Supplementary materials

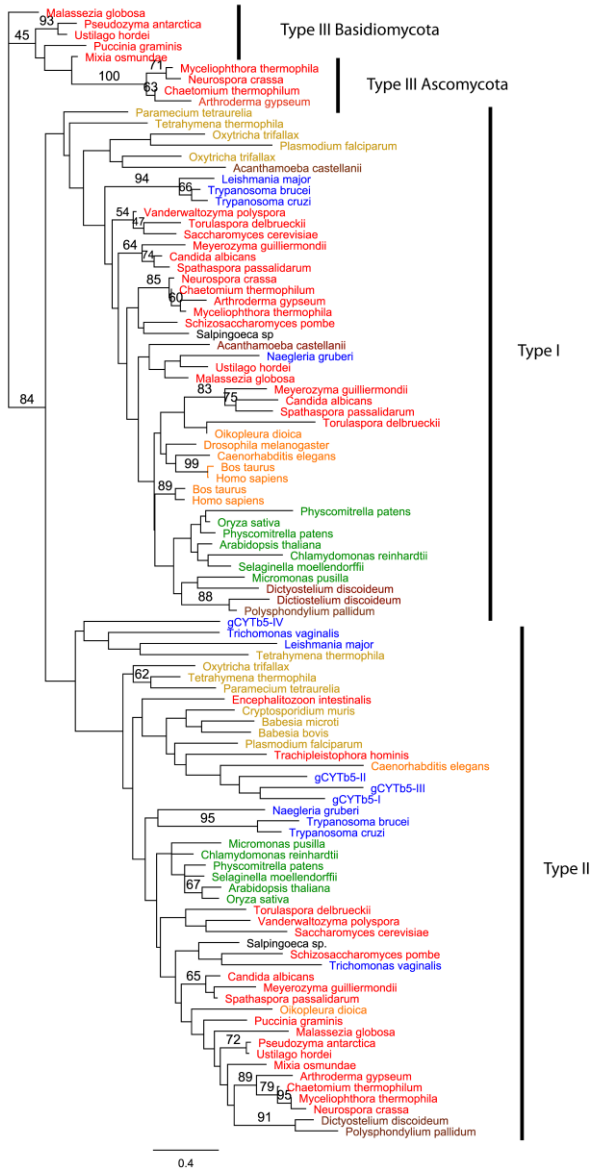

Figure S1. Gene tree of eukaryotic cytb5 proteins.

The PHYML tree of cy*tb5* proteins and their homologues TAX-2 proteins. Numbers on the branches display statistical support in the form of bootstrap values and the scale bar indicates estimated number of amino acid substitutions per site. Only bootstrap support values greater than 50 are shown. gCYTb5s are members of type II clade. Green color was used for Viridiplantae clade, red for Fungi, orange for Metazoa, yellow for SAR supergroup, brown for Amoebozoa, and blue for Excavata.

