

# Supplementary Materials: Transcriptome-Wide Analysis of Human Liver Reveals Age-Related Differences in the Expression of Select Functional Gene Clusters and Evidence for a *PPP1R10*-Governed ‘Aging Cascade’

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**Table S1.** Demographic and clinical characteristics of patient groups I and II in order of gender and increasing age. Prior to surgery, all patients tested negative for the hepatitis viruses HAV, HBV, HCV, and HDV as well as HIV-1. All patients underwent surgery before the onset of the COVID-19 pandemic and, thus, were not tested for SARS-CoV-2. None of the patients had received radiotherapy. (Abbreviations: AHT, arterial hypertension; BEP, bleomycin + etoposide + cisplatin; CA, carcinoma; CCA, cholangiocellular carcinoma; CP/PT, carboplatin + paclitaxel; f, female; FOLFOX, folinic acid + fluorouracil + oxaliplatin; FNH, focal nodular hyperplasia; HCC, hepatocellular carcinoma; HCA, hepatocellular adenoma; HHEx, hemihepatectomy; m, male; mets, metastases; NASH, non-alcoholic steatohepatitis; pre-OP, preoperatively; RES, resection; SEG, segment or segment; T1DM or T2DM, type 1 or type 2 diabetes mellitus; TC, testicular cancer.).

Group I: Young									
Demographic Information				Medication		Comorbidities or Additional Information			
Gender	Age	Underlying Disease	Surgical Intervention	Preoperative Chemotherapy	Diabetes	Steatosis (Grade)	NASH	Fibrosis (Grade)	Other
f	23	HCA	Right HHEx	–	–	–	–	–	Bronchial asthma
f	26	FNH	Right HHEx + Atypical RES: SEG I, II, III	–	–	–	–	–	–
f	26	FNH	RES: SEG II, III, VII	–	–	2	Yes	–	–
f	34	FNH	Typical RES: SEG IV	–	–	–	–	–	Obesity
f	43	Cervical CA: Mets	Left HHEx	CP/PT	T1DM	–	–	–	–
f	47	CCA	Right HHEx	–	–	–	–	–	Substituted hypothyreosis after hemithyroidectomy 3 yrs. pre-OP
m	25	TC: Mets	RES: SEG V, VI + atypical RES: SEG III	BEP	–	–	–	–	–
m	46	HCA	RES: SEG II, III	–	T2DM	2	Yes	–	AHT
m	48	Colon CA: Mets	RES: SEG II, III, IVa, VI, VIII	FOLFOX	–	–	–	–	–
Group II: Old									
Demographic Information				Medication		Comorbidities or Additional Information			
Gender	Age	Underlying Disease	Surgical Intervention	Preoperative Chemotherapy	Diabetes	Steatosis (Grade)	NASH	Fibrosis (Grade)	Other

f	79	Colon CA: Mets	Right HHEx	–	–	–	–	–	Multinodular goiter
f	79	CCA	Right HHEx	–	–	2	–	F1	AHT
m	75	HCC	RES: SEG VI	–	–	1	–	F4	AHT
m	76	Colon CA: Mets	Right HHEx + atypical RES: SEG II	–	T2DM	–	–	–	AHT
m	76	Sigma CA: Mets	RES: SEG VIb, V	–	–	–	–	F0–F1	AHT
m	78	Colon CA: Mets	Right HHEx	–	–	2	–	F2	AHT
m	78	CCA	RES: SEG II, III	–	–	–	–	F1	AHT, obesity
m	78	CCA	Extended right HHEx	–	–	–	–	–	In-situ split 2 wks. pre-OP

**Table S2.** Human target transcripts for qRT-PCR. The list starts with the reference gene, followed by genes of the transcripts investigated in alphabetical order.

Genes <sup>1</sup>	Sequences	Genes <sup>1</sup>	Sequences
<i>PPIA</i> (reference)			
Main target	ENST00000468812		
Amplicon length	114		
Number of targets	4		
<i>CFLAR</i>		<i>HSD17B14</i>	
Main target	ENST00000309955	Main target	ENST00000596349
Amplicon length	109	Amplicon length	86
Number of targets	7	Number of targets	4
<i>DUSP1</i>		<i>IGFALS</i>	
Main target	ENST00000239223	Main target	ENST00000415638
Amplicon length	116	Amplicon length	67
Number of targets	1	Number of targets	1
<i>EGR1</i>		<i>KIAA0040</i>	
Main target	ENST00000239938	Main target	ENST00000563563
Amplicon length	127	Amplicon length	131
Number of targets	1	Number of targets	6
<i>FAH</i>		<i>LIPC</i>	
Main target	ENST00000537726	Main target	ENST00000560664
Amplicon length	117	Amplicon length	84
Number of targets	10	Number of targets	6
<i>FLNA</i>		<i>PPP1R10</i>	
Main target	ENST00000420627	Main target	ENST00000376511
Amplicon length	141	Amplicon length	103
Number of targets	7	Number of targets	2
<i>GATA4</i>		<i>TFF3</i>	
Main target	ENST00000526716	Main target	ENST00000398431
Amplicon length	81	Amplicon length	68
Number of targets	1	Number of targets	4

<sup>1</sup> *CFLAR*, CASP8- and FADD-like apoptosis regulator; *DUSP1*, dual specificity phosphatase 1; *EGR1*, early growth response 1; *FAH*, fumarylacetoacetate hydrolase; *FLNA*, filamin A; *GATA4*, GATA-binding protein 4; *HSD17B14*, hydroxysteroid 17- $\beta$  dehydrogenase 14; *IGFALS*, insulin-like growth factor-binding protein acid labile subunit; *KIAA0040*, uncharacterized protein KIAA0040; *LIPC*, lipase C, hepatic type; *PPP1R10*, protein phosphatase 1 regulatory subunit 10; *PPIA*, peptidyl-prolyl *cis-trans* isomerase A; *TFF3*, trefoil factor 3.

**Table S3.** Gene expressions of interest identified by applying EFS software. Transcripts are listed from most relevant to least relevant according to their EFS scores (>0.3: 16 transcripts / >0.2: 28 further transcripts). For full names of protein products, cf. Table 2. Increases in EFS scores indicate increasing relevance when comparing young and old patients. Ratios between Group (Grp.) I and Group II differing by a factor of at least  $\times 2$  (i.e.,  $\leq 0.5$  or  $\geq 2.0$ ) are boxed (red data in Figure 2), while ratios between >0.5 and <2.0 are underlined (blue data in Figure 2). Data for all 44 transcripts are at least statistically significant ( $p < 0.05$ ). (Abbreviations: Grp., Group; TPM, transcripts per million.).

