PLASMODIUM EXOERYTHROCYTIC PARASITES REDIRECT TRAFFICKING OF HUMAN PROTEINS TO THE PARASITOPHOROUS VACUOLE

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KEYWORDS

Hypnozoite, malaria, *P. vivax*, Hepatocyte.

ABBREVIATIONS USED

PVR, Parasitophorous Vacuole Region; NR4A3, Nuclear receptor subfamily 4 group A member 3, HCI, High Content Imaging; qRT-PCR quantitative Reverse Transcriptase Polymerase Chain Reaction; ATQ, Atovaquone; EEF, Exoerythrocytic form; FC, flow cytometry; FACS, Fluorescence-Activated Cell Sorting; hpi, hours post infection; TRAP, Thrombospondin Related Anonymous Protein; GOLGA8A, Golgin subfamily A member 8A; MUC13, Mucin 13; CGA, Glycoprotein hormones alpha chain; Binding immunoglobulin protein, BiP; CXCL2, Chemokine

35 (C-X-C Motif) Ligand 2; CSP, Circumsporozoite protein; UIS4, Up-regulated in infective
36 sporozoites gene 4; Lipopolysaccharide, LPS.

ABSTRACT

Changes in host cell morphology and transcription after apicomplexan parasite infection have long been noted, but there have been few studies of the functional consequences of host cell remodeling. Here we show, using time-dependent immunofluorescence microscopy of multiple human cell lines (HepG2, HC-04, Huh7.5.1 and primary human hepatocytes), infected with multiple *Plasmodium* species (*Plasmodium berghei, P. falciparum* and *P. vivax* (hypnozoites and schizonts)), and antibodies to multiple human proteins (HsNR4A3, HsMUC13, HsGOLGA8A, HsCGA, HsBiP, HsCXCL2), that human protein trafficking is extensively modified in *Plasmodium* infected cells. Using conventional as well as ultrastructure expansion microscopy we show that newly-synthesized human proteins are trafficked to the parasitophorous vacuole instead of the infected-cell plasma membrane, nucleus or extracellular space. Universal redirection of human signaling proteins cells the parasitophorous vacuole may provide a mechanistic explanation for how apicomplexan parasites can block host cells response to infection.

INTRODUCTION

Malaria remains a worldwide problem with up to 241 million cases reported in 2021[1]. Given the economic and humanitarian burden of malaria, and the continual emergence of parasite resistance to drugs that act against the symptomatic stages of the infection, new strategies to treat, prevent or control malaria are needed.

Liver cells are the primary cellular target of the *Plasmodium* parasite's exoerythrocytic stage. After injection into the vertebrate host by the bite of an infected Anopheline mosquito, the parasites (sporozoites) travel to the liver where they migrate and cross through several barriers. These barriers include endothelial cells, dermal fibroblast, liver sinusoidal endothelial cells (LSECs) and highly phagocytic Kupffer cells, that allows sporozoites to pass all barriers to find and infect hepatocytes[2,3]. When a suitable hepatocyte is reached, they inject the contents of their apical organelles using their specialized secretory organelles, the micronemes and rhoptries, into the hepatocyte membrane creating a molecular machine that enables the formation of a vacuole in the host cell cytoplasm[4]. The vacuole will enclose and protect the parasite and is surrounded by a host-derived parasitophorous vacuole membrane (PVM)[5]. Similar vacuoles are formed when

65 blood stage malaria parasites invade erythrocytes and by other apicomplexan parasites, including
66 *Toxoplasma gondii* when they invade fibroblasts, for example[6]. Some malaria parasite species can
67 also remain in the liver in a dormant state, called a hypnozoite[7]. Hypnozoite-forming species,
68 especially *P. vivax*, represent barriers to malaria eradication because if they are not eliminated by
69 drug treatment, they can reawake months to years later, leading to patient relapse, as well as active
70 malaria transmission in an area that was once cleared of malaria. Despite their importance,
71 hypnozoites are poorly understood.

72 There are no clinical symptoms associated with liver stage infection[8]. Fever and chills that
73 are characteristic of malaria only develop after the merozoites are released from the schizont and
74 move to the circulatory system scanning for their next target cells, the erythrocytes.

75 Pre-erythrocytic stages are the primary target for the development of malaria vaccines. It
76 has been suggested that vaccines directed at this stage will be more effective because they will
77 suppress the spread of vaccine-resistance because of low parasite numbers at this stage (hundreds
78 versus billions in blood stages) and the only licensed vaccine, RTS, S/AS01, Mosquirix™, consists
79 of a target, circumsporozoite protein (CSP), which is highly expressed in *Plasmodium*
80 sporozoites[9-11]. Irradiated sporozoites also provide strong protection against malaria and have been
81 used in vaccine trials[12-14]. Irradiated sporozoites invade hepatocytes, form a trophozoite, but then
82 the host cell will apoptose before merozoite maturation is complete, allowing the display of
83 antigens and potentially elicit an improved immune response that is missing with unirradiated
84 sporozoites[15]. Vaccination with parasites that have mutations in PV-localized UIS3 and UIS4
85 result in an unprotective infection and sterile protection against challenge[16].

86 In the course of their development, erythrocytes will have shed many of their organelles,
87 including their nuclei, before they become susceptible to invasion by malaria parasites.
88 Hepatocytes, on the other hand, have nuclei and remain transcriptionally active after parasite
89 invasion. We had previously performed a dual RNAseq study on flow-sorted, *P. berghei*-infected
90 liver cells[17]. The experiments, which were conducted in a variety of time points and on different
91 cell types showed concerted patterns of gene upregulation, including genes involved in the immune
92 response, such as the mucin, *HsMUC13*[17]. Curiously, we observed that HsMUC13 protein
93 colocalized with parasite UIS4, a protein inserted into the PVM in infected cells[17]. To further

94 investigate these phenomena, here we examine other human genes that were upregulated after
95 parasite infection, including the orphan human nuclear hormone receptor, *HsNR4A3*.

96 Surprisingly, we find that the cognate protein for all upregulated human genes that we
97 examine show colocalization with the parasite parasitophorous vacuole (PV) marker, UIS4[18]. We
98 show this colocalization is observed not only for *P. berghei* and *P. falciparum* liver stages, but for
99 both *P. vivax* schizonts and hypnozoites as well. Furthermore, using ultrastructure expansion
100 microscopy, a recently developed sample preparation method that enabled the isotropic expansion
101 of *Plasmodium* parasites up to 4.5-fold[19], we revealed the association of HsNR4A3 proteins with
102 the PVM of *P. berghei* parasite. Our data indicate that in all these parasites, host proteins are being
103 redirected to the PV. We propose that apicomplexan parasites manipulate host secretion apparatus
104 to favor their development and to hide from the immune system.

105 RESULTS

106 *Host proteins are transcriptionally upregulated during P. berghei infection of liver cells*

107 A previous dual (parasite and human) RNA-seq study with the rodent malaria parasite, *P.*
108 *berghei* (*Pb*GFP-SM_{CON} (*Pb*-GFP)), identified a series of human transcripts that were upregulated
109 during parasite exoerythrocytic infection (Fig. 1a, 1b). Multiple time points, replicates, and
110 different hepatoma host cells (HepG2s, Huh7.5.1s, HC-04s) were used in these experiments in
111 which human cells were individually sorted into infected and noninfected pools based on the
112 presence of parasite-encoded GFP signal. Oddly, the most upregulated and statistically significant
113 genes across cell lines at 48 hours did not seem to be functionally related to one another. *HsMUC13*
114 encodes a secreted membrane localized mucin that is usually expressed in the gut. It is normally
115 upregulated after infection and is asymmetrically localized to luminal side of gut epithelial cells[20].
116 We had previously shown that it colocalized to the parasite vacuole in mature *P. vivax* and *P.*
117 *berghei* schizonts[17]. Also upregulated in multiple hepatocyte-derived lines was *HsNR4A3* ($p =$
118 2.8×10^{-30}). The NR4A subfamily encodes orphan nuclear receptors that belong to the larger nuclear
119 receptors (NRs) superfamily of eukaryotic transcription factors. The NR4A subfamily includes
120 three members, namely NUR77 (HsNR4A1), NURR1 (HsNR4A2) and NOR1 (HsNR4A3), which
121 are gene regulators and participate in diverse biological functions. Although exact function of

122 HsNR4A3 is not known, data suggest a potential role in controlling glucose in some cell types[21].
123 NR4A3 knockout mice show expansion of the pancreatic beta cells, needed for insulin production,
124 and NR4A3 overexpression lines increase glucose production in response to some stimuli, and act
125 as activator of beta cell proliferation and insulin production[22,23]. It has also been reported as an
126 NLRP3 inflammasome activation-responsive gene[24]. In the cancer arena, it has been shown that
127 NR4A3 overexpression attenuates proliferation of cancer cells and promotes apoptosis by
128 augmenting the expression of pro-apoptotic genes via a p53 dependent mechanism[25]. *HsCGA*
129 (adjusted $p = 4.4\text{x}10^{-13}$) encodes the shared alpha chain of the heterodimeric human glycoprotein
130 hormones; thyrotropin/thyroid stimulating hormone/TSH, lutropin/luteinizing hormone/LH,
131 follitropin/follicle stimulating hormone/FSH and choriogonadotropin/CG. HsCGA, along with its
132 partner, would normally be secreted into the bloodstream where it would bind rhodopsin-like G
133 protein-coupled receptors on the target cell[26]. *HsCGA* is expressed at high levels in the placenta
134 but is also upregulated in breast cancer and is an estrogen receptor alpha (ERα)-responsive gene
135 in human breast tumors[27]. *HsTAS2R4* encodes a G-protein coupled receptor that is normally
136 expressed on the surface of tongue epithelial cells. It is responsive to bitter molecules, including,
137 interestingly, the antimalarial, quinine but may also play a role in small molecule-sensing in
138 extraoral cell types[28].

139 In addition, the set of statistically significant upregulated genes included several noncoding
140 genes. The lncRNA, *HsNEAT1* (adjusted $p = 1.8\text{x}10^{-34}$), promotes activation of inflammasomes in
141 macrophages[29]. *HsMALAT1*, also known as *HsNEAT2* is also a large, non-coding RNA, which is
142 highly conserved amongst mammals and highly expressed. Overexpression of *HsMALAT1*
143 suppresses breast cancer metastasis in transgenic, xenograft, and syngeneic models of cancer[30] and
144 is also strongly associated with inflammasome activation[31].

145 Because the RNAseq studies had been performed on model hepatoma cells lines we used
146 qRT-PCR to confirm the upregulation of *HsNR4A3, HsMUC13, HsCGA, HsSLC22A8,*
147 *HsMALAT1, HsRASSSF9, HsSDHA, HsSLC25A27, HsNEAT1,* and *HsTAS2R4* in *P. berghei*-
148 infected primary human hepatocytes, as well as in *P. berghei* infected Huh7.5.1, HepG2 and HC-
149 04 cells. Cells were infected with *P. berghei* expressing GFP and flow sorted. RNA was extracted
150 from infected cells, as well as from control, parallel non-infected cells. In these four cell lines, we
151 observed consistent 13-20-fold upregulation of *HsNR4A3* in all cell lines except for HepG2 cells

152 (Fig. 1c). We also observed upregulation of other transcripts via qRT-PCRs but not as consistently
153 across all lines.

154 To further ensure that the patterns were not specific to parasites, we performed Western
155 blot analysis. We obtained several commercial antibodies to some of the different human proteins
156 that showed the highest, most significant, and reproducible upregulation. These included
157 HsNR4A3, HsCGA, and HsMUC13. HsGOLGA8A, was also included (probability of
158 upregulation of chance = 4.04×10^{-10}). It encodes a member of the Golgin family of membrane
159 proteins which play a role in tethering vesicles to the Golgi apparatus[32],[33]. All 21 annotated Golgins
160 were amongst the set of upregulated genes in infected cells, 13 significantly (hypergeometric mean
161 probability of enrichment by chance = 5.5×10^{-5}), with an average upregulation of 2.3×. The bulk
162 cultures as well as uninfected HC04 control cultures were infected with both *P. berghei* and *P.*
163 *falciparum*. All the evaluated antibodies showed recognition of a single band corresponding to the
164 native protein (Fig. S1a, S1b). In some cases, upregulation was observed relative to human control
165 protein (Hs-vinculin, Figure S1b), but given that only a small proportion of cells are infected in
166 bulk cultures (1 to 2%), especially with *P. falciparum*, we did not attempt to further quantify this.
167 The antibody to CGA1 recognized a larger than expected protein. CGA1 is heavily glycosylated
168 which may contribute to the size difference.

