Supplementary Materials: Toxicity of Recombinant Necrosis and Ethylene-Inducing Proteins (NLPs) from *Neofusicoccum parvum*

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A

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B

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C

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CDNANprvNep3   AACGTCCCCGGCGATGCCATTGGCAACACGTTGAAGAGATTCGAGCCGTTCCTGCACATC 180

DNANprvNep3    GCCCACGGCTGCCAATCCTACCCTGCCGTCGACGGCGAGGGCAACACCGGCTGAGCCGCA 240
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DNANprvNep3    AAGAACACAGGCAGCCCGTCGGGCGGGTGCCGCGACCTCTCCAAGGGCCAGACGTACGTG 360
CDNANprvNep3   AAGAACACAGGCAGCCCGTCGGGCGGGTGCCGCGACCTCTCCAAGGGCCAGACGTACGTG 300

DNANprvNep3    CGGGCCGACTACTACAACGGCAAGTACGGCATCATGTACGCGTGGTACTTCCCCAAGGAC 420
CDNANprvNep3   CGGGCCGACTACTACAACGGCAAGTACGGCATCATGTACGCGTGGTACTTCCCCAAGGAC 360

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DNANprvNep3    GACCCCACCGCCGCCGAGCCACAGCTGCTGGGCGCCGCCGCCTCCGGCCACGGCGGCTAC 540
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D

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E

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DNANprvNep5     TACGAGCCGTTCCTCCACATTGCTCACGGCTGCCAGTCGTACACCGCCGTCAACGCCCGC 240
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DNANprvNep5     GGTGACACCAGGTGAGAACTCTCCTCCGCCCCCTTCGCAATTGCGCATCAACTGACATTC 300
CDNANprvNep5    GGTGACAC-------------------------------------------------- 248

DNANprvNep5     AACACAGCGGTGGTCTTCAGAACTCCGGCGGTGCCACTGCCGGCTGCCGTGATGACCGCA 360
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DNANprvNep5     GGGGCCAGACCTACGCCAGAGGTGCTTGGCACAATGGCCGCTACGCCATCATGTACTCCT 420
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DNANprvNep5     GGCAGCCGCCTCCAGGTCGAATACATGACCAACTTCCCCAGGAACCACGAGCTTCAGTTC 720
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DNANprvNep5     TTCGAGAGCAACCTCGGCAAGGCCTGGATCTAA   873
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Figure 1. Alignment of the DNA and cDNA sequence of *Neofusicoccum parvum* NLPs; **A**: NprvNep1, **B**: NprvNep2, **C**: NprvNep3, **D**: NprvNep4, and **E**: NprvNep5. Red boxes indicate the absence and presence of the introns in cDNA and DNA, respectively.

