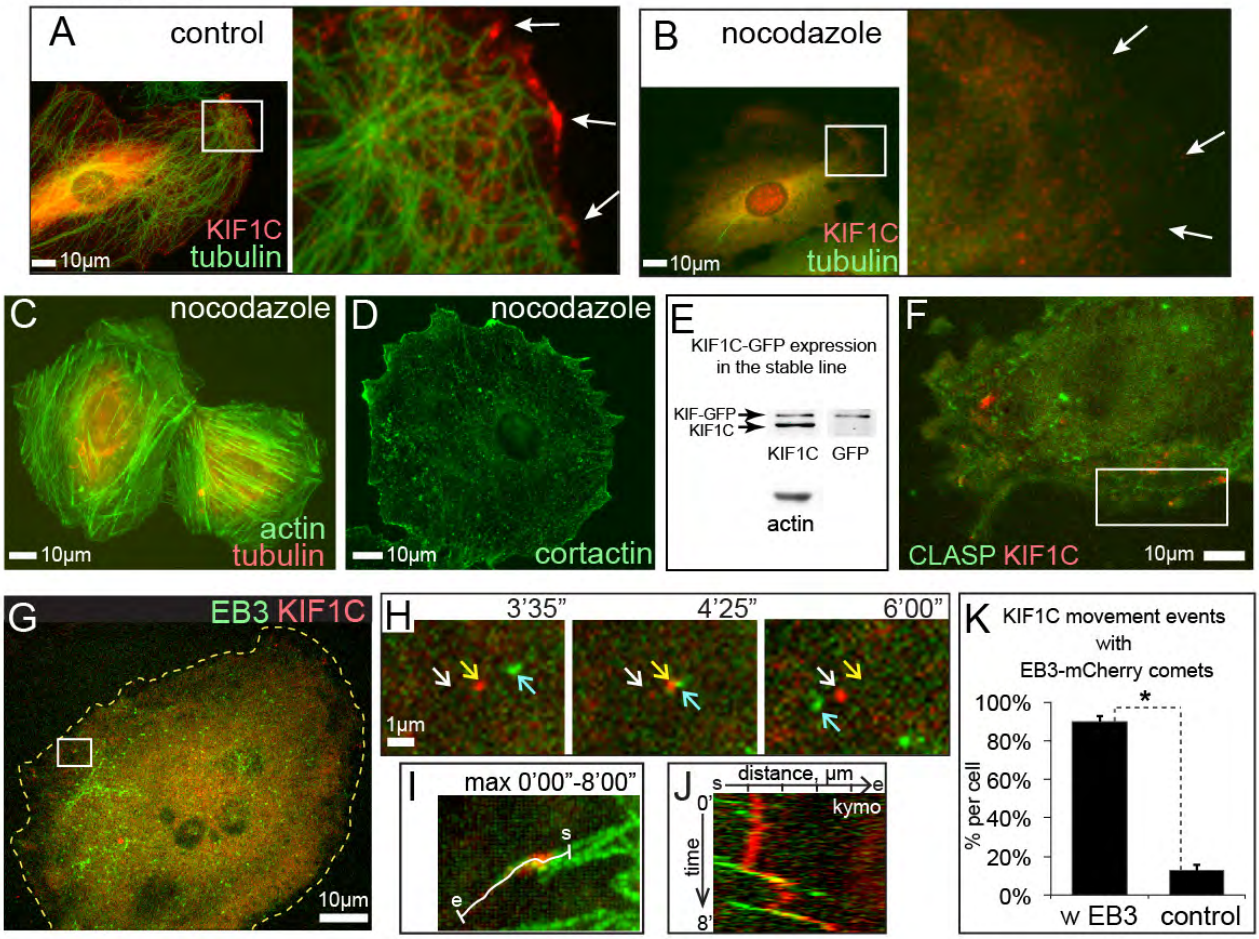
