SUPPLEMENTARY INFORMATION APPENDIX

Selection of *Plasmodium falciparum* cytochrome B mutants by putative PfNDH2 inhibitors

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Supplemental Methods

Selection of resistant parasites.

Fifty mL cultures were grown to 5% parasitemia, split into 4 flasks, supplemented to 5% hematocrit in CM, and incubated until they again reached 5 – 15% parasitemia for a total of 4 – 12 × 10^8 total parasites/flask. CM containing indicated concentrations of ATQ, CK-2-68, or RYL-552 was changed daily for the first 5 days (no change on sixth day). On day 7, 50% of the erythrocytes were replaced, maintaining 5% hematocrit. Selection medium was changed every third day thereafter, and every other change was accompanied by 50% replacement of erythrocytes. Giemsa-stained thin smears were examined at each change for emergence of resistant parasites. Control cultures without antimalarial compound were maintained in parallel to monitor quality of culture conditions.

Sequencing of *pfndh2* and *pfcytB*. The entire open reading frames of *pfndh2* (type II NADH: ubiquinone oxidoreductase gene, PF3D7_0915000) and *pfcytB* (cytochrome B gene, mal_mito_3) were amplified from parasite DNA extracted by recommended methods (1). Amplicons were treated with Exo-SAP-IT (US Biochemical, Cleveland, OH) and sequenced on an ABI-3730xl DNA analyzer (Applied Biosystems, Foster City, CA) using internal primers listed in Table S4. DNA sequences were assembled and analyzed using Sequencher version 5.4 (GeneCodes, Ann Arbor, MI).

Dose response assays.

Two-fold serial dilutions of drug in complete media were made across a 96 plate (1.2 mL wells; ThermoScientific, Asheville, NC). In a fresh 96-well plate, 50 µL of each dilution was placed in wells 1–10, leaving wells 11 and 12 as drug-free controls wells for each row (2). Cultures containing at least 70% ring stage parasites were diluted to 1% parasitemia in 1% hematocrit solution and 150 µL was plated atop the drugs to be tested. Plates were incubated at 37°C for 72 hours in an atmosphere of 90% nitrogen, 5% carbon dioxide, and 5% oxygen. After 72 hours, plates were frozen at -20°C until processing. Plates to be processed were thawed at 37°C and 95 µL of SYBR Green I (Molecular Probes, ThermoScientific) lysis buffer (20 mM Tris, 5 mM EDTA, 0.008% saponin w/v, 0.08% Triton X v/v) (3) was added to each well. Plates were incubated at room temperature 1 – 2 hours in the dark, then read on a FluoStar (BMG Labtech Inc, Durham, NC) plate reader at excitation 490 nM/emission 540 nM. Half-maximal effective concentrations were determined using the variable slope sigmoidal function feature from Prism version 7 (GraphPad Software Inc., La Jolla, CA). EC_{50} data are the result of 3 – 7 independent experiments and reported ± standard deviation.

Gene copy number determinations

Parasite populations were expanded with or without ETC inhibitor to 5% parasitemia (predominantly trophozoites and schizonts) in 10 ml culture volumes. For each copy number determination, at least three independent pellets were

collected from saponin-lysed cells (0.15%, Sigma-Aldrich, St Louis MO) followed by detergent removal with three CM washes. DNA was extracted by the QIAGEN DNeasy Blood and Tissue kit (QIAGEN, Louisville, KY) following the manufacturer's protocol except that DNA was eluted with 50 µL molecular grade water (Takara Bio USA Inc., Mountain View, CA). DNA concentrations were approximated by a ND-1000 Spectrophotometer (NanoDrop Technologies). Primers for the control single copy *P. falciparum* serine tRNA synthetase gene (*pfserRS*, PF3D7_1216000) were designed and verified by BLAST search to be unique in the *P. falciparum* genome (Table S4). Primers to *pfcytB* and *pfdhod* were designed to amplify a unique 122 – 142 base pair product similar in size to the *pfserRS* amplicon. Each primer set was subjected to a temperature gradient PCR reaction to determine optimum temperature. The initial qPCR reaction for efficiency used 25 ng, 2.5 ng, and 0.25 ng of 106/1 DNA. The results were graphed as log dilution plotted against Ct value for each dilution. Efficiency was calculated as $10^{(1/\text{slope of the line})}$ -1, acceptable amplification efficiency was between 80 – 120% (4). Primer pair efficiencies were calculated to be 81% (*pfdhod*), 83% (*pfserRS*), 85% (*pfcytB*), 92% (*pfcoxI*), and 99% *pfcoxIII*). All qPCR reactions were carried out with an IQ5 Multicolor Realtime PCR Detection System (Bio-Rad; Hercules, CA) using 96-well plates and 25 µL reaction volumes (12.5 µL of 2× SensiFAST SYBR No-ROX master mix (Bioline; Taunton, MA), 400 nM of each primer, and 25 ng DNA in molecular grade water). After initial denaturation at 95°C for 7 minutes, amplifications were performed by 40 cycles of (a) denaturation for 20 seconds at 95°C, (b) annealing for 20 seconds at 58.5°C, and

(c) extension for 20 seconds at 68°C. At least three independent DNA extractions were performed from each clone and three technical triplicate wells were run with each DNA extraction. Copy numbers were calculated as $2^{\Delta Ct}$ (4), where. $\Delta Ct = (Ct_H - Ct_E)$ and subscripts H and E refer to the mean of technical replicates for the single-copy reference (*pfserRS*) and experimental subject gene (*pfcoxI, pfcoxIII, pfcytB* or *pfdhod)*, respectively. Statistical analysis was performed using the GraphPad Prism 7 software (GraphPad Software Inc., La Jolla, CA).

