Supplemental data

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Liver zonation index of drug transporter and metabolizing enzyme protein expressions in mouse liver acinus.

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Supplemental Methods

Sulforhodamine101 (SR-101) uptake by mouse Oatp2b1-overexpressing HEK293 cells

HEK293 cells transiently expressing Oatp2b1 were generated by transfection of myc-DDK-tagged open reading frame of mouse Oatp2b1 inserted in the pCMV6 vector (OriGene Technologies, Rockville, MD), respectively. The transfection was performed using Lipofectamine^{TM} 2000 and Opti-MEM I medium (Invitrogen) according to the manufacturer's protocol. Briefly, the cells were seeded at 90% confluence in 24-well plates using antibiotic-free medium. The complex of the vector and lipofectamine was incubated with the cells for 6 h, and then the cells were cultured in normal culture medium for 24 hrs. For uptake study, the cells were washed with normal extracellular fluid (ECF) buffer (122 mM NaCl, 25 mM $NaHCO_3$, 3 mM KCl, 0.4 mM K_2HPO_4, 10 mM glucose, 1.4 mM $CaCl_2$, 1.2 mM $MgSO_4$ and 10 mM HEPES, pH 7.4). Uptake was initiated by applying 300 μL normal ECF buffer containing 1 μM SR101 at 37◦C. At 20 min, the solution was removed to terminate uptake, and the cells were washed in ice-cold normal ECF buffer. Images were taken with a fluorescence microscope (Fluoview, Olympus, Tokyo, Japan). The cells were then homogenized in distilled water using a sonicator. The homogenate was centrifuged at 21,600xg for 5 min at 4°C and the supernatant was collected. The cell-associated fluorescence was measured with a fluorescence detector (Fluoroscan Acent FL, Thermo Fisher Scientific, Waltham, MA). The accumulation of SR-101 in the cells was expressed as the cell-associated fluorescence per well.

Supplemental Table S1 Target peptide sequences and selected/multiple reaction monitoring (SRM/MRM) transitions for quantification of each protein

Molecule	Target peptide sequence	SRM/MRM transitions (m/z)				
		Q1	Q3-1	Q3-2	Q3-3	Q3-4
Slc21a7/Oatp1a5	SENSPLYIGILESGK	803.9	1189.683	816.4828	703.3987	533.2931
	SENSPLYIGI**L*** ESGK	807.4	1196.7	823.5	710.4159	540.3103
Slc38a4/Ata3	TSVITLLFPR	573.8	859.5402	746.4561	532.3243	419.2402
	TSVITLLF**P***R	576.9	865.554	752.4699	538.3381	425.254
Abca2	LLFGPLPDLDGK	642.9	1058.552	911.4833	757.4092	432.2454
	LLFGPLPD**L***DGK	646.4	1065.569	918.5004	764.4264	439.2624
Abca3	VFQVGNK	396.2	692.3727	545.3043	318.1773	417.2457
	VFQ**V***GNK	399.2	698.3864	551.3181	318.1773	423.2594
Abca4	WIAEPAR	421.7	656.3727	543.2886	343.2089	175.119
	WIAEP**A***R	423.7	660.3798	547.2957	347.216	175.119
Abca5	NAVVPIK	370.7	357.2498	555.3866	456.3182	626.4237
	NAVVP**I***K	374.2	364.267	562.4038	463.3354	633.4409
Abca6, 8a, 9	LFPQAAR	401.7	542.3046	445.2518	317.1932	689.373
	LFPQA**A***R	403.7	546.3117	449.2589	321.2003	693.3801
Abca7	QFQSPLR	438.2	385.2559	600.3464	472.2879	747.4149
	QFQSP**L***R	441.7	392.2731	607.3637	479.3051	754.4321
Abca8a	DLTLDVYK	483.8	738.4033	637.3556	524.2715	310.1762
	DLTLD**V***YK	486.8	744.4171	643.3694	530.2853	310.1762
Abca8b	LFPQASR	409.7	705.3679	558.2995	461.2467	333.1881
	LFPQ**A***SR	411.7	709.375	562.3066	465.2538	337.1952
Abca9	LLPQEEL	421.2	227.1757	615.2986	132.102	710.3723
	LL**P***QEEL	424.2	227.1757	621.3124	132.102	716.3861
Abca12	LLAIPIPDNR	561.3	711.3784	501.2416	824.4626	614.3257
	LLAIPI**P***DNR	564.3	717.3923	507.2554	830.4764	620.3395
Abca13	NIVWDPQK	500.3	487.2512	772.3989	673.3305	372.2243
	NIVWD**P***QK	503.3	493.265	778.4127	679.3443	378.2381
Abcb4 / mdr2	IATEAIENIR	565.3	531.2886	715.4098	644.3727	844.4524
	IATE**A***IENIR	567.3	531.2886	719.4169	644.3727	848.4595
Cyp1a2	YLPNPALK	458.3	620.3647	428.2869	542.3298	331.2341
	YLPNPAL**K***	462.3	620.3647	436.3011	550.344	339.2483
Cyp2a5	GYGVVFSSGER	579.3	781.3839	682.3155	377.1822	535.2471
	GYGVVFSSGE**R***	584.3	791.3922	692.3238	377.1822	545.2554
Cyp2c29	NISQSFTNFSK	636.8	1045.495	830.4043	743.3723	596.3039
	NISQSFTNFS**K***	640.8	1053.509	838.4185	751.3865	604.3181
Cyp2d22	GTTLITNLSSALK	659.9	833.4728	486.2926	946.5569	732.4251
	GTTLITNLSSAL**K***	663.9	841.487	486.2926	954.5711	740.4393
Ugt1a1	SLSFNSDR	463.2	725.3212	638.2892	491.2208	201.1236
	SLSFNSD**R***	468.2	735.3295	648.2975	501.2291	201.1236
Ugt1a9	SFLTGSAR	419.7	235.108	604.341	491.257	390.21
	SFLTGSA**R***	424.7	245.108	614.341	501.257	400.21
Ugt2b5	GAAVALNIR	442.8	200.103	586.367	129.066	299.171
	GAAVALNI**R***	447.8	200.103	596.367	129.066	299.171
Ugt2b36	TPATLGPNTR	514.3	544.284	657.368	758.416	487.262
	TPATLGPNT**R***	519.3	554.284	667.368	768.416	497.262
HMG-CoA reductase	LAEPSSLQYLPYR	768.9	1223.642	435.2351	839.4411	314.1713
	LAEPSSLQYLPY**R***	773.9	1233.65	445.2434	849.4494	314.1713
NADPH-CPR	FAVFGLGNK	476.8	635.3513	734.4197	488.2829	805.4568
	FAVFG**L***GNK	480.3	642.3685	741.4369	495.3001	812.474
FcRn	EQLFLEALK	545.8	573.3606	460.2766	720.4291	833.5131
	EQLFLEAL**K***	549.8	581.3748	468.2908	728.4433	841.5273
Glutamine synthetase (GS)	DIVEAHYR	501.8	774.3893	675.3209	546.2783	387.6983
	DIVEAHY**R***	506.8	784.3976	685.3292	556.2866	392.7024
Actin	AGFAGDDAPR	488.7	701.3213	630.2842	458.2358	343.2089
	AGFAGDDA**P***R	491.7	707.3351	636.298	464.2496	349.2227

Bold letters with asterisks indicate amino acid residues labeled with stable isotope (13C and 15N). Conditions of SRM/MRM analysis were optimized for high signal intensity following direct injection of peptide solution into the mass spectrometer through a turbo ion spray source. Theoretical m/z values of doubly charged ions of intact peptides (Q1) were assumed for precursor ions. Four singly charged fragment ions (Q3) were derived from each precursor ion.

