Supplementary Figures

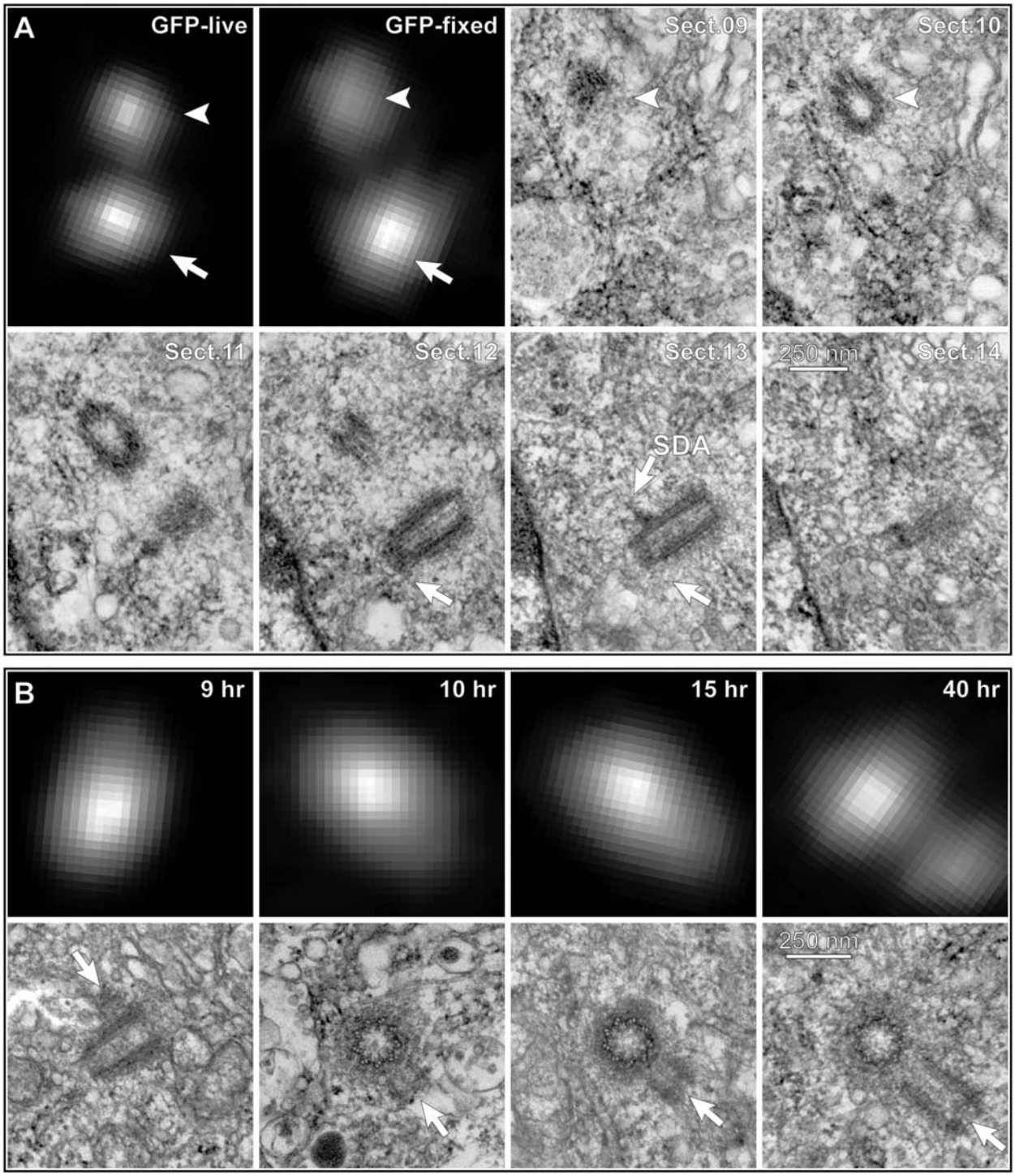

Figure S1. Centrin-GFP is a reliable live-cell marker of centriole replication. **(A)** Typical centrosome in a HeLa cell during G_1. In the live cell both centrioles appear as round spots of centrin-GFP. One of the spots is consistently brighter than the other ("GFP-live" frame).

