Supplementary Information

FGF21 modulates immunometabolic homeostasis via the ALOX15/15-HETE axis in early liver graft injury

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Supplementary figures:

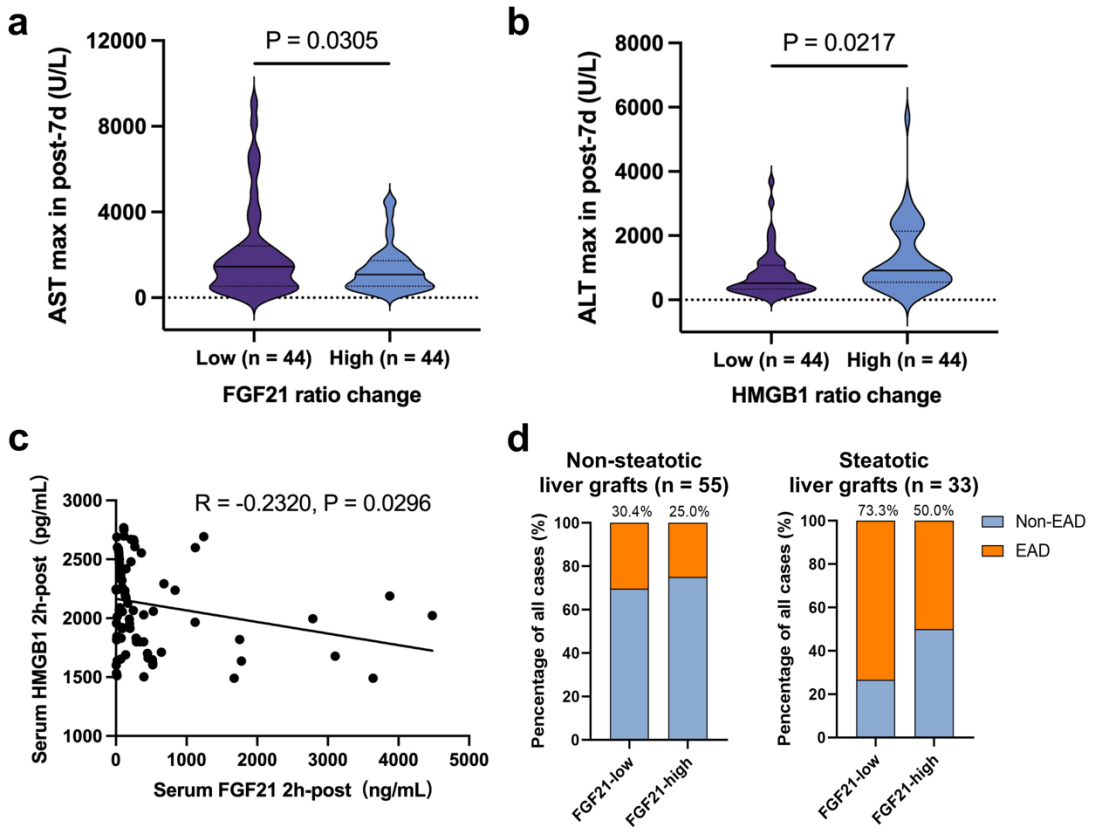

Figure S1. The association of FGF21 levels with short-term clinical outcomes in patients after liver transplantation.

(a) The association between the ratio change of peripheral FGF21 level and maximal AST within 7 days after transplantation. **(b)** The 88 patients were divided into the HMGB1-elevated group (n = 44) and non-elevated group (n = 44) according to the median value of ratio change (post-reperfusion/pre-transplant). The association between the ratio change of peripheral HMGB1 level and maximal ALT within 7 days after transplantation. **(c)** The correlation analysis between the peripheral FGF21 level and HMGB1 level 2 hours after reperfusion (n = 88). **(d)** Comparisons of EAD percentages for non-steatotic or steatotic liver grafts. Two-tailed t-test. Statistic data are presented as the mean ± SD, error bars represent the means of at least three independent experiments. *p* values are shown on the graphs, $p < 0.05$ was considered statistically significant, source data are provided as a Source Data file.

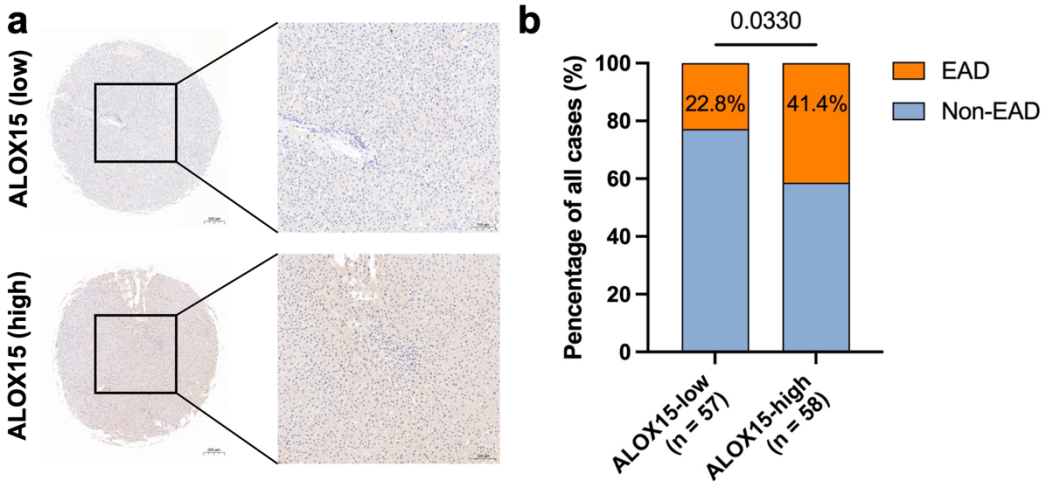

Figure S2. The association of ALOX15 levels in pre-transplant liver graft biopsies with short-term clinical outcomes in patients after liver transplantation.

(a) IHC staining for ALOX15 in pre-transplant liver graft biopsies from Cohort 2 (n = 115). (b) Comparison of the incidence of EAD in recipients between the low ALOX15 group and the high ALOX15 group. Categorical variables were compared using two-sided Pearson's chi-square test. *p* values are shown on the graphs, $p < 0.05$ was considered statistically significant, source data are provided as a Source Data file.

