

Supplementary Materials for

Signaling by the Matrix Proteoglycan Decorin Controls Inflammation and Cancer Through PDCD4 and MicroRNA-21

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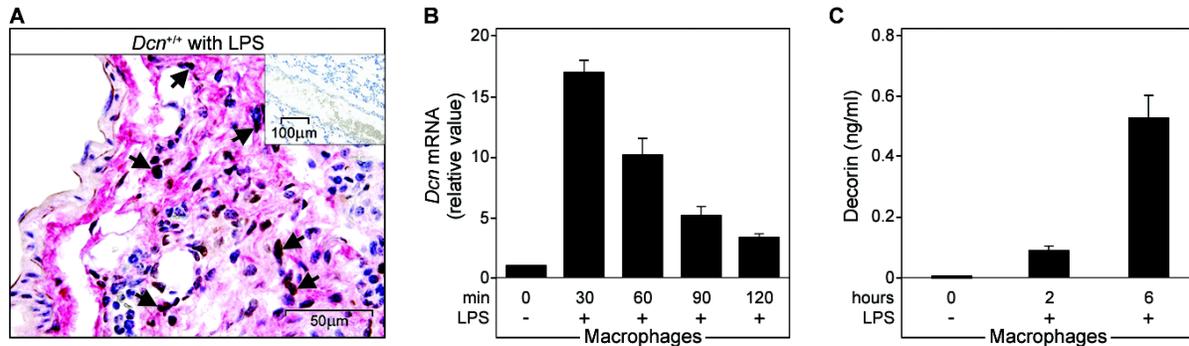


Figure S1. LPS-mediated increase in decorin abundance in macrophages in vivo and in vitro.

(A) Immunohistochemical staining for decorin (APAAP, red) and the macrophage marker F4/80 (immunoperoxidase, brown) in septic lungs from *Dcn*^{+/+} mice 2 hours after i.p. injection of 50 mg/kg LPS. Arrowheads indicate macrophages. Insert shows the negative control without the first antibody. Scale bars indicate the respective magnifications. (B) qPCR for murine *Dcn* in C57BL/6 peritoneal macrophages after stimulation with LPS (100 ng/ml) presented as fold induction relative to *Dcn* abundance in unstimulated control cells and normalized to *Gapdh*. (C) ELISA of mouse decorin in culture supernatants from C57BL/6 peritoneal macrophages stimulated with LPS (100 ng/ml) for 2 and 6 hours. Data represent the mean \pm SEM from a minimum of three independent experiments.

