

## Supplementary material

- Table S1:** Total amount of lipids measured in liver organoids from control and ASMD type B. Lipid species are shown in order from most to least abundant.\*SD: Standard deviation; \*\* P-value is referred to comparison with control organoids.

Lipid Class		Control		ASMD type B		
		Average (pmol)	SD*	Average (pmol)	SD*	p-value**
Cholesterol	Chol	5070.61	488.56	4592.23	503.14	0.175
Triacylglycerol	TAG	776.33	40.78	1544.77	175.92	0.048
Phosphatidylethanolamine	PE	1750.48	169.52	947.04	84.90	0.042
Phosphatidylcholine	PC	1151.01	98.63	693.33	46.36	0.007
Phosphatidylinositol	PI	361.83	17.42	337.68	26.10	0.469
Phosphatidylethanolamine (-ether)	PE O-	176.58	16.26	240.80	21.14	0.100
Sphingomyelin	SM	141.20	15.16	227.21	24.73	0.039
Ceramide	Cer	148.94	11.99	197.44	17.10	0.049
Phosphatidylserine	PS	273.10	32.14	170.92	19.05	0.060
Cholesterol esters	CE	13.47	0.59	160.43	17.96	0.014
Diacylglycerol	DAG	97.13	6.39	55.85	4.49	0.015
Phosphatidylglycerol	PG	18.41	1.87	49.39	5.20	0.021
Hexosylceramide	HexCer	62.00	6.41	33.46	2.94	0.058
Phosphatidylcholine (-ether)	PC O-	12.18	0.39	30.90	2.50	0.008
Lyso-Phosphatidylcholine	LPC	22.38	2.17	26.01	3.52	0.224
Phosphatidate	PA	22.70	1.14	17.19	2.07	0.079
Cardiolipin	CL	16.56	0.37	13.19	0.82	0.033
Lyso-Phosphatidylethanolamine (-ether)	LPE O-	2.32	0.22	5.05	0.48	0.034
Lyso-Phosphatidylserine	LPS	3.12	0.53	4.57	0.46	0.235
Lyso-Phosphatidylinositol	LPI	2.70	0.41	3.27	0.40	0.275
Lyso-Phosphatidylcholine (-ether)	LPC O-	1.12	0.18	2.73	0.29	0.057
Lyso-phosphatidylglycerol	LPG	1.24	0.09	2.01	0.11	0.023
Lyso-Phosphatidylethanolamine	LPE	1.39	0.14	1.00	0.08	0.131
Lyso-Phosphatidate	LPA	0.96	0.08	0.46	0.05	0.037

