SUPPLEMENTAL DATA

Supplemental Methods. Additional information on the methodology used in this manuscript.

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SUPPLEMENTAL METHODS

Rhesus macaque longitudinal infection

The longitudinal experiment included baseline collection of venous blood and bone marrow aspirates 5 days before sporozoite inoculation and daily follow-up for 100 days after the inoculation (about 100 freshly isolated salivary gland sporozoites were inoculated in the saphenous vein at day 0). Capillary blood samples were collected every day during the infection (approximately 100 μL), into EDTA-coated capillary tubes, using ear-prick procedures. Capillary blood samples were used for complete blood counts (CBCs) analysis using a Beckman Coulter AcTDiff hematology analyzer, to quantify parasitemias and stage differentials using Giemsa-stained thin and thick smears, to quantify reticulocytes by manual counting using new methylene blue staining of blood smears, and for plasma collection for cryopreservation and subsequent metabolomics. Whole blood samples (10 mL) obtained from the femoral vein and bone marrow aspirates derived from iliac crest puncture while the animals were under anesthesia were

collected into EDTA-coated vacutainer tubes at seven TPs. For parasite transcriptomics, venous blood was collected in Blood Tempus RNA tubes containing 6 mL of Stabilizing Reagent, shaken vigorously for 10 seconds and stored at -80°C until processing for RNA-Seq. For metabolomics, a 300 μL aliquot of venous blood was centrifuged, and plasma was collected and stored at -80°C until processing for HRM.

To avoid possible clinical complications, subcurative treatment with artemether, a fast-acting antimalarial drug with a short half-life, was administered intramuscularly (IM) at 1 mg/kg. This treatment was repeated when clinically warranted, and fluid support and blood transfusion were also treatment options if deemed necessary by veterinarians. Before unassignment, the macaques received a curative antimalarial regimen of 4 mg/kg of artemether IM followed by 2 mg/kg/day IM for seven days.

For bone marrow cytology, aspirate films were prepared immediately, fixed in methanol and stained with Wright-Giemsa. Total counts were determined using a Beckman Coulter AcTDiff hematology analyzer. Differential cell counts were performed by a board-certified veterinary pathologist using a blinded procedure and manual counting of 500 progenitor cells. To determine the absolute counts of erythroid and myeloid progenitor cells, they were summed for each TP and multiplied by the total nucleated cell count. This provided a cells/μL measure for both lineages. To summarize these data by phase, mean values were calculated per animal per phase.

Human sample collection

Venous blood samples were collected in heparin tubes by MVRU staff between August 2011 and August 2014. Giemsa-stained thick and thin blood smears were prepared for

parasitemia determination and species identification. PCR was additionally performed at the MVRU in Bangkok to confirm the parasite species.

Venous blood samples were also collected from the NMFI and healthy controls. Three mL was collected for a CBC test, parasite checks by microscopy, and PCR. One mL was collected in a heparin tube for plasma separation. All blood samples were kept on ice and transferred less than 4 hours later to the Tropical Disease Research Center in Kanchanaburi, or the MVRU in Bangkok. Plasma was separated by centrifugation, aliquoted and frozen at -80°C. A frozen aliquot of 200 μL from each participant was shipped on dry ice to Emory University and remained frozen until used for HRM analysis. Samples were refrozen and thawed later for Biocrates targeted validation analyses.

Annotation of untargeted metabolomics data

Initial evaluation of metabolite features has been previously discussed (1) concerning metabolite identification, for example, multiple ions can be formed from a single metabolite, and one *m/z* feature may include ions from different chemicals. For simplicity, *m/z* features are discussed as metabolites; identities of metabolites have been established for this platform by tandem mass spectrometry (MS/MS), co-elution with authentic standards and cross-platform validation(1). Accurate mass matches (10 ppm) of other ions to metabolites were obtained using the xMSannotator R package available on Source Forge (https://sourceforge.net/projects/xmsannotator/) (2, 3). xMSannotator, which uses a multi-step procedure based on clustering based on intensities across samples, retention time, adducts/isotopes, mass defect, and correlation between adducts/isotopes was used to assign

confidence levels (0: low/no confidence; 2: medium confidence; 3: high confidence) to database matches in HMDB. Metabolite identification levels are assigned according to the criteria established by the metabolomics standards initiative(4). Level 1 includes metabolites confirmed by matching accurate mass, retention time, and MS/MS relative to authentic standards. Level 2a includes metabolites confirmed by MS/MS relative to online spectral libraries. Level 2b includes computationally derived putative annotations using xMSannotator with annotation score 2 or 3, corresponding to medium to high confidence, respectively."

Targeted metabolite quantification

Targeted metabolite quantification was performed according to manufacturer instructions (Biocrates Life Sciences AG, Innsbruck, Austria). Samples were randomized into a 96-well plate for extraction. The methanolic extracts in the 96-well capture plate were split for data acquisition using two different approaches- LC-MS/MS in positive mode and flow injection analysis (FIA) in positive and negative mode. The LC-MS/MS approach uses column for metabolites separation in contrast to the FIA approach that does not utilize column. The data were acquired injecting 10 µl of extract into SCIEX LC AC chromatography system coupled to SCIEX QTRAP 5500 (AB SCIEX LLC, Framingham, MA, USA). The data acquired in multiple reaction monitor (MRM) mode were further processed and quantified using Analyst 1.6.3 and validated using MetIDQ software (Biocrates Life Sciences AG, Innsbruck, Austria).

Quality Control for Parasite Transcriptional Analysis

A set of quality control evaluations were conducted to ensure that the results of our parasite transcriptional analysis are reliable and meaningful. The results of these assessments and the steps taken to appropriately analyze this data are described below.

1. Negligible contribution of sequencing error to this dataset. We aligned reads from TP1 (pre-inoculation, no *Plasmodium* in blood) with the *P. coatneyi* genome in order to test for non-specific alignment errors that would have created non-specific reads in our data set. The vast majority of the 5,578 annotated *P. coatneyi* genes had either 0 or 1 reads assigned in TP1. Genes found to have greater than 10 reads in any animal at TP1 were removed from analysis.
2. Expression levels for housekeeping genes were used to set the lower limit of detection for further analysis of parasite transcriptomes. As RNAseq and differential gene expression analysis has previously been shown to work reliably on populations of as little as 5,000 parasites with 100,000 reads (Ngara et al 2018), we decided to perform analysis on our dataset to determine whether we could reliably detect housekeeping markers in our samples that fell in the range of 100,000 parasite reads and greater. We identified putative housekeeping genes by identifying *P. coatneyi* orthologs of genes previously published shown to be constitutively expressed in *P. falciparum*. We then analyzed expression levels of such genes and found that in the samples with at least 100,000 parasite reads, the constitutively expressed genes could be reliably identified in all samples.
3. A library size normalization was applied to account for differences in parasite read count across the acute, post-treatment and chronic phases. After the samples were selected

(9 in total: 3 animals with one sample per phase, all of which met the quality control criteria), a library normalization was applied to all samples together using DESeq2.

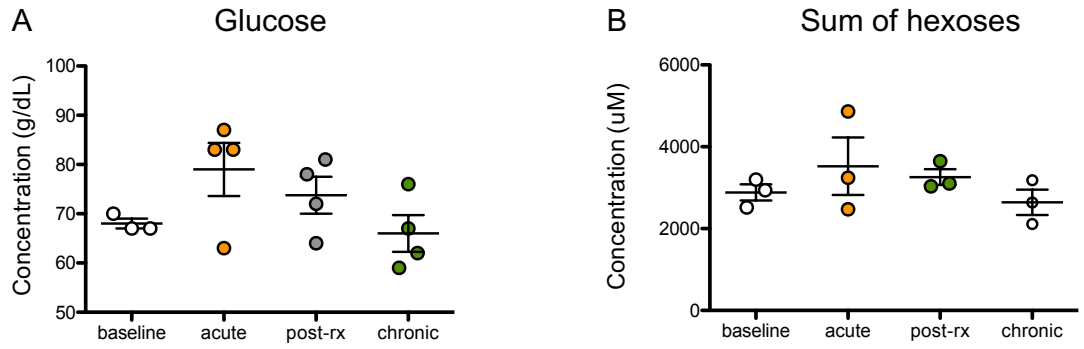
4. A base mean cutoff was used to eliminate those genes for which expression was very low. Following normalization, genes were only analyzed if they had a base mean normalized read count of greater than 4.

SUPPLEMENTAL TABLES

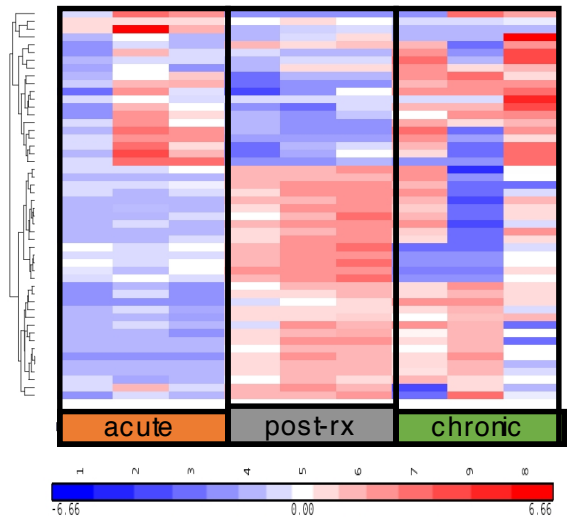
Table S1. Supplemental hematology data. Mean values per animal per phase for various hematological factors. Full dataset and metadata descriptions are available on the E03 clinical malaria data table on PlasmoDB.org (http://plasmodb.org/plasmo/mahpic.jsp).

phase	animal	parasites	WBC	RBC	hgb	hct	mcv	plt	rdw	mpv	gran	lymph	reticulocytes	RPI	G/L Ratio
Prepatent	RTi13	0	12767	5919333	14	49	82	361867	15	10	30	55	71560	1.61	0.57
Prepatent	RUn13	0	10040	7248462	16	55	76	347420	17	8	30	59	86052	3.18	0.57
Prepatent	RZe13	0	10213	6568667	15	52	79	329667	15	9	21	65	73531	2.20	0.35
Prepatent	RWr13	0	13664	6417857	15	53	83	463214	14	9	38	47	58809	1.90	0.87
Acute	RTi13	64511	8971	5485714	13	43	78	254571	14	9	35	50	122060	1.97	0.78
Acute	RUn13	47531	8338	6952500	15	51	73	266038	16	8	37	50	86546	2.29	0.83
Acute	RZe13	32510	7329	6165714	14	47	30	203857	14	8	27	56	63769	1.27	0.53
Acute	RWr13	35790	9910	5359000	13	42	79	344000	13	8	43	42	115465	1.91	1.31
Post-SubRx	RTi13	16960	16240	4153333	10	35	85	378209	20	9	31	59	319480	4.13	0.61
Post-SubRx	RUn13	7577	10070	5254048	11	39	74	331152	18	8	30	60	323070	3.99	0.54
Post-SubRx	RZe13	7280	10092	4597632	10	36	79	305789	18	8	27	56	285023	3.42	0.54
Post-SubRx	RWr13	2216	10480	3990667	10	34	85	417467	20	8	39	50	471410	5.93	1.44
Chronic	RTi13	141	22978	5238889	12	45	87	373889	17	9	17	73	97858	1.98	0.24
Chronic	RUn13	165	16817	5648000	12	42	74	314971	16	8	17	74	187800	2.71	0.24
Chronic	RZe13	131	14695	5259250	12	41	79	309710	15	8	20	70	168155	2.62	0.30
Chronic	RWr13	152	13070	4573279	11	40	87	433364	16	8	38	51	271049	4.16	0.83

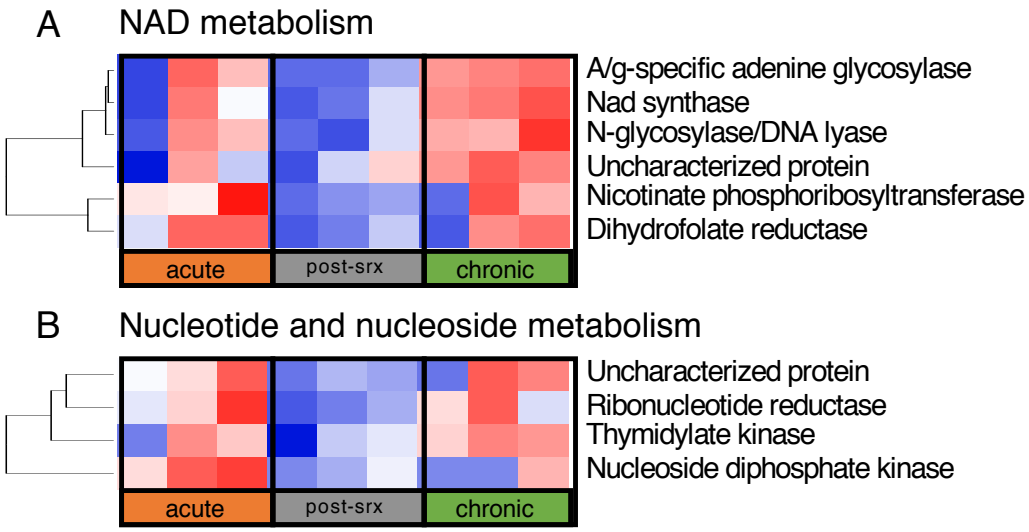
SUPPLEMENTAL FIGURES



Supplemental Figure 1. Glucose data. A. Glucose as measured by blood chemistry (i-STAT) and **B.** Sum of hexoses as measured by targeted metabolomics (Biocrates). Data reveal no significant differences across phases by ANOVA.



Supplemental Figure 2. KIR gene expression data. HCA plots of differentially expressed parasite genes of the KIR gene family with manual clustering along the x-axis (columns are grouped by clinical phase and are always in the same order of animals: RWr13, Run13, RTi13).



Supplemental Figure 3. **Differentially expressed genes with reductions in the post-SRx phase.** NAD metabolism and Nucleotide and nucleoside metabolism genes of *P. coatneyi* were downregulated in the post-SRx phase as compared to the acute and chronic phases. This indicates a reduction in the activity of those pathways, possibly coinciding with drug treatment.

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