Figure S1

Figure S1: Sequence alignment of chloroplast FtsH proteins. (a) FtsH2 and FtsH5 alignment; (b) FtsH5 and FtsH1 alignment (type A); (c) FtsH2 and FtsH8 alignment (type B). Binary alignments were obtained using SIM and LALNVIEW (http://www.expasy.ch/tools/sim-prot.html).

Figure S2

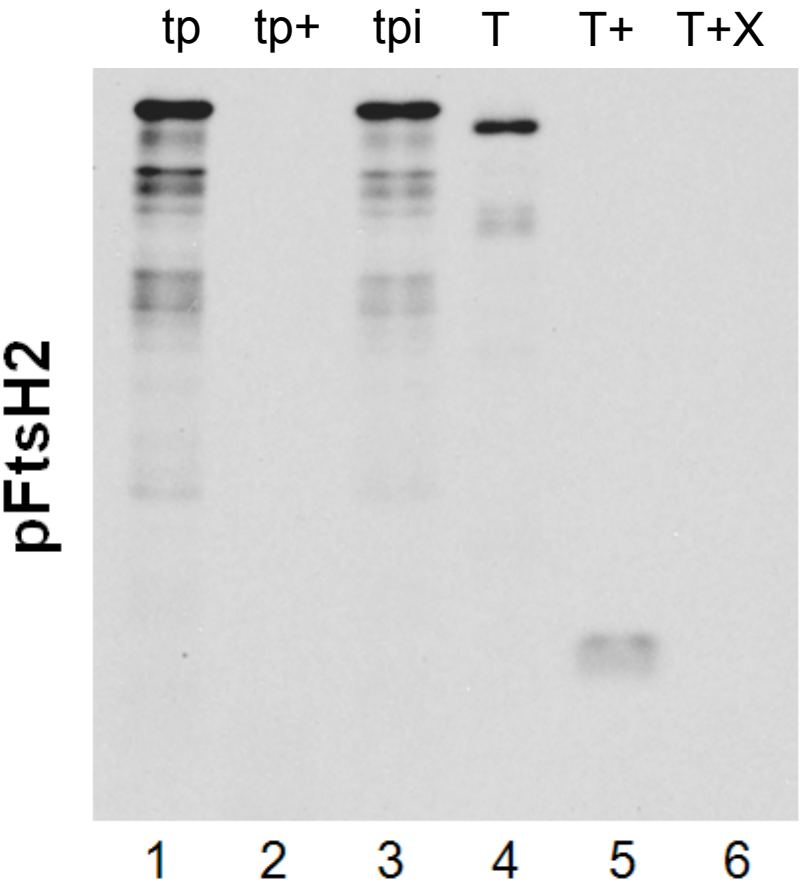

Figure S2: pFtsH2 is completely degraded by thermolysin without protection by the thylakoid bilayer. In vitro translated precursor was diluted with one volume of 60 mM leucine 2X IB. An aliquot of the precursor was used for a 110 µl thylakoid transport assay with chloroplast lysate. The remaining precursor was diluted 3-fold with IB and 25 µl aliquots were incubated at 4 °C without (TP, lane 1) or with 20 µg of thermolysin for 60 min (TP+, lanes 2). Proteolysis was terminated with 5 µl of 500 mM EDTA and samples denatured with an equal volume of 100 °C 2X SDS sample buffer for 4 min. As a control for potential proteolysis during denaturation, one untreatreated aliquot received 20 µg of EDTA-inhibited thermolysin immediately before SDS denaturation (TP_i, lane 3). Thylakoids from the transport assay were resuspended in three 25 µl aliquots in IB and either mock treated (T, lane 4), treated with 20 µg of thermolysin (T+, lane 5), or treated with thermolysin in presence of 1% Triton X-100 for 60 min at 4 °C (T+X, lane 6). Proteolysis was terminated, samples denatured as above and were analyzed by SDS-PAGE on 12.5% gels and fluorography.

