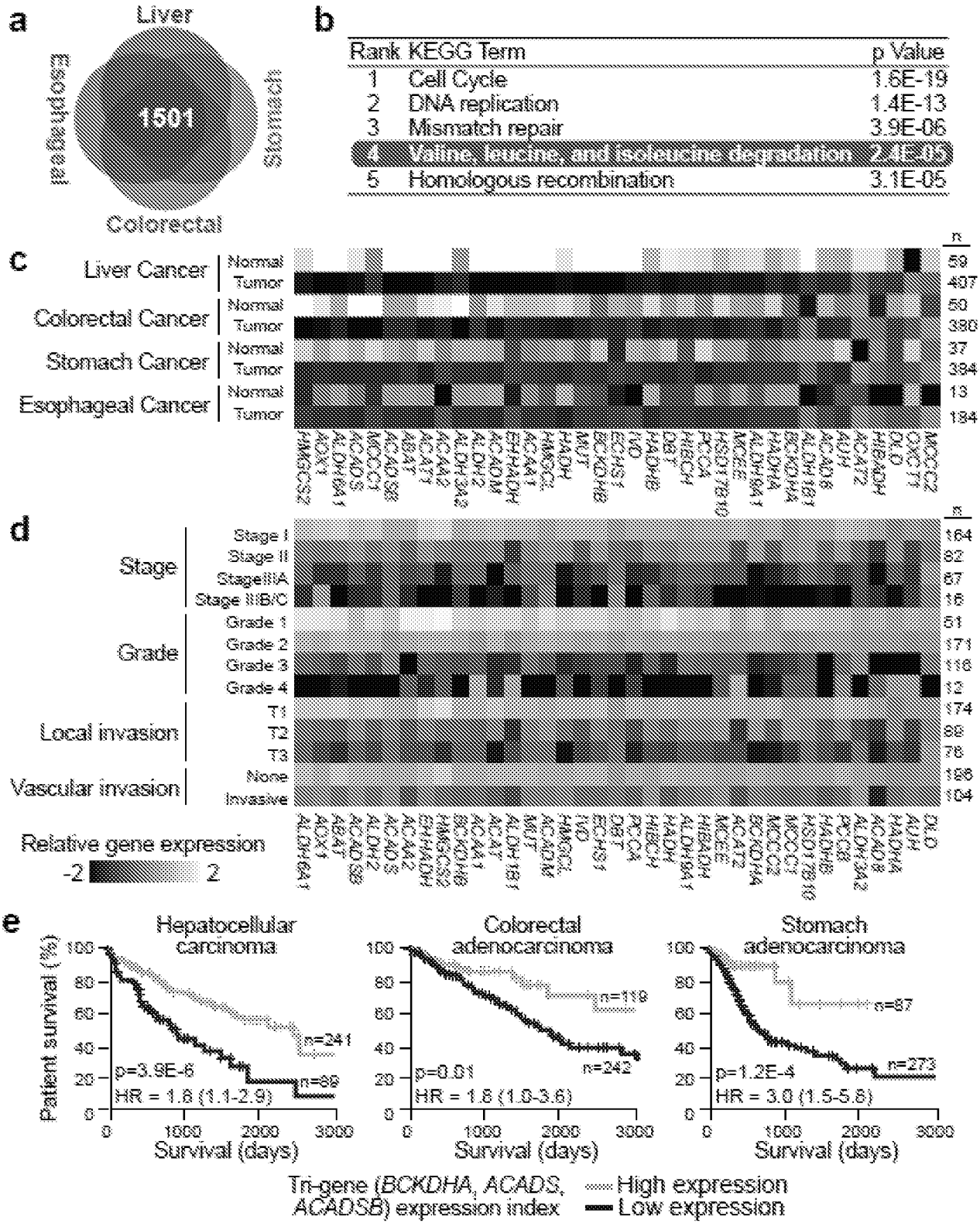

a
Liver
Esophagus
1501
Stomach
Colorectal
b
Rank KEGG Term p Value
1 Cell Cycle 1.6E-19
2 DNA replication 1.4E-13
3 Mismatch repair 3.9E-06
4 Valine, leucine, and isoleucine degradation 2.4E-05
5 Homologous recombination 3.1E-05
c
Liver Cancer
Colorectal Cancer
Stomach Cancer
Esophageal Cancer
Normal
Tumor
n
59
407
50
380
37
384
13
184
HMGCS2 AOX1 ALDH6A1 ACADS MCCC1 ACADSB ABAT ACAT2 ACAA2 ALDH3A2 ALDH2 ACADM EHHADH ACAA1 HMGCL HADH MUT BCKDHB ECHS1 IVD HADHB DBT HIBCH PCCA HSD17B10 MCEE ALDH9A1 HADHA BCKDHA ALDH1B1 ACAD8 AUH ACAT2 HIBADH OLD OXCT1 MCCC2
d
Stage
Stage I
Stage II
StageIIIA
Stage IIIB/C
Grade
Grade 1
Grade 2
Grade 3
Grade 4
Local invasion
T1
T2
T3
Vascular invasion
None
Invasive
n
164
82
67
16
51
171
116
12
174
68
76
166
104
Relative gene expression
-2
2
e
Hepatocellular carcinoma
Colorectal adenocarcinoma
Stomach adenocarcinoma
Patient survival (%)
n=241
n=89
p=3.9E-6
HR = 1.8 (1.1-2.9)
n=119
n=242
p=0.01
HR = 1.8 (1.0-3.6)
n=67
n=273
p=1.2E-4
HR = 3.0 (1.5-5.8)
Survival (days)
Tri-gene (BCKDHA, ACADS, ACADSB) expression index
High expression
Low expression

Figure 1

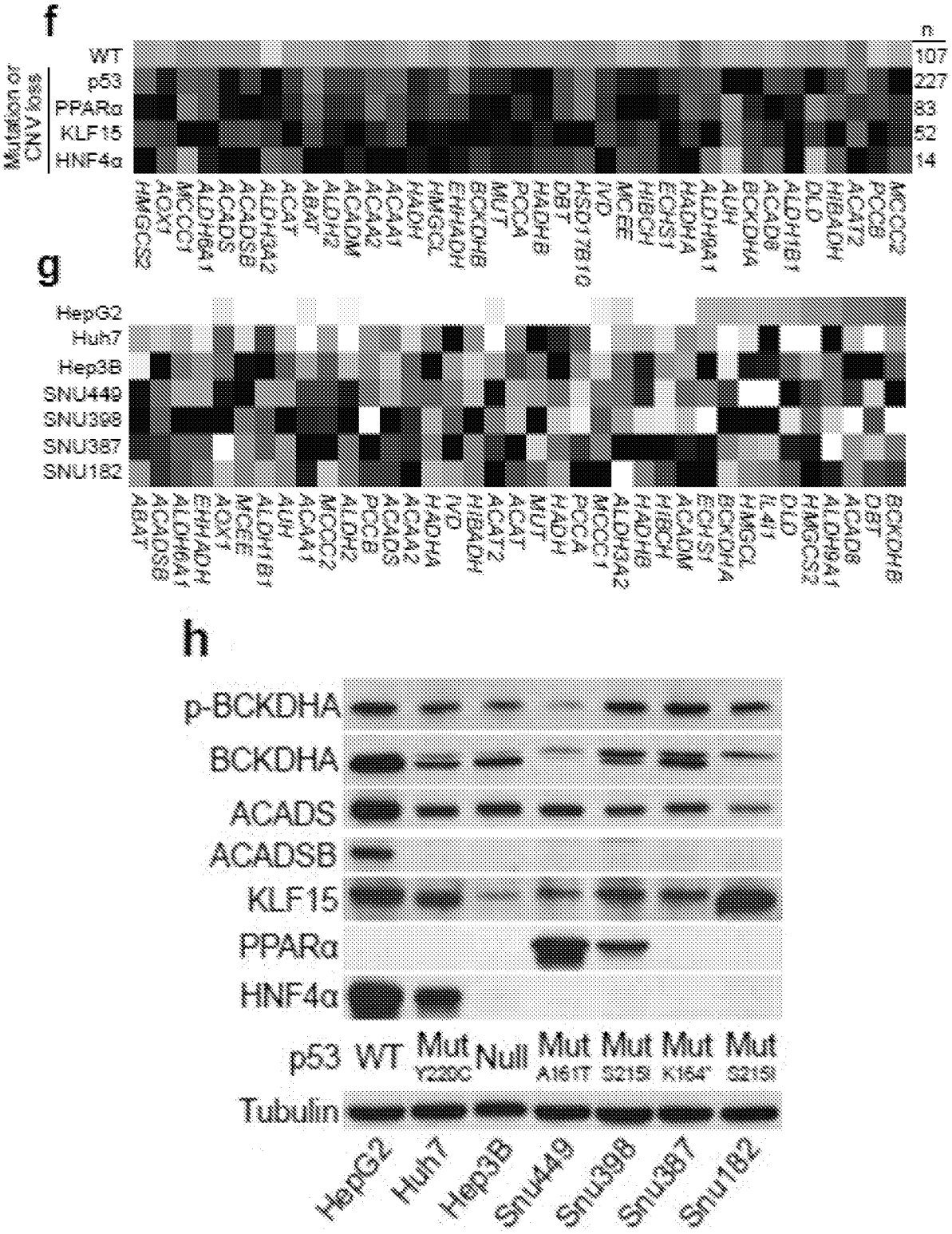
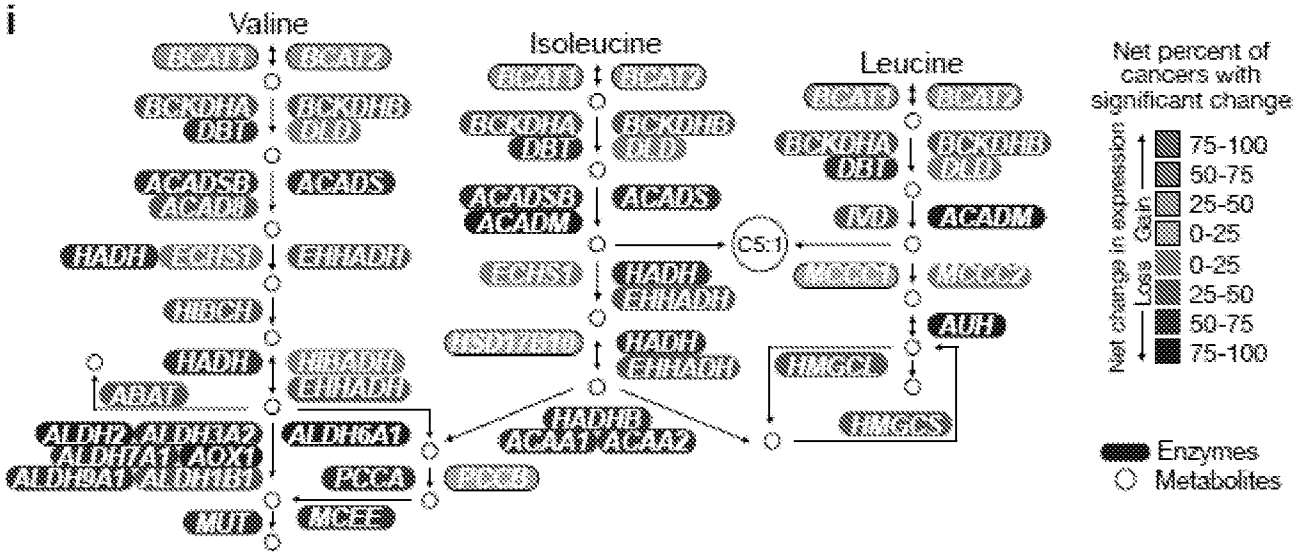


Figure 1 (cont.)

i



j

Hepatocellular carcinoma

Rank	KEGG Term	p Value
1	Valine, leucine and isoleucine degradation	7.3E-12
2	Fatty acid metabolism	1.8E-08
3	Cell cycle	1.8E-05
4	Pyrimidine metabolism	4.8E-05
5	Butanoate metabolism	5.6E-05

Cholangiocarcinoma

Rank	KEGG Term	p Value
1	Valine, leucine and isoleucine degradation	6.1E-17
2	Fatty acid metabolism	1.4E-13
3	Complement and coagulation cascades	3.7E-10
4	Retinol metabolism	1.4E-09
5	Butanoate metabolism	1.7E-09

Figure 1 (cont.)

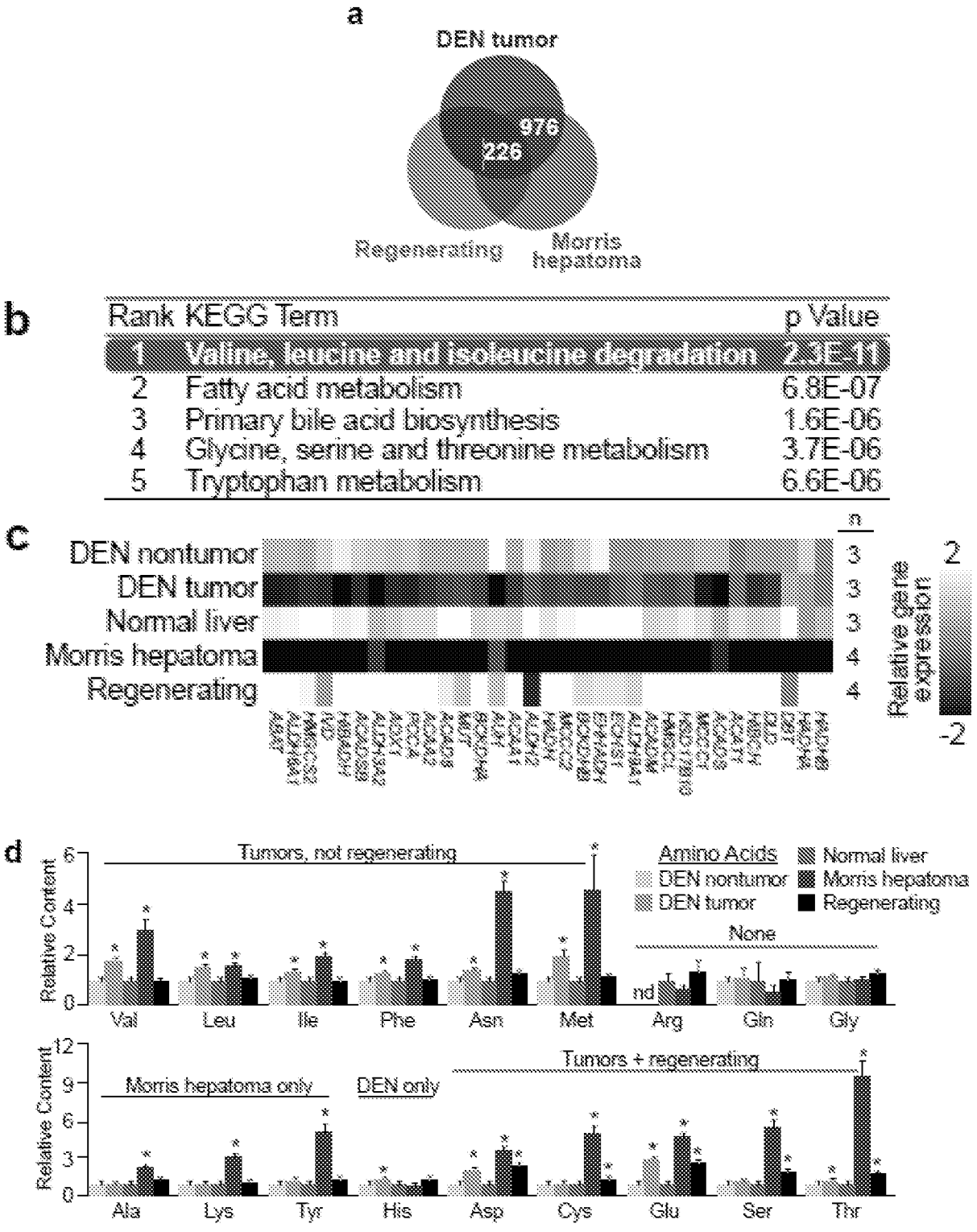

a
DEN tumor
976
226
Regenerating
Morris hepatoma
b
Rank KEGG Term p Value
1 Valine, leucine and isoleucine degradation 2.3E-11
2 Fatty acid metabolism 6.8E-07
3 Primary bile acid biosynthesis 1.6E-06
4 Glycine, serine and threonine metabolism 3.7E-06
5 Tryptophan metabolism 6.6E-06
c
n
DEN nontumor 3
DEN tumor 3
Normal liver 3
Morris hepatoma 4
Regenerating 4
Relative gene expression
2
-2
d
Tumors, not regenerating
Amino Acids
Normal liver
DEN nontumor
Morris hepatoma
DEN tumor
Regenerating
None
Relative Content
Val Leu Ile Phe Asn Met Arg Gln Gly
nd
Morris hepatoma only
DEN only
Tumors + regenerating
Ala Lys Tyr His Asp Cys Glu Ser Thr

Figure 2

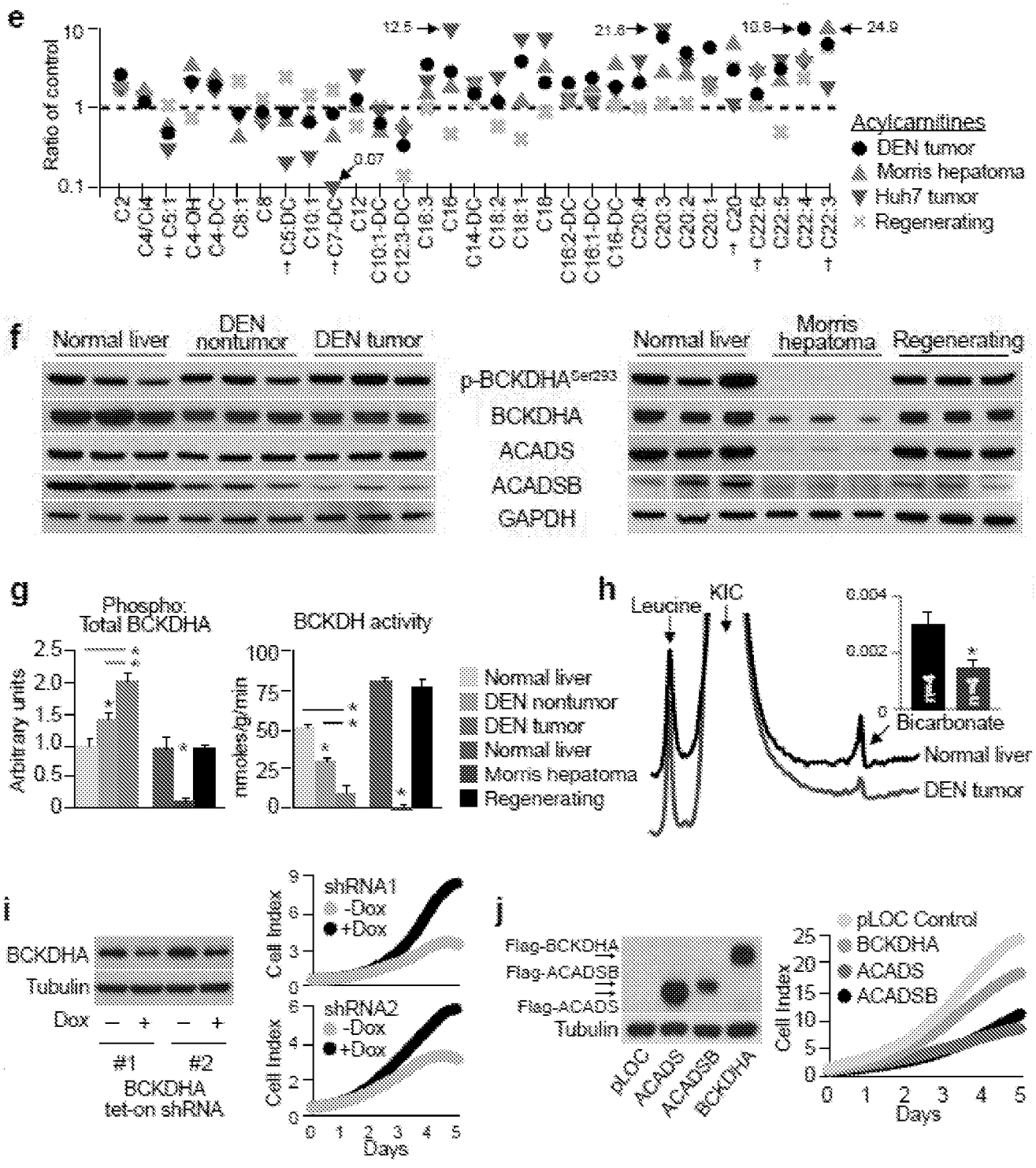


Figure 2 (cont.)

