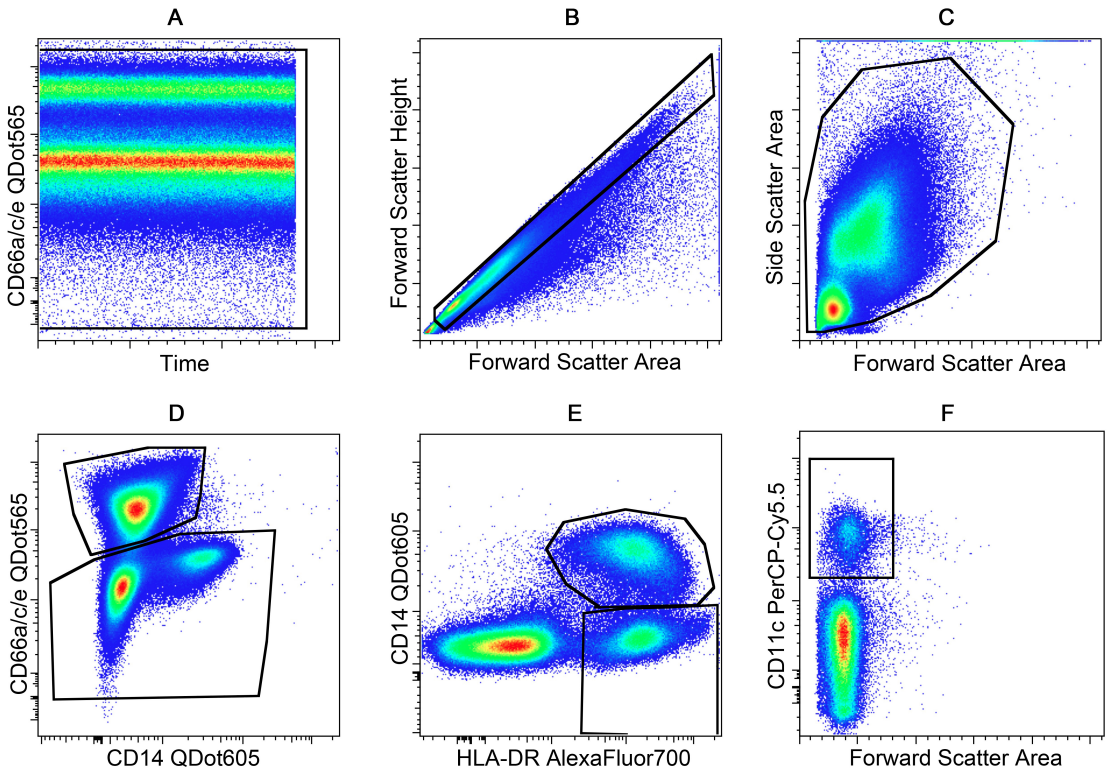
Supplementary Table I: Pro-inflammatory responses induced by LPS.

Cell subset	Outcome	Age of participants (weeks)			Overall p value
		0	**10**	**36**	
		Median (IQR)	Median (IQR)	Median (IQR)	
Monocytes	IL-12 (iMFI)	94 (7-273)	1,032 (268-3417)	2,808 (1,516-8,388)	<0.0001
	IL-6 (iMFI)	71,884 (28,205-133,938)	222,762 (55,449-354,329)	273,949 (165,056-353,423)	0.0003
	TNF-α (iMFI)	5,960 (917-11,397)	77,121 (21,797-138,869)	89,839 (44,320-187,420)	<0.0001
	IL-10 (iMFI)	251 (121-614)	295 (116-696)	481 (169-941)	0.4
	Polyfunctional cells (%)*	0.02 (0.01-0.05)	0.13 (0.01-1.21)	1.23 (0.21-3.91)	<0.0001
	Bifunctional cells (%)*	1.12 (0.41-2.01)	10.21 (3.13-18.32)	12.40 (5.91-19.19)	<0.0001
	Monofunctional cells (%)*	36.79 (15.91-52.80)	53.20 (31.71-62.83)	50.24 (40.24-57.11)	0.1
Myeloid DC	IL-12 (iMFI)	3 (0-683)	1046 (430-3,251)	774 (416-3,412)	0.0007
	IL-6 (iMFI)	77,009 (45,854-127,398)	151,246 (55,226-249,850)	95,355 (33,245-176,531)	0.2
	TNF-α (iMFI)	26,253 (10,483-65,334)	154,201 (53,866-285,420)	48,437 (27,523-170,017)	0.0001
	IL-10 (iMFI)	110 (7-326)	74 (38-248)	73 (26-219)	0.7
	Polyfunctional cells (%)*	0.14 (0.04-0.29)	0.48 (0.18-2.60)	0.77 (0.30-3.18)	0.004
	Bifunctional cells (%)*	4.62 (3.40-10.37)	19.56 (8.22-30.86)	9.97 (3.30-24.54)	0.008
	Monofunctional cells (%)*	34.90 (20.30-39.42)	25.25 (17.04-32.54)	17.87 (5.23-28.69)	0.0006

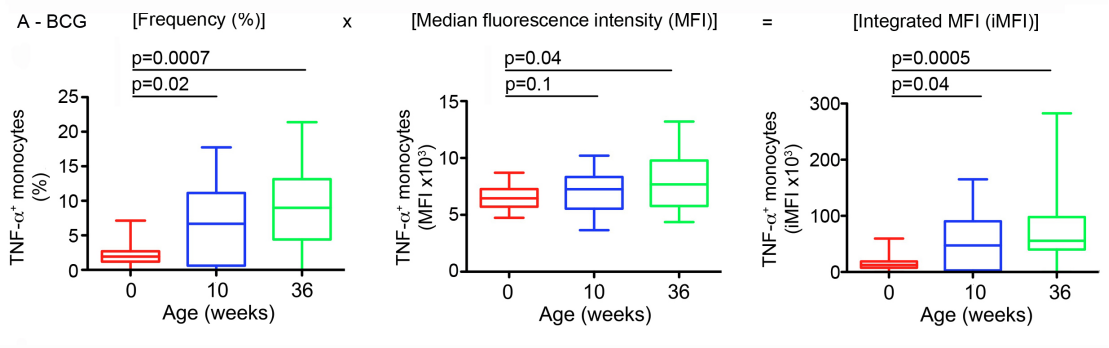
*Amongst pro-inflammatory cytokine^{+} cells. The Kruskal-Wallis test was used for statistical analyses (overall p-values). iMFI, integrated median fluorescence intensity.

Supplementary Figure 1



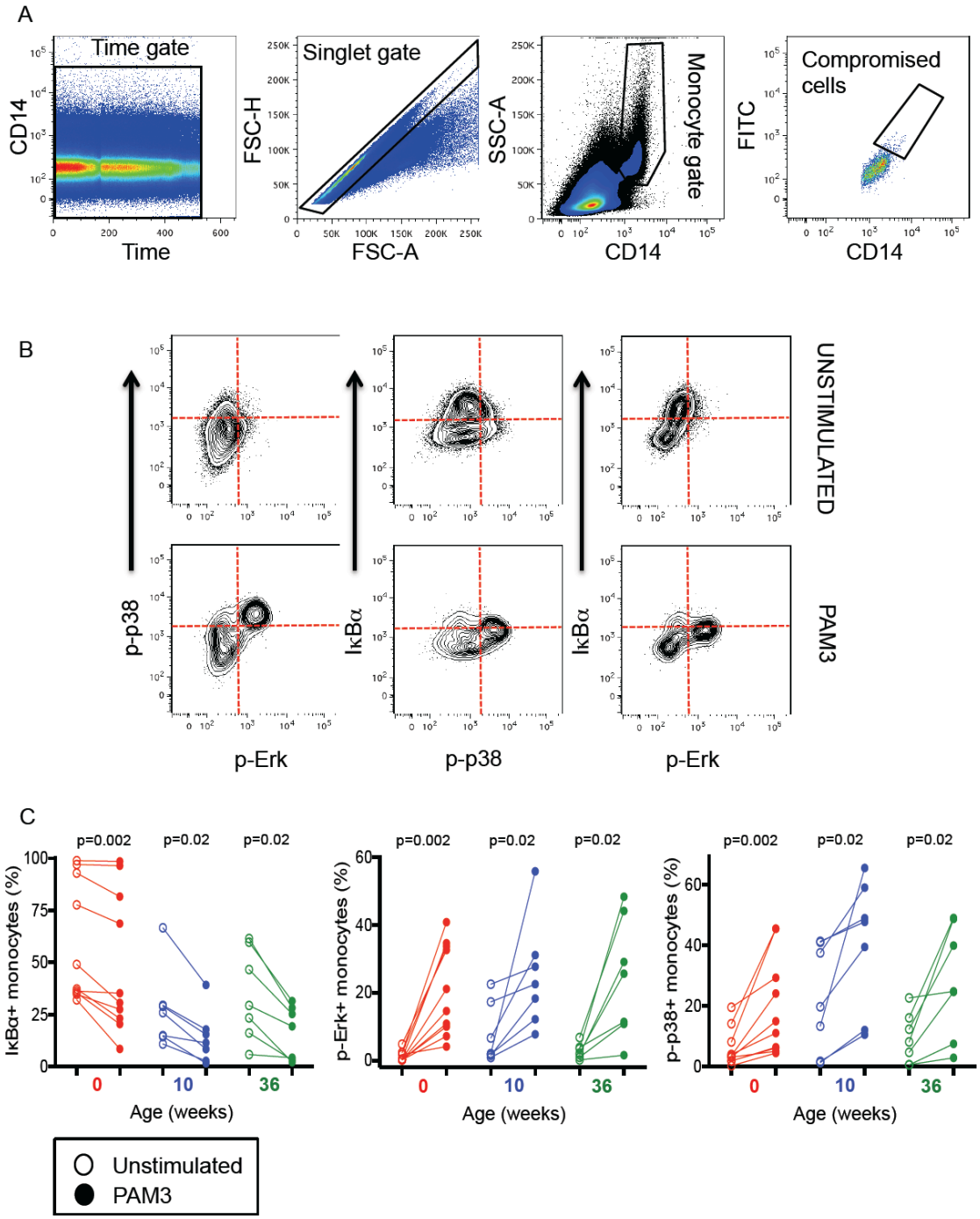
Supplementary Figure 1: Gating strategy of whole blood innate ICS assay. (**A**) Time gate to ensure uniform fluorescence over time during acquisition. (**B**) Singlet gate to exclude cell doublets. (**C**) Leukocyte gate. (**D**) Identification of CD66a/c/e^{+} granulocytes. (**E**) Identification of CD14^{+} HLA-DR^{+} monocytes. (**F**) Identification of CD14^{-}HLA-DR^{+}CD11c^{+} mDC.

Supplementary Figure 2



Supplementary Figure 2: Integrated median fluorescence intensity (iMFI) of BCG-induced cytokine expressing cells. iMFI of BCG-induced cytokine expression by innate cells was calculated by multiplying the frequency of cytokine$^+$ innate cell subsets with the MFI of cytokine$^+$ innate cell subsets for each participant. Shown are frequencies, MFI and iMFI of TNF-α-expressing monocytes in whole blood from newborns, 10 and 36-week old infants. Horizontal lines represent the median, boxes represent the interquartile range (IQR) and whiskers represent the range (n = 25 for each group). Group comparisons were done using the Kruskal-Wallis test (Overall effect), followed by the Mann-Whitney test.

Supplementary Figure 3



Supplementary Figure 3: Gating strategy for monocyte signaling assay and overall response to PAM3. Cryopreserved PBMC were thawed, rested and incubated with medium or PAM3 for 30 minutes, stained and analysed by flow cytometry. (**A**) Hierarchical gates were set as shown: time gate to ensure

uniform fluorescence over time during acquisition; singlet gate to exclude cell doublets; monocyte gate; autofluorescent, compromised cells were identified as shown and excluded from further analyses. (**B**) Representative example of activation of pro-inflammatory signaling molecules (degradation of IkBa, phosphorylation of Erk and p38) in monocytes incubated with medium (unstimulated) or PAM3. Boolean combinations of gates identifying monocytes expressing IkBa, p-Erk and p-p38 were generated to assess simultaneous activation of different signaling pathways, shown in **Fig. 7C and 7G**. (**C**) Frequencies of monocytes expressing IkBa, p-Erk and p-p38 when unstimulated (open circles) or upon PAM3 stimulation (solid circles) from newborns (red; n=10), 10-week-old (blue; n=7) and 36-week-old (green; n=7) infants. Lines connect samples from the same infants; p values were calculated using Wilcoxon matched-pairs signed rank test.