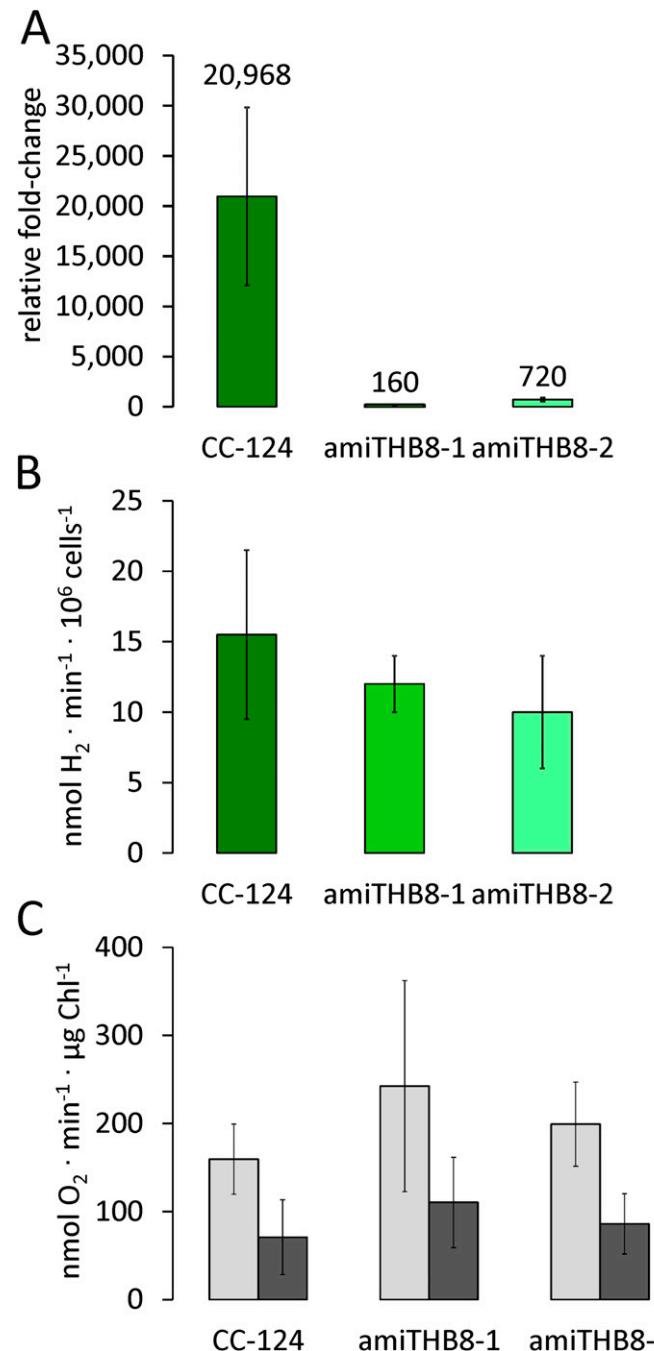


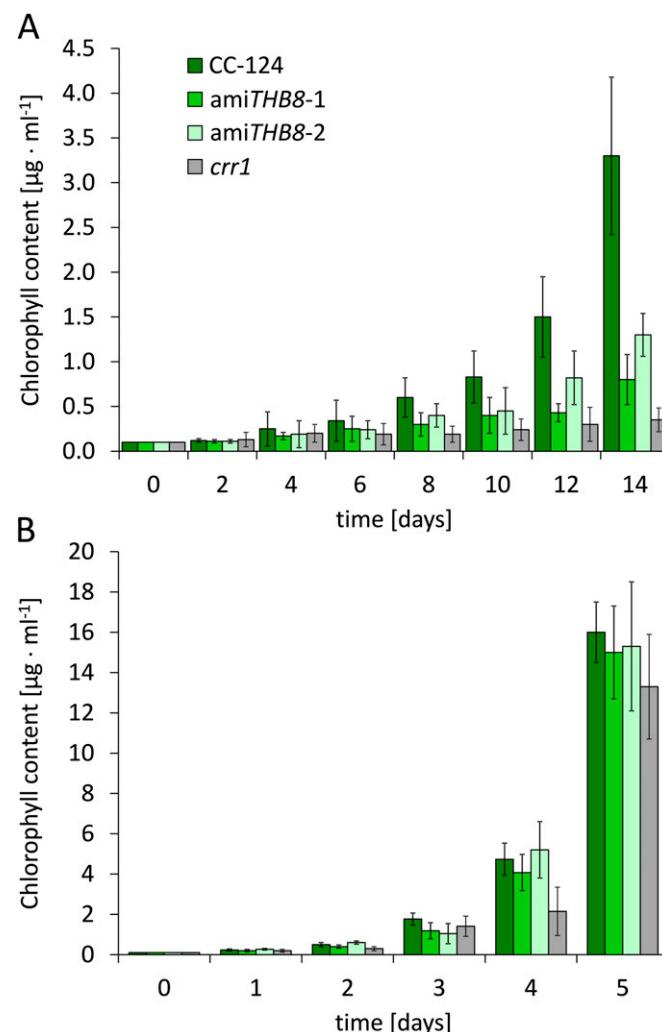
# Supporting Information

Hemschemeier et al. 10.1073/pnas.1302592110



**Fig. S1.** Additional characteristics of the transformants in which *THB8* transcript amounts were reduced by applying the artificial microRNA (amiRNA) technology, ami*THB8*-1 and -2. (A) *Chlamydomonas reinhardtii* wild-type CC-124 and transformants ami*THB8*-1 and -2 were grown aerated in the light and then incubated anaerobically in the dark for 7 h. RNA was extracted from the precultures and after dark-anoxic incubation. Quantitative real-time PCR (qPCR) analyses of relative *THB8* transcript amounts were done as described before (1). Fold-changes were calculated using the  $\Delta\Delta C(t)$  method. (B) In vitro hydrogenase activity of transformants ami*THB8*-1 and ami*THB8*-2 in comparison with strain CC-124 determined after 7 h of anaerobiosis. (C) O<sub>2</sub> evolution upon illumination (light gray bars) and O<sub>2</sub> uptake in the dark (dark gray bars) of the indicated strains grown aerobically in the light. (B and C) Hydrogenase activity and O<sub>2</sub> exchange rates were determined as described previously (2). (A–C) Bars show the average of two independent experiments. Error bars indicate the SD.

1. Pape M, Lambertz C, Happe T, Hemschemeier A (2012) Differential expression of the *Chlamydomonas* [FeFe]-hydrogenase-encoding *HYDA1* gene is regulated by the copper response regulator1. *Plant Physiol* 159(4):1700–1712.
2. Philipp G, Happe T, Hemschemeier A (2012) Nitrogen deprivation results in photosynthetic hydrogen production in *Chlamydomonas reinhardtii*. *Planta* 235(4):729–745.



**Fig. S2.** Growth of the *C. reinhardtii* wild type and transformants *amiTHB8-1* and -2, as well as of the COPPER RESPONSE REGULATOR 1 (*crr1*) mutant under hypoxic conditions in dim light. (A) Algal cultures were inoculated at a chlorophyll concentration of  $0.1 \mu\text{g}$  of Chl·mL $^{-1}$  in 100 mL of Tris Acetate Phosphate (TAP) medium in squared glass bottles. These bottles were sealed with Suba Seals and incubated on a magnetic stirrer in dim light ( $15 \mu\text{mol}$  of photons·s $^{-1}$ ·m $^{-2}$ ). The medium was continuously purged with 0.1% CO $_2$  in N $_2$ . After the indicated time points, samples were withdrawn for determining the chlorophyll content. The *crr1* mutant was used as a control for hypoxia as it grows poorly under hypoxic conditions (1). Cellular chlorophyll content in units of  $\mu\text{g}$  of chlorophyll per 1 million cells was similar in all strains analyzed:  $3.2 \pm 0.2$  (CC-124),  $2.9 \pm 0.3$  (strain *amiTHB8-1*),  $3.0 \pm 0.5$  (strain *amiTHB8-2*), and  $2.6 \pm 0.4$  (*crr1* mutant). (B) Cells were equally inoculated and incubated under the same conditions, but purged with 0.1% CO $_2$  in air.

1. Eriksson M, et al. (2004) Genetic dissection of nutritional copper signaling in *Chlamydomonas* distinguishes regulatory and target genes. *Genetics* 168(2):795–807.

**Table S1. List of Hb sequences used as queries to detect Hb encoding genes**

Query	Hb type	Hits <i>C. reinhardtii</i> *	Hits <i>Volvox carteri</i> *
>gi 44955888 ref NP_976312.1  myoglobin [Homo sapiens]	3/3Hb (Mb)	None	None
>gi 56749856 sp P68871.2 HBB_HUMAN RecName: Full = Hemoglobin subunit beta; AltName: Full = Beta-globin; AltName: Full = Hemoglobin beta chain; Contains: RecName: Full = LVV-hemorphin-7	3/3Hb (Gb)	None	None
>gi 57013850 sp P69905.2 HBA_HUMAN RecName: Full = Hemoglobin subunit alpha; AltName: Full = Alpha-globin; AltName: Full = Hemoglobin alpha chain	3/3Hb (Gb)	None	None
>gi 19549331 ref NP_599030.1  cytoglobin [Homo sapiens]	3/3Hb (Cb)	None	None
>gi 10864065 ref NP_067080.1  neuroglobin [Homo sapiens]	3/3Hb (Nb)	None	None
>gi 168823435 ref NP_078970.3  androglobin [Homo sapiens]	3/3Hb (Ab)	None <sup>†</sup>	None
>gi 58332516 ref NP_001011196.1  x globin [Xenopus (Silurana) tropicalis]	3/3Hb (GbX)	None	None
>gi 14600601 ref NP_147118.1  protoglobin ApPgb [Aeropyrum pernix K1]	3/3Hb (PGb)	None	None
>gi 73670387 ref YP_306402.1  hypothetical protein Mbar_A2927 [Methanoscincus barkeri str. Fusaro]	3/3Hb (PGb)	None	None
>gi 154351341 gb ABS73420.1  HemAT [Bacillus amyloliquefaciens FZB42]	3/3Hb (GCS)	None	None
>gi 300817675 ref ZP_07097890.1  flavohemoprotein [Escherichia coli MS 107-1]	3/3Hb (FHb)	None	None <sup>‡</sup>
gi 3980177 emb CAA38024.1  leghemoglobin [Medicago sativa]	3/3Hb (sHb)	None	None
>gi 1518444 emb CAA90868.1  leghemoglobin B [Vicia faba]	3/3Hb (sHb)	None	None
>gi 441461 emb CAA54331.1  leghemoglobin II [Lupinus luteus]	3/3Hb (sHb)	None	None
>gi 313503 emb CAA23731.1  leghemoglobin [Glycine max]	3/3Hb (sHb)	None	None
>gi 18015 emb CAA37898.1  hemoglobin [Casuarina glauca]	3/3Hb (nsHb-1)	None	None
>gi 1707907 sp Q08753.1 TRHN1_CHLMO RecName: Full = Group 1 truncated hemoglobin LI637; Short = CtrHb; Short = Truncated Hb; AltName: Full = Globin LI637; Flags: Precursor	2/2Hb-I	12 hits <sup>§</sup>	10 hits <sup>¶</sup>
>gi 1707909 sp P52334.1 TRHN2_CHLMO RecName: Full = Group 1 truncated hemoglobin LI410; Short = Truncated Hb; AltName: Full = Globin LI410; Flags: Precursor	2/2Hb-I	10 hits <sup>  </sup>	9 hits <sup>**</sup>
>gi 307107379 gb EFN55622.1  hypothetical protein CHLNCDRAFT_35393 [Chlorella variabilis]	2/2Hb-I	None	None
>gi 18418064 ref NP_567901.1  hemoglobin 3 [Arabidopsis thaliana]	2/2Hb-I	None	Vocar20005548m.g, E-value 1.62e-2
>gi 75318578 sp O65532 O65532_ARATH Hypothetical protein F4D11.110 (Hypothetical protein AT4g32690)	2/2Hb-II	None	None
>gi 55379358 ref YP_137208.1  cyanoglobin [Haloarcula marismortui ATCC 43049]	2/2Hb-I	10 hits <sup>  </sup>	9 hits <sup>**</sup>
>gi 170078229 ref YP_001734867.1  cyanoglobin [Synechococcus sp. PCC 7002]	2/2Hb-I	11 hits <sup>  </sup>	10 hits <sup>**</sup>
>gi 16330583 ref NP_441311.1  cyanoglobin [Synechocystis sp. PCC 6803]	2/2Hb-I	12 hits <sup>§</sup>	10 hits <sup>**</sup>
>gi 15608680 ref NP_216058.1  glbN gene product [Mycobacterium tuberculosis H37Rv]	2/2Hb-I	11 hits <sup>  </sup>	10 hits <sup>**</sup>

Protein sequences used for BlastP analyses on Phytozome v8.0 *Chlamydomonas reinhardtii* v4.3 and *Volvox carteri*. Ab, androglobin; Cb, cytoglobin; Gb, animal  $\alpha$ - or  $\beta$ -globin; GbX, globin X; FHb, flavohemoglobin; 2/2Hb, 2-on-2 helices ("truncated") Hbs; 2/2Hb-I, -II, -III, 2/2Hbs group I, II, III; 3/3Hbs, 3-on-3 helices Hbs (myoglobin-fold); Mb, myoglobin; Nb, neuroglobin; nsHb, nonsymbiotic hemoglobin (nsHb-1, -2, nsHb class1, 2); PGb, protoglobin; sHb, symbiotic hemoglobin.

\*Phytozome v8.0, *Chlamydomonas reinhardtii* v4.3, and *Volvox carteri*, BlastP using default settings.

<sup>†</sup>Two none-Hb domain containing proteins were detected, Cre12.g519950 (Flagellar Associated Protein, adenylate/guanylate kinase-like, E-value: 1.4e-8, and Cre10.g448850, Calpain cystein protease and Flagellar Associated Protein, E-value: 1.48e-3).

<sup>‡</sup>One hit Vocar20012052m.g, oxidoreductase, no hit using only the globin domain (1-148).

<sup>§</sup>All 12 (putative) 2/2Hbs identified as shown in Fig. 1 in the main article.

<sup>¶</sup>Vocar20011139m, Vocar20010321m, Vocar20010250m, Vocar20010617m, Vocar20010444m, Vocar20001214m, Vocar20005615m, Vocar20005548m, Vocar20002059m, Vocar20001900m.

<sup>||</sup>Included in the list shown in Fig. 1.

<sup>\*\*</sup>Included in ¶.

Table S2. List of 2/2Hb sequences used for the phylogenetic analysis

Organism	Abbreviation	Accession no.	Database	Total protein length (aa)/position 2/2Hb-domain* [length 2/2Hb domain (aa)]
<b>Prokaryota</b>				
<i>Acetobacter pasteurianus</i> IFO 3283–01	<i>A. past.</i>	YP_003189286	GenBank	193 /60–193(134)
<i>Bacillus cereus</i> ATCC 10987	<i>B. cereus</i>	NP_977638	GenBank	132 /7–132(126)
<i>Bordetella pertussis</i> Tohama I	<i>B. pertussis</i>	NP_880766	GenBank	134/1–124 (124)
<i>Brachyspira murdochii</i> DSM 12563	<i>B. murdochii</i>	YP_003632527	GenBank	124/(124)
<i>Bradyrhizobium japonicum</i> USDA 110	<i>B. japonicum</i>	NP_772862	GenBank	154/23–154 (132)
<i>Burkholderia graminis</i> C4D1M	<i>B. graminis</i> C4D1M	ZP_02883481	GenBank	180/11–130 (120)
<i>Burkholderia multivorans</i> ATCC 17616	<i>B. multivorans</i>	YP_001579609	GenBank	184/14–134 (121)
<i>Campylobacter coli</i> RM2228	<i>C. coli</i> RM2228	ZP_00367734	GenBank	150 /22–150(129)
<i>Campylobacter fetus</i>	<i>C. fetus</i>	YP_892161	GenBank	124 /(124)
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i> 1336	<i>C. jejuni</i>	ZP_06373377	GenBank	127 /(127)
<i>Campylobacter lari</i> RM2100	<i>C. lari</i> RM2100	YP_002574763	GenBank	127 /(127)
<i>Campylobacter upsaliensis</i> RM3195	<i>C. upsaliensis</i>	ZP_00370192	GenBank	129 /(129)
<i>Chloroflexus aggregans</i> DSM 9485	<i>C. aggregans</i>	YP_002462570	GenBank	125 /(125)
<i>Corynebacterium diphtheriae</i> NCTC 13129	<i>C. diphtheriae</i>	NP_940138	GenBank	130 /6–130(125)
<i>Flavobacteria bacterium</i> BAL38	<i>Flavob. bact..</i> BAL38	ZP_01733305	GenBank	123 /(123)
<i>Flavobacteria bacterium</i> BBFL7	<i>Flavob. bact.</i> BBFL7	ZP_01200962	GenBank	131 /(131)
<i>Flavobacteriaceae bacterium</i> 3519–10	<i>F. bacterium</i>	YP_003094561	GenBank	125 /(125)
<i>Frankia alni</i> ACN14a	<i>Frankia</i> GlbN	YP_712694	GenBank	118 /2–118(117)
<i>Frankia alni</i> ACN14a	<i>Frankia</i> HbO	YP_712079	GenBank	135 /14–135(122)
<i>Frankia</i> sp. Eul1c	<i>Frankia</i> sp. Eul1c	YP_004014801	GenBank	150/8–150 (143)
<i>Helicobacter bilis</i> ATCC 43879	<i>H. bilis</i>	ZP_04580628	GenBank	132 /3–132(130)
<i>Helicobacter canadensis</i> MIT 98–5491	<i>H. canadensis</i>	ZP_04869854	GenBank	127 /(127)
<i>Kordia algicida</i> OT-1	<i>K. algicida</i>	ZP_02163608	GenBank	129 /(129)
<i>Legionella longbeachae</i> D-4968	<i>L. longbeachae</i>	ZP_06187916	GenBank	128 /(128)
<i>Methylobacterium chloromethanicum</i> CM4	<i>M. chloromethanicum</i>	YP_002423259	GenBank	130 /(130)
<i>Mycobacterium avium</i> 104	<i>M. avium</i>	YP_883162	GenBank	154 /5–127(123)
<i>Mycobacterium leprae</i> TN	<i>M. leprae</i> GlbO	NP_301903	GenBank	128 /4–128(125)
<i>Mycobacterium</i> sp. MCS	<i>Myc. sp.</i> MCS	YP_642448	GenBank	162 /19–162 (144)
<i>Mycobacterium tuberculosis</i> H37Ra	<i>M. tub.</i> H37Ra	ZP_02552072	GenBank	131 /7–131(125)
<i>Mycobacterium tuberculosis</i> H37Rv	<i>M. tub.</i> H37Rv	NP_216058	GenBank	136 /14–136(123)
<i>Nitrobacter</i> sp. Nb-311A	<i>N. sp.</i> Nb 311A	ZP_01045872	GenBank	140 /5–140(136)
<i>Nostoc punctiforme</i> PCC 73102	<i>N. punctif.</i>	YP_001864679	GenBank	174 /58–174(122)
<i>Oceanobacter</i> sp. RED65	<i>Oceanobacter</i>	ZP_01305408	GenBank	121 /(121)
<i>Ochrobactrum anthropi</i> ATCC 49188	<i>O. anthropi</i>	YP_001370024	GenBank	139 /6–139(134)
<i>Paracoccus denitrificans</i> PD1222	<i>P. denitrificans</i>	YP_917377	GenBank	140 /9–140(132)
<i>Pedobacter heparinus</i> DSM 2366	<i>P. heparinus</i>	YP_003092108	GenBank	129 /(129)
<i>Pseudomonas syringae</i> pv. <i>syringae</i> B728a	<i>P. syringae</i>	YP_237008	GenBank	142 /27–142 (116)
<i>Rhizobium leguminosarum</i> bv. <i>viciae</i> 3841	<i>R. leg. viciae</i>	YP_765868	GenBank	155 /22–155(134)
<i>Ralstonia eutropha</i> H16	<i>R. eutropha</i> H16	YP_725533	GenBank	138 /15–138(124)
<i>Rhodococcus equi</i> 103S	<i>R. equi</i>	YP_004008063	GenBank	128 /6–128(123)
<i>Roseobacter</i> sp. MED193	<i>Ros. sp.</i> MED193	ZP_01057748	GenBank	144 /11–144(134)
<i>Roseiflexus castenholzii</i> DSM 13941	<i>R. castenholzii</i>	YP_001431358	GenBank	133 /(133)
<i>Ruegeria pomeroyi</i> DSS-3	<i>R. pomeroyi</i> DSS-3	YP_165021	GenBank	139 /8–139(132)
<i>Sacharophagus degradans</i>	<i>S. degradans</i>	ABD80803	GenBank	141 /15–141(127)
<i>Sagittula stellata</i> E-37	<i>S. stellata</i>	ZP_01747349	GenBank	134 /3–134(132)
<i>Silicibacter</i> sp. TrichCH4B	<i>Sil. sp.</i> TrichCH4B	ZP_05741205	GenBank	137 /6–137(132)
<i>Sinorhizobium meliloti</i> 1021	<i>S. meliloti</i>	NP_386684	GenBank	155 /22–155(134)
<i>Sphingomonas</i> sp. SKA58	<i>Sph. sp.</i> SKA58	ZP_01304894	GenBank	145 /1–121(121)
<i>Synechocystis</i> sp. PCC 6803	<i>S. CyGLB</i>	NP_441311	GenBank	124 /(124)
<i>Tetrahymena pyriformis</i>	<i>T. pyr HbN</i>	P17724	GenBank	121/6–121 (116)
<i>Thiomonas intermedia</i> K12	<i>T. intermedia</i>	YP_003643170	GenBank	162 /43–162(120)
<i>Xanthomonas axonopodis</i> pv. <i>punicae</i> str. LMG 859	<i>X. axonopodis</i>	ZP_10260820	GenBank	142 /27–142(116)
<b>Eukaryota</b>				
<i>Paramecium caudatum</i>	<i>P. caudatum</i>	P15160	GenBank	117/2–116 (116)
<b>Chlorophyta</b>				
<i>Chlamydomonas meowusii</i>	<i>CmHBN</i>	Q08753	GenBank	164 /44–164(121)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB1</i>	Cre14.g615400	Phytozome	136/10–129 (120)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB2</i>	Cre14.g615350	Phytozome	147/ 27–147 (121)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB3</i>	Cre04.g218800	Phytozome	297/105–225(121)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB4</i>	Cre04.g218750	Phytozome	214/ 59–178 (120)

