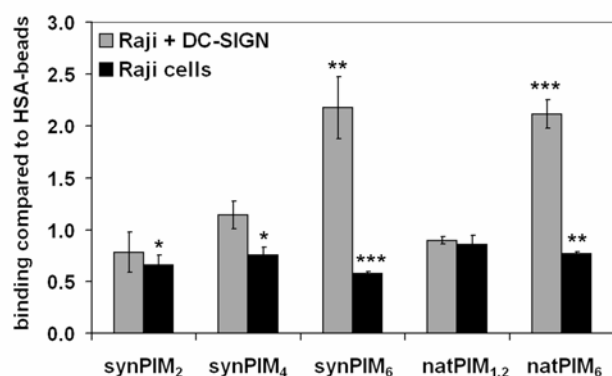
Driessen *et al.*, SUPPLEMENTARY MATERIAL



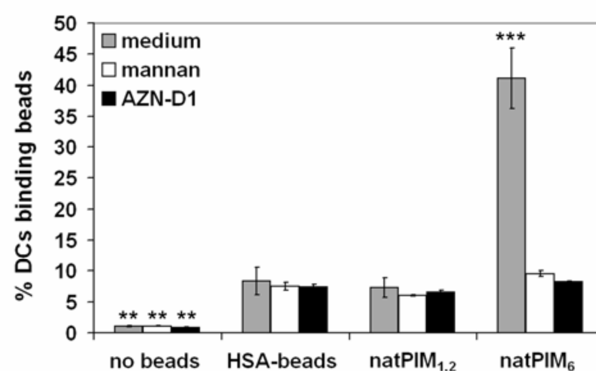
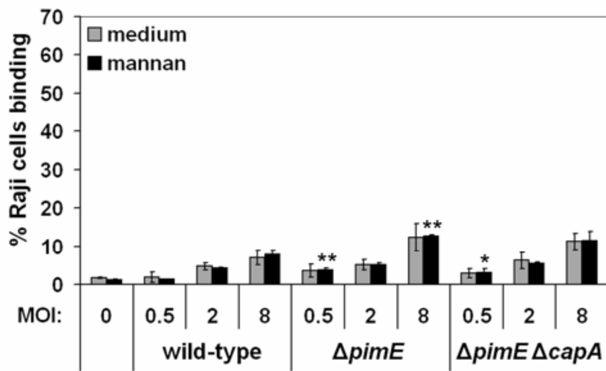


FIG. S1. Binding assay with Raji cells and Raji + DC-SIGN cells. Cells were incubated with beads coated with synthetic and purified phosphatidylinositol mannosides (synPIMs and natPIMs, respectively) at a multiplicity of infection (MOI) of 50 for 45 minutes at 37°C. Percentage of the cell population binding PIM-coated beads was measured using flow cytometry and compared to beads incubated only in human serum albumin (HSA). Shown are means of triplicates and standard deviation. One representative experiment out of five (synPIMs) and two (natPIMs) is shown. These experiments were performed in addition to the binding experiments with dendritic cells (DCs) as described in article (Fig. 2B). *** is $P < 0.0005$; ** is $P < 0.005$; * is $P < 0.05$ compared to control HSA-beads.

FIG. S2. Binding assay with dendritic cells (MoDCs). MoDCs were pre-incubated with 50 µg mL^{-1} DC-SIGN-blocking antibody AZN-D1 (5) or 2 mg mL^{-1} mannan for 30 minutes followed by incubation with beads coated with natPIMs at a MOI of 50 for 45 minutes at 37°C. Percentage of the cell population binding PIM-coated beads was measured using flow cytometry. Shown are means of triplicates and standard deviation. *** is $P < 0.0005$; ** is $P < 0.005$ compared to control HSA-beads (plus medium).