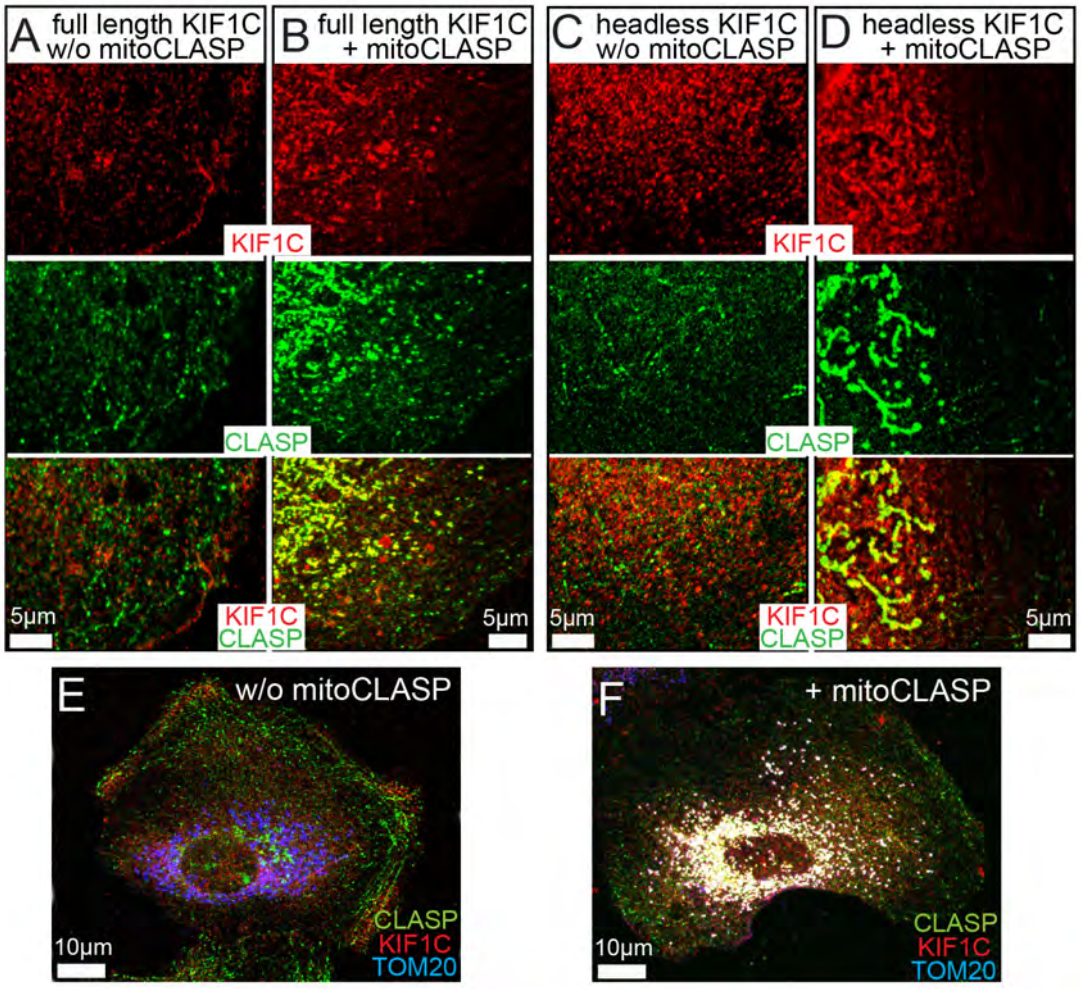
Supplemental figures

