ONLINE APPENDIX - Supplementary Materials
Experimental Procedures

Immunofluorescence staining and flowcytometric analysis— To determine the purity of isolated $CD4^+$ T lymphocytes, $CD4^+$ T cells from the spleen, thymus and LN were treated with Fc receptor blocking CD16/32 antibody and subsequently incubated with PE anti-CD3, APC anti-CD4 or PerCP anti-CD8 antibodies (BioLegend, San Diego, CA) for 30 mins at 4°C. Following washing steps, cells were analyzed with an LSRII and a FACS Diva software (BD Biosciences, San Diego, CA).

Rac1 pull-down activation assay— GTP-bound Rac1 was measured using a Rac1 activation assay kit (Upstate Technology) according to the manufacturer's instructions. Briefly, DPP-IV (100 mU/ml), GIP (100 nM) or GLP-1 (100 nM) treated $CD4^+$ T lymphocytes were lysed in ice-cold magnesium lysis buffer and cleared with glutathione-agarose beads. Cell extracts were then incubated with p21-binding domain (PBD, residues 67-150) of human PAK-1 agarose beads, the resulting beads were resuspended and separated on 15 % sodium dodecyl sulfate (SDS)/polyacrylamide gel. GTP-bound Rac1 was detected using an anti-Rac1 antibody.

Preparation of nuclear extracts— Nuclear proteins were isolated as described by Schreiber *et al* (1). Briefly, LN $CD4^+$ T cells were washed with PBS, and disrupted with 200 µl ice-cold buffer A (10 mM HEPES pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 0.1 % Nonidet P40 and protease inhibitors). Following centrifugation, the resulting pellet was re-suspended in 20 µl buffer B (20 mM HEPES, pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 20 % glycerol and protease inhibitors) and incubated on ice for 10 min. After clarification of the mixture by centrifugation, the supernatant (nuclear extract) was collected and subjected to an NFκB transcription factor activity assay.

NFκB transcription factor activity assay— An NFκB transcription factor activity assay kit (Millipore, MA) was used to measure DNA binding activity of NFκB transcription factor in nuclear extracts of LN $CD4^+$ T cells, according to the manufacturer's protocols. Briefly, nuclear extracts of LN $CD4^+$ T cells were incubated with biotinylated oligonucleotide containing the consensus sequence for NFκB binding (5'-GGGACTTTCC-3'), and the biotinylated oligonucleotide bound by active NFκB protein was immobilized. The bound NFκB transcription factor subunit p50, was detected with a specific primary antibody, α-NFκB p50 (active form), and subsequently with an HRP-conjugated secondary antibody. Nuclear NFκB activity is presented as relative activity normalized to protein concentration.

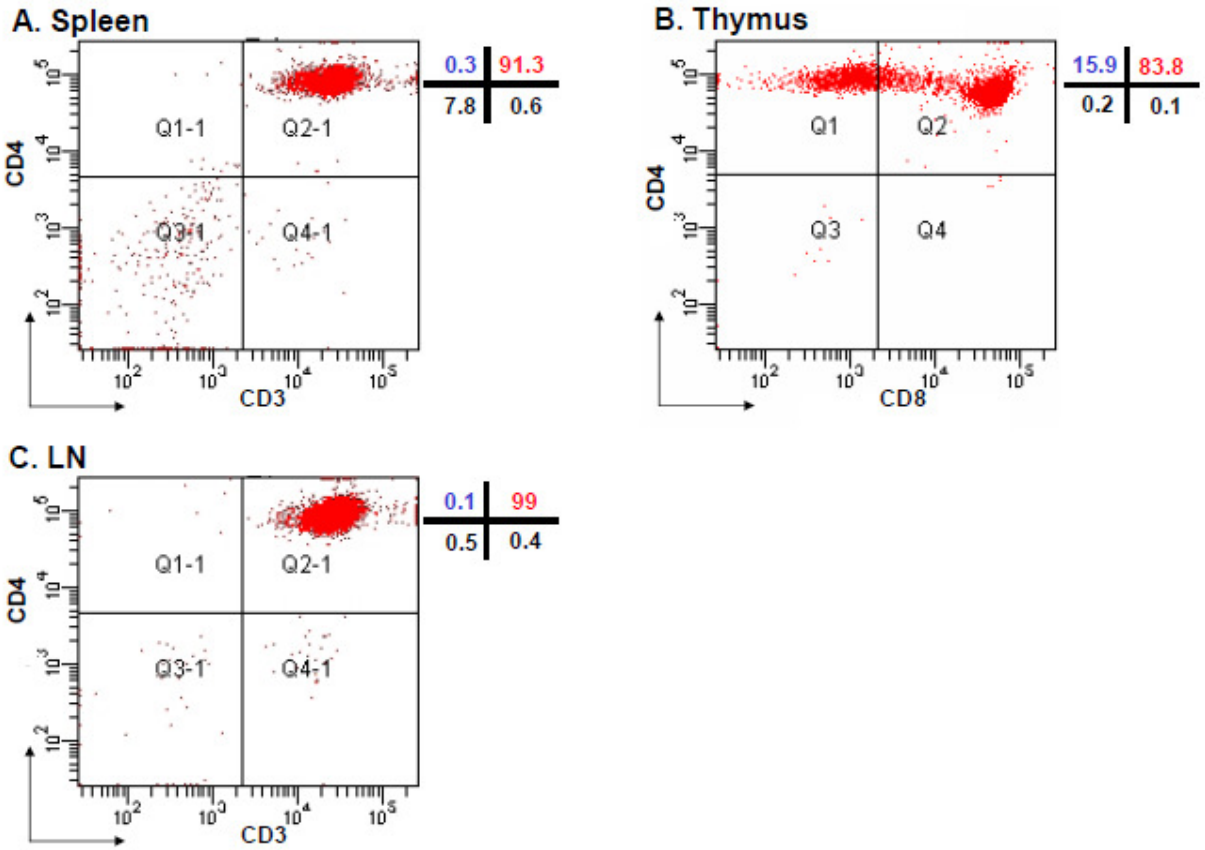
Western blot analysis— Total cellular or nuclear extracts were separated on a 15% sodium dodecyl sulfate (SDS)/polyacrylamide gel and transferred onto nitrocellulose membranes (Bio-Rad). Probing of the membranes was performed with antibodies against phospho-Cofilin (Serine3), Cofilin, Phospho-Ezrin (Threonine567)/Radixin (Threonine564)/Moesin (Threonine558), Ezrin/Radixin/Moesin, phospho-VASP (Serine157), phospho-VASP (Serine239), VASP, phospho-IκBα (Ser32), IκBα, phospho-NFκB p65 (Ser536), NFκB p65, phospho-IKKα/β (Ser176/180), IKKα, IKKβ, NFκB p50 and β-actin (Cell Signaling Technology, Beverly, MA), as identified in the figures. Immunoreactive bands were visualized by enhanced chemiluminescence

