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Supplemental Information

Opposing Kinesin and Myosin-I Motors Drive Membrane Deformation and Tubulation along Engineered Cytoskeletal Networks

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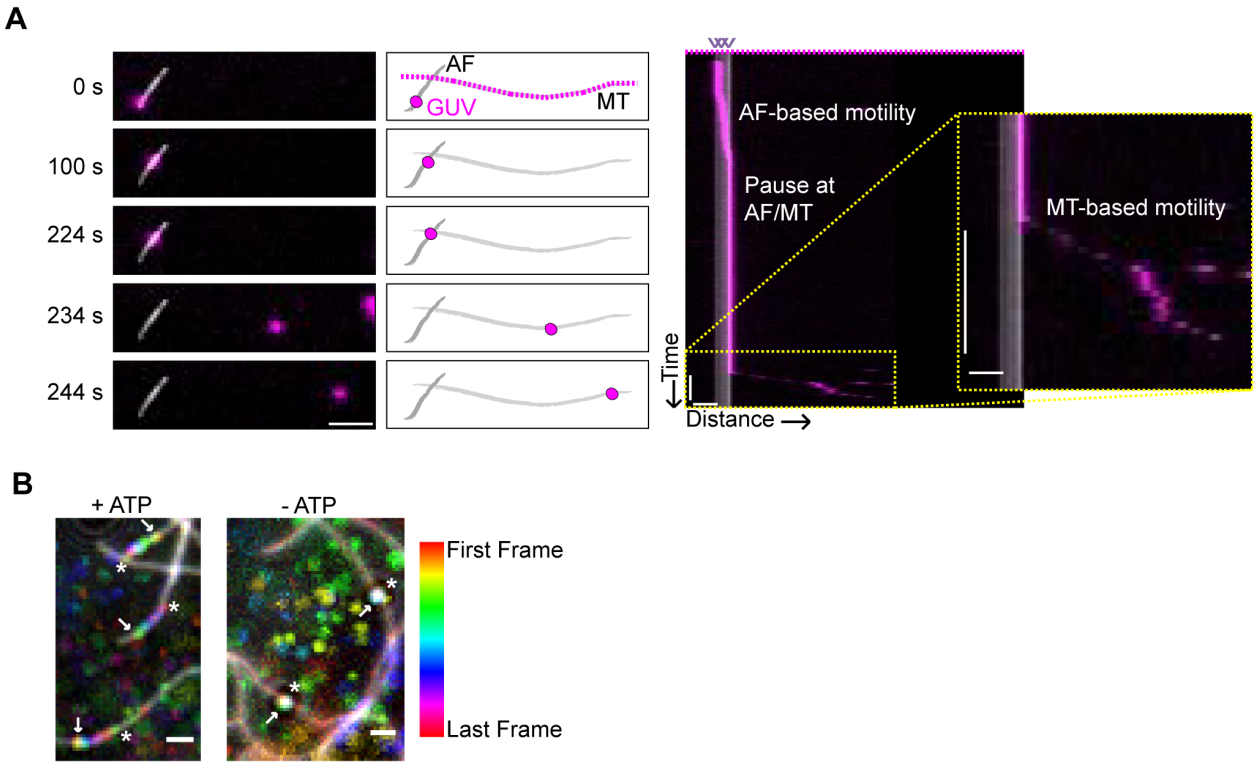

Figure S1: PIP_2-GUV transportation along AF to MT. Related to Figure 1, Movies S1 and S2.

(A) Example time series, illustrations, and kymograph of a PIP_2-GUV switching from AF-based to MT-based motility. Different modes of motility are indicated along the kymograph. Magnified region highlights MT-based motility. This example shows a sparse AF geometry to allow clear visualization of an AF to MT transition. Dashed magenta line in illustration indicates region plotted in kymograph; purple arrowheads above kymograph indicate AFs. Scale bars = 5 μm, 20 sec. See also Movie S1.

(B) Myo1c bound to membrane-coated beads composed of 2% $PI(4,5)P_2$ and 98% PC interacts with AFs in the presence (left) and absence (right) of 1 mM MgATP. These temporally color-coded hyperstacks show Myo1c-driven motility of membrane-coated beads in the presence of MgATP. Membrane-coated bead motility is illustrated as initiating at the arrow, and terminating at the asterisk. Membrane-coated beads specifically bound to AFs in the absence of MgATP, but did not show motility. Scale bar = 1 μm. See also Movie S2.

