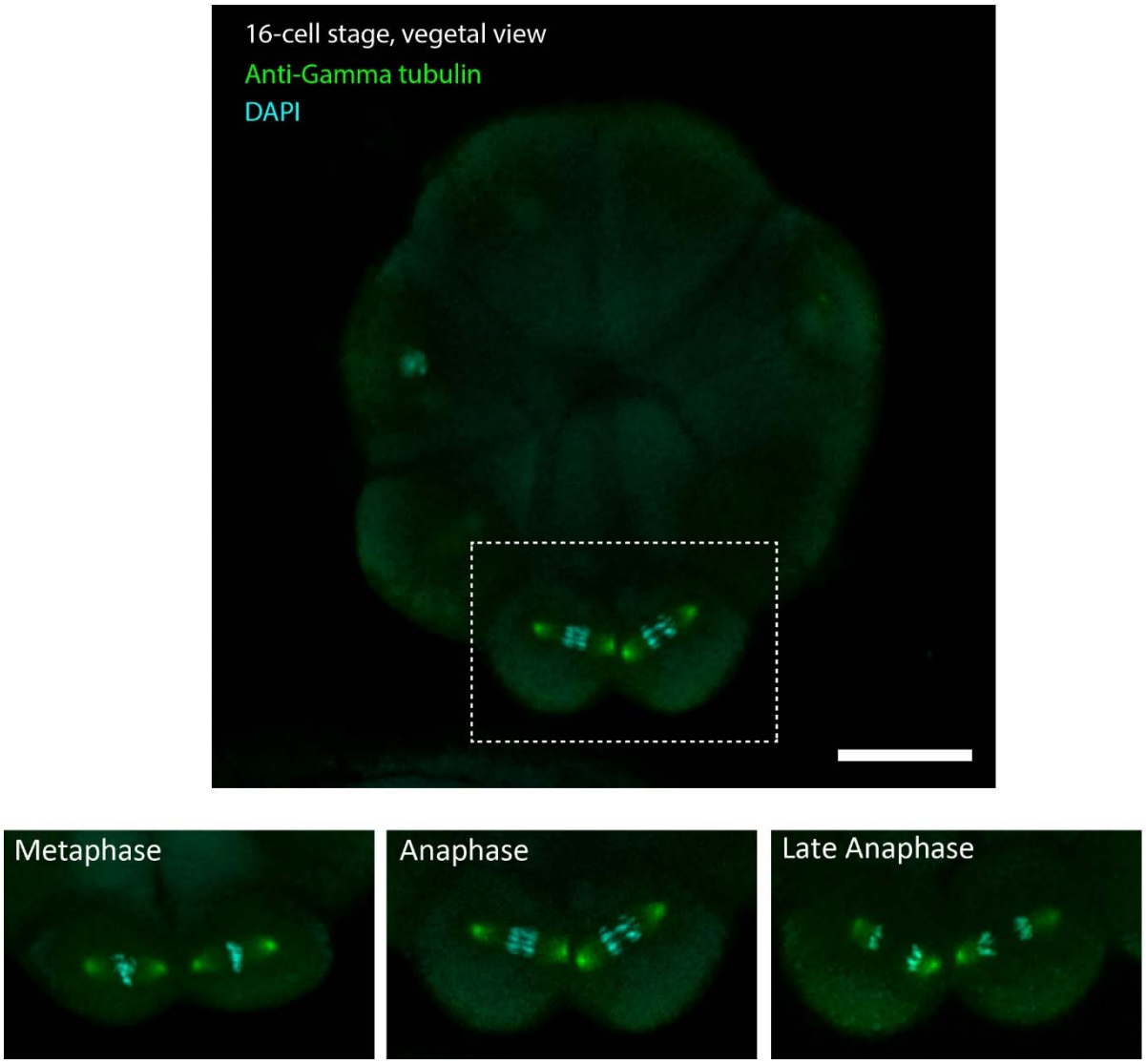