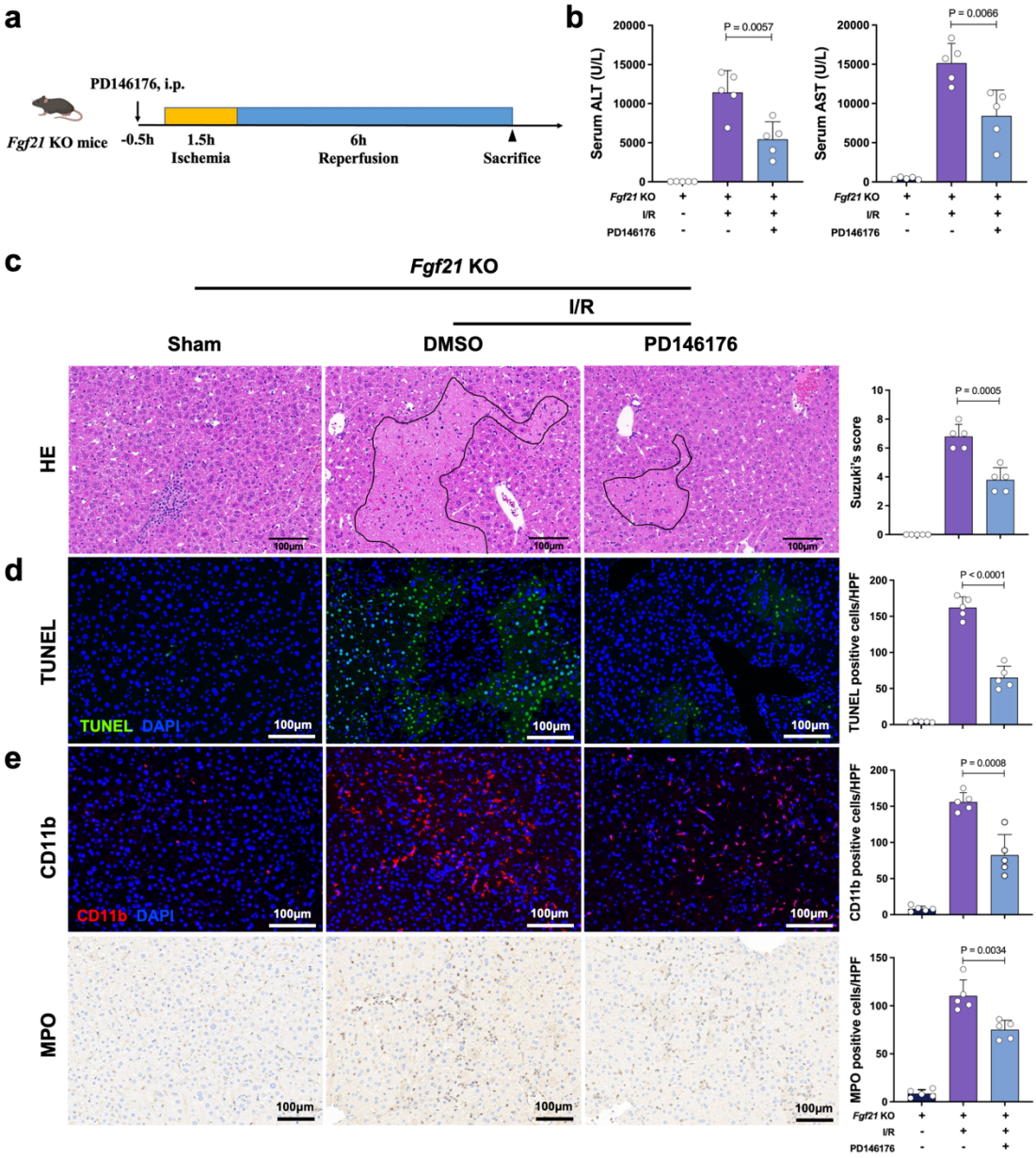

Figure S3. A repression of ALOX15 alleviated I/R injury in in *Fgf21* KO mice.

(a) The experimental schema for establishing the mouse I/R injury model. The *Fgf21* KO mice were intravenously administered PD146176 (10 mg/kg), n = 5 mice/group. **(b)** Serum ALT and AST levels for each group 6 hours after reperfusion (n = 5, per group). **(c-e)** Representative H&E staining, TUNEL staining, CD11b and MPO IHC staining of liver sections from each group and quantification assessment (n = 5, per group). Two-tailed t-test. Statistic data are presented as the mean ± SD, error bars represent the means of at least three independent experiments. *p* values are shown on the graphs, $p < 0.05$ was considered statistically significant. Source data are provided as a Source Data file. Figure S4a created with BioRender. com

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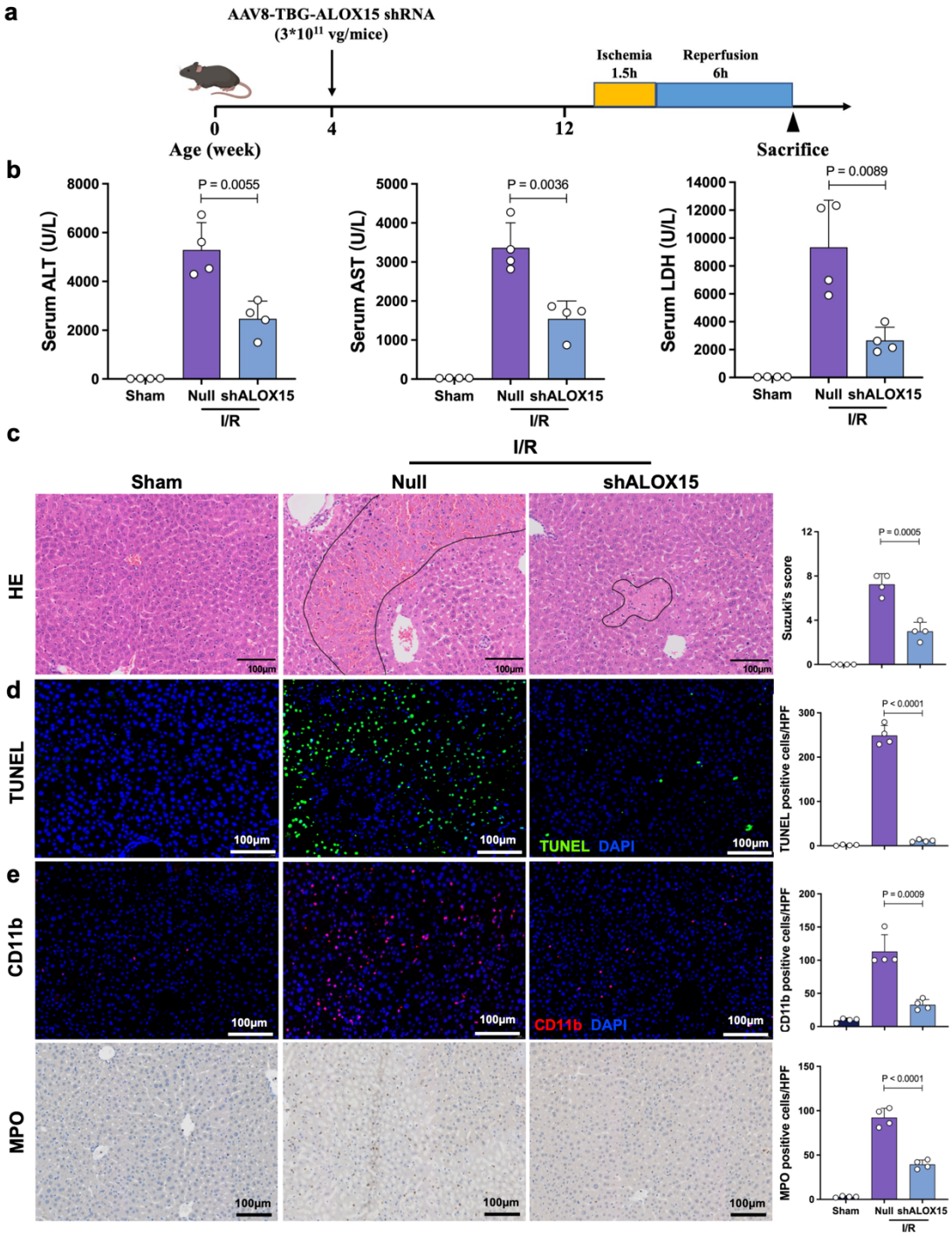

Figure S4. The shRNA-mediated knockdown of *Alox15* alleviated I/R-induced hepatocellular injury.

(a) Experimental schema for establishing mouse I/R injury model pre-treated with shAlox15. **(b)** Serum ALT and AST levels of sham, I/R with and without shAlox15 groups 6 h following reperfusion (n = 4, per group). **(c-e)** representative H&E staining, TUNEL staining, CD11b and MPO IHC staining of liver sections 6 h following reperfusion (n = 4, per group). Two-tailed t-

test. Statistic data are presented as the mean ± SD, error bars represent the means of at least three independent experiments. p values are shown on the graphs, $p < 0.05$ was considered statistically significant. Source data are provided as a Source Data file. Figure S5a created with BioRender. com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license (https://creativecommons.org/licenses/by-nc-nd/4.0/deed.en).

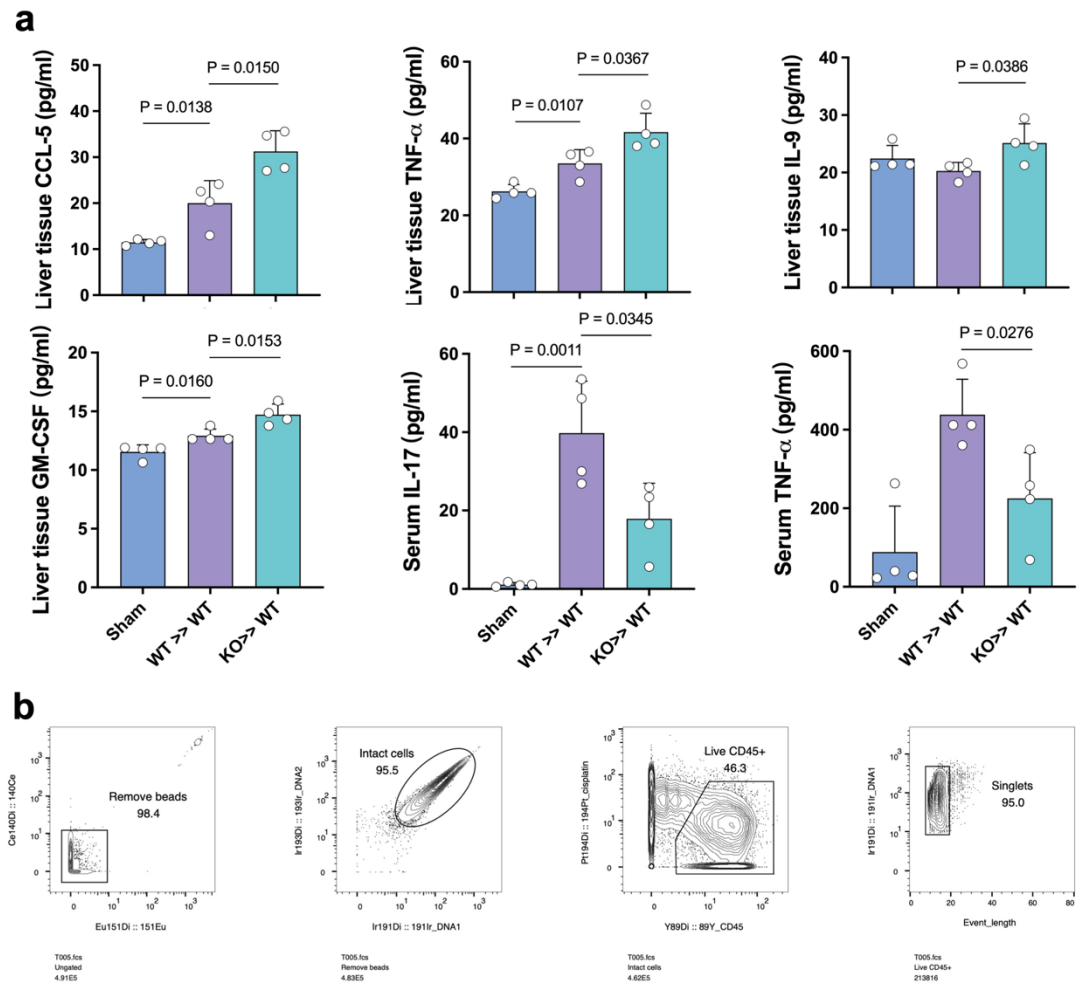

Figure S5. Cytokines and immune cells in mouse liver tissues after transplantation.

(a) Comparative analysis of multiplex cytokines in mouse serum after liver transplantation (n = 4, per group). **(b)** Gating strategy for immune cells analyzed with mass cytometry. Two-tailed t-test. Statistic data are presented as the mean ± SD, error bars represent the means of at least three independent experiments. *p* values are shown on the graphs, $p < 0.05$ was considered statistically significant. Source data are provided as a Source Data file.

