Supplementary Figure 1

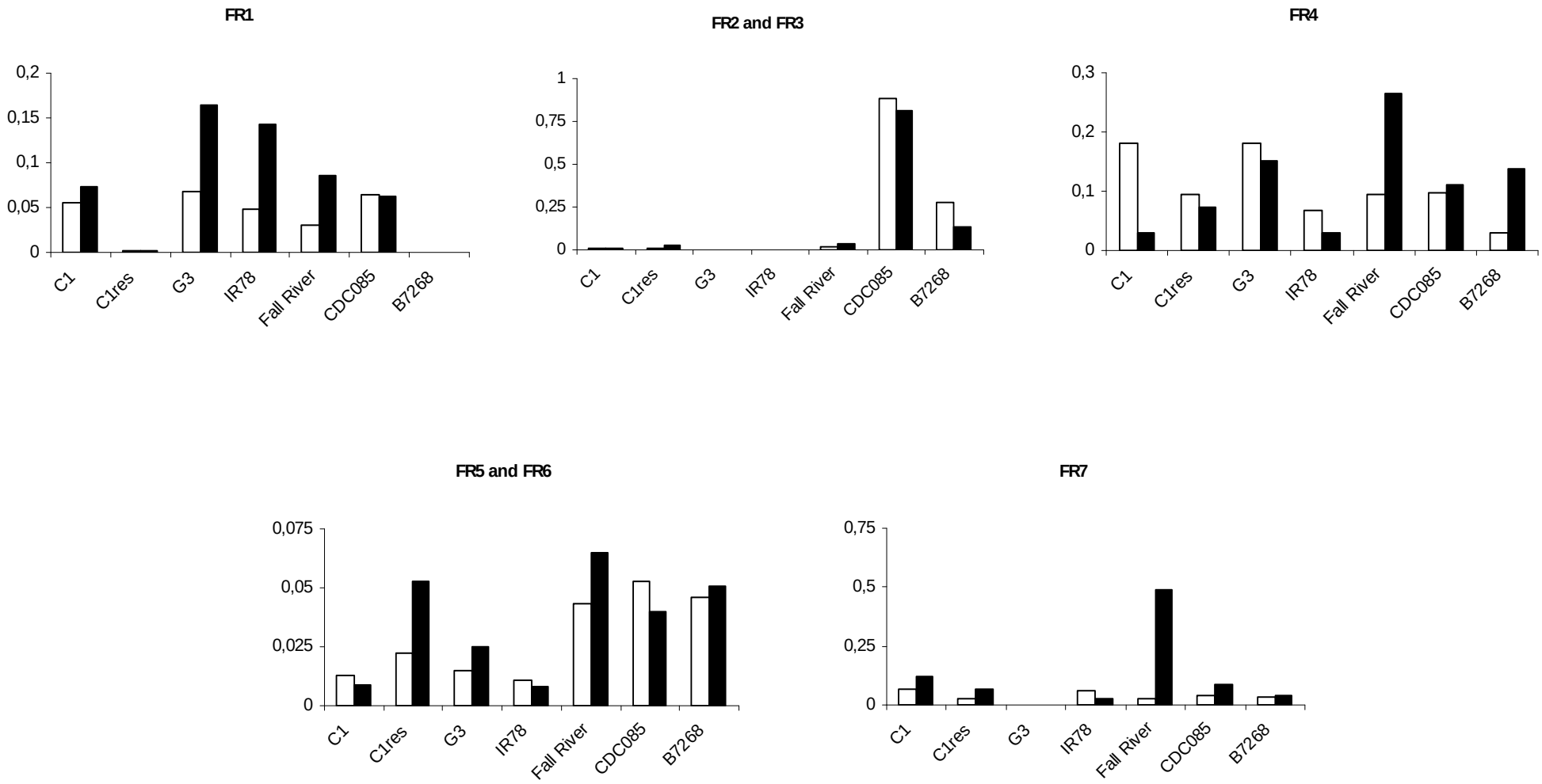


Figure Legend:
Expression of all FRs was measured at the mRNA level in strains C1, C1res, G3, IR78, Fall River, CDC085, and B7268. Flavin reductases 2 and 3 and FR5 and 6 were measured with one primer pair each. Levels of mRNA (y-axis) are given as relative abundances (arbitrary units) as compared to cytosolic TrxR mRNA (1 = 100%). White bars: first experiment; black bars: second experiment

