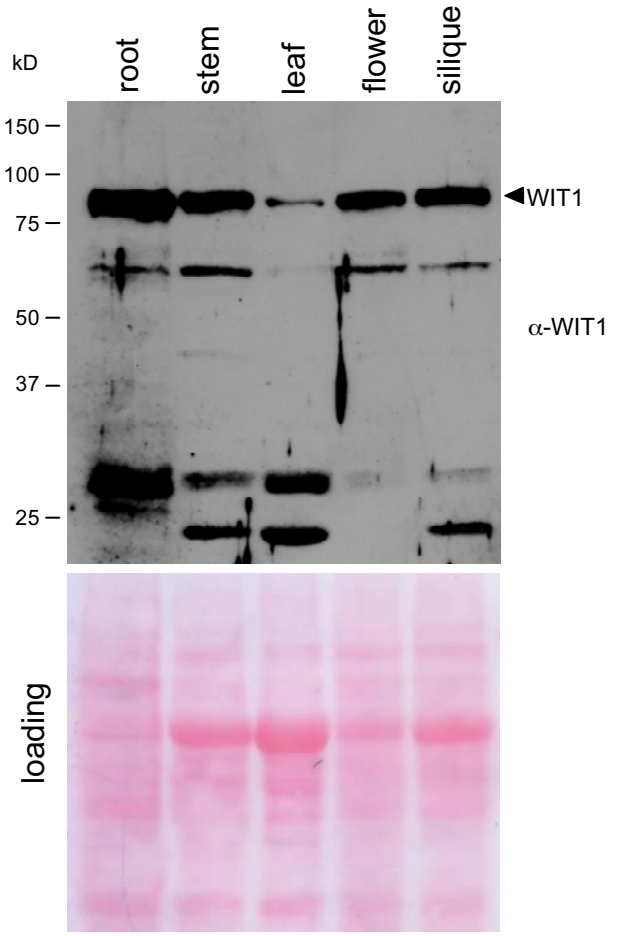
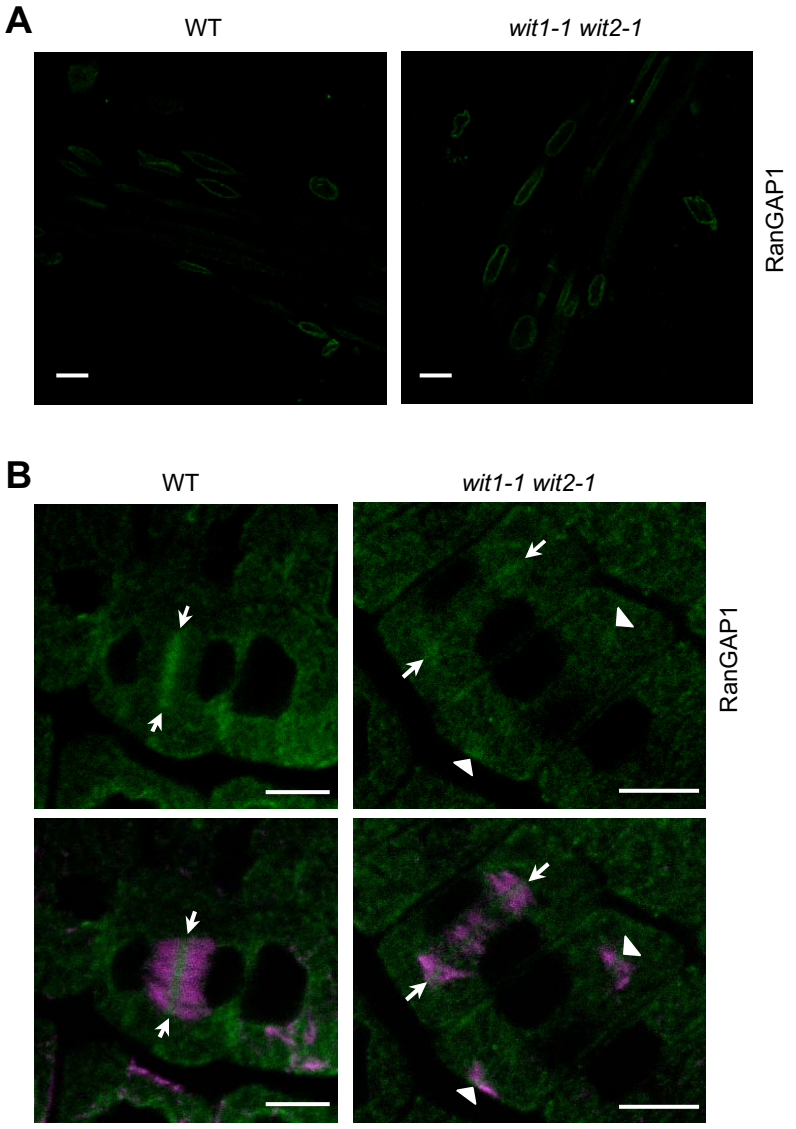
Supplemental Data Zhao et al. (2008). Two distinct, interacting classes of nuclear envelope-associated coiled-coil proteins are required for the tissue-specific nuclear envelope targeting of Arabidopsis RanGAP.

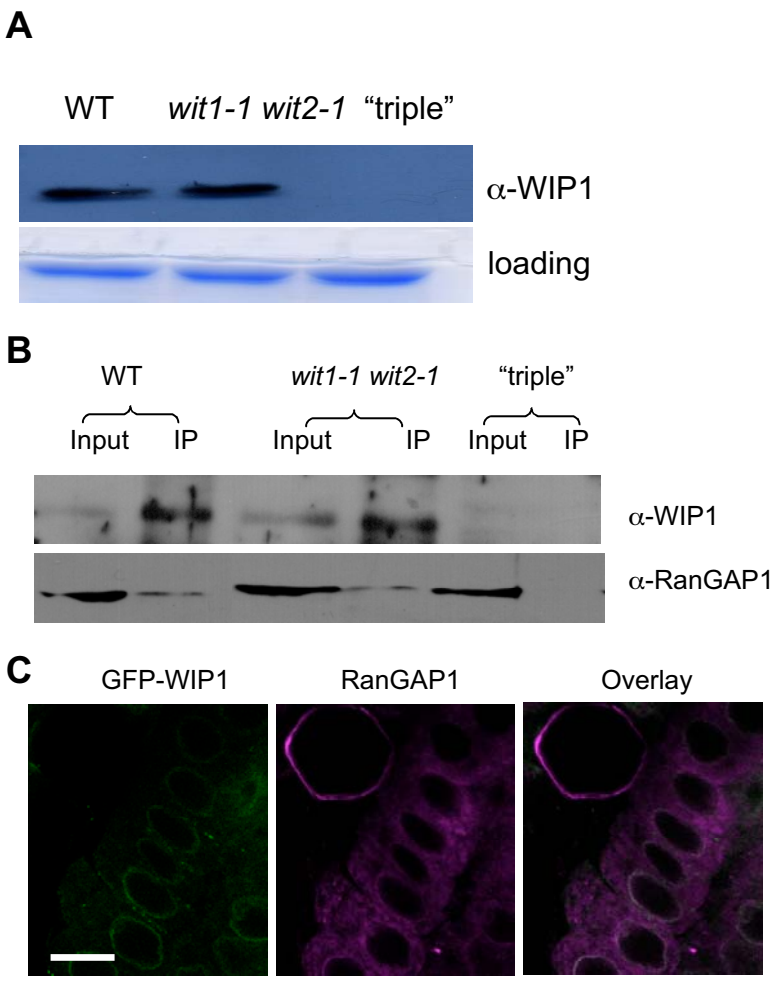


Supplemental Figure 1. WIT1 is Expressed Ubiquitously in Arabidopsis.

Protein extracts from roots, stems, rosette leaves, flowers and green siliques of wild-type Arabidopsis (ecotype Columbia) were probed with the anti-WIT1 antibody. Ponceau S staining is shown as loading control.

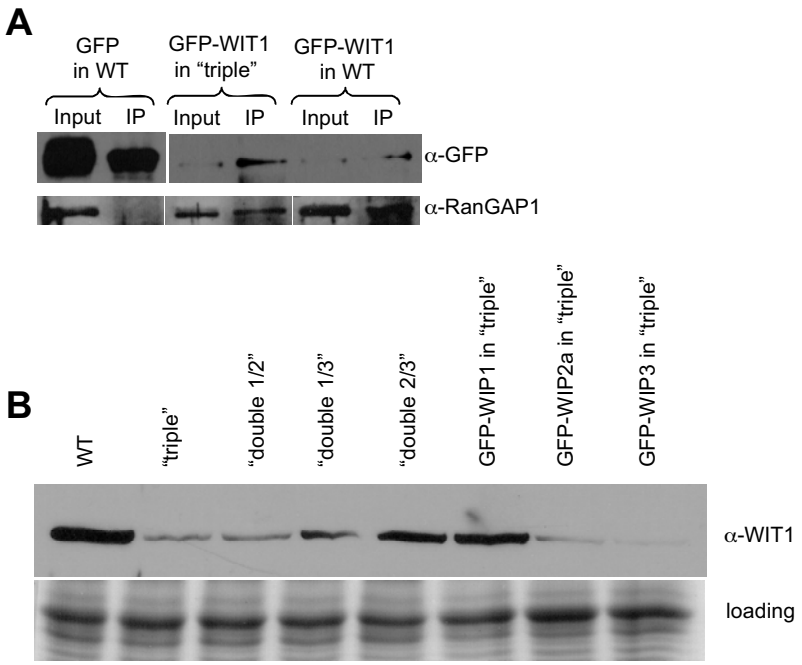


Supplemental Figure 2. RanGAP1 is targeted to the NE in the differentiation zone and during cytokinesis in *wit1-1 wit2-1* roots. (A) Immunofluorescence localization of RanGAP1 in differentiated cells of Arabidopsis wild-type (WT) and the *wit1-1 wit2-1* double mutant. All scale bars: 10 μm. **(B)** Double immunofluorescence localization of RanGAP1 (green) and α-tubulin (magenta) in the root tip cells of Arabidopsis wild-type (WT) and *wit1-1 wit2-1*. Arrows and arrowheads are depicting the cell plate during early and late stage of cytokinesis, respectively. All scale bars: 10 μm.



Supplemental Figure 3. The fate of WIP1 in *wit1-1 wit2-1*.

(A) WIP1 protein level is not changed in the *wit1-1 wit2-1* double mutant. Protein extracts from wild-type (WT), *wit1-1 wit2-1* double mutant and *wip1-1 wip2-1 wip3-1* triple mutant ("triple") were probed with the anti-WIP1 antibody. A coomassie brilliant blue stained replica gel is shown as loading control. **(B)** The interaction between endogenous WIP1 and RanGAP1 in Arabidopsis is not changed in *wit1-1 wit2-1*. Samples immunoprecipitated from WT, *wit1-1 wit2-1* and *wip1-1 wip2-1 wip3-1* ("triple") with the anti-WIP1 antibody, were probed with the anti-WIP1 antibody and co-immunoprecipitated RanGAP1 was detected with the anti-RanGAP1 antibody. The *wip1-1 wip2-1 wip3-1* triple mutant ("triple") serves as a negative control for WIP1 immunoprecipitation. **(C)** Double immunofluorescence localization of GFP-WIP1 (green) and RanGAP1 (magenta) in the root tip cells of *wit1-1 wit2-1* transformed with GFP-WIP1. All scale bars: 10 μm.



Supplemental Figure 4. WIT1-RanGAP interaction in *wip1-1 wip2-1 wip3-1* and WIT1 abundance in different WIP double mutant combinations.

(A) Overexpressed GFP-WIT1 interacts with RanGAP1 in the *wip1-1 wip2-1 wip3-1* triple mutant. Samples were immunoprecipitated with anti-GFP from Arabidopsis wild-type (WT) seedlings expressing GFP or GFP-WIT1 or from *wip1-1 wip2-1 wip3-1/*GFP-WIT1 seedlings (top lane) and were probed with the anti-RanGAP1 antibody (bottom lane). **(B)** WIT1 level is reduced in the *wip1-1 wip2-1* and *wip1-1 wip3-1* double mutants and in *wip1-1 wip2-1 wip3-1* expressing GFP-WIP2a or GFP-WIP3. Protein extracts from wild-type (WT); *wip1-1 wip2-1* ("double 1/2"), *wip1-1 wip3-1* ("double 1/3") and *wip2-1 wip3-1* ("double 2/3") double mutants; *wip1-1 wip2-1 wip3-1* triple mutant ("triple") expressing GFP-WIP1, GFP-WIP2a or GFP-WIP3 were probed with anti-WIT1 antibody. A coomassie brilliant blue stained replica gel is shown at the bottom as loading control.

Supplemental Table 1. Selected organ-specific expression profiles of *WIT1* and *WIT2* from Genevestigator expression analysis.

	seedling	inflorescence	rosette	roots	root tip	elongation zone	root hair zone
WIT1	656	1192	585	1248	1480	1558	1520
WIT2	202	167	237	241	65	215	119

Complete expression profile of *WIT1* is available in https://iii.genevestigator.ethz.ch/at/index.php?page=tair&option=atlas&agi=AT5g11390 and complete expression profile of *WIT2* is available in https://iii.genevestigator.ethz.ch/at/index.php?page=tair&option=atlas&agi=AT1g68910

Supplemental Table 2. **List of plasmids used in this study.**

pDEST22	N-terminal fusion with GAL4 AD; TRP1 Amp^R	Invitrogen
pDEST32	N-terminal fusion with GAL4 BD; LEU2 Gm^R	Invitrogen
TOPO pENTR	Blunt-end PCR product entry vector; Kan^R	Invitrogen
pK7WGF2,0	N-terminal fusion with GFP; Sp^R Kan^R	Karimi et al., 2002
pH2GW7,0	Overexpression or antisense; Sp^R Hyg^R	Karimi et al., 2002
pEarleyGate 202	N-terminal fusion with FLAG; Kan^R Bar^R	Earley et al., 2006
NTAPi	N-terminal fusion with TAP tag; Sp^R Bar^R	Rohila et al., 2004
pDEST17	N-terminal fusion with His; Amp^R	Invitrogen
pIM1021	*WIT1* in TOPO pENTR; Kan^R	This study
pIM1022	N-*WIT1* (AA 1-660) in TOPO pENTR; Kan^R	This study
pIM1023	TMD-*WIT1* (AA 661-703) in TOPO pENTR; Kan^R	This study
pIM1024	*WIT1* in pK7WGF2,0; Sp^R Kan^R	This study
pIM1025	N-*WIT1* (AA 1-660) in pK7WGF2,0; Sp^R Kan^R	This study
pIM1026	TMD-*WIT1* (AA 661-703) in pK7WGF2,0; Sp^R Kan^R	This study
pIM1027	*WIT1* in pDEST22; TRP1 Amp^R	This study
pIM1028	*WIT1* in pDEST32; LEU2 Gm^R	This study
pIM1029	*WIT1* in pEarleyGate 202; Kan^R Bar^R	This study
pIM1030	*WPP2* in NTAPi; Sp^R Bar^R	This study
pIM1031	N-*WIT1* (AA 1-317) in pDEST17; Amp^R	This study
pIM1032	*WIP1* in pH2GW7,0; Sp^R Hyg^R	This study
pIM2054	*WPP1* in pDEST32; LEU2 Gm^R	Xu et al., 2007
pIM2058	*WPP2* in pDEST32; LEU2 Gm^R	Xu et al., 2007
pIM2061	*WPP3* in pDEST32; LEU2 Gm^R	Xu et al., 2007
pIM2002	*WIP1* in pK7WGF2,0; Sp^R Kan^R	Xu et al., 2007
pIM2015	*WIP2a* in pK7WGF2,0; Sp^R Kan^R	Xu et al., 2007
pIM2009	*WIP3* in pK7WGF2,0; Sp^R Kan^R	Xu et al., 2007
pIM2072	*RanGAP2* in pK7WGF2,0; Sp^R Kan^R	Xu et al., 2007
pIM2109	*RanGAP1* in pFGC1008; Cm^R Hyg^R	Jeong et al., 2005
pIM2116	*RanGAP1* (WPP/AAP) in pFGC1008; Cm^R Hyg^R	Jeong et al., 2005

Abbreviations: Amp^R, ampicillin resistance; BD, DNA-binding domain; AD, activation domain; Kan^R, kanamycin resistance; Sp^R, spectinomycin resistance; Hyg^R, hygromycin resistance; Bar^R, Basta resistance; Gm^R, gentamicin resistance; Cm^R, chloramphenicol resistance.