Microsatellite typing

Microsatellite typing was performed using primers and methods as described (5). Polymerase chain reactions (25 µL volumes) were performed in 96-well plates with 1 µL template DNA (20 ng/µL), 200 nM forward primer, 200 nM reverse primer with the 5' fluorescent label 6-FAM, 12.5 µL 2× MyTaq Mix (Takara Bio USA Inc., Mountain View, CA and 10.5 µL molecular grade water. Initial denaturation was for 2 minutes at 94°C, and amplification was by 42 cycles of denaturation for 20 seconds at 94°C, annealing for 10 seconds at 45°C plus 10 seconds at 42°C, and extension at 60°C for 30 seconds; after the 42 cycles, a final extension was performed at 60°C for 5 minutes. To each well in an optical 96-well plate, 0.1 µL PCR product was added to 9.7 µL of Hi-Di Formamide and 0.2 µL GeneScan 500XL ROX Size Standard (both products from Life Technologies Corporation, now ThermoFisher Scientific, Asheville, NC). The PCR products were analyzed by capillary electrophoresis on a ABI-3730xl DNA Analyzer (Applied Biosystems, now ThermoFisher Scientific, Asheville, NC).

Base-pair length for the highest peak was used to determine microsatellite length using GeneMapper v 4.1 software (ThermoFisher, Asheville, NC).

AutoDock calculations for molecular modeling studies

The *P. falciparum* bc1 complex (Pfbc1) and yeast bc1 sequences are homologous and highly similar in the sequence of the Q_o pocket (6). We used the yeast bc1 crystal structure, PDB ID 1KB9 and 4PD4, for the ligand docking studies. We remodeled the ScCytB Q_o pocket by making amino acid replacements ScCytB L275F, M295V, F296L, and I299L (corresponding to the residues at PfCytB positions 264, 284, 285, and 288) in the unbound PDB ID 1KB9, to resemble the PfCytB Q_o pocket; this pocket was used for the remainder of the studies. Compounds were docked in the predicted PfCytB Q_o pocket using the Lamarckian genetic algorithm to minimize both steric clashes and docking energies, then to redock the molecules. Lowest root-mean-square deviation (RMSD) docking to the protein model determined the choice of ligand orientation and position.

In all cases, a grid box size of 60 × 60 × 60 points with a grid-spacing of 0.375 Å was centered on the ligand to cover the whole pocket. We generated thirty docked structures (i.e. 30 runs) using genetic algorithm searches and applied a default protocol, with an initial population of 50 randomly placed individuals, a maximum number of 2.5×10^5 energy evaluations, and a maximum number of 2.7×10^4 generations. A mutation rate of 0.02 and a crossover of 0.8 were used.

Results differing by less than 1.0 Å in positional RMSD were clustered together. Estimated energies of free binding and inhibition constants were generated by the AutoDock software package. Figure S4 identifies the individual atoms of each drug structure for which the docking results, binding energies, and calculations are summarized in Table S3.

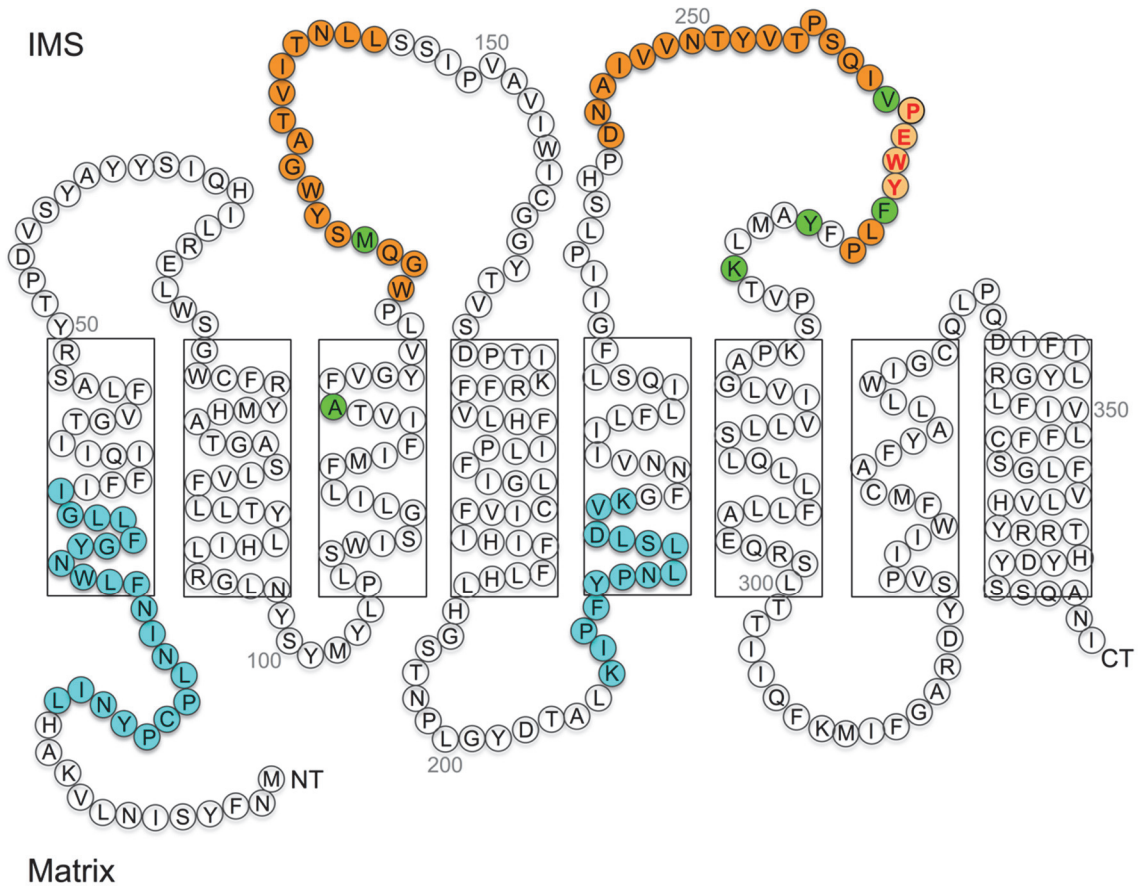

Figure S1. *Plasmodium falciparum* cytochrome B (PfCytB) primary structure with the predicted transmembrane domains (wildtype sequence). Green-filled circles identify residues that have known mutations in or near the Q_o site selected by drug pressure. The Q_o (orange fill) and Q_i (cyan fill) sites are located at opposite sides of the mitochondrial inner membrane. Residues in red font identify the catalytic PEWY motif. IMS, intermembrane space; NT, N-terminus; CT, C-terminus.

