Lymphocyte Subsets		Dutch		P value pre- vs post-CHMI[d]	Tanzanian		P value pre vs post-CHMI[d]	P value D vs. T pre-CHMI[e]	P value D vs. T post-CHMI[e]
		Pre	Post		Pre	Post			
CD4+	**total[a]**	43.4 [39.5-50.4]	44.4 [41.1-49.3]	*0.57*	36.5 [30.9-41.6]	37.1 [33-41.3]	**0.06**	**0.02**	**0.01**
	Tem[b]	11.6 [7.1-20.8]	12.3 [6.4-17.5]	***0.03***	22.2 [17-25.3]	20.8 [18-23.5]	0.40	**0.003**	**0.0004**
	Tcm[b]	27 [20.4-36.4]	25.7 [18.2-36.8]	*0.36*	40 [36.4-45.2]	37.7 [31.8-42.6]	**0.05**	**0.003**	**0.01**
	T_N[b]	44.4 [39.5-66.1]	47.5 [42.9-66.3]	*0.73*	35.2 [29.3-40.7]	37 [29.4-43.7]	0.18	**0.01**	**0.006**
CD8+	**total[a]**	19.9 [18.8-24.2]	20.2 [18.5-22.6]	*0.16*	20.4 [17.6-25.1]	19.2 [18.3-25.5]	0.73	0.89	0.89
	Tem[c]	18.7 [10-19.6]	13.6 [9.2-20]	*0.36*	20.4 [12.9-27.2]	16.4 [11.9-23]	**0.04**	0.39	0.4
	Tcm[c]	6.5 [4-10.5]	9.8 [3.7-11.1]	*0.91*	6.4 [4.6-8.9]	6.5 [4.5-8.5]	0.85	0.75	0.62
	T_N[c]	60.2 [47.7-68.9]	62.2 [53-73.1]	***0.06***	52 [40.5-57.5]	53.8 [43.6-63.6]	**0.02**	0.09	0.1
NKT-γδT[a*]		7.1 [5.2-8.3]	11.5 [7.7-13.3]	***0.01***	8.8 [6-12.7]	9.2 [5.7-12]	0.13	0.12	0.3
CD56dim NK[a]		8.1 [4.9-10.5]	4.2 [2.8-7.2]	***0.004***	12.6 [9.5-14.5]	8.3 [5.5]	**0.003**	**0.01**	**0.003**
CD56bright NK[a]		0.6 [0.5-0.7]	0.6 [0.4-1.0]	*1.00*	0.5 [0.4-0.8]	0.5 [0.3-0.8]	0.47	0.37	0.2
CD4+CD45RO+ FoxP3+[#]		2.4 [1.8-3.1]	2.2 [1.6-3.5]	*0.44*	2.3 [1.5-3.5]	1.6 [1.2-3.4]	**0.008**	0.66	0.28

Table S1: Lymphocyte subset frequencies in Dutch and Tanzanian volunteers

Data represents the median and [IQR] of the lymphocyte subset frequencies in Dutch and Tanzanian volunteers pre- and post-challenge.

a = % of total viable lymphocytes

b = % of CD4+ T-cells

c = % of CD8+ T-cells

d = Responses pre- versus post-CHMI were compared using Wilcoxon matched-pairs signed rank test

e = Responses between groups were compared using Mann-Whitney U-test

* = NKT and γδ T were combined in the same gate

= Analysis was done for n=6 Dutch and n=8 Tanzanian volunteers for which sufficient cells were available; expressed as % of CD4 T-cells

Tem - effector memory T-cell (CD45RO+ CD62L-); Tcm - Central memory T-cell (CD45RO+ CD62L+); T_N - Naive T-cell (CD45RO- CD62L+)

