Supplementary Table 1

Supplementary Table 1: Lists of VOLs generated in each PUFA+AAPH, or AAPH/hemin induced peroxidation system (*VOLs identified using NIST library. **Peaks that were not identified in the NIST library search were designated as VOL-n).

FA18:2 derived VOLs

ID	Compounds	Quantitative ion, *m/z*	t_R, min	MS peak area (n=3) control, Average	AAPH, Average	AAPH/hemin, Average	AAPH, Fold change (vs control)	AAPH/hemin, Fold change (vs control)
1	pentanal	44	7.9	1186.666667	30302	144943	25.53539326	122.1429775
2	hexanal	56	11.4	59275.33333	2398940.333	7034259	40.47114033	118.6709311
3	2-butylfuran	81	14.8	0	10289	47046.33333	>2	>2
4	heptanal	70	15.15	1124.333333	19685.66667	31869.66667	17.50874592	28.34538986
5	hexanoic acid methyl ester	74	15.9	3394.666667	6317.333333	86528	1.860958366	25.48939513
6	2-heptenal	83	17.1	8251	138875.6667	933575.6667	16.83137397	113.1469721
7	1-octen-3-one	55/70	17.9	17506	270442.6667	1428053	15.44857001	81.57505998
8	1-octen-3-ol	57/72	17.9	12334.33333	122347.3333	824935.6667	9.919249791	66.88125287
9	2,3-octadiene	43	18.1	47120.66667	122347.3333	825389.6667	2.596468641	17.5165108
10	VOL-1**	81	18.4	20450	84571	328353	4.135501222	16.05638142
11	2-pentylfuran	138	18.4	0	11599	2379570.333	>2	>2
12	VOL-2**	55	18.55	16418.33333	60772.66667	191248.6667	3.701512537	11.64848239
13	2-octenal	70	20.2	3488	681484.6667	792424.6667	195.3797783	227.1859709
14	2-octen-1-ol	57	20.9	8645.333333	243601.6667	412111.3333	28.17724399	47.6686459
15	3-nonenal*	55	21.8	7207.666667	452889.3333	30127.33333	62.83438931	4.179901031
16	2-nonenal	55	23.8	5176	162270	26170	31.35046368	5.056027821
17	2,4-nonadienal*	81	25.5	3948.666667	72127.66667	345105.3333	18.26633463	87.39794023
18	2,4-decadienal*	81	27.8	7532	68594	492304	9.10701009	65.36165693
19	cis-4,5-epoxy-(E)-2-decenal*	68	29.75	4282.333333	42790.33333	417043.6667	9.992293921	97.38701642

FA20:4 derived VOLs

ID	Compounds	Quantitative ion, *m/z*	t_R, min	MS peak area (n=3) control, Average	AAPH, Average	AAPH/hemin, Average	AAPH, Fold change (vs control)	AAPH/hemin, Fold change (vs control)
1	pentanal	44	7.9	1534	44181.33333	130602.6667	28.8013907	85.13863538
2	hexanal	56	11.4	139583	4377453	5464536	31.36093221	39.14900812
3	2-butylfuran	81	14.8	1689.333333	21459.66667	136144	12.70303867	80.59037096
4	heptanal	70	15.15	7274.333333	59714.66667	111120.3333	8.208953856	15.27567246
5	hexanoic acid methyl ester	74	15.9	1569.666667	11581.66667	88935.66667	7.378424294	56.65895094
6	2-heptenal	83	17.1	6788.666667	120687.3333	374261.6667	17.77776687	55.13036433
7	1-octen-3-one	55/70	17.9	60963.33333	2237188.667	14343587.67	36.69728252	235.2822079
8	1-octen-3-ol	57/72	17.9	33034.66667	1735519.667	8091854	52.53631539	244.9503754
9	2,3-octadiene	43	18.1	2743.333333	1734750.333	8086030.667	632.3512758	2947.520292
10	VOL-1**	81	18.4	8926.666667	12570.66667	36220	1.408215086	4.057505601
11	2-pentylfuran	138	18.4	11536	46507.33333	227335	4.031495608	19.70657074
12	VOL-2**	55	18.55	4216.333333	103046	323192	24.43971855	76.65238359
13	2-octenal	70	20.2	17178.66667	832372.6667	4284639	48.4538575	249.4162721
14	2-octen-1-ol	57	20.9	30003.66667	383090	1226464.667	12.76810612	40.87715946
15	3-nonenal*	55	21.8	16424	763947.3333	94464.33333	46.51408508	5.751603345
16	2-nonenal	55	23.8	2227.666667	91644.33333	37539	41.13915906	16.8512644
20	4-decenal	84	24.9	2644.666667	79859	127442.6667	30.19624401	48.18855558
17	2,4-nonadienal*	81	25.5	1974	82198	155455	41.64032421	78.75126646
18	2,4-decadienal*	81	27.8	28546.33333	1701590.333	9110973.667	59.60801738	319.1644111
21	Nona-3,5-dien-2-one, isomer-1*	95	29.4	7547.333333	695041.3333	412288.6667	92.09098136	54.62706475
19	cis-4,5-epoxy-(E)-2-decenal*	68	29.8	5386.666667	48035.66667	749836.3333	8.917512376	139.2022896
22	Nona-3,5-dien-2-one, isomer-2*	95	30.2	2645.666667	148015.3333	105153	55.94632733	39.74536979
23	2,4-dodecadienal*	81	30.6	433	9865	70837.33333	22.78290993	163.5966128
24	2,6-dodecadien-1-al*	69	31.1	1670.333333	46372	42995.66667	27.76212333	25.74077031
25	2,4,7-tridecatrienal*	91	33.3	1394.333333	26802.33333	89024	19.22232847	63.84699976

