

**Supplementary materials****Hepatic CEACAM1 Expression Indicates Donor Liver Quality and Prevents Early Transplantation Injury**

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## Recipients' and surgical parameters

Variables	low CEACAM1	high CEACAM1	p value
	[/ $\beta$ -actin<0.85 (median) ] n=30	[/ $\beta$ -actin>0.85 (median) ] n=30	
Age (years)	60 (29-75)	56 (30-73)	0.074
Gender (M/F)	22 (73.3%) / 8 (26.7%)	19 (63.3%) / 11 (36.7%)	0.58
Race			0.207
White	10 (33.3%)	13 (43.3%)	
Hispanic	10 (33.3%)	14 (46.7%)	
Black	2 (6.7%)	1 (3.3%)	
Asian	7 (23.3%)	1 (3.3%)	
Others	1 (3.3%)	1 (3.3%)	
BMI (kg/m <sup>2</sup> )	25.5 (16.6-44.3)	28.5 (14.5-47.5)	0.139
Disease ethiology			0.16
Viral hepatitis	20 (66.7%)	12 (40.0%)	
EtOH	2 (6.7%)	8 (26.7%)	
Cryptogenic cirrhosis / NASH	3 (10.0%)	6 (20.0%)	
ALF	1 (3.3%)	1 (3.3%)	
PBC	1 (3.3%)	2 (6.7%)	
Others	3 (10.0%)	1 (3.3%)	
ABO			
identical	30 (100%)	30 (100%)	N / A
MELD score	20.6 (7.5-41.6)	33.6 (6.4-44.1)	0.158
Pre-transplant AST (IU/L)	54 (23-1523)	82.5 (22-1918)	0.07
Pre-transplant ALT (IU/L)	37 (11-868)	40.5 (11-3705)	0.784
T-Bil (g/dl)	2.55 (0.4-59.1)	15.0 (0.3-49.2)	0.086
PT-INR	1.65 (1.1-2.8)	1.95 (1.0-3.6)	0.195
Pre-operative hospital stay (days)	1.0 (0-49)	10.5 (0-76)	0.081
CIT (min)	407.5 (163-816)	433.5 (150-747)	0.819
WIT (min)	52 (25-79)	50.5 (36-78)	0.848
RBC transfused during surgery(ml)	4000 (750-15250)	4125 (500-18000)	0.515
FFP transfused during surgery(ml)	3662.5 (0-16500)	5000 (250-18000)	0.61

Table S1

## Donors' parameters

Variables	low CEACAM1 [/ $\beta$ -actin<0.85 (median) ] n=30	high CEACAM1 [/ $\beta$ -actin>0.85 (median) ] n=30	p value
Age (years)	51 (16-74)	41 (7-76)	0.141
Gender (M/F)	14 (46.7%) / 16 (53.3%)	14 (46.7%) / 16 (53.3%)	>0.999
Race			0.78
White	18 (60.0%)	19 (63.3%)	
Hispanic	8 (26.7%)	9 (30.0%)	
Black	1 (3.3%)	1 (3.3%)	
Asian	3 (10.0%)	1 (3.3%)	
Others	0	0	
BMI (kg/m <sup>2</sup> )	25.6 (19.6-38.4)	26.2 (13.4-37.5)	0.496
Pre-transplant AST (IU/L)	49 (10-306)	32.5 (10-314)	0.53
Pre-transplant ALT (IU/L)	29.5 (8-403)	22.5 (8-669)	0.391
T-Bil (g/dl)	0.5 (0.2-4.9)	0.7 (0.3-2.8)	0.217
PT-INR	1.235 (1.0-2.0)	1.20 (1.0-1.8)	0.765
DCD	4 (13.3%)	0	0.112

Table S2

Values	Sensitivity		
	Sensitivity	Specificity	+ Specificity
-0.878	1.000	0.000	1.000
0.225	0.978	0.000	0.978
0.356	0.957	0.000	0.957
0.415	0.935	0.000	0.935
0.446	0.913	0.000	0.913
0.514	0.891	0.000	0.891
0.586	0.870	0.000	0.870
0.602	0.848	0.000	0.848
0.623	0.848	0.071	0.919
0.637	0.826	0.071	0.898
0.646	0.826	0.143	0.969
0.653	0.804	0.143	0.947
0.661	0.804	0.214	1.019
0.675	0.804	0.286	1.090
0.688	0.804	0.357	1.161
0.695	0.804	0.429	1.233
0.695	0.804	0.500	1.304
0.696	0.783	0.500	1.283
0.698	0.761	0.500	1.261
0.700	0.761	0.571	1.332
<b>0.710</b>	<b>0.761</b>	<b>0.643</b>	<b>1.404</b>
0.722	0.739	0.643	1.382
0.727	0.717	0.643	1.360
0.730	0.696	0.643	1.339
0.744	0.674	0.643	1.317
0.758	0.652	0.643	1.295
0.762	0.630	0.643	1.273
0.766	0.609	0.643	1.252
0.803	0.587	0.643	1.230
0.840	0.565	0.643	1.208
0.850	0.543	0.643	1.186
0.872	0.522	0.643	1.165
0.886	0.500	0.643	1.143
0.892	0.478	0.643	1.121
0.906	0.457	0.643	1.099
0.917	0.435	0.643	1.078
0.921	0.413	0.643	1.056
0.938	0.391	0.643	1.034
0.961	0.391	0.714	1.106
0.976	0.370	0.714	1.084
0.996	0.348	0.714	1.062
1.032	0.348	0.786	1.134
1.056	0.326	0.786	1.112
1.061	0.304	0.786	1.090
1.080	0.283	0.786	1.068
1.104	0.261	0.786	1.047
1.118	0.239	0.786	1.025
1.146	0.239	0.857	1.096
1.173	0.217	0.857	1.075
1.187	0.217	0.929	1.146
1.208	0.196	0.929	1.124
1.227	0.174	0.929	1.102
1.239	0.152	0.929	1.081
1.278	0.130	0.929	1.059
1.325	0.109	0.929	1.037
1.350	0.087	0.929	1.016
1.422	0.065	0.929	0.994
1.498	0.043	0.929	0.972
1.696	0.043	1.000	1.043
1.998	0.022	1.000	1.022
3.117	0.000	1.000	1.000

Table S3

## Recipients' and surgical parameters

Variables	CEACAM1/ $\beta$ -actin< 0.71 n=20	CEACAM1/ $\beta$ -actin>0.71 n=40	p value
Age (years)	60 (47-71)	57 (29-75)	0.096
Gender (M/F)	13 (65.0%) / 7 (35.0%)	28 (70.0%) / 12 (30.0%)	0.772
Race			<b>0.037</b>
White	6 (30.0%)	17 (42.5%)	
Hispanic	5 (25.0%)	19 (47.5%)	
Black	2 (10.0%)	1 (2.5%)	
Asian	6 (30.0%)	2 (5.0%)	
Others	1 (5.0%)	1 (2.5%)	
BMI (kg/m <sup>2</sup> )	14.6 (16.6-36.3)	28.5 (14.5-47.5)	0.082
Disease ethiology			0.452
Viral hepatitis	13 (65.0%)	19 (47.5%)	
EtOH	1 (5.0%)	9 (22.5%)	
Cryptogenic cirrhosis / NASH	3 (15.0%)	6 (15.0%)	
ALF	0	2 (5.0%)	
PBC	1 (5.0%)	2 (5.0%)	
Others	2 (5.0%)	2 (5.0%)	
ABO			
identical	20 (100%)	40 (100%)	N / A
MELD score	16.6 (7.5-41.6)	33.3 (6.4-44.1)	0.312
Pre-transplant AST (IU/L)	59.5 (23-163)	71.5 (22-1918)	0.401
Pre-transplant ALT (IU/L)	39 (14-229)	35.5 (11-3705)	0.351
T-Bil (g/dl)	2.45 (0.7-41.8)	8.5 (0.3-59.1)	0.182
PT-INR	1.65 (1.1-2.2)	1.95 (1.0-3.6)	0.99
Pre-operative hospital stay (days)	0.5 (0-49)	10.5 (0-76)	<b>0.032</b>
CIT (min)	413 (163-762)	426.5 (150-816)	0.672
WIT (min)	41 (25-79)	50.5 (36-78)	0.919
RBC transfused during surgery(ml)	3500 (750-15250)	4500 (500-18000)	0.338
FFP transfused during surgery(ml)	3250 (0-16500)	5000 (250-18000)	0.495

