Kidney VISTA prevents IFNγ-IL-9 axis-mediated tubulointerstitial fibrosis after acute glomerular injury

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Supplementary Table 1. Antibodies and materials used in this study

| Antibodies                                      | Source     | Clone   |
|------------------------------------------------|------------|---------|
| Anti-mouse/human CD11b antibody-BV785          | BioLegend  | M1/70   |
| Anti-mouse F4/80 antibody-BV711                 | BioLegend  | T45-2342|
| Anti-mouse CD3 antibody-BV711                   | BioLegend  | 17A2    |
| Anti-mouse CD8 antibody-BV650                   | BioLegend  | 53-6.7  |
| Anti-mouse CD62L antibody-BV605                 | BioLegend  | MEL-14  |
| Anti-mouse I-A/E antibody-BV605                 | BioLegend  | M5/114.15.2|
| Anti-mouse CD44 antibody-AmCyan                 | BioLegend  | 1M7     |
| Anti-mouse CD4 antibody-AmCyan                  | BioLegend  | RM4-5   |
| Anti-mouse CD45.2 antibody-PerCP-Cy5.5         | BioLegend  | 104     |
| Anti-mouse B220 antibody-FITC                   | BD Biosciences | RA3-6B2 |
| Anti-mouse CD72 antibody-FITC                   | BD Biosciences | K10.6   |
| Anti-mouse IL-4 antibody-PE                     | BD Biosciences | 11B11   |
| Anti-mouse IL-17 antibody-PE                    | eBioscience | 17B7    |
| Anti-mouse CD19 antibody-PE-Cy7                 | BioLegend  | 6D5     |
| Anti-mouse CD4 antibody-PE-Cy7                  | BioLegend  | GK1.5   |
| Anti-mouse Ly6C antibody-PE-Cy7                 | BioLegend  | HK1.4   |
| Anti-mouse CD11b antibody-PE-Cy7                | eBioscience | M1/70   |
| Anti-mouse IFNγ antibody-APC                    | BioLegend  | XMG1.2  |
| Anti-mouse IL-9 antibody-APC                    | BioLegend  | RM9A4   |
| Anti-mouse VISTA antibody-APC                   | BioLegend  | MH5A    |
| Anti-mouse NK1.1 antibody-APC                   | BioLegend  | PK136   |
| Anti-mouse Ly6G antibody-APC-Cy7                | BioLegend  | 1A8     |
| Anti-mouse CD8 antibody-APC-Cy7                 | BioLegend  | 53-6.7  |
| Anti-mouse CD45.1 antibody-APC-Cy7              | BioLegend  | A20     |
| Anti-mouse CD4 antibody-APC-Cy7                 | BioLegend  | GK1.5   |
| Anti-mouse NK1.1 antibody-APC                   | BioLegend  | PK136   |
| Anti-mouse F4/80 antibody-APC                   | BioLegend  | BM8     |
| Anti-mouse/human VISTA antibody-AF488          | Pharmabcine | 2C12    |
| Human IgG1 isotype control antibody-AF488       | Pharmabcine | 2G4     |
| Antibody Name                        | Manufacturer               | Clone/Isotype          |
|-------------------------------------|----------------------------|------------------------|
| Anti-mouse CD16/CD32 antibody       | eBioscience                | 93                     |
| Anti-human Fc receptor binding inhibitor | eBioscience              | Polyclonal             |
| Fixable Viability Stain 450         | BD Biosciences             |                        |
| Fixable Viability Stain 780         | BD Biosciences             |                        |
| Anti-mouse IFNγ antibody            | BioXcell                   | XMG1.2                 |
| Anti-mouse IL-9 antibody            | BioXcell                   | 9C1                    |
| Anti-mouse Thy1 antibody            | Ours                       | T24                    |
| Anti-mouse IL-9 antibody            | Abcam                      | Polyclonal             |
| Anti-mouse IL-9 receptor antibody   | Thermo Fisher Scientific   | Polyclonal             |
| Anti-mouse αSMA antibody            | Thermo Fisher Scientific   | 17HCLC                 |
| Anti-mouse VISTA antibody           | Cell Signaling             | D5L5T                  |
| Anti-mouse VISTA antibody           | BioXcell                   | 13F3                   |
| Anti-mouse F4/80 antibody           | Abcam                      | BM8                    |
| Anti-mouse CD3 antibody             | Abcam                      | SP162                  |
| Anti-mouse CD72 antibody            | R&D Systems                | Polyclonal             |
| Anti-mouse KIM-1 antibody           | Abcam                      | Polyclonal             |
| Anti-human IL-9 antibody            | Abcam                      | Polyclonal             |
| Anti-human VISTA antibody           | Abcam                      | Polyclonal             |
| Anti-human CD14 antibody            | Novus Biologicals          | Polyclonal             |
| Anti-human CD3 antibody             | Abcam                      | PS1                    |
| Anti-rabbit IgG antibody-HRP        | Abcam                      |                        |
| Anti-mouse CD11b antibody-biotin    | BD Biosciences             | M1/70                  |
| Anti-mouse CD45R/B220 antibody-biotin | BD Biosciences         | RA3-6B2                |
| Anti-mouse NK1.1 antibody-biotin    | BD Biosciences             | PK136                  |
| Anti-mouse CD8a antibody-biotin     | BD Biosciences             | 53-6.7                 |
| Anti-mouse CD4 antibody-biotin      | BD Biosciences             | RM4-5                  |
| Rabbit IgG isotype control antibody | Thermo Fisher Scientific   |                        |
| Anti-rat IgG cross-adsorbed secondary antibody-AF 594 | Thermo Fisher Scientific | Polyclonal             |
| Anti-rat IgG cross-adsorbed secondary antibody-AF 488 | Thermo Fisher Scientific | Polyclonal             |
| Anti-goat IgG cross-adsorbed secondary antibody-AF 568 | Thermo Fisher Scientific | Polyclonal             |
| Anti-rabbit IgG cross-adsorbed secondary antibody-AF 488 | Thermo Fisher Scientific | Polyclonal             |
# Supplementary Table 2. Primers used for real-time qPCR

| Genes | Forward (5′–3′) | Reverse (5′–3′) |
|-------|-----------------|-----------------|
| **Ifng** | ACAGCAAGGCGAAAAAGGAT | TGAGCTCATTTGAATGCTTGG |
| **Gzma** | AGACCGTATATGGCTCTACT | CCCTCACGTGATATTCACT |
| **Tnf** | CCTCACACTCACAACCACCA | GTGAGGAGCACGTAGTCGG |
| **Il1b** | ATGCCACCTTTTGAAGCGTGATG | AGCTTCTCCACAGCCCAAT |
| **Il2** | GCAGGATGGAGAATTACAGGA | TCAGAGCCCTTATGTTTAC |
| **Il4** | TGAACGAGGCACAGGGAAGAA | CGAGCTCAGTCTCTGTGGT |
| **Il5** | AGCAATGAGACAGATGGGAC | ACACTTCTCTTTTGGGCCG |
| **Il6** | TGATGCACTTGCAGAAAAACA | ACCAGAGAAAATTTTCAATAG |
| **Il8** | GATTCACCTCAAGAACACATCCGAGA | GGACACCTTTTGAACATTTTGG |
| **Il9** | ATGTTGGTGACATACATCCTTGCC | TGACGGAATGATCATCTTCAG |
| **Il10** | ATCGATTCTCCCCTGTTGA | TGTCATATTCATTCATTCAG |
| **Il12b** | GATTCAGACTCCAGGGGACA | GGAGACACCAGCAAACAGAT |
| **Il13** | ACATCACACAAGACCAGACTCC | GAGGCCATGCAAATATCCTCT |
| **Il17a** | TCTCCACCGAATGAGACC | CACACCCACGACATCTTC |
| **Il22** | TGAGGATGGTCCACTTTCTTCA | AGCCGGACGTCTGTGGT |
| **Il33** | TGGCCTCACCATAAGAAAGG | GACTTGCAGGACAGGGAGAC |
| **Tgfb1** | TGAGGTCACCTGGAGTTGTACCG | GGTCATGGCTATGGGATG |
| **Atp5o** | TGACCACAGCATCTCCTCTA | GTCACTCTTGAATCTCCAGTT |
| **Atp6voc** | CCCTAGAGTTGCTCTGTGATAAA | GCTCCACAGACCATGAATAG |
| **Hsp90aa1** | CATCGGAGCCTCCTGGAATAA | CTGTGTCGGGAATGAGATT |
| **Odc1** | CTTGAGGGAGCTGTGTGATAAT | GCAGTCAAACCTGCTTTAGT |
| **18S rRNA** | CGGCTACCACATCCAAGGAA | GCTGGAATTACCCCGGCT |
Supplementary Figure 1. Baseline comparison between WT and Vsir$^−/−$ mice (n = 3 per group). (A) Kidney damage markers such as blood urea nitrogen (BUN), serum creatinine (Cr), and random urine protein-to-creatinine ratio (uPCR). (B) Representative PAS staining images of kidneys in WT (upper image) and Vsir$^−/−$ (lower image) mice. Scale bar = 100 μm. Glomerular and tubular injury scores. (C) Representative Sirius red images of kidneys in WT (upper image) and Vsir$^−/−$ (lower image) mice. Scale bar = 100 μm. Comparison of the Sirius red$^+\$ area between WT and Vsir$^−/−$ kidneys.
(C)
Supplementary Figure 2. Serum albumin and cholesterol levels in WT and $Vsir^{-/-}$ mice ($n = 8$ per group). Hypoalbuminemia and hypercholesterolemia are the features of aggravated glomerulonephritis (i.e., signs of nephrotic syndrome). $***P < 0.001$. Data were obtained from three independent experiments.
Supplementary Figure 3. Metabolic gene expression in infiltrated T cells. (A) Representative genes related with oxidative phosphorylation. (B) Representative genes related with fatty acid metabolism. Infiltrated T cells were isolated using magnetic bead sorting method ($n = 3$ per group). $P$ values are calculated using the unpaired Student’s $t$ test. *$P < 0.05$, **$P < 0.01$. 

(A) 

(B)
Supplementary Figure 4. Proportions of IFNγ+ cells in CD4+ and CD8+ cells after the use of anti-VISTA antibody (n = 4 per group). Either 300 μg of anti-VISTA antibody or control antibody was treated via intraperitoneal injection every 3 days from day 0 of NTN induction. P values are calculated using the unpaired Student’s t test. *P < 0.05. Data represent two independent experiments.
Supplementary Figure 5. Proportions of IFNγ+ cells in CD4+ and CD8+ cells after the treatment with the inhibitors of oxidative phosphorylation and fatty acid metabolism (n = 4 per group). 10 ng of oligomycin (OM) and etomoxir (Eto) were treated via subcapsular injection at day 7 after NTN induction to inhibit the oxidative phosphorylation and fatty acid metabolism, respectively. P values are calculated using analysis of variance with Tukey’s test. *P < 0.05, **P < 0.01. Data represent two independent experiments.
Supplementary Figure 6. T cell-dependency of the NTN model in Vsir⁻/⁻ mice. (A) Flow cytometry confirmation of T-cell depletion after anti-Thy1 antibody. (B) Kidney damage markers after the anti-Thy1 antibody. The isotype control or anti-Thy1 antibody was used before NTN induction. *$P < 0.05$, **$P < 0.01$. 

(A)

(B)
Supplementary Figure 7. Expression of the IL-9 receptor (IL-9R) in NTN-induced kidneys. (A) Representative images of kidney sections immunostained for the IL-9R in WT (upper) and Vsir<sup>−/−</sup> (lower) kidneys. (B) Comparison of the IL-9R<sup>+</sup> area in NTN-induced WT and Vsir<sup>−/−</sup> kidneys.
Supplementary Figure 8. Figure. Kidney damage markers in NTN-induced $Rag1^{-/-}$ mice with adoptive transfer of T cells. Phosphate buffered saline (PBS) was used as a vehicle. **$P < 0.01$. Data represent two independent experiments.
Supplementary Figure 9. Expression of VISTA protein in adoptively transferred T cells from WT mice to NTN-induced \( RagI^{-/-} \) mice. A representative flow cytometry plot of VISTA at day 8 after the NTN induction is displayed as obtained from two independent experiments.
Supplementary Figure 10. Volcano plot showing gene expression changes in two mononuclear phagocyte (MNP) clusters from human kidneys.
Supplementary Figure 11. Representative image of normal human kidney sections immunostained for VISTA. Scale bar = 200 μm. The image was selected from two independent samples.