A “molecular guillotine” reveals an interphase function of Kinesin-5

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Summary statement

We design a general strategy for conditional motor mutants by inserting a protease cleavage site between head and stalk domain of the motor protein, making it susceptible to specific proteolytic cleavage. We demonstrate the feasibility of the approach with the motor Kinesin-5 and the protease TEV in Drosophila embryos. This approach can potentially be applied to motor proteins kinesin and myosin due to the conserved structures.
Abstract

Motor proteins are important for transport and force generation in a variety of cellular processes and morphogenesis. Here we design a general strategy for conditional motor mutants by inserting a protease cleavage site into the “neck” between the head domain and the stalk of the motor protein, making the protein susceptible to proteolytic cleavage at the neck by the corresponding protease. To demonstrate the feasibility of this approach, we inserted the cleavage site of TEV protease into the neck of the tetrameric motor Kinesin-5. Application of TEV protease led to a specific depletion and functional loss of Kinesin-5 in Drosophila embryos. With our approach, we revealed that Kinesin-5 stabilized the microtubule network during interphase in syncytial embryos. The “molecular guillotine” can potentially be applied to many motor proteins due to the conserved structures of kinesins and myosins with accessible necks.

Introduction

Cytoskeletal motor proteins, including myosins, dyneins and kinesins, convert the chemical energy of ATP hydrolysis into mechanical work. Motor proteins are widely involved in multiple fundamental cellular processes such as intracellular transport, cell division, cell shape change and migration (Schliwa and Woehlke, 2003). The structure of motor proteins is conserved. They contain a motor domain, referred to as “head”, which catalyzes ATP and binds microtubules or F-actin. The catalytic cycle links ATP hydrolysis to a conformational change of the protein that translates into unidirectional movement of the motor protein on the filament. A second part of the protein, the stalk, links the head to the cargo binding site, contains coil-coiled structures for oligomerization or associates with other subunits. Head and stalk are parts
of the same polypeptide, which is functionally relevant as a tight link of head and stalk is essential for transmission of mechanical force (Kent and Lele, 2016).

Genetic analysis of the physiological function of motor proteins has been hampered by their essential function for the cell or organism. For example, Kinesin-5 serves indispensable functions during mitosis (Heck et al., 1993), making an analysis of its function in interphase or in terminally differentiated cells difficult. Conditional mutations, such as temperature sensitive alleles, can overcome these limitations of genetic analysis (Enos, 1990). Gene knock down by RNAi approaches relies on protein turnover, leading to insensitivity of stable proteins. Pharmacological approaches with small molecules inhibitors or specific antibodies provide an alternative and have been applied for motor protein inhibition (Peterson and Mitchison, 2002; Sharp et al., 2000; Sharp et al., 1999). However, chemical approaches cannot be generalized, and need to be developed case by case.

Kinesin-5 proteins are members of kinesin superfamily, consisting of a motor domain and a coiled-coil rod with the central bipolar assembly (BASS) domain. Forming bipolar homo tetramers, Kinesin-5 can crosslink anti-parallel aligned microtubules (van den Wildenberg et al., 2008). The motor activity enables filament sliding, e. g. during formation and elongation of the mitotic spindle (Kapitein et al., 2005; Shimamoto et al., 2015a; Shimamoto et al., 2015b; Waitzman and Rice, 2014). In Drosophila syncytial embryos, Kinesin-5 is enriched at mitotic spindles (Barton et al., 1995) and is essential for spindle formation and chromosome segregation. Injection of antibodies specific for Kinesin-5 into embryos leads to a collapse of newly formed spindles and the formation of mono-asters (Sharp et al., 2000; Sharp et al., 1999).
Making proteins susceptible to proteolytic cleavage represents a generally applicable strategy for generation of conditional alleles (Harder et al., 2008; Oliveira et al., 2010; Pauli et al., 2008). Here we apply this concept to motor proteins by inserting a proteolytic site between the head and stalk region (“neck”). We designated this strategy a “molecular guillotine” (Fig. 1A). We chose the well-characterized Kinesin-5 in order to demonstrate the feasibility of this approach. As a protease, we employ TEV, which is highly specific. No match of TEV recognition motif within the Drosophila proteome has been identified, and flies expressing TEV are viable and fertile (Harder et al., 2008).

