Brownlee et al., http://www.jcb.org/cgi/content/full/jcb.201107086/DC1

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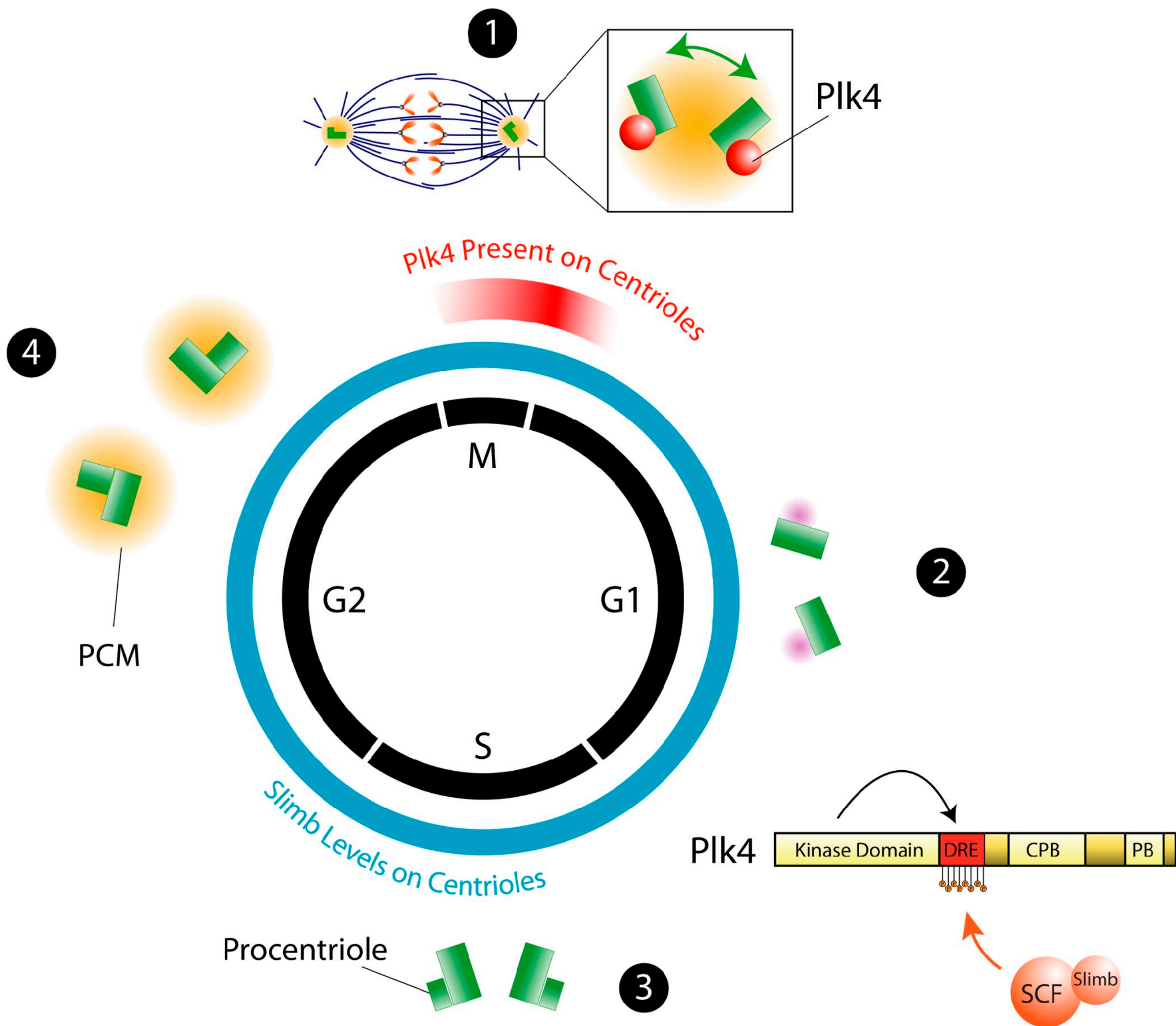

Figure S1. **Illustration of the centrosome duplication cycle and the role and regulation of Plk4 in this process.** Adapted from Rogers et al. (2009). (1) During mitosis, spindles normally have one centrosome at each spindle pole containing a pair of tightly associated or "engaged" mother–daughter centrioles. During late anaphase, the centriole pair separates or "disengages" as shown (green arrows). During this stage, Plk4 (red dot and red stripe) is stabilized and localizes to each centriole as an asymmetric spot (Rogers et al., 2009). Plk4 is required for centriole duplication (an event that occurs later during S phase). Although the molecular mechanism is unknown, Plk4's kinase activity may modify or "license" these centrioles to be competent for duplication. Notably, the F-box protein Slimb localizes to centrioles throughout the cell cycle (blue stripe; Rogers et al., 2009). SCF^{Slimb} is responsible for ubiquitinating the autophosphorylated form of Plk4 (Rogers et al., 2009), which leads to Plk4 degradation. However, even though Slimb is present on mitotic centrioles, an unknown mechanism prevents the destruction of the Plk4 spot. In this study, we demonstrate that the PP2A along with its regulatory subunit Tws (or SV40 ST) counteracts Plk4 autophosphorylation, thus stabilizing Plk4 and promoting centriole duplication. (2) During G1, Tws protein decreases and PP2A no longer counters Plk4 autophosphorylation. Consequently, autophosphorylation of Plk4's downstream regulatory element (DRE) triggers SCF^{Slimb} binding and ubiquitin-mediated proteolysis of Plk4. A linear map of the Plk4 polypeptide is shown, including its cryptic polo box (CPB) and polo box (PB) motifs. Though Plk4 is persistently degraded, centrioles retain their duplication license (purple dots). (3) During S phase, duplication of licensed centrioles begins as procentrioles assemble. Plk4 is expressed during S phase, but its continuous degradation by SCF^{Slimb} blocks centriole reduplication. (4) During G2, centrioles recruit pericentriolar material (PCM), allowing the maturing centrosomes to nucleate microtubules that facilitate mitotic spindle assembly in the next cell cycle phase. Plk4 is continuously degraded by SCF^{Slimb} during G2.

