

Fig. S1. Control experiments for single germ cell lineage marking. (A-C) Lineage-labeled (YFP⁺) germ cell clones were generated using *Complete* mice (*CAGCreER*^{+/+}; *R26R*^{YFP/YFP}) injected with Tamoxifen (Tmx) at E10.5 (A). No cells expressing YFP were detected in the gonads of mice that received an injection of vehicle rather than Tmx (B), or in *R26R*^{YFP/YFP} mice that received Tmx but that lacked the *CreER* construct (C). (D,E) Quantitation of the experiments in A-C, which were carried out for E10.5 injections scored at E11.5 (1 day after Tmx injection) or at P4 (14 days after Tmx injection). (F) Sex of pups obtained from *Complete* mice receiving Tmx injection at E10.5. No distortion of the sex ratio is evident. (G) The fraction of gonads scored 1 day after Tmx injection that contained zero (blue), one (green) or more than one (red) germ cell clone. (H) An example of a rare E14.5 ovary that contained two lineage-labeled germ cells, showing that both clones are still easily recognized and resolved. (I) The average number of clones per gonad following Tmx injection at E10.5 does not change with subsequent development.

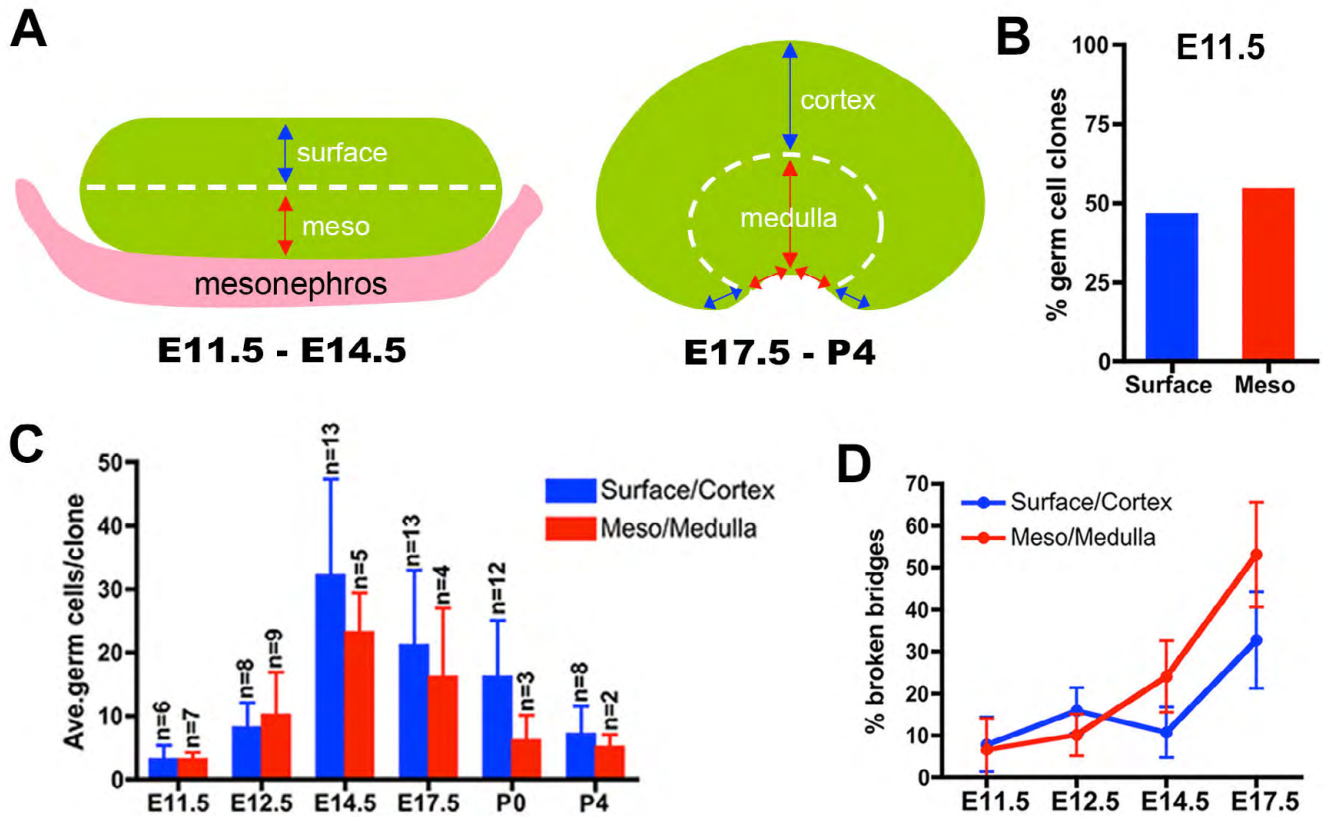


