

Supporting Table S1. Clinical and Serological Characteristics of 98 Biopsy-Proven NAFLD Patients Used for RNA Sequencing Analysis

Variables	NAFL	NASH	<i>P</i> Value
Number	51	47	
Matteoni classification (1/2/3/4)	33/18/0/0	0/0/11/36	
Age (y)	50.1 ± 11.6	58.3 ± 13.8	<0.05
Sex (M/F)	43/8	21/26	<0.05
BMI (kg/m <sup>2</sup> )	26.6 ± 4.04	27.6 ± 4.37	N.S.
AST (U/L)	37.1 ± 13.7	49.3 ± 24.1	<0.05
ALT (U/L)	66.6 ± 33.1	79.7 ± 57.3	N.S.
GGT (U/L)	88.9 ± 67.1	69.8 ± 48.2	N.S.
ALP (U/L)	255.8 ± 77.9	260.9 ± 64.7	N.S.
Triglyceride (mg/dL)	161.5 ± 95.7	166.0 ± 91.1	N.S.
LDL-C (mg/dL)	128.2 ± 28.3	121.2 ± 33.6	N.S.
Fasting blood sugar (mg/dL)	103.6 ± 16.1	110.3 ± 35.5	N.S.
HbA1c (%)	5.84 ± 0.62	6.05 ± 1.18	<0.05
AFP (ng/mL)	3.34 ± 1.66	3.91 ± 1.99	N.S.
Albumin (g/dL)	4.63 ± 0.27	4.51 ± 0.36	N.S.
Platelet count (X 10 <sup>4</sup> /μL)	24.6 ± 6.16	20.8 ± 6.32	<0.05
FIB-4 index	1.07 ± 0.80	1.88 ± 1.21	<0.05

Abbreviations: AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; GGT, γ-glutamyltranspeptidase; LDL-C, low-density lipoprotein cholesterol; M, male; N.S., not significant.

Supporting Table S2. 137 Significantly Upregulated Genes in NASH Compared to NAFL Among 98 Biopsy-Proven NAFLD Patients (More Than 2.5-Fold)

Symbol	Row Names	Fold Change	Adjusted P Value	Gene Name	PANTHER Protein Class	NAFL vs NASH F3 in European NAFLD Patients (Govareo O et al., Sci Transl Med 2020)	
						Fold change	adjusted p value
UBD	ENSG00000213886	3.50	2.5E-19	Ubiquitin D		3.20	3.0E-05
THBS2	ENSG00000186340	2.72	8.4E-19	Thrombospondin-2		2.55	6.6E-10
LOXL4	ENSG00000138131	2.76	4.5E-17	Lysyl oxidase homolog 4	Oxidase (PC00175)	3.27	3.9E-09
DTNA	ENSG00000134769	5.02	9.9E-17	Dystrobrevin alpha	Ubiquitin-protein ligase (PC00234)	4.62	2.4E-11
LAMC3	ENSG00000050555	2.76	3.3E-15	Lamin subunit gamma-3	Extracellular matrix protein (PC00102)	2.77	1.2E-07
TNFRSF12A	ENSG00000006327	2.53	1.8E-14	Tumor necrosis factor receptor superfamily member 12A	Transmembrane signal receptor (PC00197)	2.67	1.5E-08
COL10A1	ENSG00000123500	4.98	9.8E-14	Collagen alpha-1(X) chain	Extracellular matrix structural protein (PC00103)	5.19	8.9E-09
KLHL29	ENSG00000119771	2.89	1.1E-13	Kelch-like protein 29	Scaffold/adaptor protein (PC00226)	1.77	3.2E-03
GPC3	ENSG00000147257	5.95	2.9E-13	Glypican-3		3.86	1.5E-04
SPATA21	ENSG00000187144	5.95	3.7E-13	Spermatogenesis-associated protein 21	Calmodulin-related (PC00061)	4.11	4.2E-08
DUSP8	ENSG00000184545	3.46	3.7E-13	Dual specificity protein phosphatase 8	Protein phosphatase (PC00195)	3.07	5.8E-06
COMP	ENSG00000105664	11.02	1.3E-12	Cartilage oligomeric matrix protein		3.96	7.9E-07
CR12	ENSG00000074410	8.44	1.0E-11	Carbonic anhydrase 12		4.34	1.8E-04
MMIP9	ENSG00000100395	3.53	1.8E-11	Matrix metalloproteinase-9	Metalloproteinase (PC00153)	2.29	1.4E-02
NALCN	ENSG00000102452	2.98	2.7E-11	Sodium leak channel nonselective protein	Ion channel (PC00133)	3.03	3.1E-09
FND1C1	ENSG00000164694	2.96	6.3E-11	Fibronectin type III domain-containing protein 1		2.64	9.7E-05
AKR1B10	ENSG00000198074	6.43	6.5E-11	Aldo-keto reductase family 1 member B10	Reductase (PC00198)	5.50	2.4E-05
CPZ	ENSG00000109625	2.53	6.6E-11	Carboxypeptidase Z	Protease (PC00190)	ns	ns
ELK2AP	ENSG00000213140	2.99	1.3E-10			ns	ns
THY1	ENSG00000154096	2.71	1.5E-10	Thy-1 membrane glycoprotein	Cell adhesion molecule (PC00069)	3.99	5.2E-10
ITGBL1	ENSG00000198542	2.77	5.9E-10	Integrin beta-like protein 1	Integrin (PC00126)	4.24	2.2E-13
OLR1	ENSG00000173391	3.57	6.0E-10	Oxidized low-density lipoprotein receptor 1		3.54	1.9E-04
HKDC1	ENSG00000156510	4.79	8.4E-10	Hexokinase HKDC1	Kinase (PC00137)	4.43	1.8E-06
FAM111B	ENSG00000189057	2.85	9.6E-10	Protein FAM111B		2.21	3.3E-03
LPL	ENSG00000175445	3.31	1.7E-09	Lipoprotein lipase	Lipase (PC00143)	3.94	8.3E-04
SPPI	ENSG00000118785	2.71	1.9E-09	Osteopontin	Cytokine (PC00083)	3.05	3.5E-05
RRM2	ENSG00000125222	2.52	2.3E-09	Reductase	Reductase (PC00198)	1.78	4.4E-02
SPR4	ENSG00000106483	4.40	4.9E-09	Ribonucleoside-diphosphate reductase subunit M2		3.31	9.1E-06
EFEMP1	ENSG00000115380	2.76	7.3E-09	Secreted frizzled-related protein 4	Transmembrane signal receptor (PC00197)	3.63	1.4E-07
PADI1	ENSG00000142623	8.48	8.0E-09	EGF-containing fibulin-like extracellular matrix protein 1	Extracellular matrix structural protein (PC00103)	3.63	1.4E-07
KIF23	ENSG00000137807	2.50	1.3E-08	Protein-arginine deiminase type-1		ns	ns
SLC22A12	ENSG00000197891	5.18	1.5E-08	Kinesin-like protein KIF23	Microtubule binding motor protein (PC00156)	ns	ns
LOXL1	ENSG00000129038	2.67	1.9E-08	Solute carrier family 22 member 12	Secondary carrier transporter (PC00258)	3.23	5.0E-04
KIF20A	ENSG00000112984	2.54	2.3E-08	Lysyl oxidase homolog 1	Oxidase (PC00175)	3.32	4.6E-08
CFAP221	ENSG00000163075	2.81	2.3E-08	Kinesin-like protein KIF20A	Microtubule binding motor protein (PC00156)	1.72	4.7E-02
CIDEA	ENSG00000187288	3.51	3.2E-08	Cilia- and flagella-associated protein 221	Structural protein (PC00211)	3.25	4.8E-10
MCM10	ENSG00000065328	2.94	3.3E-08	Cell death activator CIDE-3		2.78	7.9E-04
E2F8	ENSG00000129173	3.22	4.1E-08	Protein MCM10 homolog		2.16	1.2E-03
MFAP2	ENSG00000171222	2.85	5.2E-08	Transcription factor E2F8	General transcription factor (PC00259)	1.82	3.0E-02
FOXN4	ENSG00000139445	3.50	5.3E-08	Microtubular-associated protein 2		2.74	1.6E-04
FCGR1A	ENSG00000150337	3.50	8.2E-08	Forhead box protein N4		2.47	1.7E-02
CDC45	ENSG00000093039	2.79	9.6E-08	High-affinity immunoglobulin gamma 1c receptor 1	Immunoglobulin receptor superfamily (PC00124)	ns	ns
ATP5VD2	ENSG00000147614	3.82	1.0E-07	Cell division control protein 45 homolog	Replication origin binding protein (PC00199)	2.11	6.1E-04
CHI3L1	ENSG00000133048	2.95	1.5E-07	V-type proton ATPase subunit d 2	ATP synthase (PC00002)	ns	ns
TRIM9	ENSG00000100505	3.94	1.6E-07	Chitinase-3-like protein 1	Glycosidase (PC00110)	3.37	1.2E-04
DCDC2	ENSG00000146038	2.54	1.8E-07	Ubiquitin-protein ligase TRIM9	Ubiquitin-protein ligase (PC00234)	2.52	3.5E-02
TNFRSF9	ENSG00000049249	2.58	1.8E-07	Doublecortin domain-containing protein 2		2.33	8.5E-07
STMN2	ENSG00000104435	5.22	1.9E-07	Tumor necrosis factor receptor superfamily member 9	Transmembrane signal receptor (PC00197)	ns	ns
PAPP2A	ENSG00000116183	4.80	2.0E-07	Stathmin-2		17.54	5.7E-17
RASL11B	ENSG00000128045	2.87	2.3E-07	Pappalysin-2		ns	ns
MOXD1	ENSG00000079931	2.86	2.4E-07	Ras-like protein family member 11B		3.03	3.7E-06
WBSCR17	ENSG00000137269	3.54	6.7E-07	DBH-like monoxygenase protein 1	Hydroxylase (PC00122)	3.72	1.2E-07
LRR1C1	ENSG00000100510	2.53	7.1E-07	Leucine-rich repeat-containing protein 1		4.91	5.3E-10
MEOX1	ENSG00000005102	3.90	7.2E-07	Homeobox protein MOX1	Homeodomain transcription factor (PC00119)	2.49	1.9E-05
ACS10	ENSG00000169743	2.79	7.4E-07	Acyl-coenzyme A synthetase ACS10-mitochondrial	Ligase (PC00142)	2.86	2.0E-04
RAB3B	ENSG00000169213	3.29	7.9E-07	Ras-related protein Rab-3B		2.96	2.6E-03
EEF1A2	ENSG00000101210	4.22	1.1E-06	Elongation factor 1-alpha 2	Translation factor (PC00223)	5.71	3.3E-04
HECW1	ENSG000001002746	3.19	1.1E-06	E3 ubiquitin-protein ligase HECW1	Ubiquitin-protein ligase (PC00234)	2.99	3.0E-06
ROR2	ENSG00000169071	3.09	1.2E-06	Tyrosine-protein kinase transmembrane receptor ROR2	Transmembrane signal receptor (PC00197)	3.30	8.5E-07
CCL19	ENSG00000172724	2.78	1.2E-06	C-C motif chemokine 19	Cytokine (PC00083)	2.60	5.1E-03
CDCA2	ENSG00000184661	2.72	1.7E-06	Cell division cycle-associated protein 2		1.80	1.4E-02
TMEM92	ENSG00000167105	4.08	2.1E-06	Transmembrane protein 92		2.89	5.6E-05
CLEC5A	ENSG00000258227	3.14	2.9E-06	C-type lectin domain family 5 member A		1.91	5.9E-03
CFH25	ENSG00000138135	2.91	3.2E-06	Cholesterol 25-hydroxylase	Oxidase (PC00175)	2.70	2.0E-04
EPCAM	ENSG00000119888	2.57	3.2E-06	Epithelial cell adhesion molecule		3.57	9.9E-07
MDF1	ENSG00000112559	2.72	3.4E-06	MyoD family inhibitor		3.23	4.3E-06
LINC00319	ENSG00000188660	3.22	3.8E-06			ns	ns
DLGAP5	ENSG00000126787	2.58	4.5E-06	Disks large-associated protein 5	Scaffold/adaptor protein (PC00226)	2.03	8.0E-03
RAB25	ENSG00000132698	3.70	4.7E-06	Ras-related protein Rab-25		2.48	3.3E-03
CHRNA1	ENSG00000138435	4.58	6.8E-06	Acetylcholine receptor subunit alpha	Ligand-gated ion channel (PC00141)	ns	ns
CLIP2	ENSG00000180161	3.42	8.2E-06	Cartilage intermediate layer protein 2		1.89	2.1E-02
PDZK1IP1	ENSG00000162366	2.66	8.3E-06	PDZK1-interacting protein 1		4.61	1.2E-08
EAD2R	ENSG00000131080	2.50	9.8E-06	Tumor necrosis factor receptor superfamily member 27		2.90	6.6E-05
TRIM31	ENSG00000204616	2.87	1.0E-05	E3 ubiquitin-protein ligase TRIM31	Ubiquitin-protein ligase (PC00234)	3.05	3.5E-03
SLC2A14	ENSG00000173262	2.67	1.3E-05	Solute carrier family 2, facilitated glucose transporter member 14		2.25	1.1E-04
SLC2A5	ENSG00000142583	2.84	1.3E-05	Solute carrier family 2, facilitated glucose transporter member 5		ns	ns
CDH16	ENSG00000166589	5.65	1.4E-05	Cadherin-16	Cadherin (PC00057)	2.62	4.0E-04
GXYL2	ENSG00000172986	4.06	2.2E-05	Glucoside xylosyltransferase 2		1.88	3.8E-02
CDCA7	ENSG00000144354	2.85	2.6E-05	Cell division cycle-associated protein 7		ns	ns
CTSL1	ENSG00000131094	2.90	3.6E-05	C1q-related factor		2.44	4.3E-05
KLHL4	ENSG00000197705	2.69	3.7E-05	Kelch-like protein 14		ns	ns
ADAMDEC1	ENSG00000134028	3.09	4.9E-05	ADAMDEC1	Metalloproteinase (PC00153)	2.44	3.6E-03
FAP	ENSG00000178088	2.98	6.2E-05	Folyl endopeptidase FAP	Serine protease (PC00203)	5.08	9.7E-10
HCN1	ENSG00000164588	2.78	6.0E-05	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 1	Ion channel (PC00133)	ns	ns
SLC6A11	ENSG00000132164	9.91	6.0E-05	Sodium- and chloride-dependent GABA transporter 3	Primary active transporter (PC00068)	3.92	1.2E-04
CALCA	ENSG00000110880	3.72	7.4E-05	Calcitonin	Peptide hormone (PC00179)	2.12	7.6E-03
C6orf223	ENSG00000181577	6.04	7.9E-05	Uncharacterized protein C6orf223		9.57	2.0E-07
CLIC6	ENSG00000159212	2.58	9.9E-05	Chloride intracellular channel protein 6	Ion channel (PC00133)	3.48	1.3E-08
CCL20	ENSG00000115009	3.40	1.0E-04	C-C motif chemokine 20	Cytokine (PC00083)	4.46	7.1E-06
OSR1	ENSG00000143867	3.37	1.3E-04	Protein odd-skipped-related 1	Zinc finger transcription factor (PC00244)	2.50	7.1E-04
MPP2	ENSG00000108852	2.52	1.4E-04	MAGUK p55 subfamily member 2	Scaffold/adaptor protein (PC00226)	1.71	4.5E-02
KRT18	ENSG00000167767	2.86	1.6E-04	Keratin, type II cytoskeletal 18		3.50	2.6E-07
ACKR1	ENSG00000213088	2.53	1.8E-04	Atypical chemokine receptor 1	Transmembrane signal receptor (PC00197)	2.05	7.2E-03
NTM	ENSG00000182667	3.36	2.2E-04	Neurotrimin	Immunoglobulin (PC00123)	ns	ns
CASP5	ENSG00000137757	3.37	2.8E-04	Caspase-5	Protease (PC00190)	ns	ns
KCNH2	ENSG00000135118	2.71	3.5E-04	Potassium voltage-gated channel subfamily H member 2	Ion channel (PC00133)	ns	ns
TMEM178B	ENSG00000281115	2.72	3.7E-04	Transmembrane protein 178B		2.72	8.8E-04
LDLRAD1	ENSG00000203985	3.73	3.9E-04	Low-density lipoprotein receptor class A domain-containing protein 1		ns	ns
COL22A1	ENSG00000189438	4.23	3.9E-04	Collagen alpha-1(XII) chain	Extracellular matrix structural protein (PC00103)	ns	ns
EGOT	ENSG00000235947	3.98	4.4E-04			2.49	6.8E-05
PAGE4	ENSG00000101951	4.85	5.0E-04	P antigen family member 4		2.12	9.5E-03
KRT6C	ENSG00000170465	4.02	5.7E-04	Keratin, type II cytoskeletal 6C		2.25	1.6E-02
AKR1B15	ENSG00000227471	5.21	6.2E-04	Aldo-keto reductase family 1 member B15	Reductase (PC00198)	2.46	2.5E-02
TRPC6	ENSG00000137672	2.88	6.2E-04	Short transient receptor potential channel 6	Ion channel (PC00133)	ns	ns
KRT23	ENSG00000263309	2.83	7.1E-04	Keratin, type II cytoskeletal 23		ns	ns
BDRR2	ENSG00000168398	2.52	8.1E-04	B2 bradykinin receptor	G-protein coupled receptor (PC00021)	3.08	4.1E-05
KIRREL3	ENSG00000149571	2.90	8.1E-04	Kin of IRRE-like protein 3		ns	ns
ITGB6	ENSG00000115221	2.72	8.4E-04	Integrin beta-6	Integrin (PC00126)	3.88	1.2E-07
RTRN2	ENSG00000162010	2.52	9.3E-04	Rhotekin-2	Nonmotor actin binding protein (PC00165)	ns	ns
ANKK2P3	ENSG00000216740	2.89	1.1E-03			ns	ns
APLP1	ENSG00000105290	2.38	1.2E-03	Amyloid-like protein 1	Protease inhibitor (PC00191)	ns	ns
CAPN13	ENSG00000162949	4.57	1.3E-03	Calpain-13	Cysteine protease (PC00081)	ns	ns
GPR84	ENSG00000139572	3.09	1.3E-03	G-protein coupled receptor 84	G-protein coupled receptor (PC00021)	ns	ns
GABBR2	ENSG00000136928	3.27	1.4E-03	Gamma-aminobutyric acid type B receptor subunit 2	G-protein coupled receptor (PC00021)	ns	ns
LYPD1	ENSG00000150551	3.34	2.1E-03	Lyb6/PLAUR domain-containing protein 1		2.10	1.7E-02
FGF23	ENSG00000118972</						

