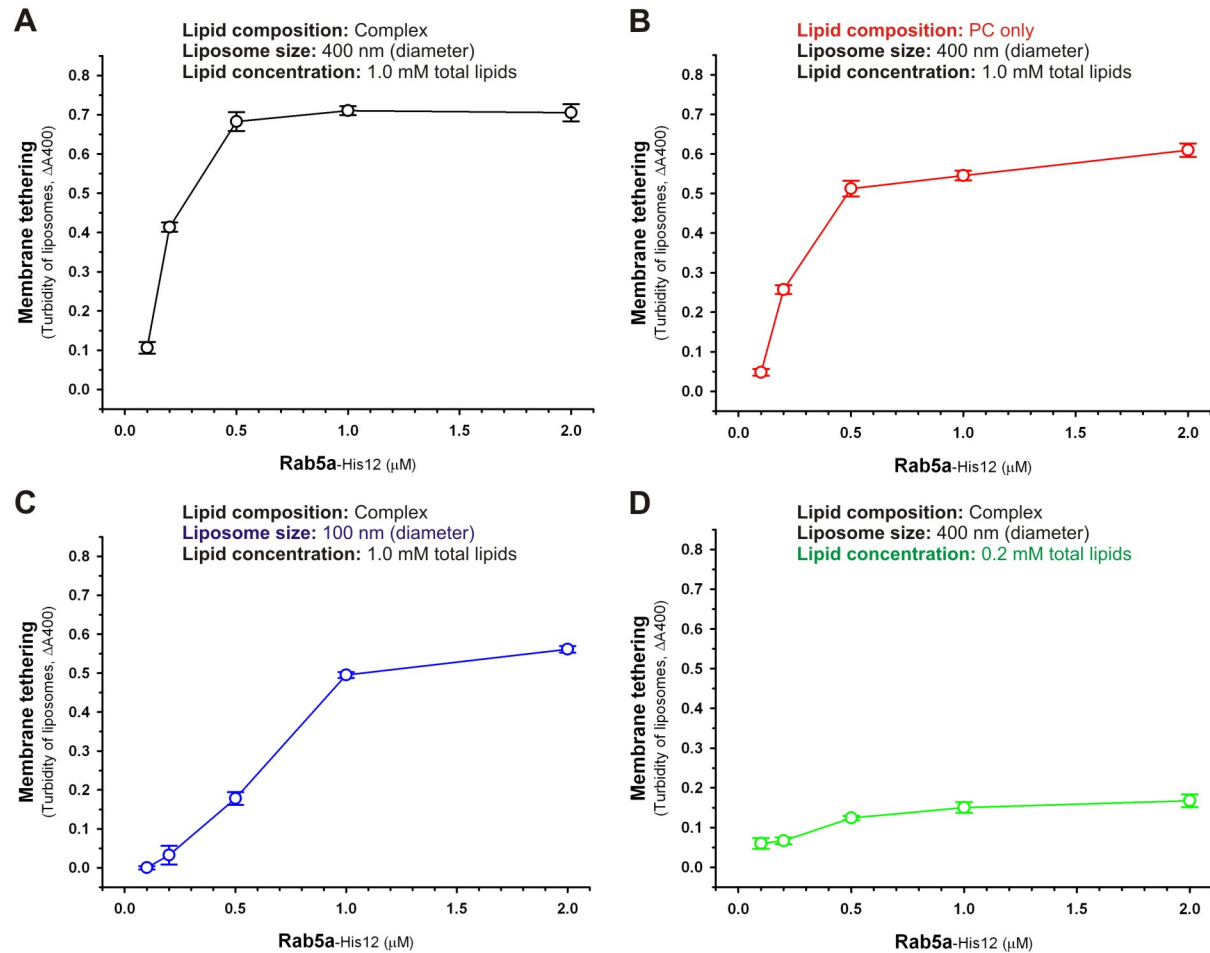


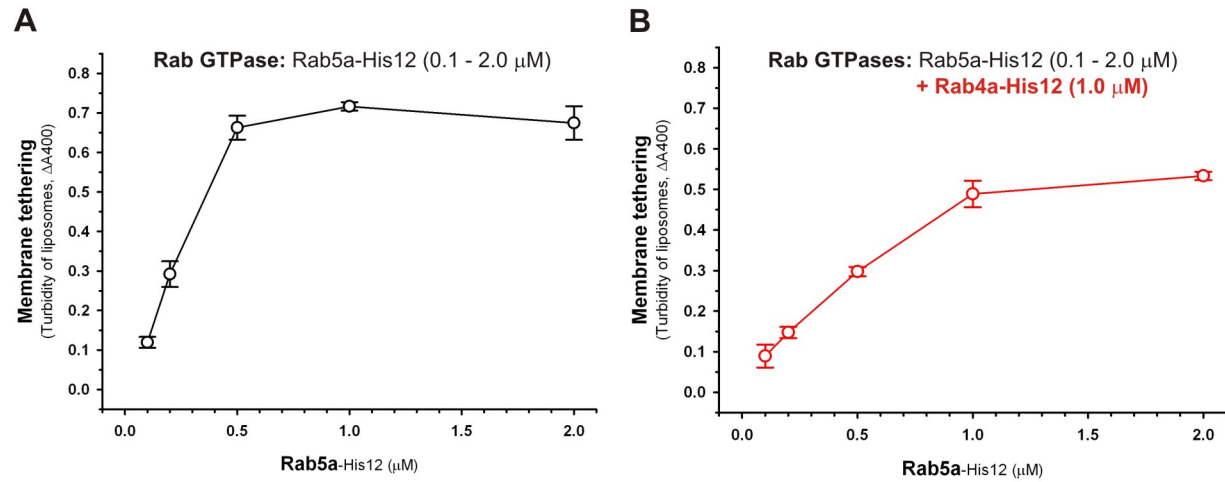
**Figure S1.** Inoshita & Mima



**Figure S1. Effects of lipid composition, liposome size, and total lipid concentration on reconstituted liposome tethering mediated by Rab5a-His12.**

Rab5a-His12 proteins (0.1 - 2.0  $\mu\text{M}$  final) were mixed with synthetic liposomes in RB150 containing 5 mM  $\text{MgCl}_2$  and 1 mM DTT, and then incubated at 30°C for 30 min. After the 30 min incubation, turbidity changes of the Rab-liposome mixed reactions were measured with the absorbance at 400 nm ( $\Delta A_{400}$ ), using the SpectraMax Paradigm plate reader with the ABS cartridge (Molecular Devices) and a black 384-well plate (Corning no. 3544; Corning). Liposomes used bore the physiological mimic complex lipid composition (Complex) (A, C, D), as in Figure 2, or the PC-only lipid composition [PC only; POPC (92.5%), DOGS-NTA (6%), Rh-PE (1.5%)] (B). Liposome sizes used were 400 nm (A, B, D) or 100 nm (C) in diameter. Total lipid concentrations tested in these turbidity reactions were 1.0 mM (A, B, C) or 0.2 mM (D) in final. Means and standard deviations of the  $\Delta A_{400}$  values were determined from three independent experiments.

