SUPPLEMENTAL MATERIAL

Means et al., http://www.jem.org/cgi/content/full/jem.20082109/DC1

The Journal of Experimental Medicine

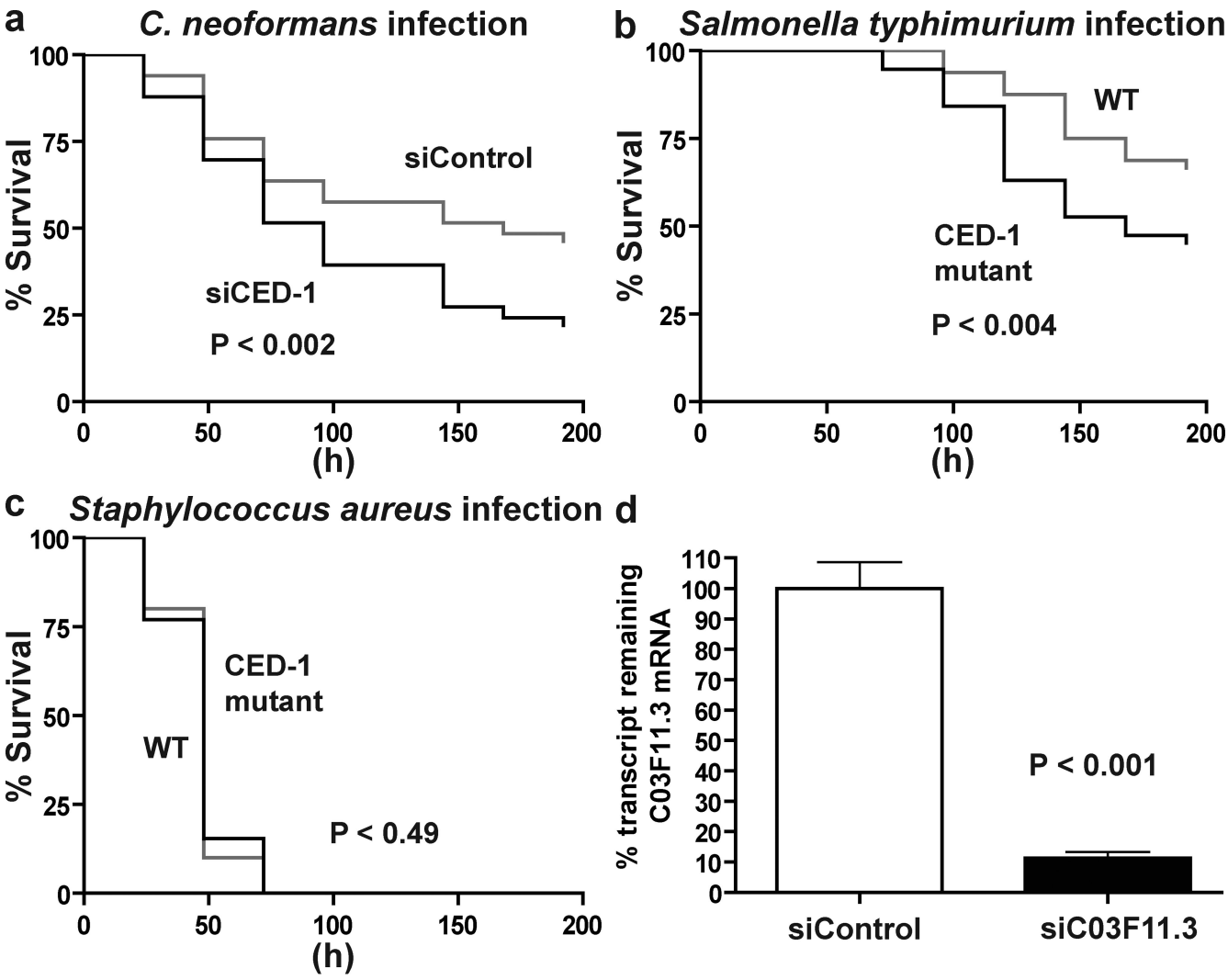

Figure S1. Survival of CED-1–deficient *C. elegans* to several pathogens. (a) Survival of shRNA control L4440 strain (gray) and siCED-1 (black) nematodes after exposure to *C. neoformans* strain H99. (b) Survival of WT N2 strain nematodes (gray) and CED-1 mutant strain MT4933 (black) after exposure to *S. typhimurium*. (c) Survival of WT N2 strain nematodes (gray) and CED-1 mutant strain MT4933 (black) after exposure to *S. aureus*. (d) *C03F11.3* mRNA levels were measured by QPCR 24 h after exposure of L440 and siC03F11.3 nematodes to *C. neoformans*. Survival was determined as described in Materials and methods, and p-values were determined using the Kaplan-Meier survival statistical test ($n > 100$ worms per strain). Data are representative of three independent experiments with similar results. Means ± SEM are shown.