Supplemental Table S2 Molecules under the limit of quantification

Alias	Limit of quantification (fmol/mm^2)	
	SR101(+)	SR101(-)
Slc transporter		
Slc2a1/Glut1	0.287	0.309
Slc6a2/Net	1.11	1.10
Slc7a5/Lat1	0.565	0.551
Slc16a7/Mct2	0.860	0.872
Slc21a2/Oatp2a1 (Pgt)	1.94	1.48
Slc21a5/Oatp1a4	3.00	4.11
Slc21a7/Oatp1a5	2.41	2.29
Slc21a9/Oatp2b1	1.29	1.24
Slc21a11/Oatp3a1	1.43	1.36
Slc21a12/Oatp4a1	0.635	0.604
Slc21a13/Oatp1a6	2.21	2.08
Slc21a14/Oatp1c1	0.685	0.649
Slc21a17/Oatp17	1.03	1.15
Slc21a18/Oatp18	2.30	2.23
Slc38a4/Ata3	0.375	0.402
Slc47a1/Mate1	1.18	1.14
Abc transporter		
Abca1	0.448	0.427
Abca2	1.15	1.14
Abca3	0.332	0.329
Abca5	0.315	0.316
Abca6, 8a, 9	0.839	0.868
Abca7	0.323	0.298
Abca8a	1.47	1.40
Abca8b	0.445	0.461
Abca9	0.487	0.493
Abca12	0.834	0.853
Abca13	0.547	0.529
Abcb1a/Mdr1a	0.425	0.406
Abcb1b/Mdr1b	0.566	0.544
Abcc3/Mrp3	0.677	0.651
Abcc5/Mrp5	0.220	0.225
Abcc12/Mrp9	0.503	0.464
Abcg8	0.257	0.264
Enzyme		
Cyp1a2	32.3	32.1
Cyp2a5	0.399	0.385
Ugt2b5	1.93	1.82
HMG-CoA reductase	0.620	0.594

Supplemental Table S3 Summery of the zonated expressions of transporters and metabolizing enzymes in peri-portal vein (PP) or peri-central vein (PC) regions of the liver in rodents

Molecule		Species	Method	mRNA Protein	Distribution in PP and PC regions	Reference
Slc transporter						
Slc10a1	Ntcp	Male Sprague-Dawley Rat	Quantitative PCR/Isolated hepatocytes by ante- or retrograde perfusion with digitonin Immunohistochemistry	mRNA Protein	PP=PC PP=PC	(Baier et al., 2006)
		Male Sprague-Dawley Rat	Immunohistochemistry	Protein	PP=PC	(Donner et al., 2007)
Slc21a1	Oatp1a1	Male Sprague-Dawley Rat	Immunoblot/Isolated hepatocytes enriched by digitonin/collagenase perfusion	Protein	PP=PC	(Abu-Zahra et al., 2000)
		Male Sprague-Dawley Rat	Quantitative PCR/ Isolated hepatocytes by ante- or retrograde perfusion with digitonin Immunohistochemistry	mRNA Protein	PP=PC *mRNA expression tends to be greater in PC region although there is no statistical significance. PP=PC *Protein expression is excluded in the midzonal area.	(Baier et al., 2006)
		Male Sprague-Dawley Rat	Immunohistochemistry	Protein	PP=PC	(Donner et al., 2007)
Slc21a10	Oatp1b2	Male Sprague-Dawley Rat	Immunohistochemistry	Protein	PP<PC	(Donner et al., 2007)
Slc22a1	Oct1	Rat	Immunohistochemistry	Protein	PP<PC	(Meyer-Wentrup et al., 1998)
Slc22a7	Oat2	Rat	Quantitative PCR/Laser capture microdissection	mRNA	PP=PC	(Fork et al., 2011)
Abc transporter						
Abcb11	Bsep	Male Sprague-	Quantitative PCR/Isolated hepatocytes by ante- or	mRNA	PP<PC	(Baier et al., 2006)

		Dawley Rat	retrograde perfusion with digitonin Immunohistochemistry	Protein	PP<PC * Predominant pericentral cytoplasmatic staining.	
		Male Sprague-Dawley Rat	Immunohistochemistry	Protein	PP=PC	(Donner et al., 2007)
Abcc2	Mrp2	Male Wistar Rat	Immunohistochemistry	Protein	PP>PC	(Micuda et al., 2008)
		Male Sprague-Dawley Rat	Quantitative PCR/ Isolated hepatocytes by ante- or retrograde perfusion with digitonin Immunohistochemistry	mRNA Protein	PP=PC PP=PC	(Baier et al., 2006)
Abcc4	Mrp4	Male Sprague-Dawley Rat	Immunohistochemistry	Protein	PP=PC	(Donner et al., 2007)
Enzyme						
Cyp2c29	Cyp2c29	Male C3H/He Mouse	Microarray/Isolated hepatocytes enriched by digitonin/collagenase perfusion	mRNA	PP<PC	(Braeuning et al., 2006)
Cyp2e1	Cyp2e1	Male C3H/He Mouse Male Alko mixed strain Rat	Quantitative PCR/Isolated hepatocytes enriched by digitonin/collagenase perfusion Immunohistochemistry	mRNA Protein	PP<PC PP<PC	(Braeuning et al., 2006) (Buhler et al., 1992)
Other						
Glul	GS	Mouse C3H/He Mouse	Microarray and Immunoblot/Isolated hepatocytes enriched by digitonin/collagenase perfusion	mRNA Protein	PP<PC PP<PC	(Braeuning et al., 2006)

Protein or mRNA expressions of transporters and metabolizing enzymes in liver PP or PC regions are summarized according to the previous reports. PP=PC: Even/Homogenous distribution in PP and PC regions, PP>PC: PP region-predominant distribution, PP<PC: PC region-predominant distribution

