## **Supplementary figures**

**Supplementary Figure 1**. Monocyte purity of different isolation steps. The proportion of T-cells was determined in whole blood, the PBMC fraction after Ficoll density gradient, the purified monocyte fraction after Percoll density gradient and after 1 hour adherence and washing of the purified Percoll isolated monocytes. T-cell contamination is approximately 5% after adherence and washing.



**Supplementary Figure 2.** Increased pro-inflammatory cytokine production is dependent on both training and resting time. **(A)** IL-6 production after re-stimulation in pg/ml; cells were trained for 2h, 4h or 24h with  $\beta$ glucan (upper panel), BCG (middle panel) or oxLDL (lower panel), and rested for 24h, 3d or 6d. **(B)** TNF $\alpha$  production after re-stimulation in pg/ml; cells were trained for 2h, 4h or 24h with  $\beta$ glucan (upper panel), BCG (middle panel) or oxLDL (lower panel), and rested for 24h, 3d or 6d. **(B)** TNF $\alpha$  production after re-stimulation in pg/ml; cells were trained for 2h, 4h or 24h with  $\beta$ glucan (upper panel), BCG (middle panel) or oxLDL (lower panel), and rested for 24h, 3d or 6d (n=6, \* p<0.05, \*\* p<0.01, \*\*\* P<0.001).



**Supplementary Figure 3.** Training induces changes in cell morphology. **(A)** Cell morphology of cells from 2hr-T; 24hr-R trained with RPMI (negative control) βglucan, BCG or oxLDL. Pictures were taken before re-stimulation (20x). **(B)** Cell morphology of cells from 2hr-T; 3d-R **(C)** Cell morphology of cells from 2hr-T; 6d-R **(D)** Cell morphology of cells from 4hr-T; 24hr-R **(E)** Cell morphology of cells from 4hr-T; 3d-R **(G)** Cell morphology of cells from 24hr-T; 24hr-R **(H)** Cell morphology of cells from 24hr-T; 24hr-R **(H)** Cell morphology of cells from 24hr-T; 3d-R



**Supplementary figure 4.** Anti-inflammatory cytokine production is increased in trained monocytes. **(A)** Production of the anti-inflammatory cytokine IL-10. **(B)**. Production of the anti-inflammatory cytokine IL-1Ra increases upon training, not only after restimulation, but also on baseline. Cells were trained for 2h, 4h or 24h with  $\beta$ glucan (upper panel), BCG (middle panel) or oxLDL (lower panel), and rested for 24h, 3d or 6d (n=6,^=p0,06 \* p<0.05, \*\* p<0.01, \*\*\* P<0.001).



**Supplementary Figure 5.** Morphological changes after 24hr training and 6 days resting persist after 24h of restimulation. Cell morphology of cells trained with either RPMI,  $\beta$ -glucan, BCG or oxLDL and restimulated with RPMI, LPS or Pam3Cys.

