Supplemental Figure 1. Il7 and Tpx2 transcript stability during oocyte maturation

A. CEOs were cultured for 17 hrs in milrinone (GV) or in absence (IVM) or presence of FSH (IVM+FSH). At the end of the incubation oocytes were denuded, the RNA extracted and the cDNA used for qPCR. The bar graph represents the ΔΔCT (mean ± s.e.m.) of Il7 and Tpx2 normalized by Dppa3 and Rpl19 in the different treatments (N=3). No differences were observed (One-Way ANOVA).

B. CEOs were collected from Pten<sup>fl/fl</sup> and Pten<sup>fl/fl</sup>:Zp3cre mice, cultured for 17 hrs with (GV) or without milrinone (IVM). At the end of the incubation oocytes were denuded, the RNA extracted and the cDNA used for qPCR. The bar graph represents the ΔΔCT (mean ± s.e.m.) of Il7 and Tpx2 normalized by Dppa3 and Rpl19 in the different treatments (N=3). No differences were observed (One-Way ANOVA).
Supplemental Figure 2: Summary of the luciferase assay data in WT oocytes collected over a period of two and a half years.

cRNA was injected in oocytes still enclosed in the cumulus cells and cultured for 16 hrs in Milrinone (GV) or different IVM treatments (IVM, IVM+AREG, IVM+FSH). At the end of the incubation oocytes were denuded, homogenized and the luciferase activity measured in the oocyte extract. The graphs represent the ratio between the renilla luciferase activity, translated under the control of the Tpx2 3'UTR (A-B-C), II7 3'UTR (D-E-F), and the firefly luciferase activity used for injection normalization. The same data are presented with different type of plots.

A-D. Bar graphs showing the mean ± s.e.m. * and ** indicate significant differences (P<0.05 and P<0.01, respectively) in the luciferase activity between IVM+AREG and IVM+FSH compared to IVM (One-Way ANOVA followed by Tukey’s multiple comparison test).

B-E. Scatter plots showing the data distribution and the median. * and ** indicate significant differences (P<0.05 and P<0.01, respectively) in the luciferase activity between IVM+AREG and IVM+FSH compared to IVM (One-Way ANOVA followed by Tukey’s multiple comparison test).

C-F. Paired scatter plots showing IVM and IVM+FSH pairing. * and ** indicate significant differences (P<0.05 and P<0.01, respectively) in the luciferase activity (paired t-test).
Supplemental Figure 3. Time course of AKT activation in the oocyte
CEOs were cultured for 1.5-2.5-4 hrs in different IVM treatments (IVM, IVM+AREG, IVM+FSH). At the end of the incubation oocytes were denuded, homogenized and extracts used for Western blot. Representative immunoblots of phosphorylated (p-AKT) and total AKT (AKT) are reported. The line graph represents the ratio p-AKT/AKT (mean ± SEM) in the different time/treatment (N=5). * indicates significant differences (P<0.05) in AKT phosphorylation at 2.5 hrs between IVM+AREG and IVM+FSH compared to IVM (Kruskal-Wallis test followed by Dunn’s multiple comparison test).
Supplemental Figure 4. Tpx2 reporter translation and AKT activation in Areg^{−/−} oocytes

A. CEOs were collected from wild type or Areg^{−/−}. Oocytes still enclosed in the cumulus cells were injected with Tpx2 reporter and cultured for 16 hrs in milrinone (GV) or in different IVM conditions (IVM, IVM+AREG, IVM+FSH). At the end of the incubation oocytes were denuded, homogenized and the luciferase activity measured in the oocyte extract. The bar graph represents the ratio (mean ± s.e.m.) between the renilla luciferase activity, translated under the control of the Tpx2 3'UTR and the firefly luciferase activity used for injection normalization (N=3). *** and ** indicates significant differences (P<0.001 and P<0.01 ) in the luciferase activity between IVM+AREG and IVM+FSH compared to IVM both in wild type mice and Areg^{−/−} mice (Two-Way ANOVA followed by Tukey’s multiple comparison test).

B. CEOs were collected from wild type or Areg^{−/−} mice and cultured for 2.5 hrs in different IVM conditions (IVM, IVM+AREG, IVM+FSH). At the end of the incubation oocytes were denuded, homogenized and extracts used for Western blots. Representative immunoblots of phosphorylated (p-AKT) and total AKT (AKT) are reported. The bar graph represents the ratio p-AKT/AKT (mean ± s.e.m.) in the different treatment/genotype (N=3). No differences were observed in between IVM+AREG and IVM+FSH in both genotypes (Kruskal-Wallis test followed by Dunn’s multiple comparison test).
Supplemental Figure 5. The constitutive AKT activation does not affect oocyte growth in vivo and the dynamic of meiotic resumption in vitro

A. CEOs were collected from Pten<sup>ffe</sup> (n=40) and Pten<sup>ffe</sup>:Zp3cre mice (n=42), denuded and individually imaged under an inverted microscope. The oocyte diameter was measured using ImageJ. The bar graph represents the diameter (mean ± SEM) in the two genotypes. No differences were observed (P>0.05, unpaired t-test).

B. CEOs were collected from Pten<sup>ffe</sup> and Pten<sup>ffe</sup>:Zp3cre mice, denuded and cultured for 90 mins. The oocytes were imaged every 15 mins and classified according to the presence or absence of the GV (GVBD). The line graph represents the percentage (mean ± SEM) of oocytes undergoing GVBD in the different time/genotype (N=3). No differences were observed (P>0.05, unpaired t-test).