# Supplemental Data

## Supplemental Figure Legends

Fig S1. **Mlx is required for nuclear import of ChREBP in 832/13 cells.** 832/13 cells were transfected with an expression plasmid for FLAG-tagged ChREBP overnight and subsequently incubated in RPMI media containing 2.5 mM (low) glucose for 4 h. Leptomycin B (3.6 μM) was added to cells and incubation was continued for 2 h in 2.5 mM glucose or in 25 mM (high) glucose. Immunofluorescence was performed using an anti-FLAG primary antibody and a FITC-conjugated secondary antibody. Nuclei were stained with TO-PRO3 and images were obtained on a confocal microscope. Note that in both low and high glucose ChREBP remains predominantly cytoplasmic even in the presence of leptomycin B. This is in contrast with the results shown in Fig 1b, in which ChREBP is localized to the nucleus after treatment with leptomycin B in cells co-transfected with both ChREBP and Mlx. From this result, we conclude that Mlx is required for nuclear localization of ChREBP.

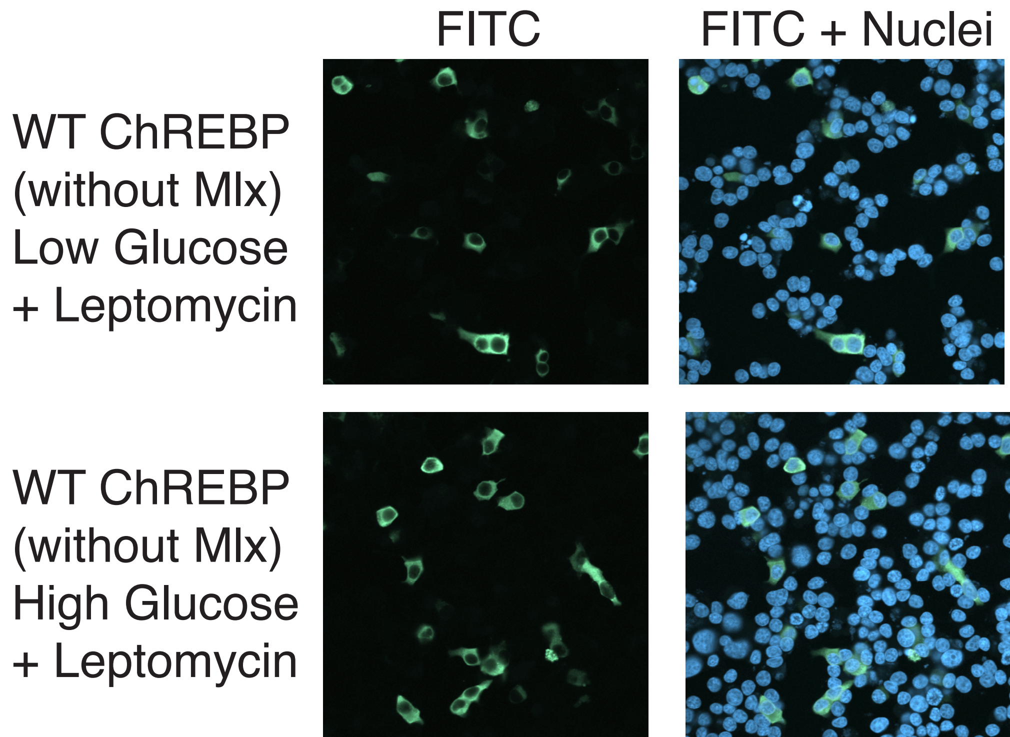
Fig S2. **Expression of various ChREBP mutants.** HEK293 cells were transfected with an expression plasmid for FLAG-tagged ChREBP (wild-type or mutant) (1.6 μg). Cells were incubated for 24 h and then cellular extracts were prepared as described previously (29). Aliquots from each cell extract were subjected to SDS-polyacrylamide gel electrophoresis and transferred to Immobilon-P membranes. ChREBP was detected using a monoclonal antibody to FLAG and a secondary horseradish peroxidase-conjugated anti-mouse IgG antibody (Santa Cruz). ChREBP ran at approximately 95 kDa, as previously observed (29). *a.* ChREBP mutant tested in Fig 3. *b.* ChREBP mutants tested in Fig 5. *c.* ChREBP mutants tested in Fig 6. *d.* ChREBP mutants tested in Fig 7.

Fig S3. **Rate of nuclear entry of ChREBP mutants T85A and L95A.** 832/13 cells were co-transfected with expression plasmids for FLAG-tagged ChREBP mutants and Mlx overnight. Cells were treated and counted as described in legend to Fig 2. Note that both of these mutant ChREBP forms show similar kinetics of nuclear accumulation in low glucose conditions, reaching half-maximal in approximately 25 to 30 min. In high glucose, both forms show a modest reduction in nuclear accumulation at 15 min, but by 30 to 45 min these mutants are observed in the nucleus of transfected cells. Since functional assays are carried out for 24 h, it is unlikely that this delay at early time points would account for the lack of activity of these mutants.

Fig S4. **Functional activity of several ChREBP mutants tested in primary hepatocytes.** To test whether the phenotype of ChREBP mutants observed in 832/13 cells was cell-specific, we tested several mutants using the same rescue assay in primary rat hepatocytes, as described previously (29). Note that the activity of all mutants followed a similar pattern to that observed in 832/13 cells. The combined F90A/275-277 and F90A/289-291 mutants did not show an additive effect in high glucose conditions, perhaps because the transcriptional response has been maximized in these cells. However, the synergistic effect of these combined mutations in low glucose conditions is evident.

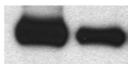
Fig S5. **Cellular distribution of MCR3 and MCR5 domain mutants.** 832/13 cells were co-transfected with FLAG-tagged ChREBP mutants and Mlx and cells were treated as described in the legend to Fig 1. The distribution of FLAG-tagged ChREBP was scored as either predominantly cytoplasmic, both cytoplasmic and nuclear or predominantly nuclear after incubation in low or high glucose for 2 h. Means (±SEM) from two independent experiments, each was scored by two observers, are displayed. Note that mutants 275-277 and 289-291 have higher proportion of cells with nuclear staining, especially in high glucose conditions. The basis of the increased nuclear accumulation is not known, but has been observed previously for other mutations that caused increased transcriptional activity (29). Also, mutant W130A shows a much higher localization to the nucleus than wild type ChREBP in either low or high glucose conditions. This mutation causes a disruption of the interaction between ChREBP and 14-3-3, which may account for this effect.

**Figure S1**



# Figure S2

**a**

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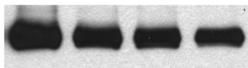


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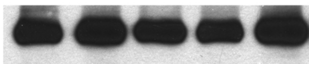


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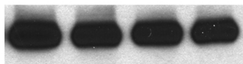

**Figure S3**

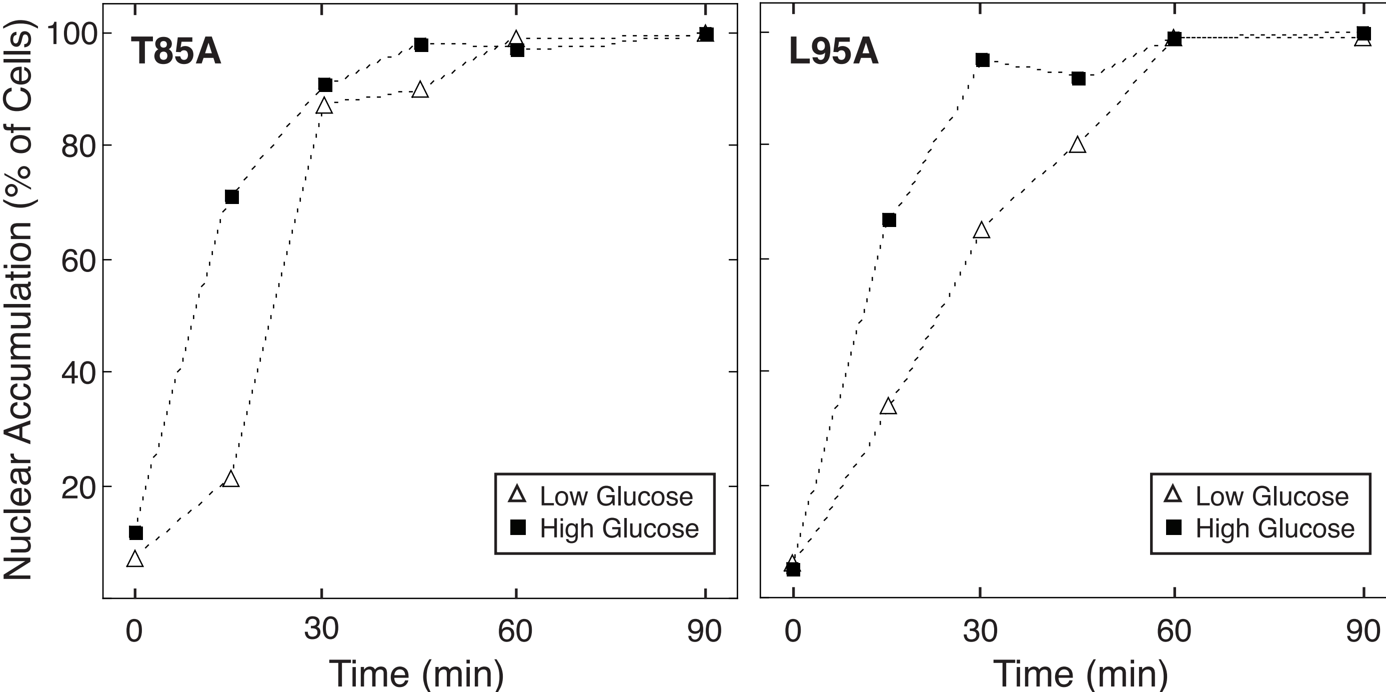

**Figure S4**

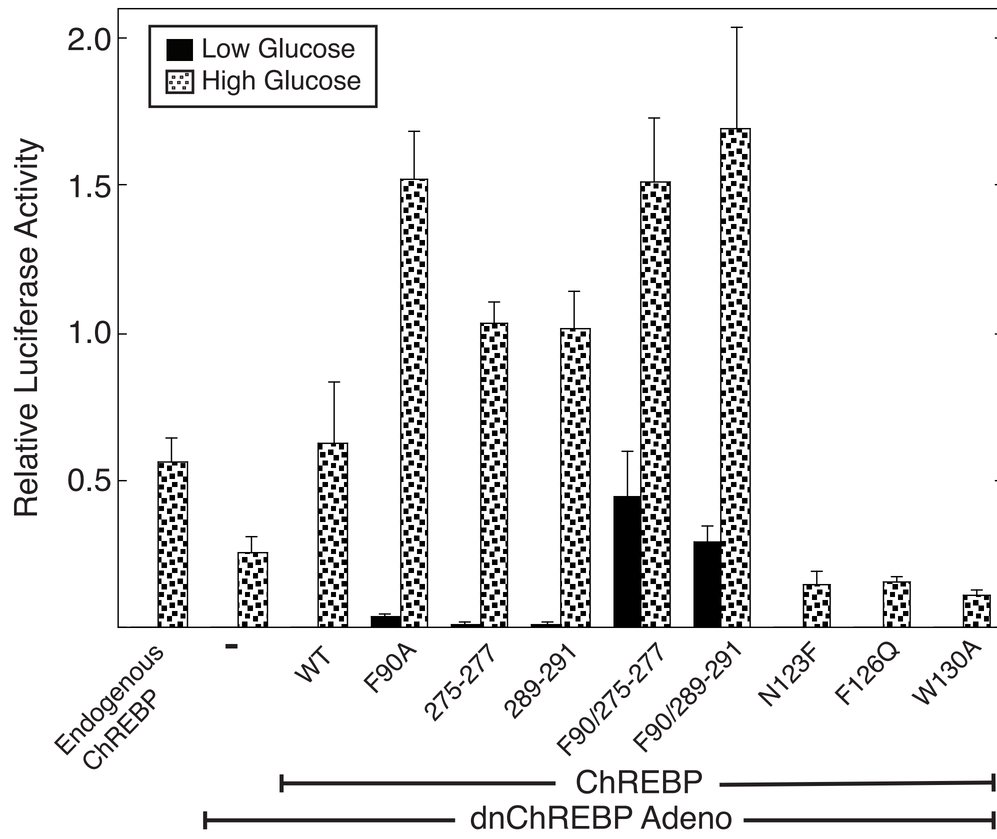

**Figure S5**