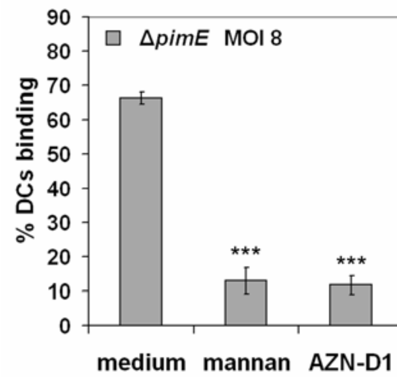
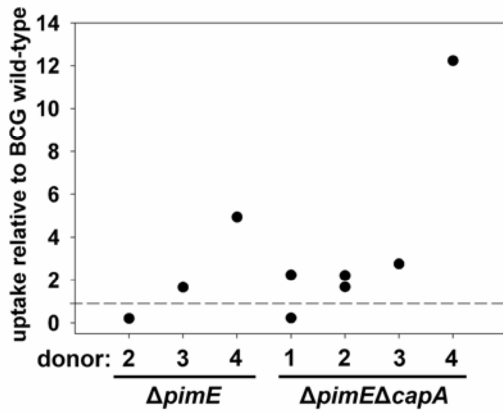


FIG. S3. Binding assay with Raji cells. Cells were incubated with *Mycobacterium bovis* BCG wild-type, Δ*pimE* or Δ*pimE*Δ*capA* at a MOI of 0.5, 2 or 8 for 45 minutes at 37°C in presence or absence of mannan (2 mg mL^{-1}). Percentage of the cell population binding *M. bovis* BCG was measured using flow cytometry. Shown are means of triplicates and standard deviation. One representative experiment out of three is shown. These experiments were performed simultaneously with the binding experiments on Raji + DC-SIGN cells as described in article (Fig. 5). ** is $P < 0.005$; * is $P < 0.05$ compared to wild-type BCG strain at equal MOI.

FIG. S4. Binding assay with MoDCs. MoDCs were pre-incubated with 50 μg mL^{-1} DC-SIGN-blocking antibody AZN-D1 (5) or 2 mg mL^{-1} mannan for 25 minutes followed by incubation with *M. bovis* BCG Δ*pimE* at a MOI of 8 for 45 minutes at 37°C. Percentage of the cell population binding *M. bovis* BCG Δ*pimE* was measured using flow cytometry. One representative experiment out of two is shown. Shown are means of triplicates and standard deviation. *** is $P < 0.0005$ compared to binding of *M. bovis* BCG Δ*pimE* to MoDCs pre-incubated with medium only.



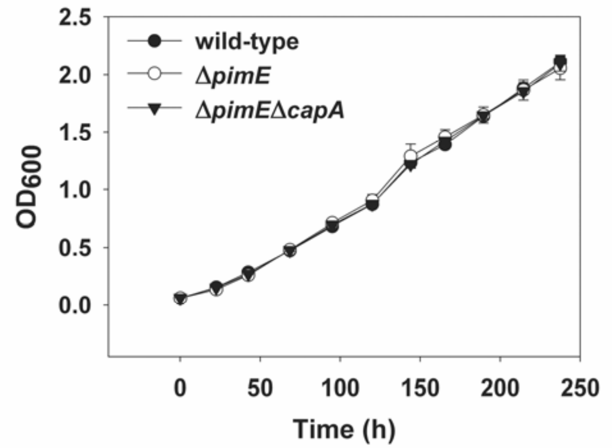


FIG. S5. Relative phagocytic uptake of *M. bovis* BCG Δ*pimE* and Δ*pimE*Δ*capA* as compared to *M. bovis* BCG wild-type. MoDCs were co-cultured with mycobacteria for three hours after which the extracellular mycbacteria were killed by amikacin, followed by washing and lysis of the DCs. The released mycobacteria were plated in serial dilutions for CFU counting. Six experiments were performed with MoDCs from four different donors (two experiments were performed in duplicate with DCs from one donor but with independently grown and prepared bacterial suspensions). The data on the uptake of *M. bovis* BCG Δ*pimE* comprises results from three independent experiments due to unreliable results in the CFU counting of the inoculum in the other three experiments.

FIG. S6. Representative growth curve (out of three) of *M. bovis* BCG wild-type (●), Δ*pimE* (○) and Δ*pimE*Δ*capA* (▼) in Middlebrook 7H9 broth supplemented with 10% Middlebrook ADC enrichment and 0.05% Tween-80 at 37ºC. Shown are means of triplicates and standard deviation.

