

Figure S1. Aging-associated histological alterations in rhesus monkey testes.

Immunofluorescence staining of P16 (senescence marker), and Collagen IV (marker of basal membrane of seminiferous tubules). Left panels: representative images. Right panels: quantification of P16-positive cells, as well as basement membrane (BM) thickness based on collagen IV staining, presented as fold changes (mean $\pm$ SEM). Scale bars, 20 μ m for P16 and 50 μ m for collagen IV staining.

Figure S2. Pseudotime analysis of germ cell development across age and seasons

A Pseudotime analysis depicting germ cell development from SCto SPD.e across age and seasonal conditions. **B** Heatmaps and curve charts showing the dynamic expression profiles of genes along the pseudotime trajectory of meiosis. The heatmap provides a visual representation of the expression levels of these prominent DEGs. Line plots illustrate gene expression trends, and bar charts summarize the enrichment of associated GO terms for each gene cluster.

Figure S3. Somatic cell subtypes and functional programs in the rhesus testis across age and reproductive seasons

A UMAP plot showing subtypes of Sertoli cells. **B** Ro/e value indicates the enrichment levels of each Sertoli cell subtype across age/seasonal conditions. **C** UMAP plots showing the expression pattern of SOX9 and AMH. **D** Lollipop plot displaying significantly enriched upregulated (right) and downregulated (left) pathways in sertoli.a cells compared with sertoli.p. **E** Lollipop plot showing enriched pathways in adult Sertoli.a cells (breeding versus non-breeding seasons). **F** UMAP plot illustrating subtypes of Leydig cells. **G** Ro/e value indicates the enrichment levels of each Leydig cell subtype across different age/seasonal conditions. **H** Dotplot illustrating the distinct expression patterns of subtype-specific marker genes across Leydig cell populations. **I** Lollipop plot displays significantly enriched pathways in adult Leydig-3 cells (breeding versus non-breeding seasons). **J** Lollipop plots illustrating specifically enriched upregulated (right) and downregulated (left) pathways in Leydig-1&2, Leydig-3, and leydig-4 cells. **K** UMAP plot illustrating subtypes of PMC cells. **L** Ro/e value indicates the enrichment levels of PMC cell subtypes across different age/seasonal conditions. **M** Dotplot illustrating the distinct expression patterns of subtype-specific marker genes across PMC cell populations. **N** Lollipop plot displaying significantly downregulated pathways in PMC1_1 cells in aged monkeys compared with prepuberty and adult

groups. **O** Lollipop plot displaying significantly upregulated pathways in adult PMC1_2 cells (breeding versus non-breeding seasons).

Figure S4. Gene expression and intercellular communication among immune, germ, and somatic cells across age and seasonal conditions

A Interaction strength among immune cells, germ cells, and somatic cells under different age/seasonal conditions. **B** Chord diagram showing directional ligand (immune cells)-receptor (germ cells) interactions across adult breeding, adult non-breeding, aged breeding and aged non-breeding seasons. **C** Chord diagram visualizes directional ligand (immune cells)-receptor (somatic cells) interactions across adult breeding, adult non-breeding, aged breeding, and aged non-breeding seasons. **D** Chord diagram visualizes directional ligand (somatic cells)-receptor (germ cells) interactions across adult breeding, adult non-breeding, aged breeding, and aged non-breeding seasons.

Figure S5. U3 definition, regulatory programs, and cross-species comparison

A Circular plot showing Augur algorithm-based ranking of spermatogenic cell types by responsiveness to age and seasonal effects. **B** UMAP visualization showing the distribution of SSC subpopulations (U1–U3, dif, end) across age and seasonal conditions. **C** Bar plots showing the counts of enriched GO terms for U3 versus u1/u2 in adult testes during the non-breeding season. **D** Heatmap showing regulon activity across SC subpopulations. Regulon activity was scaled by row (z-score) and grouped into five modules (c1–c5) using hierarchical clustering (complete linkage). For each module, representative enriched GO terms of regulon target genes are shown. Dot plots display enrichment significance ($-\log_{10} P$ value), and bar plots indicate the corresponding gene counts for each GO term. **E** UMAP visualization showing distribution of SSC subpopulations in mouse and human. Dot plots showing expression patterns of subtype-specific marker genes. **F** Cross-species gene expression correlation analysis of SSC subpopulations, comparing rhesus U3 with reported human and mouse SSC states.

