Klebba et al., http://www.jcb.org/cgi/content/full/jcb.201410105/DC1

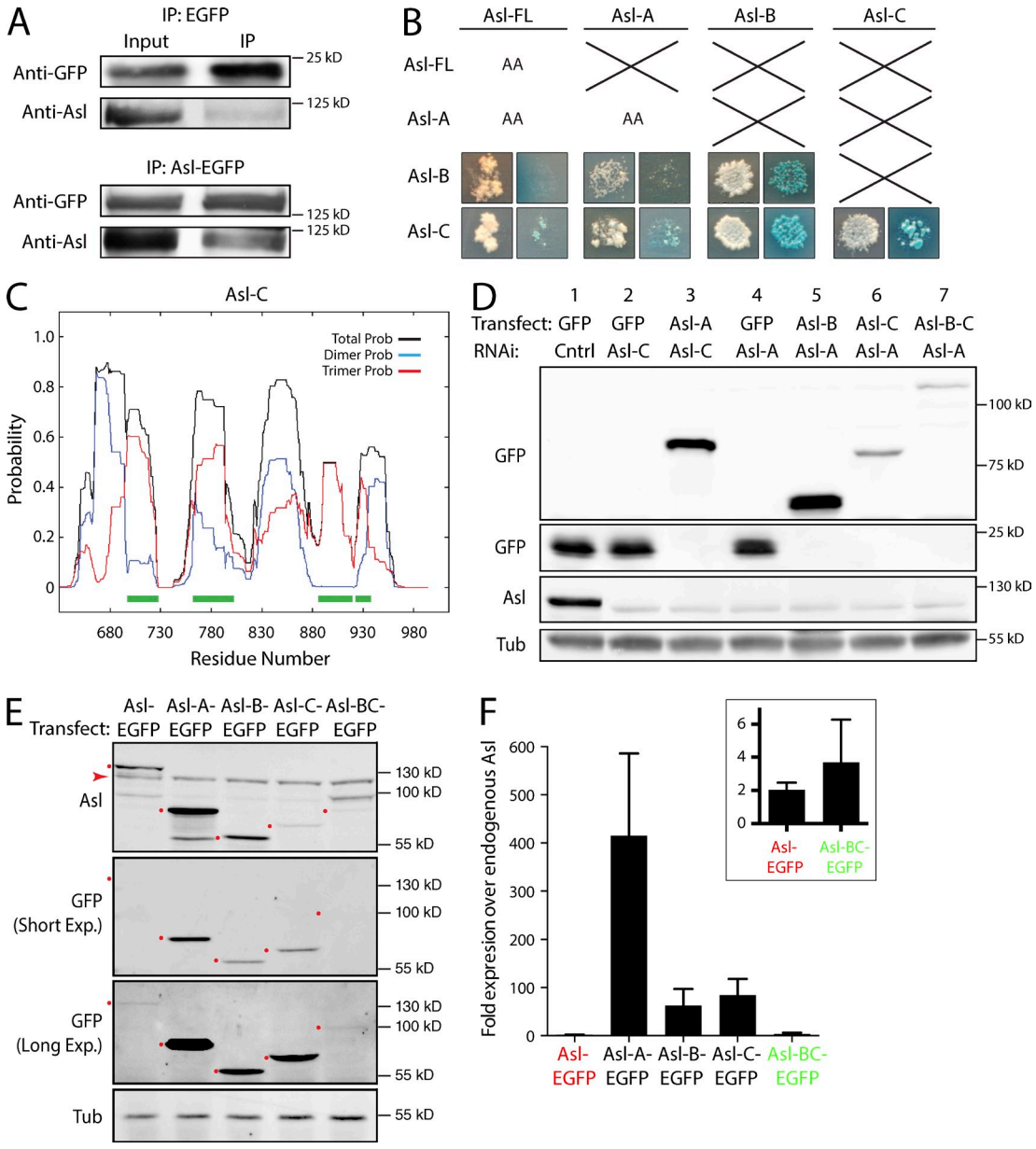

Figure S1. **Asl self-interacts.** (A) Asl-EGFP associates with endogenous Asl. Anti-GFP immunoprecipitates (IPs) were prepared from lysates of S2 cells transiently coexpressing either inducible EGFP or FL Asl-EGFP constructs. Blots of the input lysates and IPs were probed for GFP and Asl. (B) Y2H analysis of Asl identified several regions of self-interaction. Colonies from replica plating are shown in each panel. (left image) Growth indicates presence of both bait and prey. (right image) Growth on DDOXA, and color indicates an interaction. AA indicates that one or both protein fragments autoactivated the Y2H reporters on their own and could not be tested. Note that Asl-B and Asl-C self-interact. Asl-C also strongly interacts with Asl-B and weakly interacts with Asl-A. (C) MultiCoils algorithm analysis of Asl-C predicts both double- and triple-stranded coiled-coils throughout the domain. Stretches of high trimer coiled-coil prediction are underlined in green. Prob, probability. (D) Immunoblots of S2 cells shown in Fig. 1 D. Cells were depleted of endogenous Asl using the indicated exon-targeting RNAi for 3 d and transfected with the indicated inducible Asl-EGFP construct (or control EGFP). While under continuous RNAi treatment, transfected cells were allowed to recover for 24 h and then induced to express for an additional 3 d. Cells were immunostained to visualize centrioles, and in parallel, lysates were prepared for immunoblotting with the indicated antibody. Cntrl, control. (E) Immunoblots of S2 cells show the levels of transgenic Asl-EGFP constructs compared with endogenous Asl. 1.0 µg of Asl-EGFP expression plasmids was transfected and induced with 0.25 mM $CuSO_4$ for 24 h, and