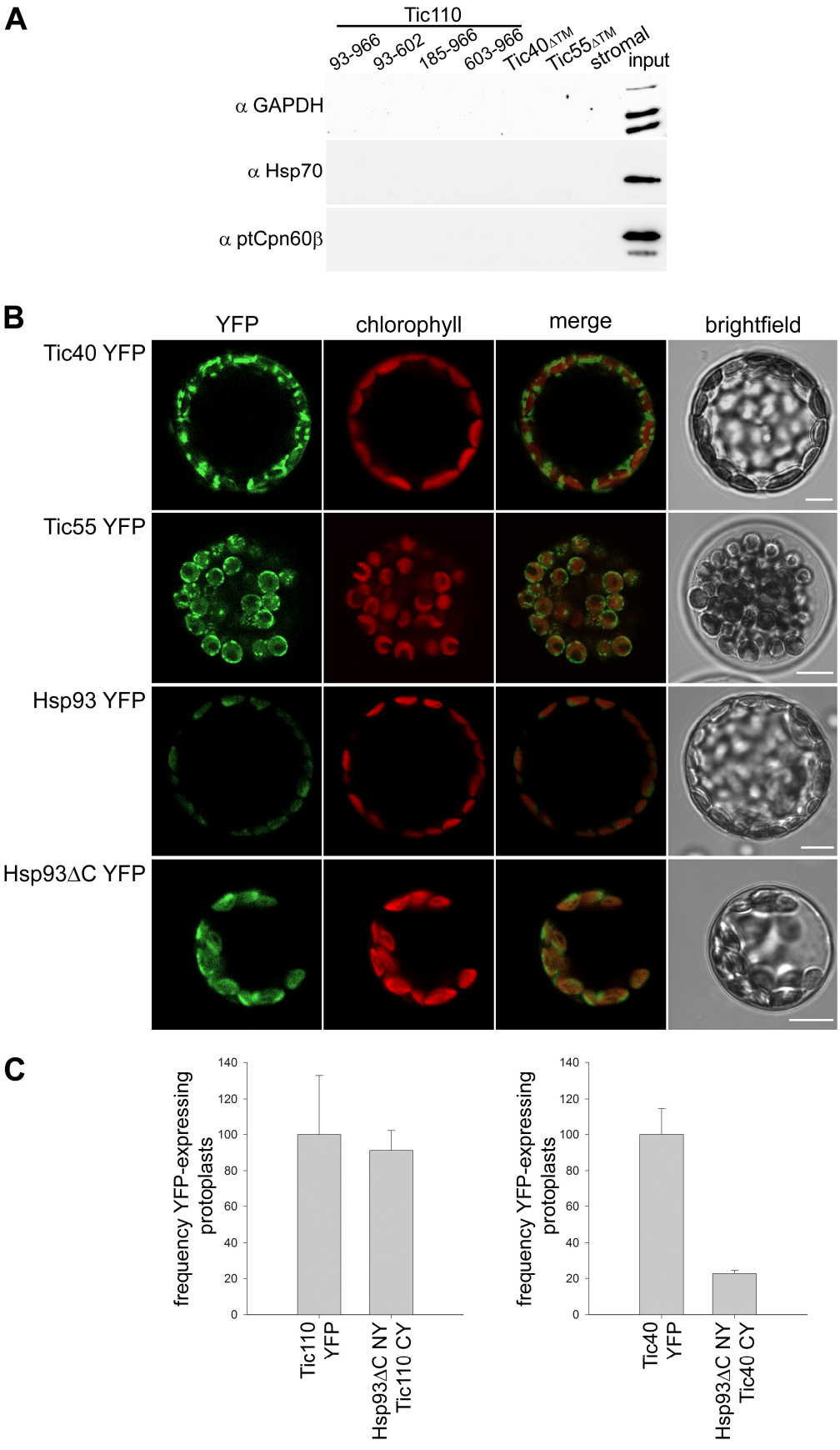
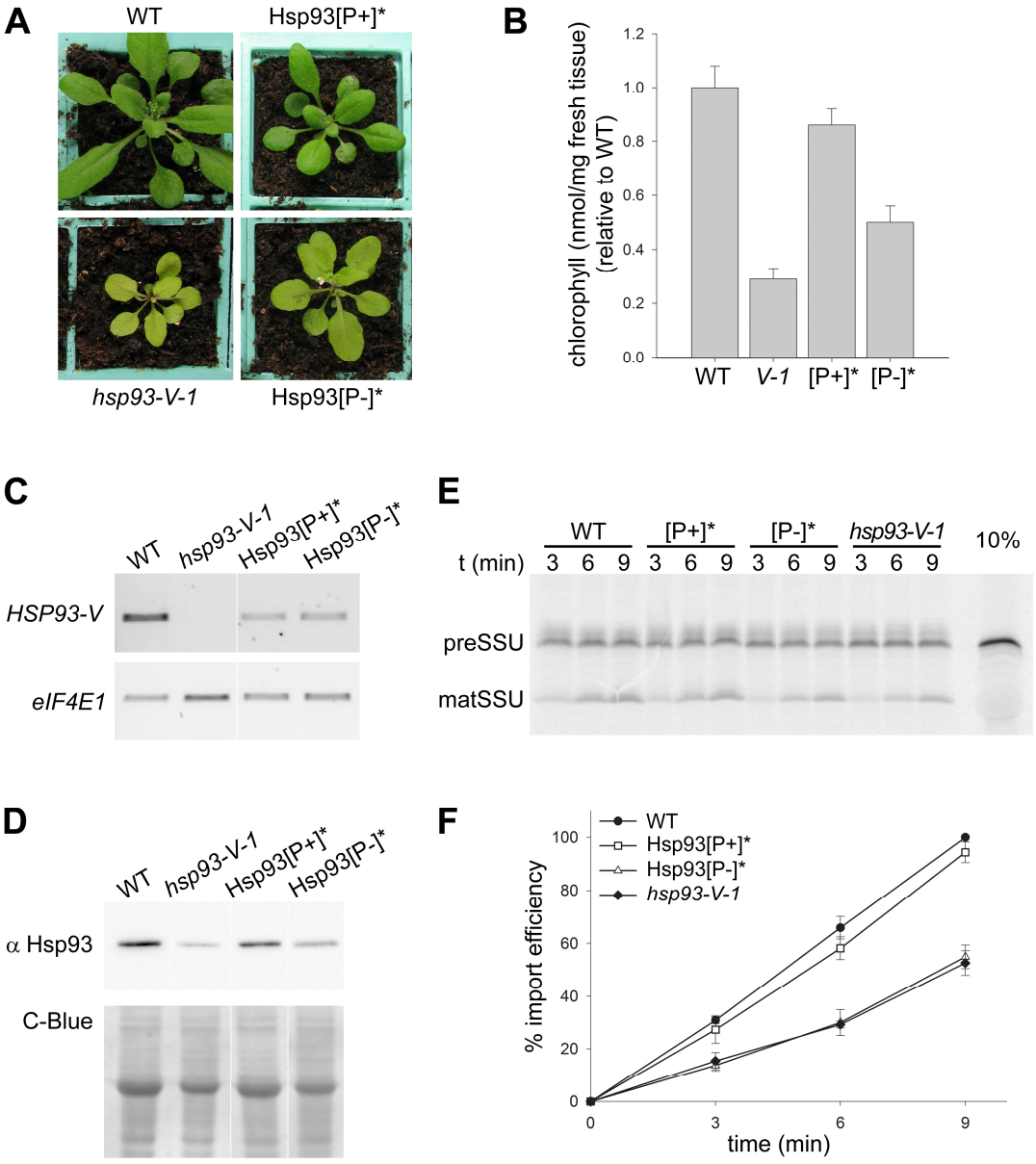
Supplemental Materials



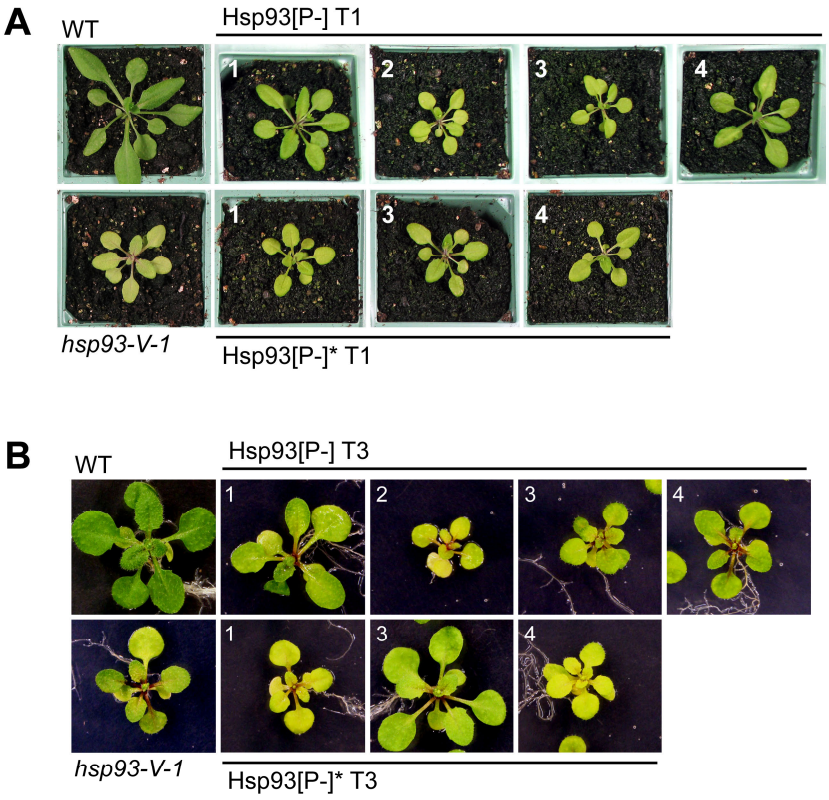
Supplemental Figure S1. Pull-down and BiFC controls, and quantitative analyses associated with the BiFC studies.
(A) Controls for the *in vitro* pull-down studies. To test for possible non-specific and/or chaperone-substrate interactions with the Tic110, Tic40 and Tic55 recombinant proteins in our *in vitro* pull-down system, the pull-down eluates were analysed by immunoblotting using antibodies against three additional stromal proteins. Primary antibodies used were against GAPDH (glyceraldehyde 3-phosphate dehydrogenase), the chloroplast chaperone cpHsp70, and the plastid chaperonin 60β (ptCpn60β). As in Fig. 1B, this figure shows immunoblot analysis of 30% of the eluted proteins and a sample (~2 µg) of the stromal fraction input. (B) Controls for the BiFC studies. Analysis of control constructs in which the proteins of interest were fused to full-length YFP. Wild-type protoplasts were transfected and analysed by confocal microscopy for YFP fluorescence and chlorophyll autofluorescence (merged images of these two are also presented), and by brightfield illumination (as described in relation to Fig. 1C). The fusion proteins were localised accordingly at their respective chloroplast compartments: YFP fluorescence was associated exclusively with the envelope in protoplasts transfected with Tic40 or Tic55 constructs, and was mainly in the stroma in protoplasts transfected with Hsp93 or Hsp93ΔC constructs. Bars = 10 µm. (C) Quantitative analysis of the frequency of YFP fluorescence in the BiFC studies presented in Fig. 1C. The frequency of protoplasts that displayed YFP reconstitution via heterodimerization (*i.e.*, Hsp93ΔC+Tic110, or Hsp93ΔC+Tic40) was calculated. Values for formation of the heterodimers are normalized relative to the frequency observed for the respective full-length YFP construct. All values shown are means, and error bars indicate SD (n=3).



Supplemental Figure S2. Hsp93 PBM mutant complementation analysis using non-tagged constructs.

Transgenic lines carrying non-FLAG-tagged constructs (denoted with an asterisk, Hsp93[P+]* and Hsp93[P-]*) in the *hsp93-V* single-mutant background were also analysed to evaluate the effect of the I772E mutation on Hsp93 function and protein import. (A) Visible phenotypes of typical transgenic plants (T2 generation) after 27 days growing on soil. Wild-type and *hsp93-V* mutant plants were grown together as controls. (B) Relative chlorophyll concentrations in the plant genotypes described in A. Analysed leaves were from 35-day-old plants grown on soil, the transgenic plants were from segregating families that had been pre-screened on selective medium before transfer to soil. (C) Analysis of the expression of the transgenes by RT-PCR using gene-specific primers for *HSP93-V*, and for translation initiation factor *eIF4E1* as a control. Analysed RNA samples were isolated from 10-day-old seedlings grown *in vitro*. (D) Immunoblot analysis of the expression levels of the Hsp93[P+]* and Hsp93[P-]* proteins. Analysed samples were total protein lysates from 11-day-old plants grown *in vitro*. Coomassie Blue (C-Blue) staining served as an equal loading control. (E, F) Analysis of protein import into chloroplasts isolated from the Hsp93[P+]* and Hsp93[P-]* transgenic lines and from the indicated controls. For these experiments we isolated chloroplasts from plants grown *in vitro*

to developmental similar growth stages (*i.e.*, 14-day-old wild-type and Hsp93[P+]* plants, and 21-day-old Hsp93[P-]* and *hsp93-V* plants). A typical experiment is shown in E. preSSU, precursor protein form; matSSU, mature protein form; 10%, ten percent of the translation product added to each import reaction. The amount of protein imported into chloroplasts of each genotype in E (and in additional, similar experiments) was quantified by measuring the mature SSU band (F). The data are presented as a percentage of the amount of imported protein for the wild type at the last point of the time course, and expressed as "% import efficiency". All values shown are means, and error bars indicate SD (n=3).



Supplemental Figure S3. High level expression of the Hsp93[P-] protein in transgenic plants negatively affects plant vigour.
(A, B) Visible phenotypes of typical plants from each of several independent transgenic lines (line numbers are indicated) in the *hsp93-V* single-mutant background, both in the T1 generation following growth on soil (A), and in the T3 generation following growth *in vitro* (B). Wild-type and *hsp93-V* mutant plants were grown together as controls. Note that transgenic lines expressing non-FLAG-tagged proteins are highlighted with an asterisk: Hsp93[P-]*. (B) Immunoblot analysis of the expression levels of the Hsp93[P-] and Hsp93[P-]* proteins. Analysed samples (10 µg per lane; or 20 µg for Coomassie staining) were total protein lysates from typical rosette leaves of 48-day-old T3 plants grown on soil. Coomassie Blue (C-Blue) staining served as an equal loading control. The lines selected for further analysis on the basis of partial complementation (*i.e.*, Hsp93[P-] #4 and Hsp93[P-]* #3) were shown to be low level expressors, whereas the lines that displayed higher levels of transgene expression (*i.e.*, Hsp93[P-] #2 and Hsp93[P-]* #1 and #4) exhibited more severe chlorosis and reduced stature relative to the *hsp93-V* single mutant.