Supplementary Table 1. Markers and lines used in this study. Arabidopsis plants stably expressing fluorescent-tagged markers (At line) were used for *Hyaloperonospora arabidopsidis* (*Hpa*) infections; transient expression of the respective markers (construct) in *N. benthamiana* (Nb) was used for *Phytophthora infestans* (*Pi*) infections; plasma membrane (PM), endoplasmatic reticulum (ER), trans-Golgi network (TGN), extrahaustorial membrane (EHM).

Gene/Marker	Localization	At line/construct	Reference	Interaction tested	
PIP1;4	PM	WAVE138y	1	At-*Hpa*	Nb-*Pi*
ACA8	PM	ACA8-GFP/YFP	2	At-*Hpa*	Nb-*Pi*
REM1*	PM	YFP-StREM1	3		Nb-*Pi*
SYT1	PM/ER	SYT1-GFP	4		Nb-*Pi*
EFR	PM/ER	EFR-YFP	5		Nb-*Pi*
PEN1	PM/Endosome	GFP-PEN1	6	At-*Hpa*	Nb-*Pi*
FLS2	PM/Endosome	FLS2-GFP	7	At-*Hpa*	Nb-*Pi*
RPW8.2	Golgi/ER/EHM	RPW8.2-YFP	8	At-*Hpa*	
Rab D2a	Golgi/ER	WAVE29y	1		Nb-*Pi*
SYP32	Golgi	WAVE22y	1	At-*Hpa*	Nb-*Pi*
Got1p	Golgi	WAVE18y	1	At-*Hpa*	Nb-*Pi*
Rab E1d	Golgi/Post-Golgi	WAVE27y	1	At-*Hpa*	Nb-*Pi*
VAMP722	Post-Golgi	GFP-VAMP722	9		Nb-*Pi*
Rab A5d	TGN/Post-Golgi	WAVE24y	1	At-*Hpa*	Nb-*Pi*
VTI12	TGN/Early endosome	WAVE13y	1	At-*Hpa*	Nb-*Pi*
VPS28-2	TGN/Early endosome	Y/RFP-VPS28-2	this study	At-*Hpa*	Nb-*Pi*
VPS37-1	TGN/Early endosome	YFP-VPS37-1	this study		Nb-*Pi*
Rab A1e	Endosome	WAVE34y	1	At-*Hpa*	
Rab C1	Endosome	WAVE3y	1	At-*Hpa*	
Rab F2b (ARA7)	Endosome	mRFP-ARA7	10	At-*Hpa*	
Rab F2a (Rha1)	Endosome	WAVE7y	1		Nb-*Pi*
Rab F1 (ARA6)	Late endosome	ARA6-mRFP	10	At-*Hpa*	
FYVE**	Late endosome	GFP-2xFYVE	11	At-*Hpa*	Nb-*Pi*
Rab G3f	Vacuole/Late endosome	WAVE5y	1	At-*Hpa*	
Rab G3c	Vacuole/Late endosome	WAVE11y	1		Nb-*Pi*
VAMP711	Vacuole	WAVE9y	1	At-*Hpa*	

