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Supplementary Materials for

Neutrophil extracellular traps drive inflammatory pathogenesis in malaria

Sebastian Lorenz Knackstedt, Athina Georgiadou, Falko Apel, Ulrike Abu-Abed, Christopher A. Moxon, Aubrey J. Cunnington, Bärbel Raupach, Deirdre Cunningham, Jean Langhorne, Renate Krüger, Valentina Barrera, Simon P. Harding, Aase Berg, Sam Patel, Kari Otterdal, Benjamin Mordmüller, Evelin Schwarzer, Volker Brinkmann, Arturo Zychlinsky, Borko Amulic*

*Corresponding author. Email: borko.amulic@bristol.ac.uk

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Other Supplementary Material for this manuscript includes the following:

(available at immunology.sciencemag.org/cgi/content/full/4/40/eaaw0336/DC1)

Data file S1. Raw data in Excel spreadsheet.

Figure S1

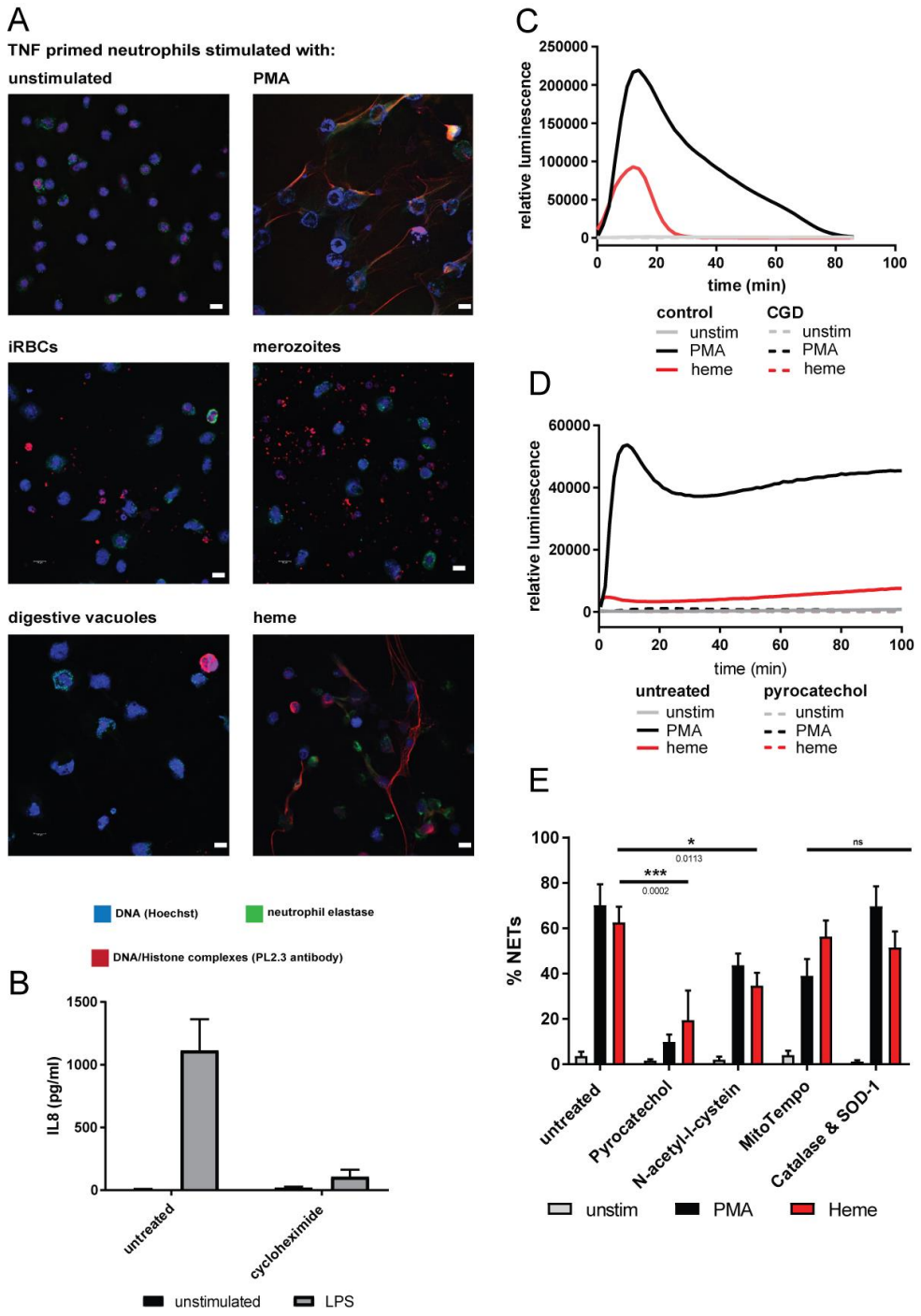

Fig. S1. In vitro stimulations of human neutrophils. (A) Confocal microscopy images of TNF primed neutrophils stimulated with malaria associated PAMPs and DAMPs; PMA serves as a positive control. Scale bars represent 20 µm. **(B)** IL-8 ELISA of supernatants from neutrophils treated with LPS. IL-8 is not stored in the neutrophil and its release requires translation. **(C)** Production of ROS measured by luminol assay **(D)** Production of ROS by neutrophils treated with pyrocatechol. Cells are unable to produce ROS in response to both PMA or heme. **(E)** Quantification of NET formation induced by PMA or heme in healthy human neutrophils pretreated with the indicated ROS scavenging agents at the following concentrations: 30 µM pyrochatecol, 10 mM N-acetyl-l-cysteine. 10 µM MitoTempo, 10 ng/µl catalase & 10 ng/µl SOD-1. Data is presented as the mean ± SEM.

Figure S2:

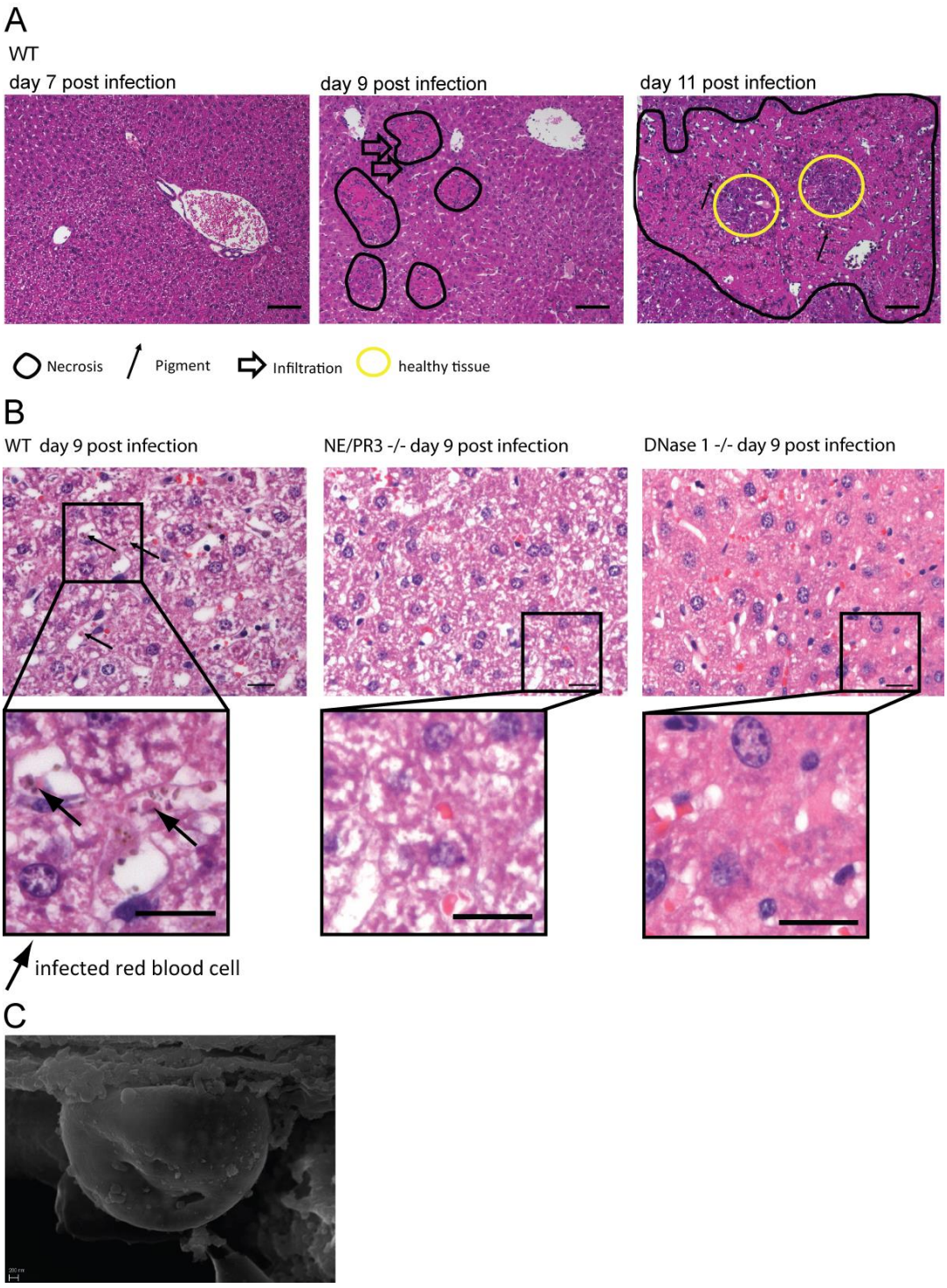

Fig. S2. Necrosis and sequestration in the livers of *P. chabaudi*–infected mice. (A) Sample images of H&E stained liver sections used to quantify liver damage. Black outlines indicate areas containing necrotic liver cells, large arrows indicate immune cell infiltration and yellow outlines indicated healthy tissue surrounded by necrosis. Analysis was performed blinded by a pathologist, scale bar equals 100 µm. **(B)** Representative H&E stained images of livers from infected animals, arrows indicate iRBCs bound to the endothelium, scale bar equals 20 µm. **(C)** Electron micrograph of an infected red blood cell attached to the vasculature in the liver of a WT mouse.

Figure S3

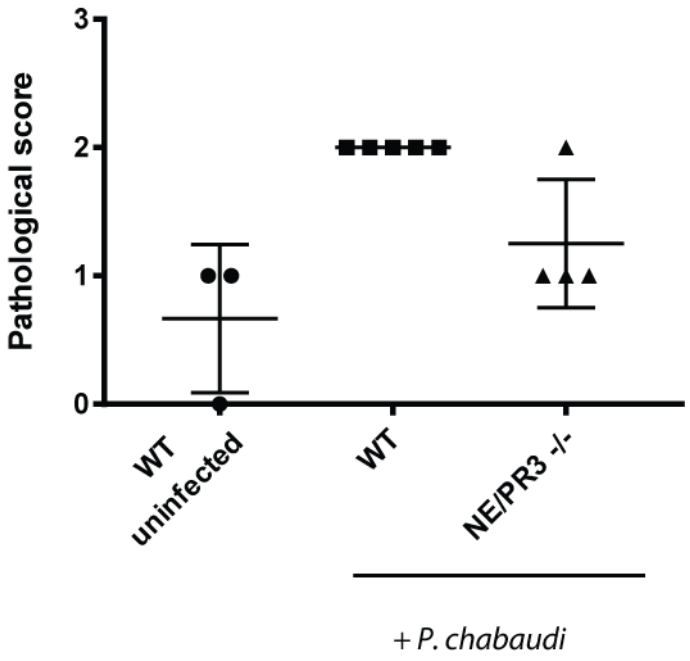

Fig. S3. Pathology in the lungs of *P. chabaudi*–infected animals. Blinded scoring of lungs from infected animals at peak parasitemia, according to score sheet presented in Methods.

Figure S4:

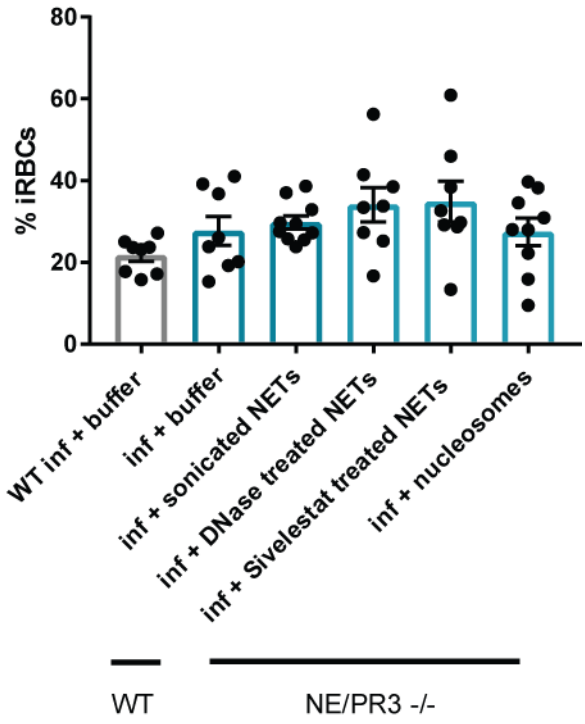

Fig. S4. Parasitemia of NET fragment–injected mice. Parasitemia of infected animals quantified by counting Giemsa-stained thin blood smears.

Figure S5:

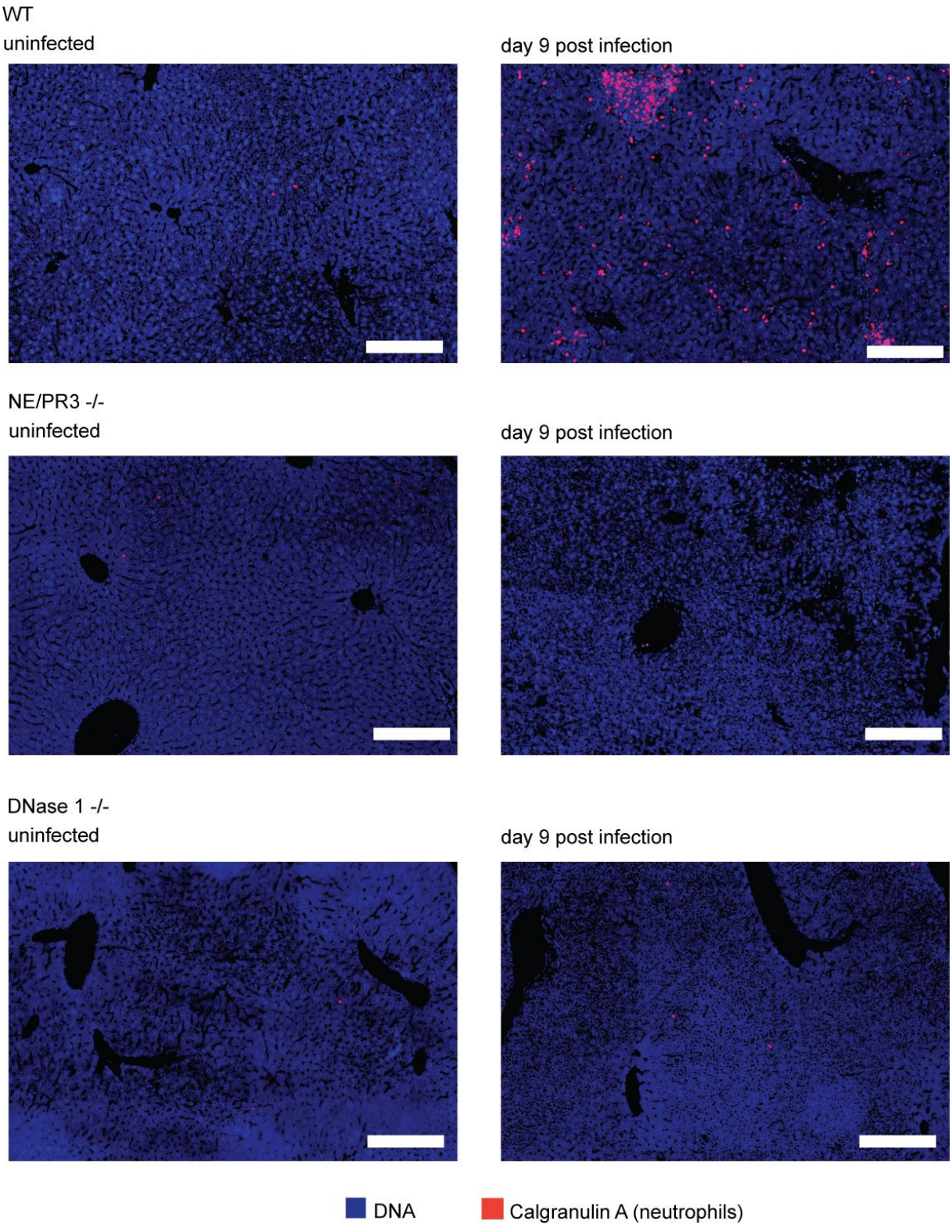

Fig. S5. Immunofluorescence images used to quantify neutrophils in livers of infected mice. Sample images of livers from infected animals of indicated genotypes stained with Hoechst (blue) and a neutrophil specific anti-calgranulin A antibody (red).

Figure S6

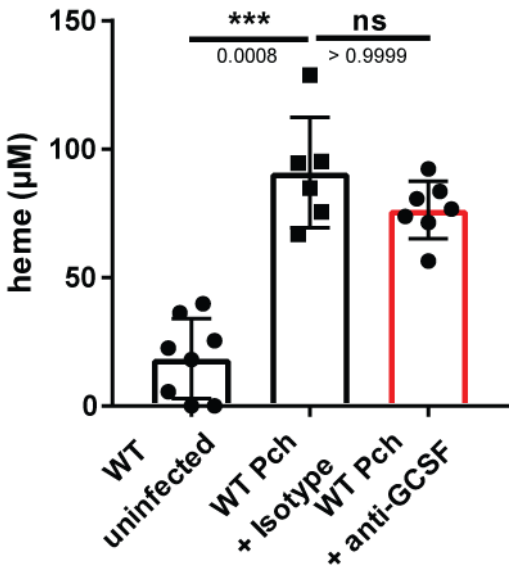

Fig. S6. Quantification of free circulating heme in plasma of uninfected and infected animals, treated with a G-CSF neutralizing antibody or isotype control antibody.

Table S1. Patient information of cohort 1 and 2 in Gabon.

	Cohort 1	Cohort 2	
	Uncomplicated	Uncomplicated	Severe
n	43	10	23
	Mean (min - max)		
Age (years)	1 (1 - 84)	4 (3 - 7)	4 (1 - 10)
Parasitemia (pf/µl)	1635 (59 - 260113)	7595 (2100 - 20000)	335743 (2600 – 1100000)
Temperature (°C)	38 (37 - 40)	n.a.	n.a.
Hematocrit (%)	33.45 (19.2 - 44.9)	n.a.	n.a.
Hemoglobin (g/dl)	10.1 (4.2 - 17.7)	10.1 (9.1 - 12.2)	8.76 (5 - 13.2)

Table S2. Clinical characteristics of the patient population in Mozambique.

	Uncomplicated malaria		Severe malaria	
n	28		27	
	Mean (min-max)	% proportion	Mean (min – max)	% proportion
Age	36 (18-73)		42 (20-65)	
Sex (females)		35.7 (10/28)		44.4 (12/27)
Hb	11.7 (5.9-15.7)		10.8 (3.2-17.0)	
WBC	6.1 (2.2-12.4)		7.5 (1.3-15.5)	
Platelets	119 (24-324)		127 (11-452)	
Creatinine	108 (57-203)		152 (72-357)	
Se-glucosis	9.0 (4.2-34.2)		9.0 (3.7-40.5)	
Systolic BP	123 (90-240)		119 (70-160)	
Respiratory rate	20 (14-28)		25 (16-68)	
Liver failure[a)]		0		11.1 (3/27)
Coagulat. disturb.[b)]		0		3.7 (1/27)
Cerebral disturb.[c)]		14.3 (4/28)		29.6 (8/27)
Case fatality rate		0[d)]		3.7 (1/27)

[a)] Defined as jaundice and/or bilirubin >43 µmol/L

[b)] Defined as bleeding disturbaces and/or hemolysis

[c)] Defined as Glascow Coma Scale ≤11, repeated convulsions and/or confusion

[d)] Two patients missing

Continuous variables given in mean (min-max), not rounded numbers.

Table S3. Clinical diagnosis and classification of subjects whose ocular tissue was used for immunofluorescence experiments.

Sample	Before post-mortem clinical diagnosis	Post-mortem classification	Retinopathy
1	Clinical CM	1	Yes
2	Clinical CM	1	Yes
3	Clinical CM	1	Yes
4	Clinical CM	1	Yes
5	Clinical CM	1	Yes
6	Clinical CM + SMA	2	Yes
7	Clinical CM	2	Yes
8	Clinical CM, severe pneumonia, severe meningoencephalitis	2	Yes
9	Clinical CM	2	Yes
10	Clinical CM – pneumonia, Reye's syndrome	3	No
11	Clinical CM, likely cause of death is anemia	3	No
12	Clinical CM + SMA, hepatitis	3	No
13	Clinical CM; Severe pneumonia	3	No
14	Clinical CM, severe pneumonia with spread to meninges	3	No
15	Clinical CM, left ventricular failure with pulmonary edema	3	No
16	Clinical CM, fatal pneumonia	3	No
17	Salmonella sepsis	7	No

Class 1 & 2 (CM1 & 2) are the “true CM” while Class 3 (CM3) are the “Faux CM”. CM3 mimics CM during life so these children are control for being really sick and for premorbid events but in fact there is no sequestration and there is an alternative cause of death – suggesting a different pathogenic process - so they represent an excellent comparator for sequestration driven pathology. Class 7= Non-malarial encephalopathy, infectious. SMA, severe malarial anemia.