Non-glycemic effect of DPP4/CD26

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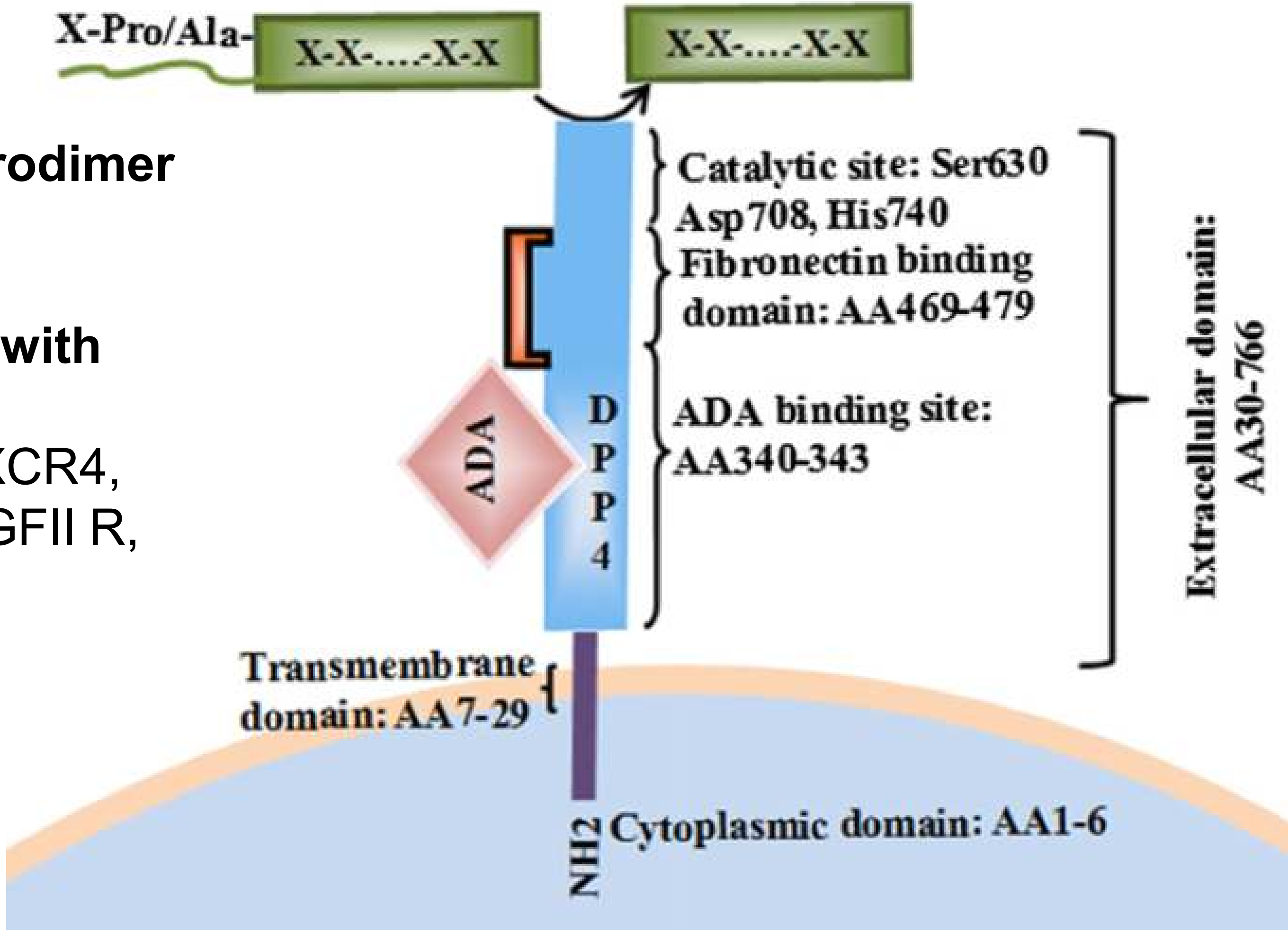
Dipeptidyl peptidase-4 (CD26)

From 1966 to 2006

Can form heterodimer with FAPα

Can associate with plasminogen 2, ADA, CD45, CXCR4, mannose-6-P/IGFII R, fibronectin collagen



ADA, adenosine deaminase
J. Zhong et al. Atherosclerosis 226 (2013) 305-314

Known DPP-4 substrates

- **Regulatory peptides**

  GLP1, GLP2, GIP
  Gastrin-releasing peptide (GRP)
  B-type natriuretic peptide (BNP)
  Growth-hormone-releasing factor (GHRF)
  Pituitary adenylate-cyclase-activating polypeptide (PACAP)-(1–38)
  Peptide YY(1–36)

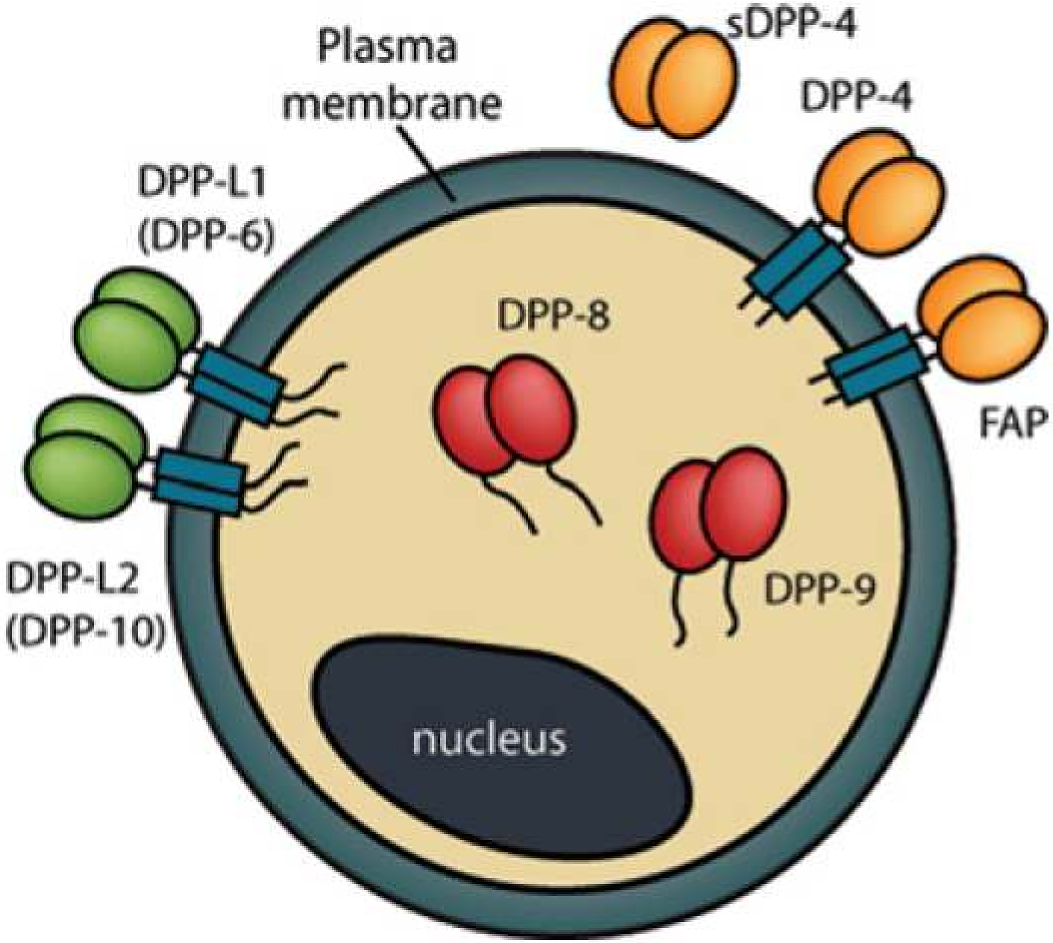
- **Neuropeptides**

  Neuropeptide-Y (NPY), Vasoactive intestinal peptide (VIP)
  Substance P

- **Chemokines**

  Eotaxin (CCL11), IP10 (CXCL10), ITAC (CXCL11)
  Macrophage-derived chemokine (MDC, CCL22)
  Monokine induced by IFN-γ (Mig, CXCL9)
  RANTES (CCL5)
  Stromal-cell-derived factor (SDF-1, CXCL12)

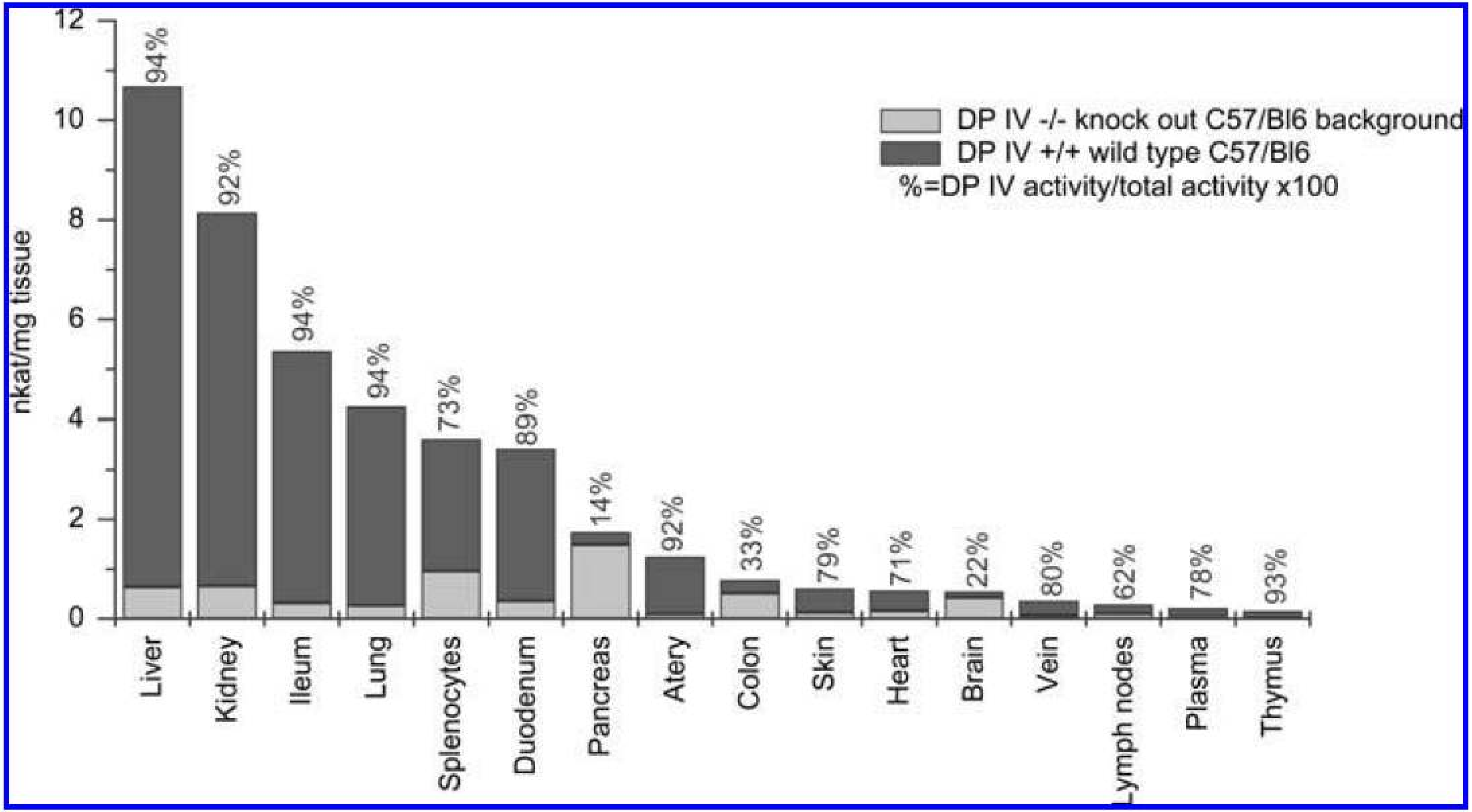
DPP-4 activity and/or structure homologues (DASH Family)



sFAP

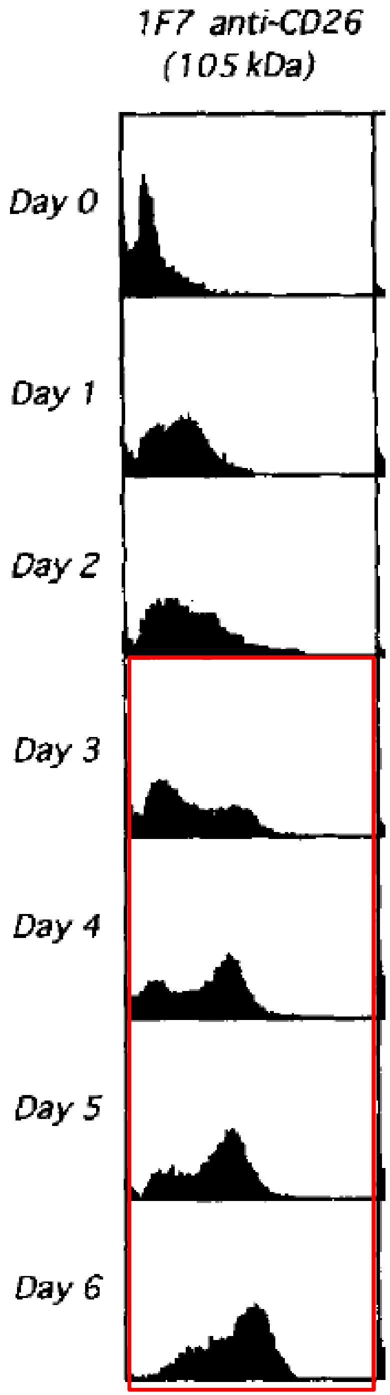
Kirby M et al. Clin Sci (Lond). 2009 Sep 28;118(1):31-41.

