

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 (⁺⁷⁷⁸ACTCAA.. ◀..CAAATG⁺⁷⁸⁹); *HR2-ko1*: SALK_093095 (⁺⁵⁸²TATGGC.. ►..CAAGAT⁺⁵⁹³); *HR3-ko1*: SALK_122954 (⁺⁹⁷²AAGAAA.. ►..ACGGAA⁺⁹⁸³); *HR3-ko2*: WiscDsLox420C08 (⁺³⁶¹AATTTA.. ► ..AATAGTT⁺³⁷²).



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 (OD₆₀₀=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.

HR3-ko1/S5



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-*gl* 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.

S5

Name of construct Gene name (ID) Purpose Primers (5' to3') for amplification Vector Cloning Method HR1-YFP HR1 (At3g50450) localization geggatecGAXGCGGTTGAAGCACCGATCTGGTG pZPYFP23' BamHI digestion HR2-YFP HR2 (At3g50460) localization geggatecGAAGATGAACCGAATTCCGTGAT pZPYFP23' BamHI digestion HR3-YFP HR3 (At3g50470) localization geggatecGCTAAAGAGCAAATTCGGTGTAATGAAGCAC pZPYFP23' BamHI digestion geggatecGCTTCAATGAACAACCAAACGAAGCGAATTCCGTGAC pZPYFP23' BamHI digestion geggatecGCTTCAATGAAATTCGGTCCA pZPYFP23' BamHI digestion geggatecGCTTCAATGAAAGAGCTAATTCTGTGACAGT geggatecGCATAAGAGCTAATTCGTGACCA pZPYFP23' BamHI digestion geggatecGCTTCAATGAAAGAGCTAATTCTGTGACCAGT geggatecGCATAAGAGCTAATTCGTTGACCCA pZA'A cloning geggatecGCATAAGAGCTAATTCGGTCCA pZA'A cloning geggatecGCATATTCAAGAGCTCATTCTGACCCA pZA'A cloning geggatecGCATATTCACGACTCTTGACCCA pCA'A cloning geggatecGCATATTCACGACTCTTACCGA pCA'A cloning geggatecTCATTGAAGACTCATTCGG geggatecTCATTGAAGACTCATTCGG geggatecTCATTGAAGACTCATTCGGA gegatecGCATTCAGGATTATCGCG geggatecTCATTTCAAAACGAAAGCGAATTCCGGA	Supplemental Table 1. Information about the DNA constructs used in this study						
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HR321-IMIK SIERCING HR1/2/3 galGTITAGIGTICATATCGGACtetetettingattee PBTEX <i>Kpn UBam</i> HI	UD221_1;D		silensing UD1/2/2		- DTEV		
LID 221 2miD ciloncing HD1/2/2 CTCCCATATCAACACTAAACAA	HK321-IMIK		silencing HR1/2/3		PBIEA	Kpn I/Bam HI	
Intszi-zink Silenting mk12/5 gas [CCGATAIGAACACTAAACA(caaagagaacaatga pBTEA Kpr // Dam mi UB201_2miD cilopting mk12/5 gas [CCGATAIGAACA(TAAACA(caaagagaacaatga pBTEA Kpr // Dam mi	HK321-2IIIK		silencing HR1/2/3	gaGTCCGATATGAACACTAAACAtcaaagagaatcaatga	PDIEA PDTEV	Kpn I/Dam HI	
http://www.miterious.com/miterio	HR321-5IIIR HR321-4miP		silencing HP1/2/3		PDIEA	Kpn I/Dam HI	
HK521-4HIIK SHERICING HK12/5 gazdri HAGAGH CATALCOTAC Catacatatatatect pBTEA Kpr // Badmini			silencing HK1/2/5		PETEA	T/A alamina	
R82p-fik3g fik3 expression cagatactartocontractoractartococa pcx-bg-s2p f/A cloning	кө2р-пкэд	пкэ	expression		pCA-DG-02p	1/A cioning	
LIP2 n LIP2(A+2)(EQ470) LIP2 NF domainCCTAAGCAACCGAATCGGATCTCACGA_ nPC7DVED22' DamUL digortion	HD2n		HD2 Nt domain		pD7DVED22/	BamHI digastion	
nKSII nKS(At3g50470) HKS Nt domain gegateGet AAGAGCTAATCA TTO A COTTACTO	пкзн	HK3(AL3g50470)			przr i rr 25		
LP2c HP2(At2gE0470) HP2 Ct domain constantCC AACTCATTCTATTCTATTCATCAACGA pP7DVED22' PamH digestion	HP2c		HP2 Ct domain		pD7DVED22/		
TINSC TINS(A(5))0470/ TINS CLOUTIANT Calculation and the transfer and the	TINSC	TIK5(AL5g50470)			przr i rr 25	Barrin uigestion	
P82n HP2(At3o50470) PDW8 2 Nt domain transfor CACCGAAATGATCACG nP7DVED23' BamHi direction	R82n	HR3(A+3a50470)	RPW/8 2 Nt domai	transtanTCACCGAAATTGTTAGTATTCACG	nD7DVFD22	BamHI digestion	
No211 InS(A(5250470) AV W0.2 At domain degrating CAGTATAGTAGTAGTAGTAGTAGT	10211	111(3(At3g30470)	Kr Wo.2 Nt domai		prz. 11123	barrin digestion	
P82c HP2(A+2g50470) PDW8 2 Ct domain appointerCCTCAACCGAACTTCAAACCGA pD7DVED23' BamHi direction	R87c	HR3(A+3a50470)	RPW/8 2 Ct domain		nD7DVFD22	BamHI digestion	
	Noze	111(3(At3g30470)	Kr W8.2 Ct domai		prz. 11123	barrin digestion	
HB3n_B82c_VEP HB1(At3g_) localization & functional contract of Decoder and Contract of Decoder and Decoder	HR3n_R82c_VEP	HR1(A+3a)	localization & fun		nP7PVFP23'	BamHI digestion	
Institute of the second static	11107-1020-111	TINI(AUSg)		geggatetet CACTTGTACAGCTCGTCCATG	przi 11125	barrin digestion	
R82n-HR3c-YEP HB1(At3g) localization & functionation CCCCAAATTGTAGCATTCACG nPZPVEP23' BamHI direction	R82n-HR3c-VFP	HR1(At3g)	localization & fun	ttggatecTCACCGAAATTGTTAGTATTCACG	nPZPYFP23'	BamHI digestion	
acagatetCACTTGTACAGCTCGTCCATG	102n-11030-11-1	·····(///.5g /		acagastetCACTTGTACAGCTCGTCCATG	pr 21 111 25	Samin algestion	
$\frac{g_{agal}(CACHORACOCCAGO}{HR3n_R82c_YEP} = HR1(At3g) = \frac{g_{agal}(CACHORACOCCAACCCAACCCCAGO}{HR3n_R82c_YEP} = \frac{HR1(At3g)}{HR3n_R82c_YEP} = \frac{HR1(At3g)}{$	HR3n_R82c_VEP	HR1(At3g)	localization & fun		pCX_DG_{2n}	T/A cloning	
maining person a function of the gegateter Action and the formation of th	111.51-1.020-111	·····(///.5g /		geagatetCACTTGTACAGCTCGTCCATG	μελ-μα-02μ	1/A CIONINg	
$R82n_HR3c_YEP$ HB1(At3g) localization & functional transfer TCACCGAAATTGTTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTA	R82n_HR3c_YEP	HR1(At3g)	localization & fun	ttggatecTCACCGAAATTGTTAGTATTCACG	pCX-DG-82n	T/A cloning	
gcagatetCACTIGTACAGCTCCATG	1020-1100-111			gcagatetCACTTGTACAGCTCGTCCATG	pen-bG-02p	1/1 Croning	