Gene ID	Gene	Transcription Rates [TPM]				$p$ (Two-tailed)		EFS Score
		Maximum	Mean	Mean	Ratio			
			Grp. I	Grp. II	$\frac{\text{Grp. II}}{\text{Grp. I}}$			
ENSG00000204569	<i>PPP1R10</i>	159.20	107.86	67.79	<u>0.629</u>	0.0003	> 0.3	0.61415
ENSG00000120129	<i>DUSP1</i>	557.40	375.44	212.30	<u>0.565</u>	0.0055		0.55957
ENSG00000087076	<i>HSD17B14</i>	90.77	47.32	12.74	0.269	0.0010		0.53103
ENSG00000148120	<i>AOPEP</i>	179.15	114.07	66.16	0.580	0.0010		0.40353
ENSG00000185745	<i>IFIT1</i>	70.04	23.61	41.94	<u>1.776</u>	0.0360		0.36984
ENSG00000235750	<i>KIAA0040</i>	61.92	27.85	14.67	<u>0.527</u>	0.0016		0.36721
ENSG00000196924	<i>FLNA</i>	92.67	56.39	33.07	0.586	0.0016		0.35981
ENSG00000003402	<i>CFLAR</i>	123.14	93.58	65.16	<u>0.696</u>	0.0006		0.33686
ENSG00000085563	<i>ABCB1</i>	54.81	29.32	42.30	<u>1.442</u>	0.0206		0.33654
ENSG00000103876	<i>FAH</i>	301.97	166.06	250.17	1.506	0.0111		0.32981
ENSG00000153250	<i>RBMS1</i>	52.92	38.16	25.51	<u>0.668</u>	0.0152		0.32777
ENSG00000099769	<i>IGFALS</i>	471.76	261.59	126.81	<u>0.485</u>	0.0037		0.32758
ENSG00000117616	<i>RSRP1</i>	162.29	108.48	73.63	0.679	0.0274		0.32683
ENSG00000089127	<i>AC004551.1</i>	58.72	21.41	32.94	1.538	0.0206		0.32390
ENSG00000144642	<i>RBMS3</i>	85.14	44.54	29.68	<u>0.666</u>	0.0055		0.31905
ENSG00000160180	<i>TFF3</i>	397.12	81.92	1.25	<u>0.015</u>	0.0055		0.31574
ENSG00000126012	<i>KDM5C</i>	51.25	30.87	19.87	0.643	0.0111	> 0.2	0.28884
ENSG00000152818	<i>UTRN</i>	207.84	125.80	70.80	0.563	0.0010		0.28580
ENSG00000101335	<i>MYL9</i>	64.72	41.33	22.73	0.550	0.0079		0.26749
ENSG00000183963	<i>SMTN</i>	65.16	36.07	21.85	0.606	0.0152		0.26602
ENSG00000143248	<i>RGS5</i>	337.12	131.96	60.40	<u>0.458</u>	0.0152		0.25855
ENSG00000074276	<i>CDHR2</i>	73.29	20.56	47.55	2.313	0.0111		0.25525
ENSG00000100234	<i>TIMP3</i>	276.12	168.16	99.01	<u>0.589</u>	0.0206		0.25389
ENSG00000166035	<i>LIPC</i>	163.41	85.65	124.67	1.456	0.0360		0.24764
ENSG00000111077	<i>TNS2</i>	118.89	78.25	52.68	0.673	0.0206		0.24403
ENSG00000105676	<i>ARMC6</i>	113.94	78.93	51.58	0.654	0.0464		0.23935
ENSG00000075240	<i>GRAMD4</i>	167.99	62.66	21.86	0.349	0.0274		0.23830
ENSG00000132254	<i>ARFIP2</i>	60.99	33.71	22.60	0.670	0.0206		0.23406
ENSG00000184226	<i>PCDH9</i>	104.44	43.80	21.61	0.493	0.0274		0.23297
ENSG00000099998	<i>GGT5</i>	92.41	42.52	27.77	<u>0.653</u>	0.0464		0.22929
ENSG00000010322	<i>NISCH</i>	61.59	37.57	22.94	0.611	0.0206		0.22850
ENSG00000120738	<i>EGR1</i>	727.96	381.72	174.46	<u>0.457</u>	0.0360		0.22582

ENSG00000149541	<i>B3GAT3</i>	54.18	22.17	15.52	0.700	0.0464	0.22514
ENSG00000136574	<i>GATA4</i>	120.06	44.12	12.75	<u>0.289</u>	0.0274	0.22168
ENSG00000154258	<i>ABCA9</i>	64.27	37.59	24.27	<u>0.646</u>	0.0274	0.22112
ENSG00000054282	<i>SDCCAG8</i>	66.80	45.26	31.39	0.693	0.0037	0.22020
ENSG00000054654	<i>SYNE2</i>	229.66	146.72	94.88	0.647	0.0274	0.21864
ENSG00000257017	<i>HP</i>	52678.43	16805.05	29002.98	<u>1.726</u>	0.0360	0.21811
ENSG00000163660	<i>CCNL1</i>	249.51	185.39	131.54	<u>0.710</u>	0.0274	0.21788
ENSG00000058668	<i>ATP2B4</i>	85.89	40.48	27.22	0.673	0.0464	0.21358
ENSG00000107796	<i>ACTA2</i>	82.20	51.34	21.13	0.411	0.0055	0.21218
ENSG00000156234	<i>CXCL13</i>	78.00	1.79	14.03	<u>7.858</u>	0.0360	0.20827
ENSG00000117318	<i>ID3</i>	113.79	74.94	45.82	0.611	0.0111	0.20143
ENSG00000110719	<i>TCIRG1</i>	88.64	57.28	39.21	<u>0.684</u>	0.0206	0.20042

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**Table S4.** Specifications for gene transcripts identified by EFS software. Genes related to the 44 hepatic transcripts matching the EFS score cutoff of >0.2 are tabulated according to their chromosome (Chr) location, wherein listed alphabetically according their gene designations. Especially high ortholog/paralog<sup>1</sup> ratios (with ≤9 paralogs) are boxed. As summarized in the bottom section, gene transcripts of relevance were expressed from all human chromosomes, except for Chr 18 and Chr Y. Hence, no statistically significant age-dependent transcript differences were attributable to the male sex chromosome. Transcript numbers (##) with relevant age-dependent differences were six for Chr 1; four for Chr 22; three for Chr 3, 5, and 11; two for Chr 2, 6, 10, 12, 15, 16, 19, and X; and one for Chr 4, 7, 8, 9, 13, 14, 17, 20, and 21, respectively. Information was compiled from [https://www.ensembl.org/Homo\\_sapiens/Info/Index](https://www.ensembl.org/Homo_sapiens/Info/Index) (accessed on 2021-04-16).

Gene ID	Gene	Full Name of Protein Product (Comments)	Location	Gene Information			
				Transcripts	Alleles	Orthologs <sup>1</sup>	Paralogs <sup>1</sup>
ENSG00000058668	<i>ATP2B4</i>	ATPase plasma membrane Ca <sup>2+</sup> -transporting 4	Chr 1	7	0	221	22
ENSG00000117318	<i>ID3</i>	Inhibitor of DNA binding 3 (a so-called 'bHLH protein') <sup>2</sup>		3	1	201	3
ENSG00000235750	<i>KIAA0040</i>	KIAA0040 (uncharacterized)		6	0	5	0
ENSG00000143248	<i>RGS5</i>	Regulator of G protein signaling 5		12	0	202	23
ENSG00000117616	<i>RSRP1</i>	Arginine- and serine-rich protein 1		22	0	114	9
ENSG00000054282	<i>SDCCAG8</i>	SHH signaling and ciliogenesis regulator SDCCAG8		11	1	90	0
ENSG00000003402	<i>CFLAR</i>	CASP8- and FADD-like apoptosis regulator	Chr 2	25	0	222	15
ENSG00000153250	<i>RBMS1</i>	RNA-binding motif single-stranded interacting protein 1		15	0	265	24
ENSG00000163660	<i>CCNL1</i>	Cyclin L1	Chr 3	26	0	213	6
ENSG00000010322	<i>NISCH</i>	Nischarin		14	0	205	1
ENSG00000144642	<i>RBMS3</i>	RNA-binding motif single-stranded interacting protein 3		20	0	261	24
ENSG00000156234	<i>CXCL13</i>	C-X-C motif chemokine ligand 13	Chr 4	2	0	111	12
ENSG00000074276	<i>CDHR2</i>	Cadherin-related family member 2	Chr 5	7	0	118	34
ENSG00000120129	<i>DUSP1</i>	Dual specificity phosphatase 1		1	0	204	30
ENSG00000120738	<i>EGR1</i>	Early growth response 1		1	0	225	4
ENSG00000204569	<i>PPP1R10</i>	Protein phosphatase 1 regulatory subunit 10	Chr 6	7	1	197	2
ENSG00000152818	<i>UTRN</i>	Utrophin		13	0	261	36
ENSG00000085563	<i>ABCB1</i>	ATP-binding cassette subfamily B member 1	Chr 7	11	0	159	11
ENSG00000136574	<i>GATA4</i>	GATA-binding protein 4	Chr 8	9	1	206	7
ENSG00000148120	<i>AOPEP</i>	Aminopeptidase O (putative)	Chr 9	21	0	224	22
ENSG00000107796	<i>ACTA2</i>	Actin α <sub>2</sub> (smooth muscle)	Chr 10	6	0	149	27
ENSG00000185745	<i>IFIT1</i>	Interferon-induced protein with tetratricopeptide repeats 1		2	0	507	4
ENSG00000149541	<i>B3GAT3</i>	β-1,3-Glucuronyltransferase 3	Chr 11	6	0	194	2

ENSG00000132254	<i>ARFIP2</i>	ADP ribosylation factor interacting protein 2		10	0	<div>266</div>	<div>2</div>
ENSG00000110719	<i>TCIRG1</i>	T-cell immune regulator 1, ATPase H <sup>+</sup> -transporting V0 subunit a <sub>3</sub> (a.k.a. V-type proton ATPase 116 kDa subunit a <sub>3</sub> )		16	0	<div>233</div>	<div>3</div>

ENSG00000089127	<i>AC004551.1</i>	2'-5'-Oligoadenylate synthetase 1	Chr 12	33	0	<div>148</div>	<div>3</div>
ENSG00000111077	<i>TNS2</i>	Tensin 2		20	0	<div>115</div>	<div>1</div>

ENSG00000184226	<i>PCDH9</i>	Protocadherin 9	Chr 13	6	0	220	34
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ENSG00000054654	<i>SYNE2</i>	Spectrin repeat-containing nuclear envelope protein 2	Chr 14	31	0	308	36
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ENSG00000103876	<i>FAH</i>	Fumarylacetoacetate hydrolase	Chr 15	14	0	<div>204</div>	<div>3</div>
ENSG00000166035	<i>LIPC</i>	Lipase C (hepatic type)		7	0	<div>228</div>	<div>9</div>

ENSG00000257017	<i>HP</i>	Haptoglobin	Chr 16	18	0	137	17
ENSG00000099769	<i>IGFALS</i>	Insulin-like growth factor-binding protein acid labile subunit		3	0	187	22

ENSG00000154258	<i>ABCA9</i>	ATP-binding cassette subfamily A member 9	Chr 17	8	0	105	11
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–	–	–	Chr 18	–	–	–	–
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ENSG00000105676	<i>ARMC6</i>	Armadillo repeat containing 6	Chr 19	20	0	<div>201</div>	<div>0</div>
ENSG00000087076	<i>HSD17B14</i>	Hydroxysteroid 17-β-dehydrogenase 14		4	0	<div>162</div>	<div>26</div>

ENSG00000101335	<i>MYL9</i>	Myosin light chain 9	Chr 20	2	0	<div>229</div>	<div>7</div>
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ENSG00000160180	<i>TFF3</i>	Trefoil factor 3	Chr 21	3	0	0	0
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ENSG00000099998	<i>GGT5</i>	γ-Glutamyltransferase 5	Chr 22	6	0	<div>342</div>	<div>7</div>
ENSG00000075240	<i>GRAMD4</i>	GRAM domain containing 4		7	0	<div>265</div>	<div>0</div>
ENSG00000183963	<i>SMTN</i>	Smoothelin		24	0	<div>125</div>	<div>0</div>
ENSG00000100234	<i>TIMP3</i>	TIMP metallopeptidase inhibitor 3		1	0	<div>211</div>	<div>3</div>

ENSG00000196924	<i>FLNA</i>	Filamin A	Chr X	21	0	198	36
ENSG00000126012	<i>KDM5C</i>	Lysine demethylase 5C		15	0	171	11

–	–	–	Chr Y	–	–	–	–
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Chr	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
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##	6	2	3	1	3	2	1	1	1	2	3	2	1	1	2	2	1	–	2	1	1	4	2	–
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<sup>1</sup> *Ortholog*: Gene that diverged upon evolutionary separation of species lineages.

*Paralog*: Gene derived from the same ancestor by duplication within the same species.

<sup>2</sup> Negative transcriptional regulator of basic helix-loop-helix (bHLH) transcription factors.





Ursodeoxycholic acid      -      -      -      -      +      -      -      -      -

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<sup>1</sup> According to the Comparative Toxicogenomics Database, North Carolina State University, Raleigh, NC 27695-7617, USA. Source: <http://ctdbase.org/> (accessed on 20 July 2021, which applies to all footnotes).

<sup>2</sup> Specifics for *PPP1R10*: <http://ctdbase.org/detail.go?type=gene&acc=5514&view=ixn>.

<sup>3</sup> Specifics for *IGFALS*: <http://ctdbase.org/detail.go?type=gene&acc=3483&view=ixn>.

<sup>4</sup> Specifics for *DUSP1*: <http://ctdbase.org/detail.go?type=gene&acc=1843&view=ixn>.