lysates were prepared for immunoblotting with the indicated antibody. Endogenous Asl is indicated with the red arrowhead. The positions of the various Asl-GFP proteins are indicated with red dots. Note that the anti-Asl antibody also recognizes a non-Asl polypeptide, which migrates under the 100-kD marker. Exp., exposure. (F) Graph of the levels of the transgenic Asl-EGFP proteins in D relative to endogenous Asl levels. Asl proteins were measured by densitometry of the anti-Asl and GFP immunoblots and then normalized to the measured amounts of α-tubulin (the loading control). Calculation of the Asl fragments relative to endogenous Asl required two steps: first, FL Asl-EGFP relative to endogenous Asl was measured on the Asl immunoblot, and then, each Asl fragment-EGFP was measured relative to FL Asl-EGFP on the GFP immunoblot. (inset) The calculated values for only FL Asl-EGFP and Asl-BC-EGFP are replotted at a different scale. *n* = 3 experiments; error bars indicate SEM. Tub, tubulin.

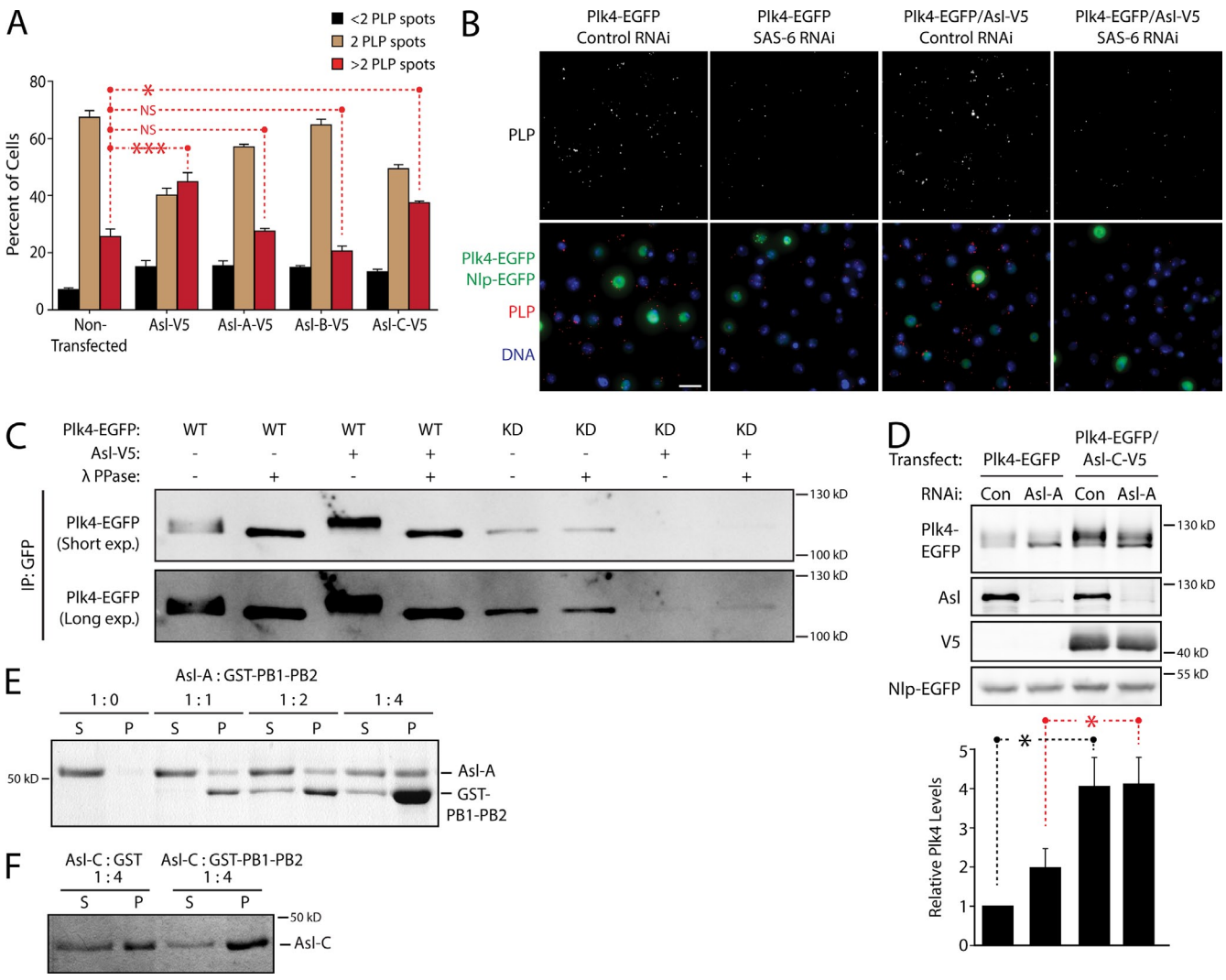

Figure S2. **Asl-C binds Plk4 PB1-PB2 and is sufficient to induce centriole amplification and stabilize Plk4.** (A) Asl-C expression induces centriole amplification. S2 cells were transiently transfected with the indicated Asl-V5 constructs and induced to express for 3 d, and then, centrioles visualized by anti-PLP immunostaining. Each bar shows the mean percentage of cells containing the indicated number of centrioles (n = 3 experiments; 300 cells counted per treatment, per experiment). Asterisks mark treatments with significant differences (relative to control), whereas NS marks treatments with insignificant differences for selected comparisons. *, 0.05 > P ≥ 0.01; ***, P < 0.001. Error bars indicate SEM. (B) SAS-6 depletion decreases centriole numbers in cells expressing Plk4 and Asl. S2 cells were treated with control or SAS-6 RNAi for 6 d. On day 4, cells were transfected with inducible Plk4-EGFP alone or with Asl-V5 and then induced to express the next day for 24 h. Centriole-PLP immunostaining confirms a reduction in centriole number in SAS-6–depleted cultures. Coexpressed Nlp-EGFP (a nuclear protein) was used to identify transfected cells. Bar, 10 µm. (C) Asl overexpression induces Plk4 autophosphorylation. Plk4-EGFP was immunoprecipitated from lysates of cells transiently expressing EGFP-tagged WT-Plk4 or kinase-dead (KD) Plk4 either alone or with Asl-V5. Immunoprecipitates (IPs) were either mock or λ phosphatase treated, and blots of the IPs were probed for GFP. Note that coexpression with Asl-V5 induces WT-Plk4 to shift to a slower migrating species compared with WT-Plk4 expressed alone. Plk4 shifts from a broad band to a sharper, faster migrating single band after phosphatase treatment, but this treatment does not affect negative control KD-Plk4. The immunoblot is shown at short and long exposures (exp.). (D) Asl-C expression stabilizes Plk4 in the absence of endogenous Asl. S2 cells were control or Asl depleted for 6 d using RNAi targeting to an exon within the Asl-A region. On day 4, cells were transfected with inducible Plk4-EGFP either alone or with Asl-C–V5 and then induced to express the next day for 24 h. The relative stability of the Plk4-EGFP protein was analyzed by immunoblotting cell lysates with anti-GFP, Asl, and V5 antibodies. Cotransfected Nlp-EGFP was used as a loading control. Amounts of Plk4-EGFP were determined by densitometry of the anti-GFP immunoblots (all Plk4 polypeptides within the smear) and normalized to the amount of Plk4 present in the control RNAi-treated cells (lane 1). n = 3 experiments; error bars indicate