Figure S3

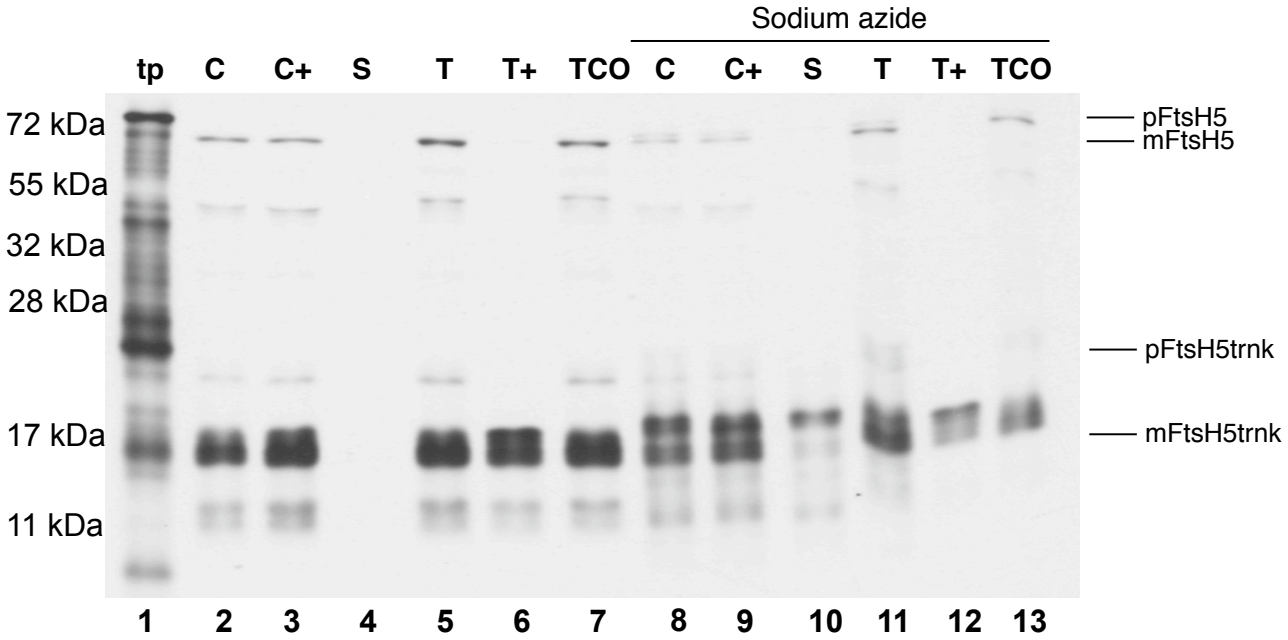

Figure S3: In vitro translation of pFtsH5 RNA produces a truncated precursor in addition to the full-length pFtsH5. In vitro translated pFtsH5 (tp) yielded full-length pFtsH5 (75-kDa) and a band at ~ 25-kDa. After incubation with intact pea chloroplasts in a reaction containing 5mM ATP and light at 25 °C for 20 min two imported and processed products were detected, the expected FtsH5 protein at 67-kDa and a smaller band at 17-kDa. The 17-kDa import product behaved similarly to FtsH5 with respect to its localization to thylakoids and its accumulation as a larger band in the stroma during import reactions containing sodium azide. This indicates that it is a truncated FtsH5 containing the lumenal domain and the transmembrane domain and that the 25-kDa translation product is its truncated precursor. In the figure, translation products were imported into chloroplasts in the absence or presence of 10 mM sodium azide, as depicted above the panels. Intact chloroplasts were recovered (C, lanes 2 and 8) and treated with thermolysin (C+, lanes 3 and 9). Untreated intact chloroplasts were fractionated into stroma (S, lanes 4 and 10) and thylakoids. Thylakoid aliquots were washed with import buffer (T, lanes 5 and 11), submitted to treatment with thermolysin (T+, lanes 6 and 12) or treated with 200 mM Na_2CO_3 (TCO, lanes 7 and 13). Samples were analyzed by SDS-PAGE on 12.5% gels and fluorography.

