Supplementary Information

Host macrophages/monocytes promote malaria transmission by modulating mosquito microbiota via SR-A-mediated phagocytosis

Biao He[1#], Meilin Li[1#], Shuai Guo[1#], Jiaqin Fang[1], Yongling Fan[1], Nie Tan[1], Taiping Liu[1], Jianyong Li[1], Feng Zhu[1], Jian Zhang[1], Wenyue Xu[1,2*]

[1]Department of Pathogenic Biology, Army Medical University, Chongqing 400038, China.

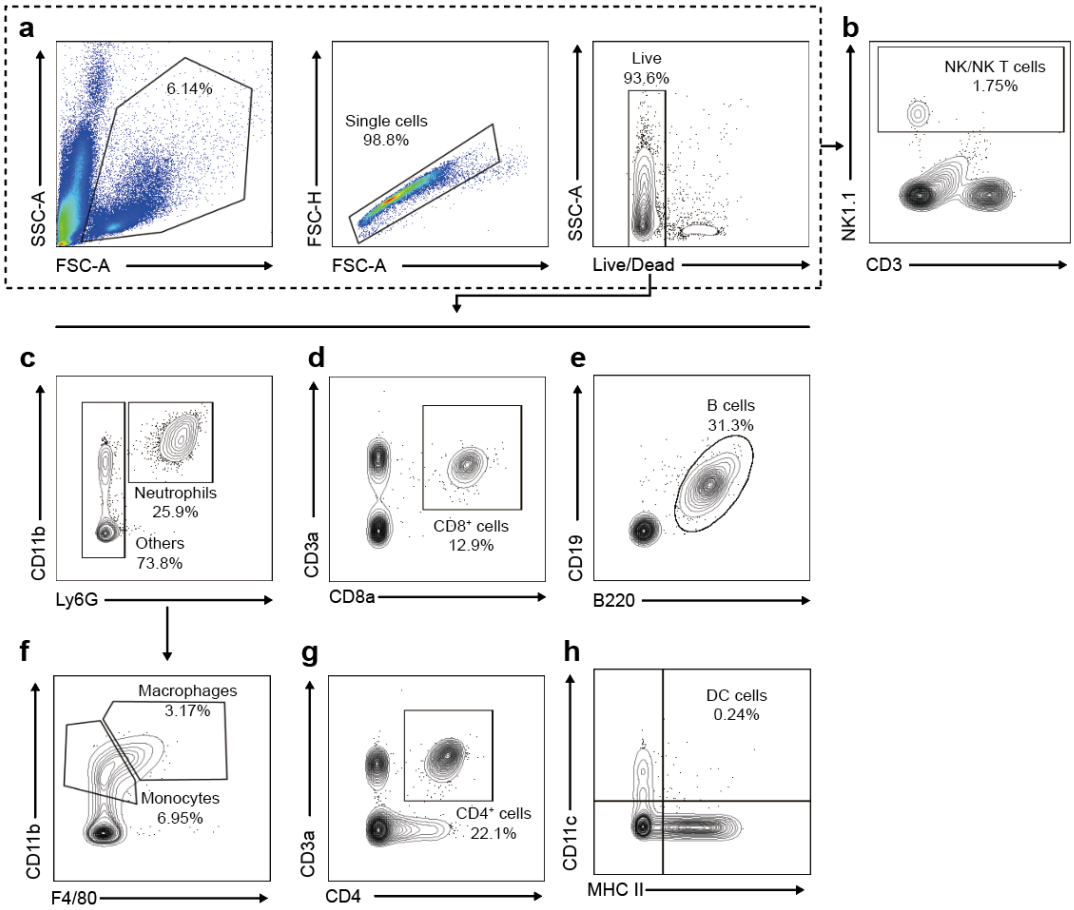
[2]Key Laboratory of Extreme Environmental Medicine, Ministry of Education of China, Chongqing, 400038, China.

*Corresponding author. E-mail: xuwenyue@tmmu.edu.cn

List of Supplementary Information

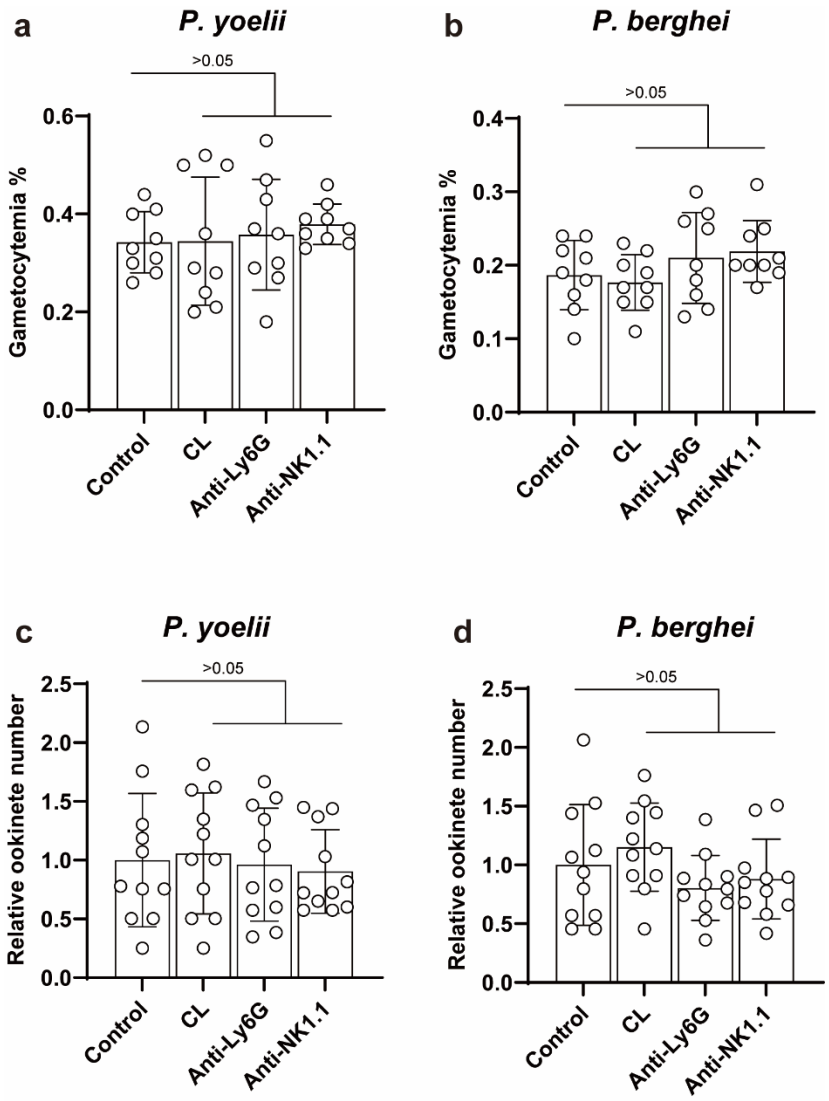
Supplementary Figs. 1-4

Supplementary Table. 1



Supplementary Fig. 1. Gating strategy

a-h Gating strategy; gating strategy **a-h** was used in main figure 1 **a-i**; gating strategy **a-c**, **f**, and **h** was used in main figure 2 **a**; gating strategy **a, c** and **f** was used in main figure 5 **a** and **c**; gating strategy **a-h** was used in Supplementary Fig 3 **a-h**.

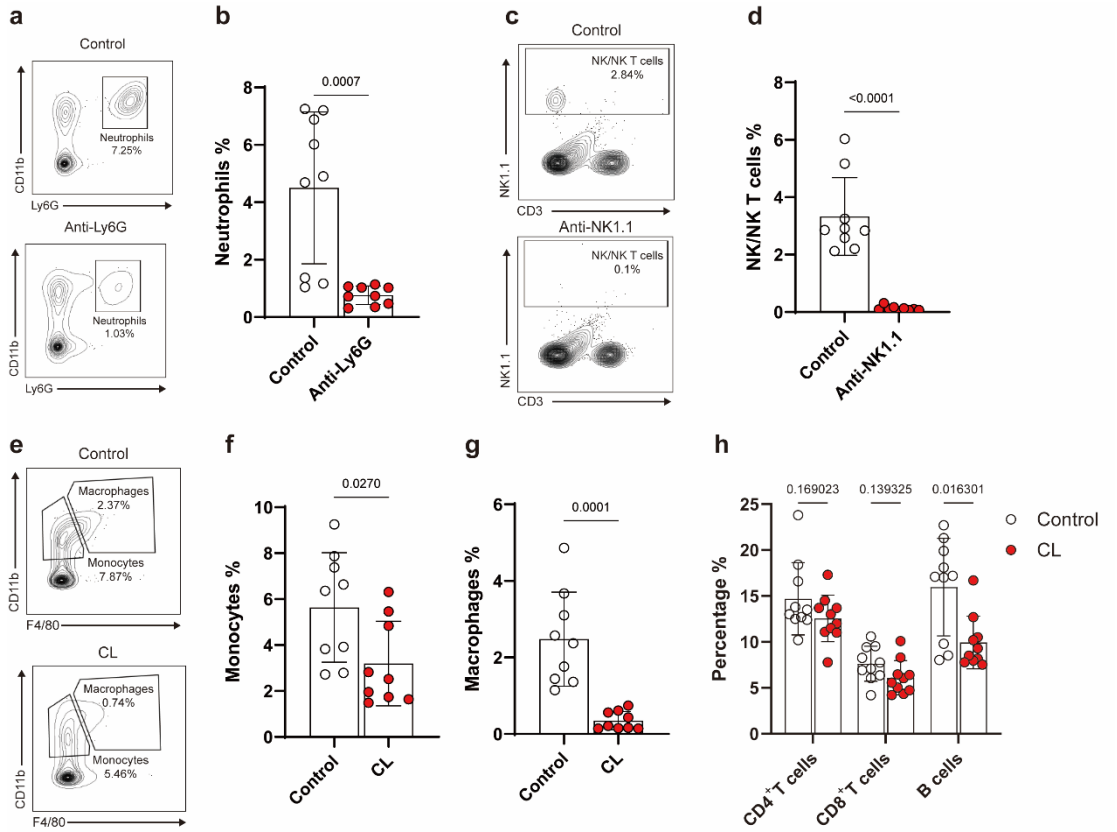


Supplementary Fig. 2. CL, anti-Ly6G, and Anti-NK1.1 treatment did not change gametocytemia and its conversion rate from gametocytes to ookinetes

a-b The gametocytemia of mice (n=9) after treated with CL, or anti-Ly6G, or anti-NK1.1 or without at 3 days post *P. yoelii* (**a**) or *P. berghei* (**b**) infection.

c-d The relative ookinetes number generated from gametocytes in *P. yoelii* (**c**) or *P. berghei* (**d**) infected mice (n=11) treated with CL, or anti-Ly6G, or anti-NK1.1 or without were determined.

Data were presented as mean ± SD. Three independent experiments were performed for each experiment and the data were pooled. Source data are provided as a Source Data file. Significance was determined by unpaired *t*-test (two-tailed). Number between each group represent the *P* value, $P<0.05$ was consider as statistically significant.



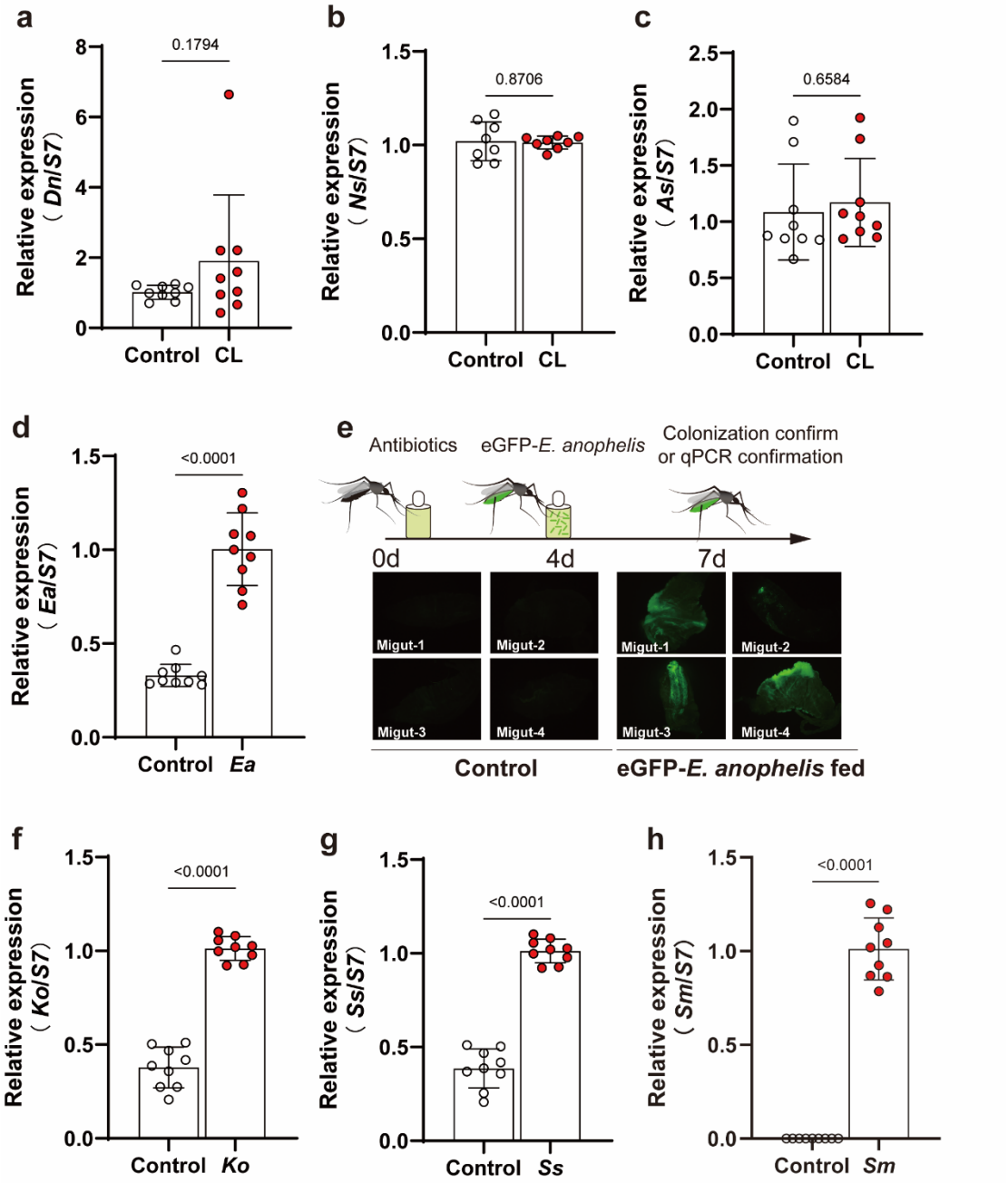
Supplementary Fig. 3. FACS analysis of the effects of the treatment of anti-ly6G, anti-NK1.1 or CL on immune cells in peripheral blood of the infected-mice

a-b Mice (n=9) were infected with *P. yoelii*, and 3 days later mice were i.v injected with anti-Ly6G or isotype control IgG. Twenty-four hours later, neutrophils in peripheral blood were analyzed using FACS (**a**), and the percentage of neutrophils in peripheral blood of the parasite-infected mice treated with or without anti-ly6G was statistically analyzed (**b**).

c-d Mice (n=9) were infected with *P. yoelii*, and 3 days later mice were i.v injected with anti-NK1.1 or isotype control IgG. Twenty-four hours later, neutrophils in peripheral blood were analyzed using FACS (**c**), and the percentage of NK/NK T cells in peripheral blood of the parasite-infected mice treated with or without anti-NK1.1 was statistically analyzed (**d**).

e-h Mice (n=9) were infected with *P. yoelii*, and 3 days later mice were i.v injected with CL or control liposomes. Twenty-four hours later, monocytes, macrophages, $CD4^+$ T cells, $CD8^+$ T cells and B cells in peripheral blood were analyzed using FACS (**e**), and the percentage of monocytes (**f**), macrophages (**g**) and other immune cells ($CD4^+$ T,

54 $CD8^+$ T and B cells) (**h**) in peripheral blood of the parasite-infected mice treated with
55 or without CL was statistically analyzed.
56 Data were presented as mean ± SD. Three independent experiments were performed for
57 each experiment and the data were pooled. Source data are provided as a Source Data
58 file. Significance was determined by unpaired *t*-test (two-tailed). Number between each
59 group represent the *P* value, $P<0.05$ was consider as statistically significant.
60



Supplementary Fig. 4. Verification of the abundance change of four bacteria by qPCR and the colonization of *E. anophelis*, *K. oxytoca*, *S. sonnei*, and *S. marcescens* in mosquito midgut

a-c Mosquito fed on CL or control liposomes treated mice infected with *P. yoelii*, 12 hours post blood meal, the abundance of *D. natronolimnaea* (**a**), *N. salmonea* (**b**), or *A. spathodeae* (**c**) in mosquito midgut (n=9) was detected by qPCR.

d-e Following four consecutive days of antibiotic treatment to clear the midgut microbiota, mosquitoes were provided with 10% sugar solution containing 1 × 10^6

CFUs/ml *E. anophelis*. The successful colonization was confirmed by qPCR (n=9) (**d**) and fluorescence microscope (**e**).

f-h Four days post fed on antibiotics sugar, mosquito was fed with 10 % sugar supplement with 1×10^6/ml *K. oxytoca* (**f**), *S. sonnei* (**g**), or *S. marcescens* (**h**), three days later, the successful colonization was confirmed by qPCR (n=9).

Twenty mosquito midguts were pooled as one biological replicate, and three biological replicates were performed. Data were presented as mean ± SD. Three independent experiments were performed for each experiment and the data were pooled. Source data are provided as a Source Data file. Significance was determined by unpaired *t*-test (two-tailed) for (**a-d**) and (**f-h**). *Dn*: *D. natronolimnaea*; *Ns*: *N. salmonea*; *As*: *A. spathodeae; Ea*: *E. anophelis*; *Ss*: *S. sonnei*; *Ko*: *K. oxytoc*a; *Sm*: *S. marcescens;* Number between each group represent the *P* value, $P<0.05$ was consider as statistically significant.

Supplementary Table 1. Primer used in this study.

Primer name	Forward primer	Reverse primer
Total bacteria	TGCGGGACTTAACCCAACA	TGCGGGACTTAACCCAACA
D. natronolimnaea	AGCGGCGGAGCATGTGGATTA	CACGACACGAGCTGACGACAAC
N. salmonea	GGTTTGCCACGTCTGCTGTGAA	TTCGCCATCGGTGTTCCTCCT
A. spathodeae	TCGTCAGCTCGTGTCGTGAGAT	CCACTGTCACCGCCATTGTAGC
D. tsuruhatensis	GAACACCGATGGCGAAGGCAAT	TAGGGCGTGGACTACCAGGGTA
E. anophelis	CAAGCGGTGGAGCATGTGGTT	ACGGCACGAGCTGACGACAA
S. marcescens	GCCACACTGGAACTGAGACACG	CACCACCTTCCTCCTCGCTGAA
S. sonnei	GCCACACTGGAACTGAGACACG	GGTGCTTCTTCTGCGGGTAACG
K. oxytoca	GCGGTCTGTCAAGTCGGATGTG	ACAAGACTCCAGCCTGCCAGTT
SOAP	TGGATGGCGATGGCAGTAATCC	GCGGCGGCATTAAGACTGTGT
WARP	AAAGCGACGGTGATTGTCCA	ACCTGTTCTGCTGCACGTAT