### Supplementary video titles and legends

# Video S1 Continuous calcium signal within the migrasome during its formation.

L929-T4-BFP cells were stained with Fluo-8 and then subjected to time-lapse imaging. Time interval, 180 s. Scale bar,  $10 \ \mu m$ .

#### Video S2 VAMP2 vesicles fuse with the migrasome membrane during migrasomes grow.

L929-GFP-VAMP2 cells were subjected to time-lapse imaging. Time-lapse images were acquired at intervals of 30 s. Scale bar, 2 µm.

#### Video S3 Myosin5a transports to the site of migrasome formation.

Time-lapse imaging of L929 cells stably expressing GFP-Myo5a and T4-mCherry. Time interval, 90 s. Scale bar, 20 μm.

#### Video S4 Two modes of the movement of Myosin5a transported into migrasomes.

Grazing Incidence-Structured Illumination Microscopy (GI-SIM) imaging of L929- GFP-Myo5a cells. Time-lapse images were acquired at intervals of 30 s. Scale bar, 5 µm.

## Video S5 Myosin5a displays polarized distribution during cell migration.

L929 cells stably expressing mCherry-Myo5a and T4-BFP, treated with or without 10  $\mu$ M GLPG0187, were subjected to time-lapse imaging. Time interval, 10 min. Scale bar, 20  $\mu$ m.

# Video S6 Cell migration causes the polarization of Rab8a vesicles to the rear end of the cell.

L929 cells stably expressing GFP-Rab8a and T4-BFP, treated with or without 10  $\mu$ M GLPG0187, were subjected to time-lapse imaging. Time interval, 10 min. Scale bar, 20  $\mu$ m.

# Video S7 Cell migration causes the polarization of Rab11a vesicles to the rear end of the cell.

L929 cells stably expressing GFP-Rab11a, treated with or without 10  $\mu$ M GLPG0187, were stained with WGA and then subjected to time-lapse imaging. Time interval, 10 min. Scale bar, 20  $\mu$ m.