Supplementary Table, Figure and Movie Legends

Table S1.

MS analysis of STIL co-immunoprecipitates in HeLa and HEK293T cells. Sequence coverage (%), the number of detected peptides and unique peptides of STIL and RBM14 are shown. We show the list of 21 proteins that were identified as high-rank candidates for STIL or HsSAS-6-interacting proteins in our MS experiments. In these experiments, we could not find CPAP peptides in the STIL co-immunoprecipitates. Please note that we addressed their function for centriole formation in human U2OS and HeLa cells by targeting each protein with 3 different siRNAs and found that RBM14 depletion was the only case to show a defect in centriole biogenesis.

Figure S1. RBM14 depletion induces amplification of centriolar protein complexes.

A. Soluble cytosolic fractions of U2OS cells treated with RBM14 siRNA or control siRNA were analyzed using antibodies against RBM14, α-tubulin and centrin. Note that whereas RBM14 protein level was significantly reduced by treatment of RBM14 siRNA (~15.4% of that in control cells), centrin protein level was not affected.

B–D. Amplification of centrin foci was observed during mitosis in RBM14-depleted HeLa cells (B), RPE-1 cells (C) or during interphase in RBM14-depleted U2OS cells (D). Histograms represent frequency of mitotic cells with excess centrin or Cep164 foci. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition). **P < 0.01. *P < 0.05. n.s., not significant (one tailed t-test).

E and F. Ectopic formation of centriolar protein complexes in RBM14-depleted cells is not a consequence of centriole overduplication induced by cell cycle arrest. (E) FACS profiles of RBM14-depleted U2OS cells demonstrate no remarkable cell cycle arrest at least within 24 hours after transfection. U2OS cells treated with RBM14 siRNA were fixed at 0, 24 hours after transfection, and the DNA contents were analyzed using flow cytometry after propium iodide (PI) staining. Values are mean percentages ± SEM from three independent experiments (n=50,000 for each

condition). (F) Amplification of centrin foci is detectable even 24 hours after transfection in U2OS cells. Histograms represent frequency of mitotic cells with excess centrin foci. Values are mean percentages ± SEM from three independent experiments (n=30 for each time point). **P < 0.01 (one tailed t-test).

G. U2OS cells were treated with RBM14 siRNA or control siRNA, thereafter treated without or with Cycloheximide, a translation inhibitor, (final concentration of 0.5 mM) at 8 hours after siRNA transfection, followed by incubation for 16 hours and fixed. Histograms represent frequency of interphase cells with excess centrin foci in the indicated conditions. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition). Note that the addition of Cycloheximide did not affect the extent to which the number of cells with excess centrin foci was increased by RBM14 depletion. **P < 0.01. n.s., not significant (one tailed t-test).

H. Mislocalization of RBM14 from the nuclear paraspeckles does not induce amplification of centrin foci. U2OS cells treated with NONO siRNA or control siRNA were stained with antibodies against centrin-2 (green) and RBM14 (magenta). DNA is shown in blue. Bar, 10 μm. Histograms represent the frequency of cells with robust RBM14 signal in the nuclear paraspeckles (left) or mitotic cells with excess centrin foci (right) in the indicated condition. Values are mean percentages ± SEM from three independent experiments (n=50 for counting paraspeckle, and n=30 for counting excess centrin foci). **P < 0.01, n.s., not significant (one tailed t-test). RBM14 is known to act as a component of the nuclear paraspeckle. Note that although NONO siRNA efficiently disassembled the paraspeckle puncta and displaced RBM14 in the nucleus, centriole formation was not affected.

I. Mitotic U2OS cells treated with control or RBM14 siRNA were stained with antibodies against centrin-2 and Cep164. Histograms represent frequency of mitotic cells with excess foci of centrin and Cep164. Values are mean percentages ± SEM from three independent samples (n=30 for each condition). **P < 0.01, n.s., not significant (one tailed t-test).

