Supplementary Material

Potent Suppression of Hydrophobic Bile Acids by Aldafermin, an FGF19 Analogue, Across Metabolic and Cholestatic Liver Diseases

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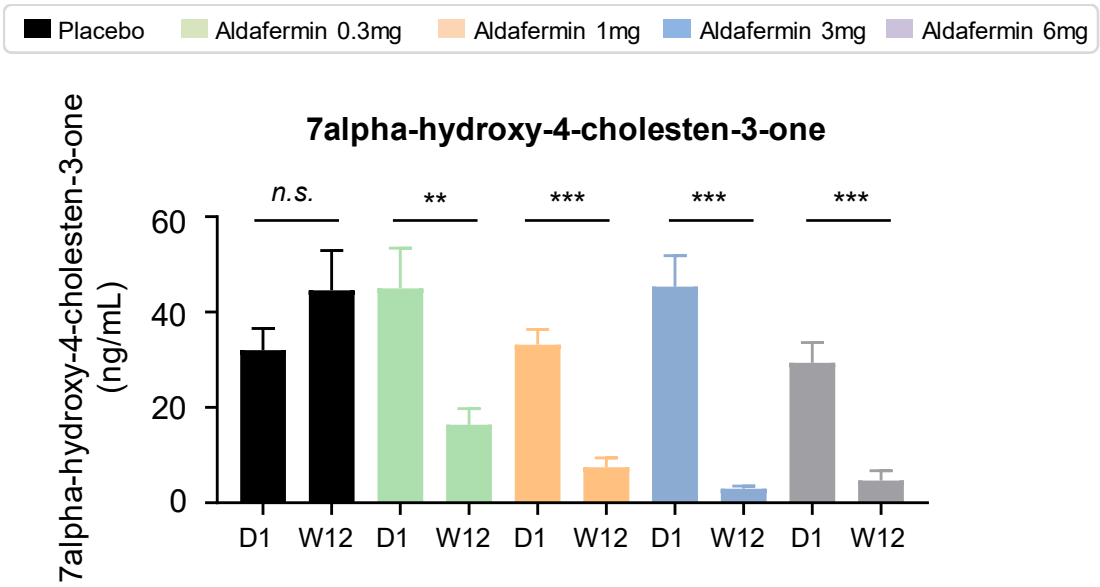

Fig. S1. Potent Suppression of 7alpha-Hydroxy-4-Cholesten-3-One by Aldafermin in Patients with NASH.

176 patients with biopsy-proven NASH received 0.3 mg (n=23), 1 mg (n=49), 3 mg (n=49), 6 mg (n=28) aldafermin or placebo (n=27) for 12 weeks. Serum concentrations of 7alpha-hydroxy-4-cholesten-3-one were determined using mass spectrometry methods. ***P < .001, **P < .01 vs baseline (Wilcoxon test). *n.s.*, not significant. D1, day 1; W12, week 12.

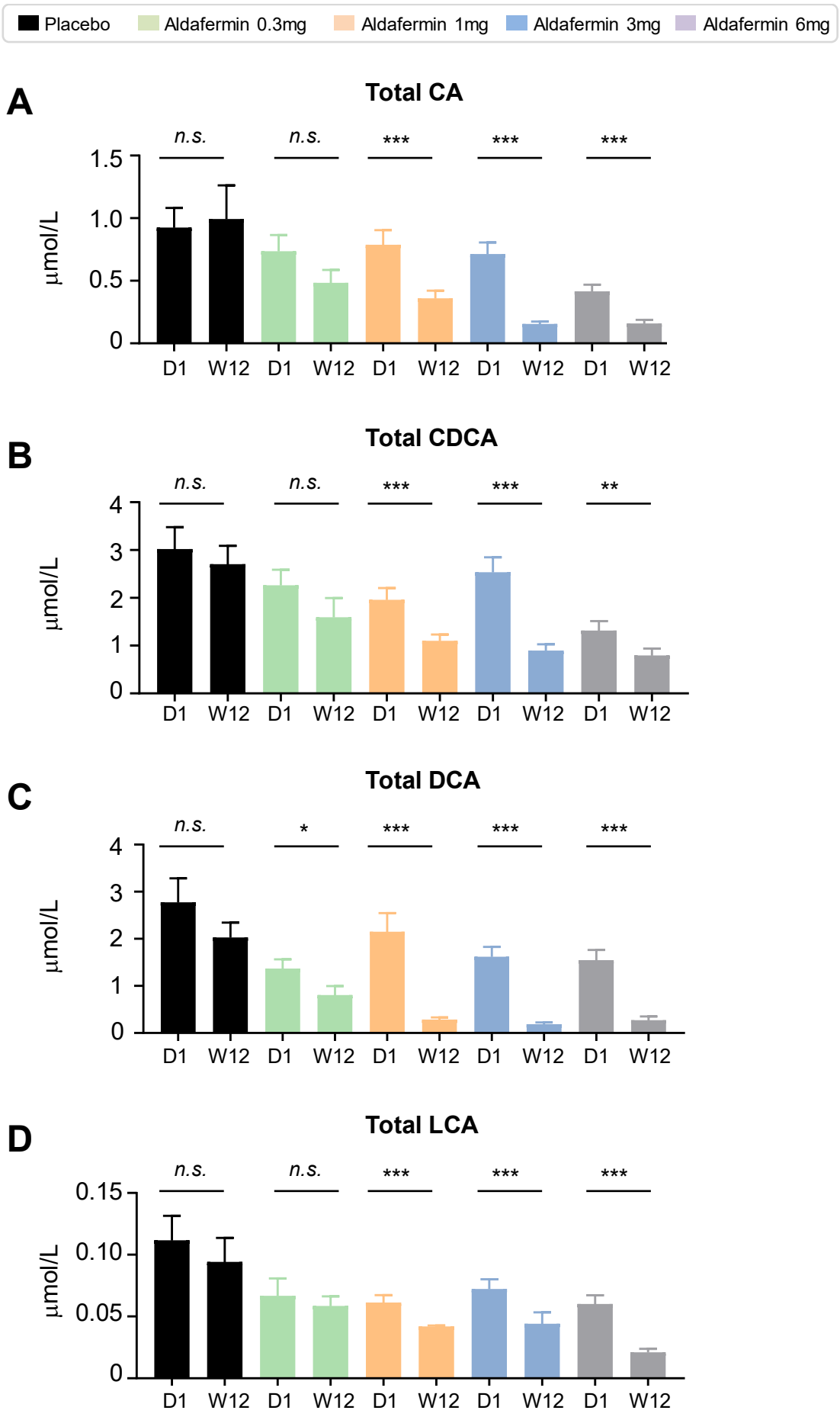
Placebo
Aldafermin 0.3mg
Aldafermin 1mg
Aldafermin 3mg
Aldafermin 6mg
A
Total CA
n.s.
n.s.
***
***
***
µmol/L
1.5
1.0
0.5
0
D1
W12
B
Total CDCA
n.s.
n.s.
***
***
**
µmol/L
4
3
2
1
0
C
Total DCA
n.s.
*
***
***
***
µmol/L
D
Total LCA
n.s.
n.s.
***
***
***
µmol/L
0.15
0.10
0.05

Fig. S2. Aldafermin Reduces Total Cholic Acid, Total Chenodeoxycholic Acid, Total Deoxycholic Acid and Total Lithocholic Acid in Patients with NASH.

176 patients with biopsy-proven NASH received 0.3 mg (n=23), 1 mg (n=49), 3 mg (n=49), 6 mg (n=28) aldafermin or placebo (n=27) for 12 weeks. Serum samples were collected at baseline (D1, day 1) and week 12 (W12, end of treatment) for bile acid profiling. **(A)** Concentrations of total CA at baseline and week 12. Total CA includes conjugated and unconjugated CA. **(B)** Concentrations of total CDCA at baseline and week 12. Total CDCA includes conjugated and unconjugated CDCA. **(C)** Concentrations of total DCA at baseline and week 12. Total DCA includes conjugated and unconjugated DCA. **(D)** Concentrations of total LCA at baseline and week 12. Total LCA includes conjugated and unconjugated LCA. ***$P < .001$, **$P < .01$, *$P < .05$ (Wilcoxon test). *n.s.*, not significant. CA, cholic acid; CDCA, chenocholic acid; DCA, deoxycholic acid; LCA, lithocholic acid.

