The volatilome signatures of Plasmodium falciparum parasites during the intraerythrocytic development cycle in vitro under exposure to artemisinin drug

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Supplementary Material

Parasite Strain	Country	Mutation (KELCH13)	Lab of origin
NF54	Country information not available	none	F. Ariey
NF54 C580Y	Country information not available	C580Y	F. Ariey
3D7	Country information not available	none	F. Ariey
MRA-1169	Tanzania	VBRF010000024.1:1,727,849T>G	M. Fried
MRA-1251	Cambodia	C580Y	DA. Fidock
MRA-464	Sudan	VBRF01000025.1: 1,727,988 Insertion of ATTATT	TE Wellmans

Table S1: Summary information table of parasite strains used for this study.

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	Putative identification	CAS #	Similarity	Rings, schizonts, and trophozoites	Artemisinin treated wild-type and resistant parasites	Lab-adapted field isolates with different genotypes
1	2-Butanamine, (S)-	513-49-5	88%			
2	Pentanal	110-62-3	87%			
3	Octane, 3-methyl-	2216-33-3	89%			
4	Pentane, 2,3,3-trimethyl-	560-21-4	94%	**X**		
5	Heptane, 3-ethyl-5-methylene-	537680 (PubChem CID)	79%			
6	Heptane, 4-methyl-	589-53-7	90%	**X**	**X**	**X**
7	Hexane, 2,2,4-trimethyl-	16747-26-5	91%	**X**		**X**
8	Hexanal	66-25-1	93%	**X**	**X**	**X**
9	2,4-Dimethyl-1-heptene	19549-87-2	95%	**X**	**X**	**X**
10	Dimethyl sulfide	75-18-3	85%			
11	Ethylbenzene	100-41-4	89%	**X**	**X**	
12	o-Xylene	95-47-6	85%			**X**
13	Cyclohexanol	108-93-0	95%	**X**		

14	Styrene	100-42-5	97%	**X**	**X**	**X**
15	Hexanoic acid, 2-pentenyl ester, (Z)-	74298-89-8	84%	**X**		
16	2,5-Dimethylhexane-2,5-dihydroperoxide	3025-88-5	80%	**X**	**X**	**X**
17	2-Heptanone, 4-methyl-	6137-6-0	92%	**X**	**X**	**X**
18	1-Octanol, 2,7-dimethyl-	15250-22-3	85%	**X**		**X**
19	Benzaldehyde	100-52-7	91%	**X**	**X**	**X**
20	Benzene, 1,3,5-trimethyl	108-67-8	85%	**X**		**X**
21	2-tert-Butoxytetrahydrofuran	1927-59-9	86%			
22	Benzene, 1-ethyl-3-methyl-	620-14-4	91%	**X**	**X**	**X**
23	n-Caproic acid vinyl ester	3050-69-9	91%			
24	Benzene, 1,2,3-trimethyl-	526-73-8	83%	**X**	**X**	**X**
25	Heptane, 2,5,5-trimethyl-	1189-99-7	90%	**X**		**X**
26	3-Ethyl-3-methylheptane	17302-1-1	90%	**X**	**X**	**X**
27	1-Octene, 3,7-dimethyl-	4984-01-4	88%	**X**	**X**	**X**
28	1-Hexanol, 2-ethyl-	104-76-7	94%	**X**	**X**	**X**
29	Indane	496-11-7	91%	**X**	**X**	**X**
30	1-Octene, 2,6-dimethyl-	6874-29-9	75%			
31	Decane	124-18-5	75%	**X**	**X**	**X**
32	Benzene, 4-ethyl-1,2-dimethyl-	934-80-5	91%	**X**		**X**
33	Benzenemethanol, .alpha.,.alpha.-dimethyl-	617-94-7	83%	**X**	**X**	
34	Benzoic acid, methyl ester	93-58-3	92%			**X**
35	Nonanal	124-19-6	93%	**X**	**X**	
36	Benzene, 1,4-diethyl-	105-5-5	83%	**X**		
37	Benzene, 1,2,4,5-tetramethyl-	95-93-2	90%	**X**		
38	o-Cymene	527-84-4	95%	**X**		
39	4-Nonanone, 7-ethyl-	40237-88-5	79%			**X**

40	Cyclopropane, 1-methyl-1-phenyl-	2214-14-4	82%	**X**		
41	Nonane, 3,7-dimethyl-	17302-32-8	91%			**X**
42	3-Ethyl-4-methyl-3-heptanol	66719-39-9	86%	**X**		**X**
43	4-Methyldocosane	25117-30-0	82%	**X**		**X**
44	Octanoic acid, 7-oxo-	14112-98-2	82%	**X**		
45	Azulene	275-51-4	85%			**X**
46	Dodecane	112-40-3	96%	**X**	**X**	**X**
47	Sulfurous acid, decyl 2-ethylhexyl ester	6420775(PubChem CID)	90%	**X**		**X**
48	Decane, 2,3,5,8-tetramethyl-	192823-15-7	87%			
49	Oxalic acid, bis(2-ethylhexyl) ester	13675-20-2	90%	**X**		**X**
50	Benzene, 1,3-bis(1,1-dimethylethyl)-	1014-60-4	96%	**X**	**X**	
51	1-Undecene, 4-methyl-	74630-39-0	94%			
52	1-Iodo-2-methylundecane	73105-67-6	90%			
53	Nonane, 5-(2-methylpropyl)-	62185-53-9	90%			
54	Tridecane	629-50-5	96%	**X**	**X**	
55	1-Heptanol, 2-propyl-	10042-59-8	85%			
56	Octadecane, 1-(ethenyloxy)-	930-2-9	84%			
57	2,4-Decadienal, (E,E)-	25152-84-5	91%			**X**
58	2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.0.1(1,7)]decan-5-one	121747-63-5	80%	**X**	**X**	
59	Cyclopentene, 5-hexyl-3,3-dimethyl-	61142-66-3	85%			
60	Not Identified	-	0%	**X**		**X**
61	Tetradecane	629-59-4	92%	**X**	**X**	**X**
62	2,5-di-tert-Butyl-1,4-benzoquinone	2460-77-7	86%			

Table S2: List of VOCs detected in at least one sample. For each VOC, the putative name and the corresponding CAS is reported. The similarity is the score in percentage of the correspondence of the detected mass spectra with NIST20R library. Crosses indicate the VOCs that was found in all replicas of at least one group of samples in the each of the three experiments.

		ALBUMAX							HUMAN SERUM						
		bkg	3D7	Cam	K13	Sud	Tan	WT	bkg	3D7	Cam	K13	Sud	Tan	WT
1	Heptane, 4-methyl-														
2	Hexane, 2,2,4-trimethyl-														
3	Hexanal														
4	2,4-Dimethyl-1-heptene														
5	o-Xylene														
6	'Styrene '														
7	2,5-Dimethylhexane-2,5-dihydroperoxide														
8	2-Heptanone, 4-methyl-														
9	1-Octanol, 2,7-dimethyl-														
10	benzaldeide														
11	Mesitylene														
12	Benzene, 1-ethyl-3-methyl-														
13	Benzene, 1,2,3-trimethyl-														
14	Heptane, 2,5,5-trimethyl-														
15	3-Ethyl-3-methylheptane														
16	1-Octene, 3,7-dimethyl-														
17	1-Hexanol, 2-ethyl-														
18	Indane														
19	Dodecane														
20	Benzene, 4-ethyl-1,2-dimethyl-														
21	Benzoic acid, methyl ester														
22	4-Nonanone, 7-ethyl-														
23	Nonane, 3,7-dimethyl-														
24	3-Ethyl-4-methyl-3-heptanol														
25	4-Methyldocosane														
26	Azulene														
27	Dodecane														
28	Sulfurous acid, decyl 2-ethylhexyl ester														
29	'Oxalic acid, bis(2-ethylhexyl) ester'														
30	2,4-Decadienal, (E,E)-														
31	UNIDENTIFIED														
32	Tetradecane														

Supplementary Table 3: Presence (green) and absence (white) of all volatile organic compounds found in Albumax cultured samples and Human serum cultured samples.

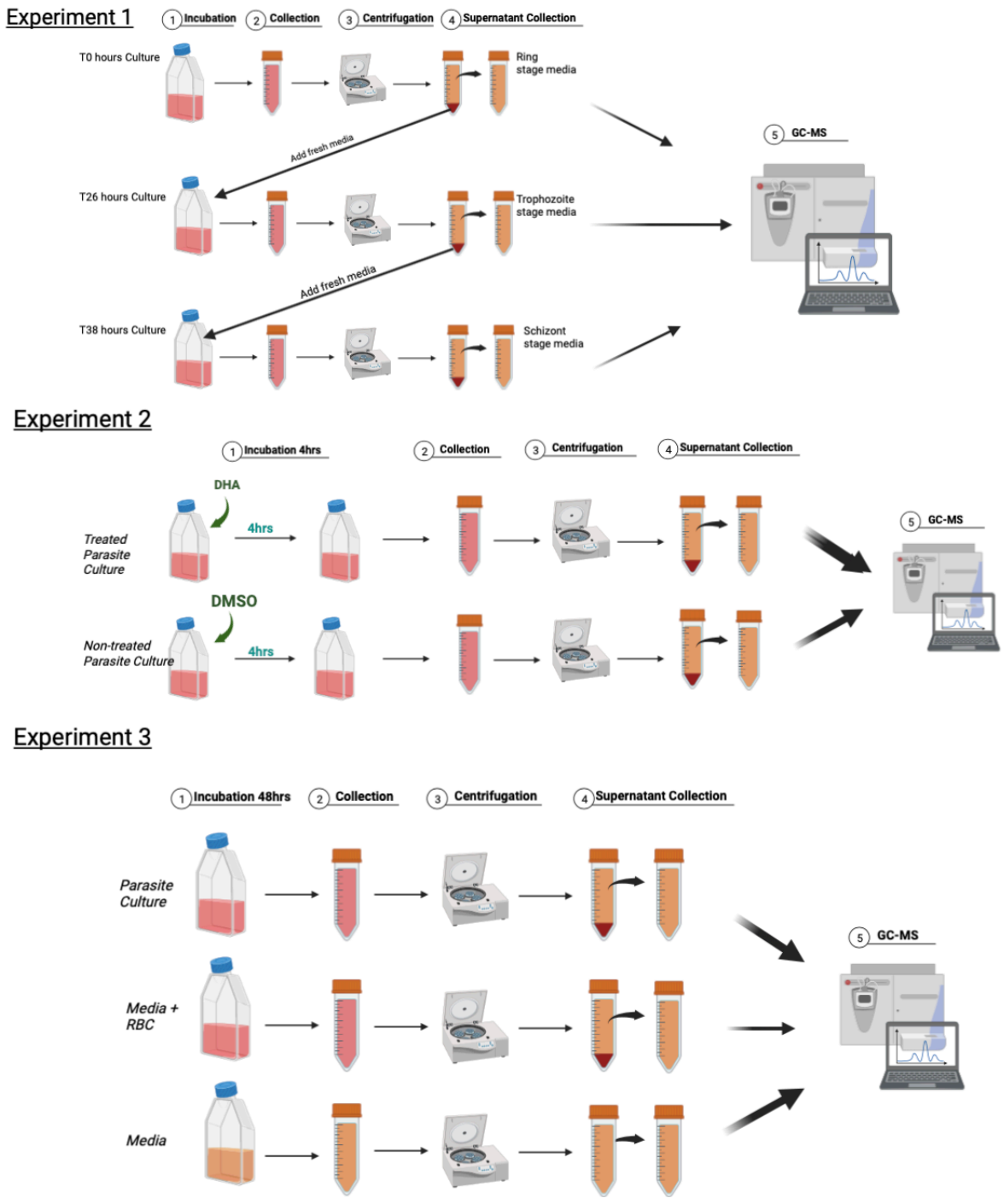

Figure S1: Experimental set up. Experiment 1: Synchronized asexual parasite cultures had fresh media added at T0 hpi. Supernatant was collected at 26hpi, fresh media was added on top of remaining iRBC culture and then collected at 38hpi. This process was repeated for final supernatant collection timepoint at 48hpi. Collected supernatants were measured using GC-MS. Experiment 2: Synchronized asexual parasite cultures at 6hpi ring stage were treated with

dihydroartemisin or DMSO for 4 hours. Supernatants were collected and measured using GC-MS. Experiment 3: Synchronized asexual parasite cultures of field isolate and lab strains were cultured for 48hrs, supernatants were collected and measured using GC-MS.

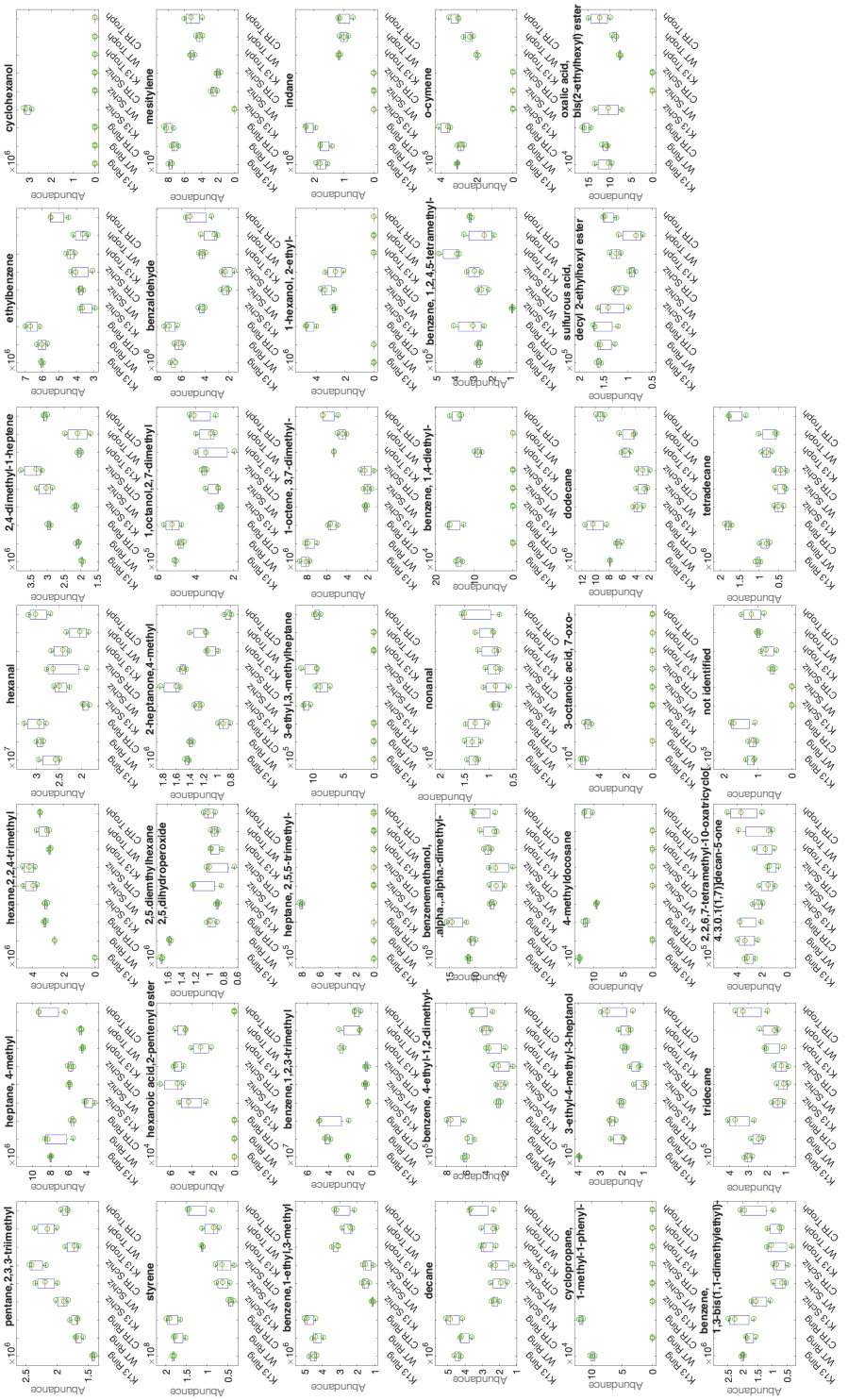

Figure S2: Distribution of the abundance of the compounds composing the Volatilomic profiles of rings, schizonts, and trophozites stages.

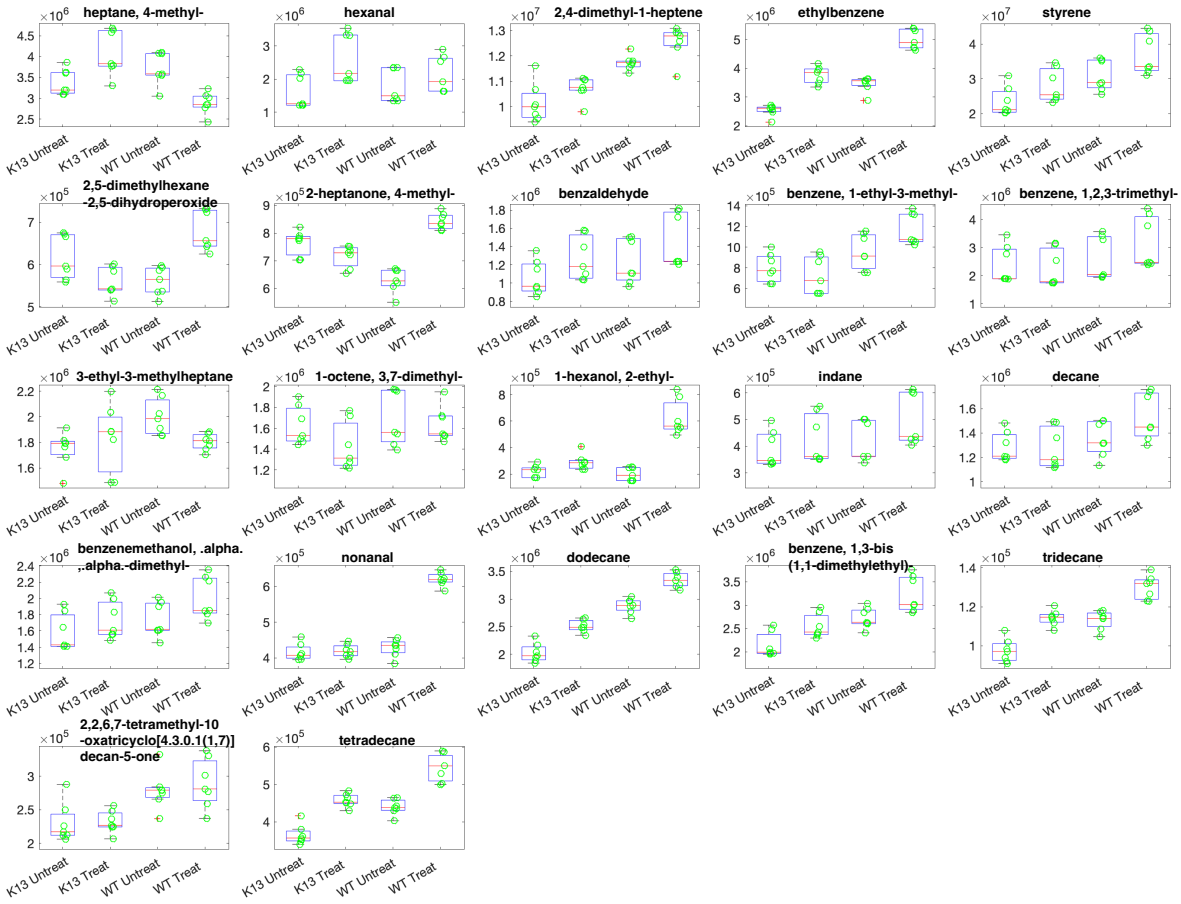

Figure S3: Distribution of the abundances of the compounds composing the Volatilome profile of artemisinin treated and untreated wild-type and resistant parasites.

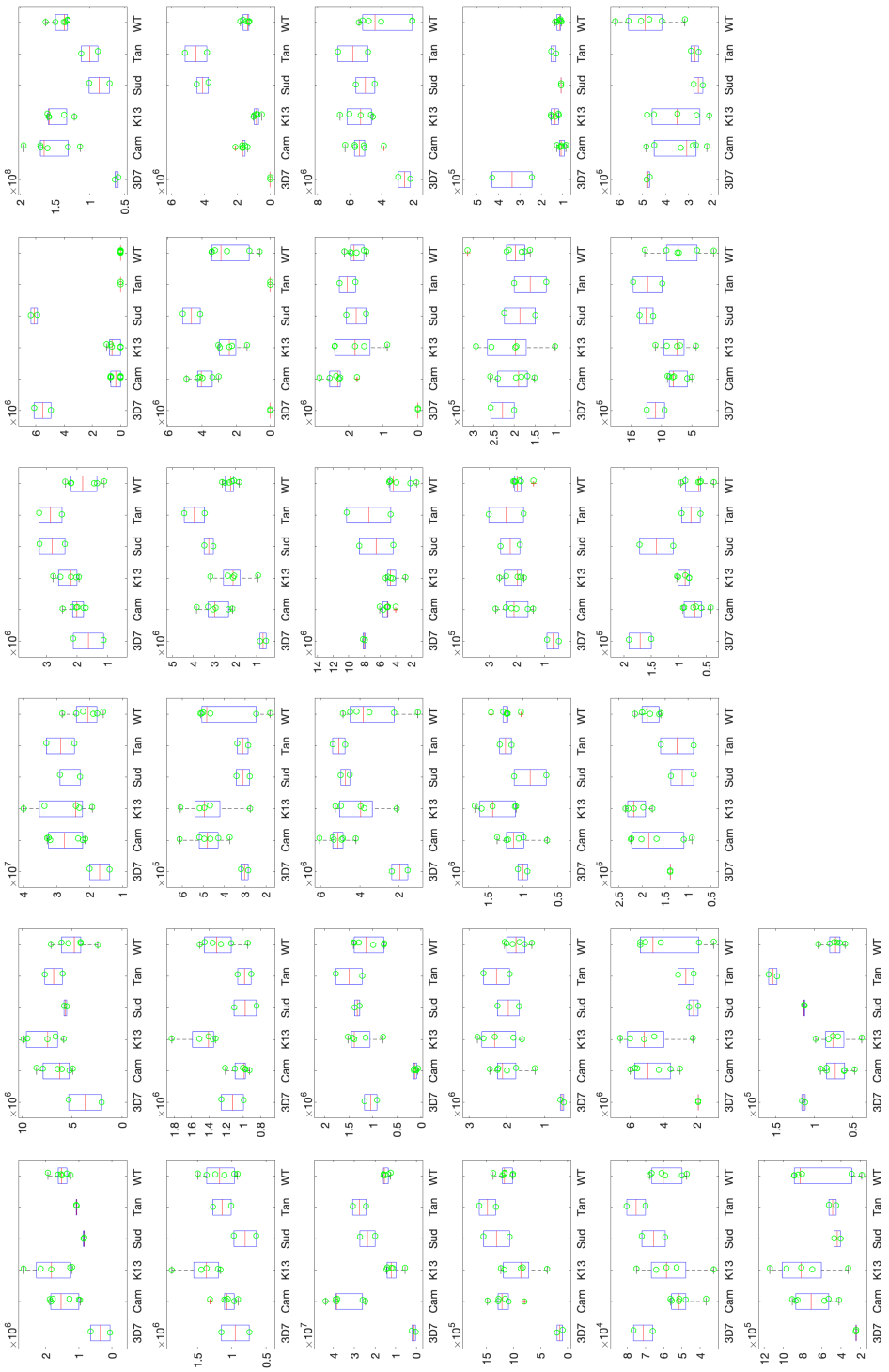

Figure S4 Distribution of the abundances of the compounds composing the Volatilome profile of lab-adapted and field isolated parasites cultured in Albumax.


3D7
Cam
K13
Sud
Tan
WT

Figure S5: Distribution of the abundances of the compounds composing the Volatilome profile of lab-adapted and field isolated parasites cultured in Human Serum.