

# Supporting Information

El-Hayek et al. 10.1073/pnas.1414648111

## SI Methods

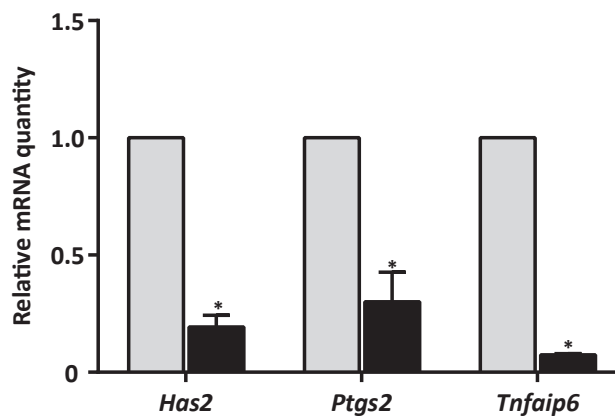
**Histological Sections.** The ovaries were those described in ref. 1. Sections were scanned visually to identify large follicles and were photographed using a Leica DM6000 microscope.

**GC Monolayer.** Ovaries were harvested from *Fshb*<sup>+/-</sup> and *Fshb*<sup>-/-</sup> females at P21, and granulosa were isolated (see *Methods* in the main text). Cells then were seeded onto glass chamber-culture slides (Becton Dickinson) and cultured in MEM (Gibco) supplemented with 5% (vol/vol) FBS (Life Technologies) at 37 °C in an atmosphere of 5% CO<sub>2</sub> in air. Bright-field images of the

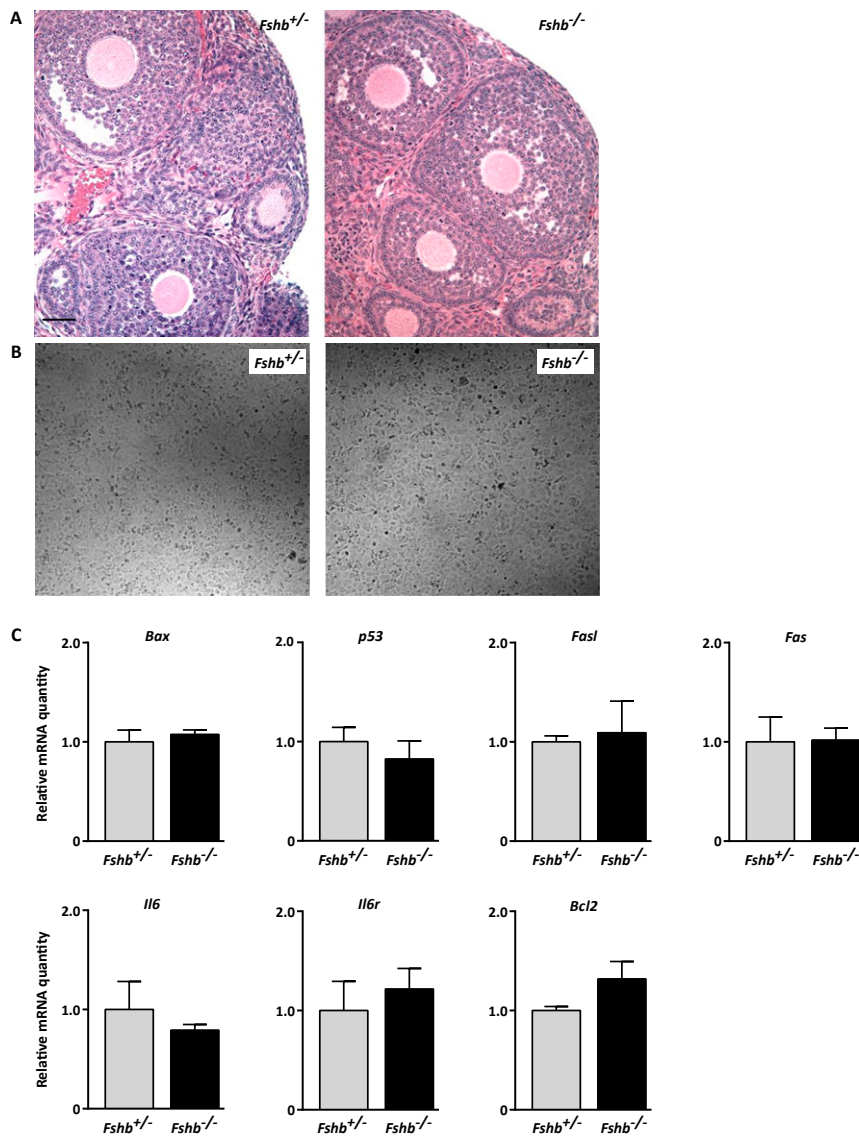
monolayers were taken with a Zeiss LSM 510 confocal microscope.

