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Supplemental Information

Sectm1a Facilitates Protection against Inflammation-Induced Organ Damage through Promoting TRM Self-Renewal

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Supplemental figures

Figure S1

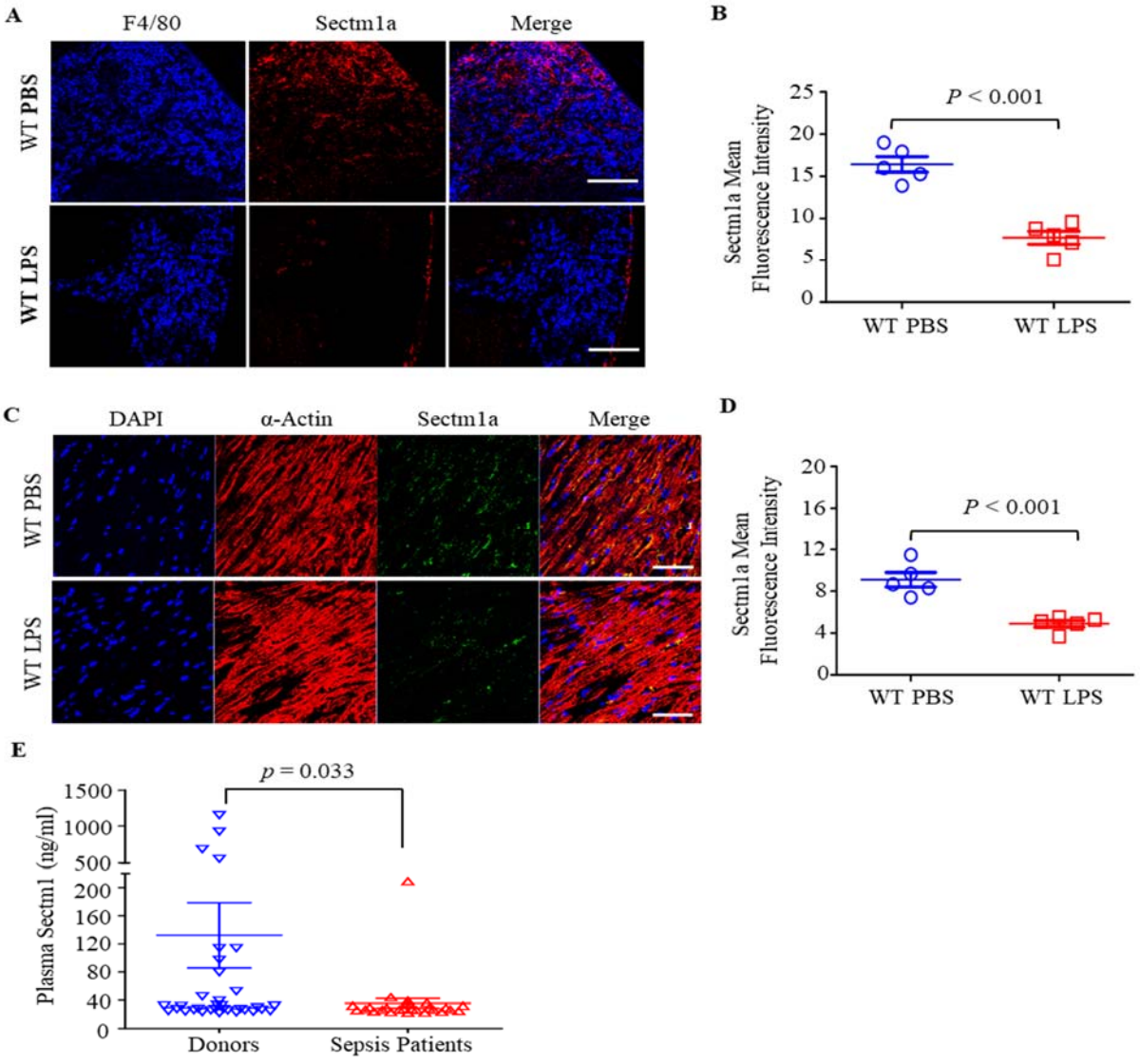

Figure S1. The spleen and heart tissues in WT mice were collected at 18 h after PBS or LPS injection and subjected to immunofluorescent staining. (A) Representative immunofluorescence staining image for Sectm1a in the spleen. Sectm1a (Red), F4/80 (Blue). Scale bar: 100 μm (B) The mean of red fluorescence intensity in view field was quantified with Image J. n = 5. (C) Representative immunofluorescence staining image for Sectm1a (Green) in the heart. α-Actin (Red), Sectm1a (Green), DAPI (Blue). Scale bar: 50 μm (D) The mean of green fluorescence intensity in view field of heart was quantified with Image J. n = 5. (E) Plasma SECTM1 levels were quantified by ELISA in septic patients (n = 27) and healthy donors (n = 34).

