Nutrient sensing modulates malaria parasite virulence

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The lifestyle of intracellular pathogens, such as malaria parasites, is intimately connected to that of their host, primarily for nutrient supply. Nutrients act not only as primary sources of energy but also as regulators of gene expression, metabolism and growth, through various signalling networks that enable cells to sense and adapt to varying environmental conditions. Canonical nutrient-sensing pathways are presumed to be absent from the causative agent of malaria, Plasmodium, thus raising the question of whether these parasites can sense and cope with fluctuations in host nutrient levels. Here we show that Plasmodium blood-stage parasites actively respond to host dietary calorie alterations through rearrangement of their transcriptome accompanied by substantial adjustment of their multiplication rate. A kinase analysis combined with chemical and genetic approaches identified KIN as a critical regulator that mediates sensing of nutrients and controls a transcriptional response to the host nutritional status. KIN shares homology with SNF1/AMPK and yeast complementation studies suggest that it is part of a functionally conserved cellular energy-sensing pathway. Overall, these findings reveal a key parasite nutrient-sensing mechanism that is critical for modulating parasite replication and virulence.

To establish the effect of host-derived nutrients on the dynamics of malaria infection, we applied a long-term moderate caloric restriction (CR) protocol (30–40% reduction in calorie intake, without changes in any dietary component, for 2–3 weeks before infection) on different rodent malaria models. CR leads to a consistent reduction in body weight, blood glucose levels, lipids and hormones (for example, insulin), associated with improved health and longevity (Fig. 1a, Extended Data Fig. 1a–c). CR-fed mice infected with the rodent malaria parasite Plasmodium berghei (by mosquito bite or blood passage) showed a significant suppression of peripheral parasitaemia and total parasite load relative to the control ad libitum (AL) regimen (Fig. 1b, Extended Data Fig. 1d–f). Attenuated parasitaemia and virulence was consistently observed regardless of mouse or parasite genetic background (Extended Data Fig. 1g–i), implying a common effect of host diet on Plasmodium spp. infections.

As previously reported for short-term dietary restriction, our CR-fed mice did not develop experimental cerebral malaria, resulting in extension of survival (Fig. 1c). Experimental cerebral malaria protection under short-term CR has been linked to CR-induced changes in inflammation and immunomodulation, resulting in reduced parasite accumulation in peripheral tissues and increased parasite clearance in the spleen of mice, with no effect on parasitaemia. However, in the long-term CR condition, a general reduction in parasite load was observed with no alterations in parasite body distribution or spleen accumulation (Extended Data Fig. 1l–m). Moreover, severe immunocompromised SCID mice under CR had attenuated parasitaemia (Extended Data Fig. 1k), precluding potential effects of increased clearance.

Having excluded potential redistribution of parasites or increased clearance, we next focused on parasite growth as a possible cause of reduced parasite load in mice under the CR regimen. Plasmodium parasites replicate inside erythrocytes by schizogony to generate the infectious merozoite forms. Microscopic analysis of parasite development in mice under CR revealed a significant decrease in the mean number of merozoites formed per schizont (Fig. 1d) in P. berghei ANKA and P. berghei K173 (an isolate that naturally presents a high percentage of circulating schizonts). This observation was recapitulated in an in vitro setting, where early-stage rodent malaria parasites, as well as the human malaria parasite (Plasmodium falciparum), were cultured in medium supplemented with either CR or AL sera (Fig. 1e). Reduction of merozoite formation under CR was further corroborated by other methods such as flow cytometry and luminescence analysis (Extended Data Fig. 2a–f). Altogether, these data imply that Plasmodium spp. have an intrinsic capacity to respond to a nutrient-poor environment by reducing their replicative fitness, thereby lowering total parasite load (Extended Data Fig. 2g–i).

To understand the molecular basis of parasite response to CR, we performed global gene-expression profiling from synchronized P. berghei parasites under CR or AL regimens, which revealed a transcriptional reprogramming across the analysed developmental stages (Extended Data Fig. 3a–c). Differential expression was validated by quantitative PCR (qPCR) (Extended Data Fig. 3d–e) and functional enrichment analysis revealed a dynamic parasite response to CR (Extended Data Fig. 3f). Whereas functions related to regulation of gene expression and signalling (including a number of kinases) were induced, functions related to parasite maturation and replication, such as ion transport, DNA replication, and cell cycle were repressed. The repression of functions critical to intraerythrocytic development is consistent with the reduced-growth CR phenotype and is in agreement with a response to nutrient-limiting environments observed for other organisms.

Given the overrepresentation of protein kinases in the transcriptomic analysis (Extended Data Fig. 3f) and their prominent role in eukaryotic nutrient sensing, we sought to identify parasite kinases implicated in the CR response. We used the in vitro maturation assay to screen P. berghei kinase mutant lines and identified NEK4, PK7 and KIN as unresponsive to CR (Fig. 2a, Extended Data Fig. 4a). Whereas Δnek4 and Δpk7 (ref. 15) produced a reduced number of merozoites regardless of AL or CR conditions, Δkin parasites in CR generated merozoite numbers comparable to wild-type parasites in AL (Fig. 2a, Extended Data Fig. 4b). We further examined Δkin parasites in vivo under both dietary regimens and observed no significant differences in parasitaemia during the first days of infection, when parasite growth is linear (Fig. 2c). From day 4 onwards, Δkin parasites outgrew wild-type in both dietary conditions, but presented lower parasitaemia in CR (Extended Data Fig. 2g–i).
Host diet affects survival and parasite replication. a, Body weight change (mean ± s.d.; n = 8 mice per group) of C57BL/6 mice under long-term CR, normalized to initial weight. b, c, Parasitaemia (mean ± s.e.m.; two-way ANOVA) (b) and survival (c) of C57BL/6 mice infected via injection of infected red blood cells (iRBCs) obtained from AL mice (ANKA, circles, AL n = 10, CR n = 10; K173, triangles, AL n = 8, CR n = 9). d, e, Box plot of merozoite numbers per schizont (Mann–Whitney). AL CR Pb ANKA (AL n = 105, CR n = 137) and K173 (AL n = 70, CR n = 50, representative images shown) in mice (d) and P. berghei ANKA (AL n = 110, CR n = 106) and P. falciparum (AL n = 71, CR n = 74) after in vitro culture with AL or CR sera (e).

