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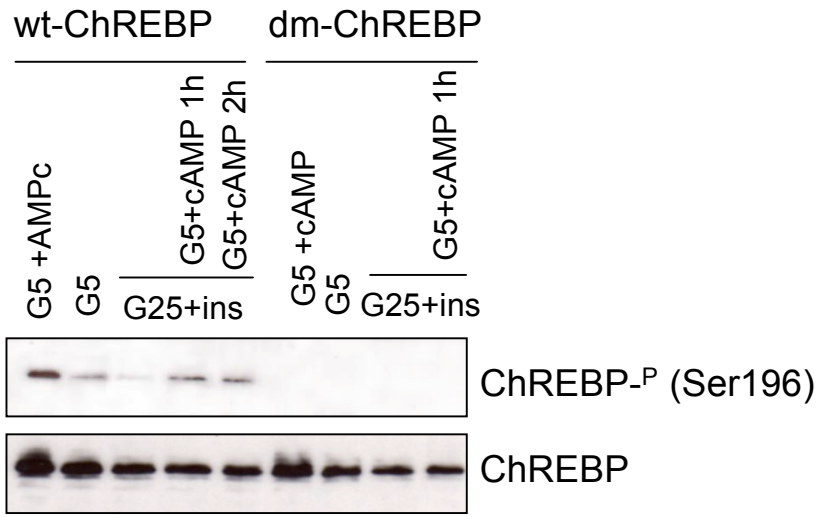