Figure S2

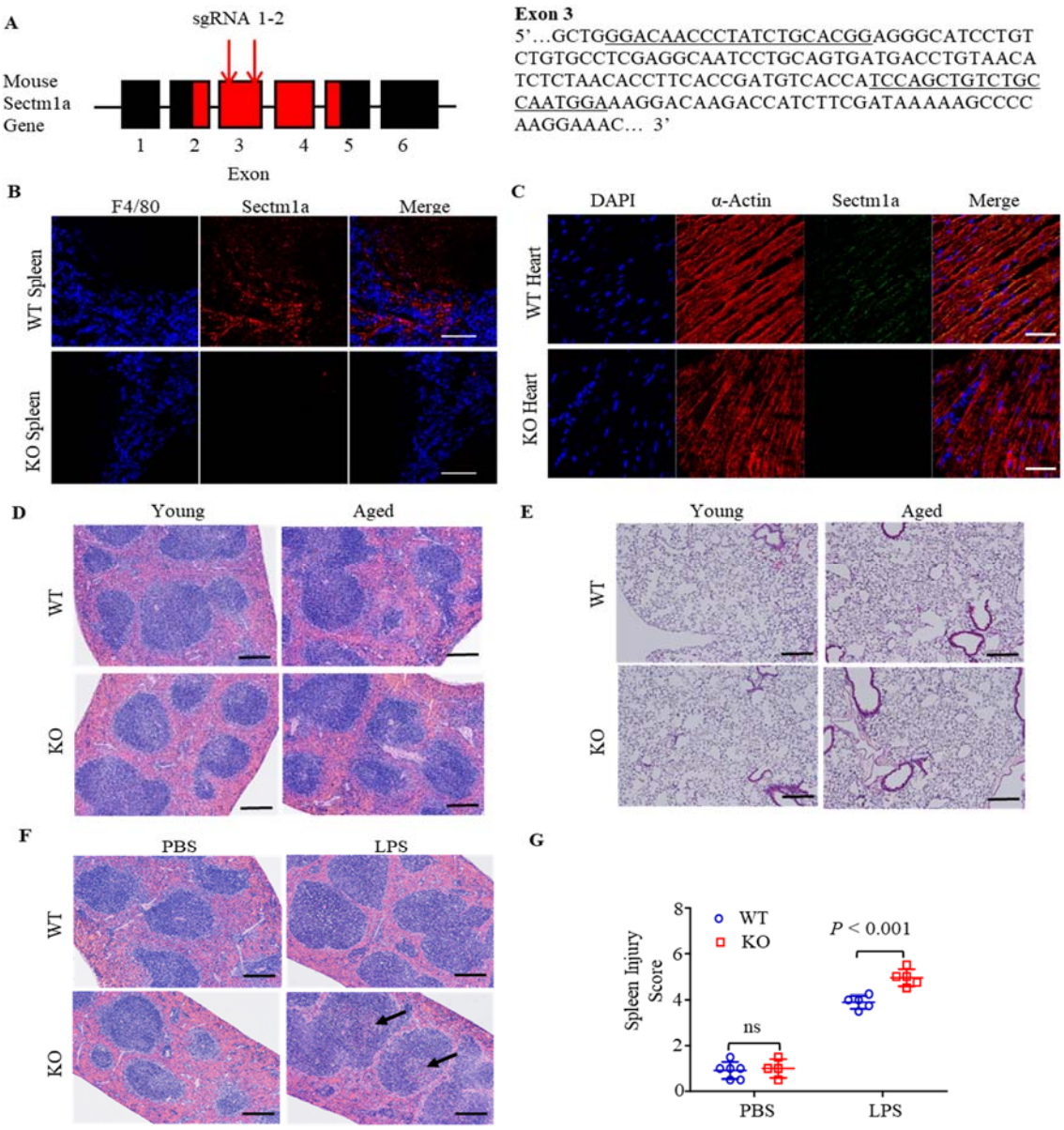

Figure S2. (A) Generation of Sectm1a-knockout mouse model. Two sgRNAs targeting to Exon3 of Sectm1a were selected to inject with Cas9mRNA into one-cell embryos and sequence between the sgRNA targeting sites were deleted. The cutting site of Cas9 is indicated by arrow and the targeting sequence is underlined. (B) Representative immunofluorescence stain images for Sectm1a in spleens of WT and KO mice. F4/80 (Blue) and Sectm1a (Red). Scale bar: 100 μm. (C) Representative immunofluorescence stain images for Sectm1a (Green) in hearts of WT and KO mice. α-Actin (Red), Sectm1a (Green), DAPI (Blue). Scale bar: 50 μm. (D) Representative H&E staining images of spleen sections collected from WT and KO mice at different ages. Young (2 months old), Aged (12 months old). Scale bar: 200 μm. (E) Representative H&E staining images of lung sections collected from WT and KO mice at different ages. Young (2 months old), Aged (12 months old). Scale bar: 200 μm. (F) Representative H&E staining images of spleen sections collected from WT and KO mice at 24 h after PBS or LPS injection. Scale bar: 200 μm. Arrows indicate germinal centers. (G) Injury scores for spleen.

