Plasmodium UIS3 sequesters host LC3 to avoid elimination by autophagy in hepatocytes

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The causative agent of malaria, Plasmodium, replicates inside a membrane-bound parasitophorous vacuole (PV), which shields this intracellular parasite from the cytosol of the host cell. One common threat for intracellular pathogens is the homeostatic process of autophagy, through which cells capture unwanted intracellular material for lysosomal degradation. During the liver stage of a malaria infection, Plasmodium parasites are targeted by the autophagy machinery of the host cell, and the PV membrane (PVM) becomes decorated with several autophagy markers, including LC3 (microtubule-associated protein 1 light chain 3). Here we show that Plasmodium berghei parasites infecting hepatic cells rely on the PVM transmembrane protein UIS3 to avoid elimination by host-cell-mediated autophagy. We found that UIS3 binds host LC3 through a non-canonical interaction with a specialized surface on LC3 where host proteins with essential functions during autophagy also bind. UIS3 acts as a bona fide autophagy inhibitor by competing with host LC3-interacting proteins for LC3 binding. Our work identifies UIS3, one of the most promising candidates for a genetically attenuated vaccine against malaria, as a unique and potent mediator of autophagy evasion in Plasmodium. We propose that the protein-protein interaction between UIS3 and host LC3 represents a target for antimalarial drug development.

One key function of microtubule-associated protein 1 light chain 3 (LC3) is to facilitate the delivery of autophagic membranes to lysosomes. Intriguingly, most LC3-decorated hepatic parasites seem to escape this fate. This implies that Plasmodium exo-erythrocytic forms (EEFs) actively disrupt the autophagic flux to avoid the deleterious effects of fusing the vacuole with lysosomes. The parasitophorous vacuole membrane (PVM) is ideally positioned to carry out this task, and several genetic studies point to the critical contribution of PVM-resident proteins to parasite survival. Nevertheless, a putative autophagy subversion activity in Plasmodium has yet to be discovered. Of the few PVM proteins identified thus far, UIS3, has the greatest impact on Plasmodium survival inside host hepatocytes. Parasites lacking UIS3 (uis3−) infect host cells, but disappear rapidly thereafter and fail to complete development. To determine whether UIS3 influences Plasmodium’s susceptibility to host autophagy, we allowed uis3− and uis3+ P. berghei parasites to infect HepG2 cells that had been depleted of Atg5 or Rab7 to arrest the autophagic flux in early (LC3 membrane conjugation) and late (lysosomal fusion) stages, respectively. Consistent with previous reports, uis3− parasites were virtually undetectable in control HepG2 cells, and the few hepatic schizonts observed 65 h after infection did not express MSP1, the merozoite surface marker seen in fully mature uis3(+) parasites (Fig. 1a,b). Strikingly, depletion of Atg5 or Rab7 in HepG2 cells was sufficient to fully revert the phenotype of uis3− mutants to a wild-type one (Fig. 1a,b and Supplementary Fig. 1c). With the autophagy flux of the host cell arrested, uis3− parasites behaved in the same way as their uis3(+) counterparts with respect to schizont abundance as well as MSP1 expression (Fig. 1a,b and Supplementary Fig. 1c). Parasites that complete the liver stage successfully induce the release of blood-infective merozoite-filled sacs (merosomes) from infected cells. To test the viability of the uis3− progeny released by HepG2-infected cells, we collected the cell culture supernatants (HepG2 SN) 65 h after infection, inoculated them into mice and monitored the onset of blood-stage infection by collecting blood samples daily (Fig. 1a). HepG2 SN from Atg5- or Rab7-depleted cells infected with uis3− mutants caused patent blood-stage infections within 4–5 days of mouse inoculation, similar to mice inoculated with HepG2 SN derived from uis3(+)-infected cells (Fig. 1d-f). In stark contrast, HepG2 SN collected from control uis3− infected cells did not cause infection in mice (Fig. 1d-f). Thus, loss of autophagy during the liver stage fully restores uis3− infectivity. Additionally, the survival rates of uis3− and uis3+ parasites in Atg3, Atg5 or Atg7 knockout mouse embryonic fibroblasts (MEFs) were also identical, whereas few uis3− mutants survived in wild-type MEFs (Fig. 1g). To obtain additional evidence that uis3− parasites are removed by host autophagy, newly invaded hepatoma cells were treated with chloroquine (CQ), a lysosomotropic alkalizing agent that impairs the lysosomal degradation of autophagic cargo. CQ treatment, like genetic impairment of autophagy, rescued uis3− mutants (Fig. 1h). On rare occasions we were able to observe uis3− parasites that exhibited distinctive signs of having undergone fusion with LAMP1-positive lysosomes, most prominently, the presence of LAMP1 within the vacuole and loss of the PVM marker, UIS4 (Supplementary Fig. 1d). Thus, we conclude that uis3− mutants normally fail to establish liver-stage infections because they are promptly eliminated by host autophagy and that the critical function of UIS3 is to protect Plasmodium from this innate defence mechanism.

