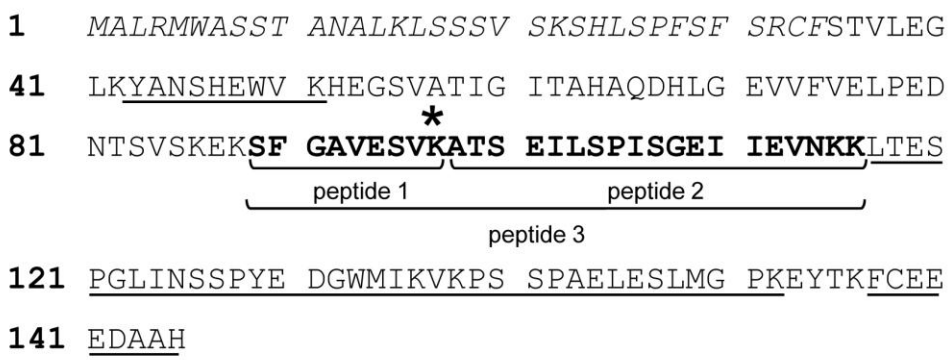
**Supplemental Figure S3.** Mass spectrometric analysis of H-protein lipoylation. H-protein-containing protein bands were excised from a Coomassie-stained gel of mitochondrial extracts, reduced and alkylated with iodoacetamide and digested with trypsin. The resulting peptide mixtures were analyzed by MALDI-TOF mass spectrometry.

**A**, Amino acid sequence of the H-protein GLDH1 from *Arabidopsis thaliana* (Swiss-Prot accession number P25855). The sequence stretch that includes the lipoyl binding site (K97, marked by an asterisk) is printed in bold letters. The occurrence of tryptic peptides 1, 2, or 3, respectively, enabled differentiation between lipoylated and unlipoylated H-protein. Further sequence stretches covered by tryptic peptides are underlined. The predicted mitochondrial targeting peptide is shown in italics.

**B**, Cutouts of MALDI mass spectra from wild type (upper panel) and *mtkas-1* mitochondrial proteins (lower panel). The ion signals at *m/z* 923.4 and *m/z* 2028.0 correspond to peptides 1 and 2, indicating that K97 is not lipoylated in the mutant. Binding of lipoate to K97 prevents tryptic cleavage at this site. Thus, peptides 1 and 2 are missing in the wild type. The ion signal at *m/z* 3236.5 that corresponds to lipoylated peptide 3 is only visible in the wild type.

A




B