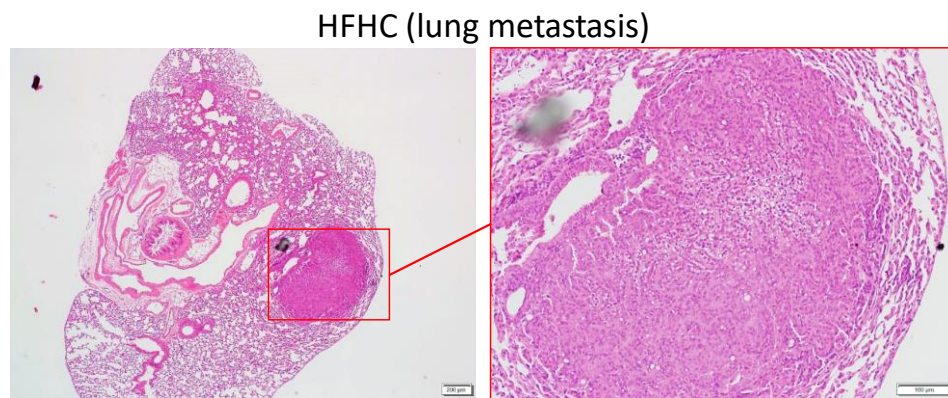


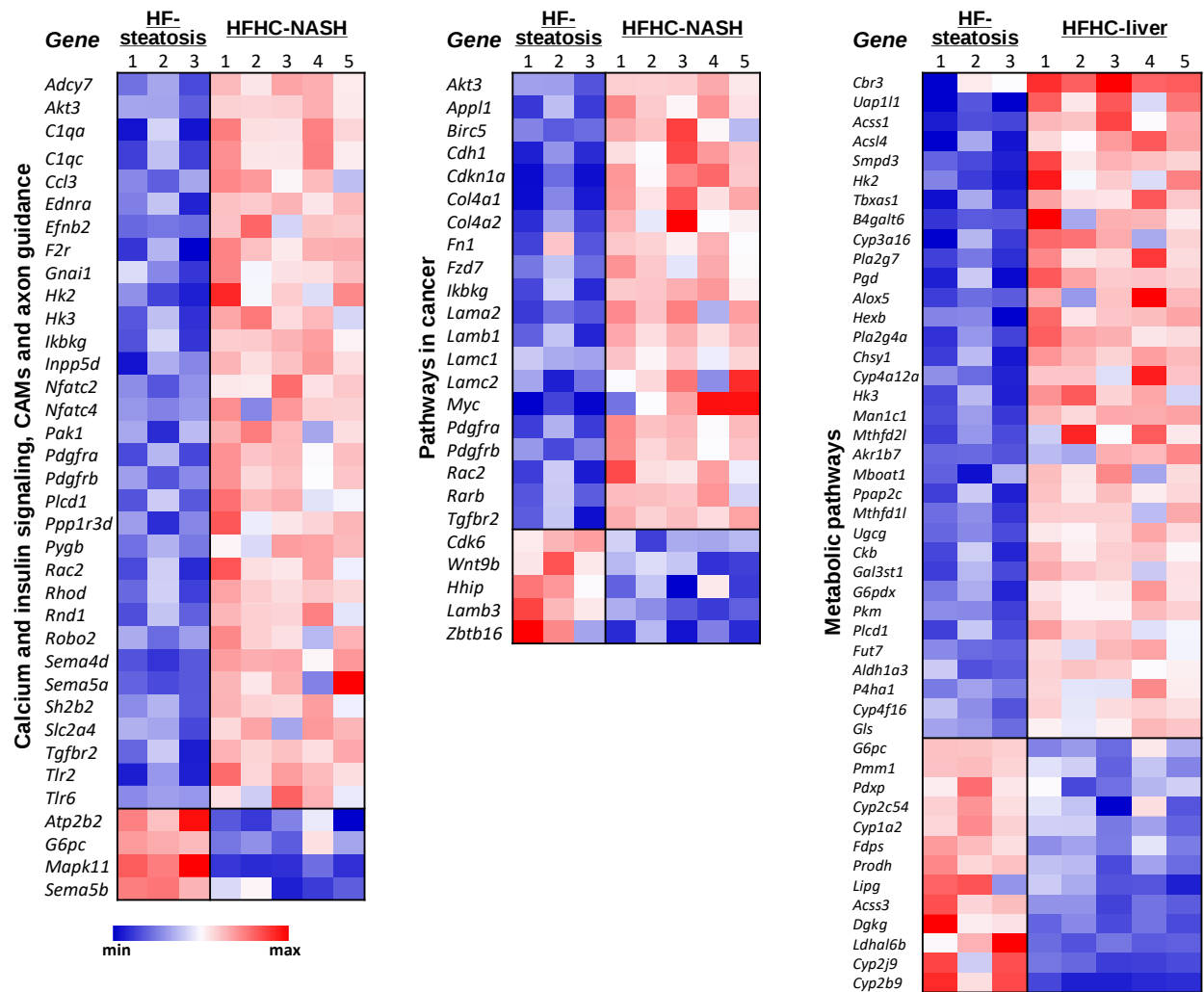
Supplementary Figures and Tables

Supplementary Fig. 1



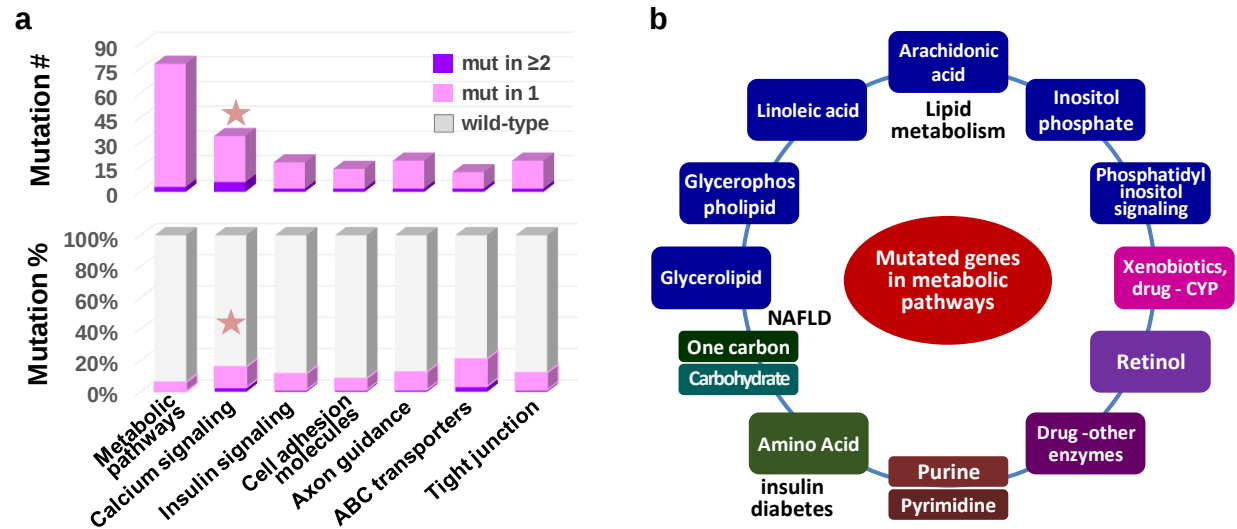
Supplementary Fig. 1. Representative H&E staining histological images of lung metastasis from a HFHC-fed mouse.

Supplementary Fig. 2



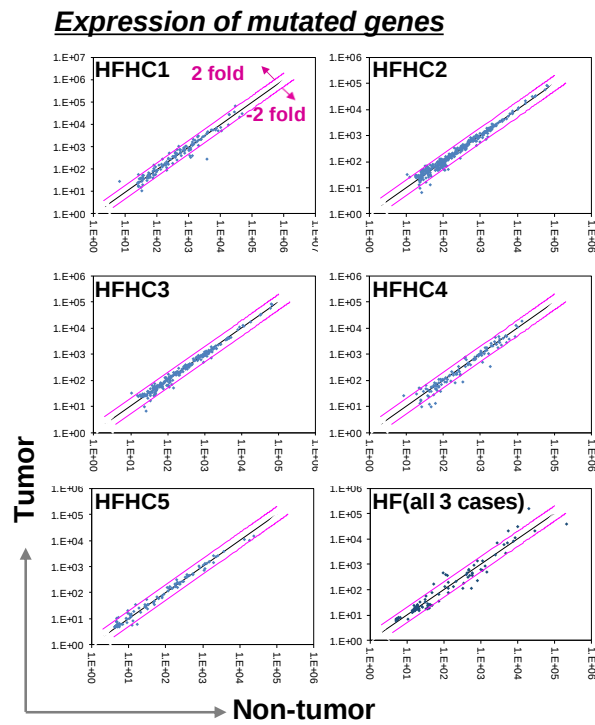
Supplementary Fig. 2 Expressional aberration of cancer- and metabolism-related genes associated with NASH development in HFHC-fed livers. Expression levels were normalized to the mean level of each gene among all samples shown.

