## SUPPLEMENTAL TABLES AND FIGURES

# unique peptides										
Protein name	Mn₁	Fe1	Mn <sub>1</sub>	Fe₁	Accession	Function				
Lhca3	х	х	4	7	gi: 326510901 PSI antennae					
Lhca4	х	х	4	4	gi: 9624495	PSI antennae				
PsaA	х	х	3	4	gi: 152032676	PSI core				
PsaB	x	х	6	8	gi: 158512663	PSI core				
PsaC	x	х	3	5	gi: 60392949	PSI core				
PsaD	х	х	14	16	gi: 548603	PSI core				
PsaE	х	х	5	8	gi: 131176	PSI core				
PsaF	х	х	7	9	gi: 326498793	PSI core				
PsaG	х	х	5	3	gi: 131192	PSI core				
PsaH (1)		х		5	gi: 131196	PSI core				
PsaH (2)	х		6		gi: 326534178	PSI core				
PsaK	х	х	4	4	gi: 268612172	PSI core				
PsaN	х	х	4	7	gi: 326506464	PSI core				
Lhcb1	x		3		gi: 326520097	PSII antennae				
Lhcb4 (CP29)	x	х	9	9	gi: 445116	PSII antennae				
Lhcb5 (CP26)	х	х	13	5	gi: 326511355	PSII antennae				
Lhcb6 (CP24)	х	х	8	7	gi: 326529461	PSII antennae				
PsbA (D1)	х	х	8	7	gi: 131248	PSII core				
PsbB (CP47)	х	х	11	12	gi: 11594	PSII core				
PsbC (CP43)	х	х	11	10	gi:126302587	PSII core				
PsbD (D2)	х	х	10	12	gi: 12592	PSII core				
PsbE (cyt b559α)	x	х	4	4	gi: 60392973	PSII core				
PsbO	х	х	22	17	gi: 326494020	PSII OEC				
PsbQ	х	х	10	6	gi: 326516042	PSII OEC				
PsbQ-like	х	х	4	3	gi: 326503564	PSII OEC				
PsbR	х	х	3	5	gi: 326510039	PSII OEC				
PsbS	х	x	8	5	gi: 326523967	PSII antennae quencher				

Table S1: Mass spectrometric identification of protein subunits present in the collected Mn1 and Fe1 fractions

Protein subunits belonging to either PSI or PSII are listed. The proteins are given by their trivial name. Positive identifications in a given fraction is indicated by (x) along with the number (#) of unique peptides, gi number, and the protein function.

		Antonia (M	n inefficient)		Vanessa (Mn efficient)			
			Mn deficiency	/		Mn deficiency		
Treatment	control	mild	moderate	severe	control	mild	moderate	severe
Chl a/b ratio	2.39±0.15 <sup>a</sup>	2.14±0.02 <sup>b</sup>	2.05±0.02 <sup>bc</sup>	2.03±0.01 <sup>bc</sup>	2.32±0.00 <sup>a</sup>	2.10±0.00 <sup>bc</sup>	2.02±0.01 <sup>bc</sup>	1.95±0.04°
Chl a+b	70.9±3.8ª	56.2±2.0 <sup>bc</sup>	60.6±1.6 <sup>b</sup>	61.4±3.1 <sup>b</sup>	77.6±2.5ª	71.7±1.3ª	61.4±0.7 <sup>b</sup>	47.7±5.6 <sup>c</sup>
Fv/Fm	0.82±0.00 <sup>a</sup>	0.72±0.01 <sup>b</sup>	0.64±0.00 <sup>d</sup>	0.58±0.01 <sup>e</sup>	0.82±0.00 <sup>a</sup>	0.69±0.01 <sup>c</sup>	0.62±0.01 <sup>d</sup>	$0.54 \pm 0.00^{f}$
F0	264±6 <sup>f</sup>	322±31 <sup>de</sup>	384±21 <sup>bc</sup>	397±14 <sup>bc</sup>	279±6 <sup>ef</sup>	373±23 <sup>cd</sup>	430±9 <sup>b</sup>	545±25ª
Fm	1441±33ª	1169±73 <sup>bc</sup>	1075±48 <sup>cd</sup>	964±37 <sup>d</sup>	1514±25ª	1223±55 <sup>b</sup>	1123±22 <sup>bc</sup>	1179±57 <sup>bc</sup>

 Table S2: Photosynthetic parameters for Antonia and Vanessa grown under control conditions or exposed to mild,

 moderate and severe Mn deficiency

The ChI composition of thylakoids was determined using HPLC. The content of ChI *a*+*b* is expressed in pmol  $\mu$ g<sup>-1</sup> protein. Fluorescence parameters were measured on the youngest fully emerged leaf after 25 min. of dark adaptation. Fv/Fm, maximum quantum yield of PSII. F0, minimal fluorescence. Fm, maximal fluorescence. Values are means ± SE (*n* = 3). Values followed by the same letter are not significantly different (*p*≥0.05).



**Figure S1:** Background corrected chromatographic signals for <sup>55</sup>Mn<sup>+</sup> (upper chromatogram) and <sup>40</sup>Ca<sup>+</sup> (lower chromatogram) from four independent size-fractionated thylakoid preparations.



**Figure S2:** Measured molar Mn to Ca ratio as a function of the retention time for four independent size-fractionated thylakoid preparations. The least overlap with additional chromatographic Mn and Ca peaks is observed in the early part of the chromatographic signal (retention time 475 to 510 s). Here an approximate molar Mn to Ca ratio between 4 and 5 is detected.



**Figure S3:** Peak fittings (for repetition 1 in Fig. S1 and S2) using EMG profile for Mn (upper panel) and Ca (lower panel). Background corrected chromatographic ion intensity data (signal), individual peak fits (peak #), the sum of all peak fits (fit), and signal minus fit (residual). The unexplained hump observed at approximately 450 s in the residual for both the Mn and Ca fit is most likely due to PSII fragments not sufficiently solubilized. The overall peak shape observed in the presented chromatograms was consistent across the four repetitions and the multiple fitting iterations.



**Figure S4:** Spectroscopic characteristics. Absorption spectra of fractions containing the largest Mn and Fe peaks (Mn<sub>1</sub> and Fe<sub>1</sub>), corresponding to the size exclusion chromatogram in Fig. 3A.



**Figure S5:** Elution profiles for <sup>55</sup>Mn<sup>+</sup> (upper chromatogram) and <sup>72</sup>FeO<sup>+</sup> (lower chromatogram) of size-fractionated thylakoids from the barley PSI-less mutant, *viridis zb63* and the corresponding wild type (WT).



Figure S6: Elution profiles for <sup>55</sup>Mn<sup>+</sup>, <sup>56</sup>Fe<sup>+</sup>, and <sup>40</sup>Ca<sup>+</sup> measured in H<sub>2</sub>-mode of size-fractionated barley thylakoids.