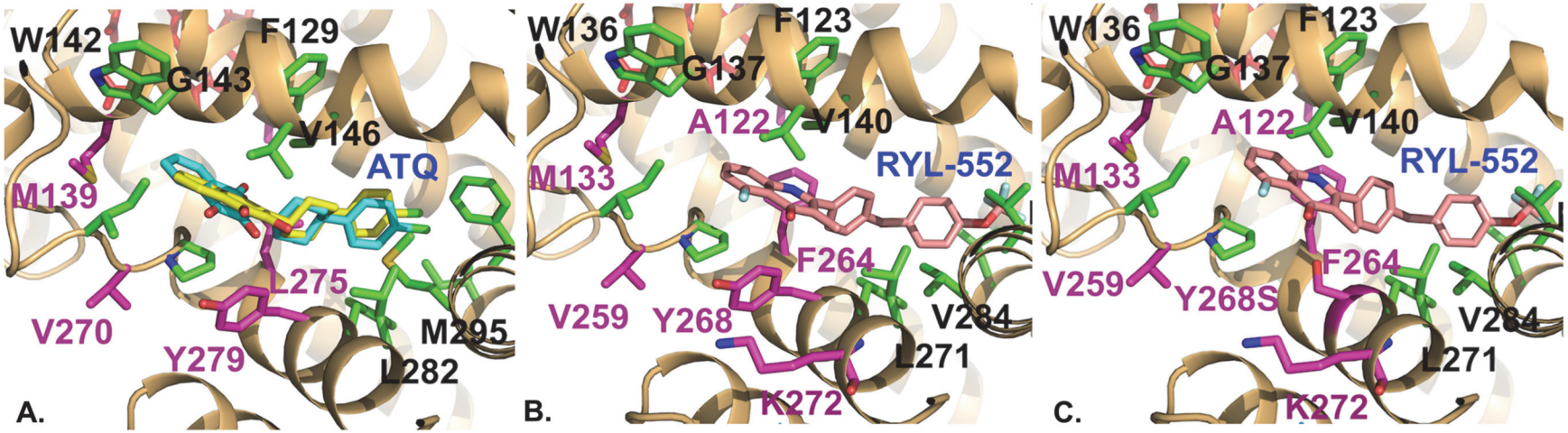

Figure S2. Locations of ATQ bound to *Saccharomyces cerevisiae* CytB (ScCytB) compared with docked RYL-552 in PfCytB. (A) Structural alignment showing the position of ATQ (yellow) in the solved ATQ-ScCytB crystal structure (PDB ID 4PD4) relative to the predicted position for ATQ (cyan) docked in the unbound yeast enzyme (PDB ID 1KB9). Residues are numbered according to the *S. cerevisiae* sequence. (B, C) Predicted docking of RYL-552 (pale red) is in nearly the same location in wildtype PfCytB as in the Y268S mutant. Residues subject to known mutations (magenta) and ATQ binding residues (green) are highlighted.