Suppl Figure S1



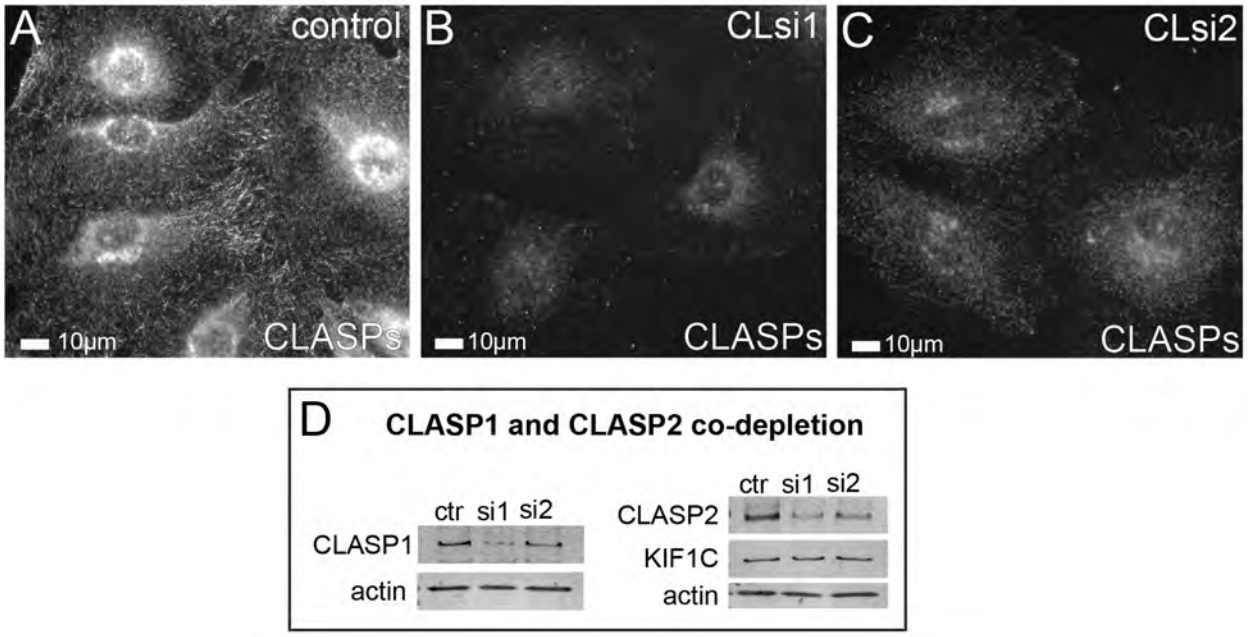
Supplemental Fig. S1. KIF1C localization depends on MTs and MT +TIPs. **A,B,** Wide-field epifluorescence microscopy images of KIF1C (red) and tubulin (green). In control cells (A), KIF1C (red) is accumulated at the cell edge, either at MT plus tips (arrows) or without MT association. In nocodazole-treated cell (B), neither MTs nor KIF1C accumulations at the cell edge are detected (arrows). Boxes shown in the overview images (left) are enlarged at the right. **C-D**, MT depolymerization by nocodazole does not facilitate podosome formation. Wide-field epifluorescence microscopy. Actin (phalloidin, green) and tubulin (red) (C). Cortactin (D). **E**, Western blotting indicating low expression levels (47% of endogenous) of KIF1C-GFP in the stable line. KIF1C and GFP are detected at the same gel in two fluorescent channels. Actin, loading control (the same for both channels). **F.** KIF1C-mCherry (red, white arrows) move with the ends of GFP-CLASP2 (green, blue arrows)-associated MTs in PDBu-treated cell. The box is enlarged in Fig. 4C. Live-cell confocal image. See Movie 6. **G-I,** KIF1C-GFP (red)-associated vesicle is transported in association with EB3-mCherry (green)-labeled growing MT tips. Boxed area from the cell overview (G) is enlarged as video frames (H) and a maximum intensity projection over 8 minute sequence (I). Yellow arrow, initial position of KIF1C vesicle. White arrow, final position of KIF1C vesicle. Cyan arrow, EB3 comet. White line in max shows direction of the kymograph shown in I**. J**, Kymograph indicated that KIF1C (red) is stationary at times when it does not co-localize with moving EB3 comets (green). See also Movie 3 and 4. **K**, KIC1C-GFP relocation events co-localized with EB3-mCherry comets as compared to the same events superimposed on a spatially shifted EB3 video sequence. Based on data as in Movies 3, 4. Student's t-test: $p<0.001$. N=12 cells.