Supplementary Fig. 3



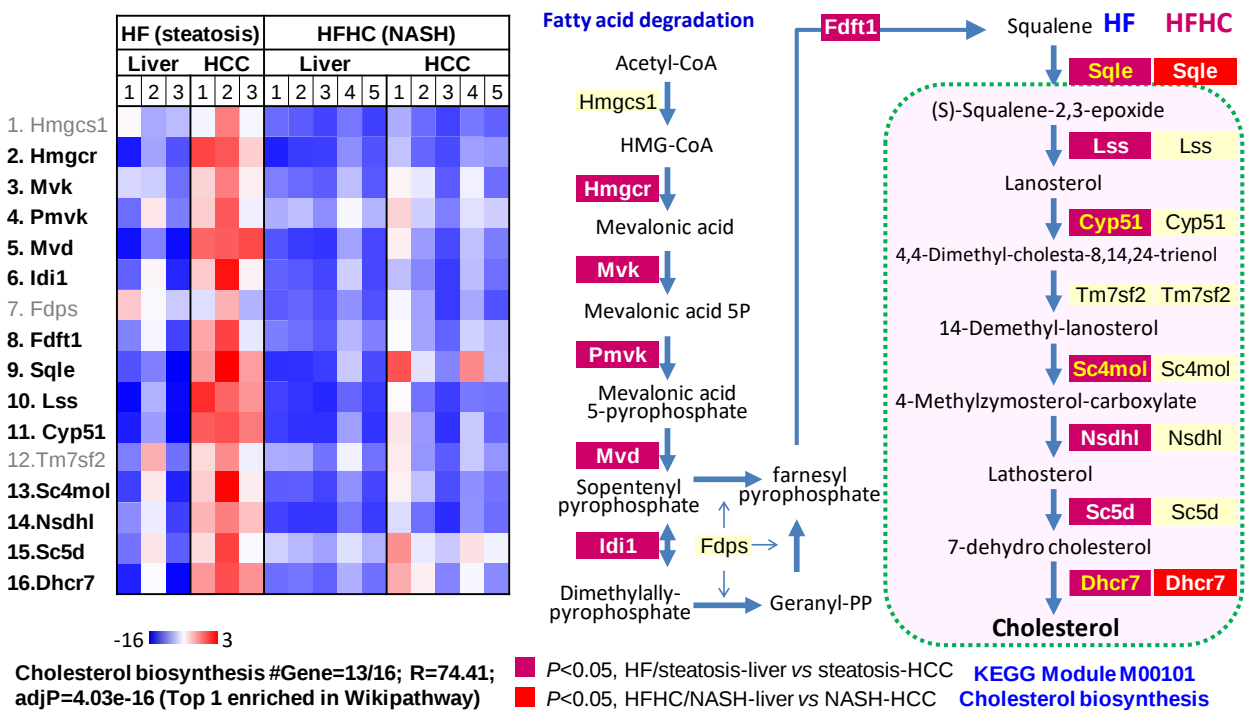
Supplementary Fig. 3 a Mutated gene numbers and proportions of affected pathways.
b Forty-five of the 75 mutated genes in metabolic pathways are involved in specific metabolic pathways, 6 of which are associated with lipid metabolism.