PHEX	ENSG00000102174	2.72	3.7E-03	Phosphate-regulating neutral endopeptidase PHEX	Metalloprotease (PC00153)	ns	ns
TUBB3	ENSG00000258947	2.72	5.2E-03	Tubulin beta-3 chain	Tubulin (FC00229)	ns	ns
COL11A1	ENSG00000060718	3.57	6.2E-03	Collagen alpha-1(XI) chain	Extracellular matrix structural protein (PC00103)	1.87	3.9E-02
SMC1B	ENSG00000077935	2.70	7.5E-03	Structural maintenance of chromosomes protein 1B		ns	ns
PF4V1	ENSG00000109272	2.60	8.1E-03	Platelet factor 4 variant	Chemokine (PC00074)	2.16	3.7E-03
ASCL1	ENSG00000139352	3.53	9.1E-03	Achaete-scute homolog 1	Basic helix-loop-helix transcription factor (PC00055)	ns	ns
TNFRSF13C	ENSG00000159958	2.53	1.0E-02	Tumor necrosis factor receptor superfamily member 13C	Transmembrane signal receptor (PC00197)	ns	ns
TMEM132C	ENSG00000181234	2.65	1.2E-02	Transmembrane protein 132C		1.95	7.6E-03
FCGR3C		2.79	1.3E-02			ns	ns
KCNA2	ENSG00000177301	2.60	1.7E-02	Potassium voltage-gated channel subfamily A member 2	Voltage-gated ion channel (PC00241)	ns	ns
KRTAP5-AS1	ENSG00000233930	2.50	1.7E-02			3.04	5.2E-05
JPH1	ENSG00000104369	2.52	2.0E-02	Junctophilin-1		ns	ns
CDX1	ENSG00000113722	2.50	2.1E-02	Homeobox protein CDX-1	Homeodomain transcription factor (PC00119)	ns	ns
FAM130A		2.54	2.5E-02	ALK and LTK ligand 1		ns	ns
GLIS1	ENSG00000174332	2.84	2.5E-02	Zinc finger protein GLIS1	DNA-binding transcription factor (PC00218)	ns	ns

Abbreviation: ADAM, a disintegrin and metalloproteinase domain-like protein; DBH, dopamine beta-hydroxylase; EGF, epidermal growth factor; GABA, gamma-aminobutyric acid; MAGUK, membrane-associated guanylate kinase; ns, not significant; PLAUR, plasminogen activator, urokinase receptor.

Supporting Table S3. Clinical and Serological Characteristics of 98 Biopsy-Proven NAFLD Patients Used for RNA Sequencing Analysis

Variables	Fibrosis Stage		
	0	1–2	3–4
Number	8	77 (F1:51, F2:26)	13 (F3:9, F4:4)
NAFL/NASH	8/0	43/34	0/13
Age (y)	47.5 ± 7.03	53.4 ± 13.7	62 ± 10.6
Sex (M/F)	8/0	51/26	5/8
BMI (kg/m <sup>2</sup> )	26.3 ± 3.52	26.7 ± 3.91	29.8 ± 5.41
AST (U/L)	31 ± 8.62	42.7 ± 20.2	52.1 ± 22.2
ALT (U/L)	58.1 ± 22.8	74.4 ± 49.6	73.2 ± 38.3
GGT (U/L)	105.4 ± 79.9	74.5 ± 54.9	94.5 ± 68.9
ALP (U/L)	247.5 ± 48.7	257.8 ± 70.3	267.6 ± 92.6
Triglyceride (mg/dL)	189.5 ± 124.3	165.9 ± 94.1	134.1 ± 59.1
LDL-C (mg/dL)	118.1 ± 43.2	126.2 ± 30.3	120.8 ± 28.4
Fasting blood sugar (mg/dL)	106.1 ± 25.2	105.5 ± 26.9	114.8 ± 31.3
HbA1c (%)	6.00 ± 1.14	5.84 ± 0.84	6.5 ± 1.21
AFP (ng/mL)	2.99 ± 1.23	3.45 ± 1.56	4.99 ± 2.91
Albumin (g/dL)	4.60 ± 0.20	4.60 ± 0.31	4.39 ± 0.36
Hyaluronic acid (ng/mL)	11.7 ± 12.8	26.1 ± 24.7	56.6 ± 34.5
Platelet count (X 10 <sup>4</sup> /μL)	24.3 ± 2.26	23.7 ± 6.43	16.0 ± 4.29
NAFLD Fibrosis Score	-2.95 ± 0.77	-2.52 ± 1.51	-0.44 ± 1.20
FIB-4 index	0.81 ± 0.20	1.31 ± 0.91	2.73 ± 1.49

Abbreviations: AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; GGT, γ-glutamyltranspeptidase; LDL-C, low-density lipoprotein cholesterol; M, male.

Supporting Table S4. Correlation Between THBS2 mRNA Levels and Clinicopathological Parameters of 98 Biopsy-Proven NAFLD Patients Used for RNA Sequencing Analysis

Variables	<i>r</i>	<i>P</i> Value
Age (y)	0.237	N.S.
BMI (kg/m <sup>2</sup> )	0.264	<0.05
AST (U/L)	0.387	<0.05
ALT (U/L)	0.173	N.S.
GGT (U/L)	-0.068	N.S.
ALP (U/L)	0.061	N.S.
Triglyceride (mg/dL)	-0.0293	N.S.
LDL-C (mg/dL)	-0.009	N.S.
Fasting blood sugar (mg/dL)	0.188	N.S.
HbA1c (%)	0.193	N.S.
AFP (ng/mL)	0.23	N.S.
Albumin (g/dL)	-0.156	N.S.
Hyaluronic acid (ng/mL)	0.395	<0.05
Platelet count (10 <sup>4</sup> /μL)	-0.331	<0.05
FIB-4 index	0.446	<0.05
NAFLD Fibrosis Score	0.401	<0.05

Abbreviations: AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT,  $\gamma$ -glutamyltranspeptidase; LDL-C, low-density lipoprotein cholesterol; N.S., not significant.

Supporting Table S5. REACTOME Pathways Correlated With Intrahepatic THBS2 Level in 98 Biopsy-Proven NAFLD Patients

REACTOME Pathways	Correlation Coefficient With Intrahepatic THBS2 Level
EXTRACELLULAR_MATRIX_ORGANIZATION	0.905
INTEGRIN_CELL_SURFACE_INTERACTIONS	0.904
COLLAGEN_DEGRADATION	0.899
CROSSLINKING_OF_COLLAGEN_FIBRILS	0.896
ASSEMBLY_OF_COLLAGEN_FIBRILS_AND_OTHER_MULTIMERIC_STRUCTURES	0.895
ECM_PROTEOGLYCANS	0.895
COLLAGEN_FORMATION	0.894
DEGRADATION_OF_THE_EXTRACELLULAR_MATRIX	0.890
ELASTIC_FIBRE_FORMATION	0.877
COLLAGEN_CHAIN_TRIMERIZATION	0.870
ANCHORING_FIBRIL_FORMATION	0.868
NON_INTEGRIN_MEMBRANE_ECM_INTERACTIONS	0.866
SIGNALING_BY_RECEPTOR_TYROSINE_KINASES	0.864
COLLAGEN_BIOSYNTHESIS_AND_MODIFYING_ENZYMES	0.858
MOLECULES_ASSOCIATED_WITH_ELASTIC_FIBRES	0.856
SIGNALING_BY_PDGF	0.844
MET_ACTIVATES_PTK2_SIGNALING	0.837
PLATELET_ADHESION_TO_EXPOSED_COLLAGEN	0.835
MET_PROMOTES_CELL_MOTILITY	0.830
O_GLYCOSYLATION_OF_TSR_DOMAIN_CONTAINING_PROTEINS	0.822
PLATELET_ACTIVATION_SIGNALING_AND_AGGREGATION	0.820
RESPONSE_TO_ELEVATED_PLATELET_CYTOSOLIC_CA2	0.819
DISEASES_OF_GLYCOSYLATION	0.818
GP1B_IX_V_ACTIVATION_SIGNALING	0.818
HS_GAG_DEGRADATION	0.818
HEMOSTASIS	0.817
DEFECTIVE_EXT2_CAUSES_EXOSTOSES_2	0.814
PLATELET_AGGREGATION_PLUG_FORMATION	0.813
SIGNALING_BY_MET	0.813
SEMAPHORIN_INTERACTIONS	0.812
DISEASES_ASSOCIATED_WITH_GLYCOSAMINOGLYCAN_METABOLISM	0.812
NCAM1_INTERACTIONS	0.802
POST_TRANSLATIONAL_PROTEIN_MODIFICATION	0.796
GLYCOSAMINOGLYCAN_METABOLISM	0.795
DISEASE	0.791
L1CAM_INTERACTIONS	0.785
METABOLISM_OF_FAT_SOLUBLE_VITAMINS	0.785
HS_GAG_BIOSYNTHESIS	0.784
LAMININ_INTERACTIONS	0.783
NUCLEAR_EVENTS_KINASE_AND_TRANSCRIPTION_FACTOR_ACTIVATION	0.782
CASPASE_MEDIATED_CLEAVAGE_OF_CYTOSKELETAL_PROTEINS	0.780
HEPARAN_SULFATE_HEPARIN_HS_GAG_METABOLISM	0.778
SYNDECAN_INTERACTIONS	0.776
SCAVENGING_BY_CLASS_A_RECEPTORS	0.775
MAPK_FAMILY_SIGNALING_CASCADES	0.773
O_LINKED_GLYCOSYLATION	0.770
CHONDROITIN_SULFATE_DERMATAN_SULFATE_METABOLISM	0.770
CELL_CELL_COMMUNICATION	0.770
CELL_SURFACE_INTERACTIONS_AT_THE_VASCULAR_WALL	0.770
DISSOLUTION_OF_FIBRIN_CLOT	0.770
DISEASES_OF_METABOLISM	0.766
EPH_EPHRIN_SIGNALING	0.762
NCAM_SIGNALING_FOR_NEURITE_OUT_GROWTH	0.760
NERVOUS_SYSTEM_DEVELOPMENT	0.751
REGULATION_OF_INSULIN_LIKE_GROWTH_FACTOR_IGF_TRANSPORT_AND_UPTAKE_BY_INSULIN_LIKE_GROWTH_FACTOR_BINDING_PROTEINS_IGFBPS	0.751

