2013 ICDM
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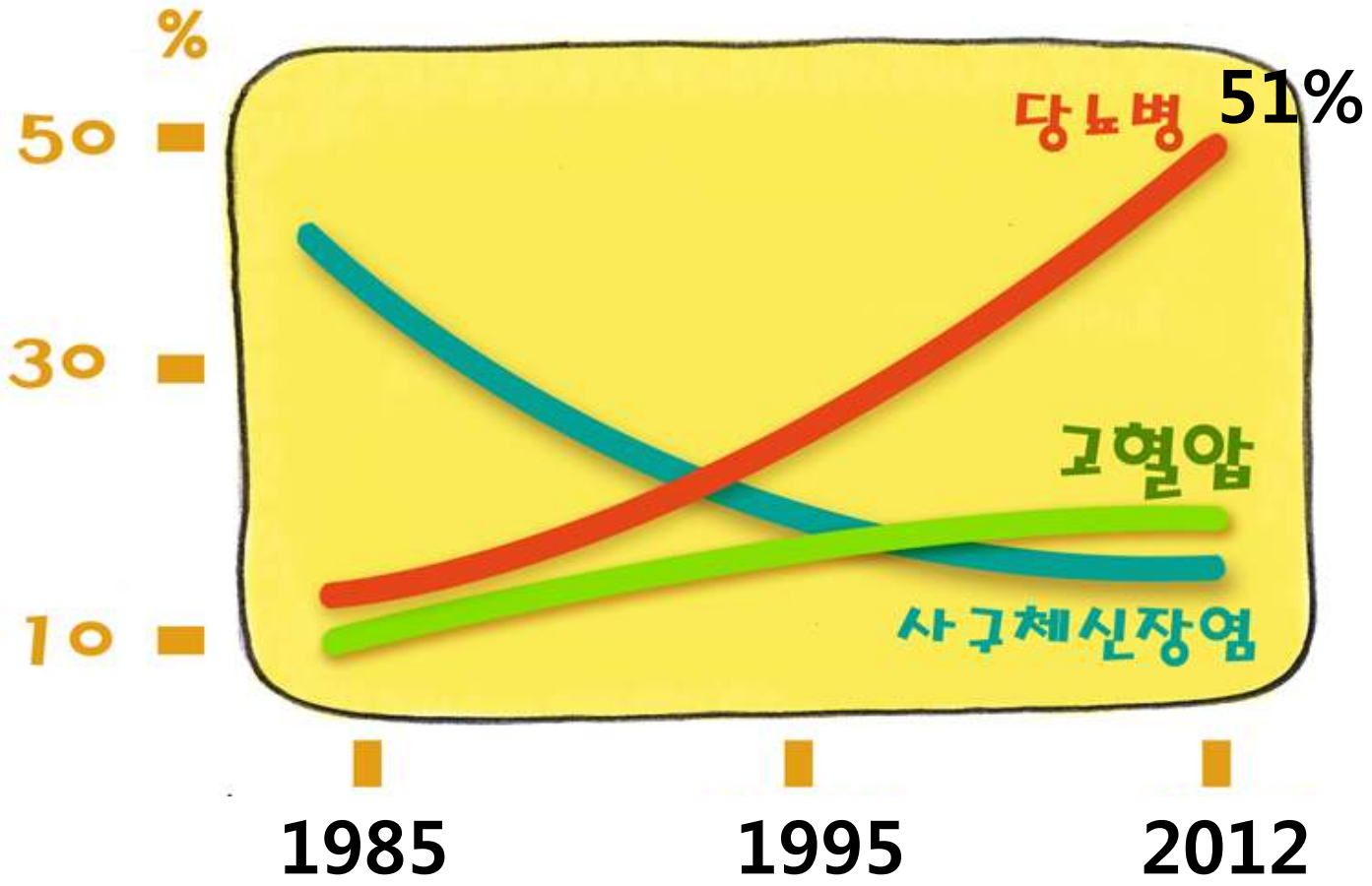
송경희 박제현박사

Direct effect of fractalkine on ECM accumulation in diabetic kidneys

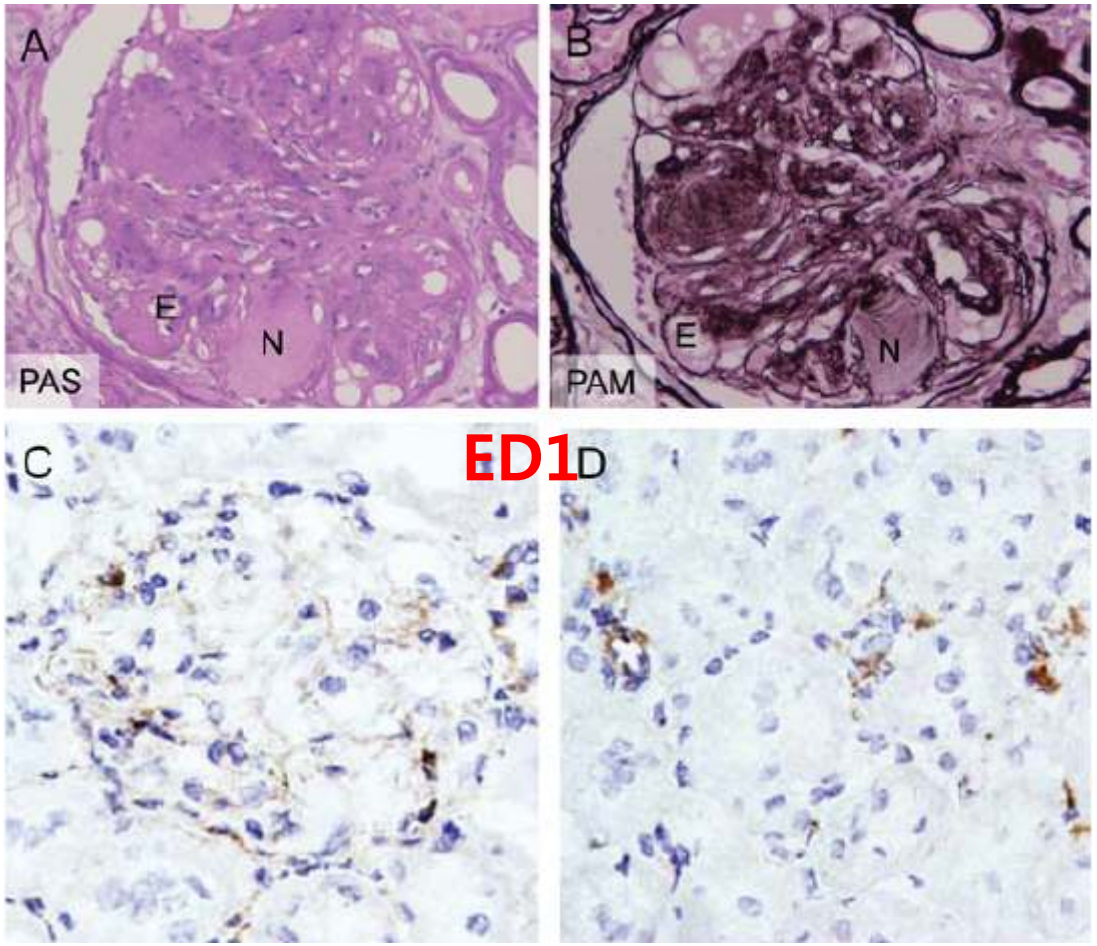
Hunjoo Ha

Department of Pharmaceutical Science, Graduate School
College of Pharmacy, Ewha Womans University, Seoul, Korea

The most common cause of ESRD: diabetes

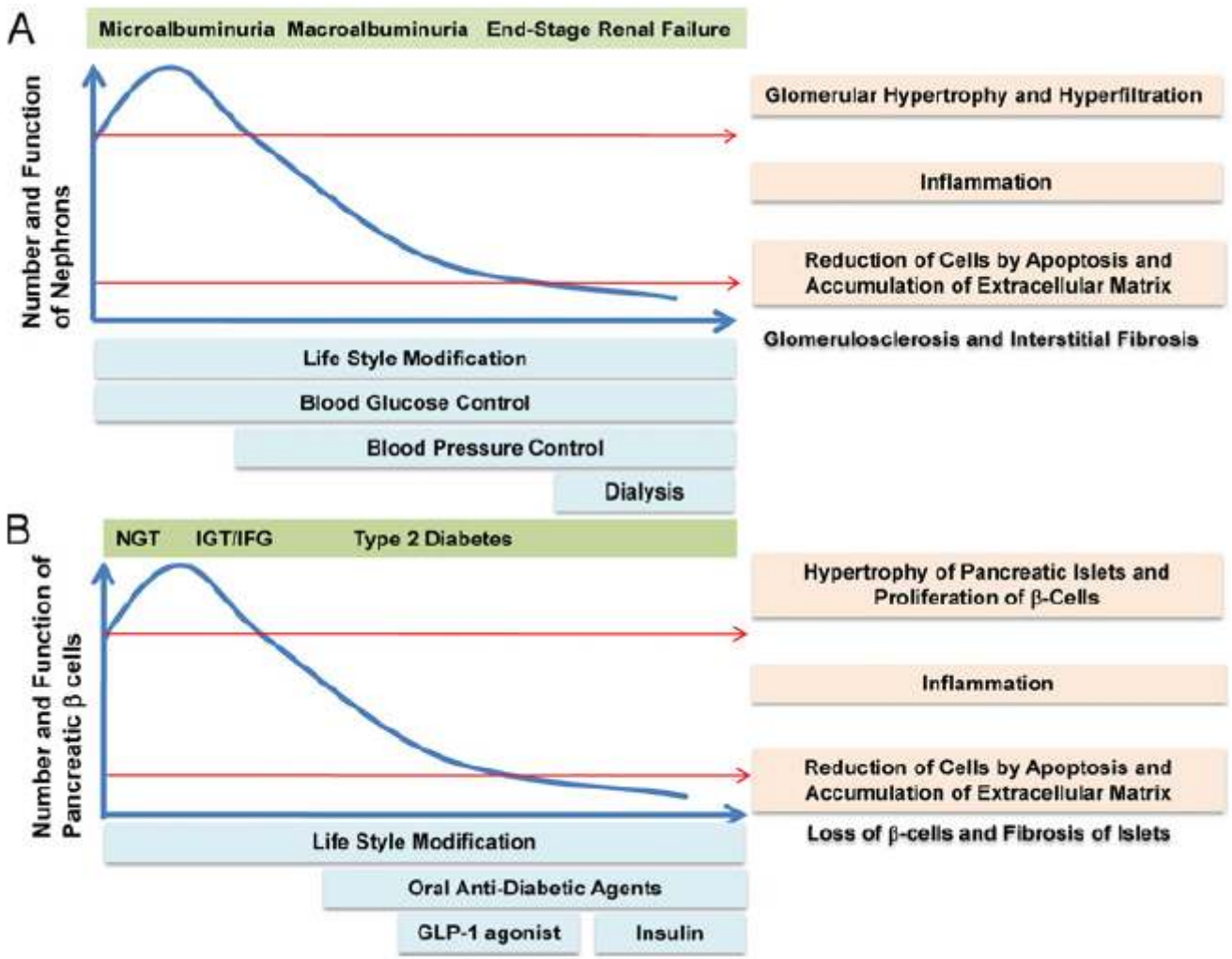


Pathological changes in diabetic nephropathy



Wada J & Makino H. Diabetes 2006

Similarity of the natural history of Type 2 diabetes and diabetic nephropathy



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Inflammatory molecules in diabetic nephropathy

Category	Molecule
Transcription factors	NF-κB
Pro-inflammatory cytokines and signalling molecules	IL-6
	IL-18
	IL-1
	TNF
	JAK2 and STAT-1, -3 and -5
Chemokines	CCL2 (MCP-1) and CCR2
	CXCL12 (stromal-cell-derived factor-1)
	CX3CL1 (fractalkine) and CX3CR1
Adhesion molecules	Intercellular adhesion molecule 1 (ICAM1)
	Vascular cell adhesion protein 1 (VCAM1)
	E-selectin (SELE)
TLRs	TLR2
	TLR4
Adipokines	Adiponectin
	Leptin
Nuclear receptors	VDR
	NR1H4 (FXR)
	PPARα
	PPARγ
	PPARδ

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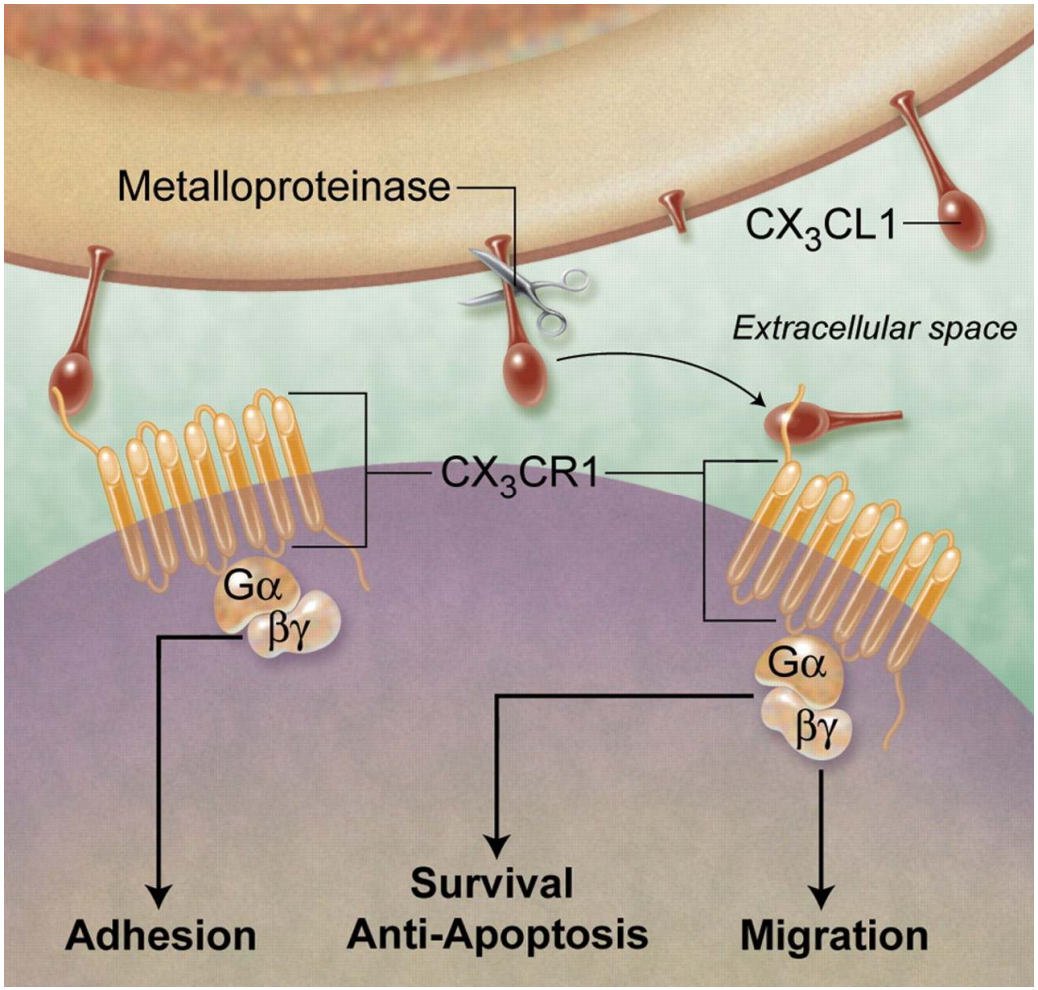
Fractalkine (FKN: CX3CL1) and CX3CR1



- unique member of CX3C chemokine
- 2 forms: soluble, membrane-bound

FKN/CX3CR1 in chronic kidney disease

- Fractalkine expression is upregulated in human crescentic glomerulonephritis.

