Wei et al., http://www.jcb.org/cgi/content/full/jcb.200809090/DC1

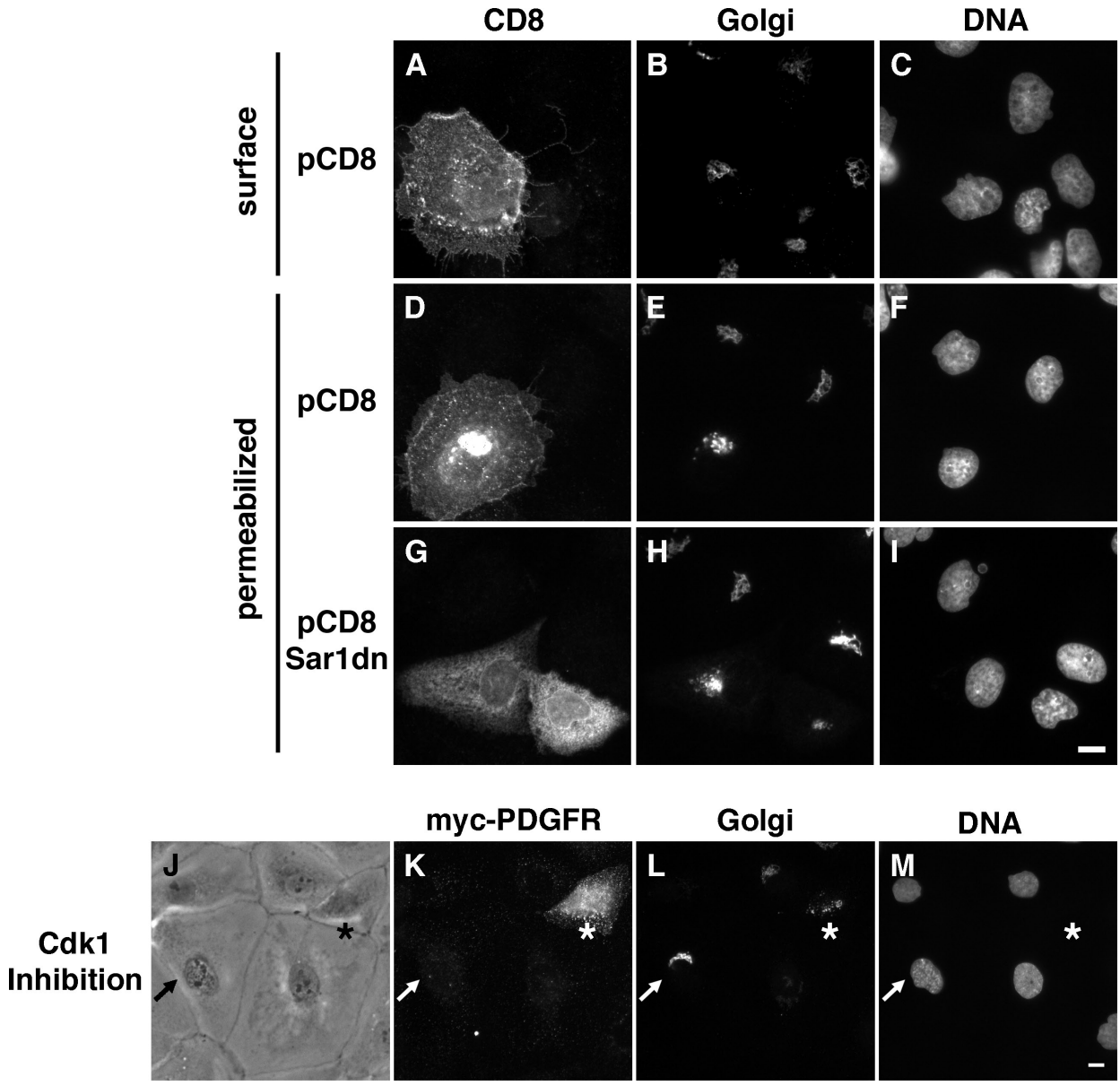


Figure S1. **Transport of newly synthesized proteins to the cell surface.** (A–I) PtK1 cells stably expressing NAGT I–GFP were microinjected with a plasmid encoding CD8 alone (A–F) or together with Sar1dn protein (G–I). 3 h after expression, cells were labeled for CD8 on the cell surface without permeabilization (A–C) or fixed and permeabilized before staining for CD8 (D–I). The Golgi was visualized by GFP, and DNA was stained with Hoechst. (A–C) CD8 was detected on the cell surface of nonpermeabilized cells. (D–F) In permeabilized cells, CD8 was detected in the Golgi in addition to the plasma membrane. (G–I) Sar1dn blocked transport to the cell surface, and CD8 was retained in the ER. (J–M) Secretion is functional in the cytoplasts. Asymmetrical cell division was induced by Cdk1 inhibition and followed by video microscopy (J). The cytoplasts were microinjected with the mRNA of myc-tagged protein A fused to the transmembrane domain of the PDGF receptor (PDGFR). 2 h after expression, cells were stained for myc without permeabilization. The Golgi was visualized by GFP, and DNA was stained with Hoechst. Cytoplasts are marked by asterisks, and the arrows indicate karyoplasts. Note that myc–PDGF receptor was transported to the plasma membrane. Bars, 10 µm.

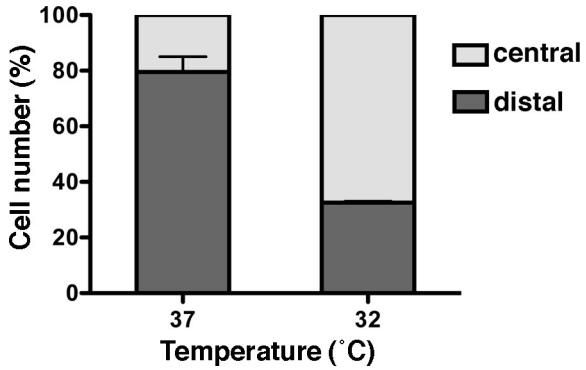
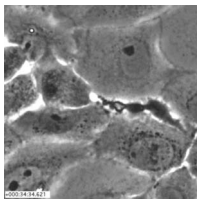
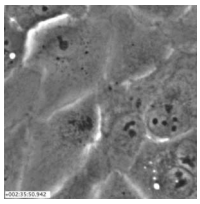


Figure S2. **Temperature affects spindle positioning during division.** Unsynchronized PtK1 cells were treated with trityl-cysteine for 2 h. Cell division was followed by time-lapse phase-contrast microscopy after addition of purvalanol A. In cells that entered mitosis at 37°C, the monopolar spindle was positioned distal to the cleavage furrow. At 32°C, the spindle was positioned centrally in close proximity to the cell division plane. For each experiment, 285 nonoverlapping microscopic fields were monitored to ensure unbiased sampling. Error bars represent SEM from two independent experiments with a mean of 50 cells per condition.



Video 1. **At 37°C, the spindle is positioned distal to the cleavage furrow.** PtK1 cells arrested with trityl-cysteine (2 h) were induced to divide asymmetrically with purvalanol A at 37°C and followed by time-lapse phase-contrast microscopy. Images were captured every 10 min for 2 h and displayed at 2.5 frames per s.



Video 2. **At 32°C, the spindle is positioned close to the cleavage furrow.** PtK1 cells arrested with trityl-cysteine (2 h) were induced to divide asymmetrically with purvalanol A at 32°C and followed by time-lapse phase-contrast microscopy. Images were captured every 12 min for 2 h and displayed at 2.5 frames per s.