Supplementary Table I

Gene Target	dsRNA Size	Oligonucleotide Sequences (5'-3')
BiP	95/115 nt* 246/266 nt*	CCGGCCAAGTGGAGATCATCC/GGATGATCTCCACTTGGCCGG CCTTATCGGGCGCAAGTTTGA/TCAAACTTGCGCCCGATAAGG
	823/847 nt**	CA*GGATCC*ACCAACACAGGAGATAAGGCAAAGG/ TC*GCATGC*CCTTTGCCTTATCTCCTGTGTTGGT
	1/760 nt***	CA*GGATCC*AATCATGTCCCCAGAGGAGATTTCCG/ TC*GCATGC*GAGCTGACGAATCTTCGGTATAC
α-Tubulin	114/168 nt* 1283/1307 nt*	GTTCAACACGTTCTTCTCGGAGACG/CGTCTCCGAGAAGAACGTGTTGAAC TCGAGAAGGACTACGAGGAGATCGG/CCGATCTCCTCGTAGTCCTTCTCGA
	1283/1307 nt**	CA*GGATCC*TCGAGAAGGACTACGAGGAGATCGG/ TC*GCATGC*CCGATCTCCTCGTAGTCCTTCTCGA
	1/1000 nt***	CACGGGATCCAGCACGACGGCCAGATGCCGTCC/ GTAT*GCATGC*GTAGGCGTCGTCCTCCTCCATGTC
µ1	177/201 nt* 1305/1329 nt*	CAAGCATACAGACTGTTATTTGGTG/CACCAAATAACAGTCTGTATGCTTG GTCACTGACTGGAATGGTTGTCATA/TATGACAACCATTCCAGTCAGTGAC
	1305/1329 nt**	CA*GGATCC*CAAGCATACAGACTGTTATTTGGTG/ GTAT*GCATGC*CACCAAATAACAGTCTGTATGCTTGTC
	1/1000 nt***	CACGGATCCATGATAAGCTCTCTGCTAATCATTCAC/ CCA*GCATGC*CTTGACAACTTGCTCATGAGGGCAATA
µ2	26/48 nt* 519/539 nt*	ATGATGTAGGTGAGCTTATCTTG/CAAGATAAGCTCACCTACATCAT CGTCGTTGAGTCTGTCAATGC/ GCATTGACAGACTCAACGACG
	519/539 nt**	GTT*GGATCC*ATGATGTAGGTGAGCTTATCTTG/ GGT*GCATGC*CAAGATAAGCTCACCTACATCAT
	1/1000 nt***	GTT*GGATCC*ATGATCAAGGCGGTCATTCTTTTGGAT/ GGT*GCATGC*CCACCTAATCTGATAAGTCCCCGCGCT
RdRP	74/96 nt* 1334/1354 nt*	GTGCCTTAGTCTTCAAAGTTATC/GATAACTTTGAAGACTAAGGCAC GCGTTCCGGATGATGTCTTTC/GAAAGACATCATCCGGAACGC
	85/107 nt**	CAC*GGATCC*TTCAAAGTTATCATAGTCATAAT/ CCA*GCATGC*ATTATGACTATGATAACTTTGAA
	1/3420 nt***	CAC*GGATCC*ATGTCTGGCCCCCACATCCACTCGCCT / CCA*GCATGC*GGCTACAGATTTAGAGTGGTAAATTAT
eGFP	101/125 nt* 264/288 nt*	AGAAGCGCGATCACATGGTCC/GGACCATGTGATCGCGCTTCT CCGGGGTGGTGCCCATCCTG/CAGGATGGGCACCACCCCGG
	641/665 nt**	CAC*GGATCC*AGAAGCGCGATCACATGGTCCTGCT/ CCT*GCATGC*AGCAGGACCATGTGATCGCGCTTCT
	1/1000 nt***	CAC*GGATCC*GTGAGCAAGGGCGAGGAGCTGTTCA/ CCT*GCATGC*GTGGTTGTCGGGCAGCAGCACGGGG
CWP2	101/125 nt* 264/288 nt*	GTGCTAATTGGAAGTCGAATAACTG/CAGTTATTCGACTTCCAATTAGCACC GTCGCTGTACCTTAATAATAATGAC/GTCATTATTATTAAGGTACAGCGAC
	824/848 nt**	CAC*GGATCC*AGAGGAAATGCAACATGCCAAACAG/ CCT*GCATGC*CTGTTTGGCATGTTGCATTTCCTCT
	1/1000 nt***	GACA*GGATCC*AATTGGAAGTCGAATAACTGGCTT/ GTA*GCATGC*TTTCCTCTGCGCATTGTGTGCACTG

*oligonucleotide sequence were designed by using siDirect sequences (http://genomics.jp/sidirect/) and the sRNA duplex produced exogenously by HiScribe RNAi Transcription Kit. **oligonucleotide sequence to produce endogenous siRNA: 10 µl of both strands were combined and incubated at 95°C in a 200-ml water bath. After 5 min, the water bath was allowed to gradually cool to room temperature over a period of 1 h. ***oligonucleotide sequence to produce endogenous dsRNA: oligonucleotides were used to amplify the 5' sequences of specific genes from genomic *Giardia* DNA using the High Fidelity Taq polymerase (Invitrogen). Restriction sites are denoted in italics. All oligonucleotides were designed to target the 5' sequence of the selected genes.

Supplementary figure 2

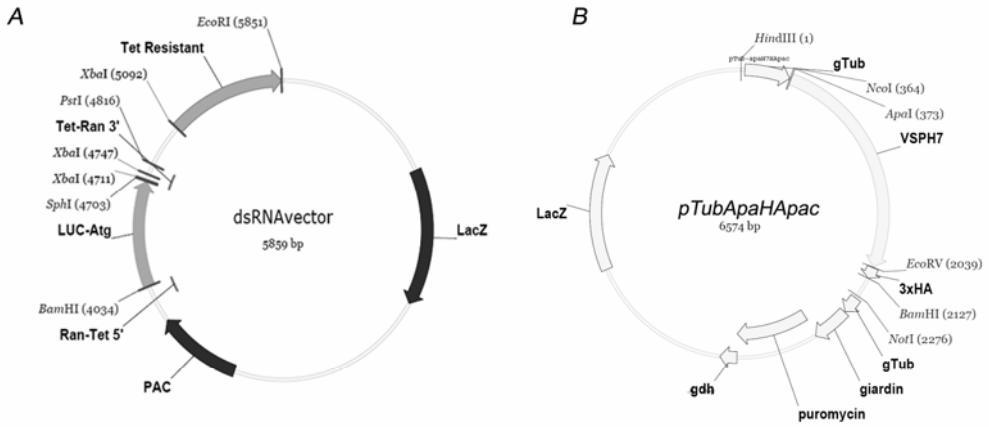

Figure S2: Giardia vectors. (A) dsRNA Tet-inducible vector. This vector was developed for inducible expression of double-stranded RNAs in Giardia. It contains opposing Giardia ran promoters with tetracycline (Tet) operator elements and is designed for insertion and double-stranded expression of PCR products. It also has a puromycin cassette under the control of an endogenous non-regulated gdh promoter. Gene targeted sequences were amplified by PCR from genomic Giardia DNA and introduced between opposing tetracycline-inducible Giardia ran promoters. To cotransfect trophozoites and to be able to select each vector, the puromycin acetyl transferase was exchanged for the neomycin acetyl transferase gene present in pdsRNA by using the QuikChange mutagenesis kit (Stratagene) (Touz et al., 2004). (B) The vector pTubApaHApac was engineered and constructed for constitutive over-expression of transgenes under the control of endogenous alpha-tubulin promoters. It has a puromycin cassette under the control of an endogenous non-regulated gdh promoter (Touz et al., 2003).