169 *Upregulated host proteins show colocalization with PVM via immunofluorescence analysis.*

170 Given the challenges of analyzing level by Western we next used immunofluorescence and
171 confocal microscopy to confirm our RNA-seq results. First, assays were performed with mature
172 *P. berghei*, the species on which hepatocyte flow sorting was performed. Isolated *P. berghei*
173 sporozoites were incubated with a permissive human liver cell line, HC-04. After 48 hours, the
174 cultures were fixed and stained with a set of primary commercial antibodies against various
175 upregulated hepatocyte proteins (HsNR4A3, HsCGA, HsGOLGA8A, and HsMUC13), and to
176 parasite UIS4 (Fig. 2a), a parasite protein highly expressed in sporozoites and liver stages of
177 multiple *Plasmodium* species, and which localizes to the PVM in both hypnozoites and schizonts[34].
178 All antibodies (against HsNR4A3, HsCGA, HsGOLGA8A, and HsMUC13 proteins) showed high
179 expression of their cognate proteins in *P. berghei*-infected but not -uninfected HC-04 cells.
180 Interestingly, all four host proteins colocalized with the parasite marker, UIS4 (Fig. 2a). HsNR4A3

181 is normally localized to the nucleus[35] and indeed staining was observed in the nucleus in uninfected
182 cells visible in the image. A zoom in (Fig. 2a1), a panoramic view (Fig. 2a2), and a panoramic
183 overexposed view (Fig. 2a3) is also shown for the HsNR4A3 protein. GOLGA8A encodes protein
184 with an N-terminal coiled-coil domain and a single C-terminal transmembrane domain and is also
185 known as golgin-67. It localized to the Golgi apparatus which in most human cells is adjacent to
186 the nucleus[36]. As expected, our staining showed weak HsGOLGA8A surrounding the nucleus in
187 uninfected cells. MUC13 and CGA are both expected to be secreted into the extracellular space.
188 Uninfected cells showed some weak diffuse cytoplasmic staining with antibodies to these proteins.

189 To determine if similar staining would be observed in human cells infected with the human
190 pathogen, *P. falciparum,* we performed experiments using dissected *P. falciparum* sporozoites and
191 primary hepatocytes. Sporozoites were placed on primary hepatocytes in 96-well plates and
192 cultures were incubated for 120 hours, necessary because of the longer *P. falciparum* liver
193 development time. Immunofluorescence was performed using a *P. vivax* antibody to PvUIS4[34]
194 (which is cross-reactive with PbUIS4, and PfUIS4) (Fig. 2b) and anti-human primary antibodies.
195 These experiments confirmed upregulation of the HsNR4A3, HsCGA, HsGOLGA8A, and
196 HsMUC13 proteins with staining, as with *P. berghei*, occurring in the PVM region, overlapping
197 largely with UIS4 staining. Importantly, these data showed that the staining patterns were not
198 associated with the use of a hepatoma cell line and were conserved in primary human hepatocytes.

199 We next examined another human pathogen, *P. vivax,* which can remain in an infected
200 liver cell as a hypnozoite that can be activated months or years after the primary infection. As *P.*
201 *vivax* cannot be maintained in cell culture, patients diagnosed with *P. vivax* malaria, were recruited,
202 and asked to provide 20mL of whole blood, which was used to feed fasted, female *Anopheles*
203 *darlingi* mosquitoes using a standard membrane feeding assay. After 14 days the mosquitoes were
204 dissected to obtain sporozoites, which were used to infect HC-04 cell lines (see Methods). After
205 five days, the cells were fixed and stained with our primary antibodies (against HsNR4A3,
206 HsCGA, HsGOLGA8A, and HsMUC13 proteins). Again, all antibodies showed strong
207 colocalization with the parasites (Fig. 2c) for all proteins.

208 To determine whether this pattern would be observed for hypnozoites we searched for UIS4
209 positive objects that were smaller than 10μm and which showed characteristic circular patterns

210 associated with UIS4 signal, the bonafide way to identify hypnozoites[34]. For our *P. vivax* infected
211 samples, we identified hypnozoites at a rate of 1 per 5,000 hepatocytes, and schizonts at a
212 frequency of ~1:1,000, some of which are shown in Fig. 2d. Interestingly, the same
213 immunofluorescent micrographs also showed colocalization with HsNR4A3, HsCGA,
214 HsGOLGA8A, and the HsMUC13 (Fig. 2d), a zoom-in for HsNR4A3 protein in hypnozoites
215 stages is shown (Fig. 2d1). In fact, previous parasite-associated immunolocalization had been
216 observed for HsMUC13[17]. Analysis of the images showed a strong correlation between UIS4 and
217 the different tested proteins for all species (Fig. S2).

218 While the relocalization of one protein could be functional, the relocalization of all was
219 puzzling. To rule out cross hybridization or other antibody specific effects we next performed
220 staining with an antibody to the parasite cytosolic markers *P. berghei* and *P. vivax* HSP70 (*Pb*-
221 *Pv*HSP70)[17] in infected HC-04 cells (Fig. S3a) instead of UIS4. These micrographs showed similar
222 localization of the human proteins, ruling out the possibility of the species-specific secondary
223 antibodies cross-reacting with the primary UIS4 antibody. In addition, we repeated IFAs with a
224 variety of different primary antibodies using the *P. berghei* infection model and in all cases, the
225 same patterns were obtained (Figure S4).

226 *HsNR4A3 edited lines show decreased NR4A3 staining in Westerns and IFA*

227 To further assess the question of antibody specificity as well as HsNR4A3 functionality,
228 we next performed knockouts on *HsNR4A3*. We used CRISPR/*Cas9*-based methods to disrupt
229 *HsNR4A3* in clonal HC-04 cell lines using two different approaches (two plasmid or single
230 plasmid) and different guide RNA sets (Fig. S5a and S5b), obtaining six different stably edited
231 clones (GL1, GL2, and GL3 and NM1, NM2, and NM3 created using the two approaches)).
232 Western blot analysis showed lower expression of HsNR4A3 in five of six clones (clones GL1 and
233 GL3 and NM1, NM2, and NM3)) relative to native HC-04 cells (Fig. S5c, S5d).

234 To evaluate antibody specificity, *HsNR4A3* knockout cells were infected with *P. berghei*
235 (*Pb*-ANKA-GFP-Luc SMCON (*Pb*-Luc)) sporozoites. After 48hpi, the infected and uninfected
236 cells were evaluated by immunofluorescence and confocal microscopy. For HC-04 HsNR4A3
237 unmodified cells (WT) we observed colocalization with the parasite markers UIS4 (Fig. S5e) and
238 HSP70 (Fig. S5e, S5f). In contrast, no NR4A3 staining was observed in *HsNR4A3* knockout cells

239 (Fig. S5e) and pattern quantification showed no correlation ($p < 0.0005$) Fig. S5f). Staining was
240 similar to the negative control in which the NR4A3 antibody was not used. These data largely rule
241 out the possibility of NR4A3 antibody cross-hybridization with an as-yet unspecified parasite
242 protein.

243 To confirm our findings in *P. vivax*, we also performed *in vitro* infection tests using cloned
244 GL1 and GL3 *HsNR4A3* knockout cells and *P. vivax* parasites obtained as described above. We
245 observed a marked expression of the HsNR4A3 protein in the HC-04 HsNR4A3 WT cells in the
246 two stages of the parasite (hypnozoites and schizonts) showing the expected co-staining with UIS4.
247 For clone GL1, we did not identify any parasites (see below) while for clone GL3 (Fig. S5g), we
248 observed no signal or co-staining with UIS4. A statistically-significant loss of staining correlation
249 in *HsNR4A3* knockouts was confirmed by image quantitation (Fig. S5h, S5i).

250 *Role of the protein HsNR4A3 in exoerythrocytic infections*

251 To determine whether HsNR4A3 contributes to parasite growth and development we
252 investigated both schizont size, schizont number and schizont morphology in the *HsNR4A3*
253 knockout clones in *P. berghei* and *P. vivax*. First, *in vitro* infections with *P. berghei* parasites were
254 carried out. We performed a qRT-PCR experiment using primers to the *P. berghei* 18S RNA gene
255 as previously described[37]. These data showed that a higher number of cycles were needed to detect
256 parasites in NM1, NM2 and NM3 clones, with NM3 requiring almost as many cycles for detection
257 as treatment with 5 nM atovaquone, a drug which completely blocks parasite exoerythrocytic
258 development (Fig. S6a). Next, high content imaging analysis was performed on *P. berghei* infected
259 cells and used to determine the number of UIS4 positive objects per field ($0.8cm^2$). These data
260 showed a ~70% reduction in the relative number of parasites in all three *HsNR4A3* knockout clones
261 (Fig. S6b). We also observe that most of the positive objects identified in *HsNR4A3* knockout cells
262 (NM1-NM3) have a diameter of 5 µm compared with 40µm the WT HC-04 cells (Fig. S6c).

263 To investigate the role of HsNR4A3 in *P. vivax* development, we infected our GL1 and
264 GL3 *HsNR4A3* knockout lines with *P vivax* sporozoites, obtained as described above, and allowed
265 the parasites to develop for 120 hours. Cultures were co-stained with the UIS4 parasite marker to
266 identify and count infected cells. Hypnozoites were distinguished from schizonts based on parasite
267 size (Fig. S6d, S6e). In both clones we observed fewer schizonts, although the reduction in the

268 number of hypnozoites was not as marked. This suggests that the expression of the HsNR4A3
269 protein is needed for the *P. vivax* parasite to complete the schizont maturation process. To
270 determine if this was true for *P. berghei* as well, we also infected the *HsNR4A3* knockout clones
271 with GFP-expressing *P. berghei* sporozoites and performed flow sorting of GFP-positive objects
272 on GL1 and WT HC-04 cells after 48 hours incubation (Fig. S6f). We observed a marked reduction
273 in the number of GFP positive objects in GL1. These data suggest that HsNR4A3-depleted HC-04
274 cells may be less able to support parasite invasion and maturation but also highlight that different
275 clones have different levels of gene disruption in diploid human cells, and the phenotype is slightly
276 inconsistent.

277 On the other hand, all experiments described above were normalized to the number of
278 human cells (at either the cell counting level or via total RNA in the qPCR studies) and if human
279 cells grew faster this would contribute to lower relative parasite numbers after a 48-hour incubation
280 and an even greater reduction with P. vivax infections. Because it has been reported that
281 HsNR4A3-edited human breast and lung cancer cells (MDA-MB-231 and H1299, respectively)
282 show increased cell proliferation[25], we measured growth rates in the NM1 and NM3 clones and
283 indeed observed 3 to 6 × more cells in comparison to the HC-04 HsNR4A3 WT cells 36 hours
284 after plating. Thus, although we do find that HsNR4A3 expression may help the parasites survive
285 and grow, the impact could be partially indirect.

286 *HsNR4A3 is trafficked to the parasitophorous vacuole membrane.*

287 As a nuclear hormone receptor, HsNR4A3 is expected to traffic between the nucleus and
288 cytoplasm. To study the temporal dynamics of HsNR4A3 relocalization in the context of a parasite
289 infection we next conducted a time course experiment in *P. berghei,* monitoring subcellular
290 localization of HsNR4A3 during exoerythrocytic infections. *P. berghei* sporozoites were placed
291 on HC-04 cells and samples were collected and fixed for immunofluorescence analysis every three
292 hours. At three hours, HsNR4A3 staining was observed in the host cell nucleus, overlapping with
293 host cell DAPI for both infected and uninfected cells, as expected for a nuclear hormone orphan
294 receptor, which are constitutively-active transcription factors (Fig. 3). In contrast, the parasite-
295 specific marker, UIS4, was clearly in the host cell cytoplasm, surrounding the parasite and only
296 found in infected cells. At later time points, HsNR4A3 was no longer found in the host nucleus,

297 and was now found in the cytoplasm of infected cells. By 12 hours, weak HsNR4A3 staining was
298 observed in the nucleus of uninfected cells, but strong staining was observed in infected cells in
299 the PVR. In late stages (24-72 hours), HsNR4A3 expression overlapped with the parasite marker,
300 UIS4 (Fig. 3a, 3b). Given the clear upregulation in NR4A3 gene expression in infected cells, these
301 patterns are consistent with newly synthesized NR4A3 being trafficked directly into the PVR
302 instead of to the host nucleus. Image quantification (Fig. S7a) showed little PbUIS4:HsNR4A3
303 correlation at early times (<0.1 at 3 hours) but strong correlation at later times (>0.9 at 96 hours),
304 and an inverse relationship with DAPI:HsNR4A3 correlation (Fig. S7b). Similar relocalization
305 patterns were also observed over the course of *P. berghei* exoerythrocytic development for
306 HsGOLGA8A (Fig. S8a, S8b) with the staining observed around the HC-04 cell nucleus at time
307 zero and then moving to co-localize with PbUIS4 by 48 hours in infected cells only. As a control,
308 we examined a gene that did not show differential regulation after infection. HsBiP, also known
309 as HsHSPA5 or GRP-78 is a highly expressed heat shock protein that is typically found in the
310 lumen of the ER and is a well-established marker of ER. It shows little evidence of differential
311 regulation after infection in our dual RNAseq study ($p = 0.97$, log2fold change = -0.006) but is
312 one of the most abundant transcripts (ranking at 96 of ~25,000 transcripts in terms of base mean
313 expression). Although in early stages of development it surrounded the host nuclei (Fig. S8c) as
314 predicted in all eukaryotic cells, it completely colocalized with PbUIS4 by 72 hours of infection
315 and image analysis showed strong correlation with PbUIS4 (Fig. S8d).

316 *Expansion immunofluorescence confocal microscopy localizes hsNR4A3 to the parasitophorous*
317 *vacuole.*

318 To further characterize the interaction of HsNR4A3 with the PVM we turned into
319 ultrastructure expansion microscopy (U-ExM) coupled with Airyscan super-resolution
320 microscopy[19,38]. We fixed and expanded Huh7.5.1. cells after 48 hours of infection with *P. berghei*
321 parasites. Previous work in blood-stage parasites demonstrated that the higher resolution achieved
322 post-expansion allows to distinguish the PVM from the parasite plasma membrane both stained by
323 Bodipy-TR-ceramide dye[38]. Using anti-UIS4 antibody in combination with Bodipy-TR ceramide
324 staining we expectedly localized UIS4 at the PVM and observed numerous UIS4-positive lipid-
325 enriched vesicles within the PV space or in the host-cytoplasm (Fig. S9). After the validation of
326 UIS4 as U-EXM compatible PVM marker, we used primary antibodies against HsNR4A3 and

327 UIS4 proteins and confirmed the close association of HsNR4A3 with the PVM. HsNR4A3
328 localized at the host-cytosolic side of the PVM in foci that predominantly are uniformly distributed
329 around the PV and occasionally concentrated in pockets associated with the PVM with some
330 staining in the PV (Fig. 4a).

331

332 *Secreted immune signaling molecules are also redirected to the PVR*

333 One unifying hypothesis that could explain the colocalization that was observed with many
334 of our examined proteins would be if all newly synthesized inter and intracellular host signaling
335 molecules were redirected away from their usual cytoplasmic or cell membrane location and into
336 the parasite vacuole membrane region PVM, potentially as a way to avoid immune signaling or
337 instructions to undergo apoptosis. To further investigate this, we examined the list of upregulated
338 genes to identify well-characterized cytokines and selected HsCXCL2 (Fig S10a, S10b), which
339 was expressed and upregulated in infected HC-04 cells in particular ($\log_2$ fold change = 2.76, p =
340 1.46×10^{-07}). *HsCXCL2* encodes a chemokine ligand that is secreted by monocytes and
341 macrophages at the site of inflammation and is strongly responsive to LPS stimulation. It binds to
342 the chemokine receptor, HsCXCR2 and plays a role in early stage of neutrophil recruitment during
343 tissue inflammation[39]. To test the hypothesis that HsCXCL2 would migrate to the PVR after
344 immune stimulation HC-04 cells were first infected with *P. berghei* and then after 24 hours treated
345 with LPS. Immunofluorescence assays using a CXCL2 antibody were performed at 2, 24 and 48
346 hours (26, 48 and 72hpi). These data showed a strong upregulation of CXCL2 expression in LPS-
347 treated, infected and uninfected HC-04 cells. As with other host proteins, HsCXCL2 staining
348 colocalized with the PVR and PbUIS4 in the infected cells (Fig. 5a, 5b). The specificity of
349 commercial antibodies against HsCXL2, HsBiP (Fig S11a) and the controls (Fig S11b) are
350 showed.

351 DISCUSSION

352 Here we have shown that five different upregulated proteins (HsNR4A3, HsCGA,
353 HsGOLGA8A, HsMUC13, and HsCXCL2), each with different, intracellular trafficking patterns,
354 all end up in the PV or PVR, surrounding the parasite. In addition, this pattern has also been

355 observed by others with unrelated proteins: Posfai and coworkers observed upregulation of
356 HsAQP3 in a *P. berghei* dual RNAseq study (this was confirmed in our dataset, $p = 1.81x10^{-19}$)
357 and also showed that this transporter is localized to the PVM[40]. Normally HsAQP3, a membrane
358 transporter of water and glycerol, would be trafficked to the human cell plasma membrane. The
359 host autophagosome marker, LC3, as well as other autophagosome markers, P62, NDP52, and
360 NBR1 and LAMP1 also colocalize with UIS4[41-45]. The timing of relocalization is also similar,
361 occurring here as early as 12 hours post infection, as observed for LC3[43]. While each protein that
362 was examined here and by others could have a distinct role in supporting parasite development,
363 we favor a model in which newly synthesized proteins are redirected to the vacuole and away from
364 their natural home, potentially as a universal neutralization mechanism to block cell signaling and
365 apoptosis in the infected host.

366 It has been proposed that one mechanism by which the malaria parasite may accomplish
367 the host cell remodeling is through modification of the host autophagy pathways[46]. Here the host
368 cell is relatively passive, and the parasite now directs the formation of lysosomes which are used
369 to capture pieces of the host cytoplasm and redirect them toward the parasite. This has been termed
370 the *Plasmodium*-associated autophagy-related (PAAR) response[46]. Support for this model comes
371 from the demonstration that many autophagosome markers colocalize with the PVM and some are
372 also needed for parasite development[46]. However, this does not explain why a variety of
373 completely unrelated proteins, including MUC13, AQP3[40], NR4A3 or CXCL2, all show the same
374 behavior. This model, which is based on what is observed in infected erythrocytes, does not
375 necessarily take into account the massive transcriptional changes which are observed in infected
376 hepatocytes. This autophagy model also suggests that lysosome cargo that is directed to the PV
377 consists of digested proteins.

378 We propose an alternative but possibly complementary model in which newly synthesized
379 host proteins are now secreted into the PV because the host secretion apparatus has been
380 extensively modified. This model is supported by the fact that many of the antibodies we have
381 used in our study are to full length proteins and their appearance in the PVR is associated with
382 their transcriptional upregulation in the infected host cells. Our model is also based on our
383 micrographs as well as those of others[47-49] showing that the endoplasmic reticulum (ER) is also
384 relocalized to the PVR. In fact, the upregulation and association of HsGOLGA8A with the PVR

385 may be a consequence of the relocalization of the ER and Golgi apparatus around the PV and may
386 be the easiest to understand in light of recent reports showing that the Golgi and ER move from
387 around the host nucleus in uninfected cells to around the parasite[47-49].

388 Most of the proteins that we examined bear signal sequences that would direct them to the
389 ER and Golgi. On the other hand, HsNR4A3, which presumably does not bear a signal sequence,
390 would normally be translated in the cytoplasm and imported to the nucleus via the nuclear pore
391 complex (NPC) by virtue of its nuclear localization signal. In early stages of the infection,
392 HsNR4A3 is found in the nucleus and only later relocates to the PV. Do nuclear pore proteins
393 somehow relocate from the nuclear membrane to the PVM? It is possible that nuclear pore complex
394 proteins find their way to the PVM, as well. It was recently shown that infection with LS parasites
395 results in the host microtubule organizing center (MTOC) relocalizing to the PVM. The authors of
396 the paper suggested that during infection MTOCs reorganize the host microtubule network around
397 the developing LS parasites[50]. It may be that there is an active contact site between the host nucleus
398 and the PVM through the NPCs. Active contact sites between nucleus and mitochondria[51] or
399 multiple inter-organelle contact sites[52] have been shown in mammalian cells.

400 Despite questions about how it occurs, the redirection of transcription factors away from
401 the host nucleus into to the PV could provide physiological advantages to the parasite. It could
402 render the infected cell less able to transcriptionally respond to external stimuli such as signals to
403 divide, differentiate, or undergo apoptosis, all of which would likely be unfortunate events for the
404 parasite. This could be particularly important for cells that must harbor hypnozoites for many
405 years. The redirection of HsNR4A3 away from the nucleus could also have consequences for
406 immune evasion. Proliferation and IL-2 production levels of T cells cocultured with HsNR4A3
407 knocked-down DCs were significantly lower than that of T cells cocultured with control DCs[53].

408 In contrast, HsCGA, HsMUC13, and HsCXCL2 are all secreted proteins (one membrane
409 localized and two not) and would presumably be trafficked via the endoplasmic reticulum to the
410 Golgi apparatus where they would be packaged into secretory vesicles for export. Further work
411 and mutagenesis of reporters may be needed to determine whether there are specific signals that
412 result in export to the PVM or PV.

413 The redirection of secretory vesicles to the PV versus to the extracellular space or cell
414 membrane in infected cells could have strong immunological consequences. A number of human
415 cytokines and proteins from the fever-causing NLRP3 inflammasome[54] including ATM, CDCA8,
416 CXCL10, CXCL2, ESPL1, H2AFX, HMOX1, LIG1, MDM2, NFKBIE, OGG1, PRIM1, PTTG1,
417 RAD50, RAD51, RELB, TDP1, TP53, TYMS, and XRCC1 are differentially regulated in late
418 infected hepatocytes via pathway analysis ($p = 0.0093$) and the redirection of secreted proteins to
419 the PV could be a mechanism to both improve parasite nutrition and evade host immune responses.
420 The antigens that would normally be displayed on the major histocompatibility complex on the
421 infected hepatocyte cell surface and present parasite antigens to T cells are now being secreted into
422 the PV space, where they cannot be recognized by CD8+ T cells.

423 Although both infected and uninfected cells were treated identically in our expression
424 dataset, the use flow sorted hepatoma cells, could create artefactual patterns. Recently a single cell
425 RNA-seq study was published[55]. Although a comprehensive comparison of the mouse and human
426 transcriptomes is outside the scope of this study, the patterns in our cell culture dual RNAseq
427 dataset are supported by single RNA-seq data from mouse liver infected with *P. berghei*. In
428 general, both datasets showed upregulation of genes involved in immune modulation and
429 decreased expression of metabolism associated genes. The single cell mouse study shows that
430 MmNR4A3 is clearly upregulated in infected mouse hepatocytes ($q = 9.18928E-06$) as is
431 MmCXCL2 ($q = 0$).

432 The reason for clinical silence of the malaria parasite infected liver cells has long been a
433 source of discussion. Bertolino and Bowen speculated that one reason CD8+ T cells poorly
434 recognize infected hepatocytes in a natural infection is because of either the low numbers of
435 infected hepatocytes, or because of natural tolerance[56]. An alternative explanation is that secretion
436 is modified in infected cells to direct products to the PV, as supported by our data. Our data may
437 also explain why irradiated sporozoites, which can complete the early stages of invasion but do
438 not progress to liver schizonts could create a better immune response. It would be interesting to
439 evaluate the distribution of organellar markers such as HsBiP, and HsGOLGA8A with irradiated
440 sporozoites.

441 How the parasite accomplishes host cell remodeling is unknown. Our data support the
442 Golgi as key to host cell remodeling. Much of the Golgi structure and function is controlled by
443 small Rab GTPases, which are key regulators of intracellular transport and membrane trafficking
444 in eukaryotic cells GTPases[57]; De Niz et al. demonstrated the functional importance of the
445 GTPases ARF1 and RAB1A, in *P. berghei* exoerythrocytic stage development, when they
446 expressed dominant negatives (with gain-of-function mutations in the nucleotide binding sites)
447 and showed that these mutants proteins caused an arrest and lower survival of the parasite in HeLa
448 cells[48]. In the related apicomplexan parasite, *T. gondii*, genetic screens have shown that the parasite
449 releases effector molecules into the host cell cytoplasm when it invades[58]. Although parasite
450 proteins that are needed for exoerythrocytic stages to develop have been identified in genome wide
451 genetic screens[59], it is not clear which of these are released into the host cell cytoplasm and which
452 are retained within the parasite. Screens of these mutants using host markers will likely be needed
453 to identify these factors.

454 One of the most interesting discoveries is that host cell remodeling is also observed in *P.*
455 *vivax* hypnozoites, as this now presents a way to disturb the PV and potentially provide a radical
456 cure. *P. vivax* hypnozoites, which presumably are not replicating their DNA and are not growing,
457 have traditionally been challenging to eliminate. The 8-aminoquinoline family of compounds that
458 can eliminate hypnozoites were discovered by injecting hundreds of macaques with random
459 compounds and testing to see which were permanently cured of malaria[60,61]. Recently, phenotypic
460 screening systems have begun to identify other types of compounds that are critical to hypnozoite
461 survival[62]. Our work shows that early-stage *P. berghei*, and *P. vivax* EEFs both redirect
462 upregulated proteins to the PVR, and this presents a way to study the process. This could
463 potentially lead to the identification of drug targets for the elusive hypnozoite.

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469 **MATERIALS AND METHODS**

470 Note: Requests for materials should be addressed to Elizabeth A. Winzeler:
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472 *Parasites*

473 *P. berghei*-ANKA-GFP-Luc-SMCON (*Pb*-Luc)[63] and *P. berghei*-GFP (*Pb*-GFP)[63]
474 sporozoites were obtained by dissection of infected *Anopheles stephensi*[64,65] mosquito salivary
475 glands. Dissected salivary glands were homogenized in a glass tissue grinder and filtered twice
476 through nylon cell strainers (20µm pore size, Millipore SCNY00020) and counted using a
477 Neubauer hemocytometer. The sporozoites were kept on ice until needed. *Pb*-Luc and *Pb*-GFP-
478 infected *An. stephensi* mosquitoes were also obtained from the Insectary Core Facility at New
479 York University or from the Sporocore at the University of Georgia, Athens (Grant number R01
480 AI090141-08).

481 For *Pb*-Luc sporozoite production, female 6–8-week-old Swiss Webster mice (UCSD
482 Vivarium) were injected with blood stage *Pb*-Luc parasites to begin the growth cycle. Animal
483 handling was conducted according to the UCSD Institutional Animal Care and Use Committee-
484 approved protocols. *An. stephensi* mosquitoes raised at UCSD insectary were allowed to feed on
485 infected mice with gametocytemia of ~10%. Dissected thorax sporozoites were isolated using a
486 standard protocol at day 14 or 15 post-blood meal. The sporozoites were washed with DMEM
487 incomplete media to remove debris from the thorax. The sporozoites were centrifuged 15,000 × g
488 for 5 min at 4°C to pellet and resuspended in the desired volume of complete medium.

489 *P. falciparum* sporozoites strain NF54 were isolated from infected *An. stephensi* mosquito
490 salivary glands. The sporozoites were isolated at TropIQ Health Sciences. Transistorweg 5, The
491 Netheherlands.

492 *Cell culture*

493 HC-04[66], Huh7.5.1[67] and HepG2-A16-CD81^{EGFP} [68,69] cells were cultured at 37°C with 5%
494 CO_2 in complete media (DMEM (Invitrogen, Carlsbad, USA) supplemented with 10% FBS,
495 0.29mg/mL glutamine, 100 units of penicillin, and 100µg/mL streptomycin). During and after the
496 infection with *Pb*-Luc, or *Pb*-GFP, or *P. vivax* sporozoites, complete DMEM media was

497 supplemented with antibiotics 50µg/mL gentamycin, 50µg/mL neomycin, 100 units of penicillin,
498 and 100µg/mL streptomycin, also antimycotics 50µg/mL 5-fluorocytosine, 100µg/mL
499 posaconazole and 100µg/mL neomyxin. All cell lines and source are described in Table S1.

500 *Infection of hepatocytes for qRT-PCR*

501 Hepatocytes were seeded into 24-well plates (120,000 cells per well) 24h before infection,
502 and infected with *Pb*-GFP sporozoites obtained from freshly dissected and infected *An. stephensi*
503 mosquitoes at a ratio of 0.3 sporozoites per seeded cell. Plates were centrifuged at 330 × g for 3
504 min to bring sporozoites closer to cells, and plates were then incubated at 37°C in 5% CO_2 for 2h
505 to promote sporozoite invasion[70]. After 2h, the cells were washed, fresh complete DMEM media
506 were supplemented with 12µM 5-fluorocytosine (Cayman, #11635), 50µg/mL gentamicin sulfate
507 (Gibco, #15710072), and 100µg/mL neomycin trisulfate salt hydrate (Sigma-Aldrich, #72133),
508 and the cells were returned to the incubator.

509 *Separation of infected and uninfected hepatocytes, RNA isolation and gene expression*
510 *quantification by qRT-PCR*

511 HepG2, Huh7.5.1 and HC-04 cells, in addition to primary human hepatocytes, were
512 dissociated from plates at time zero (uninfected hepatocytes and sporozoites before infection), 24
513 and 48hpi by the addition of 500µL TrypLE Express (1×, ThermoFisher, #12605010), washed
514 once using 1× PBS (Gibco, #14190144) and resuspended in FACS buffer (1× PBS supplemented
515 with 1mM EDTA, 25mM HEPES and 0.5%FBS), then passed through a 40µm cell strainer prior
516 to sorting (BD Falcon). Uninfected and infected cells were isolated by FACS using a BD Influx
517 cell sorter with GFP mean fluorescence intensity (MFI) used to determine infection status. Overall
518 gating strategy is as previously reported[17], and shown in Fig. S12 with total cells identified via
519 Forward Scatter (FSC) vs Side Scatter (SSC) (P1 gate), individual cells identified via tandem
520 gating of FSC-Height vs FSC-width and SSC-Height vs SSC-Width (gates P2 and P3), then GFP
521 positive and negative cells were collected as indicated (gates P4, and P5 respectively). For RNA
522 extraction, cells were directly sorted into Eppendorf tubes containing 600µL Qiazol reagent
523 (Qiagen) and total RNA was extracted using the Qiagen miRNEasy Mini kit (Qiagen, #217004)
524 according to manufacturer's instructions. Gene expression differences were measured using SYBR
525 green qRT-PCR with PerfeCTa SYBR® Green FastMix (Quanta Bio, #95054), using the primers

526 indicated in Table S2, and a Bio-Rad CFX96 qRT-PCR system (Bio-Rad). Changes were
527 quantitated according to the ΔΔCT method and normalized using Human Beta-2-Microglobulin
528 (*B2M*) gene.

529 *Dual RNA-sequencing analysis and code availability*

530 We analyzed the gene differential expression using data previously generated and obtained
531 in our group[17]. Reads can be found in the short read archive (http://www.ncbi.nlm.nih.gov/sra),
532 under accession number PRJNA390648. We took the dual RNA-seq read count tables from the
533 same study, and used DESeq2[71] with a multifactorial design, to calculate gene differential
534 expression at both 24 and 48h for infected versus uninfected cells, in HepG2s, Huh7.5.1s and HC-
535 04s cell lines as well as using HC-04 cells alone.

536 *Determination of infection in hepatocytes through RNA quantification by qRT-PCR*

537 Quantification of *Pb*-Luc infection was as previously described[72]. Briefly, 90,000 HC-04
538 HsNR4A3 WT and CRISPR/*Cas*9 edited cells were plated 24h before infection in 24-well plates.
539 *Pb*-Luc parasites were added at a 1:2 ratio and complete DMEM media was changed 2h later.
540 After 48h, infected cells were harvested and washed gently with PBS. RNA was isolated using
541 Qiagen miRNEasy Mini Kit (Qiagen, #217004) according to manufacture instructions. RNA was
542 quantified using a Nanodrop (Thermo Scientific) and cDNAs were synthetized using
543 SuperScript™ II Reverse Transcriptase kit (Invitrogen, #18064022). Gene expression levels for
544 parasite 18S RNA were then assessed by SYBR green qRT-PCR using perfecta SYBR Green
545 FastMix (Applied Biosystems, #4367659), the set of primers described in Table S2, a Bio-Rad
546 CFX realtime PCR system and quantitated using the ΔΔCT method and normalized against Human
547 Hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) gene.

548 *Western blot of CRISPR/Cas9 edited cell lines*

549 Cells were first plated, infected, and sorted for GFP as described above. After sorting,
550 1,000 infected or 150,000 uninfected cells were washed twice with cold 1× PBS. Cells were lysed
551 via the addition of 200µL RIPA buffer (Teknova, #R3792) plus 1:100 protease inhibitor (Halt –
552 Thermo Fisher Scientific, #78429). Equal numbers of cells were loaded onto the gel in 2× Laemmli
553 Buffer (BioRad, #1610737), and proteins were loaded onto 18-well BioRad anyKD Criterion TGX
554 gels (Bio-Rad, #5671124). For validation of HsNR4A3 protein knockout, 10µg of protein from

555 uninfected HC-04 WT and CRISPR/*Cas*9 edited cell lines were lysed in RIPA buffer as above and
556 loaded into identical gels.

557 Approach 1: Proteins were transferred to membranes and were probed with primary α-
558 HsNR4A3 (dilution 1:100; #ab56340), and α-HsVinculin (dilution 1:100; #ab129002) antibodies
559 overnight at 4°C, probed with goat α-rabbit HRP secondary (dilution 1:5,000; #G21234), and
560 detected using Super Signal West Pico, and Femto Chemiluminescent Substrate (Thermo Fisher,
561 #34577 and #34090). Densitometry was calculated using ImageJ (http://rsbweb.nih.gov/ij/) after
562 image inversion and is shown relative to loading control.

563 Approach 2: Proteins were transferred to membranes and were probed with primary α-
564 HsNR4A3 (dilution 1:100; #TA804893), and α-HsVinculin (dilution 1:100; #4650S) antibodies
565 overnight at 4°C, probed with goat α-mouse HRP secondary (dilution 1:5,000; #7076P2) or goat
566 α-rabbit HRP secondary (dilution 1:5,000; #7074S), and detected using SuperSignal West Pico
567 and Femto Chemiluminescent Substrate (Thermo Fisher, #34577 and #34095), images were
568 acquired using a gel documentation and analysis system (Syngene, G:Box) and the software Gen
569 Sys V.1.4.3.0. Densitometry was calculated using ImageJ (http://rsbweb.nih.gov/ij/) after image
570 inversion and is shown relative to loading control.

571 *Plasmodium exoerythrocytic form (EEF) culture for cellular localization*

572 For *Pb*-Luc imaging, 96-well glass bottom plates (MatTek Corporation) or 8-well Nunc
573 Lab-Tek slides (Thermo Scientific, #125658) were coated with Poly-L-Lysine 0.01% (v/v)
574 (Sigma, #P4707) and subsequently seeded with HC-04 cells (2,500 or 25,000 cells per well,
575 respectively) 24h before infection. *Pb*-Luc sporozoites were freshly isolated from infected *An.*
576 *stephensi* mosquitoes as above and resuspended in screening DMEM media (DMEM media
577 supplemented with 5% FBS, 1.46mg/mL glutamine, 500 units of penicillin, and 500μg/mL
578 streptomycin), and additionally we added 0.5μM posaconazole (Cayman), 12μM 5-fluorocytosine
579 (Cayman), 50μg/mL gentamicin sulfate (Gemini Bio-Products), and 100μg/mL neomycin
580 trisulfate salt hydrate (Sigma-Aldrich). Pre-seeded well plates were infected with *Pb*-Luc
581 sporozoites using a 1:2 infection ratio (sporozoite to cell) and incubated for 2h at 37°C in 5% CO_2.
582 After 2h infection, medium was replaced, and plates were incubated for 48h.

583 *P. falciparum experiments*

584 Sixty thousand human primary hepatocytes were seeded in 60 wells of a collagen-coated
585 96 well microtiter plate. Two days after seeding, cells were infected with fifty thousand sporozoites
586 of *Plasmodium falciparum* strain NF54 (isolated from infected *An. Stephensi* mosquitos). Five
587 days post-infection, cells were fixed with methanol, permeabilized, blocked, incubated with
588 different primary antibodies, and evaluated by immunofluorescence and confocal microscopy.

589 *P. vivax experiments*

590 *P. vivax* sporozoites were isolated from infected *An. darlingi* mosquitoes from a laboratory-
591 established colony in the Peruvian Amazon region[73,74]. Briefly, 30 patients (18 years and older)
592 from the Iquitos region, who were diagnosed with *P. vivax* malaria by blood smear and who had
593 not received treatment yet were enrolled. Approximately 20mL of whole blood was collected.
594 After centrifugation at 500 × *g* for 5 min at 37°C, plasma was removed from the *P. vivax*-infected
595 packed red cells and replaced with an equal volume of heat inactivated O^+ plasma. Then the red
596 cells were used to feed fasted female *An. darlingi* mosquitoes using a standard membrane feeding
597 assay as previously described[73,74]. After seven days, a group of ten mosquitos were midgut
598 dissected and stained by mercurochrome to evaluate oocyst development, and 14 days after the
599 first infection mosquitoes were dissected to obtain sporozoites. Dissected salivary glands were
600 homogenized in a 1.5mL tube, crushed with a homogenizer, filtered twice using nylon cell strainers
601 (20µm pore size, Millipore SCNY00020), centrifuged, and resuspended in a 500µL final volume.
602 The number of sporozoites were counted using Neubauer hematocytometer. The sporozoites were
603 kept on ice until needed.

604 For Western blot assays, 6-well glass bottom plates (Thermo Scientific, #NUNC-140675)
605 were coated with Poly-L-Lysine 0.01% (v/v) (Sigma, #P4707) and seeded with HC-04 WT cells
606 (800,000 cells per well) 24h before *P. berghei* or *P. falciparum* NF54 sporozoites infection
607 (400,000 sporozoites per well). *P. berghei* or *P. falciparum* NF54 were diluted in DMEM media
608 using 1:2 infection ratio (sporozoites to HC-04 cells) and incubated 4h at 37°C under 5% CO_2
609 atmosphere. After this initial incubation period, infection media was replaced with fresh screening
610 DMEM media, plates with HC-04 cells infected with *P. berghei* were incubated for two days, and
611 the plates with HC-04 cells infected with *P. falciparum* were incubated for five days, the screening
612 DMEM media was replaced every 24h.

613 Uninfected and infected cells were lysed via the addition of 200µL RIPA buffer (Fisher
614 Scientific, #P189900) plus protease inhibitor cocktail (cOmplete Mini, EDTA-free – Roche,
615 #11836170001). Proteins (50µg) were mixed with 2× Laemmli Buffer (BioRad, #1610737), and
616 loaded onto 12-well BioRad Criterion TGX gels (Bio-Rad, #5671083). Proteins were transferred
617 to nitrocellulose membranes and were different primary antibodies overnight at 4°C (Table S2),
618 probed with goat α-mouse HRP secondary antibody (dilution 1:2,000; #7076P2), goat α-rabbit
619 HRP secondary antibody (dilution 1:2,000; #7074S) or rabbit α-goat HRP secondary antibody
620 (dilution 1:2,000; #A27014), and detected using SuperSignal West Pico or Femto
621 Chemiluminescent Substrate (Thermo Fisher, #34577 and #34095), images were acquired using a
622 gel documentation and analysis system (Syngene, G:Box) and the software Gen Sys V.1.4.3.0.

623 For IFA analysis 96-well glass bottom plates (MatTek Corporation) or 8-well Nunc Lab-
624 Tek chamber slides (Thermo Scientific, #125658) were coated with Poly-L-Lysine 0.01% (v/v)
625 (Sigma, #P4707) and seeded with HC-04 WT or CRISPR/*Cas*9 edited cells (2,500 or 25,000 cells
626 per well) 24h prior to mosquito dissection and infection. *P. vivax* sporozoites were diluted in
627 screening DMEM media (supplemented as above). Slides were infected using a 1:2 infection ratio
628 (sporozoites to HC-04 cells) and incubated for 4h at 37°C under 5% CO_2 atmosphere. After this
629 initial incubation period, infection media was replaced with fresh screening DMEM media, and
630 plates or slides were incubated for five days, the screening DMEM media was replaced every 24h.

631 *Plasmodium EEF and HsNR4A3 immunofluorescence microscopy and high content imaging*

632 All primary and secondary antibodies used in this study are given in Table S3-S5. For
633 HsNR4A3 antibody #1 (dilution 1:100; #TA84893) or #2 (dilution 1:100; #ab56340) or #3
634 (dilution 1:100; #ab155535), we used HC-04 HsNR4A3 wild type, CRISPR/*Cas*9 edited cells lines
635 and the *Pb*-Luc parasites[75]. We seeded the cells in 8-well chamber slides and infected them with a
636 1:2 infection ratio (sporozoite to HC-04 cell). After 48h of infection, cells were fixed with 4%
637 paraformaldehyde-PBS (Affymetrix, #19943) for 20 min at RT, permeabilized with 0.1% TritonX-
638 100 (Fisher, #BP151500) for 10 min at RT, blocked with 1% BSA for 1h and incubated with two
639 antibodies overnight at 4°C. The first antibody was a α-PvUIS4 or α-PbUIS4 (mouse monoclonal
640 antibody for *P. vivax* UIS4 or goat polyclonal antibody for *P. berghei* UIS4; dilution 1:500;
641 PvUIS4 Ab donated by Kappe's Lab or PbUIS4, #LS-C204260), and for human host proteins the

642 following set of antibodies were used: α-HsNR4A3 (dilution 1:100; #TA804893, or #ab56340, or
643 #ab155535), α-HsCGA (dilution 1:50; #LS-C293331), α-HsGOLGA8A (dilution 1:50; #PA5-
644 35036), or α-HsMUC13 (dilution 1:50, #ab65109). Then, the following secondary antibodies were
645 used for plasmodium parasites (PvUIS4 or PbUIS4) 1) Donkey α-Mouse (1:1,000; #715297003)
646 or Bovine α-Goat (1:1,000; #805297008), Rhodamine Red™-X - Red, and for the human host
647 cells: 2) Donkey α-Mouse or Goat α-Rabbit (dilution 1:1,000; #A32766, or #111545046, Alexa
648 Fluor 488-Green) and incubated for 2h at RT. Hepatocyte plasma membrane was detected using
649 CellMask deep red (Thermo Fisher Scientific, #C10046) at 1×. After IFA, chambers were
650 removed from *Pb*-Luc-infected Lab-Tek systems, slides were mounted with Vectashield with
651 DAPI (Vector Labs, #H-1500) and #1.5 glass coverslips were affixed using nail polish. Images
652 were acquired using a Zeiss LSM880 with Airyscan Confocal Microscope (63× Plan-Apochromat
653 /1.4 NA DIC oil immersion objective); laser power was set to 3% for 405, 488, 561, and 640nm.
654 The images were processed using the confocal ZEN software (Black edition, Zeiss). The level of
655 colocalization between the two fluorescent proteins (α-PvUIS4/α-HsNR4A3, α-PvUIS4/α-
656 HsCGA, α-PvUIS4/α-HsGOLGA8A, α-PvUIS4/α-HsMUC13, or α-PvUIS4/α-HsCXCL2 were
657 determined by calculating the product of the difference from the mean (PDM) indicated a mean
658 Pearson coefficient r (Pearson correlation coefficient - PCC) in PV, α-PvUIS4 was on channel A
659 vs human host protein on Channel B. The PCC were analyzed using Volocity 3D/4D rendering
660 software for three identified objects from each of three biological replicates.

661 *Ultrastructure Expansion Microscopy (U-ExM)*

662 Clean coverslips (Fisher Scientific, #NC1129240) pretreated with poly-l-lysine for 1h at
663 37°C, washed with MilliQ water, dried, and placed in the wells of a 12-well plate, were used to
664 seed human liver cells (HC-04 or Huh7.5.1 cells, 200,000) resuspended in 1ml of DMEM complete
665 media and incubated at 37°C, 5% CO_2, for 24h. The infection was performed with *P. berghei*
666 sporozoites (100,000) at ratio 2:1 (two cells to one parasite), incubated during 48h, 37°C, 5% CO_2.
667 Supernatants were removed, and the cells were fixed with 1 mL of 4% PFA (EMS #15710)/PBS
668 for 20 min at 37°C. then stored and shipped overnight to the Absalon lab at Indiana University
669 School of Medicine. At reception, coverslips were proceeded for expansion microscopy as
670 described in Liffner and Absalon 2021[38]. Gels were incubated overnight with primary antibodies

671 with the following dilution 1:250 for α-PvUIS4 and 1:100 for α-HsNR4A3 at room temperature
672 on a shaker. All secondary antibodies were diluted 1:500 in PBS (Donkey anti-Goat-Alexa488,
673 ThermoFisher Scientific #A-11055 and Donkey anti-Mouse-Alexa555, ThermoFisher Scientific
674 #A-31570), 1:250 for NHS ester-Alexa405 (ThermoFisher Scientific #A7573) and 1:1000 for
675 Sytox™ Deep red (ThermoFisher Scientific #S11380) and incubated room temperature on a shaker
676 for 2h and 30 min. Staining with 1:1000 Bodipy-TR-Ceramide (ThermoFisher Scientific #D7540)
677 required an additional overnight room temperature on a shaker.

678 For imaging, small sections of the larger gel were cut and gently dried before being placed
679 into 35 mm #1.5 coverslips bottomed imaging dishes (Cellvis; Fisher Scientific #NC0409658)
680 precoated with poly-D-lysine. The images were acquired using a Zeiss LSM900 AxioObserver
681 microscope with Airyscan 2 detector, with 63x Plan-Apochromat (NA 1.4) objective lens. All
682 images were acquired in multiplex mode 2 as Z-stacks with and XY pixel size 0.035 µm and a Z-
683 step size of 0.13 µm, and processed using Zen Blue software (Version 3.5, Zeiss, Oberkochen,
684 Germany).

685 *High content imaging (HCI) of edited lines*

686 HC-04[66] CRISPR/*Cas*9 edited cell lines were seeded in 384-well plates or 8-well Lab-Tek
687 slides and infected with *Pb*-Luc parasites[63] at a ratio 2:1 (cells/sporozoites). After 48h cells were
688 fixed, permeabilized, blocked and incubated with PbUIS4 polyclonal antibody (dilution 1:500;
689 #LS-C204260) and HsNR4A3 monoclonal antibody (dilution 1:100; #TA804893). The data were
690 acquired (image analysis) using a High Content Imaging System (Operetta®, PerkingElmer).
691 Nuclei (DAPI) were detected using an excitation wavelength of 360-400nm, emission 410-480nm,
692 with exposure time at 200 milliseconds (ms), and a height 40µm. PbUIS4 protein was detected
693 using the Alexa 488 channel (excitation 460-490, emission 500-550nm), exposure time of 200ms,
694 and height of 82µm. HsNR4A3 protein was detected using the Alexa 568 channel (excitation 560-
695 580, emission 590-640nm) with exposure time 200ms and height 82µm. Plasma membranes were
696 detected using the DRAQ5 channel (excitation 620-640nm, emission 650-760nm) with exposure
697 time of 200ms and a height 80µm.

698 To evaluate and determined the size of *P. berghei* schizonts forms we use a splitting
699 coefficient of 0.40; common threshold, 0.40, split factor 72.0; individual threshold, 0.50; contrast,

700 0.50. Schizonts were also evaluated using different sets for diameter: 5, 20, 30 and 40µm^2. The
701 images were captured using a 10× long WD/0.3 NA objective, 10× magnification, 10 fields, 1
702 plane and 1 time point.

703 *Cytokine HsCXCL2 stimulation with lipopolysaccharide (LPS)*

704 To evaluate the expression of human chemokine HsCXCL2 (GROß), *in vitro* infections
705 were performed using the human liver line HC-04 with *P. berghei* sporozoites in 8-well Lab-Tek
706 slides. Cells were stimulated by treatment with 1µg/mL lipopolysaccharide (LPS), from *E. coli*
707 O55: B5 (Sigma Aldrich, # L6529). Cells were treated with LPS at 24hpi and collected for confocal
708 microscopy at 24, and 48h. The expression and localization of HsCXCL2 protein in HC-04 cells
709 were visualized through the use of the anti-HsCXCL2 primary antibody (dilution 1:100; Table S3).
710 *P. berghei* parasites were visualized α-PbUIS4 polyclonal antibody (dilution 1:500; #LS-
711 C204260), and their respective secondary antibodies. Plasma membranes were detected using
712 CellMask deep red, and nucleus were stained with DAPI.

