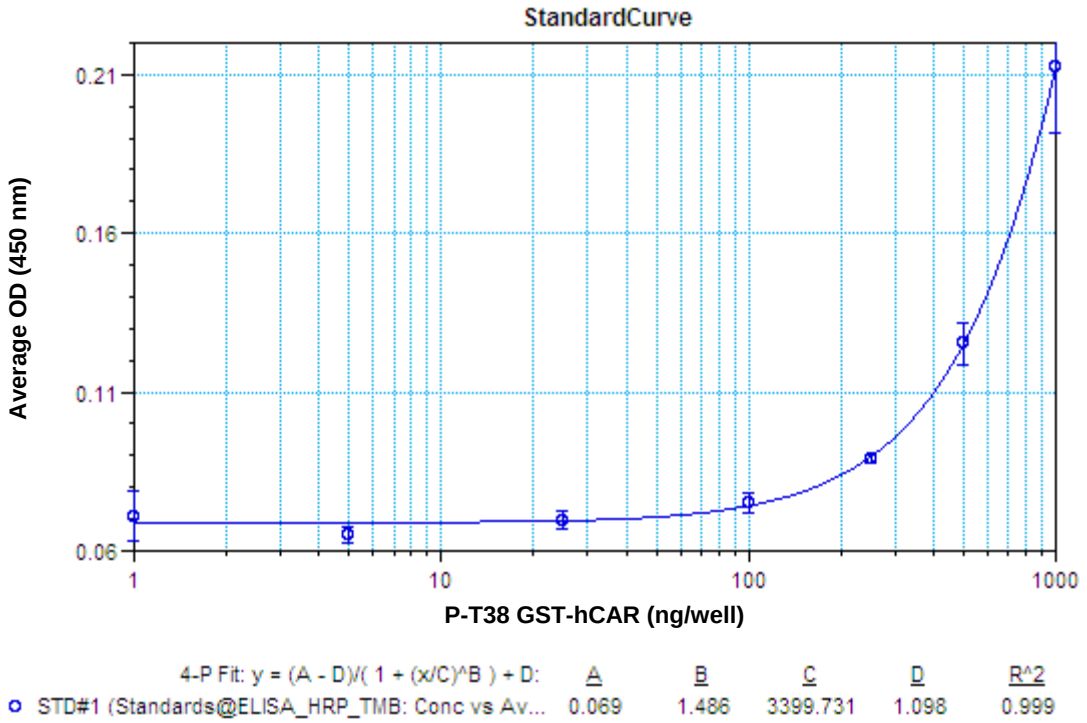
Supplemental Figures

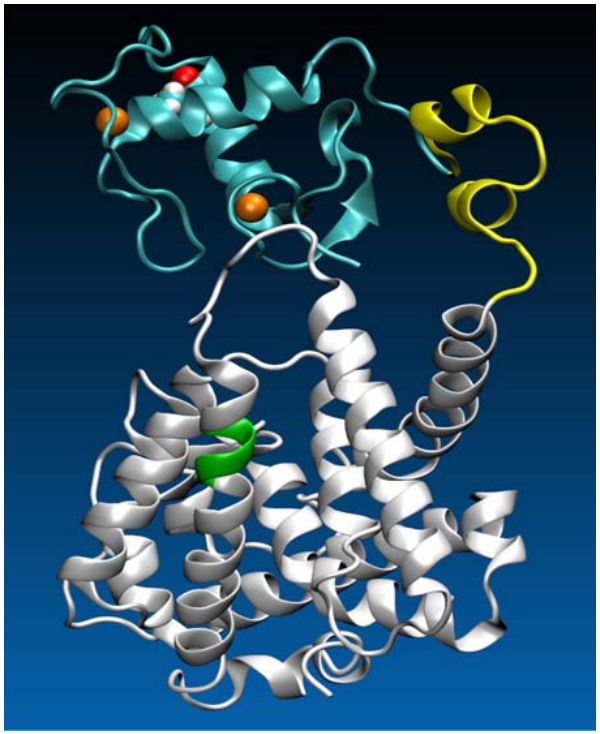
Makoto Osabe and Masahiko Negishi

Supplemental Figure 1



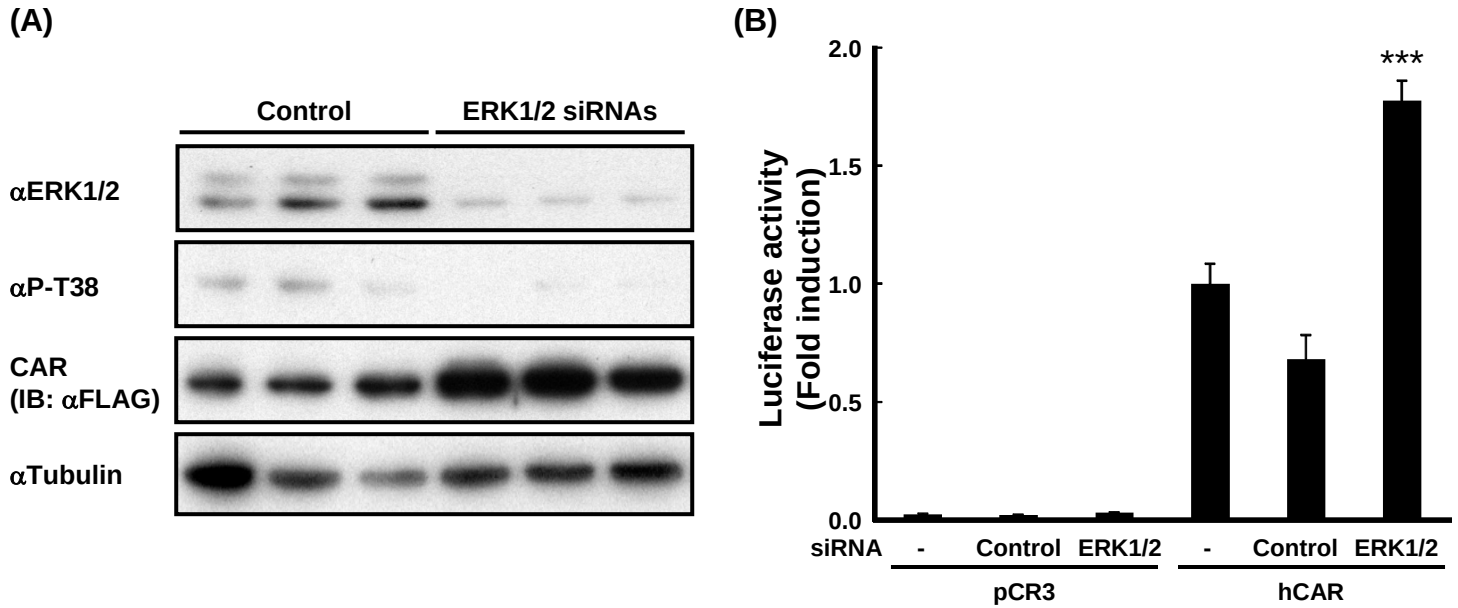
Supplemental figure 1. Standard curve generated from diluted phosphorylated GST-hCAR by PKC. Bacterially expressed GST-hCAR was incubated in 30 µl of assay buffer containing 20 mM HEPES (pH 7.4), 1.67 mM $CaCl_2$, 1 mM DTT, 10 mM $MgCl_2$, 0.6 mg/ml phosphatidyl serine and 100 µM ATP in the presence of 1 µl (1.6 units) of PKC at 30 ºC for 30 min (14). This phosphorylated GST-hCAR protein was serially diluted and then detected phosphorylation of threonine 38 by ELISA, as described in the Experimental Procedure section. Absorbance was read on a Molecular Devices SpectraMax® Plus^{384} at 450 nm.

Supplemental Figure 2



Supplemental figure 2. Localization of the ERK1/2-interacting site on the hCAR structure. The simulated structure of the hCAR molecule which contains DBD (cyan), hinge (gold), and LBD (in gray), was modeled based on our previous model structure of hCAR DBD (14) and the published X-ray crystal structure of PPARγ (17). Details of this modeling will be published elsewhere. The P-ERK1/2-interacting site (residues 312 to 315) is shown in green and threonine 38 in the DBD in CPK representation along with the two Zn ions bound to DBD.

Supplemental Figure 3



Supplemental figure 3. De-phosphorylation of T38 by ERK1/2 siRNAs leads to increase CAR-mediated NR-1 transcriptional activity. Control siRNA or a mixture of hERK1 and hERK2 siRNAs (20 nM each, Sigma-Aldrich) was transfected into Huh-7 cells for 24 h, which were subsequently transfected with an expression plasmid for FLAG-hCAR WT for additional 24 h . Whole cell extracts were prepared as described in the Experimental Procedure section (*A*). Control siRNA or a mixture of hERK1 and hERK2 siRNAs (20 nM each) was transfected into Huh-7 cells for 24 h, which were subsequently cotransfected $(NR1)_5$-luciferase reporter plasmid along with pRL-tk, plus or minus a plasmid expressing hCAR (pCR3-hCAR) (*B*). Results are expressed as the fold induction of luciferase activity relative to values obtained in siRNA non-transfected cells expressing hCAR. All firefly luciferase activity values were standardized with the *Renilla* luciferase activity measured in each determination. *** denotes significantly different ($P < 0.001$) from hCAR treated with control siRNA (unpaired *t*-test). Results are the means ± S.E. of two different experiments, with each determination performed in quadruplicate.