Appendix 2

Immunoprecipitation of PGC-1α and FOXO1 for acetylation.

Preparing mouse liver extracts
Buffer A
10mM KHEPES pH 7.9,
10mM KCl,
1.5mM MgCl2,
0.5mM DTT,
1mM PMSF,
protease inhibitors (Roche),
10mM Nicotinamide,
1μM TSA,
phosphatase inhibitors

RIPA Buffer
1% NP-40
0.1% SDS
0.5% NaDeoxycholate
In PBS

Pulverize mouse liver on liquid nitrogen store at -80°C
Incubate ~300mg pulverized liver in 500uL Buffer A for 20 minutes on ice.
Homogenize tissue with Tissue Miser (Fisher) for 10 seconds on a medium speed
Spin 3 minutes at 4K RPM
Aspirate Supernatant and Resuspend Pellet in 300uL RIPA Buffer
Vortex 3x 15sec
Spin 10 minutest at 16K RPM
Save the supernatant
Quantitate protein

Immunoprecipitation.

Dilute 500ug of liver extracts with at least 3 volumes of PBS (with Nam, TSA, and protease inhibitors)
Add anti-PGC-1α (Santa Cruz, H-300, K-15, or P-19) or anti-FOXO1 (Santa Cruz, H-128) at 1:50-1:20.
IP overnight rotating at 4°C
Add 10uL of 50% Protein A Agarose and 10uL of 50% Protein G agarose.
Rotate 2-4 hours at 4°C
Wash IPs at least 3x with 500uL PBS (with Nam and TSA)
after final wash add 100uL 2x SDS sample buffer and boil.

Notes.
Perform a control IP (IgG or no antibody) and an Antibody alone IP.
A positive IP control can be performed using liver extracts from mice overexpressing PGC-1a. Or by adding whole cell extracts from 293 overexpressing PGC-1a to the liver extracts-this will yield a very strong PGC-1a band on the western blot.
PGC-1a runs at ~120kDa, its best to run a 7% gel until the 120kDa is in the middle; to get good separation as sometimes there is an IgG band ~110kDa.
When western blotting for anti-acetylated lysine (Cell Signaling): block in 5% milk TBSt for 1 hour and probe overnight with anti-acetylated lysine (1:2000) in 5% BSA TBSt.