

Supplemental Figure 1. Genomic structure of *CYG56* and domains of the CYG56 protein. (A) The complete 6111-bp cDNA of *CYG56* was sequenced and compared to the genomic *Chlamydomonas* sequence at the JGI v3.0 (http://genome.jgi-psf.org/Chlre3/Chlre3.home.html). UTRs are represented by light grey boxes, exons by black boxes and introns by a line. The scale is represented on the figure. (B) The CYG56 sequence was analyzed with the Smart and Expasy softwares (see Methods section). Positions of the conserved NO-binding HNOB, HNOBA domains and catalytic GC domain are indicated. A line corresponding to the 957-residues at the N-terminal fragment of the protein expressed in the pQE80 vector as a 6xHis tagged protein is schematized.

Α		10	20	30	40	50	60	70	80 90
	Cr CYG56 Cr CYG57*	NAGIINEAAKLEVL	DSYGQEVWDKIVE	ETTGVDPHFI		KILGAVAEL ISTLATVAED	KGEALGDVME	ACLHONTVY	
	Cr CYG38*	MIGWINLSVEAFIC	DSF GRD AWLKI IF	EQAHVDCNW	SSCPYPDKVT	DLVITGAGI	GVTVPQALE GVTPADALE	YGAY -VNY	TIKO GYLPLLOS
	Cr CYG12				CCCD D TIT	MLGSNT	EATPHOLLE	FCEYE-VSYL	
	Ls GCb1	MYGEVNY LELVL	RNF GEE AWKQ IK(FIZE CEE TWEEL KI	EAYLDMEGDFI		DLVDAACKV	HVDAATVLQ	FGKME-FEY	VESGYDKILKV
	Hs GCb3	MGEVNHALELVI	RNYGPEV WED IKF	EAQLDEEGQFI	VRIIYDDSKT	DLVARASKV	NLNAGEILQ	FGKMP-FVF	
	MM GCD3		KNUGPL V WED LIKE	(EAQEDEEGQEI	VKIIMUSKII	ULVARASAV	NLNAGEL	T GRIT-F VF	VES 510 11 9KV
		100	110 	120 	130		150 	160 	170
	Cr CYG57*	MGGNLVHELQNLNV	YHLHLSMIFKEL(PPA INVSEVTI	PQ-STIFTIAS, PD-SEVERYASI	RPG TRF AV	LLR GAARTL	G-KEVTVSI	GLASD
	Cr CYG15		LHLHLGMSFPAM	LAPA FICTD VGI	PT-CLT LHY HS	RPALGPIVV	VLKCLACOY		ELV KS - ELL RG -
	Cr CYG12 Cr CYG11	IGSIM RELEND	LHLHLSMSFPSM		PT-CLT HYHS	IRPALGPIVV	VLKGLACOY	GLGGEQLQVE	ILL RG-
	Rn GCb2	LG NL EFIENLD	LHSYLALSYQEM	TAPS TRUEE GAI	G-AMLLHYYSD	RHGLCHIVP		D-TDVAMSI	JDMNEE
	Mm GCb3	LGS NV REELQNED	LHDHLATIYPGM LHDHLATIYPGM	CAPS FROTD ALL CAPS FROTD ALL	KGKGLI LHYYSE	REGLODIVI	IIKIVAQQII IIKIVAQQII	IG-TEIDMKVI IG-TEIDMKVI	IQQ RNE IQQ RNE
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В		10	20	30 	40 5	0 (50 7 	0 8 	0 90
