Supporting information

Materials and Methods

(Fig S3) Subplastidial localization of PHSL or ΔPHSL**

Chloroplasts were isolated from transplastomic tobacco leaves and fractionated as previously described[16] on the top of a discontinuous sucrose gradient. After centrifugation at 70 000 ×*g* and 4°C for 1 h, the soluble stromal fraction was separated from the pelleted thylakoids. For further thylakoid purification, the pellet was washed with 10 volumes of washing buffer and centrifuged at 110,000 ×*g* and 4°C for 1 h, and then the thylakoid fraction was resuspended in a minimal volume of washing buffer. To verify the association between thylakoids and PHSL* or ΔPHSL*proteins, the thylakoid pellet was washed with solution containing DTT 0.1 M, NaCl 2 M or washing buffer alone, centrifuged at 14 000 ×*g* and 4°C for 10 min, and the supernatant was recovered. The soluble fraction and the pellet derived from these experiments were analyzed by immunoblot assay using anti-phaseolin antiserum (1:10,000), anti-Rubisco (1:7500; Jackson ImmunoResearch Inc., West Grove, PA) or anti-CP47 (1:5000) antiserum.

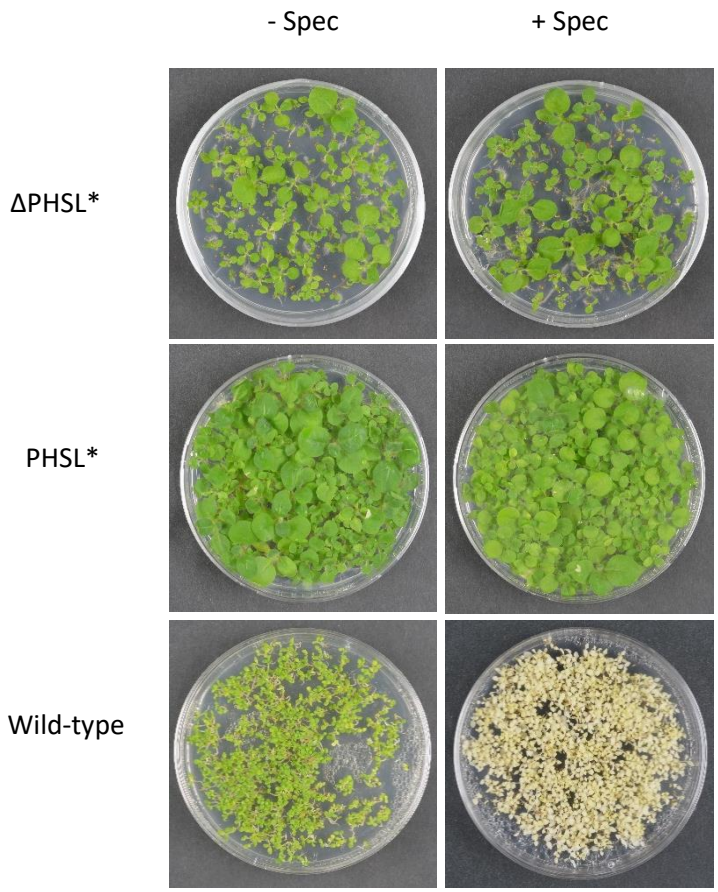

Figure S1. Germination of seeds on spectinomycin to confirm homoplasmy. Seeds from the PHSL* and ΔPHSL* T0 transplastomic plants, together with seeds from a wild type plant as a control, were germinated on MS medium with (+Spec) or without (-Spec) spectinomycin 500 mg l^{1}. The homoplasmic state of the PHSL* and ΔPHSL* T0 plants is confirmed by the resistence of the T1 seeds to spectinomycin.

