

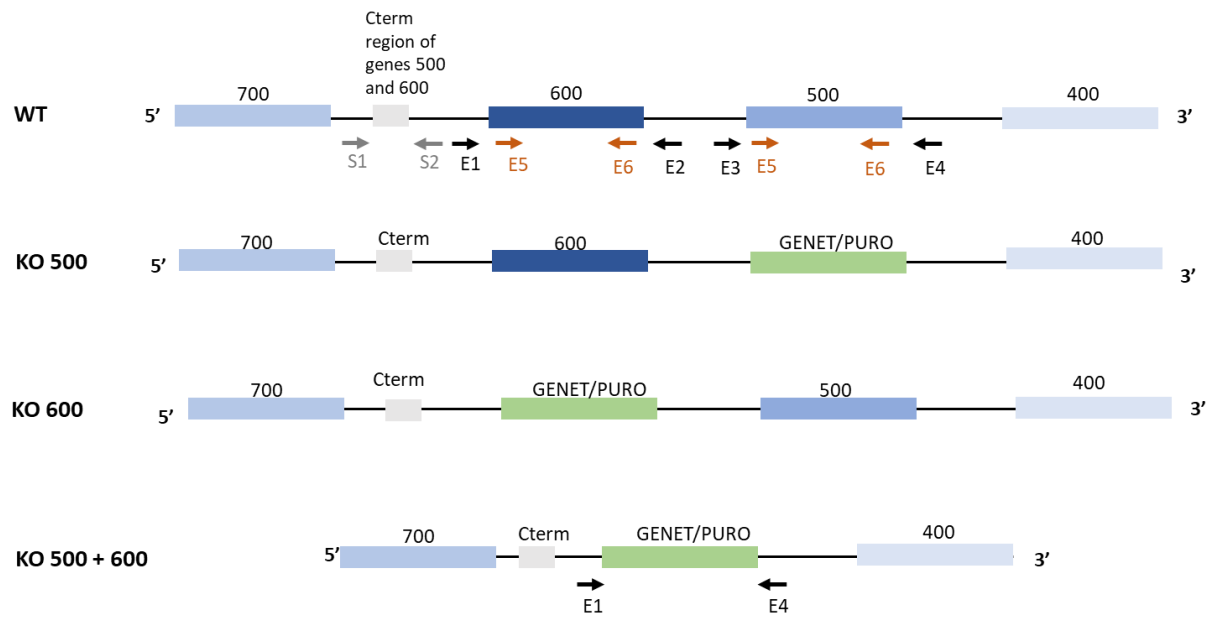


Figure S1: KO construction. Each KO is orientated in 5' – 3'. Abbreviations: WT: wild type, KO 500: LINF_310039500 KO; KO 600: LINF_310039600 KO; KO 500 + 600: LINF_310039500 and LINF_310039600 KO; S1, S2, E1, E2, E3, E4, E5, E6: enzymes used; GENET/PURO: plasmid, 400: LINF_310039400; 500: LINF_310039500; 600: LINF_310039600; 700: LINF_310039700.

