Supplemental Figure legends

Supplemental Figure 1. DNA sequence alignment of putative CYP7A1 FoxO1 binding sites.
The DNA sequence alignment of the FoxO1 recognition sites identified here for the rat promoter and the corresponding regions of the CYP7A1 promoters for mouse and human genes. The insulin response element consensus sequence that binds FoxO1 was taken from O'Brien et al. (O'Brien et al., 2001).

Supplemental Figure 2. EMSA Studies of FoxO1 binding sites in the CYP7A1 promoter.
A). Complementary oligonucleotides encompassing a putative FoxO1 binding site, designated FoxO1/1 in supplemental figure S1, were annealed, labeled with ^{32}P, and incubated with nuclear extracts from 293T cells either mock transfected or transfected with an expression vector for FoxO1. Where indicated, an antibody against the FLAG epitope attached to the FoxO1 coding sequence or a 100-fold molar excess of the indicated unlabeled probe (Comp.) were also included in the binding reactions with the labeled probe. Samples containing only the oligonucleotide probe were loaded in lanes 1, 8, and 12. W and C denote the oligonucleotide pairs from the wild type FoxO1/1, or the control FoxO1 binding site present in the IGFBP-1 promoter (Guo et al., 1999), respectively. M denotes the use of an oligonucleotide pair corresponding to a mutant version of the FoxO1/1 site that was predicted to not bind FoxO1. The wild type FoxO1/1 was labeled and used in lanes 1–7, the mutant FoxO1/1 probe was used in lanes 8-11, and the control FoxO1 binding site from the IGFBP-1 promoter was used as the labeled probe in lanes 12-18. The arrow denotes the position of the FoxO-1–DNA complex. B). Similar experiments were performed analyzing the second putative FoxO1 binding site from the CYP7A1 promoter, designated FoxO1/2 in supplemental figure S1.

Supplemental Figure 3. One of the FoxO1 sites is essential for FoxO1 activation of the CYP7A1 promoter.
A). Schematic diagrams of promoter-luciferase reporter constructs of a wild type and mutants of the CYP7A1 promoter are shown. The wild type and mutant FoxO1 binding

sites are represented as an open oval and a filled oval, respectively. B). HepG2 cells were transfected with an expression vector for FoxO1 (0.5 μg) along with the indicated promoter-luciferase fusion construct (2 μg). Results are expressed as corrected luciferase light units divided by the internal control signal for ß-galactosidase activity. Fold activation was calculated relative to the normalized luciferase activity for cells transfected with the indicated reporter alone. The data represent the mean of duplicates for three individual experiments and include *error bars*. DR1, hepatocyte nuclear factor-4 (HNF-4) binding site. LRH, liver receptor homologue-1 (LRH-1) binding site. DR4, liver X receptor (LXR) binding site.

Supplemental Figure 4. FoxO1 activation of the human and rat CYP7A1 promoters in 293T Cells.

293T cells were transfected with an expression vector for wild type FoxO1 or the FoxO1 TSS mutant (0.5 μg) along with the rat or human CYP7A1 promoter-luciferase fusion construct (2 μg) and processed as described in the legend of supplemental Fig. S3. Results are expressed as corrected luciferase light units divided by the internal control signal for ß-galactosidase activity.

Reference

O'Brien, R.M., Streeper, R.S., Ayala, J.E., Stadelmaier, B.T., and Hornbuckle, L.A. (2001). Insulin-regulated gene expression. Biochemical Society transactions 29, 552-558.

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Supplemental Fig. 1

FoxO1/2

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Rat CYP7A1     -294 TGTGCTCATCTGTTTACTTCTTTTTC -269
Mouse CYP7A1   -307  TCTGTAGTGTGTTTACTTCTTTTTCTACA -279
Humnan CYP7A1  -297 AAACAGGTTTATTTGTTCTTTTTACA -272
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FoxO1/1

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Rat CYP7A1          -89 AGGACAAATAGTGTTTGCTTTGGTCACTCA -60
Mouse CYP7A1    -93 AGGACAAATAGTTAGTGTTTGCTCTGGTCACCCAAG -58
Human CYP7A1    -93  AACAAATGGCTAATTGTTTGCTTTGTCAACCAAGCT -58
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FoxO1 Consensus Sequence T(G/A)TTT(T/G)(G/T)

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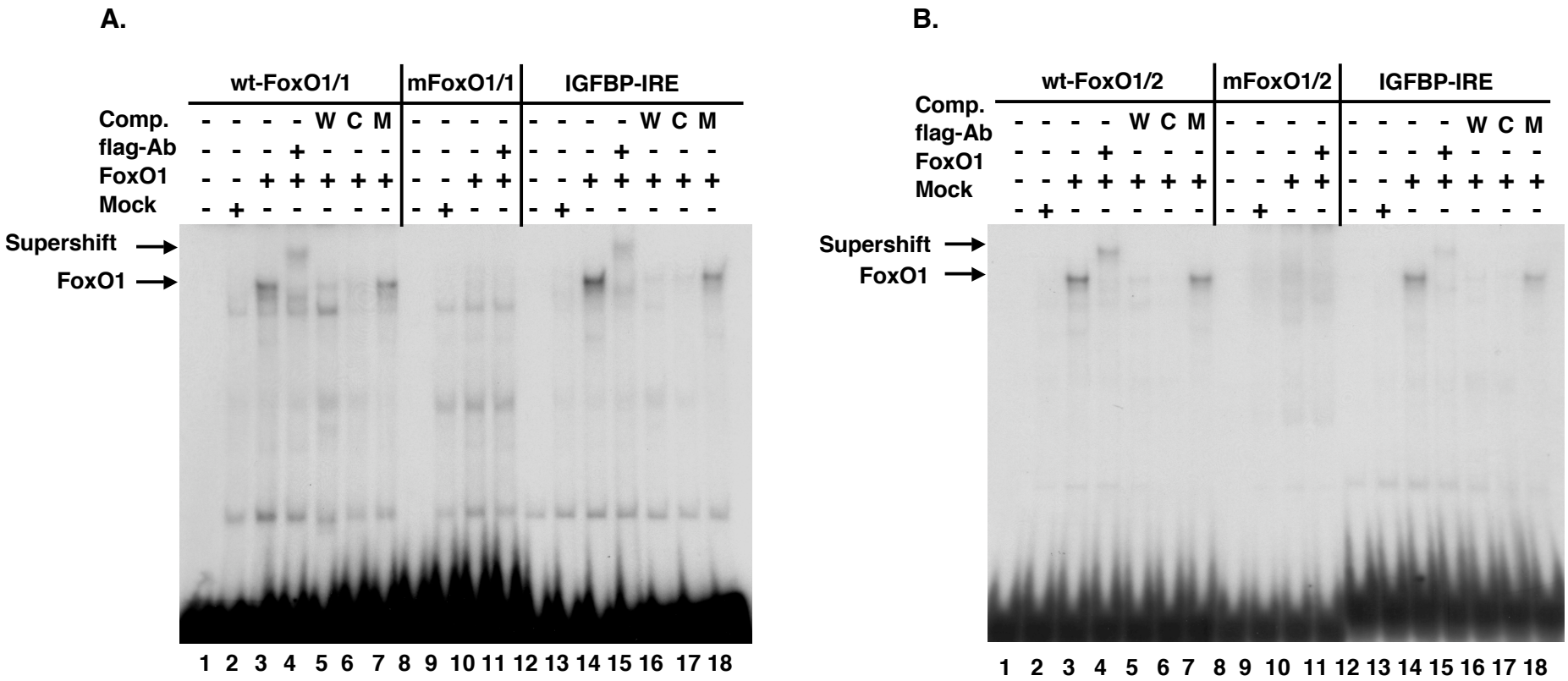
Supplemental Fig. 2



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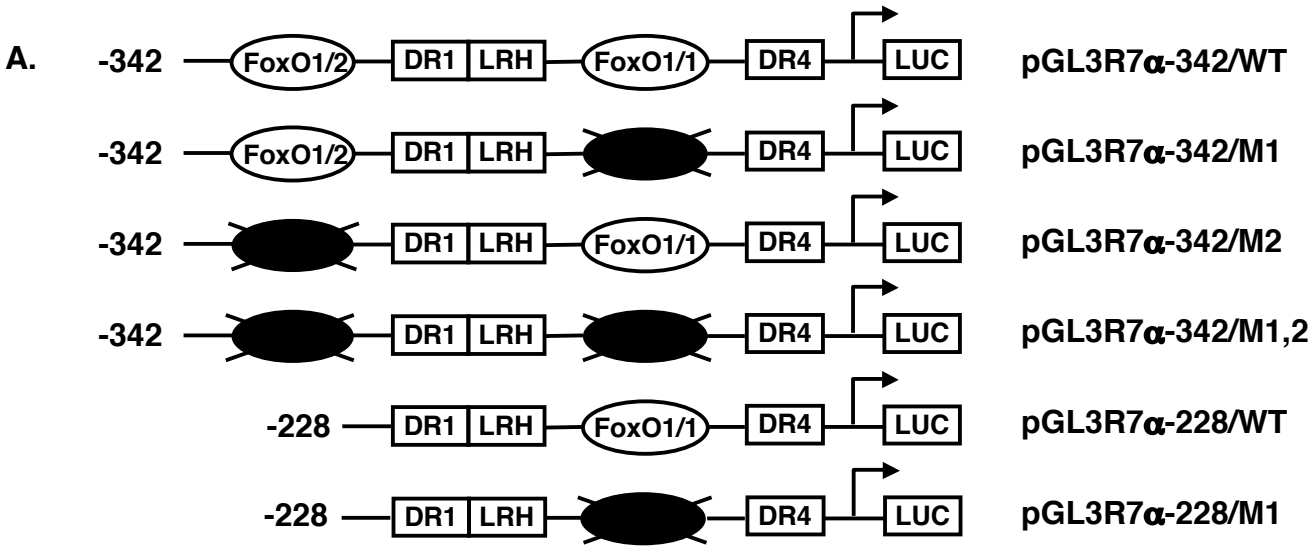
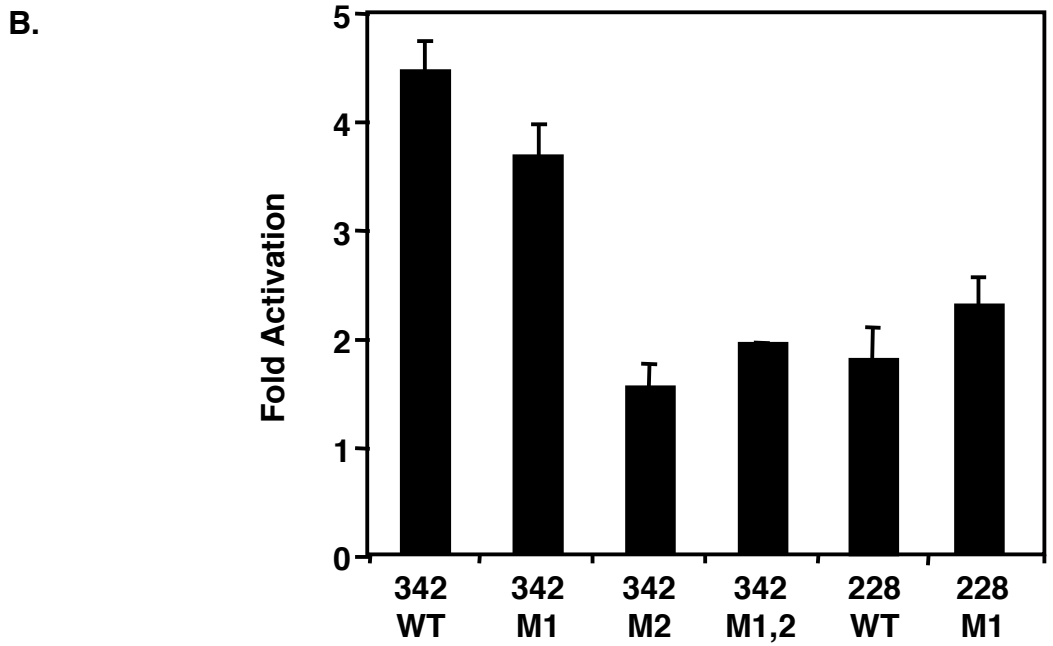
Supplemental Fig. 3





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Supplemental Fig. 4