Pigment composition in *Marchantia polymorpha* genotypes grown at 100 μmol quanta m⁻²s⁻¹ (normal light, NL) and at 200 μmol quanta m⁻²s⁻¹ (high light, HL)



Pigments were extracted by incubation in 100% acetone. Mean values with standard error are given (n=3). Stars indicate significant differences, compared to the control condition, based on Student's t-test (* p<0.05).

Pigment determination was done in accordance to Lichtenthaler (1987). Ref.: Lichtenthaler H. (1987) Methods in Enzymology 148, pp 350-382

Plants grown in different light regimes.

a: Arabidopsis thaliana (2 weeks old) in long day or for 1 week in fluctuating light condition (LL = 55 μ mol quanta m⁻²s⁻¹, HL = 850 μ mol quanta m⁻²s⁻¹)



b: Marchantia polymorpha (2 weeks old), grown for 1 week in fluctuating light (5 min 55 μ mol quanta m⁻²s⁻¹, 1 min 850 μ mol quanta m⁻²s⁻¹)



Sequence alignment of MpPTOXa and Nter truncated MpPTOXb catalytic sites by MUSCLE.

MpPTOXa	FTSQFVGSGNSLQLLLTSSISK
MpPTOXb	ASLRWRMPLDCSLIRSEDHLLKLSVTGLSRREKFGSSSCSNLSVRALOKNAISTEDDSSL
- <u>-</u>	
MpPTOXa	FRRRMTVP
MpPTOXb	MSSVAGKVPEHEVELKSORLGSDESSADHSGTASEEALPKRIOFDYGFOARFLRTGPTVP
	*** *. ** ***
MpPTOXa	KSVL
MpPTOXb	ONVFKLAFENFGREWRALRRSYLFRVLKPIRPAEIEGGPFOLVGAYTGRGLILFLRGLDK
	1.*1
MpPTOXa	AEEPIIENEESPOGLEKWVIAAEHGFNTFATETVVK
MpPTOXb	FLTLYDGLKEIOPVKOOPNIEODELREOLKKLKLSNKKVWEREKAREOVEAPWWILGPYY
	11* **11* 1 *1* 11 11 11 1
	E truncated sequence
MpPTOXa	ILETLYAKRIYARFYVLETIARVPYFAFVSVLHMYESFGWWRR-ADYIKIHFAESW
MpPTOXb	FLCWMLDVIFEDRFIORFWFLETVARMPYFSYISMLHLYETLGWWRSGAEVRKVHFAEEW
- <u>-</u>	:*:.:: .* **:.**:**:**:**:***::****
	E*HHLL E
MpPTOXa	NELHHLLVMEALGGDERWIDRFLAOHIAVAYYLLTVLMYLLSPRMAYHFSECVEKHAFST
MpPTOXb	NEMHHLKIMESLGGDLEWGDRFFAOHAAFFYYWTLNAMFLISPTVAYNFSELIESHAVDT
	****** ****** * ****** * ** ***********
MpPTOXa	YDKFIKSHGDELKLLPAPEVAVOYYTKGDLYMFDEFOTAIEPNTRRPKIENLYDVFVNIR
MpPTOXb	YGEFADENEELLKTLPPSPVAVAYYESGDLYMYDEFOTSRPPESRRPKMGSLYDVFMAIC
	*. ** * * ** *** ** . **********
	E**H
MpPTOXa	EDEAOHCKTMHACOSGKSLRSPHRDAPLTEIADDEKIPPPADCEGLFECATTATSFADRA
MpPTOXb	GDEGEHVKTMVACOOI.DTOVVSPNRVKVSGOKVETEVET PDRL
МрРТОХа	RKLGVENLVAKTDGSEL
MpPTOXb	P
The round	•

