

Figure S1. Overexpression of Ensconsin increases mitotic spindle length in a Kinesin-1-independent manner but efficiently rescues *khc*-dependent centrosome separation defects and in brain NBs.

A) Dividing neuroblasts were imaged in four different genotypes: control, *khc*²⁷/*khc*⁶³, Ensconsin-Venus overexpression (Ens OE) and in *khc*²⁷/*khc*⁶³ overexpressing Ensconsin (*khc*²⁷/*khc*⁶³; Ens OE). Scale bar: 10 μ m. Time is min:sec. B) Box plot showing the mean (\pm s.d.) mitotic spindle length analysis for control ($11.3 \pm 0.8 \mu\text{m}$, $n=14$), *khc*²⁷/*khc*⁶³ ($10.9 \pm 0.5 \mu\text{m}$, $n=17$), Ens OE ($13.6 \pm 1.9 \mu\text{m}$, $n=24$), *khc*²⁷/*khc*⁶³; Ens OE ($13.5 \pm 2.0 \mu\text{m}$, $n=24$) NBs. ***, $P < 0.001$ (Wilcoxon test). C) Analysis of centrosome separation angle (α) at NEBD for the indicated genotypes. D) Box plot showing the mean (\pm s.d.) centrosome separation angle for control ($151.4 \pm 19.0^\circ$, $n=17$), *khc*²⁷/*khc*⁶³ ($114.1 \pm 20.7^\circ$, $n=21$), Ens OE ($134.0 \pm 40.3^\circ$, $n=26$), *khc*²⁷/*khc*⁶³; Ens OE ($159.4 \pm 16.2^\circ$, $n=29$) NBs, corresponding to panel C. ****, $P < 0.0001$ (Wilcoxon test). E) Scatter box blot showing the mean value (\pm s.d.) of Ens-Venus centrosomal signal (a.u) 30 sec before NEBD in WT (15659 ± 2188 , $n=29$) or *khc*²⁷/*khc*⁶³ (16038 ± 3255 , $n=22$). F) Western blot showing Ensconsin, KHC and actin (as a loading control) protein levels in WT, *khc*²⁷/*khc*⁶³ mutant and *ensc* mutant brain extracts. KHC levels are stable in *ensc* mutants (and vice versa). G) Endogenous Ensconsin localization during interphase in control (left) and in *khc*²⁷/*khc*⁶³ mutant NBs (right). Tubulin is red in the merge panels and monochrome in the middle panels. Ensconsin is Green in the merge panels and Monochrome in the bottom panels. Scale bar: 5 μ m.