SEM. Con, control. (E) Asl-A binds GST-Plk4 PB1-PB2 as shown by an in vitro pull-down assay. An increasing amount of purified GST-Plk4 PB1-PB2 was mixed with a constant amount of purified Asl-A–His_6; glutathione-coated beads were added to recover GST-Plk4 PB1-PB2. Proteins in the supernatant (S) and the washed bead pellet (P) are shown in the Coomassie-stained protein gel after mixing at the indicated molar ratios of Asl-A/GST–PB1-PB2. Lanes 1 and 2 show Asl-A mixed with beads as a control. Note that increasing amounts of GST–PB1-PB2 pull-down an increasing amount of Asl-A in the pellet. (F) Asl-C binds GST-Plk4 PB1-PB2 in vitro. For technical reasons, it was necessary to first cross-link GST–PB1-PB2 (and control GST) to the glutathione-coated beads before adding Asl-C. Proteins in the supernatant and bead pellet are shown in the Coomassie-stained protein gel after mixing at the indicated molar ratios. Although some Asl-C binds the GST-coated beads, Asl-C is enriched ninefold in the bead pellet coated with GST–PB1-PB2.

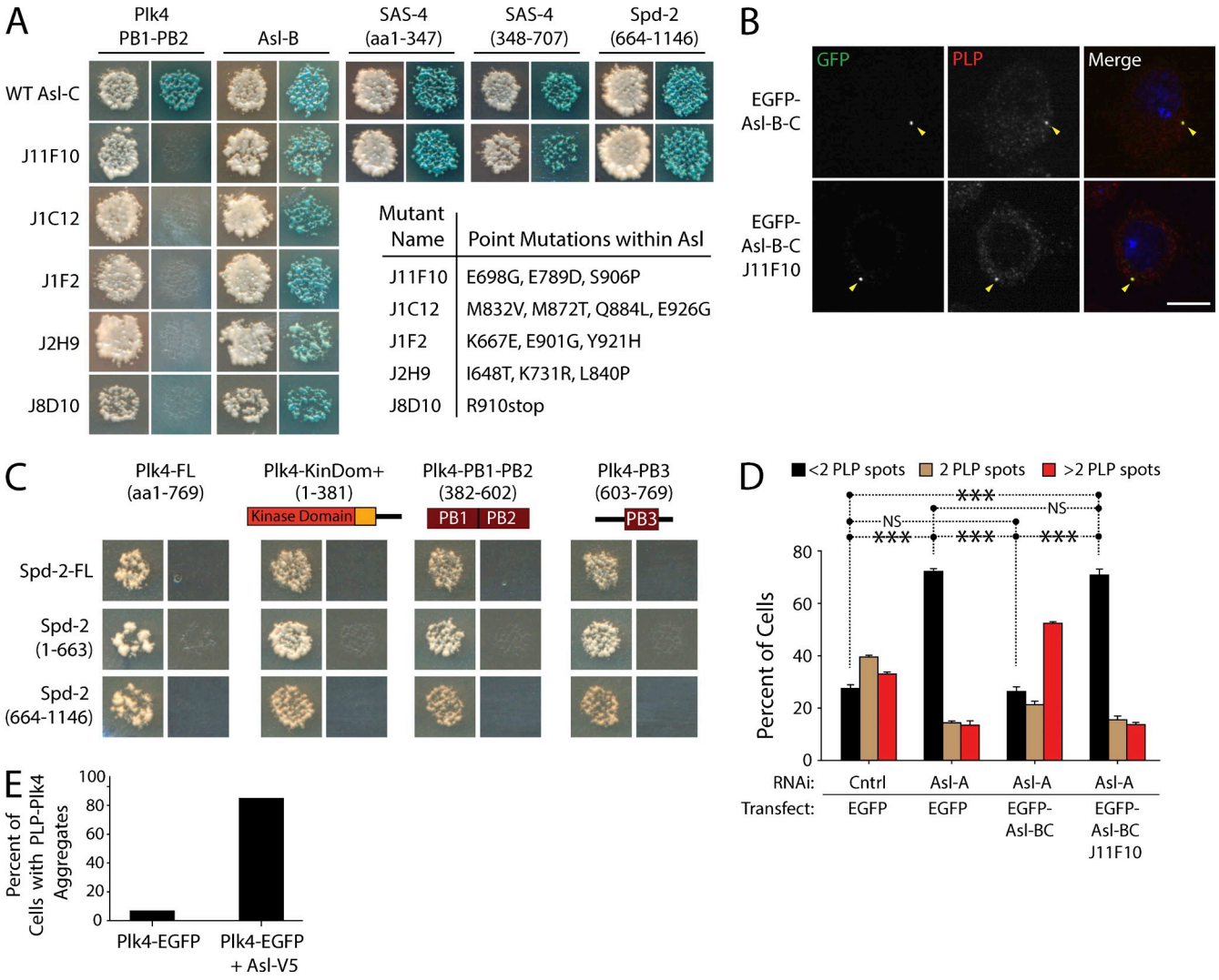

Figure S3. **The J11F10 mutant of Asl-C localizes to centrioles but fails to rescue centriole duplication in Asl-depleted cells.** (A) Selected Asl-C mutants do not interact with Plk4 PB1-PB2 by Y2H. Five mutants generated by random PCR mutagenesis of Asl-C fail to interact with PB1-PB2 by Y2H. Mutant J11F10 was chosen for further characterization. Both WT–Asl-C and J11F10 positively interact with Asl-B, two SAS-4 fragments that collectively span the FL protein (aa 1–347 and 348–707), and the Spd-2 C terminus (aa 664–1,146). In each panel, colonies from replica plating are shown. (left image) Growth indicates presence of both bait and prey. (right image) Growth on DDOXA, and color indicates an interaction. (B) The J11F10 mutation does not prevent