Supplementary Materials

Recombinant Relaxin Protects Liver Transplants from Ischemia Damage via Hepatocyte Glucocorticoid Receptor: From Bench-to-Bedside

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Fig. S1

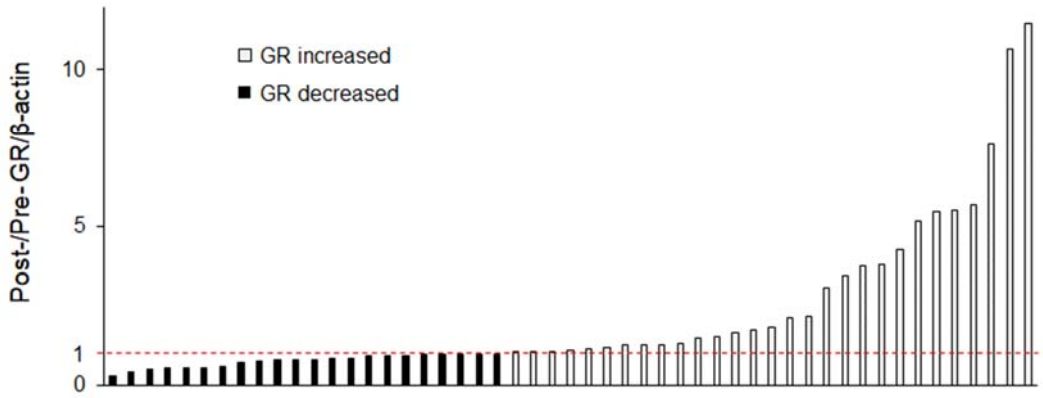

Fig. S1: Peri-transplant GR expression in human OLT.

Pre-transplant and post-transplant liver biopsy (Bx) samples were collected from fifty-one OLTs. Bx samples were analyzed by Western blots with β-actin normalization and post-/pre-transplant GR ratio was calculated.

Fig. S2

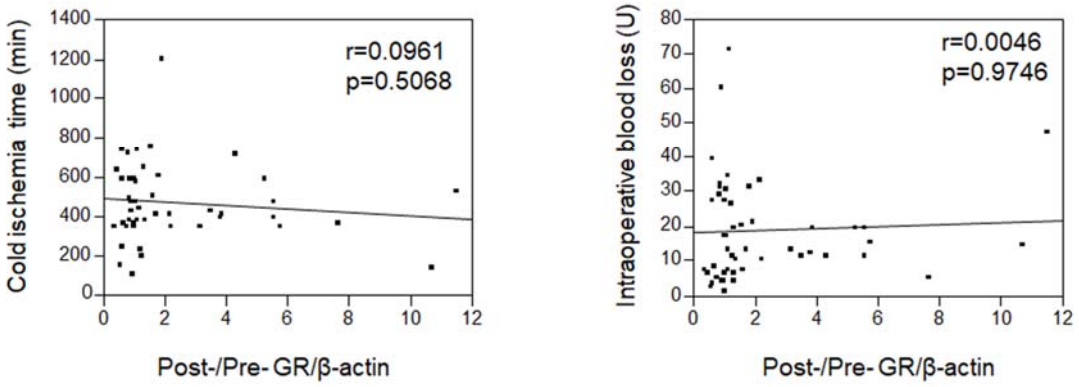

Figure S2: Correlation between peri-transplant GR increase in human OLT and cold ischemia time/intraoperative blood loss.

Post-/pre-transplant GR ratio was calculated from fifty-one clinical OLT cases and relationship with cold ischemia time and intraoperative blood loss was analyzed. r: Spearman's correlation coefficient.

Fig. S3

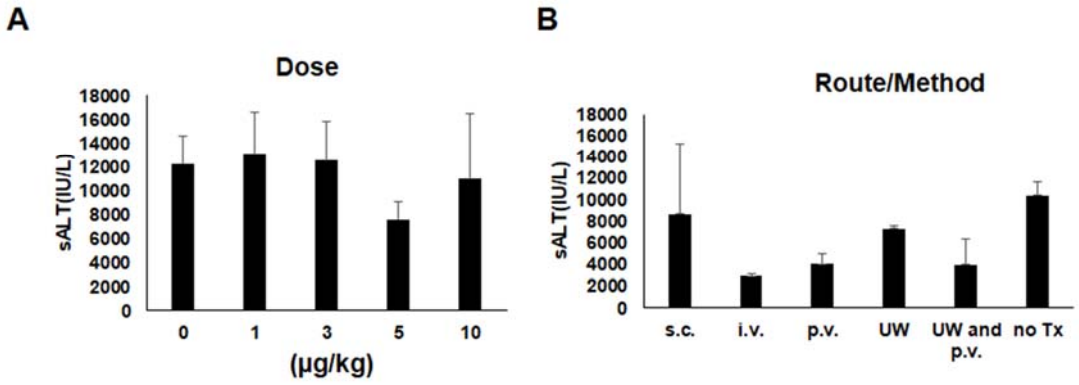

Figure S3: rhRLX dose-response and route of administration in a mouse IRI-OLT model.

