**Supplementary**

**Table S1.** Trained immunity induced per training stimulus, compared to non-trained cells. after TLR7/8 (R848) stimulation (IL-6 production).

| donor | β-glucan | Raw milk | Bovine lactoferrin | Bovine IgG |
|---|---|---|---|---|
| 1 | + | + | + | + |
| 2 | + | + | + | + |
| 3 | + | - | + | + |
| 4 | + | + | 0 | + |
| 5 | + | + | - | + |
| 6 | - | 0 | - | - |
| 7 | + | 0 | 0 | + |
| 8 | + | + | - | + |
| 9 | + | + | | + |
| 10 | + | - | - | - |
| 11 | + | + | - | + |
| 12 | 0 | - | - | - |
| | | | | |
| Training | 10/12 | 7/12 | 3/11 | 9/12 |

+: >10% higher IL-6 production of training stimulus compared to non-trained cells: trained immunity; -: <−10% lower IL-6 production of training stimulus compared to non-trained cells: tolerance. 0: comparable (−10 < % < 10%) IL-6 production of training stimulus compared to non-trained cells.

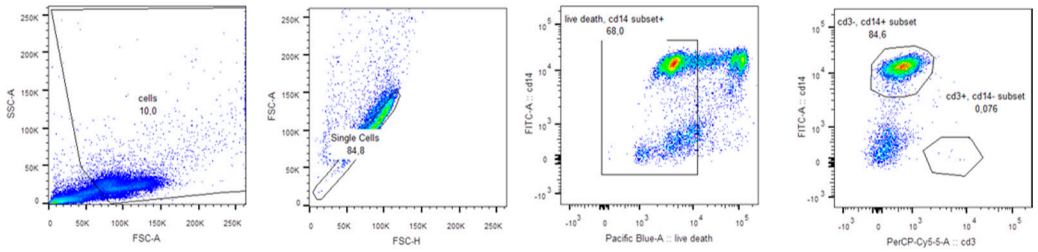


**Figure S1.** Gating strategy and purity of isolated monocytes (84.6%). Staining was performed with live/death staining, CD3 and CD14.

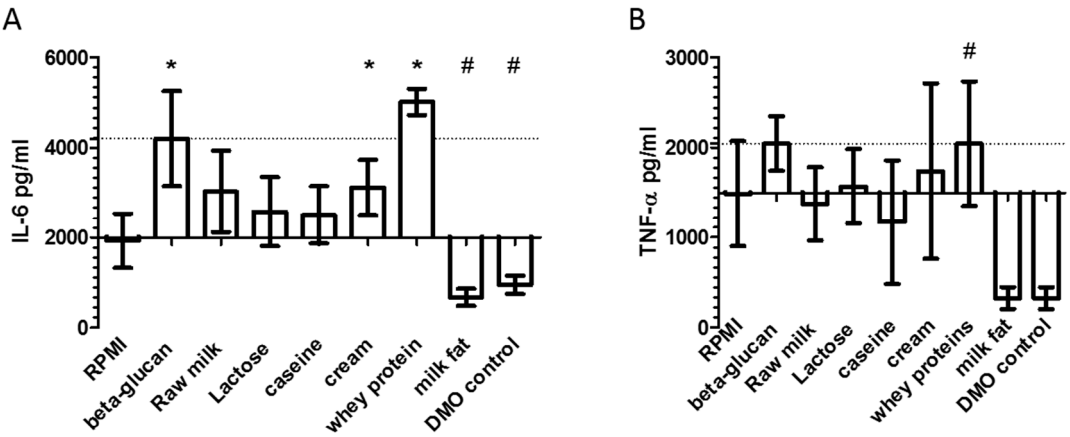


**Figure S2.** Induction of trained immunity or tolerance is dependent on the training of monocytes. Monocytes were stimulated 24 h in the presence or absence of β-glucan (1 μg/mL), Pam3Cysk4 (10 μg/mL), LPS (0.1 μg/mL), or R848 (10 μg/mL); after five days of

rest the differentiated macrophages were stimulated for 24 h with R848 (10 μg/mL. After stimulation with R848, the produced IL-6 and TNF-α (pg/mL) was measured in the supernatant. Data is shown as mean ± SEM, with the IL-6 and TNF-α production of non-trained cells (RPMI) as the *x*-axis. RPMI and β-glucan: $n = 5$; TLR stimuli: $n = 3$. Data was statistically analysed using a paired t-test. * $p < 0.05$,.