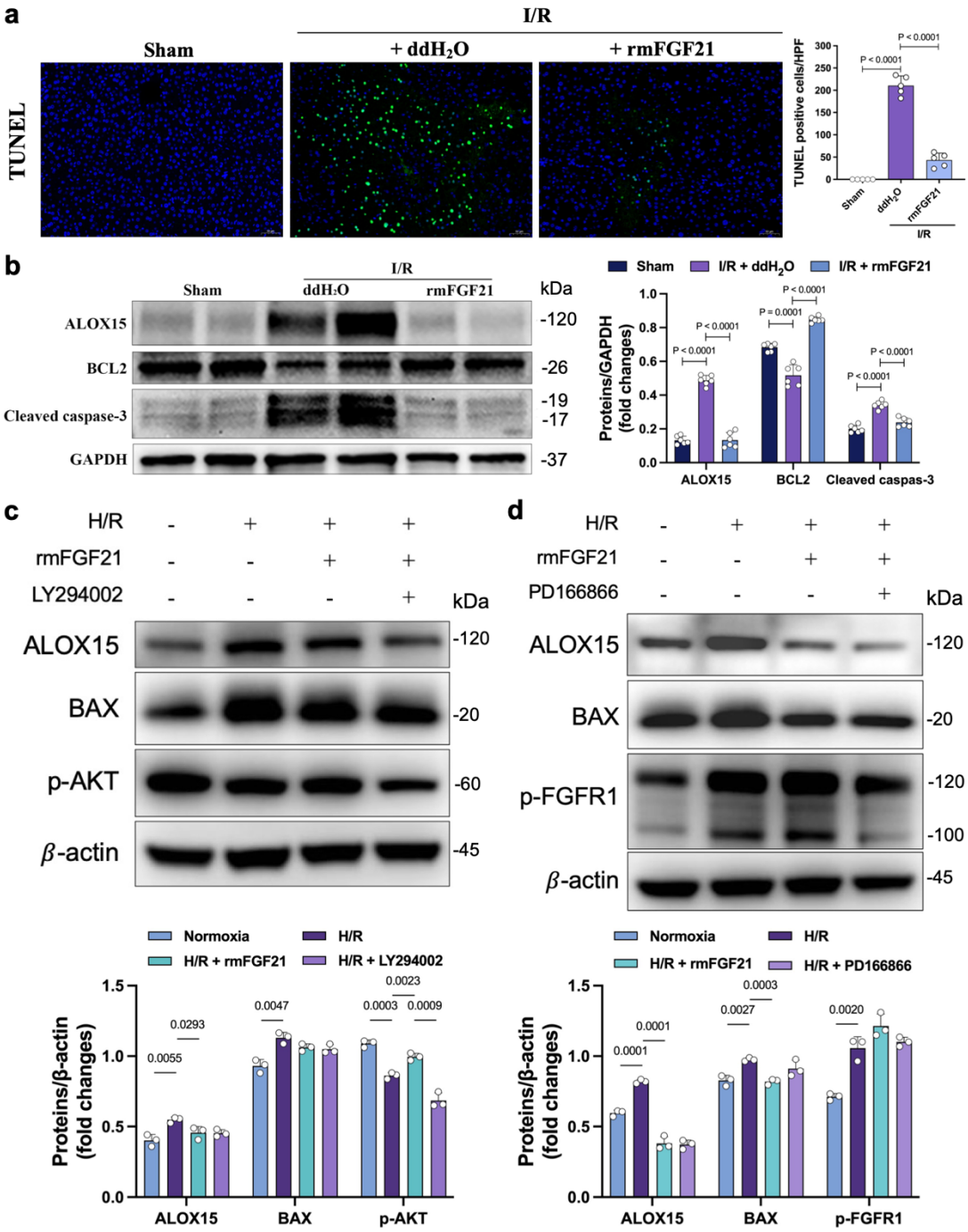

Figure S6. rmFGF21 treatment protected hepatocytes against I/R-insult.

(a) TUNEL immunofluorescent staining 6 hours after reperfusion (n = 5, per group). **(b)** Western blot analysis (left) and quantification (right) of ALOX15, BCL-2 and Cleaved caspase-3 in livers from each group. **(c)** Western blot analysis (up) and quantification (down) of ALOX15, BAX and p-AKT in AML12 from each group. **(d)** Western blot analysis (up) and quantification (down) of ALOX15, BAX and p-FGFR1 in AML12 from each group. **b-d**, three

independent biological mice samples. Two-tailed t-test. Statistic data are presented as the mean ± SD, error bars represent the means of at least three independent experiments. *p* values are shown on the graphs, $p < 0.05$ was considered statistically significant. Source data are provided as a Source Data file.

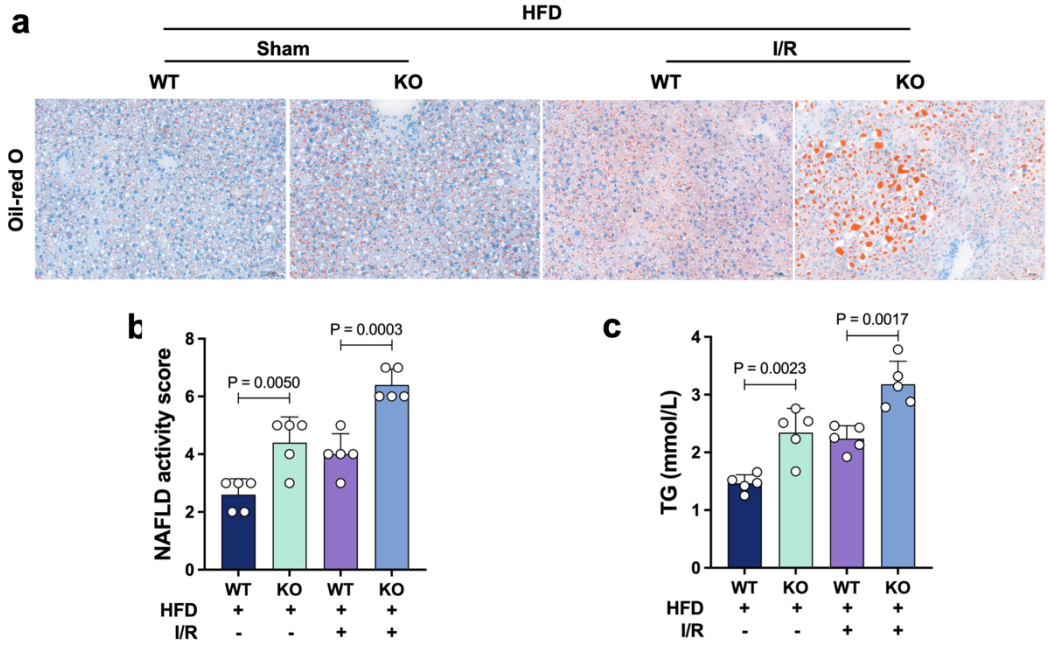

Figure S7. FGF21 deficiency deteriorated hepatic steatosis after I/R treatment.

(a) Oil red O staining of liver 6 h following reperfusion. **(b)** NAFLD activity score was evaluated 6h after reperfusion (n = 5, per group). **(c)** Serum TG 6 h following reperfusion were determined (n = 5, per group). Two-tailed t-test. Statistic data are presented as the mean ± SD, error bars represent the means of at least three independent experiments. *p* values are shown on the graphs, $p < 0.05$ was considered statistically significant. Source data are provided as a Source Data file.