Fixation with 2.5% glutaraldehyde results in only minor degradation of the image which occurs primarily due to the increase in non-specific green fluorescence in the cytoplasm (an effect which decreases the signal-to-noise ratio). However, it was not unusual for the centrioles to slightly change their orientation during the time necessary for fixation (cf. “GFP-live” and “GFP-fixed” frames). This change is likely due to the high mobility of centrioles in the cytoplasm. Both GFP images are maximal-intensity projections of a Z-series through the entire centrosome (200-nm steps). Serial-section EM analysis of the same cell revealed that the brighter centrin-GFP spot corresponded to the mother centriole (arrow in each frame) as evidenced by the fact that this centriole bears sub-distal appendages (labelled SDA in “sect.13” frame). The dimmer centrin-GFP spot corresponds to the daughter centriole (arrowhead in each frame). **(B)** Changes in the appearance of centrin-GFP spots during centriole replication. Maximal intensity projections of GFP-fluorescence series (200-nm steps) recorded immediately after fixation are shown in the top frame of each pair while the bottom frame presents a single EM section (80-nm) selected from the complete series through the same centrosome. Cells with the minimal distortion of the centrin-GFP spots contained 50-70-nm “procentrioles”; these appear as electron-dense material lacking developed microtubule triplets (arrow in lower “9 hr” frame). Daughter centrioles as small as ~150 nm create a prominent distortion of the centrin-GFP spot (arrow in lower “10 hr” frame). At later stages, centrin-GFP distortions become even more pronounced as daughter centrioles elongate (arrow in lower “15 hr” frame). Finally, the centrin-GFP spots become resolvable when daughter centrioles reach lengths of >250 nm (arrow in lower “40 hr” frame). The time shown on each frame reflects when each individual cell was fixed (after mitotic shake-off).

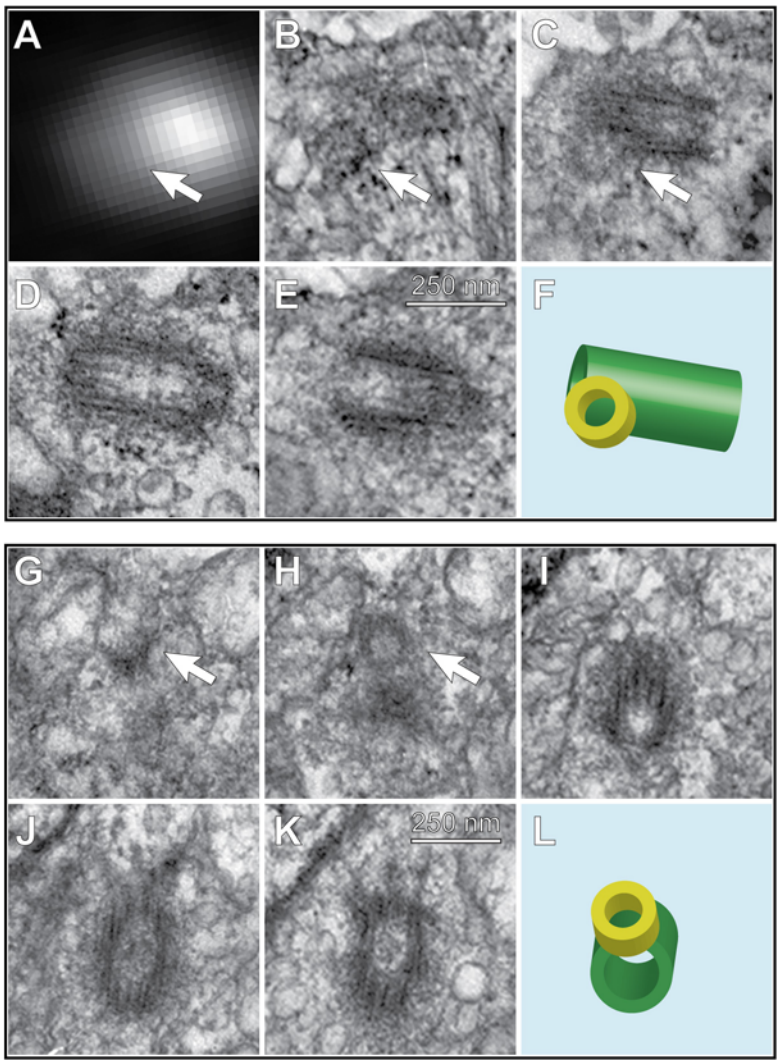

Figure S2. Procentrioles are not always oriented orthogonal to the mother centriole. Two examples (A-F and G-L) of early stages of centriole replication are shown. This stage of procentriole development is manifested by a slight distortion in the appearance of centrin-GFP spots (arrow in A). Such a distortion consistently corresponds to short (<100 nm) procentrioles (arrows in B-C and G-H). Surprisingly, young procentrioles are positioned at random angles with respect to mother centrioles (schematized in F and L).

