	2.14E-36	Antigen binding; immunoglobulin receptor binding; innate immune response
	Ighm	3.13E-01	Antibody; immune response
	Apoe	4.83E-14	Key regulator of active cholesterol uptake in ovarian follicle cells for steroidogenesis
	Lyz2	1.04E-08	Lysozyme with primarily bacteriolytic function
	Cpe	1.05E-93	Directs prohormones to the regulated secretory pathway
	Ctsd	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
	Rps24	3.24E-121	Ribosomal protein
	Rps3a1	1.65E-114	Ribosomal protein
	Ldhb	2.93E-110	Oxidoreductase involved in synthesizing lactate from pyruvate
	Inhbb	1.15E-12	Inhibin; inhibits the secretion of FSH by the pituitary gland; oocyte development and maturation
	Rpl37	1.77E-16	Ribosomal protein
	Padi6	2.96E-09	Oocyte cytoskeletal organization; cytoplasmic lattice (CPL) component in oocytes
	Inhba	2.42E-31	Inhibin; inhibits the secretion of FSH by the pituitary gland; oocyte development and maturation
	Greb1	1.91E-21	Estrogen-stimulated cell proliferation
CL-P			
	Hsd17b7	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
	Hsp90b1	4.11E-04	Molecular chaperone that functions in the processing and transport of secreted proteins
	Timp1	5.65E-03	Inhibits metalloproteinases, such as collagenases, by binding to their catalytic zinc cofactor
	Clu	2.87E-41	Extracellular chaperone that prevents aggregation of non-native proteins; inhibits apoptosis when associated with the mitochondrial membrane; following stress, promotes apoptosis
	Pcolce	1.06E-02	Binds C-terminal pro-peptide of type I procollagen and enhances procollagen C-proteinase activity
	Fth1	4.77E-05	Stores iron in a soluble, non-toxic, readily available form; important for iron homeostasis
	mt-Co1	3.82E-02	Mitochondrial electron transport chain; oxidative phosphorylation
	Spp1	2.15E-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
	Gbp2	4.23E-146	Hydrolyzes GTP to GMP, major product is GDP
	Upk3b	1.82E-144	Negative regulation of glucose import
	Ly6e	6.00E-108	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
	Crip1	1.05E-88	Zinc intracellular transport and absorption
	Laptm4b	2.70E-70	Required for lysosomal function; blocks EGF-stimulated EGFR intraluminal sorting and degradation; negative regulator of TGFβ1 production in regulatory T-cells
	Krt7	2.75E-02	Keratin; constitutes Type II intermediate filaments of the intracytoplasmic cytoskeleton
	Lgals1	1.68E-46	Cell-cell and cell-matrix interactions; apoptosis, cell proliferation and cell differentiation
	Ptma	1.71E-17	Immune function; apoptosis
Epi2			
	Dynlrb2	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
	Ccdc153	1.06E-76	Coiled-coil domain containing protein
	Tppp3	1.06E-74	Regulator of microtubule dynamic that has microtubule bundling activity
	Fam183b	1.07E-66	Cytoskeleton component
	Ovgp1	6.95E-52	Binds oocyte zona pellucida *in vivo*; may play a role in the fertilization process
	Mt3	3.18E-50	Binds heavy metals
	Elof1	3.65E-04	Transcription elongation factor implicated in maintenance of chromatin structure in actively transcribed regions
	Hsp90aa1	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
	Cnn1	1.82E-34	Thin filament-associated protein implicated in the regulation and modulation of smooth muscle contraction
	Pdlim3	1.44E-29	Organization of actin filament arrays within muscle cells
	Actg2	1.12E-28	Gamma actins exist in most cell types as components of the cytoskeleton and as mediators of internal cell motility
	Ctla2a	4.76E-27	Function unknown; expressed in activated T-cells
	Csrp1	2.24E-21	Neuronal development
	Myl9	2.33E-20	Myosin regulatory subunit important in regulation of smooth muscle and non-muscle cell contractile activity via phosphorylation
	Tagln	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
	Ccl21a	5.32E-04	Inhibits hemopoiesis and stimulates chemotaxis; chemotactic in vitro for thymocytes and activated T-cells