Suppl Figure S2



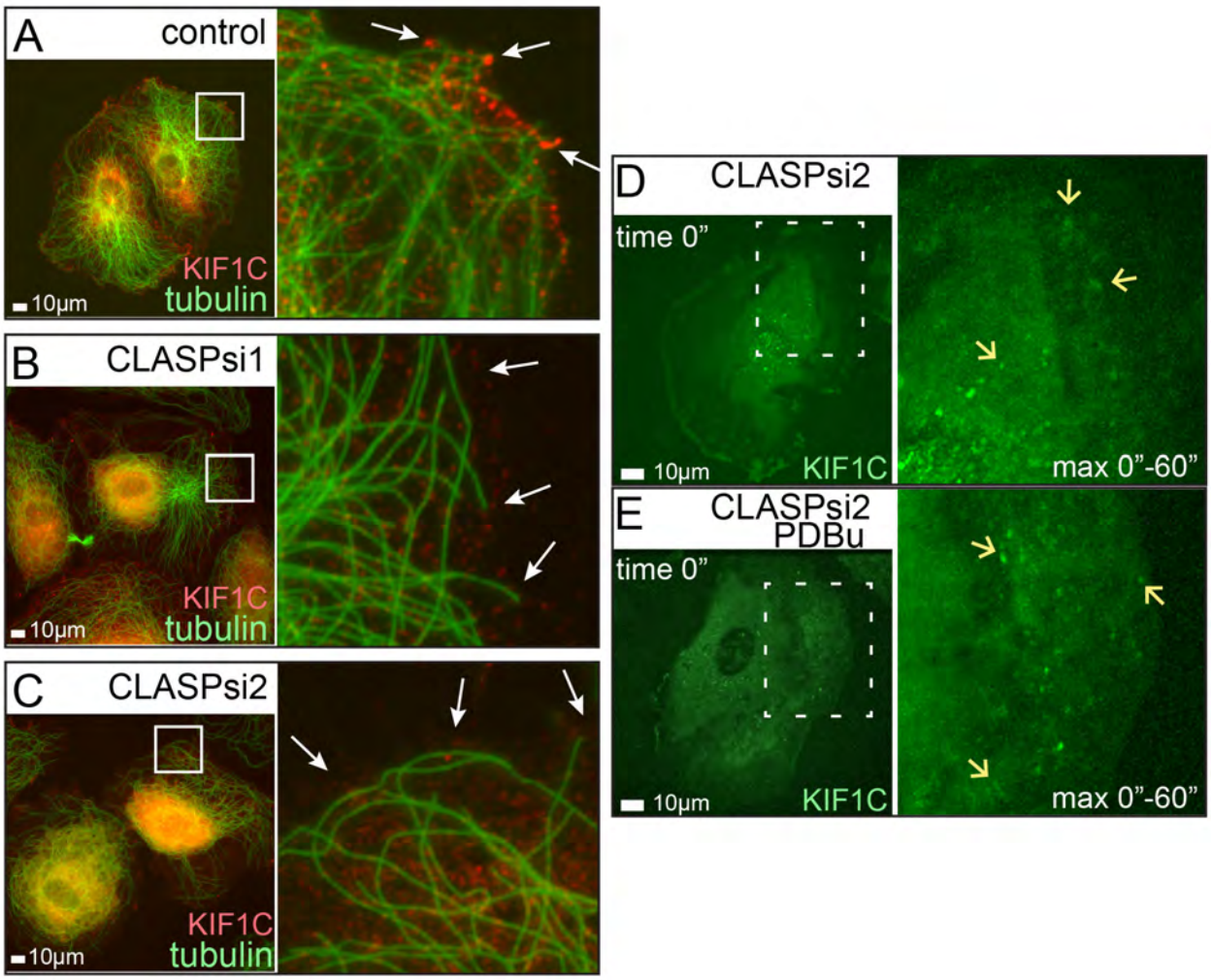
Supplemental Fig. S2. KIF1C is recruited by mito-CLASP. **A, B,** Co-localization of KIF1C (red) and CLASP(green) corresponding to Fig. 4G, H. **C, D**. Co-localization of KIF1C tail domain (red) and CLASP (green) corresponding to Fig. 4I, J. **E, F,** Visualization of KIF1C (red), CLASP (green) and mCherry TOM20 corresponding to Fig. 4K,L.

Suppl Figure S3



Supplemental Fig. S3. A-C, CLASPs immunostaining in cells transfected with non-targeted siRNA (**A**) and anti-CLASP1/CLASP2 siRNA combination 1 (**B**) or 2 (**C**). **D**. Western blotting in cells depleted by siRNA combinations 1 and 2. KIF1C levels are not influenced by anti-CLASP siRNA. Actin, loading control. See also Fig. 5A.

