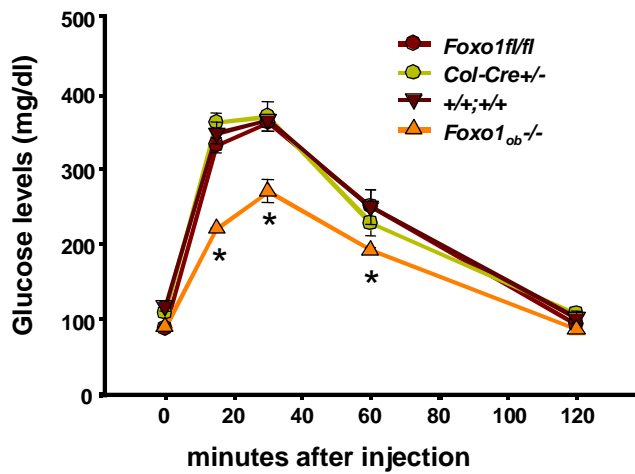
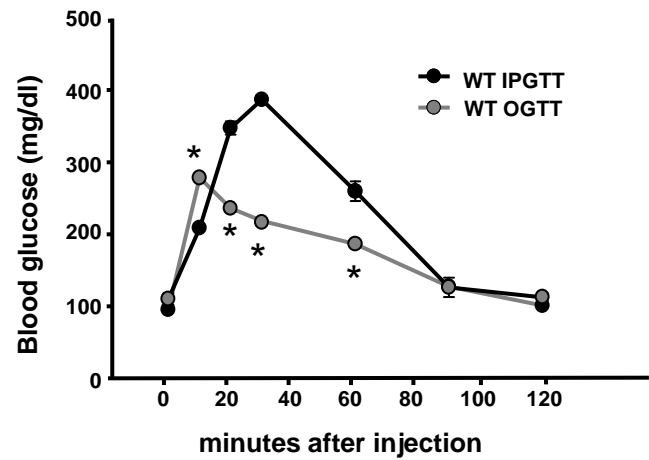
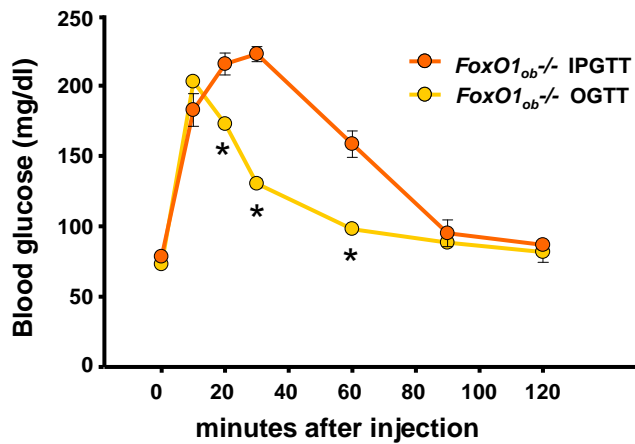
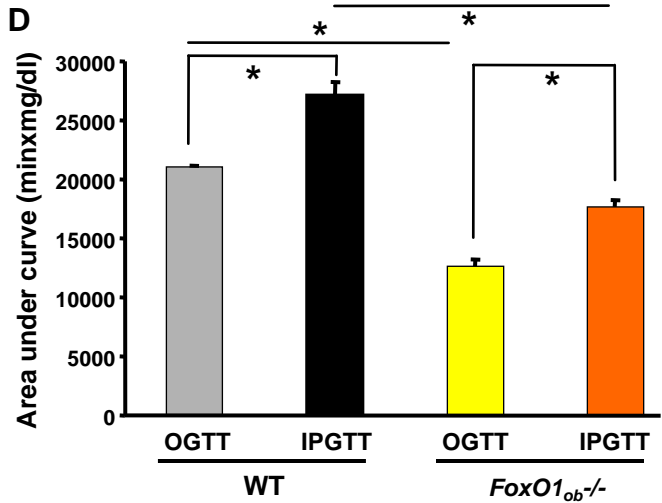
