# Expanded View Figures

**Figure EV1. Histological analyses of cy fetal ovaries *in vivo* and in xenotransplanted rOvaries.**

A Size of developing cy fetuses and ovaries at 8–18 wpf [8/10/12/14/16/18 wpf ($n$ = 26/3/3/5/4/3)]. Lines indicate LOESS curves fitted to the sample populations.

B, C IF of a cy fetal gonad at 8 wpf (B) and ovarian cortexes in cy fetuses at 10–18 wpf (C). The results of staining with FOXL2 (granulosa-cell marker, green), DDX4 (germ-cell marker, magenta), and DAPI (nucleus, white) are shown. The ovarian cortexes at 10–18 wpf were divided into an outer and inner cortex at the line bisecting the ovarian cortex. Scale bars = 40 μm (B) and 50 μm (C).

D The granulosa/germ cell ratios at each developmental stage assessed from the IF data are shown [8–18 wpf ($n$ = 1)].

E IF for oogonia (NANOG and PDPN), cell proliferation (Ki67), apoptosis (cleaved PARP and cleaved CASPASE 3), extracellular matrix (LAMININ), and oogenic (FIGLA, NOBOX, and NLRP5) markers during cy fetal ovary development. Representative images for each marker are shown. Germ cells were marked with DDX4 (magenta). Nuclear DAPI staining is shown in white. The upper-right magnified images of each panel show the expression of key markers (green) co-stained with DDX4 (magenta) except for NLRP5. The lower-right magnified images of each panel show DDX4 expression (magenta) co-stained with DAPI (white). The numbers written in the upper-left corner indicate the stage (wpf) of the fetus. Scale bar = 20 μm.

F Percentages of cells positive for individual markers among $DDX4^+$ germ cells from the IF of the cy fetal ovarian outer/inner cortex at 8–18 wpf. The mean values from more than three biological replicates are shown [8 wpf ($n$ = 4), 10/12/14/16/18 wpf ($n$ = 3)].

G H&E staining of transplanted cy rOvaries at 3-/6-/9-/12-/15-week post-transplantation (w-ptp). Dashed lines indicate the transplanted cy rOvaries beneath the kidney capsule of KSN/Slc mice. K, mouse kidney. Scale bar = 200 μm.

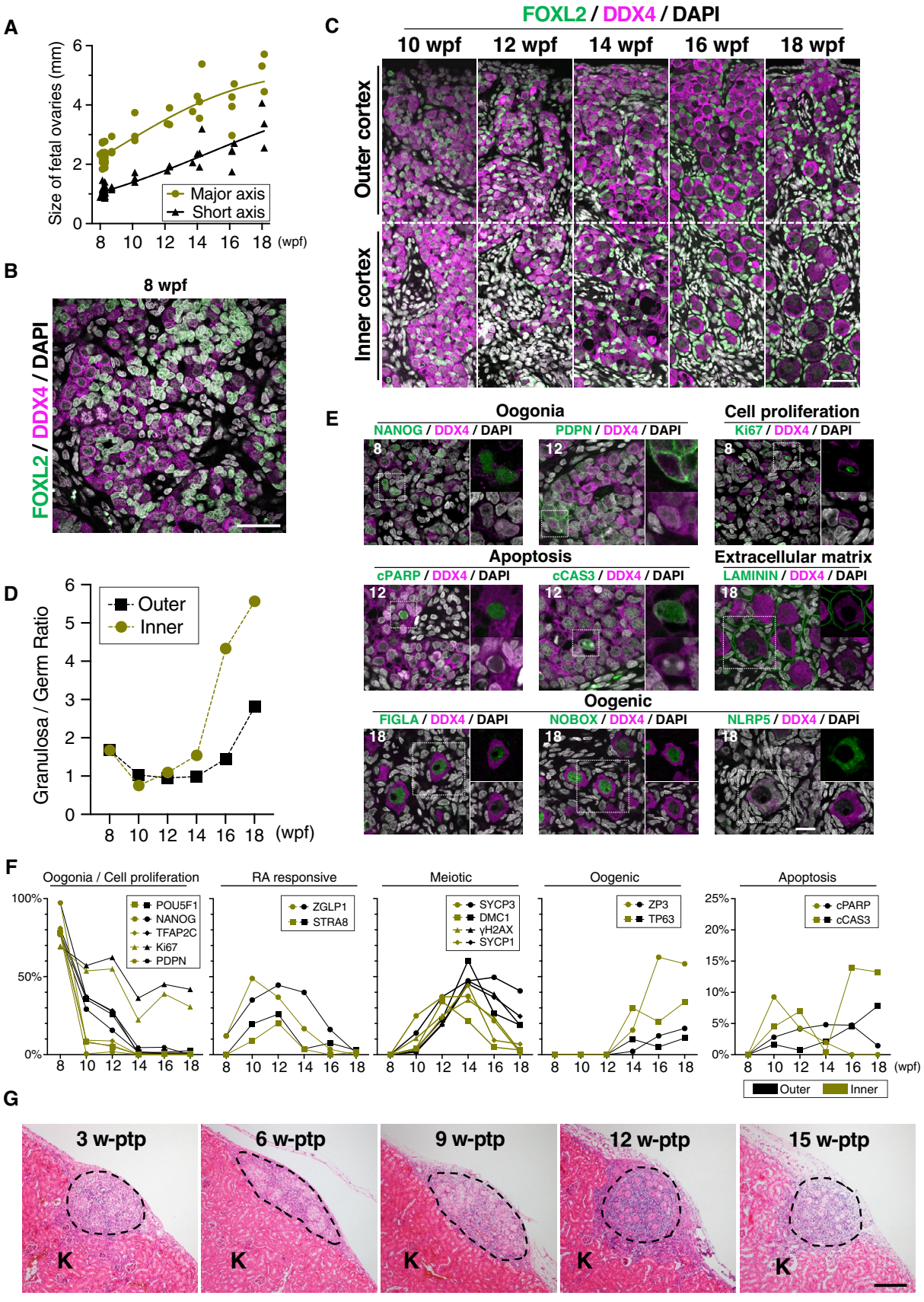
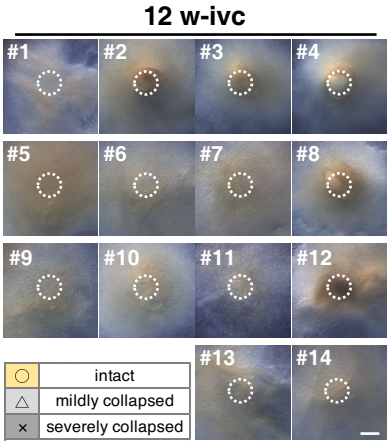


