

1 ***P. falciparum* gametocyte density and infectivity in peripheral blood and**  
2 **skin tissue of naturally infected parasite carriers**

3

4 **Running title:** Gametocytes in the skin of malaria patients

5

6 **Authors:** Elamaran Meibalan<sup>1,2\*</sup>, Aissata Barry<sup>3,4\*</sup>, Matthew P. Gibbins<sup>5\*</sup>, Shehu Awandu<sup>4</sup>,  
7 Lisette Meerstein-Kessel<sup>4</sup>, Fiona Achcar<sup>5</sup>, Selina Bopp<sup>1</sup>, Christopher Moxon<sup>5</sup>, Amidou  
8 Diarra<sup>3</sup>, Siaka Debe<sup>3</sup>, Nicolas Ouédraogo<sup>3</sup>, Ines Barry-Some<sup>3</sup>, Emilie Badoum<sup>3</sup>, Traoré  
9 Fagnima<sup>6</sup>, Kjerstin Lanke<sup>4</sup>, Bronner P. Gonçalves<sup>7</sup>, John Bradley<sup>8</sup>, Dyann Wirth<sup>1</sup>, Chris  
10 Drakeley<sup>7</sup>, Wamdaogo Moussa Guelbeogo<sup>3</sup>, Alfred B.Tiono<sup>3</sup>, Matthias Marti<sup>1,5#</sup>, Teun  
11 Bousema<sup>4,7#</sup>

12

13 **Author affiliation:**

14 <sup>1</sup>Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public  
15 Health, Boston, MA, USA.

16 <sup>2</sup>Center for Excellence in Vascular Biology, Department of Pathology, Brigham and Women's  
17 Hospital, Boston, MA, USA

18 <sup>3</sup>Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso

19 <sup>4</sup>Radboud Institute for Health Sciences, Radboud University Medical Center, Netherlands.

20 <sup>5</sup>Wellcome Centre for Integrative Parasitology, Institute of Infection, Immunity and  
21 Inflammation, University of Glasgow, Glasgow, UK.

22 <sup>6</sup>Centre Hospitalier Universitaire Régional de Ouahigoua, Université de Ouahigouya, Burkina  
23 Faso

24 <sup>7</sup>Immunology and Infection Department, London School of Hygiene and Tropical Medicine,  
25 London, UK.

26 <sup>8</sup>MRC Tropical Epidemiology Group, London School of Hygiene and Tropical Medicine,  
27 London, UK.

28

29 \* These authors contributed equally to this work

30 # Corresponding author. Email: [matthias.marti@glasgow.ac.uk](mailto:matthias.marti@glasgow.ac.uk);

31 [teun.bousema@radboudumc.nl](mailto:teun.bousema@radboudumc.nl)

32

33

34

35

36 **ABSTRACT**

37 Transmission of *Plasmodium falciparum* depends on the presence of mature gametocytes that  
38 can be ingested by mosquitoes taking a bloodmeal when feeding on human skin. It has long  
39 been hypothesised that skin sequestration contributes to efficient transmission. Although skin  
40 sequestration would have major implications for our understanding of transmission biology  
41 and the suitability of mosquito feeding methodologies to measure the human infectious  
42 reservoir, it has never been formally tested. In two populations of naturally infected  
43 gametocyte carriers from Burkina Faso, we assessed transmission potential to mosquitoes and  
44 directly quantified male and female gametocytes and asexual parasites in: i) finger prick  
45 blood, ii) venous blood, iii) skin biopsies, and in pools of mosquitoes that fed iv) on venous  
46 blood or, v) directly on the skin. Whilst more mosquitoes became infected when feeding  
47 directly on the skin compared to venous blood, concentrations of gametocytes in the  
48 subdermal skin vasculature were identical to that in other blood compartments. Asexual  
49 parasite densities, gametocyte densities and sex ratios were identical in the mosquito blood  
50 meals taken directly from the skin of parasite carriers and their venous blood.  
51 We also observed sparse gametocytes in skin biopsies from legs and arms of gametocyte  
52 carriers by microscopy. Taken together, we provide conclusive evidence for the absence of  
53 significant skin sequestration of *P. falciparum* gametocytes. Gametocyte densities in  
54 peripheral blood are thus informative for predicting onward transmission potential to  
55 mosquitoes. Quantifying this human malaria transmission potential is of pivotal importance  
56 for the deployment and monitoring of malaria elimination initiatives.

57

58 **IMPORTANCE**

59 Our observations settle a long-standing question in the malaria field and close a major  
60 knowledge gap in the parasite cycle. By deploying mosquito feeding experiments and stage-

61 specific molecular and immunofluorescence parasite detection methodologies in two  
62 populations of naturally infected parasite carriers, we conclusively reject the hypothesis of  
63 gametocyte skin sequestration. Our findings provide novel insights in parasite stage  
64 composition in human blood compartments, mosquito bloodmeals and their implications for  
65 transmission potential. We demonstrate that gametocyte levels in venous or finger prick blood  
66 can be used to predict onward transmission potential to mosquitoes. Our findings thus pave  
67 the way for methodologies to quantify the human infectious reservoir based on conventional  
68 blood sampling approaches to support the deployment and monitoring of malaria elimination  
69 efforts for maximum public health impact.

70

71

## 72 INTRODUCTION

73 Significant reductions in malaria burden in recent decades have stimulated malaria  
74 elimination initiatives (1). It is widely accepted that malaria elimination with current tools is  
75 unlikely for the majority of African settings (2). Therefore, novel interventions are needed and  
76 approaches that specifically reduce malaria transmission may be of key importance (3).  
77 Transmission of malaria depends on the presence of mature male and female gametocytes that  
78 circulate in the bloodstream and may be ingested by mosquitoes from the subdermal  
79 capillaries upon blood feeding. For *P. falciparum*, these circulating mature gametocytes are  
80 the product of a prolonged developmental process that starts with commitment of asexual  
81 parasites to the sexual pathway upon activation of AP2-G(4, 5). Developing gametocytes are  
82 sequestered for 10-12 days, primarily to the bone marrow and spleen (6), until their release  
83 into the blood circulation as mature gametocytes. Mosquitoes may become infected when  
84 feeding and ingesting mature male and female gametocytes, even if their densities in the  
85 peripheral blood are low (7). Interestingly, mosquito infections have been observed from  
86 gametocyte donors whose low gametocyte density appears incompatible with transmission  
87 (8). Mosquito infection rates are typically higher when mosquitoes feed directly on the skin of  
88 gametocyte carriers, as compared to feeding on venous blood through an artificial membrane  
89 (9, 10). In addition to a strategic adjustment of gametocyte sex-ratio to maximize transmission  
90 success (7, 11, 12), gametocyte aggregation and sequestration may facilitate mosquito  
91 infections from low gametocyte densities. Aggregation of gametocytes in blood meals has  
92 been observed (13) and may increase the chance that both male and female gametocytes are  
93 ingested. Gametocyte sequestration in the skin tissue may further increase transmission rates  
94 and would parallel sequestration patterns for other human parasites. The importance of skin  
95 sequestration for transmission to invertebrate vectors was recently demonstrated for skin-  
96 dwelling *Trypanosoma brucei* (14), as was previously reported for *Onchocerca volvulus*,

97 different species of *Mansonella*, *Leishmania infantum* and *L. donovani*, where parasite burden  
98 in the skin is the best predictor of infectiousness (15-18).

99 Indirect evidence for skin sequestration of mature gametocytes in the microvasculature  
100 of the skin was first described following surveys conducted in the 1940s and 1950s in DR  
101 Congo: gametocyte prevalence in a survey using skin scarification was 3-fold higher  
102 compared to a survey 5 years earlier using finger prick blood (19). In a follow up study with  
103 1243 paired samples, a more modest 13.4% increase in *P. falciparum* parasite prevalence and  
104 15.6% increase in gametocyte prevalence was observed when blood and dermal fluids from  
105 skin scarification were used for sample preparation instead of finger prick blood (20). The  
106 hypothesized skin sequestration of intra-erythrocytic *P. falciparum* gametocytes may be  
107 related to mechanical retention in cutaneous capillaries (21, 22), analogous to the reversible  
108 rigidity that likely prevents immature gametocytes from entering circulation (23, 24).  
109 Alternatively, sequestration may be related to gametocyte cytoadhesive properties (25)  
110 mediated by parasite proteins that are present on the infected red blood cell (iRBC) surface,  
111 analogous to adhesion of asexual *P. falciparum* parasites to receptors on human vascular  
112 endothelial cells by *P. falciparum* erythrocyte membrane-1 (PfEMP1)(26).

113 Whilst sequestration of mature gametocytes in the skin of naturally infected  
114 individuals remains speculative, it may play an important role in determining *Plasmodium*  
115 transmission efficiency (8, 22). Here, we report on two independent studies in naturally  
116 infected gametocyte carriers from Burkina Faso where we quantified mature *P. falciparum*  
117 gametocytes in skin tissue, blood samples and mosquito blood meals in association with  
118 onward transmission to *Anopheles* mosquitoes.

119

## 120 RESULTS

121 A total of 31 individuals aged 15-48 (median 29) participated in experiments with paired skin  
122 feeding (27) and membrane feeding (28). The median number of dissected mosquitoes per  
123 experiment was 35 (interquartile range (IQR) 33-37) for direct skin feeding and 73 (IQR 69-  
124 82) for membrane feeding. Of 31 paired experiments, 18 (58.1%) direct skin feeding and 22  
125 (71.0%) membrane feeding experiments resulted in at least one infected mosquito ( $p=0.289$ ).  
126 Total gametocyte density, quantified in venous blood by quantitative reverse transcriptase  
127 PCR (qRT-PCR) targeting female-specific *Pfs25* mRNA and male-specific *Pfmget* mRNA  
128 (29), was positively associated with the proportion of mosquitoes that became infected  
129 following direct skin feeding (Spearman  $\rho=0.415$ ,  $p=0.0204$ ) or membrane feeding (Spearman  
130  $\rho=0.596$ ,  $p = 0.0004$ ) (Figure 1A). The proportion of infected mosquitoes was higher by direct  
131 skin feeding as compared to membrane feeding assays (odds ratio 2.01; 95% CI 1.21 – 3.33,  $p$   
132 = 0.007), in line with previous studies (9, 10, 30). The median number of oocysts was 4 (IQR  
133 2-7.5; maximum 38) for mosquitoes that became infected after feeding directly on the skin  
134 and 2 (IQR 1-5; maximum 24) for mosquitoes that became infected after feeding on venous  
135 blood through a membrane feeder.

136 To examine whether this higher infectivity in direct skin feeding assays was related to  
137 higher ingested gametocyte densities, or to a higher gametocyte fraction in the blood meal, we  
138 directly quantified gametocytes and asexual parasites in mosquito blood meals. The blood  
139 content of individually fed mosquitoes was released into an RNA preservative 15 minutes  
140 after starting the feeding; RNA was then extracted and quantified from pools of 4 mosquitoes.  
141 We quantified asexual parasites by skeleton-binding protein 1 *sbp1* qRT-PCR (31) and  
142 gametocytes (*Pfs25* and *Pfmget* qRT-PCR) in a median of 3 mosquito pools per participant,  
143 each containing 4 individual mosquitoes, from skin-feeding (range=2-3) and 4 pools per  
144 participant, each containing 4 individual mosquitoes, from membrane feeding (range=2-4).  
145 We observed strong correlations between parasite quantities in pools of mosquitoes that fed

146 on skin or venous blood through artificial membranes for asexual ring-stage parasites  
147 ( $r=0.921$ ,  $p<0.0001$ ), male ( $r=0.790$ ,  $p<0.0001$ ) and female gametocytes ( $r=0.655$ ,  $p=0.0001$ )  
148 (Figure 1B). We also expressed gametocytes as a fraction of the total parasite biomass. This  
149 fraction ranged from very low ( $<1\%$  gametocytes in an individual with 21,086 ring-stage  
150 asexual parasites/ $\mu\text{L}$  and 179 gametocytes/ $\mu\text{L}$ ) to 100% in 3 individuals without asexual  
151 parasites detected by qRT-PCR (Figure 1C). We observed no tendency towards a higher  
152 fraction of gametocytes in skin-fed mosquitoes or capillary blood compared to venous blood  
153 (Figure 1D).

154 In a complementary study, 9 adult gametocyte carriers participated in skin biopsy  
155 sampling. After a screening visit, participants were seen on 2 occasions spaced 4 days apart.  
156 One participant came on day 5 for the return visit instead of day 4; one other participant  
157 withdrew consent prior to the second visit. On each occasion, venous blood, finger prick  
158 blood and 4 small skin biopsy punches were taken from the leg ( $n=2$ ) and arm ( $n=2$ ). Half of  
159 these biopsies were used for RNA extraction; the other half for histological assessments. Male  
160 and female gametocytes and ring-stage asexual parasites were quantified by qRT-PCR to  
161 calculate the gametocyte fraction in finger prick blood (16 observations; 9 donors), venous  
162 blood ( $n=16$ ; 9 donors), as well as skin tissue from the arm ( $n=13$ ; 7 donors) and leg ( $n=12$ ; 8  
163 donors). Gametocytes were detected in all tissue and all blood samples by qRT-PCR; asexual  
164 parasites were detected in 17/25 tissue and in 30/32 blood samples. The gametocyte fraction  
165 was highly variable between donors (and between time-points) whilst estimates from the  
166 different compartments from the same donor and time-point showed strong correlation: the  
167 gametocyte fraction in venous blood was strongly associated with that in finger prick blood  
168 (Spearman  $\rho = 0.947$ ,  $p<0.0001$ ), arm skin tissue (Spearman  $\rho = 0.928$ ,  $p < 0.0001$ ) and leg  
169 skin tissue (Spearman  $\rho = 0.870$ ,  $p=0.0002$ ) (Figure 2A). Parasite density estimates per  
170 microliter of blood or tissue were generally lower in the skin tissue compared to blood



171 samples (Figure 2B) and not significantly different between venous or finger prick blood  
172 ( $p \geq 0.121$ ) or between leg skin tissue or arm skin tissue ( $p \geq 0.116$ ). The same RNA aliquots  
173 were also processed for analysis by Nanostring expression array, a highly sensitive probe-  
174 based expression platform that we have optimized for use in *P. falciparum* (32, 33). Using a  
175 previously defined stage-specific marker set for asexual rings and mature gametocytes (33,  
176 34), there was no evidence for higher gametocyte transcripts in skin samples compared to  
177 blood samples (Figure 2C). The two approaches to quantify gene expression also showed a  
178 strong correlation for *sbp1* and *Pfs25* (Figure 2D).

179 To directly detect gametocytes in subcutaneous tissue, skin biopsy samples that were  
180 stored in formalin were processed for imaging. Given the low densities of gametocytes  
181 predicted based on the qRT-PCR quantification (estimated median of 55.0 gametocytes in arm  
182 tissue samples (IQR 28.2-153.0) and 36.9 gametocytes in leg tissue samples (IQR 11.6-98.3);  
183 we established a protocol to image 10 $\mu$ m sections by confocal microscopy, hence maximizing  
184 the detectability of sparse gametocytes (Figure 3A). Skin sections were initially analysed by  
185 haematoxylin and eosin staining and labelled with the endothelial marker CD31 (Figures 3B)  
186 to confirm integrity of the tissue. Evaluation of gametocyte markers identified Pfs16  
187 antibodies (6, 35) as highly specific and sensitive using the confocal imaging protocol (Figure  
188 3C), while antibodies against Pfs48/45 and Pfs230 were unable to detect gametocytes in  
189 formalin fixed parasites and therefore not evaluated further. Screening of at least 12 sections  
190 per skin snip in arm and leg samples from each participant identified several putative  
191 gametocytes. A Pfs16 positive cell with a characteristic crescent shape, three-dimensional  
192 structure and nuclear stain is shown in close association with a vessel (Figure 3D and  
193 Supplementary movies 1 and 2). Based on these results, with low success gametocyte  
194 detection rates by this highly sensitive fluorescence microscopy protocol, no further  
195 gametocyte carriers were recruited as tissue donors.

196

## 197 **DISCUSSION**

198 Here, we tested a long-standing hypothesis of *P. falciparum* gametocyte sequestration in skin  
199 tissue in two populations of naturally infected individuals in Burkina Faso. By combining  
200 mosquito feeding assays and direct quantification of parasite populations in skin tissue,  
201 mosquito blood meals and blood compartments, we conclude that there is no evidence for  
202 significant skin sequestration of mature gametocytes.

203 Parasite sequestration in skin tissue is an intuitive explanation for how vector-borne  
204 parasites can maximize the likelihood of update by blood-feeding insects. This phenomenon,  
205 well demonstrated for a range of helminths (15-18) and protozoic trypanosomes (14), has  
206 remained speculative for *Plasmodium* parasites (22). Two recent studies in Cameroonian  
207 parasite carriers that used microscopy as diagnostic tool yielded conflicting results: one  
208 observed higher *P. falciparum* parasite prevalence in finger prick capillary blood compared to  
209 venous blood from hospital patients (36), the other found no differences for asexual parasites  
210 or gametocytes in gametocyte carriers (37). The utility of finger prick blood to estimate  
211 parasite biomass in skin tissue is uncertain. Studies published in the 1940s and 50s reported  
212 superiority of skin scarification as compared to finger prick blood samples for parasite  
213 detection (19, 20, 38). In the most extensive of these studies, in 1243 natural infections, 1 cm<sup>2</sup>  
214 skin of the scapular region was very slightly scarified with 4-5 light incisions, expressing a  
215 mixture of dermal fluids and capillary blood, with the first drop appearing richest in parasites  
216 (20). This study demonstrated a 10-20% increase in prevalence of asexual parasites and  
217 gametocytes of *P. vivax*, *P. malariae* and *P. falciparum* but not *P. ovale*. Also parasite  
218 density, expressed as parasites per 15,000 examined white blood cells, appeared increased  
219 (20). In the current study, we therefore not only collected venous blood and finger prick blood  
220 but we also directly quantified parasite stage composition in skin tissue of naturally infected

221 donors and in blood meals of mosquitoes that naturally fed on the skin of the corresponding  
222 donor. We used the absolute quantity of gametocytes and the fraction of the total parasite  
223 biomass that is gametocyte as indicators of sequestration. In skin biopsy samples, we only  
224 sporadically encountered gametocytes by histology. We chose a fluorescence imaging  
225 protocol to image thick sections by confocal microscopy. This method allowed capturing of  
226 entire parasites and three-dimensional reconstruction of parasite and surrounding tissues.  
227 Using Pfs16 labelling we classified gametocytes by crescent shape, three-dimensional  
228 structure (as opposed to non-specific speckles and autofluorescence, which is an inherent  
229 issue of this approach), nuclear stain and presence of a surrounding red blood cell. The  
230 frequency of immunofluorescence-detected gametocytes in our tissue samples was lower than  
231 that by molecular methods in a tissue sample taken during the same visit. The quality of the  
232 skin tissue, tested by analysing the tissue sections by haematoxylin and eosin staining, as well  
233 as by labelling for endothelial cells, clearly indicates they were processed and preserved well.

234 In contrast, molecular detection of gametocytes was successful for all tissue samples  
235 by qRT-PCR and for the majority of samples by Nanostring. Because the volume of blood is  
236 unknown in tissue samples and specifically gametocytes are hypothesized to be enriched in  
237 skin tissue (19, 20, 22), we compared the gametocyte fraction between different blood  
238 compartments and found no evidence for a biased gametocyte fraction. Gametocyte  
239 quantification in mosquito blood meals corroborated this finding and allowed a direct  
240 comparison of parasite densities. Again, we observed no evidence for higher concentrations of  
241 gametocytes in mosquitoes that fed directly on the skin of gametocyte donors compared to  
242 venous blood and observed a very strong association between gametocyte fractions from the  
243 different blood compartments. There must therefore be an alternative explanation for the  
244 higher infection rates that we, in line with other studies (9, 10), observed in direct skin feeding  
245 experiments compared to membrane feeding experiments using venous blood. Gametocyte

246 activation may occur following phlebotomy and may reduce infection rates observed  
247 following membrane feeding. In addition, anticoagulants used in phlebotomy can have a  
248 pronounced effect on mosquito infection rates (39). Although heparin is the preferred  
249 anticoagulant (39), it may still have a disadvantageous impact on sporogonic development. In  
250 malaria-naïve individuals in whom *P. falciparum* gametocytes were induced during controlled  
251 human malaria infection studies, replacement of heparin plasma by serum resulted in  
252 increased mosquito infection rates (10). Since human immune responses are unlikely to be of  
253 relevance in these gametocytaemic volunteers, this observation provides additional indirect  
254 evidence for a transmission modulatory effect of heparin.

255         We conclude that there is no evidence for gametocyte sequestration in skin tissue. Our  
256 findings argue against a long-standing hypothesis that never had a solid evidence base or  
257 proposed mechanism. Since the deformability of erythrocytes infected with mature  
258 gametocytes is similar to that of uninfected erythrocytes (23, 40) and there is no evidence for  
259 antigens on the surface of mature gametocyte-infected erythrocytes (41, 42), it is perhaps  
260 unsurprising that gametocyte concentrations are similar in the different blood compartments.  
261 While direct skin-feeding assays tend to result in higher infectivity compared that observed in  
262 indirect feeding procedures using venous blood, our data demonstrate that any differences  
263 observed are based on technical rather than biological differences in the feeding procedure.  
264 Our findings also indicate that gametocyte levels in venous or finger prick blood can be used  
265 to predict onward transmission potential to mosquitoes. Our findings thus pave the way for  
266 methodologies to quantify the human infectious reservoir based on conventional blood  
267 sampling approaches to support the deployment and monitoring of malaria elimination efforts  
268 for maximum public health impact.

269

## 270 **MATERIALS AND METHODS**

271 **Ethics statement**

272 Ethical approval for the studies was granted by the Ethical Review Committee of the Ministry  
273 of Health of Burkina Faso (Deliberation numbers 2016-03-033 and 2017-02-018) and the  
274 Ethics Committee of the London School of Hygiene and Tropical Medicine (#10489 and  
275 #11962). Individual written informed consent was obtained from each participant prior to  
276 enrolment. Malaria cases were treated according to the National guidelines in Burkina Faso  
277 (43).

278

279 **Study site and population**

280 Study participants were recruited in the village of Balonghin, located in Saponé district, in  
281 Burkina Faso. Malaria transmission is seasonal and intense. The main malaria vectors are  
282 *Anophele gambiae s.s.*, *An. coluzzii*, *An. arabiensis* and *An. funestus*. *P. falciparum* parasite  
283 carriage and gametocyte carriage by molecular methods in the study area are 51-84% and 49-  
284 75%, respectively (44).

285

286 **Study design**

287 **Paired skin feeding and membrane feeding study**

288 This study was conducted in October-December 2017. Individuals from the eligible age range  
289 (15-50 years) in the study area were invited to study information meetings based on a village  
290 census list and, if expressing an interest to participate, invited for screening at Balonghin  
291 health facility. Eligible participants had *P. falciparum* gametocyte densities  $\geq 1$   
292 gametocyte/500 leucocytes by microscopy ( $\geq 16$  gametocytes/ $\mu\text{L}$  when assuming 8000  
293 leucocytes/ $\mu\text{L}$ ). Exclusion criteria were: signs of acute or chronic disease that required  
294 immediate clinical care; haemoglobin concentration  $< 8$  g/dL; current or previous participation  
295 in malaria vaccine trials; recent blood transfusion or administration of blood products; use of

296 antimalarials in the last 2 weeks; co-infection with *P. malariae* or *P. ovale*. Eligible  
297 participants were provided transport to the Centre National de Recherche et de Formation sur  
298 le Paludisme (CNRFP) in Ouagadougou for membrane feeding and skin feeding. Immediately  
299 after venipuncture in lithium heparin and EDTA tubes (BD Vacutainer™), 400-500µL of  
300 heparinized blood in duplicate (for infectivity) and 400-500µL EDTA blood (for gametocyte  
301 quantification in blood meals) was offered to 60 starved 4–5-day-old female *An. coluzzii*  
302 mosquitoes via an artificial membrane attached to a water-jacketed glass feeder maintained at  
303 37°C (28). After exactly 15 minutes of feeding in the dark, fully fed mosquitoes from heparin  
304 blood were transferred to storage cups by aspiration and maintained with glucose solution at  
305 27-29°C for 6-8 days before dissection with 1% mercurochrome staining and examination for  
306 oocysts by two independent microscopists. From mosquitoes that fed on EDTA blood, 16  
307 fully fed mosquitoes were sacrificed after feeding for exactly 15 minutes by sharp needle  
308 puncture of their midguts to release the blood contents into 50µl of RNAprotect cell reagent;  
309 blood meal material was stored for individual mosquitoes at -80°C. Immediately following  
310 membrane feeding, direct skin feeding took place. The participant's calves were exposed to  
311 60 mosquitoes distributed over 2 paper cups that were allowed to feed for exactly 15 minutes.  
312 From this group, 12 fully fed mosquitoes were immediately sacrificed and their midguts  
313 punctured as described above. Remaining mosquitoes were maintained on glucose solution  
314 before dissections for oocyst presence, as above. In addition to the membrane and direct skin  
315 feeding assays, K2EDTA blood was collected by venipuncture (BD Vacutainer™) and finger  
316 prick (BD Microtainer®).

317

### 318 **Skin biopsy study**

319 In the period September 2016-March 2017, adults (aged 18-50 years) were invited for study  
320 participation as described above. Participants were eligible if they had *P. falciparum*

321 gametocyte densities  $\geq 1$  gametocyte/500 leucocytes by microscopy ( $\geq 16$  gametocytes/ $\mu\text{L}$ ).  
322 For skin biopsy sampling, exclusion criteria were signs of acute or chronic disease that  
323 requires immediate clinical care; haemoglobin concentration  $< 11$  g/dL; skin infections or  
324 conditions; history of vasovagal responses to blood sampling or biopsies; allergy to lidocaine/  
325 prilocaine. Eligible individuals were invited to the CNRFP central lab in Ouagadougou on  
326 two occasions, 4 days apart for sample collection. At each occasion, skin biopsy samples  
327 including the dermis and hypodermis were taken from under the arm ( $n=2$ ) and leg ( $n=2$ )  
328 using single use punchers (4mm Biopsy Punch; Miltex Inc. York, US). This procedure was  
329 performed 1 hour after applying local anaesthetic by means of a xylocaine-adrenaline by a  
330 qualified dermatologist. Half of the biopsy samples (one each from arm and leg) were  
331 immediately immersed in 2 mL of 10% formalin and placed at  $4^{\circ}\text{C}$  overnight; following  
332 washing, samples were stored in 2 mL of 70% ethanol and stored at  $4^{\circ}\text{C}$  until further  
333 processing. Other biopsy samples were transferred to 1000  $\mu\text{L}$  RNALater stabilization  
334 reagent (Qiagen), incubated overnight at  $2-8^{\circ}\text{C}$  and then transferred to  $-80^{\circ}\text{C}$ . Finger prick  
335 and venous blood samples were collected in EDTA-coated tubes, as above.

336

### 337 **Molecular analysis**

338 Mosquito homogenates were pooled (4 mosquitoes in a total of  $200\mu\text{L}$  of RNAprotect per  
339 pool) with 4 pools (16 mosquitoes) for membrane feeding experiments and 3 pools per skin  
340 feeding experiment (12 mosquitoes). Mosquitoes where no blood was released into  
341 RNAprotect (upon visual expectation upon thawing) were not used for extraction and, as a  
342 result, fewer pools of mosquitoes were extracted. Nucleic acids from these  $200\mu\text{L}$  mosquito  
343 pools and from  $100\mu\text{L}$  venous and finger prick whole blood samples in RNAprotect Cell  
344 Reagent were isolated using the bead-based MagNAPure LC automatic extractor (Total  
345 Nucleic Acid Isolation Kit—High Performance, Roche Applied Science) and eluted in  $50\mu\text{L}$

346 of water. In these samples, ring-stage asexual parasites, female gametocytes and male  
347 gametocytes were quantified by individual quantitative reverse-transcription PCR (qRT-PCR)  
348 assays targeting *sbp1* (31); *Pfs25* (45) and *PfMGET* (29), respectively. Skin biopsy samples  
349 were immediately stored in RNAlater solution after collection. RNA extraction from skin  
350 tissue was performed using the Qiagen RNeasy Plus Mini kit (Qiagen). First, the tissue  
351 samples were removed from RNAlater solution and then homogenized in RLT lysis buffer  
352 (Qiagen) using Polytron Homogenizer (Kinematica). The homogenized lysate was passed  
353 through genomic DNA eliminator columns (Qiagen) and subsequently applied to RNeasy spin  
354 columns. Following several washes, RNA was eluted in nuclease-free water according to the  
355 manufacturer's instructions.

356 The NanoString nCounter custom code set included differentially expressed genes to  
357 distinguish specific *P. falciparum* parasite stages as defined from our previous study (34). A  
358 total of 456 parasite genes were included in the custom probe set including housekeeping  
359 genes. 161 genes representing asexual circulating stages, 147 genes representing asexual  
360 sequestering stages, 26 genes representing gametocyte rings, 27 immature gametocytes and 29  
361 mature gametocyte genes. The remaining set was not annotated for any of these parasite  
362 stages. For NanoString analysis, 5 µl of purified total RNA was used for initial hybridization  
363 reaction. RNA from each sample was allowed to hybridize with reporter and capture probes at  
364 65°C for 20 hours according to the nCounter gene expression assay protocol (NanoString  
365 Technologies). RNA-probe complexes were immobilized to nCounter cartridge followed by  
366 scanning in the nCounter Digital Analyzer. Data was first normalized by applying background  
367 subtraction and then normalized to expression of housekeeping genes using the R package  
368 "NanoStringNorm". The dataset was then quantile normalized using the R package  
369 "aroma.light" and rank scaled. Mature gametocyte and asexual marker genes, as defined in<sup>33</sup>,  
370 were then averaged per patient, per tissue and per visit.



371

372 **Histological analysis of skin samples**

373 Skin biopsies were processed by passing through an increasing alcohol gradient and xylene  
374 before embedded in paraffin wax. 10µm sections of biopsy samples were cut on a microtome  
375 and placed on adhesion slides (SuperFrost® Plus Gold, VWR). Slides were dried at room  
376 temperature for at least one hour then baked overnight at 42°C. The slides were allowed to  
377 reach room temperature before proceeding with the staining protocol. Slides were incubated at  
378 60°C to melt the wax around the section; sections were cleared with xylene and rehydrated by  
379 passing through a decreasing alcohol gradient (xylene: 5 minutes twice; 100% ethanol: 3  
380 minutes, twice; 90% ethanol: 3 minutes, twice; 70% ethanol: 3 minutes, twice). After  
381 incubation in distilled water for 3 minutes, heat induced antigen retrieval was performed using  
382 citrate buffer pH 6.0 (TCS Biosciences) in a table top autoclave. Slides were immersed in  
383 buffer using a metal rack in an empty tip box (without lid) and autoclave initiated until it  
384 reached 126°C, at which point the autoclave was unplugged and slides allowed to incubate in  
385 the autoclave for a further 10 minutes. Subsequently, the slides were removed and cooled in  
386 their buffer in a running water bath. Once at room temperature, slides were transferred to  
387 distilled water and then TBST (Tris Buffered Saline with 0.05% Tween 20) for 3 minutes  
388 each. Slides were then blocked with goat block containing 2.5% normal goat serum (Vector  
389 Laboratories) complemented with 2.5% normal human serum (ThermoFisher Scientific). All  
390 blocking and staining were performed in a humidified chamber. All staining solutions were  
391 removed by tapping the side of the slide gently on tissue paper. Excess liquid was removed by  
392 wicking away with tissue paper, being careful not to touch the sections. This was done to  
393 maintain intact, well-formed skin sections which are particularly delicate. After 30-60 minutes  
394 blocking at room temperature, the slides were incubated in primary antibodies diluted in goat  
395 block. Sections were stained with 1:20 (1.12µg/ml) mouse anti-CD31 (Cell Marque: clone

396 JC70) at 4°C overnight or 1:1250 (1.04µg/ml) rabbit anti-Pfs16 (6) at room temperature for  
397 one hour. The slides were then washed with TBST for 3 minutes thrice before adding 1:100  
398 goat anti-mouse IgG-AlexaFluor488 (ThermoFisher, A-11029) or 1:250 goat anti-rabbit IgG-  
399 AlexaFluor647 (ThermoFisher, A-21245) secondary antibody diluted in goat block and  
400 incubated at room temperature for 30 minutes. Following secondary antibody staining, the  
401 sections were washed twice with TBST and then once with TBS for 3 minutes each, before  
402 incubation with 2.5nM final concentration of DAPI diluted in TBS for 10 mins at room  
403 temperature. Sections were washed twice more in TBS for 3 minutes, before addition of  
404 TrueView autofluorescence quenching reagent (Vector Laboratories) and incubation for 3  
405 minutes at room temperature. Sections were washed once more in TBS for 5 minutes before  
406 mounting with Vectashield Vibrance mountant (Vector Laboratories). Slides were viewed on  
407 a Nikon A1R inverted confocal microscope with Piezo Z-drive to acquire z-stacks. In addition  
408 to skin biopsies, clots of cultured *P. falciparum* parasites (strains Pf2004, 3D7 and NF54)  
409 were generated to act as positive and negative controls. Asexual and mixed asexual-immature  
410 gametocyte clots and mature gametocyte clots were generated as described previously (6).  
411 Sections of formalin fixed paraffin embedded blocks were used to optimise Pfs16 antibody  
412 and DAPI staining and determine the staining of mature gametocytes. Using these controls  
413 gametocytes in the skin were determined by their circumferential staining with Pfs16 and  
414 obvious outline of a red blood cell. Red blood cells were determined by their bright  
415 autofluorescence under 488nm laser light. Images and movies were generated using Image J  
416 software.

417

#### 418 **Sample size justification**

419 For the paired skin feeding-membrane feeding study, we assumed an average of 15% infected  
420 mosquitoes in patent gametocyte carriers with a standard deviation of 20% and a within

421 subject correlation of the outcome of 0.5 (9, 46, 47). If we then expected two-fold higher  
422 mosquito infection rates in direct skin feeding, 17 paired membrane feeding and skin-feeding  
423 experiments on patent gametocyte carriers would give 80% power to detect this difference at  
424 an alpha of 0.05. Sample size justification for skin-biopsy sampling was based on a paired  
425 comparison of the proportion of the total parasite population that is mature gametocyte. We  
426 expected that 73% of the skin snip biopsy samples had higher gametocyte concentrations,  
427 based on a meta-analysis that demonstrated enhanced infectivity following skin feeding  
428 compared to venous blood membrane-feeding (9). When assuming that 70% of infected adults  
429 have detectable malaria parasites in skin tissue and allow quantification of the proportion of  
430 parasites that is gametocyte, and a lower limit of the 95%-CI >50%, 45 paired skin snip  
431 samples and venous/finger prick blood samples would give 83% power with an alpha of 0.05  
432 to detect a different in parasite stage composition. A go/no-go criterion was defined where an  
433 initial 10 gametocyte carriers were recruited for biopsy samples and additional participants  
434 would only be recruited if gametocytes were detected in  $\geq 50\%$  of all samples.

435

### 436 **Statistical analysis**

437 All statistical analyses were performed in STATA version 15.0 (Statacorp; College Station,  
438 TX, US). The proportion of infectious gametocyte carriers was compared between paired  
439 feeding experiments using McNemar's test; the proportion of infected mosquitoes was  
440 compared between direct skin feeding and membrane feeding using logistic regression  
441 controlling for study participant as a fixed effect. Spearman non-parametric correlation  
442 coefficients were calculated to assess associations between continuous variables; the paired  
443 Wilcoxon rank-sum test was used to compare parasite densities between blood or tissue  
444 samples from the same participants. The gametocyte fraction was calculated as the sum of

445 male and female gametocytes, expressed as a proportion of the total parasite biomass of  
446 asexual ring-stage parasites and gametocytes.

447

#### 448 **Supplemental data**

##### 449 **Supplementary movie 1** (3D movie):

450 3D projection of Z-stack of mature gametocyte in skin snip. This movie shows the 3D  
451 reconstruction of the z-stack (step-size 0.2 micron) to illustrate the localisation of a mature  
452 gametocyte. The gametocyte is stained with Pfs16 (magenta), denoted by DAPI staining  
453 (cyan), and within an RBC (yellow). It is in close proximity to skin vasculature. Movie was  
454 generated using Image J software.

455

##### 456 **Supplementary Movie 2** (Z stack):

457 Z-stack of mature gametocyte in skin snip. Confocal z-stack of mature gametocyte taken  
458 across the whole thickness of the section (step-size 0.2 micron). Gametocyte stained with  
459 Pfs16 (magenta), with DAPI (cyan) nuclear staining. Movie generated using Image J  
460 software.

461

#### 462 **Data availability**

463 Data underlying this manuscript are available through  
464 [https://datadryad.org/stash/share/\\_Di1z3S3jl2ahewKXHAXHfAtI7slSBGNAZmgueslqbl](https://datadryad.org/stash/share/_Di1z3S3jl2ahewKXHAXHfAtI7slSBGNAZmgueslqbl).

465

#### 466 **Funding statement**

467 This work was supported by a fellowship from the European Research Council (ERC-2014-  
468 StG 639776) to T.B and by the Bill and Melinda Gates Foundation (INDIE OPP1173572).

469 T.B is further supported by the Netherlands Organization for Scientific Research through a

470 VIDJ fellowship grant to T.B. (no. 016.158.306). The work was further supported by a grant  
471 from the US National Institutes of Health (NIH; R21AI117304-01A1 to M.M.) and a Royal  
472 Society Wolfson Merit award to M.M. T.B and M.N. This project is also supported through  
473 funding from the Radboud-Glasgow Collaboration fund.

474

#### 475 **Acknowledgements**

476 We would like to thank all study participants from Balonghin, Burkina Faso, for their  
477 participation. We further thank Fiona McMonagle for her guidance and assistance in the  
478 histology work.

479

#### 480 **References**

- 481 1. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, Battle K, Moyes  
482 CL, Henry A, Eckhoff PA, Wenger EA, Briet O, Penny MA, Smith TA, Bennett A,  
483 Yukich J, Eisele TP, Griffin JT, Fergus CA, Lynch M, Lindgren F, Cohen JM, Murray  
484 CLJ, Smith DL, Hay SI, Cibulskis RE, Gething PW. 2015. The effect of malaria  
485 control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 526:207-  
486 211.
- 487 2. Rabinovich RN, Drakeley C, Djimde AA, Hall BF, Hay SI, Hemingway J, Kaslow  
488 DC, Noor A, Okumu F, Steketee R, Tanner M, Wells TNC, Whittaker MA, Winzeler  
489 EA, Wirth DF, Whitfield K, Alonso PL. 2017. malERA: An updated research agenda  
490 for malaria elimination and eradication. *PLoS Med* 14:e1002456.
- 491 3. Reservoir mRCPoCt, Measuring T. 2017. malERA: An updated research agenda for  
492 characterising the reservoir and measuring transmission in malaria elimination and  
493 eradication. *PLoS Med* 14:e1002452.

- 494 4. Sinha A, Hughes KR, Modrzynska KK, Otto TD, Pfander C, Dickens NJ, Religa AA,  
495 Bushell E, Graham AL, Cameron R, Kafsack BFC, Williams AE, Llinas M, Berriman  
496 M, Billker O, Waters AP. 2014. A cascade of DNA-binding proteins for sexual  
497 commitment and development in Plasmodium. *Nature* 507:253-257.
- 498 5. Kafsack BF, Rovira-Graells N, Clark TG, Bancells C, Crowley VM, Campino SG,  
499 Williams AE, Drought LG, Kwiatkowski DP, Baker DA, Cortes A, Llinas M. 2014. A  
500 transcriptional switch underlies commitment to sexual development in malaria  
501 parasites. *Nature* 507:248-52.
- 502 6. Joice R, Nilsson SK, Montgomery J, Dankwa S, Egan E, Morahan B, Seydel KB,  
503 Bertuccini L, Alano P, Williamson KC, Duraisingh MT, Taylor TE, Milner DA, Marti  
504 M. 2014. Plasmodium falciparum transmission stages accumulate in the human bone  
505 marrow. *Sci Transl Med* 6:244re5.
- 506 7. Bradley J, Stone W, Da DF, Morlais I, Dicko A, Cohuet A, Guelbeogo WM, Mahamar  
507 A, Nsango S, Soumare HM, Diawara H, Lanke K, Graumans W, Siebelink-Stoter R,  
508 van de Vegte-Bolmer M, Chen I, Tiono A, Goncalves BP, Gosling R, Sauerwein RW,  
509 Drakeley C, Churcher TS, Bousema T. 2018. Predicting the likelihood and intensity of  
510 mosquito infection from sex specific Plasmodium falciparum gametocyte density.  
511 *Elife* 7.
- 512 8. Lawniczak MK, Eckhoff PA. 2016. A computational lens for sexual-stage  
513 transmission, reproduction, fitness and kinetics in Plasmodium falciparum. *Malar J*  
514 15:487.
- 515 9. Bousema T, Dinglasan RR, Morlais I, Gouagna LC, van Warmerdam T, Awono-  
516 Ambene PH, Bonnet S, Diallo M, Coulibaly M, Tchuinkam T, Mulder B, Targett G,  
517 Drakeley C, Sutherland C, Robert V, Doumbo O, Toure Y, Graves PM, Roeffen W,  
518 Sauerwein R, Birkett A, Locke E, Morin M, Wu Y, Churcher TS. 2012. Mosquito

- 519 feeding assays to determine the infectiousness of naturally infected *Plasmodium*  
520 *falciparum* gametocyte carriers. *PLoS One* 7:e42821.
- 521 10. Collins KA, Wang CY, Adams M, Mitchell H, Rampton M, Elliott S, Reuling IJ,  
522 Bousema T, Sauerwein R, Chalon S, Mohrle JJ, McCarthy JS. 2018. A controlled  
523 human malaria infection model enabling evaluation of transmission-blocking  
524 interventions. *J Clin Invest* 128:1551-1562.
- 525 11. Paul RE, Coulson TN, Raibaud A, Brey PT. 2000. Sex determination in malaria  
526 parasites. *Science* 287:128-31.
- 527 12. Babiker HA, Schneider P, Reece SE. 2008. Gametocytes: insights gained during a  
528 decade of molecular monitoring. *Trends Parasitol* 24:525-30.
- 529 13. Gaillard FO, Boudin C, Chau NP, Robert V, Pichon G. 2003. Togetherness among  
530 *Plasmodium falciparum* gametocytes: interpretation through simulation and  
531 consequences for malaria transmission. *Parasitology* 127:427-35.
- 532 14. Capewell P, Cren-Travaille C, Marchesi F, Johnston P, Clucas C, Benson RA,  
533 Gorman TA, Calvo-Alvarez E, Crouzols A, Jouvion G, Jamonneau V, Weir W,  
534 Stevenson ML, O'Neill K, Cooper A, Swar NK, Bucheton B, Ngoyi DM, Garside P,  
535 Rotureau B, MacLeod A. 2016. The skin is a significant but overlooked anatomical  
536 reservoir for vector-borne African trypanosomes. *Elife* 5.
- 537 15. Courtenay O, Carson C, Calvo-Bado L, Garcez LM, Quinnell RJ. 2014.  
538 Heterogeneities in *Leishmania infantum* infection: using skin parasite burdens to  
539 identify highly infectious dogs. *PLoS Negl Trop Dis* 8:e2583.
- 540 16. Doehl JSP, Bright Z, Dey S, Davies H, Magson J, Brown N, Romano A, Dalton JE,  
541 Pinto AI, Pitchford JW, Kaye PM. 2017. Skin parasite landscape determines host  
542 infectiousness in visceral leishmaniasis. *Nat Commun* 8:57.

- 543 17. Lustigman S, Makepeace BL, Klei TR, Babayan SA, Hotez P, Abraham D, Bottazzi  
544 ME. 2018. *Onchocerca volvulus*: The Road from Basic Biology to a Vaccine. *Trends*  
545 *Parasitol* 34:64-79.
- 546 18. Macfie JWS, Corson JT. 1922. A new species of filarial larva found in the skin of  
547 natives in the Gold Coast. *Ann Trop Med Parasitol* 16:465-471.
- 548 19. Chardome M, Janssen PJ. 1952. Inquiry on malarial incidence by the dermal method  
549 in the region of Lubilash, Belgian Congo. *Ann Soc Belg Med Trop (1920)* 32:209-11.
- 550 20. Van Den Berghe L, Chardome M, Peel E. 1952. Superiorite des preparations de  
551 scarification du derme sur les pre´parations de sang peripherique pour le diagnostic de  
552 malaria. *An Inst Med Trop* 9:553-62.
- 553 21. Nacher M. 2004. Does the shape of *Plasmodium falciparum* gametocytes have a  
554 function? *Med Hypotheses* 62:618-9.
- 555 22. Nixon CP. 2016. *Plasmodium falciparum* gametocyte transit through the cutaneous  
556 microvasculature: A new target for malaria transmission blocking vaccines? *Hum*  
557 *Vaccin Immunother* 12:3189-3195.
- 558 23. Tiburcio M, Niang M, Deplaine G, Perrot S, Bischoff E, Ndour PA, Silvestrini F,  
559 Khattab A, Milon G, David PH, Hardeman M, Vernick KD, Sauerwein RW, Preiser  
560 PR, Mercereau-Puijalon O, Buffet P, Alano P, Lavazec C. 2012. A switch in infected  
561 erythrocyte deformability at the maturation and blood circulation of *Plasmodium*  
562 *falciparum* transmission stages. *Blood* 119:e172-80.
- 563 24. Naissant B, Dupuy F, Duffier Y, Lorthiois A, Duez J, Scholz J, Buffet P, Merckx A,  
564 Bachmann A, Lavazec C. 2016. *Plasmodium falciparum* STEVOR phosphorylation  
565 regulates host erythrocyte deformability enabling malaria parasite transmission. *Blood*  
566 127:e42-53.



- 567 25. Sutherland CJ. 2009. Surface antigens of Plasmodium falciparum gametocytes--a new  
568 class of transmission-blocking vaccine targets? Mol Biochem Parasitol 166:93-8.
- 569 26. Baruch DI, Pasloske BL, Singh HB, Bi X, Ma XC, Feldman M, Taraschi TF, Howard  
570 RJ. 1995. Cloning the P. falciparum gene encoding PfEMP1, a malarial variant  
571 antigen and adherence receptor on the surface of parasitized human erythrocytes. Cell  
572 82:77-87.
- 573 27. Brickley EB, Coulibaly M, Gabriel EE, Healy SA, Hume JCC, Sagara I, Traore SF,  
574 Doumbo O, Duffy PE. 2016. Utilizing direct skin feeding assays for development of  
575 vaccines that interrupt malaria transmission: A systematic review of methods and case  
576 study. Vaccine 34:5863-5870.
- 577 28. Ouédraogo AL, Guelbéogo WM, Cohuet A, Morlais I, King JG, Gonçalves BP,  
578 Bastiaens G, Vaanhold M, Sattabongkot J, Wu Y. 2013. A protocol for membrane  
579 feeding assays to determine the infectiousness of P. falciparum naturally infected  
580 individuals to Anopheles gambiae. MWJ 4:1-4.
- 581 29. Stone W, Sawa P, Lanke K, Rijpma SR, Oriango R, Nyaurah M, Osodo P, Osofi V,  
582 Mahamar A, Diawara H, Woestenenk R, Graumans W, Bradley J, Chen I, Brown JM,  
583 Gosling R, Dicko A, Drakeley C, Bousema T. 2017. A molecular assay to quantify  
584 male and female *P. falciparum* gametocytes: results from two randomised controlled  
585 trials using primaquine for gametocyte clearance. J Infect Dis in press.
- 586 30. Diallo M, Toure AM, Traore SF, Niare O, Kassambara L, Konare A, Coulibaly M,  
587 Bagayogo M, Beier JC, Sakai RK, Toure YT, Doumbo OK. 2008. Evaluation and  
588 optimization of membrane feeding compared to direct feeding as an assay for  
589 infectivity. Malar J 7:248.
- 590 31. Tadesse FG, Lanke K, Nebie I, Schildkraut JA, Gonçalves BP, Tiono AB, Sauerwein  
591 R, Drakeley C, Bousema T, Rijpma SR. 2017. Molecular markers for sensitive

- 592 detection of Plasmodium falciparum asexual stage parasites and their application in a  
593 malaria clinical trial. *The American journal of tropical medicine and hygiene* 97:188-  
594 198.
- 595 32. Van Tyne D, Tan Y, Daily JP, Kamiza S, Seydel K, Taylor T, Mesirov JP, Wirth DF,  
596 Milner DA, Jr. 2014. Plasmodium falciparum gene expression measured directly from  
597 tissue during human infection. *Genome Med* 6:110.
- 598 33. De Niz M, Meibalan E, Mejia P, Ma S, Brancucci NMB, Agop-Nersesian C, Mandt R,  
599 Ngotho P, Hughes KR, Waters AP, Huttenhower C, Mitchell JR, Martinelli R,  
600 Frischknecht F, Seydel KB, Taylor T, Milner D, Heussler VT, Marti M. 2018.  
601 Plasmodium gametocytes display homing and vascular transmigration in the host bone  
602 marrow. *Sci Adv* 4:eaat3775.
- 603 34. Pelle KG, Oh K, Buchholz K, Narasimhan V, Joice R, Milner DA, Brancucci NM, Ma  
604 S, Voss TS, Ketman K, Seydel KB, Taylor TE, Barteneva NS, Huttenhower C, Marti  
605 M. 2015. Transcriptional profiling defines dynamics of parasite tissue sequestration  
606 during malaria infection. *Genome Med* 7:19.
- 607 35. Eksi S, Williamson KC. 2011. Protein targeting to the parasitophorous vacuole  
608 membrane of Plasmodium falciparum. *Eukaryot Cell* 10:744-52.
- 609 36. Njunda AL, Assob NJC, Nsagha SD, Kamga FHL, Mokenyu MD, Kwenti TE. 2013.  
610 Comparison of capillary and venous blood using blood film microscopy in the  
611 detection of malaria parasites: A hospital based study. *Sci J Microbiol* 2:89-94.
- 612 37. Sandeu MM, Bayibeki AN, Tchioffo MT, Abate L, Gimonneau G, Awono-Ambene  
613 PH, Nsango SE, Diallo D, Berry A, Texier G, Morlais I. 2017. Do the venous blood  
614 samples replicate malaria parasite densities found in capillary blood? A field study  
615 performed in naturally-infected asymptomatic children in Cameroon. *Malar J* 16:345.

- 616 38. Peel E, Van Hoof L. 1948. Comportement de Plasmodium falciparum dans le derme  
617 chez l'enfant indigène. Ann Soc Belg Med Trop (1920) 28:273-7.
- 618 39. Solarte Y, Manzano Mdel R, Rocha L, Castillo Z, James MA, Herrera S, Arevalo-  
619 Herrera M. 2007. Effects of anticoagulants on Plasmodium vivax oocyst development  
620 in Anopheles albimanus mosquitoes. Am J Trop Med Hyg 77:242-5.
- 621 40. Aingaran M, Zhang R, Law SK, Peng Z, Undisz A, Meyer E, Diez-Silva M, Burke  
622 TA, Spielmann T, Lim CT, Suresh S, Dao M, Marti M. 2012. Host cell deformability  
623 is linked to transmission in the human malaria parasite Plasmodium falciparum. Cell  
624 Microbiol 14:983-93.
- 625 41. Chan JA, Drew DR, Reiling L, Lisboa-Pinto A, Dinko B, Sutherland CJ, Dent AE,  
626 Chelimo K, Kazura JW, Boyle MJ, Beeson JG. 2018. Low Levels of Human  
627 Antibodies to Gametocyte-Infected Erythrocytes Contrasts the PfEMP1-Dominant  
628 Response to Asexual Stages in P. falciparum Malaria. Front Immunol 9:3126.
- 629 42. Dantzer KW, Ma S, Ngotho P, Stone WJR, Tao D, Rijpma S, De Niz M, Nilsson  
630 Bark SK, Jore MM, Raaijmakers TK, Early AM, Ubaida-Mohien C, Lemgruber L,  
631 Campo JJ, Teng AA, Le TQ, Walker CL, Hermand P, Deterre P, Davies DH, Felgner  
632 P, Morlais I, Wirth DF, Neafsey DE, Dinglasan RR, Laufer M, Huttenhower C, Seydel  
633 K, Taylor T, Bousema T, Marti M. 2019. Naturally acquired immunity against  
634 immature Plasmodium falciparum gametocytes. Sci Transl Med 11.
- 635 43. Tiono AB, Ouédraogo A, Ogutu B, Diarra A, Coulibaly S, Gansané A, Sirima SB,  
636 O'Neil G, Mukhopadhyay A, Hamed K. 2013. A controlled, parallel, cluster-  
637 randomized trial of community-wide screening and treatment of asymptomatic carriers  
638 of Plasmodium falciparum in Burkina Faso. Malaria journal 12:79.
- 639 44. Gonçalves BP, Kapulu MC, Sawa P, Guelbéogo WM, Tiono AB, Grignard L, Stone  
640 W, Hellewell J, Lanke K, Bastiaens GJ. 2017. Examining the human infectious

- 641 reservoir for *Plasmodium falciparum* malaria in areas of differing transmission  
642 intensity. *Nature Communications* 8:1133.
- 643 45. Wampfler R, Mwingira F, Javati S, Robinson L, Betuela I, Siba P, Beck HP, Mueller  
644 I, Felger I. 2013. Strategies for detection of *Plasmodium* species gametocytes. *PLoS*  
645 *One* 8:e76316.
- 646 46. Dicko A, Brown JM, Diawara H, Baber I, Mahamar A, Soumare HM, Sanogo K,  
647 Koita F, Keita S, Traore SF, Chen I, Poirot E, Hwang J, McCulloch C, Lanke K, Pett  
648 H, Niemi M, Nosten F, Bousema T, Gosling R. 2016. Primaquine to reduce  
649 transmission of *Plasmodium falciparum* malaria in Mali: a single-blind, dose-ranging,  
650 adaptive randomised phase 2 trial. *Lancet Infect Dis* 16:674-684.
- 651 47. Ouedraogo AL, Goncalves BP, Gneme A, Wenger EA, Guelbeogo MW, Ouedraogo  
652 A, Gerardin J, Bever CA, Lyons H, Pitroipa X, Verhave JP, Eckhoff PA, Drakeley C,  
653 Sauerwein R, Luty AJ, Kouyate B, Bousema T. 2016. Dynamics of the Human  
654 Infectious Reservoir for Malaria Determined by Mosquito Feeding Assays and  
655 Ultrasensitive Malaria Diagnosis in Burkina Faso. *J Infect Dis* 213:90-9.

656

## 657 **FIGURE LEGENDS**

### 658 **Figure 1. The density and infectivity of gametocytes in different blood compartments. A.**

659 Gametocyte density in venous blood in association with the proportion of mosquitoes that  
660 become infected when feeding directly on the skin of the blood donor (blue) or on venous  
661 blood offered through an artificial membrane feeder (red). Size indicates the number of  
662 examined mosquitoes; error bars indicate the 95% confidence interval around the proportion  
663 of infected mosquitoes. **B.** The density of ring stage asexual parasites (green), male  
664 gametocytes (blue) and female gametocytes (red) in mosquito blood meals when feeding  
665 directly on the skin (X-axis) versus venous blood offered through an artificial membrane

666 feeder (Y-axis). Error bars indicate the standard error of density estimates in pools of  
667 mosquitoes fed directly on the skin (median 3 pools) or venous blood (median 4 pools). **C.**  
668 The fraction of the total parasite biomass that is gametocyte in finger prick capillary blood  
669 (red), mosquitoes that fed directly on the skin (green), mosquitoes that fed on venous blood  
670 (blue) or venous blood (purple). The box plot indicates median, interquartile range and range;  
671 dots indicate individual samples. **D.** The gametocyte fraction in venous blood (X-axis)  
672 versus on the Y-axis finger prick capillary blood (red; Spearman  $\rho=0.970$ ;  $p<0.0001$ ),  
673 mosquitoes that fed directly on the skin (green; Spearman  $\rho=0.916$ ;  $p<0.0001$ ), mosquitoes  
674 that fed on venous blood (green; Spearman  $\rho=0.912$ ;  $p<0.0001$ ).

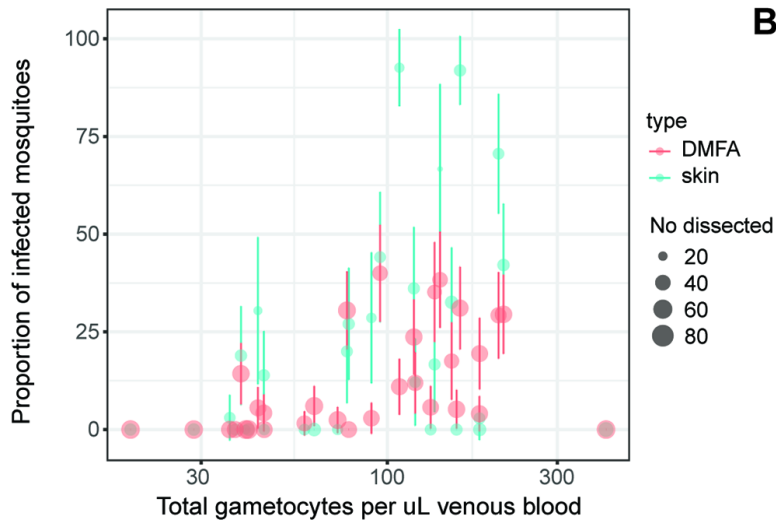
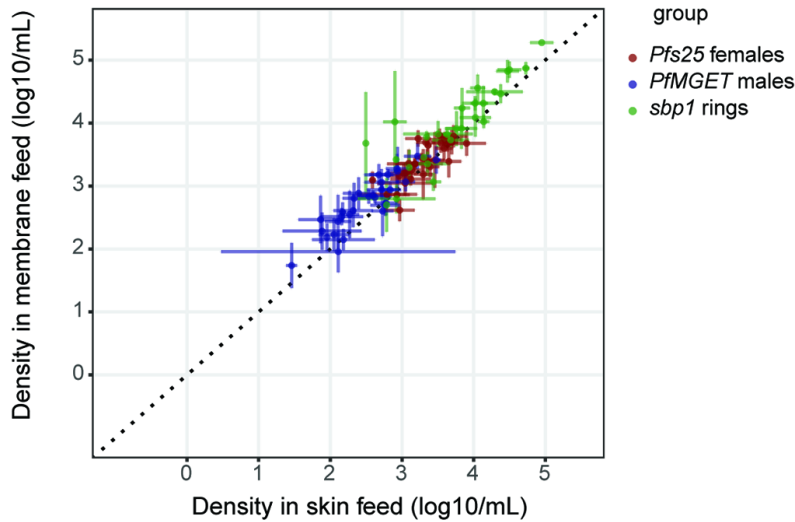
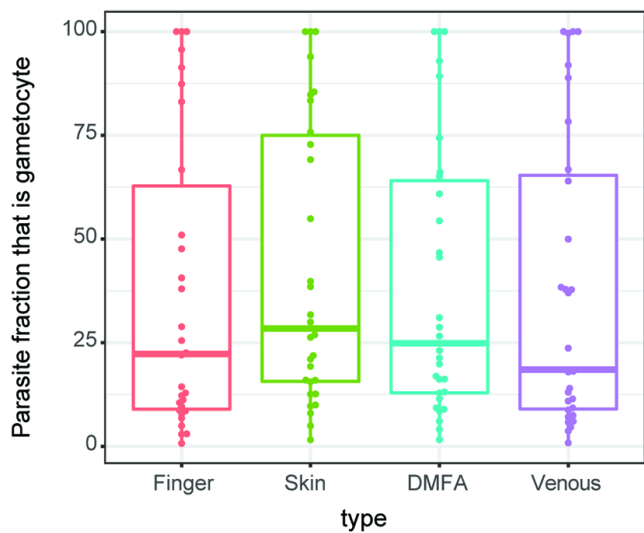
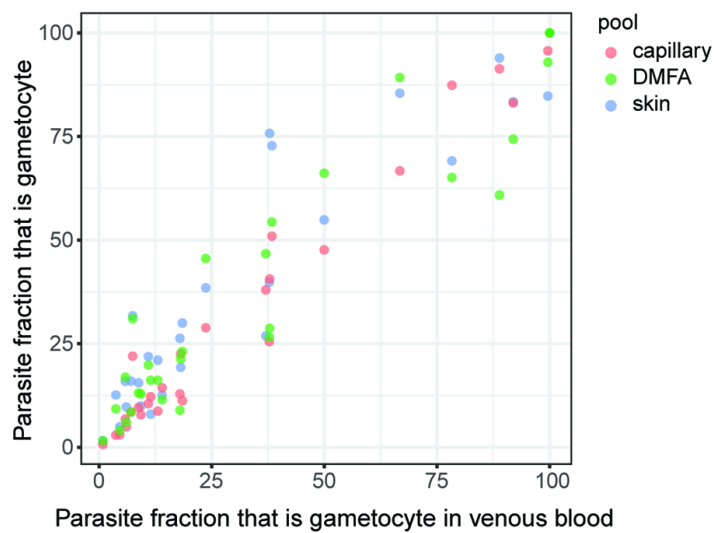
675

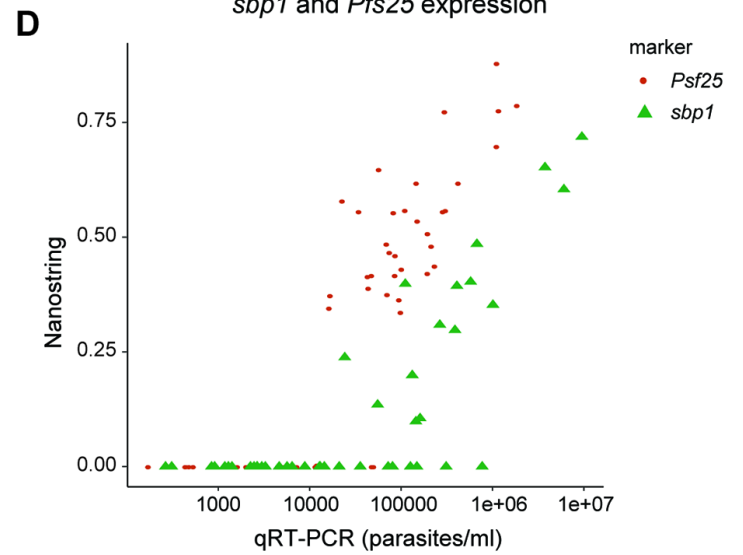
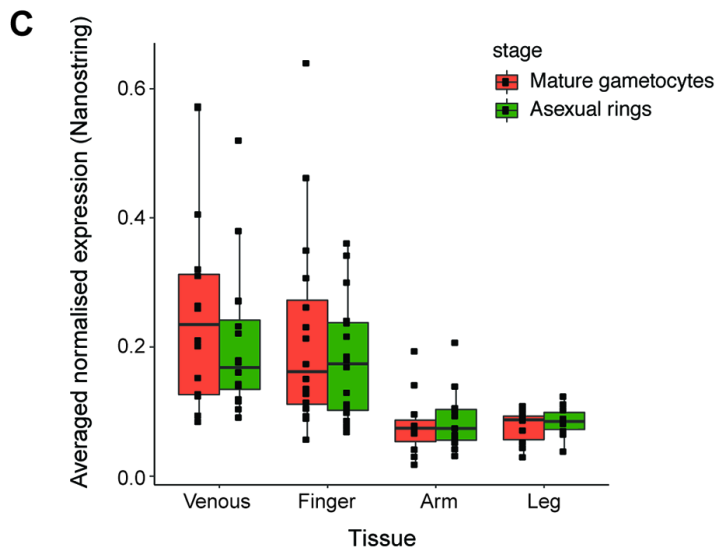
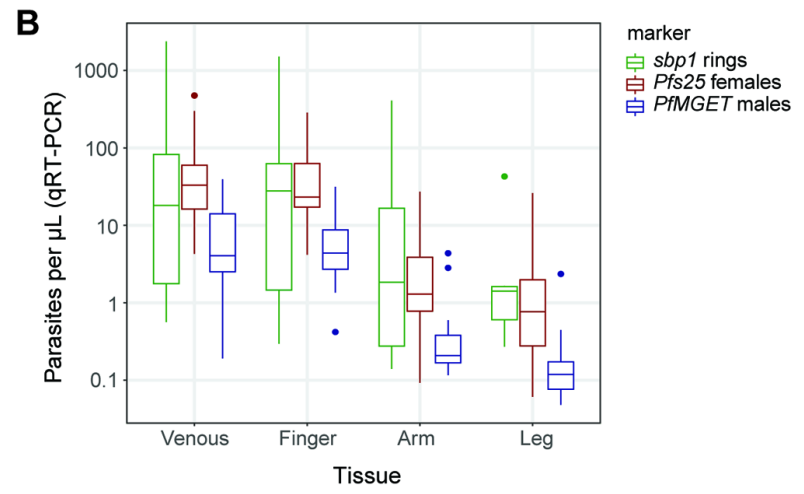
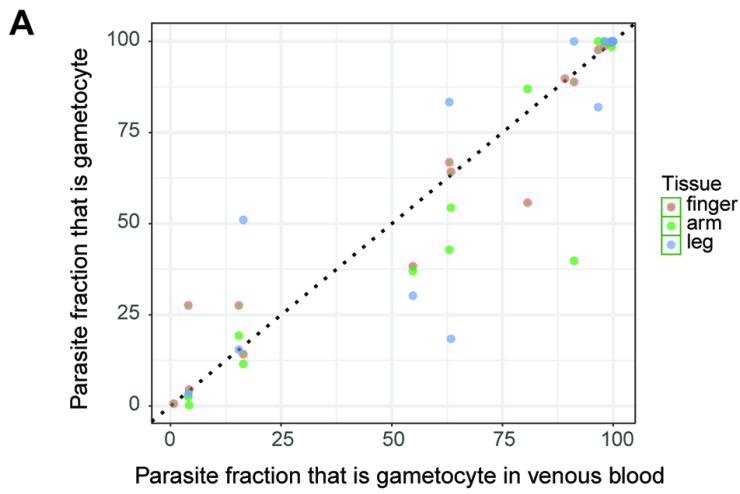
676 **Figure 2. qRT-PCR and Nanostring comparison of parasite densities in skin biopsy**  
677 **samples and blood samples. A.** Gametocyte fractions (the proportion of gametocytes in the  
678 total parasite biomass assessed by *sbp1*, *Pfs25* and *PfMGET* qRT-PCR) across compartments.  
679 **B-D.** Relative numbers of asexual parasites and gametocytes in skin tissue from the arm, skin  
680 tissue from the leg, finger prick and venous blood based on qRT-PCR (**B**) and Nanostring (**C**).  
681 Nanostring data were normalized on the basis of background subtraction and expression of  
682 housekeeping genes. **D.** Correlation between estimates of ring-stage asexual parasites by *sbp1*  
683 and female gametocytes by *Pfs25* for qRT-PCR (X-axis) and Nanostring (Y-axis) showing  
684 good agreement but higher sensitivity of qRT-PCR.

685

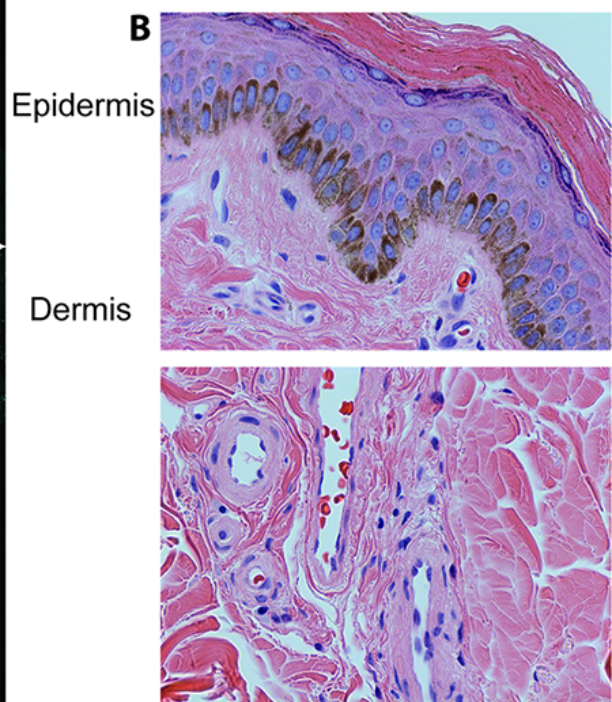
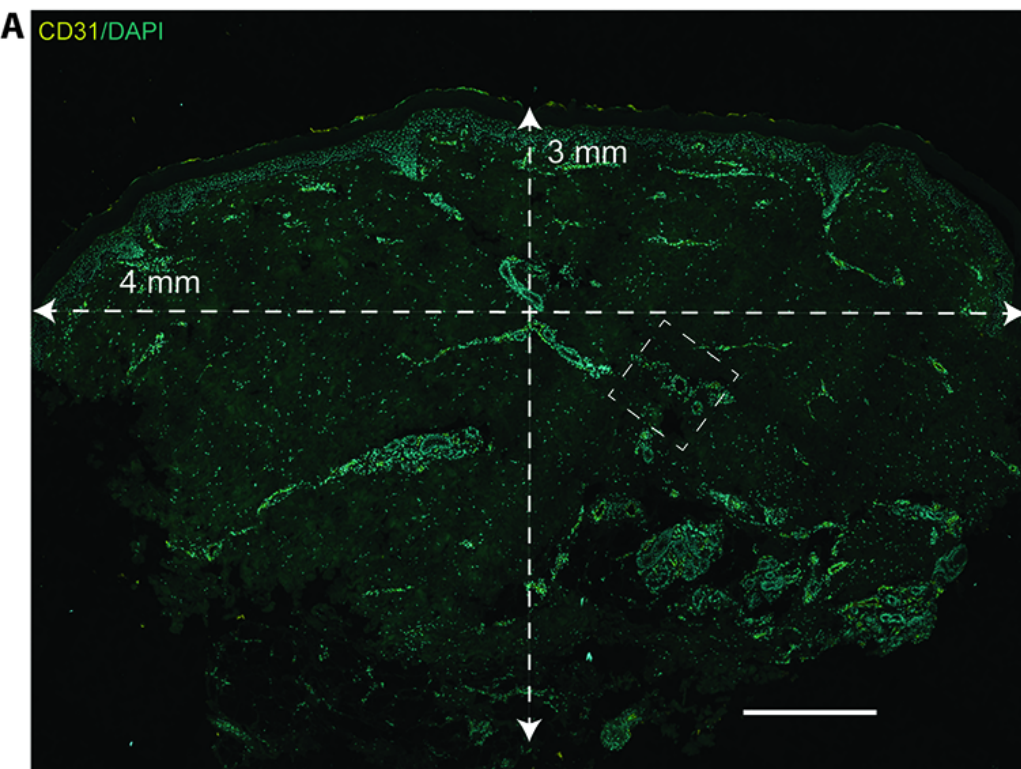
686 **Figure 3. Histological analysis of skin samples. A.** 10 $\mu$ m cross section of a skin snip from  
687 leg with dimensions indicated. Sample was stained with CD31 and DAPI and a maximum  
688 projection across the depth of the section is shown. The insert represents a small section  
689 including several vessels stained with CD31. Scale bar = 500 $\mu$ m, insert = 10 $\mu$ m. **B.** 3 $\mu$ m  
690 section of a skin snip from arm stained with haematoxylin and eosin. Sections in A and B

691 show the different layers of the epidermis on top, followed by the dermis with multiple  
692 vessels. **C.** Samples were stained with DAPI (cyan) and Pfs16 (magenta) for gametocytes.  
693 Representative images of asexual parasite (left), an immature (middle) and mature (right)  
694 gametocyte images from control blood clots. Scale bar = 10 $\mu$ m. **D.** Representative image of a  
695 gametocyte in skin samples from arm. DAPI staining indicates several vessels in the vicinity  
696 of a gametocyte stained with Pfs16. XZ and YZ orientations are included to demonstrate the  
697 three-dimensional nature of the tissue section and the gametocyte. Scale bar = 10 $\mu$ m.  
698

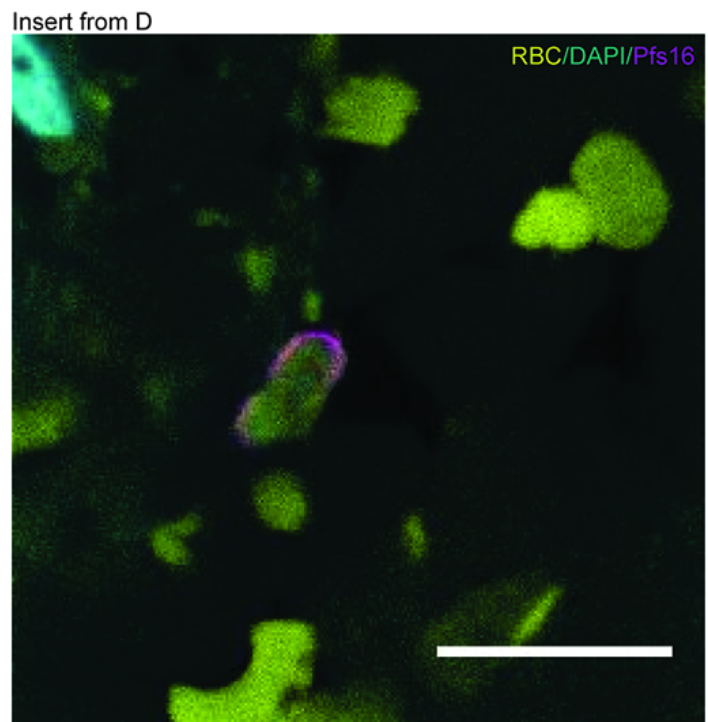
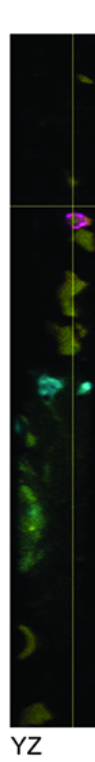
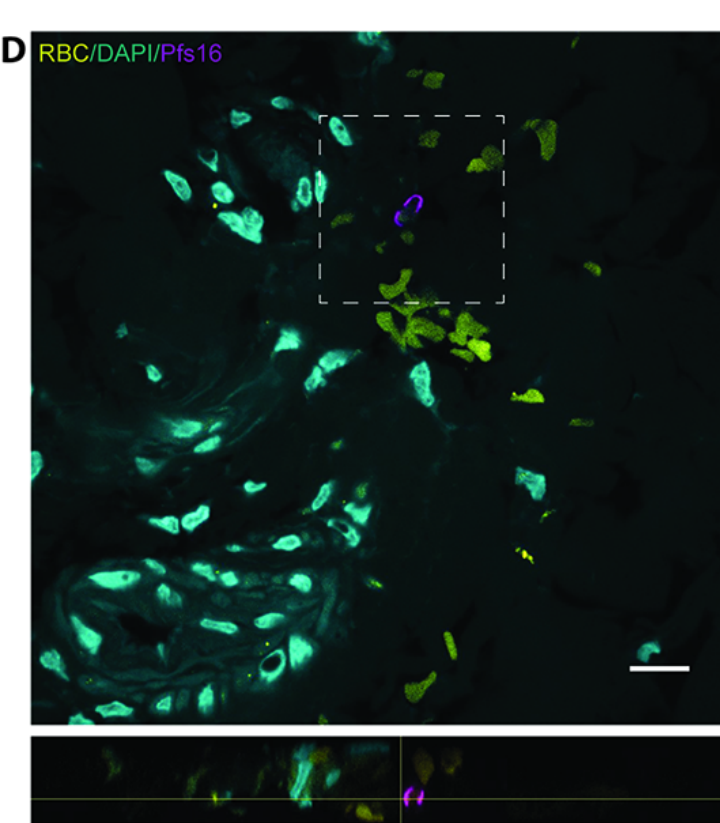
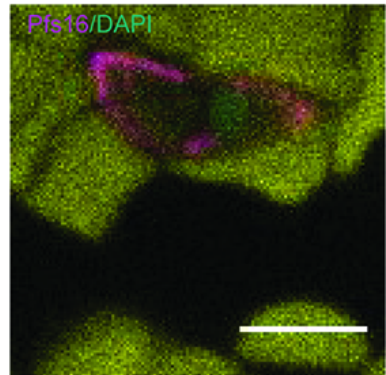
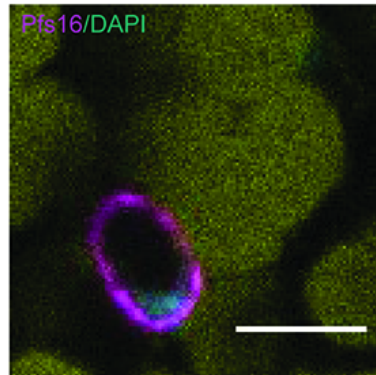
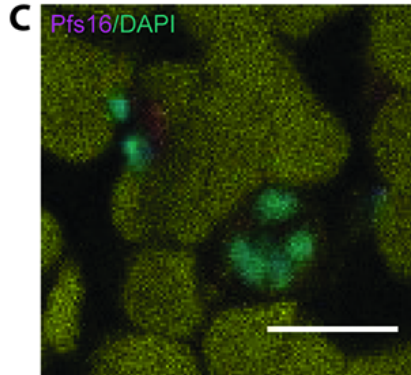
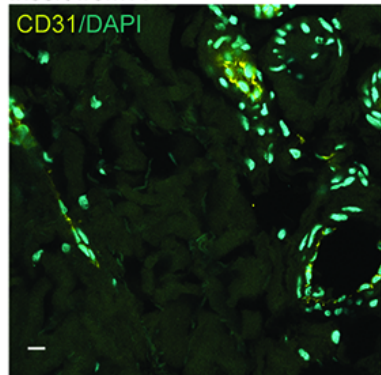
**A****B****C****D**







Insert from A



XZ