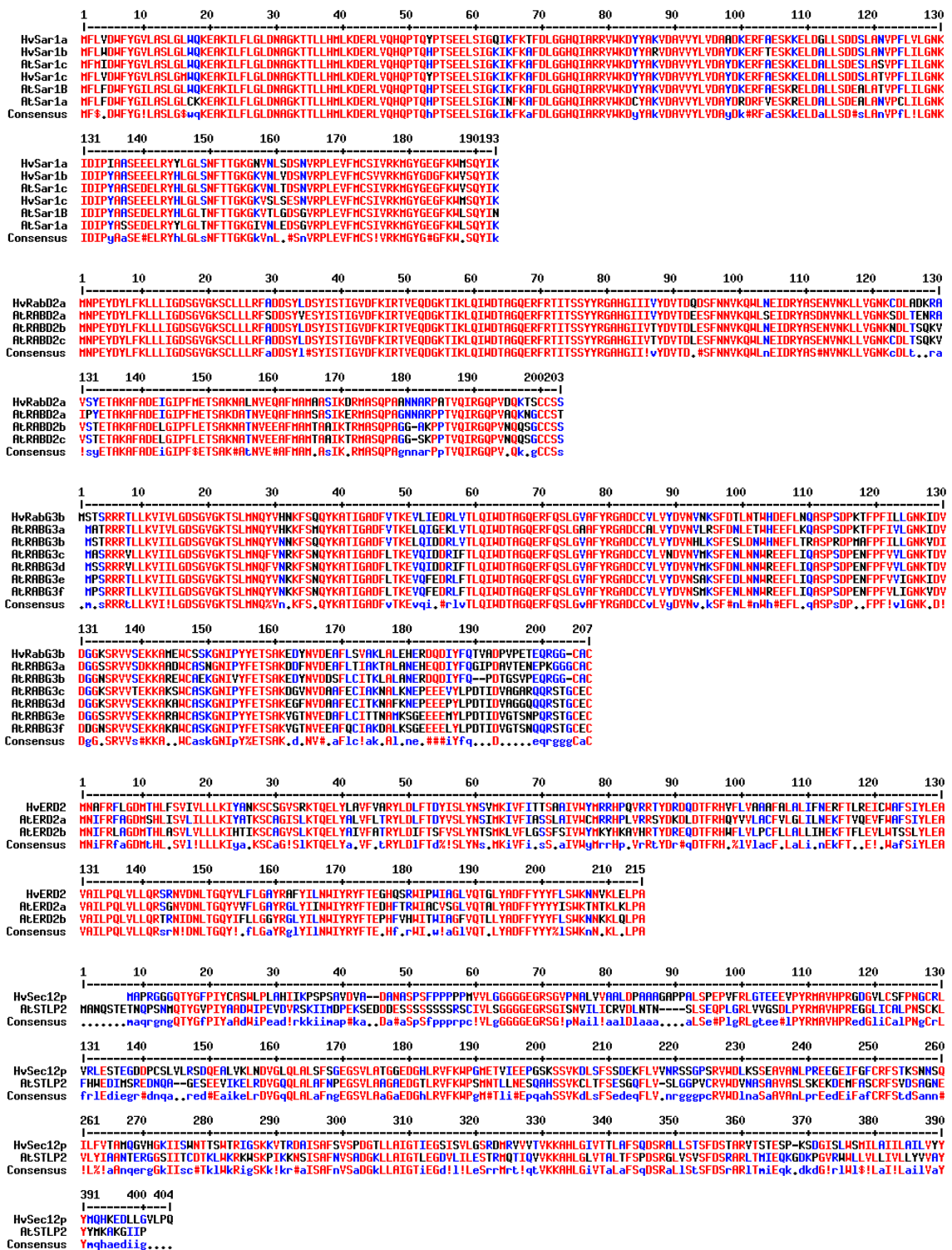


Supplementary data

The plant membrane surrounding powdery mildew haustoria shares properties with the endoplasmic reticulum

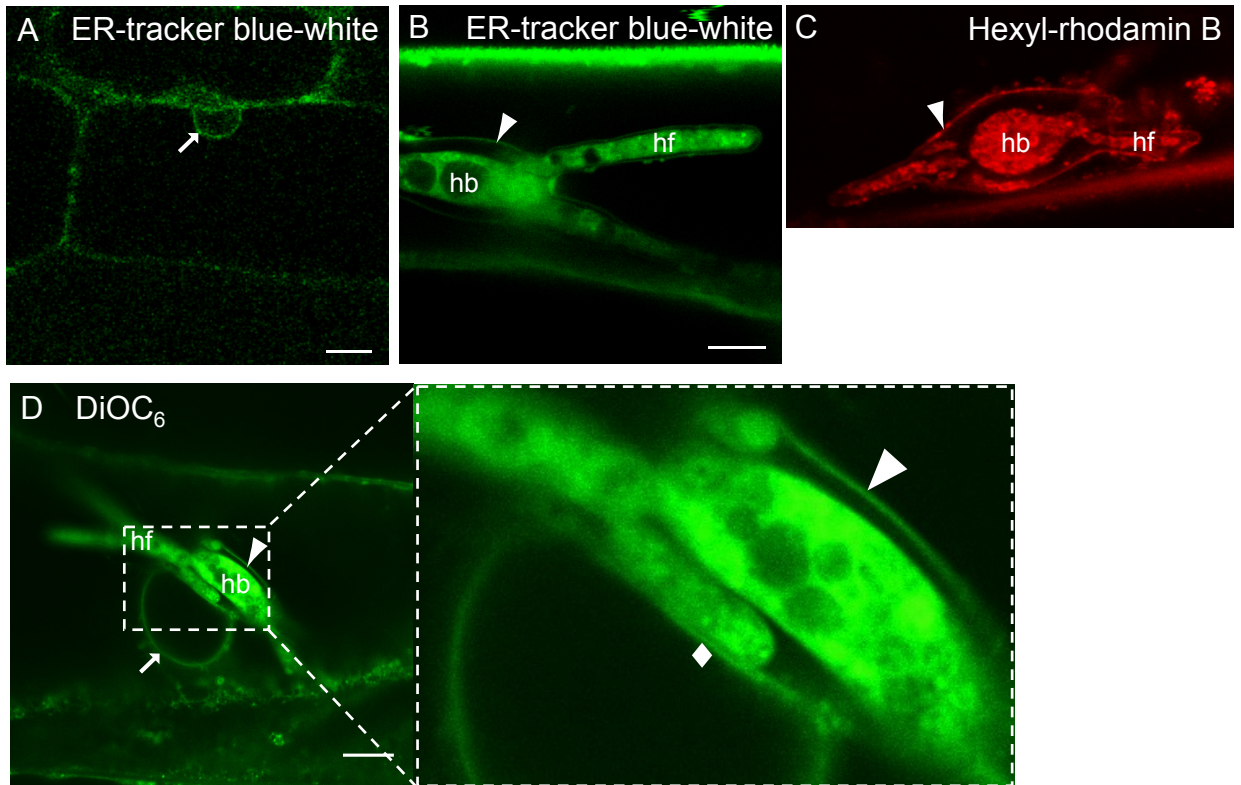
Mark Kwaaitaal, Mads Eggert Nielsen, Henrik Böhlenius and Hans Thordal-Christensen

Kwaaitaal et al. Supplementary Figure S1



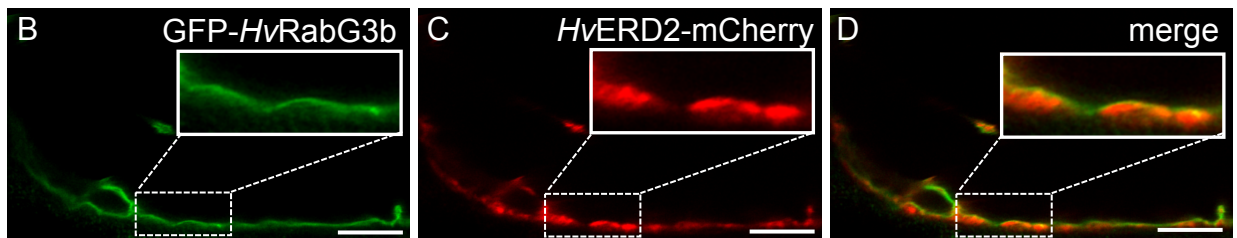
Supplementary Figure S1. Alignments of barley proteins used in this study to their closest relatives in Arabidopsis.

These are, AtSar1a: At1G09180, AtSar1b: At5G56330, AtSar1c: At4G02080, AtRabD2a: At1G02130, AtRabD2b: At5G47200, AtRabD2c: At4G17530, AtRabG3a: At4G09720, AtRabG3b: At1G22740, AtRabG3c: At3G16100, AtRabG3d: At1G52280, AtRabG3e: AtAt1G49300, AtRabG3f: At3G18820, AtERD2a: At1G29330, AtERD2b: At3G25040, AtSTLP2: At2G01470. The alignments were made using <http://multalin.toulouse.inra.fr/multalin/cgi-bin/multalin.pl>.



Supplementary Figure S2. ER-membrane dyes and the extrahaustorial membrane.

(A) Non-inoculated barley cell stained with ER-tracker blue-white. (B-D) Barley cells containing *Bgh* haustoria 2 days after inoculation (dai) stained with ER-tracker blue-white (B), Hexyl-rhodamin B (C) and DiOC₆ (D). ER membrane around the nucleus (arrows), membrane around haustoria (arrowhead), membrane between nucleus and haustorium (diamond), haustorial bodies (hb), haustorial fingers (hf). Scale bar, 10 μm.