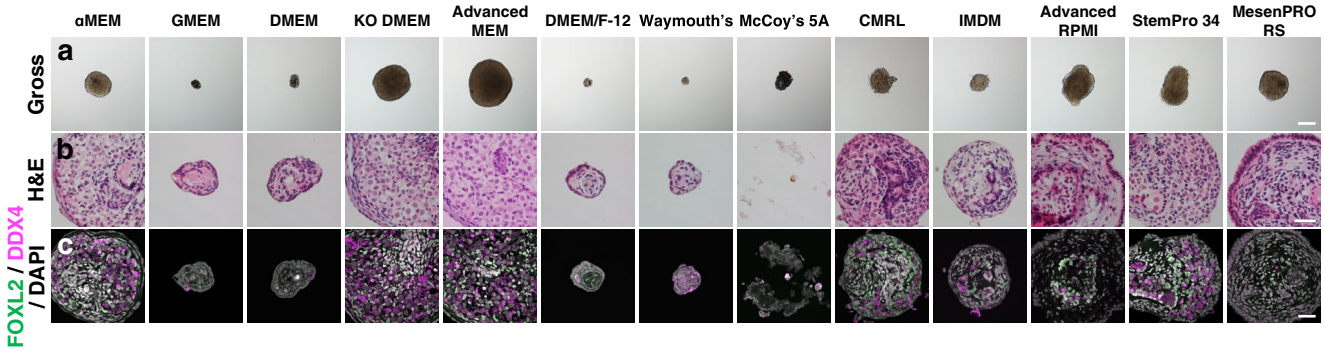

**Figure EV1.**

**A**

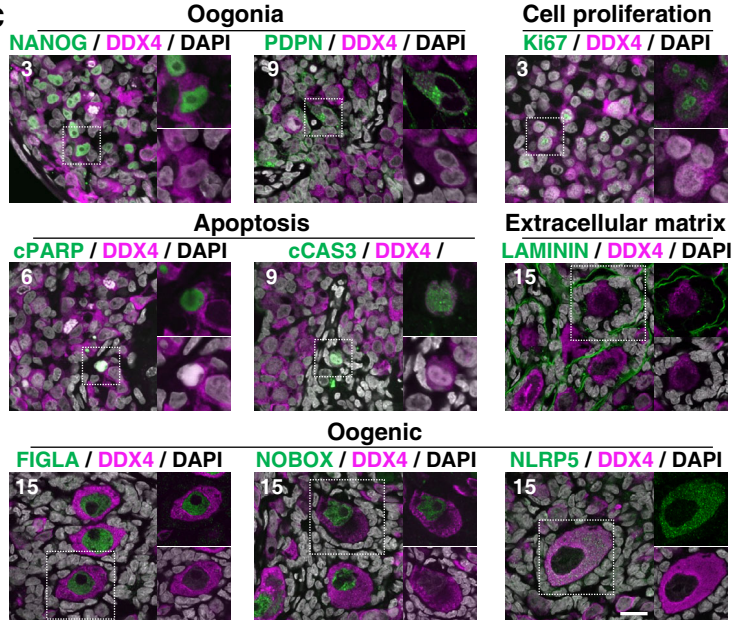
| # | Membrane | Coating reagent | *in vitro* culture period (w-ivc) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 12 |
| 1 | Transwell-COL | - | ○ | △ | △ | × | × | × | × | × | × |
| 2 | VECELL | - | ○ | ○ | ○ | △ | △ | × | × | × | × |
| 3 | | hCollagen type I + III | ○ | ○ | ○ | ○ | ○ | △ | × | × | × |
| 4 | | hCollagen type IV | ○ | ○ | △ | △ | △ | △ | △ | × | × |
| 5 | | hCollagen type V | ○ | △ | △ | △ | × | × | × | × | × |
| 6 | | hCollagen type VI | ○ | △ | △ | △ | △ | × | × | × | × |
| 7 | | hCollagen type I + III + IV | ○ | ○ | ○ | △ | △ | △ | × | × | × |
| 8 | | hCollagen type I + III + IV + V + VI | ○ | ○ | ○ | ○ | ○ | ○ | △ | × | × |
| 9 | | hFibronectin | ○ | × | × | × | × | × | × | × | × |
| 10 | | iMatrix | ○ | ○ | ○ | ○ | ○ | ○ | △ | × | × |
| 11 | | Laminin (111/121/211/221/411/421/332/521) | ○ | ○ | △ | × | × | × | × | × | × |
| 12 | | Matrigel (Growth factor reduced) | ○ | ○ | △ | △ | △ | △ | × | × | × |
| 13 | | Laminin + hCollagen (ALL types) | ○ | ○ | △ | △ | × | × | × | × | × |
| 14 | | Laminin + hCollagen (ALL types) + hFibronectin | ○ | × | × | × | × | × | × | × | × |
| 15 | Floating culture | | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |




**B**




**C**




**D**

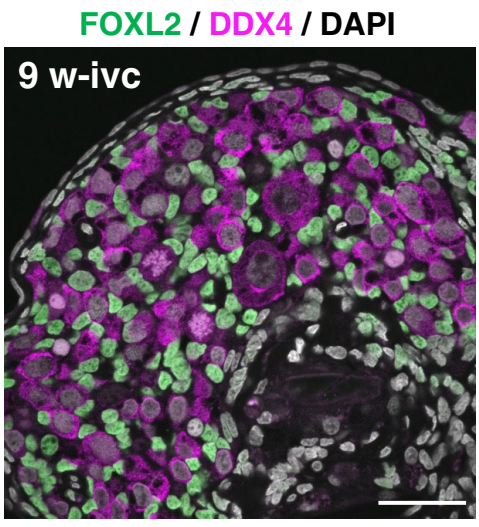



**Figure EV2.**

**Figure EV2. Establishment of a novel *in vitro* culture system for cy oocyte differentiation.**

A (Left) Summary table of coating conditions tested for cy rOvaries. (Right) Representative images of cy rOvaries cultured on membranes at 12 w-ivc. Dotted circles show the edge of cy rOvaries at the starting point of air–liquid interface culture. Scale bar = 500 μm.