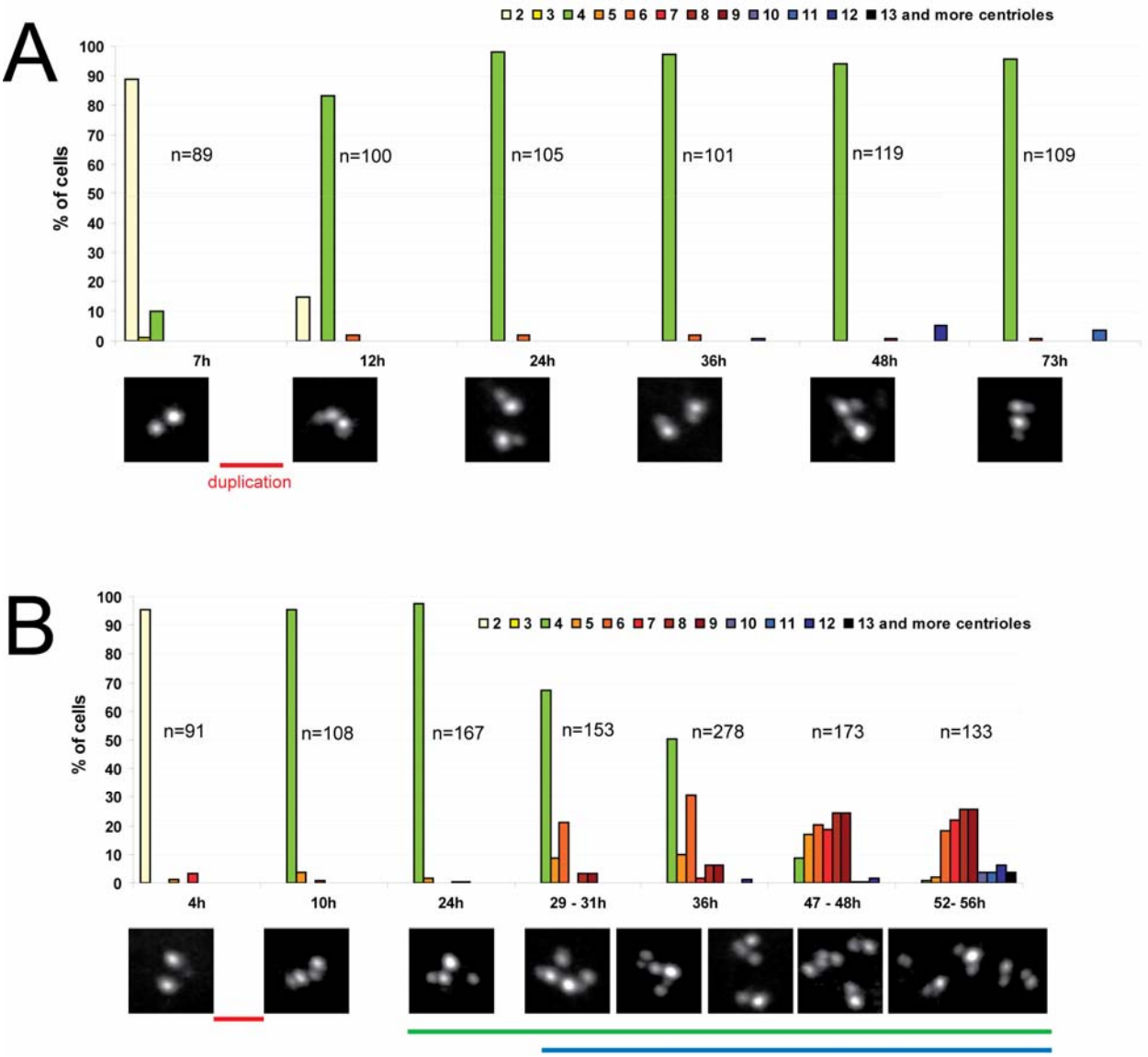

Figure S3. Dynamics of centriole duplication in S-arrested HeLa and CHO cells. Mitotic cells were collected by shake-off from untreated cultures and plated on coverslips in full growth medium supplemented with 2-mM hydroxyurea. Complete Z-series of centrin-GFP fluorescence were collected in live cells. Polyploid cells (recognized by exceedingly large nuclei and/or multiple nuclei) were excluded from analyses. Plots present percentages of cells with different numbers of centrioles in HeLa **(A)** and CHO **(B)** cells; typical centriolar configurations at the various time points are also shown (time after mitotic shake-off).

