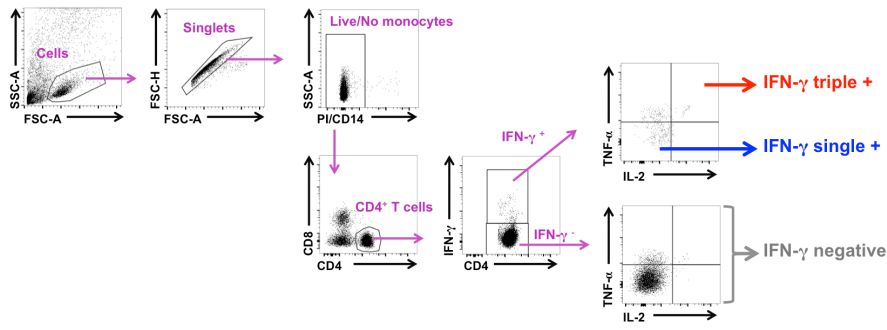


**Supplemental Figure 1. Identification of single, double and triple cytokine**

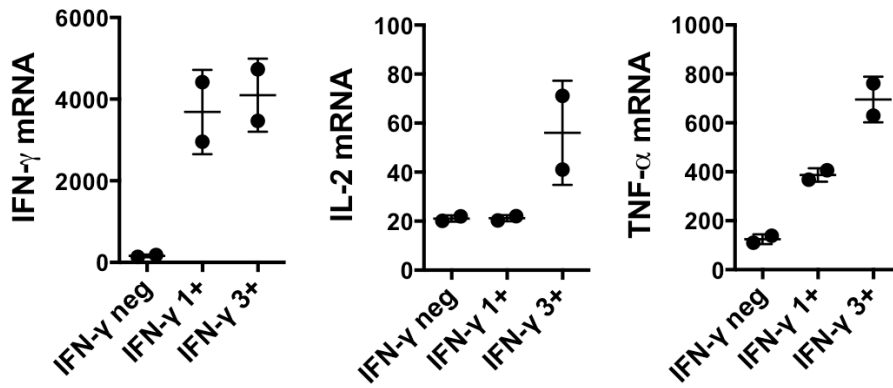
**producing CD4<sup>+</sup> T cells.** (A) Representative staining for IFN- $\gamma$ , IL-2 and TNF

production by CD4<sup>+</sup> T cells measured by flow cytometry and intracellular cytokine staining from whole blood samples stimulated with uninfected red blood cell extract (nRBC) or *P. falciparum* infected red blood cell extract (Pf RBC).

(B) Boolean gating strategy to identify single, double and triple cytokine producing CD4<sup>+</sup> T cells using FlowJo.



**Supplemental Figure 2. Gating strategy to identify and sort viable IFN- $\gamma$  negative, IFN- $\gamma$  single positive and IFN- $\gamma$ /IL-2/TNF triple positive CD4<sup>+</sup> T cells.** Whole blood was collected four weeks post-infection with *P. falciparum*, *in vitro* stimulated with *Pf* pRBC parasite antigen extract overnight, and *P. falciparum*-reactive cytokine producing CD4<sup>+</sup> T cells sorted using the three-color polyfunctional cytokine secretion assay. Cytokine producing CD4<sup>+</sup> T cells were gated as PI/CD14/CD8/ CD4<sup>+</sup> cells. Plots show representative staining of one volunteer.



**Supplemental Figure 3. Expression of IFN- $\gamma$ , IL-2 and TNF cytokines in IFN- $\gamma$  negative, IFN- $\gamma$  single positive and IFN- $\gamma$ /IL-2/TNF triple positive CD4<sup>+</sup> T cells determined by microarray analysis.** Whole blood was collected four weeks post-infection with *P. falciparum*, *in vitro* stimulated with parasite antigen extract overnight, and *P. falciparum*-reactive cytokine producing CD4<sup>+</sup> T cells sorted using the three-color polyfunctional cytokine secretion assay. Cytokine expression was determined with an Affymetrix Human Gene ST 2.0 gene array from mRNA pools from sorted cells subsets of four independent volunteers per array. Data derived from two independent microarray experiments, with four independent volunteers per experiment.

