# Supplemental Information

## Loss of microRNA-7a2 induces hypogonadotropic hypogonadism and infertility

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**Supplemental Figure 1**
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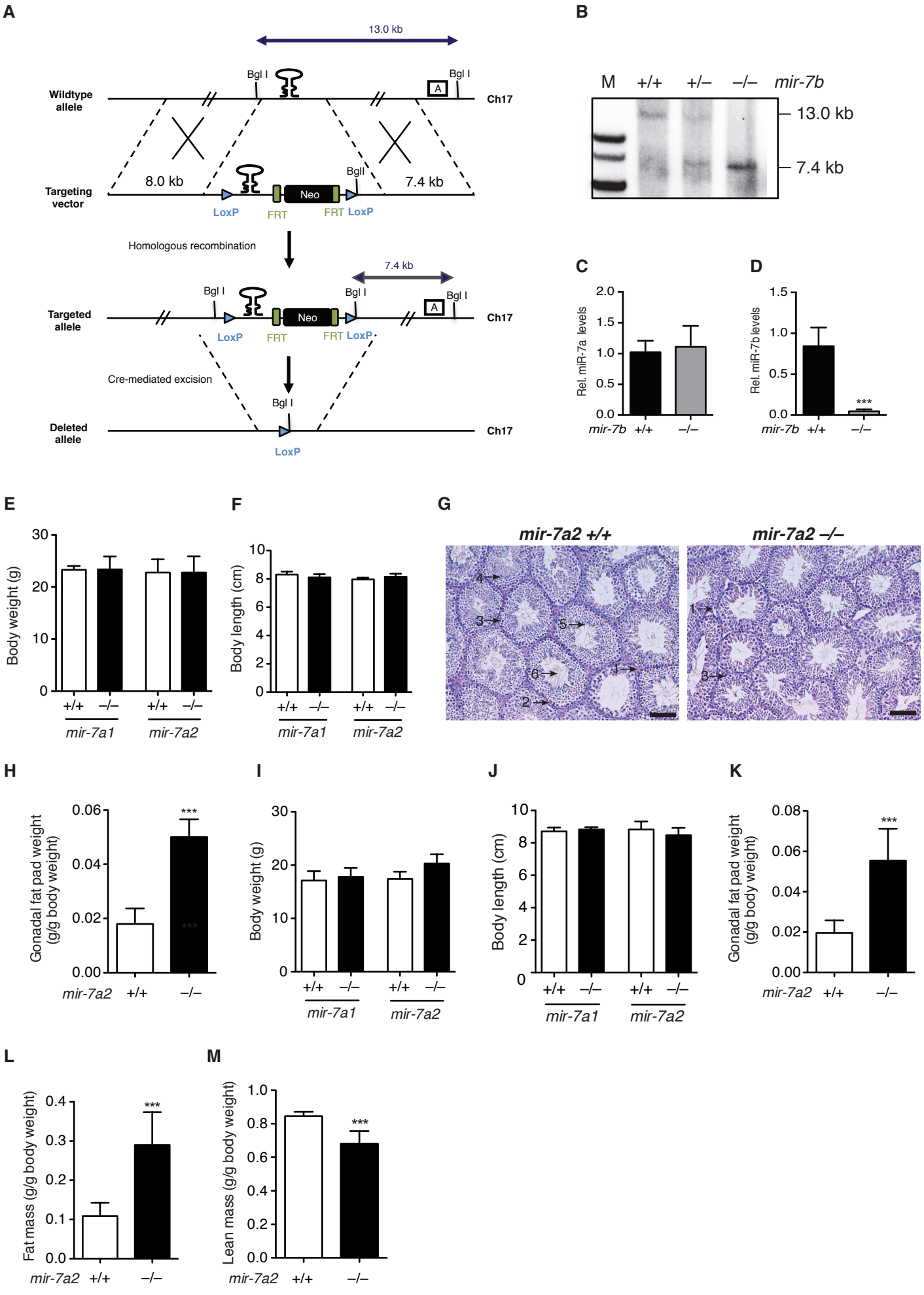
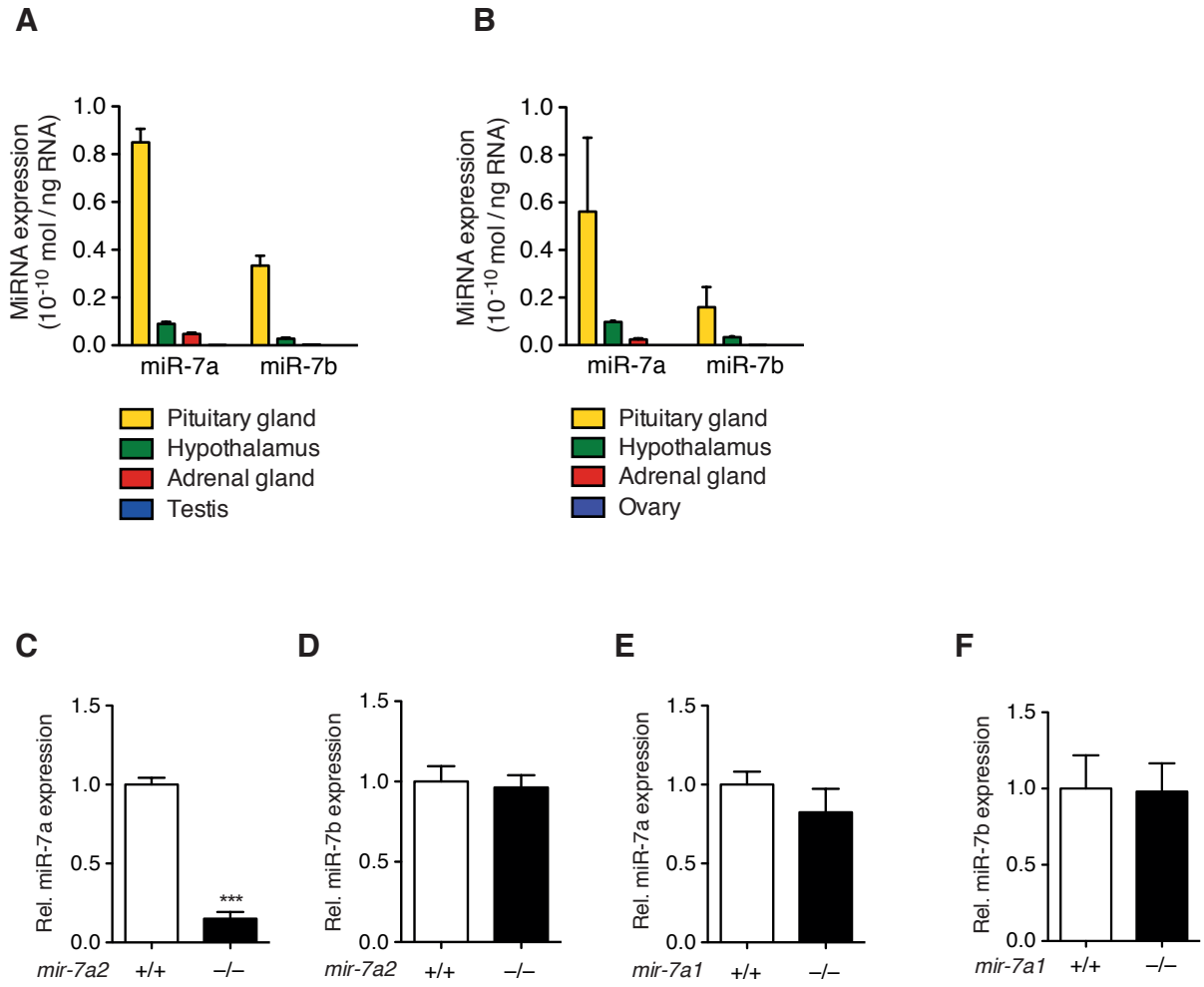
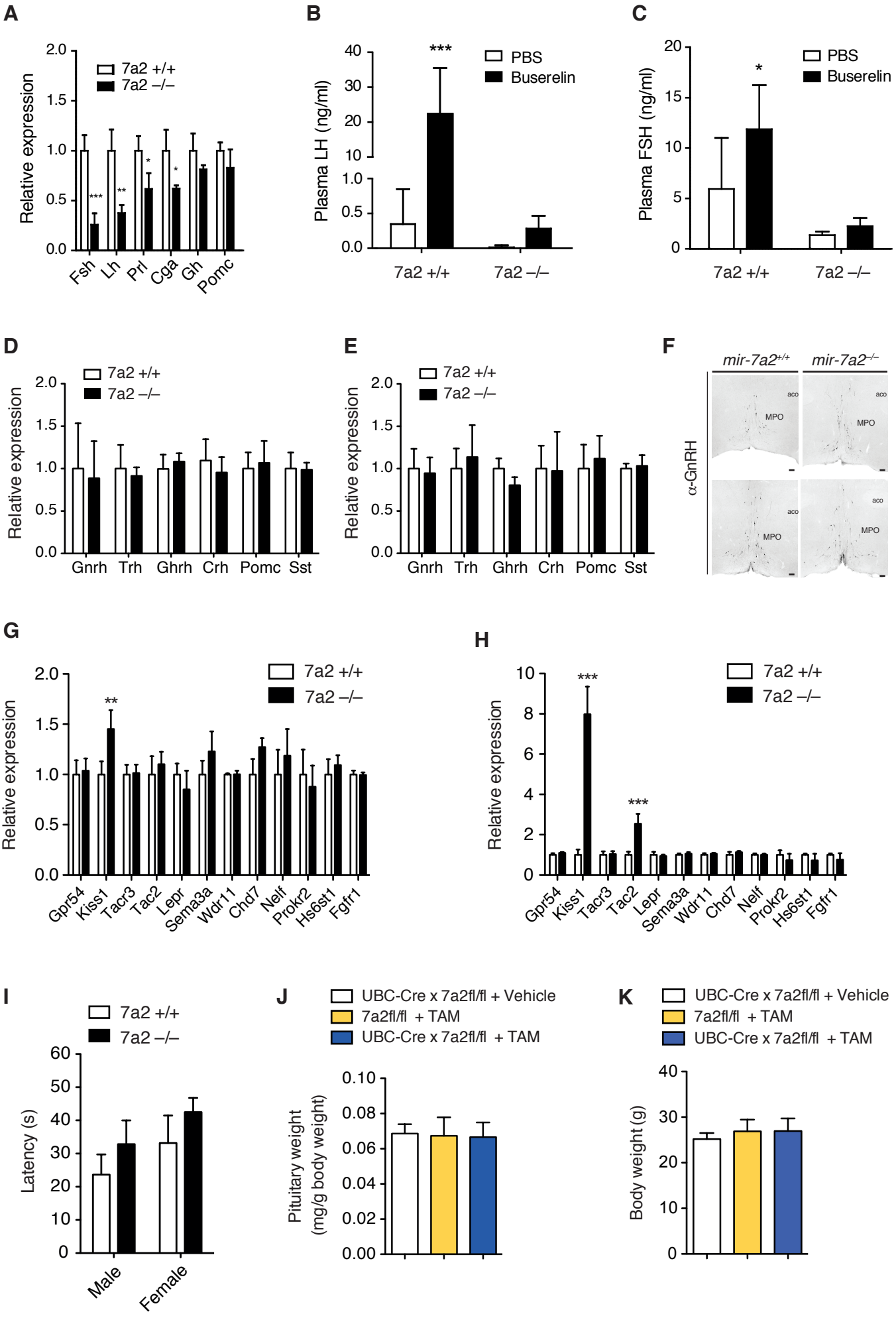