Fig.S6 Age-Associated expansion and functional attenuation of DNT cells in the rhesus testis

68 **A** UMAP plot depicting the distribution of T cell populations. **B** Bar plot with nested
69 percentage information showing T cell subpopulation composition across age and
70 seasonal conditions. Percentages shown at the right of each bar indicate the proportion
71 of T cells among total cells for each condition. Percentages within the stacked bars (and
72 pie chart) indicate the relative proportions of CD4, CD8, and DNT subsets within the T
73 cell compartment. The pie chart summarizes overall T cell subtype composition. **C**
74 Representative flow cytometry plots for *CD3* expression in testicular cells within each
75 condition. **D** qPCR analysis of *CD3D* expression in adult testes relative to aged group
76 in breeding or nonbreeding seasons. **E** Lollipop plot displaying showing pathways
77 downregulated in DNT cells in aged versus prepubertal/adult testes.

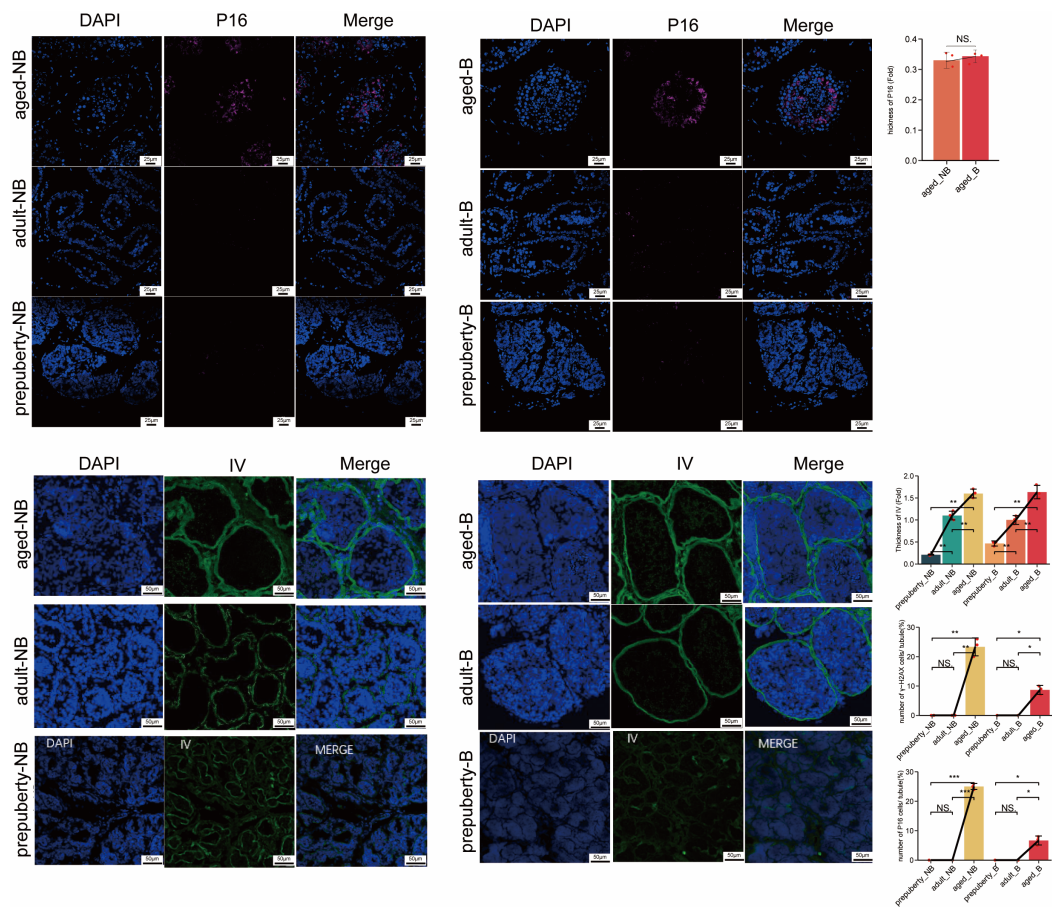
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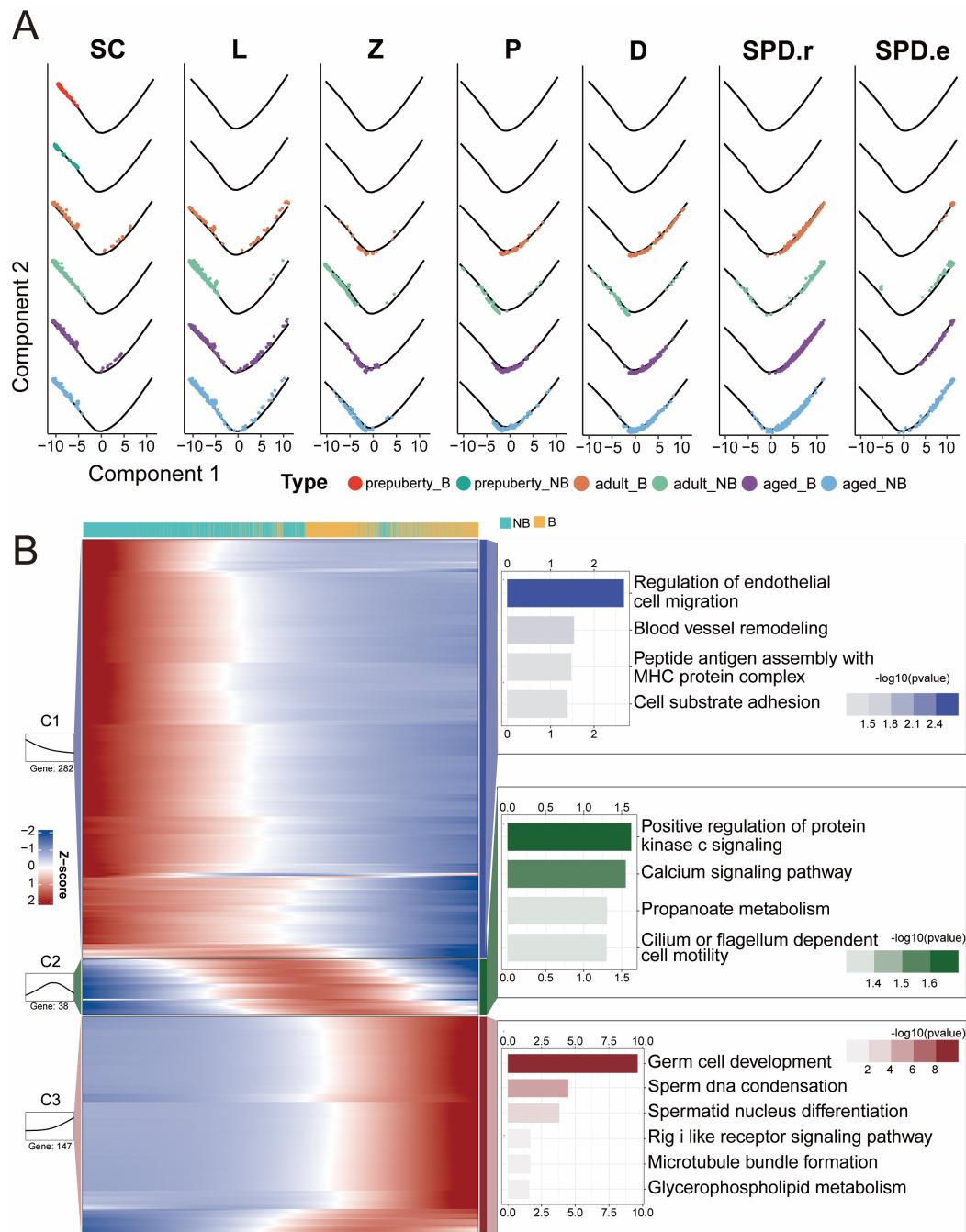
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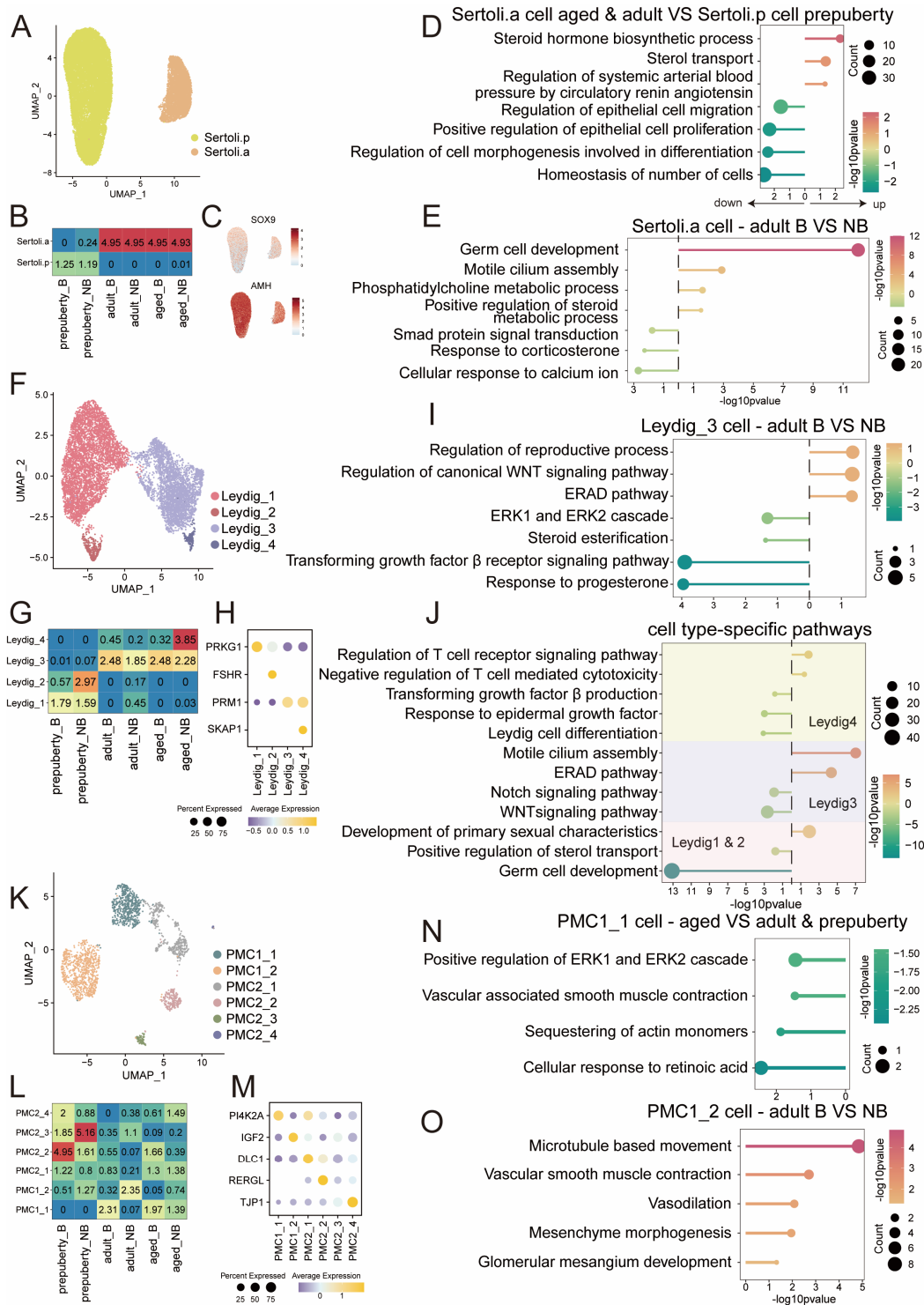