	Cr CYG57*	AEACTYPEATILES	VVDI		EFDSL	ELEKYQSLY	KVETIGD IM	VANVIEPCVE VANVIKPCHD	
	Cr CYG15	APAQEHPEATVLES	IVGETRISARSI IVGETEIASRSS	PLEVCSL	ID LYORFDGA	EEYPOLY	KVETIGD YM	VCNVTVPCDD	HADVLLEFAL
	Cr CYG12 Cr CYG11	APAGENPEATULES	TVGF TELASRSS	PLEVCSL	LU LY RFD	E-EYPOLY	KVETIGD YM	VCNVIVPCDD	HADVLLEFAL
	Rn GCb2	VAAGEFETCTILES	VVIETNICAACE	PIQIVNM	INSMYSKEDRL	EDEVENIENTE ESVHDVY	KVETUGD YM KVETIGD YM	VGGVPVPVES	HAQRVANFAL
	Mm GCb3	VPAKRYDNVTILFS	FIVGENAE CSKHA FIVGENAE CSKHA	SGEGA <mark>MKI</mark> VNL	LNDLYTRFDTL.	IDSRKNPFVI IDSRKNPFVI	KVETVGD MAN	VSGLPE <mark>PC</mark> IH	HARSTCHLAL
		100	110	120	130 1	40 1	50 <u>1</u>	60 <u>1</u> '	10 180
	Cr CYG56 Cr CYG57*	DMRVCSRVKT-NR DMRVCSRVRT-NR	OPL II VGIHTG OPI II IGVHSG	SVVGGIVGRRM	PRF LEGDTVN PRF LEGDTVN	IT SRIES - GV SRIES - GV	P GSVHIS GASI PGS VHMS GASI	RETRHP	ORYLTTERGE SOYLLTERGE
	Cr CYG38* Cr CYG15	RMQD A ARQVKN – AQ RMHEE ASRVAS – SL	GEPVOIRIGIHTG GEPVRIRVGMHSG	PVV GVLG K VV GVVGRKM	PRFSTYGDTVN PRFCLFGDTVN	/TSRMESHGL TSRMESHGE	GKIHISGASY Goihisfacy	ARTVDK SCIRSK	KY V RGN
	Cr CYG12 Cr CYG11	RMHEE ASRVAS – SLO RMHEE ASRVAS – SLO	GERVEIEVGMHSG GERVEIEVGMHSG	VV GVVGRKM VV GVVGRKM	PRF CLFGD TVN PRF CLFGD TVN	SR ES G SR ES G	GOIHIS GOIHIS	CCURSK (SCURSK	RF IR <mark>RG</mark> N
	Ls GCb1 Rn GCb2	DLMDISRELKD-PN GMRISAKEVMNPVT	GDPIMITIGIHSG GEPIOURVGIHTG	VV GVIGKKM VI GVVG KM	PRY_LFGNTVN PRY_LFGDTVN	SRETG SRESG	K <mark>GRINVS</mark> EYAY PS KVHLS PTAI	RFT QD STNSD	KSF KFDY <mark>RG</mark> E (G-F E T VR <mark>RG</mark> E
	Hs GCb3 Mm GCb3	DMMEIAGQVQVD DMMEIAGQVQVD	GESVOITIGIHTG GESVOITIGIHTG	EVVI GVI GORM	PRY_LFGNTVN PRY_LFGNTVN	SRETG SRETG	(GKINVSEYT) (GKINVSEYT)	RCCMSPENSD RKCGSTVPFG	POF HLEHRGP AQRPSVYEGO
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	Cr CYG56	ISVKGKGL-METFL							
	Cr CYG57* Cr CYG38*	IAVKGKGL-MELDF IAVKGRGN-MTTY-							
	Cr CYG15 Cr CYG12	ITVKGKGT-MRTF- ITVKGKGT-MRTY-							
	Cr CYG11 Ls GCb1	ITVKGKGT-MRTY- VVMKGKRTPMKCY-							
	Rn GCb2 Hs GCb3	IEVKGKGK-MTTY- VSMKGKKEPMQVW-							
	Mm GCb3	EGTNASLVPTQEK-							

Supplemental Data. Montaigu et al. (2010). Plant Cell 10.1105/tpc.108.062380

Supplemental Figure 2. Sequence comparison annealing of HNOB (**A**) and GC (**B**) domains. Proteins compared are Cr CYG56 (Accession number EU841916), Cr CYG11[au5.g13848_t1 (ID 524363)], Cr CYG15 [au5.g13857_t1 (ID 524374)], Cr CYG12 [au5.g13849_t1 (ID 524364)], Cr CYG38 [au5.g6042_t1 (ID 515997)] and Cr CYG57 [au5.g4360_t1 (ID 514215)] from *Chlamydomonas reinhardtii*, Ls GCb1 (AAC95432.1) from *Lymnaea stagnalis*, Rn GCb2 (AAF86581.2) from *Rattus norvegicus*, Hs GCb3 (NP_000848.1) from *Homo sapiens* and Mm GCb3 (NP_001155268.1) from *Mus musculus*. See Supplemental Figure 4 for further details. In the HNOBA domain (**A**), histidine binding heme is boxed in red, conserved YxSxR residues in green, and conserved cysteines in blue.

	10 20	30	40 5	0 60	70	80	90
Cr CYG56			DCDDICEFET		DOUDDAT O	NCMET RCONTH	INTE INC.
Cr CYG57*	TODATLE AL TOTALAE OSECAL		DCDI I CDFICC	KINGLE ENGE ERVIKER V		NGMELLAGUMIN	UVUL 1VI
Cr CYG38*	MODELEEV NOVELLE TODACE		DCCTVCDTPUT			COLETER	
Cr CVG15	I NUMBER OF STREET		COCCUT CDURAL	KINCTIFE ENVELLE			
Cr CVG12						TCLDIRG	
Cr CVG11	LSINDEIQL INTELLORSCRV		GREGUELGEVIQU	KARSETTSE DSLITAA	TADIVE RARA	CCLERKG	
	LARADIZUL 1237 LLLDRSCRV	VUAGAMLERLEPELK	GRSGVPLGEVIQU	KHEHGPLDEDHLVSD	LUNA ALIKARA	SGLELKG	007
LS GCD1	ISPASECRS FIGHLIED RNL VI	UUTGMSTGRVLPKVY	U-NCCIT-ELEDM	IRPHMEESEDNILSH	INTVOVILTRŲ	GLENSEELNDA	USA
RI GCD2	VEEEVECDA FIJEHI VEDE ALRV	KUAGVNIUKYVPGIL	TUKFALD-EYESI	THEUVTENTSSUCKE	INSURVIKTRK	EMMP	K
Hs GCb3	ISPYTECKAEPPHILEDROLVV	TUCGNALYRVLPULU	PGNCSLL-SVESL	VREHLDISEHGILSH	INTV TV RSKE	GLLDVEKLECE	DEL
Mm GCb3	TSEX.I.SCKA SIMPLITED RUPAN	TUCCNALYRVLPULU	PGNCSLL-SVES	WREHLDISEHGELSH	INTV W RSKE	GLLDVEKLECE	DEL
	100 110	120	130 14	10 150	160	170	180
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Cr CYG56	PAGTGDHNPARSYTRMVVPPTG	LEVIHSALARMHEPG	TSGAASAHGASSY	YNAAPESTVASLTGS	LACPHMASLSQ	QQQQQTRFQGQ	isqq
Cr CYG57*		G	VWGGGS		CPSLPTTAM	SQPV	AQQ
Cr CYG38*					QMVKTQM	P	
Cr CYG15					QM V AVPL	PLHPG	
Cr CYG12					QVLPVRV	LASPEPVTATQ	CAE
Cr CYG11					QMVAVPL	PLHPG	
Ls GCb1	VLAE				QQQ M R L KG	QMIYVL	
Rn GCb2	ARKS				QPMLKLRG	QMIWME	
Hs GCb3	TGTE				ISCLRLKG	QMIYLP	