Next, we aimed to elucidate how UIS3 interferes with host autophagy. To that end, we complemented uis3− parasites with the UIS3 gene fused C-terminally to c-Myc (this parasite line will be referred
**Fig. 1** *Plasmodium UIS3 protects liver-stage parasites from host autophagy.* **a,** Timeline of HepG2 infection and cell supernatant (HepG2 SN) transfer into C57BL/6 mice. **b, c,** HepG2 cells treated with siRNAs to silence Atg5 or Rab7 expression and infected with *uis3* (+) or *uis3* (−) parasites were fixed 65 h after infection and immunostained with anti-MSP1 (red), anti-PbHsp70 (green) and Hoechst (blue). Panel **b** shows differentiated progeny surrounded by a ring of MSP1. Scale bar, 10 μm. Panel **c** shows the number of exo-erythrocytic forms (EEFs), quantified by immunofluorescence (IF) microscopy. Mean ± s.e.m. of pooled replicates from two (Atg5, Rab7) to four (control) independent experiments normalized to the control. **d**, Representative images of Giemsa-stained blood smears from C57BL/6 mice 10 days after receiving the indicated HepG2 SN. Animals that received control *uis3* (−) HepG2 SN remained parasite free up to 30 days after inoculation. Data represent the per cent of mice (out of 5 to 10; two independent experiments) with positive blood smears. **e**, Onset of blood-stage parasitaemia following inoculation of C57BL/6 mice with the indicated HepG2 SN. Wild-type and knockout *uis3* genomic loci were amplified with specific primer sets. One representative mouse from each experimental group is shown. **f**, Genotype of blood-stage parasites collected 10 days after mice inoculation with the indicated HepG2 SN. Wild-type and knockout *uis3* genic loci were amplified with specific primer sets. One representative mouse from each experimental group is shown. **g, h,** Huh7 cells treated with chloroquine (CQ) from 1 h to 24 h after infection with *uis3* (+) or *uis3* (−) parasites were immunostained with anti-PbUIS4, anti-PbHsp70 and Hoechst. The numbers of EEFs were quantified by microscopy in three independent experiments. Statistical significance was assessed using non-parametric two-tailed Mann-Whitney test. NS, *P > 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
to as uis3-myc) (Supplementary Fig. 2). Immunofluorescence analysis of liver-stage schizonts revealed that UIS3-myc co-localized with host LC3 and UIS4 to the PVM (Fig. 2a,b and Supplementary Fig. 3a,b). Of note, immuno-electron microscopy showed LC3 in direct association with the PVM, with no evidence of autophagosomal double membranes—a hallmark of canonical autophagy—forming around the PV (Fig. 2c). Prompted by these observations, we asked whether UIS3 might form a molecular complex with LC3. We allowed uis3-myc parasites to infect HeLa cells stably expressing GFP-LC3 and, 24 h after infection, proceeded to immunoprecipitate UIS3-myc. Strikingly, GFP-LC3 was found to co-immunoprecipitate with UIS3 (Fig. 2d). We further confirmed this association in P. berghei-infected mouse primary hepatocytes (Supplementary Fig. 3c), thus ruling out the possibility of a potential artefact linked to GFP-LC3 over-expression. LC3 immunoprecipitation could not be detected in cells infected with uis3(−) mutants, which do not express UIS3 (Fig. 2d and Supplementary Fig. 3c). Next, we used recombinant UIS3 and LC3 to test whether the two proteins directly

Fig. 2 | Plasmodium UIS3 binds to host LC3. a, Timeline of in vitro infection and analysis. b, Representative confocal images (two independent experiments) of Huh7 cells infected with P. berghei expressing UIS3-myc (uis3-myc). Cells were fixed 24 h (top) and 48 h (bottom) after infection and immunostained with anti-myc (white), anti-UIS4 (red), anti-LC3 (green) and Hoescht (blue). Scale bars, 10 μm. c, Representative immunoelectron microscopy (IEM) micrograph of the host–parasite interface in a Huh7 cell 24 h after infection with P. berghei. PbUIS4 and host LC3 are shown labelled with 12 nm (arrowhead) and 18 nm (arrow) gold particles, respectively. The EEF cytosol is marked with an asterisk. The PbUIS4-positive structures correspond to the PVM and tubular-vesicular network (TVN) of the parasite. Scale bar, 200 nm. Seventeen randomly selected cells were analysed. d, Lysates of GFP–LC3 HeLa cells infected with uis3-myc or uis3(−) P. berghei were immunoprecipitated with anti-myc. Analysis of the precipitates was carried out by western blot with anti-LC3 and anti-myc antibodies (top and middle panels). Total cell lysates (TCL) were immunoblotted with anti-LC3 (bottom panel). e, Purified GST or GST-LC3 were used as bait to pull down recombinant His-tagged sPfUIS3. Pull-down fractions were immunoblotted with anti-GST and anti-His antibodies. One of four independent experiments is shown (d,e). f, Binding of GST–LC3 to sPfUIS3 immobilized on a sensor chip was analysed by surface plasmon resonance (SPR). The indicated concentrations of GST-LC3 were perfused over the sPfUIS3-coated sensor. Normalized response sensorgrams were calculated by subtracting the unspecific binding contribution of the same molar concentrations of GST alone. g, SPR response units (RU) at equilibrium plotted as a function of LC3 concentration. The dissociation constant of the interaction, $K_D$, is shown.
associate. Full-length UIS3 comprises a signal peptide, a short intravacular N-terminal domain, a transmembrane anchor and a C-domain that spans residues 83–229 and is predicted to be exposed to the host cytosol. Previous studies reported that the C-terminal domain of P. falciparum UIS3 is prone to dimerization, but undergoes proteolytic cleavage in solution, yielding a stable and soluble monomeric fragment consisting of residues 130–229 (hereafter referred to as soluble UIS3, sPfUIS3)\(^\text{15}\). Using recombinant GST-LC3, we were able to pull down recombinant His-tagged sPfUIS3 (Fig. 2c). The dissociation constant \((K_d)\) for the complex measured by surface plasmon resonance was 0.244 \mu M (Fig. 2f,g). Despite being co-localized with both UIS3 and UIS4 on the PVM, LC3 was shown to co-immunoprecipitate with sPbUIS3, but not with the corresponding C-terminal domain of PbUIS4 (Supplementary Fig. 3d). We also did not observe co-immunoprecipitation of L-FABP with recombinant sPfUIS3 (Supplementary Fig. 3e), in line with previous work suggesting that the reported interaction between the C-terminus of Plasmodium yoelii UIS3 and mouse L-FABP (ref. \(^\text{14}\)) might be species-specific\(^\text{15}\). Collectively, these data demonstrate that the distal C-terminal subdomain of UIS3, spanning amino acids 130–229, binds to LC3 directly. Our data establish that UIS3 forms a molecular complex with LC3, but the association of LC3 with the PVM was found to be host-driven (two core components of the LC3 conjugation system transfer LC3 in a ROS-dependent manner to pathogen-containing phagosomes\(^\text{18}\)). Intriguingly, the pathway leading to Plasmodium targeting by autophagy appears to

Fig. 3 | Non-canonical binding of Plasmodium UIS3 to the LIR pocket of LC3. a–d, Representations of the putative complex between the soluble domain of UIS3 and LC3 obtained by molecular docking. The LC3 areas depicted in green and orange represent the two hydrophobic pockets responsible for the recognition of LIR motifs and overlap significantly with the surface recognized by UIS3 (Supplementary Fig. 5d,e). All key interacting amino acids are highlighted in black (LC3) and red (UIS3). P. falciparum (a,b) and P. berghei complexes (c,d) are shown. e–f, Lysates of GFP-LC3 HeLa cells expressing the indicated sPfUIS3 (e) or sPbUIS3 (f) myc-tagged proteins (amino acid substitutions shown on the left) were immunoprecipitated with anti-myc. Precipitates were analysed by western blot with anti-LC3 (top panels) and anti-myc (middle panels). TCL were immunoblotted with anti-LC3 (bottom panels). g, Relative contribution of single amino acid substitutions (shown on the left) to the binding of luciferase-tagged sPfUIS3 to recombinant GST-LC3. Inset: outline of the pulldown assay. The average \± s.e.m. of two independent experiments is shown. h, CD spectra of sPfUIS3 recombinant proteins (wild-type and indicated mutants). Each spectrum is the average of 10 technical replicates, corrected for the buffer background. i, Immunoprecipitation of myc-tagged sPfUIS3\(^\text{17}\) from HeLa cell lysates co-expressing the indicated HA-tagged LC3 proteins (amino acid substitutions shown on top). Precipitates were probed with anti-HA (top panel) and anti-myc (middle panel). The bottom panel shows the levels of LC3 in TCL. One representative immunoblot of at least three independent experiments is shown in e, f and i. Statistical significance was assessed using an unpaired two-tailed t-test. *\(P<0.05; **P<0.01; ****P<0.0001\).
Fig. 4 | UIS3 acts as a bona fide autophagy inhibitor by competing with host LC3-interacting proteins for LC3 binding. a, Western blot analysis of p62 levels in HeLa cells expressing the indicated sPbUIS3 proteins and kept for 8 h in complete (control) or amino-acid-depleted medium (amino acid starvation). Whole-cell lysates were analysed by western blot for p62, tubulin and UIS3-myc. Immunoblots representative of five independent experiments are shown. b, The levels of p62 before and after amino acid starvation were calculated as the ratio of p62 to tubulin. Bars represent mean ± s.e.m. of five independent experiments. c, Outline of LUMIER competition assay. The assay measures the amount of p62 LIR peptide necessary to displace luciferase-tagged p62 or sPfUIS3 from their complexes with recombinant GST–LC3. Inset: input levels (detected with anti-Renilla luciferase) of luciferase-tagged p62 and sPfUIS3 in one (of three) representative experiments. d, Binding of luciferase-tagged p62 or sPfUIS3 to recombinant GST–LC3 in the presence of the indicated concentrations of p62 LIR. The mean ± s.e.m. of three independent experiments is shown. For the data points corresponding to 2 and 5 µM of p62 LIR, n = 2. e, Recombinant GST–LC3 was used to pull down extracts of HEK 293T cells co-transfected with the indicated plasmids. The ratio of PLEKHM1 and sPfUIS3 in the pulldown versus input was calculated from the GFP and luminescence signals of each fraction. The bottom panels show the levels of PLEKHM1 and UIS3 in the input extract. Bars represent average ± s.e.m. of three independent experiments. Statistical significance was assessed using unpaired two-tailed t-tests in b and e and two-way ANOVA in d. NS, P > 0.05; ***P < 0.001; ****P < 0.0001. f, Summary of the observed effects of Plasmodium UIS3 on host autophagy. Antiplasmodial autophagy depends on the LC3 conjugation machinery and on lysosome-associated Rab7, with unidentified LIR-motif proteins mediating one or several steps leading to lysosomal fusion. The C terminus of UIS3 binds to the LIR-docking site on LC3, rendering the site inaccessible for autophagy-promoting interactions.
be inhibited byULK1. Indeed, we observed significantly higher levels of PVM-associated LC3 upon knockdown ofULK1 expression (Supplementary Fig. 4f). These results confirm the unconventional nature of the host autophagy response against Plasmodium and imply that the association of LC3 to the PVM precedes the UIS3–LC3 interaction.

Whereas LC3 binding partners typically contain an LC3-interacting region (LIR) with the consensus sequence W/F-x-x-x/I/L/V (refs 19,20), UIS3 lacks this canonical LIR (Supplementary Fig. 5a). To gain further insight on the specific mode of binding of UIS3 to LC3, we modelled the three-dimensional structure of the putative complex between sPfUIS3 or sPbUIS3 and LC3 using a molecular docking algorithm and the published X-ray structures of sPfUIS3 (ref. 15) and LC3B (ref. 22) (Fig. 3a-d and Supplementary Fig. 5). Our in silico analysis predicted that the interaction is likely to be driven by a flexible turn between helices α2 and α3 of UIS3 (N181, M182 and E183 in P. falciparum UIS3) and the β2 strand of LC3 (Fig. 3a,b). The complex appears also to be stabilized by polar interactions involving K213 and Q217 (Fig. 3b). In the P. berghei complex, the residues located in equivalent positions are D173, Y174 and D175, with E205 and K209 stabilizing the complex through electrostatic interactions (Fig. 3c,d). To validate these predictions, we substituted the above amino acids for alanine to measure the effect of the substitutions on UIS3 binding to LC3. Simultaneous or individual mutations on all five residues abolished the interaction of sPfUIS3 with LC3 (Fig. 3c,g). Similarly, alanine substitutions of the equivalent positions in sPbUIS3 abrogated binding to LC3 almost completely (Fig. 3i). Of note, the circular dichroism (CD) spectra of recombinant sPfUIS3 proteins harbouring the above mutations did not reveal any significant effect of the amino acid substitutions on protein conformation (Fig. 3h). Detailed analysis of the predicted LC3–UIS3 complexes from P. berghei and P. falciparum (Fig. 3a-d and Supplementary Fig. 5d,e) indicated that the LC3 surface where UIS3 putatively binds coincides with the surface recognized by autophagy proteins containing canonical LIR motifs, such as p62 (refs 22,23). Mutations of key residues in this LIR-binding LC3 surface to alanine abolished the interaction between sPfUIS3 and LC3 (Fig. 3j), as predicted. Thus, although UIS3 lacks a canonical LIR, our data demonstrate that the LIR-binding surface of LC3 can accommodate this atypical interaction.

