Additional file 2

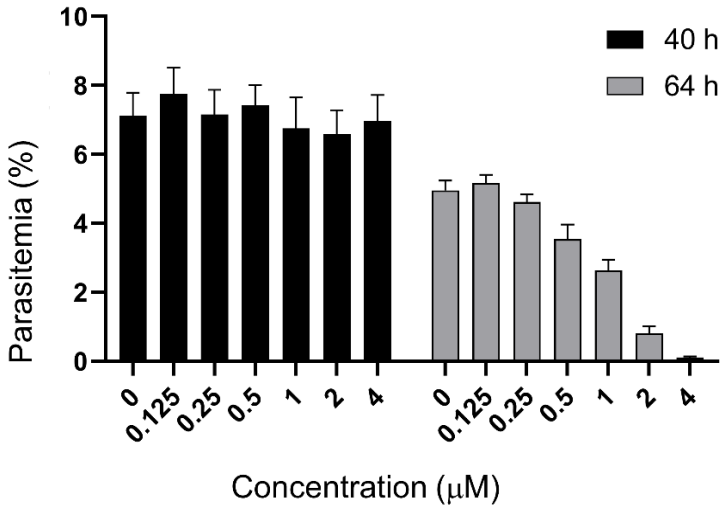

Figure S1. Percentage of infected red blood cells exposed to different concentrations of fosmidomycin. At 40 h into the first development cycle, parasitemia was unaffected by fosmidomycin. At this time point, a tenth of each late-stage culture was transferred to fresh culture medium without drug. At 64 h, the parasitemia of the diluted cultures were found to be negatively affected by prior exposure to fosmidomycin concentrations of 1 μM and above. Error bars indicate the standard error of the mean from two independent experiments conducted in quadruplicate.

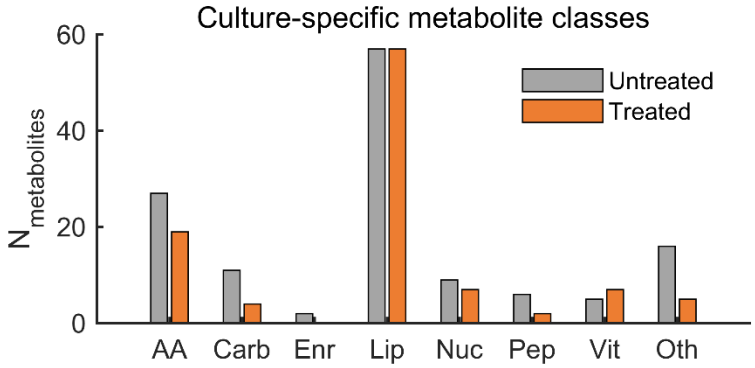

Figure S2: Number of metabolites ($N_{metabolites}$) detected exclusively in untreated and treated cultures under each metabolic class. Metabolites found exclusively in the untreated cultures and treated cultures are grey and orange, respectively. Abbreviations: AA, amino acids; Carb, carbohydrates; Enr, energy; Lip, lipids; Nuc, nucleotides; Pep, peptides; Oth, other.

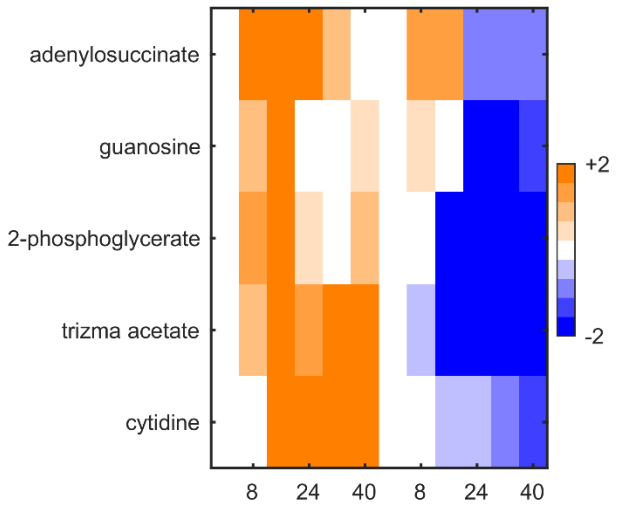

Figure S3. Metabolites of Cluster 1 in Fig. 2A of the main text under untreated (CTL) and treated (FOS) cultures of infected erythrocytes

