

**Fig S1. Motifs of the mouse TEX14 protein.** (Adapted from Iwamori T et al., Molecular and Cellular Biology, 2010; 30(9):2280-92).



**Fig S2. Single-PGC lineage labeling efficiency.** The efficiency of single PGC-lineage labeling shown by the percentage of fetal gonads containing YFP positive germ cell clones in wild type and *Tex14* mutant mouse fetal gonads.



**Fig S3. Clustered SYCP3-positive cells observed in the E14.5 homozygous mutant ovary.** (n=6 ovaries).



**Fig S4. The germ cell clone revealed by single-PGC lineage tracing in E14.5 testes.** (A) A YFP-positive germ cell clone containing a germline cyst in the E14.5 wild type testis (n=22 testes). (B) A YFP-positive germ cell clone containing many smaller cysts in the E14.5 homozygous mutant testis (n=20 testes).



**Fig S5. 3D images of TEX14 antibody-stained intercellular bridges and the models of these bridges observed in E14.5** *Tex14* **heterozygous mutant ovaries.** (A, B) Ring-shaped intercellular bridges. (C-F) Fragmented intercellular bridges. (N=6 ovaries were examined).



**Fig S6. The expression of cytokinesis proteins.** (A) The expression of TEX14, MKLP1, ALIX, RacGAP, CEP55 and TSG101 in E14.5 wild type (+/+), heterozygous mutant (+/-) and homozygous mutant (-/-) gonads examined by western blot. N=40 gonads of each genotype were pooled for each western blot sample. N=3 experiments were conducted. (B) The relative protein level of TEX14 in E14.5 wild type and mutant gonads. Error bars present standard deviations



**Fig S7. The fold of germ cell volume increase from E14.5 to P4 and the percentage of E14.5 germ cells differentiate into primary oocytes.** N=6 ovaries (approximately 100 germ cells/ovary) at each time point were used for germ cell volume analyses. Total number of the germ cells per ovary at each time point were used for germ cell number analyses.



**Fig S8. Number of germ cells in the fetal ovary during oocyte differentiation.** N=6 ovaries at each time point were used for analyses. One-way ANOVA was used for statistical analyses, \* indicates significant difference with P value <0.05. Error bars present standard deviations.



**Fig S9. Number of follicles in the adult ovary from 1 month to 8 months.** N=6 ovaries at each time point were used for analyses. One-way ANOVA was used for statistical analyses, \* indicates significant difference with P value <0.05. Error bars present standard deviations.



**Fig S10.** Proportion of primary oocytes based on cell diameter (prop <sub>oocyte size</sub>) in the ovarian reserve of P4 ovaries. The percentage of primary oocytes at each size range (percentage <sub>oocyte size</sub>) was calculated based on germ cell diameter measurements in P4 ovaries (Fig 3H). The prop <sub>oocyte size</sub> = average number of primordial follicles per ovary x percentage <sub>oocyte size</sub> at each size range.

Antibodies	Vendors and antibody validation profile	Dilution
TEX14	Protein Tech (18351-1-AP; Lot# was not provided by the vendor) https://www.ptglab.com/products/TEX14-Antibody-18351-1-AP.htm	1:200 (IF) 1:1000 (WB)
RacGAP1 (A-6)	Santa Cruz Biotechnology (sc 271110; Lot# F0612) https://www.scbt.com/p/rac-gap1-antibody-a- 6?requestFrom=search	1:100
GM130	BD Biosciences (610822; Lot# 7163678) https://www.bdbiosciences.com/us/reagents/research/antibodies- buffers/cell-biology-reagents/cell-biology-antibodies/purified-mouse- anti-gm130-35gm130/p/610822	1:100
MVH	Abcam (ab13840; Lot# GR3218256) https://www.abcam.com/ddx4mvh-antibody-ab13840.html	1:200
Sodium Potassium ATPase	Abcam (ab76020; Lot# GR3184452-3) https://www.abcam.com/sodium-potassium-atpase-antibody- ep1845y-plasma-membrane-loading-control-ab76020.html	1:200
TRA98	B-Bridge (73-003; Lot# 170819B) https://www.asone-int.com/wp-content/uploads/2017/05/73-003_73- 003_Datasheet.pdf	1:500
Cleaved Parp (Asp214)	Cell Signaling (94885S; Lot# 1) https://www.cellsignal.com/products/primary-antibodies/cleaved- parp-asp214-d6x6x-rabbit-mab-rodent- specific/94885?Ntk=Products&Ntt=94885	1:200
GFP	Aves Labs (GFP-1020; Lot# GFP697986) https://www.aveslabs.com/products/green-fluorescent-protein-gfp- antibody?_pos=1&_sid=8140431bd&_ss=r	1:1000
EdU (labeling kit)	Invitrogen (C10337; Lot# 1741650) https://www.thermofisher.com/order/catalog/product/C10337#/C103 37	1:200 for EdU incubation
SCP3	Abcam (ab15092; LotT# 823899) LEI, L. & SPRADLING, A. C. 2013b. Mouse primordial germ cells produce cysts that partially fragment prior to meiosis. <i>Development</i> , 140, 2075-81.	1:200
MKLP1	Santa Cruz Biotechnology (sc-867; Lot# B2310) https://www.scbt.com/p/mklp-1-antibody-n-19?requestFrom=search	1:500
ALIX	BioLegend (634502; Lot# was not provided by the vendor) https://www.biolegend.com/en-us/products/purified-anti-alix- antibody-4469	1:500

Table S1. Antibodies used in the manuscript.

RacGAP1	Protein Tech (13739-1-AP; Lot# was not provided by the vendor) https://www.ptglab.com/products/RACGAP1-Antibody-13739-1- AP.htm	1:500
CEP55	Abcam (ab170414; Lot# GR128676) https://www.abcam.com/cep55-antibody-epr11944b-ab170414.html	1:500
TSG101	Santa Cruz Biotechnology (sc-7964; Lot# H1319) https://www.scbt.com/p/tsg-101-antibody-c-2?requestFrom=search	1:500
GAPDH	Cell Signaling (5174; Lot# 7) https://www.cellsignal.com/products/primary-antibodies/gapdh- d16h11-xp-rabbit-mab/5174?Ntk=Products&site-search- type=Products&N=4294956287&Ntt=gapdh&fromPage=plp	1:10000