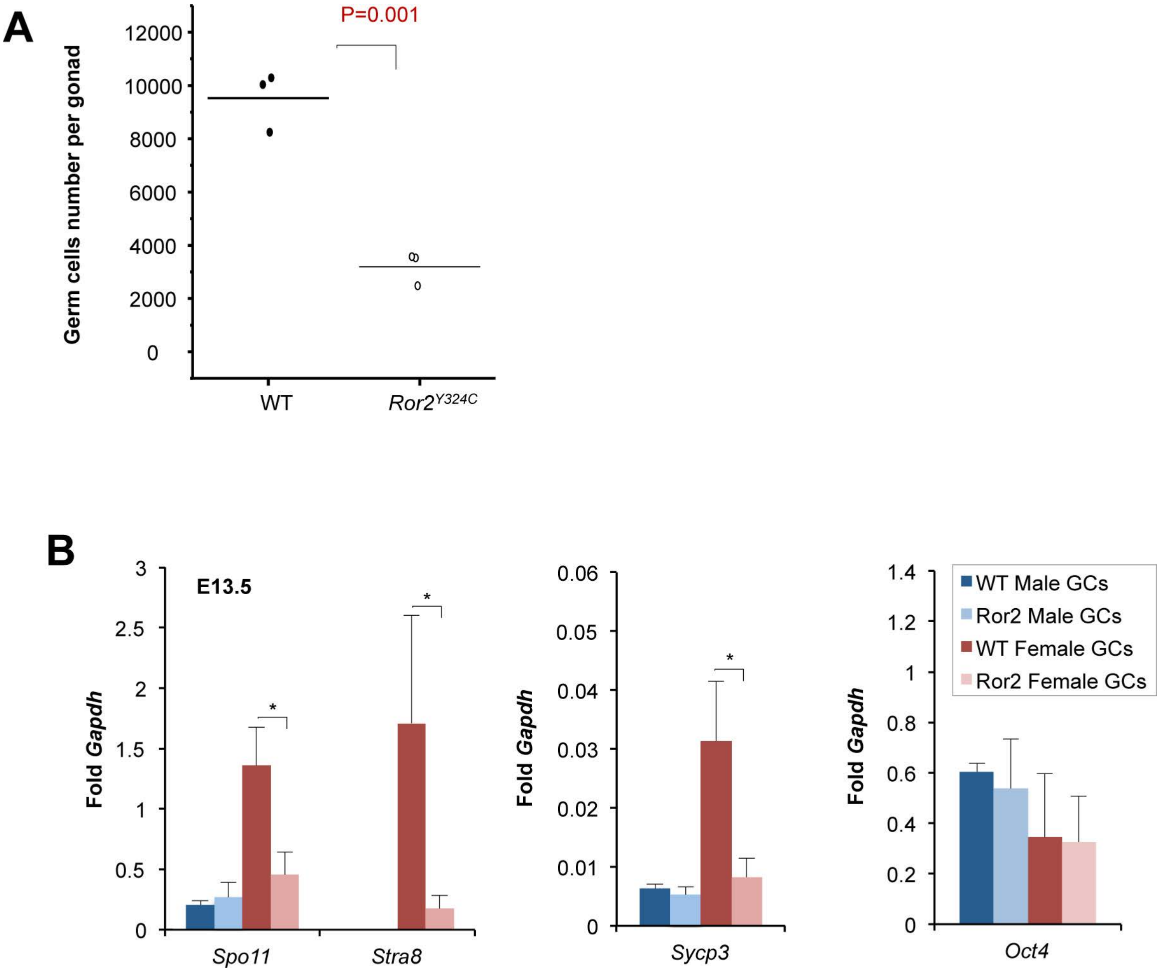

**Figure S1. (A)** Comparison of the number of germ cells per gonad between *Ror2*$^{Y324C}$ and WT control littermates at E14.5. **(B)** Expression of meiosis genes is decreased in Oct4-GFP+ germ cells sorted from *Ror2*$^{Y324C}$ ovaries compared to mixed wild-type and heterozygous controls at E13.5. qRT-PCR results shown are from pooled cells run in technical triplicate as compared to *Gapdh* levels.