(Amersham Pharmacia Biotech) using horseradish peroxidase-conjugated IgG secondary antibodies.

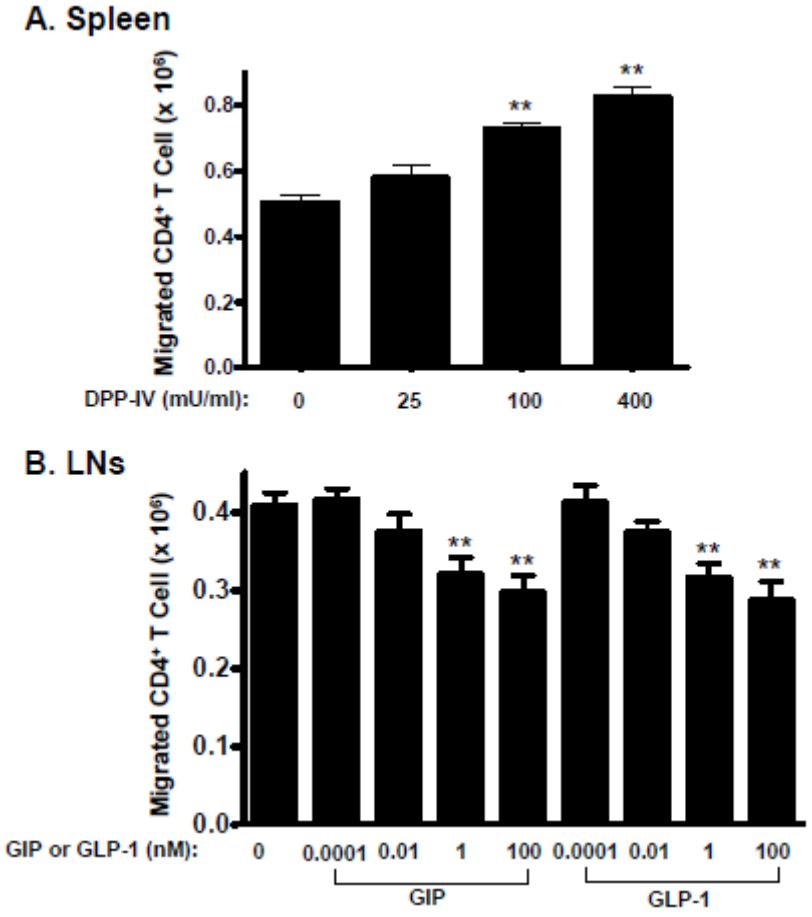
REFERENCES

1. Schreiber E, Matthias P, Müller MM, Schaffner W: Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. *Nucleic Acids Research* 17: 6419, 1989.