Supplementary Fig. 4



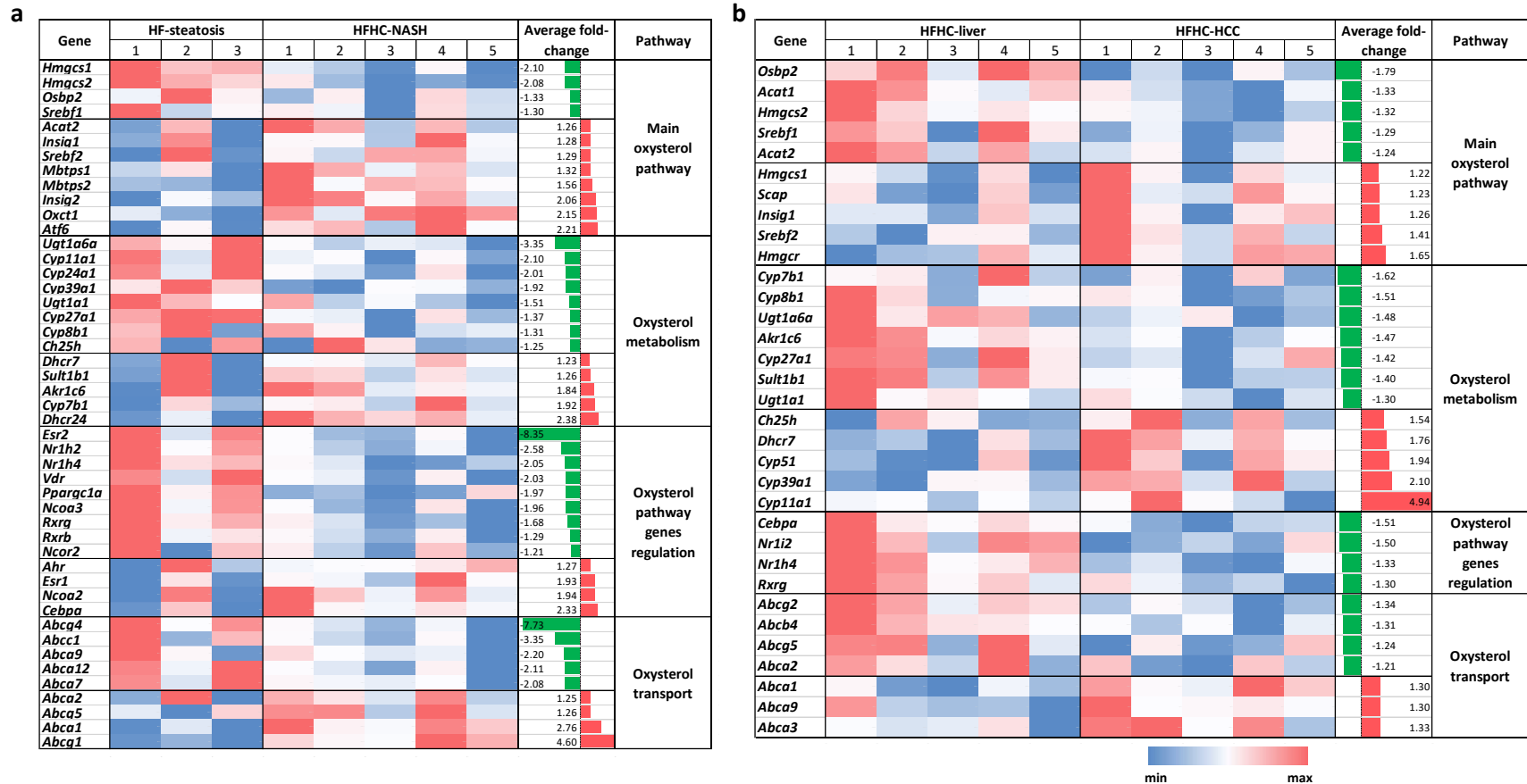
Supplementary Fig. 4 Dot plots showing the expression of mutated genes in HCCs as compared to adjacent non-tumorous livers.

Supplementary Fig. 5



Supplementary Fig. 5 Genome-wide gene expressional analysis by microarray showed that 13 of the 16 genes in cholesterol biosynthesis pathway were significantly up-regulated in HF-induced steatosis-HCCs as compared with adjacent non-tumorous livers, while only two of these genes were up-regulated in HFHC-induced NASH-HCCs.

Supplementary Fig. 6



Supplementary Fig. 6. Expressional aberration of oxysterol pathway genes in NASH-livers compared to steatosis-livers (a), and in NASH-HCCs compared to adjacent non-tumor livers (b) in HFHC-fed mice. Expression levels were normalized to the mean level of each gene among all samples shown.

Supplementary Table 1. Mutated genes in calcium signaling

Sample	Gene#	adjP*	Mutated genes									
HFHC_87	7	0.0003	Ryr1	Ryr2	P2rx1	Nos2	Cacna1s	Itpr2	Itpr3			
HFHC_88	9	0.0004	Ryr1	Ryr2	Grin2c	Itpr1	Cacna1h	Cacna1d	Erb3	Drd1a	Adcy8	
HFHC_89	10	1.10E-05	Ryr1	Ntsr1	Ptafr	Plcb2	Phka2	Cacna1d	Slc25a4	Atp2a3	Adcy4	Gna15
HFHC_93	5	0.0043	Ryr1	Plcg2	Htr7	Atp2a1	Cacna1h					
HFHC_96	5	0.0003	Plcd3	Pdgfra	P2rx1	Itpr1	Chrna7					
Recurrent	6	3.60E-06	Ryr1	Ryr2	P2rx1	Itpr1	Cacna1h	Cacna1d				

*adjP, p value adjusted by multiple test adjustment.

Supplementary Table 2. Mutated genes in metabolism-related pathways

#	PathwayName	Mutated genes (EntrezGene#)	Mutated Gene #	Gene # in pathway	adjusted P
1	Glycerolipid metabolism	110197 67800 50784 215456 102247	5	51	1.34E-08
2	Glycerophospholipid metabolism	110197 75320 50784 215456 102247 27388 665270	7	80	3.40E-11
3	Arachidonic acid metabolism	13096 107141 665270 404195 13098 71522 435802 13089 13090 631304 208285 11687	12	90	1.69E-20
4	Linoleic acid metabolism	13096 107141 665270 404195 13098 337924	6	46	7.44E-11
5	Phosphatidylinositol signaling system	72469 234779 18796 104015 110197	5	78	1.04E-07
6	Inositol phosphate metabolism	72469 234779 18796 104015	4	57	1.22E-06
7	Metabolism of xenobiotics by cytochrome P450	13096 337924 13089 404195 13090 107141 13098 94284	8	77	3.18E-13
8	Drug metabolism - cytochrome P450	13096 337924 13089 404195 13090 107141 13098 94284 213043	9	87	1.11E-14
9	Retinol metabolism	13096 337924 13089 404195 13090 107141 13098 94284 435802	9	77	4.72E-15
10	Drug metabolism - other enzymes	103149 337924 94284 382053	4	59	1.29E-06
11	Purine metabolism	18973 18971 18969 94041 231327 328099	6	168	1.40E-07
12	Pyrimidine metabolism	18973 18971 18969 103149	4	99	9.57E-06
13	Amino Acid metabolism	17448 18263 18948 15109 21990	5	112	7.92E-07
14	One carbon metabolism and related pathways	232087 108037 75320 246277	4	49	7.92E-07
15	Carbohydrate digestion and absorption	15277 226413 14377 18796	4	39	2.82E-07

Supplementary Table 3. Effective data and sequencing depth by whole-exome sequencing

Sample ID*	Effective data (Mb)	Mean depth	Coverage
HF_82T	5641.7	109.3	99.80%
HF_82N	5698.7	110.4	99.80%
HF_83T	5848.0	113.3	99.80%
HF_83N	5507.9	106.7	99.80%
HF_85T	5693.6	110.3	99.80%
HF_85N	5662.9	109.7	99.80%
HFHC_87T	6411.5	124.2	99.70%
HFHC_87N	5964.5	115.6	99.80%
HFHC_88T	5628.9	109.1	99.80%
HFHC_88N	5724.6	110.9	99.80%
HFHC_89T	5552.5	107.6	99.80%
HFHC_89N	5777.4	111.9	99.80%
HFHC_93T	5795.1	112.3	99.70%
HFHC_93N	5627.4	109.0	99.70%
HFHC_96T	5639.6	109.3	99.80%
HFHC_96N	5706.0	110.5	99.80%
Mean	5742.5	111.3	99.78%

*T, Tumor; N, adjacent non-tumor

Supplementary Table 4. Primers used in this study

Gene/target name	Primer	Nucleotide sequence (5'->3')	Size (bp)
<i>CFD</i>	Forward	CTTGATGTGCGCGGAGAG	139
	Reverse	GTAGATCCCGGGCTTCTTG	
<i>DDIT3</i>	Forward	AAGGCACTGAGCGTATCATGT	127
	Reverse	TGCTTTCAGGTGTGGTGATG	
<i>ALDH18A1</i>	Forward	TGTGGAGGGGAAGAAAGTTG	157
	Reverse	CAGATCAGCCAGATGATGGA	
<i>PSPH</i>	Forward	TCTTGCTCTTGCAGAATCCA	162
	Reverse	GCTCTGAGTGGGAGACCATC	
<i>ITGA6</i>	Forward	CGTGATCCGGAATATGGAG	137
	Reverse	TGGCTCTCTGCAGTGGAAG	
<i>CHKA</i>	Forward	TCCAGTGCTCCCTACCTGAC	122
	Reverse	AGCTTGTTCCGATCCCTCTT	
<i>SQLE</i>	Forward	GCAAGCTTCCTTCCTCCTTC	103
	Reverse	CAACAGTCATTCCTCCACCA	
<i>Beta-actin</i>	Forward	GTCTTCCCCTCCATCGTG	113
	Reverse	AGGGTGAGGATGCCTCTCTT	