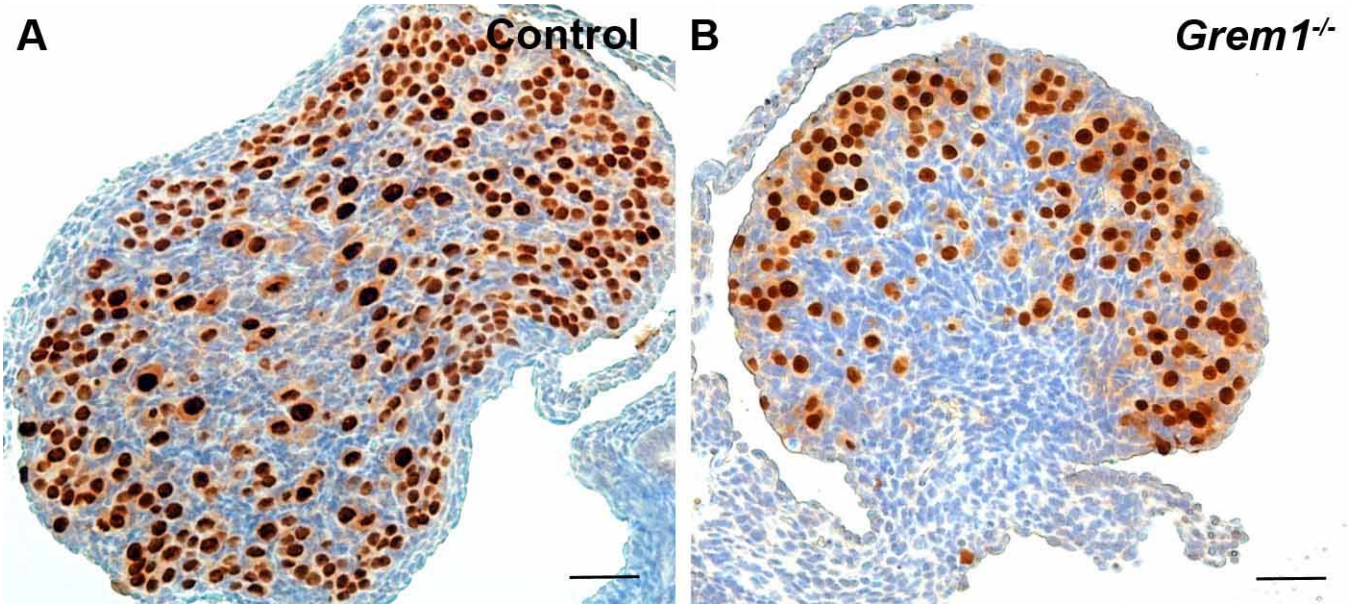
BOR-Papers in Press. Published as DOI: 10.1095/biolreprod.111.091728

Supplemental Data

Supplemental Figure S1



Supplemental Figure S1. NOBOX immunostaining in newborn control and *Grem1*$^{-/-}$ ovaries.

Newborn control (**A**) and *Grem1*$^{-/-}$ ovaries (**B**) immunostained with an antibody against NOBOX, an oocyte specific transcription factor highly expressed in nuclei of germ cell cysts and primordial follicles, as well as later stage oocytes [1]. NOBOX immunostaining (brown) was used to facilitate counting of germ cells (Text, Fig. 1C, D). Ovaries were counterstained with hematoxylin. Scale bar, 50 μm.

