Supporting Information

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SI Materials and Methods

Hepatocyte Culture and TPO Measurement. Livers from WT or *Urah*$^{plt2/plt2}$ mice aged 5 to 6 wk were perfused retrogradely via the inferior vena cava to isolate individual hepatocytes, as previously described (1). Then, 6×10^6 hepatocytes were cultured in RPMI containing 10% vol/vol bovine calf serum (HyClone) and 5×10^{-5} M 2-mercaptoethanol for 60 h at 37 °C in 10% (vol/vol) CO_2 in air before culture supernatant collection and concentration with a centrifugal filter unit (Amicon Ultra-15 10000 MWCO; Millipore). The number of hepatocytes in individual culture flasks was determined by measurement of the total genomic DNA content of each flask using a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen). TPO content of individual flasks was then corrected for the number of cultured hepatocytes as measured by the total dsDNA content of the cell lysate. TPO concentration in serum, liver lysates, and cell culture supernatant was determined by a Quantikine murine TPO ELISA (R&D Systems).

Production of *Urah* Transgenic Mice. Transgenic mice were generated via injection of *C57BL/6* zygotes with a DNA construct in which the *Urah*^{long} cDNA was linked to the ubiquitin C promoter (2). DNA extracted from tail tips of founder mice was screened for the presence of the transgene by Southern blot analysis. Colonies were maintained by mating transgene-positive mice with *C57BL/6* partners. Transgenic rescue was performed by mating *Urah* transgenic mice with *Urah*$^{plt2/plt2}$ mice and then backcrossing to additional *Urah*$^{plt2/plt2}$ mice.

1. Brysha M, et al. (2001) Suppressor of cytokine signaling-1 attenuates the duration of interferon gamma signal transduction in vitro and in vivo. *J Biol Chem* 276: 22086–22089.

2. Schorpp M, et al. (1996) The human ubiquitin C promoter directs high ubiquitous expression of transgenes in mice. *Nucleic Acids Res* 24:1787–1788.

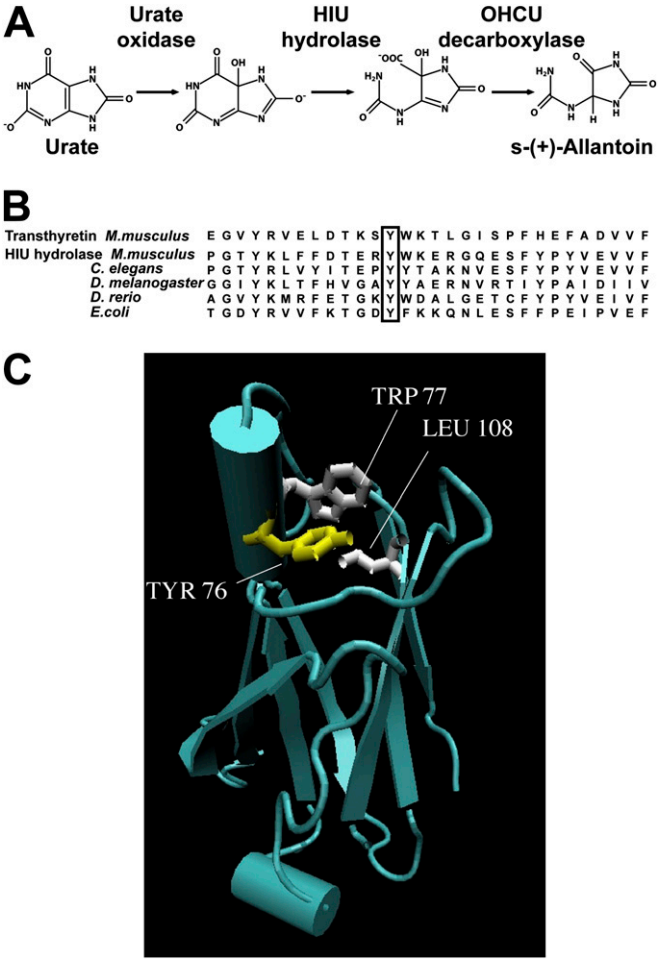

Fig. S1. HIU hydrolase structure and function. (*A*) HIU hydrolase catalyzes the second step of the three-step conversion of urate to allantoin in the mouse and most other nonhuman mammals. (*B*) Protein homology of HIU hydrolase in the region of the *plt2* mutation with predicted genes from other species (*Mus musculus*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Danio rerio*, and *Escherichia coli*) demonstrating that the Tyr substituted in *Urah*$^{plt2/plt2}$ mice (boxed) has been conserved throughout evolution. (*C*) Structure of zebrafish HIU hydrolase (1) showing the tyrosine (TYR76 according to numbering as in ref. 1) substituted in *Urah*$^{plt2/plt2}$ mice making strong hydrophobic contacts with conserved neighboring residues.

1. Zanotti G, et al. (2006) Structure of zebra fish HIUase: Insights into evolution of an enzyme to a hormone transporter. *J Mol Biol* 363:1–9.

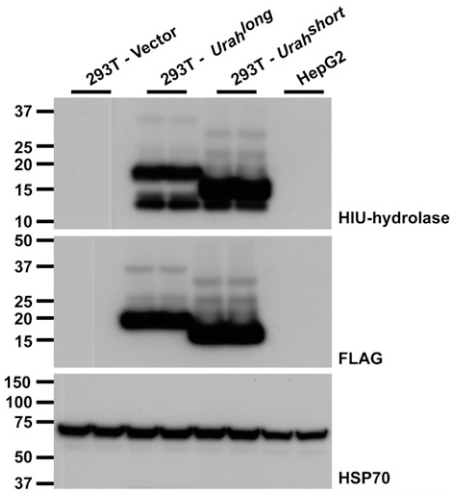

Fig. S2. Anti-HIU-hydrolase antiserum detects protein in cells transfected with murine *Urah* cDNA but not in human Hep-G2 cells. 293T cells were transiently transfected with vectors containing the short or long form of the murine *Urah* cDNA, including an N-terminal FLAG epitope tag and lysates examined by Western blot analysis with a rabbit anti-mouse HIU hydrolase polyclonal antiserum, anti-FLAG antibody, or control antibodies to HSP70. Sizes of molecular weight markers are shown in kilodaltons.

Table S1. Hematological and serum biochemical analysis in *Urah*$^{plt2/plt2}$ mice

	WT	*Urah*$^{plt2/plt2}$
Hematology		
Red cell count, 10^{12}/L	10.3 ± 0.4	10.1 ± 0.4
White cell count, 10^{9}/L	9.0 ± 1.7	9.1 ± 2.1
Neutrophils	0.9 ± 0.3	0.9 ± 0.8
Lymphocytes	7.7 ± 1.6	7.6 ± 1.3
Monocytes	0.1 ± 0.1	0.1 ± 0.1
Eosinophils	0.2 ± 0.1	0.2 ± 0.1
Platelets, 10^{9}/L	1,424 ± 139	2,234 ± 838*
Serum biochemistry		
Total protein, g/L	46 ± 7	46 ± 6
Albumin, g/L	30 ± 2	30 ± 2
Bilirubin, mmol/L	3.1 ± 0.6	2.6 ± 0.5
Alanine aminotransferase, IU/L	28 ± 10	35 ± 9
Aspartate aminotransferase, IU/L	69 ± 34	67 ± 15
γ-Glutamyl transferase, IU/L	0.5 ± 0.8	0.3 ± 0.6

n = 8–11 mice for biochemical analyses and n = 18–24 mice for hematology.
*$P < 0.01$.