Table S2. Cont.

Organism	Abbreviation	Accession no.	Database	Total protein length (aa)/position 2/2Hb-domain* [length 2/2Hb domain (aa)]
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB5D1</i>	Cre07.g351100	Phytozome	959 /62–182 (121)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB5D2</i>	Cre07.g351100	Phytozome	959/203–326(124)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB6</i>	Cre16.g654250	Phytozome	347 /99–224 (126)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB7</i>	Cre16.g661000	Phytozome	292 /54–182 (129)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB8</i>	Cre16.g661200	Phytozome	519 /46–166 (121)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB9</i>	Cre16.g661250	Phytozome	712 /53–176 (124)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB10</i>	Cre16.g661300	Phytozome	483 /53–175 (123)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB11</i>	Cre16.g662750	Phytozome	395 /51–163 (113)
<i>Chlamydomonas reinhardtii</i>	<i>CrTHB12</i>	Cre16.g663000	Phytozome	432 /46–166 (121)
<i>Chlorella sp. NC64A</i>	<i>C64THB1</i>	EFN57932	GenBank	107/(107)
<i>Chlorella sp. NC64A</i>	<i>C64THB2</i>	EFN56894.1	GenBank	151 /28–151 (124)
<i>Chlorella sp. NC64A</i>	<i>C64THB3</i>	EFN59290.1	GenBank	217 /15–141 (127)
<i>Chlorella sp. NC64A</i>	<i>C64THB4</i>	EFN55622.1	GenBank	244 /59–180 (122)
<i>Chlorella sp. NC64A</i>	<i>C64THB5</i>	EFN59229	GenBank	124 /7–124 (118)
<i>Micromonas pusilla CCMP1545</i>	<i>MpTHB1</i>	XP_003064586	GenBank	111 /2–111 (110)
<i>Volvox carteri f. nagariensis</i>	<i>VcTHB1</i>	Vocar20001214m	Phytozome	142 /22–142(121)
<i>Volvox carteri f. nagariensis</i>	<i>VcTHB2</i>	Vocar20001900m	Phytozome	290/142–270(129)
<i>Volvox carteri f. nagariensis</i>	<i>VcTHB3</i>	Vocar20002059m	Phytozome	161 /6–127 (122)
<i>Volvox carteri f. nagariensis</i>	<i>VcTHB4</i>	Vocar20005548m	Phytozome	462 /69–184 (116)
<i>Volvox carteri f. nagariensis</i>	<i>VcTHB5</i>	Vocar20005615m	Phytozome	319 /64–184 (121)
<i>Volvox carteri f. nagariensis</i>	<i>VcTHB6</i>	Vocar20010250m	Phytozome	399/146–280(135)
<i>Volvox carteri f. nagariensis</i>	<i>VcTHB7</i>	Vocar20010321m	Phytozome	521 /51–171 (121)
<i>Volvox carteri f. nagariensis</i>	<i>VcTHB8</i>	Vocar20010444m	Phytozome	729 /25–145 (121)
<i>Volvox carteri f. nagariensis</i>	<i>VcTHB9</i>	Vocar20010617m	Phytozome	528 /54–174 (121)
<i>Volvox carteri f. nagariensis</i>	<i>VcTHB10</i>	Vocar20011139m	Phytozome	244 /85–208 (124)
Planta				
<i>Arabidopsis thaliana</i>	<i>A.thal.</i>	NP_567901	GenBank	175/26–147 (122)
<i>Glycine max</i>	<i>G.max</i>	NP_001237344	GenBank	172/26–147 (122)
<i>Vitis vinifera</i>	<i>V.vinifera</i>	XP_002284484	GenBank	169/26–147 (122)

From left to right, columns indicate sequence origin, sequence abbreviation used in Fig. 2 in the main article, accession number of the polypeptide sequence, and data bank source, as well as total protein length, position, and length of compared 2/2Hb domains.

\*No numbers are indicated when the whole protein was used.

**Table S3.** Relative transcript abundances of the indicated genes in anaerobic *C. reinhardtii* wild-type CC-124 and transformants ami*THB8*-1 and -2

Gene	Relative transcript abundance				
	CC-124	ami <i>THB8</i> -1	ami <i>THB8</i> -2	ami <i>THB8</i> -1 vs. CC-124	ami <i>THB8</i> -2 vs. CC-124
<i>HYDA1</i>	53	67	47	1.3	0.9
<i>HYDA2</i>	6	1	3	0.2	0.5
<i>HYDEF</i>	4	3	6	0.8	1.4
<i>HYDG</i>	13	5	10	0.4	0.8
<i>PFR1</i>	3.9	2.5	1.9	0.6	0.5
<i>PFL1</i>	65	29	45	0.4	0.7
<i>CRR1</i>	40	31	48	0.8	1.2
<i>CPX1</i>	483	429	525	0.9	1.1
<i>CYC6</i>	16	79	65	4.8	4.0
<i>FDX5</i>	646	443	497	0.7	0.8
<i>CAT1</i>	11	19	19	1.8	1.8
<i>MSD3</i>	0.4	0.6	1.5	1.5	3.5
<i>HCP4</i>	14	3	9	0.2	0.6
<i>CYG12</i>	0.6	0.1	0.2	0.2	0.3
<i>THB8</i>	6	0.03	0.1	0.01	0.02
<i>RBOL2</i>	0.1	0.06	0.08	0.5	0.7
<i>HSP22A</i>	474	770	1043	1.6	2.2
<i>DES6</i>	0.1	0.4	0.4	2.9	2.4
<i>NAR1.3</i>	1.6	0.6	1.0	0.4	0.6
<i>Cre12.g512400</i>	5	4	5	0.7	1.0
<i>Cre01.g013450</i>	0.01	0.01	0.007	1.0	0.6
<i>Cre16.g668550</i>	3	5	2	1.4	0.7
<i>Cre03.g209200</i>	2.4	4.3	4.2	1.8	1.7

*C. reinhardtii* wild-type CC-124 and transformants ami*THB8*-1 and -2 were incubated in the absence of O<sub>2</sub> in the dark for 7 h. Relative transcript abundances were determined by qPCR as described by Pape et al. (1). The calculated values were multiplied by 1,000. Indicated genes encode the following proteins: *CAT1*, catalase; *CPX1*, coproporphyrinogen III oxidase; *Cre01.g013450*, hemerythrin HHE cation binding domain containing protein; *Cre03.g209200*, RRM (RNA recognition motif) containing protein; *Cre12.g512400*, protein containing Sigma-54 interaction domain; *Cre16.g668550*, RING- and FYVE-type zinc finger containing protein; *CRR1*, Copper Response Regulator 1; *CYC6*, cytochrome c<sub>6</sub>; *CYG12*, heme/nitric oxide-binding guanylate cyclase; *DES6*, omega-6-fatty acid desaturase, chloroplast isoform; *FDX5*, ferredoxin 5; *HCP4*, hybrid cluster protein 4; *HSP22A*, small heat-shock protein 22A; *HYDA1* and *HYDA2*, [FeFe]-hydrogenase isoforms HYDA1 and HYDA2; *HYDEF* and *HYDG*, [FeFe]-hydrogenase assembly proteins HYDEF and HYDG; *MSD3*, Mn superoxide dismutase; *NAR1.3*, formate nitrite transporter; *PFL1*, pyruvate:formate lyase 1; *PFR1*, pyruvate:ferredoxin oxidoreductase 1; *RBOL2*, respiratory burst oxidase 2; *THB8*, truncated hemoglobin 8.

1. Pape M, Lambertz C, Happe T, Hemschemeier A (2012) Differential expression of the *Chlamydomonas* [FeFe]-hydrogenase-encoding *HYDA1* gene is regulated by the copper response regulator1. *Plant Physiol* 159(4):1700–1712.

**Table S4.** Oligonucleotides used for qPCR analyses

Locus	Name	Forward primer (5' → 3')	Reverse primer (5' → 3')
Cre13.g599400	<i>RCK1</i>	GCCACACCGAGTGGGTGTCGTGCG	CCTTGCCGCCGAGGCCACAGCG
Cre03.g199800	<i>HYDA1</i>	GCGATTGACGTTGGTAGG	GTGCTTACAAGCGGCTGATG
Cre09.g396600	<i>HYDA2</i>	CGCTCCACCGACAAGCAGAT	AGCTGGTTGACCGCCCTCGTT
Cre06.g296750	<i>HYDEF</i>	CTGCATGATTGACGCCAGA	AGCGGCCCAAGAGAAGAAC
Cre06.g296700	<i>HYDG</i>	CCGGTCAATGACGCTGACTT	ATCTGGCTCATGCCGCACTT
Cre11.g473950	<i>PFR1</i>	CGATGTGGAGGAGTGGCTGA	CAGCTCGTGGTGCAGCTTGT
Cre01.g044800	<i>PFL1</i>	CAAGTACGGCAACGACGATG	ACGTTGGAGGTGATGGTCAG
Cre02.g125250	<i>CRR1</i>	TGCGTGTGTTGTTTCAGG	GCCAGGTGTGATGGAGAGAC
Cre02.g085450	<i>CPX1</i>	CCAGACCTCAAGCGTGTGT	GCGTTGCGGATCACCTTCTC
Cre16.g651050	<i>CYC6</i>	GCGGCTGCCAAGCGCGTGCAGGATG	CTGCTCAAGGGCGGCCCTGTCCAG
Cre17.g700950	<i>FDX5</i>	CGGCTTCATCCTCATGTGCT	ACGCTGACACGAATGGTACG
Cre09.g417150	<i>CAT1</i>	GATGGTATGGCCTGGTGC	CAATCGGATGGGCTCCCTAA
Cre16.g676150	<i>MSD3</i>	CAGCCCCAACCGAGATAAC	ACCAGACCCACCCAGGAG
Cre09.g391650	<i>HCP4</i>	AGTGGAAGTGGTGCCTGTCATTG	AGAGTACACCTAGCGCATTGCA
Cre07.g320750	<i>CYG12</i>	CGAAGGCTGTTGCTTGAGA	GGTGGCATTGCGTGTGGTAA
Cre16.g661200	<i>THB8</i>	CGGGAGTCAGCAAGCTGTCAAC	CCGCCCCTACACAAACAAGCAC
Cre03.g188400	<i>RBOL2</i>	GGAGAGCGTGTGATACATTG	CTGCACCCCTACCATTGTCTTG
Cre07.g318800	<i>HSP22A</i>	TGCCGGCTGTGTTCGTGAC	GGCGGCCACAATTGATAG
Cre13.g590500	<i>DES6</i>	TTGCGCGGGCGGCTTGTTC	CACCGCTGCTCCACAGACAC
Cre03.g210900	<i>NAR1.3</i>	GCCTGCTGGCGGGTTCTACA	ACAGATCCGCGCCAACCACC
Cre12.g512400	n.a.	ACACTAGCGCACTACCTACC	CCGCAGTCAGCAATGAGATG
Cre01.g013450	n.a.	CCTTCAGTGCCAAGGAGCTGAC	ATGCCGTGCTGCTGCATCATGG
Cre16.g668550	n.a.	TGAGGGCCGTCTGACTGAG	GAAC TGACGCCGTGAGAAGC
Cre03.g209200	n.a.	GAGGCCTGATGCAGACCTT	TGTCTCCGCGTGCCTCGAA

Locus ID on Phytozome ([www.phytozome.net/chlamy](http://www.phytozome.net/chlamy)), gene name (n.a., not applicable), and forward and reverse oligonucleotide sequences are shown. Except for *RCK1* (receptor of activated protein kinase C), gene names are the same as indicated in the legend of Table S3.