Kachaner et al., http://www.jcb.org/cgi/content/full/jcb.201408081/DC1

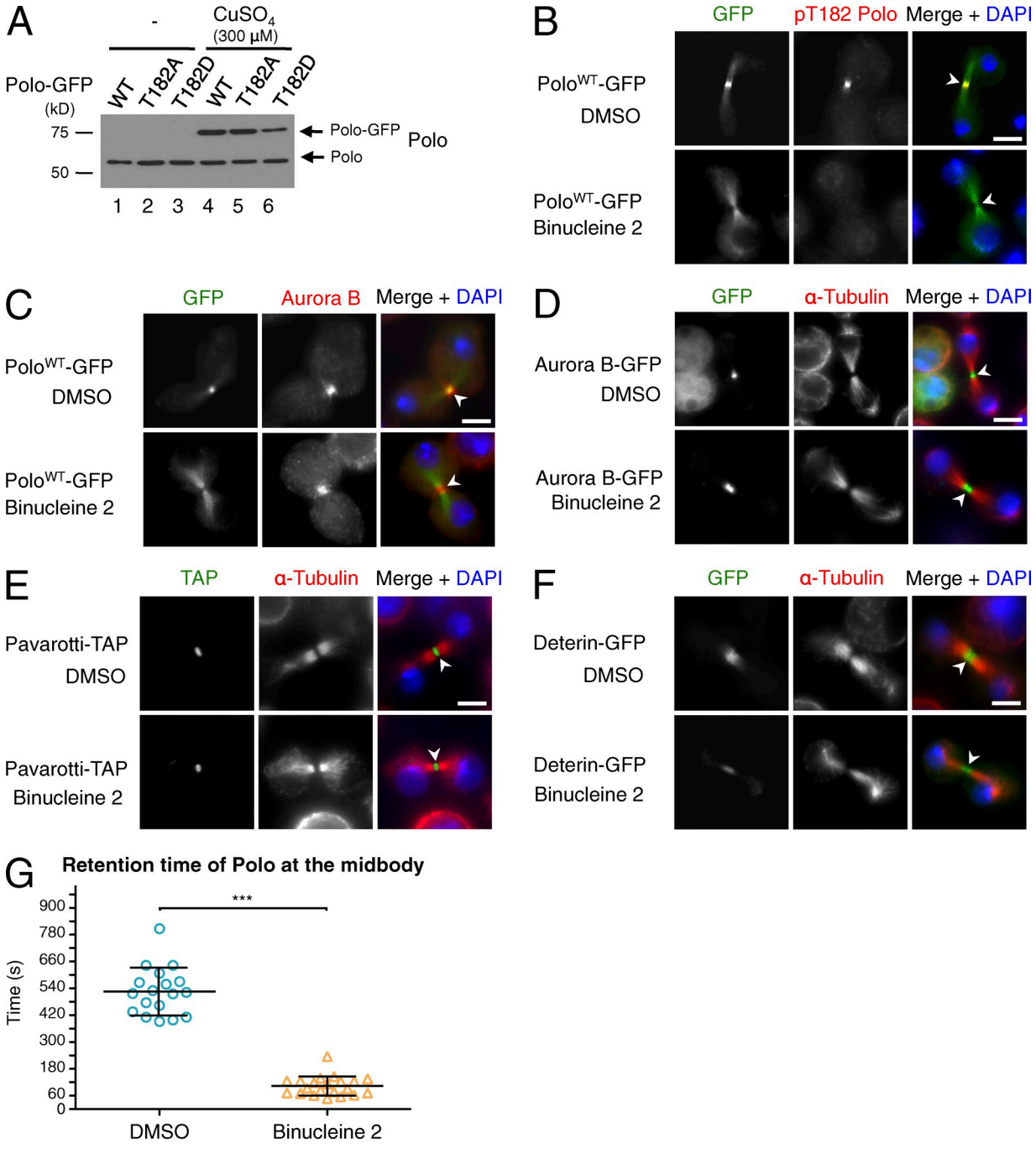


Figure S1. **Additional experiments and controls accompanying Fig. 1.** (A) Expression of Polo^{WT}-GFP, Polo^{T182A}-GFP, and Polo^{T182D}-GFP was induced with $CuSO_4$ (300 µM). The next day, protein extracts were analyzed by immunoblotting with an anti-Polo monoclonal antibody. (B) The localization of Polo-GFP and pT182-Polo at the midbody depends on Aurora B. Cells expressing Polo-GFP were treated with DMSO or Binucleine 2, and stained for pT182-Polo (red). DNA is stained with DAPI, and colocalization of GFP (green) and pT182 Polo (red) appears in yellow. Arrowheads indicate the midbody. Bar, 5 µm. (C–F) Inhibition of Aurora B does not affect the localization at the midbody of Aurora B, Pavarotti, and Deterin. Cells expressing Polo^{WT}-GFP, Aurora B-GFP, Pavarotti-TAP, or Deterin-GFP were treated with DMSO or Binucleine 2, and examined by immunofluorescence as indicated. DNA is stained with DAPI. Arrowheads indicate the midbody. Bars, 5 µm. (G) The activity of Aurora B is required to maintain Polo-GFP at the midbody during cytokinesis. Polo-GFP–expressing cells having just formed a midbody were treated with Binucleine 2 or DMSO and imaged by time-lapse microscopy. The retention time of Polo-GFP was measured for at least 18 cells in each condition.