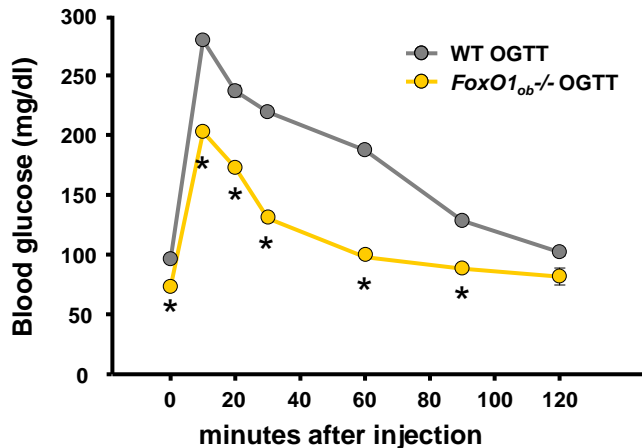
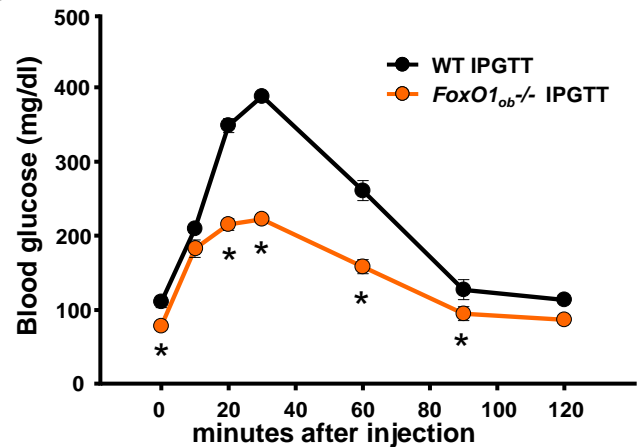
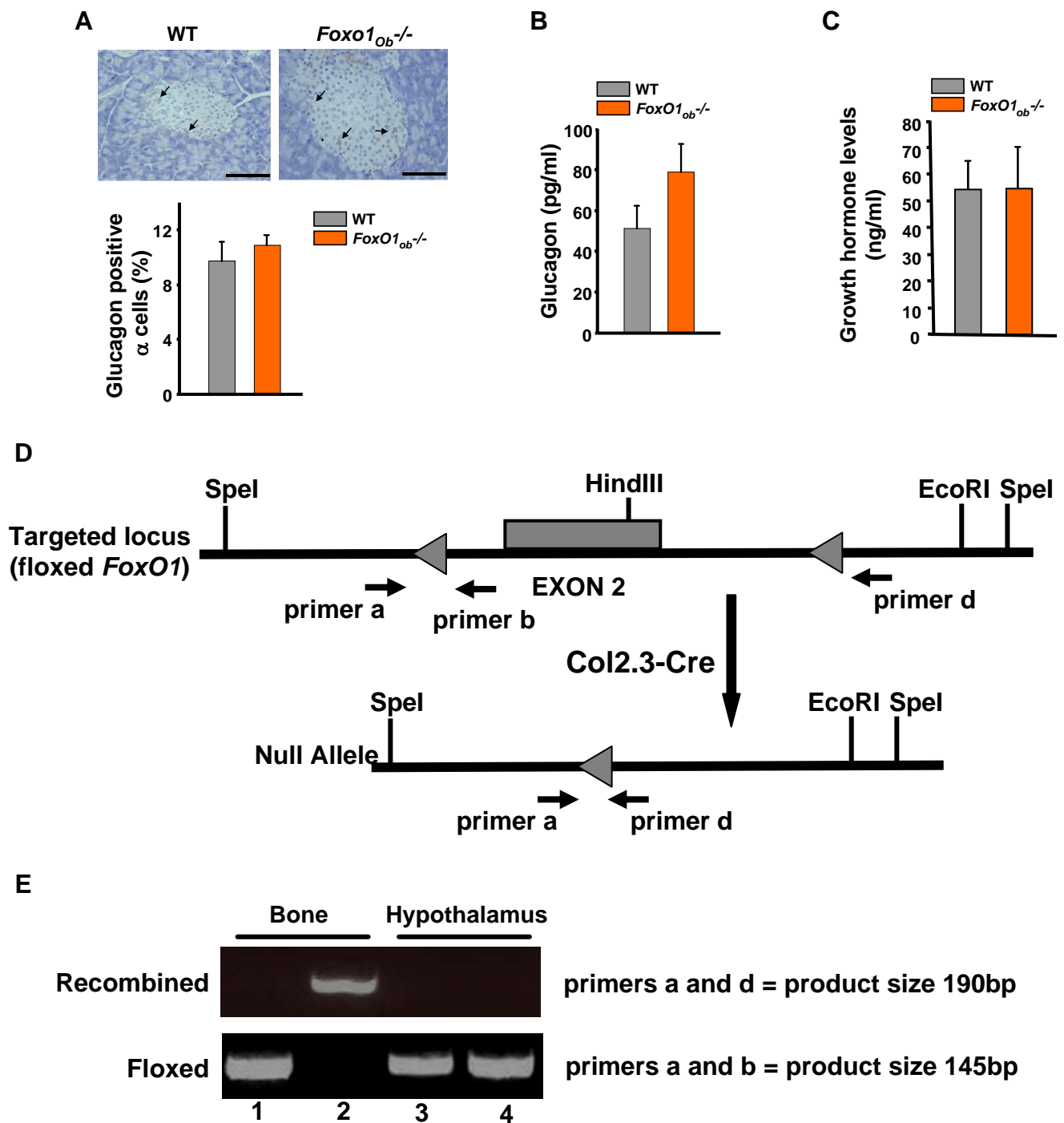


A**B****C****D****E****F**

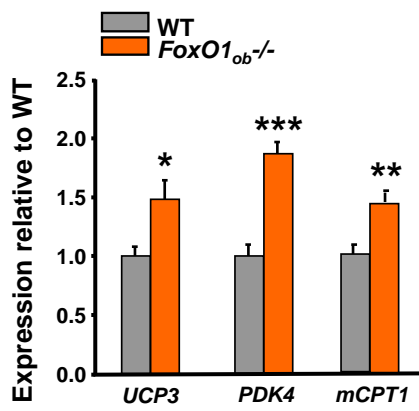
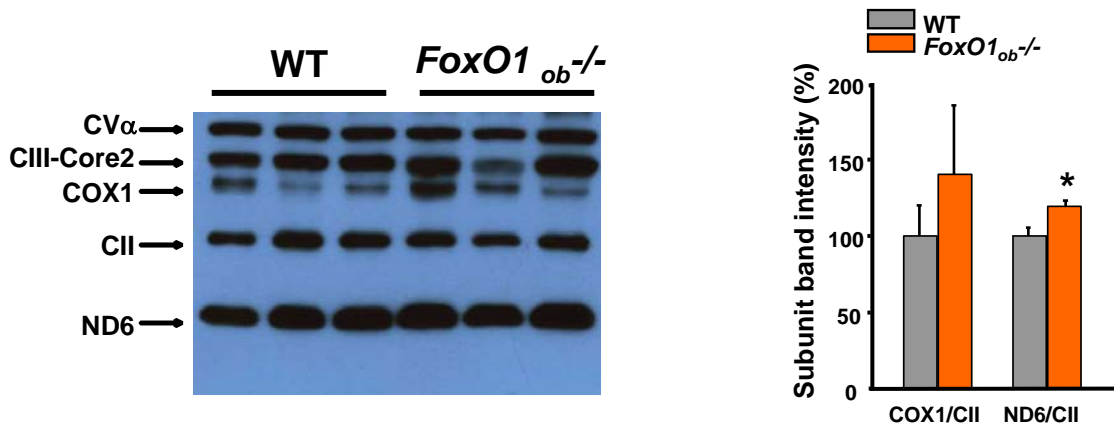
Supplementary Figure 1. Improved glucose tolerance in *FoxO1*^{ob}^{-/-} mice.