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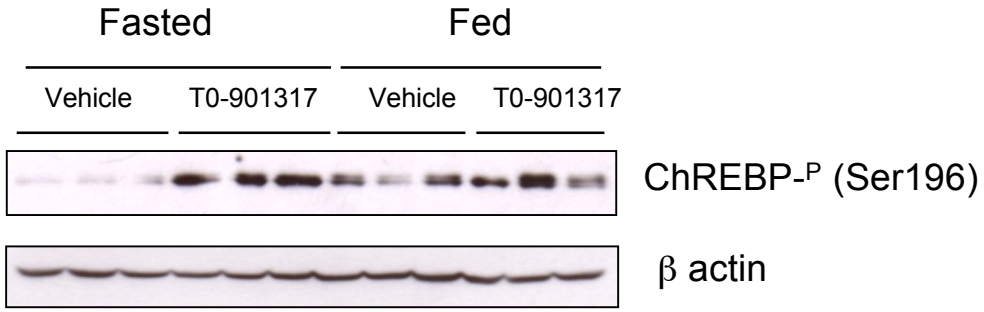
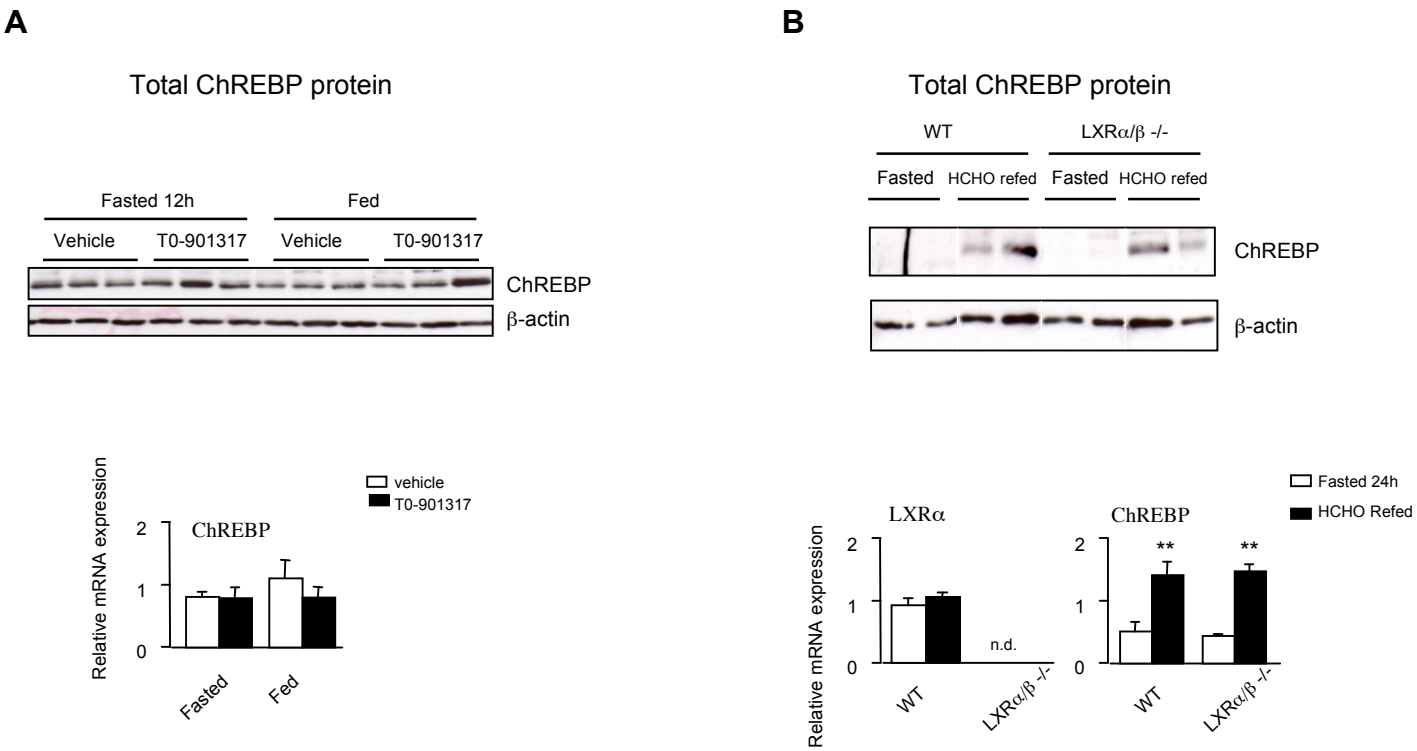
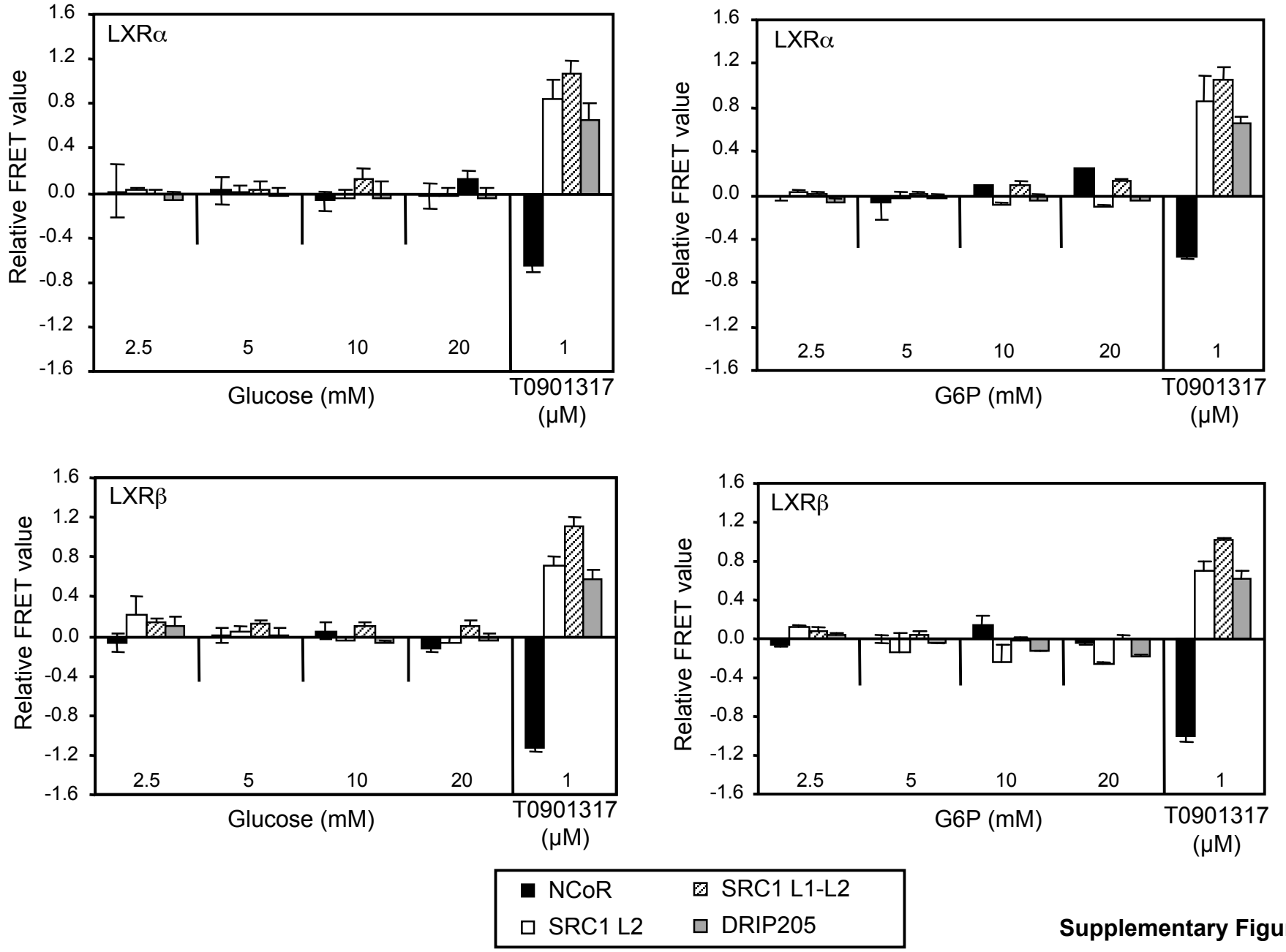


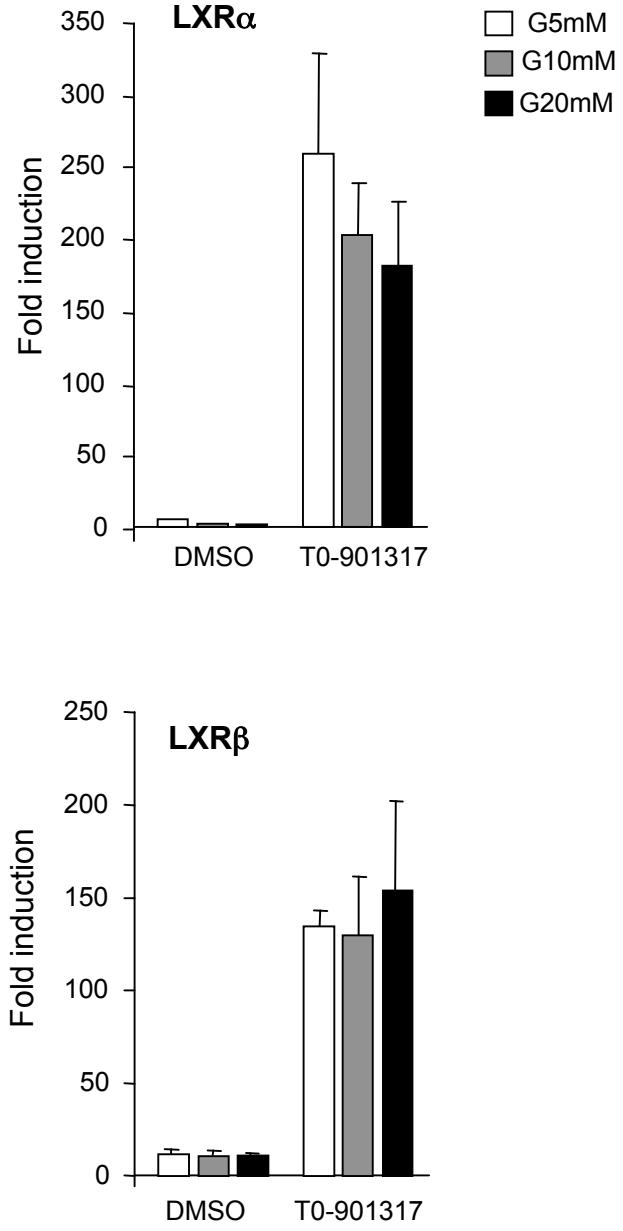
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

Supplementary Figure 1. (A). Modulation of Ser^{196} phosphorylation in vitro. Mouse hepatocytes were transfected using the PEI/adenofection method (1) with 2 µg of pTZ18R expression vector containing the full-length wild type ChREBP-ζ isoform or the isoform mutated on Ser^{196} and Thr^{666} (2) in 5 mM glucose M199 medium. After transfection (5h), hepatocytes were cultured for 24h in M199 medium. Then, specific conditions were performed for 24 h. *Lane 1*: glucose 5 mM (G5) + cAMP (10^{-4}M); *Lane 2*: glucose 5 mM (G5), *Lane 3*: glucose 25 mM (G25) + insulin (100 nM); *Lanes 4 and 5*: glucose 25 mM (G25) + insulin (100 nM) followed by glucose 5 mM (G5) + cAMP (10^{-4}M) for 1h (*Lane 4*) or 2 h (*Lane 5*). A representative Western blot is shown, n=3 idependant cultures. **(B). Ser-196 phosphorylation status of the endogenous ChREBP protein in liver.** Ser-196 phosphorylation level of the endogenous ChREBP protein in liver lysates from C57BL/6J mice treated for 3 days with either vehicle or 50 mg/kg body weight of the synthetic LXR agonist T0-901317. After treatment, mice were fasted overnight or maintained in the fed states. β-actin was used as loading control. A representative Western blot is shown, n=6 / group.

Supplementary Figure 2. (**A**). **ChREBP expression is not induced by T0-901317 treatment in white adipose tissue.** *Upper panel*: Total ChREBP protein content in white adipose tissue lysates from vehicle- and T0-901317-treated fasted (12h) and fed mice. β-actin was used as loading control. A representative Western blot is shown, n=6 / group. *Lower panel:* qRT-PCR analysis of ChREBP in white adipose tissue from C57BL/6J mice treated for 3 days with either vehicle or 50 mg/kg body weight of the synthetic LXR agonist T0-901317. After treatment, mice were fasted overnight or maintained in the fed state. Results are the mean ± S.E., n = 6/group. **(B). ChREBP is induced upon HCHO refeeding in the absence of LXR.** *Upper panel*: Total ChREBP protein content in white adipose tissue lysates from fasted and HCHO refed wild type and LXRα/β knockout mice. β-actin was used as loading controls. A representative Western blot is shown, n=5-8 / group. Lanes were run on the same gel but were noncontiguous. *Lower panel:* qRT-PCR analysis of ChREBP in white adipose tissue from control and LXRα/β knockout mice either fasted overnight or challenged with a HCHO diet for 18h. All error bars represent s.d., n=5-8 mice / group. **, P<0.001 vs. fasted mice.

Supplementary Figure 3. Glucose does not influence cofactor interaction with LXR. Glucose and glucose 6-phosphate (G6P) do not influence the interaction of cofactor peptides with either LXRα or LXRβ, as determined in cell-free FRET assays. In contrast, T0901317 induces the recruitment of the coactivator peptides SRC1 L2, SRC1 L1-L2, and DRIP205, and induces the release of the corepressor peptide NCoR from both LXRα and LXRβ. Values are presented as the relative cofactor recruitment measured as a change in 670/635 nm emission relative to vehicle. Data are representative of 2-4 experiments performed in triplicate; error bars indicate standard deviations.

Supplementary Figure 4. Gal4 transactivation assays in HepG2 cells. 24h prior to transfection, HepG2 cells were transferred to glucose-free DMEM containing 2% Ultroser. The following day, cells in the same medium were transfected in suspension using jetPEI™, with 136.4 ng/well of the reporter plasmid Gal4 (5xRE)_TK_pGL3-Basic and 13.6 ng/well of pcDNA3-based expression plasmids (Gal4 DBD alone, or Gal4

DBD – LXRα/β LBD chimeric proteins). Transfected cells were seeded into 96-well plates at 40.000 cells/well, and incubated for 16h at 37°C, 5% CO_2. The next day, the transfection medium was discarded and replaced with glucose-free DMEM without Ultroser, with the addition of test compounds at the appropriate concentrations in 0.1% DMSO. After 24h of incubation, cells were lyzed and luciferase activity was assessed using the Steady-Glo Luciferase Assay System (Promega, Southampton, UK). Relative luciferase activity (RLU) was measured using a luminometer (GeniosPro, Tecan), and fold inductions were determined by calculating the ratio between RLU values obtained in the presence of test compounds (2 μM T0901317 and glucose) and those obtained in the presence of 0.1% DMSO only. Data are representative of 2-6 experiments performed in triplicate; error bars indicate standard deviations.

References

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2. Stoeckman, A.K., Ma, L., and Towle, H.C. 2004. Mlx is the functional heteromeric partner of the carbohydrate response element-binding protein in glucose regulation of lipogenic enzyme genes. *J Biol Chem* 279:15662-15669.