Table S1. Names and sequences of PCR primers.

Name of Primer	Sequence (5' - 3')
35S promoter primer	
forward	AGGAAACAGCTATGACCATG
reverse	GAACTTCCTTATATAGAGGAAGG
actin primer	
forward	GCCATGCCCTTTTAAATGGC
reverse	CAGTTGGAGGGGACTGAATC
SLDCL1	
forward	GCATAAGGAACGGGGTTGTA
reverse	ATCTCTTCCACCACGACCAC
SlDCL2a	
forward	GCTCGGGACGAGACAAAATA
reverse	GGAAGGCACATCCAAACAGT
SlDCL2b	
forward	GAATGTCCATCTAATGCTACTGAAC
reverse	AACGTGTCGTCTCACAAAGC
SlDCL2c	
forward	TTGTTTGTGGAAACCAGCTAG
reverse	TCTTTCCAGAACCAGTCTCCA
SlDCL2d	
forward	CAGGGCGTATTACTACGTTCAA
reverse	CAAGCCCTCTTCAAGAATCG
SlDCL3	
forward	CGAAAAGCGCAAAAAGAAAC
reverse	CAGTGTGGCACATCAATTCC
SlDCL4	
forward	GGAAGAGGTCGTAGAGCGATT
reverse	CACACCAATAACAGGCCACA
SlActin	
forward	TGGGATGATATGGAGAAGATATGG
reverse	GGCTTCAGTTAGGAGGACAGGA

Table S2. Relative ROS production and relative ROS scavenging activity of the 5th and 7–8th true leaves.

5th leaves

	80 sec	120 sec	Reactive light unit (RLU)※1	averase control RLU	ROS scavenging rate (%)※2	avrease of ROS scavenging rate (%)	relative value	t value
control_1	6.80E+08	1.23E+09	5.50E+08					
control_2	8.94E+08	1.52E+09	6.22E+08					
control_3	1.03E+09	1.69E+09	6.59E+08	5.56E+08				
control_4	6.53E+08	1.13E+09	4.81E+08					
control_5	6.64E+08	1.13E+09	4.68E+08					
Empty_H_1	5.98E+08	1.03E+09	4.32E+08		22.3		1.12	
Empty_H_2	6.09E+08	1.06E+09	4.47E+08		19.7	19.9	0.99	
Empty_H_3	6.36E+08	1.09E+09	4.58E+08		17.7		0.89	
Empty_PS_1	5.32E+08	9.49E+08	4.17E+08		25.0		1.26	
Empty_PS_2	5.58E+08	9.61E+08	4.03E+08		27.4	25.7	1.38	0.0681
Empty_PS_3	5.29E+08	9.47E+08	4.18E+08		24.8		1.25	
72E_H_1	5.03E+08	8.85E+08	3.82E+08		31.3		1.57	
72E_H_2	4.80E+08	8.15E+08	3.35E+08		39.7	37.0	2.00	0.0530
72E_H_3	4.26E+08	7.60E+08	3.34E+08		39.8		2.00	
72E_PS_1	4.84E+08	7.37E+08	2.54E+08		54.4		2.73	
72E_PS_2	4.27E+08	6.64E+08	2.37E+08		57.3	54.6	2.88	0.0022
72E_PS_3	4.24E+08	6.91E+08	2.67E+08		52.0		2.61	

7-8th leaves

	80 sec	120 sec	Reactive light unit (RLU)※1	averase control RLU	ROS scavenging rate (%)※2	avrease of ROS scavenging rate (%)	relative value	t value
control_1	6.80E+08	1.23E+09	5.50E+08					
control_2	8.94E+08	1.52E+09	6.22E+08					
control_3	1.03E+09	1.69E+09	6.59E+08	5.56E+08				
control_4	6.53E+08	1.13E+09	4.81E+08					
control_5	6.64E+08	1.13E+09	4.68E+08					
Empty_H_1	6.21E+08	1.08E+09	4.54E+08		18.3		0.92	
Empty_H_2	6.31E+08	1.09E+09	4.57E+08		17.7	18.5	0.89	
Empty_H_3	5.21E+08	9.69E+08	4.48E+08		19.4		0.97	
Empty_PS_1	6.50E+08	1.10E+09	4.54E+08		18.3		0.92	
Empty_PS_2	6.70E+08	1.12E+09	4.47E+08		19.6	19.7	0.98	0.1838
Empty_PS_3	5.96E+08	1.03E+09	4.37E+08		21.3		1.07	
72E_H_1	6.59E+08	1.09E+09	4.30E+08		22.7		1.14	
72E_H_2	6.87E+08	1.14E+09	4.48E+08		19.4	20.7	0.97	0.1776
72E_H_3	7.08E+08	1.15E+09	4.44E+08		20.1		1.01	
72E_PS_1	7.20E+08	1.16E+09	4.43E+08		20.4		1.03	
72E_PS_2	8.19E+08	1.27E+09	4.46E+08		19.8	21.3	1.00	0.0585
72E_PS_3	6.82E+08	1.11E+09	4.25E+08		23.6		1.19	

