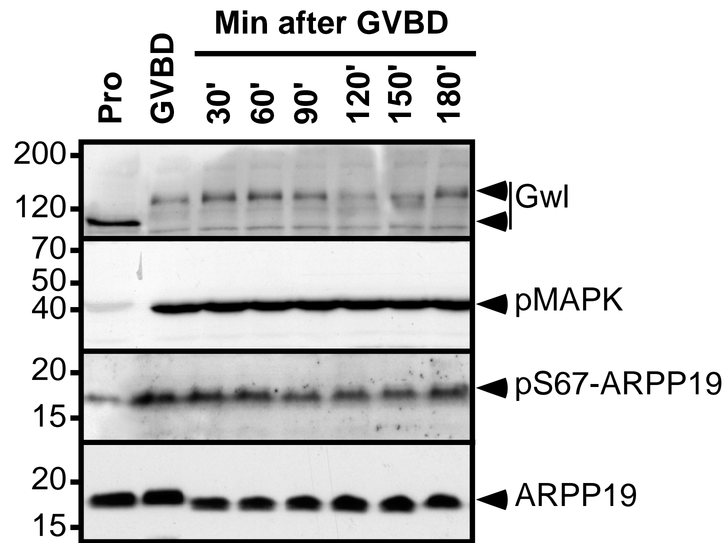

Fig. S1. ARPP19 phosphorylation at S67 after GVBD.

Oocytes (Pro) treated with progesterone were collected at GVBD or at the indicated times following GVBD, then analyzed by western blot for Greatwall (Gwl), phosphorylated MAPK (pMAPK), S67-phosphorylated ARPP.

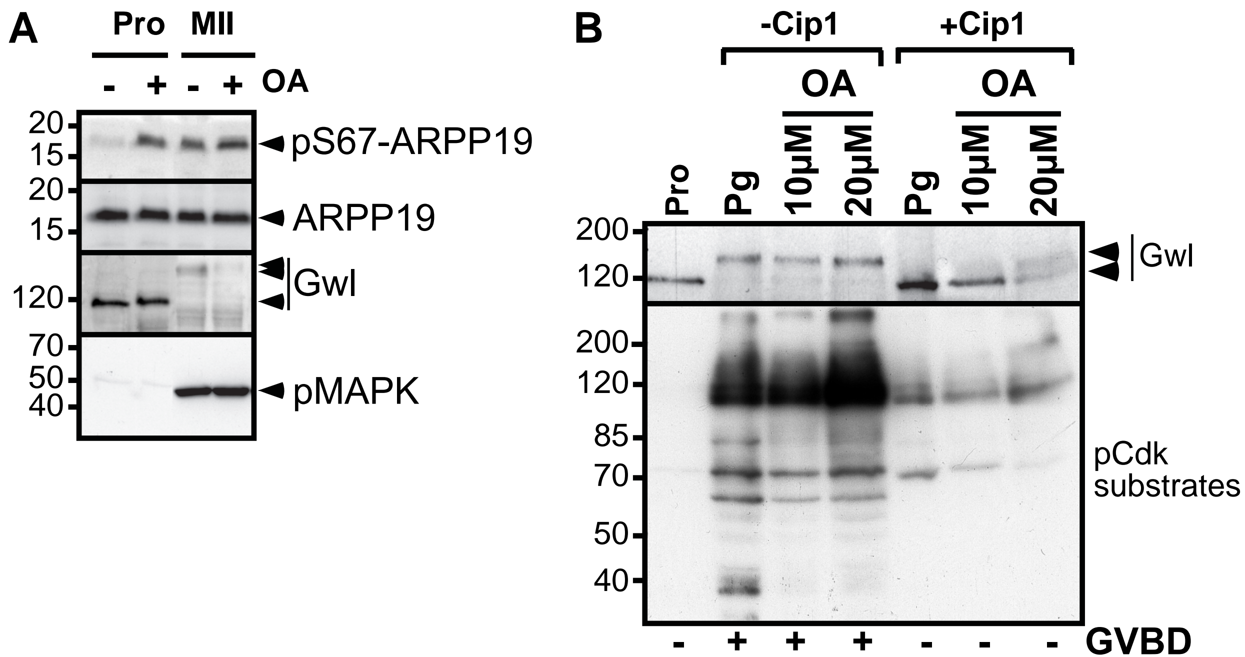

Fig. S2. Phosphorylations of Gwl and ARPP19 are sensitive to an okadaic acid-sensitive phosphatase in prophase-arrested oocytes.

In order to set up the best conditions to preserve ARPP19 phosphorylation level at S67 during oocyte homogenization, prophase and MII oocytes were lyzed in the presence or in the absence of okadaic acid (OA), a phosphatase inhibitor with high affinity towards the main phosphatase activities, PP1 and PP2A (Fig. S2A). Although MAPK and Gwl phosphorylation were insensitive to OA, phosphorylation of ARPP19 et S67 was strongly increased in prophase lysates containing OA (Fig. S2A). This effect was not reproduced in lysates from MII oocytes. OA is known to indirectly promote the activation of many kinases, including Cdk1. As shown in Fig. S2B, injection of OA in prophase oocytes induced the activation of Cdk1 (as judged by the level of Cdk1 substrates) and Gwl . When Cdk1 activation was blocked by p21^{Cip1} injection, 20 μM OA still promoted a partial phosphorylation of Gwl despite the absence of Cdk1 activity (Fig. S2B). Therefore, both S67 phosphorylation level of ARPP19 and Gwl activity are under the control of an OA-sensitive phosphatase in prophase oocyte extracts.

(A) S67 phosphorylation of ARPP19 is controlled by an okadaic acid-sensitive protein phosphatase. Prophase-arrested oocytes (Pro) and metaphase II-arrested oocytes (MII) were homogenized in the presence (+) or in the absence (-) of 1 μM okadaic acid, then immunoblotted using antibodies directed against S67-phosphorylated ARPP19 (pS67-ARPP19), ARPP19, Gwl and phosphorylated MAPK (pMAPK).

(B) Okadaic acid injection leads to a partial phosphorylation of Gwl in the absence of Cdk1 activation. Prophase-arrested oocytes (Pro) were injected or not with the Cdk1 inhibitor, p21^{Cip1} (Cip1), then treated by progesterone (Pg) or injected with 10 or 20 μM okadaic acid (OA). Oocytes were collected at GVBD and lysates were western blotted for either Gwl or phosphorylated-Cdk substrates (pCdk susbtrates).

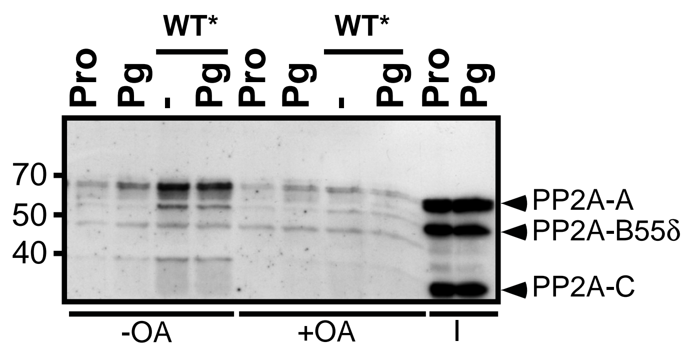

Fig. S3: Association of S67-thiophosphorylated ARPP19 with PP2A-B55δ is disrupted by OA. Prophase oocytes (Pro) were injected or not with S67-thiophosphorylated WT-GST-ARPP (WT*) then treated or not with progesterone (Pg). Oocytes were collected at time of GVBD in the presence (+OA) or in the absence (-OA) of 1 μM okadaic acid. S67-thiophosphorylated WT-GST-ARPP was then pulled down and its binding to PP2A was analyzed by western blot using antibodies against PP2A-A, PP2A-C and PP2A-B55δ. Extracts from oocytes (containing OA) before the pull-down were also immunoblotted (I).

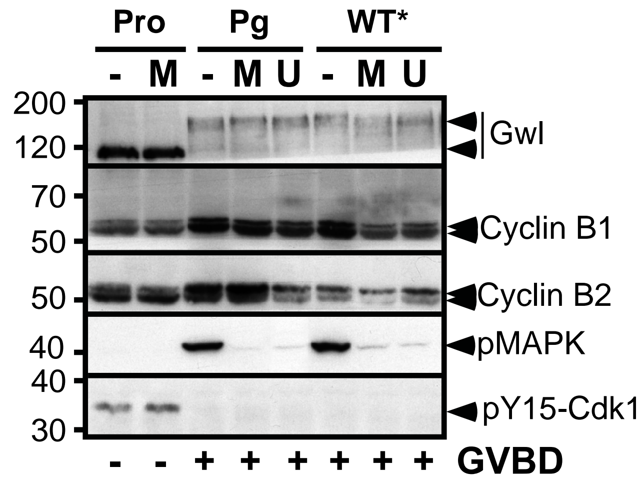

Fig. S4. Cdk1 activation induced by S67-thiophosphorylated ARPP19 occurs independently of the Mos/MAPK pathway.

Prophase-arrested oocytes (Pro) were injected with antisense morpholinos directed against Mos mRNA (M) or incubated in the presence of U0126 (U). Oocytes were then either treated with progesterone (Pg) or injected with S67-thiophosphorylated WT-GST-ARPP (WT*). Oocyte were collected at time of GVBD and lysates were analyzed by western blot with antibodies directed against Gwl, Cyclin B1, Cyclin B2, phosphorylated MAPK (pMAPK) and Y15-phosphorylated Cdk1 (pY15-Cdk1).

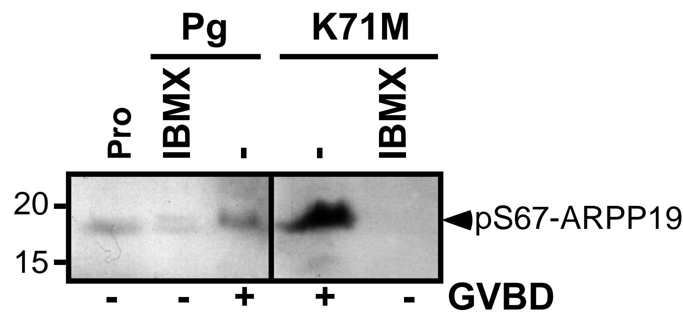

Fig. S5. S67 phosphorylation of endogenous ARPP19 in response to progesterone or K71M-Gwl overexpresssion.

Prophase oocytes (Pro) were incubated or not with IBMX. 16 hours later, oocytes were induced to mature either by adding progesterone (Pg) or by injecting K71M-Gwl mRNA (K71M). Oocytes were collected at time of GVBD and analyzed by western blot for S67-phosphorylated ARPP19 (pS67-ARPP19). The phosphorylation level of endogenous ARPP19 by endogenous Gwl activated in response to progesterone is lower than the phosphorylation achieved by overexpressed K71M-Gwl.

To visualize both phosphorylation states, the western blot membrane was cut in two parts: the one corresponding to progesterone treatment was exposed for a long period (left panel), the other one corresponding to K71M-Gwl overexpression was exposed for a shorter period (right panel).

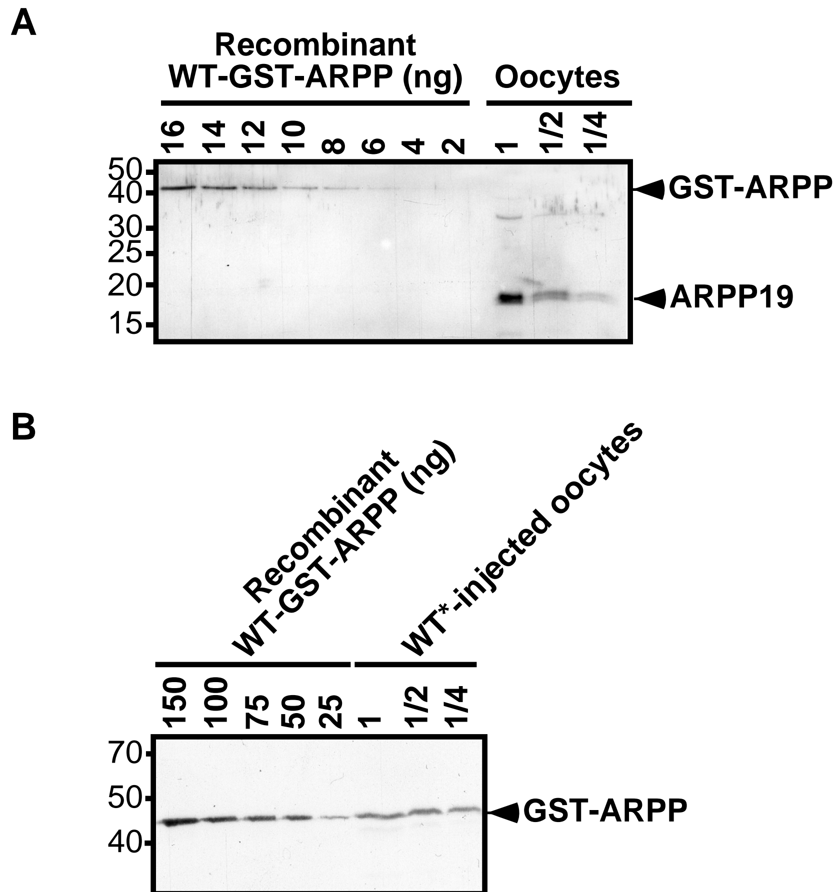

Fig. S6. Intracellular concentrations of endogenous ARPP19 and of injected S67-thiophosphorylated ARPP19.

(A) Recombinant WT-GST-ARPP (16, 14 12, 10, 8, 6, 4 and 2 ng per lane) and prophase oocyte lysates (1, 1/2 or 1/4 oocyte equivalent) were western blotted using the ARPP19 antibody that recognizes the recombinant protein (GST-ARPP) and the endogenous one (ARPP19). After quantification of the signals by the ImageJ software, the oocyte concentration of endogenous ARPP19 is estimated to be 2.3 μM.

(B) Recombinant WT-GST-ARPP (150, 100, 75, 50 and 25 ng per lane) and lysates of S67-thiophosphorylated WT-GST-ARPP (WT*) injected oocytes (1, 1/2 or 1/4 oocyte equivalent) were western blotted using an anti-GST antibody (GST-ARPP). After quantification of the signals by the ImageJ software, the oocyte concentration of the injected protein was estimated to be 3 μM.