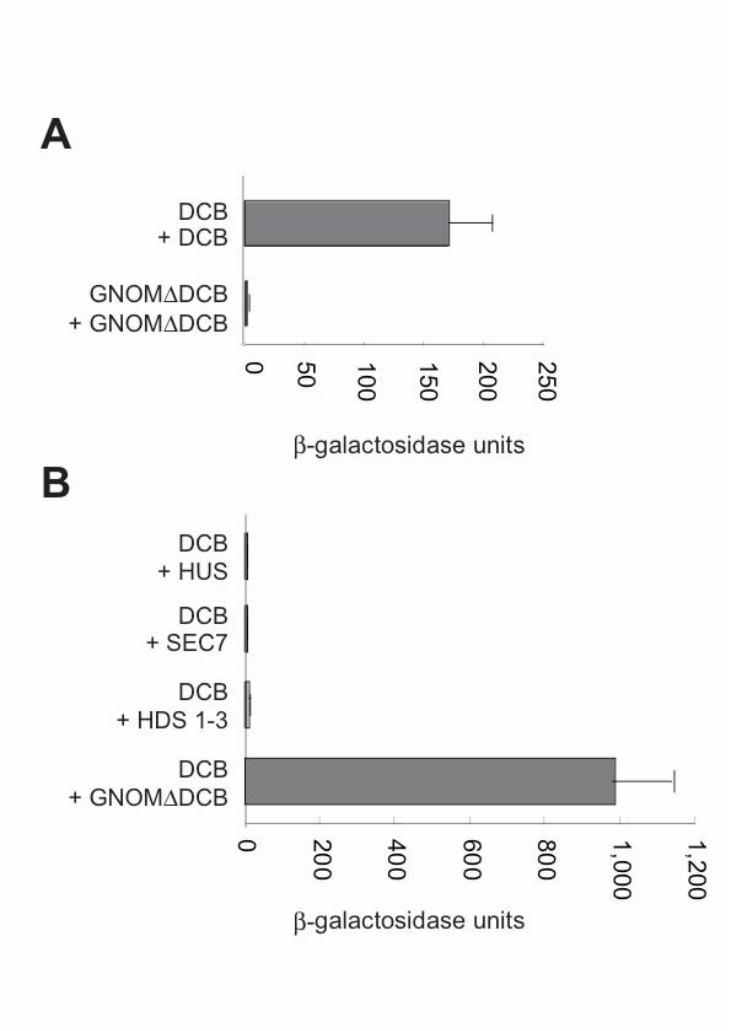
Supplemental Data. Anders et al. (2008). Membrane Association of the *Arabidopsis* ARF Exchange Factor GNOM Involves Interaction of Conserved Domains.

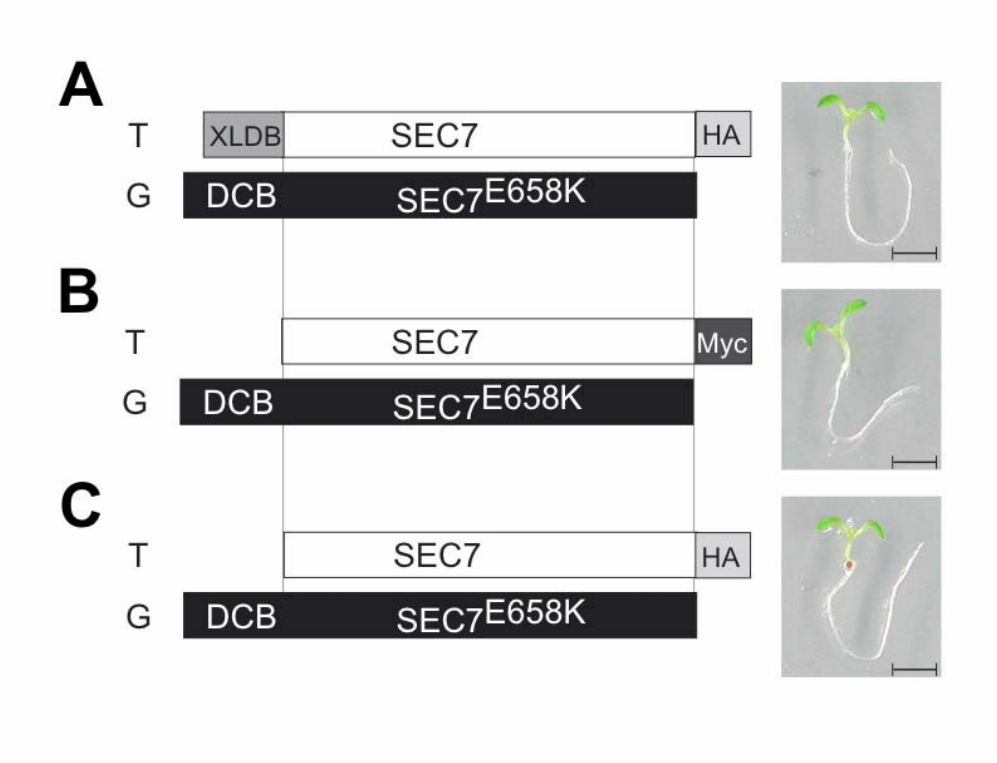


Supplemental Figure 1. Domain interactions of the GNOM protein.

Yeast two-hybrid analysis, quantitative assay each of at least 6 independent transformants; error bars indicate standard deviation.

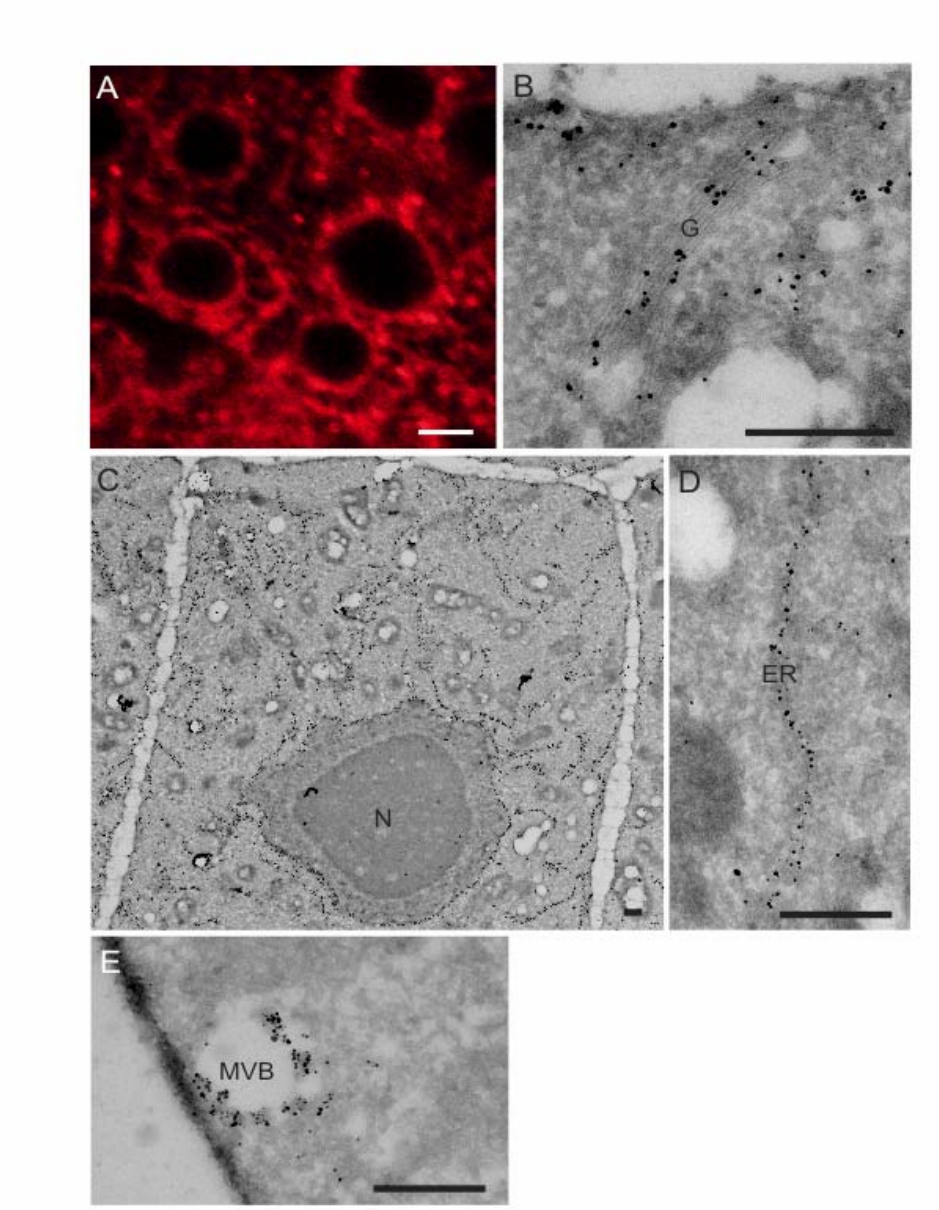
(**A**) Homotypic interaction of GNOMΔDCB and the DCB domain (positive control). Note that GNOMΔDCB does not interact with itself.

