

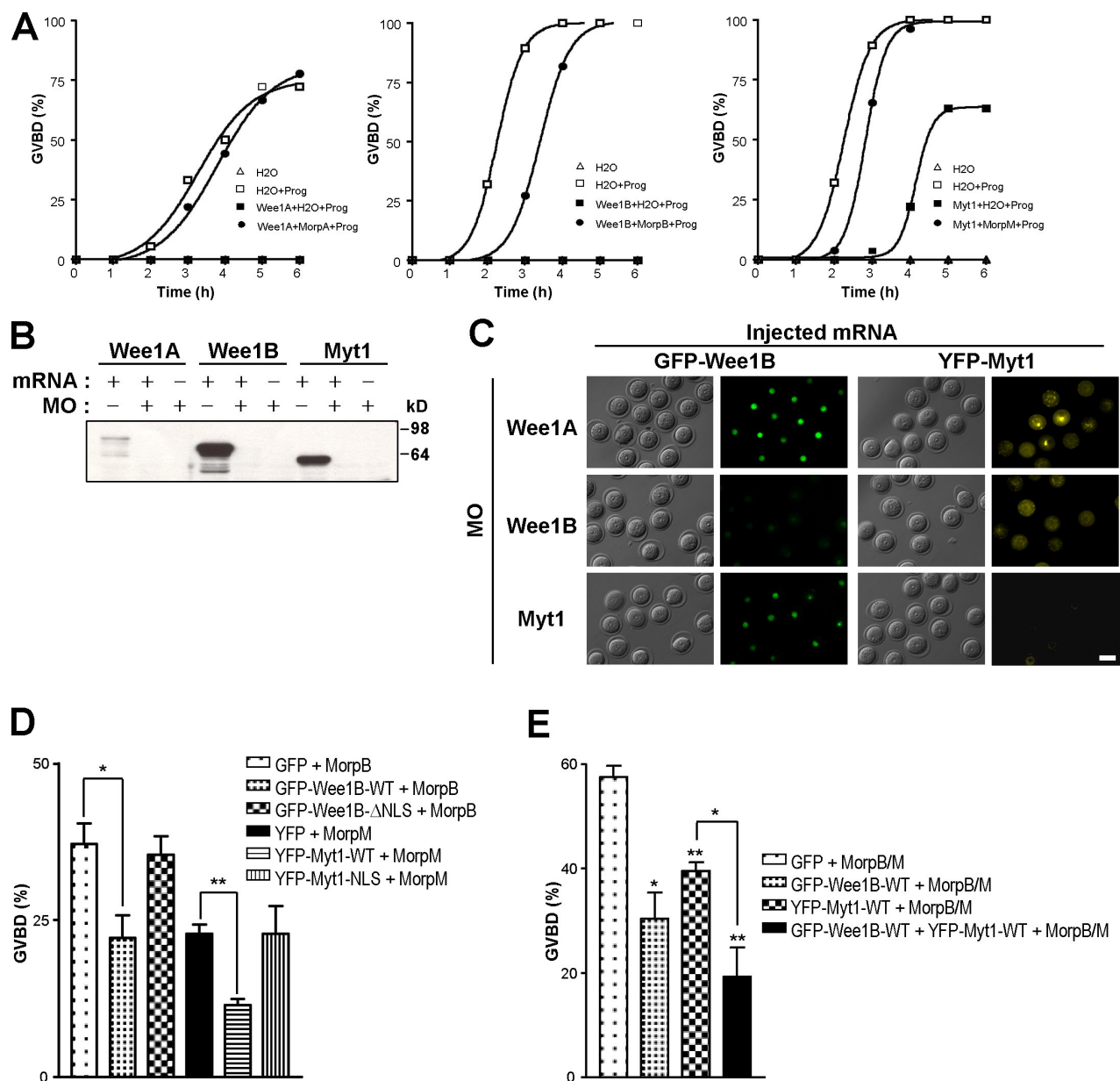
Oh et al., <http://www.jcb.org/cgi/content/full/jcb.200907161/DC1>

