

Supplementary information

Selective PPAR α modulator Pemafibrate and sodium-glucose cotransporter 2 (SGLT2) inhibitor Tofogliflozin combination treatment improves histopathology in experimental mice model of non-alcoholic steatohepatitis

Kentaro Murakami, Yusuke Sasaki, Masato Asahiyama, Wataru Yano, Toshiaki Takizawa, Wakana Kamiya, Yoshihiro Matsumura, Motonobu Anai, Tsuyoshi Osawa, Jean-Charles Fruchart, Jamila Fruchart-Najib, Hiroyuki Aburatani, Juro Sakai, Tatsuhiko Kodama and Toshiya Tanaka

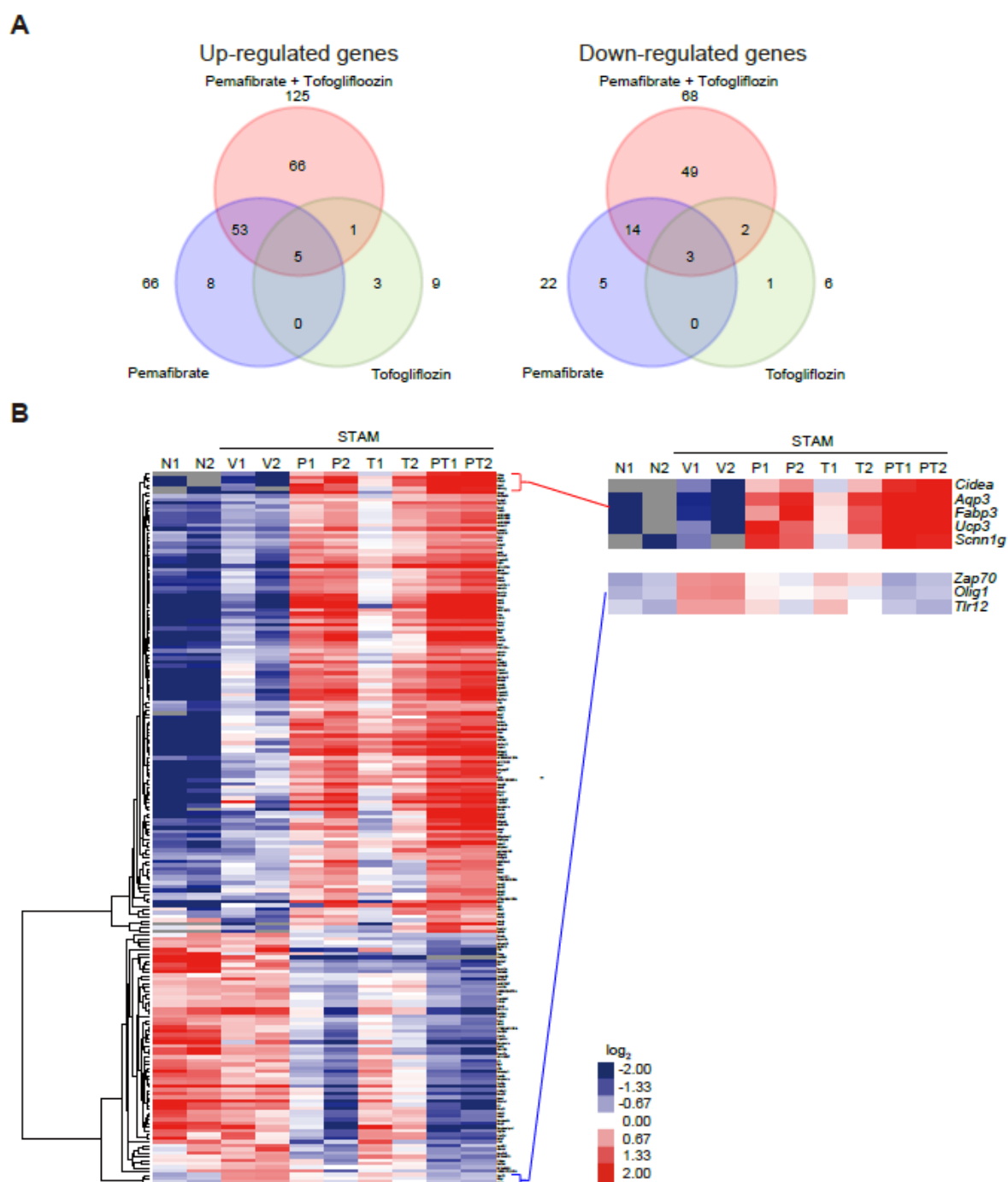


Figure S1. Pemafibrate and tofogliflozin combination regulated genes in STAM mouse liver. (A) Venn diagrams illustrating the overlap of either pemafibrate-, tofogliflozin-, and pemafibrate and tofogliflozin combination-treated STAM mouse liver. (B) Shown are genes whose transcript abundance is increased more than 2-fold or decreased greater than 2-fold in pemafibrate and tofogliflozin combination-treated STAM mice compared to control STAM mice group. N1 and N2: normal, C1 and C2: control, P1 and P2: pemafibrate, T1 and T2: tofogliflozin, PT1 and PT2: pemafibrate and tofogliflozin combination-treated group. For reference, a color intensity scale is included at the bottom.



mouse liver. Schematic representation of the fatty acid metabolism pathways in the liver is shown in left panel. Heat

map showing changes in expression of selected fatty acid metabolism-related markers in normal (N1 and N2) or STAM control (C1 and C2) or pemafibrate-treated (P1 and P2) or tofogliflozin-treated (T1 and T2) or pemafibrate and tofogliflozin combination-treated (PT1 and PT2) STAM mouse liver.

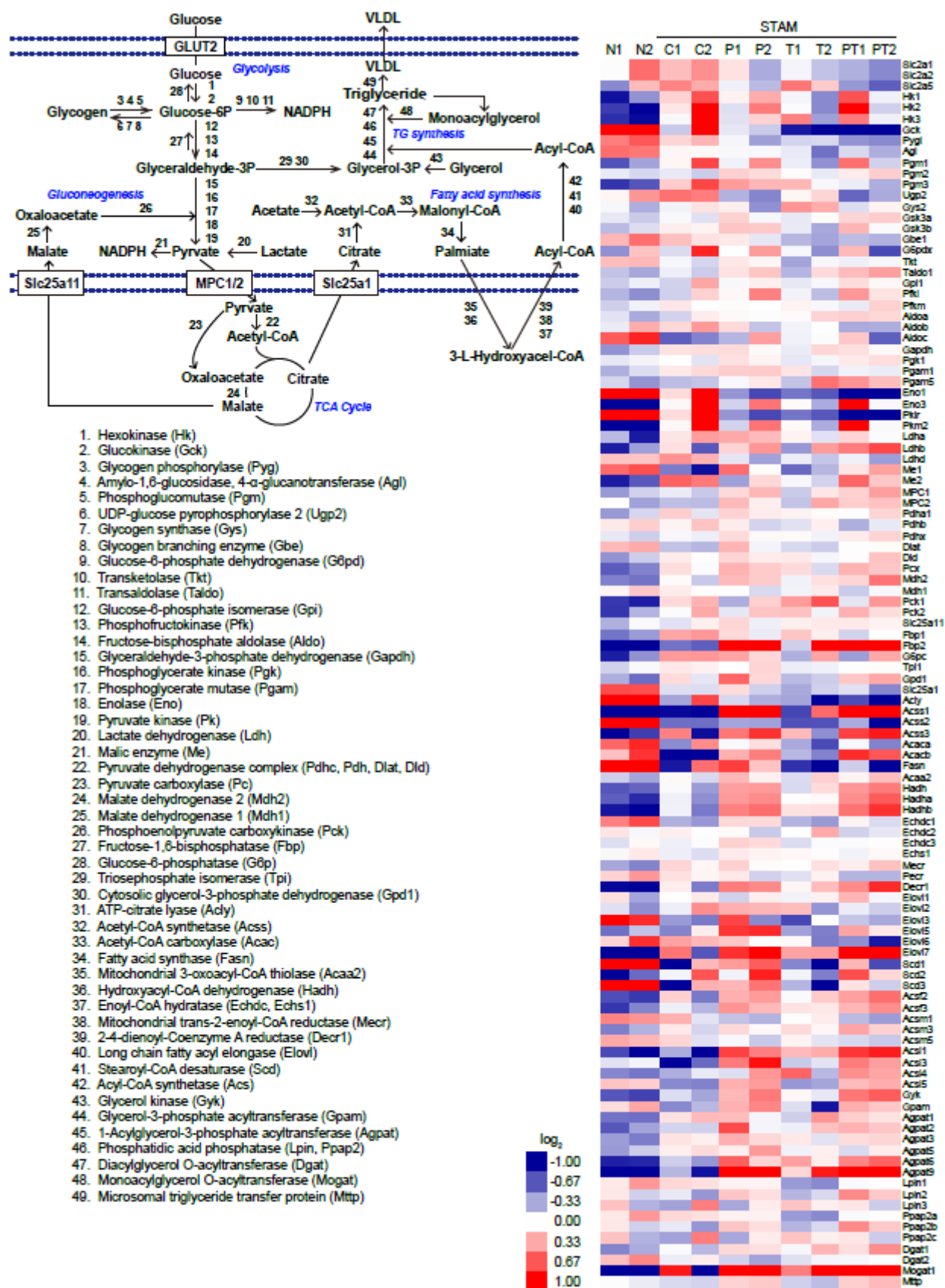


Figure S3. Effect of pemafibrate and tofogliflozin combination on glucose metabolism and triglyceride synthesis-related genes expression in STAM mouse liver. Schematic representation of the glycolytic, gluconeogenic, and triglyceride synthesis pathway in the liver is shown in left panel. Heat map showing changes in expression of selected glucose metabolism and triglyceride synthesis-related markers in normal (N1 and N2) or STAM control (C1 and C2) or pemafibrate-treated (P1 and P2) or tofogliflozin-treated (T1 and T2) or pemafibrate and tofogliflozin combination-treated (PT1 and PT2) STAM mouse liver.

Table S1. qPCR Primer lists.

Gene		Sequence
<i>Slc2a2</i>	Forward	5'- TGAGTTCCTTCCAGTTCGGC -3'
	Reverse	5'- AGCTTTCCGGTCATCCAGTG -3'
<i>Hk1</i>	Forward	5'- CAGTGTGAAGTCGGCCTGAT -3'
	Reverse	5'- GGCTCCCCATTCCGTGTAA -3'
<i>Gck</i>	Forward	5'- CGGATGGTGGATGAGAGCTC -3'
	Reverse	5'- AGCACAAGTCGTACCAGCTC -3'
<i>Gpi</i>	Forward	5'- GCTTGTCCCCTGAGACTTCC -3'
	Reverse	5'- CGCTTCGAGAAACCACTCCT -3'
<i>Pfk1</i>	Forward	5'- GCACTGACATGACCATTGGC -3'
	Reverse	5'- CCGTCCCATCACCTCCAAA -3'
<i>Aldob</i>	Forward	5'- CCACGAGACCCTCTACCAGA -3'
	Reverse	5'- TGTTTGTTCCTGCAAGCGGG -3'
<i>Pck1</i>	Forward	5'- TGC GGATCATGACTCGGATG -3'
	Reverse	5'- AGGCCCAGTTGTTGACCAAA -3'
<i>Tpi</i>	Forward	5'- CAGCAGGCACAGGAAGTACA -3'
	Reverse	5'- GCTCCAGTCACAGAACCTCC -3'
<i>Gpd1</i>	Forward	5'- GACCTGATGCAGACACCCAA -3'
	Reverse	5'- AAGCCAAGCCCATCACAGAA -3'
<i>Gpam</i>	Forward	5'- ACACAAGGCACAGAGGATGG -3'
	Reverse	5'- TTGCCTCTTGGACTCTGCTG -3'
<i>Agpat6</i>	Forward	5'- CCCTGCCATCTTTGGAGTGT -3'
	Reverse	5'- CCTCTCCTTGGCTCCTCTCT -3'
<i>Agpat9</i>	Forward	5'- TCCCTATCTGGCACCATCCA -3'
	Reverse	5'- GAGAGGTGTGATTGGCGACA -3'
<i>Lpin2</i>	Forward	5'- TTTCACGTACGCTTCGGGAA -3'
	Reverse	5'- TCCACAGCACTGCCATTGAT -3'
<i>Ppap2b</i>	Forward	5'- CAATTGCTCCGAGGGCTACA -3'
	Reverse	5'- AACATGGAGAAGGAGGCGTG -3'
<i>Dgat1</i>	Forward	5'- CTCAACTTTCCTCGGTCCCC -3'
	Reverse	5'- GATCAGCCCCACTTGAAGCT -3'
<i>Mttp</i>	Forward	5'- AGAGGACAGCTTTGTCACCG -3'
	Reverse	5'- CTTCCCGGGGATCATCCTTG -3'
<i>Lipe</i>	Forward	5'- TCCAGTTCACACCTGCCATC -3'
	Reverse	5'- GTCACACTGAGGCCTGTCTC -3'