Donor mouse (C57/Bl6) livers subjected to 18h of cold storage were transplanted to recipient mice. Serum ALT levels were analyzed at 6h of reperfusion. Groups of OLT recipients were treated with (**A**) increasing rhRLX doses (0 - 10µg/kg, iv) at time of reperfusion; (**B**) optimal rhRLX dose (5µg/kg) by different route (s.c., subcutaneous; i.v., intravenous; p.v., portal vein; UW, rhRLX supplement in UW during cold storage; no Tx, no treatment). Mean±SD are shown (n=2/group).

Fig. S4

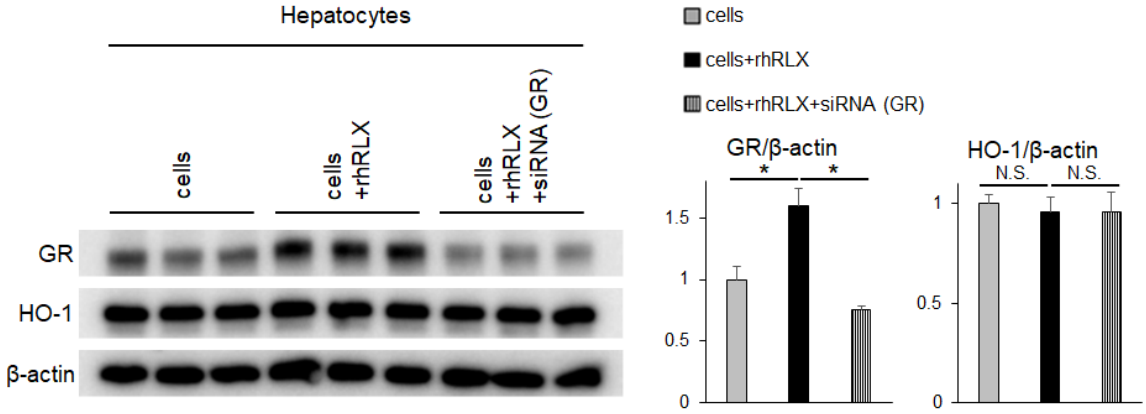

Figure S4: Neither rhRLX treatment nor GR knockdown by siRNA affects HO-1 expression in cultured hepatocytes.

Primary mouse hepatocytes were treated with or without rhRLX (1µg/ml; 24h) and siRNA against GR. Western blot-assisted detection and relative intensity ratio of GR and HO-1. β-actin expression served as an internal control and used for normalization (n=3/group). Data are shown as means±SD. *p<0.05, N.S.: not significant (Student t-test).

Fig. S5

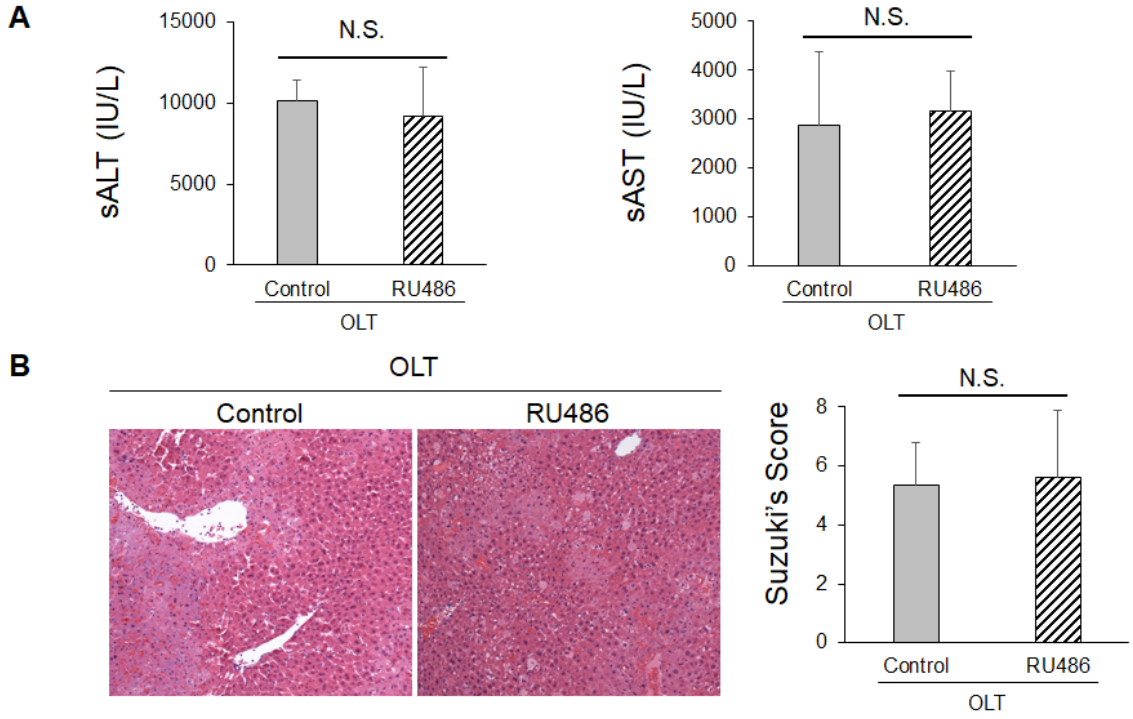

Figure S5: RU486 pretreatment alone does not affect IRI severity in mouse OLT.