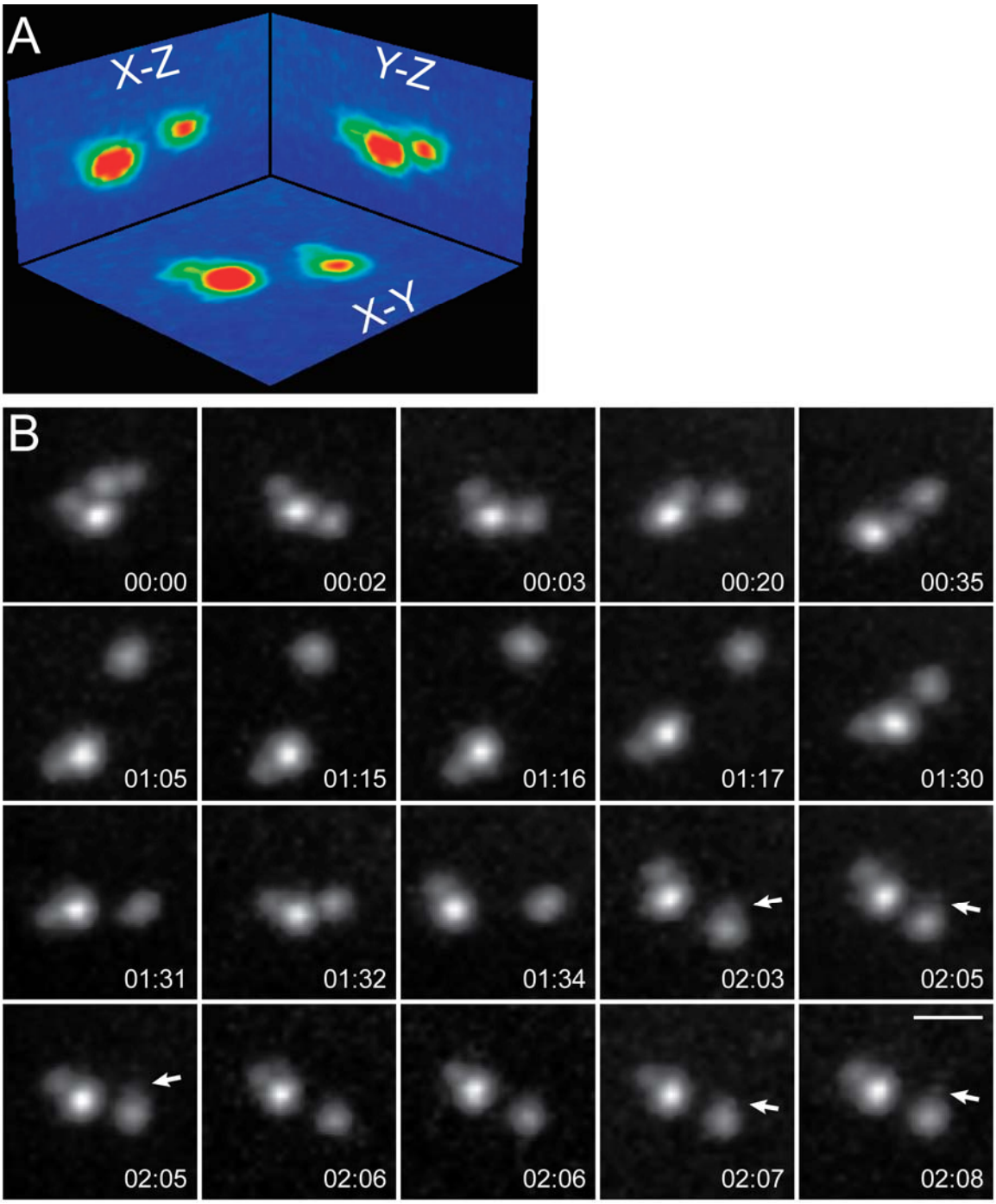

Figure S4. Complete set of fluorescence images illustrating the experiment presented in Fig. 1A. **(A)** Daughter centrioles can sometimes be masked by their mothers in planar projections. Notice that in this 3-D reconstruction (computed from the dataset presented in panel B "02:08") daughter centrioles in both diplosomes are clearly seen in the XY

projection. However, only one of the daughters is seen in the YZ, and none in the XZ projection. This implies that the ability to detect the initial stages of daughter centriole formation depend on the orientation of the diplosome. **(B)** To circumvent problems associated with unfavourable orientation of centrioles we collected several fluorescence datasets (30-60 seconds apart) at a single time point. Because centrioles continuously tumble in live cells, these multiple datasets allowed us to record the same diplosome in different orientations. Notice that the newly formed daughter centriole (arrows) is seen in five of seven datasets recorded at 2 hours after ablation of the original daughter. Time in hours : minutes. Scale bar represents 1 µm.

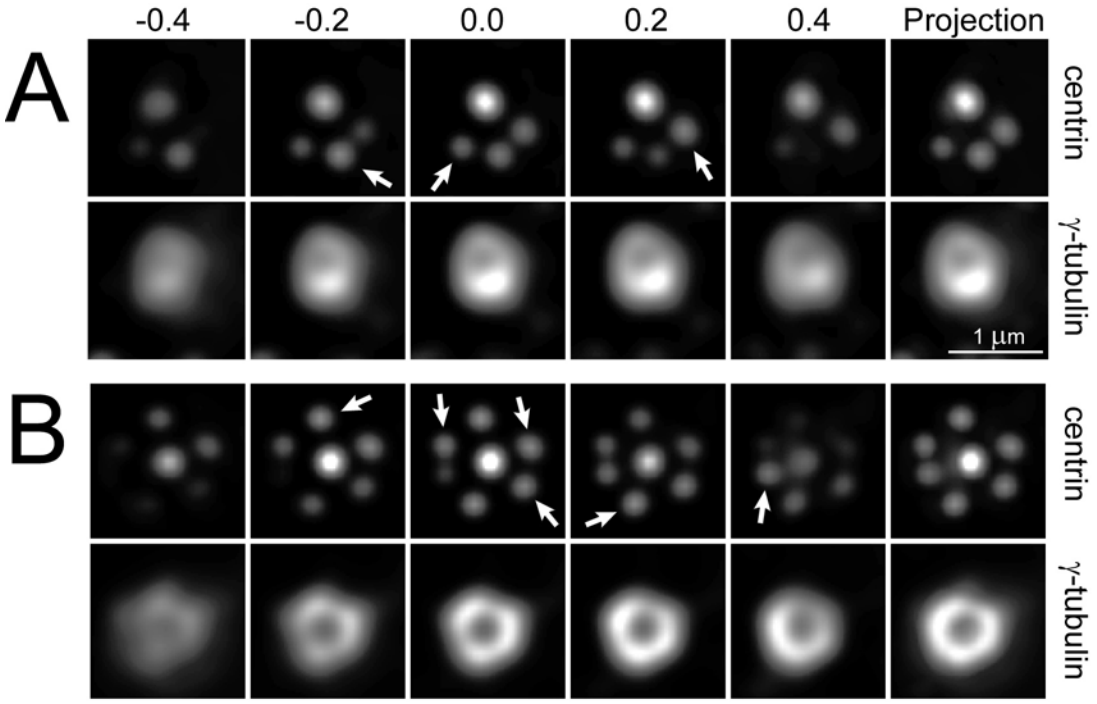

Figure S5. Organization of centriolar rosettes in CHO cells. **(A)** In this example four daughter centrioles form simultaneously in association with a single mother. Arrows indicate maximal intensities of centrin-GFP fluorescence of each daughter centriole. The middle Z-plane (0.0 µm) corresponds to the maximal intensity of centrin-GFP in the mother centriole. **(B)** another example of multiple daughter centrioles associated with a single mother. In this case there are 6 daughter centrioles. Notice that the 3-D distribution of centrin GFP in the daughter centrioles indicates that different daughters reside at different heights along the mother centriole.

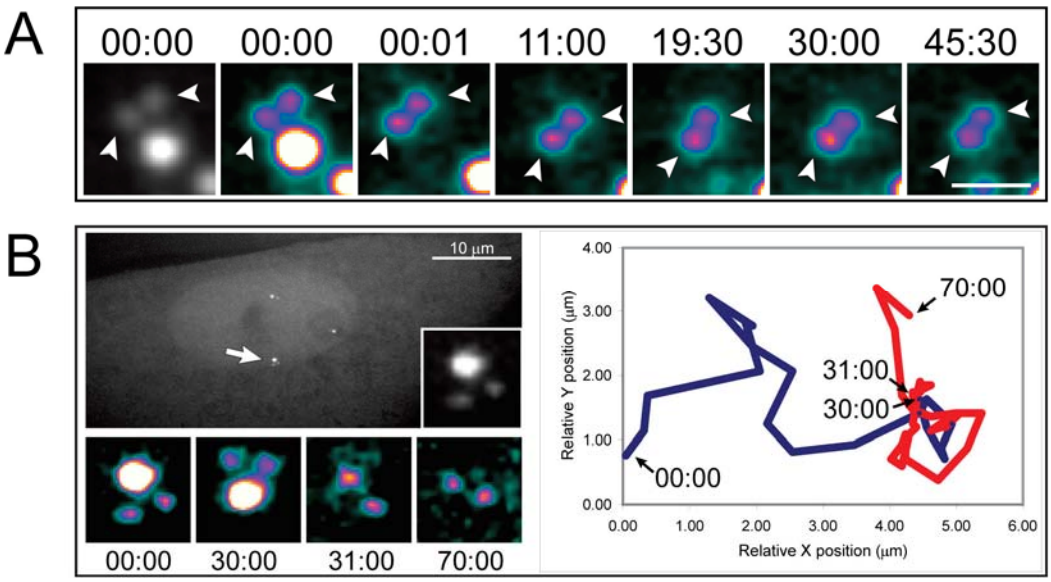

Figure S6. Daughter centrioles remain connected after ablation of the mother. **(A)** Example of mother-centriole ablation in a triplosome. The two daughters (arrowheads) remained in a common complex for ~45 min, at which point the cell was fixed. Serial-section EM analysis confirmed that the mother centriole was destroyed (not shown). **(B)** Another example of mother centriole ablation in a triplosome. In this cell, the centrioles did not form a common complex in the centre of the cell. Instead, individual diplosomes and a triplosome (arrow and inset in the first image) moved extensively through the cytoplasm. The trajectory of the triplosome is depicted by the blue line in the graph on the right. 30 min after the initiation of time-lapse recording, the mother centriole was ablated (cf. 30:00 and 31:00 frames). The remaining daughters continued to move in the cytoplasm (red line) as a single common complex for at least 40 min (cf. 31:00 and 70:00 frames). Scale bar in A = 1 µm. Same LUT as in Fig.1. Time stamps in minutes : seconds.