Figure S1. **Specificity and efficacy of MOs.** (A and B) Specific amounts of indicated mRNAs (for Wee1A, 0.4 ng mRNA; for Wee1B and Myt1, 10 ng mRNA) were injected with corresponding MOs (for Wee1A, MorpA; for Wee1B, MorpB; for Myt1, MorpM) in stage IV *Xenopus* oocytes. After a 16-h incubation, oocytes were collected for immunoblot analysis (B) or treated with 500 nM progesterone (Prog) to induce oocyte maturation (A). The percentage of GVBD was determined at different times after the addition of progesterone. Immunoblot analysis was performed with V5 antibodies. (C) GFP-Wee1B or YFP-Myt1 mRNA was microinjected into GV-stage oocytes with Wee1A, Wee1B, or Myt1 MO. The intensity of the fluorescent signal was quantified after a 20-h culture in 5 μ M cilostamide-supplemented medium. (D and E) Indicated mRNAs and MOs were microinjected into GV-stage oocytes, and GVBD was monitored after a 22-h culture in 1 μ M cilostamide-supplemented medium. Error bars represent the mean \pm SEM of three independent experiments. *, $P < 0.05$; and **, $P < 0.005$. Bar, 60 μ m.

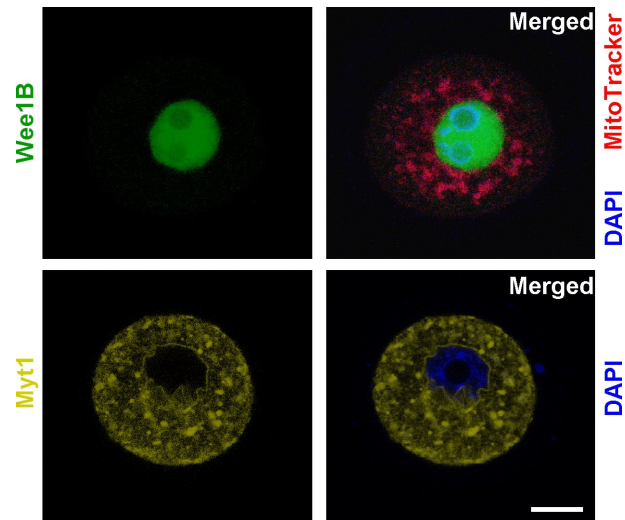


Figure S2. **Subcellular localization of Wee1B and Myt1.** GFP-Wee1B or YFP-Myt1 mRNA was injected into the GV oocytes, and a confocal microscope was used to determine their localization. DAPI and MitoTracker (Invitrogen) were used to counterstain DNA present in the nucleus and mitochondria, respectively. Bar, 20 μ m.