Mouse livers were transplanted to syngeneic recipients with or without pre-incubation with UW containing GR antagonist (RU-486, 500nM) for 18h during cold storage. (A) Serum ALT and AST levels (IU/L, n=4-5/group). (B) Representative hematoxylin and eosin staining (original magnification, x100) and Suzuki's histological IRI grading (n=4-5/group). Data shown as mean±SD. N.S.: not significant (Student t-test).

Table S1: Primer Sequences Used for Real-Time Quantitative PCR

Gene	Forward	Reverse
TLR4	5'-GGACTCTGATCATGGCACTG-3'	5'-CTGATCCATGCATTGGTAGGT-3'
RAGE	5'-GGACCCTTAGCTGGCACTTAGA-3'	5'-GAGTCCCGTCTCAGGGTGTCT-3'
IL1β	5'-TGTAATGAAAGACGGCACACC-3'	5'-TCTTCTTTGGGTATTGCTTGG-3'
CCL2	5'-CATCCACGTGTTGGCTCA-3'	5'-GATCATCTTGCTGGTGAATGAGT-3'
CXCL10	5'-GCTGCCGTCATTTTCTGC-3'	5'-TCTCACTGGCCCGTCATC-3'
TNFα	5'-GCCTCTTCTCATTCCTGCTTGT-3'	5'-GATGATCTGAGTGTGAGGGTCTG-3'
CXCL1	5'-ACCCAAACCGAAGTCATAG-3'	5'-TTGTATAGTGTTGTCAGAAGC-3'
CXCL2	5'-ACTTCAAGAACATCCAGAG-3'	5'-CTTTCCAGGTCAGTTAGC-3'
HPRT	5'-TCAACGGGGGACATAAAAGT-3'	5'-TGCATTGTTTTACCAGTGTCAA-3'

Table S2

Donor	ΔGR-low (n=26)	ΔGR-high (n=25)	p value
Age (years)	37.5 (13-67)	47 (19-74)	p=0.20
Gender (M/F)	11/15	11/13	p=0.57
Weight (kg)	70.5 (47.2-106.0)	81.9 (52.3-127)	p=0.16
BMI (kg/m^2)	25.4 (19.6-34.9)	27.0 (19.7-42.6)	p=0.06
Pre-transplant ALT (IU/L)	26.5 (11-403)	22.5 (8-887)	p=0.07
Pre-transplant AST (IU/L)	44.5 (14-360)	26 (10-749)	p=0.36
Cold Ischemic Time (min)	465 (120-750)	420 (150-1213)	p=0.94
Donation status	All of the donors were donation after brain death		

Recipient	ΔGR-low (n=26)	ΔGR-high (n=25)	p value
Age (years)	59 (23-73)	59 (30-73)	p=0.76
Gender (M/F)	20/6	17/8	p=0.54
Weight (kg)	83.5 (51.3-151.6)	79.6 (36.0-146.1)	p=0.27
BMI (kg/m^2)	28.6 (17.8-44.3)	25.6 (14.5-47.5)	p=0.41
Disease ethiology			p=1.00
Alchol	4	5	
HCV	10	10	
NASH	3	2	
other	9	8	
HCC (with/without)	10/16	10/15	p=1.00
ABO			p=0.11
identical	22	25	
compatible	4	0	
MELD score	30.5 (9-44)	33 (17-42)	p=0.19
Intraoperative blood loss (U)	18 (2-72)	14 (5-48)	p=0.98
Pre-transplant ALT (IU/L)	52.5 (9-6168)	36 (11-617)	p=0.54
Pre-transplant AST (IU/L)	63 (22-6967)	74 (25-283)	p=0.70
Pre-operative hospital stay (days)	1 (0-33)	9 (0-78)	p=0.11
Pre-operative ICU stay (days)	0 (0-32)	3 (0-54)	p=0.14

Table S2. Donor and recipient demographics.

Pre- and post- transplant liver biopsy samples were collected from fifty-one human liver transplantation cases and analyzed by Western-blots. Peri-operative GR increase was determined by calculating post-/pre- GR expression (ΔGR), and then 51 cases were divided into ΔGR-low (<1.1, n=26) or ΔGR-high (>1.1, n=25) groups. Correlations between donor and recipient demographic parameters and peri-transplant ΔGR were analyzed using Fisher's exact test for categorical variables and Mann–Whitney U test for continuous values. Values are expressed as median (range).