Table S1. qPCR Primer lists (continued)

Gene		Sequence
<i>Mgll</i>	Forward	5'- ATCATCCCGGAGTCAGGACA -3'
	Reverse	5'- TTGGTCACTTCCGGAAGCTC -3'
<i>Mogat1</i>	Forward	5'- ACATCGTATCTCCACGTGGC -3'
	Reverse	5'- CTCCATCCTTGCTCAGCACA -3'
<i>Vldlr</i>	Forward	5'- TCAACTGCCCTTCTCGAACC -3'
	Reverse	5'- AGCCATCAACACAGTCTCGG -3'
<i>Aqp9</i>	Forward	5'- CGAGCCAAGAAGAACCTCGT -3'
	Reverse	5'- CCACCAGCCTTTTCTCGACT -3'
<i>Gyk</i>	Forward	5'- TGGCCTAATGAAAGCTGGGG -3'
	Reverse	5'- AGCACCTGTTCATACGTG -3'
<i>Aldoa</i>	Forward	5'- ACCCAGCAACAGACAGAGTT-3'
	Reverse	5'- CAGACAGCTCCTTCTTCTGC-3'
<i>Slc2a5</i>	Forward	5'- ATGGGTACAACGTAGCTGCC-3'
	Reverse	5'- GCGTCAAGGTGAAGGACTCA-3'
<i>Khk</i>	Forward	5'- GCTGGTGGTGGTGGACAT-3'
	Reverse	5'- AAGGAAAGGACAGTGCAGGA-3'

<i>Dak</i>	Forward	5'- TACGTTCTGACCTGGACACC-3'
	Reverse	5'- CTGCGATGACACCTGTTAGC-3'
<i>Dgat2</i>	Forward	5'- AAAGGATCTGCCCTGTCACG-3'
	Reverse	5'- CCTGCAGCTGTTTTTCCACC-3'
<i>Perk</i>	Forward	5'- TAGATGGACGAATCGCTGCA-3'
	Reverse	5'- GAGGAAGTTTTGTGGGTGCC-3'
<i>Irela</i>	Forward	5'- AGAAGATCCAGTCCTGCAGG-3'
	Reverse	5'- CCTTCGTTGTTCTTGCCTCC-3'
<i>Atf6</i>	Forward	5'- TGTCACCTGGTCCTGGAAACA-3'
	Reverse	5'- GGCGCTGCTGGAAGTAATAA-3'
<i>Grp78</i>	Forward	5'- CGAGGAGGAGGACAAGAAGG-3'
	Reverse	5'- GTTGGCTATGATCTCCACGC-3'
<i>Pdia1</i>	Forward	5'- AGAGCAACTTCGAGGAGGC-3'
	Reverse	5'- CTTTGGCATACTCGGGAGC-3'
<i>Nrf2</i>	Forward	5'- TGCCACATTCCCAAACAAG-3'
	Reverse	5'- GATGAGGGGCAGTGAAGACT-3'
<i>Bak1</i>	Forward	5'- TGCCTACGAACTCTTCACCA-3'
	Reverse	5'- GTAGACGTACAGGGCCAGAC-3'

Table S1. qPCR Primer lists (continued).

Gene		Sequence
<i>Bax</i>	Forward	5'- TCATGAAGACAGGGGCCTTT-3'
	Reverse	5'- AGACACTCGCTCAGCTTCTT-3'
<i>Traf2</i>	Forward	5'- ACTGTGCTGCCTGTGTCTAT-3'
	Reverse	5'- CCCTTCCAAGTGCATCCATC-3'
<i>Xbp1</i>	Forward	5'- GAAAGAAAGCCCGGATGAGC-3'
	Reverse	5'- CCATTCCCAAGCGTGTCTT-3'
<i>Phlda3</i>	Forward	5'- CGCCACATCTACTTCACGC-3'
	Reverse	5'- TCTTGAACCTTGACCAGGCCC-3'
<i>Cide A</i>	Forward	5'- CATACATCCAGCTCGCCCTT-3'
	Reverse	5'- CCAGGCCAGTTGTGATGACT-3'
<i>Cide C</i>	Forward	5'- TCATGGCTCACAGCTTGGAG-3'
	Reverse	5'- TCTGTCTCCACGATTGTGCC-3'