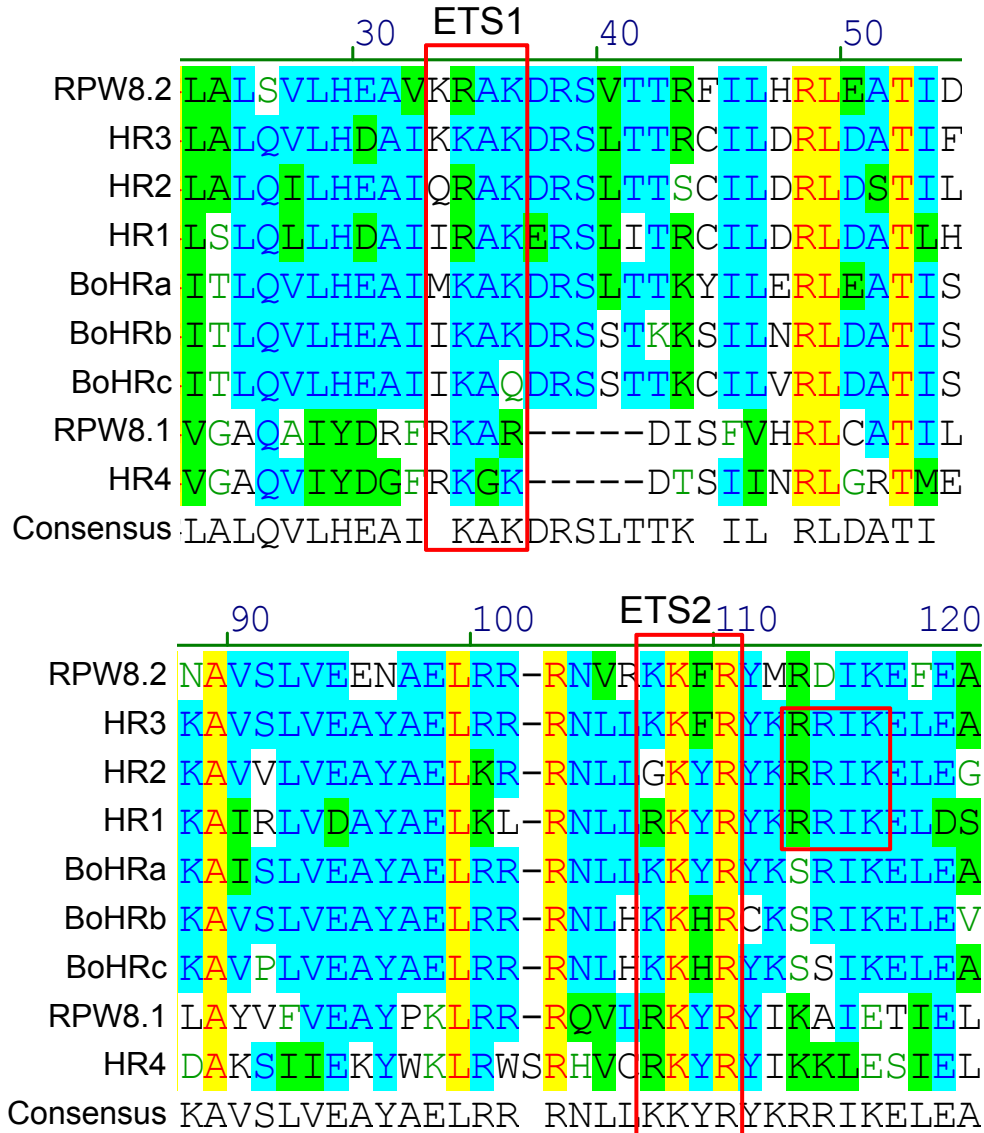
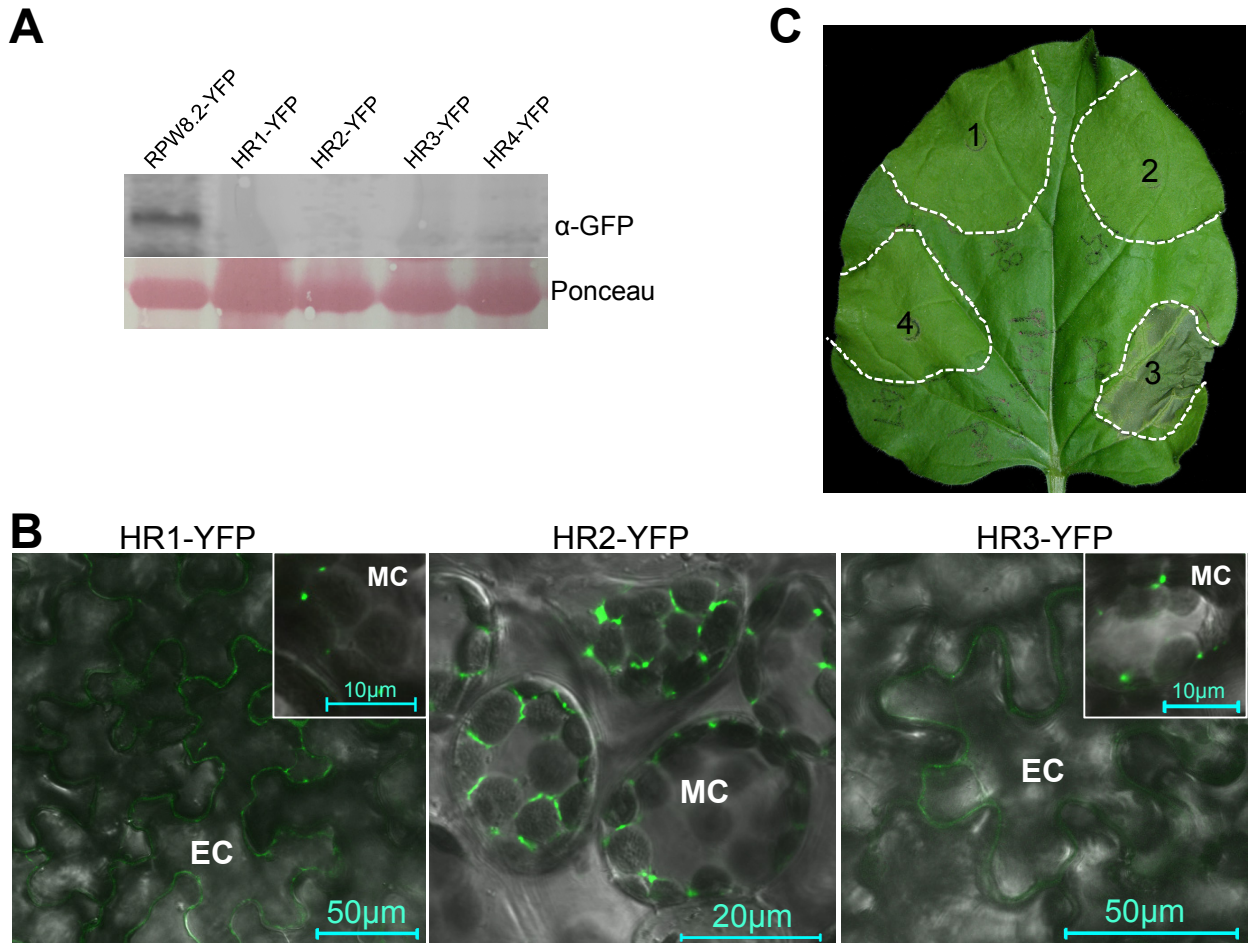


**Supplemental Fig. S1.** Schematic gene structures and location and direction of the T-DNA insertions (arrowheads) in *HR1*, *HR2* and *HR3*. *HR1-kd1*: SALK\_056764 (+<sup>778</sup>ACTCAA..◀..CAAATG<sup>+789</sup>); *HR2-ko1*: SALK\_093095 (+<sup>582</sup>TATGGC..▶..CAAGAT<sup>+593</sup>); *HR3-ko1*: SALK\_122954 (+<sup>972</sup>AAGAAA..▶..ACGGAA<sup>+983</sup>); *HR3-ko2*: WiscDsLox420C08 (+<sup>361</sup>AATTTA..▶..AATAGTT<sup>+372</sup>).



**Supplemental Fig. S2.** Protein sequence alignment of the RPW8 family. Six family members (RPW8.1, RPW8.2, HR1, HR2, HR3, and HR4) from *Arabidopsis thaliana* and three family members (BoHRa, BoHRb and BoHRc) from *Brassica oleracea* were aligned. The two core EHM-targeting signals (ETSs) R/K-R/K-X-R/K identified in Wang et al., 2013 were highlighted in red boxes. Note that the ETS2 is more conserved than the ETS1 and there is a third R/K-R/K-X-R/K motif downstream of ETS2 in HR1, HR2 and HR3.



**Supplemental Fig. S3.** Expression of HR1-YFP, HR2-YFP and HR3-YFP is detectable in *N. benthamiana* when transiently co-expressed with a viral RNAi suppressor. **A.** A Western blotting showing that HR1-YFP, HR2-YFP, HR3-YFP and HR4-YFP was not detectable in T4 Arabidopsis lines transgenic for each of the DNA constructs under control of their respective native promoter (NP). An Arabidopsis line expressing RPW8.2-YFP was used as control. An anti-GFP antibody (Abcam, A290) capable of recognizing YFP was used to detect the YFP fusion proteins. Equal loading of the microsomal fraction was shown by Ponceau staining of the gel. **B.** Confocal images showing expression and subcellular localization of the indicated fusion proteins in epidermal cells (EP) and/or mesophyll cells (MC) of *N. benthamiana*. Agrobacterial cells harboring either *NP::HR1-YFP*, *NP::HR2-YFP*, *NP::HR3-YFP* or *NP::HR4-YFP* were mixed in equal amount with Agrobacterial cells containing a DNA construct for expressing the *Turnip crinkle virus* (TCV) coat protein. The Agrobacterial suspension mixture ( $OD_{600}=0.5$ ) was infiltrated to leaves of *N. benthamiana*. Infiltrated leaf sections were subjected to confocal imaging at 48 hrs after infiltration. Note HR4-YFP was not detectable and is not shown. **C.** A picture a *N. benthamiana* leaf infiltrated with Agrobacterial cell mixtures harboring the TCV coat protein construct and either *NP::HR1-YFP* (1), *NP::HR2-YFP* (2), *NP::HR3-YFP* (3) or *NP::HR4-YFP* (4). The picture was taken at 72 hrs after infiltration.



**Supplemental Fig. S4.** HR3 is not required for RPW8.1 and RPW8.2-mediated resistance. To test if loss of HR3 affects RPW8.1 and RPW8.2-mediated resistance, the *HR3-ko1* alleles was cross into S5, a *Col-gI* line transgenic for a single copy of a genomic fragment containing *RPW8.1* and *RPW8.2* with their native promoters. Plants of the indicated genotypes were inoculated with *Gc UCSC1* and pictures were taken at 10 dpi.

**Supplemental Table 1. Information about the DNA constructs used in this study**

Name of construct	Gene name (ID)	Purpose	Primers (5' to 3') for amplification	Vector	Cloning Method
HR1-YFP	HR1 (At3g50450)	localization	cgggatccGAGCGGTTTGAGCATCTTGTT cgggatccGAAGATGAACCGGATCTCGTGCT	pPZPYFP23'	BamHI digestion
HR2-YFP	HR2 (At3g50460)	localization	cgggatccGCATCTCAATGGACTTTAACAACACCA cgggatccAAAACGAAGCGAATCCCGTGAT	pPZPYFP23'	BamHI digestion
HR3-YFP	HR3 (At3g50470)	localization	cgggatccGCTAAAGAGCTAATCTCTTGACCCA cgggatccCTTCAAGACAAATCGGATCTCACGGT	pPZPYFP23'	BamHI digestion
HR4-YFP	HR4 (At3g50480)	localization	cgggatccCATTCGTTGGAGTTGTGTAATGAGGA cgggatccGCCTTCATTTCTTTGATATCGGTCCA	pPZPYFP23'	BamHI digestion