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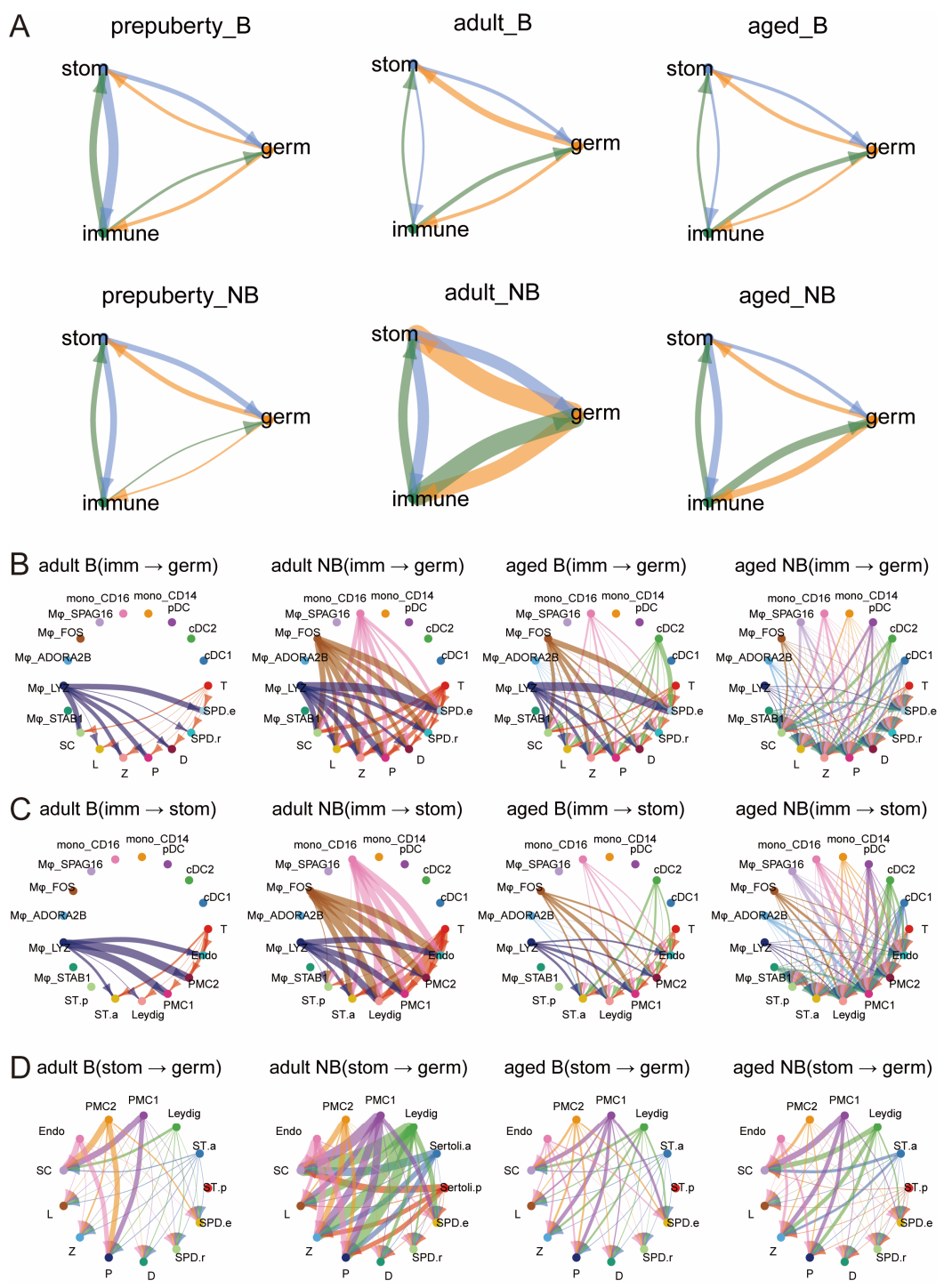
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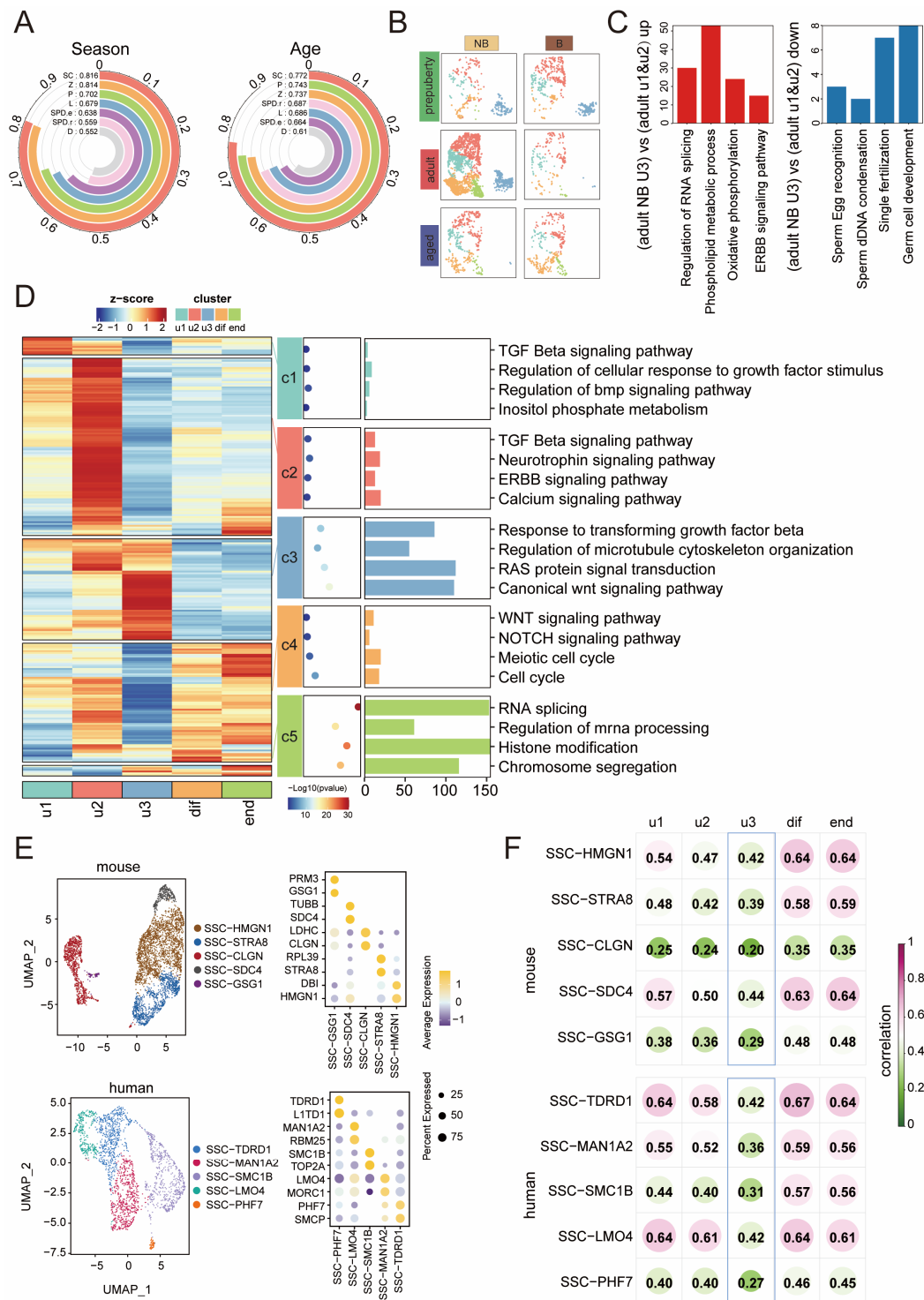
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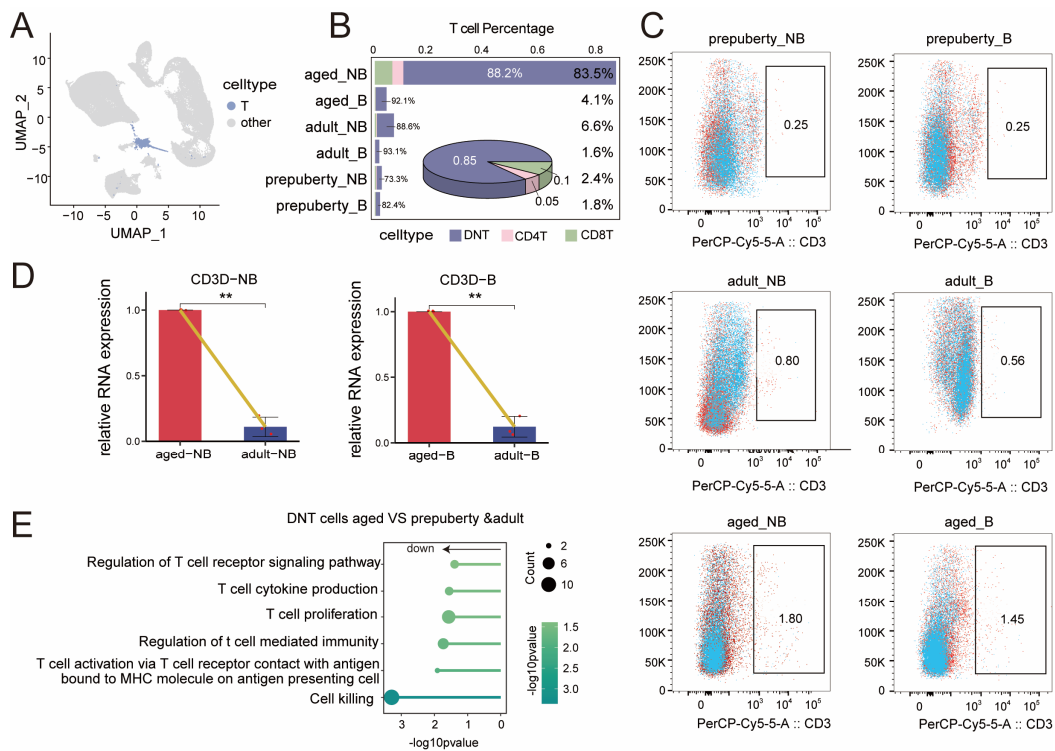


