New Phytologist

***New Phytologist* Supporting Information**

Article title: Tomato SlGSTU38 interacts with the PepMV coat protein and promotes viral infection
Authors: Eduardo Méndez-López, Livia Donaire, Blanca Gosálvez, Pedro Díaz-Vivancos, M. Amelia Sánchez-Pina, Jens Tilsner, Miguel A. Aranda
Article acceptance date: 19 December 2022

The following Supporting Information is available for this article:

Fig. S1 Yeast two-hybrid screening of a cDNA normalized tomato library against the CP of PepMV and identification of SlGSTU38 as a possible CP interacting protein.

Fig. S2 Phylogenetic relationship of SlGSTU38 and its homologs.

Fig. S3 Mock-inoculated and PepMV-infected WT and *gstu38* Micro-Tom plants at 14 days post inoculation.

Fig. S4 Subcellular localization and colocalization of CP and SlGSTU38.

Fig. S5 RNA-seq data validation.

Fig. S6 PepMV or SlGSTU38 knock out do not induce lipid peroxidation in Micro-Tom plants.

Table S1 Primer sequences.

Table S2 Construct summary.

Table S3 BLASTp output using the SlGSTU38 protein sequence as query in the Sol Genomics Network database.

Table S4 RNA-seq results: Number of expressed genes per treatment and in all treatments together.

Table S5 RNA-seq results: Genes exclusively expressed in mock-inoculated *gstu38* (gstu38_M),

mock-inoculated wild type (WT_M), and PepMV-inoculated wild type plants (WT_PepMV).

Table S6 RNA-seq results: Number of differentially expressed genes (DEGs) for all the possible pairwise comparisons between treatments.

Table S7 Differentially expressed genes (DEGs) comparing mock-inoculated *gstu38* with mock-inoculated wild type (WT) plants.

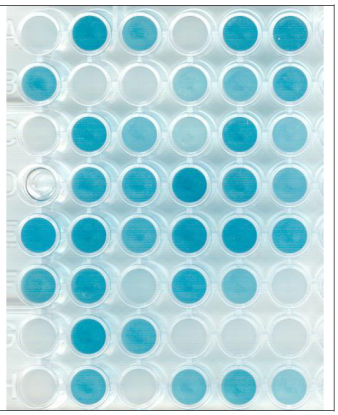
Methods S1 Tomato cDNA library and yeast two-hybrid screening.

Fig. S1 Yeast two-hybrid screening of a cDNA normalized tomato library against the CP of PepMV and identification of SlGSTU38 as a possible CP interacting protein. (a) Beta-galactosidase assay of the 48 histidine autotroph co-transformed yeast clones obtained after 5 passaging rounds in liquid screening medium (SD-his-leu-trp + 1 mM 3-AT). Blue stained cultures in the plate correspond to the 35 co-transformant that activated the second reporter gene, *LacZ*. The table on the left of the plate corresponds with numbers in each well. (b) Alignment of the *SlGSTU38* mRNA and the cDNA inserts of clones 21, 27, 29, 31 and 36. The length of each sequence is indicated after the sequence IDs. In red and yellow are highlighted the start and stop codons, respectively.

Fig. S1

a

	1	2	3	4	5	6
A	1	9	17	25	33	41
B	2	10	18	26	34	42
C	3	11	19	27	35	43
D	4	12	20	28	36	44
E	5	13	21	29	37	45
F	6	14	22	30	38	46
G	7	15	23	31	39	47
H	8	16	24	32	40	48



