

Supporting information for:

Adipose tissue macrophage-derived microRNA-210-3p disrupts systemic insulin sensitivity by silencing GLUT4 in obesity

Short Title: ATM-derived miR-210-3p loaded EVs promote insulin resistance

Debarun Patra¹, Palla Ramprasad¹, Shivam Sharma³, Upalabdhya Dey², Vinod Kumar³, Satpal Singh⁴, Suman Dasgupta², Aditya Kumar², Kulbhushan Tikoo³, Durba Pal^{1*}

¹Department of Biomedical Engineering, Indian Institute of Technology Ropar, Punjab 140001, India

²Department of Molecular Biology & Biotechnology, Tezpur University, Assam 784028, India

³Department of Pharmacology and Toxicology, NIPER, S.A.S. Nagar, Punjab 160062, India

⁴Department of Gastro Surgery, DMC&H, Ludhiana, Punjab 141001, India

Contact information:

*Correspondence should be addressed to: Durba Pal (durba.pal@iitrpr.ac.in)

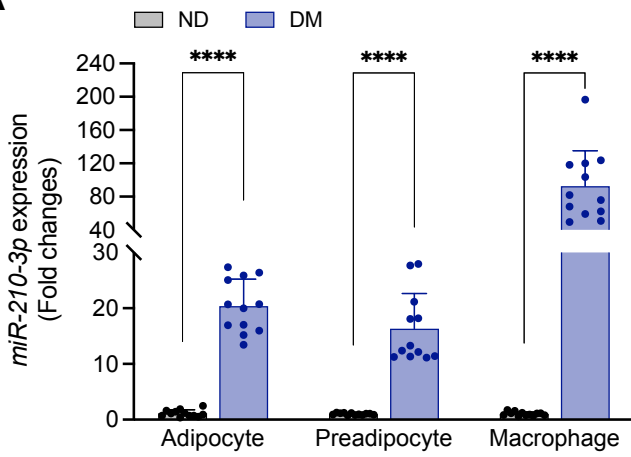
Department of Biomedical Engineering, Indian Institute of Technology Ropar, Rupnagar-140001, Punjab, India

Phone: +91-1881-23-2506

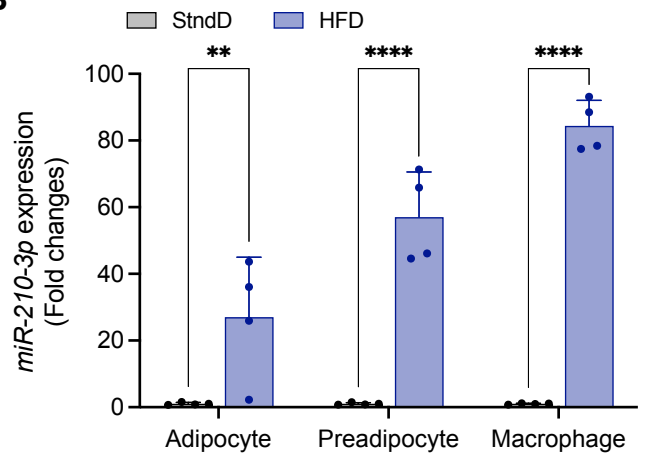
ORCID: 0000-0001-7672-3529

Figure S1

A



B



C

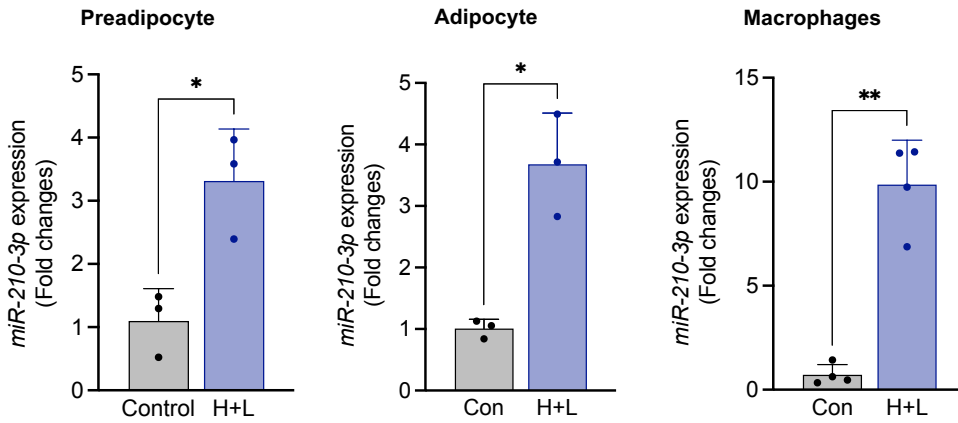


Figure S1. miR-210-3p expression in macrophages, adipocytes and preadipocytes.

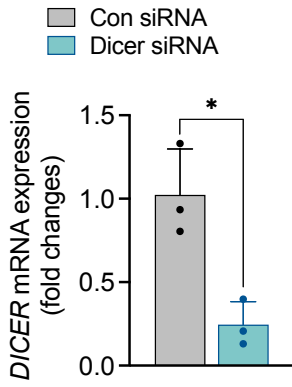
(A) qRT-PCR analyses of *miR-210-3p* in the preadipocytes, adipocytes, and macrophages isolated from VAT of ND and DM human patients. (n= 14-16) **** $P < 0.0001$ by Student's *t* test.

(B) qRT-PCR analyses of *miR-210-3p* in the preadipocytes, adipocytes, and macrophages isolated from VAT of StndD and HFD mice. (n=4) ** $P < 0.01$, **** $P < 0.0001$ by Student's *t* test.