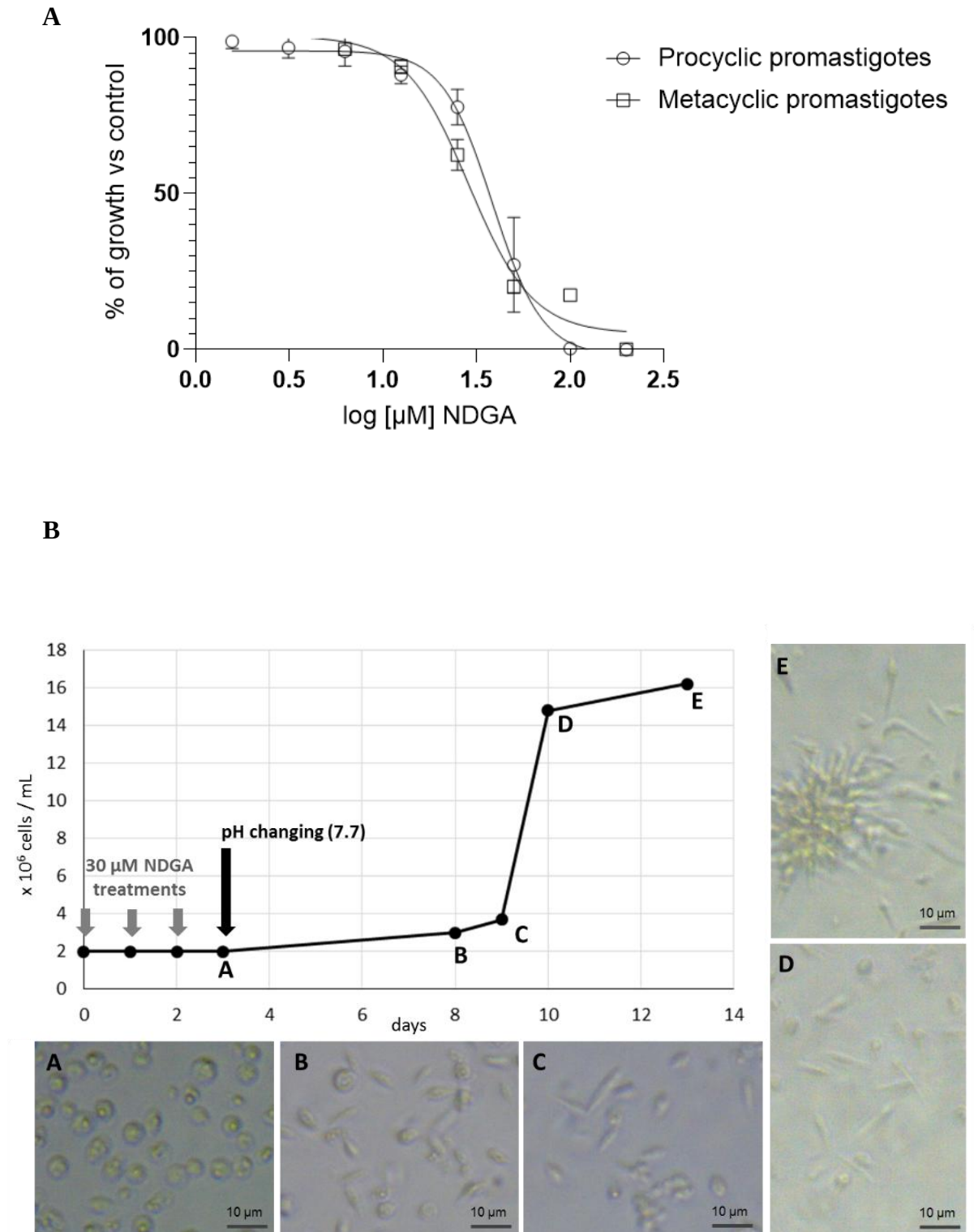


Figure S2: NDGA treatment modifies metacyclic promastigotes morphology. (A) Parasites percentage of viability depending on NDGA dose (μM) used, for procyclic promastigotes (circle) and metacyclic promastigotes (square). Data are represented as mean $\pm$ SEM from 3 independent experiments. IC₅₀ values were calculated by non-linear regression analysis processed on dose response curves, using GraphPad Prism (version 8.0) software. **(B)** Effect of NDGA treatment on procyclic and

metacyclic promastigotes morphology. Cells were treated with 30 μ M of NDGA added every 24 h over a 72-hour period.

Table S1: Expression of specific amastigote stage genes amastin and A2 protein MP NDGA vs MP, MP NDGA vs A and A vs MP, expressed in log2FC: log2 (fold change).

