

Supplementary Figures S1-S8

Evolution of a guarded decoy protease and its receptor in solanaceous plants

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SpRcr3: ATGGCTATGAAATGTGATTTATGATATTTAATACATATTAATACGATGTCAACATCAAAACAGAGTGCAGCCCGCCACCGCCAAAGCCAAATG
 NrRcr3a: ATGGCTATGAAATCAATTTGATGAGTGTTTAAATTTATGCTTTGTAGTTCGATGTACAGTTCTCATGCAATGTGCGCAAGCTGCGACCACTG
 NrRcr3b: ATGGCTATGAAATCAATTTGATGAGTTTAAATTTATGCTTTGTAGTTCGATGTACAGTTCTCATGCAATGTGCGCAAGCTGCGACCACTG

SpRcr3: AACTGTCCTGCTGTGAGAGACACGAGCTGTGATGTACAGTCATGCGAGCCGTTTACAGAGCAGCATGTAGCAAAAGGAGAACCATTCATGATTTTCAAG
 NrRcr3a: AATTTGTTGTGTGAGAGACACGAGCTGTGATGTACAGTCATGCGAGCATTACAGAGTGAAGAGAAAGGAGAACCACTCATGATATTTAAAG
 NrRcr3b: AATTTGTTGTGTGAGAGACACGAGCTGTGATGTACAGTCATGCGAGCATTACAGAGTGAAGAGAAAGGAGAACCACTCATGATATTTAAAG

SpRcr3: AATCATGATTTCTTGGTTCGGTCAATAAGCGAGGAAATCTGCTTTCACAGTATGGGAGATGAATTTGCGATATTTACTTCCACAGAGTTTTTGGC
 NrRcr3a: AAACATCATTTCTTGGTTCGGTCAATAAGCGAGGAAATCTTCTTAAAGTAAAGTGAATTTGCGATATTTACTTCCACAGTTTTTTGGC
 NrRcr3b: AAACATCATTTCTTGGTTCGGTCAATAAGCGAGGAAATCTTCTTAAAGTAAAGTGAATTTGCGATATTTACTTCCACAGTTTTTTGGC

SpRcr3: AATTTATCTGATTAACATATCTATCTTCAATCTTACCTTTCCCAATGTCAACAGCGAATTTAAAGAAATTAATGATCTTACGAGCATATCATGT
 NrRcr3a: AGCACTGCTATTAAACAATATGCTTCAACACCTCACTTCCCACTTCAATTTAAAGTAAATTAATGATCTTAAAGCATATCATGT
 NrRcr3b: AGCACTGCTATTAAACAATATGCTTCAACACCTCACTTCCCACTTCAATTTAAAGTAAATTAATGATCTTAAAGCATATCATGT

SpRcr3: GGTCTAACTTGGACTGGAGAGAGTGTGGGCGTCACTCAAGTGAACATCAAGGCTGATGCGATGTGCTGGGCGTTTGTGCTTGGATCACTAG
 NrRcr3a: GGTCTAACTTGGATGGAGAGAGAGAGAGTGCCTCACTCGTGAAGTGTATCAAGGCTCACGTGTGTTTGGTGGGGAATCTCTGCTTGGATCACTAG
 NrRcr3b: GGTCTAACTTGGATGGAGAGAGAGAGAGTGCCTCACTCGTGAAGTGTATCAAGGCTCACGTGTGTTTGGTGGGGAATCTCTGCTTGGATCACTAG

SpRcr3: AGGAGCGCTACAAAATTTGCACAGCGCACTTGTGGAAATTTCTGAACAGCAACTTTCTCGATGCAACCACCAAAATATGGCTTCAAGCGAGTTTTC
 NrRcr3a: AGGAGCGCTACAAAATTTGCACACGCAAGTGAATTTCTGAACAGCAACTTTCTCGATGCAACCACCAATTAATGGCTTCAAGCGAGTTTTC
 NrRcr3b: AGGAGCGCTACAAAATTTGCACAGCGCACTTGTGGAAATTTCTGAACAGCAACTTTCTCGATGCAACCACCAATTAATGGCTTCAAGCGAGTTTTC

SpRcr3: ACGAATCGATTCGATTTATCATAGAAAATGGTGGAAATTCAGGGAATCAGATATGAATACCTAGGTCAACAGTACACATCGAGAGCCACAGAGAA
 NrRcr3a: ACGAATCGATTCGATTTATCATAGAAAATGGTGGAAATTCCTCATGTCAGTCACCCCTTACGAAAGCAGCAAGCACATCGAGAGCCACAGGCT
 NrRcr3b: ACGAATCGATTCGATTTATCATAGAAAATGGTGGAAATTCCTCATGTCAGTCACCCCTTACGAAAGCAGCAACATCGAGAGCCACAGGCT

SpRcr3: ACACGACAGCTGCAAAATAGTAGTTATCAAGCTGCGCTCGAGGTGAAGCACTTCACTATGCAAGCGGTAACTTAACACAGCAGTCTCCATTTGGAATAG
 NrRcr3a: ACACGACAGCTGCAAAATAGTAGTTATCAAGTTTGTGCTCGAAGTAGTGAAGCACTTCACTATGCAAGCGGTAACTTCAACAGCAGTCTCCATTTGGAATAG
 NrRcr3b: ACACGACAGTGAAGAAATAGTAGTTATCAAGTTTGTGCTGAAGTAGTGAAGCACTTCACTATGCAAGCGGTAACTTCAACAGCAGTCTCCATTTGGAATAG

SpRcr3: TGTGACCGAAGTTTACAGTTTACGGGCGGAGTGTGACGGAAATCTGGCCGATGCGATTAACCGATGTGCTGTAACGACGGATAGGATATGGAATCGAT
 NrRcr3a: TGTGACCGAAGTTTACAGTTTACGAGGAGCAATTAATCTGGAATTTGCGAGATCGAATTAACCGATGTGCTGTAACGACGGATAGGATATGGAATCGAT
 NrRcr3b: TGTGACCGAAGTTTACAGTTTACGAGGAGCAATTAATCTGGAATTTGCGAGATCGAATTAACCGATGTGCTGTAACGACGGATAGGATATGGAATCGAT

SpRcr3: GAGGAGGTCAGAAATATTGGTTGTGAGGAATCTATGGGGAACAGTTTGGGTCAGAAATGGGTATATGAAAAATTATAAGAGATTATGGGATCCCTTCT
 NrRcr3a: GAGGAGGTCAGAAATATTGGTTGTGAGGAATCTATGGGGAACAGTTTGGGTCAGAAATGGGTATATGAAAAATTATAAGAGATTATGGGATCCCTTCT
 NrRcr3b: GAGGAGGTCAGAAATATTGGTTGTGAGGAATCTATGGGGAACAGTTTGGGTCAGAAATGGGTATATGAAAAATTATAAGAGATTATGGGATCCCTTCT

SpRcr3: GTCTTTGTGATATCGACAAAGATGTCTTCTTATCCAAATCATGCGTAG
 NrRcr3a: GTCAATTTGTGATATCAACAGTTGTCTTCTTATCCAAATCATTTAA
 NrRcr3b: GTCAATTTGTGATATCAACAGTTGTCTTCTTATCCAAATCATTTAA

SpRcr3 **KAMKVDIMNIIITLFFVVISVNTQGR-SQP-KLSVSRHELHMSRHRGVYKDEVEKGERFMIFKENKFIESVYNKAGNLSYKLGNEFADITSQFFL**
NbRcr3a **KAMKINLSVVT-LEFFVGMVKSHATAANLPAE-LSVSRHELHMSRHRGVYKDEVEKGERFMIFKENKFIESINAGMSYKLGNEFADITSQFFL**
NbRcr3b **KAMKINLISFIM-LEFFVGMVKSHATAANLPAE-LSVSRHELHMSRHRGVYKDEVEKGERFMIFKENKFIESINAGMSYKLGNEFADITSQFFL**

SpRcr3 **KFTGLNIPNLSYSPSPMSSTE-FKINDLDDYMFNLDWRESGAVTVQVHKVRCGGCWFASVAVSGLEGAYKIATGNLMEFSEQELDDCTNNYCGNGL**
NbRcr3a **RYTGLNMFHSPISSEET-SMEFKINDLDD-MFNLWDREKGAVTVQVHKVRCGGCWFASVAVGLEGAYKIATRKVLEFSEQELDDCTNNYCGNGL**
NbRcr3b **RYTGLNMFHSPISSEET-SMEFKINDLDD-MFNLWDREKGAVTVQVHKVRCGGCWFASVAVGLEGAYKIATRKVLEFSEQELDDCTNNYCGNGL**

SpRcr3 **TNAFDFTIENGGISRESDEYELGPQYTCRS-EKTAAVQISSYKVVPEGETSLQAVTKQPVSIGAASQDLQFYAGGTYDGNCAI-INHVAITAGYTD**
NbRcr3a **TNAE**
NbRcr3b **TNAE**

SpRcr3 **EGQKYWLKNSWGTSGWENGFMKIIRDSGPGLCLDIAMSSYPNIA**
NbRcr3a **EGQKYWLKNSWGTSGWENGFMKIIRDSGPGLCLDIAMSSYPNIA**
NbRcr3b **EGQKYWLKNSWGTSGWENGFMKIIRDSGPGLCLDIAMSSYPNIA**

SIPlp1 ATGGCTTCAATTTTTCCTTCAGAAATTAACCTGTGCTCTCTCTCTTTTTCATCTCTAGCCATATACCCCTTATAGTAAGTCCGCCCACTTAAAGT
NbPlp1 ATGGCTTCAATTTTTCCTTAAAA-----TTGCTCTCTCTCTTTTTCATCTCTTAATCTAGCTGTGTTTGAAGTACATCCCTGACCTACAG

SIPlp1 AATTATCATGCTTGGAAGCATTGAAATTTGGATGGTTCTCATGGAGCTGTATATCAAAAGATGATAGAAAAGAACACCGCTTCAAAATTTCAAGGA
NbPlp1 AAGCTATCGATCAGAAAGCATGGA-RAATGGATATTTCTCATGGTGTATATCAAAAGATGATAGAAAAGAAC-CCATTCCTAAAATATTTTAGGA

SIPlp1 AAGCGTTGAGTTATCATCGATTTTCAACAGAAGCGGAATCAACGTTATPAGCTAGCCATCAATAAATATGCTGATCTGACCATGGAAGATTCACAACTA
NbPlp1 AAGCGTTGAGTTATCATGAATCATTCATCAATGAATGGAATCAAGCTATATPAGTGTGAGCCATCAATAAATATGCTGATCTACCACTGAGGAATTTCTGGCTA

SIPlp1 TCGATTATGGGGCTTCGACATCTTCATTACTATCCCGACGAAGTCAACAGCACTACACAAACGCTGTCTTAAATATGATAGTGT-----TGACTGAAGTTCGAAATTA
NbPlp1 TCGATTATA-GGGGCTTCGACATCTT-----CCAGCAAAATCAAGTCTGACACAG-----STCTTTAAATATGATAGCAAGCTGATGATATCTCTCTTA

SIPlp1 GCATGGACTGGAGAAAGAGAGGAGGATGTCACAGAGTCAAGGATCAAGATGTATGTGGATGTGTTGGGCATTTTCTGGCGCGCGGCTATPAGAAGGAGC
NbPlp1 GCATGGACTGGAG-AGAGAG-GGCAGTGTACAGAGATCAAGGATCAAGTGTGATGTGGATGTGTTGGGCATTTTCTGGCGCTGGAGCTATPAGAAGGAGC

SIPlp1 ATATCAAAATTGCAAAACCAACTAATCTCTATTTCCAGCAACAACATTTGGATTGCTCTAGCCAGA-----ACAAAGGTTTGGAGGCGGGCATTAATGAT
NbPlp1 ATATCAATTTG-AAACATCAACTAATCTCTATTTCCAGCAACA-CAACTTCTAGATTGCAACAGTTCACAGTTCATGTTAGGGCGGGCATTAATGAT

SIPlp1 TGGGCTTACAGTCTTACTTTCAGAAACATGGCGGGGGCTATTACACAGAGACTAATTAATCTTATGAAGAGAGTCAAAAGTTTGCAGAGCAAGCAAC
NbPlp1 CAAGCTACGACTTCTACTTCAAAATGGCGGGCG-----CACTACCAACAGAGACTAATTAATCTTATCAAAATTCAAAGCTTTGGCACTCAACAACT

SIPlp1 -----CAGCAGCATTTACTATCAATGGCTACGAAGCTGTACCATCTGACGAGTCATCGTGTGTAAAGCTGTAGTGAATCAACTATTTCTGTTGGTAT
NbPlp1 TATCTCTACAGCAATTAATCACTGTGTATTAATAATATGAGTCAAGCGAGTCCGGTCTACGCAAGCTGTAGCTAATCAACATAATCTGTTGGTAT

SIPlp1 TGGCGCTCAAGCATGGCTTTCATATGTACGGAAGTGGGAATATATGATGGGAAGTGTAAATCTPAGACTGAATCAAGCTGACAGTATATAGGTTATGGGACA
NbPlp1 TGGCTCTCATTTGAGGTTTCATTTGTATGGAATGGGAATATATGATGGGAGTGTGACACCTCATCGAATCATGCACTGACAGTATATAGGTTATGGGACA

SIPlp1 AGTGAAGAGATGTTTCAAAATATTTGATATGAAGATTTATGGGGGAGTGTGGGGTGAAGAGGATATATGAAATGCTAGAGATGTGGAGTGTGGAGT
NbPlp1 AGTGAAG---TGTCAAAATTAATGGCTAGTCAAGAACTCATGGGGGCTACATGGGGTGAAGAGGATACATGAAATGTTAGAGATGCCGGGATG

SIPlp1 ATGGTGGCATTTGGGCATTCGAAGGTTGCTTCGTCCCTACCTTTGA
NbPlp1 AAGGTGGGCACTGGGCATGGCCAGGTGTCTGACAGAGTACCTGAGGCTCAATAATTTCAATGTTAAATTAAG

7/17 Protein alignment of *Sil1* p17 vs NbP17

<i>Sil1</i> p17	MA ¹ SNF ¹ FL ¹ FL ¹ TV ¹ LL ¹ FS ¹ LS ¹ LP ¹ VT ¹ VS ¹ NR ¹ NK ¹ LS ¹ MR ¹ EN ¹ NM ¹ WH ¹ GR ¹ VY ¹ KD ¹ IE ¹ KH ¹ RP ¹ T ¹ K ¹ EN ¹ VF ¹ ES ¹ FN ¹ KG ¹ Q ¹ RY ¹ KL ¹ AK ¹ AD ¹ LT ¹ EE ¹ FT ¹
NbP17	MA ¹ N ¹ F ¹ C ¹ ---K ¹ IG ¹ V ¹ ES ¹ LI ¹ N ¹ C ¹ CP ¹ VS ¹ RS ¹ D ¹ CA ¹ SM ¹ ER ¹ HK ¹ IK ¹ Y ¹ TH ¹ GR ¹ RP ¹ YK ¹ IE ¹ KE ¹ R ¹ FK ¹ IK ¹ EN ¹ VF ¹ ES ¹ FN ¹ KG ¹ Q ¹ RY ¹ KL ¹ AK ¹ AD ¹ LT ¹ EE ¹ FT ¹

<i>Sil1</i> p17	S ¹ Y ¹ GL ¹ SL ¹ DS ¹ LS ¹ Q ¹ SE ¹ AT ¹ TS ¹ FK ¹ YD ¹ -V ¹ TE ¹ VNS ¹ MD ¹ WR ¹ K ¹ GS ¹ VT ¹ GK ¹ VG ¹ KG ¹ CG ¹ GC ¹ W ¹ AF ¹ SA ¹ AA ¹ IE ¹ GAY ¹ QI ¹ AN ¹ EL ¹ LS ¹ ST ¹ Q ¹ LL ¹ DC ¹ ST ¹ Q ¹ -NKG ¹ CE ¹ GL ¹
NbP17	S ¹ Y ¹ GL ¹ SL ¹ DS ¹ FQ ¹ Q ¹ -S ¹ N ¹ Y ¹ TS ¹ FK ¹ YD ¹ QL ¹ -V ¹ TE ¹ NS ¹ MD ¹ WR ¹ ES ¹ SV ¹ TK ¹ KD ¹ Q ¹ KG ¹ CG ¹ GC ¹ W ¹ AF ¹ SA ¹ AA ¹ IE ¹ GAY ¹ QI ¹ AN ¹ EL ¹ LS ¹ ST ¹ Q ¹ LL ¹ DC ¹ ST ¹ Q ¹ -FHD ¹ SG ¹ KG ¹ GL ¹

<i>Sil1</i> p17	V ¹ AY ¹ DE ¹ L ¹ Q ¹ N ¹ GG ¹ IT ¹ Y ¹ EEA ¹ Q ¹ NCK ¹ TEQ ¹ -A ¹ AT ¹ Y ¹ EN ¹ EV ¹ VP ¹ DS ¹ ES ¹ LL ¹ KAV ¹ NS ¹ PI ¹ SV ¹ GA ¹ IA ¹ AN ¹ DE ¹ FH ¹ Y ¹ SG ¹ SI ¹ YD ¹ GS ¹ CN ¹ T ¹ NH ¹ AV ¹ TV ¹ GY ¹ GT ¹
NbP17	Q ¹ AY ¹ DE ¹ L ¹ Q ¹ N ¹ -GG ¹ IT ¹ Y ¹ EEA ¹ Q ¹ NCK ¹ TEQ ¹ -A ¹ AT ¹ Y ¹ EN ¹ EV ¹ VP ¹ DS ¹ ES ¹ LL ¹ KAV ¹ NS ¹ PI ¹ SV ¹ GA ¹ IA ¹ AN ¹ DE ¹ FH ¹ Y ¹ SG ¹ SI ¹ YD ¹ GS ¹ CN ¹ T ¹ NH ¹ AV ¹ TV ¹ GY ¹ GT ¹

<i>Sil1</i> p17	E ¹ ED ¹ G ¹ KY ¹ W ¹ V ¹ K ¹ NS ¹ W ¹ GS ¹ DW ¹ EE ¹ GY ¹ MR ¹ IA ¹ RD ¹ V ¹ GD ¹ GG ¹ CH ¹ AK ¹ VA ¹ FS ¹ TV ¹ -----
NbP17	S ¹ -E ¹ ED ¹ G ¹ KY ¹ W ¹ V ¹ K ¹ NS ¹ W ¹ GS ¹ DW ¹ EE ¹ GY ¹ MR ¹ IA ¹ RD ¹ V ¹ GD ¹ GG ¹ CH ¹ CG ¹ CT ¹ GS ¹ TV ¹ GS ¹ NN ¹ FN ¹ V ¹ N ¹

Supplementary Figure

1 Nucleotide and protein alignments of *NbRcr3s* and *NbPip1*. Sequences were aligned using Clustal Omega.

Frameshift mutations are highlighted (red). Used accession codes are:

AF493232 (SpRcr3);

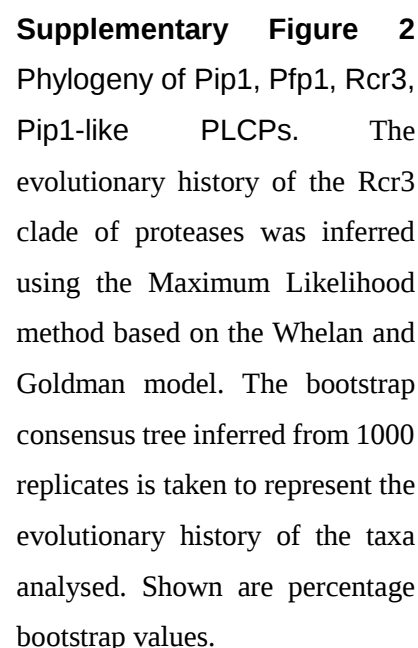
Niben101Scf08921-
g77214 (NbRcr3a);

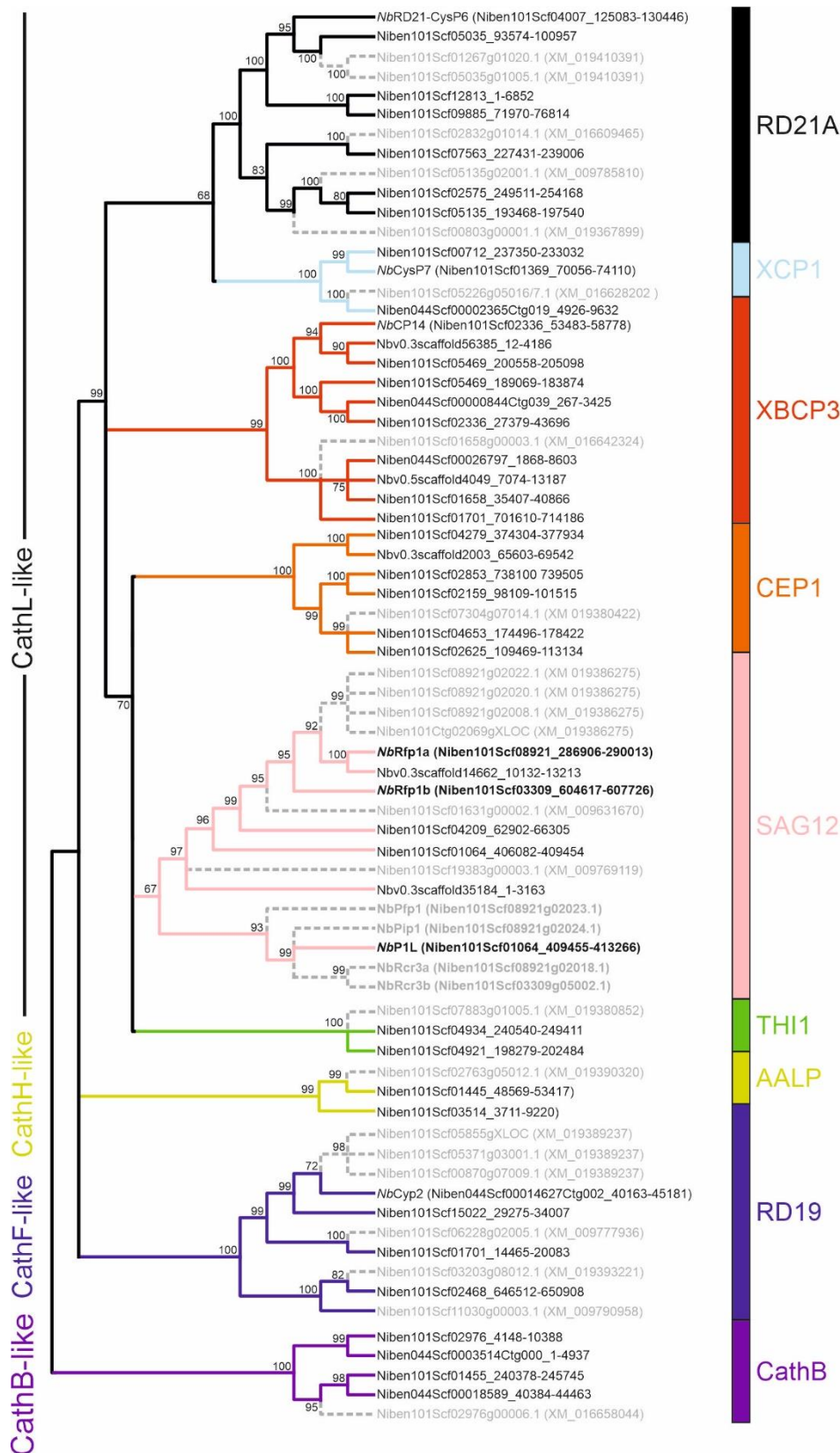
Niben101Scf03309-
g49741 (*NbRcr3b*)

NM_001247020.2

(S/Pip1); and

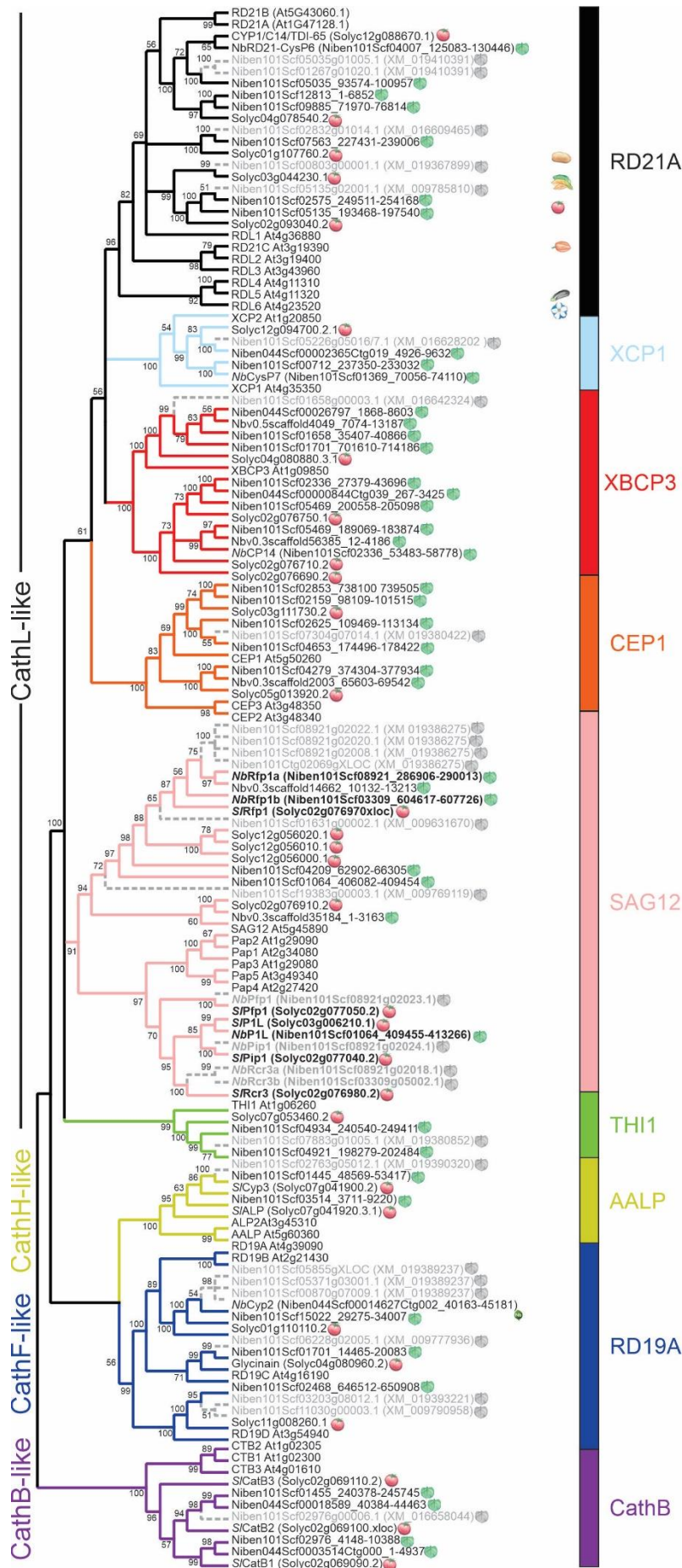
Niben101Scf08921-
g77212 (*NbPip1*).





Supplementary Figure 3

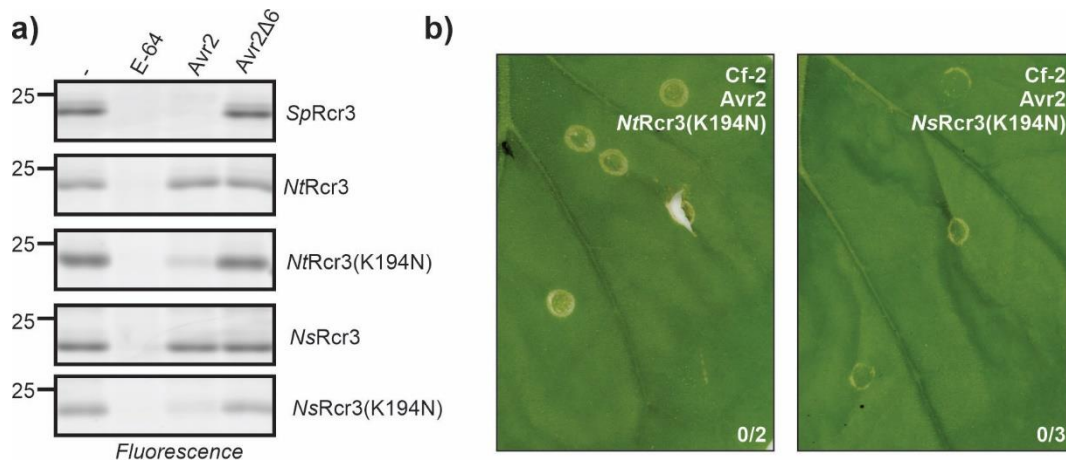
Phylogeny of *N. benthamiana* PLCPs. The evolutionary history of the PLCP family was inferred using the Maximum Likelihood method based on the Whelan and Goldman model. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed. Shown are percentage bootstrap values. Putative pseudogenes are indicated in grey. Naming of PLCP clades according to (Richau *et al.*, 2012).



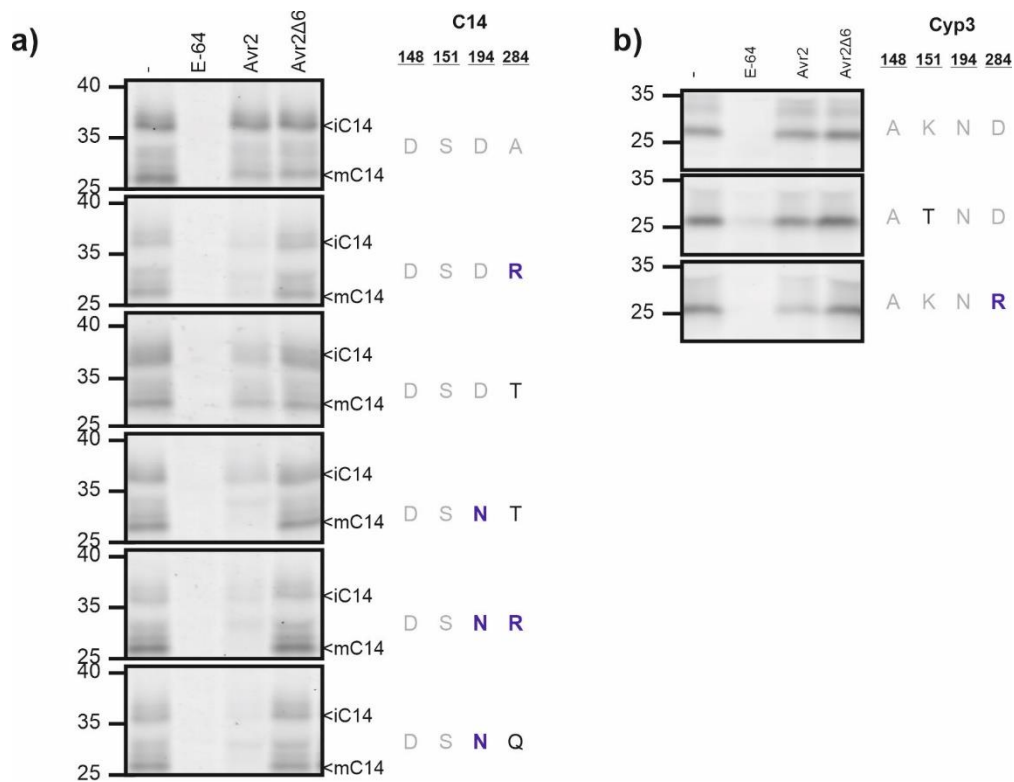
Supplementary Figure 4

Phylogeny of tomato, Arabidopsis, and *N. benthamiana* PLCPs.

The evolutionary history of the PLCP family was inferred using the Maximum Likelihood method based on the Whelan and Goldman model. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed. Shown are percentage bootstrap values. Putative pseudogenes are indicated in grey. Naming of PLCP clades according to (Richau *et al.*, 2012).



Supplementary Figure 5 *Nicotiana* Rcr3 K194N mutants can be inhibited by Avr2, but do not trigger HR. **a)** Activity profiling of *SpRcr3*, and the *Nicotiana tabacum* and *Nicotiana sylvestris* Rcr3 homologs (*NtRcr3* and *NsRcr3*, respectively) and the K194N mutants thereof. These proteins were co-expressed with silencing inhibitor P19 in *N. benthamiana* leaves by agroinfiltration. Apoplastic fluids were isolated at 4 dpi, pre-incubated for 45 min with or without 100 μ M E-64, 2 μ M Avr2 or Avr2 Δ 6, or DMSO, and labelled for 3 hours with 2 μ M MV201. Samples were separated on SDS-PAGE gels and scanned for fluorescence. **b)** Co-expression of Cf-2, Avr2 and the *NtRcr3(K194N)* or *NsRcr3(K194N)* mutant protease in *N. benthamiana* by agroinfiltration. Binary vectors contained the silencing inhibitor P19 in the backbone, and the *Agrobacterium* cultures were diluted to $OD_{600} = 0.25$ each in 1:1:1 mix. Pictures taken at 5 dpi. Numbers indicate the number of leaves showing HR.



Supplementary Figure 6 Mutating C14 and Cyp3 results in Avr2 inhibition.

a) C14 and, **b)** Cyp3 mutants were co-expressed with the silencing inhibitor P19 by agroinfiltration of *N. benthamiana* leaves. Apoplastic fluids were isolated at 2dpi, pre-incubated for 45min with or without 100μM E-64, 2μM Avr2 or Avr2Δ6, or DMSO, and labelled for 3 hours with 2μM MV201. Samples were separated on SDS-PAGE gels and scanned for fluorescence. The granulin-domain and proline-rich region are removed from active C14 generating an intermediate C14 (iC14) and mature C14 (mC14), respectively (Gu *et al.*, 2012).