※1 120sec-80sec

※2 averase control (4.62+E08) RLU - sample RLU x 100 / averase control RLU

Reactive light unit (RLU) in this table means ROS production. ROS scavenging rate means ROS scavenging activity. AccuFLEX Lumi400 (Hitachi, Japan) was used for the mesurement.

Figure S1. Schematic diagram of the artificial chimera gene (SlDCL2&4). Based on the alignment of three tomato DCL2 genes and one DCL4 gene sequences registered in the database, two regions (A and B) from DCL 2 and one region (C) from DCL 4, as shown in the top of the figure, were selected. An Artificial chimeric gene in which A, B and C were conected were created, and then arranged by

placing parts of DCL2 (A and B, 620 bp) and DCL4 (C, 300 bp) in head-to-head orientation across an intron sequence to create an IR sequence. SlartDCL2&4 IR was inserted into the SacII/SalI site of pBluescript II SK (+) plasmid, re-cloned into the BglII/KpnI site of binary vector pIG121-Hm downstream of the CaMV-35S promoter (35S:SlartDCL2&4 IR), and introduced into Agrobacterium tumefaciens strain EHA105 to transform tomato cv Moneymaker by the leaf disc method.

Supplementary figure 2

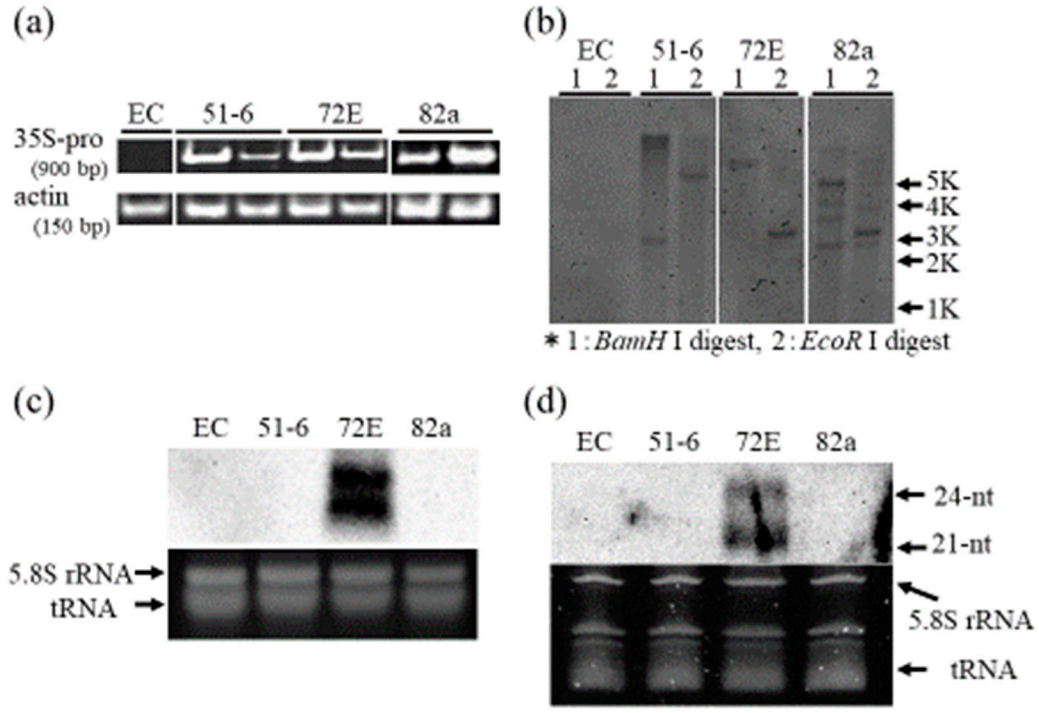

Figure S2. Northern-blot hybridization analysis of transgene transcripts (a), transgene siRNA (b) in three transgenic lines (hairpin DCL2/4-51-6, -72E, -82a) and the EC control line. (a) Total RNAs (~10 μg), extracted from three transgenic lines and the control line were separated in 1.2% agarose gel, transferred to nylon membrane and northern hybridized with a DIG-DCL2/4 cRNA probe. The upper panel shows the hybridization signal and the lower panel shows the loading control stained with ethidium bromide. The 72E line showed a dense hybridization signal. (b) Total RNAs (~10 μg), extracted from three transgenic lines and the control line were separated in 8M-urea 12% polyacrylamide gel, transferred to nylon membrane and northern hybridized with a DIG-DCL2/4 cRNA probe. The upper panel shows the hybridization signal and the lower panel shows the loading control stained with ethidium bromide. The 72E line showed two dense hybridization signals of the size ~21- and 24-nt.

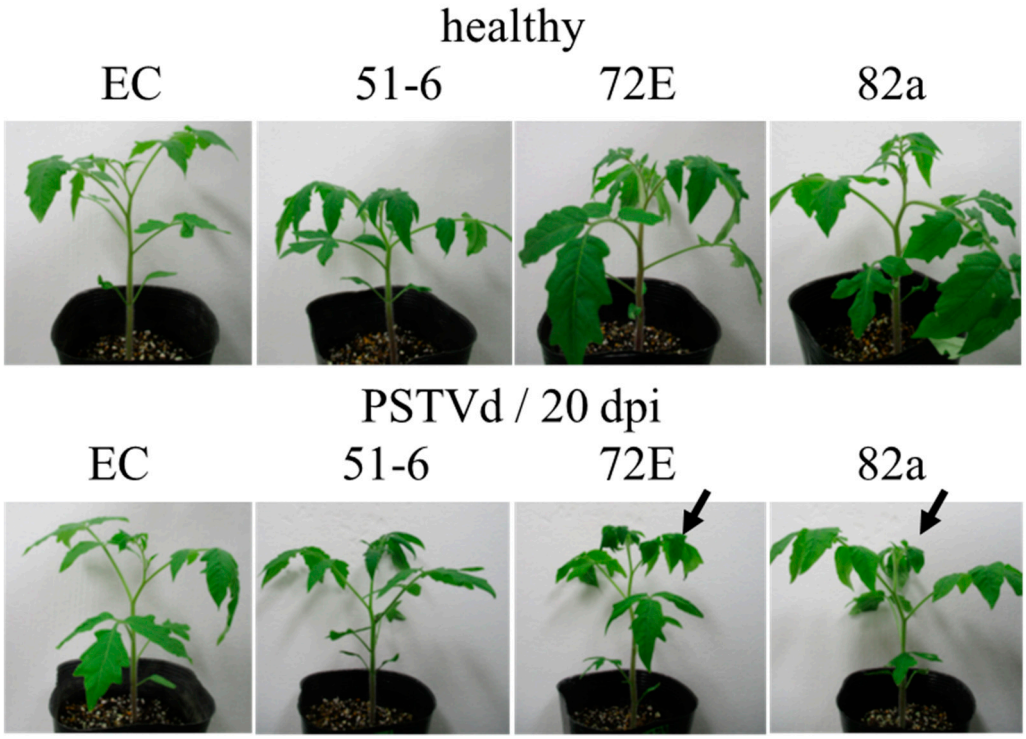

Figure S3. Primary symptoms associated with PSTVd infection in the DCL2/4i-72E and 82a Moneymaker tomato line. The arrows indicates mild leaf curling ~20 dpi.

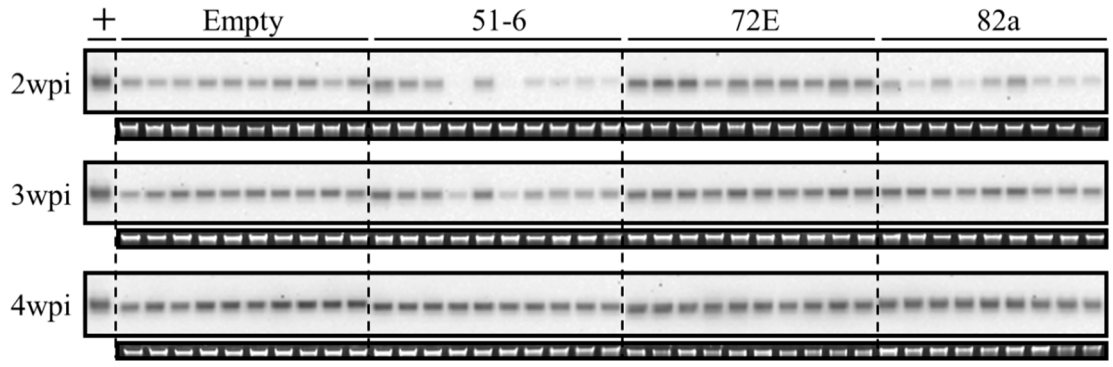

Figure. S4 Northern-blot hybridization analysis to monitor the levels of PSTVd accumulation in DCL2/4i-51-6, 72E, and 82a lines. Total RNA extracted from the upper leaves of nine to ten individual tomato plants from each line at 2, 3, and 4 wpi was used to monitor the levels of PSTVd accumulation. PSTVd was detected in almost every inoculated plant, even at 2 wpi (10/10 plants infected in line EC, 8/10 in line 51-6, 10/10 in line 72E, and 9/9 in line 82a. One or two weeks later, all plants were infected.

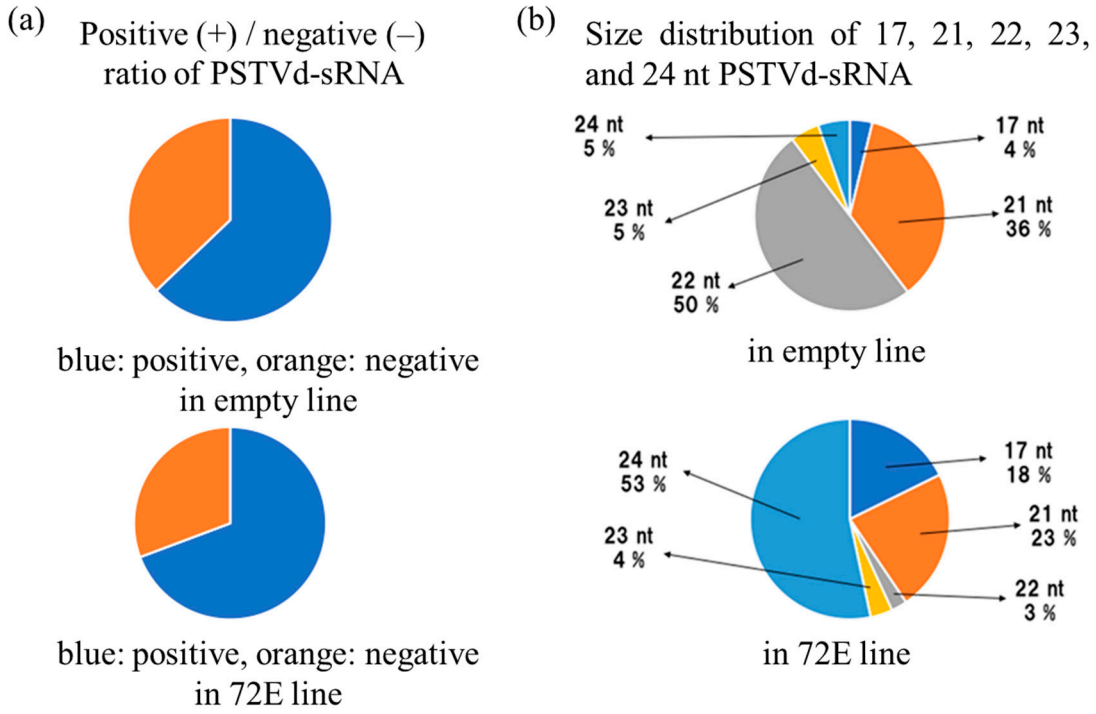

Figure S5. Overview of the changes in PSTVd-sRNA reads between PSTVd-72E and PSTVd-EC preparations. (a) PSTVd-sRNAs in PSTVd-72E comprised 383,947 reads (69%) from plus-strand and 170,091 reads (31%) from the minus-strand. This ratio was very similar in line EC; i.e., 318,585 reads (63%) to 188,092 reads (37%). (b) The most abundant size class in line EC was 22-nt (50%), followed by 21-nt (36%), 24-nt (5%), and 23-nt (5%), however, the most abundant size class in line 72E, was 24-(53%), followed by 21-nt (23%), 17-nt (18%), 23-nt (4%), and 22-nt (3%).

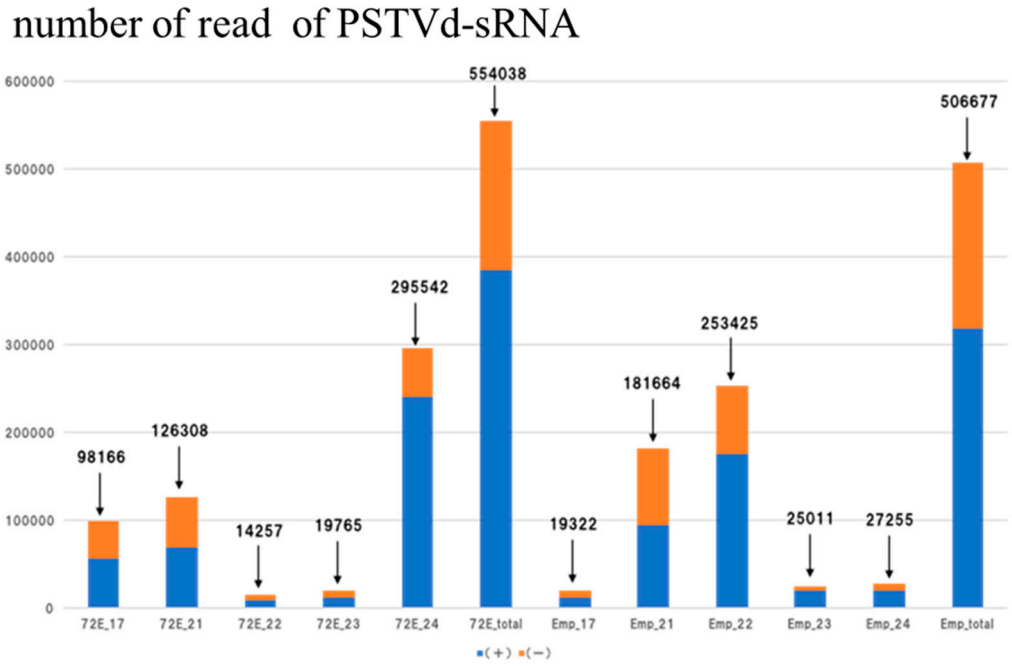

Figure S6. Histograms of the number of PSTVd-sRNA reads in 72E and EC (Emp in the figure). Five major PSTVd-sRNA size classes (i.e., 17, 21, 22, 23, and 24) were presented. The most abundant size class in line EC was 22-nt (50%), followed by 21-nt (36%), 24-nt (5%), and 23-nt (5%), however, the most abundant size class in line 72E, was 24-nt (53%), followed by 21-nt (23%), 17-nt (18%), 23-nt (4%), and 22-nt (3%).

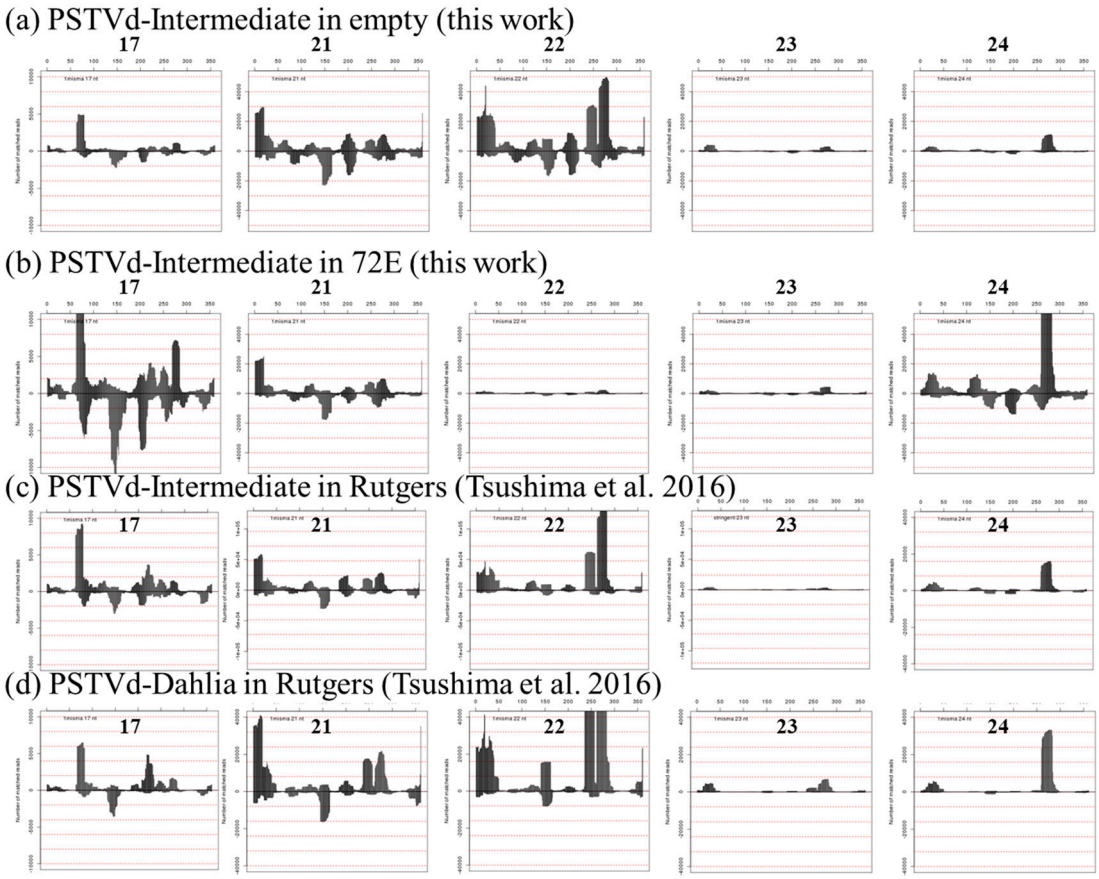

Figure S7. A comparison of hotspot pattern (size distribution and positive (+) / negative (–) ratio) of PSTVd-sRNAs of the size 17-nt, 21-nt, 22-nt, 23-nt, and 24-nt accumulated in the EC (a) and 72E (b) lines in this work, and in a similar analysis performed previously in Tsushima et al. [11]. Both of the analysis was performed by Hokkaido System Science Co., Ltd. (Sapporo, Japan) for small RNA sequence analysis (2Gb scale, paired end) using an Illumina HiSeq (Illumina, San Diego, CA, USA). Moneymaker tomato infected with PSTVd-intermediate was used in this work, and Rugers tomato infected with PSTVd-Intermediate and PSTVd-Dahlia was used in the previousl work. Nevertheless, the hotspot patterns seen in this work (a) was very similar to those in the earlier report (b and c), indicating a high degree of reproducibility among multiple deep sequencing analyses.

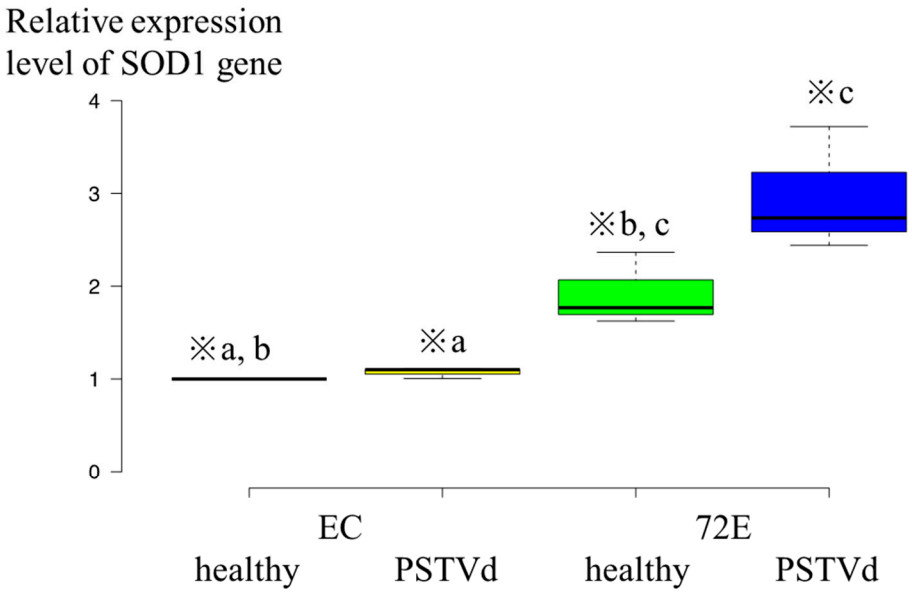

Figure S8. Statisctical analysis of expression levels of SOD1 in healthy and PSTVd-infected EC and 72E lines. Band signals obtained from Fig. 6c and d by ChemiDoc XAR (BioRad) was quantified with Quantity One software (Bio-Rad) and normalized the value of healthy EC as 1.0. The analysis was repeated three. “a”, “b”, and “c” were statistically significant at 5% level ($P < 0.05$).