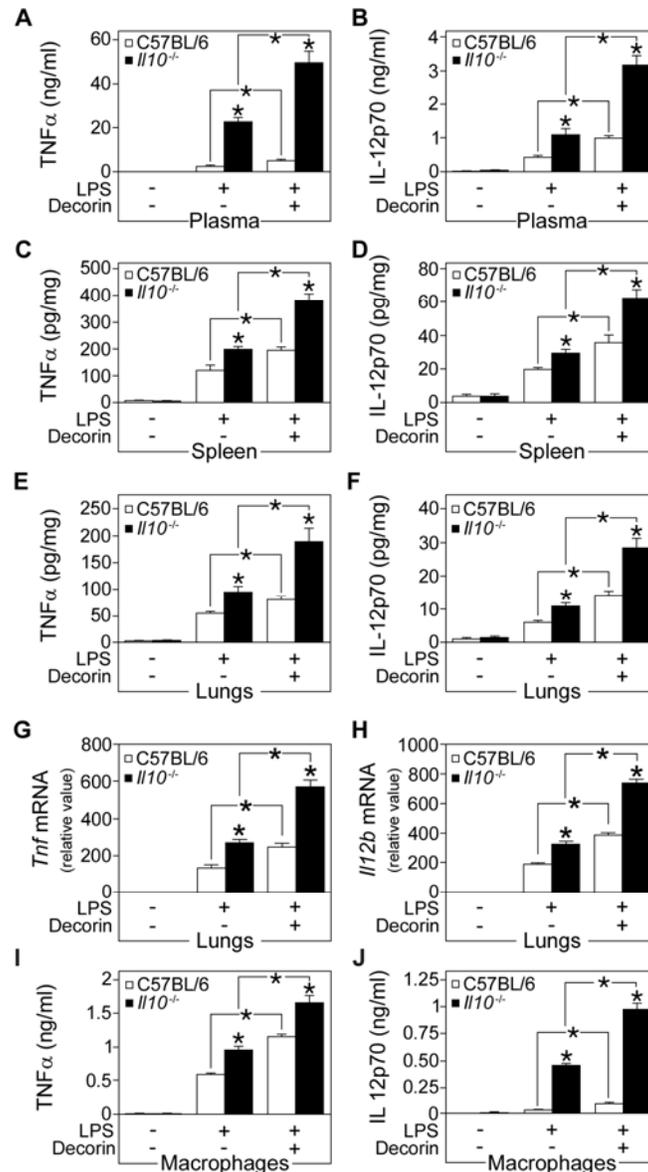


Figure S2. Role of negative feedback signaling through the IL-10 receptor in the effects of decorin and LPS on TNF α and IL-12p70 protein and mRNA abundance in vivo and in vitro. (A and B) ELISA of plasma TNF α (A) and IL-12p70 (B) in septic C57BL/6 and *Il10*^{-/-} compared to decorin-treated C57BL/6 and *Il10*^{-/-} septic mice, 2 hours after i.p. injection of 50 mg/kg LPS and intravenous administration of 5 mg/kg recombinant human decorin. (C to F) Tissue cytokine ELISA of splenic TNF α (C) and IL-12p70 (D) as well as pulmonary TNF α (E)

and IL-12p70 (F). (**G** and **H**) qPCR of pulmonary *Tnf* (G) and *Il12b* (H). Asterisks over bars indicate statistical significance for septic *Il10*^{-/-} compared to septic C57BL/6 mice with and without administration of decorin. (**I** and **J**) ELISA for TNF α (I) and IL-12p70 (J) in media from C57BL/6 and *Il10*^{-/-} macrophages stimulated with decorin (8 μ g/ml), LPS (50 ng/ml), or both for 6 hours. qPCR data are presented as fold induction normalized to *Gapdh*. Data represent the mean \pm SEM. $n = 4$ in each group. *P<0.05.

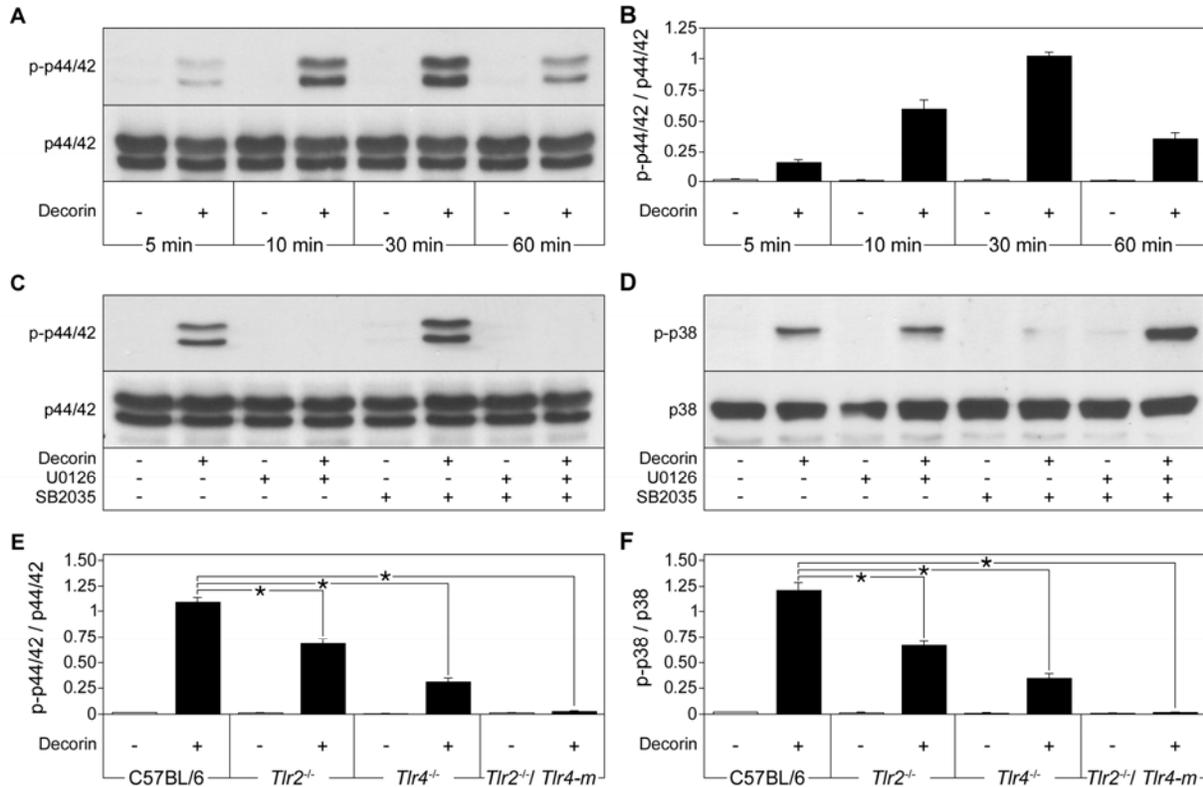


Figure S3. Decorin treatment triggers the phosphorylation of the MAPKs p44p42 and p38 in thioglycolate-elicited macrophages in a TLR2- and TLR4-dependent manner. (A and B) Representative Western blots (A) and quantifications (B) indicating decorin-mediated (8 μ g/ml, 5–60 min) phosphorylation of p44p42 in C57BL/6 macrophages. (C and D) Immunoblots showing the effects of 1 hour preincubation with U0126 (MEK-1–2 inhibitor) or SB203580 (inhibitor of p38 MAPK) (both 10 μ M) on decorin-mediated (8 μ g/ml, 30 min) phosphorylation of p44p42 (C) and p38 (D) MAPKs in C57BL/6 macrophages. (E and F) Quantification of immunoblots demonstrating decorin-dependent (8 μ g/ml, 30 min) phosphorylation of p44p42 (E) and p38 (F) MAPKs in C57BL/6, *Tlr2*^{-/-}, *Tlr4*^{-/-}, and *Tlr2*^{-/-}/*Tlr4*^{-/-} macrophages. OD of phosphorylated p44p42 or p38 protein bands were normalized to the OD of total p44p42 or p38. Data represent the mean \pm SEM from a minimum of three independent experiments. *P<0.05.

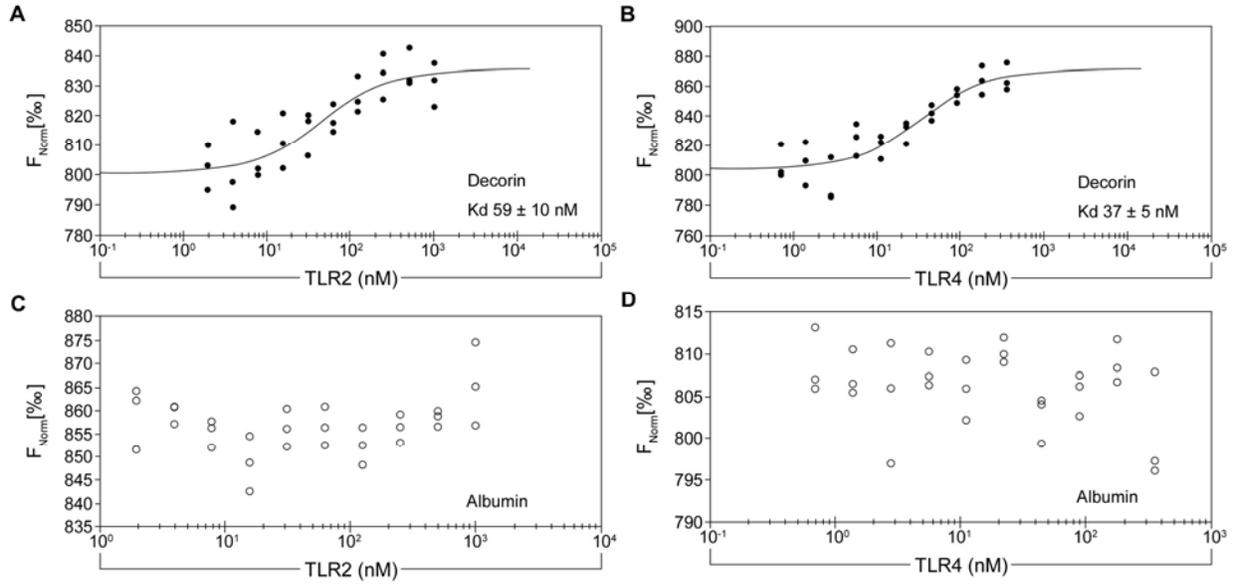


Figure S4. Analysis of decorin binding to recombinant TLR2 and to the TLR4-MD2 complex with microscale thermophoresis. (A to D) Binding of NT-647-labeled human decorin to recombinant human TLR2 (A) and to the complex of TLR4 and MD2 (B). Binding of NT-647-labeled albumin (10 nM) to TLR2 (C) and to the complex of TLR4 and MD2 (D) was used as negative control. K_d was calculated from three independent thermophoresis measurements. $F_{\text{Norm}} [\%]$ indicates normalized fluorescence per mill.