Mm GCb3	TGAE				ISCLRLKG	QMTYLP	
	190 200	210	220 23	2 2 4 0	250	260	270
				$\cdots \cdots \mid \cdots \mid \cdots \mid \cdots \mid \cdots \mid$.		$\cdot \cdot 1$
Cr CYG56	PTLPLHLLAPSVAMDLLGTSTG	TGQDTGSAFLATKGT	GTGTGTGGFMASG	GGCGGANPLSSRSSS	FGSLGGGGHSS	QLCGGSQSQGA	GGG
Cr CYG57*							
Cr CYG38*	DG	TPAL					
Cr CYG15	AG	CPAT					
Cr CYG12	HVRTEHGGHQRQLAEHVGQRDG	RPEQDG					
Cr CYG11	AG	CPAT					
Ls GCb1							
Rn GCb2							
Hs GCb3							
Mm GCb3							
	280 290	300	310 32	20 330	340	350	360
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Cr CYG56	RGLEPVMLFLGSPRIGNVDELR	TYGLYVSDLPLFDNS	REMTL SMEQRTAF	AA KOSFER SMOLE		EPRHRTQQ	LLY
Cr CYG57*	QEPVMLFLGTPRIGSVDELR	TYGLFVSDLPLFDNS	REMVL MAEQHITAE	AA KOTFER ISLOLE		EPRRRSD	LLY
Cr CYG38*	IFLGSPRLAN LEEMN	GH <mark>GLYLSDIPLHD</mark> MS	RD FVLLAEQROAF	AD KERFICKMAVELR	QANER ELTTR	WLDEFKIRSDA	LLY
Cr CYG15	QEGLIFMGTARLAGLDDMR	HH <mark>GHTISDIP</mark> HDIN	RDYVLLAEQROAF	AQ IQERLEY ITREIK	DTNSR	DEERRRSDR	LLY
Cr CYG12	EQGELLELGSPWLAGLAGMR	QHGICISDIARHDST	RD FVLL EQRHAF	TQ KERLET TLEIK	DANSR <mark>E</mark> GEMAG	WLDEERRRSDG	LLY
Cr CYG11	QEGLIEMGTARLAG LDDMR	HH <mark>GTIFTSDIP</mark> HHDIN	RDYVLLAEQROAF	AQ IQERLES TREFK	DANSR <mark>E</mark> GEMAG	WLD AE <mark>RRRSD</mark> G	LLY
Ls GCb1	ECDSILFLCSPSVMNLDDIS	RR <mark>GLYL</mark> SDIPLHDAT	RELVLLSPHWEAP	YKTAQNLEV TDQIQ	QTYRE E	DEKKKTDR	LMY
Rn GCb2	SLRCMIFMCSPNVRSLQELE	ESKMHL <mark>SDI</mark> APHDTT	RDLTLLNQQRLAF	MERSCQLICKKKEEPR	VLSNH <mark>U</mark> A	I	LLY
Hs GCb3	EADSILFLCSPSVMN LDDLT	RR <mark>GLYLSDIP</mark> LHDAT	RD LVLL GEQEREE	YK TQELEI TORIQ	LTLRA <mark>E</mark> E	D KKKTD	LLY
Mm GCb3	EADSILFLCSPSVMNLDDLT	RR <mark>GLYLSDIP</mark> LH D AT	RD LVLL GEQFREE	YKUTQELEI (TDRIQ	LTLRA <mark>E</mark> E	D KKKTDT	LLY
					-		
	370						
Cr CYG56	QMIPRRIADVIRSGER						
Cr CYG57*	QMTPPHVADTURRGER						
Cr CYG38*	RMEPADIAADEREGHK						
Cr CYG15	OMLPPEVASCLKNEER						
Cr CYG12	OMLPPEVASCLKNEER						
Cr CYG11	OMLPPEVASCLKNEER						
Ls GCb1	SILPPSVANELRHRRP						
Rn GCb2	AMLPEHVANOLKEGRK						
Hs GCb3	SVLPPSVANELRHKRP						
Mm GCh3	SVLPPSVANETRHKRP						
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Supplemental Figure 3. Sequence comparison annealing of HNOBA domain. Proteins compared are the same as in Supplemental Figure 2.

