Supporting Information

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SI Materials and Methods

Generation of Transgenic Rice. All enzymes used in the construction of plasmids were purchased from New England Biolabs. A 1,241-bp *Gt13a* promoter and signal peptide sequence (GenBank accession no. AP003256) were amplified from the rice genome of TP 309 (1) and fused into the mature HSA gene optimized with a rice codon bias. The gene was synthesized by Blue Heron Biotechnology, with a SchI and an XhoI site at the 5′ and 3′ end, respectively. The synthesized HSA gene was digested by SchI and XhoI and cloned into a *pOsPMP01* plasmid digested with NaeI/XhoI. The resulting construct was designated *pOsPMP04*. To construct the Agrobacterium binary vector, *pOsPMP04* was digested with HindIII and EcoRI, and a 2,832-bp fragment was cloned into the binary vector pCambia1301 using the same enzyme sites. The resulting binary vector was designed *pOsPMP114* and transformed into Agrobacterium strain EHA105. All plasmids were described in previous investigations (1, 2).

pOsPMP114 and *pOsPMP02* were cotransformed into calli derived from scutellum of the variety TP309 or Zhonghua 11 by the Agrobacterium-mediated transformation. A forward primer (5′-GAGGGTGTGGAGGCTCTTGT-3′) from the *Gt13a* signal peptide and a reverse primer (5′-GTCACTTCACGTCTGGA-CA-3′) from the *HSA* gene were used to identify successful cotransformants with PCR. The PCR program used was the following: denaturation at 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s. All PCR-positive plantlets were maintained in a greenhouse until maturation.

Western Blot Analysis. The total soluble protein from 10 seeds was extracted using extraction buffer [50 mM Tris (pH 8.0)]. Approximately 50 μg of protein was loaded and separated on a 12% polyacrylamide gel, and Western blotting analysis was performed according to the manufacturer's instructions (Bio-Rad). The HSA antibody (Bethel Laboratory) and a goat anti-rabbit IgG conjugated to alkaline phosphatase antibody (BioLegend) were used for Western blot analysis. Blot images were developed with 5-bromo-4-chloro-3-indolyl phosphate-nitrobluetetrazolium chloride.

Recombinant HSA Quantification Assay. Crude protein (500 μL per seed) was obtained by grinding of seeds in extraction buffer [50 mM Tris (pH 8.0), 100 mM NaCl] and clarification by centrifugation at 12,000 × *g* for 10 min. The protein concentration was measured with the BCA protein assay (Pierce). HSA was quantized with ELISA using a human albumin quantification kit (Bethyl Laboratories). To determine OsrHSA expression levels, the crude protein from the transgenic rice seeds was diluted with dilution buffer [50 mM Tris (pH 8.0), 180 mM NaCl, 1% BSA, 0.5% Tween-20] at ratios of 1:500, 1:1,000, 1:2,000, and 1:4,000. The procedure was followed based on the manufacturer's instructions.

Purification Protocol for OsrHSA from Rice Seeds. Rice seeds were ground into powder and homogenized in a phosphate buffer (PB) [25 mM PB with 50 mM NaCl (pH 7.5)] at a ratio of 1:10 (wt/vol) at room temperature for 1 h, and the mixture was precipitated for 2 h at pH 5.0 by adjusting the pH with acetic acid. The crude extract was clarified by centrifugation. For large-scale purification, a pressure filter was used. The clarified extracts were then loaded onto Capto-MMC (GE Healthcare) at pH 5.0, and eluted with PB buffer [25 mM PB with 100 mM NaCl (pH 7.0)]. The resulting collected fractions were adjusted to pH 7.5 and further purified with Q Sepharose Fast Flow (GE Healthcare, www.gelifesciences.com). OsrHSA was eluted with PB buffer [25 mM PB with 50 mM NaCl (pH 7.5)]. OsrHSA fractions from Q Sepharose were finally purified through Phenyl HP (GE Healthcare) using 0.45 M ammonium sulfate. The resulting OsrHSA fraction was concentrated and desalted using ultrafiltration with Pellicon (Millipore). The purity of OsrHSA was determined with silver stain of an SDS/PAGE and analysis using an Agilent 1200 HPLC system (Agilent Technologies) equipped with TSKgel G3000 SW or Symmetry 300 C_4, 5 μm (Waters); purity was calculated by size-exclusion HPLC. Large-scale production of OsrHSA was performed on BPG 300, BPG 200 (GE Healthcare), and Easypack 100 columns (Bio-Rad) packed with the same resins used in the laboratory-scale purification.

Spectroscopic Measurements. Circular dichroism (CD) spectra were measured on a JASCO J-820 automatic spectropolarimeter with a concentration of 4 μM and 20 μM in PB buffer [25 mM PB with 150 mM NaCl (pH 7.4)] used for analysis in the far-UV region and the near-UV region, respectively. Data were recorded from 190 to 360 nm with a scan speed of 50 nm/min. The melting point was determined by measuring the change in ellipticity at 222 nm from 20 to 90 °C with a protein concentration of 10 μM. Intrinsic fluorescence spectra of 3 μM OsrHSA in PB buffer [25 mM PB with 150 mM NaCl (pH 7.4)] were obtained using an F-4500 fluorescence spectrometer (Hitachi) equipped with a thermostatically controlled 1-cm quartz cell, with an excitation wavelength of 295 nm with excitation and emission bandwidths of 5 nm. UV absorbance scans of OsrHSA and pHSA were taken from 200 to 450 nm at different concentrations using a UV probe (Shimazu). All of the spectra were normalized by using the corresponding buffer as a baseline.

