

Supplementary Materials: Ribosome Inactivating Proteins from Rosaceae

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Type 1 RIPs

1. *Malus domestica* (MDP0000918923)

MALSFSIKNATTTYRTFIELRQLTAGGSTSHGIPVLRRQDVKDDQRFVLVNLTNYDSYTITVA
IDVVNAYVVGYCAGTRSYFLRDPATHPPPLHRLFPGBTTRTLPFAGDYLGLGRAAQEALQQNTNRNR
AAGSRIHENISMREIPLGPGEVDNAISQLRYAESASSQAAAFIVIIQIVSEAARFRYIQGQVRDRI
RDGTSAEPDPAMLSLENSWSNLSEQIQMV PANQLLFINNGSVQIRKADNSIVLVKSVDSDAVRGAF
LLYCGGNPPAPNSESARTSKVTVKPTLAKKKK

2. *Malus domestica* (MDP0000223290)

MSIPFTLIDATPDSYSRFIDQLRARLTGTTSQGIRVLPPSRQVGNNARFIYVDLTNYDGVTVTIGI
DVVNAYVMGYEQGEQNYPLQTLPPDDPAPVELLPNTRSAGELPFTGHYASLGHEYARRMQNEQPNRRD
QOALNRLSNPMRQNIGLGPSSLHSAIDMLERAATPLSQAGAILVIIQMVSEAARYPYIERQVRESIQ
TGNSFLPDPRMLSLENNWSNLSRQIMGATRAGRESFSTSVSLLDAYQSHGAPPLVVNSVRDSFIQDM
EIALLLHDRGDDRGTDQGNDPENCTPGPSGSGIGRRGXKKPQQHE

3. *Malus domestica* (MDP0000134012)

MSISFTLIGATPDSYSTFINQLRDRLTGTTSQGIPVLPPSRQVGNNDRFIYVNLTNYDGVTVTIGI
DVVNAYVMGYEQGGQNYLLGTLPEAATVFPNTRAAGELPFRADYGSLQYARGMPNEQPNRRDQQ
SVNRLRNDMRENIALGPSSLHWAIHMLVHAATSSQASAIIVIIQMVSEAARYPYIERVRESIQTAN
SFIPDPRMLTLENHWSTLSRQIMEATRAGRESFSTSVSLLDAYQSHGAPPLVVNSVRDSFVQDMEIA
LLLHDRGDDRGTDQGNDPENCTPGPSVSGRGGKPHDEL

4. *Prunus mume* (XP_016652174.1)

MALSFSTKNTNPQKYRDFIESLRQRLTAGRPKSHDI PVLPRREDVPDAQLFLLDLTNSGNNTIRLA
IDVVNAYVVGYAAGGRSYFLKENARDNPPPPIHTLFRDTTRMPPLDFDGTYTGLSRAAQEAVKRNIAR
DRARNPAVAGLHKDTPILERIPLGRNELDDAINLLSLAPSQSDQAI GFIVVIQMCEAARFRFIEGL
LRNSMKDVYDPTIPGPATRSLETHWSDLSEEIQRVPANQTQFQKAVV LHNIRNERVEVRSDVSDVVR
GVAMLLYDQNQNANPGPSAKPPLLKNQKPHIGKPTK

5. *Prunus mume* (XP_016652175.1)

MILSFSTKNATPETYRGFIQALRDQLTAGRPTSHGIPVLPRREDVPDAQRFLVLDLTNSQGNTIRLA
IDVVNAYVVGYAADGRAYLLQENARDNRPPIHTLFREATTRIDLGFDSYSGLSRVAREAVERNTPR
NRARNRAGASAHDNTPVLEQIPMGRNELDTAISLLRSASSPTNQALGFIVVIQMLSEAARFRAIEGL
VRTTMRETYDPLMRGIAMESLETHWSDLSEEIQRVPANQTQFQKAVV LHNIRNERVEVRSDVSDVVR
RGVAMLLYDRNGNCNPFGSPHRHDEL

6. *Prunus mume* (XP_008243880.1)

MALVFSTRNATPQTYRTFIDALRLRTAGRPTSHGIPVLPRKEDVQNAQRFLVLDLTNSENNNTITVA
IDVVNAYVVGYAAGGRSYFLAENAPDDKPPIHVLFPGBTTRVPTLRFNGTYSGLTRGAVEAVRRRAG
NRDPNIDEKTPVLEQIFLGRNQLDEAIRLLRSAVSQPEQALGFVVIQMLSEAARFRQKL RDWSALP

7. *Prunus mume* (XP_008243881)