Supporting Table 6. Gene List in the Module That THBS2 Gene Belongs to Identified by WGCNA Analysis

Gene	Ensemble Gene ID	Gene Name	PANTHER Protein Class
ADAM28	ENSG0000042980	Disintegrin and metalloproteinase domain-containing protein 28	Metalloprotease (PC00153)
AEBP1	ENSG00000106624	Adipocyte enhancer-binding protein 1	
ANTXR1	ENSG00000169604	Anthrax toxin receptor 1	Cell adhesion molecule (PC00069)
BHLHE41	ENSG00000123095	Class E basic helix-loop-helix protein 41	Basic helix-loop-helix transcription factor (PC00055)
BOC	ENSG00000144857	Brother of CDO	
C7	ENSG00000112936	Complement component C7	
CACNA1C	ENSG00000151067	Voltage-dependent L-type calcium channel subunit alpha-1C	
CCDC80	ENSG00000091986	Coiled-coil domain-containing protein 80	
CCL19	ENSG00000172724	C-C motif chemokine 19	Cytokine (PC00083)
CCL21	ENSG00000137077	C-C motif chemokine 21	Cytokine (PC00083)
CD1E	ENSG00000158488	T-cell surface glycoprotein CD1e, membrane-associated	
CD79A	ENSG00000105369	B-cell antigen receptor complex-associated protein alpha chain	Immunoglobulin receptor superfamily (PC00124)
CDH11	ENSG00000140937	Cadherin-11	
CH25H	ENSG00000138135	Cholesterol 25-hydroxylase	Oxidase (PC00175)
CLDN11	ENSG00000013297	Claudin-11	Tight junction (PC00214)
CLSTN2	ENSG00000158258	Calsyntenin-2	Membrane traffic protein (PC00150)
COL10A1	ENSG00000123500	Collagen alpha-1(X) chain	Extracellular matrix structural protein (PC00103)
COL12A1	ENSG00000111799	Collagen alpha-1(XII) chain	Extracellular matrix structural protein (PC00103)
COL14A1	ENSG00000187955	Collagen alpha-1(XIV) chain	Extracellular matrix structural protein (PC00103)
COL15A1	ENSG00000204291	Collagen alpha-1(XV) chain	Extracellular matrix structural protein (PC00103)
COL16A1	ENSG00000084636	Collagen alpha-1(XVI) chain	Extracellular matrix structural protein (PC00103)
COL1A1	ENSG00000108821	Collagen alpha-1(I) chain	Extracellular matrix structural protein (PC00103)
COL1A2	ENSG00000164692	Collagen alpha-2(I) chain	Extracellular matrix structural protein (PC00103)
COL4A1	ENSG00000187498	Collagen alpha-1(IV) chain	Extracellular matrix structural protein (PC00103)
COL4A3	ENSG00000169031	Collagen alpha-3(IV) chain	Extracellular matrix structural protein (PC00103)
COL4A4	ENSG00000081052		
COL5A1	ENSG00000130635	Collagen alpha-1(V) chain	Extracellular matrix structural protein(PC00103)
CPXM2	ENSG00000121898	Inactive carboxypeptidase-like protein X2	
CPZ	ENSG00000109625	Carboxypeptidase Z	
CRISPLD2	ENSG00000103196	Cysteine-rich secretory protein LCCL domain-containing 2	Defense/immunity protein (PC00090)
DKK3	ENSG00000050165	Dickkopf-related protein 3	
DTNA	ENSG00000134769	Dystrobrevin alpha	
EFEMP1	ENSG00000115380	EGF-containing fibulin-like extracellular matrix protein 1	Extracellular matrix structural protein (PC00103)
ELK2AP	ENSG00000213140		
EMP1	ENSG00000134531	Epithelial membrane protein 1	Cytoskeletal protein (PC00085)
EPHA3	ENSG00000044524	Ephrin type-A receptor 3	
F13A1	ENSG00000124491	Coagulation factor XIII A chain	Transferase (PC00220)
F3	ENSG00000117525	Tissue factor	Transmembrane signal receptor (PC00197)
FAP	ENSG00000078098	Prolyl endopeptidase	Serine protease (PC00203)
FBLN5	ENSG00000140092	Fibulin-5	Extracellular matrix structural protein (PC00103)
FCRL5	ENSG00000143297	Fc receptor-like protein 5	Immunoglobulin receptor superfamily (PC00124)
FHL2	ENSG00000115641	Four and a half LIM domains protein 2	Transcription cofactor (PC00217)
FMO2	ENSG00000094963	Dimethylaniline monooxygenase [N-oxide-forming] 2	Oxygenase (PC00177)
FNDC1	ENSG00000164694	Fibronectin type III domain-containing protein 1	
FZD7	ENSG00000155760	Frizzled-7	Transmembrane signal receptor (PC00197)
GEM	ENSG00000164949	GTP-binding protein GEM	
GLIS2	ENSG00000126603	Zinc finger protein GLIS2	C2H2 zinc finger transcription factor (PC00248)
GPC3	ENSG00000147257	Glypican-3	
GPC4	ENSG00000076716	Glypican-4	
GSN	ENSG00000148180	Gelsolin	Nonmotor actin binding protein (PC00165)
IgL5	ENSG00000254709	Immunoglobulin lambda-like polypeptide 5	Immunoglobulin (PC00123)
IGSF10	ENSG00000152580	Immunoglobulin superfamily member 10	
IRF4	ENSG00000137265	Interferon regulatory factor 4	Winged helix/forkhead transcription factor (PC00246)
ITGBL1	ENSG00000198542	Integrin beta-like protein 1	Cell adhesion molecule (PC00069)
ITIH5	ENSG00000123243	Inter-alpha-trypsin inhibitor heavy chain H5	Protease inhibitor (PC00191)
JCHAIN	ENSG00000132465	Immunoglobulin J chain	Immunoglobulin (PC00123)
KIAA1211		Cancer-related regulator of actin dynamics	
LAMA2	ENSG00000196569	Laminin subunit alpha-2	Extracellular matrix protein (PC00102)
LAMC3	ENSG00000050555	Laminin subunit gamma-3	Extracellular matrix protein (PC00102)
LOXL1	ENSG00000129038	Lysyl oxidase homolog 1	
LOXL4	ENSG00000138131	Lysyl oxidase homolog 4	
LRRC1	ENSG00000137269	Leucine-rich repeat-containing protein 1	
LTBP2	ENSG00000119681	Latent-transforming growth factor beta-binding protein 2	Extracellular matrix structural protein (PC00103)
LUM	ENSG00000139329	Lumican	
MAP1A	ENSG00000166963	Microtubule-associated protein 1A	Nonmotor microtubule binding protein (PC00166)
MAP1B	ENSG00000131711	Microtubule-associated protein 1B	Nonmotor microtubule binding protein (PC00166)
MFAP4	ENSG00000166482	Microfibril-associated glycoprotein 4	Intercellular signal molecule (PC00207)
MMP2	ENSG00000087245	72 kDa type IV collagenase	Metalloprotease (PC00153)
MOXD1	ENSG00000079931	DBH-like monooxygenase protein 1	
MZB1	ENSG00000170476	Marginal zone B- and B1-cell-specific protein	
NALCN	ENSG00000102452	Sodium leak channel non-selective protein	
NAV3	ENSG00000067798	Neuron navigator 3	
NEXN	ENSG00000162614	Nexilin	Scaffold/adaptor protein (PC00226)
NFASC	ENSG00000163531	Neurofascin	
OSMR	ENSG00000145623	Oncostatin-M-specific receptor subunit beta	Cytokine (PC00083)
PAQR5	ENSG00000137819	Membrane progesterin receptor gamma	G-protein coupled receptor (PC00021)
PLCXD3	ENSG00000182836	PI-PLC X domain-containing protein 3	
PODN	ENSG00000174348	Podocan	
PODXL	ENSG00000128567	Podocalyxin	Cell adhesion molecule (PC00069)
PTK7	ENSG00000112655	Inactive tyrosine-protein kinase 7	Tyrosine protein kinase receptor (PC00233)
ROR2	ENSG00000169071	Tyrosine-protein kinase transmembrane receptor ROR2	

RRAD	ENSG00000166592	GTP-binding protein RAD	
SEMA3C	ENSG00000075223	Semaphorin-3C	Membrane-bound signaling molecule (PC00152)
SFRP4	ENSG00000106483	Secreted frizzled-related protein 4	Transmembrane signal receptor (PC00197)
SGCD	ENSG00000170624	Delta-sarcoglycan	Cytoskeletal protein (PC00085)
SLIT2	ENSG00000145147	Slit homolog 2 protein	
SPON1	ENSG00000262655	Spondin-1	Cell adhesion molecule (PC00069)
SPP1	ENSG00000118785	Osteopontin	Cytokine (PC00083)
SSC5D	ENSG00000179954	Soluble scavenger receptor cysteine-rich domain-containing protein SSC5D	Serine protease (PC00203)
STMN2	ENSG00000104435	Stathmin-2	
SUSD2	ENSG00000099994	Sushi domain-containing protein 2	
THBD	ENSG00000178726	Thrombomodulin	Transmembrane signal receptor (PC00197)
THBS1	ENSG00000137801	Thrombospondin-1	
THBS2	ENSG00000186340	Thrombospondin-2	
THY1	ENSG00000154096	Thy-1 membrane glycoprotein	Cell adhesion molecule (PC00069)
TNXB	ENSG00000206258	Tenascin-X	Extracellular matrix protein (PC00102)
VCAN	ENSG00000038427	Versican core protein	Extracellular matrix glycoprotein (PC00100)
VWF	ENSG00000110799	von Willebrand factor	Extracellular matrix protein (PC00102)
WNT4	ENSG00000162552	Protein Wnt-4	Intercellular signal molecule (PC00207)

Abbreviation: CDO, cysteine dioxygenase; DBH, dopamine beta-hydroxylase; EGF, epidermal growth factor; GTP, guanosine triphosphate; ID, identifier; PI-PLC, phosphoinositide phospholipase C; WGCNA, weighted correlation network analysis.



Supporting Table S7. Correlation Between mRNA Levels of THBS2 and Collagen Genes in the Module

Variables	<i>r</i>	<i>P</i> Value
COL1A1	0.899	<0.05
COL1A2	0.912	<0.05
COL4A1	0.863	<0.05
COL4A3	0.558	<0.05
COL4A4	0.741	<0.05
COL5A1	0.871	<0.05
COL10A1	0.77	<0.05
COL12A1	0.742	<0.05
COL14A1	0.808	<0.05
COL15A1	0.675	<0.05
COL16A1	0.793	<0.05

Supporting Table S8. Clinical and Serological Characteristics of 213 Biopsy-Proven NAFLD Patients

Variables	NAFL	NASH	<i>P</i> Value
Number	121	92	
Matteoni classification (1/2/3/4)	94/27/0/0	0/0/35/57	
Age (y)	58.0 ± 12.5	66.3 ± 12.1	<0.05
Sex (M/F)	47/74	31/61	N.S.
BMI (kg/m <sup>2</sup> )	27.4 ± 4.04	27.8 ± 5.61	N.S.
AST (U/L)	48.4 ± 24.6	68.7 ± 32.2	<0.05
ALT (U/L)	72.8 ± 40.5	88.3 ± 56.6	<0.05
GGT (U/L)	84.1 ± 74.2	94.8 ± 69.4	N.S.
ALP (U/L)	278.0 ± 102.5	285.1 ± 103.9	N.S.
Triglyceride (mg/dL)	166.5 ± 82.6	178.3 ± 77.4	N.S.
LDL-C (mg/dL)	131.4 ± 34.3	126.1 ± 30.7	N.S.
Fasting blood sugar (mg/dL)	114.7 ± 33.3	124.7 ± 52.3	N.S.
HbA1c (%)	6.30 ± 0.92	6.61 ± 1.63	N.S.
AFP (ng/mL)	3.84 ± 1.83	5.35 ± 2.99	<0.05
Albumin (g/dL)	4.42 ± 0.28	4.29 ± 0.36	<0.05
Hyaluronic acid (ng/mL)	55.7 ± 67.6	130.2 ± 138.6	<0.05
Platelet count (X 10 <sup>4</sup> /μL)	25.0 ± 7.10	19.5 ± 6.63	<0.05
FIB-4 index	1.56 ± 1.16	2.91 ± 1.61	<0.05
NAFLD activity score	2.50 ± 0.93	4.25 ± 1.21	<0.05

Abbreviations: AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; GGT, γ-glutamyltranspeptidase; LDL-C, low-density lipoprotein cholesterol; M, male; N.S., not significant.

