

Supplementary Materials for

Disrupting CD147-RAP2 interaction abrogates erythrocyte invasion

by *Plasmodium falciparum*

Meng-Yao Zhang^{1,2,3,4†}, Yang Zhang^{1,3†}, Xiao-Dong Wu^{1,3†}, Kun Zhang^{1,3,5†},
Peng Lin^{1,3†}, Hui-Jie Bian^{1,3}, Min-Min Qin¹, Wan Huang^{1,3}, Ding Wei^{1,3}, Zhao Zhang^{1,3},
Jiao Wu^{1,3}, Ruo Chen^{1,3}, Fei Feng^{1,3}, Bin Wang^{1,3}, Gang Nan^{1,3},
Ping Zhu^{1,2*} & Zhi-Nan Chen^{1,3*}

correspondence to: znchen@fmmu.edu.cn; zhuping@fmmu.edu.cn.

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Supplementary Methods

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Extended Figure 1

Supplementary Methods

The vectors construction

Total RNA was extracted from FCC1 and Nf54 parasites using the QIAamp RNA Blood Mini Kit (Qiagen, German). cDNA fragments were PCR-amplified, using primer pairs of RAP2-F(5'- CAATCGATATGGGTTTAAAATTTTATG -3')/RAP2-R (5'- ATGGTACCGAAAGAACATTTAATTCTC -3') for subcloning into p3XFLAG-CMV14 (Sigma-Aldrich), and of RAP2-pET32a-F(5'- TAGGTACCATGGGTTTAAAATTTTATG -3')/RAP2-pET32a-R (5'- GCCTCGAGAAGAACATTTAATTCTCTA-3') for subcloning into pET32a vector (Novagen) for mammalian and *E. coli* expression, respectively.

Acute toxicity studies

HP6H8 and the buffer control were intravenously administered to *cynomolgus* monkeys in three groups, each group containing two male monkeys and female monkeys of 3~6 years old and 3.1~3.2 kg's in body weights. All the monkeys used in this study were treated in accordance with institutional guidelines.

In vitro cytotoxicity assay of HP6H8 in inflammatory setting in vitro

Peripheral blood mononuclear cells (PBMCs) from healthy volunteers were used to perform the cytotoxicity assay *in vitro*. The PBMCs and RBCs were obtained from the same donor. The PBMCs were cultured *in vitro* and were activated by antibodies and cytokines, while the RBCs were cultured and infected with *P. falciparum* 3D7 strain. Then, the activated PBMCs and infected RBCs were co-cultured with or without HP6H8 at the concentration of 100 µg/ml for 4 hours to observe the immune mediated hemolysis.

Two effector cells (PBMCs): target cells (RBCs) ratios of 1:1000 and 1:100 were tested, reflecting PBMC:RBC ratios in healthy human and 10x worse conditions, respectively. The immune mediated hemolysis effect of HP6H8 was determined by CytoTox 96[®] Non-Radioactive Cytotoxicity Assay (Promega, G1780) and the absorbance signal is measured at 490nm by Tecan Spark[™] 10M.

Determination of the immune mediated hemolysis effect of HP6H8 in patients-derived inflammatory setting in vitro

The whole blood was obtained from three patients whose immune system had been activated due to infection or surgery. The whole blood was incubated with HP6H8 at 100 µg/ml for 4 hours and the hematology parameters were determined by SYSMEX XP-100.

Determination the immune mediated hemolysis effect of HP6H8 on lipopolysaccharide (LPS) treated Rhesus monkeys model

Eight Rhesus monkeys, half male and female, aged at 3-5 years, weighted at 3-5 kilograms, were divided into two groups. One was vehicle control, and the other was HP6H8 group. All the monkeys were tested for body temperature, body weight, food consumption, electrocardiogram, clinical chemistry indicators, hematology indicators and so on. After that, they were injected with LPS at 25 µg/kg (Sigma, L2880), followed by intravenous injection of HP6H8 at 1 mg/kg or vehicle control. Data were collected at 0h, 2h, 4h, 8h, to test the immune activated effect of LPS and 0h, 8h, D2, D4, D6 to test the toxicity of HP6H8.

Data collection	
Wavelength (Å)	0.97926
Resolution range (Å)	43.34-2.50
Data completeness (%)	97.0 (84.9)
Unique reflections	33808
Redundancy	1.0 (1.0)
R _{merge} (%)	6.9 (34.7)
I/σ(I)	20.0 (7.1)
Refinement Statistics	
Resolution range (Å)	43.34-2.50
Space group	P2 ₁
Cell parameters: a, b, c (Å)	47.0, 83.5, 130.2
Cell parameters: β (°)	92.9
r.m.s.d. bonds(Å)	0.006
r.m.s.d. angles(°)	1.046
R _{work}	0.246
R _{free}	0.301

Note: values in parentheses indicate the corresponding statistics in the highest resolution shell

Table S1. Data collection and refinement statistics of the CD147D1-6H8Fab interface details.

Group	clinical diagnosis	WBC (10 ⁹ /L)	NEUT (10 ⁹ /L)	LYMPH (10 ⁹ /L)	MONO (10 ⁹ /L)	BASO (10 ⁹ /L)	EOS (10 ⁹ /L)	RBC (10 ¹² /L)	HGB (g/L)	MCH (pg)	RDW- CV
Before incubation	Liver abscess	17.23	13.00	3.05	1.04	0.05	0.09	4.71	145	30.8	0.120
	After PCI	12.05	7.72	3.4	0.72	0.1	0.11	4.72	139	29.4	0.133
	Fever	13.36	9.26	3.03	1.01	0.06	0	5.01	160	31.9	0.132
After incubation	Liver abscess	15.84	11.91	2.88	0.9	0.06	0.09	4.78	148	31.0	0.125
	After PCI	11.73	7.49	3.32	0.73	0.09	0.1	4.85	140	28.9	0.143
	Fever	12.42	8.49	2.94	0.91	0.08	0	4.76	153	32.1	0.143
Baseline	Reference value	3.5-9.5	1.8-6.3	1.1-3.2	0.1-0.6	0-0.06	0.02-0.52	4.3-5.8	130-175	27-34	0.04-0.15

Table S2. Individual Data of Hematology. WBC: White Blood Cell Count; NEUT: Neutrophils Count; LYMPH: Lymphocytes Count; MONO: Monocytes Count; BASO: Basophils Count; EOS: Eosinophils Count; RBC: Red Blood Cell Count; HGB: Hemoglobin; MCH: Mean Corpuscular Hemoglobin; RDW: Red Cell Volume Distribution Width; The value marked in red represents it is higher than the reference value, while the value marked in green represents it is lower than the reference value.

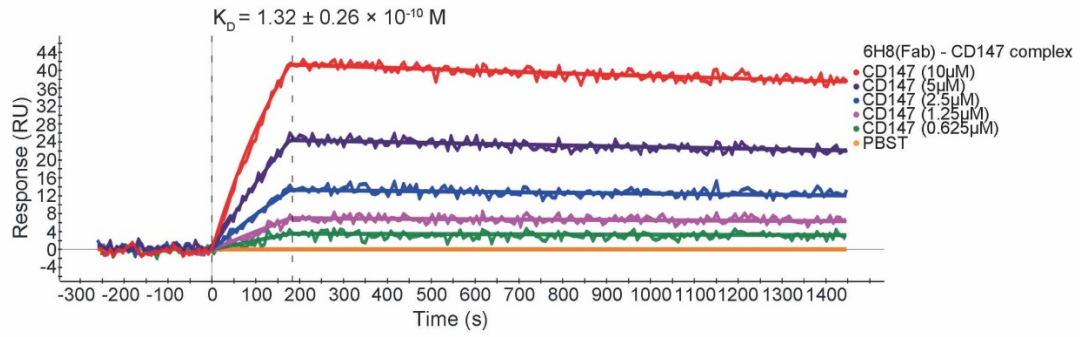


Figure S1. SPR analysis of the HP6H8's affinity to CD147. The calculated K_D value was represented as Mean \pm SD.

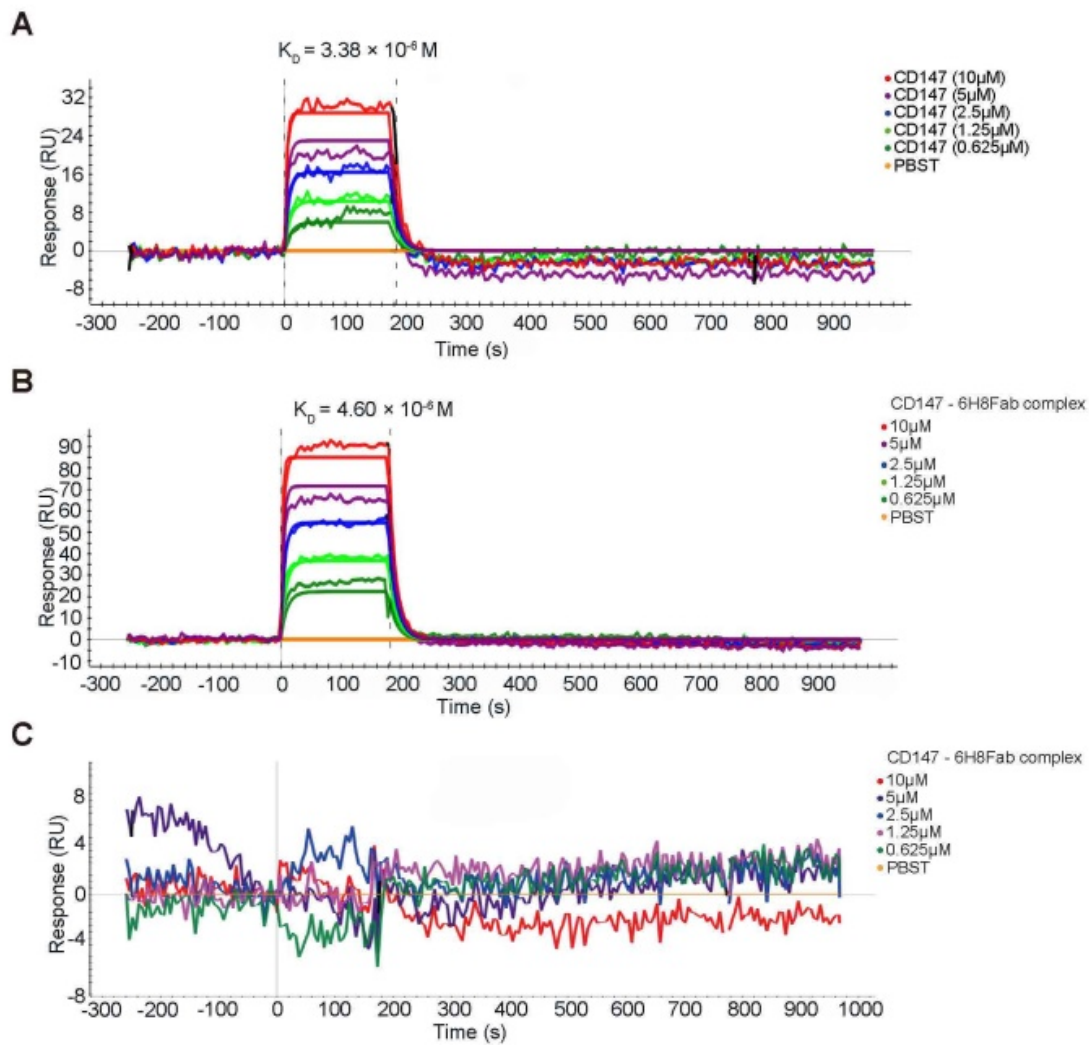


Figure S2. SPR analysis of competitive inhibition. (A) The CD147-Pfrh5 interaction. (B) Interruption of the CD147-Pfrh5 interaction by 6H8Fab. (C) Interruption of the CD147-RAP2 by 6H8Fab.

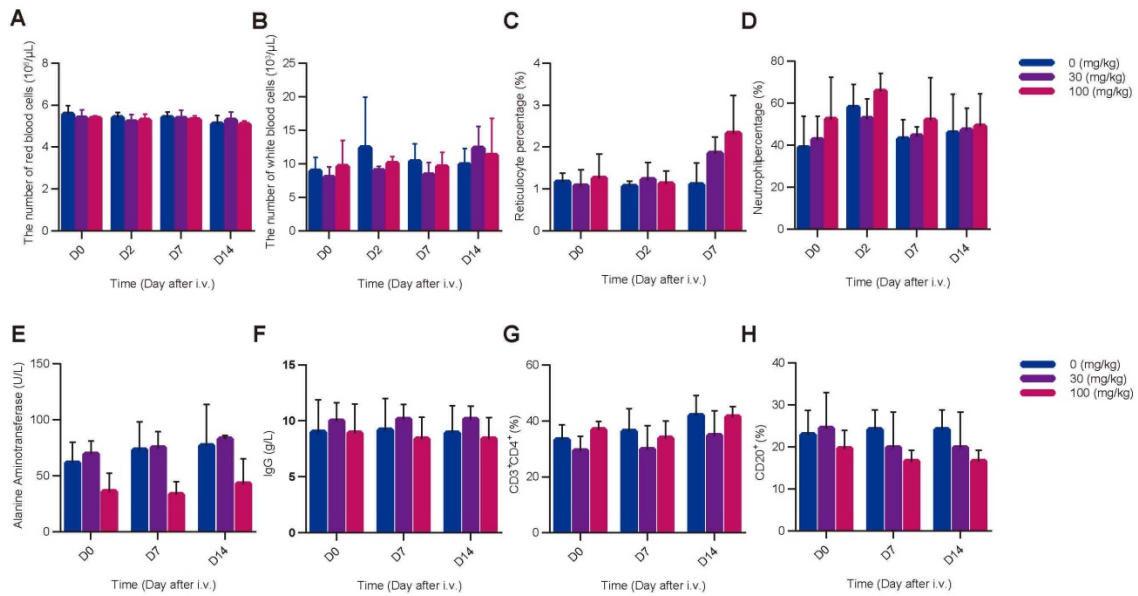


Figure S3. Representative parameters from the acute toxicity study of HP6H8 on *cynomolgus* monkeys. (A) The number of red blood cells ($10^6/\mu\text{L}$). (B) The number of white blood cells ($10^3/\mu\text{L}$). (C) The percentage of reticulocytes (%). (D) The percentage of neutrophil (%). (E) The serum concentration of alanine aminotransferase (U/L). (F) The percentage of CD3⁺CD4⁺ cells (%). (G) The percentage of CD20⁺ cells (%). The black, purple, pink column represented 0, 30, 100 mg/kg of HP6H8 respectively. Data of the D0, D2, D7, D14 were shown as Mean \pm SD.

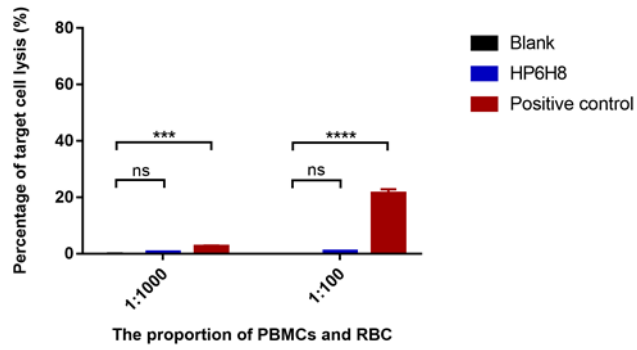


Figure S4. The effect of immune mediated hemolysis by HP6H8. Black, blue, red columns represent blank control, HP6H8, positive control, respectively. ANOVA test was used to determine statistical significance. Mean and SD are shown.

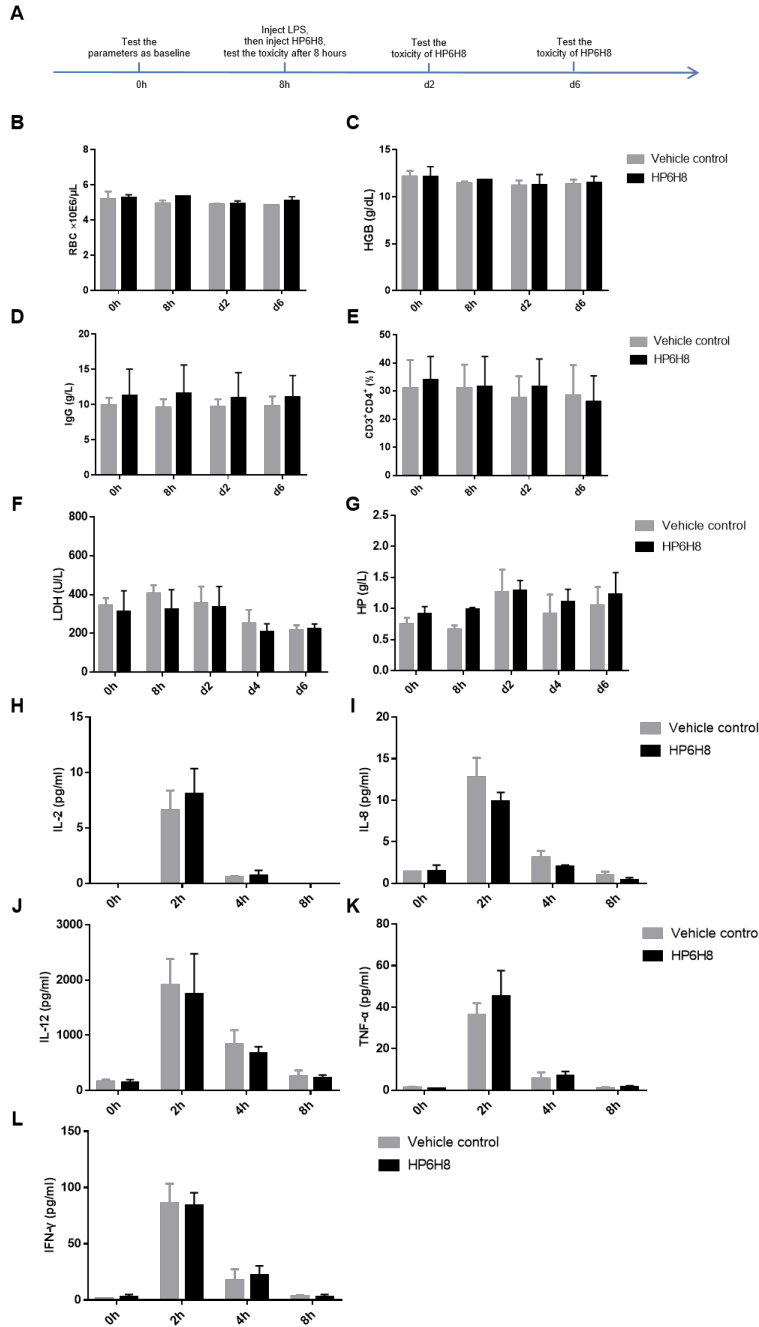
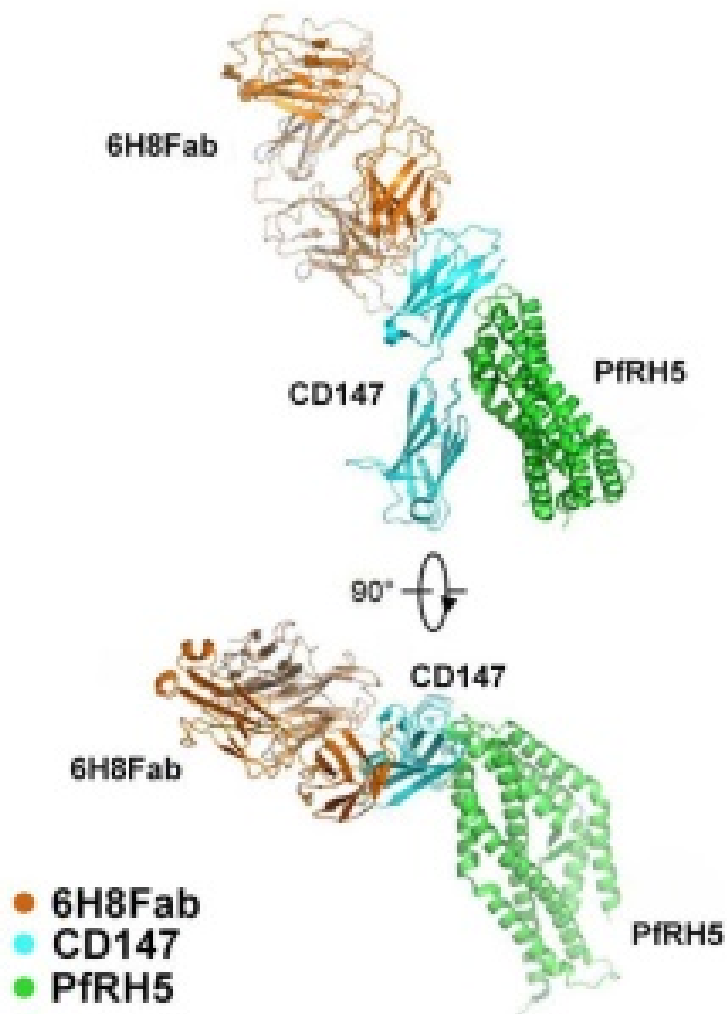


Figure S5. Representative parameters from the toxicity study of HP6H8 on LPS treated Rhesus monkeys model. (A) The experiment flow chart. (B) The number of red blood cells ($10^6/\mu\text{L}$). (C) The serum concentration of hemoglobin (g/dL). (D) The concentration of IgG (g/L). (E) The percentage of $\text{CD3}^+\text{CD4}^+$ cells (%). (F) The concentration of LDH (U/L). (G) The concentration of haptoglobin (g/L). (H) The concentration of IL-2 (pg/ml). (I) The concentration of IL-8 (pg/ml). (J) The concentration of IL-12 (pg/ml). (K) The concentration of $\text{TNF-}\alpha$ (pg/ml). (L) The concentration of $\text{IFN-}\gamma$ (pg/ml). The black and gray column represented HP6H8 and vehicle control, respectively. Data were shown as Mean \pm SD.



Extended Fig. 1. The crystal structures of the CD147-6H8Fab and CD147-PfRh5 complex composed from the CD147-6H8Fab data presented in the manuscript and from the CD147-PfRh5 data in the published paper.¹¹