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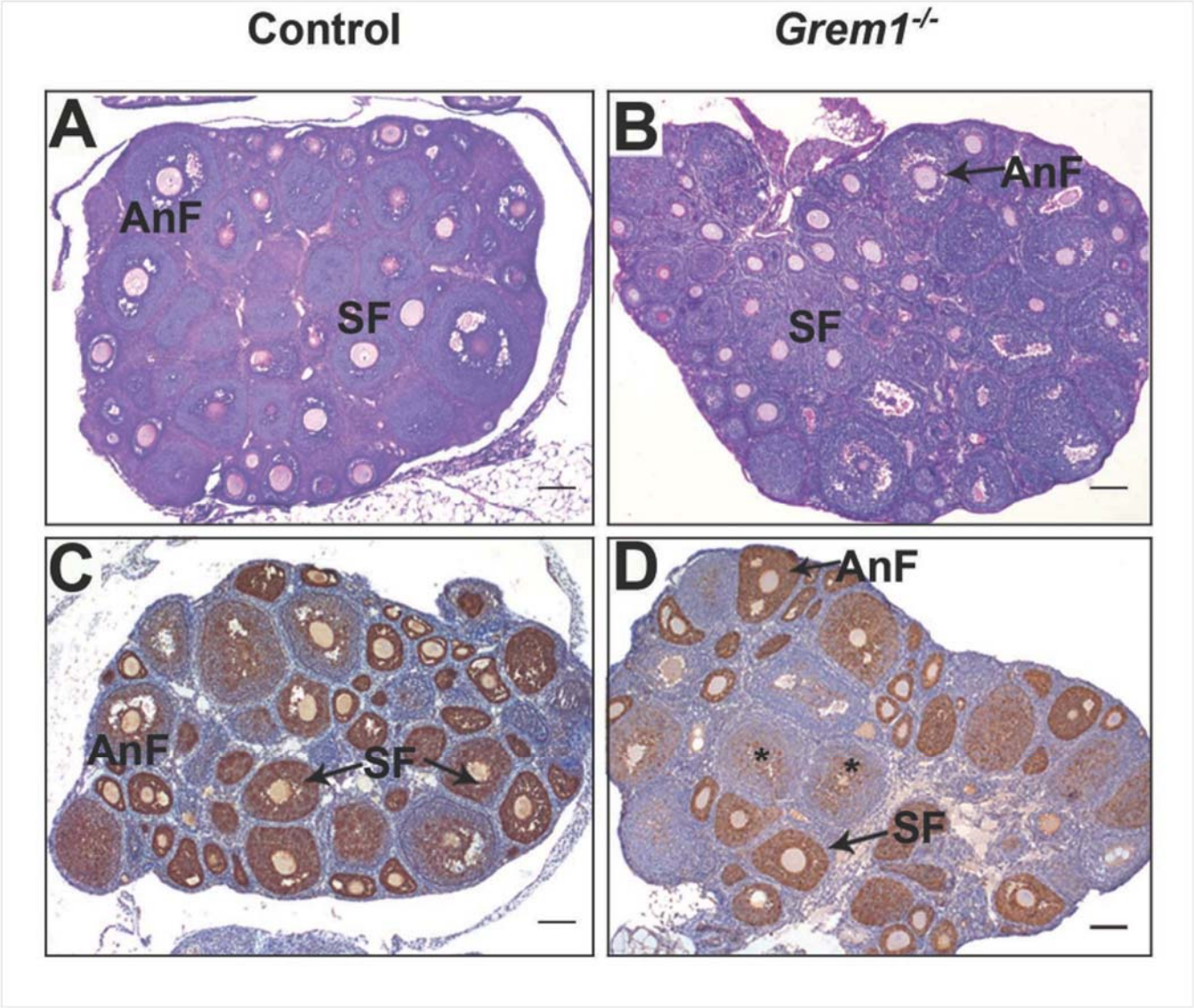
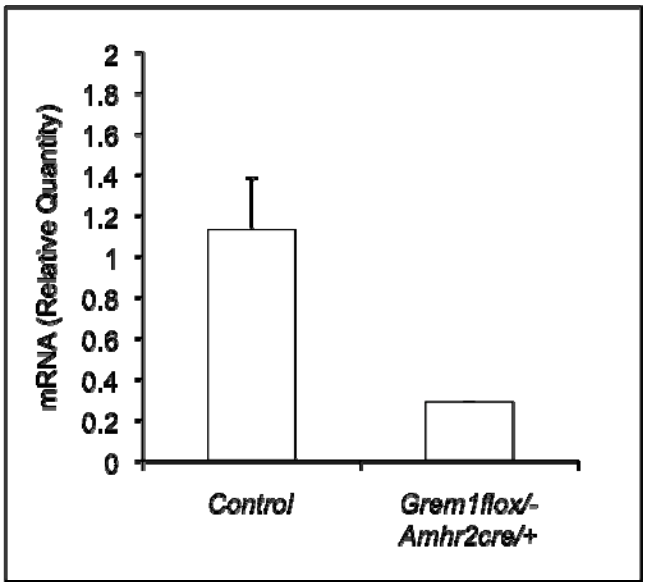
Supplemental Figure S2



Supplemental Figure S2: Gross histology reveals normal ovarian morphology in a 3-week old *Grem1*$^{-/-}$ postnatal ovary.

A, B. PAS and hematoxylin stained ovarian sections from a 3-week old control mouse (**A**) displays numerous secondary follicles (SF) and follicles with small antral cavities (AnF). Similar follicle types are also observed in an age-matched *Grem1*$^{-/-}$ ovary (**B**) from a mouse that survived postnatal lethality. **C.** Positive immunoreactivity for AMH in control ovaries is evident in secondary (SF) and small antral follicles (AnF), with loss of expression as the follicle matures and as follicles become atretic. **D.** Comparable staining patterns of AMH are also evident in the *Grem1*$^{-/-}$ ovary with positive immunoreactivity in secondary (SF) and small antral follicles (AnF). Follicles beginning to lose their AMH expression are also evident in the *Grem1*$^{-/-}$ ovary (*). Scale bars, 100μm.

