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Pathophysiological implication of Autotaxin on osteoclast function

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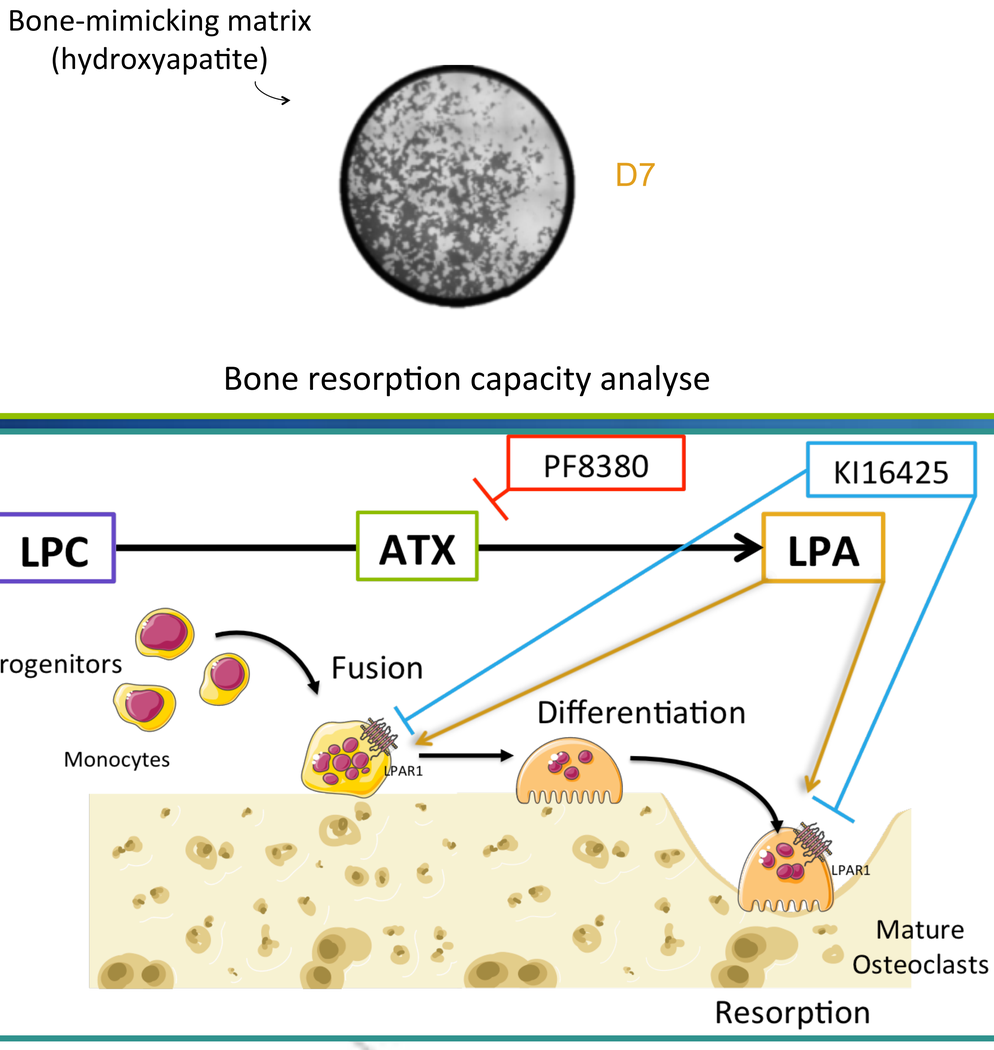
Introduction

Autotaxin (ATX) is a secreted protein produced by various tissues in the body including the liver, adipose tissue and bone. Autotaxin is an enzyme with a lysophospholipase D activity responsible for cleavage of lysophosphatidyl choline (LPC) in lysophosphatidic acid (LPA). LPA is a bioactive phospholipid, which acts as a growth factor, affecting cell proliferation, migration and differentiation. It has been shown that biological effects of LPA could be the direct consequence of local production of Autotaxin (ATX) in a given tissue or cell type[1]. Recently, we have shown that LPA controls two steps of osteoclastogenesis: the fusion of monocyte progenitors and bone resorption capacity of osteoclasts[2].