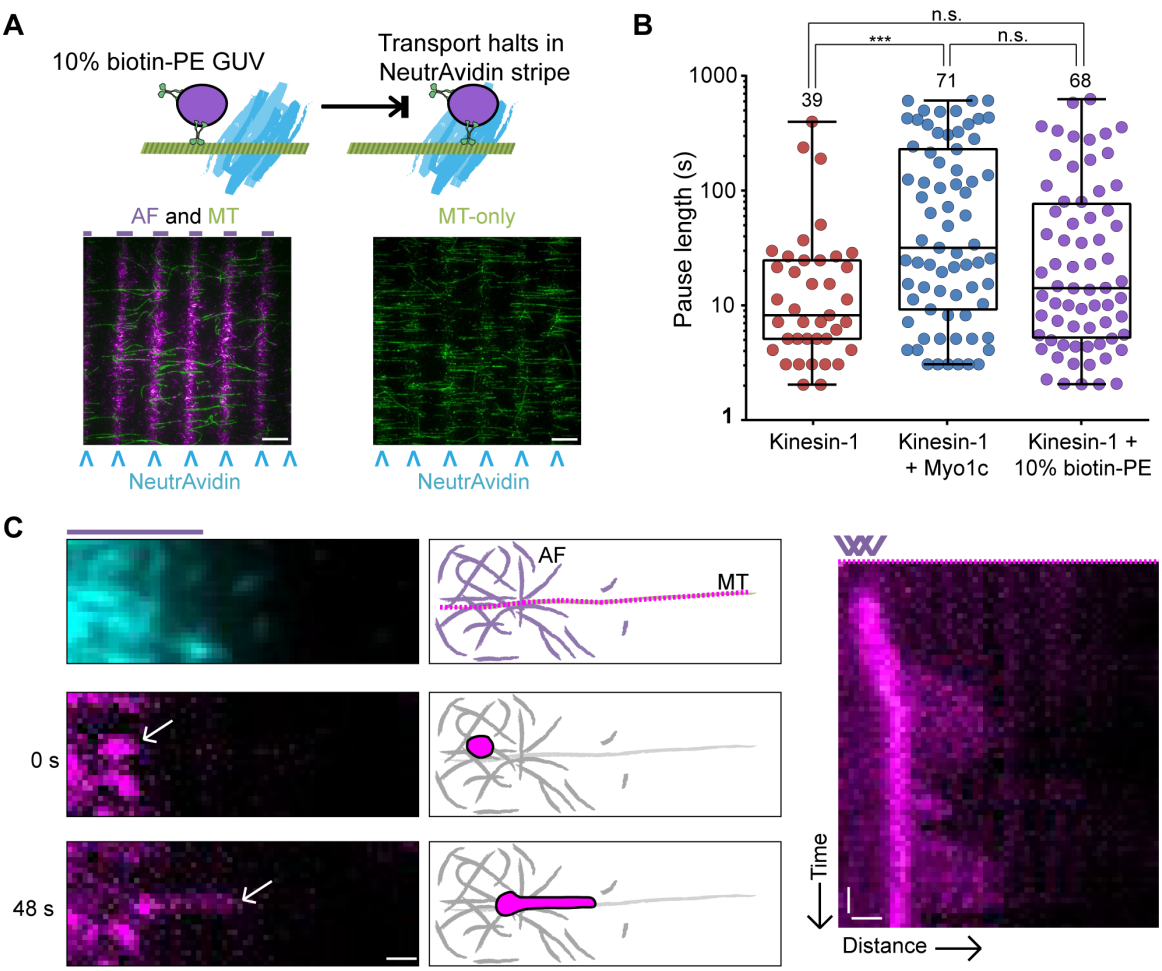

Figure S2: A non-Myo1c-tethered-GUV control experiment, and additional exploration of endophilin-stimulated tubulation. Related to Figure 3.

(A) Experimental design for biotin-PIP_2-GUV experiments. 10% biotin-PE lipid was added to PIP_2-GUVs, which interacted with the NeutrAvidin patterned on the coverslip (indicated by cyan arrowheads) in the presence of MTs, but not AFs. Example cytoskeletal patterning of AFs and MTs (left) and MTs-only (right), where purple lines above indicate micropatterned region of AF binding. Scale bar = 10 μm.

(B) Box plot of the log of PIP_2-GUVs pause time as a function of motor and biotin-PE content. The number of GUV pauses observed is listed above each box plot. Myo1c tethered to AFs pause GUVs significantly longer than non-specific pausing of kinesin-1-only GUVs at AF intersections. Biotin-PE tethers GUVs to NeutrAvidin for pause lengths that are between those of kinesin-1-only and kinesin-1+Myo1c. In this plot, error bars show the range of values (smallest to largest), and the box indicates the interquartile range (middle 50% of data) with the median indicated by the central line.

(C) Fluorescence micrographs (left), cartoon (center), and kymograph (right) showing tubulation in the presence of kinesin-1+Myo1c and 80 nM endophilin. In the time lapse, AFs (cyan) and PIP_2-GUVs (magenta) are shown at GUV shortest (0.74 μm, t = 0s) and longest (3.51 μm, t = 48s) lengths. In time lapse, purple line above indicates micropatterned region of AF binding, white arrows indicate leading edge of tube; purple arrows above kymograph indicate AF intersections. Magenta dotted line in illustration represents the line where the kymograph was drawn. Scale bars = 1 μm, 5 s.

Supplemental Movie Legends

Movie S1: PIP_2-GUVs can switch from AF- to MT-based transport. Related to Figure 1C.