b

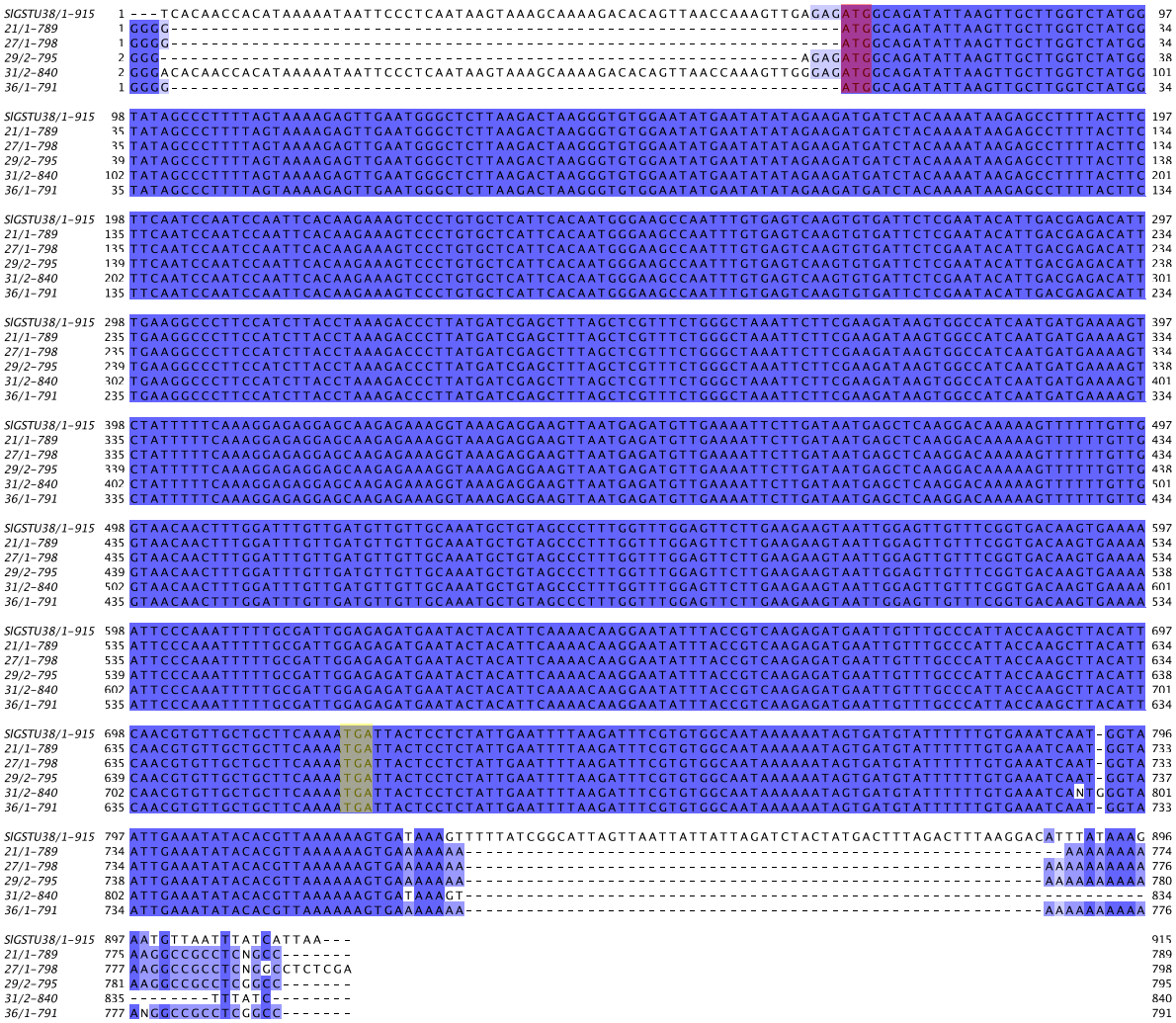
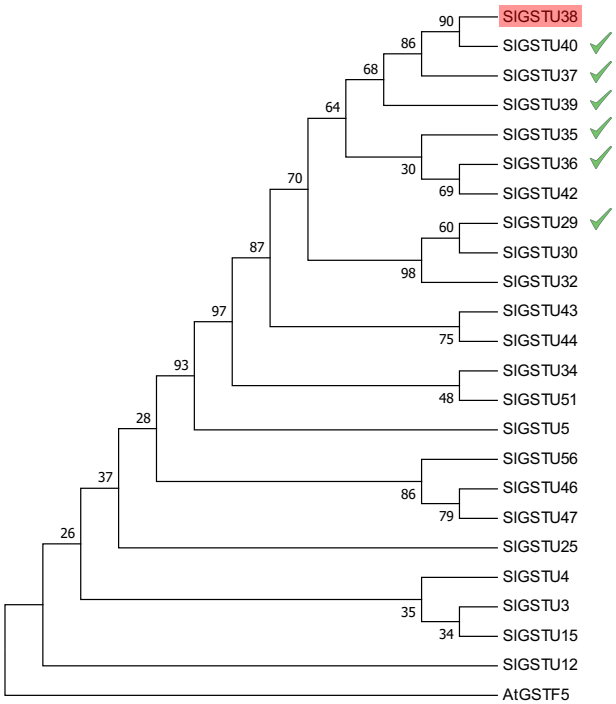