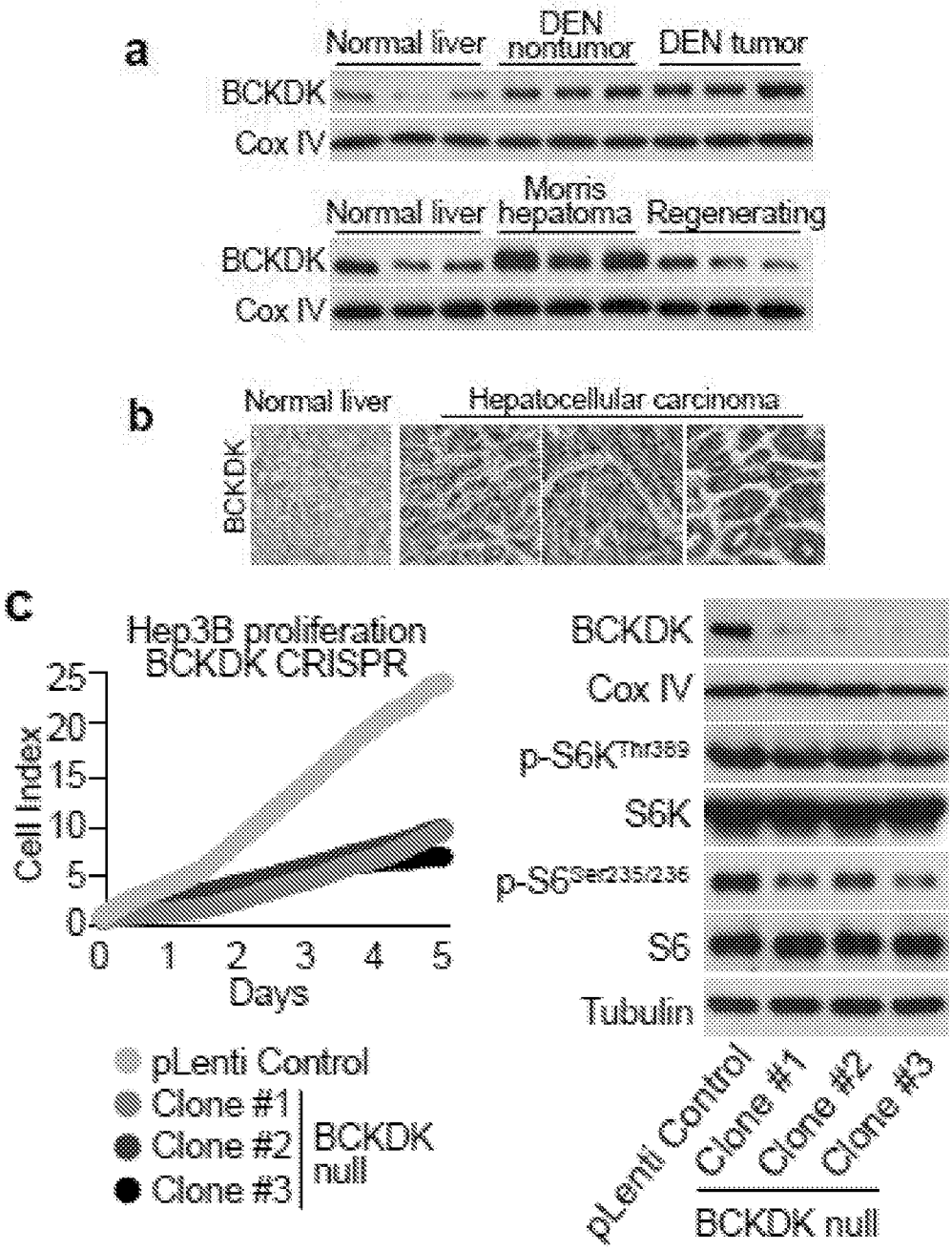

a
Normal liver
DEN nontumor
DEN tumor
BCKDK
Cox IV
Normal liver
Morris hepatoma
Regenerating
BCKDK
Cox IV
b
Normal liver
Hepatocellular carcinoma
BCKDK
c
Hep3B proliferation
BCKDK CRISPR
Cell Index
25
20
15
10
5
0
0 1 2 3 4 5
Days
pLenti Control
Clone #1
Clone #2
Clone #3
BCKDK null
BCKDK
Cox IV
p-S6K Thr389
S6K
p-S6 Ser235/236
S6
Tubulin
pLenti Control
Clone #1
Clone #2
Clone #3
BCKDK null

Figure 3

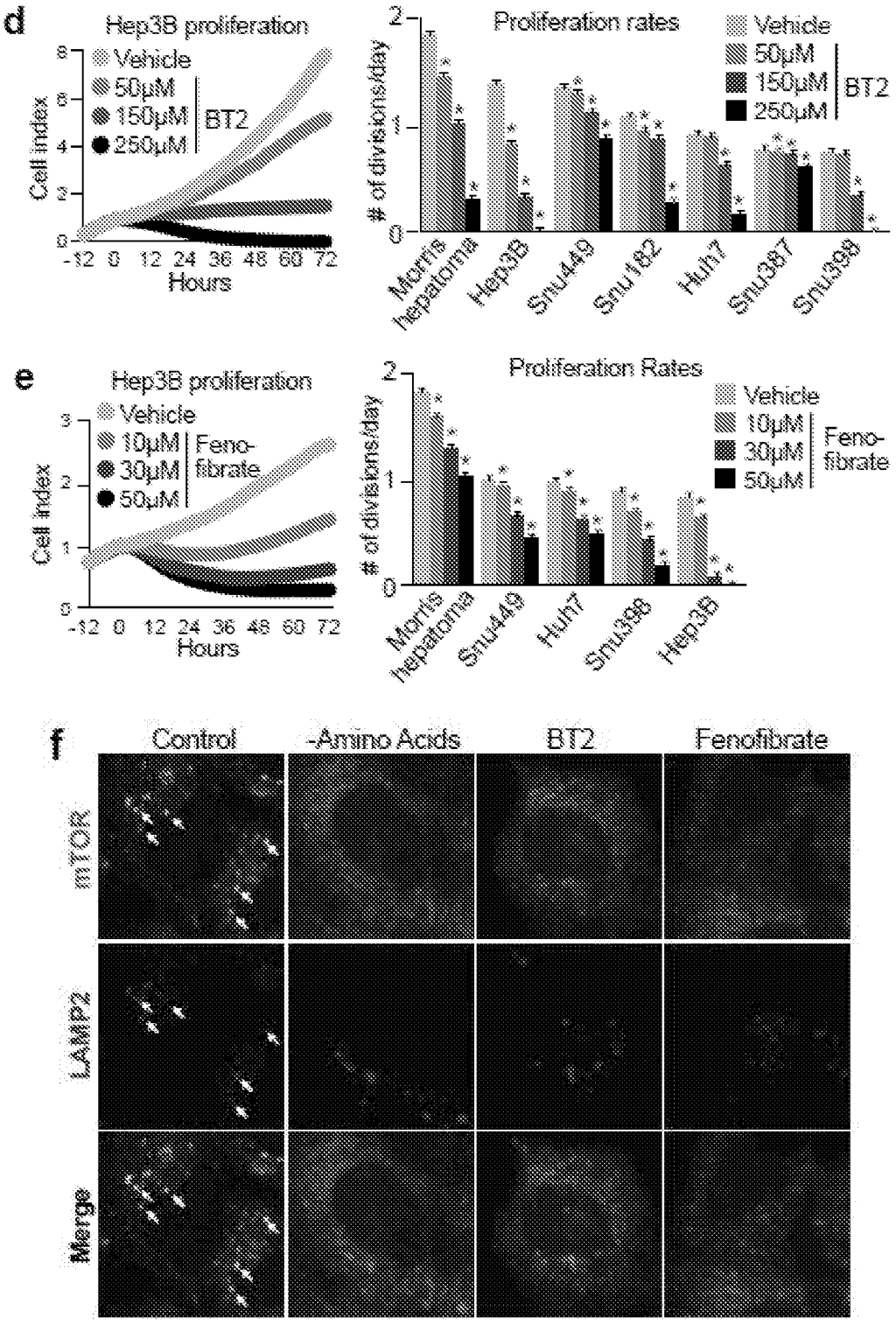


Figure 3 (cont.)

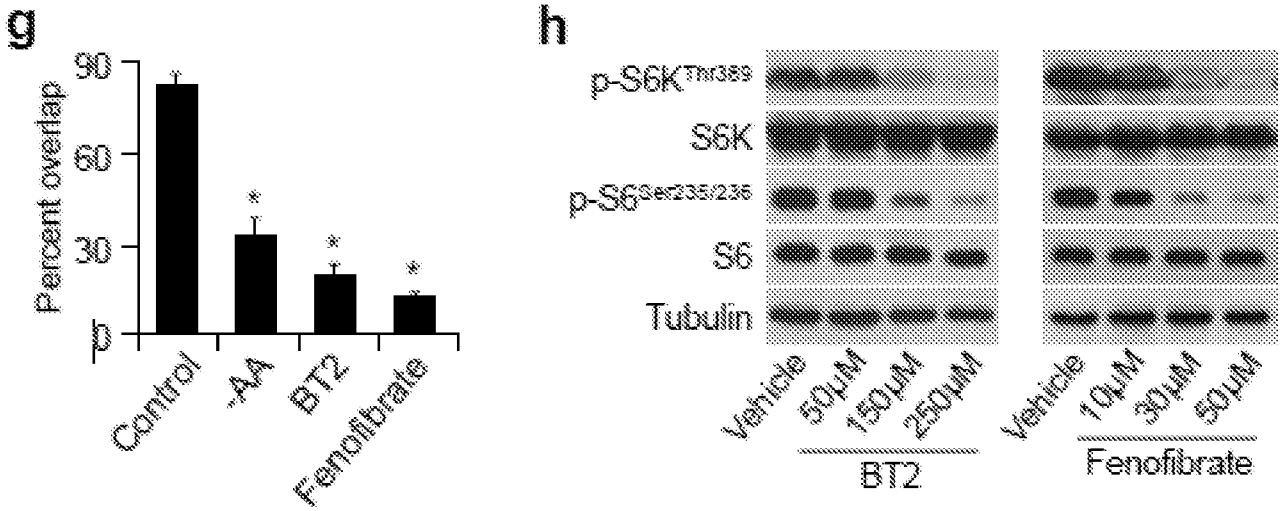


Figure 3 (cont.)

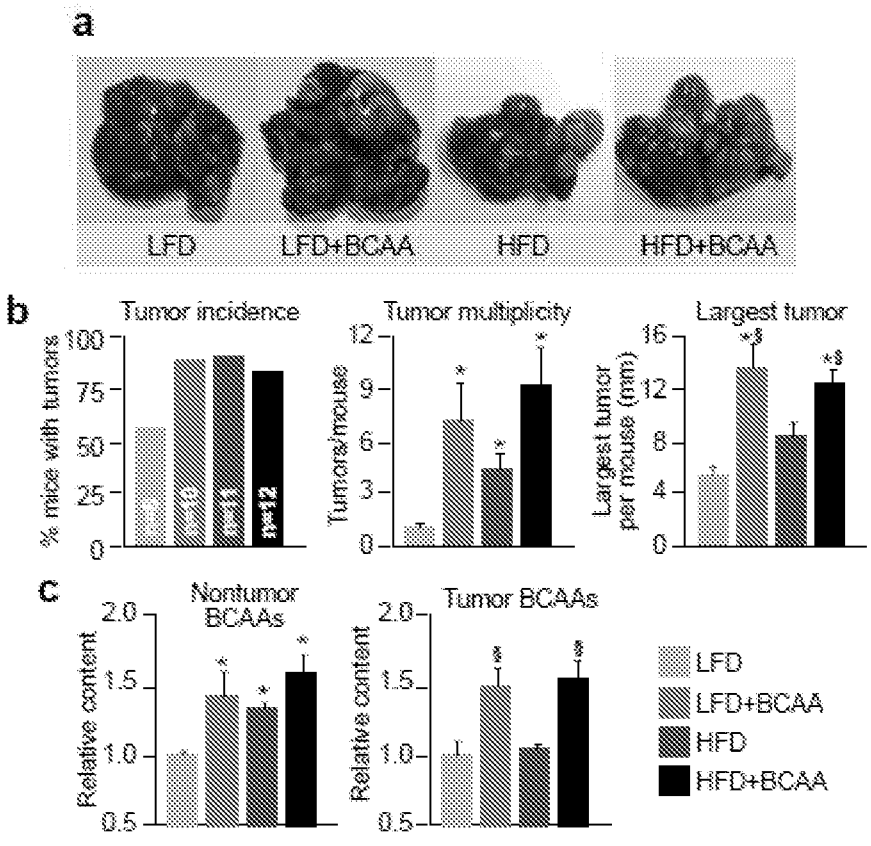


Figure 4

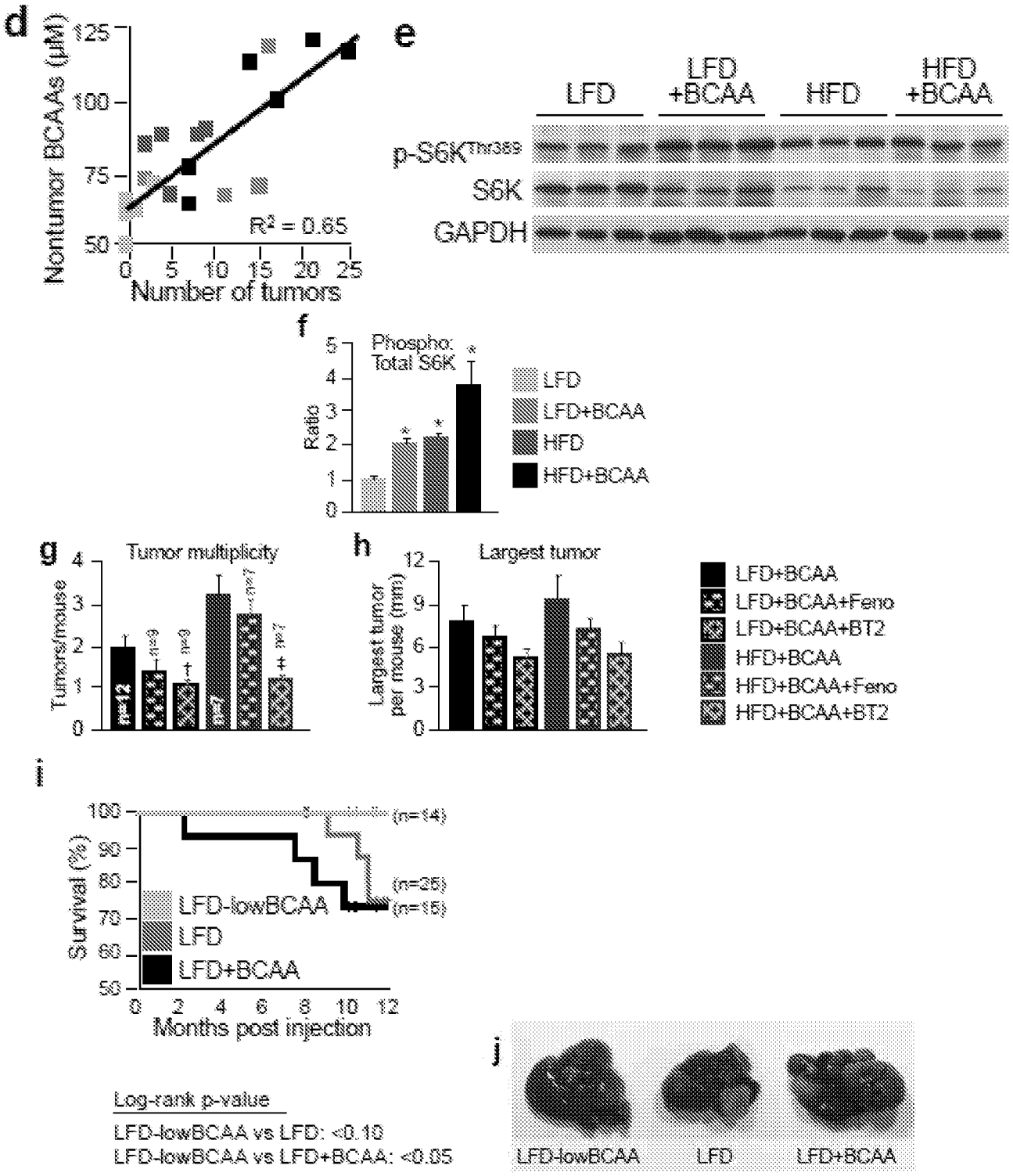
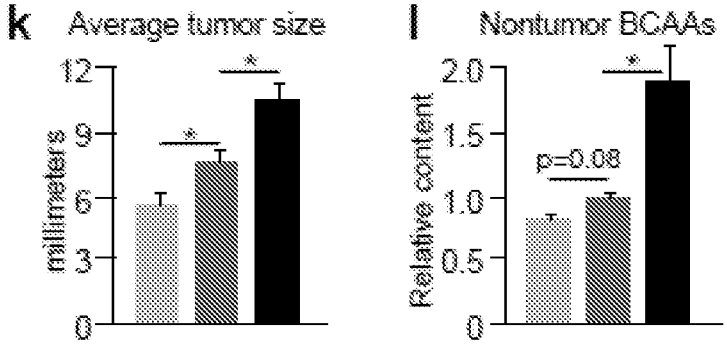


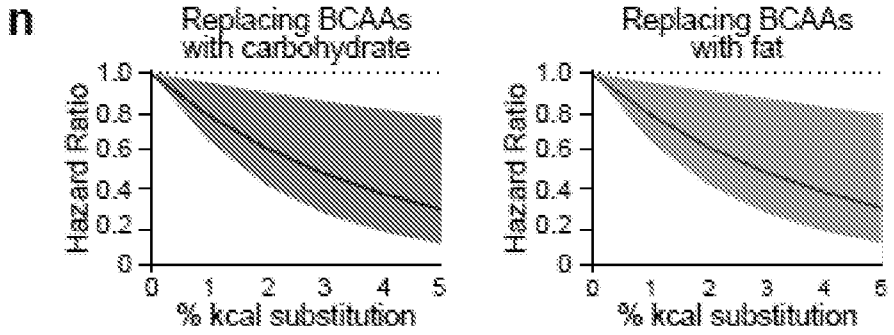
Figure 4 (cont.)



m

NHANES III cancer mortality risk

Tertile	BCAA Intake (% kcal)	Adjusted HR[1]	Adjusted HR[2]
1	Low (<1.73%)	1.0	1.0
2	Medium (1.73-3.89%)	1.82 (0.90-3.68)	1.68 (0.82-3.44)
3	High (>3.89%)	3.65 (1.62-8.24)	2.99 (1.20-7.46)



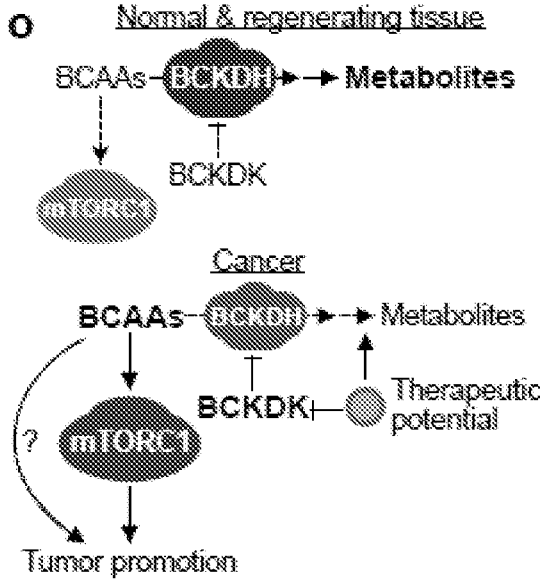


Figure 4 (cont.)

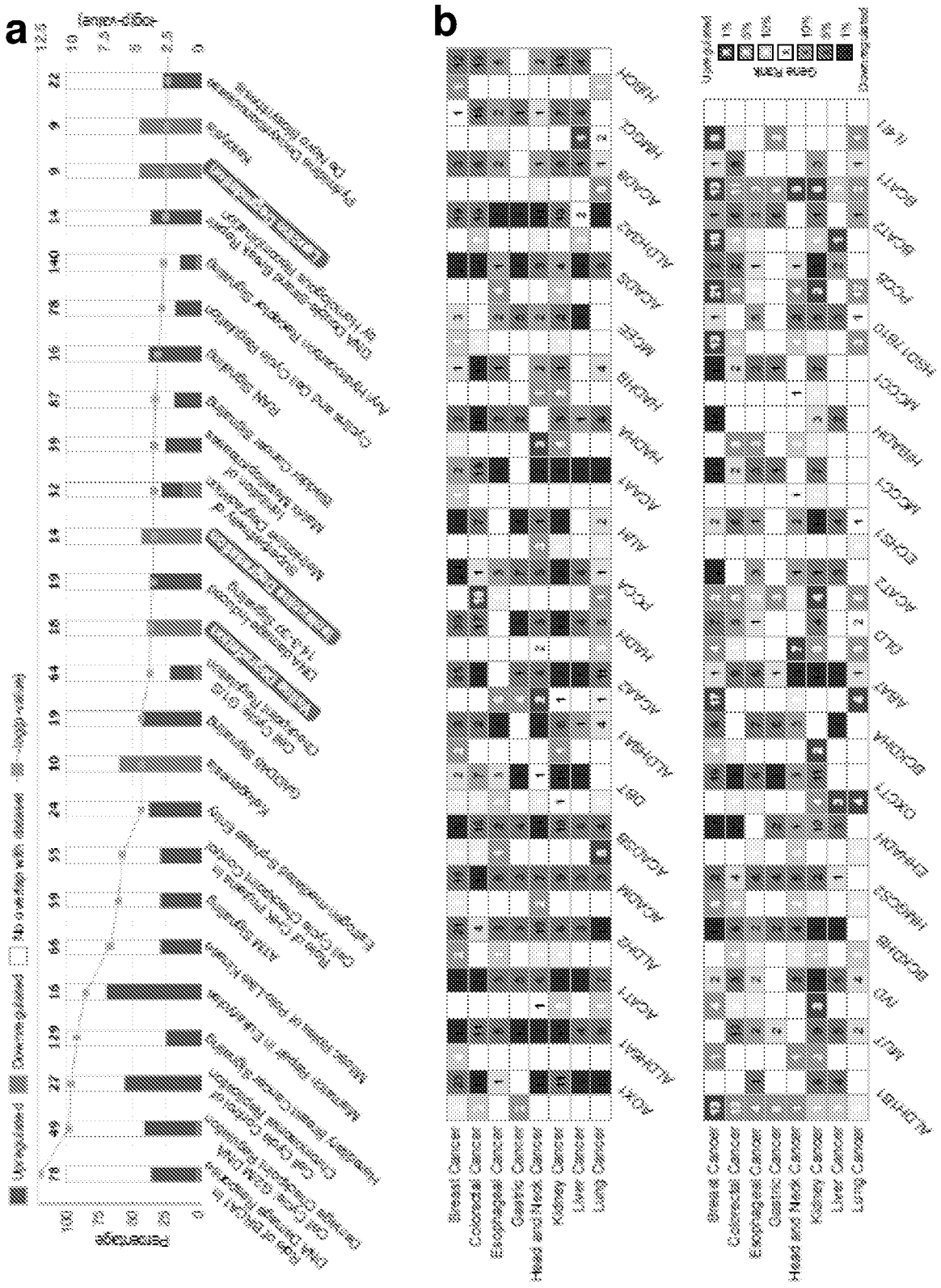
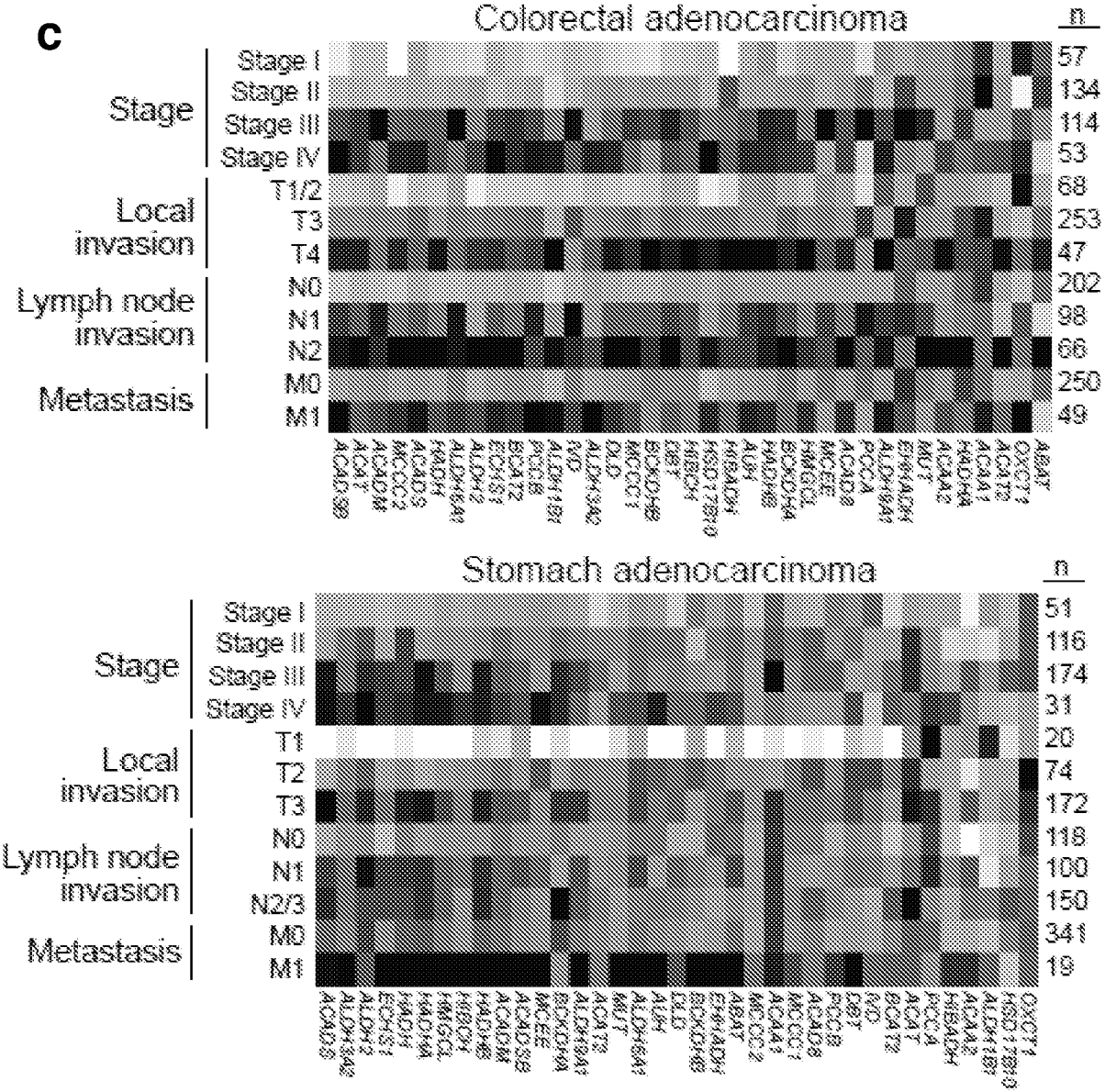

a
b
Percentage
Breast Cancer
Colorectal Cancer
Esophageal Cancer
Gastric Cancer
Head and Neck Cancer
Kidney Cancer
Liver Cancer
Lung Cancer
Gene Rank