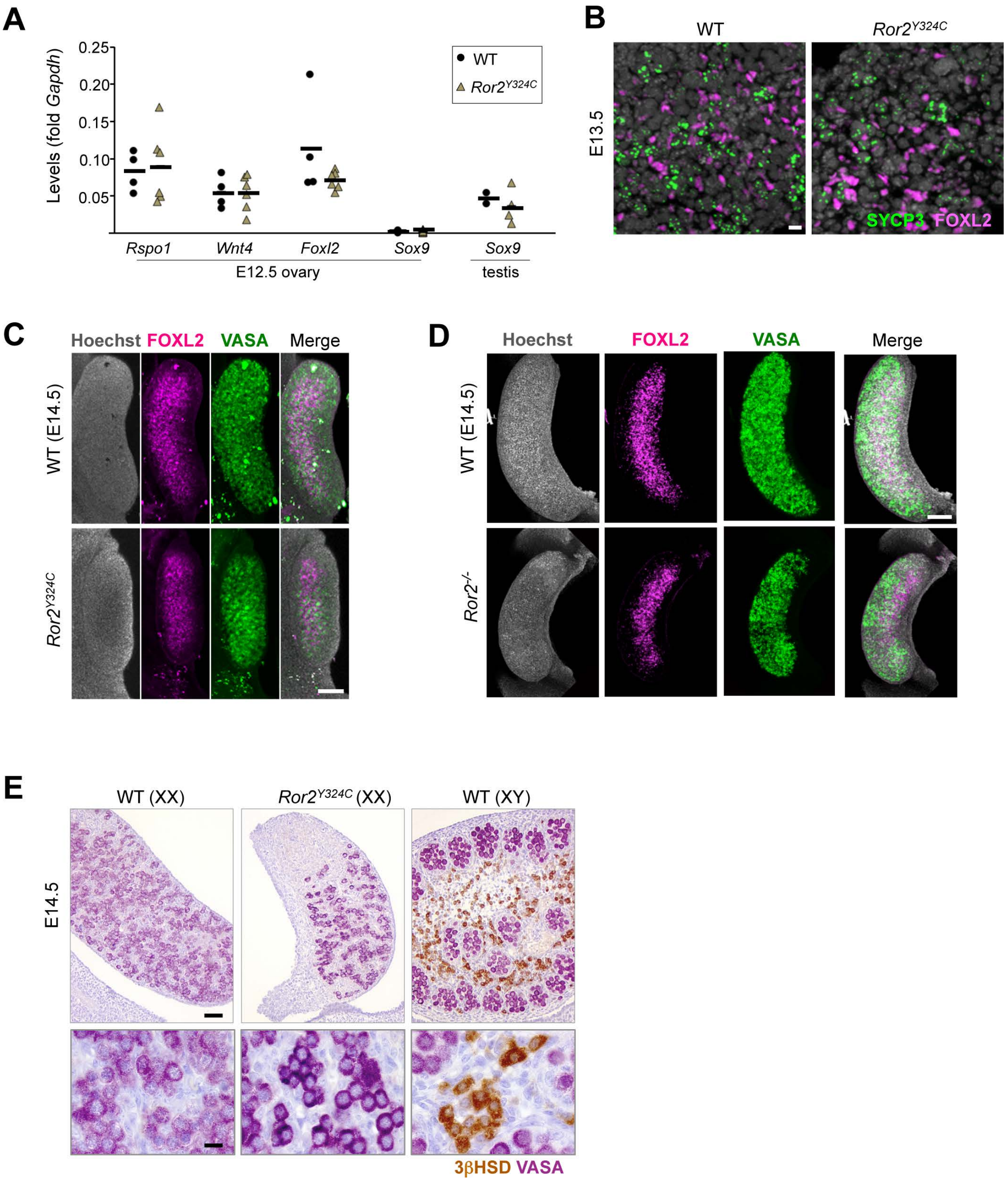

**Figure S2.** Meiotic entry delay in *Ror2*$^{Y324C/Y324C}$ mutants is not due to aberrant sex differentiation. (A) qPCR showing comparable levels of *Wnt4, Rspo1* and *Foxl2* transcript in WT and mutant ovaries at E12.5. *Sox9* is expressed in the testis at this time but not in the WT or mutant ovaries. (B) Comparable expression of *Foxl2* in WT and mutant ovaries at E13.5 by immunofluorescence. (C,D) Whole-mount staining showing similar distribution of FOXL2-positive somatic cells in WT and *Ror2*$^{Y324C/Y324C}$ (C) ovaries and *Ror2*$^{-/-}$ *ovaries* at E14.5 (D). (E) Absence of 3βHSD expression in WT and mutant ovaries, with WT testis shown as a positive control. Scale bars in B,E (lower panel)=10µm; C,D=100µm, E (upper panel) = 40 µm.

