

Supplemental Research Design and Methods

Evolution Analysis

Following proteins were used in evolutionary analysis with MEGA3 package (1): human MondoA (genbank accession no. NP_055753), fish MondoA (CAG08439), mouse MondoA (AY968204), human ChREBP (AF245470), mouse ChREBP (NP_067430), rat ChREBP (AB074517), fish ChREBP (CAF98521), *D. melanogaster* Mio (AAF53988) and *C. elegans* myc- and mondo-like protein (AAA19059). The sequences were aligned with the Clustal-W algorithm (2). Unequal rate of evolution was assumed for distance calculation. Phylogenetic tree was reconstructed with Neighbor-joining method (3).

Quantitative RT-PCR

Full-length ChREBP or ChREBP Δ 1-196 was cloned into pMSCV-puro retroviral vector (Clontech). Retrovirus made with these constructs or empty vector were infected into 832/13 cells. Infected cells were selected with puromycin in media containing either 2.5 mM or 27.5 mM glucose for 48 hours so that all non-infected cells were killed. mRNA was extracted from these cells with RNeasy RNA miniprep kit (Qiagen), and cDNA was synthesized with Omniscript RT kit (Qiagen). The levels of L-PK (genbank: NM_012624) and ACC (NM_022193) were determined by Real-time PCR with cDNA from these samples as templates. The primer sequences for L-PK, ACC, and internal control eEF-1G (XM_215165) are as follows. L-PK: forward, 5'-tgccttctccagcagcag-3'; reverse, 5'-agaagttgagtcgtgcaatgt-3'; ACC: forward, 5'-tgtgaggtggatcagagattt-3'; reverse, 5'-agccccaaggtacaggtg-3'; eEF-1G: forward, 5'-ggccaaaccaaccgcacc-3'; reverse, 5'-cgatgtcactgtcagcaaag-3'.

Supplemental figure legend

Supplemental Figure 1. Amino acid sequence alignment of Mondo gene family

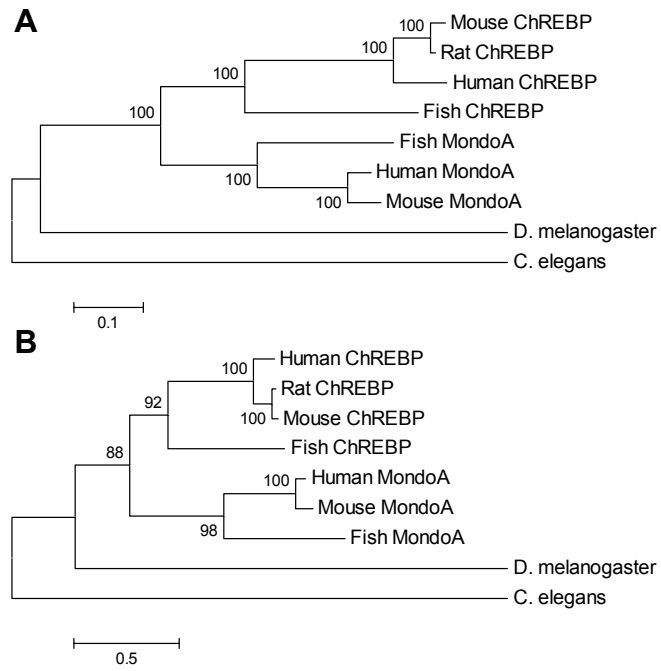
Alignment of amino acid sequences of GSM region of the Mondo family genes. Amino acids were colored according to the following rule: Acidic amino acids, Red; Basic, Dark blue; Hydrophilic, Green; Hydrophobic, Brown; Proline, Pink.

Supplemental Figure 2. Phylogentic tree of Mondo family. Based on sequence of full length (A) or GSM region (B) of Mondo proteins. Scale bar denotes the percent of distance. Bootstrap values (degree of confidence) are indicated at each node.

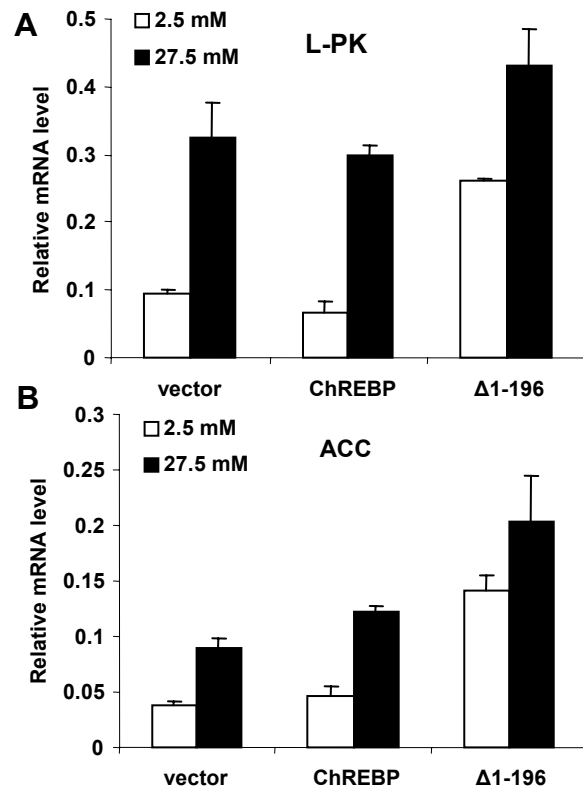
Supplemental Figure 3. Constitutively-active ChREBP activates endogenous glucose target genes. mRNA level of (A) L-PK and (B) ACC in 832/13 cells infected with empty virus or those harboring full-length ChREBP or ChREBP Δ 1-196, and treated with low or high glucose media.

Supplemental References

1. Kumar S, Tamura K, Nei M: MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* 5:150-163, 2004
2. Thompson JD, Higgins DG, Gibson TJ: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673-4680, 1994
3. Saitou N, Nei M: The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406-425, 1987



Supplemental Figure 2



Supplemental Figure 3