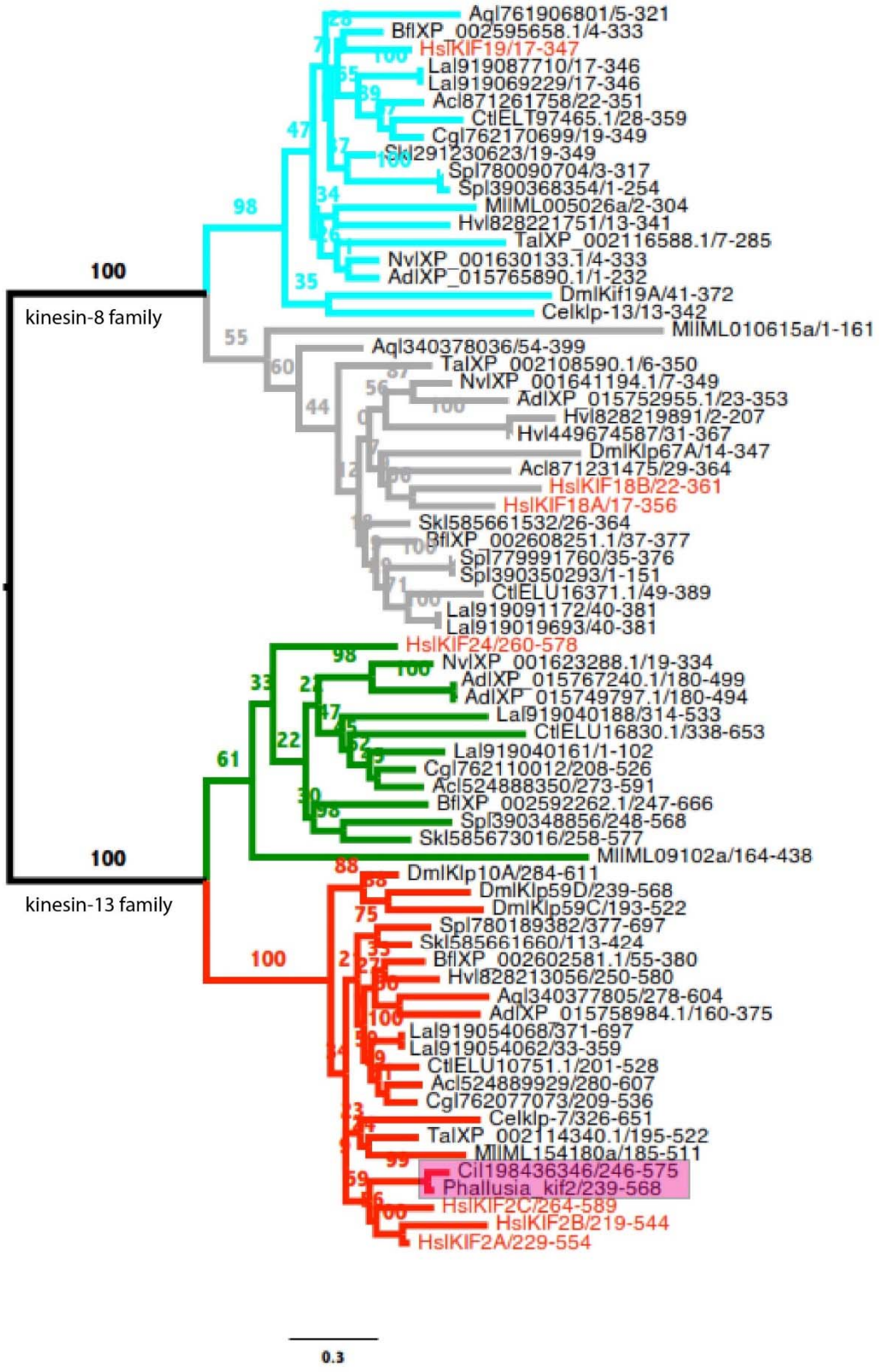