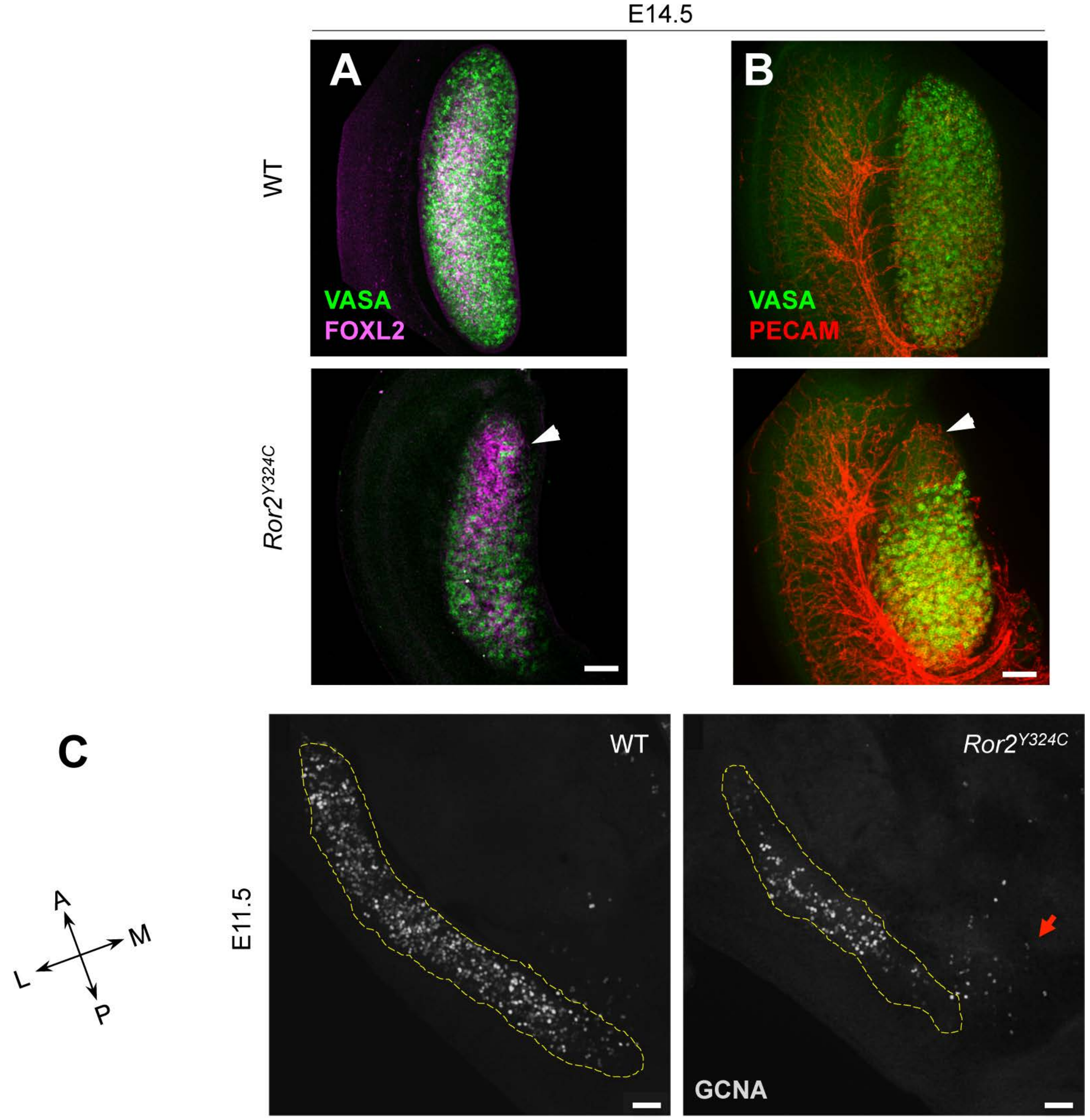

**Figure S3.** Anterior loss of germ cells in *Ror2$^{Y324C}$* ovaries (A,B) Whole ovaries show anterior GC loss at E14.5 despite normal morphology of FOXL2+ granulosa cells (magenta) (A) and PECAM+ endothelial cells (red) (B). White arrowheads indicate anterior defects. All images are oriented with anterior at the top. Scale bars in A,B=100µm. (C) Ectopic GCs are found in midline tissues at various locations along the anterioposterior axis (red arrow) without an anterior bias. GCs are shown in whole-mount immunofluorescence in *Ror2$^{Y324C}$* right gonad and littermate control at E11.5. The gonad periphery is marked in yellow. Scale bar = 70 mm. A, anterior; P, posterior; L, lateral; M, medial.

**Table S1.** Per gonad frequency of SYCP3+ germ cells in *Ror2*$^{Y324C}$ and *Ror2*$^{-/-}$ mutants and their control littermates. The presence of an anterior germ cell defect is indicated.

| **Genotype** | **sections counted** | **% SYCP3+** | **total germ cells** | **Ant defect?** |
|---|---|---|---|---|
| WT 7a | 2 | 81% | 686 | no |
| WT 7b | 2 | 82% | 697 | no |
| WT 20c | 2 | 78% | 692 | no |
| WT 20d | 2 | 70% | 871 | no |
