

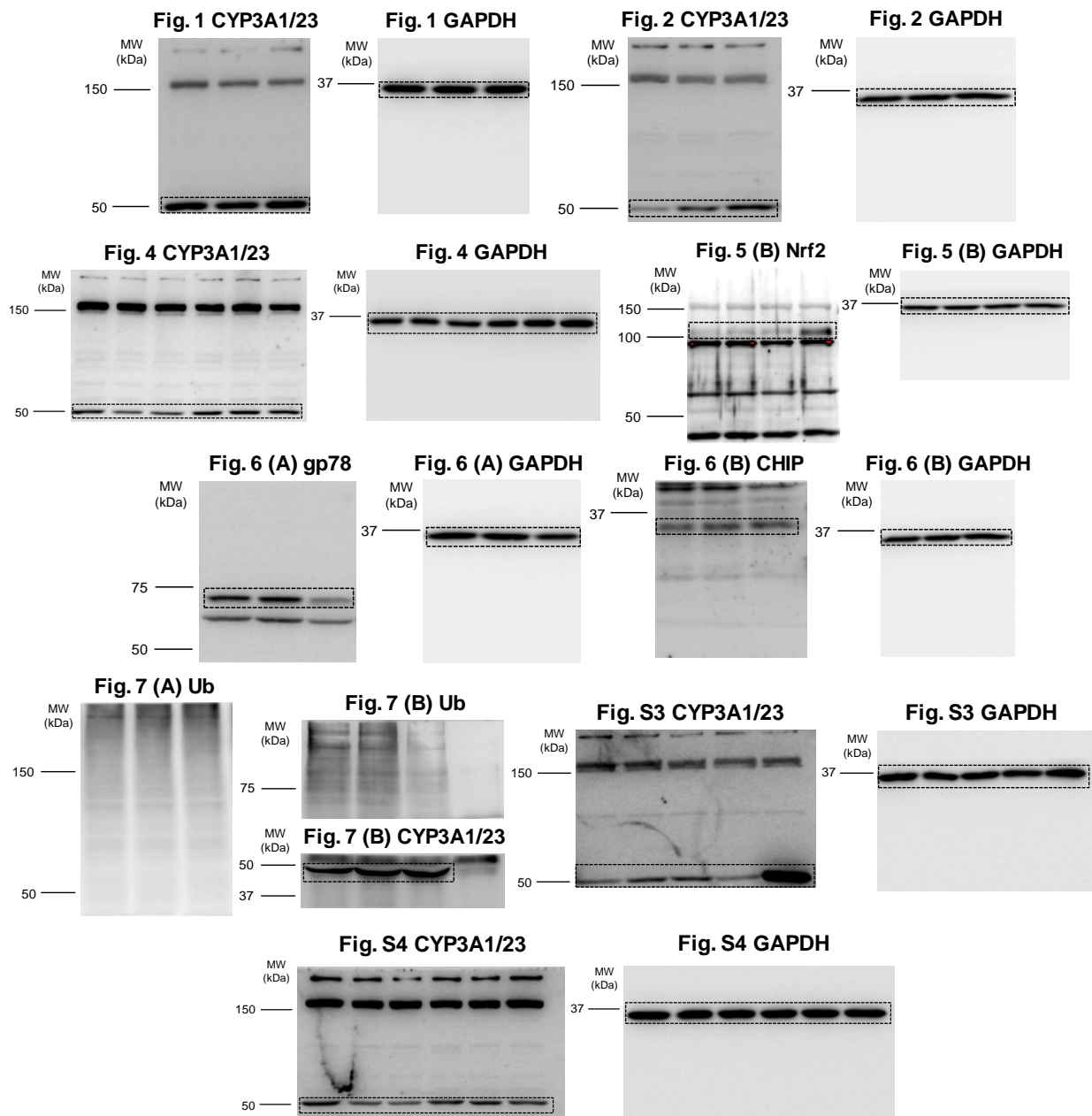
## **Supplementary Information**

### **Acetaminophen induces accumulation of functional rat CYP3A via polyubiquitination dysfunction**

Masataka Santoh<sup>1</sup>, Seigo Sanoh<sup>1,\*</sup>, Masashi Takagi<sup>1</sup>, Yoko Ejiri<sup>2</sup>, Yaichiro Kotake<sup>1</sup>,  
and Shigeru Ohta<sup>1</sup>

<sup>1</sup>Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3  
Kasumi, Minami-ku, Hiroshima, 734-8553, Japan, <sup>2</sup>Molding Component Business  
Department, New business Development Division, Kuraray Co., Ltd. 1-1-3 Otemachi,  
Chiyoda-ku, Tokyo, 100-8115, Japan.

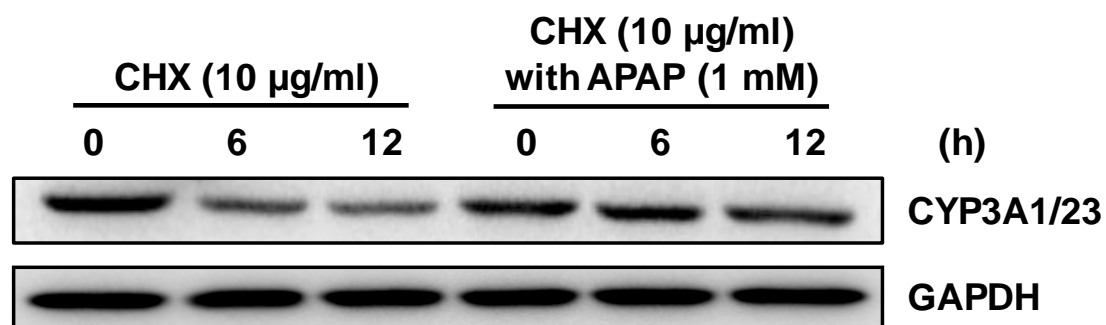
\*Corresponding author. E-mail address: [sanoh@hiroshima-u.ac.jp](mailto:sanoh@hiroshima-u.ac.jp)



**Figure S1.** Images of full-length blots. Dotted lines show cropped images used in the figures.



**Figure S2.** Evaluation of specificity of anti-CYP3A1 IgG using cell lysates from Dex-induced rat hepatocyte spheroids. Rat hepatocyte spheroids (day 5) were exposed to APAP (1 and 10 mM) and Dex (20  $\mu$ M) for 24 h. The results of CYP3A1/23 and GAPDH immunoblotting analyses of cell lysates (5  $\mu$ g of protein) are shown in the top panel. GAPDH was measured as a loading control. Cropped blots were shown and the full-length blots were presented in Supplementary Fig. 1.



**Figure S3.** Effects of APAP (1 mM) on CYP3A1/23 protein degradation. Rat hepatocyte spheroids (day 5) were exposed to cycloheximide (10 µg/ml) with or without APAP (1 mM) for 6 or 12 h before harvesting. The results of CYP3A1/23 and GAPDH immunoblotting analyses of cell lysates (5 µg of protein) are shown. GAPDH was measured as a loading control. Cropped blots were shown and the full-length blots were presented in Supplementary Fig. 1.