Supporting Table S9. Clinical and Serological Characteristics of 213 Biopsy-Proven NAFLD Patients Stratified by Fibrosis Stage

Variables	Fibrosis Stage				
	0	1	2	3	4
Number	86	55	21	34	17
NAFL/NASH	82/4	24/31	4/17	8/26	3/14
Age (y)	58.6 ± 13.5	61.0 ± 13.3	65.0 ± 12.8	65.3 ± 10.1	67.1 ± 11.0
Sex (M/F)	35/51	18/37	5/16	15/19	5/12
BMI (kg/m <sup>2</sup> )	26.8 ± 3.89	27.7 ± 4.24	27.3 ± 3.44	28.2 ± 5.57	30.6 ± 8.27
AST (U/L)	45.5 ± 21.9	63.3 ± 33.9	75.4 ± 29.2	65.1 ± 31.1	57.6 ± 29.5
ALT (U/L)	70.5 ± 38.7	95.9 ± 62.6	94.0 ± 41.1	79.2 ± 47.8	54.6 ± 31.4
GGT (U/L)	78.7 ± 70.5	87.7 ± 71.1	123.2 ± 74.0	104.6 ± 82.9	67.9 ± 38.2
ALP (U/L)	274.1 ± 104.2	283.5 ± 105.3	298.3 ± 99.3	290.5 ± 104.3	268.1 ± 96.4
Triglyceride (mg/dL)	172.8 ± 82.5	179.2 ± 75.6	173 ± 67.6	181.1 ± 95.2	120.4 ± 50.5
LDL-C (mg/dL)	132.4 ± 34.8	137.2 ± 30.3	120.1 ± 31.9	123.4 ± 29.5	107.8 ± 27.6
Fasting blood sugar (mg/dL)	118.1 ± 38.7	116.7 ± 46.9	106.6 ± 30.1	129.6 ± 47.0	125.2 ± 51.1
HbA1c (%)	6.27 ± 0.89	6.50 ± 1.50	6.08 ± 0.83	6.91 ± 1.63	6.52 ± 1.66
AFP (ng/mL)	4.03 ± 2.56	3.96 ± 2.03	5.04 ± 2.67	5.52 ± 2.34	6.10 ± 2.94
Albumin (g/dL)	4.42 ± 0.28	4.44 ± 0.30	4.26 ± 0.23	4.27 ± 0.43	4.15 ± 0.33
Hyaluronic acid (ng/mL)	51.5 ± 72.8	53.9 ± 56.4	127.4 ± 102.2	142.9 ± 140.9	225 ± 178.7
Platelet count (X 10 <sup>4</sup> /μL)	25.3 ± 6.96	23.8 ± 6.82	21.2 ± 9.0	19.2 ± 4.16	13.7 ± 4.44
FIB-4 index	1.45 ± 0.87	1.92 ± 1.26	2.94 ± 1.62	2.70 ± 1.42	4.29 ± 2.19
NAFLD Fibrosis Score	-2.17 ± 1.45	-1.67 ± 1.43	-1.25 ± 1.45	-0.60 ± 1.05	0.80 ± 1.13

Abbreviations: AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; GGT, γ-glutamyltranspeptidase; LDL-C, low-density lipoprotein cholesterol; M, male.

Supporting Table S10. Correlation Between Serum TSP-2 Levels and Clinicopathological Parameters Among 213 Biopsy-Proven NAFLD Patients

Variables	<i>r</i>	<i>P</i> Value
Age (y)	0.143	N.S.
BMI (kg/m <sup>2</sup> )	0.153	N.S.
AST (U/L)	0.349	<0.05
ALT (U/L)	0.085	N.S.
GGT (U/L)	0.108	N.S.
ALP (U/L)	0.055	N.S.
Triglyceride (mg/dL)	0.067	N.S.
LDL-C (mg/dL)	-0.216	<0.05
Fasting blood sugar (mg/dL)	0.059	N.S.
HbA1c (%)	0.105	N.S.
AFP (ng/mL)	0.282	<0.05
Albumin (g/dL)	-0.222	<0.05
Hyaluronic acid (ng/mL)	0.378	<0.05
Platelet count (10 <sup>4</sup> /μL)	-0.201	<0.05
FIB-4 index	0.435	<0.05
NAFLD Fibrosis Score	0.335	<0.05

Abbreviations: AFP, alpha-fetoprotein; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; GGT, γ-glutamyltranspeptidase; LDL-C, low-density lipoprotein cholesterol; M, male; N.S., not significant.

1 **Supporting Figure S1.** Pathway analysis focusing on gene ontology biological  
2 processes enriched in the RNA sequencing data of liver tissues from 98 patients  
3 with biopsy-proven NAFLD. Significantly upregulated and downregulated  
4 pathways in NASH patients compared to NAFL patients are shown in red and  
5 green, respectively, with *P* values.

6

7 **Supporting Figure S2.** CIBERSORT analysis of the RNA sequencing data of  
8 liver tissues from 98 patients with biopsy-proven NAFLD. **(A)** Estimated  
9 intrahepatic population of each immune cell type in 98 NAFLD patients. **(B)**  
10 Absolute immune score and estimated intrahepatic relative fraction of M1  
11 macrophages, M2 macrophages, NK cells, mast cells, and dendritic cells in NAFL  
12 ( $n = 51$ ) and NASH ( $n = 47$ ) patients ( $*P < 0.05$ ).

13

14 **Supporting Figure S3.** Heatmap showing the intrahepatic expression of 63  
15 genes registered in the cytokine–cytokine receptor interaction pathway in the  
16 Kyoto Encyclopedia of Genes and Genomes database in 98 patients with biopsy-  
17 proven NAFLD.

18

19 **Supporting Figure S4. (A)** mRNA expression levels of *THBS2* were evaluated  
20 by qPCR in 98 NAFLD patients ( $*P < 0.05$ ). **(B)** Receiver operating characteristic  
21 curve for qPCR-based intrahepatic *THBS2* mRNA levels for the diagnosis of  
22 NASH among 98 NAFLD patients. The AUROC is shown.

23

24 **Supporting Figure S5.** Total 98 NAFLD patients were diagnosed based on  
25 NAFLD activity score (NAS). Patients with NAS score of equal or above 5 were  
26 defined as NASH. **(A)** mRNA expression levels of *THBS2* by RNA sequencing  
27 ( $*P < 0.05$ ). **(B)** Receiver operating characteristic curve for intrahepatic *THBS2*  
28 mRNA levels for the diagnosis of NASH among 98 NAFLD patients. The AUROC  
29 is shown.

30

31 **Supporting Figure S6. (A)** mRNA expression levels of *THBS2* by RNA  
32 sequencing in 206 NAFLD patients ( $*P < 0.05$ ) (GSE135251). **(B)** Receiver  
33 operating characteristic curve for intrahepatic *THBS2* mRNA levels for the  
34 diagnosis of NASH among 206 NAFLD patients. The AUROC is shown.

35

36 **Supporting Figure S7.** Principle component analysis of the RNA sequencing  
37 data of liver tissues from 98 patients with biopsy-proven NAFLD stratified by  
38 fibrosis stage.

39

40 **Supporting Figure S8.** Venn diagram showing comparisons among significantly  
41 upregulated genes in NAFLD patients (stage F1–2 versus F0; stage F3–4 versus  
42 F1–2) and predicted secreted proteins registered in the Human Protein Atlas  
43 database.

44

45 **Supporting Figure S9. (A)** mRNA expression levels of *THBS2* were evaluated  
46 by qPCR in 98 NAFLD patients ( $*P < 0.05$ ). **(B)** Receiver operating characteristic  
47 curve for qPCR-based intrahepatic *THBS2* mRNA levels for the diagnosis of  
48 advanced fibrosis (F3–4) among 98 NAFLD patients. The AUROC is shown.

49

50 **Supporting Figure S10. (A)** mRNA expression levels of *THBS2* were evaluated  
51 by RNA sequencing in 206 NAFLD patients ( $*P < 0.05$ ) (GSE135251). **(B)**  
52 Receiver operating characteristic curve for RNA sequencing–based intrahepatic

53 *THBS2* mRNA levels (**left**) and FIB-4 index (**right**) for the diagnosis of advanced  
54 fibrosis (F3–4) among 206 NAFLD patients. The AUROC is shown.

55

56 **Supporting Figure S11.** Heatmap showing 10 modules identified by the  
57 weighted correlation network analysis of the transcriptome data of liver tissues  
58 from 98 patients with biopsy-proven NAFLD. *THBS2* belonged to module 4  
59 highlighted in the red box.

60

61 **Supporting Figure S12. (A–B)** Correlation between intrahepatic *THBS2* and  
62 *COL1A1* (**A**) or *COL1A2* (**B**) mRNA levels examined by qPCR in 98 NAFLD  
63 patients.

64

65 **Supporting Figure S13. (A–B)** Correlation between intrahepatic *THBS2* and  
66 *COL1A1* (**A**) or *COL1A2* (**B**) mRNA levels examined by RNA sequence in 206  
67 NAFLD patients (GSE135251).

68

69 **Supporting Figure S14.** Correlation of serum TSP-2 levels with hepatic *THBS2*  
70 mRNA levels in 27 NAFLD patients.



71

72 **Supporting Figure S15.** Total 213 NAFLD patients were diagnosed based on  
73 NAFLD activity score (NAS). Patients with NAS score of equal or above 5 were  
74 defined as NASH. **(A)** Relative serum TSP-2 levels ( $*P < 0.05$ ). **(B)** Receiver  
75 operating characteristic curve of the performance of serum TSP-2 levels in the  
76 diagnosis of NASH among 213 patients with biopsy-proven NAFLD. The AUROC  
77 is shown.

78

79 **Supporting Figure S16. (A)** Correlation of serum TSP-2 levels with serum AST  
80 levels in 51 NAFLD patients with advanced fibrosis (F3–4). **(B)** Serum TSP-2  
81 levels in NAFLD patients based on the existence of hepatocyte ballooning ( $*P <$   
82  $0.05$ ).

83

## **Supplementary Materials and Methods**

### **qPCR**

Total RNA was reverse transcribed using the ReverTra Ace qPCR RT Kit (Toyobo, Tokyo, Japan) to synthesize complementary DNA. The TaqMan gene expression assay was used to analyze the expression of the following mRNAs: human *ACTB* (Hs01060665), human *THBS2* (Hs01568063), human *COL1A1* (Hs00164004), and human *COL1A2* (Hs01028969). Human gene expression levels were normalized to *ACTB* expression levels.

### **Enzyme-linked immunosorbent assay**

Serum TSP-2 levels were examined by enzyme-linked immunosorbent assay using human TSP-2 enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) following the manufacturer's protocols.

### **Laboratory testing**

The FIB-4 index was calculated as reported (age [years] x AST [U/L]/platelet count [x10<sup>9</sup>/L]/√ALT [U/L]).<sup>(1)</sup> NFS was calculated as reported (−1.675 + 0.037 x

age [years] + 0.094 x body mass index [kg/m<sup>2</sup>] + 1.13 x impaired fasting glycemia/diabetes mellitus [yes = 1, no = 0] + 0.99 x AST [U/L]/ALT [U/L] – 0.013 x platelet count [x10<sup>9</sup>/L] – 0.66 x Alb [g/dL]).<sup>(2)</sup>

### **Bioinformatic analysis**

Unsupervised hierarchical clustering, principal component analysis, detection of differentially expressed genes, generally applicable gene set enrichment pathway analysis focusing on gene ontology biological processes, and weighted correlation network analysis of the RNA sequencing data were performed using iDEP92 (<http://bioinformatics.sdstate.edu/idep92/>).<sup>(3-5)</sup> Single-sample gene set enrichment analysis<sup>(6)</sup> was performed using Gene Pattern (<https://cloud.genepattern.org/gp/pages/index.jsf>)<sup>(7)</sup>. CIBERSORT analysis<sup>(8)</sup> using RNA sequencing data was performed to estimate intrahepatic immune cell infiltration.