The aim of this study is to test if ATX produced by osteoclasts could play a direct role on osteoclastogenesis and in bone mass control.

Methods

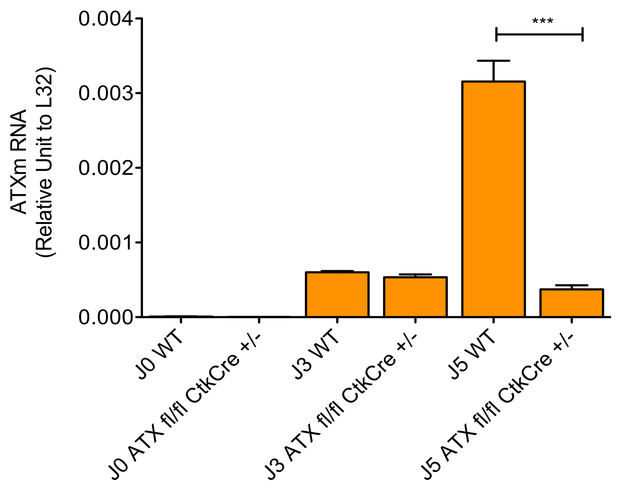
We generated Ctsk-Cre+ ATXfl/fl mice and use these animals as a source of osteoclasts progenitors and ATX-deficient mature OC. Because LPA,LPC and ATX are massively present in sera, to explore either LPA or ATX putative effect in culture, the use of delipidated serum was mandatory.



Results

Figure 1 : ATXm RNA expression is decreased in ATX fl/fl CtskCre +/- mature OC compared to WT mature OC.

Bone marrow derived monocytes of WT or ATX fl/fl CTK-Cre + mice were maintained in αMEM medium during 3 and 5 days with M-CSF and RANK-L for differentiation into osteoclasts. At day 0, 3 and 5, mRNA was extracted from cells. Expression of Enpp2/ATX mRNA was measured by real-time quantitative by real time PCR. Quantifications were normalized to corresponding L32 RNA values. Results represent the mean with SD (*p<0.05, ** p<0.01, ***p<0.001).



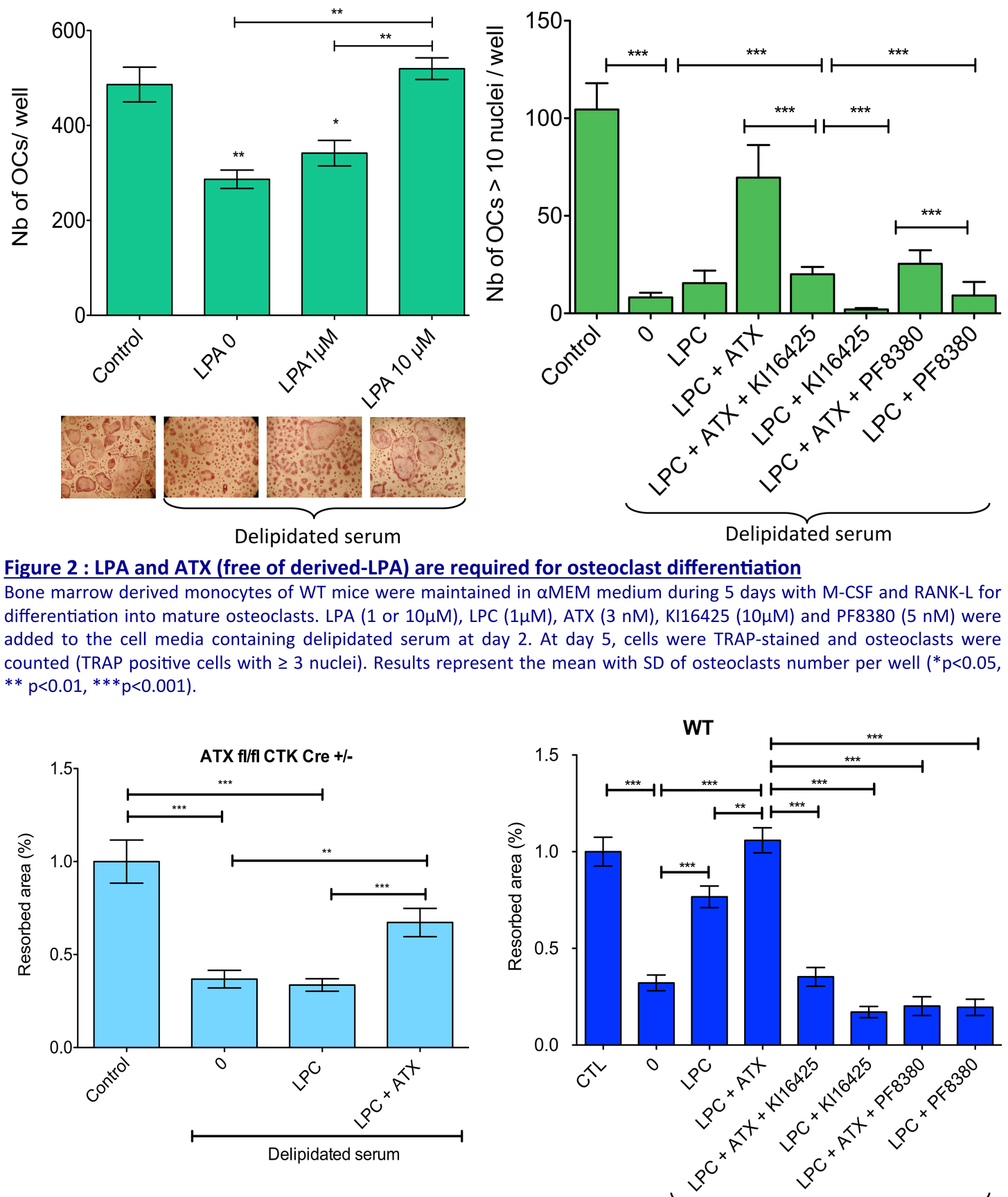


Figure 2 : LPA and ATX (free of derived-LPA) are required for osteoclast differentiation

Bone marrow derived monocytes of WT mice were maintained in αMEM medium during 5 days with M-CSF and RANK-L for differentiation into mature osteoclasts. LPA (1 or 10µM), LPC (1µM), ATX (3 nM), KI16425 (10µM) and PF8380 (5 nM) were added to the cell media containing delipidated serum at day 2. At day 5, cells were TRAP-stained and osteoclasts were counted (TRAP positive cells with ≥ 3 nuclei). Results represent the mean with SD of osteoclasts number per well (*p<0.05, ** p<0.01, ***p<0.001).

