Article – Supplementary Files

Truncated hemoglobins 1 and 2 are implicated in the modulation of phosphorus deficiency-induced nitric oxide levels in *Chlamydomonas*

Valentina Filina, Alexandra Grinko and Elena Ermilova

Biological Faculty, Saint-Petersburg State University, Universitetskaya nab. 7/9, Saint-Petersburg 199034, Russia; filina.valya@yandex.ru (V.F); st047636@student.spbu.ru (A.G.)
* Correspondence: e.ermilova@spbu.ru

Supplementary Figures 1, 2 and 3:

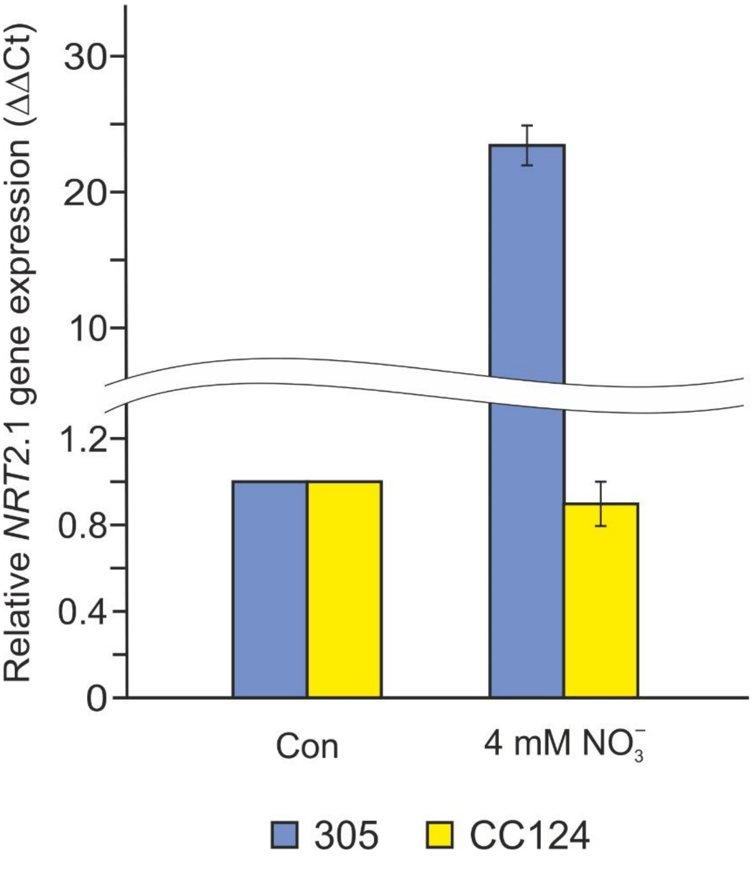

Figure S1. Expression analysis of the *NRT2.1* gene in strains 305 and CC124. Cells were grown in ammonium-containing medium (Con) and then transferred to a medium containing 4 mM KNO_3 for 1 h. Levels of gene transcripts are calculated as times of relative abundance with respect to the housekeeping control gene (*RACK1*) that has a value of 1. Data are the means ± SE from three biological and two technical replicates obtained by quantitative RT-PCR. Con