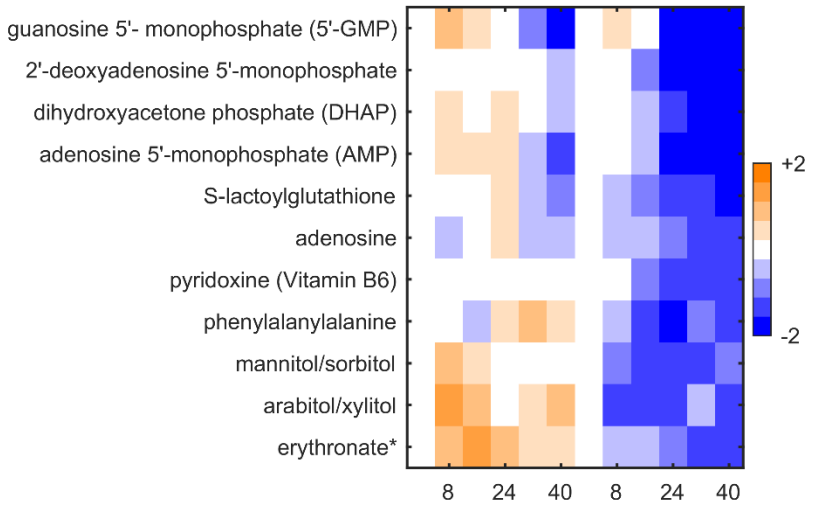

Figure S4. Metabolites of Cluster 2 in Fig. 2A of the main text under untreated (CTL) and treated (FOS) cultures of infected erythrocytes

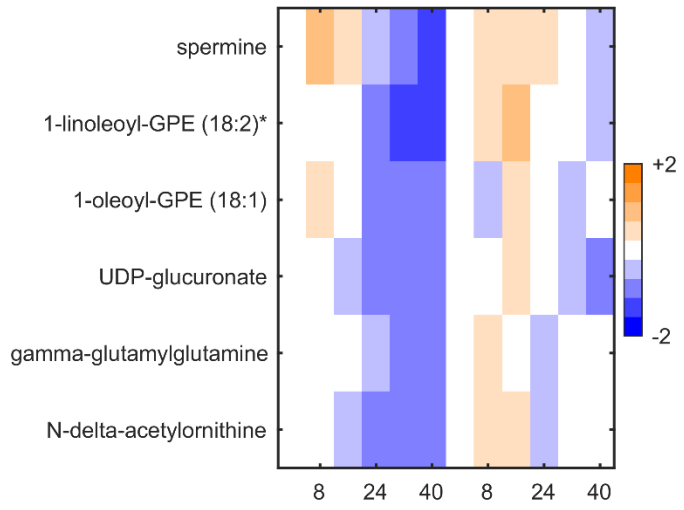

Figure S5. Metabolites of Cluster 3 in Fig. 2A of the main text under untreated (CTL) and treated (FOS) cultures of infected erythrocytes

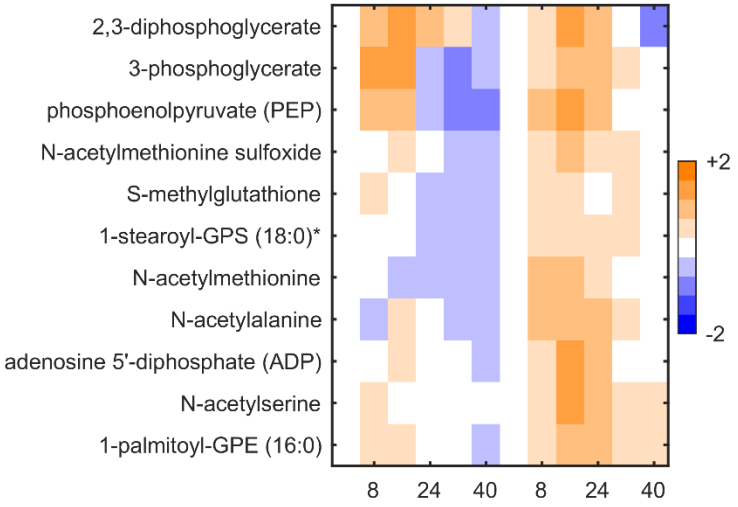

Figure S6. Metabolites of Cluster 4 in Fig. 2A of the main text under untreated (CTL) and treated (FOS) cultures of infected erythrocytes