Gene		Log2 FC MP-NDGA vs MP	Log2 FC MP NDGA vs Am	Log2 FC Am vs MP
Amastin, putative	LINJ_29_3000	1.797		1.553
	LINJ_29_3010	1.870	0.537	1.326
	LINJ_29_3030	1.433	0.438	0.988
	LINJ_31_0460	2.085	1.042	1.037
	LINJ_08_0690	1.183	-1.035	2.212
	LINJ_08_0720	2.575	-0.87	3.439
Amastin-like protein	LINJ_08_0760	0.402	-0.542	0.937
	LINJ_08_0780	0.841	-0.536	1.37
	LINJ_08_0790	0.578	-0.494	1.064
	LINJ_08_1320	1.033	-0.429	1.456
	LINJ_08_1330			1.608
	LINJ_28_1510	0.402		0.208
	LINJ_29_1450	1.579	0.415	1.157
	LINJ_34_1040	0.386	0.607	-0.232
	LINJ_34_4340	-0.327	-0.888	0.555
	LINJ_34_4350		-0.728	0.661
Amastin-like surface protein, putative	LINJ_34_4370	0.268		0.439
	LINJ_24_1280	-0.512		-0.458
	LINJ_24_1290	0.282	0.257	
	LINJ_24_1300	1.212	0.255	0.948
	LINJ_24_1310	-1.088		-0.883
	LINJ_30_0930	-0.806		-0.678
	LINJ_34_1010		-0.757	0.959
	LINJ_34_1020	1.042	-0.849	1.884
	LINJ_34_1030	0.711	-0.886	1.589
	LINJ_34_1150	1.267		1.542
	LINJ_34_1670		-0.892	0.717
	LINJ_34_1680	1.398		1.506
	LINJ_34_1690	0.412	-0.811	1.215
	LINJ_34_1700	1.397		1.462
	LINJ_34_1710	1.727		1.88
	LINJ_34_1720	1.4		1.376
	LINJ_34_1730	1.3		1.432
	LINJ_34_2650	0.997		1.176
	LINJ_34_2660	1.339		1.381
Putative surface protein amastin	LINJ_30_0920	-0.462		-0.34
A2 protein	LINJ_22_0670	3.831	0.401	3.422

Table S2: Identified DEG for TCA cycle pathway. Expression of TCA cycle enzymes analysis, for PP vs MP and MP vs A, expressed in log2FC: log2 (fold change).

Metabolic pathway	Enzyme	KEGG identifiants	log2FC MP NDGA vs MP	log2FC MP NDGA vs Am	log2FC Am vs MP
TCA cycle	2-oxoglutarate dehydrogenase subunit/	LINJ_27_0740		0,223	
	2-oxoglutarate dehydrogenase E1 component	LINJ_36_3630	0,293	0,486	-0,202
	2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide succinyltransferase	LINJ_28_2600	-0,317	0,182	-0,507
	Aconitase	LINJ_18_0510	-0,721	0,196	-0,926
	Citrate synthase	LINJ_18_0690	-0,269	0,296	-0,573
		LINJ_18_0700	0,486	-0,224	0,702
	Fumarate hydratase/fumarate hydratase, class I	LINJ_29_2080	-0,771	-0,562	-0,217
		LINJ_24_0310	0,153	0,178	
	Glycosomal malate dehydrogenase/malate dehydrogenase	LINJ_34_0140	-0,313	-0,334	
		LINJ_34_0150		0,642	-0,733
		LINJ_19_0710	-2,073	-0,852	-1,229
	Isocitrate dehydrogenase [NADP], mitochondrial precursor/isocitrate dehydrogenase	LINJ_33_2680	-0,589	-0,490	
		LINJ_10_0310	-1,228	-0,415	-0,821
	Succinyl-CoA synthetase alpha subunit	LINJ_25_2220	-0,855		-0,852
		LINJ_25_2230	-0,852	0,280	-1,140

Table S3: Identified DEG for glycolysis. Expression of glycolysis enzymes analysis, for PP vs MP and MP vs A, expressed in log2(fold change).