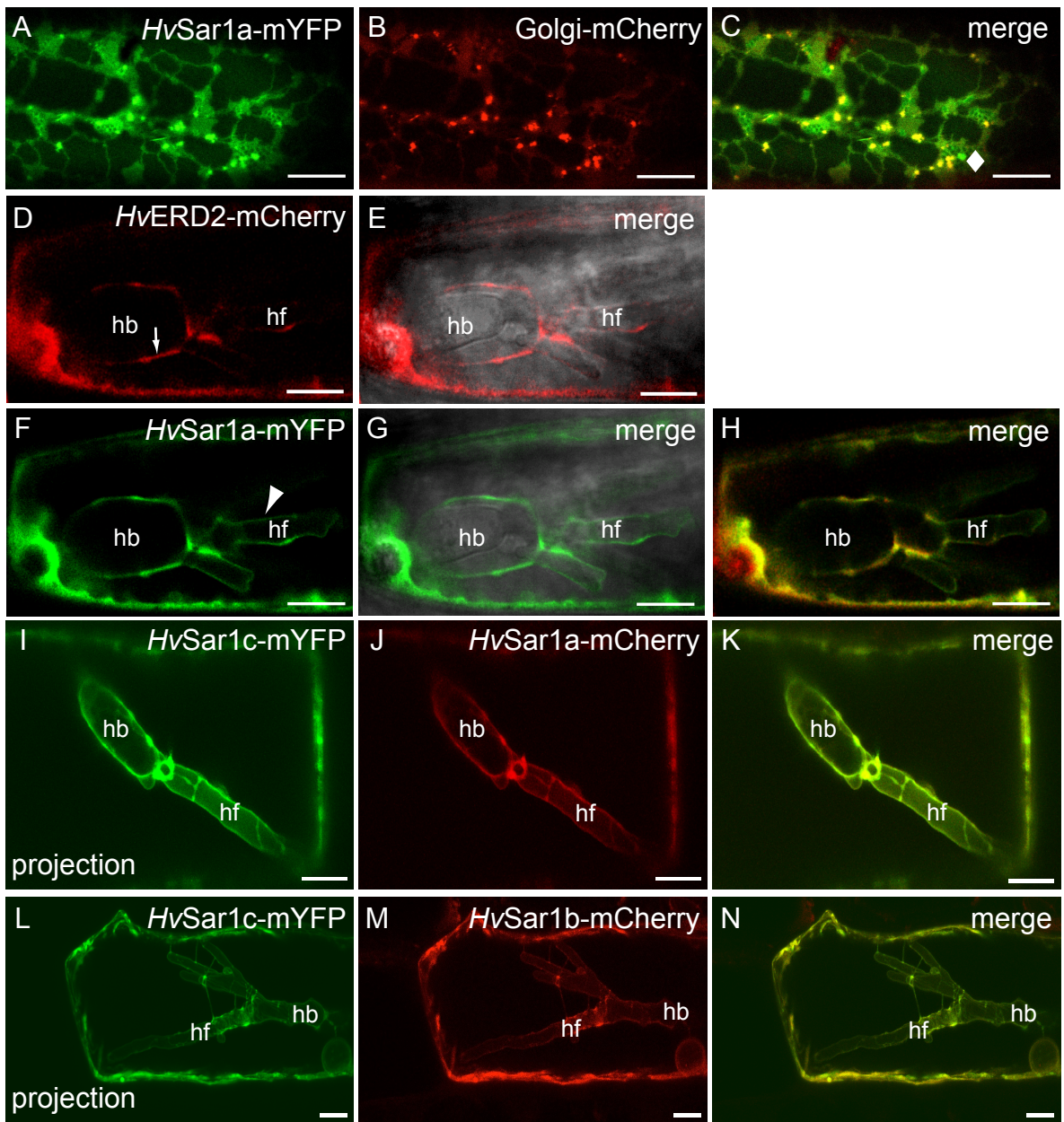
Supplementary Figure S3. Barley Rab GTPases.

(A) Phylogenetic analysis of members of the Arabidopsis and barley Rab GTPase family. Arabidopsis Rab protein sequences were obtained from the Genbank database and named according to Rutherford and Moore (2002) and Vernoud et al. (2003). Yeast ScYpt1 is used as an outgroup. The barley Rab GTPases were named according to their closest homologues in Arabidopsis. *HvRabD2a* and *HvRabG3b* are circled. Protein sequence comparison and subsequent bootstrapping (1000 iterations) was performed with the CLC sequence viewer (Qiagen). (B-D) Micrographs of a non-inoculated barley epidermal cell co-expressing the vacuolar marker GFP-*HvRabG3b* and the ERES/Golgi marker *HvERD2-mCherry*. Inserts show magnified area where GFP-*HvRabG3b* marks a membrane towards the inside of the cell and *HvERD2-mCherry* marked the ERES/Golgi in the cytosol. This observation confirms that GFP-*HvRabG3b* marked the tonoplast. Scale bar, 10 μm .

