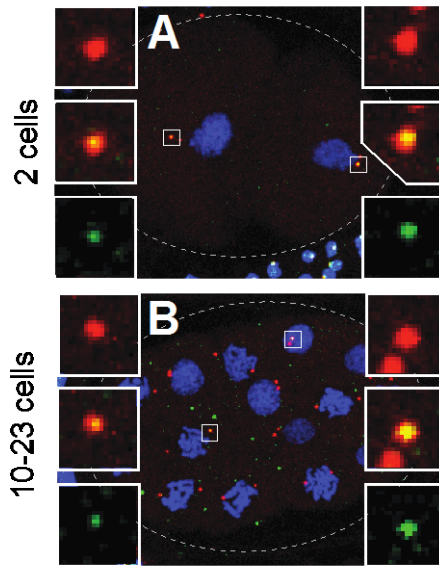


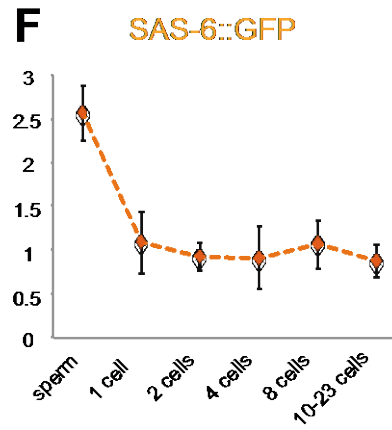
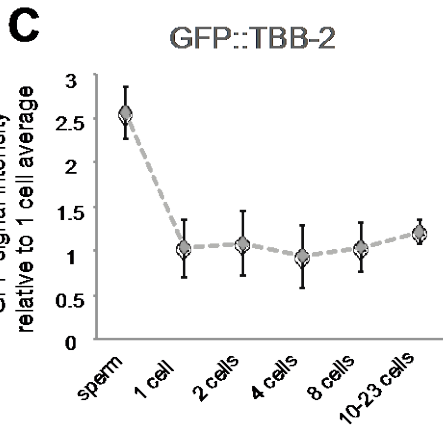
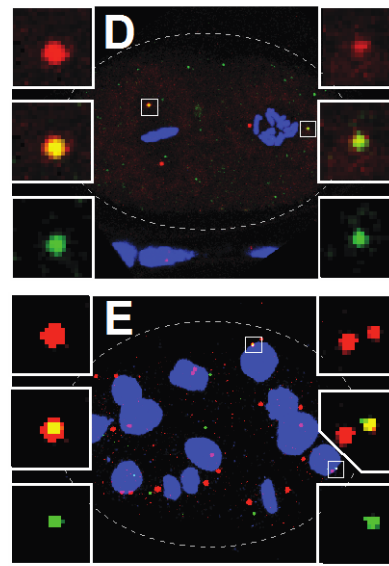
### GFP::TBB-2

GFP DNA IFA



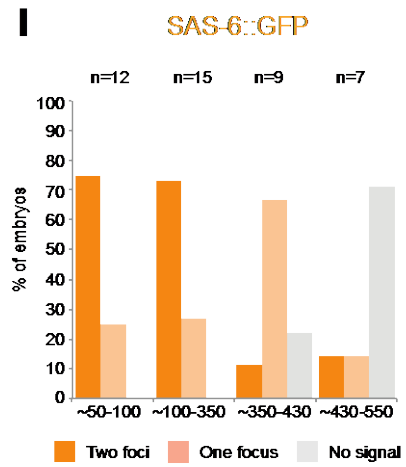
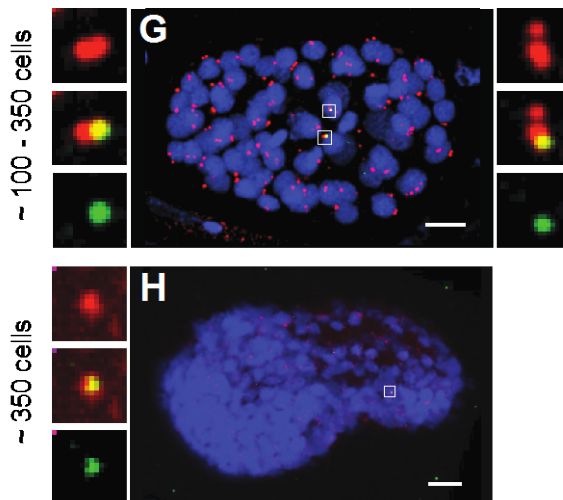
### SAS-6::GFP