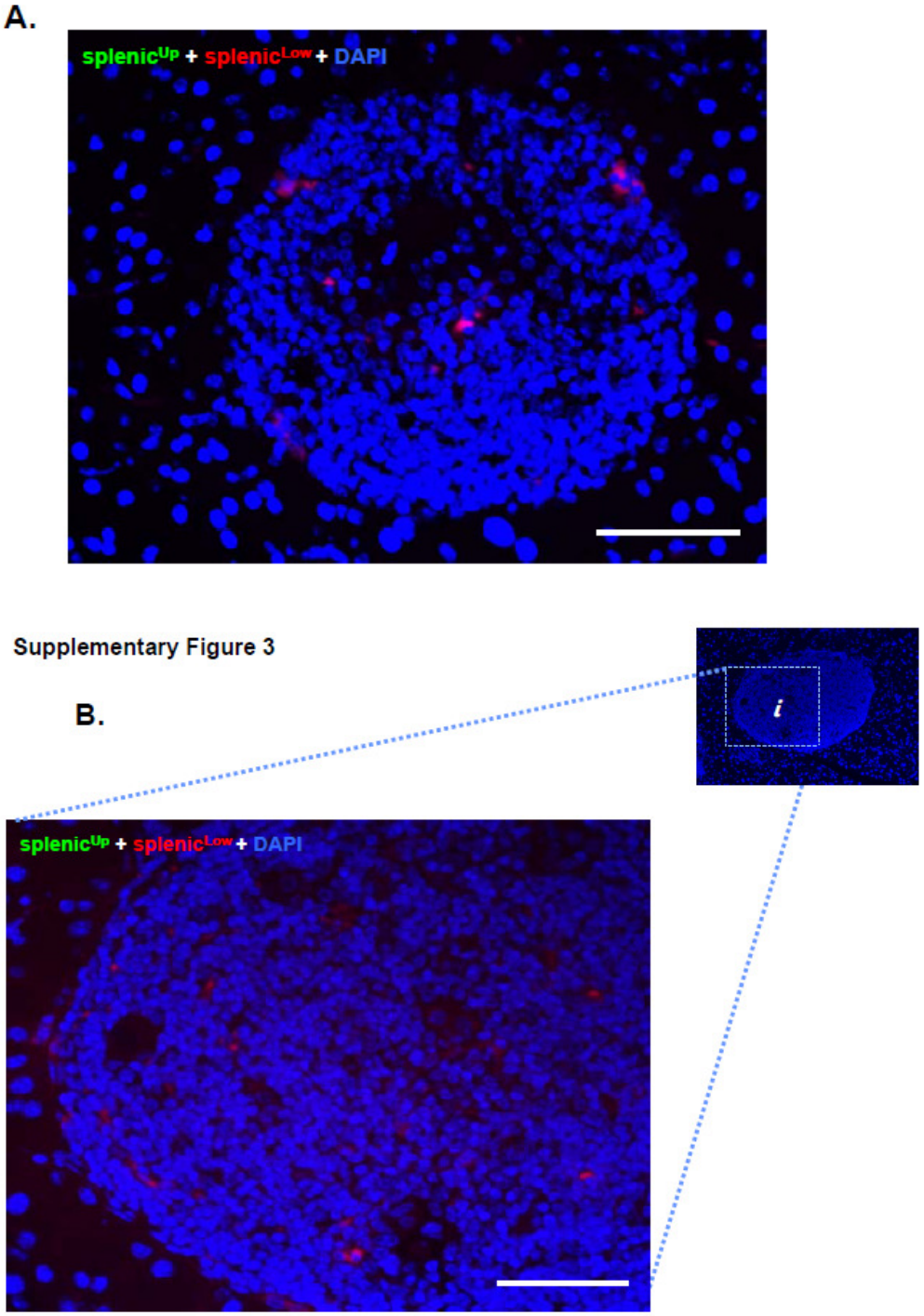
Supplementary Figure 1. Representative FACS profiles of purified CD4$^+$ T cells. For these, and all subsequent, studies presented as Supplementary Figures, CD4$^+$ T cells were positively isolated from non-diabetic female NOD mice receiving NCD. FACS profile of CD4$^+$ T cells from Spleen (A) and LN (C) in CD3$^+$CD4$^+$-gated events, and Thymus (B) in CD4$^+$ CD8$^+$-gated events.



Supplementary Figure 2. Concentration dependent effects of sDPP-IV and incretins on the migration of CD4$^+$ T cells. Splenic (A) or LN (B) CD4$^+$ T cells isolated from non-diabetic female NOD mice were incubated with indicated concentrations of sDPP-IV, GIP or GLP-1. The migration of CD4$^+$ T cells was determined using Transwell chamber (Corning) as described in *Experimental Procedures*. All data represent the mean ± S.E.M. and significance was tested using ANOVA with a Newman-Keuls post hoc test, where ** represents $p<0.05$ *vs* Control.



Supplementary Figure 3. Islet localization of labeled splenic (A-D) and LN (E-H) CD4$^+$ T lymphocytes following sDPP-IV or incretin stimulation. splenic^{Up} and splenic^{Low} (A-D) and LN^{Up} and LN^{Low} (E-H) = Splenic or LN lymphocytes from upper and lower chambers, respectively. Mixtures of DiI- and CFSE- labeled splenic (A-D) and LN (E-H) CD4$^+$ T lymphocytes were intravenously injected into diabetic recipient mice and pancreatic homing of labeled lymphocytes determined by confocal fluorescent microscopy. Infiltrated islets are represented with an *i*, and the scale bar indicates 25 μm.



Supplementary Figure 3

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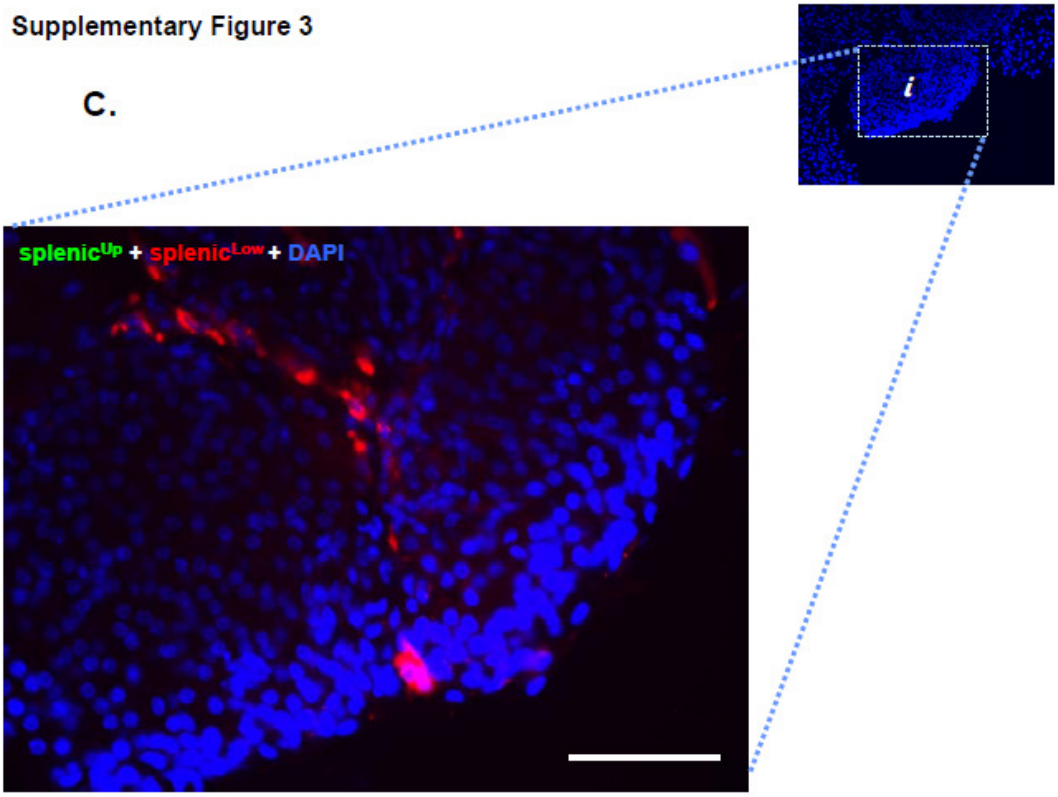