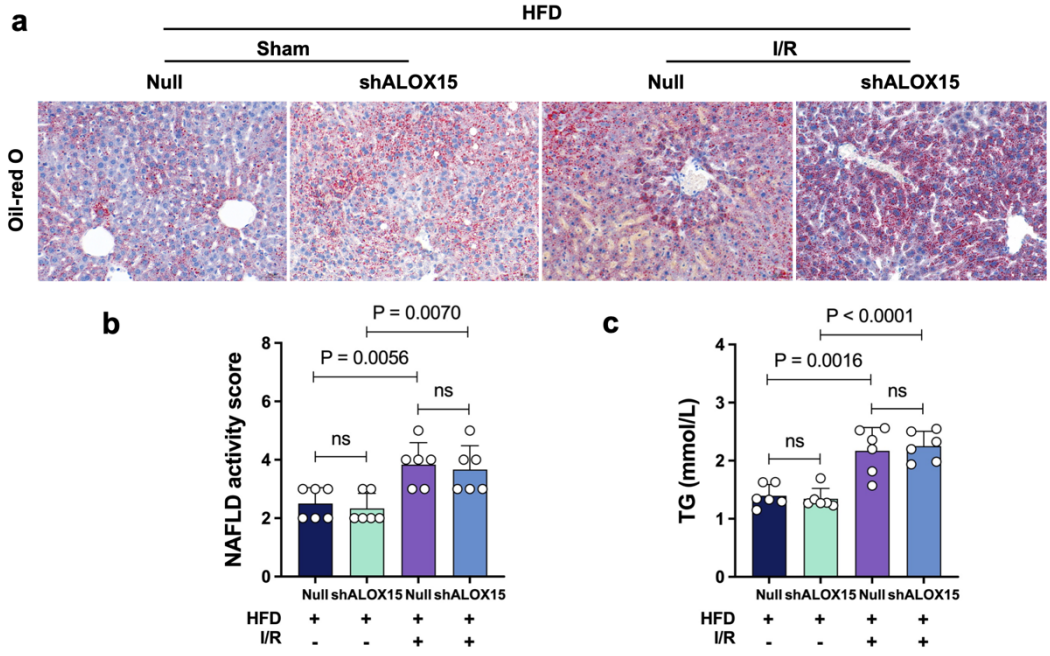

Figure S8. Transgenic overexpression of hepatic *Alox15* attenuated hepatic steatosis after I/R treatment.

(a) Oil red O staining of liver 6 h following reperfusion. **(b)** NAFLD activity score was evaluated 6 h after reperfusion (n = 6, per group). **(c)** Serum TG 6 h following reperfusion were determined (n = 6, per group). Two-tailed t-test. Statistic data are presented as the mean ± SD, error bars represent the means of at least three independent experiments. *p* values are shown on the graphs, $p < 0.05$ was considered statistically significant. Source data are provided as a Source Data file.

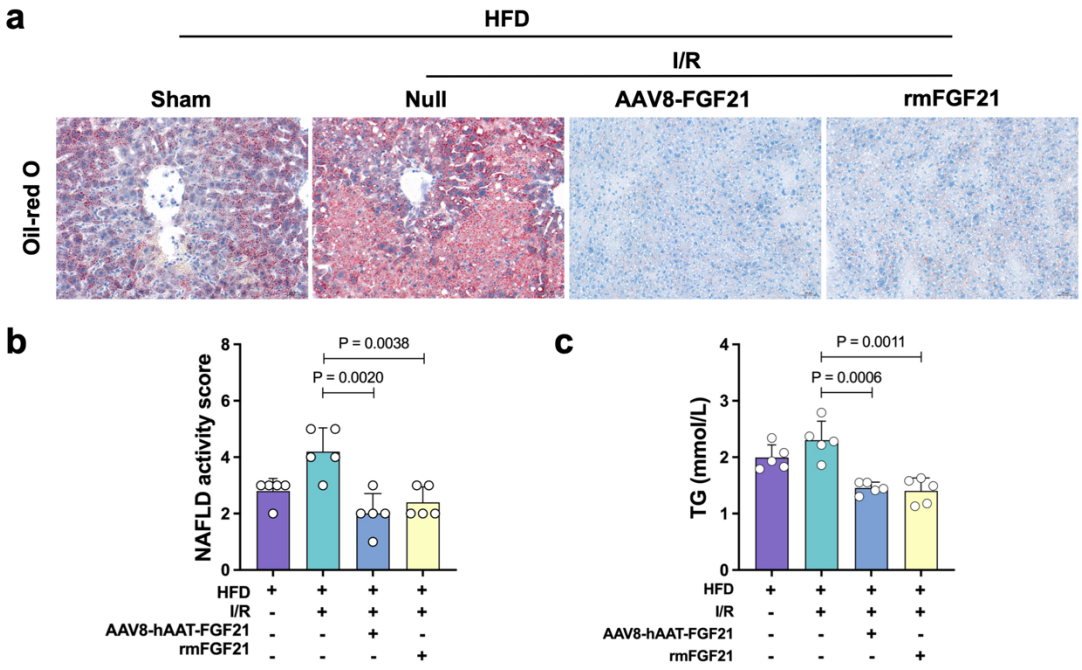

Figure S9. Transgenic overexpression of hepatic *Fgf21* or infusion with rmFGF21 attenuated hepatic steatosis after I/R treatment.

(a) Oil red O staining of liver 6 h following reperfusion. **(b)** NAFLD activity score was evaluated 6 h after reperfusion (n = 5, per group). **(c)** Serum TG 6 h following reperfusion were determined (n = 5, per group). Two-tailed t-test. Statistic data are presented as the mean ± SD, error bars represent the means of at least three independent experiments. *p* values are shown on the graphs, $p < 0.05$ was considered statistically significant. Source data are provided as a Source Data file.