Supplementary Figure 1. Gamma-tubulin presence at spindle poles during mitosis.

16-cell stage *Phallusia mammillata* embryos fixed and labelled with anti-γ-tubulin (green) antibody and DAPI (blue). Inset showing γ-tubulin at metaphase, anaphase and late anaphase of the posterior pair of blastomeres (B5.2). γ-tubulin is present at both spindle poles throughout mitosis. Scale bar = 30µm.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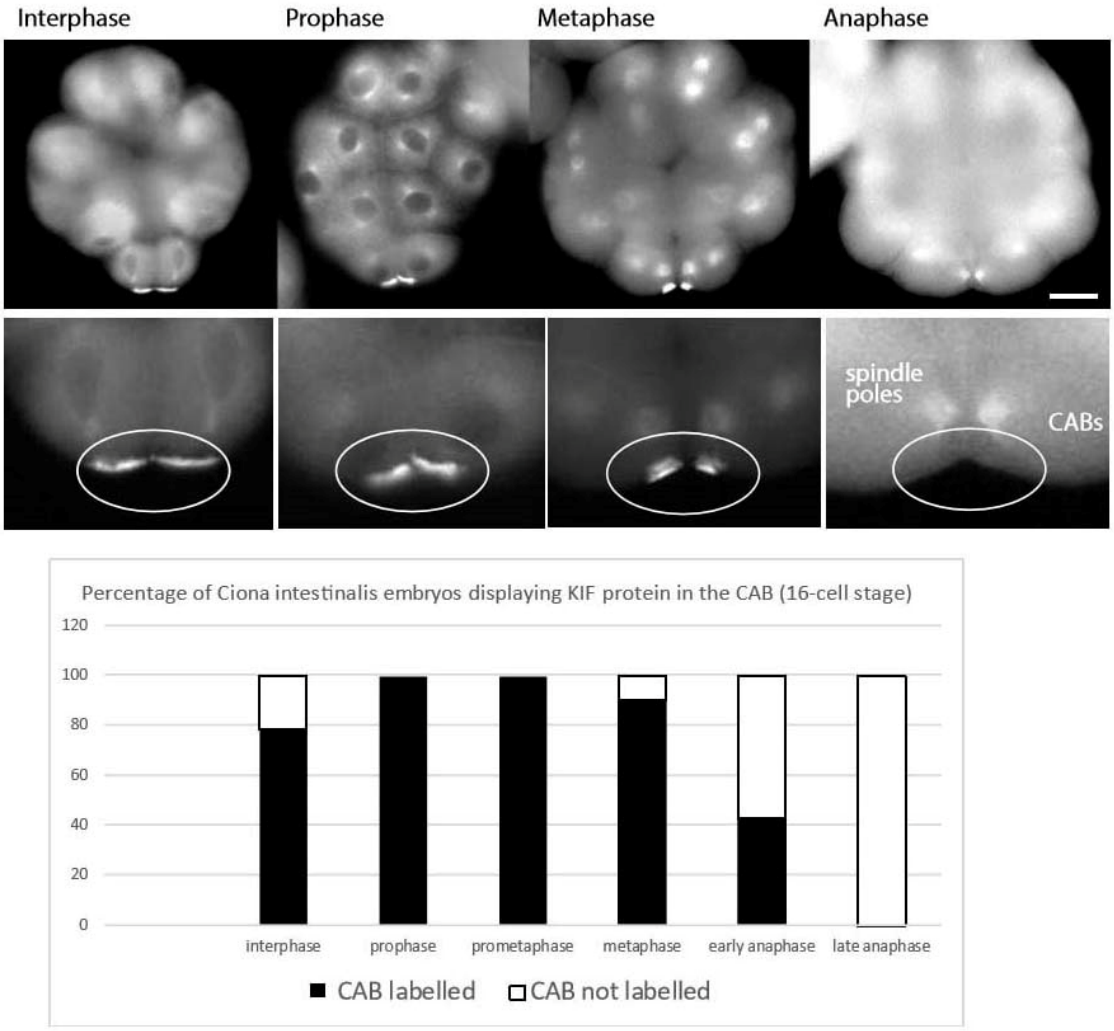
100
kinesin-8 family
100
kinesin-13 family
0.3

