Supporting Information

Table S1: Oligonucleotides used in this study

The following abbreviations are used: 3' rep, 3' replacement; cDNA, complementary DNA; DHFR, dihydrofolate reductase; fwd, forward; LIC, ligation independent cloning; rvs, reverse.

No.	Primer Name	Primer Sequence
1.	UroD 3' LIC fwd	5'-TACTTCCAATCCAATTTAGCCAGAACGGTGGAGATAAGAGACAGC
2.	UroD 3' LIC rvs	5'-TCCTCCACTTCCAATTTTAGCAACTGTTGAGGTATCTTGGCCCTC
3.	UroD 3'flank fwd	5'-GTCAAGATCTAAAATGCAGGCGTCCTCCTCCTCTCC
4.	UroD 3'flank rvs	5'-GACTGCGGCCGCGTCAGTTGATCTTAATCTACCTCTCC
5.	UroD 5'flank fwd	5'-GACTTTAATTAACACCAAGAAACCGTTCGAGAGC
6.	UroD 5'flank rvs	5'-GACTATGCATGGCGTAGCGACTCTCTCAAAGC
7.	UroD screen fwd	5'-CTTTGACTTCTCTTCGCGTGAAG
8.	UroD screen rvs	5'-GACATCTGCCTATCTGTCTACGC
9.	t7s4 fwd	5'- ACGCAGTTCTCGGAAGACG
10.	UroD 3'rep fwd	5'-GATCAGATCTCGTGACGATGATTGCGTTCCC
11.	UroD 3'rep rvs	5'-GATCCCTAGGAACTGTTGAGGTATCTTGGCCCTCC
12.	UroD cDNA fwd	5'-GTCAAGATCTAAAATGCAGGCGTCCTCCTCCTCTCC
13.	CytC-A 3'rep fwd	5'-GATCAGATCTCCAAATGCTCGCAATGCCACACC
14.	CytC-A 3'rep rvs	5'-GATCCCTAGGCTTGTTGGAGGCATCAACAAGGTAC
15.	CytC-B 3'rep fwd	5'-GATCAGATCTCCTTCATTTTCAGGTACTGAACTCCG
16.	CytC-B 3'rep rvs	5'-CAGCGGCCGCGCCTGTTCATGTCGCACACTGG
17.	CytC1 3'rep fwd	5'-TCGAAGATCTGCTGCGGAGTACGACGTGACC
18.	CytC1 3'rep rvs	5'- GATCCCTAGGCAAATACTTCAGCTTTCCGAAATCG
19.	CCHL1 3'flank fwd	5'-GATCCTTAAGCCCGGGATGGCCACCGCGGCAACTTGCCCT
20.	CCHL1 3'flank rvs	5'-CGATGCGGCCGCGTTTTTCCAAGTTCTCTCATTGACC
21.	CCHL1 5'flank fwd	5'-CGATGGGCCCTTGTCGAAATCGCACTGGGCATC
22.	CCHL1 5'flank rvs	5'-CTGACATATGAGAAGAACCGAGAAAACGCAGACAG
23.	CCHL1 screen fwd	5'-GGTTCAAGCTTTTCGACGG
24.	CCHL1 screen rvs	5'-GTGCAGTGCATTCTCCCAGT
25.	CCHL2 3' flank fwd	5'-CATGCCTAGGATGGCGGTGAACGATG
26.	CCHL2 3' flank rvs	5'-GTCAGCGGCCGCAGTCAGGAAACCTGCTC
27.	CCHL2 5' flank fwd	5'-GATCGGGCCCCGGTTCTACACAAGAAGTGGCTTTCTG
28.	CCHL2 5'flank rvs	5'-GACTCATATGGTGAAAACGGGAATTTGACAGGTCG
29.	CCHL2 3'screen fwd	5'- CTACACTGTAGAGAGGGTTCTGCC
30.	CCHL2 3'screen rvs	5'- CGCTCGCGTTACCTGAGAGTC
31.	CCHL2 5'screen fwd	5'- CGTTACGAAGCACCGACAGGAG
32.	CCHL2 5'screen rvs	5'- TGACGCTGGCAGAACCCTC
33.	DHFR cassette rvs	5'- GGTGTCGTGGATTTACCAGTCAT

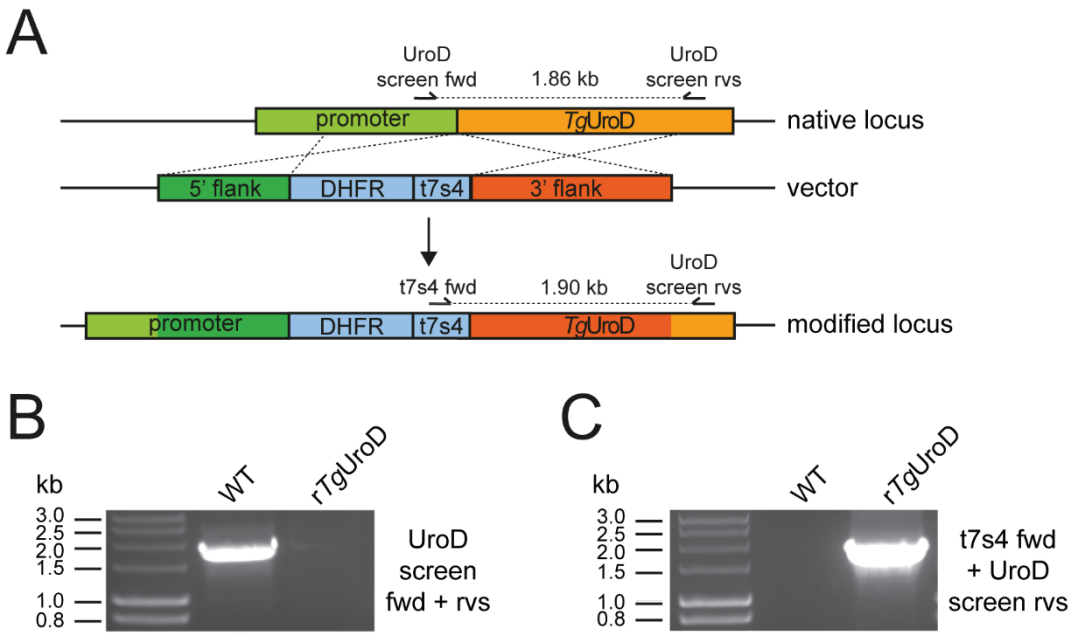

Figure S1. Generation of r*Tg*UroD parasites. (A) Schematic of the genetic strategy employed for generating the r*Tg*UroD cell line. The native promoter of *Tg*UroD (light green) was replaced by an ATc-regulatable tet operator × 7/sag4 (t7s4) promoter and pyrimethamine-resistant dihydrofolate reductase (DHFR) marker by homologous recombination using 5' and 3' flanking regions around the *Tg*UroD start codon (dark green and dark orange respectively). The relative positions of primers used in subsequent PCR screens are indicated. **(B-C)** Polymerase chain reaction (PCR) screens to test for the presence of **(B)** the native locus or **(C)** the genetically modified locus using the indicated primers and genomic DNA extracted from wild type (WT) or clonal rTgUroD parasites. These screens indicated successful modification of the *Tg*UroD locus with the ATc-regulatable promoter.

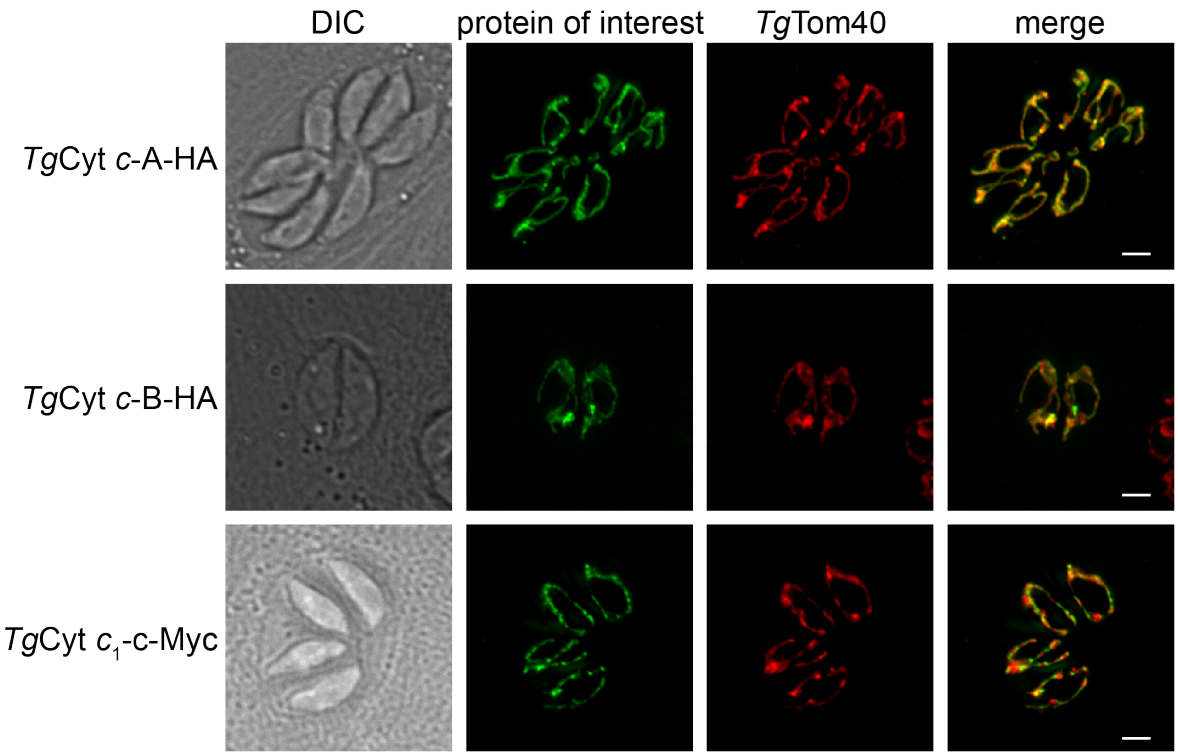

Figure S2. Localisation of epitope-tagged *c*-type cytochrome proteins in *T. gondii*.
Immunofluorescence assays examining the localisation of epitope-tagged c-type cytochromes (green) in *T. gondii*. Parasites were-co-labelled with antibodies against the mitochondrial marker *Tg*Tom40 (red). Included are HA tagged *Tg*Cyt *c*-A (top), HA-tagged *Tg*Cyt *c*-B (middle), and c-Myc-tagged *Tg*Cyt c_1 (bottom). DIC, differential interference contrast image. Scale bars are 2 μm.

