Supplementary data

Supplementary material and methods

Microarray analysis

Microarray analysis was performed as described previously (de Wit et al., PloS one, 2016). Briefly, RNA quality was verified, and the Affymetrix Human Gene 1.1 ST array plate was used. Scans of arrays were analyzed using a package from Bioconductor project. Raw signal intensities were obtained by Robust Multi-Array Average method. Probe sets were defined using remapped chip definition file (CDF) based on Entrez gene database. Individual genes were included in the analysis if the UPC value >0.5 (Piccolo et al., PNAS, 2013); the absolute fold change >1.8; and have a false discovery rate $p<0.05$ on at least one array. Data was interpreted using Ingenuity pathway analysis. Microarray data are available upon request.

Supplementary Figures

Supplementary table 1 Differential transcription of cytokine and chemokine genes that were analyzed for protein secretion

Genes	M1	M2	yeast-βG	apple-RGI	shiitake-βG	wheat-AX	Chemoattractant for[1]
CCL1	26.7*	-1.0	8.9*	23.8*	57.0*	238.9*	Monocytes but not neutrophils
CCL5	18.3*	1.6	2.1	9.0*	2.8*	9.6*	Monocytes, memory T-helper cells and eosinophils
CCL20	6.2*	-1.5	2.6	7.3*	4.3*	69.9*	Lymphocytes and, neutrophils, but not monocytes
CCL24	-1.7	7.3*	22.2*	18.9*	121.6*	150.1*	Resting T-lymphocytes, and eosinophils
CXCL8	1.3	-3.6*	13.3*	33.9*	17.2*	51.2*	Neutrophils, basophils, and T-cells, but not monocytes
IL1B	-1.4	-3.7*	8.8*	19.4*	10.7*	32.8*	Neutrophils and macrophages
TNF	11.4*	-1.2	1.5	1.0	2.3*	5.7*	Neutrophils and macrophages
MMP1	-1.0	1.2	3.5	19.4*	6.1*	106.5*	Muscle Cells
CXCL9	481.2*	2.3	2.5	2.2	6.6	1.6	Activated T-cells
CXCL10	239.0*	-1.5	6.1*	2.0	2.3	-2.2	Monocytes and T-lymphocytes
CXCL11	304.0*	1.1	1.9	1.7	1.3	-1.1	Interleukin-activated T-cells but not unstimulated T-cells, neutrophils or monocytes.

Values represent fold changes of gene transcription of M1-like or M2-like macrophages or yeast-βG, apple-RGI, shiitake-βG or wheat-AX-treated macrophages compared to non-polarised macrophages of n=3 different donors as determined with microarray. Statistically significant differences were calculated with IBMT regularised paired *t*-test: * FDR P<0.05.

[1] Adapted from GeneCards (http://www.genecards.org/).

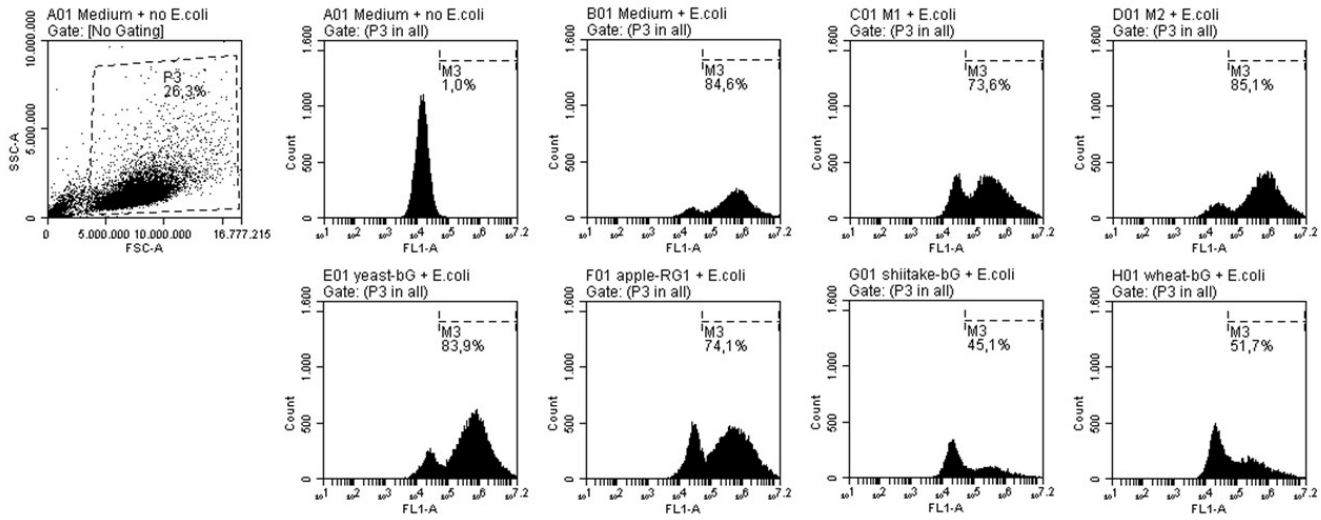
Supplementary table 2 Differential transcription of genes involved in antigen processing

Genes	M1	M2	yeast-βG	apple-RGI	shiitake-βG	wheat-AX
CD80	11.7*	1.7*	2.4*	4.7*	2.2*	4.9*
CD83	2.9*	1.4*	1.1	1.1	1.7*	2.0*
CD274	12.8*	3.0*	2.2*	4.0*	2.5*	5.9*
HLA-DMA	1.3*	1.2	1.0	-1.5*	-1.5*	-1.9*
HLA-DMB	1.3	-1.0	-1.2	-2.2*	-1.7*	-3.1*
HLA-DOA	3.6*	1.7*	-1.2	-1.8*	-1.7*	-3.5*
HLA-DOB	5.6*	1.3	1.1	1.1	1.1	1.1
HLA-DQA2	11.4*	1.1	2.2	2.2	1.5	2.2
HLA-DQB2	3.0*	1.1	1.2	1.3	1.0	-1.1
HLA-F	2.8*	-1.0	1.1	1.2	-1.0	1.0
HLA-L	3.3*	1.1	-1.3	-1.0	-1.1	-1.0

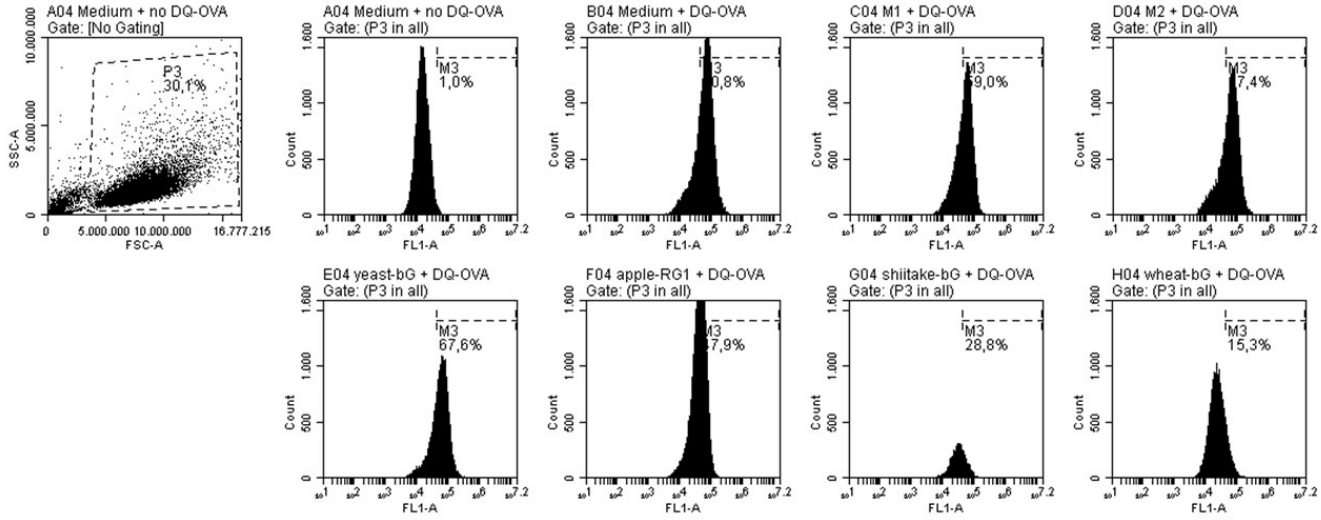
Values represent fold changes of gene transcription of M1-like or M2-like macrophages or yeast-βG, apple-RGI, shiitake-βG or wheat-AX-treated macrophages compared to non-polarised macrophages of n=3 different donors as determined with microarray. Statistically significant differences were calculated with IBMT regularised paired *t*-test: * FDR $P<0.05$.

Supplementary figure 1

A



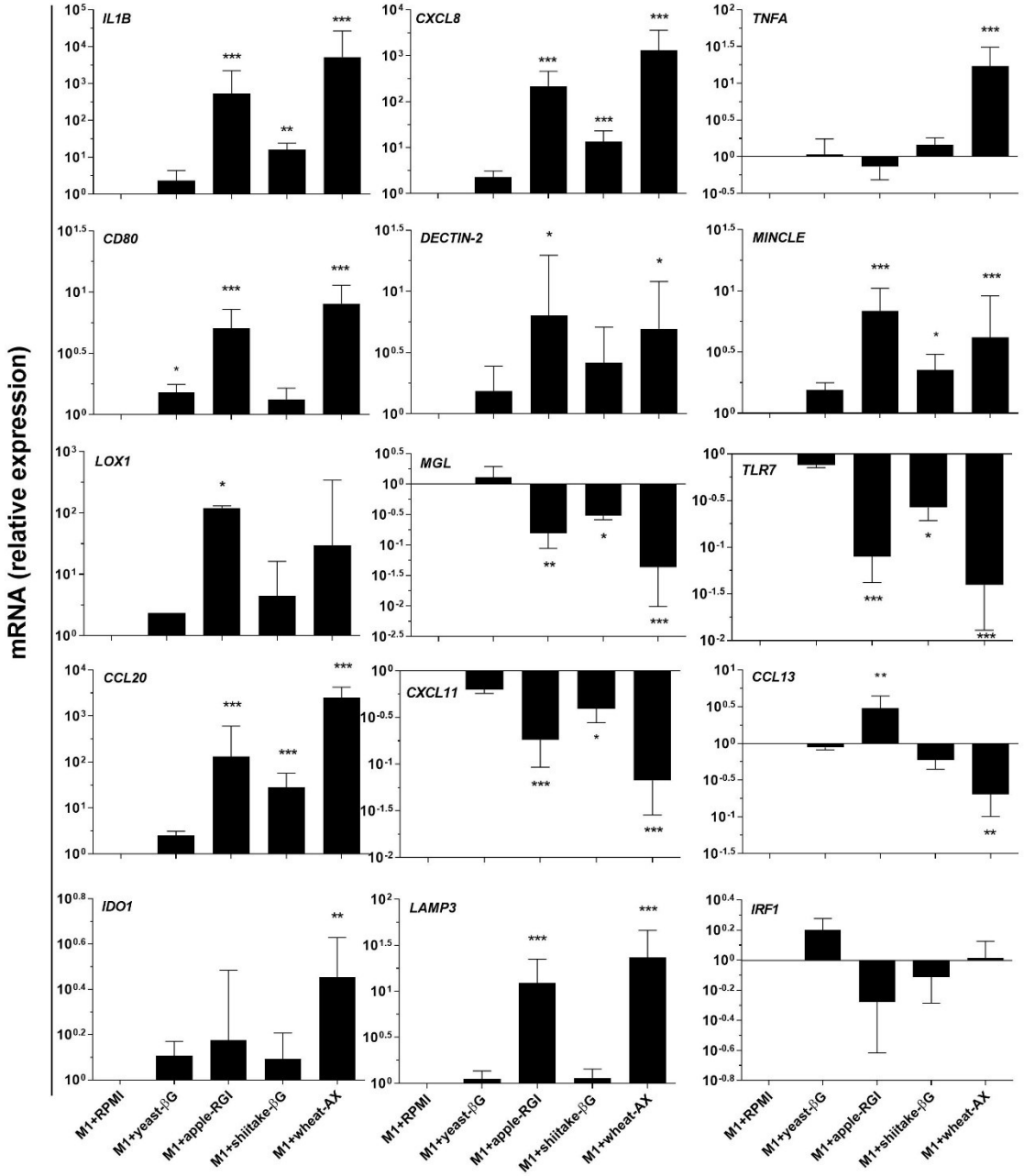
B



NDPs modify antigen processing and endocytosis capacity of non-polarized macrophages. Macrophages were non-treated (medium), polarized towards M1-like or M2-like macrophages or treated with 500 μg/ml yeast-βG, apple-RGI, shiitake-βG or wheat-AX for 18 hours and

subsequently incubated for 1 h with fluorescent *E. coli*-fragments (A) or DQ-OVA (B). Macrophages of n=4 independent donors were measured with flow cytometry, of which representative images are displayed.

Supplementary figure 2



NDPs induced an M(NDP) transcriptional phenotype in M1-like macrophages. M1-like macrophages were stimulated with medium, yeast-βG, apple-RGI, shiitake-βG or wheat-AX at 500 μg/ml for 24 h and analyzed for gene transcription using qPCR. Bars represent mean fold

change ± SD of n=2-3 different donors. Statistically significant differences compared to medium control were analyzed by one-way ANOVA: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.