

## Supplemental material

### Legends for supplemental figures

**Figure S1** *In vitro* binding of Arf6-FLAG to the Sec7 domain of Fbx8. Cos-7 cells were transfected with Arf1-FLAG and Arf6-FLAG. Twenty-four h after transfection, cell lysates (300 µg) were prepared and incubated with the Fbx8 Sec7 domain, fused to GST (GST-Fbx8, 5 µg), or GST alone, as indicated. Proteins pulled-down with these GST proteins were then analyzed using an anti FLAG antibody (Pull-down). Amounts of the pulled down GST proteins are also shown by an anti-GST blot. Amounts of Arf1-FLAG and Arf6-FLAG in total cell lysate (20 µg) are also shown (Total).

**Figure S2** Specificity of the polyclonal anti-Arf6 antibody. Cos-7 cells were transfected with Arf isoforms (Arf1-6), each tagged with FLAG. Twenty-four h after transfection, cell lysates (20 µg) were prepared and subjected to immunoblotting analysis using an affinity-purified rabbit polyclonal anti-Arf6 antibody. The membrane was then reblotted with an anti-FLAG antibody to show the amounts of each FLAG-tagged Arf isoform (lower panel). Asterisks indicate endogenous Arf6.

**Figure S3** Protein levels of Arf6 in Cos-7 and NMuMG cells

Twenty µg of total cell lysate were subjected to immunoblotting analyses using anti-Arf6 and anti-β-actin antibodies.

**Figure S4** Expression of cDNA products in the experiments shown in Figure 6D.

Fifty µg of total cell lysate were subjected to immunoblotting analyses, as indicated. Each lane corresponds to each experiment with the same set of transfections as described in Figure 6D.

**Figure S5** Ubiquitination of Arf6 (Q67L) and Arf6 (T27N) by GST-Fbx8. Cos-7 cells were transfected with wild type Arf6-FLAG (WT), or its Q67L or T27N mutants, together with HA-ubiquitin and GST-Fbx8 or GST, as indicated. Ubiquitination of Arf6-FLAG was then assayed as in Figure 1B. Immunoblots of 20 µg of total cell lysate are also shown (Total). Open triangle indicates IgG heavy chain; closed triangle, IgG light chain.

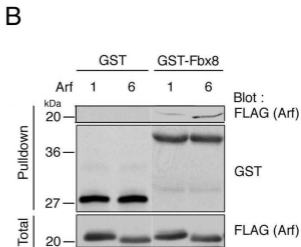
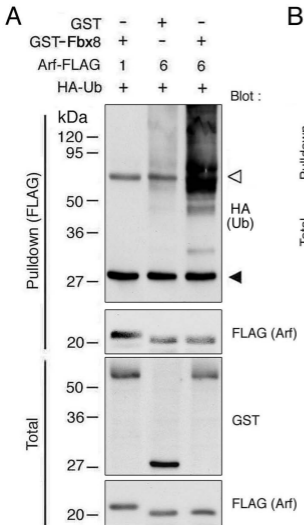


Figure S1 Yano et al.

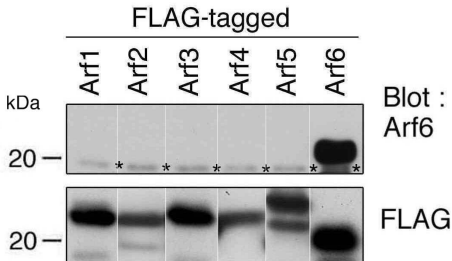


Figure S2 Yano et al.

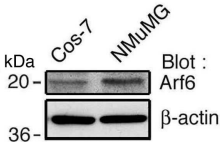


Figure S3 Yano et al.

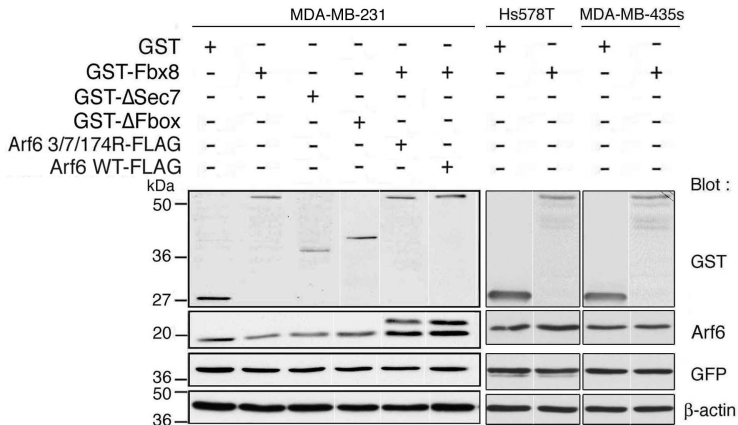


Figure S4 Yano et al.

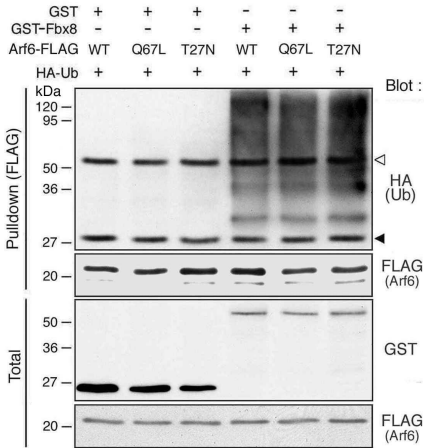


Figure S5 Yano et al.