R82p-HR3-YFP	HR3 (At3g50470)	localization	cgggatccGCTAAAGAGCTAATCTCTTGACCCA gcagatctCACTGTACAGCTCGTCCATG	pCX-DG-82p	T/A cloning
35S-YFP-HR1	HR1 (At3g50450)	localization	caccATGCCTCTTGTGAGCTTCTTACAA cgggatccTCATTTGAAGATGAACCGGATCTCGT	pEG104	Gateway cloning
35S-YFP-HR2	HR2 (At3g50460)	localization	caccATGCCCTTACCGAGATTATCGCA cgggatccCTAATTCAAAACGAAGCGAATCCCGT	pEG104	Gateway cloning
35S-YFP-HR3	HR3 (At3g50470)	localization	caccATGCCGGTTAGTGAGATTATG cgggatccTCACTTCAAGACAAATCGGATCTCA	pEG104	Gateway cloning
35S-YFP-HR4	HR4 (At3g50480)	localization	caccATGCCGATGCTGAGCTTGCT cgggatccTCATTTATATGCTTTTCAGATATCTGGG	pEG104	Gateway cloning
NP-HR3 <sup>D116G</sup>	HR3 (At3g50470)	defense function	TCATTAAGATGGATGGTAGGTGGGATGTCCAAGTCAAT ATTGACTTGGACATCCACACCTACCATCTTAATGA	pPZPYFP23'	BamHI digestion
NP-HR3 <sup>AC165</sup>	HR3 (At3g50470)	defense function	cgggatccAAAACGAAGCGAATCCCGTGAT cgggatccTCATGTTGACGAGTGATTTCATCAAGT	pPZPYFP23'	BamHI digestion
NP-HR3- <sup>AC126/G186D</sup>	HR3 (At3g50470)	defense function	cgggatccAAAACGAAGCGAATCCCGTGAT cgggatccAGAGTCATCACTCGAACATCAACA	pPZPYFP23'	BamHI digestion
R82p-HR3- <sup>AC165</sup>	HR3	expression	caggatccATGCCGGTTAGTGAGATTATGGCA cgggatccTCATGTTGACGAGTGATTTCATCAAGT	pCX-DG-82p	T/A cloning
HR321-1miR		silencing HR1/2/3	gaTGTTTAGTGTTCATATCGGACtctctctttgtattcc	pBTEX	<i>Kpn I/Bam HI</i>
HR321-2miR		silencing HR1/2/3	gaGTCCGATATGAACACTAAACAtcaaaagagaatcaatga	pBTEX	<i>Kpn I/Bam HI</i>
HR321-3miR		silencing HR1/2/3	gaGTACGATATGAACCTAAACtccacaggtcgtgatag	pBTEX	<i>Kpn I/Bam HI</i>
HR321-4miR		silencing HR1/2/3	gaAGTTTAGAGTTCATATCGTACTctacatatattctct	pBTEX	<i>Kpn I/Bam HI</i>
R82p-HR3g	HR3	expression	caggatccATGCCGGTTAGTGAGATTATGGCA cgggatccTCACTTCAAGACAAATCGGATCTCACGGT	pCX-DG-82p	T/A cloning
HR3n	HR3(At3g50470)	HR3 Nt domain	cgggatccGCTAAAGAGCTAATCTCTTGACCCA caccatggTTGACGAGTGATTTCATCAAGTTTAGTGT	pPZPYFP23'	BamHI digestion
HR3c	HR3(At3g50470)	HR3 Ct domain	caccatggCCAAGTATGTTATTTGTTTCAAGAGCA cgggatccTCACTTCAAGACAAATCGGATCTCA	pPZPYFP23'	BamHI digestion
R82n	HR3(At3g50470)	RPW8.2 Nt domain	ttggatccTCACCGAAATGTTAGTATTACAG caccatggCATTATTTTGTCAAGTTTAGTGTGATT	pPZPYFP23'	BamHI digestion
R82c	HR3(At3g50470)	RPW8.2 Ct domain	caccatggCCTCAACCGAAGTTTGAATCCA ttggatccTCAAGAATCATCACTGCAGAAC	pPZPYFP23'	BamHI digestion
HR3n-R82c-YFP	HR1(At3g )	localization & func	cgggatccGCTAAAGAGCTAATCTCTTGACCCA gcagatctCACTGTACAGCTCGTCCATG	pPZPYFP23'	BamHI digestion
R82n-HR3c-YFP	HR1(At3g )	localization & func	ttggatccTCACCGAAATGTTAGTATTACAG gcagatctCACTGTACAGCTCGTCCATG	pPZPYFP23'	BamHI digestion
HR3n-R82c-YFP	HR1(At3g )	localization & func	cgggatccGCTAAAGAGCTAATCTCTTGACCCA gcagatctCACTGTACAGCTCGTCCATG	pCX-DG-82p	T/A cloning
R82n-HR3c-YFP	HR1(At3g )	localization & func	ttggatccTCACCGAAATGTTAGTATTACAG gcagatctCACTGTACAGCTCGTCCATG	pCX-DG-82p	T/A cloning