FA20:5 derived VOLs

ID	Compounds	Quantitative ion, *m/z*	t_R, min	MS peak area (n=3) control, Average	AAPH, Average	AAPH/hemin, Average	AAPH, Fold change (vs control)	AAPH/hemin, Fold change (vs control)
26	1-penten-3-ol	57	7.4	2576.666667	30531	224762.6667	11.84902975	87.23001294
27	1-penten-3-one	55	7.6	3551	98426.33333	721566.6667	27.71791983	203.2009763
28	2,3-pentanedione	57	7.8	553.6666667	3280	16811.66667	5.924142083	30.36423841
29	2-ethylfuran	81	8	18224	72185.33333	275873	3.961003805	15.13789508
30	2-pentenal-1*	55	9.4	1652.333333	13369	104522.6667	8.090982449	63.25761549
31	2-pentenal-2*	55	9.8	2911	12058.66667	63016.66667	4.142448185	21.64777282
32	3-hexenal	69	11.3	1292	110205.6667	33472.66667	85.29850361	25.90763674
33	2-hexenal	83	13.1	0	5592.333333	2667	>2	>2
34	4-heptenal	68	15.1	814.6666667	15848.66667	40711	19.45417349	49.97258592
35	2,4-heptadienal-1*	110	18.5	1027	22201.66667	164259.3333	21.61798117	159.9409283
36	2,4-heptadienal-2*	110	19	0	2908	36025.33333	>2	>2
37	VOL-3**	95	20.05	0	2679.333333	27529.33333	>2	>2
38	VOL-4**	95	20.15	0	5838.666667	85601.66667	>2	>2
39	VOL-5**	95	20.25	0	8458.666667	110186	>2	>2
40	VOL-6**	95	20.55	562.3333333	5970	75949	10.61647896	135.0604624
41	2,6-nonadienal*	69	23.6	2202	56041.66667	21195.33333	25.45034817	9.625491977
42	VOL-7**	79	24.4	1541.333333	12569	56323	8.154628028	36.54173875
43	VOL-8**	79	25	6056.666667	22089.33333	38979.66667	3.647110622	6.435828288
44	VOL-9**	57	27.1	324	781.3333333	32239.33333	2.411522634	99.50411523
45	VOL-10**	79	27.7	3072.666667	43002.66667	252450.3333	13.99522673	82.16001302
46	VOL-11**	81	28.4	3297.666667	2446	23995.33333	0.741736581	7.276458102
47	VOL-13**	79	33.4	823	9089	28503	11.04374241	34.63304982

FA22:6 derived VOLs

ID	Compounds	Quantitative ion, *m/z*	t_R, min	MS peak area (n=3) control, Average	AAPH, Average	AAPH/hemin, Average	AAPH, Fold change (vs control)	AAPH/hemin, Fold change (vs control)
26	1-penten-3-ol	57	7.4	7778	39968	156286.6667	5.13859604	20.0934259
27	1-penten-3-one	55	7.6	4169.666667	105402	666545.6667	25.27827964	159.8558638
28	2,3-pentanedione	57	7.8	1684.333333	6300	17785	3.740352266	10.55907382
29	2-ethylfuran	81	8	25161.66667	73825.66667	148571.3333	2.934053123	5.904669802
30	2-pentenal-1*	55	9.4	1968.666667	9879.333333	95089	5.018286488	48.3012191
31	2-pentenal-2*	55	9.8	5809.666667	16746.33333	58260	2.882494693	10.02811406
32	3-hexenal	69	11.3	5351.333333	74368	13590.33333	13.8970973	2.539616295
33	2-hexenal	83	13.1	394.3333333	4237	846.3333333	10.74471682	2.146238377
34	4-heptenal	68	15.1	3982.333333	34688	36182.33333	8.710471248	9.085711894
35	2,4-heptadienal-1*	110	18.5	14537	50349	158462.3333	3.463506913	10.9006214
36	2,4-heptadienal-2*	110	19	3512.666667	8538.666667	34039.33333	2.430821788	9.690453597
37	VOL-3**	95	20.05	905.6666667	5155.333333	35042	5.692307692	38.69193964
38	VOL-4**	95	20.15	2341.666667	13123	109454	5.604128114	46.74192171
39	VOL-5**	95	20.25	1381	15733.66667	154084.3333	11.39295197	111.5744629
40	VOL-6**	95	20.55	1927.666667	10143	96683.33333	5.261801833	50.15562857
41	2,6-nonadienal*	69	23.6	26604	81364	28353.66667	3.058337092	1.065767053
42	VOL-7**	79	24.4	2119	20169.66667	66517	9.518483561	31.39075035
43	VOL-8**	79	25	10297.33333	85905.33333	48415	8.342483491	4.701702706
44	VOL-9**	57	27.1	864.3333333	1896.333333	91858	2.193983803	106.276128
45	VOL-10**	79	27.7	27706	114355.3333	263125.3333	4.12745735	9.497052383
46	VOL-11**	81	28.4	12733	7912	36248.66667	0.621377523	2.846828451
48	VOL-12**	79	30.1	34146.66667	108721	84915.66667	3.18394182	2.486792269
47	VOL-13**	79	33.4	4326.666667	22743	42729	5.256471495	9.875731895

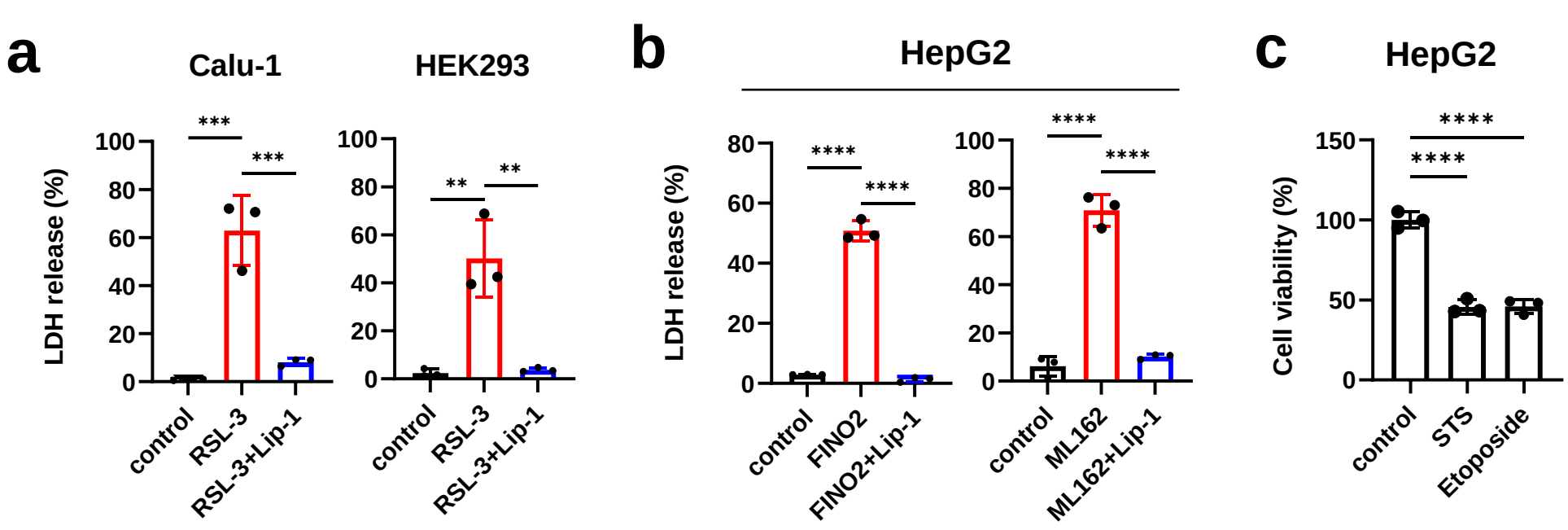
Supplementary Table 2

Supplementary Table 2: Patient characteristics.