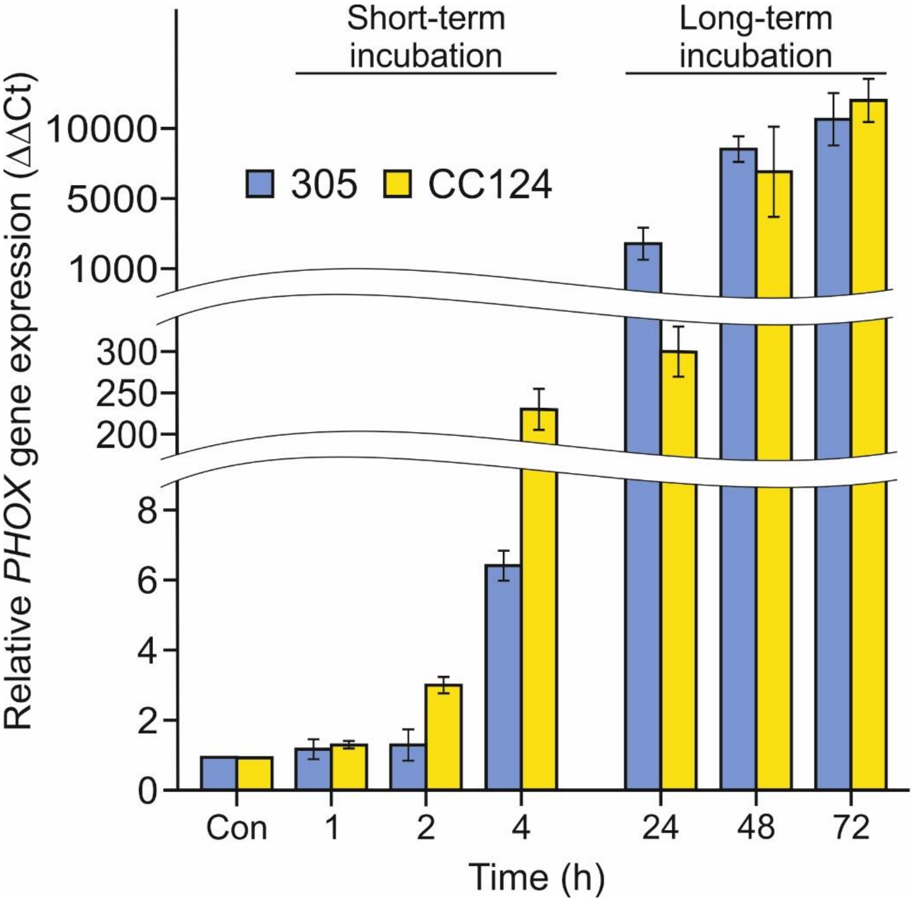

Figure S2. Expression analysis of the *PHOX* gene in nitrate reductase deficient mutants during P starvation. Levels of gene transcripts are calculated as times of relative abundance with respect to the housekeeping control gene (*RACK1*) that has a value of 1. Data are the means ± SE from three biological and two technical replicates obtained by quantitative RT-PCR.