Supplementary Figure 3

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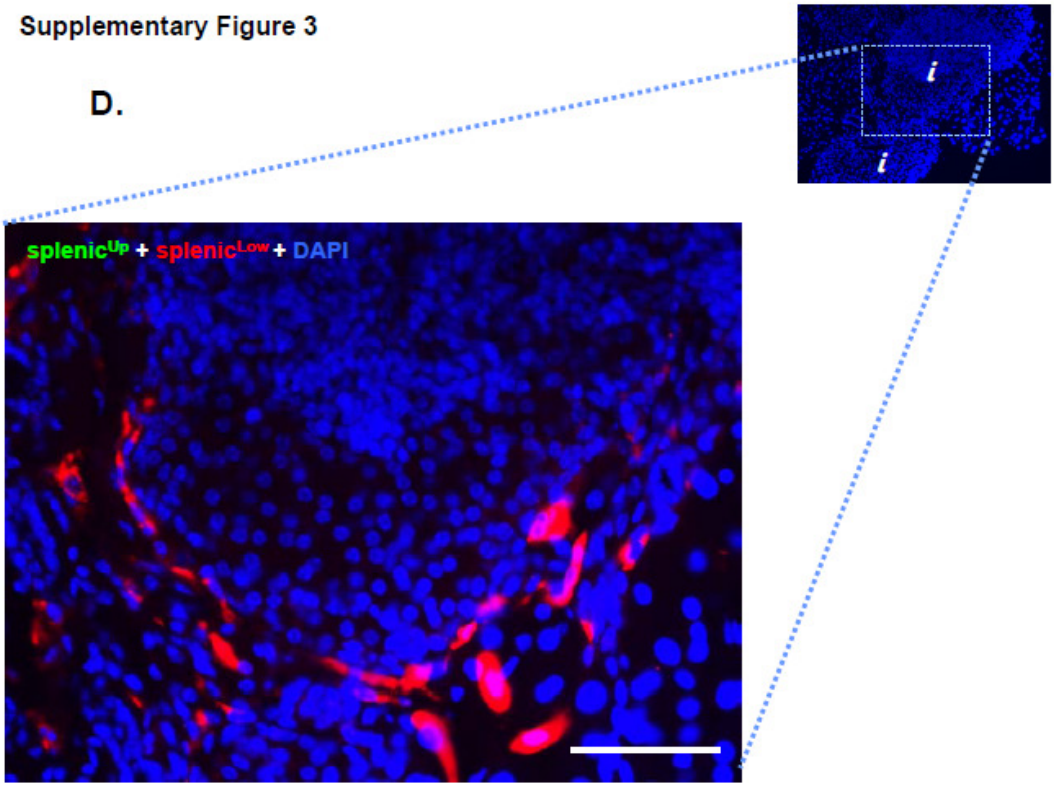

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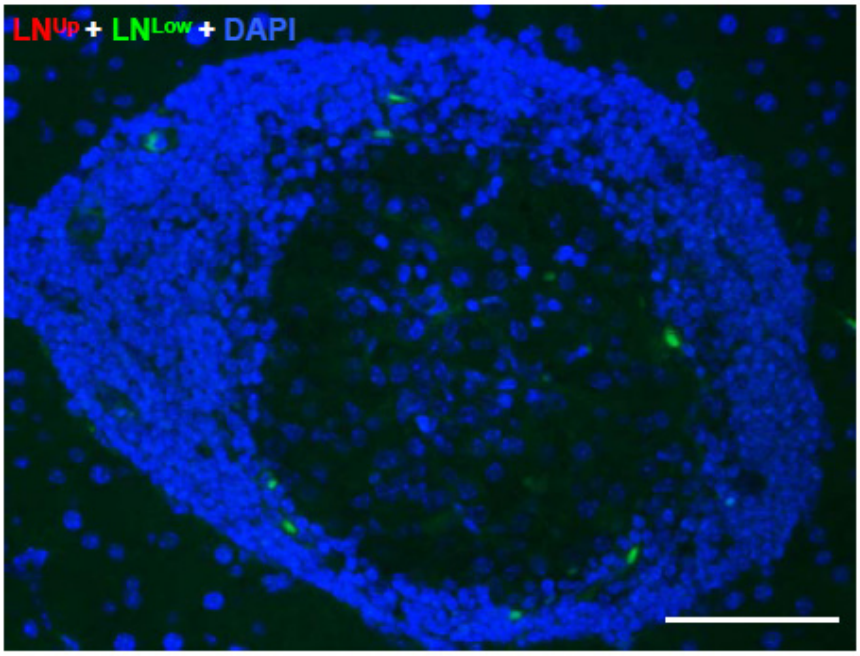

