SUPPLEMENTARY DATA

Quantitative Real Time PCR (RT-qPCR)

First strand cDNA was synthesized and RT-qPCR was performed using RT2 first strand kits (Cat#330401) and RT2 qPCR Master Mix (SABioscience, Frederick, MD). Experiments were performed on an Applied Biosystems 7300 Real-time PCR System, using SYBR Green as the detected fluorophore and ROX as the passive reference. For mRNA studies, ACTB was used as internal controls. Primers for mRNA were purchased from SABioscience, Frederick, MD. Primers for mature miRNAs were purchased from OriGene (Rockville, MD). miR-103 was used as an internal control as its expression levels are stable in adipocytes. Relative fold change of mRNA and miRNA expression of targeted genes and miRNA were calculated by using the ΔΔCt method.

Differentiation of human preadipocytes to adipocytes

To induced differentiation, preadipocytes were cultured to full confluence and then maintained in differentiation medium (Cat# DM-2, ZenBio Inc., Research Triangle Park, NC) for one week (day 7 of differentiation) before being cultured in adipocyte medium (Cat# AM-1, ZenBio Inc., Research Triangle Park, NC) for an additional week (day 14 of differentiation).

Differentiation of mouse 3T3-L1 preadipocytes to adipocytes

3T3-L1 preadipocytes (Cat# SP-L1-F) were purchased from Zenbio Company. Maintenance and differentiation of 3T3-L1 preadipocytes to adipocytes was followed the instructions in the 3T3-L1 Cell Care Manual. Briefly, 3T3-L1 preadipocytes were maintained in preadipocyte medium (Cat# PM-1-L1, ZenBio Inc., Research Triangle Park, NC) until 100% confluent in a humidified incubator, 37°C, with 5-10% CO2. Cells fed with PM-1-L1 every other day. Once the cells were confluent, incubate an additional 48 hours before initiating differentiation. Two days (day 0 of differentiation) after the cells have been confluent, removed the preadipocyte medium and replaced with an appropriate volume of 3T3-L1 differentiation medium (Cat# DM-2-L1, ZenBio Inc., Research Triangle Park, NC). After 3 days (day 3 of differentiation) cultured in differentiation medium, the differentiation medium was removed and replaced with 3T3-L1 adipocyte maintenance medium (Cat#AM-1-L1, ZenBio Inc., Research Triangle Park, NC). The cells were incubated for another 3 days in adipocyte maintenance medium. Cells were fed every 2-3 days using 3T3-L1 adipocyte maintenance medium until ready for assay. Assays were done on day 7-14 of differentiation.

Transfection of vectors

Six well plates of differentiated human (on day14) or 3T3-L1 (on day 7) adipocytes were used to do transfection by following instructions in the insert of transfection reagent. Briefly, for each well, 2 µg of vectors were diluted in 100 µl of Opti-MEM (Cat#51985, Life Technologies, Carlsbad, CA). Six µl of MegaTran 1.0 (Cat# TT200002, OriGene, Rockville, MD) was added to the diluted DNA and vortex the solution immediately for 10 seconds. The mixture was incubated for 10 minutes at room temperature. The mega Tran1.0/DNA mixture was added to well (already containing 900 ul culture medium) gently. The plate was rocked to achieve even distribution of the complexes and incubated at 37 ºC for 48 hours. The medium was replaced with fresh culture medium at 3 hours post-transfection. The assays were done at 48 hours post-transfection.

Transfection of inhibitors

Six well plates of differentiated human (on day14) or 3T3-L1 (on day 7) adipocytes were used to do transfection by following instructions in the insert of transfection reagent. Briefly, for each well, an appropriate amount of inhibitor (30 nM final concentration) was diluted in 250 µl of Opti-MEM (Cat#51985, Life Technologies, Carlsbad, CA). Five µl of Lipofectamine®RNAiMAX reagent (Cat# 13778-100, Life Technologies, Carlsbad, CA) was diluted in another tube with 250 µl of Opti-MEM. The diluted inhibitor and Lipofectamine®RNAiMAX reagentwere combined, mixed and incubated for 15 minutes at room temperature. The mixture was added to well (already containing 2.5 ml culture medium) gently. The plate was rocked to achieve even distribution of the complexes and incubated at 37

ºC for 48 hours. The medium was replaced with fresh culture medium at 4 hours post-transfection. The assays were done at 48 hours post-transfection.

SUPPLEMENTARY DATA

Supplementary Table 1. Differentially expressed microRNA from array of adipocytes derived from Lean PCOS patients vs. matched control women.

Probeset ID	Fold-Change (PCOS vs. Control)	p-value	Species
hsa-miR-183-star_st	-1.65	0.005	human
hsa-miR-548a-3p_st	1.86	0.007	human
hsa-miR-574-5p_st	-2.33	0.007	human
hsa-miR-199a-3p_st	-2.64	0.012	human
hsa-miR-27a-star_st	-1.58	0.014	human
hsa-miR-1825_st	-1.96	0.017	human
hsa-miR-147_st	1.52	0.021	human
hsa-miR-548c-3p_st	1.73	0.023	human
hsa-miR-499-5p_st	2.11	0.026	human
hsa-miR-10a_st	-1.84	0.027	human
hsa-miR-150_st	-2.71	0.028	human
hsa-miR-1201_st	1.95	0.028	human
hsa-miR-30d_st	1.63	0.030	human
hsa-miR-34c-3p_st	-1.55	0.034	human
hsa-miR-768-3p_st	-1.92	0.035	human
hsa-miR-203_st	-1.66	0.036	human
hsa-miR-510_st	-1.72	0.041	human
hsa-miR-181c_st	-2.46	0.042	human
hsa-miR-154_st	3.62	0.042	human
hsa-miR-585_st	1.75	0.043	human
hsa-miR-93-star_st	2.10	0.046	human
hsa-miR-223_st	1.70	0.047	human
hsa-miR-422a_st	-6.48	0.048	human
hsa-miR-1270_st	-1.57	0.048	human
hsa-miR-498_st	-1.58	0.049	human
hsa-miR-125b-2-star_st	-2.55	0.049	human
hsa-miR-195_st	-2.71	0.050	human
hsa-miR-23b_st	-1.70	0.050	human
mmu-miR-382-star_st	1.57	0.002	mouse
mmu-miR-433-star_st	1.59	0.003	mouse
mmu-miR-540-3p_st	1.54	0.006	mouse
mmu-miR-29b-star_st	-1.68	0.010	mouse
mmu-miR-379_st	-3.19	0.013	mouse
mmu-miR-139-5p_st	-7.59	0.013	mouse
mmu-miR-484_st	1.92	0.015	mouse
mmu-miR-702_st	1.61	0.019	mouse
mmu-miR-433_st	2.33	0.020	mouse
mmu-miR-199b-star_st	-1.51	0.022	mouse
mmu-miR-135a-star_st	-1.72	0.025	mouse
mmu-miR-615-3p_st	-1.62	0.026	mouse
mmu-miR-689_st	-3.45	0.031	mouse
mmu-miR-28-star_st	1.80	0.032	mouse
mmu-miR-505_st	1.52	0.032	mouse
mmu-miR-709_st	-4.23	0.036	mouse
mmu-miR-10b_st	-1.98	0.037	mouse
mmu-miR-30e_st	2.45	0.038	mouse
mmu-miR-10a_st	-1.59	0.038	mouse
mmu-miR-293_st	2.52	0.039	mouse
mmu-miR-467b_st	2.66	0.040	mouse

SUPPLEMENTARY DATA

mmu-miR-330_st	1.78	0.043	mouse
mmu-miR-101a-star_st	1.57	0.046	mouse
mmu-let-7e_st	-2.32	0.046	mouse
mmu-miR-467e_st	1.82	0.049	mouse
mmu-miR-495_st	1.55	0.050	mouse
mml-miR-30a-5p_st	1.54	0.006	primate
ppa-miR-29b_st	1.53	0.006	primate
mml-miR-150_st	-4.00	0.011	primate
mml-miR-18b_st	1.82	0.013	primate
ppa-miR-224_st	-6.18	0.016	primate
ppy-miR-198_st	-2.46	0.017	primate
ppa-miR-98_st	-1.82	0.018	primate
mml-miR-18_st	4.95	0.019	primate
mml-miR-99a_st	-1.59	0.022	primate
mml-miR-219-5p_st	-1.56	0.022	primate
mml-miR-211_st	3.53	0.024	primate
mml-miR-128a_st	-1.97	0.024	primate
ppy-miR-199a_st	-2.77	0.025	primate
ppy-miR-154_st	2.31	0.026	primate
ptr-miR-23b_st	-1.92	0.028	primate
mml-miR-653_st	3.87	0.031	primate
mml-miR-888_st	1.50	0.032	primate
ppy-miR-135_st	1.61	0.032	primate
mml-miR-553_st	2.09	0.032	primate
ppa-miR-141_st	1.70	0.033	primate
ppy-miR-23a_st	-1.92	0.034	primate
mml-miR-369-3p_st	-1.85	0.034	primate
mml-miR-133b_st	1.91	0.034	primate
ptr-miR-105_st	1.60	0.037	primate
mml-miR-886-5p_st	3.55	0.037	primate
ppa-miR-134_st	-3.02	0.039	primate
ppa-miR-196_st	-1.63	0.042	primate
ppa-miR-195_st	-2.45	0.043	primate
mml-miR-20b_st	5.43	0.044	primate
ppy-miR-23b_st	-1.71	0.046	primate
ptr-miR-194_st	-2.35	0.047	primate
mml-miR-505_st	1.52	0.048	primate
mml-miR-455-5p_st	1.54	0.050	primate
rno-miR-425_st	2.26	0.001	rat
rno-miR-30e_st	4.35	0.007	rat
rno-let-7i_st	-1.57	0.020	rat
rno-miR-369-5p_st	2.68	0.022	rat
rno-miR-150_st	-5.35	0.022	rat
rno-miR-379_st	-2.51	0.034	rat
rno-miR-451_st	2.77	0.038	rat
rno-miR-195_st	-2.48	0.042	rat
rno-miR-23b_st	-1.91	0.042	rat
rno-miR-128_st	-1.61	0.049	rat

SUPPLEMENTARY DATA

Supplementary Table 2. Differentially expressed human miRNA in PCOS (2-fold change, $p < 0.05$).

miRNA	**Fold change (PCOS vs. Controls)**	**p-value**
hsa-miR-422a_st	-6.48	0.048
hsa-miR-150_st	-2.71	0.028
hsa-miR-195_st	-2.71	0.050
hsa-miR-199a-3p_st	-2.64	0.012
hsa-miR-125b-2-star_st	-2.55	0.049
hsa-miR-181c_st	-2.46	0.042
hsa-miR-574-5p_st	-2.33	0.007
hsa-miR-93-star_st	2.10	0.046
hsa-miR-499-5p_st	2.11	0.026
hsa-miR-154_st	3.62	0.042

SUPPLEMENTARY DATA

Supplementary Table 3. Differentially Expressed miRNA in PCOS adipose tissue.

miRNA	Fold-Change (PCOS vs. Control)	p-value	miRNA confirmed
miR-93	+2	0.04	Yes
miR-30d	+1.6	0.02	Trend
miR-574-5p	-2.3	0.007	Yes
miR-223	2	0.003	Yes
miR-21	-2.8	0.03	Yes
miR-133a	+2	0.03	Yes
miR-133b	+2	0.02	Yes