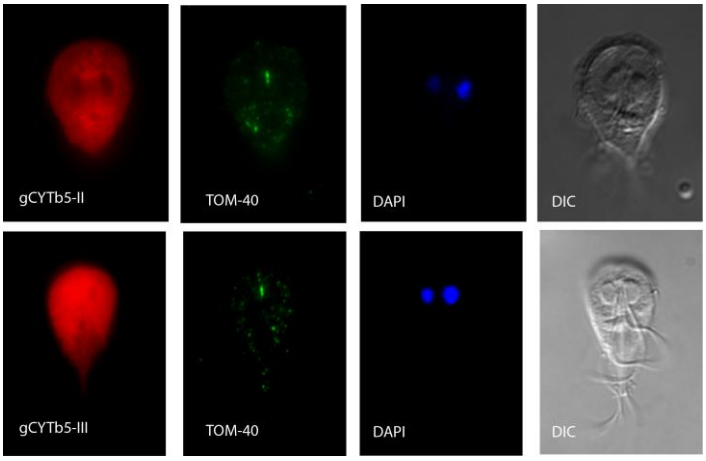

Figure S2. Localization of C-terminal HA-tagged gCYTb5 in *G. intestinalis*

The cytosolic localization of C-terminal HA-tagged gCYTb5 II and III visualized using immunofluorescence microscopy. gCYTb5 was detected using rat anti-HA-tag and anti-rat Alexa Flour 594 (red) antibodies. A mitosomal marker, TOM40, was visualized using mouse anti-TOM40 and anti-rabbit Alexa Fluor 488 (green) antibodies. The nuclei were stained with DAPI (blue). DIC, differential interference contrast.

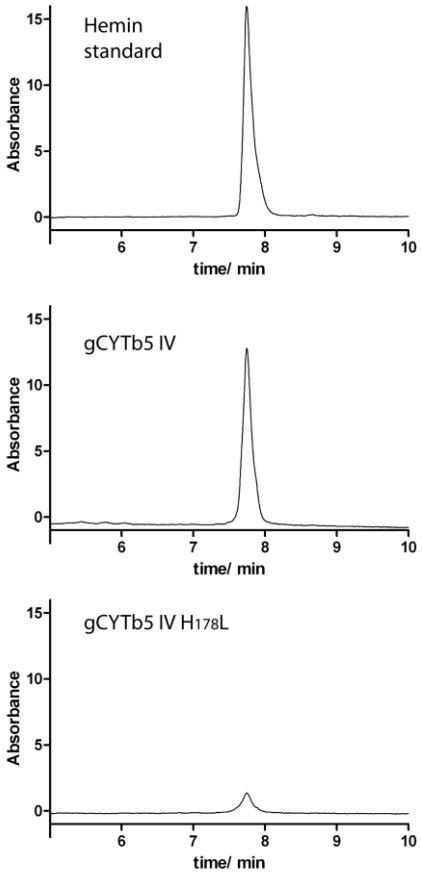

Figure S3. Heme determination in recombinant proteins.

HPLC traces of heme extracted from the recombinant gCYTb5-IV compared with that of the gCYTb5-IVH_{178}L mutant. Hemin was used as a standard. Retention time for heme was 7.8 minutes. 10 µg of each recombinant protein was used for the extraction.

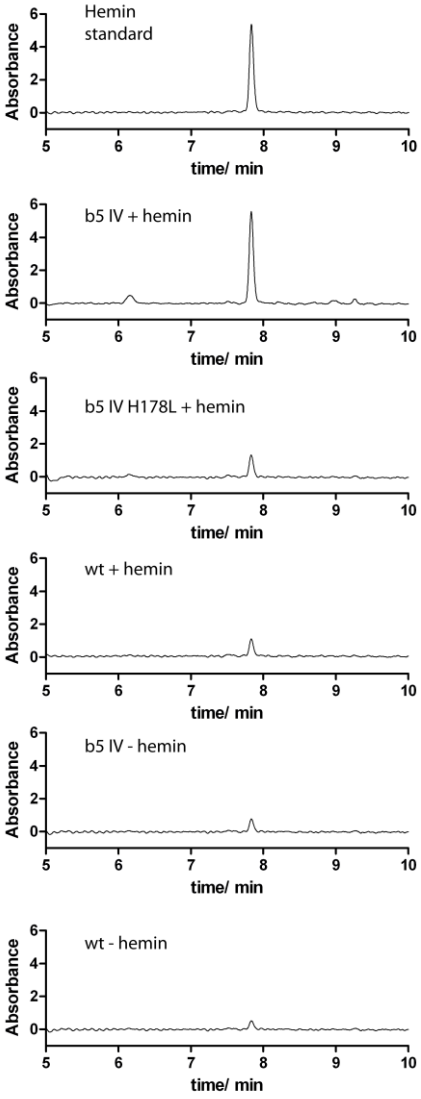

Figure S4. Heme determination in *G. intestinalis*. Representative HPLC traces of heme samples extracted from cytosolic fractions of the wild type strain (wt), the gCYTb5-IV expressing strain (b5-IV), and the strain expressing gCYT-IVH_{178}L (b5-IVH_{178}L). For each extraction, we used 125 µg of cytosolic proteins. Cells were cultivated in medium supplemented with hemin (+) or in standard medium (-). Hemin was used as a standard. Retention time for heme was 7.8 minutes