Figure S4

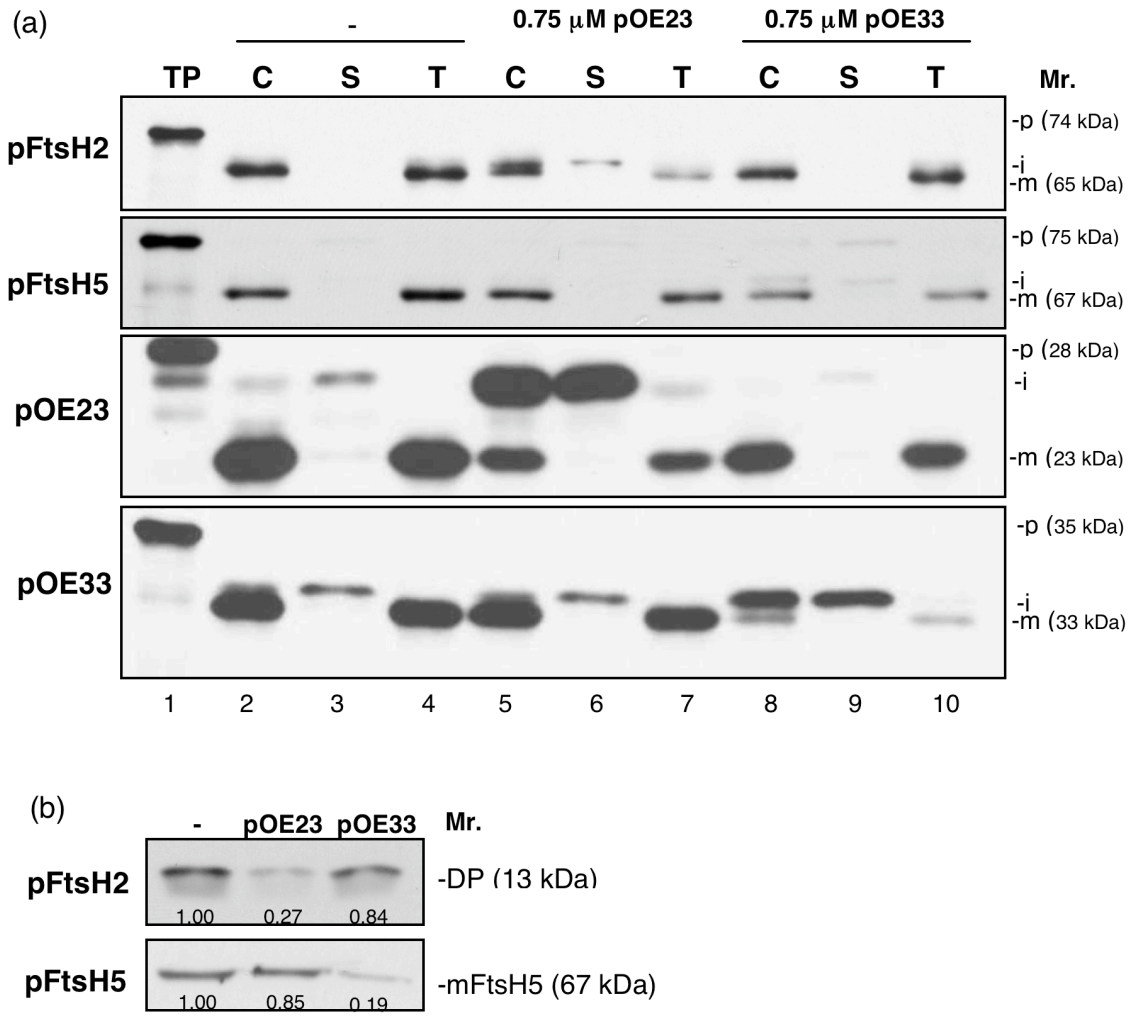

Figure S4: pFtsH2 integration is competed *in organello* by a Tat precursor while FtsH5 is competed by a Sec precursor. For *in-organello* competition intact pea chloroplasts were pre-incubated without (-) or with 0.75 μM of unlabeled iOE23 or iOE33 for 7 min at 25 °C in the light and then radiolabeled pFtsH2, pFtsH5, pOE23 or pOE33 were added and the reaction continued for 10additional min. (a), intact chloroplasts recovered from reactions (C, lanes 2, 5 and 8) were subfractionated into stroma (S, lanes 3, 6 and 9) and thylakoids (T, lanes 4, 7 and 10). (b), aliquots of thylakoids from samples imported with pFtsH2 and pFtsH5 in (a) were treated with thermolysin or 200 mM Na_2CO_3, respectively. All samples were analyzed by SDS-PAGE and fluorography on 7.5% gels (pFtsH2 and pFtsH5, (a)) or 12.5% gels (pOE23, pOE33, and FtsH2-DP). In panel (b), bands were quantified with Image J software and relative amounts depicted below the bands.