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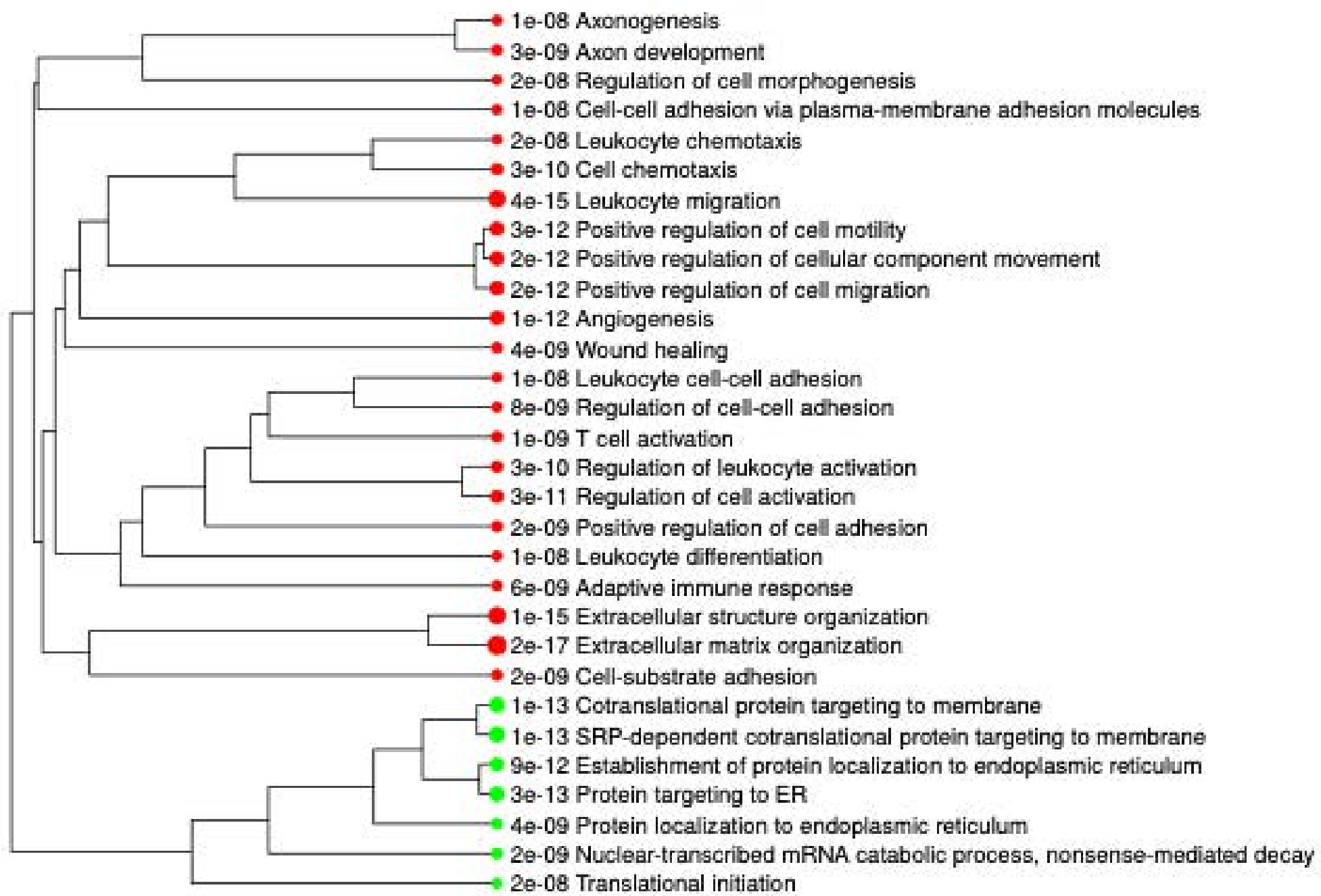
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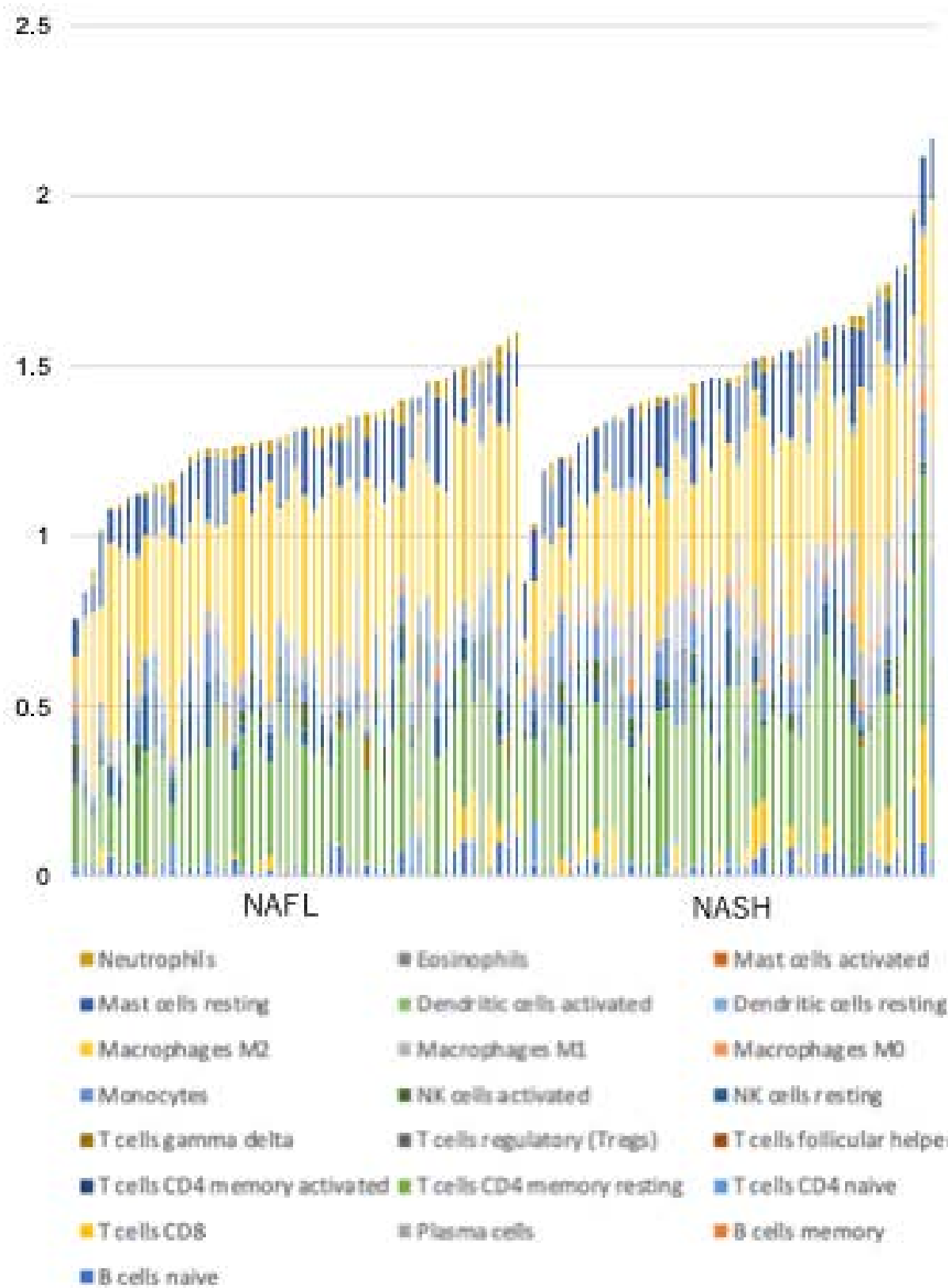
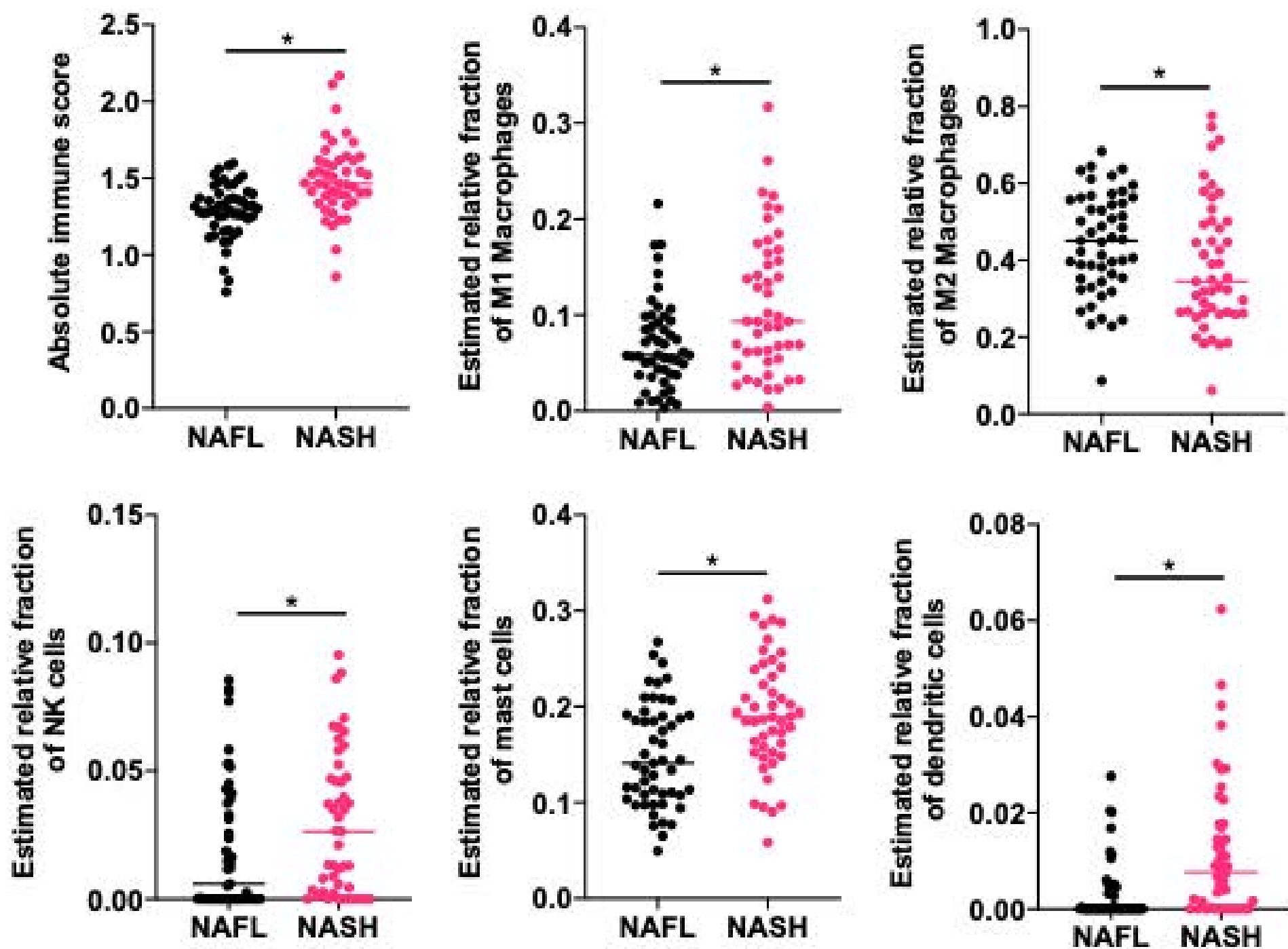
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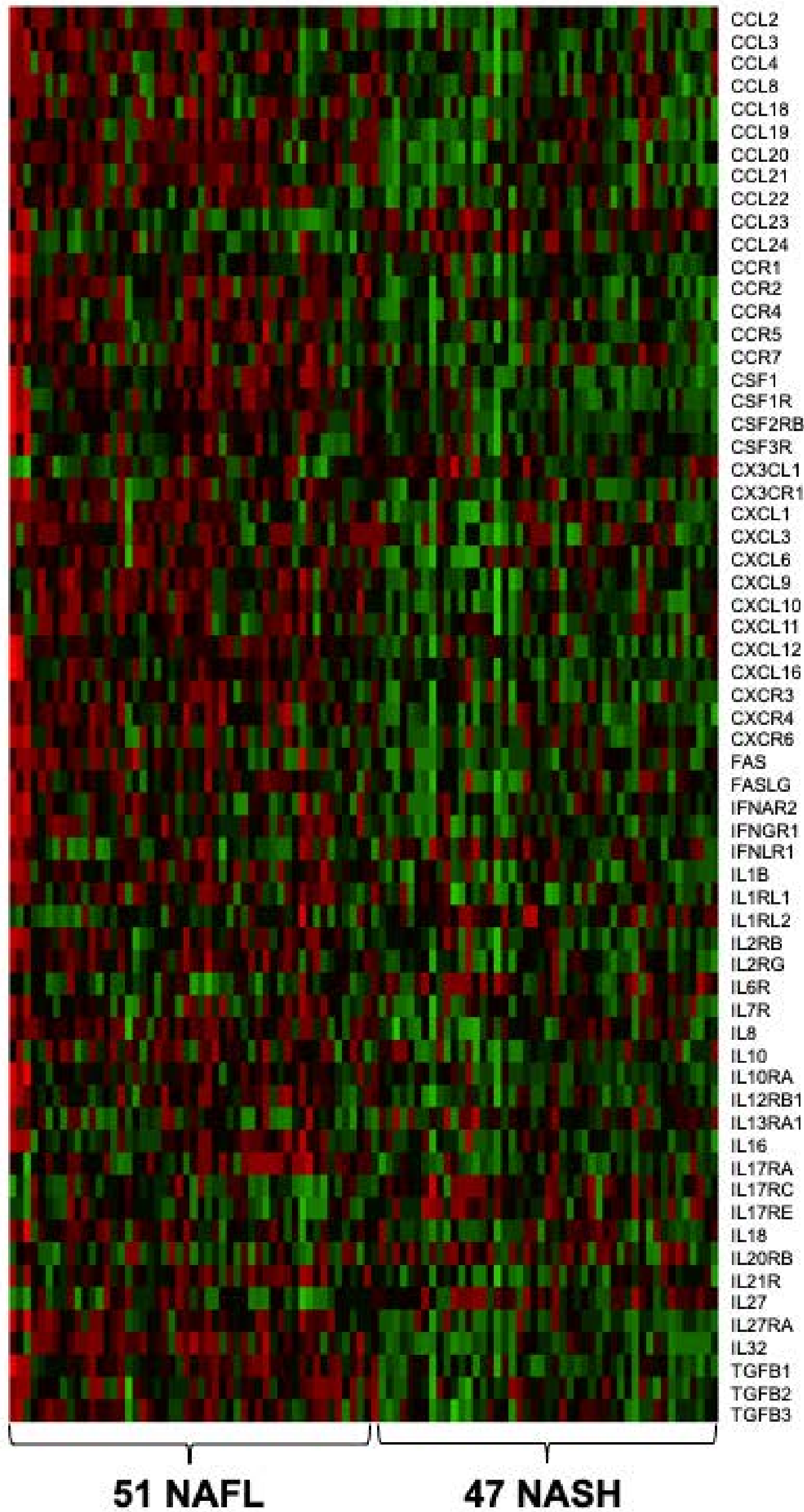
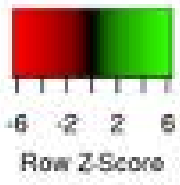
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● Down ● Up



