Supporting information for Lee *et al.* (2002) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.232308999

Supporting Text

Target RNA Preparation and High-Density Oligonucleotide Array Hybridizations. Total RNA was extracted from frozen tissue by using TRIZOL reagent (Life Technologies, Grand Island, NY). Polyadenylate [poly(A)$^+$] RNA was purified from the total RNA with oligo(dT)-linked oligotex resin (Qiagen, Valencia, CA). One microgram of poly(A)$^+$ RNA was converted into double-stranded cDNA (dscDNA) by using the SuperScript Choice System (Life Technologies) with an oligo(dT) primer containing a T7 RNA polymerase promoter (Genset, La Jolla, CA). dscDNA was extracted with phenol-chloroform-isoamyl alcohol and precipitated with pellet paint coprecipitant (Novagen). Biotin-labeled RNA was synthesized *in vitro* by using the BioArray High Yield RNA Transcript Labeling Kit (Enzo, Farmingdale, NY). The biotin-labeled antisense cRNA was purified using the RNeasy affinity column (Qiagen), and fragmented randomly. The hybridization cocktail (200 μl) containing 10 μg of fragmented cRNA was injected into the MG-U74A array (Affymetrix, Santa Clara, CA). The gene chip was placed in a 45°C oven at 60 rpm for 16 h. After hybridization, the gene chips were washed and stained in a fluidic station (Model 800101, Affymetrix) with signal amplification protocol using antibody. DNA chips were scanned at a resolution of 3 μm twice by using a Hewlett–Packard GeneArray Scanner (Model 900154, Affymetrix), and the averaged images were used for further analysis.

Preliminary Data Analysis by Affymetrix Algorithms. Affymetrix software determines the presence of mRNA in samples and computes the signals of probe sets. The software calculates differences and ratios between perfect match and mismatch signals, which are representative of the hybridization levels of their targets in each probe set. These values are integrated into a decision matrix to determine whether the transcript is detected in the sample. The average of the differences between perfect match and mismatch signals (after removing the outliers beyond 3 standard deviations) is used to estimate relative mRNA levels of the transcripts. Signals in each image are normalized to minimize an overall variability in hybridization intensities by a global scaling method. Global scaling is the computational technique in which the average signal of all probe sets in an image is scaled to a target average intensity by multiplying a scaling factor. This scaling factor is multiplied to each probe set signal for normalization. Because the probe set signal is directly related to its expression level, the ratio between two images becomes the fold change (FC). To calculate FC between caloric restriction (CR) data and control data, the following formulae are used by the software:

$$\text{FC} = \frac{(\text{Signal}_{\text{CR1}} - \text{Signal}_{\text{Control1}})}{(\text{the smaller of either Signal}_{\text{CR1}} \text{ or Signal}_{\text{Control1}})} + 1 \text{ if Signal}_{\text{CR1}} \geq \text{Signal}_{\text{Control1}}$$

$$(\text{Signal}_{\text{CR1}} - \text{Signal}_{\text{Control1}})$$

$$FC = \frac{}{\text{(the smaller of either } Signal_{CR1} \text{ or } Signal_{Control1})} \quad - 1 \text{ if } Signal_{CR1} < Signal_{Control1},$$

where $Signal_{CR1}$ is the signal of a probe set for a gene 1 from a mouse on the CR diet, and $Signal_{Control1}$ is the signal of the same probe set for the same gene 1 from a mouse in control diet. Alternatively, if the denominator is less than the noise, the greater of the two scaled-noise values from the two images substitutes the denominator in the calculation of FC. Noise is the average of mean adjusted standard deviation of pixel signals in the background, which represents mechanical variation of the scanner.