Figure 2 | KIN mediates parasite response to CR. a, Merozoite numbers (mean ± s.d.) in AL and CR sera of 15 kinase knockout lines. Parasites in green (Δkin; PBANKA_031030), red (Δpk7; PBANKA_031030) and yellow (Δnek4; PBANKA_061670) do not reduce replication in CR. b, Box plot of wild-type (AL n = 79, CR n = 105), Δkin (AL n = 62, CR n = 85) and Δkin+kin (AL n = 108, CR n = 124) cultured as in a (Mann–Whitney). c, Parasitaemia (mean ± s.e.m.; two-way ANOVA) of C57BL/6 mice infected with wild-type (AL n = 10, CR n = 11), Δkin (AL n = 13, CR n = 12) and Δkin+kin (AL n = 10, CR n = 6). d, RNA-sequencing analysis of wild-type and Δkin (n = 3 mice per group).

KIN is a putative serine/threonine kinase, with limited homology to the conserved family of sucrose non-fermenting 1 (SNF1) and AMP-activated kinases (AMPK).14,17 that regulate cellular energy homeostasis in yeast and mammalian cells, respectively.18,19 AMPK is a heterotrimeric complex comprising an α-catalytic subunit and two regulatory subunits β and γ. Although avian Plasmodium spp. genomes encode homologues to α, β and γ subunits,20,21 mammalian Plasmodium spp. seem to have lost the heterotrimeric complex and only retained KIN as a plausible AMPK-α-related protein kinase14,17. It is still possible that cryptic β and γ subunits exist but remain to be identified or that the poorly conserved N- and C-terminal extensions serve these functions (Extended Data Fig. 5a). Homology of P. berghei and P. falciparum KIN to the AMPK subunit is confined to the kinase domain, which includes a conserved threonine residue in the activation loop (T616 in P. berghei KIN, Extended Data Fig. 5b, b), the phosphorylation of which is essential for SNF1 and AMPK activity19, and replacement of this threonine by an aspartic acid residue has been validated as a phosphomimetic.21 To investigate the possible effect of KIN activation on parasite replication, we engineered a parasite in which the T616 was replaced by aspartic acid. Parasites expressing KIN(T616D) generated fewer merozoites and lower parasitaemia in mice under AL conditions,
appearing to closely phenocopy the CR response of wild-type parasites (Fig. 2e, f, Extended Data Fig. 5c–e). Moreover, the T616D phosphomimetic mutation was essential to complement the yeast Δsnf1 mutant. Expression of the KIN(T616D) variant in Δsnf1, but not the wild-type KIN or N-terminal truncated versions, rescued yeast growth nearly to the extent of wild-type SNF1 (Fig. 2g, h, Extended Data Fig. 5f–g). Although these results imply that targets downstream of KIN are at least partly conserved, the failure to rescue Δsnf1 with wild-type KIN indicates that upstream regulatory kinases have likely diverged or that the active complex is not correctly assembled in yeast. Nevertheless, the results point towards a functional conservation of KIN with the SNF1 and AMPK family of kinases.

We next tested whether parasites would respond to direct AMPK-activating compounds. Despite the lack of a clear homologue for the AMPKδ3 subunit (known to interact with salicylate and A769662 to promote and maintain phosphorylation in the AMPKδ activation loop), both P. berghei and P. falciparum parasites responded to treatments in a dose-dependent manner in vitro, with a reduction in merozoite formation in wild-type but not in Δkin parasites. Other kinase-deficient parasite lines, as well as Δkin+kin, responded to treatment as wild-type parasites (Fig. 2i, Extended Data Fig. 6a–e). In vivo daily administration of salicylate led to decreased parasitaemia in mice infected with wild-type and Δkin+kin parasite lines, but not in mice infected with Δkin parasites (Extended Data Fig. 6f, g).

SNF1/AMPK kinases are activated under conditions of energy deficiency. Sugars that are metabolized, such as glucose, suppress their activation. The effect of CR sera on parasite replication was abolished by glucose addition in a KIN-dependent manner (Fig. 3a, b). Glucose also reverted the salicylate effect in P. berghei and P. falciparum (Fig. 3a, Extended Data Fig. 7a, b). Supplementation with other nutrients (vitamins, essential and non-essential amino acids, and iron) or leptin (altered under CR) did not affect parasite sensitivity to salicylate or A769662 (Extended Data Fig. 7a–d). Furthermore, in vivo glucose supplementation, which increased blood glucose levels and mouse weight in CR-fed mice, led to increased parasitaemia and antagonized the CR-induced parasite gene repression (Fig. 3c, d, Extended Data Fig. 7e–g). These data demonstrate that glucose can suppress a nutrient-sensing signalling cascade that requires KIN activation.

P. falciparum malaria cases arising in patients shortly after their arrival at a hospital have been associated with increased parasitaemia and the onset of premature death owing to onset of experimental cerebral malaria (Fig. 3g). These results together with previous observations in humans provide strong evidence that malaria parasites rapidly adjust their replicative capacity on the basis of nutrient availability. The data presented here reveal that despite the lack of the canonical nutrient-sensing pathways, Plasmodium parasites encode an unusual SNF1/AMPKδ homologue (KIN) that acts as a nutrient sensor, driving a fast and coordinated response, before any nutrient becoming limiting. This raises the question of what triggers the immediate response of the parasite to diminishing nutrient availability. Levels of intracellular and extracellular nutrients and micronutrients such as sugars, amino acids, lipids and surrogate metabolites, iron and zinc, or global energy levels are known to serve as signals for cells to sense their environment. Although the rescue by glucose supplementation could suggest that glucose is the primary signal for parasite growth, the range of variation of glucose levels in rodent blood is small (6 and 4 mM for AL and CR, respectively) and only high levels of glucose (50 mM) revert the effects of CR in vitro. By contrast, parasites in AL can develop in low glucose (<2.5 mM), indicating that AL serum contains the components that are necessary and sufficient to suppress KIN activity and allow high levels of parasite replication. Altogether, the data favours a model in which energy alterations caused by changes in the nutrient status of the host are sensed by the parasite through KIN (Extended Data Fig. 8).

