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Supplemental information

Protective effects of S100A8 on sepsis mortality:

Links to sepsis risk in obesity and diabetes

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Figure S1

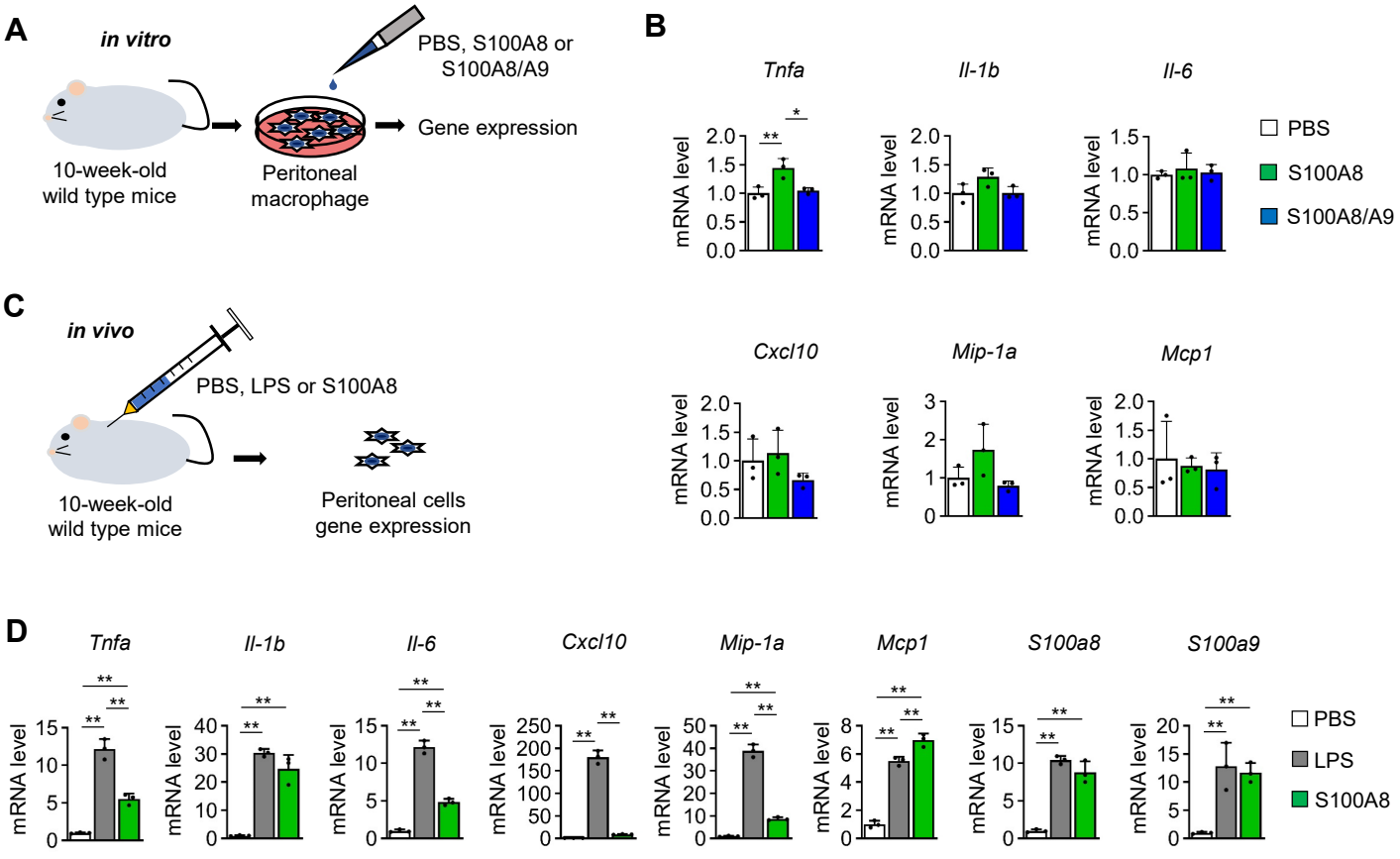

Figure S1. S100A8 administration upregulated inflammatory cytokine expression in peritoneal macrophages. Related to Figure 1.

Wild-type mice at 10 weeks of age were used for *in vitro* and *in vivo* experiments.

(A) Experimental protocol for an *in vitro* study. Peritoneal macrophages from mice were incubated with PBS, S100A8 (0.3 μg/mL), or S100A8/A9 (0.65 μg/mL) and analyzed by quantitative PCR at 2 hours.

(B) mRNA expression of the indicated inflammatory cytokine genes in peritoneal macrophages (n = 3).

(C) Experimental protocol for an *in vivo* study. Mice were injected with PBS, LPS (12.5 μg/gBW), or S100A8 (0.1 μg/gBW), and then peritoneal cells were analyzed by quantitative PCR at 4 hours after injection.