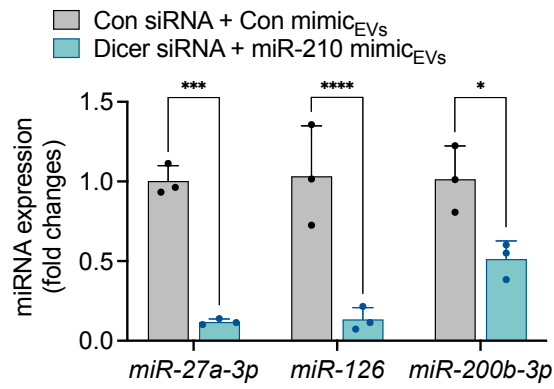
(C) *miR-210-3p* expression in the *in-vitro* cultured murine preadipocyte, adipocyte, and macrophage in the presence or absence of hypoxia and lipid. (n=3) * $P < 0.05$; ** $P < 0.01$ by Student's *t* test.

Figure S2

A



B



C

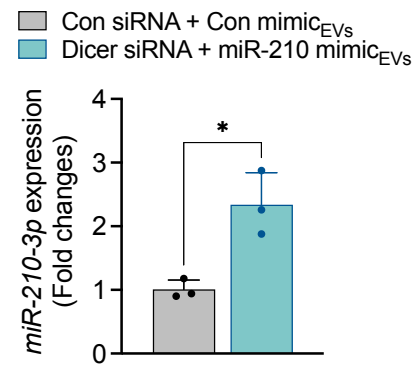
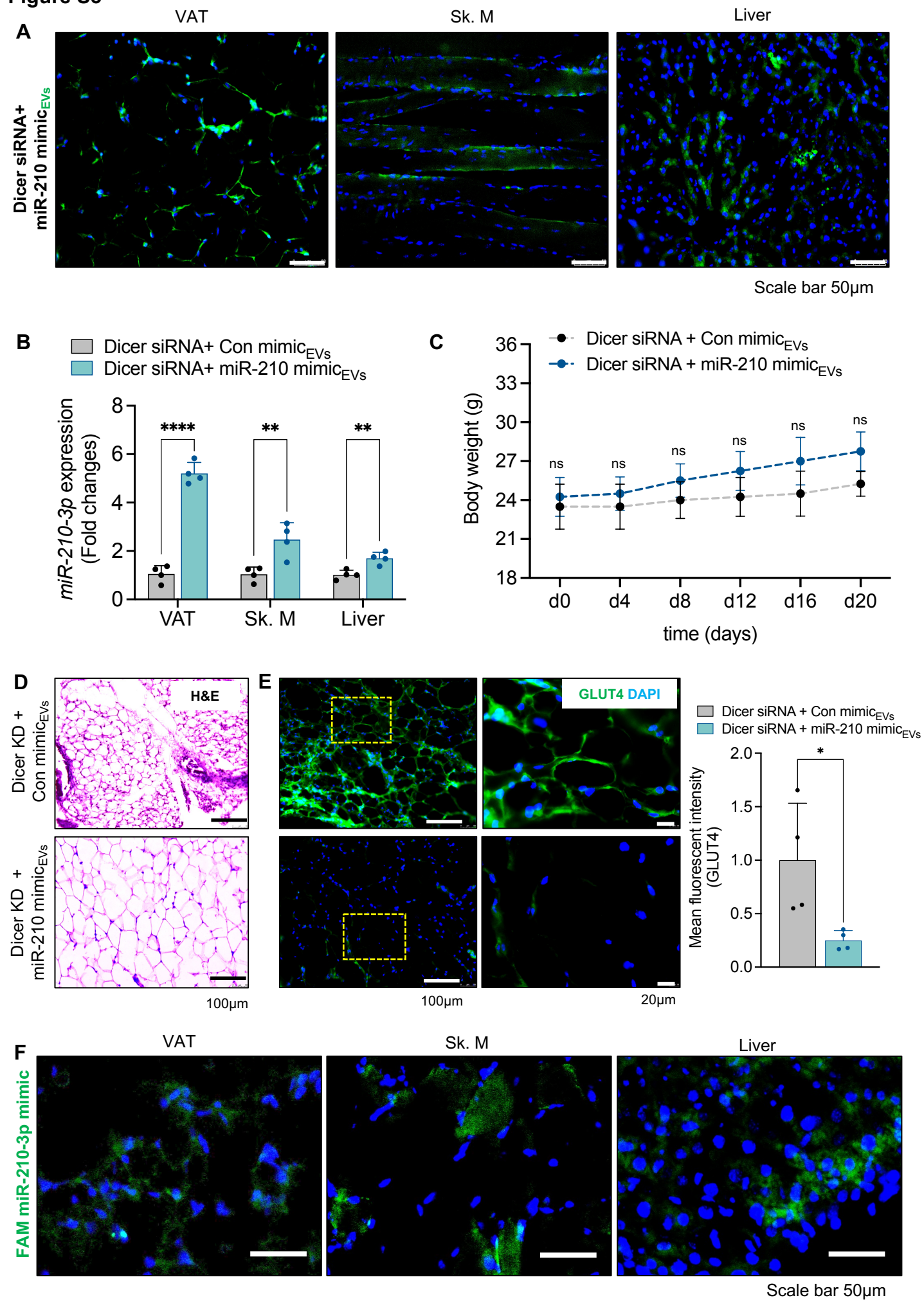


Figure S2. miR-210-3p targets GLUT4

(A) qRT-PCR analyses of *DICER* mRNA expression in BMderived-macrophages (BMDMs) transfected with control siRNA and DICER siRNA (n=3) * $P < 0.05$ by student's *t*-test.

