Supporting Information

Babbitt et al. 10.1073/pnas.1209823109

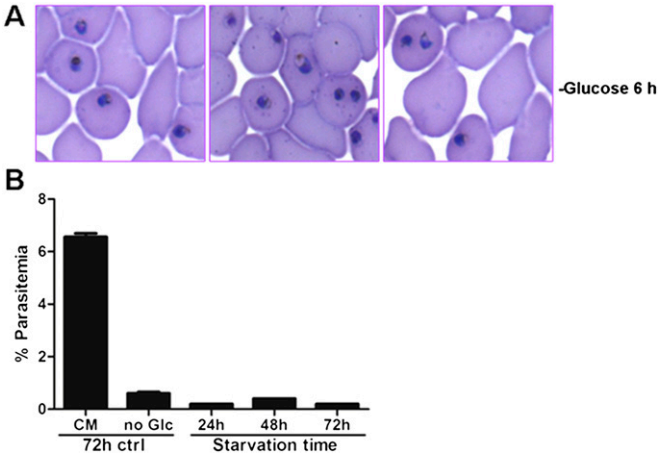

Fig. S1. Glucose-starved parasites do not recover growth. (*A*) Representative images of Giemsa-stained thin blood smears prepared from parasites incubated in glucose-free medium for 6 h. (*B*) Growth recovery following glucose resupplementation of parasites starved for indicated times. A control set of parasites was fed (complete medium, CM) or glucose starved (no Glc) for 72 h. Parasitemia of all cultures was measured by flow cytometry after 72 h of recovery. Data shown represent the mean parasitemia ± SEM; $n = 3$.

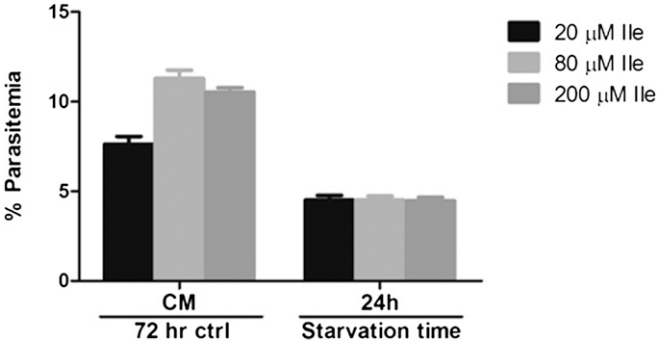

Fig. S2. Parasite recovery does not depend on preexisting isoleucine (Ile) stores. Synchronous 3D7 parasites, previously maintained in RPMI medium containing various concentrations of isoleucine, were starved for isoleucine for 24 h and then were resupplemented. Parasitemia of all cultures was measured by flow cytometry after 72 h of recovery. Data shown represent the mean parasitemia ± SEM; $n = 3$.

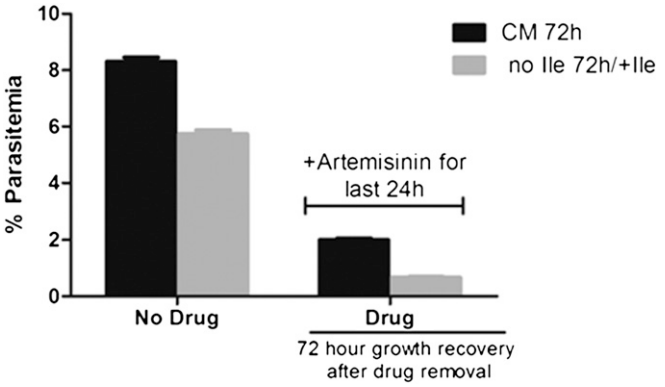

Fig. S3. Hibernating parasites remain susceptible to artemisinin. Synchronous 3D7 parasites were fed (black bars) or starved for isoleucine (gray bars) for 72 h with 50 nM artemisinin present for the last 24 h of the incubation. After drug removal, each culture was replated in CM for recovery. A control culture was incubated in the absence of drug for 72 h in CM or isoleucine-free RPMI (no Ile) for 72 h, followed by isoleucine supplementation and recovery. Parasitemia was measured by flow cytometry after 72 h of recovery. Data shown represent the mean parasitemia ± SEM; $n = 3$.

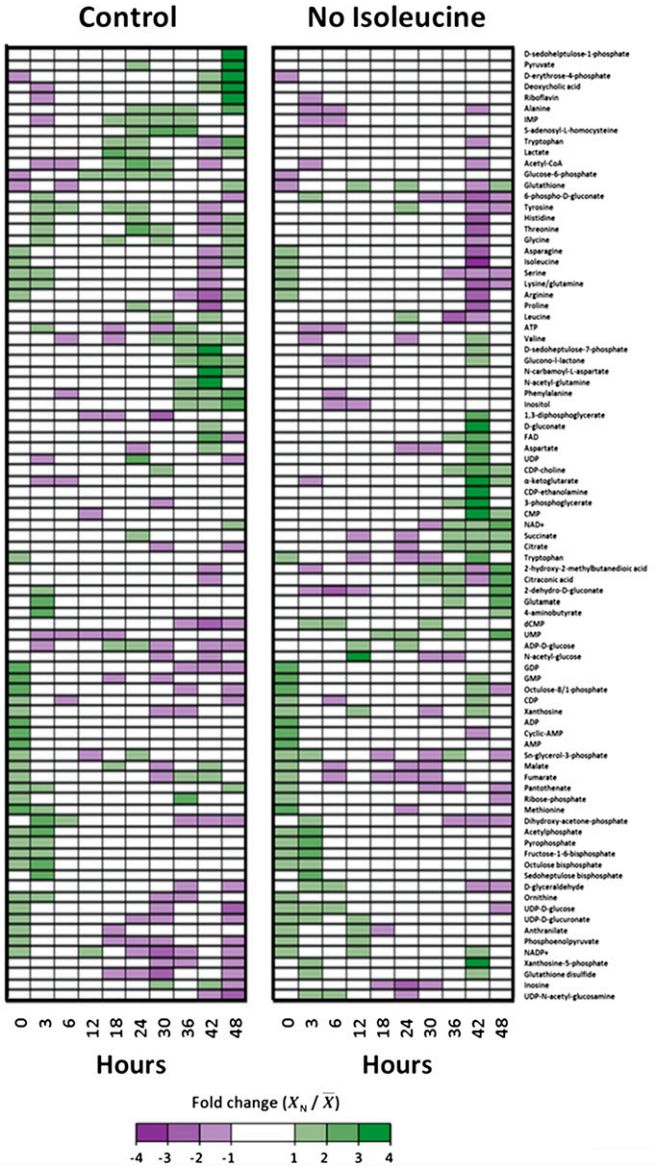

Fig. S4. Metabolite profile of infected-erythrocyte cultures under standard and isoleucine-starved conditions. Profiles of 87 intracellular metabolites for 3D7 *Plasmodium falciparum*-infected erythrocytes over 48 h. Infected erythrocytes were cultured under standard conditions (Control) and isoleucine-depleted conditions (No Isoleucine). Relative levels are expressed as the mean-centered ratio of the normalized signal intensity in the infected erythrocyte extract at each time point ($X_N/\bar{X}$) from two independent replicates.

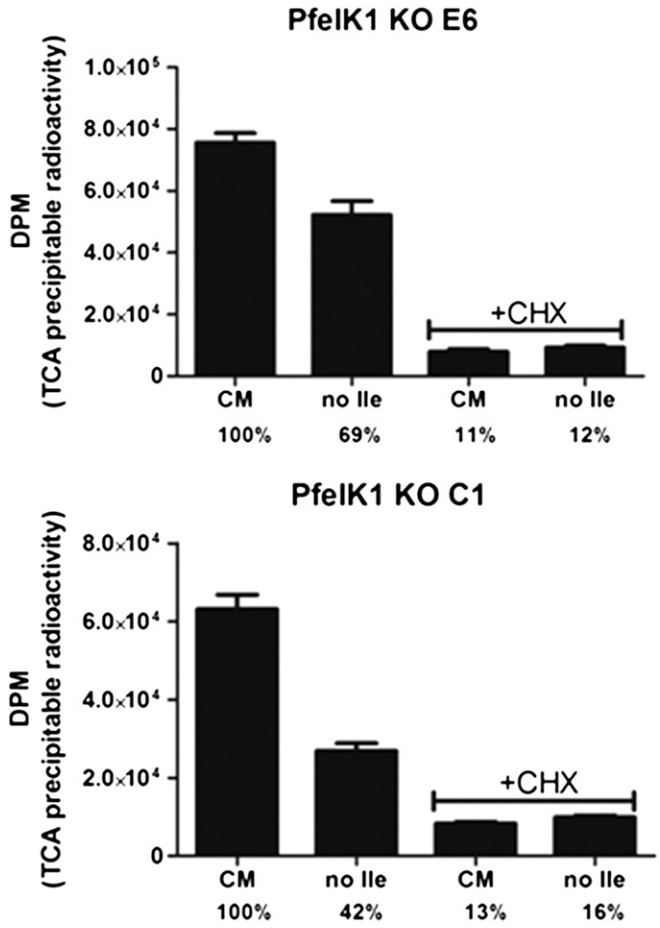

Fig. S5. Protein translation is reduced in PfeIK1 mutants during isoleucine starvation. Protein synthesis in starved parasites. Synchronous clonal *pfeik1*$^{-}$ parasites were fed or starved for isoleucine for 6 h and labeled with [^{35}S]methionine/cysteine for the last hour while incubated in complete (CM) or isoleucine-free (no Ile) labeling RPMI medium in the presence or absence of the protein synthesis inhibitor cycloheximide (CHX). Parasite proteins were tricarboxylic acid (TCA)-precipitated, and the amount of incorporated radioactivity was determined in a scintillation counter. Data shown represent the mean disintegrations per minute (DPM) of incorporated radioactivity ± SEM, n = 6.

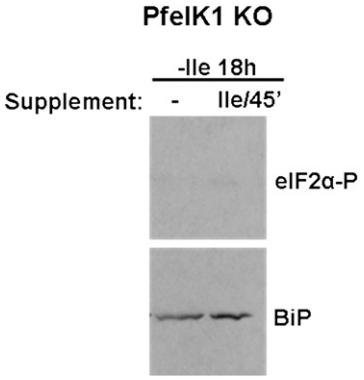

Fig. S6. PfeIF2α remains unphosphorylated in PfeIK1-KO parasites during prolonged starvation. Synchronous clonal *pfeik1*$^{-}$ parasites were maintained in isoleucine-free RPMI medium for 18 h, followed by resupplementation with isoleucine for 45 min. Parasite lysates were prepared for SDS/PAGE followed by immunoblotting with antibodies against phosphorylated eIF2α (eIF2α-P) and with BiP as a loading control.

Table S1. R^2 correlation of gene expression between fed (+) and isoleucine-starved (–) parasites

	0 h	3 h+	6 h+	12 h+	18 h+	24 h+	30 h+	36 h+	42 h+	48 h+	3 h–	6 h–	12 h–	18 h–	24 h–	30 h–	36 h–	42 h–	48 h–
0 h																			
3 h+	0.6473																		
6 h+	0.4188	0.8637																	
12 h+	0.0488	0.2812	0.5455																
18 h+	0.0924	0.0169	0.0088	0.3638															
24 h+	0.0733	0.2273	0.1961	0.0384	0.1831														
30 h+	0.1541	0.0042	0.0145	0.1415	0.0753	0.2540													
36 h+	0.7558	0.5164	0.2568	0.0018	0.1871	0.0336	0.3803												
42 h+	0.6160	0.9464	0.8836	0.3350	0.0091	0.2058	0.0036	0.4972											
48 h+	0.1709	0.4786	0.7582	0.8586	0.1647	0.0963	0.0894	0.0475	0.5584										
3 h–	0.6895	0.8805	0.6718	0.1811	0.0467	0.2005	0.0207	0.6154	0.8667	0.3350									
6 h–	0.5440	0.8110	0.7292	0.2873	0.0038	0.1673	0.0016	0.4388	0.8395	0.4438	0.8443								
12 h–	0.3176	0.6241	0.7088	0.4522	0.0141	0.1212	0.0185	0.2012	0.6817	0.5690	0.6139	0.8490							
18 h–	0.2538	0.6068	0.7942	0.6521	0.0725	0.1065	0.0514	0.1148	0.6477	0.7501	0.5142	0.6978	0.8562						
24 h–	0.1232	0.3407	0.5139	0.6394	0.1496	0.0358	0.0600	0.0437	0.4021	0.6321	0.3187	0.5495	0.7856	0.8321					
30 h–	0.0435	0.2270	0.4345	0.8136	0.3534	0.0152	0.1137	0.0013	0.2704	0.7038	0.1719	0.3244	0.5292	0.7274	0.8165				
36 h–	0.0007	0.0680	0.2074	0.6796	0.6035	0.0065	0.1029	0.0198	0.0902	0.4938	0.0419	0.1384	0.2934	0.4563	0.6215	0.8570			
42 h–	0.0091	0.0020	0.0390	0.3117	0.5475	0.0964	0.0302	0.0411	0.0062	0.1751	0.0009	0.0643	0.1879	0.2180	0.4651	0.5065	0.7254		
48 h–	0.0533	0.0250	0.0001	0.2027	0.6611	0.2275	0.0258	0.1218	0.0138	0.0780	0.0182	0.0005	0.0243	0.0618	0.1902	0.3164	0.5756	0.6750	

Yellow: best correlation between fed control samples; green: worst correlation between fed control sample; turquoise: best correlation between fed and starved samples; pink: worst correlation between fed and starved samples; orange: best correlation between starved samples; purple: worst correlation between starved samples; gray: point at which gene expression starts to deviate significantly between fed and starved sample.

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLSX)
Dataset S3 (XLS)