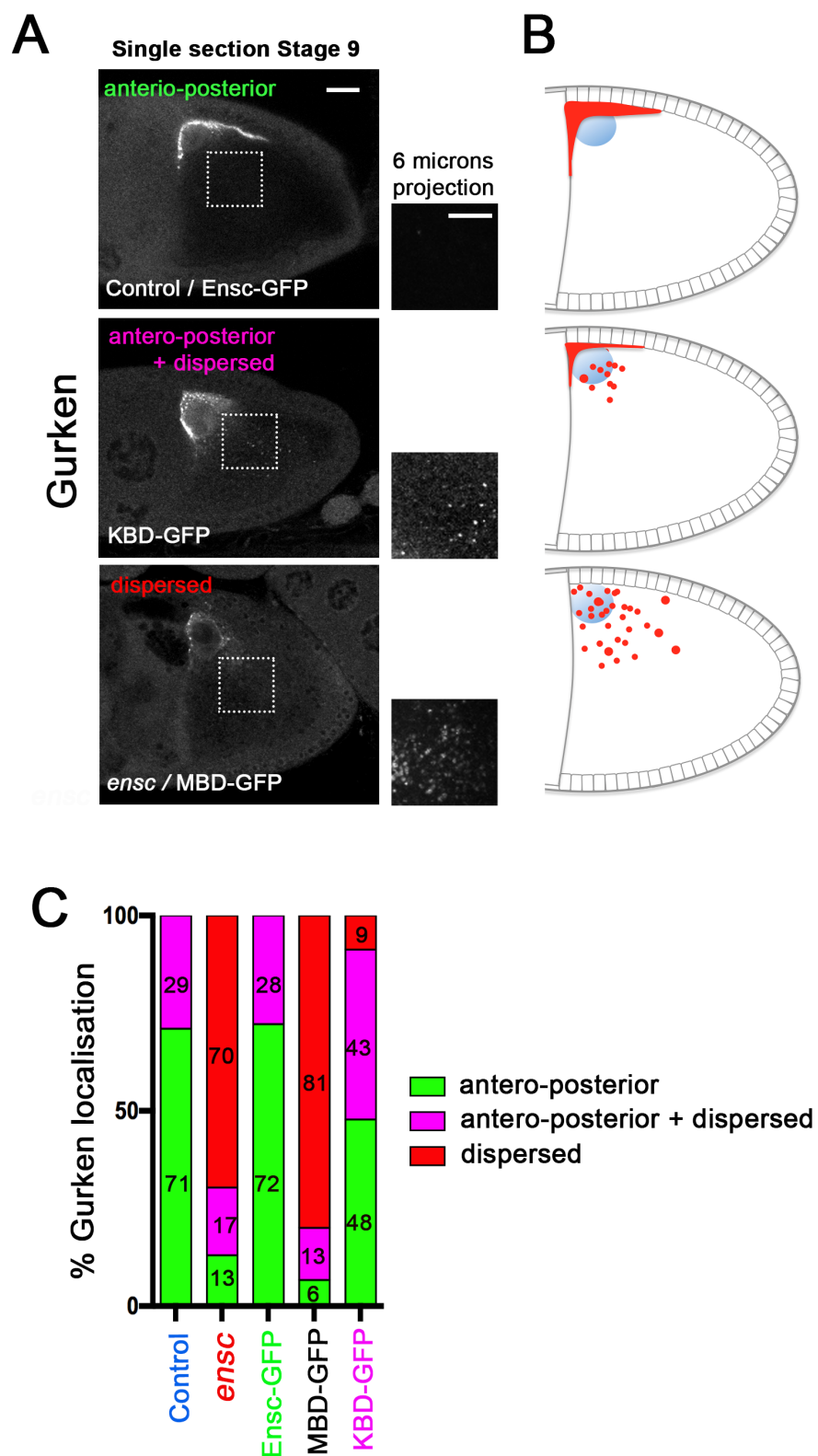


Figure S2. Gurken localization in oocytes (revoir les numerous avec le texte)

A) Gurken localization patterns at stage 9 in different genetic backgrounds. Gurken is mainly located at the dorsal corner side of the nucleus in both control and *Ensc*-GFP. Note that it can also appear as tiny dots around the nucleus. In *ensc* and MBD-GFP, Gurken signal was dispersed or appeared as tiny spots around the nucleus (bottom). In KBD-GFP oocytes (middle), some oocytes showed a WT Gurken distribution (11/23), a small portion of the oocytes has a Gurken localization pattern comparable to *ensc* mutant (2/23) and the remaining oocytes displayed an intermediate phenotype with a punctiform distribution at the dorsal side corner region (10/23). B) Schematic diagram of Gurken localization (red) in stage 9 oocytes that summarises a representative oocyte for WT and *Ensc*-GFP (top), KBD-GFP (middle) and in *ensc* and MBD-GFP backgrounds (bottom). The nucleus is shown as blue sphere. C) Histogram showing the percentage distribution of Gurken localization in stage 9 oocytes for controls ($n=14$), *ensc* ($n=23$), MBD-GFP ($n=15$), KBD-GFP ($n=23$) and *Ensc*-GFP ($n=18$). Numbers in the columns correspond to percentages.