B Gross appearances (a), H&E staining (b), and IF for FOXL2/DDX4/DAPI (c) of cy rOvaries cultured in 13 different basal media at 6 w-ivc. The rOvary cultured in Advanced MEM maintained its size, and $DDX4^+$ germ and $FOXL2^+$ granulosa cells were found within the rOvaries. Scale bars = 500 μm (a) and 40 μm (b and c).

C IF analyses of key markers as described in Fig EV1E in the cultured cy rOvaries. Germ cells and nuclei were marked with DDX4 (magenta) and DAPI (white), respectively. The upper-right magnified images of each panel show the expression of key markers (green) co-stained with DDX4 (magenta) except for NLRP5. The lower-right magnified images of each panel show DDX4 expression (magenta) co-stained with DAPI (white). The numbers written in the upper-left corner indicate the period of IVC (w-ivc). Scale bar = 20 μm.

D IF analysis for FOXL2 (granulosa cells)/DDX4 (germ cells) stained with DAPI (nucleus) in the cultured cy rOvary at 9 w-ivc. Scale bar = 40 μm.

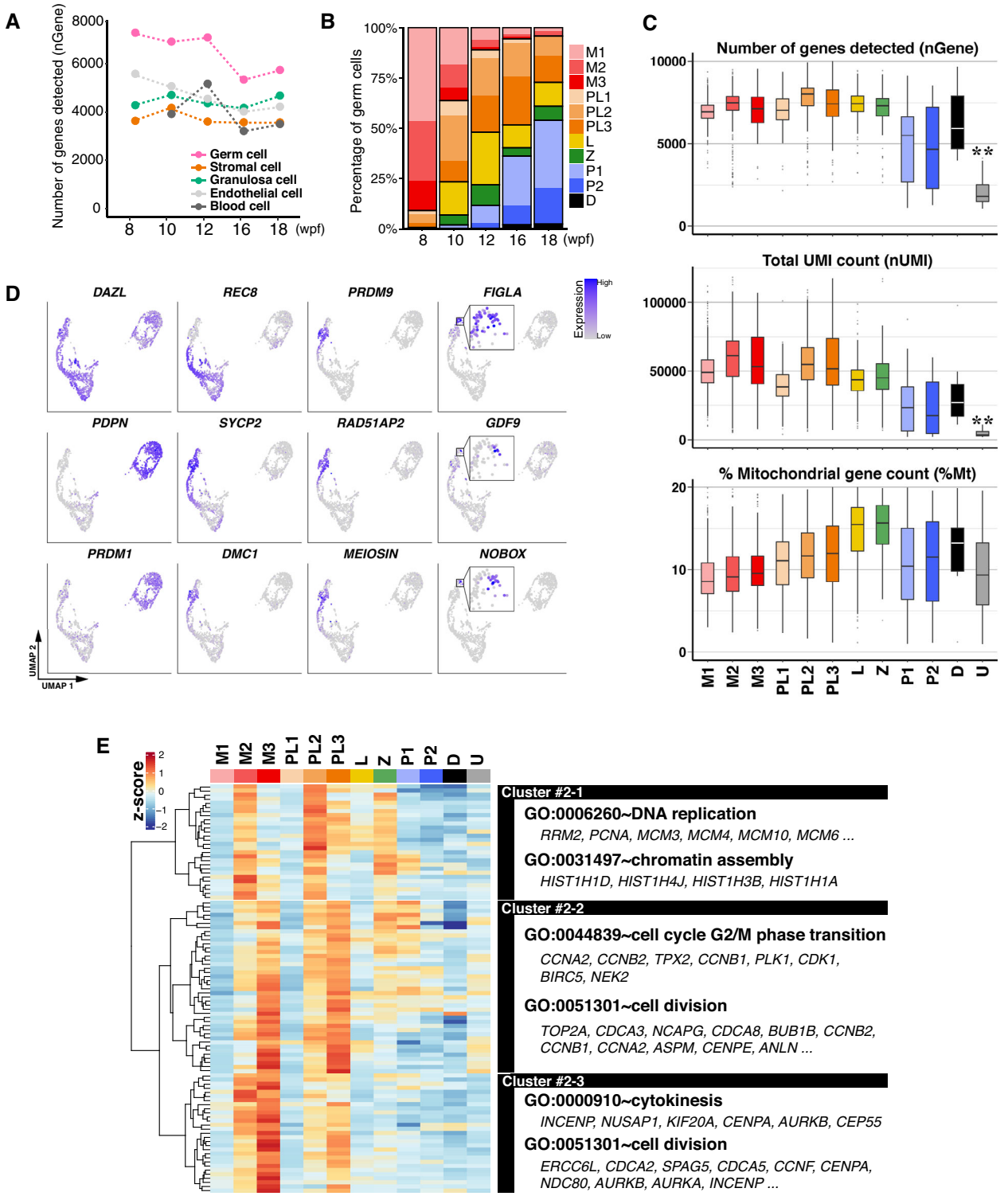

A
Number of genes detected (nGene)
8000
6000
4000
2000
0
8 10 12 16 18 (wpf)
Germ cell
Stromal cell
Granulosa cell
Endothelial cell
Blood cell
B
Percentage of germ cells
100%
75%
50%
25%
0%
8 10 12 16 18 (wpf)
M1
M2
M3
PL1
PL2
PL3
L
Z
P1
P2
D
C
Number of genes detected (nGene)
10000
5000
0
Total UMI count (nUMI)
100000
50000
0
% Mitochondrial gene count (%Mt)
20
10
0
M1 M2 M3 PL1 PL2 PL3 L Z P1 P2 D U
D
DAZL
REC8
PRDM9
FIGLA
PDPN
SYCP2
RAD51AP2
GDF9
PRDM1
DMC1
MEIOSIN
NOBOX
Expression
High
Low
UMAP 2
UMAP 1
E
z-score
2
1
0
−1
−2
M1 M2 M3 PL1 PL2 PL3 L Z P1 P2 D U
Cluster #2-1
GO:0006260~DNA replication
RRM2, PCNA, MCM3, MCM4, MCM10, MCM6 ...
GO:0031497~chromatin assembly
HIST1H1D, HIST1H4J, HIST1H3B, HIST1H1A