Table S4

## Donors' parameters

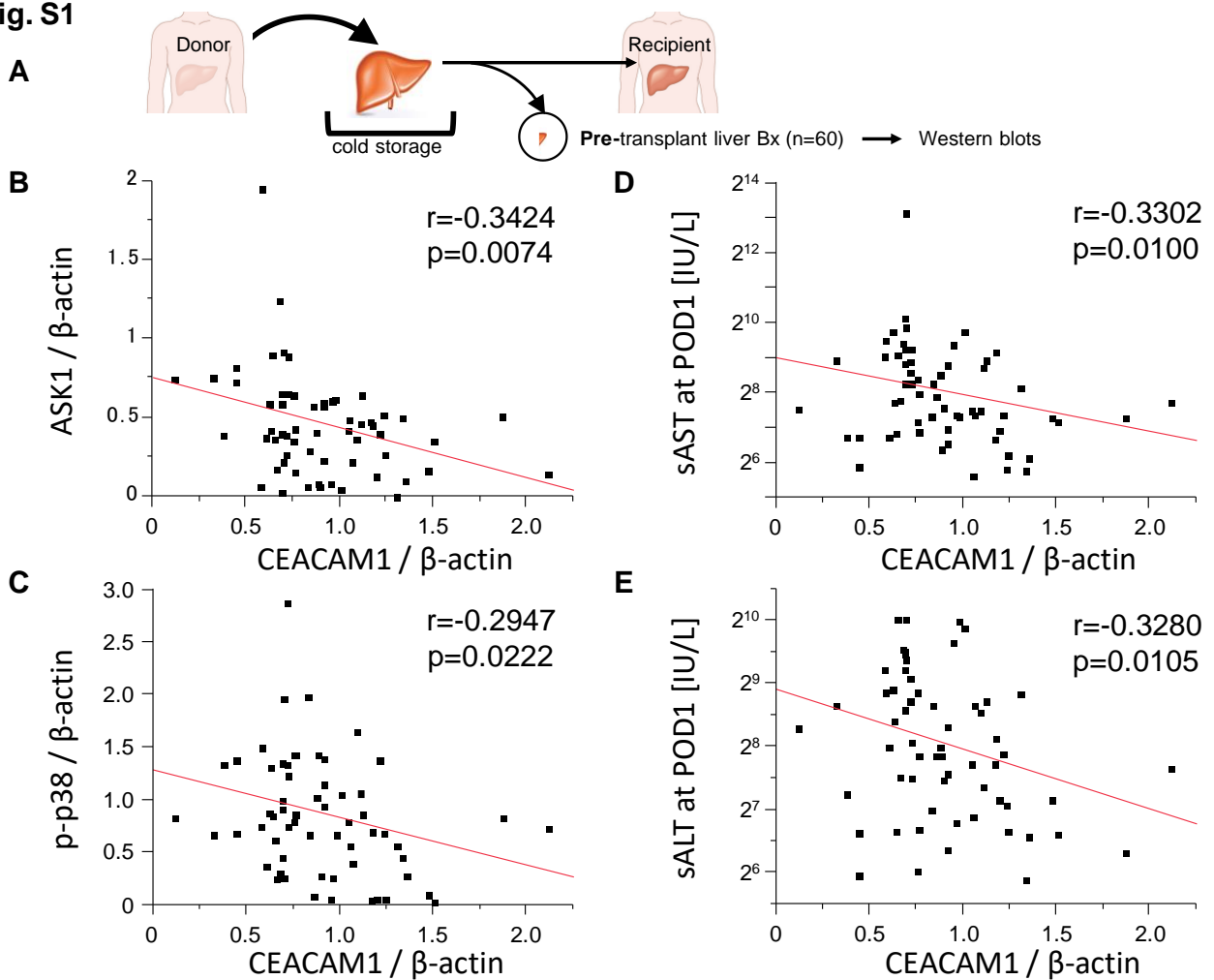
Variables	CEACAM1/ $\beta$ -actin< 0.71 n=20	CEACAM1/ $\beta$ -actin>0.71 n=40	p value
Age (years)	53 (16-74)	41 (7-76)	0.062
Gender (M/F)	8 (40.0%) / 12 (60.0%)	20 (50.0%)	0.585
Race			0.694
White	14 (70.0%)	23 (57.5%)	
Hispanic	4 (20.0%)	13 (32.5%)	
Black	1 (5.0%)	1 (2.5%)	
Asian	1 (5.0%)	3 (7.5%)	
Others	0	0	
BMI (kg/m <sup>2</sup> )	25.6 (21.3-38.4)	26.2 (13.4-37.5)	0.323
Pre-transplant AST (IU/L)	36 (10-306)	35 (10-314)	0.79
Pre-transplant ALT (IU/L)	29.5 (8-403)	24.5 (8-669)	0.701
T-Bil (g/dl)	0.6 (0.2-1.9)	0.7 (0.3-4.9)	0.263
PT-INR	1.3 (1.0-2.0)	1.2 (1.0-1.8)	0.584
DCD	2 (10.0%)	2 (5.0%)	0.595