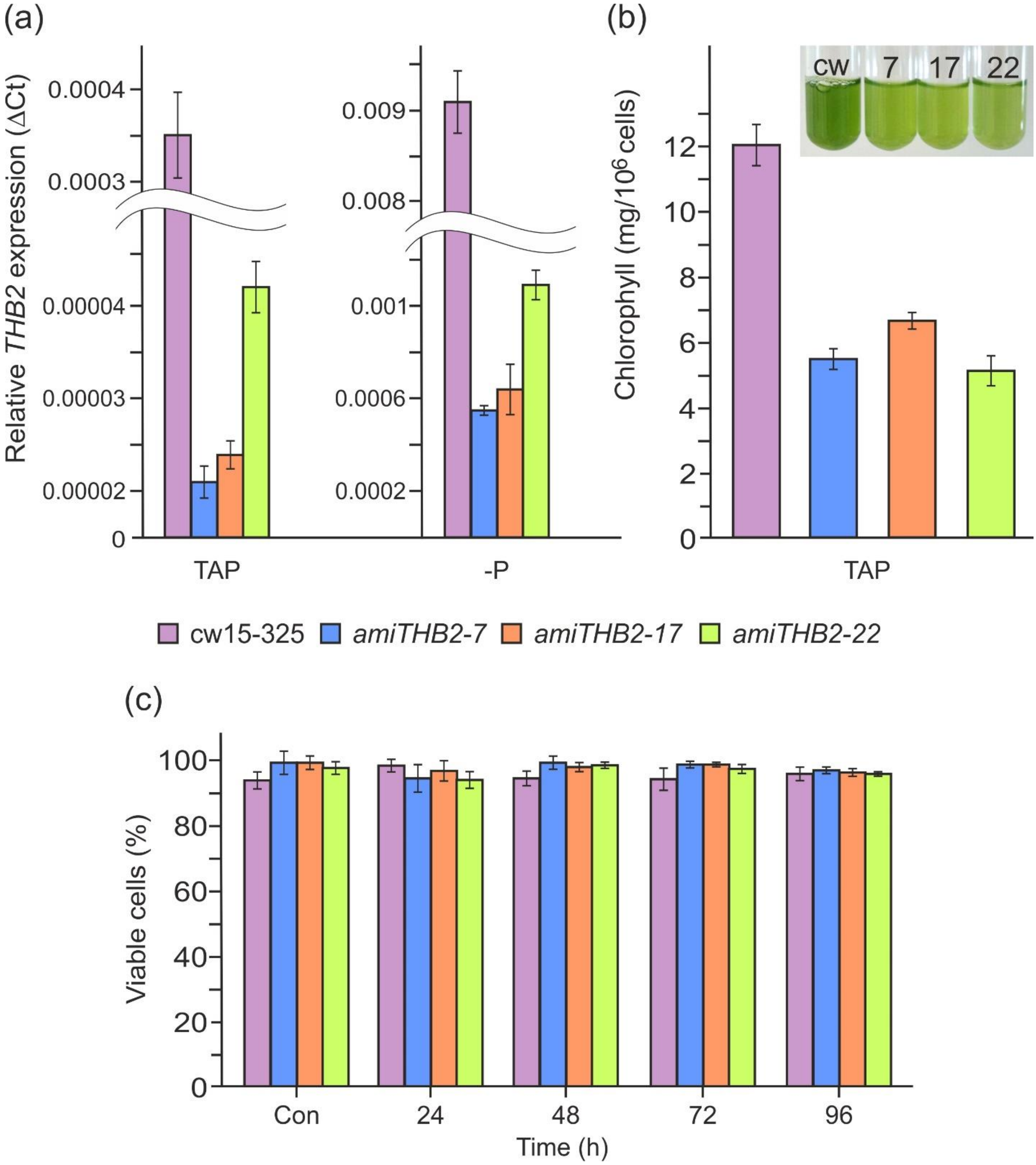

Figure S3. Characterization of *amiRNA-THB2* strains: **(a).** Expression analysis of the *THB2* gene in cw15-325 and *ami*RNA-*THB2* cells grown in TAP or incubated in P-free medium for 24 h. Relative expression levels were normalized with the gene expression of *RACK1* and calculated using ΔC_T; all measurements were done in triplicate; **(b)** Comparative chlorophyll contents of parental strain cw15-325 and *ami*RNA-*THB2* strains. Vegetative cells were grown in TAP medium. Insert shows test tubes with the same cell density of cultures ($2\ 10^6$ cells/ml) in TAP; **(c)** Viability of parental strain cw15-325 and *ami*RNA-*THB2* strains. Vegetative cells were grown in TAP medium and transferred to TA medium for 24h, 48h, 72h or 96h. A viability dye was used to distinguish viable from nonviable cells as explained in the Materials and Methods section. Values are means ± SD (n = 3).

Table S1: *Chlamydomonas* strains used

Strain	**Genotype**	**References**
cw15-325	*mt+, cw15, arg7*	[37]
CC124	*mt*$^-$*, nit1, nit2*	[37]
305	*mt*$^-$*nit1*	[44]
*ami*THB1-11	*mt+, cw15*	[9]
*ami*THB1-14	*mt+, cw15*	[9]
*ami*THB1-23	*mt+, cw15*	[9]
*ami*THB2-7	*mt+, cw15*	This work
*ami*THB2-17	*mt+, cw15*	This work.
*ami*THB2-22	*mt+, cw15*	This work

Table S2. Primers for RT-qPCR analysis

Target gene/Accession number	Primer name	Sequence (5′–3′)	Reference
THB1/Cre14.g615400	*THB1*F *THB1*R	ATGAAGAAGCAGCGCCGCAAAC ACCAGGTCAAAGTGGTGGTGGTTC	[10]
THB2/Cre14.g615350	*THB2*F *THB2*R	GCCGGTTGATCCGCGACAAG CGATCCAACTTTTACACCCGCTCAA	[10]
THB3/Cre04.g218800	*THB3*F *THB3*R	TCACTTATCGCCAGTCTAGAGGAC CGCTCAGGATGTCGTCTATAAGC	[10]
THB4/Cre04.g218750	*THB4*F *THB4*R	GCTTCAAGGAGACGGTGTGAAGTCTAC ACATCCACCGTTGCTGCCACA	[10]
THB5/Cre07.g351100	*THB5*F *THB5*R	GGCGTTTTATCGCAAGTTGT CTTGAACGTATCCAGCAGCA	[9]
THB6/Cre16.g654250	*THB6*F *THB6*R	CCTGGACTCGATAGCAGAGG TGTCGTGAGAGACGGAACTG	[9]
THB7/Cre16.g661000	*THB7*F *THB7*R	CATGGTGCCGTGCTCGTACA CGACCAGCACTGCCTACTTG	[4]
THB8/Cre16.g661200	*THB8*F *THB8*R	CGGGAGTCAGCAAGCTGTCAAC CCGCCCGTACACAAACAAGCAC	[4]
THB9/Cre16.g661250	*THB9*F *THB9*R	GCTCTCTCTGGTTTTGAAGCAT AGCTGCTCATCTGCGTACAAT	[9]
THB10/Cre16.g661300	*THB10*F *THB10*R	TGCTGCGGAGGTGTTCCTTG CATTGCCGCCTCTGCTGATG	[4]
THB11/Cre16.g662750	*THB11*F *THB11*R	TTGCGTGCGTCCATGCTGTC CCGGTTGCGGATACACCTCT	[4]
THB12/Cre16.g663000	*THB12*F *THB12*R	GACCCCTCACTCATAAAGTTCCT AAGTACTTCATGCCCAGATCAAA	[9]
RACK1/Cre06.g278222	*RACK1*F *RACK1*R	CTTCTCGCCCATGACCAC CCCACCAGGTTGTTCTTCAG	[38]
PHOX/Cre04.g216700	*PHOX*F *PHOX*R	TTCCGTTTCCGTTCTCTGAC CCCTGCATCTTGTTCTCCAG	[24]
NRT2.1/Cre09.g410850	*NRT2.1F* *NRT2.1R*	CGCCGTGGCAACTGACCCTGAG CGCCACCTCCTCCGCACTCCAC	[15]

Sequences were obtained from Phytozome 12, *Chlamydomonas reinhardtii v5.5*