J. Mitotic U2OS cells treated with RBM14 siRNA were stained with antibodies against CPAP, CP110, Cep192, γ-tubulin, acetylated tubulin or HsSAS-6 as well as centrin. Histograms represent frequency of ectopic centrin foci co-localized with the indicated proteins. Values are mean percentages ± SEM from three independent samples (n=30 for each condition).

K and L. U2OS cells treated with RBM14 siRNA or control siRNA were stained with antibodies against CP110 (magenta, K) or Cep192 (magenta, L) as well as centrin-2 (green). DNA is shown in blue. Insets show two-fold magnified images of fluorescent foci. Bar, 5 μm. Histograms represent the frequency of mitotic cells with excess foci of the indicated protein. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition).

Figure S2. Subcellular localization of RBM14.

A. U2OS cells expressing Flag–RBM14 full-length (FL), RBM14[N] or RBM14[C] and treated with siRNA against endogenous RBM14 were stained with antibodies against Flag (magenta) and centrin-2 (green); DNA is shown in blue. Insets show approximately two-fold magnified views of fluorescent foci around the centrosome and scale bars are 5 μm.

B. U2OS cells or U2OS cells expressing FLAG-RBM14FL, FLAG-RBM14[N], FLAG-RBM14[C], FLAG-RBM14-NES or GFP-RBM14-PACT were stained with antibodies against centrin-2 and FLAG or GFP (for RBM14-PACT). We counted only cells that had adequate intensity of FLAG or GFP signals. Histograms represent frequency of mitotic cells with the indicated number of centrin in each condition from three independent experiments (n=20 for each condition).

C. U2OS cells or U2OS cells expressing FLAG-RBM14 full-length were stained with antibodies against centrin-2 and γ-tubulin. Histograms represent frequency of mitotic cells with the indicated number of γ-tubulin from three independent experiments. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition). n.s., not significant (one tailed t-test).

D. Histograms represent frequency of interphase U2OS cells with centriolar rosettes in each condition. The cells were stained with antibodies against FLAG-tag, HA-tag or HsSAS-6 as well as RBM14. The formation of centriolar rosettes was judged from the distribution of FLAG-Plk4, HA-STIL or Myc-HsSAS-6 signals at centrioles. We counted only cells that had expressed adequate amount of the exogenous proteins. Values are mean percentages ± SEM from three independent samples (n=30 for each condition). n.s., not significant (one tailed t-test).

E. U2OS cells treated with control siRNA or RBM14 siRNA were stained with antibodies against RBM14 (magenta) and centrin-2 (green); DNA is shown in blue. Insets show approximately three-fold (interphase) or two-fold (mitotic) magnified views of fluorescent foci around the centrosome and scale bars are 10 μm. The percentages of U2OS cells with centrosomal localization of endogenous RBM14 are shown below the immunofluorescence images. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition).

F. Cytoplasmic and nuclear extraction was performed from U2OS cells treated with RBM14 siRNA or control siRNA. The isolated fractions were analyzed by western-blotting using antibodies against RBM14 and Histone H4, as a nuclear marker. Note that there is a substantial amount of endogenous RBM14 proteins in the cytoplasmic fraction.

G. U2OS cells expressing Flag–RBM14-NES, or RBM14-PACT and treated with siRNA against endogenous RBM14 were stained with antibodies against Flag and centrin-2. Histograms represent frequency of mitotic cells with excess number of centrin foci from three independent experiments. Values are mean percentages ± SEM from three independent samples (n=30 for each condition). **P < 0.01, **P < 0.05 (one tailed t-test). Consistent with Fig. 2D, expression of Flag–RBM14-NES rescued the centrin amplification phenotype provoked by RNAi-mediated reduction of endogenous RBM14.

Figure S3. RBM14[C] interacts with STIL[N].

