SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURE 1. Sample histograms of Khc-73 single molecule processive motility behavior. A) Histogram of velocities of dimerized, GFP-tagged Khc-73 1-387-LZ motor (n=119). A single-peak Gaussian curve was fit to the histogram of velocities and showed a peak corresponding to an average of 1.56 μm/sec and a standard deviation of 0.15 μm/sec. B) Histogram of run lengths of dimerized, GFP-tagged Khc-73 1-387-LZ motor (n=119). An exponential decay curve was fit to the data and generated an average run length of 1.98 μm and a standard deviation of 0.20 μm. The decay curve assumed that run lengths existed below our threshold of detection due to sampling error (i.e. our inability to differentiate diffraction-limited spots).

SUPPLEMENTAL FIGURE 2. Co-expression of fluorescently labeled Khc-73 constructs and alpha tubulin in S2 cells. *Drosophila* S2 cells were transiently transfected with constructs containing mCherry-tagged α-tubulin and GFP-tagged, Khc-73 constructs shown under the control of the metallothinein promoter.

SUPPLEMENTAL FIGURE 3. Co-expression of fluorescently labeled full-length Khc-73 and Rab GTPases. *Drosophila* S2 cells were transiently transfected with constructs containing mCherry-tagged Khc-73 under the control of the metallothinein promoter and GFP-tagged Rab GTPases under the control of the actin promoter. Images shown are individual frames of time-lapse movies.

SUPPLEMENTAL FIGURE 4. Co-expression of fluorescently labeled Khc-73 constructs and Rab5. *Drosophila* S2 cells were transiently transfected with constructs containing mCherry-tagged Khc-73 constructs under the control of the metallothinein promoter and GFP-tagged Rab5 under the control of the actin promoter. Images shown are individual frames of time-lapse movies.

SUPPLEMENTAL FIGURE 5. A) Knockdown of Khc-73 in BG2 cells. *Drosophila* BG2 cells were incubated for 7 days with RNAi targeting the 3'-UTR of Khc-73 mRNA, the region corresponding to amino acids 910-1044, or mock treated. After 7 days, culture medium was removed and cells were centrigured and analyzed by SDS-PAGE gel. Gel was transferred to nitrocellulose and probed for Khc-73 and alpha-tubulin (loading control). B) *Drosophila* S2 cell expressing Rab5-GFP and incubated for one hour with 5 mM cytochalasin D. C) Histogram of plus-end directed velocities of Rab5-GFP puncta in mock-treated *Drosophila* S2 cells. Application of a Gaussian curve to the histogram yielded a mean velocity of 0.78 ± 0.29 μm/sec (mean ± SD). D) Histogram of plus-end directed velocities of Rab5-GFP puncta in *Drosophila* S2 cells treated for 7 days with RNAi targeting Khc-73. Application of a Gaussian curve to the histogram yielded a mean velocity of 0.66 ± 0.31 μm/sec (mean ± SD).

SUPPLEMENTAL FIGURE 6. RNAi-mediated knockdown of Khc-73 in BG2 cells expressing fluorescently labeled Khc-73 constructs and Rab5. *Drosophila* BG2 cells were subjected to 7-day RNAi treatment with an RNAi construct targeting the 3'-UTR of Khc-73. On day 4, cells were transfected with constructs containing the mCherry-tagged portion of Khc-73 shown under the control of the metallothinein promoter and GFP-tagged Rab5 under the control of the actin promoter. On day 6, cells were incubated with 50 μM copper sulfate for 16 hours. On day 7, time-lapse imaging was performed. Images shown are single frames of time-lapse movies.

SUPPLEMENTAL MOVIE 1. *Drosophila* S2 cell expressing full-length Khc-73-GFP and mCherry-α-tubulin as described for Figure 4A. Individual channels are merged into a single image with Rab5-GFP shown in green and Khc-73-mCherry shown in red. Movie runs at 20 frames per second, corresponding to roughly 20X real time.

SUPPLEMENTAL MOVIE 2. *Drosophila* S2 cell expressing Rab5-GFP and full-length Khc-73-mCherry as described for Figure 4B. Individual channels are merged into a single image with Rab5-GFP shown in green and Khc-73-mCherry shown in red. Movie runs at 20 frames per second, corresponding to roughly 20X real time.

SUPPLEMENTAL MOVIE 3. *Drosophila* BG2 cell expressing Rab5-GFP and full-length Khc-73-mCherry as described for Figure 4B. Individual channels are merged into a single image with Rab5-GFP shown in green and Khc-73-mCherry shown in red. Movie runs at 20 frames per second, corresponding to roughly 20X real time.