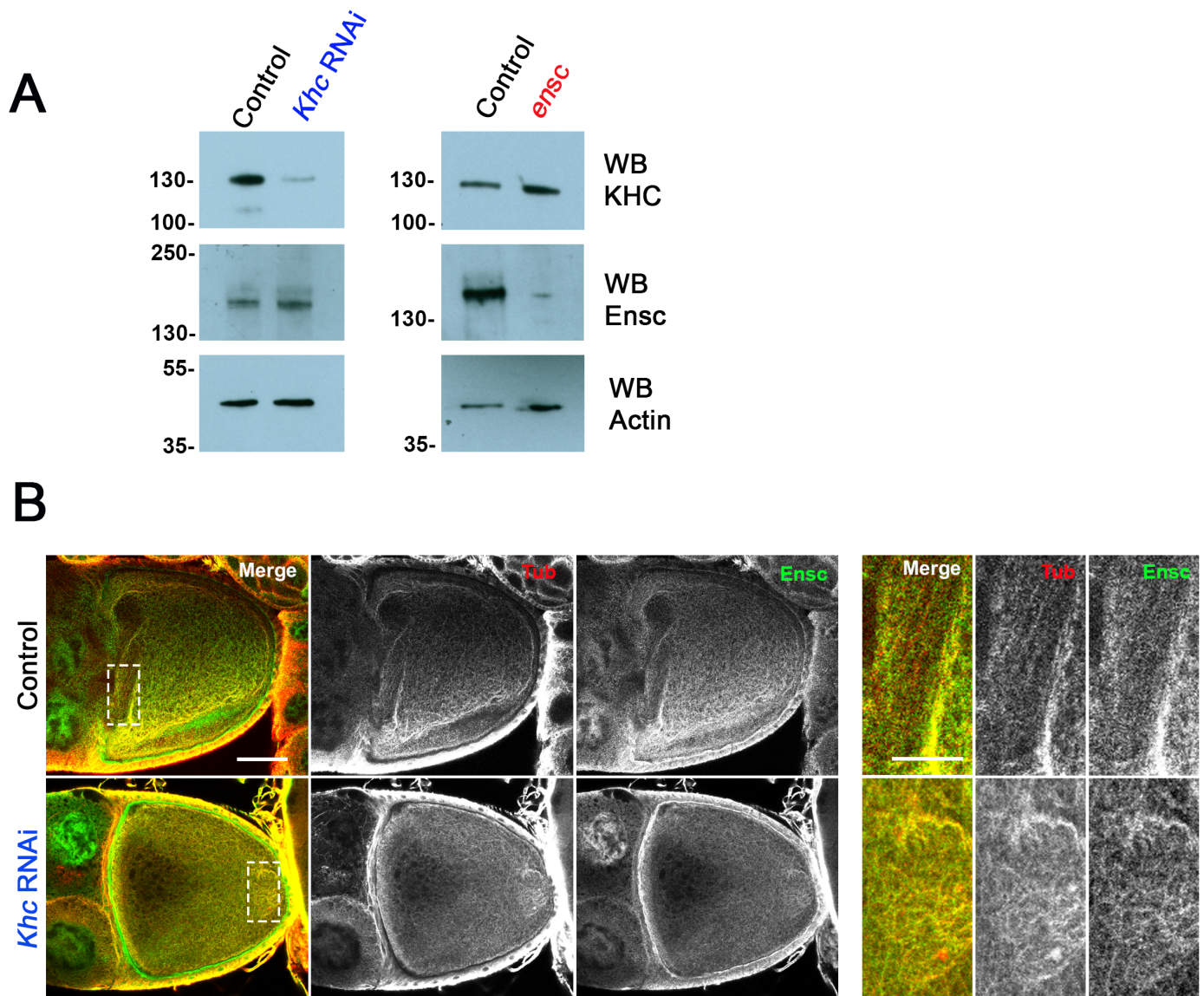


Figure S3. Ensconsin localization in *khc* RNAi oocytes and Western blot analyses.