	Healthy control	MASLD	Liver cirrhosis (LC)	*P* values
No. of patients	48	44	10	-
Age median [range] years	49.5 [22-78]	58.5 [32-84]	69.4 [58-81]	****0.0001[a-1] **0.0038[b-1] *0.0486[c-1]
Male/Female	17/31	16/28	5/5	-
BMI	22.0 [16.2-29.5]	26.9 [21.1-33.3]	29.5 [19.3-35.4]	****<0.0001[a-1] 0.0004[b-1] 0.7768[c-1]
ALT, IU/L	23.0 [11-55]	55.1 [11-155]	28.7 [9-48]	****<0.0001[a-1] 0.8407[b-1] *0.0269[c-1]
AST, IU/L	53.4 [23-89]	46.2 [17-194]	38.1 [18-71]	0.5002[a-1] 0.3041[b-1] 0.7084[c-1]
ALBI	-	-2.96 [-3.46 - -2.33]	-2.37 [-3.33 - -1.65]	****<0.0001[c-2]
FIB-4 index	-	1.70 [0.30-6.61]	4.78 [1.77-10.86]	****<0.0001[c-2]
M2BPGi	-	0.97 [0.26-2.62]	2.91 [0.54-5.73]	****<0.0001[c-2]

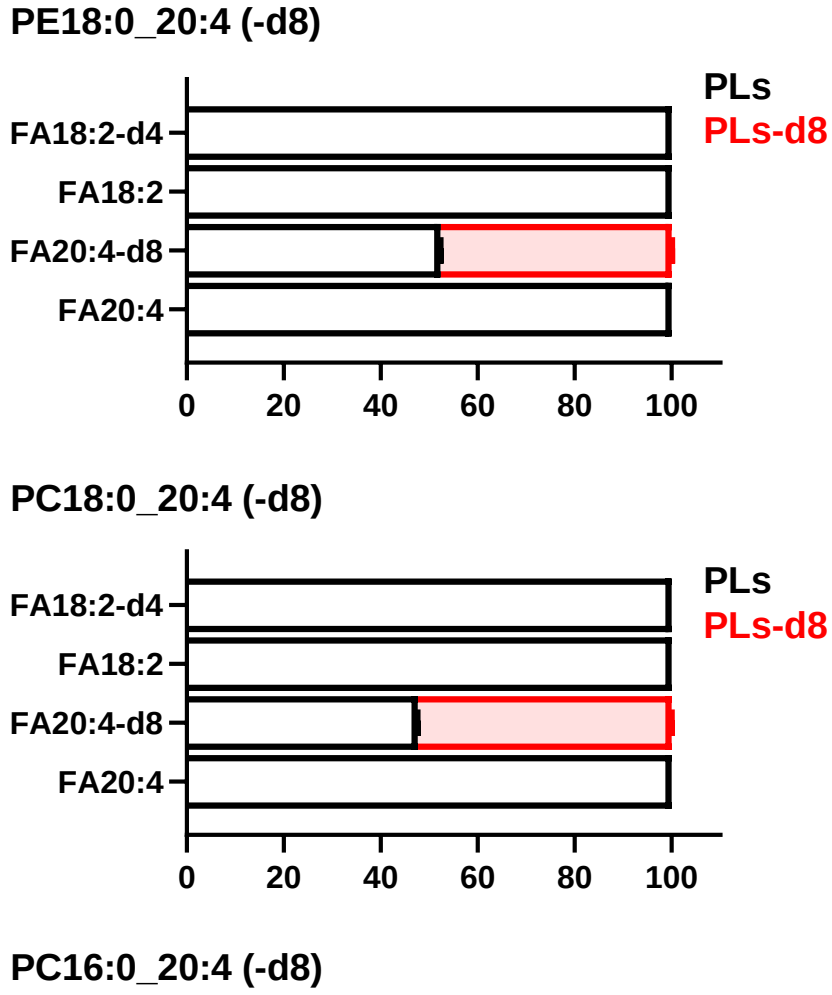
a: Healthy control vs MASLD. b: Heathyl control vs LC. c: MASLD vs LC.
1: One-way ANOVA with Tukey's multiple comparisons test. 2: Student's t-tests. * p<0.05; ** p < 0.01; *** p<0.001; **** p<0.0001.