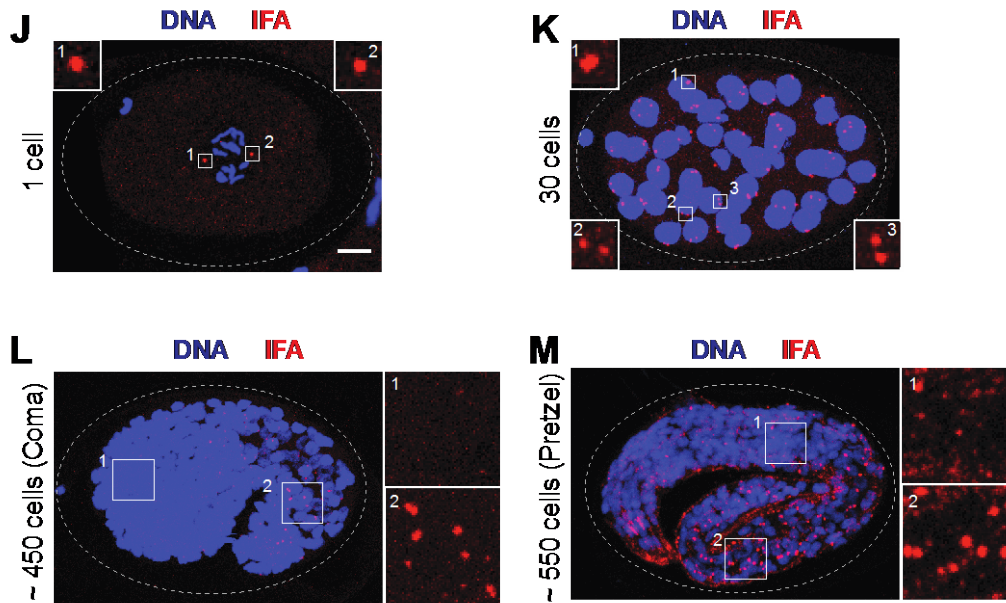
GFP DNA IFA



### SAS-6::GFP

GFP DNA IFA





**Supplementary information, Figure S1.** Distribution of centriolar components in *C. elegans* embryos. **(A, B, D, E)** Images of representative embryos at two developmental stages from marked mating experiment for GFP::TBB-2 (A, B) and SAS-6::GFP (D, E), stained with antibodies against GFP (green), the pan-centriolar marker IFA (red), as well as with Hoechst to reveal DNA (blue). Note that the GFP::TBB-2 signal intensity often differs between the two paternally contributed centrioles in the marked mating experiment for GFP::TBB-2 (A, B) **(C, F)** Quantification by immunofluorescence analysis of the GFP signal at paternally contributed centrioles at the indicated stages of early development for GFP::TBB-2 (C) and SAS-6::GFP (F). Signal intensity is expressed relative to the average signal of the 1 cell-stage embryos. In C, only the brightest centriole in each embryo was quantified. Note also that although levels of centriolar SAS-6 fluctuate somewhat during the first embryonic division [6], the majority of centriolar SAS-6 remain stable, and this is likely the protein fraction that is quantified here. Number of centrioles analyzed: (C): sperm n=35, 1 cell n=6, 2 cells n=9, 4 cells n=9, 8 cells n=5, 10-23 cells n=2; (F) sperm n=99, 1 cell n=5, 2 cells n=6, 4 cells =12, 8 cells n=8, 10-23 cells n=4. **(G, H)** *C. elegans* embryos at the indicated stages from marked mated experiments for SAS-6::GFP stained with antibodies against GFP (green), the pan-centriolar marker IFA (red), as well as with Hoechst for DNA

(blue). **(I)** Percentage of embryos with 1, 2 or no GFP positive paternally contributed centrioles from marked mating experiments with SAS-6::GFP at indicated stages of embryogenesis. **(J-M)** Representative images of *C. elegans* embryos stained with the pancentriolar marker IFA (red), as well as with Hoechst to reveal DNA (blue). Scale bar corresponds to 5  $\mu$ m and insets are 5 times magnified views of centrioles.

## **Supplementary information, Data S1** Materials and Methods

Marked mating experiments.

Nematodes were cultured according to standard procedures [1]. The following strains were used: *fog-2(q71)* [2], GFP::SAS-4 [3], SAS-6::GFP [4], GFP::TBB-2 (a generous gift from Julien Dumont). To ensure that cross-progeny were examined in marked mating experiments, we used the feminized *fog-2(q71)* mutant background, in which hermaphrodites do not produce sperm [2]. *fog-2(q71)* L4 animals were mated with heterozygous males carrying one integrated copy of a given gfp-containing transgene. The progeny of such a cross is either homozygous transgene negative (50% of embryos) or heterozygous, carrying one copy of the gfp-containing transgene (the remaining 50% of embryos). This latter half enabled us to monitor the behavior of GFP-tagged paternally contributed centriolar components throughout embryogenesis, without the confounding effect of protein synthesized following zygotic transcription.

Indirect immunofluorescence, image acquisition, and quantification.

Adult worms were dissected in 4  $\mu$ l dH<sub>2</sub>O to release the embryos on microscope slides coated with 2  $\mu$ g/ $\mu$ l poly-L-lysine in PBS. Embryos were fixed in ice-cold methanol for 2 min, washed in PBS and incubated with primary antibodies overnight at 4°C. Slides were then washed in PBS and incubated with secondary antibodies for 1h at room temperature. Slides were washed and then counterstained with 1  $\mu$ g/ml Hoechst 33258 (Sigma) to reveal DNA; samples were mounted in 4% n-Propyl-Gallate and 90% Glycerol in PBS. Primary antibodies were: mouse anti IFA 1:50 [5] and rabbit anti GFP 1:1000 (a gift from Viesturs Simanis). Secondary antibodies were goat anti-mouse coupled to Alexa 568 (Life Technologies, A-11004) and goat anti-rabbit coupled to Alexa 488 (Life Technologies, A11034), both used at 1:500. Images were acquired with a Zeiss

LSM 700 confocal microscope using a 63x, NA 1.0 oil objective. Images were taken as z-stacks every 0.36  $\mu\text{m}$ ; maximal projections of relevant planes were used during analysis with ImageJ (National Institute of Health). Non-saturated GFP and IFA signal intensities at centrioles were determined by selecting a circular area of 0.78  $\mu\text{m}^2$  for individual centrioles in the embryo or of 1.96  $\mu\text{m}^2$  for pairs of centrioles in sperm cells. GFP signal intensities in the figures are expressed relative to the average GFP signal intensity observed for centrioles in one cell-stage embryos in each experiment. Analogous conclusions were reached by expressing GFP signal intensities relative to IFA signal intensities within each centriole.

### **Supplementary References**

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