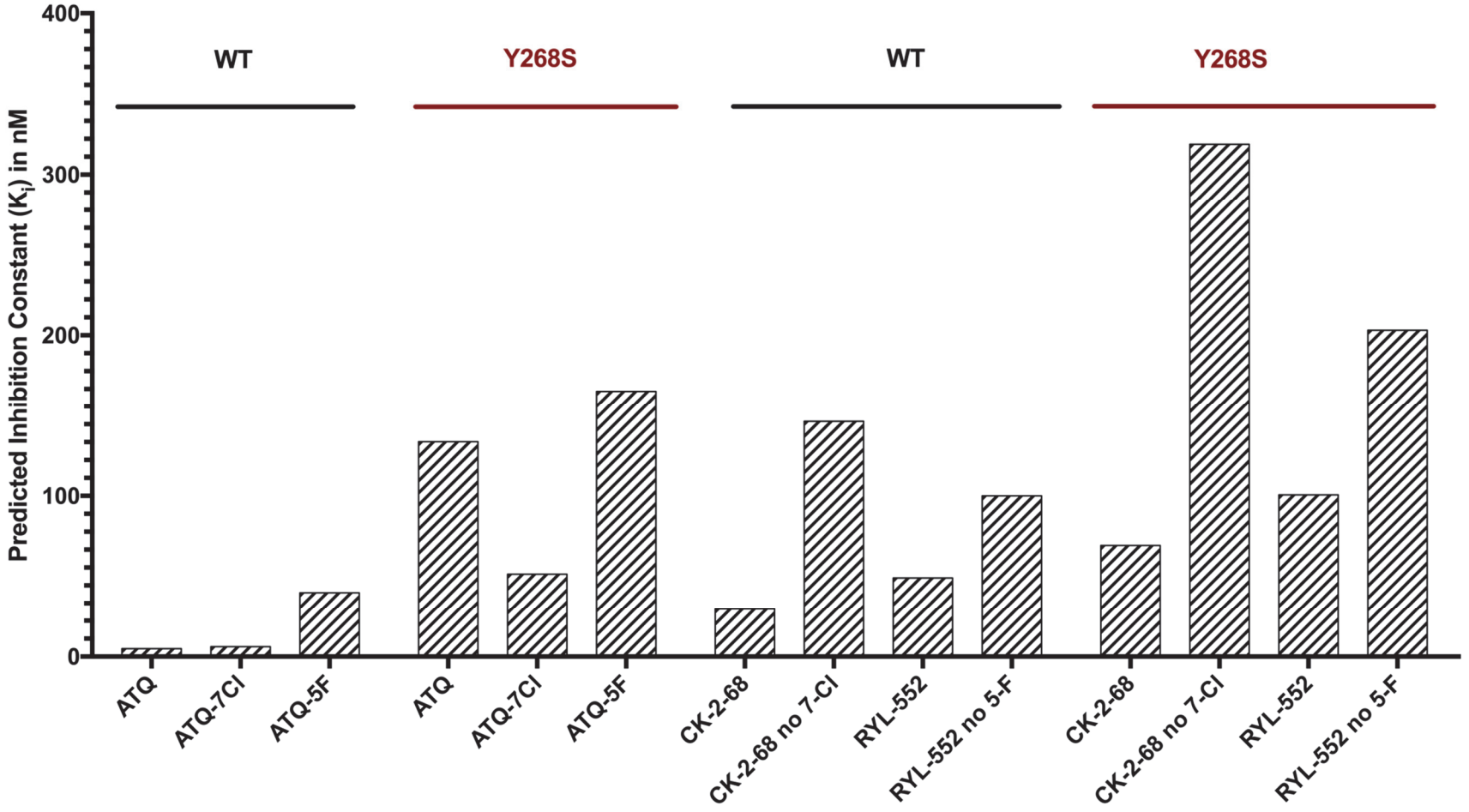

Figure S3. Predicted inhibition constants for ATQ, CK-2-68, RYL-552 and their derivatives, with or without halogen substituents. AutoDock calculations were performed with the wildtype (WT) and mutant (Y268S) models of PfCytB.

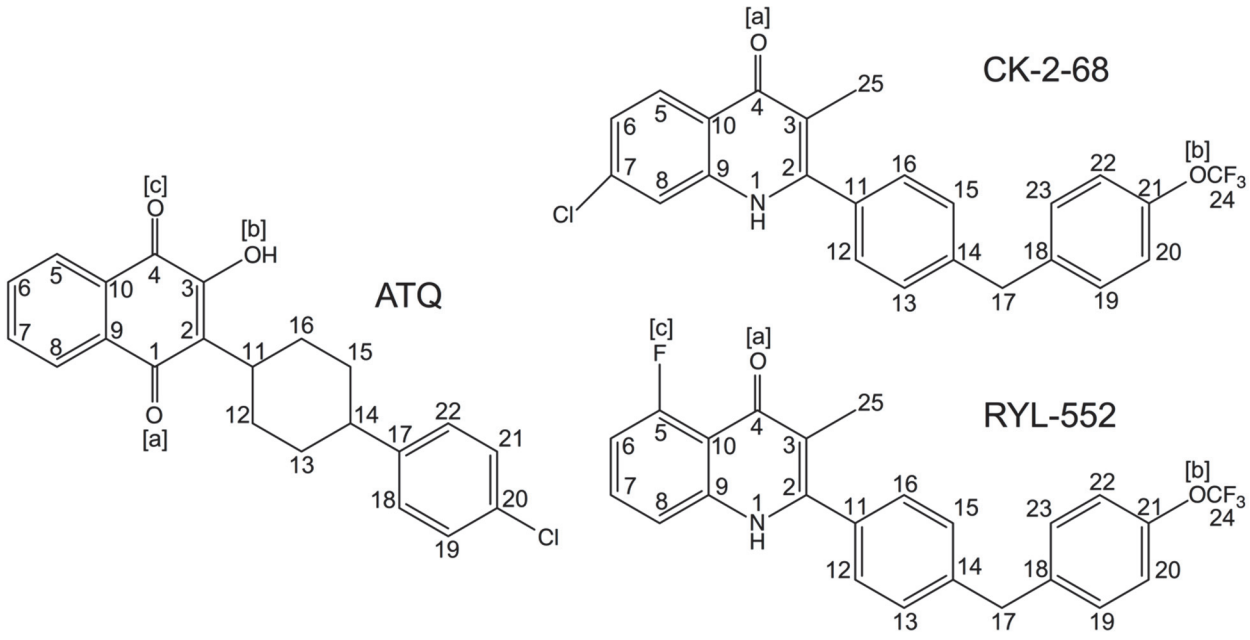

Figure S4. ETC inhibitor structures showing the atom numbers listed in Table S3.

Table S1. Microsatellite sizes (base pairs) in the genomes of Dd2, 106/1, and selected clones.

	Microsatellite Markers											
Clone	C3M64	C3M54	C4M30	B5M3	TAA81	TAA87	PE14D	TA127	C9108	401780	1451458	2549455
Dd2 lineage												
Dd2	174	235	165	123	121	106	114	132	114	80	-	179
DA-3H6^{M133I}	174	235	165	-	-	106	114	132	113	80	203	179
DA-4^{K272R}	174	235	165	-	121	106	114	132	113	80	203	178
DR-4H5^{F264L}	174	235	165	123	121	107	115	132	114	80	203	179
106/1 lineage												
106/1	107	259	179	121	115	103	114	116	114	80	198	174
6A-4F12^{Y268S}	107	259	173	121	115	103	114	116	114	80	198	175
6C-2A7^{A122T}	107	259	179	121	115	103	114	116	114	80	198	174
6R-3H8^{V259L}	107	-	173	121	-	103	114	115	114	80	198	174
6R-4E5^{A122T}	107	259	173	120	115	103	114	116	114	80	198	175

Table S2. *pfcoxI, pfcoxIII, pfcytB,* and *pfdhod* copy number determinations.

Line	*pfcoxI*	*pfcoxIII*	*pfcytB*	*pfdhod*
HB3	38 ± 5	43 ± 3	35 ± 2	1
3D7	31 ± 3	54 ± 10	41 ± 2	1
Dd2	31 ± 1	40 ± 11	34 ± 6	1
DR-4H5^{F264L}	20 ± 7	30 ± 1	26 ± 3	1
DA-3H6^{M133I}	24 ± 4	42 ± 8	48 ± 10	1
DA-4^{K272R}	20 ± 6	29 ± 2	28 ± 2	1
106/1	21 ± 3	31 ± 3	25 ± 2	1
6R-3H8^{V259L}	20 ± 2	30 ± 4	27 ± 1	1
6R-4E5^{A122T}	28 ± 5	42 ± 5	27 ± 3	1
6C-2A7^{A122T}	20 ± 6	38 ± 7	33 ± 5	1
6A-4F12^{Y268S}	22 ± 7	30 ± 7	33 ± 3	1
Group Mean	**26 ±6**	**33 ± 7**	**38 ± 8**	**1**

Copy numbers (mean ± standard deviation) are relative to the single-copy serine tRNA synthase gene, *pfserRS*. The *pfcoxI* and *pfcoxIII* genes encoding two subunits of Complex IV are transcribed in opposite directions, whereas *pfcoxIII* and *pfcytB* transcription are in the same direction (7).