(**B**) Heterotypic interaction of the DCB domain with the HUS, SEC7, HDS1-3 domain(s) or GNOMΔDCB (positive control). Note that the heterotypic interaction partner of DCB cannot be mapped to a single domain.



Supplemental Figure 2. Functional analysis of GNOM mutant proteins.

(A-C) Inter-allelic complementation of endogenous (G) and transgenic (T) GNOM variants: schematic showing heterologous dimerization module XLDB, Myc or HA tag, and amino-acid exchange mutations (*left*). Phenotypes of homozygous *gnom* mutant seedlings harboring the respective *GNOM* transgene (*right*). Scale bar, 3mm.

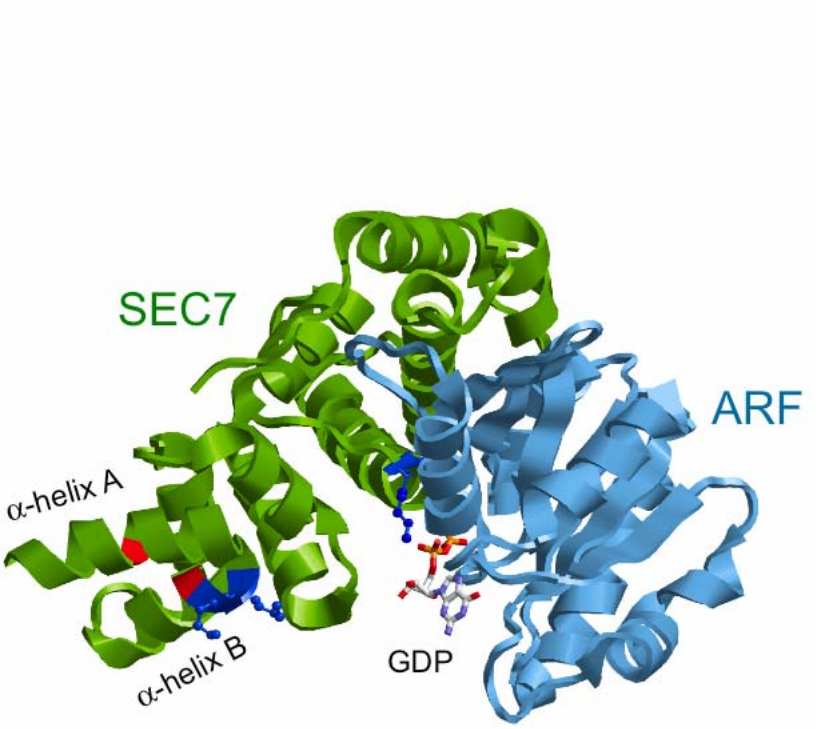


Supplemental Figure 3. At CYP19-4 localizes to the secretory pathway.

Subcellular localization of At CYP19-4-Myc with anti-Myc antibody.

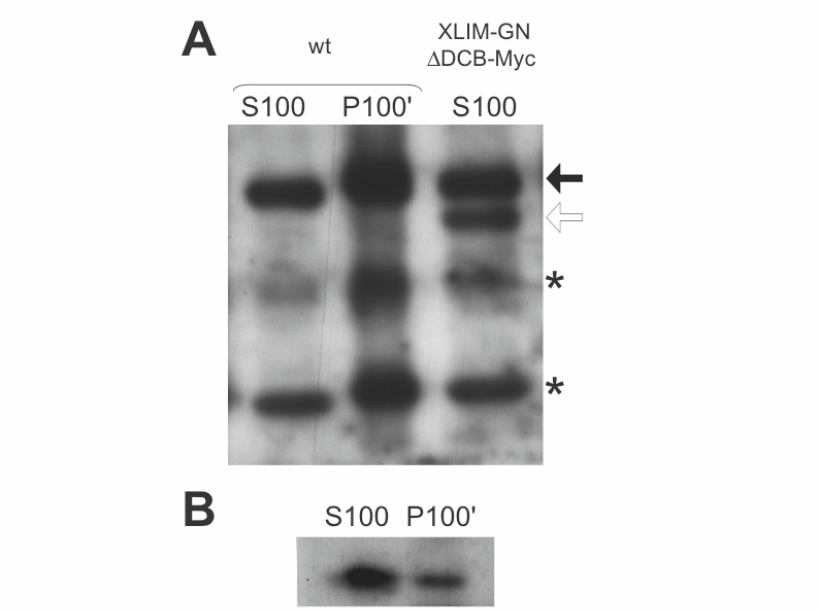
(**A**) Confocal immunofluorescence imaging; scale bar, 4μm.

