SUPPLEMENTAL MATERIAL

Targeting xenobiotic nuclear receptors PXR and CAR to prevent cobicistat hepatotoxicity

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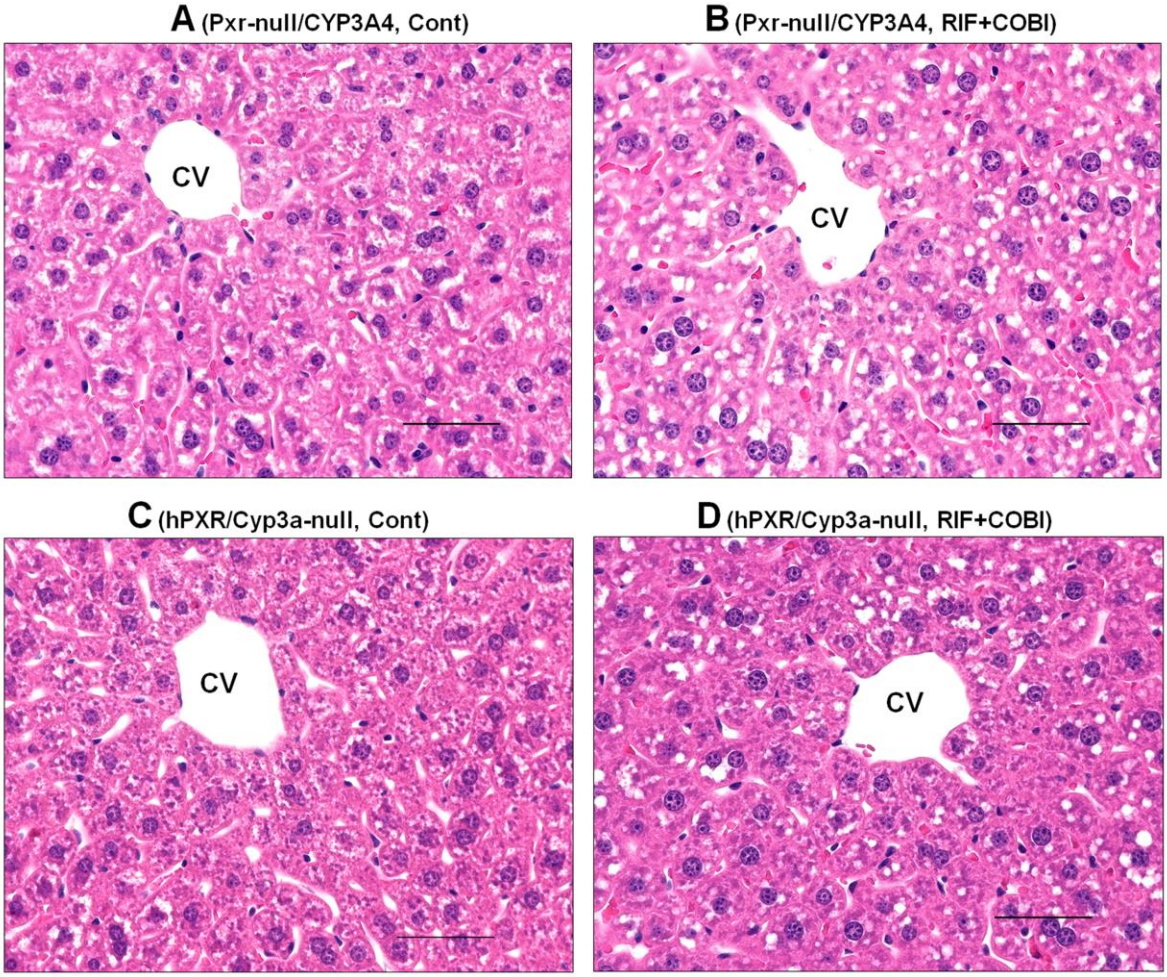
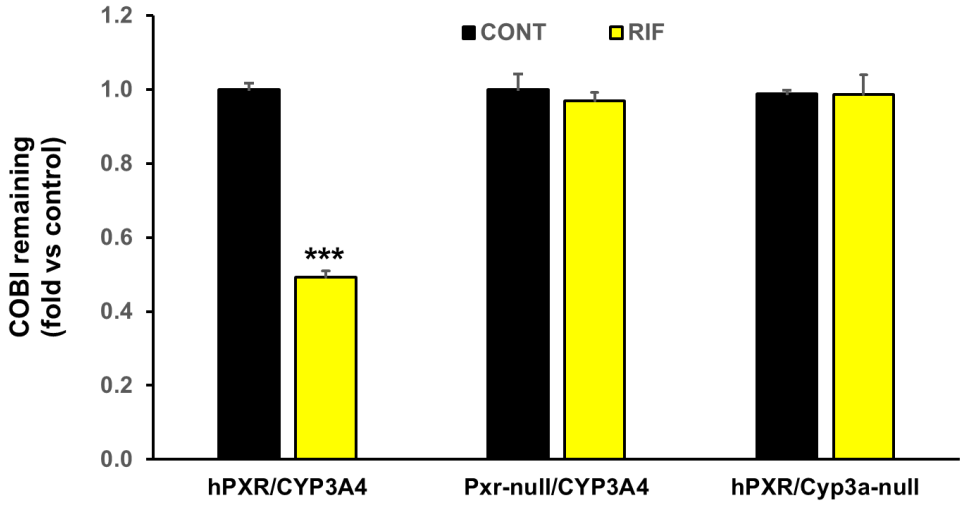
Supplemental Fig. 1



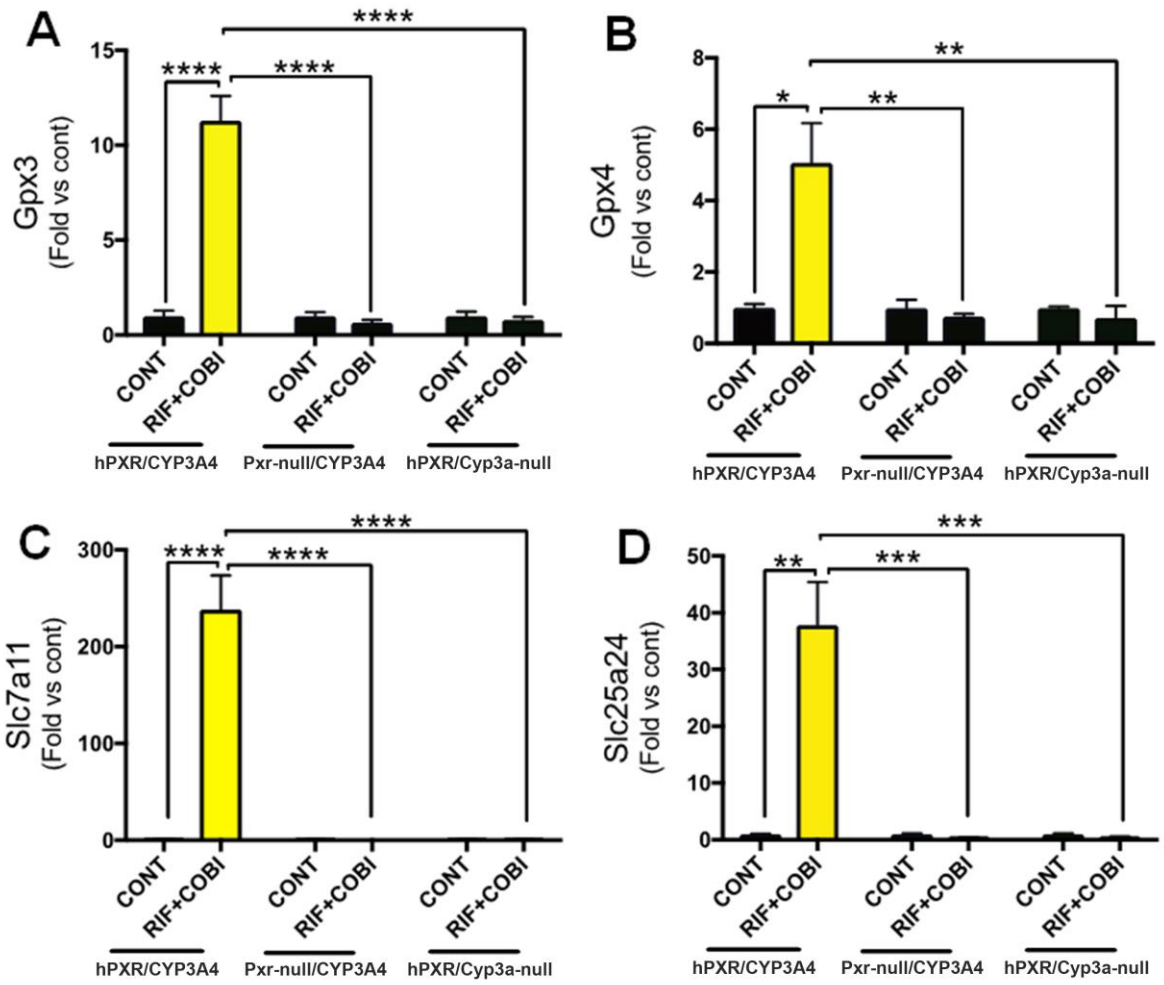
Supplemental Fig. 1. Deficiency of PXR or CYP3A4 protects against liver injury caused by the adverse drug-drug interactions between RIF and COBI. Pxr-null/CYP3A4 and hPXR/Cyp3a-null mice were pretreated with RIF for 7 days followed by COBI for 1 day. No injury was observed in histological analysis of livers from control (**A, C**) and RIF+COBI (**B, D**) groups. H&E staining. CV, central vein. Scale bars: 50 μm.

Supplemental Fig. 2



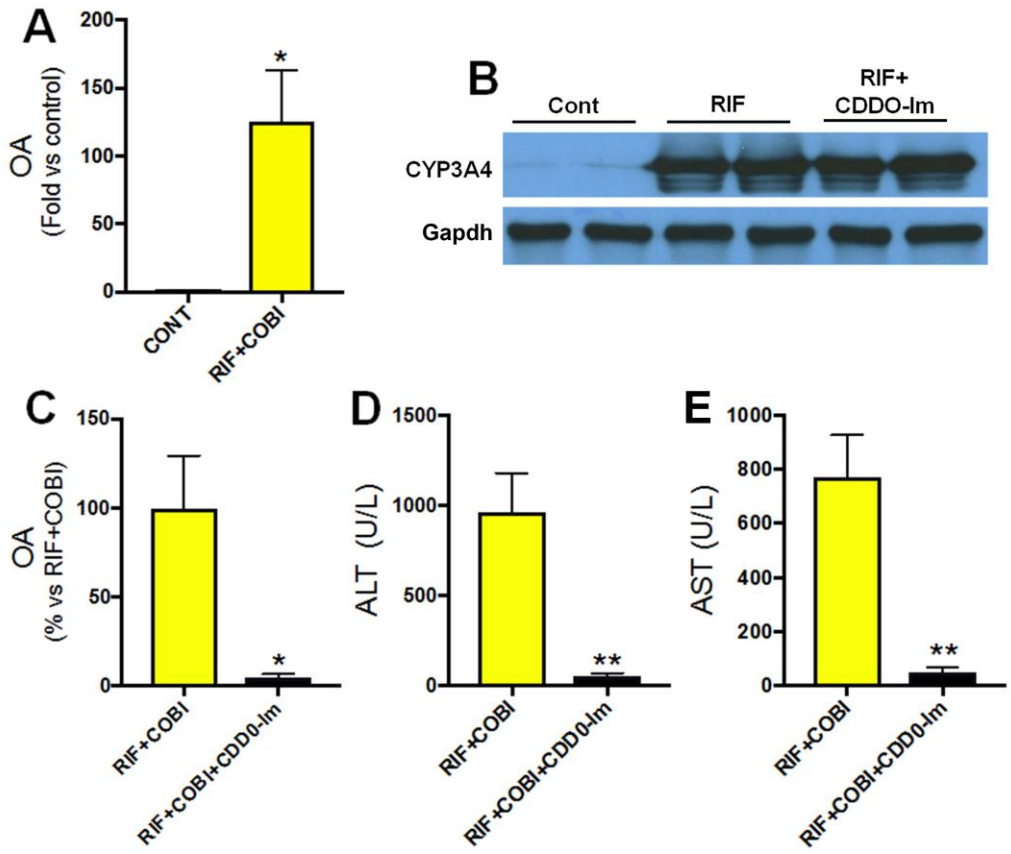
Supplemental Fig. 2. Pretreatment with RIF accelerates COBI metabolism in the liver microsomes of hPXR/CYP3A4 mice. The incubation was carried out in 1 x PBS containing 30 μM of COBI and 1 mg of liver microsomes for 30 min. The remaining COBI in the incubation system was analyzed by UPLC-qTOFMS. All data are expressed as mean ± SD (n = 3-4). Data in control groups are set as 1, respectively. ***$P < 0.001$.

Supplemental Fig. 3



Supplemental Fig. 3. The adverse drug-drug interactions between RIF and COBI cause oxidative stress in the liver in a PXR- and CYP3A4-dependent manner. hPXR/CYP3A4, Pxr-null/CYP3A4, and hPXR/Cyp3a-null mice were pretreated with RIF followed by COBI. *Gpx3* (**A**), *Gpx4* (**B**), *Slc7a11* (**C**), and *Slc25a24* (**D**) mRNAs in the liver were analyzed by qPCR. All data are expressed as mean ± SEM (n = 3-7). Data in control groups are set as 1, respectively. Statistical significance was determined by two-way ANOVA with Tukey's post hoc test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

Supplemental Fig. 4



Supplemental Fig. 4. CDDO-Im, an antioxidant, prevents liver injury caused by the adverse drug-drug interactions between RIF and COBI in hPXR/CYP3A4 mice. The mice were pretreated with RIF and antioxidant CDDO-Im followed by COBI. (**A**) The levels of ophthalmic acid (OA), a biomarker of oxidative stress, in the liver of hPXR/CYP3A4 mice pretreated with RIF followed by COBI. (**B**) Impact of CDDO-Im on RIF-mediated CYP3A4 induction in the liver of hPXR/CYP3A4 mice. CYP3A4 was analyzed by Western blotting. Gapdh was used as a loading control. (**C**) The effects of CDDO-Im on OA levels in the liver of hPXR/CYP3A4 mice pretreated with RIF followed by COBI. OA in the liver was analyzed by UPLC-qTOFMS. (**D, E**) The effects of CDDO-Im on the liver injury in hPXR/CYP3A4 mice

pretreated with RIF followed by COBI. Serum activities of ALT (**D**) and AST (**E**) were analyzed by a biochemical approach. All data are expressed as mean ± SEM (n = 3-7). Statistical significance was determined by the two-tailed Student's t-test. $*P < 0.05$, $**P < 0.01$.