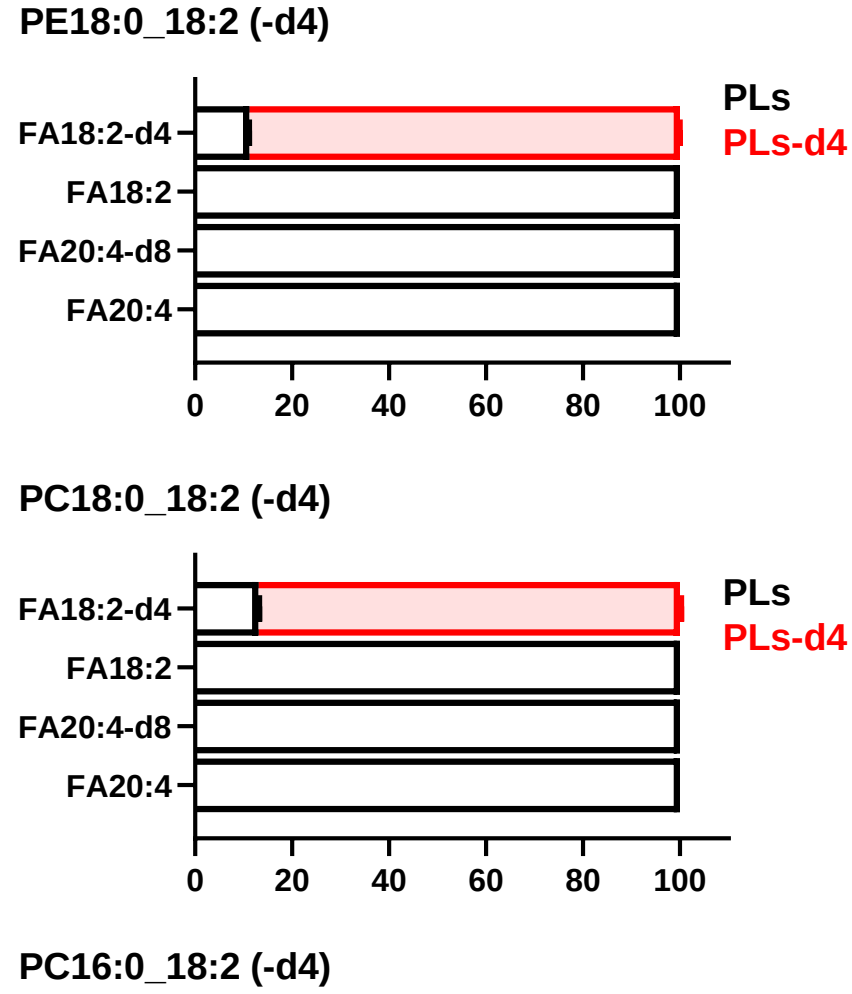
Extended Data Figure 1

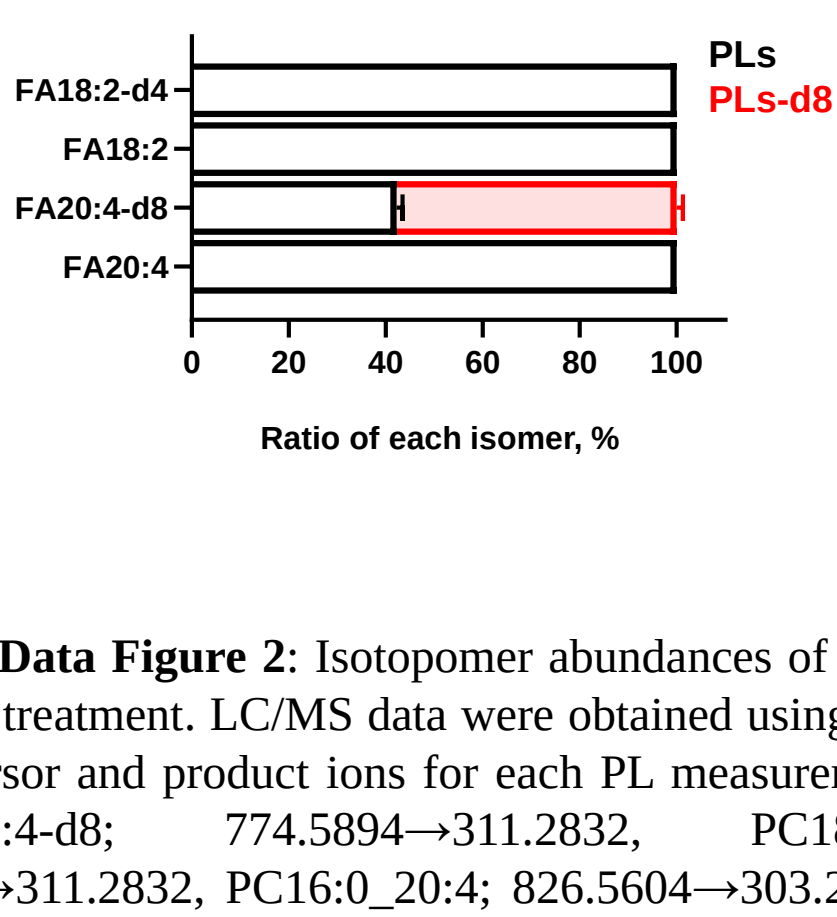


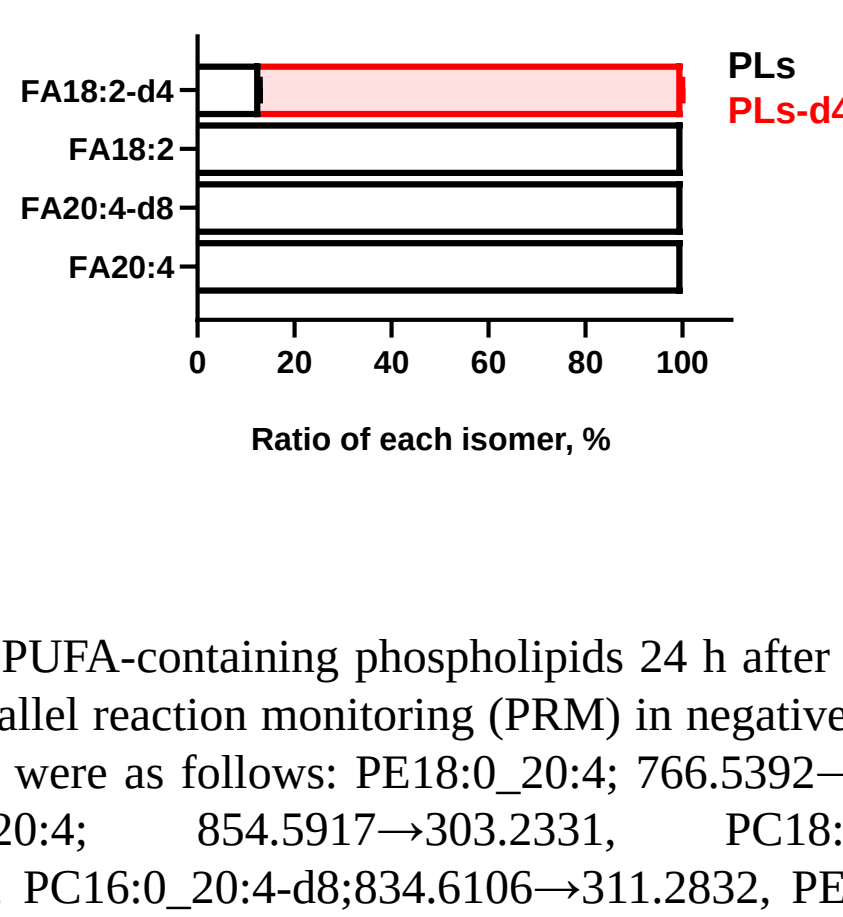
Extended Data Figure 1: (a) LDH release assay of Calu-1 and HEK293 cells treated with 1.0 μM RSL-3 for 4 h. (b) LDH release assay in HepG2 cells treated with 50 μM FINO2 or 5.0 μM ML162 for 4 h. (c) Cell viability of HepG2 cells treated with 10 μM STS or 100 μM etoposide for 6 h, measured using the MTT assay. (d) Flow cytometric analysis of annexin V-633 and propidium iodide fluorescence. HepG2 cells were treated with 10 μM STS or 100 μM etoposide for 6 h. Data are presented as the mean ± standard deviation of three repeated experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Source Data are provided as source data files.

