Figure S1. Rab binding specificity of Rab11-FIP2-C, Rab11-FIP4-C, and RBD11, and Arf6 binding activity of RBD11 (related to Figures 1 and 3)

(A) Rab binding specificity of Rab11-FIP2-C and Rab11-FIP4-C as determined by yeast two-hybrid assays. Rab11-FIP2-C and Rab11-FIP4-C were subcloned into the pGAD-C1 vector, and it was then transformed into yeast cells expressing pGBD-C1 vector carrying a constitutively negative form, which contains a Ser/Thr-to-Asn substitution, of each Rab. Yeast cells were grown on SC-LW (growth medium) and SC-AHLW (selection medium).

(B) Rab binding specificity of RBD11 as determined by yeast two-hybrid assays. CN# indicates the constitutively negative mutants with a Ser/Thr-to-Asn substitution shown in (A).

(C) Arf6 binding activity of Rab11-FIP4-C and Rab11-FIP4/2-C1 as determined by yeast two-hybrid assays. The pGAD-C1 vector carrying pGAD-C1-Rab11-FIP4-C (or -Rab11-FIP4/2-C1) was transformed into yeast cells expressing pGBD-C1 vector carrying a CN or CA form of Arf6.
Figure S2. Neither RBD11 nor 2×RBD11 colocalized with the Golgi, early endosomes (EEs), late endosomes (LEs), lysosomes (LYs), or TfR (related to Figures 3 and 4).

(A) MDCK cells stably expressing EGFP-RBD11 (green) were fixed with 4% PFA and immunostained with antibodies against GM130 (Golgi marker), EEA1 (EE marker), LBPA (LE marker), LAMP2 (LY marker), and TfR (recycling endosome marker) (magenta). Scale bars, 20 µm.

(B) MDCK cells stably expressing EGFP-2×RBD11 (green) were fixed with 4% PFA and immunostained with the antibodies shown in (A) (magenta). Scale bars, 20 µm.