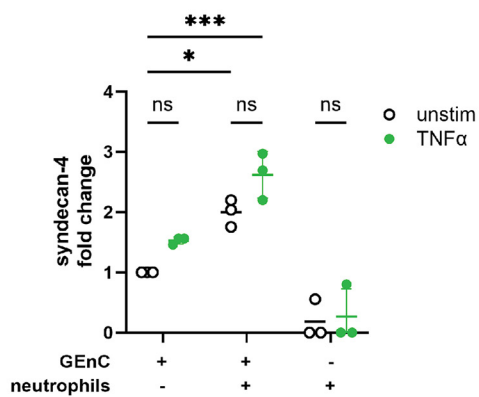
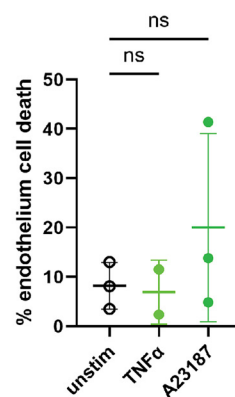
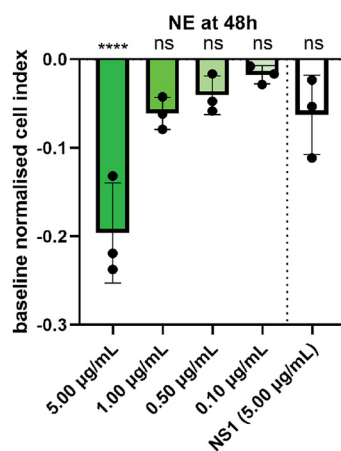
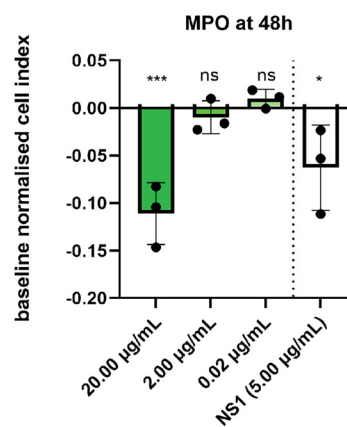
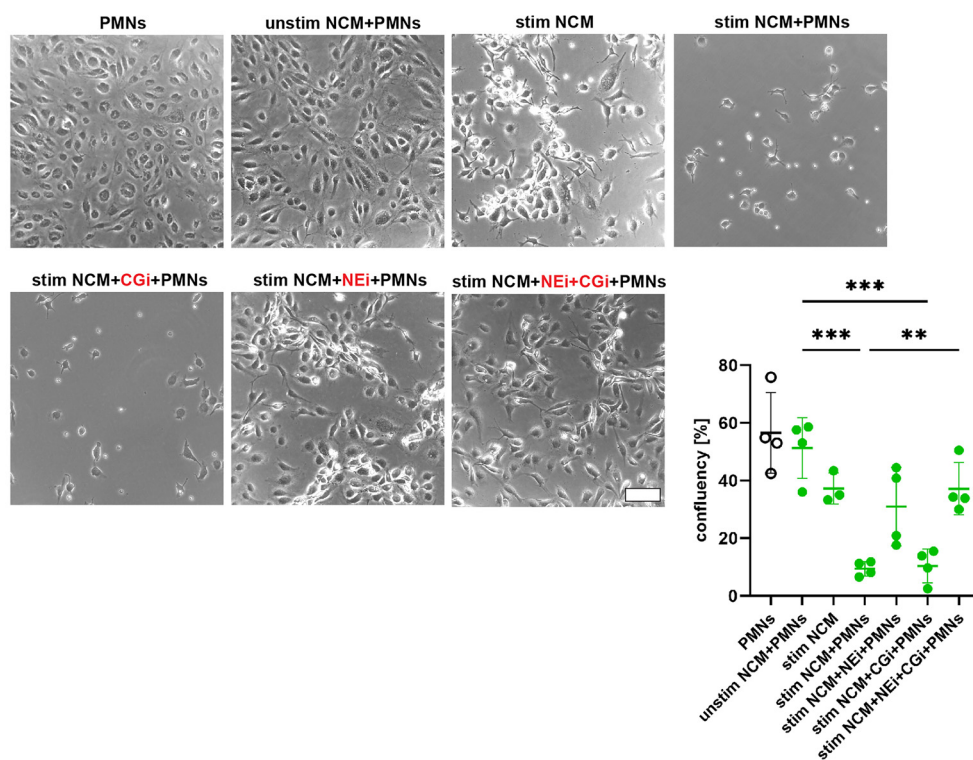


Expanded View Figures

Figure EV1. Additional analyses of endothelial permeability and glycocalyx shedding.

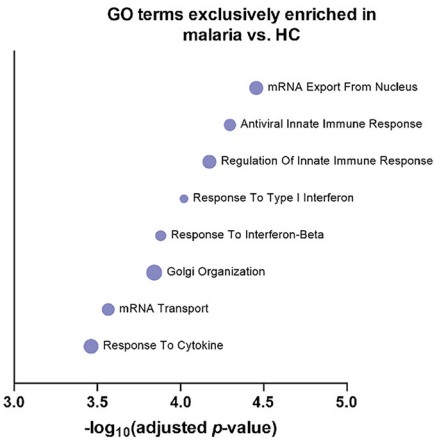
(A) Fold change of syndecan-4 in GEnC culture supernatants following co-culture with peripheral blood neutrophils for 5 h, with or without treatment with TNF α (2 ng/mL), $n = 3$. (B) Percentage of GEnC death following culture with untreated, TNF α (2 ng/mL) and A23187 (2.5 μ M)-treated neutrophils for 5 h, $n = 3$. (C) TEER cell index at 48 h following treatment of HUVECs with NE (0.10–5.00 μ g/mL) and positive control (Dengue virus NS1 protein; 5.00 μ g/mL) and compared to baseline, $n = 3$. (D). TEER cell index at 48 h following treatment of HUVECs with MPO (0.02–2.00 μ g/mL) and positive control (Dengue virus NS1 protein; 5.00 μ g/mL) and compared to baseline, $n = 3$. (E) Representative phase contrast images of HUVECs treated with neutrophil condition media (NCM) for 5 h followed by incubation with freshly isolated neutrophils for 1 h (PMNs) and measured cell confluency after the treatment, $n = 4$. unstim NCM – NCM obtained by incubating neutrophils for 4 h with DMSO; stim NCM – NCM obtained by incubating neutrophils for 4 h with 2.5 μ M A23187; NEi – neutrophil elastase inhibitor BAY-678; CGi – cathepsin G inhibitor I; scale bar 100 μ m. Data information: Data are presented as mean $\pm$ SD. ns not significant, * $P \leq 0.05$, *** $P \leq 0.001$, **** $P \leq 0.0001$, assessed using two-way ANOVA with Tukey's post-hoc test (A) and one-way ANOVA with Dunnett's (B–D) and Tukey's post-hoc tests (E).

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

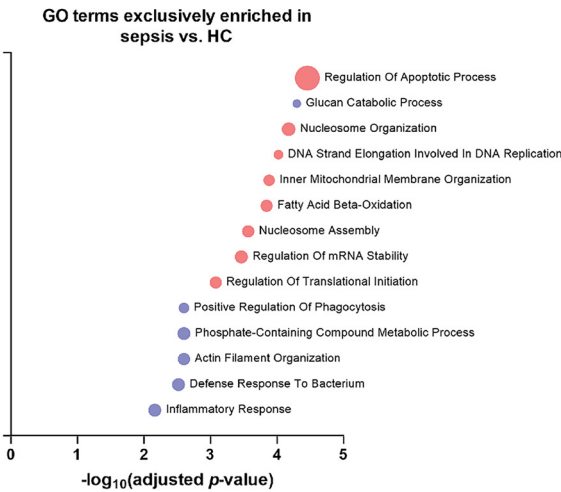
A

	age	gender	sampling day at ICU				Gram	survival?
			1/T1	4/T2	10/T3	25/T4		
patient 1	68	F					-	Y
patient 2	78	M					+	N
patient 3	62	M					-	Y
patient 4	49	M					+	Y

B

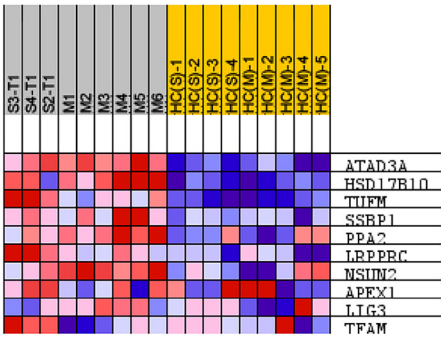


C



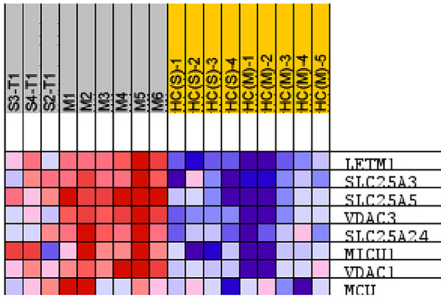
D

mitochondrial central dogma



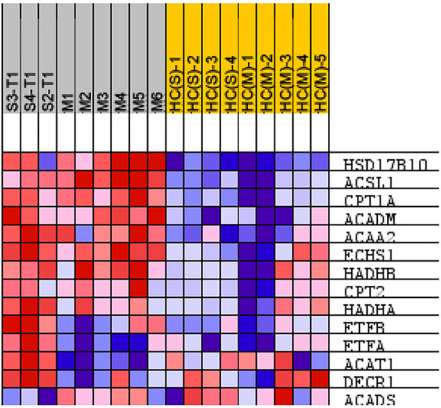
F

calcium homeostasis

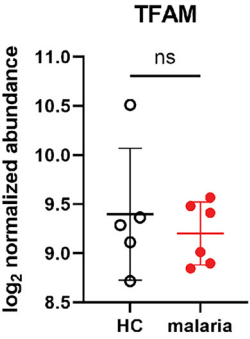


E

fatty acid oxidation



G



H

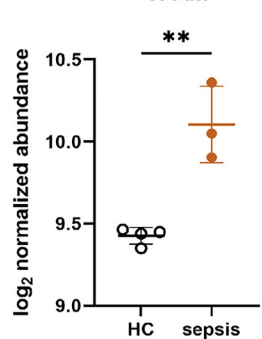
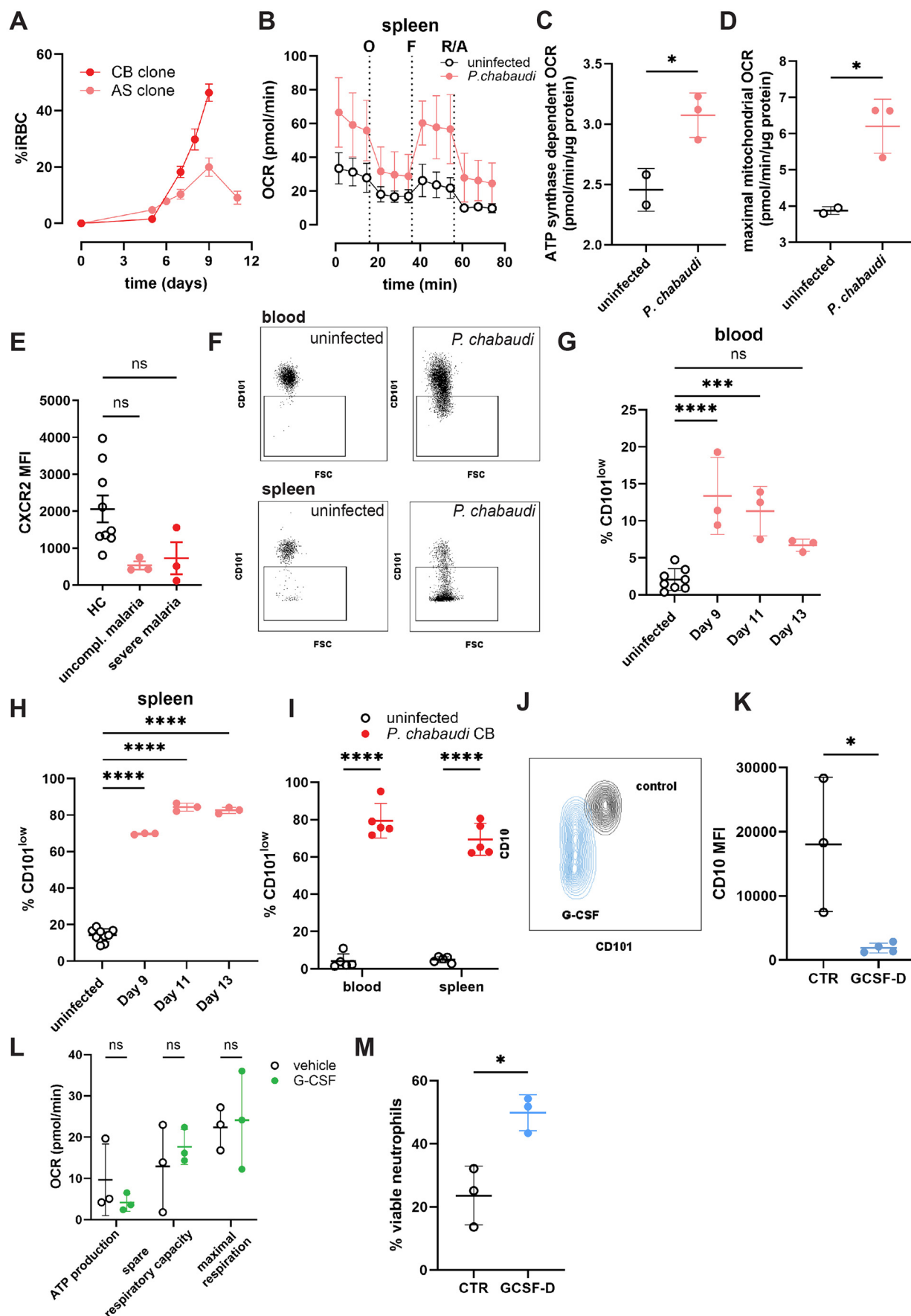


Figure EV2. Additional bioinformatics analysis.

(A) Detailed schematic of blood collection from sepsis patients. (B, C) Gene Set Enrichment Analysis (GSEA) results showing GO terms exclusively enriched in malaria vs. HC (B) and sepsis vs. HC (C) datasets. (D–F) GSEA results showing enriched MitoCarta terms. (G, H) Individual normalized abundances of TFAM peptide in malaria vs. HC (G) and sepsis vs. HC (H) neutrophils. Samples from malaria patients were collected at admission; these patients are designated M1–M6. Samples from sepsis patients were collected on days 1, 4, 10 and 25 following admission to the intensive care unit and are designated T1–T4 for each patient (patients S1–S4). Healthy controls on (D–F) are indicated HC(S) for sepsis dataset and HC(M) for malaria dataset. Data information: Data are presented as mean $\pm$ SD. ns not significant, $^{**}P \leq 0.01$, assessed using hypergeometric test with P values adjusted using the Benjamini-Hochberg method (B, C) or unpaired t test (G, H).



◀ **Figure EV3. Additional analyses of mitochondrial function in malaria-elicited and GCSF-D neutrophils.**

(A) Percentage of infected red blood cells with *P. chabaudi* clone AS ($n = 3$) and CB ($n = 5$) in the circulation of mice. (B) OCR of spleen neutrophils of *P. chabaudi* clone AS-infected mice ($n = 3$). Seahorse port injections are indicated as: O – oligomycin (1.5 μ M), F – FCCP (400 nM), R/A – rotenone (1 μ M) and antimycin A (1 μ M). (C) ATP synthase dependent OCR of splenic neutrophils isolated from *P. chabaudi* clone AS-infected mice on day 9 post-infection ($n = 3$). (D) Maximal mitochondrial OCR of splenic neutrophils isolated from *P. chabaudi* clone AS-infected mice on day 9 post-infection ($n = 3$). (E) Median fluorescence intensity of CXCR2 on peripheral blood neutrophils from HC, and patients with uncomplicated and severe malaria, $n = 9$ (HC), 3 (uncomplicated malaria), 3 (severe malaria). (F) Representative flow cytometry plots showing CD101 expression on peripheral blood and splenic neutrophil from mice at day 9 post-infection with *P. chabaudi* clone AS. (G) Percentage of CD101^{low} peripheral blood neutrophils from *P. chabaudi* clone AS-infected mice at days 9, 11 and 13 post-infection. $n = 8$ (uninfected mice), 3 (infected mice). (H) Percentage of CD101^{low} splenic neutrophils from *P. chabaudi* clone AS-infected mice at days 9, 11 and 13 post-infection. $n = 8$ (uninfected mice), 3 (infected mice). (I). Percentage of CD101^{low} neutrophils in peripheral blood and spleen from mice infected with *P. chabaudi* clone CB at day 9 post-infection, $n = 5$. (J) Representative flow cytometry contour plot for CD10 and CD101 expression on peripheral blood neutrophils derived from control (CTR) and GCSF-treated (GCSF-D) donors. (K) Median fluorescence intensity of CD10 on peripheral blood neutrophils from CTR and GCSF-D donors, $n = 3$ (CTR), 4 (GCSF-D). (L) ATP production, spare respiratory capacity, and maximal respiration of CTR neutrophils treated ex vivo with 10 ng/mL G-CSF for 30 min, $n = 4$. (M) Percentage of viable CTR and GCSF-D neutrophils incubated overnight in complete media, $n = 3$. Data information: Data are presented as mean $\pm$ SD. ns – not significant, * $P \leq 0.05$, *** $P \leq 0.001$, **** $P \leq 0.0001$, assessed using unpaired t test (C, D, K, M), one-way ANOVA with Dunnett's post-hoc test (E, G, H), and two-way ANOVA with Šidák's post-hoc test (I, L).

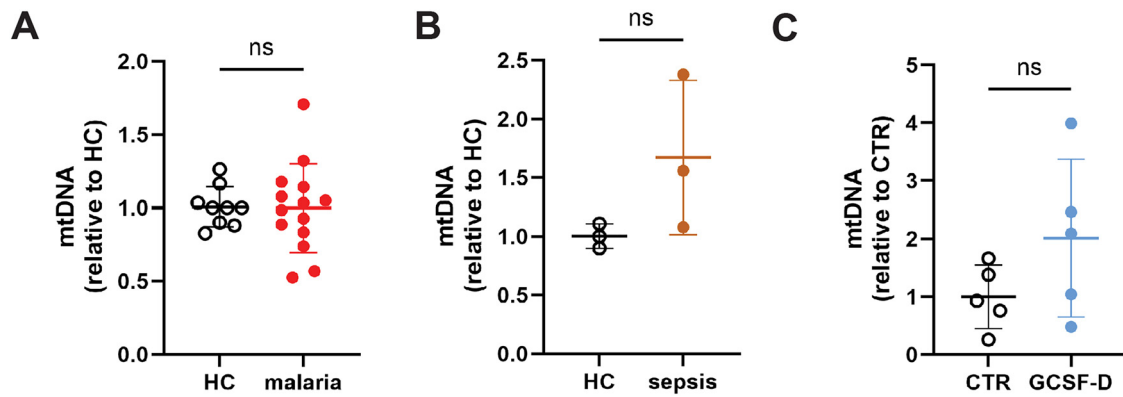
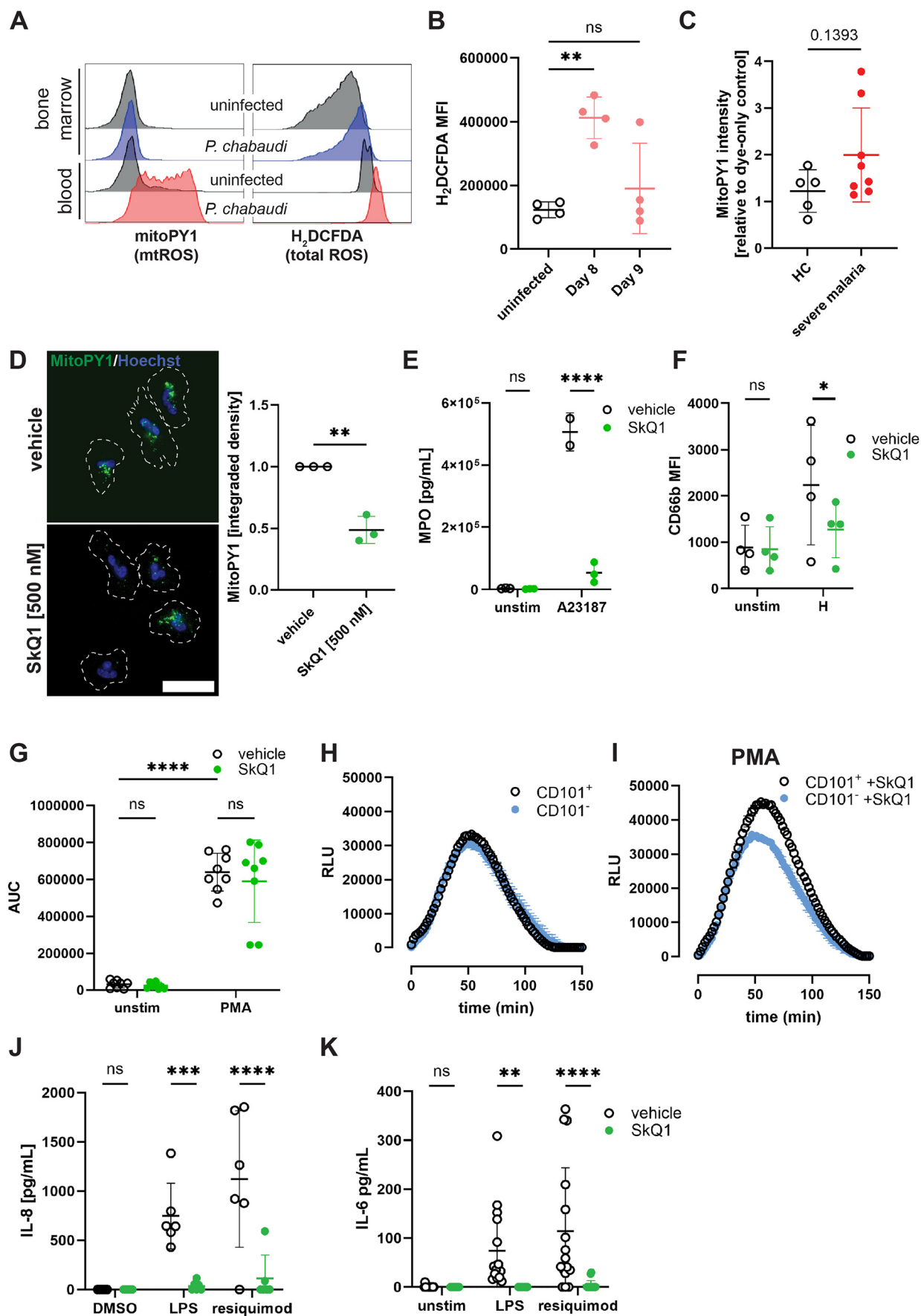


Figure EV4. Additional analyses of mitochondria in neutrophils.

(A) Relative mtDNA content in neutrophils from malaria patients ($n = 14$) compared to HC ($n = 9$). (B). Relative mtDNA content in neutrophils from sepsis patients ($n = 3$) compared to HC ($n = 3$). (C) Relative mtDNA content in neutrophils from GCSF-D donors ($n = 5$) compared to CTR neutrophils ($n = 5$). Data information: Data are presented as mean $\pm$ SD. ns - not significant, assessed using unpaired t test.



◀ **Figure EV5. mtROS regulates neutrophil degranulation, NOX2-dependent oxidative burst and cytokine release.**

(A) Representative FACS histograms showing mtROS (MitoPY1) and total ROS (H₂DCFDA) profiles of blood and bone marrow neutrophils from uninfected and *P. chabaudi* clone AS-infected mouse. (B) Median fluorescence intensity of H₂DCFDA in neutrophils from uninfected and *P. chabaudi* clone AS-infected mice at day 8 and 9 post-infection, *n* = 4. (C) Fluorescence intensity of MitoPY1 in neutrophils from HC (*n* = 5) and cerebral malaria patients (*n* = 8) relative to dye-only control. (D) Left: Representative widefield fluorescence images of CTR neutrophils stained with MitoPY1 and Hoechst, pre-incubated with vehicle (DMSO) or SkQ1 (500 nM), scale bar 10 μm. Dashed borders outline cell boundaries. Right: MitoPY1 integrated density, *n* = 3. (E). Myeloperoxidase (MPO) levels in supernatants of CTR neutrophils stimulated with calcium ionophore A23187 (2.5 μM), pre-incubated with vehicle (DMSO) and SkQ1 (500 nM), *n* = 3. (F) Median fluorescence intensity of CD66b on peripheral blood neutrophils from CTR donors stimulated with heme (30 μM), *n* = 4. (G). Quantification of oxidative burst (as area under curve, AUC) of CTR neutrophils stimulated with PMA (100 nM), pre-incubated with vehicle (DMSO) and SkQ1 (500 nM) *n* = 8. (H) Representative luminol-based measurement of ROS production in FACS-sorted mouse CD101[−] and CD101⁺ neutrophils stimulated with PMA (100 nM). (I) Representative luminol-based measurement of ROS production in FACS-sorted mouse CD101[−] and CD101⁺ neutrophils stimulated with PMA (100 nM) and pre-stimulated with SkQ1 (500 nM). (J) Interleukin 8 (IL-8) levels in supernatants of CTR neutrophils stimulated overnight with LPS (100 ng/mL; *n* = 6) and resiquimod (5 μM; *n* = 5), (K) Interleukin 6 (IL-6) levels in supernatants of CTR neutrophils stimulated overnight with LPS (100 ng/mL) and resiquimod (5 μM), *n* = 13 (LPS), 15 (resiquimod). Data information: Data are presented as mean ± SD. ns not significant, **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001, *****P* ≤ 0.0001, assessed using one-way ANOVA with Dunnett's post-hoc test (B), unpaired *t* test (C, D) and two-way ANOVA with Tukey's (E–G, J, K).