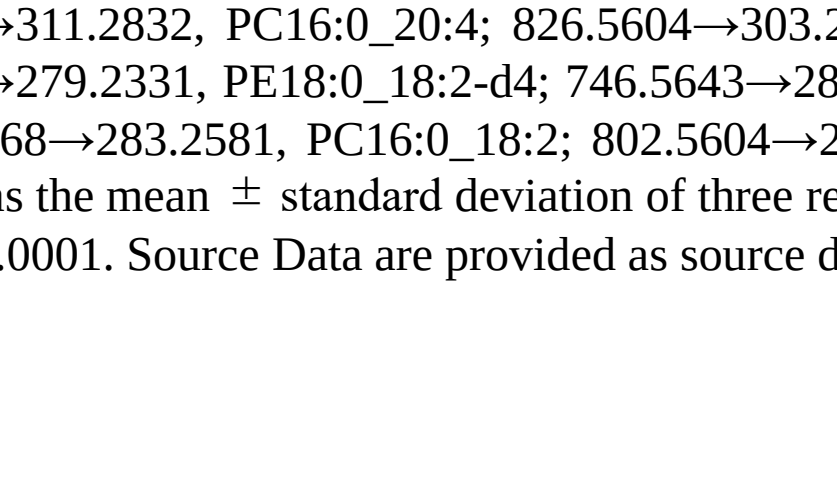
Extended Data Figure 2

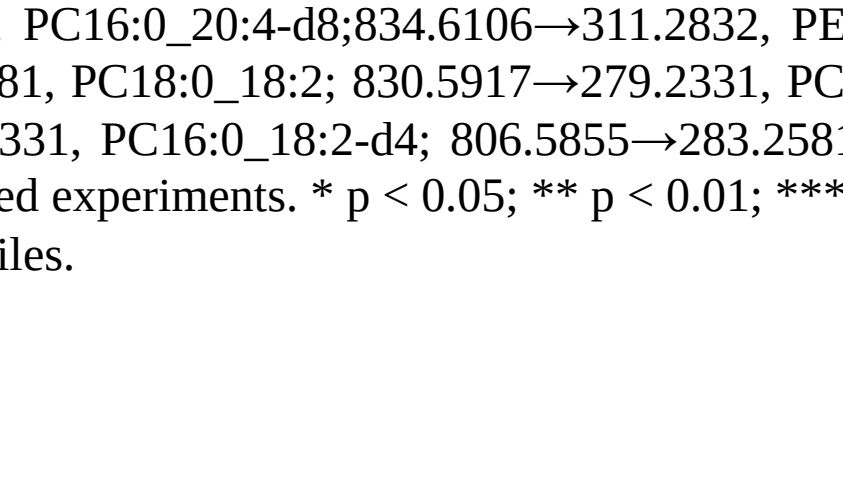






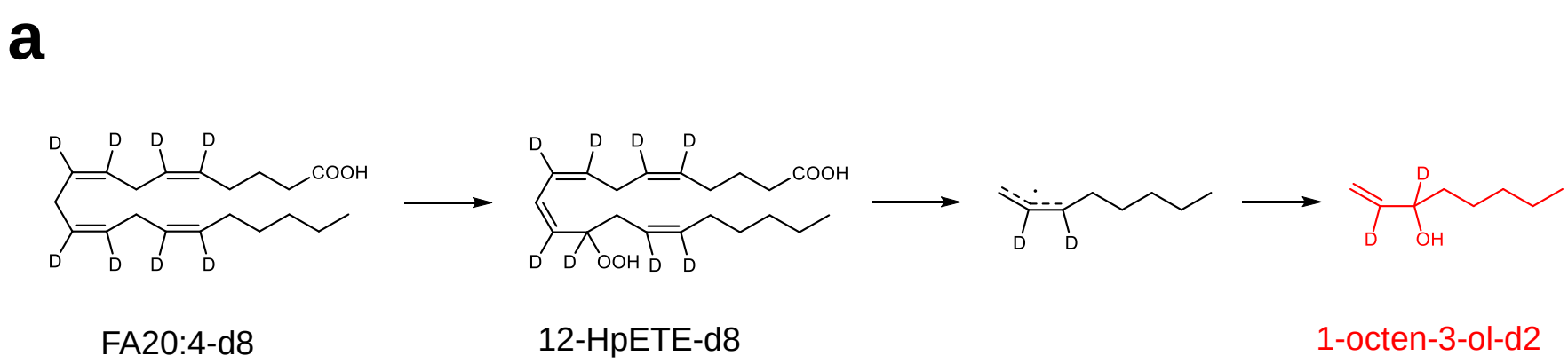






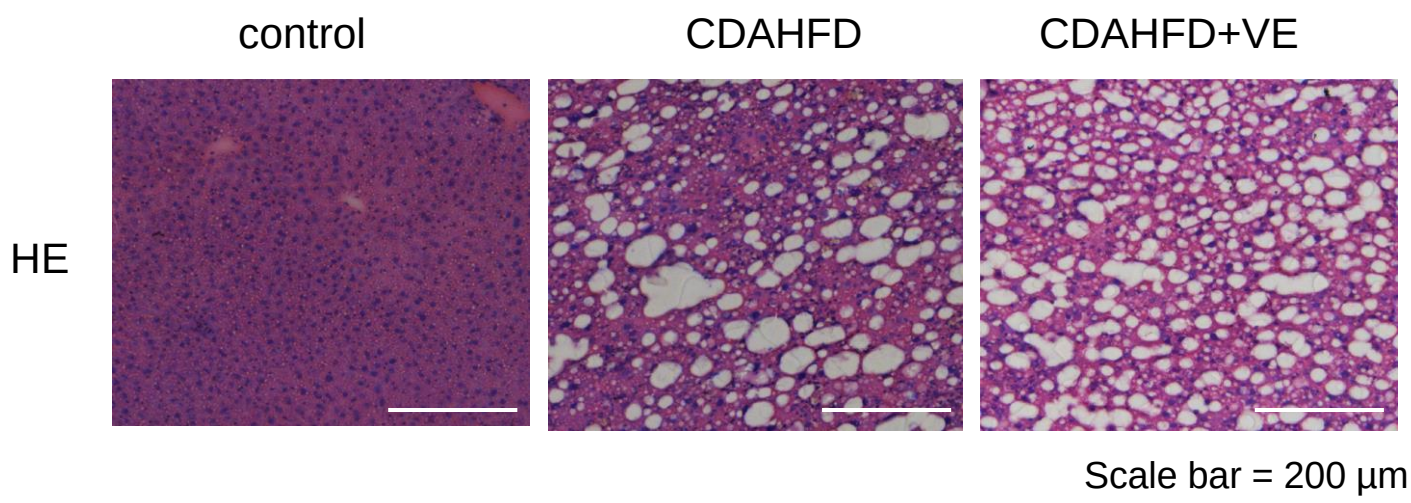
Extended Data Figure 2: Isotopomer abundances of ω-6 PUFA-containing phospholipids 24 h after (d-labeled) ω-6 PUFA treatment. LC/MS data were obtained using parallel reaction monitoring (PRM) in negative ion mode. The precursor and product ions for each PL measurement were as follows: PE18:0_20:4; 766.5392→303.2331, PE18:0_20:4-d8; 774.5894→311.2832, PC18:0_20:4; 854.5917→303.2331, PC18:0_20:4-d8; 862.6419→311.2832, PC16:0_20:4; 826.5604→303.2331, PC16:0_20:4-d8;834.6106→311.2832, PE18:0_18:2; 742.5392→279.2331, PE18:0_18:2-d4; 746.5643→283.2581, PC18:0_18:2; 830.5917→279.2331, PC18:0_18:2-d4; 834.6168→283.2581, PC16:0_18:2; 802.5604→279.2331, PC16:0_18:2-d4; 806.5855→283.2581. Data are presented as the mean ± standard deviation of three repeated experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Source Data are provided as source data files.