Suppl. Figure S4

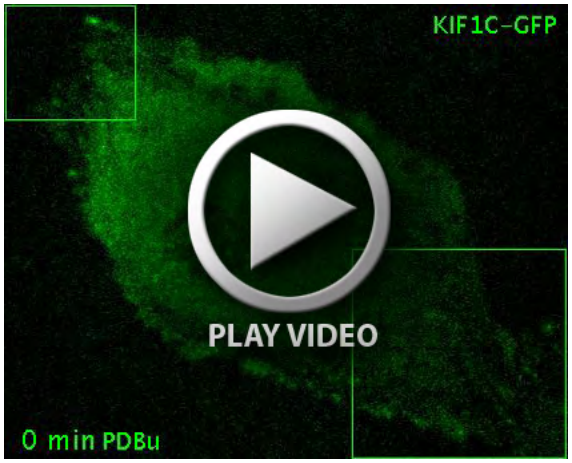


Suppl. Fig. S4. A-C, Wide-field epifluorescence microscopy of KIF1C (red) and tubulin (green). KIF1C-GFP (red) localizes to the ends of MTs (tubulin, green) and the cell edge (arrow) in cell treated with non-targeted control siRNA (A) but not in cells treated with siRNA against CLASPs (B,C). Boxes outlined on the left are enlarged on the right. **D, E**, KIF1C-GFP (green) trafficking visualized by a single plane confocal image sequence in cells depleted of CLASPs by siRNA combination 2. Single frame cell overviews are shown of the left. Video sequences from the boxed regions are shown on the right as enlarged maximal intensity projections over time. Arrows indicate tracks of KIF1C particles, which lack directional movement in PDBu-treated (D) or untreated (E) cells.

Movie legends

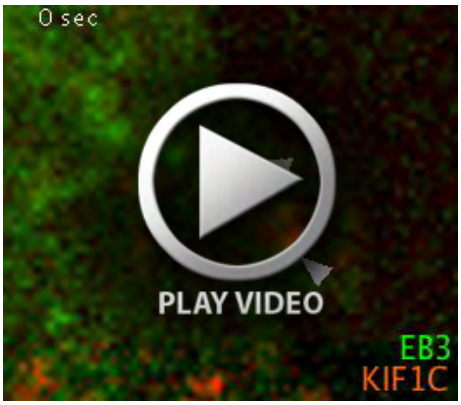
Movie 1. KIF1C accumulates at the cell periphery in response to PDBu treatment. Pseudo-colored map of KIF1C intensity (purple/low to white/high). Time indicates PDBu treatment duration. Corresponds to Fig. 3A.
Movie 2. KIF1C accumulates at the cell periphery in response to PDBu treatment. KIF1C-GFP, green. Time indicates PDBu treatment duration. Corresponds to Fig. 3B.
Movie 3. KIF1C-positive vesicle (white arrow) moves with MT plus ends toward the cell edge (not shown). Cyan vesicle indicates vesicle transport. EB3-mCherry, green. KIF1C-GFP, red. Corresponds to Supplemental Figure S1F-I.
Movie 4. MT plus ends deposit KIF1C accumulations (arrowheads) to the cell edge. EB3-mCherry, green. KIF1C-GFP, red.
Movie 5. KIF1C-mCherry (red, arrows) is delivered by GFP-CLASP2-associated MTs (green) in a PDBu-treated cell. Corresponds to Figure 4 C. Overview is shown in Movie 6.
Movie 6. KIF1C-mCherry (red) is moved by GFP-CLASP2-associated MTs (green) in a PDBu-treated cell. Multiple events of KIF1C movements are indicated by arrows. Corresponds to Suppl. Figure S1F. An inset is shown in Move 5 and Figure 4 C.
Movie 7. KIF1C trafficking in PDBu requires CLASP. Left, KIF1C-associated tubes and vesicles (arrows) actively move in a control cell. Right, most vesicles are immobile (arrows) in a CLASP-depleted cell. KIF1C-GFP, green. Corresponds to Figure 5 G, I.

Movie 1.



Movie 2.

Movie 3.



Movie 4.

Movie 5.

Movie 6.

Movie 7.