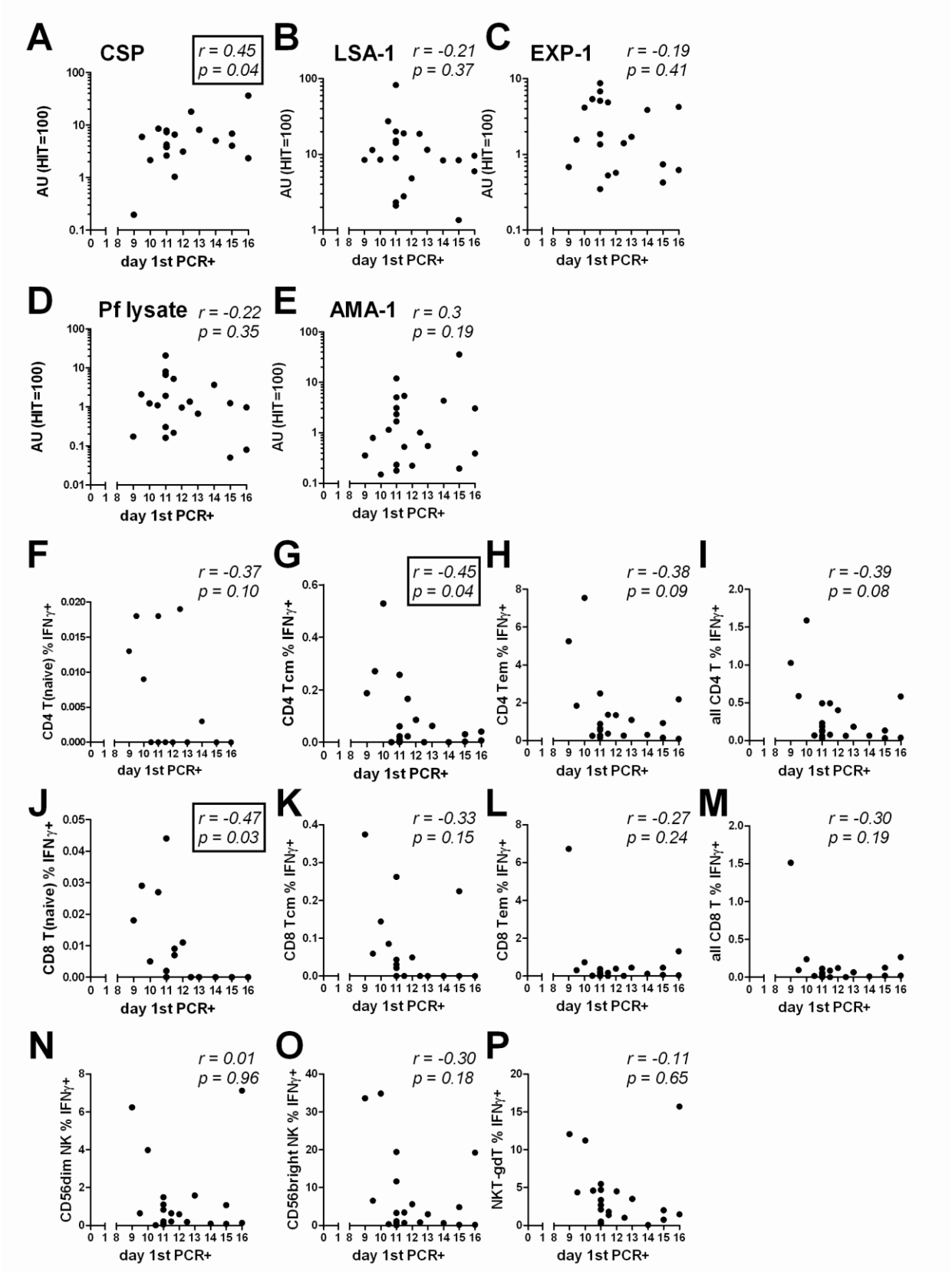

Figure S1: Correlations between baseline Pf-specific immune responses and outcome of CHMI in Tanzanian volunteers. The relationship between pre-patency by qPCR and pre-CHMI **(A-E)** humoral and **(F-P)** cellular immune responses was analyzed by Pearson correlation.

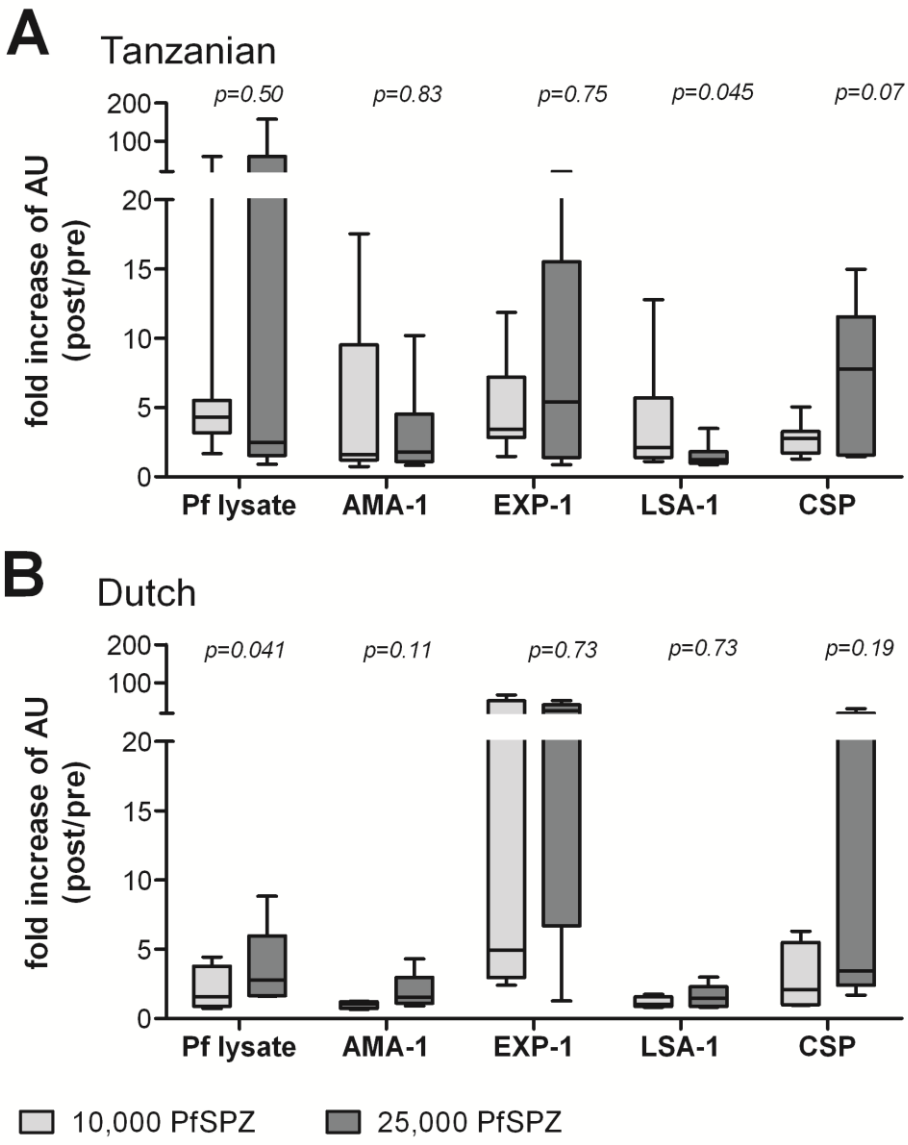

Figure S2: Changes in malaria-specific humoral responses after CHMI in relation to PfSPZ Challenge dose. The fold increase in antibody titers four weeks (Tanzanians) or five weeks (Dutch) after intradermal injection with PfSPZ Challenge was calculated for Pf lysate, AMA-1, EXP-1, LSA-1 and CSP in (**A**) Tanzanian (n=21) and (**B**) Dutch volunteers (n=9) inoculated with either 10,000 (light grey) or 25,000 (dark grey) PfSPZ. Data are shown as whisker plots with boxes indicating the median and IQR, and whiskers the min/max values. Responses between the two dose groups were compared using Mann-Whitney U-test.

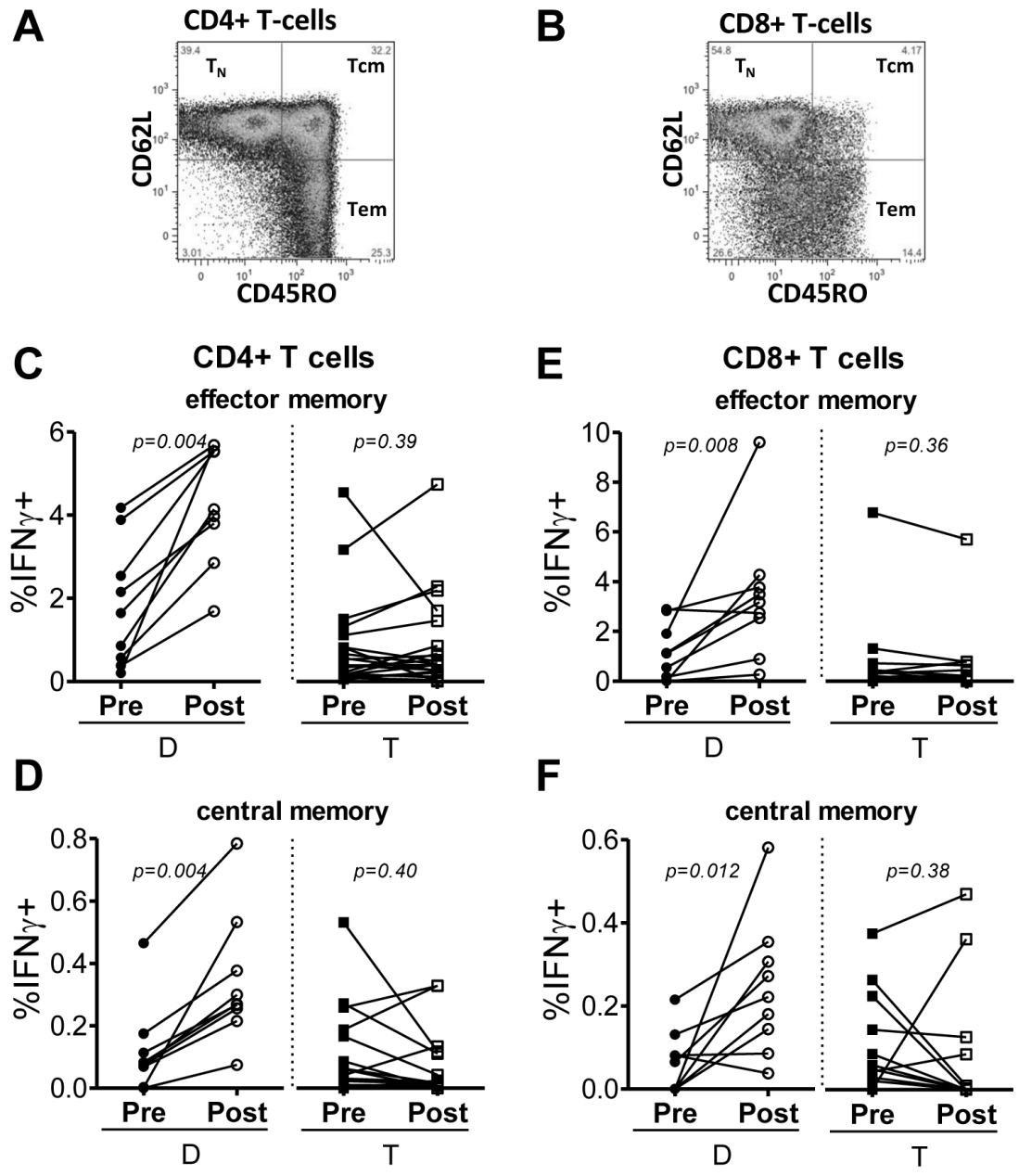

Figure S3: **Dutch, but not Tanzanian volunteers show increased Pf-specific *in vitro* IFNγ-production by memory T-cell subsets post CHMI.** PBMCs collected pre- and post-administration of PfSPZ Challenge from Dutch (D; circles; n=9; post=35 days after CHMI) and Tanzanian (T; squares; n=21; post=28 days after CHMI) volunteers were stimulated with PfRBC for 24h and IFNγ production assessed by intracellular cytokine staining. Effector (Tem) and central memory T-cells (Tcm) within (**A**) CD4+ and (**B**) CD8+ subsets were gated based on CD62L and CD45RO surface expression. The proportions of cells with Pf-specific IFNγ-production pre- and post-CHMI within each cohort are shown for **(C**) CD4+ Tem, **(D**) CD4+ Tcm, **(E**) CD8+ Tem and **(F**) CD8+ Tcm. For each volunteer both time points were measured in a single experiment. Data are shown as individual data points with lines connecting the two time points for each volunteer. All responses were corrected for background by subtracting responses to uRBC. Responses pre- versus post-CHMI were compared using Wilcoxon matched-pairs signed rank test.

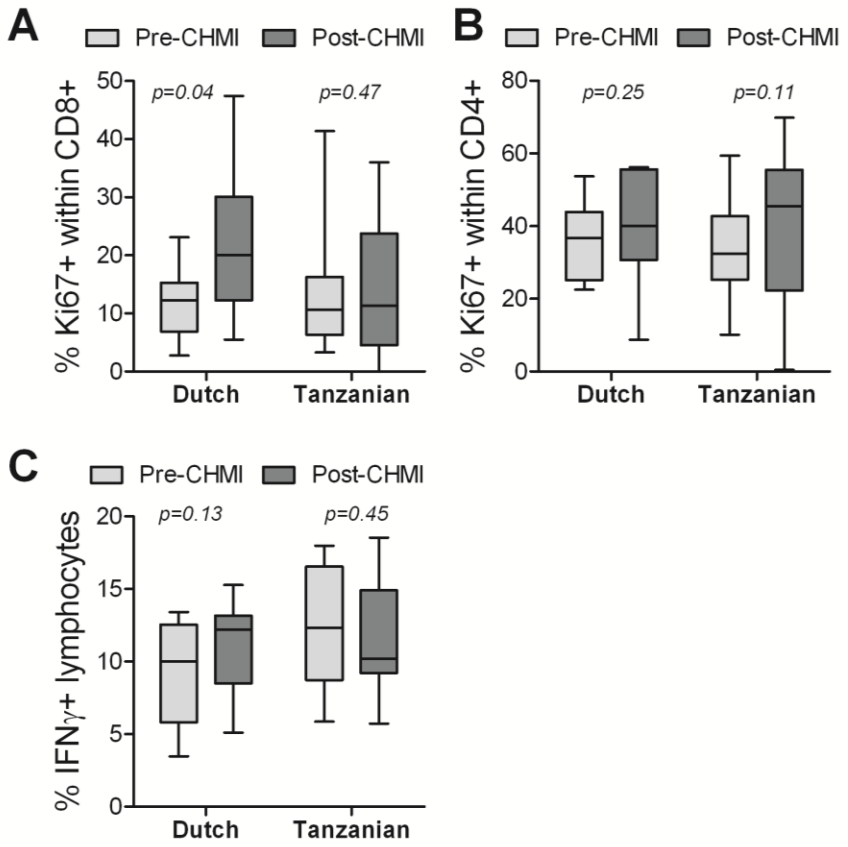

Figure S4: CHMI-induced changes in Pf-specific proliferative responses and non-specific IFNγ production. PBMCs collected pre- and post-administration of PfSPZ Challenge from Dutch (n=9) and Tanzanian (n=21) volunteers were stimulated with PfRBC for 6 days. After stimulation cells were stained for intracellular Ki67 expression and surface expression of CD3, CD4 and CD8. Graphs show the proportions of Ki67-expressing cells pre- (light grey) and post-CHMI (dark grey) in (**A**) CD8+ T-cells and (**B**) CD4+ T-cells. (**C**) shows PMA/ionomycine-induced IFNγ production by total lymphocytes after 4 hours stimulation. Data are shown as whisker plots with boxes indicating the median and IQR, and whiskers the min/max values of responses corrected for background by subtracting responses to uRBC. Responses pre- versus post-CHMI were compared using Wilcoxon matched-pairs signed rank test.