Table S3. Summary table of docking results, listed using the atom identifications shown in Figure S4.

ATQ docking into WT PfCytB model

Estimated Free Energy of Binding [=(1)+(2)+(3)-(4)] = -11.34 kcal/mol
Estimated Inhibition Constant, Ki = 4.84 nM [Temperature = 298.15 K]

(1) Final Intermolecular Energy = -11.94 kcal/mol
vdW + Hbond + desolv Energy = -11.97 kcal/mol
Electrostatic Energy = +0.03 kcal/mol
(2) Final Total Internal Energy = -0.89 kcal/mol
(3) Torsional Free Energy = +0.60 kcal/mol
(4) Unbound System's Energy [=(2)] = -0.89 kcal/mol

		vdW	Elec	q	sum
WT-Y268	ATQ-all atoms	-9.32	0.04	0.003	-11.917
	quinone-ring	-5.31	0.03	-0.037	-5.317
	remainder	-6.65	0.01	0.04	-6.6

	PDB coordinates						
	x	y	z	vdW	Elec	q	RMS
C1	-50.729	25.2	10.35	-0.28	-0.01	0.194	1.287
O [a]	-51.944	25.563	10.424	-0.24	0.01	-0.287	1.287
C2	-50.001	25.127	9.149	-0.38	0	0.045	1.287
O [b]	-47.93	24.671	8	-0.42	0.01	-0.187	1.287
C3	-48.667	24.742	9.158	-0.15	-0.01	0.169	1.287
C4	-48.07	24.406	10.346	-0.26	-0.03	0.221	1.287
C5	-48.162	24.141	12.761	-0.59	0	0.003	1.287
C6	-48.864	24.193	13.957	-0.61	0	0	1.287
O [c]	-46.879	24.045	10.32	-0.45	0.07	-0.284	1.287
C7	-50.188	24.588	13.917	-0.56	0	0	1.287
C8	-50.802	24.916	12.724	-0.47	0	0.003	1.287
C9	-50.099	24.858	11.54	-0.43	0	0.042	1.287
C10	-48.776	24.471	11.546	-0.47	-0.01	0.044	1.287
C11	-49.82	26.646	7.114	-0.62	0	0.005	1.287
C12	-50.527	25.475	7.807	-0.47	0	0.028	1.287
C13	-50.527	24.169	7.03	-0.42	0	0.005	1.287
C14	-51.018	24.384	5.62	-0.44	0	0.005	1.287
C15	-50.032	25.353	5.002	-0.54	0	0.022	1.287
C16	-50.233	26.716	5.659	-0.65	0	0.005	1.287
C17	-50.285	25.408	3.556	-0.48	0	-0.022	1.287
C18	-51.6	25.615	3.157	-0.39	0	-0.003	1.287
C19	-51.913	25.668	1.808	-0.4	0	0.011	1.287
C20	-50.885	25.515	0.886	-0.4	0	0.058	1.287
C21	-49.561	25.308	1.29	-0.52	0	0.011	1.287
C22	-49.254	25.245	2.643	-0.57	0	-0.003	1.287
Cl	-51.298	25.597	-0.849	-0.75	0.01	-0.082	1.287

ATQ docking into Y268S PfCytB model

Estimated Free Energy of Binding [=(1)+(2)+(3)-(4)] = -9.38 kcal/mol
Estimated Inhibition Constant, Ki = 133.64 nM [Temperature = 298.15 K]

(1) Final Intermolecular Energy = -9.97 kcal/mol
vdW + Hbond + desolv Energy = -9.97 kcal/mol
Electrostatic Energy = -0.00 kcal/mol
(2) Final Total Internal Energy = -0.96 kcal/mol
(3) Torsional Free Energy = +0.60 kcal/mol
(4) Unbound System's Energy [=(2)] = -0.96 kcal/mol

		vdW	Elec	q	sum
Y268S mutant	ATQ-all atoms	-7.6	0.02	0.008	-7.572
	quinone-ring	-4.32	0.02	-0.037	-4.337
	remainder	-5.66	0	0.04	-5.62

	PDB coordinates						
	x	y	z	vdW	Elec	q	RMS
C1	-49.847	24.768	8.102	-0.26	-0.05	0.194	1.457
O [a]	-49.152	24.199	7.204	-0.3	0.11	-0.287	1.457
C2	-50.921	25.636	7.835	-0.34	0	0.045	1.457
O [b]	-52.704	27.045	8.644	-0.25	0	-0.187	1.457
C3	-51.644	26.2	8.877	-0.23	0	0.169	1.457
C4	-51.288	25.925	10.172	-0.19	0	0.221	1.457
C5	-49.871	24.777	11.78	-0.47	0	0.003	1.457
C6	-48.807	23.934	12.067	-0.46	0	0	1.457
O [c]	-51.943	26.462	11.085	-0.17	-0.01	-0.284	1.457
C7	-48.11	23.38	11.009	-0.48	0	0	1.457
C8	-48.454	23.662	9.701	-0.42	0	0.003	1.457
C9	-49.509	24.503	9.423	-0.39	-0.02	0.042	1.457
C10	-50.226	25.068	10.456	-0.36	-0.01	0.044	1.457
C11	-50.32	26.387	5.478	-0.48	0	0.005	1.457
C12	-51.41	26	6.484	-0.4	0	0.028	1.457
C13	-52.299	24.842	6.059	-0.37	0	0.005	1.457
C14	-52.849	25.069	4.673	-0.37	0	0.005	1.457
C15	-51.633	25.145	3.773	-0.4	0	0.022	1.457
C16	-50.897	26.446	4.08	-0.6	0	0.005	1.457
C17	-52.089	25.098	2.378	-0.34	0	-0.022	1.457
C18	-53.409	24.723	2.162	-0.32	0	-0.003	1.457
C19	-53.905	24.648	0.869	-0.36	0	0.011	1.457
C20	-53.053	24.961	-0.183	-0.41	0	0.058	1.457
C21	-51.724	25.339	0.037	-0.47	0	0.011	1.457
C22	-51.228	25.403	1.333	-0.44	0	-0.003	1.457
Cl	-53.705	24.873	-1.842	-0.7	0	-0.082	1.457