| WT 46.7 | 4 | 88% | 1068 | no |
| WT 46.9 | 4 | 75% | 699 | no |
| WT 25.6 | 13 | 78% | 5885 | no |
| WT 38.10 | 7 | 88% | 4355 | no |
| WT 36.2 | 8 | 85% | 3214 | no |
| | | | | |
| Y324C 15b | 2 | 80% | 442 | no |
| Y324C 46.4b | 3 | 76% | 347 | no |
| Y324C 46.4 | 3 | 80% | 461 | no |
| | | | | |
| Y324C 46.2a | 4 | 52% | 344 | yes |
| Y324C 8 | 3 | 85% | 485 | yes |
| Y324C 27a | 3 | 64% | 582 | yes |
| Y324C 27b | 2 | 71% | 177 | yes |
| Y324C 46.1b | 3 | 60% | 170 | yes |
| Y324C 46.2b | 5 | 68% | 551 | yes |
| | | | | |
| Ror2$^{-/-}$ 38.1 | 9 | 86% | 1550 | no |
| Ror2$^{-/-}$ 38.5 | 5 | 95% | 1315 | no |
| Ror2$^{-/-}$ 38.3 | 4 | 90% | 1351 | no |
| Ror2$^{-/-}$ 25.3 | 13 | 87% | 5048 | no |
| Ror2$^{-/-}$ 39.2 | 3 | 56% | 291 | yes |

**Table S2.** A summary of the SYCP3+ germ cell frequencies presented in Table S1, with standard deviation and results of the students' T test.

| **Genotype** | **mean** | **SD** | **T-test (vs. WT)** |
|---|---|---|---|
| Wild-type | 81% | 5% | |
| Y324C (all) | 71% | 10% | 0.022 (*) |
| Y324C (no ant. defect) | 79% | 13% | 0.622 (ns) |
| Y324C (anterior defect) | 67% | 10% | 0.006 (**) |
| Ror2$^{-/-}$ | 83% | 14% | 0.694 (ns) |

