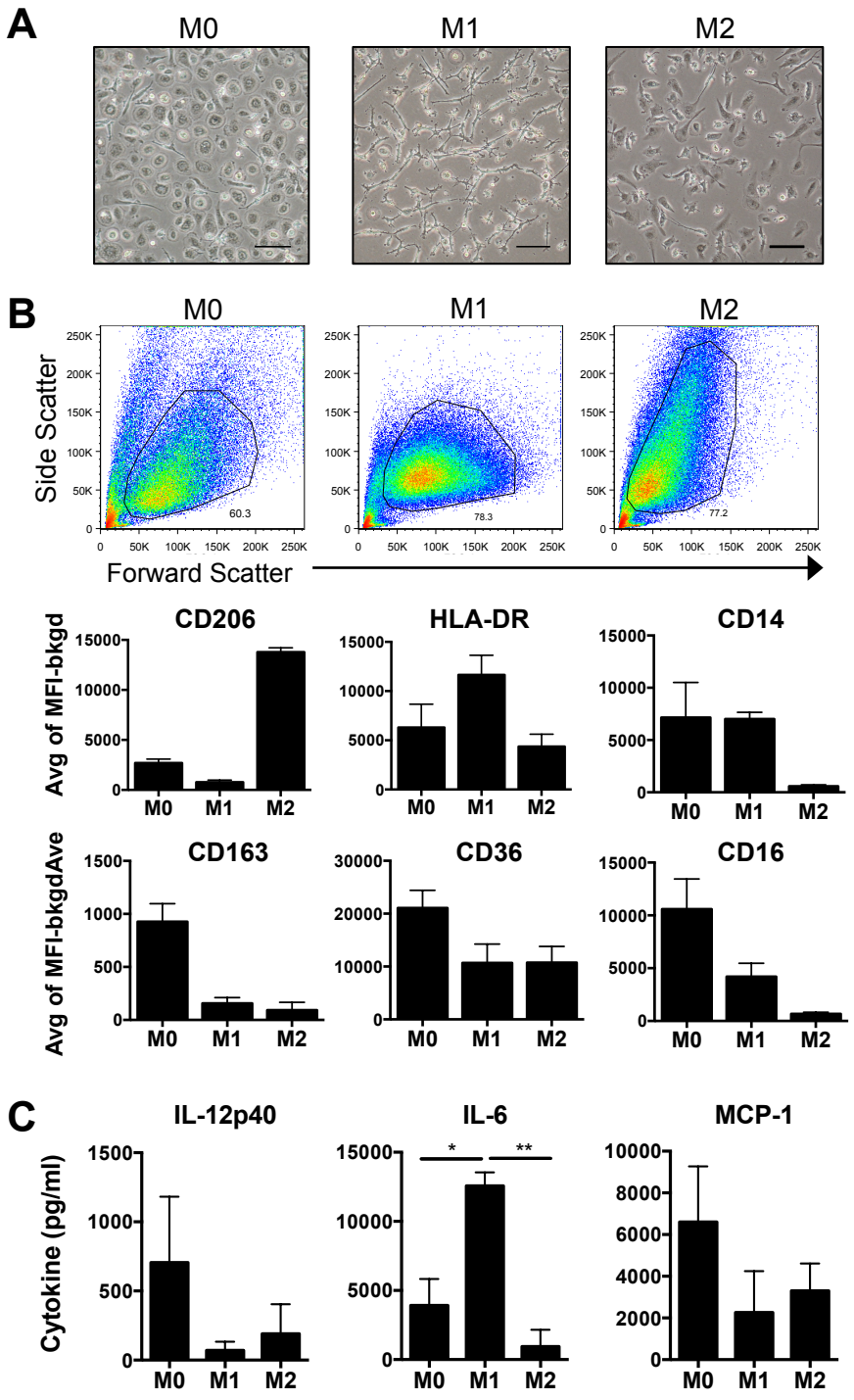

Fig S1 Phenotype of human monocyte-derived macrophages differentiated with M-CSF alone (M0), or with addition of IFNγ and LPS (M1) or IL-4 (M2). (A) Phase contrast images of cells differentiated under M0, M1 and M2 conditions for 7 days. Scale bar = 100 μM (B) Flow cytometry analysis of cell surface markers after 7 days of differentiation. Cells were gated on forward and side scatter as shown, and then for CD33 and CD11b. The geometric mean fluorescence of each marker was measured, and the fluorescence of unstained cells was subtracted. The mean of 3 independent experiments ±SD is shown. (C) Measurement of cytokines in cell culture supernatants 12 hours after infection with SL1344, minus values from mock-infected controls. Mean ±SD of 3 independent expts. *p<0.05, **p<0.01 by one-way ANOVA.

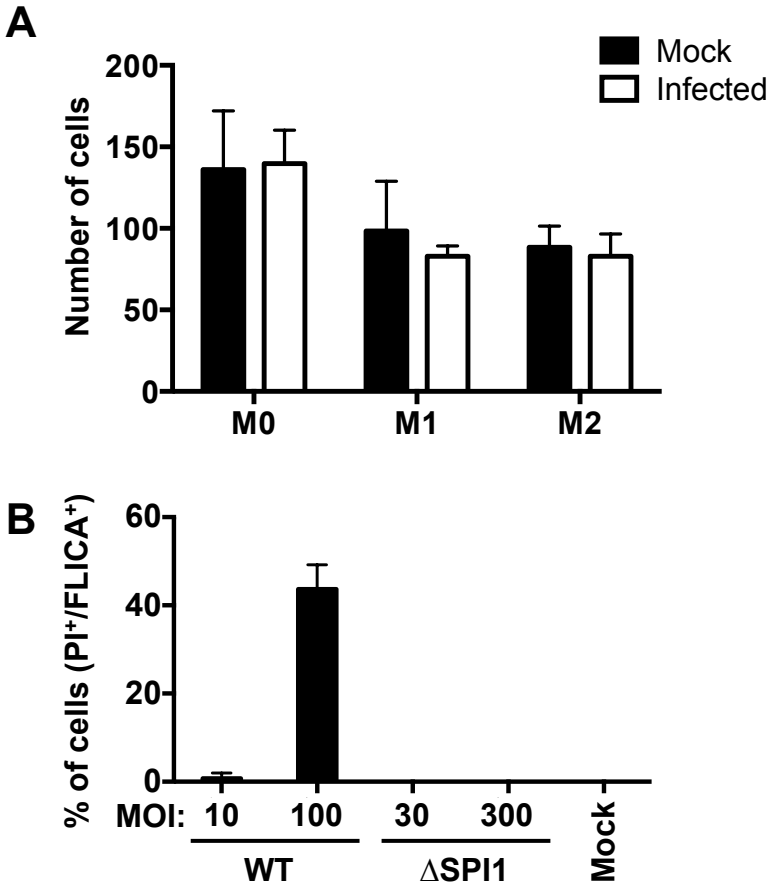

Fig S2 Infection of macrophages with SPI1-expressing bacteria does not induce pyroptosis at a low MOI. (A) Total cell numbers counted in 9 randomly selected fields by DAPI staining of macrophages of the indicated phenotypes, 2 h after infection with WT SL1344 bacteria, or mock infection. Average of 3 independent experiments, ±SD. No statistically significant differences were noted between infected and mock-infected cells. (B) Macrophages of the "M0" phenotype were infected with either WT SL1344 or an isogenic mutant lacking SPI1 at the indicated MOI (see *Materials and Methods*). At 2 h post-infection the cells were examined for propidium iodide (PI) permeability and staining with the FLICA reagent (specific for cleaved caspase-1) as a sign of caspase-1 dependent cell death (pyroptosis). The percentage of total cells scored positive for both is reported. For each condition, in each of three experiments using different donors, 30-50 cells were counted in 6 randomly selected fields. The mean of three independent experiments ±SD is shown.

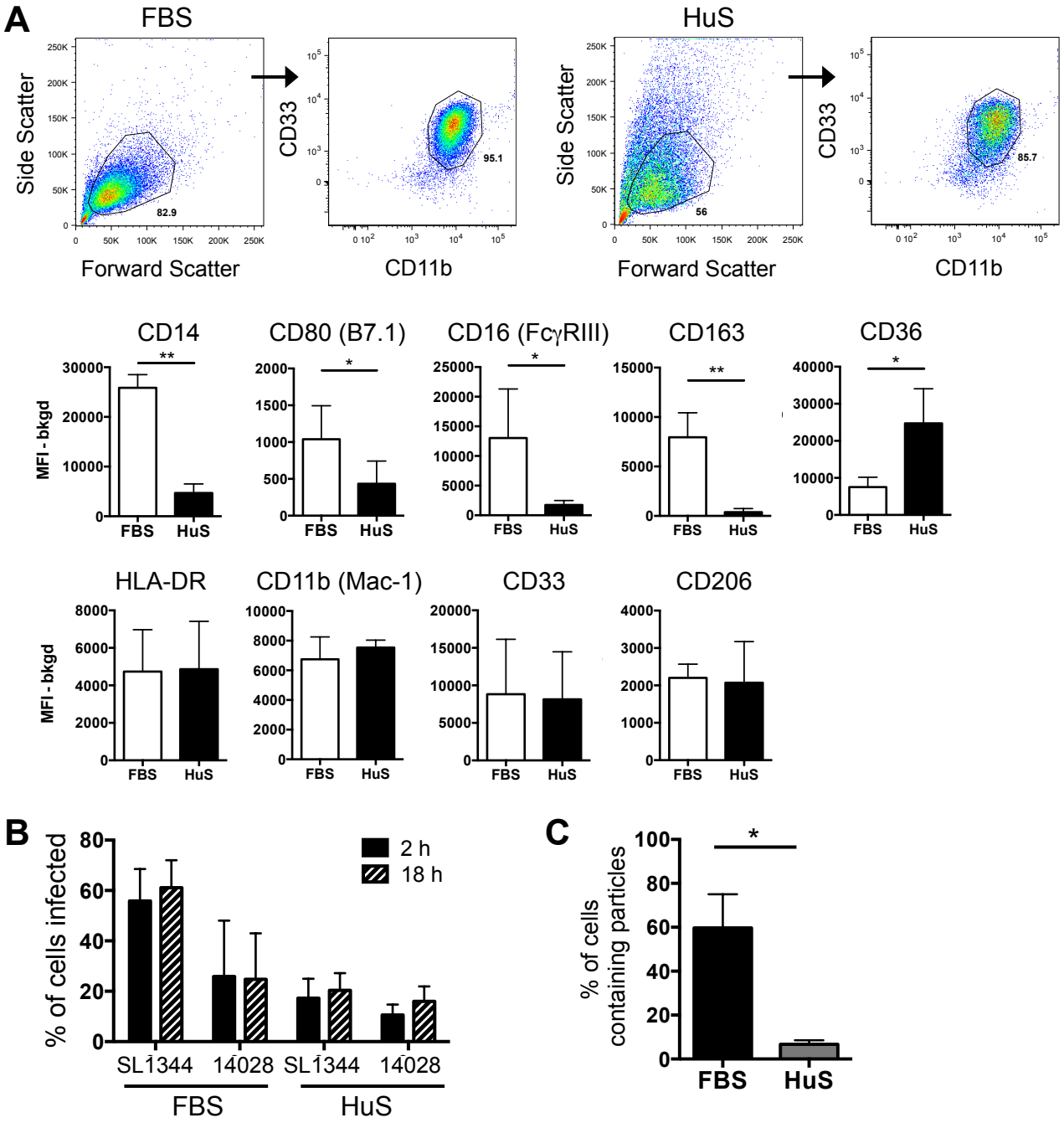

Fig S3 Comparison of cells differentiated with fetal bovine serum (FBS) or human serum (HuS). (A) Cells were differentiated in the presence of FBS or HuS, and after 7 days harvested and stained for flow cytometry. Representative plots show the gating scheme used. The geometric mean fluorescence intensity (MFI) was measured for the indicated marker, and the background fluorescence (bkgd) of unstained cells subtracted. Mean of 4 experiments using cells from different donors is shown, ±SD. *p<0.05, **p<0.01 using paired t test. (B) Cells were infected with strain SL1344 or 14028. At 2 and 18 h p.i. the cells were fixed and stained for IF microscopy. The percentage of cells that were infected at each time point is shown. No statistically significant differences between 2 and 18 h were detected. (C) Phagocytic ability of cells cultured with FBS or HuS. The percentage of cells that internalized killed Alexa488-labled *E. coli* K12 was assessed by IF microscopy. n=3, mean ±SD. *p<0.05 by paired t test.

Movie S1 Live cell microscopy of infection by WT *Salmonella*. Human MDM were infected with WT SL1344 constitutively expressing mCherry. Images were collected every 15 min from 1 h p.i. to 21 h p.i. mCherry and DIC images were taken using a 40x oil objective. Field shows a representative infected cell in 1 of 3 experiments. Time stamp represents hours post-infection.

Movie S2 Live cell microscopy of infection by ΔSPI2 *Salmonella.* Human MDM were infected with ΔSPI2 SL1344 constitutively expressing mCherry. Images were collected every 15 min from 1 h p.i. to 21 h p.i. mCherry and DIC images were taken using a 40x oil objective. Field shows representative infected cells in 1 of 3 experiments. Time stamp represents hours post-infection.