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Materials and Methods Supplement

Quantification of urine albumin and creatinine

Urine albumin was quantified using the Mouse Albumin ELISA Quantification Set (Bethyl Laboratories) according to the manufacturer's instructions. Urine creatinine was measured enzymatically using LabAssay^{TM} Creatinine (FUJIFILM).

Serum creatinine was measured by Oriental Yeast Co., Ltd (Tokyo, Japan).

Immunostaining

Paraffin-embedded tissue or frozen sections and cultured cells were fixed in 4% paraformaldehyde. After blocking with Protein blocking solution (DAKO), the samples were stained with primary antibody at 4^{o}C overnight. The next day, sections were incubated with appropriated secondary antibody 1h at room temperature. Primary antibodies used include rabbit polyclonal anti-ZO-1 (Invitrogen, 40-2200), rabbit polyclonal anti-WT1 (C-19) (Santacruz, sc192), goat polyclonal anti-mouse kidney injury marker (KIM)-1 (R&D, AF1817), rabbit polyclonal anti-IL-17 (Abcam, ab79056), and rabbit polyclonal anti-Klebsiella *spp* (FITC) (Abcam, ab69467), MAVS (CST, #4983), rabbit anti-CD3 (Nichirei Bio, 413591), purified Rat Anti-Mouse CD45R/B220 (BD, 01121A), purified Rat Anti-Mouse Ly-6G and Ly-6C (Gr-1) (BD, 550291).

Western blot and MAVS aggregation

Total cellular lysates were extracted from cultured cells in RIPA buffer (Merck Millipore) with protease and phosphatase inhibitors (Roche). Cellular lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). For MAVS aggregation, lysates were run on 1.5% agarose, 0.1% SDS gel, then transferred to PVDF (Thermo Fisher Scientific). After incubation in PVDF-blocking reagent (TOYOBO, Japan), membranes were incubated with primary antibody overnight at 4 °C followed by incubation with appropriate secondary antibodies for 1 h at room temperature. Then, membrane-derived protein bands were detected using the enhanced chemiluminescent substrate (Thermo Fisher Scientific). The used primary antibodies for Western blot include MAVS (CST, #4983), Stat3 (79D7) Rabbit mAb (CST, #4904), Phospho-Stat3 (Tyr 705) (D3A7) (D3A7) XP® Rabbit mAb (CST, #9145), p44/42 MAPK (Erk1/2) (137F5) rabbit mAb (CST, #4695), Phospho-p44/42 MAPK (Erk1/2) (Thr 202/Tyr 204) (197G2) rabbit mAb (CST, #4377), and β-actin Rabbit Ab (CST, #4967)

Intestinal permeability assay with FITC-dextran (FD) 4 kDa

Fluorescence intensity in the serum, and urine was measured at an excitation wavelength of 490 nm and emission wavelength of 510–570 nm using the GloMax®-Multi Detection System.

Flow cytometry

The kidney was collected and digested using collagenase I (1mg/ml) (Worthington Biochemical Corporation) and DNAse I (200 mg/ml) (Roche). The isolated cells were resuspended in 5% FBS–PBS, stained with CD45-PE and CD11b-APC antibodies (BD Bioscience) for 30 min on ice. After washing, the stained cells were resuspended in PBS, mixed with PI (Immunostep Biotech), run on a BD FACS Calibur cytometer, and analyzed with FlowJo software.

Assessment of bacterial translocation

The spleen, kidney, and mesenteric lymph nodes were aseptically removed, minced with sterile PBS under sterile conditions, divided into two Tryptic soy broth (TSB) (Nippon Becton Dickson) liquid medium tubes, and placed in an aerobic and anaerobic incubator at 37°C. After 3 d, 10 µl of the aerobic culture medium was inoculated onto blood (Nissui), and Drigalski Lactose Agar (BTB) agar (Nippon Becton Dickson) and aerobically incubated for 24 h. Then, 10 µl of the anaerobic culture medium was streaked on BHK Brucella agar (KYOKUTO) and anaerobically incubated for 48 h. The colonies growing on the plates were identified using Bruker Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) biotype identification system.

Blood was aseptically collected, 0.5 mL of the sample was inoculated (through a safety device Vacutainer) into a BD BACTEC™ Peds Plus™ Medium (Becton Dickson), then incubated for 7 d. For bottles flagged positive, an inoculum was sub-cultured, cloned, and identified.

Bone marrow-derived macrophages (BMDMs) culture

Conditioned medium from L929 (ATCC) fibroblast cells was used as a crude source of macrophage colony-stimulating factor (M-CSF or CSF-1) for the culture of macrophages. Bone marrow cells were isolated from femurs of WT or MAVS KO mice (6–12 weeks), cultured in L929 cell-conditioned medium (RPMI 1640 (Gibco) with 20% L929 medium,

10% FBS (Sigma-Aldrich), and 1% P/S (FUJIFILM)) to separate adherent differentiated cells. Non-adherent, immature cells were removed and cultured for 3–4 days. Glucose concentration was controlled at 5.6 mM D-glucose (normal glucose [NG]), plus 25mM D-glucose (FUJIFILM) (high glucose [HG]) or 25mM L-glucose (Sigma Aldrich) (osmotic control [OSM]). For long-term exposure with NG, HG, or OSM, bone marrow cells were cultured and differentiated in different media immediately after being isolated for 7 d. For short-term exposure, bone marrow cells were cultured in NG medium, then stimulated with HG or OSM media at the indicated time.

T cell isolation and T cell–BMDMs coculture

Remove the spleen from WT mice. The plunger of a 1ml syringe was used to grin the spleen on a sterile 100 μm cell strainer mesh in a Petri dish containing 2 ml ice-cold RPMI/FBS to obtain a single-cell suspension. Pass through all cells through a cell strainer into a 50-ml tube. Wash the cell culture strainer and dish with 5 ml of RPMI twice and combine all cells. Centrifuge the cells and remove the supernatant. Resuspend the cell pellet into 5 ml of 1X BD Pharm Lyse™ lysing solution (BD Bioscience). Centrifuge the cells and re-suspend the cell pellet in 0.5% BSA, 2mM EDTA in PBS. Count the cells, then isolate $CD4^+$ T cells using $CD4^+$ T cell Isolation Kit (Miltenyi Biotec) as manufacturer's protocol. Isolated cells were resuspended in RPMI with 10% FBS (Sigma Aldrich), 1% P/S (FUJIFILM), 10mM Hepes (Dojindo), 1 mM Sodium Pyruvate (FUJIFILM). All procedures were performed aseptically. The purity of isolated cells was confirmed by flow cytometry over 90%.

WT BMDMs (in NG condition) were seeded on 12-well plates at $1x10^5$ cells/ well. 24 h after *K. oxytoca* supernatant stimulation, the medium was changed to fresh medium and isolated $CD4^+$ T cells were added to BMDMs with a 1:1 ratio. After 72 h, T cells in the supernatant were collected and extracted RNA.

Macrophage sorting

Kidneys were isolated from mice after perfusion with cold PBS and cut into small pieces. Kidneys were digested in 5 ml of RPMI (Gibco) with 0.25 mg/ml Liberase (Gibco) and 0.2 mg/ml DNAse I (Roche). After digestion, cells were passed through a 70-μm strainer and washed once with 5% FBS-PBS. Mononuclear cells were enriched using a 65%–25% Percoll (GE Healthcare) gradient by centrifugation (1000 g for 20 min at room temperature). After Percoll enrichment, cells were washed once and resuspended in FACS buffer (PBS with

1% BSA (Sigma Aldrich), 1 mM EDTA (Nippon Gene), 25 mM Hepes (Dojindo)). For surface staining, cells were incubated with purified anti-mouse CD16/32 Fc Block (BD Bioscience, 553142) for 10 min on ice. Additional antibodies including anti-mouse CD45-PE (103106), CD11b-FITC (101206), and F4/80-APC (17-4801-80) (BD Bioscience) were then added in FACS buffer to a final volume of 100 µl at 4°C for 30 min. After staining, cells were washed twice and resuspended in FACS buffer adding 7-AAD (BioLegend), then sorted using FACS Aria Fusion. The purity was confirmed over 95%.

RNeasy Micro Kit (QIAGEN) was used to extract RNA from sorted cells.

DNA extraction

DNA from whole blood samples was extracted using the DNeasy PowerLyser Microbial kit (QIAGEN) or Nucleospin Tissue (Takara).

Droplet digital PCR

Droplet digital PCR (ddPCR) was performed using the QX200 AutoDG Droplet Digital PCR System (Bio-Rad) according to the manufacturer's protocol. Each 22 µl ddPCR reaction contained 11 µl of the 2x ddPCR SuperMix for probes (no dUTP) (Bio-Rad), template DNA, *K. oxytoca* primer, and probes (Genesig), and *16S rDNA* TaqMan primers and probes (catalog Ba04230899_s1; Applied Biosystems TaqMan Gene Expression Assays). at defined concentrations. Reactions were prepared in a semi-skirted 96-well plate (Bio-Rad). Following droplet generation on the AutoDG system, the plate was sealed with a pierceable foil heat seal (Bio-Rad) and PCR was performed on the C1000 Touch^{TM} thermal cycler (Bio-Rad). After the PCR reaction was completed, the plate was read using a QX200 droplet reader (Bio-Rad) and then analyzed using QuantaSoft software.

siRNA transfection and stimulation experiments for cultured cells.

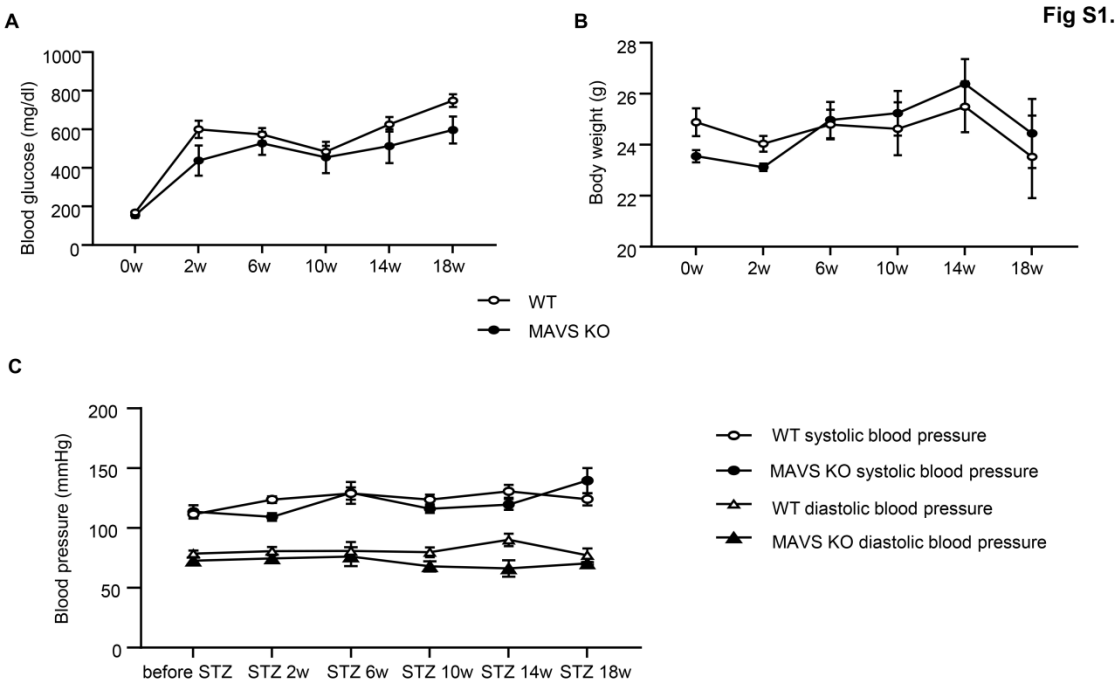
Human and murine siMAVS were obtained from Invitrogen and transiently transfected using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions. The efficiency of transfection was tested by using qPCR with a more than 50% transduction rate.

For high glucose stimulation, cultured cells were stimulated with the cultured medium containing 25 mM D-glucose (FUJIFILM) (high glucose - HG) or 25 mM L-glucose (Sigma Aldrich) (osmotic control - OSM) at the indicated time. For *K. oxytoca* stimulation, human

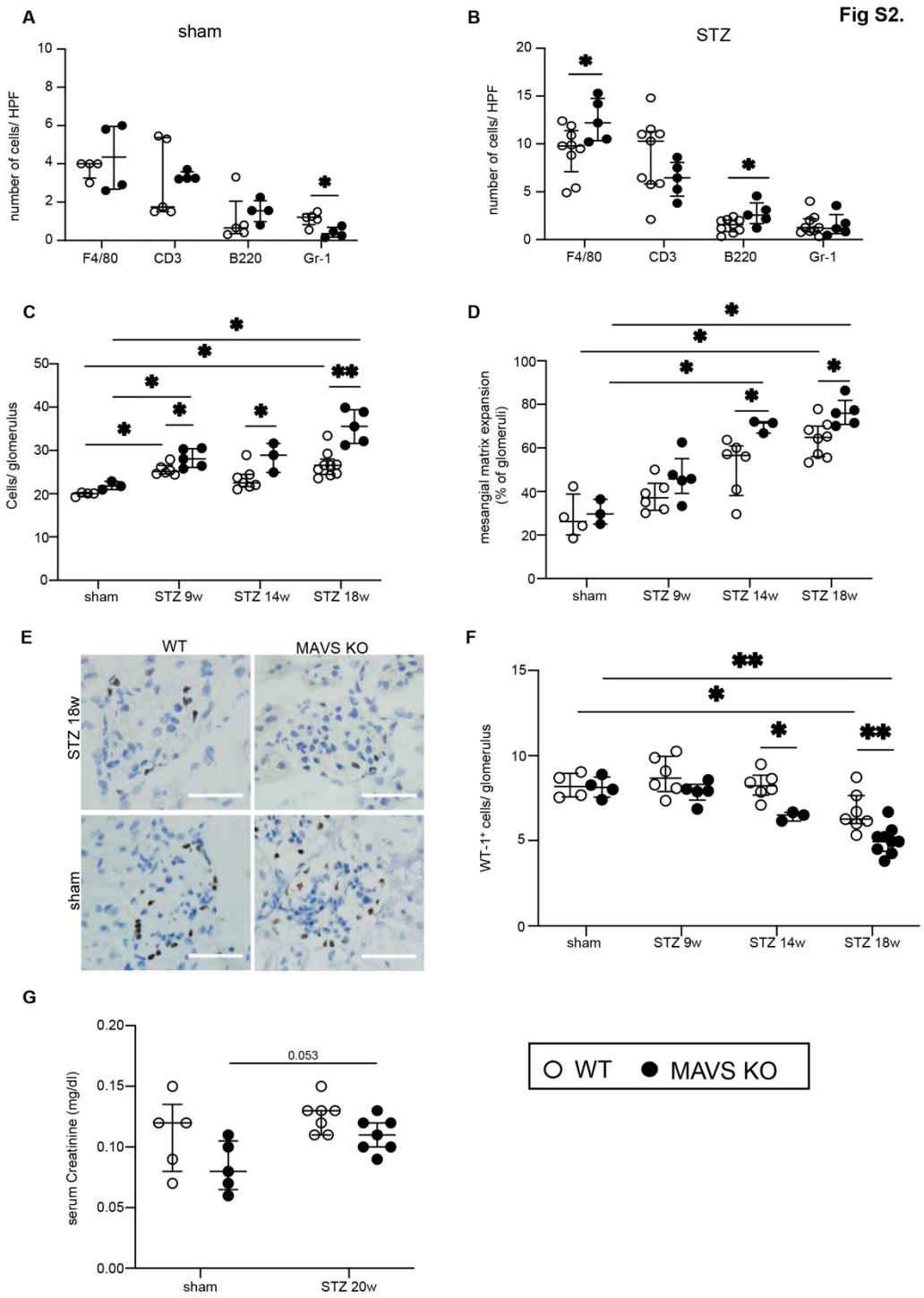
IECs were challenged with live bacteria. For IL-6 or IL-17 induction, cultured cells were stimulated with recombinant human/murine IL-6 or IL-17 (R&D) at the indicated concentration and time.

Assessment of podocyte cell death

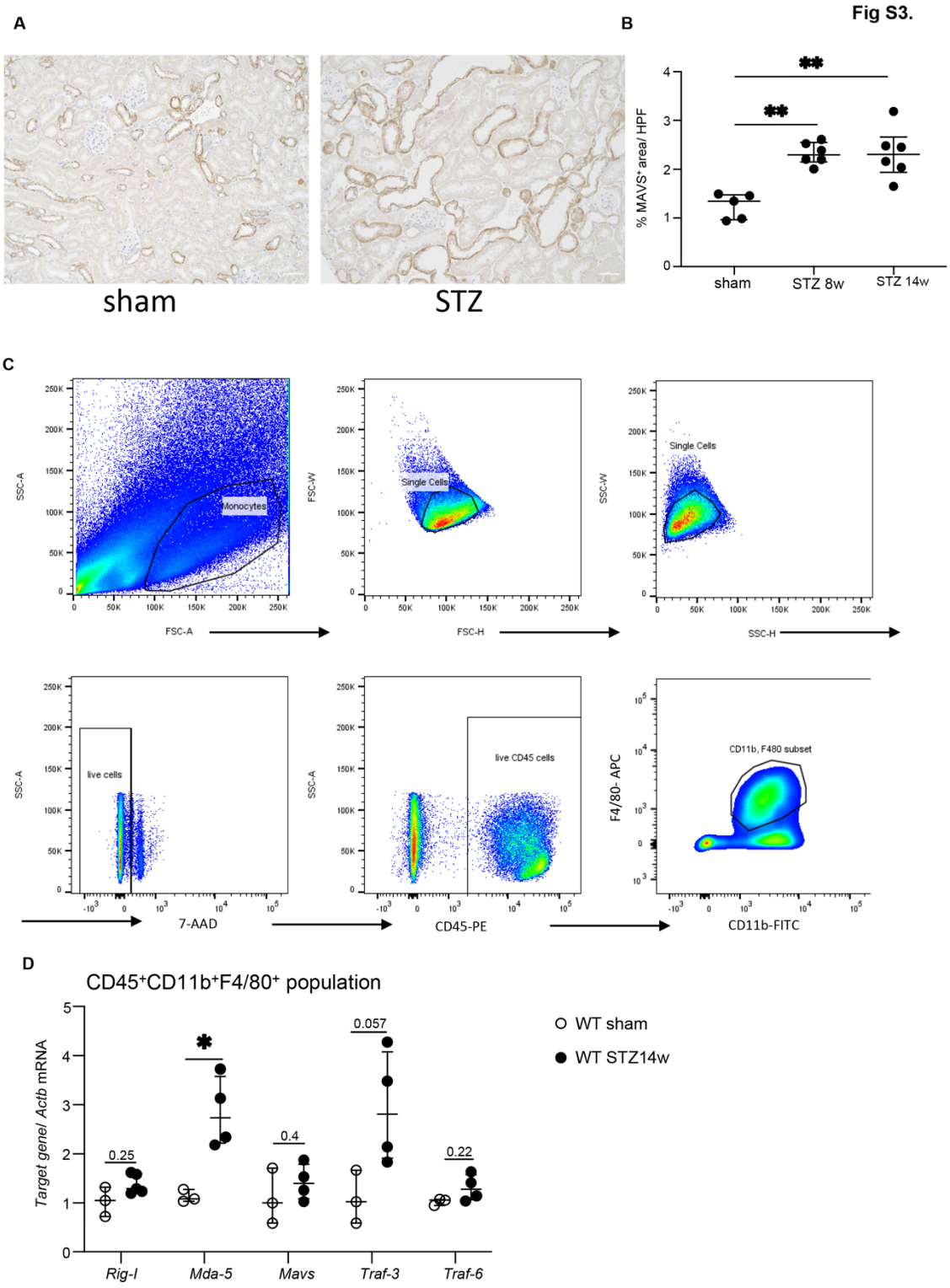
After indicated time for cell treatment, cells were harvested and resuspended 1×10^5 cells in 200 µL of Annexin V binding buffer (10 mM HEPES, 150 mM NaCl, 2.5 mM $CaCl_2$ in PBS (pH 7.4)), containing 1 µL of Annexin V-FITC (Medical & Biological Laboratories) and 1 µL of PI (Immunostep Biotech) incubate in 15 min then run on a BD FACS Calibur cytometer. PI^-Annexin V^+ cells = apoptotic cells. PI^+ Annexin V^+ cells = necrotic cells.



Supplemental Figure 1. WT and MAVS KO mice developed equivalent degrees of hyperglycemia. Blood glucose (A), body weight (B) and blood pressure (C) were shown. Data are shown as median ± IQR; *$p < 0.05$; **$p < 0.01$



Supplemental Figure 2. Mitochondrial antiviral signaling protein (MAVS) deficiency promotes glomerular injury in diabetic conditions. F4/80, CD3, B220, and Gr-1 cells were examined by immunohistochemistry staining in sham and diabetic mice (A, B). Glomerular hypercellularity (C), and mesangial expansion (D). The loss of podocytes is assessed by Wilms tumor 1 (WT-1) staining (1000 X, scale bar = 50 μm) (E, F). Serum creatinine levels (G). Data are shown as median ± IQR; *$p < 0.05$; **$p < 0.01$

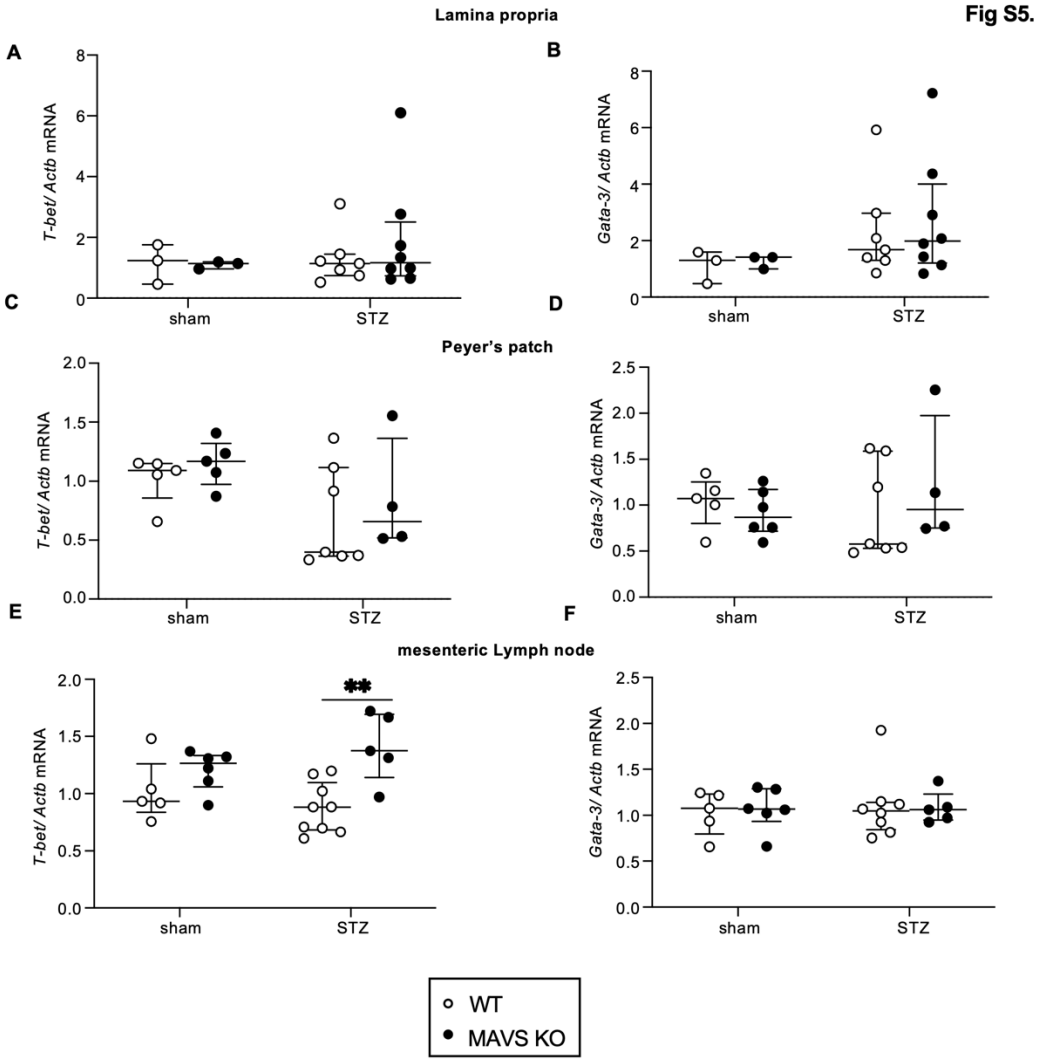


Supplemental Figure 3. MAVS expression in the kidney and macrophages of WT diabetic mice. MAVS expression in the kidney of WT sham and WT diabetic mice (A, B). FACS sorting strategy for renal macrophages (C). *Rig-I, Mda-5, Mavs, Traf-3, Traf-6* mRNA expression in kidney-isolated macrophages from WT sham and diabetic mice (D). Data are shown as median ± IQR; *$p < 0.05$; **$p < 0.01$

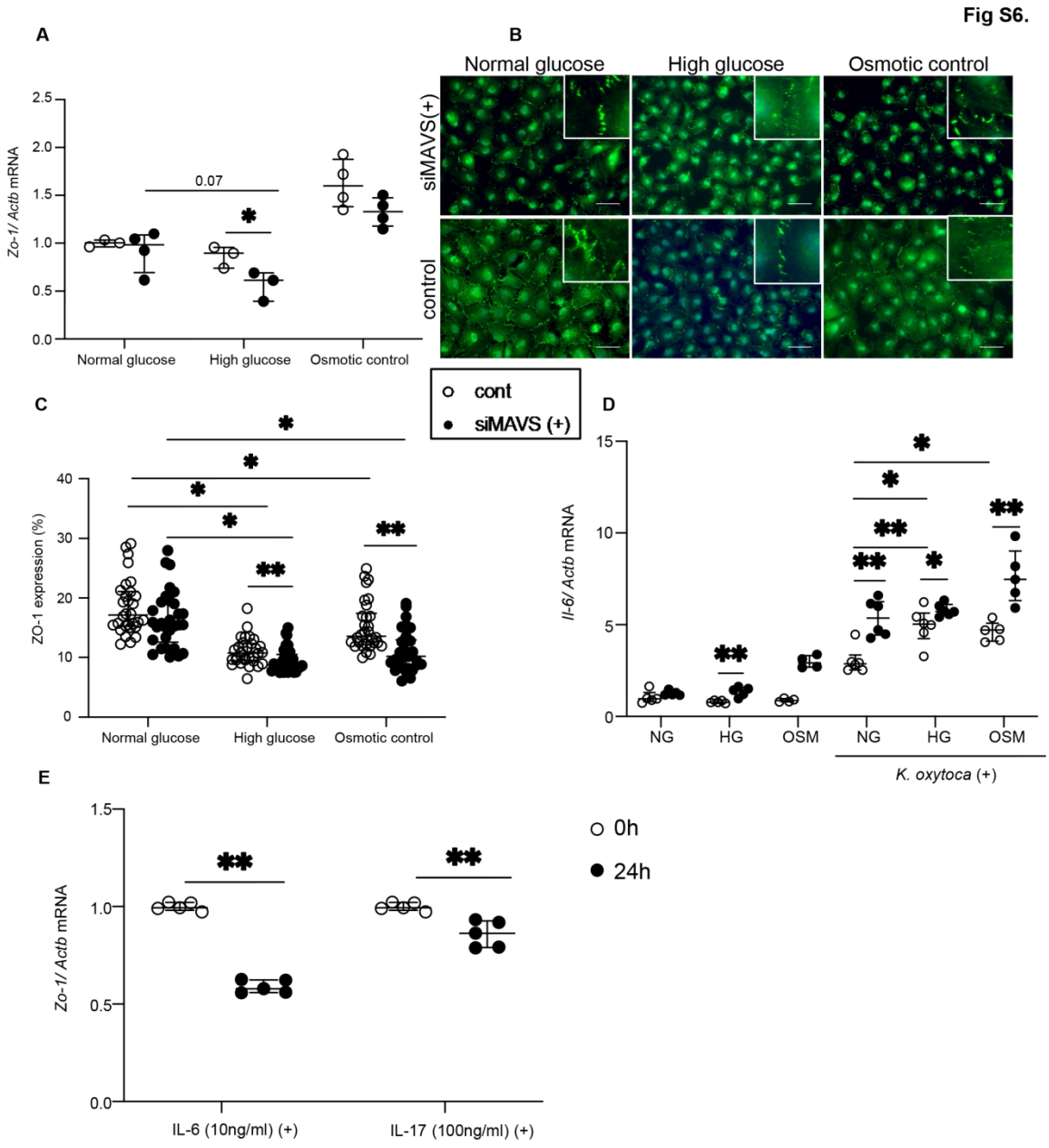
Fig S4.

	Spleen				Mesenteric lymph nodes			
	WT	MAVS KO	WT + STZ	MAVS KO + STZ	WT	MAVS KO	WT + STZ	MAVS KO + STZ
Klebsiella oxytoca								
Enterococcus faecalis								
Escherichia coli								
Providencia rettgeri								
Proteus mirabillis								
Klebsiella pneumonia								
Myroides odoramimus								
Aerococcus viridans								
Lactococcus lactis								
Vagococcus fluvialis								

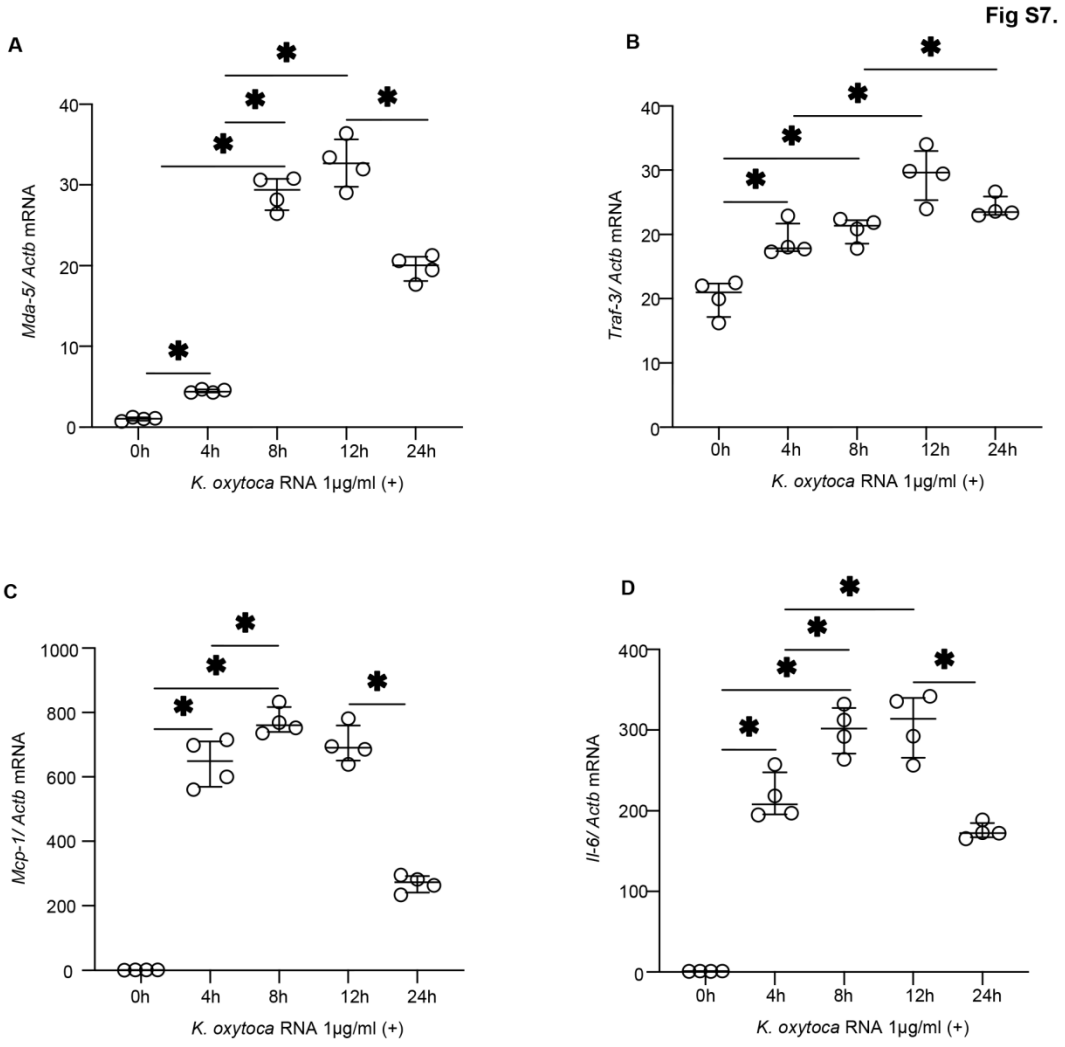
Supplemental Figure 4. Bacterial strains in the spleen and mesenteric lymph node culture



Supplemental Figure 5. An intestinal inflammatory condition in the control and diabetic mice. The expression of the transcription factor *T-bet* and *Gata-3* in lamina propria (A, B), Peyer's patch (C, D), and mesenteric lymph node (E, F) of WT and MAVS KO sham or diabetic mice. Data are shown as median ± IQR; *$p < 0.05$; **$p < 0.01$

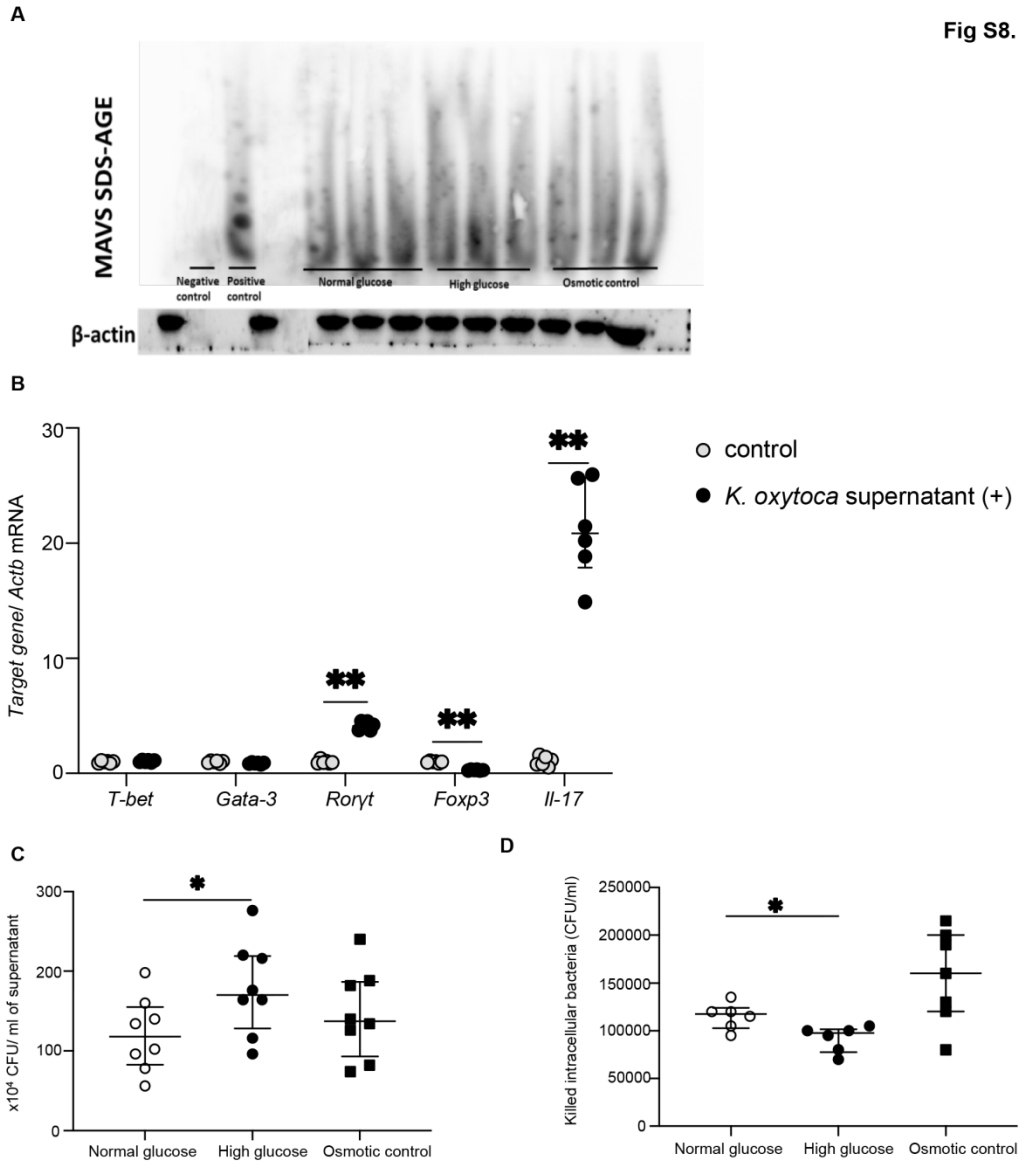


Supplemental Figure 6. Experiments with human intestinal epithelial cells. Zonula occludens (*Zo-1)* mRNA expression (24 h stimulation) (A) and ZO-1 staining (48 h stimulation) (B, C) under normal glucose (NG), high glucose (HG), and osmotic control (OSM) conditions. mRNA expression of *Il-6* (D) under NG, HG, and OSM conditions with and without coculture with *Klebsiella oxytoca* for 4 h. *Zo-1* expression 24h after IL-6 or IL-17 stimulation (E). Data are shown as median ± IQR; **p* < 0.05; ***p* < 0.01

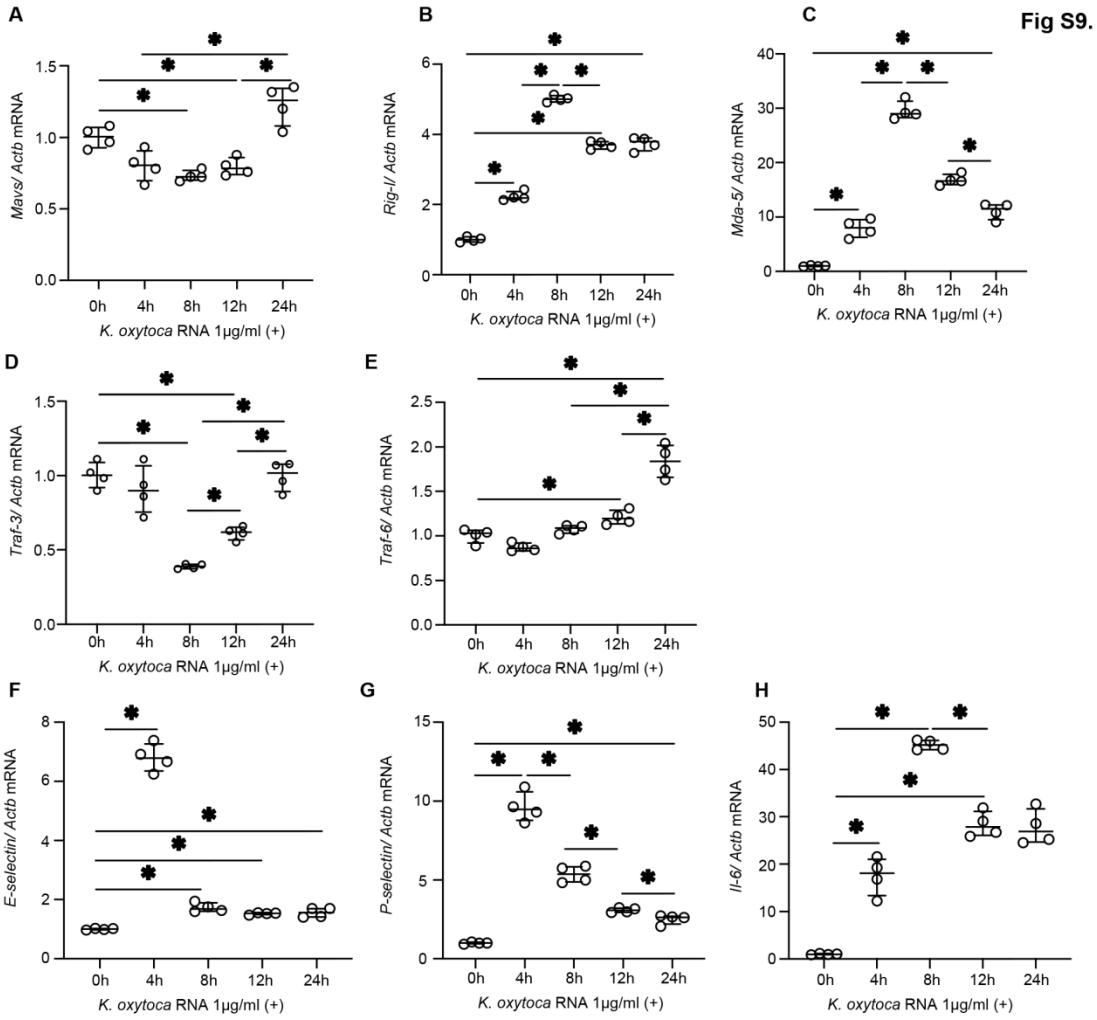


Supplemental Figure 7. *K. oxytoca* activates RIG-I/MDA-5–MAVS–TRAF-3/TRAF-6 signaling pathway in TECs. *Mda-5* (A), *Traf-3* (B), *Mcp-1* (C), *Il-6* (D) mRNA were shown under *K. oxytoca* RNA transfection. Data are shown as median ± IQR; *$p < 0.05$; **$p < 0.01$

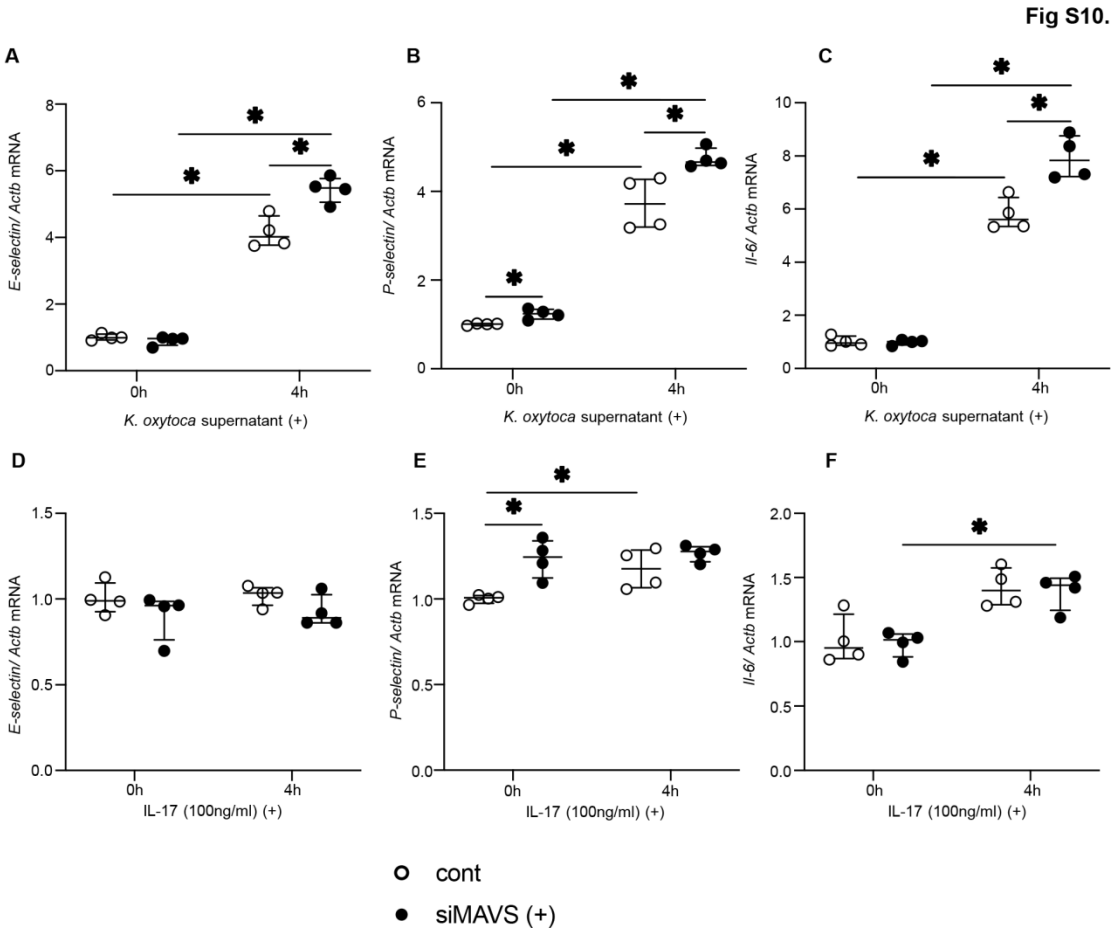
Fig S8.



Supplemental Figure 8. The dysfunction of bone marrow-derived macrophages (BMDMs). Detect MAVS aggregation by SDS-AGE after high glucose stimulation for 8 h in BMDMs (A). *T-bet, Gata-3, Rorɣt, Foxp3, Il-17* mRNA expression in T cells at 72 h after coculture with *K. oxytoca* supernatant- activated or control BMDMs (B). Phagocytic activity (C) and intracellular bacterial killing activity (D) to *K. oxytoca* of long-term NG, HG, or OSM-exposured BMDMs. Data are shown as median ± IQR; * $p < 0.05$; ** $p < 0.01$

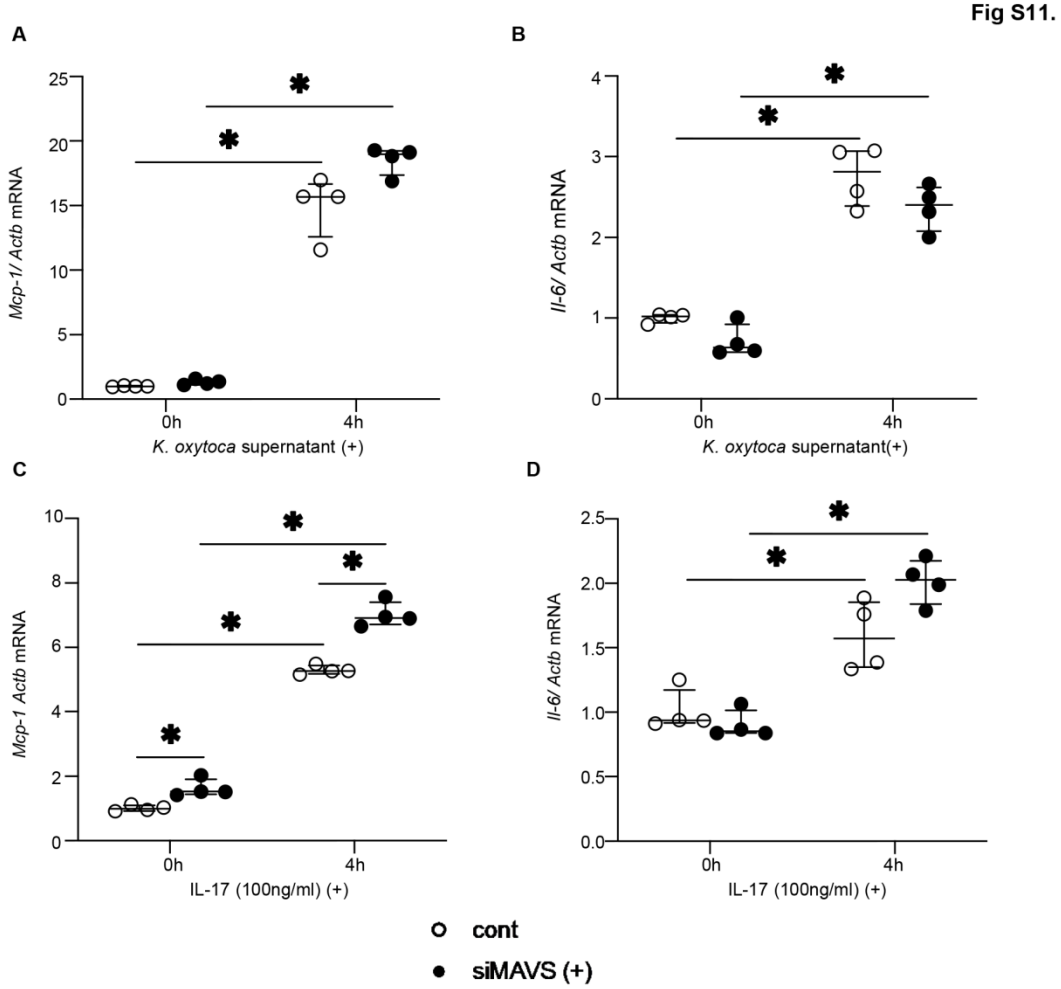


Supplemental Figure 9. *K. oxytoca* supernatant activates RIG-I/MDA-5–MAVS–TRAF-3/TRAF-6 signaling pathway in glomerular endothelial cells (GECs). *Mavs* (A), *Mda-5* (B), *Rig-I* (C), *Traf-3* (D), *Traf-6* (E) mRNA were upregulated under *K. oxytoca* RNA transfection. Activated markers *E-selectin* (F), *P-selectin* (G), *Il-6* (H) were shown. Data are shown as median ± IQR; * $p < 0.05$; ** $p < 0.01$

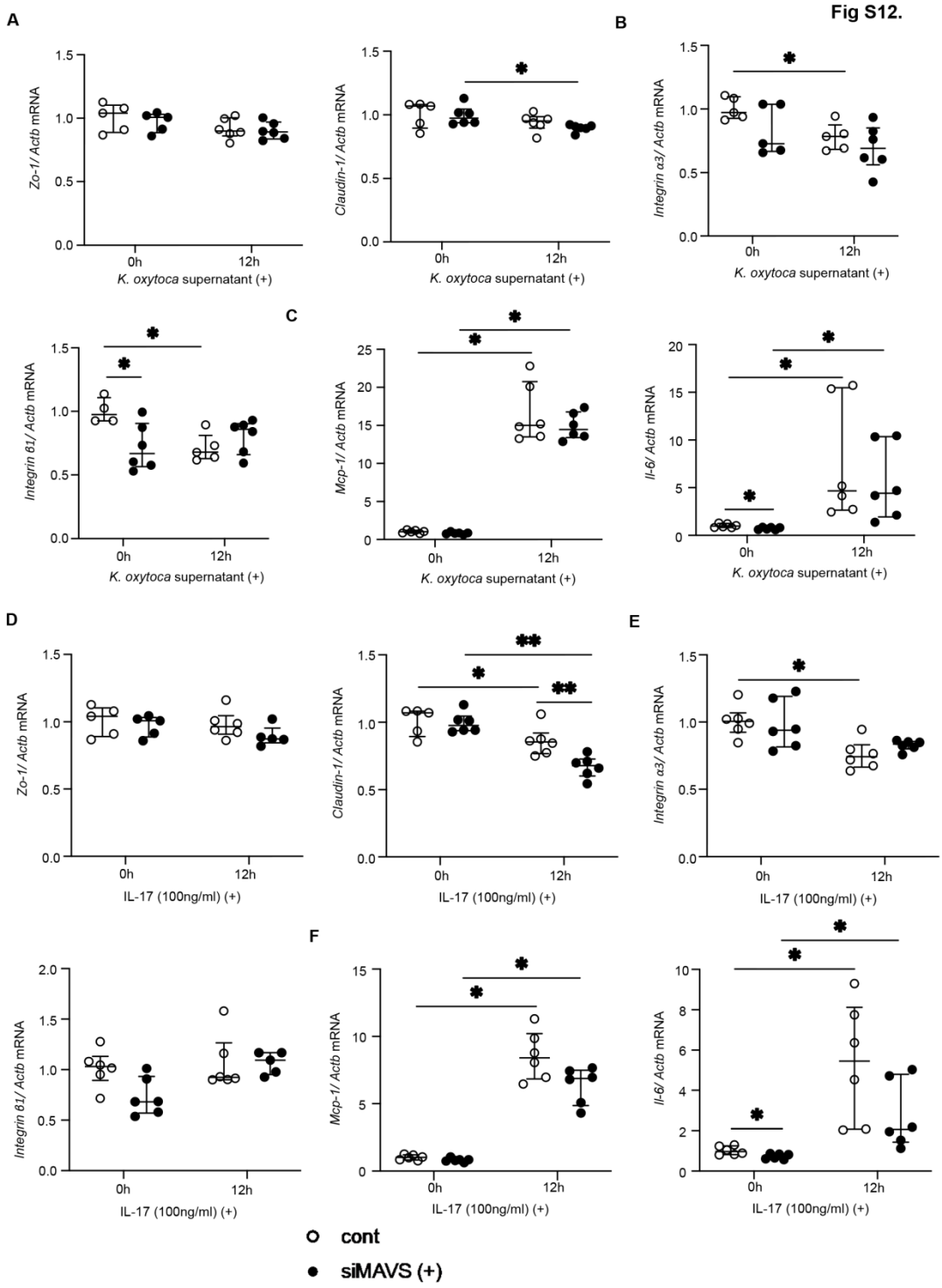


Supplemental Figure 10. The effect of *K. oxytoca* supernatant and IL-17 on glomerular endothelial cells (GECs) under normal and MAVS knockdown conditions. Levels of *E-selectin*, *P-selectin*, *Il-6* mRNA after 4h *K. oxytoca* supernatant stimulation (1:20) (A–C) or IL-17 stimulation (100ng/ml) (D–F). Data are shown as median ± IQR; *p<0.05; **p<0.01

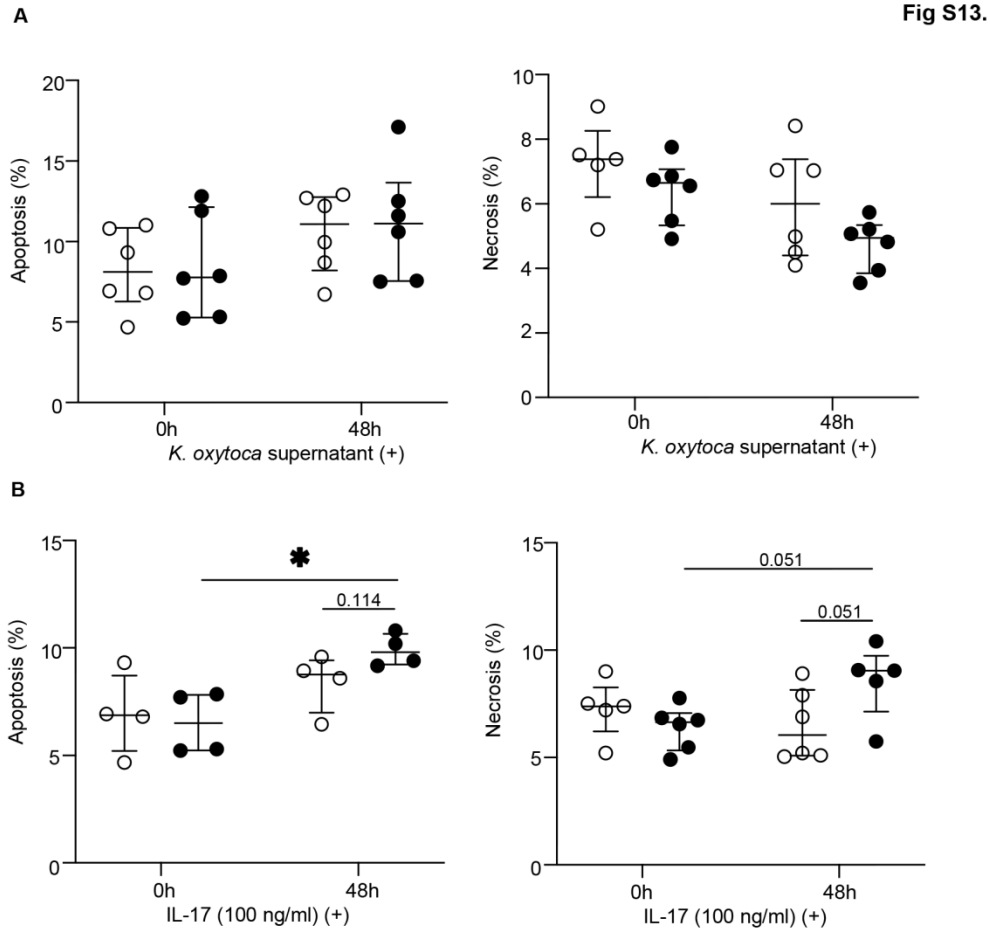
Fig S11.



Supplemental Figure 11. The effect of *K. oxytoca* supernatant and IL-17 on mesangial cells under normal and MAVS knockdown conditions. Levels of pro-inflammatory cytokines *Mcp-1*, *Il-6* after 4 h of *K. oxytoca* supernatant stimulation (1:20) (A, B) or IL-17 stimulation (100ng/ml) (C, D). Data are shown as median ± IQR; *p<0.05; **p<0.01

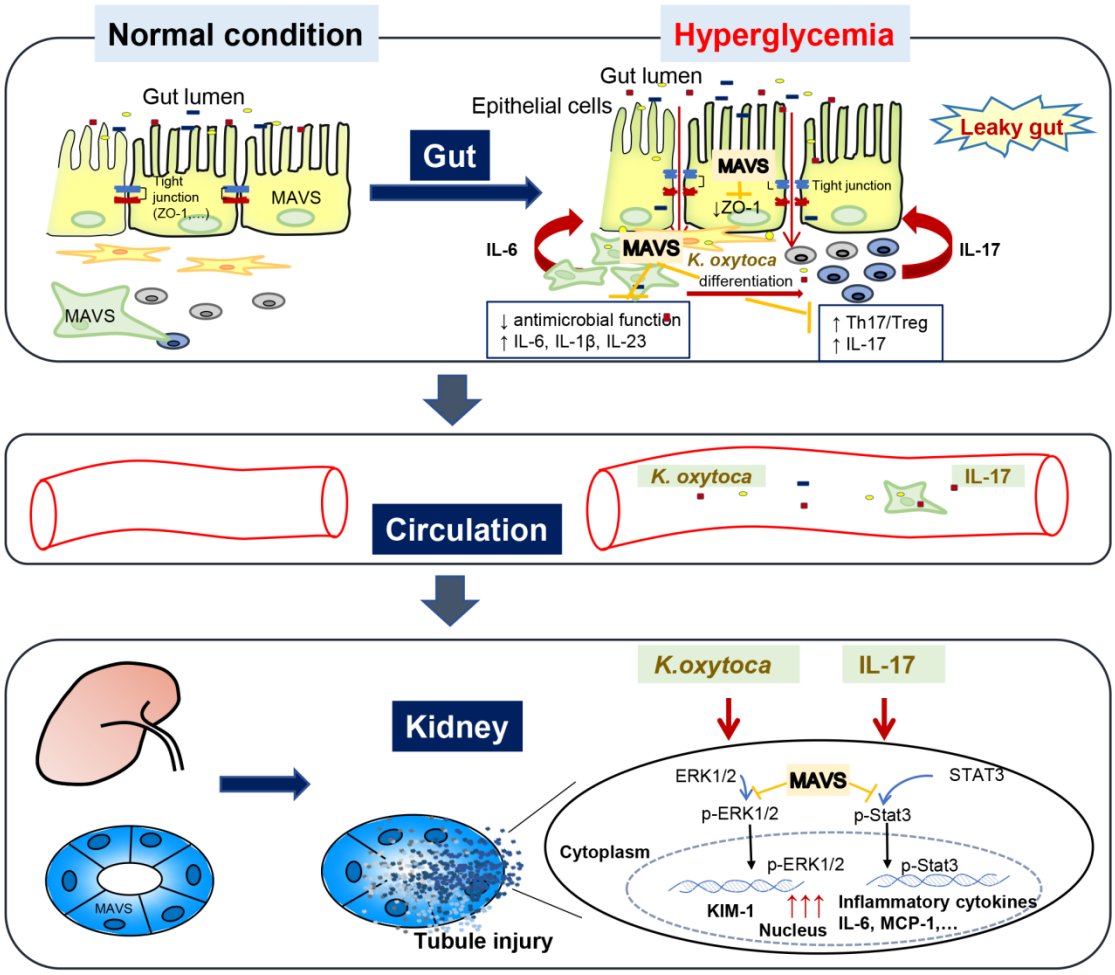


Supplemental Figure 12. The effect of *K. oxytoca* and IL-17 on immortalized conditional culture podocytes. *Tight junction* (*Zo-1*, *claudin-1*) (A), *integrin (α3, β1)* (B), *proinflammatory cytokines* (*Mcp-1*, *Il-6*) (C) mRNA expression after *K. oxytoca* supernatant stimulation (1:20) with and without siMAVS. *Tight junction (Zo-1, claudin-1)* (D), *integrin (α3, β1)* (E), *proinflammatory cytokines (Mcp-1, Il-6)* (F) mRNA expression after IL-17 treatment (100ng/ml) with and without siMAVS. Data are shown as median ± IQR; *p < 0.05; **p < 0.01



Supplemental Figure 13. The effect of *K. oxytoca* and IL-17 to cell death on immortalized conditional culture podocytes. Apoptotic and necrotic cells were measured after *K. oxytoca* supernatant (1:20) treatment (A) or IL17 treatment (B). Data are shown as median ± IQR; *p<0.05; **p<0.01

Fig S14.



Supplemental Figure 14. Schematic graphic: Chronic hyperglycemia leads to a "leaky gut" and an inflammatory intestine with notably IL-17 up-regulation to promote the translocation of bacteria, including *K. oxytoca* and IL-17 to extraintestinal sites and blood circulation. Then, bacteremia and IL-17 turn back to worsen gut dysfunction and accelerate kidney injury. Systemic MAVS is reno-protective in DKD mice by an intestinal protective role and the dominant inhibitory effects on suppressing KIM-1 production under *K. oxytoca* supernatant or IL-17 stimulation.

Supplemental Table 1 List of primers used for RT-qPCR

Mouse		Forward	Reverse
	Mcp-1	CTTCCTCCACCACCATGCA	CCAGCCGGCAACTGTGA
	Il-17	CTCCAGAAGGCCCTCAGACTAC	AGCTTTCCCTCCGCATTGACACAG
	Il-6	TGGCTAAGGACCAAGACCATCCAA	AACGCACTAGGTTTGCCGAGTAGA
	Tnf-α	ATCCGCGACGTGGAACTG	ACCGCCTGGACTTCTGGAA
	Il-23p19	TGCTGGATTGCAGAGCAGTAA	ATGCAGAGATTCCGAGAGA
	Zo-1	AGCTCATAGTTCAACACAGCCTCCAG	TTCTTCCACAGCTGAAGGACTCACAG
	Kim-1	CTATGTTGGCATCTGCATCG	AAGGCAACCACGCTTAGAGA
	Rorγt	CAGCAGCAACAGGAACAAGTG	CCCATCTGAGAGCCCTAAAGTG
	Foxp3	GGCGAAAGTGGCAGAGAGG	AAGGCAGAGTCAGGAGAAGTTG
	Tgfβ	GAGCGCTCATCTCGATTTTTA	TGAGGCTCTGACACCAAGGT
	Il-1β	GGATGAGGACATGAGCACCT	AGCTCATATGGGTCCGACAG
	Mavs	GCGAGGTCCACTGAGCTATC	CAGGTCAGGAGCAATGGAGG
	Rig-I	GCGGCGCCGAATAGTTT	GTTTAACCCCTCCCCACCAT
	Mda-5	GTGATGACGAGGCCAGCAGTTG	ATTCATCCGTTTCGTCCAGTTTCA
	Traf-3	GCCCGTCCTCCCTTTTAGTG	GGCCACCTCGCATAGGAA
	Traf-6	CCCAGTTGCACATGAGACTGTT	CGGACGCAAAGCAAGGTTAA
	Claudin-1	CTGGAAGATGATGAGGTGCAGAAGA	CCACTAATGTCGCCAGACCTGAA
	Integrinα3	AGCAACCTGCAGATGCGAGC	CTCATGCGCATCTTCCCCAG
	Integrinβ1	TTTTGCAACACCAAGCTCAC	TTTCCAAACCTGCATGTGAA
	E-selectin	AGCTACCCATGGAACACGAC	ACGCAAGTTCTCCAGCTGTT
	P-selectin	GTCCACGGAGAGTTTGGTGT	AAGTGGTGTTCGGACCAAAG
	β-actin	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA
human	*Zo-1*	TGCCATTACACGGTCCTCTG	GTTGATGATGCTGGGTTTGTTT
	Il-6	TGCAGAAAAAGGCAAAGAATCTAG	CGTCAGCAGGCTGGCATTT
	β-actin	AGGCACCAGGGCGTGAT	GCCCACATAGGAATCCTTCTGAC