**Supplementary Figure 1**

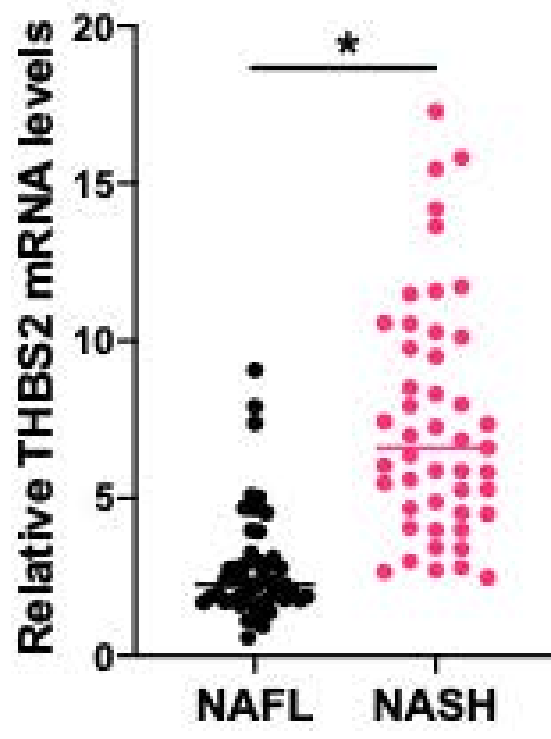
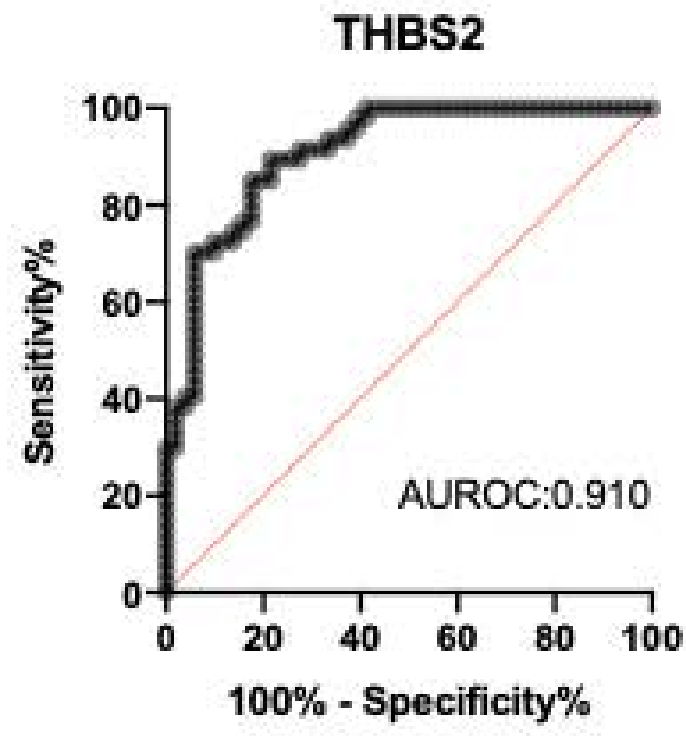
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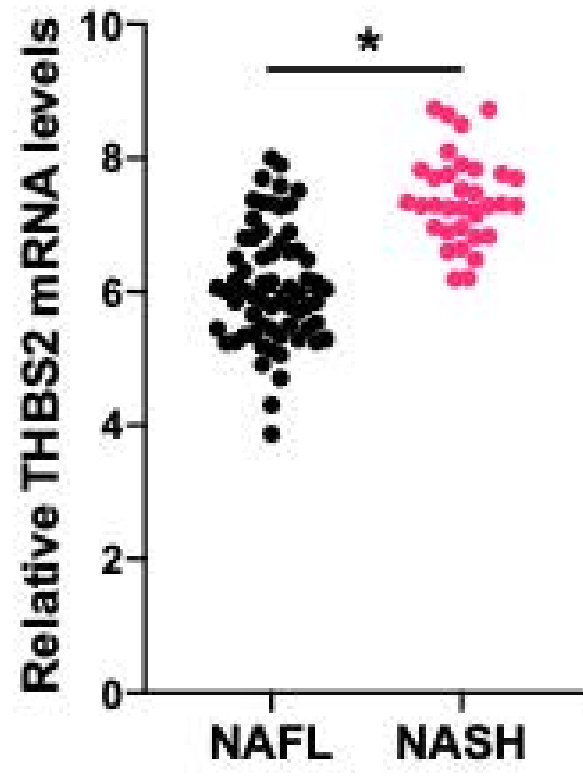
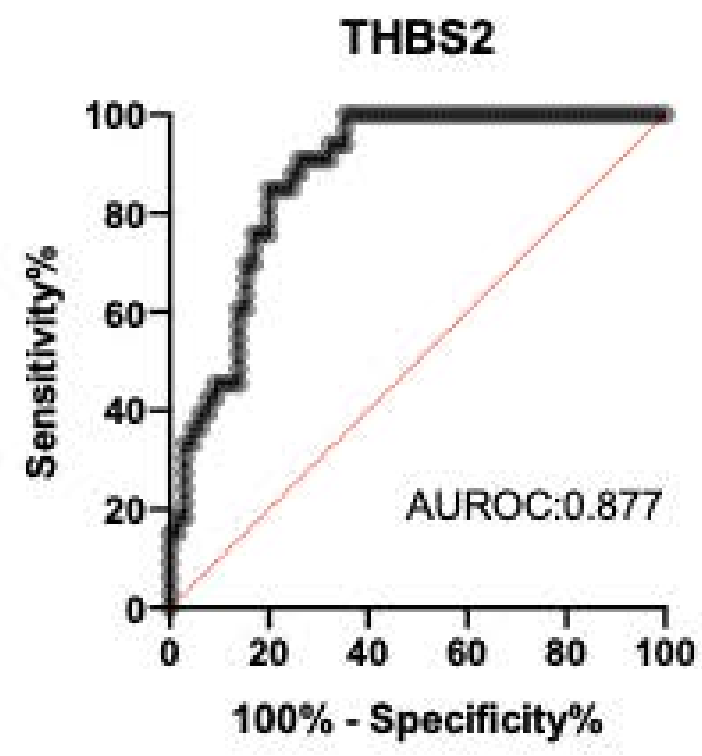


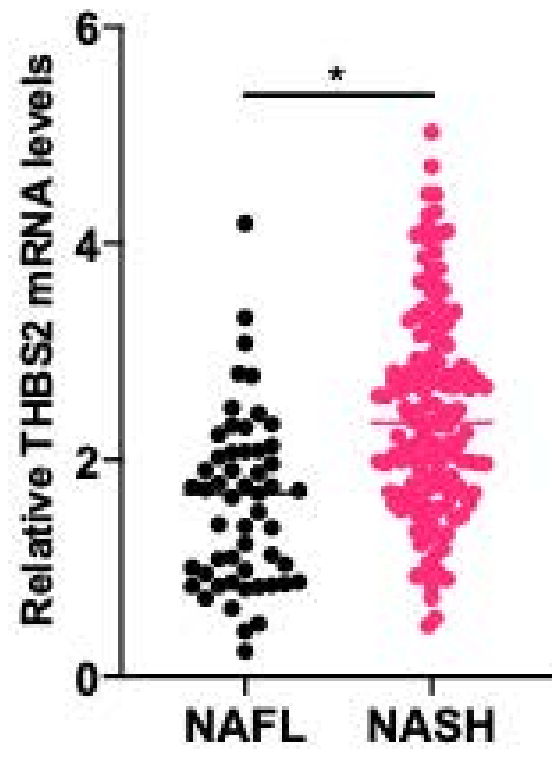
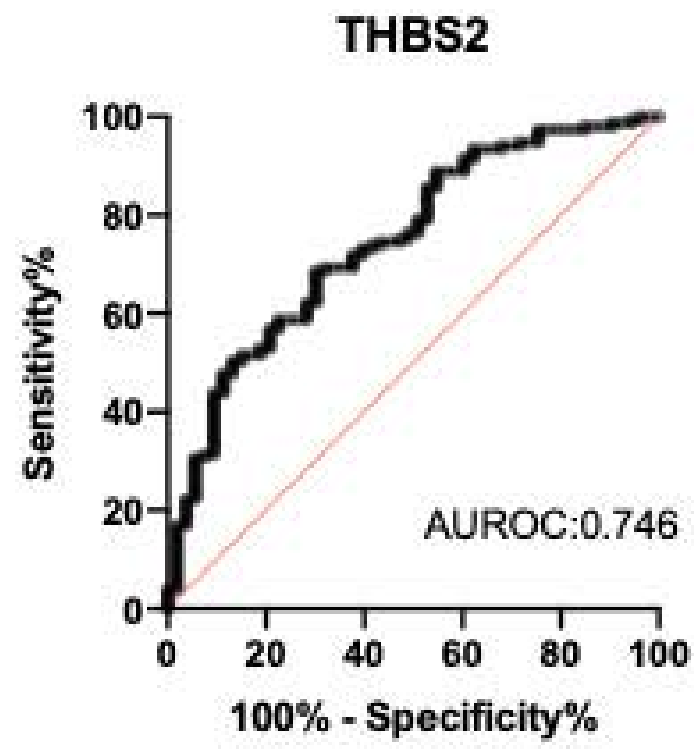
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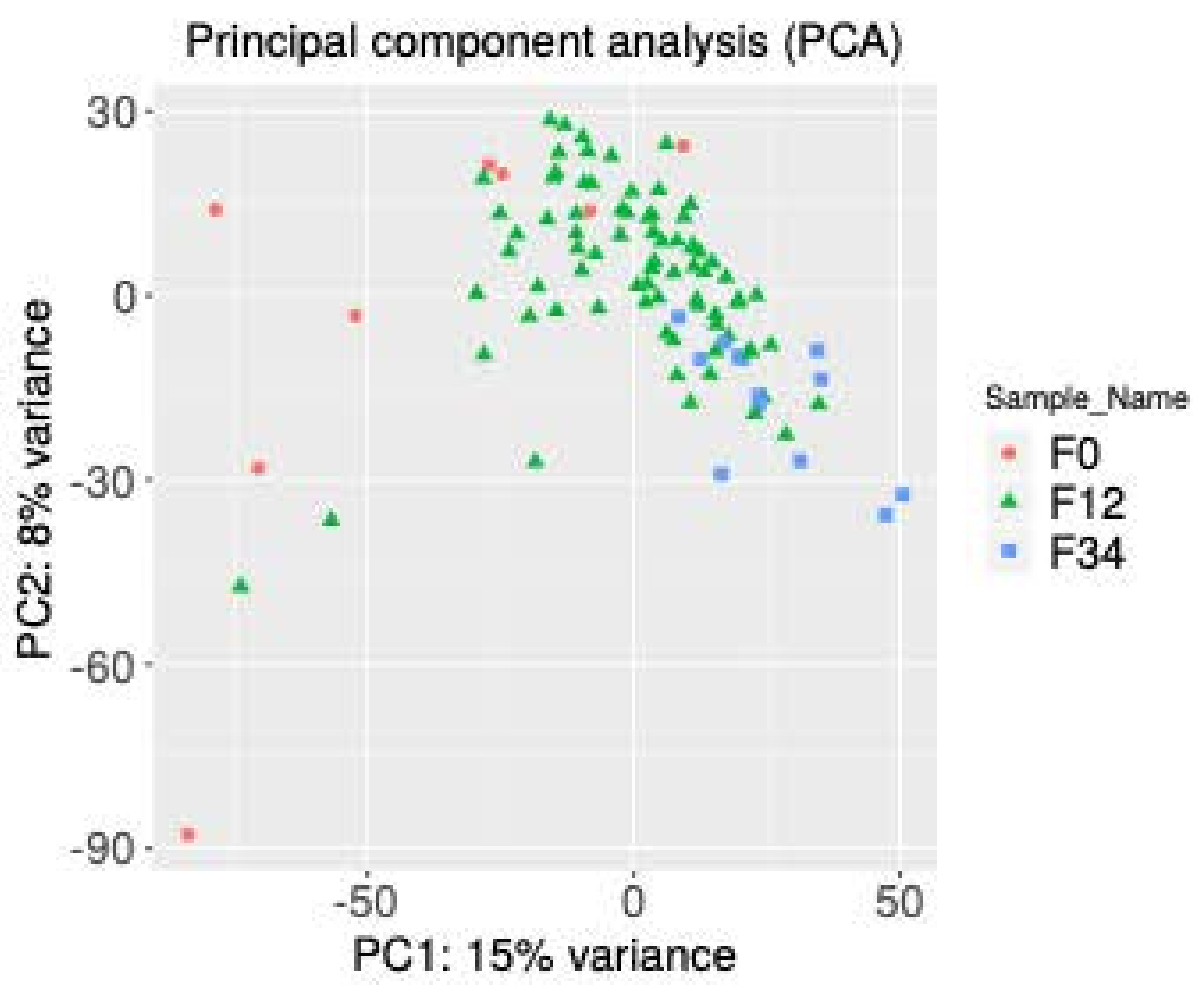
Supplementary Figure 3



