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Supplemental material

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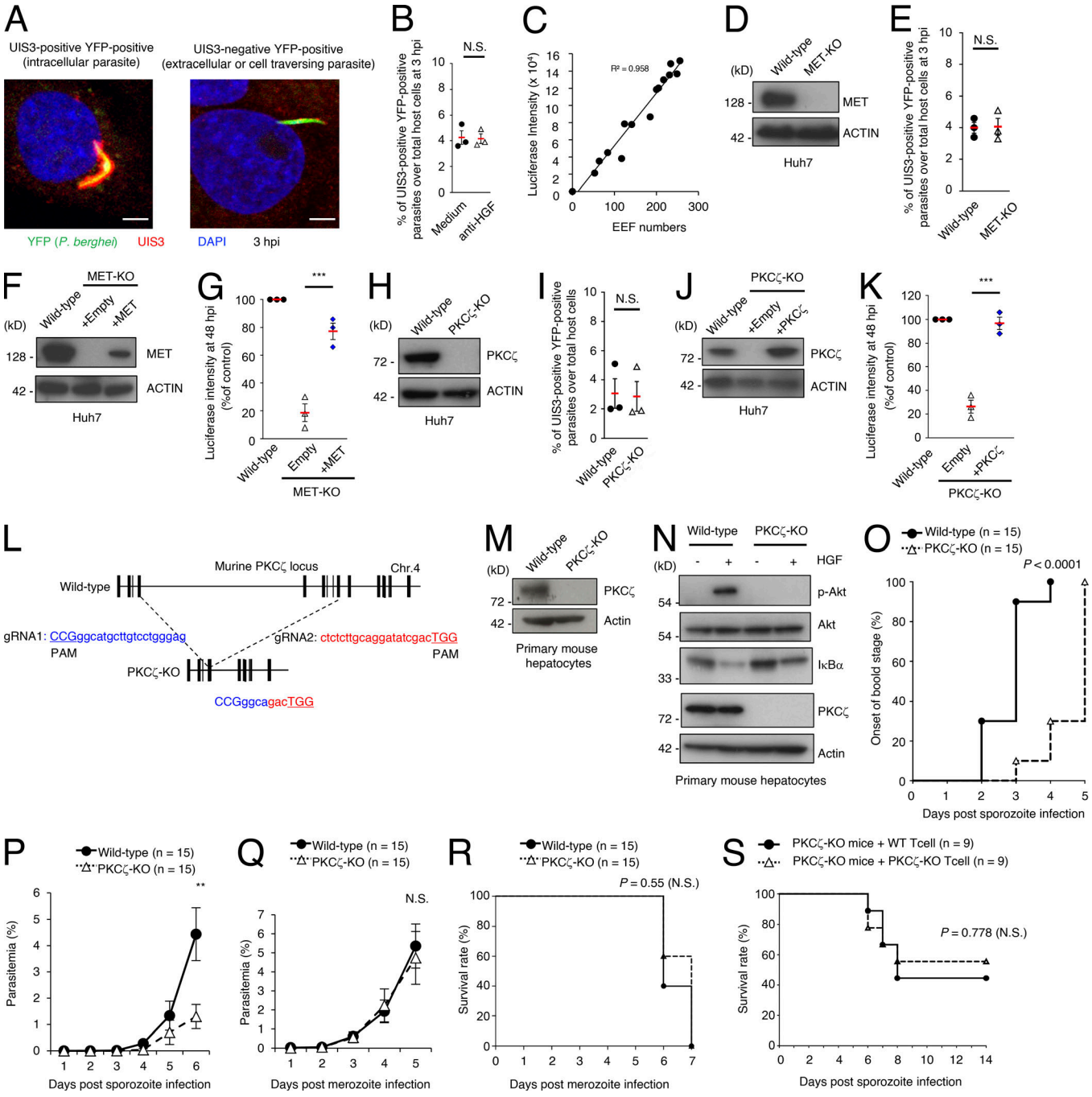
A
UIS3-positive YFP-positive (intracellular parasite)
UIS3-negative YFP-positive (extracellular or cell traversing parasite)
YFP (P. berghei) UIS3 DAPI 3 hpi
B
% of UIS3-positive YFP-positive parasites over total host cells at 3 hpi
N.S.
Medium
anti-HGF
C
Luciferase Intensity (x 10⁴)
R² = 0.958
EEF numbers
D
(kD)
Wild-type
MET-KO
128
MET
42
ACTIN
Huh7
E
% of UIS3-positive YFP-positive parasites over total host cells at 3 hpi
N.S.
Wild-type
MET-KO
F
MET-KO
(kD)
Wild-type
+Empty
+MET
128
MET
42
ACTIN
Huh7
G
Luciferase intensity at 48 hpi (%of control)
***
Wild-type
Empty
+MET
MET-KO
H
(kD)
Wild-type
PKCζ-KO
72
PKCζ
42
ACTIN
Huh7
I
% of UIS3-positive YFP-positive parasites over total host cells
N.S.
Wild-type
PKCζ-KO
J
PKCζ-KO
(kD)
Wild-type
+Empty
+PKCζ
72
PKCζ
42
ACTIN
Huh7
K
Luciferase intensity at 48 hpi (%of control)
***
Wild-type
Empty
+PKCζ
PKCζ-KO
L
Murine PKCζ locus
Chr.4
Wild-type
gRNA1: CCGggcatgcttgtcctgggag
PAM
gRNA2: ctctcttgcaggatatcgacTGG
PAM
PKCζ-KO
CCGggcagacTGG
M
(kD)
Wild-type
PKCζ-KO
72
PKCζ
42
Actin
Primary mouse hepatocytes
N
Wild-type
PKCζ-KO
(kD)
- + - +
HGF
54
p-Akt
54
Akt
33
IκBα
72
PKCζ
42
Actin
Primary mouse hepatocytes
O
Wild-type (n = 15)
PKCζ-KO (n = 15)
P < 0.0001
Onset of boold stage (%)
Days post sporozoite infection
P
Wild-type (n = 15)
PKCζ-KO (n = 15)
**
Parasitemia (%)
Days post sporozoite infection
Q
Wild-type (n = 15)
PKCζ-KO (n = 15)
N.S.
Parasitemia (%)
Days post merozoite infection
R
Wild-type (n = 15)
PKCζ-KO (n = 15)
P = 0.55 (N.S.)
Survival rate (%)
Days post merozoite infection
S
PKCζ-KO mice + WT Tcell (n = 9)
PKCζ-KO mice + PKCζ-KO Tcell (n = 9)
P = 0.778 (N.S.)
Survival rate (%)
Days post sporozoite infection

Figure S1. **PKCζ is not essential for *P. berghei* sporozoite invasion into hepatocytes. (A)** Representative IFA images of intracellular and extracellular sporozoites expressing YFP (green). Nuclei are stained with DAPI (blue), and parasites are stained with anti-UIS3 antibodies (red). UIS3-positive/YFP-positive sporozoites are defined as intracellular parasites (left). UIS3-negative/YFP-positive sporozoites are defined as extracellular parasites (right). Bars, 5 µm. Data are representative of three independent experiments. **(B)** Effect of addition of anti-HGF antibody to the culture medium on *P. berghei* sporozoite development in Huh7 cells. The percentage of intracellular parasites at 3 h after infection was assessed by IFA. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated (Student's t test). **(C)** Huh7 cells were infected with *P. berghei* sporozoites. One aliquot of the culture was used to count EEFs at 48 h after sporozoite infection using confocal laser microscopy. Another aliquot was used to measure luciferase intensity at 48 h after sporozoite infection. The correlation between EEFs number and luciferase intensity is shown on the x and y axes, respectively. **(D)** Expression of the genes indicated to the right was detected in WT or MET-KO Huh7 cell lysates by Western blotting. Numbers (in kilodaltons) to the left indicate the position of size markers. Data are representative of three independent experiments. **(E)** WT or MET-KO Huh7 cells were infected with *P. berghei* sporozoites. The percentage of intracellular parasites at 3 h after infection was determined by IFA. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated (Student's t test). **(F)** Expression of the genes indicated to the right were detected by Western blotting in WT and in MET-KO Huh7 cells that were transfected with an empty vector (+Empty) or with a vector expressing MET (+MET) lysates. Numbers (in kilodaltons) to the left indicate the position of size markers. Data are representative of three independent experiments. **(G)** WT, MET-KO+Empty, or MET-KO+MET Huh7 cells were infected with *P. berghei* sporozoites, and luciferase intensity was measured 48 h after sporozoite infection. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated. ***, P < 0.001 (Student's t test). **(H)** Expression of the genes indicated to the right was detected by Western blotting in WT or PKCζ-KO Huh7 cell lysates. Numbers (in kilodaltons) to the left indicate the position of size markers. Data are representative of three independent experiments. **(I)** WT or PKCζ-KO Huh7 cells were infected with *P. berghei* sporozoites. The percentage of intracellular parasites at 3 h after infection was measured by IFA. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated (Student's t test). **(J)** Expression of the genes indicated to the right detected by Western blotting in WT and in PKCζ-KO Huh7 cells transfected with an empty vector (+Empty) or a vector expressing PKCζ (+PKCζ) lysates. Numbers (in kilodaltons) to the left indicate the position of size markers. Data are representative of three independent experiments. **(K)** Luciferase intensity 48 h after infection in WT, PKCζ-KO+Empty, or PKCζ-KO+ PKCζ Huh7 cells. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated. ***, P < 0.001 (Student's t test). **(L)** Schematic of CRISPR/Cas9-mediated disruption of PKCζ-KO mice. Vertical bars represent exons. PAM, protospacer adjacent motif. **(M)** Expression of the genes indicated to the right was detected by Western blotting in WT or PKCζ-KO Huh7 cell lysates. Numbers (in kilodaltons) to the left indicate the position of size markers. Data are representative of three independent experiments. **(N)** WT or PKCζ-KO primary mouse hepatocytes were treated with HGF (50 ng/ml) for 1 min. Expression of the genes indicated to the right was detected in the cell lysates by Western blotting. Numbers (in kilodaltons) to the left indicate the position of size markers. Data are representative of three independent experiments. **(O and P)** WT or PKCζ-KO mice (n = 15 per each group) were infected with *P. berghei* sporozoites. **(O)** Onset of blood-stage parasitemia was assessed by Giemsa staining. Data are cumulative of three independent experiments. P < 0.0001 (log-rank test). **(P)** The parasitemia was assessed by Giemsa staining. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated. **, P < 0.01 (Student's t test). **(Q and R)** WT or PKCζ-KO mice (n = 15 per each group) were infected with *P. berghei* parasitized RBCs. **(Q)** Onset of blood-stage parasitemia as a function of time was assessed by Giemsa staining. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated (Student's t test). **(R)** Mouse survival as a function of time. Data are cumulative of three independent experiments. P < 0.55 (nonsignificant by log-rank test). **(S)** T cells were purified from the spleens of WT or PKCζ-KO mice. 2×10^6 T cells were transferred into PKCζ-KO mice (n = 9 per each group) for 12 h. Mice were then infected with *P. berghei* sporozoites, and survival rates were analyzed. Data are cumulative of three independent experiments. P < 0.778 (nonsignificant by log-rank test). N.S., not significant; hpi, hours post-infection.

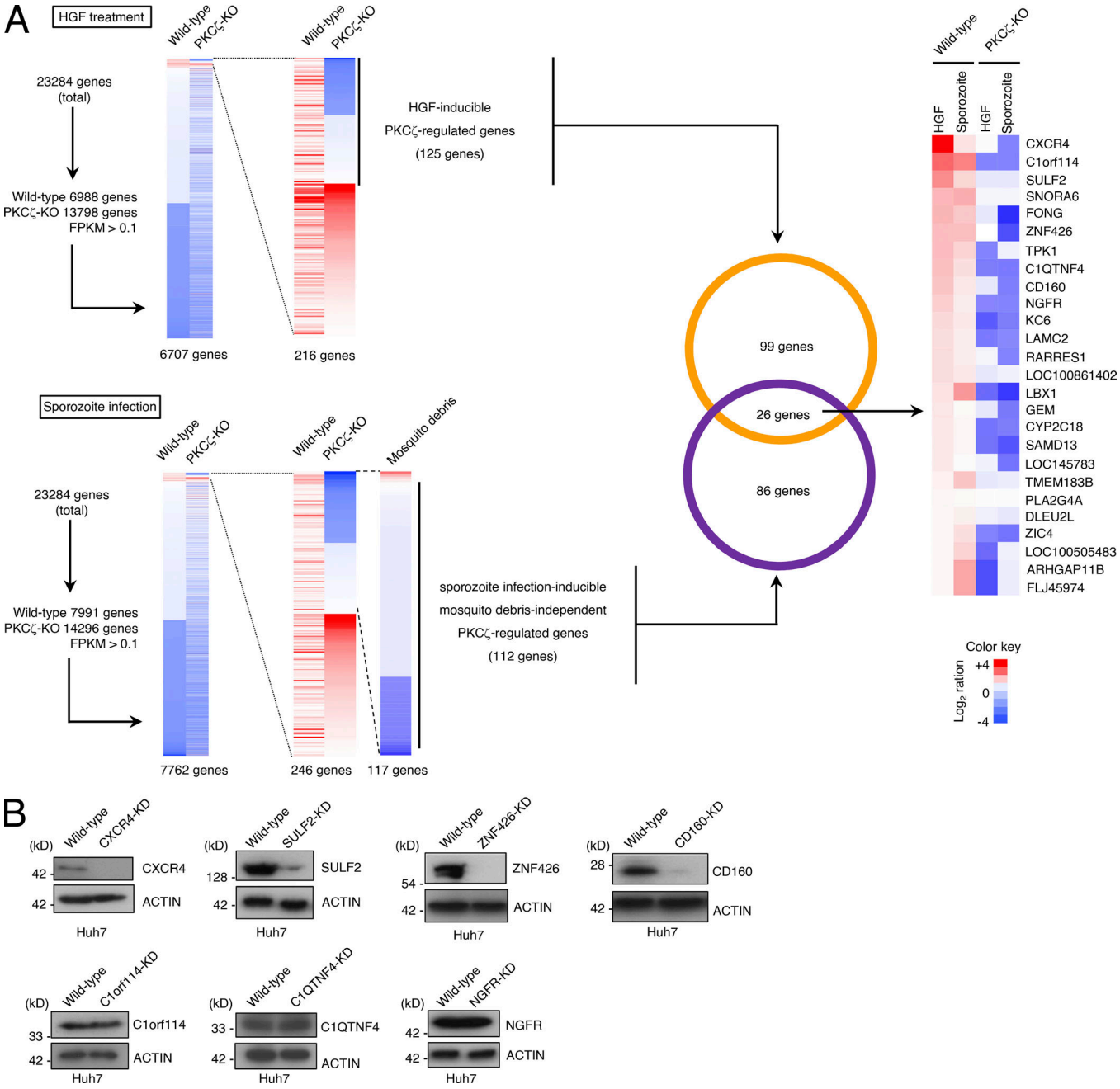

Figure S2. **Transcriptional changes in *P. berghei* sporozoite–infected or HGF-treated Huh7 cells. (A)** RNA-seq analysis of HGF- or *P. berghei* sporozoite infection–induced PKCζ-regulated genes. Heatmap showing the changes in gene expression between WT and PKCζ-KO Huh7 cells 6 h after HGF (50 ng/ml) treatment or *P. berghei* sporozoite infection. For extraction of PKCζ-dependent HGF-inducible genes among 23,284 total genes, 6,707 common genes (FPKM>0.1) in WT or PKCζ-KO Huh7 cells treated with HGF were selected from 6,988 and 13,798 genes, respectively (upper panel, left). Next, HGF-inducible genes that were changed by more than +1.5-fold in WT Huh7 cells after HGF treatment yields 216 genes (upper panel, middle). Among them, PKCζ-dependent genes that were altered by less than +1.5-fold change in PKCζ-KO Huh7 cells yields 99 genes (upper panel, orange circle). For extraction of PKCζ-dependent sporozoite infection–inducible genes among total 23,284 total genes, 7,762 common genes (FPKM >0.1) in WT or PKCζ-KO Huh7 cells infected with sporozoite were selected from 7,991 and 14,296 genes, respectively (lower panel, left). Next, sporozoite infection–inducible genes that were altered by more than +1.5-fold in WT Huh7 cells after sporozoite infection yields 246 genes (lower panel, middle). Among them, PKCζ-dependent genes that were altered by less than +1.5-fold in PKCζ-KO Huh7 cells yields 117 genes (lower second panel). In addition, mosquito debris–inducible genes that were changed by more than +1.5-fold in Huh7 cells after mosquito debris treatment yields five genes (lower third panel). Thus, sporozoite infection–inducible genes that were altered by more than +1.5-fold in Huh7 cells yields 112 genes (lower panel, purple circle). The Venn diagram shows common PKCζ-dependent genes (26 genes) that are shared by HGF- and sporozoite infection–inducible genes (right). The 26 genes were ranked by fold induction in WT cells after HGF stimulation. The top 10 PKCζ-regulated HGF-inducible genes shown in Fig. 2 A were further analyzed. Up-regulated genes are marked in red, and down-regulated genes are marked in blue. Expression values are scaled up to the rows and range from −4 to +4. Data are from one experiment. **(B)** Expression of the genes indicated to the right was detected in WT, CXCR4-KD, SULF2-KD, ZNF426-KD, CD160-KD, C1orf114-KD, C1QTNF4-KD, or NGFR-KD Huh7 cell lysates by Western blotting. Numbers (in kilodaltons) to the left indicate the position of size markers. Data are representative of three independent experiments.

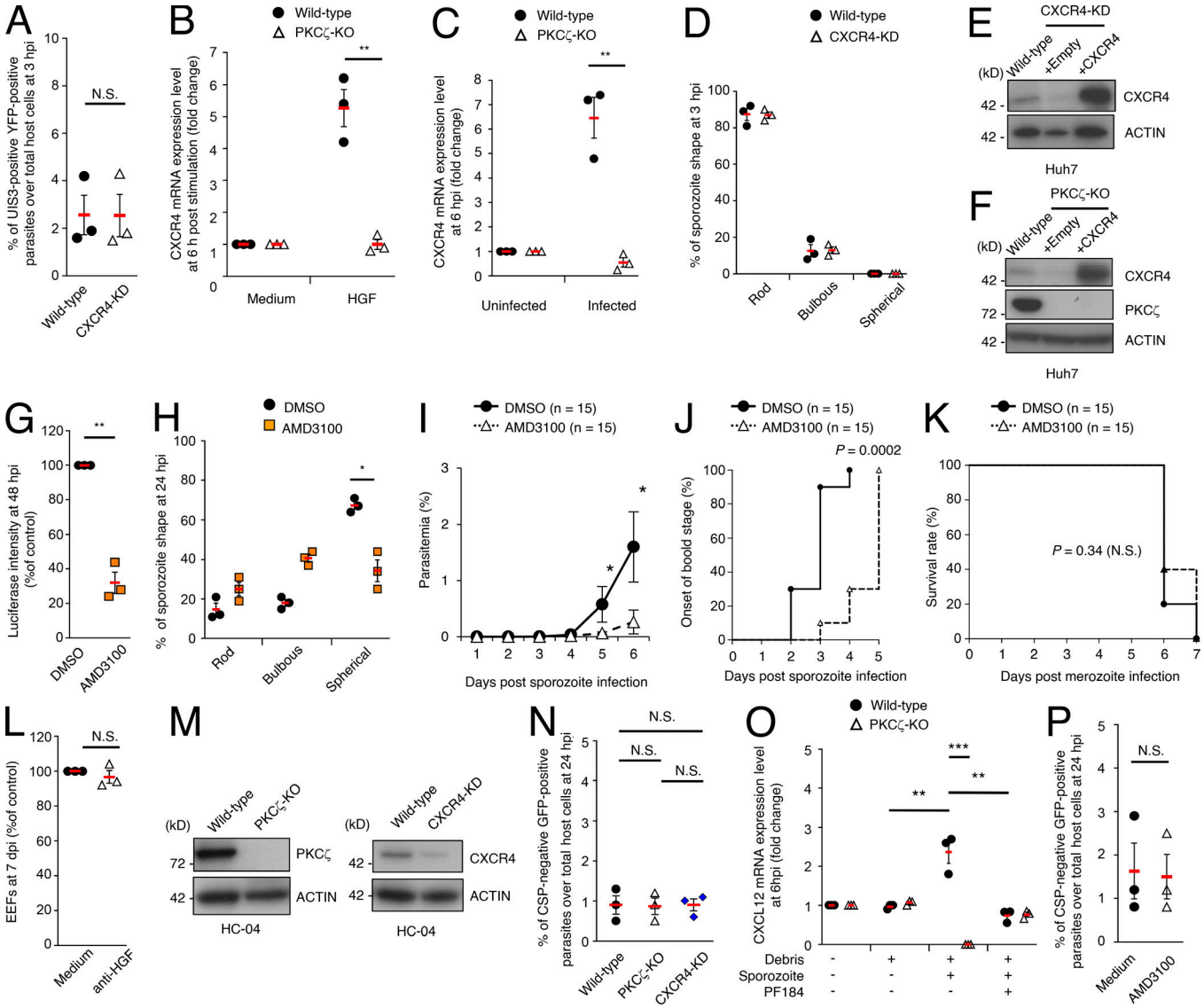
A
% of UIS3-positive YFP-positive parasites over total host cells at 3 hpi
N.S.
Wild-type
CXCR4-KD
B
Wild-type
PKCζ-KO
CXCR4 mRNA expression level at 6 h post stimulation (fold change)
**
Medium
HGF
C
Wild-type
PKCζ-KO
CXCR4 mRNA expression level at 6 hpi (fold change)
**
Uninfected
Infected
D
Wild-type
CXCR4-KD
% of sporozoite shape at 3 hpi
Rod
Bulbous
Spherical
E
CXCR4-KD
(kD)
Wild-type
+Empty
+CXCR4
42
CXCR4
42
ACTIN
Huh7
F
PKCζ-KO
(kD)
Wild-type
+Empty
+CXCR4
42
CXCR4
72
PKCζ
42
ACTIN
Huh7
G
Luciferase intensity at 48 hpi (%of control)
**
DMSO
AMD3100
H
DMSO
AMD3100
% of sporozoite shape at 24 hpi
*
Rod
Bulbous
Spherical
I
DMSO (n = 15)
AMD3100 (n = 15)
Parasitemia (%)
*
*
Days post sporozoite infection
J
DMSO (n = 15)
AMD3100 (n = 15)
P = 0.0002
Onset of boold stage (%)
Days post sporozoite infection
K
DMSO (n = 15)
AMD3100 (n = 15)
Survival rate (%)
P = 0.34 (N.S.)
Days post merozoite infection
L
EEFs at 7 dpi (%of control)
N.S.
Medium
anti-HGF
M
(kD)
Wild-type
PKCζ-KO
72
PKCζ
42
ACTIN
HC-04
(kD)
Wild-type
CXCR4-KD
42
CXCR4
42
ACTIN
HC-04
N
% of CSP-negative GFP-positive parasites over total host cells at 24 hpi
N.S.
N.S.
N.S.
Wild-type
PKCζ-KO
CXCR4-KD
O
Wild-type
PKCζ-KO
CXCL12 mRNA expression level at 6hpi (fold change)
***
**
**
Debris
Sporozoite
PF184
P
% of CSP-negative GFP-positive parasites over total host cells at 24 hpi
N.S.
Medium
AMD3100

Figure S3. **Pharmacological CXCR4 inhibition abrogates *P. berghei* sporozoite development. (A)** WT or CXCR4-KD Huh7 cells were infected with *P. berghei* sporozoites. The percentage of intracellular parasites at 3 h after infection was counted by IFA. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated (Student's t test). **(B and C)** WT or PKCζ-KO Huh7 cells were untreated or treated with HGF (50 ng/ml) for 6 h (B), uninfected or infected with sporozoites for 6 h (C). Quantitative RT-PCR analysis of CXCR4 mRNA levels is reported. Indicated values are means (n = 3) of ± SEM. Each point represents the mean of one experiment. Three experiments were repeated. **, P < 0.01 (Student's t test). **(D)** WT or CXCR4-KD Huh7 cells were infected with *P. berghei* sporozoites. Parasite shape 3 h after sporozoite infection was assessed by IFA. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated. **, P < 0.01 (Student's t test). **(E)** The expression of the genes indicated to the right were detected in WT and CXCR4-KD Huh7 cells that were transfected with an empty vector (+Empty) or a vector expressing CXCR4 (+ CXCR4) Huh7 cell lysates by Western blotting. Numbers (in kilodaltons) to the left indicate the position of size markers. Data are representative of three independent experiments. **(F)** Expression of the genes indicated to the right was detected in WT and PKCζ-KO Huh7 cells that were transfected with an empty vector (PKCζ-KO+Empty) or a vector expressing CXCR4 (PKCζ-KO + CXCR4) Huh7 cell lysates by Western blotting. Numbers (in kilodaltons) to the left indicate the position of size markers. Data are representative of three independent experiments. **(G and H)** Effect of the addition of AMD3100 (1 µM) on *P. berghei* sporozoite development in Huh7 cells. **(G)** Luciferase intensity 48 h after sporozoite infection. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated. **, P < 0.01 (Student's t test). **(H)** Parasite shapes 24 h after sporozoite infection were assessed by IFA. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated. *, P < 0.01 (Student's t test). **(I and J)** Untreated or AMD3100 (10 mg/kg) treated mice (n = 15 per each group) were infected with *P. berghei* sporozoites. **(I)** Parasitemia was assessed by Giemsa staining. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated. *, P < 0.05 (Student's t test). **(J)** Onset of blood-stage parasites was assessed by Giemsa staining. Data are cumulative of three independent experiments. P = 0.0002 (significant by log-rank test). **(K)** Mice were infected with *P. berghei* sporozoites. 48 h after infection, mice were untreated or treated with AMD3100 (10 mg/kg), and the survival rate was analyzed. Data are cumulative of three independent experiments. P = 0.34 (nonsignificant by log-rank test). **(L)** Effect of the addition of anti-HGF (20 µg/ml) antibody to the culture medium on *P. falciparum* sporozoite development in HC-04 cells. EEFs were counted using IFA. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated (Student's t test). **(M)** Expression of the genes indicated to the right detected by Western blotting in WT, PKCζ-KO, or CXCR4-KD HC-04 cell lysates. Numbers (in kilodaltons) to the left indicate the position of size markers. Data are representative of three independent experiments. **(N)** WT, PKCζ-KO, or CXCR4-KD HC-04 cells were infected with *P. falciparum* sporozoites. The percentage of intracellular parasites at 3 h after infection was counted by IFA. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated (Student's t test). **(O)** Effect of addition of AMD3100 (1 µM) on *P. falciparum* sporozoite development in HC-04 cells. The percentage of intracellular parasites at 3 h after infection was assessed by IFA. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated. **, P < 0.01; ***, P < 0.001 (Student's t test). **(P)** HC-04 cells were treated or not with PF184 (500 nM) for 4 h, as indicated, and then infected or not with *P. falciparum* sporozoites or treated with mosquito debris for 6 h, as indicated. The graphs report the quantitative RT-PCR analysis of CXCL12 mRNA levels. Indicated values are means (n = 3) ± SEM. Each point represents the mean of one experiment. Three experiments were repeated (Student's t test). N.S., not significant; hpi, hours post-infection.

Table S1 is provided online as a separate Excel file and lists details of primers used in this study.