**Quantitative PCR Analysis.** Ovaries were harvested from *Fshb*<sup>+/-</sup>, *Fshb*<sup>-/-</sup>, eCG-injected *Fshb*<sup>-/-</sup>, and eCG-injected *Fshb*<sup>+/-</sup> females at P16, P18, or P21/23, depending on experiment. Follicles, COCs, or GCs, depending on experiment, were collected and treated accordingly. RNA extraction followed by cDNA synthesis and quantitative PCR then were performed (see *Methods* in the main text). Primer sequences are given in Table S1.

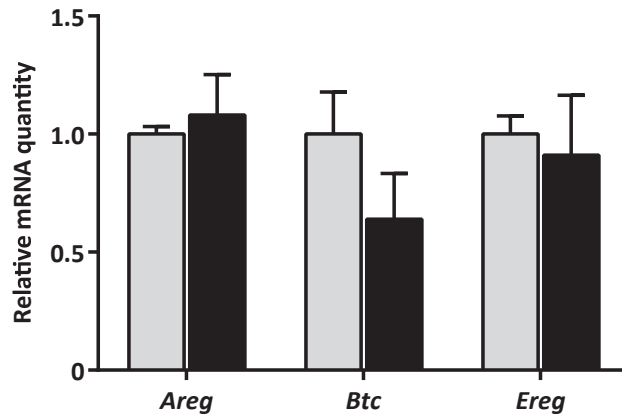
1. Demeestere I, et al. (2012) Follide-stimulating hormone accelerates mouse oocyte development in vivo. *Biol Reprod* 87(1):3, 1-11.



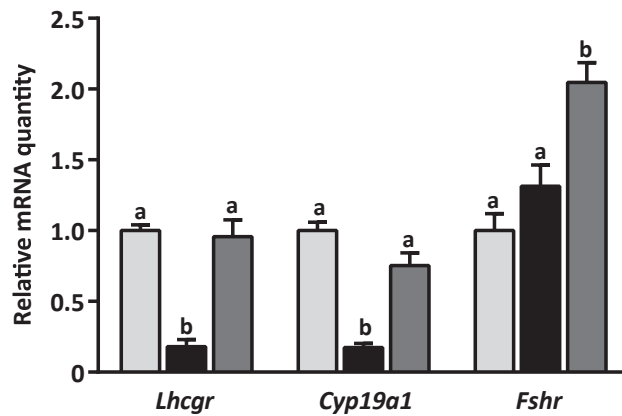
**Fig. S1.** Quantities of *Has2*, *Ptgs2*, and *Tnfaip6* are higher in the COCs of *Fshb*<sup>+/-</sup> females (gray bars) than in the COCs of *Fshb*<sup>-/-</sup> females (black bars) at P21/23. Data were analyzed using a single-sample t test. \**P* < 0.05.



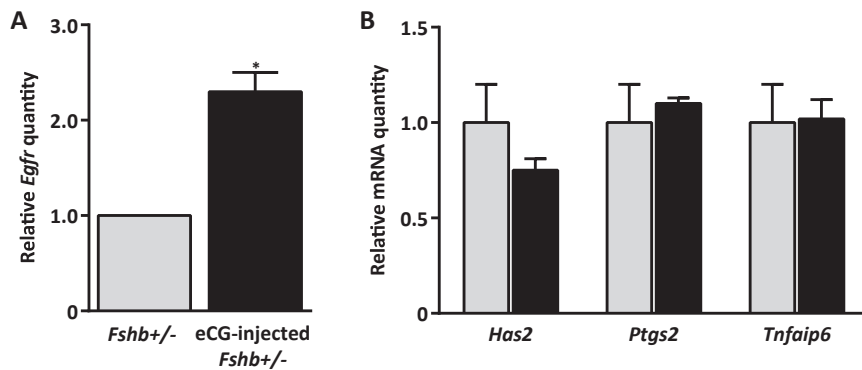
**Fig. S2.** (A) Histological sections of ovaries of *Fshb*<sup>+/-</sup> and *Fshb*<sup>-/-</sup> females at P24. Ovarian follicles of the *Fshb*<sup>-/-</sup> females do not contain an increased number of pycnotic or otherwise abnormal nuclei. (B) GCs of *Fshb*<sup>+/-</sup> and *Fshb*<sup>-/-</sup> females form morphologically indistinguishable monolayers in vitro. Shown are bright-field images of monolayers formed by GCs of *Fshb*<sup>+/-</sup> and *Fshb*<sup>-/-</sup> females collected at P21 and cultured for 3 d. (C) Quantities of mRNAs encoding proapoptotic and antiapoptotic factors are similar in follicles of *Fshb*<sup>+/-</sup> and *Fshb*<sup>-/-</sup> females. Quantitative PCR analysis of the proapoptotic factors *Bax*, *p53*, *FasI*, and *Fas* and the antiapoptotic factors *Il6*, *Il6r*, and *Bcl2* in follicles of *Fshb*<sup>+/-</sup> females (gray bars) and *Fshb*<sup>-/-</sup> females (black bars) at P18. Data were analyzed using a two-sample t test. *P* values <0.05 were considered statistically significant.