Because UIS3 and canonical LIR motifs interact with the same region of LC3, we next hypothesized that UIS3 might interfere with the binding of LIR-containing proteins to LC3, thus interrupting the autophagic flux. To examine whether the UIS3–LC3 interaction represents a physiological autophagy subversion mechanism, we quantified the impact of heterologous UIS3 expression on the autophagic flux of HeLa cells. As a readout for autophagy, we monitored p62 degradation in response to amino acid starvation22,24. Strikingly, sPfUIS3WT inhibited the degradation of p62 induced by amino acid depletion, whereas UIS3 mutants had no effect (Fig. 4a,b). The data demonstrate that binding of UIS3 to LC3 disrupts the autophagic flux, hinting at the possibility that UIS3 functions as an autophagy inhibitor, through a hindrance effect on the LC3 surface. To explore this hypothesis, we set up a LUMIER-based competition assay22 in which sPfUIS3 or p62 was complexed to GST–LC3 and subsequently incubated with increasing concentrations of the p62 LIR (ref. 22) (Fig. 4c). We found that higher concentrations of p62 LIR were necessary to disrupt the UIS3–LC3 complex compared to p62–LC3 (Fig. 4d). This is consistent with the lower $K_{d}$ measured for the UIS3–LC3 interaction (Fig. 2g) compared to similar measurements for canonical LIR sequences22. To determine how pervasive the UIS3 hindrance effect was, we examined the interaction of sPfUIS3 on the association between the LIR-containing Rab7 effector PLEKH1M1 (ref. 2) and recombinant GST–LC3. When sPfUIS3WT and PLEKH1M1 were co-expressed, LC3 pulldowns were significantly depleted of PLEKH1M1 and enriched in UIS3, whereas mutations that reduced the affinity of UIS3 for LC3 restored the PLEKH1M1–LC3 association (Fig. 4e). Taken together, these observations not only demonstrate that Plasmodium UIS3 antagonizes LIR-mediated LC3 interactions by binding to a conserved LIR-interacting surface on LC3, but they also confirm UIS3 as a bona fide autophagy inhibitor.

In this study, we show that Plasmodium parasites escape autophagy by engaging host LC3 in an inhibitory interaction with the cytosolic domain of the PVM transmembrane protein UIS3. Our observations demonstrate that UIS3 competitively inhibits the binding of LIR-containing host proteins to the LIR-anchoring surface of LC3. This strongly suggests that the UIS3-mediated protection against antiplasmodial autophagy is a direct consequence of LC3 being sequestered into UIS3–LC3 complexes on the PVM (Fig. 4f and Supplementary Fig. 6). Although UIS3 is necessary to avert the potential deleterious effects of the association of LC3 to the PVM, whether Plasmodium succeeds in evading autophagy probably depends on how much of the PVM-associated LC3 is bound by UIS3. This, in turn, is determined by the levels of UIS3 expression and the robustness of the antiplasmodial response, both of which may diverge significantly between individual parasites and hosts, and could explain the vulnerability of P. berghei parasites to autophagy observed in previous reports22.

Evidence that interferon (IFN)-γ enhances LC3 recruitment onto the PVM of Plasmodium vivax, causing parasite elimination25, supports the idea that host autophagy may be chemically enhanced to suppress the protective effect of UIS3 expression. Repurposing autophagy-inducing drugs for malaria prophylaxis might thus be a safe and cost-effective way of breaking the transmission cycle in endemic areas. Whether clinically available autophagy inducers activate the non-canonical autophagy pathway targeting Plasmodium in hepatic cells remains to be determined. Of note, neither metformin nor rapamycin, two of the best known autophagy-inducing drugs, affect the number of infected cells in vitro or in vivo26,27, supporting our observations that antiparasitic autophagy is mechanistically distinct from the AMPK- and mTOR-regulated canonical autophagy pathway.

Recent reports underscored the risk of breakthrough infections in mice immunized with a $\mu$s3(−) P. berghei ANKA whole-organism vaccine28. The data presented here suggest that host genetic or metabolic factors compromising the autophagy response might be the cause of such breakthrough infections. Although insufficiently safe on its own, UIS3 deletion is likely to improve the safety of any genetically attenuated whole-organism vaccine against malaria by enhancing the susceptibility of hepatic parasites to host autophagy. Additionally, with the spread of drug resistance casting a shadow over malaria eradication efforts, the protein–protein interaction between UIS3 and LC3 constitutes a promising new target for tailored pharmacological interventions aiming at controlling Plasmodium liver infection and disease.

Methods

Chemicals. RPMI 1640, DMEM, EBSS and other cell culture reagents were purchased from Gibco Invitrogen. All chemicals were from Sigma-Aldrich, unless specified otherwise. Lipofectamine (Invitrogen) was used for transfection of HepG2 cells. siRNAs were transfected using Lipofectamine RNAiMAX in OptiMEM (Invitrogen).

Cell lines. Huh7, HepG2 and HEK 293T (ATCC) cells were cultured under standard conditions in RPMI 1640 (Huh7) or DMEM (HepG2 and HEK 293T) medium supplemented with 10% FCS, 1% non-essential amino acids (RPMI 1640), 1% glutamine, 1% penicillin/streptomycin, and 1% Hepes. Mouse embryonic fibroblasts (MEFs) were obtained from the Riken BioResource Center (Ag$_{5}{\text{A}}$ (RC27110) and Ag$_{5}{\text{B}}$ (RC27211))29, M. Komatsu, Tokyo Metropolitan Institute of Medical Science (Ag$_{5}{\text{A}}$ and Ag$_{5}{\text{B}}$)30 and T. Finkel, NIH National Heart, Lung, and Blood Institute (Ag$_{5}{\text{A}}$ and Ag$_{5}{\text{B}}$). HELa cells expressing GFP–LC3 were a gift from T. Hyman, MIP–CRC. MEFs and HEla cells were maintained in DMEM supplemented as before. MEFs were validated through quantitative reverse transcription PCR (qRT–PCR) and HEla cells were tested for the expression of
GFP–LC3 by western blot. All cell lines were routinely tested for mycoplasma contamination.

**Mice.** All mice in this study were C57BL/6j males, between 6 and 8 weeks of age, housed in the animal facility of the Instituto de Medicina Molecular in Lisbon. All protocols were approved by the internal animal care committee of the Instituto de Medicina Molecular and were performed according to national and European regulations.

**Mouse primary hepatocytes.** Mouse primary hepatocytes were isolated using a modified two-step perfusion protocol followed by a Percoll purification step. Briefly, mice were sacrificed by CO₂ inhalation and immediately processed for cannulation of the portal vein using a 26-gauge needle. Next, the inferior vena cava (IVC) was cut to allow fluid to drain. Liver perfusion medium (LPM) was perfused at 8–9 ml min⁻¹ for 30 s followed by liver digestion medium (LDM) also at a rate of 8–9 ml min⁻¹ for 10 min. Intermittent clamping of the IVC (3 s clamp every 30 s) was performed during LDM perfusion to improve tissue digestion. After digestion, the liver was excised and the cells were liberated by tearing and shaking of the liver with forceps. The cell suspension was then sequentially filtered through a 100 μm and a 70 μm cell strainer and spun at 50g for 3 min. The cell pellet was resuspended in William’s Medium E with 10% of fetal calf serum (FBS) and carefully overlaid on a 60% Percoll solution (1:1). The cell suspension was fractionated by centrifugation at 750g for 20 min, without break, at 20 °C. Viable hepatocytes deposited in the pellet were washed with William’s Medium E with 10% FBS, spun at 50g for 3 min and resuspended in complete William’s Medium E (supplemented with 4% FBS and 1% penicillin/streptomycin). Viability and yield were assessed by trypan blue staining. Viable hepatocytes were plated on collagen-coated 24-well plates.

**RNAi.** HepG2 cells were reverse-transfected with target-specific SMARTpool siRNAs, as previously described. Non-targeting SMARTpool siRNA was used as a negative control (Dharmacon, GE Healthcare). The efficiency of knockdown was assessed 48 h after transfection by qRT–PCR using kits from Applied Biosystems. Gene expression levels were normalized against those of hypoxanthine guanine phosphoribosyltransferase (Hprt1). The primer pairs used for qRT–PCR reactions are listed in Supplementary Table 3.

**Parasite lines.** P. berghei sporozoites were obtained through dissection of the salivary glands of infected female Anopheles stephensi mosquitoes bred at the Instituto de Medicina Molecular. The following parasite lines were used: GFP-expressing P. berghei ANKA (2932cl, Leiden Malaria Research Group), uis3(–), uis3(+), and uis3-myc P. berghei NK65 (generated for this study).

**Plasmodium transfection.** P. berghei uis3-myc and uis3(+) were obtained by complementing the uis3(–) parasite line via single crossover recombination as described in Supplementary Fig. 2. Transfection was carried out by electroporation of purified schizonts as previously described. Parasites were harvested at 36 h, washed with fresh medium (Wilms medium) and cultured for another 65 h in standard medium supplemented with 0.3% Fungizone. At 65 h, the entire volume of cell culture was used as a negative control (Dharmacon, GE Healthcare) during the whole time period. The smears were analysed daily from day 1 to day 10 after transfection, each sample being scored by two independent observers. The smears were analysed daily from day 1 to day 10 after inoculation, when most animals suffered from hyperparasitaemia and had to be killed. On day 10, parasite genomic DNA was extracted from the blood of each experimental animal for parasite genotyping. The primer pairs used to probe the uis3 wild-type and knockout genomic loci are listed in Supplementary Table 2.

For ROS-scavenging experiments, Huh7 cells were incubated for 2 h in complete medium supplemented with 20 μM N-acetylcysteine (NAC), before immunofluorescence. Chlороquine (50 μM) was added to hepatoma cells 1 h after sporozoite infection.

**Infections.** Hepatoma cells, MEFs and mouse primary hepatocytes were plated on 24-well culture plates at a density of 65,000, 50,000 and 120,000, per well, respectively. Cells were infected with freshly dissected P. berghei sporozoites (30,000 per well) 1 day after seeding and processed for analysis at 24 h or 48 h. siRNA-treated HepG2 cells were infected with uis3(+) or uis3(–) sporozoites 36 h after siRNA transfection and cultured for another 65 h in standard medium supplemented with 0.3% Fungizone. At 65 h, the entire volume of cell culture medium (HepG2 SN) in each well was collected and immediately inoculated into mice (1 well for each animal). The onset of blood-stage parasitaemia was monitored through Giemsa-stained thin blood smears by two blinded independent observers. The smears were analysed daily from day 1 to day 10 after inoculation, when most animals suffered from hyperparasitaemia and had to be killed. On day 10, parasite genomic DNA was extracted from the blood of each experimental animal for parasite genotyping. The primer pairs used to probe the uis3 wild-type and knockout genomic loci are listed in Supplementary Table 2.

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**Plasmids.** The plasmids used in this study are listed in Supplementary Table 1. All Plasmodium sequences (except single point mutants) and human LC3B were chemically synthesized and subcloned into pCMV-Myc-N or pCMV-HA-N expression vectors (Clontech) using the EcoRI and KpnI restriction sites. GeneArt, Invitrogen). The Rennil luciferase sequence was chemically synthesized (GeneArt, Invitrogen) and subcloned into pCMV-Myc–puU13 using the EcoRI restriction site. Single point mutants of puU13 were obtained by site-directed mutagenesis using the primers listed in Supplementary Table 4 and the QuickChange II site-directed mutagenesis kit (Agilent Technologies), following the manufacturer’s instructions. For production of Hs6-spuU13 (WT and mutants), the soluble domain of P. falciparum UIS3 was amplified from pCMV-Myc–puU13 by PCR (forward primer: 5′-CCCGGCTGTACCGATCGGCAAGAGCCCTG-3′, reverse primer: 5′-CCCGGCTGTACCGATCGGCAAGAGCCCTG-3′) and cloned into pET28a (Novagen). All plasmids were verified by sequencing. Plasmodium sequences were optimized for expression in mammalian cells.

**Immunofluorescence.** Cells plated on glass coverslips were fixed in 4% paraformaldehyde (Chem Cruz) for 10 min at room temperature (RT) and permeabilized/blockaded with a solution of 0.2% saponin-1% BSA. For immunostaining, samples were incubated with primary antibodies diluted in blocking solution (2 h, RT), washed with PBS, incubated for 1 h at RT with Alexa Fluor-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories) and Hoechst 3342 (Invitrogen) and washed again. The coverslips were then mounted on microscope slides with Fluoromount (SouthernBiotech). The following primary antibodies were used for confocal microscopy: LC3 (mouse monoclonal, MBL, M192-3), PbHSP70 (2E67), PbUIS4 (goat polyclonal, SicGen, AB0042-200), LAMP1 (rabbit polyclonal, Sigma, L1418) and PbMSP1 (rabbit polyclonal, GenScript, custom-made). All images were acquired on Zeiss confocal microscopes (LSM 510 META or LSM 710) and processed in ImageJ. For co-localization analysis, the region of interest (ROI) corresponding to the PVM was defined by thresholding the UIS4 signal, after which the Manders coefficients M1 and M2 within the ROI were calculated using the Colocalisation Threshold plugin from ImageJ. For infection quantifications, circles were detected with anti-UIS3 (2626) and anti-PbMSP1 and imaged on a Zeiss Axiovar 200M wide-field microscope equipped with an automated stage. All images (42 per coverslip) were processed and analysed using ImageJ.