N-Terminal Sequence Determination, Peptide Mapping, and Molecular Mass Determination. Total protein extracts from the transgenic grain were separated using 10% SDS/PAGE. N-terminal sequencing was performed using Edman degradation at the Genome Center Proteomics Core Facility at the University of California (Davis, CA).

For peptide mapping, protein samples were first separated using 12% SDS/PAGE. The gel bands corresponding to OsrHSA were excised, washed, reduced, alkylated, and in-gel tryptic digested. Tryptic-digested peptides extracted from the gel were concentrated and reconstituted in 10 μL of 5% formic acid followed by LC-MS/MS analysis. A CapLC (Waters) equipped with a Magic C_{18} column (5 μm, 100 μm id × 150 mm; Michrom Bioresources) was used to separate the proteolytic peptides. Solvent A was 5% CH_3CN + 0.1% formic acid + 0.01% TFA, and solvent B was 85% CH_3CN + 20% isopropanol + 5% H_2O + 0.075% formic acid + 0.0075% TFA. Peptides were eluted using a 70-min linear gradient of solvent B from 10% to 50%, and the MS/MS was performed using a Q-TOF II mass spectrometer (Waters/Micromass). The in-house software, MASCOT 2.2, from Matrix Science was used to for the interpretation of MS/MS spectra against the SwissProt human subdatabase.

Ligand-Binding Assay. Warfarin sodium (19272A; Adamas), naproxen sodium (M1275; Sigma), pHSA (A3782, 089K7561; Sigma), and defatted OsrHSA were used in the binding assays. The drug-binding constants were measured at 25 °C with isothermal titration calorimetry (MicroCal). The concentration of HSA was maintained at 40 μM protein in PB buffer [25 mM PB (pH 7.4),

150 mM NaCl] and 1.0 mM of warfarin or naproxen loaded into the injector; proteins were then titrated with 10 μL ligands at each time point with a 5-min equilibration time. Titration of ligand to buffer [25 mM PB (pH 7.4), 150 mM NaCl] was used as a blank control. A one-site binding model was used to calculate the binding affinity for warfarin, and a three-site binding model was used for naproxen. The K_1 value was used to compare the affinities of OsrHSA and pHSA.

Identification of Lipids on OsrHSA. Lipid extraction from human serum albumin was performed as indicated in the literature (3). Briefly, lipid samples were dissolved in a mixture of chloroform, methanol, and water (4:4:1 vol/vol). A solution of 10 mg/mL 2,5-dihydroxybenzoic acid was prepared with a mixture of water and acetonitrile (1:4 vol/vol). A 0.25-μL aliquot of the lipid sample was deposited directly on the target and covered with the same amount of matrix solution. The extracted lipid was analyzed using 4800 MALDI-TOF/TOF in the positive ion mode (Applied Biosystems). Four hundred scans were accumulated for each MS spectrum. Data were acquired in reflectron mode and processed using Data Explorer (Applied Biosystems).

HPLC-MS was performed on a 4000 Q-Trap instrument (Applied Biosystems/MDS Sciex) coupled to an UltiMate 3000 HPLC (Dionex). Liquid chromatography was performed on a TSKgel Amide-80 (HILIC) column (150 × 1 mm, 5 μm; Thermo Scientific) at a flow rate of 50 $\mu L \cdot min^{-1}$. Mobile phase A consisted of 4% MeOH aqueous solution with 10 mM ammonium acetate, and mobile phase B consisted of 96% THF with 10 mM ammonium acetate. The following gradient was used: 10% solvent B from 0 to 4 min, increasing to 70% B for 20 min with a hold at 70% B for 10 min, finishing with 5% solvent B. In precursor ion scan experiments, the collision energy was ramped from −30 to −45 eV for experiments in the negative ion mode and from +30 to +50 eV for the positive ion mode, respectively. For neutral loss scan in the positive ion mode, the collision energy was also ramped from +30 to +50 eV. The enhanced product ion scan was used to obtain fragment ion spectra for structure confirmation.

Crystallization and X-Ray Data Collection. OsrHSA from large-scale production was solubilized in buffer [50 mM KPO_4 (pH 7.5), 150 mM NaCl], and the protein was further purified using size-exclusion chromatography to remove any dimers. The peak corresponding to the monomeric species was collected and the protein was concentrated to 100 mg/mL in 50 mM KPO_4 (pH 7.5) and 150 mM NaCl. Results from dynamic light scattering showed the presence of only the monomeric species.

