Prosser et al., http://www.jcb.org/cgi/content/full/jcb.201412099/DC1

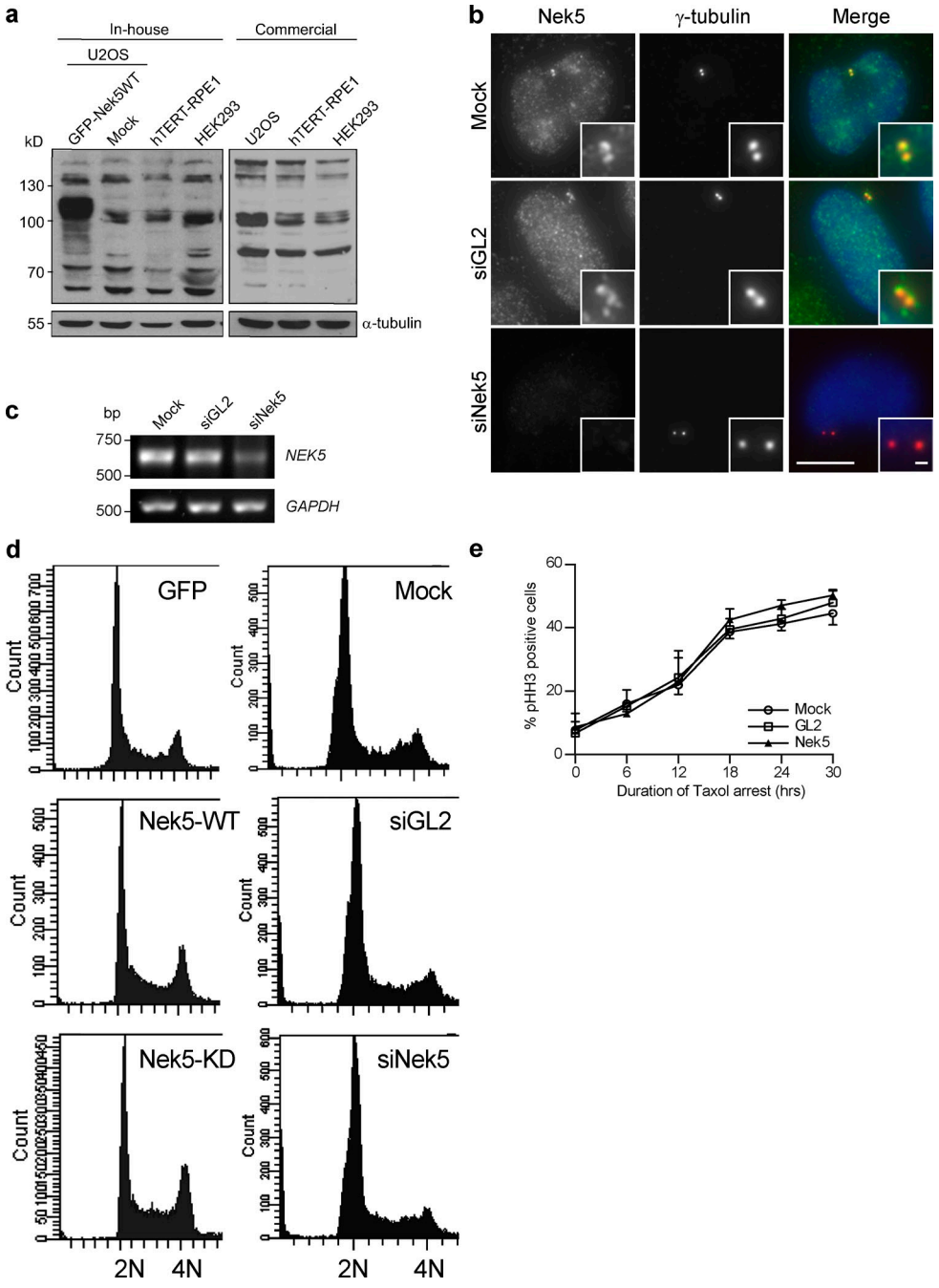
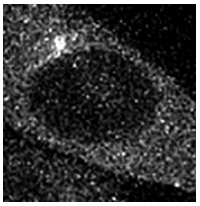
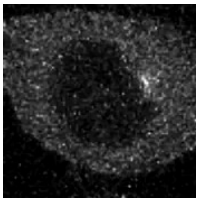