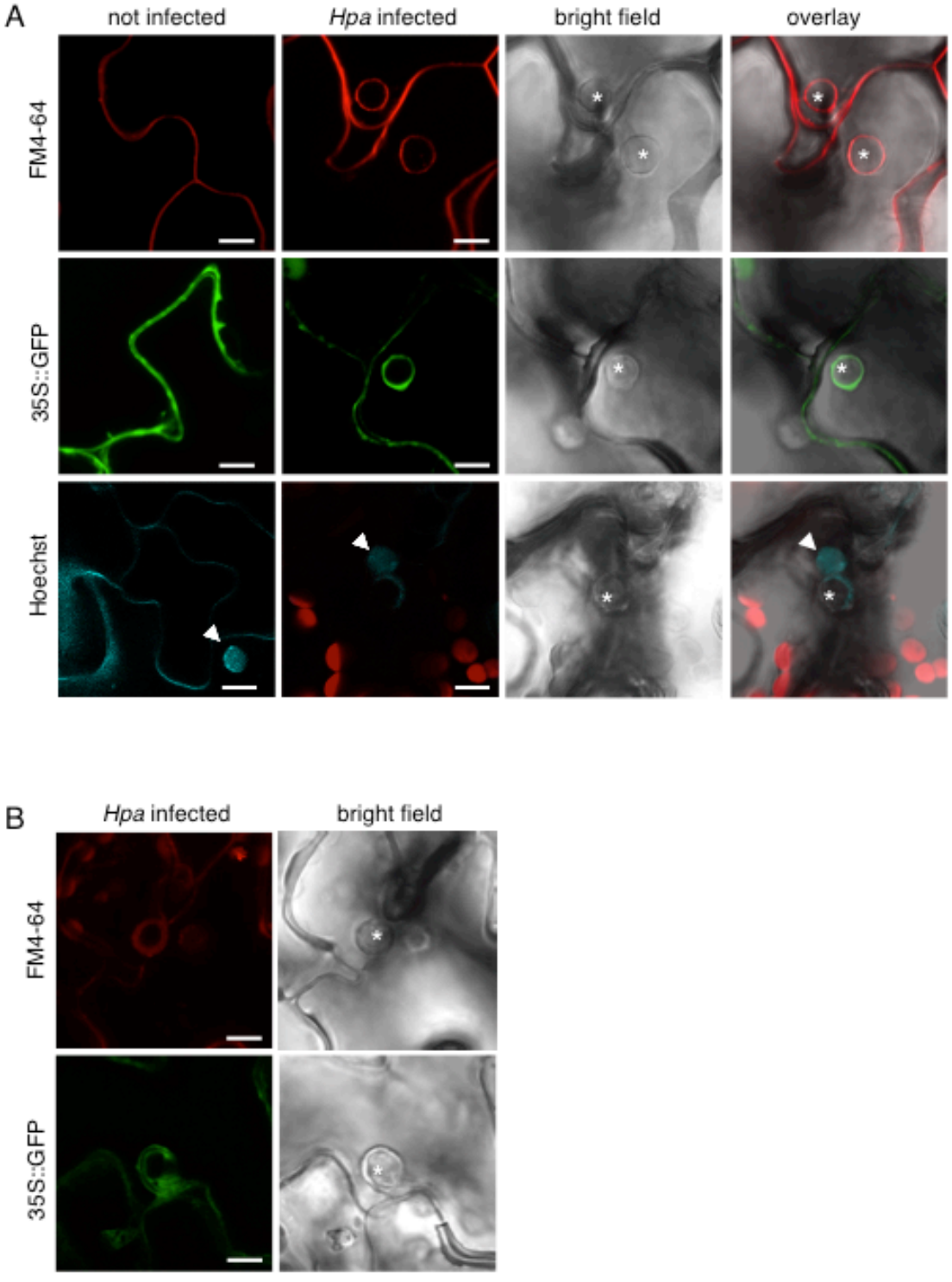
Markers without asterisk are originating from Arabidopsis; * potato; ** FYVE domain from the mouse hepatocyte growth factor-regulated tyrosine kinase substrate; [1]Geldner et al., 2009; [2]Frei dit Frey et al., 2011; [3]Lefebvre et al., 2010; [4]Schapire et al., 2008; [5]Häweker et al., 2010; [6]Meyer et al., 2009; [7]Göhre et al., 2008; [8]Wang et al., 2009; [9]Lipka et al., 2005; [10]provided by K. Schumacher; [11]Vermeer et al., 2006

Supplementary Table 2. Summary of marker localization in the absence and presence of *Hyaloperonospora arabidopsidis* (*Hpa*) and *Phytophthora infestans* (*Pi*) infection in *A. thaliana* (At) and *N. benthamiana* (Nb). Detection of fluorescent signals: clear discrete signal (discrete); weak widespread signal (diffuse); at cell periphery (periphery); at spots (vesicles); not at haustoria (excluded); at haustoria (surrounding); at extrahaustorial membrane (EHM); at haustorial encasements (encased); accumulates at haustoria (+); reduced (-); no difference in localization between uninfected and infected (no difference).

Marker	At uninfected	At infected	*Hpa* haustoria	Nb uninfected	Nb infected	*Pi* haustoria
PIP1;4	Periphery discrete	Periphery discrete	Excluded Encased	Periphery discrete	Periphery diffuse	Excluded -
ACA8	Periphery discrete	Periphery discrete	Excluded Encased	Periphery discrete	Periphery discrete	Excluded
REM1				Periphery discrete	Periphery discrete	Surrounding
SYT1				Periphery discrete	Periphery discrete	Surrounding +
EFR				Periphery discrete	Periphery discrete	Excluded
PEN1	Periphery discrete	Periphery discrete Vesicles	Surrounding EHM Encased	Vesicles discrete		
FLS2	Periphery discrete	Periphery discrete	Surrounding EHM	Periphery discrete	Periphery discrete	Excluded
RPW8.2	Not expressed	Vesicles discrete	Surrounding + Encased			
Rab D2a				Periphery Vesicles diffuse	Periphery Vesicles diffuse	Surrounding
SYP32	Vesicles discrete	Vesicles discrete	Surrounding + Encased	Vesicles discrete	Vesicles discrete	No difference
Got1p	Vesicles discrete	Vesicles discrete	Surrounding Encased	Vesicles discrete	Vesicles discrete	No difference
Rab E1d	Periphery Vesicles discrete	Periphery Vesicles discrete	Surrounding Encased	Vesicles discrete	Vesicles discrete	Surrounding +
VAMP722				Vesicles	Vesicles	Surrounding

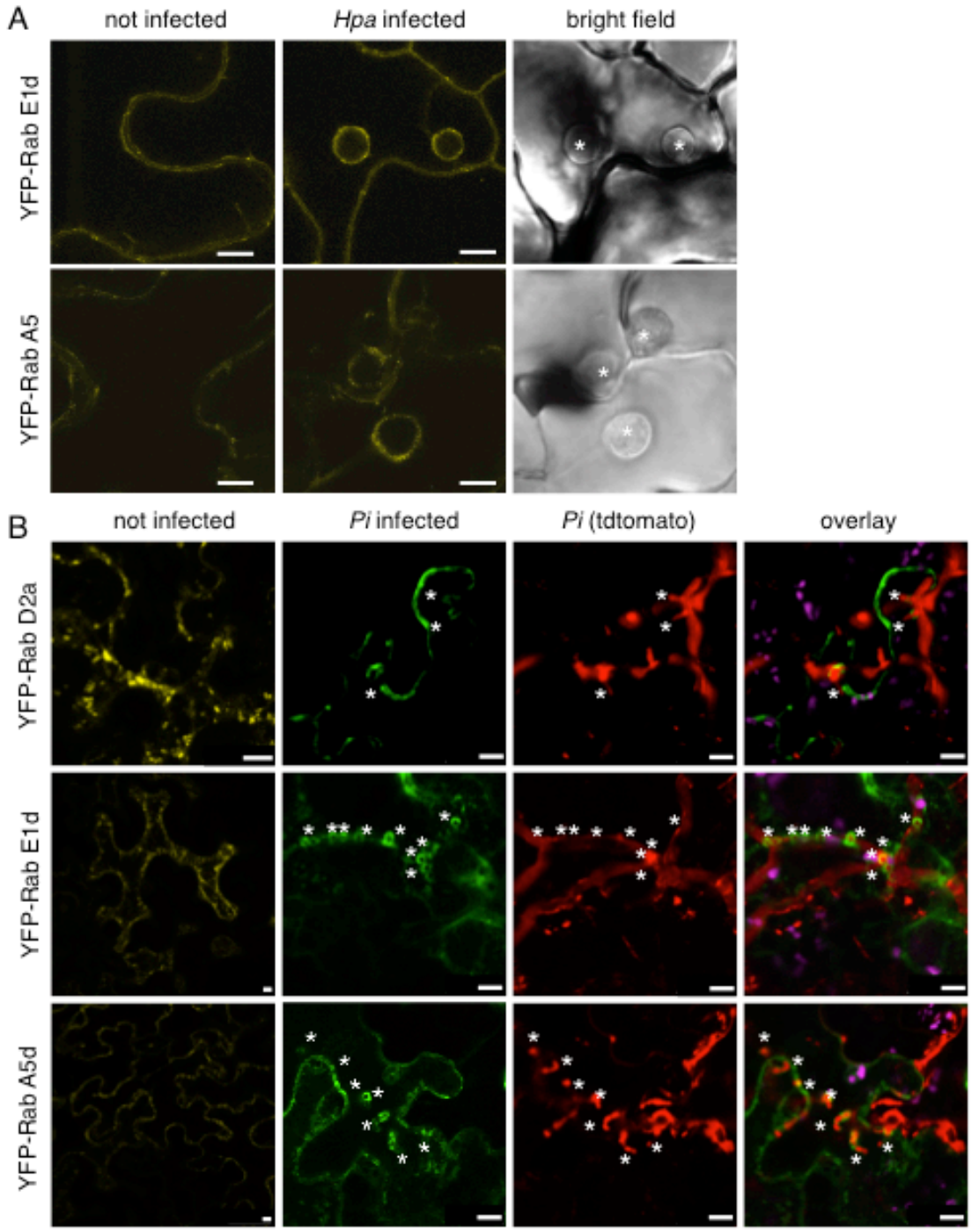
				discrete	discrete	(callosic) +
Rab A5d	Periphery diffuse	Periphery diffuse	Surrounding Encased	Vesicles diffuse	Vesicles diffuse	Surrounding +
VTI12	Vesicles diffuse	Vesicles diffuse	Surrounding Encased	Vesicles diffuse	Vesicles diffuse	No difference
VPS28-2	Vesicles distinct	Vesicles distinct	Surrounding	Periphery Vesicles distinct	Periphery Vesicles distinct	Surrounding
VPS37-1				Periphery Vesicles distinct	Periphery Vesicles distinct	Surrounding
Rab A1e	Periphery Vesicles diffuse	Periphery diffuse	Surrounding Encased	Vesicles/ ER?	Vesicles diffuse	Surrounding +
Rab C1	Periphery Vesicles discrete	Periphery Vesicles discrete	Surrounding Encased			
Rab F2b ARA7	Vesicles discrete	Periphery diffuse	Surrounding Encased			
Rab F2a Rha1				Vesicles diffuse	Vesicles diffuse	Surrounding +
Rab F1 ARA6	Periphery Vesicles discrete	Periphery Vesicles + discrete	Surrounding Encased			
FYVE	Periphery Vesicles discrete	Periphery Vesicles - discrete	Surrounding Encased	Periphery Tonoplast Vesicles discrete	Tonoplast Vesicles discrete	Surrounding
Rab G3f	Periphery discrete	Periphery discrete	Surrounding Encased			
Rab G3c	Periphery diffuse	Periphery diffuse	Surrounding Encased	Tonoplast discrete	Tonoplast Vesicles discrete	Surrounding +
VAMP711	Periphery discrete	Periphery discrete	Surrounding Encased			