Fig. S2. Ovarian regional effects on cyst development. (A) Illustration of how the ovarian surface/cortex region and mesonephros/medullar region used to examine ovarian regional effects were defined. (B) In the E11.5 gonad, germ cells within the surface and meso regions were lineage labeled at similar frequency. Note that at E11.5 the volumes of the two regions are similar. (C) During cyst formation (E11.5-E14.5) and follicle formation (E17.5-P4), germ cell clones located within the surface or cortex regions and the meso or medullar regions differed little in average size, indicating that cyst development in the whole ovary is relatively homogeneous. (D) Germ cell clones in both the surface/cortex region and meso/medulla regions showed similar rates of cyst fragmentation.

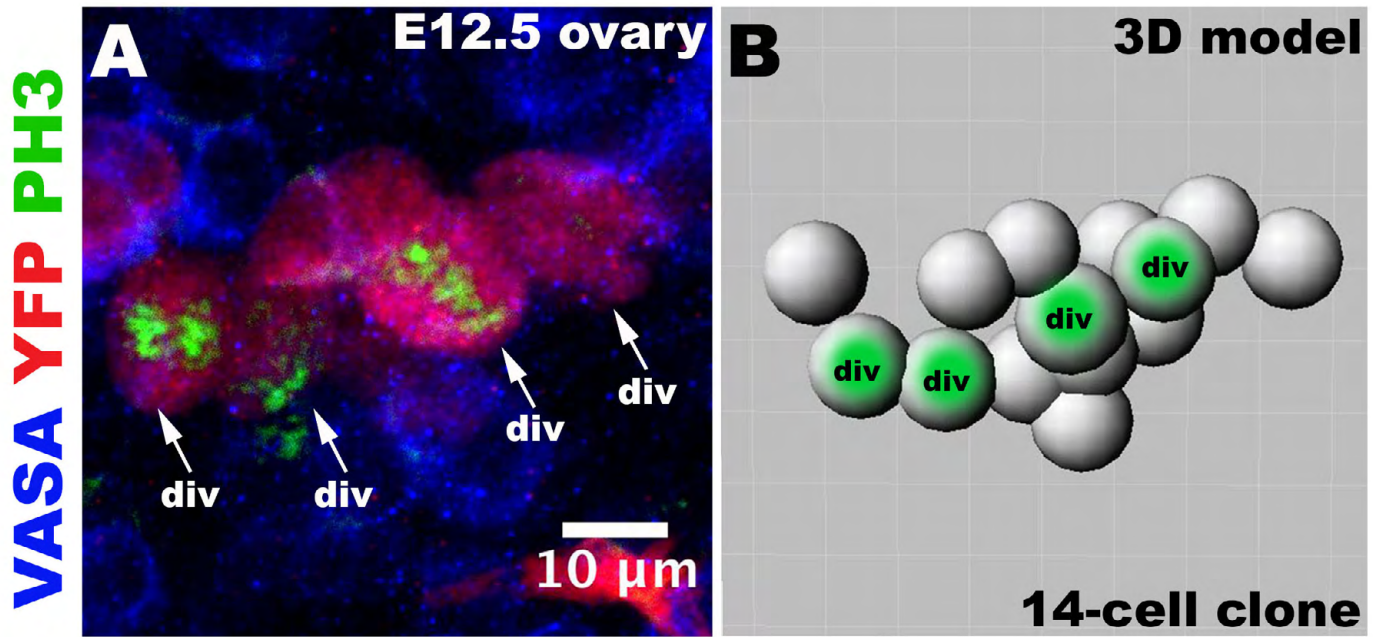


Fig. S3. Mitotic synchrony can be lost prior to cell separation. A 14-cell germ cell clone from an E12.5 mouse fetal ovary (A) and three-dimensional model (B). Two pairs of germ cells show synchronous mitosis and are positive for the mitotic marker phospho-histone H3 (PH3). However, the remaining clonal and adjacent germ cells were not dividing, nor did they exhibit PH3, suggesting that, rarely, intercellular bridges close functionally prior to the physical separation of cyst fragments.

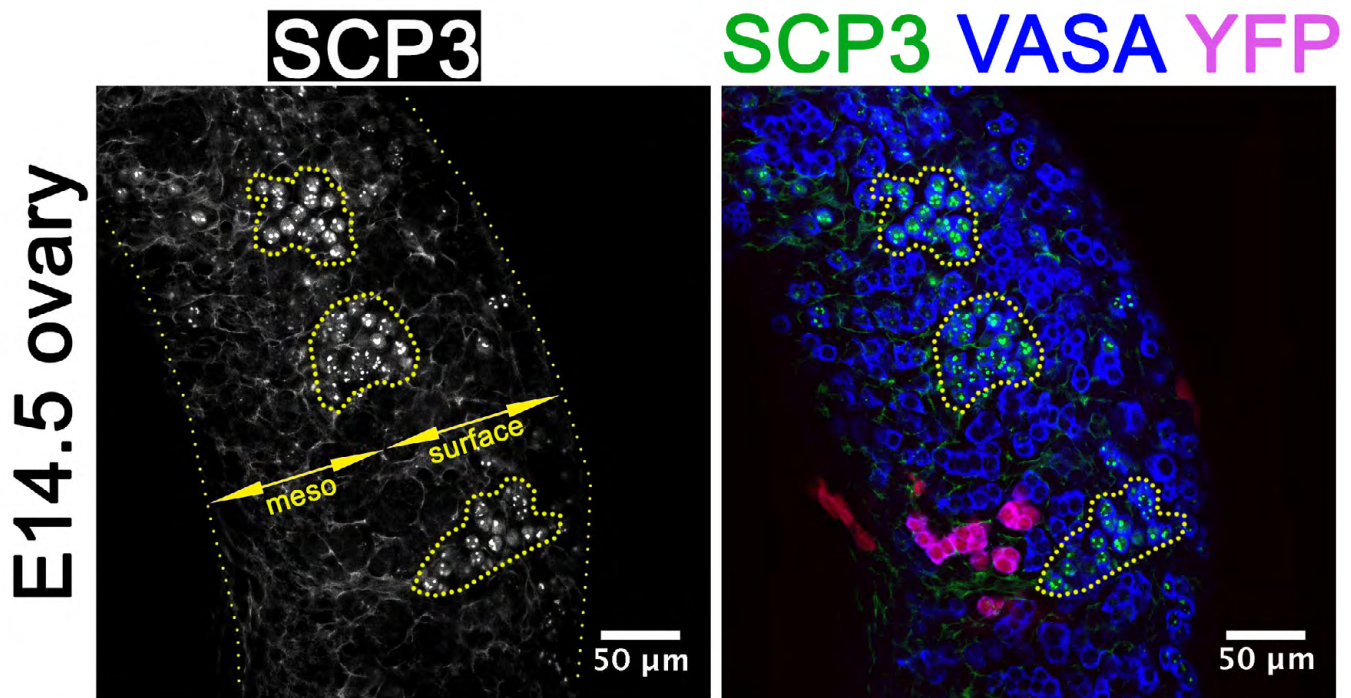
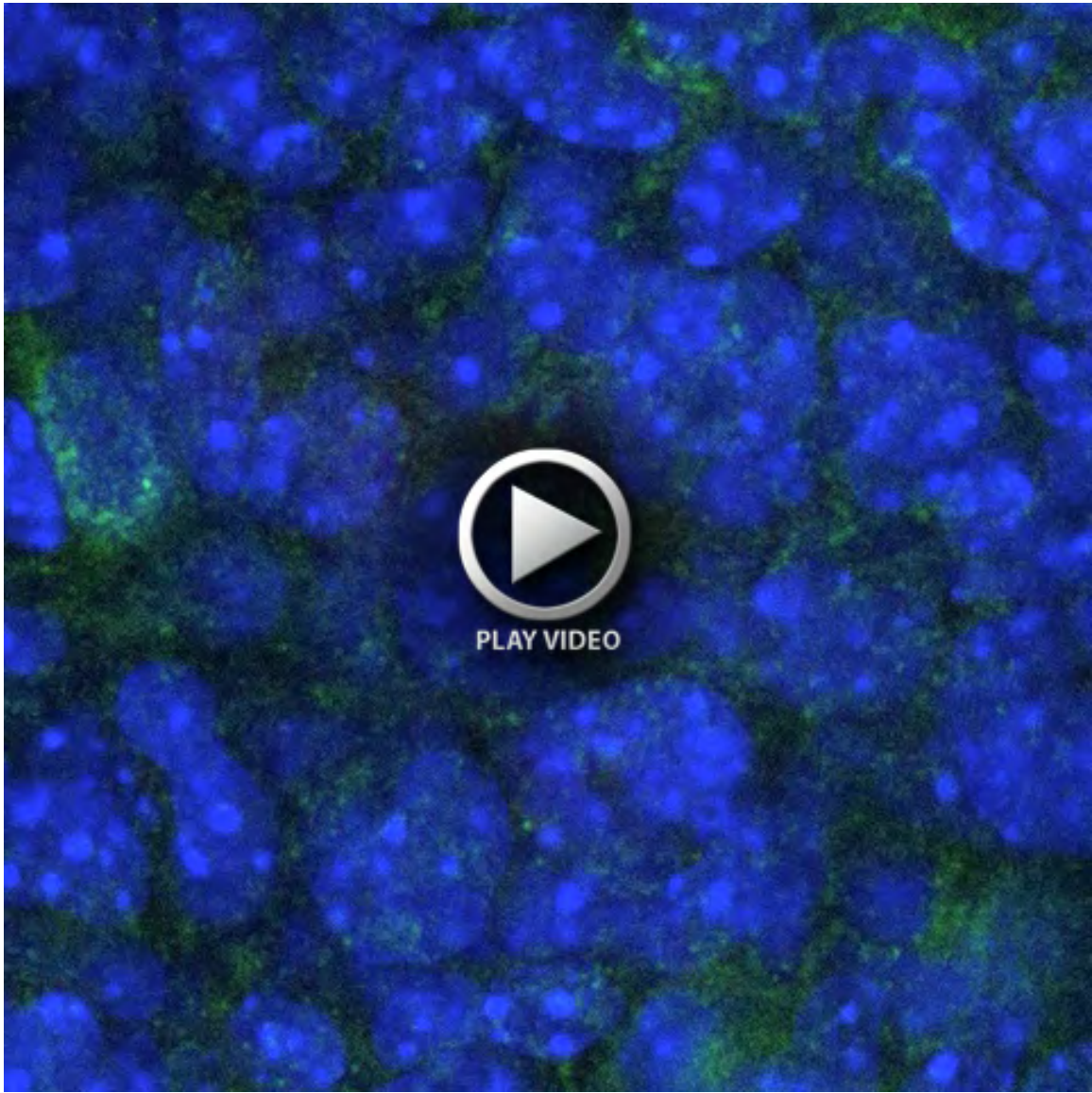


