
Activation DWG TrueView 2015 Key

In summary, these findings demonstrate that Notch1 activation in adipose tissue is sufficient to promote insulin resistance and inflammatory adipokine secretion. We show that Notch1 activation in adipose tissue may be sufficient to cause insulin resistance and diabetes. These are important findings that may have significant implications for understanding the relationship between Notch1 and adipose tissue inflammation during obesity. Tissues were snap frozen in Optimal Cutting Temperature Compound (OCT). Cryosections were obtained with a thickness of 10 or 20 microns and mounted on cover slips. Sections were fixed in 10% buffered formalin, pH 7.4 for 20 minutes and washed twice in PBS for 10 minutes. Sections were incubated for 5 minutes in TrueView autofluorescence quenching solution containing PowerBlock (Biogenex) followed by three washes for 5 minutes with 1x wash buffer (Vector Laboratories, SK-4700). Sections were incubated overnight with primary antibodies diluted in 1% BSA/PBS. Primary antibodies were used at the following concentrations: 5, 12.5, 25, and 50 microliters per slide. 8-8, 20 microliters of primary antibodies were applied per slide. Slides were incubated in primary antibodies for 16 hours at 4C. Slides were washed 4 for 15 minutes in wash buffer and incubated for 45 minutes with the appropriate secondary antibody, 20 microliters per slide. Sections were washed 4 for 15 minutes in wash buffer and incubated for 45 minutes with the appropriate secondary antibody (see figure legends), 20 microliters per slide. Slides were washed 4 times in wash buffer, counterstained with DAPI for 10 minutes, and covered with Vectashield. Confocal images were captured using a Zeiss Axiovert 200M wide-field microscope (20x/0.75 objective) with red (580/20 nm) and green (475/40 nm) emission filters.

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