Fig. 7. Model of Foxo3 regulation by PI3K-Akt signaling pathway within oocytes in the control of primordial follicle activation. In resting state, absence of a presumptive ligand engaging receptor tyrosine kinase (RTK) complex or other cell surface receptor is associated with diminished Akt signalling in primordial oocytes. Foxo3 is unphosphorylated and thus localized to the nucleus, where Foxo3 acts to suppresses primordial follicle activation. Binding of presumptive ligand activates PI3K (consisting of p85 regulatory and p110 catalytic subunits), which phosphorylates the 3'OH group of PI[4,5]P2 (PIP2) to form PI[3,4,5]P3 (PIP3) at the oocyte membrane. Pten acts as a potent negative regulator of this pathway by dephosphorylating PIP3. Pten inactivation results in accumulation of PIP3 at the oocyte membrane and hyperactivation of Akt, resulting in Foxo3 phosphorylation and nuclear export, thereby triggering primordial follicle activation. In this model, Pten inactivation is functionally equivalent to the binding of presumptative ligand at a cell surface receptor.

**Supplementary information**

Fig. S1. Expression of Foxo1 and Foxo4 in wild-type adult ovary (6 weeks of age); tissue sections counterstained with hematoxylin. Foxo1 is strongly expressed in granulosa cells in large preantral follicles but not in oocytes or other ovarian compartments, whereas Foxo4 is not abundant in any ovarian compartment. Size bar = 100 μ.

Fig. S2. Validation of *Vasa-Cre*$^{ERT2}$ Cre deletor mouse line. (A) Northern analysis with *Cre* probe shows gonad-specific expression of *Vasa-Cre*$^{ERT2}$ transcript (2.1 kb). Membrane was reprobed with *GAPDH* (1.2 kb transcript) as loading control. (B-I), X-gal stained wholemounts and tissue sections from *Vasa-Cre*$^{ERT2}$; *R26R* male and female mice treated with tamoxifen for three days at 3 weeks of age. (*B*) Testes. Testis from untreated mouse (left) shows no Cre-mediated recombination, whereas testis from +tam

sibling (right) shows widespread recombination. This demonstrates that the $Cre^{ERT2}$ protein is maintained in an inactive state prior to tamoxifen administration. **(C)** Testis. Section from untreated mouse shows absence of Cre activity. (D) Testis. Section of +tam mouse shows widespread recombination in intratubular germ cells but not in somatic interstitial cells (arrow). (E) Ovaries. Untreated ovary (left) shows no Cre-mediated recombination, whereas ovary from +tam sibling (right) shows widespread recombination in primordial and growing oocytes. (F) Higher magnification of +tam ovary surface shows widespread recombination in primordial and growing oocytes. Primordial oocytes are excluded in areas occupied by larger follicles (asterisks). (G) Ovary. Section from untreated mouse shows absence of Cre activity. (H) Ovary. Section from +tam mouse shows Cre-mediated recombination in both primordial oocytes in plane of section and in secondary follicle oocyte. *Note*: For unknown reasons, β-galactosidase within oocytes becomes tightly associated within the Balbiani body (Gallardo et al., 2007; Pepling et al., 2007). (I) Ovary. Section from +tam mouse with cluster of primordial follicles in upper right shows high efficiency of recombination. Serial ovarian sections showed β-galactosidase activity in >90-95% of oocytes in *Vasa-Cre$^{ERT2}$*; *R26R* mice treated at 3 or 6 weeks of age.

**Supplemental References**

Gallardo, T., et al., 2007. Generation of a germ cell-specific mouse transgenic Cre line, Vasa-Cre. Genesis. 45**,** 413-7.

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