Fig. S4. Germ cells enter meiosis in synchronous groups in the E14.5 ovary. The meiotic marker synaptonemal complex protein 3 (SCP3) in an E14.5 ovary is first detected in groups of adjacent germ cells (dotted lines). In this example, the lineage-labeled (YFP⁺) clone was negative for SCP3 expression.



Movie 1. Serial confocal microscopy images of an eight-germ cell clone in an E12.5 mouse fetal ovary.

Table S1. Germ cell clones in E11.5 gonads (Tmx at E10.5)

Region	Size of clone	No. of cysts	Size of cysts	Samples
surface	8	2	6, 2	
meso	4	1	4	
meso	4	1	4	
surface	4	2	2(2)	
meso	4	2	2(2)	
meso	2	1	2	
meso	2	1	2	
surface	2	1	2	
surface	2	1	2	
surface	2	1	2	
surface	2	1	2	
meso	2	1	2	
meso	1	1	1	
Total:	39	16		13
Average:	3.0	1.2	2.4	

This and each of the following tables summarizes germ line clones induced in the ovary or testis by Tamoxifen (Tmx) injection at a specified age (E10.5 or E11.5) followed by the developmental age at analysis. Based on three-dimensional microscopy analysis, the size of the clone, the number of separate fragments and the sizes of the fragments are listed. When n fragments of size m were observed, this is indicated as $m(n)$.