Discussion:

Since the FR1 antiserum cross-reacted with all FRs, we used quantitative mRNA analyses by RT-qPCR as an alternative strategy to identify the expressed FRs. The following strains were selected for the analysis: C1 and G3 as normally susceptible strains, C1res as a highly metronidazole-resistant cell line of C1 which totally lacks FR activity and expression at the protein level, IR78 which displays reduced expression levels of FRs, Fall River and CDC085 which displays elevated FR levels despite reduced FR activity, and B7268, a metronidazole-resistant isolate which lacks FR activity and expression (Figure 5B). Five primer pairs were designed to measure mRNA levels of all FRs (amplified fragments and primer sequences shown in Supplementary Table 2). Flavin reductases 2 and 3, as well as FR5 and 6 mRNA levels were assessed with one primer pair each; whereas FR 1, 4, and 7 were each measured separately. The gene for cytoplasmic thioredoxin reductase (XM_001316888) was chosen as the internal standard because it is present as a single copy in the genome and is expressed at similar levels in all strains studied by us so far (Leitsch et al., 2012). Two separate experiments, including RNA isolation, reverse transcription, and qPCR were conducted in duplicate with each strain. Relative abundances of FR mRNAs with standard error of the mean of duplicate measure points are given below. Strikingly, all isoforms of FR were transcribed in all strains tested, with the notable exception of FR1, whose mRNA was virtually absent from C1res and B7268. This result is consistent with the total absence of FR activity in these strains (Figure 1). Further, FR7 mRNA was barely detectable in G3. This isoform was also exceptional in the sense that its mRNA levels in strain Fall River varied almost 15-fold between the two experiments (Figure 5), far exceeding the variation observed for other mRNA, including the internal standard, *i.e*. TrxR mRNA, which was highly reproducible between the two separate experiments and the different strains (see below). This suggests biological rather than technical variation of results. Flavin reductase 2 and 3 mRNAs were 10 to 30 times more abundant in CDC085 than in any other strain, with the exception of B7268 which likewise displayed very high levels. This result was seemingly consistent with the very high levels of alternative FR protein expressed in CDC085 (Figure 5B). However, as B7268 expresses high levels of FR2 and 3 mRNAs with no FR protein detectable in Figure 5B, qRT-PCR of FR mRNA levels does not appear to be an appropriate read-out to compare FR expression between *T*. *vaginalis* strains.

Materials and Methods:
Quantitative mRNA analysis by RT-qPCR

Sets of primers for amplification of all FR genes in RT-qPCR experiments were designed as shown below. The gene for cytosolic thioredoxin reductase (XM_001316888) was chosen as internal standard because this gene is expressed in all strains analyzed at a comparable rate (Leitsch *et al*., 2012). All primer pairs were tested on genomic DNA and cDNA from all five strains before RT-qPCR was performed (not

shown). RNA was isolated from *T*. *vaginalis* cultures using the GeneJET™ RNA purification kit (Fermentas) according to the manufactuter's protocol for "mammalian cultered cells". RNA isolations from one strain for two separate experiments were never conducted on the same day. Contaminating DNA was digested using DNAse I (Fermentas) and first-strand cDNA synthesis was performed using the RevertAid™ premium first strand cDNA synthesis kit (Fermentas). Quantitative mRNA analysis was performed in a Roche Light Cycler 480 using the software provided and applying the following program: 95°C, 15 min as denaturation step, and [95°C, 15 sec; 50°C 30 sec; 72°C, 20 sec] × 45 for amplification. Reactions were prepared with FIREPol® EvaGreen® qPCR Mix Plus (Solis Biodyne). Cytosolic thioredoxin reductase (TrxR) mRNA was chosen as standard and analyzed in dilutions of 50, 5, 0.5 and 0.05 ng RNA in volumes of 20 µl each. Only experiments in which the slope between TrxR mRNA dilutions ranged between 3 and 3.4 (3.2 being optimal) were considered. The other analytes were measured applying RNA at a concentration of 50 ng/20 µl. Relative abundances of FR mRNAs were calculated in relation to the abundance of TrxR mRNA.