This study reveals a missing link between the host nutrient status and parasite growth, which might be highly relevant given the trend of global increase in overweight populations, including malaria endemic regions. It puts forward the possibility of targeting parasite nutrient-sensing mechanisms as an approach to attenuate parasite replication and virulence. It also uncovers a potential strategy used by the parasite to modulate its transmission. Dry seasons create parasite transmission bottlenecks, with the endemic populations carrying, asymptomatically, low parasite burden. It is tempting to propose that parasite nutrient-sensing may be critical to ensure parasite survival and persistence in hosts living in areas where Plasmodium is seasonally transmitted.
Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions L.M.S. and M.M.M. conceived and led the study. O.B., M.L., R.T. and E.B.G. contributed ideas and interpretation. Animal work was conducted by L.M.S., K.S., M.T.G.R., I.M.V. and J.S.D. Parasite transfections and analysis were performed by L.M.S., K.S., M.T.G.R. and A.R.Go. Transcriptomic analysis was carried out by A.R.Gr., K.K.M., C.R.M., L.M.S. and M.T.G.R. Yeast complementation studies were performed by K.S., M.T.G.R. and M.A. AMPK modelling was performed by PC.

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parasites were collected from the tail of infected mice at 0.5–2% parasitemia, washed in warmed incomplete glucose-free RPMI, and added to 96-well plates with complete culture medium (glucose free RPMI supplemented with 4–5 mM glucose, 25 mM HEPES, 2 g l⁻¹ NaHCO₃, 50 μg l⁻¹ gentamicin and 10–25% AL or CR sera) at 1% haematoctit (final volume 100 μl). Plates were incubated at 37 °C in an atmosphere of 5% O₂, 5% CO₂ and 90% N₂ for 22–30 h. Most of the experiments, including the kinetic screen (Fig. 2a), were performed with 25% sera and 4 mM glucose, which makes the final concentration of glucose in the medium of approx. 1 mM. The replicates (and Fig. 3b) were performed with 10% sera and 5 mM glucose in the base medium, making the final glucose concentration 5.1 mM for AL and 4.9 mM for CR.

Mature schizonts were visualized by microscopy after DAPI or Giemsa staining and quantified manually or automatically using ImageJ counter tools (30–50 segmented schizonts with a single hemoglobin fragment and clearly separated merozoites were scored per condition). Some experiments were scored by different investigators blindly. Alternatively, the GFP-expressing parasites were analysed by flow cytometry (as described in Extended Data Fig. 2e), or luciferase-expressing parasites (in schizont stage only) were assessed by luminescence assays (as described below).

For P. falciparum parasites, we replaced the lipid-rich bovine serum albumin (Albunax II) by AL or CR rat sera. Mixed-stage parasites were allowed to mature into schizonts in RPMI-based medium containing 5 mM glucose, 25 mM HEPES, 2 g l⁻¹ NaHCO₃, 100 μM hypoxanthine, 50 μg l⁻¹ gentamicin and 10% AL or CR sera, in 96-well plates. Initial parasitaemia was set to 1% and haematoctit to 2%. Plates were incubated at 37 °C as described above. Parasite samples were collected and Giemsa stained at multiple time-points (24–30 h). Blood smears were examined by microscopy as above detailed for rodent parasites.

Drug treatments in vitro. Drugs and vehicles (as control) were dispensed into a 96-well plate in duplicates with a series of twofold dilutions. The parasite suspension was then added to the plates and incubated at 37 °C in 5% O₂, 5% CO₂ and 90% N₂ for 24 h (P. berghei) or 48 h and 96 h (P. falciparum). P. berghei parasites were cultured in glucose-free RPMI-supplemented with 5 mM glucose, 25 mM HEPES, 2 g l⁻¹ NaHCO₃, 50 μg l⁻¹ penicillin–streptomycin and 25% FBS at 1% final haematoctit. The drug effect on schizont development was examined at 24 h by microscopy, or luminescence as detailed below. Ring stages of P. falciparum were cultured in standard RPMI (11 mM glucose), 25 mM HEPES, 100 μM hypoxanthine, 2 mM l-glutamine, 50 μg l⁻¹ gentamicin and 0.5% albumin II at 2% haematoctit and starting parasitaemia adjusted to 0.1%. Parasitaemia after 48 h and 96 h was analysed by flow cytometry after SYBR green staining and EC₅₀ values calculated by fitting the data to a log dose–response curve using GraphPad (Prism).

Luminescence assay. Supernatants were carefully removed from the 96-well plates at 24 h after incubation and the cells were re-suspended in 50 μl of lysis buffer (Firefly Luciferase Assay Kit, Biotium). Plates were then shaken for 15 min. Luminescence was determined by adding 50 μl of n-luciferin (200 ng ml⁻¹) in Firefly Luminescence Buffer to 30 μl of total lysate in white 96-well plates and immediately measured using a multiplate reader (Infinite 200 M, Tecan). Values of luciferase activity are expressed as relative luminescence units.

Flow cytometry. Flow cytometry analysis was used to determine the parasitaemia in mice (for P. berghei GFP-expressing parasites), parasitaemia in P. falciparum in vitro experiments (after staining with SYBR green), and schizont development (for P. berghei GFP-expressing parasite lines after in vitro maturation). P. berghei GFP-expressing parasites were analysed on BD FACSCalibur or BD LSRFortessa equipment. The number of total acquired events ranged from 1–2 million (days 1–3 of infection) to 100,000–200,000 (onwards). To assess schizont development 100,000–200,000 events per condition were acquired. P. falciparum SYBR Green stained samples were analysed on CyFlow SL Blue or BD Accuri C6 instrument (100,000–200,000 events per condition). The data was further analysed on FlowJo (TreeStar). RBCs were selected on the basis of their size by gating first on FSC. The number of total acquired events ranged from 1–2 million (days 1–3 of infection) to 100,000–200,000 (onwards). To assess schizont development 100,000–200,000 events per condition were acquired. P. falciparum SYBR Green stained samples were analysed on CyFlow SL Blue or BD Accuri C6 instrument (100,000–200,000 events per condition). The data was further analysed on FlowJo (TreeStar). RBCs were selected on the basis of their size by gating first on FSC and SSC and, subsequently, on FITC FL1 and PE FL3 channels (to eliminate false positives associated to RBCs auto-fluorescence). iRBCs were detected in the FITC FL1 channel.

Teast complementation assays. A codon-optimized version of P. berghei KIN ORF (GenScript, USA Inc.) was used for expression in Saccharomyces cerevisiae Δsnf1 mutant¹⁰. The expression plasmid was constructed by cloning the KIN ORF without the stop codon as BamHII–NotI fragment into the expression vector pRS426, containing the Ura selectable marker. Subsequently, the GFP coding sequence was cloned in frame with KIN ORF as a NotI–SacI fragment. The T616D mutation was introduced in the codon-optimized KIN ORF by site-directed mutagenesis. All of the generated plasmids were transformed into Δsnf1 using a previously described Li⁺-acetate method and selected on s. medium lacking uracil¹⁰. The sequences of primers used for cloning are given in Supplementary Table 1.

