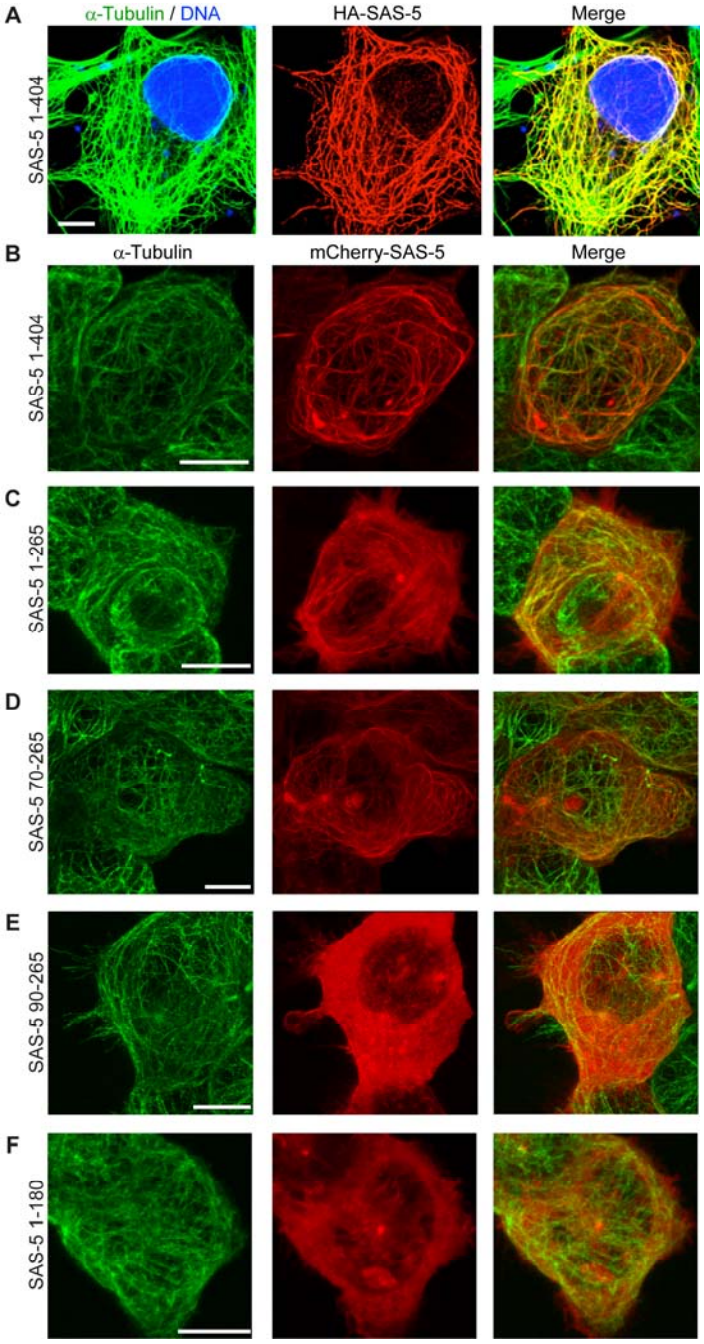
Supplemental Materials

Molecular Biology of the Cell

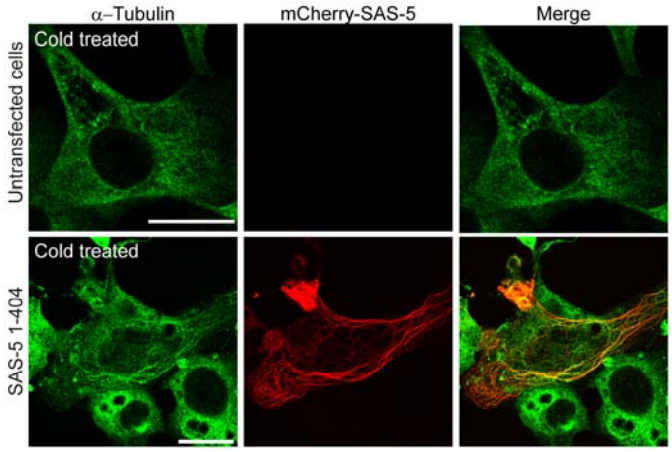
Bianchi et al.