Data Source:

Relative abundancies of FR mRNA in relation to TrxR mRNA (± SEM).

FR1

C1	C1res	G3	IR78	Fall River	CDC085	B7268
0,055 ± 0,0037	0,0012 ± 0,00002	0,067 ± 0,0084	0,048 ± 0,0083	0,031 ± 0,002	0,064 ± 0,005	0,00022 ± 0,00003
0,073 ± 0,002	0,0015 ± 0,00003	0,164 ± 0,004	0,143 ± 0,006	0,085 ± 0,0003	0,063 ± 0,002	0,0004 ± 0,000001

FR4

C1	C1res	G3	IR78	Fall River	CDC085	B7268
0,18 ± 0,0057	0,095 ± 0,0017	0,182 ± 0,009	0,068 ± 0,022	0,095	0,097 ± 0,01	0,03 ± 0,007
0,03 ± 0,00063	0,074 ± 0,011	0,151 ± 0,0026	0,03 ± 0,0033	0,264 ± 0,0112	0,11 ± 0,068	0,137 ± 0,0093

FR7

C1	C1res	G3	IR78	Fall River	CDC085	B7268
0,07 ± 0,07	0,028 ± 0,0006	0,00016 ± 0,00016	0,06 ± 0,0002	0,03 ± 0,0002	0,041 ± 0,0013	0,032 ± 0,0003
0,123 ± 0,0343	0,068 ± 0,0029	0,00064 ± 0,00003	0,027 ± 0,001	0,486 ± 0,119	0,09 ± 0,029	0,041 ± 0,0039

FR2 und FR3

C1	C1res	G3	IR78	Fall River	CDC085	B7268
0,0058 ± 0,01	0,013 ± 0,00014	0,00092 ± 0,00021	0,0015 ± 0,00012	0,018 ± 0,0026	0,883 ± 0,1	0,274 ± 0,0058
0,0045 ± 0,00094	0,028 ± 0,003	0,00096 ± 0,000003	0,0011 ± 0,00012	0,035 ± 0,00006	0,812 ± 0,023	0,133 ± 0,0019

FR5 und FR6

C1	C1res	G3	IR78	Fall River	CDC085	B7268
0,013 ± 0,0006	0,022 ± 0,0016	0,015 ± 0,0018	0,011 ± 0,001	0,043 ± 0,0015	0,053 ± 0,0023	0,046 ± 0,0093
0,0087 ± 0,0005	0,053 ± 0,007	0,025 ± 0,00009	0,0078 ± 0,0015	0,065 ± 0,0008	0,04 ± 0,0027	0,051 ± 0,0009

Ct values obtained for TrxR mRNA (internal standard) in two separate experiments in duplicates (four measurements in total):

	Ct TrxR mRNA
C1	17,23 ± 1,3
C1res	18,1 ± 0,64
G3	16,01 ± 0,27
IR78	16,39 ± 0,15
Fall River	16,42 ± 0,39
CDC085	18,01± 0,25
B7268	17,42 ± 0,34

Primers used:

	Sequence	Amplified fragment
FR1 forward	CTTGATGTCTCACATGCACG	77 bp
FR1 reverse	TTGGCTGAATCAGCGAAACG	
FR2 + 3 forward	CAGAGCGTGTATTTTCA	118 bp
FR2 + 3 reverse	AAGAACATTGGGGCAAC	
FR4 forward	GGTCGCACATCCTGATCC	93 bp
FR4 reverse	CTAACCTCATTTCCTGCTG	
FR5 + 6 forward	GTTCTTTTCCTCGTCGC	84 bp
FR5 + 6 reverse	CTCTAAGGCTGCCTTTGC	
FR7 forward	CAGATTCGCTGATTCAGCTA	104 bp
FR7 reverse	CCTTTGTTGTAAGTGTTCTGTC	

