Supplementary Materials

Neuregulin 4 downregulation induces insulin resistance in 3T3-L1 adipocytes through inflammation and autophagic degradation of GLUT4 vesicles

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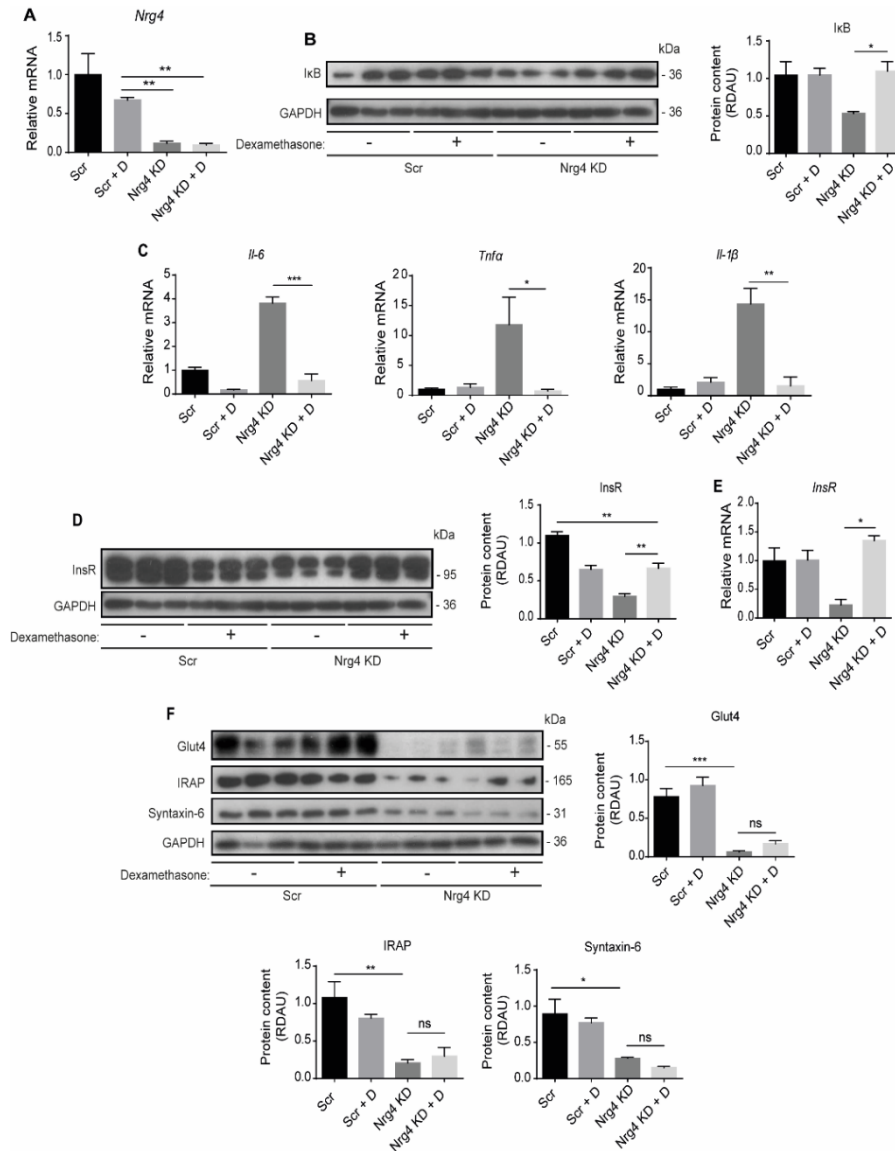
Supplementary Figure S1

Figure S1. Dexamethasone treatment reverses the cell-autonomous inflammatory phenotype and recovers InsR protein content upon Nrg4 knockdown. Adipocytes were treated with 200 nM dexamethasone (D) at D5 for 48 h. (**A**) *Nrg4* expression in non-treated or D-treated Scr and Nrg4 KD adipocytes at day 7 (D7) of differentiation (n=3); (**B**) Western blot bands and quantification of IκB in D-treated or non-treated Scr and Nrg4 KD adipocytes at D7 (n=3); (**C**) *Tnfα, Il-6* and *Il-1β* expression in non-treated or D-treated Scr and Nrg4 KD adipocytes at D7 (n=3); (**D**) Western blot bands and quantification of InsR in D-treated or non-treated Scr and Nrg4 KD adipocytes at D7 (n=3). The lower band of the InsR blot was quantified. Western blots from panel B and D were performed simultaneously with a common loading control, GAPDH; (**E**) *InsR* expression in non-treated or D-treated Scr and Nrg4 KD adipocytes at D7 (n=3). All the qPCR values were normalised to those in non-treated Scr D7 adipocytes; (**F**) Western blot bands and quantification of Glut4, IRAP and Syntaxin-6 in D-treated or non-treated Scr and Nrg4 KD adipocytes at D7 (n=3). Each lane of the blots represents an independent experiment. Overall contrast and brightness of the western blots were adjusted to clarify the images. Data represent mean ± SEM. *p≤0.05, **p≤0.01, ***p≤0.001, ns (not significant).

Supplementary Table S1

Table S1. Primer sequences used for quantitative-PCR analyses.

Target gene	Forward sequence	Reverse sequence
Adiponectin	CGGCAGCACTGGCAAGTT	CCGTGATGTGGTAAGAGAAGTAGTAGA
Arp	AAGCGCGTCCTGGCATTGTCT	CCGCAGGGGCAGCAGTGGT
Glut4	GTGACTGGAACACTGGTCCTA	CCAGCCACGTTGCATTGTAG
Il-1β	GCACTACAGGCTCCGAGATGAAC	TTGTCGTTGCTTGGTTCTCCTTGT
Ifnβ	CCCTATGGAGATGACGGAGA	CCCAGTGCTGGAGAAATTGT
Il-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Il-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
Irap	TTTACCAATGATCGGCTTCAGC	ICGAACCTCGGGGCTCATATT
Lpl	GGGAGTTTGGCTCCAGAGTTT	TGTGTCTTCAGGGGTCCTTAG
InsR	ATGGGCTTCGGGAGAGGAT	GGATGTCCATACCAGGGCAC
Nrg1	TTCCCATTCTGGCTTGTCTAGT	CCAGGGTCAAGGTGGGTAG
Nrg4	CACGCTGCGAAGAGGTTTTTC	CGCGATGGTAAGAGTGAGGA
Pparγ	CCAGAGCATGGTGCCTTCGCT	CAGCAACCATTGGGTCAGCTC
Syntaxin-6	ACAGGCCGTCATGCTAGATG	GGATGGCTATGGCACACCAC
Tnfα	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG