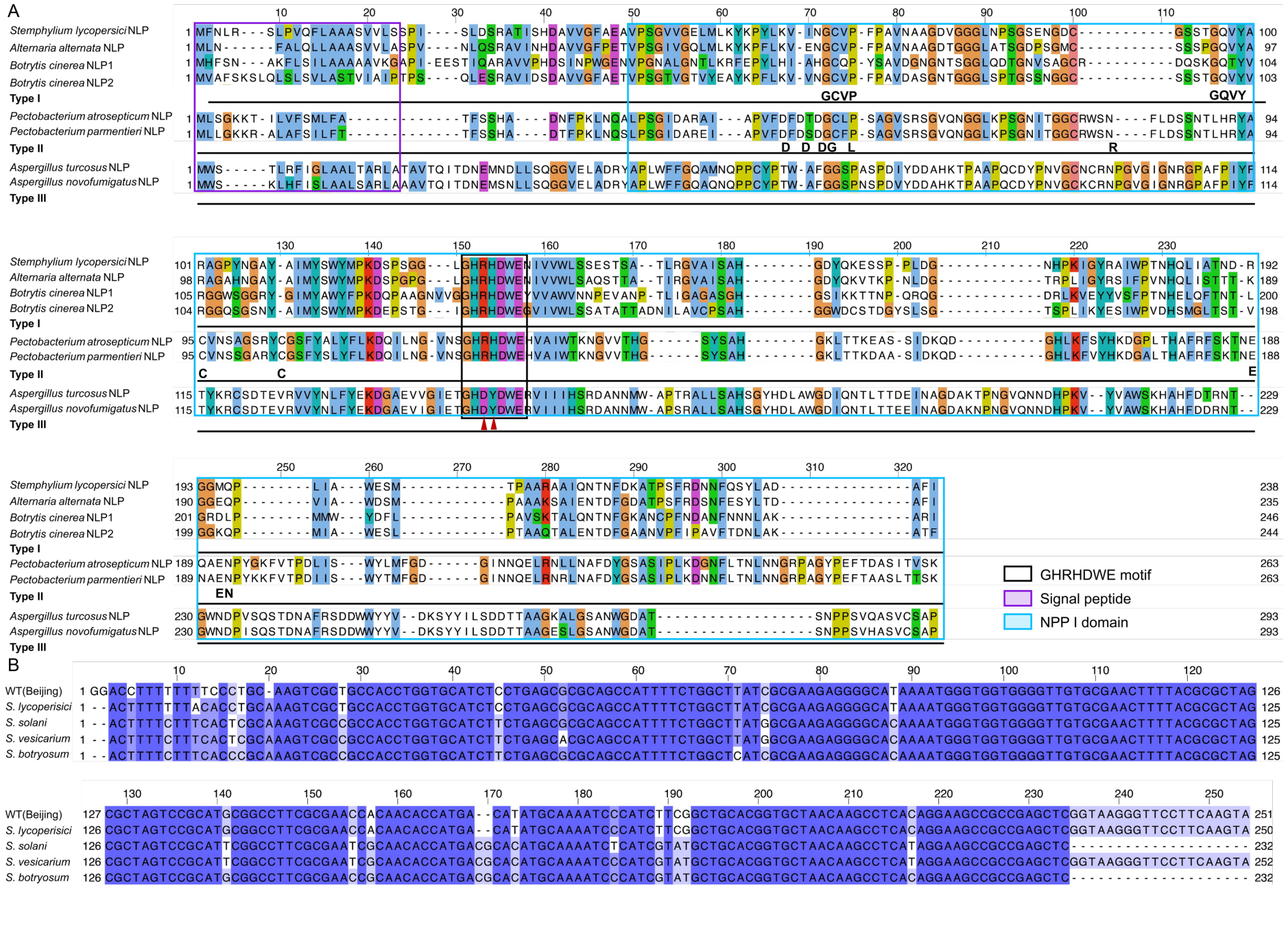
A
Stemphylium lycopersici NLP
Alternaria alternata NLP
Botrytis cinerea NLP1
Botrytis cinerea NLP2
Type I
GCVP
GQVY
Pectobacterium atrosepticum NLP
Pectobacterium parmentieri NLP
Type II
D D DG L
R
Aspergillus turcosus NLP
Aspergillus novofumigatus NLP
Type III
C
C
E
EN
GHRHDWE motif
Signal peptide
NPP I domain
B
WT(Beijing)
S. lycoperisici
S. solani
S. vesicarium
S. botryosum

**Figure S1 .** Alignment of NLP proteins shows that *S. lycopersici* NLP is a type I NLP protein. (**A**) The *S. lycopersici* NLP protein sequence was aligned with typical type I, type II, and type III NLP proteins. Purple and blue boxes represent the signal peptide and NPP I domain, respectively. The black box highlights the most conserved heptapeptide motif (GHRHDWE) in the NPP1 domain; red triangles indicate the two amino acids (RH) which were conserved in type I and type II but divergent (DY) in type III NLPs. The conserved DxDxDG motif specific to type II NLPs is also noted. (**B**) A nucleotide alignment use *EF1α* gene in *Stemphylium* spp. to confirm the WT strain is a *S. lycopersicis* strain. The alignment was performed by the Jalview with Muscle method (http://www.jalview.org, accessed on 15 May 2022). *EF1α* gene sequence of WT (Beijing) strain used in this study, *S. lycopersici*, *Stemphylium solani*, *Stemphylium vesicarium,* and *Stemphylium botryosum* f. sp. *Lycopersici* were aligned.

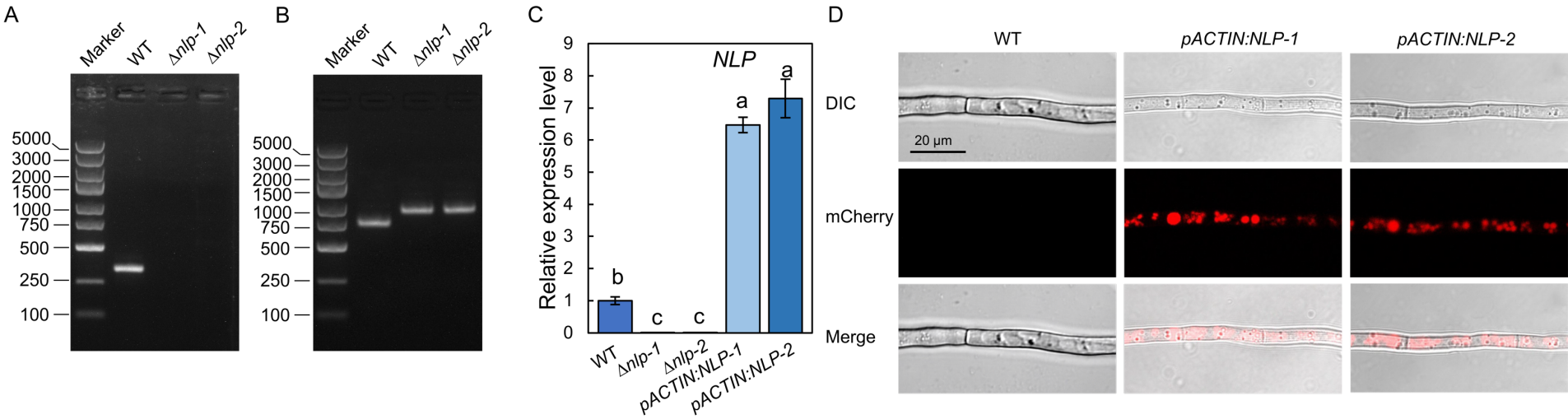


**Figure S2**. Confirmation of *NLP* gene knockout and overexpression *S. lycopersici* strains. (**A**) PCR results showing the targeted replacement of *NLP* gene in Δ*nlp-1* and Δ*nlp-2*. (**B**) PCR results of full-length amplification with primers (NLP-full-check) designed outside the gene indicate the length of *NLP* gene has changed in Δ*nlp-1* and Δ*nlp-2*. (**C**) Relative expression of *NLP* gene in the WT, Δ*nlp-1*, Δ*nlp-2*, *pACTIN:NLP-1*, and *pACTIN:NLP-2* strains determined by RT-qPCR. (**D**) mCherry fluorescence in *pACTIN:NLP* strains. Lowercase of a, b, and c denotes significant difference among multiple groups ($p < 0.05$) by Duncan's new multiple range test.

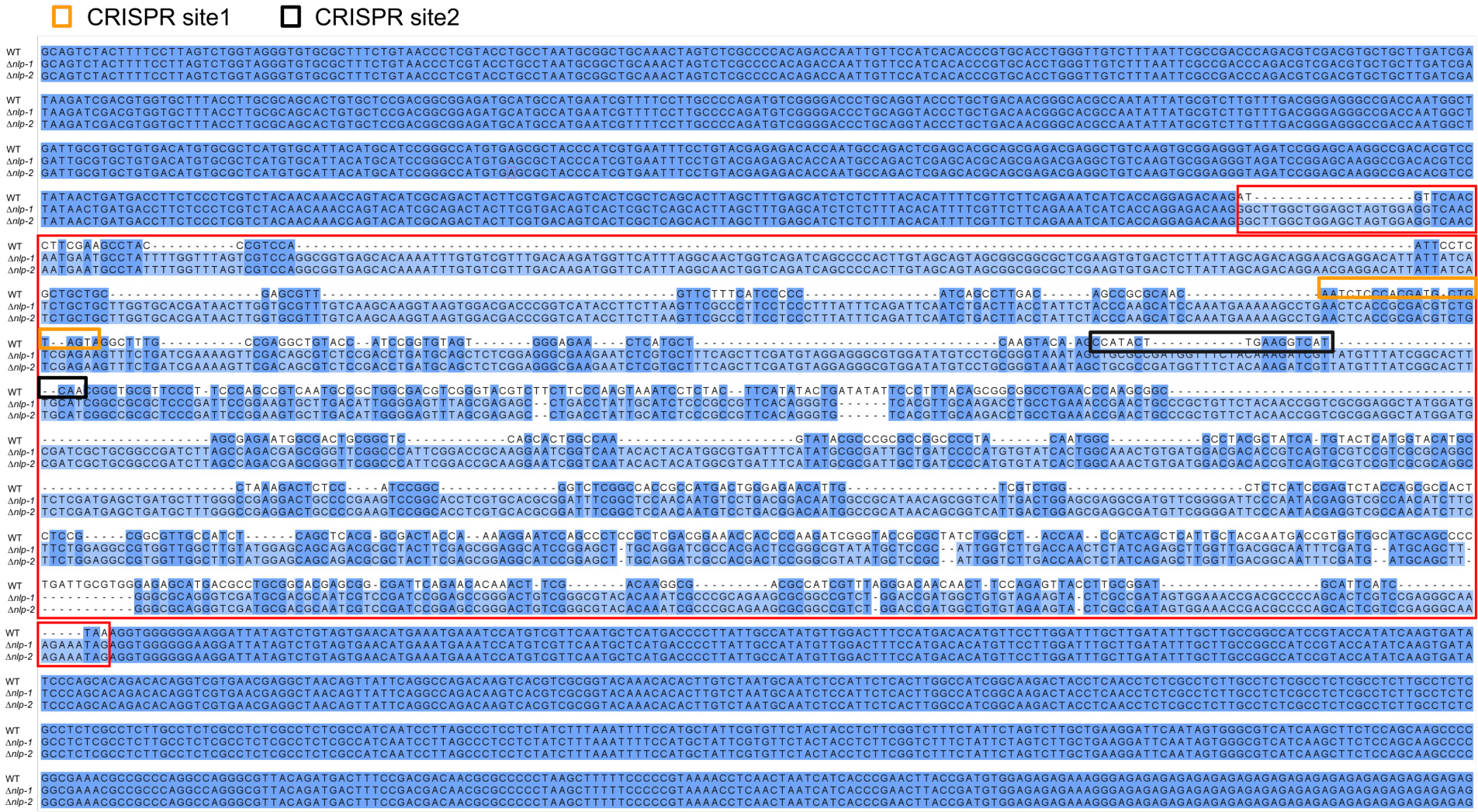


**Figure S3.** Confirmation of *NLP* gene knockout strains by sequence alignment. The red box represents the regions where the *NLP* gene has been replaced by the hygromycin B resistance marker gene (*hph*). The yellow and dark boxes represent CRISPR site 1 and 2 respectively.

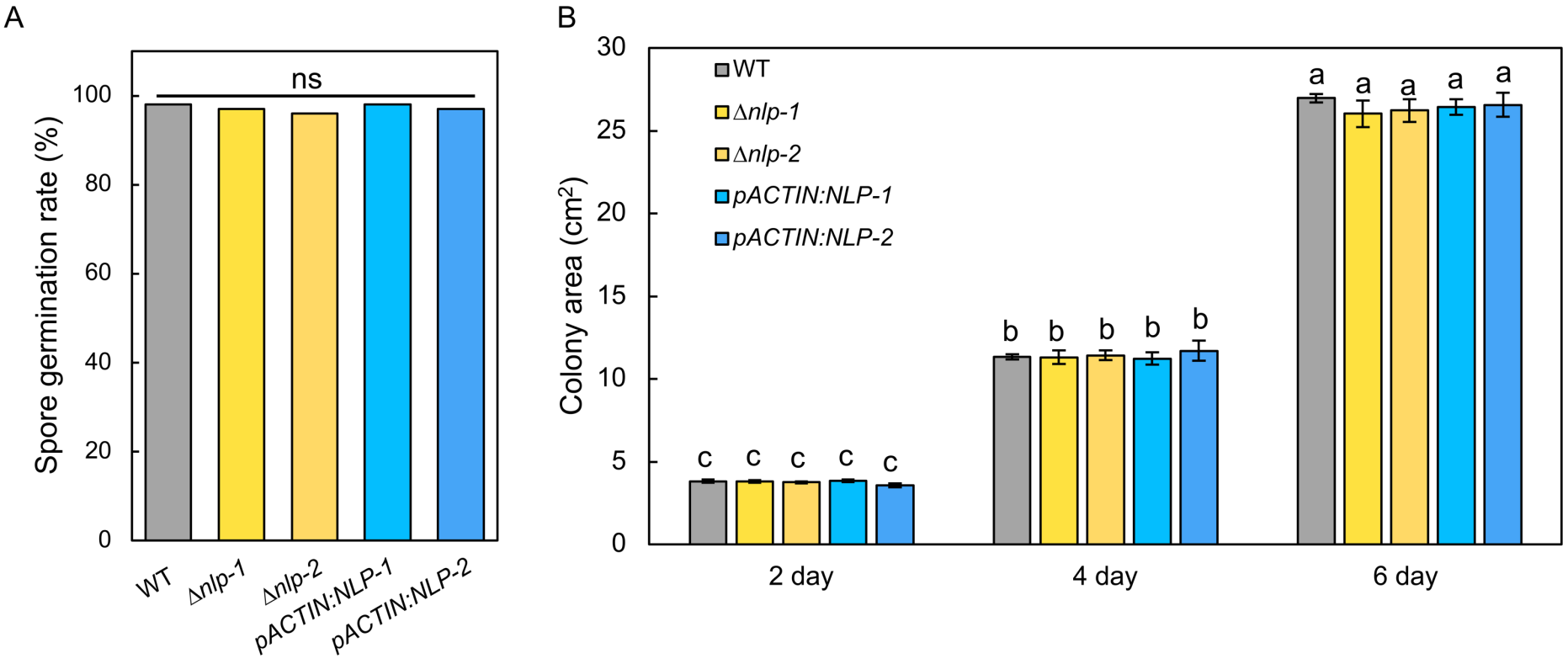


**Figure S4.** NLP does not affect spore germination and colony growth. (**A**) Conidial germination rate of the WT, *NLP* gene knockout mutants, and overexpression strains. (**B**) Colony growth of WT, *NLP* gene knockout mutants, and overexpression strains. Strains were grown on CM medium and colony growth was measured at days 2, 4, and 6 after inoculation. Statistical analyses were conducted using the DPS software; a, b, and c designate statistically significant differences. Lowercase of a, b, and c denotes significant difference among multiple groups ($p < 0.05$) by Duncan's new multiple range test. ns denotes no significant differences.

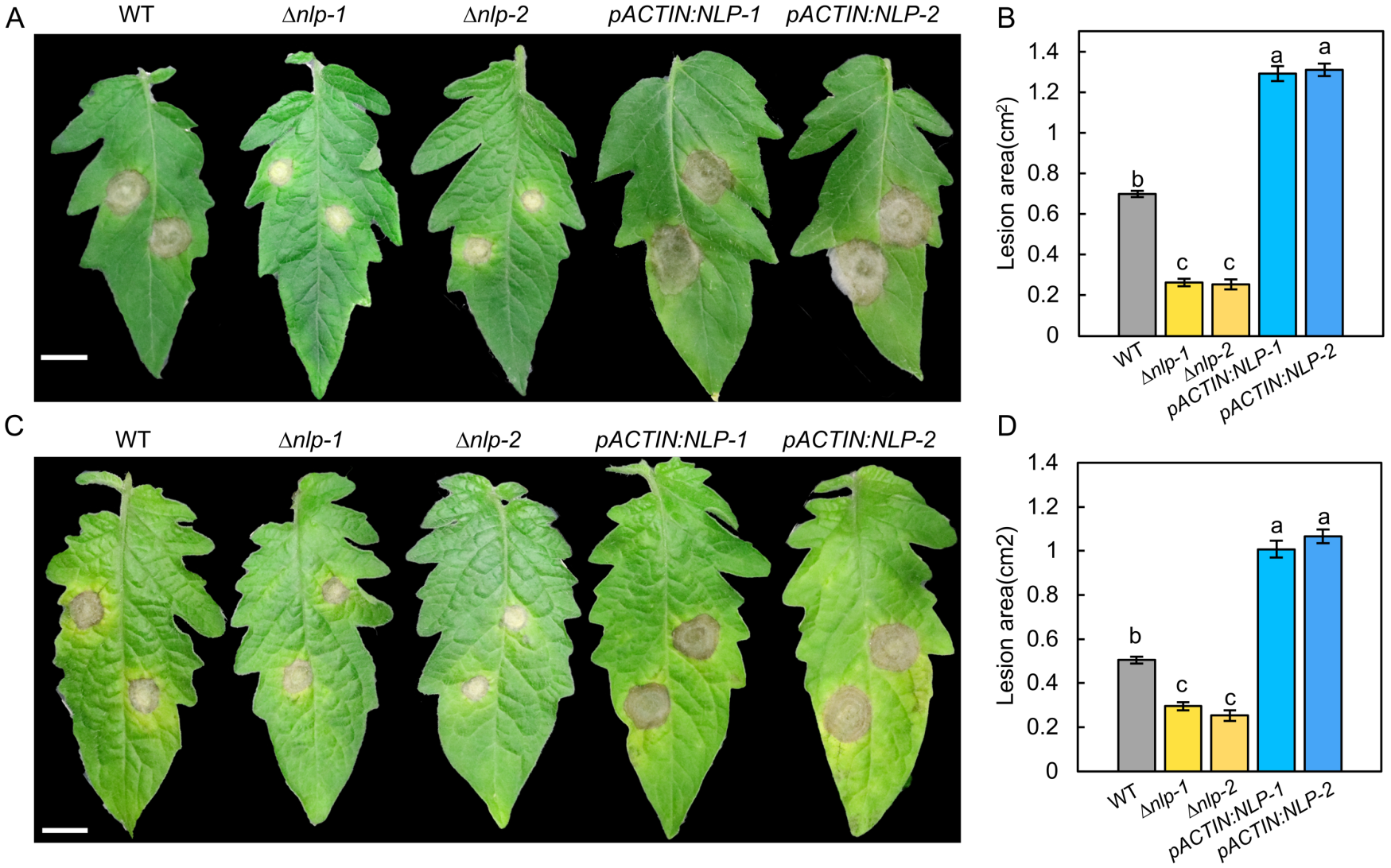


**Figure S5.** NLP is is a key virulence factor of *S. lycopersici* during infection on other tomato cultivars. (**A**) Infected leaves of tomato cultivar AC (Ailsa Craig) by the WT, Δ*nlp*, and overexpression strains at 5 days post inoculation (dpi). (**B**) Lesion area of tomato leaves of AC resulting from *S. lycopersici* infection of WT, Δ*nlp*, and overexpression strains. (**C**) Infected leaves of tomato cultivar E6203 by the WT, Δ*nlp*, and overexpression strains at 5 days post inoculation (dpi). (**D**) Lesion area of tomato leaves (E6203) resulting from infection of WT, Δ*nlp*, and overexpression strains. Lowercase of a, b, and c denotes significant difference among multiple groups ($p < 0.05$) by Duncan's new multiple range test.

Table S1 Primers for fungi NLP identification and gene manipulation

| ID | Primers sequences |
|---|---|
| ITS1 | TCCGTAGGTGAACCTGCGG |
| ITS4 | TCCTCCGCTTATTGATATGC |
| EF1-α-F | CATCGAGAAGTTCGAGAAGG |
| EF1-α-R | TACTTGAAGGAACCCTTACC |
| gpd-F | CAACGGCTTCGGTCGCATTG |
| gpd-R | GCCAAGCAGTTGGTTGTGC |
| NLP U6-1 -crispr -F | TTTATTGAGCTTCTCTTCGTCTCCCACGATGCTGTAGTGTTTTAGAGCTAGAAATAGCA |
| NLP U6-1 -crispr -R | CTATTTCTAGCTCTAAAACTTGATGACCTTCAAGTATGCGAAGAGAAGCTCAATAAAGT |
| NLP-left-F | GGAGGCAGTTTGGATGAATGAC |
| NLP-lef-R | CTTGTCTCCTGGTGATGATTTCTG |
| NLP-right-F | AGGTGGGGGGAAGGATTATAGT |
| NLP- right-R | AAAGTCTGCGGGGAGAGGTG |
| NLP-TG-check-F | AGCGTTGTTCTTTCATCCCC |
| NLP-TG-check-R | AGCCGCAGTCGCCATTCT |
| NLP-full-check-F | ACTACTTCGTGACAGTCACTCGCTC |
| NLP-full-check-R | CGACATGGATTTCATTTCATGTTCACTACAG |
| Fungi-NLP-OE-F | TGAGAGAGCCCGTTTCTGACAGCTCTGCAG ATGTTCAACCTTCGAAGCCTAC |
| Fungi-NLP-OE-R | CATGTTATCCTCCTCGCCCTTGCTCACCATGATGAATGCATCCGCAAGGT |

Table S2 Primers for *NLP* gene overexpression in tomato

| ID | Primers sequences |
|---|---|
| Tomato-NLP-OE-F | ACAAGTTTGTACAAAAAAGCAGGCTTCCTCGAGATGTTCAACCTTCGAAGCCTACC |
| Tomato-NLP-OE-R | ACCACTTTGTACAAGAAAGCTGGGTTGAATTCGATGAATGCATCCGCAAGGTA |

Table S3 Primers Used for RT-qPCR

| ID | Primers sequences |
|---|---|
| NLP-qRT-F | GCTCACGGCGACTACCAAA |
| NLP-qRT-R | TGCTCTCCCACGCAATCAG |
| Fungi-ACTIN-qRT-F | TCCGTGACATCAAGGAGAAGC |
| Fungi-ACTIN-qRT-R | CAAGACAGAAGGCTGGAAAAGA |
| Tomato-ACTIN2-qRT-F | TTGCTGACCGTATGAGCAAG |
| Tomato-ACTIN2-qRT-R | GGACAATGGATGGACCAGAC |
| PR-STH2-qRT-F | GAAGGGGATCCATTGGGACAA |
| PR-STH2-qRT-R | TTCCCATAGCACTATCTTTTCCA |
| ERF.C3-qRT-F | TGCTGAAGGATCATCGCAAG |
| ERF.C3-qRT-R | ACCTAGCCATACACGAACACC |