Supplemental Figures

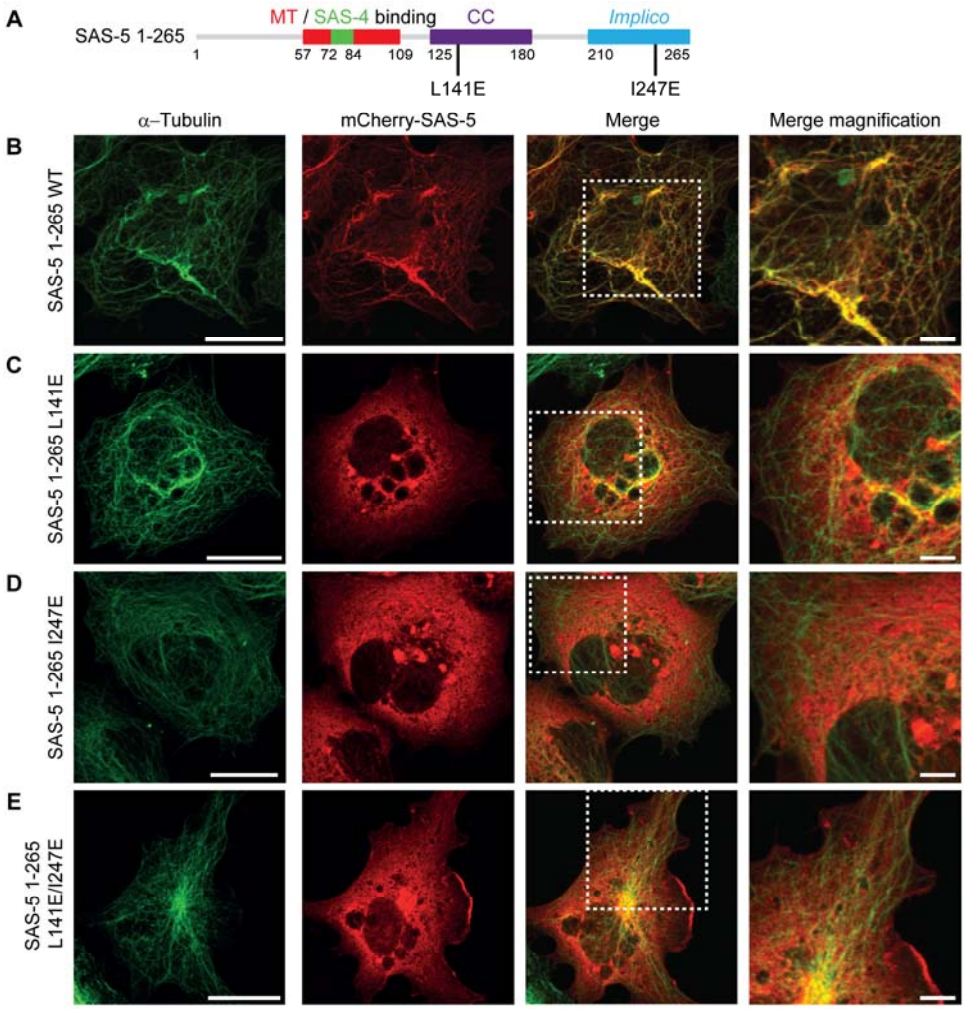


Supplemental Figure S1: SAS-5 variants co-localise with cytoplasmic microtubules. Shown are representative fluorescence images of transfected COS-7 cells (A) expressing full-length HA-SAS-5 or HEK293T cells (B-F) expressing mCherry-SAS-5 full-length or fragments thereof, as indicated. Microtubules were visualised by immunofluorescence using

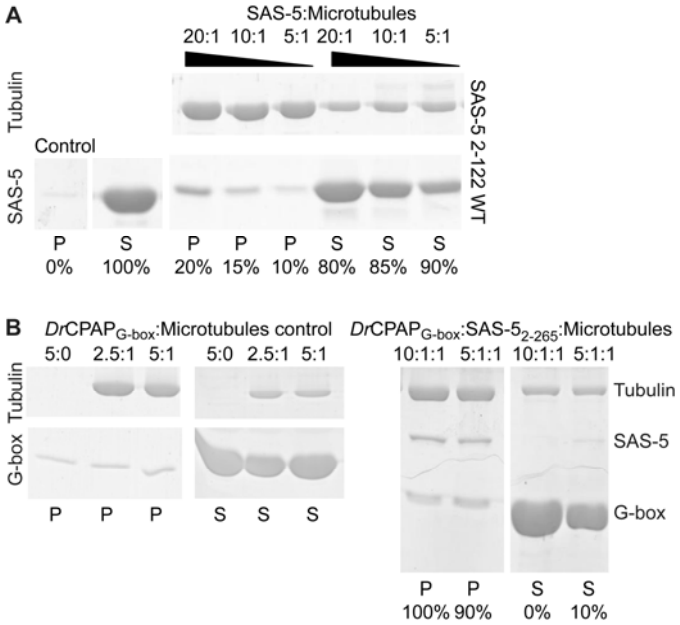
α–tubulin antibodies; SAS-5 fusion proteins were visualised using HA antibodies (A) or mCherry fluorescence (B-F). DNA was visualised by DAPI staining in (A). Scale bars, 10 μm. Assays were performed once.



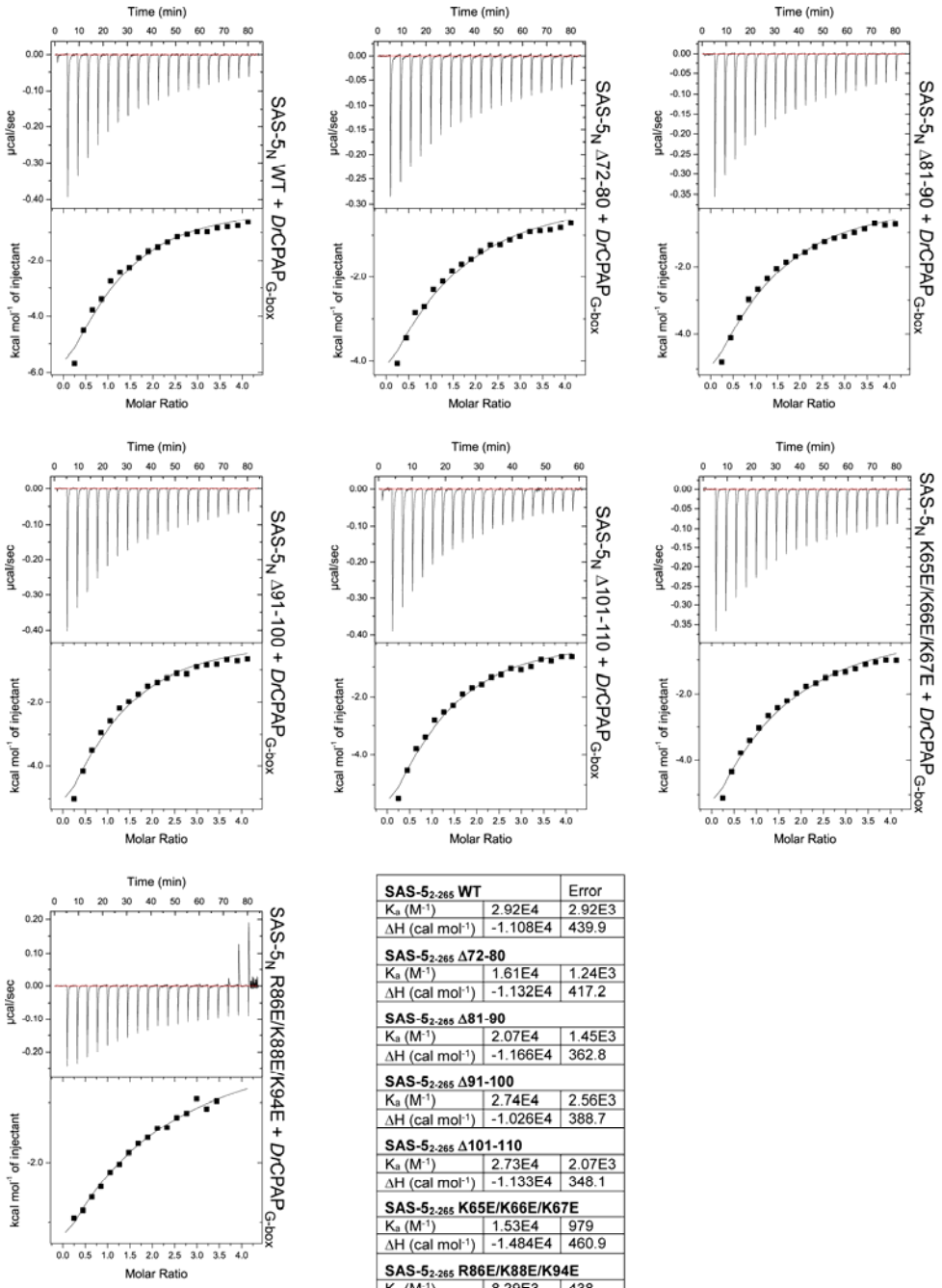
Supplemental Figure S2: SAS-5 stabilises microtubules. Shown are representative fluorescence images of untransfected COS-7 cells (top row) or transfected with full-length mCherry-SAS-5 (bottom row) following cold-treatment to induce microtubule de-polymerisation. Microtubules were visualised by immunoflurescence using α–tubulin antibodies; SAS-5 was visualised by mCherry fluorescence. Scale bar, 20 μm. Assays were performed once.



Supplemental Figure S3: SAS-5 oligomerisation affects its co-localisation with microtubules. (A) Schematic representation of SAS-5 constructs used in these transfection assays, annotated as in Figure 3A. (B-E) Representative fluorescence images of COS-7 cells transiently expressing variants of an mCherry-SAS-5 fragment spanning amino acids 1-265. Row B corresponds to cells expressing SAS-5 WT; rows C-E correspond to SAS-5 variants that comprise protein dimers (C), trimers (D) or monomers (E). Microtubules were visualised by immunoflurescence using α–tubulin antibodies; SAS-5 was visualised by mCherry fluorescence. Scale bar, 20 μm. The rightmost column corresponds to digital magnification of boxed areas in merged images. Scale bar, 5 μm in magnified images. Assays were performed once.



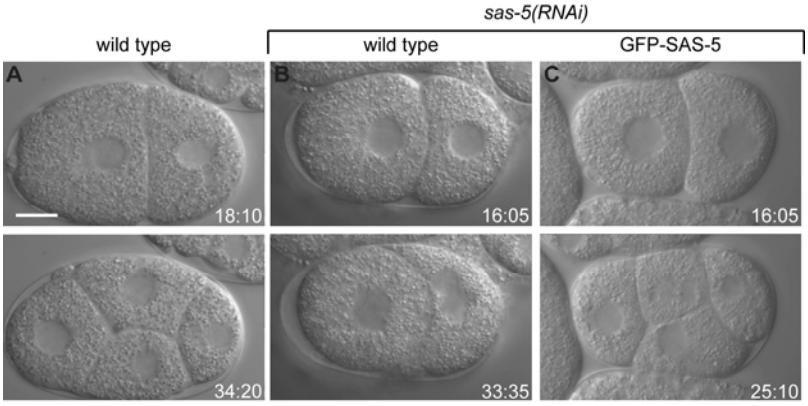
Supplemental Figure S4: Microtubule pelleting assays of SAS-5$_N$ and SAS-5 with DrCPAP$_{G\text{-}box}$. Shown are relevant sections of Coomassie-stained SDS-PAGE from the supernatant (S) and pellet (P) fractions of microtubule-pelleting assays. (A) Pelleting assays performed twice using three different stoichiometric ratios of SAS-5$_N$ versus tubulin in Taxol-stabilised microtubules, as indicated. The SAS-5$_N$ control shown here was performed using 60 μM SAS-5$_N$ alone. (B, left) Control pelleting assays of *Dr*CPAPG-box alone or with Taxol-stabilised microtubules performed using the indicated stoichiometric ratios. (B, right) Pelleting assays of *Dr*CPAPG-box in two ratios with stoichiometric amounts of SAS-5$_{2\text{-}265}$ and tubulin in Taxol-stabilised microtubules, as indicated. Fractional values under each lane correspond to the percentage of SAS-5 constructs present in the supernatant versus the pellet of assays. Tubulin concentration was 3 μM throughout. Control assays for microtubules are shown in Figure 3B. Assays were performed once.



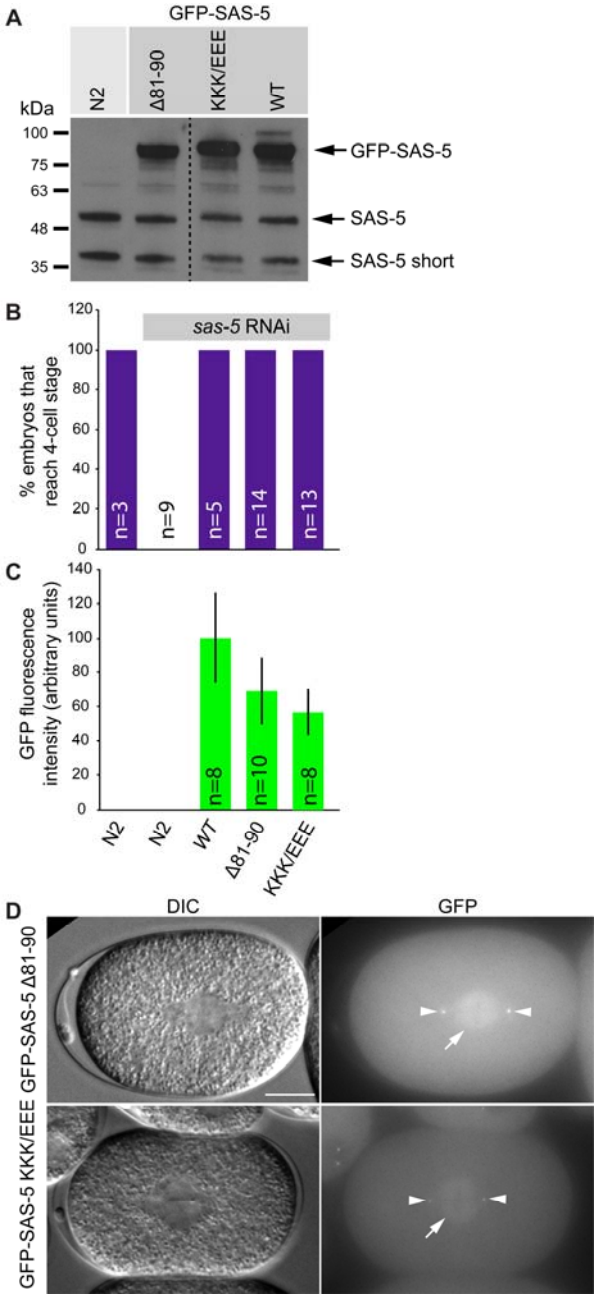
SAS-5$_{2-265}$ WT		Error
K_a (M^{-1})	2.92E4	2.92E3
ΔH (cal mol^{-1})	-1.108E4	439.9
SAS-5$_{2-265}$ Δ72-80		
K_a (M^{-1})	1.61E4	1.24E3
ΔH (cal mol^{-1})	-1.132E4	417.2
SAS-5$_{2-265}$ Δ81-90		
K_a (M^{-1})	2.07E4	1.45E3
ΔH (cal mol^{-1})	-1.166E4	362.8
SAS-5$_{2-265}$ Δ91-100		
K_a (M^{-1})	2.74E4	2.56E3
ΔH (cal mol^{-1})	-1.026E4	388.7
SAS-5$_{2-265}$ Δ101-110		
K_a (M^{-1})	2.73E4	2.07E3
ΔH (cal mol^{-1})	-1.133E4	348.1
SAS-5$_{2-265}$ K65E/K66E/K67E		
K_a (M^{-1})	1.53E4	979
ΔH (cal mol^{-1})	-1.484E4	460.9
SAS-5$_{2-265}$ R86E/K88E/K94E		
K_a (M^{-1})	8.29E3	438
ΔH (cal mol^{-1})	-1.426E4	444.1