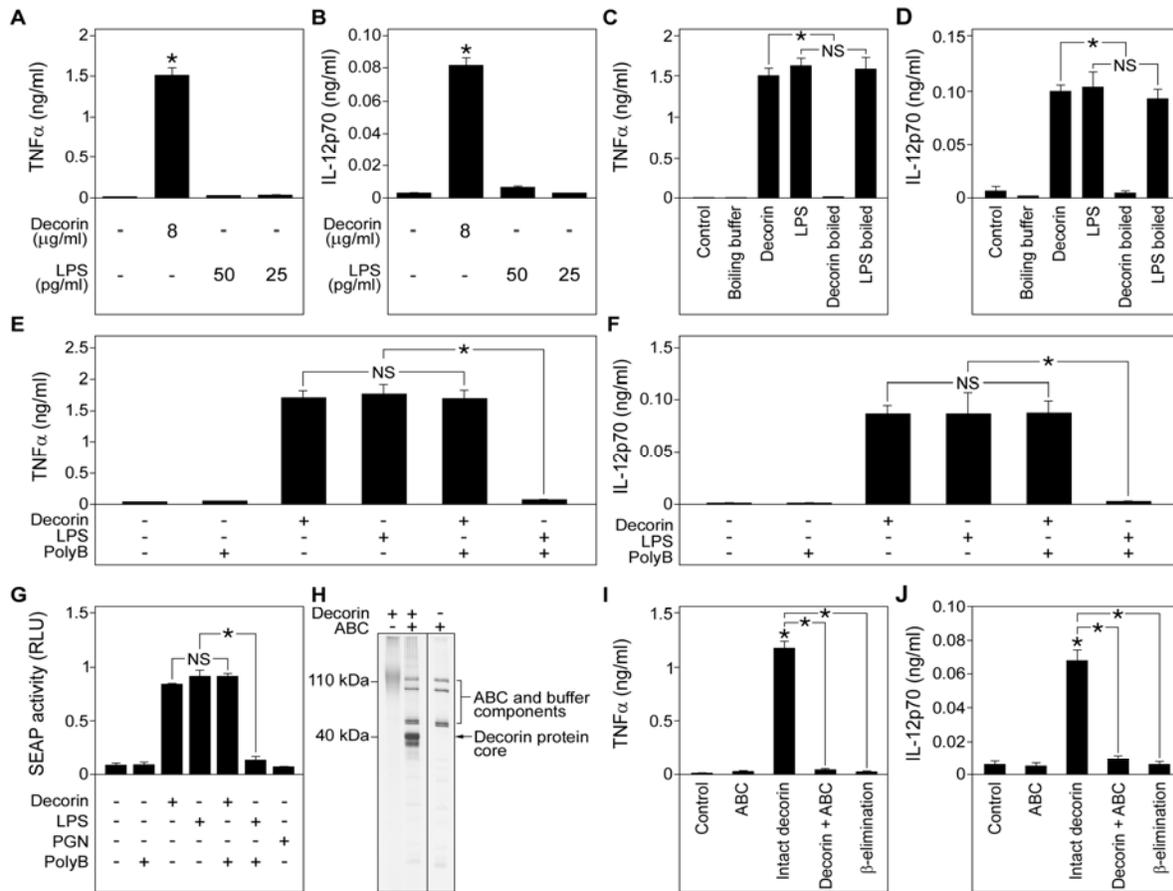


Figure S5. Procedures to rule out contamination of decorin. (A to F) ELISA of media from *Dcn*^{-/-} macrophages stimulated with decorin (8 μg/ml) or LPS for 6 hours. ELISA for TNFα (A) and IL12p70 (B) after stimulation with decorin or LPS (25 and 50 pg/ml). Endotoxin contamination of 8 μg/ml decorin corresponded to 16 pg/ml of LPS. ELISA for TNFα (C) and IL12p70 (D) after stimulation with boiled (for 30 min) decorin or LPS (100 ng/ml). ELISA for TNFα (E) and IL12p70 (F) after stimulation of macrophages with decorin or LPS (100 ng/ml) preincubated with polymyxin B (PolyB, 50 μg/ml, 1 hour). (G) SEAP activity assay in HEK-Blue-hTLR4 cells stimulated with decorin, LPS (100 ng/ml), and peptidoglycan (PGN, 5 μg/ml) with or without preincubation with polymyxin B. (H) SDS-PAGE of intact and chondroitinase ABC-digested decorin followed by silver staining. (I and J) ELISA for TNFα (I) and IL12p70 (J) in media from macrophages stimulated with decorin or GAGs (β-elimination). Decorin+ABC

indicates protein core. ABC denotes ABC chondroitinase and buffer components. Data represent the mean \pm SEM. $n \geq 3$ experiments. *P<0.05.

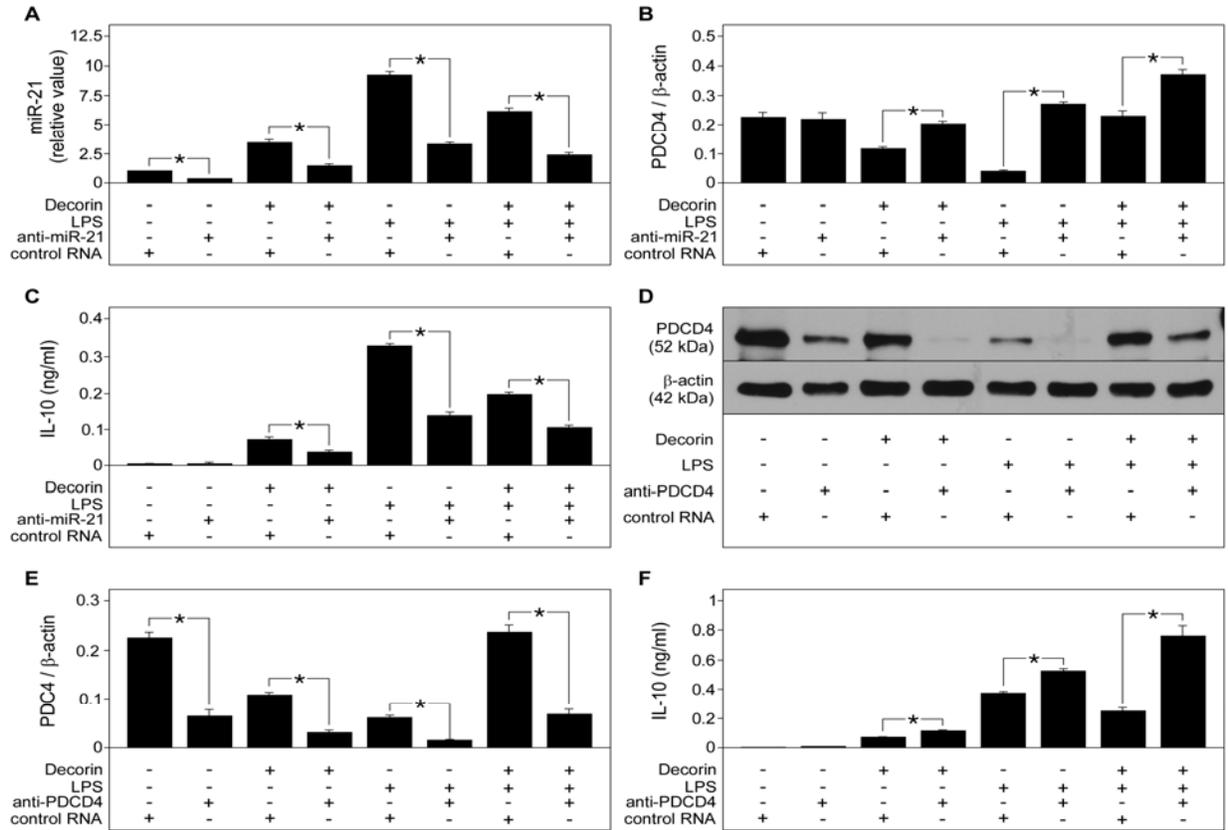


Figure S6. Decorin- and LPS-dependent regulation of IL-10 release is mediated by miR-21 and PDCD4 in thioglycolate-elicited macrophages. (A) qPCR of miR-21 in C57BL/6 macrophages transfected for 48 hours with anti-miRTM miRNA-21 inhibitor (60 nM) or anti-miRTM negative controls followed by stimulation with LPS (100 ng/ml) and decorin (8 μ g/ml) for 8 hours. Data are presented as fold induction normalized to *RNU6B*. (B and C) Quantification of Western blots for PDCD4 normalized to β -actin in cell lysates (B) and ELISA for IL-10 in culture media (C) from transfected and stimulated C57BL/6 macrophages as described in (A). (D to F) Western blots (D) and quantification of PDCD4 normalized to β -actin in cell lysates (E) and ELISA for IL-10 in the culture media from C57BL/6 macrophages transfected for 48 hours with small interfering RNA (siRNA) for PDCD4 (100 nM) and non-targeting control siRNA

followed by stimulation (F) as described in (A). Data represent the mean \pm SEM. $n \geq 3$ experiments. *P<0.05.

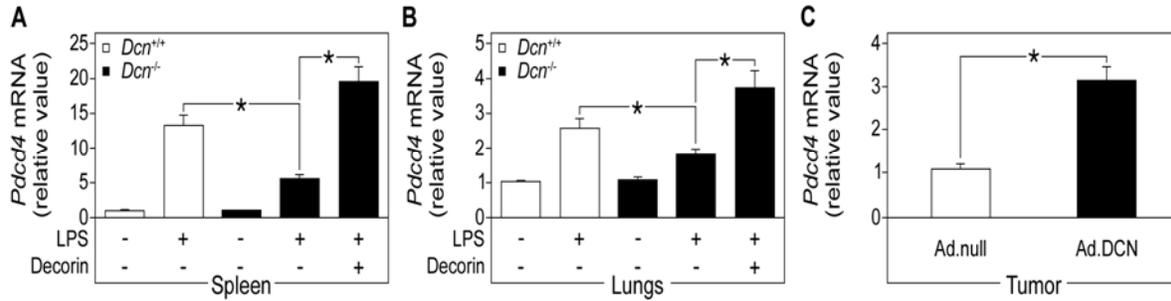


Figure S7. Decorin-dependent regulation of *Pdc4* expression in LPS-induced septic spleen and lungs and in Ad.DCN-transfected tumor xenografts. (A and B) qPCR for murine *Pdc4* in the spleen (A) and lungs (B) from septic (LPS, 50 mg/kg, i.p.) *Dcn*^{+/+}, *Dcn*^{-/-}, and decorin-treated (human decorin, 5 mg/kg, i.v.) *Dcn*^{-/-} mice compared to control animals presented as fold induction relative to the respective mRNA in the control spleen (A) or lungs (B) and normalized to *Gapdh*. (C) qPCR for murine *Pdc4* at day 10 in athymic nude (*nu/nu*) mouse tumor xenografts intratumorally injected with Ad.DCN or Ad.null vector at days 0, 3, and 6. Results are presented as fold induction relative to the respective mRNA in control Ad.null-injected tumor xenografts normalized to *Gapdh*. Data in (A) to (C) represent the mean \pm SEM of a minimum of three independent experiments. *P<0.05.

Table S1. Characteristics of patients with sepsis and healthy controls.

Characteristics	Septic patients	Healthy controls
Number of patients or volunteers	15	10
Age (mean \pm SEM in years)	54 \pm 4.4	52 \pm 6.4
Gender (F/M)	7/8	5/5
Apache II score (mean \pm SEM)	27.8 \pm 0.60	-
Infection type, n (%)		
Gram-negative	9 (60%)	-
Gram-positive	6 (40%)	-