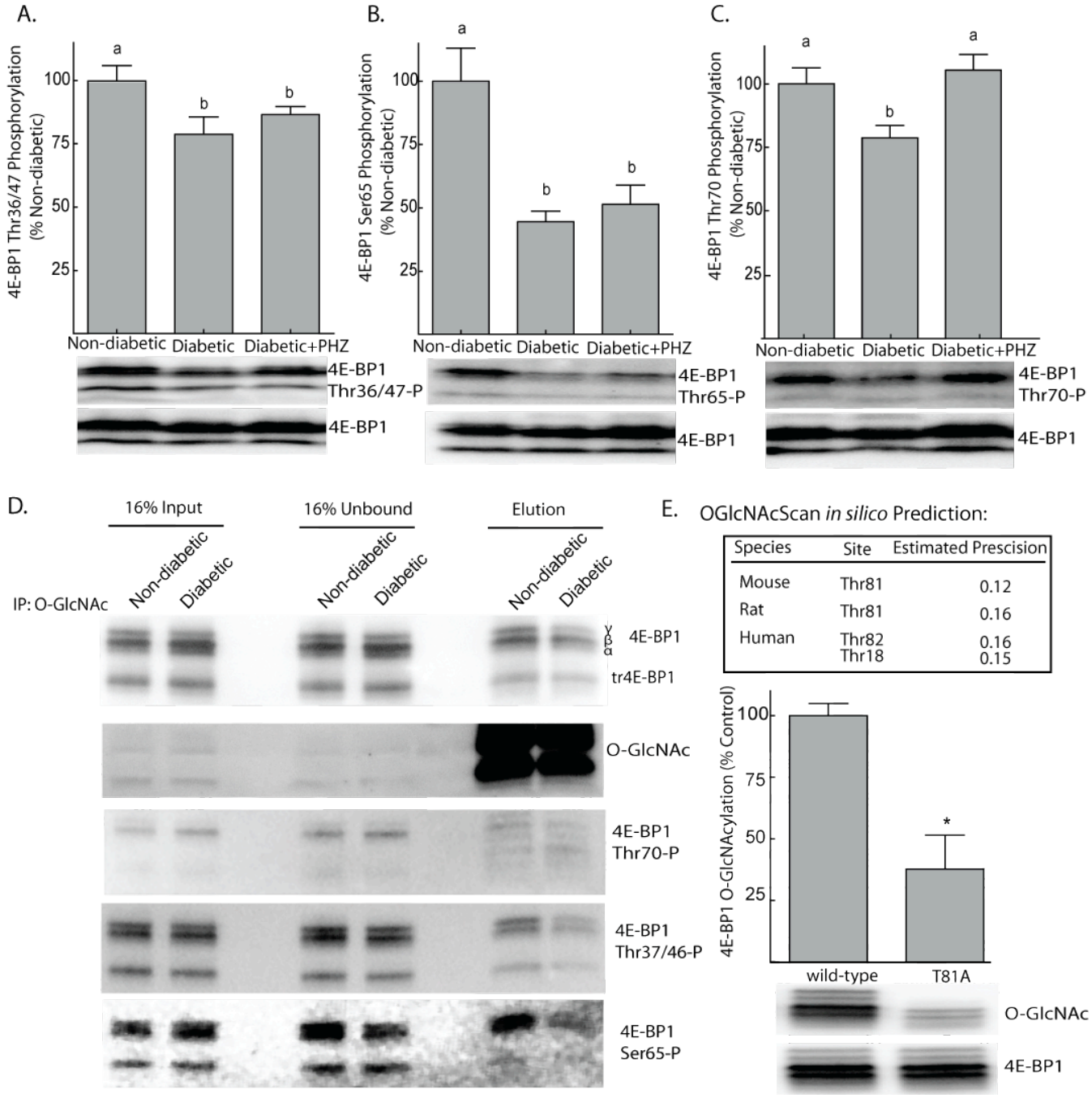
Fig S4.



Supplemental Fig 4. Phlorizin treatment of diabetic mice increases phosphorylation of 4E-BP1 at Thr70. $Ins2^{Akita/+}$ diabetic mice and $Ins2^{+/+}$ littermates were treated with phlorizin (PHZ) or solvent only twice daily for 7-days. 4E-BP1 phosphorylation was assessed in supernatants from liver whole cell lysates by Western blot analysis with phosphospecific antibodies for Thr36/47 (A), Ser65 (B), and Thr70 (C) Representative blots are shown. Values are means ± SE, *n*= 5. Statistical significance is denoted by the presence of different letters above the bars on the graphs. Bars not sharing a letter are statistically different, $p < 0.05$. (D) O-GlcNAcylated 4E-BP1 was immunoprecipitated from liver supernatants using anti-O-GlcNAc antibody and modification of 4E-BP1 in the input, unbound, and bound fractions was analyzed by Western blotting for total 4E-BP1, O-GlcNAcylation, and phosphorylation at Thr70, Thr37/46, and Ser65. (E) The amino acid sequence of 4E-BP1 of mouse (Swiss-Prot: Q60876.3), rat (Swiss-Prot: Q62622.3), and human (Swiss-Prot: Q13541) 4E-

BP1 was subjected to *in silico* analysis using OGlcNAcScan (http://cbsb.lombardi.georgetown.edu/hulab/OGAP.html) to identify residues likely to be modified by O-GlcNAcylation. From this analysis, Thr81 was identified as the most likely O-GlcNAcylation site. Therefore, using site-directed mutagenesis mouse 4E-BP1 Thr81 was mutated to Ala. Cultures of HEK293T cells were grown to ~80% confluency in Dulbeco's modified Eagle's medium lacking sodium pyruvate and containing 25 mM glucose supplemented with 10% heat-inactivated fetal bovine serum (Atlas) and 1% penicillin/streptomycin (Gibco). Wild-type and T81A 4E-BP1 were overexpressed in HEK293T cells using FugeneHD (Roche) according to the manufacturer's instructions. Cells were exposed to 40 μM PUGNAc to disrupt O-GlcNAc cycling via inhibition of OGA. O-GlcNAcylation of 4E-BP1 bound to eIF4E was measured by Western blot analysis.