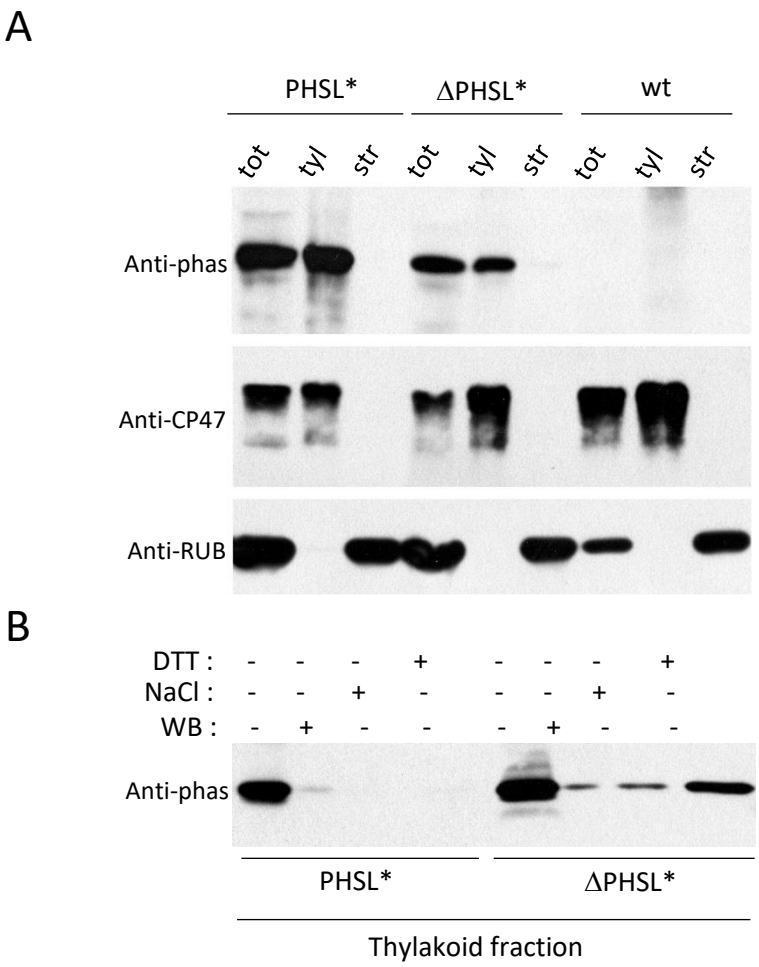

Figure S2. The signal peptide triggers the integration of PHSL* protein in the thylakoidal membranes. (A) Purified chloroplasts derived from wt or transplastomic plants expressing PHSL* and ΔPHSL* were lysed (tot) and fractionated into the stroma (str) and thylakoid fractions (tyl). (B) The thylakoid fractions were subjected to two sequential washes and then to a buffer containing 0.1 M DTT, 2 M NaCl or Washing Buffer (WB). Samples were analysed by immunoblot using antibodies against phaseolin, CP 47 and Rubisco (A) or using only antiserum against phaseolin (B).