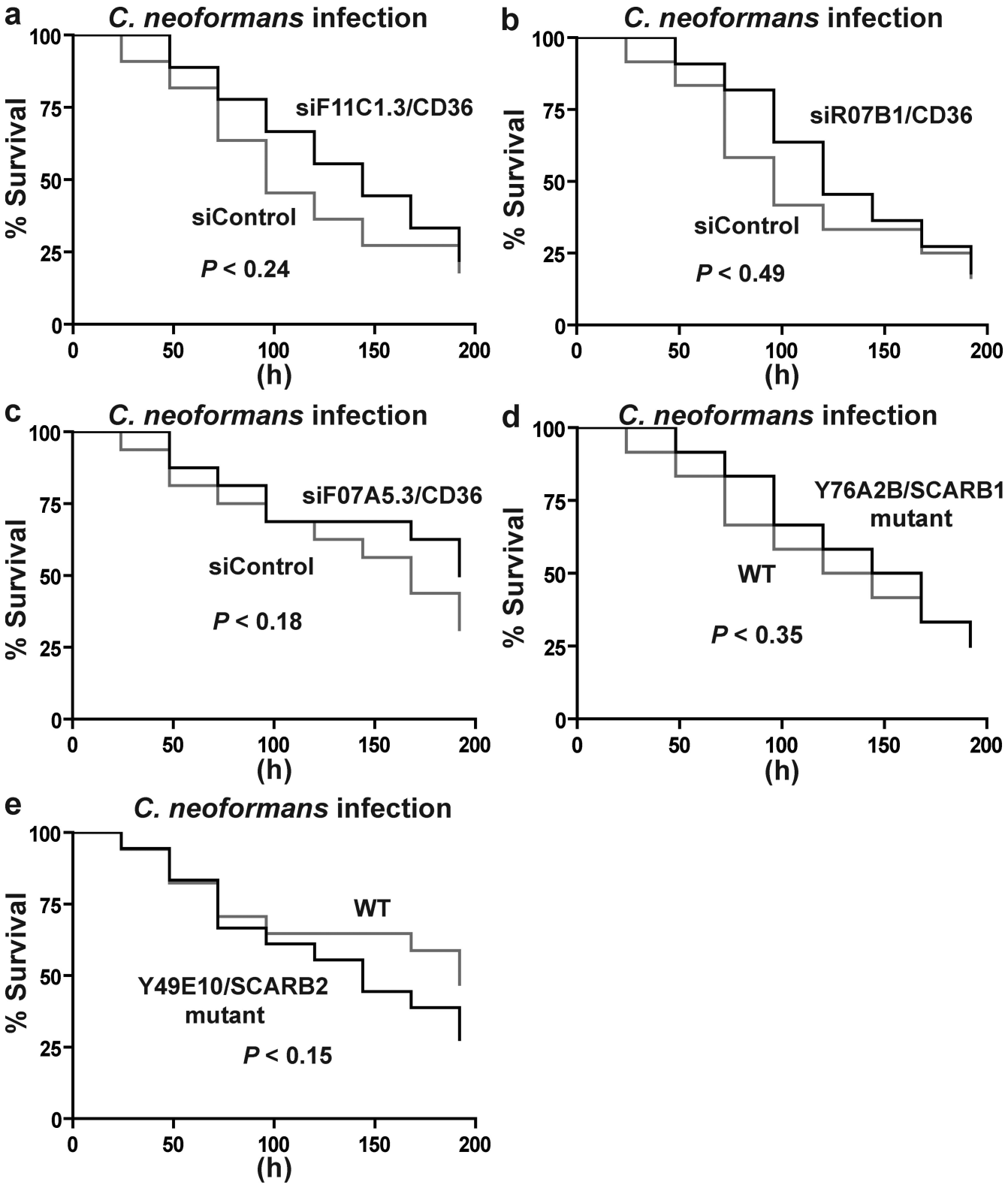

Figure S2. Survival of CD36, SCARB1, and SCARB2 orthologue-deficient *C. elegans* infected with *C. neoformans*. (a–c) Survival of shRNA control L4440 strain (gray), siF11C1.3/CD36, siR07B1/CD36, and siF07A5.3/CD36 (black) nematodes after exposure to *C. neoformans* strain H99. (d and e) Survival of WT N2 strain (gray), *Y76A2B/SCARB2* mutant strain (black), and *Y49E10/SCARB1* mutant strain (black) nematodes after exposure to *C. neoformans* strain H99. Survival was determined as described in Materials and methods, and p-values were determined using the Kaplan-Meier survival statistical test ($n > 100$ worms per strain). Data are representative of three independent experiments with similar results.

