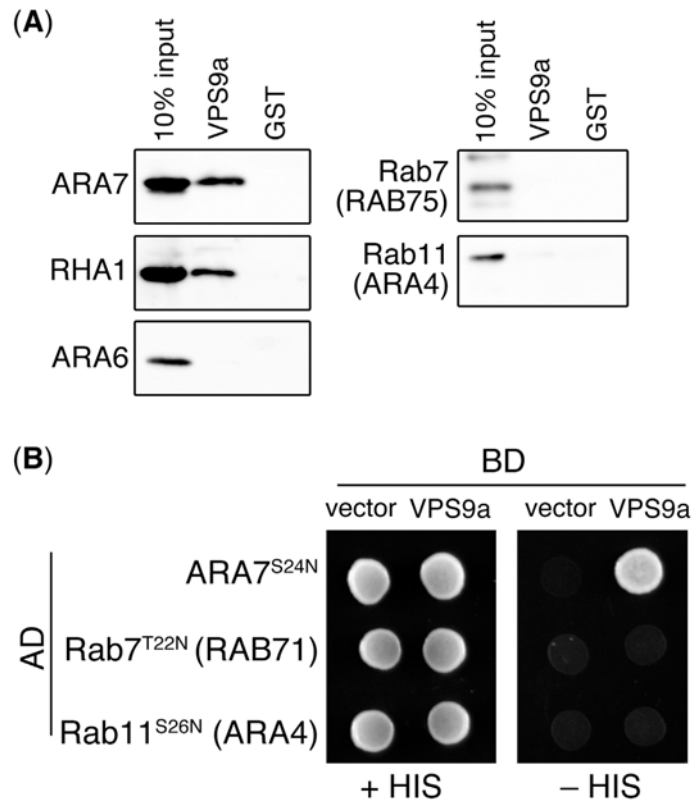


Supplemental Figure 1. VPS9a Does Not Activate Rab1, Rab2, Rab11 and Rab18 in Vitro.

The conformational changes of GST-fusions of Rab1 (ARA5), Rab2 (RAB2A), Rab11 (ARA4) and Rab18 (RAB18-1) upon GDP/GMP-PNP exchange were measured by monitoring the tryptophan autofluorescence in the absence (light blue) or presence of 0.25 μM (pink), 0.5 μM (orange), or 1 μM (red) GST-VPS9a.

