Supplementary Figure S1: Expression and purification of full length *Pf*Ddi1 gene

a

Full length *Pf*Ddi1


b

c

d

Cathepsin D substrate
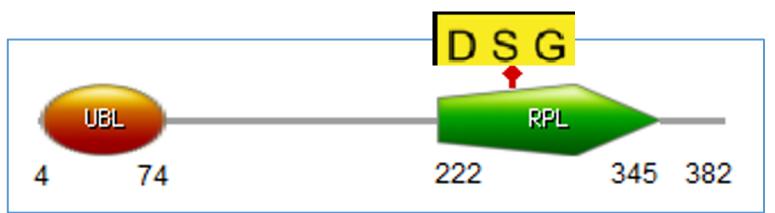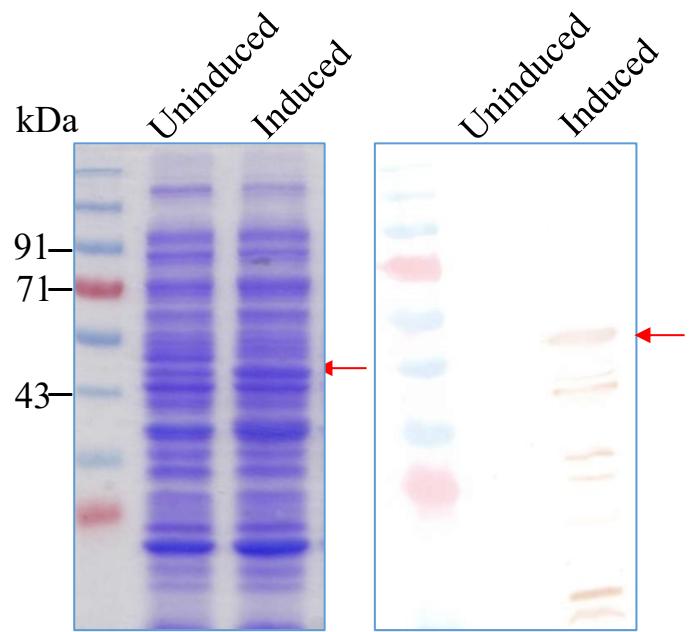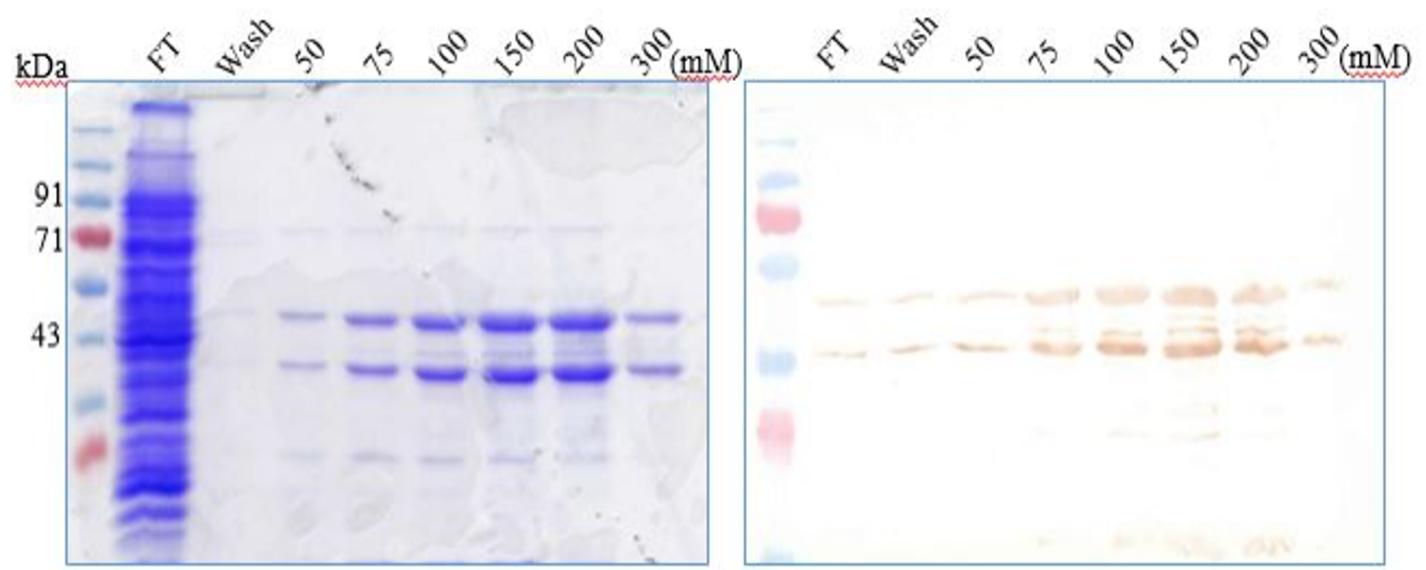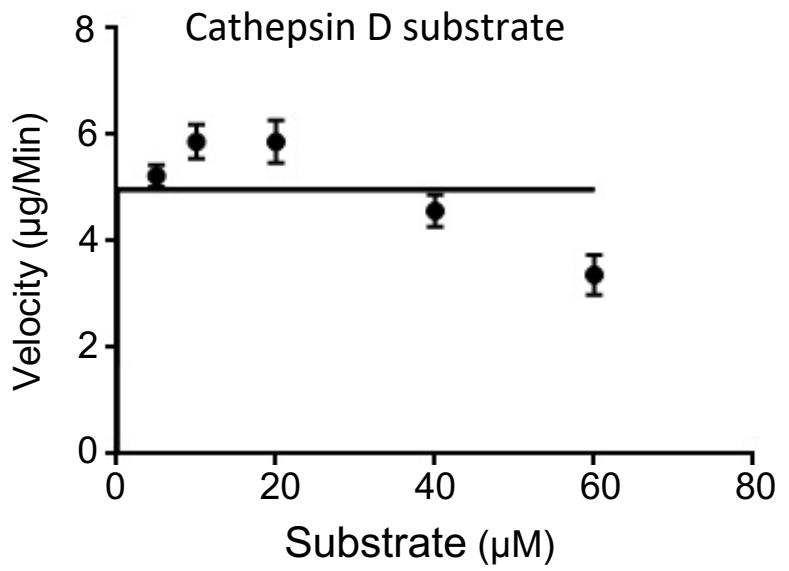
Supplementary Figure S2: LC-MS/MS proteome analysis of purified *Pf*Ddi1 fragments

~44 kDa *Pf*Ddi1

a

Sample Peptide reads

Accession	Description	Score	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI
PF3D7_14 09300	organism=Plasmodium_falciparum_3D7 | product=DNA damage-inducible protein 1, putative (DDI1) | location=Pf3D7_14_v3:363092-364240(+) | length=382 | sequence_SO=chromosome | SO=protein_coding	2072.85	75.92	1	48	48	691	382	43.8	5.06

Sample Peptide reads:
FIYENIYKNEINNnLALAQEHFPEAFGVVFMLYI PVEInKNTVHAFVDSGAQSSImSK

KISADImNDnVnNmSALNNILSTNNNVGNIGN IGNNLNNENVQNLLNNPAFK

LQDEPLYNAIFSQNLEEIK

FIYENIYKNEINnnLALAQEHFPEAFGVVFMLYI PVEInK

FIYENIYKNEINnnLALAQEHFPEAFGVVFMLYI PVEInKNTVHAFVDSGAQSSIMSK

~34 kDa *Pf*Ddi1

b

Sample Peptide reads

Accession	Description	Score	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI
PF3D7_140 9300	organism=Plasmodium_falciparum_3D7 | product=DNA damage-inducible protein 1, putative (DDI1) | location=Pf3D7_14_v3:363092-364240(+) | length=382 | sequence_SO=chromosome | SO=protein_coding	2200.58	73.56	1	46	46	700	382	43.8	5.06

Sample Peptide reads:
LQDEPLYNAIFSQNLEEIK

LQDEPLYnAIFSQNLEEIK

FIYENIYKNEINNNLALAqEHFPEAFGVVFMLYI PVEInKnTVHAFVDSGAQSSImSK

FIYENIYKnEInNNLALAQEHFPEAFGVVFMLYI PVEINK

KISADIMNDNVNNmSALNNILSTNNNVGNIG NIGNNLNNEnVQNLLNNPAFK

Supplementary Figure S3: Hydrolysis of substrates by the recombinant *Pf*Ddi1 enzyme
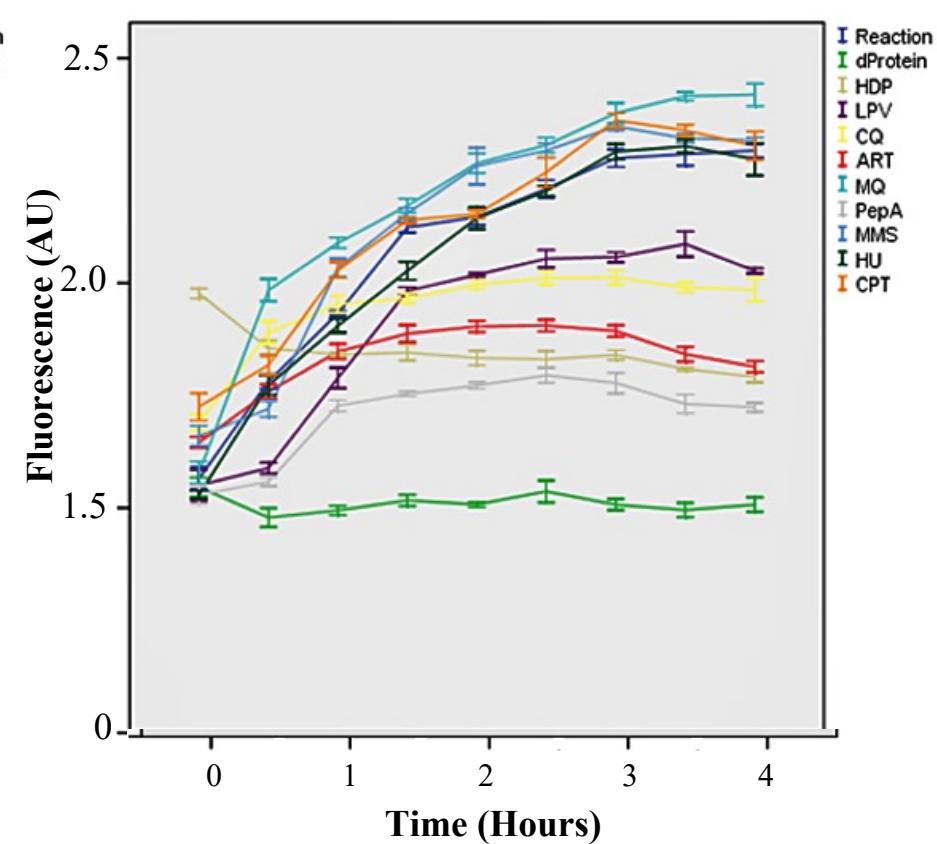
Supplementary Figure S4: Artemisinin treatment leads to increased recruitment of *Pf*Ddi1 into the nucleus
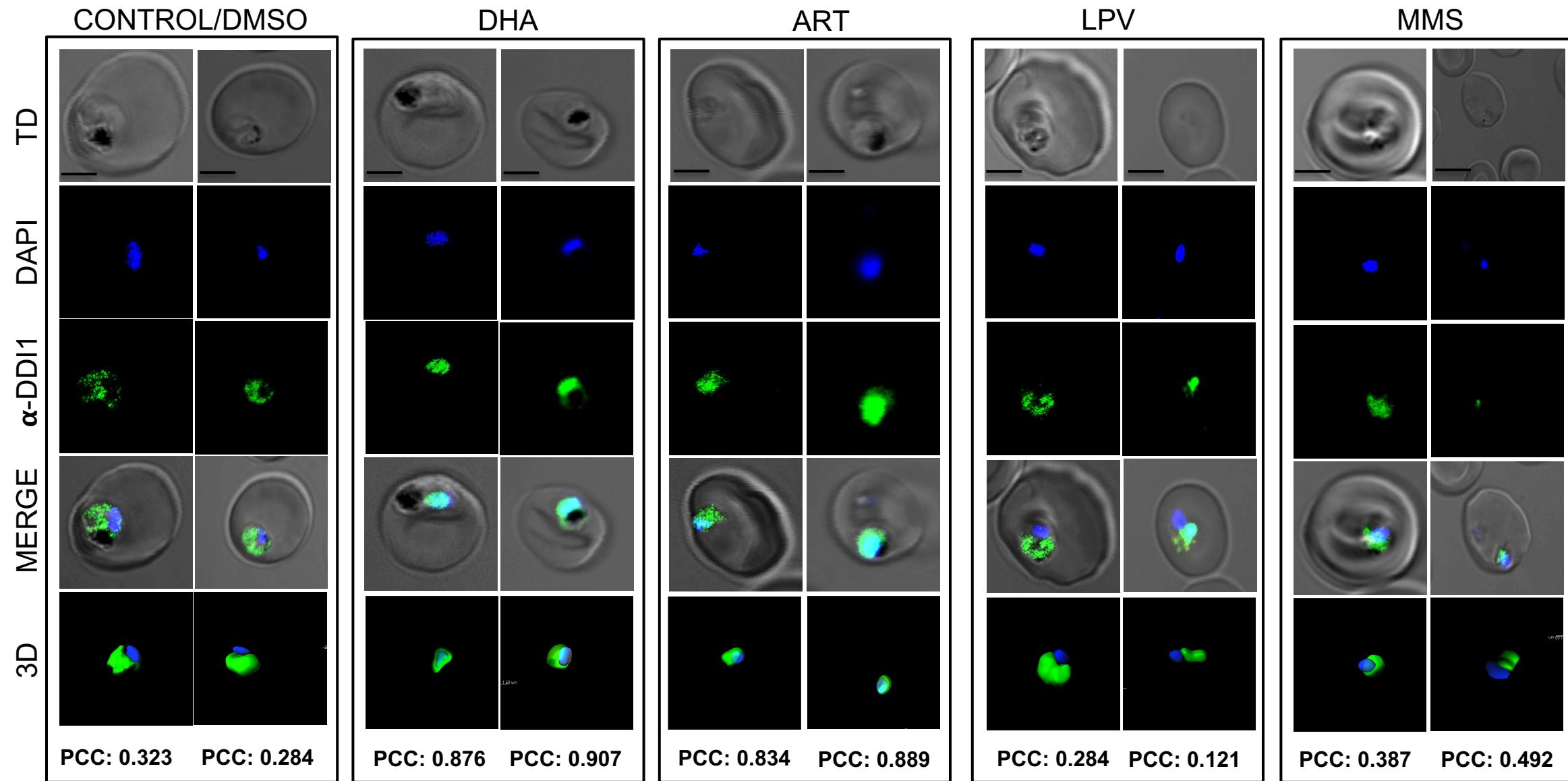
Supplementary Figure S5: Microscopic analysis of parasite morphologies under artemisinin treatment

Pyknotic cell

Pyknotic cells
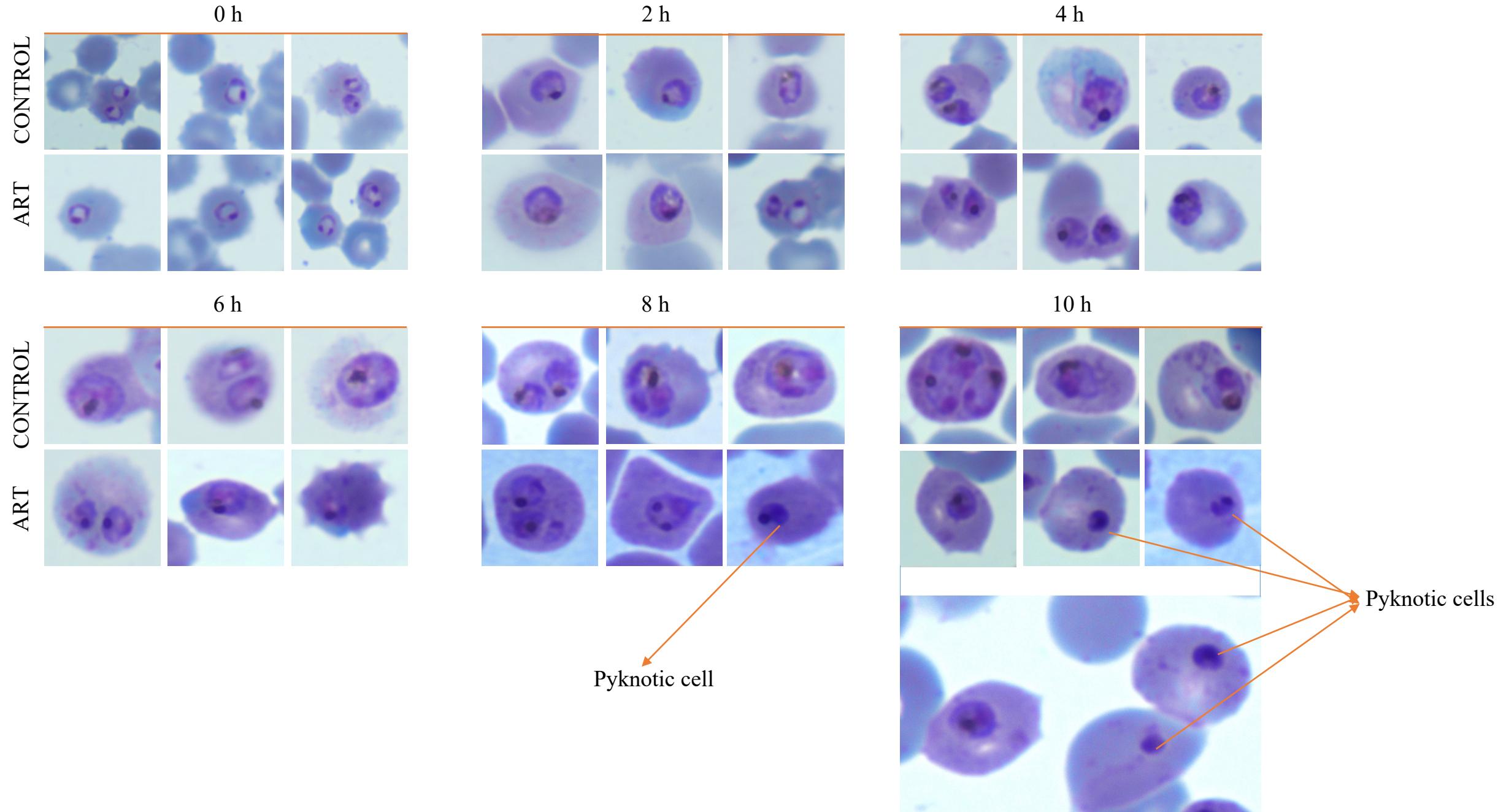
Supplementary Figure S6: SPR binding analysis of the compounds with heme detoxification protein (HDP)

Compounds	Binding Energy (kcal/mol)
	Site Specific Docking
Artemisinin	-5.81
lopinavir	-1.43
Methyl	-2.67
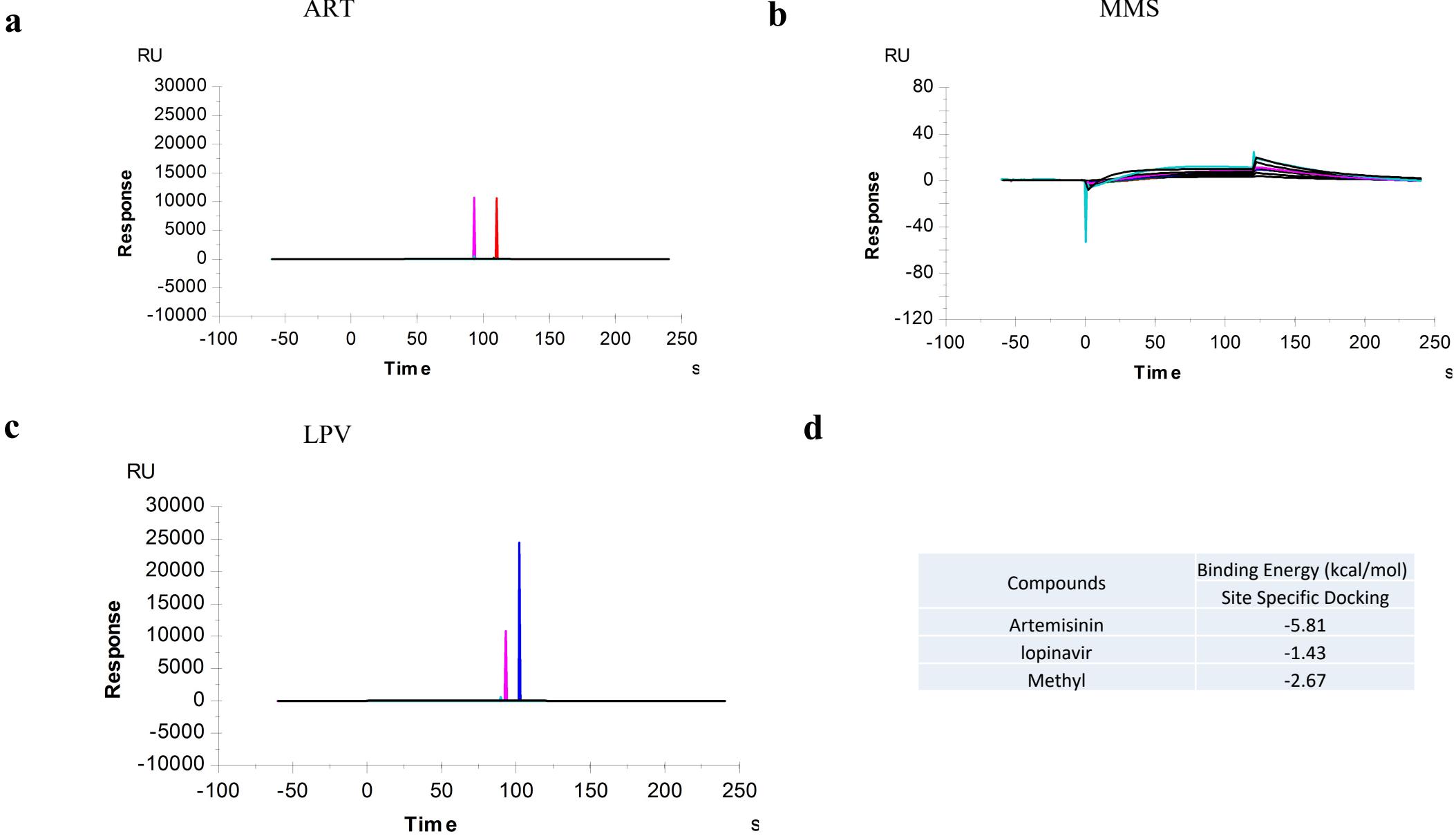
Supplementary Figure S7: Multiple sequence analysis of the *Pf*Ddi1 gene

a

Ddi1_P. falciparum

Ddi1_S. cerevisiae

Ddi1_H. sapiens Homolog 2

Ddi1_H. sapiens Homolog 1

Ddi1_L. major
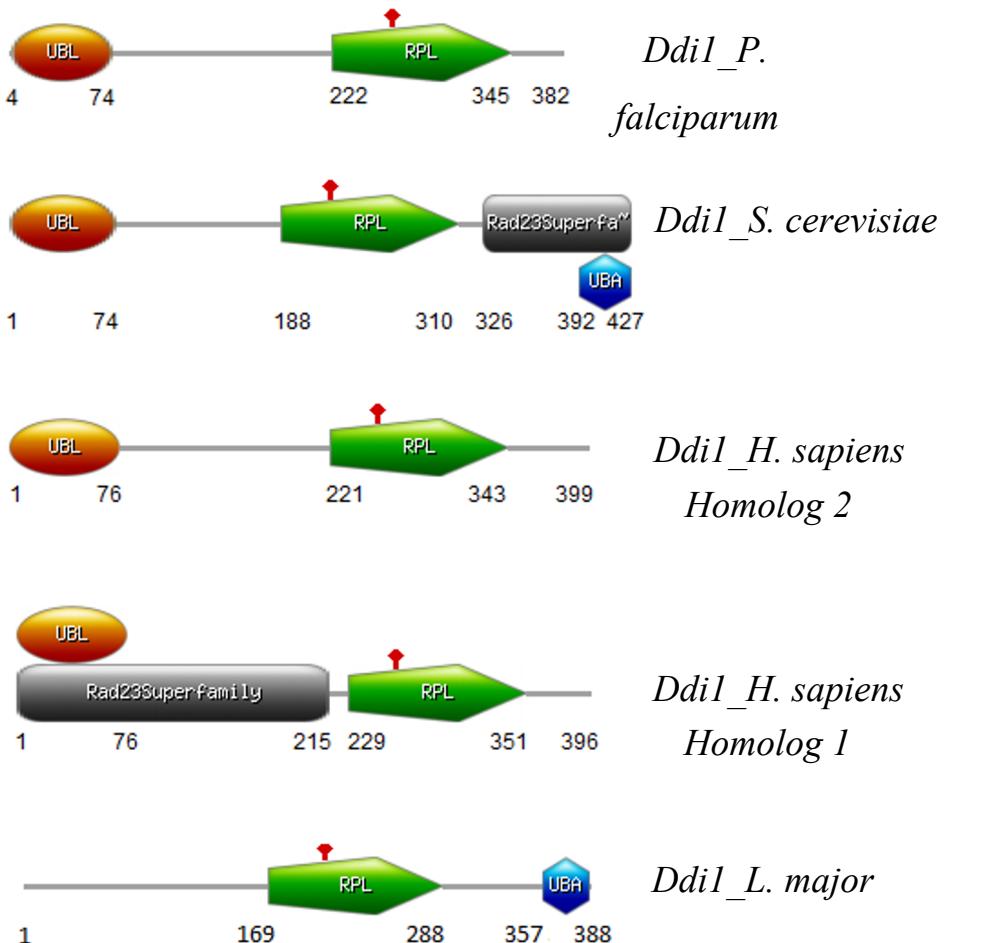
b

```
P. falciparum   MVF----ITISDDNNIITSLDVHEDTEIWTIT--NIIENDFSLNMNINELTYNGNAV--D     52
S. cerevisiae   MDLT---ISNELTGEIYGPIEVSEDMALTDL--IALLQADCGFDKTKHDLYYNMDILDSN     55
H. sapiens      MLLTVYCVRRDLS-EVTFSLQVDADFELHNFR--ALCELESGIPAAESQIVYAERPLT-D     56
L. major        --------------------MVQLTIDNARGVTLCRVSLPANATVQQLLL--------     30
                                            :  :       : . .        ::

P. falciparum   KFDTIKKLNIKEGDLLFVRKKISADIMNDNVNNMSALNN--------------------     91
S. cerevisiae   RTQSLKELGLKTDDLLLIRGKISNSIQ---------TDAAT-----------------     87
H. sapiens      NHRSLASYGLKDGDVVILRQKENADPRPPVQFPNLPRIDFSSIAVPGTSSPRQRQPPGTQ    116
L. major        -------------------QLTVA--KPELRQAQAIRNDVRHV-------THRLTPAST     61
                                        :

P. falciparum   --------ILSTNNNVGNIGNIGNNLNNENV--QNLLNNPAFKTLLDQFKVYQENEYIKK    141
S. cerevisiae   --LSDEAFIEQFRQELLNN---------QMLRSQLILQIPGLNDLVN-------------    123
H. sapiens      QSHSSPGEITSSPQ---------GLDNPALLRDMLLANPHELS----------------    150
L. major        TTTTTTSVVSSNAQTLLQAGLVGQGATAETLVVLMAADAPAAAS---------------    105
                       : .  :                      :             *

P. falciparum   ESEILLEMKNDKSKMAVLKLQDEPLYNAIFSQNLEEIKKIVKEKYETEKKEKEKEQQMYE    201
S. cerevisiae   -------------DPLLFRERLGPLI---LQR---RYGGYNTA---MNP--FGIPQDEYT    159
H. sapiens      ----------------LLKERNPPLAEALLSGDLEKFSRVLVE---QQQDRARREQERIR    191
L. major        ----------------SAAAAAPSPTKAVAAQILDLFGCASA-----SPSAGVRSQASVV    144
                                                     .                  *

P. falciparum   NALKNPLSEDSQKFIYENIYKNEINNNLALAQEHFPEAFGVVFMLYIPVEINKNTVHAFV    261
S. cerevisiae   KLMANPDDPDNKKRIAELLDQQAIDEQLRNAIEYTPEMFTQVPMLYINIEINNYPVKAFV    219
H. sapiens      LFSADPFDLEAQAKIEEDIRQQNIEENMTIAMEEAPESFGQVVMLYINCKVNGHPVKAFV    251
L. major        PSTMDERQLELQRRIYAQIQQQQIDENLANALEYTPEAFAKVTMLYVPCTINQVLVKAFV    204
                 :  .  : :    *     : :: *::::  * *  ** **  *  *** :  :*    *:***

P. falciparum   DSGAQSSIMSKKCAQKCNILRLMDKRFTGIAKGVGTKTILGKIHMIDIKIGNYFYAVSLT    321
S. cerevisiae   DTGAQTTIMSTRLAKKTGLSRMIDKRFIGEARGVGTGKIIGRIHQAQVKIETQYIPCSFT    279
H. sapiens      DSGAQMTIMSQACAERCNIMRLVDRRWAGIAKGVGTQKIIGRVHLAQVQIEGDFLPCSFS    311
L. major        DSGAQNSIMNKRTAERCGLMRLVDVRMRDVAVGVGRQEICGRIHMTPVNLAGMYIPFAFY    264
                *:*** :**.    *:: .: *::* *  .  * ***    * *::*    :::   :  ::

P. falciparum   IIEDYDIDFIFGLDLLKRHQCLIDFKQNALIIEDN--KIPFLSEKDVISISTQSIDIDAN    379
S. cerevisiae   VLD-TDIDVLIGLDMLKRHLACVDLKENVLRIAEV--ETSFLSEAEIPKSFQEGLPAPTS    336
H. sapiens      ILEEQPMDMLLGLDMLKRHQCSIDLKKNVLVIGTTGSQTTFLPEGELPECARLAYGAGRE    371
L. major        VIEDQAMDLIIGLDQLKRHQMMIDLKHNCLTIDNI--NVPFLPENDLPALAALGDDENAM    322
                :::    :*.::*** ****   :*:*.* * *     : ** * ::          .

P. falciparum   NDL---------------------------------------     382
S. cerevisiae   VTT---SSDKPLTPTKTSSSLPPQPGAVPALAPRTGMGPTPTGRSTAGATTATGRTFPEQ    393
H. sapiens      DVRPEEIADQELA-EA------LQKSAEDA----------ERQKP--------------    399
L. major        HA--PRHQDPATTATTAS--NPAAPVLSEG---------ERQARIEGFMTVSGITDPTQ    368

P. falciparum   ------------------------------------     382
S. cerevisiae   TIKQLMDLGFPRDAVVKALKQTNGNAEFAASLLFQ-     428
H. sapiens      ------------------------------------     399
L. major        AAEL--------------LEAADWNPNVAAALLFDT     390
```

Supplementary Table S1: List of primer sequences

Deletion and confirmation primers				
Primer name	Sequence (5'-3')			
Ddi1_DEL_F	AGAAAGTATACTTAACGTAGTTAACAAAGTACATACCAAACATAACAGCAAAAATATACGTAAAGCCTTCCGC			
Ddi1_DEL_R	CTTATACTTATCTATTTGTGTTATGGGCTACATACGTAGAGGCCGATCACAATATCAGTGGTTGCTGCGGCCG(			
Ddi1_CON_1	GAGCAACCTCTAGAGGTCGAGAC			
Ddi1_CON_2	CCTACCAGGGGCCAAGTACCACC			
Ddi1_CON_3	ACTAGACTAATGGCCAACCCCGATG	COMMON PRIMERS FOR DELETION GENOTYPE ANALYSIS		
Ddi1_CON_4	GGTGGGTGTTAGGGGCTTATCAGAC			
Ddi1_CON_5	TGCGCACGTCAAGACTGTCAAGG			
Ddi1_CON_6	TGTGAATGCTGGTCGCTATACTGC			
Cloning Primers in pGPD2				
Ddi1_*Sc*_F	CGCGAACTAGTATGGATTTAACAATTTCAAACG			
Ddi1_*Sc*_R	CGCGACTCGAGTCATTGGAAAAGGAGGGATGCAGC			
Ddi1_*Pf*_F	CGCGAACTAGTATGGTTTTTATTACAATATCAGAC			
Ddi1_*Pf*_R	CGCGACTCGAGTTATAAATCATTGTTTGCATCAATGTC			