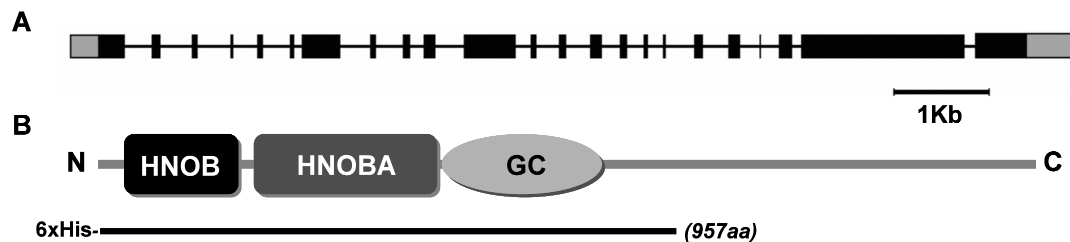
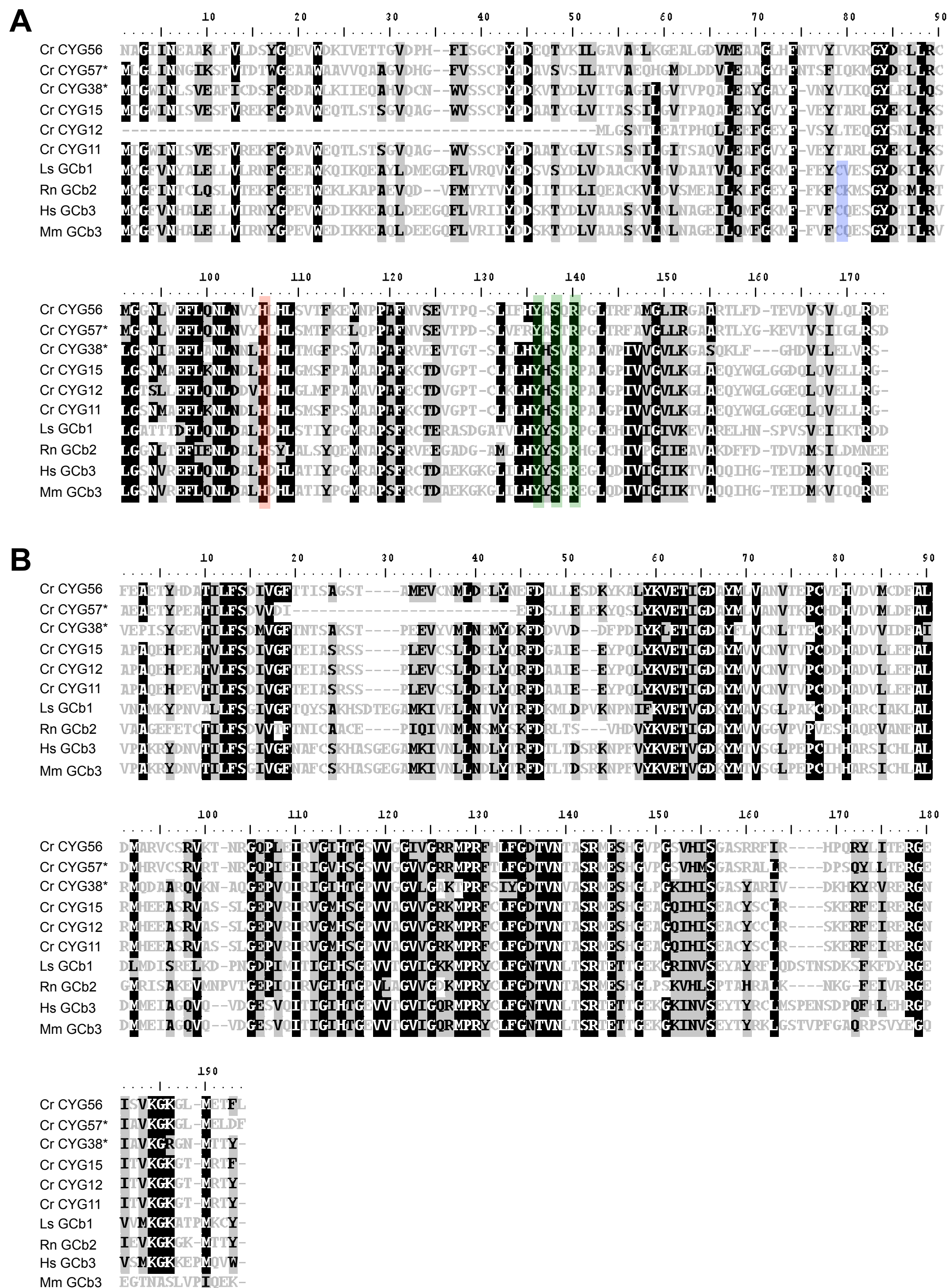
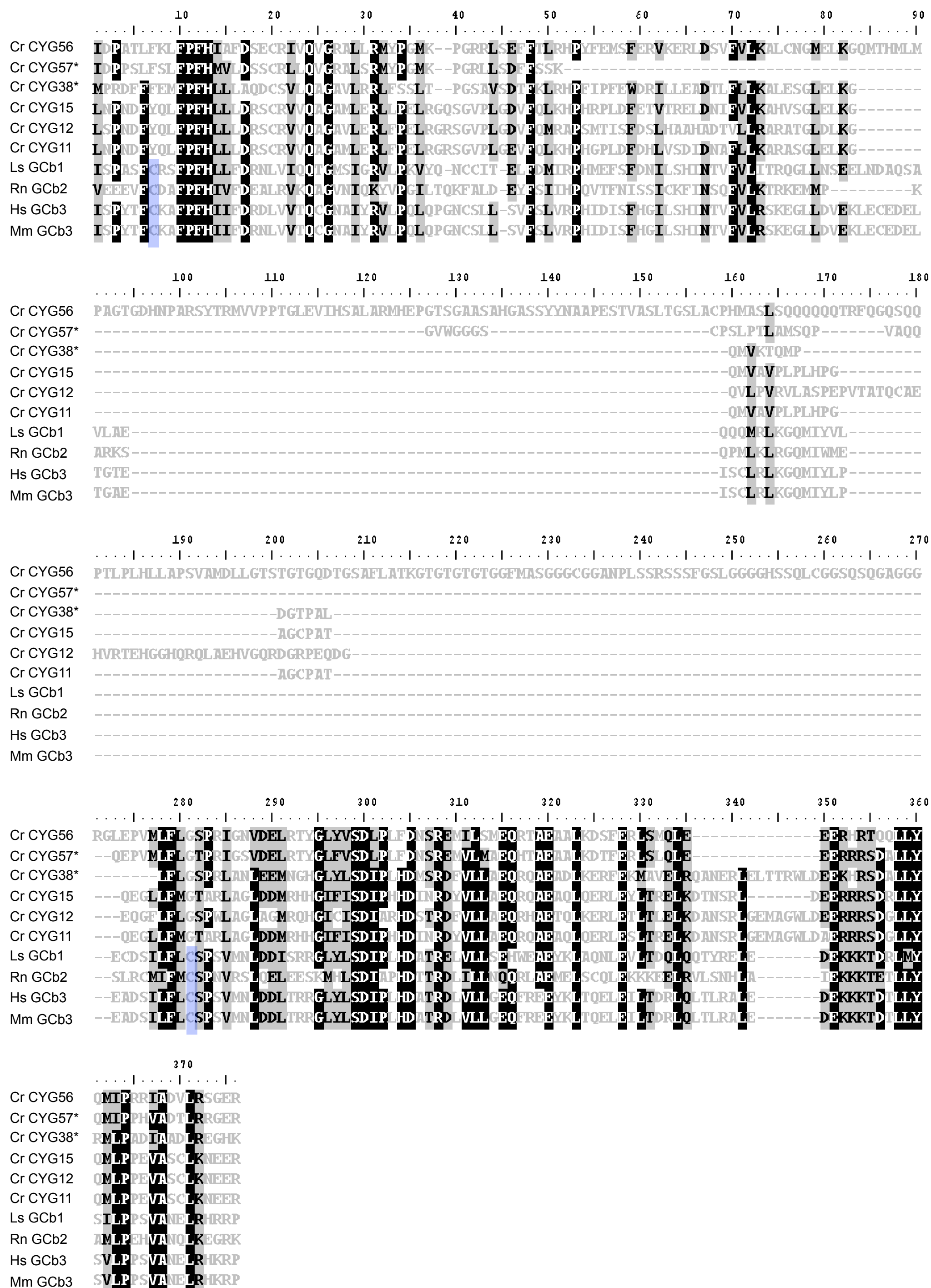


Supplemental Figure 1. Genomic structure of *CYG56* and domains of the CYG56 protein. **(A)** The complete 6111-bp cDNA of *CYG56* was sequenced and compared to the genomic *Chlamydomonas* sequence at the JGI v3.0 (http://genome.jgi-psf.org/Chlre3/Chlre3.home.html). UTRs are represented by light grey 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.



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.



Supplemental Figure 3. Sequence comparison annealing of HNOBA domain. Proteins compared are the same as in Supplemental Figure 2.

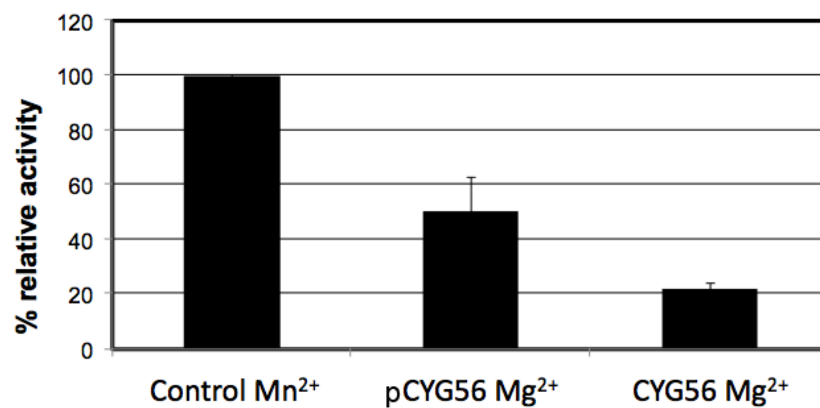
Percent Identity

Divergence

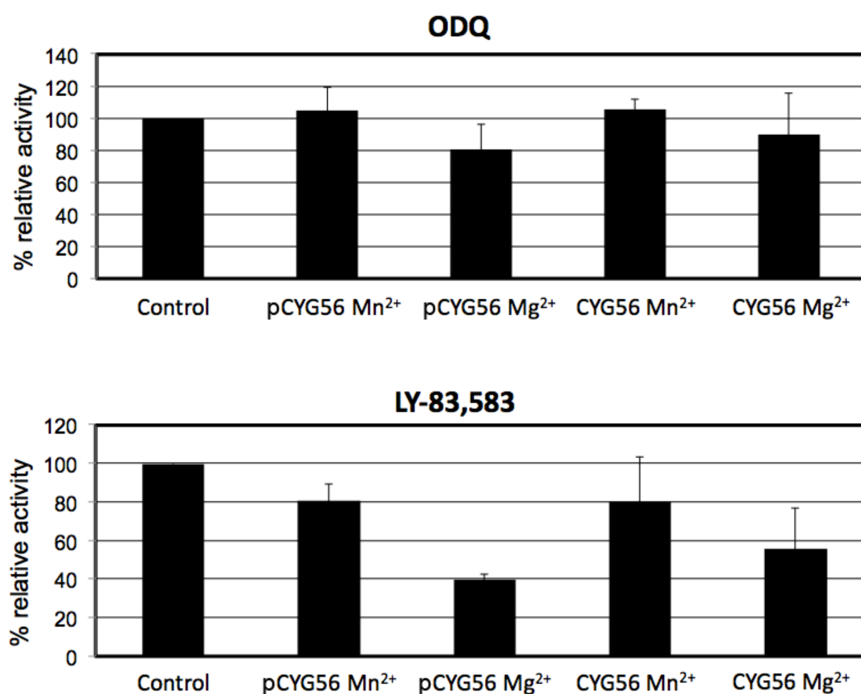
	1	2	3	4	5	6	7	8	9	10		
1		46.0	30.2	30.4	24.4	32.4	21.5	26.4	23.9	18.9	1	Cr CYG56
2	63.8		28.5	29.9	24.3	31.2	22.4	19.9	23.3	19.0	2	Cr CYG57*
3	136.6	122.1		37.8	30.6	48.7	28.7	25.1	29.1	29.1	3	Cr CYG38*
4	118.4	99.5	91.6		55.8	91.5	28.2	27.9	30.4	30.7	4	Cr CYG15
5	140.9	107.6	117.9	47.9		69.9	24.5	24.8	27.8	27.8	5	Cr CYG12
6	98.7	84.2	70.6	7.3	25.8		29.5	31.4	30.2	30.2	6	Cr CYG11
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 GCb3
	1	2	3	4	5	6	7	8	9	10		

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*

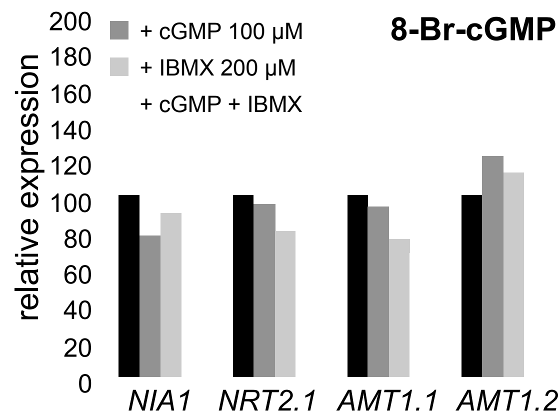
Supplemental Figure 4. Degree of identity and divergence among compared NO-dependent type GC sequences. Percentages of identity among compared sequences were obtained by using the MegAlign program of the DNASTAR software package. Either Accession numbers or identity of predicted *Chlamydomonas* sequences are indicated.



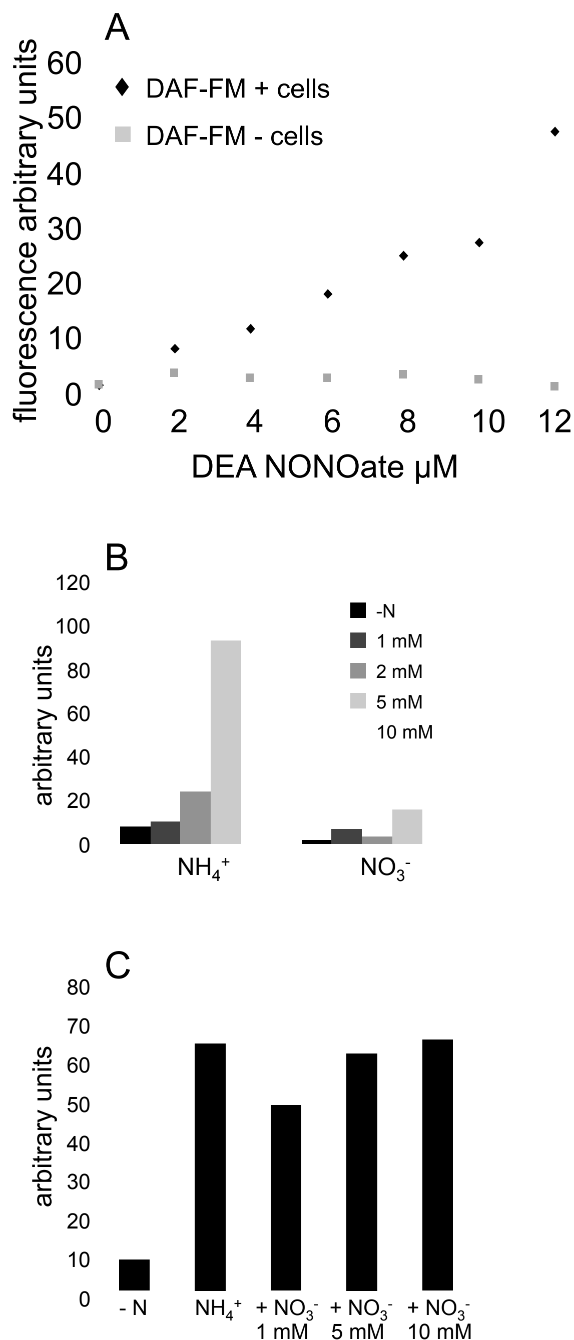
Supplemental Figure 5. GC activity assayed with Mg^{2+} and Mn^{2+} of truncated (pCYG56) and full-length CYG56 purified preparations. Standard assays conditions indicated in this paper were used except that 4mM of either Mg^{2+} or Mn^{2+} was used. Activities are referred to 100% of the activity of each enzyme with Mn^{2+} as a control. Data correspond to mean values from three independent experiments ± 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 μM and 100 μM, 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 NH_4Cl. After three days of growth, cells were induced for 1 h in minimum medium containing 100 μM KNO_3 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 1μM 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.

NIA1	**704**	***cyg56***
NO_3^- 100µM	100	100
SNP 1mM	ND	43,12 ± 8,37
FeCN 1mM	3,47 ± 2,62	ND
DEA NONOate 10µM	2,20 ± 0,31	5,38 ± 1,38
IBMX 2mM	0,81 ± 0,41	3,38 ± 1,85
SNAP 2mM	32,13 ± 17,98	84,99 ± 35,51
A23187 10µM	24,85 ± 6,83	58,06 ± 7,24
NRT2.1	**704**	***cyg56***
NO_3^- 100µM	100	100
SNP 1mM	2,37 ± 2,51	48,69 ± 20,07
FeCN 1mM	0,47 ± 0,35	0,091 ± 0,06
DEA NONOate 10µM	2,00 ± 0,33	4,55 ± 1,52
IBMX 2mM	3,16 ± 0,80	2,20 ± 0,69
SNAP 2mM	40,86 ± 22,61	82,97 ± 19,90
A23187 10µM	35,10 ± 8,15	78,94 ± 26,29

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.