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### Supplemental Information

### Spontaneous Formation of a Globally Connected Contractile Network

#### in a Microtubule-Motor System

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#### **Supporting Materials and Methods**

#### **Measurement of single motor movements**

The movements of GFP-fused kinesin proteins were observed using total internal reflection-fluorescence microscopy, as described previously (1). To immobilize fluorescently labeled MTs on a glass surface coated with diphenyldimethoxysilane, 10 μg/mL anti-β-tubulin antibody (sc-58884, Santa Cruz, Dallas, TX) diluted in BRB80 buffer (80 mM PIPES-KOH, 1 mM MgSO4, and 1 mM EGTA) was flowed into the chamber and incubated for 5 min. The chamber was blocked with 1% (w/v) Pluronic F127 dissolved in BRB80 buffer and incubated for 5 min. Following a wash with 0.6–0.7 mg/mL casein (Nacalai) solution, the chamber was filled with ATTO647N-labeled MTs diluted in BRB80 buffer, supplemented with 10 μM taxol and 0.6–0.7 mg/mL casein, and incubated for 5 min. Unbound microtubules were washed away with BRB80 buffer supplemented with 10 μM taxol and 0.6–0.7 mg/mL casein, after which assay buffer (80 mM PIPES-KOH, 2 mM MgSO<sub>4</sub>, 1 mM EGTA, 2 mM dithiothreitol, 42.5 U/mL glucose oxidase (Sigma), 42.5 U/mL catalase, 25 mM glucose, and 1 mM ATP) containing kinesin molecules was injected. The images were recorded at time intervals of 100 ms, and rolling averages were determined over 4 frames. The positions of motor molecules were determined by 2D Gaussian fitting with custom software (2), and velocities and durations were calculated from each single trace. Velocities were determined by linear fitting to the traces.

#### **Microtubule sliding assay**

The sliding movements of crosslinked MTs driven by Eg5 were observed by total internal reflection fluorescence microscopy or confocal laser scanning microscopy (Nikon A1 and Ti-E; Nikon, Tokyo, Japan), using a CFI Plan Apo VC 60X (NA = 0.95, Nikon). To immobilize biotinylated ATTO647N-labeled MTs on the glass chamber coated by Teflon (Furuta, in preparation), 1 mg/mL streptavidin (Wako, Osaka, Japan) in 20 mM PIPES-KOH buffer (pH 6.8) was flowed into the chamber and incubated for 3 min. Then, chamber was blocked with 1% (w/v) Pluronic F127 in BRB80 buffer and incubated for 5 min. Following a wash with 0.6–0.7 mg/mL casein solution, the chamber was filled with ATTO647N-labeled MTs diluted in BRB80 buffer with 10 μM taxol and 0.6–0.7 mg/mL casein, and incubated for 5 min. Eg5 solution (20 nM) in BRB80 buffer with 10 μM taxol was injected into the chamber and incubated for 5 min. The chamber was filled with ATTO565-labeled MTs diluted in BRB80 buffer with 10 μM taxol and 0.6–0.7 mg/mL casein, followed by a 5-min incubation. After washing the chamber with BRB80 buffer containing 10 μM taxol and 0.6–0.7 mg/mL casein, assay buffer was applied to the chamber. The images were recorded at time intervals of 100 ms. Displacement of MTs was traced by manual tracking with custom software (2). The velocity of each MT was determined by a linear fitting to the trajectory.

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### **Supporting Figures**



#### **Figure S1. Motile properties of Eg5 used in this study.**

(A) Kymograph of GFP-fused Eg5 along a taxol-stabilized GTP-MT in the presence of 1 mM ATP. (B) Trajectories of GFP-fused Eg5 along MTs in the presence of 1 mM ATP (gray lines,  $n = 239$  from 3 experiments). The orange line represents the average trajectory of all traces. (C) Distribution of the interval velocity ( $\Delta t = 1.0$  s) obtained from all traces shown in (B). The data were analyzed by Gaussian fitting (orange line, mean =  $8.9 \pm 0.4$  nm/sec). (D) Representative time-lapsed images of MT sliding by Eg5 in the presence of 1 mM ATP. The images of MTs immobilized on glass surface (green) and sliding MTs (red) were merged. The surface MTs were biotinylated and immobilized via biotin-avidin interactions. (E) Kymograph depicting a relative sliding movement between the crosslinked MTs shown in (E). The images of MTs immobilized on a glass surface (green) and sliding MTs (red) were merged. (F) Velocity distribution of MT sliding by Eg5 (n  $= 56$  from 3 experiments). The velocity-distribution data were fitted by Gaussian fitting, and the mean  $\pm$  SD are shown.

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#### **Figure S2. Spatiotemporal dynamics of a static network**

(A) Three-dimensional representations of the time-dependent evolutionary patterns of MTs and motors in the static network shown in Figure 1C ( $[Eg5] = 8.91 \text{ nM}$ ,  $[tubulin] = 1 \mu M$ ). (B) Time-series images showing the formation of bundled MT structures under low MT and Eg5 concentrations ([tubulin] = 1  $\mu$ M, [Eg5] = 4.5 nM). Yellow circles indicate Eg5-accumulated nodes and yellow arrow heads represent MT bundles. The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and GFP-fused Eg5 (cyan) were merged. The times displayed in the images represent the time elapsed following mixing. (C) Image of ATTO647N-labeled MTs shown in (B). (D) Temporal evolution patterns of the total cluster size and the largest cluster. (E) The temporal evolution of the velocity correlation functions in the static network pattern shown in Figure 1. Note that several horizontal stripes appeared in  $C_{xx}$  ( $C_{yy}$ ) are the artifacts created by small drift component in *x*(*y*)-direction left even after drift corrections by the software ImageJ. Note also that several blanks appeared within the small distances  $(< 50 \text{µm}$ ) are due to shortage of the number of nodes for averaging.

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#### **Figure S3. Spatiotemporal dynamics of an active network**

(A) Three-dimensional representations of the time-dependent evolutionary patterns of MTs and motors in the active network shown in Figure 2A ( $[Eg5] = 22.5$  nM,  $[tubulin] = 1 \mu M$ ). (B) The temporal evolution of the velocity correlation functions in the active network pattern shown in Figure 2. (C) Time-series images

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showing occasional self-rupturing of a network ( $[Eg5] = 22.5$  nM and  $[tubulin] = 1 \mu M$ ). The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and GFP-fused Eg5 (cyan) were merged. The times displayed in the images represent the time elapsed following mixing. The yellow dashed line indicates the point of rupture. (D) Time-series images showing that different spatiotemporal patterns emerged under the same concentration of Eg5 and MT ( $[Eg5] = 22.5$  nM and  $[tubulin] = 1 \mu M$ ). The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and GFP-fused Eg5 (cyan) were merged. The times displayed in the images represent the time elapsed following mixing.

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**Figure S4. Active networks under various motor concentrations.**

Time-series images showing spatiotemporal dynamics of active network under various Eg5 concentrations. The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and GFP-fused Eg5 (cyan) were merged. The times displayed in the images represent the time elapsed following mixing. The temporal evolutionary pattern shown in left displayed a self-rupturing.

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**Figure S5. Different spatiotemporal patterns from the same concentration of components** (A) Images of motor channels at the initial dynamics state (5 min following mixing). (B) Distribution of intensities at the early stage of dynamics (5 min).

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#### **Figure S6. Spatiotemporal dynamics of aggregation**

(A) Three-dimensional representations of spatiotemporal dynamics of MTs and motors in the aggregation shown in Figure 3A ( $[Eg5] = 44.5$  nM,  $[tubulin] = 1 \mu M$ ). (B) The image showing the formation of astral structures following the rapid contraction of the network ( $[Eg5] = 44.5$  nM,  $[tubulin] = 1 \mu M$ ). The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and GFP-fused Eg5 (cyan) were

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merged. The inset displays the three-dimensional representation of the astral structure indicated by the yellow square. (C) The temporal evolution of the velocity correlation functions in the aggregation pattern shown in Figure 3. (D) Time-series images showing the examples of temporal evolutionary pattern of aggregation  $(Eg5] = 44.5$  nM,  $[tubulin] = 1 \mu M$ ). The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and GFP-fused Eg5 (cyan) were merged. The times displayed in the images represent the time elapsed following mixing.