**Supplemental Figure 1.** Generation of *mir-7b* KO and phenotypic characterization. The generation of *mir-7a1* KO and *mir-7a2* KO was described previously (21). (**A**) Strategy used to generate *mir-7b* KO mice by homologous recombination. miRNA sequences were flanked with loxP sites and recombination induced by breeding mice with DeleterCre transgenics. Bgl1 digested DNA and southern blotting using the indicated miR-7b Probe was used to identify targeted clones. Wildtype allele: 13.0 kb; Mutant allele: 7.4 kb. (**B**) Southern blotting of genomic DNA from wild type (+/+), heterozygotes (+/–) and homozygotes (–/–) *mir-7b* mutant mice. DNA was digested with Bgl*I* and blotting performed with probe A**.** M, Molecular weight marker. **(C** and **D**) Relative miR-7a (**C**) and miR-7b (**D**) Expression in pituitary glands of controls (+/+) and *mir-7b* KO (–/–) mice (n = 4). (**E** and **F**) Body weights (**E**) and length (**F**) of male *mir-7a1* KO, *mir-7a2* KO or respective control mice (*mir-7a1* control, *mir-7a2* control n = 11; *mir-7a1* KO, *mir-7a2* KO, n = 7). Body length measured as distance from nose to base of tail. (**G**) Histological testes sections stained with hematoxylin-eosin of control (left image) or *mir-7a2* KO mice (right image). Arrows indicate: 1: Leydig cells, 2: Basement membrane, 3. Spermatogonia, 4: Spermatids, 5: Spermatozoa, 6: Spermatozoa tails, 7: Sertoli cell. Shown are representative images of three mice per genotype, scale bar, 100 µm. (**H**) Gonadal fat pad weights normalized to body weight of 16 week-old male control or *mir-7a2* KO mice (*mir-7a2* control n = 20; *mir-7a2* KO, n = 5). (**I** and **J)** Body weights (**I**) and length (**J**) of female *mir-7a1* KO, *mir-7a2* KO or respective control mice (*mir-7a1* control, *mir-7a1* KO, n = 4; *mir-7a2* control, n = 21; *mir-7a2* KO, n = 7). The measurements were performed at 2 months of age. (**K)** Gonadal fat pad weights normalized to body weight of 16 week-old female control or *mir-7a2* KO mice (*mir-7a2* control, n = 21; *mir-7a2* KO, n = 7). (**L** and **M**) Magnetic resonance imaging analysis revealing fat mass **(L)** and lean mass **(M)** in 16 week-old control or *mir-7a2* KO female mice (*mir-7a2* control n = 6; *mir-7a2* KO, n = 5). All data are mean ± SD. *** $P < 0.001$ by *t*-test.




**Supplemental Figure 2.** (**A** and **B**) Absolute expression levels of miR-7a or miR-7b in indicated organs of male (**A**) and female (**B**) wildtype mice (n = 4). (**C** and **D**) Relative expression levels of miR-7a (**C**) or miR-7b (**D**) in pituitary of male control or *mir-7a2* KO mice (*mir-7a2* control, *mir-7a2* KO, n = 3). (**E** and **F**) Relative expression levels of miR-7a (**E**) or miR-7b (**F**) in pituitary of male control or *mir-7a1* KO mice (*mir-7a1* control, *mir-7a1* KO, n = 4). All data are mean ± SD. *** $P < 0.001$ by *t*-test.


A
Relative expression
7a2 +/+
7a2 –/–
Fsh
Lh
Prl
Cga
Gh
Pomc
B
Plasma LH (ng/ml)
PBS
Buserelin
7a2 +/+
7a2 –/–
C
Plasma FSH (ng/ml)
PBS
Buserelin
7a2 +/+
7a2 –/–
D
Relative expression
Gnrh
Trh
Ghrh
Crh
Pomc
Sst
E
Relative expression
F
mir-7a2+/+
mir-7a2–/–
α-GnRH
aco
MPO
G
Relative expression
Gpr54
Kiss1
Tacr3
Tac2
Lepr
Sema3a
Wdr11
Chd7
Nelf
Prokr2
Hs6st1
Fgfr1
H
Relative expression
I
Latency (s)
Male
Female
J
UBC-Cre x 7a2fl/fl + Vehicle
7a2fl/fl + TAM
UBC-Cre x 7a2fl/fl + TAM
Pituitary weight (mg/g body weight)
K
Body weight (g)

**Supplemental Figure 3.** (**A**) Relative mRNA levels in pituitaries of 14 day old mice measured by qPCR (*mir-7a2* control, n = 4; *mir-7a2* KO, n = 3). (**B** and **C**) Plasma LH (**B**) and FSH (**C**) levels of miR-7a2 KO (7a2–/–) and littermate control mice (7a2+/+) that were injected with Buserelin of PBS 15 min prior to blood collection (n = 6 for each group). (**D** and **E**) Relative expression levels of genes encoding pituitary releasing hormones in hypothalamus of male (**D**) and female (**E**) *mir-7a2* KO or control mice (males, *mir-7a2* control, *mir-7a2* KO, n = 3, females, *mir-7a2* control, *mir-7a2* KO, n = 5). (**F**) Representative immuno-histological images of hypothalamic sections stained for GnRH of control (left images) or *mir-7a2* KO mice (right images), (WT, *mir-7a2* KO, n = 2); scale bar, 100 um. (**G** and **G**) Relative expression levels of hypothalamic genes involved in GnRH-neuronal functions in male (**G**) and female (**H**) control or *mir-7a2* KO mice (male, *mir-7a2* control, *mir-7a2* KO, n = 3; female, *mir-7a2* control, *mir-7a2* KO, n = 5). (**I**) Time elapsed in a food-seeking olfaction test in male and female *mir-7a2* KO or control mice (WT, *mir-7a2* KO, n = 6). (**J** and **K**) Pituitary weight (**J**) and body weight (**K**) in male UBC-Cre x *mir-7a2flox* or Cre-negative *mir-7a2flox* mice 10 weeks after a 5-day treatment with tamoxifen (TAM) or vehicle (UBC-Cre x *mir-7a2flox* + vehicle, n = 5; *mir-7a2flox* + TAM, UBC-Cre x *mir-7a2flox* + TAM, n = 4. All data are mean ± SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by *t*-test (A,D,E,G,H) and ANOVA (B,C,I)