Figure 5



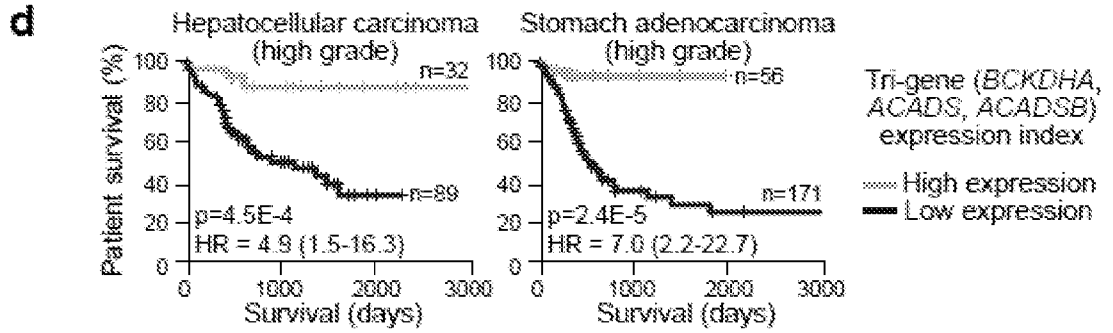


Figure 5 (cont.)

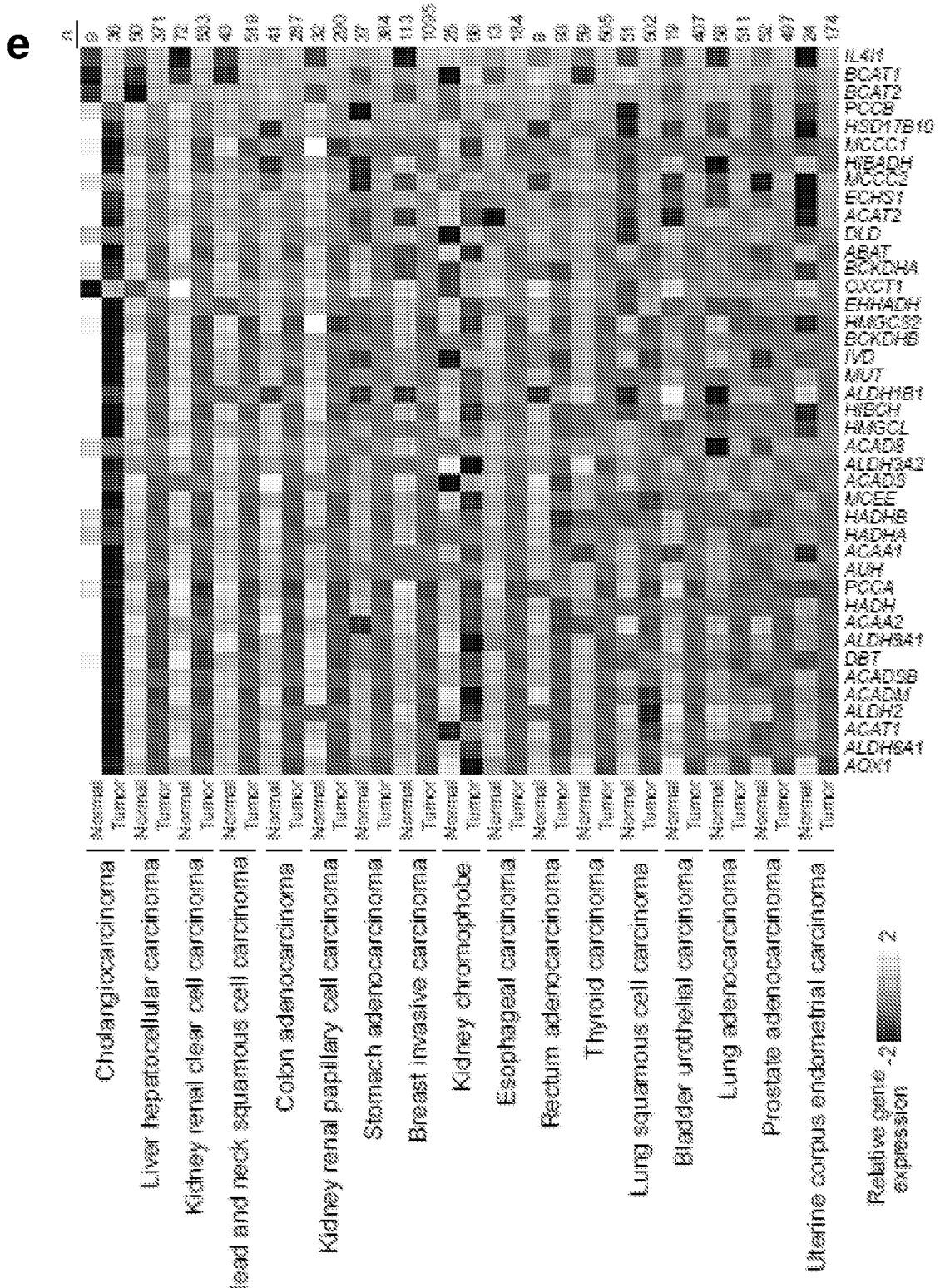


Figure 5 (cont.)

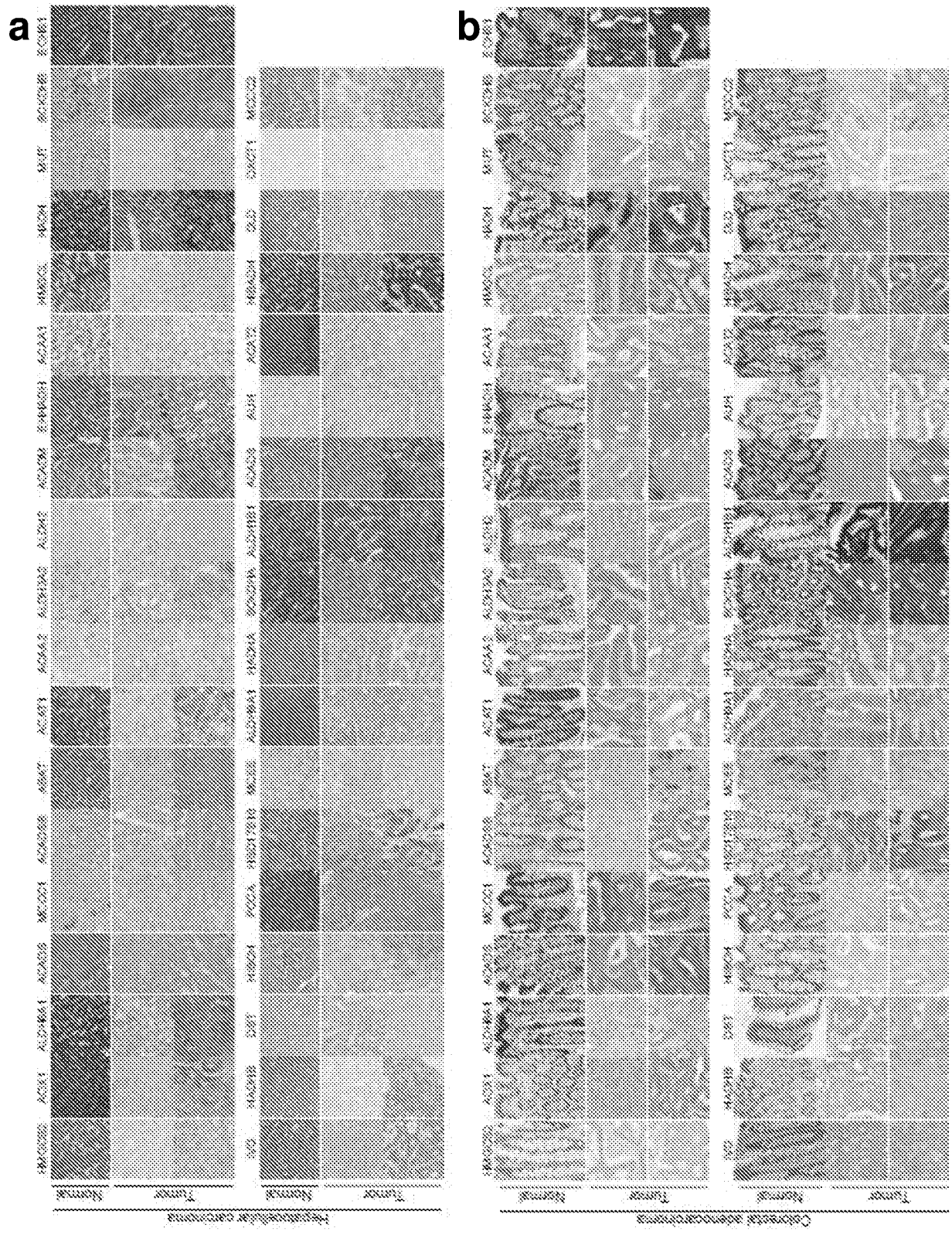

a
b
Normal
Tumor
Hepatocellular carcinoma
Cholangiocarcinoma

Figure 6

c

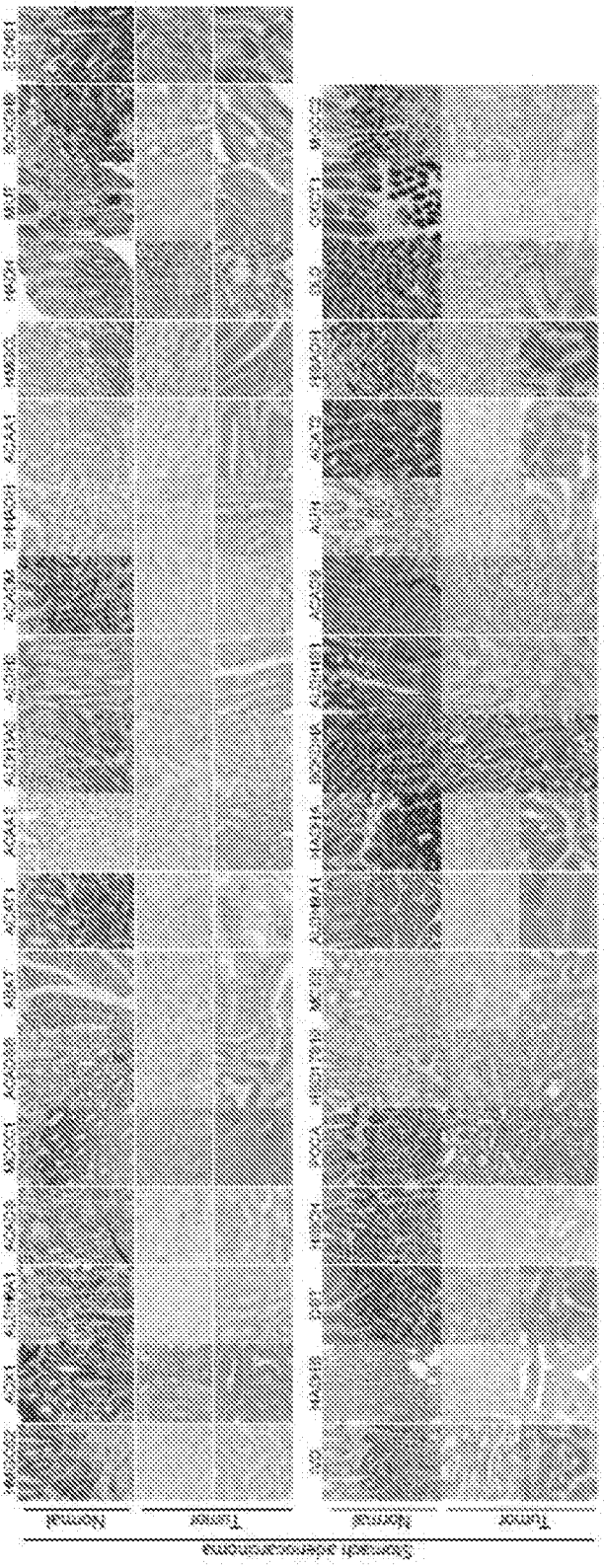


Figure 6 (cont.)

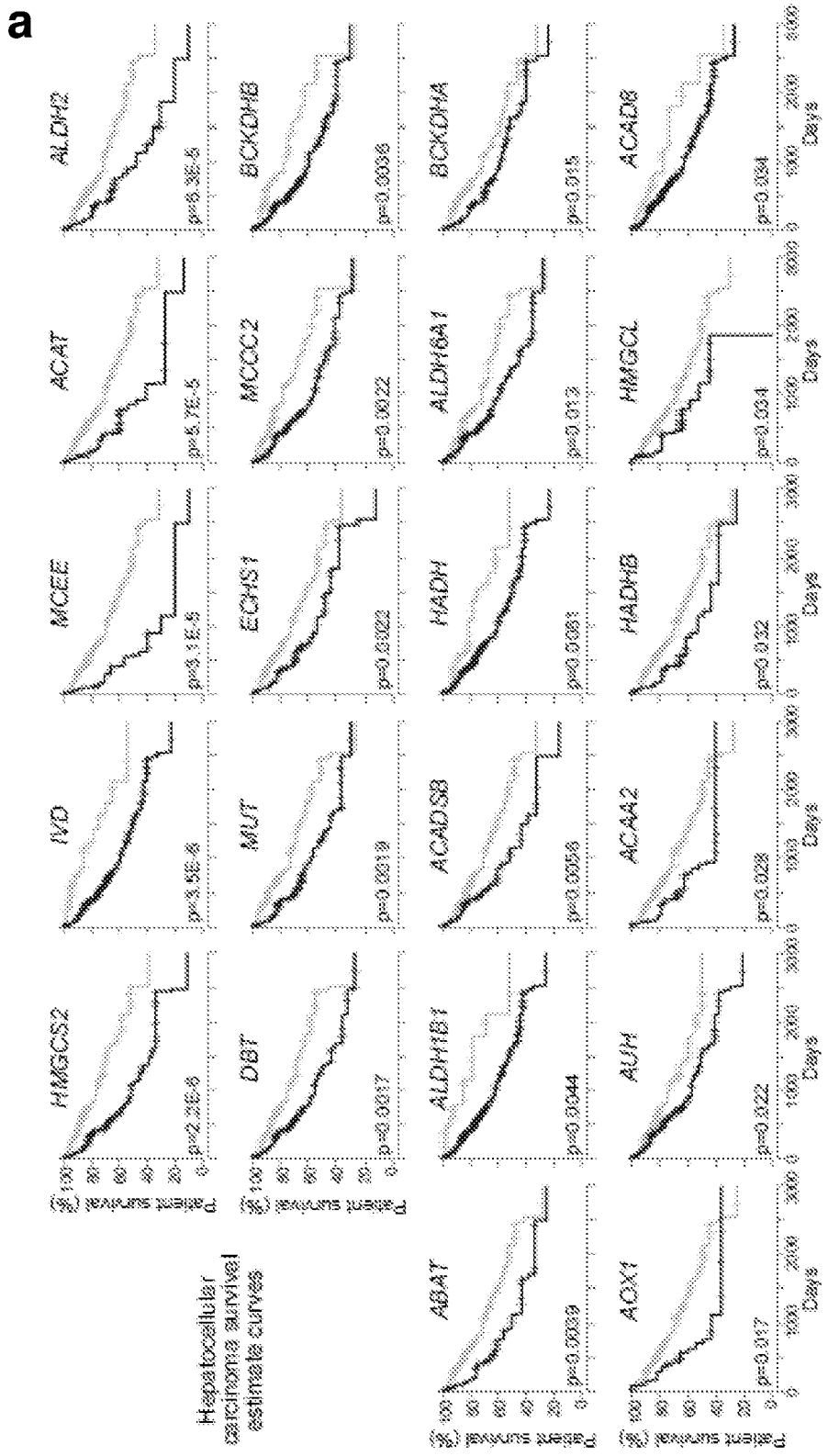

a
Hepatocellular carcinoma survival estimate curves
HMGCS2
IVD
MCEE
ACAT
ALDH2
DBT
MUT
ECHS1
MCCC2
BCKDHB
ABAT
ALDH1B1
ACADSB
HADH
ALDH6A1
BCKDHA
AOX1
AUH
ACAA2
HADHB
HMGCL
ACAD8
Patient survival (%)
Days
p=2.2E-6
p=3.5E-6
p=3.1E-5
p=5.7E-5
p=8.3E-5
p=0.0017
p=0.0019
p=0.0022
p=0.0022
p=0.0036
p=0.0039
p=0.0044
p=0.0056
p=0.0061
p=0.013
p=0.015
p=0.017
p=0.022
p=0.028
p=0.032
p=0.034
p=0.034

Figure 7

Hepatocellular carcinoma hazard ratios

Gene	Adjusted HR[1]	Adjusted HR[2]
Tri-gene[3], all grades	2.3 (1.6-3.5)	1.8 (1.1-2.9)
Tri-gene[3], high grade	6.2 (1.9-20.2)	4.9 (1.5-16.3)
ALDH1B1	2.7 (1.3-5.6)	2.7 (1.1-6.0)
IVD	2.6 (1.5-4.5)	2.2 (1.2-4.0)
MCEE	2.6 (1.5-4.3)	2.5 (1.4-4.3)
HMGCS2	2.4 (1.6-3.6)	2.2 (1.4-3.4)
ALDH2	2.3 (1.5-3.5)	1.6 (1.0-2.6)
ACAT	2.3 (1.5-3.6)	1.9 (1.2-3.2)
ACADS	2.1 (1.4-3.1)	1.8 (1.1-2.9)
HMGCL	2.1 (1.2-3.3)	1.6 (0.8-3.1)
HADH	2.0 (1.2-3.3)	1.7 (1.0-2.9)
ACAD8	2.0 (1.1-3.5)	1.8 (1.0-3.2)
ECSH1	2.0 (1.3-2.9)	1.7 (1.1-2.7)
DBT	1.9 (1.3-2.8)	1.7 (1.1-2.6)
MUT	1.9 (1.3-2.8)	1.7 (1.1-2.6)
BCKDHB	1.8 (1.2-2.8)	1.6 (0.9-2.5)
MCCC2	1.8 (1.2-2.7)	1.6 (1.0-2.6)
ABAT	1.8 (1.2-2.7)	1.4 (0.9-2.3)
BCKDHA	1.7 (1.2-2.5)	1.7 (1.1-2.6)
ACADSB	1.7 (1.1-2.6)	1.2 (0.7-1.9)
ACAA2	1.7 (1.0-2.8)	1.1 (0.6-2.0)
AUH	1.6 (1.1-2.4)	1.4 (0.9-2.1)
AOX1	1.6 (0.9-2.8)	1.4 (0.7-2.7)
ALDH6A1	1.6 (1.1-2.3)	1.4 (0.9-2.2)
PCCA	1.6 (1.0-2.5)	1.2 (0.7-2.0)
HADHB	1.5 (1.0-2.4)	1.4 (0.8-2.4)
ACAA1	1.5 (1.0-2.2)	1.3 (0.9-2.0)
DLD	1.5 (1.0-2.2)	1.3 (0.8-2.0)

[1]Adjusted for age, sex

[2]Adjusted for age, sex, tumor stage, tumor grade, radiation therapy, prescription therapy, additional therapies

[3]*BCKDHA*, *ACADS*, and *ACADSB*

Figure 7 (cont.)