Table S2. Germ cell clones in E12.5 ovaries (Tmx at E10.5)

Region	Size of clone	No. of cysts	Size of cysts	Samples
meso	24	4	12, 6, 4, 2	
meso	16	2	13, 3	
surface	16	4	6(2), 2(2)	
meso	15	5	6, 3(2), 2, 1	
surface	8	1	8	
meso	8	2	7, 1	
meso	8	2	5, 3	
surface	8	3	5, 2, 1	
meso	8	2	4(2)	
surface	8	2	4(2)	
surface	8	2	4(2)	
surface	7	2	5, 2	
meso	4	1	4	
meso	4	1	4	
meso	4	1	4	
surface	4	2	2(2)	
surface	2	1	2	
Total:	152	37		17
Average:	8.9	2.2	4.2	

Table S3. Germ cell clones in E14.5 ovaries (Tmx at E10.5)

Region	Size of clone	No. of cysts	Size of cysts	Samples
surface	63	15	31, 8, 5, 3(3), 2, 1(8)	
surface	60	2	59, 1	
surface	39	5	17(2), 3, 1(2)	
surface	38	8	14, 8, 6, 4, 3, 1(3)	
surface	32	1	32	
surface	32	3	29, 2, 1	
surface	31	3	24, 6, 1	
meso	31	7	12, 7, 4, 2(4)	
surface	30	4	12, 11, 4, 3	
surface	29	3	27, 1(2)	
meso	24	6	8(2), 2(3), 1(2)	
surface	22	5	8(2), 2(3)	
meso	20	10	5, 4(2), 1(7)	
meso	18	1	18	
surface	18	2	16, 2	
surface	16	4	7, 4, 3, 2	
meso	16	5	7, 4, 3, 1(2)	
surface	12	2	8, 4	
Total:	531	86		18
Average:	30	4.8	9.5	

Table S4. Germ cell clones in E17.5 ovaries (Tmx at E10.5)

Region	Size of clone	No. of cysts	Size of cysts	Samples
cortex	52	14	25, 4(2), 3, 2(6), 1(4)	
cortex	43	17	9, 8, 6, 4, 2(3), 1(10)	
medulla	32	8	13, 5(2), 3(2), 1(3)	
cortex	28	2	27, 1	
cortex	24	5	11, 9, 2, 1(2)	
cortex	23	8	7, 6, 3, 2(2), 1(3)	
cortex	23	10	7, 4, 3, 2(2), 1(5)	
cortex	14	4	10, 2, 1(2)	
cortex	14	6	5(2), 1(4)	
cortex	13	2	12, 1	
cortex	13	9	4, 2, 1(7)	
cortex	12	4	9, 1(3)	
medulla	12	6	5, 2(2), 1(3)	
medulla	12	10	2(2), 1(8)	
cortex	11	4	7, 2, 1(2)	
medulla	9	6	3, 2, 1(4)	
cortex	9	7	2(2), 1(5)	
Total:	344	122		17
Average:	20	7.2	3.7	

Table S5. Germ cell clones in P0 ovaries (Tmx at E10.5)

Region	Size of clone	No. of cysts	Size of cysts	Samples
cortex	33	17	8, 4, 3(2), 2(2), 1(11)	16
cortex	27	22	3, 2(3), 1(18)	
cortex	22	16	7, 1(15)	
cortex	22	15	4, 3, 2(2), 1(11)	
cortex	19	13	4, 2(3), 1(9)	
cortex	19	9	3(3), 2(4), 1(2)	
cortex	19	17	3, 1(16)	
cortex	14	12	2(2), 1(10)	
cortex	13	11	2(2), 1(9)	
medulla	11	7	3(2), 1(5)	
cortex	9	9	1(9)	
cortex	8	6	2(2), 1(4)	
medulla	5	5	1(5)	
cortex	5	5	1(5)	
medulla	3	3	1(3)	
cortex	3	3	1(3)	
Total:	232	170		
Average:	15	11	1.3	

Table S6. Germ cell clones in P4 ovaries (Tmx at E10.5)

Region	Size of clone	No. of cysts	Size of cysts	Samples
cortex	17	17	1(17)	10
cortex	9	8	2, 1(7)	
cortex	7	7	1(7)	
cortex	7	7	1(7)	
medulla	6	6	1(6)	
cortex	5	5	1(5)	
cortex	4	4	1(4)	
cortex	4	4	1(4)	
medulla	3	3	1(3)	
cortex	2	2	1(2)	
Total:	64	63		
Average:	6.4	6.4	1.0	

Table S7. Germ cell clones in 4-week adult ovaries (Tmx at E10.5)*

Region	Size of clone	No. of cysts	Size of cysts	Samples
cortex	7	7	1(7)	4
cortex	4	4	1(4)	
cortex	4	4	1(4)	
cortex	1	1	1	
Total:	16	16		
Average:	4.0	4.0	1.0	

*A single clone per ovary was assumed.