Results

Design of a “molecular guillotine”

We inserted three copies of the TEV recognition motif at one of two positions, G394 or Q499, into the stalk region. G394 and Q499 are located within conserved coiled-coil regions next to the head domain (Fig. 1B, C). In addition, we fused GFP to the C-terminus, which does not affect the function of Kinesin-5, as previously reported (Cheerambathur et al., 2008). These constructs were expressed as transgenes in levels comparable to the endogenous allele with Ubiquitin promoter, as assayed by western blot (Fig. 1D). Due to the C-terminal GFP moiety, the constructs showed a slower mobility in SDS-PAGE than wild type Kinesin-5. The TEV sites do not affect the functionality of Kinesin-5 as the construct with the insertion at G394 (Kin-5[G394tev]-GFP) complemented the lethality of a Kinesin-5 (Klp61f07012) mutation. For this, we recombined Kin-5[G394tev]-GFP with a Kinesin-5 mutation. The resulting flies only expressed Kin-5[G394tev]-GFP, were viable and fertile and can be kept as a homozygous stock. In embryos from this line, Kinesin-5 was detected only at the molecular weight corresponding to transgenic Kin-5[G394tev]-GFP, which confirms the absence of endogenous Kinesin-5 (Fig. 1E).
**Kinesin-5 cleavage in vivo**

We expressed TEV protease in stripes in embryos under the control of the *engrailed* promoter. Control embryos with no TEV expression showed uniform Kin-5[G394tev]-GFP expression. In contrast, the GFP signal was strongly depleted in stripes with TEV expression (Fig. 2A). Next we turned to syncytial embryos, which are characterized by their rapid and synchronous nuclear division cycles and the associated remodeling of the cytoskeleton. During mitosis, microtubules and their motors are important for formation and function of mitotic spindles and chromosome segregation, whereas they function in nuclear arrangement and stabilization of the nuclear array in interphase (Kanesaki et al., 2011; Winkler et al., 2015). Kinesin-5 localizes to the mitotic spindle and is involved in chromosome segregation during mitosis (Cheerambathur et al., 2008; Sharp et al., 2000; Sharp et al., 1999). We microinjected TEV protease into syncytial embryos and recorded GFP fluorescence. Following injection of TEV protease but not buffer, GFP fluorescence rapidly dropped (Fig. 2B). Correspondingly, the specific staining pattern, such as labelling of mitotic spindles or cytoplasmic asters was lost in TEV injected embryos (Fig. 2D). Quantification of total GFP fluorescence provided an estimate for an approximate half life of about 30 min (Fig. 2C). Kinesin-5 was specifically cleaved, since the electrophoretic mobility of Kin-5[G394tev]-GFP was higher in TEV than buffer injected embryos (Fig. 2E). Kin-5[G394tev]-GFP embryos were lysed about 30 min after injection and extracts analyzed by western blot against the C-terminus of Kinesin-5. The observed difference in electrophoretic mobility was consistent with proteolytic cleavage at the TEV sites at the neck and corresponding loss of the head domain. As we
detected a single band, proteolytic cleavage was close to complete under our experimental conditions (Fig. 2E).

**Cleavage of Kinesin-5 leads the loss-of-function in mitosis**

Next we analyzed the functional consequences of Kinesin-5 cleavage. To track the nuclear cycles and behavior of chromosomes, we co-injected fluorescent labelled histone-1 and TEV protease into *Kinesin-5* null embryos expressing the Kin-5[G394tev]-GFP transgene (ΔKin5, Kin-5-tev-GFP). Following TEV injection, we observed a failure of chromosome separation and monoastral spindles (Fig. 3A, B). These phenotypes were observed in individual spindles interspersed between normally appearing spindles. These phenotypes were consistent with the previously reported mitotic defects following Kinesin-5 antibody injection (Sharp et al., 1999). We quantified the percentage of failed spindle in the following embryos with TEV injection: 1) ΔKin-5, Kin-5-tev-GFP, 2) Kin-5-tev-GFP and 3) wild type (Fig. 3C). Whereas not defects were observed in wild type embryos, about 3/4 of the spindles did not form or collapsed in embryos containing only the TEV-cleavable Kinesin-5 protein. The proportion of abnormal spindles was about 10%, if both TEV-cleavable and wild type Kinesin-5 was present. This indicates that the TEV-cleavable form of Kinesin-5 exerts a dominant-negative activity on the wild type protein, consistent with Kin-5 acting as a homo tetramer.

**Interphase function of Kinesin-5 in syncytial embryos**

Compared to the well-established mitotic function, much less is known about how Kinesin-5 is involved in microtubule organization and function in interphase. In interphases of syncytial embryos, nuclei and the associated centrosomes form a regular arrangement (Fig. 4A), and Kinesin-5-GFP is strongly enriched at the centrosomes and associated asters. In addition, dynamic extended structures between adjacent asters were detected (Fig. 4B). These transient
signals may represent microtubules coated with Kinesin-5 and possibly antiparallel aligned microtubules. We noticed that in Kinesin-5 depleted embryos, two nuclei originating from different spindles fused together after successful separation from their own sister nuclei (Fig. 4C), which implies an interphase function of Kinesin-5 in keeping nuclei in distance and separated. As hypothesized previously (Kanesaki et al., 2011; Winkler et al., 2015), Kinesin-5 may be involved in nuclear positioning and formation of the nuclear array in syncytial Drosophila embryos. We examined nuclear positions in interphase carefully (Fig. 4D and E). We quantified the numbers of next neighbors for each nucleus (Fig 4F). In control embryos, 50% nuclei have 6 next neighbors, while 33% and 13% have 5 and 7 next neighbors, respectively. In contrast, the distribution was different in the Kinesin-5 depleted embryos, with significantly fewer nuclei with 6 next neighbors and significantly more with fewer than 5 or more than 7 next neighbors (Fig. 4F). As a measure for the regularity of the nuclear arrangement, we calculated the variation of next neighbor distances (standard deviation (σ) normalized to the distance average (μ)). We detected a much higher variation in Kinesin-5 depleted embryos compared to the control embryo (Fig. 4G). In summary our data indicate that Kinesin-5 is involved in interactions between adjacent asters leading to a normal arrangement of the nuclei in interphase.

Kinesin-5 bound to anti-parallel aligned microtubules may push adjacent asters away from each other and thus generate a repulsive force, which may lead to uniform internuclear distances. In this model, Kinesin-5 would promote movements of centrosome and their associated asters. Alternatively, Kinesin-5 may crosslink microtubules from adjacent asters and stabilize the syncytial microtubule network. In this alternative model Kinesin-5 would suppress movement of centrosomes and associated asters (Fig. 5A). To distinguish
these two models, we recorded the dynamics of centrosomes in the scale of seconds and calculated second-scale fluctuations of the centrosomes defined as previously reported (Fig. 5B) (Winkler et al., 2015). We calculate fluctuations, $D_i(t_j)$, as the deviation from slow (minute-scale) drift movements averaged over time and multiple centrosomes. These fluctuations have the dimension of a diffusion constant and do not contain slow drift movements. Comparing buffer and TEV injected embryos, we find that the fluctuations are strongly increased (about three fold) in Kin-5 depleted embryos. The baseline for passive, non-ATP-dependent fluctuations is about 5 times below the wild type fluctuations (Winkler et al., 2015) (Fig. 5D). Due to the increased mobility of centrosomes in Kin-5 depleted embryos we conclude that Kinesin-5 functions in the stabilization of the array of microtubule asters by crosslinking, for example.

Discussion
The function of Kinesin-5 in spindle formation and elongation during mitosis is well established (Heck et al., 1993, Sharp et al., 2000). Consistently, depletion of Kinesin-5 by our guillotine method induced defects in chromosome segregation in syncytial Drosophila embryos consistent with previous reports (Sharp et al., 1999). Beside its role for the mitotic spindle, our insights are limited into Kinesin-5 functions during interphase. Studying interphase function is hampered by the difficulty that interphase functions are obscured by mitotic defects. In the case of Drosophila, embryos depleted of Kinesin-5 cannot be obtained, since Kinesin-5 has an essential function during oogenesis (Radford et al., 2017). Employing small molecule inhibitors, Kinesin-5 was found to be involved in neuron dendritic structure maintenance (Kahn et al., 2015) and intracellular transport from Golgi to the cell surface in cultured cells (Wakana et al., 2013). As another approach for conditional interference, we developed a method for decapitating motor proteins by proteolysis (“molecular guillotine”).
This method is potentially suitable for many motor proteins. We demonstrate the feasibility of this approach with Kinesin-5 and TEV protease. We revealed an interphase function of Kinesin-5 in syncytial Drosophila embryos for the stabilization of the microtubule network and keeping adjacent nuclei in distance.

In syncytial embryos, the microtubule asters originating from centrosomes can directly interact with neighboring asters, since they are not physically separated by plasma membranes. These interactions lead to formation of an extended network covering the embryonic cortex. The phenotypic behavior of centrosomes and their associated nuclei reflect their intrinsic properties but also, as part of the network, the influences from the neighbors. Adjacent microtubule asters potentially interact via crosslinkers such as Feo/Ase1p, bundling proteins or motors with sliding activity, such as Kinesin-5. Here we tested the hypothesis that Kinesin-5 generates repulsive forces between adjacent astral microtubules in interphase. We expected that a loss of force generation would have led to a reduced mobility of the network and its nodes, the centrosomes. Using the fluctuations of centrosomes as an indicator of network dynamics, we rejected our hypothesis, because we measured an increased mobility of the centrosomes, when Kinesin-5 was inactivated. We interpret this data in that the function of Kinesin-5 as a crosslinker is more dominant in vivo than its function for sliding of anti-parallel aligned microtubules and thus pushing apart adjacent microtubule asters. The in vivo function of Kinesin-5 is similar to Kinesin-1, which is enriched at the cortex and F-actin and actin caps (Winkler et al., 2015). Both may be involved in anchoring microtubule asters to the cortex and in this way counteract fluctuation movements of centrosomes. Having identified a stabilizing function of Kinesin-5, the questions remains about the origin of the forces driving centrosome fluctuations. Fluctuations are due to an active
component, since ATP depletion leads to loss of fluctuations. The (−)-end directed motor Kinesin-14 may serve as a force generator (Sharp et al., 2000).

The “molecular guillotine” is potentially a versatile method for conditional inactivation of motor proteins. TEV protease has been employed for inactivation of cohesin in yeast (Uhlmann et al., 2000) and in fly (Pauli et al., 2008), as well as Drosophila claudin (Harder et al., 2008). However, this approach had not been applied for inactivation of motor proteins to our knowledge. The approach of a “molecular guillotine” as reported in this study can be applied widely to members of the motor protein families. Unlike using the small molecules inhibitor (Engelke et al., 2016; Peterson and Mitchison, 2002), TEV protease can be specifically and conditionally expressed using UAS-GAL4 system and its temperature-dependent variants in any genetically tractable cell type. This allows “decapitation” of the selected motor protein in a tissue and developmental stage specific manner. The “molecular guillotine” offers an alternative approach to other previously reported conditional approaches based on protein degradation such as Auxin-inducible degradation system (Gray et al., 2001), the degron (Dohmen et al., 1994) or deGradFP systems (Caussinus et al., 2012), which rely on the ubiquitin-mediated protein degradation machinery. In the case of TEV cleavage a single peptide bond is cleaved, whereas in the degron or deGradFP systems the protein complex is subjected to complete proteolysis in the proteasome. Direct comparison of these systems will reveal the respective advantages and disadvantages with respect to kinetics and effectiveness of motor depletion. As second intrinsic difference to degradation based systems is a potential dominant effect of the “molecular guillotine”. The single cut generates two parts, which may interact with uncut counterparts and thus induce a dominant effect. This detected evidence for such a dominant effect, as a phenotype by TEV protease is detected also in heterozygous
embryos containing a Kinesin-5 wild type allele and a copy of the Kinesin-5-tev-GFP transgene.

In summary, the novel approach of a “molecular guillotine” enabled us to investigate a specific function of the motor protein Kinesin-5 in interphase. Potentially, the decapitation approach can be correspondingly applied to other kinesin motors as well as myosins, as they have a related domain structure in common.

Materials and methods

Genetics

Fly stocks (en-Gal4, α-Tubulin-mCherry, His2Av-GFP, Sas6-GFP, Klp61f 07012) (Peel et al., 2007; Spradling et al., 1999) were obtained from the Bloomington Stock Center, if not otherwise noted, and genetic markers and annotations are described in Flybase (Gramates et al., 2017). Transgenes of ubi-Kin5-tev-GFPQ499, ubi-Kin5-tev-GFPG394 and sqh-Kin5-GFP were generated by P element mediated random genome integration. We isolated multiple insertions on the third chromosome with varying expression. The ubi-Kin5-tev-GFPG394 line with strongest GFP fluorescence was recombined with an amorphic Kinesin-5 mutation (Klp61f 07012) and kept as a homozygous line. Similarly, transgenes of spq-Kin5-GFP (expression driven by the spaghetti-squash (spq) promoter) without TEV sites complemented the lethality of the Kinesin-5 mutation (Klp61f 07012) and were kept as homozygous stocks. TEV protease was expressed from a UAS-t-TEV transgene (Harder et al., 2008) or injected as a purified recombinant protein.
Cloning

A sequence coding for three recognitions sites of TEV protease (PS ENLYFQG PR ENLYFQG GS ENLYFQG PR) was inserted behind the codons of G394 or Q499 of the Kinesin-5 cDNA (SK-Klp61f, Drosophila genomic resource center, Bloomington). The resulting coding sequence (Kinesin-tev) was cloned behind the ubiquitin promoter (Lee et al., 1988; Oda and Tsukita, 2001) and 5’ to eGFP into the multiple cloning site of a pUASt vector derivative lacking the UAS and hsp70TATA sites. The Kinesin-5-GFP fusion constructs were generated by cloning the Kinesin-5 cDNA (from SK-klp61f) with GFP inserted at the C-terminus into the transformation vector sGMCA (Kiehart et al., 2000), which contains the spaghetti-squash promoter for ubiquitous expression. Sequence information and details of the cloning procedure are available upon request.

Western blotting, Kinesin antibody

The Kinesin5 coding sequence corresponding to the C-terminal tail (aa 600–1066) was cloned by PCR with SK-Klp61f (Drosophila genomic resource center, Bloomington) as template into a protein expression vector with a N-terminal 9xHis tag. The His9-Kinesin-5-C600 protein with an apparent molecular weight of about 70 kd in SDS polyacrylamide gel electrophoresis (SDS-PAGE) was purified under denaturing conditions (Trenzyme, Konstanz) and used for immunization of rabbits (BioGenes, Berlin). Embryonic extracts were analyzed by SDS-PAGE and immunoblotting as previously described (Wenzl et al., 2010). Briefly, proteins were blotted to nitrocellulose filters by wet transfer (100 mA per mini gel, overnight). The blots were blocked with 5% fat-free milk in phosphate buffered saline (PBS), incubated with following primary antibodies in PBT (PBS with 0.1% Tween20), rabbit-α-Kinesin-5, (1:5000, this study), mouse-α-Tubulin (1:100000, B512, Sigma), rabbit-α-GFP (1:5000, Torrey Pines Biolabs) and
fluorescently labelled secondary antibodies (LiCOR, 1:20000, 0.05 µg/ml in PBT) for each two hours at room temperature. The developed blots were imaged with a LICOR system.

Microinjection
Embryos were dechorionated and aligned on a coverslip, desiccated for 10 min, and covered with halocarbon oil (Voltalef 10S, Lehmann & Voss). We injected TEV protease at 10 µM purified from overexpressing E. coli (a gift from Dirk Görlich). Histone1-Alexa-488 protein (ThermoFisher) was injected at a concentration of 2 mg/ml.

Microscopy
Images were recorded with a Zeiss microscope equipped with a spinning disc (25x/NA0.7 multi immersion, 40x/NA1.3oil). Centrosome movement was recorded in Sas6-GFP expressing embryos as previously described with a frame rate of 1 Hz (Winkler et al., 2015). Kin-5-GFP distribution in interphase was recorded with a confocal microscope (Zeiss LSM780 with airy scan unit, 63xNA1.4/oil). Images were processed with Fiji/ImageJ (Schindelin et al., 2012).

Fluctuation analysis
The centrosomes tracking and measurement of fluctuation were carried out as previously described (Winkler et al., 2015). The custom made software code is available on request.

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**Competing interests**
The authors declare no competing or financial interests.

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**Data availability**
Original data are available from the authors on reasonable request. Source data as shown in the figures are summarized in Table S1 within the supplemental materials.

**Supplemental information**
Supplementary information available online at .........
References


During Rapid Syncytial Cell Cycles in Drosophila. *G3 (Bethesda)* 7, 2305–2314.
Figure 1. Design of a molecular guillotine for Kinesin-5. (A) Schematic illustration of motor protein molecular guillotine by inserting a protease substrate site next to the head domain of a motor. (B) TEV cleavage site in three copies (3x) is inserted in the coiled-coil region in stalk domain at position G394 or Q499. Domain structure of Kinesin-5 (UniProtKB P46863, motor domain, red, coiled-coil regions, orange) and secondary structure prediction (α-helix in blue, coiled coil (cc) in red) are indicated. (C) Sequence alignment of the insertions sites at G394 and Q499. (D, E) Western blots with embryonic extracts (0–4 h) from wild type and flies with the Kin-5[Q499tev]-GFP and Kin-5[G394tev]-GFP transgene, probed as indicated with antibodies for Kinesin-5, GFP, α-tubulin. Apparent molecular weight (MW) in kilo Dalton.
Figure 2. Kin-5[G394tev]-GFP is cleaved by TEV protease. (A) Image of living embryos expressing Kin-5[G394tev]-GFP with or without TEV protease expressed in striped pattern. Scale bar 50 µm. Region marked by squares in yellow are shown in high magnification. Scale bar 10 µm. Quantification of GFP signal along the anterior-posterior body axis (line in green). (B–D) TEV protease or buffer was injected into syncytial embryos mutant for Kinesin-5 and expressing Kin-5[G394tev]-GFP. (B) Images from time lapse recording. Time in minute:second. (C) GFP fluorescence following TEV injection. Plotted are the mean (solid) and standard deviation (dashed). N, number of quantified embryos for each experimental condition. (D) Images of living embryos before and 30 min after injection. Scale bar 10 µm. (E) Western blot with extracts from embryos 30 min after injection with TEV or buffer probed with Kinesin-5 and α-Tubulin antibodies. Apparent molecular weight in kilo Dalton.
Figure 3. Phenotype of Kin-5[G394tev]-GFP cleavage by TEV protease. (A) Images from time lapse recording of embryos mutant for *Kinesin*-5 expressing the Kin-5[G394tev]-GFP, His2Av-GFP, α-Tublin-mCherry transgene and injected with TEV protease. Arrow head in yellow points to defective mitotic figure. (B) Images from the controlled experiment. TEV protease was injected into the embryo expressing His2Av-GFP, α-Tublin-mCherry, without “Kin-5 guillotine”. Schematic drawing of the mitotic figures. Scale bar: 10 µm. (C) Proportion of abnormal spindles after TEV injection. Mean with standard deviation. N=148 spindles in 3 embryos for ΔKin-5, Kin-5-[tev]-GFP, 348 spindles in 3 embryos for Kin-5-[tev]-GFP and 1384 spindles in 3 embryos for wild type. Source data are listed in Tab. S1.
Figure 4. Kinesin-5 is important for nuclear positioning in interphase. (A) Projected image of an embryo expressing Histone 2Av from selective plane illumination microscopy in side view and cross section (position indicated by lines in blue). Magnified section illustrate the interactions between the nuclei and between nuclei and cortex. Dots in yellow indicate centrosome pairs. (B) Image of living embryo expressing Kin-5-GFP (apical position) Scale bar 5 µm. (C) Images from time lapse recording of embryos mutant for Kinesin-5 expressing the Kin-5[G394tev]-GFP transgene and co-injected with TEV protease and fluorescent labeled Histone H1. The green and red arrow heads indicate 2 nuclei which underwent nuclear division cycle. After successful daughter nuclei separation, the two non-daughter nuclei fused as indicated in yellow arrow head. Scale bar 10 µm. (D) Nuclei labeled with injected Histone H1. Embryos (Kinesin-5 mutant with Kin-5[G394tev]-GFP transgene) injected
with buffer or TEV protease and fluorescently labeled Histone H1 to label nuclei. Scale bar 10 µm. (E) Segmented images from (D). Color coding for the number of next neighbors as indicated. The nuclei labeled by empty circles were not included in the calculation. (F) Proportion of nuclei according to number of next neighbors and (G) irregularity of nuclear arrangement in embryos injected with buffer or TEV protease. The irregularity parameter σ/μ in Kinesin-5 depleted and control embryo. Mean with standard deviation. N=105 nuclei in 3 embryos for TEV injection and 152 nuclei in 3 embryos for buffer injection. Statistical significance calculated by Student T test, * indicated P<0.05, ns, not significant. Source data are listed in Tab. S1.
Figure 5. Kinesin-5 suppresses centrosome fluctuation in interphase. (A) Illustration of microtubule asters with overlapping microtubules in anti-parallel orientation. Kinesin-5 may slide microtubules apart (Model 1) or crosslink adjacent asters (Model 2). (B) Definition of the fluctuation parameter $D$ as the deviation from slower “drift” movement. The fluctuation parameter has the dimension of a diffusion constant and can be regarded as apparent diffusion. (C) Images from living embryo mutant for Kinesin-5 expressing Kin-5[G394tev]-GFP and SAS6-GFP and injection with TEV protease or buffer. Trajectories of centrosomes over 220 s on an image from time lapse recoding. Scale bar 5 µm. (D) Box plot displaying time-averaged fluctuation of centrosomes in embryos expressing SAS-6-GFP injected with buffer (wild type, 5 embryos, 1757 centrosomes), sodium azide (2 embryos, 228 centrosomes) or TEV protease
for cleavage of Kin-5[G394tev]-GFP (2 embryos, 660 centrosomes). Source data are listed in Tab. S1.
Table S1

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