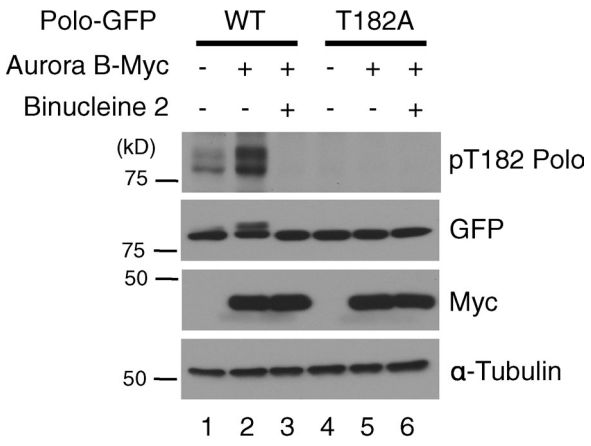


Figure S2. **Polo hyperphosphorylation at Thr182 induced by Aurora B overexpression depends on Aurora B kinase activity.** Cells expressing Polo^{WT}-GFP or Polo^{T182A}-GFP were transfected with Aurora B–Myc and treated with Binucleine 2 as indicated. 24 h after transfection, cells were treated for 1 h with okadaic acid (100 nM) and protein extracts were analyzed by Western blotting for pT182 Polo, GFP, Myc, and α-Tubulin.

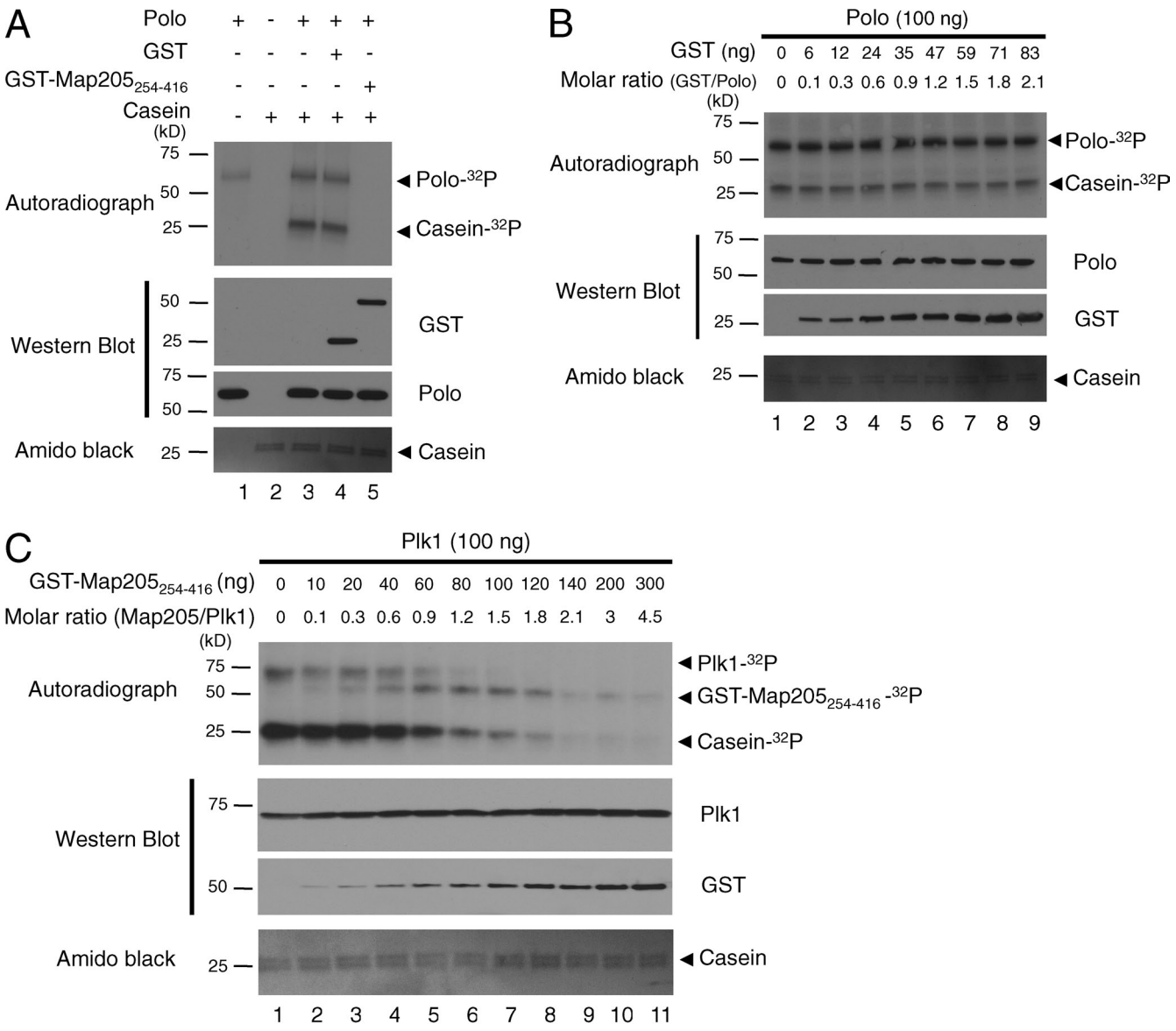
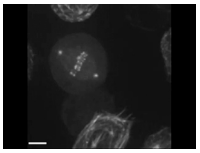
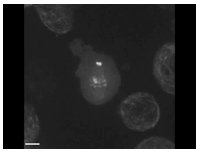