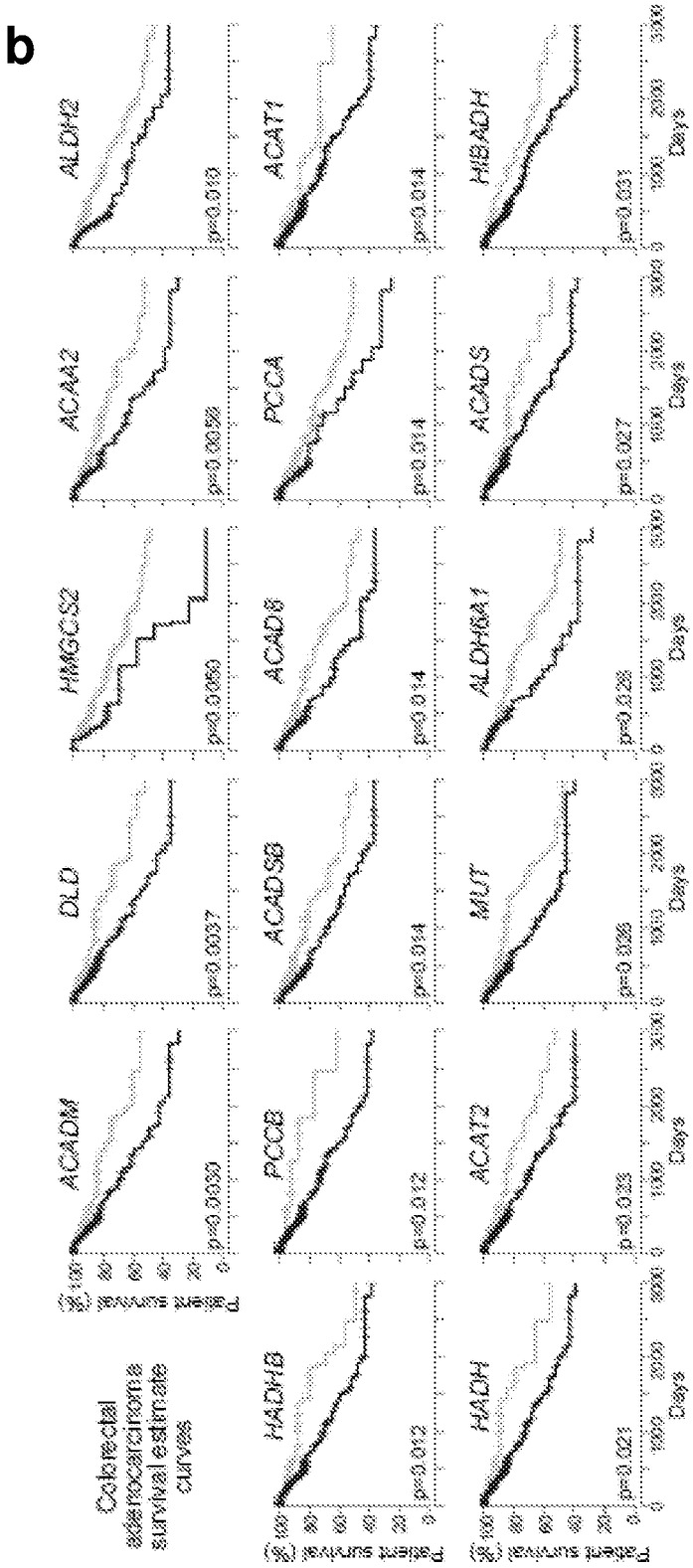


Figure 7 (cont.)

Colorectal adenocarcinoma hazard ratios

Gene	Adjusted HR[1]	Adjusted HR[2]
Tri-gene[3]	2.2 (1.2-4.1)	1.8 (1.0-3.6)
PCCB	2.9 (1.3-6.8)	1.7 (0.7-4.2)
HMGCL	2.5 (1.4-4.5)	2.7 (1.4-5.4)
HMGCS2	2.3 (1.3-4.3)	2.1 (1.1-4.0)
DLD	2.1 (1.3-3.5)	1.8 (1.0-3.0)
ACAT1	2.1 (1.2-3.9)	1.8 (0.9-3.5)
HADH	2.1 (1.1-4.0)	1.6 (0.8-3.1)
ACAA2	2.0 (1.3-3.3)	2.0 (1.2-3.3)
ACADM	2.0 (1.3-3.3)	1.7 (1.0-2.8)
HADHB	2.0 (1.2-3.5)	1.7 (0.9-3.1)
IVD	1.9 (1.0-3.6)	1.4 (0.7-2.7)
ALDH2	1.9 (1.2-3.1)	2.0 (1.1-3.4)
HIBADH	1.9 (1.1-3.2)	1.9 (1.0-3.5)
ACADS	1.8 (1.1-3.2)	1.3 (0.7-2.4)
ACADSB	1.8 (1.1-3.0)	1.4 (0.8-2.5)
ALDH1B1	1.8 (1.0-3.4)	1.4 (0.7-2.6)
PCCA	1.8 (1.1-3.0)	1.5 (0.9-2.6)
ACAD8	1.8 (1.1-2.9)	1.5 (0.9-2.6)
ACAT2	1.8 (1.1-2.9)	1.9 (1.1-3.2)
MCCC2	1.8 (1.0-2.9)	1.5 (0.9-2.7)
ALDH6A1	1.8 (1.1-2.9)	1.7 (1.0-3.0)
MUT	1.7 (1.1-2.7)	1.7 (1.0-2.8)
HADHA	1.7 (1.0-2.7)	1.6 (1.0-2.8)

[1]Adjusted for age, sex

[2]Adjusted for age, sex, tumor stage and subtype, and radiation, prescription, and additional therapies

[3]*BCKDHA*, *ACADS*, and *ACADSB*

Figure 7 (cont.)

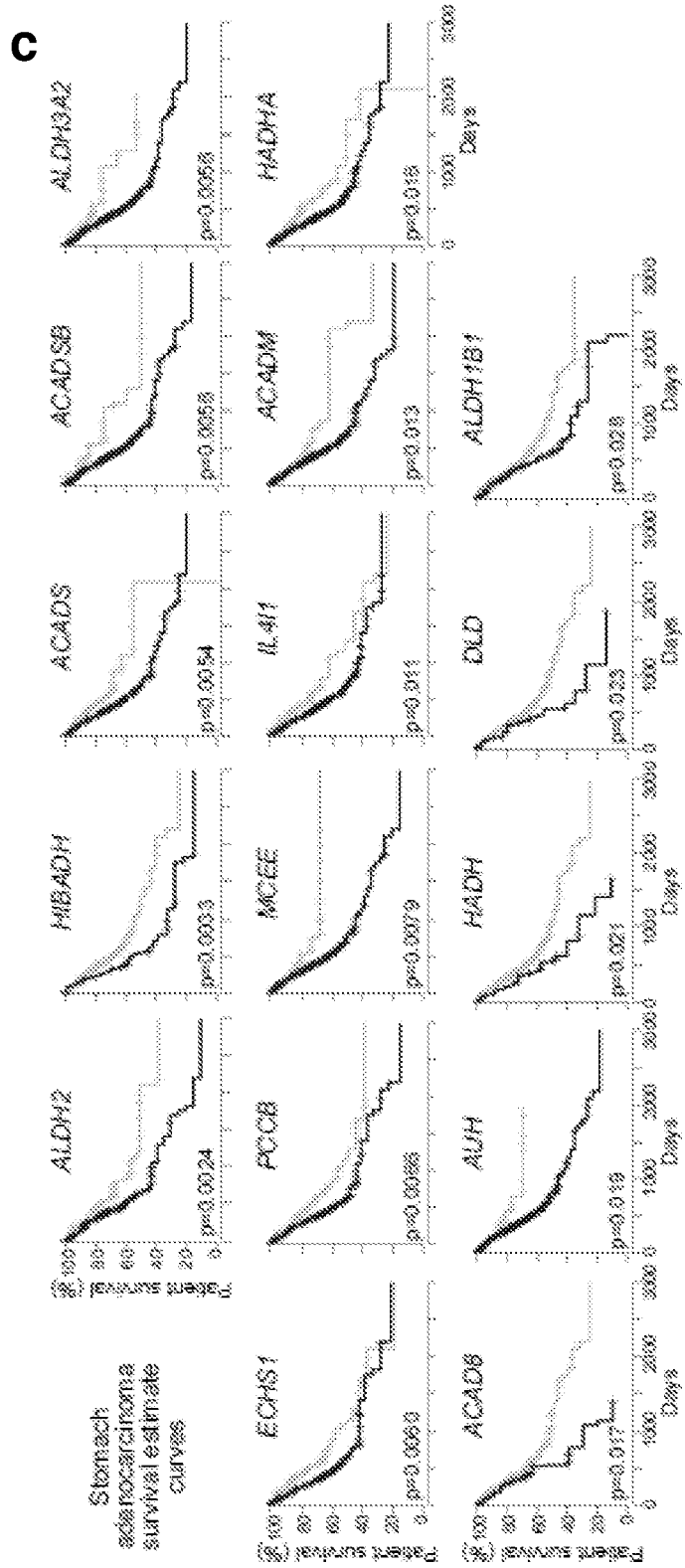


Figure 7 (cont.)

Stomach adenocarcinoma hazard ratios

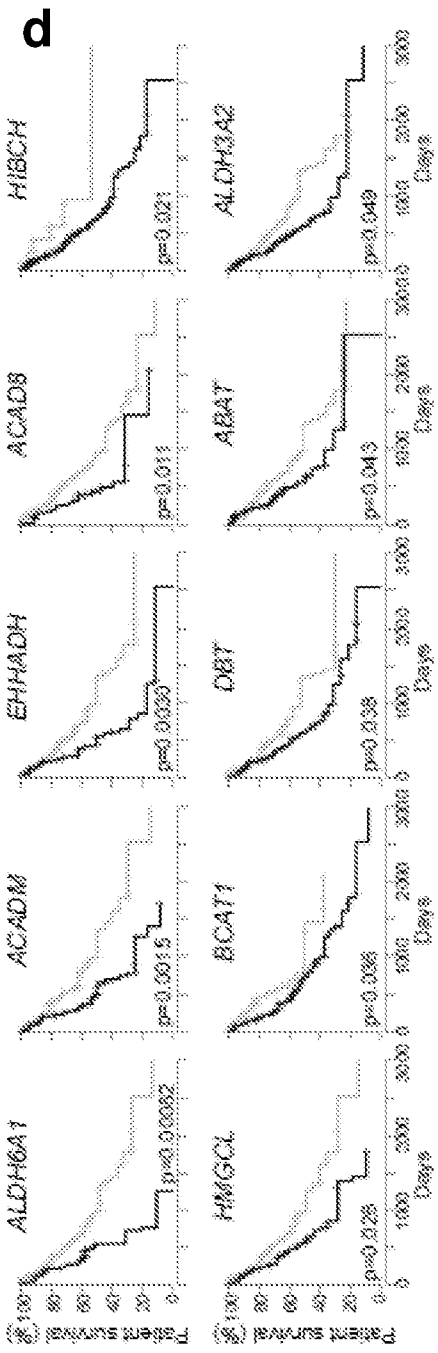
Gene	Adjusted HR[1]	Adjusted HR[2]
Tri-gene[3], all grades	3.3 (1.7-6.3)	3.0 (1.5-5.8)
Tri-gene[3], high grade	7.8 (2.5-24.9)	7.0 (2.2-22.7)
MCEE	2.1 (1.2-3.9)	1.7 (1.0-3.1)
ALDH3A2	2.0 (1.1-3.4)	1.6 (1.0-2.9)
ACADSB	2.0 (1.1-3.5)	1.6 (0.9-2.8)
IL4I1	1.9 (1.3-2.9)	2.2 (1.4-3.4)
AUH	1.9 (1.0-3.6)	1.8 (0.9-3.5)
DLD	1.8 (1.1-3.0)	1.4 (0.8-2.4)
ACADM	1.8 (1.1-3.0)	1.7 (1.0-2.9)
ACADS	1.8 (1.2-2.7)	1.8 (1.1-2.8)
PCCB	1.8 (1.2-2.6)	1.9 (1.3-2.9)
HADHA	1.8 (1.1-2.7)	1.9 (1.2-3.0)
ACAD8	1.7 (1.1-2.7)	1.5 (0.9-2.5)
HSD17B10	1.7 (1.1-2.5)	1.4 (0.9-2.2)
HADH	1.7 (1.0-2.7)	1.4 (0.8-2.5)
BCAT2	1.6 (1.1-2.4)	1.4 (0.9-2.0)
HIBADH	1.6 (1.1-2.4)	2.1 (1.3-3.2)
ECHS1	1.6 (1.1-2.4)	1.7 (1.1-2.4)
ALDH2	1.6 (1.1-2.3)	1.5 (1.0-2.2)
ALDH1B1	1.5 (1.1-2.3)	1.5 (1.0-2.2)

[1]Adjusted for age, sex

[2]Adjusted for age, sex, tumor stage, grade and subtype, and radiation, prescription, and additional therapies

[3]*BCKDHA*, *ACADS*, and *ACADSB*

Figure 7 (cont.)



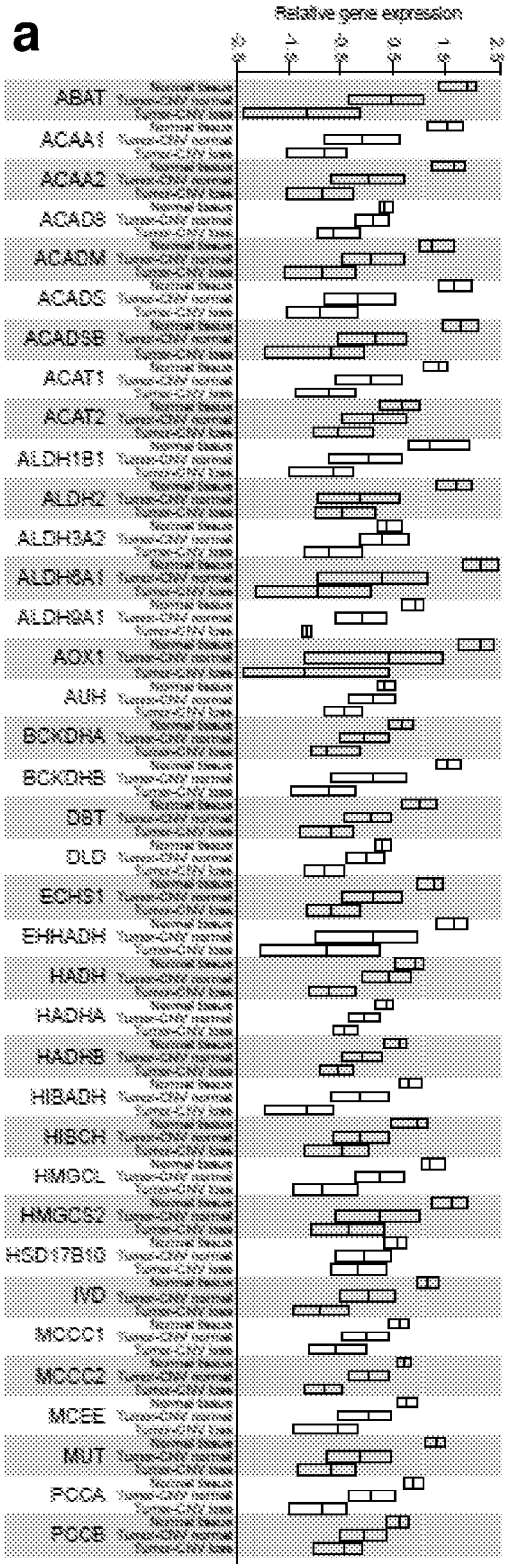
Esophageal carcinoma hazard ratios

Gene	Adjusted HR[1]
ALDH6A1	2.6 (1.5-4.5)
HIBCH	2.6 (1.1-6.0)
ACAD8	2.6 (1.4-4.7)
ACADM	2.1 (1.3-3.5)
EHHADH	2.0 (1.2-3.4)
HMGCL	2.0 (1.2-3.3)
ABAT	1.7 (1.0-2.8)
BCAT1	1.6 (0.9-2.8)
DBT	1.6 (1.0-2.7)
ALDH3A2	1.6 (1.0-2.6)

[1]Adjusted for age, sex

High expression
Low expression

Figure 7 (cont.)



b

Percent tumors with CNV loss

Gene	LIHC	CHOL	COAD	READ	STAD	ESCA
ABAT	28.1	13.9	8	6.7	21.1	28.9
ACAA1	14.3	80.6	14	10.3	30.6	62.5
ACAA2	25.4	33.3	60.7	87.3	44.2	63
ACAD8	24.9	36.1	15.1	21.8	22.2	43.5
ACADM	24.6	13.9	29.1	33.9	19.5	30.4
ACADS	14.6	19.4	12.7	13.3	13.4	23.9
ACADSB	28.6	22.2	22.7	27.9	18.6	29.9
ACAT1	24.3	33.3	16	23	20.4	40.8
ACAT2	37.8	69.4	11.8	16.4	20.6	26.6
ALDH1B1	27	47.2	12.2	13.9	27.4	51.1
ALDH2	11.9	22.2	13.6	13.3	13.2	24.5
ALDH3A2	38.1	22.2	48.7	66.1	37	45.1
ALDH6A1	34.6	58.3	33.3	41.2	29	27.2
ALDH9A1	0.8	0	10	9.1	3.2	5.4
AOX1	10.3	5.6	2.9	3	9.8	14.1
AUH	31.1	52.8	13.3	16.4	23.8	31.5
BCKDHA	10.5	8.3	9.4	10.3	17.5	26.6
BCKDHB	30	55.6	12.7	14.5	20.2	23.9
DBT	24.3	16.7	28.7	33.3	20.4	29.9
DLD	7.6	13.9	1.3	1.8	11.8	17.9
ECHS1	28.1	22.2	22.9	29.7	19	29.9
EHHADH	11.4	8.3	6.9	6.7	9.3	7.6
HADH	46.5	44.4	30	44.2	39	51.6
HADHA	7.8	5.6	1.8	7.9	7	8.2
HADHB	7.8	5.6	1.8	7.9	7.3	8.2
HIBADH	4.3	8.3	0.4	0.6	3.6	7.1
HIBCH	10	5.6	2.7	3.6	10.7	11.4
HMGCL	37.3	80.6	36.4	44.2	25.9	36.4
HMGCS2	19.2	2.8	22.4	26.1	16.3	28.1
HSD17B10	26.8	47.2	14.2	18.2	14.7	33.7
IVD	19.5	19.4	38.7	47.3	27.2	33.7
MCCC1	11.4	8.3	6.9	6.7	8.4	7.1
MCCC2	13	22.2	23.8	32.7	37.9	62
MCEE	10	8.3	1.5	7.3	4.3	3.8
MUT	2.7	11.1	7.5	6.1	9.3	13
PCCA	25.7	41.7	2.7	6.1	10.9	37
PCCB	11.1	13.9	7.6	6.7	10.7	10.9
Overall	20	26.5	16.3	20.7	18.5	29

Figure 8

c All significant, differentially expressed genes: predicted upstream regulators

Rank	Upstream regulator	Activation state	p-value				
			LIHC	CHOL	COAD	READ	STAD
1	TP53	Inhibited	9.21E-29	8.45E-30	2.34E-29	1.05E-14	1.81E-34
2	HNF4A	Inhibited	7.82E-25	3.39E-38	2.00E-38	2.24E-10	1.03E-26
3	CCND1	Activated	2.03E-12	2.30E-16	1.03E-16	1.31E-13	1.34E-23
4	NUPR1	Inhibited	3.36E-24	4.19E-21	8.46E-20	3.50E-11	5.80E-16
5	MYC	Activated	2.87E-09	1.32E-12	3.38E-24	4.27E-13	3.48E-22
6	E2F1	Activated	7.51E-16	8.54E-10	6.27E-16	6.48E-12	2.39E-23
7	TBX2	Activated	8.03E-21	5.61E-14	2.19E-10	5.58E-07	2.48E-15
8	E2F3	Activated	1.03E-11	5.75E-11	1.40E-11	2.49E-07	3.93E-14
9	CDKN2A	Inhibited	1.92E-08	3.78E-07	1.48E-13	3.99E-07	1.68E-14
10	PPARA	Inhibited	1.61E-11	9.68E-45	1.79E-07	5.82E-04	4.03E-07
11	SMARCB1	Inhibited	7.83E-09	2.68E-10	1.44E-05	2.19E-05	6.78E-11
12	E2F2	Activated	1.29E-07	2.46E-06	1.80E-06	3.22E-06	2.82E-10
13	PPARG	Inhibited	2.01E-04	3.81E-11	2.07E-07	8.29E-03	1.33E-10
14	RBL1	Inhibited	4.51E-06	7.75E-05	7.80E-07	7.52E-06	2.03E-07
15	PPARD	Inhibited	6.76E-05	2.03E-11	1.08E-03	1.12E-02	4.45E-04
16	FOXO3	Inhibited	2.05E-05	2.55E-03	4.87E-05	2.13E-03	7.43E-06
17	KLF15	Inhibited	1.73E-04	4.02E-07	3.13E-03	2.09E-04	1.01E-03
18	MXI1	Inhibited	7.89E-04	1.70E-02	1.23E-05	1.82E-02	2.49E-01

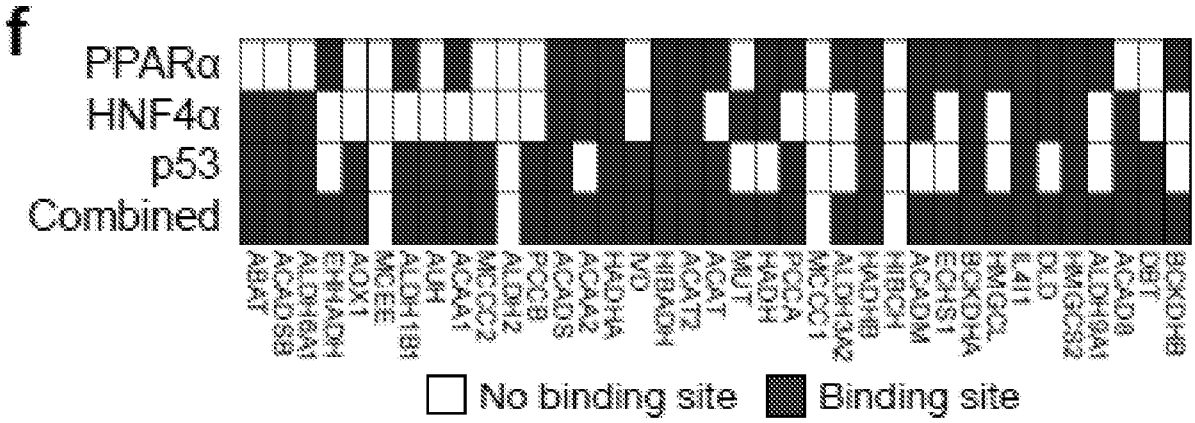
d Tumors, not regenerating tissues: predicted upstream regulators

Rank	Upstream regulator	Activation state	p-value
1	PPARA	Inhibited	1.83E-24
2	TP53	Inhibited	1.97E-14
3	HNF4A	Inhibited	8.11E-14
4	HNF1A	Inhibited	5.27E-13
5	CAR	Inhibited	1.38E-08
6	PXR	Activated	2.29E-08
7	FXR	Inhibited	3.35E-08
8	PPARG	Inhibited	3.70E-08
9	PPARD	Inhibited	3.53E-07
10	FOS	Activated	7.82E-07
11	ATF4	Activated	8.86E-07
12	SMAD4	Activated	1.21E-06
13	PDX1	Activated	2.45E-06
14	HIF1A	Activated	3.40E-06
15	NFE2L2	Activated	6.22E-06
⋮	⋮	⋮	⋮
114	KLF15	Inhibited	1.16E-02

Figure 8 (cont.)

e BCAA catabolic enzymes: predicted upstream regulators

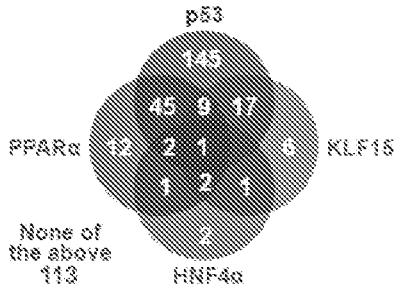
Rank	Upstream regulator	p-value
1	PPARA	7.69E-19
2	KLF15	7.54E-11
3	PPARG	1.48E-09
4	TFAM	2.67E-08
5	PPARD	3.83E-07
6	RXRG	1.66E-05
7	RXRA	5.39E-05
8	HNF4A	2.20E-04
9	TP53	2.75E-04



g Hepatocellular carcinomas

	p53	PPARα	KLF15	HNF4α
CNV loss	60.6%	21.2%	11.3%	2.8%
Mutation	31.7%	4.8%	6.0%	2.5%

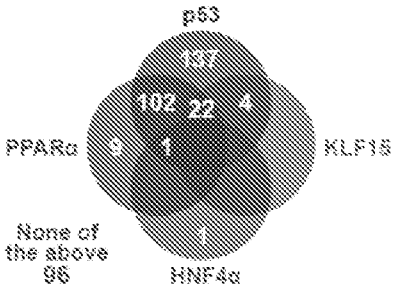
Mutation or CNV loss



Colorectal adenocarcinomas

	p53	PPARα	KLF15	HNF4α
CNV loss	59.8%	36.3%	7.0%	0.3%
Mutation	82.1%	1.7%	0.3%	1.0%

Mutation or CNV loss



Stomach adenocarcinomas

	p53	PPARα	KLF15	HNF4α
CNV loss	41.0%	36.5%	11.3%	0.5%
Mutation	49.3%	1.3%	2.4%	2.4%

Mutation or CNV loss

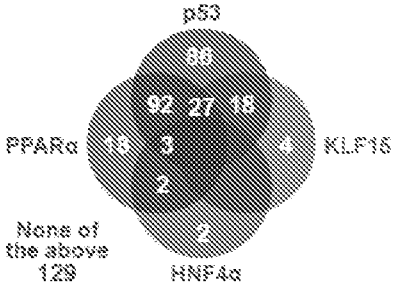


Figure 8 (cont.)