Tissue distribution of DPP4 and DPP4-like enzymes in WT mice and DPP4 KO mice.

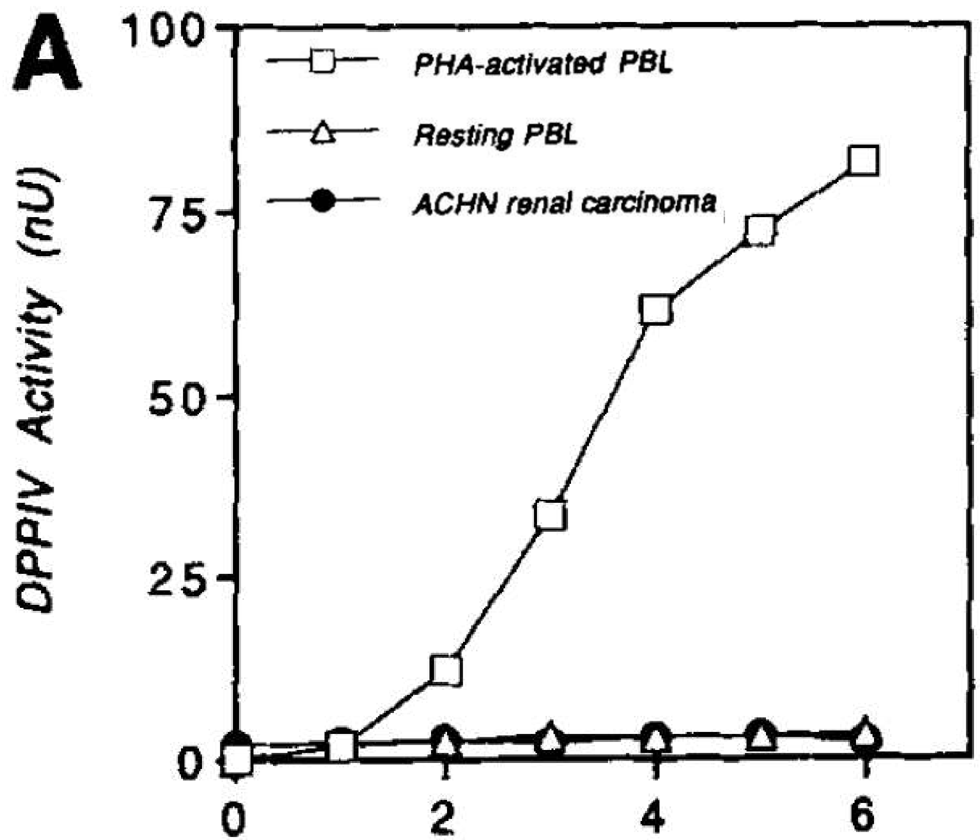


Ansorge S et al . 2009 Clin Chem Lab Med 47:253-261

T cell DPP4 and release on stimulation with PHA



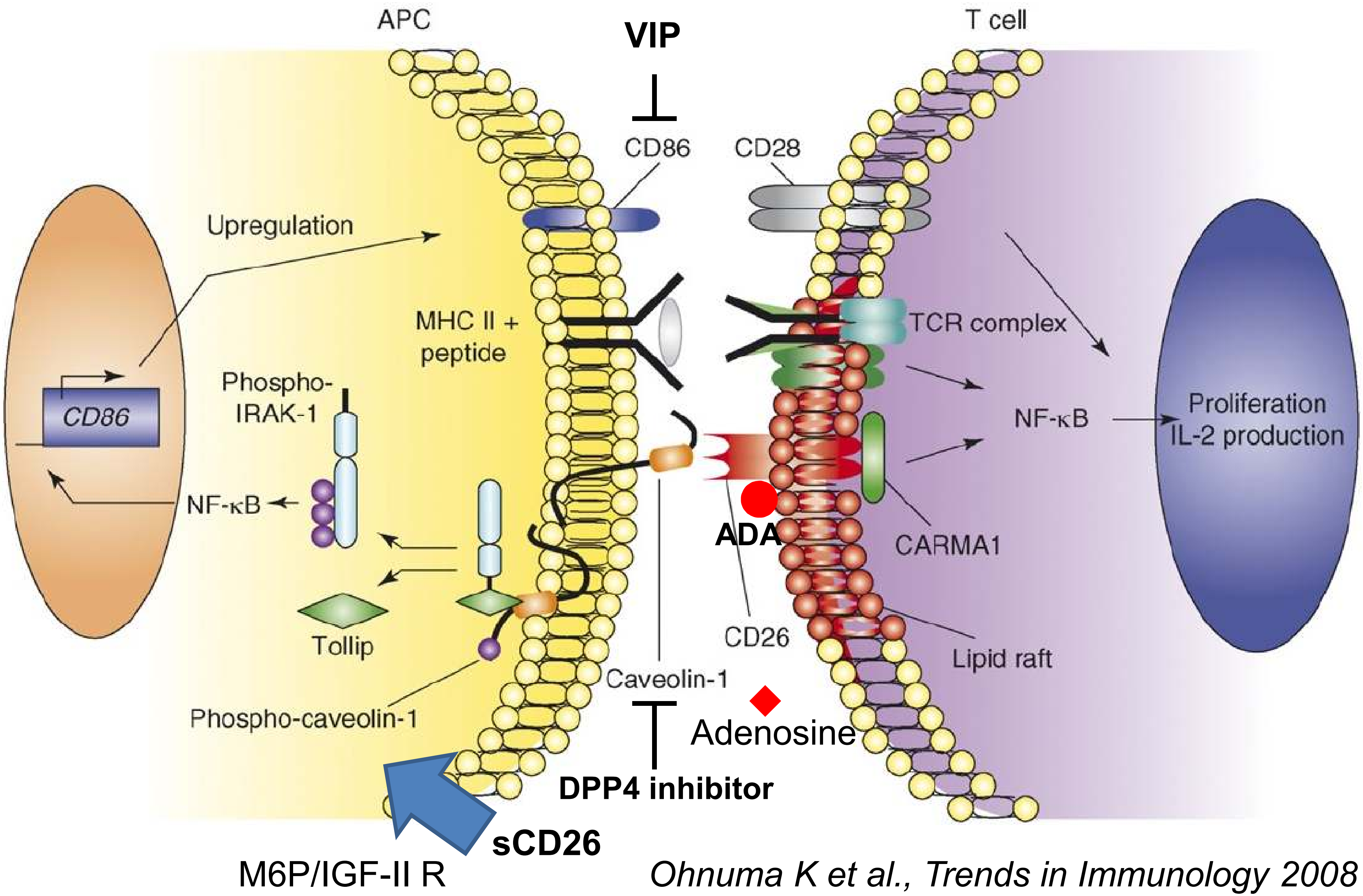
Culture media



Days

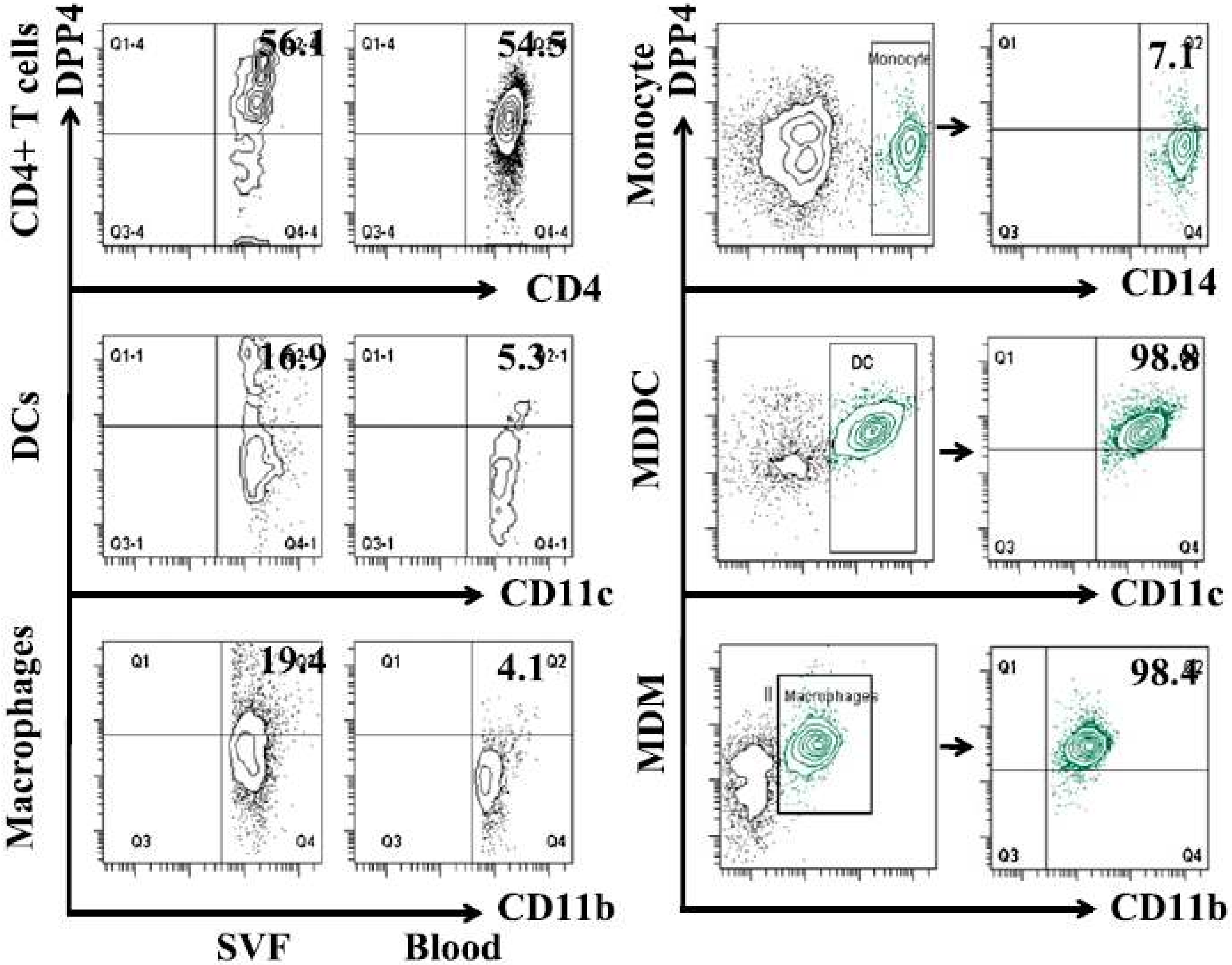
Duke-Cohan JS et al. The Journal of Immunology, 1996, 156: 1714-1721.