References

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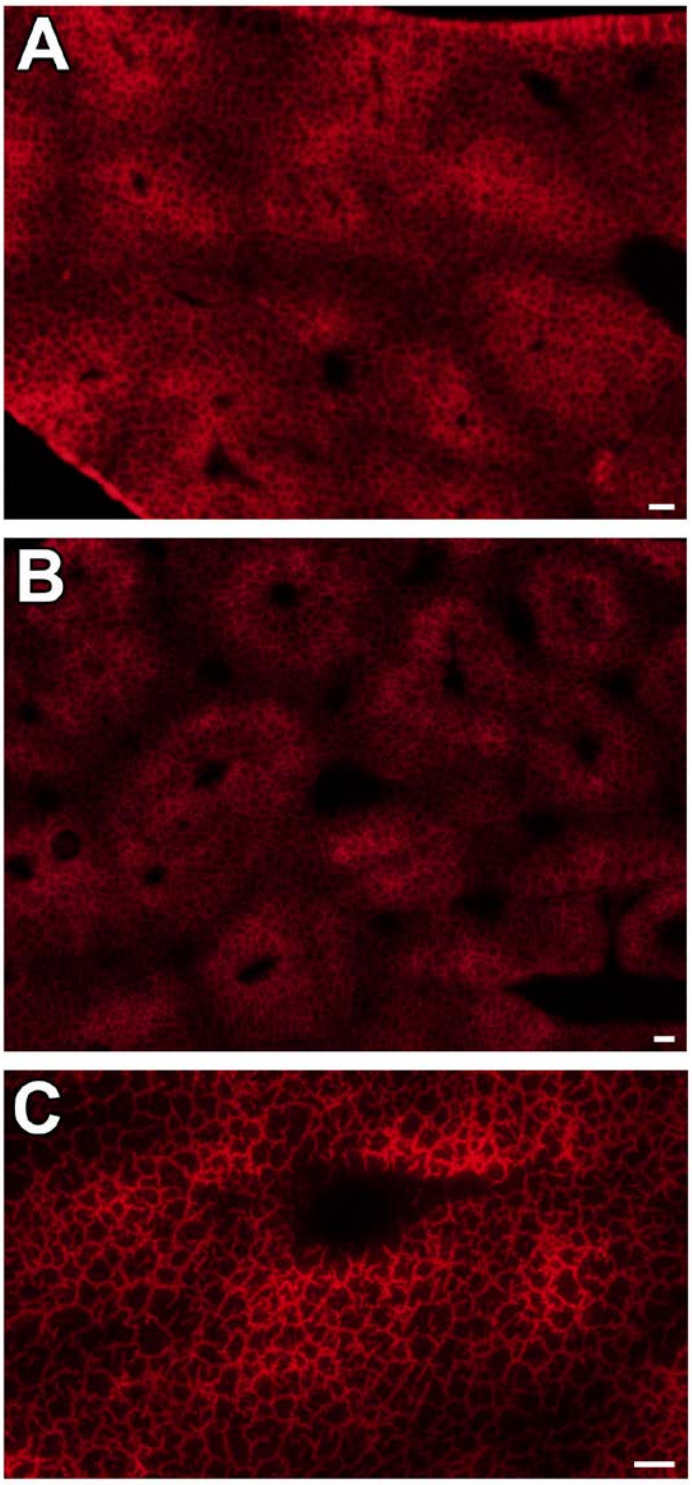
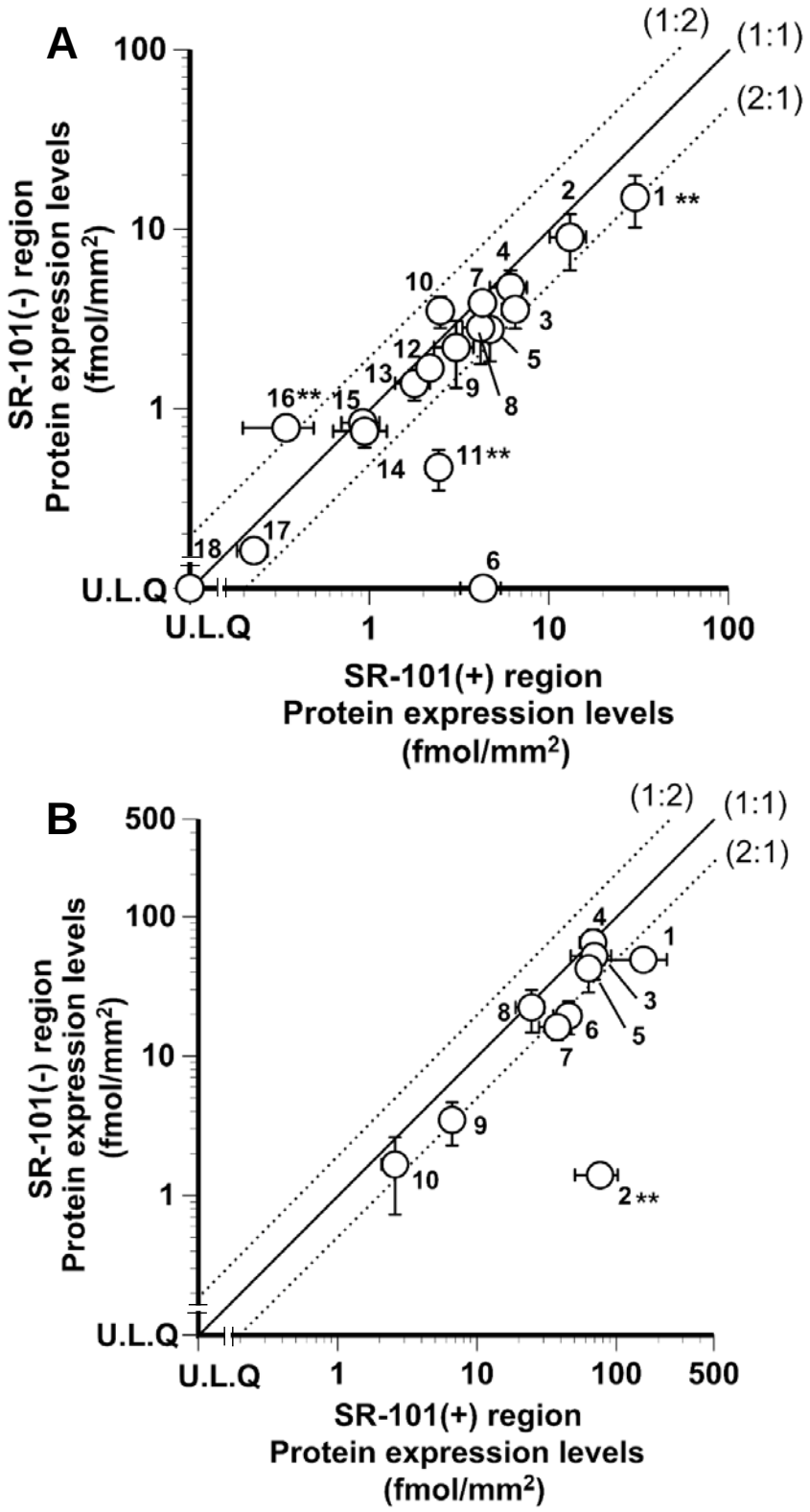
