Supplementary material to:

Gut-derived lipopolysaccharide augments adipose macrophage accumulation but is not essential for impaired glucose or insulin tolerance in mice

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Content:

Figure S1

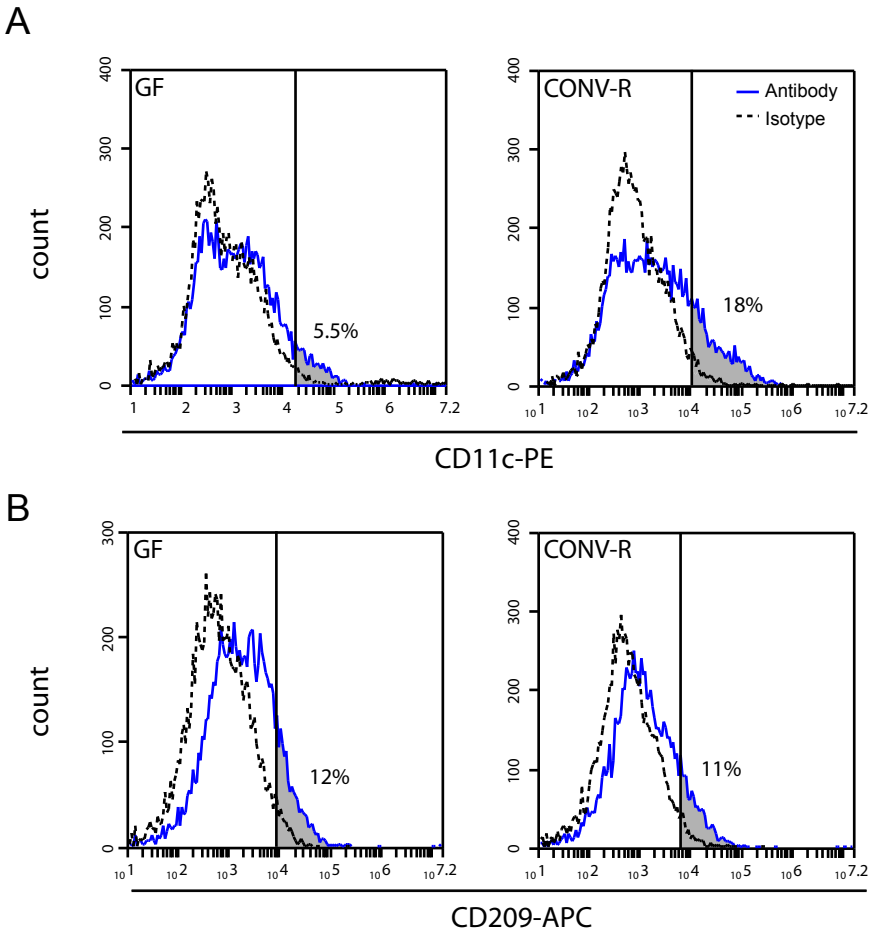

Figure S1. Flow cytometry analysis of CD11c and CD209 of WAT macrophages from GF and CONV-R mice. Flow cytometry analysis of (A) CD11c+ and (B) CD209+ macrophages isolated from epididymal WAT of GF or CONV-R mice. Data from representative samples are shown. Gray areas indicate cells positive for CD11c and CD209.

Figure S2

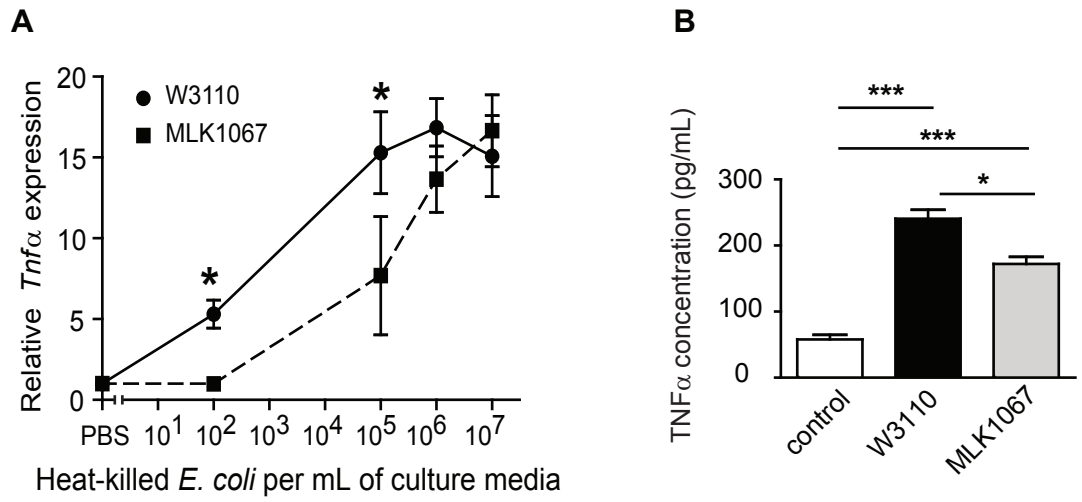

Figure S2. TNFα expression and secretion in RAW 267.7 macrophages after exposure to heat-killed *E. coli.* (A) Cells were stimulated with various concentrations of either wildtype *E. coli* or the isogenic mutant MLK1067 for 1 h and gene expression of *Tnfα* was determined by q-PCR. (n = 4; 2 independent experiments). (B) Cells were stimulated with 100 heat-killed *E. coli* per mL for 4 h and abundance of TNFα in the supernatant was determined by Mesoscale immune-analysis (n = 3). Mean values ± SEM are plotted; **P* < 0.05, ****P* < 0.001.

Figure S3

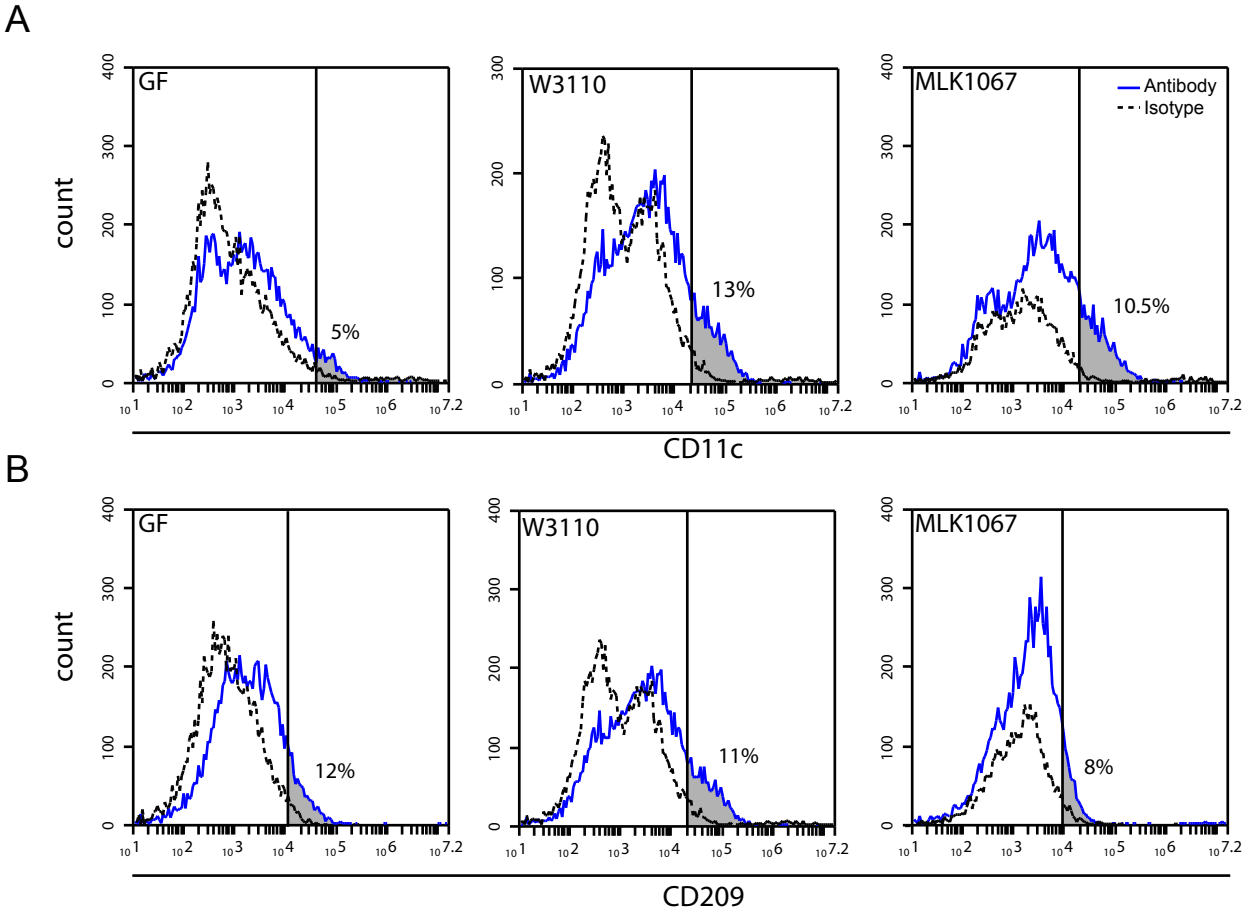

Figure S3. Flow cytometry analysis of CD11c and CD209 on WAT macrophages from GF mice and mice colonized with *E. coli* W3110 or MLK1067. Flow cytometry analysis of (A) CD11c+ and (B) CD209+ macrophages isolated from epididymal WAT of GF mice and mice colonized with *E. coli* W3110 or MLK1067. Data from representative samples are shown. Gray areas indicate cells positive for CD11c or CD209.

Table S1. List of antibodies

Primary antibodies:

Target protein	Product ID	Manufacturer
JNK	9252	Cell Signaling, Danvers, MA
JNK-P	4668P	Cell Signaling, Danvers, MA
Actin	Sc1615	Santa Cruz Biotechnology, Santa Cruz, CA
CD11c-PE	130-091-830	Miltenyi Biotec, Bergisch Gladbach, Germay
CD209-APC	17-2092-80	e-bioscience, San Diego, CA
MAC-2/galectin-3	CL8942AP	Cedarlane Laboratories, Burlington, ON

Secondary antibodies:

Description	Product ID	Manufacturer
goat anti-rabbit IgG-HRP	Sc-2054	Santa Cruz Biotechnology, Santa Cruz, CA
donkey anti-goat IgG-HRP	Sc-2056	Santa Cruz Biotechnology, Santa Cruz, CA
rabbit biotinylated anti-rat IgG	VEBA-4001	Vector lab, Burlingame, CA

Isotype controls:

Description	Product ID	Manufacturer
lgG-PE, Armenian Hamster	Sc-2875	Santa Cruz Biotechnology, Santa Cruz, CA
lgG2a κ-APC, Rat	174321-41	e-bioscience, San Diego, CA
lgG2B-PE, Rat	IC013P	R&D Systems, McKinley Place, MN

Table S2. List of primers

Gene	Direction	Sequence
Emr1	Forward	TGACAACCAGACGGCTTGTG
Emr1	Reverse	GCAGGCGAGGAAAAGATAGTGT
Tnfα	Forward	CCAGACCCTCACACTCA
Tnfα	Reverse	CACTTGGTGGTTTGCTACGAC
Mlg1	Forward	TGAGAAAGGCTTTAAGAACTGGG
Mlg1	Reverse	GACCACCTGTAGTGATGTGGG
Il-10	Forward	AAGGACCAGCTGGACAACAT
Il-10	Reverse	TCTCACCCAGGGAATTCAAA
Saa3	Forward	TGCCATCATTCTTTGCATCTTGA
Saa3	Reverse	CCGTGAACTTCTGAACAGCCT