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1		46.0	30.2	30.4	24.4	32.4	21.5	26.4	23.9	18.9	1	Cr CYG5
2	63.8		28.5	29.9	24.3	31.2	22.4	19.9	23.3	19.0	2	Cr CYG5
3	136.6	122.1		37.8	30.6	48.7	28.7	25.1	29.1	29.1	3	Cr CYG3
4	118.4	99.5	91.6		55.8	91.5	28.2	27.9	30.4	30.7	4	Cr CYG1
5	140.9	107.6	117.9	47.9		69.9	24.5	24.8	27.8	27.8	5	Cr CYG1
6	98.7	84.2	70.6	7.3	25.8		29.5	31.4	30.2	30.2	6	Cr CYG1
7	146.0	132.3	128.9	122.1	118.3	118.8		30.6	59.9	58.6	7	Ls GCb1
8	142.8	135.8	131.5	129.9	126.9	123.1	114.1		34.7	35.1	8	Rn GCb2
9	132.8	134.3	128.9	116.0	110.2	114.3	54.5	109.3		94.4	9	Hs GCb3
10	139.1	140.5	132.4	121.2	117.3	120.3	57.2	111.2	5.8		10	Mm GCb
	1	2	3	4	5	6	7	8	9	10		

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-	Name	Accession number	Organism
	Cr CYG56	EU841916	Chlamydomonas
	Cr CYG57	au5.g4360_t1 (ID 514215) Chromosome 13:417950-428744	Chlamydomonas
	Cr CYG38	au5.g6042_t1 (ID 515997) Chromosome 16:202664-213812	Chlamydomonas
	Cr CYG15	au5.g13857_t1 (ID 524374) Chromosome 7:1189275-1194213	Chlamydomonas
	Cr CYG12	au5.g13849_t1 (ID 524364) Chromosome 7:1149954-1156218	Chlamydomonas
	Cr CYG11	au5.g13848_t1 (ID 524363) Chromosome 7:1143134-1148318	Chlamydomonas
	Ls GCb1	AAC95432.1	Lymnaea stagnalis
	Rn GCb2	AAF86581.2	Rattus norvegicus
	Hs GCb3	NP_000848.1	Homo sapiens
	Mm GCb3	NP_001155268.1	Mus musculus

Supplemental Figure 4. Degree of identity and divergence among compared NOdependent type GC sequences. Percentages of identity among compared sequences were obtained by using the MegAlign program of the DNASTAR software package. Either Accession numbers or identity of predicted *Chlamydomonas* sequences are indicated.



Supplemental Figure 5. GC activity assayed with Mg^{2+} and Mn^{2+} of truncated (pCYG56) and full-length CYG56 purified preparations. Standard assays conditions indicated in this paper were used except that 4mM of either Mg^{2+} or Mn^{2+} was used. Activities are referred to 100% of the activity of each enzyme with Mn^{2+} as a control. Data correspond to mean values from three independent experiments \pm standard deviations.



Supplemental Figure 6. Effect of the inhibitors ODQ and LY 83,583 on GC activity of truncated and full-length CYG56 purified preparations. GC assays were performed under standard conditions of assay with 4mM of either Mg^{2+} or Mn^{2+} and using truncated (pCYG56) and full-length CYG56 purified preparations. Activity in the presence of ODQ and LY at final concentrations 500 μ M and 100 μ M, respectively, were included as indicated and compared to each appropriate control (100%). Data correspond to mean values from three independent experiments ± standard deviations.



Supplemental Figure 7. Effect of 8-Br-cGMP on expression of *NIA1, NRT2.1, AMT1.1,* and *AMT1.2* in the wild type 704. The effect 8-Br-cGMP on expression of *NIA1, NRT2.1, AMT1.1,* and *AMT1.2* was determined by Real Time PCR. Wild type 704 cells were grown in minimum medium containing 8 mM NH₄Cl. After three days of growth, cells were induced for 1 h in minimum medium containing 100 μ M KNO₃ supplied with the indicated pharmacological products. cDNAs corresponding to treated and untreated samples were always compared on the same PCR run and results from the treated samples were normalized to the untreated control. The error bars represent the standard deviation of at least three replicates, each replicate corresponding to a different PCR run. As IBMX is soluble in DMSO, DMSO was added in the untreated control. cGMP means 8-Br-cGMP.



Supplemental Figure 8. DAF-FM DA fluorescence is proportional to NO intracellular levels. (A) NO intracellular fluorescence levels after incubation with different concentrations of DEA NONOate in medium without nitrogen were determined using DAF-FM DA 1 μ M as described in the methods. (B) NO intracellular fluorescence levels after incubation with the indicated concentrations of ammonium and nitrate were determined in cells preincubated for 24 h in N-free medium. (C) The effect of different concentrations of nitrate on the NO levels produced by 5 mM ammonium.

NIA I	704	cyg56
NO ₃ ⁻ 100μM	100	100
SNP 1mM	ND	$43,12 \pm 8,37$
FeCN 1mM	3,47 ± 2,62	ND
DEA NONOate 10µM	$2,20 \pm 0,31$	$5,38 \pm 1,38$
IBMX 2mM	$0,81 \pm 0,41$	$3,38 \pm 1,85$
SNAP 2mM	$32,13 \pm 17,98$	84,99 ± 35,51
Α23187 10μΜ	24,85 ± 6,83	58,06 ± 7,24
NRT2.1	704	cyg56
NRT2.1 NO3 ⁻ 100μM	704	<i>cyg56</i> 100
NRT2.1 NO ₃ ⁻ 100μM SNP 1mM	704 100 2,37 ± 2,51	<i>cyg56</i> 100 48,69 ± 20,07
NRT2.1 NO ₃ ⁻ 100μM SNP 1mM FeCN 1mM	100 $2,37 \pm 2,51$ $0,47 \pm 0,35$	<i>cyg56</i> 100 48,69 ± 20,07 0,091 ± 0,06
<i>NRT2.1</i> <u>NO₃⁻ 100μM</u> <u>SNP 1mM</u> <u>FeCN 1mM</u> DEA NONOate 10μM	100 $2,37 \pm 2,51$ $0,47 \pm 0,35$ $2,00 \pm 0,33$	
NRT2.1 NO ₃ ⁻ 100μM SNP 1mM FeCN 1mM DEA NONOate 10μM IBMX 2mM	100 $2,37 \pm 2,51$ $0,47 \pm 0,35$ $2,00 \pm 0,33$ $3,16 \pm 0,80$	$cyg56$ 100 $48,69 \pm 20,07$ $0,091 \pm 0,06$ $4,55 \pm 1,52$ $2,20 \pm 0,69$
NRT2.1 NO ₃ ⁻ 100μM SNP 1mM FeCN 1mM DEA NONOate 10μM IBMX 2mM SNAP 2mM	704 100 $2,37 \pm 2,51$ $0,47 \pm 0,35$ $2,00 \pm 0,33$ $3,16 \pm 0,80$ $40,86 \pm 22,61$	cyg56 100 48,69 ± 20,07 0,091 ± 0,06 4,55 ± 1,52 2,20 ± 0,69 82,97 ± 19,90

Supplemental Table 1. Raw data used to produce Figure 10. Relative transcript levels of *NIA1* and *NRT2.1* were quantified by Real Time PCR in strains 704 and *cyg56* when treated with the indicated pharmacological products. Results from the treated samples were normalized to the untreated control for every PCR run. The untreated samples always corresponded to the relative value 100 and the standard deviation was therefore 0. Standard deviations of the treated samples correspond to the mean of at least three replicates, each corresponding to a different PCR run.