Figure S3

A

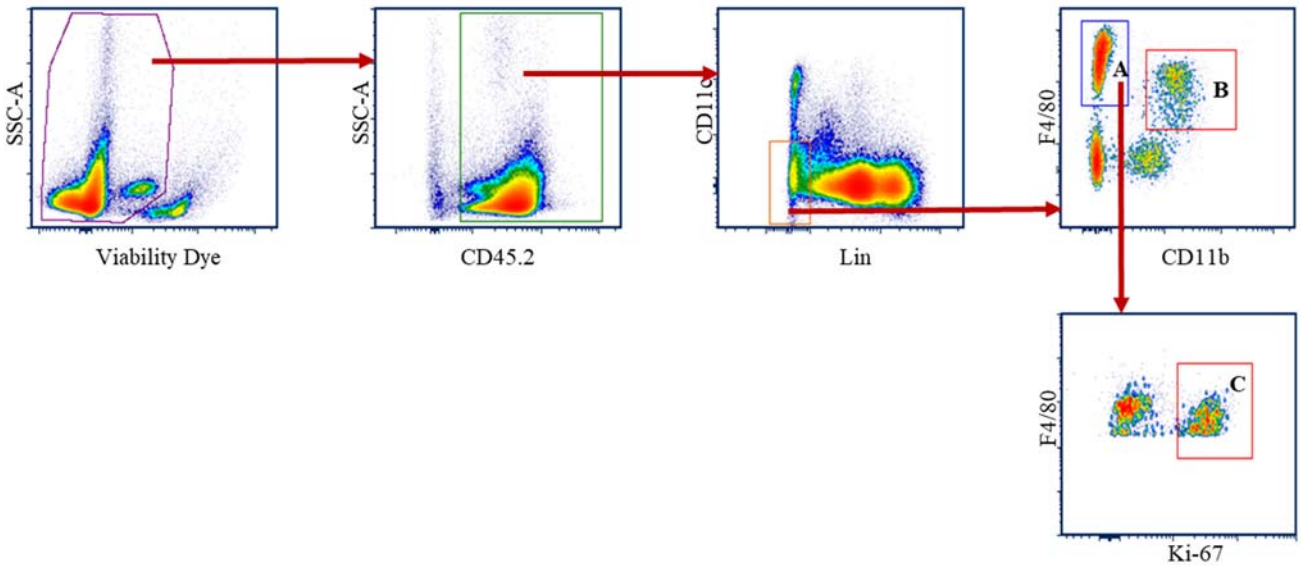

Figure S3A. Flow cytometry gating strategy used to identify leukocyte subsets in the mouse spleen following PBS or LPS injection. Single alive cell were subjected to a sequential gating strategy. Leukocytes were distinguished on the basis of CD45.2 expression and antibodies to specific markers (as outlined in Table S3) were used to identify red pulp macrophages (RPMs) (A: $CD45.2^{+}$ Lin^{-} $CD11c^{-}$ $F4/80^{++}$ $CD11b^{low/-}$) and splenic monocytes (B: $CD45.2^{+}$ Lin^{-} $CD11c^{-}$ $F4/80^{+}$ $CD11b^{++}$). RPMs undergoing proliferation were stained by Ki-67 (C: $CD45.2^{+}$ Lin^{-} $CD11c^{-}$ $F4/80^{++}$ $CD11b^{low/-}$ $Ki\text{-}67^{+}$).

Figure S3

B

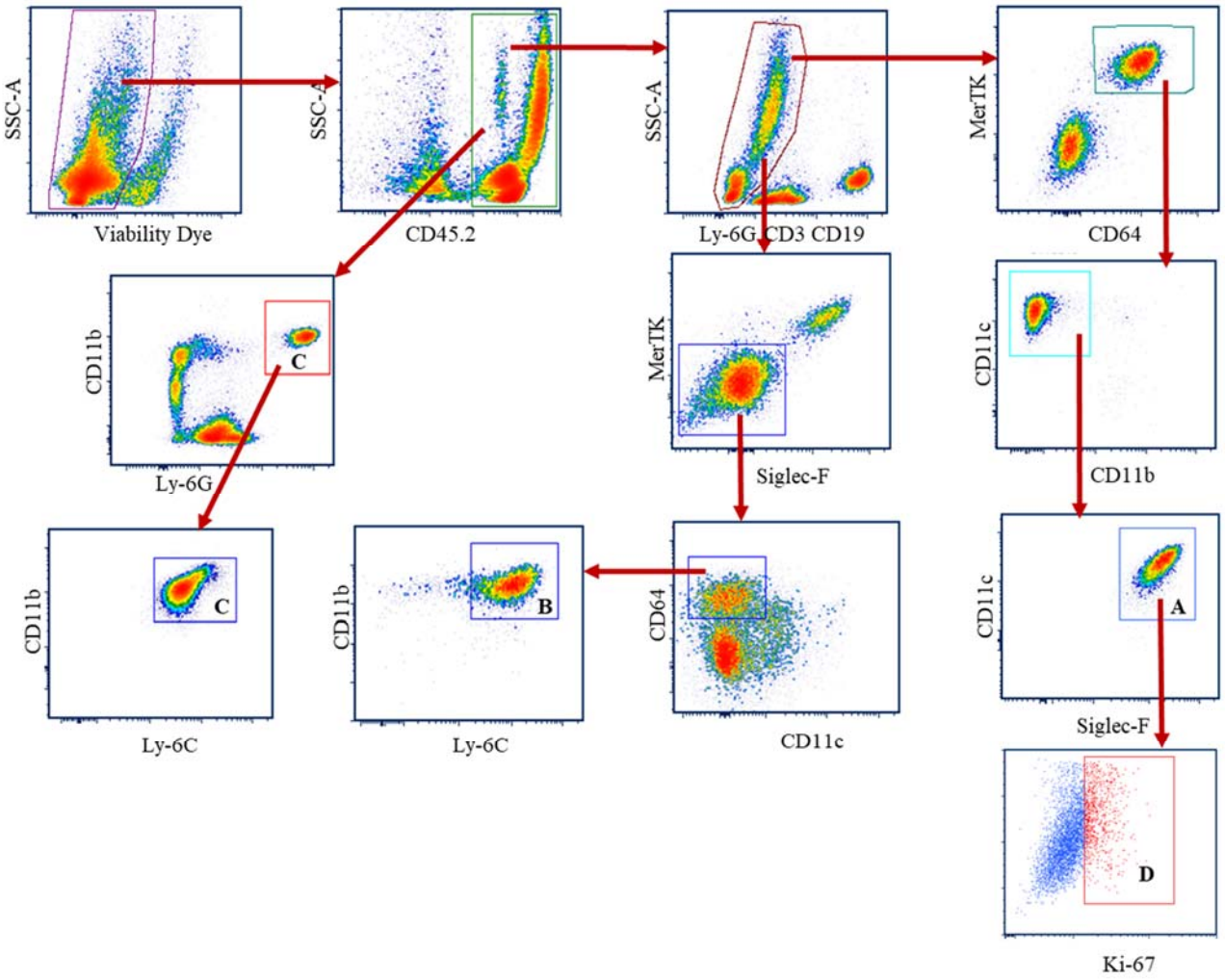

Figure S3B. (B) Flow cytometry gating strategy used to identify leukocyte subsets in the mouse lung following PBS or LPS injection. Single alive cell were subjected to a sequential gating strategy. Leukocytes were distinguished on the basis of CD45.2 expression and antibodies to specific markers (as outlined in Table S3) were used to identify alveolar macrophages (AMs) (A: $CD45.2^{+}$ $Ly\text{-}6G^{-}$ $CD3^{-}$ $CD19^{-}$ $MerTK^{+}$ $CD64^{+}$ $CD11c^{+}$ $Siglec\text{-}F^{+}$ $CD11b^{-}$), inflammatory monocytes (B: $CD45.2^{+}$ $Ly\text{-}6G^{-}$ $CD3^{-}$ $CD19^{-}$ $MerTK^{-}$ $Siglec\text{-}F^{-}$ $CD64^{+}$ $CD11b^{+}$ $Ly\text{-}6C^{+}$) and neutrophils (C: $CD45.2^{+}$ $Ly\text{-}6G^{+}$ $CD11b^{+}$ $Ly\text{-}6C^{+}$). AMs undergoing proliferation were stained by Ki-67 (D: $CD45.2^{+}$ $Ly\text{-}6G^{-}$ $CD3^{-}$ $CD19^{-}$ $MerTK^{+}$ $CD64^{+}$ $CD11c^{+}$ $Siglec\text{-}F^{+}$ $CD11b^{-}$ $Ki\text{-}67^{+}$).

