SUPPLEMENTAL INFORMATION

Fig. S1. CARD8 is expressed in a variety of human tissues and different cell lines. (A) Expression of CARD8 mRNA in adult human tissues and different cell lines. cDNA samples from different human tissues were amplified by RT-PCR as described in Material and Methods. (B) Expression of endogenous CARD8 protein was detected in different cell lines using a polyclonal antibody against CARD8. As a control CARD8 was transiently overexpressed in HEK cells. Expression of β-actin served as loading control

Fig. S2. Subcellular distribution of NOD2 and CARD8. Measurement of relative color intensities of pAmCyan1-C1-CARD8 and pZsYellow1-C1-NOD2 along an indicated line.

Fig. S3. Expression verification of FLAG-NOD2, full length GFP-CARD8, GFP-CARD8(1-320). HEK cells have been transfected as indicated and were stimulated with MDP as described in Fig 2. After medium removal for detection of IL-1β and IL-8 release the cells have been subjected to Western Blot analysis (20 µg protein lysate used for electrophoresis) to verify expression of the transfected protein with anti-FLAG-M2 antibody (Sigma-Aldrich, St. Louis, MO, USA), anti-GFP antibody (Clontech, Palo Alto, CA, USA). Expression of β-actin served as loading control.

Table S1. Oligonucleotides used for generation of MYC-tagged NOD2

Expression construct	Sequence (5' to 3'): sense/antisense	Vector
MYC-NOD2	CGAAGTCGACTATGGGGGAAGAGGGTGGTTCAG / GAAAGCGGCCGCTCAAAGCAAGAGTCTGGTGTCCCTG	pCMV-MYC (BD Biosciences, San Jose, CA)
YFP-NOD2	GCTACTCGAGCTGGGGAAGAGGGTGGTTC / CGATGGATCCTCAAAGCAAGAGTCTGGTGTC	pZsYellow-C1 (Clontech, Palo Alto, CA, USA)
CFP-CARD8	GCTACTCGAGCTATGAGACAGAGGCAGAGCC / CGATGGATCCTTACAAATTCTGCTGTCTAAGATAGG	pAmCyan-C1 (Clontech, Palo Alto, CA, USA)
GFP-CARD8 (1-320)	GCTAGCTAATGAGACAGAGGCAGAGCC / CGATGGATCCTTAGAACCAAAGTTCAGGGGTTC	pEGFP-C3 (Clontech, Palo Alto, CA, USA)

Table S2. Oligonucleotides used for RT-PCR

Name	Sequence (5' to 3')	Amplicon length
RT.FL.CARD8.S	GAGACAGAGGCAGAGCCATTATTGTTCCG	1288 bp
RT.FL.CARD8.A	CAAATTCTGCTGTCTAAGATAGGACACGAGG	

Table S3. siRNA used for endogenous CARD8 knockdown
(Invitrogen, Carlsbad, CA, USA)

Name	Sequence (5' to 3'): sense/antisense
CARD8HSS117857	CCCAUUCAACUUGAGAUUACUGAAA / UUUCAGUAAUCUCAAGUUGAAUGGG
CARD8HSS117858	CCCGAAGAUAUUAAGUUCCACUUGU / ACAAGUGGAACUUAAUAUCUUCGGG
CARD8HSS177013	GAGCCUUUCUAUGCUGUCCUGGAAA / UUUCCAGGACAGCAUAGAAAGGCUC

Supporting Materials and Methods

Cell culture and Reagents

Human embryonal kidney HEK293 cells, HeLaS3 cells, colonic carcinoma cell lines HT-29, SW480 and CACO-2, acute monocytic leukemia cell lines THP1 and Mono-Mac-6 were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). HEK293, CACO-2 and HT-29 were cultured in Dulbecco´s MEM, all other cell lines were cultured in RPMI 1640 (media from PAA Laboratories, Paschberg, Austria). Media were supplemented with 10% fetal calf serum (FCS) and penicillin/streptomycin (each at 50µg/ml). Cell lines were grown in 5% CO_2 at 37°C. MDP was from Bachem.

mRNA isolation and RT-PCR

Total RNA from respective cell lines were isolated using the RNeasy kit from Qiagen (Qiagen, Hilden, Germany) and subsequently reverse transcribed (Advantage RT-for-PCR kit, Clontech Laboratories, Palo Alto, CA). For investigation of tissue specific expression patterns, a human cDNA tissue panel have been used (Clontech, Palo Alto, CA, USA). See supplemental Table S2 for primer information. To confirm the use of equal RNA amounts all samples were checked for GAPDH mRNA expression. Amplified DNA fragments were analyzed on 1% agarose gels.

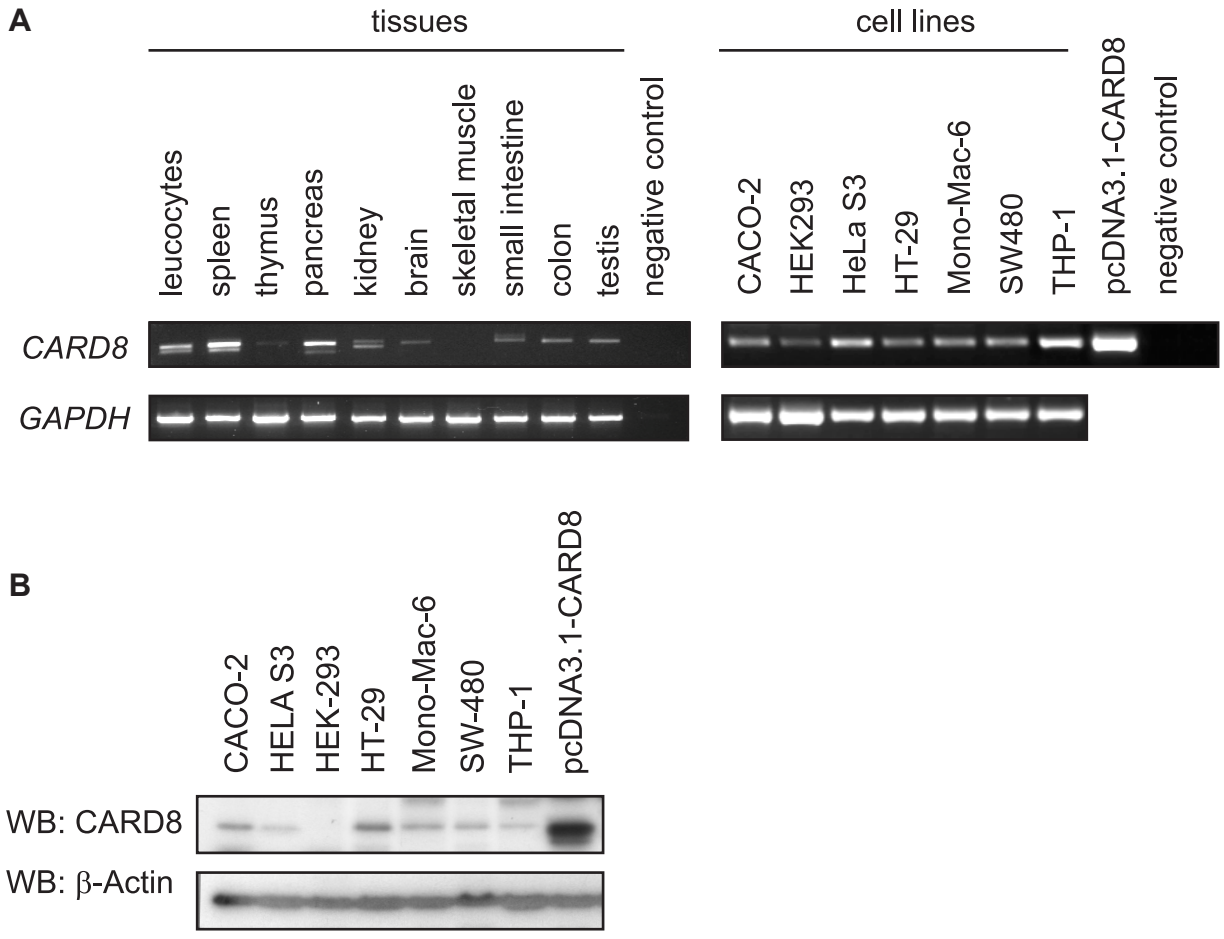
Gentamicin protection assay

HEK cells were cultured for 1 h with fresh grown culture *Listeria monocytogenes* (serotype 1/2a, strain EGD) using 100 bacteria/cultured cell. Extracellular bacteria were eliminated with gentamicin (1 h, 100 µg/ml, Sigma) followed by washing (PBS buffer) and lysing (10% Triton X100). 5 µl lysate were mixed with 20 µl LB medium and grown on antibiotic-free agar plates (quadruplicates, 24 h, 37°C) and colony-forming units (CFU) were counted. The CFU number of an untreated sample was set as 100% used as a reference for all other samples.

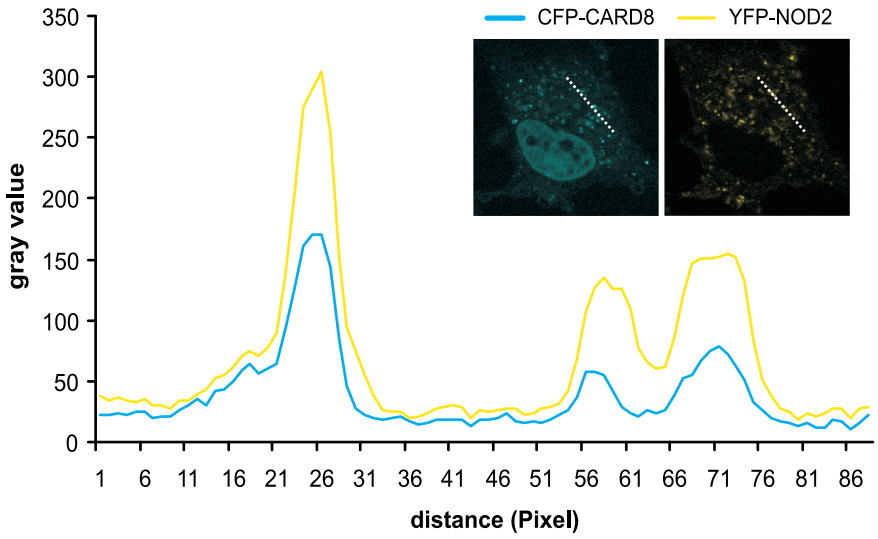
Fluorescence microscopy

HeLa expressing YFP-CARD8 and CFP-NOD2 were washed twice in ice-cold PBS, fixed (4% paraformaldehyde, Carl Roth, Karlsruhe, Germany), stained with DAPI (Roche, Mannheim, Germany) and subjected to microscopy (Axio Imager Z1 apotome-equipped, magnification 630-fold, Zeiss, Jena, Germany). Digital picture analysis was performed with ImageJ (http://rsb.info.nih.gov/ij/).

Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure 3