(D) mRNA expression of the indicated inflammatory cytokine and S100 genes in peritoneal cells (n = 3).

Data are represented as the mean ± SEM. *p < 0.05, **p < 0.01.

Figure S2

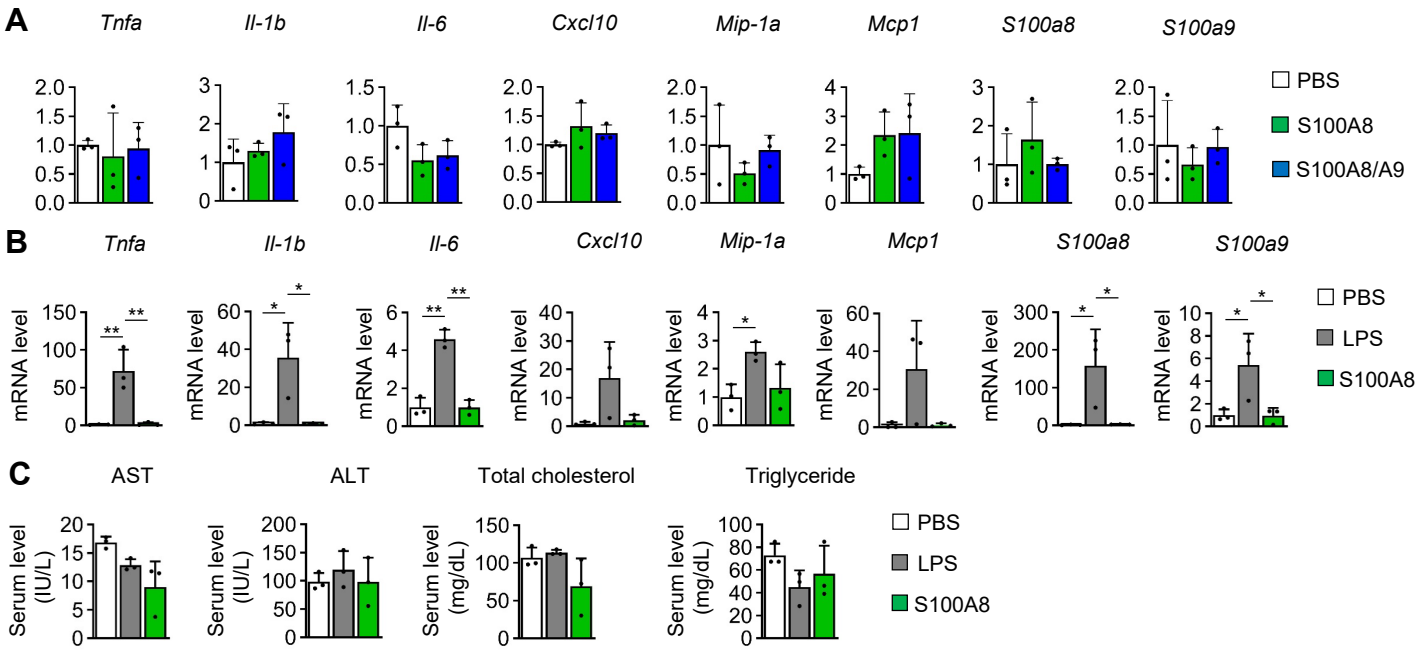

Figure S2. S100A8 administration did not induce inflammatory response in hepatoma cells. Related to Figure 1.

(A) mRNA expression of the indicated cytokine genes in Hepa1-6 mouse hepatoma cells incubated with PBS, S100A8 (0.3 μg/mL), or S100A8/A9 (0.65 μg/mL) for 4 hours (n = 3).

(B and C) Wild-type mice were intraperitoneally injected with PBS, LPS (12.5 μg/gBW), or S100A8 (0.1 μg/gBW).

(B) mRNA expression of the indicated inflammatory cytokine genes in the mouse liver at 4 hours after injection (n = 3).

(C) AST, ALT, total cholesterol and triglyceride levels in serum samples collected from mice at 4 hours after injection (n = 3).

Data are represented as the mean ± SEM. *p < 0.05, **p < 0.01.

Figure S3

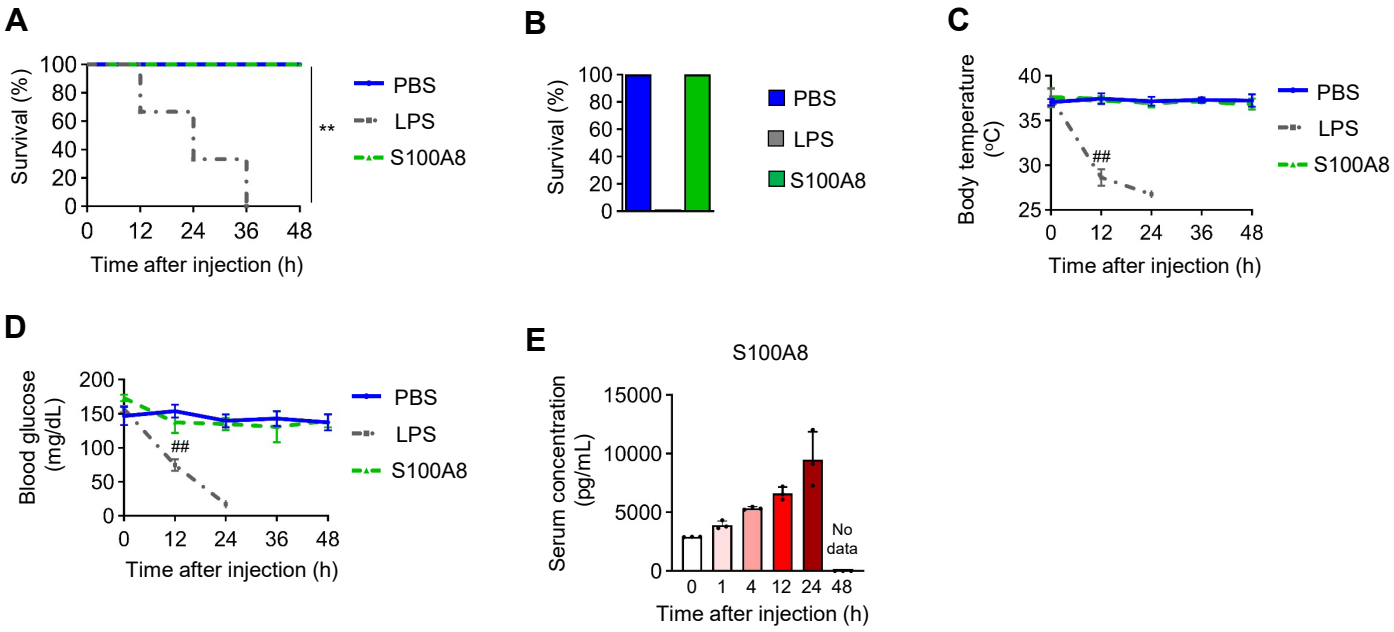

Figure S3. S100A8 administration did not induce sepsis. Related to Figure 1.
Wild-type mice at 10 weeks of age were used for experiments.
(A) Survival rates at 12, 24, 36, or 48 hours after PBS, LPS, or S100A8 injection (n = 3).
(B) Survival rates at 72 hours after PBS, LPS, or S100A8 injection (n = 3).
(C and D) Body temperature (C) and blood glucose levels (D) at 12, 24, 36, or 48 hours after PBS, LPS, or S100A8 injection (n = 3).
(E) Serum S100A8 levels at 1, 4, 12, or 24 hours after LPS (12.5 μg/gBW) injection. All mice died within 48 hours of LPS injection (n = 3).
Data are represented as the mean ± SEM. $^{**}p < 0.01$; $^{\#\#}p < 0.01$ vs. S100A8.

Figure S4

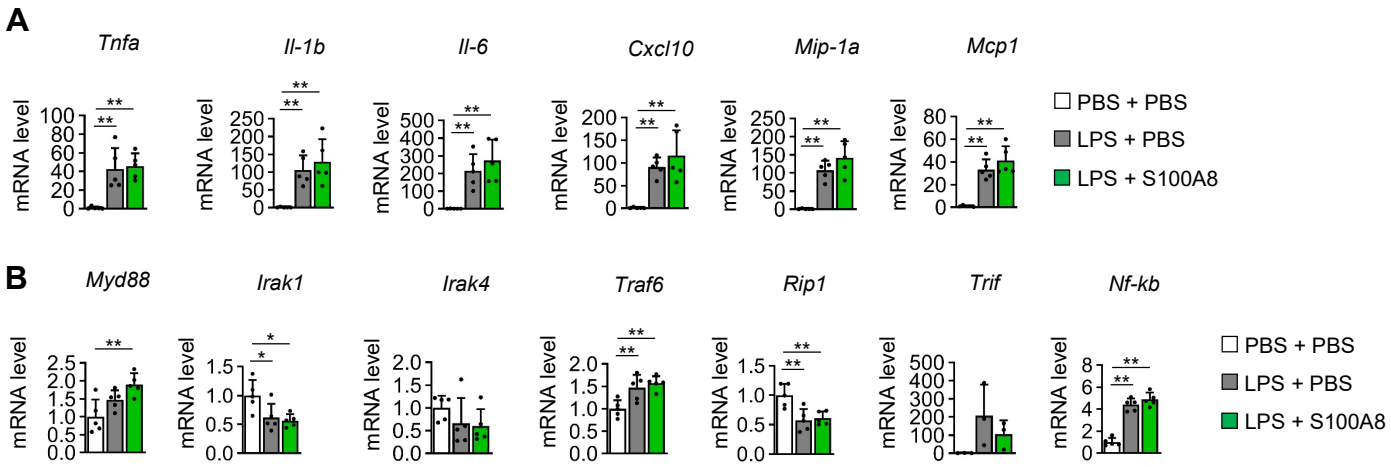

Figure S4. Administration of S100A8 to peritoneal macrophages *in vitro* after LPS stimulation, fails to suppress LPS-induced upregulation of inflammatory cytokine expressions in peritoneal macrophages. Related to Figure 1.

Wild-type mice at 10 weeks of age were used for *in vitro* experiments. Peritoneal macrophages from mice were pretreated with LPS (2 μg/mL) for 30 minutes before PBS or S100A8 (0.3 μg/mL) supplementation for 2 hours and analyzed by quantitative PCR.

(A) mRNA expression of the indicated inflammatory cytokine genes in peritoneal macrophages (n = 5).

(B) mRNA expression of the indicated TLR4 signaling pathway-related genes in peritoneal macrophages (n = 5).

Data are represented as the mean ± SEM. *p < 0.05, **p < 0.01.

