Human Dendritic cell-specific ICAM-3-grabbing non-integrin downstream signaling alleviates renal fibrosis via Raf-1 activation in systemic candidiasis

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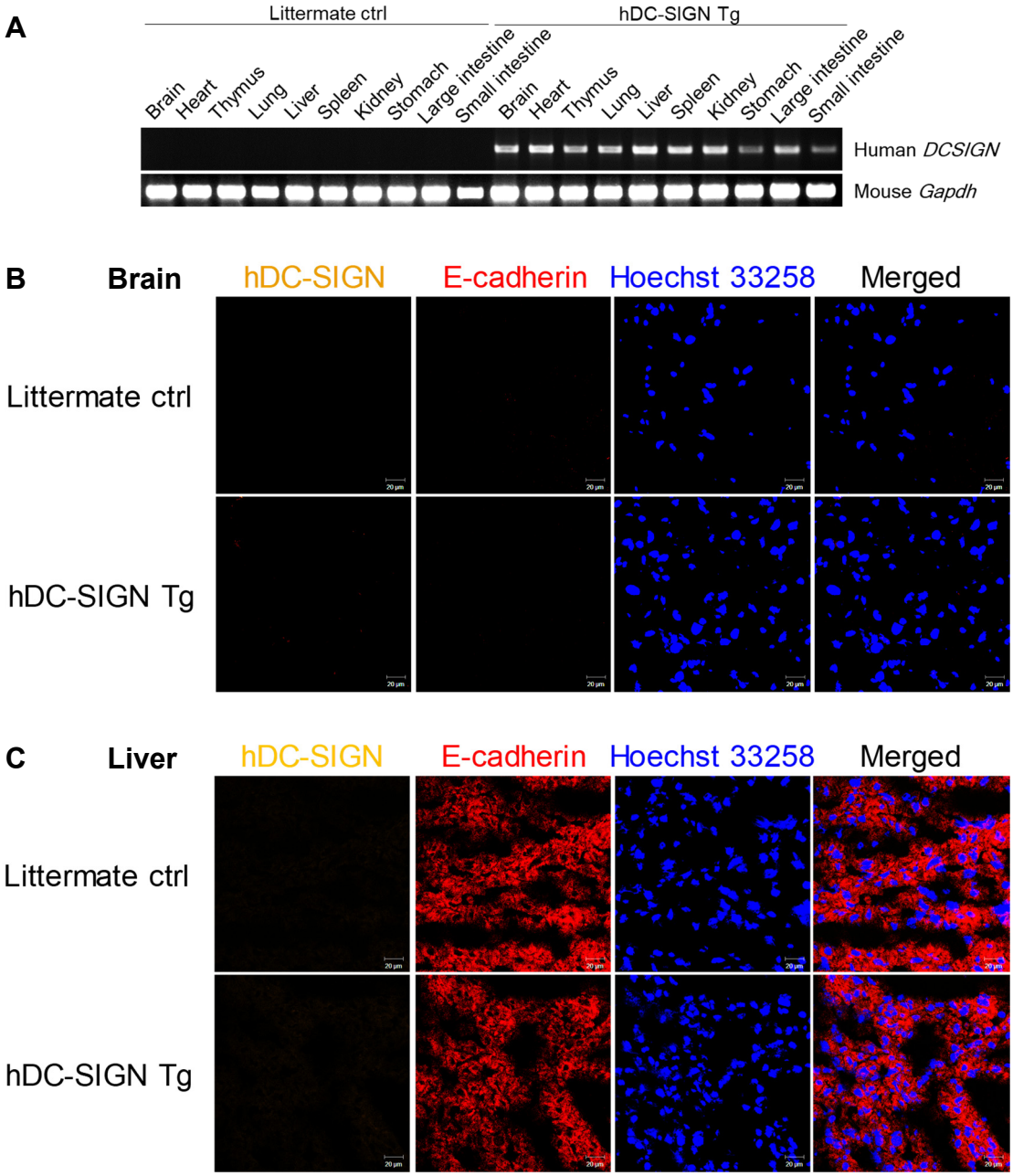
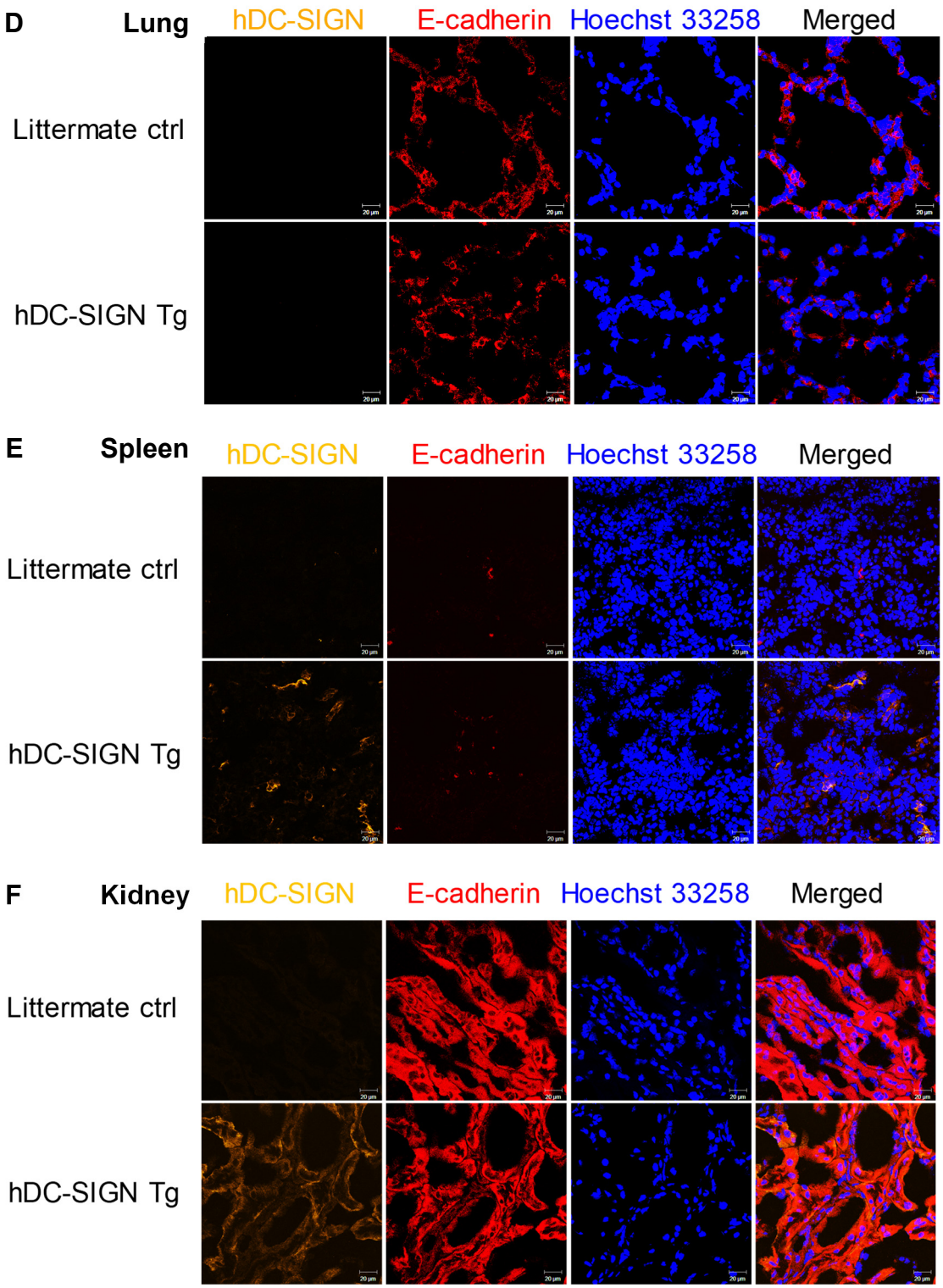
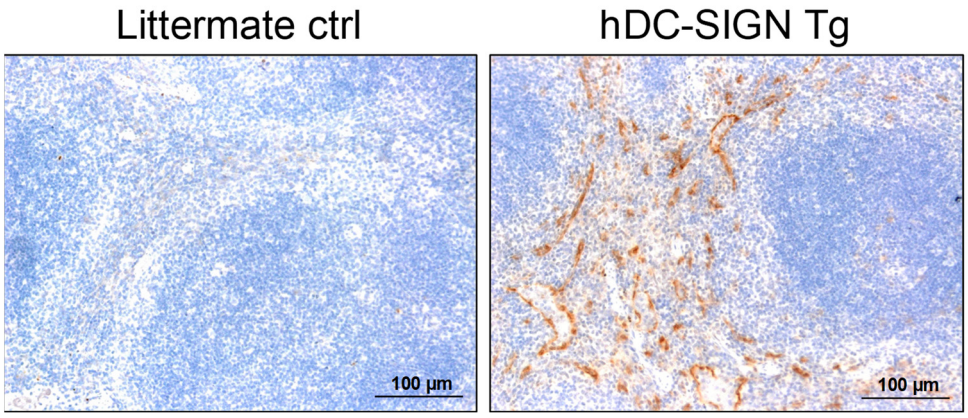
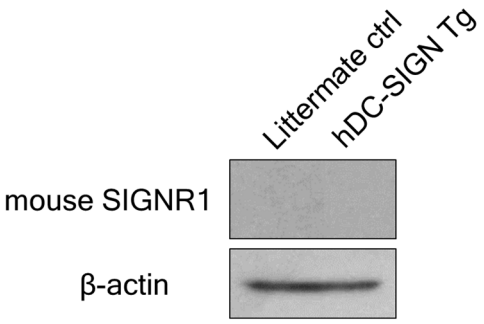




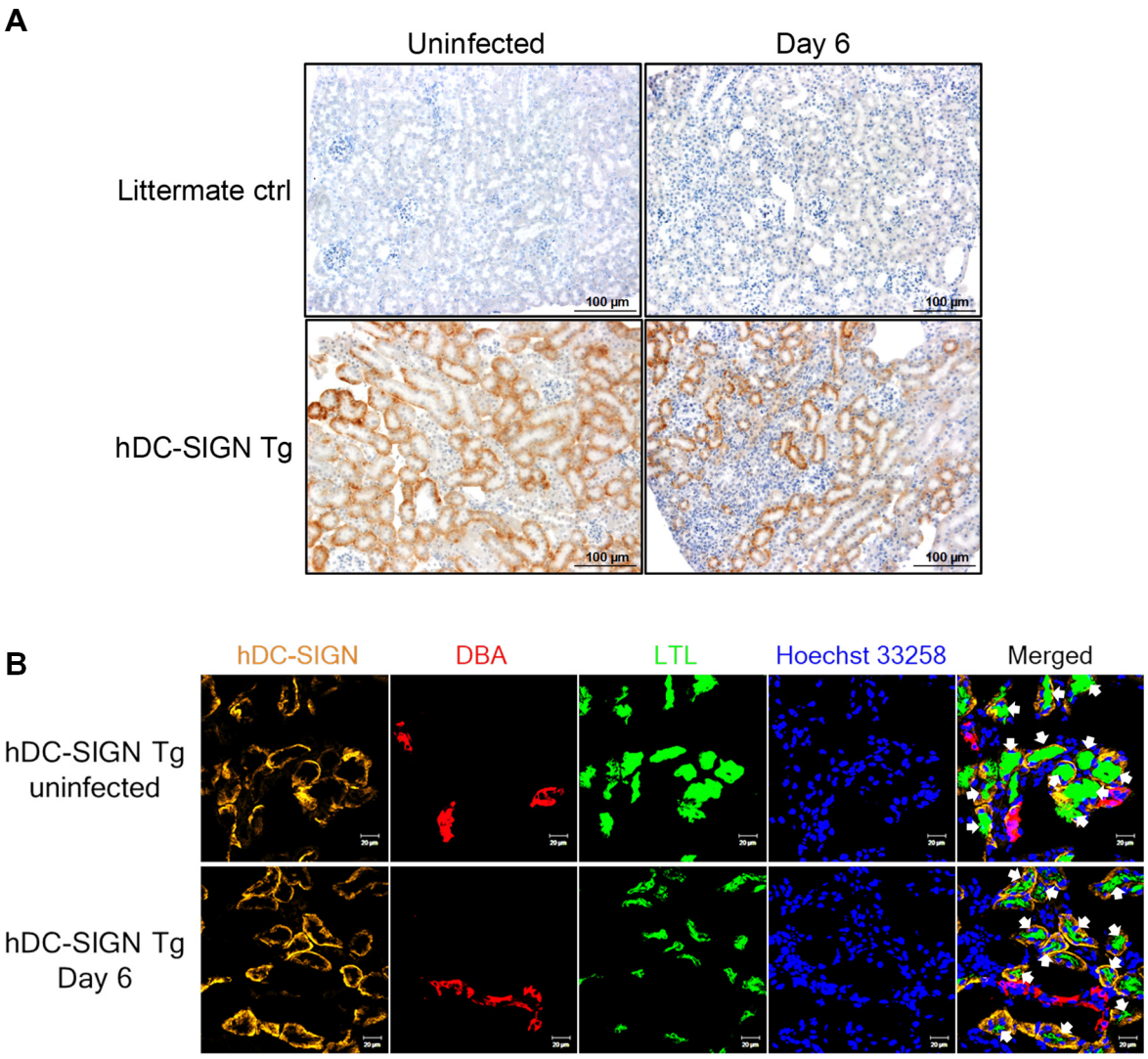
Suppl Figure 1. The expression of hDC-SIGN mRNA and protein in organs of hDC-SIGN transgenic mice. Different organs from naïve hDC-SIGN transgenic and littermate ctrl mice were collected after perfusion with PBS. **(A)** Human *DCSIGN* mRNA expression in the organs are shown. Mouse *Gapdh* mRNA expression were as an internal control. Cryosections of **(B)** brain, **(C)** liver, **(D)** lung, **(E)** spleen and **(F)** kidney were stained for hDC-SIGN (orange) and E-cadherin (red) and nuclei (Hoechst 33258, blue). Slides were read under a confocal microscope. Original magnification, × 400. Scale bar = 20 μm.