  Furuichi K et al. Nephron. 87:314, 2001

- CX3CR1 mediates renal interstitial fibrosis in ischemia-reperfusion injury.

  Furuichi K et al. Am J Pathol. 169:372, 2006

- Fractalkine/CX3CR1 mediate hypertensive interstitial fibrosis in the kidney.

  Shimizu K et al. Hypertens Res. 34:747, 2011

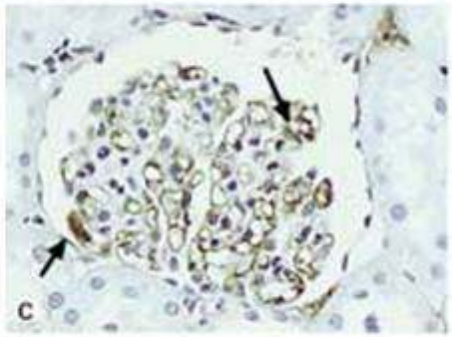
FKN/CX3CR1 in diabetic kidneys

- Fractalkine/CX3CR1 are upregulated in STZ-induced diabetic kidneys.

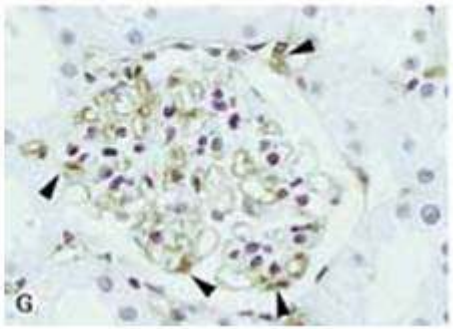
  Kikuchi Y et al. Nephron Exp Nephrol. 97:e17, 2004

- AGE induces fractalkine upregulation in normal rat glomeruli.

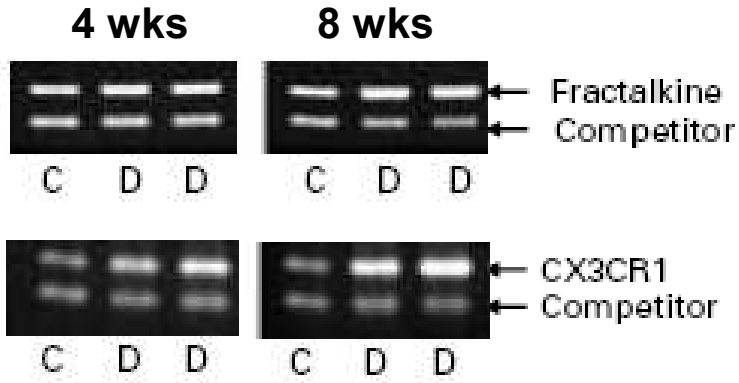
  Kikuchi Y et al. Nephrol Dial Transplant. 20:2690, 2005



FKN



CX3CR1



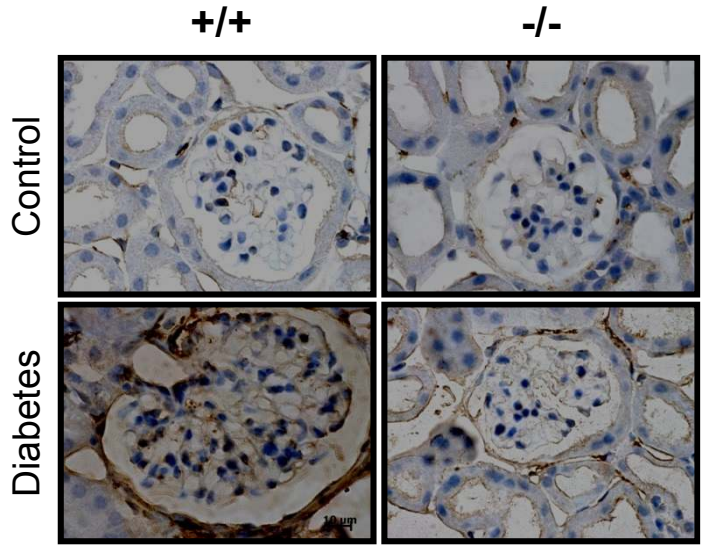
Hypothesis

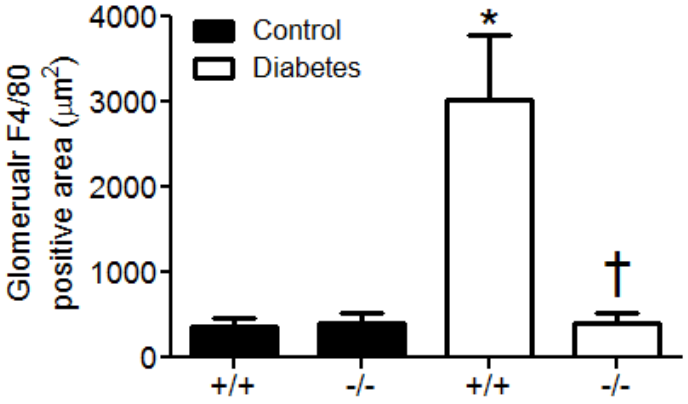
FKN/CX3CR1 system mediates renal fibrosis and inflammation during the development and progression of diabetic nephropathy.

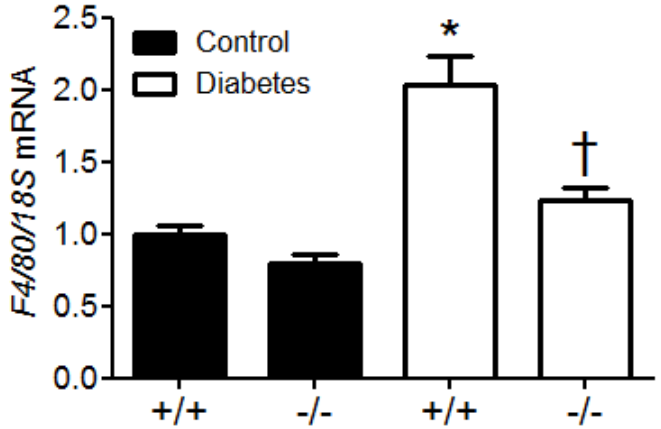
Specific aim 1.

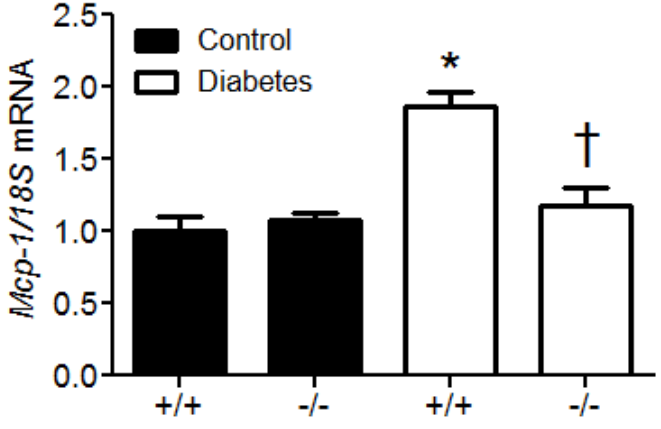
Role of FKN/CX3CR1 in inflammation

Macrophage infiltration in glomeruli was reduced in diabetic CX3CR1 KO mice



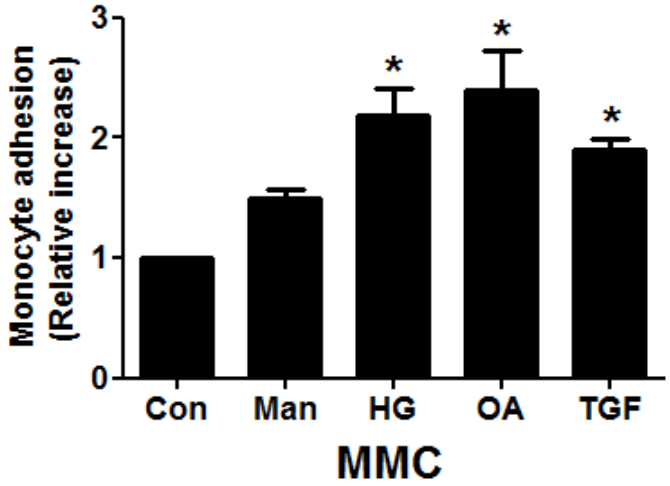


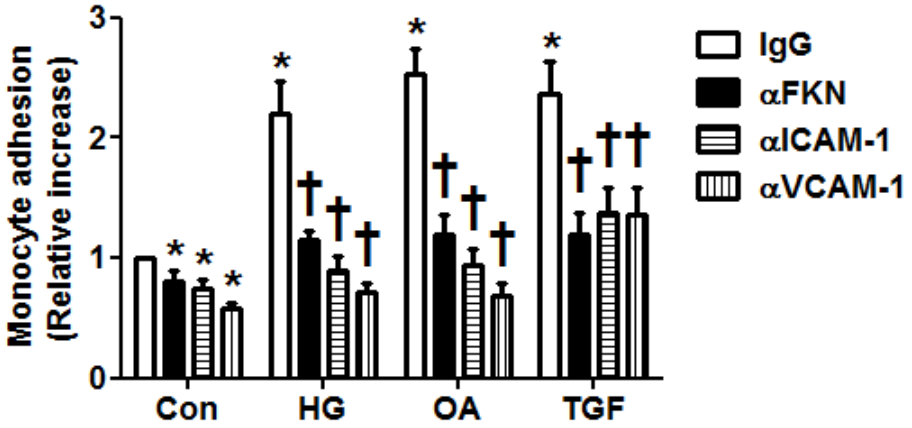


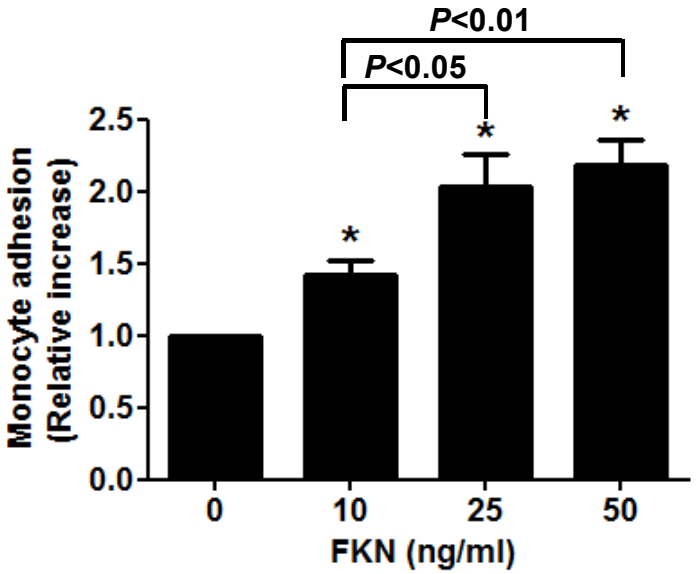


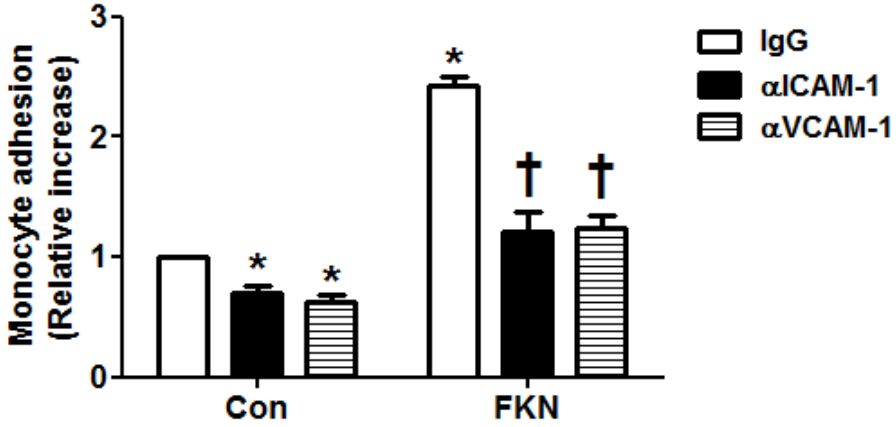
mean±SE of 8-12 mice. *P<0.05 vs control CX3CR1+/+, †P<0.05 vs diabetic CX3CR1 -/-

FKN mediated monocyte-MMC binding in diabetic conditions

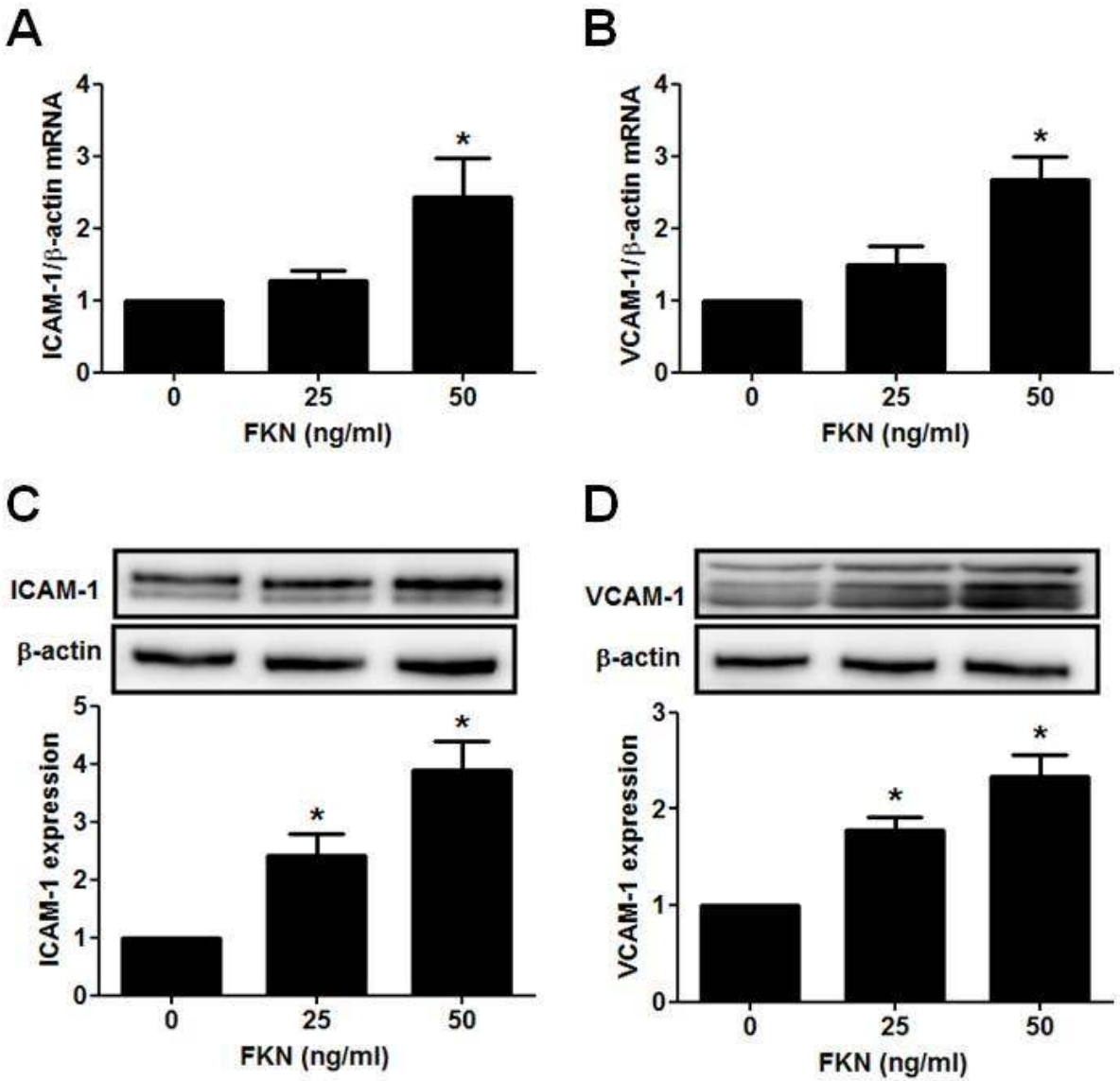




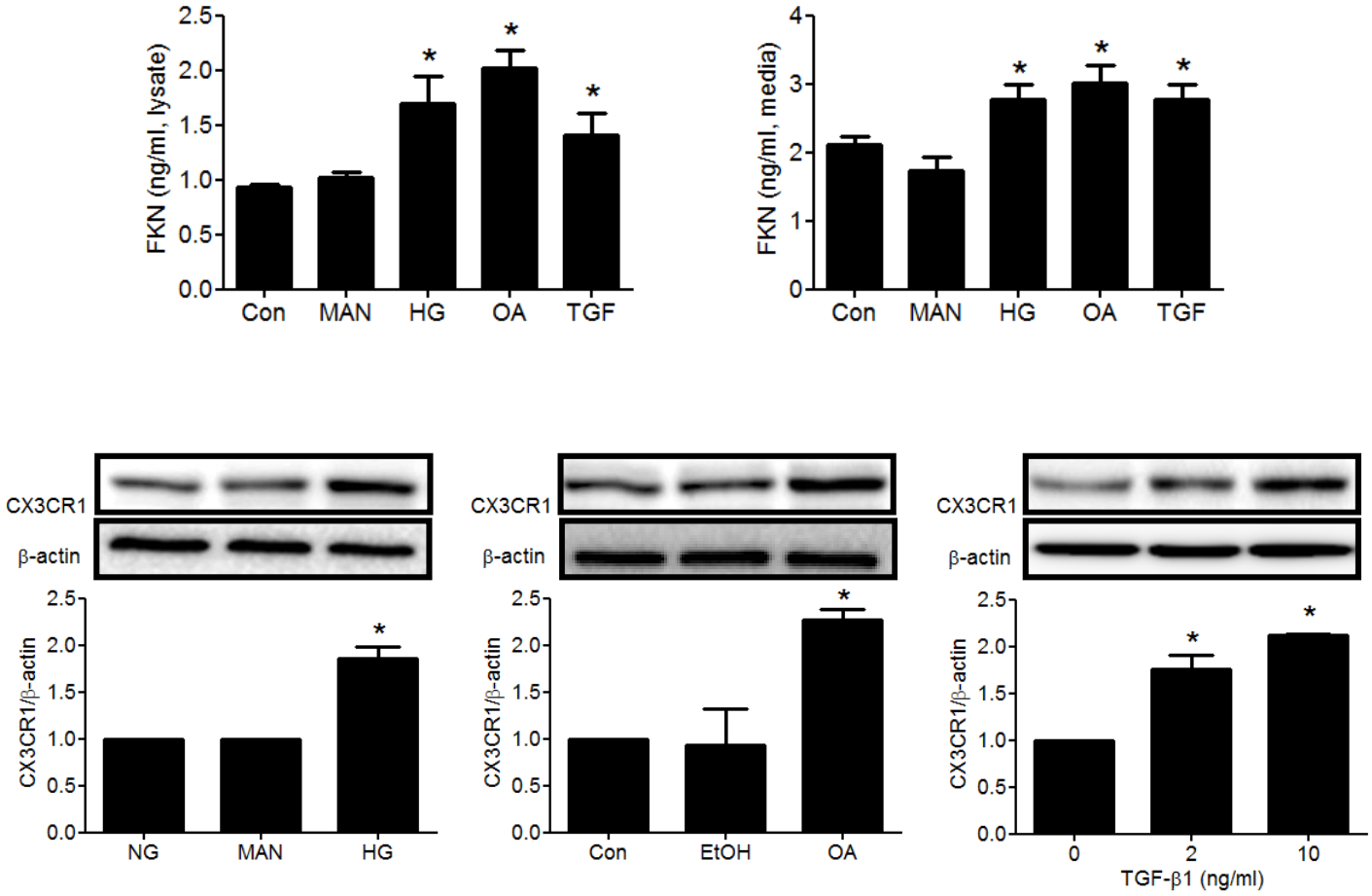




FKN upregulated adhesion molecule



Diabetic stimuli increased FKN/CX3CR1 protein production in MMCs



HG, 30 mmol/l D-glucose; OA, 100 μmol/l oleic acid; TGF, 10 ng/ml transforming growth factor-β1.

Specific aim 2.

Role of FKN/CX3CR1 in fibrosis

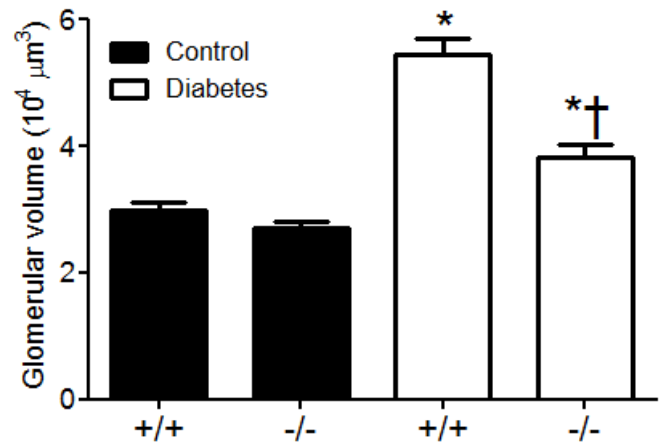
Diabetologia
DOI 10.1007/s00125-013-2907-z

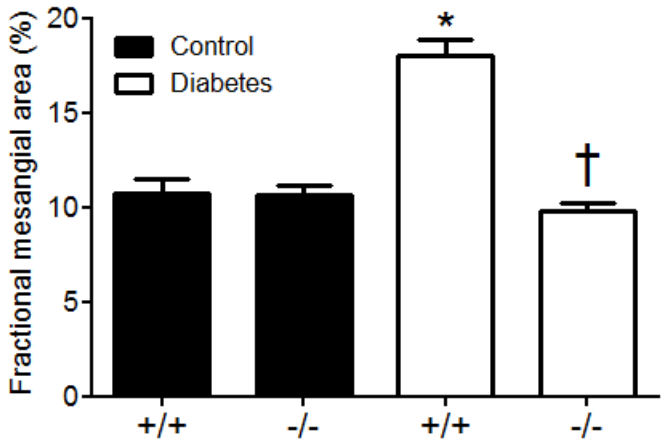
ARTICLE

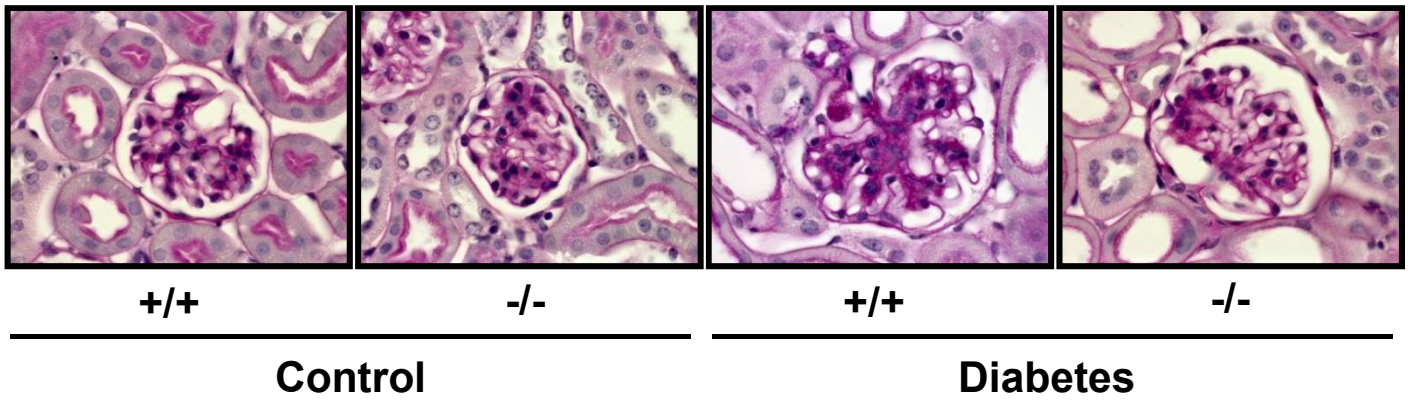
Fractalkine and its receptor mediate extracellular matrix accumulation in diabetic nephropathy in mice

K. H. Song · J. Park · J. H. Park · R. Natarajan · H. Ha

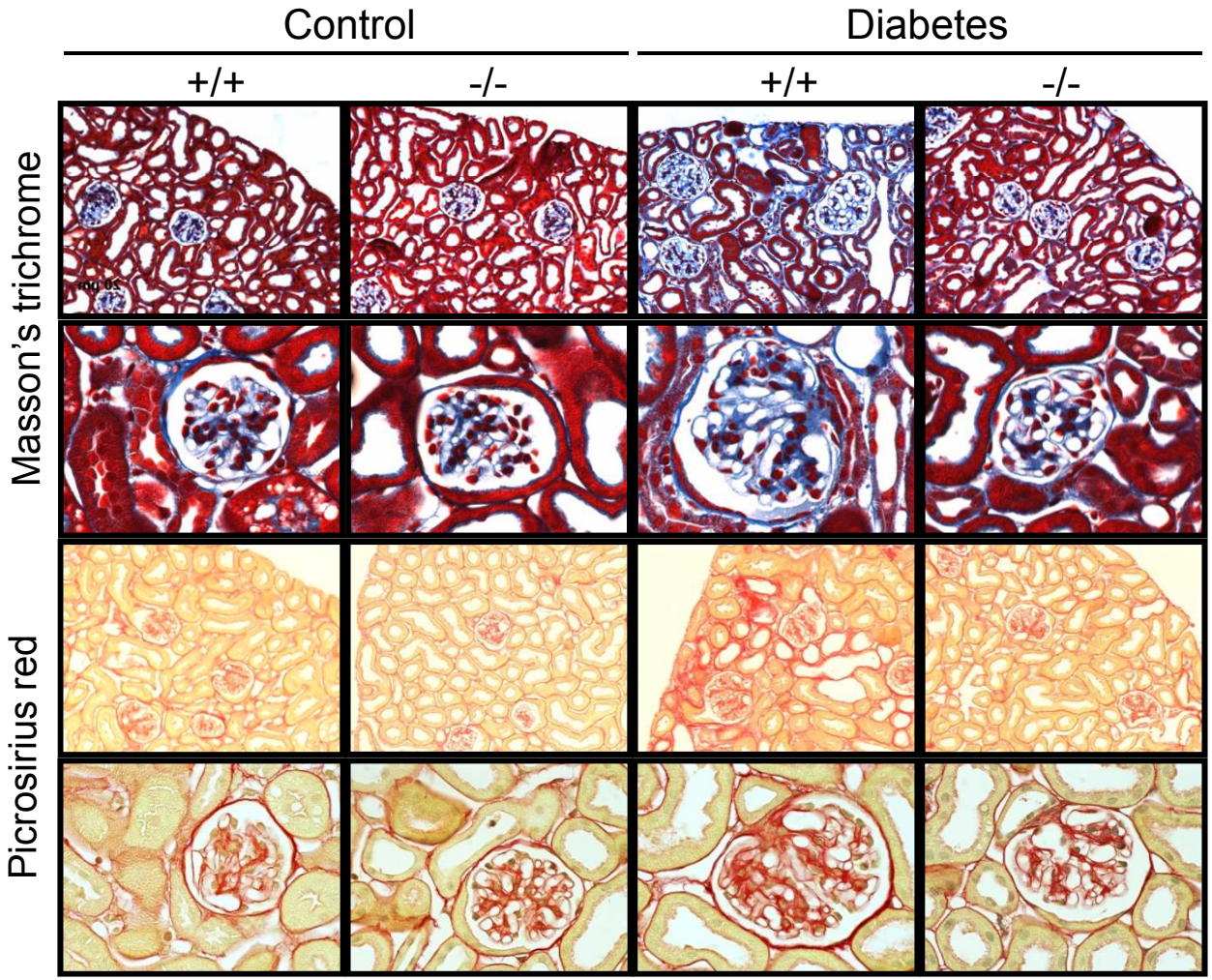
Inhibiting CX3CR1 decreased glomerular volume and fractional mesangial area in mouse kidneys



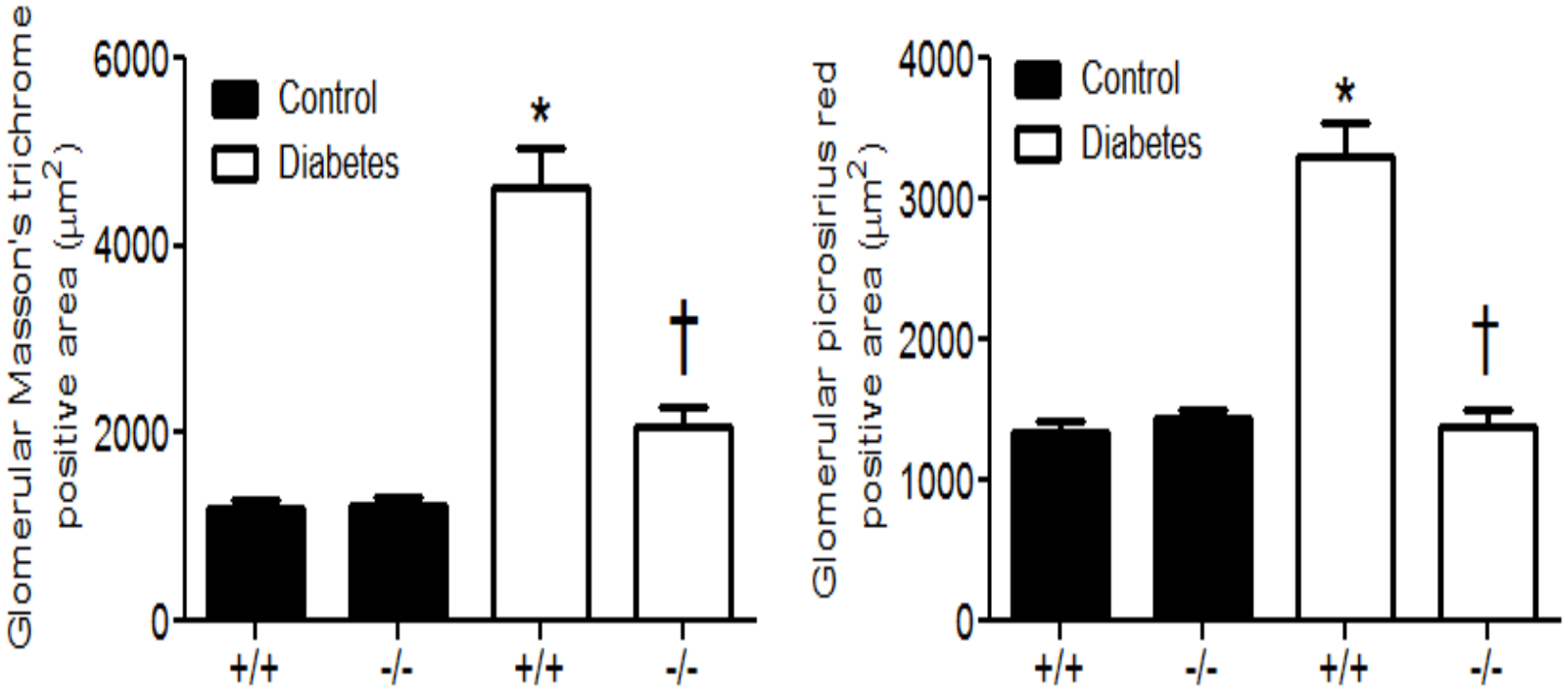




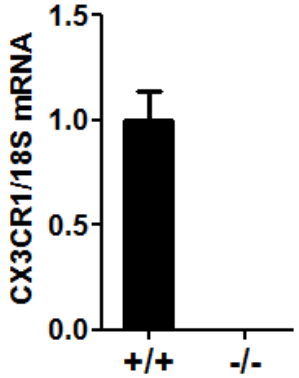
Renal fibrosis was decreased in diabetic CX3CR1 KO mice

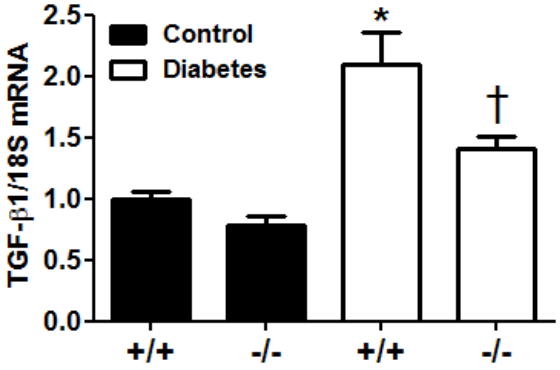


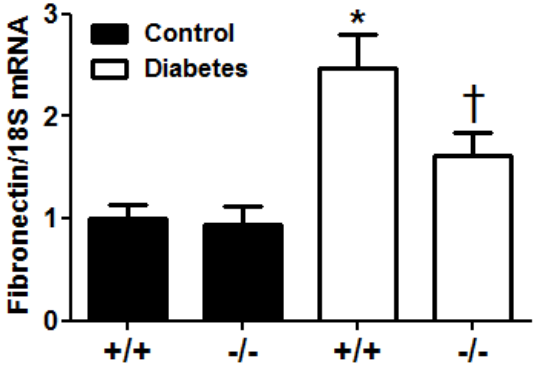
Renal fibrosis was decreased in diabetic CX3CR1 KO mice

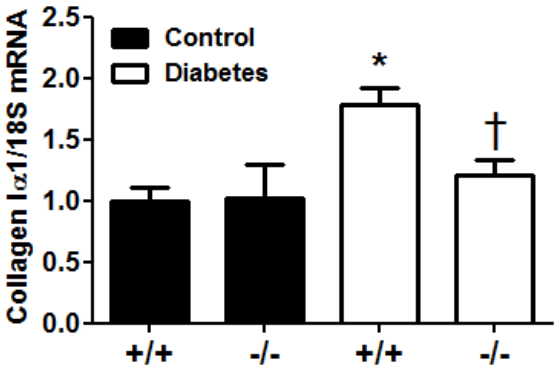


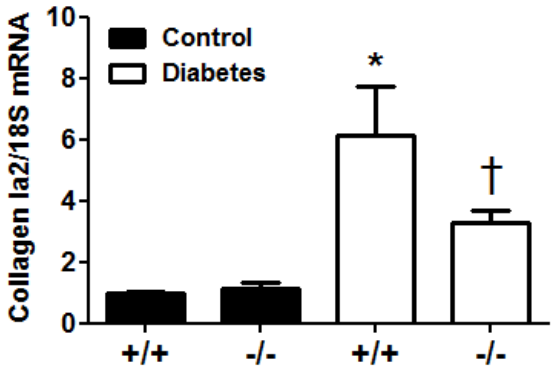
ECM markers were downregulated in diabetic CX3CR1 KO kidneys

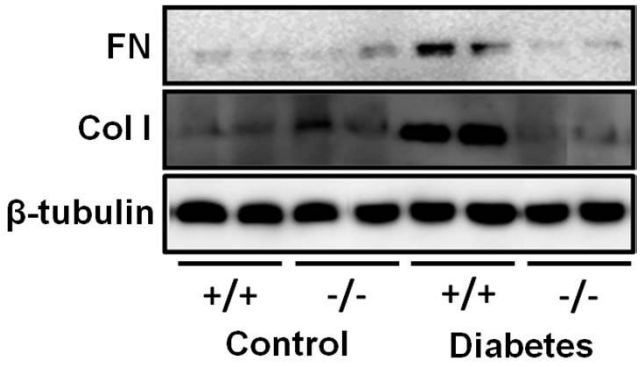






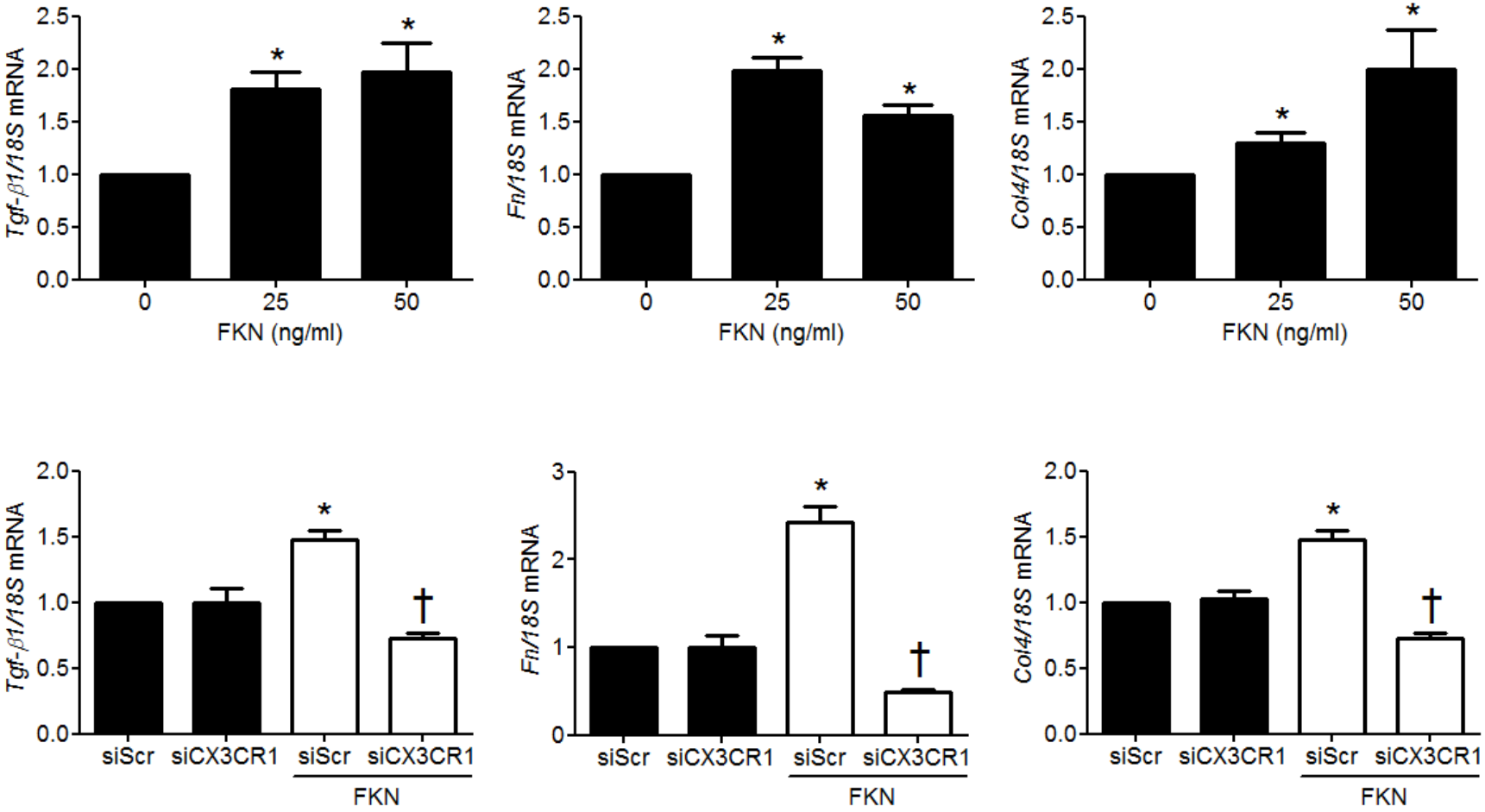




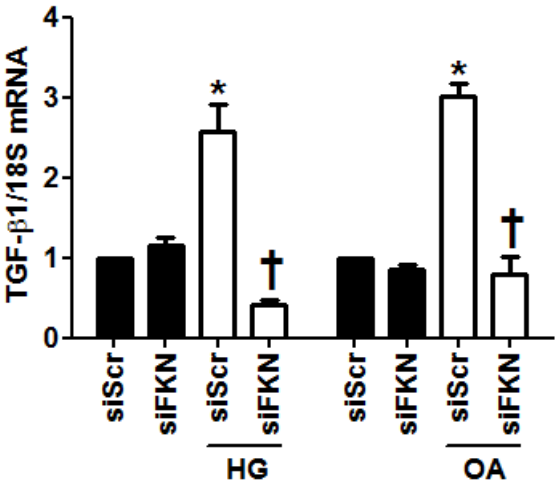


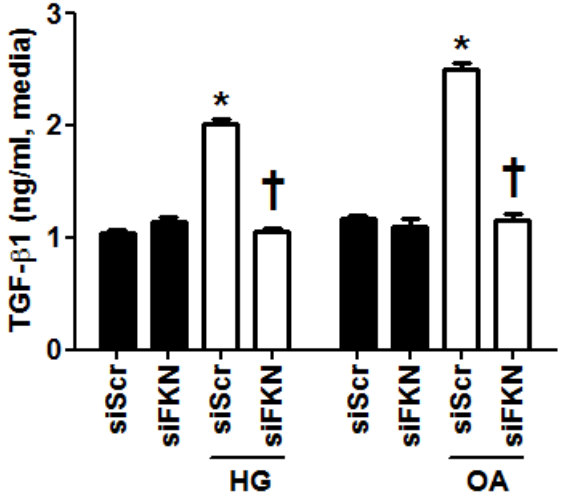
mean±SE of 8-12 mice. *P<0.05 vs control CX3CR1 +/+, †P<0.05 vs diabetic CX3CR1 -/-