**Fig. S3.** Quantities of *Areg*, *Ereg*, and *Btc* are not significantly different in the GCs of *Fshb*<sup>+/+</sup> females (gray bars) and *Fshb*<sup>-/-</sup> females (black bars) at P21/23. Data were analyzed using a two-sample t test. *P* values <0.05 were considered statistically significant.



**Fig. S4.** Quantities of *Lhcgr*, *Cyp19a1*, and *Fshr* in follicles of P18 *Fshb*<sup>+/+</sup> females (light gray bars), *Fshb*<sup>-/-</sup> females (black bars), or eCG-injected *Fshb*<sup>-/-</sup> females (dark gray bars). Data were analyzed using one-way ANOVA and Tukey HSD. Different letters above bars indicate *P* < 0.05.



**Fig. S5.** (A) Quantity of *Egfr* in follicles of *Fshb*<sup>+/-</sup> and eCG-injected *Fshb*<sup>+/-</sup> P18 females. Data were analyzed using a single-sample t test. \**P* < 0.05. (B) Quantity of *Has2*, *Ptgs2*, and *Tnfaip6* after EGF stimulation of follicles of *Fshb*<sup>+/-</sup> females (gray bars) and eCG-injected *Fshb*<sup>+/-</sup> females (black bars) P18. Data were analyzed using a two-sample t test. A *P* value <0.05 was considered statistically significant.

**Table S1. Primer sequences used for quantitative PCR analysis**

Gene/transcript	Primer sequence
<i>Actb</i>	F: 5'-GGCTGTATTCCCCTCCATCG-3' R: 5'-CCAGTTGGTAACAATGCCATGT-3'
<i>Areg</i>	F: 5'- CCTTCTGGCAGTGAACCTCCAC-3' R: 5'-GGTCCTTGTTCATCCTCGCTGTGA-3'
<i>Bax</i>	F: 5'-CGAGCTGATCAGAACCATCA-3' R: 5'-GAAAAATGCCTTCCCCTTC-3'
<i>Bcl2</i>	F: 5'-TAAGCTGTCACAGAGGGGCT-3' R: 5'-TGAAGAGTTCCCTCCACCACC-3'
<i>Bmp15</i>	F: 5'-GAGCGAAAATGGTGAGGCTG-3' R: 5'-GGCGAAGAACAACACTCCGTCC-3'
<i>Btc</i>	F: 5'-CGGGTAGCAGTGTGAGCTC-3' R: 5'-CGATGTTTCCGAAGAGGATG-3'
<i>Cyp19a1</i>	F: 5'-ACACGTCTGGTCTCCTGTAGAGT-3' R: 5'-GATCCACCGTAAGCAACTGGGTTT-3'
<i>Egfr</i>	F: 5'-GTGGAGGGACATCGTCCAAA-3' R: 5'-ATTGGGACAGCTTGGATCACAT-3'
<i>Ereg</i>	F: 5'-AACTCAGGAACAATTTACGTCTCTG-3' R: 5'-GCTTTGGTTCCTCAGTATAGAGAGAGA-3'
<i>Fas</i>	F: 5'-GAGAATTGCTGAAGACATGACAATCC-3' R: 5'-GTAGTTTTTCACTCCAGACATTGTCC-3'
<i>Fasl</i>	F: 5'-TTAGCTTCTCTGGAGCAGTCAGCGTC-3' R: 5'-CCTTCTTCTTTAGAGGGGTCAGTGCC-3'
<i>Fshr</i>	F: 5'-CAGGTCAACATACCGCTTGA-3' R: 5'-GATCCCCAGGCTGAGTCATA-3'
<i>Gdf9</i>	F: 5'-GCTCTATAAGACGTATGCTACC-3' R: 5'-CAGAGTGTATAGCAAGACCGAT-3'
<i>Has2</i>	F: 5'-AAGACCCTATGGTTGGAGGTGTT-3' R: 5'-CATTCCCAGAGGACCGCTTAT-3'
<i>Il6</i>	F: 5'-AGAGGATACCACTCCCAACAGA-3' R: 5'-ATCTCTCTGAAGACTCTGGCT-3'
<i>Il6r</i>	F: 5'-CACTCCTGGATAGCAGAGCC-3' R: 5'-GACACAGAGAAGCAACCCAAAC-3'
<i>Lhcgr</i>	F: 5'-CGGACCCTCCCAGATGTTTCG-3' R: 3'-GTGGCGATGAGCGTCTGAAGT-3'
<i>Ptgs2</i>	F: 5'-CCTTCTCCCGTAGCAGATG-3' R: 5'-ATGAACTCTCTCCGTAGAAGAACCCTT-3'
<i>p53</i>	F: 5'-GGAGTATTTGGACGCCG-3' R: 5'-TCAGTCTGAGTCAGGCC-3'
<i>Tnfai<math>\beta</math>6</i>	F: 5'-GATGGTCGTCCCTTTGCTT-3' R: 5'-TATCTGCCAGCCCGAGCTT-3'