Supplemental Data

Supplemental Figure Legends

Supplemental Figure 1. Testis histological phenotype of WT and *gilz* KO testes. (A) High-power (40x) images of testis histological section from WT and *gilz* KO mice at various age as indicated in the figure assessed by H&E staining. At 7dpp *gilz* KO tubules contain more cells compared to controls. Abnormal germ cell (arrowheads) were detectable in 10dpp *gilz* KO mice. At 14dpp and 21dpp, germ cells had almost disappeared in tubules of gilz KO mice and many abnormal spermatocytes and apoptotic cells are shown (asterisks). *Gilz* KO tubules contain only Sertoli cells (black arrows). Scale bars 50 μm. (B) Quantification of germ cells per seminiferous tubule. The numbers of germ cells in 25 seminiferous tubules per testis were counted in five mice of each genotype. The numbers of germ cells per tubule were statistically significant between WT and *gilz* KO mice at 7dpp and 14dpp. * P< 0,05, two-tailed Student's t-test. (C) Loss of the germ cell–specific markers in mutant testes as assessed by qPCR; results are shown as means ± SEM; N=5 mice per group. * P< 0,05, two-tailed Student's t-test. (D) IHC analysis of γH2AX expression in sections from WT and *gilz* KO 7dpp and 10dpp testes. Scale bars, 200 μm.

Supplemental Figure 2. Massive apoptosis in *gilz* KO spermatocytes. (A, B) Germ cell apoptosis in different stages of spermatogenesis, as reported in the figure, was evaluated by TUNEL assay. Apoptotic cells were stained in red (A). In the graphic chart (B) are reported the total number of TUNEL+ cells per image. Scale bars, 200 μm. (C, D) Expression of apoptosis-related genes did not differ in WT and *gilz* KO mice before meiosis entry (10dpp). Heat map (C) and scatter plot (D) images represent results of array analysis assessed by gene expression profiling using qPCR. Heat map shows differential expression patterns between 10 dpp WT and *gilz* KO testes (up-regulated genes are shown in red, and down-regulated genes in green); scatter plot indicate deviation of gilz KO data from WT trendline. There are no significative differences between WT and gilz KO apoptotic gene expression, the only gene strongly downregulated, represented in position D11 (green) in the heat map and indicated by in the scatter plot (arrow), was *Tsc22d3* (alias *gilz*).

Supplemental Figure 3. FSH, LH and Testosterone hormonal levels are comparable in WT and *gilz* KO testes. (A) Analysis of mRNA expression of FSH and LH in the pituitary gland from adult WT and *gilz* KO mice, as assessed by RTPCR. (B) Analysis of mRNA expression of AR, GR, ER, LHR, FSHR in the testes from adult WT and *gilz* KO mice, as assessed by RTPCR. HPRT (25 cycles) served as a normalization control. (C) Serum testosterone concentrations in 6 months old WT and *gilz* KO mice. Values are reported as means ± s.e.m.; n=3 mice per group. * P< 0,05, two-tailed Student's t-test. No significant difference was found between WT and *gilz* KO mice.

Supplemental Figure 4. Specific *gilz* KO in germ cell lineage (cKO) is sufficient to disrupt spermatogenesis. Histological analysis (A) showed that testes from 3 months old *gilz* cKO mice contain only Sertoli cells (arrowhead). Scale bars, 50 μm. (B) Loss of the germ cell–specific markers in *gilz* cKO testes assessed by qPCR. Results are shown as means ± s.e.m.; n=4 mice per group. * P< 0,05, two-tailed

Student's t-test. (C, D) Sections of *gilz* KO recipient testis after transplantation with wt germ cells were stained with hematoxylin and eosin. Repopulation of seminiferous tubuli in *gilz* KO recipient testis. Scale bars, 100 μm (C); 50 μm (D). (E) Donor germ cells were positive for L-GILZ protein as evaluated by IHC analysis. (F) Percentages of repopulated tubules in *gilz* KO transplanted testis. n=5 mice per group. The mean of each data set is plotted with a horizontal bar. At least 100 tubules were counted for each testis.

