Supplemental Data

Table 1. Sequences of oligonucleotide for Sirt2 shRNA knockdown.

Oligo Name	Sequence
S-1 Forward	GATCCCCGAAGGAGTGACACGCTACAttcaagaga TGTAGCGTGTCACTCCTTCTTTTTGGAAA
S-1 Reverse	AGCTTTTCCAAAAAGAAGGAGTGACACGCTACAtctcttgaa TGTAGCGTGTCACTCCTTCGGG

Underlined uppercase sequences represent the restriction sites, uppercase sequences without underline represent the antisense and sense sequence of the RNAi, lowercase sequences represent the folding loop region.

Table 2. Antibody list

Antibody Target	Dilution	Antibody Type	Vendor
anti-FLAG M2	1:10000	Mouse	Sigma
anti-actin	1:5000	Rabbit	Santa Cruz
anti-PPARγ	1:1000	Rabbit	Upstate
C/EBPβ	1:1000	Rabbit	Santa Cruz
C/EBPα	1:1000	Rabbit	Santa Cruz
FAS	1:2000	Rabbit	Abcam
Glut4	1:1000	Rabbit	Chemicon
SOD4	1:1000	Rabbit	Abcam
Lamin A	1:1000	Rabbit	Abcam
FoxO1	1:1000	Rabbit	Santa Cruz
Ser-253 phosphorylated FoxO1	1:1000	Rabbit	Cell Signaling
Sirt2	1:1000	Rabbit	Cell signaling
Sirt1	1:2000	Rabbit	Upstate
Akt	1:1000	Rabbit	Cell Signaling
MAPK	1:1000	Rabbit	Cell Signaling
Phosphorylated Akt Ser307	1:1000	Rabbit	Cell Signaling
Phosphorylated MAPK	1:1000	Rabbit	Cell Signaling
Phosphorylated p38 MAPK	1:1000	Rabbit	Cell Signaling
Acetylated lysine	1:1000	Mouse	Upstate

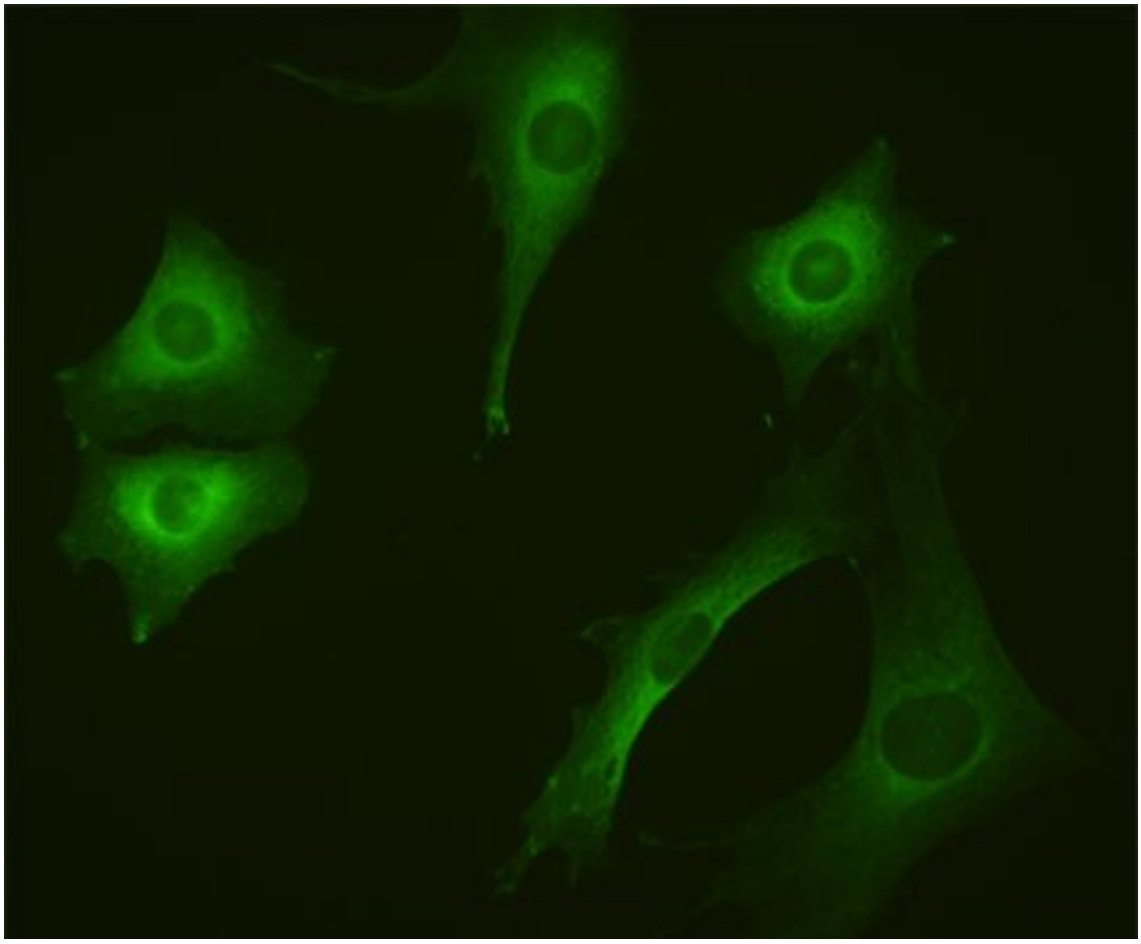
The use of antibodies for western blot and immunoprecipitation were following the instruction or recommendation by the vendors. Actual application concentrations may vary depending on the antibody sensitivity or background levels.

Table 3. Realtime quantitative PCR primer list.

Gene Name	Forward Primer Sequence 5'-3'	Reverse Primer Sequence 5'-3'
Glut4	TGATTCTGCTGCCCTTCTGT	GGACATTGGACGCTCTCTCT
C/EBPα	CAAGAACAGCAACGAGTACCG	GTCACTGGTCAACTCCAGCAC
Sirt1	AGAACCACCAAAGCGGAAA	TCCCACAGGAGACAGAAACC
Sirt2	AGCCAACCATCTGCCACTAC	CCAGCCCATCGTGTATTCTT
Sirt3	TGCTACTCATCTTGGGACCT	CACCAGCCTTTCCACACC
FoxO1	GCTTTTGTCACATGCAGGT	CGCACAGAGCACTCCATAAA
FABP4/aP2	GATGCCTTTGTGGGAACCT	CTGTCGTCTGCGGTGATTT
TBP	ACCCTTCACCAATGACTCCTATG	TGACTGCAGCAAATCGCTTGG
FAS	GGCTCTATGGATTACCCAAGC	CCAGTGTTCGTTCCTCGGA
PPARγ	TCAGCTCTGTGGACCTCTCC	ACCCTTGCATCCTTCACAAG

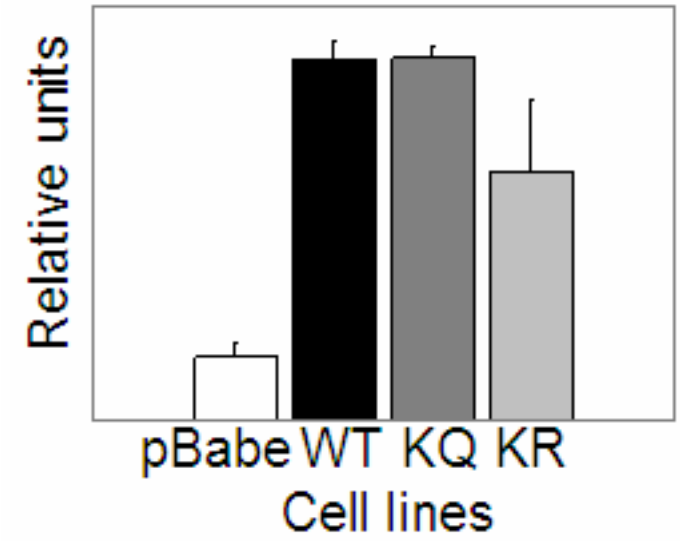
All primers were designed with Primer3 online design tool.