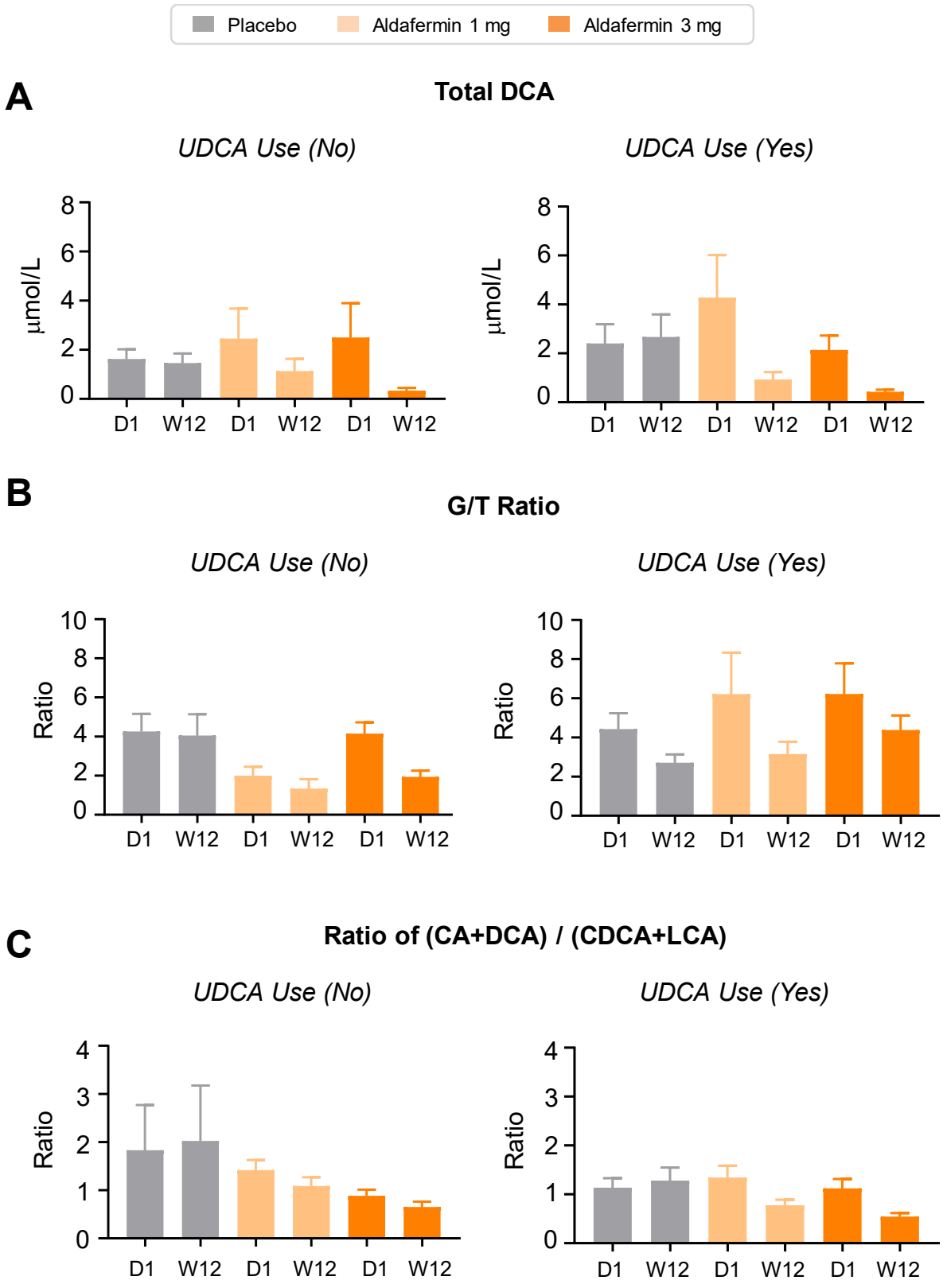
Placebo
Aldafermin 1 mg
Aldafermin 3 mg
A
Total DCA
UDCA Use (No)
UDCA Use (Yes)
µmol/L
0
2
4
6
8
D1
W12
B
G/T Ratio
Ratio
0
2
4
6
8
10
C
Ratio of (CA+DCA) / (CDCA+LCA)
0
1
2
3
4

Fig. S3. Levels of DCA, G/T Ratio and the Ratio of (CA+DCA) to (CDCA+LCA) in PSC Patients Stratified by Baseline UDCA Use.

62 patients with PSC diagnosed according to EASL criteria received 1 mg (n=21), 3 mg (n=21) aldafermin or placebo (n=20) for 12 weeks. Serum samples were collected at baseline (D1, day 1) and week 12 (W12, end of treatment). Left panels, patients not on UDCA at baseline. Right panels, patients on UDCA at baseline. **(A)** Concentrations of total DCA at baseline and week 12. **(B)** G/T ratio at baseline and week 12. **(C)** Ratio of (CA+DCA) to (CDCA+LCA) at baseline and week 12. CA, cholic acid; CDCA, chenocholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid.

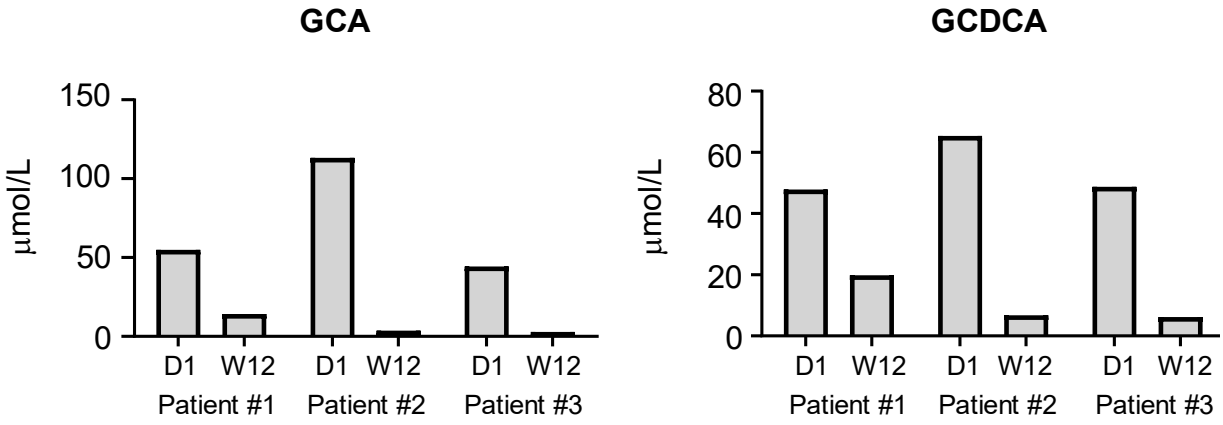

Fig. S4. Aldafermin is Efficacious in Lowering Bile Acids in PSC Patients Who Had Baseline Serum Bile Acids >100μM.

Three PSC patients had serum bile acids >100μM at baseline and received aldafermin 1 mg (patient #1 and patient #2) or 3 mg (patient #3) for 12 weeks. Serum samples were collected at baseline (D1, day 1) and week 12 (W12, end of treatment). **(A)** Concentrations of GCA at baseline and week 12. **(B)** Concentrations of GCDCA at baseline and week 12. GCA, glycocholic acid; GCDCA, glycochenocholic acid.

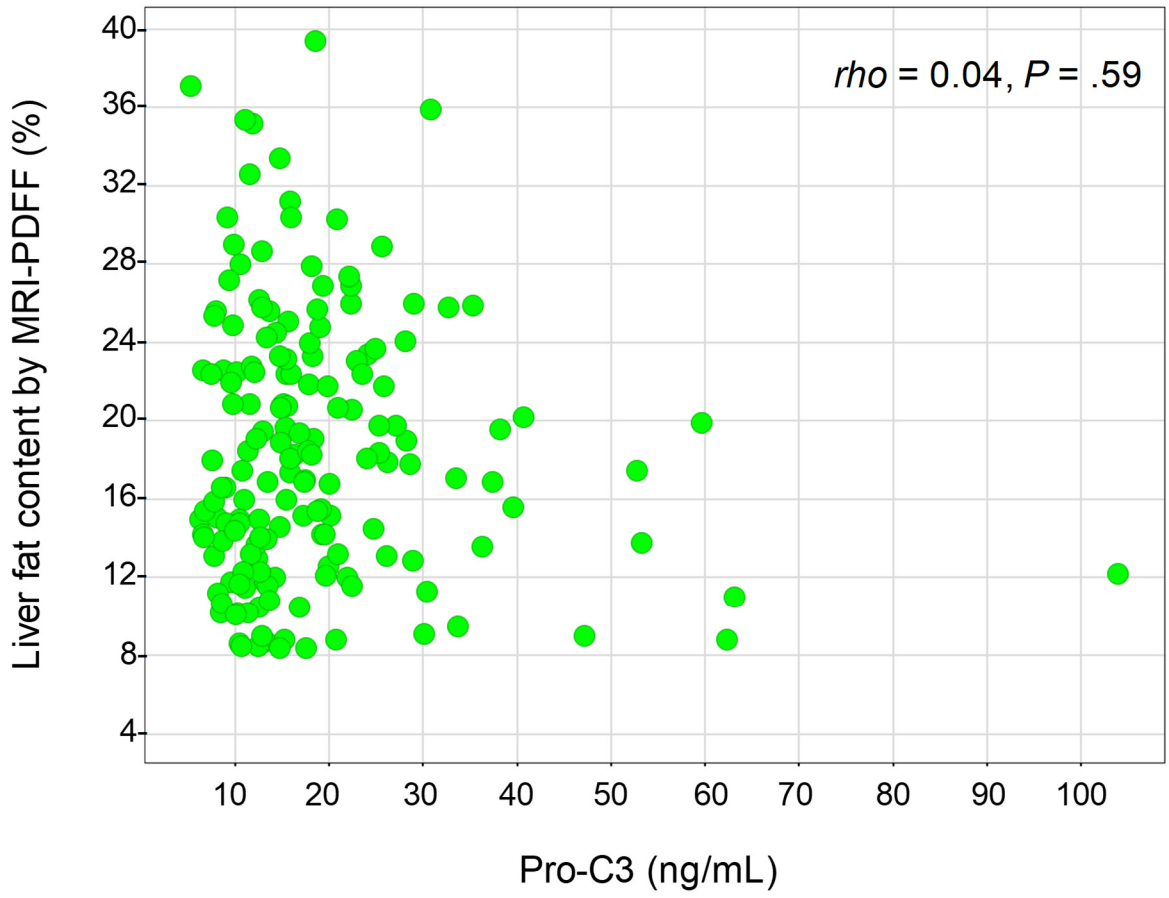

Fig. S5. Lack of Correlation Between Pro-C3 and Liver Fat Content in Patients with NASH.