Table S1: PCR primer sequences

Name of primer	primer sequence
APOA1-F	5'-AAGATGAACCCCCACAGACC-3'
APOA1-R	5'-GGACACATAGTCTTTGCCGC-3'
VIM-F	5'-GGACCAGCTAACCAACGACA-3'
VIM-R	5'-AGATTGCAGGGTGTTCTCGG-3'
PPM1E-F	5'-GCCTGCAGCAGCTCTACAAATATAA-3'
PPM1E-R	5'-AGTCCCTTCTGTGCCATCCG-3'
PRSS21-F	5'-TCAGAACCGGACAGACTGCT-3'
PRSS21-R	5'-TCCTGGAGGGTATAGGGAGATG-3'
CD3D-F	5'-GCTACCCTTCTCTCCCAAGTG-3'
CD3D-R	5'-ACCGTTCCCTCTACCCATGT-3'
CD86-F	5'-AAACCGAAGCCTGAGTGAGC-3'
CD86-R	5'-GTGCGGCCCATATACTTGGA-3'
SPAG16-F	5'-CGTTGGGCATGGGTTTGAC-3'
SPAG16-R	5'-CCACGGGAAGGATACGCTC-3'

Table S2. Animal and single-cell sequencing metadata

Sample ID	Age group	Sex	Season	Mean UMI Counts per Cell
RNA-195041	puberty	male	NB	3734.83
RNA-195041-12	puberty	male	B	4322.74
RNA-197007	puberty	male	NB	3850.52
RNA-197007-12	puberty	male	B	3803.62
RNA-145269	adult	male	NB	4243.52
RNA-145269-12	adult	male	B	3370.64
RNA-146057	adult	male	NB	3235.34
RNA-146057-12	adult	male	B	3850
RNA-98093	aged	male	NB	2464.58
RNA-98093-12	aged	male	B	2852.15
RNA-98333	aged	male	NB	2907.17
RNA-98333-12	aged	male	B	2769.66

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Table S3. SSC- monkey_human_correlation

	95% CI Lower Limit	Correlation Coefficient	95% CI Upper Limit	P value	95% CI Summary
SSC- TDRD1-u1	0.63	0.64	0.65	0	0.64 (0.63- 0.65)
SSC- MAN1A- u1	0.54	0.55	0.56	0	0.55 (0.54- 0.56)
SSC- SMC1B-u1	0.42	0.44	0.45	0	0.44 (0.42- 0.45)
SSC- LMO4-u1	0.63	0.64	0.65	0	0.64 (0.63- 0.65)
SSC- PHF7-u1	0.39	0.4	0.42	0	0.4 (0.39- 0.42)
SSC- TDRD1-u2	0.57	0.58	0.59	0	0.58 (0.57- 0.59)
SSC- MAN1A- u2	0.5	0.52	0.53	0	0.52 (0.5- 0.53)
SSC- SMC1B-u2	0.38	0.4	0.42	0	0.4 (0.38- 0.42)
SSC- LMO4-u2	0.59	0.61	0.62	0	0.61 (0.59- 0.62)
SSC- PHF7-u2	0.39	0.4	0.42	0	0.4 (0.39- 0.42)
SSC- TDRD1-u3	0.4	0.42	0.43	0	0.42 (0.4- 0.43)
SSC- MAN1A- u3	0.35	0.36	0.38	0	0.36 (0.35- 0.38)
SSC- SMC1B-u3	0.3	0.31	0.33	9.296118446 65874e-259	0.31 (0.3- 0.33)