DPP4 (CD26) in T cell Co-stimulation



Ohnuma K et al., Trends in Immunology 2008

Dendritic Cell/Macrophage-Expressing DPP4

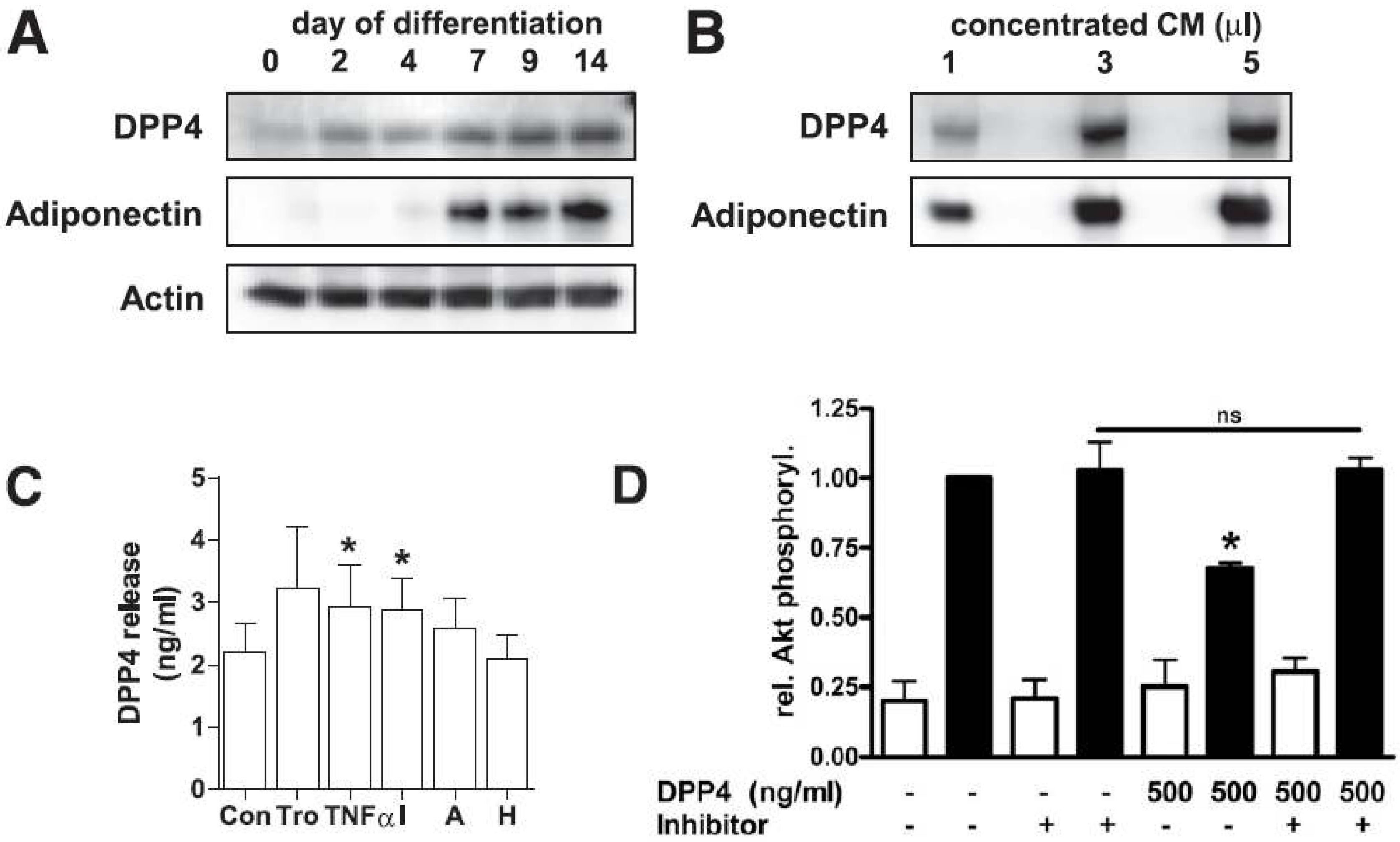


Zhong J et al, Diabetes (2012)

Regulation of DPP4 activity, expression, or release

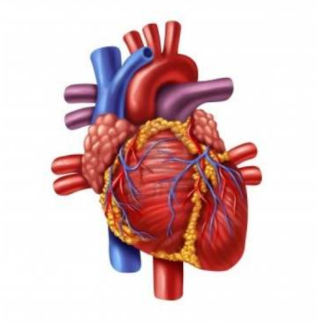
- Caco-2 cell by HNF-1α
- T cells, NK cells, and B cells: various cytokines
- Differentiated adipocyte: increased release by TNFα or insulin
- Endothelial cells (ECs): oxidative stress (release), HIF1α
- Microvascular ECs: **high glucose** (activity)
- Renal epithelial cells by IFN-γ, IL-4, IL-13
- HepG2 cell: n-butyrate (activity)
- Macrophages : LPS (?)
- CD34+ progenitors by G-CSF or GM-CSF

DPP-4 is an adipokine



Lamers D et al., Diabetes 60:1917–1925, 2011

Pathophysiology associated with DPP-4



↑M1/M2 macrophages, ↓Treg/Th1, ↓ Treg/Th17, ↓IL10

↓ SERCA 2a activity
↓ SR calcium uptake
↓ Glucose uptake
↓ Fibrin polymerization

↑AGE-RAGE pathway
↑Oxidative stress
↑eNOS uncoupling
↑MCP-1, VCAM-1, TGFβ
↑ PAI-1/TPA
↓NO/cGMP
↑PMN
↓ EPC homing
↓ Neoangiogenesis

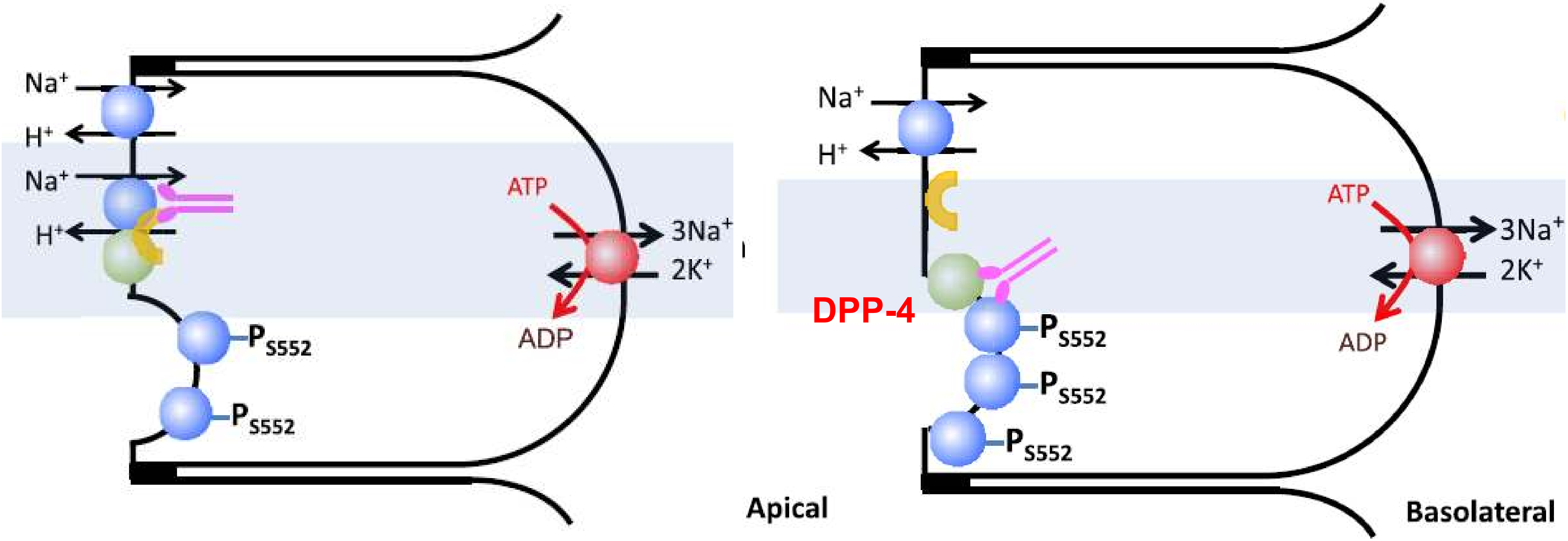
↑Podocyte apoptosis
↑Foot process retraction
↑Na^+/H^+E3 activity
PTC dysfunction

LVH, diastolic dysfunction, ↓ischemia reconditioning

Endothelial dysfunction

Hypertension Proteinuria

DPP-4 and Na^+/H^+ Exchanger type 3 (NHE3) in renal proximal tubule cells



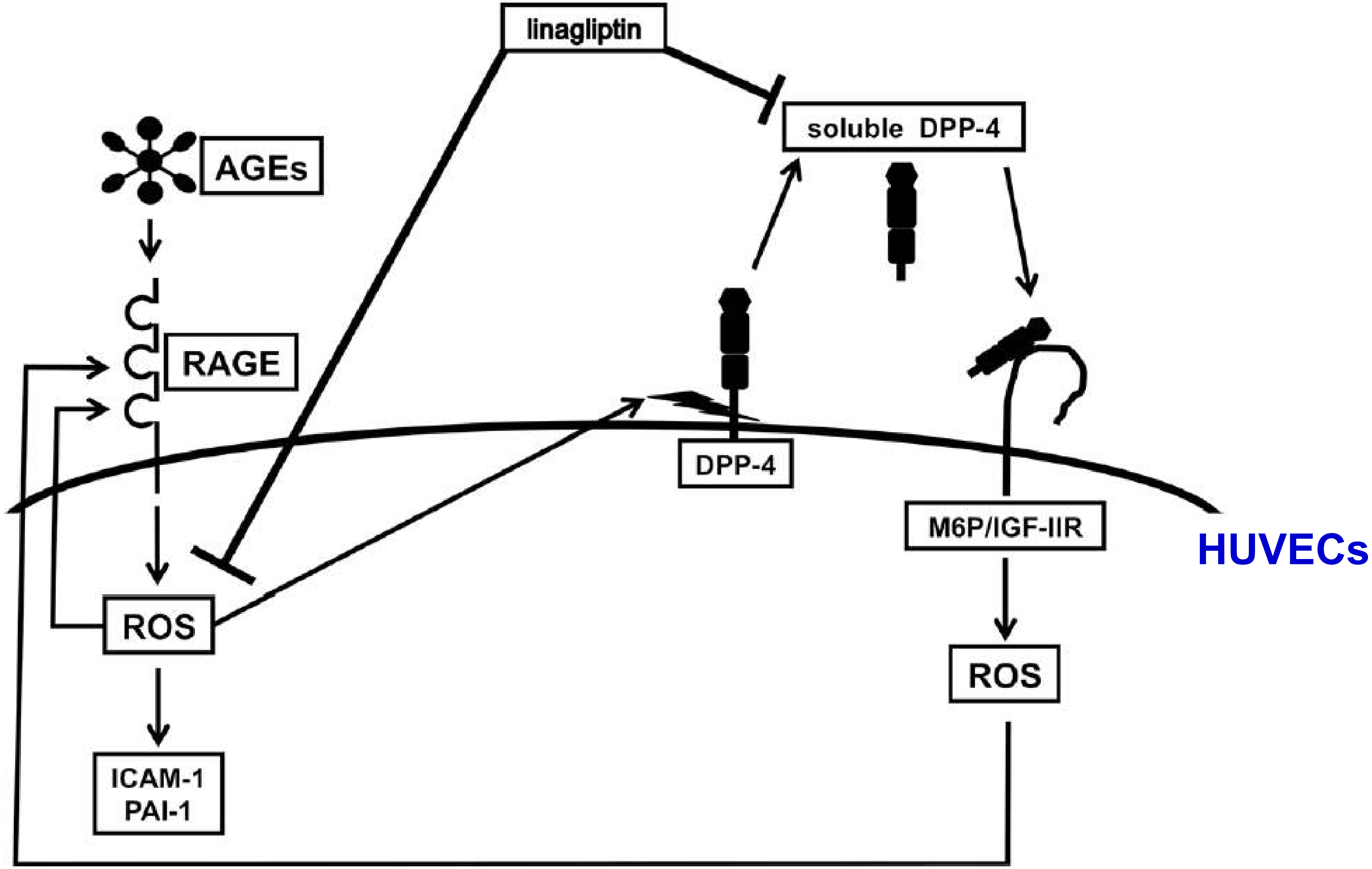
High glucose, or AG-II, or PPARγ activation increases NHE3 activity in renal proximal tubular cells

Adriana C. C. Girardi and Francesca Di Sole : *Am J Physiol Cell Physiol 302: C1569–C1587, 2012*

DPP-4 substrates and CV effects of DPP4 inhibition

SDF-1α(1-68)	**VIP(1-28)**	**BNP(1-32)**	**NPY(1-36)**
CXCR4, CXCR7 Akt Erk1/2 VEGF Stem cell homing Angiogenesis Tissue repair Cell survival ↓IRI ↓Apoptosis	VAPC 1 & 2 PAC1 Vasodilation Antiinflammation Anti-oxidative ↓PL A2 activity Inotropic Chronotropic ↓IRI	NOS/NO/cGMP Natriuresis Vasodilation Anti-aldosterone ↓IRI ↓Apoptosis	Y1 Rc Vasoconstriction VSMC Proliferation ↓ Lipolysis