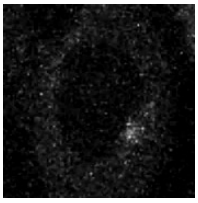
Figure S1. **Nek5 antibody characterization and demonstration that loss of Nek5 function does not substantially alter cell cycle distribution or the spindle assembly checkpoint.** (a) Western blot analysis of untransfected U2OS cells, U2OS cells transiently transfected with GFP-Nek5 for 24 h, hTERT-RPE1 cells, and HEK293 cells. Blots were probed with either the in-house or commercial Nek5 antibody as indicated. α-Tubulin was used a loading control. The GFP-Nek5 and mock-transfected samples from the in-house and α-tubulin blots also appear in Fig. 1 c. (b) Staining of Nek5 with commercial antibody and γ-tubulin in U2OS cells transfected with Nek5- or GL2-specific siRNAs for 48 h. Bars: (main images) 10 µm; (insets) 1 µm. (c) RT-PCR of Nek5 and GAPDH with RNA collected after 48 h of siRNA treatment of U2OS cells with mock-, GL2-, or Nek5-depleted siRNAs. (d) DNA profiles from flow cytometry analysis of U2OS cells transfected with GFP, GFP-Nek5WT (WT), or GFP-Nek5KD (KD) constructs and mock-, GL2-, or Nek5-depleted cells for 48 h. $n = 2$. (e) Percentage of phospho-histone H3 (pHH3)–positive cells during the time course of taxol treatment in mock-, GL2-, or Nek5-depleted cells. Data are mean ± SD; $n = 3$; 200 cells per experiment.



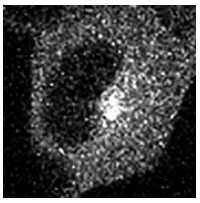
Video 1. **A control GL2-depleted HeLa::GFP–α-tubulin cell progressing through mitosis.** HeLa cells stably expressing GFP–α-tubulin were transfected with siRNA against GL2 for 40 h before the commencement of imaging. Images were captured using a laser-scanning confocal microscope (TCS SP5; Leica). Time is given in minutes and t = 00:00 is defined as the frame in which a distinct MTOC becomes visible. Frames were taken every 5 min. Stills from this video appear in Fig. 5 a.



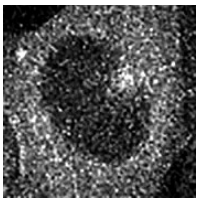
Video 2. **A control GL2-depleted HeLa::GFP–α-tubulin cell progressing through mitosis.** HeLa cells stably expressing GFP–α-tubulin were transfected with siRNA against GL2 for 40 h before the commencement of imaging. Images were captured using a laser-scanning confocal microscope (TCS SP5; Leica). Time is given in minutes and t = 00:00 is defined as the frame in which a distinct MTOC becomes visible. Frames were taken every 5 min.



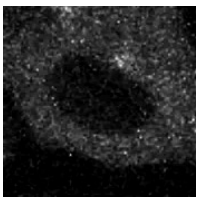
Video 3. **A control GL2-depleted HeLa::GFP–α-tubulin cell progressing through mitosis.** HeLa cells stably expressing GFP–α-tubulin were transfected with siRNA against GL2 for 40 h before the commencement of imaging. Images were captured using a laser-scanning confocal microscope (TCS SP5; Leica). Time is given in minutes and t = 00:00 is defined as the frame in which a distinct MTOC becomes visible. Frames were taken every 5 min.



Video 4. **A Nek5-depleted HeLa::GFP–α-tubulin cell progressing through mitosis.** HeLa cells stably expressing GFP–α-tubulin were transfected with siRNA against Nek5 for 40 h before the commencement of imaging. Images were captured using a laser-scanning confocal microscope (TCS SP5; Leica). Time is given in minutes and t = 00:00 is defined as the frame in which a distinct MTOC becomes visible. Frames were taken every 5 min. Stills from this video appear in Fig. 5 a.



Video 5. **A Nek5-depleted HeLa::GFP–α-tubulin cell progressing through mitosis.** HeLa cells stably expressing GFP–α-tubulin were transfected with siRNA against Nek5 for 40 h before the commencement of imaging. Images were captured using a laser-scanning confocal microscope (TCS SP5; Leica). Time is given in minutes and t = 00:00 is defined as the frame in which a distinct MTOC becomes visible. Frames were taken every 5 min.



Video 6. **A Nek5-depleted HeLa::GFP–α-tubulin cell progressing through mitosis.** HeLa cells stably expressing GFP–α-tubulin were transfected with siRNA against Nek5 for 40 h before the commencement of imaging. Images were captured using a laser-scanning confocal microscope (TCS SP5; Leica). Time is in minutes and t = 00:00 is defined as the frame in which a distinct MTOC becomes visible. Frames were taken every 5 min.