A) Western blot showing KHC, Ensconsin and actin protein levels in control and *khc* RNAi ovary extracts (left) or in control and *ensc* mutant ovary extracts (right). B) Ensconsin (green in the merge panel and monochrome in the middle panels) and MT localization (red in green in the merge panel and monochrome in the right) panels in WT (top, n=10) and *khc* RNAi oocytes (bottom, n=12). *khc* RNAi oocytes display a loss of the large cytoplasmic MTs bundles but Ensconsin is maintained on the remaining MT cytoskeleton. Insets show an enlarged view of the cytoplasmic MTs. Scale bar 50 μ m and 20 μ m in the insets.

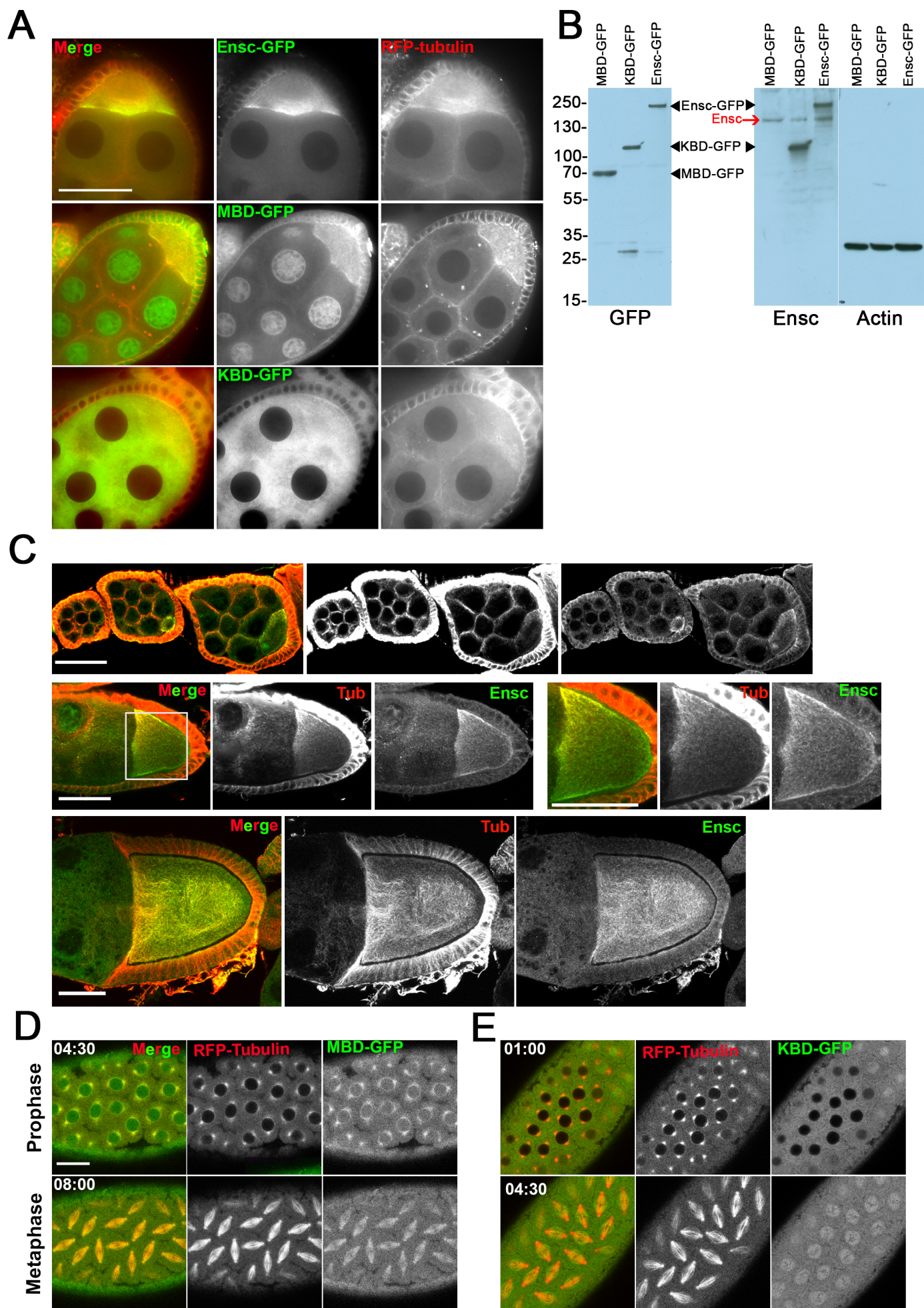


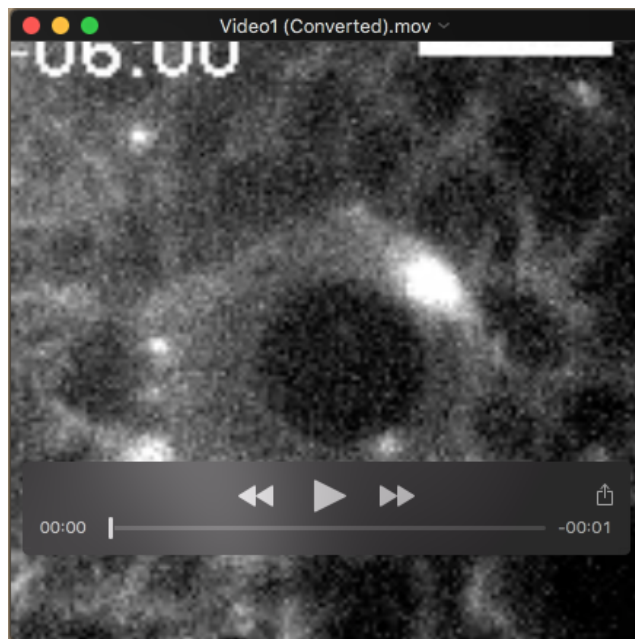
Figure S4. Localization of Endogenous Ensconsin, Ens-GFP, KBD-GFP, and MBD-GFP variants in oocytes and in live embryos.