Table S5

Table S6: Primer Sequences Used for Real-Time Quantitative PCR

Gene	Forward	Reverse
Mouse MCP1	5'-CATCCACGTGTTGGCTCA-3'	5'-GATCATCTTGCTGGTGAATGAGT-3'
Mouse CXCL1	5'-ACCCAAACCGAAGTCATAG-3'	5'-TTGTATAGTGTTGTCAGAAGC-3'
Mouse CXCL2	5'-ACTTCAAGAACATCCAGAG-3'	5'-CTTTCCAGGTCAGTTAGC-3'
Mouse CXCL10	5'-GCTGCCGTCATTTTCTGC-3'	5'-TCTCACTGGCCCGTCATC-3'
Mouse HPRT	5'-TCAACGGGGGACATAAAAGT-3'	5'-TGCATTGTTTTACCAGTGTCAA-3'
Mouse $\beta$ 2M	5'-TGACCGGCTTGTATGCTATC-3'	5'-CACATGTCTCGATCCCAGTAG-3'
Human TLR4	5'-AGACCTGTCCCTGAACCCTAT-3'	5'-CGATGGACTTCTAAACCAGCCA-3'
Human CD80	5'-AAACTCGCATCTACTGGCAA-3'	5'-GGTCTTGTACTCGGGCCATA-3'
Human CD86	5'-CTGCTCATCTATACAGGTTACC-3'	5'-GGAAACGTCGTACAGTTCTGTG-3'
Human CXCL10	5'-GTGGCATTCAAGGAGTACCTC-3'	5'-TGATGGCCTTCGATTCTGGATT-3'
Human CD68	5'-GGAAATGCCACGGTTCATCCA-3'	5'-TGGGGTTCAGTACAGAGATGC-3'
Human Cathepsin G	5'-GAGTCAGACGGAATCGAAACG-3'	5'-CGGAGTGATCTGTTCCCCTC-3'
Human CD28	QuantiTect Primer Assay (QT00001267)	
Human CD4	QuantiTect Primer Assay (QT02401812)	
Human IL17	5'-TCCCACGAAATCCAGGATGC-3'	5'-GGATGTTGAGTTGACCATCAC-3'
Human GAPDH	5'-GGAGCGAGATCCCTCCAAAAT-3'	5'-GGCTGTTGTCATACTTCTCATGG-3'

Fig. S1

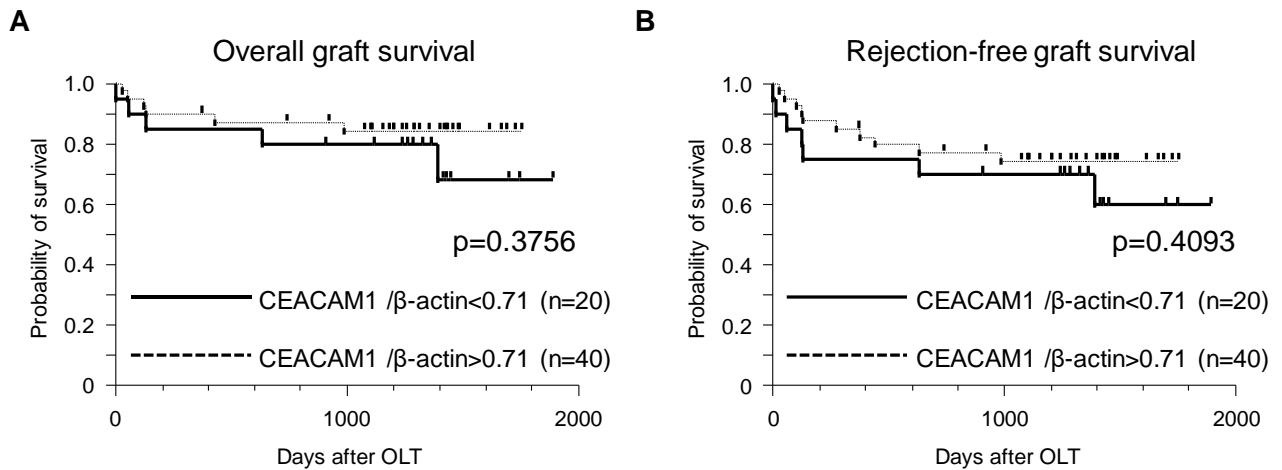


**Figure S1: CEACAM1 expression in cold-stored liver graft negatively correlates with ASK1/p-p38 level and post-transplant liver damage.**

(A) Pre-transplant (post cold storage) liver biopsies (Bx) were collected at the back table from sixty OLTs. Bx samples were analyzed by Western blots with  $\beta$ -actin normalization for CEACAM1, ASK1 and p-p38. (B) Relationship between CEACAM1 level and ASK1 expression. (C) Relationship between CEACAM1 level and p-p38 expression. (D) Relationship between CEACAM1 level and sAST level at postoperative day 1 (POD1). (E) Relationship between CEACAM1 level and sALT level at POD1. r: Spearman correlation coefficient



Fig. S2



**Figure S2: Association of graft CEACAM1 expression with long term OLT outcomes.**

Pre-transplant human liver Bx samples collected at back table prior to implantation (n=60) were screened by Western blots for CEACAM1 levels, and then sixty human OLTs were classified into CEACAM1/ $\beta$ -actin<0.71 (n=20) and CEACAM1/ $\beta$ -actin>0.71 cases (n=40). **(A)** The cumulative probability of overall graft survival. **(B)** The cumulative probability of rejection-free graft survival. Solid line indicates CEACAM1/ $\beta$ -actin<0.71, while dotted line CEACAM1/ $\beta$ -actin>0.71 human OLT patient group (Kaplan-Meier method, log-rank test).