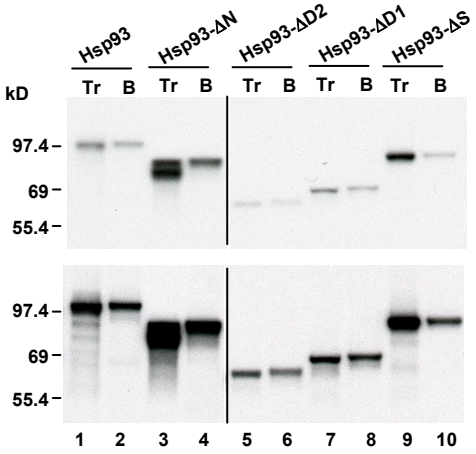
Supplemental Table

Sequences of primers used in this study.

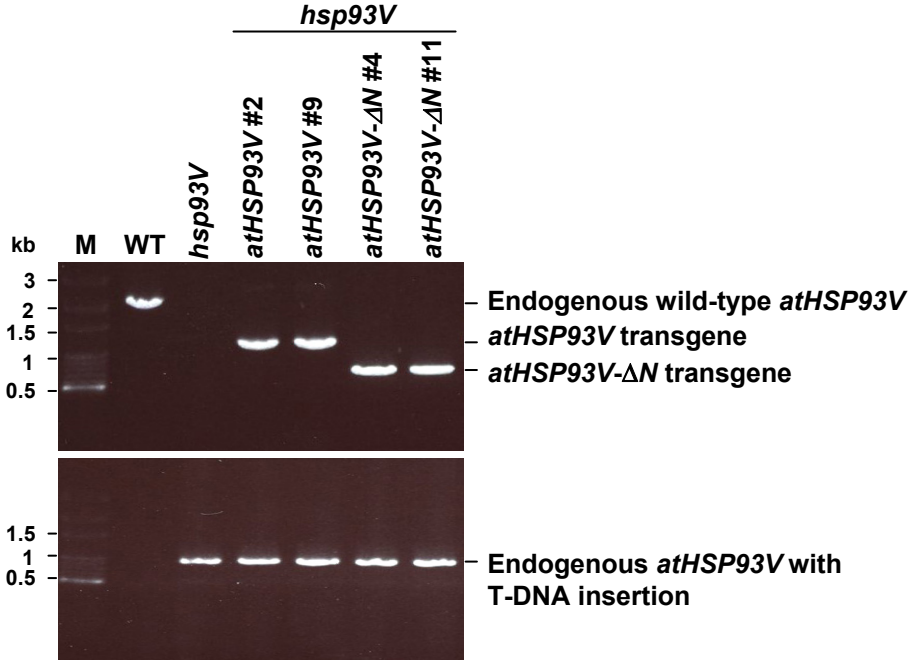
Primers	Sequences
PsprHsp93-delN-F	agtgctgacagtgttaccgctactgttggc
PsprHsp93-delN-R	tctaggaatgaatctcttagcccttgcccg
PsprHsp93-delD1-F	gaagaggcaaaagaacttgacaaggaggtc
PsprHsp93-delD1-R	ctcacccaccatgcgaataacctgtgtg
PsprHsp93-delSpacer-F	attcctgttgataaagtctcagcagatgaatc
PsprHsp93-delSpacer-R	tggcaactgtgcatgttgaagacggactc
PsprHsp93-I584amber-F	gtgtcctcctggacaggctagcctgttgataaagtctca
PsprHsp93-I584amber-R	tgagactttatcaacaggctagcctgtccaggaggacac
mHsp93-delN-F-NdeI	gcacatatgagtgctgacagtgttaccgctac
mHsp93-delN-R-NotI	cgtgcggccgcttatatagaaagagcctctgg
atHsp93V/F7-PstI	ctctgttacgcttcaatcgaactcgg
atHsp93V/R7-KpnI	gctttttttatttaagcaacagggagag
atHsp93V-delN-F	aacaatgaagtaactgctaatgttgggggaggaagc
atHsp93V-delN-R	tttcaccgtaaaccggctagctttccccttg
atHsp93V/F8-KpnI	caggtaccatggctatggccacaagggtgttg
atHsp93V/R8-PstI	cactgcagttaagcaacagggagagaatcttcc
atHsp93V-R	tctgccatactatcctctaaaagcctcat
atClpCV-R1	ctcatcgctctgtctaatttc
JL202	cattttataataacgctgcggacatctac

Supplemental Figure S1



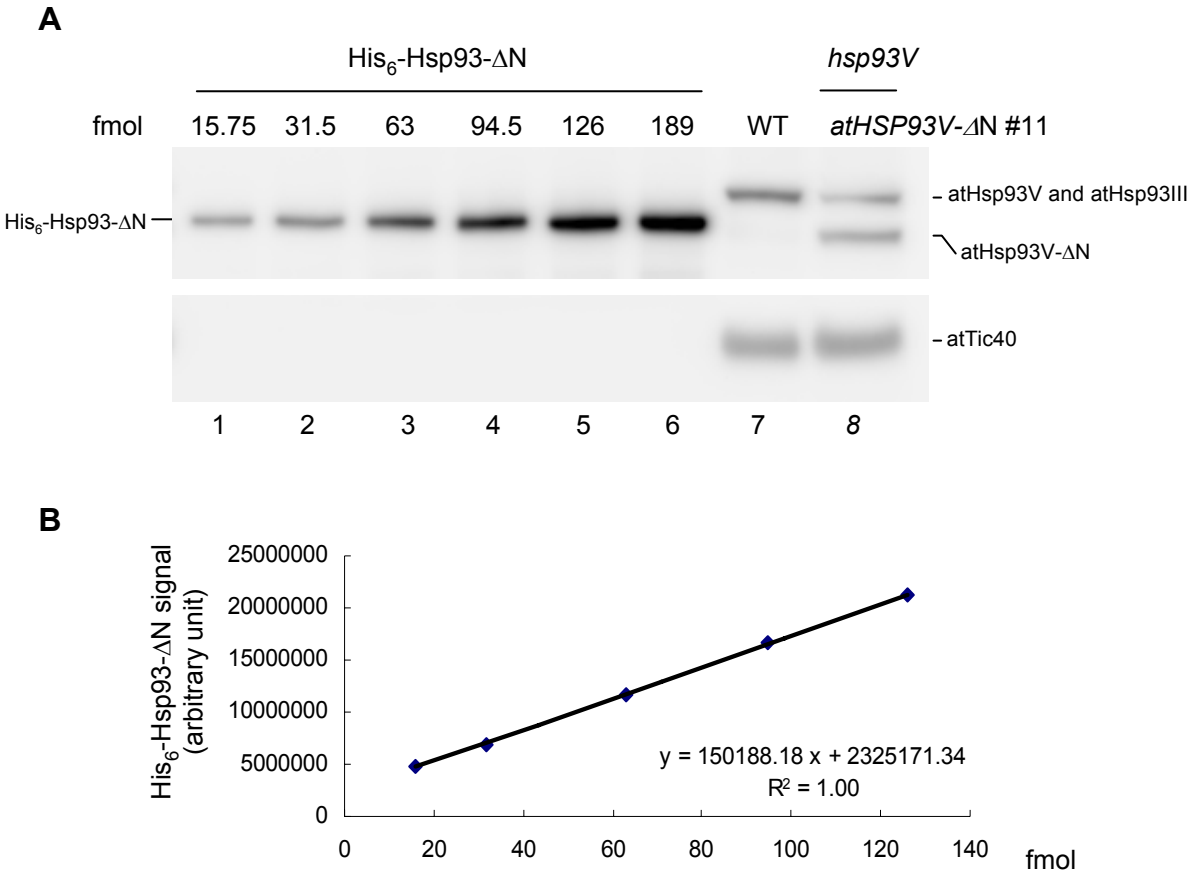
Supplemental Figure S1. Association of prHsp93 and the deletion mutants with chloroplasts under binding conditions. In vitro-translated [^{35}S]Met-labeled precursors (Tr lanes) were incubated with isolated pea chloroplasts in the presence of 3 mM ATP at 4°C for 5 min (under these conditions precursors can only bind to chloroplasts but can not be translocated across the envelope) and intact chloroplasts (B lanes) were recovered through a 40% Percoll cushion. Samples were analyzed by SDS-PAGE and fluorography. All Tr lanes contain 5% of the translation products used for the B lanes. All B lanes contain 30 μg of total chloroplast proteins in each lane. The lower panel is a longer exposure of the same gel shown in the upper panel. Lanes 1 to 10 were run on the same gels with some lanes in between lanes 4 and 5 removed.

Supplemental Figure S2



Supplemental Figure S2. Genotyping of *atHSP93V* and *atHSP93V-⊿*N transgenic plants. PCR analyses of genomic DNA of wild type (WT), *hsp93V*, and two independent lines of *hsp93V* mutant transformed with *atHSP93V* or *atHSP93V-⊿N*. Primers used for the *atHSP93V* wild-type copy and the transgenes are atHsp93V-F8-KpnI and atClpC-V-R1 and primers used for the T-DNA insertion of the *hsp93V* mutant are JL202 and atHsp93V-R. M, DNA size markers.

Supplemental Figure S3



Supplemental Figure S3. Wild type and *atHSP93V-ΔN* transgenic plants contain an equal amount of atHsp93 proteins. A, Immunoblot analyses of serial dilutions of His_6-Hsp93-ΔN proteins and proteins extracted from 15-day-old seedlings of wild type (WT) and the #11 line of the *atHSP93V-ΔN* transformant. Lanes 1 to 6 contain 15.75, 31.5, 63, 94.5,126 and 189 fmol of His_6-Hsp93-ΔN proteins, respectively. For plant extracts (lanes 7 and 8), 30 µg of total proteins were analyzed. The gel was analyzed by immunoblotting with antibodies against Hsp93 and atTic40. atTic40 was analyzed as a loading control. B, The chemiluminescence signals as shown in A were quantified and plotted against the amount of His_6-Hsp93-ΔN proteins. The formula and R^2 are also shown. Based on the formula in B, the amount of total atHsp93 in WT (lane 7 of A), and atHsp93III and atHsp93V-ΔN in *atHSP93V-ΔN* #11 (lane 8 of A) was calculated. In wild type, about 43 fmol of atHsp93 were detected in 30 µg of total proteins. In the *atHSP93V-ΔN* transformant, about 20.9 fmol of atHsp93III and 32 fmol of atHsp93V-ΔN were detected in 30 µg of total proteins. The Tic40 signal in the atHsp93V-ΔN transformant was 1.23 fold of that in the wild type.