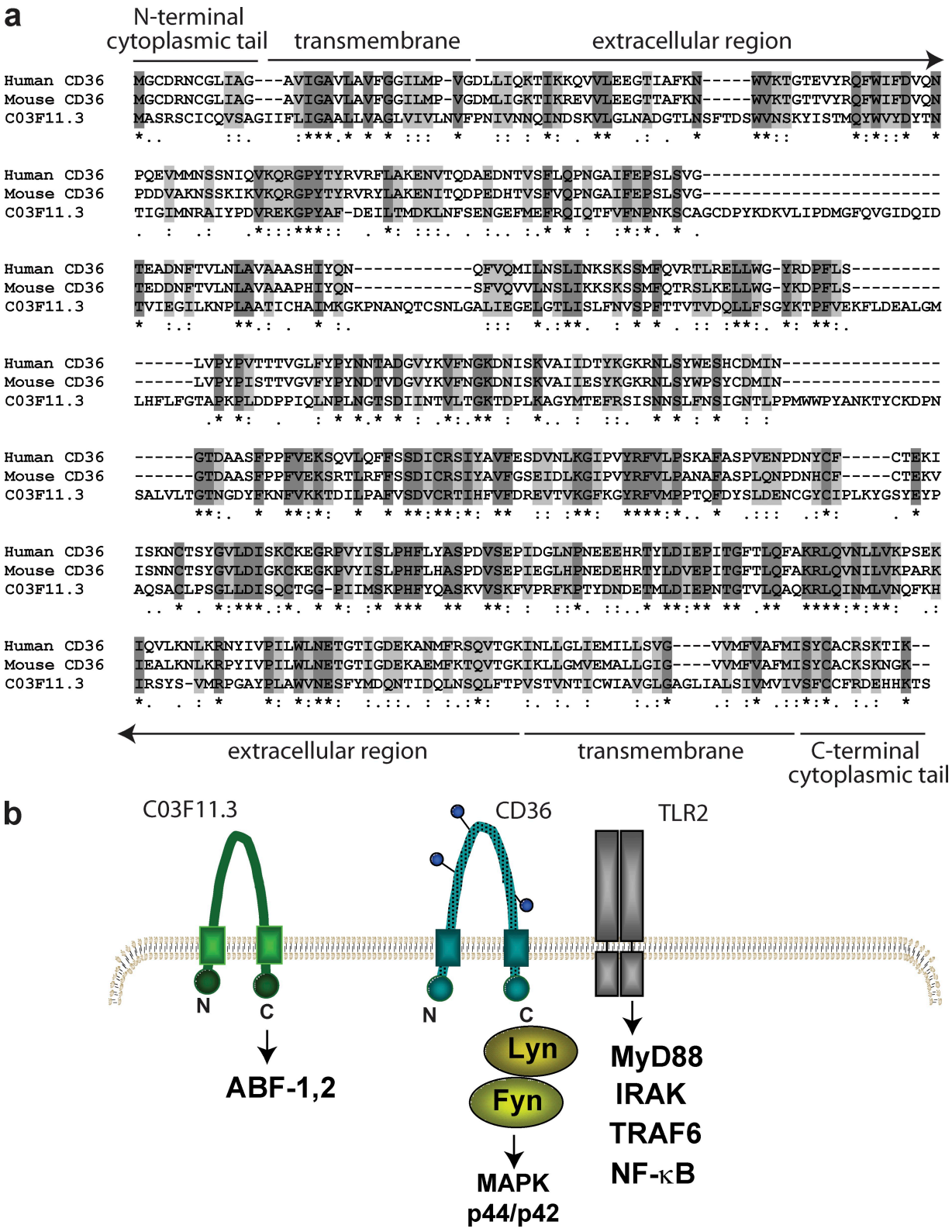

Figure S3. Alignment of *C. elegans* C03F11.1 and mouse and human CD36. (a) Alignment was performed by using ClustalW software (European Bioinformatics Institute). Asterisks (*) indicate identical or conserved residues in all sequences in the alignment, colons (:) indicate conserved substitutions, and periods (.) indicate semiconserved substitutions. *C. elegans* C03F11.3 is ~22% identical, ~42% identical with conserved substitutions, and has an overall similarity of ~53% with mouse and human CD36. (b) The *C. elegans* C03F11.3 gene is located on chromosome X and encodes a predicted protein of 563 aa. C03F11.3, like its mammalian orthologues, has two short cytoplasmic tails, one on the N terminus (1–11 aa) and another on the C terminus (554–563 aa), and a large extracellular domain (35–530 aa).