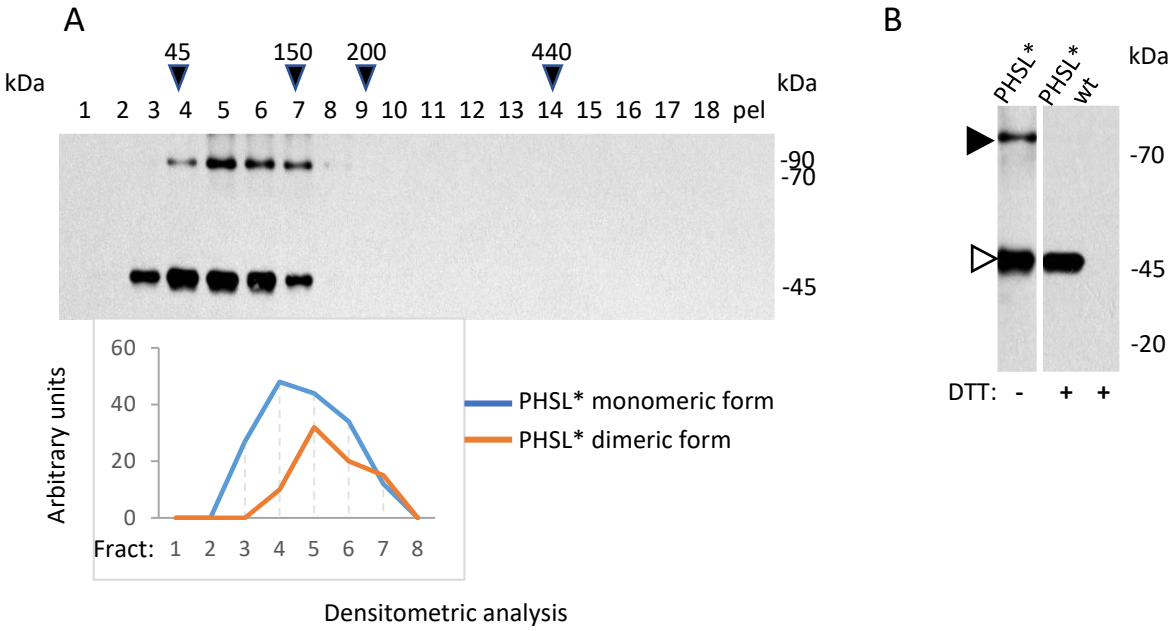

Figure S3. PHSL* can form disulfide bonds when expressed in tobacco chloroplast. (A) Pellet of the sucrose gradient of total proteins extracted from PSHL* transplastomic leaves was solubilized by homogenation buffer containing SDS in order to break down the phaseolin trimers into monomers, and then fractionated by another velocity sucrose gradient. Different fractions were collected, and each fraction was analyzed by SDS PAGE and western blotting using antiphaseolin antiserum. Numbers on top and at the right indicate molecular mass in kDa. The putative dimers of PHSL* were detected at about 90 kDa and the graphic of the fraction densitometric analysis below the figure shows the shift (fraction 4 to 5 due to the increase in the molecular weight of the dimer in comparison to the monomer). (B) An aliquot of fraction 5 of the velocity sucrose gradient shown in (A) was subjected to the action of the reducing agent DTT, and then analysed by SDS PAGE and western blot as described above.The

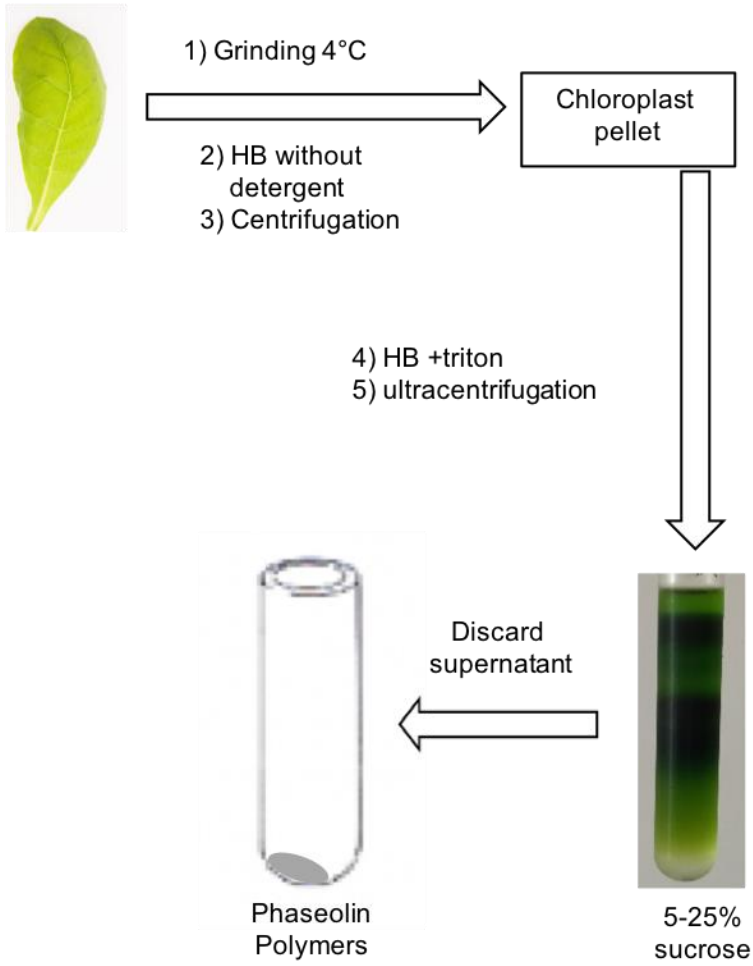

Figure S4. Schematic representation of PHSL* purification from transplastomic tobacco leaves. Detailed description is reported in the Materials and Methods section.

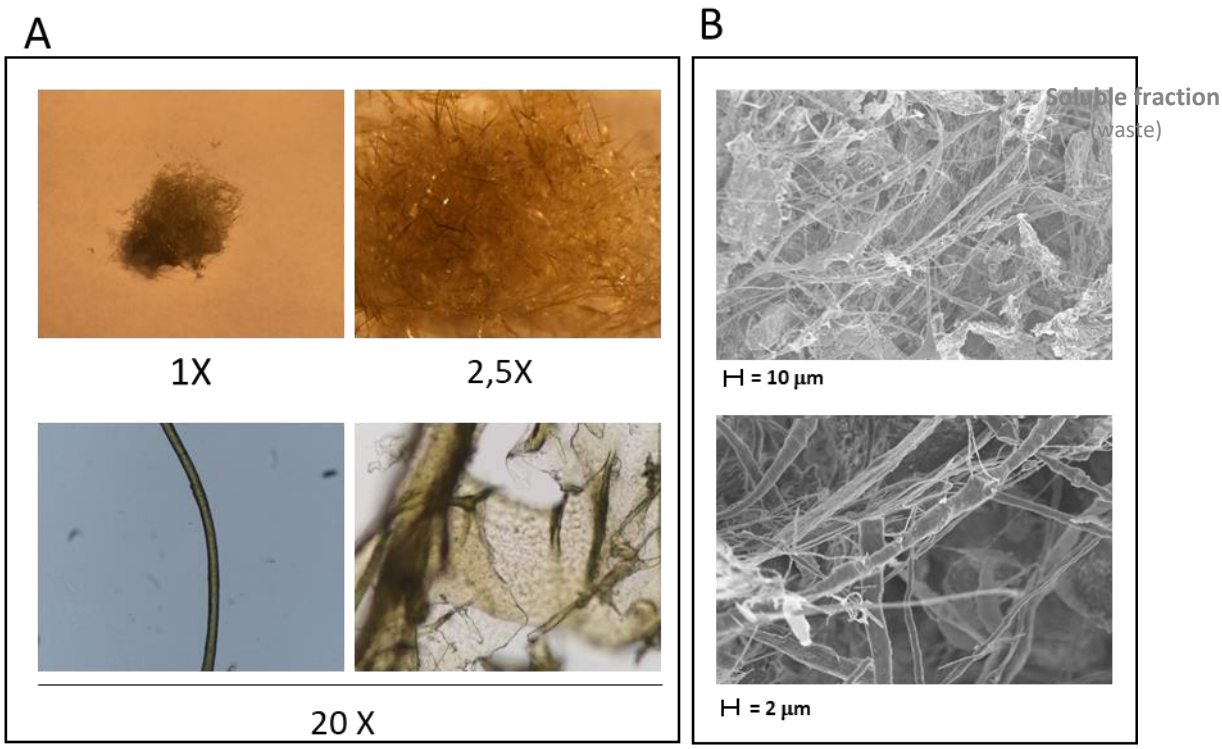

Figure S5. Macroscopic and microscopic analysis of PHSL* purified from tobacco chloroplasts. (A) Powder of PHSL* obtained by freeze-drying of aqueous solutions, observed at the optical microscope at different magnifications (2.5 and 20X). (B) Powder of PHSL* obtained by freeze-drying of aqueous solutions, observed at the scanning electron microscope.

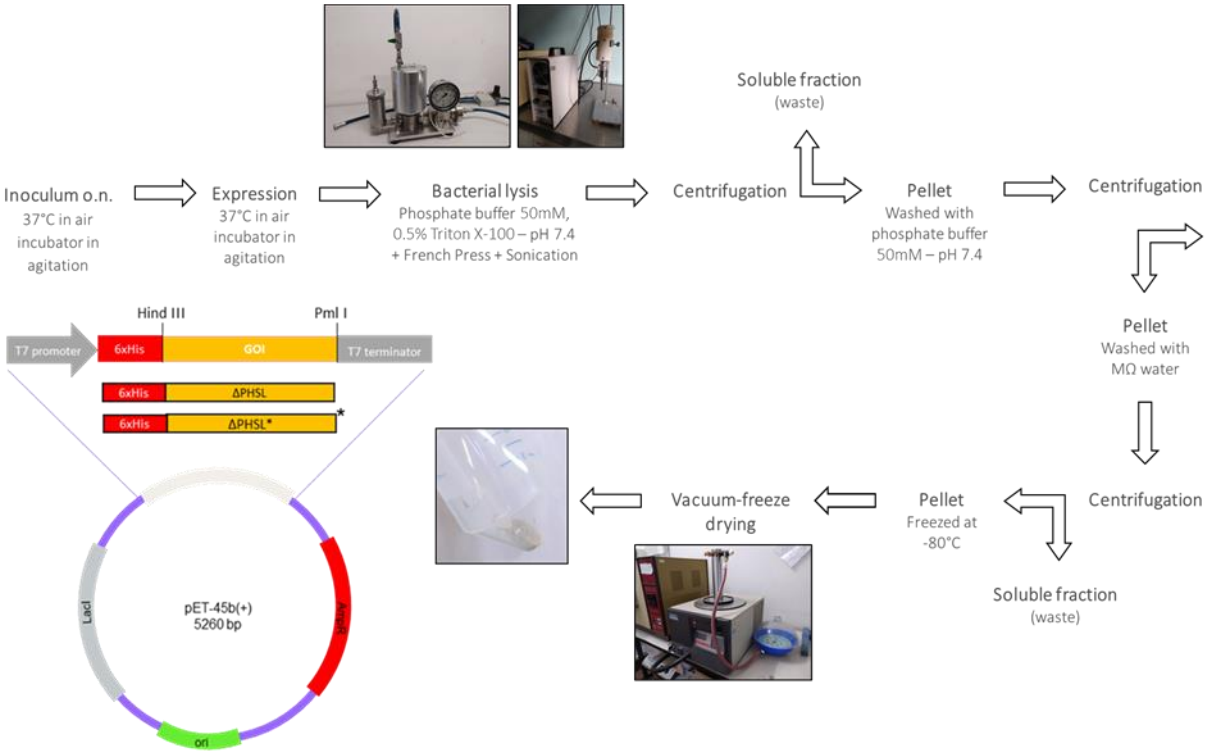

Figure S6. Schematic representation of the backbone vector and the cloned constructs. Overview of the protocol used for expression and extraction of ΔPHSL and ΔPHSL* from *E. coli* cells, as described in details in the Material and Methods section.

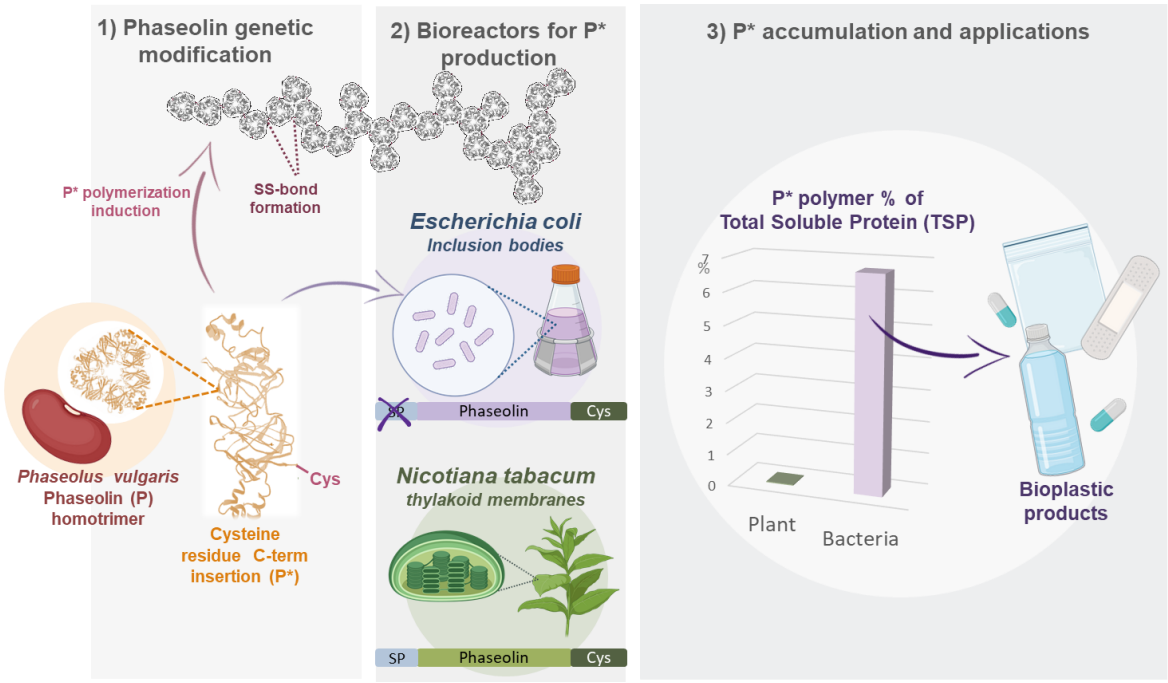

Figure S7. Schematic representation of protein based biopolymers production systems. Cys residue was introduced into the wt phaseolin sequence via genetic engineering in order to allows the formation of disulfide bridges that lead to a polymerization event. Two bioreactors were analyzed with two constructs differentiated by the presence of SP sequence. Bacteria show an interesting overproduction capacity of P* biopolymer for possible applications in bioplastic field (P, wt phaseolin; P*, phaseolin + cysteine residue; Cys, cysteine; SP, signal peptide; SS-bond, disulfide bridge; TSP, total soluble protein).