

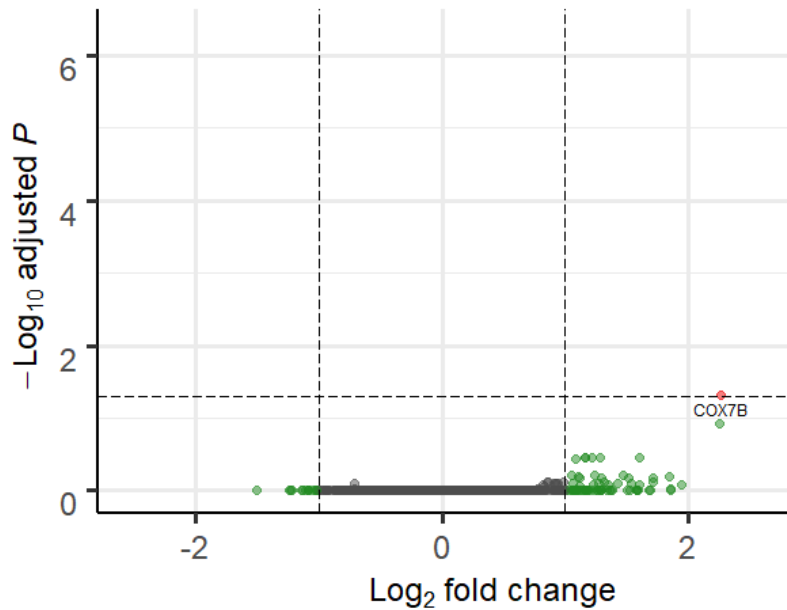
Supplementary Table S1: Participant Characteristics.

Variables	Uninfected control (n=7)	Asymptomatic (n=25)	Symptomatic (n=12)	P-value
Age in years,	11 (10-11)	9 (8-10)	10 (7-12)	0.30 ^a
No. (%) of male subjects	3 (43)	16 (64)	6 (50)	0.38 ^b
Haemoglobin (g/dl)	11.9 (10.6-12.8)	10.6 (9.8-12.0)	11.5(10.3-13.6)	0.07 ^a
WBC (x10 ⁹ /l)	8.9 (6.2-9.1)	6.2 (5.1-8.3)	7.0 (5.1-9.7)	0.33 ^a
Neutrophils (x10 ⁹ /l)	2.2 (1.4-4.1)	1.8 (1.5-2.5)	4.5 (2.8-7.4)	<0.0001 ^a
Neutrophils (%)	29.3 (21.7-45.5)	28.1 (20.9-33.0)	72.9 (69.1-78.8)	<0.0001 ^a
Lymphocytes (x10 ⁹ /l)	4.2 (4.1-5.2)	3.3 (2.5-4.8)	1.1 (0.7-2.0)	<0.0001 ^a
Lymphocytes (%)	58.0(46.5-67.6)	55.9 (48.6-65.6)	17.5 (9.8-27.8)	<0.0001 ^a
Monocytes (x10 ⁹ /l)	0.6 (0.5-0.8)	0.6 (0.4-0.7)	0.5 (0.20-0.60)	0.41 ^a
Monocytes (%)	8.1 (6.9-9.2)	8.7 (6.8-11.6)	6.1 (4.0-11.0)	0.26 ^a
Platelets (x10 ⁹ /l)	229 (159-262)	232 (201-305)	125 (59-245)	0.06 ^a
Parasite density/uL	Not detected	320 (160-640) ¹¹	9402 (5679-68889)	<0.0001 ^c
PfHRP2 (ng/mL)	Not detected	8.7 (3.9- 17.8) ²⁴	213.5 (6.6-391.1)	0.01