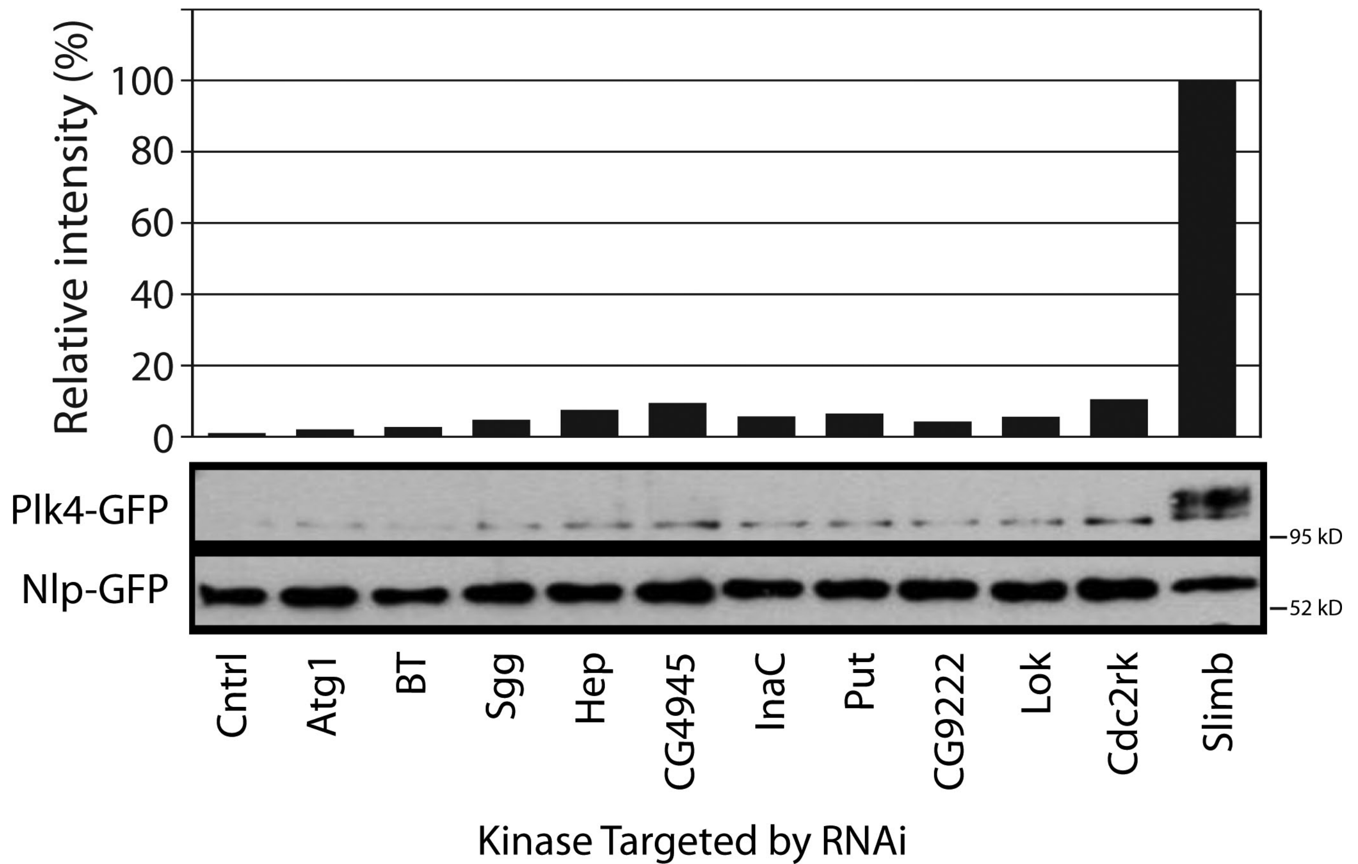

Figure S2. **Plk4 protein levels are unaffected in an RNAi screen of the *Drosophila* kinome.** S2 cells were cotransfected with inducible Plk4-GFP and Nlp-GFP (a nuclear marker used as a loading control and driven by its endogenous promoter; Rogers et al., 2009) and seeded into 96-well plates, in which each well contained dsRNA against one of 222 different kinases (Table S1). After 4 d of RNAi, cells were induced to express Plk4-GFP for 24 h, lysed, and then analyzed by quantitative anti-GFP immunoblotting. (bottom) A representative immunoblot is shown containing both negative control and Slimb RNAi-treated samples as well as lysates from 10 different kinase RNAi treatments. (top) Plk4-GFP levels were measured using densitometry (ImageJ), normalized to the negative RNAi loading control, and compared with the Slimb RNAi treatment, which produces maximal Plk4 accumulation. Cntrl, control; BT, bent; sgg, shaggy; Hep, hemipterous; InaC, inactivation no afterpotential C; Put, punt; Lok, loki.

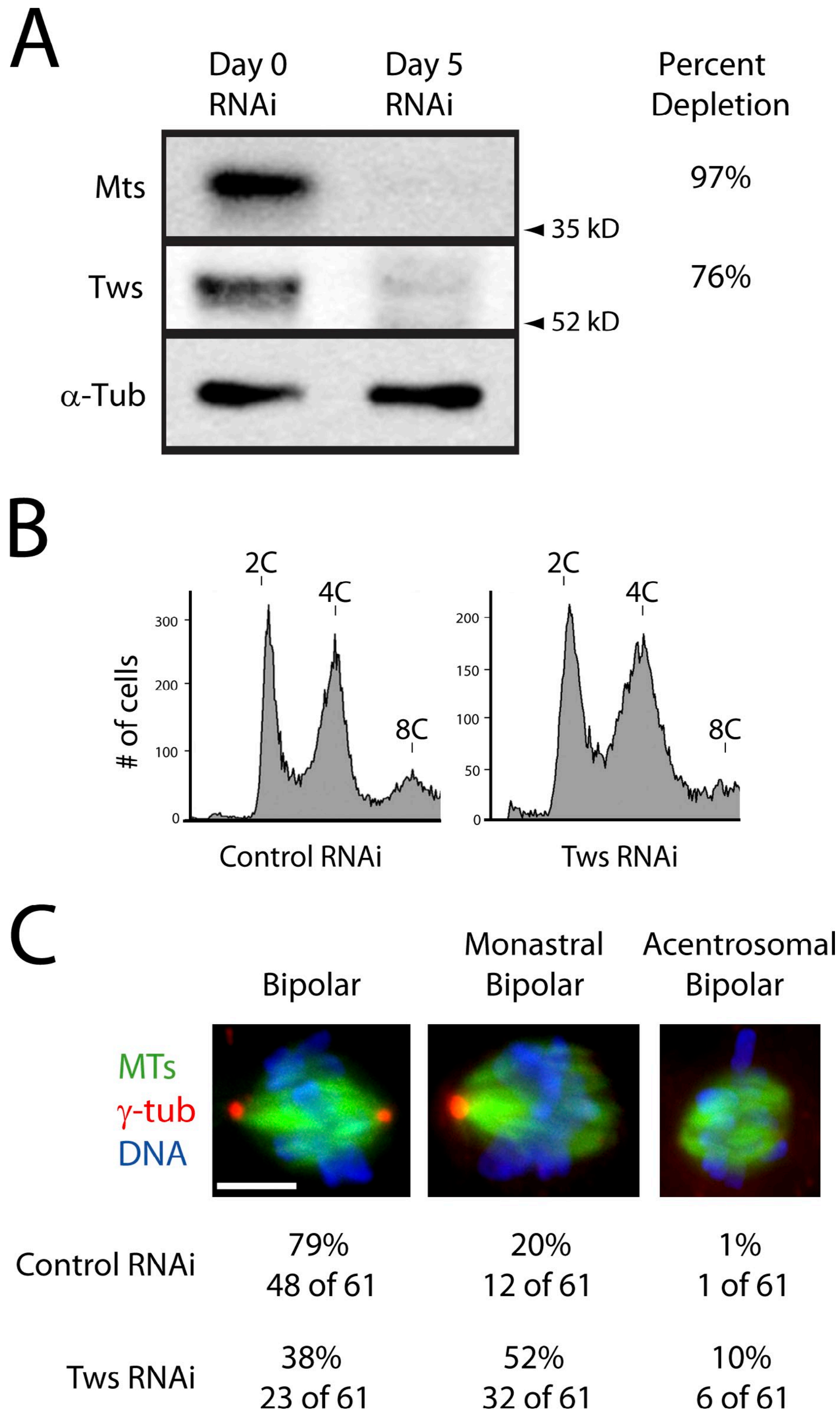

Figure S3. **Tws RNAi eliminates mitotic centrosomes but does not affect cell cycle progression.** (A) Immunoblotting demonstrates the efficiency of RNAi-mediated protein depletion from S2 cells. For each treatment, samples of S2 cell lysates from day 0 (before depletion) and day 5 (after completion of RNAi) are shown. Equal total protein amounts were loaded. Percent depletion of the target protein was calculated from the ratio of the day 5 and day 0 band intensities (measured using integrated densitometry [ImageJ] and normalized with similar measurements of the α-tubulin bands). (B) Cell cycle progression is unaffected by Tws RNAi. Histograms of DNA fluorescence intensity (x axis) and cell number (y axis) of 7,000 cells with 2C, 4C, and 8C populations indicated. (C) Tws RNAi reduces mitotic centrosome number. Control and Tws RNAi cells were stained for γ-tubulin (γ-tub), α-tubulin (green), and DNA. The bipolar spindles of RNAi-treated cells were categorized based on the presence of centrosomes (indicated by the presence of γ-tubulin foci). The percentages of mitotic cells belonging to each of the three different categories are shown. MTs, microtubules. Bar, 2.5 µm.

Table S1. **Primer sequences used to generate dsRNA**

Gene	Celera gene number	Sense primer	Antisense primer	Expected size
				bp
Control	NA	5′-ATGGATAAGTTGTCGATCG-3′	5′-ACCAGGTTCACATGCTTGCG-3′	900
Plk4/sak	7186	5′-CCACCAGATCTTCAAATACCAAGCGG-3′	5′-TAATAAGGCGTTGTCGTTTTGGG-3′	914
FBXW1/slimb	3412	5′-GGCCGCCACATGCTGCG-3′	5′-CGGTCTTGTTCTCATTGGG-3′	912
Mts/PP2A^C	7109	5′-CCGGTGACAGTGTGCGG-3′	5′-AAGGTGTAACCGGCGCC-3′	509
PP2A-29B	8998	5′-GACATGGCTGATGATCC-3′	5′-TCCCATGCGCTGAATGG-3′	518
Tws	6235	5′-CCAATCGCACGAGCCCG-3′	5′-CGGATGGAATTCGGCCG-3′	478
Well rounded	7913/7901	5′-CGGAGGAGGATGAGCCC-3′	5′-GCTAGGATCCTTCTCCAGG-3′	482
Widerborst	5643	5′-CTTGCCCCTGAAGGCCG-3′	5′-TGGAGGTAAGCTCGTCG-3′	583
PR72	8711	5′-TAATACGACTCACTATAGGGCCAC-AACAACGACCACC-3′	5′-TAATACGACTCACTATAGGGGCCTGA-TCTGGCACGGG-3′	505

All primers began with the T7 promoter sequence 5′-TAATACGACTCACTATAGGG-3′ immediately followed by a gene-specific sequence. In most cases, a large single exon was PCR amplified from a cDNA template to generate dsRNA; otherwise, genomic DNA was used. The control template was generated from a region of the plasmid pBluescript SK that is dissimilar to any *Drosophila* coding sequence.

Table S2 shows a list of *Drosophila* kinases depleted in an RNAi screen and their effects on Plk4 protein levels and is provided in an Excel file.

Reference

Rogers, G.C., N.M. Rusan, D.M. Roberts, M. Peifer, and S.L. Rogers. 2009. The SCF^{Slimb} ubiquitin ligase regulates Plk4/Sak levels to block centriole reduplication. *J. Cell Biol.* 184:225–239. http://dx.doi.org/10.1083/jcb.200808049