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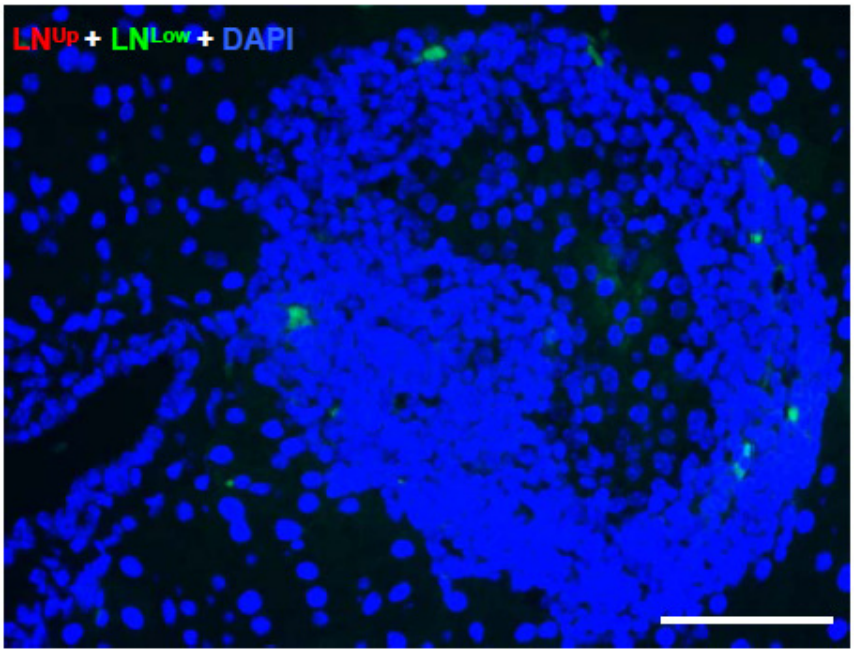

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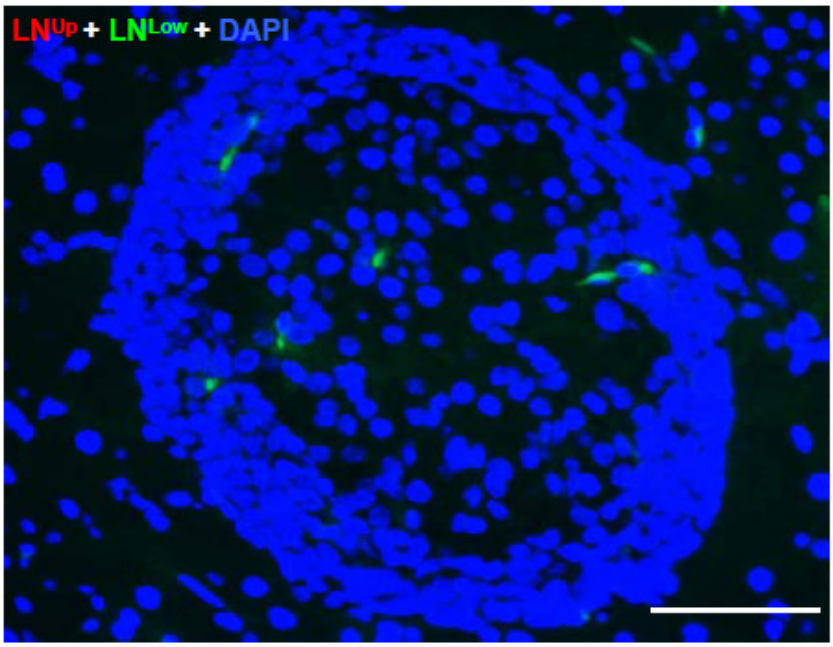