176 patients with biopsy-proven NASH received 0.3 mg (n=23), 1 mg (n=49), 3 mg (n=49), 6 mg (n=28) aldafermin or placebo (n=27) for 12 weeks. At baseline, patients underwent magnetic resonance imaging—proton density fat fraction (MRI-PDFF) to measure liver fat content. Serum samples were collected for determination of Pro-C3 concentrations using an ELISA method. Correlation between baseline liver fat content and Pro-C3 concentration was performed using Spearman's method. Correlation coefficient (*rho*) and *P* value are shown on the graph. Pro-C3, neoepitope-specific N-terminal pro-peptide of type III collagen.

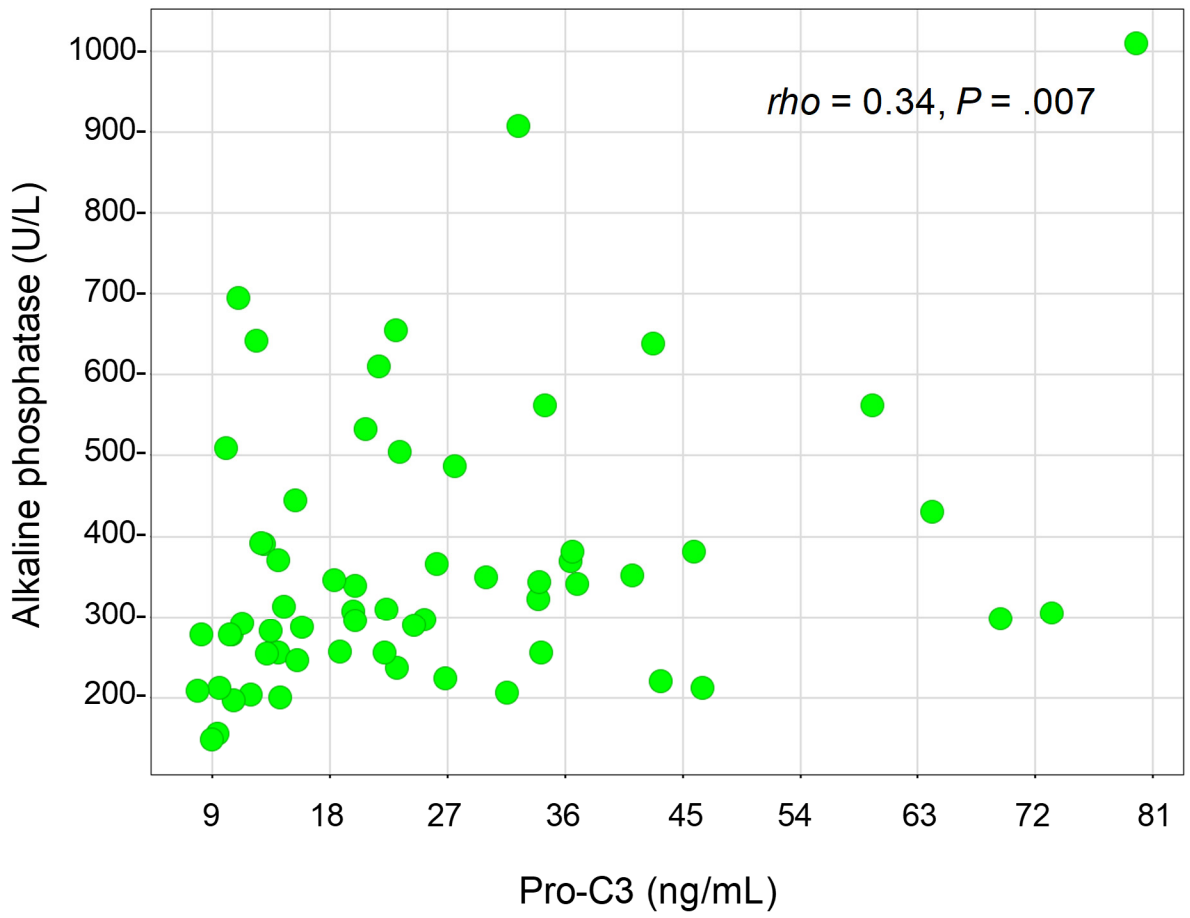

Fig. S6. Serum Concentrations of Pro-C3 Correlate with Alkaline Phosphatase in Patients with PSC.