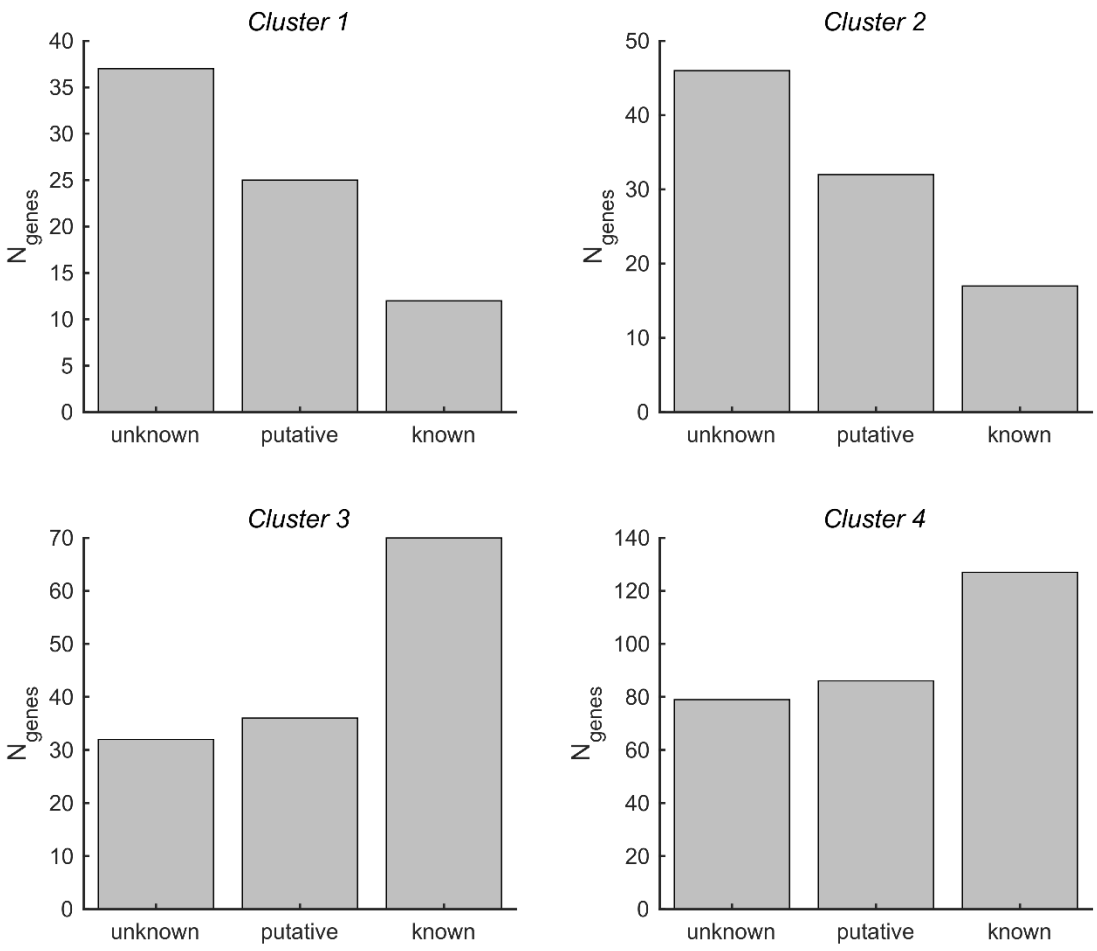

Figure S7. *Plasmodium* genes of unknown, putative, and known functions (obtained from plasmaodb.org) in Clusters 1–4 of Figure 3A in the main text.