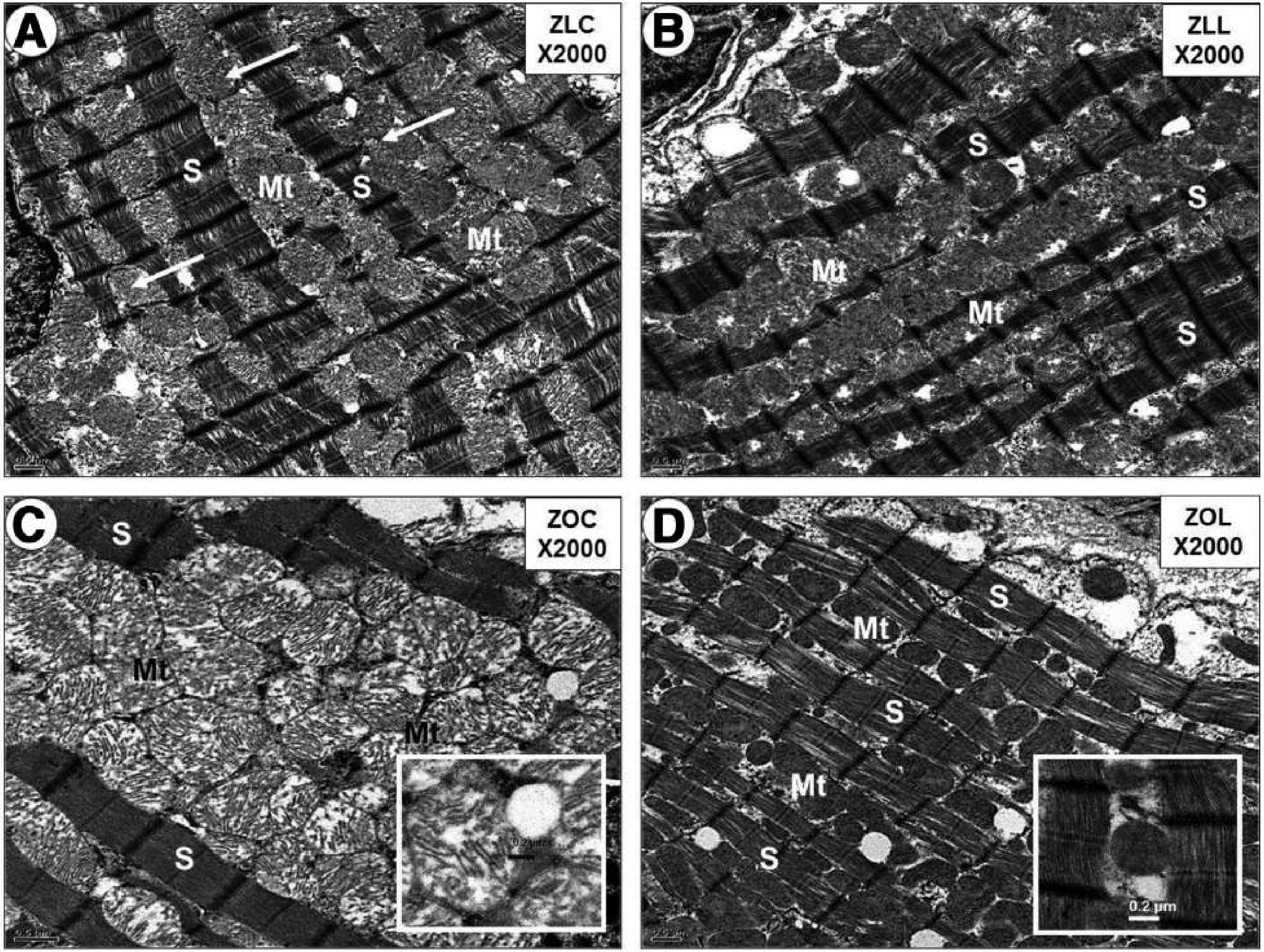
AGE-RAGE axis and soluble DPP-4



* Serum levels of AGEs are independently correlated with serum DPP4 in humans., Tahara N et al., 2013

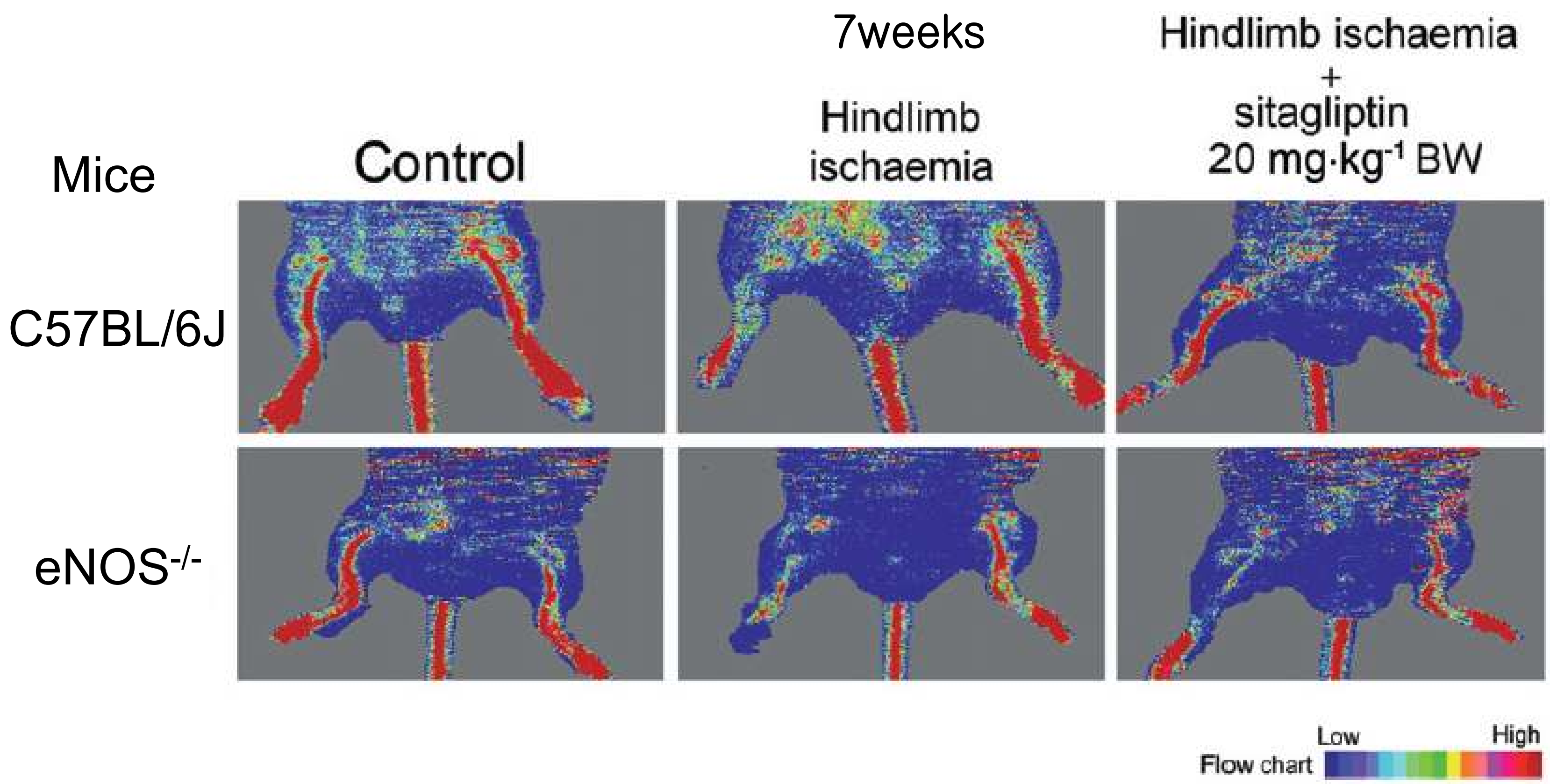
Ishibashi Y et al. Cardiovascular Diabetology 2013, 12:125

Linagliptin on Zucker Obese rats (Heart)



Aroor AR et al., *Endocrinology* 154:2501–2513, 2012

DPP-4 inhibitor improves neovascularization



Increases SDF-1
Increase circulating levels of EPCs
Enhanced the expression of CD 34 and eNOS in ischaemic muscle.

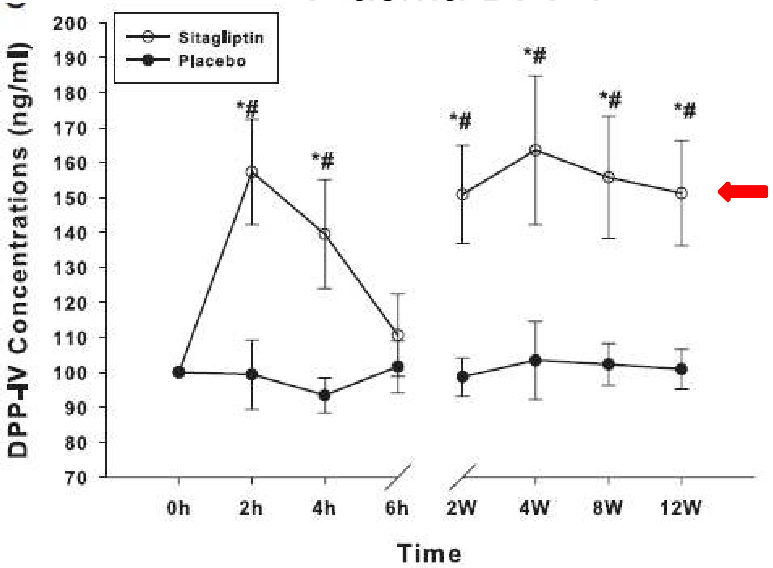
Huang CY et al., British Journal of Pharmacology (2012) 167 1506–1519

CV effects of DPP-4i in patients with T2DM

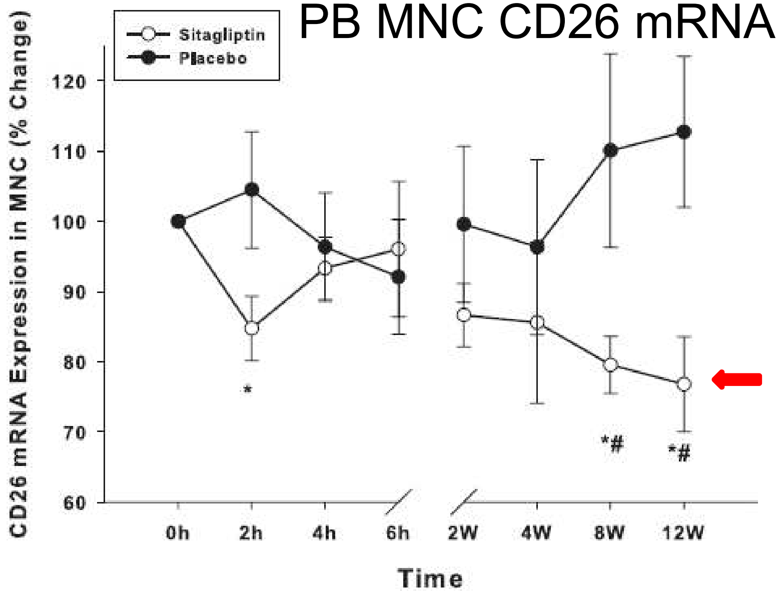
- Modest reduction or no change in blood pressure
- Reduction in postprandial lipemia, and T-cholesterol
- Improvement of liver fibrosis index (linagliptin)
- Reduction in the levels of hsCRP, IL-6, and IL-18
- Reduction in the level of nitrotyrosine
- Increases circulating endothelial progenitor cells
- Improved flow-mediated dilatation
- Reduction in platelet aggregation
- No positive results in EAMINE and SAVER-TIMI53 trials

Anti-inflammatory actions of Sitagliptin (6h, 12W)