Western blot analysis. Whole-cell extracts were prepared by the following procedure: overnight cultures of Δsnf1-transfected strains were collected by
centrifugation and washed by shaking at 30°C for 15 min in buffer containing 0.1 M Tris pH 9.4, 50 mM 2-mercaptoethanol and 0.1 M glucose; cells were then treated with lyticase to digest the cell wall in buffer containing 0.9 M sorbitol, 0.1 M glucose, 50 mM Tris pH 8.5, 5 mM dithiothreitol and 0.5-× 5× SDS for 1 h at 30°C. After washing in 1 M sorbitol, cells were lysed in NuPAGE LDS loading buffer and 5 μg of protein were separated by SDS–PAGE in an Any kD mini-protein premade gel (Biorad). Protein transfer to a nitrocellulose membrane was performed overnight at 4°C in buffer containing 6.0× g−1 Tris and 3.09 g−1 35 tide, at 30 V with constant voltage. Blocking was performed in TBS-5%-milk Tween 0.2% for 1 h at room temperature. The membrane was then incubated with primary anti-GFP antibody diluted 1:1,000 in TBS-5%-milk Tween 0.2% for 3 h at room temperature. Secondary antibody was goat anti-mouse HRP conjugated antibody (Jackson ImmunoResearch), diluted 1:5,000 in TBS-5%-milk Tween 0.2% and incubated for 1 h at room temperature.

**Microarray analysis.** Transcriptional profiling of synchronized parasites across the developmental cycle in AL and CR was performed using DNA microarrays. To prepare the samples, we started by transferring the blood of a BALB/c infected mouse (>10% parasitaemia) into 3 Wistar rats by intraperitoneal injection. When the parasitaemia was 1–3%, the blood was collected by cardiac puncture (with heparin) and cultured overnight (standard RPMI supplemented with 25 mM HEPES, 50 μg 1−1 gentamicin and 25% FBS). The mature schizonts obtained in vitro were separated from uninfected cells by Nycodenz-density gradient centrifugation and injected intravenously (tail) into BALB/c mice that were under AL or CR, as outlined in Extended Data Fig. 3a. The parasites were allowed to go through one developmental cycle in the new hosts and samples started to be collected with 4 h intervals on the second cycle. Giemsa staining on thin blood smears was performed for each collecting point and parasite area (as proxy for parasite age) was scored from three mice per condition and by cartilage puncture (with heparin) and cultured overnight (standard RPMI, filtered through Plasmodipur (EuroProxima) to remove mouse leukocytes, lysed in PureZOL (BioRad) and stored at −80°C. RNA was then extracted with chloroform, precipitated overnight at −20°C with isopropanol, washed with ethanol, and stored at −80°C.

Total RNA was primed with a mix of OligoDT and random primers for 10 min at 70°C, and reverse-transcribed with Superscript III (Life Technologies) with amino-allyl dUTP (Ambion) for 2 h at 37°C. After purification, samples and references (pool of cDNA samples) were labelled with cy5 and cy3 (Amersham), respectively, and hybridized on the Agilent nuclear expression array. The samples were selected using an absolute fold-change higher than two and FDR adjusted p values cutoff of 0.01. Significant expression alterations were graphically represented using an MA-plot, showing the log-fold-changes on CR versus AL samples. The expression data sets were normalized using the Gene Expression Omnibus database under the accession number GSE69629.

**RNA sequencing.** Transcription profiling of synchronized *P. berghei* wild-type and Δkin parasites was performed by RNA sequencing. The samples were obtained as described above (mouse > rat > ex vivo culture > mice in AL or CR), but collected at single time-point (10h after invasion). The total mRNA was extracted using the PureZOL reagent (Bio-Rad) and Magnetic mRNA Isolation Kit (NEB). First-strand cDNA was synthesized with Random Hexamer primers and Superscript III Reverse Transcriptase (Life Technologies). The DNA–RNA hybrids were purified using Agencourt AMPure XP beads and the second strand was synthesized using dUTP nucleotide mix, DNA polymerase I (E. coli) and RNase H by 2.5 h of incubation at 16°C. cDNA was fragmented to approximately 200 nucleotides using Covaris S220 System. The fragments were end-repaired, TA-tailed and ligated to Illumina adapters with index barcodes using NEBNext kits according to the manufacturer’s instructions. Excess adapters were removed with two rounds of clean up with 50 μl of Agencourt AMPure XP beads (Beckman Coulter). Final libraries were eluted in water, quantified on an Agilent Bioanalyzer 2100 High Sensitivity DNA chip and quantified by qPCR. A pool of the indexed libraries was sequenced on an Illumina HiSeq2500, with 100bp paired-end reads. RNA-seq reads were mapped to the PlasmodDB reference *P. berghei* ANKA genome (http://www.plasmodb.org) with TopHat2 software using default parameters15. Gene expression levels were obtained using PlasmoDB genome annotations and genes with strong gametocyte signatures (K.K.M. and O.B., unpublished work) were removed from the analysis. Differential expression was assessed using edgeR and limma R packages30,31. First, the normalization factors were calculated to scale the raw library sizes using edgeR. Second, linear modelling and empirical Bayes moderation were applied to detect differentially expressed genes (thresholds of absolute fold-change higher than two and FDR adjusted p values lower than 0.01). Normalized expression values (RPKM) were obtained using edge R package38.

**qPCR analysis.** Samples prepared above were tested by RT–qPCR analysis for data comparison. Additionally, RNA extracted with PureZOL (BioRad) was coupled with purification on columns including DNase treatment (NZY Total RNA Isolation kit, NZY Tech). Second-strand-treated RNA was primed with random hexamers and reverse-transcribed using NZY First-Strand cDNA Synthesis Kit (NZYTech). Real-time PCR was performed on ABI 7500 or 7900HT systems (Applied Biosystems), using Universal SYBR Green Supermix (Bio-Rad) and the primers listed in Supplementary Table 1. Relative gene expression was normalized to the geometric mean of reference genes PBANKA_061540 and PBANKA_011140 for *P. berghei*, using the ΔAC method. *P. falciparum* data was normalized to PF03D7_071700 gene. Total RNA from infected spleens was extracted with NZY Total RNA Isolation kit (NZYTech), primed with random hexamers, reverse-transcribed and analysed by qPCR as above. Total parasite load was determined by detecting expression of *P. berghei* 18S-RNA, normalized to mouse *Hprt* housekeeping gene using the ΔAC method. Primers listed in Supplementary Table 1.

**Statistics.** Significance was calculated using different tests on the GraphPad (Prism) software. Mann–Whitney U tests for comparisons between two conditions, and two-way ANOVA was used to compare parasitaemia between CR or drug treatments and the control group mice. The log-rank (Mantel–Cox) test was used to compare the survival distributions of two groups. Significance was considered for P values below 0.05. The outliers in the box plots represent 10% of data points. Biological replicates (n) indicated in figure legends refers to the number of mice, number of schizonts, or number of independent cultures that were pooled from 1 to 3 experiments performed independently. Sample sizes were chosen on the basis of historical data, no statistical methods were used to predetermine sample size.

**Data availability.** All expression data (microarray and RNA-seq) are available from the Gene Expression Omnibus database under the accession number GSE69629.

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Extended Data Figure 1 | Effect of calorie restricted diet in rodent malaria models. a, b, Body weight change (a) and blood glucose levels (b) of C57BL/6 male mice under long-term calorie restriction (CR) or ad libitum (AL). Body weight data (n = 4 per group) was normalized to the initial weight for each animal. The average daily food consumption for AL and CR mice was 2.93 ± 0.48 g and 1.76 ± 0.11 g, respectively (mean ± s.d.). c, Number of RBCs per ml in infected C57BL/6 male mice in AL and CR diet regimens. Cell density was determined in 1 μl of blood collected from the tail and counted on a haemocytometer chamber (n = 4 per group). d–f, Full course of parasitaemia from infected mice infected by mosquito bite (d) or by intraperitoneal (i.p.) injection of 1 x 10^6 iRBCs (e, f). Values (mean ± s.e.m.) represent one of two independent experiments. Parasitaemia of GFP-expressing *P. berghei* ANKA (mosquito bite, n = 5 per group; iRBCs, n = 4 per group) determined by flow cytometry analysis. Parasitaemia of *P. berghei* K173 (n = 4 per group) obtained by microscopy analysis of blood smears. g, h, Parasitaemia of male BALB/c mice infected by i.p. injection of 1 x 10^6 *P. yoelii* 17XNL iRBCs (n = 4 per group) or 1 x 10^6 *P. chabaudi* AS iRBCs (n = 3 per group). Parasitaemia obtained by microscopy analysis represents 1 of 2 independent experiments. i, BALB/c mice infected with *P. berghei* ANKA expressing luciferase under the *ama1* schizont-specific promotor and imaged on day 4 after infection (AL n = 10, CR n = 5; 2 independent experiments pooled). j, k, Parasitaemia of *P. berghei* ANKA infection in BALB/c (j) and BALB/c scid (severe combined immune deficiency) mice (k). Mice were infected by i.p. injection of 1 x 10^6 GFP-expressing parasites and parasitaemia was assessed by flow cytometry (mean ± s.e.m.; BALB/c, n = 8 per group; BALB/c scid, AL n = 7, CR n = 8; 2 independent experiments pooled). l, BALB/c mice infected with *P. berghei* ANKA expressing luciferase under the *ama1* schizont-specific promotor, to allow imaging of the sequestering parasite stage. Mice were imaged 25 h after i.v. infection with purified mature schizonts. AL, top animals; CR, bottom animals. Spleen weight and parasite load measured 72 h after infection with mature schizonts. Parasite load was determined by qPCR analysis of *P. bergheri* 18S rRNA. Spleen weight was normalized to body weight for each mouse (AL n = 3, CR n = 4). m, Relative abundance of circulating young and mature parasites in AL and CR C57BL/6 mice infected with 1 x 10^6 GFP-expressing *P. berghei* ANKA iRBCs. Tail blood from infected mice was analysed by flow cytometry. After excluding false GFP-positive events, the total GFP population was separated in low (GFP+) and high (GFP++) GFP-expressing cells, corresponding to young and mature parasites, respectively. The same gates were applied to AL and CR blood samples on days 4, 5, and 6 after infection. The observed marked reduction in the percentage of mature parasites overtime in both diet conditions is indicative of sequestration of infected RBCs. Bars are mean ± s.e.m. (7 mice per group; 2 independent experiments pooled).
Extended Data Figure 2 | Effect of CR in vitro. a, Workflow representation of the in vitro parasite maturation assay. Experiments were conducted in a glucose-free RPMI medium supplemented with HEPES, antibiotic, 10–25% rodent sera and glucose at the concentration indicated in the corresponding figure and/or legend. b, c, Box plots showing microscopic quantification of the number of P. berghei ANKA (b) or P. yoelii 17XNL (c) merozoites per segmented schizont after in vitro culturing in the presence of 25% AL or CR mouse sera (glucose, 4 mM). Culture for 30 h showed similarly reduced merozoite numbers in the CR condition, suggesting that parasite development is not delayed in CR. Only segmented schizonts with a single pigmented digestive vacuole were imaged and scored (Mann–Whitney test). Total numbers of schizonts analysed in two independent experiments are as follows: P. berghei AL 22 h, 111; CR 22 h, 78; AL 30 h, 74; CR 30 h, 94; P. yoelii AL, 58; CR, 107. d, Flow cytometry analysis of P. berghei ANKA schizonts prepared as in b and stained with SYBR Green to quantify the DNA content (two independent experiments). e, Representative flow cytometry plots and gating strategy for analysis of GFP-expressing P. berghei ANKA parasites after 24 h in vitro culture with 25% AL or CR rat sera (glucose, 4 mM). Cells were selected on FSC and SSC and then on FITC (green) and PE (red) channels. As shown in left and middle panels, mature schizonts express strong GFP signal detected in the FITC channel. Histogram plot show fluorescent intensity comparison between AL and CR. Data represents 1 of 3 independent experiments. f, Luminescence analysis of schizont-specific luciferase-expressing parasites after in vitro maturation (mean ± s.e.m.; n = 5; Mann–Whitney). g, Comparing parasitaemia levels (left axis, black) and the estimated parasite numbers (right axis, blue) using a geometric progression (y = nx^2) in which the basis is the mean merozoite number for AL and CR (9 and 6, respectively) for the first days of parasite linear growth. Parasitaemia data was obtained from Fig. 2c (mean ± s.e.m.; n = 11 per group; AL, closed circles, CR open circles). Mathematical modelling of parasitaemia taking into account only the number of merozoites appears to be sufficient to predict the observed growth difference of P. berghei during early infection. h, i, Box plot of merozoite numbers (h) and parasitaemia (i) of wild-type P. berghei ANKA and Δpk7 parasites. Number of schizonts analysed after in vitro maturation in AL conditions in 2 independent experiments are as follows: wild type, 36; Δpk7, 85. Parasitaemia determined by microscopic examination of blood smears from C57BL/6 mice infected with 1 × 10^6 iRBCs of P. berghei ANKA Δpk7 (n = 4) and the parental wild type (AL n = 3; CR n = 4). The data show that parasites producing fewer merozoites lead to similar low parasitaemia to that of parasites under CR.
Extended Data Figure 3 | Microarray analysis of *P. berghei* parasites under CR. **a**, Schematic representation of parasite sample preparation for microarray time-course analysis. *P. berghei* ANKA parasites collected with 4 h intervals 30 h after intravenous injection of purified schizonts into AL and CR mice. **b**, Microscopy analysis of parasite size (as proxy for parasite age) in the samples used in the microarrays show no apparent morphological differences in parasite development under AL and CR across the different time-points. The parasite area (a.u., arbitrary units) is defined by the Giemsa staining on thin blood smears and was scored using ImageJ. Histograms of parasite size distribution (3 mice per group per time point). The total number of parasites analysed are as follows: 6 h, AL n = 150, CR n = 143; 10 h, AL n = 158, CR n = 159; 14 h, AL n = 158, CR n = 157; 18 h, AL n = 180, CR n = 121). The indicated time-points correspond to the parasite developing time after RBC re-invasion, in the second cycle. **c**, Scatter plots of log2 fold change (y axis) and mean expression levels (x axis) of parasites in CR versus AL (CR/AL) of 3 mice per group at the indicated time-points. Genes differentially induced or repressed in CR (with log2(fold change) of 1 and FDR adjusted *P* < 0.01) are highlighted in red and blue, respectively. The number (and relative percentage) of genes altered for each time-point is given in the graphs in red (induced) and blue (repressed). **d**, Correlation plot between microarray and qPCR analysis for the 14-h samples. 20 genes were selected on the basis of the highest fold-changes, analysed by qPCR and compared to the values obtained in the microarray analysis. Validation rate was 80%. Values are mean of 3 mice per group. The list of genes analysed and their fold-changes in qPCR and microarray are provided in the Source Data. **e**, qPCR analysis of repressed genes in independent biological samples collected at 14 h (AL n = 4, CR n = 3). Each circle represents one mouse. Gene IDs are shown without the 'PBANKA_' prefix. Microarray hybridization was performed one time and confirmed by qPCR for a subset of genes in the same RNA samples (**d**), as well as independently collected samples (**e**). **f**, Gene ontology enrichment analysis (Molecular Function) of the genes showing significant alterations for each time point using PlasmoDB (http://www.plasmodb.org) tools and considering Benjamini–Hochberg adjusted *P* < 0.05. The graph highlights the top four of terms with highest significance for each time point and/or terms that appear more than once. Red, induced; blue, repressed. The full list of terms (including biological process analysis) for each time point are provided in the Source Data.
Extended Data Figure 4 | See next page for caption.
Extended Data Figure 4 | Screen of *P. berghei* kinase mutants and characterization of Δkin and complemented parasite lines. a, Screening of kinase mutants using the CR in vitro assay. Screen performed in media supplemented with 25% of AL or CR sera (glucose, 4 mM). The graph shows the relative reduction of merozoite formation in CR (unfilled bars) in comparison to AL (filled bars). Values are mean ± s.d. of 3 independent experiments for wild type, Δnek4, Δpk7, Δkin, Δcdkl, Δcdpk3, Δgak, Δtkl5 and 2 independent experiments for other knockout lines. The total number of schizonts analysed is as follows: wild type, AL n = 150, CR n = 167; Δkin, AL n = 80, CR n = 86; Δpk1, AL n = 108, CR n = 89; Δpk7, AL n = 134, CR n = 154; Δnek2, AL n = 99, CR n = 86; Δnek4, AL n = 117, CR n = 79; Δcdpk3, AL n = 46, CR n = 81; Δcdpk4, AL n = 55, CR n = 63; Δgak, AL n = 46, CR n = 54; Δcdkl, AL n = 79, CR n = 62; Δtkl5, AL n = 99, CR n = 93; Δsrpk, AL n = 59, CR n = 86; Δeik1, AL n = 110, CR n = 71; Δeik2, AL n = 116, CR n = 84; Δmap1, AL n = 90, CR n = 59; Δmap2, AL n = 50, CR n = 42. b, Box plot of microscopic analysis of merozoite numbers for *P. berghei* ANKA wild-type (AL n = 49, CR n = 77) and a second independent clone of Δkin (AL n = 54, CR n = 73) in the same conditions as in a (Mann–Whitney test). c, Schematic of Δkin complementation strategy. Double crossover recombination at *kin* 5′ and 3′ UTR was used to genetically delete the previously introduced *Toxoplasma gondii* dhfr (tgdhfr) and complement with codon-altered *kin* gene and human dhfr. Transgenic parasites were selected by WR99210 treatment of mice (4 subcutaneous daily injections, 16 mg per kg per day). Annealing sites for genotyping primers are illustrated (left) and primer sequences are given in Supplementary Table 1. Agarose gel image (representative of 3) showing diagnostic PCR products from Δkin and Δkin+kin extracted genomic DNA, after dilution cloning of the complemented parasite line (right). d, Flow cytometry analysis of GFP-expressing *P. berghei* ANKA wild type, Δkin and complemented Δkin parasites after in vitro maturation to schizonts with medium supplemented with AL and CR sera as in a and analysed as in Extended Data Fig. 2c. Histograms represent 2 independent experiments. e, Full course parasitaemia (mean ± s.e.m.) and survival of C57BL/6 mice AL and CR infected by i.p. injection of 1 × 10⁷ IRBCs of *P. berghei* wild type (AL n = 7, CR n = 7), Δkin (AL n = 9, CR n = 8) and Δkin+kin (AL n = 10, CR n = 6). AL, closed circles; CR, open circles. f, Analysis of parasite area (arbitrary units, a.u.) on Giemsa-stained smears of the samples used for RNA sequencing, as in Extended Data Fig. 3b. Histograms of parasite size distribution (3 mice per group). The total number of parasites analysed as follows: wild type, AL n = 172, CR n = 148; Δkin, AL n = 112, CR n = 129. g, Correlation plot between microarray and RNA-seq analysis for the wild-type samples at 10 h. Analysis of top 500 genes with *P* < 0.01 in CR versus AL and expression levels higher than the first quartile in both platforms are shown in the graph. Despite the use of different platforms to analyse gene expression, there is 0.45 correlation between microarray and RNA-seq data from the two independently obtained wild-type samples (*P* < 0.001). h, Comparison of GO term enrichment analysis of CR-altered genes between RNA-seq and microarray platforms for the 10 h time-point. The GO ‘Molecular Function’ graph highlights the location and relation of significantly enriched terms. As indicated in the key, node size refers to the level of significance of each GO term, while the colour of the node represents if a particular term was detected in one or both platforms. The graph is split into two halves; where the top half represents the enrichment of terms from upregulated genes and the bottom half that of downregulated genes. Although the overlap between different transcriptomic methods was, as expected incomplete, this GO term enrichment analysis of the data sets revealed consistency in the functions of the genes that responded to CR. i, qPCR analysis of wild-type *P. berghei* and Δkin in independent biological samples (wild type, *n* = 3 per group; Δkin, *n* = 5 per group). Data normalized to AL of the correspondent genotype. Each circle represents 1 mouse. Gene IDs are given without the ‘PBANKA_’ prefix. The genes analysed were experimentally validated in Extended Data Fig. 3d, e and encode proteins related to lipid metabolism, members of transcriptional regulators (ApiAP2), and several transporters.
Extended Data Figure 5 | KIN and KIN(T616D) phosphomimetic mutation. a, Schematic diagram of yeast SNF1 and human AMPKα showing the conservation of *P. falciparum* and *P. berghei* KIN kinase catalytic domain (red). This kinase domain is flanked by an unusually long N-terminal region and a poorly conserved C terminus, both with no obvious domains. Amino acid sequence alignment of the activation loop reveals a high degree of similarity and the conservation of the T-loop threonine (red line) whose phosphorylation is essential for kinase activity. AID, autoinhibitory domain; KA1, kinase associated domain 1. b, Model of KIN on AMPKα (top) and KIN catalytic domain (bottom). The predicted amino acid sequence of KIN kinase domain (455–712) was used to generate a model by Phyre (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) using the human AMPK structure (PDB: 4RER). The model is shown in cartoon representation and depicts the small lobe (455–539) in green, the large lobe (540–712) in blue and the T-loop (601–626) in red with the T616 in sticks. An ATP molecule (stick representation) was docked to illustrate the catalytic site with Lysine 489 (stick representation). Both the AID and the KA1 domain were also modelled but it appeared not to be conserved. Figures were generated with PyMol (http://www.pymol.org). c, Schematic for the generation of *P. berghei* KIN and KIN(T616) mutant. Double crossover recombination at 5′ and 3′ UTR was used to genetically delete endogenous *kin* and complement with codon-altered *kin* gene encoding wild-type (KIN(T616)) or phosphomimetic mutation (KIN(T616D)). Codon-altered sequence of *kin* was obtained from GenScript, which was then used in the site-directed mutagenesis reaction to introduce the T616D mutation. Presence of codon-altered *kin* and *kin*<sub>T616D</sub> was confirmed by sequencing of the locus of the transgenic parasites. Annealing sites for genotyping primers are illustrated and primer sequences are given in Supplementary Table 1. d, Agarose gel image (representative of 2) showing diagnostic PCR products from *P. berghei* KIN(T616), KIN(T616D) and parental wild-type. e, Survival of C57BL/6 mice infected by i.p. injection (1 × 10<sup>6</sup> iRBCs per mouse) of *P. berghei* KIN(T616) (*n* = 9) and KIN(T616D) (*n* = 9). f, Complementation of the Δsnf1 yeast mutant with GFP-fused yeast-optimized sequences of N-terminal truncated *P. berghei* kin, *kin*<sub>T616D</sub> or yeast *snf1* without GFP tag. Truncation is indicated with ‘t’. Growth of transformed Δsnf1 cells in culture, inoculated at a density of 0.05 OD<sub>600</sub> in s.d. medium supplemented with glucose or raffinose as a carbon source and grown for 42 h. Shown are OD values obtained in the raffinose condition, normalized to those obtained in glucose condition for each cell line (*snf1* *n* = 6, t.**kin**, *n* = 6, t.**kin**<sub>T616D</sub> *n* = 6; mean ± s.e.m.; Mann–Whitney test). g, Western blot analysis of Δsnf1 expressing full length (left) or N-terminal truncated (right) KIN(T616)–GFP or KIN(T616D)–GFP. Predicted size of full-length KIN–GFP is 147 kDa and of N-terminal truncated version is 93 kDa. Membranes were probed with anti-GFP antibody. A representative blot from 2 independent lysates is shown.
Extended Data Figure 6 | Effect of AMPK agonists. a, Dose-dependent effect of AMPK activators (salicylate and A769662) on \( P.\) \( b e r g h e i \) ANKA that express luciferase (under \( a m a 1 \) schizont-specific promoter). Parasites were cultured for 24 h with increasing concentrations of the compounds (media supplemented with 20% FBS and 5 mM glucose). Analysis of schizont development was performed by measuring luminescence. Values are mean ± s.e.m. (salicylate, \( n = 5 \); \( A 7 6 9 6 6 2 , n = 6 \)). EC\(_{50}\) values determined by using GraphPad Prism nonlinear regression variable slope (normalized) analysis. The calculated EC\(_{50}\) values are as follows: salicylate, 2.4 ± 0.9 mM; \( A 7 6 9 6 6 2 , 2 5 6 . 5 ± 6 0 . 6 \mu M \). b, Dose-dependent effect of \( A 7 6 9 6 6 2 \) on \( P.\) \( b e r g h e i \) ANKA wild-type, \( \Delta k i n \) and complemented parasites analysed by microscopy after Giemsa staining (Mann–Whitney test). Box plots show the data for the following number of schizonts (vehicle, \( A 7 6 9 6 6 2 \) 62.5 and 125 μM): \( P.\) \( f a l c i p a r u m \), 59, 18, 48; wild-type \( P.\) \( b e r g h e i \), 44, 47, 40; \( \Delta k i n + k i n \), 34, 57, 37. c, Dose-dependent effect of \( A 7 6 9 6 6 2 \) and salicylate treatments on \( P.