**Supplemental Figure 4**
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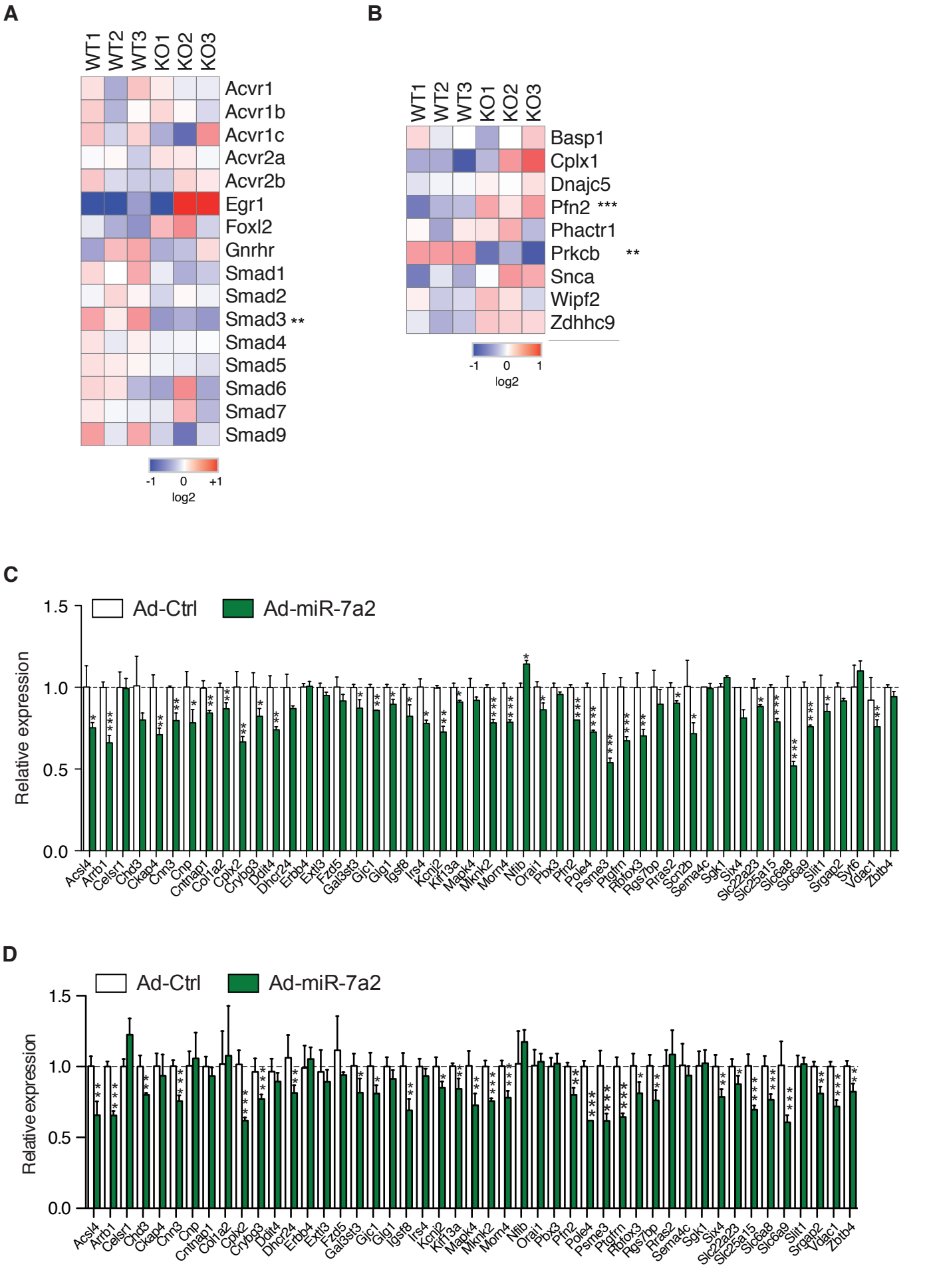
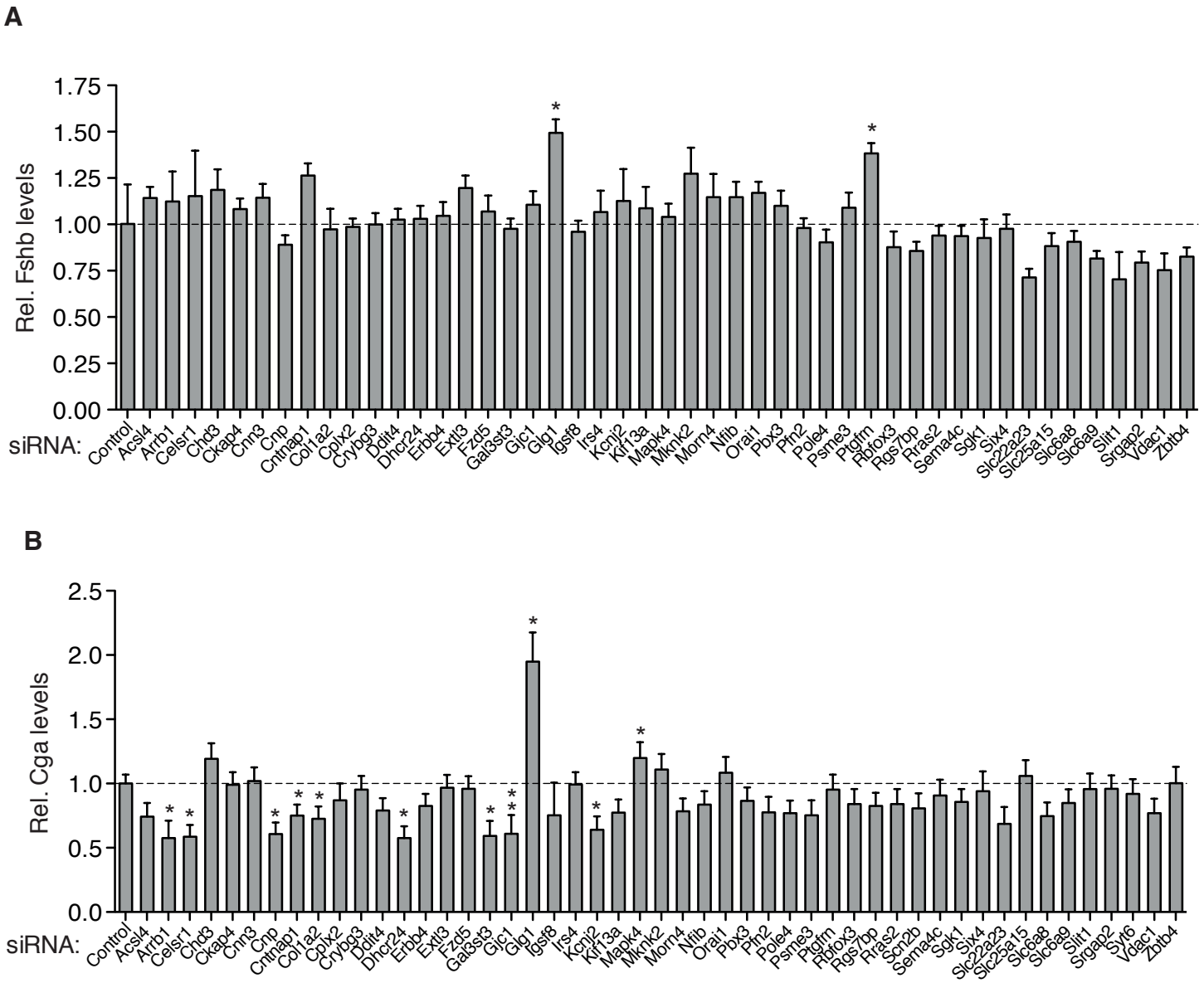
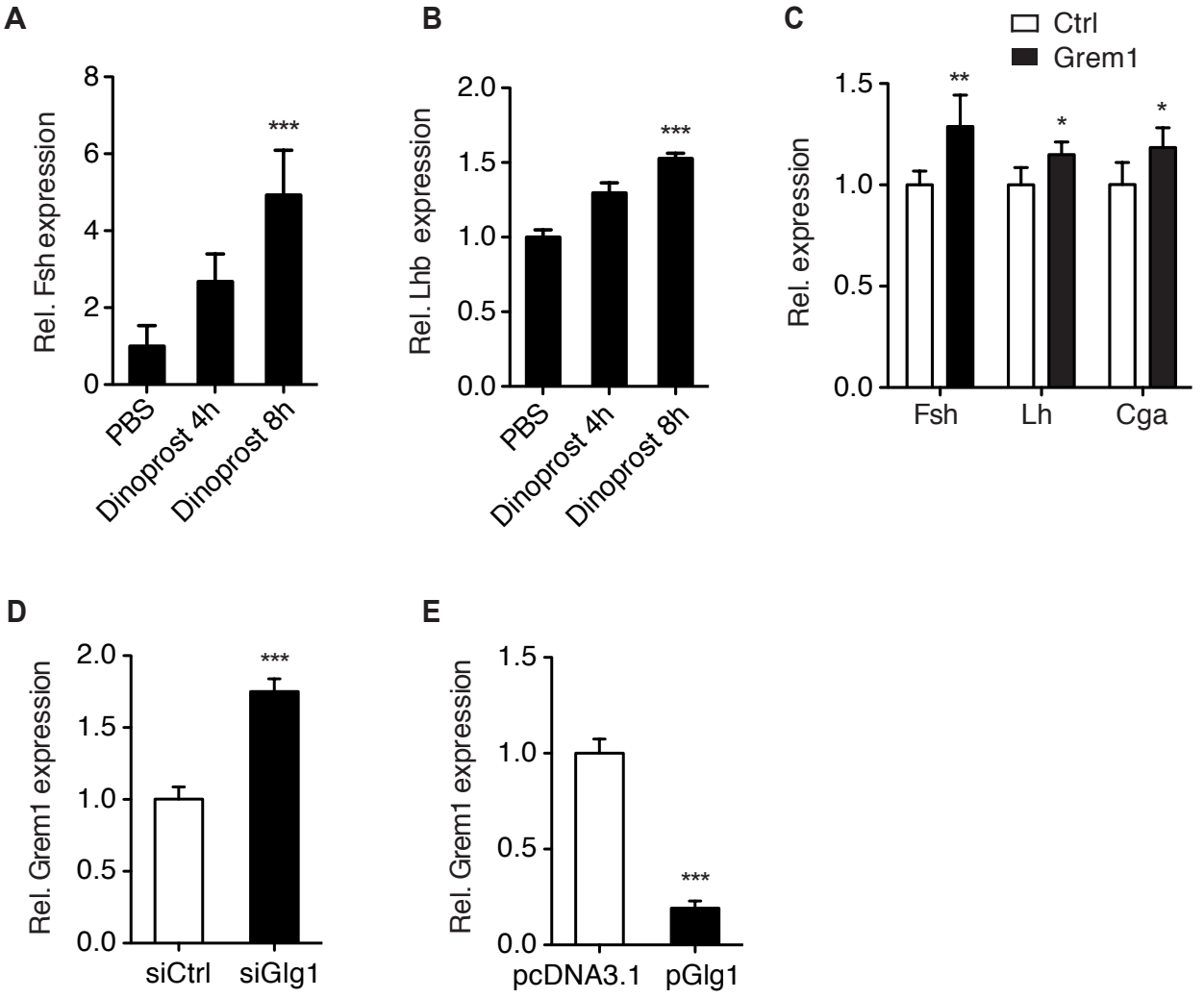


**Supplemental Figure 4.** (**A**) Expression levels of established key factors of early pituitary function. Data from RNA seq of *mir-7a2* KO or control mice shown as heat map (n = 3). (**B**) Expression levels of predicted miR-7 targets in pituitaries of *mir-7a2* KO mice that were previously shown to be upregulated in pancreatic islets implicated in insulin granule exocytosis. Data from RNA seq of *mir-7a2* KO or control mice shown as heat map (n = 3). (**C** and **D**) Relative expression of predicted miR-7 target genes that were more than 1.3-fold upregulated in RNA Seq in gonadotroph cell lines aT3 (**C**) and LbT2 (**D**) transduced with adenoviral constructs overexpressing mir7a2 (Ad-mir-7a2) or control (Ad-Ctrl). *Fgf1, Kcna1, Prelp, Rgs8, Scnb2, Slc4a4, Snca*, and *Syt6* were only lowly expressed in aT3 and/or LbT2 cells and could not be analyzed. (aT3, n = 3; LbT2, n = 4). All data are mean ± SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by *t*-test.




**Supplemental Figure 5**. (**A** and **B**) Effect of siRNA-mediated silencing of predicted miR-7 targets that were more than 1.3-fold upregulated in RNA Seq in the gonadotroph cell lines LbT2 (**A**) or aT3 (**B**) on mRNA expression of Fshb (A) or Cga (B) 48 h after transfection (n = 3). All data are mean ± SD. * $P < 0.05$; ** $P < 0.01$ by *t*-test.




**Supplemental Figure 6**. (**A** and **B**) Relative expression of gonadotroph hormones Fshb (**A**) and Lhb (**B**) in LbT2 cells treated with or without 100 nM dinoprost for 4 h or 8 h (n = 4). (**C**) Relative expression of gonadotropic hormones Fshb, Lhb and Cga in LbT2 cells overexpressing Grem1 (n = 4). (**D** and **E**) Relative expression of Grem1 in LbT2 cells silenced for Glg1 (**D**) and over-expressing Glg1 (**E**) (n = 4). All data are mean ± SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by *t*-test.

| | ♀ | | | | ♂ | | | |
|---|---|---|---|---|---|---|---|---|
| Genetic background | 7a1 | 7a2 | 7a2 | 7b | 7a1 | 7a2 | 7a2 | 7b |
| Genotype | –/– | +/– | –/– | –/– | –/– | +/– | –/– | –/– |
| Number of animals | 7 | 6 | 6 | 5 | 7 | 7 | 5 | 7 |
| Pregnancies/mated mouse (%) | 81 | 75 | 0 | 78 | 69 | 79 | 0 | 81 |
| Average number of progeny/pregnancy | 8.3 | 7.5 | 0 | 7.8 | 9.1 | 8.9 | 0 | 7.8 |

**Table 1**. Fertility assessment of *mir-7a1*, *mir-7a2* and *mir-7b* KO mice.

## Supplemental Table 2

| **Primers for generation of the *mir-7b* targeting vector and genotyping of *mir-7b* mutant mice** | | |
|---|---|---|
| **Sequence** | **Forward (5'-3')** | **Reverse (5'-3')** |
| mir-7b (15.7) kb locus | GCTCCTGTTCATGTTTGA GCGTGGTCTCAGTGCTT GAGTACACTATATTTGCT CTCCGAGTAGGACAAATC | AAATTCTGGGATTGTTTTTG CTACTTTCCATCTTTACTGT GTGTCCATGTTCACAGCTTG TCTGTAAGCGGATG |
| mir-7b Geno PCR1 | atcccacgttggtgatgtgccaggg | cctgtttgtgtatttcaggaga |
| mir-7b Geno PCR2 | atcccacgttggtgatgtgccaggg | gatttgaactctggaccttgcggttcag |

