```
CAH4      MSSRNVATALRMFATLGRSQAGEASAMMGTGSALLAQRAAALGGASAVNKGCSCRCGRVA  60
CAH5      MSSRNVATALRMFATLGRSQAGEASAMMGTGSALLAQRAAALGGASAVNKGCACRCGRVA  60
          ****************************************************:*******

CAH4      CMGACMPMRHLHAHPNPPSDPDQALEYLREGNKRFVNNKPHDSHPTRNLDRVKATAAGQK  120
CAH5      CMGACMPMRHLHAHPNPPSDPDQALEYLREGNKRFVNNKPHDSHPTRNLDRVKATAAGQK  120
          ************************************************************

CAH4      PFAAFLSCADSRVPVEIIFDQGFGDVFVTRVAGNIVTNEITASLEFGTAVLGSKVLMVLG  180
CAH5      PFAAFLSCADSRVPVEIIFDQGFGDVFVTRVAGNIVTNEITASLEFGTAVLGSKVLMVLG  180
          ************************************************************

CAH4      HSACGAVAATMNGAAVPGVISSLYYSISPACKKAQAGDVDGAIAENVKVQMEQLKVSPVL  240
CAH5      HSACGAVAATMNGAAVPGVISSLYYSISPACKKAQAGDVDGAIAENVKVQMEQLKVSPVL  240
          ************************************************************

CAH4      QGLVKEGKLKIVGGVYDLATGKVTEIA-  267
CAH5      QGLVKEGKLKIVGGVYDLATGKVTEIA*  267
          ***************************
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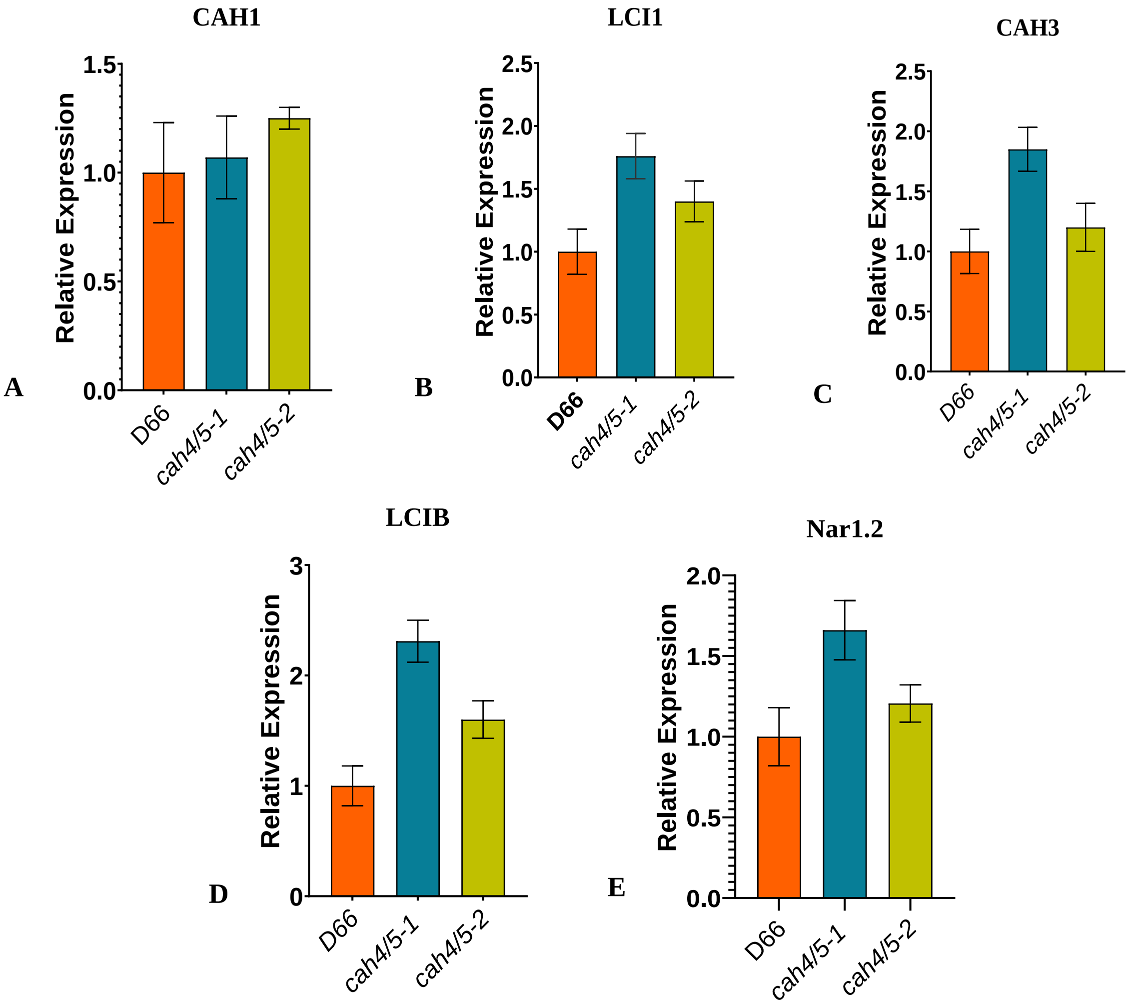
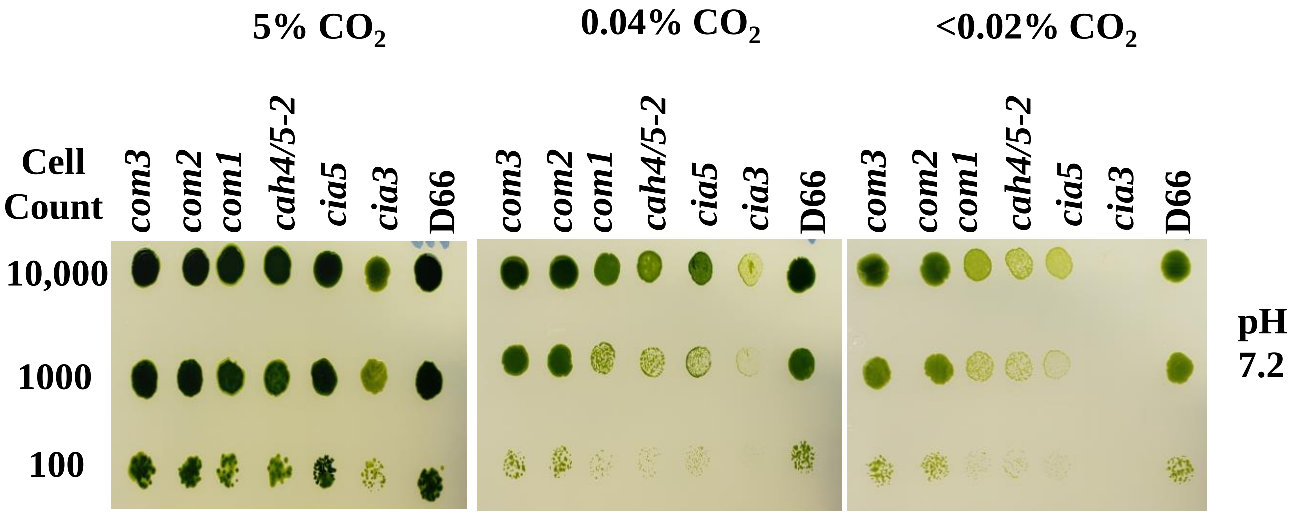
Supplemental Figure S1. Multiple sequence alignment of the CAH4 and CAH5 protein sequence. Arrow indicates the transit peptide cleavage site.



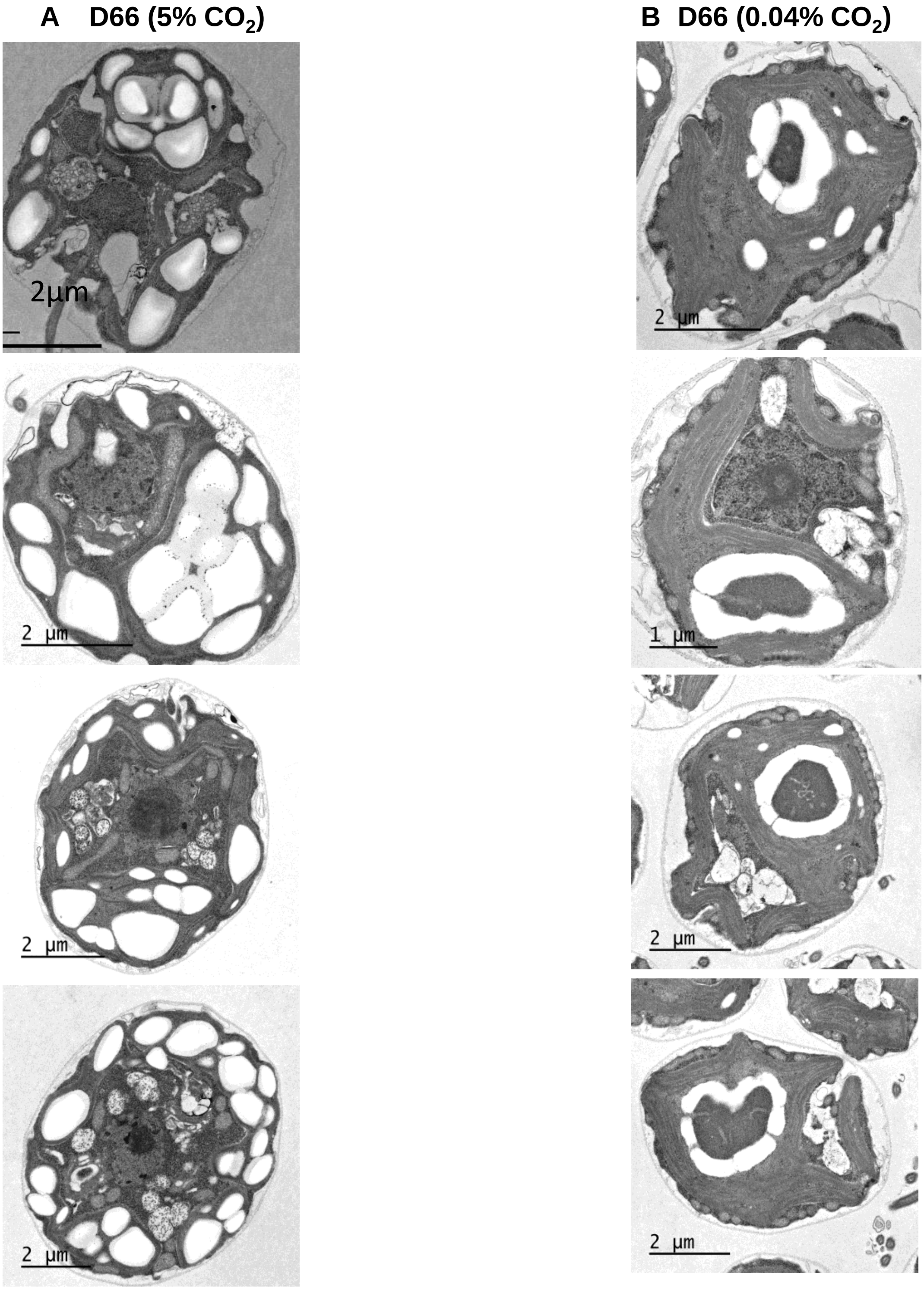
Supplemental Figure S2.

Supplemental Figure S2. RT-qPCR of other CCM genes. The figure shows comparative relative expression of CCM genes in wild type (D66) and RNAi knockdown strains for (A) *CAH1* (B) *LCI1* (C) *CAH3* (D) *LCIB* (E) *Nar1.2.* Error bars represent standard deviation from three biological replicates. For all the CCM genes, transcript levels were calculated using $2^{-\Delta\Delta C_T}$ relative to the reference gene *CBLP* and reported relative to the corresponding wild type D66 cells.



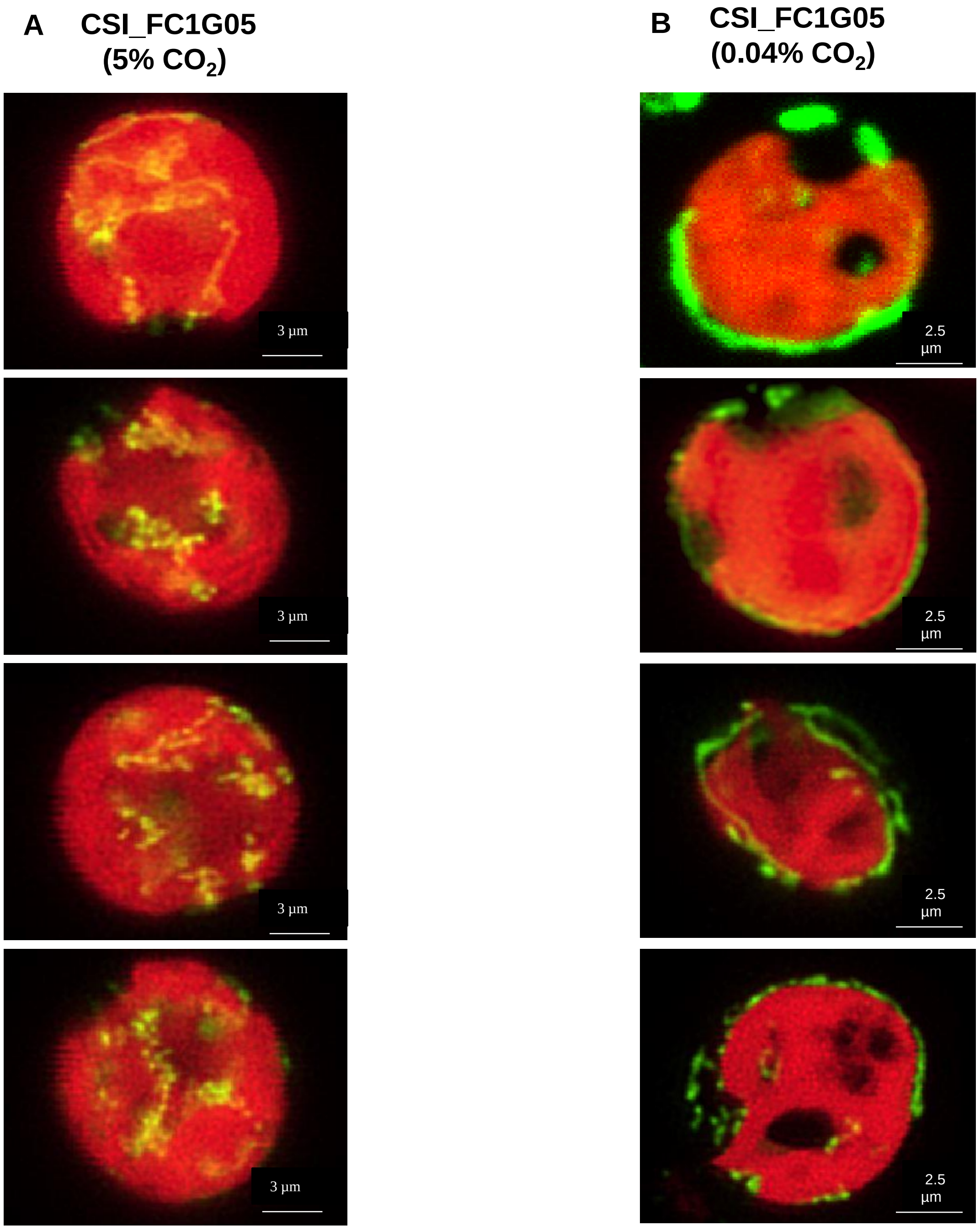
Supplemental Figure S3.

Supplemental Figure S3. Growth of strains complementing the *cah4/5* RNAi knockdown line at different CO_2 levels. Growth analysis showing D66, *cah4/5-2*, *cia3*, *cia5*, *com1*, *com2* and *com3* . Cells were diluted to 6.6 × 106 cells ml^{-1}, followed by 1:10 serial dilution 3 times at very low CO_2, ambient CO_2 and high CO_2 at pH 7.2. Cells were grown for 6 days. The *cia3* and *cia5* mutants were included as a CCM-deficient control. Cells were initially grown in TAP media at low CO_2 in the light before spotting them onto plates.



Supplemental Figure S4.

Supplemental Figure S4 . Additional TEM images of whole cells of the D66 strain. Images were from (A) high CO$_2$ and (B) ambient CO$_2$ levels. At ambient levels of CO$_2$, the mitochondria are arranged along the plasma membrane of the Chlamydomonas cell.



CSI_FC1G05 (5% CO_2)

CSI_FC1G05 (0.04% CO_2)

Supplemental Figure S5.

Supplemental Figure S5 . Additional confocal images of whole cells of the CSI_FC1G05 expressing pLM005-*CAH5*-Venus-3xFLAG. Images were from (A) high CO_2 and (B) ambient CO_2 levels. At ambient levels of CO_2, the CAH5 proteins are localized along the periphery of the Chlamydomonas cell.

Supplemental Table S1 . List of primers used in this study. For the two miRNA primers the underlined nucleotides are the ones used to target the *CAH4* and *CAH5* gene. Please move the table heading so that it is located above the table.

miRNA	**Sequence**
CAH4/5F	ctagtCCGAGTCTAGATAGCTGTCAAtctcgctgatcggcaccatgggggtggtggtgatcagcgctaTTGAGAGCTATCTAGACTCGG
CAH4/5R	ctagcCCGAGTCTAGATAGCTCTCAAtagcgctgatcaccaccacccccatggtgccgatcagcgagaTTGACAGCTATCTAGACTCGGA

qPCR	**Sequence**
CAH4/5 F	TCAGCCCGGCCTGCAAGAAGG
CAH4/5 R	CGCACACGCCCACGATCT
CBLP F	ATGTGCTGTCCGTGGCTTTC
CBLP R	CAGACCTTGACCATCTTGTCCC
CAH3 F	ATTCGATTTCGCCTACGGCA
CAH3 R	CATGTTGCCAATGAACGCCA
LCIB F	TGATCAAGCACTTCCCCTCG
LCIB R	GAGCCGAAGATGGCCTCAAT
CAH1 F	ACGCGCACCTGGCCTCAAT
CAH1 R	GTTGGCGTACCTGGGGGTTGCGGAA
CCP1 F	ACCAAGGCCGACCTCAACTACCTG
CCP1 R	CAGTCCATCGTGGACGAGTACTGGGG
HLA3 F	AGCAGACCATCAAGAAGGTGTTCGACG
HLA3 R	GCAGCTTCGAGGGCGACTCG
NAR1.2 F	GTGTGCTGCGCCGTGTGGTC
NAR1.2 R	GCCCAGCATCATGCCCAGAGGAATCA