\) \( f a l c i p a r u m \) for two developmental cycles. Synchronized cultures were set at 0.1% initial parasitaemia (rings) and analysed at 48 h and 96 h by flow cytometry after SYBR Green labelling of parasite DNA. A new generation of rings was observed at 48 and 96 h in the treated conditions, suggesting no growth delay. Data (mean ± s.e.m.) was normalized to the untreated control on each experiment at 48 h or 96 h. The EC\(_{50}\) values (determined as in a) are as follows: 133.1 ± 3.4 μM at 48 h (\( n = 6 \)) and 70.1 ± 18.9 μM at 96 h (\( n = 5 \)) for \( A 7 6 9 6 6 2 \); 2.2 ± 0.2 mM at 48 h (\( n = 6 \)) and 1.25 ± 0.2 mM at 96 h (\( n = 6 \)) for salicylate. d, qPCR analysis of \( P.\) \( f a l c i p a r u m \) parasites treated with salicylate for 72 h (\( n = 2 \) per condition). Data normalized to the untreated control. The genes analysed correspond to the \( P.\) \( b e r g h e i \) homologues experimentally validated in Extended Data Fig. 3. Gene IDs are shown in the figure without the ‘PF3D7_’ prefix. e, Dose-dependent effect of salicylate on other \( P.\) \( b e r g h e i \) ANKA kinase mutants. Box plot of parasites treated and analysed as in b (Mann–Whitney test). The number of schizonts analysed for vehicle, 0.6 mM and 1.25 mM are as follows: \( \Delta n e k 2 , 4 3 , 3 3 , 3 7 ; \Delta c d p k 3 , 3 5 , 4 4 , 3 0 . f, g, \) Salicylate effect \( i n v i v o \). Mice were treated daily with 250 mg kg\(^{-1}\) salicylate (sal) or 0.9% NaCl (vehicle, veh) starting at day 1 after infection. Parasitaemia (mean ± s.e.m.; two-way ANOVA test) and survival (log-rank Mantel–Cox test) of C57BL/6 mice infected by i.p. injection of \( 1 \times 10^8 \) wild-type (veh \( n = 1 5 \); sal \( n = 1 6 \)), \( \Delta k i n \) (veh \( n = 8 \); sal \( n = 8 \)) and complemented \( \Delta k i n + k i n \) iRBCs (veh \( n = 1 0 \); sal \( n = 1 0 \)).
Extended Data Figure 7 | Supplementation studies in vitro and in vivo. a, Effect of supplementation with extra glucose, vitamins, essential amino acids (EAA), non-essential (NEAA), leptin and iron (FeSO₄) on salicylate- or A769662-treated parasites. *P. berghei* expressing luciferase at schizont stage were cultured in 20% FBS supplemented medium (5 mM glucose, n = 5) with increasing concentrations of salicylate and extra glucose (50 mM, n = 5), vitamins (1 ×, n = 3; 5 ×, n = 2), EAA (100 μM, n = 3; 500 μM, n = 2), NEAA (100 μM, n = 3; 500 μM, n = 2), and leptin (50 ng ml⁻¹, n = 2; 150 ng ml⁻¹ n = 2). FeSO₄ was added in the presence of equal amount of ascorbic acid (AA), an iron absorption enhancer. A769662 treatments were conducted in 20% FBS supplemented medium (5 mM glucose, n = 5) and leptin (50 ng ml⁻¹, n = 2; 150 ng ml⁻¹ n = 2). Analysis of schizont development was performed by measuring luciferase activity as in Extended Data Fig. 2f. Values normalized to the vehicle control. Replicates are shown as individual data points for all conditions except 50 mM glucose, AA and salicylate alone, which are shown as mean ± s.e.m. Salicylate EC₅₀ values in these experiments were 3.4 ± 0.3 mM and 5.7 ± 1.3 mM for 5 and 50 mM glucose, respectively (P = 0.0081, two-way ANOVA test). b, Box plot of microscopic analysis of *P. berghei* ANKA wild-type segmented schizonts obtained in vitro after 24 h maturation in the presence of AL or CR sera. Recombinant leptin was added to the culture medium at the indicated concentration. Total number of schizonts analysed in two independent experiments as follows: AL, 87; CR, 85; AL + Leptin, 69; CR, 86 (Mann–Whitney test). c, Dose-dependent effect of salicylate and A769662 on *P. berghei* parasites in the presence of AL or CR sera. Parasites were incubated for 24 h with increasing concentrations of the compounds in media supplemented with 10% of AL or CR sera (glucose 5 mM). Analysis of schizont development was performed by measuring luminescence (A769662, n = 3; salicylate, n = 5). These compounds appear to have additive effects, suggesting that under CR conditions, A769662/salicylate might activate KIN through distinct and/or complementary mechanisms. This could be related to different binding sites on the kinase, as previously demonstrated for other AMPKs²¹,²². d, Effect of glucose supplementation on salicylate-treated *P. falciparum* parasites. Ring-stage synchronized cultures were set at 0.1% infected blood and 0.1% parasitemia after 4 days of culture. Parasite populations treated with glucose (50 mM glucose) were compared to parasite populations treated with glucose free medium supplemented with 5 and 25 mM glucose. Values in the graph are parasitaemia (mean ± s.e.m.; n = 5 per condition) determined by flow cytometry. Shown is parasite relative gene expression (normalized to the mean of the correspondent AL condition at day 5, AL = C57BL/6 male mice in AL or CR) with glucose in drinking water. Shown is parasite relative gene expression in CR normalized to the mean of the correspondent AL condition at day 5 after i.p. infection. Each mouse is plotted as an individual data point. Representative of two independent experiments (n = 3 per group). Each mouse is plotted as an individual data point. g, qPCR analysis of *P. berghei* wild-type parasites in AL- or CR-fed C57BL/6 mice supplemented (diamonds; n = 5) or not (circles; n = 4) with glucose in drinking water. Shown is parasite relative gene expression in CR normalized to the mean of the correspondent AL condition at day 5 after i.p. infection. Each mouse is plotted as an individual data point. Representative of two experiments performed independently. Gene IDs are given without the ‘PBANKA_’ prefix as in Extended Data Fig. 3e.
Extended Data Figure 8 | Schematic representation of the observed effect of dietary nutrients or AMPK agonists on *Plasmodium* intraerythrocytic replication. The data supports the idea that parasites can replicate in higher or fewer numbers depending on host nutrient availability. This active parasite response mediated by an AMPKα-related kinase, KIN, which is expected to become active by an increase of the AMP/ATP ratio in parasites facing nutrient deficiency. KIN upstream regulators and downstream targets remain to be determined, as well as other potential molecular factors that might also contribute to this nutrient sensing mechanism.