MALSFSTKNTNPQKYRDFIESLRQRLTAGRPKSHGIPVLPRREDVPDAQRFLVLDLTNSGNNTIRLA
IDVVNAYVVGYAAGGRSYFLKENARDNPPPPIHTLFRDTTRMPPLDFDGTYTGLSRAAQEAVKRNIAR
DRARNPAVAGLHKDTPILERIPLGRNELDDAINLLSLAPSQSDQAVGFIVVIQMCEAARFRFIEGL
LRNSMKDVYDPII PGLAIRSLETHWSDLSEEIQRVPANQTQFQKAVV LHNIRNERVEVRSDVSDVVR
GVAMLLYDRNQNANPGPSAKPPLLKNQKPHIGKPTK

8. *Prunus persica* (ppa009409mg)

MILSFSTKNATPETYRDFIQALRDLTAGRPTSGIPVLPRREDVPDAQRFLVLDLTSQGNTIRLA
IDVVNAYVVGYAADGRAYLLQENARDNRPIHTLFRDATTRIDLGFDGSYGLSRVAREAVERNTPR
NRARNRAGASAHDNTPVLEQIPMGRNELDTAISLLRSASSPTNQALGFIVIIQMLSEAARFRAIEGL
VRTTMRETYDPLMRGLAMESLETHWSDLSEQIQRAQQRNETGFDRTIVLHNVGNERREVNSVDSPFV
RGVAMLLYDRNGNCNPNGSGPHRHDEL

9. *Prunus persica* (ppa009637mg)

MELSFSTKNTTPQKYRDFIESLRQRLTAGRSKSHGIPVLPRREDVPDAQRFLVLDLTSQGNTIRLA
IDVVNAYVVGYAAGGRSYFLKENARENPPPIHTLFRDTRMPPLDFDGSYTGLSRAAQEAVKRNIA
DRARNPNAVGLHPDTPILERIPLGRNELDDAINLLRAPSQSDQAVGFIVVIQMVCEAASMKDVYDP
TIPGPATRSLENHWDISKEIQRVPANQTQFKAVVLHNINKERVEVRSVDSVVGVAMLLYDRNQ
NANPGPSAKKPLLKN

10. *Pyrus bretschneideri* (XP_009374990.1)

MALALSLLNATPKTYTAFIEALRDLTAGRPTSHGIAVLPRREDVPDAQRFLVLDLTSQGNTIRVA
IDVVNVYVVGYRSGNKSYILANNAENPAPTHILFPTAPGAGQSTRMLPFTGDYPALGAYARRTAQP
SASGARNPGSRIHEDIPMLEIPLGRNELDNAITKLHYAASHSDQAAAFIVIIQMVSEAARYRIES
QVGNRMGIDNRPYIPDPAMRSLETNWSALSEQIQKVPANGNRFNRPQLTTVNNRPLEVNSVEADM
QRGGIAMLLYAR

11. *Pyrus bretschneideri* (XP_009375039.1)

MALALSLLKATPKTYTAFIEALRARIKDVPAQRFLVLDLTSQGNTIRVA
IDVVNVYVVGYRSGNKSYILANDAKKPAPTHILFPTALGATQSTRTVLPFTGDYPELGPHARRTAQS
SASGAPGSRIHENIPMLEQIPLGRNELDNAISKLHYAASHSDQAAAFIVIIQMVSEAARFRYIES
GTRMGIDNPPYIPDPAMRSLENEWSALSEQIQVNVPANGNRFSRSIQLTTVNYRPLVVDSVEADM
RGIVMLLNAS

12. *Pyrus bretschneideri* (XP_009346751.1)

MALALSLLKETPKTYTAFIEALRARIKDVPAQRFLVLDLTSQGNTIGVA
IDVVNVYAVGYRSGNKSYILANDAKKPAPTHILFPTALGATQSTRTVLPFTGDYPELGPHARRTAQS
SASGAPGSRIHENIPMLEQIPLGRNELDNAISKLHYAASHSDQAAAFIVIIQMVSEAARFRYIES
GTRMGIDNPPYIPDPAMRSLENEWSALSEQVNVPANGNRFSRSIQLTTVNYRPLVVDSVEADM
RGIVMLLNAS

13. *Pyrus bretschneideri* (XP_009346753.1)

MGTGAAPKRRRARAAALSYVDLTNQGDTIRVAIDVVNVYVVGYRSGNKSYILANNAENPAPTHILF
PTAPGAAQSTRMLPFTGDYPALGAYARRTAQPSASGARNPGSRIHEDIPMLEQIPLGRNELDNAIT
KLHYAASHSDQAAAFIVVIQMVSEAARFRYIESQVGTRMGIDNPPYIPDPAMRSLETNWSALSEQIQ
KVPANGKRFSRPIQLTTVNNRPLKVDSEADMVQRRGIAMLLYAR

14. *Pyrus communis* (PCP001408.1)