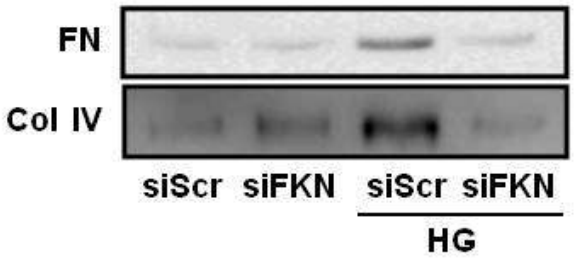
FKN directly induced ECM synthesis through CX3CR1 in MMCs

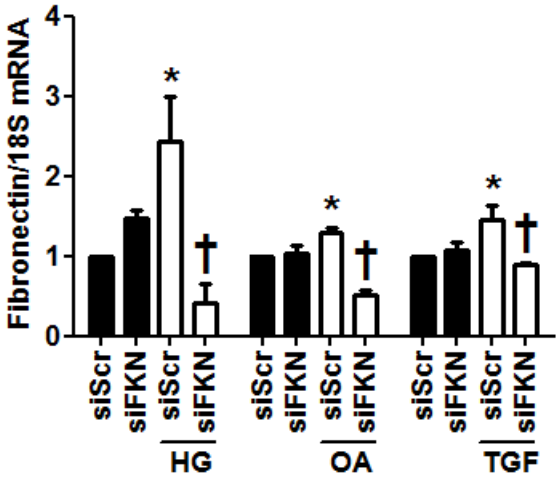


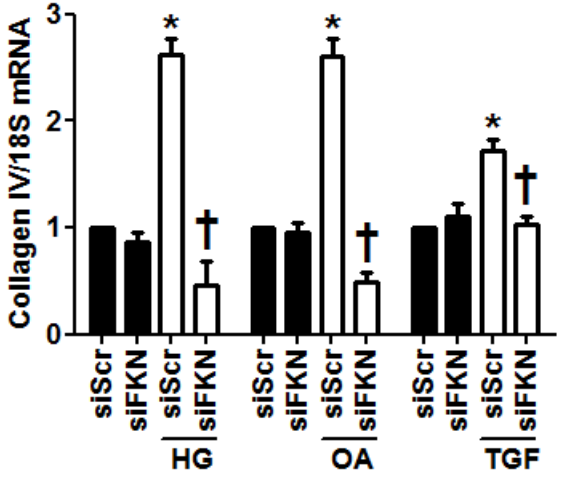
FKN siRNA inhibited diabetes-induced ECM synthesis in MMCs





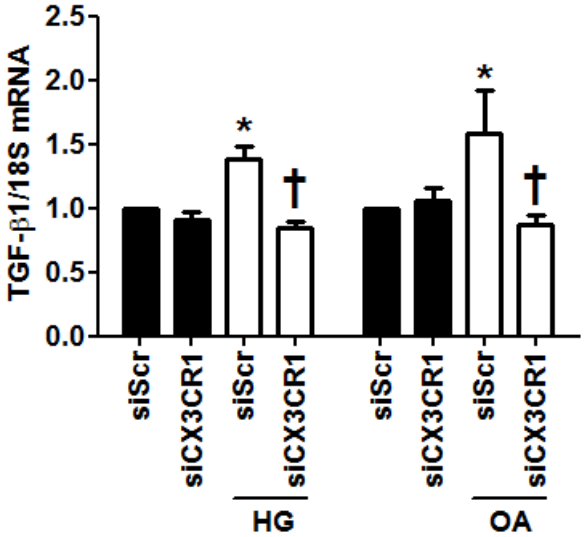


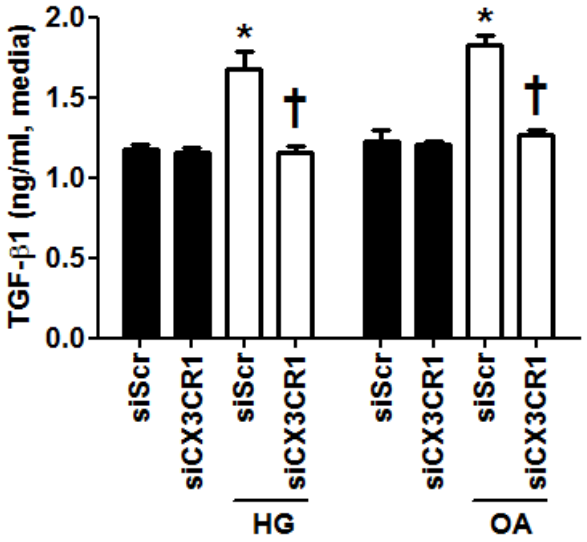


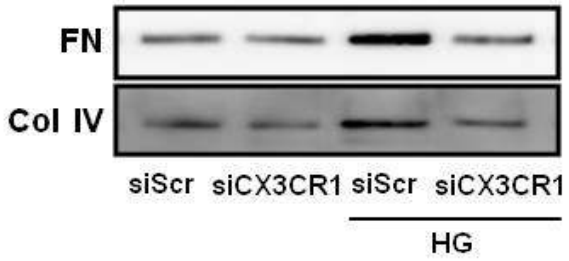


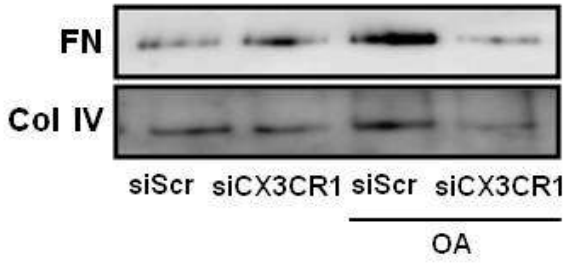
mean ± SE of 4 experiments. * $P<0.05$ vs siScr, † $P<0.05$ vs HG, OA, or TGF siScr

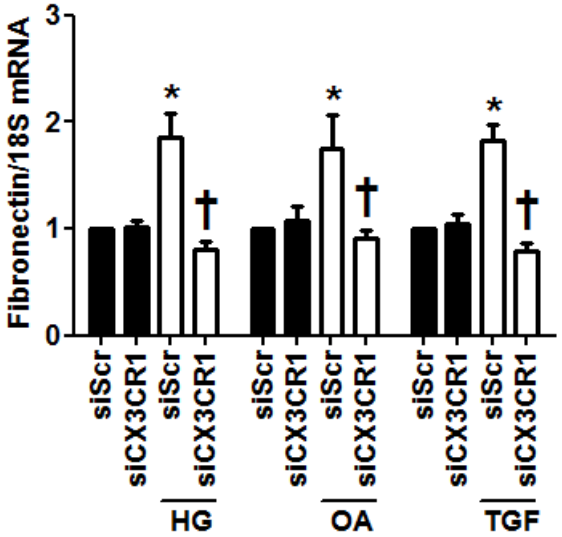
CX3CR1 siRNA inhibited diabetes-induced ECM synthesis in MMCs

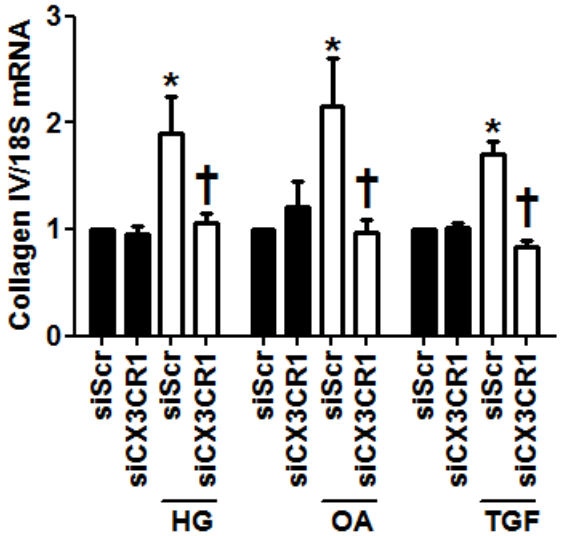


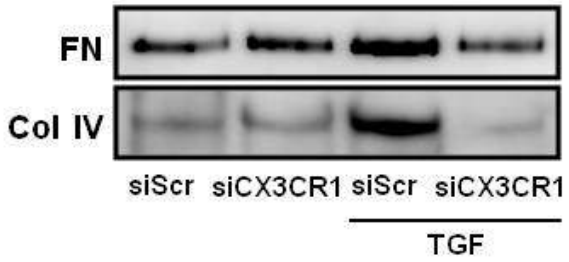






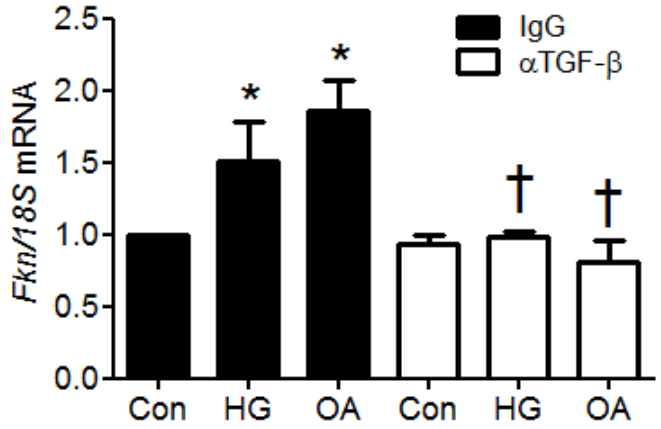


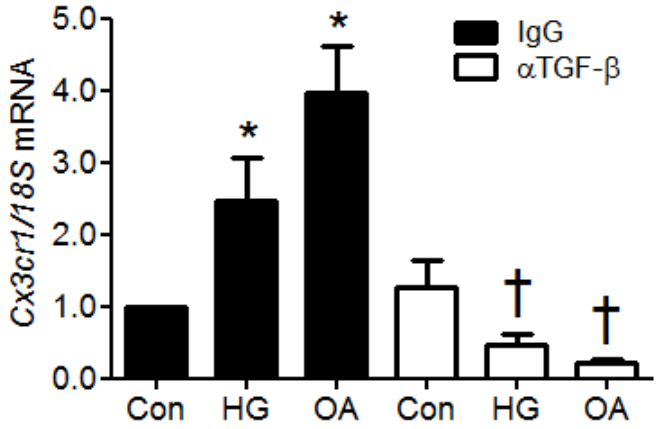


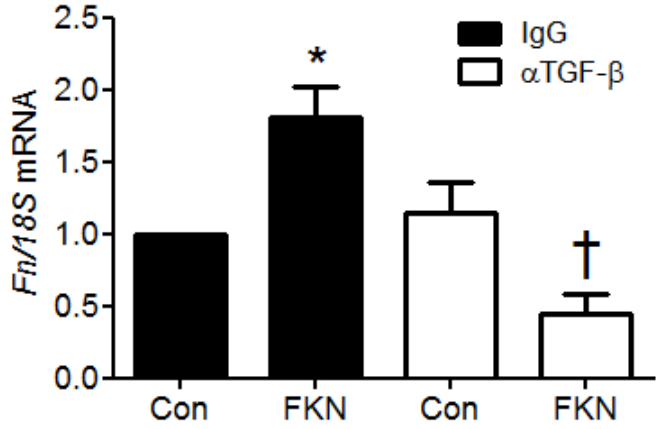


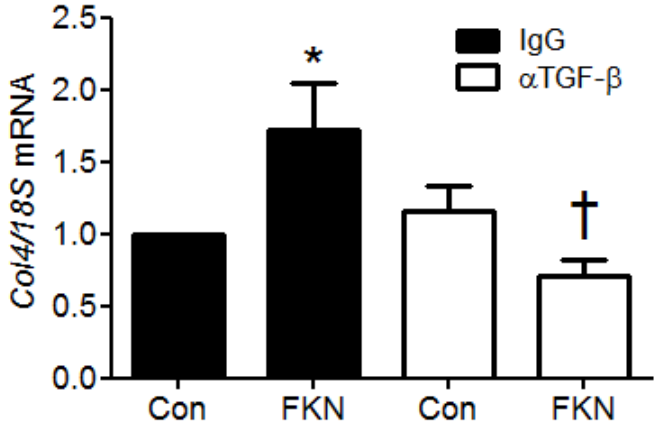
mean ± SE of 4 experiments. * $P<0.05$ vs siScr, † $P<0.05$ vs HG, OA, or TGF siScr

Inhibition of diabetic stimuli-induced FKN/CX3CR1 and FKN-induced ECM secretion with anti-TGFβ aby

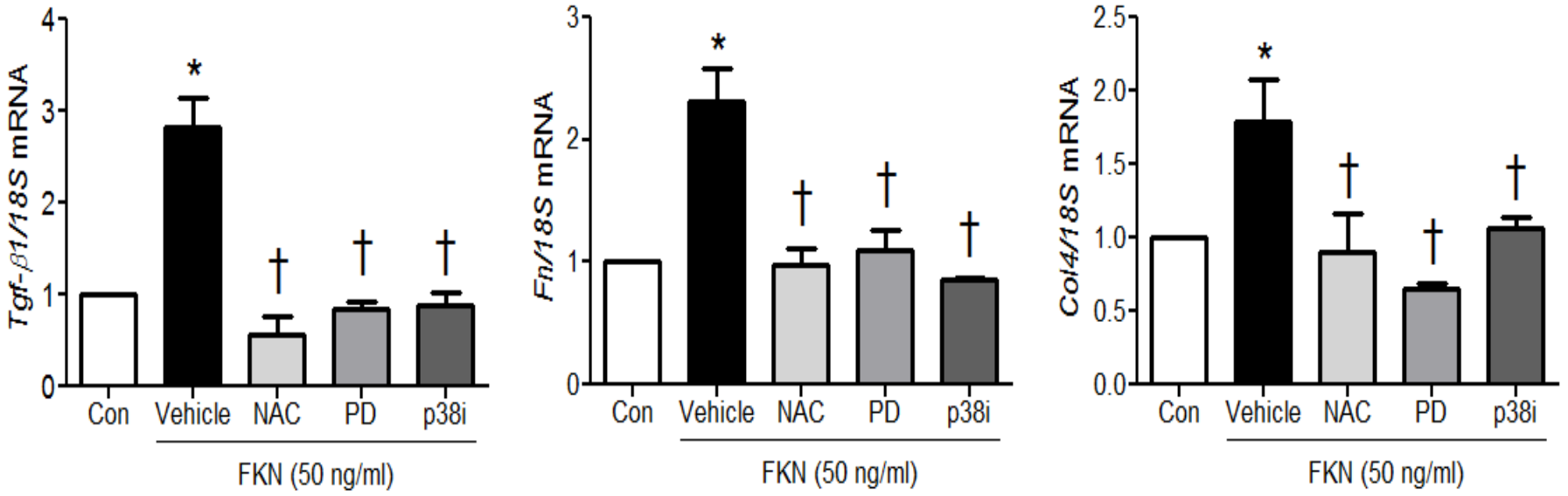




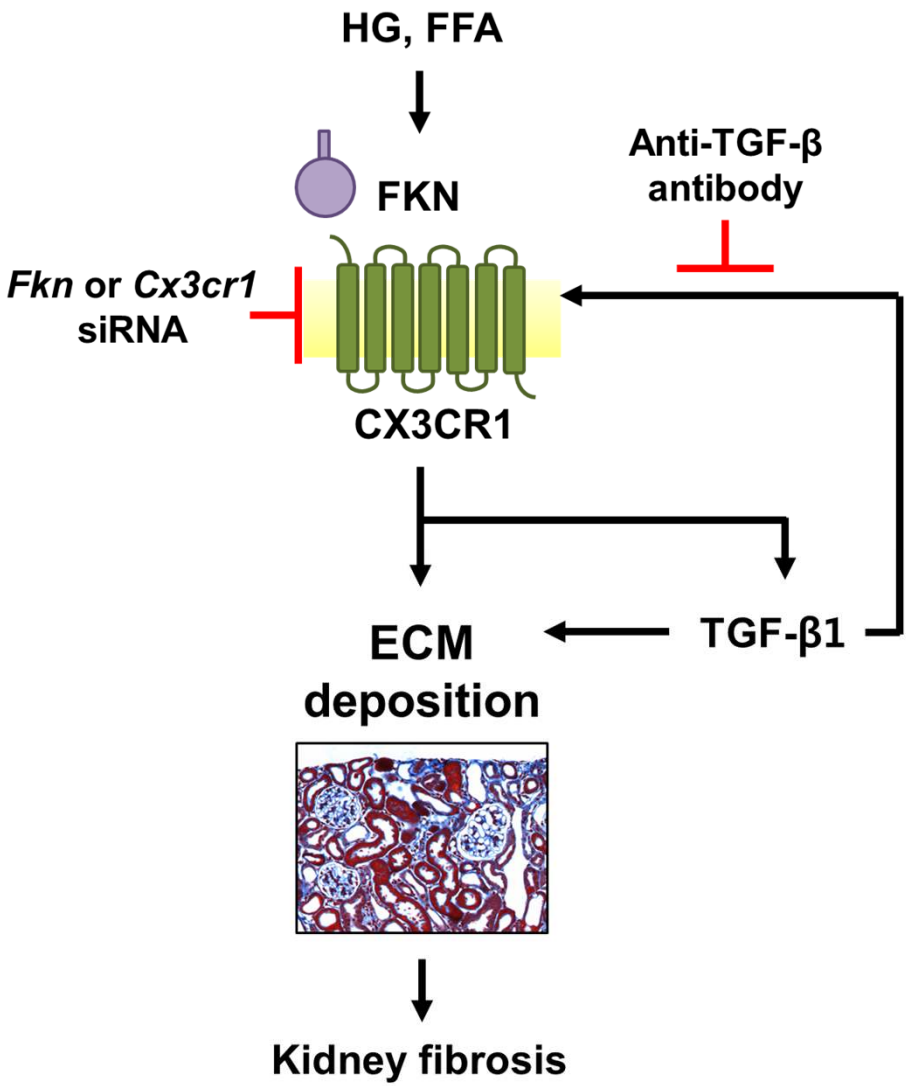




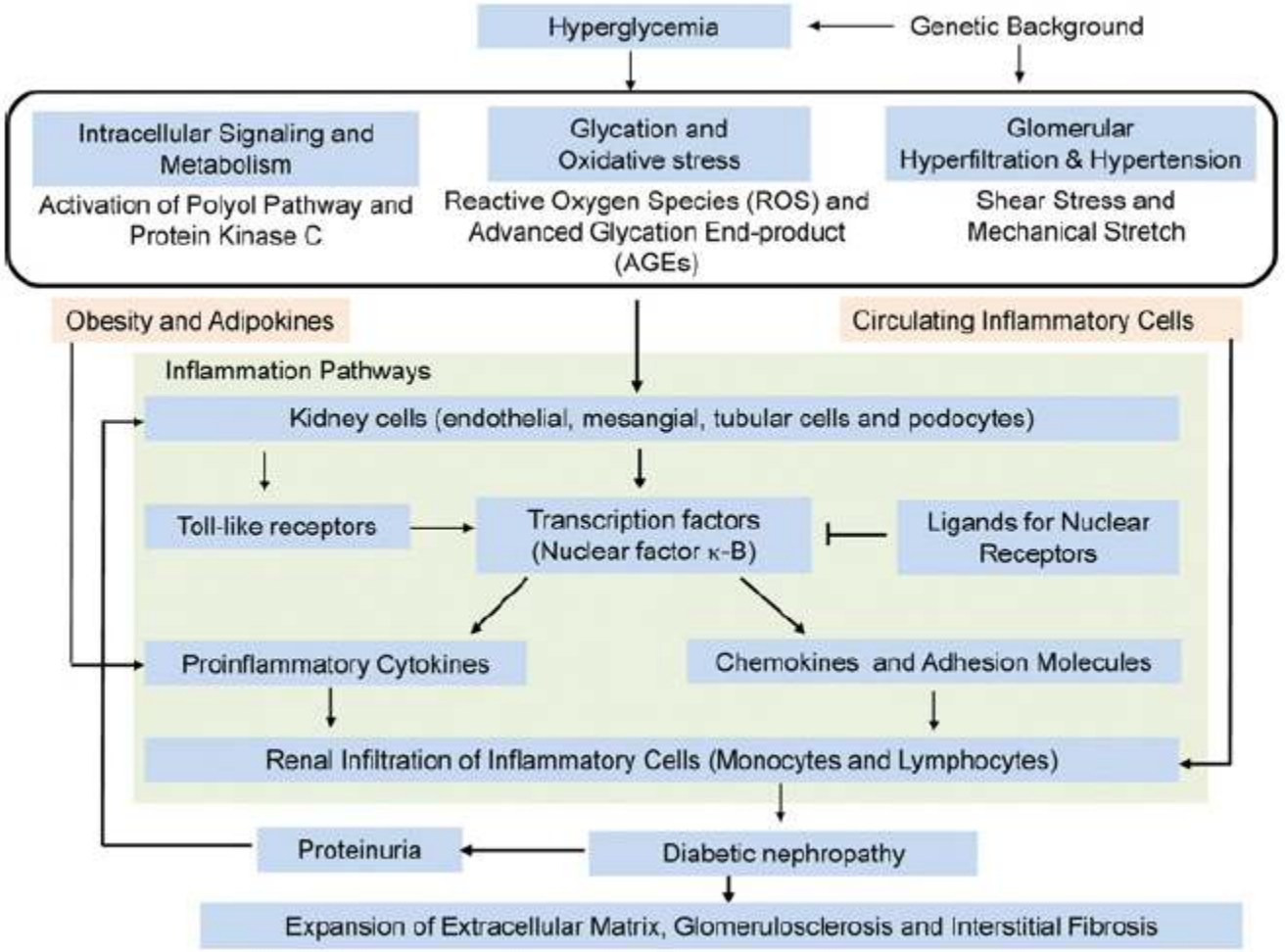
Inhibition of FKN-induced ECM levels with inhibitors of ROS, ERK, or p38 MAPK



Suggested model for FKN/CX3CR1 in the regulation of diabetic nephropathy



Inflammatory pathways in the pathogenesis of diabetic nephropathy



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감사합니다

Collaborators & Acknowledgment

Ewha Womans University
- Lee KJ
- Jeong LS
- Lee HJ
- Bai YS

Yonsei University
- Kim YS
- Jung M

Sung Kyun Kwan University
- Chung MH

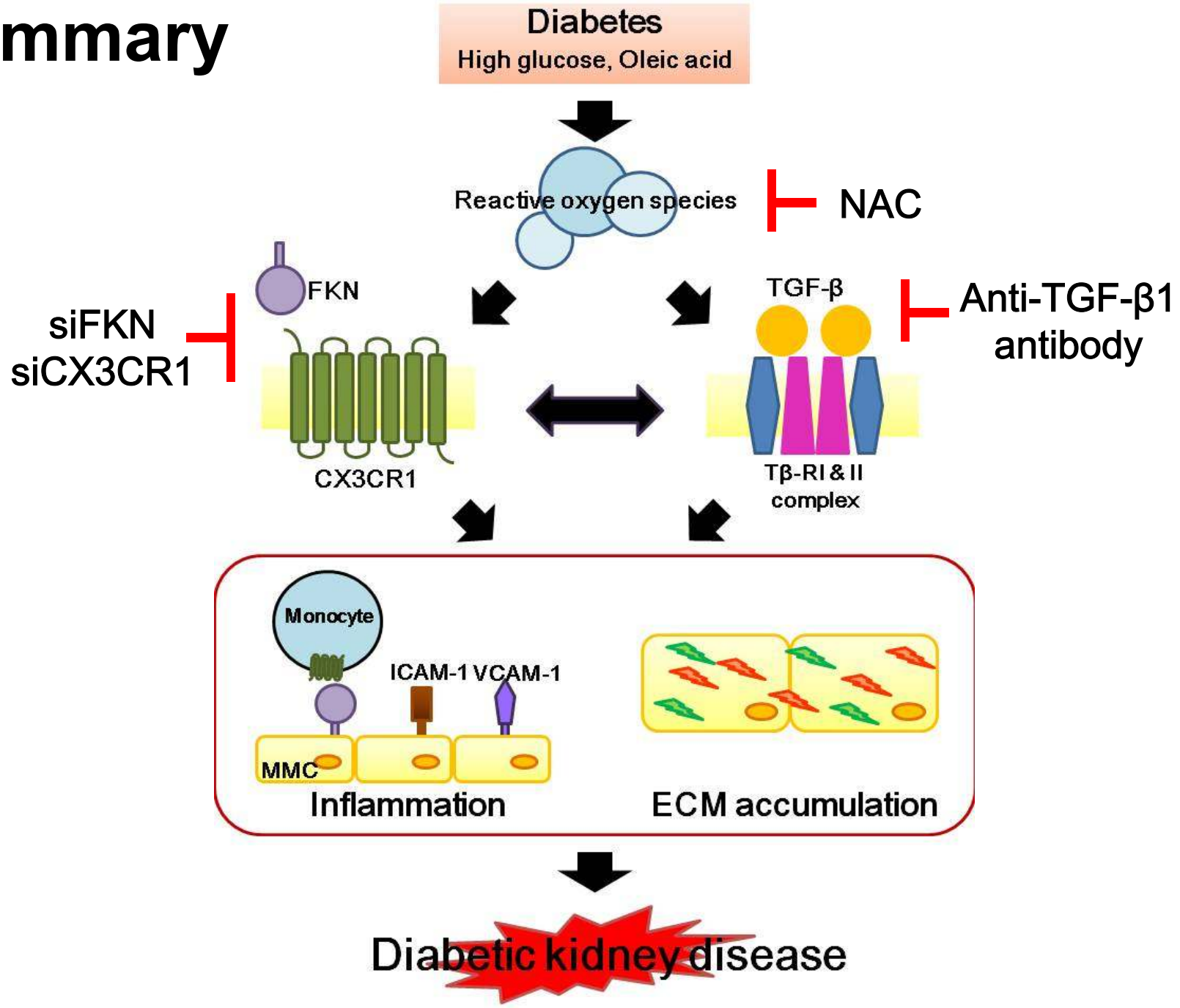
Busan National University
- Chung HY

Tohoku University
- Miyata T

Emory University
- Jo H

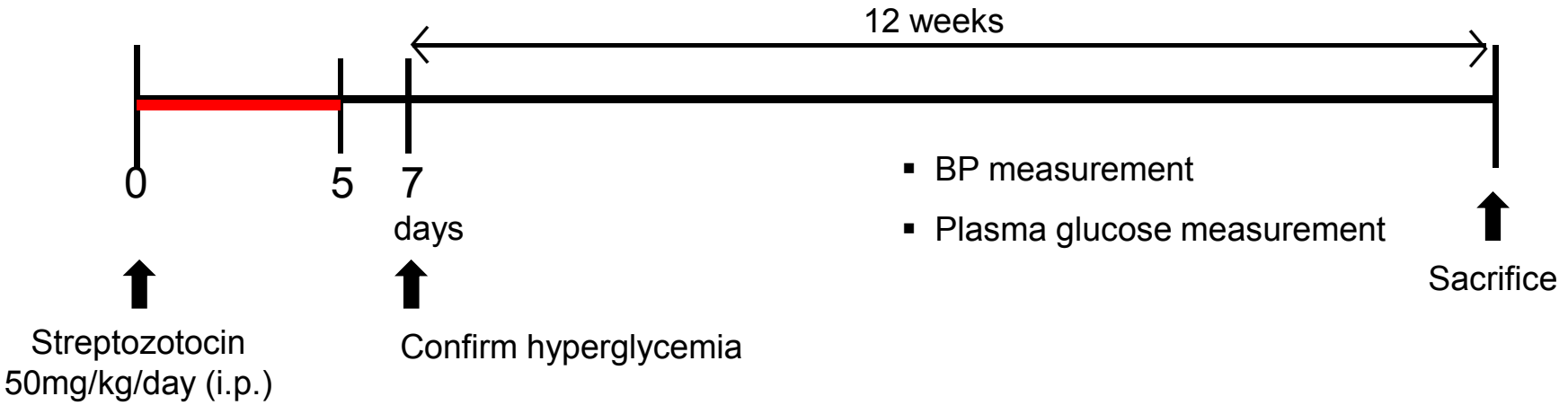
Supported by National Research Foundation, Korea

Summary


Diabetes
High glucose, Oleic acid
Reactive oxygen species
NAC
FKN
siFKN
siCX3CR1
CX3CR1
TGF-β
Anti-TGF-β1
antibody
Tβ-RI & II
complex
Monocyte
ICAM-1 VCAM-1
MMC
Inflammation
ECM accumulation
Diabetic kidney disease

Methods and materials

- In vivo
  - 8-week-old CX3CR1 KO mice and age-matched WT C57BL/6J mice (Jackson Lab, USA)



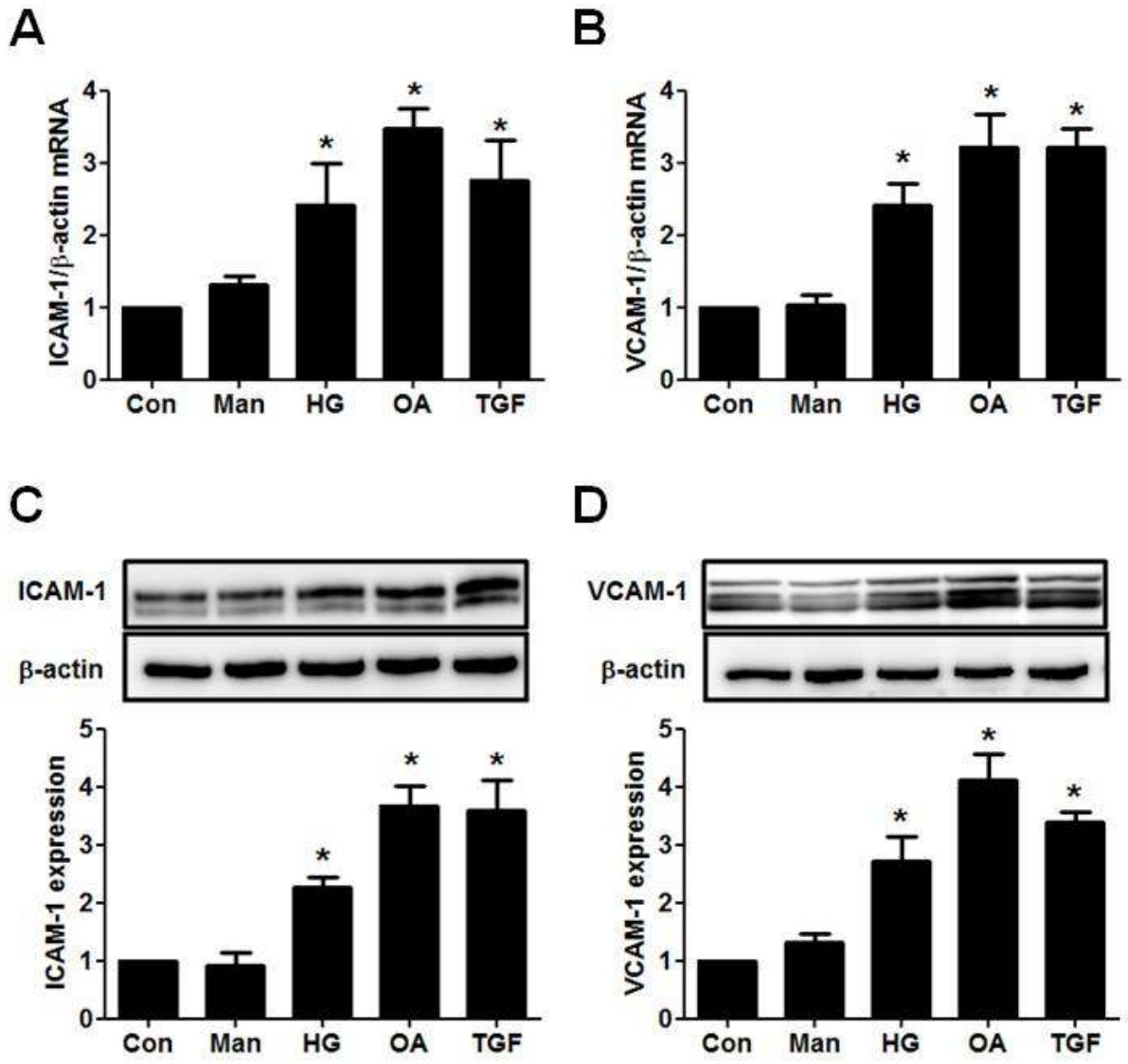
- In vitro
  - Mouse mesangial cells (MMC, SV-40 transformed)
  - Monocytes; WEHI78/24 (Dr. Rama Natarajan in Beckman Research Institute, CA, USA)

Characteristics of experimental animals

	Control		Diabetes	
	CX3CR1 +/+	CX3CR1 -/-	CX3CR1 +/+	CX3CR1 -/-
Body weight (g)	30±1	29±2	22±1 *	20±1 *
Blood glucose (mg/dl)	162±11	172±9	549±13 *	531±22 *
HbA1c (%)	4.53±0.09	4.46±0.10	10.00±0.42 *	11.00±0.66 *
Kidney weight (g)	0.19±0.01	0.18±0.01	0.22±0.01 *	0.24±0.01*
Urine protein excretion (mg/24h)	1.0±0.3	0.9±0.2	4.0±0.7*	3.8±1.0*

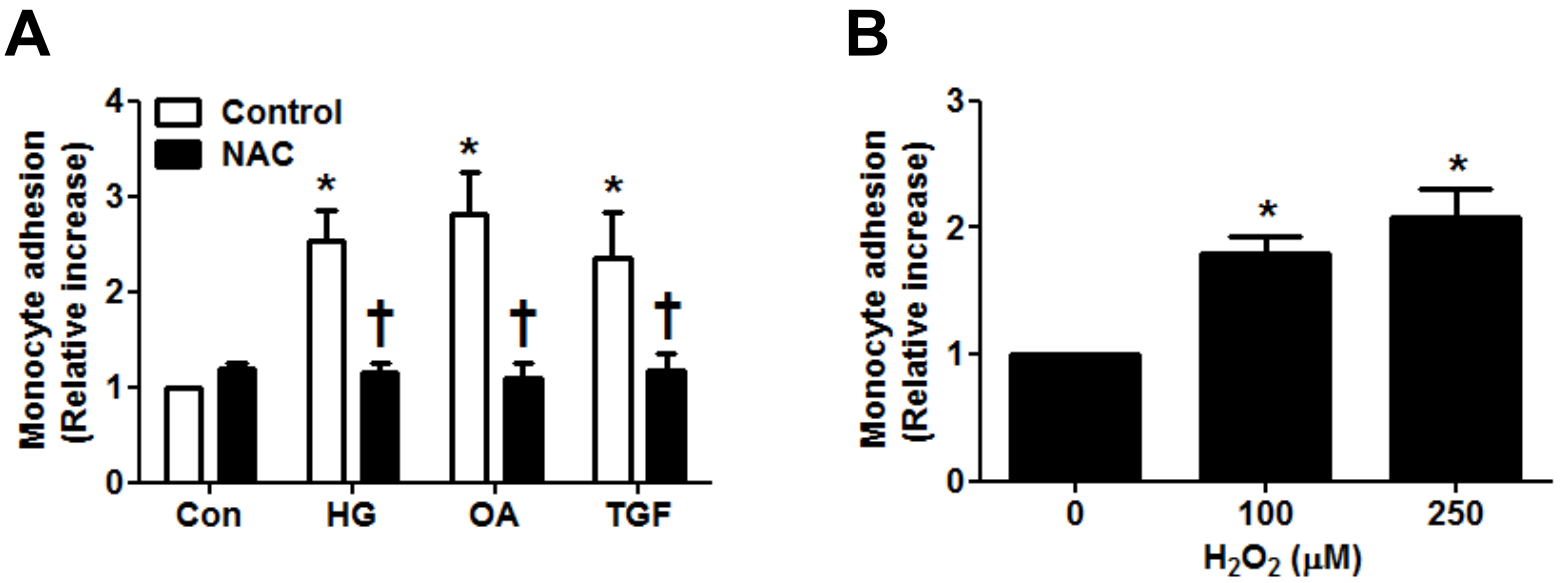
mean±SE of 8-12 mice. *P<0.05 vs control CX3CR1+/+

Adhesion molecule upregulation in diabetic condition-treated MMCs



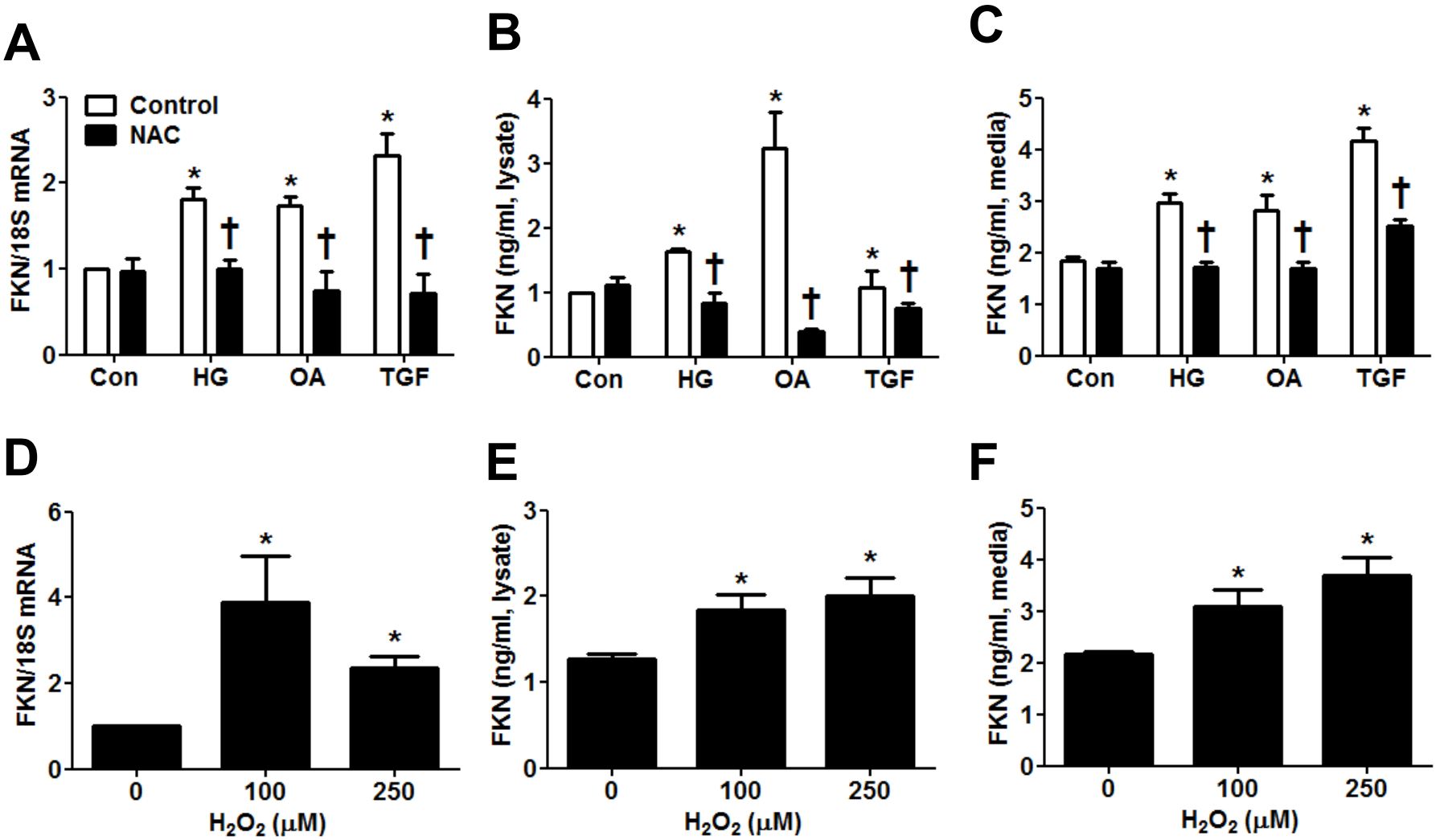
Data are presented as mean ± SE of 4 experiments. * *P*<0.05 vs Con

ROS were involved in diabetic condition-induced monocyte adhesion to MMCs



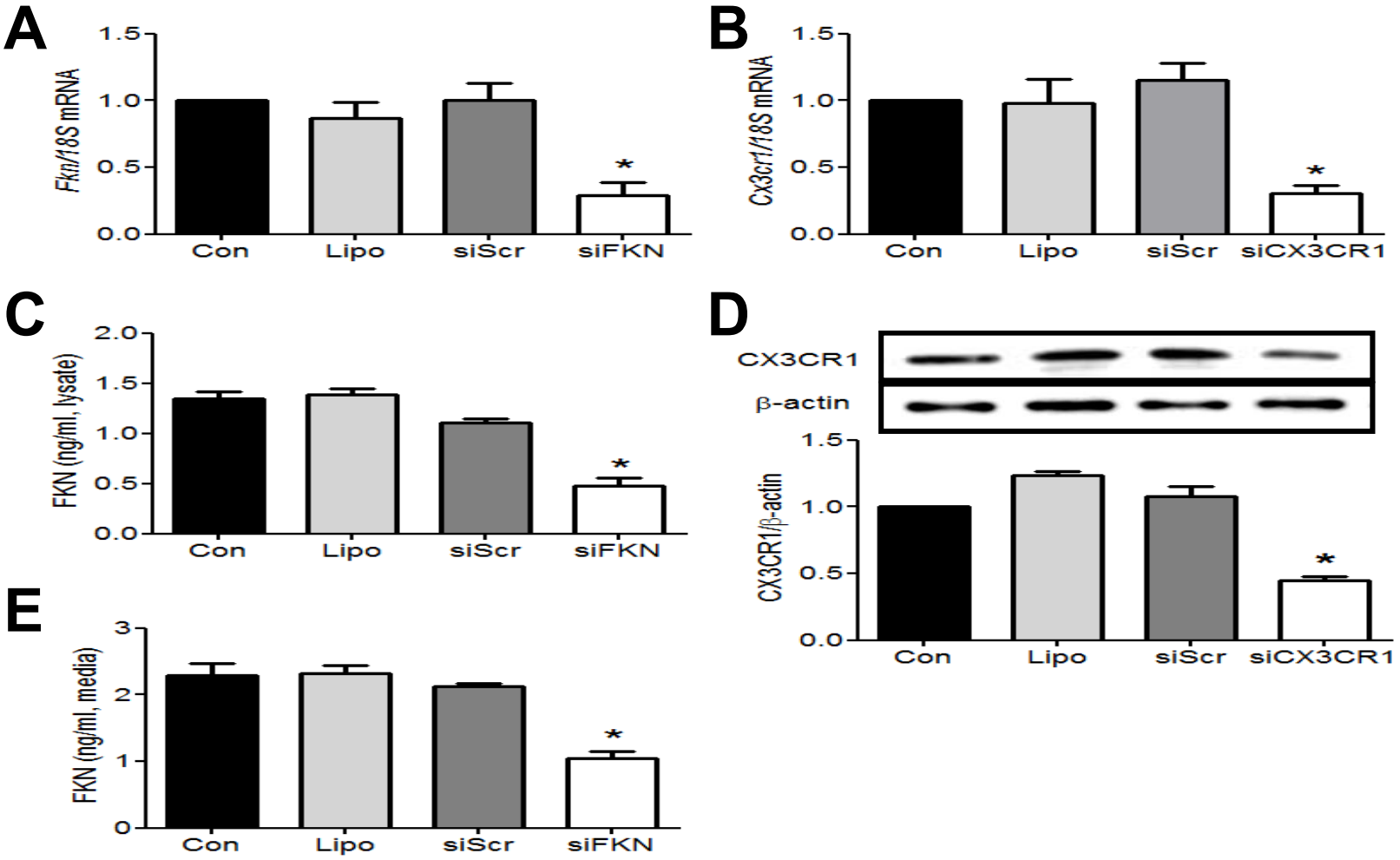
Data are presented as mean ± SE of 4 experiments. * *P*<0.05 vs Con, † *P*<0.05 vs HG, OA, or TGF

ROS were involved in diabetic condition-induced FKN expression in MMCs



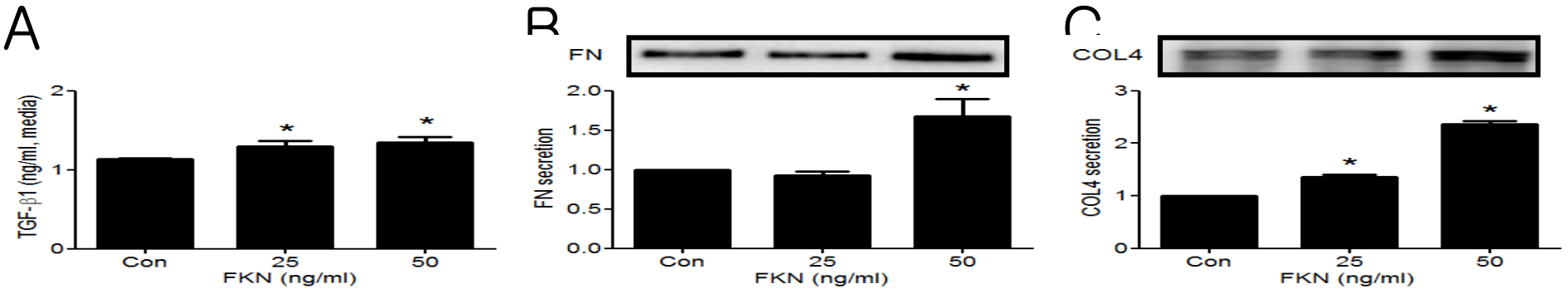
Data are presented as mean ± SE of 4 experiments. * *P*<0.05 vs Con or 0 μM H_2O_2, † *P*<0.05 vs HG, OA, or TGF

ESM Figure 1. *Fkn* and *Cx3cr1* siRNA, respectively, blocked each mRNA expression and protein levels in MMCs. *Fkn* and *Cx3cr1* siRNA transfected MMCs, *Fkn* (A) and *Cx3cr1* mRNA expression (B) were measured by real-time PCR. FKN protein levels in cell culture lysates (C) and supernatants (E) were measured by ELISA. CX3CR1 protein levels in *Cx3cr1*siRNA transfected MMCs (D) were measured by Western blot analysis. Data were shown as mean ± SE or representative Western blots of 4 experiments. * $P<0.05$ vs Con or siScr, Con: control, Lipo: lipofectamin, siScr: negative siRNA, siFKN: *Fkn* siRNA, siCX3CR1: *Cx3cr1* siRNA.



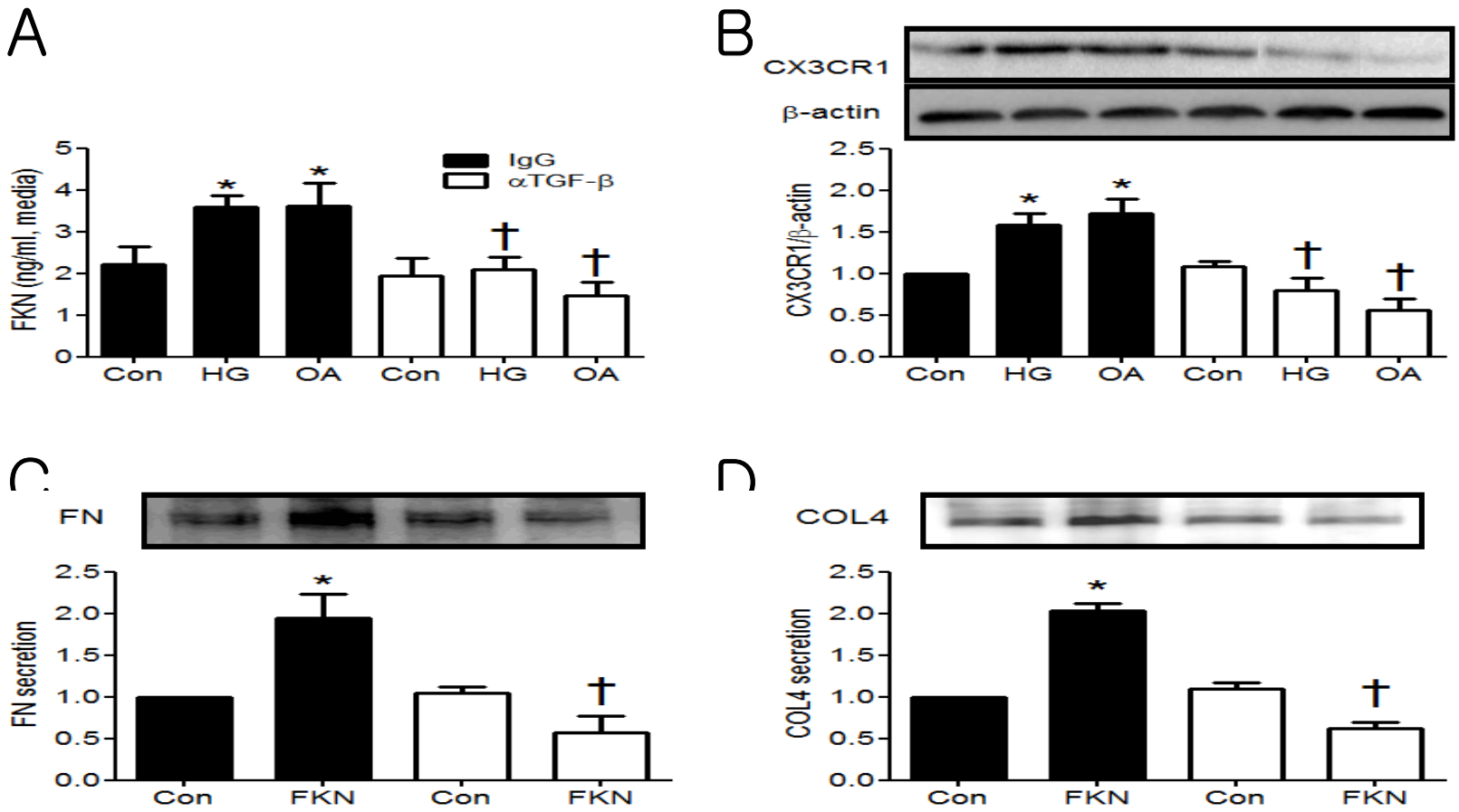
Song et al. ESM Figure 1.

ESM Figure 3. FKN directly induced ECM synthesis through CX3CR1 in MMCs. Protein secretion of TGF-β1 (A), FN (B), and COL4 (C) were determined by ELISA or Western blot analysis. Data are mean ± SE or representative Western blots of four experiments. *P < 0.05 vs. Con; Con, control or FKN 0 ng/ml.



Song et al. ESM Figure 3.

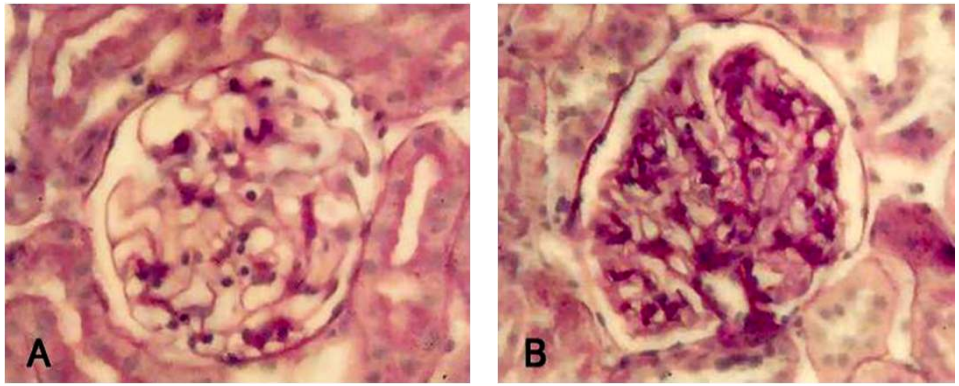
ESM Figure 5. Inhibition of diabetic stimuli-induced FKN/CX3CR1 protein production and FKN-induced ECM secretion in MMCs treated with TGF-β neutralizing antibody. FKN (A) and CX3CR1 (B) protein levels were measured in MMCs exposed to HG and OA with or without a TGF-β neutralizing antibody. ECM markers such as FN (C) and COL4 (D) protein production were assessed in MMCs incubated with a TGF-β neutralizing antibody before stimulation with FKN. Data are mean ± SE or representative Western blots of four experiments. *P < 0.05 vs. Con, †P < 0.05 vs. HG, OA or FKN; Con, control; HG, 30 mmol/l D-glucose; OA, 100 μmol/l oleic acid; αTGF-β, 5 μg/ml anti-TGF-β neutralizing antibody; FKN, 50 ng/ml fractalkine.



Song et al. ESM Figure 5.

Functional and structural characteristics of diabetic kidney

- Glomerular hyperfiltration
- Altered glomerular filtration barrier: Albuminuria
- Renal and glomerular hypertrophy
- Accumulation of extracellular matrix (ECM) in the glomeruli and the tubulointerstitium



Normal glomeruli Diabetic glomeruli