SSC-LMO4-u3	0.4	0.42	0.43	0	0.42 (0.4-0.43)
SSC-PHF7-u3	0.25	0.27	0.28	2.732782874 17653e-187	0.27 (0.25-0.28)
SSC-TDRD1-dif	0.66	0.67	0.68	0	0.67 (0.66-0.68)
SSC-MAN1A-dif	0.58	0.59	0.6	0	0.59 (0.58-0.6)
SSC-SMC1B-dif	0.56	0.57	0.59	0	0.57 (0.56-0.59)
SSC-LMO4-dif	0.63	0.64	0.65	0	0.64 (0.63-0.65)
SSC-PHF7-dif	0.45	0.46	0.48	0	0.46 (0.45-0.48)
SSC-TDRD1-end	0.63	0.64	0.65	0	0.64 (0.63-0.65)
SSC-MAN1A-end	0.55	0.56	0.57	0	0.56 (0.55-0.57)
SSC-SMC1B-end	0.55	0.56	0.57	0	0.56 (0.55-0.57)
SSC-LMO4-end	0.6	0.61	0.62	0	0.61 (0.6-0.62)
SSC-PHF7-end	0.43	0.45	0.46	0	0.45 (0.43-0.46)

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Table S3. SSC- monkey_mouse_correlation

	95% CI Lower Limit	Correlatio n Coefficien t	95% CI Upper Limit	P value	95% CI Summary
SSC-HMGN1-u1	0.53	0.54	0.56	0	0.54 (0.53-0.56)
SSC-STRA8-u1	0.46	0.48	0.49	0	0.48 (0.46-0.49)
SSC-CLGN-u1	0.24	0.25	0.27	1.56142145 328725e- 149	0.25 (0.24-0.27)
SSC-SDC4-u1	0.56	0.57	0.58	0	0.57 (0.56-0.58)

SSC-GSG1-u1	0.36	0.38	0.39	0	0.38 (0.36-0.39)
SSC-HMGN1-u2	0.46	0.47	0.49	0	0.47 (0.46-0.49)
SSC-STRA8-u2	0.41	0.42	0.44	0	0.42 (0.41-0.44)
SSC-CLGN-u2	0.22	0.24	0.26	4.65641114 525116e-134	0.24 (0.22-0.26)
SSC-SDC4-u2	0.48	0.5	0.51	0	0.5 (0.48-0.51)
SSC-GSG1-u2	0.34	0.36	0.38	2.04932366 175282e-310	0.36 (0.34-0.38)
SSC-HMGN1-u3	0.41	0.42	0.44	0	0.42 (0.41-0.44)
SSC-STRA8-u3	0.38	0.39	0.41	0	0.39 (0.38-0.41)
SSC-CLGN-u3	0.18	0.2	0.22	1.19645194 289886e-90	0.2 (0.18-0.22)
SSC-SDC4-u3	0.43	0.44	0.46	0	0.44 (0.43-0.46)
SSC-GSG1-u3	0.27	0.29	0.31	4.82718608 477216e-196	0.29 (0.27-0.31)
SSC-HMGN1-dif	0.63	0.64	0.65	0	0.64 (0.63-0.65)
SSC-STRA8-dif	0.57	0.58	0.59	0	0.58 (0.57-0.59)
SSC-CLGN-dif	0.33	0.35	0.36	6.89267699 458207e-286	0.35 (0.33-0.36)
SSC-SDC4-dif	0.62	0.63	0.64	0	0.63 (0.62-0.64)
SSC-GSG1-dif	0.46	0.48	0.49	0	0.48 (0.46-0.49)
SSC-HMGN1-end	0.62	0.64	0.65	0	0.64 (0.62-0.65)
SSC-STRA8-end	0.58	0.59	0.61	0	0.59 (0.58-0.61)
SSC-CLGN-end	0.33	0.35	0.37	6.28615626 760923e-294	0.35 (0.33-0.37)

SSC-SDC4- end	0.63	0.64	0.65	0	0.64 (0.63- 0.65)
SSC-GSG1- end	0.46	0.48	0.49	0	0.48 (0.46- 0.49)

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