MALSFISIKATTTYRTFIEALRAQLTAGGSTSHGIPVLRRQDVTDQRFVVLVNLTNYDSYTITVA
IDVVNAYVVGYCAGTRSYFLRDPATHPPPLHRLFPGBTTRTLPFAGDYLGLGRAAQEALQQNTNRNR
AAGSRIHENIPMRERIPLGPGEILDNAISQLRYAESASSQAAAFIVIIQIVSEAARFRYIQQVQRDRI
RDGTSAVPDPAMSLLENSWSNLSEQIQMV PANQLLFINNGSVQIRKADNSIVLVKSVDSDAVRGAF
LLYCGGNPPAPNSESARTSKVTVKPCTLAKKK

15. *Pyrus communis* (PCP026877.1)

MALALSLLKATPKTYTAFIEALRARIKDVPAQRFLVLDLTSQGNTIRVA
IDVVNVYVVGYRSGNKSYILANDAKKPAPTHILFPTALGATQSTRTVLPFTGDYPELGPHARRTAQS
SASGAPGSRIHENIPMLEQIPLGRNELDNAISKLHYAASHSDQAAAFIVIIQIVSEAARFRYIES
GTRMGIDNPPYIPDPAMRSLENEWSALSEQIQVNVPANGNRFSRSIQLTTVNYRPLVVDSVEADM
RGIVMLLN

16. *Pyrus communis* (PCP011148.1)

MALALSLLYVTPEKYSAFIEALRARIKDVPAQRFLVLDLTSQGNTISVA
IDVVNVYVAGYCSGNKSYILKDAENRARTQILFPTAPSATQSTPIQLPFTGDYGEGLGGYARRIAQP
SAARYPGSHSHERIPTLEIPLGRNELDNAITMLHYAASRSDQASSDQAAAFIVIIQMVSEAARFRY
IENQVTRMEENYCPYIPDPAMRSLENNWSALSEQIQVNVPANGSRFNRPIQLTNIRNSPHVVD
DIVQRRGIAMLLYSR

Type 2 RIPs

1. *Malus domestica* (MDP0000711911)

MTRVLAIYITLAFSLFLCGTECNISFSTSGATSN SYNTFIKALRAQLTNGATAIYDI PVLNPSVPDS QRFLLVDSLNNGNNTITVAIDVVNASVVAYRARAARPYFLADAPDEALDILFNDTRGF FLPFTSNV DLEKAAEKS RDKI PLGLTPLHNAITS LWNHESEAAVSLVIIQTVFEAARFRVIEQRVRNSISSKA NFIPDPAMLSLENNWLAISWETQHALNGVFSKSIQLRSTNNNLFLVDSVSSSIMAGVAFLFYNCVTF PNIIKMPVN VV MGKEIDNEICAVQNRTTHISGLEGLCVDVKNGLDSDG NLVQIWPCGQQRNQKWTQ PDETIRSMEK CMTAYSTSSPEN YVM IYNCTTAVLEATK WALSTDGTI THRRSGLVLT AHEATRGTTL TIATNSHSPKQGWRVADDVEPTVTSIIGYNDMCLTANDDKSRVWMEYCVPSKNQQQWALYSEGTIRV NSDRTLCVTSNGHNSSNVIIILKCELKRGDQRWVF KTDGSILNPNAELVMDVKNSDVYL RQIILY PY GTPNQQWLPFF

2. *Pyrus communis* (PCP031611)

MLAIYITLAFSLFLGYGTECNISFSTNGATSN SYNKFIKALRAQLTNGATRIYDI PVLNPSVPDS QRF LLVDSLNNGNNTITVAIDVVNVSVVAYRTRAARSYFLADAPDEALDILFNDTRGF FLPFTSNYIDLE KAAEKS RDKI PLGLTPLHNAITS LWNHESEAAASLLVIIQTVFEAARFRVIEQRVRNSISSKANFR SDHAMLSLENNWLAISWETQHALNGVFSKSIQLRSTNNNLFLVDSVSSSIMAGVAFLFYNCHAVTFP NI IKMPVN VV MGKEIDNEICAVQNRTTRISGLEGLCIDVKNGLDSDG NLVQIWPCGQQRNQKWTQ PDETIRSMEK CVTAYSTSSLKNYVMIDNCTTAVPEATK WALSTDGTI THRRSGLVLT AHEATQGTTL TIATNSHSPRQGWRVGDDVEPTVTSIIGYNDMCLTANDDKSRVWMEYCVPSKNQQQWALYSEGTIRV NSDRTLCVTSNGHNSSNVIIILKCELRRGDQRWVF KTDGSILNPNAELVMDVKNSDVYLREIILY PY GTPNQQWLPFF

Figure S1. Amino acid sequences of type 1 and type 2 RIPs from the Rosaceae species *Malus domestica*, *Prunus mume*, *Prunus persica*, *Pyrus bretschneideri* and *Pyrus communis*. The RIP domain is indicated in black, the signal peptide is indicated in red and the lectin domain is indicated in blue.

Gene A	MALSFSIKNATTYYRTFIEALRQLTAGGSTSHGI PVLRRQDVKDDQRFV L VNLTNYD	60
Gene B	MSIPFTLIDATPDSYSRFIDQLRARLTFG-TTSQGIRVLPPSRQVGNNARFIYVDL TYD	59
Gene C	MSISFTLIGATPDSYSTFINQLRDRLTFG-TTSQGIPVLPSSRQVGNNDRFIYVN LTNYD	59
	****.*: .**. :* **: ** ;** * :***: ** ** ::* : **: *:*****	
Gene A	SYTITVAIDVVNAYVVGYCAGTRS YFLR-DPATHPPLHRLFP GTT RT-TLPFAGDYI GL	118
Gene B	GVTVTIGIDVVNAYVMGYE QGEQNYPLQ-TLPDDPAPVELLFPNTRSA GELPFTGHYASL	118
Gene C	GVTVTIGIDVVNVYVMGYE QGGQNYLLGGTL PDEAA--TVFPNTRAAGE L PFRADYGS L	116
	. *: :.*****.**: ** * :.* * :**. * : *** ..* .*	
Gene A	GRAAQEALQ QNTNR-NRAAGSRIHENISM RERIPLGP GELDNA ISQLRYAESASSQAA AF	177
Gene B	GEYARRMQNEQP NRRDQQALNRLS N--PMRQNIGLGPSLHS AIDMLERAATPLSQAGAI	176
Gene C	GQYARGMPNEQP NRRDQQSVNRLS N--DMRENIALGPSSLHWAIHMLVHAAT-SSQASAI	173
	*. *: : : .** : : .*: : **: .* ***..*. ** * * : ***. *:	
Gene A	I VIIQIVSEAARFRYIQGQVRDRIRDGTSAEPDPAMLSLENSWSNL SEQI QMVP-ANQ LL	236
Gene B	L VIIQMVSEAARYPYI ERQVRESI QTGNSFLPDPRMLSLENNWSNLSRQIMGATRAGRES	236
Gene C	I VIIQMVSEAARYPYIERRVRESI QTANSFIPDP PRMLTLENHWSTLSRQIMEATRAGRES	233
	:****:*****: **: :** : *: ..* *** **: ** ** .**.** .. *: .	
Gene A	FINNGSVQIRKADN-SIVLVKSVDSDAVRGVAFLLYCGGNPPAPNSESARTSKVTVQKP	294
Gene B	FSTSVSLDDAYQSHGAPP LVVNSVRDSFIQDMEI ALLLHD RGDDRG TDQGNDPENCTPGP	296
Gene C	FSTSVSLVDAYQSHGAPP LVVNSVRDSFVQDMEI ALLLHD RGDDRG TDQGIDPKNCTAGP	293
	* .. *: .. . : :** .. : .: : * *	
Gene A	TLAKKK----K----- 301	
Gene B	SGSGIGRRGGKKP RRQHE 314	
Gene C	SVSGRG----KKPHDEL- 306	
	: : * :	

Figure S2. Alignment of the deduced amino acid sequences of type 1 RIPs from apple (Md1RIP, A-MDP0000918923, B-MDP0000223290, C-MDP0000134012). “**” Means that the amino acids are identical in all sequences; “.” means conserved amino acid conversions, and “..” semi-conserved amino acid substitutions.