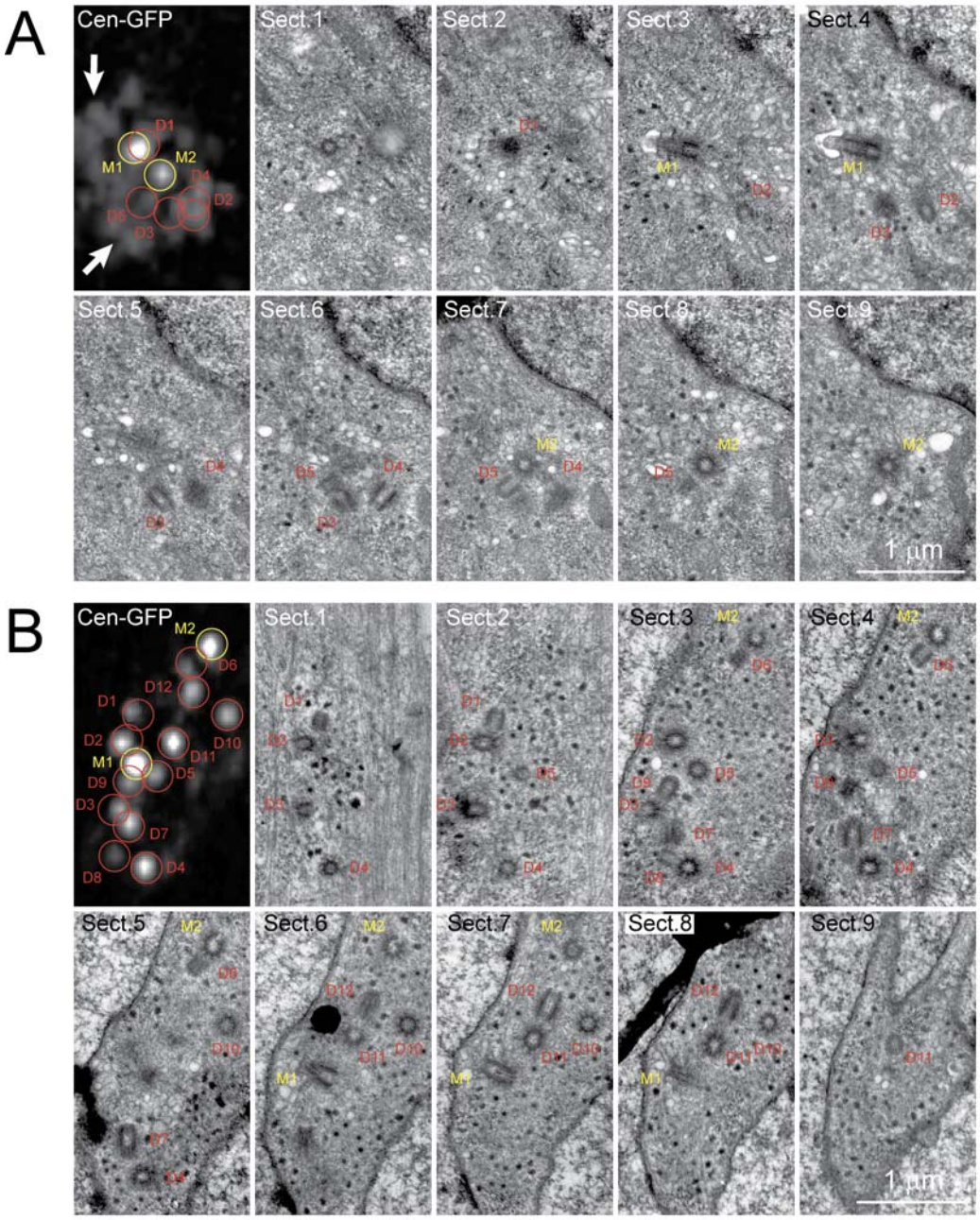

Figure S7. Many of centrin-GFP aggregates that form in S-arrested CHO cells upon overexpression of pericentrin are centrioles. Maximal-intensity projection of a 3-D fluorescence dataset (first frame) and serial 80-nm EM sections of two cells fixed 25 hr (A) and 45 hr (B) after transfection. See text for details.