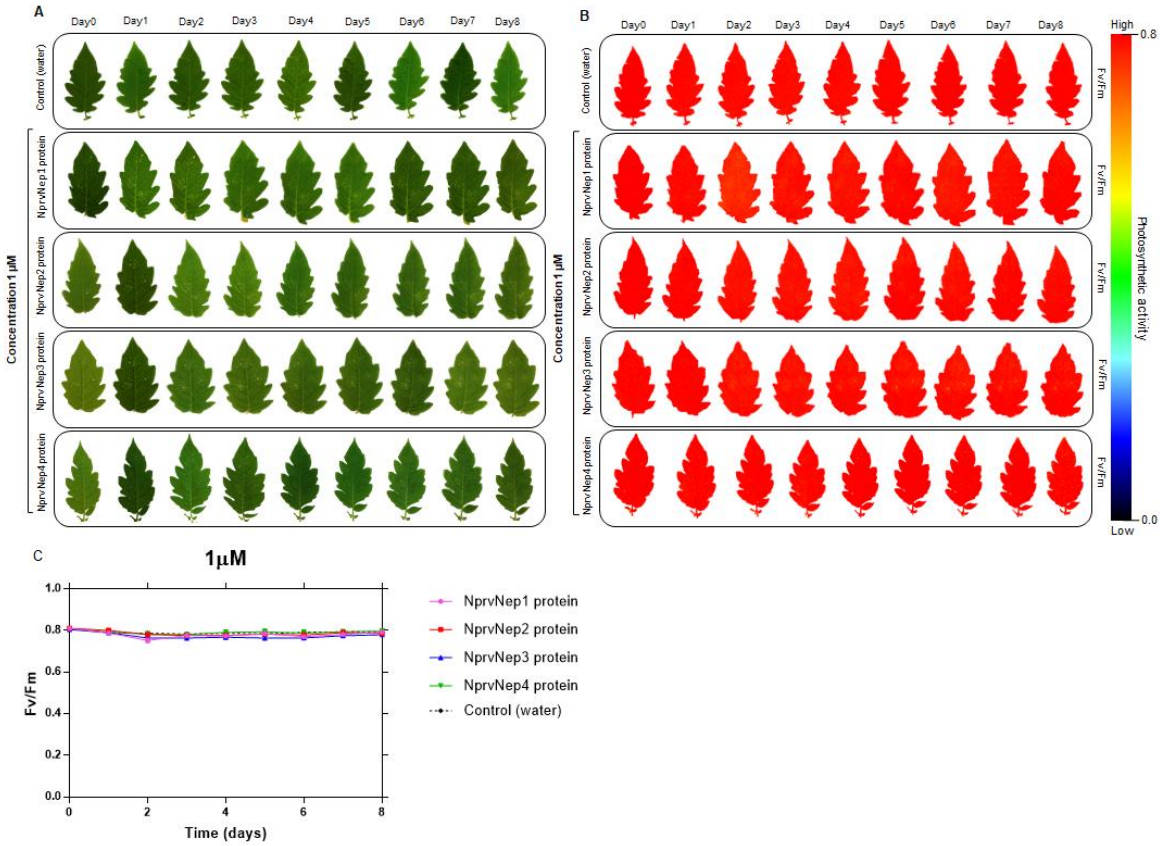

Figure 2. Effect of 1 µM recombinant NprvNeps on detached tomato leaves symptoms development. (**A**), chlorophyll fluorescence (**B**), and F_v/F_m (**C**). Ultra-pure water was used as a control. The color scale bar indicates the F_v/F_m intensity of the leaf pixels given in false colors from high (red) to low (black) values. All measurements were performed in biological triplicates and error bars show the standard deviation. Two-way ANOVA, followed by a Dunnett's multiple comparison test was used to determine the statistical significance of phytotoxicity of each protein within the same concentration against the control (**C**).