For preparation of the HSA–myristic acid complex, myristic acid at a concentration of 2.5 mM was resuspended in 20 mM KPO_4 (pH 7.5) and warmed to 50 °C. The sample was then cooled to ∼30 °C, and 0.2 mM of rHSA was added. The sample was incubated for 20 min, cooled to 4 °C, and centrifuged at 12,000 × g for 4 min at 4 °C to pellet any excess myristic acid. The supernatant was concentrated to ∼120 mg/mL and buffer exchanged in 20 mM KPO_4 (pH 7.5) and 0.1 mM myristic acid. Crystals of HSA–myristic acid complex were obtained with the hanging diffusion method in a 28% PEG 3350, 50 mM phosphate buffer (pH 7.5), and 150 mM KCl. The crystals of OsrHSA were flash-frozen in liquid nitrogen, and the X-ray diffraction data were collected at the synchrotron beamline to 2.05 Å resolution with the crystal maintained at the temperature of 100 K.

Cell Culture. CHO-K1 and Vero cell lines were obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). The hybridoma cell line SP2/0 secreting IgGκ was purchased from Beijing Cowin Biotech Ltd. Co. All cell lines were seeded at 100 μL/well into a 96-well plate at a concentration of 5,000 cell/mL and cultured for 10 d under standard conditions (37 °C, 5% CO_2/95% air atmosphere). All cell lines were grown in DMEM (Invitrogen) supplemented with 5% FBS (Luoshen Biotech), 5% FBS + 1 g/L OsrHSA, and 5% FBS + 1 g/L pHSA (Wuhan Institute of Biological Products). The MTT assay and cell number determination were performed 24 h after seeding to investigate cell viability and proliferation, respectively. Antibody secretion of the SP2/0 cell line was determined using ELISA.

Efficacy of OsrHSA on Rats with Liver Cirrhosis. Specific pathogen-free male Wistar rats weighing 200 ± 20 g from the Center of Diseases Control of Hubei province were used, and the animals were housed in a temperature- and light-controlled environment at the Center of Animal Experiment of Wuhan University, China. Animals were used after acclimatization for 1 wk. The OsrHSA used for these experiments was obtained from Healthgen Biotechnology Co. Ltd. (lot no. 20100621), and pHSA was purchased from Wuhan Institute of Biological Products (lot no. 200910023). Urinary protein test kits and plasma serum albumin test kits were obtained from Nanjing Jiancheng Technology Co.

Rats with liver cirrhosis were prepared according to previous reports. Briefly, animals were fed with a modified high-fat diet (89.5% corn flour, 10% lard, and 0.5% cholesterol) with 5–10% ethanol in drinking water. From the eighth day onward, each rat received s.c. injections of 40% (vol/vol) carbon tetrachloride (CCl_4) in olive oil at an initial dosage of 5 mL/kg and then 1–3 mL/kg twice a week for 12–16 wk. Normal group rats received the same dosage of olive oil with a normal diet and access to pure water. From the eighth week onward, the abdominal circumference and body weight were monitored every 3 d, and a ratio of abdominal circumference to body weight of more than 60 cm/kg was set as the criteria for evaluating retention of ascite. Liver cirrhosis was confirmed by the liver index (liver weight over body weight in g/kg), and liver tissue was examined histologically with H&E staining. Animals with a urinary protein excretion >51.3 mg/kg per 20 h and those with a body weight decrease of more than 10 g/day were excluded from this study.

Rats with liver cirrhosis were divided into five groups: (*i*) a liver cirrhosis model group, (*ii*) a group treated with 0.25 g/kg OsrHSA, (*iii*) a group treated with 0.5 g/kg OsrHSA, (*iv*) a group treated with 1.0 g/kg OsrHSA, and (*v*) a group treated with 1.0 g/kg pHSA. Eight to 10 rats were selected for each group. Administrations of OsrHSA or pHSA were performed according to previous investigations (4). Dosages of 0.25, 0.5, and 1.0 g/kg every 2 h were used for the OsrHSA-treated groups, and a dose of 1.0 g/kg every 2 h was used for the pHSA-treated group. The volumes for each administration were maintained at 10 mL/kg using peristaltic pumps to maintain a delivery speed of 1 mL/min. The same volume of saline solution was injected through the tail veins of the control group for 2 d.

Collection of urine samples and measurement of abdominal circumference and body weight were performed on day 0 (before administration) and day 3 (24 h after treatment for 2 d). Blood samples were obtained immediately before the rats were killed and stored at −70 °C for further use. The serum colloid osmotic pressure was determined on a Fiske Model 210 instrument (Advanced Instruments, Inc.). Experimental results are displayed as the mean + SD. Data were analyzed with a one-way ANOVA followed by Scheffé's test. The statistical significance level was set at $P < 0.05$.

Immunogenicity Evaluation of OsrHSA. OsrHSA was obtained from Healthgen Biotechnology Co. Ltd. (lot no. 20101031), and pHSA was purchased from Wuhan Institute of Biological Products. Freund's adjuvant, complete (F5881), Freund's adjuvant, incomplete (F5506), goat anti-rabbit IgG (whole molecule) conjugated to peroxidase (A0545, lot no. 039K4840), and Evens blue (lot no. 076K3664) were purchased from Sigma. ELISA kits for

rabbit IgG, IgM, IgA, IgE, complement C_3, and complement C_4 were obtained from Shanghai Yuanye Biotechnology Co.

New Zealand rabbits aged 5–6 mo with 3.6 ± 0.3 kg body weight were randomly divided into three groups, with each group containing five rabbits. The rabbits were immunized on days 0, 14, and 28 with OsrHSA (1 mg/0.25 mL per kg), pHSA (1 mg/0.25 mL per kg), or saline (0.25 mL/kg, negative control) by s.c. injection. Freund's adjuvant (complete) was used in the first immunization, and Freund's adjuvant (incomplete) was used for the second and third immunizations. Animal death, animal state, and allergic reactions were monitored every morning (10:00 AM) and afternoon (4:00 PM). Body weights were monitored, and blood samples were collected from the central auricular arteries before immunization and 7 d after every immunization.

Serum samples were collected before immunization and 3 and 4 wk after the first immunization. The titer of anti-HSA IgG was measured using ELISA with a sample dilution of 1:200. The total IgG, IgM, IgA, IgE, complement C_3, and C_4 levels were detected according to the manufacturer's instructions. Double-immunodiffusions were performed at 37 °C for 24–48 h to test the titers of OsrHSA and pHSA antiserum against OsrHSA or pHSA (using a twofold dilutions of serum and 0.02 mg/mL antigen concentration) and to test the antibody purity of OsrHSA antiserum and pHSA antiserum against pHSA (using a stock solution of serum and 0.05 mg/mL of antigens).

Female 10-wk-old guinea pigs were used for passive cutaneous anaphylaxis (PCA) assay. Anti-pHSA and anti-OsrHSA sera neutralized by pHSA were inoculated intradermally. Meanwhile, saline, antiserum against pHSA neutralized by pHSA, and antiserum against OsrHSA neutralized by OsrHSA were inoculated as negative controls, with antiserum against pHSA and OsrHSA used as positive controls; administration of 50 μL at an independent spot was used. After 4 h of sensitization, the animals were challenged with 0.5 mL OsrHSA (at a concentration of 1 mg/mL) and 0.2 mL 2% Evan's blue (Sigma). After 12 h, the dorsal skin was removed 30 min postsensitization, and the amount of Evan's blue was evaluated. The P, P', and P'' values were calculated by t test using two tails.

1. Ning T, et al. (2008) Oral administration of recombinant human granulocyte-macrophage colony stimulating factor expressed in rice endosperm can increase leukocytes in mice. *Biotechnol Lett* 30:1679–1686.
2. Xie T et al. (2008) A biologically active rhIGF-1 fusion accumulated in transgenic rice seeds can reduce blood glucose in diabetic mice via oral delivery. *Peptides* 29: 1862–1870.
3. Belgacem O, Stübiger G, Allmaier G, Buchacher A, Pock K (2007) Isolation of esterified fatty acids bound to serum albumin purified from human plasma and characterised by MALDI mass spectrometry. *Biologicals* 35:43–49.
4. Horie S, Nagai H, Yuuki T, Hanada S, Nakamura N (1998) Effectiveness of recombinant human serum albumin in the treatment of ascites in liver cirrhosis: evidence from animal model. *Gen Pharmacol* 31(5):811–815.