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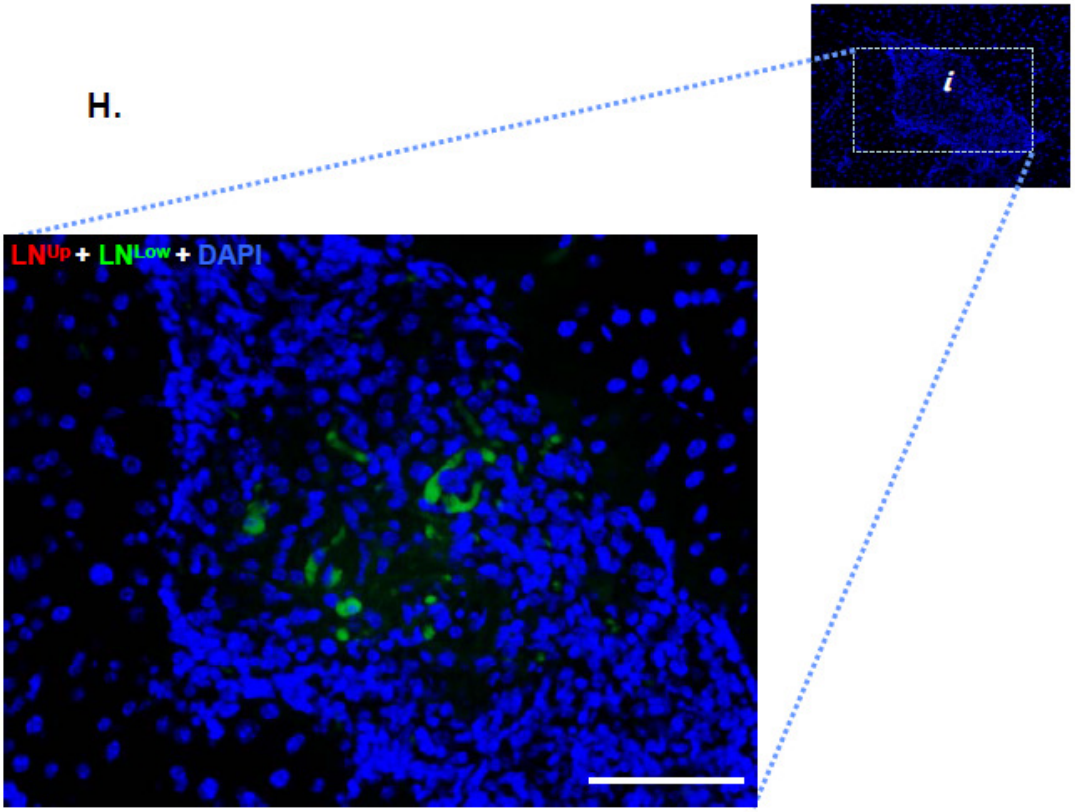
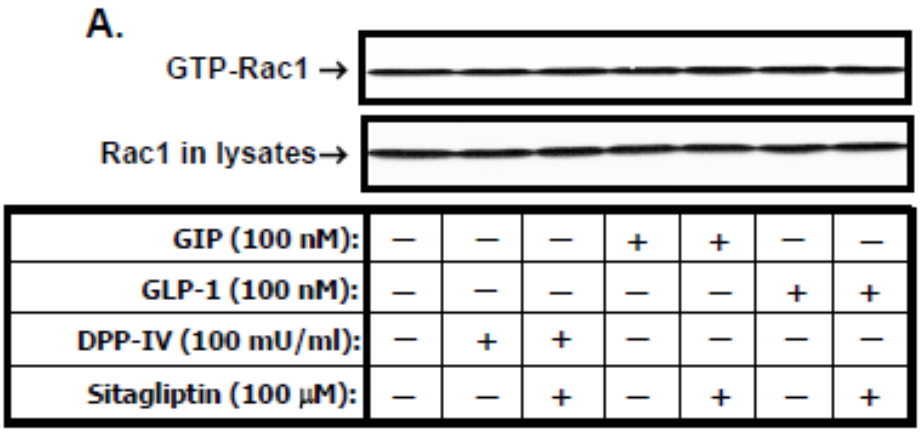
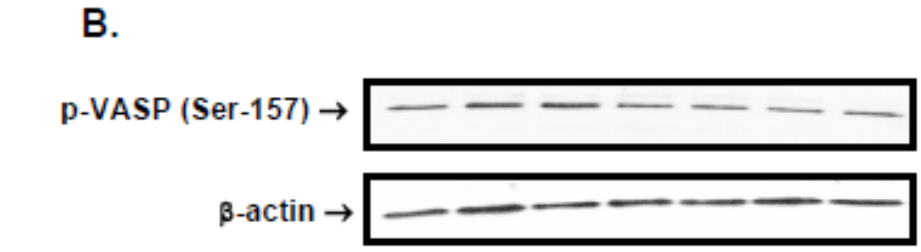


Supplementary Figure 4. The effect of Sitagliptin on phosphorylation levels of cytoskeletal organizing protein in thymic and LN CD4$^+$ T cells. CD4$^+$ T cells were stimulated for 1 h with sDPP-IV (100 mU/ml), GIP or GLP-1 (100 nM) in the presence or absence of DPP-IV inhibitor (100 μM). Total cellular extracts were isolated from thymic (A-D) or LN (E-H) CD4$^+$ T cells and Rac1 activation assays (A, E) or Western blot analyses performed with antibodies against phospho-VASP (Ser157) (B, F), Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558), Ezrin/Radixin/Moesin (C, G), phospho-Cofilin (Ser3), Cofilin (D, H), and β-actin. Western blots are representative of $n = 3$.



GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 μM):	–	–	+	–	+	–	+



GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 μM):	–	–	+	–	+	–	+

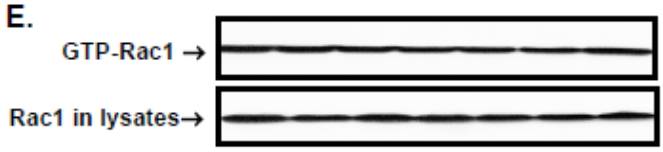
C.

GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 μM):	–	–	+	–	+	–	+

D.

GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 μM):	–	–	+	–	+	–	+

E.

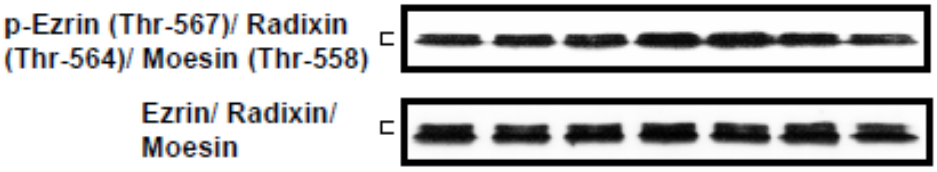


GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 μM):	–	–	+	–	+	–	+

F.