Supplemental Figure S3



Supplemental Figure S3. Expression of *Grem1* in control and *Grem1* cKO granulosa cells. Granulosa cells were collected from superovulated (n=7) control (*i.e.*, mice that were heterozygous for *Grem1*: $Grem1^{flox/-}$ or $Grem1^{flox/+}$ $Amhr2^{cre/+}$) ovaries and cKO (n=2) ($Grem1^{flox/-}$ $Amhr2^{cre/+}$) ovaries and processed qPCR as indicated in the Materials and Methods. The level of *Grem1* transcript in the cKO granulosa cells is reduced 75% compared to the controls. The relative quantity was calculated using the ΔΔCT method with *Gapdh* as a control, and the mean CT value of the control ovaries was used as the "calibrator" sample [2].

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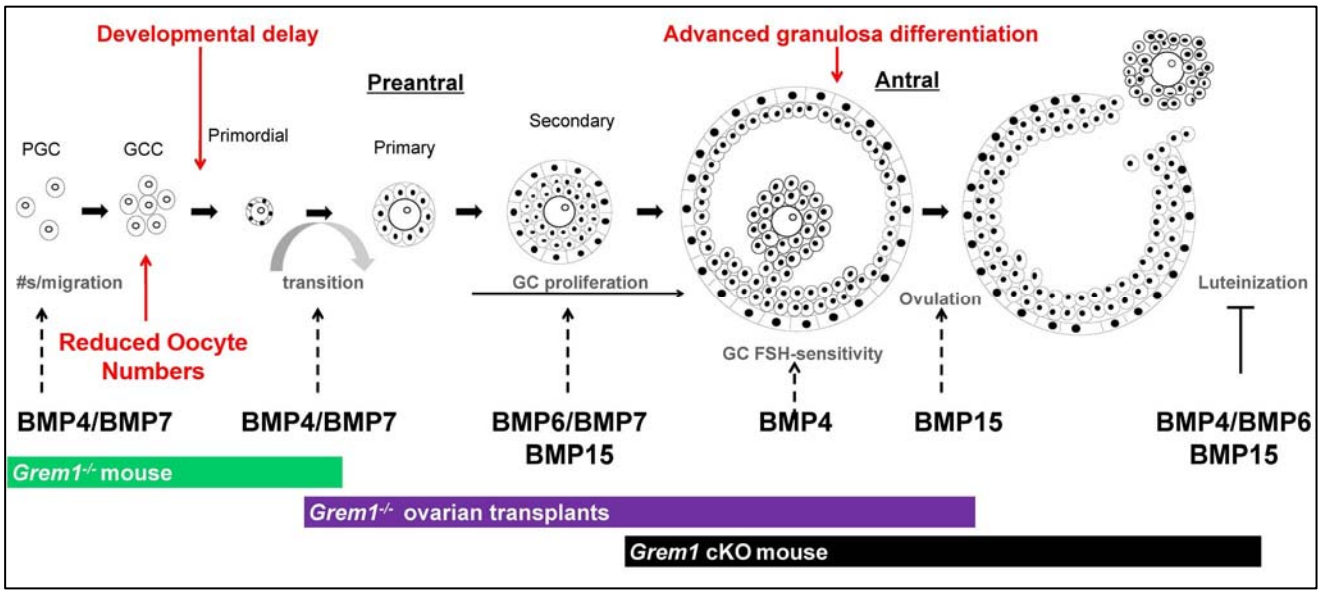
Supplemental Figure S4



Supplemental Figure S4: Genetic mouse models to study the role of gremlin in folliculogenesis

Three models were used to investigate stage specific roles of gremlin during follicular development. Putative BMP ligands involved in specific follicular processes are listed along the bottom. Left to right, primordial germ cells (PGCs) proliferate and colonize to the developing gonad where they differentiate into oogonia. In the late fetal and early neonatal mouse ovary germ cell cysts (GCC) undergo nest breakdown and surrounding pregranulosa cells are recruited to form primordial follicles. The primordial to primary transition marks the beginning of follicular growth, which in entails both oocyte growth and granulosa cell (GC) proliferation. As secondary follicles progress to the antral stage, granulosa cells become FSH-sensitive and can respond to the gonadotropin surge. Once the preovulatory follicle has ovulated, the resulting granulosa cells will luteinize and form the corpus luteum. In the absence of gremlin, our mouse models display reduced oocyte numbers at birth, delayed primordial follicle formation and alterations in the timing of granulosa cell differentiation (red font/arrows). Dashed black lines represent positive actions and solid black t-bar represents inhibition.

Supplemental Reference

1. Rajkovic A, Pangas SA, Ballow D, Suzumori N, Matzuk MM. NOBOX deficiency disrupts early folliculogenesis and oocyte-specific gene expression. Science 2004; 305: 1157-1159.
2. Pangas SA, Li X, Robertson EJ, Matzuk MM. Premature luteinization and cumulus cell defects in ovarian-specific Smad4 knockout mice. Mol Endocrinol 2006; 20: 1406-1422.