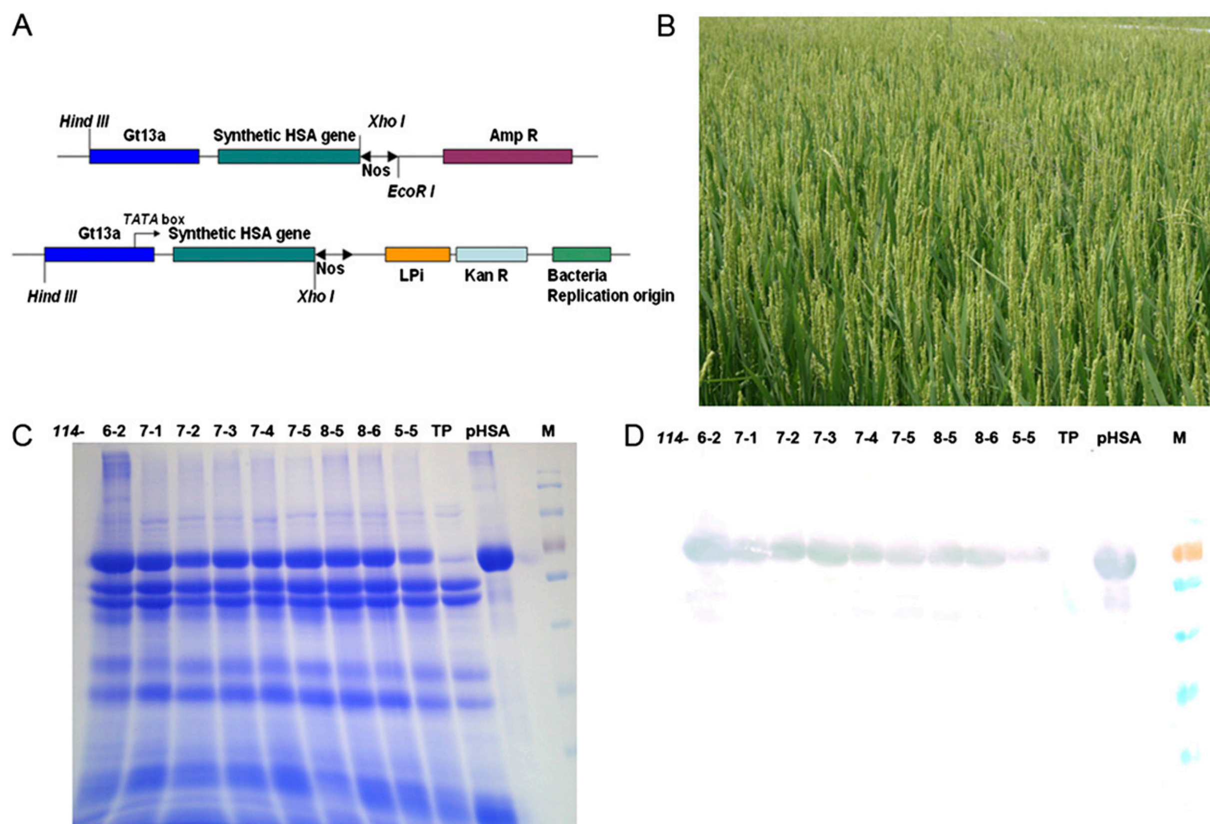

Fig. S1. Expression of OsrHSA in transgenic rice seeds. (*A*) Plasmids used for the expression of OsrHSA. The final transformed plasmid (*Lower*) originated from the expression plasmid (*Upper*). (*B*) Transgenic rice expressing OsrHSA in the field. (*C* and *D*) Expression of OsrHSA in transgenic rice seeds as characterized using SDS/PAGE (*C*) and Western blotting (*D*), with lanes 1–9 showing protein from transgenic lines 6-2, 7-1, 7-2, 7-3, 7-4, 7-5, 8-5, 8-6, and 5-5, respectively. Sample TP is a negative control using nontransgenic TP309 seeds; sample pHSA is a positive control of plasma-derived human serum albumin; and sample M is the molecular weight marker.

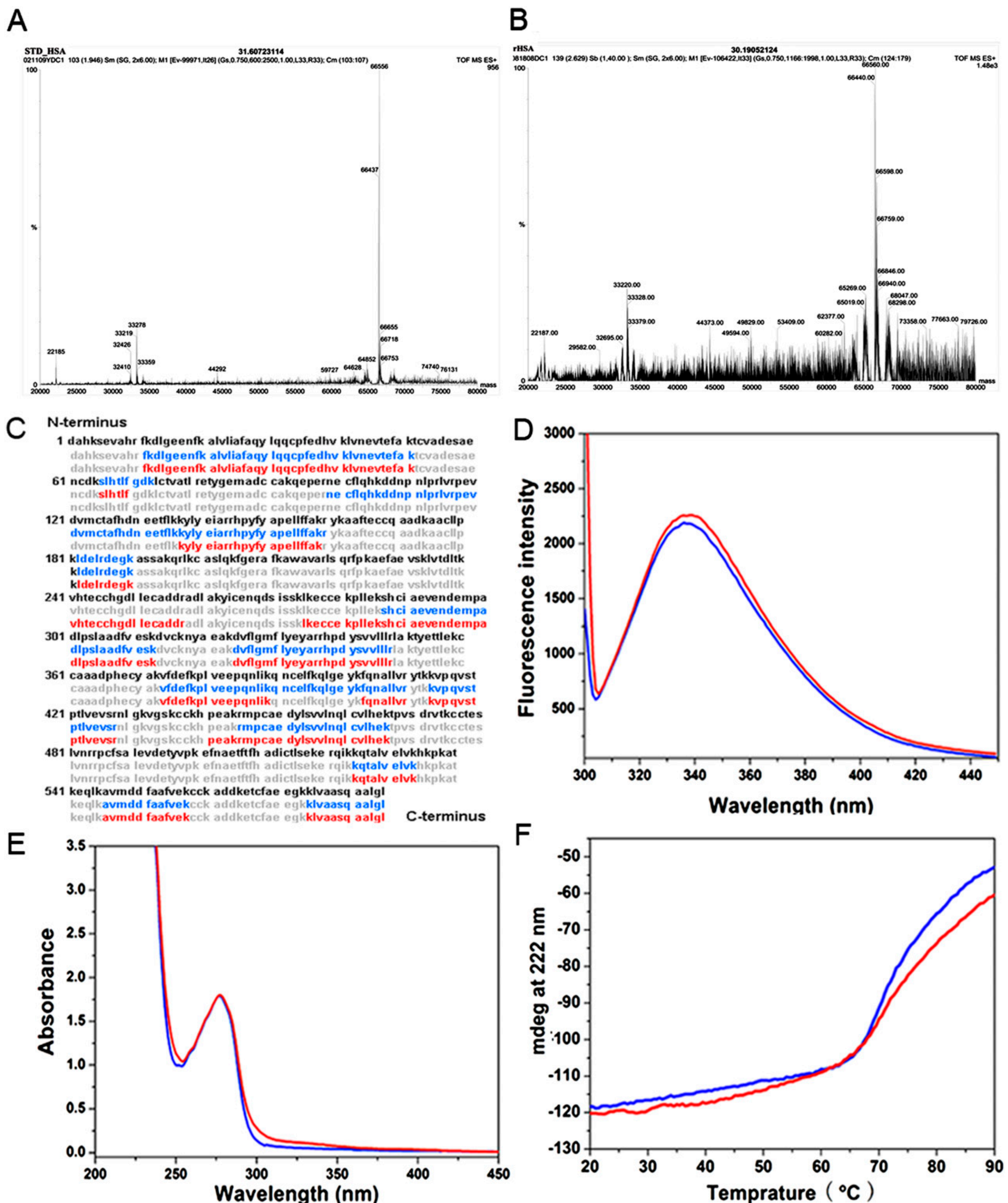

Fig. S2. Biochemical and biophysical characterization of OsrHSA. Molecular weight of OsrHSA (*A*) and pHSA (*B*) as determined using MALDI-TOF. (*C*) Peptide mapping of OsrHSA and pHSA. Peptide sequences highlighted in bold indicate peptide sequences of HSA sequence in a database. Blue and red letters show the amino acids in OsrHSA and pHSA matched with database sequence, respectively. (*D*) Intrinsic fluorescence emission spectra of OsrHSA (red) and pHSA (blue) excited at 295 nm (4 μM). (*E*) The UV absorbance of OsrHSA (red) and pHSA (blue) between 220 and 500 nm (3 mg/mL). (*F*) The change in ellipticity measured from CD spectra of OsrHSA (red) and pHSA (blue) at increasing temperatures (10 μM). The melting point was determined by thermal denaturation of OsrHSA and pHSA by monitoring the CD signal at 222 nm.

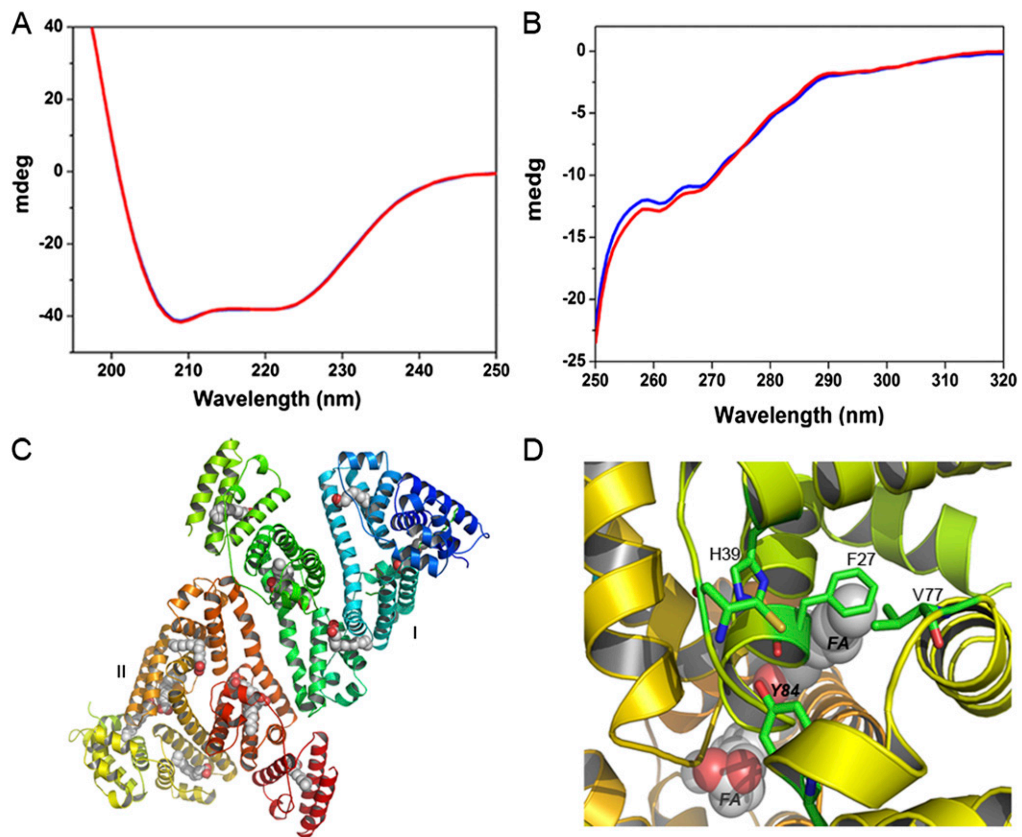

Fig. S3. Structural analysis of OsrHSA. (*A* and *B*) Secondary (*A*) and tertiary (*B*) structure of OsrHSA (red) and pHSA (blue) as characterized by CD spectra in the near- and far-UV regions. Protein concentrations were 4 and 20 μM for near- and far-UV CD measurements, respectively. (*C*) Two OsrHSA molecules obtained in an asymmetric unit, with 15 myristic acids binding (seven in molecule I, eight in molecule II). Myristic acids are presented as spheres. (*D*) Free cysteine at position 34 in OsrHSA.

