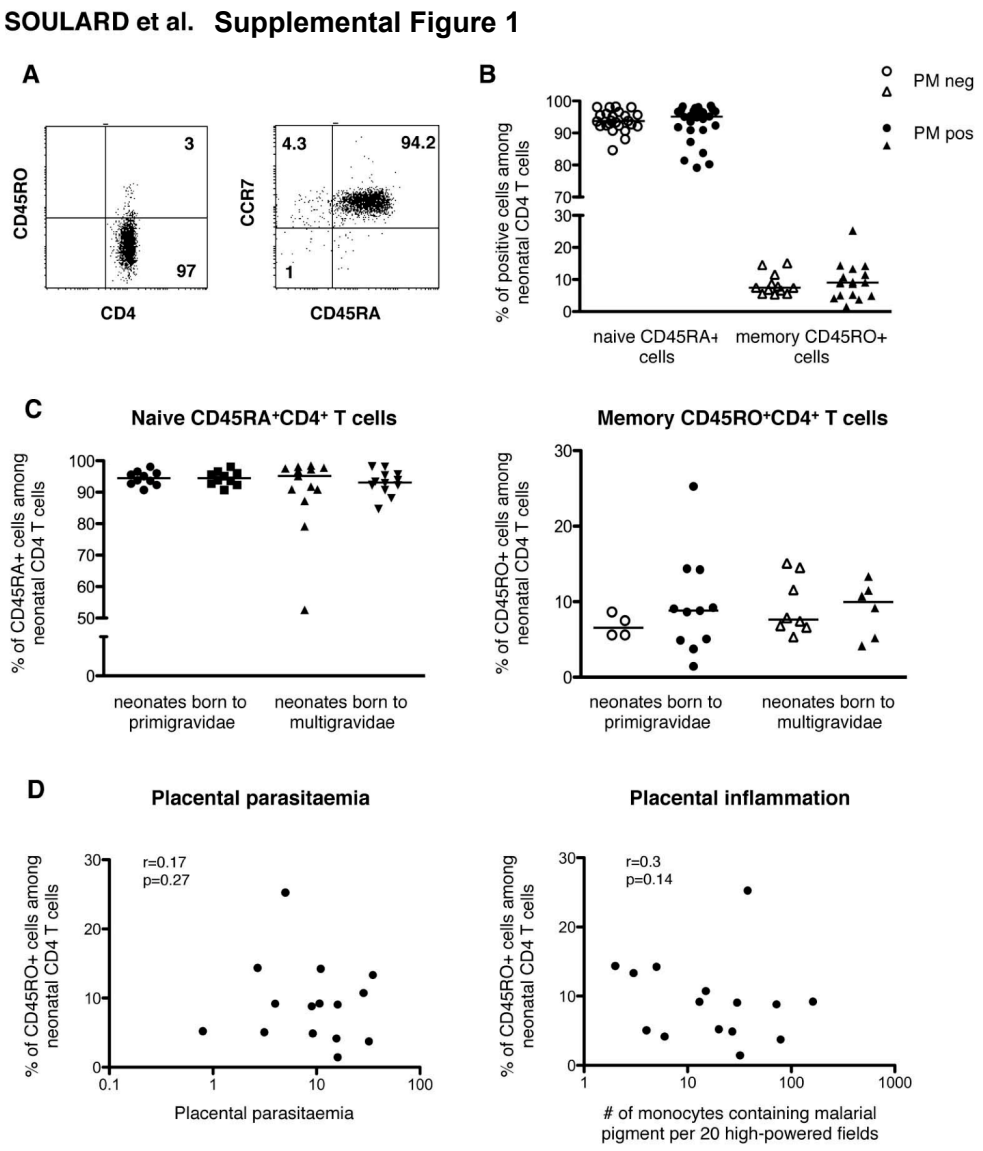
Supplemental material to Soulard V. et al. manuscript.

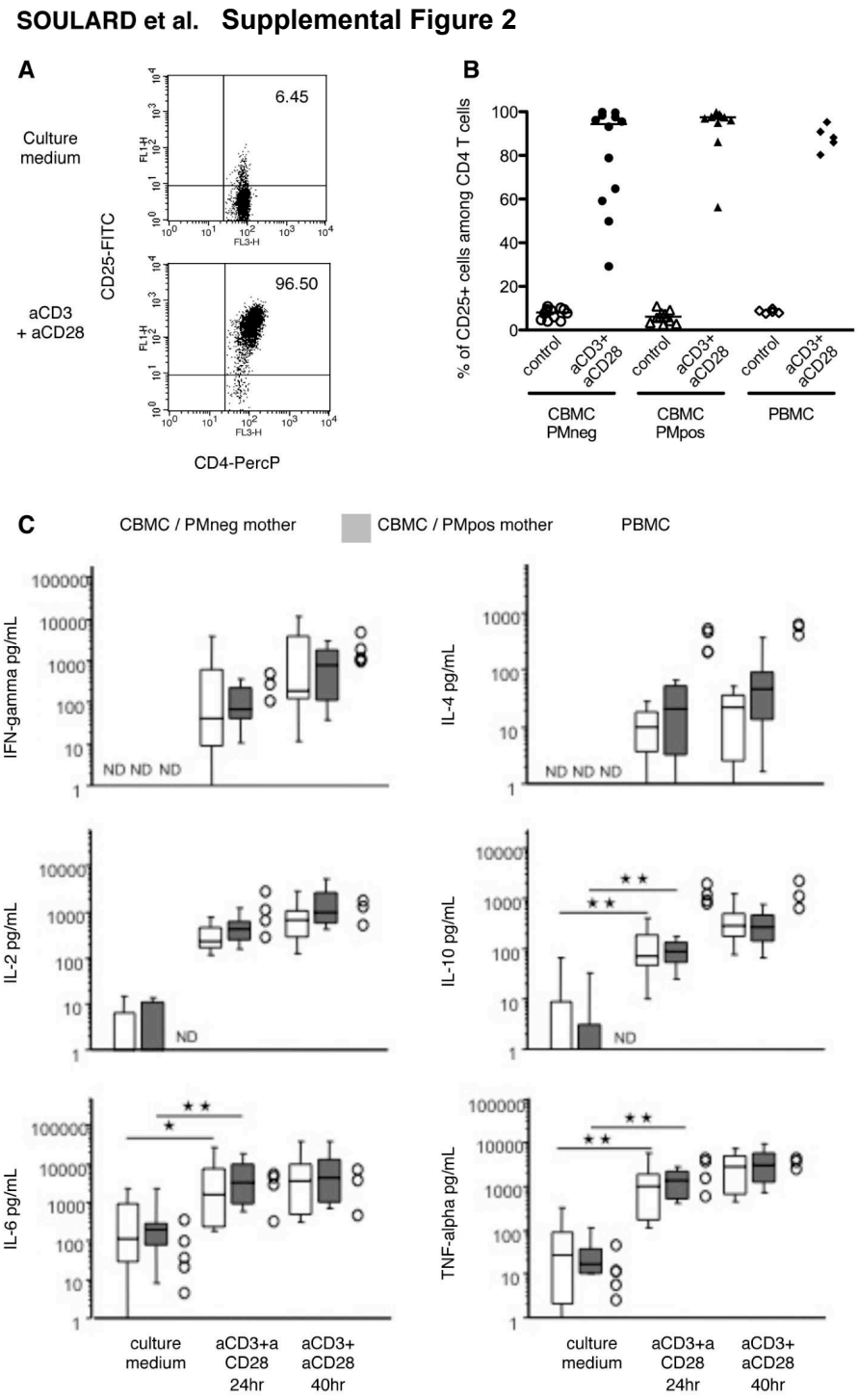
In vitro polyclonal stimulation of CMBC and co-culture with live P. falciparum-infected RBC

Freshly isolated CBMC were cultured in 24-well plates at a concentration of 1 million cells per mL of complete medium (RPMI 1650 containing 25 mM Hepes, supplemented with 2 % heat-inactivated FBS, 2 mM L-Gln, 100 UI Penicillin-Streptomycin and 1 mM Sodium Pyruvate). For polyclonal stimulation, cells were incubated with anti-CD3 mAb (clone UCHT1, eBiosciences) at 5 mg/mL in complete medium, on ice, for 30 min. Then, cells were washed and resuspended in 5 mL of complete medium containing anti-CD28 mAb (clone CD28.2, BD Biosciences) at 2.5 mg/mL, and transferred into culture wells precoated with goat anti-mouse IgG (5 mg/mL, 1031-01, Clinisciences). As a negative control, cells were cultured in complete medium alone. After 24 hr of culture, 300 mL of culture supernatant were harvested, aliquoted, and stored at -80°C. After 35 hr of culture, monensin (Golgi Stop, BD Biosciences, used following manufacturer's instructions) and brefeldin A (Sigma Aldrich, used at a final concentration of 10 mg/mL) were added to the wells. 5 hr later, supernatants were harvested, aliquoted and stored at -80 °C. Cells were then washed once in PBS-3%FBS before performing surface and intracellular staining.



Supplemental Figure 1: No association between PM and frequency of naive or memory neonatal CD4 T cells

(A) FACS dot plot showing a representative analysis for expression of CD45RO, CD45RA and CCR7 among neonatal CD4 T cells. Percentages of cells are shown. **(B)** Percentages of naive $CD45RA^+$ and memory $CD45RO^+$ cells among neonatal $CD4^+$ T cells were determined by FACS analysis on freshly isolated CBMC from neonates born to PM negative (n=23 and n=12 for CD45RA and CD45RO stainings respectively) and PM-positive (n=22 and n=17 for CD45RA and CD45RO stainings respectively) mothers. Individual (symbols) and median (bars) values are shown. **(C)** The same results are shown, according to mothers' gravidity (CD45RA: n=10 PMneg and n=10 PMpos primigravidae, n=13 PMneg and n=12 PMpos multigravidae; CD45RO: n=4 PMneg and n=11 PMpos primigravidae; n=8 PMneg and n=6 PMpos multigravidae); Empty symbols: PMneg, Filled symbols: PMpos. **(D)** The same results are shown, according to placental parasitemia and inflammation (n=15). Individual (symbols) and median (bars) values are shown. Spearmann correlation test.



Supplemental figure 2: Identical frequencies of activated CD4$^+$ CD25$^+$ T cells and cytokine responses following polyclonal stimulation between CBMC from neonates born to PM-negative and PM-positive mothers and PBMC from healthy Beninese adults.

(A) FACS dot plots showing the expression of CD25 on neonatal CD4$^+$ T cells after culture of CBMC in medium only or in presence of anti-CD3+anti-CD28 mAbs. **(B)** Frequencies of activated CD25$^+$ cells among neonatal CD4$^+$ T cells from all CBMC samples cultured in medium alone (control) or in presence of anti-CD3+anti-CD28 mAbs, and segregated according to the presence or absence of PM. Data obtained with PBMC from 5 healthy adult Beninese donors are shown for comparison. Results are expressed as individual (symbols) and median (bars) values.

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(C) Supernatants were collected at 24h and 40h of culture, and concentrations of IFN-γ, IL-4, IL-2, IL-10, IL-6 and TNF-α were determined by Multiplex analysis. CBMC from PMneg mothers (n=12); CBMC from PMpos mothers (n=10); PBMC (n=4). Results are expressed as box plots showing the medians with 25th, 75th, and whiskers for 10th and 90th percentiles, except for PBMC samples which are shown as individual values. ND: Not Detected; ★ $p < 0.05$; ★★ $p < 0.005$; Mann-Whitney U test.