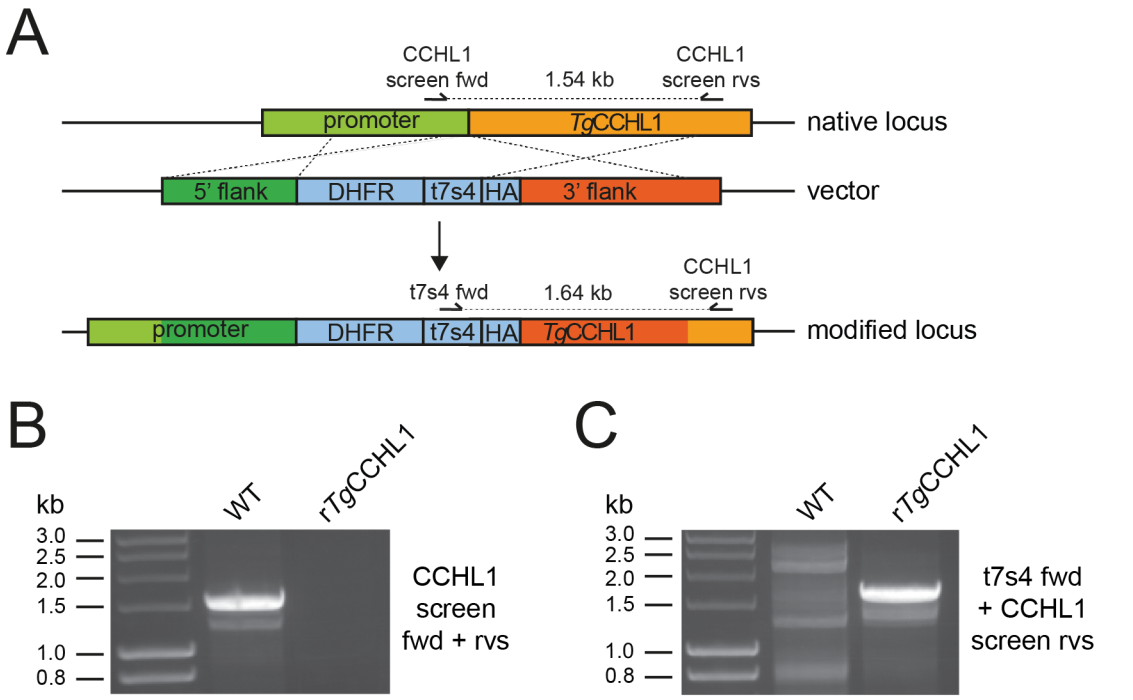

Figure S3. Generation of r*Tg*CCHL1 parasites. (A) Schematic of the genetic strategy employed for generating the r*Tg*CCHL1 cell line. The native promoter of *Tg*CCHL1(light green) was replaced by an ATc-regulatable t7s4 promoter, a 3 × HA tag, and a pyrimethamine-resistant dihydrofolate reductase (DHFR) marker by homologous recombination using 5' and 3' flanking regions around the *Tg*CCHL1 start codon (dark green and dark orange respectively). The relative positions of primers used in subsequent PCR screens are indicated. **(B-C)** Polymerase chain reaction (PCR) screens to test for the presence of **(B)** the native locus or **(C)** the genetically modified locus using the indicated primers and genomic DNA extracted from wild type (WT) or clonal r*Tg*CCHL1 parasites. These screens indicate successful modification of the *Tg*CCHL1 locus with the HA-tag and ATc-regulatable promoter.