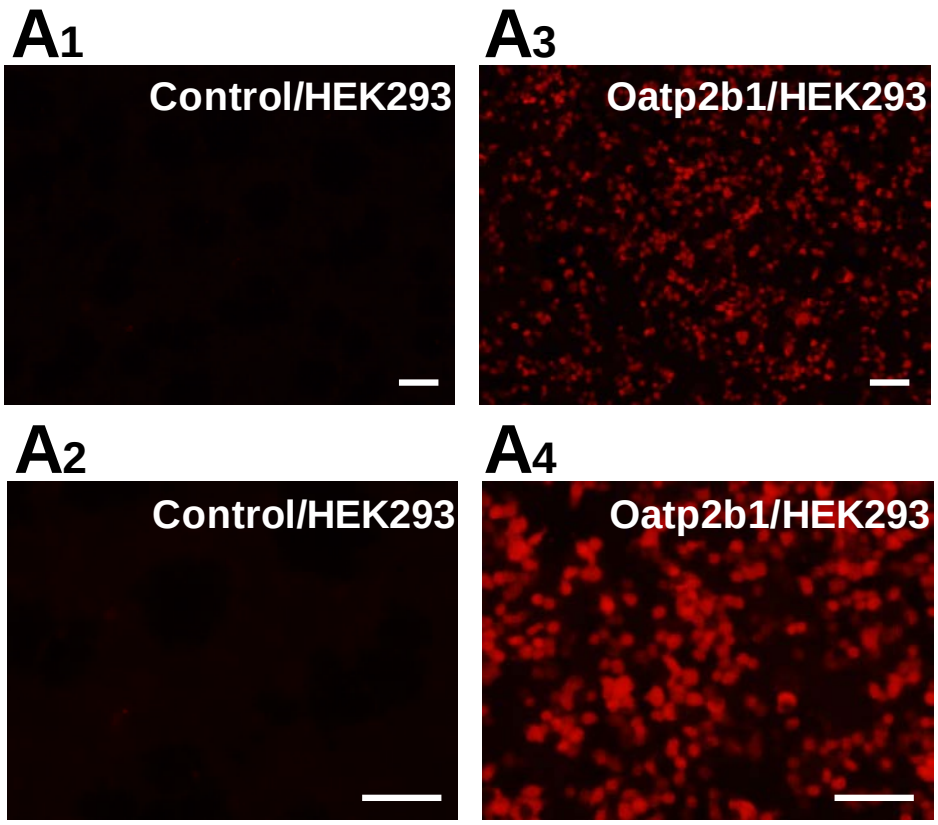
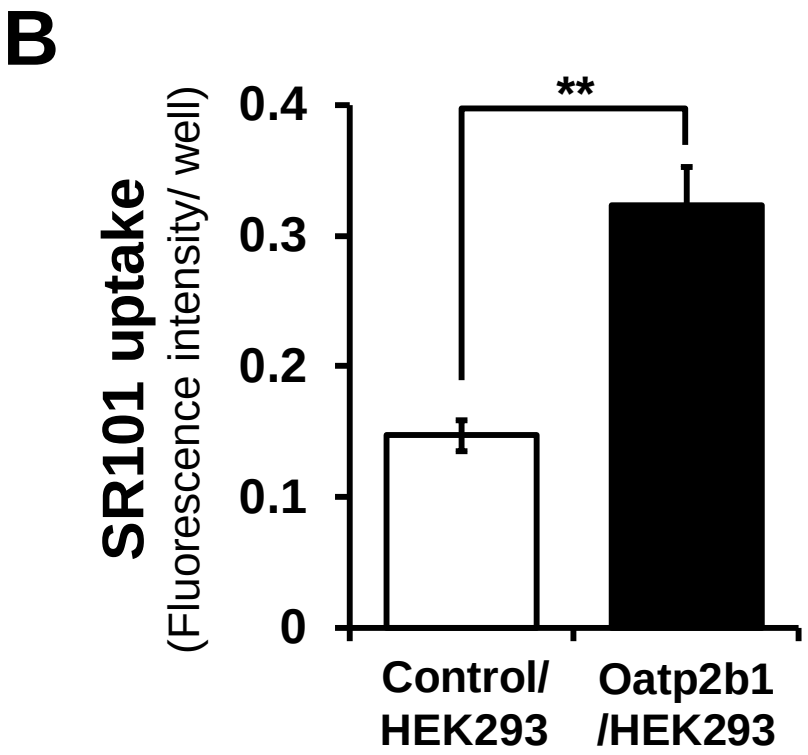