Figure 1. Subcellular distribution of Sirt2-FLAG overexpression in 3T3-L1 preadipocytes.



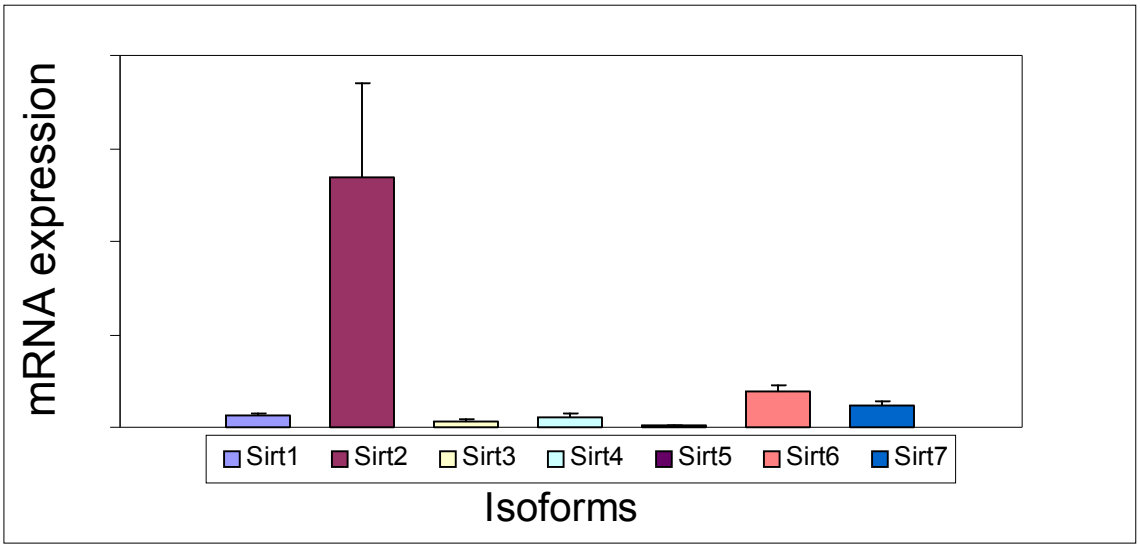
Immunocytochemistry was done with anti-FLAG-FITC. Exogenous Sirt2 overexpression showed similar distribution pattern to previous reports regarding endogenous Sirt2 subcellular localization, which is mainly in the cytoplasm. There is lighter signal in the nucleus with very localized pattern, which suggests that Sirt2 may trafficking to the nucleus.

Figure 2. Overexpression of recombinant FoxO1 wildtype and mutants.



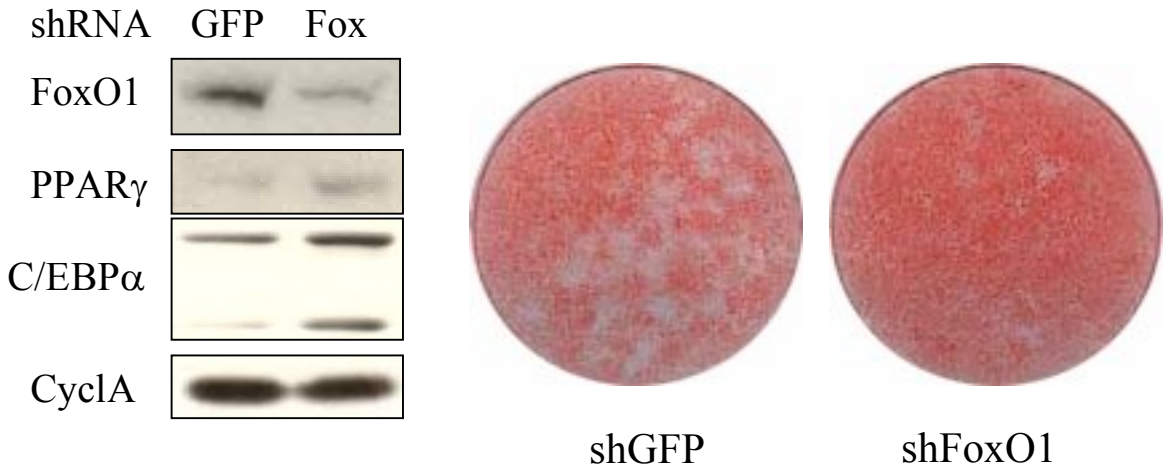
Realtime PCR of FoxO1 indicated there was 5 fold increase of recombinant FoxO1 mRNA expression in different stable cell lines.