Supplemental Text

Supplemental Materials and Methods

Generation of specific germ cells *gilz* KO mice. To generate specific germ cells *gilz* KO mice (*gilz*-cKO), male *gilz* flox/Y mice were crossed with transgenic mice in which Cre-recombinase is expressed under the control of the Mvh promoter (53), which become active specifically in germ cells starting from embryonal male primordial germ cells development.

TUNEL assay. Apoptotic cells were detected with ApopTag Red In Situ Apoptosis Detection Kit (Millipore), following manufacturer's instructions. The samples were observed by fluorescence microscopy, using a Leica microscope equipped with Diagnostic Instruments RT Color camera. TUNEL-positive cells were counted on every section and plotted in the graphic chart.

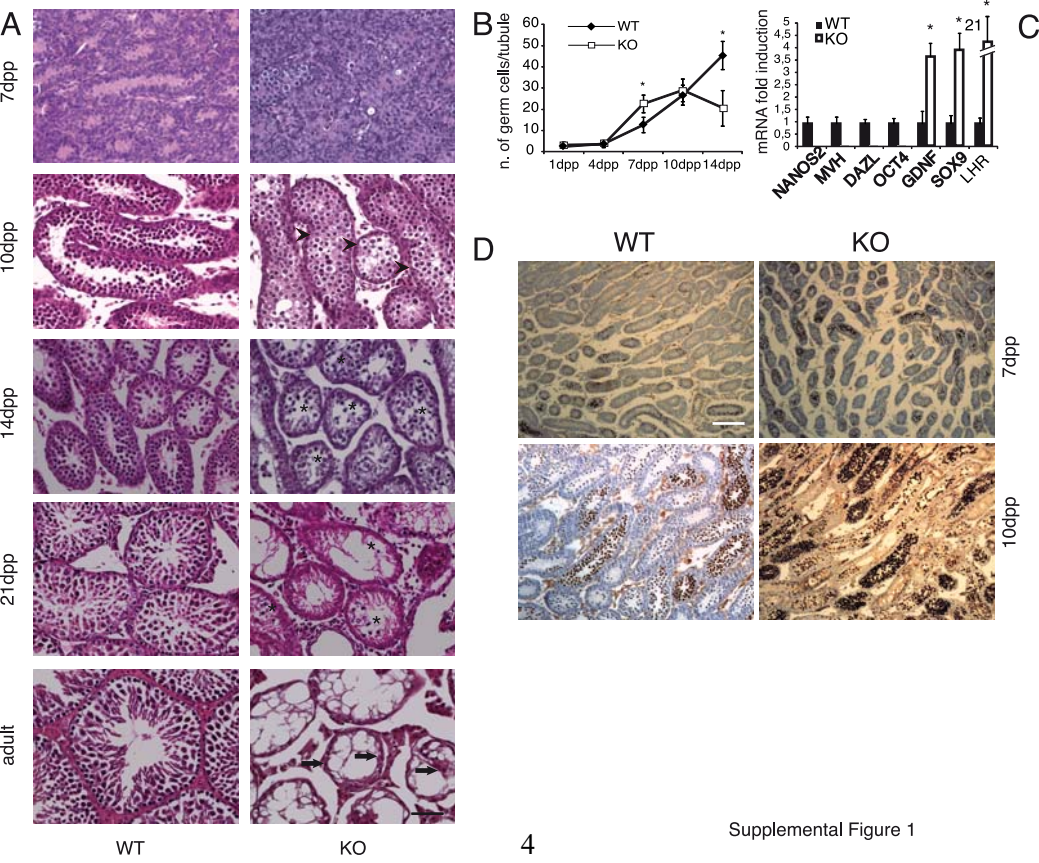
Apoptosis PCR Array analysis. For Apoptosis Signalling Pathway PCR Array analysis RNAs from 10dpp wt and *gilz* KO mice (n=3) were pooled and treated with DNase (Turbo DNA-Free; Ambion). A total of 1 μg of pooled RNA was retrotranscribed by reverse transcription-PCR (RT-PCR) kit (SABiosciences Corp.). The quality of cDNAs was tested by using RT2 RNA QC-Array (SABiosciences). Mouse Apoptosis RT2 Profiler PCR Array was purchased from SABioscience. PCR was performed according to the manufacturer's instructions on an ABI 7300 Real Time (Applied Biosystems). The data were imported into an Excel database and submitted to SABiosciences PCR Array Data Analysis Web Portal that automatically perform calculation and interpretation of the raw data. This data were analyzed using the comparative cycle threshold method with normalization to 4 houskeeping genes. The results are presented as n-fold changes versus the values in wt control mice.

Radioimmunoassay (RIA). Mice were anesthetized with isoflurane and immediately dissected to access the heart. Blood was collected and spun down at 10,000 g for 10min at 4°C to isolate the serum. Serum testosterone concentrations from individual mice were measured by radioimmunoassay after ether extraction as previously described (54).

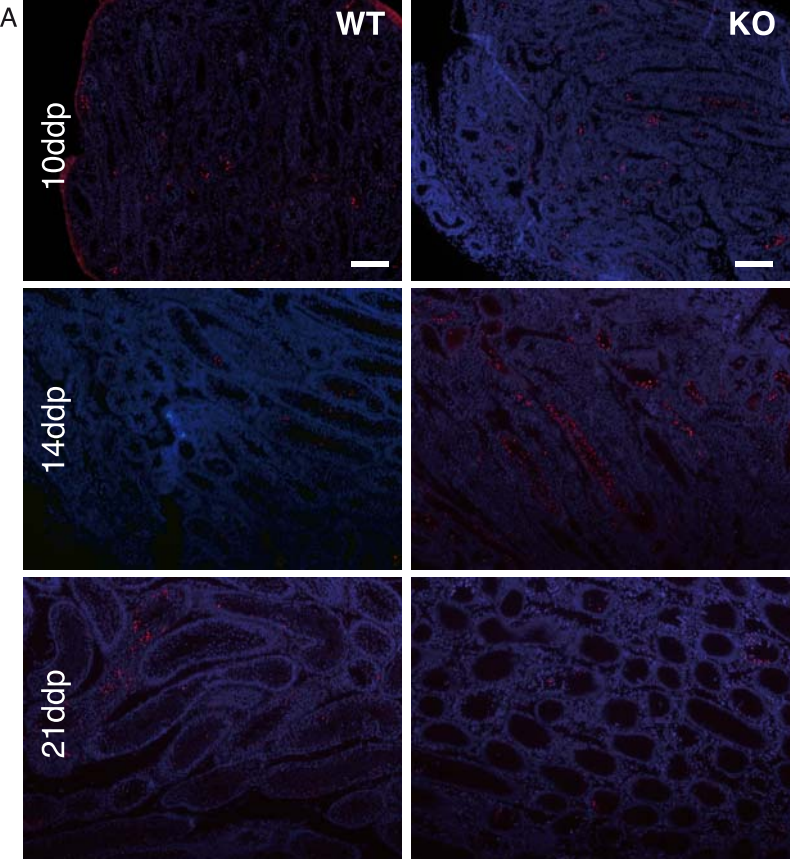
Germ Cell Transplantation. Donor cells were isolated from testis of C57BL/6 WT mice as previously described (34). Purified germ cells were transplanted into recipient 3 months old *gilz* KO male mice at a concentration of 10^6 cells/ml. Three months after trasplantation, the number of donor colonies was quantified for each recipient testis by microscopy analysis. To quantify the percentage of tubules repopulated by the donor, we analyzed at least 200 tubules throughout each testis.

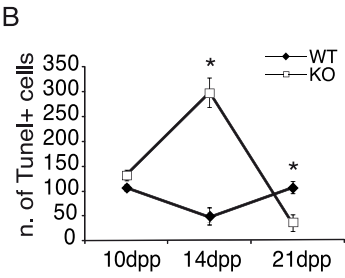
Supplemental References

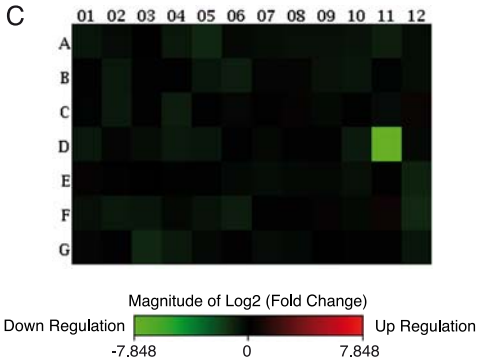
53. Gallardo, T., Shirley, L., John, G. B., and Castrillon, D. H. (2007) *Genesis* **45**, 413-417
54. Resko, J. A. (1969) *Science* **164**, 70-71

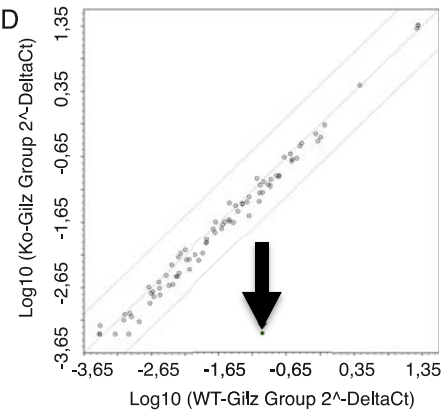


Supplemental Figure 1

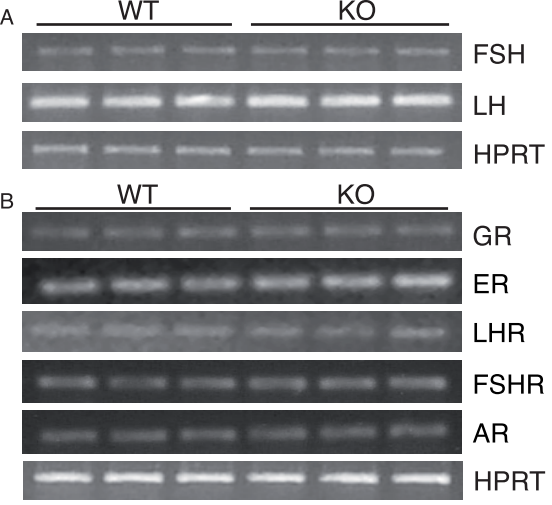


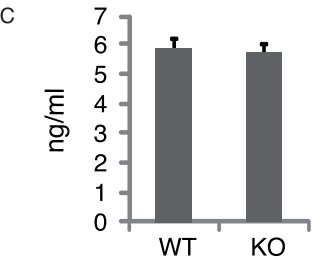




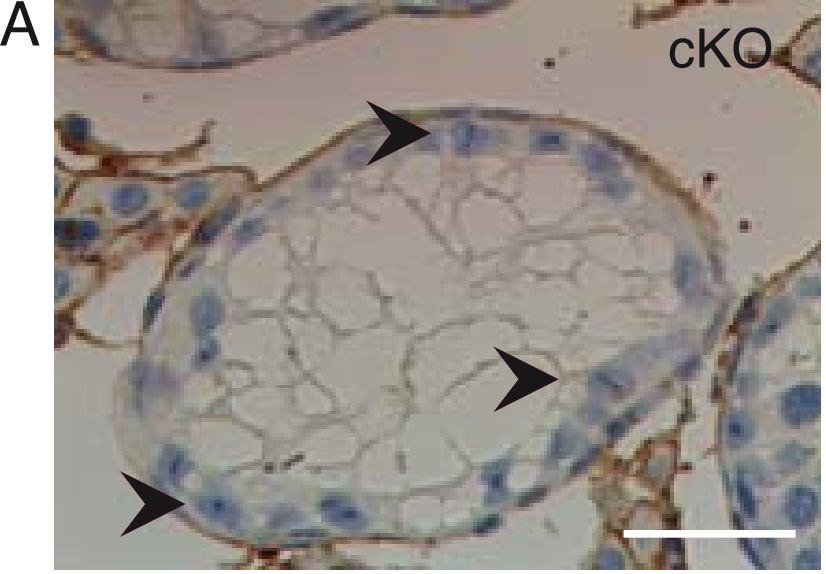


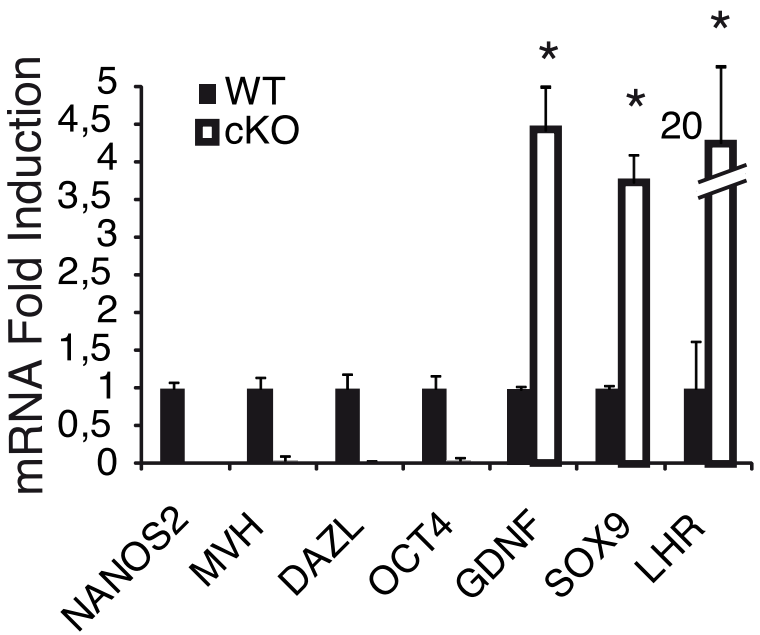
Supplemental Figure 2

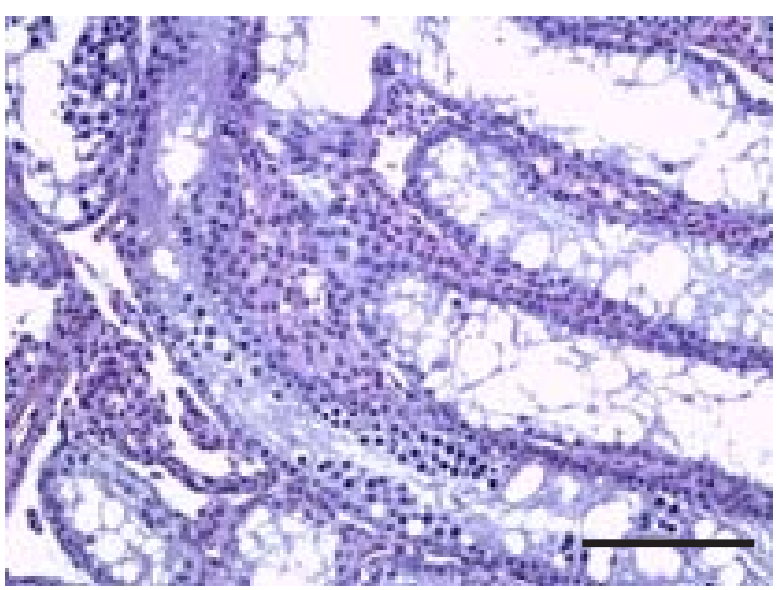


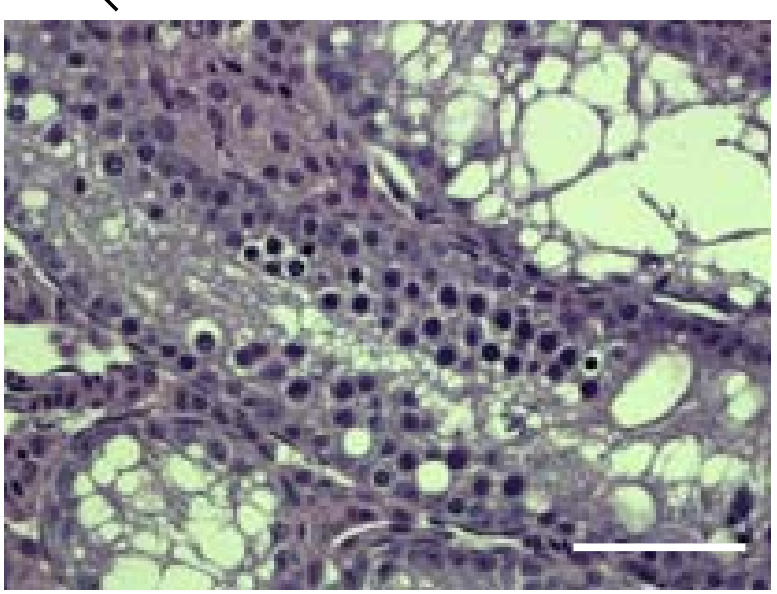


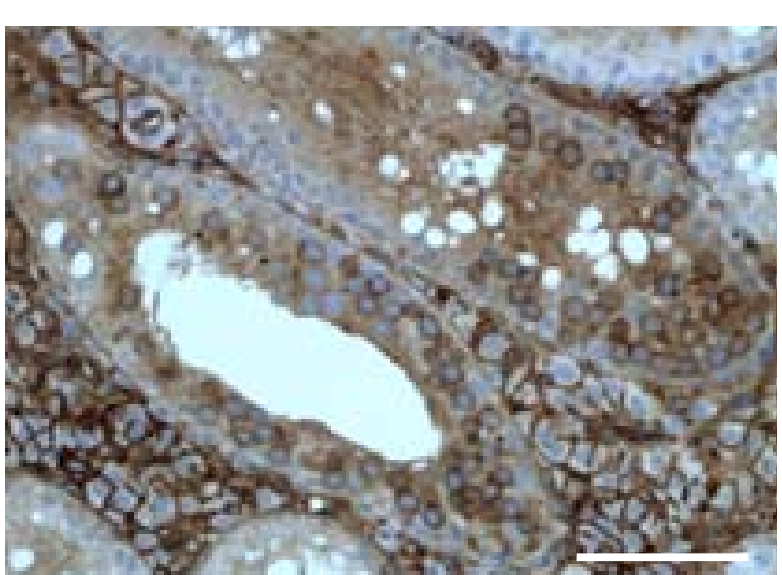
Supplemental Figure 3

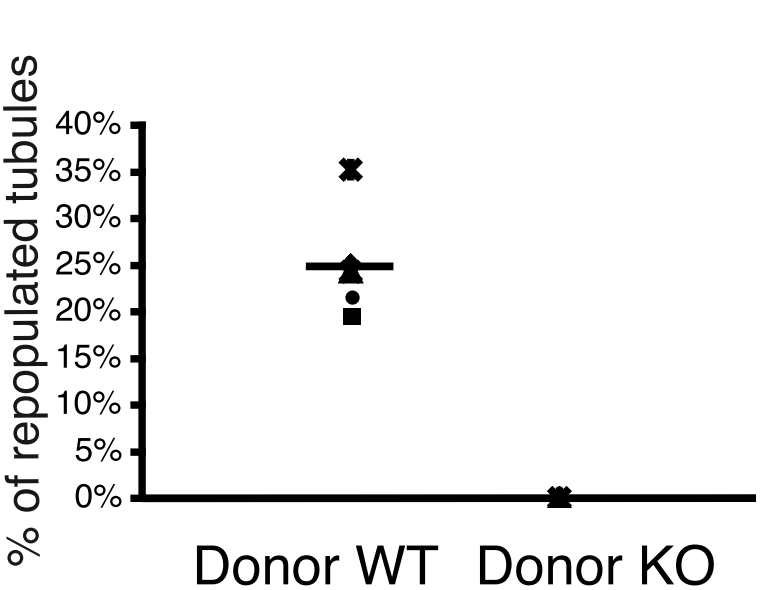












Supplemental Figure 4

Supplementary table 1: Array gene list

Probeset ID	Gene Symbol	RefSeq	Fold-Change(KO vs. WT)
10606989	Tsc22d3	NM_001077364	-2,37505
10576049	Foxf1a	ENSMUST00000098351	-1,74256
10576046	Foxf1a	NM_010426	-1,61412
10576051	Foxc2	NM_013519	-1,59418
10547227	Ret	NM_001080780	-1,59082
10428388	Rspo2	NM_172815	-1,5699
10466886		---	-1,51568
10468722	Gfra1	NM_010279	-1,48612
10598251	Dgkk	NM_177914	-1,461
10550181		---	-1,38541
10508907		---	-1,38478
10467979	Scd1	NM_009127	-1,36834
10364542	Cfd	NM_013459	-1,34956
10416725	Tdrd3	ENSMUST00000022596	-1,33408
10399581	3110053B16Rik	AK148766	-1,30283
10563706	EG668725	DQ386867	-1,29228
10489909	Ube2v1	NM_023230	-1,13801
10372730	Iltifb	NM_054079	1,04814
10573054	Gypa	NM_010369	1,19587
10545026	V1rc24	NM_134179	1,25169
10403816		---	1,25261
10418986		---	1,27584
10602372	Alas2	NM_009653	1,29731
10484600	Olfr1062	NM_147078	1,33056
10608226			1,35665
10608492	1700040F15Rik	AK076905	1,3567
10399710	Rsad2	NM_021384	1,3905
10608523	LOC380994	BC099537	1,3969
10578136		---	1,42112
10467840	C130021O09Rik	AK081501	1,42563
10608184	Zfy2	NM_009571	1,42646
10488465	Zfp345	NM_001034900	1,4795
10599917	Fmr1nb	NM_174993	1,49604
10605633	Pet2	NM_008821	1,58541
10603926	EG382275	NM_001025607	1,6217
10603936	EG382275	NM_001025607	1,6287
10603914	EG546272	NM_001100444	1,63487
10603953	382277	NM_001040669	1,70127
10566205	Dub2a	NM_001001559	1,71279
10607454	Magea1	NM_020015	1,71553
10599064	OTTMUSG00000018086	NM_001110250	1,78919
10603986	OTTMUSG00000018086	NM_001110250	1,79064
10604023	OTTMUSG00000018086	NM_001110250	1,79064
10599096	ENSMUSG00000059047	NM_001109969	1,81248
10603975	OTTMUSG00000018086	NM_001110250	1,81248
10599075	OTTMUSG00000018086	NM_001110250	1,81257
10599107	OTTMUSG00000018086	NM_001110250	1,81257
10603964	OTTMUSG00000018086	NM_001110250	1,81257
10603997	OTTMUSG00000018086	NM_001110250	1,81257
10604008	OTTMUSG00000018086	NM_001110250	1,81257
10599673	4930527E24Rik	ENSMUST00000088740	1,90453
10603911		---	1,94255