Sequence alignment of MpPTOXa and Nter truncated MpPTOXb catalytic sites by MUSCLE. The six helices α -1 (red), α -2 (green), α -4 (yellow), α -5 (magenta) and α -6 (cyan) are framed and the fourth iron binding sites motifs with the six iron binding sites determined by McDonald *et al.*, 2003, are indicated in bold above sequences. The site of truncation of the recombinant protein is indicated by a flash

Ref.: McDonald AE, Amirsadeghi S, Vanlerberghe GC. Prokaryotic orthologues of mitochondrial alternative oxidase and plastid terminal oxidase. *Plant Mol Biol.* **53**(6):865-76. (2003) Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**(5):1792-7. (2004)

Donor side limitation Y(ND) in fluctuating light condition (LL = 55 µmol quanta $m^{-2}s^{-1}$, HL = 850 µmol quanta $m^{-2}s^{-1}$) for all Marchantia genotypes. Additional data sets to Fig. 3a



Acceptor side limitation Y(NA) and non-photochemical quenching (NPQ) as a function of the light intensity for all Marchantia genotypes. Additional data to Fig. 3a



Fig. S6 Donor-side limitation (Y(ND)) at 1290 μmol quanta m⁻²s⁻¹



Arabidopsis thaliana

Marchantia polymorpha



mRNA expression levels of ptox in 2-week-old plants determined by RT-PCR. : Arabidopsis ecotype *Landsberg erecta , immutans.* and the two lines of crosses between CRTI and *im*.



Data are from two different RNA extraction. A third RNA extraction showed the same result but all expression levels were higher likely because of harvesting at a different time of the day. Mean values with standard error are shown (n=2).

Sequencing of MpPTOXa gene, after CRISPR/Cas9 transformation of $\Delta ptoxb$ mutant, in both $\Delta ptoxaptoxb$ double mutant lines

>MpPTOXa in *Aptoxb* mutant

>MpPTOXa in *Aptoxaptoxb*_1

ATGGCGTCTGGTCTGAGGATTGCTCCTCTGAATTGTGTATATAATCGCTGTGTGCCATCGGAGTCCCAAGGTTTTGGCTG TTGCACCCGAACGGGTCGGAATGGGCCGATTTTGGCTGTGGGCGGGAAGCCCGTTCGATTCACTTCGCAGTTTGTGGGGT CAGGAAATTCTTTGCAGCTTCTTTGACGAGCTCGATTTCGAAAAGCGGGTCCTCGTGCTGGCACATTCAGAAGGCGGATG ACCGTCCCTAAGTCAGTGCTTGCAGAAGAGCCCATTATTGAAAATGAGGAATCGCCTCAAGGTTTGGAGAAAGTGGGTGAT TGCTGCGGAACATGGATTCAACACCTTTGCCACTGAGACTGTTGTGAAGAATCAGAGACTCTGTACGCGAAAGCGCTTGT ACGCAAGGTTTTACGTTTTAGAAACCATCGCGAGAGTTCCGTACTTGCATCAGAGAACTCGCTTACACATGTACGAGAAGCGCTTGT GGTGGTGGAGACGGGGGGCGGACTACATCAAAATTCACTTTGCAAGAAAGCTGGAATGAACTACATCATCATCATCTTCTGGTTA GGAGGCTCTAGGTGGAGACGGGGGGGGGACTGATAGAATCGACACATGCCGTTGCAATGAACTACATCATCTTCTGTAT GGAGGCTCTAGGTGGAGACGAGAGGTGGATCGATAGAATTTTGGCTCAGCACATTGCCGTTGCATACTATCTTCTTACTG TATTAATGTATCTCCTAGGCCCCTAGAAAAGAA

>MpPTOXa in *Aptoxaptoxb*_2

ATGGCGTCTGGTCTGAGGATTGCTCCTCTGAATTGTGTATATAATCGCTGTGTGCCATCGGAGTCCCAAGGTTTTGGCTG
TTGCACCCGAACGGGTCGGAATGGGCCGATTTTGGCTGTGGGCGGGAAGCCCGTTCGATTCACTTCGCAGTTGTGGGGT
CAGGAAATTCTTTGCAGCTTCTCTTGACGAGCTCGATTTCGAAAGCGGGTCCTCGTGCTGGCACATTCAGAAGGCGGATG
ACCGTCCCTAAGTCAGTGCTTGCAGAAGAGCCCATTATTGAAAATGAGGAATCGCCTCAAGGTTTGGAGAAGTGGGTGAT
TGCTGCGGAACATGGATTCAACACCTTTGCCACTGAGACTGTTGTGAAGATACTAGAGACTCTGTACGCGAAGCGCTTGT
ACGCAAGGTTTTACGTTTTAGAAACCATCGCGAGAGTTCCGTACTTTGCTTTTGTATCGGTTTTACACATGTACGAGAGAG
TTTGGTTGGTGGAGACGGGCGGACTACATCAAAATTCACTTTGCAGAAAGCTGGAATGAACTACATCATCTTCTGGTTAT
GGAGGCTCTAGGTGGA <u>GACGAGAGGGGGGGCGGATCGATA</u> GATTTTTGGCTCAGCACATTGCCGTTGCATACTATCTTCTTACTG
TATTAATGTATCTCCTAG-CCCCTAGAATACAAAA

The first sequence is MpPTOXa CDS. The two others are the result of CRISPR/cas9 mutations in both double mutant lines. The red frame show the NGG targeted site. The orange frame represents mutations results.

Table S1:

Chlorophyll a/b and carotenoid to chlorophyll ratio in *A. thaliana* genotypes

Sample	Chla/b	Car/(chla+chlb)
Col-0	3.78±0,11	0.41±0,02
imCRTI_1	3,95±0,05	0,41±0,01
imCRTI_2	3,94±0,09	0,41±0,01

Pigments were extracted by incubation in 100% acetone. Mean values with standard deviation are given (n=4).

Pigment determination was done in according to Lichtenthaler (1987).

Ref.: Lichtenthaler H. (1987) Methods in Enzymology 148, pp 350-382

Table S2

Primer sequences for RT-qPCR

Primer names		Primer sequences
F-PTOX	F	5'-TGCAGTGTTCTGCTTCATCA-3'
R-PTOX	R	5'-GCACCGGATATCGCAGTAAA-3'
F-UBC21	F	5'-CAGTCTGTGTGTAGAGCTATCATAGCAT-3'
R-UBC21	R	5'-AGAAGATTCCCTGAGTCGCAGTT-3'
F-YLS8	F	5'-TCATTCGTTTCGGCCATGA-3'
R-YLS8	R	5'-CTCAGCAACAGACGCAAGCA-3'

Primers used for qPCR for checking PTOX mRNA expression levels in Arabidopsis ecotype *Landsberg erecta*, *immutans* and the two lines of crosses between CRTI and *im*. YLS8 and UBC21 corresponds to housekeeping genes.

Table S3

Primer sequences for *PTOX* constructs.

Primer names	Primer sequences			
F-PTOX1-Nco1	F	5'- GAT ATA CCA TGG CTT CTG GCC TGC GCA TTG -3'		
R-PTOX1-Xho1	R	5'- GCC GGC CTC GAG TTA TAA TTC GCT GCC ATC -3'		
F-PTOX2	F	5'- TTC AGG GCG CCA TGG GCA GTC CTG CCG CAT GCA TGC TTC -3'		
R-PTOX2	R	5'- GTG GTG GTG CTC GAG TCA CGG CAG ACG GTC GGG-3'		
F-PET-PTOX2 Del	F	5'-GCTACCGACTGCACGGCTGCAGCTCGAGAACAAGTGGAA GCACCTTGG-3'		
R-PET-PTOX2 Del	R	5'- TGCAGCCGTGCGTCGGTAGCAGCGGAACTGGGGAGGTG -3'		

Primers used for vector constructions for PTOX expression in *E.coli* as described in the Methods section.