Materials and Methods

Experimental Animals. *Plasmodium yoelii* 17XNL-EGFP was provided by Dr. Ana Rodriguez (New York University) and Dr. Wenyue Xu (Department of Pathogenic Biology, Army Medical University). The parasite strains were cultured in BalB/c or ICR mice. *P. falciparum* parasites (3D7 strain) was provided from national institute of parasitic diseases of Chinese center for disease control and prevention. All mice were purchased from the Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China) and stored in the Laboratory Animal Center of Tongji University. When the infection rate of *P. yoelii 17XNL* reached 25% - 35 %, artemether (100 mg/kg) was fed to the mice. After different time points of treatment, blood samples were collected, respectively.

The *in vitro* culture of *P. falciparum*. *P. falciparum* parasites (3D7 strain) were cultured in human O+ erythrocytes at 2% hematocrit in RPMI 1640 medium, supplemented with 0.5% (w/v) albumax I (Gibco), 0.2 mmol/L hypoxanthine (Sigma), 25 mmol/L HEPES (Gibco), 0.2% NaHCO3, and 20 μg/mL gentamicin, and were incubated in 5% CO2 and 5% O2 at 37°C, as described previously[1]. The parasites were synchronized through sterile 5% (m/v) D-sorbitol treatment for 10-15 min at 37°C for enriching ring-stage parasites, after which Giemsa-stained thin blood smears were examined by microscopy before each experiment to check for parasitaemia and parasite stages.

Cells isolation and cell sorting. At 6 h after artemether treatment, 2 - 4 drops of blood sample from the mouse infected with *P. yoelii* 17XNL-EGFP parasites were collected in 10 ml RPMI 1640 media in a 15 ml tube and immediately transferred onto ice, along with the controls for cell sorting. Cell samples from the test and control groups were sorted on a BD FACS-Aria II by using UV, blue and red lasers at 355, 488 and 633 nm, respectively. After sorting, only infected erythrocytes were

collected for the scRNA experiment. In addition, at 0 h, 3 h, 9 h and 24 h after artemether treatment, 2 - 4 drops of blood sample from the mice infected with *P. yoelii* 17XNL-EGFP parasites were collected according to the same way for the further analysis.

Chromium 10x Genomics library and sequencing. Single-cell suspensions were loaded to 10x Chromium to capture approximately 3000 - 8000 single cells according to the manufacturer's instructions of 10x Genomics Chromium Single-Cell 3' kit. The following cDNA amplification and library construction steps were performed according to the standard protocol. Libraries were sequenced on an Illumina NovaSeq 6000 sequencing system (paired-end multiplexing run, 150bp) by LC-Bio Technology Co., Ltd., (HangZhou, China) (Supplementary Data 6).

scRNA-seq. The samples were loaded onto a chromium platform (10x Genomics) to generate the barcoded cDNA from the individual cells. Then, scRNA-seq libraries were prepared according to the manufacturer's instructions in the Chromium Single Cell 3′ Reagents Kits V3 User Guide. Libraries were sequenced on Novaseq™ 6000 (Illumina). Sequencing results were demultiplexed and converted to FASTQ format using Illumina bcl2fastq software. Sample demultiplexing, barcode processing and single-cell 3' gene counting by using the Cell Ranger (version 3.1.0) and scRNA-seq data were aligned to reference genome of *P. yoelii.* These reference genomes were all obtained from https://www.ncbi.nlm.nih.gov/genome/65?genome_assembly_id=268934, (*P. yoelii yoelii* 17X), rather than https://www.ncbi.nlm.nih.gov/genome/65?genome_assembly_id=22743, (*P. yoelii yoelii* 17XNL), because the former had higher reads mapped to genome than the latter (Supplementary Data 7).

Clustering and visualization. Unsupervised clustering was performed with R (Seurat package version 3.1.1). Genes expressed in fewer than one cell were filtered out. Cells with >200 genes were further processed. Using the LogNormalize method of the "Normalization" function of the Seurat software to calculated the expression value of genes. Then, variation coefficient of genes was calculated with Seurat. Dimensionality reduction of data was performed by using principle component analysis based on the first 2000 highest variable genes. A k-nearest neighbor graph was constructed from Euclidean distances in the space of the first 10 significant principal components. Then optimize the modularity function to determine clusters and clustering results were visualized by using t-distributed Stochastic Neighbor Embedding (tSNE) project and Uniform Manifold Approximation and Projection (UMAP).

Marker gene identification and cell-type annotation. To determine the relation between cell clusters from tSNE or UMAP analysis and infection stages, unique highly-expressed genes at 6 h, 12 h, 18 h, 24 h, 30 h, 36 and 42 h post-infection with RBCs were selected from the transcriptome data of *P. vivax* http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE61252 (GEO Series accession number GSE61252) (Extended Data Table 1). Comparing with the reported marker genes, MSP8 (highly expressed in early blood stage asexual parasites) and MSP1(highly expressed in late blood stage asexual asexual parasites)[2], the selected marker genes could yield the similar results to that of MSP8 and MSP1 (Extended data Fig. 12). Thus, we used these genes to act as marker genes to determine the relation between cell clusters from the tSNE or UMAP analysis and infection stages.

Malaria parasites and hemozoin analysis with light microscope and TEM. BALb/c mice or ICR

mice were infected by intraperitoneal injections with *P. yoelii* 17XNL or *P. yoelii* 17XL. When the infection rate was above 20%, the mice were fed artemether (100 mg/kg). After 4 and 16 h of treatment, blood samples were collected, respectively. Blood smears were stained with Giemsa in accordance with the standard staining protocol and then observed the smears with a LM (Nikon 50i).

The erythrocytes infected by malaria parasites (*P. falciparum* or *P.yoelii*) were treated by using two methods, as follows. (1) TME1: Samples were treated with saponin at a final concentration of 0.15% and then centrifuged at 10 000 rpm for 5 min. The black deposit was collected and divided into two parts. Then, the deposits were fixed with 2.5% glutaraldehyde–phosphate-buffered saline (PBS) (pH 7.2) buffer, respectively. Part of them were treated with 1%–2.5% SDS for 1–3 h and rinsed three times with glutaraldehyde - PBS. Finally, both parts were dried at room temperature for observation. (2) TME2: Samples were fixed in glutaraldehyde overnight under 4°C. Then, they were washed with PBS, fixed in 1% osmium tetroxide, dehydrated in acetone and embedded in Epon812. Then, 60 nm- thick sections were cut by using ultramicrotome (EM UC6, Leica, German) and mounted on copper slot grids coated with Formvar and stained with uranyl acetate and lead citrate for examination.

Samples were observed by using a JEOL EW-1230 TEM (Japan) at an accelerating voltage of 80 kV, and the images were acquired by using a digital photodocumentation system (Gatan Bioscan Camera, Model 792).

Energy dispersive spectroscopy (EDS). TEM sections of malarial haemozoin (including HAS and crystal-like haemozoin) and merozoites were analyzed by using energy dispersive (X-ray) spectroscopy (Oxford INCA, Lyford, OX, UK) under a JEM-2010 transmission electron microscope

at an accelerating voltage of 200 kV as previously described[3]. The Oxford INCA software package was used to carry out the X-ray analysis to determine the elemental composition of the sample and generate its characteristic spectrum.

ESI-MS analysis of haem, artemether and adducts. A 10 mM haem solution was prepared immediately prior to each experiment by first dissolving haem in 1 N NaOH, diluting with Tris-HC1 buffer (50 mM pH 8.5) and adjusting the pH to 8.5 with 1 N HCl. Artemether was dissolved in methanol as a 10 mM solution and then stored at -20 °C. Equal volumes of haem (10 mM) and artemether (10 mM) were mixed and incubated in the dark at 37°C for 24 h. ESI–MS was performed on a Finnigan LCQ Deca XP equipped with an electrospray ionisation source mass ion-trap spectrometer. The HPLC analysis of haem/artemether adducts was performed using an Agilent ZORBAX SB-C_{18} (5 μm, 4.6 × 250 mm) column with a mobile phase of CH_3OH and H_2O with 0.2% acetic acid [0 - 45min, 70: 30 - 95: 5 (CH_3OH: H_2O with 0.2% acetic acid, v/v); 45-50 min, 95:5] at a flow rate of 1.0 mL min^{-1}.

Iron sucrose assay in vivo and in vitro. In vivo experiment, 60 mice were infected with (1-10) × 10^6 *P. yoelii* 17XNL-parasitized erythrocytes by intraperitoneal injection. When the infection rate of *P. yoelii* 17XNL reached 25% - 35%, they were randomly divided into three groups. Groups 1 and 2 were injected intraperitoneally with iron sucrose (Vifor (International) Inc.) (160 - 200 mg/kg) twice, at 4 h before artemether treatment and 2 h after artemether treatment, respectively. Simultaneously, group 2 was fed with 100 mg/kg of vitamin E at 1 h before and after taking artemether. In contrast, group 3 was intraperitoneally injected with an equal amount of saline. Blood samples were then collected from their tail veins every 3 h to examine the infection rates.

In vitro experiment, *Plasmodium falciparum* (3D7) were synchronized continuously three times by 5% D-sorbitol treatment and purified for late-stage parasites on a 40% / 70% Percoll (GE Health) discontinuous gradient for the enrichment of highly synchronized parasites. After purification, the late-stage parasites were washed in RPMI-1640 medium and cultured for 6 h with fresh erythrocytes. Then the cultures were treated again with 5% sorbitol to eliminate the remaining schizonts [4,5]. Tightly synchronized ring-stage (0–6 h) cultures were diluted to 2% haematocrit and about 1% parasitemia by adding fresh erythrocytes and dispensed in 96-well culture plates. Different drug combinations were added to cell culture media to observe the effect of iron sucrose, vitamin E or their combination on the antimalarial action of DHA. Negative and positive control wells corresponding to non-parasitized erythrocytes and parasite cultures in the absence of drugs were set in parallel. Iron sucrose (0.1, 0.2 and 0.3 mmol/L per well) was added first after the parasite cultures were prepared in 96-well culture plates. Then the plates were incubated for 12 h at 37°C. Subsequently, DHA (20, 25 and 30 nmol/L per well) or vitamin E (20, 30 and 40 μmol/L per well) or their combination were added into the wells simultaneously for another 6 h. Afterward, the media were refreshed at least twice in succession to remove all drugs after incubation for in total 18h. After growing for two cycles, the parasitemia was examined immediately through SYBR Green I assay[6-8]. The effects of iron sucrose, vitamin E (Ve), or their combination on antimalarial action of DHA were determined by measuring parasitemia per well. A total of 100 μL of the culture per well was transferred to a new 96-well black plate, followed by the addition of 100 μL of lysis buffer, which consisted of 20 mmol/L Tris (pH 7.5), 5 mmol/L EDTA , 0.008% (w/v) saponin, 0.08% (w/v) Triton X-100 and 0.2 μL/mL SYBR Green I. The plates were incubated at room temperature for 1 h under the condition of protection from light. The fluorescence intensity, which can quantify parasitemia,

was measured with a multi-mode microplate reader (SpectraMax iD3, Molecular Devices) with excitation and emission wavelengths of 485 nm and 535 nm, respectively. All assays were performed in at least three independent experiments with a minimum of three replicates. Statistical analysis was conducted using a T-test (unpaired) with Graphpad Prism 8.0 software. A p-value less than 0.05 was considered statistically significant.

Deferoxamine and vitamin E assay in vivo and in vitro. In vivo experiment, all mice were infected with (1-10) × 10^6 *P. yoelii* 17XNL-parasitized erythrocytes by intraperitoneal injection. When the infection rate of *P. yoelii* 17XNL reached 25%-35%, they were randomly divided into five groups. Groups 1-3 were fed with artemether (100 mg/kg). Simultaneously, group 2 was injected subcutaneously with deferoxamine mesylate (DFO) (MedChemExpress, MCE) (500 mg/kg) three times, once every 3 h after the first injection; Group 3 was fed with vitamin E (100 mg/kg) twice, 1 h before and after taking artemether, respectively; In contrast, groups 4-5 were only injected subcutaneously with an equal amount of DFO or saline, respectively.

In DFO assay in vitro, *P. falciparum* (3D7)- parasitized erythrocytes were synchronized to ring-stage (0–6 h), then 90 μl of parasitized erythrocytes suspension was put into each test well in 96-well culture plates. 10 μl of diluted DFO was dispensed into test wells to yield final concentrates ranging from 0 to 25 μmol/L. After 18 h of incubation, cultures were washed to remove the drug and resuspended with a complete medium. When parasites developed into second-generation rings, they were transferred to a new 96-well black plate, and 100 μl lysis buffer (0.2 μl of SYBR Green I/ml of lysis buffer) was added to each well, and the contents were mixed. After about 1 h of incubation in the dark at room temperature, the fluorescence intensity, which can quantify

parasitemia, was measured with a multi-mode microplate reader (SpectraMax iD3, Molecular Devices) with excitation and emission wavelengths of 485 nm and 535 nm, respectively.

In Ve assay in vitro, an equal amount of parasitized erythrocytes suspension was dispensed in 96-well culture plates. At 12-18 h post-invasion, four columns in 96-well culture plates were exposed to DHA per well, and at least one column without any drug was set in parallel. Meanwhile, vitamin E was added into three other columns with DHA to make final DHA concentrate at 20 nmol/L per well and vitamin E final concentrate at 10, 20 and 30 μmol/L per well, respectively. After 6 h of incubation, all cultures were washed and resuspended with the complete medium, up to a total volume of 100μl. When parasites developed into second-generation rings, they were transferred to a new 96-well black plate. 100 μl lysis buffer (0.2 μl of SYBR Green I/ml of lysis buffer) was added to each well and incubated for 1 h in the dark at room temperature. The fluorescence intensity, which can quantify parasitemia, was measured with a multi-mode microplate reader (SpectraMax iD3, Molecular Devices).

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