Cluster #2-2
GO:0044839~cell cycle G2/M phase transition
CCNA2, CCNB2, TPX2, CCNB1, PLK1, CDK1, BIRC5, NEK2
GO:0051301~cell division
TOP2A, CDCA3, NCAPG, CDCA8, BUB1B, CCNB2, CCNB1, CCNA2, ASPM, CENPE, ANLN ...
Cluster #2-3
GO:0000910~cytokinesis
INCENP, NUSAP1, KIF20A, CENPA, AURKB, CEP55
GO:0051301~cell division
ERCC6L, CDCA2, SPAG5, CDCA5, CCNF, CENPA, NDC80, AURKB, AURKA, INCENP ...

**Figure EV3.**

**Figure EV3. 10X scRNA-seq analysis of cy female oocyte development *in vivo*.**

A The average number of genes detected in five major cell types in cy *in vivo* fetal ovaries at 8–18 wpf.

B Percentages of germ cells at the indicated meiotic prophase substages defined in Fig 4D. The color-coding is as indicated. M, mitotic; PL, pre-leptotene; L, leptotene; Z, zygotene; P, pachytene; D, diplotene.

C The detected gene number, UMI count, and percentage of mitochondrial genes in each germ-cell cluster. The number of genes and UMI counts were significantly low in the "unclassified (U)" compared to any other germ-cell cluster. $^{**}P < 0.01$, Tukey–Kramer tests. In the boxplots, the central bands represent the median values; the lower/upper hinges represent the 25th/75th percentiles, respectively; the upper limits of the whiskers represent the largest values no further than 1.5 IQR (interquartile range) from the upper hinges; the lower limits of the whiskers represent the smallest values no further than 1.5 IQR from the lower hinges; the dots represent the outliers. The numbers of the cells used are 431/271/135/59/252/253/261/101/183/74/8/90 from 7 fetuses for M1/M2/M3/PL1/PL2/PL3/L/Z/P1/P2/D/U, respectively.

D Feature plots of key marker genes for oocyte development on the UMAP plot shown in Fig 4D.