Supplementary table 2: PCR primers list

Gene Symbol	Forward (5'-3')	Reverse (5'-3')
AR	AAGACCTGCCTGATCTGTGG	TCGTTTCTGCTGGCACATAG
Bcl6	GCAGCAGTGAAGAAGGAACC	AGCCACAGCCTCACAGTTCT
Beta Actin	CCAACCGTGAAAAGATGACC	CGTGAGGGAGAGCATAGCC
cRET	TCTTCTGTGTCTGCCACCAC	GCCTTCTCCCAGAGTTTTCC
Cyclin A1	CAGAGCTCCAAGAGTGGAG	AGTGGAGATCTGACTTGAGC
Cyclin A2	CACCTCGAGGCATTCGGG	CGGGTAAAGAGACAGCTGC
DAZL	TTCAGGCATATCCTCCTTATC	ATGCTTCGGTCCACAGACTTC
ER	GATGGGCTTATTGACCAACC	CCAGGCACACTCCAGAAGG
FSH	TCAGCTTTCCCCAGAAGAGA	CCGAGCTGGGTCCTTATACA
FSHR	CCAAGCTTCGAGTCATTCCA	ATGCAAGTTGGGTAGGTTGG
GDNF	CGGACGGGACTCTAAGATGA	CGTCATCAAACTGGTCAGGA
GFRA1	CCATGTTCCTAGCCACTCTG	CACTGGCTTTCACACAGTCC
GR	AACTGGAATAGGTGCCAAGG	GAGCACACCAGGCAGAGTTT
HPRT	CGTCGTGATTAGCGATGATG	ACAGAGGGCCACAATGTGAT
LH	AGTTCTGCCCAGTCTGCATC	TGAGGGCTACAGGAAAGGAG
LHR	TCACAAGCTTTCAGGGGACT	GCAGGTTTTTGGTGTTCTGG
LHX1	CCCAGCTTTCCCGAATCCT	GCGGGACGTAAATAAATAAAATGG
MAGEA1	GCCCAAGACAGGTATCCTCA	TGTATTTCAGGCACCCTTCC
MAGEA2	CCCTGGTATCAAGGAGCTGA	CTAATTCCTGTTGGGCCTGA
MAGEA3	ACCTGGAATACAGGCAGGTG	AGGTCAGAGGAGTTGGAGCA
MAGEA5	GCTGAAACCCTGGTAAAGCA	TGGAAATCAGCCATTGTGAC
MAGEA6	GCTGAAACCCTGGTAAAGCA	TGGAAATCAGCCATTGTGAC
MVH	GATGAAGATTGGGAGGCAGA	GTTTCCAAAGCCCTTTCCTC
NANOS2	CCTGGATGTCTGCCTACCAT	GCTGACTGCTGTTGAGTGGA
NGN3	GCTATCCACTGCTGCTTGA	CCGGGAAAAGGTTGTTGTGT
OCT4	GAGCACGAGTGGAAAGCAAC	TTCTGCAGGGCTTTCATGTC
SOHLH1	ATGCTTTGGGATGCTGGATA	GACCCACCAGGAACAATGTC
SOHLH2	AGAGACAGATGCCCATCGAG	CTCTCACTGCTCCCTCCAAA
SOX9	AGTACCCGCATCTGCACAAC	AATCGGGGTGGTCTTTCTTG
SRF-1	TCCATTCAGCACCTTCAACA	TCATCCAAATGGAAAGAGCC
SYCP1	TGTTGATCCAAAGTGCTGAGA	TTCAAGTTCTGATGTTAAATGATCC
SYCP2	TGCAGATACCATCAGATGAAAAA	GACAGCTTCCCATTGGTGAT
SYCP3	GTGTTGCAGCAGTGGGAAC	GCTCGTGTATCTGTTTGATTGC
XMR	GATAATCGGCTCTGTCCAGG	GGCGGCATATTCTCATGTTT