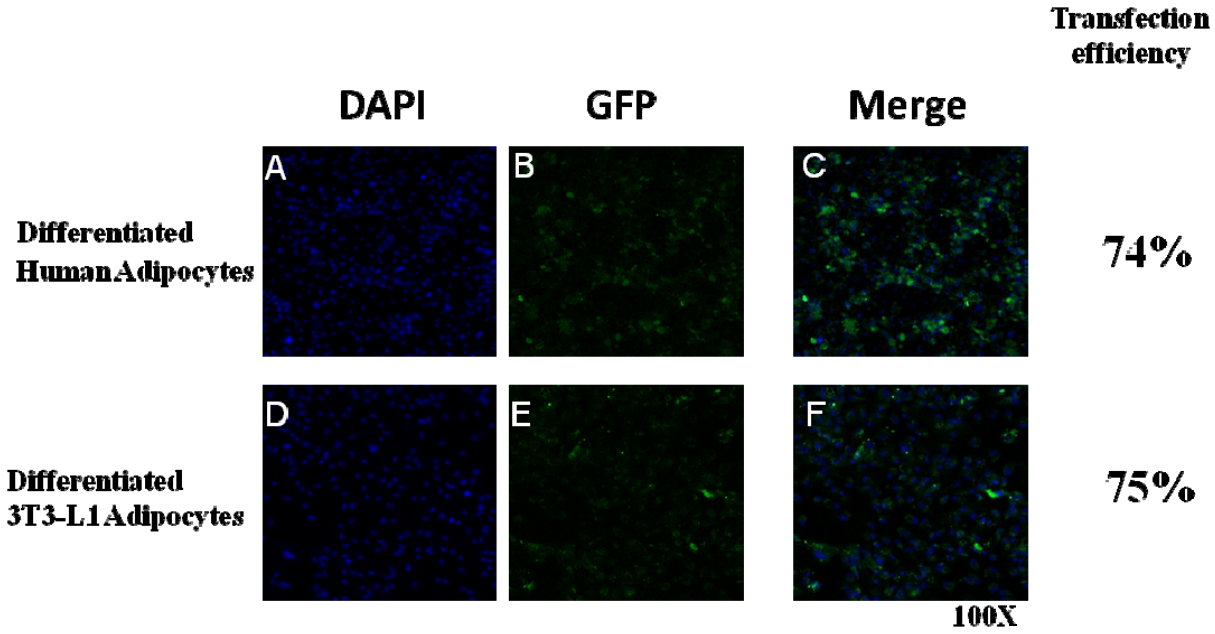
SUPPLEMENTARY DATA

Supplementary Table 4. Shared Predicted Gene Targets of miR-93 (miRanda, TargetScan, and PicTar)

ACSL4	CAMTA1	EPHA4	KPNA3	PKD2	SEMA4B	TSG101
ADAM9	CD69	EPHA5	LACE1	PLS1	SERP1	TSHZ3
ANKRD13C	CDC37L1	EPHA7	LAPTM4A	PPP2R2A	SLC17A7	TXNIP
ANUBL1	CDCA7	F3	LHX6	PPP3R1	SLC2A4 (GLUT4)	USP3
ARHGAP1	CENPO	FAM13C	LHX8	PPP6C	SLC40A1	USP32
ARHGAP12	CEP97	FAT2	LIMA1	PRR15	SMAD7	USP6
ARHGEF10	CHD9	FBXL5	LMO3	PRR16	SMOC1	WDR37
ARHGEF3	CLIP4	FBXW11	MAP3K5	PRRG1	SMOC2	ZBTB4
ARID4B	CMPK1	FEM1C	MAP7	PTPN4	SNX16	ZBTB7A
ATAD2	CNOT7	FJX1	MAPRE1	RAB5B	SOX4	ZBTB9
ATG16L1	CRIM1	FNDC3A	MASTL	RAP2C	SSX2IP	ZFPM2
ATL3	CRY2	FOXJ2	MCL1	RAPGEF4	STC1	ZFYVE9
BAHD1	DDX5	FRMD6	MED12L	RASD1	STK38	ZNF148
BAMBI	DERL2	GNB5	MINK1	RASL11B	TCTEX1D1	ZNF236
BCL11B	DNAJB9	HAS2	MKRN1	RBL1	TET1	ZNF362
BICD2	DPYSL2	HBP1	MLL4	RBL2	TGOLN2	ZNF512B
BNIP2	DRD1	HIF1A	MORF4L1	REEP3	TNFRSF21	ZNFX1
BTBD10	DUSP2	HN1	MYCN	RGL1	TNKS1BP1	
BTG3	DYNC1LI2	HPS5	NAGK	RGMA	TNKS2	
C11orf30	E2F1	KAT2B	NCOA3	RHOC	TNRC6A	
C11orf82	EGLN3	KCNJ10	NR4A3	RNF128	TOPORS	
C15orf17	EGR2	KIAA0922	NUP35	RRAGD	TRIM3	
C1orf63	EIF5A2	KIF23	PCDHA1	RSBN1	TRIM36	
C5orf41	ELK3	KLHL20	PEX5L	SAR1B	TRIP10	

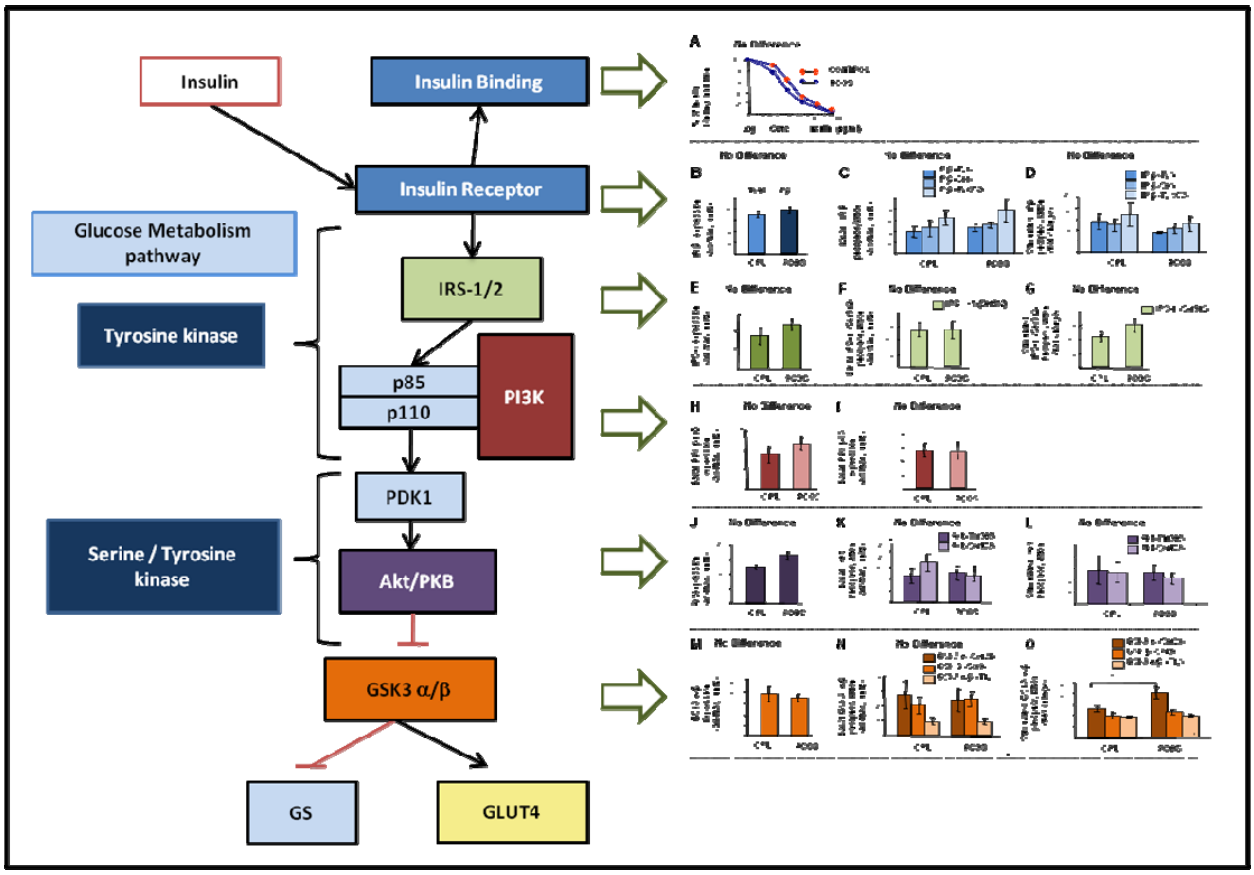
SUPPLEMENTARY DATA

Supplementary Figure 1. Transfection efficiency of human and 3T3-L1 differentiated adipocytes. Human and 3T3-L1 adipocytes were cultured in four chambers slide. After transfection, GFP positive cells were counted under microscope in three chambers of slide. Human differentiated adipocytes (A: DAPI, B: GFP, C: Merge). 3T3-L1 adipocytes. (D: DAPI, E: GFP, F: Merge).



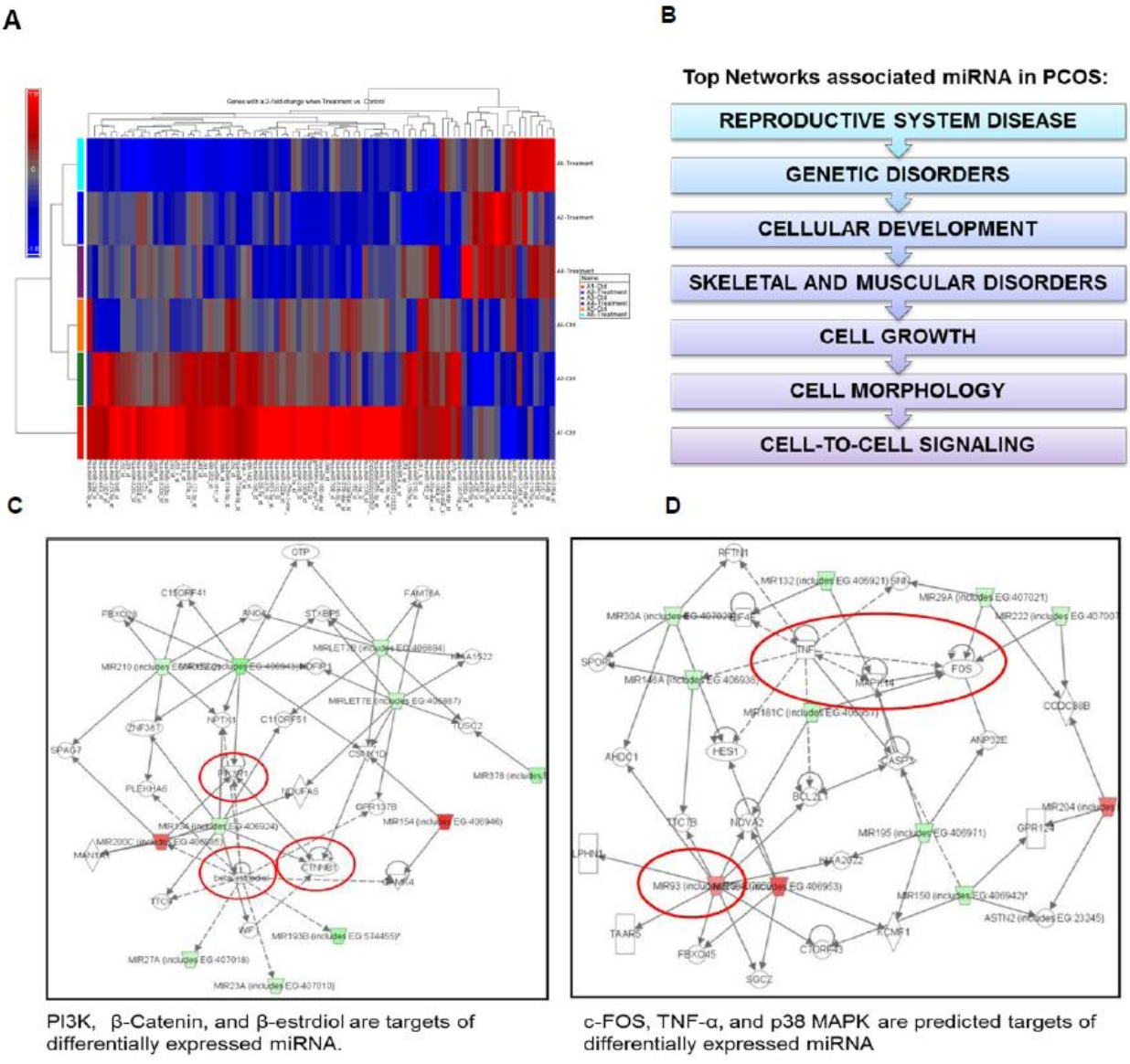
SUPPLEMENTARY DATA

Supplementary Figure 2. Simplified scheme of IRS-1/PI3K/AKT pathway and insulin binding to its receptor, with protein expression of various insulin signaling targets. 10 control (BMI range: 21.3-47.1, average: 26.7) and 13 PCOS (BMI range: 23.1-40.4, average: 30.69) were analyzed by western blotting. Isolated adipocytes were treated with 100 nM human insulin for 10 minutes. (A) Insulin binding is expressed as percentage of insulin binding inhibition. (B-O) Graphs indicate quantification of western blots. Total, basal and insulin-stimulated phosphorylated proteins of selected components are represented by bars (medium ± SE): IR (blue), IRS-1 (green), PI3K (red), AKT (violet), GSK3α/β (orange).



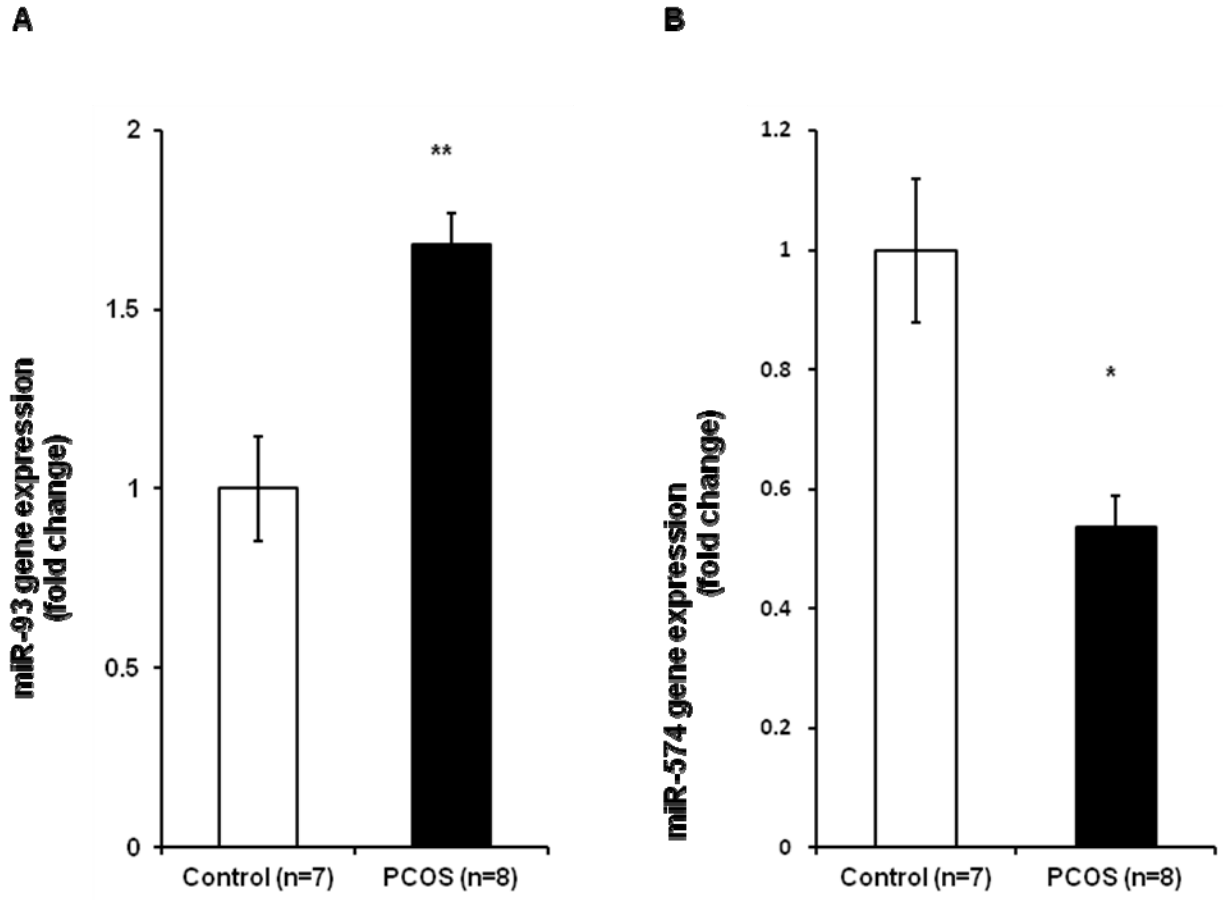
SUPPLEMENTARY DATA

Supplementary Figure 3. Differential Expression of microRNA in PCOS. Microarrays were performed to determine miRNA expression in the adipose tissue of non-obese women with PCOS and matched controls. (A) A heatmap displays miRNA array results, indicating miRNA that is up (red) or down (blue) more than 2-fold in PCOS, labeled "Treatment" (lanes A2,A4, and A6) vs. control (lanes A1, A3, and A5). (B) Gene ontology performed by IPA software analysis determined the top networks associated miRNA in PCOS, which ranked reproductive system disease and genetic disorders the highest. (C-D) Gene network analysis displaying miRNA networks related to reproductive system diseases indicate that PI3K, β-Catenin, β-estrdiol, c-FOS, TNF-α, and p38 MAPK are all targets of differentially expressed miRNA.



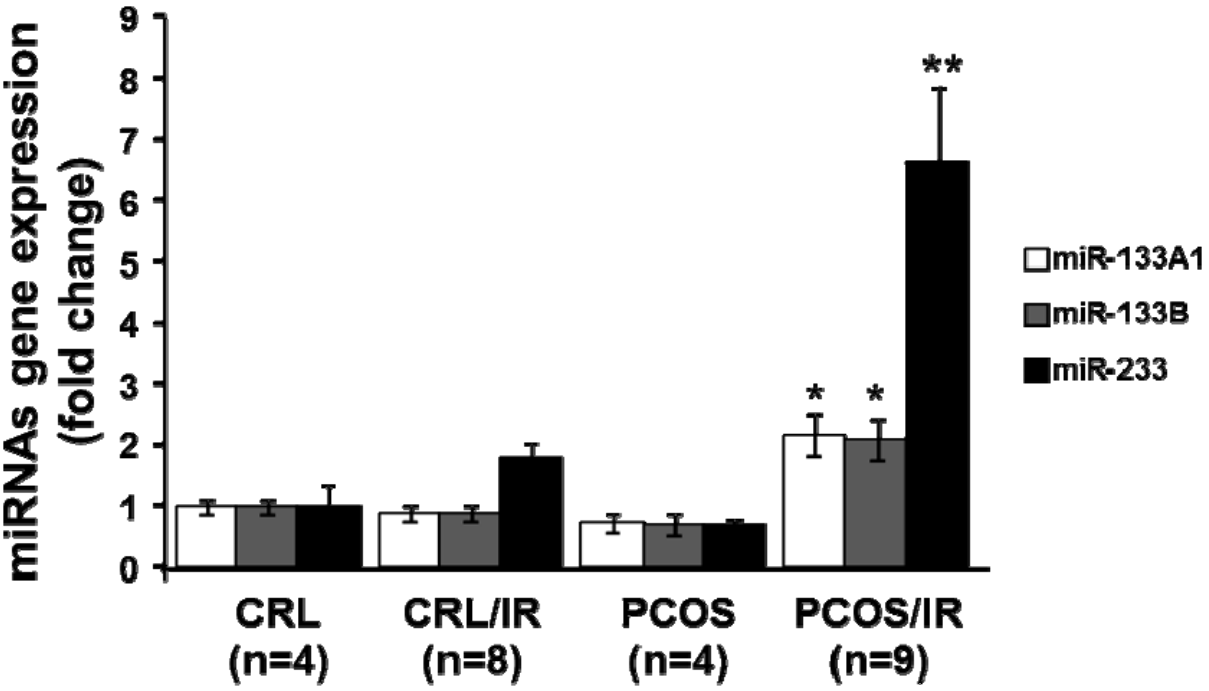
SUPPLEMENTARY DATA

Supplementary Figure 4. Confirmation of differentially expressed miRNA. Independent samples were used to confirm differential expression of miRNA in adipose from non-obese women with PCOS. (A) RT-PCR confirmed miR-93 to be significantly overexpressed ($p < 0.01$) in PCOS (n=8) vs. control (n=7). (B) In the same samples, RT-PCR confirmed miR-574 to be significantly under expressed ($p < 0.05$) in PCOS vs. controls.



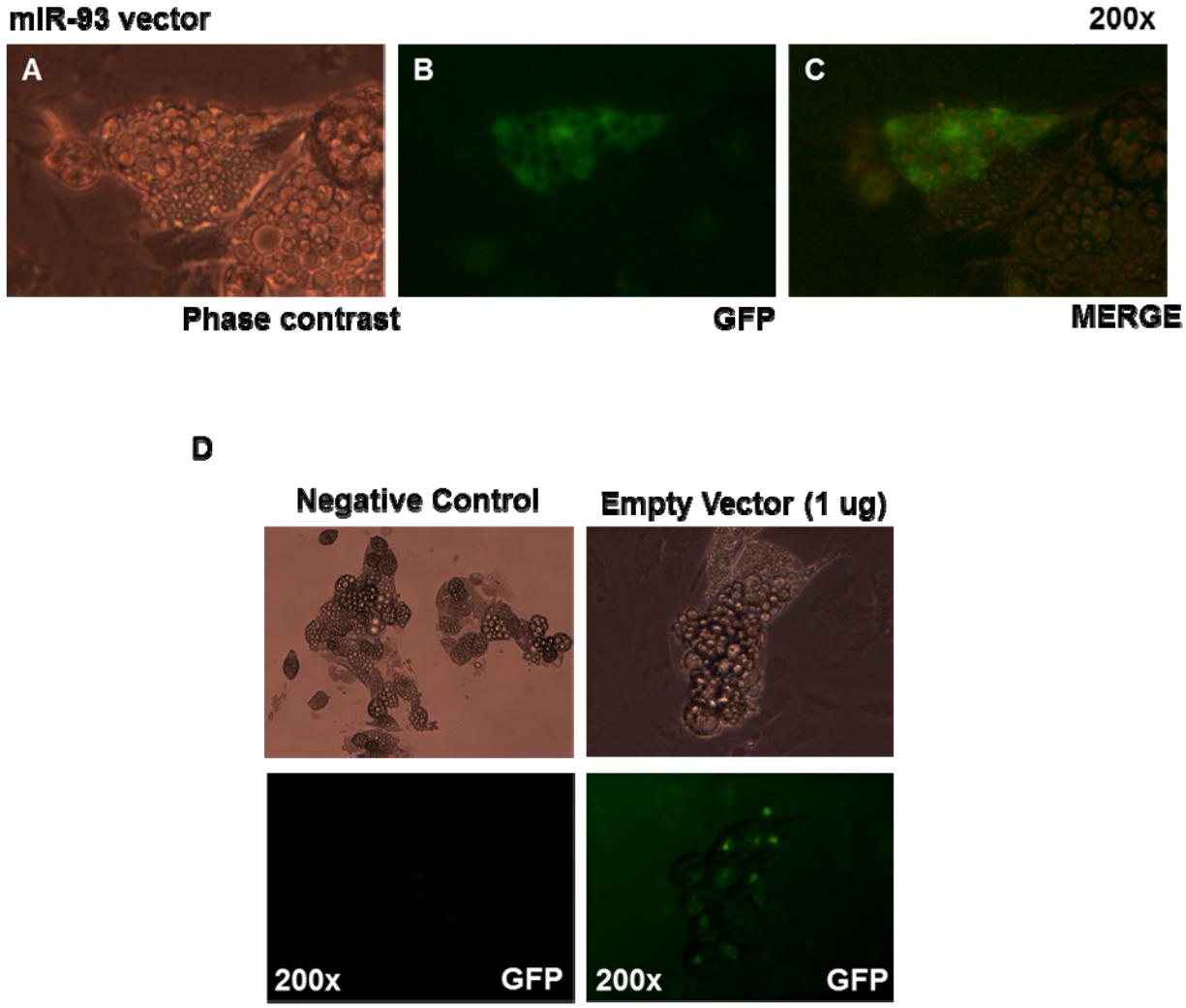
SUPPLEMENTARY DATA

Supplementary Figure 5. miR-133 and miR-223 genes expression in human adipose tissues. CRL: control, CRL/IR: control with insulin resistance, PCOS/IR: PCOS with insulin resistance. *, $p<0.05$; **, $p<0.01$ vs CRL, CRL/IR or PCOS.



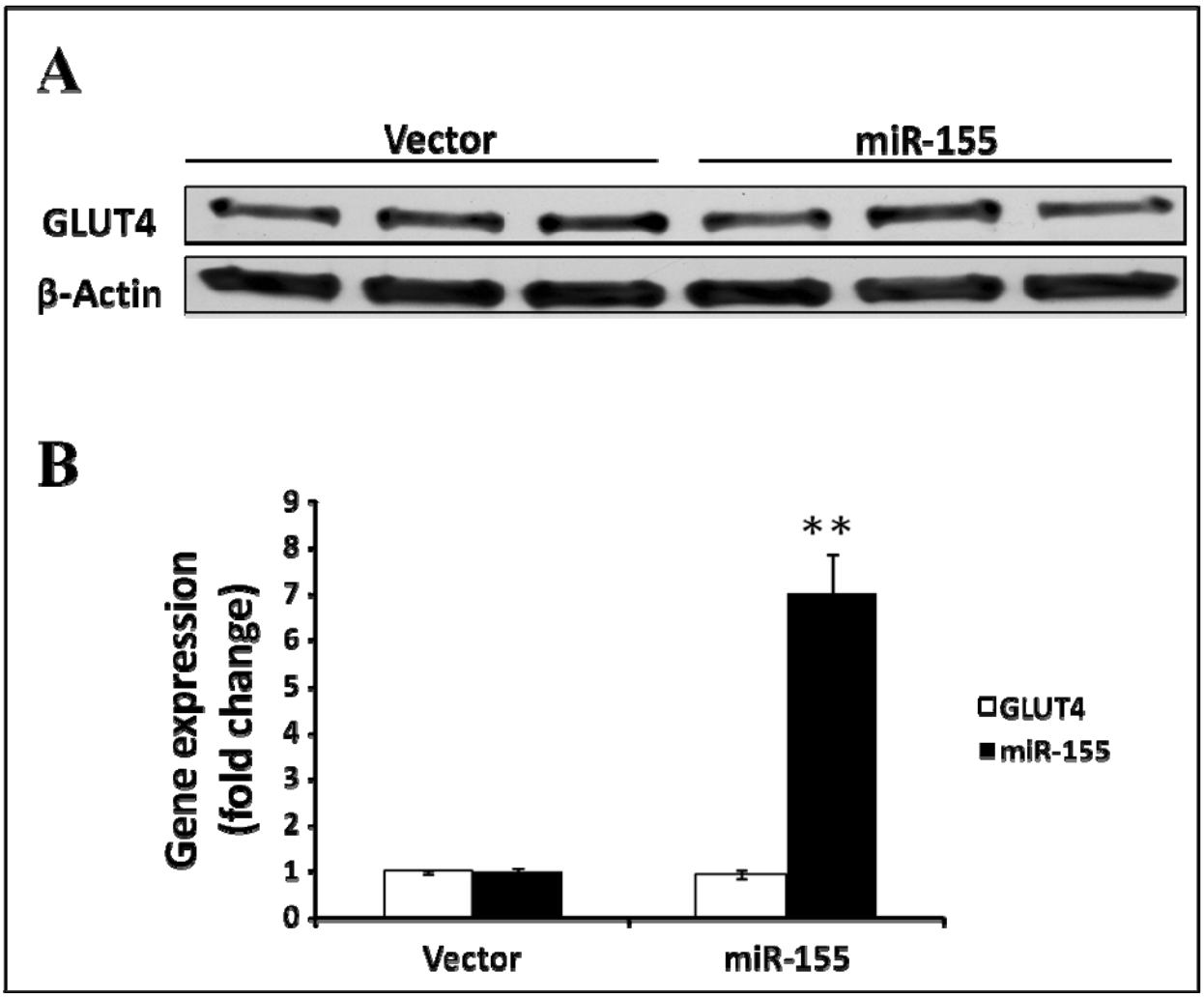
SUPPLEMENTARY DATA

Supplementary Figure 6. Transfection of miR-93 overexpression vector. (A) Differentiated human adipocytes in light phase contrast, x200. (B) GFP plasmid vector. (C) Merge of light and GFP. (D) Transfection controls.



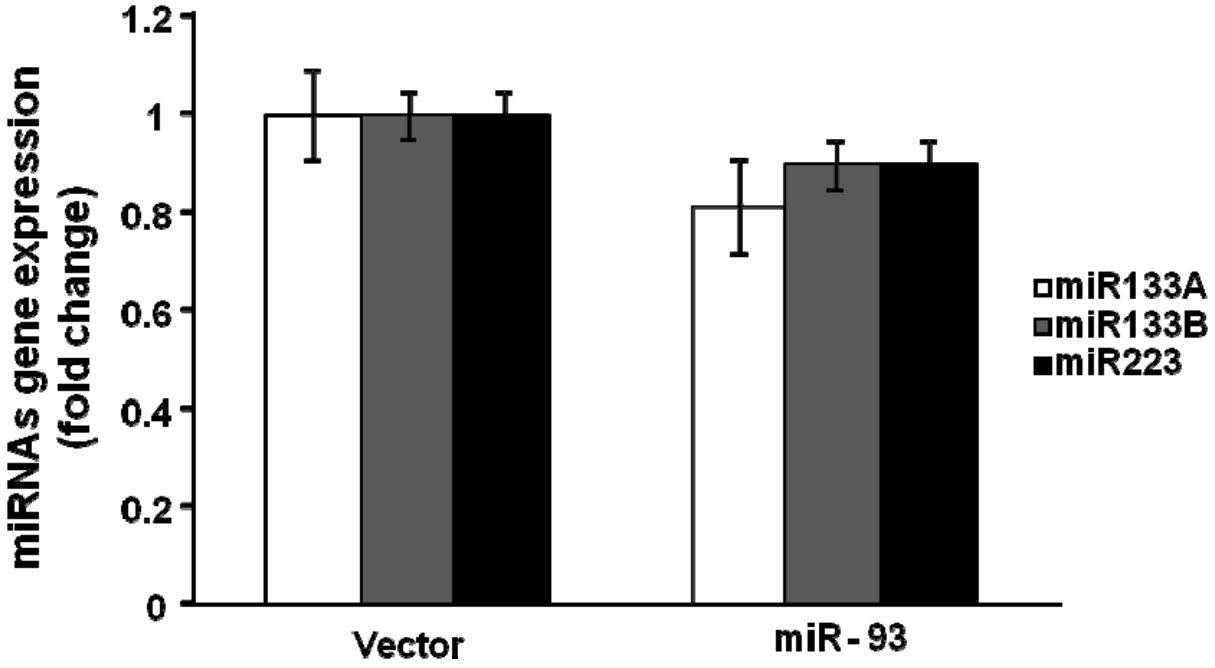
SUPPLEMENTARY DATA

Supplementary Figure 7. GLUT4 expression in miR-155 overexpressed human cultured differentiated adipocytes (n=3). (A) protein; (B) gene expression.



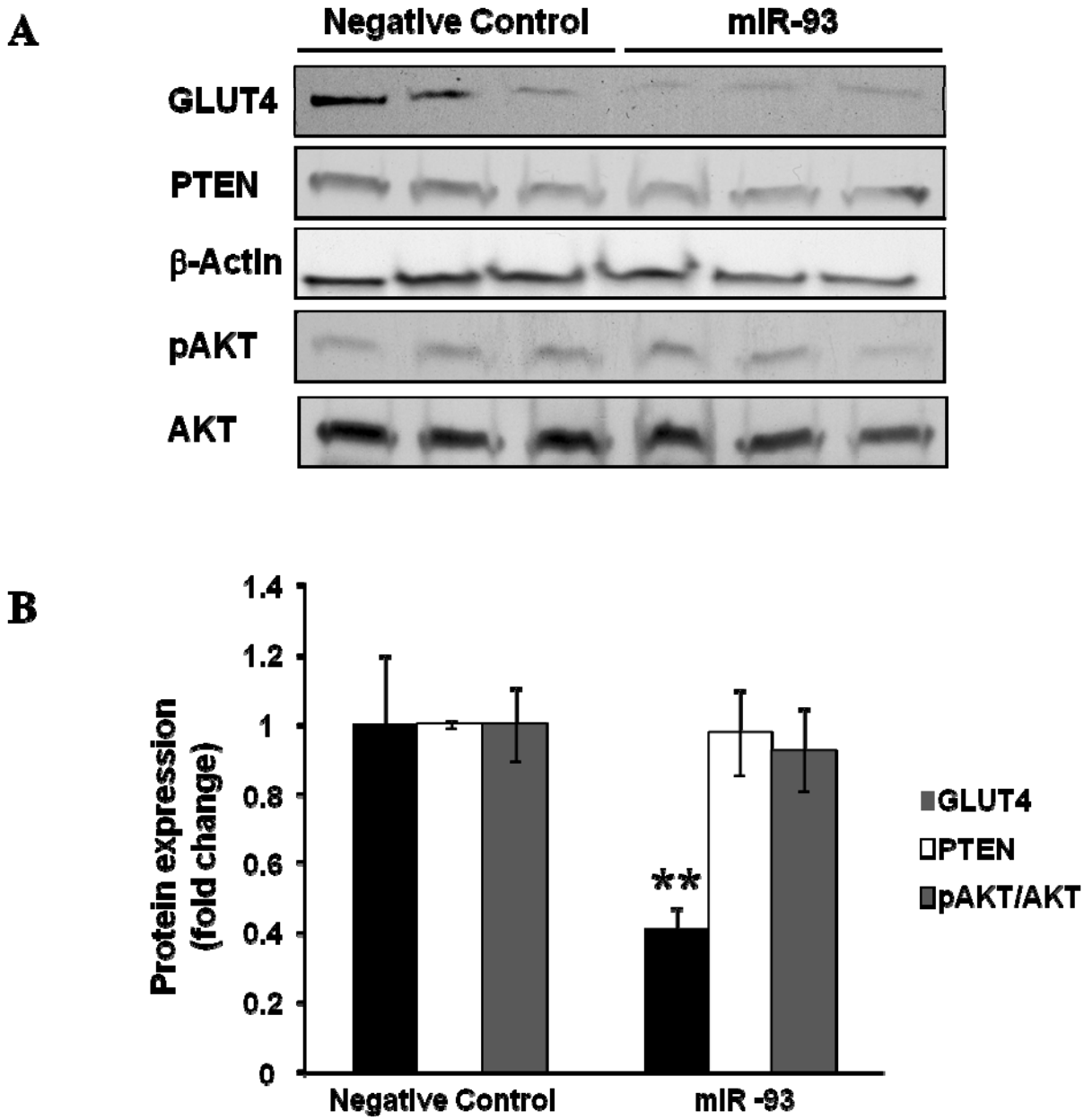
SUPPLEMENTARY DATA

Supplementary Figure 8. miR-133 and 223 genes expression in miR-93 overexpressed human cultured differentiated adipocytes (n=3).



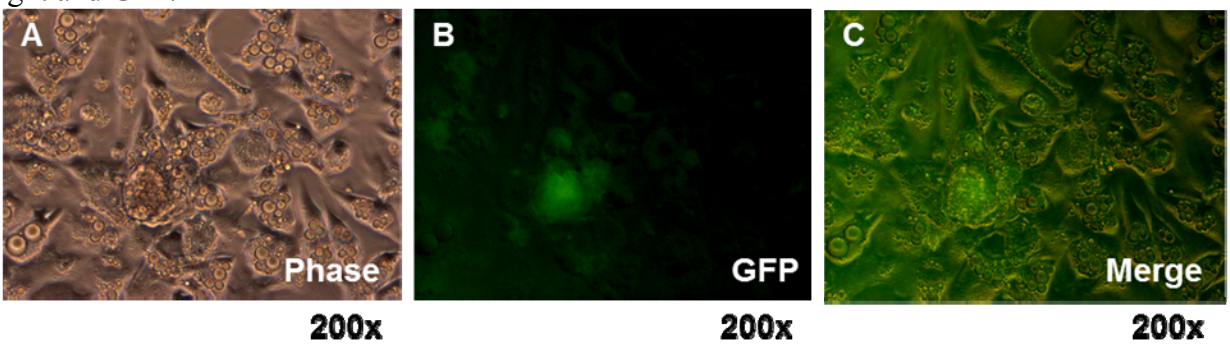
SUPPLEMENTARY DATA

Supplementary Figure 9. Protein expression of GLUT4 and PTEN in human cultured differentiated adipocytes (n=3)**.** (A) Western blot. (B) Western blot band density assay.



SUPPLEMENTARY DATA

Supplementary Figure 10. Transfection of miR-93 overexpression vector in mouse 3T3-L1 adipocytes. (A) Differentiated 3T3-L1 adipocytes in light phase contrast, x200. (B) GFP plasmid vector. (C) Merge of light and GFP.



Supplementary Figure 11. Expression vector and immunofluorescence negative controls. Imaging of GLUT4 controls demonstrate (A) cell morphology by light phase contrast; (B) GFP signal in the absence of a vector with GFP-reporter; (C) minimal background with an exposure time of 485 msec; and (D) low non-specific binding by secondary Ab.