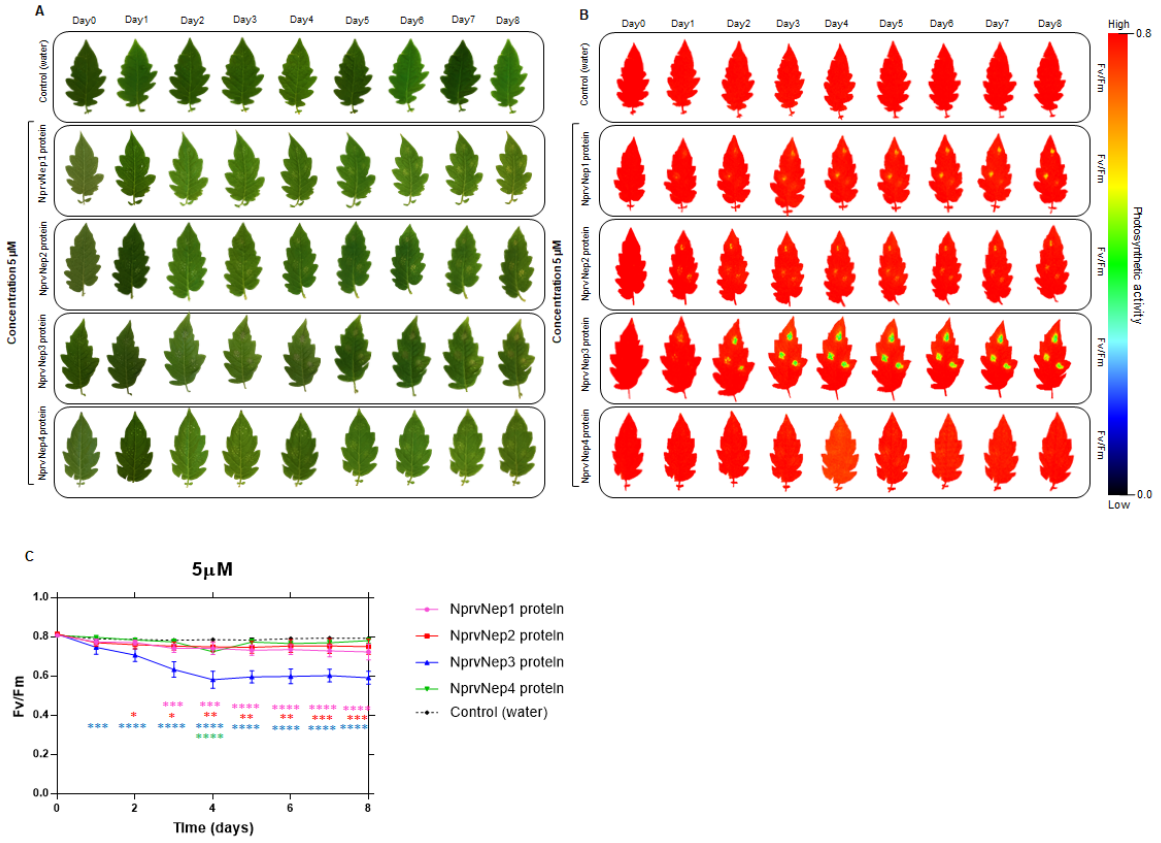

Figure S3. Effect of 5 µM recombinant NprvNeps on detached tomato leaves symptoms development. (**A**), chlorophyll fluorescence (**B**), and F_v/F_m (**C**). Ultra-pure water was used as a control. The color scale bar indicates the F_v/F_m intensity of the leaf pixels given in false colors from high (red) to low (black) values. All measurements were performed in biological triplicates and error bars show the standard deviation. Two-way ANOVA, followed by a Dunnett's multiple comparison test was used to determine the statistical significance of phytotoxicity of each protein within the same concentration against the control (**C**) (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