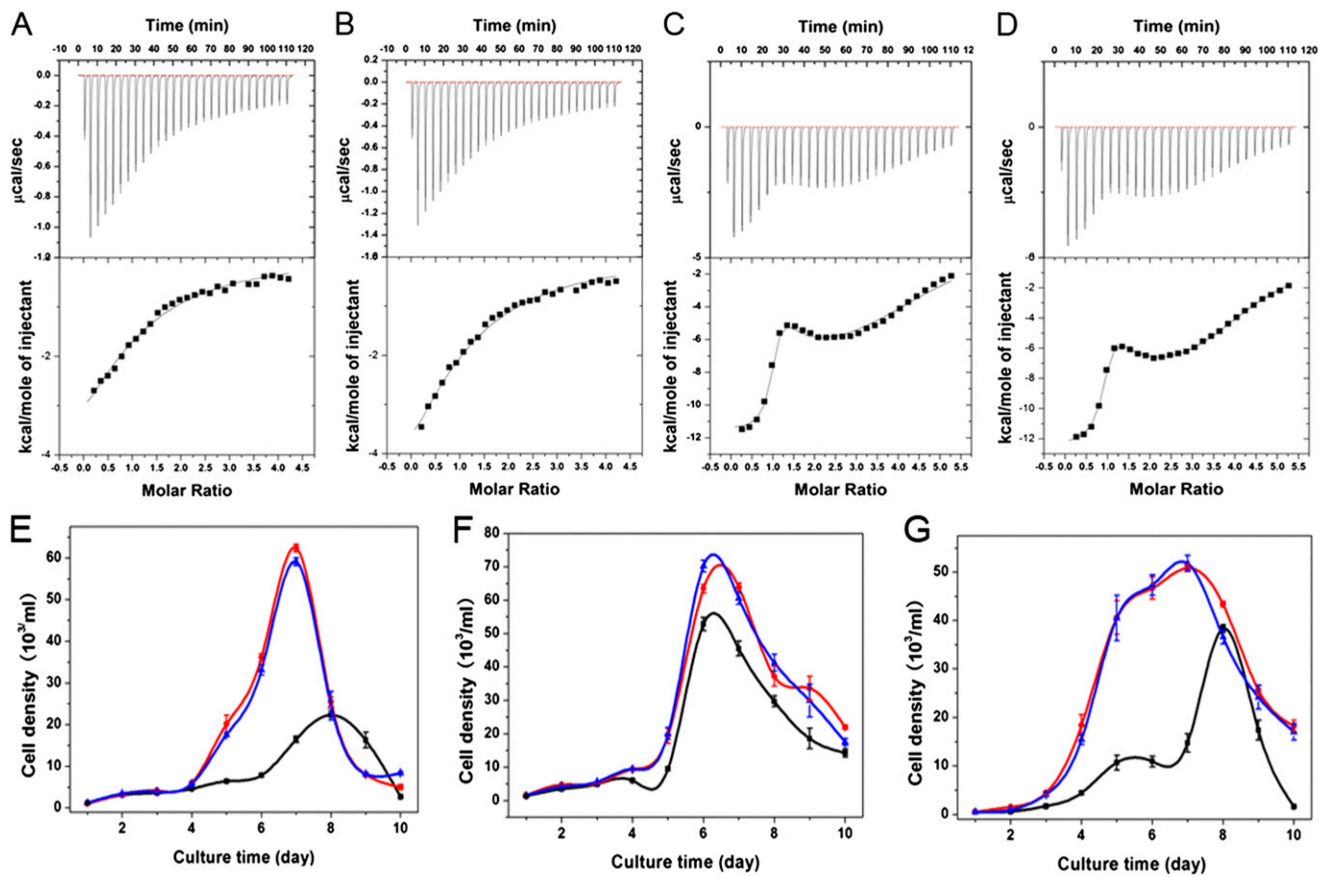

Fig. S4. Functions of OsrHSA in vitro. (*A*–*D*) Binding capacities of OsrHSA (*A*) and pHSA (*B*) to warfarin as determined using isothermal titration calorimetry (ITC). Binding capacities of OsrHSA (*C*) and pHSA (*D*) to naproxen as determined using ITC. (*E*–*G*) The effects of OsrHSA (blue) and pHSA (red) on cell density in CHO (*A*), Vero (*B*), and SP2/0 (*C*). Five percent FBS was used as a negative control (black).

Table S1. X-ray data collection and structure refinement statistics

Data set	HSA + myristoyl
Space group	$P2_1$
a, b, c, Å	95.6, 38.4, 184.0
α, β, γ	90, 104.9, 90
Resolution, Å	47.805–2.050
Last shell of resolution, Å	2.12–2.05
No. observed reflections	303,997
No. unique reflections	82,081
Completeness, %*	99.6 (99.7)
Redundancy*	3.7 (3.7)
Rsym[†]	0.064 (0.545)
I/(σI)[‡]	24.4 (2.4)
Rwork	0.234
Rfree	0.303
Wilson B, $Å^2$	32.3
B-factor (no. of atoms)	
Protein	38.298 (9,095)
Solvent	42.225 (556)
Ligand	44.447 (218)
Ramachandran	
Preferred, %	96.03
Allowed, %	3.53
Outliers, %	0.43
Rmsd	
Bond, Å	0.0204
Angles, °	1.8379

*The information for the last shell of resolution is given in parentheses.
[†]$R_{sym} = (\Sigma \mid Iobs - Iavg \mid)/\Sigma\ Iavg$.
[‡]$R_{work} = (\Sigma \mid Fobs - Fcalc \mid)/\Sigma Fobs$.

Table S2. A comparison of fatty acid bound to OsrHSA and pHSA

$[M + H]^+$	Total carbons	Total double bonds	Assignments
468.5[O]	14	0	(14:0)-lyso-PC
496.5[O,P]	16	0	(16:0)-lyso-PC
520.5[O,P]	18	2	(18:2)-lyso-PC
522.5[O,P]	18	1	(18:1)-lyso-PC
524.5[O,P]	18	0	(18:0)-lyso-PC
544.5[P]	20	4	(20:4)-lyso-PC
759.0[O,P]	34	2	(16:0/18:2)-PC
761.0[O,P]	34	1	(16:0/18:1)-PC
783.0[O,P]	36	4	(16:0/20:4), (18:2/18:2)-PC
785.0[O,P]	36	3	(16:0/20:3), (18:1/18:2)-PC
787.0[O,P]	36	2	(16:0/20:2)-PC, (18:0/18:2)-PC, (18:1/18:1)-PC
426.5[O]	14	0	(14:0)-lyso-PE
454.5[O,P]	16	0	(16:0)-lyso-PE
478.5[O,P]	18	2	(18:2)-lyso-PE
480.5[O,P]	18	1	(18:1)-lyso-PE
482.5[O,P]	18	0	(18:0)-lyso-PE
502.5[P]	20	4	(20:4)-lyso-PE
506.0[P]	20	2	(20:2)-lyso-PE
534.0[P]	22	2	(22:2)-lyso-PE
691.0[P]	30	1	(12:0/18:1)-PE
717.0[P]	32	2	(14:0/18:2)-PE
744.5[P]	34	2	(16:0/18:2)-PE
768.5[P]	36	4	(16:0/20:4), (18:2/18:2)-PE

[O]Lipids detected in OsrHSA.
[P]Lipids detected in pHSA.

Table S3. Abdominal circumference, urine volume, and urinary protein changes before and after treatments

Treatments	Dosage, g/kg	Abdominal circumference/ weight, cm/kg		Urine vol, $mL \cdot kg^{-1} \cdot h^{-1}$		Urinary protein, mg/L	
		Before Ad	After Ad	Before Ad	After Ad	Before Ad	After Ad
Normal	–	41.1 ± 3.4	42.5 ± 3.2	2.2 ± 0.3	2.3 ± 0.3	258 ± 28	278 ± 29
Saline	–	69.2 ± 5.4	67.9 ± 5.9	0.8 ± 0.6	0.8 ± 0.6	160 ± 49**	194 ± 63**
OsrHSA	0.25	68.9 ± 4.6	65.1 ± 4.7	0.6 ± 0.3	0.8 ± 0.3	197 ± 70	300 ± 41▲▲,##
OsrHSA	0.5	68.5 ± 2.7	63.1 ± 4.3▲▲	0.5 ± 0.2	1.1 ± 0.4▲▲	206 ± 87	360 ± 60▲▲,##
OsrHSA	1	71.4 ± 6.9	64.5 ± 6.4▲	0.6 ± 0.2	1.7 ± 0.6▲▲	208 ± 84	448 ± 78▲▲,##
pHSA	1	67.6 ± 2.4	61.1 ± 2.5▲▲	0.6 ± 0.3	1.6 ± 0.9▲▲	201 ± 59	402 ± 80▲▲,##

Average ± SD, n = 8–10. ▲$P < 0.05$, ▲▲$P < 0.01$ vs. before administration; **$P < 0.01$ vs. normal group; ##$P < 0.01$ vs. saline treatment.

Table S4. Production of OsrHSA in laboratory and large scale

Scale	Step	OsrHSA, g	Purity, TSP %	Yield, %	Purification fold
Lab scale (200 g)	Initial extraction	0.917 ± 0.023	10.6 ± 0.3	100	1
	IEC-1	0.575 ± 0.028	37.0 ± 2.1	62.6 ± 3.0	3.49 ± 0.20
	IEC-2	0.494 ± 0.021	85.3 ± 5.3	86.0 ± 3.7	8.05 ± 0.50
	HIC	0.418 ± 0.051	>99*	84.8 ± 10.8	9.38
	Final preparation	0.418 ± 0.005	>99*	45.6 ± 5.6	9.38
Large scale (40 kg)	Initial extraction	198.4 ± 14	9.3 ± 1.4	100	1
	Final preparation	110.0 ± 6	99.45 ± 0.2*	55.8 ± 3.2	10.64 ± 0.02

*Purity of sample from HIC was determined by reverse-phase HPLC (C_4) or size-exclusion HPLC IEC, ionic exchanger chromatography; HIC, hydrophobic interaction chromatography..

Table S5. Schedule of OsrHSA production from laboratory research to commercial production

Generation	Harvested seed	Protein level	Duration, mo	Development profile
T0	One transgenic line	μg	9	Genetic analysis
T1	10,000 seeds/200g	G	5	Purification and function assay
T2	0.05 acre/150 kg	Kg	5	Pilot scale
T3	10 acre/25 ton	200 kg	5	Large-scale production
Total duration			~24 months	