Extended Data Figure 3



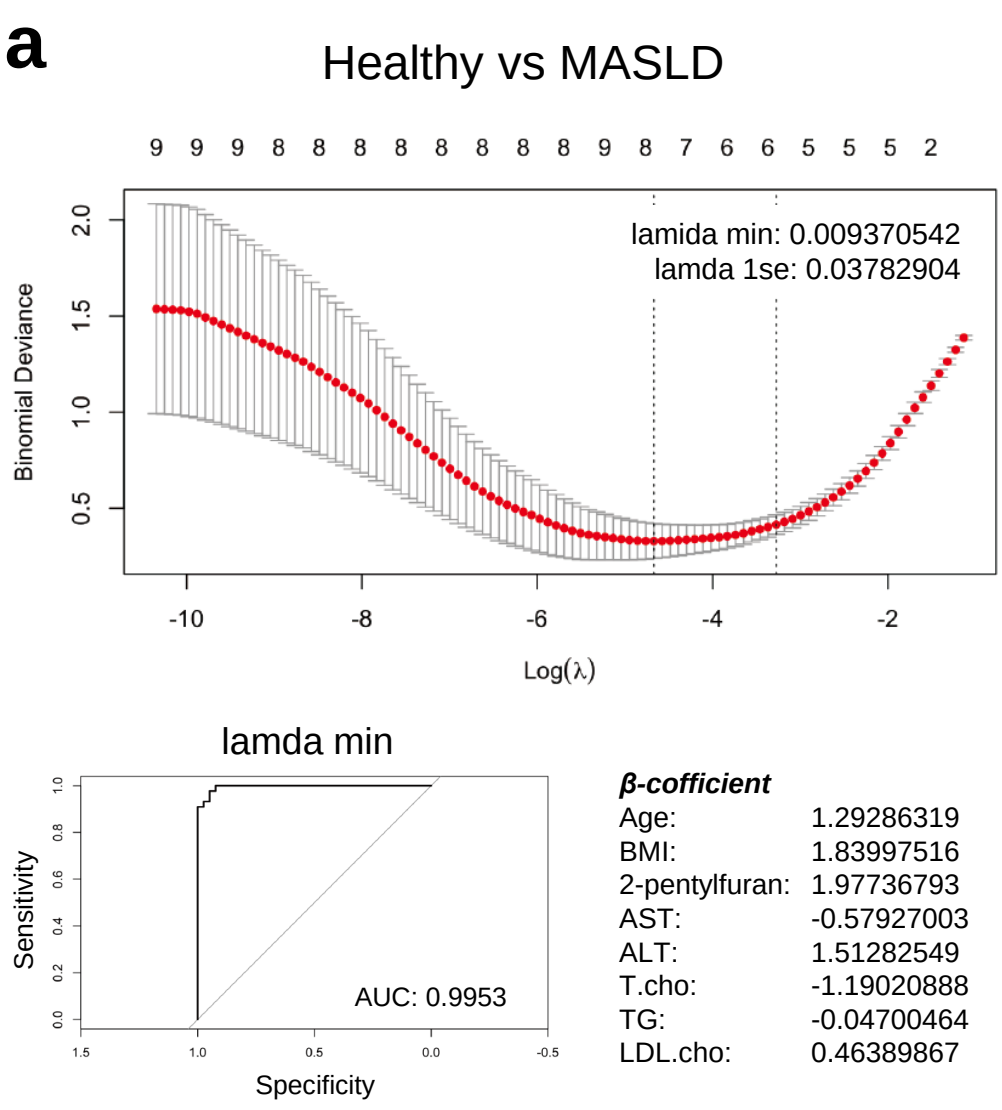
Extended Data Figure 3: Plausible production mechanisms and chemical structures of d-labeled VOLs (a,d) and their MS spectra observed in the FA20:4-d8+AAPH/hemin peroxidation system (b-c, e-f).