A) Live localization of Ens-GFP (top), MBD-GFP (middle) and KBD-GFP (bottom) in live early oocytes expressing RFP-tubulin (Red in the merge channel), in a wild type background. Ens-GFP and MBD-GFP are present on the MT network of the egg chamber and of the epithelial cells. KBD-GFP does not localize on the MT network. Scale bar: 50 μ m.

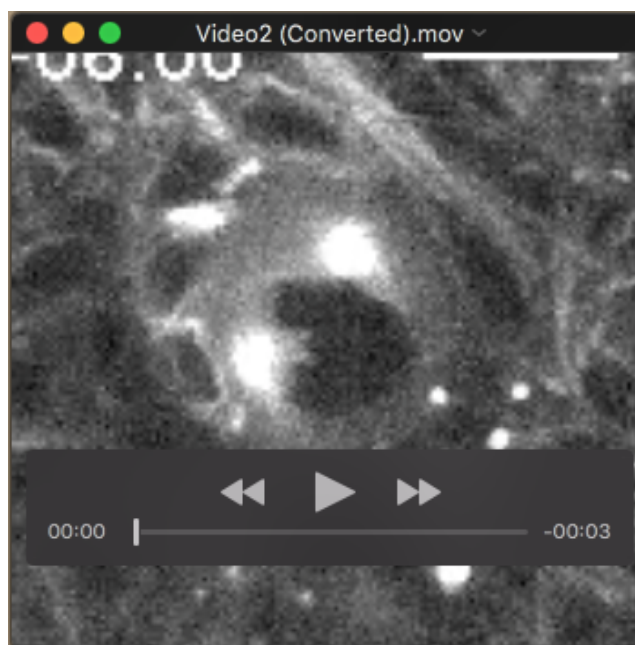
B) Western Blot showing the expression of MBD-GFP, KBD-GFP and Ens-GFP in ovary extracts from wild type flies. The membrane was probed with anti-GFP antibodies and the three GFP-tagged variants are expressed at similar levels (left). The same membrane was stripped and probed with anti-Ensconsin antibodies raised against the KBD (middle panel). The MBD is not detected. The red arrow indicates the endogenous Ensconsin. The membrane was stripped and probed with anti-actin antibodies (right) as a loading control.