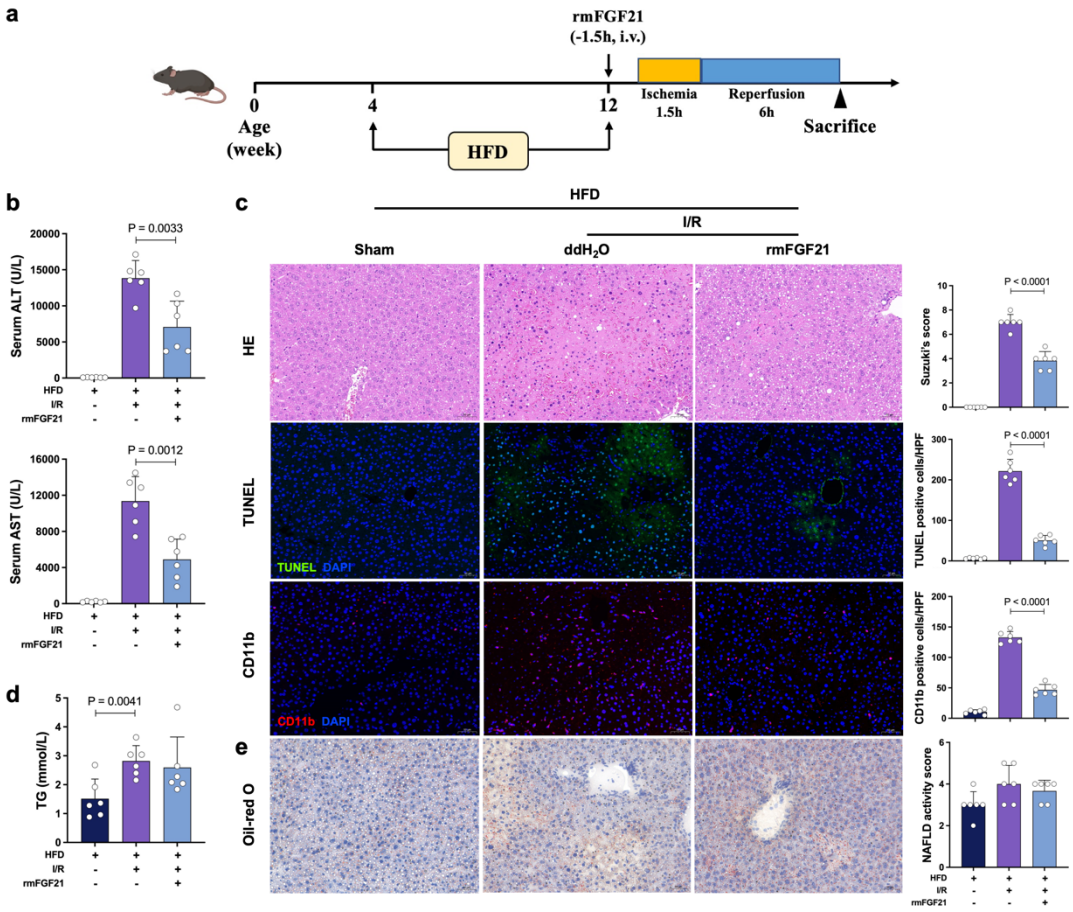

Figure S10. Treatment with rmFGF21 alleviated I/R-induced hepatocellular injury in HFD livers.

(a) Experimental schema for establishing the I/R injury mouse model. The HFD-fed mice were intravenously administered rmFGF21 (0.5 mg/kg). **(b)** Serum ALT and AST levels 6 hours after reperfusion (n = 6, per group). **(c)** H&E, TUNEL and CD11b staining and quantification results of liver sections from each group 6 hours after reperfusion (n = 6, per group). **(d)** Serum TG 6 h following reperfusion were determined (n = 6, per group). **(e)** Left: Oil red O staining of liver 6 h following reperfusion; right: NAFLD activity score was evaluated 6 h after reperfusion (n = 6, per group). Two-tailed t-test. Statistic data are presented as the mean ± SD, error bars represent the means of at least three independent experiments. *p* values are shown on the graphs, $p < 0.05$ was considered statistically significant. Source data are provided as a Source Data file. Figure S11a created with BioRender. com released under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license (https://creativecommons.org/licenses/by-nc-nd/4.0/deed.en).

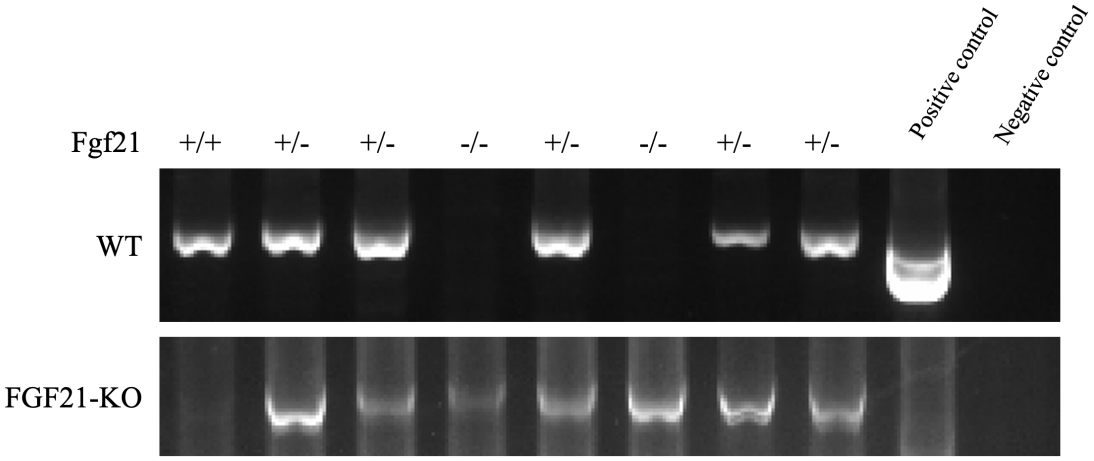

Figure S11. Representative *Fgf21* genotyping results of WT, HET and KO mice. WT, wild-type; HET, heterozygous; KO, knockout.

Table S1. Baseline characteristics and clinicopathological features of patients involving the detection of circulating FGF21.

	Total (n = 88)	High-FGF21 change (n = 44)	Low-FGF21 change (n = 44)	P
Donor characteristics				
Age (y)	42.6 ± 13.9	40.0 ± 13.3	45.1 ± 14.1	0.089
Sex				0.027
Male	72 (81.8%)	40 (90.9%)	32 (72.7%)	
Female	16 (18.2%)	4 (9.1%)	12 (27.3%)	
BMI (kg/m^2)	22.4 ± 2.4	22.3 ± 2.6	22.6 ± 2.2	0.543
Steatosis				0.367
Yes	13 (14.8%)	5 (11.4%)	8 (18.2%)	
No	75 (85.2%)	39 (88.6%)	36 (81.8%)	
Preoperative lab tests				
ALT (U/L) [a]	33.0 (18.0-55.0)	33.5 (20.5-68.0)	32.0 (15.0-50.0)	0.259
AST (U/L) [b]	51.0 (34.0-88.0)	50.0 (32.0-93.0)	57.5 (35.0-85.0)	0.699
Bilirubin (μmol/L) [c]	15.2 (10.2-24.1)	13.0 (10.2-23.5)	17.4 (10.2-24.8)	0.693
PLT (10^9/L) [d]	136.0 (65.5-209.5)	148.5 (62.0-209.5)	123.0 (67.5-211.5)	0.799
Transplant characteristics				
Donor type				0.517
DBD	37 (42.0%)	17 (38.6%)	20 (45.5%)	
DCD	51 (58.0%)	27 (61.4%)	24 (54.5%)	
CIT (h)	8.7 (6.4-11.2)	8.9 (6.5-11.2)	8.6 (6.4-11.5)	0.836
DWIT (min)	9.5 (3.0-14.8)	10.0 (1.0-15.0)	9.0 (5.0-13.0)	0.720
ABO match				0.653
Compatible	58 (65.9%)	30 (68.2%)	28 (63.6%)	
Incompatible	30 (34.1%)	14 (31.8%)	16 (36.4%)	