**Molecular docking of UIS3–LC3 complexes.** Homology models of the UIS3 soluble domains belonging to different Plasmodium species and human LC3 were built with the Phyre2 server (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) using the crystal structures of P. falciparum UIS3 (PDB code: 2WVA) and LC3B (PDB code: ZE2D) as reference models. The quality of the homology models obtained was improved by atomic coordinate refinement using a two-step atomic-level energy refinement protocol implemented in ModRefiner software. Experimental and homology-derived protein coordinates were then used to construct models of the human and mouse UIS3–LC3 complexes by flexible molecular docking with the Swamrdock algorithm. LC3 proteins were considered as rigid bodies and UIS3 as ligands. For each complex, an initial set of 100 conformational starting positions was generated in the space around the receptor, being further optimized by energy minimization. The top 10 docking solutions were ranked by increasing free energy and submitted to an additional energy minimization cycle by conjugated gradient during 1,000 steps with GROMACS, to avoid potential steric clashes between contact atoms. Visual inspection and cross comparison between the docking results obtained for the human and mouse complex showed a conserved pattern of molecular orientations for the top five docking solutions. Consensus residues involved in UIS3–LC3 interactions in the best docking solution were determined using CONS-COCOMAP application and graphically represented with LigPilot+ software.

**Co-immunoprecipitation.** HeLa cells expressing GFP–LC3 were transfected with plasmids encoding c-Myc or HA fusion proteins (Supplementary Table 1) on 10 cm dishes and collected 48 h after transfection in lysis buffer (0.5% NP-40, 150 mM NaCl, 10 mM Tris–CI pH 7.4, 0.5 mM EDTA) supplemented with Complete protease inhibitor and PhosStop phosphatase inhibitor cocktails (Roche). On some experiments, HeLa cells were co-transfected with both myc–UIS3 and HA-tagged LC3. c-Myc- and HA-tagged proteins were purified from total cell lysates using the c-Myc-tagged protein mild purification kit and the HA-tagged protein purification kit, respectively, according to the manufacturer's instructions (MBL). GFP–LC3 was immunoprecipitated from total cell lysates using GFP-Trap coupled to agarose beads (Clontech). For immunoprecipitation of parasite-encoded UIS3 from infected cells, HeLa cells or mouse primary hepatocytes were lysed 24 h after infection with uis3-myc P. berghei and total cell lysates were processed as described above. Co-immunoprecipitated proteins and total cell lysates were analysed by western blot using antibodies against LC3 (rabbit polyclonal, MBL, PM036) and c-Myc (mouse monoclonal, MBL, M192-3) or HA tags (mouse monoclonal, Biolegend, 901503) to detect UIS3 or UIS4, followed by HRP-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories). Immunoblots were developed using Luminata Crescendo Western HRP substrate reagent (Merck Millipore).
Expression and purification of recombinant proteins. *Escherichia coli* BL21 cells were transformed with GST-His-tagged LC3 or GST-His bacterial expression vectors. After overnight culture in Luria-Bertani (LB) broth, cells were diluted 1/100 in fresh medium to an optical density (OD) of ~0.7 and incubated with 0.3 mM IPTG for 5 h to induce expression of the recombinant proteins. All cultures were cultured at 37°C with vigorous shaking. For expression of His-tagged *sPfUIS3*, *E. coli* BL21 C43 bacteria were used. Cells grown overnight in LB broth were diluted 1/100 in fresh medium to an OD of ~1.2. Recombinant protein expression was induced by the addition of 0.3 mM IPTG and incubation at 25°C. His-tagged *sPfUIS3* mutants were expressed in *E. coli* BL21 bacteria. Overnight cell cultures were diluted 1/100 in LB supplemented with NZY auto-induction reagent (NZYTech). Recombinant His-tagged proteins were initially purified by metal chelating affinity chromatography using His-Trap Ni-containing columns (GE Healthcare) connected to an AKTA Explorer chromatographic system (GE Healthcare). The His-trap column (1 ml bed volume) was equilibrated with sodium phosphate buffer pH 6.8 containing 1 M NaCl and 25 mM imidazole before protein injection. His-tagged proteins were eluted by a linear gradient of imidazole from 25–500 mM in the same phosphate buffer. Fractions containing protein were collected and pooled and further polished by size exclusion chromatography purification using a Sephadex S-200 column (GE Healthcare) eluted with PBS. Fractions containing protein were analysed by SDS–PAGE, concentrated by ultrafiltration and stored at ~80°C in PBS buffer containing 15% glycerol.

Interaction between recombinant proteins. Recombinant *sPfUIS3* (5 μg) was incubated with recombinant GST-His6-LC3 (10 μg) or with GST-His6 (5 μg), pre-adsorbed to glutathione beads in lysis buffer (150 mM NaCl, 20 mM Tris–HCl (pH 7.4), 0.1% Triton-X100, 5% glycerol, 5 mM EDTA and Complete protease inhibitors (Roche)). After washing, the complex was eluted in lysis buffer supplemented with 10 mM reduced l-glutathione (Sigma-Aldrich) and analysed by western blot with antibodies against LC3 (rabbit polyclonal, MBL, PM036) and c-Myc (mouse monoclonal, MBL, M192-3).

Surface plasmon resonance. Recombinant *sPfUIS3* was immobilized on a Biacore CM5 chip surface following standard protocols provided by the manufacturer (GE Healthcare). In brief, recombinant *sPfUIS3* was chemically immobilized via amine coupling to the free carboxyl groups on the CM5 chip through standard NHS (N-hydroxy succinimide) and EDC (N-ethyl-N-(dimethylaminopropyl) carbodiimide) activation procedures. Recombinant GST–His-LC3 dissolved in PBS buffer was injected over the immobilized *sPfUIS3* at various concentrations to generate affinity sensorgrams that were collected in a single-cycle mode with no regeneration between sample injections. Unspecific binding was monitored by injecting recombinant GST-His proteins over the *sPfUIS3*-coated chip under the same conditions. Normalized response sensorgrams were calculated by subtracting the unspecific binding contribution of the same molar concentrations of GST protein alone. All biosensor data processing and analysis and *Kc* calculations were performed using the Biacore T100 Evaluation Software (GE Healthcare version 2.0.1). For *Kc* calculation, a monovalent interaction model was assumed, with both proteins interacting with 1:1 stoichiometry.

Circular dichroism. Recombinant His-tagged *sPfUIS3* proteins (wild type and mutants) used for circular dichroism were purified using a modification of the already described protocol, where the elution buffer of the size exclusion chromatography was substituted by 50 mM sodium phosphate, 200 mM NaCl, pH 7.5. CD spectra of *sPfUIS3* variants were collected between 190 and 260 nm in a JASCO J-815 spectropolarimeter (Tokyo, Japan) using 1 mm quartz cells from Hellma Analytics. Experiments were performed at 25°C. Each CD spectrum corresponds to the average of 10 technical replicates, corrected for the buffer background contribution. Mean molar residue ellipticity values, [θ], were determined through the relationship

$$[\theta] = \frac{\theta}{Nk}$$

where θ is the observed ellipticity, N is the number of amino acid residues in each protein, l is the quartz cell optical path length and c is the protein molar concentration.