E (Left) Heatmap of the standardized expression of HVGs listed in the gene cluster #2 in Fig 4H (Dataset EV3). Three gene clusters (Cluster #2–1/—2/—3) were defined according to the UHC dendrogram. (Right) Representative genes and key GO enrichments are shown.

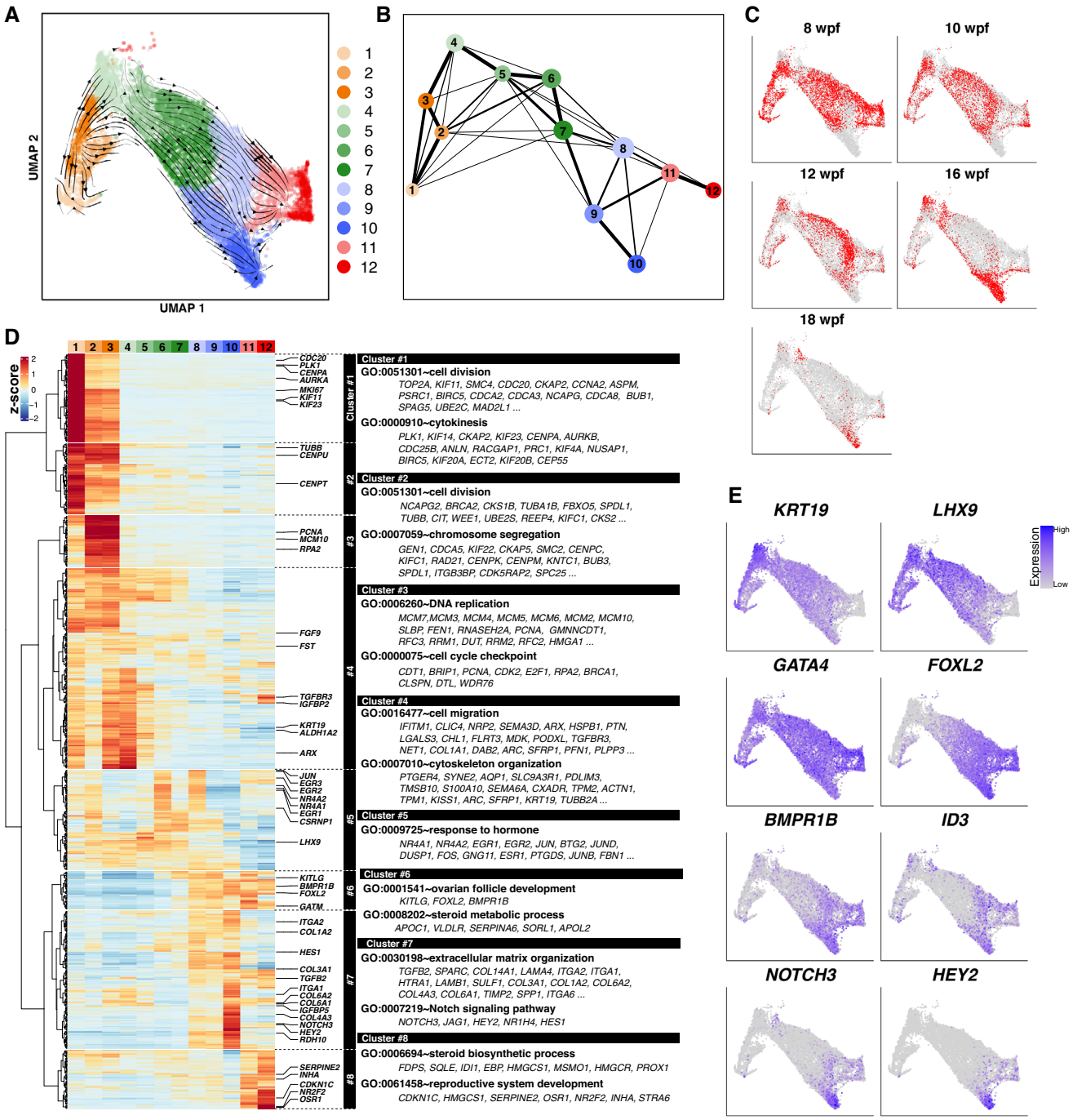



**Figure EV4. 10X scRNA-seq analysis for cy granulosa cells *in vivo* and in rOvaries.**

A, B UMAP plot with RNA velocity (A) and PAGA graph (B) of cy *in vivo* granulosa cells as defined in Fig 4A. Granulosa cells were divided into 12 subclusters by Louvain clustering. The color-coding is as indicated.

C UMAP plot shown in Fig EV4A, highlighting the cells for each fetal stage.

D (Left) Heatmap of the standardized expression of HVGs (712 genes) among *in vivo* cy granulosa-cell subclusters ordered by UHC; eight gene clusters were defined according to the UHC dendrogram. (Right) Representative genes and key GO enrichments are shown.

E Feature plots of key marker genes for cy granulosa-cell development on the UMAP plot shown in Fig EV4A.

**Figure EV5. Cross-species comparison analysis of oocyte development in humans, monkeys, and mice.**

A Heatmap of the standardized expression levels of selected genes in all three species analyzed by 10X scRNA-seq. Genes showing a conserved expression pattern in all three animal species during female germ-cell development were manually selected from the list of DEGs acquired from the SC3-seq analysis for cy/human *in vitro* cultured rOvaries (Dataset EV6). The color-coding is as indicated.

B Box plots for the gene expression levels of selected markers showing primate-specific expression dynamics during fetal germ-cell development (see also Fig 6F). In the boxplots, the central bands represent the median values; the lower/upper hinges represent the 25th/75th percentiles, respectively; the upper limits of the whiskers represent the largest values no further than 1.5 IQR (interquartile range) from the upper hinges; the lower limits of the whiskers represent the smallest values no further than 1.5 IQR from the lower hinges; the dots represent the outliers. The numbers of the cells used are 0/606/0/117/133/76/6/72/68/2/12/14/45/16/47 from 4 fetuses for humans (Chitiashvili *et al*, 2020), 0/761/2/136/286/200/14/145/158/0/44/68/143/48/23 from 7 fetuses for cynomolgus monkeys (this study), and 26/261/21/232/259/149/209/450/422/134/232/163/339/630/298 for mice (Niu & Spradling, 2020) for M1/M2/M3/M4/PL1/PL2/PL3/L1/L2/L3/Z1/Z2/P1/P2/D, respectively. Cell clusters with more than two cells were analyzed.

C IF analyses of LEIOMODIN-3 (LMOD3) with TP63 (oogenic marker) and DDX4 (germ-cell marker) in cy *in vivo* fetal oocytes at 18 wpf (top) and 13-year-old human oocytes in primordial follicles (bottom). Nuclear DAPI staining is shown in white. Scale bar = 20 μm.

D The autosome:A and X:A ratios (top) and *XIST*/*Xist* expression transitions (bottom) during female germ-cell development *in vivo* in humans (this study) and mice (Zhao *et al*, 2020; Ge *et al*, 2021) analyzed by 10X scRNA-seq. In the boxplots, the central bands represent the median values; the lower/upper hinges represent the 25th/75th percentiles, respectively; the upper limits of the whiskers represent the largest values no further than 1.5 IQR (interquartile range) from the upper hinges; the lower limits of the whiskers represent the smallest values no further than 1.5 IQR from the lower hinges; the dots represent the outliers. The numbers of the cells used are 0/275/0/40/81/40/5/20/31/0/6/1/1/2/2/3164/520 cells from 1 fetus for humans (this study), 643/1991/369/1819/306/179/490/1505/2051/523/1733/2529/2069/2863/102/0/0 for mice (Zhao *et al*, 2020), and 66/605/85/547/298/167/330/463/467/144/320/196/137/17/0/14962/5416 for mice (Ge *et al*, 2021) for M1/M2/M3/M4/PL1/PL2/PL3/L1/L2/L3/Z1/Z2/P1/P2/D/granulosa/stroma, respectively. Cell clusters with more than two cells were analyzed.

