Supporting Information

Whitney et al. 10.1073/pnas.1109503108

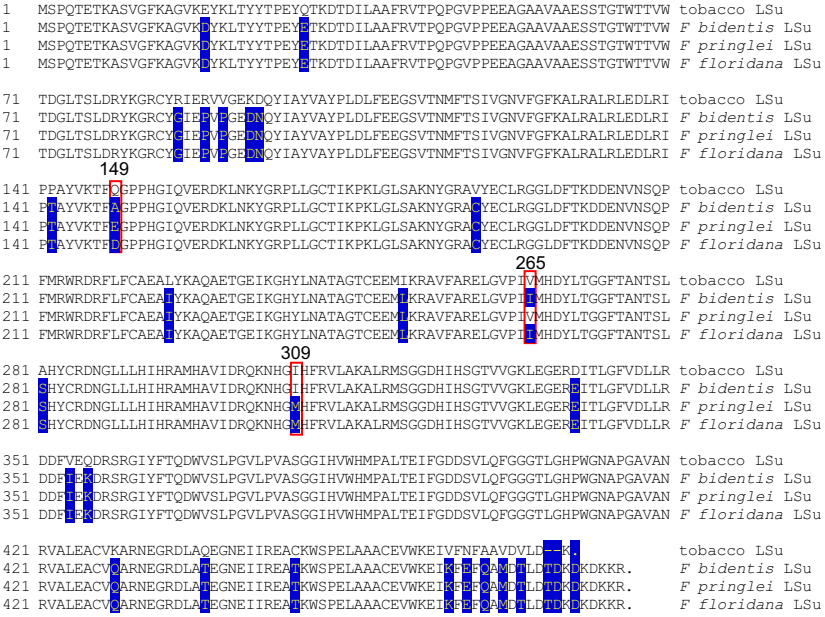

Fig. S1. Sequence comparison of tobacco and *Flaveria* ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) large subunits (L-subunits). Sequences were aligned using ClustalW (1). Amino acids residues differing from the tobacco L-subunit (GenBank accession number Z00444) are shaded blue. The positions of the divergent residues in the *Flaveria* L-subunits (at residues 149, 265, and 309) are boxed in red.

1. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680.

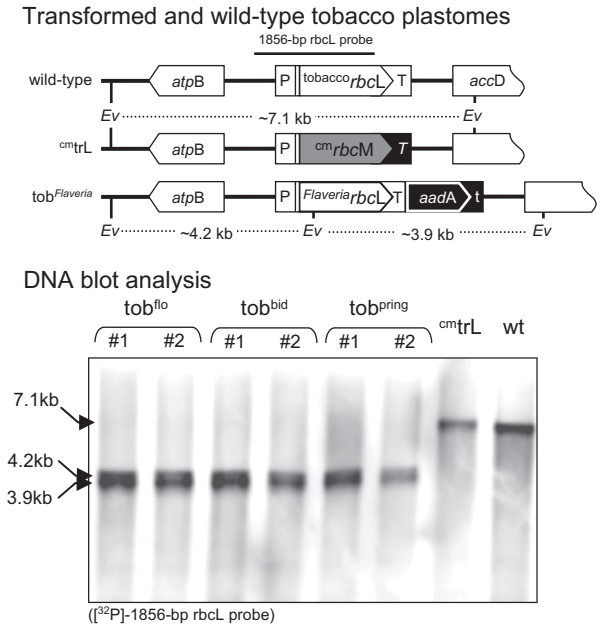

Fig. S2. DNA blot analysis of homoplasmicity. Unlike the *rbc*L in wild-type tobacco and the tobacco master line ^{cm}trL (1), the *Flaveria rbc*L genes introduced into the tobacco plastome contain an *Eco*RV site (Ev, *Lower*). Total genomic DNA from mature leaves of transformed T_1 progeny (germinated on Murashige and Skoog medium containing spectinomycin before transfer to growth in soil) and control plants (wild-type and ^{cm}trL) was isolated using the DNeasy Plant Mini Kit (Qiagen), digested with *Eco*RV, and examined by Southern (DNA) blot analysis as described (2) using a ^{32}P-labeled 1,856-bp rbcL probe as shown (*Upper*). In wild-type and ^{cm}trL plants the rbcL probe recognizes a single fragment of ~7.1 kb. Correct insertion of a *Flaveria rbc*L and *aad*A gene is indicated by hybridization to two fragments of 3.9 and 4.2 kb, respectively. No 7.1-kb band was present in the transformed lines, indicating they were homoplasmic.

1. Whitney SM, Sharwood RE (2008) Construction of a tobacco master line to improve Rubisco engineering in chloroplasts. *J Exp Bot* 59:1909–1921.
2. Whitney SM, Andrews TJ (2001) Plastome-encoded bacterial ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) supports photosynthesis and growth in tobacco. *Proc Natl Acad Sci USA* 98:14738–14743.

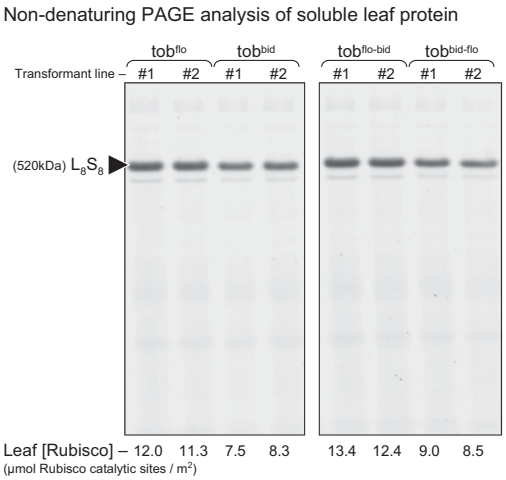

Fig. S3. Nondenaturing PAGE analysis of soluble protein from 2 mm^2 of leaf. Soluble protein from 2 mm^2 of young leaves (12 cm wide) from the same position in the upper canopy of plants ~15 cm high was separated by nondenaturing PAGE as described (1). Samples were taken from the T_2 tob^{flo} and tob^{bid} plants and the T_1 progeny of the tob$^{flo\text{-}bid}$ and tob$^{bid\text{-}flo}$ lines. In each sample, the L_8S_8 rubisco was the prominent band evident by Coomassie blue staining. The rubisco content in each sample was quantified by [^{14}C]2-carboxyarabinitol-1,5-bisphosphate binding as described (2).

1. Whitney SM, Sharwood RE (2008) Construction of a tobacco master line to improve Rubisco engineering in chloroplasts. *J Exp Bot* 59:1909–1921.
2. Ruuska S, et al. (1998) The interplay between limiting processes in C_3 photosynthesis studied by rapid-response gas exchange using transgenic tobacco impaired in photosynthesis. *Aust J Plant Physiol* 25:859–870.