PIP_2-GUVs containing both kinesin-1 and Myo1c can switch from AF-based transport to MT-based transport, as indicated by t = 0 s. This example (see also Figure S1A) illustrates an AF to MT motility transition in a simplified AF environment for clarity. This behavior was also observed in dense AF environments. Cytoskeleton shown at the beginning of the movie with the AF in purple and MT in green. During the movie, AF is shown in grey and the GUV is shown in magenta. Scale bar = 1 μm. Played back at 10x real time.

Movie S2: Myo1c can transport 2% $PI(4,5)P_2$-containing membrane-coated beads along AF in the presence (left), but not absence (right) of ATP. Related to Figure 1D.

During the movie, AFs are shown in grey (fluorescence) and 0.5 μm membrane-coated beads are white (DIC). Scale bar = 1 μm. Played back at 50x real time.

Movie S3: Kinesin-1-only PIP_2-GUVs do not deform, while PIP_2-GUVs containing both kinesin-1 and Myo1c deform at AF/MT intersections. Related to Figures 2A, 2B.

Kinesin-1-only PIP_2-GUVs are largely non-deformed at AF/MT intersections (top movie). PIP_2-GUVs containing both kinesin-1 and Myo1c (bottom movie) deform at AF/MT intersections. The arrow indicates the vesicle of interest. Cytoskeleton shown at the beginning of the movie with AFs in purple and MTs in green. During the movie, AFs are shown in grey and GUV is shown in magenta. Scale bar = 1 μm. Played back at 5x real time.

Movie S4: PIP_2-GUVs containing kinesin-1 and Myo1c rarely tubulate at AF/MT intersections. Related to Figure 2C.

Example of a PIP_2-GUV landing on an AF, undergoing AF-based transport, switching to MT-based transport, deforming along MTs, then tubulating along the MT after encountering a second AF intersection. Cytoskeleton shown at the beginning of the movie with AFs in purple and MTs in green. During the movie, AFs are shown in grey and GUV is shown in magenta. Scale bar = 1 μm. Played back at 5x real time.

Movie S5: In the presence of 8 nM endophilin, PIP_2-GUVs containing kinesin-1 and Myo1c readily tubulate at AF/MT intersections. Related to Figure 3A.

Example tubulation of a GUV at AF/MT intersections in the presence of 8 nM endophilin. The PIP_2-GUV is motile along the MT until encountering AFs patterned on the coverslip, at which point transport halts and MT-based tubulation is initiated. Cytoskeleton shown at the beginning of the movie with AFs in purple and MTs in green. During the movie, AFs are shown in grey and GUV is shown in magenta. Grey fluorescence on growing tube is a combination of fluorescence from Rhodamine-PE and GFP-kinesin-1. Scale bar = 5 μm. Played back at 10x real time.

Movie S6: In the presence of 80 nM FCHo, PIP_2-GUVs containing kinesin-1 and Myo1c frequently tubulate at AF/MT intersections. Related to Figure 3B.

Example tubulation of a GUV at AF/MT intersections in the presence of 80 nM FCHo. The PIP_2-GUV is motile along the MT until encountering AFs, at which point MT-based tubulation is rapidly initiated. The GUV continues its motility along the MT, again pausing and tubulating at the next AF intersection. Note that the FCHo-stimulated tubes are elongated rapidly and do not persist, except when stabilized by Myo1c at AF intersections. Once the tube passes AFs, Myo1c can stabilize the tube for hundreds of seconds. Note AF reorganization along tube during this long pause. Cytoskeleton shown at the beginning of the movie with AFs in purple and MTs in green. During the movie, AFs are shown in grey and GUV is shown in magenta. Scale bar = 5 μm. Played back at 5x real time.

Movie S7: In the presence of 80 nM endophilin, LM-GUV tubulation along MT, proceeded by tubulation along AFs. Related to Figure 6B.

Example tubulation event that switched from tubulation along AFs to tubulation along MTs. The cytoskeleton shown at the beginning of the movie with AFs in purple and MTs in green. During the movie, MTs are shown in grey and the GUV is shown in magenta. Scale bar = 1 μm. Played back at 20x real time.

Movie S8: Tubulation of LM-GUVs along AFs, 0 nM endophilin present. Related to Figure 6D.

Both spherical (left) and morphologically dynamic (right) LM-GUVs tubulate along AFs, even in the absence of endophilin. Cytoskeleton shown at the beginning of the movie with AFs in purple and MTs in green. During the movie, MTs are shown in grey and the GUV is shown in magenta. Scale bar = 1 μm. Played back at 20x real time.

Movie S9: Tubulation of LM-GUVs along AFs in the presence of 80 nM endophilin. Related to Figure 6E.

Both spherical (left) and morphologically dynamic (right) LM-GUVs tubulate along AFs in the presence of 80 nM endophilin. Cytoskeleton shown at the beginning of the movie with AFs in purple and MTs in green. During the movie, MTs are shown in grey and the GUV is shown in magenta. Scale bar = 1 μm. Played back at 20x real time.