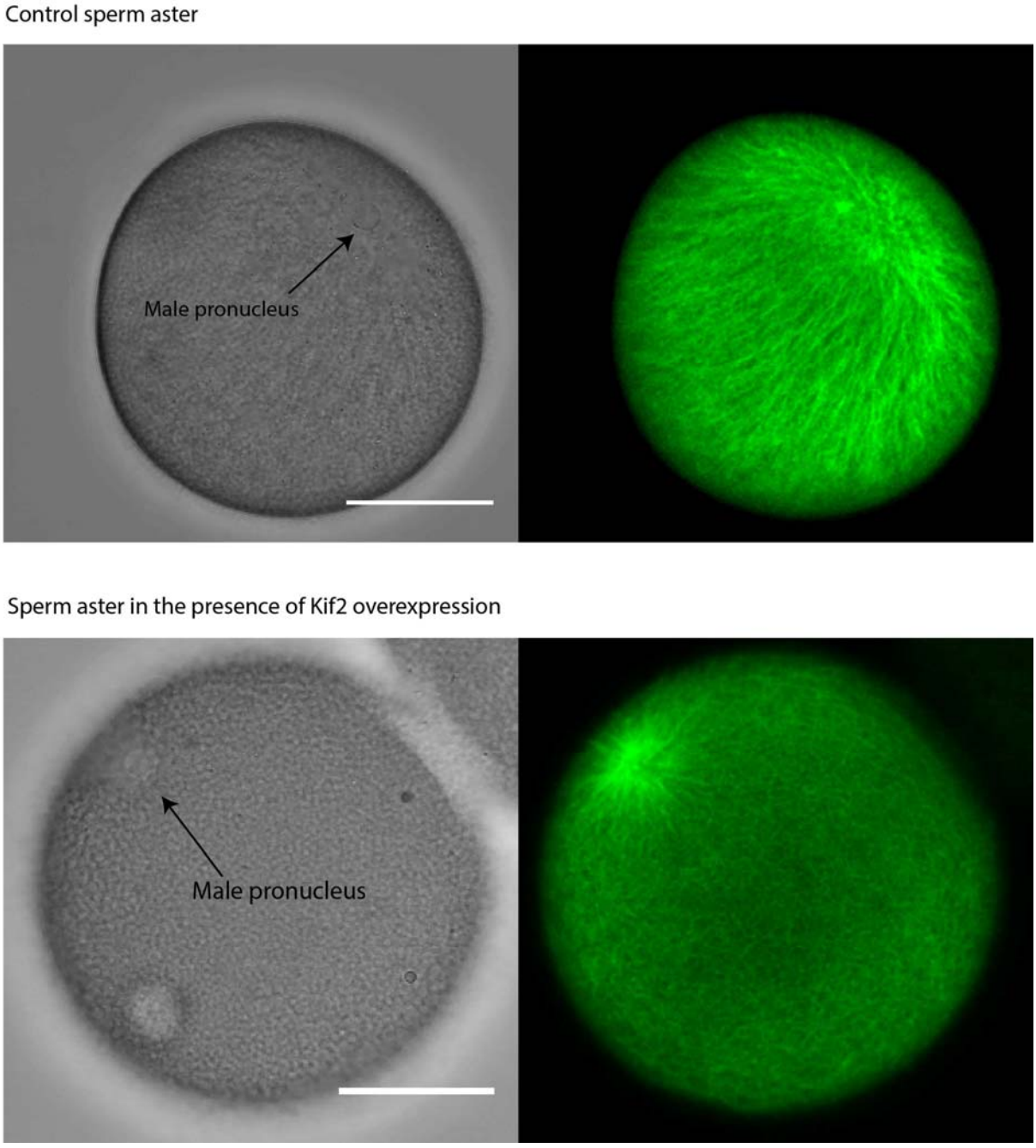
Supplementary Figure 2. Phylogenetic analysis of Kif2 (kinesin-13) family of proteins.

Phyml tree of the clade of animal KIF19, KIF18, KIF24 and KIF2A/B/C ortholog groups. Kif2 and Kif24 (bottom half of tree) are members of the Kinesin-13 family of microtubule depolymerases; Kif18 and Kif19 (top half of tree) are members of the related Kinesin-8 family [16]. With the exception of the *Phallusia* sequence, the first two letters of leaf names represent the species of the sequence (see methods for list). These are followed by identifiers retrievable from the NCBI protein sequence database. Bootstrap support is show above branches. Although within the gene groups the species phylogeny is generally not well resolved, there is high support for lineage specific duplications of Kif2 in *Drosophila* and Humans, with the majority of invertebrate taxa having only a single representative, strongly suggesting the state in the ancestral animal was a single gene. *Ciona* and *Phallusia* data are highlighted by the pink box.



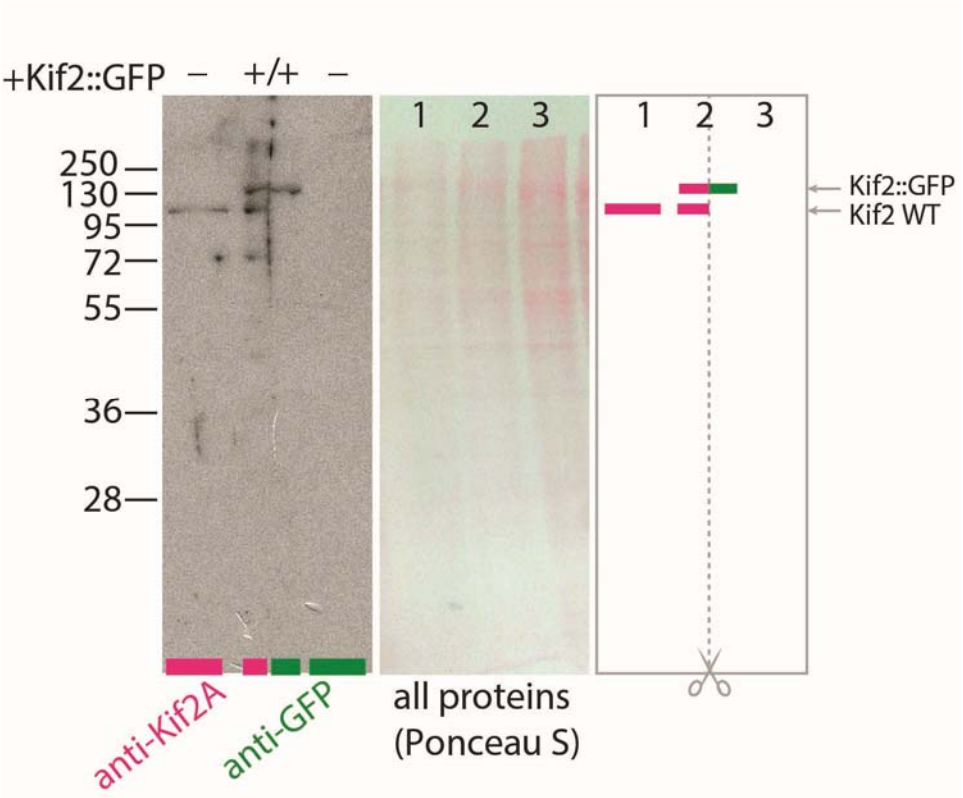
Supplementary Figure 3. Endogenous Kif2 protein localization at the CAB in *Ciona intestinalis*.

Immunolabelling with anti-Kif2 of 16-cell stage *Ciona* embryos. Kif2 labels the CAB during later interphase/early prophase and is absent at the CAB during anaphase. Scale bar = 30 μm. Graph showing percentage of embryos (total n=94) with CAB labelled at different stages of the cell cycle. Note, these percentages do not indicate amount of Kif2 at the CAB but presence or absence.



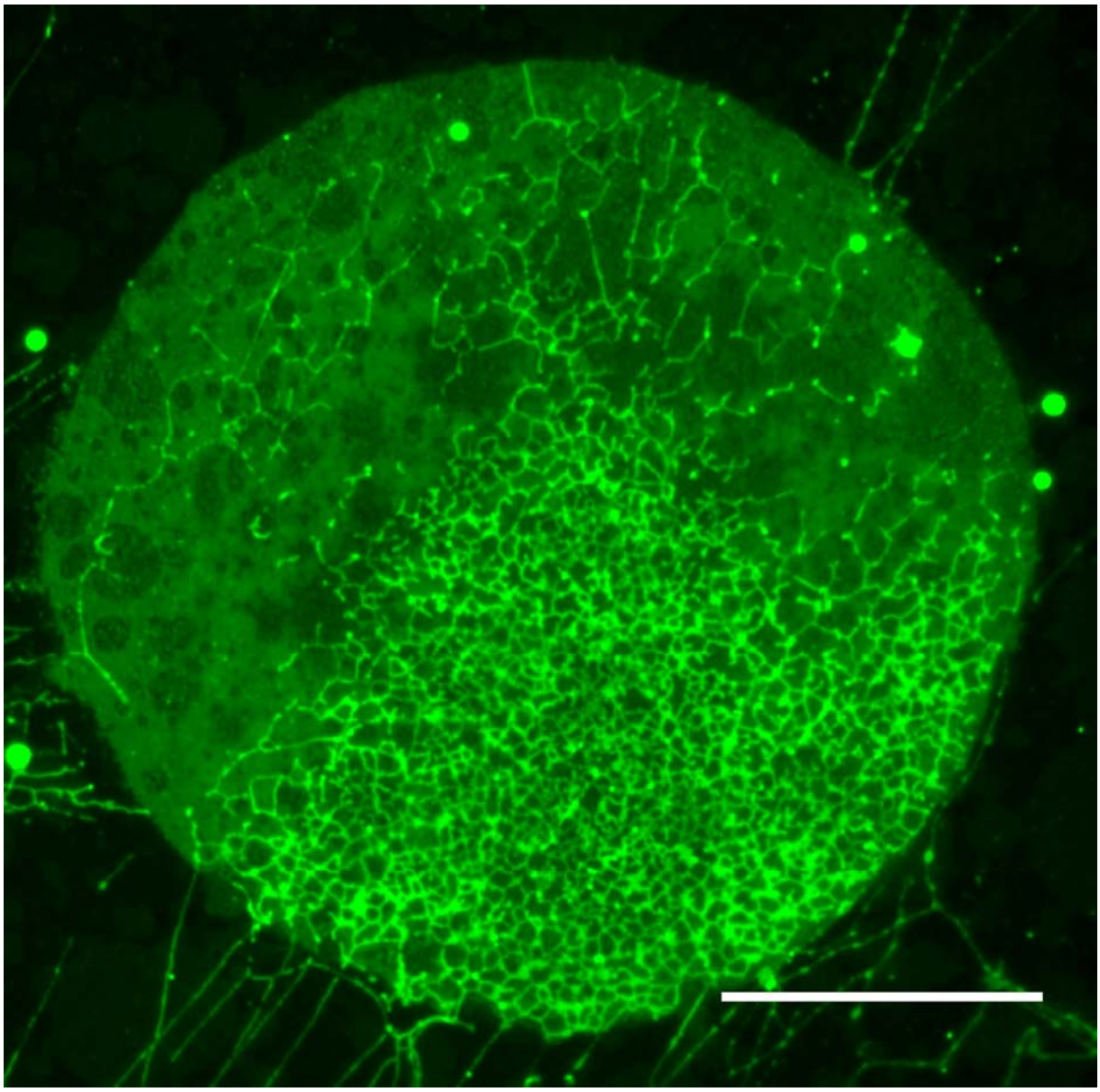
Supplementary Figure 4. Microtubule density is reduced in cells overexpressing Kif2.

Eggs injected with mRNAs encoding Ens::3GFP (top pair of images) or Kif2 (without a fluorescent tag) plus Ens::3GFP (bottom pair of images) and were left overnight then fertilized. Confocal images from a z stack showing that in eggs overexpressing Kif2 microtubule density of the sperm aster was greatly reduced (bottom fluorescence image) compared to a zygote without exogenous Kif2 (upper fluorescence image). Bright-field images showing the presence of the male pronucleus (arrows) in both zygotes. Scale bars = 50µm. See Supplementary Movie 14.



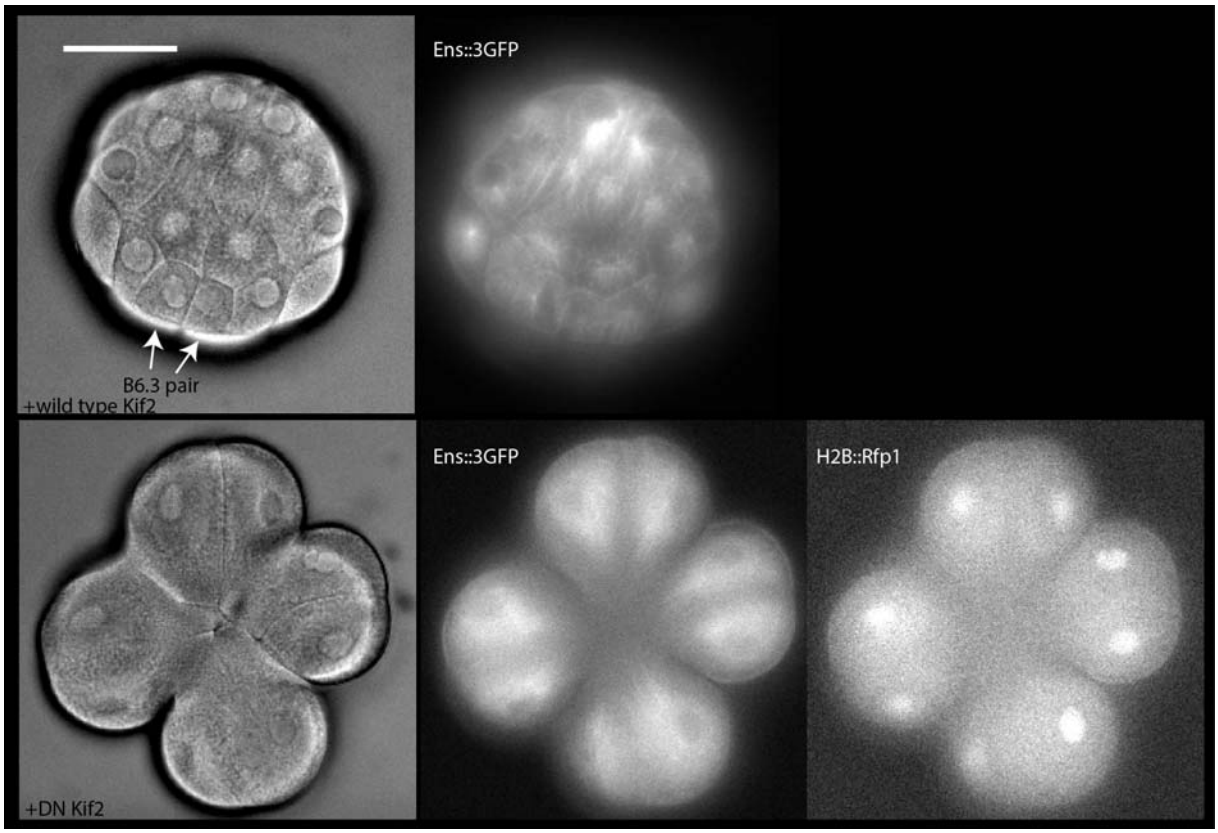
Supplementary Figure 5. Western blot of Kif2.

Western blot showing that Kif2 protein is present in unfertilized eggs. Extracts of unfertilized *Phallusia* eggs that were either uninjected (-) or injected (+) with mRNA encoding Kif2::GFP and probed with anti-Kif2 or anti-GFP (left), Ponceau S showing proteins (center) and schematic showing labelling pattern (right). Green bar represents the Kif2::GFP which migrates at a higher molecular mass than endogenous Kif2 protein (pink bar). Some degradation product can be seen in the anti-Kif2A lane.