Primers amplify the following stretches of the respective genes (in red; primer sequences in bold):

FR1:

CTTGATGTCTCACATGCACGTCTTGATCTTAGTTGCTCACCCAGACCCAACACACAAGGC**AACATCATTC**
CGTTTCGCTGATTCAGCCAAAGCTGCCCTTGAAGGTGCTGGTCACGAAGTTCGTTACGTCAACTTAATGG
AAGCTGGCTTCAATCAAACAGCTTCTCAGGATGACTTCAAGACAGTCAACAAGGAACCATTCACATACCC
AGGAAACCAAATGGTTCCAGACAATCTTGTTGATACAATCAAGGTTCAACAAGAAAACCTCAAGTGGTCT
ACACACGTTCTTGTTTTCGCACCACTTTGGTTCGGCCGTCTCCCAGCCTGCTTCTATGCTTACACAGAGC
GTGTTCTTTCTGTTGGATTCGCTTGGGACTTCCAGCACACATTTGACAAGGCTTTCCTTGCTGGCCGTAA
GGTTTCCATCATCGTTTCCGCTGGTTCACCACCAATGTACTTCGATCCAAAGAATGGAAACGGTCTCGAC
GCTTTCCTTTGGTCTGCTATGTACGGTTTCAACTATGCTGGTTTCACAATTCTCCGTTCTCTTGGTATCT
TCGGCGCTAATTCTCCAAAGAGAATTGCTCAACAACCAGAACTCCAGCAGAAGTTCAATGAGAAGTTCCT
CCAGCTTGACCAGTGGAAGACAATCGGCACAGAAAAGCTCTACTCCAAGATTGCAGAACTCGAGGAAGTC
AATTCAGAAAACATCCTCTTGTAAAATGTGTATTCAATCAAATTAGATAAAGATTCGAATATAGATTAAT
GAAATT

FR2:

ATGTCTAAAATGCACGTCTTGATCTTAGTTGCTCATCCAGATCCAACTCACAAAGCAACTGCCTTCCGCT
TCGCAGATTCTGCCAAAGCTGCTCTTGAAGAAGCTGGTCATGAAGTTAGATATGTTAACTTAATGGAAGC

TGGATTTGATGTCGTTGCCTCTATGAATGATTTCAAGAGAGTCGAAATGGATCCATTTGAGTATCCTGCA
AACCAGGCCATTCCTGACAATCTCATTGATACAATCAAAGTTCAACAGGAGAATCTCAAGTGGTCAACAC
ATGTTCTCATCTTTGCTCCACTTTGGTTTGGCCGTCTTCCAGCATGCTTTTACTCTTTCT**CAGAGCGTGT**
ATTTTCATTTGGATTCGCCTACGATGGTGAGAGTCCTCTTCATCAAAATGTCTTGAAGGGCCGCAAAGTT
GCCATCATTGTTTCAGCAGGA**GTTGCCCCAATGTTCTT**CGATCCAAAGAAAGGTAACGGTCTTGATGCTT
ACTTATGGTCTGCTATGTATGGCTTCAACTATGCAGGCTTCACAATCCTTCGTTCTCTTGGAATTTATGG
TGCAAATTCTCCAAGGAGGAAAGCAATGCAACCAGAACTTCAGAAGAAGATCAATGAAAAACTTCTCAAA
CTTGATCAATGGAAGACAGTTGATGGTGAAAGAATCTATTCCAATTTCATTAATCTTGAACAGGTCTTCC
CTGAAAACATTCTTAAAGAATGA

FR3:

ATGTCTAAAATGCACGTCTTGATCTTAGTTGCTCATCCAGATCCAACACACAAAGCAACTGCATTCCGCT
TTGCTGACTCAGCAAAGGCAGCTCTTGAAGCAGCAGGCCATGAGGTTAGATATGTTAACTTGATTGAAGA
AGGTTTTGATGTCAATGCTTCTGCTAAGGATTTCAAGAAGGTCGAAACAGATCCATTCAACTACTCAAAT
AATCAGGGTATTCCTGATAATCTCATTGATACAATCAAAGTTCAGCAAGCAAACCTGTTATGGTCAACAC
ATGTTCTCGTCTTTGCTCCACTTTGGTTTGGCCGTCTCCCGGCATGCTTCTACTCCTTTA**CAGAGCGTGT**
ATTTTCATTTGGTTTCAGCTATGATGGCGAGAAAGTATTACATCAAGGAGTCCTTGCTGGCCGCAAAGTT
GCGATCATTGTTTCAACAGGT**GTTGCCCCAATGTTCTT**TGATGTAAAGAAAGGCAACAGTCTTGATGCAT
ACTTATGGTCTGCAATGTATGCTTTCAACTACGCTGGATTCACAATCCTTCGTTCTCTTGACGTTTACGG
CGCAAATTCTCCAAAGAGAAAAGCAATGCAGCCAGAACTTCAAAAGAAGATCAATGAAAAACTCCTCAAG
CTTGATCAATGGAAGACAATTGATGGAGAAAGGATTTACTCCAATTTTGCTTATCTTGAAGAAGTCACAC
CAGAAAATATTCTTAAGGAGTGA

FR4:

ATGCGTGTCTTAATATT**GGTCGCACATCCTGATCC**AACTCATGAAGCTACATCATTTAGGTTCGCTTTGT
CTGCAAAGG**CAGCACTTGAGGCAGCAGGAAATGAGGTTAG**ATATGTAAATTTAATTGCAGAAGGCTTTGA
CAAAGCTGGCTCCGCTGGAGACTTCAAGCAATTGAAAACAGATCCATTTACATACGATGGAAACCAAATG
ATCAAAGATAATCTGATCGATTGCATCAAAGTTCAACAAGAAAATTTACTTTGGTCAACTCATGTAGTCA
TCTTCGCACCTCTTTGGTTTGGAAGACTTCCATCATGCTTTTACTGCTACACAGAGCGTGTCCTTTCTTT
CCCATTTGCTTATGATTACGAAAACTACATAGAGAAAGGATTTCTTAAAGGCAGAAAAGTAACATCAATC
ATATCAACAGGAGTAGATTCGTCATTCTTTGATCCAAAGAACGGAAATACATTGGATGCTTATGCATGGT
CAGCAATGTATGGATTCGATTACTCTGGATTTACGATACTCCGTTCTCTAGGCATATATAGCGCAGTTGT
TCCAGAGATGATCAAACAACAGCCTGAACTCATGAAAAAGATTAACGAAAAAATCGTTAAGCTCGATCAG
TGGAAGACTGTTGGAAGCAACAAATGCTTTAACGCATTATGCTCTCTCGAAGAAGTTACCCCCGATAACA
TTCTCGTTAGTGAATAA

FR5:

ATGCGC**GTTCTTTTCCTCGTCGC**ACACGTTGACCCAACACACAAGGCAACAGCATTCAGATTCGCAGACT
CA**GCAAAGGCAGCCTTAGAG**AAGGCAGGCCACGAAGTCAGGTACGTTAACCTCGTCGAGTCTGGTTTCGA
CAGAACATTGACAACAAAGGACTTCAAGAAGATCCCAGATGGCACATTCTCAATCTTCGCAGTCACAGGC
CAAGACAACTTGGTTGACGAGATCAAAGTCCAACAAGAGAACCTCAAGTGGGCAACACACGTTGTCATCT
TCGCCCCAATGTGGTACGGCAGATTCCCATCATGCGTCTACGCATACACAGAGAGAGTTCTCAACGTTCC
ATTTGCTTGGGACTTCGAACACATGCTCGACAAGGGCTACCTCGCTGGCAAGAAGGTCACATCCGTCATC
TCCACAGGCAGTGCACCAATGTTCTTCGATCCTAAGGAAGGCAACGGCCTCGACGCATACACATGGAGCG
CCCTCTACGCTTTCAACTACTCCGGCTTCACAATCCTCAGATCAATCGGCATCCACGGTGCAAACTCACC
AAAGAGAATTGCCATGCAACCAGAACTCCAGCAGAAGCTCAACGAGAAGCTCTTGAACCTCGACAACTGG
AAGGTCATCACAGACAAGAAGTTTGTTCCACTCGCAACACTCGACCAAATCACTGAACCTGAAAACTTGA
TTCAATAA

