Appendix 1

Liver nuclear 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

Buffer B
20mM KHEPES pH 7.9,
400mM NaCl,
1.5mM MgCl2,
0.5mM DTT,
0.2mM EDTA,
1mM PMSF,
protease inhibitors (Roche),
10mM Nicotinamide,
1μM TSA, and
phosphatase inhibitors

-Incubate ~300mg pulverized liver in 1.0mL Buffer A for 20 minutes on ice
-Homogenize using a Tissue Miser (Fisher) for 10 seconds on medium speed
-spin 3 minutes 4K RPM at 4°C
-Remove supernatant
-Resuspend pellet in 200uL Buffer B
-rotate for 20 minutes at 4°C
-spin for 10 minutes at 16K RPM 4°C
-Quantitate Protein.

10μg of nuclear extract were run on SDS-PAGE and probed with antibodies: SIRT1 (Upstate), anti-PGC-1α (a generous gift from the laboratory of Marc Montminy), FOXO1 (Santa Cruz, H-128), HNF-4α (Santa Cruz, H-171), and Actin (Lab Vision).