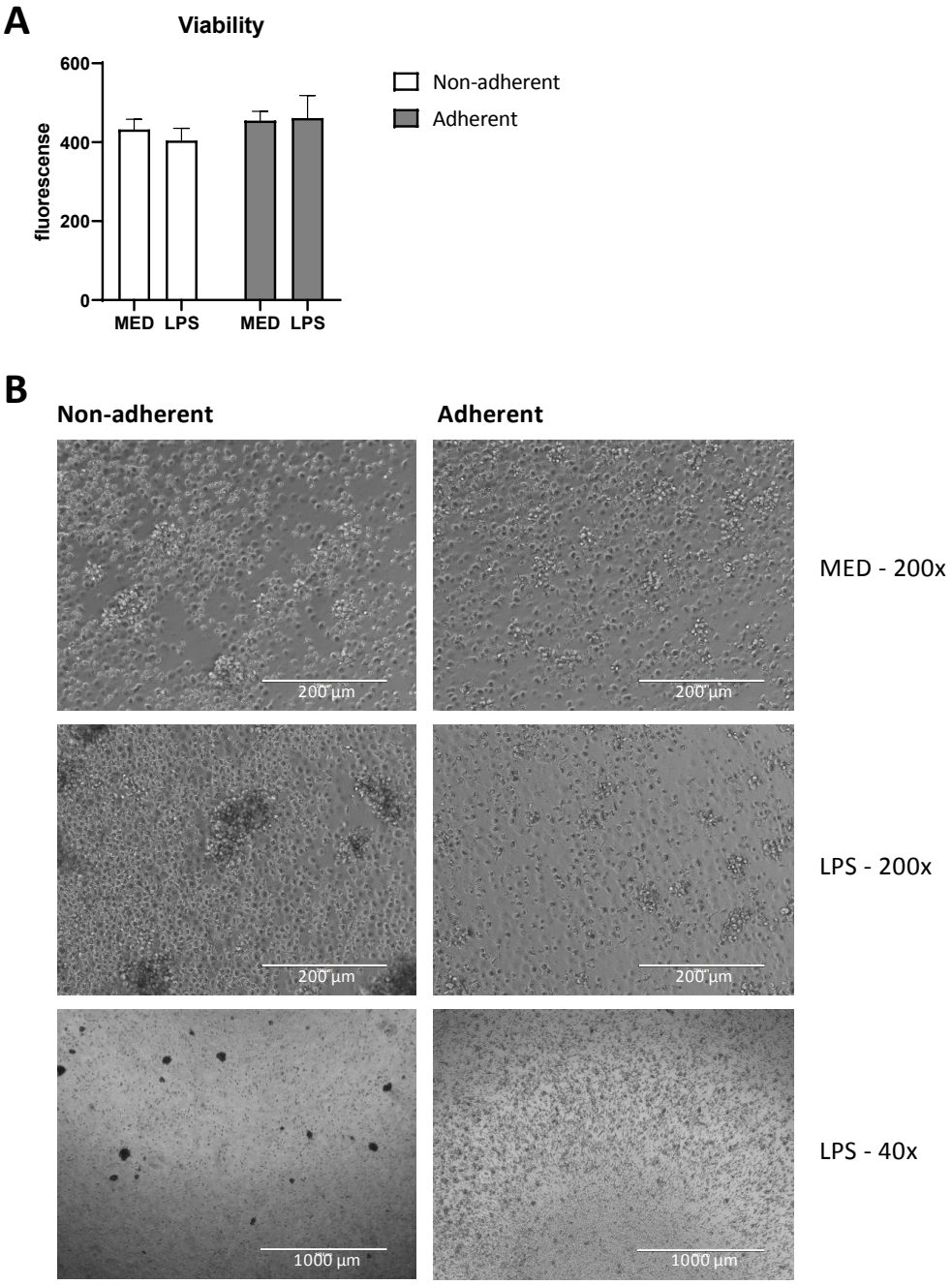
Supplementary Data

Adherence affects monocyte innate immune function and metabolic reprogramming after lipopolysaccharide stimulation in vitro