Supplemental Figure S5: SAS-5$_N$ binding to DrCPAP$_{G\text{-}box}$. Shown are titrations of SAS-5$_N$ WT and variants designed to disrupt the microtubule interaction with *Dr*CPAP$_{G\text{-}box}$ monitored by ITC. In each case the top panel represents the heat response to injections of SAS-5$_N$ at high concentration into *Dr*CPAP$_{G\text{-}box}$. The bottom panel shows integrated heat changes (closed squares) and associated curve fits (solid lines). The association affinities (K_a) and

per mole enthalpy changes derived from fits are shown in a tabular form. Assays were performed once.



Supplemental Figure S6: Phenotypic outcomes upon sas-5(RNAi). Shown are characteristic phenotypic outcomes of early *C. elegans* embryo cell divisions, adapted from (Rogala et al., 2015). (A) Wild-type (non-transgenic) embryo at the two-cell (top row) and four-cell (bottom row) stage. (B,C) Embryos in which endogenous SAS-5 protein is depleted by *sas-5*(*RNAi*) and that lack (B) or express (C) an RNAi-resistant SAS-5 WT transgene. Time stamps indicate mm:ss; scale bar: 10 μm. Embryos are oriented with the anterior on the left and the posterior on the right.



Supplemental Figure S7: Expression, centriole duplication and spindle localisation of GFP-SAS-5 WT and microtubule-binding variants. (A) Western blot of whole worm lysates from the indicated strains probed with SAS-5 antibodies. Molecular weight markers are indicated on the left in kDa. A dashed line denotes the position from which non-pertinent lanes of this Western blot have been removed. Arrows on the right indicate exogenous GFP-SAS-5 (WT or variants, upper), and endogenous SAS-5 (middle and lower). Endogenous SAS-5 exists as two isoforms: a long 404 amino acid isoform and a shorter 288 amino acid isoform, which is likely the result of trans-splicing (Wormbase, F35B12.5c). Exogenous GFP-SAS-5 expression produces the longer isoform only. Note that endogenous SAS-5 corresponds to protein in whole worm bodies; in contrast, GFP-SAS-5 WT and variants are only present in the germline, as the transgenes are driven from a germline promoter. (B,C) L4 worms from the indicated strains were subjected to *sas-5*(*RNAi*) for 24 h, or fed without

RNAi (first column), and the resulting embryos imaged by time-lapse DIC microscopy (B) or by fluorescence microscopy (C). (B) Graph shows percentage of embryos that successfully reach the 4-cell stage. (C) Average GFP fluorescence intensities of embryos from each of the strains expressed as a percentage of the GFP-SAS-5 WT. Error bars show the standard deviation. (D) DIC (left) and GFP fluorescence (right) images of embryos at metaphase of the one-cell stage from worms subjected to *sas-5*(*RNAi*) for 24 h from the indicated lines expressing GFP-SAS-5 microtubule-binding variants. White arrows indicate GFP-SAS-5 spindle fluorescence; white arrowheads indicate centrioles. GFP images are maximum intensity projections. Scale bar 10 μm. All embryos are oriented with the anterior on the left and the posterior on the right.