Ubiquitin-proteasome system in fosmidomycin-treated *P. falciparum*

Our analyses showed that components of the ubiquitin-proteasome system were perturbed by fosmidomycin, including the classic ER-associated degradation (ERAD) pathway (1). Table S1 lists genes that encode proteins of the ubiquitin-proteasome system in *P. falciparum*. The expression levels of both PfpUB and the gene encoding nedd8 (an ubiquitin-like protein) were higher after fosmidomycin treatment. Conjugate addition of nedd8 is a post-translational modification, similar to ubiquitylation, that modifies both proteins and lipids (2). Among the ubiquitin-activating and -conjugating enzymes, ubiquitin-conjugating enzyme (UBC in the Table)—the major component of the ERAD pathway (3)—showed a greater than 10-h delay in t_{peak} and an FC_{gene} of 2.5 or greater after fosmidomycin treatment. E3-ubiquitin ligases (Pf3D7_0312100 and Pf3D7_0316900), which are involved in the final step of ubiquitylation (4), showed a ~two-fold alteration in expression levels at 0 h after fosmidomycin treatment. The 26S proteasome, which ultimately degrades ubiquitylated proteins (5), showed elevated average expression levels after fosmidomycin treatment (Table S1). The DDI1 gene, which encodes a protein that transports ubiquitylated proteins to the proteasome, showed a similar response (FC_{gene} = 2), consistent with the view that fosmidomycin-treated parasites deploy compensatory mechanisms to degrade misfolded ER proteins.

Table S1: *P. falciparum* genes of the ubiquitin-proteasome system

Gene ID (PF3D7_)	Name	Protein description	t_{peak} (h of IDC)¶		FC_{gene}† (SD)
			CTL	FOS	
		Ubiquitin (Ub) and Ub-like proteins			
1211800	PfpUB	Polyubiquitin	48	48	2.67 (0.66)
1313000	NEDD8	Ubiquitin-like protein nedd8 homologue, putative	0	33	1.39 (0.96)
		E1 Ub-activating and E2 Ub-conjugating enzymes			
0817000	UBA3	NEDD8-activating enzyme E1 catalytic subunit, putative	26	30	0.89 (0.25)
1144500	AOS1	SUMO-activating enzyme subunit 1	21	25	0.83 (0.18)
1237000	UBA2	SUMO-activating enzyme subunit 2	23	30	0.60 (0.18)
1365400	Uba2	Ubiquitin-activating enzyme	13	17	0.89 (0.20)
1203900	UBC	Ubiquitin-conjugating enzyme E2	0	13	2.80 (2.25)
0319300	–	Ubiquitin-conjugating enzyme E2, putative	10	14	0.67 (0.18)
		E3 Ub ligase			
1232500	–	CG2-related protein, putative	44	45	1.32 (0.37)
1343400	RAD5	DNA repair protein RAD5, putative	0	33	0.94 (0.10)
1004300	–	E3 ubiquitin-protein ligase, putative	0	0	1.28 (0.15)
0316900	–	E3 ubiquitin-protein ligase, putative	45	0	1.13 (0.35)
0529900	–	RING zinc finger protein, putative	5	0	1.06 (0.20)
0607200	–	RING zinc finger protein, putative	27	32	1.11 (0.37)
1405700	–	RING zinc finger protein, putative	48	48	1.33 (0.14)
0415800	–	RING zinc finger protein, putative	45	48	1.70 (0.87)
1007300	–	RING zinc finger protein, putative	46	43	1.40 (0.19)
		Deubiquitinating enzymes			
1368100	RPN11	26S proteasome regulatory subunit RPN11, putative	35	38	1.25 (0.16)
1226800	ATX3	Ataxin-3, putative	35	38	1.33 (0.20)
1111900	–	Josephin domain-containing protein, putative	27	30	2.18 (1.67)
0923100	–	OTU domain-containing protein, putative	6	16	0.57 (0.34)
1317000	USP39	U4/U6.U5 tri-snRNP-associated protein 2, putative	9	15	0.66 (0.08)
0527200	USP14	Ubiquitin carboxyl-terminal hydrolase 14	33	25	1.34 (0.61)
0516700	–	Ubiquitin carboxyl-terminal hydrolase 2, putative	0	44	1.33 (0.25)
1414700	–	Ubiquitin carboxyl-terminal hydrolase, putative	5	12	1.09 (0.11)
0904600	–	Ubiquitin specific protease, putative	3	11	1.17 (0.06)
		Shuttle proteins			
1409300	DDI1	DNA damage-inducible protein 1, putative	39	41	2.03 (0.63)
1011700	RAD23	DNA repair protein RAD23, putative	21	17	0.86 (0.22)
1113400	DSK2	Ubiquitin domain-containing protein DSK2, putative	48	0	1.00 (0.31)

¶Hour (t_{peak}) of intraerythrocytic developmental cycle (IDC) when the gene was maximally expressed. †The fold-change value (FC_{gene}) of a gene was computed as the ratio of the average gene-expression level under the treated condition to that under the untreated condition. Abbreviations: CTL, untreated; FOS, fosmidomycin-treated; SD, standard deviation.

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