The cause of graft failure				0.992
Primary non-function	5 (5.7%)	2 (4.5%)	3 (6.8%)	
Hepatic artery thrombosis	4 (4.5%)	2 (4.5%)	2 (4.5%)	
Biliary complication	7 (8.0%)	3 (6.8%)	4 (9.1%)	
Chronic rejection	9 (10.2%)	4 (9.1%)	5 (11.4%)	
Recipient characteristics				
Age (y)	48.1 ± 10.1	48.1 ± 10.5	48.2 ± 9.8	0.992
Sex				0.764
Male	75 (85.2%)	37 (84.1%)	38 (86.4%)	
Female	13 (14.8%)	7 (15.9%)	6 (13.6%)	
BMI (kg/m^2)	23.1 ± 3.8	23.6 ± 4.4	22.6 ± 2.9	0.226
Preoperative lab tests				
ALT (U/L)	56.5 (20.3-162.3)	32.0 (17.0-173.0)	57.5 (25.8-157.0)	0.480
AST (U/L)	75.5 (37.0-165.0)	63.0 (36.0-145.0)	89.5 (42.0-180.8)	0.422
Bilirubin (μmol/L)	191.0 (45.3-414.0)	158.0 (47.8-414.0)	226.5 (26.0-419.8)	0.795
PT (s)	20.5 (15.8-28.2)	20.3 (15.3-28.3)	20.9 (15.9-27.9)	0.975
INR	1.8 (1.4-2.5)	1.8 (1.4-2.5)	1.9 (1.4-2.5)	0.853
PLT ($10^9/L$)	75.5 (37.0-116.8)	66.5 (33.5-116.8)	77.0 (42.0-115.8)	0.659
Indication for transplant				0.899
Cryptogenic cirrhosis	52 (59.1%)	27 (61.4%)	25 (56.8%)	
Hepatitis viruses	29 (33.0%)	13 (29.5%)	16 (36.4%)	
Cholestatic	5 (5.7%)	3 (6.8%)	2 (4.5%)	
Other	2 (2.3%)	1 (2.3%)	1 (2.3%)	
MELD	24.4 ± 9.8	23.2 ± 9.4	25.7 ± 10.2	0.231
EAD	27 (30.7%)	14 (31.8%)	13 (29.5%)	0.817

[a]: there was one case of missing data; [b]: there were three cases of missing data; [c]: there was one case of missing data; [d]: there were four cases of missing data; BMI:

body mass index; ALT: alanine transaminase; AST: aspartate transaminase; PLT: platelet; INR: international normalized ratio; CIT, cold ischemia time; DWIT, donor warm ischemia time; MELD, model for end-stage liver disease; EAD, early allograft dysfunction. Continuous variables were compared using Student's t test or Mann-Whitney U test when the variables were not normally distributed. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test. Two-sided.

Table S2. Baseline characteristics and clinicopathological features of patients from tissue microarray.

	Total (n = 115)	FGF21_low (n = 46)	FGF21_high (n = 69)	P
Donor characteristics				
Age (y)	40.2 ± 12.7	41.4 ± 13.9	39.3 ± 11.8	0.397
Sex				0.915
Male	98 (85.2%)	39 (84.8%)	59 (85.5%)	
Female	17 (14.8%)	7 (15.2%)	10 (14.5%)	
BMI (kg/m^2)	23.1 (21.3-24.5)	22.9 (20.8-26.0)	23.2 (21.5-24.2)	0.615
Preoperative lab tests				
ALT (U/L) [a]	33.0 (20.3-61.0)	35.0 (22.6-78.0)	31.5 (18.0-57.0)	0.327
AST (U/L) [b]	43.0 (31.0-75.0)	43.5 (32.0-85.0)	43.0 (28.0-72.0)	0.549
Bilirubin (μmol/L) [c]	17.0 (10.7-27.9)	16.9 (10.0-27.0)	17.0 (10.7-31.0)	0.914
PLT (10^9/L) [d]	132.0 (79.0-210.5)	154.5 (87.5-240.0)	121.0 (75.0-209.0)	0.188
Transplant characteristics				
Donor type				0.284
DBD	63 (54.8%)	28 (60.9%)	35 (50.7%)	
DCD	52 (45.2%)	18 (39.1%)	34 (49.3%)	
CIT (h)	7.2 (5.7-11.0)	7.9 (6.4-11.6)	6.8 (5.5-10.3)	0.073
DWIT (min)	9.0 (0.0-14.0)	10.5 (1.0-15.3)	8.0 (0.0-12.0)	0.060
ABO match				0.734
Compatible	83 (72.2%)	34 (73.9%)	49 (71.0%)	
Incompatible	32 (27.8%)	12 (26.1%)	20 (29.0%)	
The cause of graft loss				0.904
Primary non-function	2 (1.7%)	1 (2.2%)	1 (1.4%)	
Biliary complication	3 (2.6%)	2 (4.3%)	1 (1.4%)	

Other	15 (13.0%)	8 (17.4%)	7 (10.1%)	
Recipient characteristics				
Age (y)	51.2 ± 9.8	52.4 ± 11.3	50.5 ± 8.7	0.303
Sex				0.223
Male	91 (79.1%)	39 (84.8%)	52 (75.4%)	
Female	24 (20.9%)	7 (15.2%)	17 (24.6%)	
BMI (kg/m^2)	22.7 (20.7-24.2)	22.8 (19.8-24.5)	22.6 (21.4-24.2)	0.943
Preoperative lab tests				
ALT (U/L)	37.0 (21.0-71.0)	35.0 (20.5-68.5)	48.5 (22.5-110.8)	0.612
AST (U/L)	63.0 (33.0-124.0)	62.0 (32.5-109.0)	69.5 (34.8-138.3)	0.297
Bilirubin (μmol/L)	62.0 (21.0-319.0)	62.0 (18.5-304.5)	76.5 (26.0-373.0)	0.905
PT (s)	14.8 (12.9-23.6)	14.8 (12.8-23.5)	15.0 (12.9-23.8)	0.853
INR	1.3 (1.1-2.1)	1.3 (1.1-2.1)	1.3 (1.1-2.1)	0.828
PLT (10^9/L)	55.0 (90.0-153.0)	85.0 (57.0-156.0)	96.0 (51.8-143.3)	0.895
Indication for transplant				0.670
Cryptogenic cirrhosis	77 (67.0%)	31 (67.4%)	46 (66.7%)	
Hepatitis viruses	23 (20.0%)	10 (21.7%)	13 (18.8%)	
Cholestatic	6 (5.2%)	3 (6.5%)	3 (4.3%)	
Other	9 (7.8%)	2 (4.3%)	7 (10.1%)	
MELD	18.0 (10.0-34.0)	20.0 (11.0-40.0)	16.0 (9.0-33.5)	0.298
EAD	37 (32.2%)	16 (36.8%)	21 (30.4%)	0.625

[a]: there were six cases of missing data; [b]: there were eight cases of missing data; [c]: there were six cases of missing data; [d]: there were eleven cases of missing data; BMI: body mass index; ALT: alanine transaminase; AST: aspartate transaminase; PLT: platelet; INR: international normalized ratio; CIT, cold ischemia time; DWIT, donor warm ischemia time; MELD, model for end-stage liver disease; EAD, early allograft dysfunction. Continuous variables were compared using Student's t test or Mann-Whitney U test when the variables were not normally distributed. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test. Two-sided.

Table S3. Baseline characteristics and clinicopathological features of steatotic LT patients included in the tissue microarray.

	Total (n = 78)	Non-EAD (n = 50)	EAD (n = 28)	P
Donor characteristics				
Age (y)	41.2 ± 12.4	39.9 ± 13.3	43.6 ± 10.4	0.109
Sex				0.712
Male	63 (80.8%)	41 (82.0%)	22 (78.6%)	
Female	15 (19.2%)	9 (18.0%)	6 (21.4%)	
BMI (kg/m^2)	24.2 (22.0-26.1)	24.5 (22.0-26.7)	23.4 (22.4-25.6)	0.200
Preoperative lab tests				
ALT (U/L) [a]	40.0 (19.0-65.0)	39.0 (22.0-62.0)	49.7 (15.0-76.0)	0.745
AST (U/L) [b]	47.5 (30.0-103.0)	39.9 (29.0-111.0)	70.0 (34.4-88.0)	0.359
Bilirubin (μmol/L) [c]	14.4 (10.5-20.1)	13.8 (10.4-19.5)	14.8 (12.8-20.2)	0.337
PLT (10^9/L) [d]	129.0 (81.0-199.0)	120.0 (77.0-204.0)	131.5 (111.0-190.0)	0.606
Transplant characteristics				
Donor type				0.496
DBD	35 (44.9%)	21 (42.0%)	14 (50.0%)	
DCD	43 (55.1%)	29 (58.0%)	14 (50.0%)	
CIT (h)	10.0 (6.9-12.5)	9.8 (6.8-12.0)	10.5 (7.4-13.2)	0.274
DWIT (min)	8.0 (1.0-13.0)	8.0 (0.0-13.0)	8.5 (1.8-14.5)	0.608
ABO match				0.182
Compatible	52 (66.7%)	36 (72.0%)	16 (57.1%)	
Incompatible	26 (33.3%)	14 (28.0%)	12 (42.9%)	
The cause of graft loss				0.322
Primary non-function	4 (5.1%)	1 (2.0%)	3 (10.7%)	
Hepatic artery thrombosis	1 (1.3%)	0 (0)	1 (3.6%)	

Biliary complication	1 (1.3%)	1 (2.0%)	0 (0)	
Other	17 (21.8%)	10 (20.0%)	7 (25.0%)	
Recipient characteristics				
Age (y)	46.4 ± 13.1	45.3 ± 12.2	48.4 ± 14.8	0.899
Sex				0.709
Male	67 (85.9%)	44 (88.0%)	23 (82.1%)	
Female	11 (14.1%)	6 (12.0%)	5 (17.9%)	
BMI (kg/m^2)	22.9 (20.6-25.2)	22.8 (20.8-25.8)	23.4 (20.1-25.1)	0.937
Preoperative lab tests				
ALT (U/L)	48.0 (28.8-134.5)	37.0 (28.0-83.3)	82.0 (34.3-191.5)	0.092
AST (U/L)	74.0 (36.0-150.3)	61.0 (35.5-117.8)	93.0 (41.0-191.3)	0.162
Bilirubin (μmol/L)	203.0 (36.0-380.5)	158.5 (35.5-310.9)	293.0 (54.3-504.5)	0.040
PT (s)	20.2 (14.8-26.6)	18.8 (14.9-22.7)	24.9 (14.4-38.7)	0.045
INR	1.8 (1.3-2.4)	1.7 (1.3-2.0)	2.2 (1.2-3.3)	0.044
PLT (10^9/L)	76.5 (44.0-106.3)	80.5 (45.5-108.8)	63.0 (44.0-90.8)	0.218
Indication for transplant				0.544
Cryptogenic cirrhosis	43 (55.1%)	28 (56.0%)	15 (53.6%)	
Hepatitis viruses	21 (26.9%)	14 (28.0%)	7 (25.0%)	
Cholestatic	5 (6.4%)	4 (8.0%)	1 (3.6%)	
Other	9 (11.5%)	4 (8.0%)	5 (17.9%)	
MELD	28.0 (16.3-33.0)	26.0 (14.0-33.0)	29.0 (24.0-33.8)	0.239

[a]: there were five cases of missing data; [b]: there were eight cases of missing data; [c]: there were six cases of missing data; [d]: there were five cases of missing data; BMI: body mass index; ALT: alanine transaminase; AST: aspartate transaminase; PLT: platelet; INR: international normalized ratio; CIT, cold ischemia time; DWIT, donor warm ischemia time; MELD, model for end-stage liver disease; EAD, early allograft dysfunction. Continuous variables were compared using Student's t test or Mann-Whitney U test when the variables were not normally distributed. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test. Two-sided.

Table S4. The summary of 42 surface and intracellular immune markers used in CyTOF.

Number	Pathway	Antibody	Number	Pathway	Antibody
1	89Y	CD45	22	159Tb	F4_80
2	115ln	CD3	23	160Gd	CD274_PDL-1
3	139La	CD44	24	161Dy	iNOS
4	141Pr	CCR2	25	162Dy	CD206
5	142Nd	MHC II	26	163Dy	CD25_IL-2Ra
6	143Nd	CD366_Tim-3	27	164Dy	RORgt
7	144Nd	T-bet	28	165Ho	NK1.1
8	145Nd	GR-1	29	166Er	Arg1
9	146Nd	TER-119	30	167Er	CD16_32
10	147Sm	Ly6G	31	168Er	FoxP3
11	148Nd	Ly6C	32	169Tm	CD127
12	149Sm	CX3CR1	33	170Er	CD49a
13	150Nd	CD49b	34	171Yb	GATA3
14	151Eu	Null	35	172Yb	PD-1
15	152Sm	CD11c	36	173Yb	CD115
16	153Eu	TCRgd	37	174Yb	CTLA-4
17	154Sm	CD62L	38	175Lu	TCRb
18	155Gd	CD80	39	176Yb	MerTK
19	156Gd	CD1d	40	197Au	CD4
20	157Gd	NKp46	41	198Pt	CD8a
21	158Gd	CD19	42	209Bi	CD11b

Table S5. A list of antibodies and reagents used.

Antibody	Company	Catalog number
Anti-β-actin	Proteintech	# 66009-1-Ig
Anti-GAPDH	Abcam	# ab8245
Anti-FGF21	Abcam	# ab171941
Anti-Bcl-2	CST	# 3498-T
Anti-Cleaved caspase-3	CST	# 9661
Anti-CD11b	Invitrogen	# 14-0112-85
Anti-MPO	Proteintech	# 22225-1-AP
Anti-ALOX15	Abcam	# ab244205
Anti-FGFR1(phosphor Y654)	Abcam	# ab59194
Anti-FGFR4 (phosphor Y642)	Abcam	# ab192589
Anti-Phospho-AKT	CST	# 4060T
Anti-Phospho-ERK1/2	CST	# 9106S
Anti-BAX	Proteintech	# 60267-1-Ig
Goat Anti-rabbit HRP	Fude	# FDR007
Goat Anti-mouse HRP	Fude	# FDM007
PD-166866	MCE	# HY-101296
FGF-401	MCE	# HY-101568
SCH772984	MCE	# HY-50846
LY294002	MCE	# HY-10108