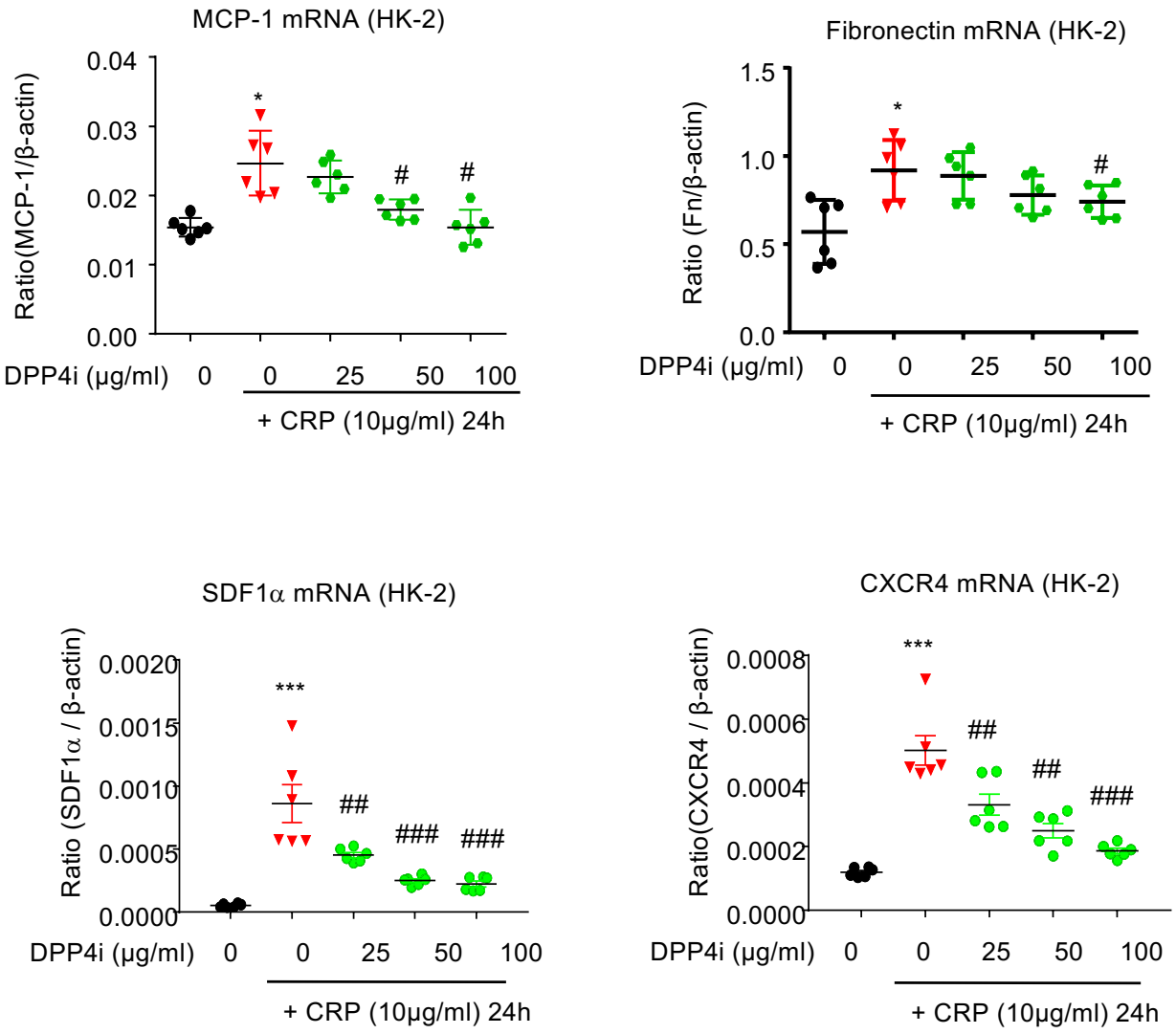
YMTHE, Volume *29*

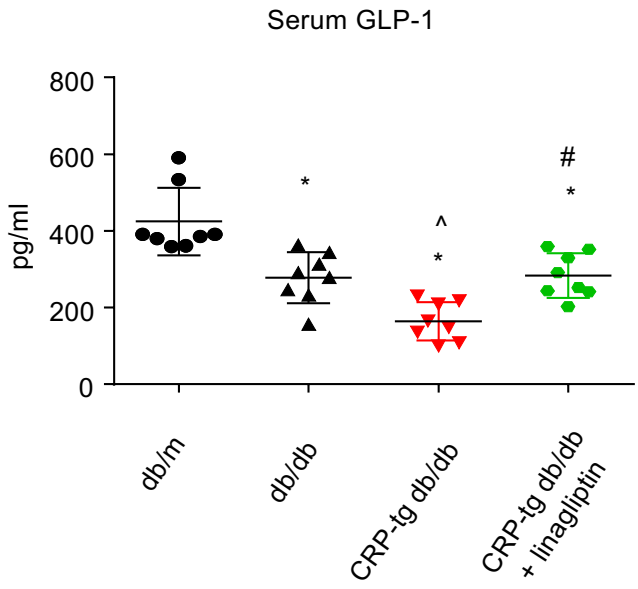
Supplemental Information

DPP4/CD32b/NF-κB Circuit: A Novel Druggable Target for Inhibiting CRP-Driven Diabetic Nephropathy

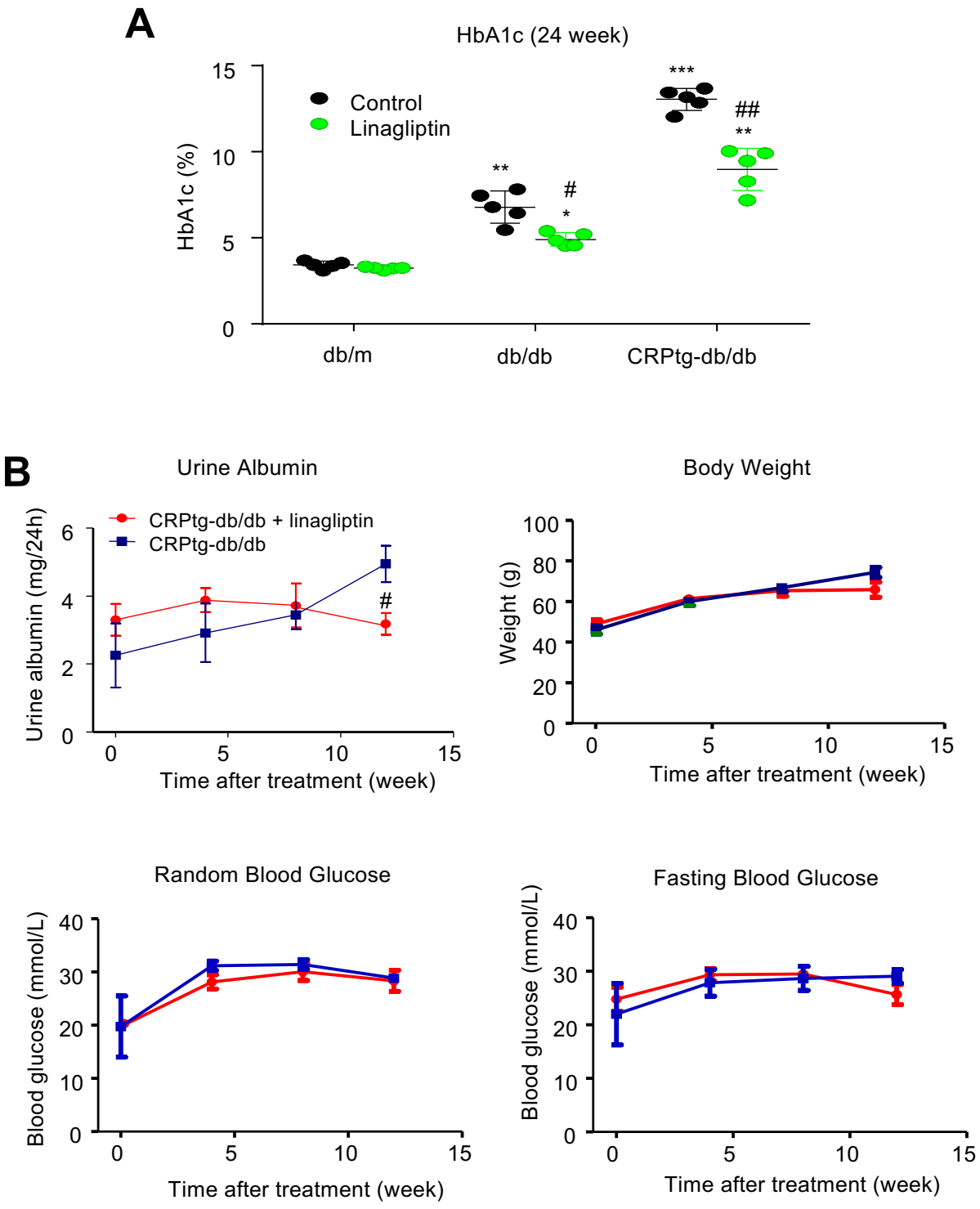
Patrick Ming-Kuen Tang, Ying-Ying Zhang, Jessica Shuk-Chun Hung, Jeff Yat-Fai Chung, Xiao-Ru Huang, Ka-Fai To, and Hui-Yao Lan



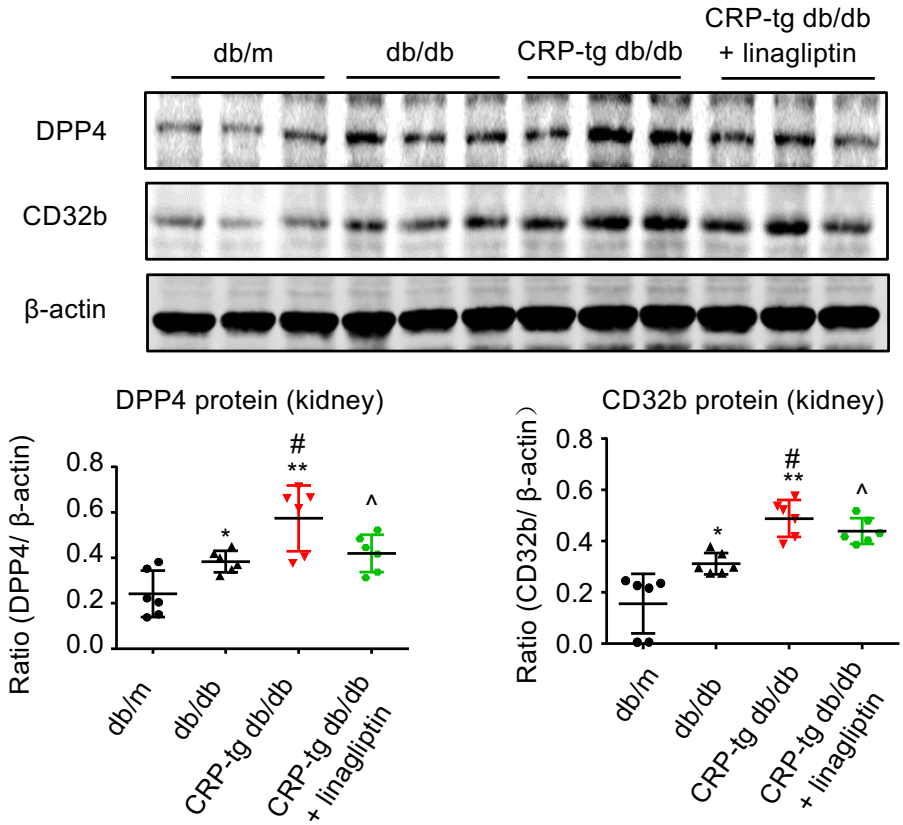
Supplementary Figure S1. Real-time PCR detects that inhibition of DPP4 with specific inhibitor Linagliptin effectively suppresses the CRP-induced expression of renal inflammation (MCP-1) and fibrosis (fibronectin) markers and activation of SDF-1α/CXCR4 pathway at mRNA levels in HK-2 cells at 24h in vitro. Data represent results from 3 independent experiments. *p<0.05 vs control; #p<0.05 vs CRP-stimulated control.



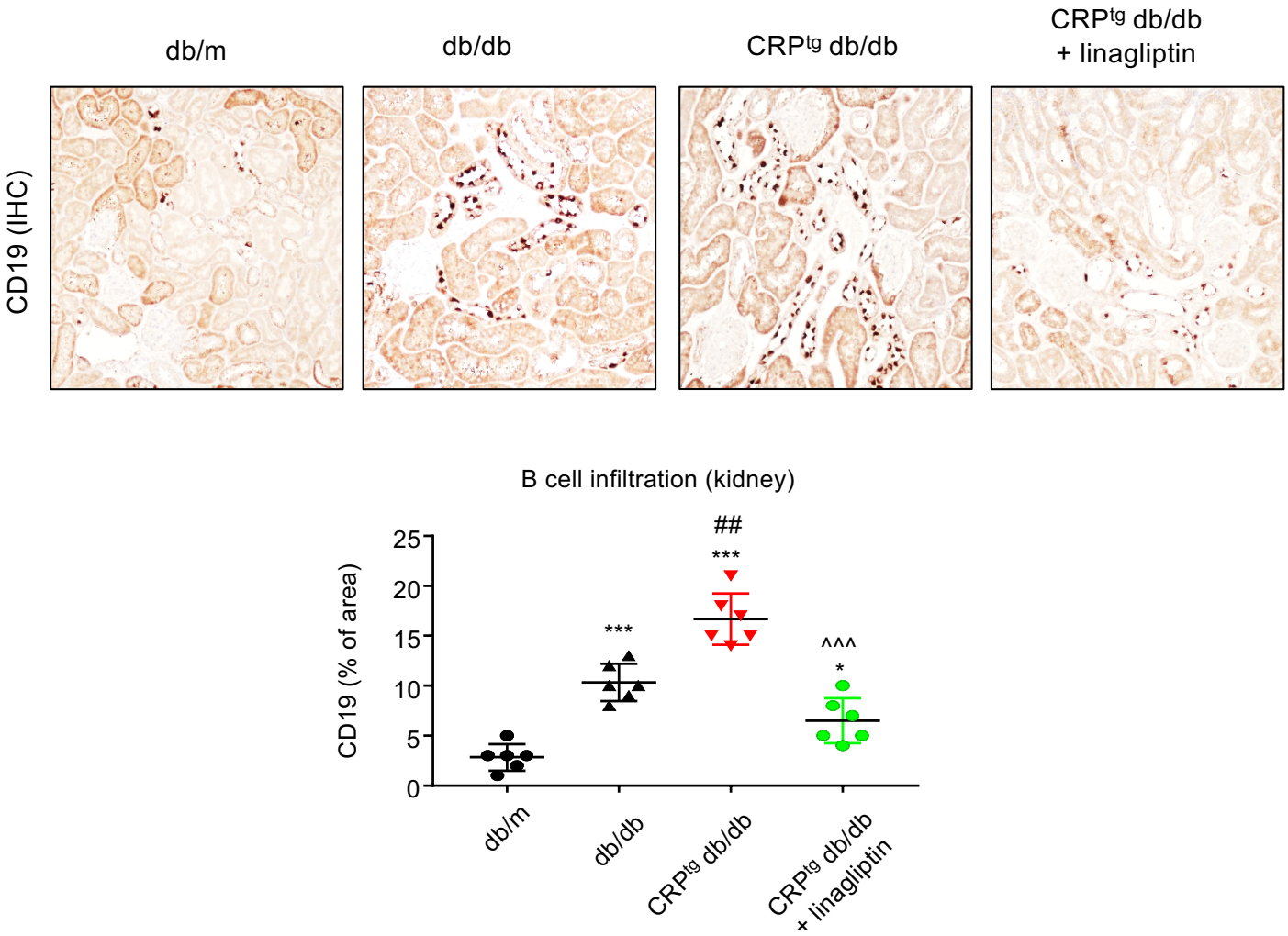
Supplementary Figure S2. ELISA detects that human CRP expression suppresses serum level of glucagon-like peptide 1 (GLP-1) in CRP-tg db/db mice compared to the parental db/db mice, which is effectively restored by the 12-week-treatment with linagliptin (3mg/kg/day orally) in vivo. Each bar represents the mean ± SEM for groups of six mice. *$p < 0.05$ vs db/m mice; ^$p < 0.05$ vs db/db mice; #$p<0.05$ vs CRP-tg db/db mice.



Supplementary Figure S3. ELISA results of **(A)** serum HbA1c of mice at 24-week-old and **(B)** the levels of urine albumin, body weight, random and fasting blood glucose of the CRPtg-db/db mice during linagliptin treatment. Data represents the mean ± SEM for five mice per group. *P<0.05, **P<0.01, *** P < 0.001 versus db/m mice; #P < 0.05 versus untreated mice.

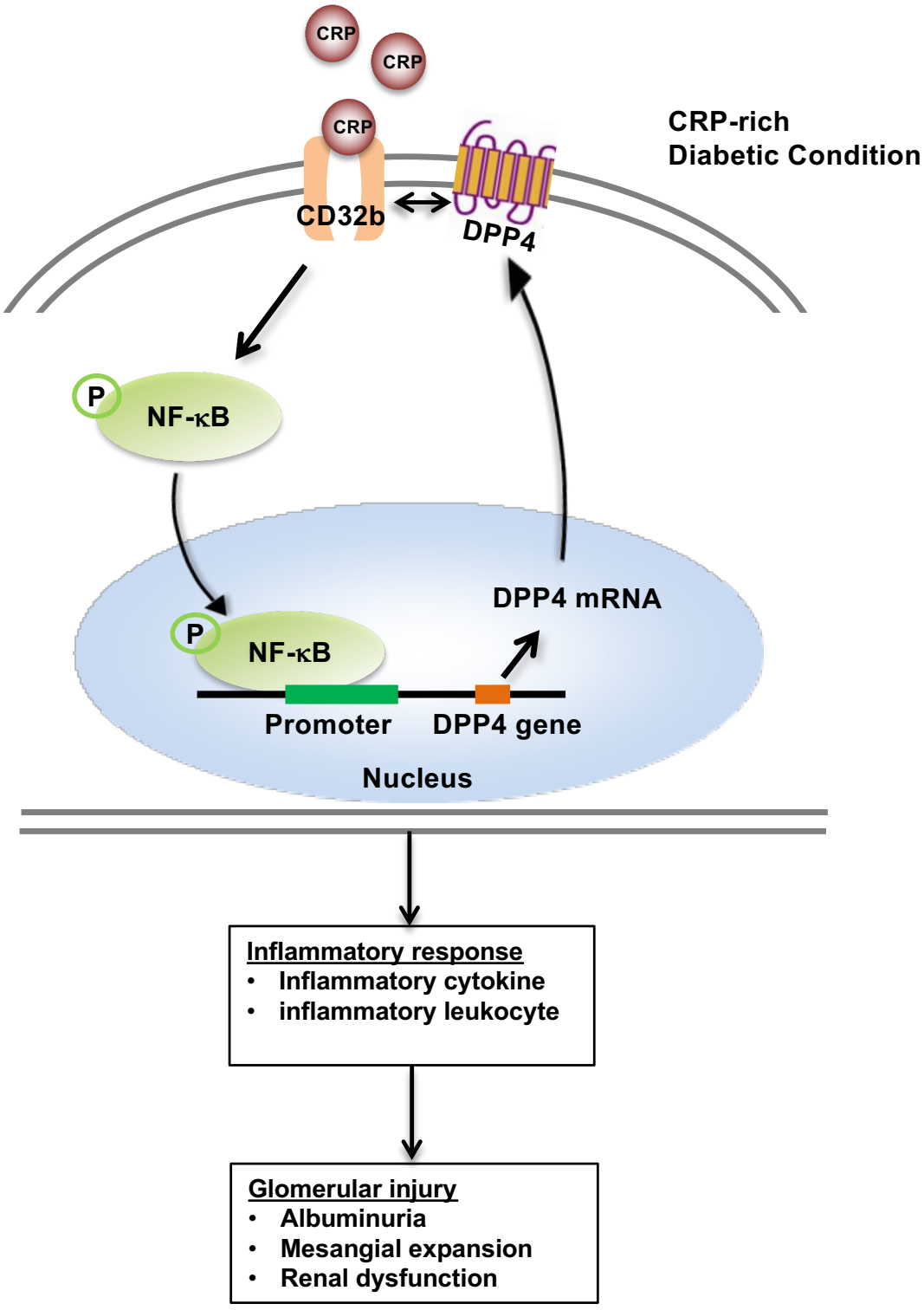


Supplementary Figure S4. Western blot shows that targeting of either CD32b, NF-κB, and DPP4 also inhibits the CRP-driven DPP4 expression in diabetic injured kidney of CRP-tg db/db mice in vivo. Each bar represents the mean ± SEM for groups of six mice. *$p < 0.05$, **$p<0.01$ vs db/m mice; #$p < 0.05$ vs db/db mice; ^$p<0.05$ vs CRP-tg db/db mice.



Supplementary Figure S5. The 12-week-treatment with linagliptin significantly suppresses the CRP-induced B cell infiltration in the diabetic kidney of CRPtg db/db mice at 24-week-old showing by the immunohistochemistry staining of CD19 (X400). Data represents the mean ± SEM for six mice per group. *P<0.05, ***P < 0.001 versus db/m mice; ##P<0.01 versus db/db mice; ^^^P < 0.001 versus untreated CRPtg db/db mice.

Schematic Graph



Supplementary Figure S6. Schematic diagram of CRP-driven DPP4/CD32b/NF-κB signaling circuit in diabetic kidney. Under diabetic conditions, CRP binds to CD32b triggers the activation and translocation of NF-κB into nucleus which then binds on the promoter region of DPP4 gene and enhances DPP4 expression at transcription level. The induced DPP4 will then dimerizes with CD32b on the membrane, which further promote the CD32b-mediated phosphorylation of NF-κB therefore forming a signaling circuit for promoting inflammatory response during CRP-driven T2DN.