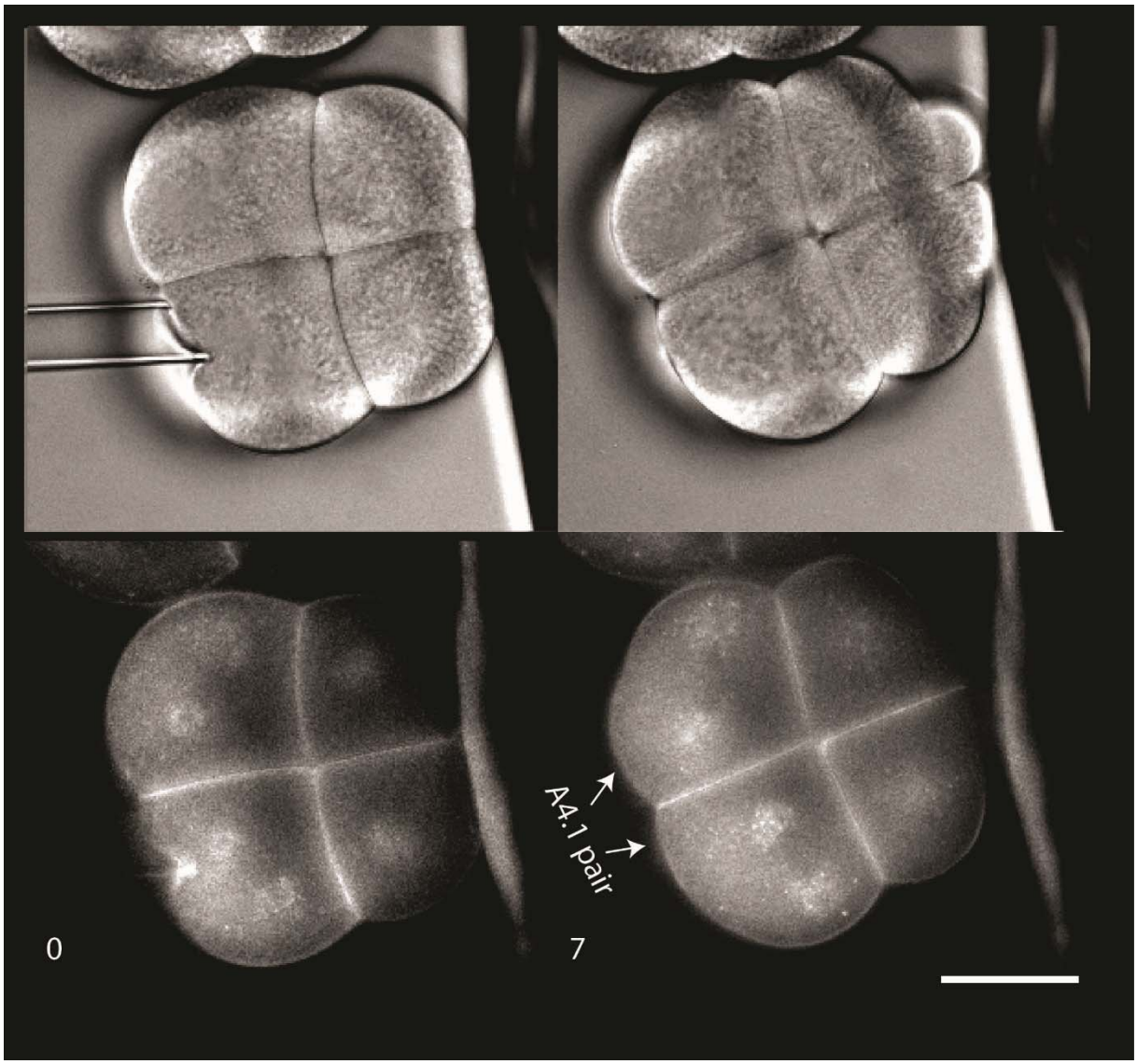
Supplementary Figure 6. Cortical preparation of DiO-labelled cortical endoplasmic reticulum in an egg.

Cortical preparations of unfertilized eggs were prepared as indicated in the material section for embryos. DiO labelling shows the reticular structure typical of the cortical ER of unfertilized eggs. Scale bar = 20 μm.



Supplementary Figure 7. Wild type versus dominant negative Kif2.

Unfertilized eggs were microinjected with mRNA encoding wild type Kif2 mixed with Ens::3GFP or dominant negative Kif2 mixed with Ens::3GFP and H2B::mRfp1. The same concentration of mRNA encoding wt Kif2 and DN-Kif2 and Ens::3GFP was used. Bright-field and fluorescence images of embryos containing wt Kif2 or DN Kif2 are shown. None of the DN Kif2-injected eggs reached the 32-cell stage (32/32 embryos scored) while 12/16 wt Kif2 embryos reached the 32-cell stage. Scale bar=50µm.



Supplementary Figure 8. Nocodazole pipette applied to non-CAB blastomeres (A4.1).

Embryos at the 8-cell stage (interphase) were bathed in Cell Mask orange to label the mitotic spindle poles and the plasma membrane and during mitosis a nocodazole pipette was applied to the surface of one A4.1 blastomere immediately following NEB. Note the exaggerated movement of the spindle pole nearest the nocodazole pipette away from nocodazole pipette. We measured the spindle pole to midline distance before and after application of the pipette. The distance to the midline before pipette was 19.2+/-2.0 µm and increased to 22.8+/-1.7 µm (mean +/- s.e.m. n=6). Time in min. Scale bar = 50µm.