0.05 - 0.10 vDW increase with mutation
0.10 - 0.15 vDW increase
0.15 - 0.20 vDW increase
more than 0.20 vDW increase
vDW decrease (more negative)

CK-2-68 docking into WT PfCytB model

Estimated Free Energy of Binding [=(1)+(2)+(3)-(4)] = -10.27 kcal/mol
Estimated Inhibition Constant, Ki = 29.64 nM [Temperature = 298.15 K]

(1) Final Intermolecular Energy = -11.76 kcal/mol
vdW + Hbond + desolv Energy = -11.75 kcal/mol
Electrostatic Energy = -0.01 kcal/mol
(2) Final Total Internal Energy = -0.95 kcal/mol
(3) Torsional Free Energy = +1.49 kcal/mol
(4) Unbound System's Energy [=(2)] = -0.95 kcal/mol

		vdW	Elec	q	sum
WT-Y268	ATQ-all atoms	-11.42	0.05	-0.222	-11.592
	quinone-ring	-4.68	0.06	-0.282	-4.902
	remainder	-6.35	-0.01	0.019	-6.341

PDB coordinates

Atom	x	y	z	vdW	Elec	q	RMS
N1	-49.873	25.31	7.92	-0.17	0.06	-0.354	6.888\par
C2	-50.964	25.679	7.165	-0.45	0	0.052	6.888\par
C3	-52.157	26.04	7.687	-0.45	0	0.017	6.888\par
C4	-52.276	26.05	9.186	-0.32	0.01	0.192	6.888\par
O [a]	-53.284	26.461	9.749	-0.23	-0.02	-0.287	6.888\par
C5	-51.23	25.411	11.369	-0.42	0	0.019	6.888\par
C6	-50.153	24.907	12.097	-0.48	0	0.025	6.888\par
C7	-48.986	24.529	11.434	-0.48	-0.01	0.036	6.888\par
Cl	-47.657	23.907	12.336	-0.89	0.06	-0.084	6.888\par
C8	-48.884	24.651	10.047	-0.42	-0.03	0.054	6.888\par
C9	-49.966	25.14	9.307	-0.37	-0.01	0.048	6.888\par
C10	-51.134	25.527	9.979	-0.39	0	0.041	6.888\par
C11	-50.763	25.626	5.705	-0.43	0	-0.009	6.888\par
C12	-50.548	26.814	4.996	-0.54	0	0.014	6.888\par
C13	-50.331	26.78	3.62	-0.44	0	0.008	6.888\par
C14	-50.298	25.559	2.933	-0.38	0	-0.054	6.888\par
C15	-50.542	24.374	3.639	-0.35	0	0.008	6.888\par
C16	-50.783	24.407	5.015	-0.36	0	0.014	6.888\par
C17	-49.945	25.525	1.464	-0.51	0.01	0.077	6.888\par
C18	-51.154	25.251	0.599	-0.43	0	-0.054	6.888\par
C19	-51.406	26.049	-0.529	-0.47	0	0.01	6.888\par
C20	-52.46	25.752	-1.39	-0.54	0	0.037	6.888\par
C21	-53.301	24.679	-1.098	-0.43	0	0.078	6.888\par
C22	-53.076	23.885	0.027	-0.31	0	0.037	6.888\par
C23	-52.004	24.172	0.874	-0.33	0	0.01	6.888\par
C24	-53.908	23.24	-2.797	-0.13	0.03	0.53	6.888\par
O [b]	-54.297	24.333	-1.97	-0.35	-0.02	-0.255	6.888\par
F1	-52.616	22.91	-2.578	-0.11	-0.01	-0.144	6.888\par
F2	-54.672	22.169	-2.487	-0.06	-0.01	-0.144	6.888\par
F3	-54.08	23.516	-4.109	-0.18	-0.01	-0.144	6.888\par
C25	-53.374	26.453	6.909	-0.46	0	0.055	6.888\par

CK-2-68 docking into Y268S PfCytB model

Estimated Free Energy of Binding [=(1)+(2)+(3)-(4)] = -9.77 kcal/mol
Estimated Inhibition Constant, Ki = 69.15 nM [Temperature = 298.15 K]

(1) Final Intermolecular Energy = -11.26 kcal/mol
vdW + Hbond + desolv Energy = -11.26 kcal/mol
Electrostatic Energy = -0.00 kcal/mol
(2) Final Total Internal Energy = -0.84 kcal/mol
(3) Torsional Free Energy = +1.49 kcal/mol
(4) Unbound System's Energy [=(2)] = -0.84 kcal/mol

		vdW	Elec	q	sum
Y268S mutant	ATQ-all atoms	-10.98	0.06	-0.222	-11.142
	quinone-ring	-4.16	0.06	-0.282	-4.382
	remainder	-6.5	0	0.019	-6.481

PDB coordinates

Atom	x	y	z	vdW	Elec	q	RMS
N1	-49.848	24.974	7.43	-0.17	0.08	-0.354	6.967\par
C2	-50.953	25.308	6.68	-0.39	-0.01	0.052	6.967\par
C3	-52.156	25.63	7.207	-0.37	0	0.017	6.967\par
C4	-52.27	25.634	8.706	-0.26	0	0.192	6.967\par
O [a]	-53.288	26.011	9.273	-0.14	-0.01	-0.287	6.967\par
C5	-51.195	25.027	10.884	-0.36	0	0.019	6.967\par
C6	-50.1	24.558	11.607	-0.45	0	0.025	6.967\par
C7	-48.923	24.219	10.94	-0.46	-0.02	0.036	6.967\par
Cl	-47.571	23.639	11.836	-0.8	0.07	-0.084	6.967\par
C8	-48.83	24.345	9.553	-0.41	-0.04	0.054	6.967\par
C9	-49.93	24.8	8.817	-0.35	-0.01	0.048	6.967\par
C10	-51.108	25.148	9.494	-0.32	0	0.041	6.967\par
C11	-50.757	25.263	5.219	-0.4	0	-0.009	6.967\par
C12	-50.546	26.455	4.515	-0.53	0	0.014	6.967\par
C13	-50.333	26.43	3.138	-0.46	0	0.008	6.967\par
C14	-50.299	25.212	2.445	-0.38	0	-0.054	6.967\par
C15	-50.538	24.023	3.146	-0.34	0	0.008	6.967\par
C16	-50.776	24.048	4.523	-0.36	0	0.014	6.967\par
C17	-49.95	25.187	0.975	-0.48	0	0.077	6.967\par
C18	-51.161	24.919	0.111	-0.43	0	-0.054	6.967\par
C19	-51.796	25.978	-0.558	-0.52	0	0.01	6.967\par
C20	-52.853	25.736	-1.432	-0.56	0	0.037	6.967\par
C21	-53.315	24.433	-1.604	-0.42	0	0.078	6.967\par
C22	-52.709	23.369	-0.934	-0.33	0	0.037	6.967\par
C23	-51.634	23.613	-0.077	-0.35	0	0.01	6.967\par
C24	-54.14	22.887	-3.106	-0.15	0.03	0.53	6.967\par
O [b]	-54.306	24.171	-2.51	-0.42	-0.02	-0.255	6.967\par
F1	-54.506	21.911	-2.247	-0.05	-0.01	-0.144	6.967\par
F2	-54.939	22.809	-4.194	-0.22	-0.01	-0.144	6.967\par
F3	-52.865	22.678	-3.502	-0.1	0.01	-0.144	6.967\par
C25	-53.389	26.004	6.433	-0.39	0	0.055	6.967\par

0.05 - 0.10 vDW increase with mutation
0.10 - 0.15 vDW increase
0.15 - 0.20 vDW increase
more than 0.20 vDW increase
vDW decrease (more negative)

RYL-552 docking into WT PfCytB

Estimated Free Energy of Binding [=(1)+(2)+(3)-(4)] = -9.97 kcal/mol
Estimated Inhibition Constant, Ki = 48.89 nM [Temperature = 298.15 K]

(1) Final Intermolecular Energy = -11.47 kcal/mol
vdW + Hbond + desolv Energy = -11.44 kcal/mol
Electrostatic Energy = -0.03 kcal/mol
(2) Final Total Internal Energy = -0.93 kcal/mol
(3) Torsional Free Energy = +1.49 kcal/mol
(4) Unbound System's Energy [=(2)] = -0.93 kcal/mol

		vdW	Elec	q	sum
WT-Y268	ATQ-all atoms	-10.94	-0.02	-0.033	-10.993
	quinone-ring	-3.98	-0.03	-0.056	-4.066
	remainder	-6.96	0.01	0.023	-6.927

	PDB coordinates						
	x	y	z	vdW	Elec	q	RMS
N1	-50.018	24.828	7.21	-0.23	0.04	-0.246	2.872\par
C2	-51.183	25.359	6.581	-0.33	0	0.08	2.872\par
C3	-52.254	26.039	7.349	-0.37	0	0.035	2.872\par
C4	-52.12	26.107	8.794	-0.26	0.01	0.197	2.872\par
O [a]	-53.004	26.64	9.451	-0.23	-0.02	-0.287	2.872\par
C5	-50.709	25.575	10.823	-0.33	-0.01	0.145	2.872\par
F [c]	-51.639	26.148	11.567	-0.12	0	-0.204	2.872\par
C6	-49.569	25.024	11.423	-0.47	-0.01	0.03	2.872\par
C7	-48.564	24.399	10.64	-0.48	0	0.004	2.872\par
C8	-48.722	24.339	9.248	-0.46	-0.01	0.019	2.872\par
C9	-49.876	24.896	8.668	-0.35	-0.02	0.087	2.872\par
C10	-50.883	25.521	9.439	-0.35	-0.01	0.084	2.872\par
C11	-51.121	25.268	5.098	-0.38	0	0.018	2.872\par
C12	-50.36	26.267	4.498	-0.48	0	0.002	2.872\par
C13	-50.207	26.278	3.132	-0.49	0	0	2.872\par
C14	-50.779	25.258	2.413	-0.45	0	0	2.872\par
C15	-51.504	24.229	3.003	-0.32	0	0	2.872\par
C16	-51.669	24.234	4.373	-0.33	0	0.002	2.872\par
C17	-50.519	25.334	0.946	-0.55	0	0	2.872\par
C18	-51.56	25.046	-0.09	-0.47	0	0	2.872\par
C19	-52.3	26.118	-0.569	-0.55	0	0.002	2.872\par
C20	-53.311	25.893	-1.489	-0.61	0	0.025	2.872\par
C21	-53.595	24.598	-1.925	-0.43	0.01	0.102	2.872\par
C22	-52.86	23.533	-1.416	-0.36	0	0.025	2.872\par
C23	-51.843	23.751	-0.495	-0.37	0	0.002	2.872\par
C24	-54.637	23.275	-3.778	-0.22	0.03	0.53	2.872\par
O [b]	-54.634	24.39	-2.881	-0.42	-0.02	-0.253	2.872\par
F2	-55.295	23.65	-4.886	-0.28	0	-0.144	2.872\par
F3	-55.272	22.217	-3.234	-0.12	-0.01	-0.144	2.872\par
F4	-53.375	22.832	-4.017	-0.13	0	-0.144	2.872\par
C25	-53.48	26.647	6.696	-0.5	0	0.036	2.872\par

RYL-552 docking into Y268S WT PfCytB

Estimated Free Energy of Binding [=(1)+(2)+(3)-(4)] = -9.55 kcal/mol
Estimated Inhibition Constant, Ki = 100.61 nM [Temperature = 298.15 K]

(1) Final Intermolecular Energy = -11.04 kcal/mol
vdW + Hbond + desolv Energy = -10.98 kcal/mol
Electrostatic Energy = -0.05 kcal/mol
(2) Final Total Internal Energy = -0.90 kcal/mol
(3) Torsional Free Energy = +1.49 kcal/mol
(4) Unbound System's Energy [=(2)] = -0.90 kcal/mol

		vdW	Elec	q	sum
Y268S mutant	ATQ-all atoms	-10.58	-0.05	-0.033	-10.663
	quinone-ring	-3.57	-0.06	-0.056	-3.686
	remainder	-7.01	0.01	0.023	-6.977