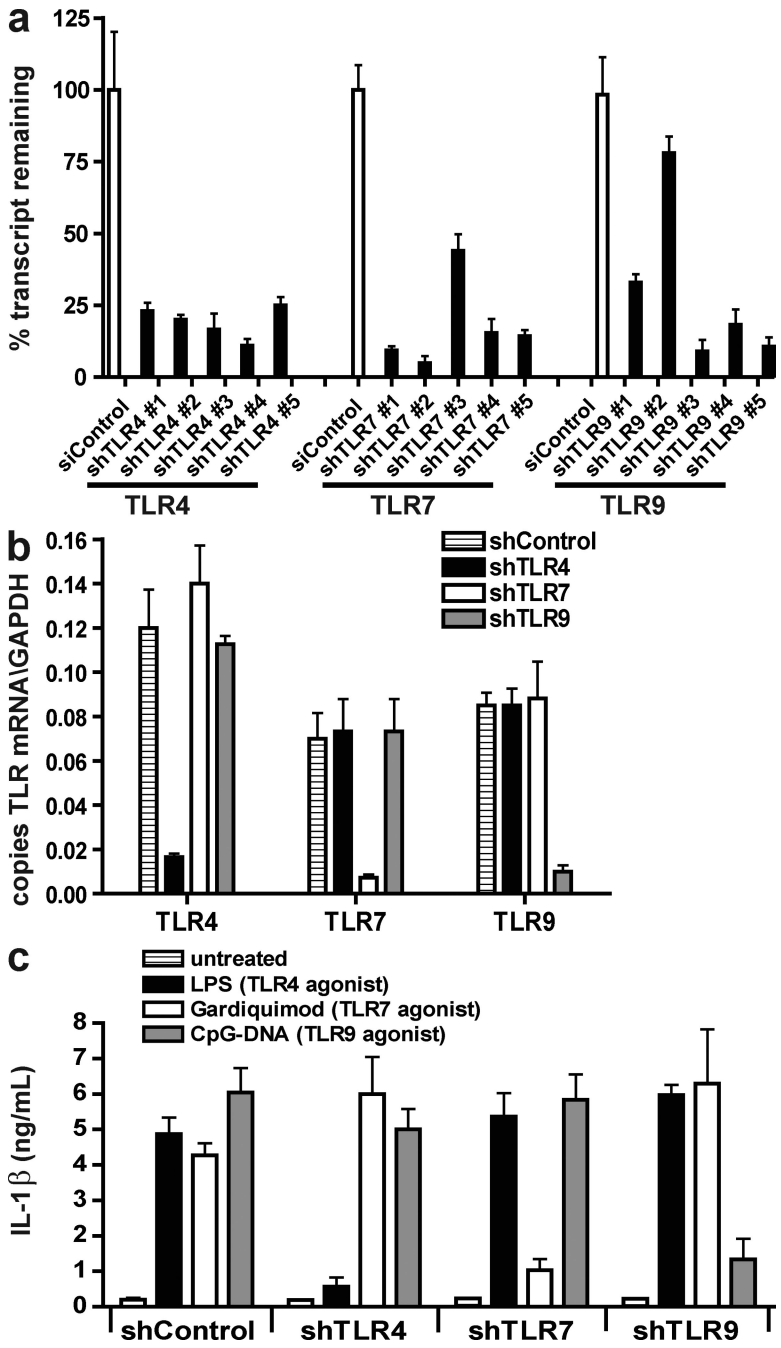

Figure S4. shRNA knockdown efficiency and specificity for *TLR4*, *TLR7*, and *TLR9*. (a) Each set of bar graphs represents mRNA knockdown of a single gene by one control shRNA virus (leftmost bar of each set) and by five gene-specific shRNA viruses measured by QPCR. (b) QPCR of *TLR4*, *TLR7*, and *TLR9* demonstrate that they are expressed in RAW cells and can be silenced by lentiviral infection of gene-specific shRNAs. (c) IL-1β levels in LPS-, Gardiquimod- (InvivoGen), or CpG-DNA–stimulated RAW cells treated with control shRNA, and RAW cells silenced for *TLR4*, *TLR7*, or *TLR9*. Data are representative of four independent experiments with similar results. Means ± SEM are shown.

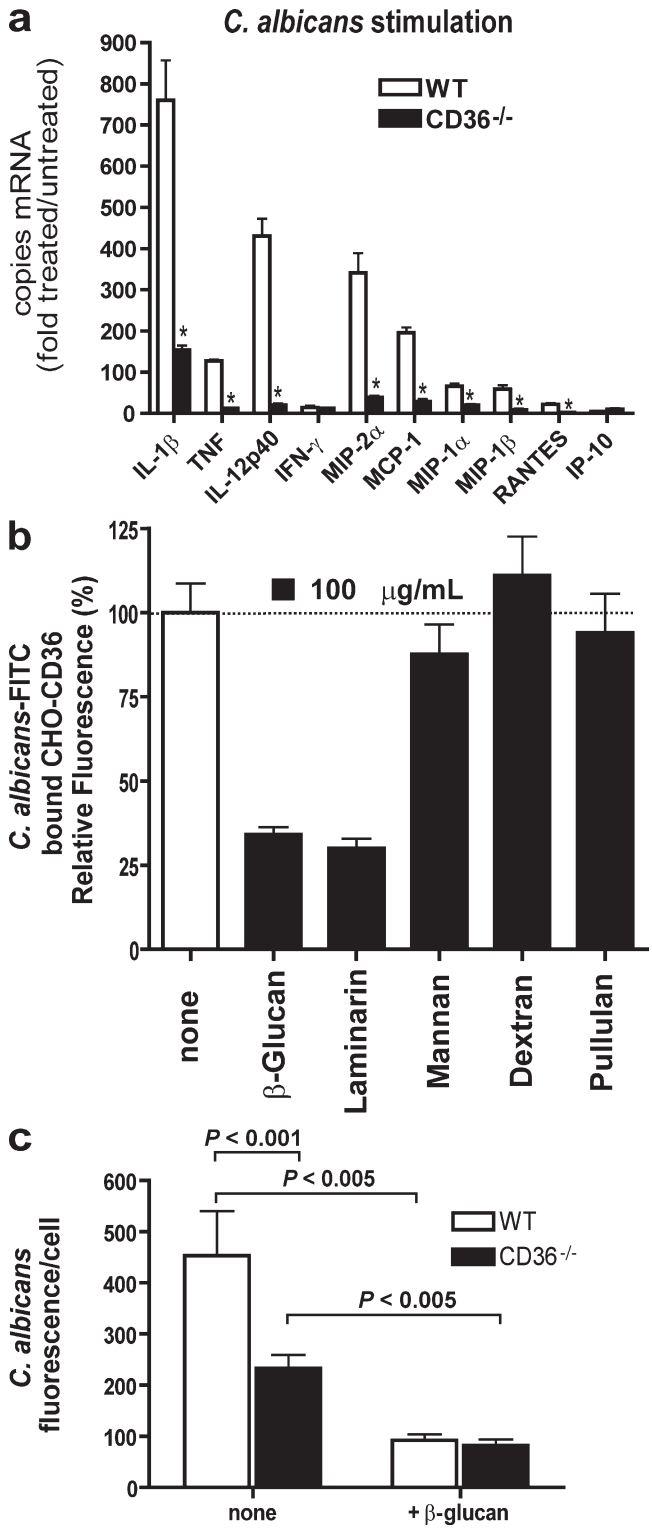

Figure S5. CD36 expression mediates recognition of *C. albicans*. (a) WT and *CD36*$^{-/-}$ macrophages were stimulated with *C. albicans* (20 per cell) for 4 h. Expression of cytokines and chemokines was determined by QPCR and is depicted as the number of copies of mRNA per copies of the control GAPDH mRNA. *, $P < 0.001$. (b) CHO-CD36 cells were pretreated with 100 μg/ml of carbohydrates before addition of fluorescently labeled *C. albicans* (20 per cell). *C. albicans* binding was quantified on a fluorescent microplate reader and is expressed relative to an uninhibited control. Background binding of *C. albicans* to control CHO cells was normally ~15–20%. Dotted lines indicate the 100% level. (c) Fluorescently labeled *C. albicans* were incubated with macrophages isolated from WT and *CD36*$^{-/-}$ mice in the presence or absence of 100 μg/ml of unlabeled β-glucan for 2 h, washed, and quantified by flow cytometry. Data in A–C are representative of four independent experiments with similar results. Means ± SEM are shown.

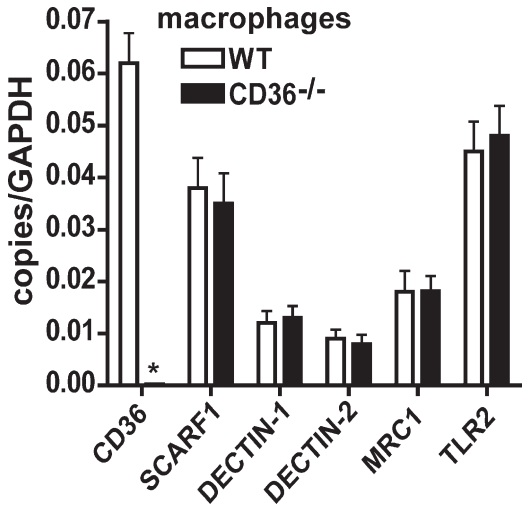

Figure S6. **Targeted deletion of *CD36* does not affect expression of other β-glucan receptors in macrophages.** mRNA levels of the β-glucan receptors *DECTIN-1*, *DECTIN-2*, mannose receptor 1 (*MRC1*), *TLR2*, and *SCARF1* were quantified by QPCR in bone marrow–derived macrophages. No statistically significant differences were detected. As expected, *CD36* mRNA was not detectable in the $CD36^{-/-}$ macrophages (*, $P < 0.01$ versus WT). Data are representative of three independent experiments with similar results. Means ± SEM are shown.

Table S1. Mouse gene identifiers and shRNA target sequences

Gene name	NCBI gene ID	Target 21–mer (5′ to 3′)
CD36	12491	CGGATCTGAAATCGACCTTAA
		GCAGGTCAACATATTGGTCAA
		GCCAAGCTATTGCGACATGAT*
TLR2	24088	CTAAGGTCTTTGTGACACAAA*
		GCCAGAATCATTTGAGATCAA
		GCAGTCTTGAACATTTGGATT
		CCCATTGAGAGGAAAGCCATT
SCARB2	12492	CGGCCTGTTCTATGAGAGAAA
		CGTTGACTTGATTAGAACAAT
		GCAGGTCAGTACATATCACTT
		GCTGTCACCAATAAGGCATAT*
SCARF1	380713	CCACGGAAACAACTGCTCTAT*
		CCGCAGTTAGACCAGAGGAAA
		CTGTCGGTGTAAACCTGGATT

Asterisks (*) indicate the target sequence for each gene that had the greatest effect on *C. neoformans*–induced cytokine induction. NCBI, National Center for Biotechnology Information.

Table S2. *C. elegans* gene identifiers and siRNA target sequences

Gene name	NCBI gene ID	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
CED-1	173064	AACTGTGAGAAGCAGGCGAT	CGGGCAGATACATAGTCCGT
C03F11.3	180812	ACGGTGCTCACAGGAAAAAC	ATCGACAAGGCAATGAGACC
F11C1.3	181429	GCAGGCTTTTTCCCACATTA	CTGTGAAACCGATTCCGAGT
F07A5.3	184107	GCTGTTTACGCAAATGCAAA	TGGTGGACTACTCCAAAGGC

The *C. elegans* RNAi feeding library was constructed by J. Ahringer and colleagues at the Wellcome CRC Institute, University of Cambridge. *C. elegans* genomic fragments were PCR amplified using the Research Genetics GenePairs primers listed and cloned into the EcoRV site of vector L4440. NCBI, National Center for Biotechnology Information.

Table S3. QPCR primer sequences

	Gene name	NCBI gene ID	Forward primer (5′ to 3′)	Reverse primer (5′ to3′)
C. elegans	*CED-1*	173064	ACTCCGTCACCGTGTGCTTC	GACAGCCATCGCCATAGCTC
	ABF-1	266827	CTGCCTTCTCCTTGTTCTCCTACT	CCTCTGCATTACCGGAACATC
	ABF-2	266826	TGTTCGTCCGTTCCCTTTTC	GGAACATCCATTCTGGCACAA
Mouse	*IL-1*β	16176	ACCTGTCCTGTGTAATGAAAGACG	TGGGTATTGCTTGGGATCCA
	TNF	21926	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
	IL-12p40	16160	TGGTTTGCCATCGTTTTGCTG	ACAGGTGAGGTTCACTGTTTCT
	*IFN-*γ	15978	AACGCTACACACTGCATCTTGG	GCCGTGGCAGTAACAGCC
	MIP-2/*CXCL2*	20310	CCAACCACCAGGCTACAGG	GCGTCACACTCAAGCTCTG
	MCP-1/*CCL2*	20296	TGGCTCAGCCAGATGCAGT	TTGGGATCATCTTGCTGGTG
	MIP-1α/*CCL3*	20302	CCAAGTCTTCTCAGCGCCAT	TCCGGCTGTAGGAGAAGCAG
	MIP-1β/*CCL4*	20303	TCTTGCTCGTGGCTGCCT	GGGAGGGTCAGAGCCCA
	RANTES*CCL5*	20304	CAAGTGCTCCAATCTTGCAGTC	TTCTCTGGGTTGGCACACAC
	IP-10/*CXCL10*	15945	GCCGTCATTTTCTGCCTCA	CGTCCTTGCGAGAGGGATC
	GAPDH	14433	GGCAAATTCAACGGCACAGT	AGATGGTGATGGGCTTCCC

NCBI, National Center for Biotechnology Information.