A. Asynchronous HeLa cells (Asy), HeLa cells arrested at G1/S phase using double-thymidine block (+DT) or HeLa cells arrested at mitotic phase using nocodazole (+Noc) were immunoprecipitated with control IgG or STIL antibodies. The resulting IPs and soluble cytosolic fractions (input) were analyzed as in Fig 3A. The DNA contents of cells from each sample were analyzed by FACS (bottom). The asterisks indicate RBM14 bands. Immuno-reactive band for actin was used as a loading control.

B. Yeast-two-hybrid assay testing interactions between full-length or the indicated fragments of STIL and RBM14[C] (aa151–669) or RBM14 FL (aa1–669).

C and D. Co-immunoprecipitation assays in 293T cells testing interactions between HA-STIL[N] (aa1–1018) or HA-STIL[C] (aa1019–1288) and Flag-RBM14 fragments. Co-immunoprecipitation performed using anti-Flag agarose beads. The resulting purified protein complexes and soluble cytosolic fractions were separated on SDS-PAGE gel and HA (white arrowheads) and Flag (black arrowheads) tagged proteins were detected using standard immunoblotting techniques. Quantification of relative protein amounts of co-immunoprecipitated HA-STIL[N], normalized by the input material of HA-STIL for each Flag-IP fraction is shown below the panel. Means ± SEM were calculated from three independent experiments.

E–G. TRBP/Ncoa6-interacting domain is responsible for STIL-RBM14 binding and required for the function of RBM14. (E) Schematic for the full-length and truncated mutants of RBM14 proteins used in this figure. (F) GST pull-down assay testing interactions between RBM14 truncated mutants and STIL[N]. (G) U2OS cells or U2OS cells expressing Flag–RBM14[C] or Flag–RBM14ΔTRBP-ID and treated with control siRNA or siRNA against N-terminal region of RBM14 targeting solely endogenous RBM14 in this experiment, were stained with antibodies against Flag as well as centrin-2. Histograms represent frequency of mitotic cells with excess foci of centrin in each condition. Values are mean percentages ± SEM from three

independent samples (n=30 for each condition). **P < 0.01, *P < 0.05, n.s., not significant (one tailed t-test).

Figure S4. The complex formation of STIL/CPAP is required for ectopic formation of centriolar protein complexes in RBM14-depleted cells

A. Soluble cytosolic fractions of asynchronous (-Noc) and nocodazole-arrested (+Noc) U2OS cells treated with RBM14 siRNA or control were analyzed using antibodies against STIL, RBM14 and α-tubulin.

B. Expression of CPAP-binding domain of STIL (STIL[CBD], aa231–781) have a dominant-negative effect on ectopic formation of centriolar protein complexes in RBM14-depleted cells. STIL FL, STIL[N], STIL[CBD], and STIL[C] were expressed in U2OS cells treated with control siRNA or RBM14 siRNA. Representative cells stained with antibodies against HA (magenta) and centrin-2 (green) are shown; DNA is shown in blue. Insets show approximately three-fold magnified views of fluorescent foci around the centrosome and scale bars are 10 μm. Histograms represent frequency of cells in interphase with excess centrin foci in the indicated conditions. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition). **P < 0.01, n.s., not significant (one tailed t-test).

C. Interaction between HA-STIL and GFP-CPAP in control or S-phase arrested 293T cells. 293T cells expressing HA-STIL and GFP-CPAP were incubated with or without Hydroxyurea for 16 hours. Soluble cytosolic fractions (input) were immunoprecipitated with anti-HA tag agarose beads (MBL, 561-8). The resulting IPs and input were analyzed by western blotting using STIL, CPAP and α-tubulin antibodies. Note that formation of cytoplasmic STIL/CPAP complex was enhanced in the S-phase arrested cells and also that expression levels of GFP-CPAP were up-regulated in S-phase arrested cells.

Figure S5. Ectopic formation of centriolar protein complexes depends on STIL, but not on HsSAS-6, Plk4 and PCM components.

A. RNAi efficiency in the experiments using double siRNAs in Fig 4A. Cytosolic fractions of the U2OS cells treated with the indicated siRNAs were analyzed by Western blotting using the antibodies against the targeted proteins.

B. HsSAS-6 depletion efficiently blocked centriole amplification provoked by HU-mediated cell cycle arrest in a similar experimental set-up of Fig 4A. U2OS cells treated with control siRNA or HsSAS-6 siRNA were arrested at S phase by addition of hydroxyurea (HU). The cells were then stained with antibodies against centrin-2 (green) and HsSAS-6 (magenta). DNA is shown in blue. Insets show three-fold magnified images of fluorescent foci. Histograms represent frequency of HU-treated cells in interphase with excess centrin foci in the indicated conditions. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition). **P < 0.01 (one tailed t-test).

C. Plk4 is dispensable for ectopic formation of centriolar protein complexes in RBM14-depleted U2OS cells. Histograms represent frequency of mitotic cells with >4 centrin foci and >1 HsSAS-6 foci in the indicated conditions. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition). **P < 0.01, n.s., not significant (one tailed t-test).

D. Cep192 depletion did not affect ectopic formation of centriolar protein complexes in RBM14-depleted cells. U2OS cells treated with the indicated combination of siRNAs were stained with antibodies against centrin-2 and γ-tubulin. Histograms represent frequency of mitotic cells with excess centrin foci or without γ-tubulin foci in each condition. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition). *P < 0.05 (one tailed t-test). Note that Cep192 depletion itself reduces the number of centrin foci presumably by affecting canonical centriole formation (Zhu et al, 2008).

E. Interaction between centriolar/centrosomal proteins and Flag-RBM14 FL or fragments. 293T cells expressing Flag-RBM14 FL, [N] or [C] were immunoprecipitated with anti-FLAG M2 agarose beads. The resulting

immunoprecipitated fractions and inputs were analyzed by western blotting using STIL, CPAP, HsSAS-6, centrin-2, γ-tubulin or Flag antibodies. Note that Flag-RBM14[C] interacts with STIL and γ-tubulin.

F. Microtubule depolymerization did not affect ectopic formation of centriolar protein complex in RBM14-depleted cells. U2OS cells transfected with RBM14 siRNA or control siRNAs were treated without or with nocodazole (200 ng/ml) for 15 hours, stained with antibodies against centrin-1 and tubulin. Histograms represent frequency of cells with excess centrin foci in each condition. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition). **P < 0.01 (one tailed t-test).

G and H. U2OS cells treated with RBM14 siRNA or control siRNAs for 24 hours were stained with antibodies against centrin-1 and PCNA to mark S-phase cells. Histograms represent frequency of PCNA-positive or negative cells with excess centrin foci (G) and CP110 foci (H) in each condition. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition). **P < 0.01, n.s., not significant (one tailed t-test). Note that when RBM14 was depleted, centrin and CP110 amplification occurs during S-phase.

I. Electron micrographs in U2OS cells depleted of RBM14. Squares indicate the ectopic centriolar protein complexes whereas circles indicate the preexisting centrioles. Bar, 500 nm. Insets show approximately 3.5-fold magnified views of a white square. Note that microtubules (white arrowheads) are observed within the electron-dense structures.

J. Excess centrin foci do not co-localize with PCM-1 foci. U2OS cells treated with RBM14 or negative control siRNA were stained with antibodies against centrin-2 (green) and PCM-1 (magenta). DNA is shown in blue. Insets show three-fold magnified images of fluorescent foci. Bar, 10 μm.

Figure S6. Ectopic formation of centriolar protein complexes that contain HsSAS-6 affects chromosome segregation and spindle formation during mitosis.

A. Selected frames from time-lapse imaging of HeLa cells expressing GFP-centrin1 (green) and RFP-H2B (magenta) and treated with RBM14 siRNA. Three types of phenotype frequently seen in the experiments are shown. Lagging chromosomes (white arrows) and multiple spindle poles (white arrowheads) are indicated. Time zero corresponding to onset of mitosis. Histograms represent the percentages of mitotic cells showing the indicated phenotype (n=45 for control, n=44 for RBM14 siRNA).

