Tomato 26S Proteasome subunit RPT4a regulates ToLCNDV transcription and activates hypersensitive response in tomato

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**Supplementary Figure 1.** Bacterial expression and purification of SlRPT4a. (A) Coomassie brilliant blue stained 12% SDS-PAGE containing induced and uninduced SlRPT4-GST fusion protein (69kDa); (B) Purified SlRPT4 protein. Band represents fractions of GSTaffinity purification of SlRPT4-GST fusion protein.

**Supplementary Figure 2.** Accumulation of Coat protein (CP) specific transcripts, (A) Northern hybridization showing the Relative accumulation of CP transcripts in the leaf samples infiltrated with empty vector (EV), SlRPT4-cmyc and RNA Pol II-3-gfp construct alone, and co-infiltrated with RNA Pol II-3-gfp and SlRPT4-muc construct. Fragment corresponding to ToLCNDV-CP gene was used as probe. Total RNA is shown as equivalent loading in the experiment. Data depicts means±SD of three independent experiments (n=3); *, P<0.05; **, P<0.01; ***, P<0.001.
Supplementary Figure 3. TRV-based VIGS in tomato and *Nicotiana benthamiana*. (A) Phenotype of tomato and *N. benthamiana* plant at 21 day post-silencing. Fragments corresponding to *Slpds* and *Nbpds* were used to produce TRV-based gene silencing constructs. After 21 day post silencing typical leaf bleaching symptoms were observed. (B), Northern blot analysis to evaluate the relative level of *pds* gene in control (without virus, mock or silencing treatments), vector infiltrated (TRV:00) and *pds* silenced (*pds-*) plants. Tubulin gene from tomato and Nicotiana were used as internal control.

Supplementary Figure 4. Accumulation of SIRPT4 in H*^T* (ToLCNDV) and H*SIRPT4+T* (TRV:SIRPT4+ToLCNDV). (A) Northern hybridization to evaluate the accumulation of *SIRPT* transcript. (B) Relative accumulation of *SIRPT4* in the experimental samples. Tomato plant infected with TRV: 00 vector was used as negative control. Bars show standard deviations (±SD). Ethidium bromide-stained total RNA has been shown as the equivalent loading control of the experiment.
Supplementary Figure 5. Phenotype of Mock and SIRPT4 silenced cv. H-88-78-1 at 21 day post ToLCNDV infection. (A) Symptom remission. Systemic leaves showed symptom recovery in mock plants, however SIRPT4 silenced cv. H-88-78-1 was failed to recover from the ToLCNDV infection. $H^{TRV:00+T}$, mock plant infected with ToLCNDV; $H^{TRV:SIRPT4+T}$, SIRPT4 silenced plant infected with ToLCNDV, UL, upper leaf; LL, lower leaf. (B) Comparison of progression of leaf curl symptom between Control ($H^{T}$) and SIRPT4 silenced cv. H-88-78-1 ($H^{TRV:SIRPT4+T}$) at 7-28 dpi of ToLCNDV.
Supplementary Figure 6. Accumulation of DNA-B specific ToLCNDV molecule. Southern blot of tomato genomic DNA from all experimental plants were hybridized with ToLCNDV-BC1 (encoding Movement proteins) gene specific probe. Replicative forms of ToLCNDV genome are designate as open circular (OC), linear (Lin), supercoiled (SC) and single strand (SS). TRV:00 infiltrated H-88-78-1 was taken as a mock control. Ethidium bromide stained DNA from each experiment were shown as equivalent loading. (C) Relative accumulation of viral DNA in the samples HT and HSRPT4+T at different time points. Data depicts means±SD of three independent experiments (n=3); *, P<0.05; **, P<0.01; ***, P<0.001.
Supplementary Figure 7. Estimation of antioxidant enzyme activity in cv. H-88-78-1. (A) Specific activity of APX was measured as 1 μmol of ascorbate oxidized min⁻¹. (B) Specific activity of CAT was measured as 1 μmol H₂O₂ oxidized min⁻¹. (C) Levels of lipid peroxidation expressed in terms of MDA concentration. (D) Percentage electrolytic leakage. Data depicts means±SD of three independent experiments (n=3); *, P<0.05; **, P<0.01; ***, P<0.001. Mock, TRV:00 infiltrated cv. H-88-78-1; TRV:SIRPT4; SIRPT4 silenced cv. H-88-78-1.
Supplementary Table-S1. List of primers used in the study

| Primer Name          | Forward Primer Sequence   | Reverse Primer Sequence   |
|----------------------|---------------------------|---------------------------|
| pGEX4-RPT4           | CCGGATCCATGGCGACCGAAGAACG | CGGAATTCCTTAATCTTGTGCAAAAAATCAG |
| **Primers used for VIGS** |                           |                           |
| pTRV-Slpds           | CCGCTCGAGCTGACGACGACTTTTCGATGC | CGGAATTCATATATGGAATGTGCAATTACAG |
| pTRV-SIRPT4          | CCGCTCGAGCTGACGACGACTTTTCGATGC | CGGAATTCCTTACTATATTACCAACCCGTTCCT |
| **Primers used for Southern blot analysis** |                           |                           |
| Coat protein         | ACAGAAAACCCAGAATGTACAGAA | CAACATTAAAGGCATTTCAGATTAG |
| BC1                  | GTTTTGTGGCTCCCCCTTCGGTCA | GTTTTGTGGCTCCCCCTTCGGTCA |
| **Primers used for transient expression analysis** |                           |                           |
| pCAMBIA1302: SIRPT4  | CATGCCATGGATAATGGGACCGAAGACG | GGACTAGTTATCTTGGCAAAAAATCAG |
| **Primers used for Northern blot analysis** |                           |                           |
| Rep gene             | TTTAAAGTGCTTTAGATTAGTG | CACCATTTAAGGTGCTTACAGAAGA |
| Coat Protein         | ATGAAATTCAGCTACATGGCGCTA | CTGGGAATGATGATCTGGCCCTTCGG |
| SIRPT4               | CCGCTCGAGCTGACGACGACTTTTCGATGC | CGGAATTCCTTACTATATTACCAACCCGTTCCT |
| Slpds                | CCGCTCGAGCTGACGACGACTTTTCGATGC | CGGAATTCATATATGGAATGTGCAATTACAG |
| Nbpds                | TAAACCCTGACGAGCTTTTCAGATGC | TTTAACCCATGAAATGTGCAATTACAG |
| αTubulin             | CAAACTTACCAAGATTCAAGATGCTACAG | ACAATTTATCCCTACCACAG |
| **Primers used for EMSA and ChIP assay** |                           |                           |
| DNA-A-IR_EMSA        | AAAACTTGTCTTTGTATT | TGGTTGAGGGCCACCTAAA |
| (2592-47)            |                           |                           |
| DNA-B-IR_EMSA        | ACACCATATGGGATTATGTGTAAT | AACGGCGTGCAATGATTACAGC |
| (2617-67)            |                           |                           |
| DNA-A-Rep_EMSA       | GACTATGCTTTATGGGCCTAAA | CCATTTTACAATTTCATCCT |
| (1939-2046)          |                           |                           |
| IR_ChIP              | AAAACTTGTCTTTGTATT | TGGTTGAGGGCCACCTAAA |
| (2592-47)            |                           |                           |
| pENTER-RPT4          | CACCATGGGCGACGAGGAAGAGACGCCG | TTCCCCAACCAGAAGAATGCTGAG |
| pENTER-RNA Pol- II-3 | CACCATGGGCGACGAGGAGGCTTTCGATCCAG | TTAACCTCCAGCATTGAGGCCCC |
| Actin 7              | CGGTGTGATGATAATAGGACG | GCTTCATCAACACATACGC |