Figure S3

C

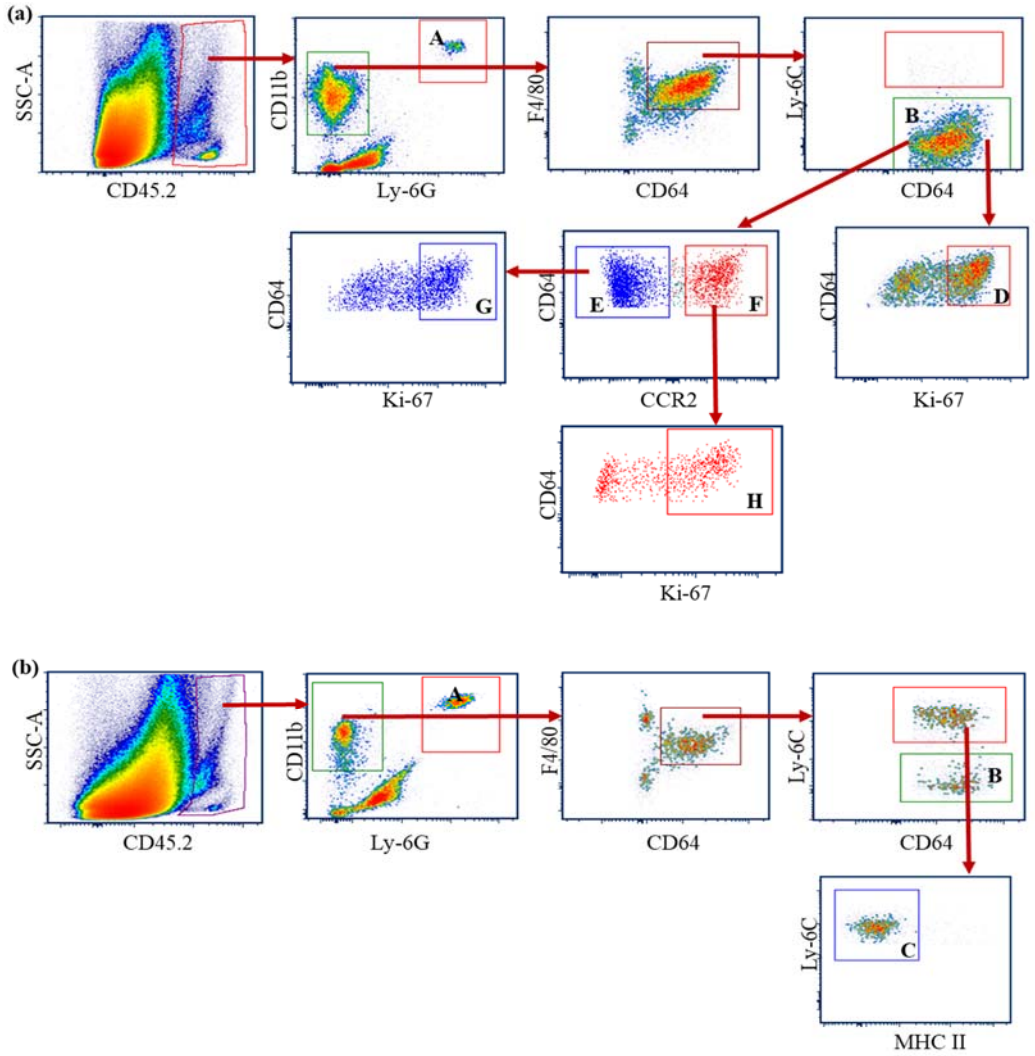

Figure S3C. (C) Flow cytometry gating strategy used to identify leukocyte subsets in the mouse heart following PBS [upper (a)] or LPS injection [lower (b)]. At 18 h post injection, cells were enzymatically and mechanically dissociated to produce single cell suspensions. Using a sequential gating strategy, leukocytes were distinguished on the basis of CD45.2 expression and antibodies to specific markers (as outlined in Table S3) were used to identify cardiac resident macrophage (B: $CD45.2^+$ $Ly\text{-}6G^-$ $F4/80^+$ $CD64^+$ $CD11b^+$ $Ly\text{-}6C^-$), inflammatory monocytes (C: $CD45.2^+$ $Ly\text{-}6G^-$ $F4/80^+$ $CD64^+$ $CD11b^+$ $Ly\text{-}6C^+$ $MHCII^-$) and neutrophils (A: $CD45.2^+$ $Ly6G^+CD11b^+$). The cardiac resident macrophages undergoing proliferation were stained by Ki-67 (D: $CD45.2^+$ $Ly\text{-}6G^-$ $F4/80^+$ $CD64^+$ $CD11b^+$ $Ly\text{-}6C^-$ $Ki\text{-}67^+$). The cardiac resident macrophages were subdivided into $CCR2^-$ macrophage (E: $CD45.2^+$ $Ly\text{-}6G^-$ $F4/80^+$ $CD64^+$ $CD11b^+$ $Ly\text{-}6C^-$ $CCR2^-$) and $CCR2^+$ macrophage (F: $CD45.2^+$ $Ly\text{-}6G^-$ $F4/80^+$ $CD64^+$ $CD11b^+$ $Ly\text{-}6C^-$ $CCR2^+$). These two subtype macrophages undergoing proliferation were identified as $Ki\text{-}67^+$ $CCR2^-$ CRM (Type G) and $Ki\text{-}67^+$ $CCR2^+$ CRM (Type H).

Figure S4

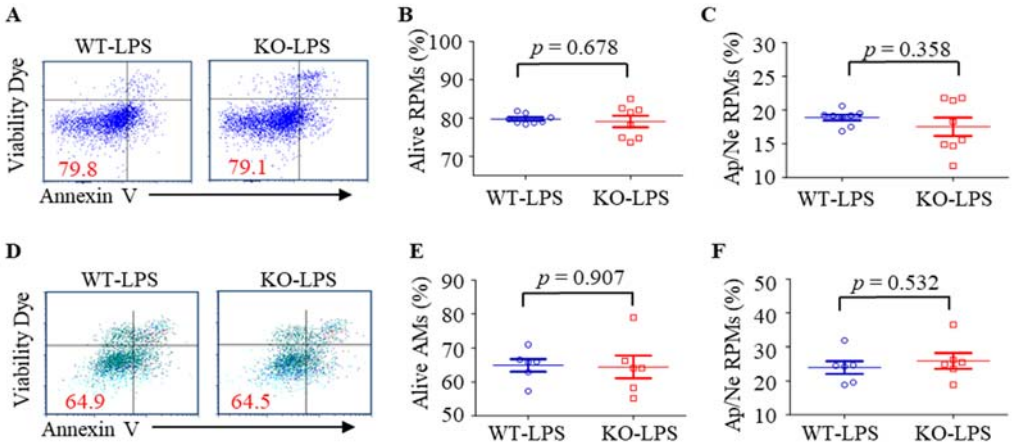

Figure S4. The spleen, lung and heart in WT and Sectm1a-KO mice were collected and subjected to flow cytometry analysis at 16 h after the administration of PBS or LPS (10 μg/g). For apoptosis/necrosis analysis, single cells isolated from the spleen and lung were stained with LIVE/DEAD fixable dye and Annexin V. (A-C) Representative flow cytometry plots (A) and quantification of live (B) as well as apoptosis/necrosis (Ap/Ne) population (C) within RPM after LPS injection. (n = 8). (D-F) Representative flow cytometry plots (D) and quantification of live (E) as well as apoptosis/necrosis population (F) within AM after LPS injection. (n = 6).

Figure S5

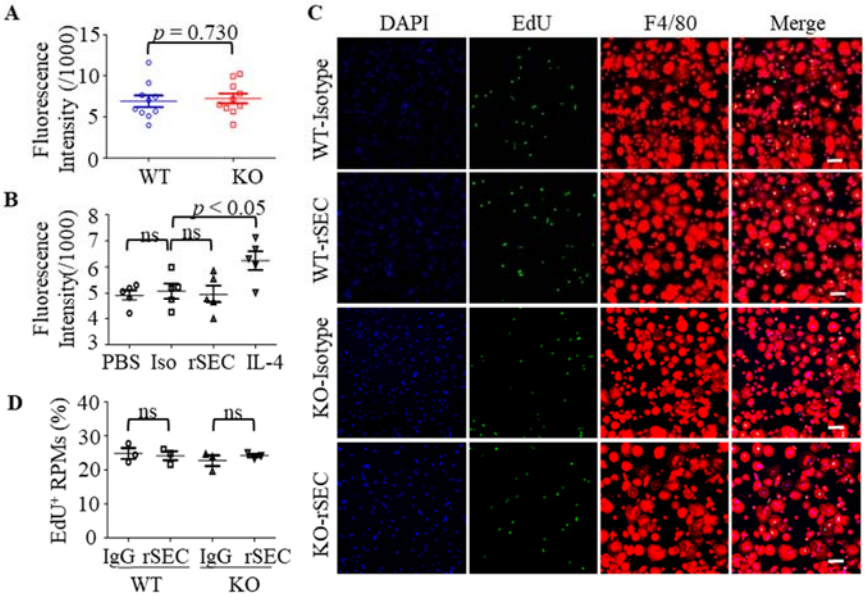

Figure S5. RPMs were isolated from WT and KO mouse spleens and stained with CellTracker™ Green CMFDA to reflect the cell number in each well. (A) After 48 h culture, fluorescence intensities of RPMs were compared between WT-RPMs and KO-RPMs (n = 10). (B) RPMs isolated from WT were stimulated with IgG2a (500 ng/ml) (Iso), rSectm1a (800 ng/ml) (rSEC) or IL-4 (200 ng/ml) for 48 h and fluorescence intensities of RPMs were compared. n = 5. (C and D) (C) EdU incorporation was assessed in RPM isolated from WT or KO mice after stimulation with IgG2a (1.25 μg/ml) or rSectm1a (2 μg/ml). Scale bar: 100 μm. (D) Percentages of EdU positive RPM in different treatment. Results are shown in means ± SEM of three independent experiments, and 400-600 cells were analyzed per experiment (n = 3).

Figure S6

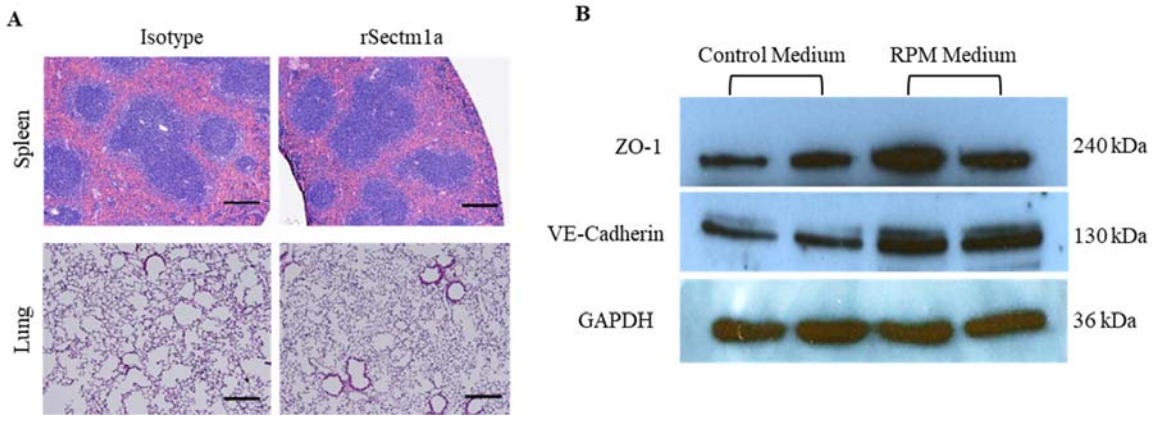

Figure S6. (A) WT mice were injected *i.v.* with IgG2a (125 μg/kg) (Isotype) or rSectm1a (200 μg/kg). The spleen and lung tissues were collected at 24 h post injection. Representative H&E staining images of spleen and lung sections. Scale bar: 200 μm. (B) MCECs were cultured in control medium or RPM conditioned medium for two days. Then, cells were collected for Western-blotting analysis of the expression levels of ZO-1 and VE-Cadherin. GAPDH was used as a loading control.

Supplemental tables

Table S1

Hematological data taken from young (2 months old) and aged (12 months old) WT mice.

	Young WT	Young KO	Aged WT	Aged KO
White blood cells ($\times 10^3/\mu l$)	2.8 ± 1.0	3.1 ± 0.7	2.5 ± 0.2	2.4 ± 0.4
Neutrophils ($\times 10^3/\mu l$)	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
Lymphocytes ($\times 10^3/\mu l$)	2.1 ± 0.7	2.3 ± 0.6	1.9 ± 0.2	1.8 ± 0.4
Monocytes ($\times 10^3/\mu l$)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
Eosinophils ($\times 10^3/\mu l$)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Red blood cells ($\times 10^6/\mu l$)	8.5 ± 0.2	8.6 ± 0.3	8.2 ± 0.2	8.6 ± 0.3
Hemoglobin (g/dL)	13.6 ± 0.2	13.4 ± 0.4	12.5 ± 0.4	12.5 ± 0.5
Hematocrit (%)	46.1 ± 1.4	45.2 ± 1.6	41.3 ± 2.6	42.9 ± 1.3
MCV (fL)	53.2 ± 3	52.6 ± 0.7	50.7 ± 3.7	50.2 ± 1.7
Platelets ($\times 10^3/\mu l$)	1046.6 ± 117.5	983.8 ± 23.6	1240.6 ± 108.9	1165.8 ± 70.3

Blood were obtained from the heart and analyzed by Hemavet. Statistical analysis was carried out with Student's t-test. MCV (mean corpuscular volume).

Table S2

Hematological data taken from IgG2a-treated and Sectm1a-treated WT mice. IgG2a (125 µg/kg) (Isotype), Sectm1a (200 µg/kg)

	IgG2a	Sectm1a
White blood cells ($\times 10^3/\mu l$)	3.4 ± 0.8	3.7 ± 1.4
Neutrophils ($\times 10^3/\mu l$)	0.6 ± 0.2	0.7 ± 0.3
Lymphocytes ($\times 10^3/\mu l$)	2.5 ± 0.6	2.8 ± 1.1
Monocytes ($\times 10^3/\mu l$)	0.2 ± 0.1	0.21 ± 0.1
Eosinophils ($\times 10^3/\mu l$)	0.0 ± 0.0	0.01 ± 0.0
Red blood cells ($\times 10^6/\mu l$)	8.0 ± 0.4	8.32 ± 0.6
Hemoglobin (g/dL)	12.5 ± 0.5	13.2 ± 0.9
Hematocrit (%)	43.3 ± 2.2	43.2 ± 2.8
MCV (fL)	52.3 ± 1.3	53.3 ± 1.2
Platelets ($\times 10^3/\mu l$)	1083.0 ± 129.7	1126 ± 171.1

Blood were obtained from the heart and analyzed by Hemavet. Statistical analysis was carried out with Student's t-test. MCV (mean corpuscular volume).

Table S3. Key resource table

Table S4. Characteristics of human subjects

	Healthy Controls	Sepsis Patients
Variable	n = 34	n = 27
Age (years) ± SD	40 ± 16	58 ± 17
Male Gender (%)	17 (50%)	16 (59%)
White Race (%)	25 (74%)	15 (56%)
Source of Infection (%)	N/A	
Urinary Tract		8 (30%)
Pneumonia		9 (33%)
Intravascular Device		3 (11%)
Other		7 (26%)
Organism (%)	N/A	
Gram Neg Bacteria		7 (26%)
Gram Pos Bacteria		4 (15%)
Unknown		9 (33%)
Other		7 (26%)
Mechanical Ventilation (%)	N/A	10 (37%)
APACHE II Score ± SD	N/A	23.4 ± 8.0
Death (%)	N/A	12 (44%)

SD standard deviation, N/A not applicable, APACHE acute physiology and chronic health evaluation

Supplemental methods

Mice

C57BL/6 mice (WT) and GITR-KO (Tnfrsf18$^{-/-}$) mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). The global Sectm1a-KO mouse model on the C57BL/6 background was generated by the Division of Developmental Biology at Cincinnati Children's Hospital Medical Center through using the CRISPR/Cas9 system (Supplemental Figure S2A).[1] Male and female mice between 6 and 10 weeks of age were used for experiments in a gender-matched manner. For generating acute inflammation model, animals received indicated dose (5 μg/g, 10 μg/g or 20 μg/g of body weight) of LPS intraperitoneally (*i.p.*) in 0.2 ml phosphate buffered saline (PBS). For survival experiments, mice immediately received IgG2a (125 μg/kg) (Isotype) or rSectm1a (200 μg/kg) through intravenous injection (*i.v.*) after LPS injection. The survival rate of WT and KO mice were monitored every 6 h for a 96 h period after LPS injection. To pre-deplete tissue-resident macrophages, WT mice were injected (*i.v.*) with clodronate-liposome (200 μl, clodronate 5 mg/ml) or vehicle-liposome (200 μl, no clodronate) (control group).[2]

Isolation of naïve CD4 T cells and CD4 T cells

We disrupt spleen in PBS containing 2% fetal bovine serum (FBS). Remove aggregates and debris by passing cell suspension through a 70 μm mesh nylon strainer. Centrifuge at 500 x g for 5 minutes and re-suspend at 1 x 10^8 nucleated cells/ml in RPMI-1640 containing 10% fetal bovine serum (heat inactivated) and 1% Penicillin/Streptomycin Solution, 100 x (Corning). Following the protocol, naïve CD4 T cells and CD4 T cell were isolated from the cell suspension by negative selection, and unwanted cells are targeted for removal with biotinylated antibodies and streptavidin-coated magnetic particles. This negative selection is fast and untouch the

isolated cells, which benefit the following experiments. After isolation, the live naïve CD4 T cells were counted with 0.2% trypan blue dye and grown in the insert of transwell (1.5×10^6/well). The live CD4 T cells were also counted and plated in 12-well plates (3×10^6/well).

Preparation of red pulp macrophages (RPMs) from spleen

Mice were terminally anesthetized, and the spleen was carefully excised and thoroughly minced in PBS containing 3% FBS. The cell suspension was filtered through 70 μm Nylon cell strainer. Then, cells were pelleted by centrifuging 500 x g for 5 min at 4°C. To lysing red blood cells (RBCs), cell pellets were suspended in RBC lysis buffer (Biolegend) for 4 min at room temperature (RT) and pelleted again. The resulting cell pellet was re-suspended in DMEM (Corning) containing 10% FBS, 10 mM HEPES and 1% penicillin/streptomycin solution (100 x). Cells were counted and seeded on 100 mm dish (5×10^7/well) or on the coverslip coated with collagen in 12-well plates at the same density (5×10^6/well). These cells were allowed to attach and grow for 6 days. Then, cells were washed with warm PBS for three times to dump these unattached cells and culture medium was replaced by fresh one. After one day, cells were collected and used for the following experiments such as FACs or co-culture experiments.

Endothelial cell culture

Mouse Cardiac Endothelial Cells (MCECs) (5×10^5 cells/well) were cultured in 12-well plate with DMEM containing 10 mM penicillin/streptomycin, 10 mM HEPES and 5% FBS or RPM conditioned medium for 2 days. After two-day culture, MCECs were washed two times with warm PBS, then lysed for RNA isolation. To observe the change of ZO-1 linear pattern after stimulation, MCECs were seeded on the coverslip coated with collagen in 24-well plates at the same density (3×10^5 cells/well) and grown for two days with normal medium or RPM conditioned medium. Then, thrombin (3 U/ml) or PMA (40 ng/ml) were used to stimulate MCECs for 0.5 h. MCECs were washed with warm PBS and fixed by 4% Paraformaldehyde (PFA) solution in PBS.

To produce RPM conditioned media, the general medium (DMEM containing 10 mM penicillin/streptomycin, 10 mM HEPES and 5% FBS) was used as a starter general culture medium. 2×10^6 RPMs (for RPM medium) and 2×10^6 MCECs (for control medium) were respectively cultured in a 100 mm cell culture dish with 12 ml general medium at 37 °C in a 5 % CO_2 incubator. After 3 days of cultivation, the cell culture supernatant was collected. Debris was removed by centrifugation at 700 x g for 5 minutes. Then, FBS was added into supernatants (RPM conditioned medium or control medium) with 1:20 ratio (FBS: supernatant) in volume. These media were aliquoted and stored at −20 °C until use.

Flow cytometry analysis

Spleen cell suspensions were prepared from the excised spleens by mechanical disruption in PBS containing 2% FBS. Remove aggregates and debris by passing cell suspension through a 70 μm mesh nylon strainer. To prepare single cell suspension from the lung and heart, mice were terminally anesthetized and perfused with 20 mL PBS via cardiac injection. Lung and heart tissues were finely minced with scissors before digestion. The lung tissue was digested with RPMI-1640 solution containing 2 mg/ml collagenase type IV (Stemcell) and Deoxyribonuclease I (DNase I), 100 U/ml (Stemcell).[3] The heart tissue was subjected to enzymatic digestion with 450 U/ ml collagenase I (Sigma-Aldrich), 125 U/ml collagenase XI (Sigma-Aldrich), 60 U/ml hyaluronidase (Sigma-Aldrich) and 50 U/ml DNase I (Stemcell).[4] After 30-40 mins incubation at 37°C under 200 rpm agitation, lung tissues were passed through 70 μm cell strainer. Heart tissues were firstly passed through 100 μm cell strainer, and, then passed through 40 μm cell strainer. After removing aggregates and debris, all cell suspension were centrifuged at 500 x g for 5 minutes at 4°C and red blood cells were lysed with 3 ml of lysis buffer (Biolegend), incubated for 4 min at room temperature, and, then, diluted and washed with PBS.

For surface marker staining, we used the following mouse antibodies: FITC-conjugated CD45.2 (clone 104), lineage markers [eFlour 450-conjugated CD19 (clone 1D3), eFlour 450-conjugated NK1.1(clone PK136), eFlour 450-conjugated CD90.2 (clone 53-2.1), eFlour 450-conjugated CD49b (clone DX5), eFlour 450-conjugated CD3 (clone 17A2), Brilliant Violet 421-conjugated Ly-6G (clone 1A8)], APC-conjugated F4/80 (clone BM8), BV786-conjugated CD64 (clone X54-5/7.1), PE-conjugated CD11b (clone M1/70), PerCP-Cyanine5.5-conjugated CD11c (clone N418), PE/Dazzle 594-conjugated Ly-6C (clone HK1.4), APC-conjugated MERTK (clone DS5MMER), Alexa Fluor 700-conjugated Siglec F (clone 1RNM44N), PE-Cyanine5-conjugated MHC Class II (clone M5/114.15.2), Alexa Fluor 700-conjugated CCR2 (clone 475301), PerCP-Cyanine5.5-conjugated CD4 (clone RM4-

5), APC-eFluor 780-conjugated CD11b (clone M1/70), Alexa Fluor 647-conjugated F4/80 (clone BM8), PerCP-Cyanine5.5-conjugated CD45.2 (clone HK1.4), PE-Cyanine7-conjugated CD11c (clone N418).

For staining of intracellular Ki-67, cells from the lung and heart were fixed and permeabilized with Foxp3 / Transcription Factor Staining Buffer Set (Invitrogen), washed with Intracellular Staining Perm Wash Buffer, and stained with BV605-conjugated anti-mouse Ki-67 (clone XMG1.2, BioLegend). For staining of intracellular Ki-67 in RPM, cells from the spleen were fixed by 2% PFA and permeabilized with 0.1% Triton in PBS, washed the cells with 2 mL of PBS (containing 0.1% triton), and stained with BV605-conjugated anti-mouse Ki-67 (clone XMG1.2, BioLegend). For measurement of intracellular cytokine expression of T cells , cells were isolated stimulated with 20 ng/ml phorbol-12-myristate 13-acetate (PMA, Stemcell) and 1 μg/mL ionomycin (Stemcell) in the presence of BD GolgiStop (BD Pharmingen) for 5 h. Cells were subsequently stained with mouse Th1/Th2/Th17 phenotyping kit (BD Pharmingen) according to the manual. The apoptosis and necrosis of RPMs and AMs were evaluated with Annexin V Apoptosis Detection Kit (eBioscience) following the manual. In all experiments, doublet cells were excluded by SSC-A and FSC-A gating followed by FSC-H and FSC-A gating. Samples were run on BD LSRII) and the data were analyzed with FCS express V6 (De Novo Software, USA).

Immunofluorescence stain

The mouse spleen was embedded in O.C.T. compound (Fisher HealthCare) and frozen in liquid nitrogen. Spleens were then sectioned at a thickness of 7 μm with a cryostat, and fixed with 4% PFA at room temperature. The sections were then permeabilized with 0.2% tween 20 (ThermoFisher Scientific) and blocked with 1% bovine serum albumin (BSA) for 1 h and incubated with the primary antibodies (Pacific Blue-conjugated anti-mouse F4/80 and Alexa Fluor 647-conjugated anti-mouse Ki-67) diluted in 1% BSA in PBS overnight at 4°C. Sections were washed three times with PBS and mounted with ProLong Diamond Antifade Mounting medium (Invitrogen). For the immunofluorescence staining of RPMs and MCECs treated with (Thrombin or PMA), cells were fixed on the coverslip, blocked with 1% BSA and incubated with primary antibody (PE-conjugated anti-mouse F4/80 or ZO-1 antibody) diluted in 1% BSA in PBS overnight at 4°C. After washing, MCECs were incubated with secondary antibody, Alexa flour 488-conjugated anti-Rabbit IgG, at room temperature for 1 h. Thereafter, cells were washed three times with PBS and mounted with ProLong diamond Antifade Mounting medium with DAPI (Invitrogen). Images were captured with Zeiss LSM710 LIVE Duo Confocal Microscope (Live Microscopy Core, University of Cincinnati)

Measurement of vascular leakage in multiple organs

The pulmonary microvascular permeability was determined in each experimental group by measuring the Evans blue (EB) accumulation in lung tissue and lung wet to dry weight ratio. The Evans blue dye assay was performed as described previously.[5] Briefly, the mice were administered 1% EB solution (Sigma, St. Louis, MO, USA) in PBS via tail vein injection. After 30 min, mice were sacrificed and perfused via the heart, and the lung tissues were collected. The lung weights were measured and placed in 1 ml of formamide (Avantor, Center Valley, PA, USA) at 60 °C for 24 h to extract EB. Absorbance was measured at 620 nm, and EB concentration was determined from a standard curve. To measure the wet to dry weight ratio of lung, the left lung was harvested and weighed to measure a wet weight in each group. The wet lung was then dried in an oven at 60 °C for 48 h and re-weighed as dry weight. The ratio of wet weight to dry weight of the lung was calculated. The wet to dry weight ratio of spleen was measured and calculated in the same way.

For the measurement of cardiac vascular permeability, EB accumulation in heart was determined as described previously.[6] Briefly, mice were sacrificed and perfused with 30 ml of PBS through the left ventricle after administration of EB, and conducted following experiment as described previously (. Heart was removed and frozen in O.C.T compound. Frozen sections (7-μm in thickness) were fixed and observed under a confocal microscope, Zeiss LSM710 LIVE Duo Confocal Microscope (Live Microscopy Core, University of Cincinnati). The intensity of Evans blue fluorescence (excitation at 620 nm, emission at 680 nm) corresponding to the amount of dye in extravascular compartment was quantified with Image J software (Wayne Rasband, National Institutes of Health, Bethesda, MD).

Spleen/lung histology and injury score

The spleen and lung tissues were collected from mice at 24 h post-LPS injection. All the lung were perfused via the heart, inflated and fixed with 10% buffered formalin for more than two days, followed by embedded in paraffin, and cut into 5-μm sections. Tissue sections were stained with hematoxylin and eosin (H&E), examined with a light microscope (Olympus, Japan), and scored by a pathologist who was blinded to the experimental groups. To evaluate

the lung jury, 6-8 independent random lung fields were evaluated per mouse for neutrophils in alveolar spaces, neutrophils in the interstitial spaces, hyaline membranes, proteinaceous debris filling the airspaces, and alveolar septal thickening and weighted according to the relevance ascribed by the official American Thoracic Society workshop report on features and measurements of experimental acute lung injury in animals.[7] Segments of spleen were separately scored for the enlargement of white pulp areas and for the presence of germinal centers according to the description in the previous report.[8]

Acquisition of human blood samples

This study was approved by the Institutional Review Board at the Medical University of South Carolina. We screened all new intensive care unit (ICU) admissions at a single tertiary-care academic hospital from July 2013 to February 2015 for the presence of severe sepsis based on the American College of Chest Physicians/Society of Critical Care Medicine consensus definition.[9] Additional inclusion criteria included age ≥18 years and admission into the ICU within the previous 24 hours. We excluded immunocompromised patients as defined by: immunosuppressive medication use, leukopenia, current hematologic malignancy, and history of stem cell transplant, and excluded patients transferred in from other hospitals if they had spent > 24 hours in an ICU at the time of screening. Patients who had transitioned to comfort measures only at the time of screening were further excluded. Healthy control subjects were recruited through local advertising. Informed consent for study participation and publication of results was obtained from all research subjects or their legally authorized representatives. Demographic and clinical information from septic patients was abstracted from the electronic medical record including the source and type of infection as well as variables required to calculate acute physiology and chronic health evaluation (APACHE) II scores (Table S4). Discharge destination and vital status were similarly captured. Basic demographics were recorded from healthy controls (Table S4). Consenting subjects had blood drawn via venipuncture or from pre-existing intravascular catheters. Blood samples from septic patients were collected within 24 hours of admission to the ICU. Samples were centrifuged at 3400 × g for 10 minutes and the plasma supernatant was collected and stored in aliquots at -80 degrees Celsius.

Supplemental references

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