Rutherford S, Moore I. 2002. The Arabidopsis Rab GTPase family: another enigma variation. *Current Opinion of Plant Biology* **5**: 518–528.

Vernoud V, Horton AC, Yang Z, Nielsen E. 2003. Analysis of the small GTPase gene superfamily of *Arabidopsis*. *Plant Physiology* **131**: 1191–1208.

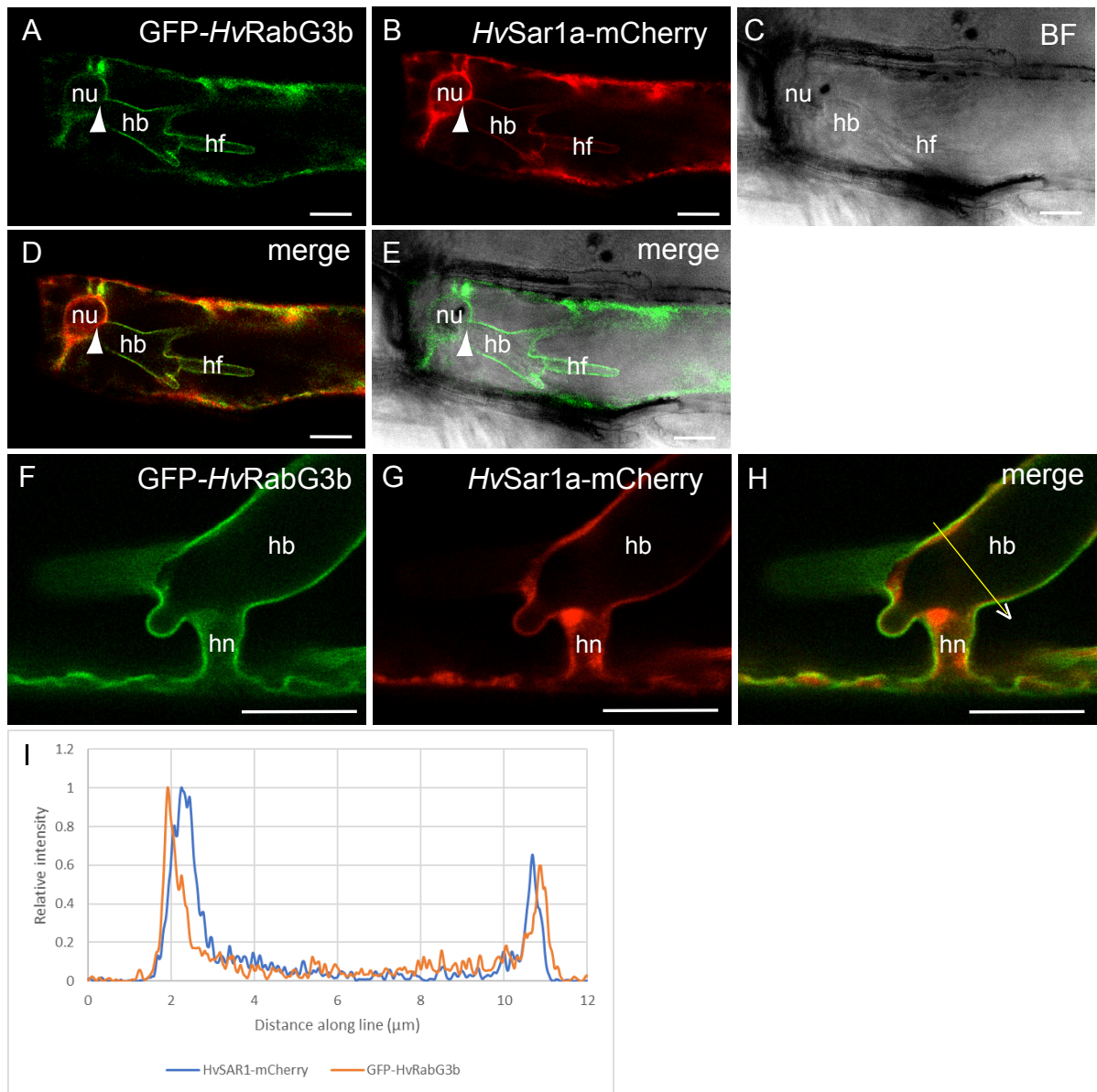
Kwaaitaal et al. Supplementary Figure S4



Supplementary Figure S4. Three Sar1 GTPases, but not the HDEL-receptor, ERD2, labeled the extrahaustorial membrane.

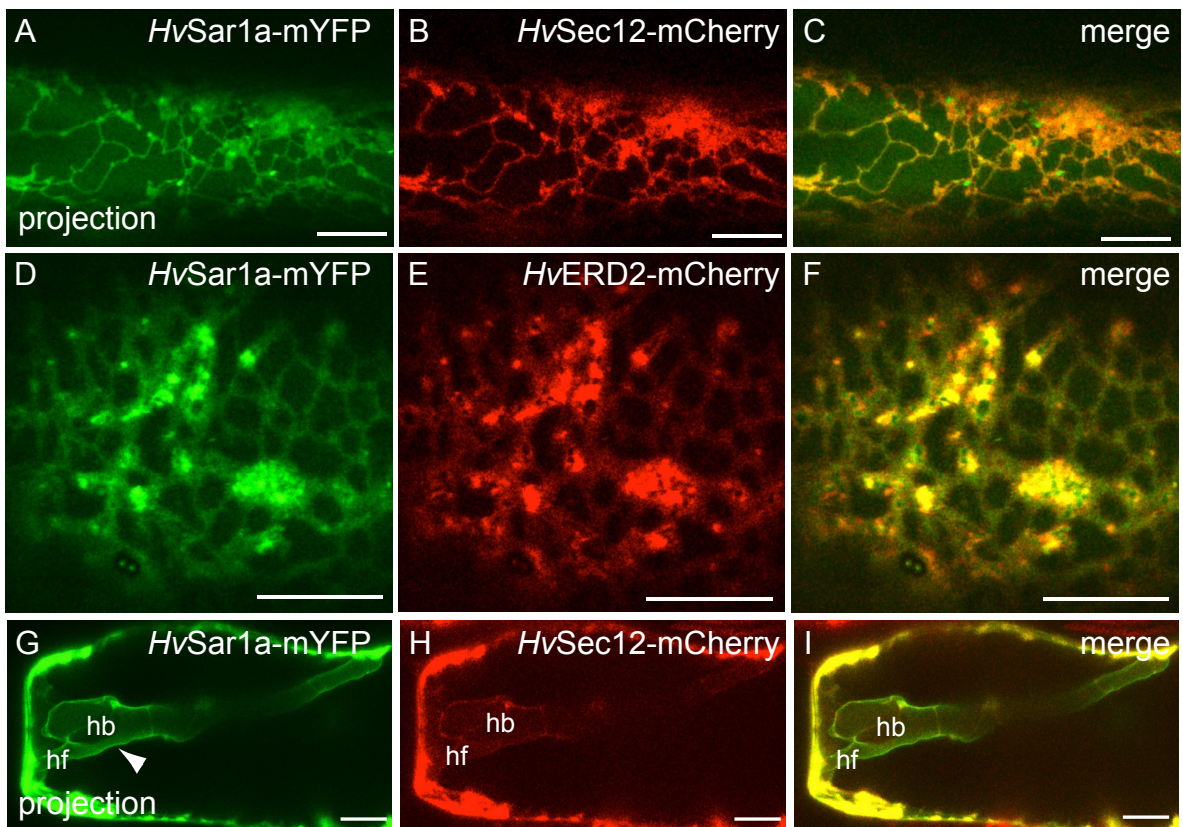
(A-C) Non-inoculated barley cell co-expressing *HvSar1a*-mYFP with the Golgi-mCherry marker (soybean α -1,2-mannosidase I fused to mCherry). (D-H) *Bgh* haustorium-containing barley cell (2 dai) co-expressing the ERES/cis-Golgi marker, *HvERD2*-mCherry, and *HvSar1a*-mYFP. Note, *HvERD2* did not labeling the EHM. Note, D and E are also shown in Figure 1, and repeated here for comparison. (I-K) *Bgh* haustorium-containing barley cell (2 dai) co-expressing *HvSar1c*-mYFP and *HvSar1a*-mCherry. (L-N) *Bgh* haustorium-containing barley cell (2 dai) co-expressing *HvSar1c*-mYFP and *HvSar1b*-mCherry. Note, localization of *HvSar1b* and *HvSar1c* at the EHM similar to *HvSar1a*. ER (arrow); EHM (arrowhead), ERES not labeled with the Golgi marker (diamond), haustorial bodies (hb), haustorial fingers (hf). Scale bars, 10 μ m.

Kwaaitaal et al. Supplementary Figure S5



Supplementary Figure S5. The tonoplast marker, *HvRabG3b*, did not label the EHM. (A-H) *Bgh* haustorium-containing barley cell (2 dai) co-expressing the GFP-*HvRabG3b* and *HvSar1a*-mCherry. (A-E) Note, GFP-*HvRabG3b* was absent between the nucleus and the haustorial body (arrowhead). (F-I) Note, ER/EHM-associated *HvSar1a*-mCherry found between GFP-*HvRabG3b* and haustorium. (I) Signal quantification of GFP-*HvRabG3b* and *HvSar1a*-mCherry along the indicated path in H. Bright field (BF), haustorial bodies (hb), haustorial fingers (hf), haustorial neck (hn). Scale bars, 10 μm.