713 *HsNR4A3 CRISPR/Cas9 (Approach 1)*

714 HsNR4A3 was edited using a sequential two plasmid CRISPR/*Cas9* approach. First, HC-
715 04 cells expressing CAS9 were generated using lentivirus containing the EF1a-*Cas9*-2A-
716 Blasticidin Lenti plasmid (Sigma Aldrich, #LVCAS9BST-1EA). HC-04 cells were plated at a
717 density of 120,000 cells per well in three independent wells of a 24 well plate. Twenty-four hours
718 after plating, media was replaced with complete media (as above) plus 5µg/mL polybrene and viral
719 particles. Cas9 lentiviral particles were added at a multiplicity of infection (MOI) of 0.5, after
720 which cells were spun at 800 × g for 30 min, then incubated overnight at 37°C. The following day
721 media, was changed to complete media and cells were again incubated overnight at 37°C. The
722 next day, cells were selected for presence of the CAS9 plasmid using complete media plus
723 2.5µg/mL blasticidin (Thermo Fisher, #A1113903). After 14 days of selection, splitting as
724 required, cells were trypsinized and plated at an average density of one cell per well in a 96 well
725 plate in complete media plus blasticidin as above. Once cells had regrown, wells were observed
726 via microscopy to identify those wells which appeared to contain single colonies. CAS9
727 expression within clones was then confirmed via PCR.

728 Once CAS9 expressing HC-04 cells were established, gRNA targeting *HsNR4A3* was
729 introduced via Lentivirus containing the LV04 U6-gRNA:hPGK-Puro-2A-BFP plasmid and a
730 gRNA sequence specific for Human *HsNR4A3*. The DNA sequence targeted by the gRNA for
731 *HsNR4A3* was CTGCAGCAGCCTGGTCAGTGGG (Sigma-Aldrich Sanger Clone ID
732 HS5000010424). Cells were infected with virus and cultured overnight as above. The following
733 day, cells were selected for presence of the gRNA plasmid using complete media plus 2.5µg/mL
734 puromycin (Gibco, #A1113803) and 2.5µg/mL blasticidin. Clonal cell lines expressing the gRNA
735 and CAS9 were generated as previously stated. The presence of *HsNR4A3* knockout within these
736 apparent monoclonal HC-04 cell lines was examined at the protein level, after being expanded into
737 25cm^2 vented tissue culture flasks, via western blot using the HsNR4A3 rabbit polyclonal antibody
738 (dilution 1:100; Abcam, #ab56340). Three edited clones (clones GL1, GL2, and GL3) were then
739 examined using confocal microscopy, all exhibited low or no host cell HsNR4A3 protein
740 expression.

741 *HsNR4A3 CRISPR/Cas9 (Approach 2)*

742 A second round of editing for *HsNR4A3* was performed using a single plasmid
743 CRISPR/*Cas9* system with the Edit-R All-in-one Lentiviral an sgRNA, targeting *HsNR4A3*,
744 packaged in a lentiviral vector (Horizon, Perkin Elmer). The gRNA sequence used was
745 TTCGACGTCTCTTGTCTACT, targeting exon 5 of *HsNR4A3* coding sequence. Briefly, HC-04
746 cells were plated at a density of 150,000 cells per well in a 6 well plate, then 24h later media was
747 replaced with complete media supplemented with 10µg/mL polybrene. Virus was added at a
748 multiplicity of infection of one and then incubated overnight at 37°C. After 48h, the cells were
749 selected for presence of the CAS9 plasmid using complete media plus 2.5µg/mL puromycin. After
750 seven days of selection, cells were trypsinized and plated at an average density of 0.5 cell per well
751 in a 96-well plate in the presence of 2.5µg/mL puromycin for clonal selection. After ~15-20 days,
752 wells were observed via microscopy to identify those wells which contained single colonies. These
753 cells were then transferred to 6-well plates and the knockout of HsNR4A3 was confirmed in these
754 apparent monoclonal HC-04 cell lines with immunoblotting.

755 Since, in the above obtained clones, there was only partial knockout of HsNR4A3, another
756 round of transductions was performed using guide RNA GCTCGAGTAGCCCTCCACGA

757 targeting at exon 4. Lenti-V2-Blast (lentiCRISPR v2-Blast plasmid, Addgene) plasmid was used
758 here. Transductions were performed, as mentioned above, and the transformed cells were selected
759 using 1µg/mL blasticidin and 2.5µg/mL puromycin. The clones obtained after clonal selection
760 were evaluated for HsNR4A3 expression by immunoblotting using the HsNR4A3 mouse
761 monoclonal antibody (dilution 1:100; #TA804893). Three knockdown clones (clones NM1, NM2,
762 and NM3) were selected to have low HsNR4A3 expression. These clones were then used for
763 further studies.

764 **ETHICS STATEMENT**

765 Human subject protocols were approved by the Human Research Protection Program of
766 the University of California, San Diego (approval number 120652) and Universidad Peruana
767 Cayetano Heredia (approval number 102357). Written informed consent was obtained from all
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AUTHOR CONTRIBUTIONS

JC performed Immunofluorescence, HCI, qRT-PCR, Western blot assays and data analysis. GL infected and sorted liver cells, isolated RNA for both infected and uninfected populations and measured gene expression levels via qRT-PCR and Western blot. JC and LG performed *P. vivax* liver stage assays. GL, BYZ, EP and NM generated *HsNR4A3* edited cell lines. JC, GL and EW wrote the manuscript. KPGM analyzed data and created figures. BC contributed concepts and helped fund the study. CTR, DG and JV developed methods and infrastructure for *P. vivax* infections. GTW performed ELISA assays for HsCXCL2 protein. SA and BL performed U-ExM and imaging. All authors assisted with editing of the manuscript. The authors declare no conflicts of interest or competing financial interests.

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1030 **FIGURE LEGENDS**

1031 **Figure 1**. ***HsNR4A3* is highly upregulated during *Plasmodium* exoerythrocytic infection. a.**
1032 Schematic representation for culture, sorting by FACs, dual RNA Seq and confocal microscopy
1033 strategies, **b.** Volcano plot of differently expressed genes, gene-expression pattern vs *p* value, with
1034 the position of *HsMUC13, HsCGA, HsSLC22A8, HsMALAT1, HsRASSF9, HsSDHA,*
1035 *HsSLC25A27, HsNEAT1, HsTAS2R4*, *HsCXCL2,* and *HsNR4A3* genes indicated at 24 and 48hpi.
1036 **c.** RT-qPCR of *HsMUC13, HsCGA, HsSLC22A8, HsMALAT1, HsRASSF9, HsSDHA,*
1037 *HsSLC25A27, HsNEAT1, HsTAS2R4,* and *HsNR4A3* at 48hpi in the indicated cell lines. *HsSDHA*
1038 was used as a control. Relative quantitation of the indicated host transcripts calculated using ΔΔCT
1039 method, in the indicated hepatocyte cell types (data presented as mean ± s.e.m, *n*=3 with individual
1040 biological replicates overlaid, * = *p* value 0.05, ** = *p* value 0.001, *** = *p* value 0.0001, **** =
1041 *p* value <0.0001. *p* values determined by two-tailed *t* test). Data from LaMonte et al.[17].

1042 **Figure 2. Immunofluorescence micrographs of *Plasmodium*-infected cells with human**
1043 **HsNR4A3, HsCGA, HsGOLGA8A, and HsMUC13 antibodies.** Representative confocal
1044 microscopy images of **a.** HC-04 liver cells infected with *P. berghei* parasites 48hpi; **a1.** Zoom in
1045 of HC-04 liver cells infected with *P. berghei* parasites and labeled with HsNR4A3 antibody, **a2.**
1046 Zoom out of a1 panel (panoramic view), **a3.** Zoom out of a1 panel (panoramic view), the signal of
1047 each channel was over exposed, **b.** primary hepatocytes infected with *P. falciparum* parasites
1048 120hpi; **c.** HC-04 liver cells infected with *P. vivax* schizonts forms 120hpi, and **d.** HC-04 liver
1049 cells infected with *P. vivax* hypnozoites 120hpi. **d1.** Zoom in of HC-04 liver cells infected with *P.*
1050 *vivax* hypnozoites and labeled with HsNR4A3 antibody. Cells were labeled using the next set of
1051 antibodies: α-HsNR4A3 (#ab56340 or #ab155535 or #TA804893), α-HsCGA (#LS-C293331), α-
1052 HsGOLGA8A (#PA5-35036), or α-HsMUC13 (#ab65109), and visualized with a donkey α-mouse
1053 or goat α-rabbit secondary antibody (#A32766, or #111545046, Alexa Fluor 488 - Green); *P.*
1054 *berghei*, *P. vivax*, and *P. falciparum* EEFs (a-d) were labeled using α-PvUIS4 (Donated by
1055 Kappe's Kab) or PbUIS4 (#LS-C204260), and visualized with a donkey α-mouse, or bovine α-
1056 goat secondary antibody (#715297003, or #805297008, Rhodamine-red). Nuclei were labeled with
1057 DAPI (blue). Merged images between HsNR4A3, HsCGA, HsGOLGA8A, HsMUC13, PvUIS4,

1058 DAPI, and Cell Membrane are shown. Scale bars 10µm; 63× oil objective, UI (Uninfected cell), I
1059 (Infected cell), PN (parasite nuclei), and HN (host nucleus).

1060 **Figure 3: Relocation of the HsNR4A3 protein in HC-04 cells infected with *P. berghei***
1061 **parasites in different periods (3-96hpi). a.** Micrographs show HC-04 cells uninfected (0h), and
1062 infected with *P. berghei* sporozoites, fixed at different times (6, 12, 18, 24, 48, and 72 hours).
1063 Cells were labeled using a α-HsNR4A3 (#TA804893) and visualized with a donkey α-mouse
1064 secondary antibody (#A32766, Alexa Fluor 488 – green); CellMask deep red was used for plasma
1065 membranes (Cell Membrane - magenta). *P. berghei* parasites were labeled using α-PbUIS4 (#LS-
1066 204260) and visualized with a bovine α-goat secondary antibody (#805297008, Rhodamine - red).
1067 Nuclei were labeled with DAPI (blue). Merged images between HsNR4A3, PbUIS4, DAPI, and
1068 Cell Membrane are shown. Scale bars 10µm; 63× oil objective. **b.** Schematic representation of
1069 HsNR4A3 relocalization during *Plasmodium* exoerythrocytic infection in infected liver cells. UI
1070 (Uninfected cell), I (Infected cell), PN (parasite nuclei), and HN (host nucleus).

1071 **Figure 4. U-ExM on huh7.5.1 cells infected with *P. berghei* parasites shows HsNR4A3 protein**
1072 **at the PVM.** Five representative micrographs show Huh7.5.1 cells after 48h of infection with *P.*
1073 *berghei* parasites prepared for U-ExM (see Methods), stained with a nuclear stain (Sytox Deep
1074 Red in cyan), α-PbUIS4 (in magenta), α-HsNR4A3 (in yellow) and a protein stain (NHS-ester, in
1075 grayscale) and visualized using Airyscan 2 microscopy. Yellow arrows indicate concentration of
1076 HsNR4A3 in pockets associated with the PVM. Scale bars as labelled in each image, solid bar =
1077 XY scale and all images are Z-projection with a combined depth of slices of 0.39 µm.

1078 **Figure 5. Relocation of HsCXCL2 protein in HC-04 cells infected with *P. berghei* parasites**
1079 **in different periods (2, 24, and 48hpi). a.** Micrographs show HC-04 cells infected with *P. berghei*
1080 parasites, treated with LPS (1µg/ml), fixed, and stained using IFA and confocal microscopy. Cells
1081 were labeled using α-HsCXCL2 (#PA5-80529) and visualized with a donkey α-rabbit secondary
1082 antibody (#711296152, Rhodamine, red); CellMask deep red was used for plasma membranes
1083 (Cell Membrane - magenta). *P. berghei* parasites were labeled using α-PbUIS4 (#LS-204260) and
1084 visualized with a donkey α-goat secondary antibody (#705546147, Alexa Fluor 488, green). Nuclei
1085 were labeled with DAPI (blue). Merged images between HsCXCL2, PbUIS4, DAPI and Cell
1086 Membrane are shown. Scale bars 10µm; 63× oil objective. Antibody controls (no α-HsCXCL2, no

1087 α-PbUIS4) and uninfected cells with and without LPS treatment are shown. Scale bars 10μm; 63×
1088 oil objective. **b.** Pearson's correlation coefficient (PCC) between α-PvUIS4 and α-HsCXCL2
1089 staining. Images were analyzed with Volocity 3D/4D Rendering Software, and PCC was
1090 calculated for identified objects. Each point represents one infected cell from one of three
1091 biological replicates. Data were analyzed with a two-tailed *t* test, * = $p < 0.0112$, **** = $p <$
1092 0.0001, UI (Uninfected cell), I (Infected cell), PN (parasite nuclei), and HN (host nucleus).

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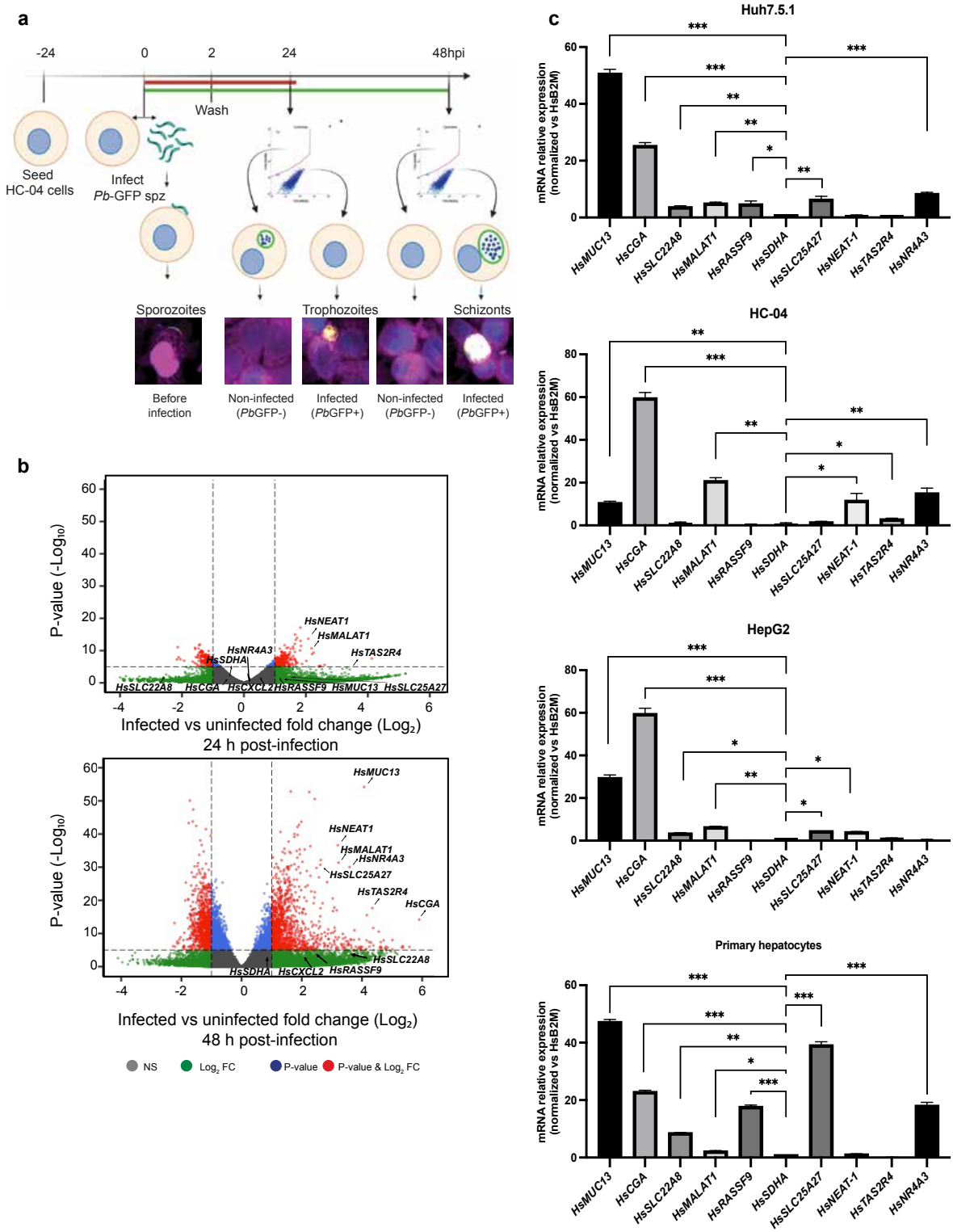
a
-24
0
2
24
48hpi
Wash
Seed
HC-04 cells
Infect
Pb-GFP spz
Sporozoites
Trophozoites
Schizonts
Before infection
Non-infected (PbGFP-)
Infected (PbGFP+)
Non-infected (PbGFP-)
Infected (PbGFP+)
b
P-value (-Log10)
Infected vs uninfected fold change (Log2)
24 h post-infection
HsNEAT1
HsMALAT1
HsNR4A3
HsSDHA
HsTAS2R4
HsSLC22A8
HsCGA
HsCXCL2
HsRASSF9
HsMUC13
HsSLC25A27
48 h post-infection
NS
Log2 FC
P-value
P-value & Log2 FC
c
Huh7.5.1
HC-04
HepG2
Primary hepatocytes
mRNA relative expression (normalized vs HsB2M)
HsMUC13
HsCGA
HsSLC22A8
HsMALAT1
HsRASSF9
HsSDHA
HsSLC25A27
HsNEAT-1
HsTAS2R4
HsNR4A3

Figure 1

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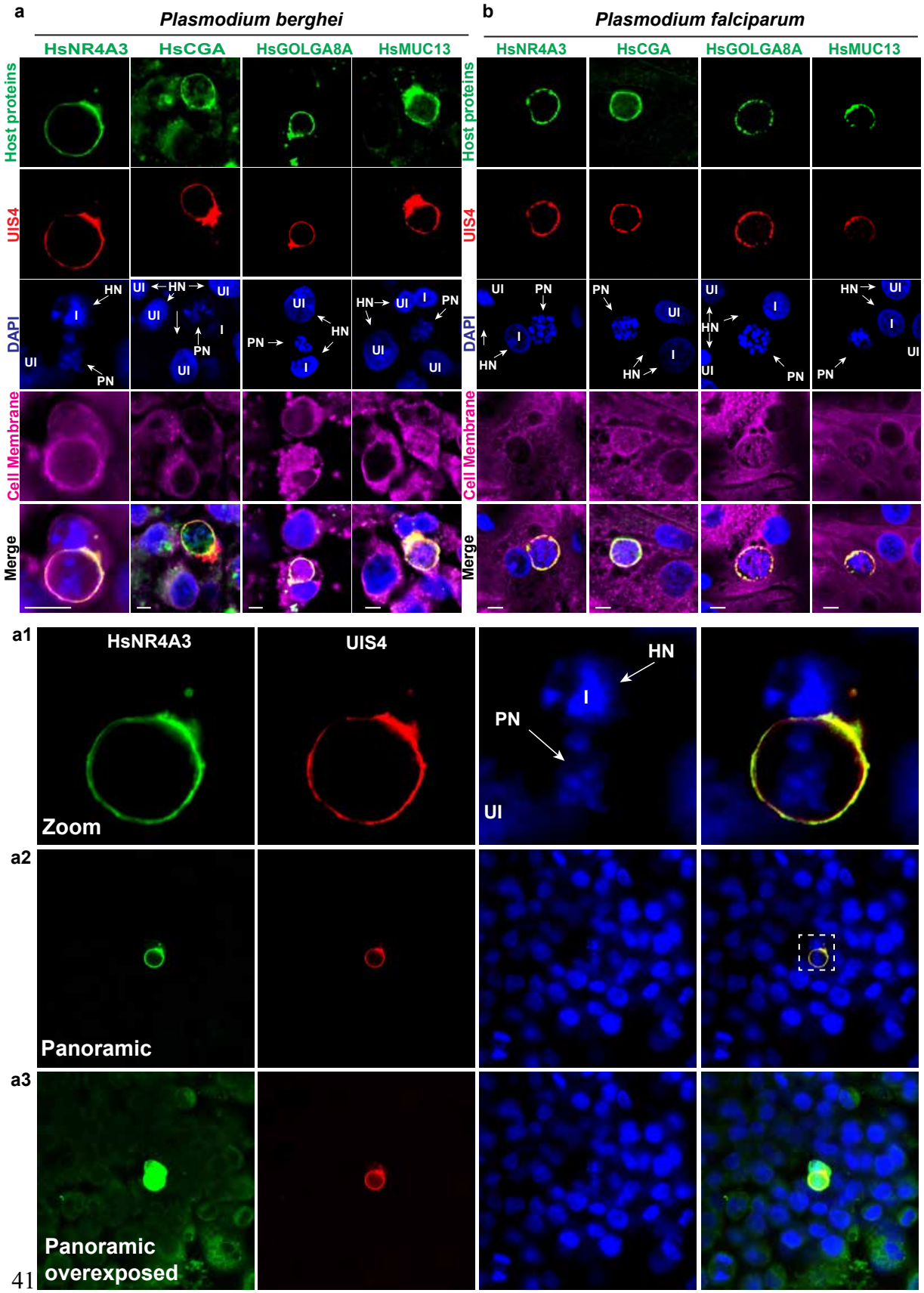
a
Plasmodium berghei
HsNR4A3
HsCGA
HsGOLGA8A
HsMUC13
b
Plasmodium falciparum
HsNR4A3
HsCGA
HsGOLGA8A
HsMUC13
Host proteins
UIS4
DAPI
Cell Membrane
Merge
HN
I
UI
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a1
HsNR4A3
UIS4
Zoom
a2
Panoramic
a3
Panoramic
overexposed

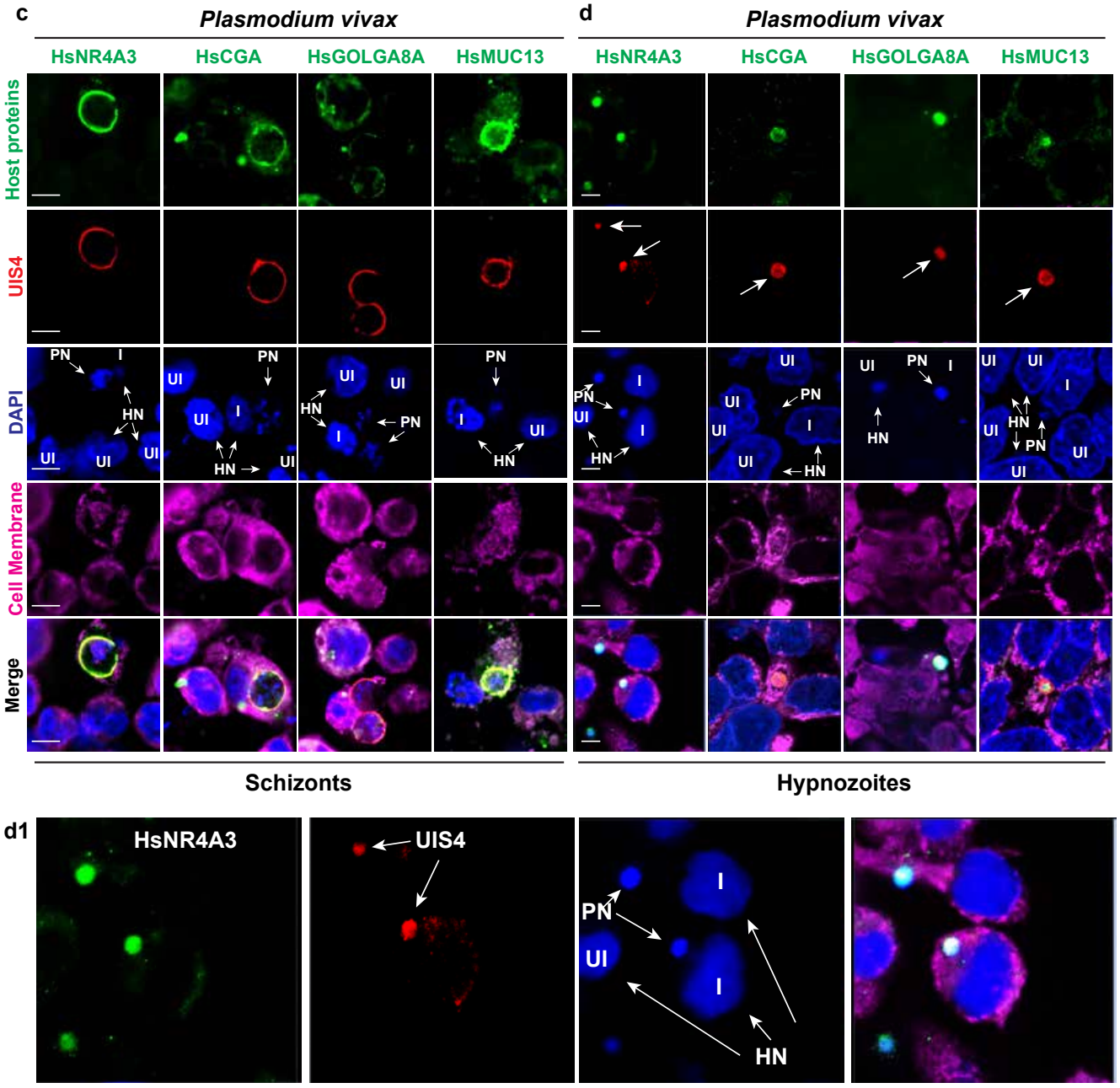
c
Plasmodium vivax
HsNR4A3
HsCGA
HsGOLGA8A
HsMUC13
d
Plasmodium vivax
HsNR4A3
HsCGA
HsGOLGA8A
HsMUC13
Host proteins
UIS4
DAPI
Cell Membrane
Merge
PN
I
HN
UI
Schizonts
Hypnozoites
d1
HsNR4A3
UIS4
PN
I
UI
HN