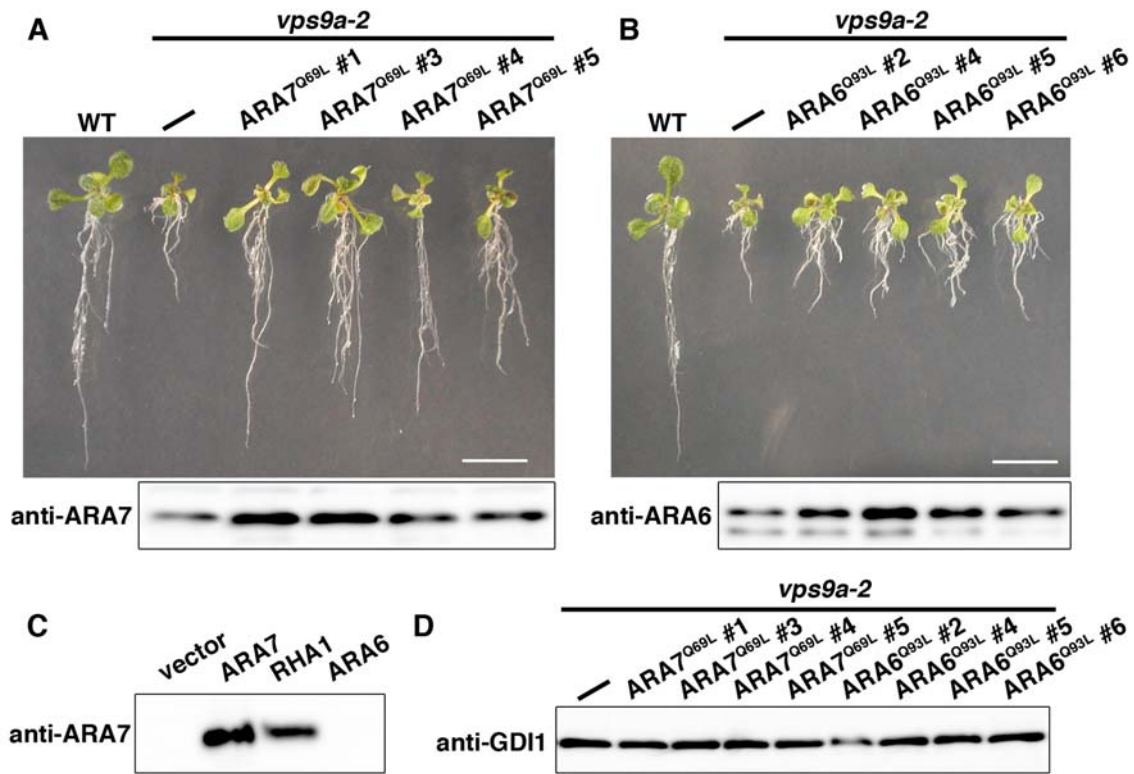


Supplemental Figure 2. VPS9a Does Not Interact with Rab7 and Rab11.

(A) Interactions between VPS9a and wild-type Rab GTPases detected by *in vitro* pull down assay.

Interactions were detected for conventional-type ARA7 and RHA1, but not for plant-unique ARA6 in this method. Rab7 (RAB75) and Rab11 (ARA4) showed no interaction with VPS9a. 10% input is also shown.

(B) VPS9a showed no interaction with either Rab7^{T22N} or Rab11^{S26N} in yeast two-hybrid assay.



Supplemental Figure 3. Constitutive Active ARA7 (ARA7^{Q69L}), but Not ARA6 (ARA6^{Q93L}), Suppresses the *vps9a-2* Mutation.

(A) and (B) ARA7^{Q69L} or ARA6^{Q93L} was overexpressed in the *vps9a-2*. 12-day-old seedlings of *vps9a-2* and four individual transgenic lines expressing ARA7^{Q69L} (A) or ARA6^{Q93L} (B) were processed to the immunoblotting to compare the expression level of ARA7^{Q69L} and ARA6^{Q93L}. Although the expression level was equivalent between ARA7^{Q69L} and ARA6^{Q93L}, ARA6^{Q93L} did not suppress the root phenotype of *vps9a-2*. ARA7^{Q69L} #4 and ARA6^{Q93L} #2 were shown in Figure 3 in the printed paper as representatives. Bars = 10 mm.

(C) Characterization of the antibody used for the detection of the conventional type of Rab5s. Lysates from yeast cells expressing ARA7, RHA1 or ARA6 were applied to the immunoblotting. This antibody was raised using GST-tagged ARA7 as an antigen, but it recognized both of ARA7 and RHA1.

(D) Amounts of GDI1 in *vps9a-2* and transgenic lines (loading control).