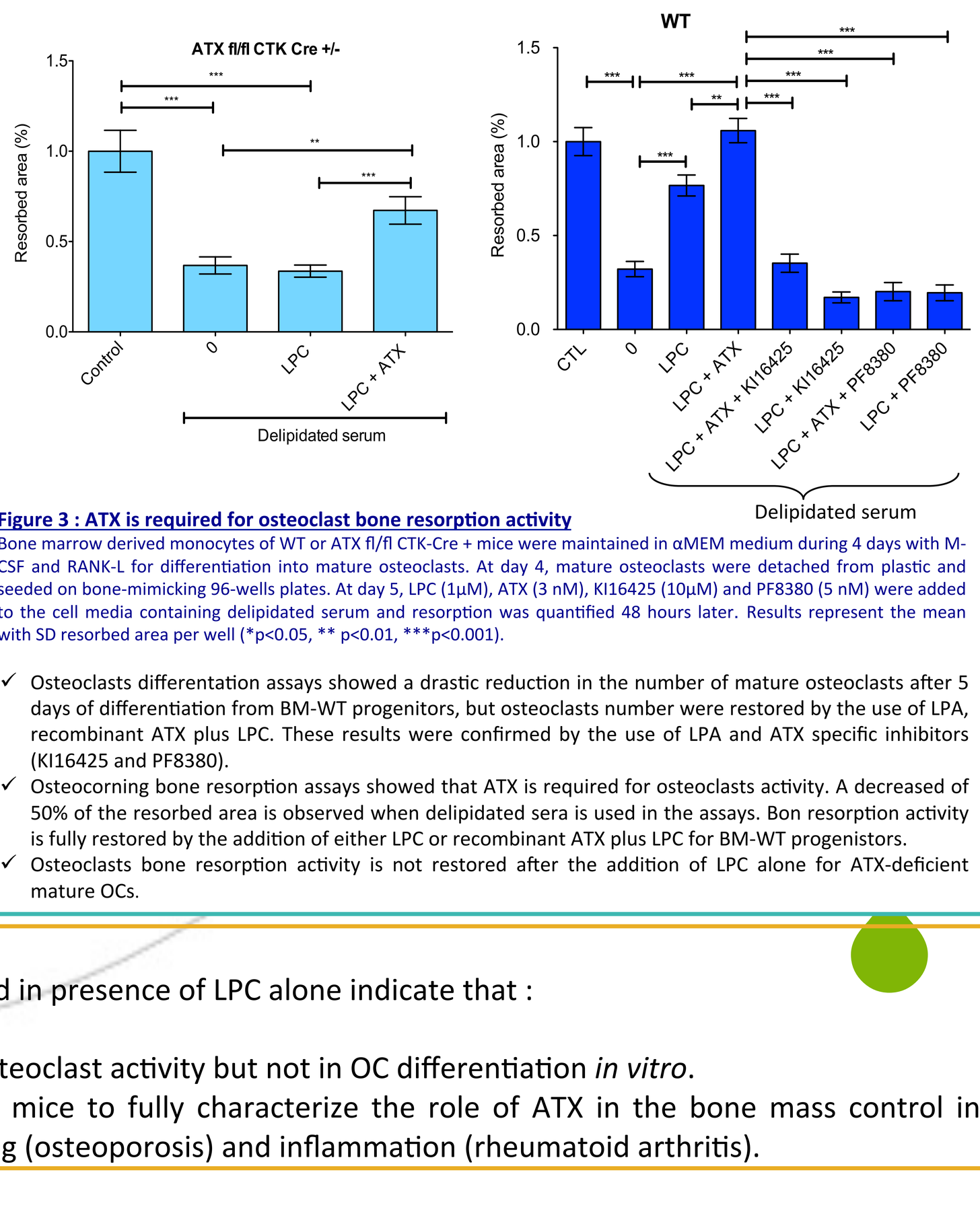


Figure 3 : ATX is required for osteoclast bone resorption activity

Bone marrow derived monocytes of WT or ATX fl/fl CTK-Cre + mice were maintained in αMEM medium during 4 days with M-CSF and RANK-L for differentiation into mature osteoclasts. At day 4, mature osteoclasts were detached from plastic and seeded on bone-mimicking 96-wells plates. At day 5, LPC (1µM), ATX (3 nM), KI16425 (10µM) and PF8380 (5 nM) were added to the cell media containing delipidated serum and resorption was quantified 48 hours later. Results represent the mean with SD resorbed area per well (*p<0.05, ** p<0.01, ***p<0.001).

- ✓ Osteoclasts differentation assays showed a drastic reduction in the number of mature osteoclasts after 5 days of differentiation from BM-WT progenitors, but osteoclasts number were restored by the use of LPA, recombinant ATX plus LPC. These results were confirmed by the use of LPA and ATX specific inhibitors (KI16425 and PF8380).
- ✓ Osteocorning bone resorption assays showed that ATX is required for osteoclasts activity. A decreased of 50% of the resorbed area is observed when delipidated sera is used in the assays. Bon resorption activity is fully restored by the addition of either LPC or recombinant ATX plus LPC for BM-WT progenistors.
- ✓ Osteoclasts bone resorption activity is not restored after the addition of LPC alone for ATX-deficient mature OCs.

Conclusions

All together, and more specifically the results obtained in presence of LPC alone indicate that :

(i) ATX is secreted by mature osteoclasts

(ii) Endogenous OC-ATX is functionally involved in osteoclast activity but not in OC differentiation *in vitro*.

Current studies are conducted on ATXfl/fl Ctsk-Cre+ mice to fully characterize the role of ATX in the bone mass control in physiological and pathological conditions due to ageing (osteoporosis) and inflammation (rheumatoid arthritis).

References :

1-Nishimura S. *et al* Diabetes. 2014 Dec;63(12):4154-64)

2-David M. *et al* J Biol Chem. 2014 Mar 7;289(10):6551-64