Supplementary Figures



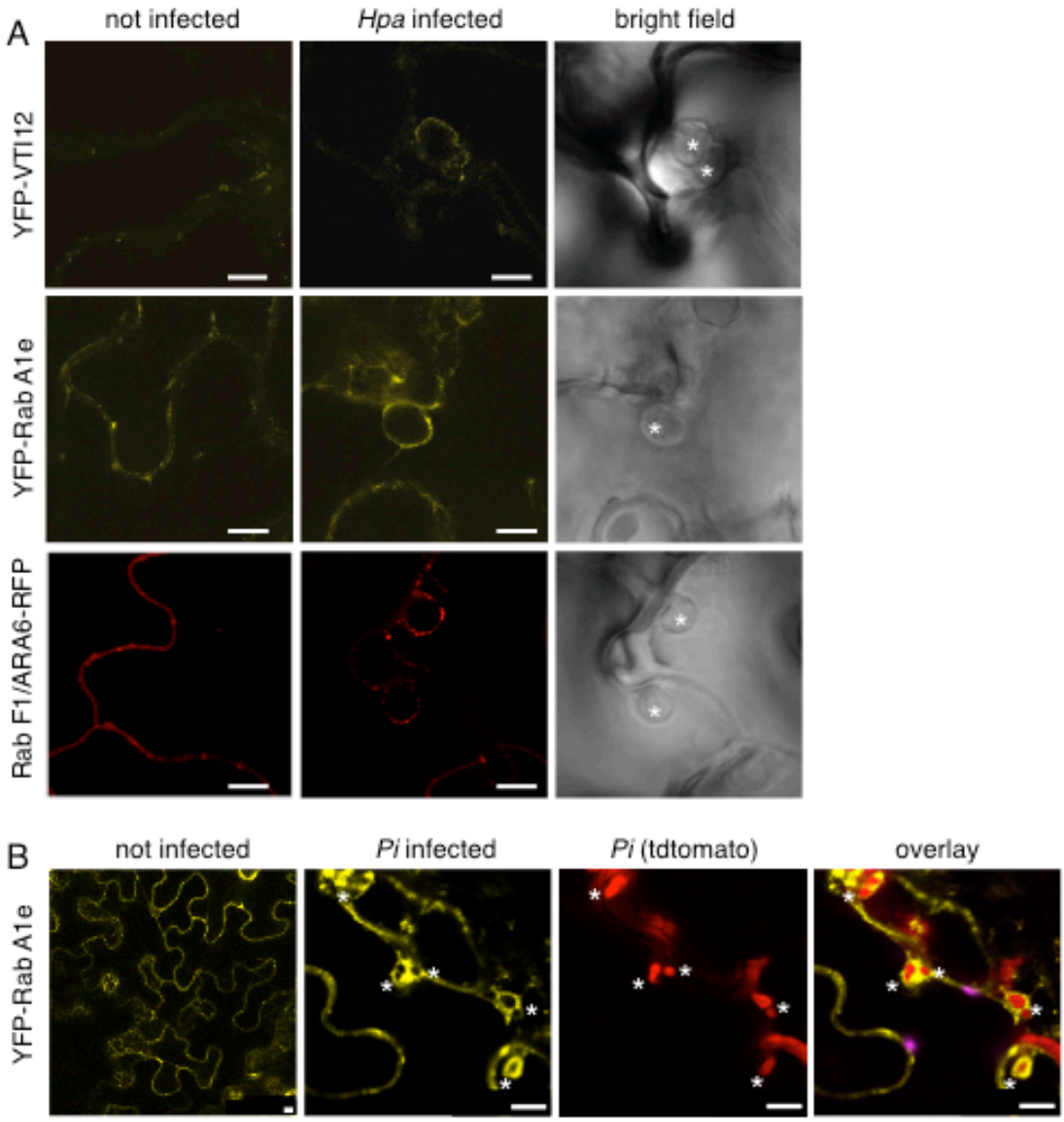
Supplementary Figure 1. Imaging Hpa haustoria. Arabidopsis Col-0 wild type and 35S::GFP transgenic lines were infected with *Hpa* isolate Waco and haustoria were imaged by confocal microscopy. At 3 dpi wild type leaves were incubated with the membrane dye FM4-64, or nuclear stain Hoechst, respectively. Confocal micrographs show cross-sections of not infected and *Hpa* infected leaves. *Hpa* haustoria are shown

in bright field images indicated by asterisks. (A) Membrane FM4-64 and cytosolic 35S::GFP signals are visible at the host cell periphery and around young haustoria. The Hoechst signal is visible in the nucleus in close proximity to the haustorium. (B) FM4-64 membrane and 35S::GFP cytosol labelling shows the double-layered structure of encasements of old *Hpa* haustoria. Bar = 10 µm.

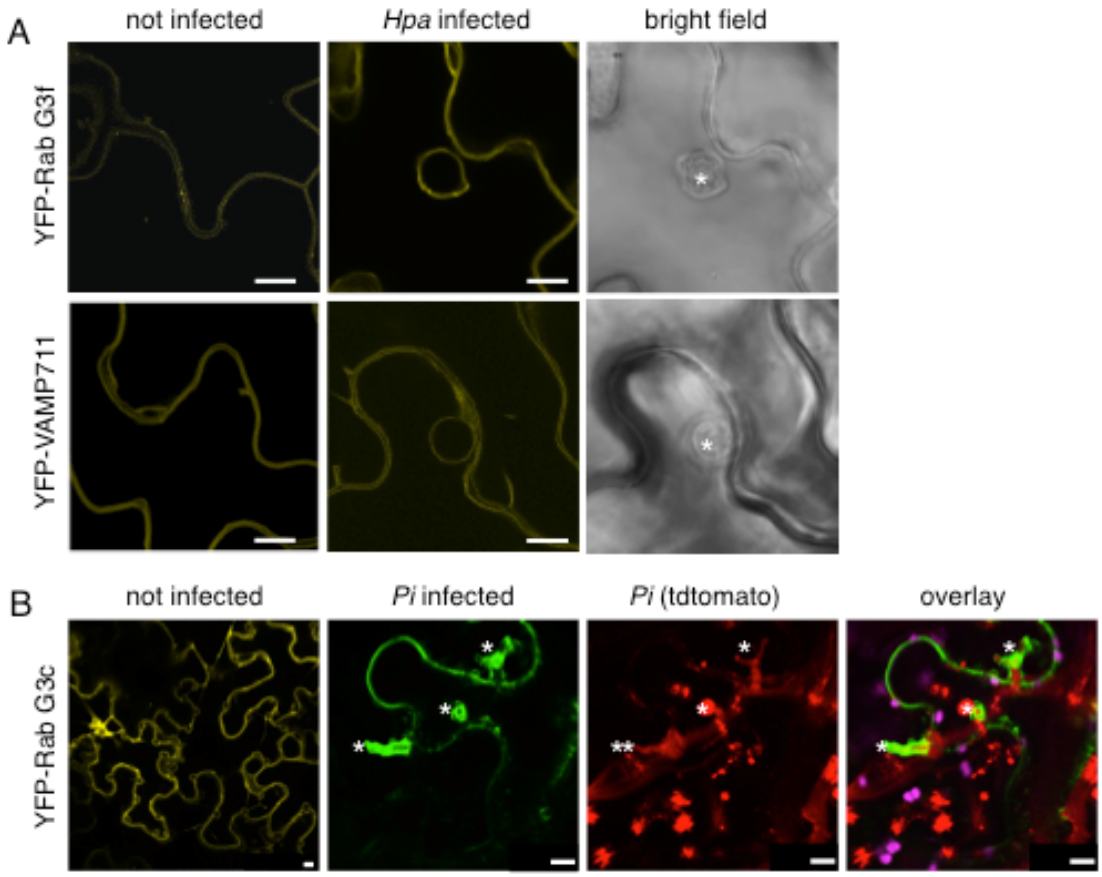


Supplementary Figure 2. Secretory vesicles differentially localize around *Hpa* and *Pi* haustoria. (A) Confocal micrographs of Arabidopsis transgenic lines expressing the indicated fluorophore fusions show cross-sections of not infected and *Hpa*-infected leaves at 3 dpi. *Hpa* haustoria are shown in bright field images indicated by asterisks. YFP-Rab E1d and YFP-Rab A5d signals are detected around *Hpa* haustoria. Bar = 10 µm. (B) Confocal micrographs of *N. benthamiana* leaves transiently expressing the indicated fluorophore fusions show cross-sections of not infected and *Pi*-infected

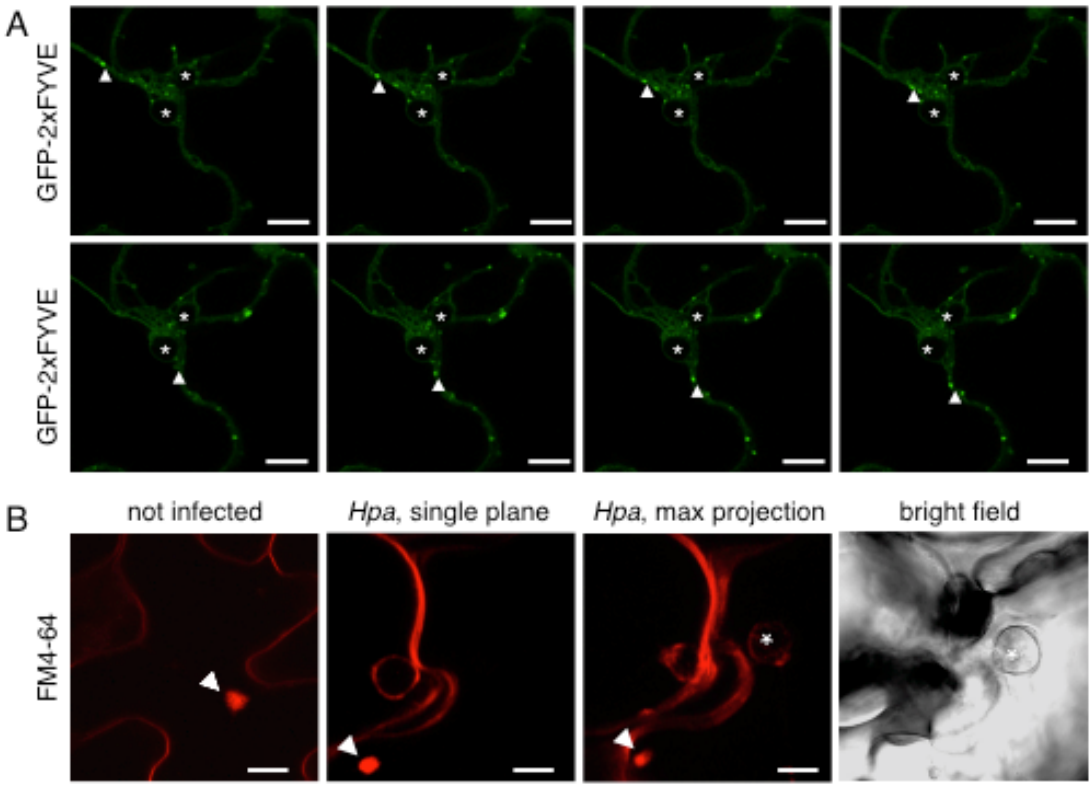
leaves at 3 dpi. *Pi* haustoria are indicated by asterisks. YFP-Rab D2a, YFP-Rab E1d and YFP-Rab A5d are surrounding *Pi* haustoria. Bar = 10 µm.