Autophagy induction and p62 degradation assay. HeLa cells were changed from normal growth medium to EBSS to induce amino acid starvation-dependent autophagy. After 7 h, cells were collected in Laemmli buffer and analysed by western blot with antibodies against p62 (rabbit polyclonal, Sigma, P0076) and gamma-tubulin (mouse monoclonal, Sigma-Aldrich, T5326). The levels of p62 were measured by quantifying the ratio of p62 to tubulin signals with ImageJ.

LUMIER competition assay. HEK 293T cells transiently transfected with Luc-p62 or Luc-*sPfUIS3* expressing plasmids (Supplementary Table 1) were lysed 24 h post-transfection in LUMIER His buffer (150 mM NaCl, 20 mM Tris–HCl (pH 7.4), 0.1% Triton-X100, 5% glycerol, 5 mM EDTA and Complete protease inhibitors (Roche)) (26). The *Renilla* luciferase activity associated with each cell lysate was assessed using the *Renilla* Luciferase Assay System (Promega). Equivalent saturating amounts of luciferase-tagged p62 or *sPfUIS3* cell lysates were incubated with recombinant GST–His6-LC3B (500 ng), pre-adsorbed to glutathione beads. After washing away unbound protein, the precipitates were incubated with increasing concentrations of synthetic p62 LIR peptide (SGGDDWTHLSSK) for 1 h with rotation, followed by elution in lysis buffer supplemented with 10 mM reduced l-glutathione (Sigma–Aldrich). The *Renilla* luciferase activity associated with LC3 after elution was quantified as described above using Tecan’s Infinite 200 multiplate reader. The expression of luciferase-tagged p62 or *sPfUIS3* was additionally detected with an anti-*Renilla* luciferase antibody (Abcam).

Statistical analysis. Data are expressed as means + s.e.m. Statistically significant differences between two different groups were determined using non-parametric two-tailed Mann–Whitney test, unpaired two-tailed t-test or two-way ANOVA, as indicated. P values < 0.05 were considered statistically significant. Significances are represented in the figures as follows: NS, *P* > 0.05; *P* < 0.05; **P* < 0.01; ***P* < 0.001. All tests were carried out in GraphPad Prism. The experiments were not randomized. Sample sizes on mice experiments were chosen on the basis of historical data, and no statistical methods were used to predetermine sample size.

Life Sciences Reporting Summary. Further information on experimental design and reagents is available in the Life Sciences Reporting Summary.

Data availability. The data supporting the findings of this study are available within the paper and its Supplementary Information.

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References

1. Liehl, P., Zazarte-Luis, V. & Mota, M. M. Unveiling the pathogen behind the vacuole. Nat. Rev. Microbiol. 13, 589–598 (2015).
2. Gomes, L. C. & Dikic, I. Autophagy in antimicrobial immunity. Mol. Cell 54, 224–233 (2014).
3. Prado, M. et al. Long-term live imaging reveals cytosolic immune responses of host hepatocytes against *Plasmodium* infection and parasite escape mechanisms. Autophagy 11, 1561–1579 (2015).
4. Thieleke-Matos, C. et al. Host cell autophagy contributes to *Plasmodium* liver development. Cell. Microbiol. 18, 437–450 (2016).
5. Mueller, A. K., Labied, M., Kappe, S. H. I. & Matuschewski, K. Genetically modified *Plasmodium* parasites as a protective experimental malaria vaccine. Nature 433, 164–167 (2005).
6. Shen, H. -M. & Mizushima, N. At the end of the autophagic road: an emerging understanding of lysosomal functions in autophagy. Trends Biochem. Sci. 39, 61–74 (2014).
7. Mueller, A. K. et al. *Plasmodium* liver stage developmental arrest by depletion of a protein at the parasite–host interface. Proc. Natl. Acad. Sci. USA 102, 3022–3027 (2005).
8. Hanson, K. K. et al. Torins are potent antimalarials that block replenishment of the parasitophorous vacuolar membrane. *Proc. Natl. Acad. Sci. USA* 110, E2838–E2847 (2013).
9. Spielmann, T., Montagna, G. N., Hecht, L. & Matuschewski, K. Molecular make-up of the *Plasmodium* parasitophorous vacuolar membrane. Int. J. Med. Microbiol. 302, 179–186 (2012).
10. Mizushima, N. et al. Dissection of autophagosome formation using Agp5-deficient mouse embryonic stem cells. J. Cell Biol. 152, 657–667 (2001).
11. Oguley, I. G., Wang, P.-M., Czonn, N. & Jiang, X. Distinct autophagosome-lysosomal fusion mechanism revealed by thapsigargin-induced autophagy arrest. Mol. Cell 42, 731–743 (2011).
12. Sturm, A. et al. Alteration of the parasite plasma membrane and the parasitophorous vacuole membrane during exo-erythrocytic development of malaria parasites. Protist 160, 51–62 (2009).
13. Sharma, A., Yogavel, M., Akhouri, R. R., Gill, J. & Sharma, A. Crystal structure of soluble domain of malaria sporozoite protein UIS3 in complex with lipid. J. Biol. Chem. 283, 24077–24088 (2008).

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14. Nikolajczak, S. A., Jacobs-Lorena, V., MacKellar, D. C., Camargo, N. & Kappe, S. H. I. L-FABP is a critical host factor for successful malaria liver stage development. *Int. J. Parasitol.* 37, 483–489 (2007).

15. Favretto, F., Assafal, M., Molinari, H. & D’Onofrio, M. Evidence from NMR interaction studies challenges the hypothesis of direct lipid transfer from L-FABP to malaria sporozoite protein UIS3. *Prot. Sci.* 22, 133–138 (2013).

16. Farré, J.-C. & Subramani, S. Mechanistic insights into selective autophagy pathways: lessons from yeast. *Nat. Rev. Mol. Cell Biol.* 17, 537–552 (2016).

17. Wacker, R. et al. LC3 association with the parasitophorous vacuole membrane of *Plasmodium berghei* liver stages follows a noncanonical autophagy pathway. *Cell. Microbiol.* 19, e12754 (2017).

18. Martinez, J. et al. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. *Nat. Cell Biol.* 17, 893–906 (2015).

19. Noda, N. N., Ohsumi, Y. & Inagaki, F. Atg8-family interacting motif crucial for selective autophagy. *FEBS Lett.* 584, 1379–1385 (2010).

20. Birgisdottir, Å. B., Lamark, T. & Johansen, T. The LIR motif—crucial for selective autophagy. *J. Cell Sci.* 126, 3237–3247 (2013).

21. Ichimura, Y. et al. Structural basis for sorting mechanism of p62 in selective autophagy. *J. Biol. Chem.* 283, 22847–22857 (2008).

22. Parkvit, S. et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J. Biol. Chem.* 282, 24131–24145 (2007).

23. Mizushima, N., Yoshimori, T. & Levine, B. Methods in mammalian autophagy research. *Cell* 140, 313–326 (2010).

24. Klionsky, D., Abdalla, F. & Abeliovich, H. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy* 8, 445–544 (2012).

25. Barrios-Rodiles, M. et al. High-throughput mapping of a dynamic signaling network in mammalian cells. *Science* 307, 1621–1625 (2005).

26. Zaffagnini, G. & Martens, S. Mechanisms of selective autophagy. *Autophagy* 1, 428–1714 (2016).

27. McEwan, D. G. et al. PLEKHM1 regulates autophagosome–lysosome fusion through HOPS complex and LC3/GABARAP proteins. *Mol. Cell* 57, 39–54 (2015).

28. Boonhok, R. et al. LAP-like process as an immune mechanism downstream of IFN-γ in control of the human malaria parasite *Plasmodium vivax* liver stage. *Proc. Natl Acad. Sci. USA* 113, E3519–E3528 (2016).

29. Ruivo, M. T. G. et al. Host AMPK is a modulator of autophagy and of the liver stage development of the malaria parasite *Plasmodium falciparum* in mice. *Mol. Biol. Cell* 19, 313–326 (2008).

30. Kumar, H. et al. Protective efficacy and safety of liver stage attenuated malaria parasites.*Cell Rep.* 16, 2539–2545 (2016).

31. Vangone, A., Oliva, R. & Cavallo, L. CONS-COCOMAPS: a novel tool to measure and visualize the conservation of inter-residue contacts in multiple docking solutions. *BMC Bioinformatics* 13(Suppl. 4), S19 (2012).

32. McEwan, D. G. et al. PLEKHM1 regulates autophagosome–lysosome fusion through HOPS complex and LC3/GABARAP proteins. *Mol. Cell* 57, 39–54 (2015).

33. Lee, I. H. et al. Atg7 modulates p53 activity to regulate cell cycle and survival. *Science* 336, 225–228 (2012).

34. Zhang, W. et al. PCB 126 and other dioxin-like PCBs specifically suppress the development of autophagic isolation membranes in mice. *Autophagy* 9, 313–326 (2013).

35. Vangone, A., Oliva, R. & Cavallo, L. CONS-COCOMAPS: a novel tool to measure and visualize the conservation of inter-residue contacts in multiple docking solutions. *BMC Bioinformatics* 13(Suppl. 4), S19 (2012).

36. McEwan, D. G. et al. PLEKHM1 regulates autophagosome–lysosome fusion through HOPS complex and LC3/GABARAP proteins. *Mol. Cell* 57, 39–54 (2015).

37. Janse, C. J., Ramesar, J. & Waters, A. P. High-efficiency transfection and drug selection of genetically transformed blood stages of the rodent malaria parasite *Plasmodium berghei*. *Nat. Protoc.* 1, 346–356 (2006).

38. Tsuji, M., Mattei, D., Nussenzweig, R. S., Eichinger, D. & Zavala, E. Demonstrations of heat-shock protein 70 in the sporozoite stage of malaria parasites. *Parasitol. Res.* 80, 16–21 (1994).

39. Fentress, S. J. et al. Phosphorylation of immunity-related GTPases by a *Toxoplasma gondii*-secreted kinase promotes macrophage survival and virulence. *Cell Host Microbe* 8, 484–495 (2010).

40. Xu, D. & Zhang, Y. Improving the physical realism and structural accuracy of protein models by a two-step atomic-level energy minimization. *Biophys. J.* 101, 2525–2534 (2011).

41. Torchala, M., Moal, I. H., Chaleil, R. A. G., Fernandez-Rejio, J. & Bates, P. A. SwarmDock: a server for flexible protein–protein docking. *Bioinformatics* 29, 807–809 (2013).

42. Prong, S. et al. GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics* 29, 845–854 (2013).

43. Barrios-Rodiles, M. et al. High-throughput mapping of a dynamic signaling network in mammalian cells. *Science* 307, 1621–1625 (2005).

44. Van Wesenbeeck, L. et al. Involvement of PLEKHM1 in osteoclastic vesicular transport and osteoporosis in incisors absent rats and humans. *J. Clin. Invest.* 117, 919–930 (2007).

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**Author contributions**

E.R. and M.M.M. conceived and led the study and wrote the manuscript. E.R., L.R., G.G.C. and J.M.-V. performed the experiments, acquired the data, performed data analysis and interpreted results. F.J.E. performed molecular docking, protein purification and SPR analysis. Animal experimentation was conducted by L.M.S., L.R., I.M.V. and V.Z.L. W.B. performed electron microscopy analysis. Circular dichroism was performed by T.N.E.G.R.M. constructed plasmids for parasite transfections. All authors read and approved the final manuscript.

**Competing interests**

The authors declare no competing financial interests.

**Additional information**

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Experimental design

1. Sample size
   Describe how sample size was determined.
   No statistical methods were used to pre-determine sample size. The sample size is specified in each figure legend and in Supplementary Table 5.

2. Data exclusions
   Describe any data exclusions.
   No data were excluded from the analysis.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
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4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   Microscopy analysis were performed on randomly acquired images. Mice were randomly assigned to each experimental group.

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   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   No blinding was performed.

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   □   □ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
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Describe the software used to analyze the data in this study.

Software used to analyze data is specified in methods section, pages 6-12.

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Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

All materials are available from standard commercial sources.

9. **Antibodies**

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

References for all antibodies are provided in the Methods sections "Immunofluorescence", "Immuno-EM", "Co-Immunoprecipitation", and "Autophagy induction and p62 degradation assay", pages 9, 10 and 11 respectively.

10. **Eukaryotic cell lines**

   a. State the source of each eukaryotic cell line used.

   Cell line source is referenced in Methods section "Cell Lines", page 7, and "Parasite lines", page 8.

   b. Describe the method of cell line authentication used.

   Authentication was performed as described in Methods section "Cell Lines", page 7

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   d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

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Details on animals used is provided in Methods section "Mice" and "Mouse primary hepatocytes", page 7.

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No human participants were used in this study.