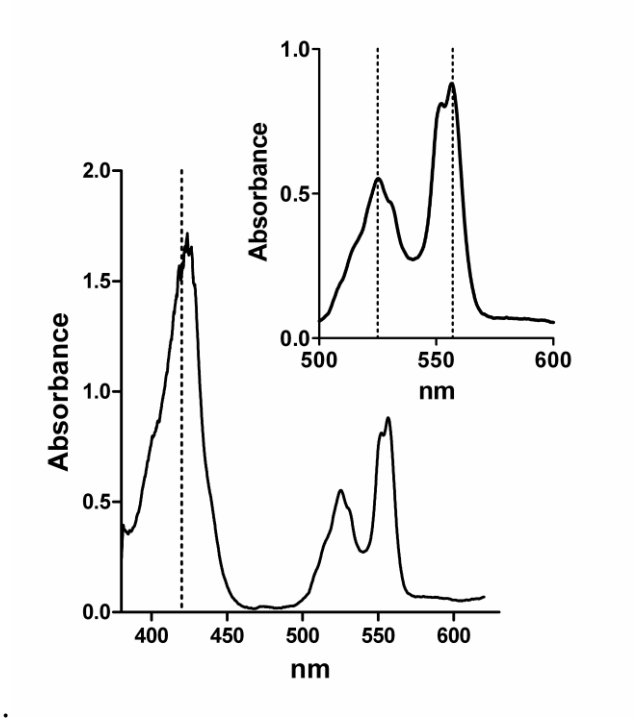

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Figure S5. Low temperature spectra of recombinant gCYTb5-IV that was produced in *Escherichia coli*. Characteristic UV-visible spectrum maxima (420, 525 and 557 nm) are indicated by dotted lines.