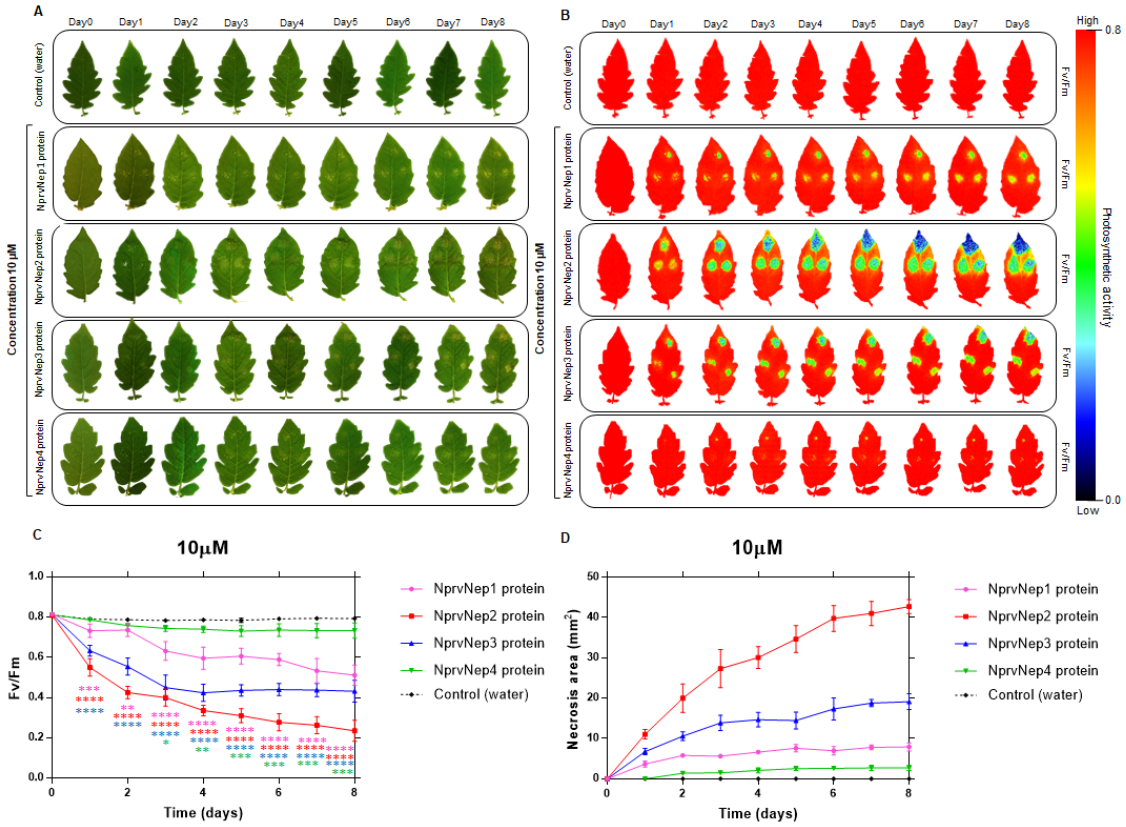

Figure S4. Effect of 10 µM recombinant NprvNeps on detached tomato leaves symptoms development (**A**), chlorophyll fluorescence (**B**), and F_v/F_m (**C**) and necrosis area (**D**). Ultra-pure water was used as a control. The color scale bar indicates the F_v/F_m intensity of the leaf pixels given in false colors from high (red) to low (black) values. All measurements were performed in biological triplicates and error bars show the standard deviation. Two-way ANOVA, followed by a Dunnett's multiple comparison test was used to determine the statistical significance of phytotoxicity of each protein within the same concentration against the control (**C**) (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

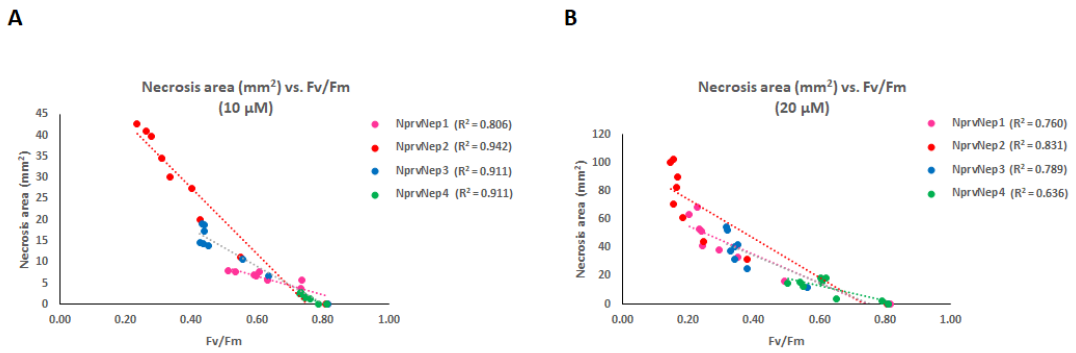

Figure S5. Scatter plot of necrosis area vs. Fv/Fm values for 8 days. The correlation between necrosis area and Fv/Fm values of detached tomato leaves treated with 10 µM (**A**) and 20 µM (**B**) recombinant NprvNeps (1-4) for 8 days is shown. Each point is the mean of biological triplicates.