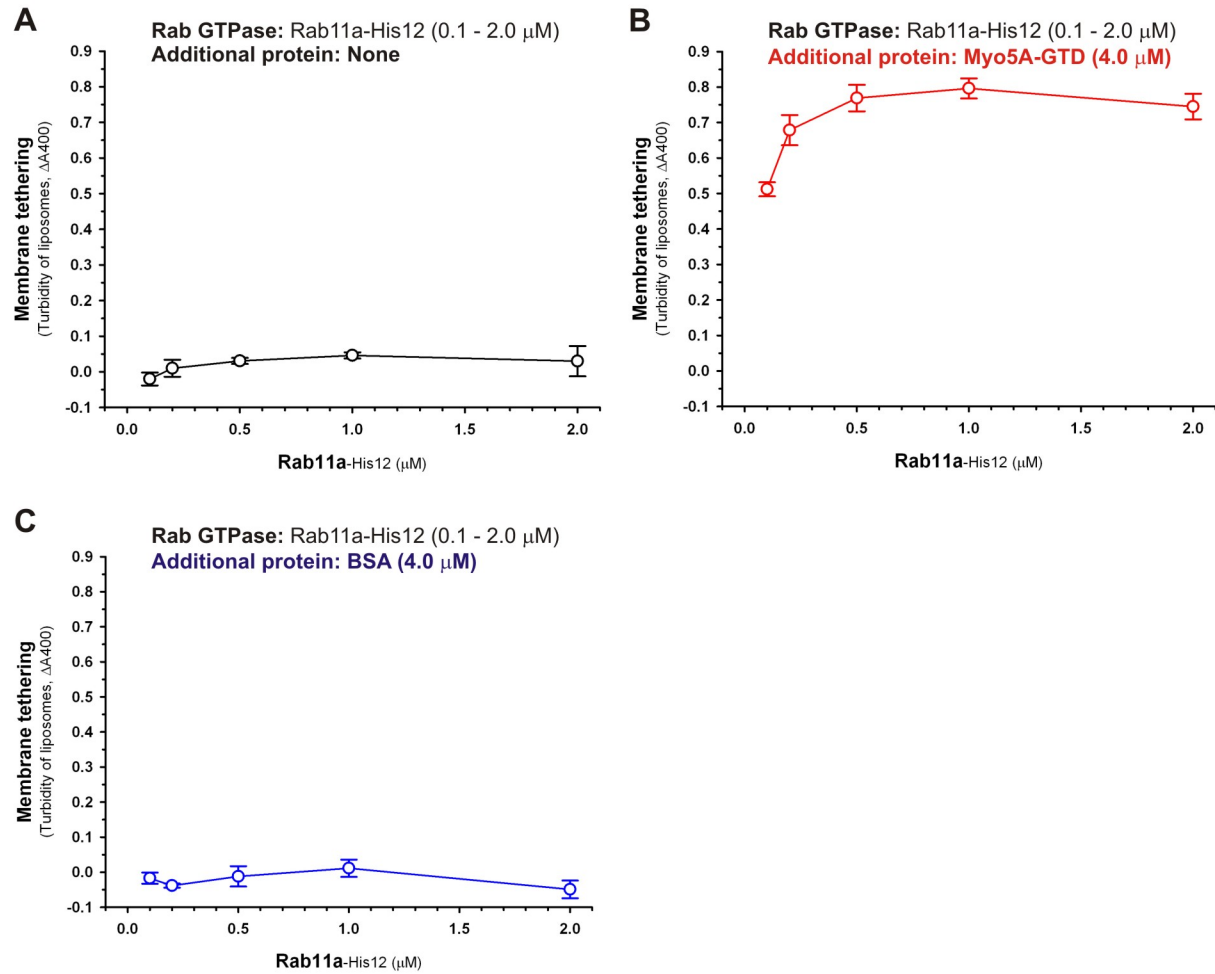
**Figure S2.** Inoshita & Mima



**Figure S2. Addition of Rab4a-His12 does not promote liposome tethering mediated by Rab5a-His12.**

Rab5a-His12 proteins (0.1 - 2.0  $\mu\text{M}$  final) were mixed with synthetic liposomes and then incubated at 30°C for 30 min as in Figure S1A, but in the absence (A) and presence (B) of Rab4a-His12 (1.0  $\mu\text{M}$  final). After the incubation, turbidity changes of the Rab-liposome mixed reactions were analyzed as in Figure S1.

**Figure S3.** Inoshita & Mima



**Figure S3. Myo5A-GTD is a specific protein component to promote liposome tethering mediated by the cognate Rab11a-His12.**

Rab11a-His12 proteins (0.1 - 2.0  $\mu\text{M}$  final) were mixed with synthetic liposomes and then incubated at 30°C for 30 min as in Figure S1A, but in the absence (A) and presence of Myo5A-GTD (4.0  $\mu\text{M}$  final) (B) or BSA (4.0  $\mu\text{M}$  final) (C). After the incubation, turbidity changes of the Rab-liposome mixed reactions were analyzed as in Figure S1.