B. Mitotic U2OS cells treated with control or RBM14 siRNA were stained with antibodies against α-tubulin and Hoechst 33258. Histograms represent the percentages of mitotic cells showing multipolar spindles and lagging chromosomes. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition). *P < 0.05 (one tailed t-test).

C. U2OS cells treated with RBM14 siRNA and stained with antibodies against acetylated tubulin as well as HsSAS-6. DNA is shown in blue. Histograms represent the frequency of mitotic cells with the indicated phenotype Values are mean percentages ± SEM from three independent experiments (n=30 for each condition).

D. Control U2OS cells or U2OS cells treated with siRNA targeting RBM14 were stained with antibodies against HsSAS-6 (magenta, upper panels) or Cep135 (magenta, lower panels) as well as centrin-2 (green). DNA is shown in blue. Bar, 5 μm. Histograms represent frequency of mitotic cells with excess foci of Cep135 at spindle poles. Values are mean percentages ± SEM from three independent experiments (n=30 for each condition). *P < 0.05 (one tailed t-test).

E. RBM14-depleted U2OS cells were stained with antibodies against Cep135 (magenta, left panel) or Cep152 (magenta, right panel) as well as HsSAS-6 (green). DNA is shown in blue. Insets show three-fold magnified images of fluorescent foci. Bar, 5 μm.

Figure S7. Ectopic centriolar protein complexes in RBM14-depleted cells recruit HsSAS-6 in the cytoplasm.

A. U2OS cells or U2OS cells expressing HsSAS-6-GFP and treated with control siRNA or RBM14 siRNA were stained with antibodies against HsSAS-6 (green) and centrin-2 (magenta). DNA is shown in blue. Histograms represent frequency of mitotic cells with the number of centrin or HsSAS-6 in the indicated conditions. Three independent experiments were performed (n=30 for each condition).

B. Schematic of the experimental design for live cell imaging conducted in (C).

C. Live imaging of RBM14-depleted U2OS cells expressing HsSAS-6-GFP (green) and treated with aphidicolin (APH). Time zero corresponding to the onset of recording. Upper panels represent the cell with ectopic HsSAS-6-GFP foci that were formed nearby pre-existing centrioles (white arrow). Lower panels represent the cell with ectopic HsSAS-6-GFP focus that was formed in the cytoplasm. Pre-existing HsSAS-6-GFP foci (white arrowheads) and ectopic HsSAS-6-GFP focus (red arrowhead) were traced throughout the time lapse recording. White broken lines represent cell shapes and blue broken lines represent nuclear shapes. Histograms represent the percentages of cells with ectopic HsSAS-6 foci in the indicated conditions. (n=57 cells for control, and n=53 cells for RBM14 siRNA).

D. Mitotic U2OS cells expressing HsSAS-6-GFP were treated with the indicated combinations of siRNAs and stained with HsSAS-6 and centrin. Histograms represent frequency of cells with excess foci of centrin and HsSAS-6 in the indicated conditions. We counted cells before metaphase/anaphase transition considering the disappearance of HsSAS-6 from anaphase in mitosis. Values are mean percentages ± SEM from three independent samples (n=30 for each condition). **P < 0.01, n.s., not significant (one tailed t-test).

Figure S8. Ectopic centriolar protein complexes can become a morphologically recognizable centriole-like structure.

A. Correlation between ectopic GFP-centrin foci and the structures on serial sectioning EM images of the corresponding region in Fig 6H. HeLa cells expressing GFP-centrin (green) and RFP-H2B (magenta) treated with RBM14 siRNA were fixed after live-imaging observation. The two left upper panels indicate Z-stacked images of the whole cell and magnified area around GFP-centrin foci. Separate Z-plane images of the magnified area around GFP-centrin foci are also aligned from bottom to top along Z-axis in upper panels. Lower panels indicate serial sections of EM images of the corresponding region and aligned from bottom to top along Z-axis. Bar, 10 μm (LM image), 1 μm (magnified LM images), and 500 nm (EM images). Labeled numbers of the GFP-centrin foci on LM images or the structures on EM images are identical to the numbers shown in Fig 6H. The asterisks indicate a vacuole-like structure showing that the serial EM sections are properly aligned from bottom to top along the Z-axis. Note that the whole configuration of structure 3 might not be shown as a few sections between the two right EM panels were lost.

B. A point diagram showing positional relationship between GFP-centrin foci along Z-axis during time-lapse recording in Fig 6H, Figure S8A, and Movie S7. We manually traced and plotted all GFP-centrin foci captured in 25 focal planes at intervals of 1.2 μm. Time zero corresponding to the onset of assembly of ectopic GFP-centrin foci. The two large GFP-centrin foci reflected preexisting centrioles and the three small foci assembled ectopically. As GFP-centrin foci move rapidly in the cytoplasm, we could not identify individual large and small foci. Note that preexisting large GFP centrin foci located very close to each other throughout time-lapse observation even in mitosis. In contrast, whereas ectopic GFP-centrin foci moved in the cytoplasm independently of each other, at the onset of mitosis, three ectopic GFP centrin foci began clustering and formed a single spindle pole.

C. HeLa cells expressing GFP-centrin (green) and RFP-H2B (magenta) and treated with RBM14 siRNA were analyzed using Live-CLEM. Individual pre-existing centrin foci (white arrowheads) and ectopic centrin foci (red circle) were traced throughout the time lapse recording and Z-stacked confocal images spanning the entire height of the cells (< 30 μm) are shown. Time zero corresponding to the metaphase onset.

Note that ectopic centrin foci formed a spindle pole and distributed to one daughter cell (b). The other daughter cell (a) containing preexisting centrioles died after mitosis. 3-fold magnified region around ectopic centrin foci (white box), and 10 and 20-fold magnified views of the region in a black box are shown in EM panels. Note that ectopic centrin foci include not only electron-dense structures (2-4, and 6-7) but also morphologically recognizable centriole-like structures (1 and 5). Bar, 10 μm (Live imaging and LM/EM images) and 500 nm (EM).

Figure S9. Depletion of mRBM14 in the early mouse embryo induces ectopic formation of centriolar protein complexes.

A. A schematic represents the design of RNAi experiments using early mouse embryos.

B. Soluble cytosolic fractions of mouse NIH3T3 cells treated with mRBM14 siRNA or control siRNA were analyzed using antibodies against RBM14, STIL, α-tubulin and centrin. Note that whereas mRBM14 protein level was significantly reduced by treatment with mRBM14 siRNA, the other protein levels were not affected.

C. NIH3T3 cells treated with mRBM14 siRNA or control siRNA were stained with the antibodies against centrin. Histograms represent the frequency of mitotic cells with excess foci of centrin. Values are mean percentages ± SEM from four independent experiments (n=20–29 for each condition).

D. E4.0 mouse embryos injected with mRBM14 siRNA were stained with antibodies against RBM14 (green), and analyzed by DIC and fluorescence microscopy. The injected blastomeres were marked with memTomato (magenta). Insets show approximately two-fold magnified views of a white square. Bar, 50 μm. Note that mRBM14 signals were decreased in the blastomeres injected with mRBM14 siRNA (red arrows) compared with the uninjected ones (white arrows).

E–G. E4.0 embryos of GFP-centrin (green) transgenic mice injected with negative control siRNA (E) or mRBM14 siRNA (F) were analyzed by DIC and fluorescence

microscopy. Injected blastomeres were marked with Tomato (magenta). Insets show approximately three-fold magnified views of a white square. GFP-centrin foci in the injected blastomeres are marked by arrowheads. Bar, 50 μm. (G) Histograms represent frequency of the injected blastomeres with the indicated numbers of GFP-centrin foci. Values are mean percentages ± SEM from 9 embryos (114 blastomeres) for control and 25 embryos (353 blastomeres) for mRBM14 siRNA. *P < 0.05 (one tailed t-test). Note that there are normally 0–2 GFP-centrin foci in the control blastomeres at this stage.

