Title page

Phenobarbital and insulin reciprocate activation of the nuclear receptor CAR through the insulin receptor

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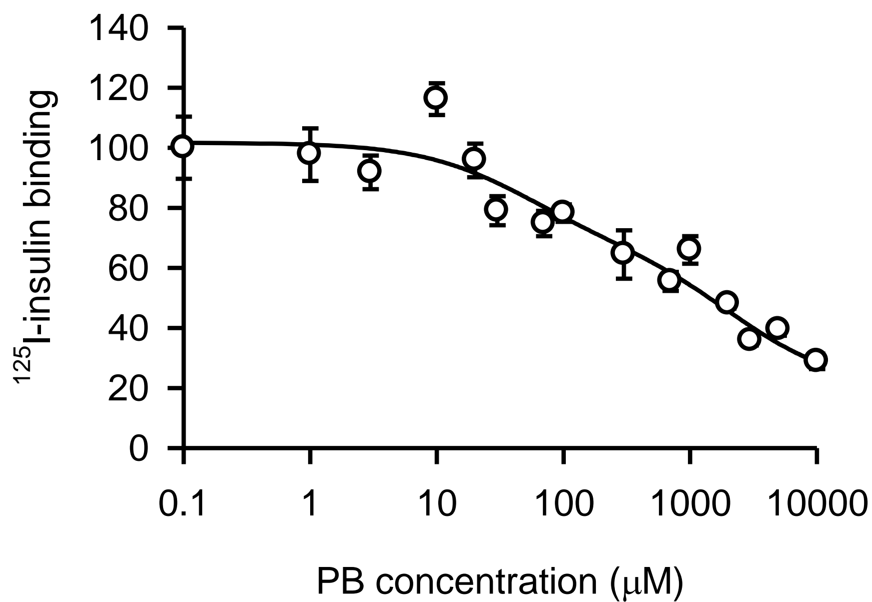
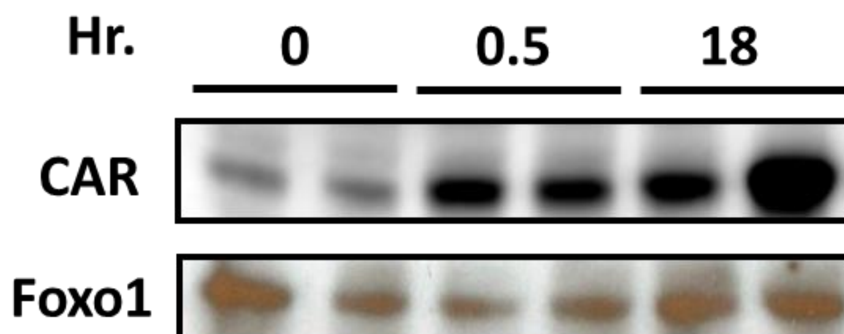
Supplemental Figure 1.



Supplemental Figure 2.



Supplemental Figure legends

Supplemental Figure 1.

PB inhibition of [^{125}I]insulin binding to the insulin receptor. HepG2 cells, which were transfected with pEGFP-N2-human insulin receptor, were co-treated with ^{125}I-rabeled insulin (1 nM) with various concentrations of PB (dissolved in pH7.4 HBSS buffer) for 30 min. After co-treatment, cells were lysed with 0.5N NaOH and transferred to counting tube to measure radioactivities by gamma counter. All plots were analyzed by the model considering that PB has dual affinity sites to the insulin receptor utilizing the equation of $B = CF_1/(1 + (C/IC_{50,1})^{n1}) + CF_2/(1 + (C/IC_{50,2})^{n2})$. In this, B is an amount of insulin binding; and CF a correction factor; C a PB concentration; IC_{50} a half maximal inhibitory concentration and n is a hill coefficient. The values of PB affinity to the insulin receptor were calculated by WinNonlin: 21.5 ± 15.5 μM for high affinity and 2.2 ± 1.4 mM for low affinity sites, respectively.

Supplemental Figure 2

For co-immunoprecipitation assays, nuclear extracts, which were prepared from the livers of CAR wild type mice after PB treatment, were incubated with a Foxo1 antibody for overnight at 4 °C and subsequently with Dynabeads protein G resin for 3 h at 4 °C. These beads were re recovered by centrifugation, washed three times with lysis buffer and treated in sample buffer for SDS-PAGE. Western blot was performed as described in the legend of Figure 2 by using an anti-CAR or FOXO1 antibody.