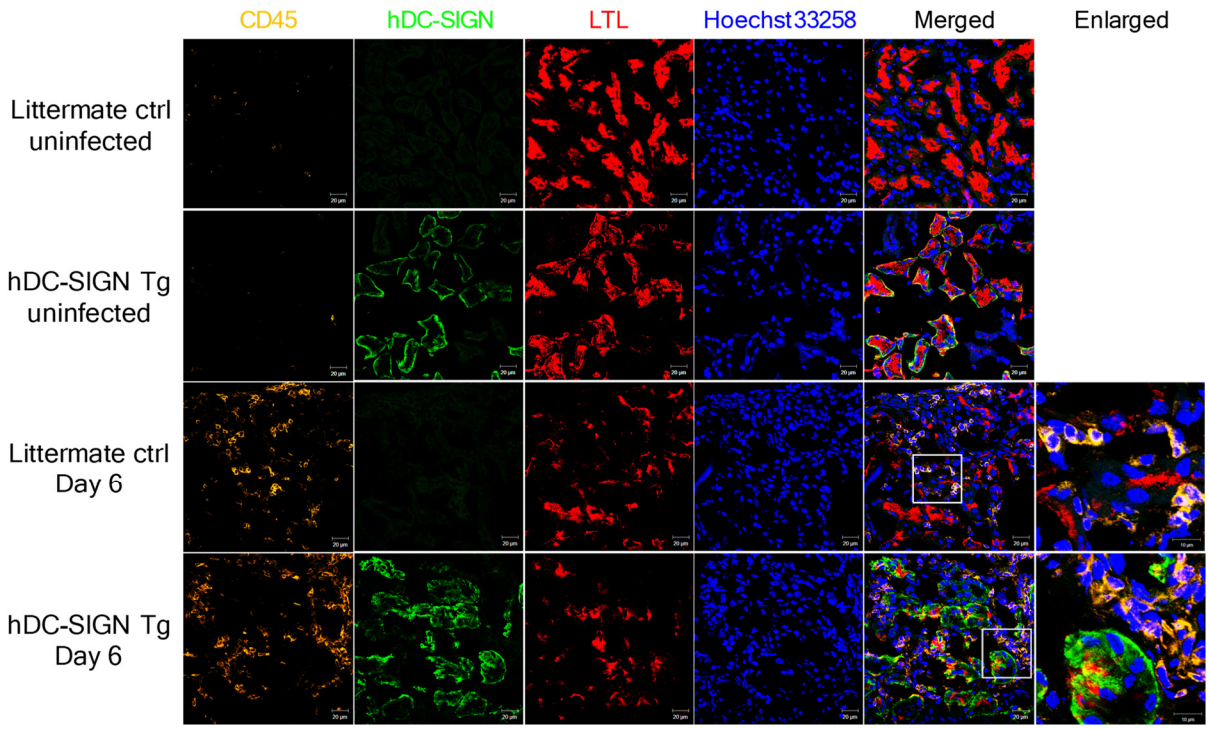
Suppl Figure 2. hDC-SIGN is expressed in the marginal zone of the spleen of hDC-SIGN transgenic mice. Spleens from naïve hDC-SIGN transgenic and littermate control mice were collected after perfusion with PBS. Paraffin-embedded spleen sections were stained for human DC-SIGN (brown). Slides were counterstained with hematoxylin (blue). Original magnification, × 200. Scale bar = 100 µm.



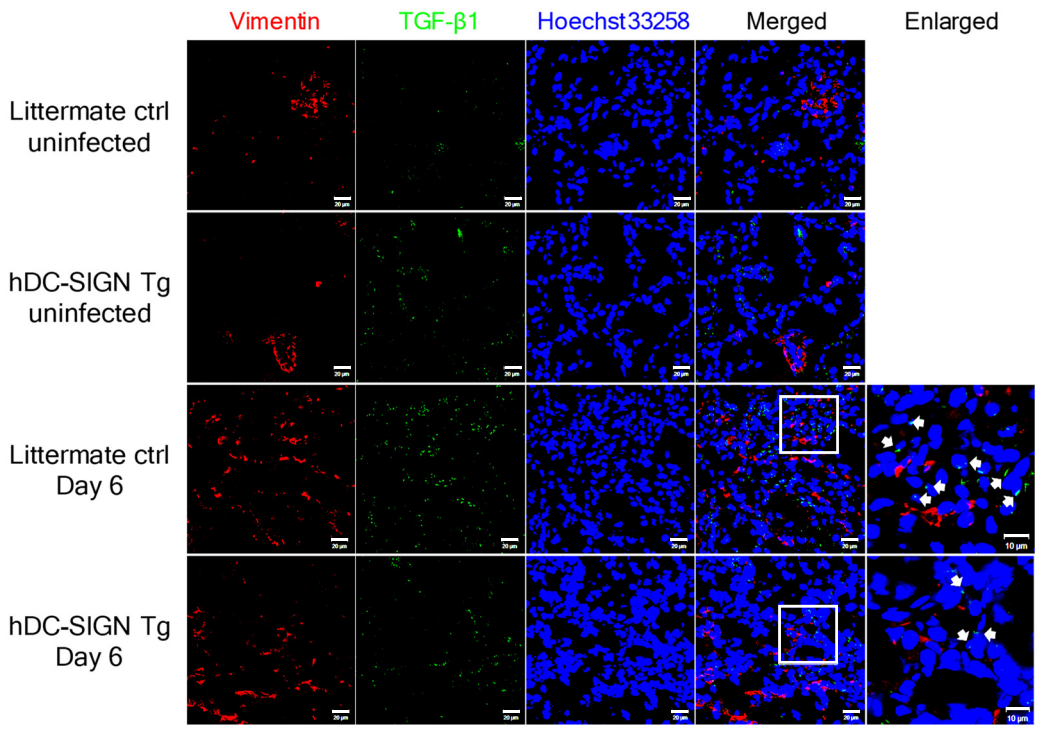
Suppl Figure 3. hDC-SIGN transgenic expression does not induce SIGNR1 expression in the kidney. After perfusion with PBS, kidneys were collected from naïve hDC-SIGN transgenic and littermate control mice. Kidney homogenates were subjected to Western blot analysis for SIGNR1 expression. β-actin was used as an internal control.



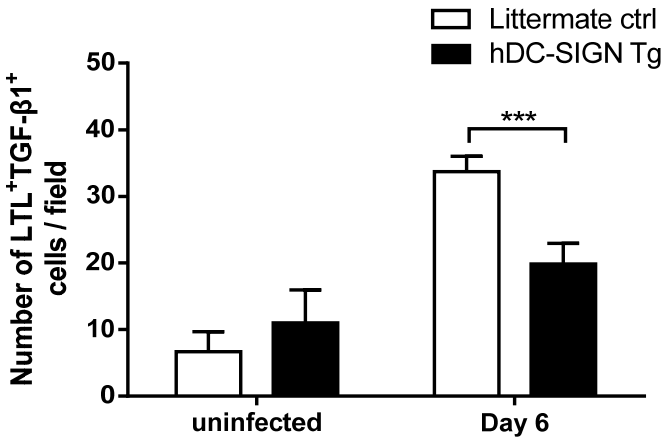
Suppl Figure 4. *C. albicans* infection does not change the intensity of hDC-SIGN expression in LTL$^+$ cells in the tubule. hDC-SIGN transgenic and littermate control mice were infected with 1×10^5 of *C. albicans* intravenously. On day 6 after infection, mice were perfused with PBS and kidneys were collected. **(A)** Paraffin-embedded kidney sections were stained for hDC-SIGN expression (brown). Slides were counterstained with hematoxylin (blue). Original magnification, × 200. Scale bar = 100 µm. **(B)** Cryosections of kidneys were stained for hDC-SIGN (orange), DBA (red), LTL (green), and nuclei (Hoechst 33258, blue). DBA and LTL mark distal and proximal tubule in renal cortex, respectively. White arrows point to hDC-SIGN$^+$ cells. Slides were read under a confocal microscope. Original magnification, × 400. Scale bar = 20 µm. Images of uninfected hDC-SIGN transgenic and littermate control mice in (A) are from Figure 1C.



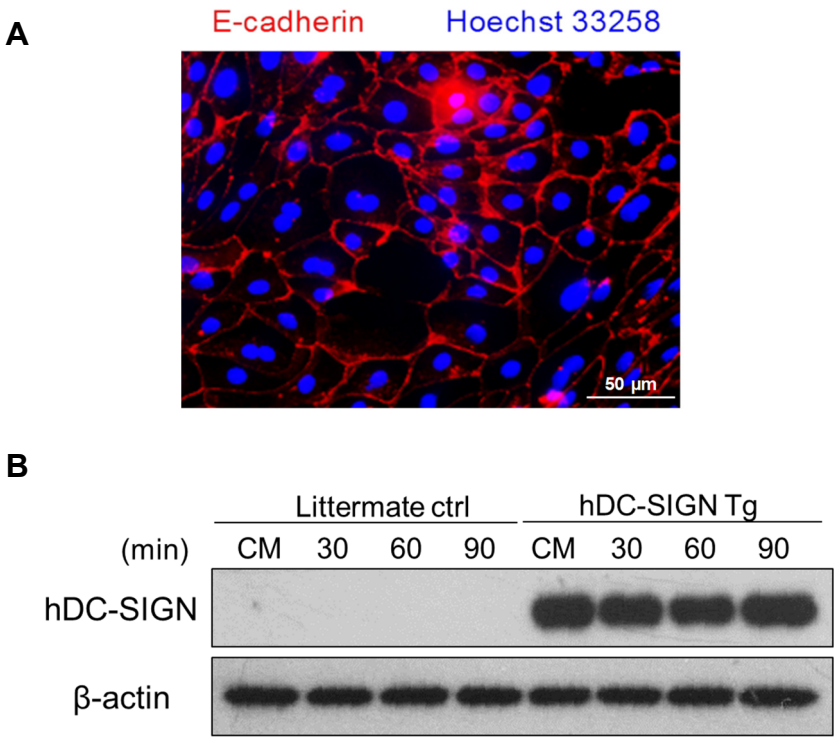
Suppl Figure 5. CD45$^+$ infiltrating cells in *C. albicans*-infected transgenic mice are hDC-SIGN-negative. hDC-SIGN transgenic and littermate control mice were infected with 1×10^5 of *C. albicans* intravenously. On day 6 after infection, mice were perfused with PBS and kidneys were collected. Cryosections of kidneys were stained for CD45 (orange), hDC-SIGN (green) and LTL (red). Hoechst 33258 was used as a nuclear counterstain (blue). Boxed areas are shown at higher magnification (enlarged) next to the corresponding image. Slides were read under a confocal microscope. Original magnification, × 400. Scale bar = 20 µm (scale bar of enlarged graph = 10 µm).



Suppl Figure 6. Mesenchymal cells are not the source of renal TGF-β1 in *C. albicans* infection. hDC-SIGN transgenic and littermate control mice were infected with 1×10^5 of *C. albicans* intravenously. On day 6 after infection, mice were perfused with PBS and kidneys were collected. Cryosections of kidneys were stained for vimentin (red), TGF-β1 (green), and nuclei (Hoechst 33258, blue). Boxed areas are shown at higher magnification (enlarged) next to the corresponding image. Slides were read under a confocal microscope. Original magnification, × 400. Scale bar = 20 µm (scale bar of enlarged graph = 10 µm). White arrows point to TGF-β1$^+$ cells.



Suppl Figure 7. The numbers of LTL$^+$TGF-β1$^+$ cells in kidneys of hDC-SIGN transgenic and littermate control mice before and after infection. Kidneys from uninfected and *C. albicans*-infected hDC-SIGN transgenic (day 6 after infection) and littermate control mice were collected after whole body perfusion. Cryosections were stained for LTL, TGF-β1 and nuclei. LTL$^+$TGF-β1$^+$ cells were counted in total of 20 fields in 2 kidney sections per group. The bars represent the mean ± SEM. Data were analyzed by two-way ANOVA followed by Tukey post-hoc test. *** $p < 0.001$.



Suppl Figure 8. *C. albicans* infection does not change the expression of hDC-SIGN in primary renal epithelial cells. (A) Primary renal tubular epithelial cells were isolated from wild type mice. Cells were seeded on cover slides, and stained for E-cadherin (red). Nuclei (blue) were stained with Hoechst 33258. Slides were read under a fluorescence microscope. Original magnification, × 400. Scale bar = 50 µm. **(B)** Primary renal tubular epithelial cells were isolated from hDC-SIGN transgenic and littermate control mice. Cells were stimulated with *C. albicans* for 0, 30, 60 and 90 min. Levels of hDC-SIGN expression in the cell lysates were analyzed by Western blotting. β-actin was used as an internal control.

Supplementary Methods

RT-PCR

Total RNA was extracted from different tissues in RNAzol RT (Molecular Research Center). cDNA was reversely transcribed in reaction mixture containing extracted RNA, first-strand buffer, DTT, dNTP, random primers (Protech), SuperScript III reverse transcriptase (Invitrogen) and RNaseOUT recombinant ribonuclease inhibitor (Invitrogen) in RNase-free H_2O. cDNA was amplified in Veriti 96-Well Thermal Cycler (Applied Biosystems) with total volume of 20 μl per reaction in buffer containing Platinum Taq DNA Polymerase (Thermo Fisher Scientific). Primers for human *DCSIGN* gene are 5'-CTGCAACTCCTCTCCTTCAC-3' (forward) and 5'-TCGTTCCAGCCATTGCCACT-3' (reverse) and for mouse *Gapdh* gene are 5'-ACCACAGTCCATGCCATCAC-3' (forward) and 5'-TCCACCACCCTGTTGCTGTA-3' (reverse). The amplicon sizes of human *DCSIGN* gene and mouse *Gapdh* gene are 965 bp and 452 bp, respectively.

Western blotting. PhosphoSafe Extraction Reagent (Millipore) were used to homogenize perfused kidneys and lyse primary renal tubular epithelial cells. Whole cell lysates and kidney homogenates were subjected to electrophoresis at 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE), and transferred to 0.45 μm PVDF membrane. The membrane was blocked with 5% non-fat milk diluted in TBST and incubated in TBST containing rabbit anti-human DC-SIGN (1:1000) (abcam), rat anti-mouse SIGNR1 (1:200) (BioLegend) or rabbit anti-mouse β-actin antibody (1:10000) (GeneTex) at 4℃ overnight, followed by addition of HRP-conjugated secondary antibody (1:20000) (GeneTex). The blot was developed by chemiluminescence using ECL solution (Millipore).