**Table S2:** List of the top lysosome related DEGs down-regulated after differentiation of liver organoids.

GENE_ID	NAME_GENE	Average Expression	log2 (fold change) <sup>1</sup>	pvalue <sup>2</sup>	padj <sup>3</sup>
<i>ATP6V0D1</i>	ATPase H <sup>+</sup> transporting V0 subunit d1	1865.15	-0.37	0.0001	0.0008
<i>CD63</i>	CD63 molecule	12104.37	-0.34	0.0000	0.0001
<i>DMXL2</i>	Dmx like 2	233.17	-0.87	0.0000	0.0000
<i>AP1G2</i>	adaptor related protein complex 1 subunit gamma 2	1121.80	-0.63	0.0000	0.0000
<i>AP3B1</i>	adaptor related protein complex 3 subunit beta 1	642.25	-1.07	0.0000	0.0000
<i>FUCA1</i>	alpha-L-fucosidase 1	2368.32	-0.69	0.0000	0.0000
<i>ARSA</i>	arylsulfatase A	940.58	-0.44	0.0004	0.0018
<i>CTSA</i>	cathepsin A	3494.65	-0.25	0.0006	0.0028
<i>CTSE</i>	cathepsin E	41030.68	-0.75	0.0000	0.0000
<i>CTSF</i>	cathepsin F	248.85	-0.75	0.0003	0.0017
<i>CTSH</i>	cathepsin H	2693.53	-0.53	0.0000	0.0000
<i>CTSV</i>	cathepsin V	425.42	-3.18	0.0000	0.0000
<i>CTSZ</i>	cathepsin Z	4617.22	-0.59	0.0000	0.0000
<i>CTNS</i>	cystinosin, lysosomal cystine transporter	144.08	-1.20	0.0006	0.0027
<i>ENTPD4</i>	ectonucleoside triphosphate diphosphohydrolase 4	1059.13	-0.50	0.0004	0.0019
<i>GALNS</i>	galactosamine (N-acetyl)-6-sulfatase	615.78	-0.80	0.0000	0.0000
<i>GM2A</i>	ganglioside GM2 activator	630.12	-0.35	0.0012	0.0053
<i>HEXB</i>	hexosaminidase subunit beta	1446.14	-0.75	0.0000	0.0000
<i>HYAL1</i>	hyaluronidase 1	5204.75	-1.38	0.0000	0.0000
<i>HYAL2</i>	hyaluronidase 2	1457.98	-0.38	0.0003	0.0017
<i>LIPA</i>	lipase A, lysosomal acid type	637.63	-0.89	0.0000	0.0000
<i>PSAP</i>	prosaposin	22061.46	-0.26	0.0003	0.0017
<i>SMPD1</i>	sphingomyelin phosphodiesterase 1	2260.37	-0.93	0.0000	0.0000
<i>SUMF1</i>	sulfatase modifying factor 1	1073.01	-0.76	0.0000	0.0000

<sup>1</sup>log2FoldChange: log2 fold change between the groups; <sup>2</sup>pvalue: Wald test p-value; <sup>3</sup>padj: Benjamini-Hochberg adjusted p-value.

**Table S3:** List of the top lysosome related DEGs up-regulated after differentiation of liver organoids.

GENE_ID	NAME_GENE	Average Expression	log2 (fold change) <sup>1</sup>	pvalue <sup>2</sup>	padj <sup>3</sup>
<i>ATP6V0B</i>	ATPase H+ transporting V0 subunit b	1473.99	0.54	0.0000	0.0000
<i>CD68</i>	CD68 molecule	3694.96	1.44	0.0000	0.0000
<i>GNPTAB</i>	N-acetylglucosamine-1-phosphate transferase subunits alpha and beta	333.86	0.56	0.0018	0.0073
<i>ASAH1</i>	N-acylsphingosine amidohydrolase 1	1421.49	0.49	0.0000	0.0000
<i>NPC1</i>	NPC intracellular cholesterol transporter 1	723.62	0.58	0.0000	0.0000
<i>TCIRG1</i>	T cell immune regulator 1, ATPase H+ transporting V0 subunit a3	2229.93	0.91	0.0000	0.0000
<i>ACP5</i>	acid phosphatase 5, tartrate resistant	198.51	4.05	0.0000	0.0000
<i>GAA</i>	alpha glucosidase	1702.46	0.71	0.0000	0.0000
<i>IDUA</i>	alpha-L-iduronidase	251.44	0.72	0.0002	0.0010
<i>NAGA</i>	alpha-N-acetylgalactosaminidase	558.86	0.91	0.0000	0.0000
<i>CTSB</i>	cathepsin B	7175.67	1.22	0.0000	0.0000
<i>CTSD</i>	cathepsin D	40423.33	0.60	0.0000	0.0000
<i>CTSL</i>	cathepsin L	750.30	1.38	0.0000	0.0000
<i>CTSS</i>	cathepsin S	1140.01	0.49	0.0000	0.0000
<i>DNASE2</i>	deoxyribonuclease 2, lysosomal	647.52	0.59	0.0000	0.0000
<i>GUSB</i>	glucuronidase beta	1161.03	0.74	0.0000	0.0000
<i>IDS</i>	iduronate 2-sulfatase	783.31	0.94	0.0000	0.0000
<i>LAMP2</i>	lysosomal associated membrane protein 2	2333.75	0.31	0.0002	0.0012
<i>MAN2B1</i>	mannosidase alpha class 2B member 1	1144.97	0.62	0.0000	0.0002
<i>MANBA</i>	mannosidase beta	883.39	0.56	0.0000	0.0000
<i>PPT1</i>	palmitoyl-protein thioesterase 1	240.13	0.90	0.0000	0.0000
<i>SCARB2</i>	scavenger receptor class B member 2	5275.19	0.97	0.0000	0.0000
<i>SLC11A2</i>	solute carrier family 11 member 2	637.07	0.55	0.0005	0.0026
<i>SLC17A5</i>	solute carrier family 17 member 5	1176.23	0.48	0.0000	0.0000
<i>SORT1</i>	sortilin 1	1832.34	0.27	0.0003	0.0016
<i>TPP1</i>	tripeptidyl peptidase 1	2157.41	0.56	0.0000	0.0000

<sup>1</sup>log2FoldChange: log2 fold change between the groups; <sup>2</sup>pvalue: Wald test p-value; <sup>3</sup>padj: Benjamini-Hochberg adjusted p-value.

**Table S4:** Oligonucleotides used for the amplification and sequencing of the 6 exons of the *SMPD1* gene and adjacent intronic regions are shown. All oligonucleotides were designed with the specific sequence of the *SMPD1* gene (genomic *SMPD1* reference sequence NG\_011780).

Exon <i>SMPD1</i> Gene	Amplicon Size (pb)	Forward Primer (5'-3')	Reverse Primer (5'-3')
1	544	agaagggtaatcgggtgtcc	tagatgccacacctccatc
2	996	tggaaatggaggccaag	ttaggggagcctcaatgaaga
3, 4	667	actgtgagctccttgaggt	tgctcaagggaatttcagc
5, 6	976	ggggaggctcctcactagaa	agctccaggaaaggagaagg
6	846	agggctcgagaaacctatgg	atgggcacctctgctgtatt
1	544	agaagggtaatcgggtgtcc	tagatgccacacctccatc

**Table S5:** Specific primers for QT-PCR amplification of genes LGR5, KRT19, ALB and APOB. TaqMan probes Universal probe library, UPL, (Roche).

Gene Name*	Forward Primer (5'-3')	Reverse Primer (5'-3')	Universal Probe Library for Human (Roche)
<i>LGR5</i>	accagactatgcctttgaaac	ttcccaggagtgattctat	#78, cat.no. 04689011001
<i>KRT19</i>	gccactactacagaccatcc	caaacttggttcggaagtcat	#71, cat.no. 04688945001
<i>ALB</i>	agaggtctcaagaaacctaggaaa	ggttcaggaccacggataga	#44, cat.no. 04688040001
<i>APOB</i>	acagctgattgaggtgtcca	agccactggaggatgtgagt	#90, cat.no. 04689151001

\*Gene Name: LGR5 (Leucine-rich repeat containing G protein-coupled receptor 5), KRT19 (Keratin 19), ALB (Albumin) and APOB (Apolipoprotein B).

**Table S6:** Specific primers of sphingomyelin metabolism genes are shown for quantitative analysis of their expression.

Gene Name	Forward Primer (5'-3')	Reverse Primer(5'-3')	Protein Name
<i>SGMS1</i>	ttctggtgtatcacactatg	gtaagatcgaggtacaattcc	Sphingomyelin Synthase 1 (SMS1)
<i>SMPD1</i>	ctcgggtgaagaaggaacca	agcgtctccacacctccacat	Acid Sphingomyelinase (ASM)
<i>SMPD2</i>	ctgttttcaaggtgcag	acgaggagtagattttcg	Sphingomyelin phosphodiesterase 2 (nSM2)
<i>GAPDH</i>	cctcgtcccgtagacaaaatg	tgaagggtcgttgatggc	Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH)