ONLINE APPENDIX – SUPPLEMENTARY FIGURES

Figure S1. Genotyping of Smad WT and KO mice. Note that while a Smad7 wild-type (WT) mouse is identified by 1.7 k bp, a mouse deficient for Smad7 is identified by a knockout (KO) band at 1.1k bp.

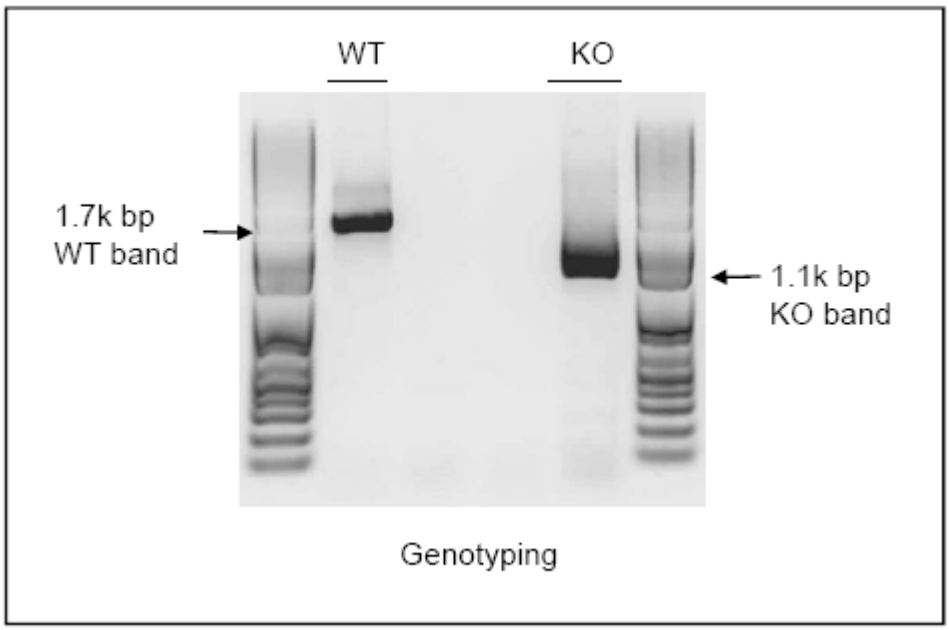

Figure S2. Fibronectin is enhanced in diabetic Smad7 KO mice. Western blot (A, B), immunohistochemistry (C, D) and real-time PCR (E) show a significant increase in fibroncetin (Fn) in diabetic Smad7 KO mice. Data represent the mean ± SEM for groups of 8 animals. *P<0.05, **P<0.01, ***P<0.001 versus normal; #P<0.05, ##P<0.01, ###P<0.001 versus WT DM mice. Magnification: x 400

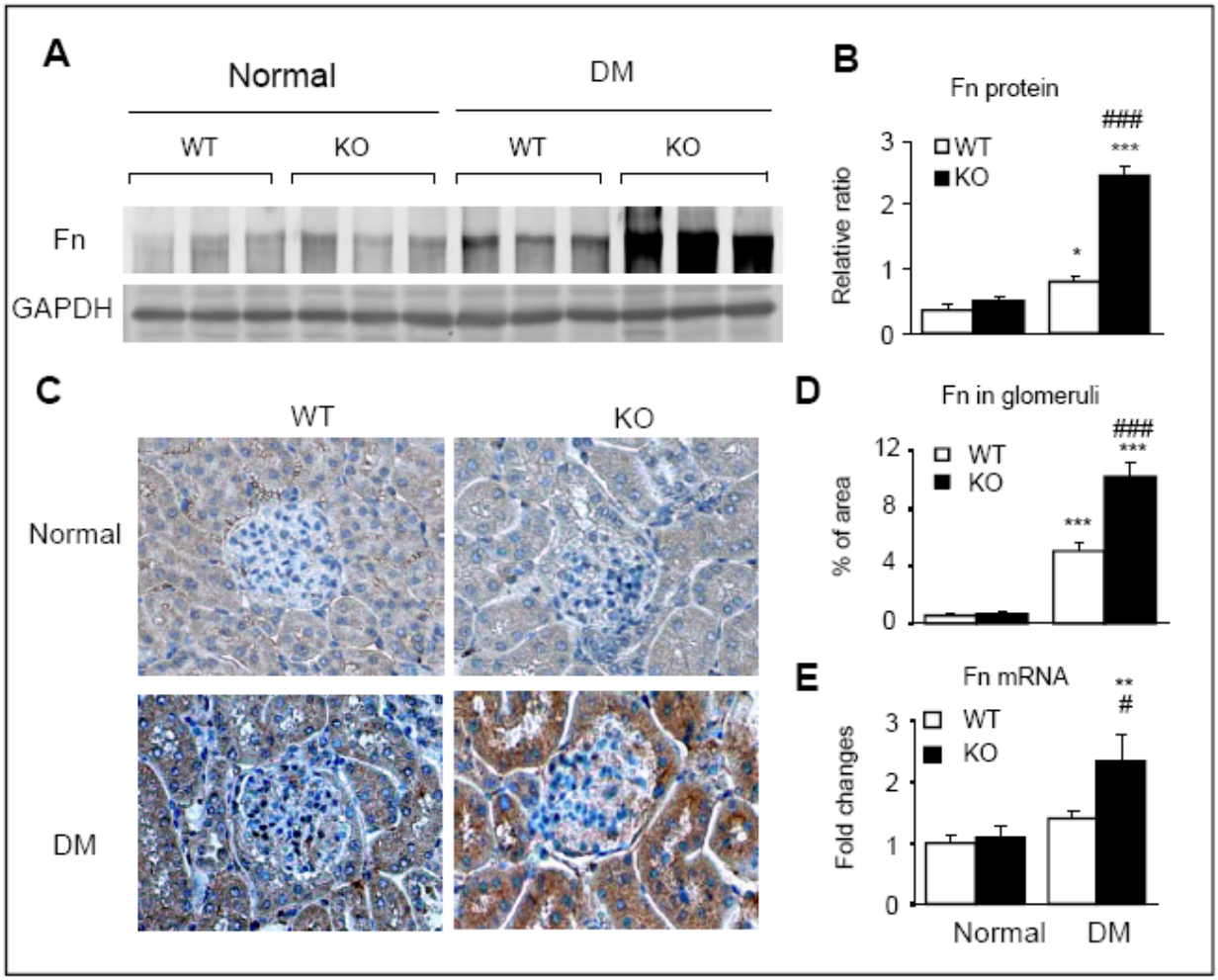

Figure S3. Renal inflammation is enhanced in diabetic Smad7 KO mice. **A-D**. ICAM-1 expression. **E-H**. MCP-1 expression. Results show that compared to the WT mice, Smad7 KO mice develop more severe renal inflammation such as enhanced renal ICAM-1 and MCP-1 expression as demonstrated in glomeruli (A,B, E, F) and in renal cortex (A,C, E,G) by immunohistochemistry and by real-time PCR (D,H). Data represent the mean ± SEM for groups of 8 animals. *P<0.05, **P<0.01, ***P<0.001 versus normal; #P<0.05, ##P<0.01, ###P<0.001 versus WT DM mice. Magnification: x 400

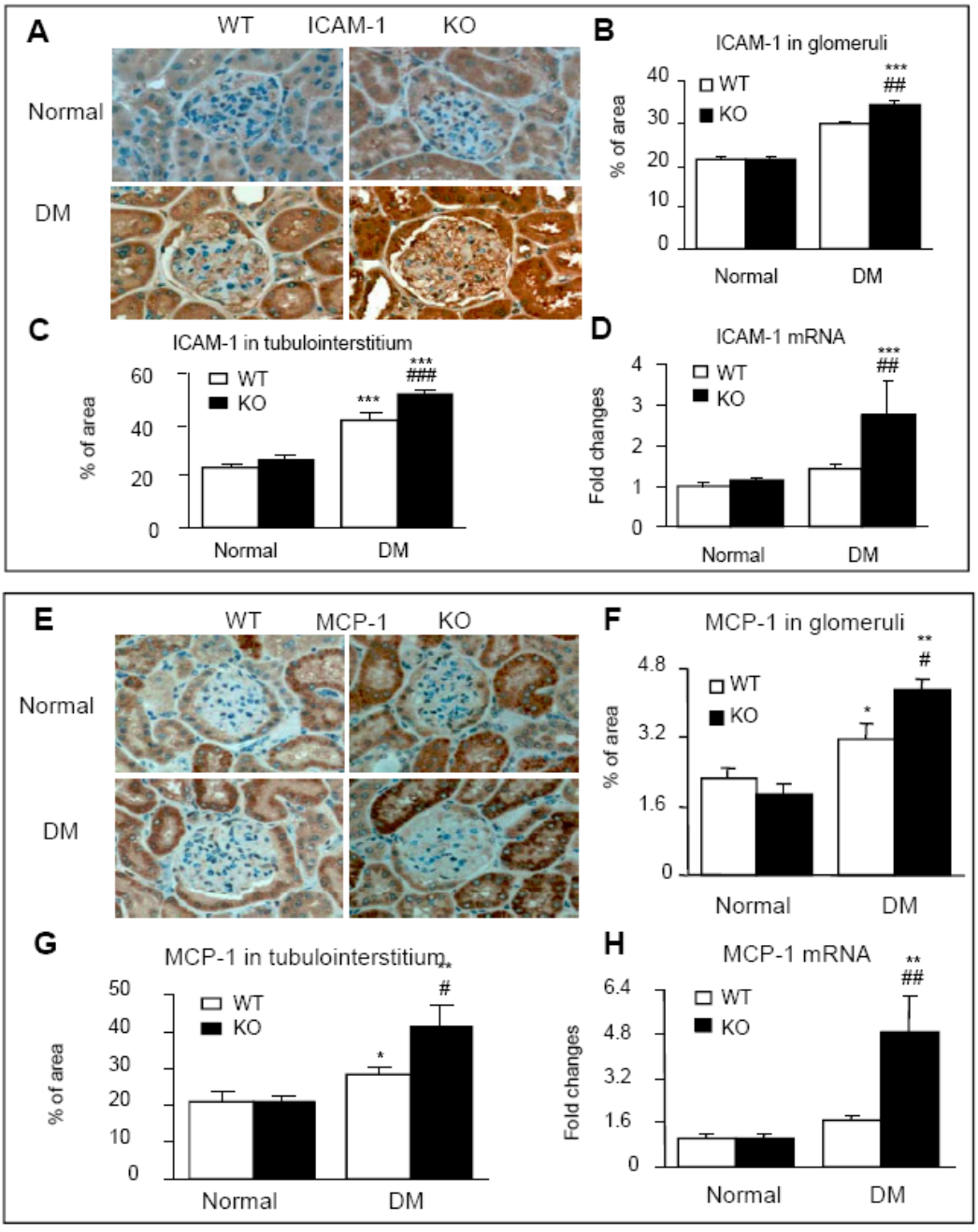

Figure S4. Smad7 gene transfection rate and transgene expression in diabetic rat kidneys. A,C.E,G. Smad7 gene transfection rate determined by monoclonal antibody against Flag-M2 protein (flag-tagged Smad7). **B, D, F, H.** Total Smad7 protein expression within the kidney determined by anti-Smad7 polyclonal antibody. Results show that compared to a normal kidney where endogenous Smad7 expression is high in both glomerulus and tubulointerstitium (B), diabetic kidney treated with control plasmids exhibits a loss of renal Smad7(D). In contrast, ultrasound-mediated Smad7 gene transfer to the diabetic left kidney (LK) results in higher Smad7 transgene expression as demonstrated by numerous flag-M2+ cells and high levels of renal Smad7 expression in both glomerulus and tubulointerstitium (E, F), while only a few flag-M2+ cells and moderate levels of Smad7 were found in the opposite right kidney (G, H). Data represent groups of 6 rats. Magnifications x400.

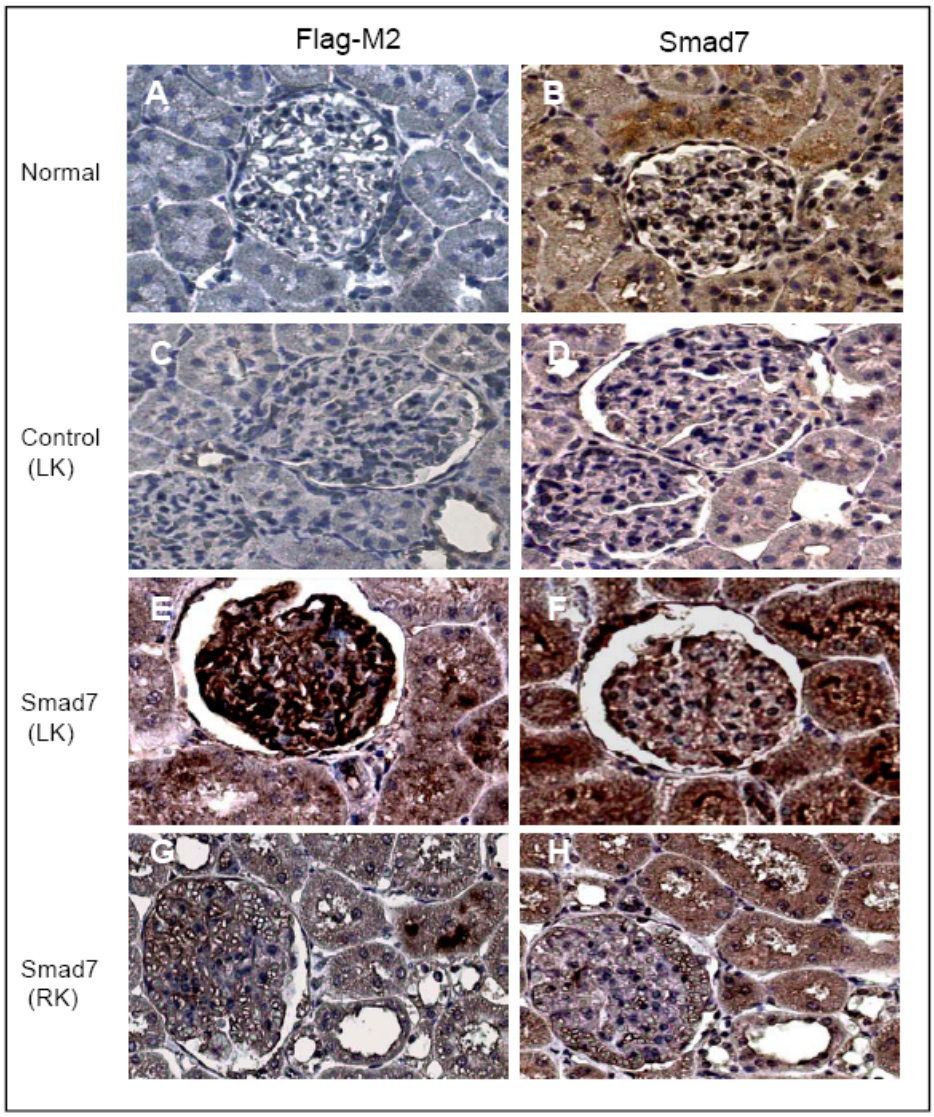

Figure S5. Smad7gene therapy inhibits renal fibrosis in diabetic rats. **A-C**. Collagen III expression. **D-G**. Fibronectin (Fn) expression. Results show that compared to diabetic rats (DM) and diabetic rats with control vector treatment (VC), Smad7 gene transfer significantly inhibits renal collagen IIII and Fn expression as demonstrated by immunohistochemistry (A, B and D, E) and real-time PCR at the mRNA levels (C and F). Data represent the mean ± SEM for groups of 6 animals. *p<0.05, **p<0.01, ***p<0.001 versus normal; #p<0.05, ###p<0.001 versus DM and VC mice. Magnification: x 400 (A,D)

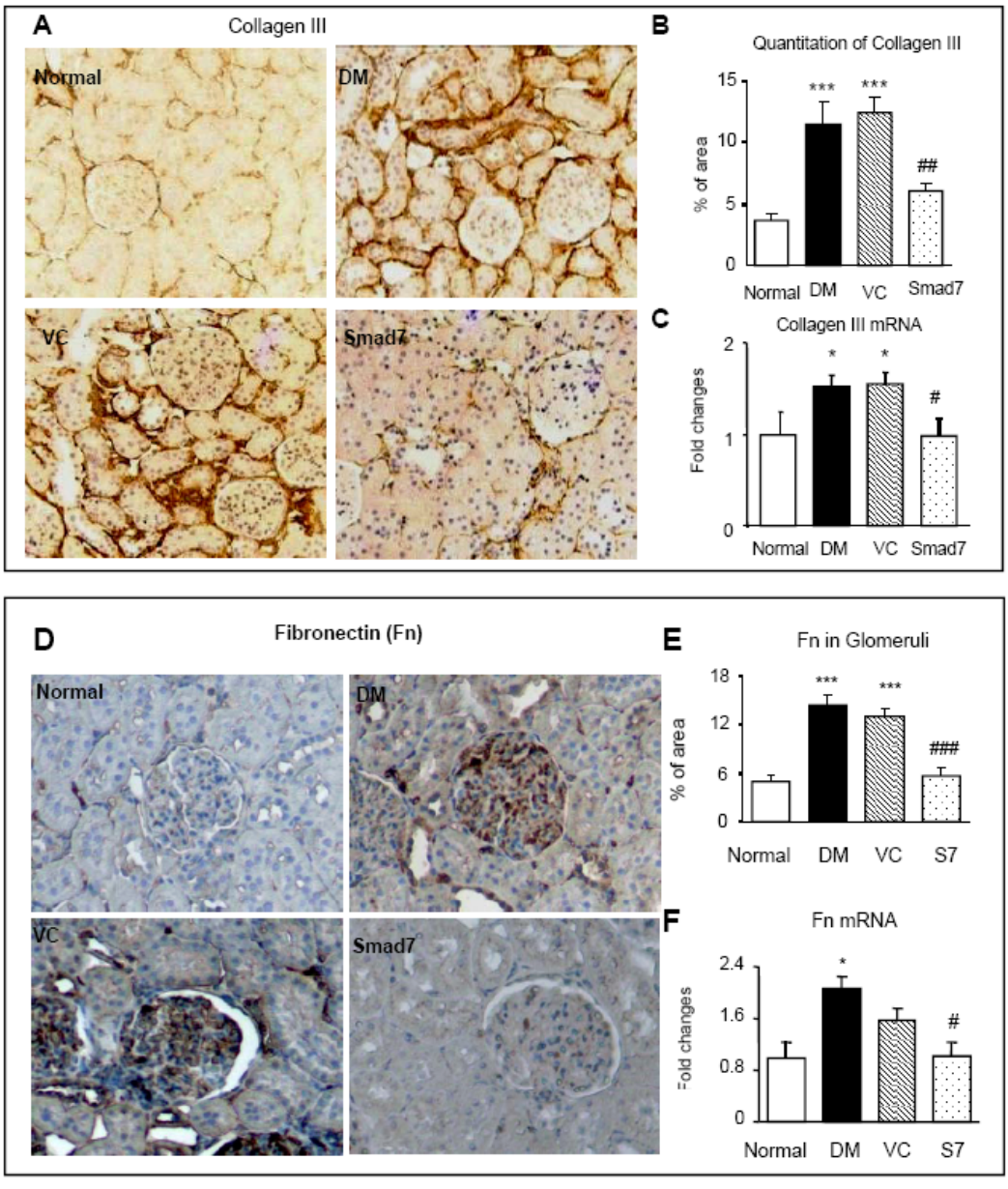

Figure S6. Smad7gene therapy inhibits renal inflsammation in diabetic rats. **A-C**. ICAM-1 expression. **D-G**. MCP-1 expression. Results show that compared to diabetic rats (DM) and diabetic rats with control vector treatment (VC), Smad7 gene transfer significantly inhibits renal ICAM-1 and MCP-1 expression in glomeruli (A,B and E,F) and tubulointerstitium (A,C and E,G) as demonstrated by immunohistochemistry and by real-time PCR (D and H). Data represent the mean ± SEM for groups of 6 animals. *p<0.05, ***p<0.001 versus normal; #p<0.05, ##p<0.01, ###p<0.001 versus DM and VC mice. Magnification: x 400 (A,D).

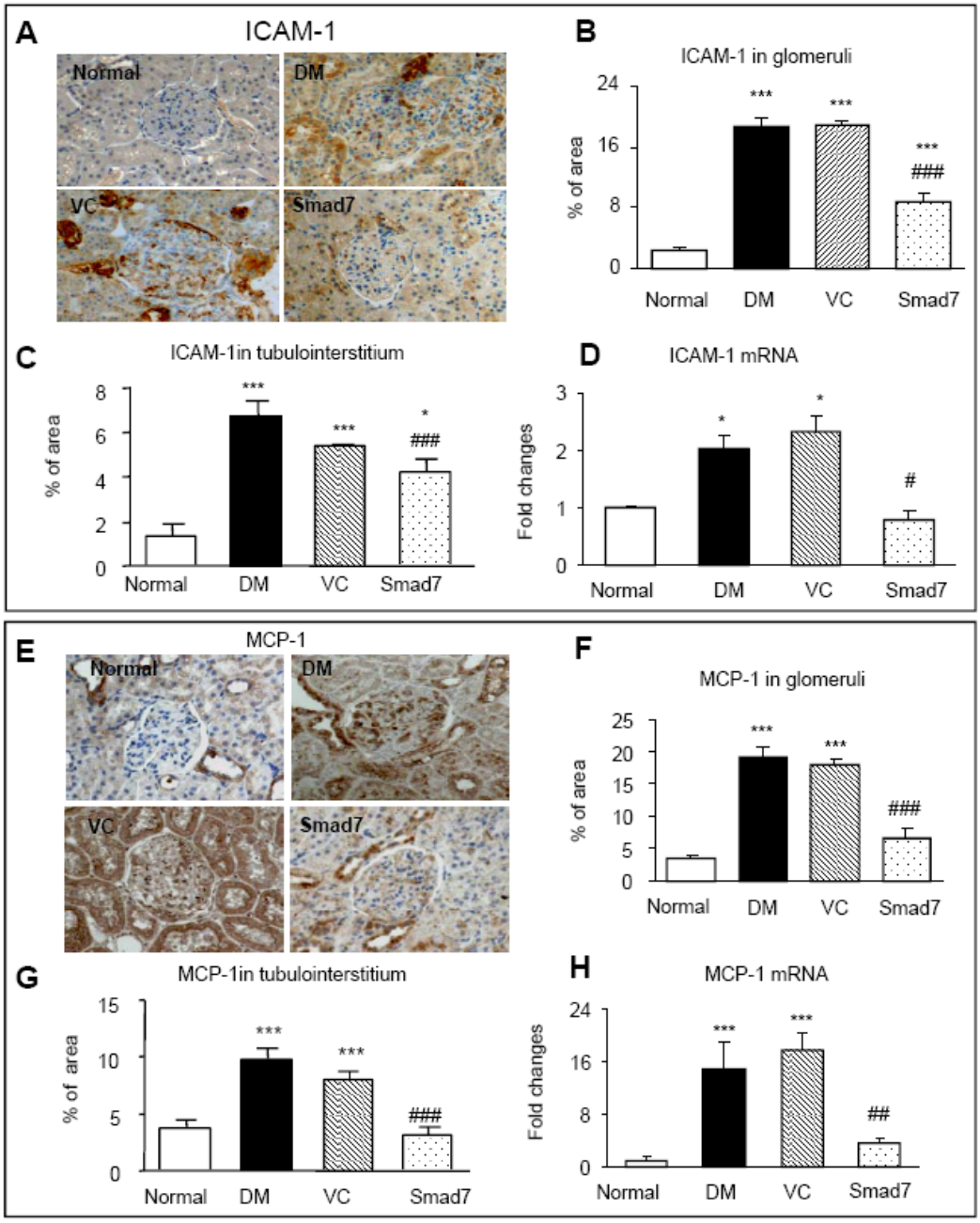

Figure S7. Overexpression of renal Smad7 attenuates Macrophages infiltration in diabetic rats. A.Immunohistochemical staining with the ED1 monoclonal antibody. (B) quantitative analysis in both glomerulus and tubulointerstitium. Data represent the mean ± SEM for groups of 6 animals. **p<0.01, ***p<0.001 versus normal; ##p<0.01, ###p<0.001 versus DM and VC mice. Magnification: x 400 (A).