Supplemental Figure 1. Import of Stisa1, Stisa2 and Stisa3 proteins into isolated pea chloroplasts.

cDNAs encoding Stisa1, Stisa2 and Stisa3 were translated in vitro (Tr). [^{35}S]-labeled proteins from the translations were incubated with pea chloroplasts (C). Following incubation of proteins and chloroplasts, samples were treated with thermolysin to look for protection of imported proteins (C+). Chloroplasts with imported proteins were also lysed and separated into stromal (S) and thylakoid (T) fractions. As controls stromal fractions from chloroplasts incubated with mock in vitro translation products (So) were analyzed. The operation of thermolysin was also checked by treating protein from each in vitro translation with thermolysin without prior incubation with chloroplasts (Tr-). Protein samples were separated by SDS-polyacrylamide gel electrophoresis. The radioactive peptides were visualized using fluorography.

In vitro translated *Stisa1* gave a number of labeled peptides, the largest of which was 89 kD. Incubation of this with intact pea chloroplasts (C) caused a reduction in size of the peptide presumably due to import of the protein and cleavage of the transit peptide. The imported protein was resistant to thermolysin treatment (C+) and upon lysis of the chloroplasts and separation of the thylakoid (T) from the stromal proteins (S) was localized in the stromal fraction (S). Similar results were obtained for *Stisa2*. *Stisa3* gave an 87 kD peptide upon in vitro translation which was imported into the chloroplast stroma. In the case of Stisa3, thermolysin treatment of the unprotected protein (Tr-) caused cleavage of the protein to a slightly smaller size but did not eliminate the labeled protein. This cleavage product was not observed when Stisa3 had been incubated with chloroplasts and then treated with thermolysin (C+), indicating that the protein was imported and protected from protease activity. The imported Stisa2 and Stisa3 proteins did not show clear reductions in size that would have confirmed transit peptide cleavage. This was probably a facet of the large size of the proteins and the limited resolution of the SDS-PAGE used. All stroma-localized proteins that have been investigated to date are imported with cleavage of their transit peptides, so we interpreted the very slight increase

in the rate of migration of imported Stisa2 and Stisa3 relative to their in vitro translation products as supportive of transit peptide cleavage.

Supplemental Figure 2. Specificity of antisera raised against peptides of the Stisa proteins.

Antisera were raised in rabbits to synthetic peptides unique to Stisa1, Stisa2, or Stisa3. These and pre-immune sera from the same rabbits were used to develop blots of SDS-polyacrylamide gels of extracts of *E. coli* expressing Stisa1, Stisa2 or Stisa3. Antisera were used at dilutions of 1/10,000 (Stisa1 and Stisa3) or 1/20,000 (Stisa2 and pre-immune sera). The positions of molecular mass makers (kD) are shown at the left of the blots, and the type of antiserum is indicated above the blots. Each panel within a blot contains three lanes, with (left to right) 1 µl, 2 µl and 3 µl of the *E. coli* extract.
A. Extracts were from *E. coli* expressing Stisa1. The preimmune serum was from the rabbit subsequently immunized with a peptide from the Stisa1 protein.
B. Extracts were from *E. coli* expressing Stisa2. The preimmune serum was from the rabbit subsequently immunized with a peptide from the Stisa2 protein.
C. Extracts were from *E. coli* expressing Stisa3. The preimmune serum was from the rabbit subsequently immunized with a peptide from the Stisa3 protein.