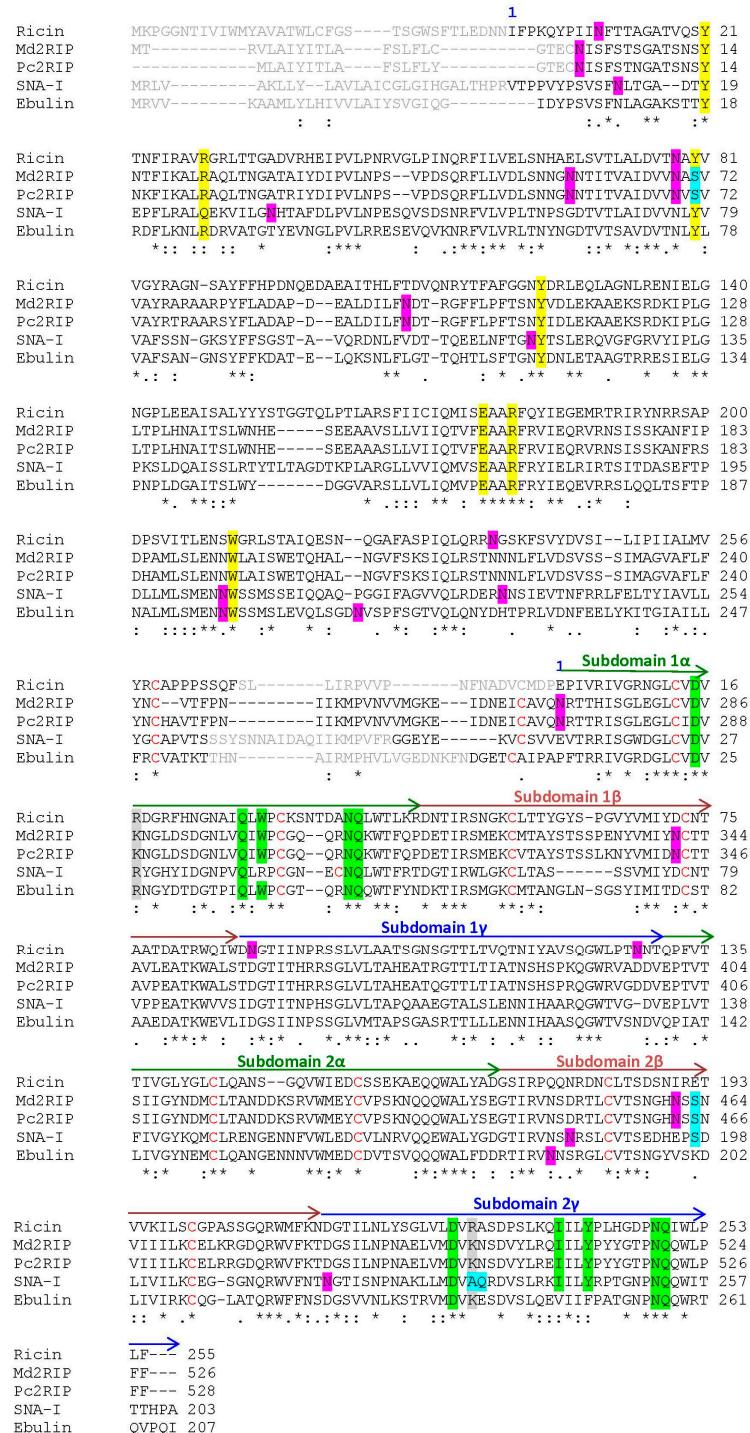


Figure S3. Sequence alignment of type 2 RIPs from *M. domestica* (Md2RIP) and *P. communis* (Pc2RIP), Ricin, Ebulin and SNA-I. “**” Means that the amino acids are identical in all sequences; “:” means conserved amino acid conversions, and “.” semi-conserved amino acid substitutions. The N-terminal signal peptide and linker are shown in gray. Amino acid residues known to be important for the carbohydrate binding activity of ricin are shown in green; Residues reported to be critical for the binding to sialic acid in the Neu5Ac(α2-6)Gal/GalNAc sequence of 2-6-sialyllectose (according to [1]) are indicated in blue. The amino acids known to be important for the catalytic activity of the N-glycosidase domain of ricin are highlighted in yellow. Cys residues involved in disulfide bridges are shown in red. Putative N-glycosylation sites are highlighted in pink. Basic residues for 6S-Gal binding [2] are highlighted in gray. Homologous subdomains (α , β , γ) are indicated by arrows.



Figure S4. Sequence information of RIPS from *Malus domestica* cv. Golden Delicious. (A) Schematic diagrams of protein domains in MdRIPs. Md1RIP sequence consists of a RIP domain (amino acids 1–301). Md2RIP consists of a signal peptide (amino acids 1–22) followed by RIP domain (amino acids 23–257) and lectin domain (amino acids 258–548); (B) Predicted amino acid sequences of apple Md1RIP (GDR accession no. MDP0000918923) and Md2RIP DNA (GDR accession no. MDP0000711911). The termination codon is marked with an asterisk (*). The signal peptide and the lectin domain within the Md2RIP sequence are highlighted in yellow and green, respectively; (C) Deduced sequence of apple type 1 RIP construct expressed in *Pichia*. Note that the apple sequence is preceded by an N-terminal signal peptide from yeast (in bold) necessary for secretion and followed by a C-terminal tag containing a c-myc epitope and a (His)₆ tag (shown in bold and italic). The cleavage sites for the α-mating factor secretion signal sequence are indicated (Kex2 protease site at position 86 and Ste 13 protease sites at positions 87 and 89). The N-terminal sequence of recombinant MdRIPs determined by Edman degradation is underlined. Putative N-glycosylation sites are highlighted in gray.