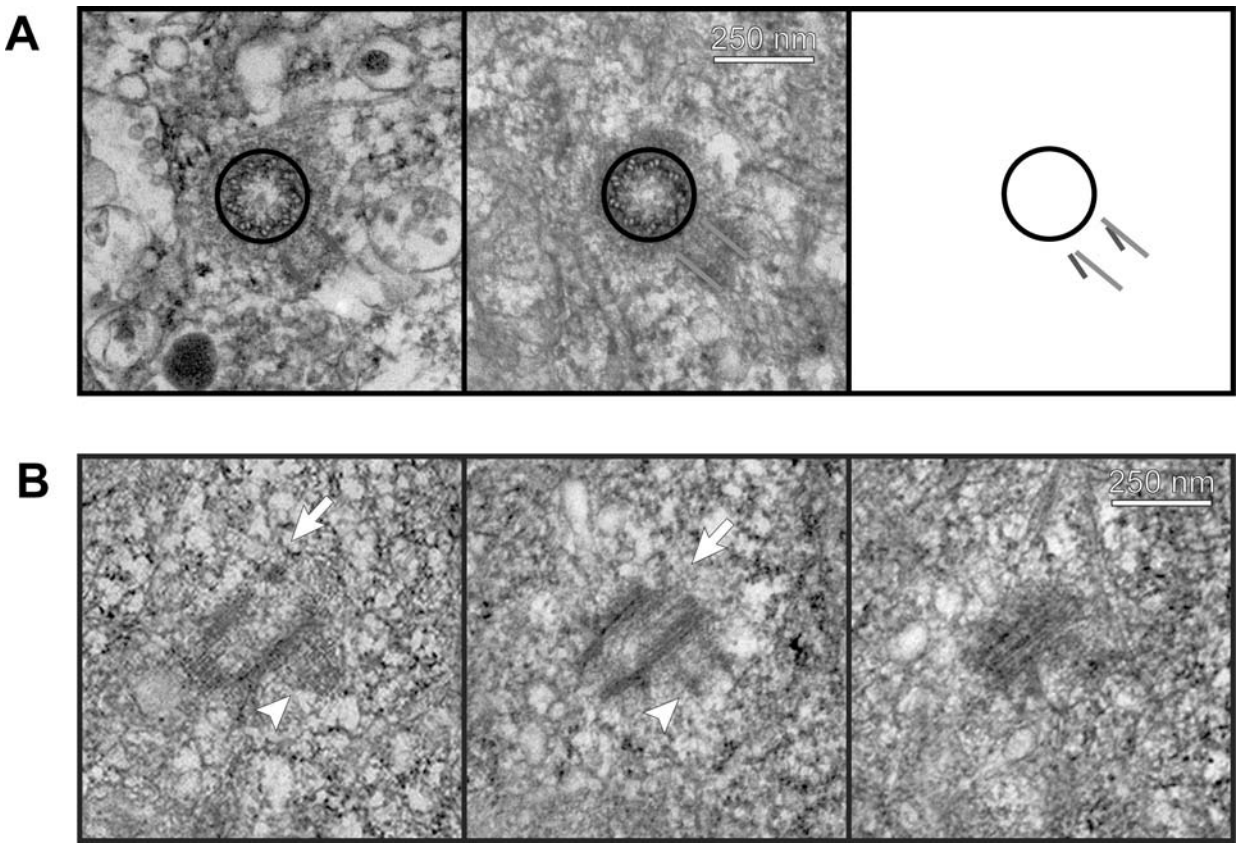

Figure S8. Additional data that suggest that daughter centrioles are not stringently attached to the wall of their mother. **(A)** The radial orientation of daughter centrioles, with respect to microtubule triplets in the mother, varies among individual diplosomes. Two diplosomes in different HeLa cells (also shown in Fig. S1B). Mother centrioles are rotationally aligned (notice position of the triplets). Positions of daughter centrioles are marked with coloured lines. Notice that because of the 9-fold radial symmetry of the centriole, the maximum variability in radial orientation of daughter centrioles cannot exceed $\pm 20^{o}$ because 40^{o} rotation will place the daughter on the next microtubule triplet in the mother. The angle between the radial positions in the two diplosomes shown is 17^{o}. **(B)** An example of a "nick" (arrow) on the mother centriole, inflicted by the laser beam during ablation of the daughter in a HeLa cell. Notice that the new daughter centriole (arrowhead) is attached to the mother on the side opposite from the nick.

Legends for Supplementary Videos

Video S1. Centriole behaviour during normal cell cycle in HeLa cells expressing centrin-GFP. The movie begins 100 min before mitosis and then follows one of the two daughter cells as it progresses through the cell cycle and into the next mitosis ~20 hrs later. Notice that the centrioles continuously move in the cytoplasm at all stages of the cell cycle. The mother centriole is consistently brighter than the daughter. Both centrioles become visually doubled ~5 hrs before the second mitosis (~900 min time point). Time in minutes; time 0 corresponds to the completion of the first mitosis. 5-min intervals, each frame is a maximal-intensity projection of the complete Z-series.

Video S2. Centriole reduplication in S-phase arrested CHO cells. The movie begins when both mother centrioles have undergone first round of duplication so that the cell contains two diplosomes. Centrioles in one diplosome disengage ~5 hr and the second diplosome breaks down ~8.5 hr after the beginning of the movie. All centrioles subsequently duplicate although it is not possible to point out the exact moment of duplication for each centriole in this movie which was recorded at a low intensity of the excitation light. Time in hours; time 0.0 corresponds to 20 hrs after mitotic shake off and addition of 2-mM hydroxyurea. 30-min intervals, each DIC frame is a single Z-plane, each fluorescence frame is a maximal-intensity projection of the complete Z-series.

Video S3. Reduplication of mother centrioles in S-phase arrested CHO cell. High temporal-resolution time-lapse recording that depicts the moment of centriole reduplication (~30 hr after mitotic shake off). Both mother centrioles (brighter spots) in this cell have duplicated once and disengaged from their daughters (dimmer spots). Notice that the mother and daughter centrioles in the centre of the frame 0.0 min reside very close to each other. However these centrioles are not engaged as evident from their uncoordinated movements.

The mother centriole in the centre of the frame develops a shadow that gradually transforms into a daughter centriole in ~30 min time period. The second mother centriole also reduplicates during this period and develops a clearly visible shadow by the “24.5 min” time point. Individual frames in this movie are aligned so that the mother centriole in the centre appears to be stationary. Each frame is a maximal-intensity projection. Fluorescence intensity is colour-coded using the lookup table presented in Fig.1.

Video S4. Movements of triplosomes and individual centrioles. Time-lapse recording of an HU-treated CHO cell (~ 47 hr after mitotic shake-off; 30-s interval; each frame is a maximal-intensity projection of the complete Z-series). During reduplication, both mother centrioles in this cell each developed two new daughters, forming ‘triplosomes’. Neither of the first-generation daughters has duplicated.

Video S5. Movements of diplosomes and triplosomes. Similar to Video S4, except that in this CHO cell, one mother centriole formed a single daughter, while the second mother centriole simultaneously developed two daughters. Both first-generation daughters also duplicated, forming diplosomes. Notice that movements of centrioles within the diplosomes and the triplosome are coordinated.

Video S6. The mother centriole and the two daughter centrioles within a triplosome are rigidly interconnected. Time-lapse recording of a triplosome in a CHO cell (60-s intervals; each frame is a maximal-intensity projection of the complete Z-series). Individual frames in this movie were aligned with respect to the mother centriole, which thus appears stationary in the movie. Daughter centrioles appear to move circumferentially around the mother centriole, an effect which is likely to be due to rotation of the entire centrosome. However, the distances between the mother and each of the daughters, as well as between the

daughter centrioles, remain constant (within the resolution of the recording). Pseudo-colour intensity lookup table, same as in Fig. 1.

Video S7. Uncoordinated movements of individual adjacent centrioles. Similar to Video S5, except that this movie presents a single centriole that is in close spatial proximity to a diplosome, in a CHO cell. Although in individual frames such a configuration may resemble a triplosome, the lack of coordination between the movements of the diplosome and the single centriole becomes apparent in the video. The sequence is aligned with respect to the mother centriole in the diplosome. Pseudo-colour intensity lookup table, same as in Fig. 1.

Video S8. Movements of daughter centrioles remain coordinated after ablation of the mother centriole in the triplosome. Individual frames are maximal intensity projections. The first frame presents a triplosome in a CHO cell immediately before laser irradiation. At this time, daughter centrioles were in the same focal plane which corresponds to the maximal apparent distance between two objects in a projection. Although the apparent distance between daughter centrioles appears to decrease in some frames, this effect is due to tumbling of the centrosome in 3D. The fact that separation between the centrioles never exceeds the original distance (indicated by yellow circle) reveals that they remain connected. The sequence is aligned with respect to the brighter daughter centriole. Pseudo-colour intensity lookup table, same as in Fig. 1.

Videos S9 – S11. Proximal ends of daughter centrioles in triplosomes (Videos S9 and S10) or in a large cloud of PCM induced _consistently reside within the cloud of γ-tubulin (PCM). Surface-rendered models presented in these movies represent centrosomes also shown in Fig.4D. Centrin-GFP which marks the distal end of centriole is shown in the green, SAS-6

which marks the proximal end of daughter centrioles is shown in the red, and γ-tubulin (PCM) is shown as isosurface corresponding to 25% of the maximal signal.