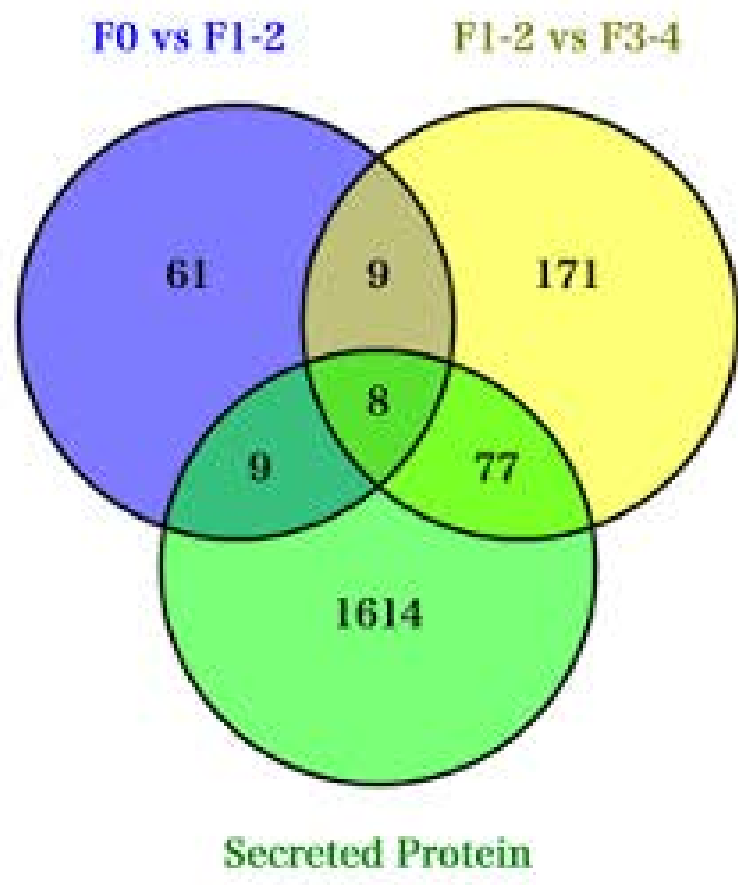
**A****B****Supplementary Figure 4**

**A****B****Supplementary Figure 5**

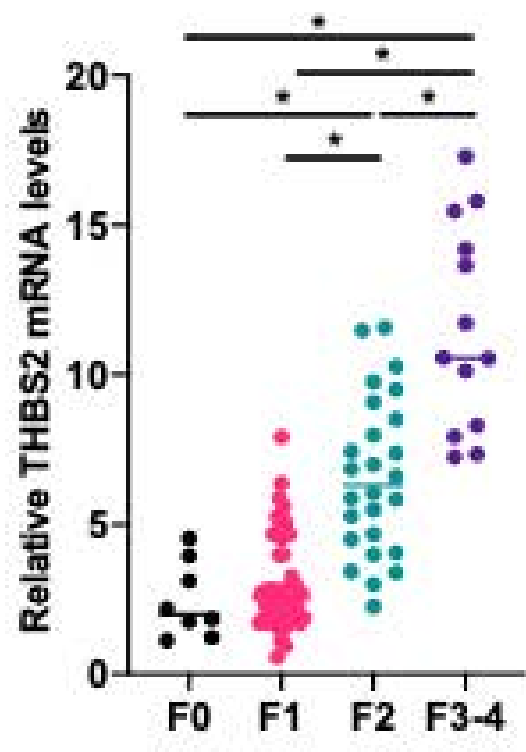
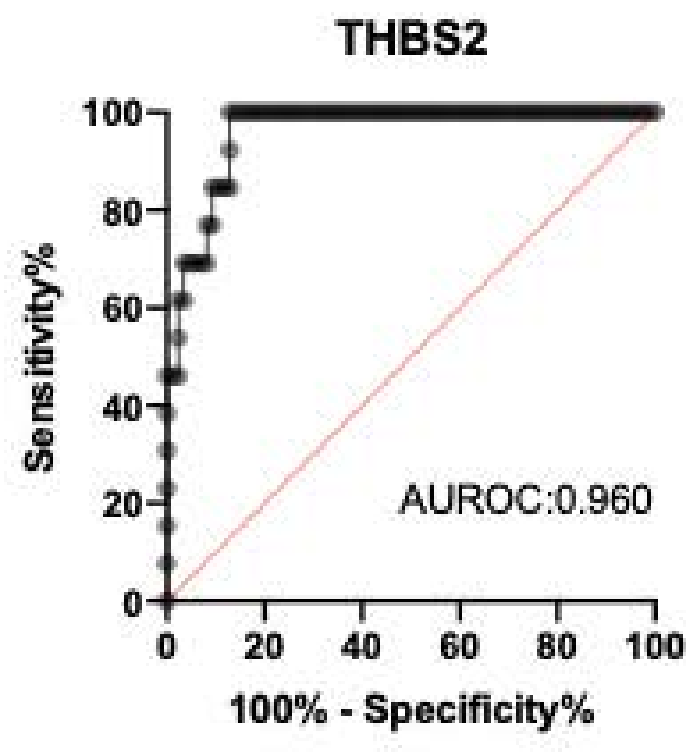
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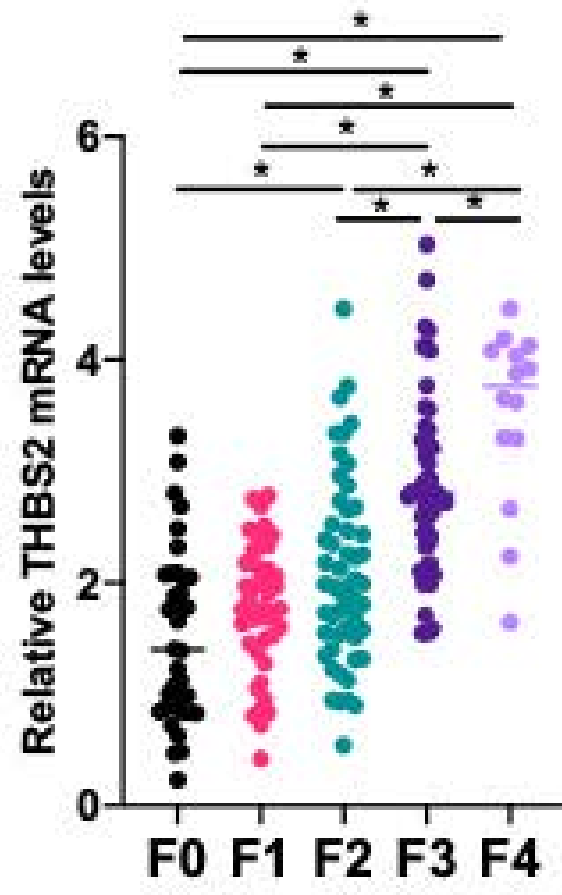
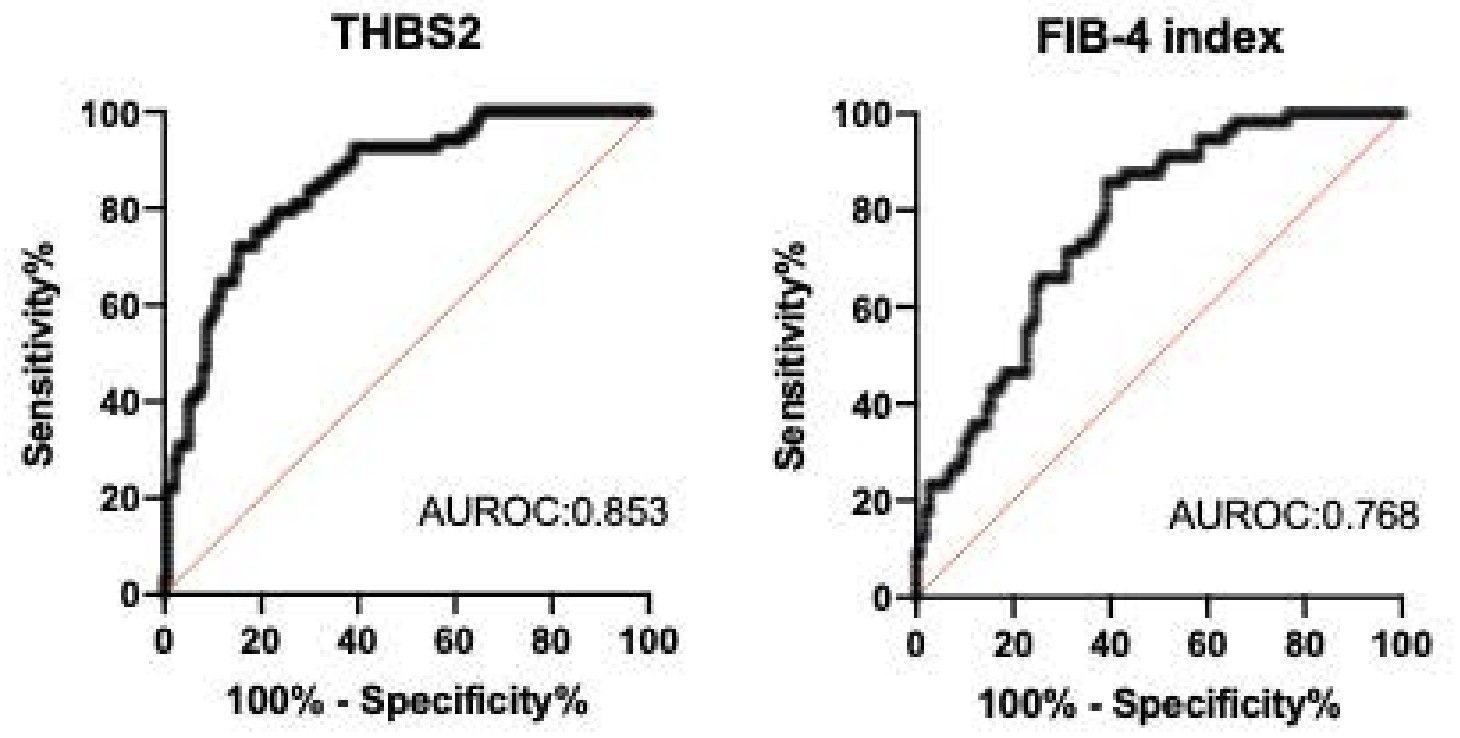


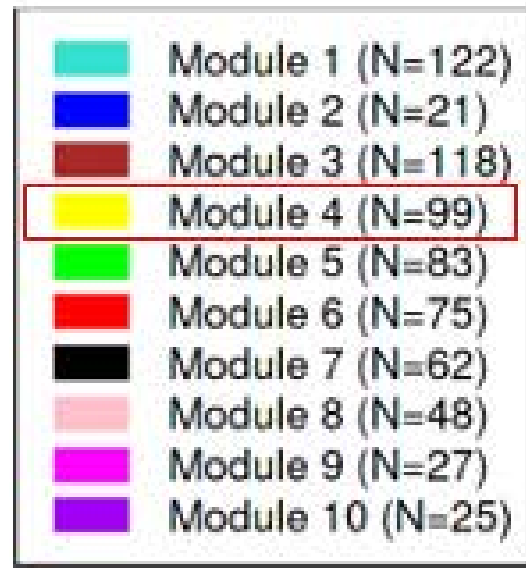
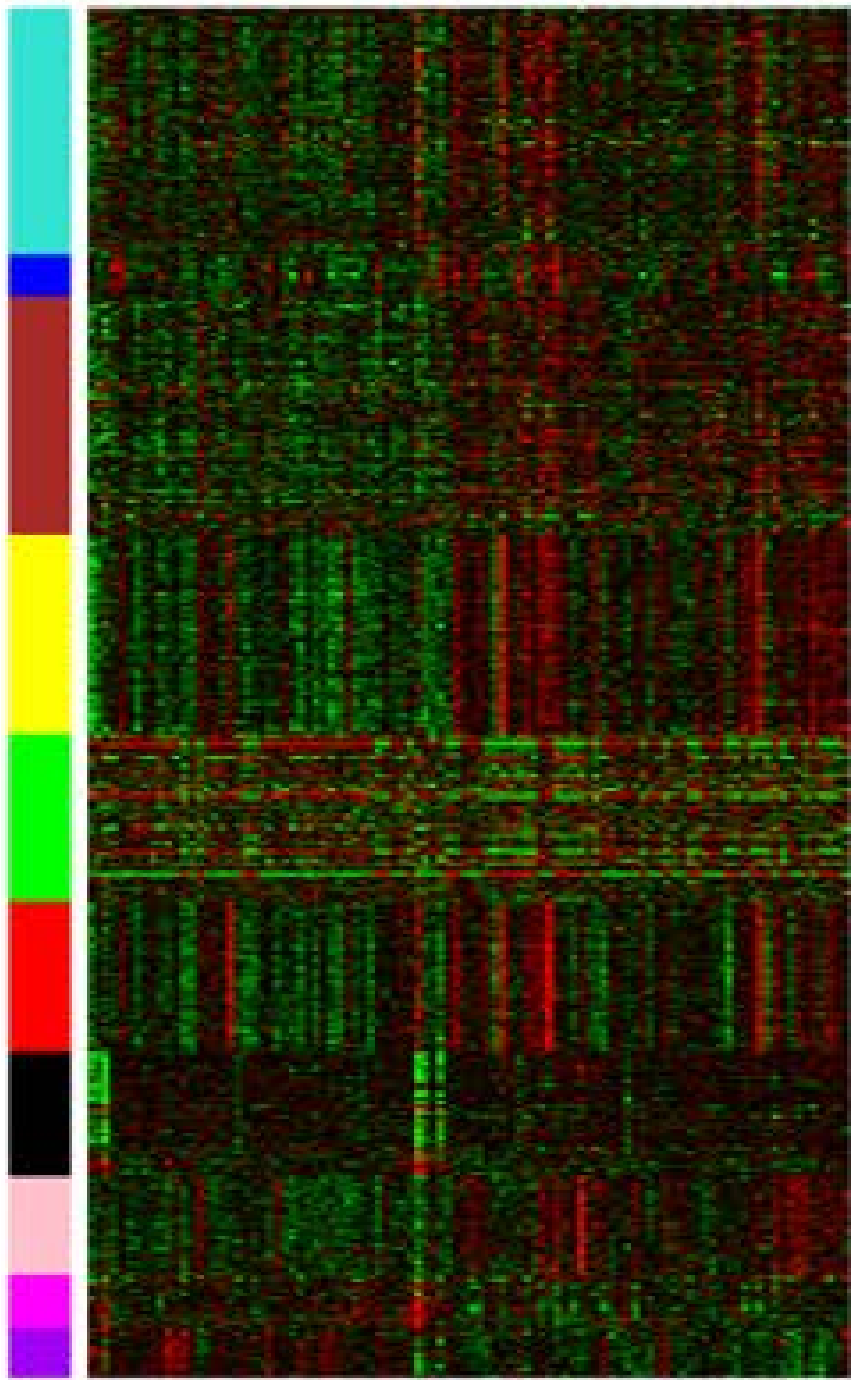
**Supplementary Figure 7**



**Supplementary Figure 8**

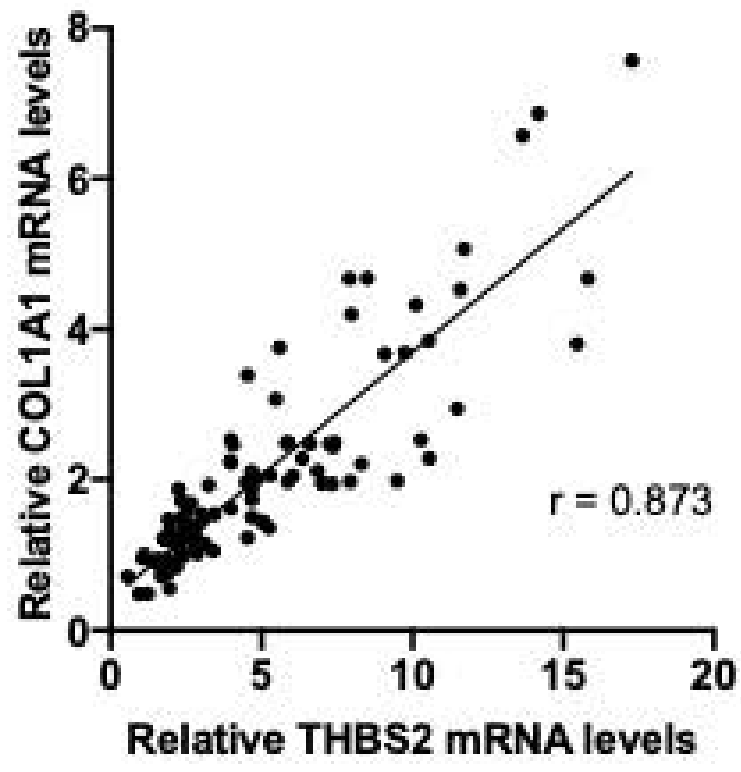
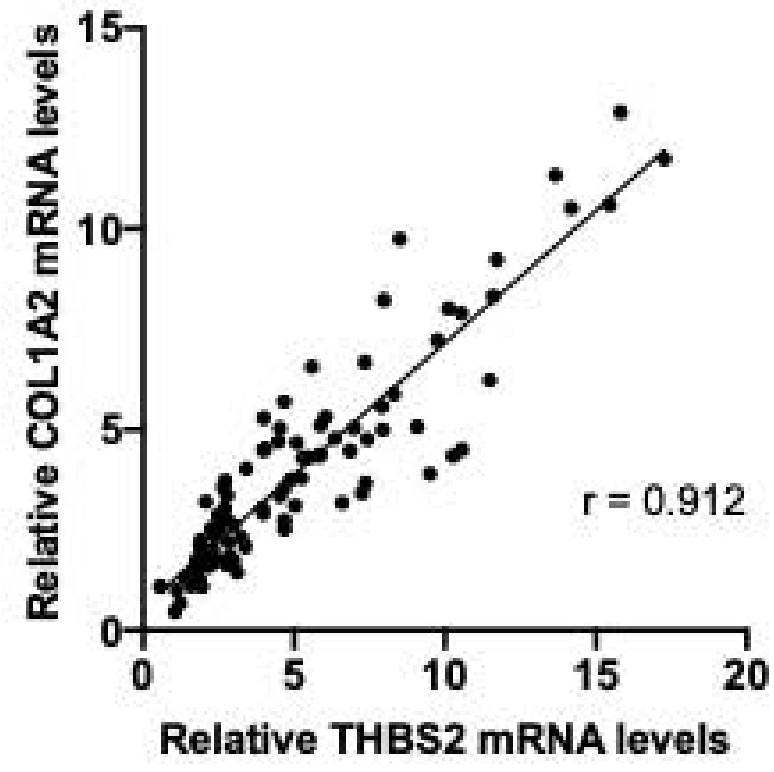
**A****B****Supplementary Figure 9**