Supplemental Figure S1 Uneven distribution of SR-101 in the liver acinus of mice at 30 min (A) or 90 min (B and C) after intravenous injection of SR-101. There are distinct regions strongly positive for SR-101 and either negative or weakly positive for SR-101, respectively. Scale bar: 50 μm.

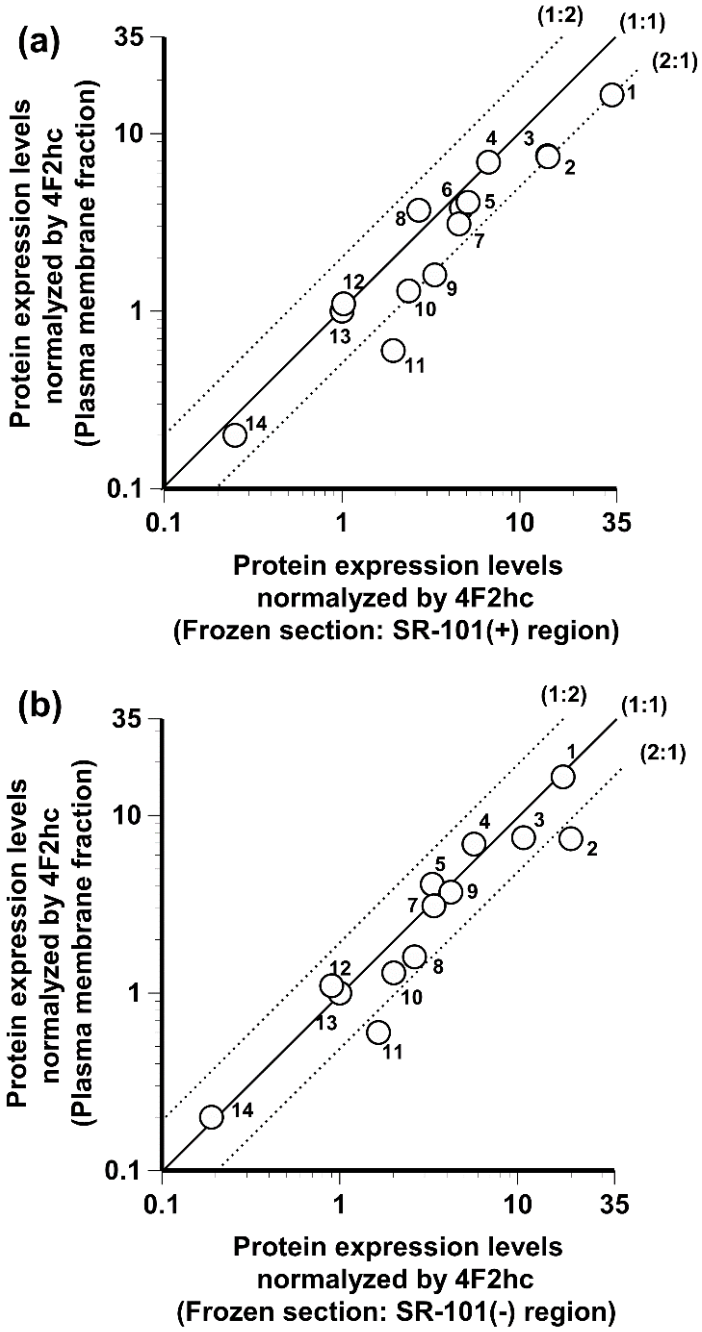


Supplemental Figure S2 A comparison of protein expression levels of transporters/receptors (A) and metabolizing enzymes (B) in the SR-101(+) and SR-101(-) liver regions of mice intravenously injected with SR-101. The solid line passing through the origin represents the line of identity, and the broken lines represent 2-fold differences. A; 1. Oatp1a1, 2. Mct1, 3. Slc22a18, 4. Ntcp, 5. Ent1, 6. Oatp1b2, 7. FcRn, 8. Bcrp, 9. Mrp6, 10. Bsep, 11. Oct1, 12. Mrp2, 13. Abcb4, 14. Abcg5, 15. 4F2hc, 16. Oat2, 17. Mrp4, 18. Oatp1a4. B; 1. Cyp2c29, 2. Cyp2e1, 3. Ugt1a9, 4. Cyp3a11, 5. Ugt2b3, 6. Ugt1a1, 7. NADPH-CPR, 8. Cyp2d22, 9. Cyp51a1, 10. Cyp8b1. Each point represents the mean±S.D. (n=3) in three independent analyses. U.L.Q; under the limit of quantification. **$p<0.01$, significantly different between the SR-101(+) and SR-101(-) regions. The individual values are shown in Table 1.





Supplemental Figure S3 Mouse Oatp2b1 mediates SR-101 uptake. (A) Representative fluorescence images of SR-101 in control HEK293 cells (Control/HEK293; A1 and A2) and mouse Oatp2b1-overexpressing HEK293 cells (Oatp2b1/HEK293; A3 and A4). Scale bars: 100 μm. (B) Comparison of the SR-101 uptake amounts in Control/HEK293 cells (open column) and Oatp2b1/HEK293 cells (closed column). Each column represents the mean±S.E.M (n=4). **p<0.01, significantly different between Oatp2b1/HEK293 and Control/HEK293 cells.



Supplemental Figure S4 A comparison of protein expression levels of transporters, normalized by the level of 4F2hc, in the plasma membrane fractions of total mouse liver and laser-microdissected samples from frozen mouse liver sections. The data on plasma membranes are taken from the previous report (Miura et al., 2017). The data on the frozen liver sections are taken from Table 1. Each point represents the mean value of each transporter protein level divided by that of 4F2hc. The solid line passing through the origin represents the line of identity, and the broken lines represent 2-fold differences. The numbers near the circles identify the transporter: 1 Oatp1a1, 2 Na^+, K^+- ATPase, 3 Mct1, 4 Ntcp, 5 Ent1, 6 Oatp1b2, 7 Bcrp, 8 Mrp6, 9 Bsep, 10 Mrp2, 11 Abcb4, 12 Abcg5, 13 4F2hc, 14 Mrp4.

Reference

Miura T, Tachikawa M, Ohtsuka H, Fukase K, Nakayama S, Sakata N, Motoi F, Naitoh T, Katayose Y, Uchida Y, Ohtsuki S, Terasaki T, Unno M (2017) Application of quantitative targeted absolute proteomics to profile protein expression changes of hepatic transporters and metabolizing enzymes during cholic acid-promoted liver regeneration. *J Pharm Sci* **106:**2499-2508.