

1 **SUPPLEMENTAL FIGURE LEGENDS**

2 **Figure S1. Bacterial strain specific induction of IL-12p70 by MDDCs**

3 IL-10 and IL-12 secretion were quantified in MDDC co-cultures with *Bifidobacterium (B.)*
4 *infantis*, *B. globosum*, *B. animalis*, *Streptococcus (S.) pyogenes*, *Staphylococcus (S.) aureus* or
5 *Pseudomonas (P.) aeruginosa*. All bacterial strains induced the secretion of IL-10 in a dose-
6 dependent manner, while only *B. infantis* did not induce the secretion of IL-12p70 at the
7 bacterial doses tested. Results are shown as the mean +/- SE, n=4 donors.

8

9 **Figure S2. *B. infantis* binding to MDDCs is associated with induction of retinoic acid**

10 **metabolism**

11 (a) *B. infantis* was labeled with PKH26, incubated with MDDCs for 24 hours at which time
12 retinoic acid metabolism was assessed by detection of BODIPY-aminoacetate (BAA) positive
13 MDDCs. PKH26 positive MDDCs, which have bound *B. infantis* and are labeled as *B.*
14 *infantis* +ve, while PKH26 negative MDDCs, which have not bound *B. infantis* are labeled *B.*
15 *infantis* -ve. (b) Following gating of the *B. infantis* +ve and *B. infantis* -ve cells, RALDH
16 enzyme activity is increased in the MDDCs, which have bound *B. infantis*. Unstimulated
17 cells were not incubated with *B. infantis*. (c) The induction of RALDH activity by *B. infantis*
18 is dose dependent, with an optimal bacterial:MDDC cell ratio of 10:1. Results from two
19 independent donors are illustrated.

20

21 **Figure S3. Microbial induction of ALDH1A2 and IDO**

22 *B. infantis*, *B. animalis*, *B. globosum* (5×10^5 or 5×10^6 cells) or LPS were incubated with
23 MDDCs (5×10^5 cells) for 16 hours at which time *ALDH1A2* (a) and *IDO* (b) gene expression
24 were quantified. Only *B. infantis* induced *ALDH1A2* gene expression, while all three
25 Bifidobacterial strains and LPS induced *IDO* expression. Results are shown as the mean +/-
26 SE, n=3 donors.

27

28 **Figure S4. *B. infantis* activates TLR-2 expressing HEK-293 cells**

29 HEK-293 cells, which do not express TLR-2 (TLR-2Neg), remained unstimulated by *B.*
30 *infantis*. HEK-293 cells, which expressed TLR-2 (TLR-2Pos), were able to respond to *B.*
31 *infantis* as demonstrated by NF- κ B activation and IL-8 secretion. Anti-TLR-2 blocking
32 antibody significantly reduced both NF- κ B activation and IL-8 secretion (results are shown as
33 the mean of 3 independent experiments). Cells were stimulated with Pam3CSK4 as the
34 positive control TLR-2 ligand. * $p < 0.01$ versus TLR-2 negative cells; *₁ $p < 0.01$ versus TLR-
35 2Pos cells.

36

37 **Figure S5. TLR-2 and TLR-6, but not TLR-1, are required for optimal NF- κ B and IL- 38 10 induction**

39 HEK-293 cells and MDDCs were stimulated with *B. infantis* plus isotype control antibody
40 (IC), anti-TLR-1, anti-TLR-2, anti-TLR-6 antibodies or combinations thereof. Results are
41 expressed as the mean +/- SE, n=4 independent experiments.

42

43 **Figure S6. *B. infantis* stimulated MDDCs induce Foxp3+ T Cells**

44 (a) *B. infantis* stimulated MDDCs were co-cultured with autologous CD4+ T cells for five,
45 seven or nine days. T cells were co-cultured with *B. infantis* stimulated MDDCs alone or
46 were re-stimulated with anti-CD2/CD3/CD28 antibodies for two days prior to analysis.
47 Induction of Foxp3 by unstimulated MDDCs was also assessed under both culture conditions
48 and at each timepoint. The data was normalized by subtracting the unstimulated MDDC-T
49 cell Foxp3+ levels from *B. infantis* stimulated MDDC-T cell at each timepoint with or
50 without re-stimulation. Peak induction of Foxp3+ lymphocytes occurred at seven days co-
51 culture with *B. infantis* stimulated MDDCs. (b) MDDCs were stimulated with three different
52 *Bifidobacteria* strains followed by co-incubation with autologous CD4+ T cells. *B. globosum*
53 and *B. animalis* induced T-bet expression while *B. infantis* did not. Results are shown as the
54 mean +/- SE, n=3 donors.*p<0.05 versus non-stimulated MDDCs.