## Supplemental Table 3

| Gene | Sequence - forward primer | Sequence - reverse primer |
|---|---|---|
| Acsl4 | gaaattcacagcatgcaatcag | tctacttggaggaacgctcaa |
| Arrb1 | gctcagtacaagtgcccagtg | agaccttgcagaatgttgagc |
| Celsr1 | ggcagtcatgaccttggacta | agctgattcccaatctgcac |
| Cga | tccctcaaaagtccagagc | gaagagaatgaagaatatgcaggaa |
| Chd3 | actttgatgagcgtcctgaag | ggcttgtccttctcatttcg |
| Chd7 | cttttcatgagccacaaacg | tcttcttcaaaagctttggtcac |
| Ckap4 | ggaggaggtccagcaggt | ttgcagggattggacctt |
| Cnn3 | ccgccgaagttaagaacaag | ggcctgtcacctcttctatcc |
| Cnp | cgctggggcagaagaatac | aaggccttgccatacgatct |
| Cntnap1 | gccgcaagaagcttcagtt | agttcacctcgtgccagaag |
| Col1a2 | gcaggttcacctactctgtcct | cttgccccattcatttgtct |
| Cplx2 | cttgcctggcggagttac | ctgcctttgggaaatgagc |
| Crh | ggaggcatcctgagagaagtc | catgttaggggcgctctc |
| Crybg3 | tggggcactgataaaagttgt | tggaaatggggcttctcata |
| Cyp11a1 | aggccaacattaccgagatg | ggttccactgcagggtcat |
| Cyp17a1 | catcccacacaaggctaaca | cagtgcccagagattgatga |
| Cyp19a1 | ccactcctgctgatcatgg | tcccagacagtagccaggac |
| Ddit4 | ccagagaagagggccttga | ccatccaggtatgaggagtctt |
| Dhcr24 | tcatgatcaacctgatggaca | ggctccactcgaacaatctg |
| Erbb4 | tggagaaaggagagcgtctg | cagcatcgatcatccaaca |
| Extl3 | ggtggctcgttactgactgg | ttgaactaatggacaccagagc |
| Fgf1 | cagcctgccagttcttcag | ggctgcgaaggttgtgat |
| Fgfr1 | gactctggcctctacgcttg | aggatgggagtgcatctga |
| Fshb | gctgccatagctgtgaattg | tgggtccttatacaccagatcc |
| Fshr | tgcctgatgatgttttccag | ggcagggaatagacctttgtc |
| Fzd5 | cagcaggatcctccgaga | cagcactcagttccacacca |
| Gal3st3 | gggtagccctgacccaag | ctctggtagcagggccagt |
| Gata2 | tcacccctaagcagagaagc | caggcattgcacaggtagtg |
| Gh | gcttggcaatggctacaga | ggaaaagcactagcctcctg |
| Ghrh | caggaagacagcatgtggac | aggcttcatccttgggaatc |
| Gjc1 | gggtaacaggagttctggtga | ctagcaggcgagtcaggaag |
| Glg1 | gccaggctctcctaccttct | tctcaccttggcactcactg |
| Gli1 | ctgactgtgcccgagagtg | cgctgctgcaagaggact |
| Gli2 | gcagactgcaccaaggagta | cgtggatgtgttcattgttga |
| Gli3 | tgcctccaggtgaagactgt | gcatgaagactgaccaccag |
| Gnrh | tcagggatctgcgaggag | gggccagtgcatctacatc |
| Gpr54 | ggtgctgggagacttcatgt | agtggcacatgtggcttg |
| Grem1 | gacccacggaagtgacaga | ccctcagctgttggcagtag |
| Hes1 | acaccggacaaaccaaagac | cgcctcttctccatgatagg |
| Hs6st1 | ggaccgaactcaccaactgt | cgcagcagggtgatgtagta |
| Hsd17b1 | gtgtgggaggcttgatgg | ggctcacatggactccaaag |
| Hsd17b2 | tcaccaagccagagcagata | gttaaccacggcccacagt |
| Hsd17b3 | aatatgtcacgatcggagctg | gaagggatccggttcagaat |
| Hsd3b1 | gaccagaaaccaaggaggaa | gcactgggcatccagaat |
| Hsd3b6 | agactgggactgctgacacc | caggaagcagatcacagttgg |
| Igsf8 | gccaaggtggagctgaga | tgcactgtgaggcgtgag |
| Irs4 | accgccacctgtagctagg | ctttgtgggcgtcttctctc |
| Isl1 | gcaacccaacgacaaaactaa | ccatcatgtctctccggact |
| Kcna1 | agactctccgccgactcag | ccctcctacccctcttcg |
| Kcnj2 | tgaagttgccctaacaagca | gctctctgggactccgttct |
| Kif13a | cccctcatgcctgtaaaaga | ttcctcctcttccgagtcct |
| Kiss1 | atgatctcaatggcttcttgg | ccaggcattaacgagttcct |