**Supplemental Table 1. Genes differentially expressed between *P. falciparum*-reactive IFN- $\gamma$  single positive and IFN- $\gamma$ /IL-2/TNF triple positive CD4<sup>+</sup> T cells**

Gene Symbol	Transcript cluster ID	Fold-change <sup>A</sup> Exp #1	Fold-change <sup>A</sup> Exp #2	Gene Symbol	Transcript cluster ID	Fold-change <sup>A</sup> Exp #1	Fold-change <sup>A</sup> Exp #2
AQP9	16801473	3.37	4.25	IRAK3	16753670	2.30	2.73
C5AR1	16863589	8.26	2.50	ITGAX	16818272	2.15	2.33
CCL3L3L1	16843627	5.70	3.75	KCTD12	16779839	2.20	2.46
CCL8	16833224	2.02	2.68	KIAA1199	16803754	4.65	2.10
CD163	16760792	5.30	3.51	LILRA1	16865383	2.40	3.10
CTSL1	17086540	3.80	2.63	LYZ	16767364	3.48	2.20
CXCL16	16840113	4.00	2.02	MS4A7	16725210	3.49	3.15
CXCL5	16976827	7.49	3.82	MT1G	16826738	16.49	3.94
CXCR3	17111955	2.06	3.69	MT1H	16819257	12.75	2.78
CYBB	17102538	3.36	2.11	MT1M	16819224	3.42	4.62
DMXL2	16809263	3.82	2.69	PLA2G7	17019728	4.67	2.47
ETS2	16922779	2.15	2.30	PLAUR	16873060	2.98	2.27
FPR1	16874945	9.69	3.78	PMS2	17054923	2.20	2.11
GNPNAT1	16784275	2.27	2.36	POLR2J	17060983	2.35	3.04
HMGAI	17117871	3.09	2.35	PTGS2	16697370	3.32	4.25
HMOX1	16929562	2.63	3.23	RN5S123	16950838	7.65	2.64
IGHM	16789782	8.44	4.76	RN5S450	16850954	2.89	2.16
IGHV4-31	16797512	2.36	2.75	RNF185	16934039	3.61	5.34
IGHV4-34	16797520	15.25	7.72	S100A9	16671139	4.78	3.27
IGKC	16900090	8.12	2.02	S1PR1	16667760	2.01	3.98
IGKV1-16	16900132	8.78	3.12	SERPINB2IB10	16852871	3.53	4.25
IGLC2	16927853	3.47	7.29	TLR2	16971631	2.05	2.12
IGLJ7/IIGLC7	16927869	2.26	2.48	TRAJ17/ITRAV3	16781830	3.58	2.02
IGLV1-44	16927858	11.84	13.29	TRAJ36	16782052	4.08	2.39
IGSF6	16824907	2.36	2.15	TRAJ42	16782040	2.67	2.81
IL1B	16901986	3.84	3.15	TRAJ54	16782018	2.03	5.08
IL2	16979570	3.22	2.02	TRAJ9	16782104	2.18	3.86
IL3	16988964	3.19	5.56	TRAV20	16781916	2.97	3.85
IL3RA	17101111	2.07	2.16	TRAV25	16781937	3.03	6.01
IL6	17044177	4.02	2.43	TRAV4	16781834	2.40	2.14
IL8	16967771	2.90	2.21				

<sup>A</sup> determined using Affymetrix Human Gene ST 2.0 array of pooled RNA samples from 4 independent volunteers

**Supplemental Table 2. Antibodies used for flow cytometry**

<b>Antigen</b>	<b>Fluorochrome</b>	<b>Clone</b>	<b>Company</b>	<b>Catalog number</b>
CD4	APC	SK3	Becton Dickinson	340672
CD4	V500	RPA-T4	Becton Dickinson	560768
CD4	BV510	OKT4	Biologend	317443
CD8	APC-H7	SK1	Becton Dickinson	560179
CD14	PE Texas Red	RMO52	Beckman Coulter	IM0650U
KLRG1	PE	2F1/KLRG1	Biologend	138407
IFN- $\gamma$	FITC	B27	Becton Dickinson	554700
IL-2	AF700	MQ1-17H12	Biologend	500319
TNF- $\alpha$	PE-Cy7	MAb11	Becton Dickinson	557647

**Supplemental Table 3. Forward and reverse primers sequences of the genes selected for validation by RT-qPCR**

<b>Gene Symbol</b>	<b>Forward Primer</b>	<b>Reverse Primer</b>
AQP9	TTGATTGTCCTTGGATGTGG	GGTTGATGTGACCACCAGAG
C5AR1	GCTTCCTGTGGCCTCTACTC	CACCACCTTGAGTGTCTTGG
CCL3L3IL1	CTCTGCAACCAGGTCCTCTC	TTTCTGGACCCACTCCTCAC
CCL8	GACTTGCTCAGCCAGATTCA	AAGATCACAGCTTCCTTGGG
CD163	TTCAGTGCAGTGGGACTGAG	CACTCTCTATGCAGGCCACA
CTSL1	AGGGAAGGGAAACACAGCTT	AAGCCCAACAAGAACCACAC
CXCL16	ATGCTTACTCGGGGATTGTG	GTGGTTTCATTGGGACGAGT
CXCL5	TTTGGACGGTGGAAACAAG	TCTCTGCTGAAGACTGGGAA
CXCR3	ACACCTTCCTGCTCCACCTA	GTTTCAGGTAGCGGTCAAAGC
ETS2	AGAGGAGTGACCCAGTGGAG	TGATGAATGACTGGCAGGAT
FPR1	CCAGGAGACCCAGACCTAGA	TGTCTGCTCCTGGACTGTTC
HMGA1	CATCCTCATTGCCTCCTGTT	GTGGCCCCTACACCCTTTAT
HMOX1	AGTGCCACCAAGTTCAAGC	CAAAGAGCTGGATGTTGAGC
IGSF6	AGTGTACCTTCTCCGCAACC	GCCTCCCTCACTGTGAACTT
IL1B	CAGATGAAGTGCTCCTTCCA	ACCAGCATCTTCCTCAGCTT
IL2	TCACCAGGATGCTCACATTT	TGCTGATTAAGTCCCTGGGT
IL3	TTTGCTGGACTTCAACAACC	CTTGACAGCCCTGTTGAATG
IL3RA	CCCAACATGACTGCAAAGTG	TCTTTCCCGGGCTCTTATTT
IL6	AATGAGGAGACTTGCCTGGT	GCAGGAACTGGATCAGGACT
IL8	AGGACAAGAGCCAGGAAGAA	ACTGCACCTTCACACAGAGC
IRAK3	TTGCAGTGTGTAGGTGACACG	GAGCATGGTTGAACGTTGTG
ITGAX	CCTGTTACAGCCTTAGCAA	CATACTGGACCACGCTGTCT
LILRA1	GACCCAGGAGTACCGTCTGT	ACTGGCCCTTCTTCACAATC
LYZ	GGAGCAGTTAATGCCTGTCA	GATCACGGACAACCCTCTTT
MS4A7	GATCATCACCCCTGCACTTT	TTAAACTGCAGCCCACTGG
MT1G	ACCTCCTGCAAGAAGAGCTG	GGAATGTAGCAAAGGGGTCA

MT1H	ACGTGTTCCACTGCCTCTTC	AGGAGCAGCAGCTCTTCTTG
MT1M	AGCAGTCGCTCCATTTATCG	AGGAGCAGCAGCTCTTCTTG
PLA2G7	GGTACGGCAAAGAGCAAAAG	CGTTGCTCCACCAAAGAAT
PLAUR	GCCTTACCGAGGTTGTGTGT	CATCCAGGCACTGTTCTTCA
PTGS2	GCTGGAACATGGAATTACCC	TGATTCATAGGGCTTCAGCA
S100A9	GGACACAAATGCAGACAAGC	TCTTGGCCACTGTGGTCTTA
S1PR1	GACTCTGCTGGCAAATTCAA	GAGGAATTGTCCGATTTGCT
SERPIN2	GTTCATGCAGCAGATCCAGA	CGCAGACTTCTCACCAAACA
SERPIN10	GAAAGACAGACCGAGGGTAAA	ATAGGGCGTTCACCAGAATC
TLR2	GGAGTTCTCCAGTGTTTGG	CCCACAGGTACCTTCACTTG
TNF	CCCATGTTGTAGCAAACCCT	TGAGGTACAGGCCCTCTGAT
Housekeeping genes		
ACTB	TGGACTTCGAGCAAGAGATG	GAAGGAAGGCTGGAAGAGTG
B2M	TGTCTTTCAGCAAGGACTGG	GCATCTTCAAACCTCCATGA
GAPDH	CATCAATGGAAATCCCATCA	GACTCCACGACGTACTCAGC
HPRT1	CTGGCGTCGTGATTAGTGAT	GAGCACACAGAGGGCTACAA