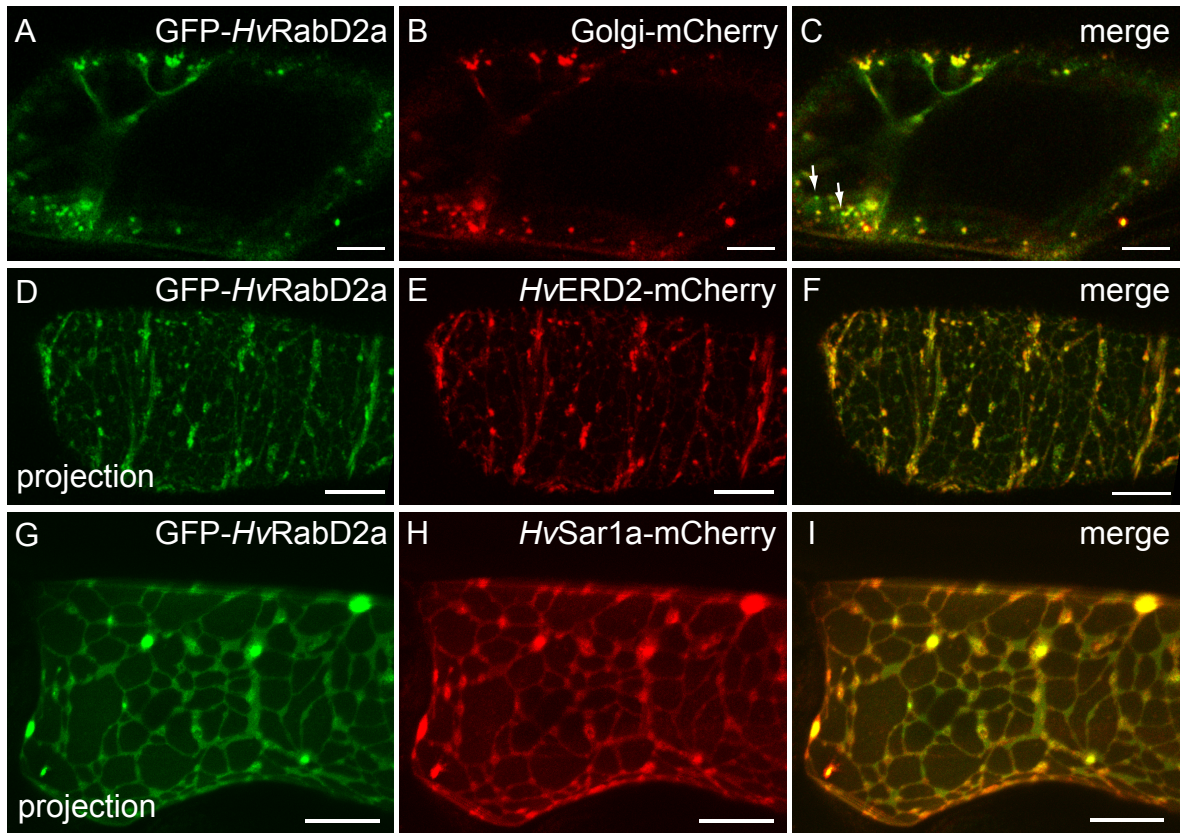
Kwaaitaal et al. Supplementary Figure S6



Supplementary Figure S6. Sar1a, ERD2, the Sar1 GEF, Sec12, and the EHM.

(A-C) Barley cells co-expressing *HvSar1a*-mYFP and *HvSec12*-mCherry. (D-F) Cells co-expressing *HvSar1a*-mYFP and *HvERD2*-mCherry. (G-I) *Bgh* haustorium-containing barley cell (2 dai) co-expressing *HvSar1a*-mYFP and *HvSec12*-mCherry. EHM (arrowhead), haustorial bodies (hb), haustorial fingers (hf). Scale bars, 10 μ m.

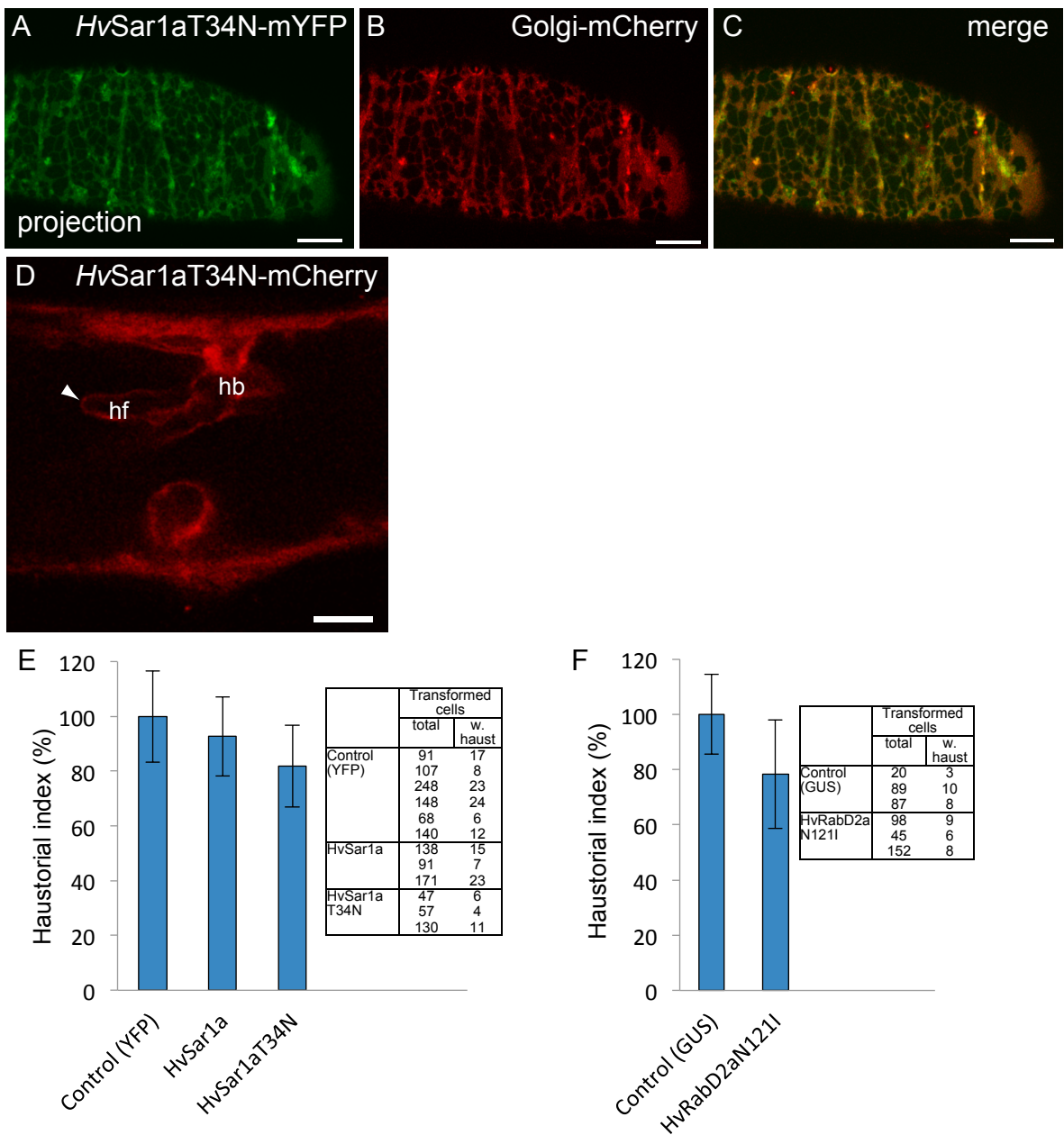
Kwaaitaal et al. Supplementary Figure S7



Supplementary Figure S7. RabD2a co-localized with Golgi-marker, ERD2 and Sar1a.

(A-I) Non-inoculated barley cells. (A-C) Barley cell co-expressing GFP-*HvRabD2a* and the Golgi-mCherry marker (soybean α -1,2-mannosidase I fused to mCherry). Arrows in C mark GFP-*HvRabD2a*-positive units not labeled as Golgi compartments. (D-F) Barley cell co-expressing GFP-*HvRabD2a* and *HvERD2*-mCherry. (G-I) Barley cell co-expressing GFP-*HvRabD2a* and *HvSar1a*-mCherry. Scale bars, 10 μ m.

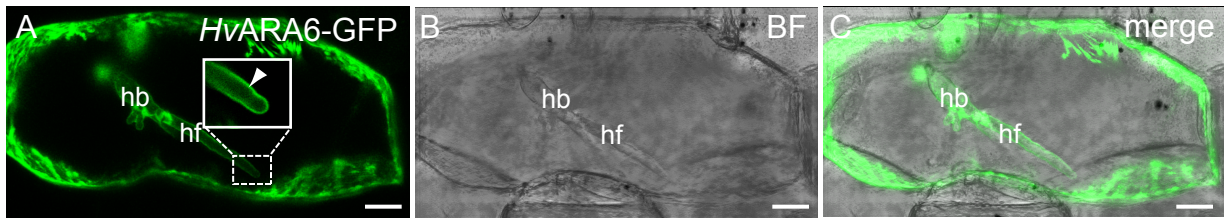
Kwaaitaal et al. Supplementary Figure S8



Supplementary Figure S8. Arresting ER-Golgi traffic did not affect haustorial formation.