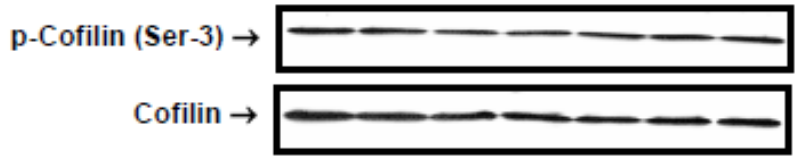
GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 μM):	–	–	+	–	+	–	+

G.



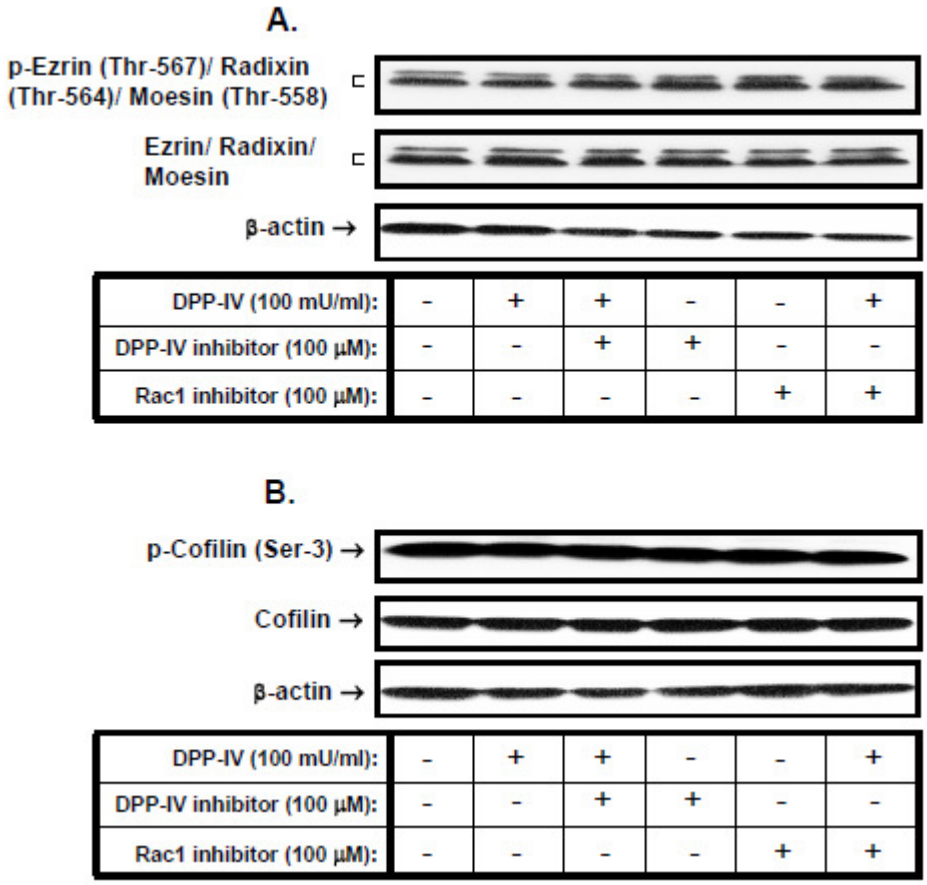
GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 µM):	–	–	+	–	+	–	+

H.



GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 µM):	–	–	+	–	+	–	+

Supplementary Figure 5. The effect of Rac1 inhibitor and PKA inhibitor on the phosphorylation of cytoskeletal organizing protein Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) and Cofilin (Ser3). Splenic $CD4^+$ T lymphocytes were stimulated for 1 h with DPP-IV (100 mU/ml) in the presence or absence of Rac1 inhibitor (100 μM) or forskolin (10 μM) ± H-89 (10 μM) and total cellular extracts were isolated A-B. Effects of treatment with Rac1 inhibitor on the level of Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) (A) and Cofilin (Ser3) (B). C-D. Effect of treatment with H-89 on the level of Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) (C) and Cofilin (Ser3) (D). Western blots are representative of $n = 3$.



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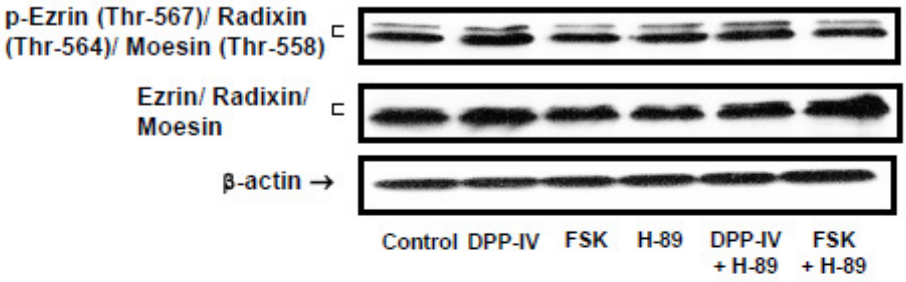