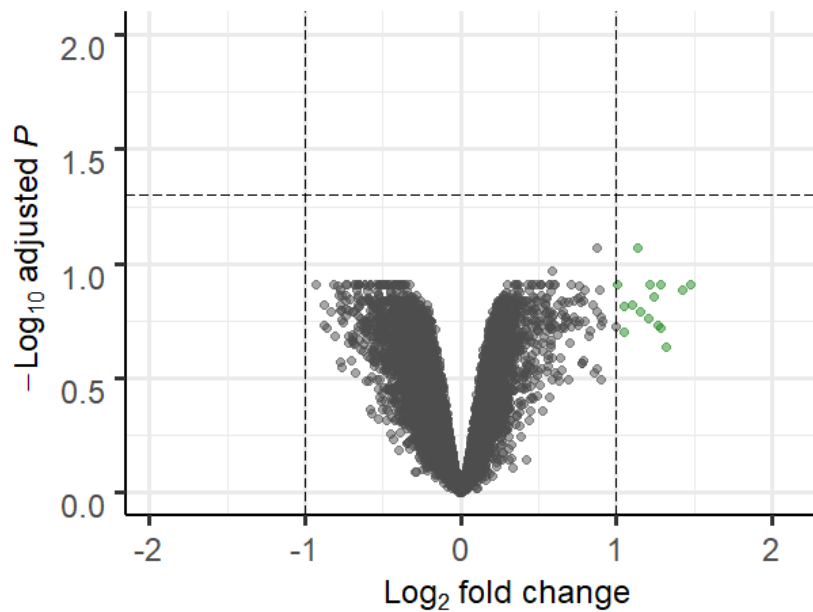
Data are median [interquartile range (IQR)], ^a Kruskal–Wallis test, ^b Chi-square test, ^c Mann–Whitney U test. Superscript superscripts indicate the number of subjects with data for each variable if less than the total. Abbreviations: PfHRP2, *P. falciparum* histidine-rich protein 2; WBC, white blood cell count.

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a) Adjusted for cell type proportions



b) Not adjusted for cell type proportions

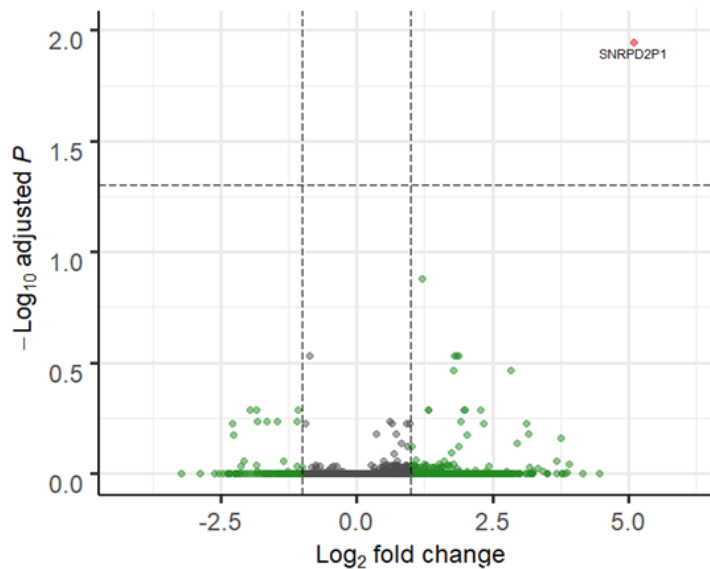


Supplementary Figure 1 Volcano plots showing the results of the differential expression analysis comparing asymptomatic to healthy uninfected control samples in the microarray cohort described in Boldt et al., 2019 (asymptomatic, n=5 pooled samples, healthy controls, n=5 pooled samples). a) and b) Genes with a $\log_2(\text{fold change})$ greater than 0 have higher expression in the asymptomatic group. Genes with a $\log_2(\text{fold change})$ less than 0 have lower expression in the asymptomatic group. The vertical dotted line on each plot represents a threshold of a Benjamini-Hochberg corrected p-value of 0.05 ($-\log_{10}$ value of 1.30). Genes on or above this line are statistically significantly differentially expressed (red). The horizontal lines represent $\log_2(\text{fold change})$ thresholds of -1 and 1 respectively. a) Immune cell types Neutrophils, CD4+ T cells, CD8+ T cells, and, CD14+ Monocytes were included as covariates in the design matrix. b) The immune cell type proportions were not included as covariates.

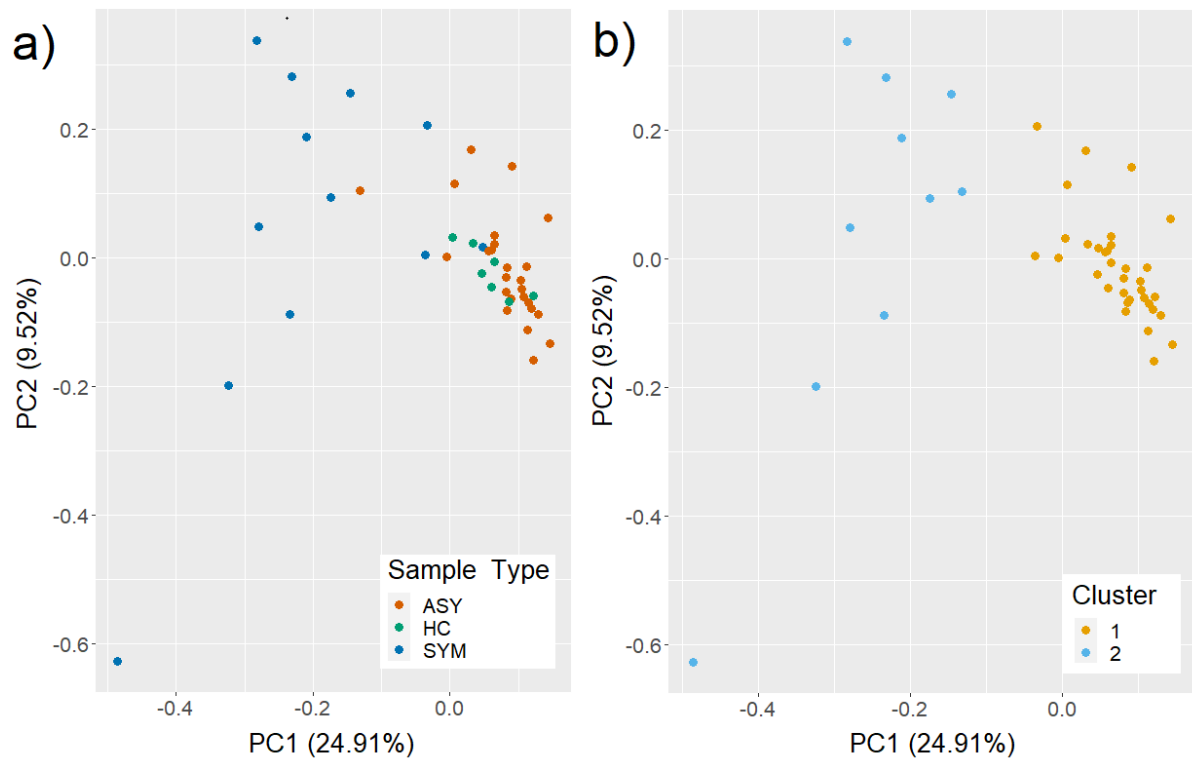
Asymptomatic vs. Healthy Control

Key

- Not statistically significant
- Log_2 Fold Change > 1
- Log_2 Fold Change > 1 & False discovery rate corrected p-value < 0.05