(B and C) *miR-27a-3p*, *miR-126*, *miR-200b-3p* (B) and *miR-210-3p* (C) expression in EVs isolated from DICER silenced macrophage (BMDMs) transfected with control mimic and miR-210-3p. (n=3) * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$ by student's *t* test.

Figure S3

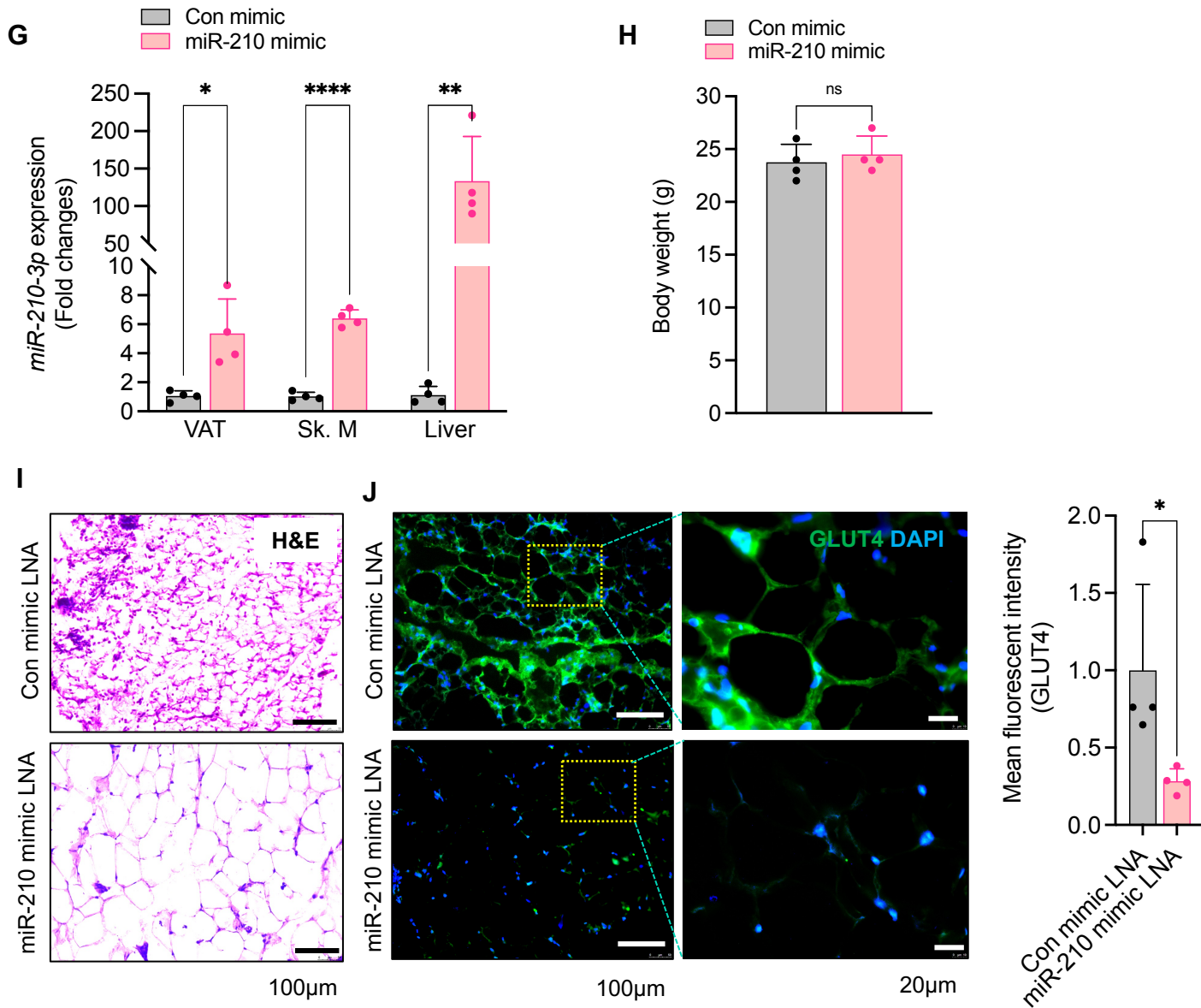


Figure S3. miR-210-3p loaded EVs disrupts systemic glucose homeostasis

(A) Fluorescence images of histological sections showing fluorochrome-labeled EVs presence in the AT, liver, and SM of StnD fed C57BL/6 mice. Scale bar 50µm.

(B) *miR-210-3p* expression in visceral adipose tissue (VAT), skeletal muscle (Sk. M) and Liver in StnD fed C57BL/6 mice administered with Dicer silenced macrophage (BMDMs) EVs having control mimic or miR-210-3p mimic LNA via the intravenous route in regular intervals for 21 days. (n=4) ** $P < 0.01$, *** $P < 0.001$ by student's *t* test.

(C) Body weight measurement in StnD fed C57BL/6 mice administered with DICER silenced macrophage EVs having control mimic or miR-210-3p mimic LNA via the intravenous route in regular intervals for 21 days. (n=4) ns-not significant by student's *t* test.