Figure S5

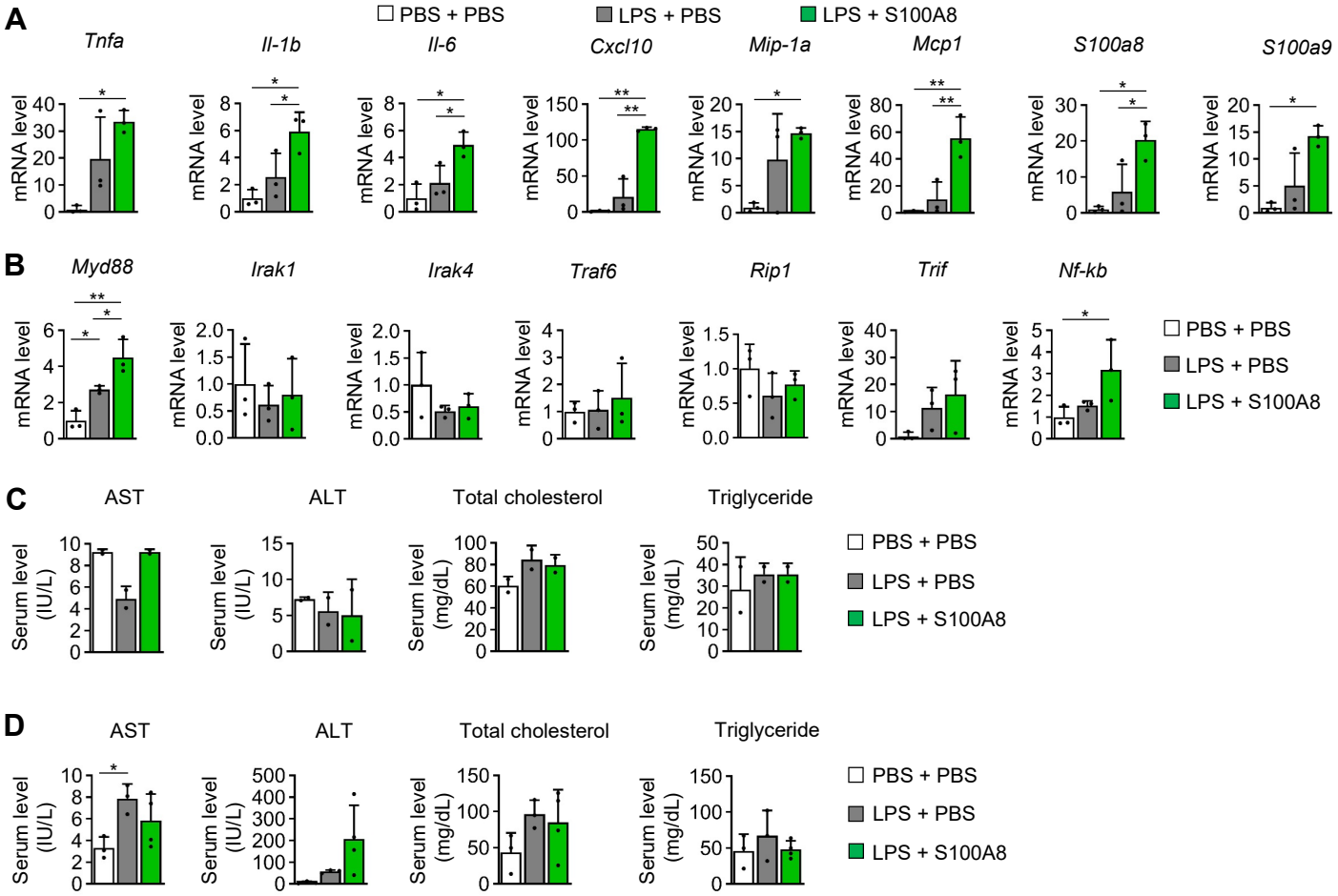

Figure S5. S100A8 promotes the expression of inflammatory cytokines in the liver by activating the TLR4-MyD88 pathway during LPS-induced sepsis. Related to Figure 1.

WT mice were intraperitoneally injected with either PBS or S100A8 (0.1 μg/gBW) at 1 hour after LPS (12.5 μg/gBW) stimulation.

(A) mRNA expression of the indicated inflammatory cytokine genes in the mouse liver at 4 hours after the first injection analyzed by quantitative PCR (n = 3).

(B) mRNA expression of the indicated TLR4 signaling pathway-related genes in the mouse liver at 4 hours after the first injection analyzed by quantitative PCR (n = 3).

(C and D) AST, ALT, total cholesterol and triglyceride levels in serum samples collected from mice at 4 hours (C) and 24 hours (D) after injection (N = 2 at 4 hours and n = 3-4 at 24 hours).

Data are represented as the mean ± SEM. *p < 0.05, **p < 0.01.

Figure S6

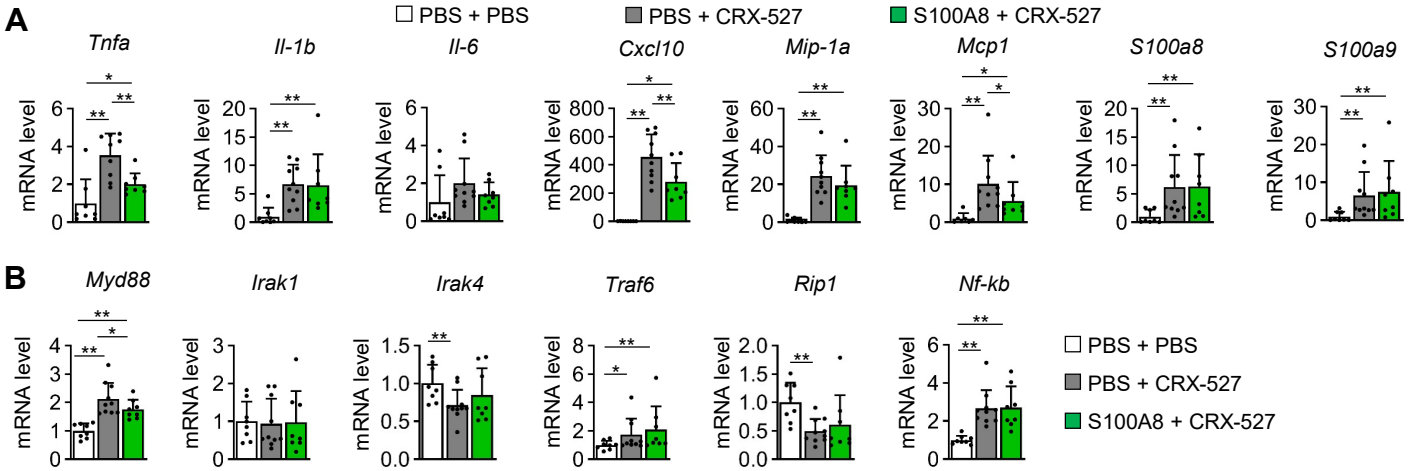

Figure S6. Intraperitoneal injection of S100A8 attenuated the upregulation of inflammatory cytokines and *Myd88* gene expression induced by CRX-527. Related to Figure 1.
C57BL/6J mice were intraperitoneally injected with either PBS or S100A8 (0.1 μg/gBW) at 30 minutes before CRX-527 (0.5 μg/gBW) stimulation.
(A) mRNA expression of the indicated inflammatory cytokine genes in the mouse peritoneal cells at 2 hours after the first injection analyzed by quantitative PCR (n = 8-10).
(B) mRNA expression of the indicated TLR4 signaling pathway-related genes in the mouse peritoneal cells at 2 hours after the first injection analyzed by quantitative PCR (n = 8-10).
Data are represented as the mean ± SEM. *p < 0.05, **p < 0.01.

Figure S7

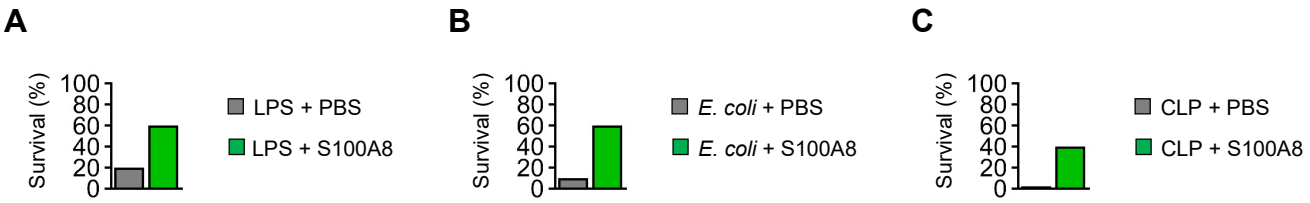

Figure S7. S100A8 improves survival rates in mice of lethal endotoxemia and sepsis models. Related to Figure 1.

Wild-type mice at 10 weeks of age were used for experiments.

(A) The survival rates of mice injected with either PBS or S100A8 (0.1 μg/gBW) at one hour after LPS (12.5 μg/gBW) stimulation at 72 hours after the first injection (n = 10).

(B) The survival rates of mice injected with either PBS or S100A8 (0.1 μg/gBW) at one hour after E. coli DH5α ($2.0*10^9$ CFU per mouse) injection were measured 72 hours later (n = 10).

(C) The survival rates of mice injected with either PBS or S100A8 (0.1 μg/gBW) twice (at 0 hour and 24 hours) after cecal ligation and puncture (CLP) treatment were measured at 72 hours later (n = 10).

Figure S8

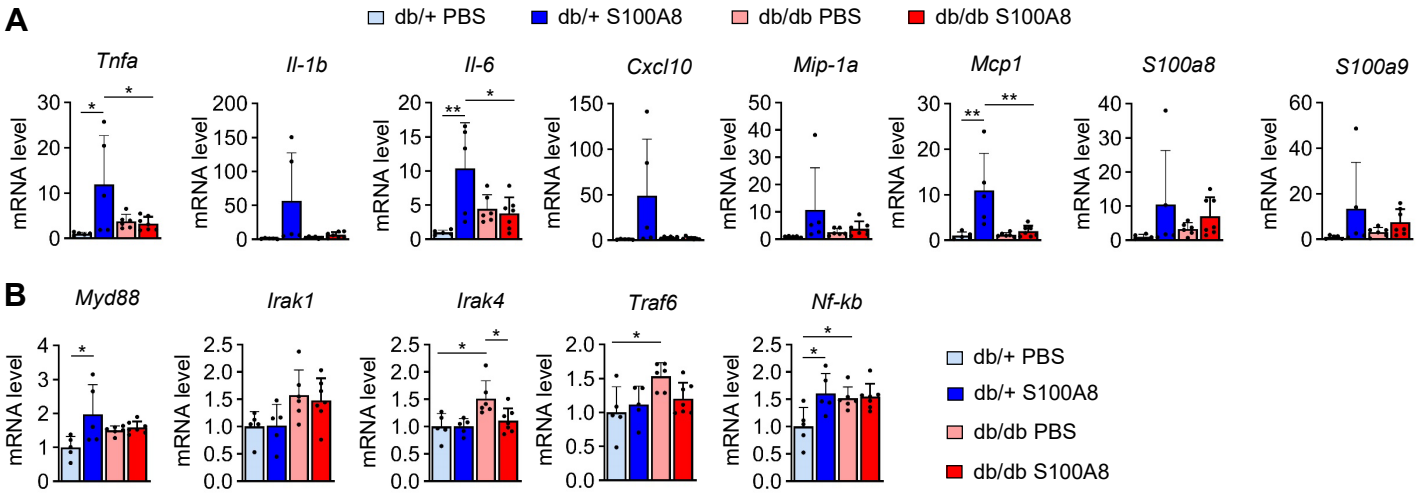

Figure S8. S100A8 administration did not alter the expression of inflammatory cytokines and TLR4 signaling pathway-related genes in peritoneal cells from db/db mice. Related to Figure 3.
db/db and db/+ mice at 11 weeks of age were used for *in vivo* experiments. Mice were injected with PBS or S100A8 (0.1 μg/gBW), and then peritoneal cells were analyzed by quantitative PCR at 4 hours after injection.
(A) mRNA expression of the indicated inflammatory cytokine and S100 genes in peritoneal cells (n = 5-7).
(B) mRNA expression of the indicated TLR4 signaling pathway-related genes in peritoneal cells (n = 5-7).
Data are represented as the mean ± SEM. *p < 0.05, **p < 0.01.

Figure S9

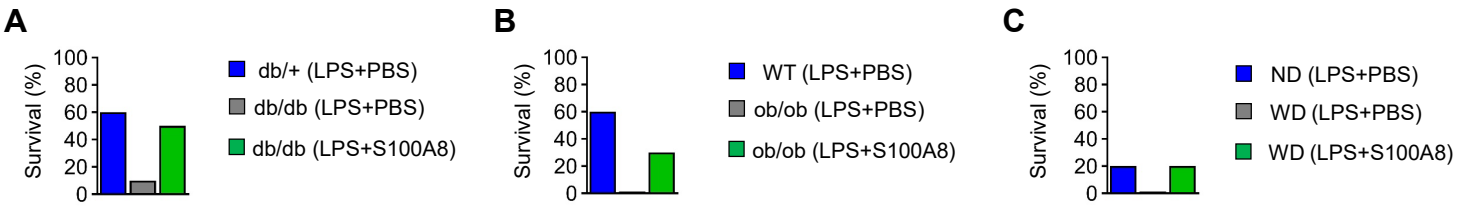

Figure S9. S100A8 improves survival rates in lethal endotoxemia with mice models of diabetes and obesity. Related to Figure 4.

(A) The survival rates of db/db and db/+ mice mice at 8 weeks of age, injected with either PBS or S100A8 (0.1 μg/gBW) one hour after LPS (12.5 μg/gBW) stimulation were measured at 72 hours (n = 13).

(B) The survival rates of ob/ob and wild-type (WT) mice at 8 weeks of age, injected with either PBS or S100A8 (0.1 μg/gBW) one hour after LPS (12.5 μg/gBW) stimulation at 72 hours after the first injection (n = 10).

(C) The survival rates of C57BL/6J mice fed Western diet (WD) or normal diet (ND) from 8 to 16 weeks of age, injected with either PBS or S100A8 (0.1 μg/gBW) at one hour after LPS (12.5 μg/gBW) stimulation were measured at 72 hours (n = 10).

Figure S10

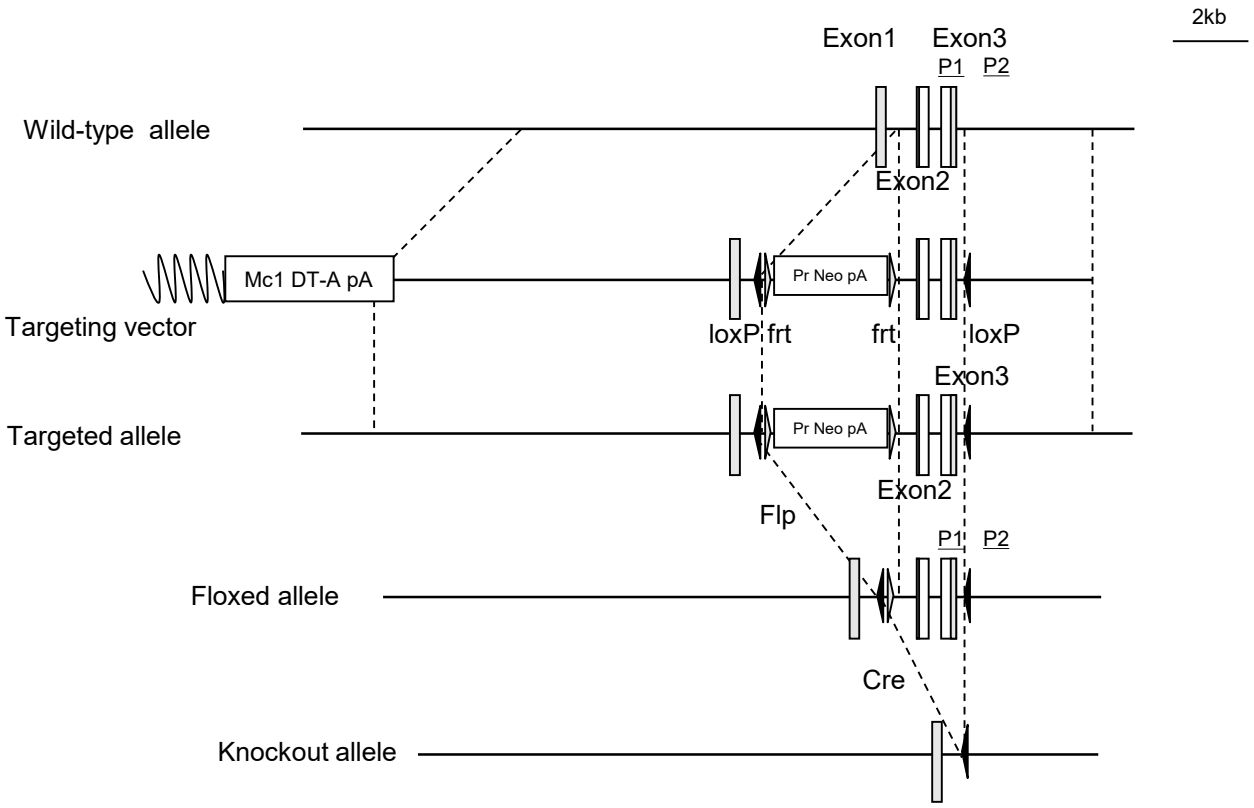

Figure S10. Generation of myelomonocytic cell-specific S100A8 knockout mice. Related to Figure 5.

Schemes show the construction of generalized myelomonocytic cell-specific S100A8 knockout mice. See the Methods for details.

Figure S11

Figure S11. LPS administration did not cause liver injury in myelomonocytic cell-specific S100A8 knockout mice. Related to Figure 5.

Myelomonocytic cell-specific S100A8 knockout (Lyz2^{cre}:S100A8$^{floxed/floxed}$) mice or S100A8-floxed (S100A8$^{floxed/floxed}$) mice at 10 weeks of age were used for experiments.

AST, ALT, total cholesterol and triglyceride levels in serum samples collected at 4 hours after PBS or LPS (12.5 μg/gBW) injection (n = 3).

Figure S12

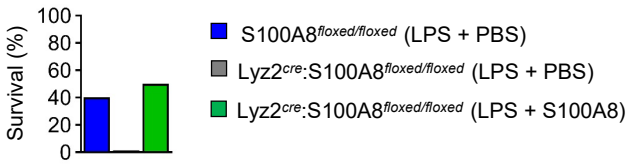

Figure S12. S100A8 improves survival rates of endotoxemia in of myelomonocytic cell-specific S100A8 knockout mice. Related to Figure 5.

Myelomonocytic cell-specific S100A8 knockout ($Lyz2^{cre}$:$S100A8^{floxed/floxed}$) mice or $S100A8^{floxed/floxed}$ mice at 10 weeks of age were injected with either PBS or S100A8 (0.1 μg/gBW) one hour after LPS (12.5 μg/gBW) stimulation. The survival rates were measured at 72 hours (n = 5-8).

Table S1. qPCR primer sequences, related to STAR Methods.

Gene (species)	Primers	
Actb (mouse)	Forward	GGCTGTATTCCCCTCCATCG
	Reverse	CCAGTTGGTAACAATGCCATGT
Tnfa (mouse)	Forward	CCCTCACACTCAGATCATCTTCT
	Reverse	GCTACGACGTGGGCTACAG
Il-1b (mouse)	Forward	GGGCCTCAAAGGAAAGAATC
	Reverse	TTGCTTGGGATCCACACTCT
Il-6 (mouse)	Forward	TAGTCCTTCCTACCCCAATTTCC
	Reverse	TTGGTCCTTAGCCACTCCTTC
Cxcl10 (mouse)	Forward	TGCTGGGTCTGAGTGGGACT
	Reverse	CCCTATGGCCCTCATTCTCAC
Mip-1a (mouse)	Forward	CCCAGCCAGGTGTCATTTTCC
	Reverse	GCATTCAGTTCCAGGTCAGTG
Mcp1 (mouse)	Forward	AAAAACCTGGATCGGAACCAA
	Reverse	CGGGTCAACTTCACATTCAAAG
S100a8 (mouse)	Forward	AAATCACCATGCCCTCTACAAG
	Reverse	CCCACTTTTATCACCATCGCAA
S100a9 (mouse)	Forward	ATACTCTAGGAAGGAAGGACACC
	Reverse	TCCATGATGTCATTTATGAGGGC
Myd88 (mouse)	Forward	CGATTATCTACAGAGCAAGGAATG
	Reverse	ATAGTGATGAACCGCAGGATAC
Irak1 (mouse)	Forward	TTCCACTCCCTGTTTCCCTC
	Reverse	AACCACCCTCTCCAATCCTG
Irak4 (mouse)	Forward	CATCGTGGCGGTGAAGAAG
	Reverse	AGCATACACTAAGCACAGGTTG
Traf6 (mouse)	Forward	TTGGAGAGTCGCCTAGTAAG
	Reverse	GTTACACTGCTGTGCTTCC
Rip1 (mouse)	Forward	CCTTCTTGCCCAGGAGAATGA
	Reverse	CTCTGAGGCGATCTGACGAC
Trif (mouse)	Forward	ACCTCCTGCATGCCATGGTTCT
	Reverse	TCAGCCAGCAGGTGGTACAA
Nf-kb (mouse)	Forward	ATTCTGACCTTGCCTATCTAC
	Reverse	TCCAGTCTCCGAGTGAAG