Figure 2

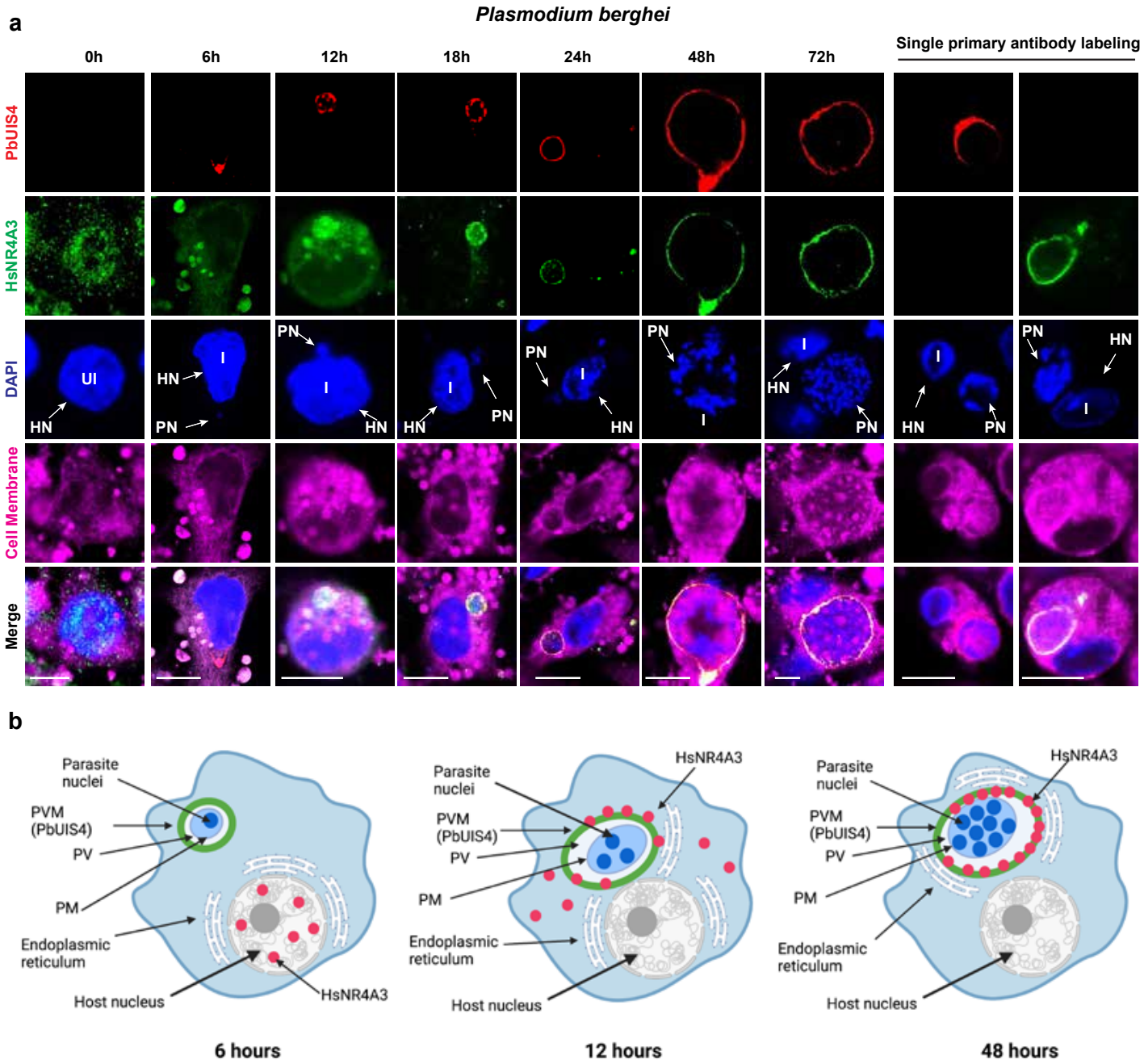
a
Plasmodium berghei
0h
6h
12h
18h
24h
48h
72h
Single primary antibody labeling
PbUIS4
HsNR4A3
DAPI
Cell Membrane
Merge
UI
HN
PN
I
b
Parasite nuclei
PVM (PbUIS4)
PV
PM
Endoplasmic reticulum
Host nucleus
HsNR4A3
6 hours
12 hours
48 hours

Figure 3

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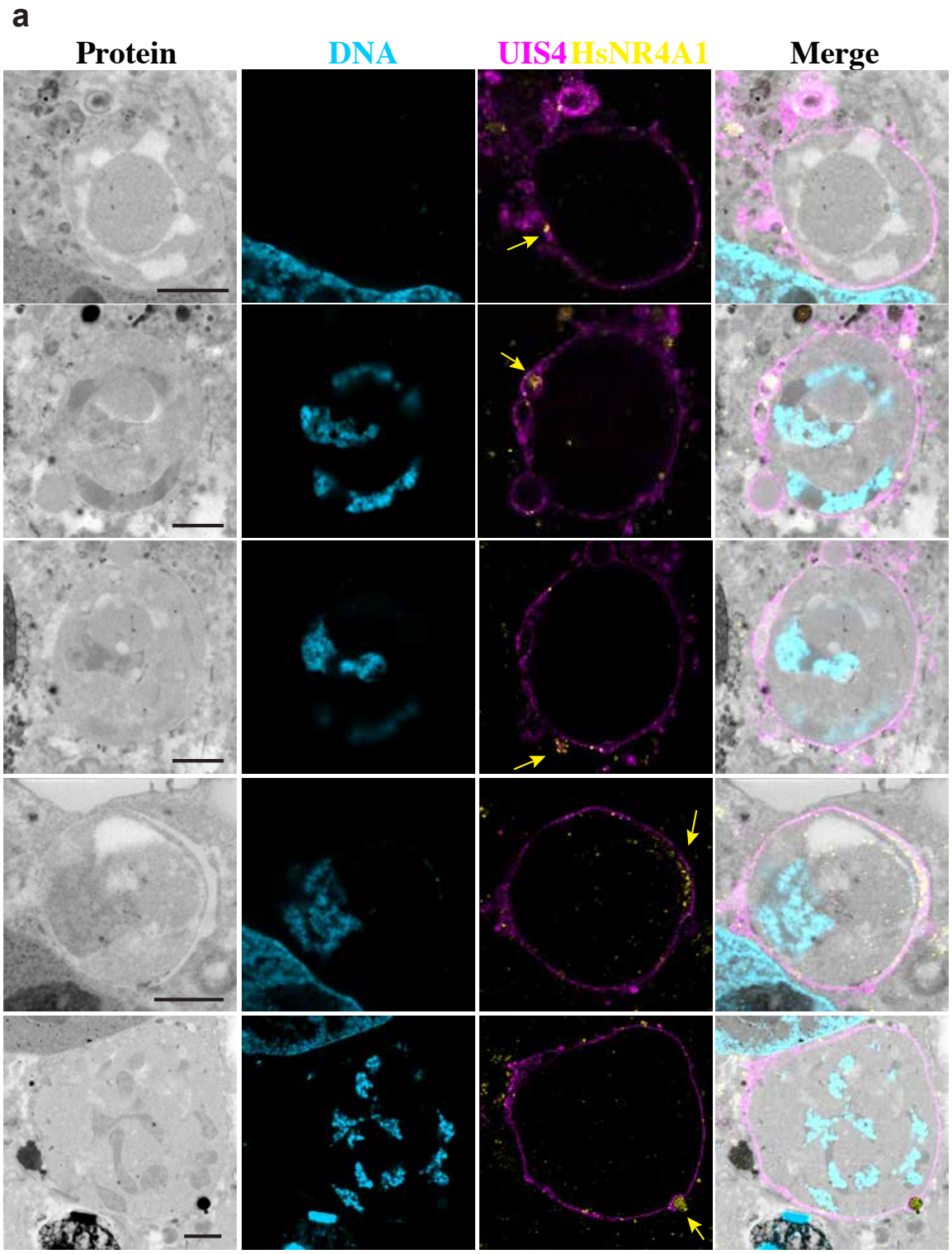

1151 Figure 4

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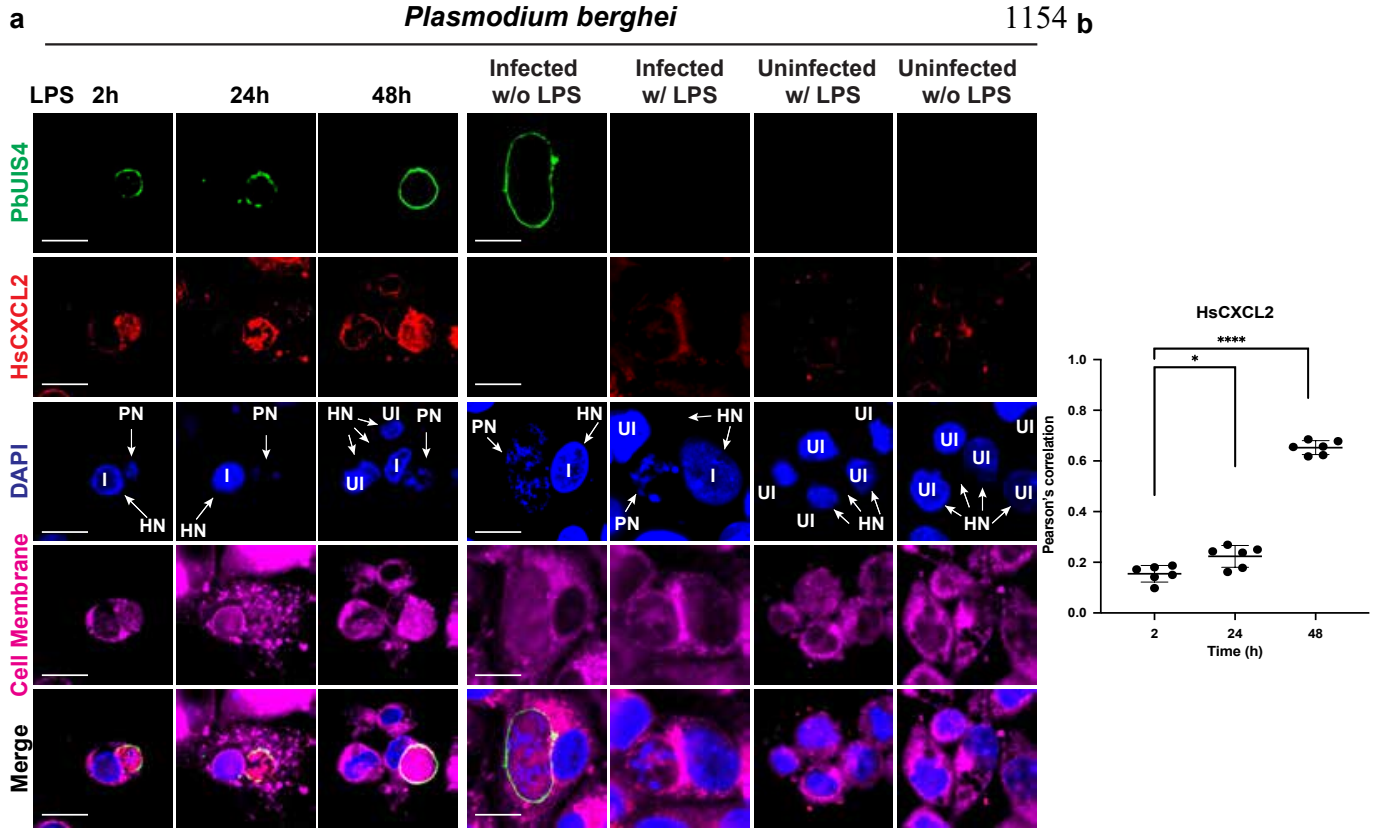

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1163 Figure 5

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