

1 **Supplementary information**

2

3 **Pemafibrate, a novel selective peroxisome proliferator-activated receptor alpha**
4 **modulator, improves the pathogenesis in a rodent model of nonalcoholic**
5 **steatohepatitis**

6

7 Yasushi Honda¹, Takaomi Kessoku¹, Yuji Ogawa¹, Wataru Tomeno¹, Kento Imajo¹, Koji
8 Fujita¹, Masato Yoneda¹, Toshiaki Takizawa², Satoru Saito¹, Yoji Nagashima³, Atsushi
9 Nakajima^{1*}

10

11 ¹Department of Gastroenterology and Hepatology, Yokohama City University Graduate
12 School of Medicine, Yokohama, Japan

13 ²Department of Pharmacology Research, Tokyo New Drug Research Laboratories,
14 Kowa Co. Ltd., Tokyo, Japan

15 ³Department of Surgical Pathology, Tokyo Women's Medical University, Tokyo, Japan

16

17 ***Corresponding author:**

18 Atsushi Nakajima, MD, PhD

- 1 Department of Gastroenterology and Hepatology, Yokohama City University Graduate
- 2 School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan
- 3 Telephone: +81-45-787-2640; Fax: +81-45-784-3546; E-mail: nakajima-ty@umin.ac.jp

1

2 **Supplementary Table 1**3 **Characteristics of model mice (methionine choline-deficient (MCD)-fed db/db mice)**

Parameter	BD (db/db)	MCD (db/db)	PEMA-L (db/db)	PEMA-H (db/db)
n	8	8	8	8
Body weight (g)	51.4 ± 3.4 †	43.0 ± 1.1	39.3 ± 1.4 *	34.6 ± 2.3 *
Liver weight (g)	2.65 ± 0.37 †	1.25 ± 0.17	1.37 ± 0.13	1.78 ± 0.12 *
Total cholesterol (mg/dL)	114.7 ± 8.7 †	136.7 ± 15.7	147.5 ± 12.8	128.7 ± 15.6
Triglycerides (mg/dL)	80.4 ± 41.8 †	28.9 ± 5.6	21.2 ± 2.5 *	15.9 ± 3.5 *
AST (IU/L)	74.8 ± 12.4 †	87.5 ± 6.5	60.9 ± 4.7 *	48.8 ± 8.1 *
ALT (IU/L)	90.8 ± 16.8	96.7 ± 25.8	61.4 ± 12.3	45.4 ± 13.7
NAS	1.6 ± 1.1 †	4.9 ± 1.0	2.3 ± 1.5	0.9 ± 1.0 *
Steatosis	0.5 ± 0.5 †	2.4 ± 0.7	1.3 ± 0.7	0.5 ± 0.5 *
Lobular inflammation	0.0 ± 0.0	0.6 ± 0.7	0.4 ± 0.5	0.0 ± 0.0 *

Hepatocyte ballooning	1.1 ± 0.6 †	1.9 ± 0.4	0.6 ± 0.9	0.4 ± 0.5
-----------------------	-------------	-----------	-----------	-----------

- 1 AST, aspartate aminotransferase; ALT, alanine aminotransferase; NAS, nonalcoholic fatty liver disease activity score.
- 2 NAS was scored according to the method described by Kleiner *et al.*⁴⁶, as outlined in Supplementary Table 2. Data are the mean ± SE.
- 3 Significance was determined using Student's *t*-test (†*p* < 0.05 *versus* MCD (db/db) mice) or Dunnett's test (**p* < 0.05 *versus* MCD (db/db)
- 4 mice).

1 **Supplementary Table 2**2 **Scoring system and fibrosis stage****NAFLD Activity Score**

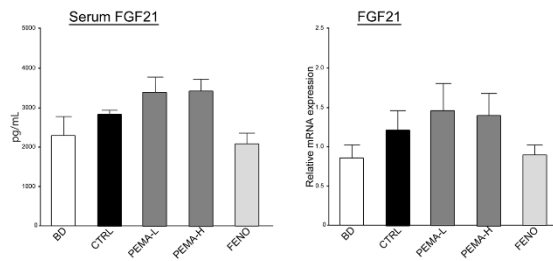
Item	Definition	Grade
Steatosis	<5%	0
	5–33%	1
	33–66%	2
	>66%	3
Lobular inflammation	None	0
	<2 foci/field	1
	2–4 foci/field	2
	>5 foci/field	3
Hepatocyte ballooning	None	0
	Few ballooned cells	1
	Many ballooned cells	2
Fibrosis stage		
Item	Definition	Stage
Fibrosis stage	Isolated perisinusoidal or portal/periportal fibrosis	1
	Perisinusoidal and portal/periportal fibrosis	2
	Bridging fibrosis	3
	Cirrhosis	4

3 **Items, definitions, grades, and stages used in this study**

1 **Supplementary Figure 1**

2 **Pemafibrate tended to increase serum and mRNA levels of fibroblast growth factor**

3 **21 (FGF21)**



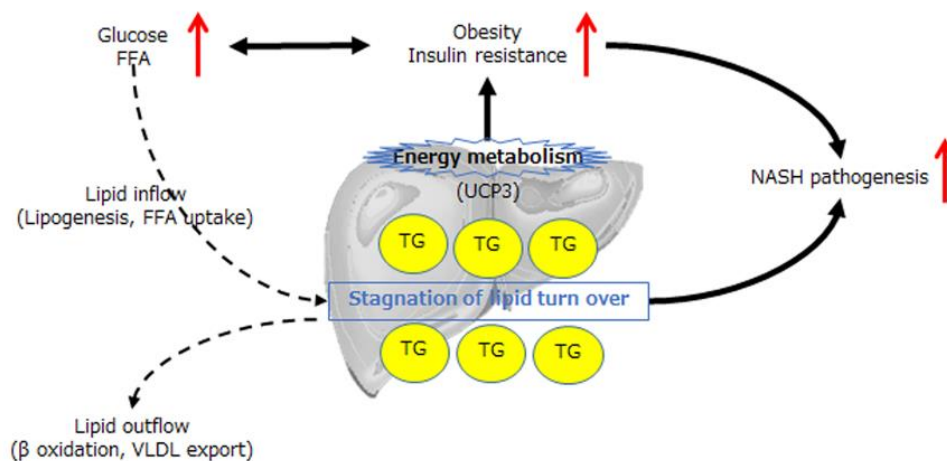
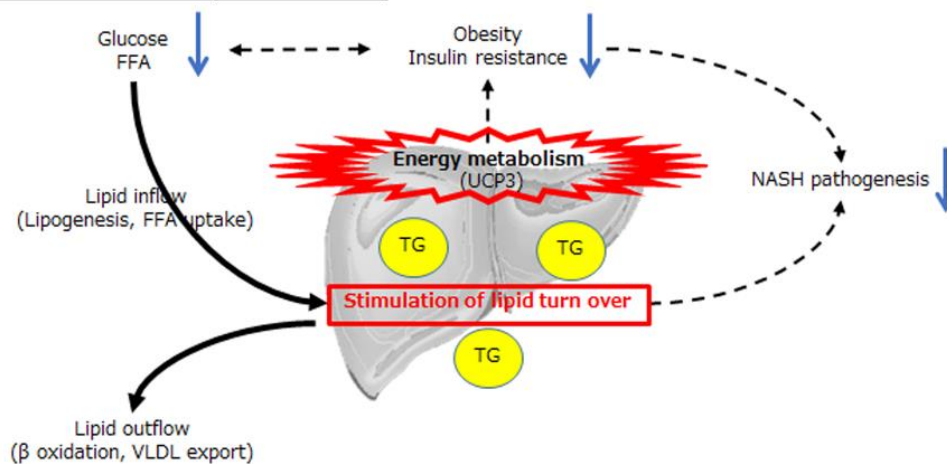
4

5 Serum levels and mRNA expression of FGF21 in BD, CTRL, PEMA-L, PEMA-H, and

6 FENO mice (n=5–10). Results are the mean \pm SE. Significance was determined using

7 Student's *t*-test ($\dagger p < 0.05$ versus CTRL mice) or Dunnett's test ($*p < 0.05$ versus CTRL

8 mice).

1 **Supplementary Figure 2**2 **Mechanism detailing the effect of pemafibrate on a rodent model of NASH**3 **(schematic)**NASH model miceAdministration of pemafibrate

4

5 NASH, nonalcoholic steatohepatitis; FFA, free fatty acid; TG, triglyceride; VLDL, very-

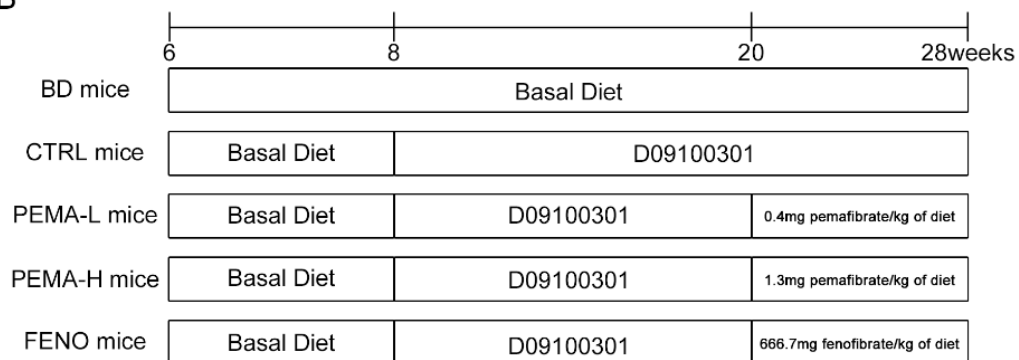
6 low-density lipoprotein; UCP3, uncoupling protein 3

1 **Supplementary Figure 3**2 **Experimental protocol**

A



B



3