62 patients with PSC diagnosed according to EASL criteria received 1 mg (n=21), 3 mg (n=21) aldafermin or placebo (n=20) for 12 weeks. At baseline, serum samples were collected for measurements of alkaline phosphase using standard laboratory methods and Pro-C3 using an ELISA method. Correlation between baseline alkaline phosphatase and Pro-C3 was performed using Spearman's method. Correlation coefficient (*rho*) and *P* value are shown on the graph. Pro-C3, neoepitope-specific N-terminal pro-peptide of type III collagen.

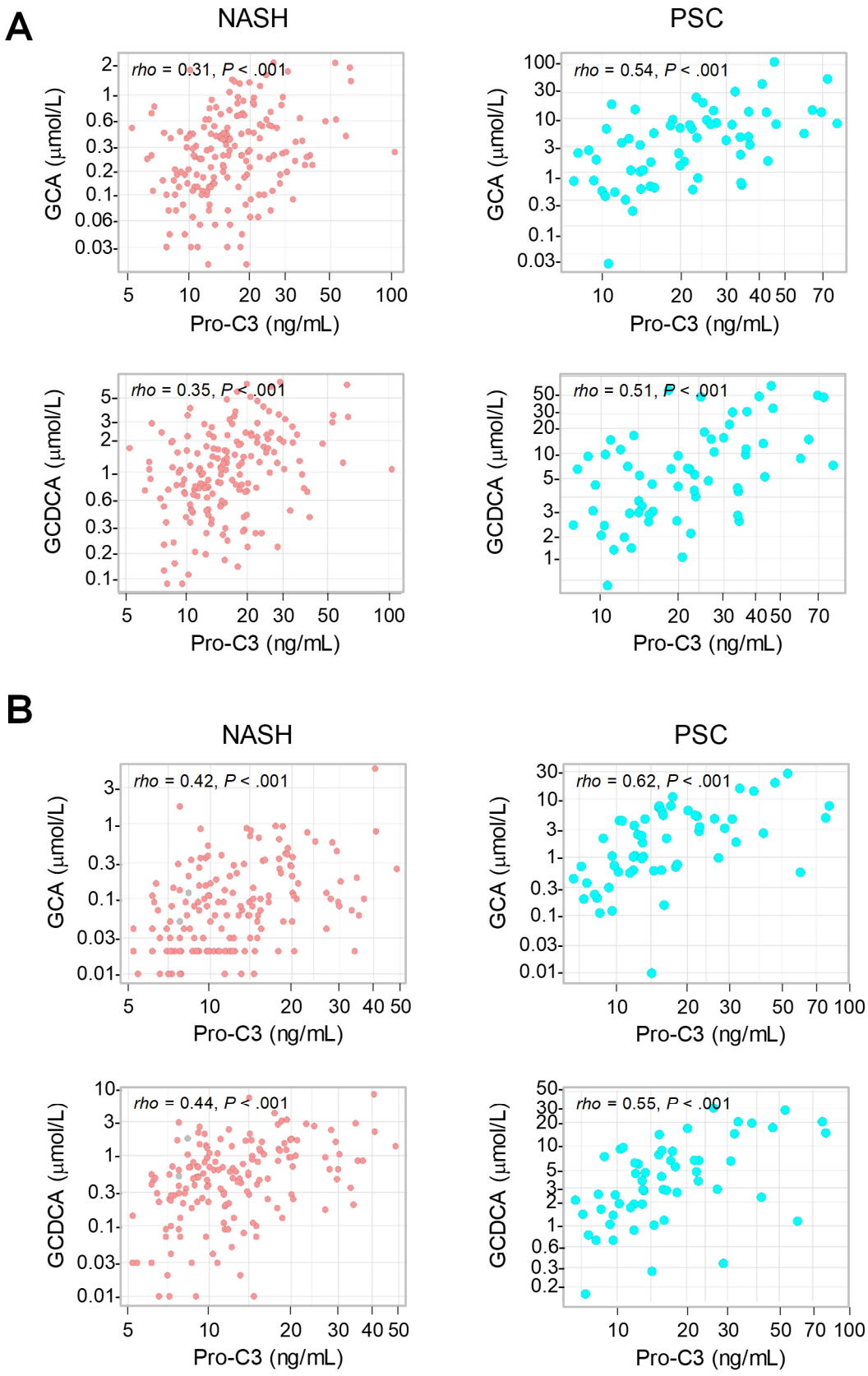
A
NASH
PSC
rho = 0.31, P < .001
rho = 0.54, P < .001
rho = 0.35, P < .001
rho = 0.51, P < .001
GCA (µmol/L)
GCDCA (µmol/L)
Pro-C3 (ng/mL)
B
NASH
PSC
rho = 0.42, P < .001
rho = 0.62, P < .001
rho = 0.44, P < .001
rho = 0.55, P < .001
GCA (µmol/L)
GCDCA (µmol/L)
Pro-C3 (ng/mL)

Fig. S7. Hydrophobic Bile Acids Correlate with the Novel Fibrogenesis Biomarker Pro-C3 in NASH and PSC Populations When Analyzed Separately.

