

Nbplc3-1 (Niben101scf02280) CTCAAAAAATCATCCTCCCGCTTTTCCGTTTTCC-----TCTTTCAAACAATGTCGAGACGAACGAGACGTACAGAGTCTGTTTCCGGCGCGGCGGTTCCGGGTAGTCGCCGCCGAGG 217 Nbplc3-2 (Niben101Scf04093) CTCCGGCGGATGTAAAGAATTTATCCAATAGATATTCCGATAACGGAGTAATGAAGGAACCTACAATGATTCTTAATTGAGGTTGAGAAAAGAGGAGAAAATGCGAGTTTAGAGGATG 352 Nbplc3-2 (Niben101Scf04093) CTCAGGGTATTTCGTTCGTCGCCTTCTGCAGTGAATCTTGCTGCAGCTGCAGCTGCAGCTGCAGTGGAACCAAATACTCTGCTCTGCTTTTCAA-GCAGCAACT 471 Nbplc3-2 (Niben101scf04093) TACAGCATATGTTGAAAAGATTTATGAAAATATTATGGGATAACTTGAAGAAGGAGTTGGGATCACTCCTTTC---CTTATGCTCCAGATTCACCATGAATGAACGAGCCTTTGTCTCAT 588 NbplC3-2 (Niben101Scf04093) TACTTCATATACACAGGACATAATTCTTATCTAACCGGGAATCAACTGAGTGATTGCAGTGATGTCCCCCATAATACAAGCCCTGCACCAAGGTGTACGAGTAATTGGATATTGGATATA 708 NbPLC3-1 (Niben101Scf02280) TGGCCAAATTCCGCCAAAGATGATGTGGAAGATTTTGCATGGACGATCAACGACCACTCCAGTTGCGCTCATCAAAGTCTAAAGTCTAAAGAACATGCCTTTTCTGTATCTGAATTT741 NbPLC3-2 (Niben101Scf04093) TGGCCAAAATCCCGCCAAAGATTACGTGGGAAGTTTGCATGGAGGAACATTGACCACTCCGGTTGCGCTCATCAAATGTCTGAGGACCATGCTTTTACTGTATCAGAGTAT 828 NbPLC3-1 (Niben101Scf02280) CCTGTTGTAATAACACTTGAAGATCATCTAACTCAAGATCTTCAGGCAAAAGTTGCGGGAGATGATCACTCAAACATTTGGAGACATGCTGTTTTCTCCCCGATTCTTGTTTGAAGGATTC 861 NbPLC3-2 (Niben1018cf04093) CCTGTTGTGGGTAACACTTGAAGATCATTTAACCCCAGATCTTCAGGCAAAAGTTGCAGAGATGATCATCTAGAGACATTGGAGACATGCTGTTTCTCCCCGATTCAAGTTGAAAGACTTT 948 Nbplc3-1 (Niben101scf02280) CAAAATGTAGCACCAGAATACAAGCGTTTAATTGCCATTCATGCTGGAAAAGGTAGAAGGTGGACTGTCAGGTTGAGGTGGAGGTTGATCCTGATAAAGTAAGACGGCGTTAATGCCAGTTGAGTGGG 1218 Nbplc3-2 (Niben101scf04093) CAAA-TGTCGCACCAGAATACA-----TATGCTGGAAAGGGAAAAGGTGGATTGTCAGATTGGCTGAGGGTTGATCCTGATAAAGTAAGACGACTTAGCTTGACCGAG 1290 NbPLC3-1 (Niben101scf02280) CAAGAACTTGGAAAAGGCTGTAGTTACTCATGGAAAAGAAATTATCAGGTTCACTAAGAGGGAATATGCCGAAAGGGCATACGTTTTGACTCATCTAATTACAACCCTCTT 1338 Nbplc3-2 (Niben101scf04093) CAAGAACTTGGAAAGGCTGTAGTTACTCATGGAAAAGAAATTATCAGGTTCACTCAGCGGAACTTGCTCAGAATATACCCCAAAGGGCATACGTTTTGACTCATCCAATTAT---CCTTTT 1407 NbPLC3-1 (Niben101scf02280) GTTGCATGGGCGCATGGAGGCCTCAAATGGTGGGCATTCAATATGCAGGGCTATGGAAGATCACTTTGGTTAATGCATGGTAATGCTAGGTATGTTCAGATACGTGGTATGTTCAGATACCA 1458 NbplC3-2 (Niben101scf04093) GTTGCATGGACGCATGGAGGCTCAAATGGTGGCATTCAATATGCAGGGCTATGGAAAATCACTTTGGTTAATGCATGGTATGTTCAGATCCAATGGTGGTGTGGGATATGTTAAGAAACCA 1527 NbPLC3-1 (Niben101scf02280) GATATACTATTGAAAGCGGGTCCCCAACAATCAGGTTTTCGATCCTGAAGCAAATTTGCCAGTCAAAACTACATTGAAGGTGACGTATTATGGGTGAAGGATGGTATTATGATTCAAG 1578 Nbplc3-1 (Niben101scf02280) TGGGATGAAAAGTTTGAGTTCCCATTAACAGTTCCTGAGTTGGCTCTACTTCGCGTCGAAGTTGATATGTCTGAAAAAGATGATTTTGCTGGCCAAAACTTGTTTACCTGTT 1818 Nbplc3-2 (Niben101scf04093) TGGGATGAAAAGTTTGAGTTCCCATTAACAGTTCCTGAATTGGCTCTACTTCGCGTCGAAAATTCATGAGTATGATATGTCTGAAAAGGATGATTTGCTGGCCAAACTTGTTTACCAGTT 1815 PLC3-1/3-2-VIGS Nbplc3-2 (Niben101scf04093) TCAGAACTAAGACAAGGTATTCGAGCAGTTTCACTCCACGACCGAAAGGGAGAGAAATA-AACTCTGTGAATCTTCTTATGCGTATCGCATTTGTCTAACTGTGA NbPlc3-2 (Niben101Scf04093) TTGTAACTTCTGTAATACATAAGCTCTGAGATGTAAATAGATGTAT--GGTAATATGGTGGAAATA-----TGTCATTCAGTGGTGGTGTTATATTGCTGCAATCAAA 2156 NbPLC3-1 (Niben101Scf02280) CTGCCG 2291

NbPLC3-1 (Niben101Scf02280) CTGCCG 2291 NbPLC3-2 (Niben101Scf04093) -----

Figure S1 Nucleotide sequences of *NbPLC1-1* **and** *NbPLC1-2.* cDNA fragments used for VIGS experiments are shown with gray backgrounds.

PI-PLC-X PI-PLC-Y C2

Α

B

Nbplc3-1	(Niben101Scf02280)	MSRQTYRVCFCFRRRFRVVAAEAPADVKNLFNRYSDNGVMNAENLQRFLI	50
Nbplc3-2	(Niben101Scf04093)		
S1PLC3	(NM001247261)	MSKQTYRVCFCFRRRFRVVAAEAPADVKNLFNRYSDNGVMSADNLHRFLI	50
NbPLC3-1	(Niben101Scf02280)	EVOKEENASLEDAHGIMNNLHDLKILNIFHRRGLHLDAFFKYLFADINPP	100
Nbplc3-2	(Niben101Scf04093)		
S1PLC3	(NM001247261)	EVQKEENATLEDAHAIMNNLHDLKILNIFHRRSLHLDAFFKYLFADINPP PI-PIC-X	100
Nbplc3-1	(Niben101Scf02280)	INPKRGIHHDMNEPLSHYFIYTGHNSYLTGNOLSSDCSDVPIIOALHRGV	150
NbPLC3-2	(Niben101Scf04093)	MLOTHHDMNEPLSHYFTYTGHNSYLTGNOLSSDCSDVPTTOALHOGV	47
S1PLC3	(NM001247261)	LNSKLGIHODMNAPLSHYFIYTGHNSYLTGNOLSSDCSDVPIIOALHRGV	150
	(,	** *** ********************************	
Nbplc3-1	(Niben101Scf02280)	RVIELDIWPNSAKDDVEVLHGRTLTTPVALIKCLKSIKEHAFSVSEYPVV	200
Nbplc3-2	(Niben101Scf04093)	RVIELDIWPNSAKDYVEVLHGGTLTTPVALIKCLRSIKEHAFTVSEYPVV	97
S1PLC3	(NM001247261)	RVIELDIWPNSAKDDVEVLHGGTLTTPVALIKCLKSIKEHAFAVSEYPVV	200
	:	******** ****** *********************	-
NbPLC3-1	(Niben101Scf02280)	ITLEDHLTQDLQAKVAEMITQTFGDMLFSPDSCLKDFPSPESLKRRVLIS	250
Nbplc3-2	(Niben101Scf04093)	VTLEDHLTPDLQAKVAEMITQTFGDMLFSPDSCLKDFPSPESLKRRVLIS	147
S1PLC3	(NM001247261)	ITLEDHLTTALQAKTAEMITQTFGDMLFTSDSCLKEFPSPESLKRRVLIS	250
	**	***** **** ****************************	
NbPLC3-1	(Niben101Scf02280)	TKPPKEYLQAKEVKEKDSKKGTESPDTEAWGREVSDLKARYNDK-DDSDD	299
Nbplc3-2	(Niben101Scf04093)	TKPPKEYLQAKEVKEKDSKKGTDSPDSEAWGREVSDLKARYNDKQDDSDE	197
S1PLC3	(NM001247261)	TKPPKEYLQAKEVKETGATKGTDDTEAWGREVSDIKARYNDK-YDSDE	297
		************** *** * ******************	
Nbplc3-1	(Niben101Scf02280)	GVGDEDELSDEGDPNSQQNVAPEYKRLIAIHAGKGKGGLSDWLRVDPDK	349
NbplC3-2	(Niben101Scf04093)	GAGEEDDGSDEGDPNSQQMSHQNTYAGKGKGGLSDWLRVDPDK	241
S1PLC3	(NM001247261)	GEADDDDEEDPTSQQNTAPEYKRLIAIHAGKGKGGLSDWLRVDPDK	344
		* * ** ** *** PI-PLC-Y ************************************	_
NbPLC3-1	(Niben101Scf02280)	RRLSLSEQELGKAVVTHGKEIIRFTKRNLLRIYPKGIRFDSSNYNPLVAW	399
Nbplc3-2	(Niben101Scf04093)	RRLSLSEQELGKAVVTHGKEIIRFTQRNLLRIYPKGIRFDSSNY-PFVAW	290
S1PLC3	(NM001247261)	RRLSLSEQELGKAVVTHGKEIIRFTQRNILRIYPKGIRFDSSNYNPFNAW	394

Nbplc3-1	(Niben101Scf02280)	<u>AHGAQMVAFNMQGYGRSLWLMHGMFRSN</u> GGCGYVKKPDILLKAGPNNQVF	449
NbplC3-2	(Niben101Scf04093)	TH <u>GAQMVAFNMQGYGKSLWLMHGMPRSN</u> GGCGYVKKPDILLKAGPNNQII	340
S1PLC3	(NM001247261)	THEAOMVAINMOEYERSIWIMHEMPRANGGCGYVKKPDILLKAGPSNQVF	444
		***************************************	-
NDPLC3-1	(Niben1018cf02280)	DPEANLPVKTTLKVTVFMGEGWYYDFKHTHFDAYSPPDFYAKIGIAGVLA	499
NDPLC3-2	(Nibeniuiseiu4093)		300
SIPLC3	(NM001247261)	DPEASLPVKTTLKVTVFMGEGWYYDFEHTHFDAY <u>SPPDFYARIGIAGVDA</u> C2 * ****** *	494
Nbplc3-1	(Niben101Scf02280)	DNIMKKTKTLEDNWIPTWDEKFEFPLTVPELALLRVEVHEYDMSEKDDFA	549
Nbplc3-2	(Niben101Scf04093)	DNVMKKTKTLEDNWIPTWDEKFEFPLTVPELALLRVEIHEYDMSEKDDFA	416
S1PLC3	(NM 00124726)	DIVMKKTKTLEDNWIPTWDEQFEFPLTVPELALLRVEVHEYDMSEKDDFA	544
	_ `	* *******	-
NbPLC3-1	(Niben101Scf02280)	GQTCLPVSELRQGIRAVSLHDRKGEKYNSVKLLMRFEFV 588	
Nbplc3-2	(Niben101Scf04093)	GQTCLPVSELRQGTRAVSLHDRKGEK 442	
S1PLC3	(NM001247261)	GOTCLPVSELROG RAVPLHSRKGDKYNSVKLLMRFEFI 583	

Figure S2 Characterization of phosphatidylinositol-specific phospholipase Cs (PI-PLCs) in Nicotiana benthamiana.

(A) Schematic representation of the PLC3 protein structure. PI-PLC-X, PI-PLC-Y and PI3K-C2 domains are the conserved domain essential for PLC activities. (B) Alignment of deduced amino acid sequences of *NbPLC3-1* (Niben101Scf02280g02004.1), *NbPLC3-2* (Niben101Scf04093g00004.1) and *Solanum lycopersicum* PLC3 (*SlPLC3*, NM001247261). Asterisks showed identical amino acids. Gray, black and white boxes indicate PI-PLC-X, PI-PLC-Y and PI3K-C2 domains, respectively.

Fig. S2



Fig. S3 continued



Estimation of expression value of *NbPLC3-1*, *NbPLC3-2*, *NbPLC1-2*, *NbPLC2-1*, NbPLC2-2, *Nbcoi1*, *NbrbohB* and *NbMEK2*.

(A) Total RNAs were isolated from empty vector control, *NbPLC3-1*- (VIGS:PLC3-1), *NbPLC3-2*- (VIGS:PLC3-2) and both *NbPLC3-1*- and *NbPLC3-3*- (VIGS:PLC3) silenced plants. Transcript levels of *NbPLC3-1* and *NbPLC3-2* were estimated by qRT-PCR. Values represent means \pm SDs from triplicate experiments. (B) Total RNAs were isolated from empty vector control and both *NbPLC3-1*- and *NbPLC3-2*- (VIGS) silenced plants. Transcript levels of *NbPLC2-1* and *NbPLC2-2* were estimated by qRT-PCR. (C) Total RNA was isolated from empty vector control (Control), *Nbcoi1* (coi1) and *NbPLC3s:Nbcoi1* double-knockdown (PLC3/coi1), *NbrbohB* (rbohB) and *NbPLC3s:NbrbohB* double-knockdown (PLC3/rbohB), *NbMEK2* (MEK2) and *NbPLC3s:NbMEK2* double-knockdown (PLC3/MEK2) plants. Transcript levels of *Nbcoi1*, *NbrbohB* and *NbMEK2* were estimated by qRT-PCR. Transcripts relative to the absolute non-treated control were normalized to internal standard genes (NbUbe35/NbNQO). Values represent means \pm SDs from triplicate experiments.

Fig. S4



Figure S4 Effects of *NbPLC3-1-* and *NbPLC3-2-*specific silencing on hypersensitive response (HR) induction by incompatible *Ralstonia solanacearum* 8107.

Control, *NbPLC3-1-* and *NbPLC3-2-*silenced plant leaves were infiltrated with *R. solanacearum* strain 8107. (A) The bacterial populations of *R. solanacearum* 8107 in Control (gray bar), *NbPLC3-1-*silenced (VIGS; black bar) and *NbPLC3-2-*silenced (VIGS; white bar) plants were determined by plating at specified time points. Values are means \pm SDs of five replicate experiments. (B) Cell death induced by *R. solanacearum* was determined in Control (gray bar), *NbPLC3-1-*silenced (VIGS; black bar) and *NbPLC3-2-*silenced (VIGS; white bar) plants by measuring the ion conductivity levels. Values represent means \pm SDs (n = 5).

Fig. S5



Figure S5. Effects of *NbPLC3s*-silencing on HR induction by *Pseudomonas cichorii*, *Pseudomonas syringae* pv. *phaseolicola*, AvrA, INF1 and TMGMV-CP with L1.

Control and *NbPLC3s*-silenced *N. benthamiana* plants were infiltrated with *Pseudomonas cichorii*, *P. syringae*, *Agrobacterium* harboring independently 35S-AvrA (AvrA) and 35S-INF1 (INF1). Cell death induction was determined by measuring the ion conductivity level. Values represent means \pm SDs (n = 5).





Figure S6. Bacterial population and hypersensitive cell death induction by incompatible *Ralstonia solanacearum* 8107 in *Nbcoi1*, *NbrbohB* and *NbMEK2*-silenced plants and NahG plant.

Control, *Ncoi1-*, *NbebohB* and *NbPLCNEK2*-silenced plant leaves were infiltrated with *R. solanacearum* strain 8107. (A) Cell death induced by *R. solanacearum* was determined in Control (gray bar), *NbPLC3-1*-silenced (VIGS; black bar) and *NbPLC3-2*-silenced (VIGS; white bar) plants by measuring the ion conductivity levels. Values represent means \pm SDs (n = 5). (B) The bacterial populations of *R. solanacearum* 8107 in Control (gray bar), *NbPLC3-1*-silenced (VIGS; black bar) and *NbPLC3-2*-silenced (VIGS; white bar) plants by measuring the ion conductivity and *NbPLC3-2*-silenced (VIGS; white bar) plants were determined by plating at specified time points. Values are means \pm SDs of five replicate experiments.

Table S1 Primers used in this paper

Primers	Sequence [5 ⁻³]	Purpose	Reference
DI C2 18		Full length cDNA	This study
PLC3-15	CGCCAGTGTTCAAATTGATTTG	Full length cDNA	This study
PLC3 2S	CAACAATCCATTATAATCCCAATTTTTATAC	Full length cDNA	This study
PLC3 2A	TATCATTTTGTTGATACTATAATTTGAACCTTG	Full length cDNA	This study
DI C3eVICSS	GTCGACGATTCACCATGATATGAATGAG	VIGS vector	This study
PI C3sVIGSR	GTCGACGAATCGGGAGAAAACAGCATG	VIGS vector	This study
PLC3-1VIGSS	GTCGACTTGTTTTCTCTCTTATGTTTCTGTGTT	VIGS vector	This study
PLC3-1VIGSA	GTCGACTTGTCCACCTAAAGCTTAGATAAAT	VIGS vector	This study
PLC3-2VIGSS	GTCGACGTCGTTTGTTTTCTCTTATGTTC	VIGS vector	This study
PLC3-2VIGSA	GTCGACTATCATTTTGTTGATACTATAATTTG	VIGS vector	This study
RV20	CAGGAAACAGCTATGAC	Sequencing	Maimbo et al. 2007
M4	GTTTTCCCAGTCACGAC	Sequencing	Maimbo et al. 2007
NbPLC3-1rtpF	GTTCCCATTAACAGTTCCTGAG	aRT-PCR	This study
NbPLC3-1rtpR	GCGACTCACAGTTAGACAAATTCGAAG	aRT-PCR	This study
NbPLC3-2rtpF	GTTCCCATTAACAGTTCCTGAA	qRT-PCR	This study
NbPLC3-2rtpR	TTTCGAATTTGTCTAACTGTGAGTCGT	qRT-PCR	This study
NbPR1rtpF	GGTCAACACGGCGAAAACC	qRT-PCR	Maimbo et al. 2007
NbPR1rtpR	GCCTTAGCAGCCGTCATGA	qRT-PCR	Maimbo et al. 2007
NbPR4rtpF	GGCCAAGATTCCTGTGGTAGAT	qRT-PCR	Maimbo et al. 2007
NbPR4rtpR	CACTGTTGTTTGAGTTCCTGTTCCT	qRT-PCR	Maimbo et al. 2007
NbICS1rtpF	ATTTCATGGTCCCTCA	qRT-PCR	Maimbo et al. 2007
NbICS1rtpR	TTCCTGGTCAAACAT	qRT-PCR	Maimbo et al. 2007
NbORPrtpF	AGGCACTAAGATTTCTC	qRT-PCR	Maimbo et al. 2007
NbOPRrtpR	GTTGATCCCATCTTTC	qRT-PCR	Maimbo et al. 2007
Nbhin1rtpF	CGGTCCACCCGAAGTCAA	qRT-PCR	Maimbo et al. 2007
Nbhin1rtpR	ACGCCGTCGATGAAGTAGTCA	qRT-PCR	Maimbo et al. 2007
NbUbe35rtpF	CTTCAGATTCGCACCGTTCT	qRT-PCR	Pombo et al. 2018
NbUbe35rtpR	CCAATGCTTCGCAATGTTCTC	qRT-PCR	Pombo et al. 2018
NbNQOrtpF	AAGGCGGTGGTCAAGAAA	qRT-PCR	Pombo et al. 2018
NbNQOrtpR	CAAACATACCAGCACCGAATG	qRT-PCR	Pombo et al. 2018
NbrbohBrtpF	AACAACCTCGGATACATTAT	qRT-PCR	Deang et al. 2015
NbrbohBrtpR	TGTAAATAGACCAGCCATAA	qRT-PCR	Deang et al. 2015
NbMEK2rtpF	GTGATTGGGCTAGTCTTAT	qRT-PCR	Deang et al. 2015
NbMEK2rtpR	AGTGGCTGGTCTATTCTGG	qRT-PCR	Deang et al. 2015
Nbcoi1rtpF	GCTCCACGCGATTACCAACGG	qRT-PCR	Song et al. 2020
Nbcoi1rtpR	CTGCCACCATCTCTTGCACACC	qRT-PCR	Song et al. 2020

Table S2 List of plasmids used in this study.

Plasmid	Relevant characteristics	Reference
pMD20	Cloning vector pMD20, Amp ^r	Takara Bio
pMD-NbPLC3-1 full	pMD20 carrying full length sequence of NbPLC3-1 cDNA	This study
pMD-NbPLC3-2 full	pMD20 carrying full length sequence of NbPLC3-2 cDNA	This study
pMD-NbPLC3s	pMD20 carrying identical sequence of NbPLC3-1 and NbPLC3-2 fragment for VIGS	This study
pMD-NbPLC3-1	pMD20 carrying NbPLC3-1 fragment for VIGS	This study
pMD-NbPLC3-2	pMD20 carrying full length NbPLC3-2 fragment for VIGS	This study
pPVX201	pPVX binary vector, Km ^r	Baulcombe et al., 1995
pPVXPLC3s	pPVX binary vector containing identical sequence of NbPLC2-1 and NbPLC3-2 fragment for VIGS	This study
pPVXPLC3-1	pPVX binary vector containing PLC3 -1 cDNA fragment for VIGS	This study
pPVXPLC3-2	pPVX binary vector containing PLC3 -2 cDNA fragment for VIGS	This study
P35S-AvrA	pMDC32-derived plasmid that contains the full-length avrA	Poueymiro et al. 2009
P35S-INF1	pGR106 binary vector carrying PR1a:Inf1 fusion gene	Huitema et al., 2005
P35S-MEK ^{DD}	pGreen binary vector carrying constitutive active form of MEK	Katou et al. 2003
P35S-TMGMV-CP	pRI101-AN vector carrying coat protein gene from TMGMV	Gupta et al. 2013
P35S-L1	pRI101-AN vector carrying <i>L1</i> gene from papper	Gupta et al. 2013