	PDB coordinates						
	x	y	z	vdW	Elec	q	RMS
N1	-49.806	24.415	7.304	-0.21	0.06	-0.246	2.866\par
C2	-50.852	25.153	6.675	-0.32	-0.01	0.08	2.866\par
C3	-51.771	26.029	7.442	-0.31	0	0.035	2.866\par
C4	-51.619	26.08	8.886	-0.21	-0.01	0.197	2.866\par
O1	-52.383	26.775	9.541	-0.17	-0.01	-0.287	2.866\par
C5	-50.326	25.303	10.914	-0.29	-0.02	0.145	2.866\par
F1	-51.127	26.047	11.657	-0.08	0	-0.204	2.866\par
C6	-49.309	24.549	11.514	-0.46	-0.01	0.03	2.866\par
C7	-48.444	23.74	10.732	-0.47	0	0.004	2.866\par
C8	-48.617	23.702	9.341	-0.42	-0.02	0.019	2.866\par
C9	-49.647	24.464	8.761	-0.33	-0.03	0.087	2.866\par
C10	-50.514	25.274	9.53	-0.3	-0.01	0.084	2.866\par
C11	-50.816	25.042	5.193	-0.38	0	0.018	2.866\par
C12	-50.578	26.247	4.537	-0.52	0	0.002	2.866\par
C13	-50.497	26.272	3.166	-0.49	0	0	2.866\par
C14	-50.608	25.08	2.495	-0.44	0	0	2.866\par
C15	-50.801	23.863	3.137	-0.34	0	0	2.866\par
C16	-50.898	23.847	4.514	-0.36	0	0.002	2.866\par
C17	-50.463	25.205	1.015	-0.55	0	0	2.866\par
C18	-51.607	25.06	0.061	-0.46	0	0	2.866\par
C19	-52.061	23.775	-0.202	-0.36	0	0.002	2.866\par
C20	-53.082	23.585	-1.119	-0.35	0	0.025	2.866\par
C21	-53.645	24.674	-1.785	-0.44	0.01	0.102	2.866\par
C22	-53.161	25.952	-1.529	-0.61	0	0.025	2.866\par
C23	-52.139	26.15	-0.609	-0.54	0	0.002	2.866\par
C24	-54.642	23.437	-3.721	-0.19	0.03	0.53	2.866\par
O [b]	-54.702	24.457	-2.719	-0.38	-0.02	-0.253	2.866\par
F2	-55.055	23.98	-4.876	-0.29	0	-0.144	2.866\par
F3	-55.454	22.405	-3.412	-0.15	-0.01	-0.144	2.866\par
F4	-53.401	22.887	-3.789	-0.16	0	-0.144	2.866\par
C25	-52.862	26.855	6.788	-0.39	0	0.036	2.866\par

0.05 - 0.10 vDW increase with mutation
0.10 - 0.15 vDW increase
0.15 - 0.20 vDW increase
more than 0.20 vDW increase
vDW decrease (more negative)

Table S4. Primers for PCR copy number assay and sequencing of *pfcoxI*, *pfcoxIII*, *pfcytB*, *pfdhod*, *pfndh2*, and *pfserRS*.

Primer Name	Type	Target	Sequence (5' – 3')
coxI-F	qPCR	*pfcoxI*	GTTTGTAGAGATGCAAAACATTCTCC
coxI-R	qPCR	*pfcoxI*	CTAGTGCATCATGTATGACTGC
coxIII-F	qPCR	*pfcoxIII*	CTGCAGCAGAATTTGGTGG
coxIII-R	qPCR	*pfcoxIII*	AGCGACTCCAGATACTAATAAACC
cytB-F	qPCR	*pfcytB*	GCACACTTAATAAATTACCCATGTCC
cytB-R	qPCR	*pfcytB*	ATGCATATGAAACATCTGGTGTATATCG
dhodh-F	qPCR	*pfdhod*	TTAAGTAAACATATTGTAGGTGTCAGTATAGG
dhodh-R	qPCR	*pfdhod*	ACATTAATAGCTATATAATCAGCGTATCTTCC
serRS-F	qPCR	*pfserRS*	GGTTCAGGTTTAGCCGTAGG
serRS-R	qPCR	*pfserRS*	CTTGTATGATGTCCTTGTTCATATATTTCC
cytochrome b-F1	Seq	*pfcytB*	AATTACCCATGTCCATTGAAC
cytochrome b-F2	Seq	*pfcytB*	TATTGTAACTGCTTTCGTTGG
cytochrome b-F3	Seq	*pfcytB*	TTTGGAATTATACCTTTATCAC
cytochrome b-R1	Seq	*pfcytB*	CATCTGGTGTATATCGACTTG
cytochrome b-R2	Seq	*pfcytB*	TGCTACTGGAATAGAGGATAAC
cytochrome b-R3	Seq	*pfcytB*	GCATAAAATGGTAGAAAGTAC
pfNDH2-F1	Seq	*pfndh2*	ATGTTAGTAAAGTTCAGGAAATGTG
pfNDH2-F2	Seq	*pfndh2*	TCGAAGTATATCAAATGTACG
pfNDH2-F3	Seq	*pfndh2*	AGTTACCGCAGAATTTGC
pfNDH2-F4	Seq	*pfndh2*	GCAAATAATGCTATTCTAAAAG
pfNDH2-F5	Seq	*pfndh2*	CACAAAATGCTAAACAAGAAG
pfNDH2-R1	Seq	*pfndh2*	TAAAACCACCCCATCCTG
pfNDH2-R2	Seq	*pfndh2*	TTGGTAATAAATTATTTCCTCC
pfNDH2-R3	Seq	*pfndh2*	GTTCATGTAATAATTTTGGTTG
pfNDH2-R4	Seq	*pfndh2*	TATATGCTAATGATCCTTTCC
pfNDH2-R5	Seq	*pfndh2*	TCATTTGATGAAAGGACGCC

qPCR, quantitative PCR; Seq, sequencing primer.

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