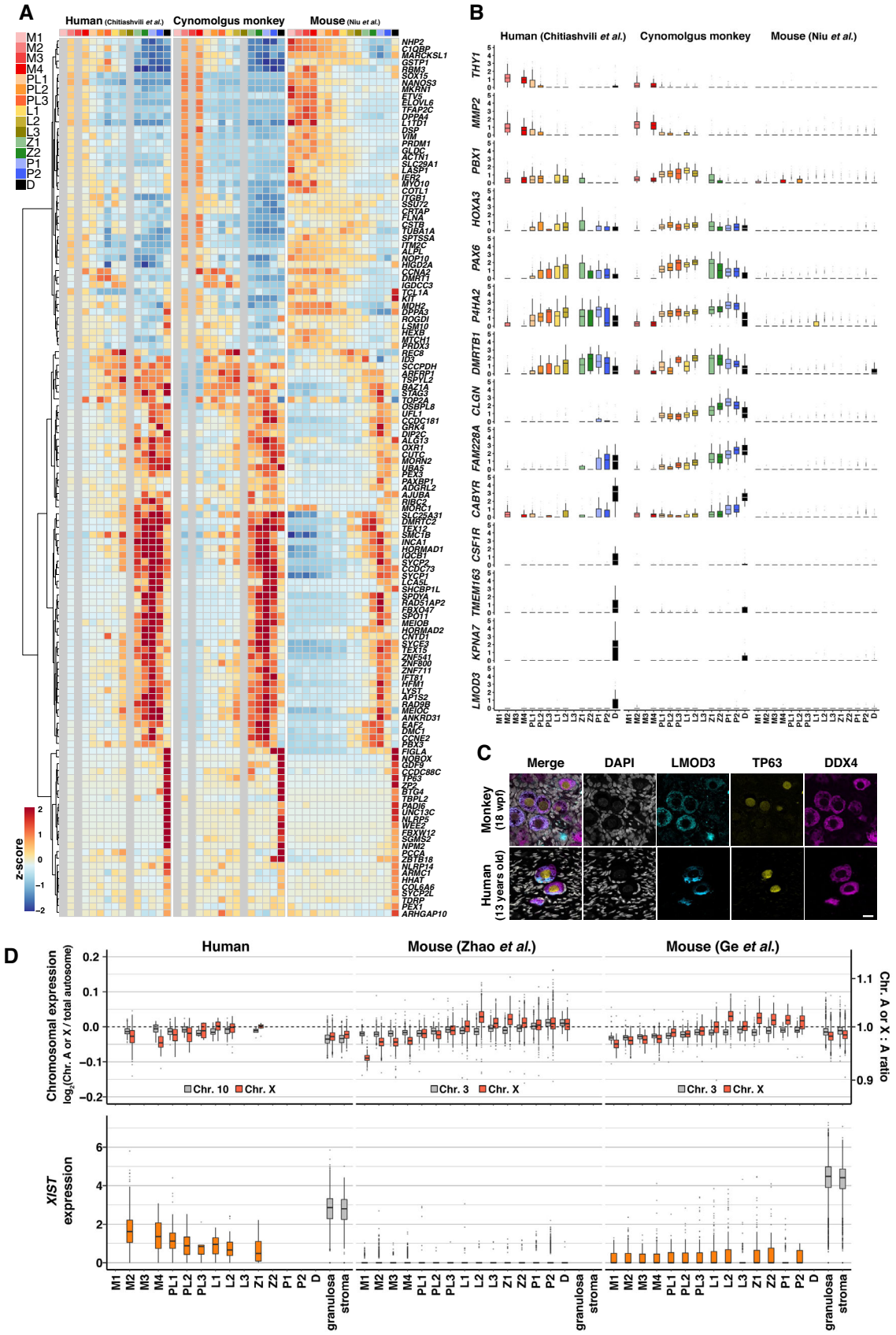

A
Human (Chitiashvili et al.)
Cynomolgus monkey
Mouse (Niu et al.)
M1
M2
M3
M4
PL1
PL2
PL3
L1
L2
L3
Z1
Z2
P1
P2
D
z-score
B
Human (Chitiashvili et al.)
Cynomolgus monkey
Mouse (Niu et al.)
THY1
MMP2
PBX1
HOXA3
PAX6
P4HA2
DMRTB1
CLGN
FAM228A
CABYR
CSF1R
TMEM163
KPNA7
LMOD3
C
Merge
DAPI
LMOD3
TP63
DDX4
Monkey (18 wpf)
Human (13 years old)
D
Human
Mouse (Zhao et al.)
Mouse (Ge et al.)
Chromosomal expression log2(Chr. A or X / total autosome)
Chr. 10
Chr. X
Chr. 3
Chr. X
Chr. A or X : A ratio
XIST expression
granulosa
stroma

**Figure EV5.**