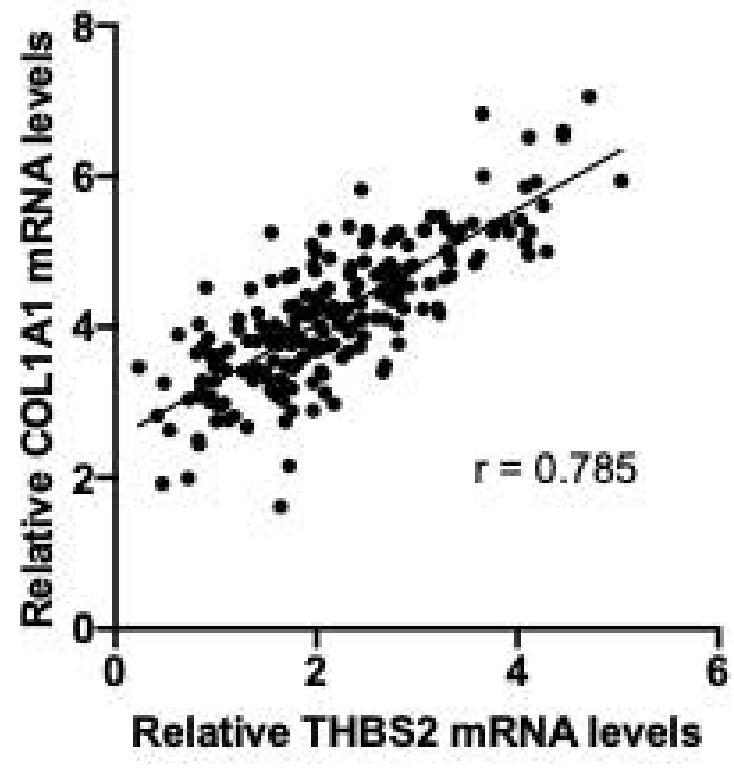
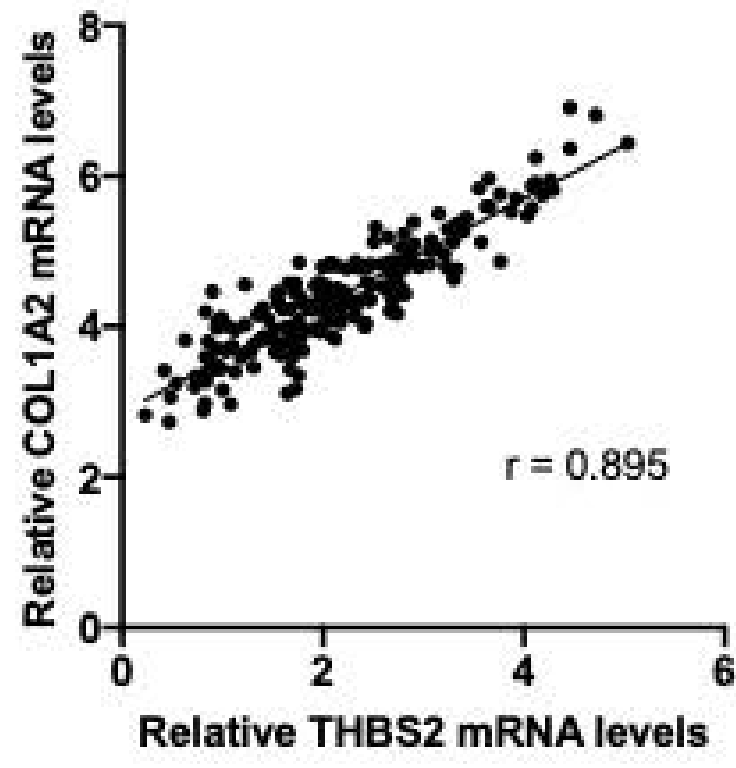
**A****B****Supplementary Figure 10**

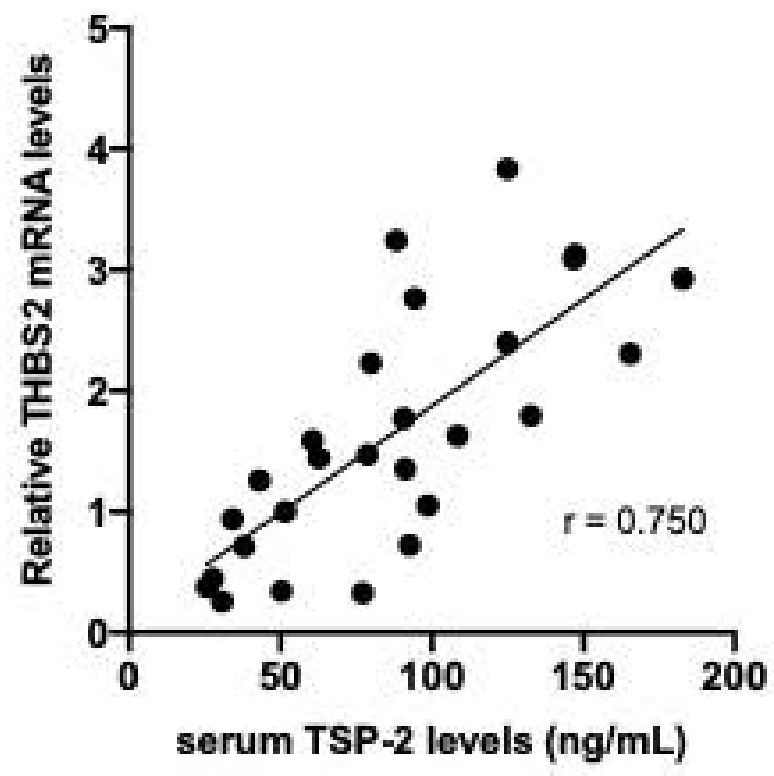


**Supplementary Figure 11**

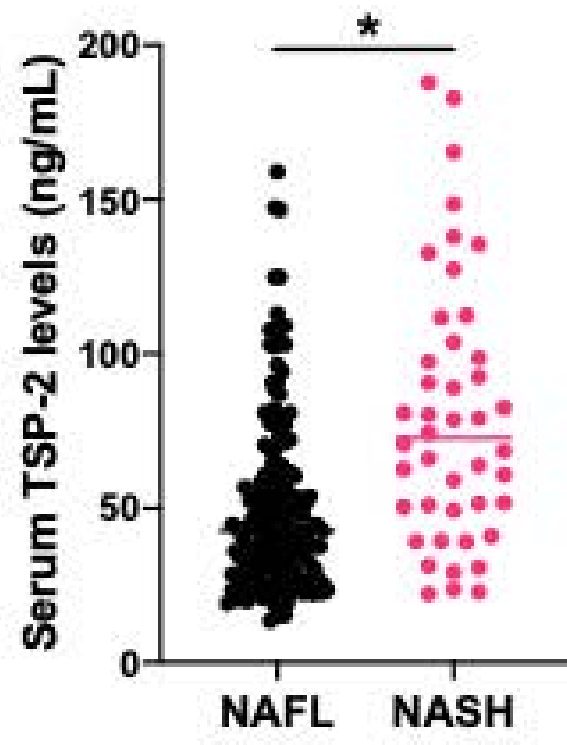
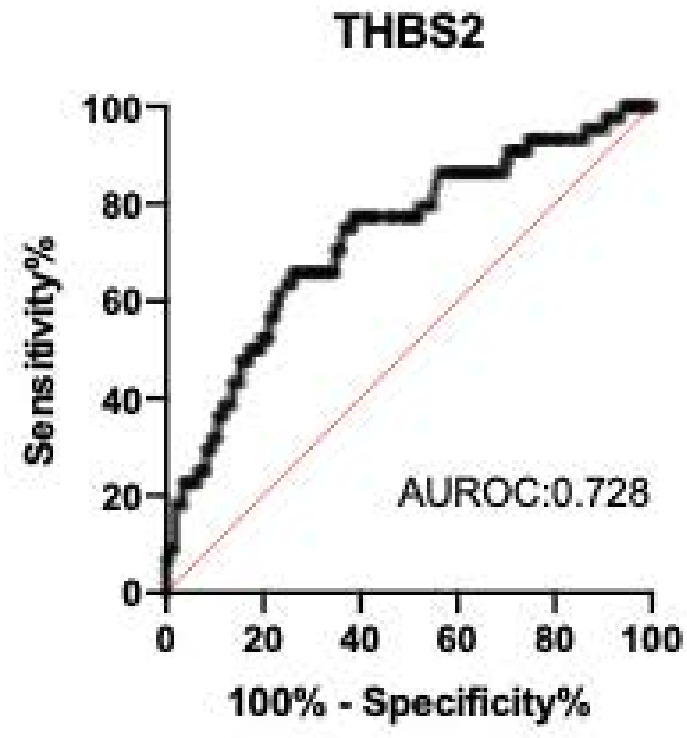


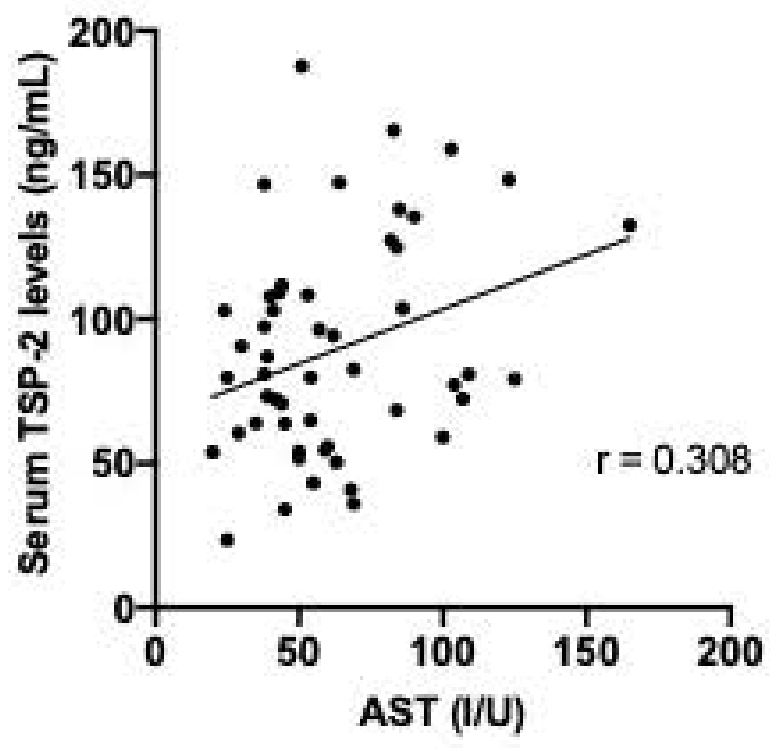
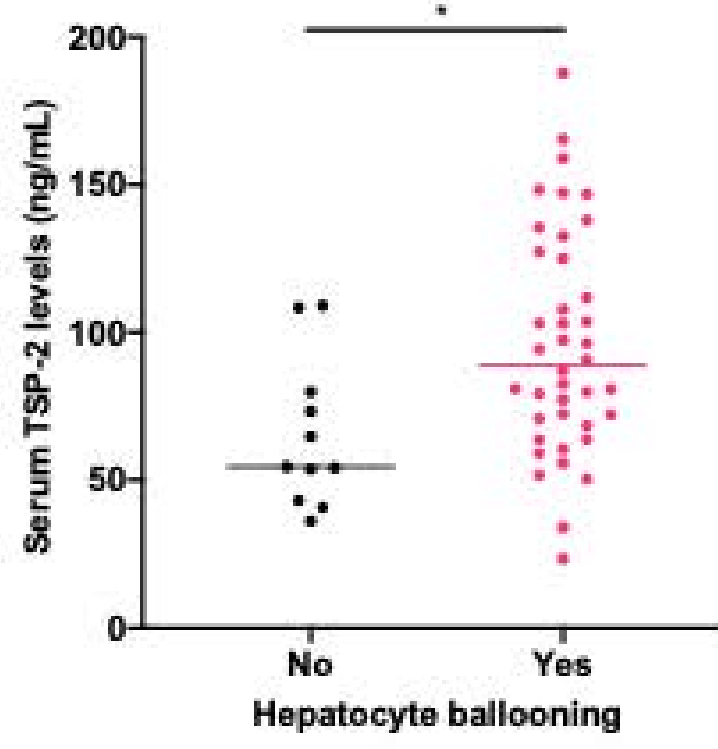
**A****B****Supplementary Figure 12**

**A****B****Supplementary Figure 13**



**Supplementary Figure 14**

**A****B****Supplementary Figure 15**

**A****B****Supplementary Figure16**