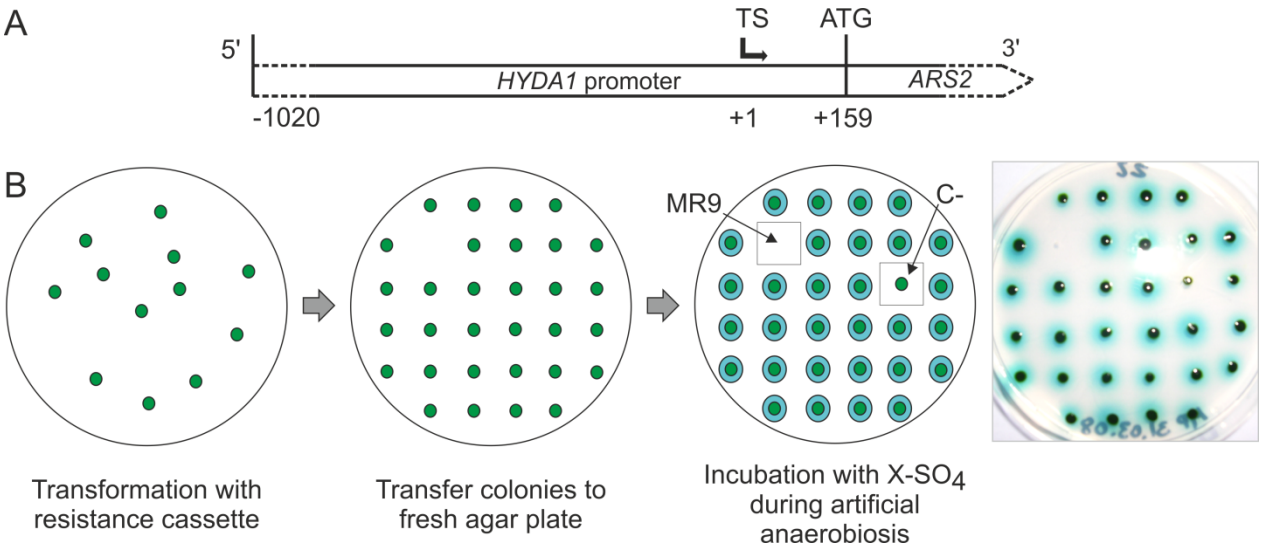
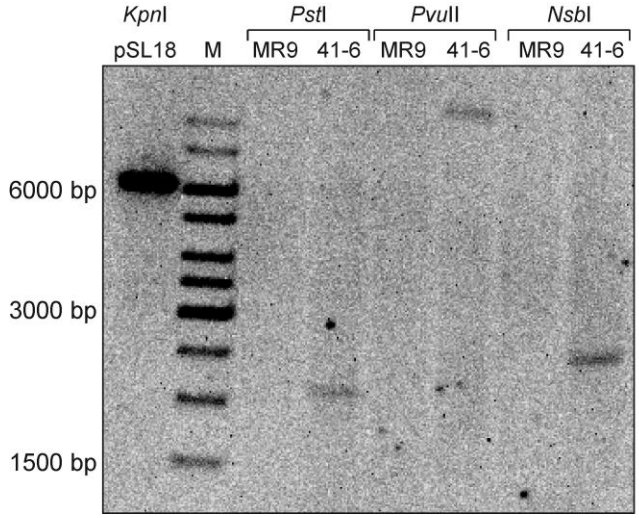
Supplemental figure S1



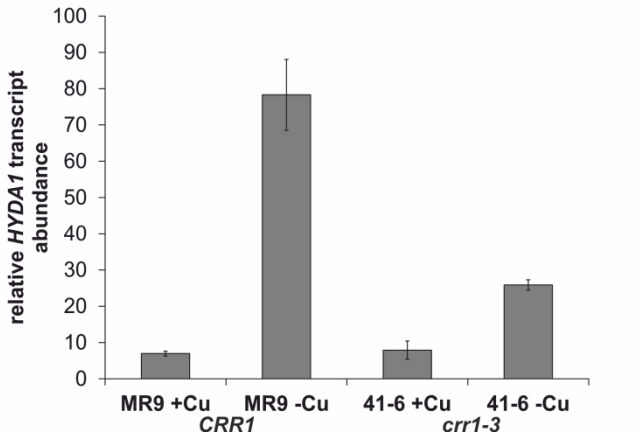
Supplemental figure S1. Scheme of the setup applied for identifying *C. reinhardtii* transformants with impaired *HYDA1* promoter activity. A, Chimeric construct consisting of the *HYDA1* promoter from position -1020 to +158 relative to the transcription start site as indicated in Stirnberg and Happe, 2004, and *ARS2* reporter gene (p*HYDA1:ARS2*) present in strain MR9, which was transformed with a paromomycin resistance cassette. B, After insertional mutagenesis of *C. reinhardtii* strain MR9, its paromomycin resistant progenies were grown on TAP-agar plates. These were transferred to an anaerobic tent and the artificial arylsulfatase (ARS) substrate X-SO_4 was dropped on each colony. After 24 h, the presence or absence of a blue staining served as an indicator for an active or inactive *HYDA1* promoter, respectively. A paromomycin resistant derivative of wild type strain CC-124 served as control for the absence of endogenous ARS activity (C-), while strain MR9 was used to ensure the paromomycin selection pressure. (TS: transcription start site, ATG: translation start site)

Supplemental figure S2



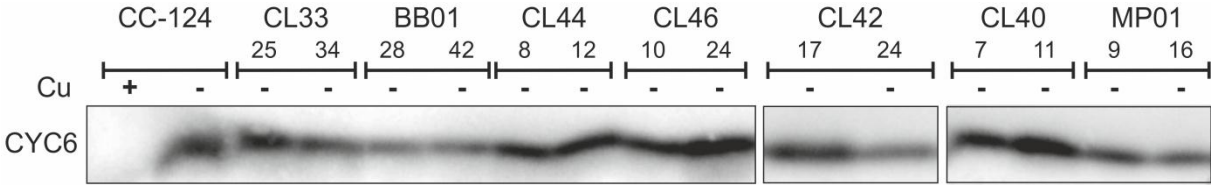
Supplemental figure S2. Southern blot analysis determining the integration frequency of the paromomycin resistance cassette in *C. reinhardtii* strain 41-6. Genomic DNA from parental strain MR9 and transformant 41-6 was digested using the indicated restriction enzymes and separated by agarose gel electrophoresis. A digoxigenin labeled *APHVIII*-specific probe was used to detect the paromomycin resistance cassette. *Kpn*I digested plasmid pSL18 served as a control.

Supplemental figure S3



Supplemental figure S3. Analysis of relative *HYDA1* transcript abundance in *C. reinhardtii* strain MR9 (*CRR1*) and *crr1-3* mutant 41-6 incubated in Cu-replete (+Cu) or Cu-deficient medium (-Cu). qPCR was done as described in details in the materials and methods section. Each depicted value represents the average from three biological experiments. Error bars indicate the standard deviation.

Supplemental figure S4



Supplemental figure S4. CYC6 protein levels in randomly selected copper deficient *C. reinhardtii* transformants used for *HYDA1* promoter analyses. Crude protein extracts were separated by Tris Tricin-PAGE, blotted on PVDF membranes and analyzed for CYC6 protein levels using anti-*C. reinhardtii*-CYC6-antibody, which was kindly donated by S. Merchant, UCLA, USA.