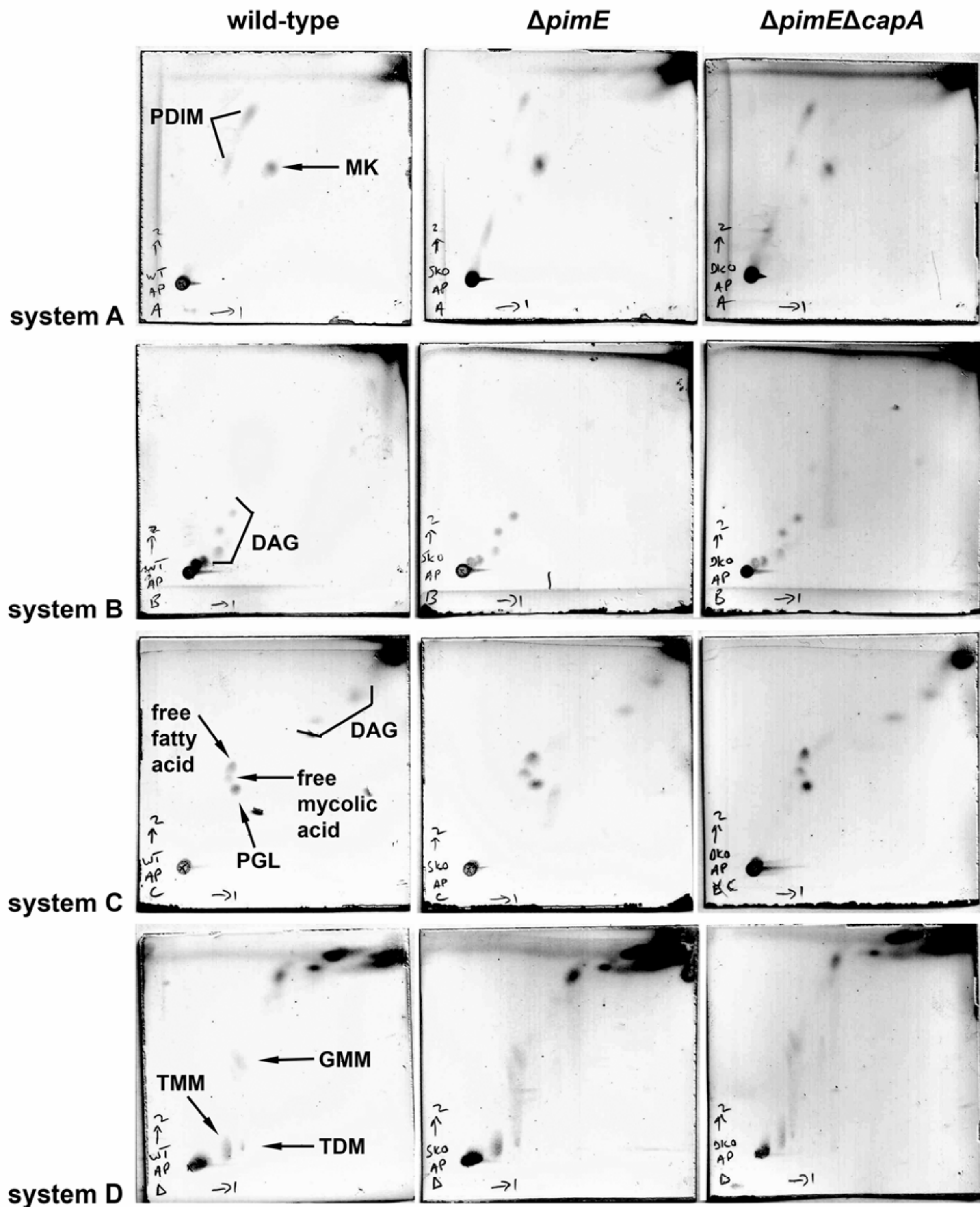


FIG. S7. Two dimensional thin-layer chromatography (2D-TLC) apolar lipid profile of *M. bovis* BCG wild-type, Δ*pimE* and Δ*pimE*Δ*capA*. Cells were harvested by centrifugation, washed once with phosphate buffered saline (PBS) and a small scale lipid extraction performed as described to afford apolar lipids (2). The apolar lipid extracts were resuspended in $CHCl_3$:CH_3OH (2:1) and crude lipids applied to the corners of 6.6 x 6.6 cm pieces of Merck 5554 aluminium backed TLC plates. The

plates were developed in a series of solvent systems, designed to cover the whole range of lipid polarities as described (2). For apolar lipid extracts these systems were named systems A–D and for polar lipid extracts; system E. System A TLCs were run thrice in direction 1 (petroleum ether 60-80: ethyl acetate 98:2) and once in direction 2 (petroleum ether 60-80: acetone 98:2). System B TLCs were run thrice in direction 1 (petroleum ether 60-80: acetone 92:8) and once in direction 2 (toluene: acetone 95:5). Petroleum ether/acetone (92:8). Systems C and D TLCs were run once in each direction using, for system C $CHCl_3$:CH_3OH (96:4) in the first direction and toluene: acetone (80:20) in the second, and for system D $CHCl_3$:CH_3OH:H_2O (100:14:0.8) in the first direction and chloroform: acetone:CH_3OH:H_2O (50:60:2.5:3) in the second. System E TLCs are described in the article. Lipids were visualized by staining with 5% molybdophosphoric acid in ethanol and briefly charring plates at 100°C until lipids appeared and compared to know standards (2). *PDIM* is phtiocerol dimycocerosate; *MK* is menaquinone; *DAG* is diaglycerol; *PGL* is phenolic glycolipid; *TMM* is trehalose monomycolate; *TDM* is trehalose dimycolate; *GMM* is glucose monomycolate.

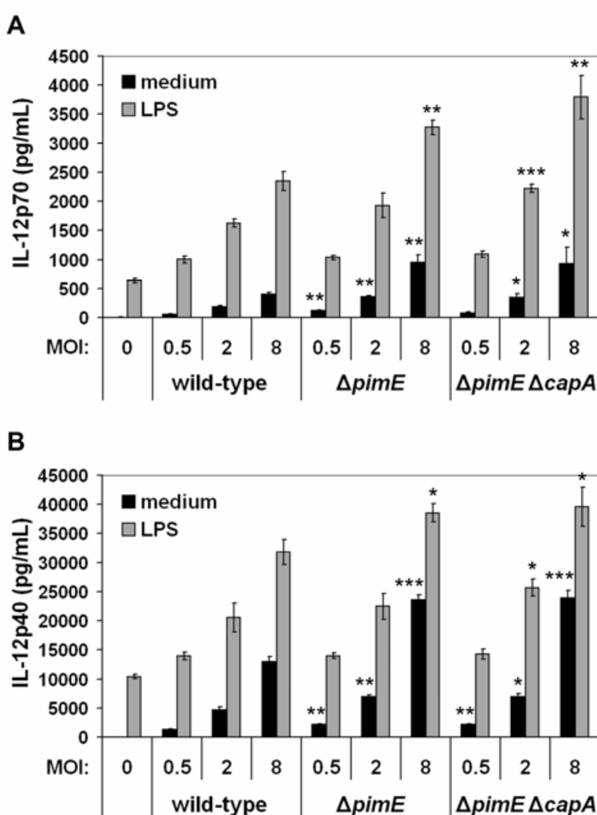


FIG. S8. Induction of IL-12p70 (A) and IL-12p40 (B) secretion in MoDCs by *M. bovis* BCG. MoDCs were co-cultured with mycobacteria for 24 h in absence or presence of LPS after which cytokine secretion was measured by ELISA. Shown are means of triplicates and the standard deviation of one representative experiment (a total of five were conducted). *** is $P < 0.0005$; ** is $P < 0.005$; * is $P < 0.05$ compared to wild-type *M. bovis* BCG at equal MOIs.

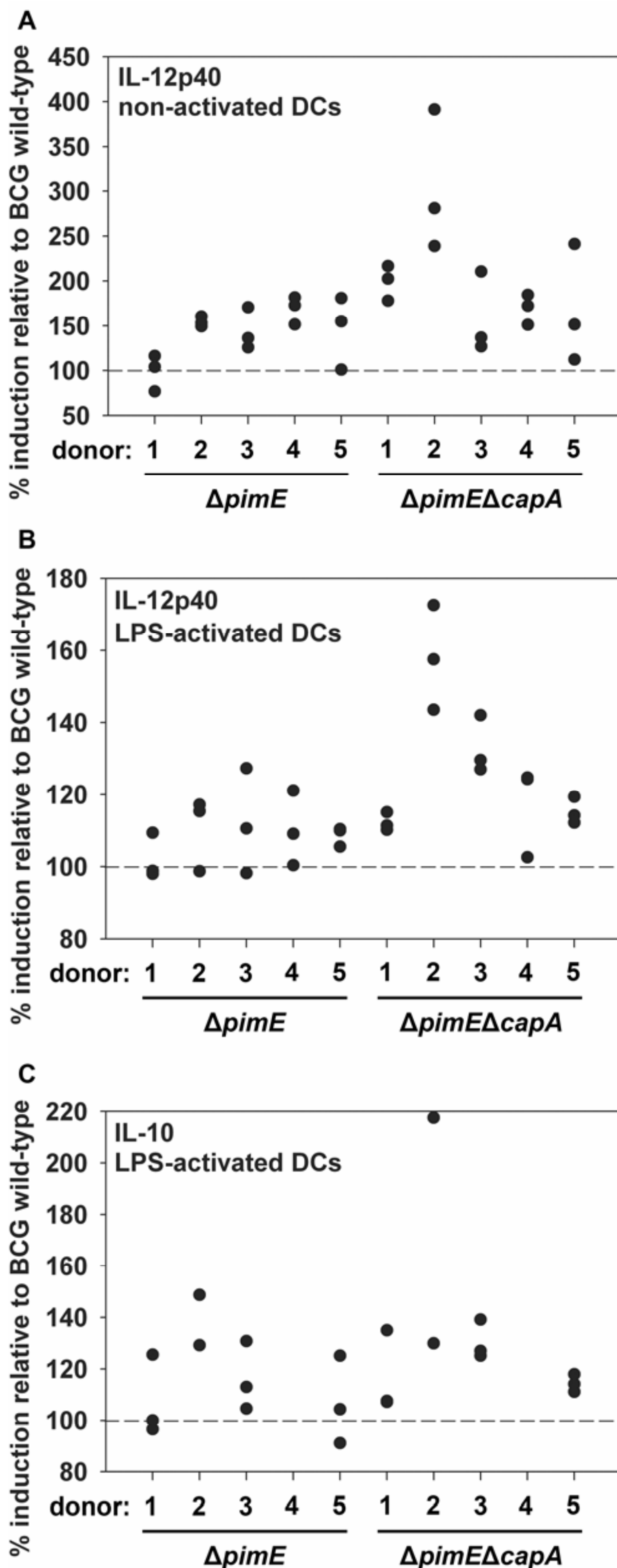


FIG. S9. Cytokine induction in DCs (both non- and lipopolysaccharide (LPS) -activated) was measured in five donors with *M. bovis* BCG wild-type, Δ*pimE* and Δ*pimE*Δ*capA* (at MOI of 0.5, 2 and 8 in triplicates). In all five donors, both non-activated and LPS-activated DCs produced interleukin (IL)-12p40 (figures A and B respectively), whereas detectable interleukin (IL)-10 secretion (> 20 pg mL^{-1}) required LPS-priming (figure C). Donor 4 even fail to produce detectable IL-10 with LPS and donor 2 only produced detectable IL-10 upon induction by BCG at MOI 2 or 8. Shown are the differences in terms of percentage of cytokine induction by BCG Δ*pimE* and BCG Δ*pimE*Δ*capA* relative to BCG wild-type (= 100 %). For each donor the means of triplicates for each of the three different MOIs is plotted (except for donor 2 and 4 in figure C). The ratios of (IL-12p40/IL-10 induced by the mutant strains) / (IL-12p40/IL-10 induced by the wild-type strain) were also calculated for LPS-primed MoDCs from four different donors (the donor that did not produce IL-10 was excluded). These ratios ranged from 0.66 to 1.33 (22 triplicate data sets) with a median of 1.01.

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