

## (SI) Supporting Information

### Legends to the supplementary figures

**Fig. S1. Wnt-4 is expressed in the somatic cells of the developing ovary.** The ovaries of newborn *Wnt-4*<sup>EGFPCre</sup>; *Rosa26*<sup>LacZ</sup> mice demonstrate LacZ staining in the somatic cells (A) while the Mvh<sup>+</sup> germ cells are not stained (B, C). The compound positive embryos of *Wnt-4*<sup>EGFPCre</sup>; *Rosa26*<sup>LacZ</sup> positive mice depicts LacZ (D) staining that is similar to the endogenous *Wnt-4* gene expression pattern. The results point that the *Wnt-4* gene is not expressed by the germ cells at any stage during the female life cycle.

**Fig. S2. The germ line cysts in the developing ovary fall prematurely apart due to *Wnt-4* deficiency.** The germ cells in wild-type and the *Wnt-4*-deficient ovaries were identified with the Mhv antibody (A-F, in green). The germ cells that have entered the ovary at E12.5 (A) are numerous also without *Wnt-4* signalling (B). At E14.5 the germ cells have migrated beneath the surface epithelium in wild-type ovary (C). However in the *Wnt-4*-deficient ovary at E14.5 the cell layers beneath the surface epithelium are devoid of germ cells (compare D to C, arrow). Note that the germ line cysts have fallen apart prematurely to aggregates composed of few cells or to single germ cells at E16.5 (compare F to E). Some germ cells in the *Wnt-4*-deficient ovary locate close to the mesonephros which does not take place in wild-type ovary (dashed line in E, F and arrows in F). Scale bar 100  $\mu$ m.

**Fig. S3. Masculinization of E-cadherin expression in germ cells of *Wnt-4*-deficient ovary.** E-cadherin is expressed similarly in the ovarian and testicular germ cells at E14.5 (A-I) and localizes around plasma membrane (B, E, H) of the Mvh<sup>+</sup> germ cells (A, D, G), (C, F,

I, merged images). At E15.5 E-cadherin has become undetectable in the germ cells of wild-type ovary (K) in the Mvh<sup>+</sup> germ cells (J, L). However E-cadherin still remains to be expressed in the *Wnt-4*-deficient ovarian germ cells at this stage (N, M) being in this respect similar to wild-type male germ cells (compare P-R and M-O to J-L). The Western blots depicting expression of the total  $\beta$ -catenin (S), the active form of  $\beta$ -catenin (T) and E-cadherin (U). Note that E-cadherin expression is elevated in the case of *Wnt-4*-deficiency in the ovaries and expression level has become closer to the intensity observed in wild-type testis (U). Scale bar 25  $\mu$ m.

**Fig. S4. Loss of expression of markers of developing follicular genes due to *Wnt-4*-deficiency.** Expression of the *Gata4* gene that encodes a transcription factor regulating gonad development is unchanged due to *Wnt-4*-deficiency as compared with the controls at E13.5 (A-C) or in newborns (D-F). (G) While *Gata4* qPCR reveals no changes in *Gata-4* expression either (G), *Fog2* gene expression at E14.5 (H) is enhanced in the *Wnt-4*-deficient ovary as compared with wild-type ovary. (I-N) The *Figla* gene, is expressed in the developing ovary but not in wild-type testis at E14.5 (compare I to K) or at the newborn stage (compare L to N). Strikingly, *Figla* expression is reduced markedly in the ovary due to *Wnt-4*-deficiency already at E14.5 (J) and in the newborn stage (M). (O) *Foxl2* gene expression is induced to a certain degree in the *Wnt-4*-deficient ovary at E14.5 as compared with the ovary of a wild-type embryo. qPCR (G, H, O). The *Gapdh* gene served as a reference in G, H and O. Scale bar 250 $\mu$ m.

**Fig. S5. Loss of expression of a female marker *Adamts19* and of meiosis markers in the *Wnt-4*-deficient ovary.** The *Adamts19* gene is expressed in the ovary at E14.5 (A), but it is