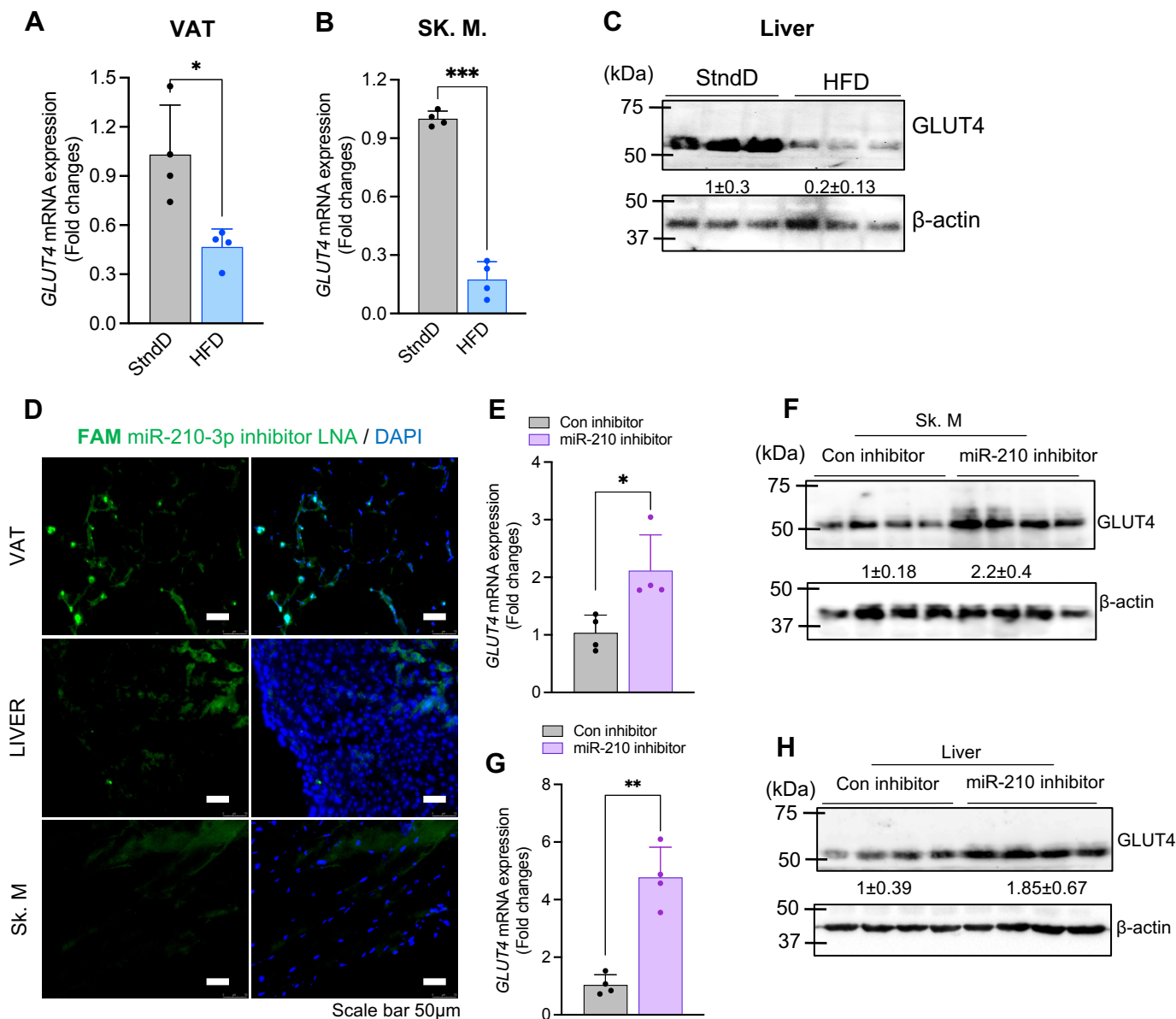
(D and E) VAT sectioning, H&E staining followed by brightfield imaging, and immunofluorescence imaging staining GLUT4, quantification analyses in StnD fed C57BL/6 mice administered with Dicer silenced and control mimic or miR-210-3p mimic co-transfected macrophage EVs administered via the intravenous route in regular intervals. Quantification analyses. (n=4) * $P < 0.05$ by student's *t* test.

(F) Fluorescence images of histological sections showing miR-210-3p presence in AT, liver, and SM of StnD fed C57BL/6 mice administered FAM conjugated miR-210-3p mimic LNA encapsulated with invivofectamine™ via the intravenous route in regular intervals for 21 days. Scale bar 50µm.

(G) *miR-210-3p* expression in visceral adipose tissue (AT), skeletal muscle (Sk. M), and Liver in StnD fed C57BL/6 mice administered control mimic or miR-210-3p mimic LNA via the intravenous route in regular intervals for 21 days. (n=4) * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$ by student's *t* test.

(H) Body weight of C57BL/6 mice administered FAM conjugated control mimic LNA and miR-210-3p mimic LNA, measured before sacrifice. (n=4) ns- not significant by student's *t* test.

(I and J) VAT sectioning, H&E staining followed by brightfield imaging (I), and immunofluorescence imaging staining GLUT4 (J) followed by quantification analyses of control mimic LNA or miR-210-3p mimic LNA. Quantification analyses (n=4) * $P < 0.05$ by student's *t* test.

Figure S4**Figure S4. miR-210-3p inhibition in obese mice rescue from insulin resistance**

(A and B) qRT-PCR analyses of GLUT4 mRNA expression in VAT and skeletal muscle of StndD and HFD mice respectively.

(C) Western blotting and quantification analyses showing GLUT4, in the liver of StndD and HFD mice. (n=4) *** $P < 0.001$, ns= not significant by two-way ANOVA; * $P < 0.01$ by Student's *t* test.

(D) Fluorescence imaging showing the presence of FAM conjugated miR-210-3p inhibitor in the histological sections of VAT, liver, and skeletal muscle from HFD mice administered with miR-210-3p inhibitor.

(E and F) qRT-PCR analyses of GLUT4 mRNA expression analyses (E) and Immunoblot analyses (F) of GLUT4 in the skeletal muscle of control inhibitor or miR-210-3p inhibitor administered HFD mice. (n=4) ** $P < 0.01$ by Student's *t* test.

(G and H) qRT-PCR analyses of GLUT4 mRNA expression analyses (E) and Immunoblot analyses (F) of GLUT4 in the liver of control inhibitor or miR-210-3p inhibitor administered HFD mice. (n=4) ** $P < 0.01$ by Student's *t* test

Table S1: List of primers and their sequences

Mouse Primers			
	Gene	F (5'-3')	R (5'-3')
1	GLUT4	CTCCGCATCTTTCCCCCTC	AGTGTTCCAGTCACTCGCTG
2	IRS1	TGGACATCACAGCAGAATGAAGA	TTCCGGTGTACAGTGCTTT
3	IRS2	TTTGCCCACAATTCCAAGCG	GTAGCGCTTCACTCTTTCACG
4	IRS3	GCTGCTACTGCTATGGGTTTC	CATGAATGCTTTGGGCCACC
5	IRS4	AGAATTCCAGGACCTTGCCG	GTGGCAGTGAATAACTCTCATTT

Cloning primer details		
		Sequence 5' - 3'
1	XbaI (Forward)	GCCGTGTAATTCTAGAGGGGCCAGGCAGGGGTGG
2	Sall (Reverse)	AAGGGCATCGGTGCGACTGTGGTCTTGGTCTCCATCTCTTC

GLUT4 3'UTR primer details			
	Gene	F (5'-3')	R (5'-3')
1	GLUT4 (mut-1)	GTTTCACTGCCCATCTGGGCTGACAC CTCC	GGAGGTGTCAGCCCAGATGGGCAGTG AAAC
2	GLUT4 (mut-2)	ATCATGGGTTTCACTGCCCATCTAGTC ACTGGGCTGACACCTCCCTCACAGAG TG	CACTCTGTGAGGGAGGTGTCAGCCCAC AGTCAAGATGGGCAGTGAAACCCATGA T

microRNA Primers				
		Sequences	Assay ID	Accession No.
1	U6 snRNA	GTGCTCGCTTCGGCAGCACATATACTAA ATTGGAACGATACAGAGAAGATTAGCAT GGCCCCTGCGCAAGGATGACACGCAAAT TCGTGAAGCGTTCCATATTTT	001973	NR_004394
2	miR-210-3p	CUGUGCGUGUGACAGCGGCUGA	000512	MIMAT0000658
3	miR-200b-3p	UAAUACUGCCUGGUAUGAUGA	002251	MIMAT0000233
4	miR-27a-3p	UUCACAGUGGCUAAGUUCGCG	000408	MIMAT0000537
5	miR-126	CAUUUUUACUUUUGGUACGCG	000451	MIMAT0000137

(F: Forward primer; R: Reverse primer)

Table S2: Patient demographic details:

Pathological features	
Non-obese non-diabetic patients:	
Number of patients	16
Age (median range)	43.6 ± 10.7 (19-65 years)
Gender	Male (n=6)
	Female (n=10)
BMI (median range)	24.3 ± 2.01 (19.4 - 27.4)
Fasting serum glucose (mmol/L)	4.7±0.78
Any other disease (Cancer and NAFLD)	No
Obese diabetic patients:	
Number of patients	14
Age (median range)	48.1 ± 12.2 (22-66 years)
Gender	Male (n=7)
	Female (n=7)
BMI (median range)	34 ± 3.6 (30.1- 44)
Fasting serum glucose (mmol/L)	8.07±3.1
Any other disease (Cancer and NAFLD)	No

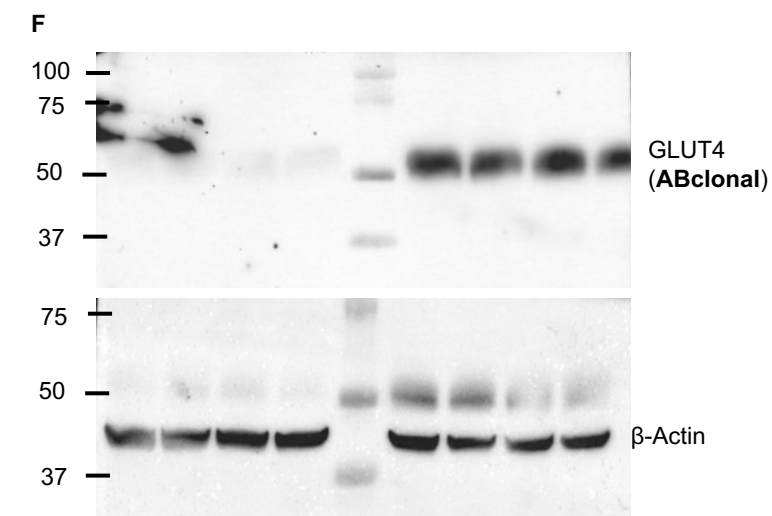
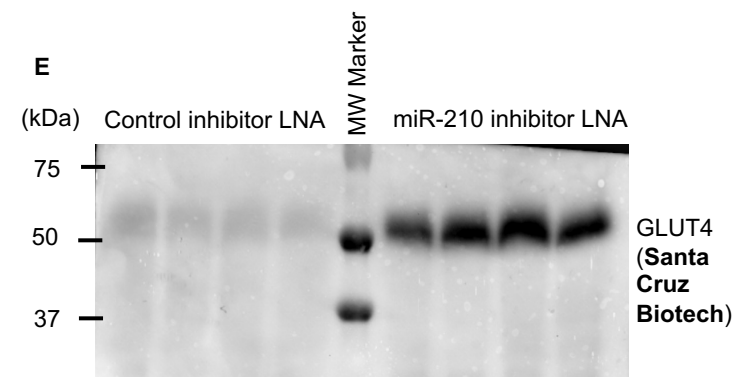
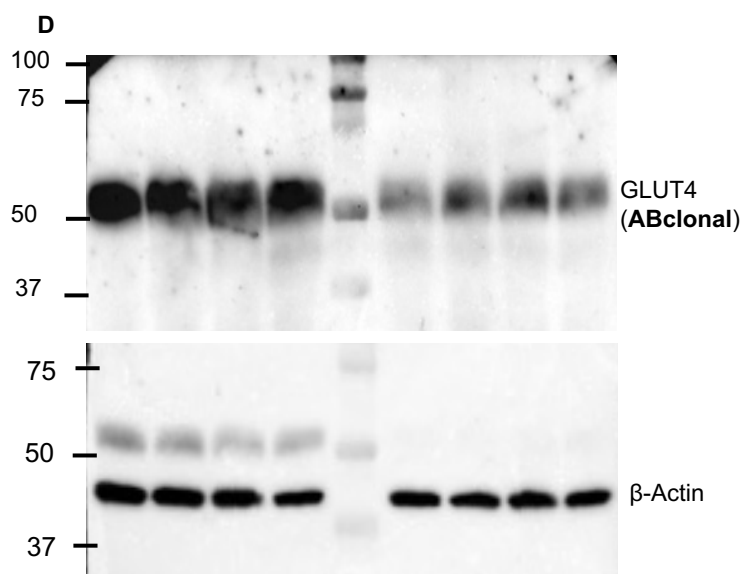
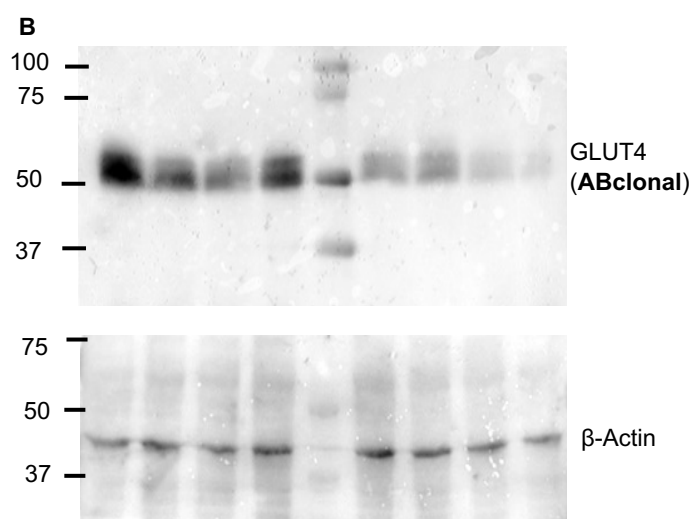
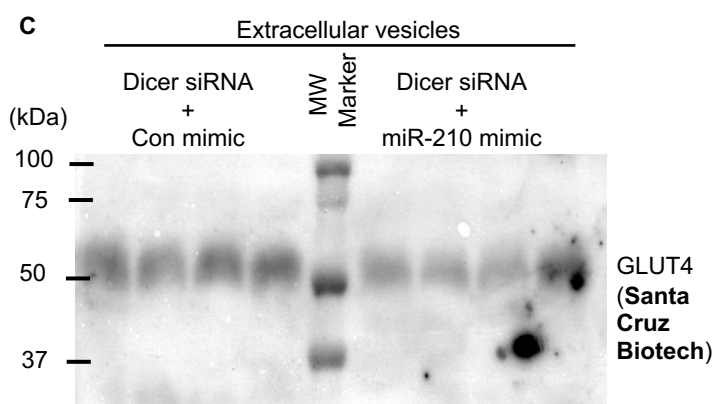
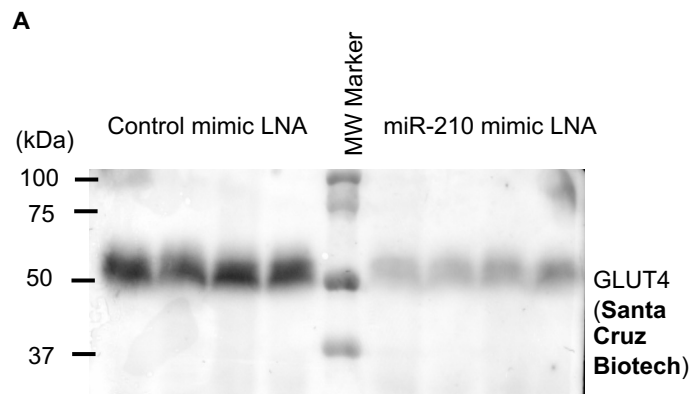


Figure 1

M

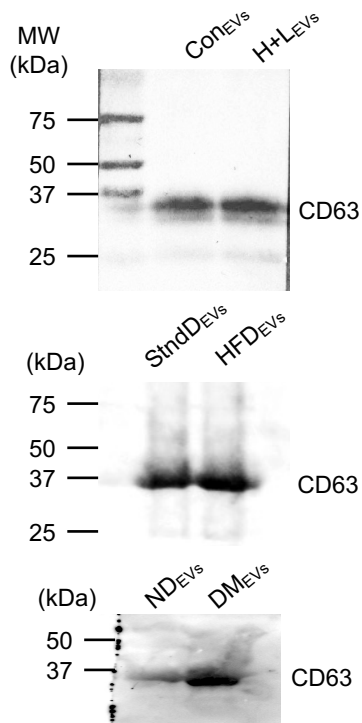
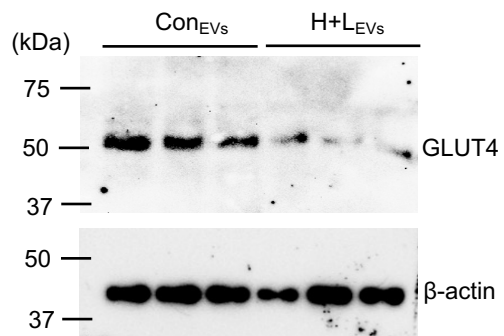
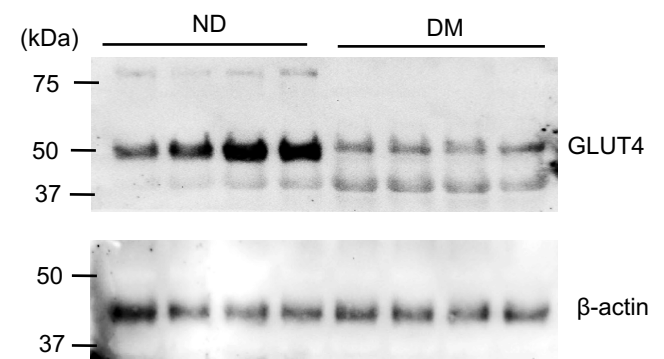


Figure 2

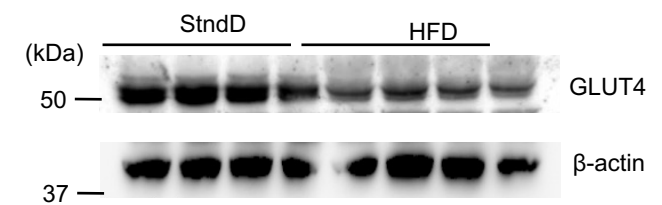
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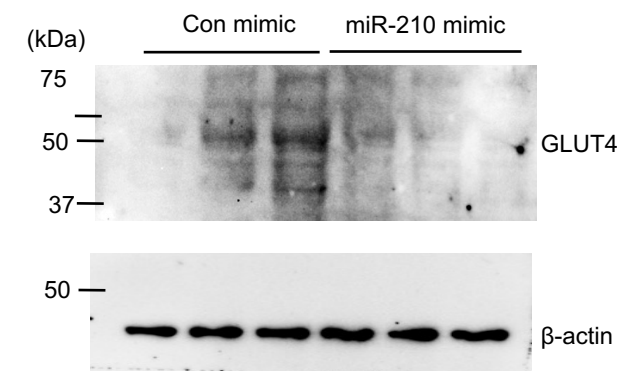
C



D



F



L

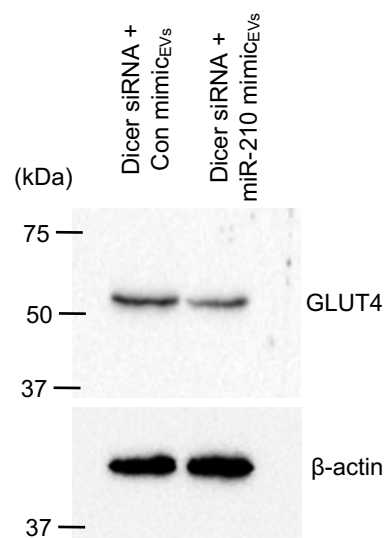
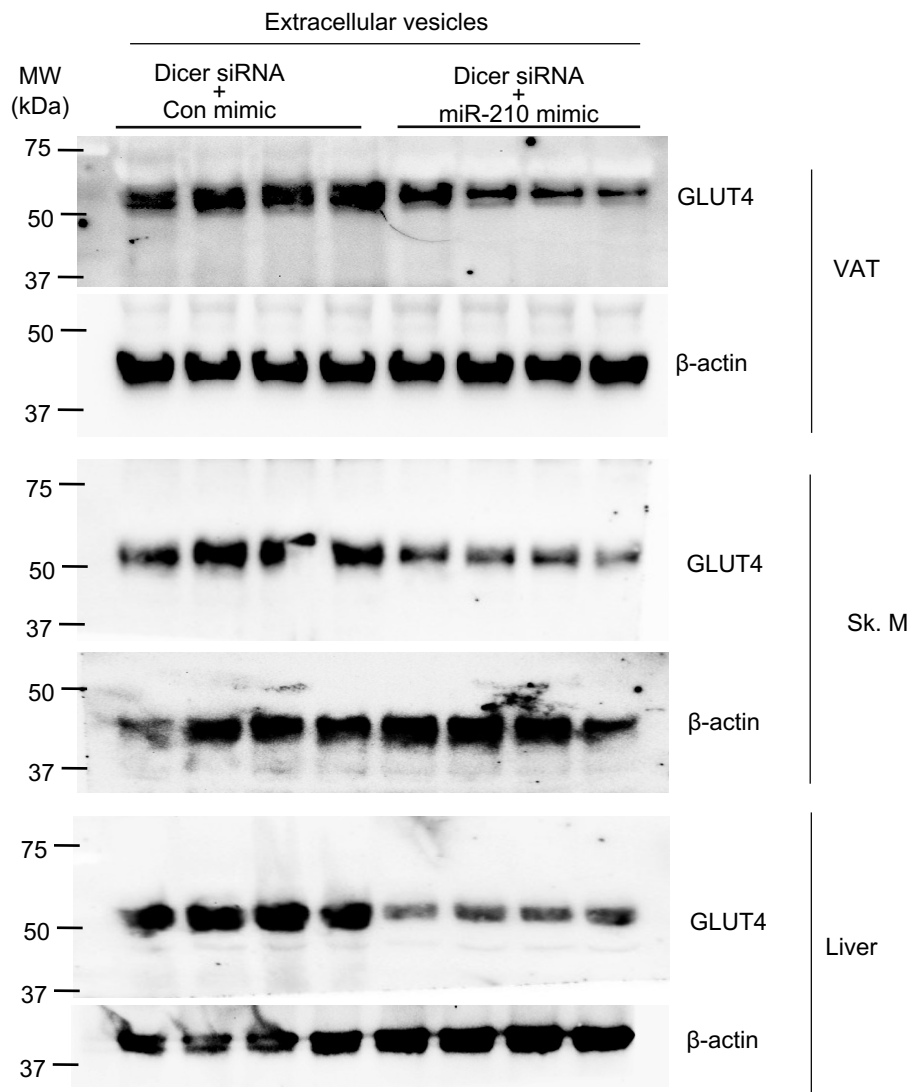