Fig. S2 Phylogenetic relationship of SlGSTU38 and its homologs. (a) The phylogenetic tree was built using the maximum likelihood method and based in the Le_Gascuel_2008 model. The percentage of trees (1000 replicates) in which proteins in a branch were grouped together is shown next to each branch. The sequences used to build the tree were identified in a BLASTp search using the SlGSTU38 sequence (e-value threshold of 1e-50) in The Sol Genomics Network database (https://solgenomics.net). Sequences with less than 200 amino acids aligned with SlGSTU38 were dismissed. AtGSTF5 was used as root. SlGSTU38 protein is highlighted in red and the homologs selected for yeast two-hybrid analysis with the CP are marked with a green tick. (b) Alignment of the protein sequences of SlGSTU38 and its homologs selected for the yeast two-hybrid analysis with the CP (SlGSTU40, SlGSTU37, SlGSTU39, SlGSTU35, SlGSTU36 and SlGSTU29). Alignment was built using T-COFFEE and visualize with Jalview. The length of each sequence is indicated after the sequence IDs and color from dark purple to white represent the % of identity.

Fig. S2

a



b

```
SlGSTU38/1-217    1 MADIKLLGLWYSPFSKRVEWALKTKGVEYEYIEDDLQNKSLLLLQSNPIHKKVPVLIHNGKPICESSVILEYID  74
SlGSTU40/1-217    1 MADVKLIGLWYSPFSRRVEWALKIKGVEYEYIEDDLHNKSLLLLQSNPIHKAVPVLIHNGKPLCESSVILEYID  74
SlGSTU37/1-210    1 MGDVKLLGLWYSPFSHRVEWALKFKGVQYEFIEQDLQNKSPILLESNPIYKKVPVLIHNGKPICESIVILEYID  74
SlGSTU39/1-217    1 MAEVKLLGLSYSPFNHRVEWALKIKGVKYEFIEEDLQNKSSLLLESNPIHKKIPVLIHNGKPICESMVILEYID  74
SlGSTU35/1-219    1 MADVKLLGLWYSPYSHRVEWALKIKGVEYEFIEEDLRNKSPLLLESNPIYKKIPVLIHNGKPICESMVIVEYID  74
SlGSTU36/1-217    1 MEEVKLLGLWYSPFCHRVEWALKVKGVKFEFIEENLQNKSPLLLESNPIHKKIPVLIHNGKSICESMVIVEYID  74
SlGSTU29/1-219    1 MVDVKLLGLWYSPASHKVEWALKLKGVKYEFIEENLQNKSPLLLESNPVHKKIPILIHNGKPICESMIILEYID  74

SlGSTU38/1-217   75 ETFEGPSILPKDPYDRALARFWAKFFEDKWPSMMKSLFFKGEEQEKGKEEVNEMLKILDNELKDKKFFVGNNFG 148
SlGSTU40/1-217   75 ETFEGPSILPKEPYDRSLARFWAKFFDDKGLAIRKSIFFKGEEQEKAKEEVYDMLKVLDNELKNKKIFVGEKFG 148
SlGSTU37/1-210   75 EVFEGPSILPKDPYNRALARFWVKFFEDKGPSMRKSILLKGEEQEKAKEEVFEMLRILDNELKGKKFFVGDKFG 148
SlGSTU39/1-217   75 EAFEGPSILPKDPYDRALARFWAKYVDDKGSAVWKSFFFKGEEQEKAKEEAYEMLKILDNEFKDKKYFVGDKFG 148
SlGSTU35/1-219   75 ETFEGPSILPKDPYDRAIARFWAKFFDEKGSSVGRSFFLKGEEQEKAKEELHEMLKVVDNELKDKKYFVVDKFG 148
SlGSTU36/1-217   75 ETFEGPSILPKDPYDRVIARFWVKFFEDKGSAVGTSFFHKS---EKAKEEVCEMLKILDNELKDKKFFVGDKFG 145
SlGSTU29/1-219   75 ETFEGPSILPKDPYDRALARFWAKFLDDKVGAMVNTFLLKGEEQEKGKKEVCEMLNVLDNELKGKKFFVGDKFG 148

SlGSTU38/1-217  149 FVDVVANAVALWFGVLEEVIGVVSVTSEKFPNFCDWRDEY--YIQNKEYLPSRDELFAHYQAYIQRVAAS---K 217
SlGSTU40/1-217  149 FVDIVANAAALWLGVLEEASGVVLVTREKYPNFCDWRDEY--CTQNKKYLPPRDELLAHYQVYIQRVTTS---K 217
SlGSTU37/1-210  149 FVDIVANAGALWLGVLEEVSGVVLVTKEKFPNFCVWRDEY--CTQNKEYLPSRDELLIRFKTYI---------- 210
SlGSTU39/1-217  149 FADIVANGAALYLGILEEVSGVVLATSEKFPNFCAWRDEY--CIQNKEYFPSRDELLIRYRAYIQPVDAS---K 217
SlGSTU35/1-219  149 FVDIVANVVALWLGVLEEASGVVLVTNEKYPNFYAWRDEYINCSENKKYLPSRNELLAKFKARILAASVA---E 219
SlGSTU36/1-217  146 FADIAANFLALWMGILEEATGIILVTKEKYPNFYAWRDEY--INGNKEYLPSRDELLAFFKARFQAAATPPYSN 217
SlGSTU29/1-219  149 YADMAANFVGYWLGVFQLEASGVVLVTSEKFSNFCVWRDEYVNCSQVKEYLPPRNDLLAFVEARTQASAS-K--A 219
```

Fig. S3 Mock-inoculated and PepMV-infected WT and *gstu38* Micro-Tom plants at 14 days post inoculation.

Fig. S3

Fig. S4 Subcellular localization and colocalization of CP and SlGSTU38. Live-cell imaging of *Nicotiana benthamiana* D. epidermal cells expressing fluorescent fusion proteins observed under the confocal laser scanning microscope. (a) Maximum projection of a Z-stack of cells expressing mRFP-CP. The fluorescent signal is localized in the cytoplasm and the nucleus (arrowhead). (b) Maximum projection of a Z-stack of cells expressing GFP-SlGSTU38. The fluorescent signal is localized in the cytoplasm and the nucleus (arrowhead). (c-h) mRFP, GFP and both channels from an image of (c-e) cells expressing both GFP-SlGSTU38 and mRFP-CP and (f-h) a nucleus from the same tissue area at higher magnification.

Fig. S4

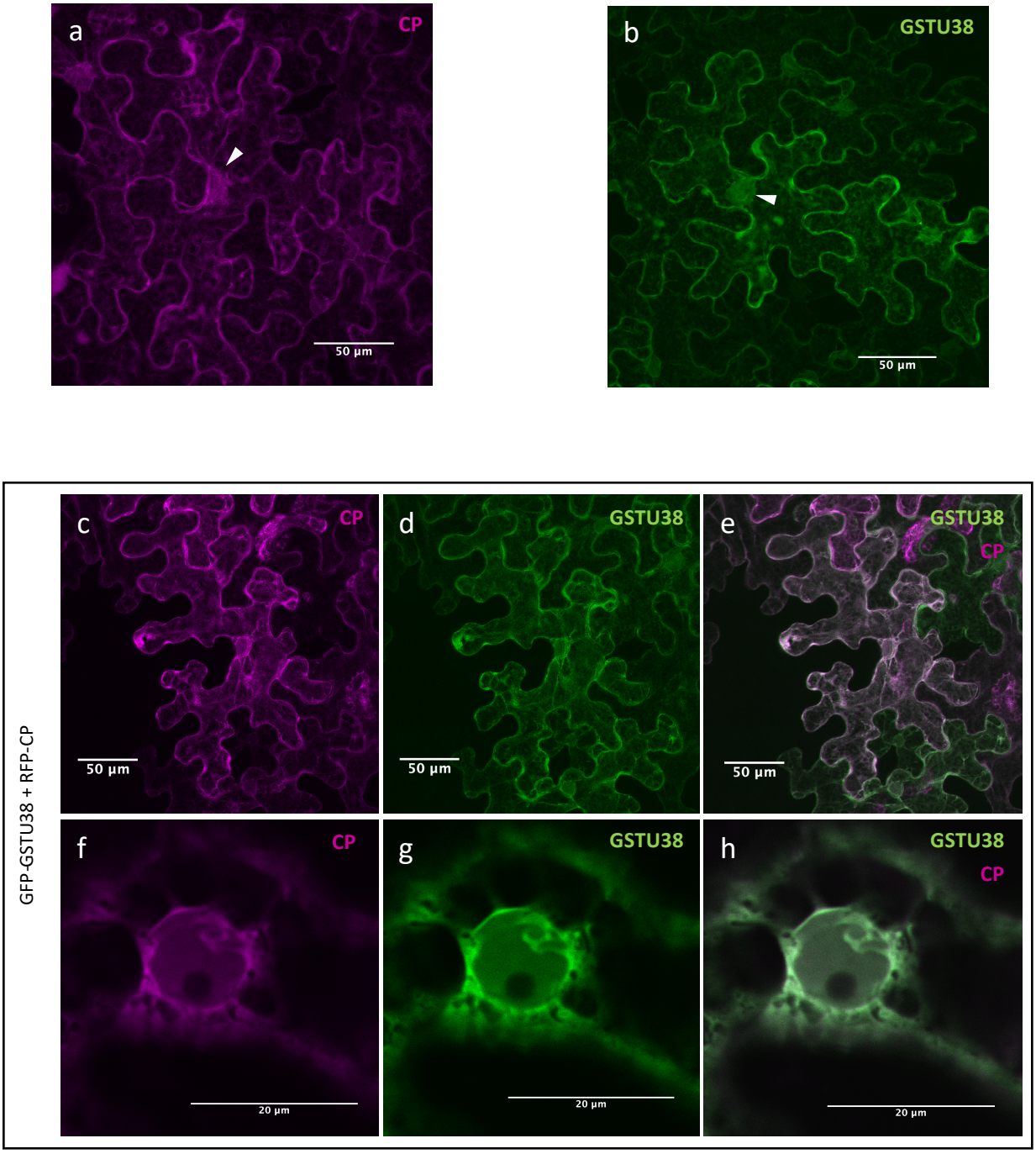
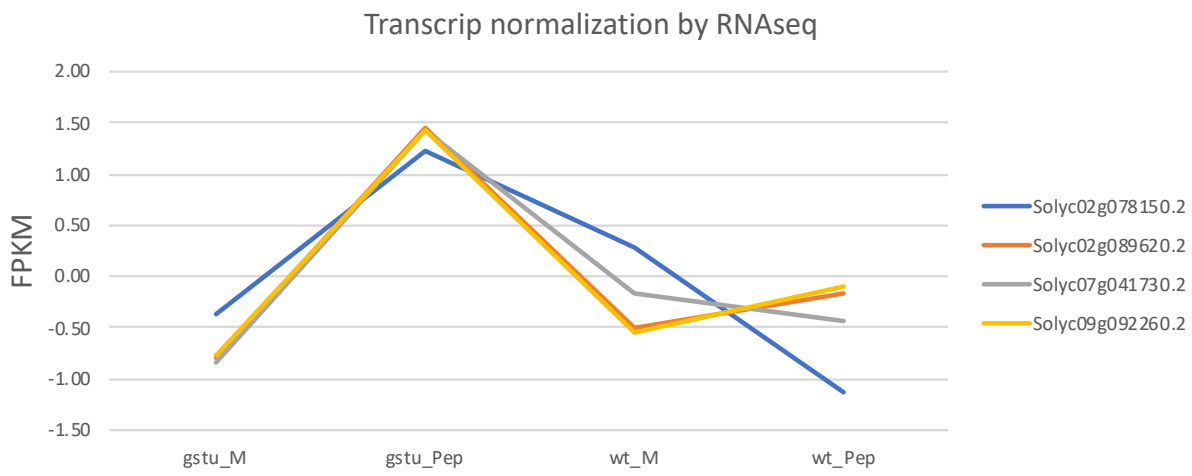

a
CP
50 µm
b
GSTU38
50 µm
GFP-GSTU38 + RFP-CP
c
CP
50 µm
d
GSTU38
50 µm
e
GSTU38
CP
50 µm
f
CP
20 µm
g
GSTU38
20 µm
h
GSTU38
CP
20 µm

Fig. S5 RNA-seq data validation. (a) Scaled fragments per kilobase per million mapped reads (FPKM) values of four genes (*Solyc02g078150.2*, *Solyc02g089620.2*, *Solyc07g041730.2*, *Solyc09g092260.2*) from cluster 6 of PepMV-specific differentially expressed genes (DEGs) for each treatment. (b) Relative quantification (RQ) by RT-qPCR of each gene transcript for each treatment. Transcript accumulation dynamics of each gene per treatment are identical regardless of RNA-seq or RT-qPCR quantification validating RNA-seq data. Treatment gstu_M: mock-inoculated *gstu38* plants; treatment gstu_Pep: PepMV-inoculated *gstu38* plants; treatment WT_M: mock-inoculated wild type plants; treatment WT_Pep: PepMV-inoculated wild type plants.

Fig. S5

a


Transcrip normalization by RNAseq
FPKM
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
gstu_M
gstu_Pep
wt_M
wt_Pep
Solyc02g078150.2
Solyc02g089620.2
Solyc07g041730.2
Solyc09g092260.2

b

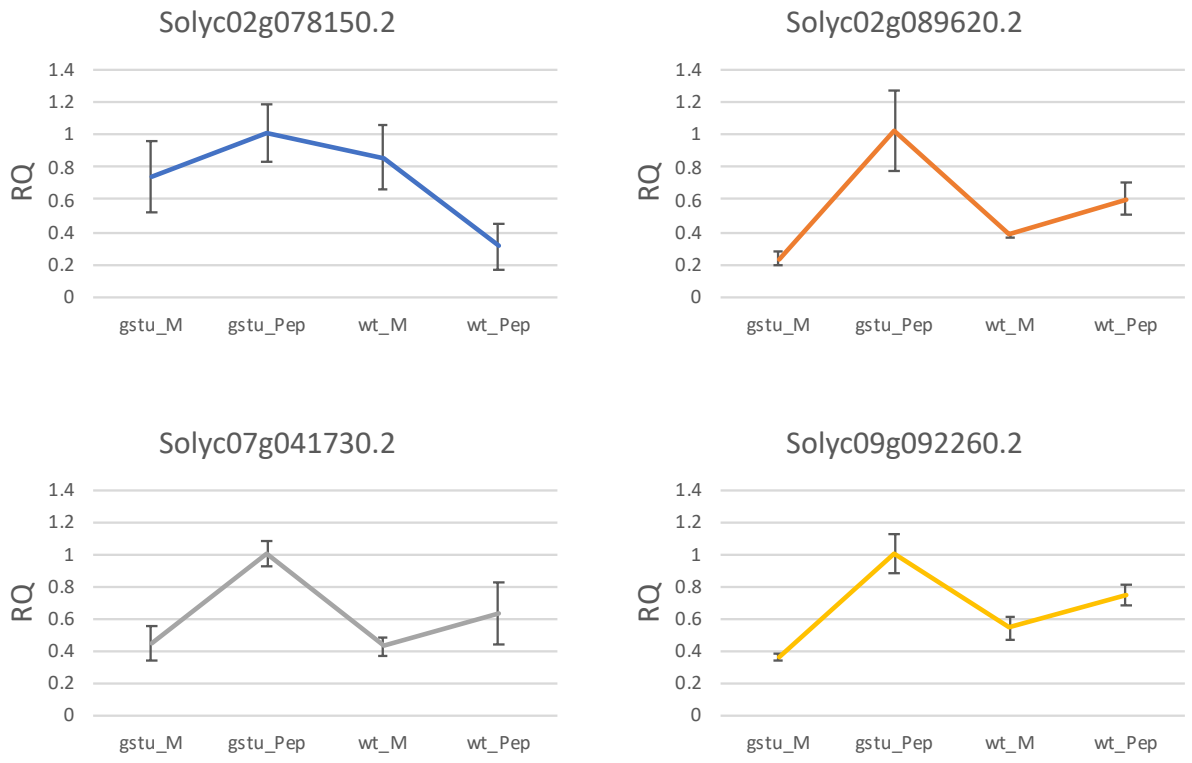

Solyc02g078150.2
Solyc02g089620.2
Solyc07g041730.2
Solyc09g092260.2
RQ
1.4
1.2
1
0.8
0.6
0.4
0.2
0
gstu_M
gstu_Pep
wt_M
wt_Pep

Fig. S6 PepMV or SlGSTU38 knock out do not induce lipid peroxidation in Micro-Tom plants. Bar plot represents the lipid peroxidation measurement means (nmol g^{-1} of Fresh Weight) of Mock-inoculated and PepMV-infected WT and *gstu38* plant leaves at 7 and 14 days post inoculation (dpi). Error bars represent standard deviation. No significative differences between treatments were computed by one-way ANOVA (7 dpi: $F_{3,12}$=0.96, P=0.441; 14 dpi: $F_{3,12}$=0.54, P=0.666)

Fig. S6

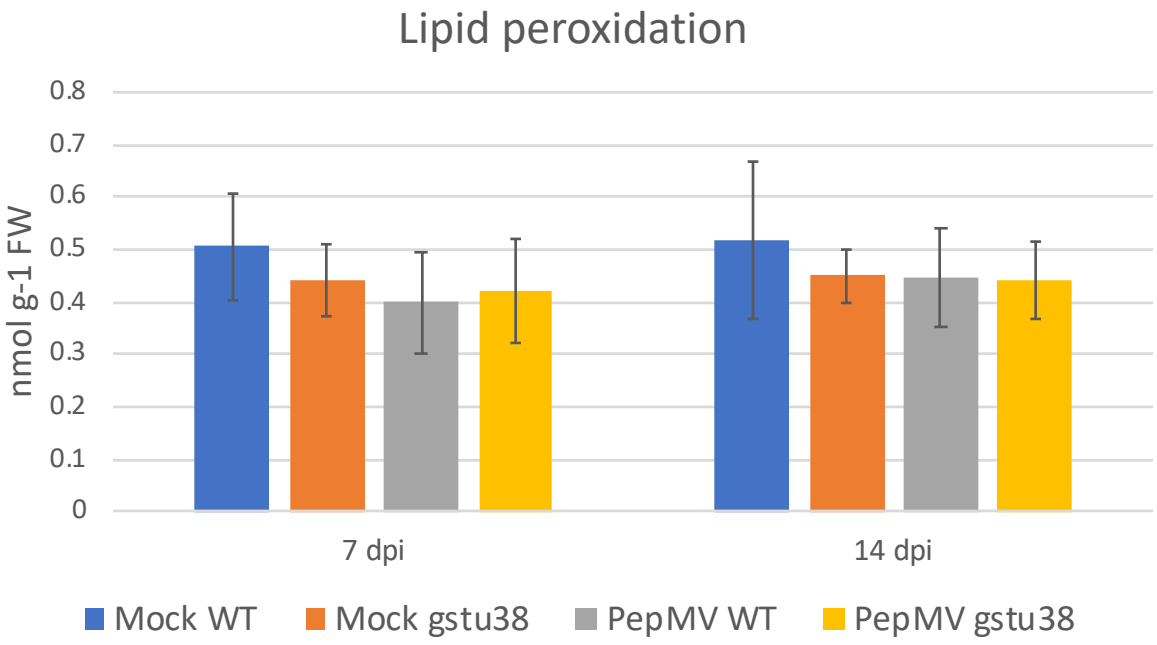


Table S1 Primer sequences.

Table S2 Construct summary. For each construct the inserted amplicon ID, the PCR template, the forward (Fw) and reverse (Rv) primer ID, the entry clone and the destination vector with a description are indicated.

Table S3 BLASTp output using the SlGSTU38 protein sequence as query in the Sol Genomics Network database. The e-value threshold was set up at 1e-50. Aln: aligned sequence length (number of amino acids).

Table S4 RNA-seq results: Number of expressed genes per treatment and in all treatments together. Treatments were mock-inoculated gstu38 (gstu38.M), PepMV-inoculated gstu38 (gstu38.P), mock-inoculated wild type (wt.M) and PepMV-inoculated wild type (wt.P) plants.

Table S5 RNA-seq results: Genes exclusively expressed in mock-inoculated gstu38 (gstu38_M), mock-inoculated wild type (WT_M), and PepMV-inoculated wild type plants (WT_PepMV).

Table S6 RNA-seq results: Number of differentially expressed genes (DEGs) for all the possible pairwise comparisons between treatments.

Table S7 Differentially expressed genes (DEGs) comparing mock-inoculated gstu38 with mock-inoculated wild type (WT) plants. Genes are sorted by log2FoldChange value. Up-regulated genes are considered those with log2FoldChange > 1, and down-regulated genes are those with log2FoldChange < -1. Genes encoding a calmodulin, two calmodulin-binding proteins, and proteins using calcium as cofactor are marked in blue. Genes encoding proteins involved in ethylene biosynthesis are marked in green. Genes encoding repressors of abscisic acid biosynthesis are marked in purple. Genes encoding factors involved in signalling transduction are marked in yellow. Genes encoding proteins related to abiotic and/or biotic stresses are marked in orange.

Methods S1. Tomato cDNA library and yeast two-hybrid screening. For the generation of the tomato cDNA normalized library used in the yeast two-hybrid (Y2H) screening, four-leaf stage tomato (cv. Money Maker) plants were mechanically inoculated with PepMV-Sp13 as in Aguilar et al. (2002). At 1 day post-inoculation (dpi), inoculated leaves of six plants and leaves from healthy plants of the same age were harvested. The same procedure was repeated at 3 and 15 dpi. In addition, at 15 dpi, upper non-inoculated leaves of 12 infected plants and 12 healthy plants were harvested. Total RNA was extracted from the harvested leaves with TRI reagent (Molecular Research Center, Inc.) and mixed. The RNA preparation was used as the template to generate a full-length cDNA library normalized using duplex-specific nuclease (DSN)-normalization (Zhulidov et al., 2004) (Evrogen, Russia). The cDNA normalized library was cloned into the prey plasmid pGAD-HA (Gietz & Woods, 2001) (Dualsystems Biotech, Switzerland), and the CP coding sequence (CDS) was PCR-amplified with primers CE802 and CE803 (Table S1) and inserted into the bait plasmid pLexA-N by ligation-independent cloning. Y2H screening was carried out following Dualsystems Biotech (Switzerland) protocol. First, DSY-5 yeast expression strain was transformed with the bait clone and a test plasmid (Gal-promotor – lexA operator – lacZ) to check if the bait enters the nucleus and binds to the LexA operators situated upstream of a reporter gene ("repression or blocking assay"). A bait which enters the nucleus and binds to the LexA operator sites decreases transcription from the downstream lacZ gene (repression) and hence decreases β–galactosidase expression on galactose medium. Most LexA fusions that enter the nucleus and bind the operators but do not activate transcription, repress β-galactosidase activity from 2 to 20 fold. >2 fold repression indicates >50% operator occupancy by the bait and signifies that the bait can be screened in the DUALhybrid assay. The assay confirmed that bait protein enters in the nucleus.Subsequently, NMY51 yeast cells were transformed with the bait construct pLexA-CP and grown in histidine, leucine and tryptophan drop-out synthetic medium (SD-(ade)-his-leu-trp) medium with different 3-amino-1,2,4-triazole (3-AT) concentration for a self-activation test of the bait to determine the optimal screening medium (SD-his-leu-trp + 1mM 3-AT). The cDNA normalized library was cloned into the prey plasmid pGAD-HA. NMY51 yeast cells bearing the bait plasmid were transformed with several aliquots of pGAD-HA-cDNA normalized library in separated transformation reactions and grown in SD-his-leu-trp + 1mM 3-AT screening plates. Out of 3.6 x 106 transformants that were screened, forty-eight prey clones activated the HIS3 reporter gene and grew under the selection. This 48 primary interacting clones were picked and

transferred to liquid screening medium, and subjected to five rounds of passages to remove non-specific interactors. Then, the selected prey clones were tested using a beta-galactosidase assay (HTX assay) using the second reporter gene lacZ. Thirty-five prey clones activated both reporter genes and the prey plasmids were isolated, amplified in E. coli and sequenced (Fig. S1a). Prey sequences were identified using the BLASTn algorithm against the Tomato Genome cDNA (release 2.40) database (https://solgenomics.net) (Fernandez-Pozo et al., 2015).Candidate interactors were classified according to the following criteria. Class A Interactors are those which have been rescued more than three times. They represent highly likely interactors of your bait. Class B Interactors are those which have been identified two times. They represent highly likely interactors of your bait. Class C Interactors are those which found only once in the screen ("singletons"). Although some of those may indeed represent true interactors of your protein of interest, others represent common false positives. Class N are considered to be non-relevant alignments (Expectation E score above 0.01). Sequences of prey clone 21, 27, 29, 31 and 36 matched with the mRNA Solyc09g011580.2.1 (ITAG release 2.40) encoding SlGSTU38 (Fig. S1b). Clone 31 was negative in the Beta-galactosidase assay, but the other 4 clones were double positive. SlGSTU38 was scored as class A interactor since it has been rescued more than three times. Clones 5, 34, 40, 46 corresponded to the PepMV CP sequence (the cDNA library was constructed from healthy and infected leaves). CP interacts with itself but the clone 46 was negative in the Beta-galactosidase assay while clones 5, 34 and 40 were positive. CP was also included in the class A. Both GSTU38 and CP were considered highly likely interactors of PepMV CP.

References

Aguilar JM, Hernández-Gallardo MD, Cenis JL, Lacasa A, Aranda MA. **2002**. Complete sequence of the Pepino mosaic virus RNA genome. *Archives of Virology* **147**: 2009–2015.

Fernandez-Pozo N, Menda N, Edwards JD, Saha S, Tecle IY, Strickler SR, Bombarely A, Fisher-York T, Pujar A, Foerster H, *et al.* 2015. The Sol Genomics Network (SGN)-from genotype to phenotype to breeding. *Nucleic Acids Research* **43**: D1036–D1041.

Gietz RD, Woods RA. **2001**. Genetic transformation of yeast. *BioTechniques* **30**: 816–831.

Zhulidov PA, Bogdanova EA, Shcheglov A, Vagner LL, Khaspekov GL, Kozhemyako VB, Matz M V, Meleshkevitch E, Moroz LL, Lukyanov SA, *et al.* 2004. Simple cDNA normalization using kamchatka crab duplex-specific nuclease. *Nucleic Acids Research* **32**: 3 e37.