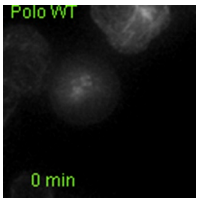
Figure S3. **Map205 inhibits the activity of *Drosophila* Polo and human Plk1.** (A) In vitro kinase assays, using casein as a substrate, were performed in the presence of purified Polo (100 ng) mixed with purified GST-Map205$_{254-416}$ (100 ng) or GST alone (control). Protein phosphorylation was analyzed by autoradiography. Western blots were performed with anti-Polo and anti-GST antibodies. Casein was stained with amido black. (B) Control experiment for results shown in Fig. 3 C. In vitro kinase assays with Polo (100 ng) and casein as a substrate are shown. Increasing amounts of GST were added. Reactions were analyzed by autoradiography, Western blots, and amido black (total protein). No Polo kinase inhibition was observed. (C) Kinase assays were performed in the presence of purified active Plk1 (100 ng) and casein as a substrate. Increasing amounts of purified GST-Map205$_{254-416}$ were added as indicated. Phosphorylation of Plk1, Casein, and GST-Map205$_{254-416}$ was analyzed by autoradiography. Western blots were performed with anti-Plk1 and anti-GST antibodies. Casein was stained with amido black.



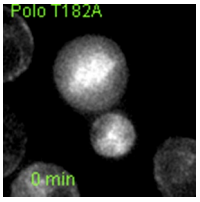
Video 1. **Time-lapse imaging of a Polo-GFP–expressing D-Mel cell in mitosis.** DMSO was added at anaphase onset. Images were acquired every 1 min on a spinning-disk confocal microscope. Bar, 3 μm.



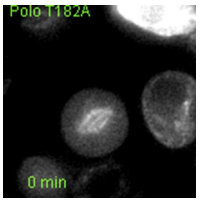
Video 2. **Time-lapse imaging of a Binucleine 2–treated Polo-GFP–expressing D-Mel cell in mitosis.** Binucleine 2 was added at anaphase onset. Images were acquired every 1 min on a spinning-disk confocal microscope. Bar, 3 μm.



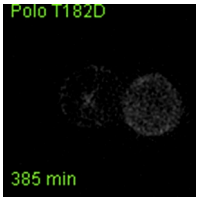
Video 3. **Time-lapse imaging of a D-Mel cell depleted of endogenous Polo and expressing Polo^{WT}-GFP.** Images were acquired every 4 min on a DeltaVision microscope (Applied Precision).



Video 4. **Time-lapse imaging of a D-Mel cell depleted of endogenous Polo and expressing Polo^{T182A}-GFP showing an early cytokinesis failure.** Images were acquired every 7 min on a DeltaVision microscope (Applied Precision).



Video 5. **Time-lapse imaging of a D-Mel cell depleted of endogenous Polo and expressing Polo^{T182A}-GFP showing a late cytokinesis failure.** Images were acquired every 4 min on a DeltaVision microscope (Applied Precision).



Video 6. **Time-lapse imaging of a D-Mel cell depleted of endogenous Polo and expressing Polo^{T182D}-GFP.** This cell successfully completes cytokinesis. Images were acquired every 7 min on a DeltaVision microscope (Applied Precision).