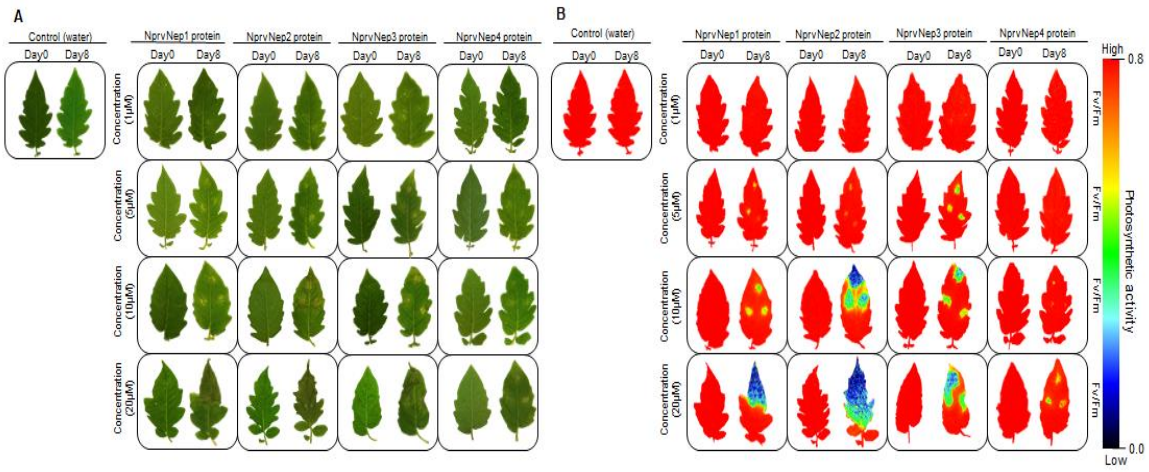

Figure S6. Toxicity of recombinant NprvNeps to detached tomato leaves evaluated by chlorophyll fluorescence. Effect of 1, 5, 10, and 20 μM recombinant NprvNeps on symptoms development (**A**), and chlorophyll fluorescence (**B**) at 0 and 8 dpi. Ultra-pure water was used as a control. The color scale bar indicates the F_v/F_m intensity of the leaf pixels given in false colors from high (red) to low (black) values. All experiments were performed in biological triplicates.

Table S1. Primers used for cloning and amplification.

Gene Name	Sequences (5′ to 3′)
NparvNep1	F: GCCCCGGTCGAGAAGCGC R: CTACAACGCCGCTAACGCTAGGTTG
NparvNep2	F: GCGGTTGTTCAACGCCGCGG R: TCACAACGACAGGCACAGGGG
NparvNep3	F: GCTGCCATCCAACGCCGC R: TCAGACCTCAGCCTTGGCGAGG
NparvNep4	F: GCTCCCACGCAGAAGCTGAACG R: TTAGACCCAAGCCTCCTCAATCTTGG
NparvNep5	F: GAGCAGATCGAGAAGCGCGCTG R: TTAGATCCAGGCCTTGCCGAGGTTG

Table S2. The data of 6 *NprvNep* genes.

Genes	GenBank No	Extracellular protein /signal peptide	Signal peptide length	Protein molecular weight (kDa)
NprvNep1*	gi|615425645	Y	18	26.5
NprvNep2*	gi|485922125	Y	21	27.6
NprvNep3*	gi|485923842	Y	21	25.8
NprvNep4*	gi|485928552	Y	18	26.2
NprvNep5*	gi|485917230	Y	28	27.3
NprvNep6	gi|615411409	N	0	17.9

A signal peptide of the NprvNep was predicted with the tool SignalP4.0. The SignalP Network predicted cleavage sites between 17 and 29 amino acid residues. * The *NprvNep* genes were selected for functional analysis. 'Y' has a signal peptide. 'N' has no a signal peptide.