Supplementary Material

Subcellular Phenotyping: Using proteomics to quantitatively link subcellular leaf protein and organelle distribution analyses of *Pisum sativum* cultivars

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Figure S1 | Morphological phenotypes of the Pisum sativum cultivars Protecta (left) and Messire (right). Length of internodes and leaf weight n = 5 biol. replicates, error bars = standard error, \*\* p < 0.01, \*\*\* p<0.001 (Kruskal Wallis).

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Figure S2 | Physiological phenotyping of the Pisum sativum cultivars Protecta and Messire. (A) Chlorophyll content, measured with a Minolta SPAD, (B) chlorophyll fluorescence (Fv/Fm), (C) non-photochemical quenching (NPQt), (D) energy loss to thermal dissipation (PhiNO), (E) leaf water content (absorbance at 940 nm) and (E) leaf surface cooling by evapotranspiration (leave temperature difference). ). n= 30, error bars = standard error, confidence intervals at 95% Kruskal-Wallis \* = p<0.05, \*\*\* = p<0.005.

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Figure S3| Mitochondrial marker peptide quality and specificity test using a Percoll gradient for mitochondria enrichment. Mitochondria were enriched according to Huang *et al.* (2014) and the marker peptide abundances [fmol µg of protein-1] of the two ATPases analysed. Fractions 7-9 and 10-14 were pooled. The latter was the region of mitochondria enrichment. F = pooled Percoll fraction.



Figure S4 |Time dependent absolute changes of RuBisCO abundances of young leaves of pea cultivars. Absolute abundance [nmol g protein-1] and [nmol (g protein g FW-1) -1] of cultivars Protecta and Messire. Asterisk if difference of abundance statistically significant, \*= p<0.05 (Kruskal-Wallis), n=5; error bars = standard error, (FW = fresh weight).

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| **Table S1 A. Selected target peptide list for the Mass Western** | | | |  |
| Protein description | Identifier | Peptide sequence | Organelle | Subcellular localization |
| RuBisCO (LS) | 3893097 | EIKFEFPAMDTL | chloroplast | stroma |
| Photosystem I iron-sulfur center | 295136986 | VYLWHETTR | chloroplast | tylakoid membrane |
| V-type proton ATPase | frv2\_80154 | ISEDVVAMLLK | vacuole | tonoplast |
| ATPase alpha subunit | 543866 | AVDSLVPIGR | mitochondrion | inner membrane |
| **Table S1 B. Heavy isotope labeled standard peptides. in brackets those amino acids that were labelled heavy isotopic.** | | | | |
| Name | Peptide Sequence | Peptide mass (light) | Peptide mass (heavy) | |
| RuBisCo | EIKFEFPAMDT(L) | 720.857603 | 724.366185 |  |
| PSI Iron Sulfur Centre | VYLWHETT(R) | 602.809105 | 607.813239 |  |
| V\_ATPase | ISEDVVAMLL(K) | 609.344131 | 613.351231 |  |
| M\_ATPase | AVDSLVPIG(R) | 513.800748 | 518.804883 |  |
| EP\_A | LVNE(L)TEF(A)K |  | 587.831102 |  |
| EP\_B | LVNELTEF(A)(K) |  | 588.32962 |  |
| QP | LVNELTEF(A)K |  | 584.32 |  |
| **concatenated:** |  |  |  |  |
| EP\_A+ M\_ATPase + RuBisCO | LVNE(L)TEF(A)KAVDSLVPIG(R)EIKFEFPAMDT(L) | |  |  |
| EP\_B + PSI + V\_ATPase | LVNELTEF(A)(K)VYLWHETT(R)ISEDVVAMLL(K) | |  |  |