

**Table S1.** Primers used for cloning of *PhENDO1*, gene expression analysis by qPCR, and making constructs for VIGS experiments.

Primer Function	Direction	Sequence
RT-PCR clone	Forward	5'- TGGAGCAAAGARGGNCA -3'
	Reverse	5'- TTTCNCCRGCTTCRACACCTT -3'
RACE	Forward	5'- TGGCGACTTATCGGCCCTCTGCGTGTGG -3'
	Reverse	5'- ACTTGGTCCGGCCACACGCAGAGGGC -3'
Full length sequence	Forward	5'- TCATTGTGCTTCTTTGAGCATGTGGG -3'
	Reverse	5'- GACTTGTCATCTCGTGTTACATGGAGAGC -3'
<i>PhENDO1</i> qPCR	Forward	5'- CCACTTGCAGCAACTTGATCAACCA -3'
	Reverse	5'- TCGACGACGACGAACGATACTTGT -3'
<i>PhCP10</i> qPCR	Forward	5'- ACTTTGTGGACTTGCAACGGAAGC -3'
	Reverse	5'- CCAAGCCTATCTCAATCCCATACA -3'
<i>PhACTIN</i> qPCR	Forward	5'- AGCCAACAGAGAGAAGATGACCCA -3'
	Reverse	5'- ACACCATCACCAGAGTCCAACACA -3'
<i>PhRPS13</i> qPCR	Forward	5'- CAGGCAGGTTAAGGCAAAGC -3'
	Reverse	5'- CTAGCAAGGTACAGAAACGGC -3'
VIGS, Exp 1	Forward	5'- catctccatggTCAAGCCCTCTCCACTTCAT -3'
	Reverse	5'- catgtggtaccGTGCAAGTTTGACTTGTGCC -3'
VIGS, Exp 2	Forward	5'- tagtttgatccTGTCTTGCTTTCATTACCCAAC -3'
	Reverse	5'- cataattctagaTGCAAGCTTCATCTGGTGTAT -3'

1 ATGTTGGGGTTAACTTCATTAAGCATTAGTTTCTTTCTGTCTT  
M L G L T S L S I S F F L C L

46 GCTTTCATTACCCAACATGATGTTGAAGCATGGAGTAAAGAGGGT  
A F I T Q H D V E A **↑**W S K E G

91 CATATGATGACTTGTGCAATCGCCCAGGAGCTCTGAATGATGAG  
H M M T C R I A Q E L L N D E

136 GCAGCTCATGCGGTTAAGATGTTGTTACCGGATTATGTTAATGGC  
A A H A V K M L L P D Y V N G

181 GACTTATCGGCCCTCTGCGTGTGGCCGACCAAGTCCGGCATTGG  
D L S A L C V W P D Q V R H W

226 TACAAGTATAGGTGGTCAAGCCCTCTCCACTTCATTGATACACCA  
Y K Y R W S S P L H F I D T P

271 GATGAAGCTTGCAACTTTGATTATGATAGGGATTGTCATGATGAA  
D E A C N F D Y D R D C H D E

316 CATGGAGTGAAGGATATGTGTGTTGCTGGTGCCATCCAAAAC TTT  
H G V K D M C V A G A I Q **□** F

361 ACCACTCAGCTTTCCCATACCCAGAGGGAAC TCCGATCGCCGA  
T T Q L S H Y P E G T S D R R

406 TATAATATGACAGAGGCCTTGTTATTCTTGTCACATTTTCATGGGA  
Y **□** M T E A L L F L S H F M G

451 GATATCCATCAACCGTTGCATGTTGGATTACAAAGTGAAGGA  
D I H Q P L H V G F T S D E G

496 GAAATACAATTAATCTGCGCTGGTTTAGGCACAAGTCAAAC T TG  
G N T I N L R W F R H K S N L

541 CACCATGTATGGGATAGAGAGATGATTCTACAAGCTGCAAAAAGAC  
H H V W D R E M I L Q A A K D

586 TATTATGCAAAGGATGTAACCTCCTTGAAGCAGACATTGAAGGA  
Y Y A K D V N L L E A D I E G

631 AACTTCACTGATGGAATCTGGACTGATGATCTTTCTTCTTGAGG  
**□** F T D G I W T D D L S S W R

676 GAATGTGGAAATCTCTTCTCCTGTGTGAATAAGTTTGCTGCGGAA  
E C G N L F S C V N K F A A E

721 AGTATAAGTATAGCTTGCAAATGGGGATACAAAGGTGTTGAGGCT  
S I S I A C K W G Y K G V E A

766 GGCGAAACATTATCAGATGATTACTTCAATTCAAGACTCCCAATT  
G E T L S D D Y F N S R L P I

811 GTGATGAAAAGAATAGCTCAAGGTGGAGTACGATTAGCTATGCTT  
V M K R I A Q G G V R L A M L

856 CTA AATCGGGTTTTTGGAGATTCTCAAGAAGATCCACTTGCAGCA  
L N R V F G D S Q E D P L A A

901 ACTTGA  
T \*

**Figure S1.** The sequences of the *PhENDO1* coding region and the predicted protein. The *PhENDO* cDNA encodes a protein of 301 amino acids. The first 25 amino acids encode a signal peptide, and the arrow indicates the cleavage site of the signal peptide. The solid lines are the 14-12 endonuclease peptides that were sequenced from 2-DE [31]. Three predicted glycosylation sites are located at 119, 137, and 211 (gray boxes).

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PhEND01      1  -MLGLTSLSISFFLCLAF---ITQHDVEAWSKEGHHMTCRIAQGLLNDEAAHAVKMLLPDYVNG-DLSAL
SlEND01      1  -MLRLTSLSSIFFLCLAF---INQHGEAWSKEGHVMTCRIAQGLLNDEAAHAVKMLLPEYVNG-DLSAL
StEN1        1  RMLRLTLLSIFFLCLAF---LNHHCAEAWSKEGHMTCRIAQGLLNDEAAHAVKMLLPEYVNG-DLSAL
NaEndonuclease
SA6          1  -MLRLTSLSSIFFLCLAF---ITQPGVKAWSKEGHMTCQIAQDLNDEAAHAVKMLLPEYVDG-DLSAL
ZEN1         1  ----MALIRLSLISCLGFFMINNYNAVQAWSKEGHVMTCRIAQGLLSPDAAHAVQMLLPDYVKG-NLSAL
nucZel       1  -----LSTVLLLLVFPVPTVRCWGVGDGHFTICKIAQGRLSQTAVDAVNSLLPEYAEQ-DLASL
nucZe2       1  ---MSHLELW-WLKSTCSIFLLSIPGVIGWGKEGHYATCKIAQSFLSEALNAVKELLPETAEG-DLASV
AtEND01      1  -MASAFRSSTRLLVGLGILILCSVSSVRSWSKEGHLITCRIAQNLLEAGPAHVVENLLPDYVKG-DLSAL
AtEND02      1  -MANQKGLH-VVMMLITVWLLYAAPNIHCWGKEGHEITICKIAQTRLDETAAKAVKELLPESAEG-DLSSL
AtEND03      1  -MGWSLRMW-IVS---ILVLTQLVNGALCWGDAGHYAVCKIAQSYFEDTVVAVKLLPEASANG-ELAAV
AtEND04      1  -MSSSLRQW-FAR---VLVLTQLINGALCWGKEGHYTVCKIAESYFEEETVAAVKLLPKSADG-DLASV
AtEND05      1  -----MRLW-IVS---VLVLTHLVHCALCWGKDGHYTVCKLAEGFFEDDTIAAVKLLPEESVDGGGLADF

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PhEND01      66  CVWPDQVRHWYKRWSSPLHFIDTPEACNFDYDRDCHDEHGKDMCVAGAIQNFITQLSHYPEGT-SDR
SlEND01      66  CVWPDQVRHWYKRWSSPLHFIDTPEACNFDYDRDCHDQHGKDMCVAGAIQNFITQLSHYREGT-SDR
StEN1        67  CVWPDQVRHWYKRWSSPLHFIDTPEACNFDYDRDCHDQHGKDMCVAGAIQNFITQLSHYREGT-SDR
NaEndonuclease
SA6          66  CVWPDQVRHWYKRWSSPLHFIDTPEACNFDYDRDCHDQHGKDMCVAGAIQNFITQLSHYREGT-SDR
ZEN1         63  CTWPDQIRHWYKRWSSPLHFIDTPEACNFDYDRDCHDQHGKDMCVAGAVHNYITQLMHYRDGT-SDR
nucZel       66  CVWPDQIRHWYKRWSSPLHFIDTPEACNFDYDRDCHDQHGKDMCVAGAIQNFITQLSHYQHG-SDR
nucZe2       59  CSWADHVKF--RYHWSALHYIDTPEACNFDYDRDCHDQHGKDMCVAGAIQNFITQLLDYK---QTS
AtEND01      69  CVWPDQIRHWYKRWSSPLHFIDTPEACNFDYDRDCHDQHGKDMCVAGAIQNFITQLSHYREGT-SDR
AtEND02      68  CLWADRVKF--RYHWSALHYIDTPEACNFDYDRDCHDQHGKDMCVAGAIQNFITQLSHYREGT-SDR
AtEND03      65  CSWDEIKKLPQWRWTSALHFADTPEACNFDYDRDCHDQHGKDMCVAGAIQNFITQLSHYREGT-SDR
AtEND04      65  CSWDEIKKLPQWRWTSALHFADTPEACNFDYDRDCHDQHGKDMCVAGAIQNFITQLSHYREGT-SDR
AtEND05      62  CSWDEIKKLSQWQWTSALHFADTPEACNFDYDRDCHDQHGKDMCVAGAIQNFITQLSHYREGT-SDR

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PhEND01      135 RYNMTEALLFLSHFMGDIHQPLHVGFSDGEGNTINLRWFRHKSNLHHVWDREIILTAAKDYYAKDVNLL
SlEND01      135 RYNMTEALLFLSHFMGDIHQPLHVGFSDGEGNTINLRWFRHKSNLHHVWDREIILTAAKDYYAKDVNLL
StEN1        136 RYNMTEALLFLSHFMGDIHQPLHVGFSDGEGNTINLRWFRHKSNLHHVWDREIILTAAKDYYAKDVNLL
NaEndonuclease
SA6          132 RYNMTEALLFLSHFMGDIHQPLHVGFSDGEGNTINLRWFRHKSNLHHVWDREIILTAADYYAKDLDAF
ZEN1         135 RYNMTEALLFLSHFMGDIHQPLHVGFSDGEGNTINLRWFRHKSNLHHVWDREIILTAASELYDKDMESL
nucZel       124 QYNLTEALLFLSHFMGDIHQPLHVGFSDGEGNTIDVHWFTRKAVLHHVWDDSIETAEERFYGSNVENL
nucZe2       137 RYNLTEALLFLSHFMGDIHQPLHVGFSDGEGNTIDVHWFTRKAVLHHVWDDSIETAEERFYGSNVENL
AtEND01      138 RYNMTEALLFLSHFMGDIHQPLHVGFSDGEGNTINLRWFRHKSNLHHVWDREIILTAALKENYDKNLDL
AtEND02      135 QYNLTEALLFLSHFMGDIHQPLHVGFSDGEGNTIDVHWFTRKAVLHHVWDDSIETAEERFYGSNVENL
AtEND03      130 HYNLTEALLFLSHFMGDIHQPLHVGFSDGEGNTIDVHWFTRKAVLHHVWDDSIETAEERFYGSNVENL
AtEND04      135 HYNLTEALLFLSHFMGDIHQPLHVGFSDGEGNTIDVHWFTRKAVLHHVWDDSIETAEERFYGSNVENL
AtEND05      132 HYNLTEALLFLSHFMGDIHQPLHVGFSDGEGNTIDVHWFTRKAVLHHVWDDSIETAEERFYGSNVENL

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PhEND01      205 EADIEGNFTDGLWSDDLASWRECGN-LFSCVNKFATESISLACKWGYKVEAGETLSDDYFNSRLPIVMK
SlEND01      205 EADIEGNFTDGLWSDDLASWRECGN-LFSCVNKFATESISLACKWGYKVEAGETLSDDYFNSRLPIVMK
StEN1        206 EADIEGNFTDGLWSDDLASWRECGN-LFSCVNKFATESISLACKWGYKVEAGETLSDDYFNSRLPIVMK
NaEndonuclease
SA6          202 QQDLQNNFTTGLWSDDLASWRECGN-LFSCVNKFATESISLACKWGYKVEAGETLSDDYFNSRLPIVMK
ZEN1         205 QKALQANFTHGLWSDDLASWRECGN-LFSCVNKFATESISLACKWGYKVEAGETLSDDYFNSRLPIVMK
nucZel       194 IDALETNIINW-WGDQVKAWENCSANQKTCPNLYATEGIIKAAACNWAYKGVTVNGSVLEDDYFNSRLPIVW
nucZe2       207 ISALEKNIITDR-WSNDISSWVNCISGEEVCPDPWASESIKYSNAYRNATPGSTLGDYFYFNSRLPIVEM
AtEND01      208 QEDLEKNIITDR-WSNDISSWVNCISGEEVCPDPWASESIKYSNAYRNATPGSTLGDYFYFNSRLPIVEM
AtEND02      205 VDALKKNIITTE-WADQVWRWETCTK-KTACPDYASEGIQAACDWAYKGVTVNGSVLEDDYFYFNSRLPIVYQ
AtEND03      200 IHELQAKLKNG-WSNDVPSWESQNLNQTACPNPYASESIDLACKYAYRNATAGTTLGDYFYFNSRLPIVEK
AtEND04      205 IEALQANLITD-WSNDVPLWESQNLNQTACPNPYASESIDLACKYAYRNATAGTTLGDYFYFNSRLPIVEK
AtEND05      202 IQALQAKLKNG-WSNDVPSWESQNLNQTACPNPYASESIDLACKYAYRNATAGTTLGDYFYFNSRLPIVEK

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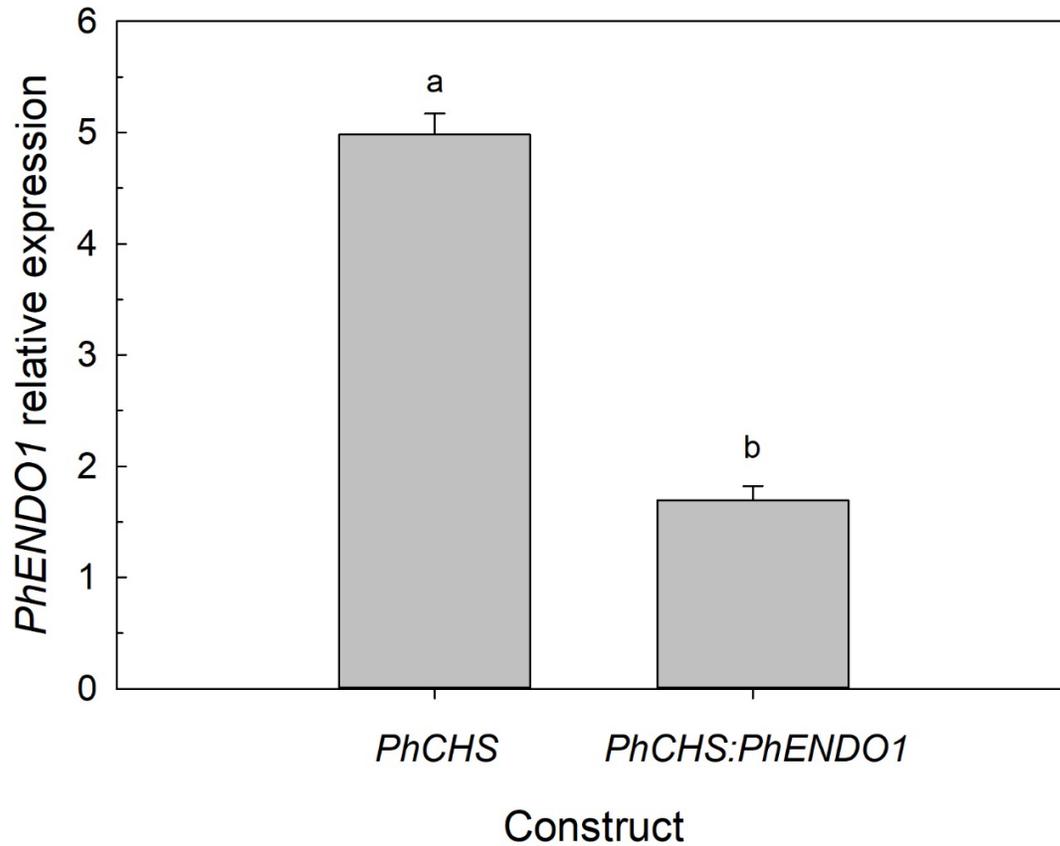
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PhEND01      274 RIAQGGVRLAMLNLRVFGDSQEDPLAAT-----
SlEND01      274 RVAQGGIRLAMLNLRVFGASQEDSVVAT-----
StEN1        275 RVAQGGIRLAMLNLRVFGVSQEDSVGAT-----
NaEndonuclease
SA6          274 RIAQGGVRLAMLNLRVFGDSQEDSVAPT-----
ZEN1         274 RIAQGGVRLAMLNLRVFGSSSSLEDALVPT-----
nucZel       263 RLAQGGVRLAANLNRIFG-----
nucZe2       276 RLAQGGVRLAATLNRIFGDPYPSVSKVAYFGKYPTCATHYLKLDVSSQTSFVN
AtEND01      277 RIVQGGVRLAMLNLRVFGSDHAIAGVAAT-----
AtEND02      273 RLAQGGVRLAATLNRIFG-----
AtEND03      269 RLAQGGIRLAGTILNRIFSAKRKLARA-----
AtEND04      274 RLAQGGIRLAGTILNRIFSSKPKHAGS-----
AtEND05      271 RLAQGGIRLAGTILNRIFSAKPKLAGL-----

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**Figure S2.** Alignment of the deduced amino acid sequences of endonuclease enzymes.

Endonuclease *PhENDO1* from petunia is compared with the amino acid sequences from *Nicotiana attenuata* NaEndonuclease1 (XP\_019234897), *Solanum tuberosum* StEN1 (AAT79582), *Solanum lycopersicum* SIENDO1 (BAL03523), *Arabidopsis thaliana* ENDO1/BFN1, ENDO2, ENDO3, ENDO4 and ENDO5 (NP\_172585, NP\_176996, NP\_001078420, NP\_001328107 and NP\_567631), *Zinnia elegans* ZEN1, ZEN2, and ZEN3 (BAA28948, AAD00694 and AAD00695), and *Hemerocallis* hybrid SA6 (AAC34856). Dark gray shading are residues that are identical in all sequences and light gray shading are residues that are functionally identical. Dashes indicate gaps introduced to produce the alignment, asterisks (\*) are residues involved in the binding of zinc atoms, plus signs (+) identify residues involved in forming disulfide bonds; and number symbols (#) are structurally important glycosylation sites. Active sites for RNase and DNase activities in nucleases (His residues at positions 85 and 157, respectively, in PhENDO1) are also indicated under the alignment [17].



**Figure S3.** *PhENDOI* transcript abundance was reduced in senescing flowers from pTRV2:*PhCHS:PhENDOI* plants. Relative abundance of *PhENDOI* in senescing corollas (48 h after pollination) was determined by qPCR in pTRV:*PhCHS* and pTRV2:*PhCHS:PhENDOI* petunias. Bars represent mean  $\pm$  SE (n=3) and least squared means were evaluated at  $P < 0.05$ . Different letters indicate significant difference between the means.