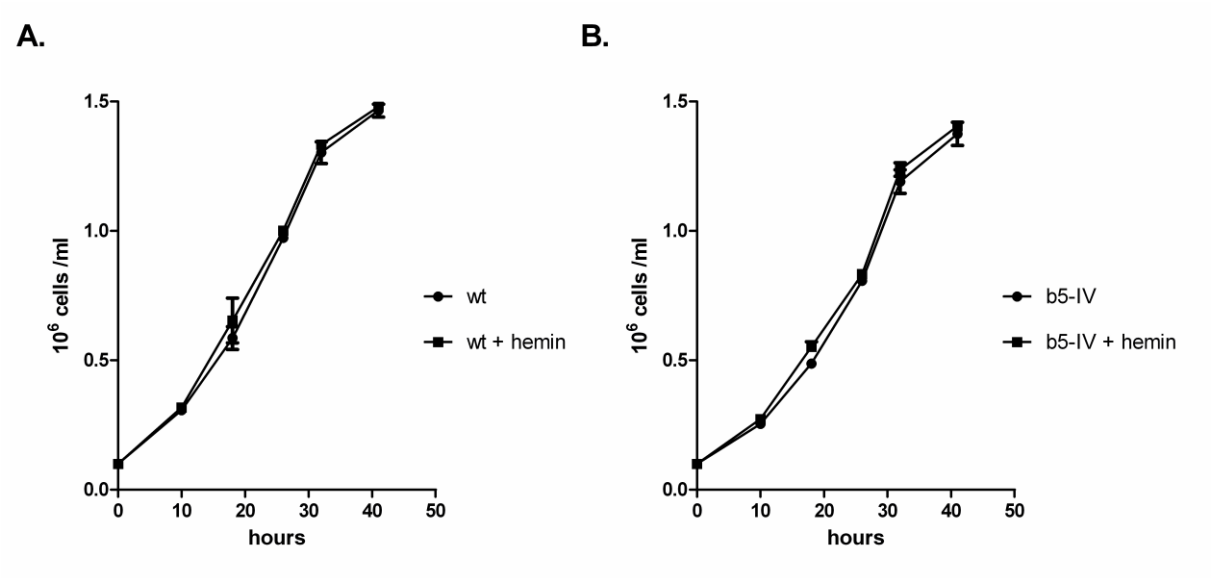

Figure S6. Effect of heme on growth of *G. intestinalis*.

Growth of the wild type strain (wt)(A) and the strain expressing recombinant CYTb5-IV (B) in the standard culture medium and in the medium supplemented with 4 μM hemin (+ hemin)

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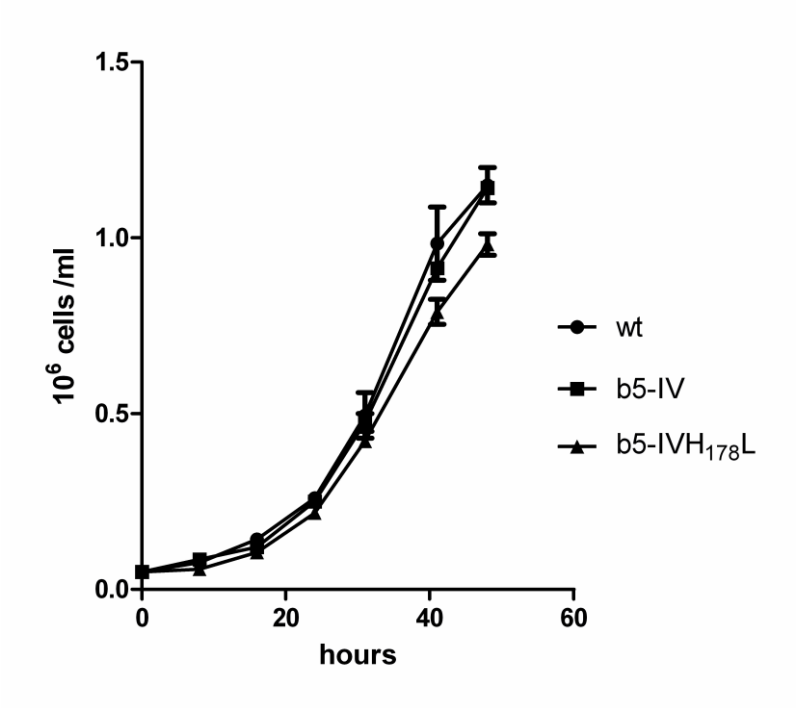

Figure S7. Effect of CYTb5-IVH$_{178}$L on growth of *G. intestinalis*. A minor decrease in growth rate of the strain expressing CYTb5-IVH$_{178}$L with impaired heme binding site was observed in comparison to the wild type strain (wt) and the strain expressing recombinant CYTb5-IV.

Table S1. Primers for the preparation of gCYTb5-IVH178L in which histidine 178 was replaced by a leucine.

Primers for the preparation of gCYTb5-IVH$_{178}$L	
F1	5'-AGTC*CATATG*GAGTCCGTATCTATG-3'
R1	5'-CAATGGAACCCTTCCGCCAGGCAGCTTATTAAGGTACTCTGTCAC-3'
F2	5'-GTGACAGAGTACCTTAATAAGCTGCCTGGCGGAAGGGTTCCATTG-3'
R2	5'-AGTC*GGATCC*TCATTCTACAACATGAAAATA-3'

In the first step, two separate PCR reactions were performed using the primers F1+R1 and F2 +R2. Subsequently, the PCR products were purified and both used as the template for a second PCR reaction using the primers F1+ R2.