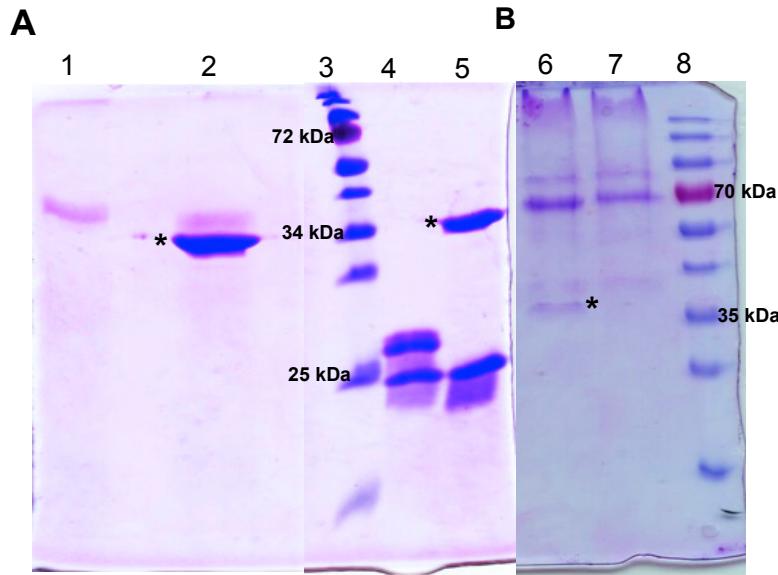


Figure S5. SDS-PAGE after PNGase F treatment of the recombinant Md1RIP (**A**) or Md2RIP (**B**). Lanes 1, 4 and 7: untreated Md1RIP, RNase B and Md2RIP; lanes 2, 5 and 6: Md1RIP, RNase B and Md2RIP treated with PNGase F. Lanes 3 and 8: protein ladder (Fermentas). In each well, 2 µg protein was loaded. The position of the polypeptide corresponding to PNGase F is indicated with an asterisk.

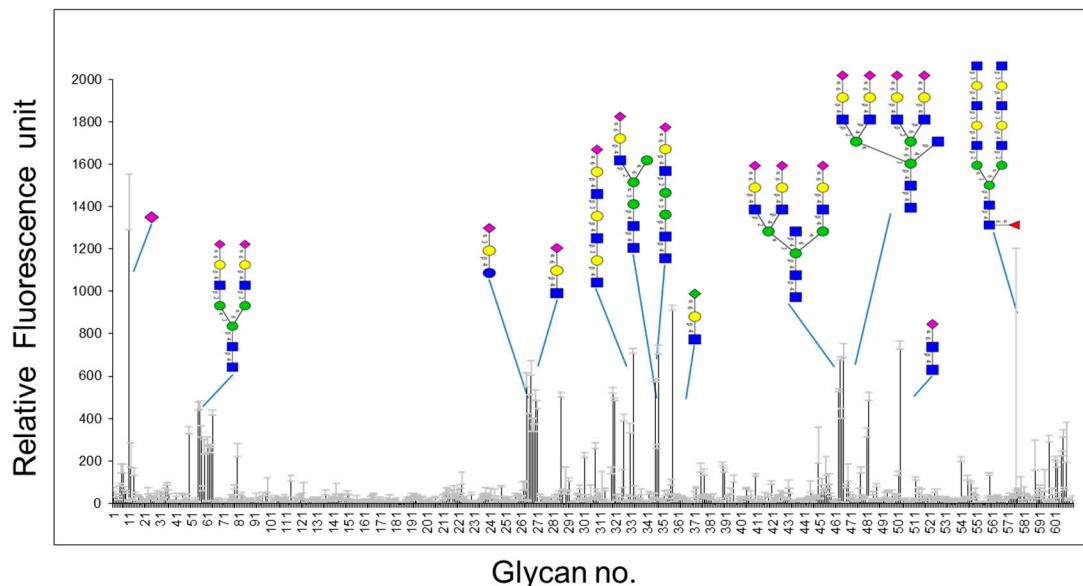


Figure S6. Glycan array analysis of recombinant Md2RIP at 300 µg/ml. The Consortium for Functional Glycomics website (<http://www.functionalglycomics.org>) supports the complete raw data for all the proteins. Sugar code used: green circles represent mannose residues, yellow circles indicate Gal, blue squares indicate GlcNAc residues, red triangles show fucose, purple diamonds indicate NeuAc and green diamonds indicate KDN.

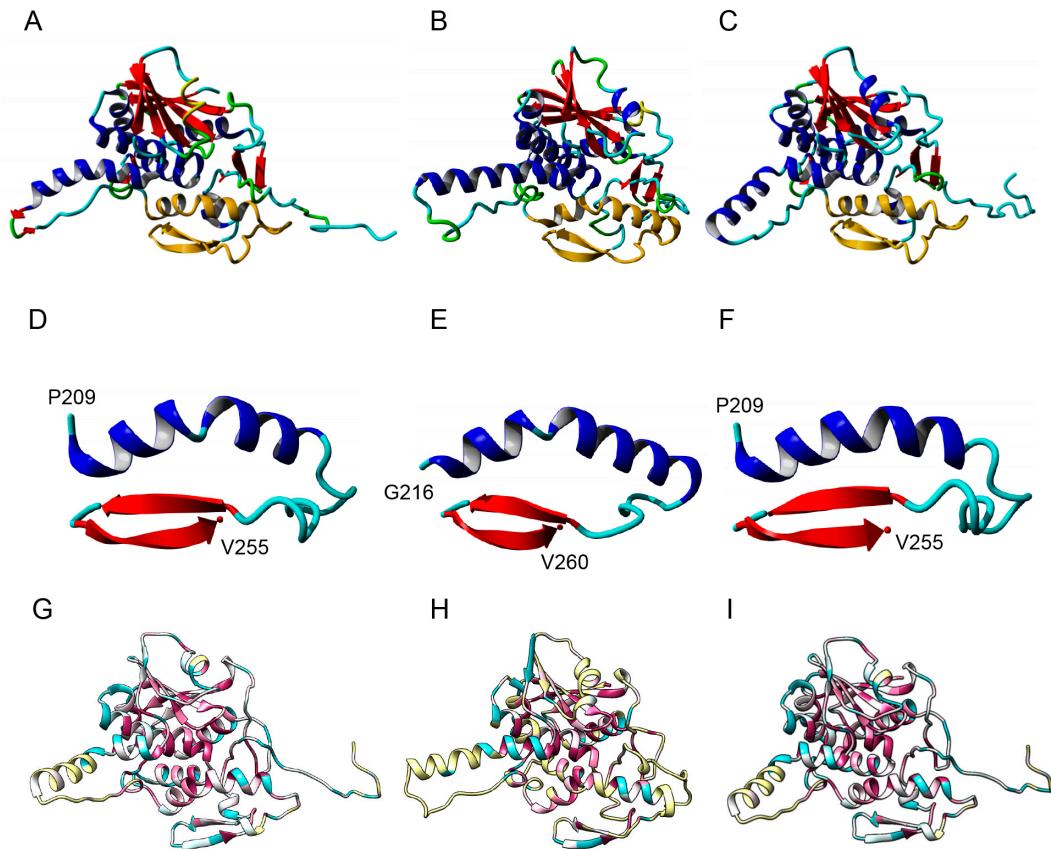


Figure S7. Ribbon diagrams of type 1 RIP from apple (A), peach (B) and pear (C), showing the α -helix- β -hairpin structure (colored orange) located in the C-terminal region of the RIPs. Structural similarity of the α -helix- β -hairpin structure of type 1 RIPs of apple (D), peach (E) and pear (F). Conservation of the secondary structural features of type 1 RIP of apple (G), peach (H) and pear (I). The code-colored conservation scale used by ConSurf is as follows:



A

BE27	201	WSKISEGIRK	A	---	VKKV	IS-PPIELV	NASNGKWTVNQV	235
Apple	220	WSNLSEQIQM	V PANQLLF	INNGSVQIRK	ADNS	I VLVKSV	258	
Peach	226	WSDLSEQIQR	AQQRN	NETGFD-RTIVLH	NVGNE	RREVNSV	263	
Pear	220	WSNLSEQIQM	V PANQLLF	INNGSVQIRK	ADNS	I VLVKSV	258	

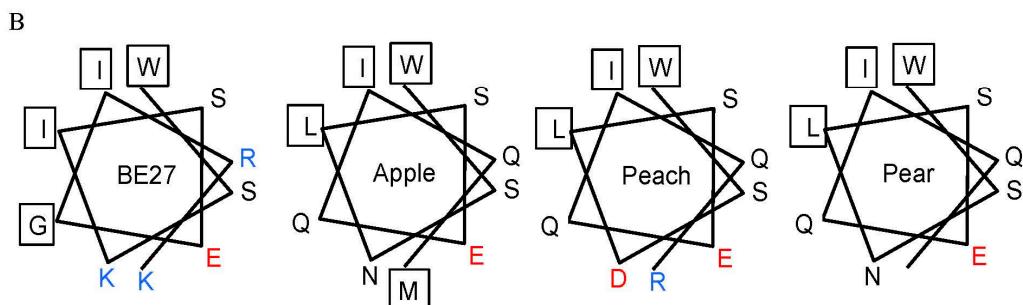


Figure S8. (A) Alignment of the α -helix- β -hairpin motif sequences from BE27 and type 1 RIPs from apple, peach and pear; (B) Helical wheel drawing of the helices of BE27, apple, peach and pear. The amino acid charge is indicated in red (negative), black (neutral) and blue (positive). Hydrophobic amino acids are boxed [3,4].

Table S1. List of genes encoding RIPs from Rosaceae.

Species	Accession Number	Size	Location in the Genome	Signal Sequence	Sequence Database Source
Type 1 RIP					
<i>Malus domestica</i>	MDP0000918923	301 aa	unanchored	No	Genome database for Rosaceae
	MDP0000223290	314 aa	unanchored	No	
	MDP0000134012	306 aa	Chr3	No	
<i>Pyrus communis</i>	PCP001408.1	301 aa	unanchored	No	
	PCP026877.1	276 aa	unanchored	No	
	PCP011148.1	283 aa	unanchored	No	
<i>Pyrus bretschneideri</i>	XP_009346751.1	278 aa	unanchored	No	NCBI database
	XP_009346753.1	246 aa	unanchored	No	
	XP_009375039.1	278 aa	unanchored	No	
	XP_009374990.1	280 aa	unanchored	No	
<i>Prunus mume</i>	XP_008243880.1	201 aa	unanchored	No	
	XP_008243881	304 aa	unanchored	No	
	XP_016652174.1	304 aa	unanchored	No	
	XP_016652175.1	294 aa	unanchored	No	
<i>Prunus persica</i>	ppa009409mg	294 aa	unanchored	No	Phytozome database
	ppa009637mg	283 aa	unanchored	No	
Type 2 RIP					
<i>Malus domestica</i>	MDP0000711911	549 aa	Chr8	Yes	Genome database for Rosaceae
<i>Pyrus communis</i>	PCP031611	547 aa	unanchored	Yes	

Table S2. Overview of the top 30 glycans interacting with the Md2RIP and comparative analysis with SNA-I. The glycan with the highest relative fluorescence unit (RFU) is assigned a value of 100. The rank is the percentile ranking. NA: not available in this glycan array version.

Glycan No.	Structure	Md2RIP 300 µg/mL		SNA-I 0.1 µg/mL	
		RFU	Rank	RFU	Rank
11	Neu5Ac β -Sp8	1421	100	25	0.09
357	KDN α 2-6Gal β 1-4GlcNAc-Sp0	922	64.9	30	0.11
502	Neu5Ac α 2-6GalNAc β 1-4(6S)GlcNAc β -Sp8	746	52.5	NA	NA
348	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3Man β 1-4GlcNAc β 1-4GlcNAc-Sp12	724	51.0	17952	65.01
466	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2)Man α 1-6(GlcNAc β 1-4)(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-4(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2)Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc-Sp21	721	50.8	NA	NA
332	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β -Sp0	719	50.6	24563	88.95
464	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-4Man α 1-6(GlcNAc β 1-4)(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-4(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2)Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc β -Sp21	683	48.1	NA	NA
267	Neu5Ac α 2-6Gal β 1-4(6S)GlcNAc β -Sp8	638	44.9	14288	51.74
576	GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-6(GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4(Fuc α 1-6)GlcNAc β -Sp24	633	44.6	NA	NA
264	Neu5Ac α 2-3Gal β 1-4Glc β -Sp8	582	40.9	18033	26.30
346	Man α 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc-Sp12	581	40.9	NA	NA
319	Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc β -Sp12	533	37.5	22956	83.13
463	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-6(GlcNAc β 1-4)(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc β -Sp21	533	37.5	NA	NA
286	Neu5G α 2-6Gal β 1-4GlcNAc β -Sp0	510	35.9	13790	49.94
270	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β -Sp0	510	35.9	20964	75.91
482	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4(Fuc α 1-6)GlcNAc β -Sp24	504	35.5	NA	NA
320	GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc β -Sp12	493	34.7	14464	52.38
271	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β -Sp0	467	32.9	25668	92.95
266	Neu5Ac α 2-6GalNAc β 1-4GlcNAc β -Sp0	466	32.8	29	0.11
56	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc β -Sp21	462	32.5	NA	NA
55	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc β -Sp12	458	32.3	18898	66.44
64	Fuc α 1-2Gal β 1-3GalNAc β 1-4(Neu5Ac α 2-3)Gal β 1-4Glc β -Sp9	429	30.2	19	0.07
465	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2)Man α 1-6(GlcNAc β 1-4)(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc β -Sp21	424	29.8	NA	NA
326	Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc β -Sp12	406	28.5	10	0.04
268	Neu5Ac α 2-6Gal β 1-4GlcNAc β -Sp0	370	28.6	27613	100
269	Neu5Ac α 2-6Gal β 1-4GlcNAc β -Sp8	355	26.0	14288	51.74
330	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-3GlcNAc β -Sp0	353	25	21014	76.10
49	Neu5,9Ac α 2-6Gal β 1-4GlcNAc β -Sp8	344	24.9	21953	79.50
57	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc β -Sp24	339	24.2	NA	NA
481	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-3)GalNAc α -Sp14	334	23.9	NA	NA

Table S3. Geometric and thermodynamic qualities of the RIP models built by homology modeling (* since ANOLEA works with 3D structures, both the numbering of residues (*) and the number of residues (**), refer to the models built for type 1 and type 2 RIPs from Rosaceae).

Models	Residues * out of the Allowed Areas in the Ramachandran Plot	Residues with Values over the Threshold in the ANOLEA Plot	QMEAN Value
Type 1 RIP apple	D44, A91, F101, N233 (4)	15 out of 292 residues **	0.60
Type 1 RIP peach	N9, D44, D81, V151, T342 (5)	11 out of 294 residues **	0.57
Type 1 RIP pear	A91, F101 (2)	5 out of 292 residues **	0.58
Type 2 RIP apple	K273, E344, S353 (3)	15 out of 538 residues **	0.56
Type 2 RIP pear	D99, H142, A245, N250, I252, E324, Y411, D420 (8)	11 out of 528 residues **	0.57

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