Figure S1.

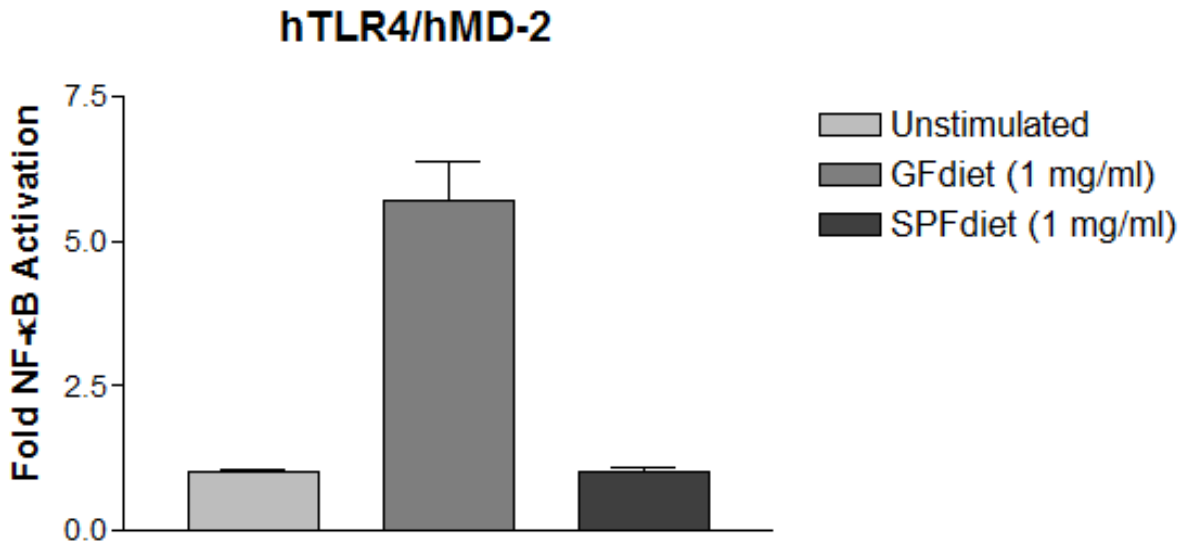


Fig S1. Related to Fig 1. GF diet but not SPF diet elicits TLR4 Activation. Recombinant TLR4 activity was measured using a HEK293 luciferase reporter assay. The relative abilities of SPF diet and GF diet to activate TLR4 were compared. The results were plotted as the mean fold induction (± standard deviation) of triplicate determinations relative to the unstimulated control.

Figure S2A.

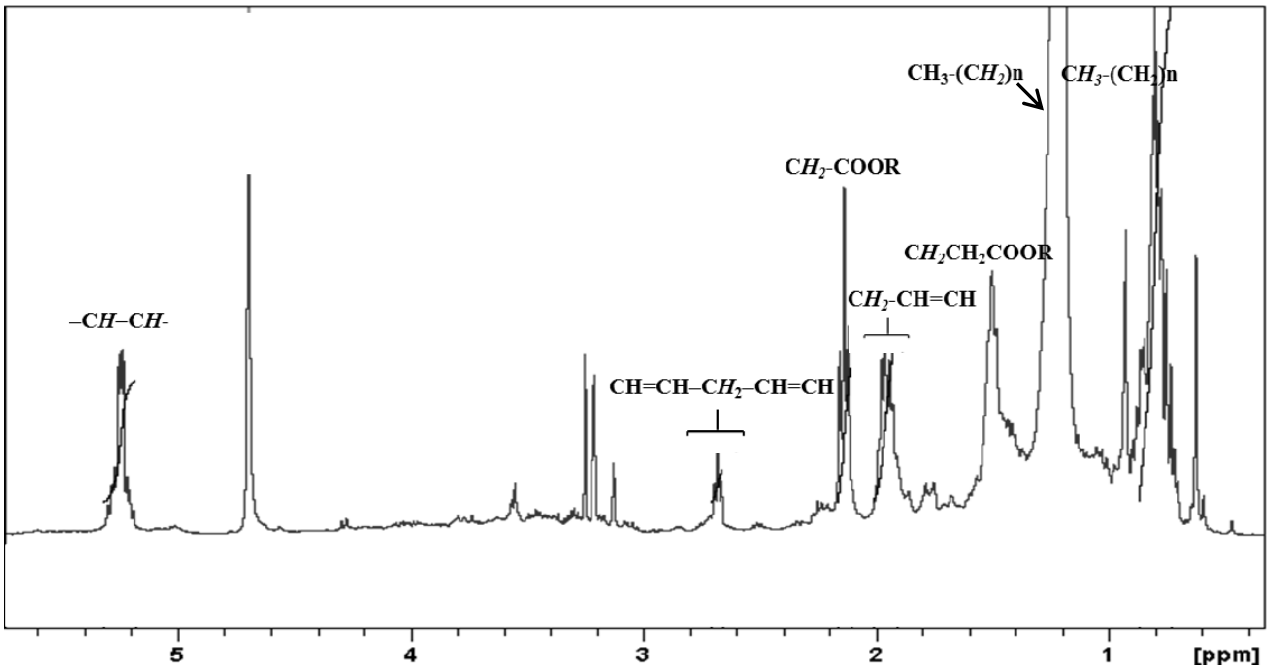


Fig S2A. Part of the ^{1}H NMR spectrum of the purified lipid extract of GF feces showing ^{1}H signals of fatty acid residues. The lipid extract was purified ToyoPearl HW- 40 column and fraction 1 was used for ^{1}H NMR analysis. The signals for protons of both saturated and unsaturated fatty acid residues are indicated in italics and assigned as shown in Table S2.

.

Figure S2B.

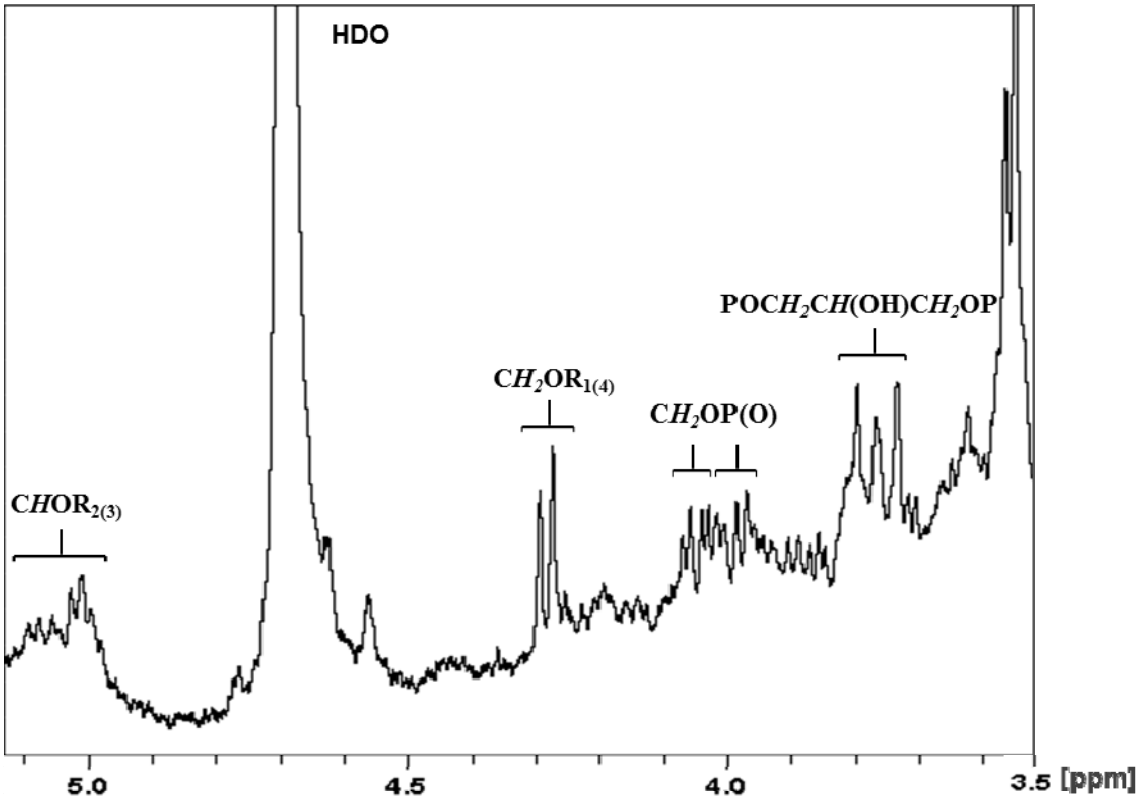


Fig S2B. Related to Fig S2A and Fig 4E. Part of the ^{1}H NMR spectrum of the purified lipid extract of GF feces which shows the signals for protons of the central and side chain glycerol residues of cardiolipin.
Signals for protons of the side chain glycerol residues and of the central glycerol residue of cardiolipin are indicated in italics. R_1- R_4 represent fatty acids residues attached to the side chain glycerol moieties (see Figure 4E). The ^{1}H chemical shifts and vicinal coupling constants $^3J_{H,H}$ are shown in Table 1.

Figure S3.

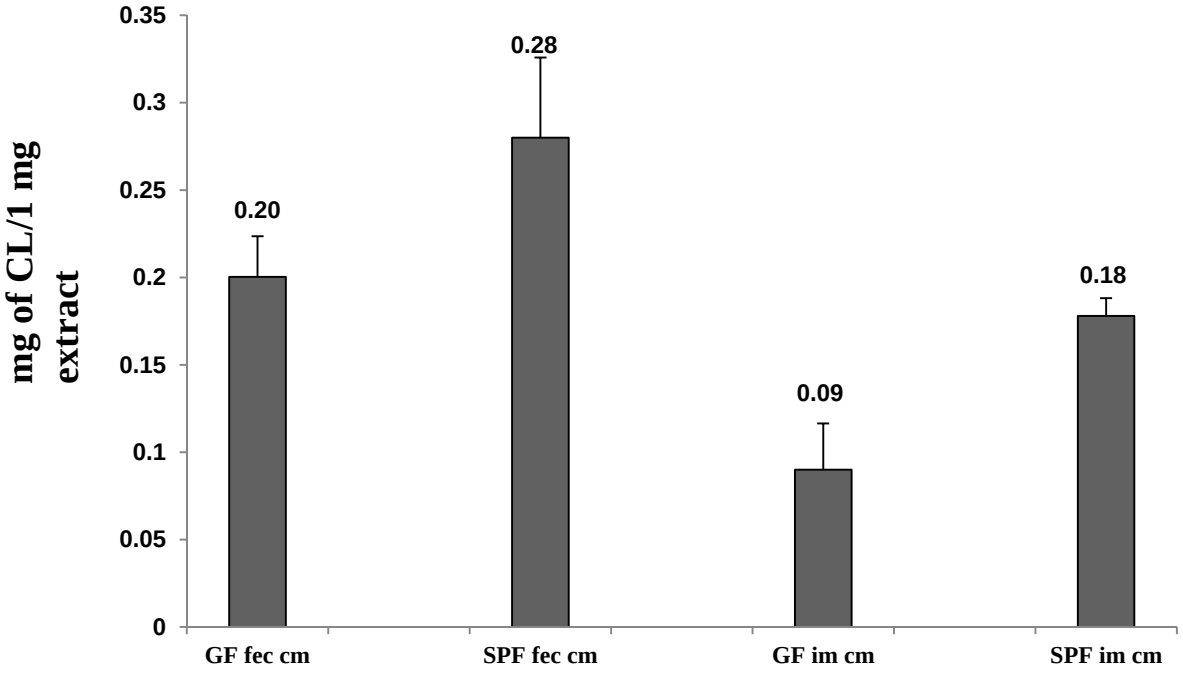


Fig S3. Cardiolipin (CL) content of chloroform-methanol lipid extracts of GF and SPF mice feces and intestinal mucosa. The CL content of $GFfec_{cm}$, $SPFfec_{cm}$, $GFim_{cm}$ and $SPFim_{cm}$ was measured fluorometrically using 10-N-nonyl-acridine orange. The results were determined by linear interpolation from the CL standard curves. The content of CL in each sample is expressed as the amount of CL (mg) in 1 mg of the total lipid extract minus the background (± standard deviation).

Figure S4.

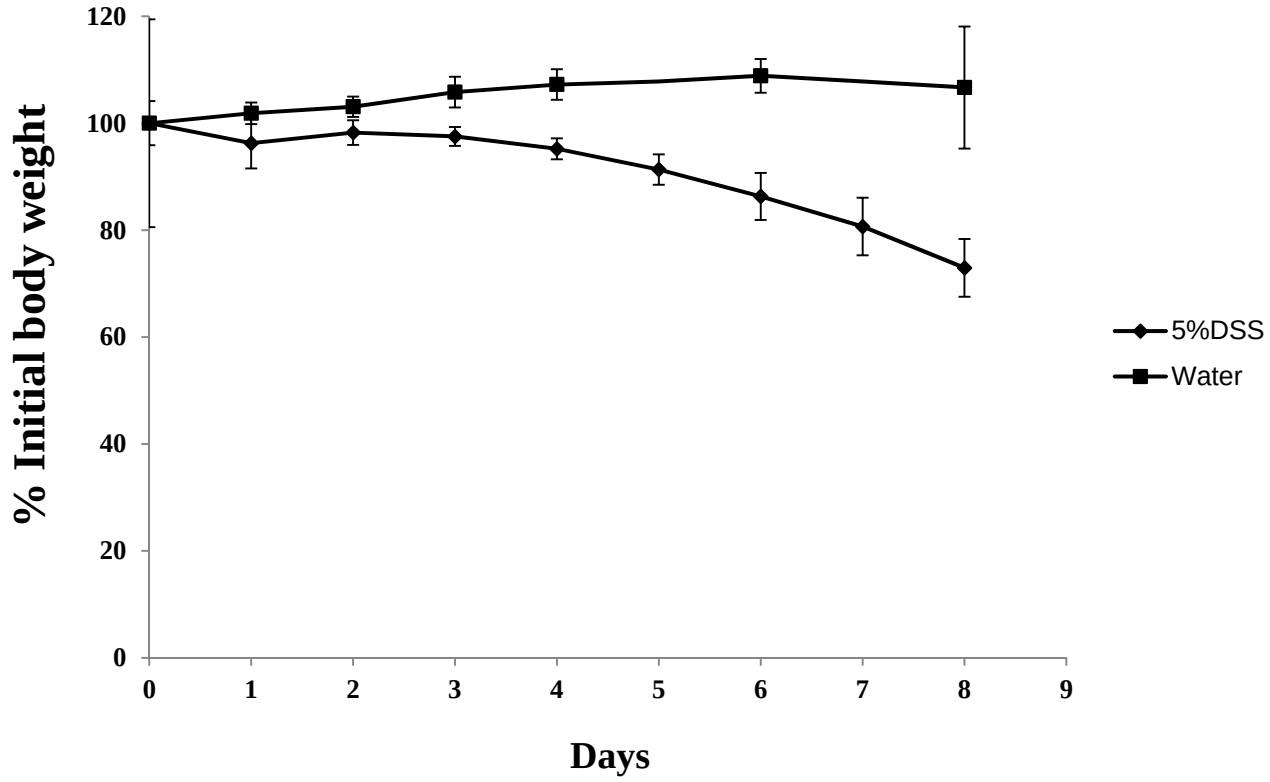
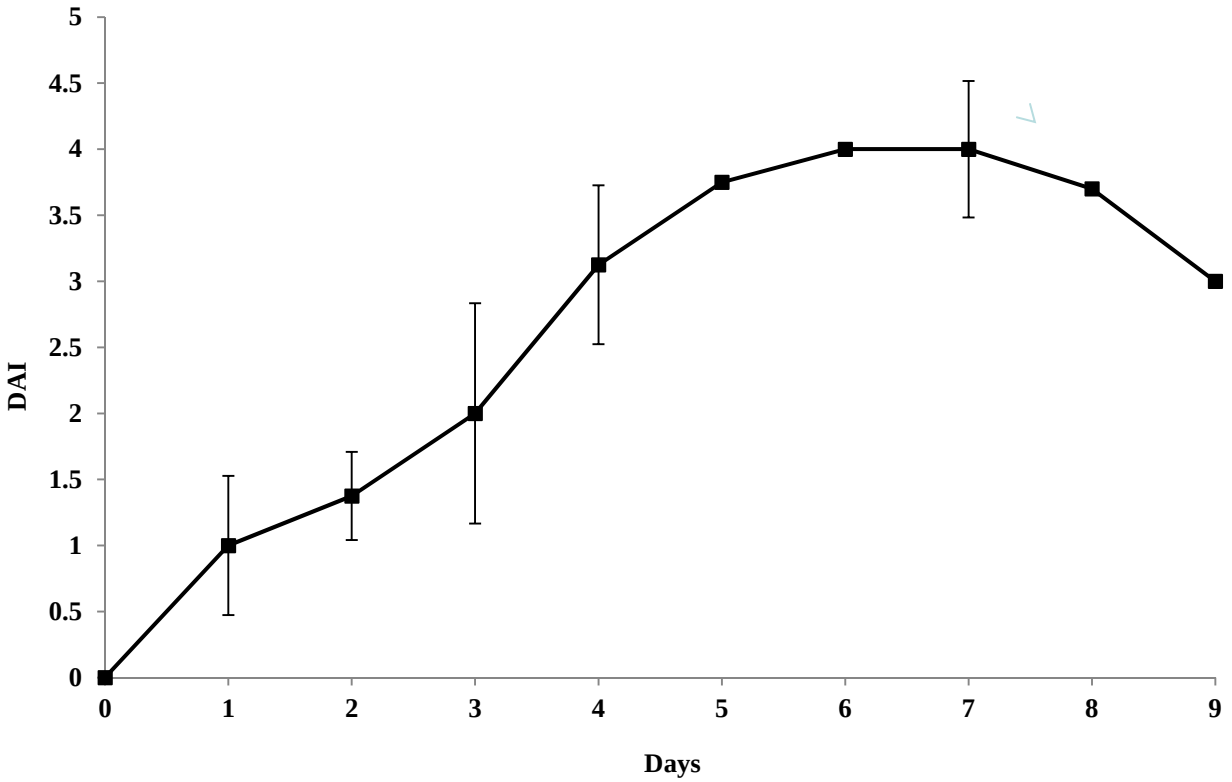


Fig S4. Daily changes in body weight of mice administered 5% dextran sodium sulphate. Changes in body weight of mice during DSS treatment for 7 days were measured daily and compared to that of control mice administered water (6 mice per group). Body weight change up to day 8 was expressed as a percentage (± standard deviation) of the weight at day 0.

Figure S5.



Score	Stool consistency	Blood in stool
0	Normal	Negative
1	Loose stool	Negative
2	Loose stool	Positive
3	Diarrhoea	Positive
4	Diarrhoea	Gross rectal bleeding

Fig S5. Determination of the disease activity index (DAI) of mice administered 5% DSS. DAI was measured for each mouse according to the scoring system outlined in the Table which was adapted from Murthy et al., Dig Dis Sci 38:1722-1734, 1993. Mice (n = 6) were given 5% DSS in drinking water for 7 days and DAI was recorded up to day 9 (± standard deviation).

Figure S6.

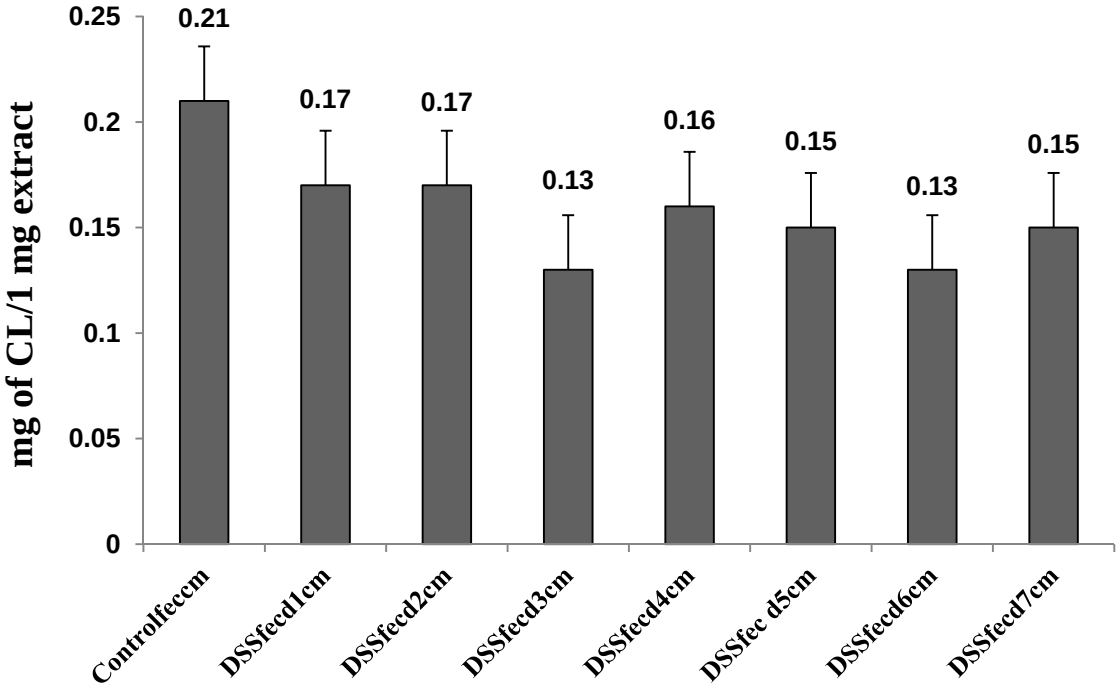


Fig S6. Related to figure 6 and Figure S4. Similar cardiolipin content of chloroform-methanol extracts derived from the feces of DSS administered mice and from controls. Feces were collected daily, up to day 7, from DSS administered mice and controls. The CL content in $DSSfec_{(d1\text{-}d7)cm}$ and control feces from water fed mice (Control fec_{cm}) was measured fluorometrically using 10-N-nonyl-acridine orange. The results were determined by linear interpolation from CL standard curves and the content of CL in each sample is expressed as the amount of CL (mg) in 1 mg of the total lipid extract minus the background (± standard deviation).

Figure S7.

Fig S7. Related to Figure 6. Lipid extracts derived from feces of DSS administered mice or SPF mice display similar abilities to antagonize LPS-dependent TLR4 signaling. Recombinant TLR4 activity was measured using a HEK293 luciferase reporter assay. The relative abilities of lipid extracts from SPF mouse feces and DSS-treated mouse feces (d1-d7) to antagonize BtLPS-dependent TLR4 activation were compared. The results of agonist (BtLPS) combined with SPFfec$_{cm}$ or DSSfec$_{(d1\text{-}d7)cm}$ lipid fractions were plotted as percent activation minus the background (± standard deviation) of triplicate determinations relative to the Bt$_{LPS}$ alone minus the background which was considered to be one hundred percent activation.

Table S1.

Fatty acid	**C16:0**, hexadecanoic acid	**iso-C16:0**, 14-Me-pentadecanoic acid	**C18:0**, octadecanoic acid	**C18:1**, 9-(trans) or 9-(cis)-octadecenoic (oleic) acid	**C18:2**, 9,12-cis/cis-octadecadienoic (linoleic) acid
Peak area	3.8	1	traces	3.8	3

Table S1. Fatty acid composition of the purified lipid extract of feces from germ-free mice ($GFfec_{cm}$) (Fraction 1, ToyoPearl HW- 40 column) determined by GC-MS.

Table S2.

Signal, δ_H	Proton	Compound
0.80	-$(CH_2)_n$-C**H**$_3$	all acids except linoleic acid
0.93	-CH=CH-CH_2C**H**$_3$	linoleic acid
1.20	-(C**H**$_2$)$_n$-	all fatty acids
1.50	-C**H**$_2$-CH_2-COOR	all fatty acid esters
1.94	-C**H**$_2$-CH=CH-	all unsaturated fatty acids
2.14	-C**H**$_2$-COOR	all fatty acids esters
2.68	-CH=CH-C**H**$_2$-CH=CH-	linoleic acid
5.25	-C**H**=C**H**-	all unsaturated fatty acids

Table S2. Related to Figure S2A. ^{1}H NMR spectral data (d_H, ppm) of the purified lipid extract from GF feces (Fraction 1, ToyoPearl HW-40). The assigned protons for the fatty acid residues which are attached to the side chain glycerol moieties of the cardiolipin species are shown in bold.