176 patients with biopsy-proven NASH received 0.3 mg (n=23), 1 mg (n=49), 3 mg (n=49), 6 mg (n=28) aldafermin or placebo (n=27) for 12 weeks (left panels). 62 patients with PSC diagnosed according to EASL criteria received 1 mg (n=21), 3 mg (n=21) aldafermin or placebo (n=20) for 12 weeks (right panels). At baseline, serum samples were collected for measurements of bile acids using mass spectrometry method and Pro-C3 using an ELISA method. Correlation between concentrations of bile acids and Pro-C3 was performed using Spearman's method. Correlation coefficient (*rho*) and *P* value are shown on the graph. **(A)** GCA and GCDCA levels correlate with Pro-C3 at baseline in the NASH and PSC populations when analyzed separately. **(B)** GCA and GCDCA levels correlate with Pro-C3 at week 12 in the NASH and PSC populations when analyzed separately. GCA, glycocholic acid; GCDCA, glycochenocholic acid; Pro-C3, neoepitope-specific N-terminal pro-peptide of type III collagen.

Supplementary Tables

Table S1. Correlation Between Changes in Bile Acids and Changes in 7alpha-Hydroxy-4-Cholesten-3-One in Patients with NASH.

Percent Change in Bile Acids from Baseline to Week 12	**Percent Change in 7alpha-Hydroxy-4-Cholesten-3-One from Baseline to Week 12**	
	rho	**P value**
Primary bile acids		
Glycine-conjugated primary bile acids		
GCA (μmol/L)	0.48	< .001
GCDCA (μmol/L)	0.34	< .001
Taurine-conjugated primary bile acids		
TCA (μmol/L)	0.08	.29
TCDCA (μmol/L)	−0.13	.10
Unconjugated primary bile acids		
CA (μmol/L)	0.36	< .001
CDCA (μmol/L)	0.41	< .001
Secondary bile acids		
Glycine-conjugated secondary bile acids		
GDCA (μmol/L)	0.38	< .001
GLCA (μmol/L)	0.26	.001
Taurine-conjugated secondary bile acids		
TDCA (μmol/L)	0.12	.15
TLCA (μmol/L)	0.05	.62
Unconjugated Secondary Bile Acids		
DCA (μmol/L)	0.48	< .001
LCA (μmol/L)	0.28	< .001

Shown are correlation coefficients. *P* values by Spearman's method.

CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; LCA, lithocholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, taurolithocholic acid.

Table S2. Baseline Demographics and Characteristics of NASH and PSC Populations.

Parameters	NASH Patients (n=176)	PSC Patients (n=62)	P Value
Age (years)	51.2 (10.6)	43.2 (13.7)	
Weight (kg)	100.2 (21.5)	78.6 (14.5)	
BMI (kg/m^2)	36.4 (7.1)	26.1 (5.1)	
Sex			
Female	121 (69%)	24 (39%)	
Male	55 (31%)	38 (61%)	
Race			
Asian	3 (2%)	1 (2%)	
Black	2 (1%)	7 (11%)	
White	166 (94%)	52 (84%)	
Other	5 (3%)	2 (3%)	
Ethnicity			
Hispanic/Latino	82 (47%)	1 (2%)	
Non-Hispanic/Latino	94 (53%)	61 (98%)	
Liver enzymes			
ALP (U/L)	99.2 (38.3)	366.7 (171.3)	< .001
ALT (U/L)	72.4 (42.9)	102.5 (63.6)	< .001
AST (U/L)	54.9 (30.4)	78.8 (49.1)	< .001
Serum bile acids			
Primary bile acids			
Glycine-conjugated primary bile acids			
GCA (μmol/L)	0.4 (0.4)	8.9 (16.8)	< .001
GCDCA (μmol/L)	1.5 (1.4)	12.0 (15.6)	< .001
Taurine-conjugated primary bile acids			
TCA (μmol/L)	0.1 (0.1)	5.0 (10.1)	< .001
TCDCA (μmol/L)	0.2 (0.3)	4.2 (7.0)	< .001
Unconjugated primary bile acids			
CA (μmol/L)	0.2 (0.4)	0.2 (0.3)	0.52
CDCA (μmol/L)	0.5 (0.8)	0.2 (0.3)	.002
Secondary bile acids			
Glycine-conjugated secondary bile acids			
GDCA (μmol/L)	0.9 (1.4)	1.9 (2.9)	< .001
GLCA (μmol/L)	0.03 (0.04)	0.19 (0.33)	< .001
Taurine-conjugated secondary bile acids			

TDCA (μmol/L)	0.3 (0.4)	0.5 (0.9)	.003
TLCA (μmol/L)	0.01 (0.01)	0.04 (0.09)	< .001
Unconjugated secondary bile acids			
DCA (μmol/L)	0.7 (0.7)	0.2 (0.3)	< .001
LCA (μmol/L)	0.03 (0.03)	0.06 (0.12)	.002

Shown are mean (SD) or n (%).

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; LCA, lithocholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, taurolithocholic acid.

Table S3. Serum Levels of Pro-C3 Correlate with Fibrosis Stage at Baseline in Patients with NASH.

	F1 (n=62)	F2 (n=56)	F3 (n=56)	P Value
Age (years)	48.7 (11.7)	50.9 (10.4)	54.1 (9.0)	.034
Weight (kg)	96.4 (21.0)	100.4 (21.3)	104.6 (22.2)	.15
BMI (kg/m^2)	35.4 (7.7)	36.2 (6.9)	37.8 (6.9)	.08
Serum parameters				
ALP (U/L)	100.6 (49.5)	93.1 (28.1)	105.6 (35.6)	.15
ALT (U/L)	63.8 (36.0)	77.0 (49.0)	77.0 (44.9)	.14
Pro-C3 (ng/mL)	13.7 (6.3)	18.6 (10.6)	23.3 (16.4)	**< .001**
Histology				
NAS	5.0 (1.0)	5.4 (1.2)	5.6 (1.2)	.011
Ballooning	1.2 (0.6)	1.5 (0.6)	1.6 (0.5)	< .001
Inflammation	1.5 (0.6)	1.8 (0.5)	1.9 (0.6)	.004
Steatosis	2.2 (0.7)	2.1 (0.8)	2.0 (0.7)	.68

Shown are mean (SD). *P* values by Kruskal-Wallis test.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; BMI, body mass index; NAS, NAFLD activity score; Pro-C3, neoepitope-specific N-terminal pro-peptide of type III collagen.

Table S4. Correlation Between Changes in Bile Acids and Changes in ELF Score in the Pooled NASH and PSC Populations.

Percent Change in Bile Acids from Baseline to Week 12	**Percent Change in ELF Score from Baseline to Week 12**	
	rho	**P value**
Primary bile acids		
Glycine-conjugated primary bile acids		
GCA (μmol/L)	0.32	< .001
GCDCA (μmol/L)	0.28	< .001
Taurine-conjugated primary bile acids		
TCA (μmol/L)	0.22	.002
TCDCA (μmol/L)	0.16	.02
Unconjugated primary bile acids		
CA (μmol/L)	0.23	.001
CDCA (μmol/L)	0.12	.08
Secondary bile acids		
Glycine-conjugated secondary bile acids		
GDCA (μmol/L)	0.33	< .001
Taurine-conjugated secondary bile acids		
TDCA (μmol/L)	0.32	< .001
Unconjugated Secondary Bile Acids		
DCA (μmol/L)	0.29	< .001

Shown are correlation coefficients. *P* values by Spearman's method.

CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; ELF, enhanced liver fibrosis; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; LCA, lithocholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, taurolithocholic acid.

Table S5. Correlation Between Changes in Bile Acids and Changes in ALT in the Pooled NASH and PSC Populations.

Percent Change in Bile Acids from Baseline to Week 12	**Percent Change in ALT from Baseline to Week 12**	
	rho	**P value**
Primary bile acids		
Glycine-conjugated primary bile acids		
GCA (μmol/L)	0.40	< .001
GCDCA (μmol/L)	0.25	< .001
Taurine-conjugated primary bile acids		
TCA (μmol/L)	0.20	.003
TCDCA (μmol/L)	−0.02	.82
Unconjugated primary bile acids		
CA (μmol/L)	0.16	.02
CDCA (μmol/L)	0.24	< .001
Secondary bile acids		
Glycine-conjugated secondary bile acids		
GDCA (μmol/L)	0.38	< .001
Taurine-conjugated secondary bile acids		
TDCA (μmol/L)	0.24	< .001
Unconjugated Secondary Bile Acids		
DCA (μmol/L)	0.42	< .001

Shown are correlation coefficients. *P* values by Spearman's method.

ALT, alanine aminotransferase; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; LCA, lithocholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, taurolithocholic acid.