(A-C) Non-inoculated barley cell co-expressing *HvSar1aT34N-mYFP* (GDP-locked) and the Golgi-mCherry marker (soybean α -1,2-mannosidase I fused to mCherry). Note Golgi-marker was prevented from progressing beyond the ER. (D) *HvSar1aT34N-mCherry* labels the EHM in barley cell co-expressing containing *Bgh* haustorium 2 dai. EHM (arrowhead), haustorial bodies (hb), haustorial fingers (hf). Scale bar, 10 μ m. (E-F) *Bgh* haustorial formation (2 dai) after overexpression of *HvSar1a* and *HvSar1aT34N* (GDP-locked) and after expression of *HvRabD2aN121I* (nucleotide free). Average haustorial indices are provided relative to the average of controls, set to 100%. Error bars show SE. Values are averages of biological replicates, as indicated in the provided raw data.

Kwaaitaal et al. Supplementary Figure S9



Supplementary Figure S9. ARA6 labels EHM.

(A-C) *Bgh* haustorium-containing barley cell (2 dai) expressing *HvARA6-GFP*. Note insert, *HvARA6-GFP* associated with EHM. EHM (arrowhead), haustorial bodies (hb), haustorial fingers (hf). Scale bar, 10 μ m.

Kwaaitaal et al. Supplementary Table S1

Supplementary Table S1. Primer sequences.

Primer Name	Sequence
<i>HvSar1a</i> (Genbank: AK252291.1)	
<u>Fluorescent fusion</u>	
HvSAR1a_GWY_KZK_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAACCATGTTTCTGGTGGATTGGTT
HvSAR1a_GWY_nst_R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTGATGTACTGGGACATCCATT
<u>Overexpression</u>	
HvSar1a_Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTC ATGTTTCTGGTGGATTGGTTCTA
HvSar1a_Rv	GGGGACCAGTTTGTACAAGAAAGCTGGGTC TCACTTGATGTACTGGGA
<u>HvSar1aT34N</u>	
HvSar1aT34N_F	GACAACGCTGGCAAGAaCACCTCCTCCACATG
HvSar1aT34N_R	CATGTGGAGGAGGGTGTCTTGCCAGCGTTGTC
<i>HvSar1b</i> (Genbank: AK252372.1)	
<u>Fluorescent fusion</u>	
HvSAR1b_GWY_KZK_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAACCATGTTCTTTGGGACTGGTT
HvSAR1b_GWY_nst_R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTGATGTACTGCGAGACCCACT
<i>HvSar1c</i> (Genbank: AK250294.1)	
<u>Fluorescent fusion</u>	
HvSAR1c_GWY_KZK_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAACCATGTTCTTGTGGACTGGTT
HvSAR1c_GWY_nst_R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAATACTGCGACATCCACT
<i>HvRabD2a</i> (Genbank: AK355333.1)	
HvRABD2a_GWY_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAATCCGGAGTATGATTACCTCTT
HvRABD2a_GWY_stop_R	GGGGACCAGTTTGTACAAGAAAGCTGGGTCTAAGAAGAGCAGCAGCTGGTCTTC
HvRabD2aN121I	
HvRabD2aN121I_F	AAGCTTCTCGTGGGGAtCAAATGTGATCTCGCT
HvRabD2aN121I_R	AGCGAGATCACATTTGaTCCCCACGAGAAGCTT
<i>RabG3b</i> (Genbank: AK368361.1)	
HvRabG3b_GWY_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCCTCGCGACGCCGCACGC
HvRabG3b_GWY_R	GGGGACCAGTTTGTACAAGAAAGCTGGGTCTAACAGCAGCCTGGTGATCTT
<i>HvERD2</i> (Genbank: AK250768.1)	
HvERD2_F	AATAAACCCCCAAAACC
HvERD2_R	GCCAATACAGACCATCAA
HvERD2_GWY_KZK_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTATACCATGAACGCCTTCCGG
HvERD2_GWY_nst_R	GGGGACCACTTTGTACAAGAAAGCTGGGTGGCAGGCAACTCGAGCTTC
<i>HvSec12</i> (Genbank: AK356828.1)	
HvSec12_F1	TAGTTGTGAACAGGAGCAG
HvSec12_R1	AGAGAGGTAAGCAGTGAAA
HvSec12_GWY_KZK_F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAACCATGGCGCCGCGCGCGGTGG
HvSec12_GWY_nst_R	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTGTGGCAACACGCCTAAGA