Supplemental Figures

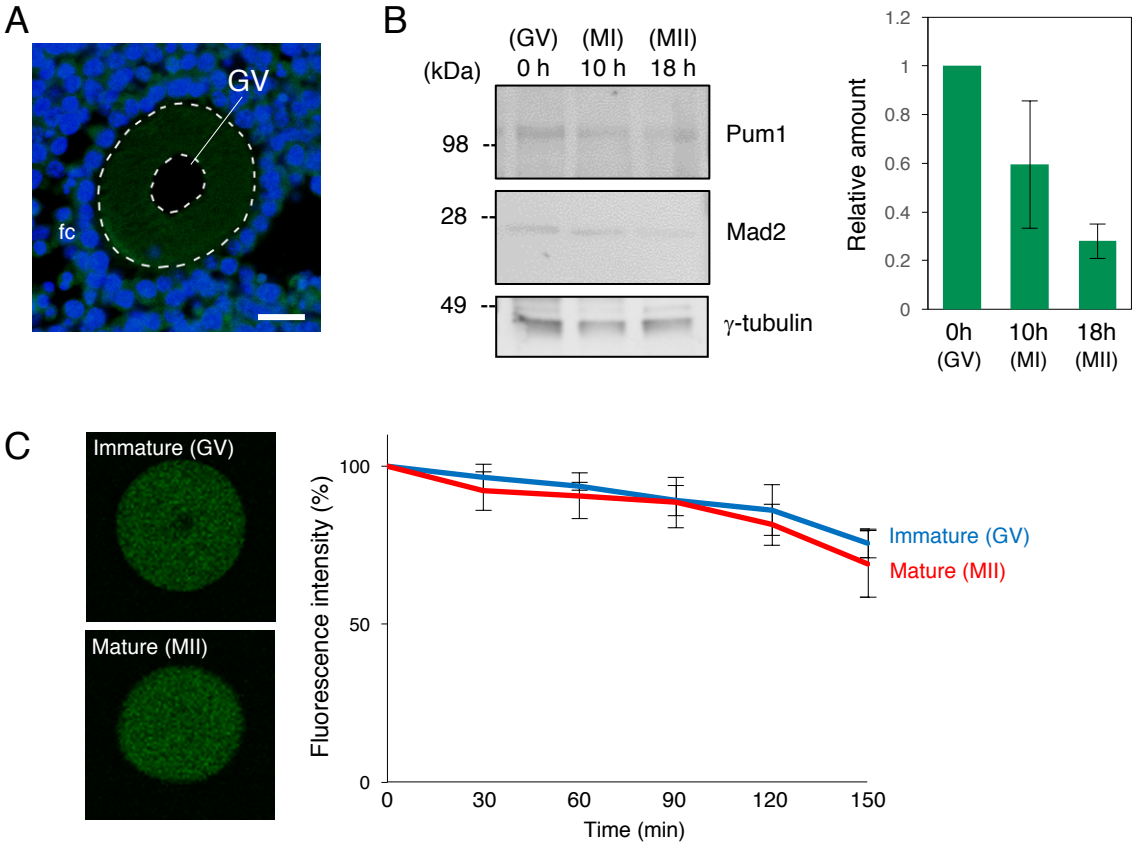

Fig. S1. Expression of *Mad2* mRNA and Mad2 protein. (A) FISH analysis of *Mad2* mRNA (green). DNA is shown in blue. No signal was detected by using the antisense RNA probe for long *Mad2*. (B) Effect of puromycin on Mad2 protein accumulation. (left) Immunoblotting of Pum1, Mad2 and γ-tubulin in oocytes incubated with puromycin at 0, 10, and 18 h after resumption of meiosis. (right) Quantitative analysis of Mad2 protein (mean ± SD; n = 2). The intensities of Mad2 were normalized by that of γ-tubulin. (C) Time course analysis of GFP-Mad2 fluorescence after treated with puromycin (mean ± SD; n = 3). GV, germinal vesicle; fc, follicle cells. Bars: 20 μm.

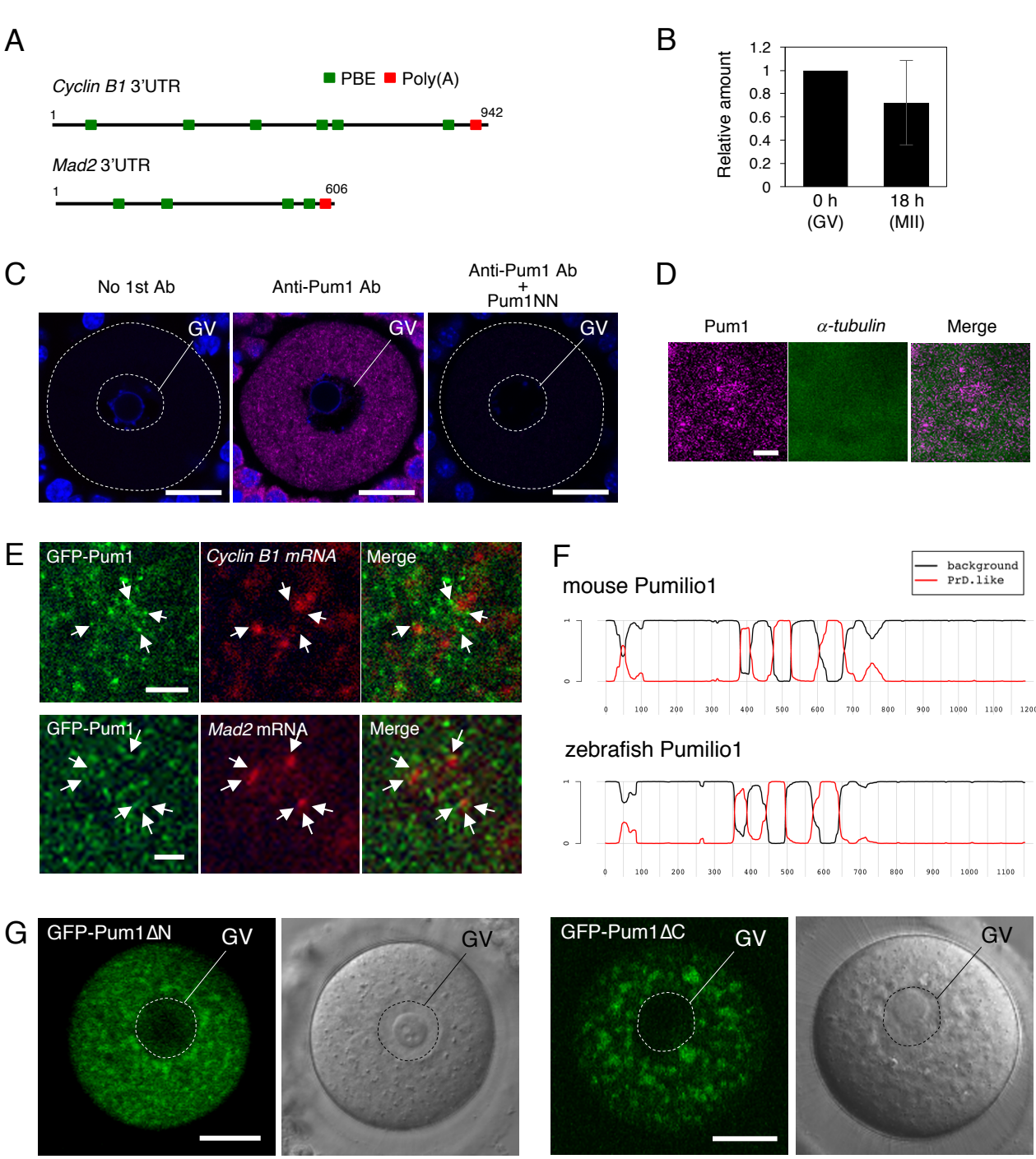

Fig. S2. Distribution of Pum1, GFP-Pum1 and mRNAs. (A) Schematic diagrams of mouse *Cyclin B1* and *Mad2* 3'UTRs. Green rectangles indicate putative Pumilio-binding elements (PBEs), and red rectangles indicate the poly(A) signal. (B) Quantitative PCR for *Mad2* mRNA in oocytes at 0 and 18 h after resumption of meiosis (mean ± SD; n = 3). (C) Immunofluorescence of Pum1 (magenta) in immature oocytes. DNA is shown in blue. Mouse ovary sections were reacted with buffer only (No 1st Ab), anti-Pum1 antibody (Anti-Pum1 Ab), or anti-Pum1 antibody incubated with recombinant Pum1 (Anti-Pum1 Ab + Pum1NN). (D) FISH analysis of *α-tubulin* mRNA (green) and immunostaining of Pum1 (magenta) in immature oocytes. (E) FISH analysis of *Cyclin B1* (top) and *Mad2* mRNA (bottom) and immunostaining of GFP in oocytes expressing GFP-Pum1. Arrows indicate aggregates of GFP-Pum1 surrounding *Cyclin B1* or *Mad2* RNA granules. Similar results were obtained from two independent experiments. (F) Identification of prion-like domains by using the PLAAC web application (http://plaac.wi.mit.edu/.). (G) Distribution of GFP-Pum1 ΔN and GFP-Pum1 ΔC in immature oocytes. Similar results were obtained from six independent experiments. GV, germinal vesicle. Bar: 20 μm in C and G, 2 μm in D and E.

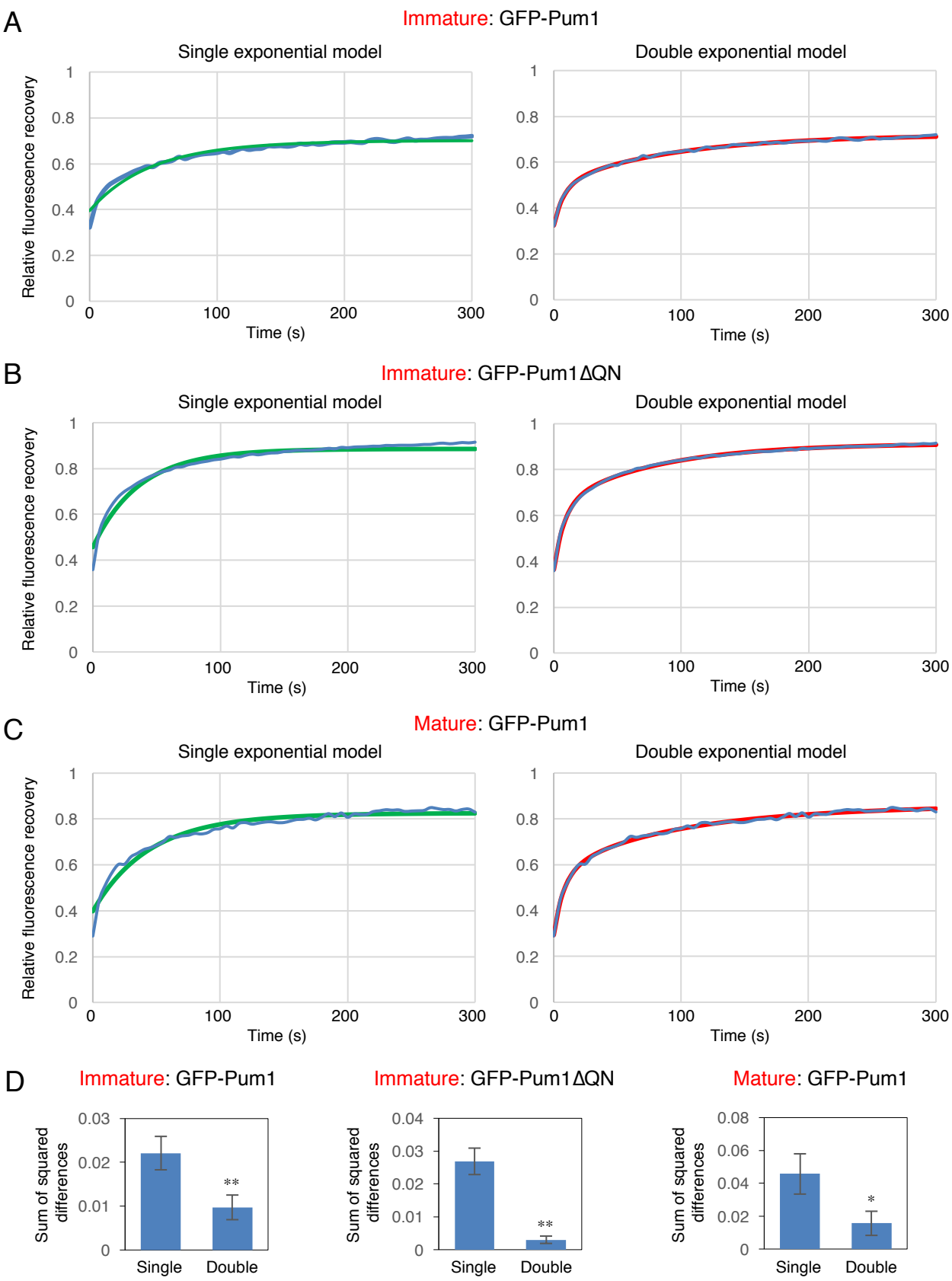

Fig. S3. FRAP analysis of GFP-Pum1 and GFP-Pum1ΔQN. (A) The average of fluorescence recovery curves (blue) of GFP-Pum1 in immature oocytes with the fittings to single (left, green) or double (right, magenta) exponential model. (B) The average of fluorescence recovery curves (blue) of GFP-Pum1ΔQN in immature oocytes with the fittings to single (left, green) or double (right, magenta) exponential model. (C) The average of fluorescence recovery curves (blue) of GFP-Pum1 in mature oocytes with the fittings to single (left, green) or double (right, magenta) exponential model. (D) Sum of squared differences between the fluorescence recovery curves and the single (Single) or double (Double) exponential model (mean ± SD). *t*-test: $*P < 0.05$, $**P < 0.01$.

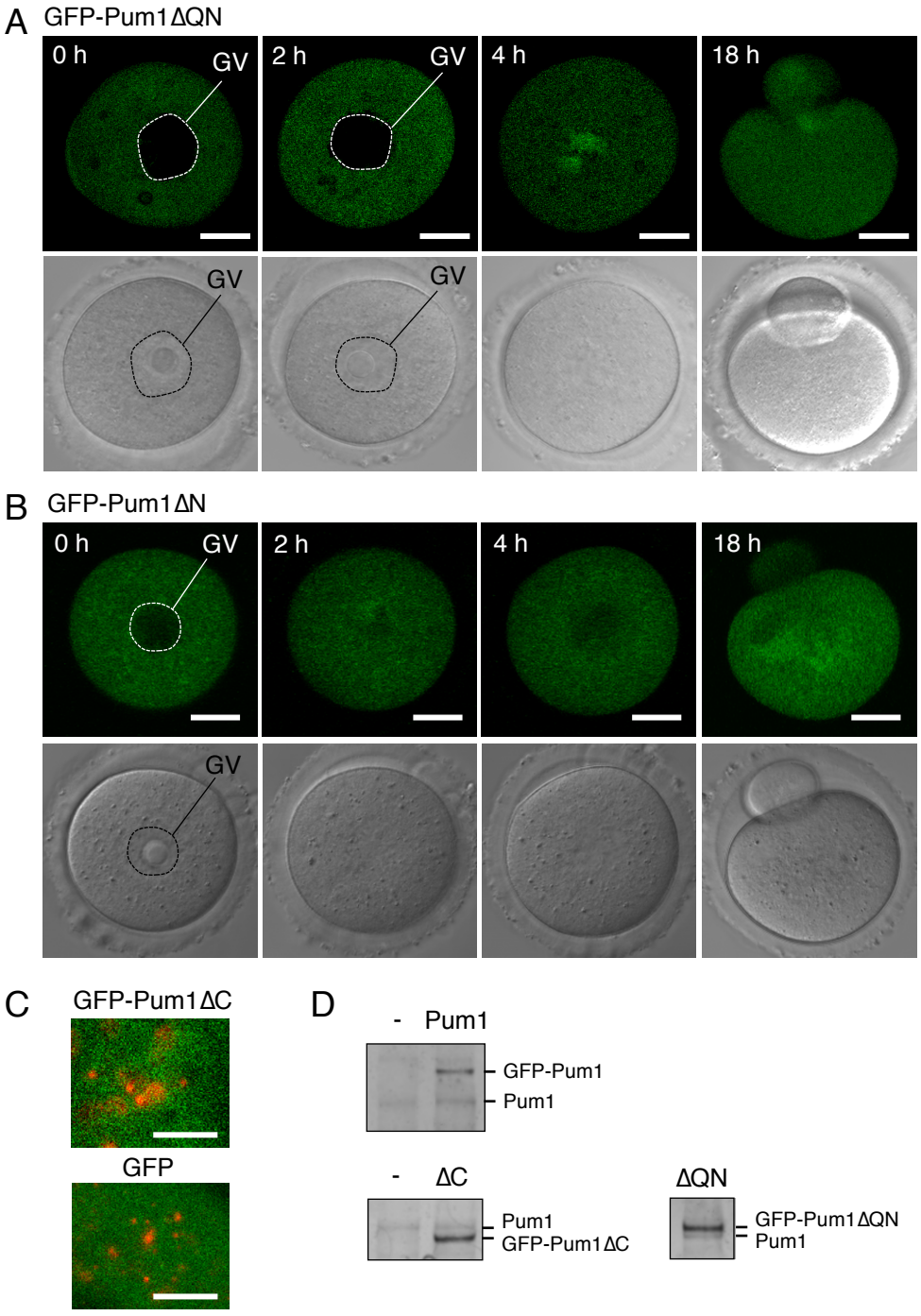

Fig. S4. Time course of GFP-Pum1ΔQN and GFP-Pum1ΔN during oocyte maturation. (A) Time course of GFP-Pum1ΔQN at 0, 2, 4 and 18 h after resumption of meiosis. (B) Time course of GFP-Pum1ΔN at 0, 2, 4 and 18 h after resumption of meiosis. Similar results were obtained from two independent experiments. (C) FISH analysis of *Cyclin B1* (red) mRNA and immunostaining of GFP-Pum1ΔC or GFP (green) in immature oocytes. (D) Immunoblotting of Pum1 in oocytes not injected (-) and injected with GFP-Pum1 (Pum1), GFP-Pum1ΔC (ΔC) and GFP-Pum1ΔQN (ΔQN). GFP-Pum1ΔN is unable to be detected since the anti-Pum1 antibody recognizes the region delated in this mutant Pum1. Bars: 20 μm in A and B, 5 μm in C.

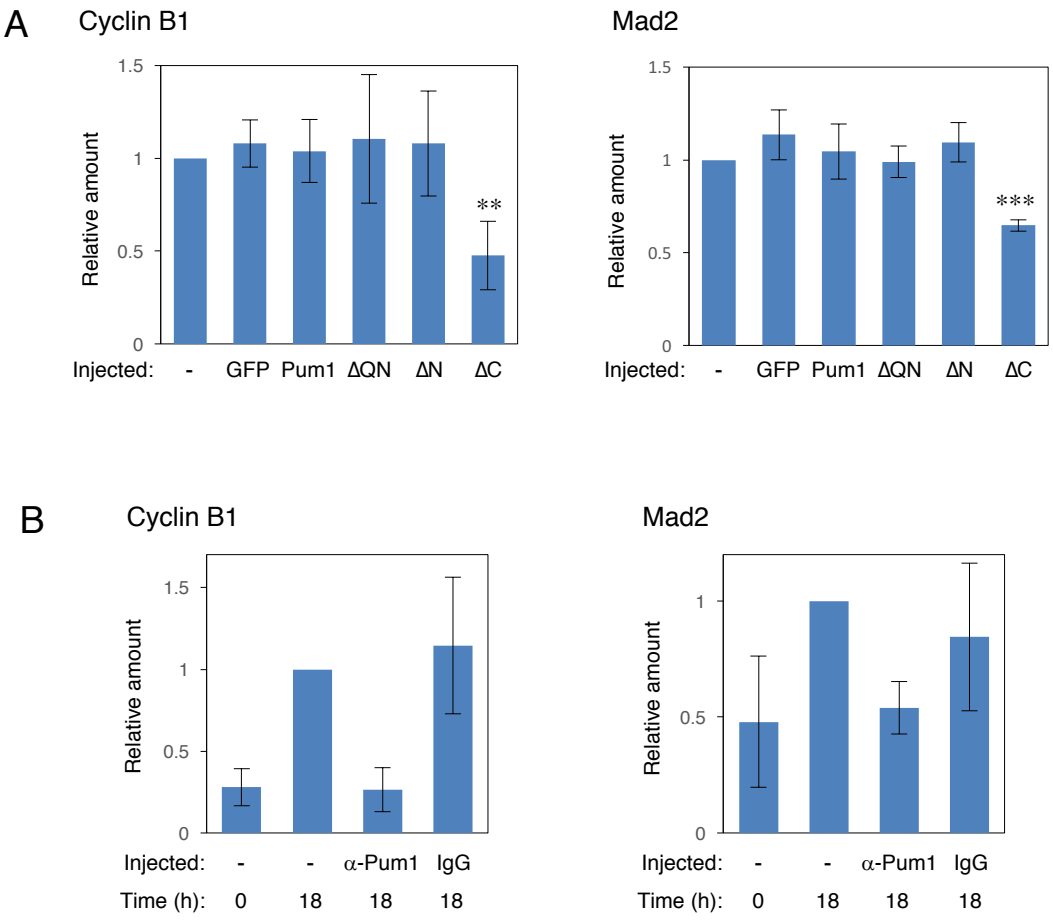

Fig. S5. Quantitative analyses of Cyclin B1 and Mad2 proteins. (A) Quantitative analysis of Cyclin B1 and Mad2 in oocytes not injected (-) and injected with GFP, GFP-Pum1 (Pum1), GFP-Pum1ΔQN (ΔQN), GFP-Pum1ΔN (ΔN), and GFP-Pum1ΔC (ΔC) (mean ± SD; n = 3). *t*-test: $^{**}P < 0.01$, $^{***}P < 0.001$. (B) Quantitative analysis of Cyclin B1 and Mad2 in oocytes not injected (-) and injected with anti-Pum1 antibody (α-Pum1) or control IgG (IgG) (mean ± SD; n = 2).

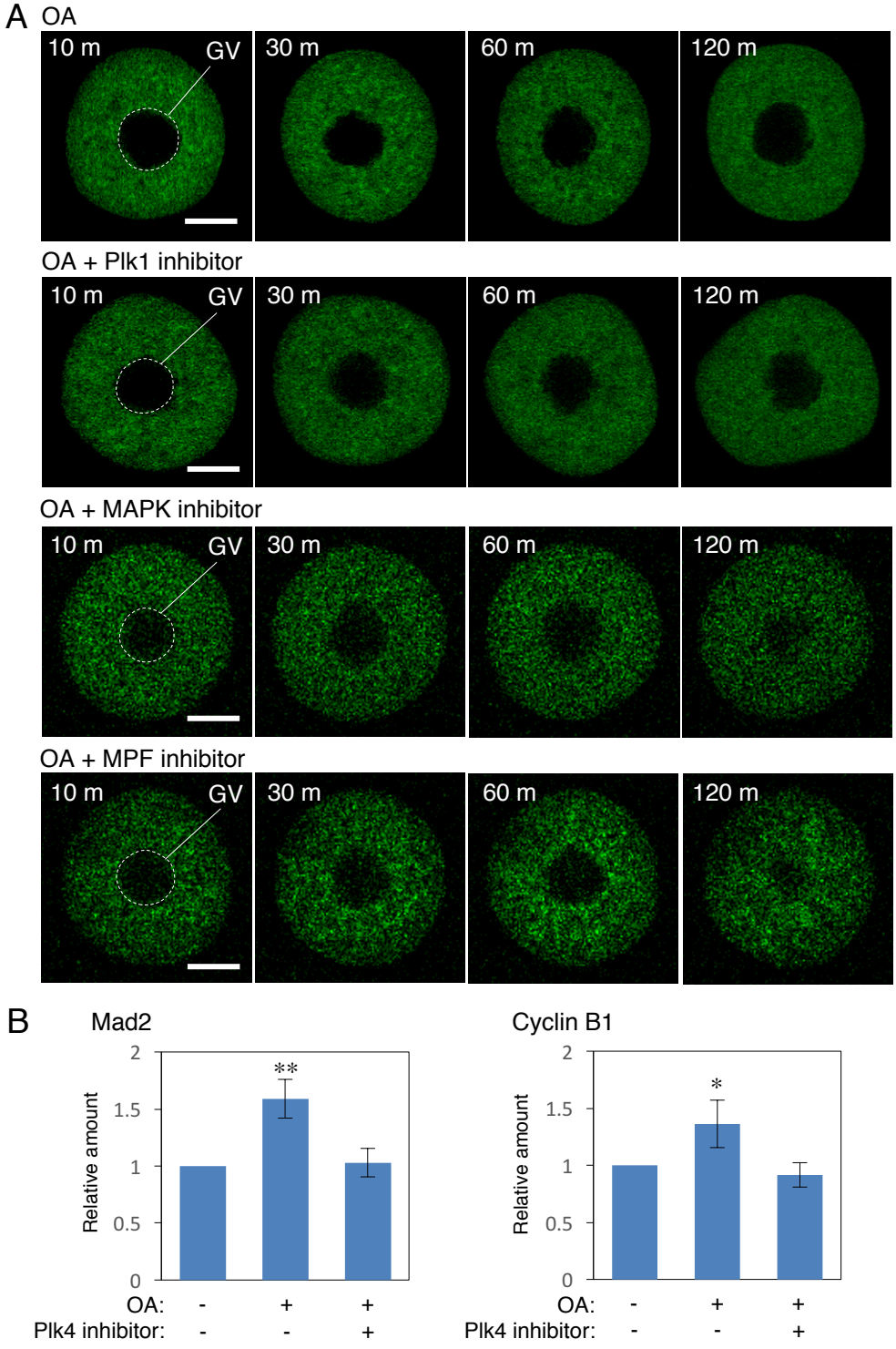

Fig. S6. Time course of GFP-Pum1 after OA treatment. (A) Time course of GFP-Pum1 in oocytes treated with OA, OA and Plk1 inhibitor, OA and MAPK inhibitor, or OA and MPF inhibitor 0-120 min after treatment. Similar results were obtained in 6 oocytes from two independent experiments. GV, germinal vesicle. Bars: 20 μm. (B) Quantitative analysis of Mad2 and Cyclin B1 in oocytes treated with (+) and without (-) OA or Plk4 inhibitor 120 min after resumption of meiosis (mean ± SD; n = 3). *t*-test: *$P < 0.05$, **$P < 0.01$.