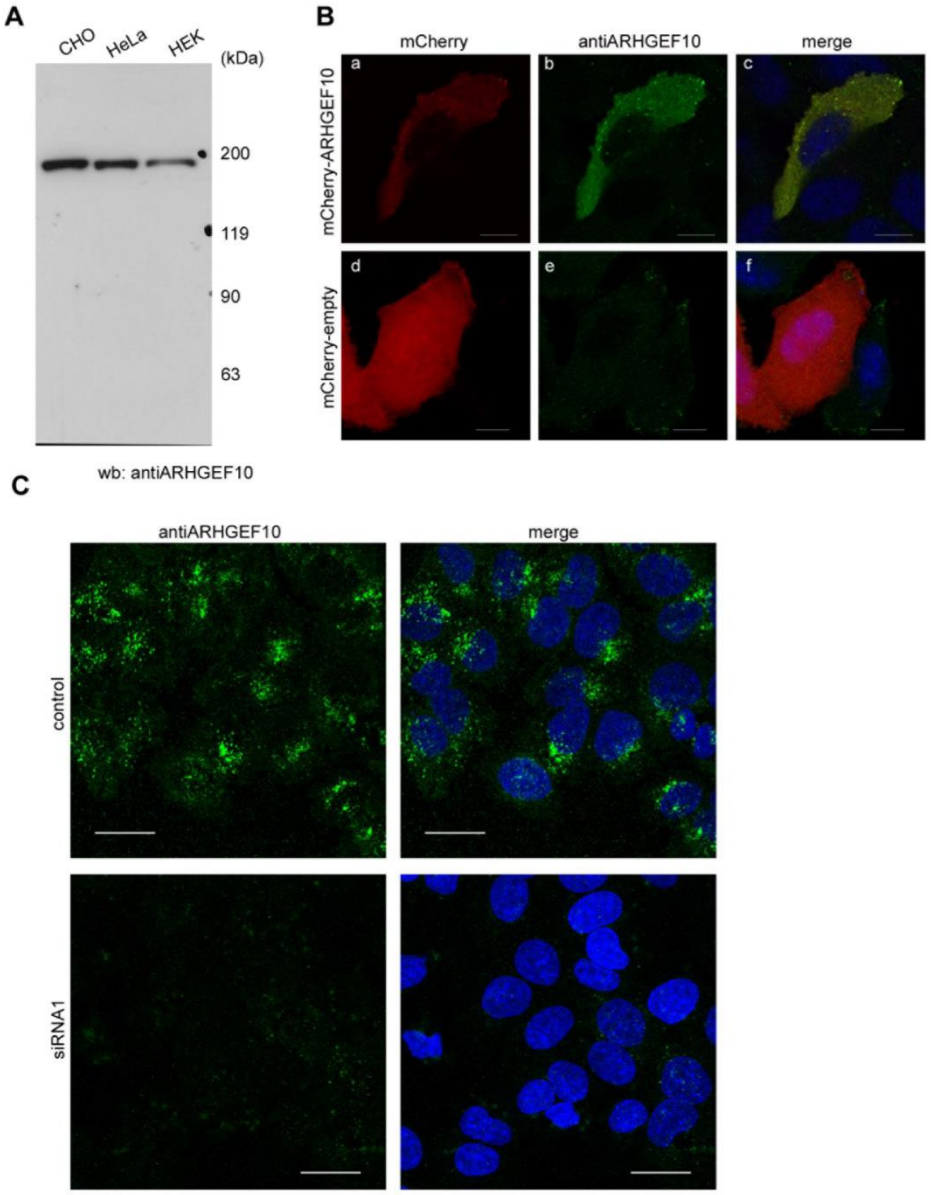


Figure S1. antiARHGEF10 specifically recognized ARHGEF10. A. Protein extract from CHO, HeLa or HEK293T cell were immunoblotted with antiARHGEF10. Bands slightly smaller than 200 kDa were specifically recognized by this antibody. Molecular weights in kDa are indicated to the right. B. HeLa cells transiently expressing mCherry-tagged ARHGEF10 (a) or mCherry (d) were stained with antiARHGEF10 (b and e). Merged images are shown in right panels. A single focal plane images were acquired on a confocal microscopy. Scale bar, 10 μm. C. HeLa cells which had been transfected with control (upper panels) or siRNA1 (lower panels) were treated with nocodazole for 1 hour and fixed by 4 % PFA. Then these cells were immunostained with antiARHGEF10 (right panels) and counterstained by Hoechst 33258. Merged images are shown in right panels. Z-stacked confocal images were acquired on a confocal microscopy. Scale bar, 20 μm.