(A) Glucose tolerance test (GTT) in WT (*FoxO1*^{fl/fl}, *α1Col1-Cre* or *+/+*) and *FoxO1*^{ob}^{-/-} mice, n=5 mice/ group. Mice were 2 months of age. (B-F) Oral (OGTT) and intraperitoneal (IPGTT) glucose tolerance tests in WT and *FoxO1*^{ob}^{-/-} mice, n=4 mice/ group. Mice were 1 month of age. In panel D, area under the curve is calculated using blood glucose at time 0 as baseline. Bars indicate means ± s.e.m *p<0.05 by Student t-test.



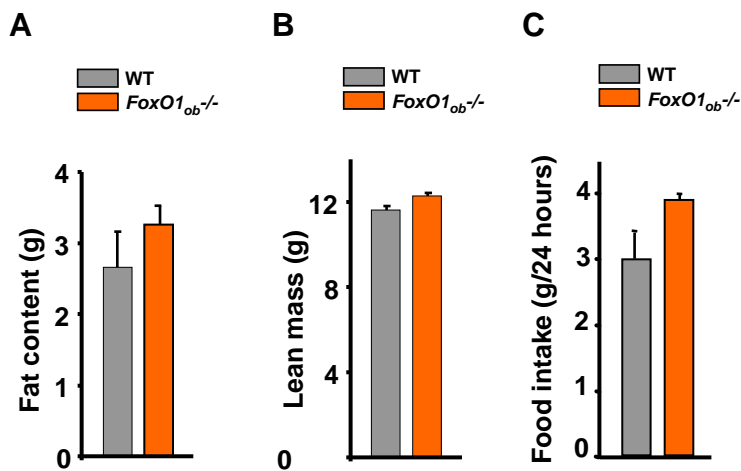
Supplementary Figure 2. Insulin counter-regulatory hormones and the hypothalamus do not contribute to the phenotype of *FoxO1_{ob}^{-/-}* mice.

(A) Glucagon immunostaining in islet sections of WT and *FoxO1_{ob}^{-/-}* mice, expressed as % of stained alpha cells out of total number of cells in the islets. Scale bars are 100 μ m. Bars indicate means \pm s.e.m. (B) Glucagon levels and (C) Growth hormone levels in the plasma of WT and *FoxO1_{ob}^{-/-}* mice. (D) Genomic structure of the floxed allele of *Foxo1* and location of the primers for PCR. (E) *FoxO1* recombination in the femur and hypothalamus of WT and *FoxO1_{ob}^{-/-}* mice; lanes 1 and 3 are *FoxO1 fl/fl* (WT) and lanes 2 and 4 are *FoxO1_{ob}^{-/-}*. Bone marrow cells were removed from the femur prior to DNA extraction. Mice were 2 months of age.

A**B**

Supplementary Figure 3. Increased expression of oxidative phosphorylation genes and mitochondrial-encoded proteins in the muscle *FoxO1_{ob}^{-/-}* mice.

(A) *UCP3* and *PDK4* mRNA expression by real-time PCR in the gastrocnemius muscle of WT and *FoxO1_{ob}^{-/-}* mice, n=3 mice/group. (B) Protein levels of mitochondrial DNA- encoded subunits in the vastus lateralis muscle by immunoblotting, from WT and *FoxO1_{ob}^{-/-}* mice. ND6 (20Kd) and Cox1 (39 Kd) protein levels are normalized to complex II subunit levels (30 Kd). Bars indicate means \pm s.e.m., *p<0.05, **p<0.01 and ***p<0.001. Mice were 2-3 months of age.



Supplementary Figure 4. Fat, lean mass and food intake are not affected in *FoxO1_{ob}^{-/-}* mice. (A-B) MRI analysis of total fat content and lean mass in WT and *FoxO1_{ob}^{-/-}* mice, n=5 mice/group. (C) Food intake analysis in WT and *FoxO1_{ob}^{-/-}* mice, n=6 mice/group. In all panels, bars indicate means \pm s.e.m. In (A) and (B) mice were 1 month-old. In (C) mice were 3 months old.