Metabolic pathway	Enzyme	KEGG identifiants	log2FC MP NDGA vs MP	log2FC MP NDGA vs A m	log2FC A m vs MP
Glycolysis	ATP-dependent phosphofructokinase	LINJ_29_2620	-1,601		-1,545
	Enolase	LINJ_32_3910	-1,147	-0,829	-0,327
	Fructose-1,6-bisphosphatase, cytosolic	LINJ_04_1170	-0,355	0,136	-0,5
	Glyceraldehyde 3-phosphate dehydrogenase, glycosomal	LINJ_30_2990	-1,192		-0,958
		LINJ_30_3000	-1,067		-1,128
	Phosphoglucumutase	LINJ_21_0700	-0,907	-0,776	-0,137
	Phosphoglycerate kinase	LINJ_30_3430	0,583	0,492	
	Pyruvate kinase	LINJ_35_5440		0,650	-0,869
		LINJ_35_5450	-0,741	0,443	-1,192
		LINJ_35_0030	-0,364	0,435	-0,808
	Pyruvate phosphate dikinase	LINJ_11_1000	-1,431	0,451	-1,889
	Triosephosphate isomerase	LINJ_24_0870	-1,312	-0,224	-1,096

Table S4: Identified DEG for pyrimidine metabolism pathway. Expression of pyrimidine metabolism enzymes analysis, for PP vs MP and MP vs A, expressed in log2FC: log2 (fold change).

Metabolic pathway	Enzyme	KEGG identifiants	log2FC MP NDGA vs MP	log2FC MP NDGA vs A _m	log2FC A _m vs MP
Pyrimidine metabolism	Aspartate carbamoyltransferase	LINJ_16_0550	-1,085	-0,619	-0,474
	Carbamoyl-phosphate synthase	LINJ_16_0590	-0,241		-0,4
	Nucleoside diphosphate kinase B	LINJ_35_3910	-0,374	-0,458	
		LINJ_32_3110	-1,089	-0,679	-0,419
		LINJ_32_3100	-1,094		-1,014
	Orotidine-5-phosphate decarboxylase/orotate phosphoribosyltransferase	LINJ_16_0560	-0,515		-0,588
	Putative ribonucleoside-diphosphate reductase small chain	LINJ_27_1970	-0,213		- 0,173
		LINJ_22_1110			-0,220
	Thymidine kinase	LINJ_21_1450	-1,126	-0,466	-0,667
	Thymidylate kinase-like protein	LINJ_10_0040	0,201	0,387	-0,194
	Uracil phosphoribosyltransferase	LINJ_34_1110	0,218		0,171
	Uridine kinase-like protein	LINJ_31_2560		-0,543	0,375

Table S5: Identified DEG for purine metabolism pathway. Expression of purine metabolism enzymes analysis, for PP vs MP and MP vs A, expressed in log2FC: log2 (fold change).

Metabolic pathway	Enzyme	KEGG identifiants	log2FC MP NDGA vs MP	log2FC MP NDGA vs A _m	log2FC A _m vs MP
Purine metabolism	Adenosine monophosphate deaminase-like protein / AMP deaminase	LINJ_35_4860	-0,866	-0,228	-0,646
		LINJ_13_0870			-0,376
		LINJ_32_2690	-0,787		-0,712
		LINJ_04_0270	-0,341	0,91	-1,255
	Adenylate kinase	LINJ_36_1410		0,455	-0,434
		LINJ_34_0120	0,621	0,535	
		LINJ_21_1490	-1,134	-0,531	-0,612
		LINJ_04_0960	-0,247	-0,277	
		LINJ_25_2480	0,471		0,615
	Guanylate kinase	LINJ_36_2390	0,435		0,381
		LINJ_33_1150	0,484		0,551
	Nucleoside diphosphate kinase B / nucleoside-diphosphate kinase	LINJ_32_3100	-1,094		-1,014
		LINJ_35_3910	-0,374	-0,458	
		LINJ_32_3110	-1,089	-0,679	-0,419
	Phosphoglucomutase	LINJ_21_0700	-0,907	-0,776	-0,138
	Ribonucleoside-diphosphate reductase small chain	LINJ_22_1110			-0,22
		LINJ_27_1970	-0,213		-0,173
	Ribose-phosphate pyrophosphokinase	LINJ_08_0520	-0,341	-0,431	