Figure 3. Sirt2 mRNA is most abundant isoform among 7 members in 3T3 L1 cells.



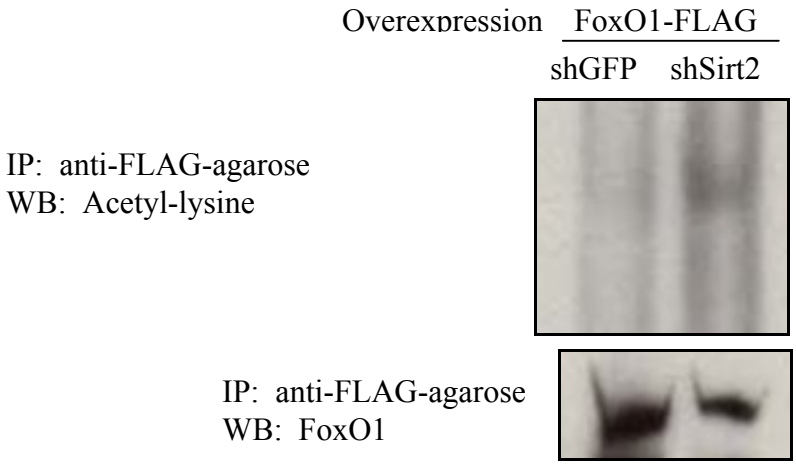
Realtime PCR was performed using different primers targeting 7 sirt isoforms. Results showed that Sirt2 mRNA is most abundant transcript among 7 family members in 3T3L1 cells. Among other Sirt transcripts, Sirt6 and Sirt7 have relatively higher mRNA expression level, while Sirt5 mRNA abundance is the lowest among 7 family members.

Figure 3. FoxO1 knockdown in 3T3L1 cells promotes adipocyte differentiation.



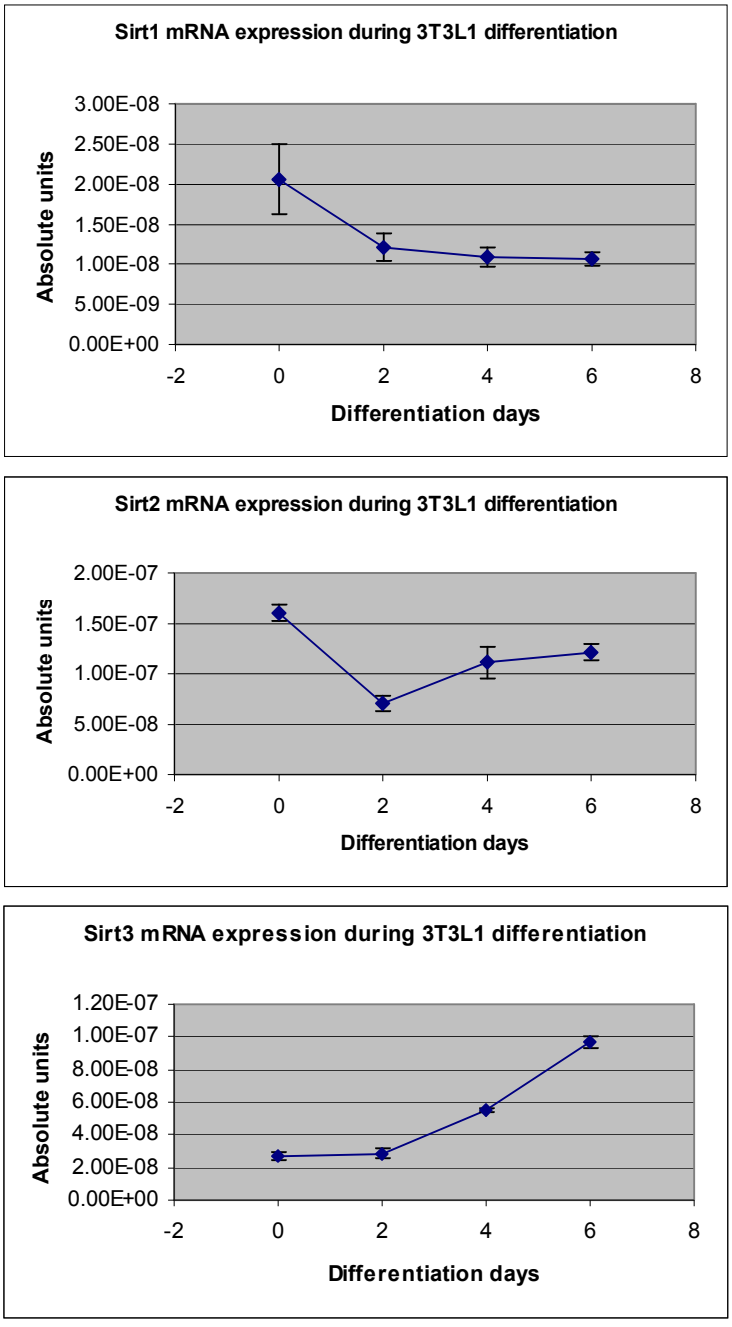
Stable cell lines overexpressing shRNA targeting either endogenous FoxO1 or GFP were generated. Cells were subjected to white adipocyte differentiation protocol after 6 days. Total cell lysate was collected and subjected to SDS gel separation and western blotting with antibodies against different adipocyte markers. There is markedly increased expression of PPARγ and C/EBPα in shFoxO1 cells after differentiation, while cyclophillinA expression is unchanged between cell lines. FoxO1 knockdown was detected using lysate from day 0 before cells were induced, western blotting showed there was about 75% reduction endogenous FoxO1 protein expression in shFoxO1 cells comparing with shGFP cells. After 6 days of differentiation, cells were fixed and stained with Oil Red O, more ORO staining in shFoxO1 cells indicated that these cells had more lipid accumulation than shGFP control cell line.

Figure 4. Endogenous Sirt2 knockdown induces hyperacetylation of exogenous FoxO1.



Cell lines overexpressing FoxO1-FLAG is also stably transfected with either shGFP or shSirt2 overexpression constructs. Lysate from these two cell lines were subjected to immunoprecipitation using anti-FLAG-agarose, thus the exogenous FoxO1 is precipitated. Then the precipitated lysate was applied to SDS gel and western blotting using either anti-acetyl-lysine or FoxO1 antibody. The results showed that FoxO1 was equally precipitated from both cell lines while cells carrying shSirt2 had higher acetyl-lysine reactivity where the signal could not be detected in cells with shGFP. The result indicated that knocking down endogenous Sirt2 lead to hyperacetylation of exogenous FoxO1, that suggested FoxO1 being potential substrate of Sirt2.

Figure 5. The mRNA expression patterns of Sirt1~3 during 3T3 L1 adipocyte differentiation.



Real time PCR was used to detect the mRNA expression patterns of Sirt1-3 during 3T3 L1 differentiation time course. During 3T3 L1 adipocyte differentiation, Sirt1 and Sirt2 showed similar expression pattern that both are decreased after induction (day 2) and stay down through out the time course, while Sirt3 displays increasing mRNA expression during differentiation time course.