nature cell biology

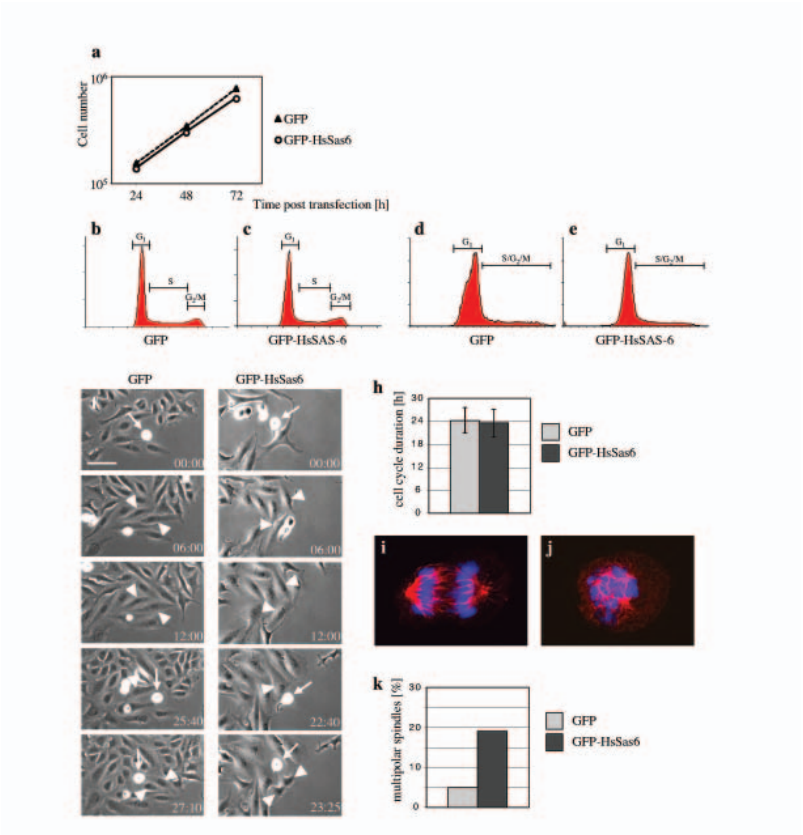

Figure S1 (a) Bulk cell proliferation assay. Approximately 0.4x10^5 U2OS cells were seeded on several 6cm diameter dishes and transfected 24h later with GFP or GFP-HsSAS-6. After an additional 24h, 48h or 72h, cells in one dish per construct were harvested and cell number determined using a Neubauer counting chamber. Note that experimental and control cell populations have identical proliferation profiles. (b-e) FACS analysis. Cells were grown in 10 cm dishes, transfected with GFP or GFP-HsSAS-6 and processed for immunofluorescence and FACS analysis 48 hours after transfection. For analysis, cells were gated to remove doublets and debris. For FACS analysis with propidium iodide (PI) (b, c), cells were trypsinized and washed in PBS. Cold 70% Ethanol was added drop-wise to pellets, cells left at 4 °C for 30min and then washed 2x in PBS. 50µl of 100µg/ml RNAseA (Sigma) was added and the cells incubated at 37 °C for 1h. 200µl of 50µg/ml propidium iodide (Sigma) was then added and cells analyzed using CellQuest on a BD FACScan. The fraction of cells in the different phases of the cell cycle (as indicated in the figure) were as follows: G1, 64% (GFP) and 67% (GFP-HsSAS-6); S: 21% (GFP) and 19% (GFP-HsSAS-6); G2/M: 15% (GFP) and 15% (GFP-HsSAS-6). For bi-parametric FACS analysis with GFP/Hoechst (d, e), cells were trypsinized, washed in PBS-3%FCS, incubated with Hoechst 33342 (Sigma) for 10min at room temperature and analyzed on a BD-LSR. Live cells were divided into groups of no/low, medium and high expression levels of GFP. Only cells with medium expressing levels are shown, but FACS profiles of cells with no/low and high expression levels of GFP were analogous. The fraction of cells in G1 versus the remainder of the cell cycle (as indicated in the figure) were as follows: G1, 77% (GFP) and 71% (GFP-HsSAS-6); S/G2/M: 25% (GFP) and 20% (GFP-HsSAS-6). (f-h) Live analysis of cell cycle progression. U2OS cells placed in a 6-well dish were transfected as in (a) and transferred 24h later to a homemade incubator chamber on an automated Zeiss Axioplan 100 microscope. The chamber was heated at 37 °C. supplemented with ~5% CO_2 and the medium was supplemented with 20mM HEPES pH 7.5. Infrared phase-images were taken through a 10x lens every 5 minutes at 5 different locations for each condition during 48 hours, controlled by Metamorph software (Universal). At the end of the sequence, a fluorescent image was taken for each of the 10 positions to reveal cells expressing GFP and thus restrict analysis to transfected cells. Cells with intermediate GFP levels were chosen as they displayed most consistently excess foci in the immunofluorescence analysis and their cell cycling time analyzed using the time-lapse recordings. Shown are 5 images from a time-lapse sequence of cells transfected with GFP (f) or GFP-HsSAS-6 (g); scale bar is 50 µm. Arrows point to relevant cells in mitosis, arrowheads to their daughters; elapsed time is shown in hours and min. Note that the average duration of the cell cycle is statistically indistinguishable when comparing cells transfected with GFP or GFP-HsSAS-6 (h; n=19 and 18, respectively; p>0.26; Student's T-test). (i-k) U2OS cells transfected with GFP (i) or GFP-HsSAS-6 (j) were fixed 48 hours thereafter and stained with antibodies against α-tubulin (red); DNA is shown in blue. Note bipolar spindle in (i) and multipolar spindle in (j); scale bar is 10 µm. Shown also is the incidence of multipolar spindles in U2OS cells transfected with GFP or GFP-HsSAS-6 and stained with antibodies against poly-glutamylated tubulin to accurately score spindle poles (k).

Movies 1 and 2 Dual GFP fluorescence and DIC time-lapse recordings of wild-type (Movie 1) or *sas-6*(*RNAi*) (Movie 2) embryos expressing GFP-TAC-1, from the time of pronuclear formation until the end of the second cell cycle. Images were captured every 10 seconds and the display rate is 10 frames per second; elapsed time indicated in minutes and seconds. Note that the intensity of GFP-TAC-1 signal is maximal during mitosis, and that centrosomes are not always in focus.

Movie 3 Spinning disc confocal time-lapse recording of a one-cell stage embryo expressing GFP-SAS-6, from the time of pronuclear formation until the end of the first cell cycle. Six confocal slices ~1 µm-apart were captured every 15 seconds and a movie reconstructed by choosing at each time-point two neighboring focal planes containing centrioles. Display rate is 10 frames per second; elapsed time indicated in minutes and seconds. Note that centrioles in both centrosomes are not in focus in all images.