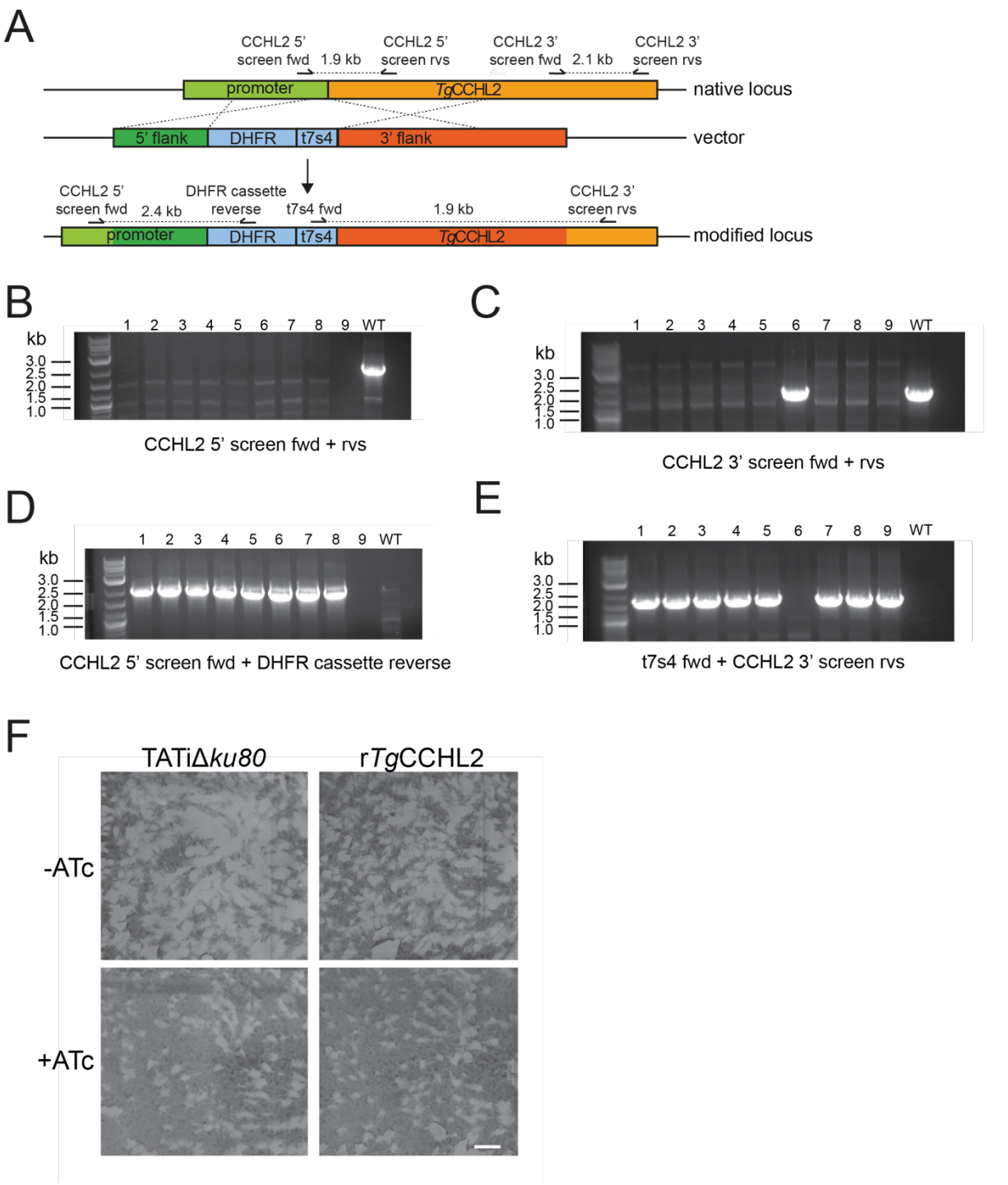

Figure S4. Generation and characterization of r*Tg*CCHL2 parasites. (A) Schematic of the genetic strategy employed for generating the r*Tg*CCHL2 cell line. The native promoter of *Tg*CCHL2(light green) was replaced by an ATc-regulatable t7s4 promoter and a pyrimethamine-resistant dihydrofolate reductase (DHFR) marker by homologous recombination using 5' and 3' flanking regions around the *Tg*CCHL2 start codon (dark green and dark orange respectively). The relative positions of primers used in subsequent PCR screens are indicated. **(B-E)** Polymerase chain reaction (PCR) screens to test for the presence of **(B)** the 5' region of the native locus, **(C)** the 3' region of the native locus, (D) the 5' region of

the genetically modified locus, or (E) the 3' region of the genetically modified locus, using the indicated primers. Genomic DNA was extracted from 9 different clonal r*Tg*CCHL2 parasite lines or from WT parasites. The screens indicate successful modification of the *Tg*CCHL2 locus in clones 1-5, 7 and 8. **(F)** Plaque assay of WT (TATiΔ*ku80*; left) and r*Tg*CCHL2 (right) parasites cultured in the absence (top) or presence (bottom) of ATc for 8 days. Data are from a single experiment and are representative of 2 independent experiments. Scale bar is 10 mm.

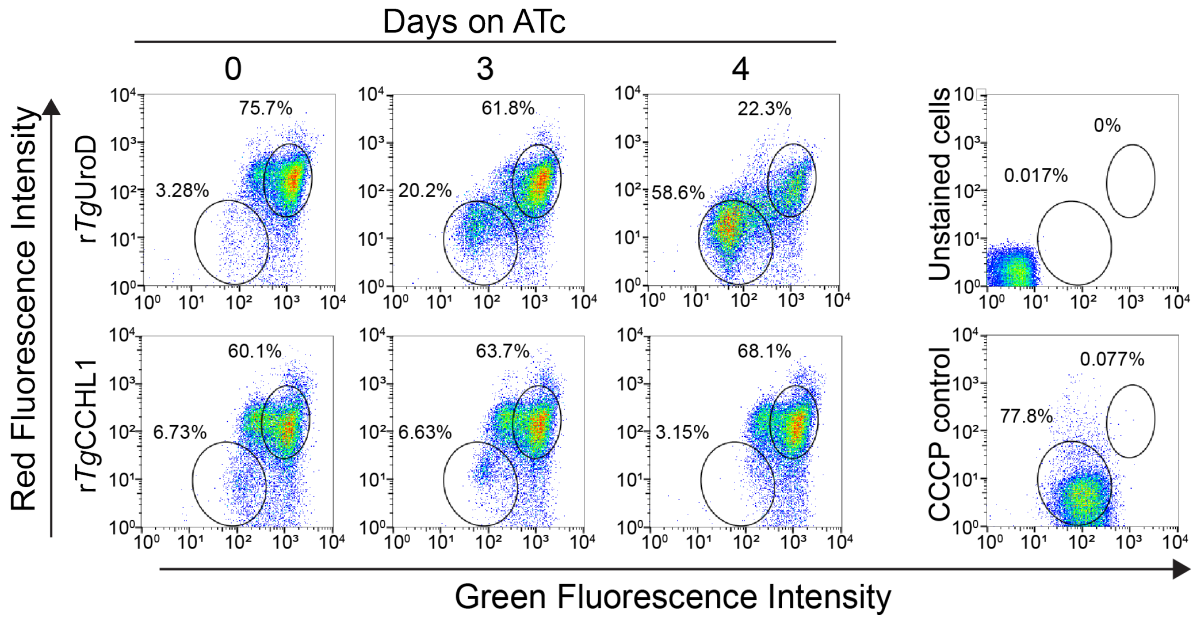

Figure S5. Knockdown of *Tg*UroD but not *Tg*CCHL1 leads to dissipation of mitochondrial membrane potential ($\Delta\Psi_m$). r*Tg*UroD parasites (top) and r*Tg*CCHL1 parasites (bottom) were grown in the absence of ATc or the presence of ATc 3-4 days, then stained with sensitive dye JC-1. Green fluorescence (x axis) and red fluorescence (y axis) were measured by flow cytometry. Dual red/green fluorescence is indicative of a high $\Delta\Psi_m$, whereas a depletion in red fluorescence is indicative of a dissipated $\Delta\Psi_m$. Controls included unstained parasites (top, right) and parasites treated with the protonophore CCCP to dissipate $\Delta\Psi_m$. Data shown is from a single experiment.

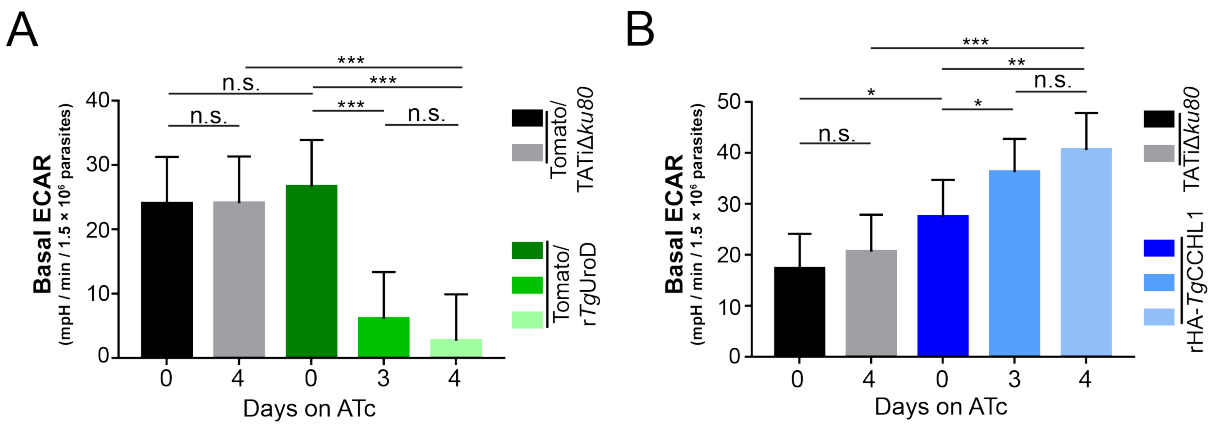

Figure S6. Knockdown of *Tg*UroD leads to a decrease in extracellular acidification rate (ECAR), whereas knockdown of *Tg*CCHL1 leads to an increase in ECAR. Basal ECAR in **(A)** parental Tomato/TATiΔ*ku80* (black/gray) and r*Tg*UroD (green) parasites, or **(B)** TATiΔ*ku80* parental (black/gray) and rHA-*Tg*CCHL1 parasites (blue). Parasites were grown in the absence of ATc, or in the presence of ATc for 3 or 4 days. Data depict the least square means from a linear mixed model ± 95% confidence limits from 3 independent experiments (*, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$; n.s. = not significant, $P > 0.05$; ANOVA with Tukey's post hoc test).