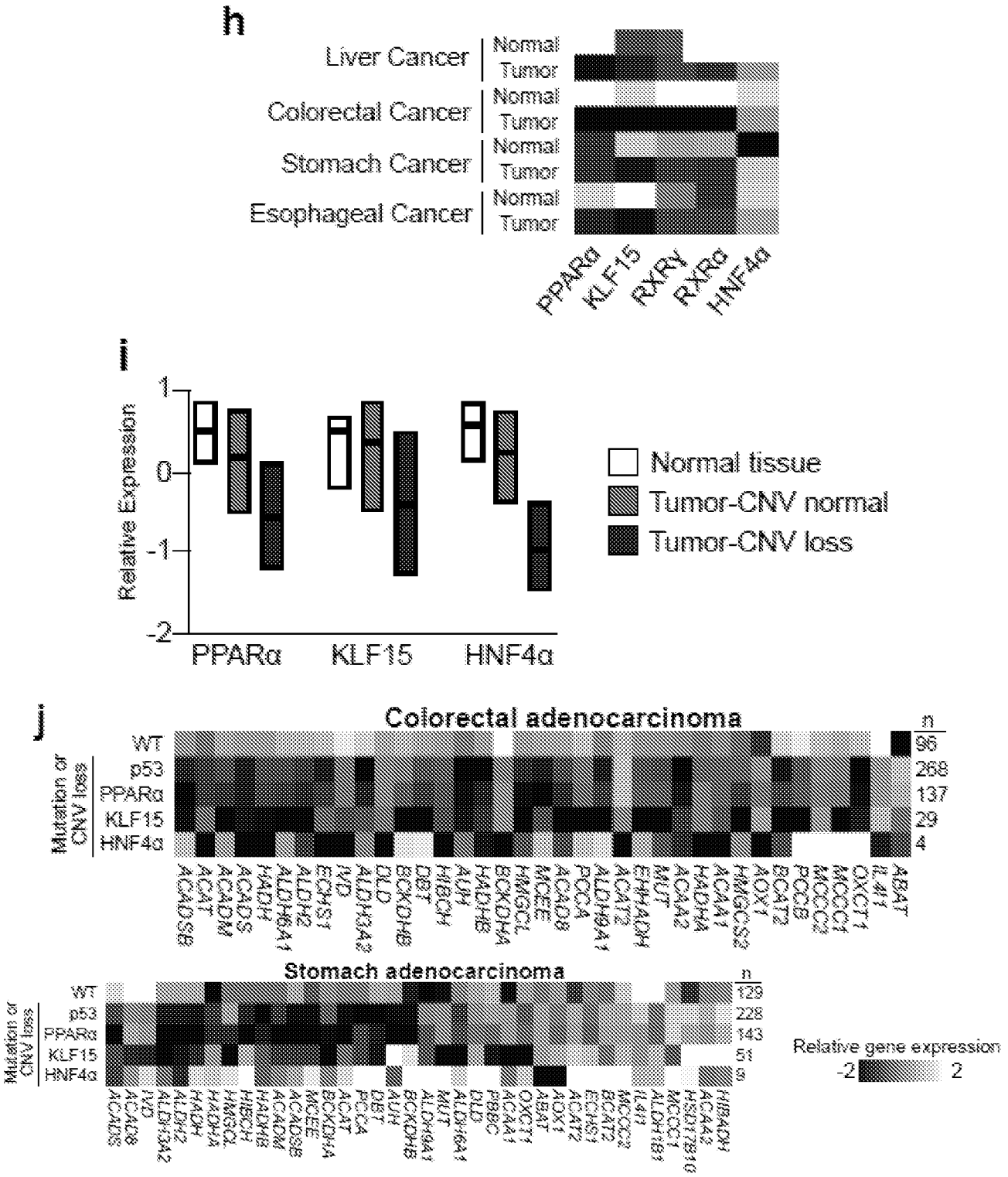


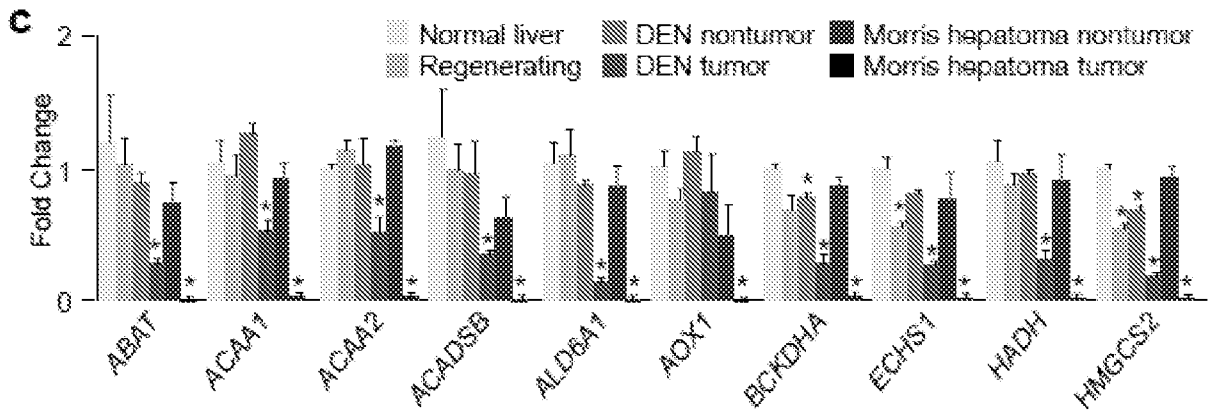
Figure 8 (cont.)

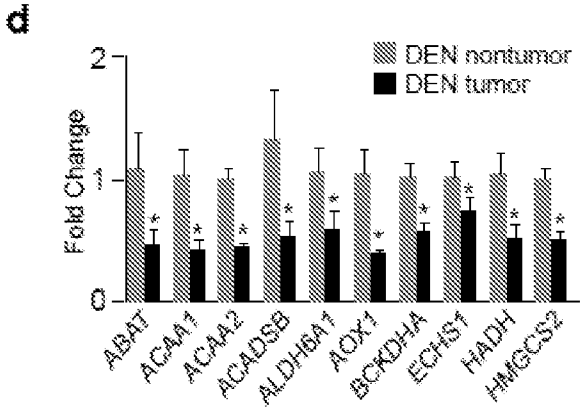
a Tumor models (1202 genes, without exclusion of regenerating tissue-related genes)

Rank	KEGG Term	p Value
1	Valine, leucine and isoleucine degradation	3.5E-09
2	Fatty acid metabolism	2.0E-06
3	Glycine, serine and threonine metabolism	5.2E-06
4	Primary bile acid biosynthesis	9.0E-06
5	Trypotophan metabolism	1.3E-05
6	Arginine and proline metabolism	1.9E-05
7	β-Alanine metabolism	2.6E-04
8	Propanoate metabolism	5.7E-04
9	Sulphur metabolism	7.3E-04
10	Complement coagulation cascades	1.2E-03

b Tumor models + regenerating tissue (226 genes)

Rank	KEGG Term	p Value
1	DNA replication	3.3E-05
2	Cell Cycle	8.1E-05
3	Amino sugar and nucleotide sugar metabolism	9.8E-04
4	Progesterone-mediated oocyte maturation	3.9E-03
5	Purine metabolism	5.4E-03
6	Pyrimidine metabolism	6.4E-03
7	Other glycan degradation	3.2E-02
8	Pancreatic cancer	3.8E-02
9	Oocyte meiosis	4.5E-02
10	Colorectal cancer	6.1E-02





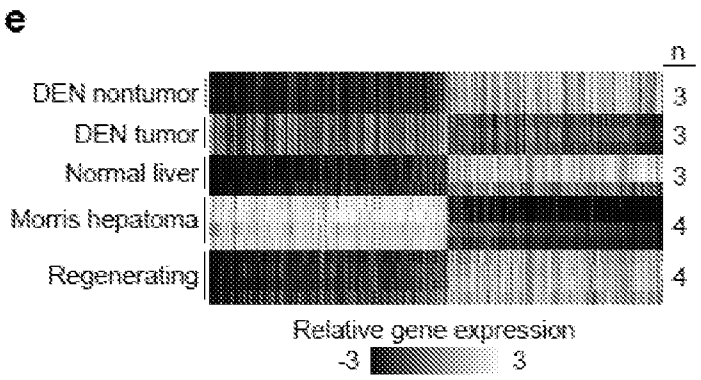


Figure 9

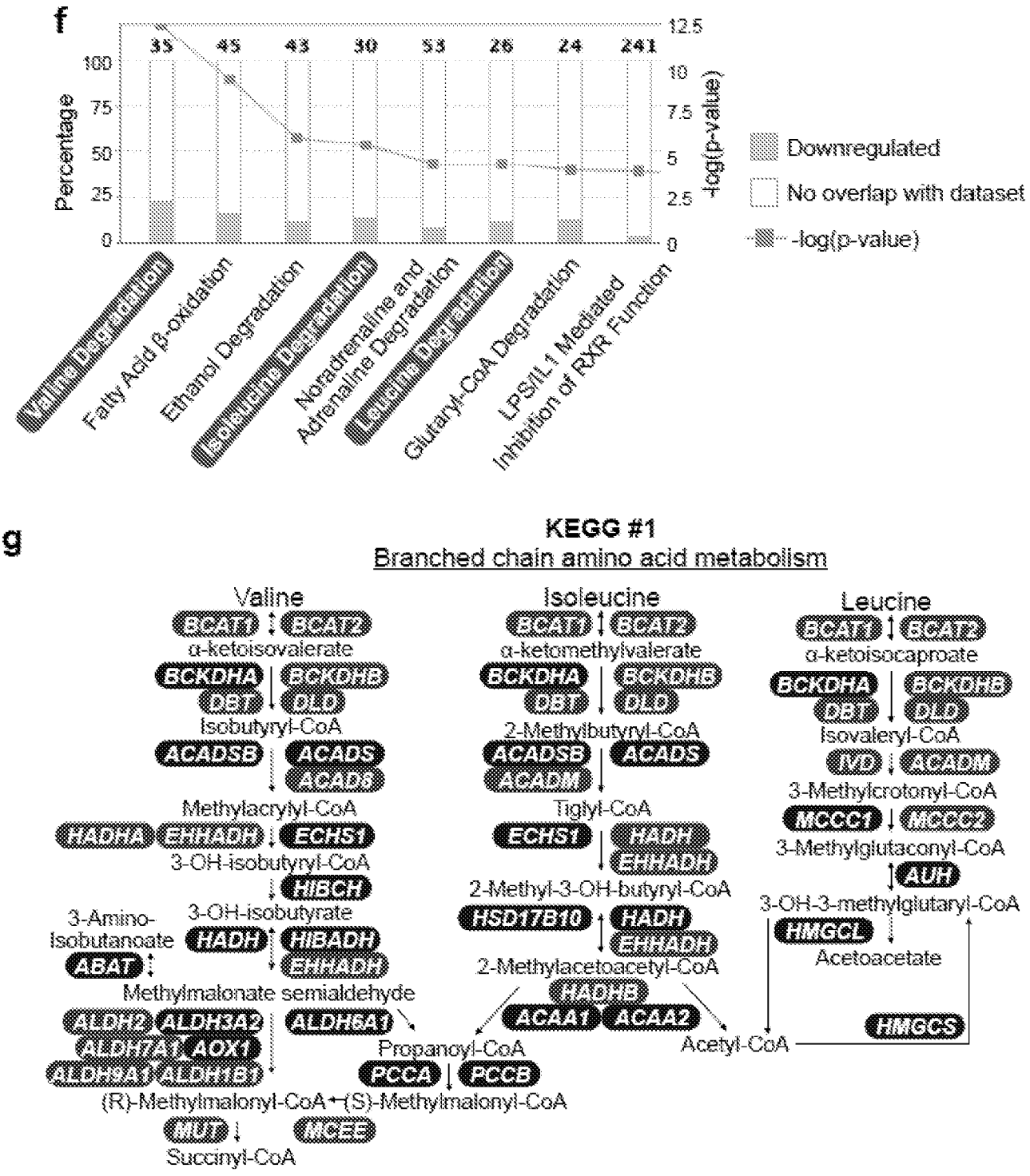


Figure 9 (cont.)

g (cont.)

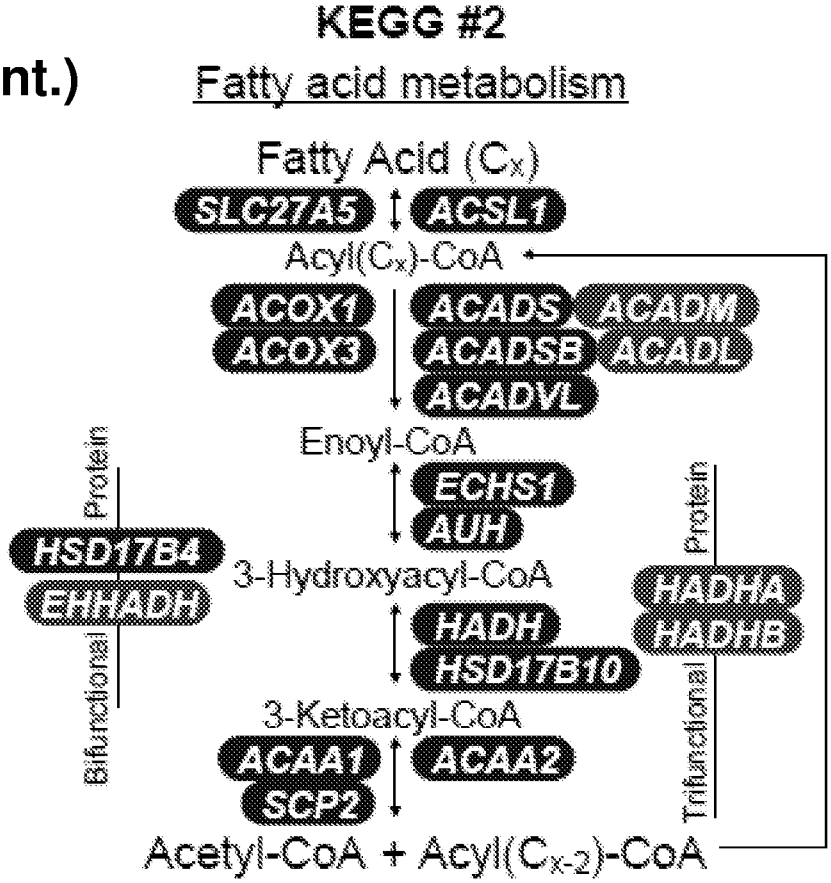


Figure 9 (cont.)

g (cont.)

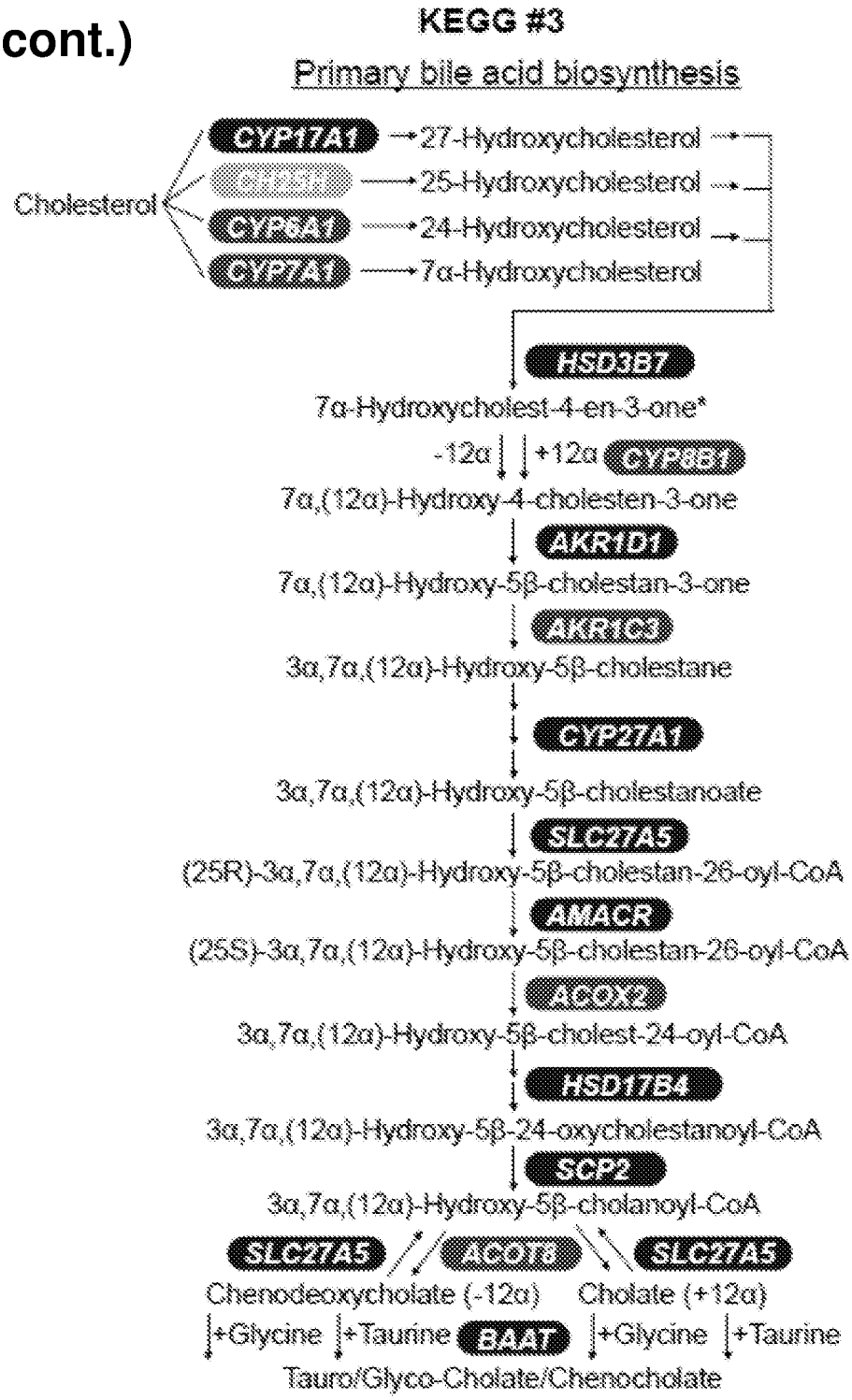
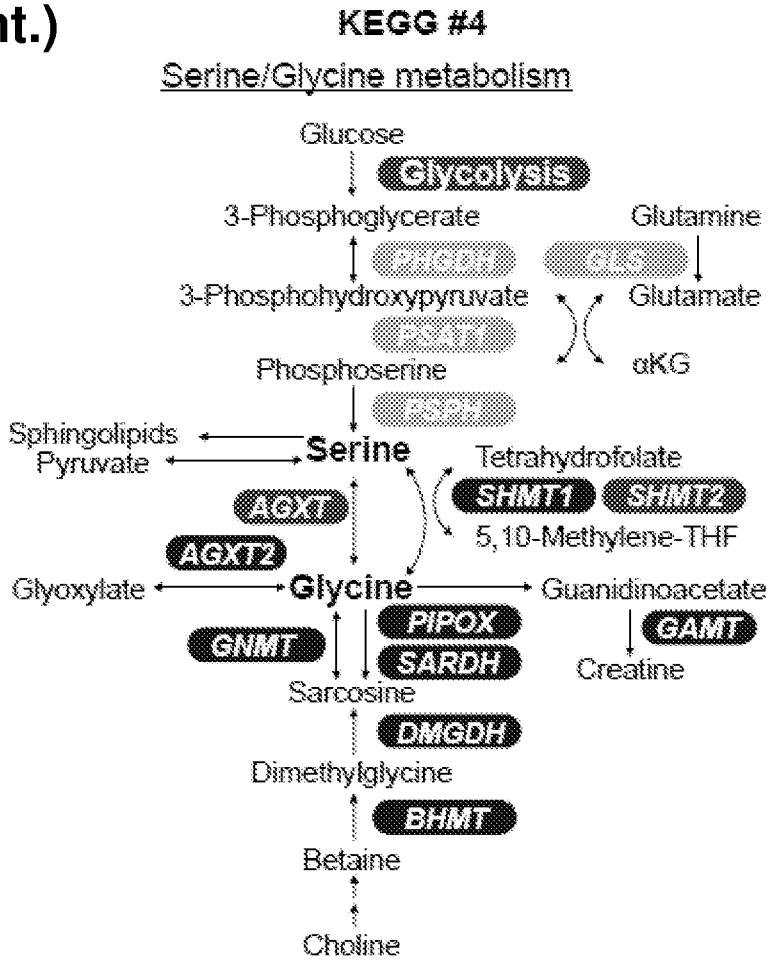


Figure 9 (cont.)

g (cont.)



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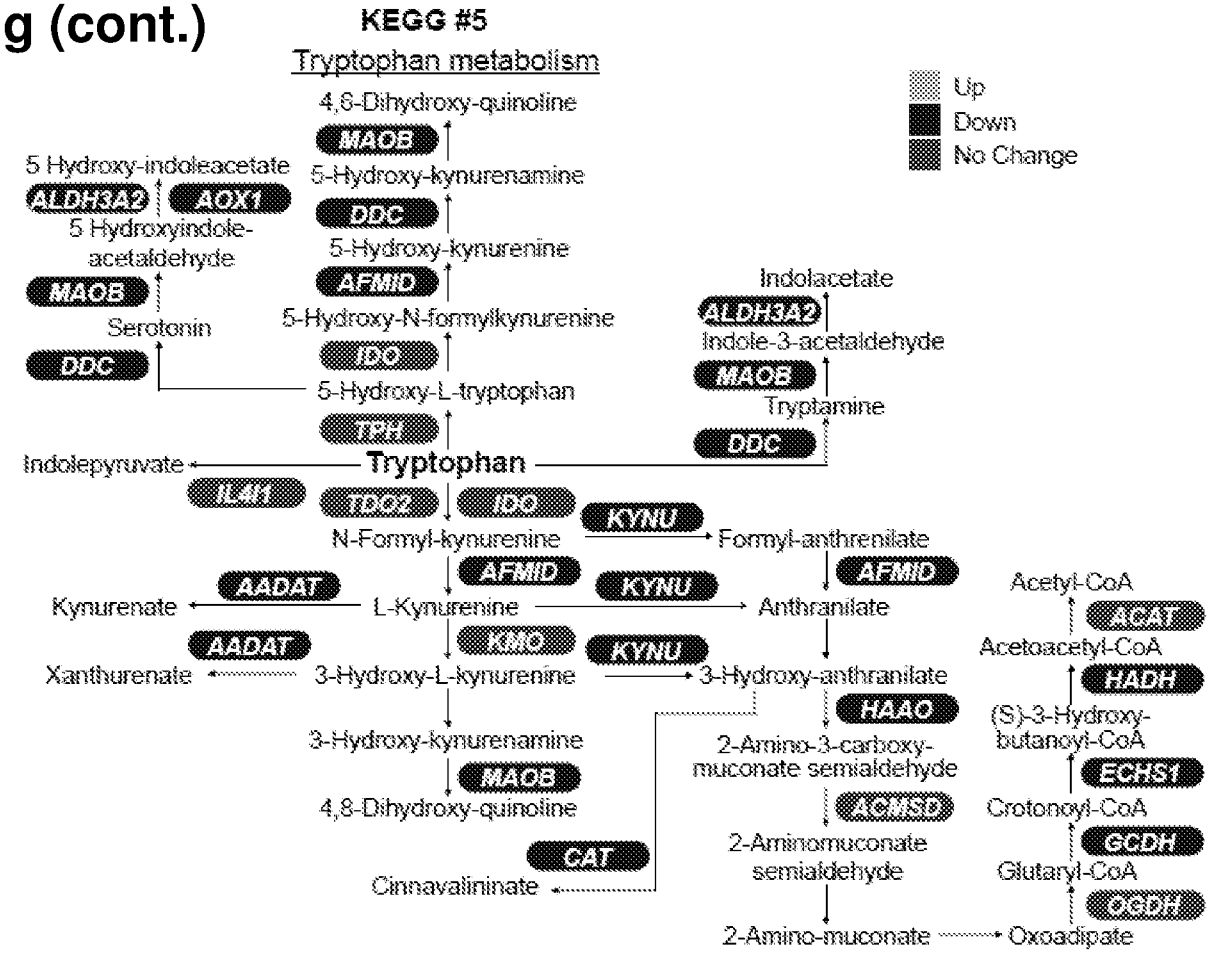

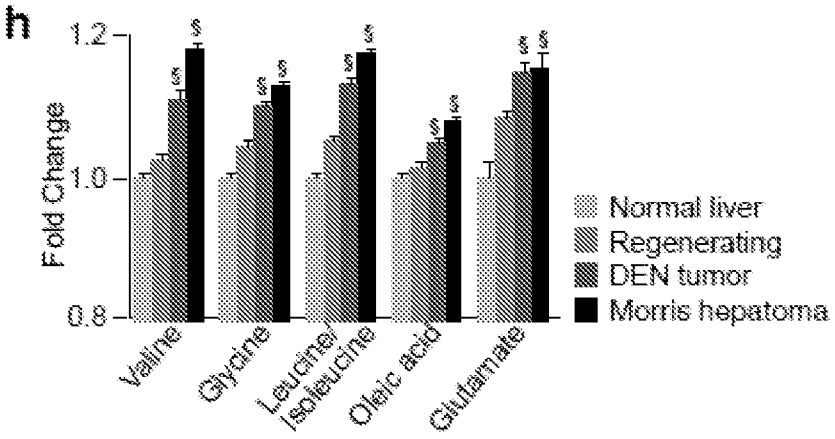

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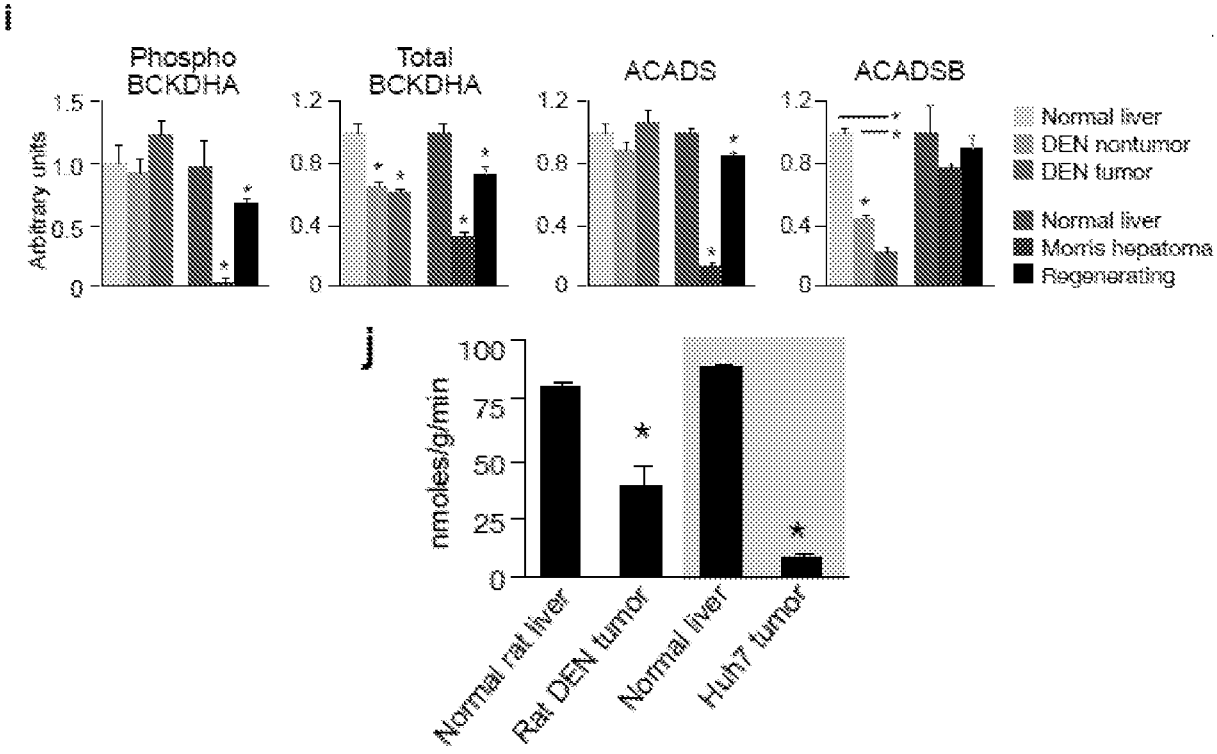
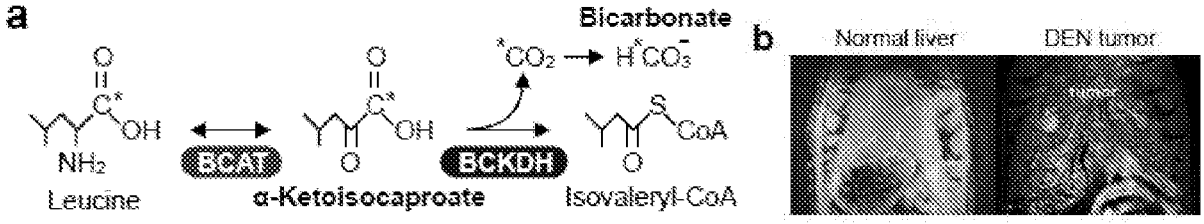


Figure 9 (cont.)



c

Hep3B CRISPR BCKDK null clones

	Allele 1	Allele 2	Allele 3 (if observed)
Clone #1	7bp deletion	8bp insertion	N/A
Clone #2	65bp deletion	1bp insertion	N/A
Clone #3	16bp deletion	1bp deletion	50bp deletion

Figure 10

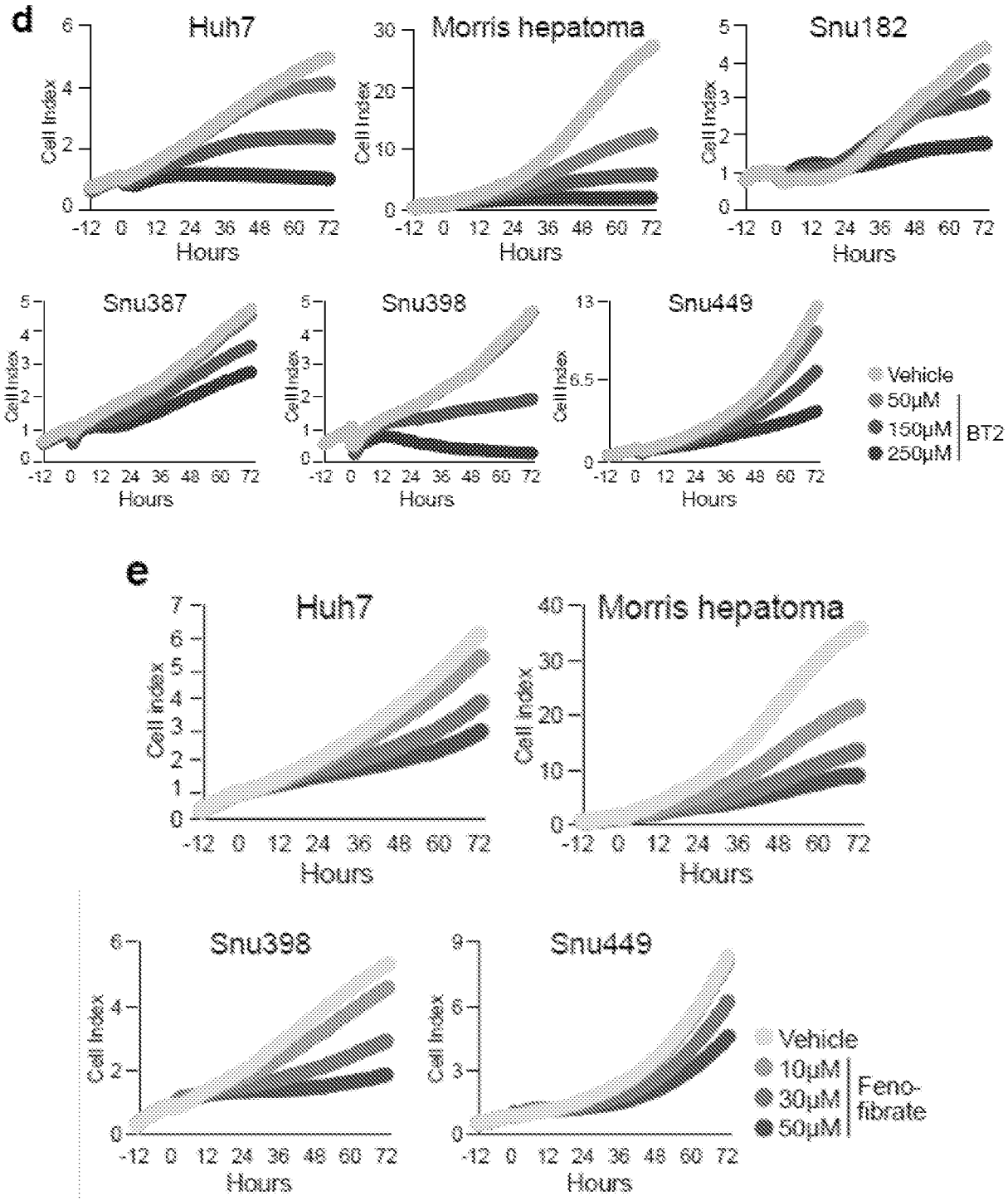


Figure 10 (cont.)

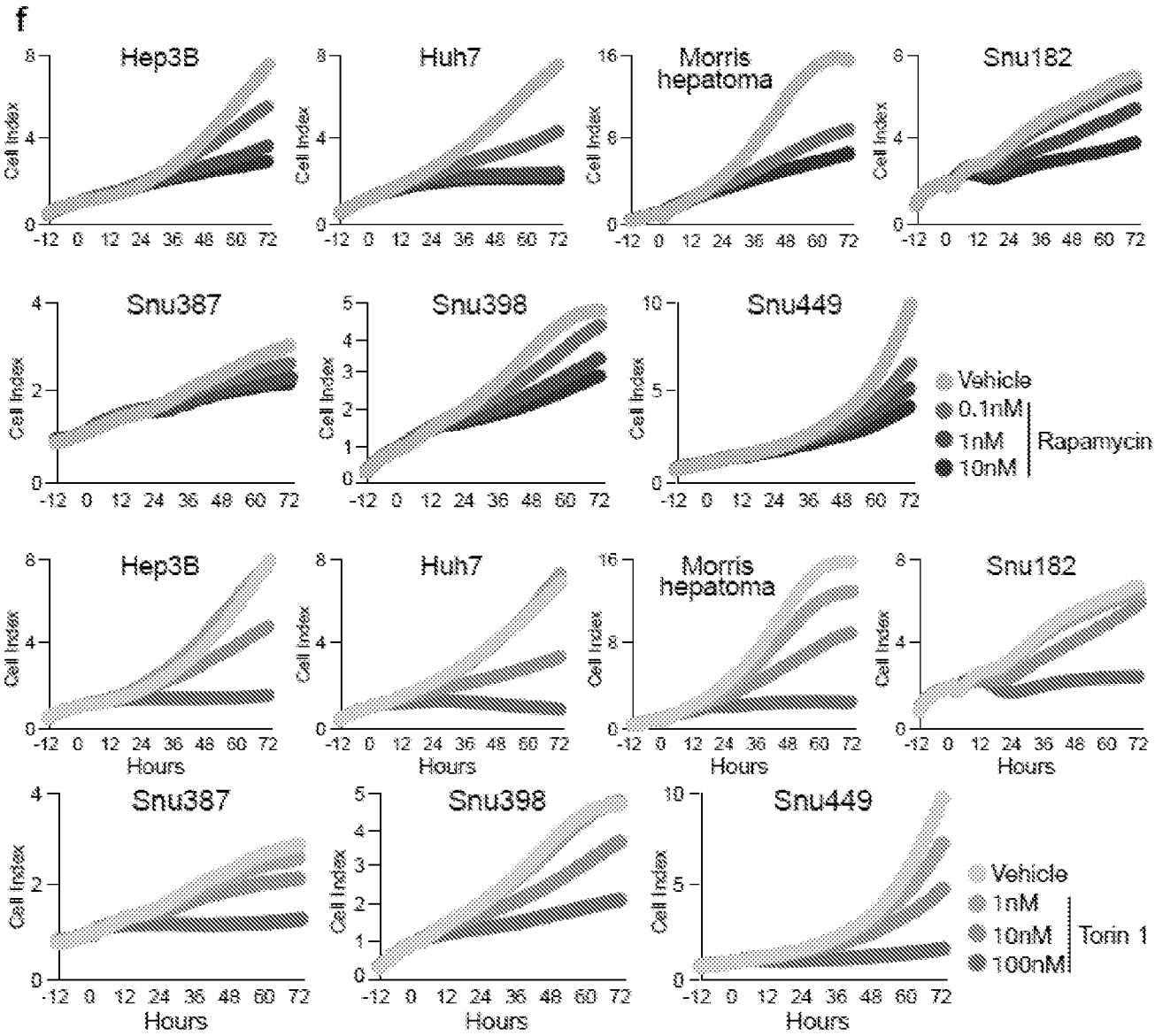
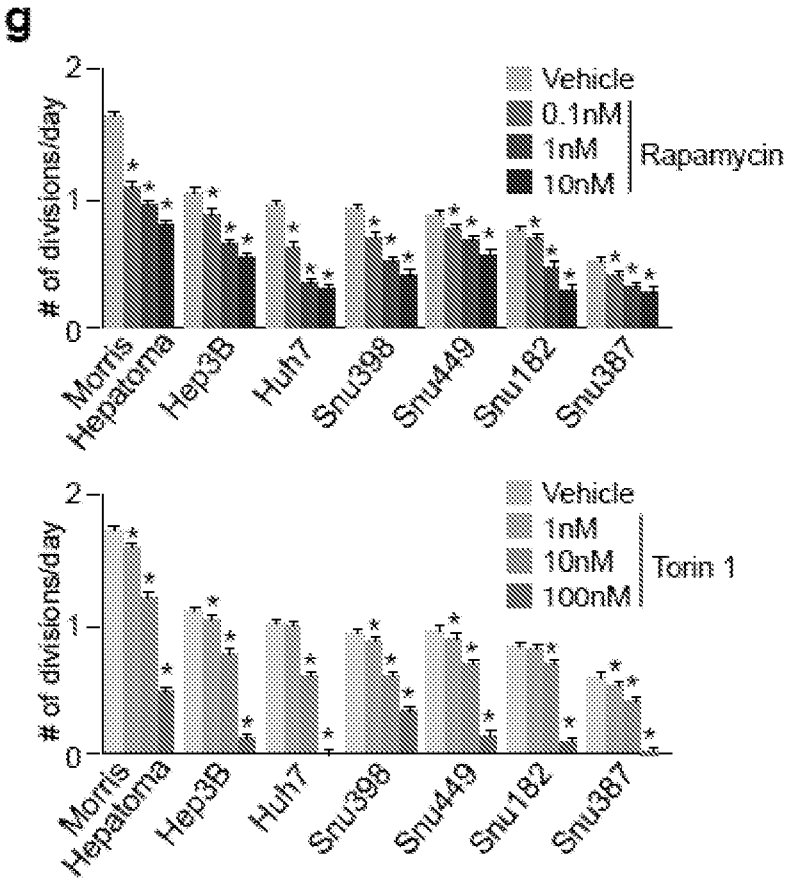


Figure 10 (cont.)



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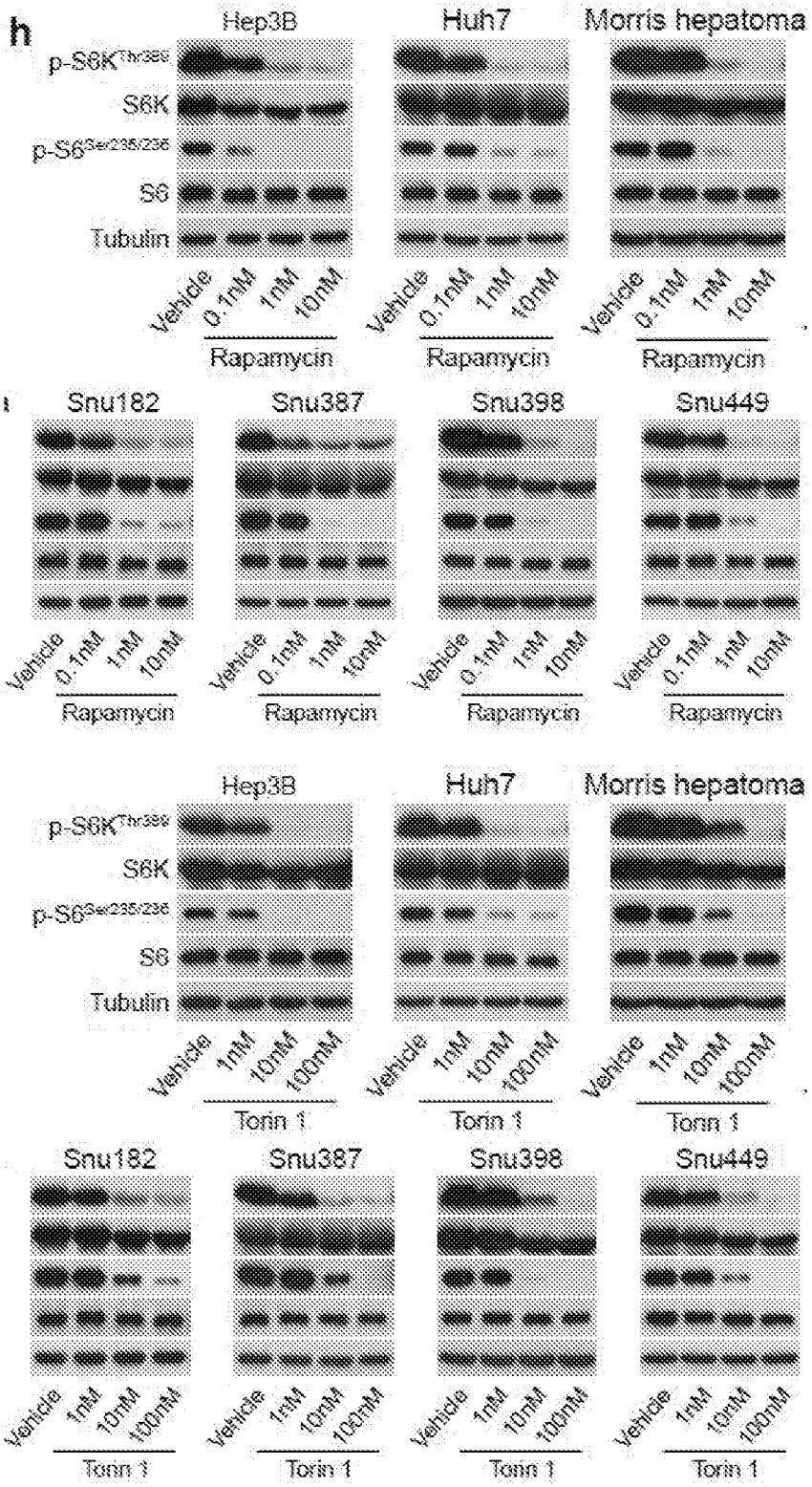
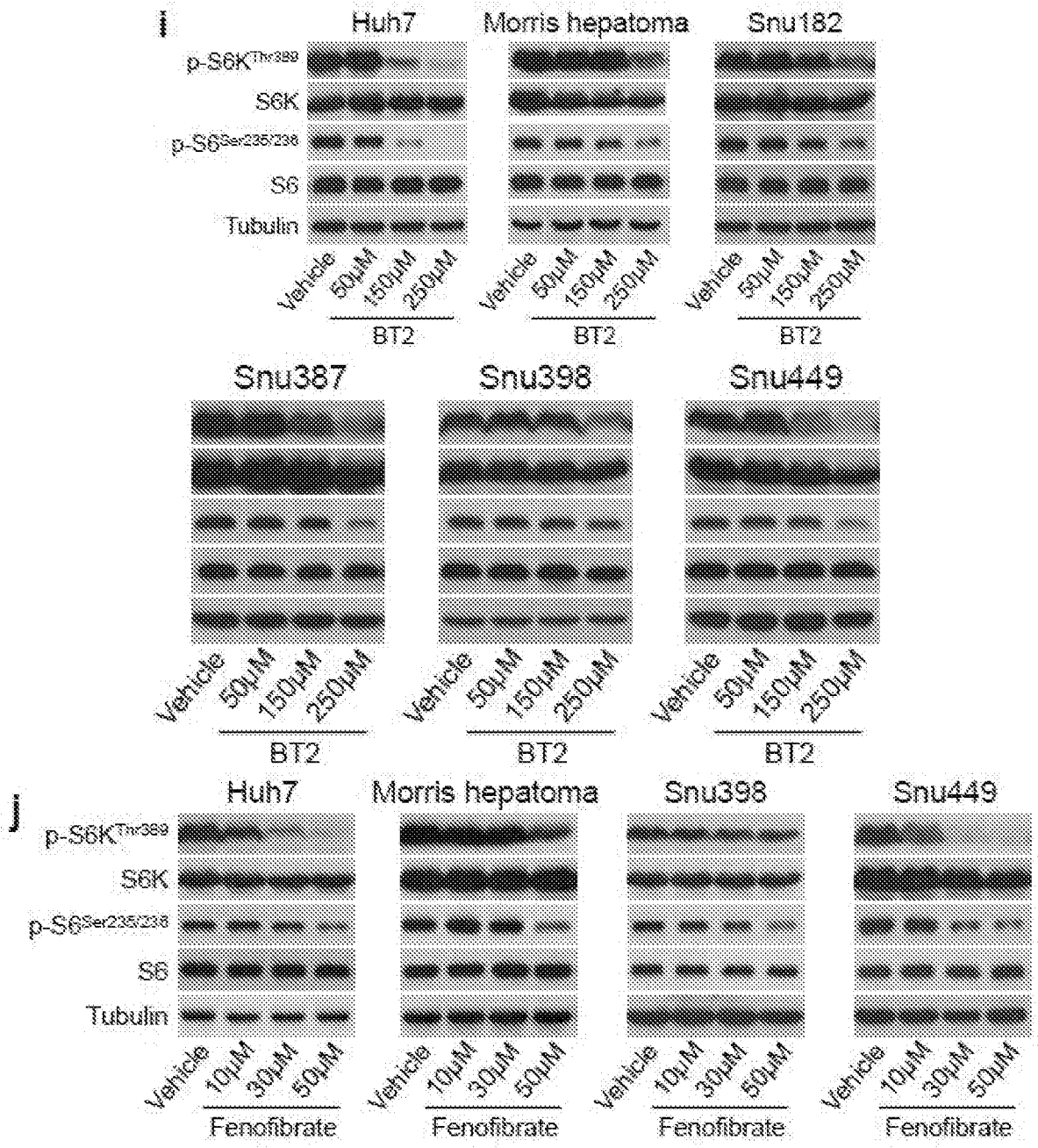


Figure 10 (cont.)



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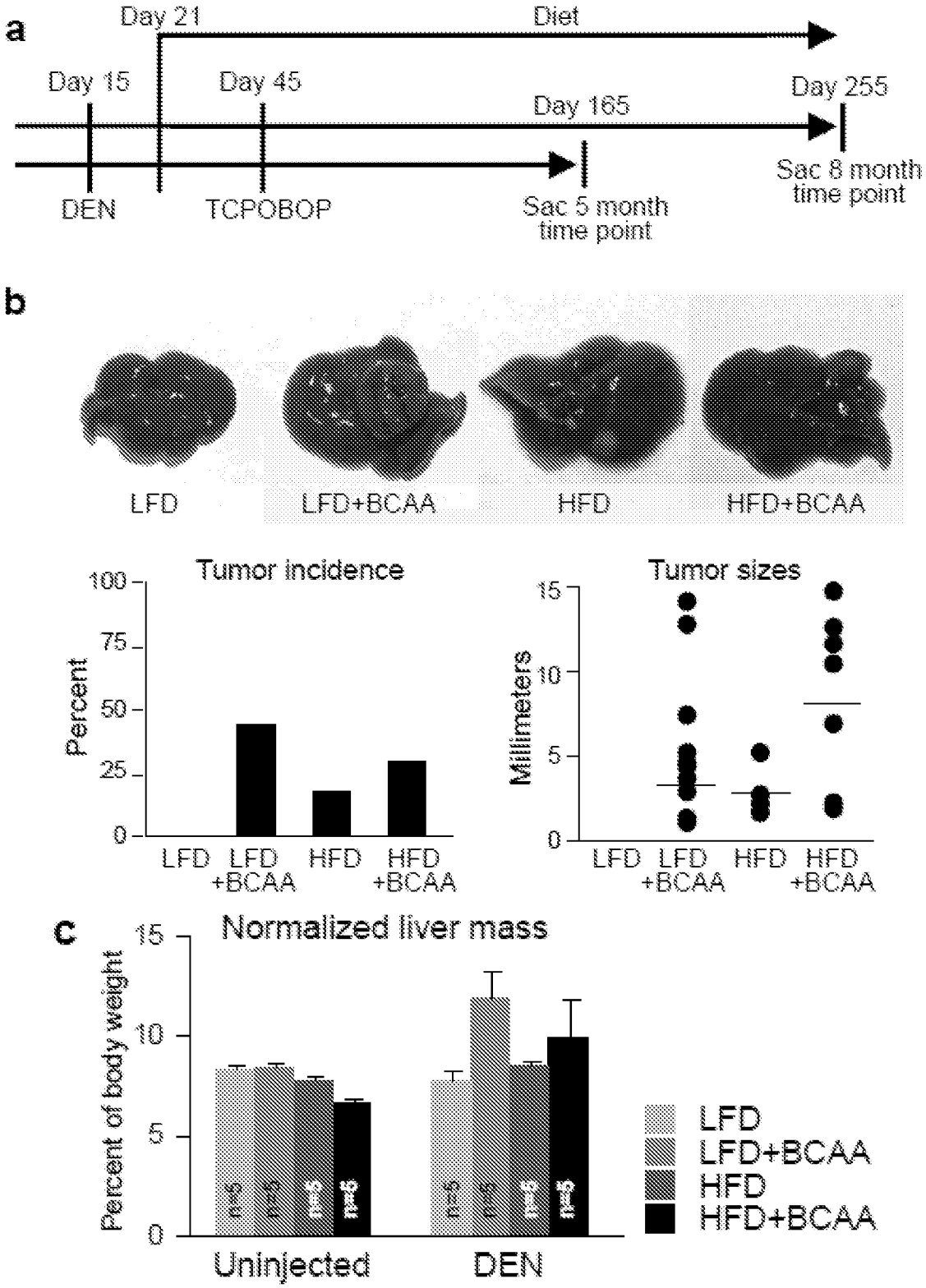

a
Day 21
Diet
Day 15
Day 45
Day 165
Day 255
DEN
TCPOBOP
Sac 5 month time point
Sac 8 month time point
b
LFD
LFD+BCAA
HFD
HFD+BCAA
Tumor incidence
Percent
100
75
50
25
0
LFD
LFD +BCAA
HFD
HFD +BCAA
Tumor sizes
Millimeters
15
10
5
0
c
Normalized liver mass
Percent of body weight
Uninjected
DEN
n=5
LFD
LFD+BCAA
HFD
HFD+BCAA

Figure 11

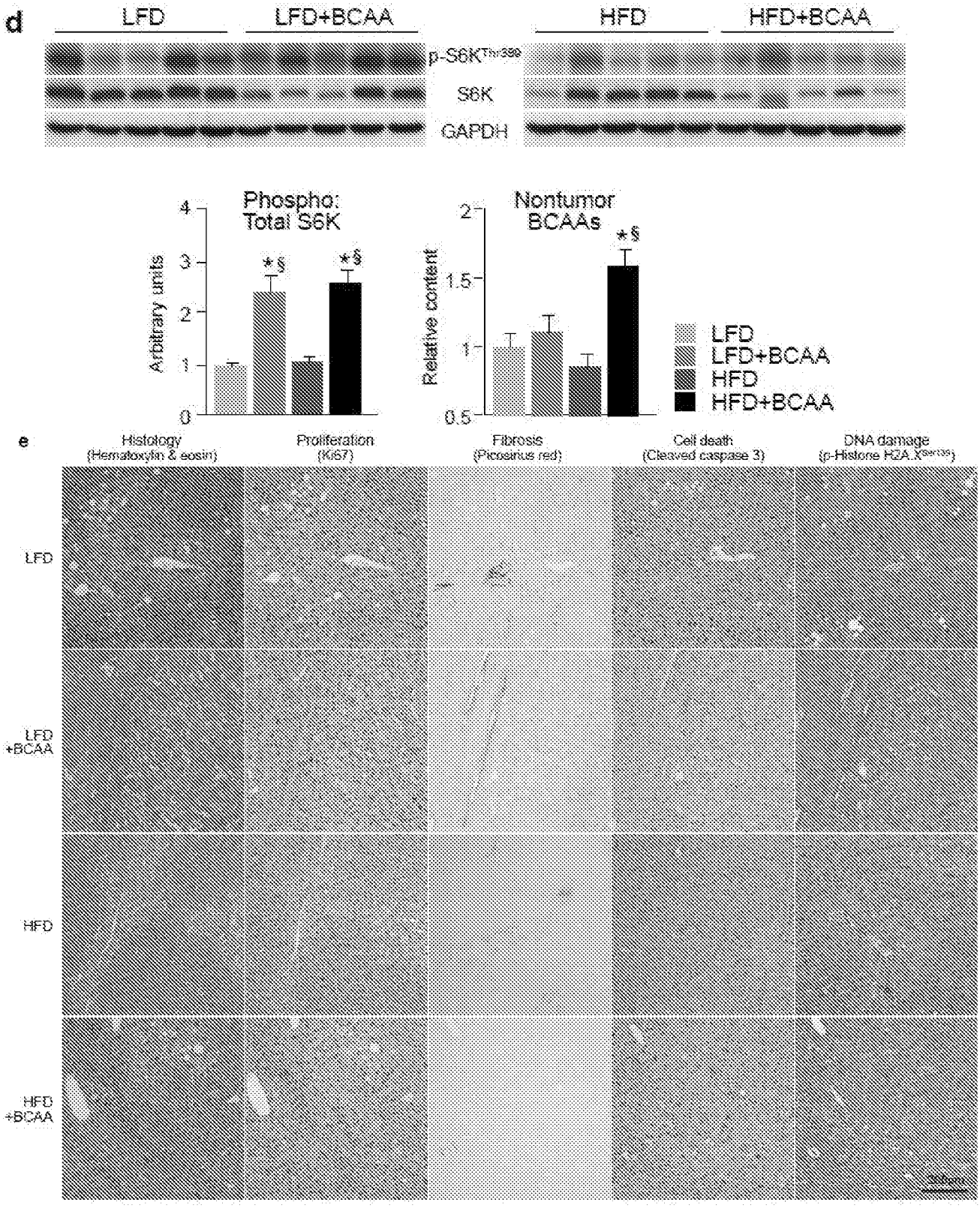


Figure 11 (cont.)

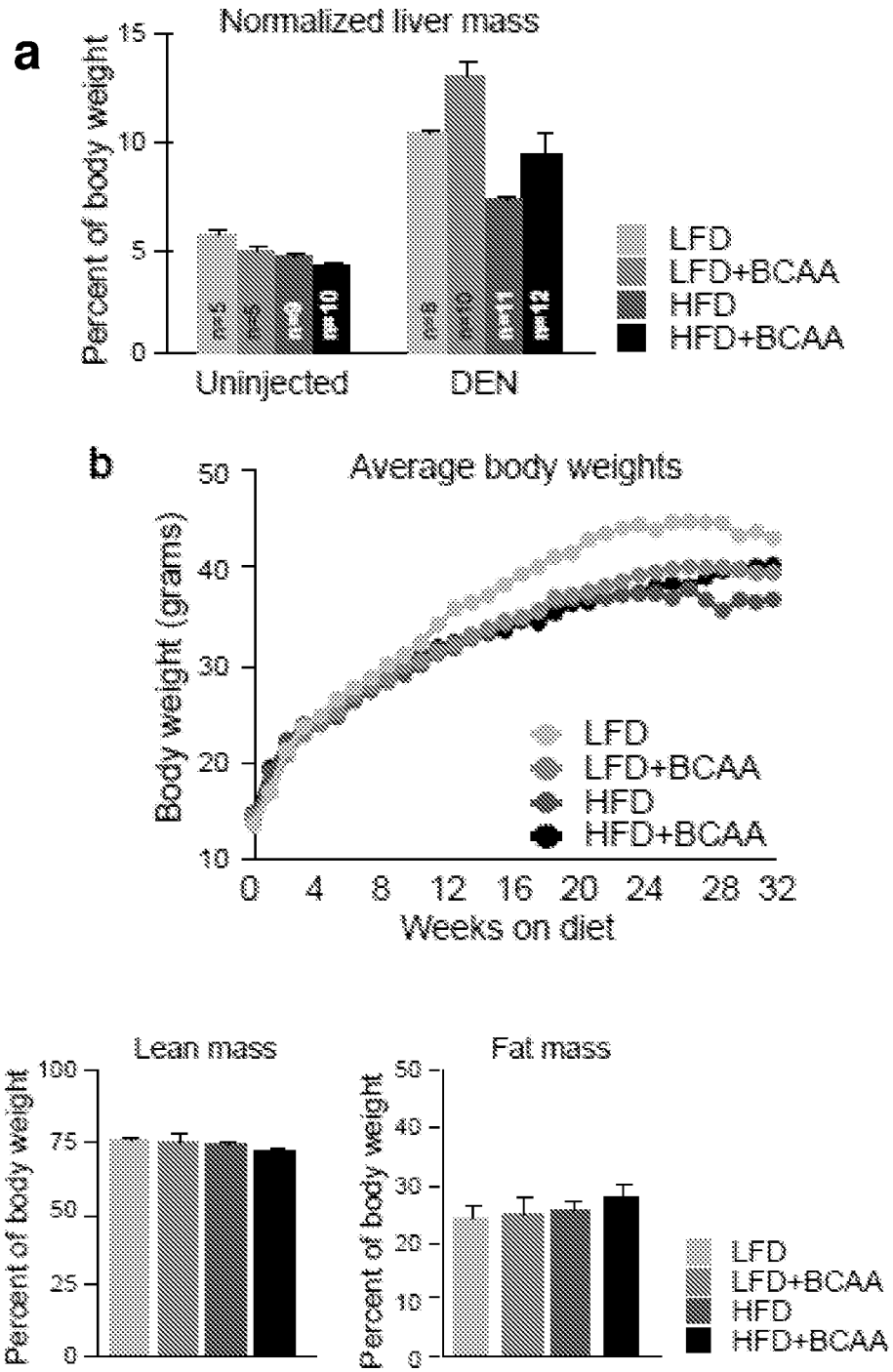

a
Normalized liver mass
Percent of body weight
15
10
5
0
n=12
n=10
n=11
n=12
Uninjected
DEN
LFD
LFD+BCAA
HFD
HFD+BCAA
b
Average body weights
Body weight (grams)
50
40
30
20
10
0 4 8 12 16 20 24 28 32
Weeks on diet
LFD
LFD+BCAA
HFD
HFD+BCAA
Lean mass
Percent of body weight
100
75
50
25
0
Fat mass
Percent of body weight
50
40
30
20
10
0
LFD
LFD+BCAA
HFD
HFD+BCAA

Figure 12

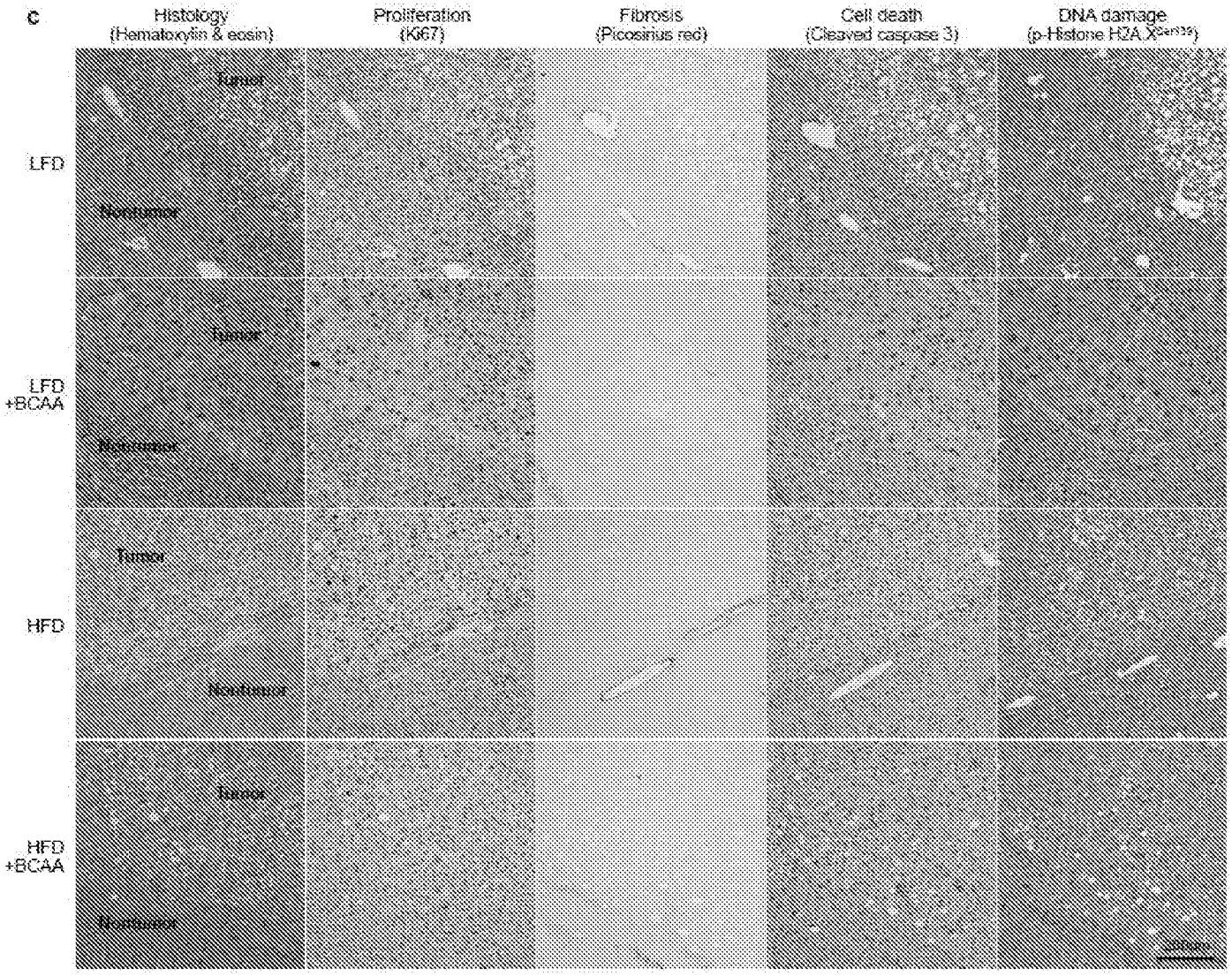


Figure 12 (cont.)

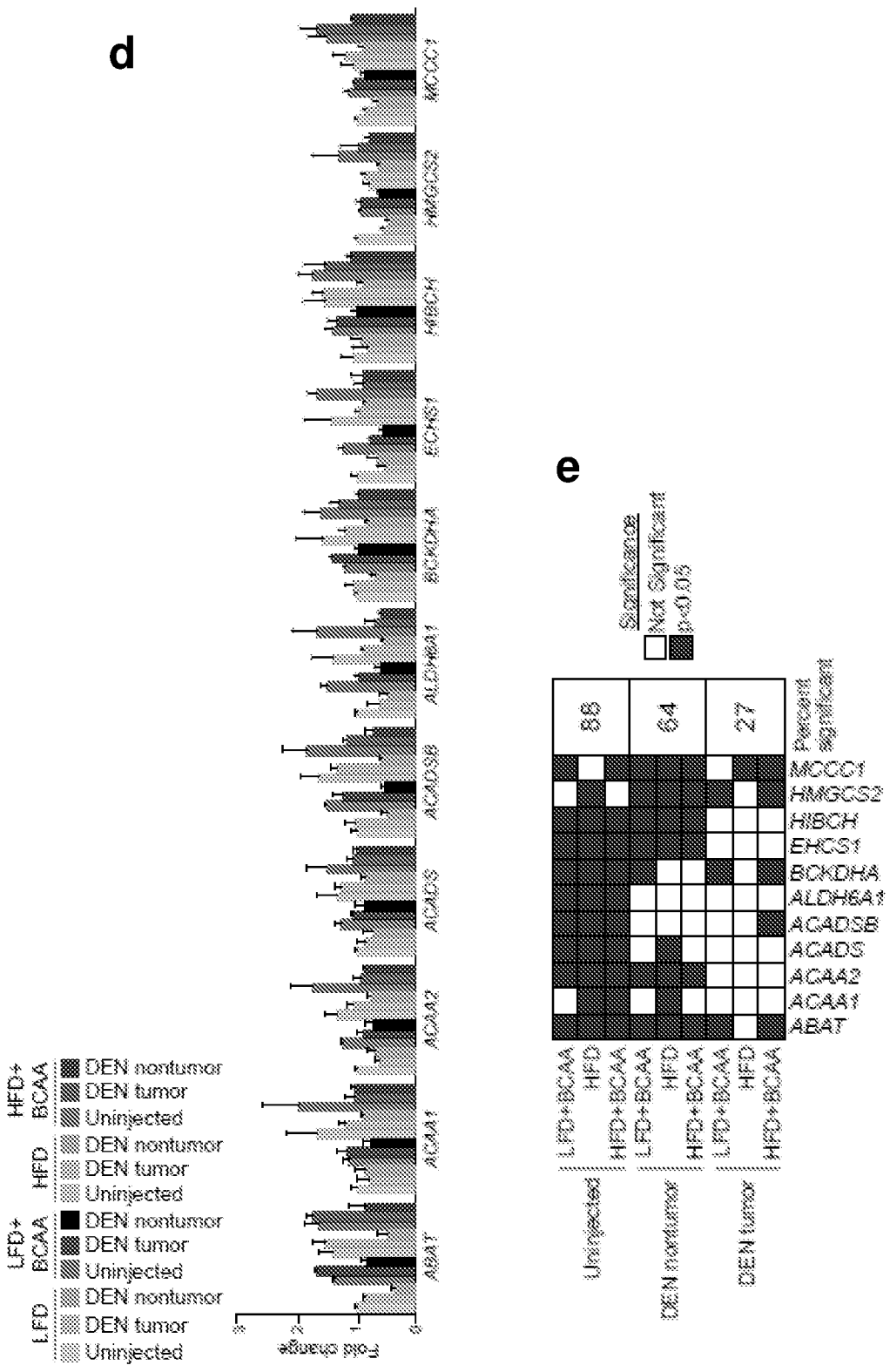


Figure 12 (cont.)

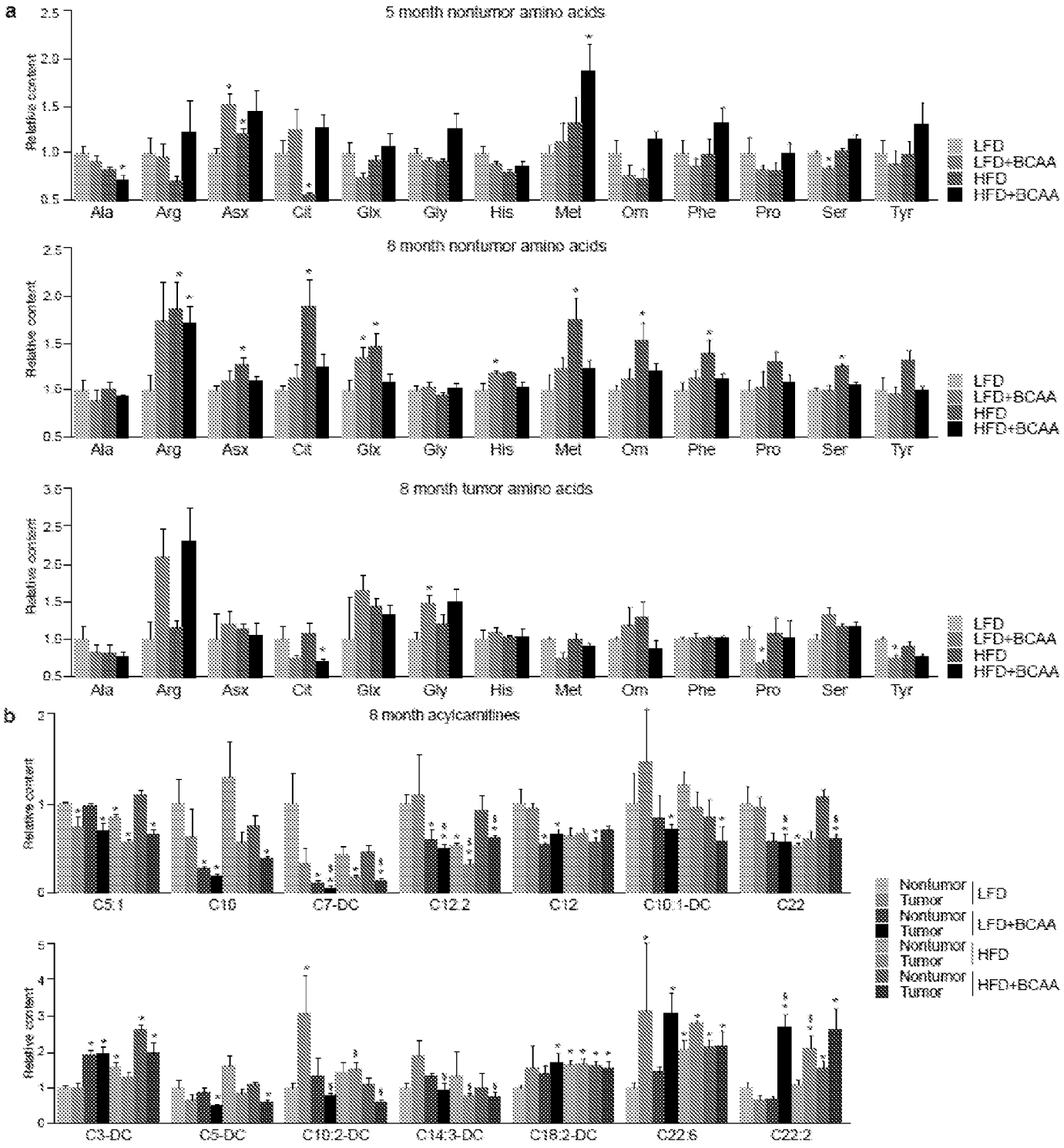

a
5 month nontumor amino acids
8 month nontumor amino acids
8 month tumor amino acids
Relative content
Ala
Arg
Asx
Cit
Glx
Gly
His
Met
Orn
Phe
Pro
Ser
Tyr
LFD
LFD+BCAA
HFD
HFD+BCAA
b
8 month acylcarnitines
C5:1
C16
C7-DC
C12:2
C12
C10:1-DC
C22
C3-DC
C5-DC
C10:2-DC
C14:3-DC
C18:2-DC
C22:6
C22:2
Nontumor
Tumor

Figure 13

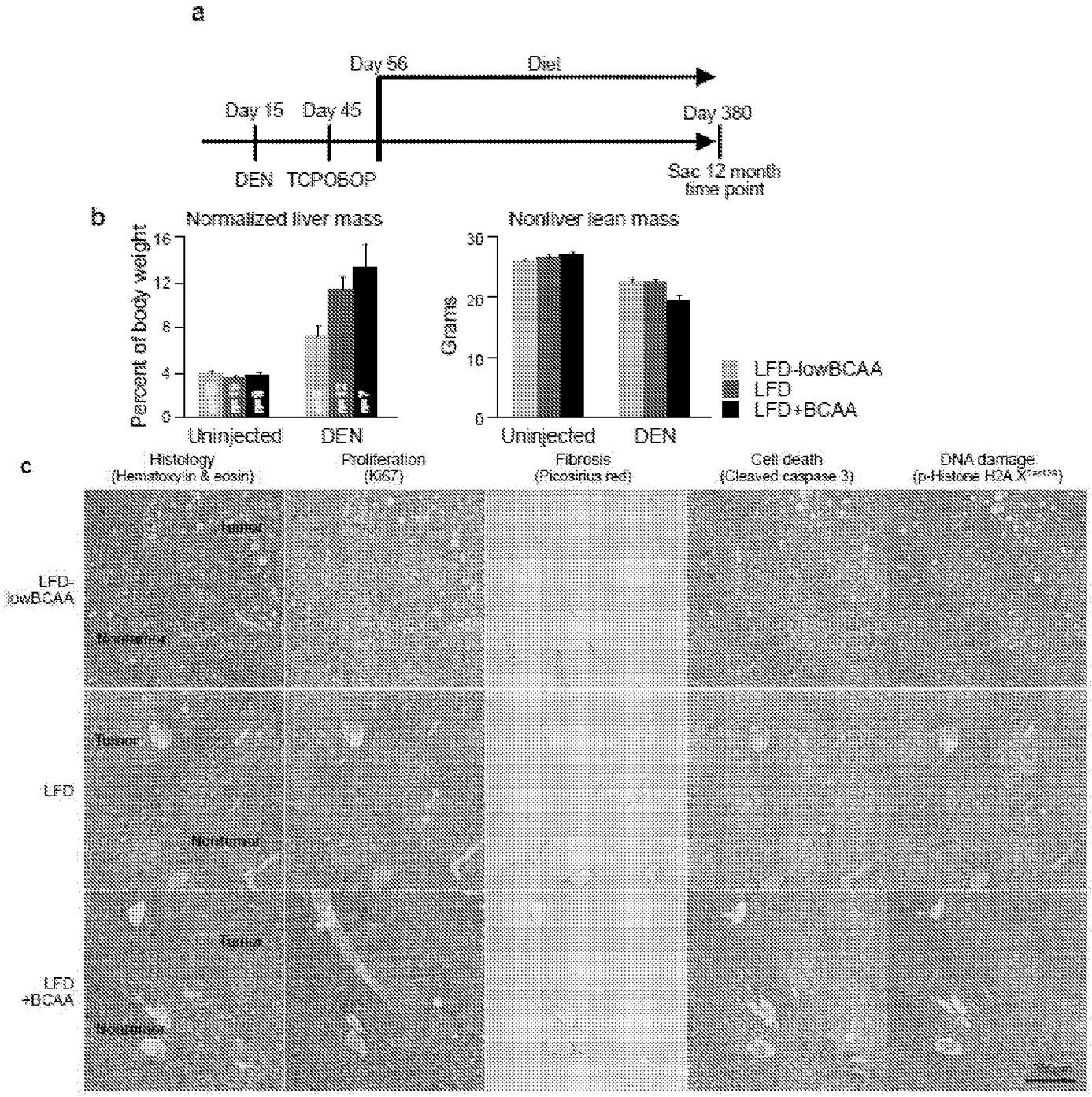
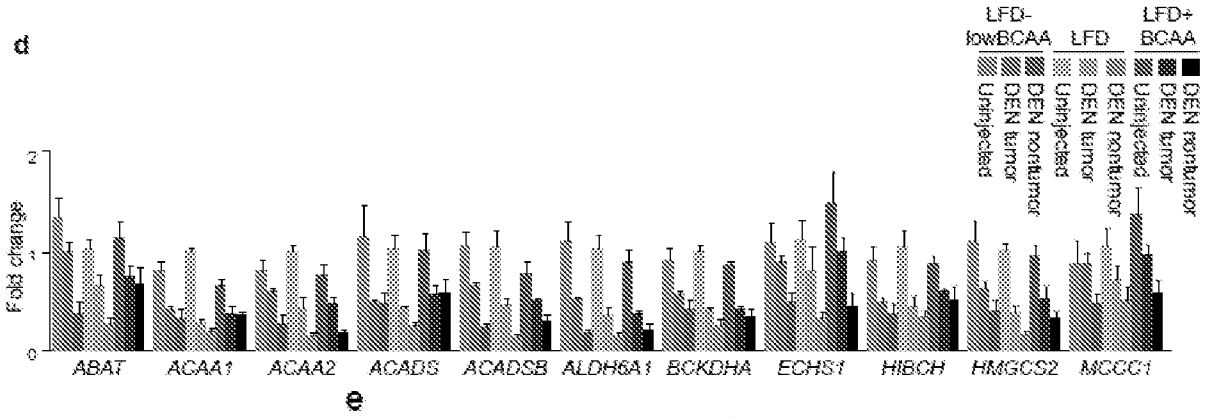
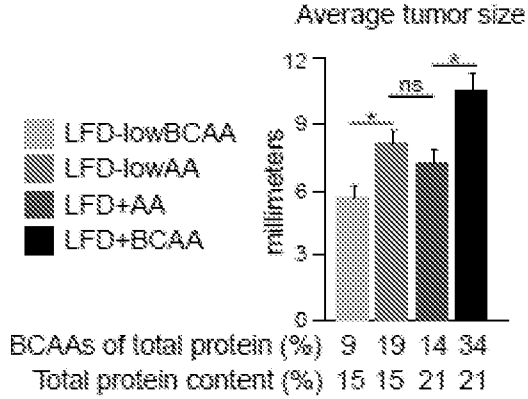

a
Day 56
Diet
Day 15
Day 45
Day 380
DEN
TCPOBOP
Sac 12 month time point
b
Normalized liver mass
Percent of body weight
16
12
8
4
0
Uninjected
DEN
Nonliver lean mass
Grams
30
20
10
0
Uninjected
DEN
LFD-lowBCAA
LFD
LFD+BCAA
c
Histology (Hematoxylin & eosin)
Proliferation (Ki67)
Fibrosis (Picosirius red)
Cell death (Cleaved caspase 3)
DNA damage (p-Histone H2A X)
LFD-lowBCAA
LFD
LFD +BCAA
Tumor
Nontumor

Figure 14





f

	Entire Sample	Low BCAA (<1.73% kcal)	Medium BCAA (1.73-3.89% kcal)	High BCAA (>3.89% kcal)
N	3156	277	2548	331
Age (years)	57.3 (0.15)	56.9 (0.41)	57.3 (0.15)	57.0 (0.38)
Female (%)	52.3	49.5	52.4	54.4
High School Graduate (%)	53.5	49.5	54.6	43.8
Non-Hispanic White (%)	45.6	43.0	46.3	42.6
Non-Hispanic Black (%)	25.7	33.9	24.8	25.4
Mexican American (%)	24.3	18.4	24.5	27.2
Current Smoker (%)	26.0	27.8	26.3	23.0
Waist Circumference (cm)	97.4 (0.38)	97.3 (1.14)	97.3 (0.42)	98.0 (0.97)
Total Physical Activity (METS)	101.2 (4.27)	84.0 (8.15)	100.4 (4.36)	120.4 (14.4)
BCAA (%)	2.78 (0.02)	1.46 (0.02)	2.69 (0.01)	4.54 (0.06)
NonBCAA (%)	13.2 (0.11)	7.53 (0.08)	12.8 (0.07)	21.0 (0.29)
Fat (%)	33.7 (0.28)	31.2 (1.02)	34.2 (0.30)	31.3 (0.75)
Carbohydrate (%)	49.6 (0.33)	56.1 (1.26)	49.8 (0.32)	42.8 (0.86)
Cancer Mortality (%)	11.9	10.5	11.9	13.0
Person Years	54,781	4,934	44,332	5,515

g

Protein Source	Adjusted HR[1]	Adjusted HR[2]
BCAA	1.29 (1.08-1.55)	1.27 (1.03-1.57)
Non BCAA	1.07 (1.03-1.11)	1.06 (1.01-1.12)

Figure 14 (cont.)

h

Tertile	BCAA Intake (% kcal)	Adjusted HR[1]	Adjusted HR[2]
1	Low (<1.73%)	1.0	1.0
2	Medium (1.73-3.89%)	0.78 (0.54-1.12)	0.94 (0.62-1.45)
3	High (>3.89%)	0.55 (0.32-0.94)	0.92 (0.40-2.12)

i

Protein Source	Adjusted HR[1]	Adjusted HR[2]
BCAA	0.82 (0.71-0.95)	1.00 (0.80-1.25)
Non BCAA	0.95 (0.92-0.98)	0.99 (0.95-1.04)

j

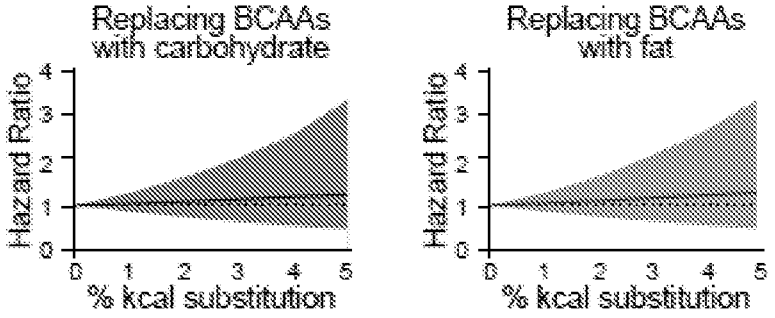


Figure 14 (cont.)

Study	Diet	Catalog Number	% kcal	Fat Composition	% kcal	Carbohydrate Composition	% kcal	Protein Composition
#1 (8 weeks, 9 mice/ group)	LFD	D07010502	10%	65% soybean oil, 35% lard	71%	43% corn starch, 6% maltodextrin, 50% sucrose	19%	53% casein, 47% purified amino acids
	LFD+BCAA	D07030503	10%		67%		22%	41% casein, 59% purified amino acids
	HFD	D09010303	45%	100% soybean oil, 60% lard	35%	31% corn starch, 20% maltodextrin, 50% sucrose	19%	53% casein, 47% purified amino acids
	HFD+BCAA	D09030307	45%		34%		23%	41% casein, 59% purified amino acids
#2 (8 weeks, time point)	LFD+BCAA	A14100108	10%	55% soybean oil, 45% lard	68%	61% corn starch, 14% maltodextrin, 25% sucrose	31%	100% purified amino acids
	LFD+BCAA+0.05%Fenofibrate	A15013010	10%		69%		21%	
	LFD+BCAA+0.02%ETZ	A15013014	10%		69%		21%	
	HFD+BCAA	A14100104	44%	100% soybean oil, 55% lard	36%	21% corn starch, 20% maltodextrin, 59% sucrose	21%	
	HFD+BCAA+0.05%Fenofibrate	A15013016	44%		36%		21%	
	HFD+BCAA+0.02%ETZ	A15013020	44%		36%		21%	
#3 (12 weeks)	LFD+oxBCAA	A14100106	10%	55% soybean oil, 45% lard	75%	64% corn starch, 13% maltodextrin, 22% sucrose	15%	100% purified amino acids
	LFD+oxAA*	A14100105	10%		75%		15%	
	LFD+AA*	A14100107	10%		69%	61% corn starch, 14% maltodextrin, 25% sucrose	21%	
(same as #2)	LFD+BCAA	A14100108	10%		69%		21%	

Figure 15

Target	Mouse Sequence	Rat Sequence
ABAT	5'-GAGGCCGTGCACTTTTTCTG-3'	5'-GAGGCCGTGCACTTTTTCTG-3'
	5'-CCAGAGCCGGATGGTTGTAA-3'	5'-CGCGTTTTGAGGCTGTTGAA-3'
ACAA1	5'-ACAGTGTTCATCGGGACTGC-3'	5'-TTACGACATTGGCATGGCCT-3'
	5'-AGATATTCCCAGGGTTCCCCA-3'	5'-CAGCCACATTCTCCGAGGTT-3'
ACAA2	5'-ACATAACTTCACGCCCCTGG-3'	5'-GGCAAAGTTCCACCGGAAAC-3'
	5'-GAGGGGCAAAAGCTTCGTTC-3'	5'-ACTGGAAACCAGAGCCACAG-3'
ACADS	5'-TTGCCGAGAAGGAGTTGGTC-3'	5'-AGCCTTTCACCAAGGAGTCG-3'
	5'-AGGTAATCCAAGCCTGCACC-3'	5'-CATCTCGGTAGTAGCGCTCG-3'
ACADSB	5'-GGACTGGCCCAAGGATGTTT-3'	5'-GGACTGGCCCAAGGATGTTT-3'
	5'-CGAGCCTAGCAGCGTTGTAT-3'	5'-ATAAATGGCCTCCCGGCTTC-3'
ALDH6A1	5'-GCGTGGGCAGACACATCTAT-3'	5'-AGTACCTGGAGCAACCATGC-3'
	5'-CTCCCAGCATGAGGGATGTC-3'	5'-CGAAGATGTACTCTCCCGCC-3'
AOX1	5'-TACGTGAATGGCCAGAAGGT-3'	5'-ACCGTACCTGAGGAAGAACCT-3'
	5'-GGATGATGCCTGATCGCCTT-3'	5'-TGCCCTCTACTGTGGTGACT-3'
BCKDHA	5'-GACCTGGTGTTTGGCCAGTA-3'	5'-CAACGATGTGTTTGCGGTGT-3'
	5'-GCCGTAGTGAACAGGCATCT-3'	5'-TTGACCTCATCCACCGAACG-3'
ECHS1	5'-CTCTTGGTGGGGGTTGTGAA-3'	5'-AGGCCATCCAATGTGCAGAA-3'
	5'-TTGCTAGCGATTTGCCGACT-3'	5'-TCCACAAAGGCAGACATCCC-3'
HIBCH	5'-AGGCGTCATAACGCTCAAC-3'	5'-TGGAACAGATTAAGTTCTCATTGAC-3'
	5'-TCCTCCGGCTCCCTTTATGA-3'	5'-TATGGTGTGCTGACCCTTGC-3'
HMGCS2	5'-GCCCAAACGTCTAGACTCCC-3'	5'-GCCCAAACGTCTAGACTCCC-3'
	5'-CTCCATTAGACGGGACACCG-3'	5'-CGGGCATATTTTCTGCGGTG-3'
MCCC1	5'-TGGGGTAGCCCGTAAATCCA-3'	5'-AAGGCATGTGGAAGTCCAGG-3'
	5'-GAGCTCCTTCTGCACTCACT-3'	5'-CAGGATCAATACCAGGCGCT-3'
β-ACTIN	5'-CAAGGTCATCCATGACAACTTTG-3'	5'-CAAGGTCATCCATGACAACTTTG-3'
	5'-GGCCATCCACAGTCTTCTGG-3'	5'-GGCCATCCACAGTCTTCTGA-3'
GAPDH	5'-CAAGGTCATCCATGACAACTTTG-3'	5'-CAAGGTCATCCATGACAACTTTG-3'
	5'-GGCCATCCACAGTCTTCTGG-3'	5'-GGCCATCCACAGTCTTCTGA-3'

Figure 16