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Supplemental Information

Immunometabolic Pathways in BCG-Induced Trained Immunity

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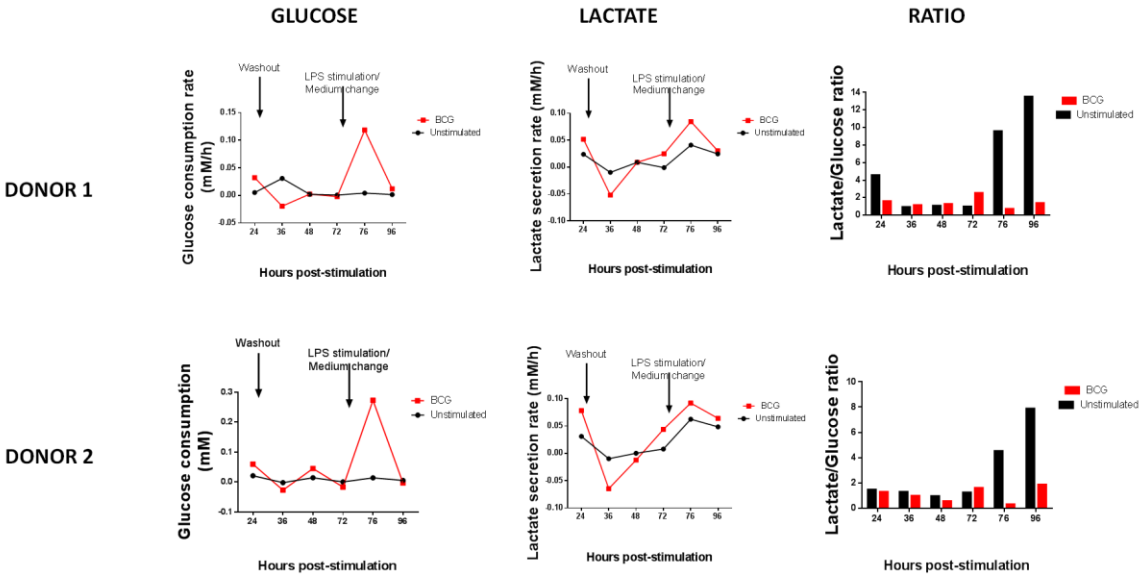


Figure S1. Glucose metabolism, Related to Figure 1. Human monocytes were trained for 24h with BCG or left in medium as control. After 2 subsequent days rest, cells were restimulated with LPS. Glucose consumption and lactate production in the medium were assessed at different time points for 2 donors and lactate:glucose ratios were calculated.

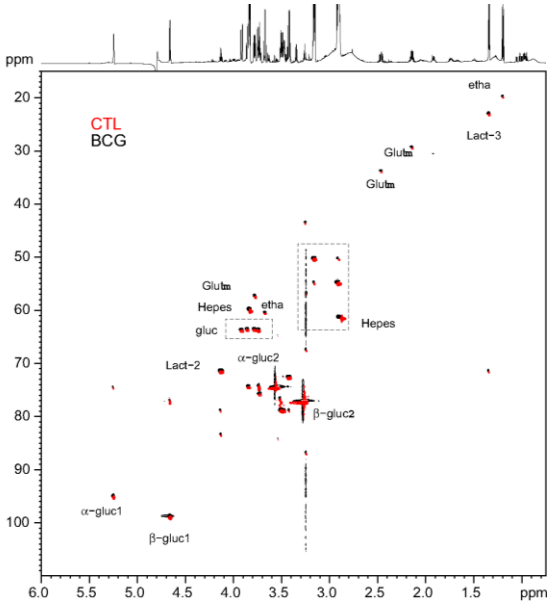
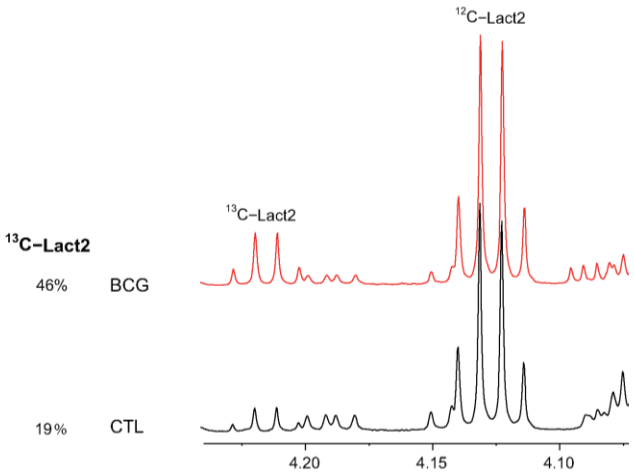
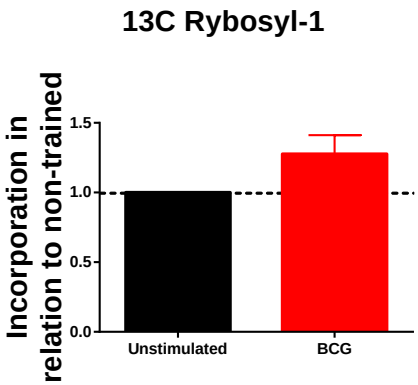


Figure S2. Related to Figure 1 and S3. (A) Spectrum of culture media. Representative ^{13}C-^{1}H-HSQC spectrum of the culture media of human monocytes trained for 24h with BCG (BCG; red) or left in medium as control (CTL; black) following a subsequent 24h incubation with LPS in the presence of 2-^{13}C-labeled glucose. The 2D-spectrum shows the proton resonances (X-axis) directly linked to ^{13}C (Y-axis), with the resonances of 2-^{13}C-glucose (Gluc-2) and 2-^{13}C-lactate (Lact-2) being the more intense (see Figure S3). The chemical shift is expressed in parts per million (ppm). The spectrum displays other peaks corresponding to other compounds that are not resulting from the metabolism of 2-^{13}C-labeled glucose. Gluc- glucose; Glutm- glutamine; Lact- lactate; etha- ethanol.

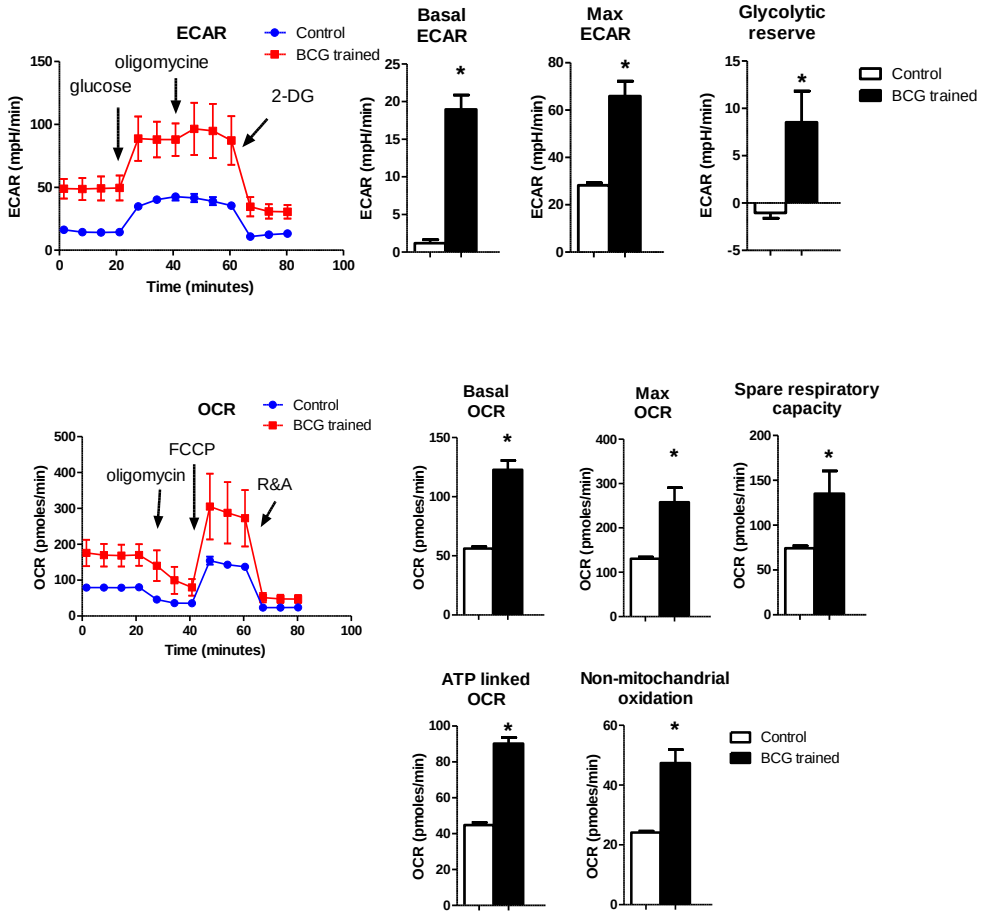


(B) Spectrum of cell extracts. Highlight of a representative 2-lactate ^{1}H-NMR spectrum of cell extracts from human monocytes trained for 24h with BCG (BCG; red) or left in medium as control (CTL; black) following a subsequent 24h incubation with LPS in the presence of 2-^{13}C-labeled glucose. The presence of a ^{13}C splits the resonances of the protons directly linked in two by a *J*-coupling constant, allowing the calculation of the ^{13}C incorporated in each position in comparison with ^{12}C-lactate abundance (≈ 4.15-4.10 ppm). In the case of the position 2 of lactate, the total incorporation of ^{13}C from the 2-^{13}C-glucose is 19 % in the control macrophages and 46% in the BCG-trained macrophages.



(C) ^{13}C-labeled glucose incorportation in Rybosyl. Human monocytes were trained for 24h with BCG or left in medium as control. After 2 subsequent days rest, cells were restimulated with LPS in medium with ^{13}C-labeled glucose. After 24h incorportation in intracellular metabolites was assessed. (Mean ± SD, n=3).

A.



B.

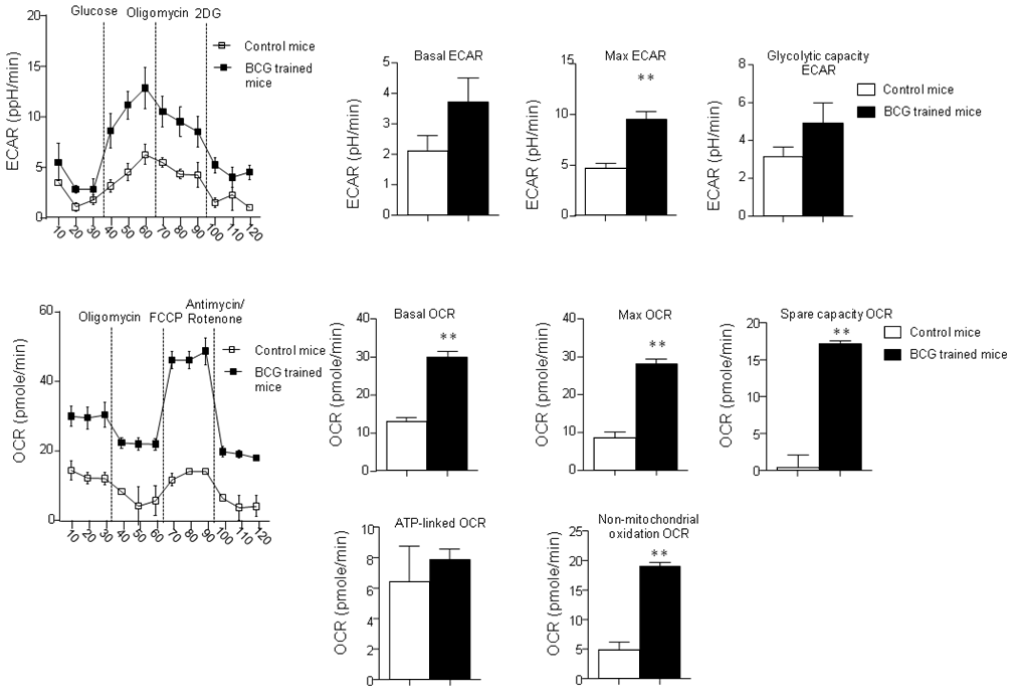


Figure S3. Related to Figure 1C,D. (A) Complete human Seahorse data. Monocytes were incubated for 24h with culture medium or BCG. At day 6 (prior to restimulation) extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were determined by Seahorse. (Mean ± SEM, n=6, * P < 0.05, Wilcoxon signed-rank test). (B) Complete mice Seahorse data. Mice were vaccinated with BCG. After 7 days splenocytes were isolated and ECAR and OCR were determined by Seahorse. (Mean ± SEM, n=6, * P < 0.05, ** P < 0.01, Mann-Whitney test).

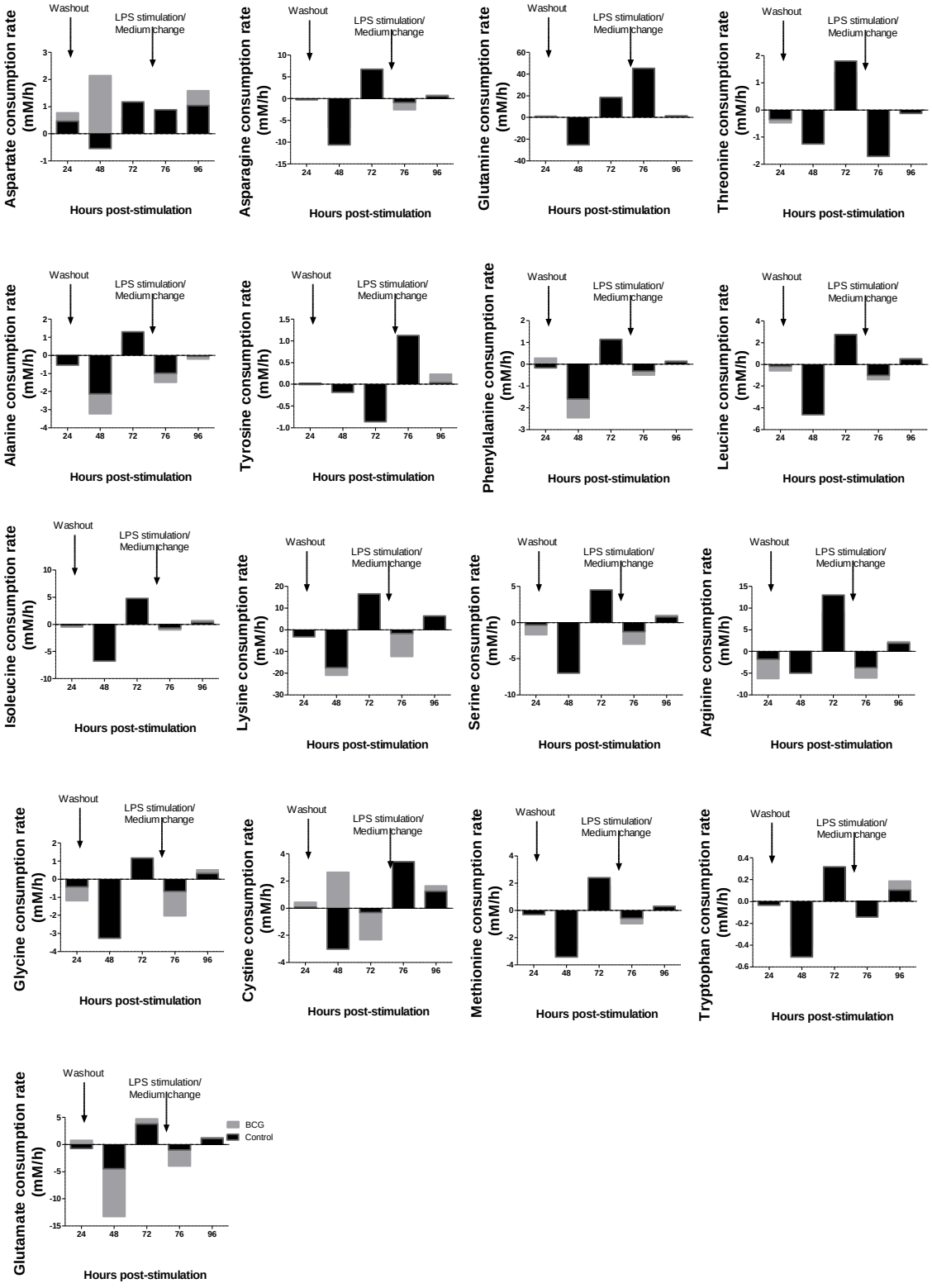


Figure S4. Related to Figure 1. Amino acid consumption from medium. Human monocytes were trained for 24h with BCG or left in medium as control. After 2 subsequent days rest, cells were restimulated with LPS. Amino acid consumption from the medium was assessed at the indicated time points.

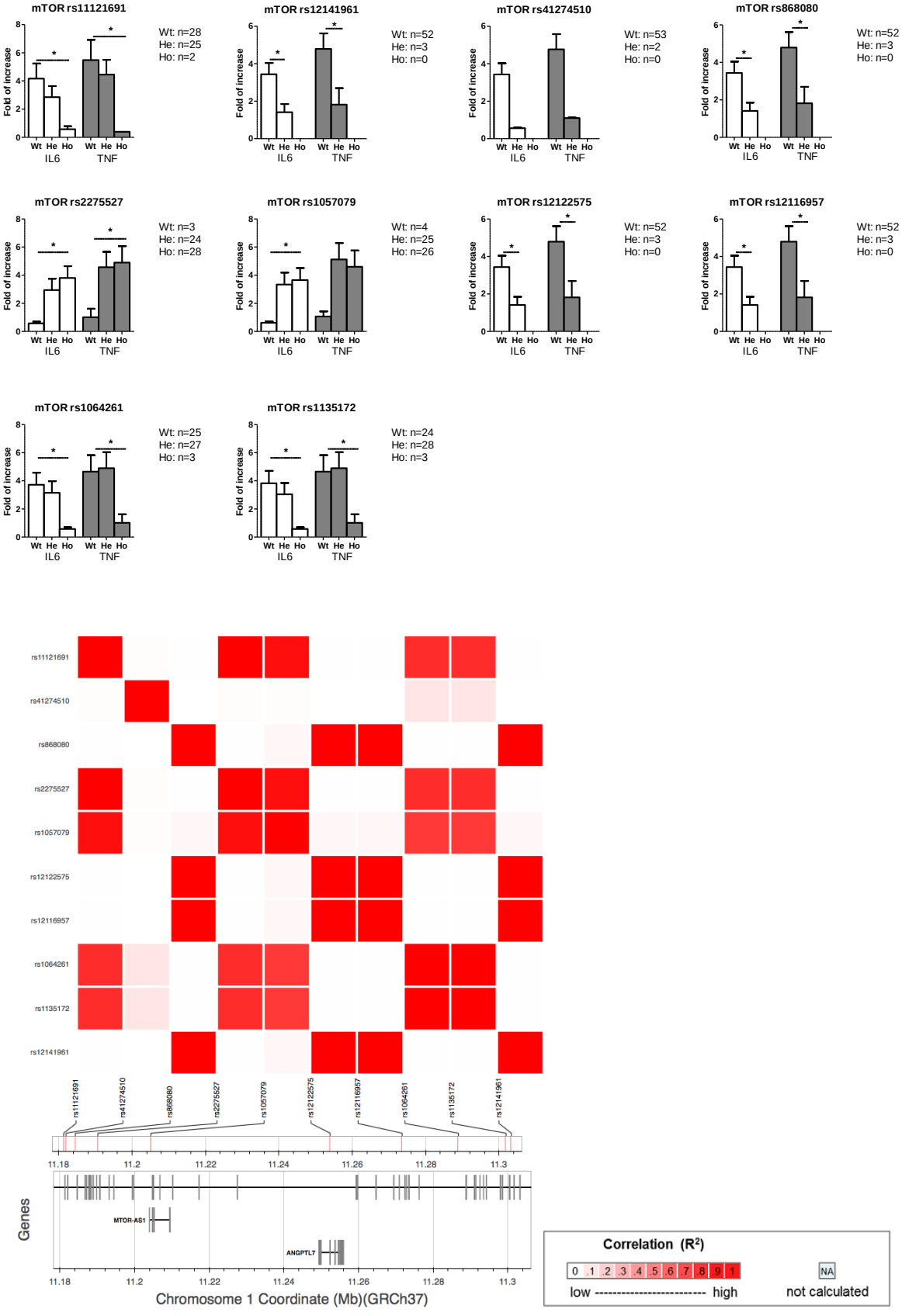


Figure S5. Related to Figure 5. Effect of mTOR SNPs on induction of trained immunity. Blood was drawn of healthy volunteers in 2011 (cohort 1) and SNPs were determined. Adherent monocytes were cultured in vitro as described in figure 1A. The indicated SNPs in mTOR were found to affect the production of IL-6 and TNF upon LPS restimulation after BCG training. (Mean ± SEM, * $P < 0.05$, Wilcoxon signed-rank test). Linkage disequilibria of the displayed mTOR SNPs. Calculated according to the methods of Machiela and Chanock. (Machiela and Chanock, 2015)

Epigenetic promoter primers		
Gene	Forward (5'-->3')	Reverse (5'-->3')
Myoglobulin	AGCATGGTGCCACTGTGCT	GGCTTAATCTCTGCCTCATGAT
H2B	TGTACTTGGTGACGGCCTTA	CATTACAACAAGCGCTCGAC
GAPDH	CCCCGGTTTCTATAAATTGAGC	AAGAAGATGCGGCTGACTGT
ZNF UTR	AAGCACTTTGACAACCGTGA	GGAGGAATTTTGTGGAGCAA
TNF	GTGCTTGTTCCTCAGCCTCT	ATCACTCCAAAGTGCAGCAG
IL6	AGGGAGAGCCAGAACACAGA	GAGTTTCCTCTGACTCCATCG
mTOR	ATAAAGAGCGCTAGCCCGAA	GACCCCTCCCGGTGTAATTC
HK2	GAGCTCAATTCTGTGTGGAGT	ACTTCTTGAGAACTATGTACCCTT
PFKP	CGAAGGCGATGGGGTGAC	CATCGCTTCGCCACCTTTC
GLS	CCAGAGCCCCTAGTACCCAA	TTGGCGATTAGGGCAGTCAA
GLUD	GAAGTCCGTCCTCCCCGTTA	TTTTAAGCCGCAGCTTCCTG
qPCR primers		
Gene	Forward (5'-->3')	Reverse (5'-->3')
HPRT	CCTGGCGTCGTGATTAGTGAT	AGACGTTCAGTCCTGTCCATAA
mTOR	TCCGAGAGATGAGTCAAGAGG	CACCTTCCACTCCTATGAGGC
HK2	TTGACCAGGAGATTGACATGGG	CAACCGCATCAGGACCTCA
PFKP	ATTGCGGTTTTCGATGCCAC	GCCACAACTGTAGGGTCGT
GLS	AGGGTCTGTTACCTAGCTT	ACGTTCGCAATCCTGTAGA
GLUD	TCGTGGAGGACAAGTTGGT	TTGCAGGGCTTGATGATCC

Table S1. Related to Figure 3. Used primers.

Supplementary references

Machiela, M.J., and Chanock, S.J. (2015). LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics *31*, 3555-3557.