Table S6: DEG exclusively modified by NDGA in MP, with $-1 \leq \log_2 \text{foldchange} \leq 1$ and p-value < 0.05 . This list was obtained by subtracting the list of DEG between MP and Am from DEGs between MP-NDGA and MP, and a cut off was made on the log2fold change to keep DEGS strongly up or down regulated. The gene highlighted was chosen to be knock-off.

Symbol	log2FoldChange	padj	Description
LINJ_21_tRNA3	-4,745	1,42E-03	tRNA
LINJ_31_3180	-4,475	3,76E-03	iron/zinc transporter protein-like protein
LINJ_21_tRNA2	-2,846	7,35E-06	tRNA
LINJ_28_0500	-2,203	6,69E-17	putative ubiquitin activating enzyme
LINJ_21_rRNA1	-1,770	4,58E-04	5S(M5) ribosomal RNA
LINJ_32_3720	-1,501	2,29E-49	conserved hypothetical protein
LINJ_05_1140	-1,447	2,09E-53	vacuolar ATPase subunit-like protein
LINJ_01_0530	-1,376	2,58E-29	putative fatty acyl CoA synthetase 2
LINJ_36_5110	-1,255	4,28E-23	conserved hypothetical protein
LINJ_30_2080	-1,245	7,05E-41	conserved hypothetical protein
LINJ_02_0110	-1,241	1,88E-50	conserved hypothetical protein
LINJ_26_snoRNA18	-1,179	2,68E-02	ncRNA
LINJ_09_0350	-1,128	1,21E-20	hypothetical protein
LINJ_31_3010	-1,126	2,99E-28	conserved hypothetical protein
LINJ_21_0280	-1,120	1,29E-33	calmodulin-like protein
LINJ_04_0250	-1,100	2,10E-14	conserved hypothetical protein
LINJ_27_1470	-1,038	4,96E-07	putative amino acid transporter
LINJ_24_1340	-1,036	1,85E-27	conserved hypothetical protein
LINJ_15_1150	-1,033	8,36E-18	conserved hypothetical protein
LINJ_32_1280	-1,032	4,01E-28	conserved hypothetical protein
LINJ_16_1190	-1,004	3,37E-24	hypothetical protein
LINJ_11_0920	-1,001	1,12E-12	conserved hypothetical protein
LINJ_16_0030	-1,001	1,54E-16	conserved hypothetical protein
LINJ_33_3060	1,091	7,31E-39	conserved hypothetical protein
LINJ_34_2330	1,094	7,70E-30	hypothetical protein
LINJ_26_2510	1,121	3,66E-25	conserved hypothetical protein
LINJ_29_1630	1,134	2,69E-08	conserved hypothetical protein
LINJ_21_1040	1,237	7,25E-47	hypothetical protein
LINJ_21_1100	1,290	1,54E-18	putative mis-match repair protein
LINJ_03_0820	1,447	1,54E-04	conserved hypothetical protein
LINJ_07_0340	1,472	1,25E-05	hypothetical protein, unknown function
LINJ_36_snRNA2	1,993	8,18E-03	ncRNA
LINJ_34_tRNA9	2,456	4,28E-02	tRNA
LINJ_33_tRNA1	2,993	2,89E-02	tRNA
LINJ_36_tRNA4	3,494	1,18E-04	tRNA
LINJ_07_tRNA1	4,243	1,30E-02	tRNA