**Appendix S1**

## SUPPLEMENTAL MATERIALS AND METHODS

*Human samples*

The chronic liver disease (CLD) patients were consecutive patients, who attended the outpatient clinic at the Department of Gastroenterology, Juntendo University Urayasu Hospital, Chiba, Japan. At the time of their visit to the outpatient clinic, they were asked to rate the VAS score as the worst pruritus intensity. None of these patients had received an oral opioid antagonist (e.g. naloxone and naltrexone), the κ-opioid receptor agonist (nalfurafine hydrochloride) or opioid pain medication, prior to blood sampling.

Blood samples were collected from 20 patients with CLD, and 22 non-liver disease controls, using blood-sampling tubes containing ethylene-diamine-tetra-acetic acid (EDTA) (Terumo Corp., Tokyo, Japan). Plasma was prepared by centrifuging the blood samples at 3,000 rpm for 20 min at 4°C and storing the resulting plasma at –80°C. Patients in the CLD group were diagnosed by physicians according to the Japanese clinical guidelines for the disease of the Ministry of Health, Labour, and Welfare of Japan. Of the 20 patients with CLD, patients were diagnosed with autoimmune hepatitis (AIH) (based on the Japanese diagnostic guidelines of AIH 2013 (S1)), viral infection (hepatitis B virus (HBV) or hepatitis C virus (HCV)), alcoholic liver cirrhosis, primary biliary cholangitis (PBC) (based on the Japanese version of the clinical practice guidelines for PBC 2012 (S2), overlap syndrome (AIH+PBC) (based on the Paris Criteria (S3)), and others (Sjogren syndrome with liver dysfunction and liver dysfunction with suspected AIH). The viral infection diagnosis was established using the CDC criteria: chronic HBV infection was diagnosed based on the HBsAg (hepatitis B surface antigen), total anti-HBs (anti hepatitis B surface antibody), and total anti-HBc (anti hepatitis B core antibody); and chronic HCV infection based on the HCVAb (hepatitis C antibody) and HCV RNA PCR (polymerase chain reaction).

To collect the serum samples, the drawn blood was stored at room temperature for 15 min and then centrifuged at 3,000 rpm for 20 min at 4°C. Serum concentrations of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), and total bilirubin (TBil) were obtained from the clinical laboratory tests. Clinical profiles and laboratory data at the time of the evaluation of pruritus are summarized in Table SI[1].

The medical ethics committee of Juntendo University Urayasu Hospital approved the study protocol, and all patients provided written and informed consent. This study was conducted according to the principles of the Declaration of Helsinki.

*Extraction of peptides from plasma*

Peptides were extracted from plasma using kits, obtained from Phoenix Pharmaceuticals (Burlingame, CA, USA), according to the manufacturer's instructions. Briefly, 500 µl of plasma was acidified with an equal volume of Buffer A (RK-BA-1) and loaded onto a SEP-COLUMN containing 200 mg of C18 (RK-SEPCOL-1). The extracted peptides were eluted with Buffer-B (RK-BB-1), dried by vacuum centrifugation and stored at −80°C.

*Enzyme-linked immunosorbent assay (ELISA)*

Concentrations of β-endorphin and dynorphin A were measured using commercial fluorescent EIA Kits (Phoenix Pharmaceuticals), according to the manufacturer`s instructions. Both ELISA Kits have < 10% intra-assay variation and < 15% inter-assay variation; the spike recovery values (average) are 91.1% for β-endorphin and 98.1% for dynorphin A. The peptide % cross-reactivity for β-endorphin ELISA kit is 100% for β-endorphin (human) and 100% for Ac- β-endorphin (human); and for dynorphin A ELISA kit is 100% for dynorphin A (human, rat, mouse, porcine), < 0.1% for dynorphin A (1–13, porcine), and 0% for dynorphin A (1–8, porcine).

*Statistical analyses*

All statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). The intergroup comparison of control, non-pruritus and pruritus patients with CLD was analyzed using the Kruskal-Wallis test; while the comparison between the pruritus and non-pruritus group (Table SI[1]) was done using the Mann-Whitney test. Correlations were determined by the Spearman rank correlation tests, with correlation coefficients (r) of 0.2 to 0.4, > 0.4 to 0.7, and > 0.7 deemed weak, moderate, and strong, respectively. In all analyses, $p < 0.05$ indicated statistical significance.

## REFERENCES

S1. Ohira H, Abe K, Takahashi A, Watanabe H. Autoimmune hepatitis: recent advances in the pathogenesis and new diagnostic guidelines in Japan. Intern Med 2015; 54: 1323–1328.
S2. Working Subgroup for Clinical Practice Guidelines for Primary Biliary C. Guidelines for the management of primary biliary cirrhosis: The Intractable Hepatobiliary Disease Study Group supported by the Ministry of Health, Labour and Welfare of Japan. Hepatol Res 2014; 44 Suppl S1: 71–90.
S3. Czaja AJ. Diagnosis and management of the overlap syndromes of autoimmune hepatitis. Can J Gastroenterol 2013; 27: 417–423.