SUPPLEMENTAL FIGURE LEGENDS

Figure 1: A) PCR detection of the floxed and recombined Foxl2 alleles in genomic DNA (150 ng) isolated from brain, pituitary, heart, lung, liver, kidney, adrenal gland, epididymis, uterus, testis, and ovary of adult control and cKO animals. B) Foxl2 mRNA levels from ovaries of adult control (n=10) and cKO (n=8) females. ns, not statistically different. C) Immunoblot (IB) analysis of FOXL2 protein expression in individual ovaries from control and cKO mice (n=5 per genotype). FOXL2 protein was not detected in testis protein extracts from two males (rightmost two lanes).

Figure 2: Mean time (in days) from pairing to first litter for control and cKO mice (n=7 per genotype, except for cKO males where one individual failed to sire any offspring). Data are means (+SEM) and were compared using t-tests (p<0.05).

Figure 3: Seminal vesicle (wet) (A) and epididymides (B) weights (in grams) normalized to body weight (in grams) for control (n=13) and cKO (n=17-21) males aged 8-13 weeks.

Figure 4: A) Uterine weight (in grams) normalized to body weight (in grams) for metestrus/diestrus-staged control (n=12) and cKO (n=10) females aged 8-13 weeks. B) Puberty onset as assessed by day of vaginal opening for control (n=23) and cKO (n=15) females.

Figure 5: A) Estrous cyclicity as assessed by vaginal cytology for three representative females per genotype. Stages are indicated as estrus (E), proestrus (P), and metestrus/diestrus (M/D). B) Average number of cycles per 5 days. C) % of days in each cycle stage for control (n=10) and cKO (n=13) females.

Figure 6: Circulating FSH levels in females from the experiment in Fig. 4D; n=10 for controls and n=6 for cKOs. * indicates statistical difference (p<0.05) by Mann-Whitney test. Individual data points are indicated with circles or squares. Means are shown with horizontal lines.

Figure 7: Circulating sex steroid levels in control and cKO mice. A) Serum 17β-estradiol levels were measured in females at metestrus/diestrus. B) Serum testosterone levels in males aged 8-13 weeks (n=10 for both genotypes) reported in log scale. In both panels, data for individuals are shown as points. The means are shown as horizontal lines and were compared by t-tests.
**Figure 8:** Growth hormone (*Gh*), prolactin (*Prl*) and thyroid-stimulating hormone β (*Tshb*) mRNA expression are normal in pituitaries of male cKO mice, as assessed by RT-qPCR (n=7-10 animals per genotype).

**Figure 9:** FSH target gene expression is altered in gonads of cKO mice. Expression of the indicated genes was examined by RT-qPCR in testes (A) or ovaries (B) of adult control and cKO mice (n=8-10 per genotype).

**Figure 10:** Secreted LH levels from male primary pituitary cultures. The data here and in Fig. 6 are means (+SEM) derived from the same five independent experiments (n=5).

**Figure 11:** Basal and activin A-stimulated *Fshb* transcription is decreased in female primary pituitary cultures following *ex vivo* recombination of the *Foxl2* locus. Mean (+SEM) relative (A) *Foxl2* and (B) *Fshb* mRNA levels and secreted (C) FSH and (D) LH levels in treated cultures from three independent experiments. Data were analyzed by two-way ANOVA followed by Tukey *post-hoc* of the significant interaction. Bars with different symbols were statistically different, whereas those sharing symbols did not differ.

**Figure 12:** Basal and activin A-stimulated *Fshb* expression is decreased in female primary pituitary cultures following *in vivo* recombination of the *Foxl2* locus. Mean (+SEM) relative (A) *Foxl2* and (B) *Fshb* mRNA levels (n=3). Normalized secreted (C) FSH and (D) LH levels in treated cultures (n=2). Data were analyzed by two-way ANOVA followed by Tukey *post-hoc* of the significant interaction. Bars with different symbols were statistically different, whereas those sharing symbols did not differ. Data in A and B are from three independent experiments. Data in C and D are from two out of the three experiments in panels A and B.
### Table 1: RT-qPCR and genotyping primer sequences

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Data are mean±SEM. * indicates statistical significance. n=11 for controls and n=8 for cKOs.
Fig. 1

A

B

C

control ovaries  cKO ovaries  testes

< IB: FOXL2

< IB: ACTB
Fig. 4

A

B

Normalized uterine weight

ns p=0.9177

control cKO

Day of opening

*p=0.0179

control cKO
Fig. 5

A

control females

E
P
M/D
0 5 10 15 20

E
P
M/D
0 5 10 15 20

E
P
M/D
0 5 10 15 20

E
P
M/D
0 5 10 15 20

cKO females

E
P
M/D
0 5 10 15 20

E
P
M/D
0 5 10 15 20

E
P
M/D
0 5 10 15 20

E
P
M/D
0 5 10 15 20

B

Number of cycles per 5 days

control cKO

ns p=0.0732

C

% days in each stage

control cKO

ns p=0.7316

ns p=0.8070

ns p=0.3942
Fig. 6

[Diagram showing FSH (ng/mL) concentrations with control and cKO groups.]

\[ p = 0.0225 \]
Fig. 7

A

Estradiol (pg/mL)

ns p=0.6439

control    cKO

B

Testosterone (ng/L)

ns p=0.3868

control    cKO
Fig. 8

Bar chart showing the relative mRNA levels of Gh, Prl, and Tshb for control and cKO conditions. The y-axis represents Relative mRNA levels ranging from 0.0 to 3.0. The x-axis includes Gh, Prl, and Tshb. The bars for Gh and Prl in the control group are marked with "ns," indicating no significant difference. The bar for Tshb in the control group is also marked with "ns." The cKO group shows a significant increase in Tshb mRNA levels compared to the control group.
Fig. 10

![Bar graph showing LH levels with and without activin A](image)

- **No ligand**
- **Activin A**

LH (mg/mL) vs. Ad-GFP and Ad-Cre conditions.