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#### **Figure S7. Comparison between GTP- and GMPCPP-MT**

(A) Representative images of taxol-stabilized GTP-MTs and GMPCPP-MTs. (B) Box plot of the mean lengths of MTs in assay solution, as measured in self-organization experiments. The mean MT lengths were obtained from 24 (normal; taxol-stabilized GTP-MTs without shear flow), 13 (shear; taxol-stabilized GTP-MTs shortened by shear flow), and 26 (GMPCPP) experiments, respectively. For each experiment, at least 300 MTs were analyzed. The p values from the paired Welch's t-test were found to be  $9.775 \times 10^{-6}$  (normal vs. shear),  $9.775 \times 10^{-6}$  (normal vs. GMPCPP), and 0.7415 (shear vs. GMPCPP), respectively. (C) MT densities in assay solution containing GTP-MTs and GMPCPP-MTs. The p value from the paired Welch's t test was found to be 0.5065.

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**Figure S8. Effects of the motile properties of Eg5 and KIF5Bhead-Eg5tail on GMPCPP-MTs**

(A) Kymograph depicting the movements of Eg5 along a GMPCPP-MT in the presence of 1 mM ATP. (B) Trajectories of Eg5 movements on GMPCPP-MTs in the presence of 1 mM ATP. The total trace number was 175 from 3 experiments. (C) Mean displacement plots of Eg5 on taxol-stabilized GTP-MTs and GMPCPP-MTs. The mean velocities obtained from a linear fitting (black line) were  $19.0 \pm 0.2$  nm/s (GMPCPP) and 9.4  $\pm$  0.1 nm/s (GTP), respectively. (D) Kymograph depicting the movements of KIF5B<sub>head</sub>-Eg5<sub>tail</sub> along a GMPCPP-MT in the presence of 1 mM ATP. (E) Trajectories of KIF5B<sub>head</sub>-Eg5<sub>tail</sub> movements on GMPCPP-MTs in the presence of 1 mM ATP. The total trace number was 228 from 3 experiments. (F) Mean displacement plots of KIF5B<sub>head</sub>-Eg5<sub>tail</sub> on taxol-stabilized GTP-MTs and GMPCPP-MTs. The mean velocities obtained from a linear fitting (black line) were  $254 \pm 1$  nm/s (GMPCPP) and  $141 \pm 1$  nm/s (GTP), respectively.

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#### **Figure S9. Effects of MT properties on network dynamics**

(A) Spatiotemporal dynamics of the Eg5 and GMPCPP-MT system. The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and Eg5 (cyan) are merged. The scale bar (1000  $\mu$ m) applies to each image shown. The tubulin concentration was  $1 \mu M$  in all experiments. (B) Spatiotemporal dynamics of the network composed of Eg5 and MTs that were shortened by shear flow. The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and Eg5 (cyan) are merged. The tubulin concentration was  $1 \mu M$  in all experiments.

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#### **Figure S10. Motile properties of the KIF5Bhead-Eg5tail protein**

 $(A)$  Schematic representations of Eg5 and a chimeric motor, KIF5B<sub>head</sub>-Eg5<sub>tail</sub>. The 6×His and FLAG tag fused to C-terminus of both constructs are not shown. (B) SDS-PAGE image of kinesin constructs. The red arrowheads indicate the purified kinesin constructs. (C) Trajectories of KIF5Bhead-Eg5tail movements along MTs in the presence of 1 mM ATP. The total trace number was 239 from 3 experiments. (D) Meandisplacement plots calculated from the traces of Eg5 (n = 239) and KIF5B<sub>head</sub>-Eg5<sub>tail</sub> (n = 249). Bars represent the standard error. The dashed lines indicate the linear fit, as determined by the least-squares method. The mean velocities calculated as the slope of fitted lines were  $9.4 \pm 0.1$  nm/s (Eg5,  $\pm$  SE of fitting) and  $141 \pm 0.1$ nm/s (KIF5B<sub>head</sub>-Eg5<sub>tail</sub>), respectively. (E) Comparison of the durations that Eg5 and KIF5B<sub>head</sub>-Eg5<sub>tail</sub> were bound to MTs. The cumulative distributions of the durations for Eg5 (black) and KIF5B<sub>head</sub>-Eg5<sub>tail</sub>-GFP (red) are shown. The decay constants derived from a 1-phase exponential decay model were  $13.6 \pm 0.1$  s ( $\pm$  SE of fitting) for Eg5 and  $5.1 \pm 0.1$  s for KIF5B<sub>head</sub>-Eg5<sub>tail</sub>, respectively. (**F**) Double plots of single-molecule velocities and durations of the kinesin constructs. Error bars for the velocities indicate the standard deviations calculated from the raw data, and error bars for the durations represent the standard errors of fitting.

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#### **Figure S11. Spatiotemporal dynamics of isolated asters**

Three-dimensional representations of the spatiotemporal dynamics of MTs and motors in the isolated asters shown in Figure 4B ( $\overrightarrow{[Eg5]}$  = 44.5 nM,  $\overrightarrow{[tubulin]}$  = 1  $\mu$ M)

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#### **Figure S12. Model schematics**

(**A**) Filament reorientation induced by the concentration gradient of motors. Black arrows indicate the local orientations of filaments. Reorientation proceeds as filaments migrate to high-concentration regions to create negative divergence in the local filament orientation, *i.e.* inward pointing asters. (**B**) A single aster-like structure of filament-motor assembly is represented using a node and links. (**C**) The contraction between 2 adjacent nodes originates from the sliding action of the motors. Cyan arrows in the top panel represent the direction of node contraction, whereas white arrows in the bottom panel indicate the direction of filament movement driven by the sliding action of motors. Plus and minus signs represent the filament polarity.

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#### **Figure S13. Formation of MT bundles around active nodes and results of a model simulation**

(A) MT bundles surrounding active nodes formed 91 min after mixing MTs and Eg5 ([tubulin] = 1  $\mu$ M, [Eg5] = 22.5 nM). Top: the images of ATTO647N-labeled MTs (magenta) and GFP-fused Eg5 (cyan) were merged. Bottom: the images of ATTO565-labeled MTs (yellow) and GFP-fused Eg5 (cyan) were merged. Bar: 100 μm. (B) Rupture dynamics of an active network.  $C<sub>ub</sub> = 0.59$ , and the other model parameters are the same as those of the active network shown in Figure 5B. (C) MTs were shuttled from their initial location to be

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exchanged between active nodes during network formation. *T* is the time measured from the point of mixing MTs and motors ([tubulin] = 1  $\mu$ M, [Eg5] = 22.5 nM). The images of ATTO565-labeled MTs (yellow) and GFP-fused Eg5 (cyan) were merged. Bar: 100 μm. (D) Simulation result of a modified model, wherein the contraction strength correlates linearly with the motor concentration:  $K_{ij} = 0.5(K - k)(C_i + C_j) + k$ .  $L_{ij} = 0.5(L - k)$  $-l(C_i + C_j) + l$ . The model parameters are the same as those of active network shown in Figure 5B. Left:  $C_{ub}$  $= 0.4$ . Right:  $C_{ub} = 0.7$ . Links are depicted by magenta lines and motors are depicted by white circles.

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## **Supporting Table**

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#### **Table S1. Quantitative analysis of spatiotemporal dynamics of MT-Eg5 network.**

MTs used in the experiments, concentrations of tubulin and Eg5, max velocities of active nodes during temporal evolutions, contractility, connectivity, types of patterns, and total observation time are shown. "GTP", "GMPCPP", and "Shear" represent taxol-stabilized MTs without shear flow, GMPCPP-MTs, and GTP-MTs shortened by shear flow, respectively. Max velocity indicates the maximum mean node velocity determined from the temporal evolutionary patterns of mean node velocities. In some experiments with  $5 \mu M$  of tubulin, the active nodes were too densely distributed to measure velocities.

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### **Movie Captions**

#### **Movie S1. Spatiotemporal dynamics of a static network**

Three dimensional reconstitution of the temporal evolution of the static network shown in Fig. 1C ( $[Eg5] =$ 8.9 nM, [tubulin] = 1  $\mu$ M). The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and GFP-fused Eg5 (cyan) are merged.

#### **Movie S2. Spatiotemporal dynamics of MTs in the static network (1800 × real-time)**

The movie displaying the global connectivity of MTs in the static network ( $[Eg5] = 1.37$  nM,  $[tubulin] = 1$ uM). The images of ATTO647N-labeled MTs were shown.

#### **Movie S3. Spatiotemporal dynamics of an active network**

Three dimensional reconstitution of the temporal evolution of the active network shown in Fig. 2A ( $[Eg5] =$ 22.5 nM, [tubulin] = 1  $\mu$ M). The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and GFP-fused Eg5 (cyan) are merged.

#### **Movie S4: Temporal evolution pattern of velocity field of the active network**

The time-dependent evolutionary pattern of node velocities in the active network shown in Fig. 2A represented by vector fields. The value of velocities are indicated by length of the vectors and colors.

#### **Movie S5: Spatiotemporal dynamics of aggregation**

Three dimensional reconstitution of the temporal evolution of the aggregation shown in Fig. 3A ( $[Eg5] = 45$ ) nM,  $[$ tubulin $] = 1 \mu$ M). The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and GFP-fused Eg5 (cyan) are merged.

#### **Movie S6: Spatiotemporal dynamics of isolated asters**

Three dimensional reconstitution of the temporal evolution of the isolated asters shown in Fig. 4B ([KIF5Bhead-Eg5<sub>tail</sub>] = 12.5 nM, [tubulin] = 1  $\mu$ M). The images of ATTO647N-labeled MTs (magenta), ATTO565-labeled MTs (yellow), and GFP-fused KIF5B<sub>head</sub>-Eg5<sub>tail</sub> (cyan) are merged.

#### **Movie S7: Spatiotemporal dynamics of MTs in the isolated asters (1800 × real-time)**

The movie displaying the lack of global connectivity in the isolated asters ([KIF5B<sub>head</sub>-Eg5<sub>tail</sub>] = 6.25 nM,  $[tubulin] = 1 \mu M$ ). The images of ATTO647N-labeled MTs were shown.

#### **Movie S8: Spatiotemporal dynamics of a static network in the coarse-grained model**

Model simulation of the static network shown in Fig. 5B. Links are represented in magenta, and motors are represented in white. Model parameters:  $C_{ub} = 0.1$ ,  $K = 15$ ,  $k = 0.5$ ,  $f = 0.01$ ,  $L = 0.008$ ,  $L_c = 0.16$ ,  $l = 0.08$ ,  $C_{\text{max}} = 1$ ,  $\tilde{C} = 0.5$ ,  $\epsilon = 0.1$ ,  $\gamma = 1$ ,  $dt = 0.01$ ,  $N_x = 100$ , and  $N_y = 58$ . The total length of the movie corresponds to 50,000 simulation steps.

#### **Movie S9: Spatiotemporal dynamics of active network in the coarse-grained model**

Model simulation of the active network shown in Fig. 5B. Links are represented in magenta, and motors are represented in white. Model parameters:  $C_{ub} = 0.4$ ,  $K = 15$ ,  $k = 0.5$ ,  $f = 0.01$ ,  $L = 0.008$ ,  $L_c = 0.16$ ,  $l = 0.08$ ,  $C_{\text{max}} = 1$ ,  $\tilde{C} = 0.5$ ,  $\epsilon = 0.1$ ,  $\gamma = 1$ ,  $dt = 0.01$ ,  $N_x = 100$ , and  $N_y = 58$ . The total length of the movie corresponds to 50,000 simulation steps.

#### **Movie S10: Spatiotemporal dynamics of aggregation in the coarse-grained model**

Model simulation of the aggregation shown in Fig. 5B. Links are represented in magenta, and motors are represented in white. Model parameters:  $C_{ub} = 0.7$ ,  $K = 15$ ,  $k = 0.5$ ,  $f = 0.01$ ,  $L = 0.008$ ,  $L_c = 0.16$ ,  $l = 0.08$ ,  $C_{\text{max}} = 1$ ,  $\tilde{C} = 0.5$ ,  $\epsilon = 0.1$ ,  $\gamma = 1$ ,  $dt = 0.01$ ,  $N_x = 100$ , and  $N_y = 58$ . The total length of the movie corresponds to 50,000 simulation steps.

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### **Supporting References**

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- 2. Furuta, K. and Y.Y. Toyoshima, *Minus-end-directed motor Ncd exhibits processive movement that is enhanced by microtubule bundling in vitro.* Curr Biol, 2008. **18**(2): p. 152-7.