Supplementary Movie Legends

Movie S1. Ectopic formation of centriolar protein complexes in RBM14-depleted HeLa cells.

This movie shows live imaging of cycling RBM14-depleted HeLa cells expressing GFP-centrin1 (green) and RFP-H2B (magenta). All Supplementary Movies in this paper were created from z-stacked images spanning the entire height of the cells (<30 μm). Time zero corresponding to the onset of excess centrin foci formation. Total elapsed time is 5 hours. In this and other Supplementary Movies, time is denoted in hours: minutes, and scale bars are 10 μm. Images were captured every 5 minutes and movies are played at 7 frames per second.

Movie S2. Shuttling of small centrin foci between the cytoplasm and the centrioles in control HeLa cells.

This movie shows live imaging of cycling HeLa cells expressing GFP-centrin1 (green) and RFP-H2B (magenta) that were transfected with control siRNA. Time zero corresponding to the onset of small centrin foci formation. Total elapsed time is 6 hours.

Movie S3. Detailed observation of ectopic formation of centriolar protein complexes in RBM14-depleted HeLa cells.

This movie shows live imaging of cycling RBM14-depleted HeLa cells expressing GFP-centrin1 (green) and RFP-H2B (magenta). Time zero corresponding to the

onset of excess centrin foci formation. Total elapsed time is 5 hours 45 minutes. Note that some of small centrin foci in the cytoplasm did not move to the centrioles but fused to each other, and grew up to the comparable size as centrin foci of preexisting centrioles.

Movie S4. Ectopic formation of centrin foci occurs approximately from late G1 to S phase.

This movie shows long-term live imaging of cycling RBM14-depleted HeLa cells expressing GFP-centrin1 (green) and RFP-H2B (magenta). Time zero corresponding to the onset of metaphase. Total elapsed time is 22 hours. Note that excess formation of centrin foci occurred at approximately 13 hours after the previous mitosis/9 hours before the next mitosis, corresponding to approximately late G1 or S phase in HeLa cells.

Movie S5. Formation of ectopic HsSAS-6 foci in the cytoplasm of RBM14-depleted U2OS cell.

This movie shows live imaging of RBM14-depleted U2OS cells expressing HsSAS-6-GFP (green) and treated with aphidicolin. Time zero corresponding to the onset of recording. Total elapsed time is 8 hours 10 minutes. Images were captured every 5 minutes and movies are played at 7 frames per second. We moved the recording area toward lower left side at 4 hours 45 minutes after the onset of recording, because the cell of interest seemed to move outside the recording area. Note that the formation of ectopic HsSAS-6 foci occurred in the cytoplasm even when the centriole disengagement was inhibited by aphidicolin treatment.

Movie S6. Ectopic centrin foci incorporate HsSAS-6 proteins in the cytoplasm of RBM14-depleted U2OS cell.

This movie shows live imaging of RBM14-depleted U2OS cells expressing HsSAS-6-GFP (green) and DsRed-centrin-2 (magenta). Time zero corresponding to the onset of recording. Total elapsed time is 7 hours. Images were captured every 5 minutes and movies are played at 7 frames per second.

Movie S7. Formation of ectopic centrin foci in RBM14-depleted HeLa cells analyzed by Live-CLEM.

This movie shows long-term live imaging of cycling RBM14-depleted HeLa cells expressing GFP-centrin1 (green) and RFP-H2B (magenta). Time zero corresponding to the onset of ectopic formation of centrin foci. Total elapsed time is 11 hours 10 minutes. Note that, in the cell, pre-existing centrin foci fail to separate each other and organize a single spindle pole whereas ectopic GFP-centrin foci form the other spindle pole. Images were captured every 10 minutes and movies are played at 7 frames per second.