switch I

	* :	:
At5g45130_RHA1/RABF2a	KDQFVEFQESTIGAA	FFSQTTLAVNDAT
At4g19640_ARA7/RABF2b	KDQFVEFQESTIGAA	FFSQTTLAVNDAT
At3g54840_ARA6/RABF1	RGQFDATSKVTVGAS	FLSQTIALQDST
At5g45750_RABA1c	KNEFSLESKSTIGVEF	ATR-SLNVDDK
At4g18800_RABA1d	RNEFSLESKSTIGVEF	ATR-SLNVNEK
At1g16920_RABA1b	KNEFNLESKSTIGVEF	ATR-TLKVDGK
At1g06400_ARA2/RABA1a	KNEFNLESKSTIGVEF	ATK-TTKVEGK
At5g60860_RABA1f	RNEFSLESKSTIGVEF	ATR-SIHVDDK
At3g15060_RABA1g	RNEFSLESKSTIGVEF	ATR-SIHVDEK
At2g33870_RABA1h	RNDFSHDSRSTIGVEF	ATRRGIQVDDK
At1g28550_RABA1i	RNDFSHDSRATIGVEF	ATR-SIQCDDK
At4g18430_RABA1e	RNEFSIESKSTIGVEF	ATR-SVHVDEK
At3g46830_RABA2c	RNEFCLESKSTIGVEF	ATR-TTQVEGK
At5g59150_RABA2d	RNEFCLESKSTIGVEF	ATR-TLQVEGK
At1g07410_RABA2b	RNEFCLESKSTIGVEF	ATR-TLQVEGK
At1g09630_RABA2a	RNEFCLESKSTIGVEF	ATR-TLQVEGR
At5g65270_RABA4a	RDEFSLDSKATIGVEF	QTR-TLVIDHK
At4g39990_RABA4b	RDEFSMDSKATIGVEF	QTR-TLSIEEK
At2g22390_RABA4e	RDEFSMDSKATIRCF	QYS-----
At5g47960_RABA4c	RNEFSIESKATIGVEF	QTR-TLEIDRK
At3g12160_RABA4d	RNEFSVDSKATIGVEF	QTK-TLVIDNK
At1g01200_RABA3	HNEFCYDSKSTIGVEF	QTR-TITLRGK
At2g31680_RABA5d	RNEFNAHSKATIGVEF	QTO-NMEIEGK
At1g05810_ARA1/RABA5e	RNEFSANSKATIGVEF	QTO-SMEIEGK
At2g43130_ARA4/RABA5c	RNEFNPNKATIGVEF	QTO-SMLIDGK
At3g07410_RABA5b	RDEFDTNSKATIGVEF	QTO-LVEIEGK
At5g47520_RABA5a	RDEFYPNSKSTIGVEF	QTO-KMDINGK
At1g73640_RABA6a	KDEFRLFDSKPTIGVEF	FAYR-NVHVGDK
At1g18200_RABA6b	RDEFRLDSKPTIGVDF	FAYR-NVRVGDK
At5g47200_RABD2b	DDSYLDSYISTIGVDF	KIR-TVEQDQK
At4g17530_RABD2c	DDSYLDSYISTIGVDF	KIR-TVEQDQK
At1g02130_ARA5/RABD2a	DDSYVESYISTIGVDF	KIR-TVEQDQK
At3g11730_RABD1	DDAYIDSYISTIGVDF	KIR-TIEQDQK
At5g59840_RABE1b	DGSFTTSFITTIGIDF	KIR-TIELDGK
At3g46060_ARA3/RABE1c	DGSFTTSFITTIGIDF	KIR-TIELDGK
At3g53610_RABE1a	DGSFTTSFITTIGIDF	KIR-TIELDGK
At5g03520_RABE1d	DDTFTTSFITTIGIDF	KIR-TVELDQK
At3g09900_RABE1e	DDTFTTSFITTIGIDF	KIR-TVELDQK
At5g03530_RABC2a	SSSVED-LAPTIGVDF	KIK-QLTVGGK
At3g09910_RABC2b	SSSVED-LAPTIGVDF	KIK-QMKVRGK
At1g43890_RABC1	SNTFDD-LSPTIGVDF	KVK-YLTIGEK
At4g35860_RABB1b	DKRFQPVHDLTIGVEF	GAR-MVTVDGR
At4g17170_RABB1c	DKRFQPVHDLTIGVEF	GAR-MITIDNK
At4g17160_RABB1a	DKRFQAVHDLTIGVEF	GAK-TITIDNK
At4g09720_RABG3a	HKKFSMQYKATIGADF	VTK-ELQIGEK
At1g22740_RABG3b	NNKFSQQYKATIGADF	VTK-ELQIDDR
At2g21880_RABG2	YKKFNKQYKATIGADF	VTK-ELHIDEK
At3g16100_RABG3c	NRKFSNQYKATIGADF	FLTK-EVQIDDR
At1g52280_RABG3d	NRKFSNQYKATIGADF	FLTK-EVQIDDR
At1g49300_RABG3e	NKKFSNQYKATIGADF	FLTK-EVQFEDR
At3g18820_RABG3f	NKKFSNQYKATIGADF	FLTK-EVQFEDR
At5g39620_RABG1	DKDFKQLHNSTIYVDL	VTK-EICIAER
At2g44610_RABH1b	YDKFDNTYQATIGIDF	LSK-TMYLEDR
At5g10260_RABH1e	YDKFDNTYQATIGIDF	LSK-TMYLEDR
At4g39890_RABH1c	YDKFDNTYQPTIGIDF	LSK-TMYLEDR
At2g22290_RABH1d	YDKFDNTYQATIGIDF	LSK-TMYLEDR
At5g64990_RABH1a	YGKFDTSYQATIGIDF	LSK-TTRYEDR

Supplemental Figure 4. The Alignment of the Switch I Region of Plant Rab GTPases.

The Phe in the switch I region (orange) are conserved throughout the *Arabidopsis* Rab members. Only Rab5 members (ARA7, RHA1 and ARA6) have a small nonacidic residue (Ala/Ser, red) preceding the invariant Phe, while other Rab members have an acidic residue at this position (Asp/Glu, green).