CXCR4 induces podocyte injury and proteinuria by activating β-catenin signaling

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Supplementary Figures

Figure S1. Colocalization of CXCR4 and β-catenin in the glomeruli of kidney biopsies from patients with proteinuric kidney diseases. Frozen sections were immunostained for CXCR4 (red) and β-catenin (green), respectively. Colocalizations of CXCR4 and β-catenin in glomeruli are indicated by arrows. Scale bar, 50 µm. Representative micrographs of normal kidney (1 case), DN (2 cases), FSGS (2 cases) and MN (2 cases). DN, diabetic nephropathy; FSGS, focal and segmental glomerulosclerosis; MN, membranous nephropathy.
Figure S2. SDF-1α does not affect the mRNA expression of various Wnt ligands in cultured podocytes. Quantitative real-time PCR (qPCR) analyses show mRNA expression of Wnt ligands in cultured podocytes (MPC5) after treatment with SDF-1α (100 ng/mL) for 24 h (n = 3).

Figure S3. Blockade of β-catenin signaling by ICG-001 preserves tight junction protein ZO-1 in cultured podocytes. Representative micrographs show ZO-1 staining in the cell-cell junctions of podocytes. Arrow indicates positive staining for ZO-1. Scale bar, 15 µm.
Figure S4. β-arrestin-1 is upregulated in various models of CKD. (A) Graphical representation shows the relative mRNA levels of β-arrestin-1 in control or ADR for 5 weeks as indicated. *$P < 0.05$ versus controls ($n = 5$). (B) Graphical representation shows the relative mRNA levels of β-arrestin-1 in control or angiotensin II infusion for 4 weeks as indicated. *$P < 0.05$ versus controls ($n = 5-6$).

Figure S5. Generation of conditional knockout mice with podocyte-specific ablation of CXCR4. (A) Genotyping of the mice by PCR analysis of genomic DNA. Lane 1 shows the genotyping of the control mice used in this study (genotype: CXCR4$^{fl/fl}$), whereas lane 2 denotes the genotyping of the podocyte-specific CXCR4 knockout mice (genotype: CXCR4$^{fl/fl}$ Cre), designated as podo-CXCR4$^{-/-}$. (B) Body weights of podo-CXCR4$^{+/+}$ and podo-CXCR4$^{-/-}$ mice at 3 months of age. $P=0.927$ ($n = 7$). (C) Representative micrographs show PAS staining in podo-CXCR4$^{+/+}$ and podo-CXCR4$^{-/-}$ mice in normal conditions. Scale bar, 20 µm.
Figure S6. Ablation of CXCR4 in podocytes reduces PAI-1 expression after ADR injury.

Quantitative real-time RT-PCR (qRT-PCR) analyses show a reduced mRNA expression of PAI-1 in podo-CXCR4-/− mice at 2 weeks after ADR, compared to podo-CXCR4+/+ mice. *P < 0.05 (n = 5-6).
## Table S1. Nucleotide sequences of the primers used for qPCR

| Mouse gene       | Primer Sequence 5’ to 3’ | Forward                        | Reverse                        |
|------------------|---------------------------|--------------------------------|--------------------------------|
| Cre recombinase  | AGGTGTAGAGAAGGACGTAGCCTAG | CTAATCGCCATCTTCCACGAGGT        |
| CXCR4            | CCACCCAGAGTGTGACTCTCTAA  | GATGGGAATTCTGTATGGAGGATTAGC    |
| Wnt2             | CGACTGTAGCAGAGAACAC      | AGCTGTAGGTACGTGCCAGCGATTG      |
| Wnt2b            | GCTCTTGACCTGCTCAACC      | CGGAGAATGGCACCGGACGAGCATG      |
| Wnt3             | CCTCGCTGCTACCCAATTT      | GCAGGACATGCTCGTCCGAGCATG       |
| Wnt3a            | GAGGCCAGCTTTCAAGGACCAGC  | ACCCATCTAGCCATGCGAGCGATG       |
| Wnt4             | ATCTCTICAGAGGGTGTCGC     | GTGCGTCCAGAGGACGCTCGGAGATG     |
| Wnt5a            | AGACCTTCAGAGGGAGATGGAC   | TCTCCGTGCACTCTTTGCGAAGATG     |
| Wnt5b            | GTGCGAGACGGGAGATGTT       | AGCTCTCTGAGGATGTCCTG          |
| Wnt6             | TCGGGGATGAGAAGTGCTCAAG   | CGGCACAGACGTTCTCCTCCTG         |
| Wnt7a            | CGGACGCCATCTAGGTCGATA    | CACTTTGATCGCTCGTCCGAGATG      |
| Wnt7b            | TCTCTACGTAAGCTCGGA       | TCCCGATCGCAATGCGAGATG          |
| Wnt8             | GCCTATCTGACATAACCCG      | GGATGTGTCGTGTCGTCGCAAGATG     |
| Wnt8b            | ACTAGAAGACTGACCCTCGG     | CTCAGGAGGCATCCACAAACT         |
| Wnt9a            | AACCTCGTGAGGGTGAGGCTAG   | TTGGTGTATTGACGTGGCCTCTG        |
| Wnt9b            | CAGGCTGCTGAGGGCTACGTA    | CTCCATTAGGACCGTGCTTGAGATG     |
| Wnt10a           | GGTAAGAGCGCTGAGGCTGAGCA | AAGATGGGCGGGTGTTGAGATG         |
| Wnt10b           | CCGAGCGCTGCAATGCAGC      | TCCATGTCGTGTTAGCAGGACCCAATG   |
| Wnt11            | ACTGTAACAGCGTGGAGGCC     | CGATGGGAGGACGATGCTTCCAG       |
| Wnt16            | TACGGCAGGTGAGGAGGCTGAGCA | GCAGGACGACGCCCAGACATA         |
| Fibronectin      | CGGAGGTAGCAGAGACCACCA    | CGGAGTGCTAGGACCACAGAC         |
| COL3A1           | AGGCCAAAGCTTGCCTCGTCTGAG | GACCTCGTCTCCAGGATGAC          |
| PAI-1            | TGGAGAAGGGCAAGATTTATCATG | GAAGTGGAGGGCAGTCACCAAG        |
| β-arrestin1      | GGCGAGAAGAGGGACAGCAG     | GTGTCAGCGATGCCTAGCTCCT         |
| β-actin          | CAGCTAGAGGGAAATCTG      | CGGAGACCAGAGGAGGCTGAGGAGTG    |
| Antibodies                  | Catalogue number | Company                        | Location              |
|----------------------------|------------------|--------------------------------|-----------------------|
| Primary antibodies         |                  |                                |                       |
| anti-CXCR4                 | sc-6190          | Santa Cruz Biotechnology       | Santa Cruz, CA        |
| anti-CXCR4                 | sc-53534         | Santa Cruz Biotechnology       | Santa Cruz, CA        |
| anti-CXCR4                 | ab2074           | Abcam                          |                       |
| anti-CXCR4                 | PA5-19856        | Fisher Scientific              |                       |
| anti-SDF-1α                | A00053-2         | Boster Biological Technology   | Wuhan, China          |
| anti-α-SMA                 | A2547            | Sigma-Aldrich                  |                       |
| anti-α-SMA                 | ab5694           | Abcam                          |                       |
| anti-active β-catenin      | 05-665           | EMD Millipore                  |                       |
| anti-active β-catenin      | #19807           | Cell Signaling Technology      |                       |
| anti-β-catenin             | ab15180          | Abcam                          |                       |
| anti-β-catenin             | #610154          | BD Transduction Laboratories   |                       |
| anti-MMP7                  | GTX32725         | GeneTex                        |                       |
| anti-MMP7                  | GTX104658        | GeneTex                        |                       |
| anti-nephrin               | 20R-NP002        | Fitzgerald Industries International |               |
| anti-WT1                   | sc-192           | Santa Cruz Biotechnology       | Santa Cruz, CA        |
| anti-podocalyxin           | AF1556           | R&D Systems                    |                       |
| anti-fibronectin           | F3648            | Sigma-Aldrich                  |                       |
| anti-ZO-1                  | 61–7300          | Thermo Fisher Scientific       | Waltham, MA           |
| anti-β-arrestin1           | 12673s           | Cell Signaling Technology      | Danvers, MA           |
| anti-Snail1                | ab82846          | Abcam                          | Cambridge, MA         |
| anti-phospho-EGFR          | sc-57542         | Santa Cruz Biotechnology       | Santa Cruz, CA        |
| anti-EGFR                  | sc-373746        | Santa Cruz Biotechnology       | Santa Cruz, CA        |
| anti-phospho-Src (Y418)    | ab40660          | Abcam                          | Cambridge, MA         |
| anti-Src                   | ab47405          | Abcam                          | Cambridge, MA         |
| anti-phospho-GSK3β (Ser9)  | 9336s            | Cell Signaling Technology      | Danvers, MA           |
| anti-GSK3β                 | 9315s            | Cell Signaling Technology      | Danvers, MA           |
| anti-phospho-p44/42MAPK(ERK1/2) (Thr202/Tyr204) | 4370s | Cell Signaling Technology | Danvers, MA |
| anti-p44/42MAPK (ERK1/2)   | 9102s            | Cell Signaling Technology      | Danvers, MA           |
| anti-p44/42MAPK (ERK1/2)   | 4695s            | Cell Signaling Technology      | Danvers, MA           |
| anti-α-tubulin             | T9026            | Sigma-Aldrich                  | St. Louis, MO         |
| anti-GAPDH                 | RM2002           | Ray Antibody Biotech           | Peachtree Corners, GA |
| Secondary antibodies                  | Code          | Supplier                        | Location            |
|---------------------------------------|---------------|---------------------------------|---------------------|
| Goat anti-mouse                       | BA1050        | Boster Biological Technology    | Wuhan, China        |
| Goat anti-rabbit                      | BA1054        | Boster Biological Technology    | Wuhan, China        |
| Rabbit anti-goat                      | BA1060        | Boster Biological Technology    | Wuhan, China        |
| Goat anti-Guinea pig                  | 106-001-003   | Sigma-Aldrich                   | St. Louis, MO       |
| Biotin-SP AffiniPure Goat Anti-Mouse IgG(H+L) | 115-065-146 | Jackson Immuno Research Laboratories | West Grove, PA |
| Biotin-SP AffiniPure Goat Anti-Rabbit IgG(H+L) | 111-065-144 | Jackson Immuno Research Laboratories | West Grove, PA |
| Cy3-AffiniPure Goat Anti-Guinea Pig IgG(H+L) | 106-165-003 | Jackson Immuno Research Laboratories | West Grove, PA |
| Cy3-AffiniPure Rabbit Anti-Goat IgG(H+L) | 305-165-003 | Jackson Immuno Research Laboratories | West Grove, PA |
| Cy2-AffiniPure Goat Anti-Rabbit IgG(H+L) | 111-225-144 | Jackson Immuno Research Laboratories | West Grove, PA |
| Cy3-AffiniPure Goat Anti-Rabbit IgG(H+L) | 111-165-003 | Jackson Immuno Research Laboratories | West Grove, PA |
| Cy2-AffiniPure Donkey Anti-Rabbit IgG(H+L) | 711-225-152 | Jackson Immuno Research Laboratories | West Grove, PA |
| Cy3-AffiniPure Donkey Anti-Mouse IgG(H+L) | 715-165-150 | Jackson Immuno Research Laboratories | West Grove, PA |