| | | |
|---|---|---|
| Lepr | gttccaaaccccaagaattg | tgctcaaatgtttcaggcttt |
| Lhb | caagaatggagaggctccag | actgggcagaactcattctctg |
| Lhcgr | gatgcacagtggcaccttc | gatgagcgtctgaatggactc |
| Lhx3 | caagtccgacaaggacagc | tagcaggccccatgtcag |
| Lhx4 | agacagccaagcaaaacgat | ggctttggggagttcttgta |
| Mapk4 | tcacggggaaaatgctctt | cagggatggtgtctaggatga |
| Mknk2 | cgtgtgcagacctgtgtca | ccagctgcttctcaatgatct |
| Morn4 | agcccagagcagcaaagat | tggtggcagcttacagaaga |
| Nelf | ccacaactatgcaagccatc | cggaatcattctcccgttt |
| Nfib | ccggaatacctggagtcg | gaaatggcaacggtgagg |
| Nr5a1 | agcatcctgctggttactgg | gcaactggagcactaactcttg |
| Orai1 | tacttaagccgcgccaag | acttccaccatcgctacca |
| Pbx3 | gccttggagcaaactcactg | agatggagttgttgcgtcct |
| Pfn2 | gtgcagcttggagagcaaa | gggaatttgatcggtatggtt |
| Pitx1 | atcgtccgacgctgatct | cttagctgggtcctctgcac |
| Pitx2 | ccttacggaagcccgagt | aaagccattcttgcacagc |
| Pole4 | gctgtggatgaattcgcttt | gggaaaggtgacagatgcag |
| Pomc | ccatagatgtgtggagctggt | agcgagaggtcgagtttgc |
| Pou1f1 | ccaccaacgtgatgtcca | tggatggctggtttccata |
| Prelp | gaacagaagagtgccccaga | atgccctcatgatccaggt |
| Prl | gttctctcaggccatcttgg | aggaggagtgtccctgcttt |
| Prokr2 | cctccgtcaactaccttcgt | gggtggacaatagcgaggt |
| Prop1 | cctcagtgaagccagaatcc | ggccgtagatagatgggcta |
| Psme3 | cactgtcacagagattgatgagaa | ggatcatgtcatggagagtgac |
| Ptgds | agtggtagccccctccacag | gagtggatgctgcccgagtg |
| Ptgfrn | ccggggagatctcatcaaa | tcgaaggccatgtcatctg |
| Rbfox3 | ggttttgggtttgtaacttttga | ggctgtggcattattgacct |
| Rgs7bp | aaccaaaggctgtgaaatgg | gatttcgggatggatctcac |
| Rras2 | aaagctgacctggaccatca | gtgaccttgagctgccttg |
| Scn2b | gccacggcaagatttacct | catcagcaccaagatgacca |
| Sema3a | atcagtgggtgccttaccaa | tccgccaaatgttttactgg |
| Sema4c | gattggagctgcacgaaag | agccagacagcccagtgt |
| Sgk1 | ggactacattaatggtggagagc | agaatcgagcccgtggtt |
| Six4 | ggagcattggattctctcca | ccgaagtgcttggggtaac |
| Slc22a2 | ccaagaaggtgtgcatcgt | gtggatcccgtaccctgtc |
| Slc25a15 | ccgtaaagtggttggattgg | agcagctgcattctgaaggt |
| Slc6a8 | ggtgaccttgtgcctgct | ggaccacgtaggggaatgta |
| Slc6a9 | tccctttaagaaagccacctg | tttcagacaacaggcctcaa |
| Slit1 | cctttcaaggacttcgttcg | gagggtggagacatcattgc |
| Sox2 | ggcagagaagagagtgtttgc | tcttctttctcccagcccta |
| Sox9 | aacgccttcatggtgtgg | tctcgctctcgttcagcag |
| Srgap2 | ccccacatgggagatatgg | accagctcactctggacagg |
| Sst | cccagactccgtcagtttct | gggcatcattctctgtctgg |
| Star | aaggctggaagaaggaaagc | ccacatctggcaccatctta |
| Tac2 | agggagggaggctcagtaag | ggcggctgtcgtagagtc |
| Tacr3 | tggaaatacatccagcaggtc | ttgaagcctgcacgaaatc |
| Tbx19 | gaagctgaccaacaagctca | cctgaggttcatatttatgcagag |
| Thbs2 | tcggacctcaagtatgagtgc | tctaagaaggggtgtttgcag |
| Trh | tgcagagtctccaccttgc | ggggataccagttagcacga |
| Tshb | agagctggggttgttcaaag | tacaaaaggatgctgcttgc |
| Vdac1 | acctttgattcgtcattctcg | tgctccctcttgtaccctgt |
| Wdr11 | ctcaacgcgcacaacaag | ggcatccatatgcaattaaacc |
| Zbtb4 | gctactgtgagaaggtgtttgc | ccagcagaagatgcactgg |

| | | |
|---|---|---|
| Acvr1 | gtggaagattacaagccacca | gggtctgagaaccatctgttagg |
| Acvr1b | gcggtcactgacaccataga | gagtcttcttgatgcgcaga |
| Acvr1c | tggtaacagaagatcacatcagtg | catgcatggtccctgttaaa |
| Acvr2a | gggacgcatttctgaggata | tcctggaggcatcctactca |
| Acvr2b | cgactttgtggctgtgaaga | tcgttccacgtgatgatgtt |
| Egr1 | tcctctccatcacatgcctg | cactctgacacatgctccag |
| Foxl2 | ggggaggagaaatgtaatgg | cacagcctaatgaagtcacc |
| Smad1 | cgctccacggcacagttaag | gccagttgatttgcgaacagaa |
| Smad2 | aacccgaatgtgcaccataagaa | gcgagtctttgatgggtttacga |
| Smad3 | gtcaacaagtggtggcgtgtg | gcagcaaaggcttctgggataa |
| Smad4 | ggacgccctaaccatttccag | ctgctaagagcaaggcagcaaac |
| Smad5 | tgcagcttgaccgtccttacc | gcagacctacagtgcagccatc |
| Smad6 | ttctcggctgtctcctcctga | gtggcctcggtttcagtgtaaga |
| Smad7 | ggcctatccacaggcttctga | gtgacaggcggcagtaagaca |
| Smad9 | cgatcattccatgaagctgacaa | tgggcaagccaaaccgata |