Table S8. Germ cell clones in E14.5 ovaries (Tmx at E11.5)

Region	Size of clone	No. of cysts	Size of cysts	Samples
meso	32	2	22, 10	9
meso	22	1	22	
meso	19	3	10, 8, 1	
meso	18	1	18	
surface	14	4	6, 4, 2(2)	
surface	12	1	12	
meso	12	3	8, 2(2)	
surface	12	5	4(2), 2, 1(2)	
surface	8	2	4(2)	
Total:	149	22		
Average:	17	2.4	9.8	

Table S9. Germ cell clones in E12.5 testes (Tmx at E10.5)

	Size of clone	No. of cysts	Size of cysts	Samples
	19	4	14, 2(2), 1	18
	17	4	13, 2, 1(2)	
	16	1	16	
	15	1	15	
	14	1	14	
	12	2	7, 5	
	8	1	8	
	8	1	8	
	8	2	7, 1	
	8	3	4, 3, 1	
	8	3	4, 2(2)	
	7	4	2(3), 1	
	6	2	5, 1	
	4	1	4	
	4	1	4	
	4	2	3, 1	
	4	2	2(2)	
	1	1	1	
Total:	163	36		
Average:	9.1	2.0	5.7	

Table S10. Germ cell clones in E14.5 testes (Tmx at E10.5)

	Size of clone	No. of cysts	Size of cysts	Samples
	63	3	57, 4, 2	
	58	7	52, 1(6)	
	55	1	55	
	51	1	51	
	51	6	45, 2, 1(4)	
	51	2	44, 7	
	51	6	39, 6, 3, 1(3)	
	49	2	29, 20	
	46	1	46	
	36	2	22, 14	
	33	2	21, 12	
	31	7	15, 7, 5, 1(4)	
	30	6	13, 9, 4, 2, 1(2)	
	30	3	26, 3, 1	
	24	6	16, 3, 2, 1(3)	
	17	5	9, 3, 2(2), 1	
	9	2	8, 1	
Total:	685	62		17
Average:	40	3.6	18	

Table S11. Germ cell clones in E17.5 testes (Tmx at E10.5)

	Size of clone	No. of cysts	Size of cysts	Samples
	62	24	21, 9, 4(2), 2(4), 1(16)	
	59	24	8(2), 6, 4, 3(4), 2(5), 1(11)	
	56	10	39, 5, 4, 2, 1(6)	
	46	14	10, 8, 7, 6, 3, 2(3), 1(6)	
	36	13	13, 6, 5, 3, 1(9)	
	28	8	12, 5, 3(2), 2, 1(3)	
	23	8	9, 5, 3, 2, 1(4)	
	16	8	7, 3, 1(6)	
Total:	326	109		8
Average:	41	14	3.1	

Table S12. Germ cell clones in P0 testes (Tmx at E10.5)

	Size of clone	No. of cysts	Size of cysts	Samples
	95	44	15, 10, 7, 6, 4(2), 2(11), 1(27)	
	79	44	9, 8, 5, 4, 3(3), 2(7), 1(30)	
	70	22	17, 5, 4(3), 3, 2(7), 1(19)	
	64	29	9, 7, 5(2), 4(2), 2(7), 1(16)	
	54	17	9, 8, 7, 5, 4, 3(4), 2, 1(7)	
	40	23	8, 6, 3, 2(3), 1(17)	
	31	17	5, 4, 2(7), 1(8)	
	29	10	17, 2(3), 1(6)	
	24	12	5, 3(2), 2(4), 1(5)	
	17	8	7, 3, 2, 1(5)	
	16	6	7, 3(2), 1(3)	
	13	7	6, 1(7)	
	13	10	3, 2, 1(8)	
	5	2	4, 1	
Total:	550	262		14
Average:	39	19	2.2	

Table S13. Germ cell clones in E14.5 testes (Tmx at E11.5)

	Size of clone	No. of cysts	Size of cysts	Samples
	57	21	11, 8, 7, 6, 4, 3, 2(3), 1(12)	
	53	5	32, 12, 6, 2, 1	
	41	4	16, 14, 10, 1	
	32	14	9, 4(2), 3, 2(2), 1(8)	
	31	10	12, 4(2), 3, 2(2), 1(4)	
	14	6	9, 1(5)	
Total:	228	60		6
Average:	38	10	5.2	