FR6:

ATGCGT**GTTCTTTTCCTCGTCGC**GCATGTTGATCCAACACACAAGGCAACAGCTTTCAGATTTGCTGACT
CC**GCAAAGGCAGCCTTAGAG**AAGGCTGGCCATGAAGTCAGATACGTTAACCTCGTTGAGTCTGGCTTTGA
CAGAACATTGACAGCCAAGGACTTCAAGAAGATCCCAGATGGCACATTCTCAATCTTCGCAGTTACAGGC
CAAGACAACTTGATTGATGAAATCAAGGTCCAGCAAGAGAATCTCAAGTGGGCAACACACGTTGTCATCT
TCGCCCCAATGTGGTACGGCAGATTCCCATCATGCGTCTATGCATATACAGAGAGAGTTCTCAACGTTCC
ATTTGCTTGGGACTTCGAGCACATGCTCGACAAGGGCTATCTCGCCGGCAAGAAGGTTACATCTGTAATC
TCTACAGGCAGTGCACCAATGTTCTTCGATCCTAAGGAAGGAAACGGCCTTGATGCTTACACATGGAGTG
CTCTCTACGCTTTCAACTACTCTGGCTTCACAATCCTCAGATCAATCGGTATTCATGGCGCAAACTCACC
AAAGAGAATTGCCATGCAGCCAGAACTTCAGCAGAAGCTCAACGATAAGCTTTTGAACCTCGATAACTGG
AAGGTCATCACAGACAAGAAGTTTGTTCCACTTGCCACACTTGACCAAATCACAGATCCAGAAAACTTGA
TCCAATAA

FR7:

ACTTTTAACAATGCGCGTTCTTTTCCTTGTCGCACACGTTGACCCAACACACAAAGCCACAGCATT**CAGA**
TTCGCTGATTCAGCTAAGAAAGCCTTAGAAGAGGCAGGACACGAAGTTAGATACGTAAATTTAGTTGAAT
CTGGTTTT**GACAGAACACTTACAACAAAGG**ACTTCAAGAAGGTTCCAGAAGGCCAATTCAATATCTTTGC
TCTTACAGGCCAAGAAAACTTAGTCGATGAAGTTAAGGTTCAGCAAGAAAACCTCAAATGGGCAACACAT
GTTGTCATCTTCACACCAATGTGGTATGGCAGATTTCCATCCTGCGTTTACGCATACACAGAGAGAGTTC
TCAACGTTCCATTCACCTGGGACTTTGAACATATGCTCGAGAAGGGCTATCTTGCCGGCAAGAAGGTCAC
ATCTATCATTTCTACAGGCAGTGCACCAATGTTCTTTGATCCAAAGCAAGGAAATGGCCTTGATGCCTAC

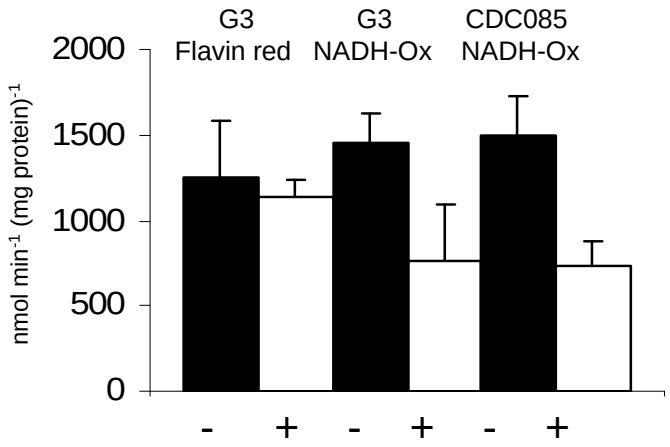
GCATGGAGCGCTCTTTACGCTTTCAACTATTCTGGCTTTACAATTCTTAGATCTATTGGTATCCATGGTG
CTAATTCTCCAAAGAGAATTGAACAACAGCCAGAACTTCAGAAGAAACTCAACCAACAACTTCTTAATCT
TGACAACTGGAAAGTAATCACAGATAAGAAGATGGGACCACTTGCTACATTAGATCAAATCACAGAACCA
GAAAACCTTATTCAATAA