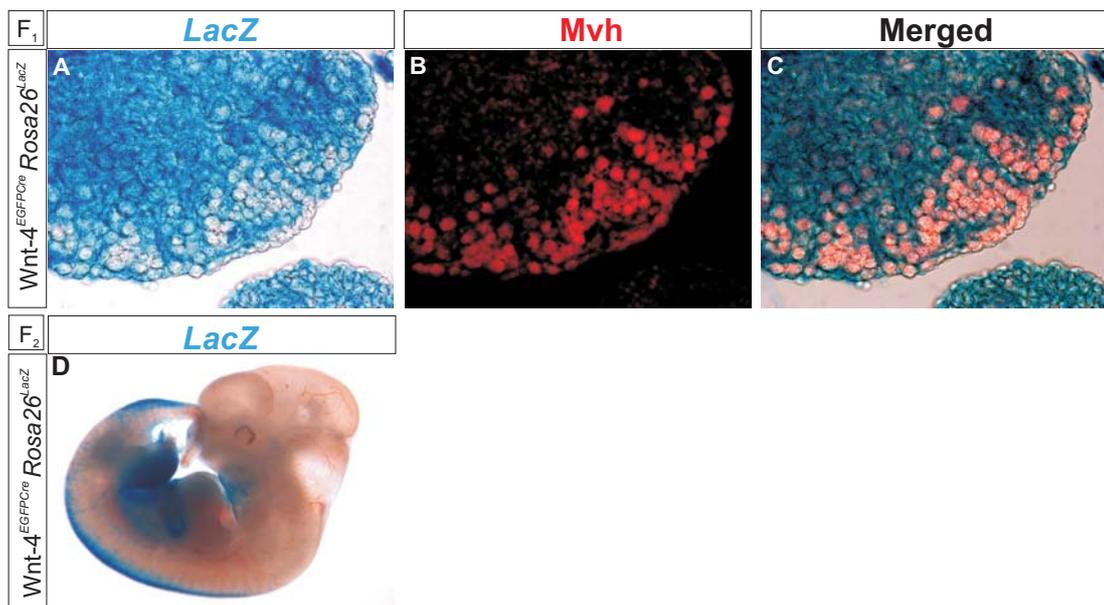
not detected in the ovary of the *Wnt-4*-deficient embryos (B) or the normal testis (C) at this stage. The *Cyp26b1* gene is not expressed in wild-type ovary (D) but it is expressed in wild-type testis at E13.5 (F) and it is also ectopic in the *Wnt-4*-deficient ovary (E, arrow). The meiotic germ cells in wild-type ovary are confined to the Mvh<sup>+</sup> germ cell layers (H, K) that are close to the ovarian cortex, and many of these germ cells express the meiotic prophase I markers  $\gamma$ H2AX and Xlr at E15.5 and E16.5 (G, J in red or yellow). Note that the Mvh<sup>+</sup> germ cells in the *Wnt-4*-deficient ovary are dispersed and the majority of them do not express  $\gamma$ H2AX or Xlr (compare H, K to G, J). As expected the germ cells in wild-type testis do not express  $\gamma$ H2AX or Xlr (I, L). A-C) Whole mount prepares. Scale bar (A-F) 250  $\mu$ m, (G-I) 50  $\mu$ m, (J-L) 100  $\mu$ m.

**Fig. S6. Development of prospermatogonia-like cells in the *Wnt-4*-deficient ovary.** At E14.5 some germ cells of the *Wnt-4*-deficient ovary have a defect in cytokinesis as indicated by the formation of a polykarion (compare C, B to A, arrowheads). D and E depict oocytes within wild-type E16.5 ovary and some of them are in meiosis as judged by their chromatin structure (arrow in E). (F) Prospermatogonia in wild-type testis at E16.5 (arrows). It is striking that the anterior part of the *Wnt-4*-deficient ovary contains prospermatogonia-like cells as judged by the cellular morphology containing condensed chromatin similarly to wild-type testis (compare H to F, black arrows). The cells have arranged to a cord-like structure surrounded by myoid-like cells (white arrows in H) that are typical for developing wild-type testis (compare F to H, white arrows). The posterior part of the *Wnt-4*-deficient ovary contains some germ cells that appear similar to those in wild-type ovary suggesting that a portion of the *Wnt-4*-deficient ovarian germ cells can still acquire some female characteristics (compare D to G and E to I, arrows). However based on the changes in their gene signature

identified in microarrays (see Table S1) such female germ cells are unlikely competent to mature to oocytes since only 10% of the germ cells remain in the ovary of *Wnt-4*-deficient newborn mice (5). Scale bar (A-C, E, F, H, I) 10  $\mu\text{m}$ , (D, G) 20  $\mu\text{m}$ .

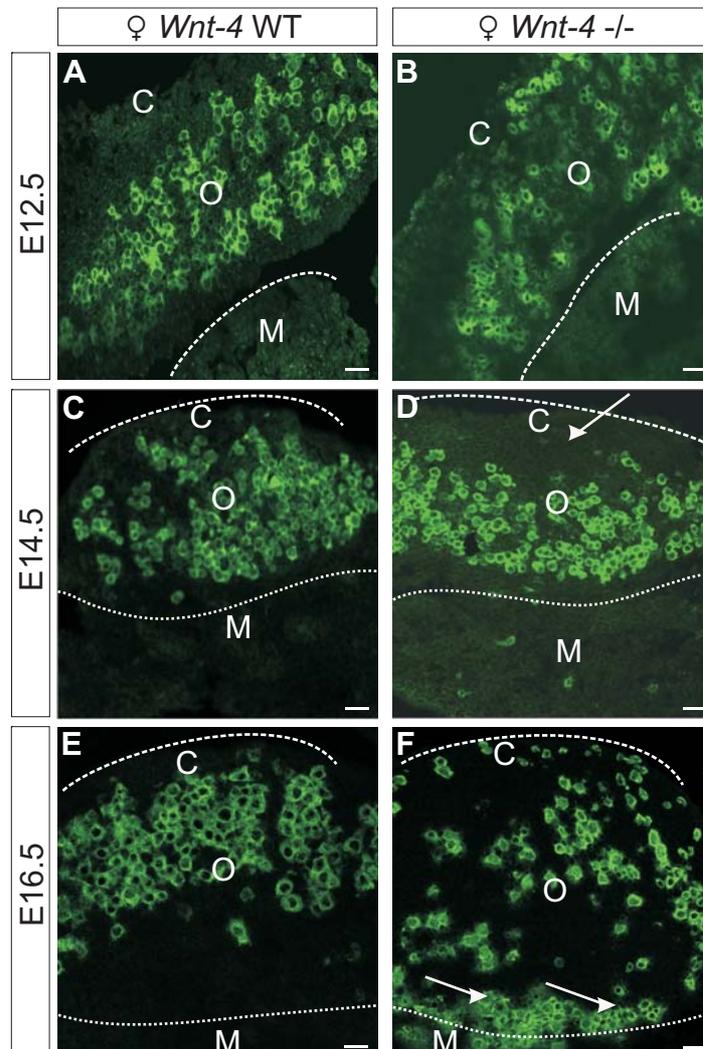
**Fig. S7. A schematic model how Wnt signalling may be involved in the control of female germ line development.** Retinoic acid (RA) that is produced by the mesonephros diffuses into the adjacent ovary, where RA promotes entry of the germ cells into meiosis via enhanced expression of *Stra8* and induction of Xlr and  $\gamma\text{H2AX}$ . By repressing the somatic expression of the *Cyp26b1* gene that encodes an enzyme that degrades RA, Wnt signalling in the somatic follicular cells in female embryo promotes germ cell entry into meiosis during early ovarian organogenesis. In the male meiosis is inhibited due to lack of Wnt signalling allowing *Cyp26b1* expression and consequently degradation of RA. Simultaneously to these signaling steps Wnt activity promotes female-type pattern of E-cadherin and  $\beta$ -catenin expression to coordinate cell adhesion in the developing follicle and to regulate Wnt target genes in the germ line cells.

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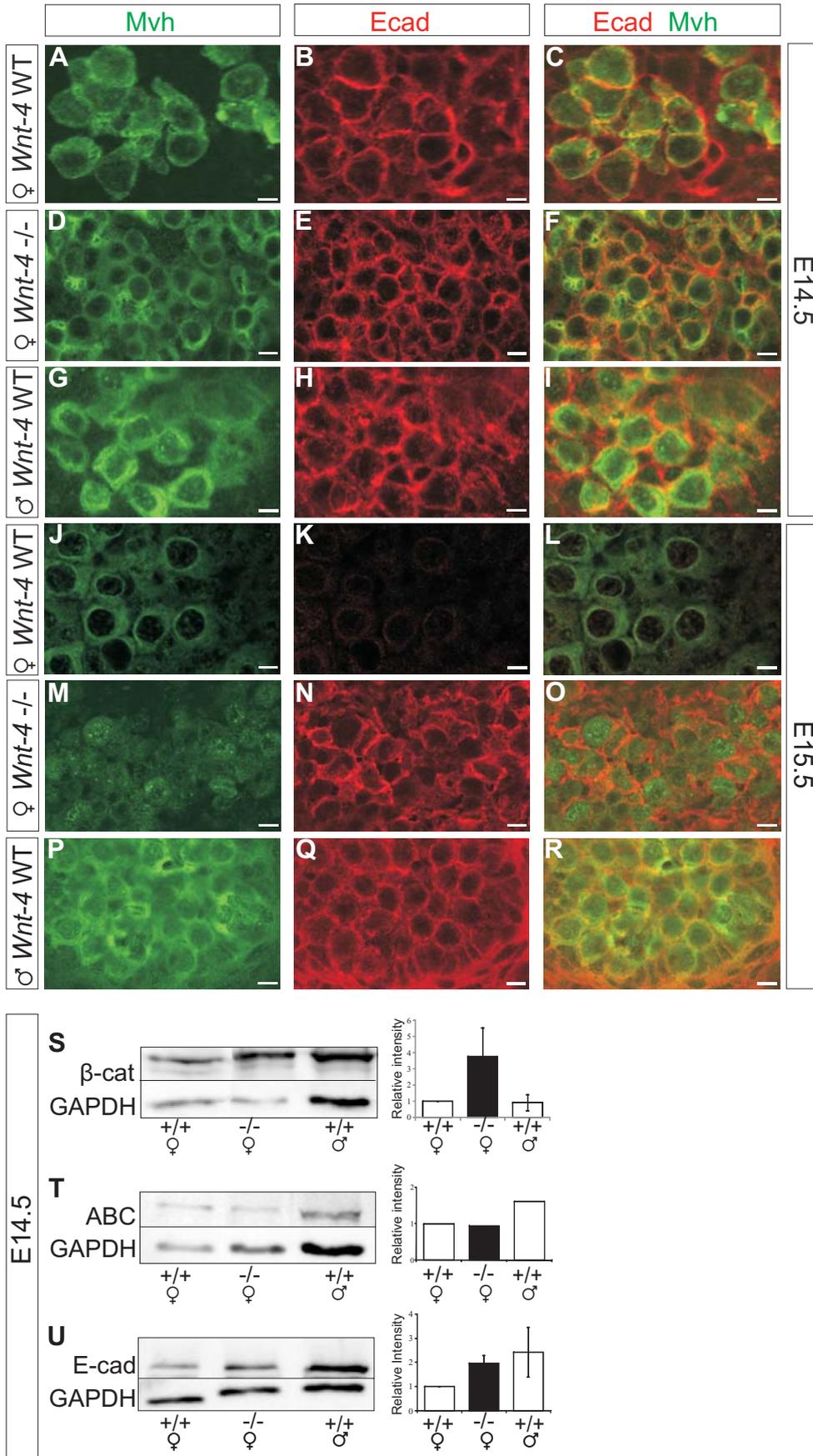


Supplementary figure 1

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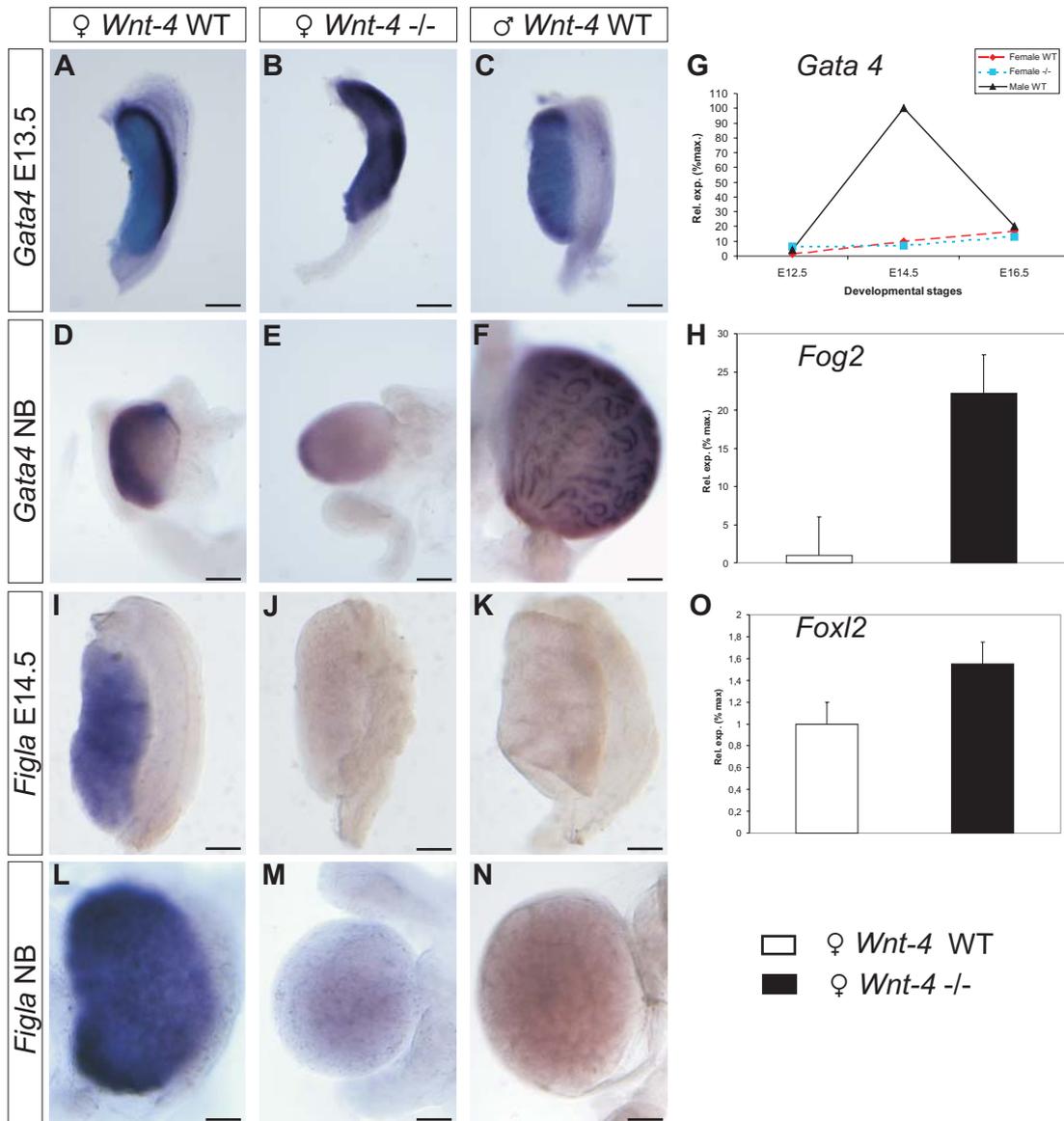


Supplementary figure 2



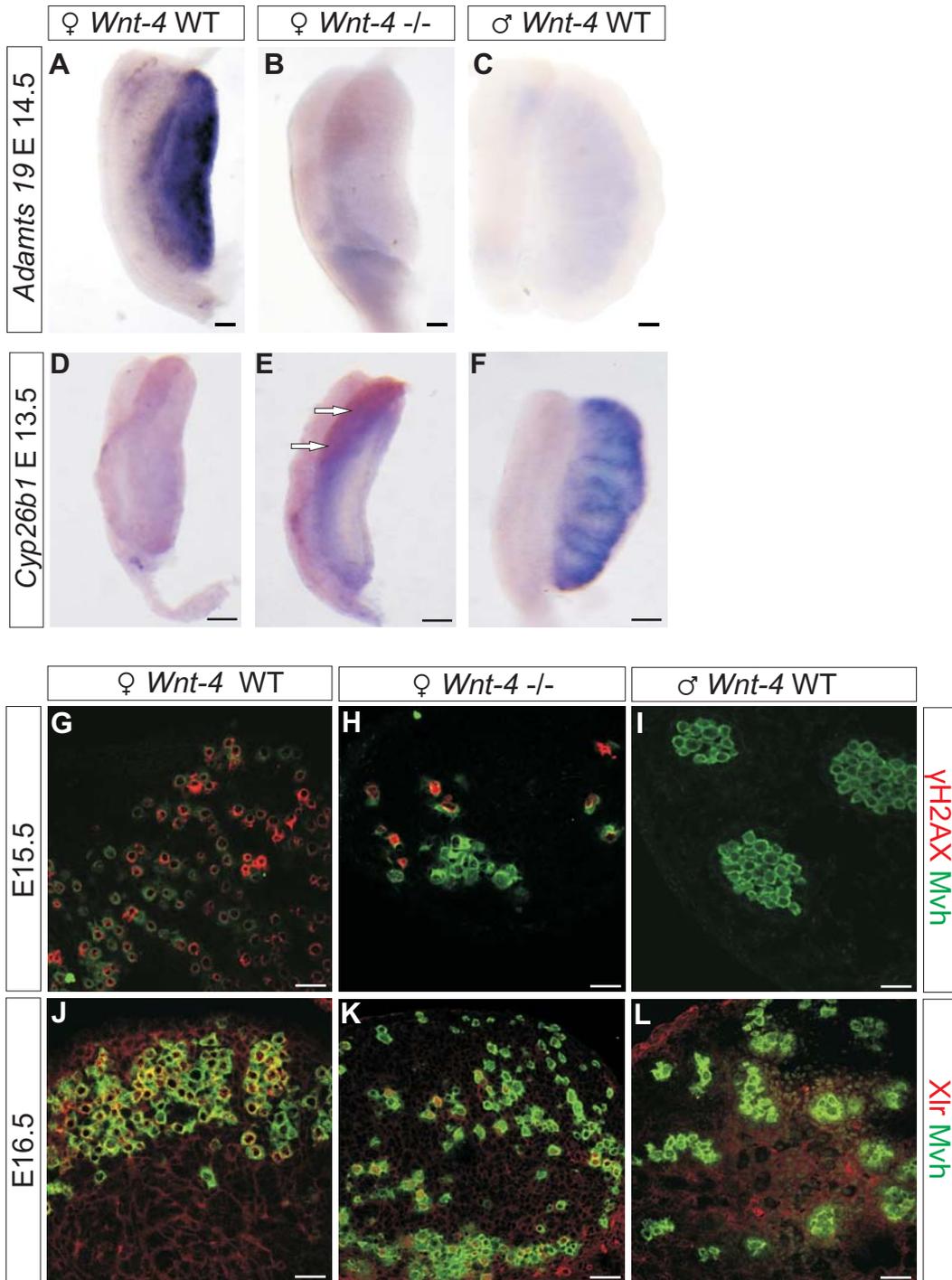
Supplementary figure 3

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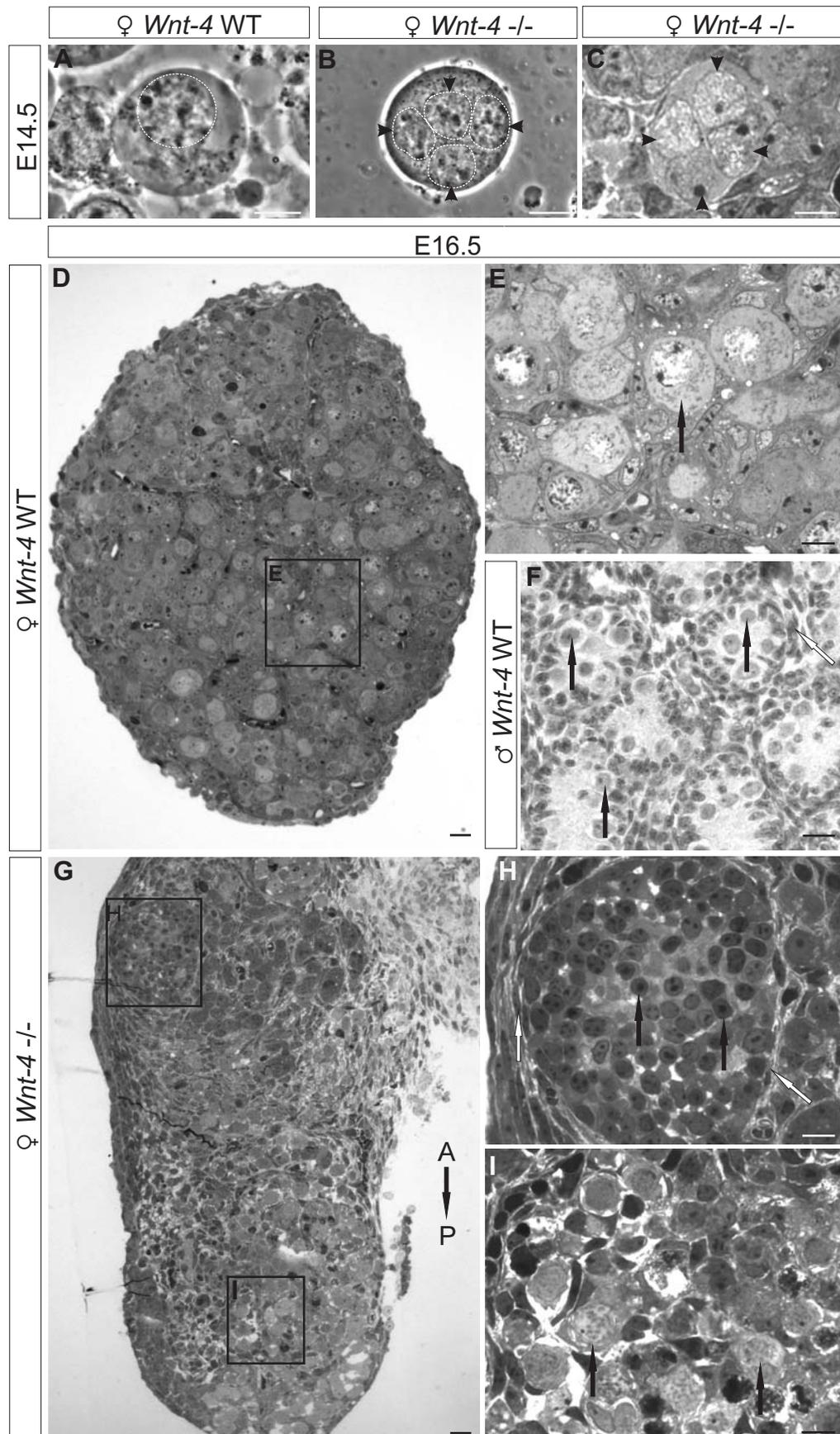
Supplementary figure 4

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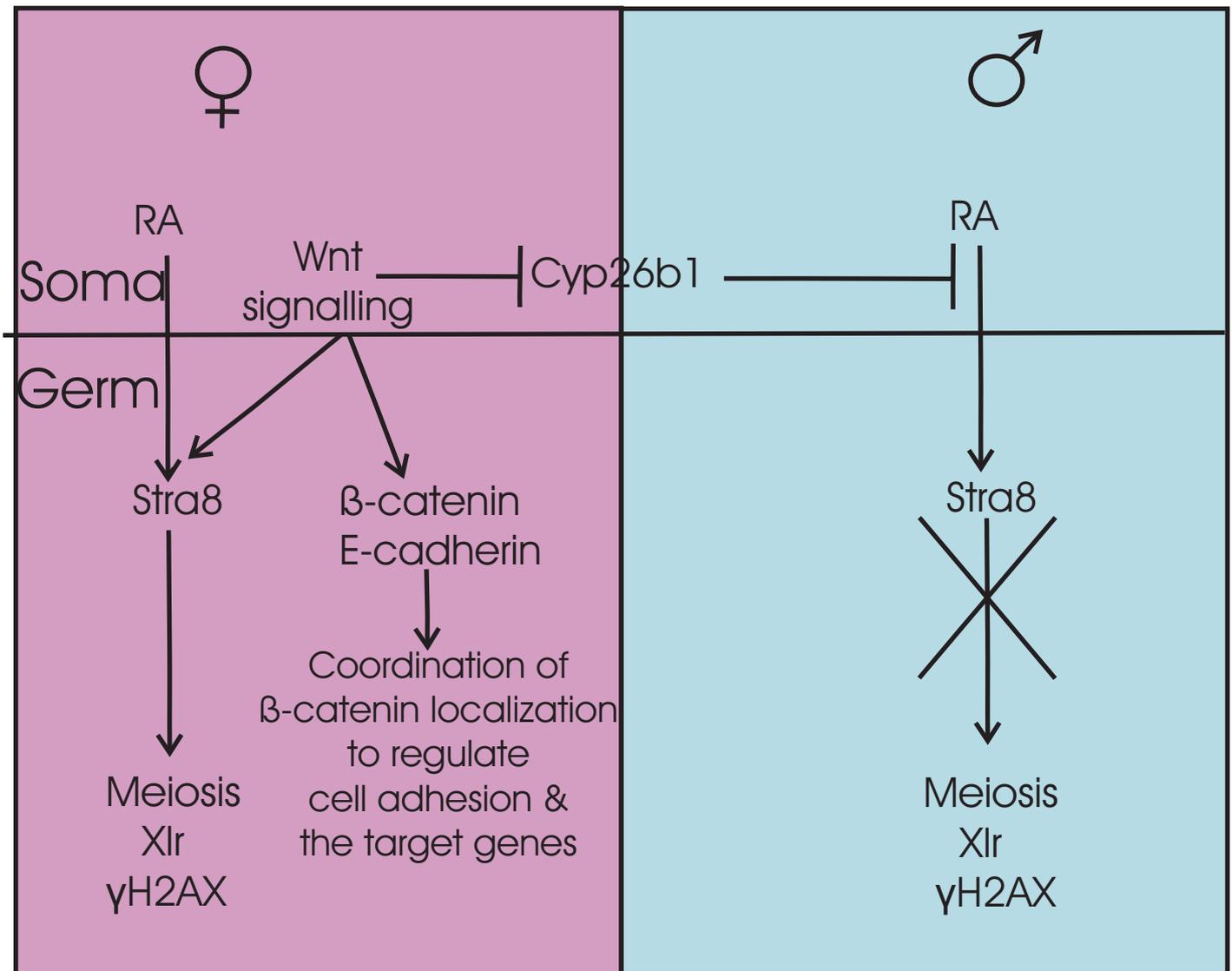
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Supplementary figure 6

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Supplementary figure 7

Supplementary Table 1

Probe set ID	Gene symbol	Assigned function	E12.5 KO/WT	E14.5 KO/WT	Newborn KO/WT
<b>Early folliculogenesis genes</b>					
1417960_at	Cpeb1	cytoplasmic polyadenylation element binding protein	NC	2,4↑	20↓
1448120_at	Gdf9	growth differentiation factor 9	NC	NC	8↓
1450369_at	Figa	factor in the germline alpha		1↓	60↓
1425759_at	Nobox	NOBOX oogenesis homeobox			128↓
1450210_at	Bmp15	bone morphogenetic protein 15	A	A	A
1423635_at	Bmp2	bone morphogenetic protein 2	3↓	2↓	2↓
1415854_at	Kitl	kit ligand	4↓	4↓	4↓
1452514_a_at	Kit	kit oncogene	NC	NC	8↓
1418517_at	Ir3	iroquois related homeobox 3 (Drosophila)	7↓	4↓	2↓
1449319_at	R-spo1	thrombospondin type 1 domain	NC	1↑	NC
1450306_at	Zp1	zona pellucida glycoprotein 1	NC	2↓	16↓
1449016_at	Zp2	zona pellucida glycoprotein 2	A	A	70↓
1417899_at	Zp3	zona pellucida glycoprotein 3	A	A	32↓
1419012_at	Zfpm2(Fog2)	zinc-finger protein FOG-2	NC	2↑	2↑
<b>Early testis genes</b>					
1421183_at	Tex12	testis protein TEX12	NC	NC	8↓
1460340_at	Piwil1	piwi like homolog 1	A	A	NC
1449103_at	Tex101	testis expressed gene 101	A	A	16↓
1417101_at	Hspa2	heat shock protein, 70 kDa 2	A	1↑	NC
1450752_at	Cyct	cytochrome c, testis	NC	4↑	NC
1422618_x_at	Xmr	Xmr Xlr-related, meiosis regulated		16↑	A
<b>Meiosis</b>					
1425101_a_at	Fkbp6	FK506 binding protein	NC	1↑	12↓
1420335_at	Dmc1	DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination (yeast)disrupted meiotic cDNA 1	A	NC	NC
1427291_at	Sycp1	synaptonemal complex protein 1	NC	2↓	20↓
1446051_at	Sycp3	synaptonemal complex protein 3	A	1↓	10↓
1421205_at	Atm	ataxia telangiectasia mutated homolog	2↓	NC	NC
1417021_a_at	Spo11	sporulation protein, meiosis-specific, SPO11 homolog (S. cerevisiae)	A	4↓	A
1449537_at	Msh5	mutS homolog 5 (E. coli)	A	A	NC
1419147_at	Rec8	meiotic cohesion	A	0.5↑	A

<b>Female meiosis</b>					
1416468_at	Aldh1a1	aldehyde dehydrogenase family 1, subfamily A1	A	2↓	NC
1422789_at	Aldh1a2	aldehyde dehydrogenase family 1, subfamily A2	1↓	NC	1↓
1420568_at	Stra8	stimulated by retinoic acid gene 8	10↓	1↑	A
1422011_s_at	Xlr	X-linked lymphocyte-regulated complex	A	NC	NC
<b>Male meiosis</b>					
1449170_at	Piwil2	piwi like homolog 2 (Drosophila)	A	2↑	12↓
1427242_at	Ddx4	DEAD (aspartate-glutamate-alanine-aspartate) box polypeptide 4	NC	1↑	12↓
1417945_at	Pou5f1(Oct4)	POU domain Class5 family 1	NC	6↑	A
<b>Sperm</b>					
1449895_at	Acr	preproacrosin	A	15↑	1↑
<b>Testis cord</b>					
1448975_s_at	Ren1	renin 1	NC	14↑	20↑
1423021_s_at	Insl3	insulin-like 3	NC	2,2↑	16↑
1418864_at	Gata4	GATA binding protein 4	NC	2,2↑	2↑
1419012_at	Zfpm2	zinc-finger protein FOG-2	NC	1,1↑	2↑
1424511_at	Aurka	serinethreonine kinase Ayk1	NC	NC	NC
<b>Wnt gene</b>					
1436791_at	Wnt5a	wingless-related MMTV integration site 5A	0,5↓	NC	NC