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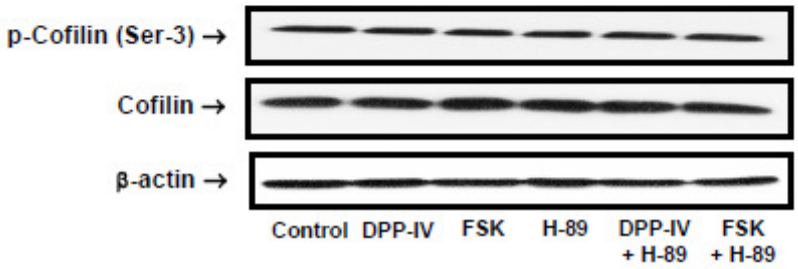
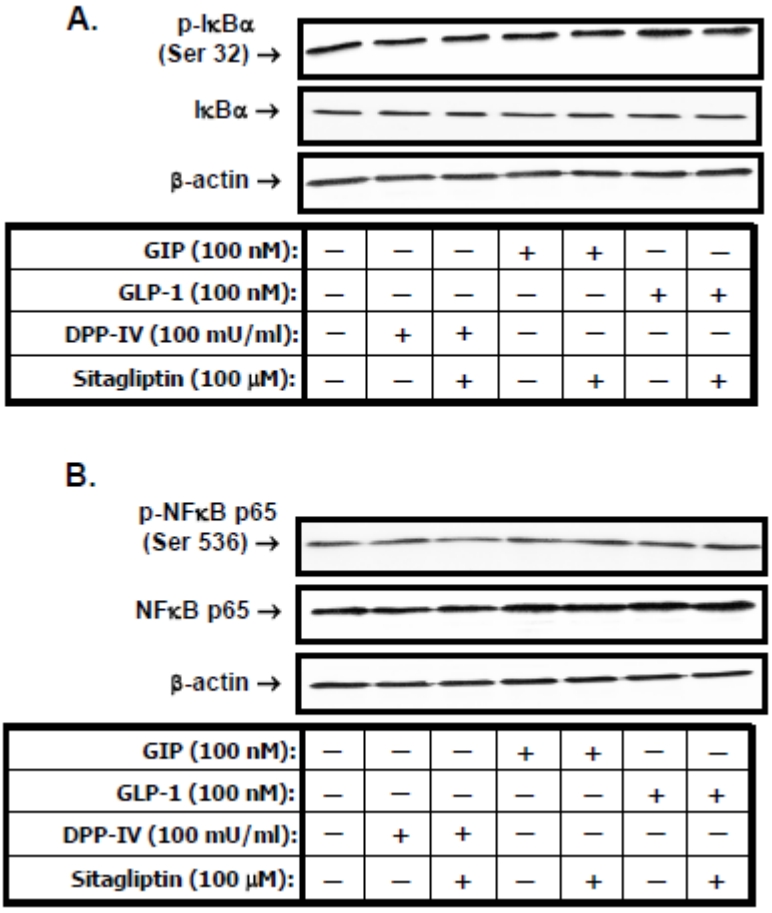


Supplementary Figure 6. The effect of Sitagliptin on phosphorylation levels of NF-κB signalling modules in splenic and thymic CD4$^+$ T cells. Total cellular extracts were isolated from splenic (A-C) or thymic (D-F) CD4$^+$ T cells and stimulated for 1 h with sDPP-IV (100 mU/ml), GIP or GLP-1 (100 nM) in the presence or absence of DPP-IV inhibitor (100 μM). Western blot analyses were performed with antibodies against phospho-IκB (Ser32), IκB (A, D), Phospho-NFκB p65 (Ser536), NFκB p65 (B, E), phospho-IKKα/β (Ser176/180), IKKα, IKKβ (C, F), and β-actin. Western blots are representative of $n = 3$.



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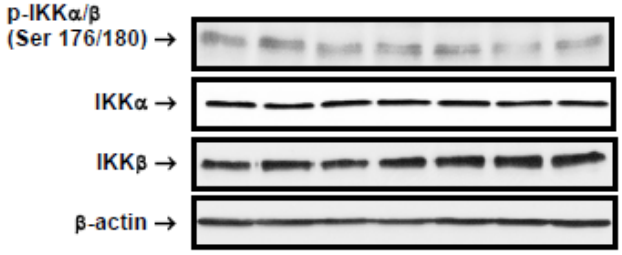
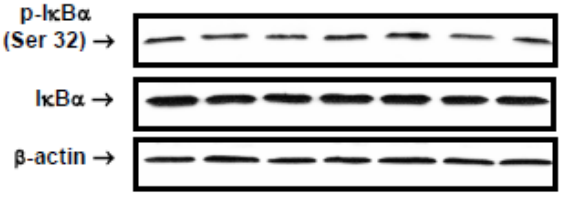


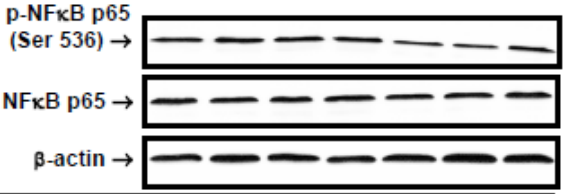
GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 μM):	–	–	+	–	+	–	+

D.



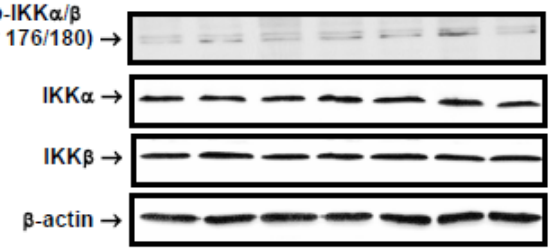
GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 μM):	–	–	+	–	+	–	+

E.



GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 μM):	–	–	+	–	+	–	+

F.



GIP (100 nM):	–	–	–	+	+	–	–
GLP-1 (100 nM):	–	–	–	–	–	+	+
DPP-IV (100 mU/ml):	–	+	+	–	–	–	–
Sitagliptin (100 μM):	–	–	+	–	+	–	+