Supplementary Figure 2

CTTG**ATG**TCTCACATGCACGTCTTGATCTTAGTTGCTCACCCAGACCCAACACACAAGGCAACATCATTCCGTTT
CGCTGATTCAGCCAAAGCTGCCCTTGAAGGTGCTGGTCACGAAGTTCGTTACGTCAACTTAATGGAAGCTGGCTT
CAATCAAACAGCTTCTCAGGATGACTTCAAGACAGTCAACAAGGAACCATTCACATACCCAGGAAACCAAATGGT
TCCAGACAATCTTGTTGATACAATCAAGGTTCAACAAGAAAACCTCAAGTGGTCTACACACGTTCTTGTTTTCGC
ACCACTTTGGTTCGGCCGTCTCCCAGCCTGCTTCTATGCTTACACAGAGCGTGTTCTTTCTGTTGGATTCGCTTG
GGACTTCCAGCACACATTTGACAAGGCTTTCCTTGCTGGCCGTAAGGTTTCCATCATCGTTTCCGCTGGTTCACC
ACCAATGTACTTCGATCCAAAGAATGGAAACGGTCTCGACGCTTTCCTTTGGTCTGCTATGTACGGTTTCAACTA
TGCTGGTTTCACAATTCTCCGTTCTCTTGGTATCTTCGGCGCTAATTCTCCAAAGAGAATTGCTCAACAACCAGA
ACTCCAGCAGAAGTTCAATGAGAAGTTCCTCCAGCTTGACCAGTGGAAGACAATCGGCACAGAAAAGCTCTACTC
CAAGATTGCAGAACTCGAGGAAGTCAATTCAGAAAACATCCTCTTG**TAA**AATGTGTATTCAATCAAATTAGATAA
AGATTCGAATATAGATTAATG

Structure of the FR1 gene in all strains tested. The start codon on the mRNA is preceded by four bases only (green background). The 3'UTR after the stop codon (in bold) is 47 bases long (light blue background). The T highlighted in red (T_{95}) is mutated to an A in strain LA1 without effect on the amino acid sequence. The G highlighted in dark blue (G_{609}) is mutated to a T in C1 (and C1res) and B7268, leading to an exchange of alanine to valine.

Supplementary Figure 3



NADH-oxidase activity was measured in *T*. *vaginalis* G3 (metronidazole-sensitive) and CDC085 (metronidazole-resistant), either untreated (-) or treated (+) with 200 µM metronidazole for two hours. Cells had been incubated in cysteine-free medium in order to guarantee elevated oxygen levels. For comparison, also flavin reductase activity was measured in G3, either in absence (-) or presence (+) of metronidazole (200 µM, 2h). All measurements were repeated at least twice. Bars indicate SEM.

Supplementary Table 1:
Proteins identified by RP-LC/MS/MS. Flavin reductase 1 is highlighted in red.

