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Supplementary Information for

C-type lectin receptor LSECtin-mediated apoptotic cell clearance by macrophages directs intestinal repair in experimental colitis

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Supplementary Information Text

Experimental Procedures.

Mice. LSECtin$^{-/-}$ were maintained as F10 generations from 129/SvJ×C57BL/6. CD45.1 mice were kindly provided by professor Mingzhao Zhu (Institute of Biophysics, CAS). LSECtin floxed mice were obtained from Mutant Mouse Regional Resource Center (MMRRC). Lyz2^{-Cre} mice were kindly provided by professor Liu Zhihua (Institute of Biophysics, CAS) and crossed with LSECtin floxed mice. All mice were on the C57BL/6 genetic background and were maintained in individually cages and were used between 6 and 12 weeks of age. As control animals, co-housed and Cre-negative littermate mice have been used in all experiments. Mice were housed in the SPF Beijing Institute of Lifeomics animal facility and all animal experiments were approved by the institutional animal care and use committee (ACUC) at Beijing Institute of Lifeomics.

Induction of colitis. Littermates from LSECtin$^{+/-}$ crosses were used for the experiments. After littermates were weaned, bedding materials from different cages were mixed twice a week so that differences in intestinal microbial environment would be further minimized. For induction of colitis, male mice received 2.5% (wt/vol) DSS (wt. 36 to 50 kDa; MP Biomedicals) in their drinking water for five days, followed by 4 days of regular water. The DSS water was replaced on days 3. On day 9, mice were sacrificed and monitored for colitis. Isolate the colon to measure length, straighten but do not stretch the colon. Then, separate the colon from the cecum (at the ileocecal junction) and quickly flush with cold PBS using 20-ml syringe with 18-G, feeding needle to remove feces and blood. For histological staining（Hematoxylin & Eosin, H&E）, colons were fixed in 10% buffered formalin.

Isolation of murine lamina propria mononuclear cells (LPMC) from colonic tissue. Colons tissue were isolated, and the residual mesenteric fat tissue was resected, opened longitudinally, washed of fecal contents and then cut into 1 cm pieces. Intestinal pieces were transferred into HBSS medium supplemented with 5% fetal bovine serum (FBS)

and 5 mM EDTA and shaken for 15 min at 37°C to remove epithelial cells. The remaining tissue was washed, cut into small pieces and subsequently incubated in digestion medium consisting of HBSS, 5% FBS, 0.5 mg/ml collagenase type VIII (Sigma), 0.5 mg/ml dispase (Roche Diagnostics), 100 IU/ml penicillin and 100 μg/ml streptomycin for 20 min at 37°C. The suspensions were filtered through a mesh and then centrifuged at 500 g for 10 min. The remaining pellets were resuspended in 6 ml of 30% Percoll (GE Healthcare), overlaid on 3 ml of 80% Percoll and centrifuged at 1000 g for 20 min at 25°C without braking. LPMCs at the interface of the two Percoll solutions were collected and subjected to flow-cytometric analysis.

Histopathology. Disease severity was determined based on weight loss, blood in the stool, and stool consistency. All mice were randomly allocated to experimental groups and mice group allocation was not recognized during the experiment. The intensity of colitis was monitored daily and clinical parameters were determined as follows: anal erosion (score 0–3; 0 = normal; 1 = mild; 2 = moderate; 3 = severe), anal bleeding (score 0–3: 0 = normal; 1 = mild; 2 = moderate; 3 = severe), diarrhea (score 0–3; 0 = normal; 1 = mild; 2 = moderate; 3 = severe), and percentage of body weight loss. Tissue sections were stained with hematoxylin & eosin (H&E). Histology was scored in a blinded manner with a scoring system as follows. Presence of ulcer: 0 = none, 1 = punctate, 2 = minimal, 3 = moderate, 4 = widespread. Presence of inflammation: 0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe. Extent of inflammation: 0 = none, 1 = mucosal, 2 = mucosal + submucosal, 3 = mucosal + submucosal + muscle penetrate, 4 = full thickness involvement. The total histopathological score was determined by summation of the scores from each category.

Generation of BM chimeras. For BM cell transfers, femurs and tibias from donor 6 to 8 week-old wild-type ($CD45.2^+$) and $LSECtin^{-/-}$ ($CD45.2^+$) mice were removed aseptically and BM cells was flushed using cold PBS. 6 to 8 week-old recipient ($CD45.1^+$) mice were irradiated with 11 Gy (5.5Gy twice) and were reconstituted 8 weeks after irradiation with 1×10^7 CD45.2 BM cells by lateral tail-vein injection. Mice were given sulfamethoxazole in the drinking water for the first 2 weeks of reconstitution before

being switched to antibiotic-free water. Chimeras were received 2.5% DSS supplied water. Prior to challenge, we confirmed that mice reconstituted with CD45.2 BM cells had achieved a satisfactory level of chimerism by assessing the number of $CD45.2^{+}$ colonic macrophages using flow cytometry.

TUNEL staining of colon. Mice were euthanized on day 5 after DSS treatment. Dissected mice colons were flushed with PBS. Colons were then fixed overnight in 10% neutral buffered-formalin prior to paraffin embedding. Proximal colon region was cut by microtome at 8μm intervals. After de-waxing, colonic sections were treated with proteinase K (10 mg/ml; Promega) for 20 min at 25℃ and then incubated with TUNEL reaction mixture for 60 min at 37°C, using the in situ cell death detection kit, TMR red (Roche) according to the manufacturer instructions. The nuclei were counterstained with DAPI.

Immunofluorescence. Mousecolon were fixed in 4% paraformaldehyde overnight at 4ºC and then embedded in OCT (Sakura). 5μm Frozen sections were prepared using a Cryotome FSE cryostat (Thermo-Fisher Scientific). The tissue sections were incubated in the blocking buffer (5% donkey serum, 0.3%Triton-X 100 in PBS) at room temperature for 1h followed by the staining with primary antibodies. The following primary antibodies were used: Rat anti-mouse F4/80 (clone CI:A3-1, ab6640, Abcam), Rabbit anti-mouse Ki67 (clone SP6, ab16667, Abcam), Rabbit anti-mouse LSECtin polyclonal antibody, Rabbit anti-human anti-CLEC4G antibody(clone EPR13724, ab181196), Mouse anti-mouse anti-pan Cytokeratin antibody (clone C-11, ab7753).Then slides were washed and incubated for 1 hour with the following secondary antibodies: Donkey anti-rat Alexa Fluor 488, Donkey anti-goat Alexa Fluor 594, Donkey anti-rabbit Alexa Fluor 647 and Goat anti-mouse TRITC (Jackson ImmunoResearch Laboratories). Sections were counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) before being mounted. All immunofluorescence staining was performed in the dark. Imaging was performed using a Zeiss LSM 880 and images were processed using Zeiss ZEN software.

Immunohistochemistry staining procedures. The immunohistochemical staining step was briefly described as follows. The paraffin section was placed in a 55℃ oven for 4 hours to melt the paraffin. The paraffin section was dewaxed in a solvent to hydrate. Tissue was quenched with three hydrogen peroxide, and antigen retrieval was performed in citrate solutions, and blocked with PBS solution containing 2% bovine serum albumin(BSA) and 5% goat serum for 1 h at room temperature. Then followed by the staining with primary antibodies(the following primary antibodies were used: Rabbit anti-mouse Ki67 (clone SP6, ab16667, Abcam), Rabbit anti-mouse LSECtin polyclonal antibody)overnight，and then incubated in secondary antibody(enzyme-labeled goat anti-rabbit IgG polymer(PV-6002，ZSGB-BIO))for 2 h. The tissue was then reacted with 3,3'-diaminobenzidine (DAB; Sigma) to visualize antibody location. The tissue was redyed by hematoxylin, then tissue was dehydrated and sealed. Imaging was performed using a NanoZoomer 2.0-RS(HAMAMATSU)and images were processed using NDP.viewer 2 software.

Flow Cytometry Analysis and FACS Sorting. The isolated cell suspension was washed with PBS and were first incubated with Fcγ receptor blocker (CD16/32, eBioscience), then stained with specific mAbs at indicated concentration in PBS solution containing 1% FBS. The following antibodies were used: fluorescently-conjugated antibodies (eBioscience) directed against mouse CD45 (30-F11), CD45.1 (A20), CD45.2 (104), CD11b (M1/70), F4/80 (BM8), Ly6C (HK1.4), Gr-1(RB6-8C5), Ly6G (1A8, BD Biosciences), CD115 (AFS98), CD11c (N418), MHC-II (M5/114.15.2), CD3 (17A2), B220 (RA3-6B2), CD4 (GK1.5), CD64 (X54-5/7.1) and their corresponding isotype controls. Antibody staining was performed at 4 °C for 30 min and the stained cells were analyzed on an LSRFortessa cell analyzer (BD Biosciences). The acquired data were analyzed with FlowJo software. For cell sorting, FACSAria III (BD Biosciences) were used. FACS sorting routinely yielded cell purity levels of over 90%.

Adoptive transfer of monocytes. To adoptively transfer wild-type or LSECtin$^{-/-}$ mice bone marrow derived CD115^{+}monocytes,viable CD11b^{+}CD115^{+} monocytes from bone marrow were sorted by FACS (using BD FACSAria III). 4×10^{6} monocytes were then

adoptively transferred into recipient mice (LSECtin$^{-/-}$) by intravenous injection one day before and three days after DSS colitis.

Phagocytosis assay. For *in vitro* flow cytometry analysis of phagocytosis, 5.0 $\times 10^5$ Raw264.7 cells were plated in the 12-well plate one day before the phagocytosis assay. Next day, the cells were starved for 30 min with 1640 containing 2% FBS and incubated with fluorescently labeled targets, such as 4.0$\times$ 10^6 CMTMR (Invitrogen)-stained apoptotic thymocytes, or 2-mm carboxylate-modified latex beads (Invitrogen) in the uptake buffer (1640 containing 2% FBS, 0.2% penicillin-streptomycin). After incubation for the indicated time, the cells were extensively washed with cold PBS, trypsinized and resuspended in cold medium containing 3% FBS, and analyzed by flow cytometry. To inhibit engulfment, 1μM of cytochalasin D (Cyto D, Sigma) was added 30 min before adding apoptotic thymocytes. For blocking PtdSer recognition we used purified recombinant Annexin V (BD Pharmingen, 556416), 15 μg Annexin V was added to 1$\times$ 10^5 CMTMR (Invitrogen)-stained apoptotic thymocytes and then resuspend cells in 1X binding buffer at a concentration of 1 x 10^6 cells/ml and incubate for 15 min at room temperature before added to the Raw264.7 cells.

For engulfment assay with peritoneal macrophages or Kupffer cells (KCs), those macrophages were plated at 5×10^5 cells in 24-well plates and incubated with CFSE-labeled apoptotic thymocytes for 30 min. The samples were analyzed by flow cytometry. Forward and side-scatter parameters were used to distinguish unengulfed targets from phagocytes. The data were analyzed using FlowJo software.

For *in vivo* phagocytosis assays of KCs, 2×10^7 CFSE labeled apoptosis cells were injected intravenously. One hour later, livers were fixed in 4% paraformaldehyde and and then embedded in OCT (Sakura) used for immunofluorescence analyses. For *in vivo* phagocytosis assays of peritoneal macrophages, 2×10^7 CFSE labeled apoptosis cells were injected intravenously. One hour later, the peritoneum was lavaged with 10 mL PBS with 1 % FBS and the collected cells were resuspended in PBS and as assessed by flow cytometer analysis.

Crypt isolation and organoid culture. Isolation of intestinal crypts was largely performed as previously described. In brief, after euthanizing the mice with CO_2 and collecting small intestines, the organs were opened longitudinally and washed with PBS. To dissociate the crypts, small intestine was incubated in collagenase type I (Sigma) supplemented with 0.05mg/ml DNase1 (Sigma) for 30 min at 37 °C to isolate the crypts. For mouse organoids, depending on the experiments, 200–400 crypts per well were suspended in Matrigel composed of 25% advanced DMEM/F12 medium (Gibco) and 75% growth-factor-reduced Matrigel (BD). After the Matrigel polymerized, complete ENR medium containing advanced DMEM/F12 (Gibco), 2 mM Glutamax (Invitrogen), 10 mM HEPES (Sigma), 100 U ml−1penicillin, 100 μg ml−1 streptomycin (Invitrogen), 1 mM N-acetyl cysteine (Sigma), B27 supplement (Invitrogen), N2 supplement (Invitrogen), 50 ng ml−1 mouseEGF (Peprotech), 100 ng ml−1 mouse Noggin (R&D), 300 ng ml−1 human R-spondin-1(R&D) and 10% culture medium from WT or KO macrophages was added to small intestine crypt cultures.

Determination of colon regeneration. For Ki67 staining, paraffin sections were stained using anti-Ki67 antibody (abcam). For each analysis, Ki67-positive cells were scored in 50 full crypts from three animals of each genotype. For BrdU staining, mice were i.p. with 100 mg/kg BrdU (Sigma) in PBS for 3 hours and then sacrificed. Paraffin sections were stained for BrdU with BrdU In-Situ detection Kit (BD PharMingen). BrdU positive cells were scored in 45 full crypts from three animals of each genotype for each analysis.

Antibiotic Treatment. Full-spectrum antibiotic treatment was supplied for 3 weeks in drinking water containing containing ampicillin (1 g/l; Sigma), gentamicin (1 g/l; Sigma), Kanamycin(1 g/l; Sigma),vancomycin (500 mg/l, Am), neomycin sulfate (1 g/l, Sigma), metronidazole (1 g/l, Sigma) and fluconazole (1 g/l, Sigma) in drinking water for 3 weeks. The antibiotic solution was refreshed every 4 days. Fecal pellets were collected and genomic DNA was isolated by using QIAamp DNA Stool Mini Kit (Qiagen) according to the manufacturer's instructions then total fecal DNA were tested for microbiota depletion by quantitative PCR. The primer following were used, Bactria 16S (8F&R357): 5’-

AGAGTTTGATCMTGGCTCAG-3', 5'-CTGCTGCCTYCCGTA-3' and fungal 18S : 5'-ATTGGAGGGCAAGTCTGGTG -3, 5'- CCGATCCCTAGTCGGCATAG -3'.

In vivo epithelial permeability assay. Mice were deprived of water and food for 3 h and then gavaged with FITC- dextran (MW 40,000; Sigma-Aldrich) at 0.6 mg/g body weight. 4 h later fluorescence was measured in sera using a spectraMax Paradigm. FITC-dextran concentration was determined from standard curves generated by serial dilution of FITC-dextran.

Lamina propria mononuclear cells stimulation and cytokine analysis. Cell suspensions were prepared for LPMC as described above. Cells were incubated with 50 ng/ml phorbol 12-myristate 13-acetate (PMA), 500 ng/ml ionomycin and 10 μg/ml Brefeldin A (eBioscience) in complete RPMI media at 37° C for 6 h. After surface staining with CD4, cells were permeabilized and intracellular cytokine staining was performed using APC-labeled anti-IFNγ mAb (eBiosciences) and PE-labeled anti-IL-17 mAb (eBiosciences) according to the manufacturer's instructions.

Generation of bone marrow derived macrophages. For mouse bone marrow–derived macrophages (BMDM), femurs and tibias were harvested from 5- to 6-week-old mice and the marrow was flushed and placed into a sterile suspension of PBS. The bone marrow suspension was cultured in RPMI-1640 medium plus 10%FBS,1%penicillin-streptomycin-glutamine with 50 ng/ml recombinant murine macrophage colony-stimulating factor (M-CSF, Peprotech). Six days later, more than 90% of the adherent cells were CD11b positive and 80% of the cells were F4/80 positive.

Real time PCR analysis. Total RNA was extracted from colon tissues or cells with the RNeasy kit (Qiagen, 74104). cDNA was synthesized with Reverse Transcription System Kit (Promega, A3500). Real-time PCR analysis via iQ SYBR Green supermix (Bio-Rad Laboratories) was performed on real-time system (Bio-Rad Laboratories). The amount of mRNA was normalized to the amount of *Gapdh*, a housekeeping control gene. The thermocycling program was 40 cycles at 95°C for 15s, 60°C for 30s, and 72°C for 30s,

with an initial cycle of 95°C for 2 min. Normalized data were used to measure relative expression levels of different genes using ΔΔCt analysis. Serially diluted samples of pooled cDNA were used as external standards in each run for the quantification and results were expressed in fold induction relative to the respective control. Primer sequences used in this study are summarized in Supplementary Table 1.

Supplementary Figures

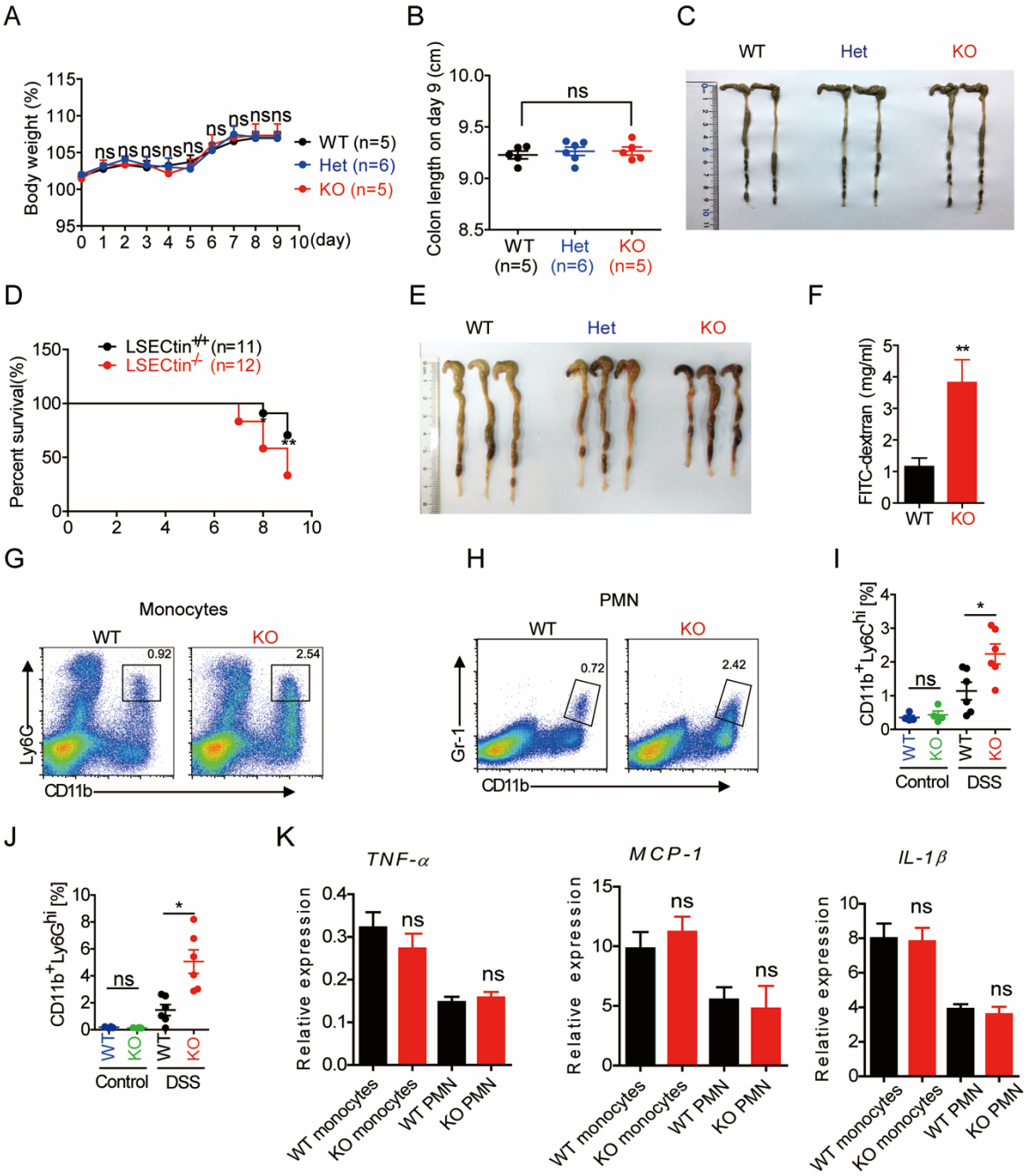


Fig. S1. LSECtin protects mice against DSS-induced colitis (related to Figure 1).

(A) Mean changes in body weight of LSECtin$^{-/-}$ (KO) and littermate LSECtin$^{+/+}$ (WT) and LSECtin$^{+/-}$ (Het) mice given the regular water. **(B-C)** Colon length **(B)** and representative morphology of colons are shown **(C)**. **(D)** Kaplan–Meier survival plot of WT and KO

mice after induction with 2.5% DSS in the drinking water. **(E)** The gross morphology of shortened and bleeding colons are shown. **(F)** DSS-challenged WT and KO mice were gavaged with FITC–dextran, and the fluorescence intensity of the serum was measured 4 hours later. **(G-H)** Flow cytometry analysis of colonic lamina propria monocytes (**G**) and neutrophils **(H)** of DSS-challenged WT and KO mice. **(I-J)** Colon lamina propria mononuclear cells (LPMCs) were isolated from DSS challenged mice, and the ratios of monocytes (**I**) and neutrophils (**J**) were analyzed by FACS. **(K)** mRNA expression of cytokines was detected in isolated cells from WT and KO mice colon with colitis. The number in parentheses represents the number of mice used in each group. The results shown are the mean ± SEM. A two-tailed unpaired t-test was used to compare experimental groups. Asterisks indicate $p < 0.05$ (WT vs. KO). Data are representative of seven independent experiments with similar results**.**

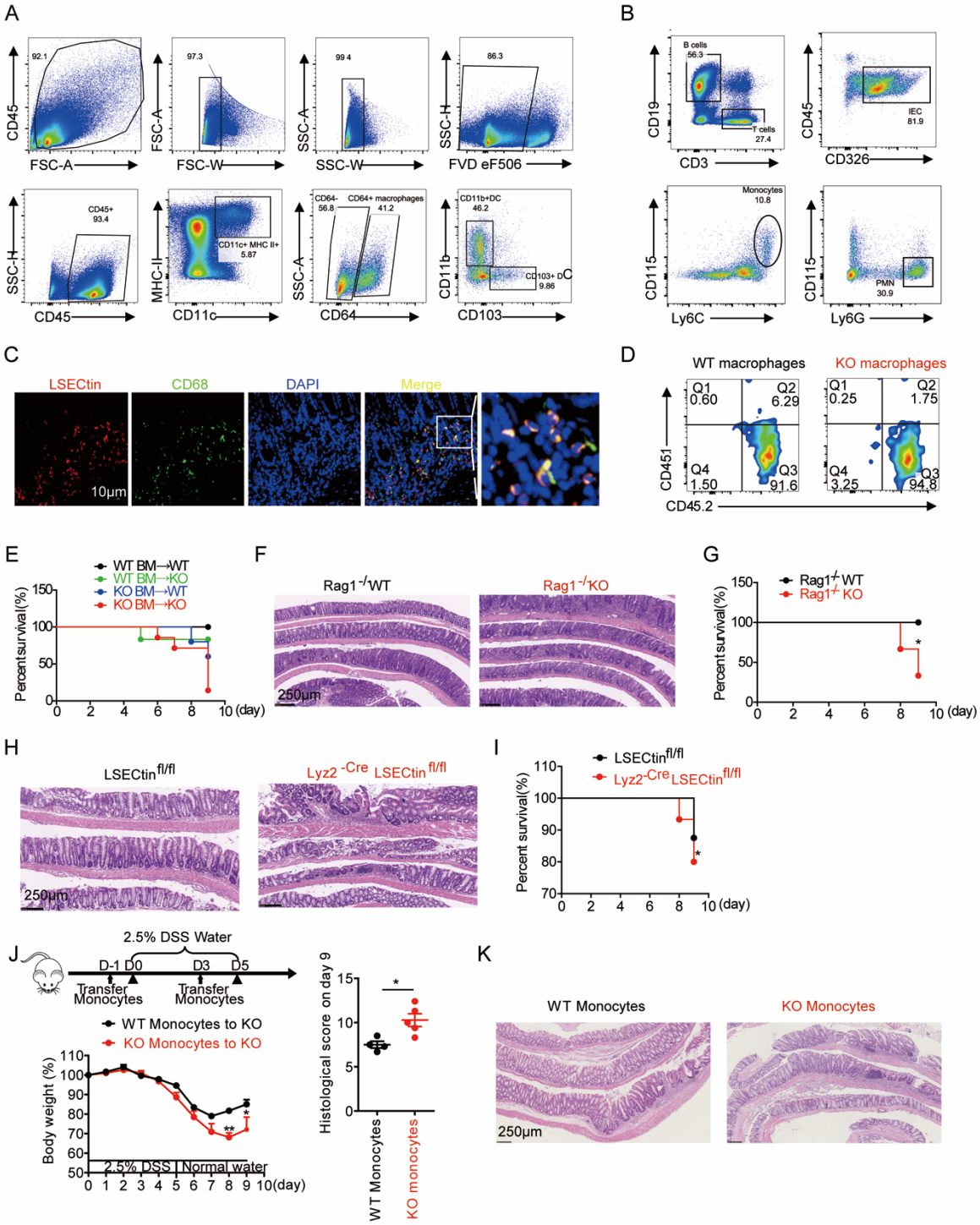


Fig. S2. LSECtin in macrophages protects mice against DSS-induced colitis (related to Figure 2).

(A) Schematic of sorting the DCs and macrophages of the colon. **(B)** Schematic of sorting the T, B, epithelial cells of the colon and the monocytes and neutrophils from the

bone marrow cells. **(C)** Immunofluorescence (IF) analysis of colon sections from human stained with anti-LSECtin and anti-CD68 Abs. Scale bar, 10 μm. **(D)** Whole wild-type or LSECtin$^{-/-}$ mice bone marrow cells from donor (CD45.2^{+}) mice were cross-transferred to irradiated recipients (CD45.1^{+}) and evaluated for engraftment 8 weeks later. Each column of the FACS plots corresponds to the subpopulation labelled at the top. Colonic macrophages were gated on MHC-II^{+} CD11c^{+} CD64^{+} and analysed for the expression of CD45.1 and CD45.2. **(E)** Kaplan–Meier survival plot of bone-marrow radiation chimaera mice. **(F, H, K)** Representative microscopic pictures of H&E-stained colons from Rag1$^{-/-}$ mice **(F),** LSECtin ΔMφ **(H)** and monocytes transfer groups **(K)** are shown. **(G, I)** Kaplan–Meier survival plot of Rag1$^{-/-}$ mice **(G)** and LSECtin ΔMφ **(I)** mice are shown. **(J)** KO mice were injected intravenously with bone marrow monocytes from WT or KO mice, and the recipients were challenged with DSS; body weight changes were monitored throughout the study.The results shown are the mean ± SEM. A two-tailed unpaired t-test was used to compare experimental groups. Asterisks indicate $p < 0.05$ (WT BM vs. KO BM; Rag1$^{-/-}$WT vs. Rag1$^{-/-}$KO; LSECtin$^{-/-}$ vs. Lyz2^{-Cre}LSECtin$^{-/-}$;WT monocytes vs. KO monocytes).**(E,G,I,J)**.

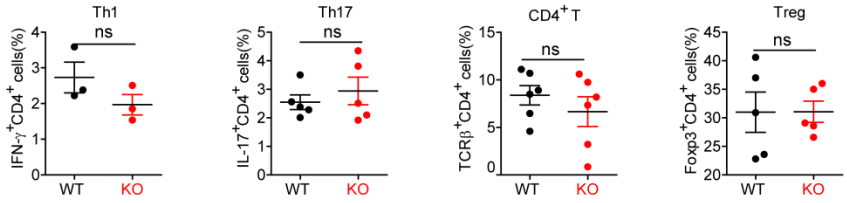


Fig. S3. LSECtin had no influence on the proportion of subpopulations of CD4$^+$ T cells constitution (related to Figure 3).

The percentage of total CD4$^+$ T cells (gated on CD45$^+$) and the different CD4$^+$ T cell subsets (gated on CD4$^+$ cells) from the mice colon with colitis were analysed. The results shown are the mean ± SEM. A two-tailed unpaired t-test was used to compare experimental groups. Asterisks indicate $p < 0.05$.

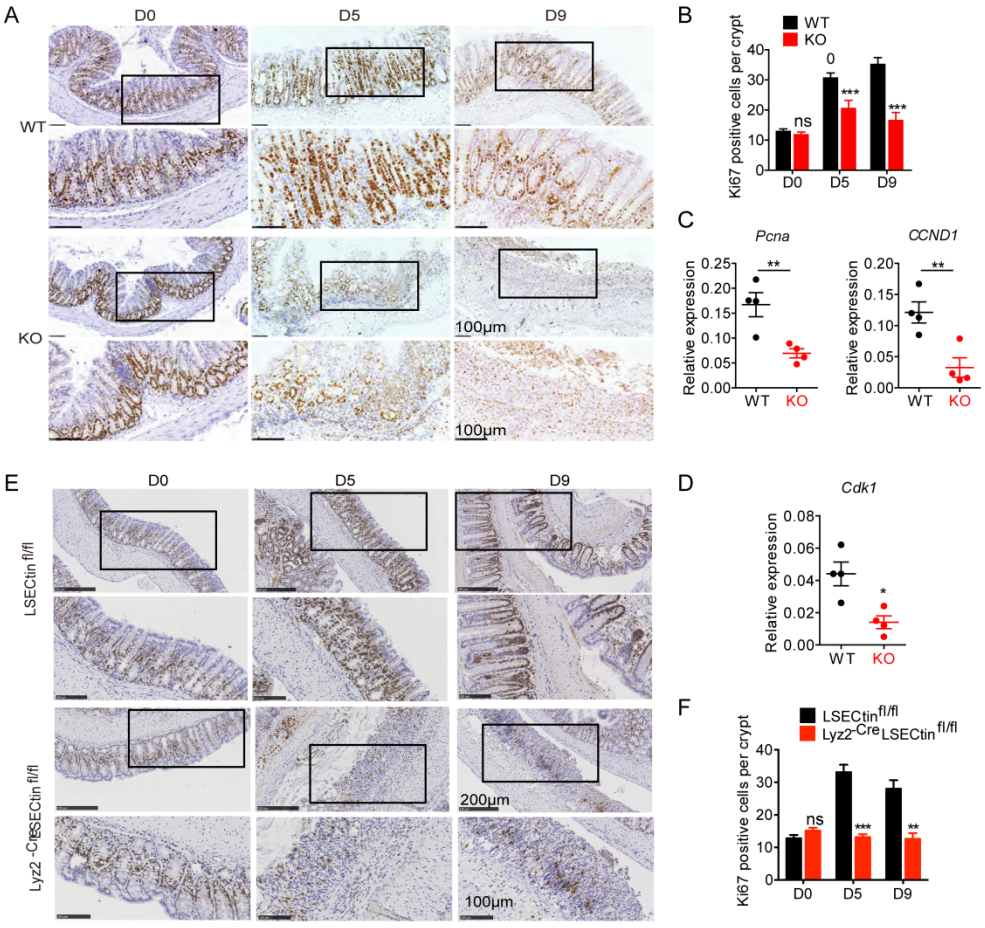


Fig. S4.LSECtin promotes the repair of the intestinal epithelium (related to Figure 3).

(A) Representative pictures of Ki-67 staining in the colon of WT and KO mouse from different times during DSS-induced colitis . **(B)** The number of Ki-67$^+$ cells/colon crypt was quantified. **(C)** Quantitative mRNA expression of PCNA and Cyclin **D**1 were detected in colons of DSS-challenged WT or KO mice. **(D)** Quantitative mRNA expression of CDK1 in the colons of WT and KO mice on day 9 of DSS-induced colitis. **(E)** Representative pictures of Ki-67 staining in the colon of WT and LSECtin ΔMφ mice from different times during DSS-induced colitis. **(F)** The number of Ki-67$^+$ cells/colon crypt was quantified. The results shown are the mean ± SEM. A two-tailed unpaired t-test was used to compare experimental groups. Asterisks indicate $p < 0.05$.

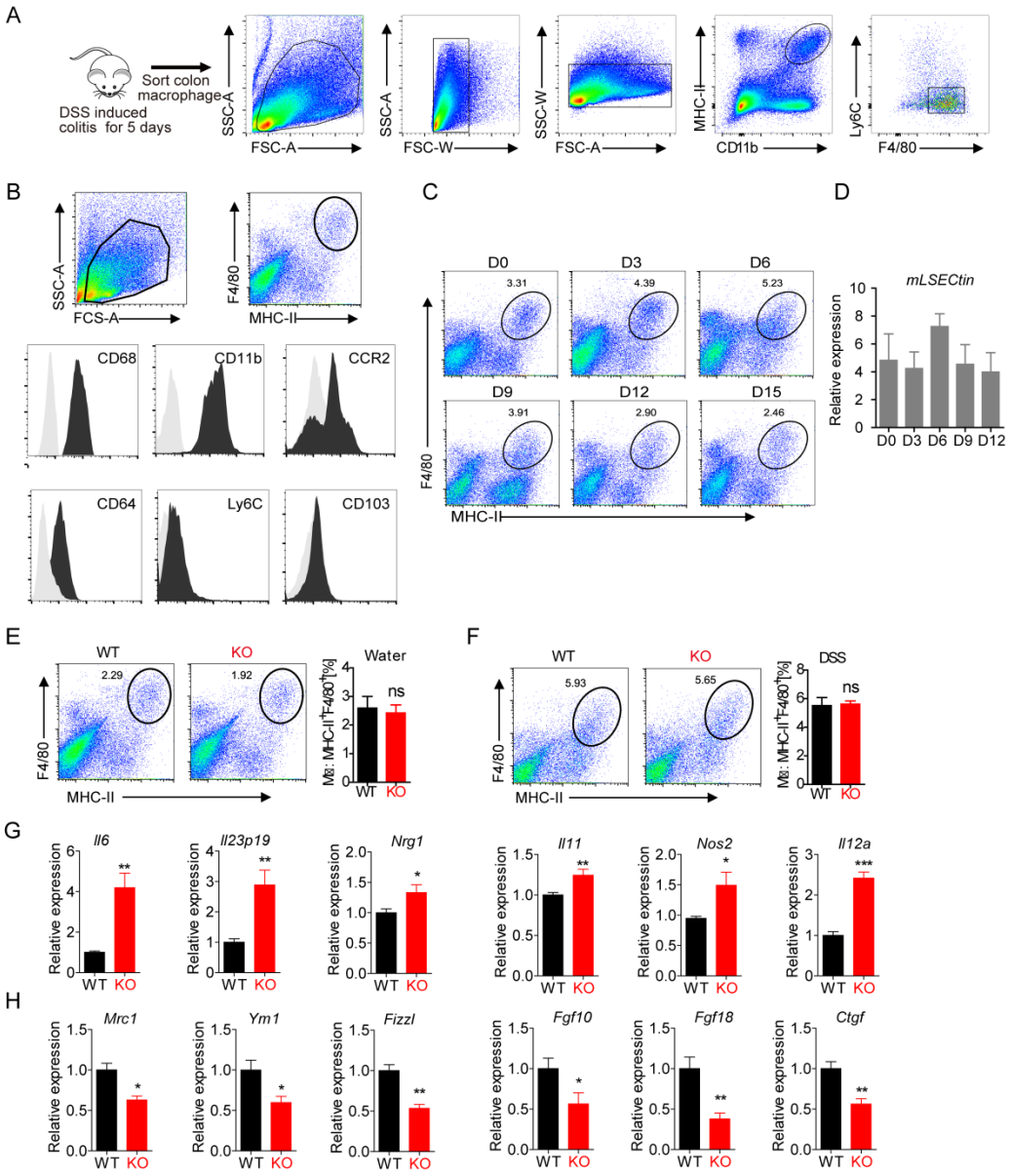


Fig. S5. The numbers of immune cells in the colon during DSS-induced colitis (related to Figure 4).

(**A**) Schematic of the sorting of colitis colon macrophages. (**B**) Identification of colonic lamina propria macrophages and the histograms show the makers CD68, CD11b, CCR2, CD64, Ly6C and CD103 expression in the intestinal gated on F4/80^{+}MHC-II^{+} subset. (**C, D**) Flow cytometry analysis of colonic lamina propria macrophages from different time of the DSS colitis **(C)** and the expression of LSECtin in indicated cell-sorted

macrophages as detected by qPCR **(D)** Mean (SEM) (from two pool data, n = 6). **(E, F)** Percentage of $F4/80^{+}MHC\text{-}II^{+}$ cells in the colon lamina propria from naive (**E**) or DSS challenged (**F**) WT and KO mice as shown by FACS analysis and graphical summary of percentage of indicated cell subset. Mean (SEM) (from three pool data, n = 10). (**G-H**) Expression of mRNA for M1-related genes **(G)** M2-related genes and tissue-protective and wound healing genes **(H)** of WT and KO colon macrophages was determined by qPCR and was presented relative to expression of the housekeeping gene *Gapdh*. Data are representative of three independent experiments, n = 15–18, with 4-5 mice as a pooled data set. The results shown are the mean ± SEM. A two-tailed unpaired t-test was used to compare experimental groups. Asterisks indicate $p < 0.05$(C-G).

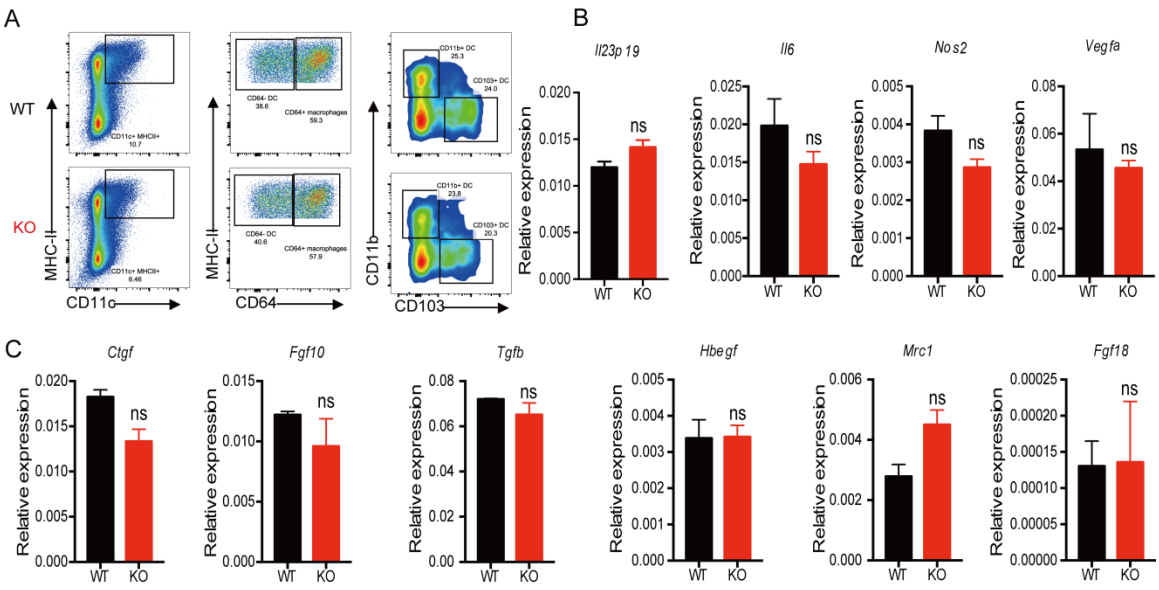


Fig. S6. Expression profile of WT and KO colonic DCs (related to Figure 4).

(**A**) Flow cytometry analysis of colonic lamina propria dentritic cells and the different subsets. (**B, C**) mRNA Expression of cytokine-related genes **(B)** and tissue-protective and wound healing genes **(C)** by WT and KO dendritic cells (gated on $CD45^{+}CD11c^{+}MHC\text{-}II^{+}CD64^{-}$ cells) were determined by qPCR. Mean (SEM) (from two pool data, n = 7). Results are representative of two or more independent experiments. Asterisks indicate $p < 0.05$.

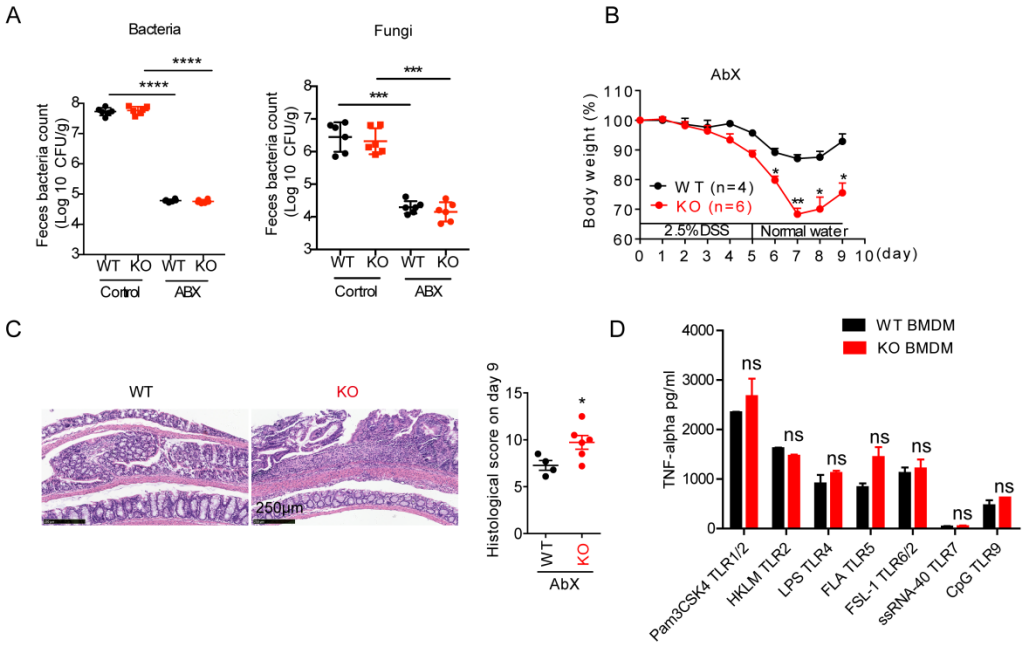
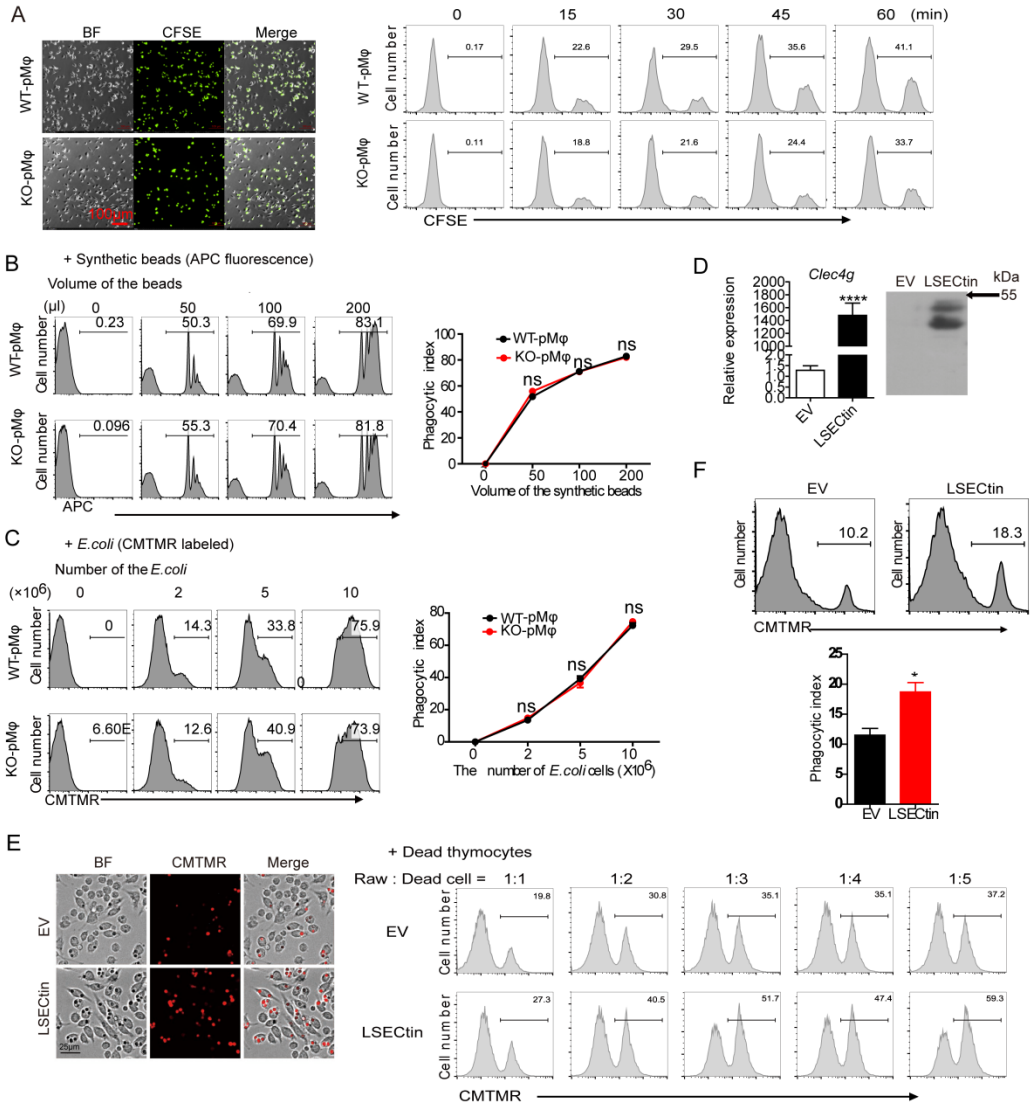


Fig. S7. LSECtin had a microbiota-independent protective function (related to Figure 5).

(A) Fecal pellets were collected and tested for microbiota depletion by quantitative PCR. **(B)** All mice were treated with a cocktail of antibiotics (AbX) and then challenged with DSS-induced colitis, the mean change in body weight (left; relative to starting weight, set as 100%) is shown. **(C)** Histological scores of the colons of 2.5% DSS-treated mice on day 9 were measured, and representative microscopic pictures of H&E-stained colons are shown. (**D**) TNF were measured in the supernatant of WT and KO BMDM treated with different ligands. The results shown are the mean ± SEM. A two-tailed unpaired t-test was used to compare experimental groups. Asterisks indicate $p < 0.05$.


A
BF
CFSE
Merge
WT-pMφ
KO-pMφ
100μm
0
15
30
45
60 (min)
Cell number
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22.6
29.5
35.6
41.1
0.11
18.8
21.6
24.4
33.7
CFSE
B
+ Synthetic beads (APC fluorescence)
Volume of the beads
(μl)
0
50
100
200
0.23
50.3
69.9
83.1
0.096
55.3
70.4
81.8
APC
Phagocytic index
WT-pMφ
KO-pMφ
ns
Volume of the synthetic beads
C
+ E.coli (CMTMR labeled)
Number of the E.coli
(×10^6)
0
2
5
10
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40.9
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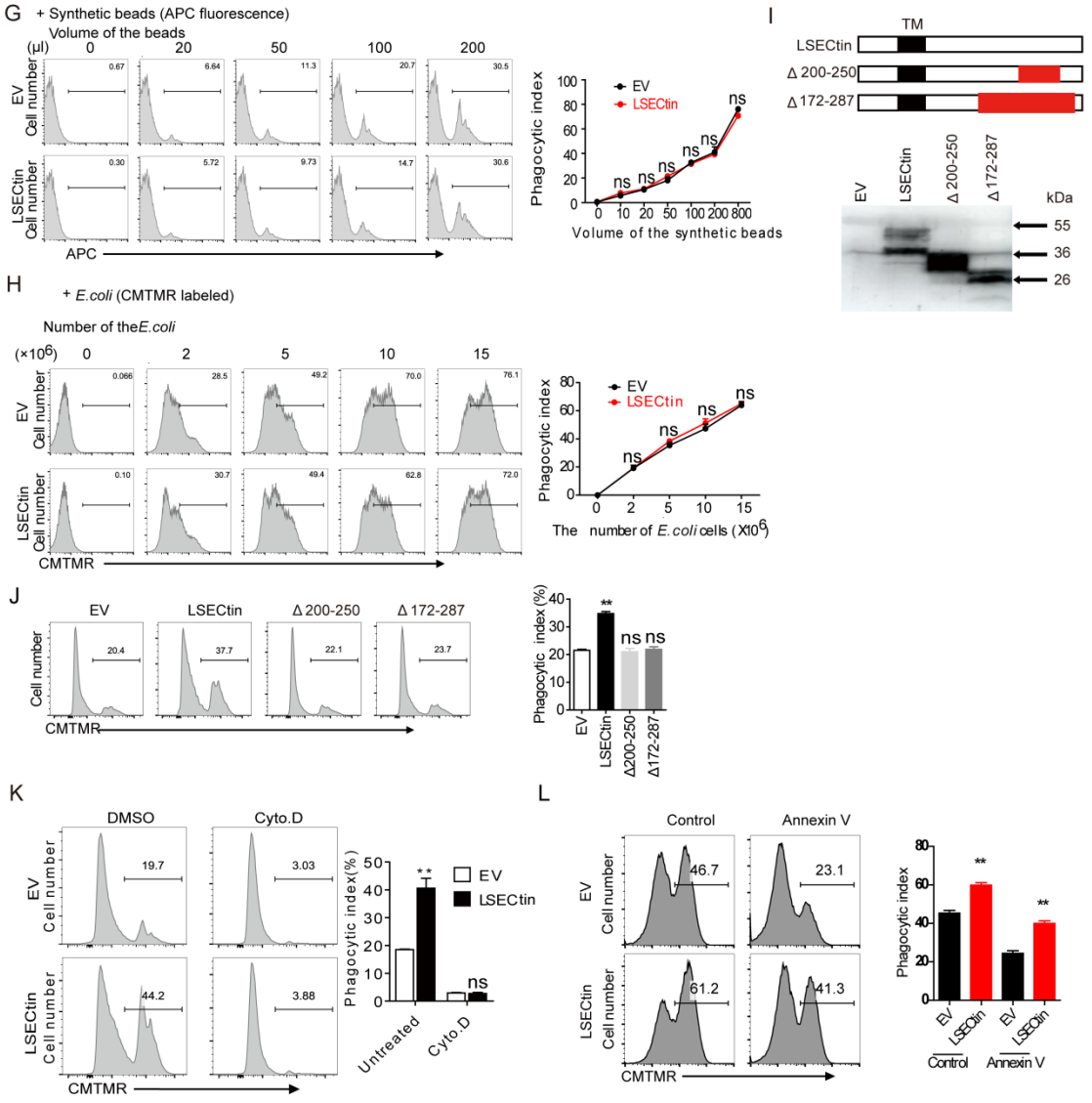

Fig. S8. LSECtin on macrophages contributes to apoptotic cell clearance. (related to Figure 5).

(A) The CFSE-labeled apoptotic thymocytes were incubated with WT and KO peritoneal macrophages (pMφs), and phagocytosis was determined by measuring the positive CFSE-containing macrophages.Apoptotic thymocytes were incubated for 30 min with pMφs, and phagocytosis was examined by immunofluorescence microscopy，scale bar,100μm. **(B, C)** WT and KO pMφs were incubated with carboxylate-modified APC fluorescent beads (synthetic beads) at the indicated volumes **(B)** and CMTMR-labelled *E.*

coli. **(C)** The phagocytosis was determined by the percentage of macrophages containing a positive CFSE or CMTMR signal. **(D)** Raw 264.7 macrophage cells were transfected with the control or LSECtin plasmid and expression was detected by WB and qPCR. **(E)** Raw 264.7 cells transfected with the empty vector control (EV) or LSECtin were incubated with CMTMR (red)–labeled apoptotic thymocytes, and phagocytosis was determined by measuring the number of positive CMTMR-containing macrophages. Phagocytosis was also examined by immunofluorescence microscopy (red fluorescence: engulfed thymocytes)，scale bar, 25μm. **(F)** Raw 264.7 cells transfected with the empty vector control (EV) or LSECtin were incubated with CMTMR (red)–labelled apoptotic CT-26 cells, and phagocytosis was determined by measuring the number of positive CMTMR-containing macrophages. **(G)** Raw 264.7 cells were incubated with carboxylate-modified APC fluorescent beads (synthetic beads) at the indicated volumes, and phagocytosis was determined by the percentage of macrophages containing a positive APC fluorescence signal. **(H)** Raw 264.7 cells were incubated with CMTMR-labeled *E. coli*, and phagocytosis was determined by the percentage of macrophages containing a positive CMTMR signal. **(I)** WT LSECtin and mutant Δ172-250 and Δ200-250 constructs were transfected to form stable expression cell lines, and expression was detected by WB. **(J)** Raw 264.7 cells transfected with EV, the full-length LSECtin or different LSECtin mutants (Δ172-250, Δ200-250) were incubated with CMTMR (red)–labeled apoptotic thymocytes, and phagocytosis was determined by FACS analysis. **(K)** Raw 264.7 cells transfected with EV or LSECtin were treated with cytochalasin D (CytoD) before incubation with CMTMR (red)–labeled apoptotic thymocytes, and phagocytosis was determined by FACS analysis. **(L)** Raw 264.7 cells transfected with the empty vector control (EV) or LSECtin were incubated with CMTMR (red)–labelled apoptotic thymocytes with or without purified recombinant Annexin V protein, and phagocytosis was determined by measuring the number of positive CMTMR-containing macrophages. The results shown are the mean ± SEM. A two-tailed unpaired t-test was used to compare experimental groups. Asterisks indicate $p < 0.05$.

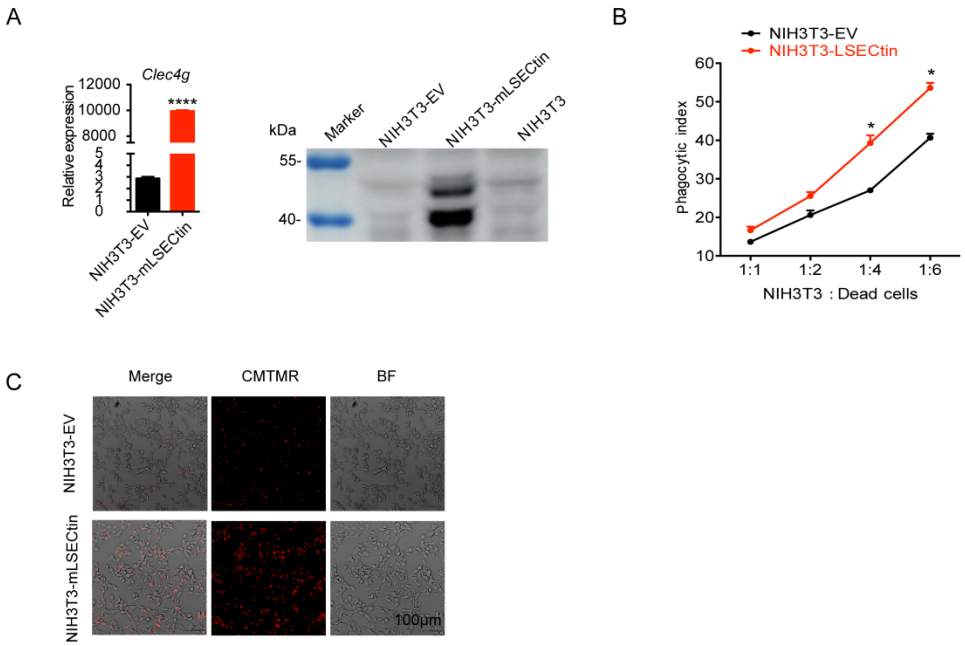

Fig. S9. LSECtin overexpressed on NIH3T3 cells contributes to apoptotic cell clearance. (related to Figure 5).

(A) NIH3T3 cells were transfected with the control or LSECtin plasmid and expression was detected by qPCR and WB. **(B, C)** NIH3T3 cells transfected with the empty vector control (EV) or LSECtin were incubated with CMTMR (red)–labelled apoptotic thymocytes, and phagocytosis was determined by measuring the number of positive CMTMR-containing NIH3T3 cells **(B)**. Phagocytosis was also examined by immunofluorescence microscopy **(C),** scale bar = 100μm. Data are representative of three independent experiments (mean ± SEM). Asterisks indicate $p < 0.05$.

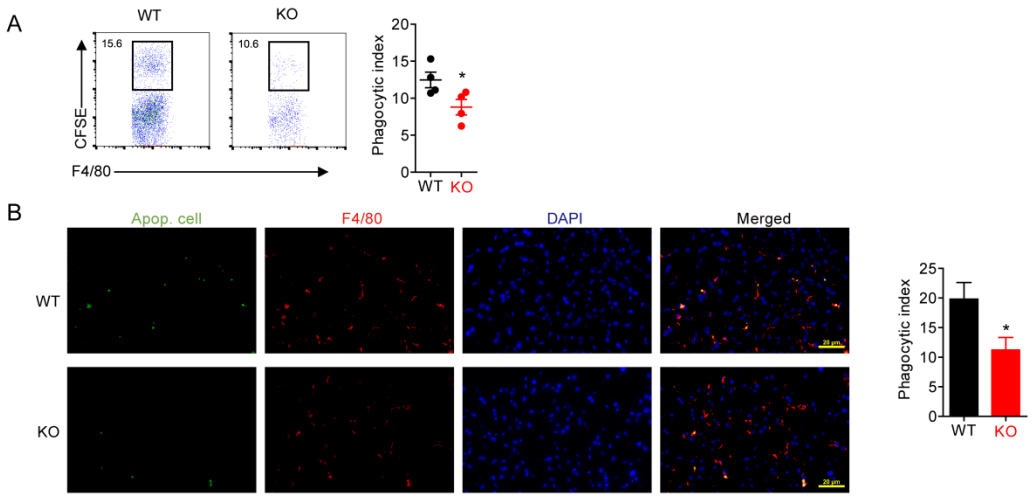

Fig. S10. LSECtin on KCs contributes to apoptotic cell clearance. (related to Figure 5).

(A) Kupffer cells (KCs) were plated at 5×10^5 cells in 24-well plates and incubated with CFSE-labeled apoptotic thymocytes for 30 min and $F4/80^+CD11b^+$ cells containing CFSE-related fluorescence was analyzed by flow cytometry. **(B)** WT and KO mice were intravenously injected with 2×10^7 CFSE labelled apoptotic thymocytes. One hour later, the livers were isolated, collected on poly-L-lysine-coated slides and incubated with PE-conjugated anti-F4/80 for observation by confocal microscopy, scale bar = 20μm. Error bars indicate SEM. Asterisks indicate $p < 0.05$.

Table S1. Primer sequences used in this study..

Gene		Sequences	Gene		Sequences
Il23p19	F	CATGCTAGCCTGGAACGCACAT	*Tgfb1*	F	TGCGCTTGCAGAGATTAAAA
	R	ACTGGCTGTTGTCCTTGAGTCC		R	CTGCCGTACAACTCCAGTGA
Il6	F	TACCACTTCACAAGTCGGAGGC	*Vegfa*	F	TGCACCCACGACAGAAGGA
	R	CTGCAAGTGCATCATCGTTGTTC		R	GTCCACCAGGGTCTCAATCG
Il11	F	CTGACGGAGATCACAGTCTGGA	*Hbegf*	F	ACCAGTGGAGAATCCCCTATAC
	R	CAGCTTGTACCAGAAGCAAGGG		R	GCCAAGACTGTAGTGTGGTCA
Nos2	F	GGGCTGTCACGGAGATCA	*Clec4g*	F	GGTGCCCATCTGGTGATTGT
	R	CCATGATGGTCACATTCTGC		R	CAGTGGCTGAAGTTGAGTGAGG
Nrg1	F	GCTCATCACTCCACGACTGTCA	*Pcna*	F	GCACGTATATGCCGAGACCT
	R	TGCCTGCTGTTCTCTACCGATG		R	CCGCCTCCTCTTCTTTATCC
Ctgf	F	TGCGAAGCTGACCTGGAGGAAA	*Cyclind1*	F	CCCTGACACCAATCTCCTCAAC
	R	CCGCAGAACTTAGCCCTGTATG		R	GCATGGATGGCACAATCTCCT
Fizzl	F	CCCTCCACTGTAACGAAGACTC	*Cdk1*	F	TCGCATCCCACGTCAAGA
	R	CACACCCAGTAGCAGTCATCC		R	GTTTGGCAGGATCATAGACTAGCA
Mrc1	F	ACAACAGACAGGAGGACTGCGT	*Fgf10*	F	ATCACCTCCAAGGAGATGTCCG
	R	AACCCATGCCGTTTCCAGCCTT		R	CGGCAACAACTCCGATTTCCAC
Chi3l3 (Ym1)	F	CAGGGTAATGAGTGGGTTGG	*Fgf18*	F	CGAGATGATGTGAGTCGGAAGC
	R	AAGTAGATGTCAGAGGGAAATGTC		R	CCGAAGGTATCTGTCTCCACTAG
Gapdh	F	AGGTCGGTGTGAACGGATTTG			
	R	TGTAGACCATGTAGTTGAGGTCA			