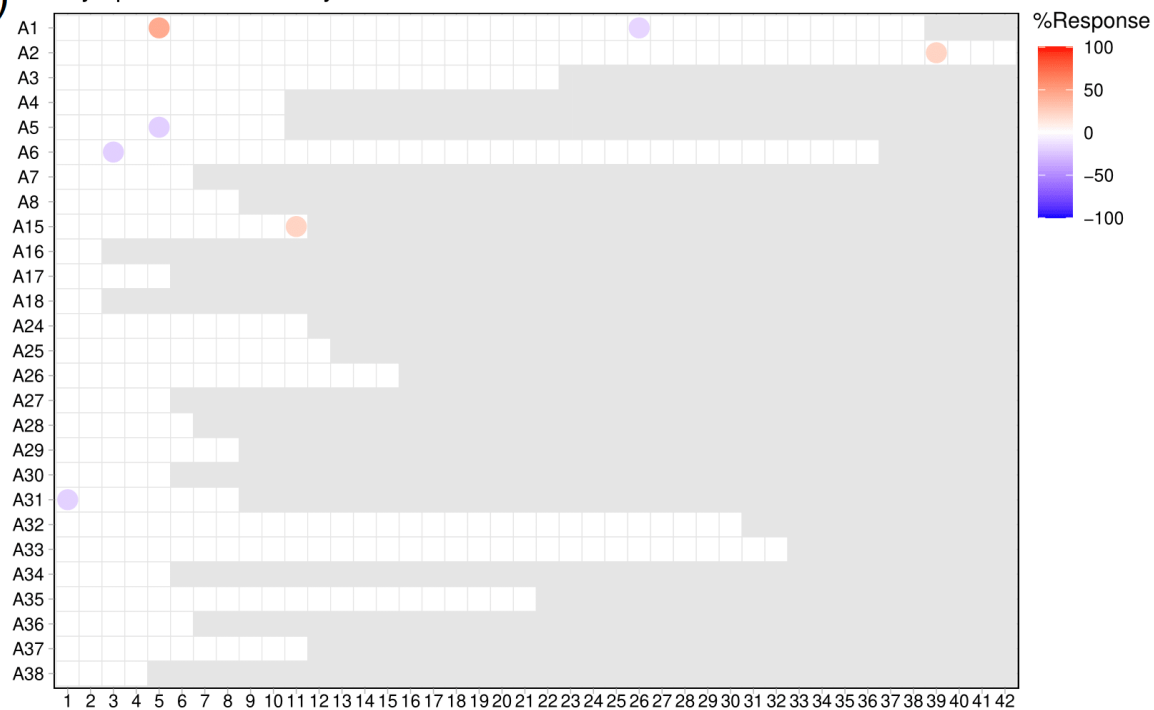


Supplementary Figure 2 Volcano plot showing the results of the differential expression analysis comparing asymptomatic to healthy uninfected control samples using all 44 samples (asymptomatic, n=25, symptomatic, n=12, healthy controls, n=7) of the RNA-Seq paediatric cohort. The only information contained in the design matrix of this analysis was the phenotype of the sample. Genes with a $\log_2(\text{fold change})$ greater than 0 have higher expression in the asymptomatic group. Genes with a $\log_2(\text{fold change})$ less than 0 have lower expression in the asymptomatic group. The vertical dotted line on each plot represents a threshold of a Benjamini-Hochberg corrected p-value of 0.05 ($-\log_{10}$ value of 1.30). Genes on or above this line are statistically significantly differentially expressed. The horizontal lines represent $\log_2(\text{fold change})$ thresholds of -1 and 1 respectively.

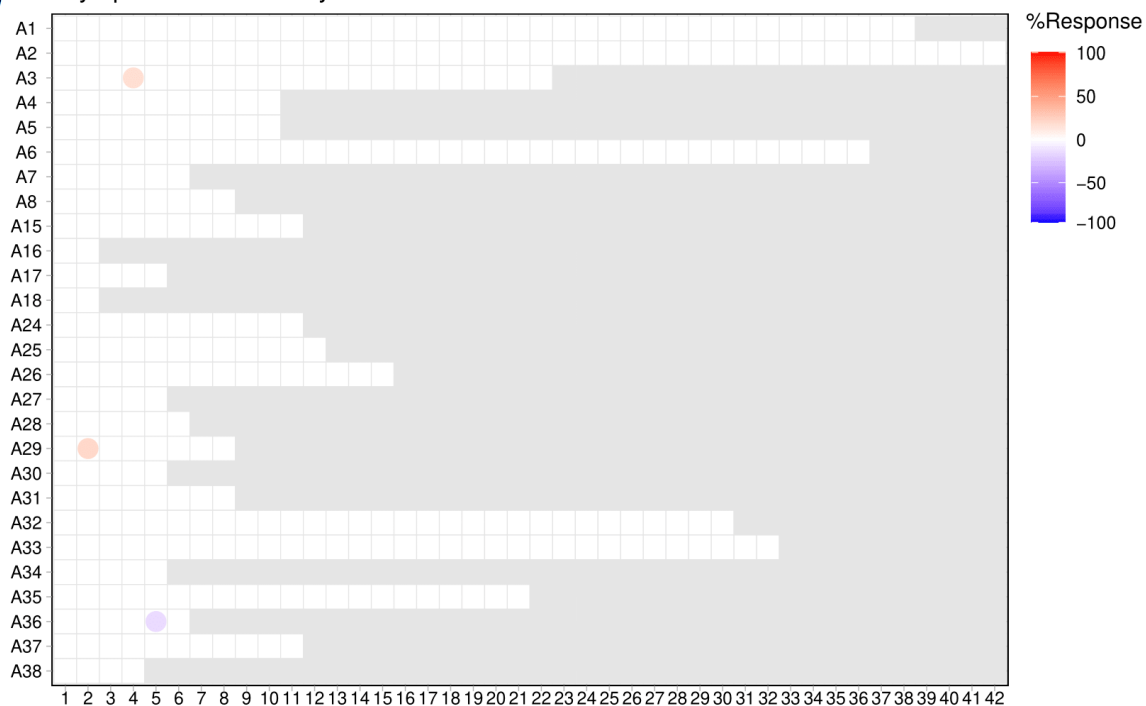


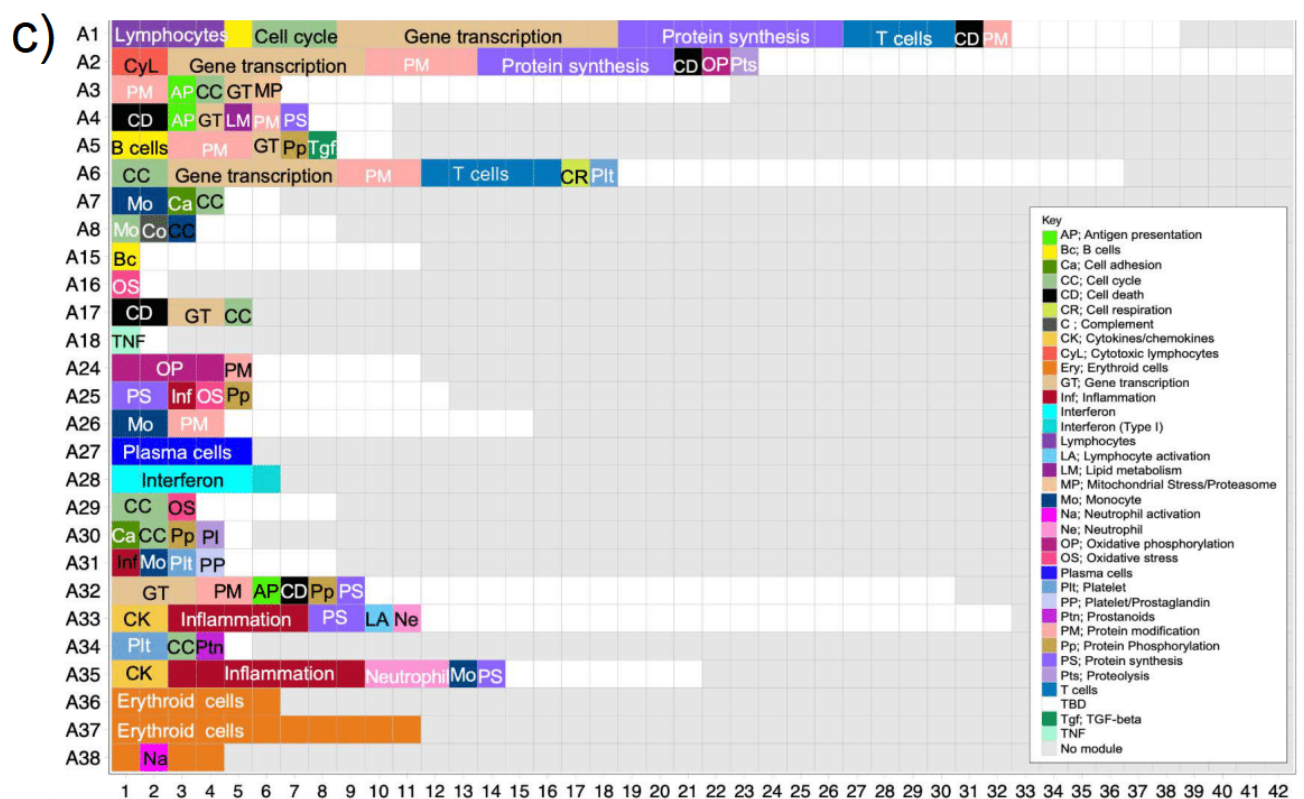
Supplementary Figure 3 A Principal Component Analysis (PCA) plot illustrating the relationship between the samples (asymptomatic, n=25, symptomatic, n=12, healthy controls, n=7) constructed using the library size normalised gene expression values that were also filtered so that only genes that had greater than or equal to 5 normalised count in 3 or more samples were retained (20,605 genes). The percentage values in the x and y axis labels represent the percentage of variation explained by the first Principal Component and second Principal Component respectively. This PCA plot has been scaled. a) samples (points are coloured by sample type. Key: Blue=symptomatic, vermilion=asymptomatic, green=healthy uninfected controls b) Samples (points) are coloured by the class to which the k-means clustering assigned them. Orange: cluster 1, blue: cluster 2. Abbreviations: ASY: Asymptomatic, SYM: Symptomatic, HC: Healthy Control, PC: Principal Component

a) asymptomatic vs healthy control



b) asymptomatic vs healthy control





Supplementary Figure 4 Module fingerprint grid plots. The differential expression of the modules is compared between the asymptomatic and control groups using t-test (a) and limma (b) methods with fold change and p-value cut-off of 0.5 and 0.05, respectively. Each block corresponds to a module position. Each row represents a 'module aggregate' including modules with the same pattern of differential expression across reference datasets. Red and blue spots represent modules with increased and decreased abundance in the asymptomatic patients vs. the healthy controls, respectively. The gradient represents 'module response' which is the percentage of genes for a given module showing significant change in abundance compared to the healthy controls. Only modules with at least 15% response have been shown. The module key (c) illustrates the biological functions attributable to the modules on the grid.