**Table S3.** Analysis of meiotic prophase in E14.5 ovaries of *Ror2*$^{Y324C}$ homozygotes and their control littermates by nuclear morphology. Classifications include gonocytes (gono), preleptotene (PL), leptotene (L), zygotene (Z), and pachytene (P). The number of cells evaluated per ovary (n), mean, and standard error of the mean (SEM) are shown.

| **Genotype** | **Gono** | **PL** | **L** | **Z** | **P** | **n** |
|---|---|---|---|---|---|---|
| WT 51.7a | 17% | 37% | 30% | 14% | 2% | 897 |
| WT 51.6a | 25% | 33% | 32% | 10% | 0% | 1011 |
| WT 5b | 18% | 39% | 33% | 9% | 1% | 1057 |
| WT 30a | 20% | 42% | 30% | 7% | 0% | 931 |
| WT 30b | 14% | 50% | 28% | 8% | 0% | 790 |
| WT 34a | 28% | 53% | 16% | 2% | 0% | 776 |
| **mean** | 20% | 42% | 28% | 9% | 0% | |
| SEM | 2% | 3% | 2% | 1% | 0% | |
| | | | | | | |
| Y324C 51.1a | 9% | 39% | 42% | 10% | 0% | 571 |
| Y324C 51.3a | 19% | 54% | 26% | 1% | 0% | 227 |
| Y324C 6 | 23% | 56% | 20% | 1% | 0% | 526 |
| Y324C 29b | 13% | 62% | 23% | 2% | 0% | 249 |
| Y324C 29a | 10% | 69% | 19% | 2% | 0% | 491 |
| Y324C 32a | 40% | 53% | 5% | 2% | 0% | 402 |
| **mean** | 19% | 56% | 23% | 3% | 0% | |
| SEM | 4% | 4% | 4% | 1% | 0% | |

**Table S4.** qRT-PCR primers used.

| Gene name | Forward | Reverse |
|---|---|---|
| *Foxl2* | TGT CAT GAT GGC CAG CTA CCC CG | GCC TCG GCC TCT TTG ACT GCG |
| *Gapdh* | GACTTCAACAGCAACTCCCAC | TCCACCACCCTGTTGCTGTA |
| *Oct4* | AGT CTG GAG ACC ATG TTT CTG AAG T | TAC TCT TCT CGT TGG GAA TAC TCA ATA |
| *Rspo1* | CGG GGG AGA CCA CAC CAC CT | ATT CTC CCT CCG GCC CTG GC |
| *Stra8* | CCTAAGGAAGGCAGTTTACTCCCAGTC | GCAGGTTGAAGGATGCTTTGAGC |
| *Spo11* | GAAGTGCCTGCCTTCACAAT | GCCGACAGAATCATCAAACAT |
| *Sox9* | TCC GGC ATG AGT GAG GTG CAC T | TTG CCA GCT TGC ACG TCG GTT |
| *Sycp3* | AAATCTGGGAAGCCACCTTTGG | TGGAGCCTTTTCATCAGCAACATC |
| *Wnt4* | CAT GAG CCC CCG TTC GTG CC | TGC TGC CCA CCG ATG ACA GC |

**Table S5.** Primary antibodies used.

| Protein | Host species | Company and catalogue # |
|---|---|---|
| DAZL | Rabbit | Abcam Cat#Ab34139 |
| FOXL2 | Goat | Novus Biologicals Cat#NB100-1277 |
| GCNA | Rat | Gift from George Enders |
| 3bHSD | Rabbit | TransGenic Inc Cat#KO607 |
| OCT4 | Mouse | Santa-Cruz Cat#Sc-5279 |
| PECAM | Rat | BD Biosciences Cat#557355 |
| SYCP3 | Mouse | Abcam Cat#Ab97672 |
| VASA | Mouse | Abcam Cat# Ab27591 |
| VASA | Rabbit | Abcam Cat#Ab13840 |