No.	Accession	Name	MW [kDa]	Meta Score	Peptides	SC [%]
1	gi|123428711	lactate dehydrogenase family protein [Trichomonas vaginalis G3]	37.1	756,61	12	35.7
2	gi|123425265	Clan MH, family M20, peptidase T-like metallopeptidase [Trichomonas vaginalis G3]	51.3	598,70	9	24.1
3	gi|123444869	actin [Trichomonas vaginalis]	40.7	416,77	7	21.9
4	gi|123473909	ribosomal protein L5 [Trichomonas vaginalis G3]	34.8	416,20	7	28.6
5	gi|123365845	Flavodoxin-like fold family protein [Trichomonas vaginalis G3]	27.0	333,38	6	34.3
6	gi|123439029	ribosomal protein [Trichomonas vaginalis G3]	28.4	282,39	4	25.5
7	gi|123431388	L-lactate dehydrogenase [Trichomonas vaginalis G3]	37.0	280,70	2	9.3
8	gi|123435358	Ser/Thr protein phosphatase [Trichomonas vaginalis G3]	35.1	275,98	3	11.4
9	gi|311303090	putative actin depolymerizing factor [Trichomonas vaginalis]	14.5	202,21	4	35.7
10	gi|123502962	dTDP-4-dehydrorhamnose 3,5-epimerase family protein [Trichomonas vaginalis G3]	38.9	132,10	2	7.2
11	gi|154413806	Serine/threonine protein phosphatase PP1-gamma catalytic subunit [Trichomonas vaginalis G3]	44.7	117,08	2	7.7
12	gi|123447599	hypothetical protein [Trichomonas vaginalis G3]	49,4	106,57	2	6,2

Abbreviations:
SC: Sequence coverage
Meta Score: calculated Score
MW: calculated molecular weight based on the database sequence

Supplementary Table 2:
Primers used for cloning of flavin reductases 1 – 7. Restriction sites are given in red letters, stop codons in blue letters, and 6 x His-tags in green letters. The mutation site in FR7 is given in bold against red background.

Flavin reductase 1 forward	TACGTACGCATATGTCTCACATGCACGTCTTGATC
Flavin reductase 1 reverse	TCATCCAGGAATTCTTAGTGATGGTGATGGTGATGCAAGAGGATGTTTTCTGAATTGACTTC
Flavin reductase 2 forward	TACGTACGCATATGTCTAAAATGCACGTCTTGATCTTAG
Flavin reductase 2 reverse	TCATCCAGCTCGAGTTAGTGATGGTGATGGTGATGTTCTTTAAGAATGTTTTCAGGGAAGACC
Flavin reductase 3 forward	TACGTACGCATATGTCTAAAATGCACGTCTTGATCTTAG
Flavin reductase 3 reverse	TCATCCAGCTCGAGTTAGTGATGGTGATGGTGATGCTCCTTAAGAATATTTTCTG GTGTGAC
Flavin reductase 4 forward	TACGTACGCATATGCGTGTCTTAATATTGGTCGCAC
Flavin reductase 4 reverse	TCATCCAGCTCGAGTTAGTGATGGTGATGGTGATGTTCACTAACGAGAATGTTATCGGG
Flavin reductase 5 forward	TACGTACGCATATGCGCGTTCTTTTCCTCGTCGC
Flavin reductase 5 reverse	TCATCCAGCTCGAGTTAGTGATGGTGATGGTGATGTTGAATCAAGTTTTCAGGTTCAGTG
Flavin reductase 6 forward	TACGTACGCATATGCGTGTTCTTTTCCTCGTCGCGC
Flavin reductase 6 reverse	TCATCCAGCTCGAGTTAGTGATGGTGATGGTGATGTTGGATCAAGTTTTCTGGATCTGTGATTTG
Flavin reductase 7 forward	TACGTACGGGATTCCGCGTTCTTTTCCTTGTCGCAC
Flavin reductase 7 reverse	TCATCCAGGAATTCTTAGTGATGGTGATGGTGATGTTGAATAAGGTTTTCTGGTTCTGTG
Flavin reductase 7 Mut fwd	CGTTCCATTCACCTGGGACTTTGAACA**C**ATGCTCGAGAAGGGCTATCTTGCCGGC
Flavin reductase 7 Mut rev	GCCGGCAAGATAGCCCTTCTCGAGCAT**G**TGTTCAAAGTCCCAGGTGAATGGAACG
Flavin reductase 7 forward 2	TACGTACGCATATGCGCGTTCTTTTCCTTGTCGCAC