Figure 3 **F**



L

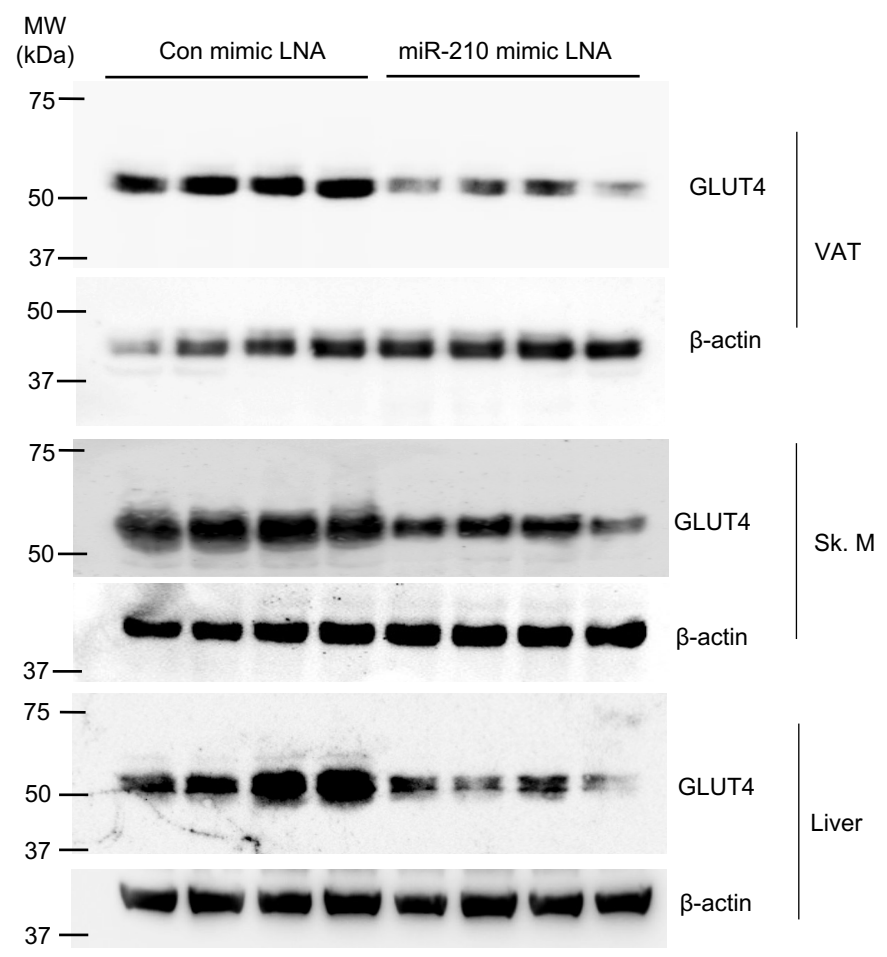


Figure 4

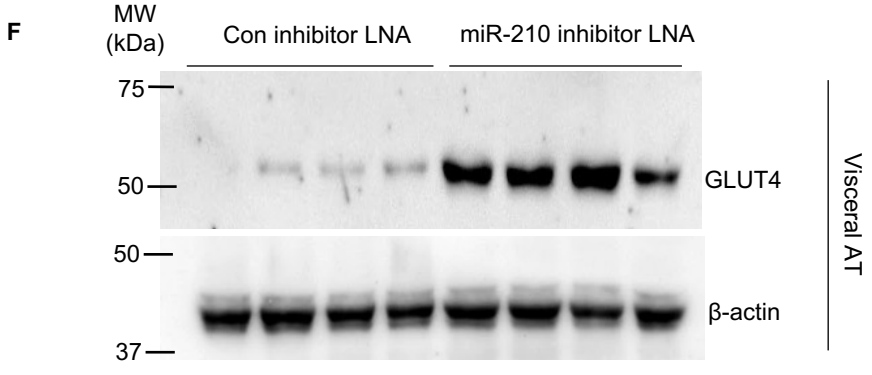


Figure S4