centriole localization. Ubi promoter–driven EGFP–Asl-BC or the same construct harboring the J11F10 mutation were transiently expressed in S2 cells and processed for PLP immunostaining. Yellow arrowheads mark centrioles in the cells. Bar, 5 µm. (C) *Drosophila* Spd-2 does not interact with Plk4 by Y2H. FL and fragments of Spd-2 and Plk4 were screened by Y2H. In each image, colonies from replica plating are shown. (left image) Growth indicates the presence of both bait and prey. (right image) Growth on DDOXA, and color indicates an interaction. The reverse bait–prey experiments between all of the combinations shown here were also all negative for an interaction. KinDom, kinase domain. (D) A J11F10 mutant of Asl-BC prevents rescue of centriole duplication after knockdown of endogenous Asl. S2 cells were transiently transfected with inducible EGFP-WT–Asl-BC, the J11F10 mutant, or EGFP and either control or Asl depleted with RNAi targeting an exon in Asl-A for 6 d. Cells were then processed for anti-PLP immunostaining, and centriole numbers were measured. Each bar shows the mean percentage of cells containing the indicated number of centrioles ($n = 5$ experiments; 200 cells counted per treatment, per experiment). Asterisks mark treatments that generate significantly different percentages of cells with less than two centrioles, whereas NS mark treatments with percentages of cells not significantly different, for selected comparisons. ***, $P < 0.001$. Error bars indicate SEM. (E) Plk4 and PLP colocalize in cytoplasmic aggregates. S2 cells were transfected with Plk4-EGFP alone or with Asl-V5 and induced to express for 24 h, and then anti-PLP was immunostained. The graph shows the frequency of Plk4-EGFP–expressing cells containing aggregates that colocalize with PLP (100 cells scored per treatment).

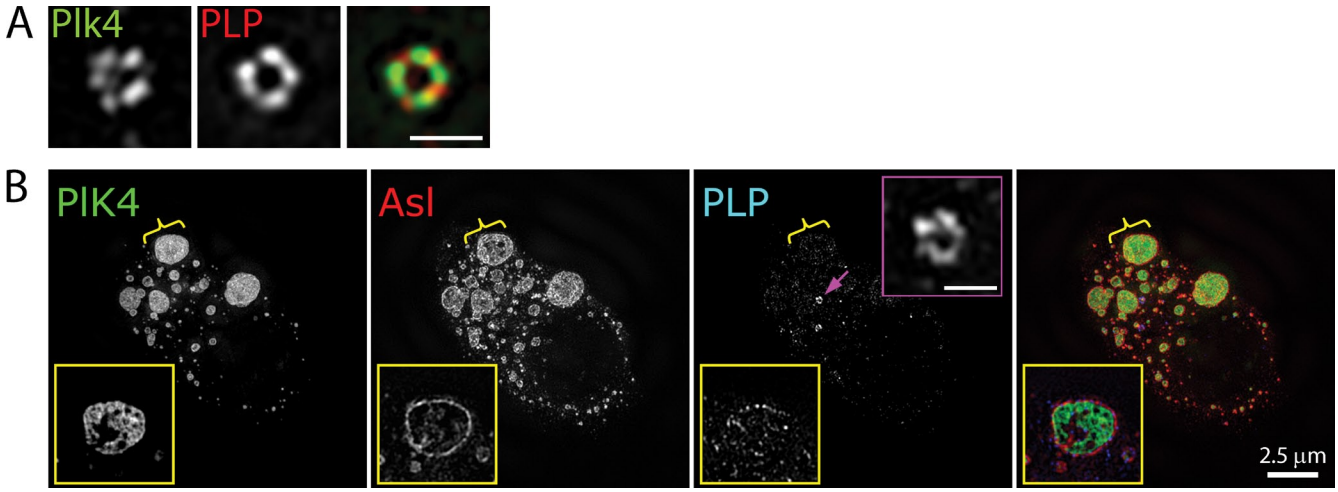

Figure S4. **Asl and Plk4 form aggregates that compromise centriolar structure.** Cells coexpressing Asl-V5 and Plk4-EGFP were analyzed by structured illumination microscopy. (A) High magnification image of a centriole in a Plk4-EGFP–expressing cell immunostained for PLP. PLP staining reveals normal quasi-ninefold symmetry. Plk4-EGFP localizes to the centriole as numerous overlapping clusters. Bar, 0.5 µm. (B) Maximum intensity projection of an S2 cell coexpressing Asl-V5 and Plk4-EGFP and immunostained for PLP. Yellow boxes show single optical sections of Plk4–Asl aggregates at higher magnification (Asl concentrates at the periphery of the aggregate forming a "shell" around Plk4; brackets). Purple box shows a PLP-labeled centriole at higher magnification (arrow). Centrioles in cells with aggregates display irregular-sized PLP clusters that vary in intensity (n = 25 centrioles examined).