C) Wild type oocytes were permeabilized for 1 h to remove cytoplasmic protein pools (see Methods). They were then fixed and immuno-labelled with an anti-tubulin antibody (red on the left, monochrome elsewhere) and an anti-Ensconsin affinity-purified antibody (green in the left panels, monochrome elsewhere). Scale bars: 50 μ m. Note that Ensconsin is first detected in the egg chamber during early stages (top). At later stages, it co-localizes with the polarised MT network (middle panels). The right panels show an enlarged view of the MT cytoskeleton of the corresponding oocyte. During stage 10B, Ensconsin also co-localizes with the MT network involved in ooplasmic streaming (bottom panels).

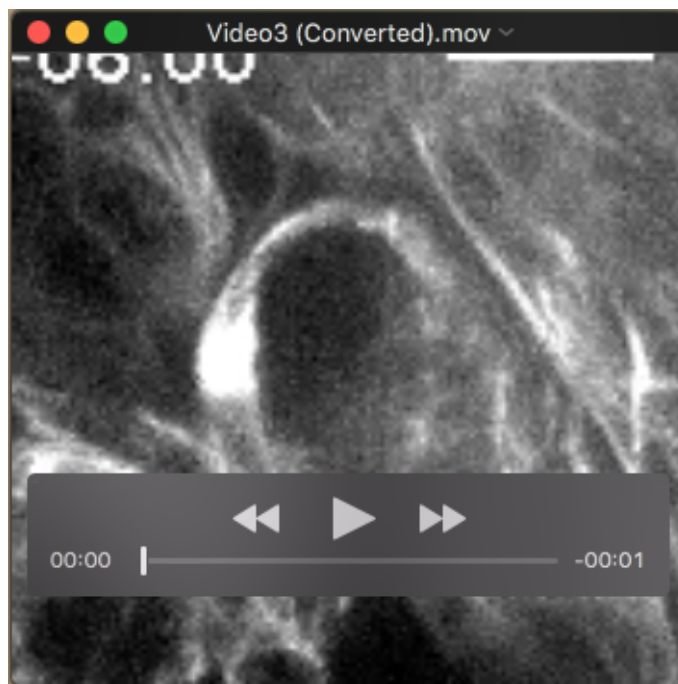
D) Selected images of a wild type embryo-expressing MBD-GFP as it divides during interphase (at 4:30, left) and during metaphase (8:00, right). E) Selected images of a wild type dividing embryo-expressing KBD-GFP, shown during prophase (1:00, left) and metaphase (4:30, right). The GFP-tagged proteins are green and RFP-tubulin is red in the merge pictures, and they are both monochrome in the other panels. Scale bars: 20 μ m. Time is indicated as min:sec. MBD-GFP co-localizes with MTs during mitosis and interphase, but KBD-GFP does not.



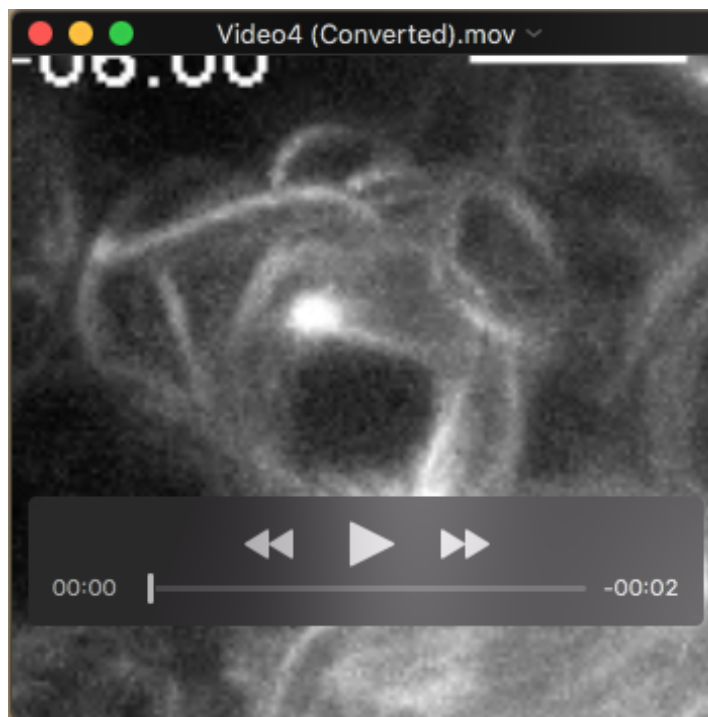
Movie 1. Control dividing NB expressing RFP-tubulin. Scale bar: 10 μ m. Time is min:sec.



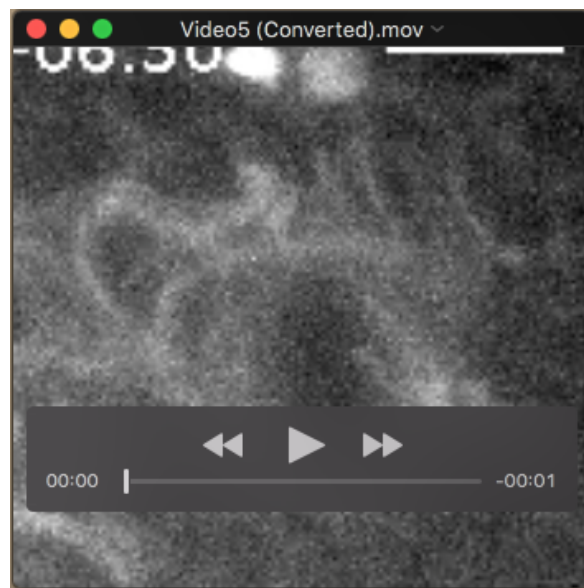
Movie 2. *khc*²⁷/*khc*⁶³ mutant NB expressing RFP-tubulin. Scale bar: 10 μ m. Time is min:sec.



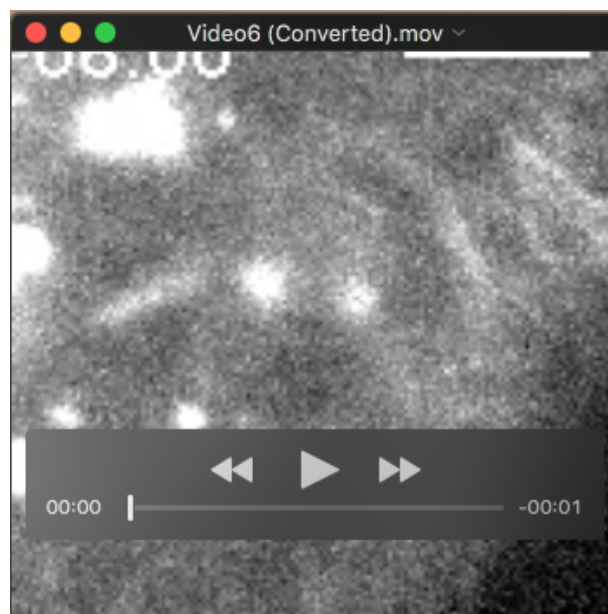
Movie 3. Dividing NB overexpressing Ensc-venus. Scale bar: 10 μ m. Time is min:sec.



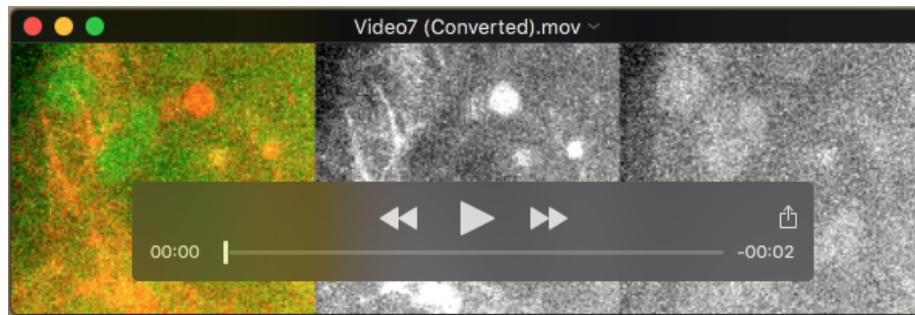
Movie 4. *khc²⁷/khc⁶³* mutant neuroblast overexpressing Ensc-Venus during cell division. Scale bar: 10 μ m. Time is min:sec. Scale bar: 10 μ m. Time is min:sec. See the rescue of the centrosome separation defect.



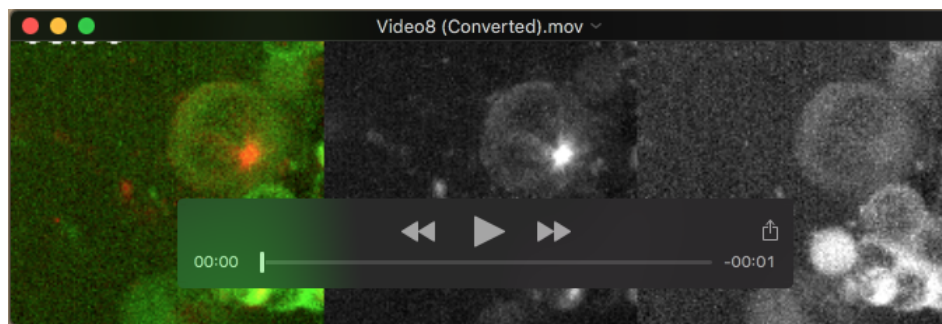
Movie 5. Control dividing NB expressing RFP-tubulin. Scale bar: 10 μ m. Time is min:sec.



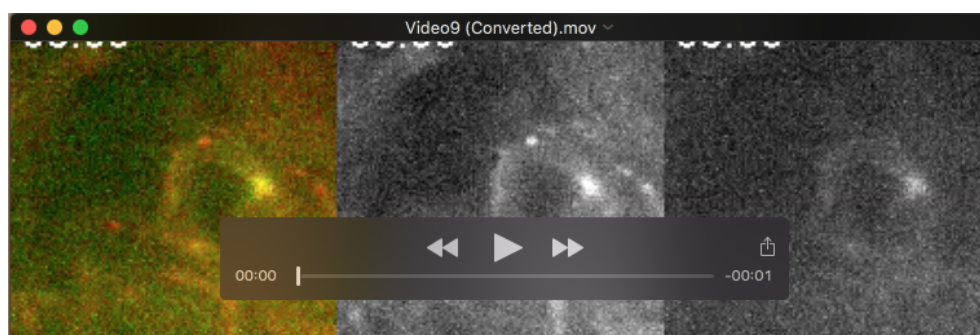
Movie 6. *ensc* mutant neuroblast expressing RFP-tubulin. See the centrosome separation defect. Scale bar: 10 μ m. Time is min:sec.



Movie 7. *ensc* mutant NB expressing RFP-tubulin (red in the merge panel and monochrome in the middle panel) and MBD-GFP (Green in the middle panel, and monochrome in the right panel). There is no rescue of the interphase centrosome separation defect. Scale bar: 10 μ m. Time is min:sec.



Movie 8. Squashed *ensc* mutant NB expressing RFP-tubulin (red in the merge panel and monochrome in the middle panel) and KBD-GFP (green in the merge panel, and monochrome in the right panel). The interphase centrosome separation defect is restored. Scale bar: 10 μ m. Time is min:sec.



Movie 9. *ensc* mutant NB expressing RFP-tubulin (red in the merge panel and monochrome in the middle panel) and full length Ensc-GFP (green in the merge panel, and monochrome in the right panel). Interphase centrosome separation is restored. Scale bar: 10 μ m. Time is min:sec.