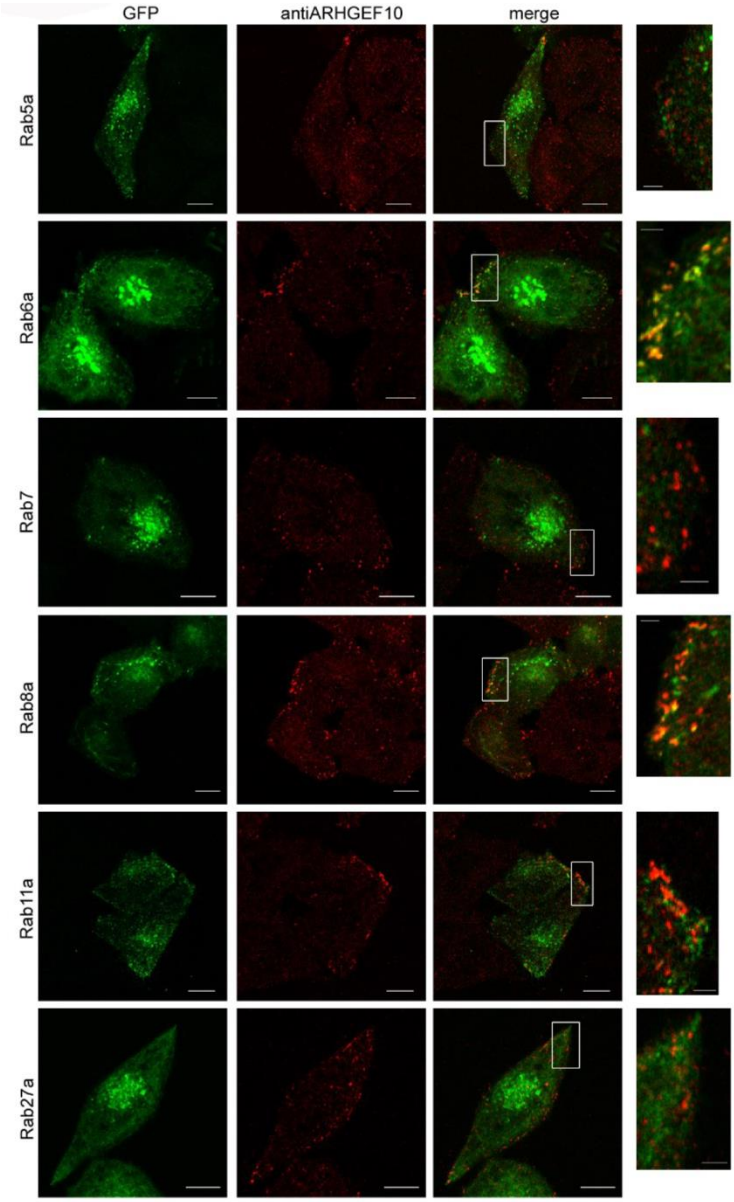


Figure S2. ARHGEF10 was colocalized with GFP tagged Rab6a- and/ or Rab8a-positive vesicles. HeLa cells transiently expressing GFP-tagged Rab5a, Rab6a, Rab7, Rab8a, Rab11a and Rab27a (left panels, green) were immunostained with antiARHGEF10 (middle panels, red). Scale bar, 10 μm. Higher magnification views of the boxed areas are shown in right panels. Scale bar, 2 μm. A single focal plane images were acquired on a confocal microscopy.

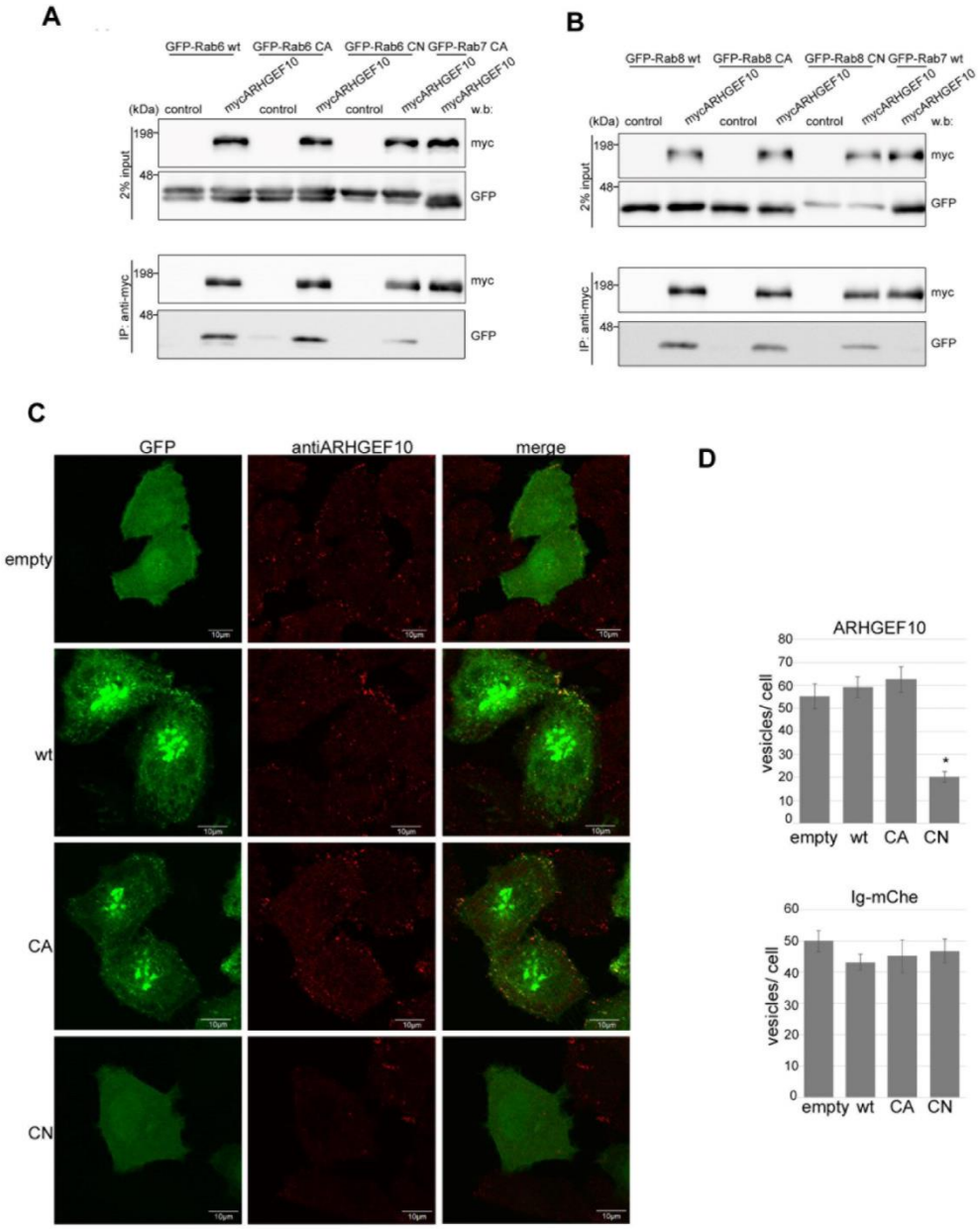


Figure S3. ARHGEF10 interacted with Rab6 and Rab8 and the localization of ARHGEF10 was regulated by activity of Rab6. A. HEK293T cells were co-transfected