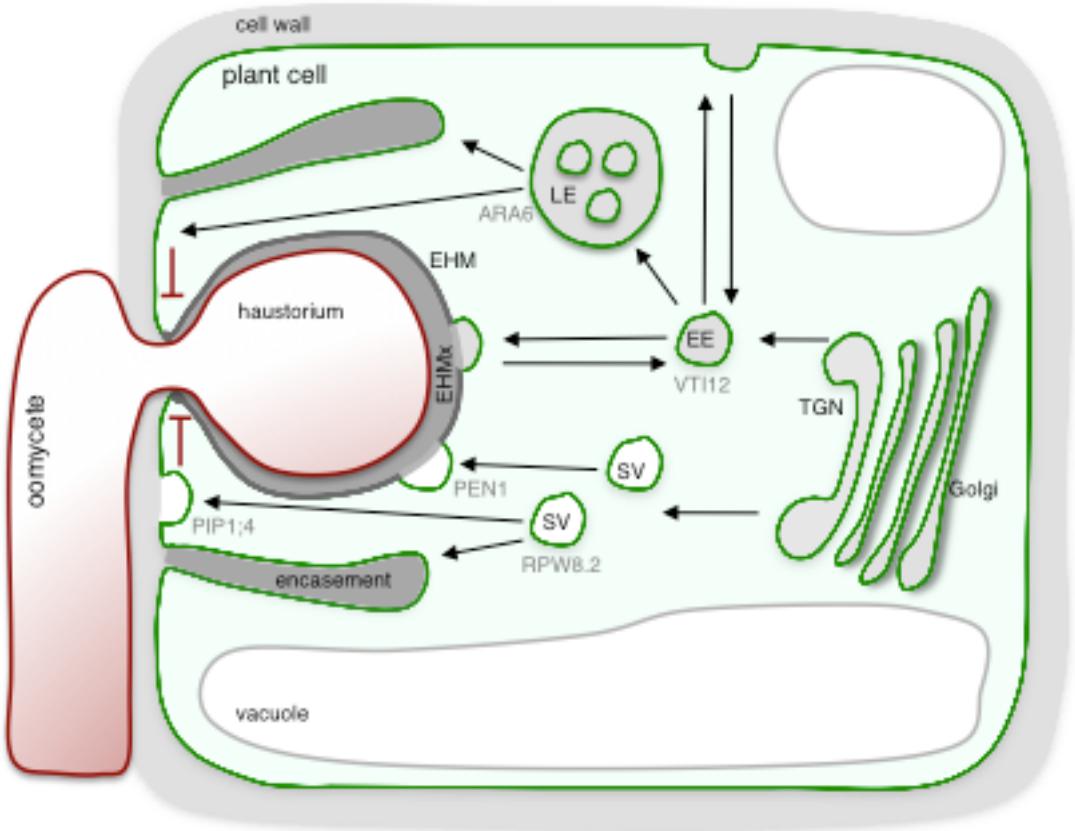
Supplementary Figure 3. Endosomal compartments differentially localize around *Hpa* and *Pi* haustoria. (A) Confocal micrographs of Arabidopsis transgenic lines expressing the indicated fluorophore fusions show cross-sections of not infected and *Hpa*-infected leaves at 3 dpi. *Hpa* haustoria are shown in bright field images indicated by asterisks. YFP-VTI12, YFP-Rab A1e, and ARA6-RFP signals are present at *Hpa* haustoria. Bar = 10 µm. (B) Confocal micrographs of *N. benthamiana* leaves transiently expressing the indicated fluorophore fusions show cross-sections of not infected and *Pi*-infected leaves at 3 dpi. *Pi* haustoria are indicated by asterisks. While YFP-VTI12 does not surround haustoria, YFP-Rab A1e accumulates around *Pi* haustoria. Bar = 10 µm.



Supplementary Figure 4. Proteins labelling the vacuolar tonoplast localize around *Hpa* and *Pi* haustoria. (A) Confocal micrographs show cross-sections of Arabidopsis transgenic leaves expressing the indicated fluorescent-tagged proteins not infected and *Hpa*-infected at 3 dpi. *Hpa* haustoria are shown in bright field images indicated by asterisks. YFP-Rab G3f and YFP-VAMP711 signals are surrounding *Hpa* haustoria. Bar = 10 µm. (B) Confocal micrographs of *N. benthamiana* leaves transiently expressing the indicated fluorophore fusions show cross-sections of not infected and *Pi*-infected leaves at 3 dpi. *Pi* haustoria are indicated by asterisks. YFP-Rab G3c accumulates around *Pi* haustoria. Bar = 10 µm.



Supplementary Figure 5. *Hpa* haustoria-containing cells maintain endosomal trafficking. (A) Arabidopsis L*er* transgenic lines expressing GFP-2xFYVE as a marker for late endosomal compartments were infected with *Hpa* isolate Cala 2 and haustoria were imaged at 3 dpi. Time-lapse imaging by confocal microscopy shows cross-sections of *Hpa* infected leaves over 815 ms. *Hpa* haustoria are indicated by asterisks. The GFP-2xFYVE signal is visible at the host cell periphery, some emerging plasma membrane strands and at the EHM of *Hpa* haustoria. The arrows indicate vesicle movement towards the haustorial site (upper panel) and away from the haustorium (lower panel). Bar = 10 µM. (B) Arabidopsis Col-0 plants were infected with *Hpa* isolate Waco 9, and at 3 dpi leaves were treated with BFA and stained with FM4-64. Confocal micrographs show cross-sections of not infected and *Hpa*-infected leaves. *Hpa* haustoria are shown in bright field images indicated by asterisks. The FM4-64 signal is visible at the plant cell plasma membrane, at BFA bodies indicated by arrows, and at the *Hpa* EHM. The BFA body is detected in a different focal plane of the haustoria-containing cell shown by single plane and maximal projection. Bar = 10 µm.



Supplementary Figure 6. Schematic representation of membrane trafficking in oomycete infected plant cells. Secretory vesicles deliver proteins such as PIP1;4 to the plasma membrane, but their lateral diffusion and incorporation into the extrahaustorial membrane must be prevented by the pathogen (EHM; red lines). Secretory vesicles may also deliver proteins like PEN1 to the EHM. Early endosomes (EE) may interact with the EHM and regulate presence of proteins at the EHM via recycling trafficking. Late endosomes (LE)/multivesicular bodies may contribute to enveloping the haustorium but proteins are sorted at the haustorial neck. As a result of recruitment of default membrane trafficking pathways, secretory vesicles, early and late endosomes contribute in the biogenesis of the encasement. TGN: *trans*-Golgi network; EHMx: extrahaustorial matrix; black arrows: proposed membrane trafficking pathways; molecular markers are highlighted in grey.

Supplementary References

Frei dit Frey, N, Mbengue, M., Kwaaitaal, M., Nitsch, L., Altenbach, D., Häweker, H., Lozano-Duran, R., Njo, M.F., Beeckman, T., Hüttel, B., Borst, J.W., Panstruga, R., Robatzek, S. (2011) A plasma membrane calcium ATPase functions in FLS2-mediated immunity. Submitted.

Geldner, N., Dénervaud-Tendon, V., Hyman, D.L., Mayer, U., Stierhof, Y.D., and Chory, J. (2009) Rapid, combinatorial analysis of membrane compartments in intact plants with a multicolor marker set. *Plant J* **59**: 169-178.

Göhre, V., Spallek, T., Häweker, H., Mersmann, S., Mentzel, T., Boller, T., *et al.* (2008) Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. *Curr Biol* **18**: 1824-1832.

Häweker, H., Rips, S., Koiwa, H., Salomon, S., Saijo, Y., Chinchilla, D., *et al.* (2010) Pattern recognition receptors require N-glycosylation to mediate plant immunity. *J Biol Chem* **285**: 4629-4636.

Lefebvre, B., Timmers, T., Mbengue, M., Moreau, S., Hervé, C., Tóth, K., *et al.* (2010) A remorin protein interacts with symbiotic receptors and regulates bacterial infection. *Proc Natl Acad Sci USA* **107**: 2343-2348.

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M. *et al.* (2005) Pre- and postinvasion defenses both contribute to nonhost resistance in Arabidopsis. *Science* **310**: 1180-1183.

Meyer, D., Pajonk, S., Micali, C., O'Connell, R., Schulze-Lefert, P. (2009) Extracellular transport and integration of plant secretory proteins into pathogen-induced cell wall compartments. *Plant J* **57**: 986-999.

Schapire, A.L., Voigt, B., Jasik, J., Rosado, A., Lopez-Cobollo, R., Menzel, D., *et al.* (2008) Arabidopsis synaptotagmin 1 is required for the maintenance of plasma membrane integrity and cell viability. *Plant Cell* **20**: 3374-3388.

Vermeer, J.E., van Leeuwen, W., Tobeña-Santamaria, R., Laxalt, A.M., Jones, D.R., Divecha, N., *et al.* (2006) Visualization of PtdIns3P dynamics in living plant cells. *Plant J* **47**: 687-700.

Wang, W., Wen, Y., Berkey, R., and Xiao, S. (2009) Specific targeting of the Arabidopsis resistance protein RPW8.2 to the interfacial membrane encasing the fungal Haustorium renders broad-spectrum resistance to powdery mildew. *Plant Cell* **21**: 2898-2913.