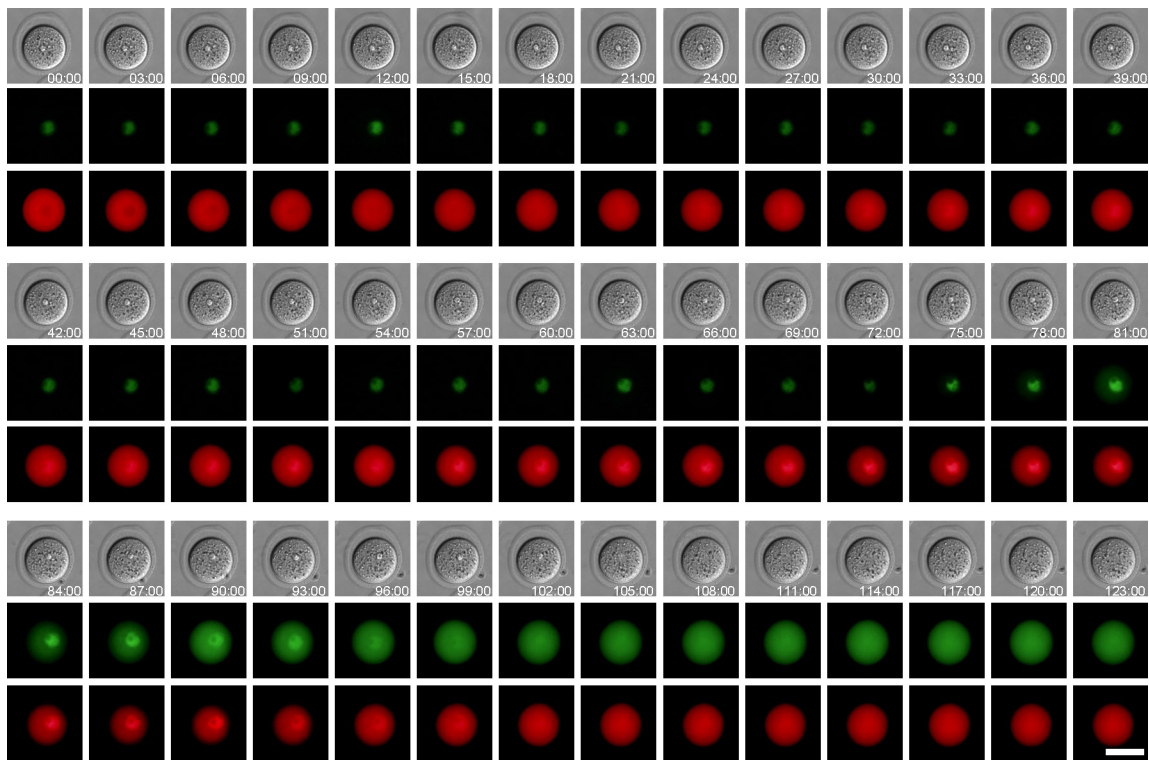
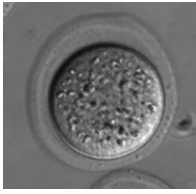


Figure S3. **Time course of nucleocytoplasmic shuttling of Wee1B and Cdc25B.** GV oocytes were microinjected with catalytically inactive GFP-Wee1B and RFP-Cdc25B mRNAs, and the time-lapse images of Wee1B (middle) and Cdc25B (bottom) are reported together with phase-contrast images (top) during meiotic resumption. Time 0.00 coincides with the removal of the phosphodiesterase inhibitor from the medium. Note that transfer of CDC25 to the nucleus is first detected at 12 min and accumulation continues to increase up to 90 min. Wee1B translocation to the cytoplasm is first detectable at 81–84 min of incubation, 22–24 min before GVBD, which in this oocyte occurs at 105–108 min. The data reported are representative of time-lapse microscopy of at least five different oocytes. This figure corresponds to Videos 1–3. Bar, 60 μ m.



Video 1. **Time-lapse image of RFP-Cdc25B- and GFP-Wee1B-coinjected oocytes during meiotic resumption.** GV-stage murine oocytes were microinjected with catalytically inactive GFP-Wee1B and RFP-Cdc25B mRNAs, and the time-lapse images of GFP-Wee1B and RFP-Cdc25B during meiotic resumption are shown. Time 0.00 coincides with the removal of the phosphodiesterase inhibitor from the medium. Frames were taken every minute for 120 min. The video is shown at 10 frames/s.



Video 2. **Time-lapse image of RFP-Cdc25B during meiotic resumption.** GV-stage murine oocytes were microinjected with catalytically inactive RFP-Cdc25B mRNA, and the time-lapse images of RFP-Cdc25B during meiotic resumption are shown. Time 0.00 coincides with the removal of the phosphodiesterase inhibitor from the medium. Frames were taken every minute for 120 min. The video is shown at 10 frames/s.



Video 3. **Time-lapse image of GFP-Wee1B during meiotic resumption.** GV-stage murine oocytes were microinjected with catalytically inactive GFP-Wee1B mRNA, and the time-lapse images of GFP-Wee1B during meiotic resumption are shown. Time 0.00 coincides with the removal of the phosphodiesterase inhibitor from the medium. Frames were taken every minute for 120 min. The video is shown at 10 frames/s.