with pCMV myc (control) or pCMV myc-ARHGEF10 and EGFP-tagged Rab6a wild type (wt), Rab6a constitutively active form (CA), Rab6a constitutively negative form (CN) or Rab7 constitutively active form (CA) expression plasmids. Total cell lysate (input) were immunoprecipitated by anti-myc antibodies. 2 % total cell lysate and the immunoprecipitates were analyzed by western blotting using anti-myc antibodies and anti-GFP antibodies. Molecular weights in kDa are indicated to the left. B. HEK293T cells were co-transfected with pCMV myc control or pCMV myc-ARHGEF10 and EGFP-tagged Rabs (Rab8a wt, Rab8CA, Rab8CN or Rab7 wt) expression plasmids. Total cell lysate (input) were immunoprecipitated by anti-myc antibodies. 2 % total cell lysate and the immunoprecipitates were analyzed by western blotting using anti-myc antibodies and anti-GFP antibodies. Molecular weights in kDa are indicated to the left. C. HeLa cells transiently transfected with GFP (empty) or GFP tagged Rab6a wild type (wt), constitutively active form (CA) or constitutively negative form (CN) expressing vector (left panels, green) were stained with antiARHGEF10 (middle panels, red). A single focal plane images were acquired on a confocal microscopy. Scale bar, 10 μm. D. The number of ARHGEF10-positive (left) and Ig-mCherry-positive vesicles (right) were counted in these cells. Error bars indicate SE. More than 10 cells were examined per an assay. (*$P < 0.01$ by Student's t-test)

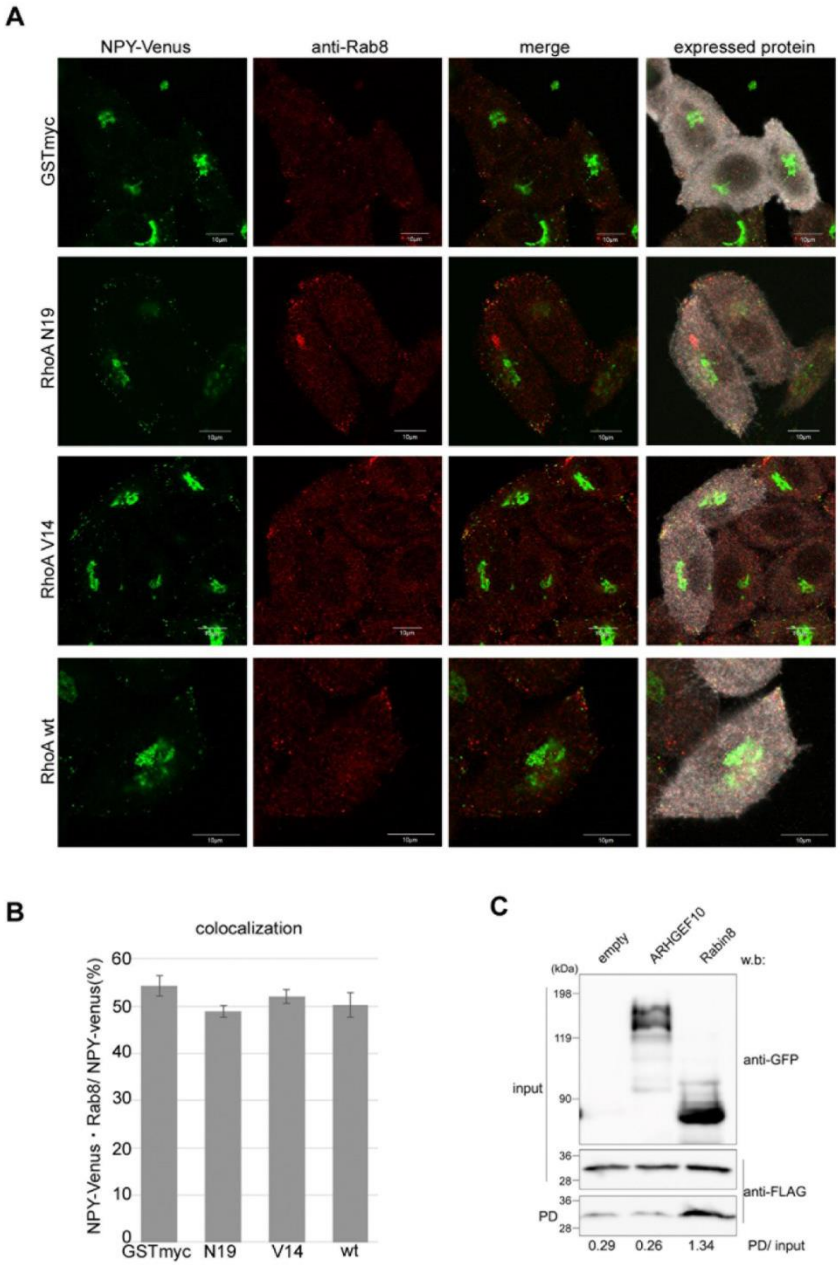
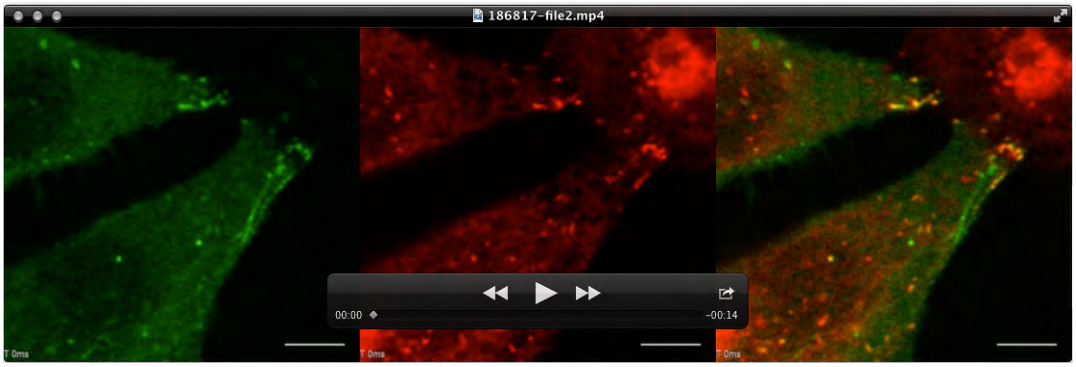


Figure S4. The localization of Rab8 to NPY-Venus-positive vesicle was not influenced by the activity of RhoA and the activation of Rab8 was not found in ARHGEF10 overexpressing cells . A. HeLa cells stably expressing NPY-Venus (green) were transfected with myc-tagged GST (GSTmyc), myc-tagged RhoA dominant negative form (T19N), HA-tagged RhoA dominant active form (G14V) and HA-tagged RhoA wild type (wt) and immunostained with anti-Rab8 antibody (red) and anti-myc antibody (GST and T19N) or anti-HA antibody (G14V and wt) (grey). A single focal plane images were acquired on a confocal microscopy. Scale

bar, 10 μm. B. Colocalization efficiency of Rab8- and NPY-Venus-positive vesicles in HeLa cells stably expressing NPY-Venus, which had been transfected with expression plasmids encoding GSTmyc, RhoA-T19N, RhoA-G14V or RhoA wt, were calculated. Percentage of both Rab8- and NPY-Venus-positive vesicles in total NPY-Venus-positive vesicles were indicated. Error bars indicate SE. More than 17 cells were examined per an assay. C. HEK293T cells were cotransfected with pEF-FLAG-Rab8 and pEGFPC2, pEGFP-C2-ARHGEF10 or pEGFP-C1-Rabin8, which are known as GEF for Rab8. Using these cells, GEF activity assay were performed according to Homma et al.'s method (Kuroda et al., 2002; Homma et al. 2016). In brief, these cells were lysed and active Rab8 was pull-downed by GST-tagged C-terminal fragment of MICAL-L2 (named MICAL-L2 C), which preferentially binds to the active forms of Rab8. The activation of Rab8 were observed in the cells which expressed GFP-Rabin8, but not GFP-ARHGEF10 or GFP. Molecular weights in kDa are indicated to the left. Quantified results are shown below each panel. Values are expressed as PD/ input. Similar results were obtained from three independent experiments.



Movie 1

HeLa cells stably expressing Ig-mCherry (middle panels) were transiently transfected with expression plasmids encoding GFP-tagged ARHGEF10 (left panels). Merged images are shown in right panels. Boxed area indicate the image used in Figure 5D. These images were obtained using confocal microscopy. Time lapse images of these cells were taken at maximum speed. Scale bar, 5 μm.

References

Homma Y. and Fukuda, M. (2016). Rabin8 regulates neurite outgrowth in both a GEF-activity-dependent and -independent manner. *Mol. Biol. Cell*, in press

Kuroda, T. S., Fukuda, M., Ariga, H. and Mikoshiba, K. (2002). The Slp homology domain of synaptotagmin-like proteins 1-4 and Slac2 functions as a novel Rab27A binding domain. *J. Biol. Chem.* **277**, 9212-9218