Supplemental Data

Supplemental Table I. Primers used in this study.

Locus name	Primer name	Sequence of primers (5' to 3')	Product size (bp)
for genotyping			
Tos17	Tos17-R	AGGTTGCAAGTTAGTTAAGA	
hsa32-1	HSA32-FG2F	GATGAGGAGCCCCTTCTACG	1214
	HSA32-FG1R	ATAAGTTTCCGGGCAGGAGT	
hsa32-2	HSA32-CD2F	TCTGGAGATAGGGCATTTGG	1030
	HSA32-CD2R	ATAAGTTTCCGGGCAGGAGT	
ACTIN1	OsACT1-F	CTGATGGACAGGTTATCACC	600
	OsACT1-R	CAGGTAGCAATAGGTATTACAG	
GUS-linker	GUS linker-F	CCGAATACGGCGTGGAT	
for semi-quantitative RT-PCR			
HSA32	HSA32-FG2F	GATGAGGAGCCCCTTCTACG	477
	HSA32-FG1R	ATAAGTTTCCGGGCAGGAGT	
for quantitative or semi-quantitative RT-PCR			
HSA32	HSA32-CD1F	CGAGGATGTTGACCTGTTGA	310
	HSA32-CD1R	TCCGAATAAGGGAGAGTTGC	
HSP101	HSP101-4F	GGCAAGGTGATCCTCCTCTTCAT	369
	HSP101-4R	GAGGTCGATCGCTTTGTCAG	
ACTIN1	ACTIN1-F	TCAGCAACTGGGATGATATGGAG	375
	ACTIN1-R	GCCGTTGTGGTGAATGAGTAAC	
18S rRNA	18S-1F	CTACGTCCCTGCCCTTTGTACA	65
	18S-1R	ACACTTCACCGGACCATTCAA	
for generating *HSP101* RNAi construct			
HSP101	HSP101-5F	ATCCACTCCGCCTTCATA	258
	HSP101-5R	CCTCCGACGCTATCTCGTG	
for southern hybridization			
HPT	HPT-F	GTCGGCATCTACTCTATTTCTTTG	1100
	HPT-R	ACGCACAATCCCACTATCCTT	

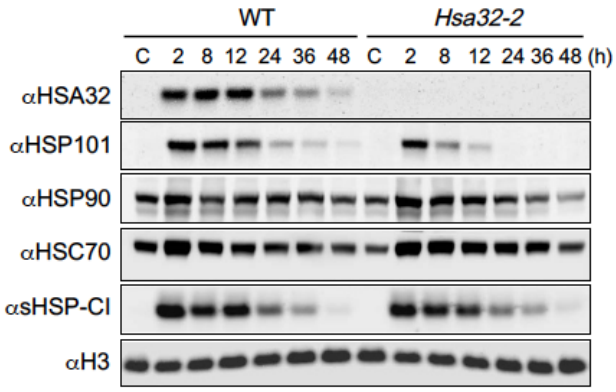

Figure S1 Faster decay of heat-induced HSP101 protein in the absence of HSA32. Immunoblot analysis of the indicated HSPs in 4-d-old wild-type and *hsa32-2* mutant seedlings, from which the crude extracts were prepared after recovery for 2-48 h following heat treatment at 42°C for 2 h. The non-heated sample was included as control (C). In each lane, 60 μg of total protein was loaded. Histone H3 (H3) is shown as a loading control. The test was repeated once and similar results were obtained.

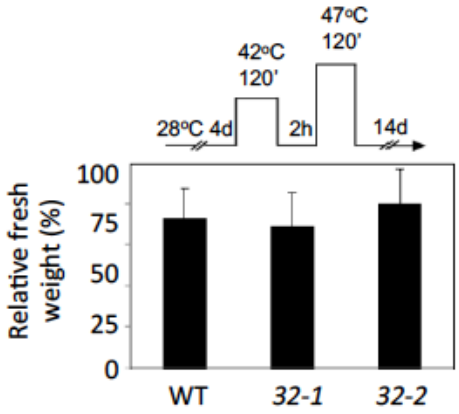

Figure S2 Analysis of fresh weight change after SAT assay. The fresh weights of wild-type (WT), *hsa32-1* (*32-1*), and *hsa32-2* (*32-2*) seedlings were measured after the SAT assay treatment described in Figure 5. The results are presented as relative fresh weight calculated by the following equation: (Fresh weigh of heat-treated seedling/mean fresh weigh of control seedlings) × 100%. The mean value of the relative fresh weight of eight seedlings is shown for each line. Results are presented as mean values of three biological replicates ± SD (n = 12). No significant differences between the relative fresh weights of WT and *hsa32* mutants were found by Student's t test.

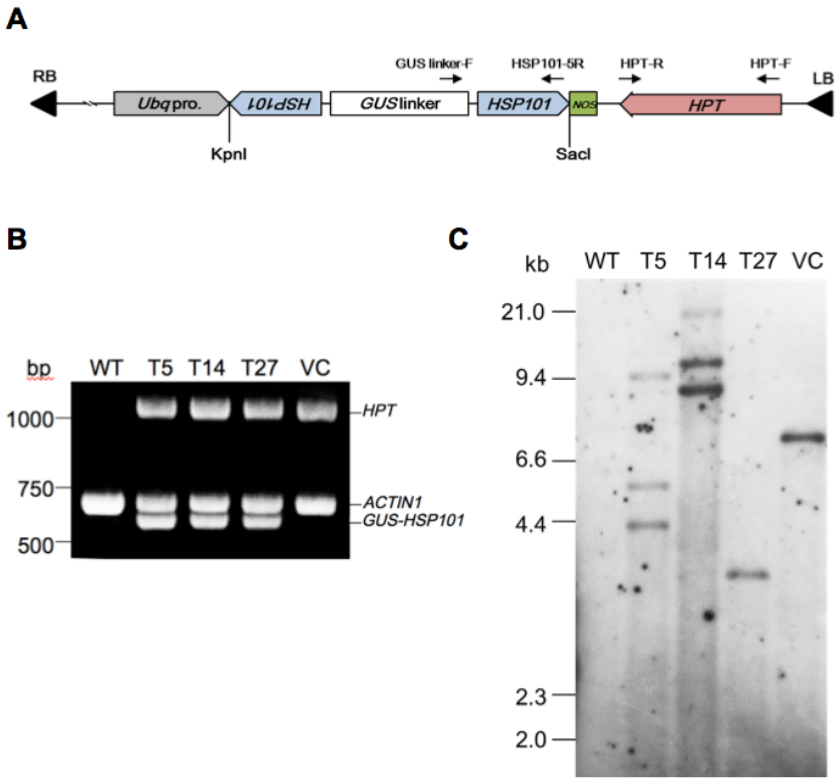

Figure S3 PCR genotyping and Southern blot analysis of RNAi lines. A, Diagram of part of the RNAi vector for *HSP101* silencing. The map is derived from that of pANDA provided by Dr. Ko Shimamoto and is not drawn to scale. The directions of the genes are shown with thick arrow heads. The small arrows indicate the locations of the primers used for PCR genotyping and preparation of the DIG-labeled probe. *HPT*, hygromycin phosphotransferase gene. B, PCR analysis of the transgenes in the genomic DNA of the wild type (WT), the empty vector control (VC), and three RNAi lines, T5, T14, and T27. Primers used in the multiplex PCR analysis are indicated in panel A. *ACTIN1* (*ACT1*) was amplified as a positive control with specific primers OsACT1-F and OsACT1-R (Supplemental Table S1). C, Southern blot analysis of the genomic DNA of WT, T5, T14, T27, and VC. Approximately 10 μg of genomic DNA was digested with restriction enzymes *Kpn*I and *Sac*I and separated on a 1% agarose gel. The blot was probed with DIG-labeled DNA annealing to *HPT*.

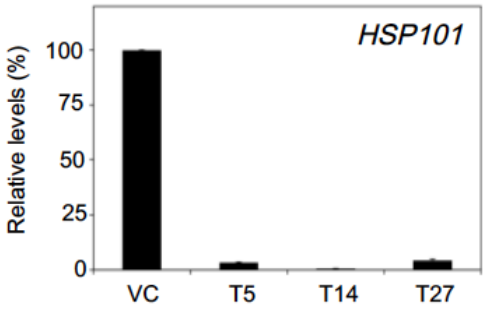

Figure S4 Quantitative RT-PCR analysis of *HSP101* transcripts in RNAi lines. VC is a transgenic line with an empty vector only; T5, T14, and T27 are independent transgenic lines with the RNAi construct targeting *HSP101*. Quantitative RT-PCR analysis of the transcript levels of *HSP101* in 4-d-old seedlings of different lines after heat treatment at 42°C for 2 h. Relative transcript levels of *HSP101* were obtained by normalization to the VC sample, which is set as 100%. Results are presented as mean values of three technical replicates ± SD. The test was repeated once and similar results were obtained.