(**B**-**E**) Electron microscopic analysis of immunogold-labeled ultrathin thawed cryo-sections; scale bar, 500nm. Silver-enhanced Nanogold markers were detected in (**B**) the Golgi (G), (**C, D**) the endoplasmic reticulum (ER), and (**E**) multivesicular bodies (MVBs). N, nucleus.



Supplemental Figure 4. The N-terminal α-helices A and B of the SEC7 domain are not in contact with the ARF substrate. Representation of the structure of ARF1(Δ17) (light blue) bound to GDP and complexed with the SEC7 domain of *Homo sapiens* ARNO^{E156K} (green) (Protein Data Bank accession number 1R8S; Renault et al., 2003). The amino acid residues of ARNO (G68 and G79) corresponding to the mutated residues G568 and G579 of GNOM that affect the heterotypic interaction are highlighted in red. Residues KK77,78 and E156 corresponding to KK677,578EE and E658K, respectively, that do not affect the heterotypic interaction are highlighted in dark blue.

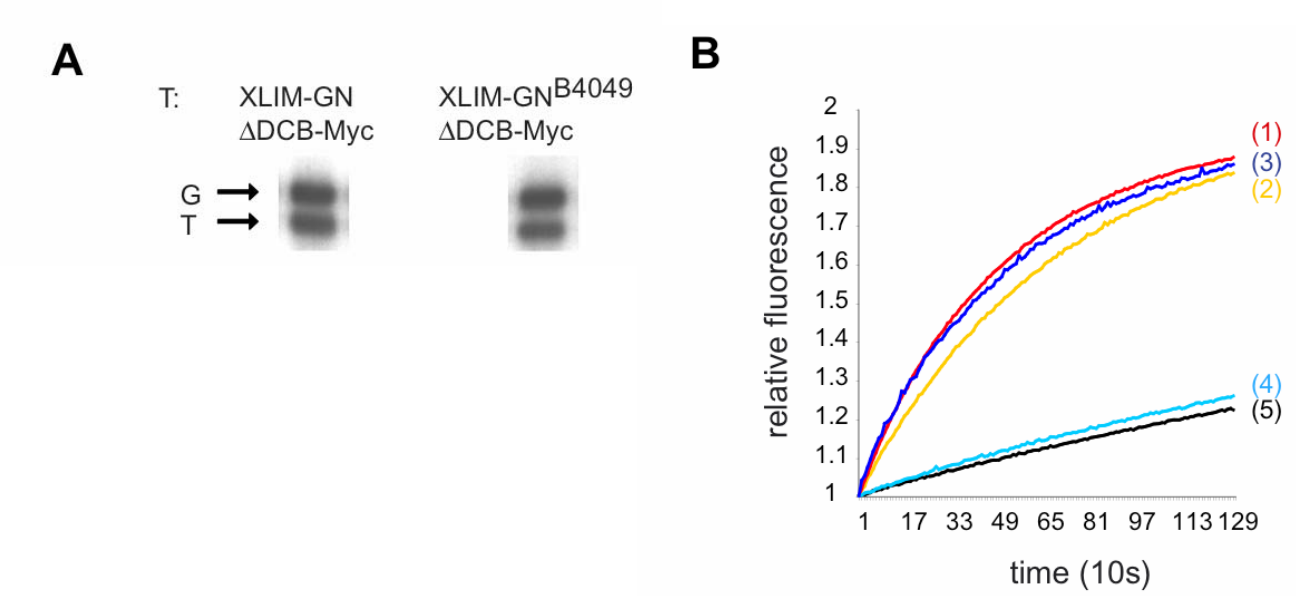
Renault, L., Guibert, B., and Cherfils, J. (2003). Structural snapshots of the mechanism and inhibition of a guanine nucleotide exchange factor. Nature 426, 525-530.



Supplemental Figure 5. Antibody detection of proteins in soluble (S100) and membrane (P100') subcellular fractions. Note P100' was loaded in 10-fold excess of S100.

(A) Specificity of the anti-SEC7 antiserum. Subcellular fractions of protein extract from wild-type (wt) and transgenic seedlings expressing truncated GNOM protein (XLIM-GNΔDCB-Myc) were probed with anti-SEC7 antiserum. Black arrow, endogenous full-length GNOM protein; white arrow, truncated GNOM protein; asterisks, cross-reacting bands. **(B)** Analysis of P100' fraction for presence of soluble (S100) proteins. Subcellular fractions of protein extract were probed with anti-POR antiserum which detects the soluble POR (TFC-C) protein (Steinborn et al., 2002), revealing contamination of the concentrated P100' fraction by soluble protein.

Steinborn, K., Maulbetsch, C., Priester, B., Trautmann, S., Pacher, T., Geiges, B., Küttner, F., Lepiniec, L., Stierhof, Y.-D., Schwarz, H., Jürgens, G., and Mayer, U. (2002). The *Arabidopsis PILZ* group genes encode tubulin-folding cofactor orthologs required for cell division but not cell growth. Genes Dev. 16, 959-971.



Supplemental Figure 6. *G579R* and the homologous mutation in BIG3 do not interfere with ARF-GEF expression level and BFA sensitivity, respectively.

(A) Expression levels of XLIM-GNOMΔDCB-Myc and XLIM-GNOM^{B4049}ΔDCB-Myc are comparable. Western blot analysis of protein extracts from seedlings. The anti-SEC7-antiserum detects endogenous full-length GNOM (G), and transgenically produced XLIM-GNOMΔDCB (T).
(B) Influence of *G579R*-homologous mutation on BFA-sensitivity of the SEC7 domain of BIG3. GDP/GTP exchange activity of SEC7^{BIG3G626R} (1; red) and *L741M* mutated SEC7^{BIG3G626R} (3; dark blue) were measured on ARF1. Addition of 100μM BFA abolished the activity of *L741M* mutated SEC7^{BIG3G626R} (4; light blue), whereas the exchange activity of SEC7^{BIG3G626R} (2; yellow) is resistant to BFA. Negative control: ARF1 alone (5; black). We used the SEC7 domain of BIG3 because that of GNOM was insoluble. As shown in Figure 4B, the *G579R*-homologous mutation of BIG3 (*G626R)* did not affect the SEC7 catalytic exchange activity on ARF1. Unlike GNOM, BIG3 is BFA-resistant (Geldner et al., 2003; Nielsen et al., 2006). To analyze whether the *G579R*-homologous mutation interferes with BFA sensitivity we introduced the *L741M* mutation predicted to render the SEC7 domain of BIG3 BFA-sensitive (Geldner et al., 2003).

Geldner, N., Anders, N., Wolters, H., Keicher, J., Kornberger, W., Muller, P., Delbarre, A., Ueda, T., Nakano, A., and Jürgens, G. (2003). The Arabidopsis GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. Cell 112, 219-230.

Nielsen, M., Albrethsen, J., Larsen, FH., Skriver, K. (2006). The Arabidopsis ADP-ribosylation factor (ARF) and ARF-like (ARL) system and its regulation by BIG2, a large ARF-GEF. Plant Science 171, 707-717.

Supplemental Table 1. Segregation rates of complementing allele combinations.

Phenotypic analysis of lines segregating endogenous *gnom* (G) and transgenic (T) GNOM variants which generated the complementing seedling phenotypes displayed in Figure 2 and Supplemental Figure 2. T1, transgene 1; T2, transgene 2; n, number of seedlings.

Figure	Transgene(s)	Parental genotype		Progeny phenotypes		
		T	G	n	Wild-type	*gnom*
2A	XLIM-GNOMΔDCB-Myc, XLDB-GNOMΔDCB-HA	T1/- T2/T2	*E658K*/+	197	100%	0%
2B	XLIM-GNOMΔDCB-Myc	T/-	*E658K*/+	296	97%	3%
Suppl. 2A	XLDB-GNOMΔDCB-HA	T/-	*E658K*/+	229	98%	2%
2D	XLIM-GNOM^{G579R}ΔDCB-Myc	T /-	*E658K*/+	142	78%	22%
2E	XLDB-GNOM^{E658K}ΔDCB-HA	T /-	*G579R*/+	187	77%	23%
2F	XLDB-GNOM^{E658K}ΔDCB-HA, XLIM-GNOM^{G579R}ΔDCB-Myc	T1/- T2/T2	*E658K*/+	459	75%	25%
Suppl. 2B	GNOMΔDCB-Myc	T/-	*E658K*/+	355	94%*	6%
Suppl. 2C	GNOMΔDCB-HA	T/-	*E658K*/+	205	94%*	6%

* Complemented seedlings may look slightly smaller than wild-type seedlings, depending on the expression level of the *GNOMΔDCB* transgene; however, comparable expression to that of the endogenous gene results in wild-type morphology.