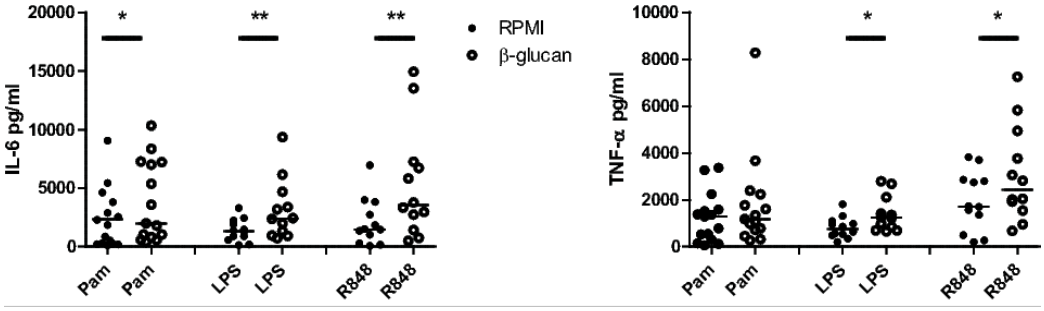


**Figure S3.** Induction of trained immunity by β-glucan. Monocytes were stimulated 24 h in the presence or absence of β-glucan (1μg/mL); after five days of rest the differentiated macrophages were re-stimulated for 24 h with Pam3Cysk4 (Pam) (10 μg/mL), LPS (0.1 μg/mL), or R848 (10 μg/mL). After stimulation with R848 the produced IL-6 and TNF-α (pg/mL) was measured in the supernatant. In 5–6 independent experiments, $n = 15$ (Pam) or $n = 12$ (LPS, R848). Data shown as dot plot with median. Statistics was done by performing a Wilcoxon signed rank test between beta-glucan and RPMI for every secondary stimulation (Pam, LPS, R848). * $p < 0.05$; ** $p < 0.01$.

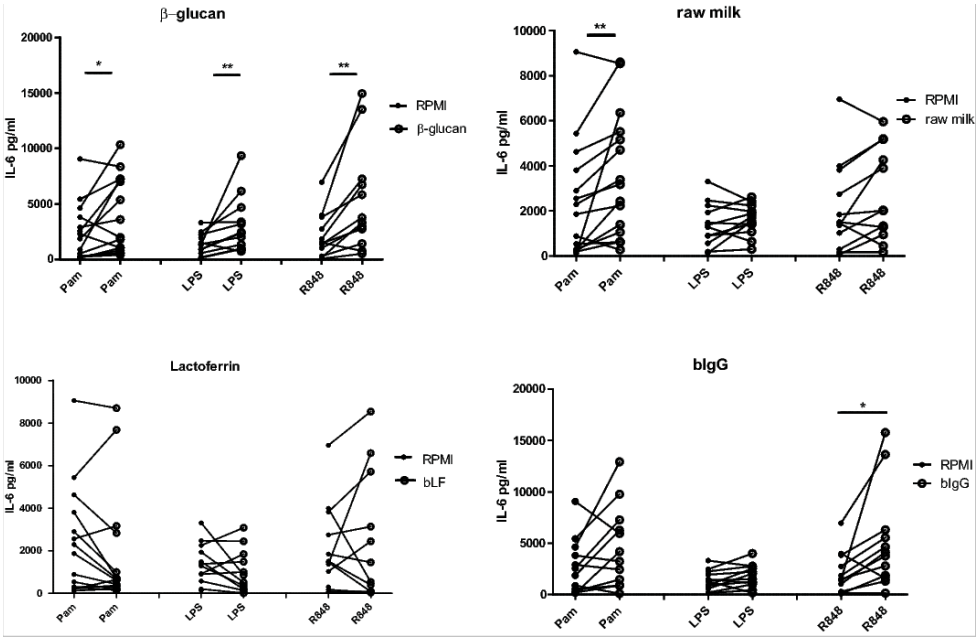


**Figure S4.** Paired analysis plots of monocytes trained with β-glucan, raw milk, bLF and bIgG, and re-stimulated with Pam, LPS, or R848 at day six. In the supernatant of day seven, IL-6 (pg/mL) was measured.