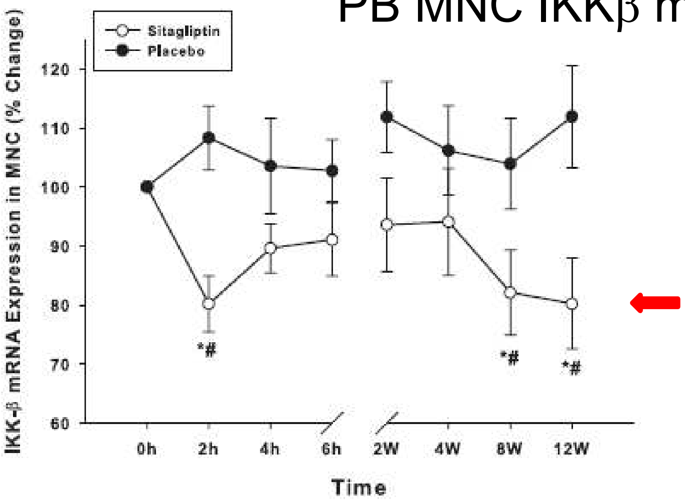
Plasma DPP4



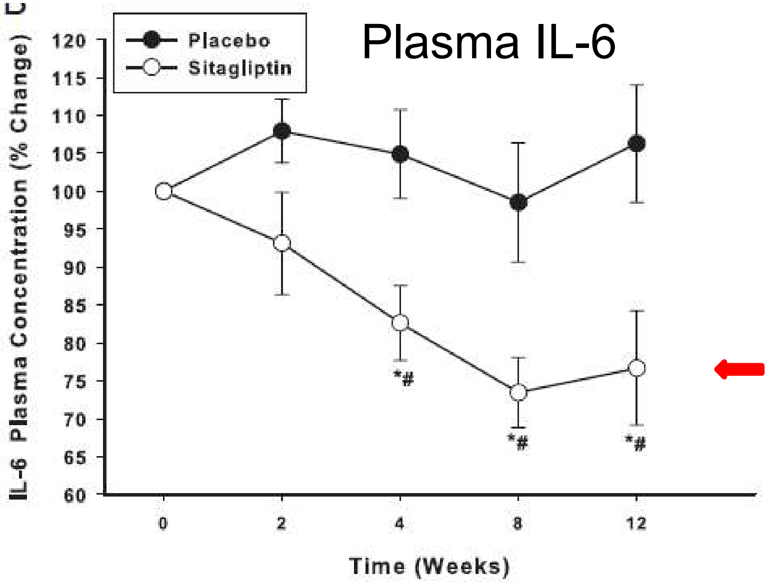
PB MNC CD26 mRNA



PB MNC IKKβ mRNA

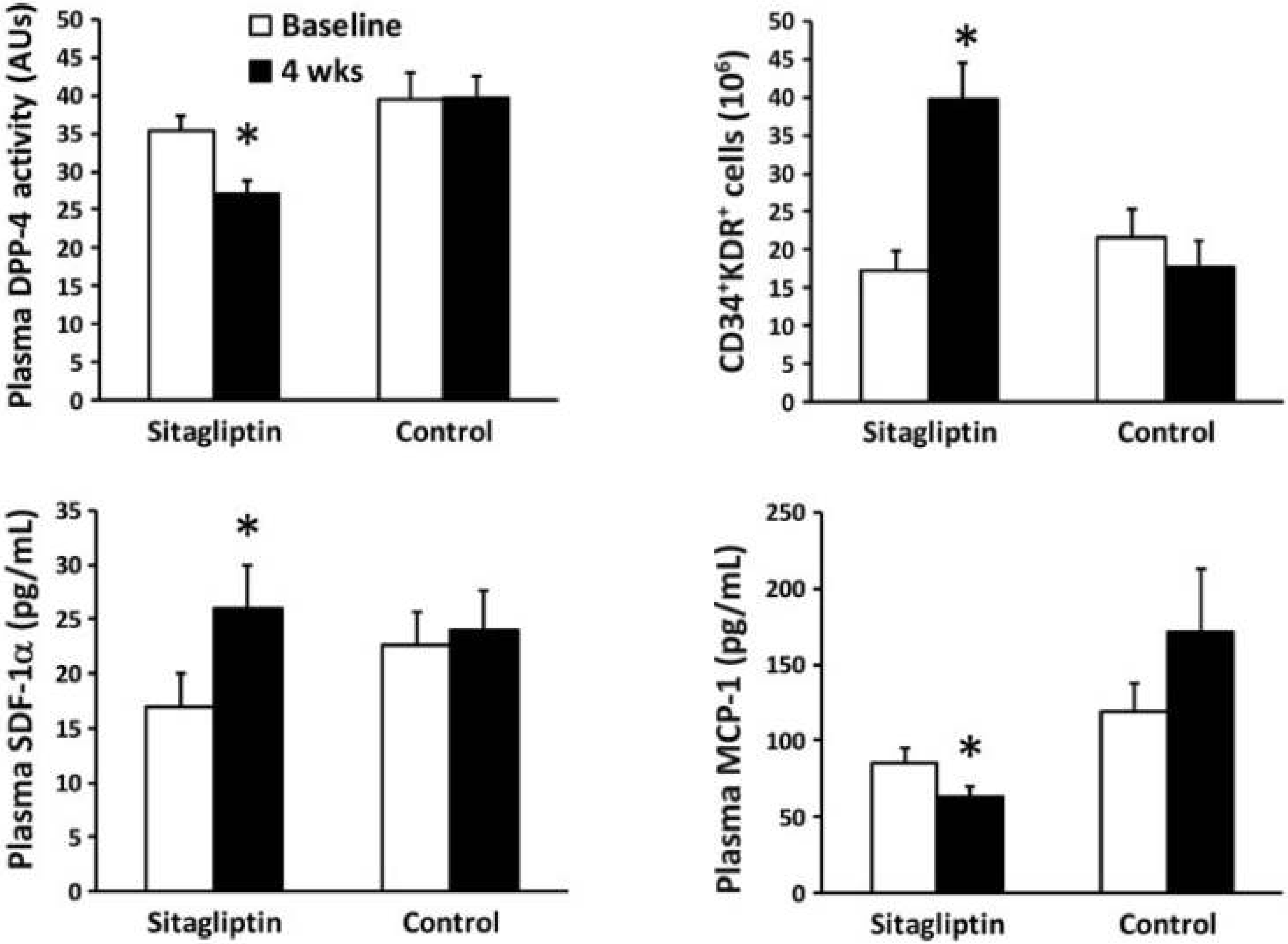


Plasma IL-6



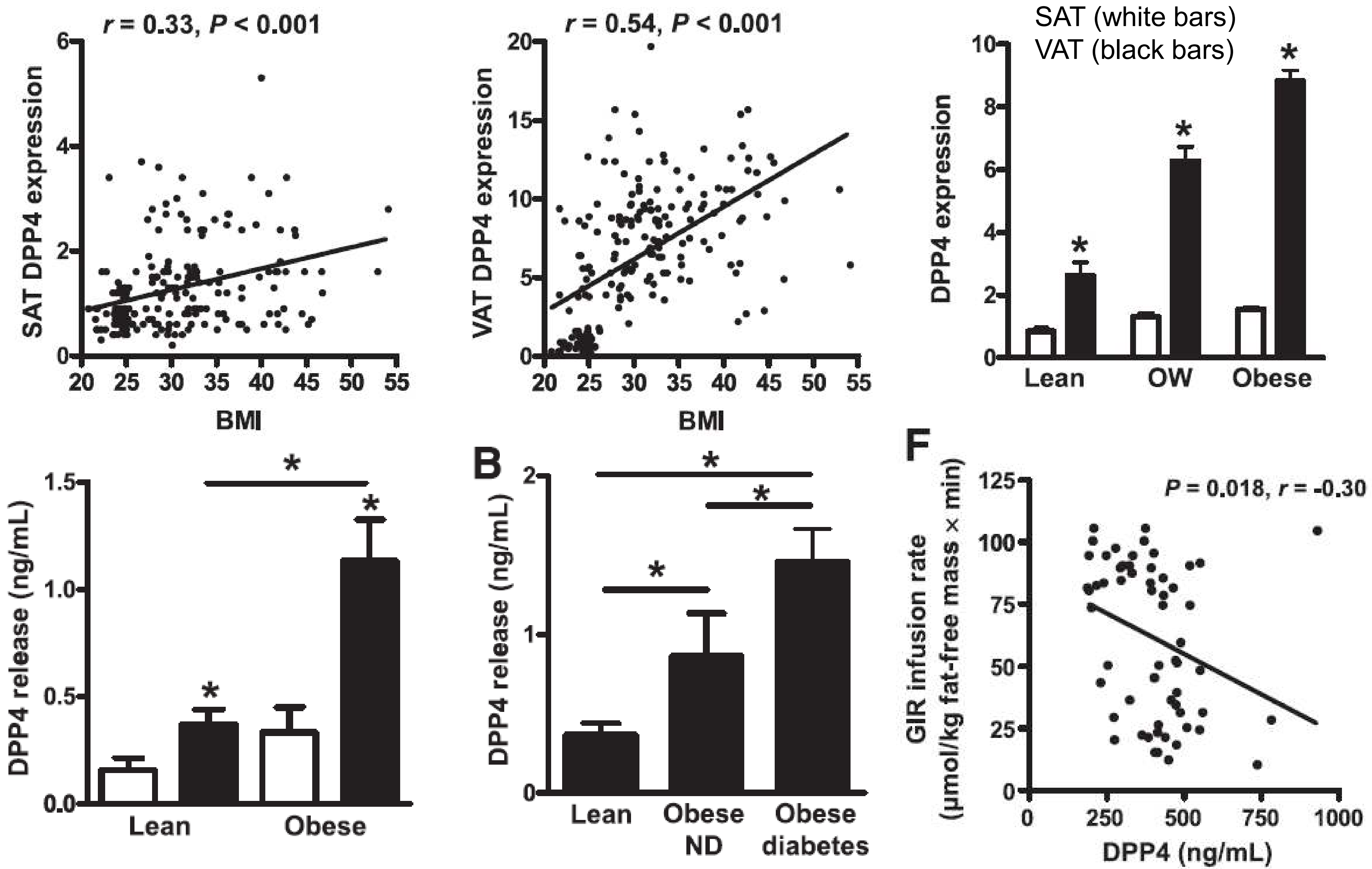
Makdissi A et al., JCEM 2012

Effects of Sitagliptin on circulating EPCs and SDF1α in patients with T2DM



Fadini GP et al Diabetes Care 33:1607–1609, 2010

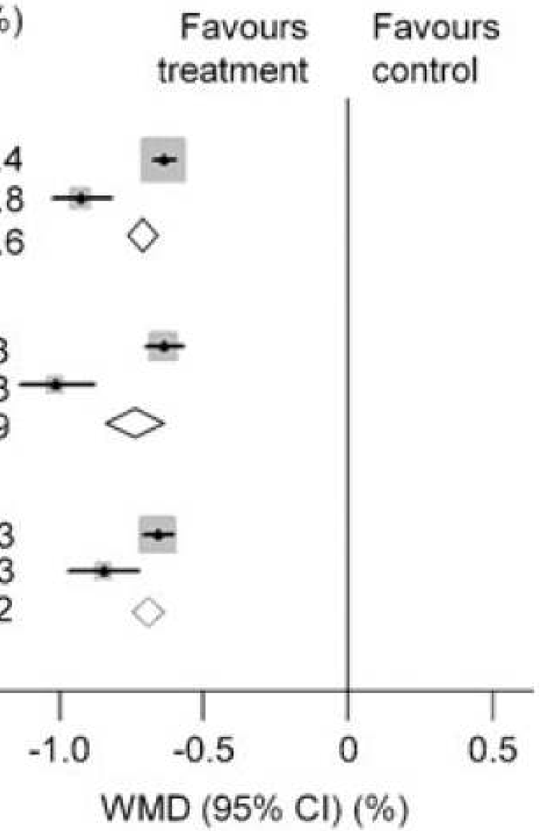
Adipose DPP-4 and Obesity

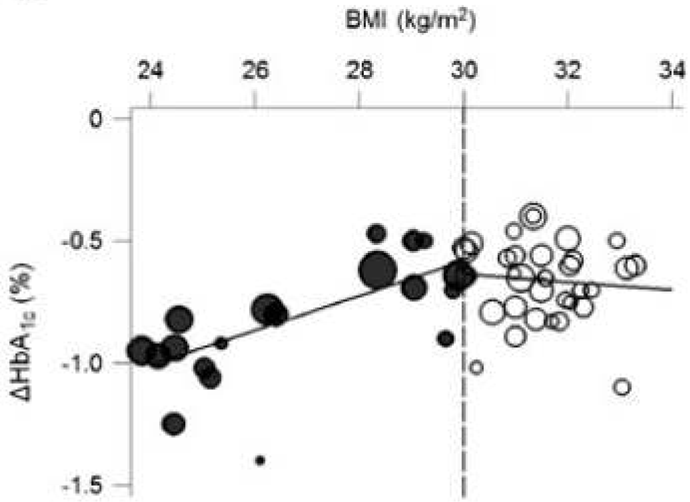


SELL H et al., Diabetes Care (in press)

BMI and DPP-4 inhibitors in Asians

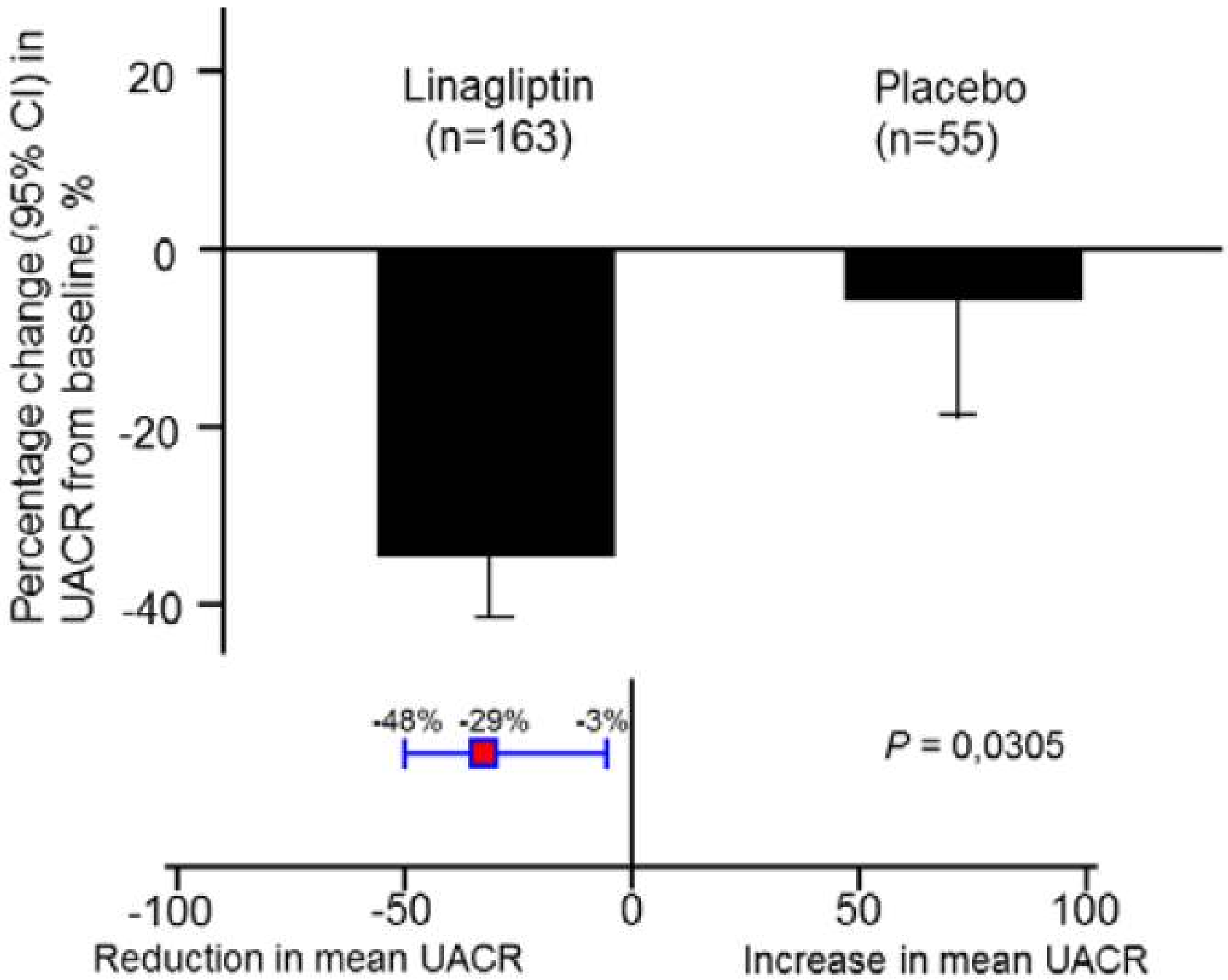
Subgroup	No. of studies (comparisons)	No. of patients Treatment	No. of patients Control	HbA_{1c} WMD (95% CI) (%)	I^2 (%)
Overall studies					
Non-Asian-dominant studies	41 (44)	7,639	6,145	–0.65 (–0.69, –0.60)	35.4
Asian-dominant studies	13 (13)	2,050	1,357	–0.92 (–1.03, –0.82)	74.8
Combined	54 (57)	9,689	7,502	–0.72 (–0.77, –0.67)	67.6
Monotherapy studies					
Non-Asian-dominant studies	15 (15)	1,944	1,533	–0.64 (–0.70, –0.57)	13.8
Asian-dominant studies	6 (6)	675	498	–1.01 (–1.14, –0.88)	58.3
Combined	21 (21)	2,619	2,031	–0.74 (–0.84, –0.64)	73.9
Oral combination therapy studies					
Non-Asian-dominant studies	27 (29)	5,695	4,612	–0.66 (–0.71, –0.60)	44.3
Asian-dominant studies	7 (7)	1,375	859	–0.85 (–0.97, –0.72)	72.3
Combined	34 (36)	7,070	5,471	–0.70 (–0.76, –0.65)	61.2





Kim YG et al, Diabetologia 2013

Effect of Linagliptin on proteinuria in patients with overt diabetic nephropathy: Pooled analysis



Hochera B et al., Kidney Blood Press Res 2012;36:65-84

CD26/DPP4 levels in Peripheral Blood and T cells in Patients with Type 2 Diabetes Mellitus

LEE SA et al., J Clin Endocrinol Metab. 2013

Objective

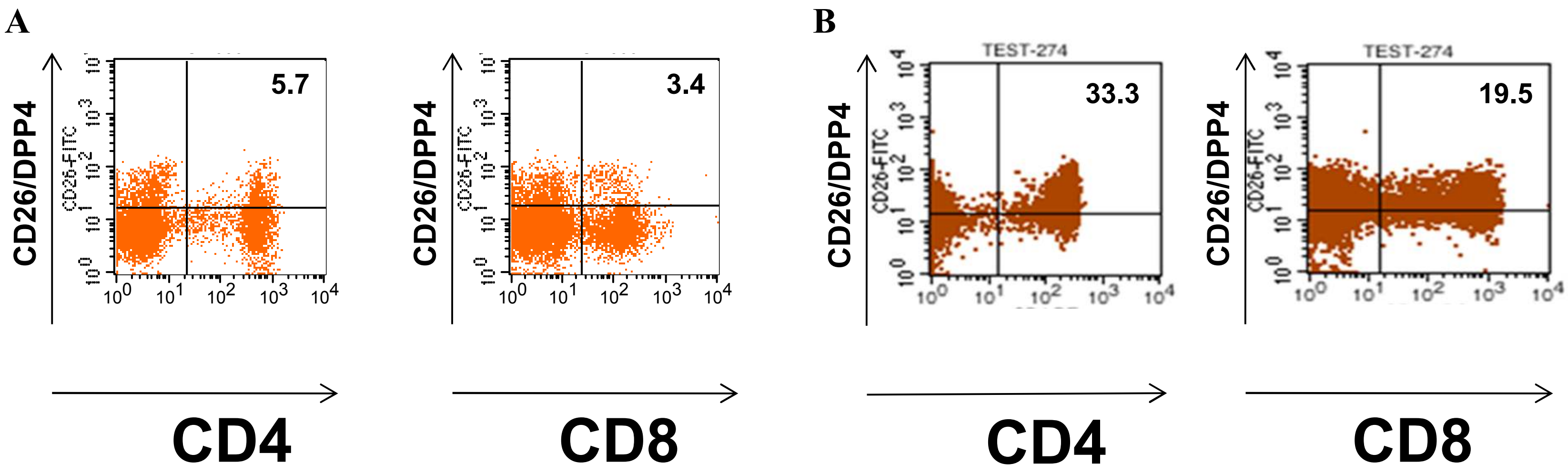
We aimed to evaluate the CD26/DPP4 expression on T cells and the serum DPP4 activity and sCD26/DPP4 level in patients with type 2 diabetes (T2DM) in relation to the degree of metabolic control.

Clinical characteristics of study groups

	Control (n= 50)	**Type2 DM** (n= 148)	***P* value**
Age (years)	44.6 ± 2.4	54.4 ± 1.1	< 0.01
Sex (female,%)	38.0	34.8	0.21
Weight (Kg)	68.1 ± 2.9	71.7 ± 1.2	0.53
BMI (kg/m^2)	25.9 ± 1.2	26.3 ± 0.4	0.89
WBC (x 10^3/mm^3)	6.3 ± 0.5	6.6 ± 0.2	0.64
Lymphocyte (x 10^3/mm^3)	1.9 ± 0.1	2.3 ± 0.1	0.04
Glucose (mg/dL)	95.8 ± 3.3	202.6 ± 6.8	< 0.01
HbA1c (%)	5.4 ± 0.1	9.7 ± 0.2	< 0.01
ALT (IU/L)	18.6 ± 2.1	35.1 ± 2.2	0.01
hsCRP (mg/dL)	0.1 ± 0.0	0.3 ± 0.1	0.16

BMI; body mass index, hsCRP; high- sensitivity C-reactive protein, WBC; white bl ood cell

Supplemental Figure 1

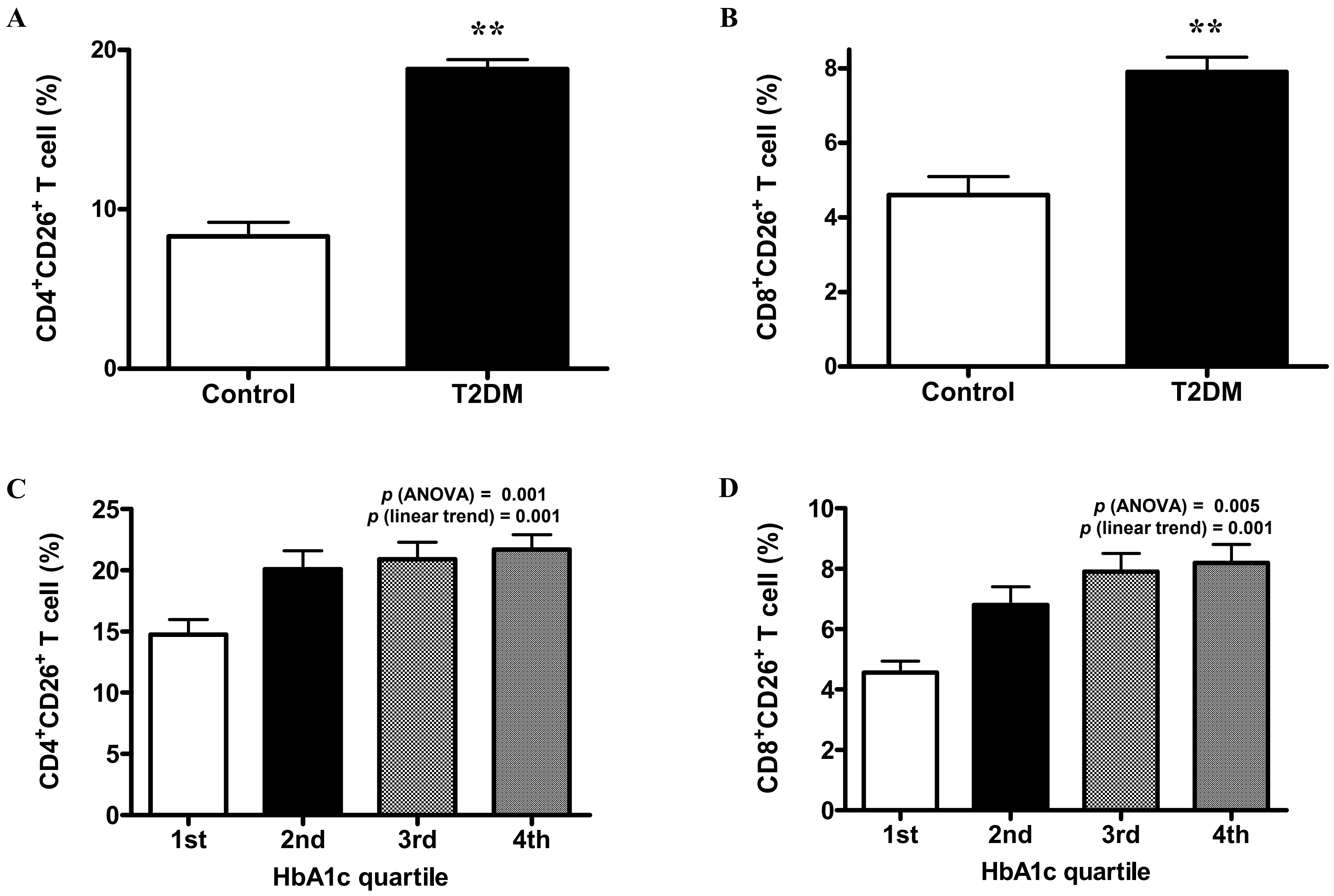


A. Healthy subjects (fasting blood glucose 103 mg/dL, HbA1c 5.2%)

B. T2DM patient (fasting blood glucose 262 mg/dL, HbA1c 11.7%)

LEE SA et al., J Clin Endocrinol Metab. 2013

The CD26 expression on CD4⁺ and CD8⁺ T cells



Serum sCD26 and DPP4 activity

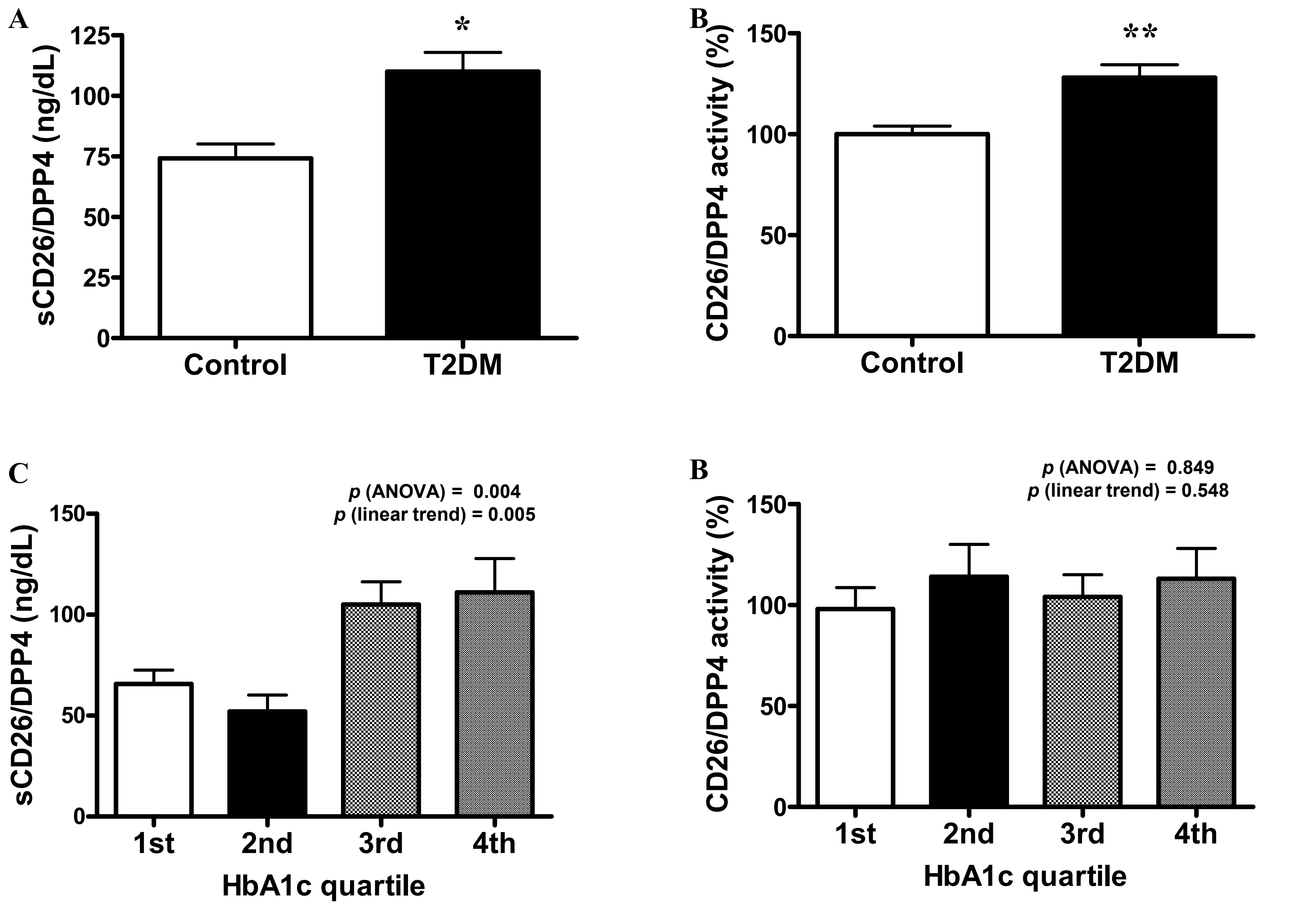
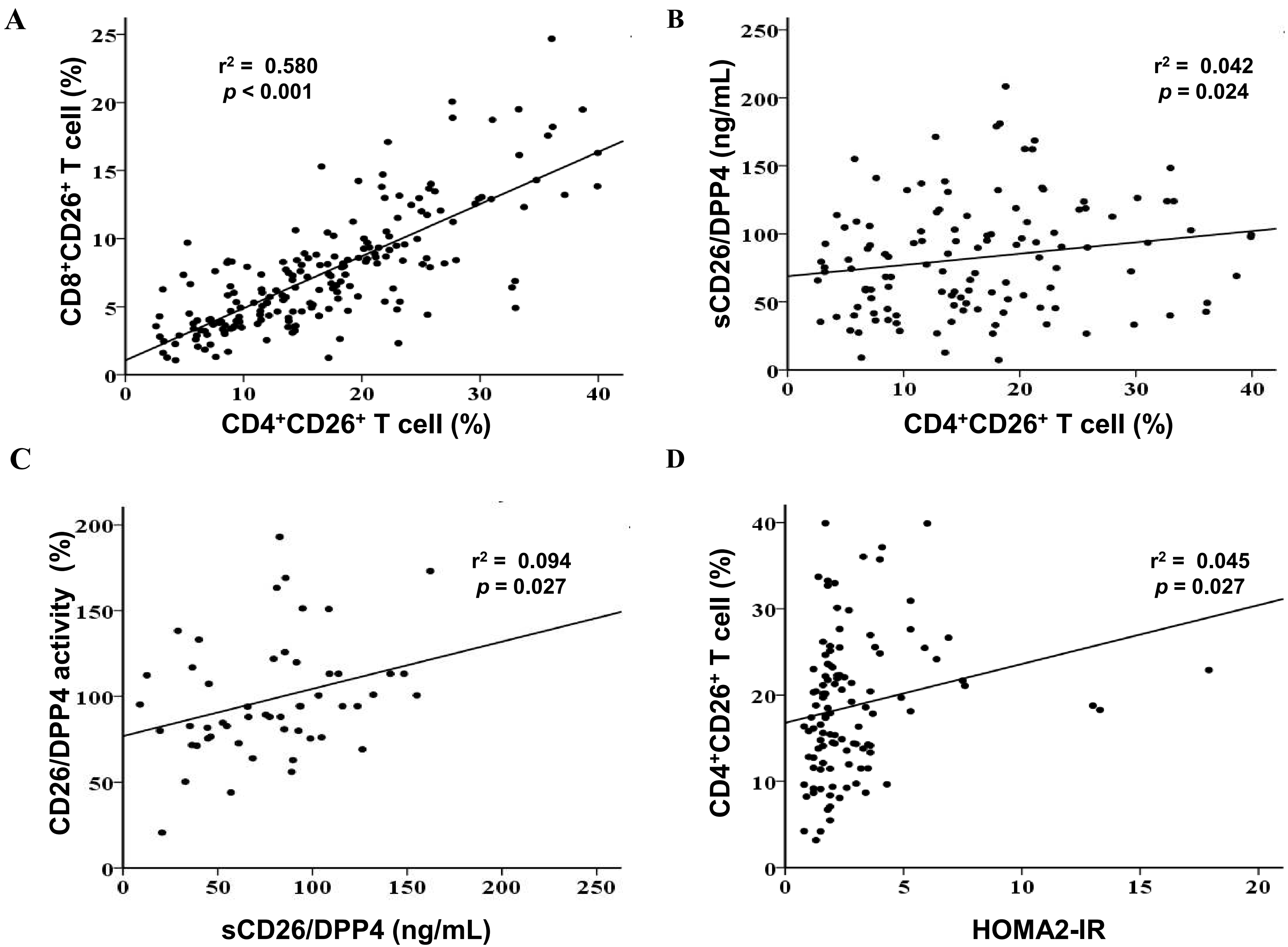


Figure 3



A
r² = 0.580
p < 0.001
CD8⁺CD26⁺ T cell (%)
CD4⁺CD26⁺ T cell (%)
B
r² = 0.042
p = 0.024
sCD26/DPP4 (ng/mL)
CD4⁺CD26⁺ T cell (%)
C
r² = 0.094
p = 0.027
CD26/DPP4 activity (%)
sCD26/DPP4 (ng/mL)
D
r² = 0.045
p = 0.027
CD4⁺CD26⁺ T cell (%)
HOMA2-IR

Independent factors associated with CD26 expression on $CD4^+$ T cells.

Multivariate analysis (dependent factor $CD4^+CD26^+$ T cells)		
Factors	β	*p* value
Age (years)	0.141	0.099
Sex (F)	- 0.032	0.714
BMI (kg/m^2)	- 0.103	0.225
HbA1c (%)	0.414	0.001*
ALT (IU/L)	0.266	0.054
Triglyceride (mg/dL)	0.142	0.092

Independent factors associated with CD26 expression on $CD8^+$ T cells.

Multivariate analysis (dependent factor $CD8^+CD26^+$ T cells)		
Factors	β	*p* value
Age (years)	0.059	0.498
Sex (F)	0.027	0.756
BMI (kg/m^2)	- 0.074	0.378
HbA1c (%)	0.351	0.001*
HDL (mg/dL)	- 0.125	0.132

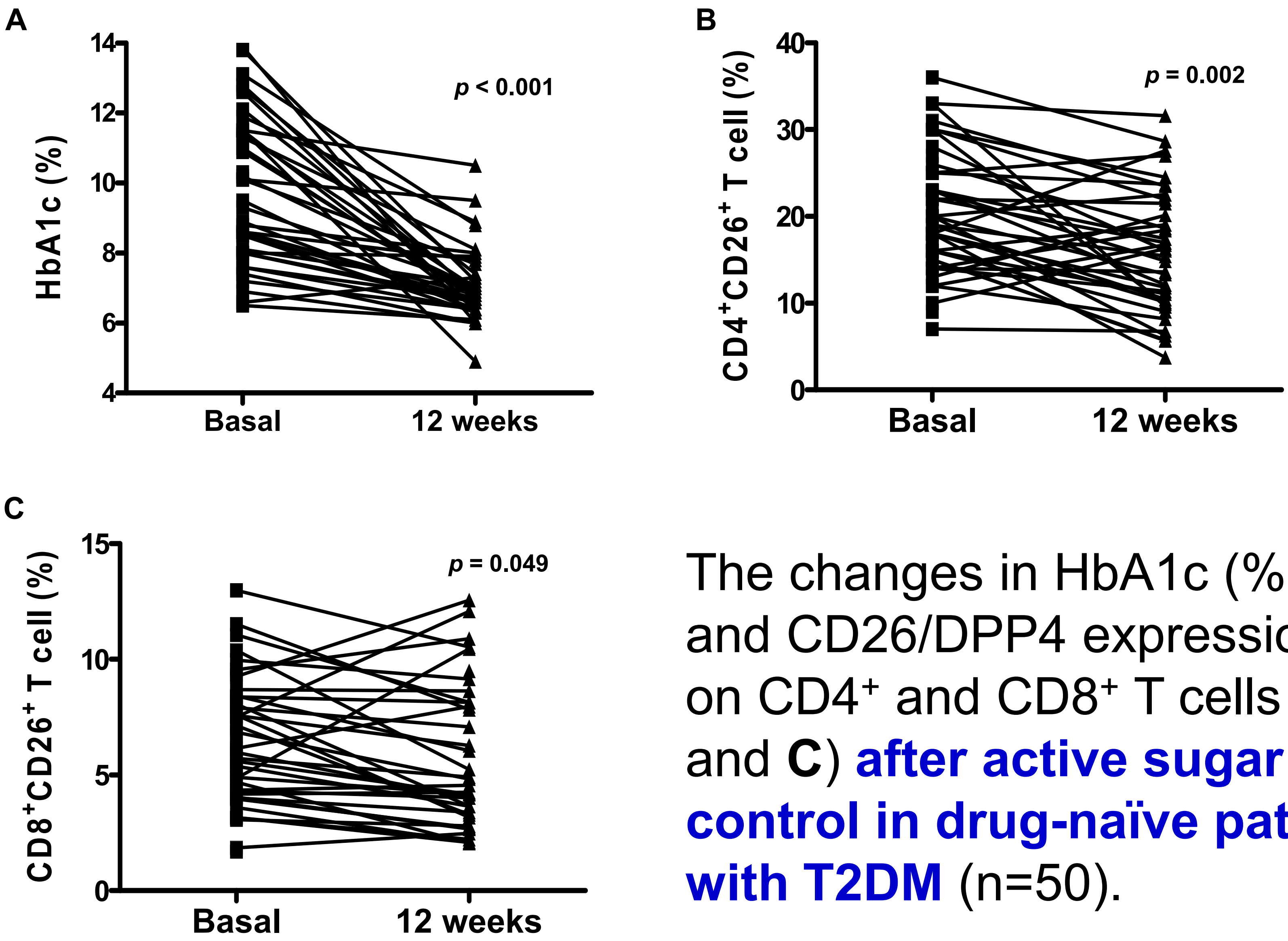
Independent factors associated with serum sCD26 level

Multivariate analysis (dependent factor sCD26/DPP4)		
Factors	β	*p* value
HbA1c (%)	0.315	0.025*
HOMA2-IR	0.281	0.042*
LDL (mg/dL)	0.166	0.160
$CD4^+CD26^+$ T cell (%)	0.112	0.534
$CD8^+CD26^+$ T cell (%)	0.180	0.297

Independent factors associated with serum DPP4 activity

Multivariate analysis (dependent factor DPP4 activity)		
Factors	β	*p* value
ALT (IU/L)	0.442	0.001*
HOMA2-IR	0.279	0.019*
γGT (IU/L)	0.549	0.001*

Figure 4



The changes in HbA1c (%) (**A**) and CD26/DPP4 expressions on $CD4^+$ and $CD8^+$ T cells (**B** and **C**) **after active sugar control in drug-naïve patients with T2DM** (n=50).

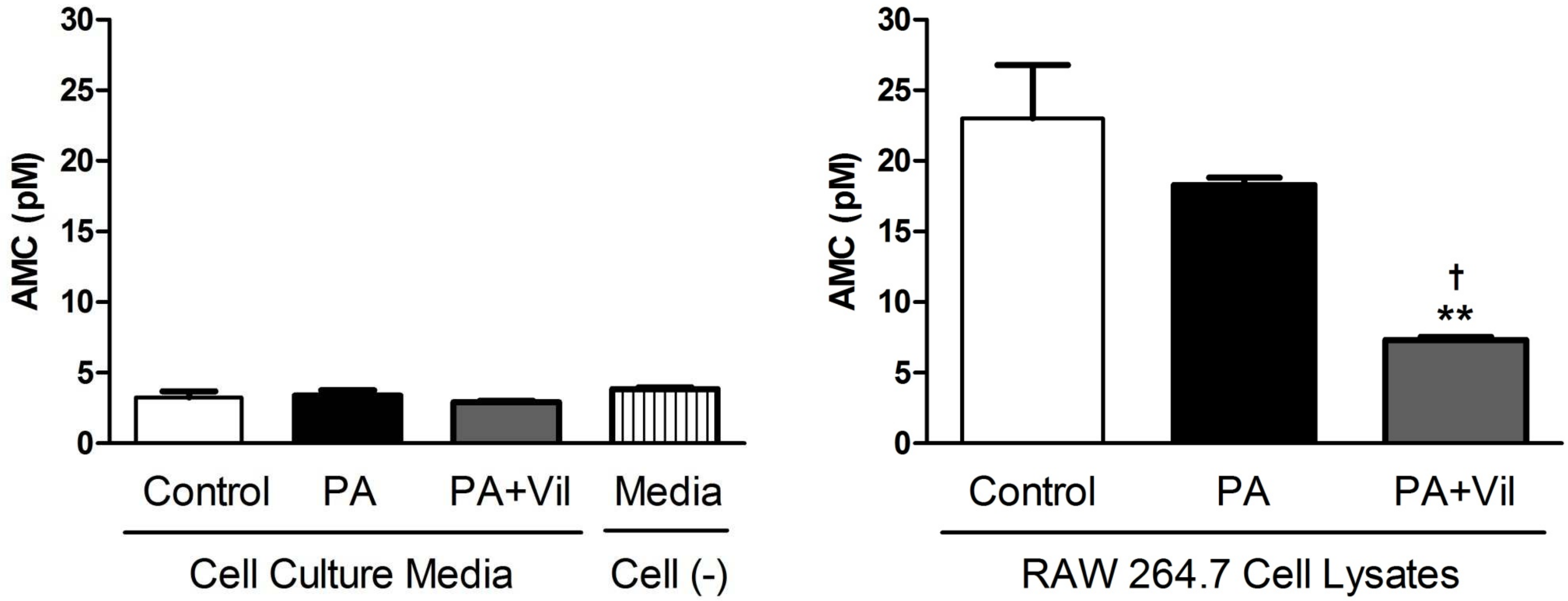
Summary

Our results suggest that CD26/DPP4 expression on inflammatory cells is not only affected by metabolic control in patients with T2DM, but also may affect metabolic control via its various non-glycemic actions

DPP-4 is involved in the inflammatory reactions of stimulated macrophages

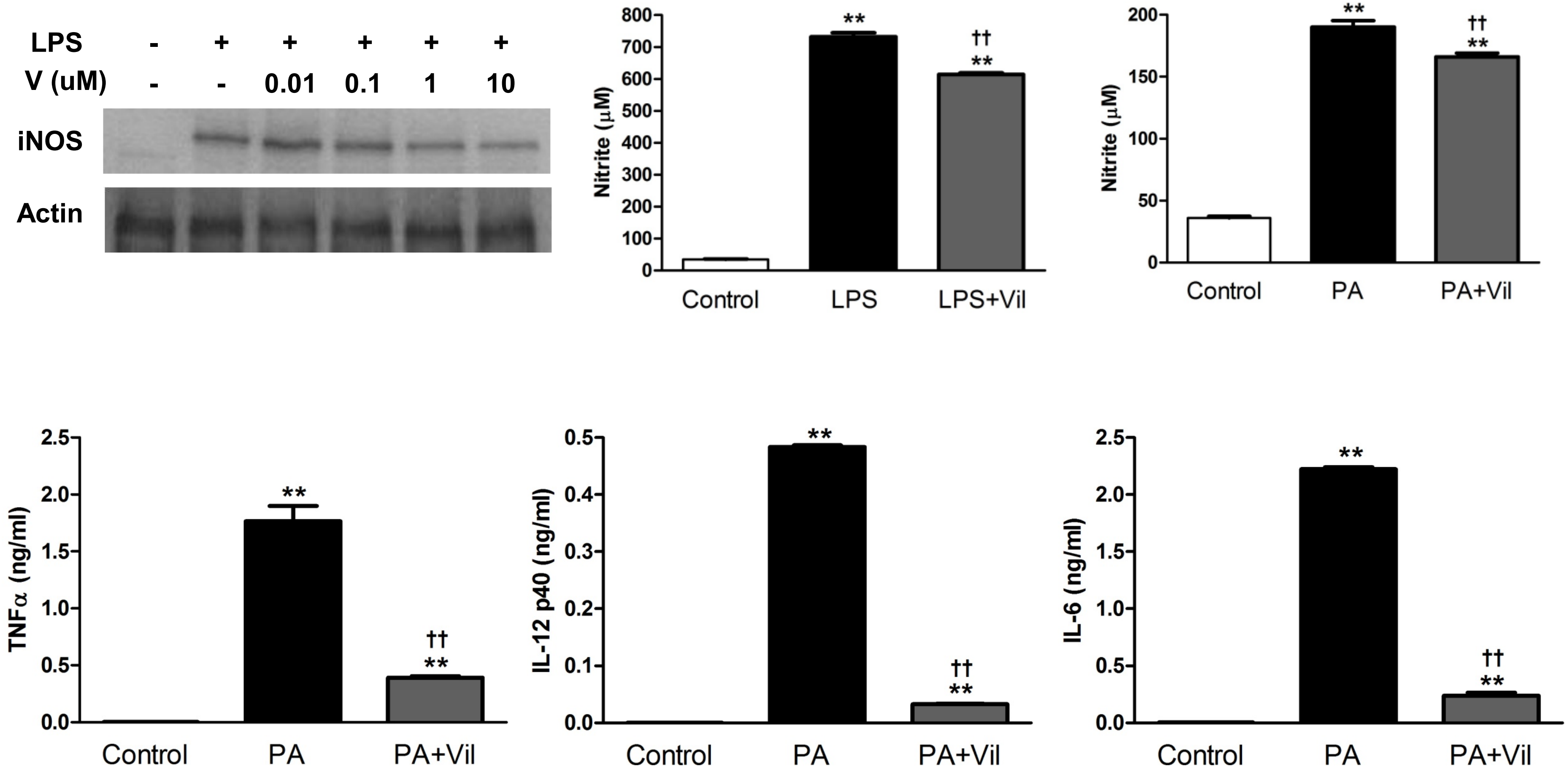
Unpublished data

DPP4 activity in Raw 264.7 cell and culture media



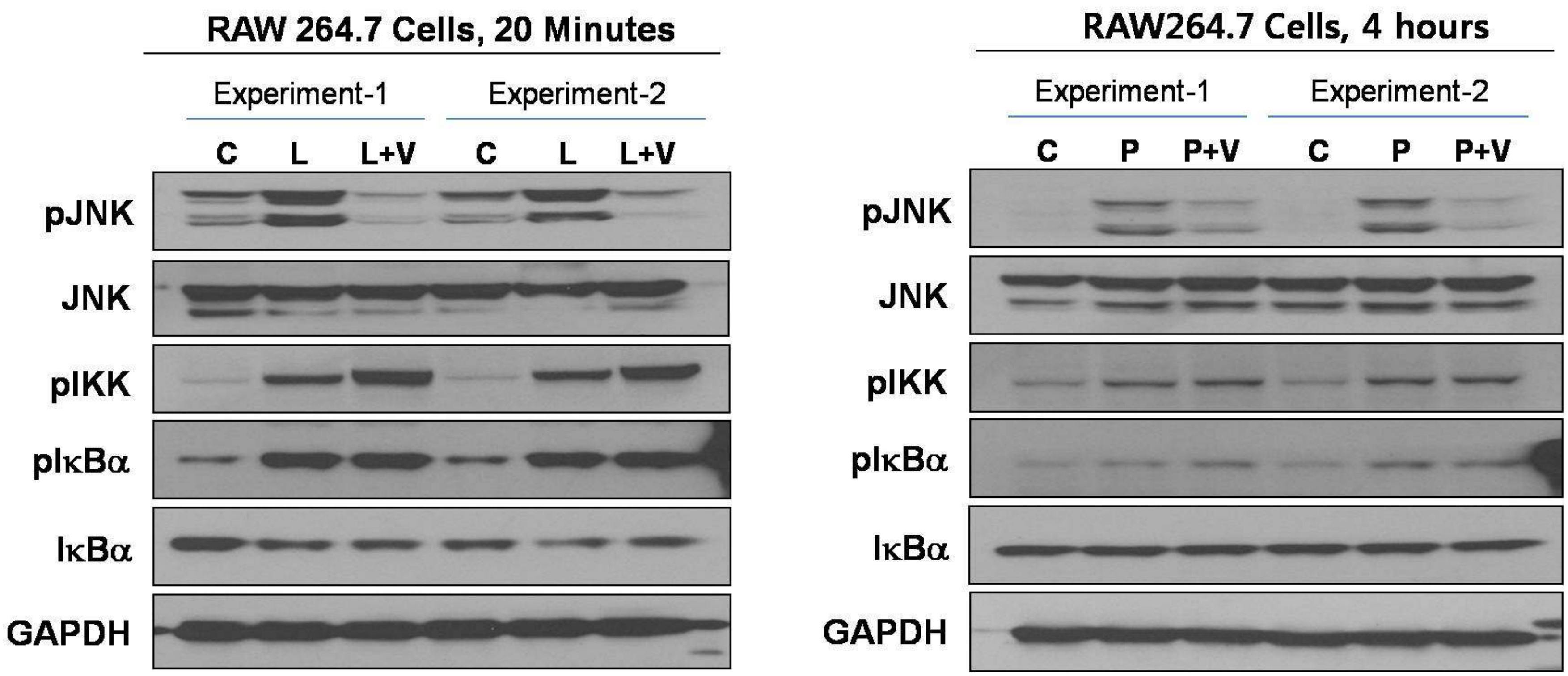
V, Vildagliptin 10 μM; PA, Palmitic acid (400 μM), 24 hr
Unpublished data

NO (Raw264.7) and Cytokine (BMDM) production in stimulated cells



LPS,LPS 10ng/ml; PA, Palmitic acid (300 μM); V, Vildagliptin 10 μM; 24 hr
Unpublished data

Effect of DPP4 inhibitor on the LPS- or Palmitate-stimulated RAW264.7 cells



L,LPS 10ng/ml; P, Palmitate 400 μM; V, Vildagliptin 10 μM
Unpublished data

Conclusions

- DPP4 has various non-glycemic actions and may be a marker of insulin resistance
- Inflammation, oxidative stress, sodium regulation, and other pathways mediated by DPP4 seem to be involved in the development of CV and renal dysfunction
- DPP4 inhibitors have pleiotropic effects including direct anti-inflammatory effect
- Further studies on the molecular mechanisms, its substrates, and other DPP members are needed.

Acknowledgements

Jeju National University School of Medicine

Sang Ah Lee, Young Ree Kim, Gwanpyo Koh

Doek Bae Park

Wonkwang University School of Medicine

Eun Sol LEE
S.KARUPPASAMY