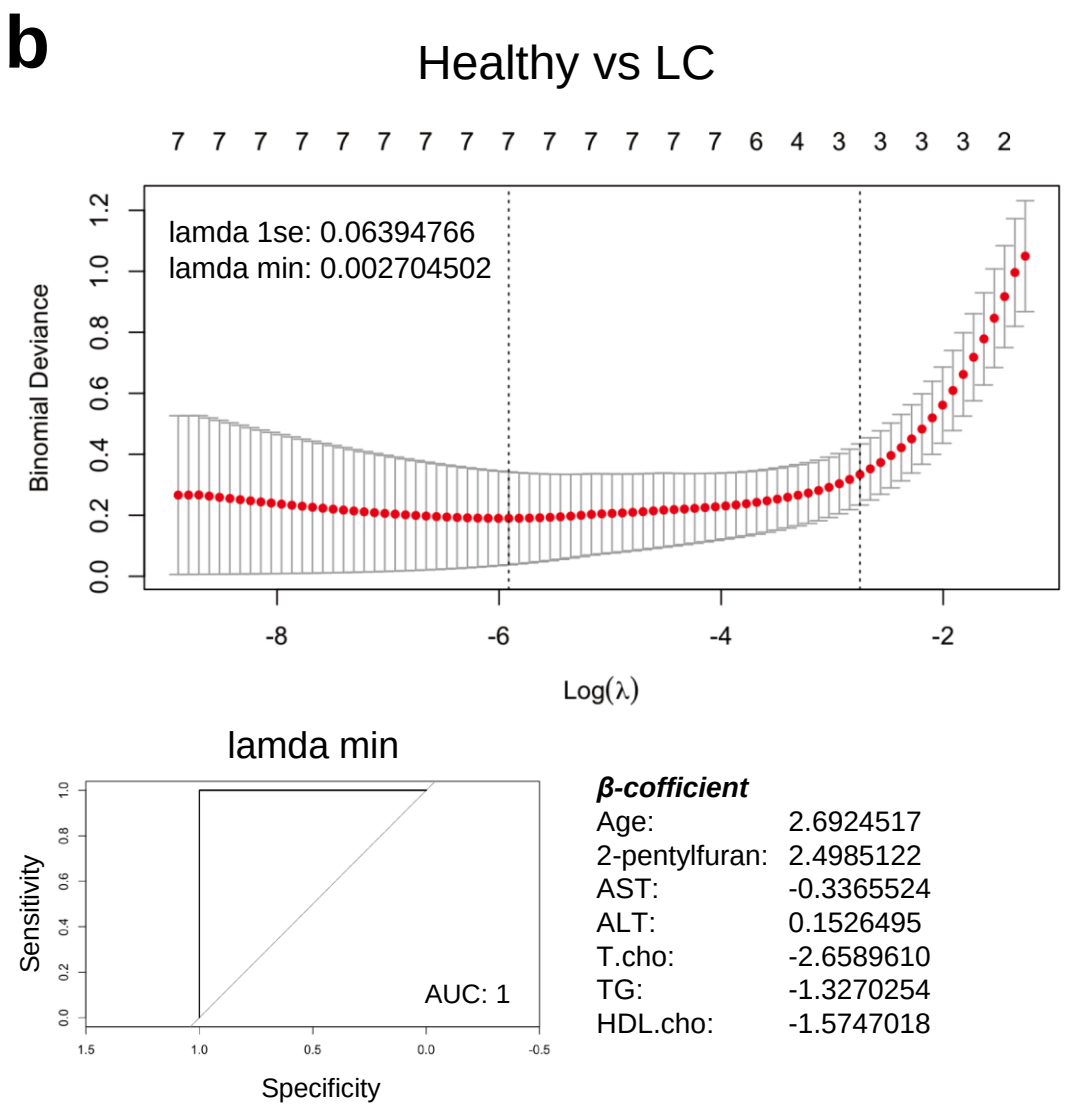
Extended Data Figure 4

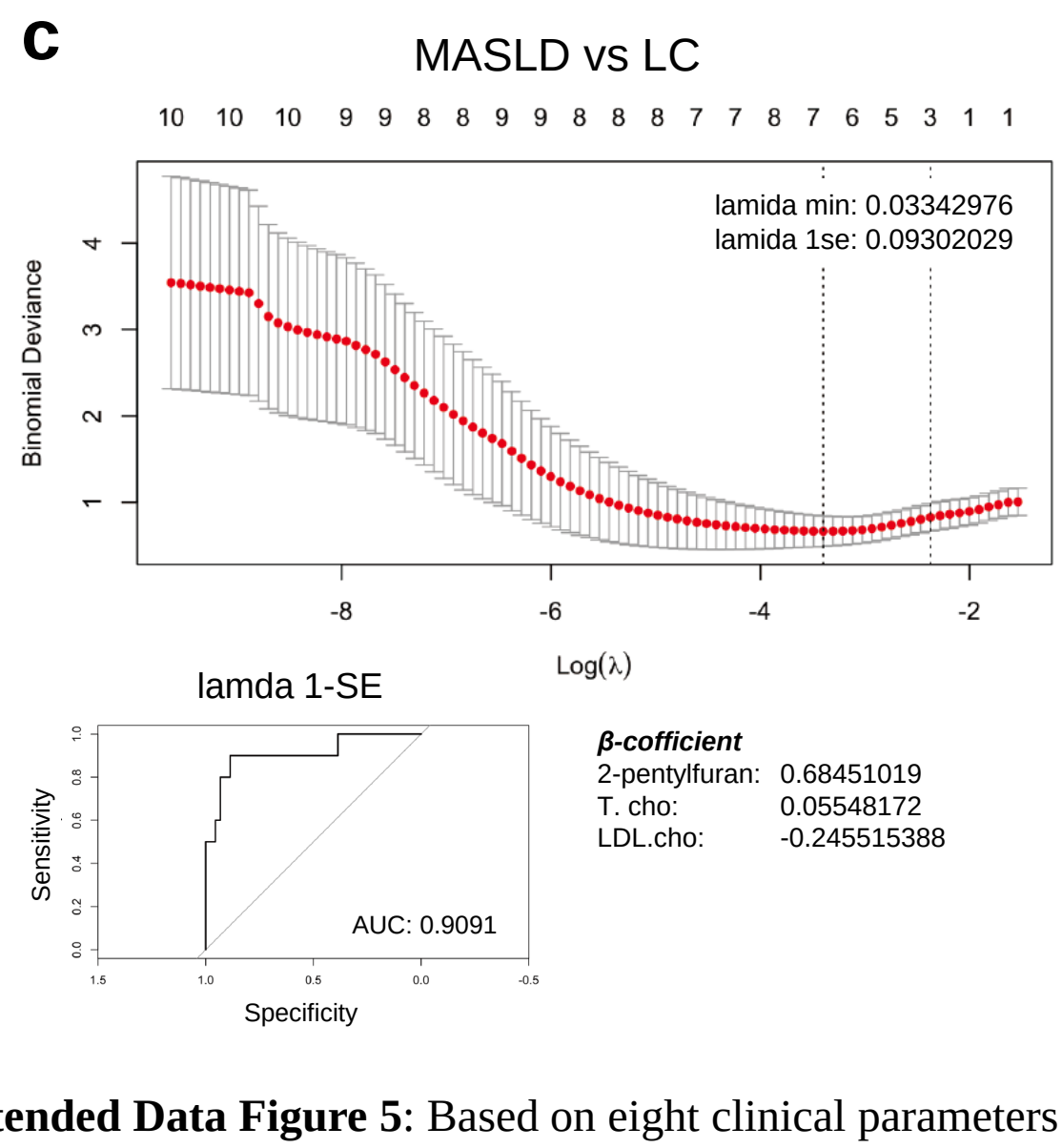


Extended Data Figure 4: Histological observation of the liver of control and CDAHFD (±VE)-fed mice (hematoxylin and eosin (H&E)). Original magnification: H&E, ×20; Scale bars: 200 μm.

Extended Data Figure 5

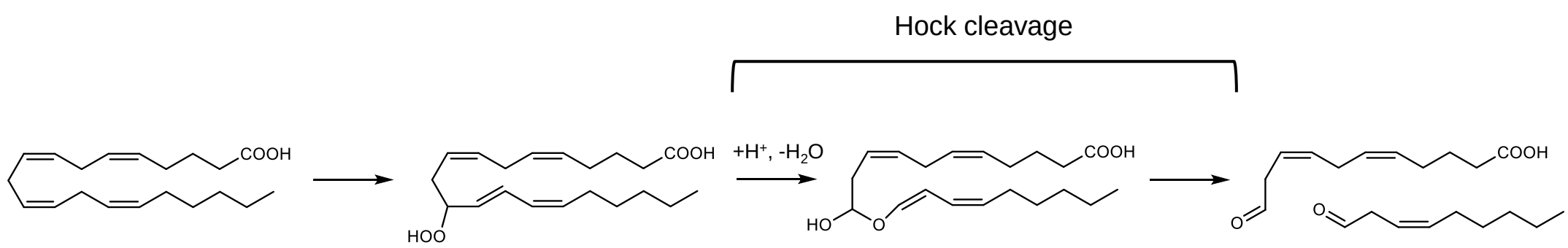






Extended Data Figure 5: Based on eight clinical parameters (Age, BMI, AST, ALT, T-cho, TG, HDL-cho, LDL-chol) and two VOL markers, we used the least absolute shrinkage and selection operator (LASSO). We used a ten-fold cross validation to test the robustness of the model. We did not use the other clinical parameters (g-GTP, T-bil, Alb, ALBI, Fib4, M2 BPGi, HbA1c) for the too many missing values. Nine subjects in the control group were excluded because there were too many missing values for clinical parameters (AST, ALT, T-cho, TG. HDL-cho, LDL-cho). Missing values for g-GTP, T-Bil, Alb, T-cho, TG, HDL-cho, and LDL-cho (n =1, 1, 1 1, 4, 3, respectively) were replaced by the mean imputation method. First, we analyzed for healthy and MASLD (n =39, 44, respectively). Lamda min selected 8 variables and lamda 1-SE selected 5 variables. ROC analysis based on each variable showed AUCs of 0.995 (**Figure 5g**) and 0.993, respectively. Next, we analyzed healthy and LC (n = 39, 10, respectively). Lamda min selected 7 variables and lamda 1-SE selected 3 variables. ROC analysis based on each variable showed AUCs of 1.00 (**Figure 5h**) and 0.997, respectively. Finally, we analyzed MASLD and LC (44, 10, respectively). Lamda min selected 7 variables and lamda 1-SE selected 3 variables. ROC analysis based on each variable yielded AUCs of 0.955 and 0.909 (**Figure 5i**), respectively.

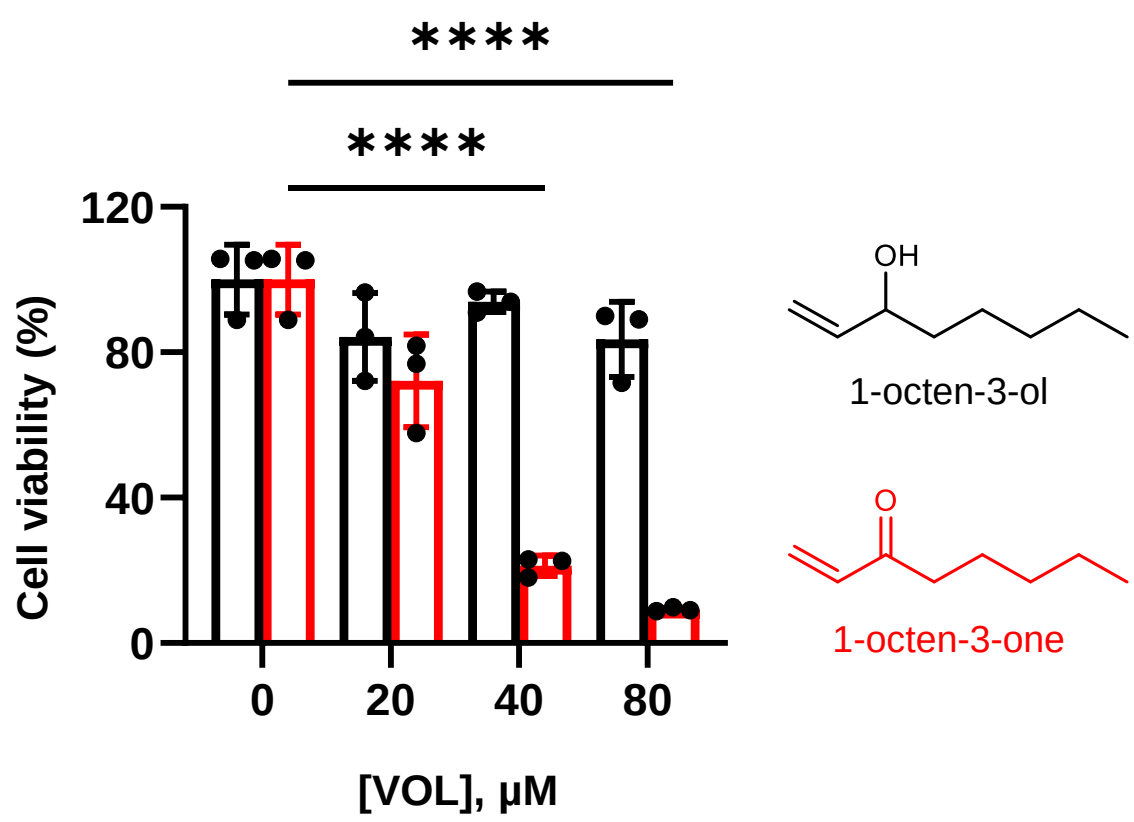
Extended Data Figure 6



FA20:4 11-HpETE 3-nonenal

Extended Data Figure 6: Plausible generation mechanism of 3-nonenal via Hock cleavage.

Extended Data Figure 7



Extended Data Figure 7: Viability of HepG2 cells treated with 1-octen-3-ol or 1-octen-3-one for 24 h, measured using the MTT assay. Data are presented as the mean ± standard deviation of three repeated experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Source Data are provided as source data files.