Natasja A. Otto*,†, Joe M. Butler*,†, Ivan Ramirez-Moral*,†, Michel van Weeghel‡,§,‖,#, Jeroen W.J. van Heijst**, Brendon P. Scicluna*,†,¶, Riekelt H. Houtkooper‡,‖,#, Alex F. de Vos*,†, Tom van der Poll*,†,††

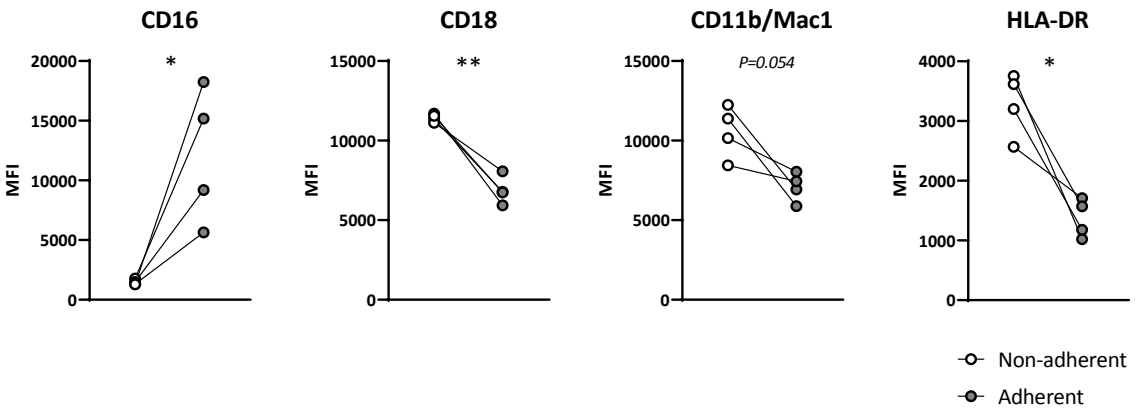
*Center for Experimental and Molecular Medicine; ‡Laboratory Genetic Metabolic Diseases; §Core Facility Metabolomics; ¶Department of Clinical Epidemiology, Biostatistics and Bioinformatics; ††Division of Infectious Diseases, Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands; **Neogene Therapeutics, Amsterdam, The Netherlands.

†Amsterdam Infection & Immunity Institute, ‖Amsterdam Gastroenterology and Metabolism and #Amsterdam Cardiovascular Sciences, Amsterdam, The Netherlands



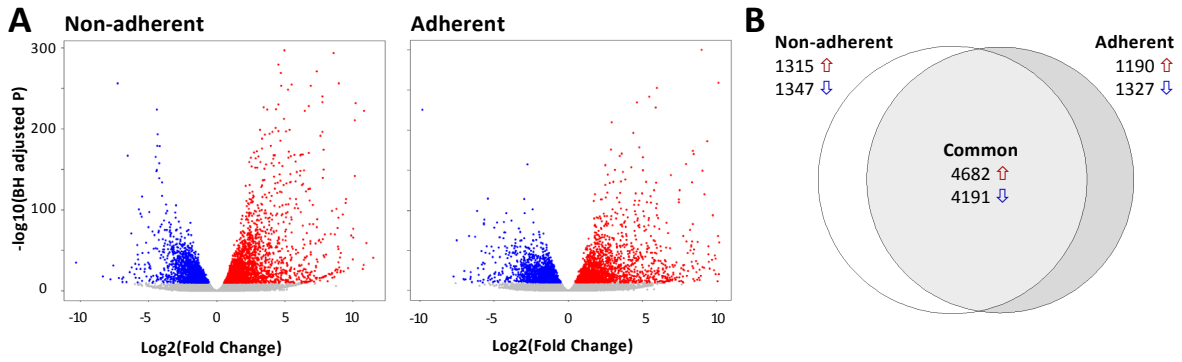
Supplementary Figure 1: Viability and visual appearance of non-adherent and adherent monocytes.

Viability as determined by CellTiterBlue assay (fluorescence (ex530/25, em590/35)) of non-adherent and adherent monocytes after 24 hours of culturing with and without LPS stimulation from 4 independent donors (**A**). Representative pictures of non-adherent and adherent monocytes cultured for 24 hours with and without LPS stimulation (**B**).



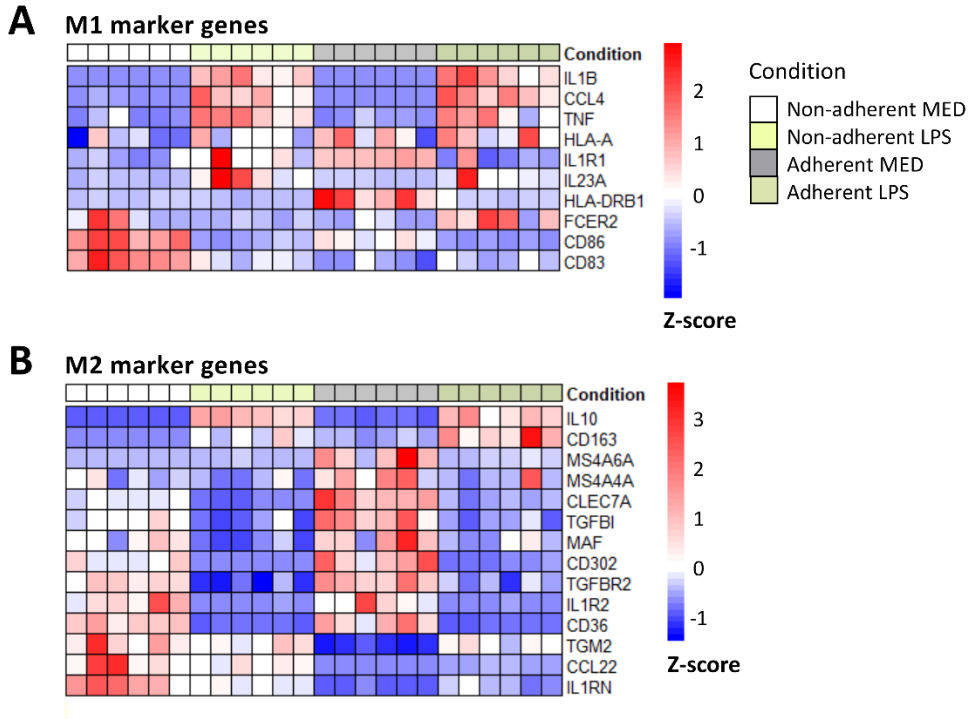
Supplementary Figure 2: Surface marker expression of monocytes under non-adherent and adherent conditions.

Surface marker expression (mean fluorescent intensity (MFI)) of CD16, CD18, CD11b/Mac1 and HLA-DR on monocytes cultured under non-adherent and adherent conditions for 20 hours. Data from 4 independent donors is shown as paired individual values. P-values were calculated using a paired Student's *t*-test. *P<0.05, **P<0.01



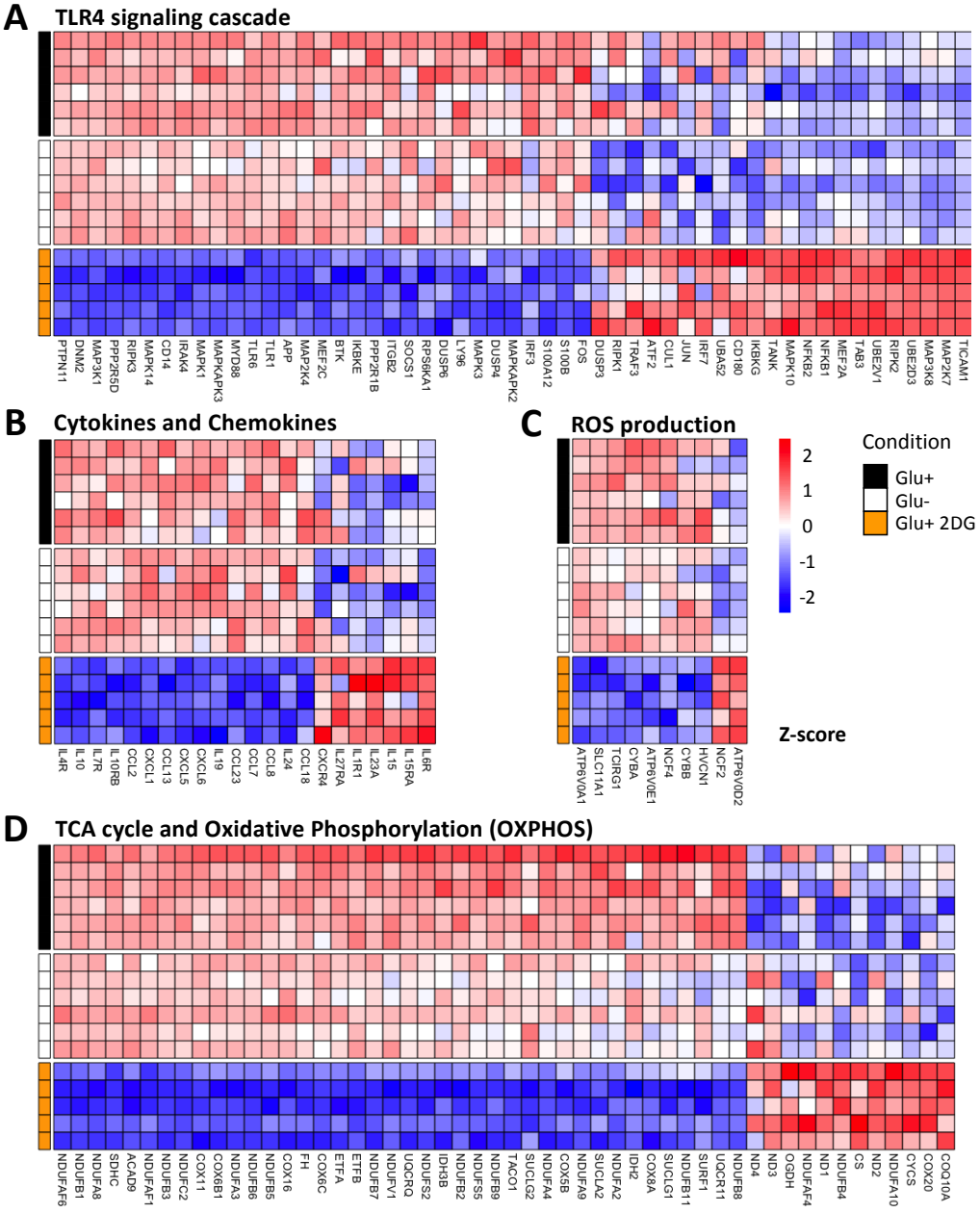
Supplementary Figure 3: Transcriptional responses of LPS-stimulated non-adherent and adherent monocytes.

Transcriptional response of non-adherent (left) and adherent monocytes (right) to LPS compared to medium control shown as volcano plots where red dots indicate significantly upregulated genes, blue depict significantly downregulated and grey dots indicate non-significant genes (**A**). Venn diagram showing the common response and unique responses between non-adherent and adherent monocytes to LPS (**B**). Material used for transcriptomic analysis was obtained from 6 donors in two independent experiments.



Supplementary Figure 4: M1 and M2 marker gene expression of non-adherent and adherent monocytes

Heatmaps comparing the expression of M1 marker genes (**A**) and M2 marker genes (**B**) of non-adherent and adherent monocytes with and without LPS stimulation. Expression is scaled per gene (Z-score). Material used for transcriptomic analysis was obtained from 6 donors in two independent experiments.



Supplementary Figure 5: transcriptional adaptation to glycolysis inhibiting interventions of adherent monocytes

Heatmaps comparing the effect of the different glucose conditions on the transcription of TLR4 signaling (**A**), Cytokine and Chemokines (**B**), ROS production (**C**), and TCA+OXPHOS (**D**) in adherent monocytes after LPS (only showing significant genes (BH adjusted p<0.0005) tested using likelihood-ratio test). Expression is scaled per gene (Z-score). Material used for transcriptomic analysis was obtained from 6 donors in two independent experiments.