

NRF2/BACH1 control of tyrosine catabolism links oxidative stress to hepatocellular carcinoma progression

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Table S1. Formula ingredients for control special diet and deficient special diet.

FORMULA INGREDIENTS G/KG	AIN93 AA Defined Control	AIN93 AA Tyr and Phe Deficient +0.3%Phe
pregelatinized cornstarch	407.1	420.5
maltodextrin	142	142
sucrose	110	110
crude cellulose	50	50
L-tyrosine	9	
L-phenylalanine	7.5	3.1
soybean oil	70	70
pre-mixture of minerals PM AIN93M_G 3,5%	35	35
pre-mixture of vitamins PV AIN 93M_G 1%	10	10
choline bitartrate	2.5	2.5
CRM (P)		

Table S2. Nutritional values of control special diet and deficient special diet.

UNIT	CALCULATED NUTRITIONAL VALUES	AIN93 AA Defined Control	AIN93 AA Tyr and Phe Deficient +0.3%Phe
%	NFE	66.57188821	67.05758221
%	Starch	34.6035	35.7425
%	Maltodextrin	12.11402	12.11402
%	Total sugars	13.80457836	13.80457836
%	Crude Protein	14.73903613	14.08771813
%	Crude Fat	7.1253915	7.1291435
g / 100 g	Ala alanine	0.34475	0.34475
g / 100 g	Arg Arginine	0.784	0.784
g / 100 g	Asp Aspartic Acid	0.784	0.784
g / 100 g	Cys Cystine	0.343	0.343
g / 100 g	Glu Glutamic Acid	3.96	3.96
g / 100 g	Gly Glycine	1.97	1.97
g / 100 g	His histidine	0.441	0.441
g / 100 g	Ile Isoleucine	0.8036	0.8036
g / 100 g	Leu Leucine	1.078	1.078
g / 100 g	Lys Lysine	1.5750627	1.5755585
g / 100 g	Met Methionine	0.4975	0.4975
g / 100 g	Phenylalanine	0.735	0.3038
g / 100 g	Pro Proline	0.3465	0.3465
g / 100 g	Ser Serine	0.34475	0.34475
g / 100 g	Thr threonine	0.8077	0.8077
g / 100 g	Trp Tryptophan	0.1782	0.1782
g / 100 g	Tyr Tyrosine	0.891	0

g / 100 g	Val Valine	0.8036	0.8036
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Table S3. MASH models characteristics.

MASH model	Diet onset age (weeks)	Experimental diet	Duration of experimental diet (weeks)	Mouse strain	Genotype	Model characteristics
WT_WT1	6	Western diet (research Diets D091000310)	42	C57BL/6N	WT	Advanced MASH, high insulin resistance, hyperglycemia and dyslipidemia
WT_WT2	4	Western diet (research Diets D091000301)	42	C57BL/6N	WT	Early MASH, high insulin resistance, hyperglycemia and dyslipidemia
OB_WD	8	Western diet (research Diets D091000310)	23	ob/ob	KO	Advanced MASH, severe macrovesicular steatosis, ballooning degeneration and advanced fibrosis. Extreme insulin resistance and dyslipidemia.
GNMT-KO	N/A	N/A	N/A	GNMT-KO	KO	High SAME content. Spontaneous MASH starting at 3 months, progressing to fibrosis and HCC

Table S4. Primers used for qRT-PCR.

Gene name	Symbol	Sequence		Species
Fumarylacetoacetate hydrolase (mouse)	<i>fah</i>	Forward	GAGAATGCGCTGTTGCCAAA	<i>Mus musculus</i>
		Reverse	GCACCATACACAGGAGGCTT	
		Reverse	TCACGTAAGTGGCAGCTGTA	
Glutathione transferase zeta 1 (mouse)	<i>gstz1</i>	Forward	CAACAAGGAGCTGCTAGCCT	<i>Mus musculus</i>
		Reverse	GGCCTACTCCCTTCCCTGTA	
Homogentisate dioxygenase (mouse)	<i>hgo</i>	Forward	GAGAGGATAGCTGACGGCAC	<i>Mus musculus</i>
		Reverse	CGGAGAGGCTCCCAACATTT	
Hydroxyphenylpyruvate dioxygenase (mouse)	<i>hpd</i>	Forward	AAGAGATGGGCGACCACTTG	<i>Mus musculus</i>
		Reverse	CGCAGTCTTCCACCTCGAAT	
		Reverse	ACGTAGATCTTGCTGCCCAC	
Ribosomal protein 9S	<i>9s</i>	Forward	GCAAGCAGGTGGTGAACATC	<i>Homo sapiens, Mus musculus</i>
		Reverse	TCTTGGCATTCTTCTTTCAC	
Ribosomal protein 18S	<i>18s</i>	Forward	TTCCACAGGAGGCCTACACG	<i>Homo sapiens, Mus musculus</i>
		Reverse	GCCCATCGATGTTGGTGTG	
Tyrosine aminotransferase (mouse)	<i>tat</i>	Forward	TCAGGACGACAGATGTCCCT	<i>Mus musculus</i>
		Reverse	TGTGTTGGATGTGACTGGGG	

Table S5. Primers used for ChIP-qPCR

Gene name	Symbol	Direction	Sequence	Specie	Position from TSS
Tyrosine aminotransferase – binding site 1 – primer pair 1	tat_1.1	Forward Reverse	ATTGCCCATGTCCAGGAGTG TTCAGGAAACTGTGGAGGGC	Mus musculus	-393 (NRF2) -394 (BACH1)
4-hydroxyphenylpyruvate dioxygenase - binding site 1- primer pair 1	hpd_1.1	Forward Reverse	TCACTCTCCCTCTGGGTCTG GGGGGAAGGGAGCTGAGATA	Mus musculus	-141 (NRF2) -142 (BACH1)
4-hydroxyphenylpyruvate dioxygenase - binding site 1 - primer pair 2	hpd_1.2	Forward Reverse	GTCACCTCCCTCTGGGTCT ATTTACCACCTGGCAAGGCA	Mus musculus	-141 (NRF2)
Homogentisate 1, 2- dioxygenase – binding site 1 – primer pair 1	hgo_1.1	Forward Reverse	CACAGGAGACAGTGGCAGAG GACTCAGGTCAGGAAGGTGC	Mus musculus	-396 (NRF2) -394 (BACH1)
Homogentisate 1, 2- dioxygenase – binding site 1 – primer pair 2	hgo_1.2	Forward Reverse	GCACCTTCTGACCTGAGTC CTCCCCAGGAAGTGGTCTCT	Mus musculus	-396 (NRF2)
Glutathione transferase zeta 1 – binding site 1 – primer pair 1	gstz1_1.1	Forward Reverse	GAGGGAGGCCATCTAGTCCA AGCTCTTCTCCGCTTCTC	Mus musculus	27 (NRF2) 26 (BACH1)
Glutathione transferase zeta 1 – binding site 1 – primer pair 2	gstz1_1.2	Forward Reverse	AGGAGGGAGGCCATCTAGTC CTTCTCCGCTTCTCAGGA	Mus musculus	27 (NRF2)
Glutathione transferase zeta 1 – binding site 2 – primer pair 1	gstz1_2.1	Forward Reverse	AGTTTTGTGCGTTTGGCTCT CGACTTAAACCTGGACGCT	Mus musculus	-527 (NRF2)
Glutathione transferase zeta 1 – binding site 3 – primer pair 1	gstz1_3.1	Forward Reverse	GTCCTTTCGCCCACTTACCA ACTTAAACGGCTGCTTGGA	Mus musculus	563 (NRF2)
Fumarylacetoacetate Hydrolase – binding site 1- primer pair 1	fah_1.1	Forward Reverse	GGCACATCTAGGGAGGATGAG TTTCTTCTCGGGCGACCTCT	Mus musculus	-322 (NRF2) -321 (BACH1)
Fumarylacetoacetate Hydrolase – binding site 1- primer pair 2	fah_1.2	Forward Reverse	ATATCCAGACCGATGAGGCG CCCTGAGACCACCCGTTTT	Mus musculus	-322 (NRF2)

Table S6. List of commercial antibodies used for Western Blot analysis.

Antibody	Host Species	Supplier	Reference	Work dilution
COQ3	<i>Oryctolagus cuniculus</i>	Invitrogen	PA5-56699	1:1000
FAH	<i>Oryctolagus cuniculus</i>	Invitrogen	PA5-42049	1:1000
GAPDH	<i>Mus musculus</i>	Invitrogen	AM4300	1:5000
GSTZ1 (Human)	<i>Oryctolagus cuniculus</i>	Invitrogen	PA5-80678	1:1000
GSTZ1 (Mouse)	<i>Oryctolagus cuniculus</i>	Bioss	bs-13442R	1:1000
HAAO	<i>Oryctolagus cuniculus</i>	Invitrogen	PA5-20774	1:1000
HGO (Human)	<i>Mus musculus</i>	Invitrogen	MA5-49214	1:1000
HGO (Mouse)	<i>Oryctolagus cuniculus</i>	Invitrogen	PA5-79360	1:1000
HPD (Human)	<i>Mus musculus</i>	Proteintech	67552-1-Ig	1:1000
HPD (Mouse)	<i>Oryctolagus cuniculus</i>	Invitrogen	PA5-97838	1:1000
HPDL	<i>Oryctolagus cuniculus</i>	Invitrogen	PA5-898	1:1000
MIF	<i>Oryctolagus cuniculus</i>	Invitrogen	pa5-81446	1:1000
NAT2	<i>Oryctolagus cuniculus</i>	Invitrogen	PA5-110014	1:1000
TAT (Human)	<i>Oryctolagus cuniculus</i>	Invitrogen	PA5-26541	1:1000
TAT (Mouse)	<i>Oryctolagus cuniculus</i>	Invitrogen	PA5-80097	1:1000

LAMIN-β	<i>Oryctolagus cuniculus</i>	Invitrogen	702972	1:1000
ACT	<i>Mus musculus</i>	Sigma	A5441	1:5000

Table S7. Metabolites of HT-1 metabolism measured by LC-MS/MS.

Analyte	Abrv	ESI	References
L-Tyrosine	L-TYR	-	Merck, T3754
4-Hydroxymandelic acid	4-HMA	-	Cymit, h944355
Succinylacetone	SA	+	Merck, D1415

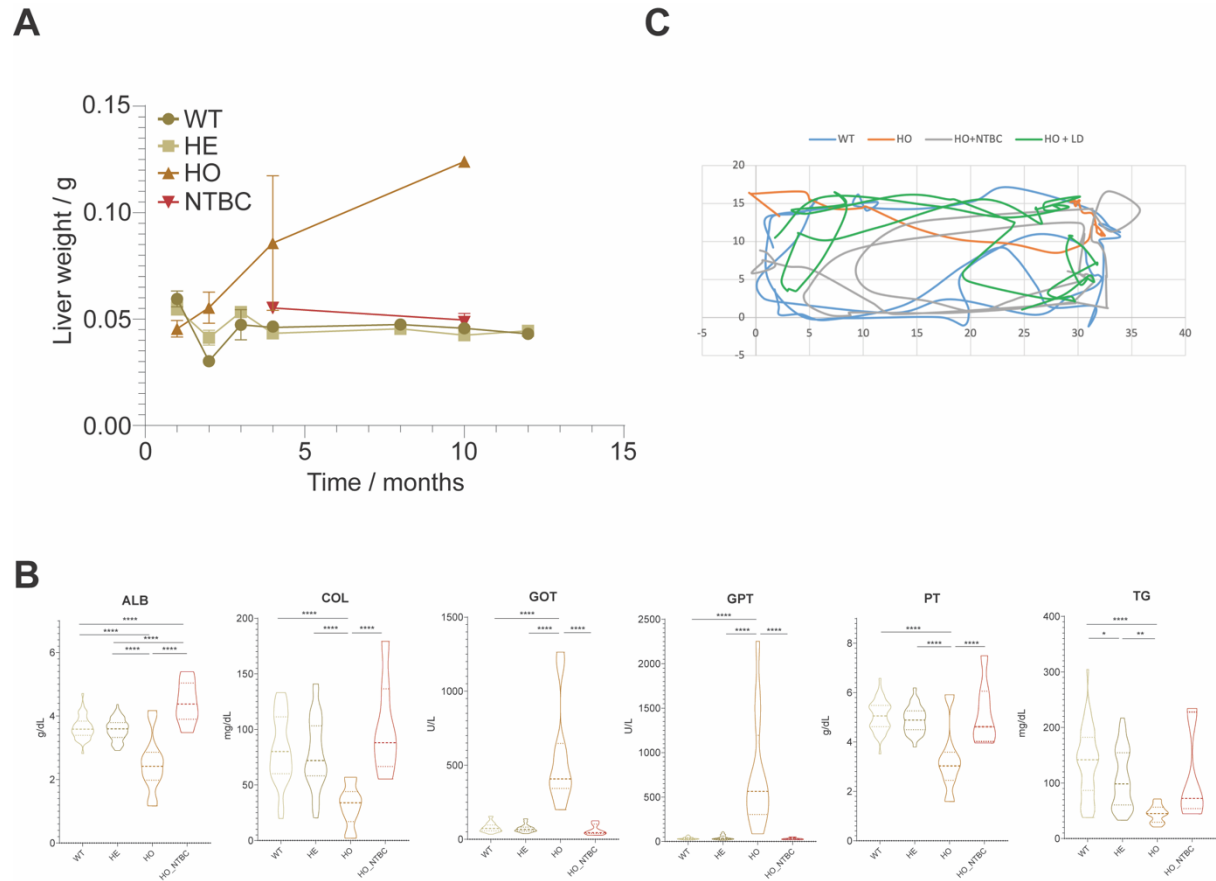


Figure S1. The G337S-FAH mouse model. **A)** Liver weight as a function of time colored according to the FAH genotype, as indicated in the legend. The error corresponds to the standard deviation obtained from 10 animals per group. **B)** Main biochemical parameters of the different mice under consideration for WT (n = 25), HE (n = 25), HO (n = 45) and NTBC-treated HO (n = 45). **C)** mobility plot of mice during 60 s, colored according to the FAH genotype, as indicated in the legend. ALB: albumin; COL: total cholesterol; GOT: glutamic-oxalacetic transaminase; GPT: glutamate-pyruvate transaminase; PT: prothrombin; TG: triglycerides. Legend for p-values: * < 0.05; ** < 0.01; **** < 0.0001.

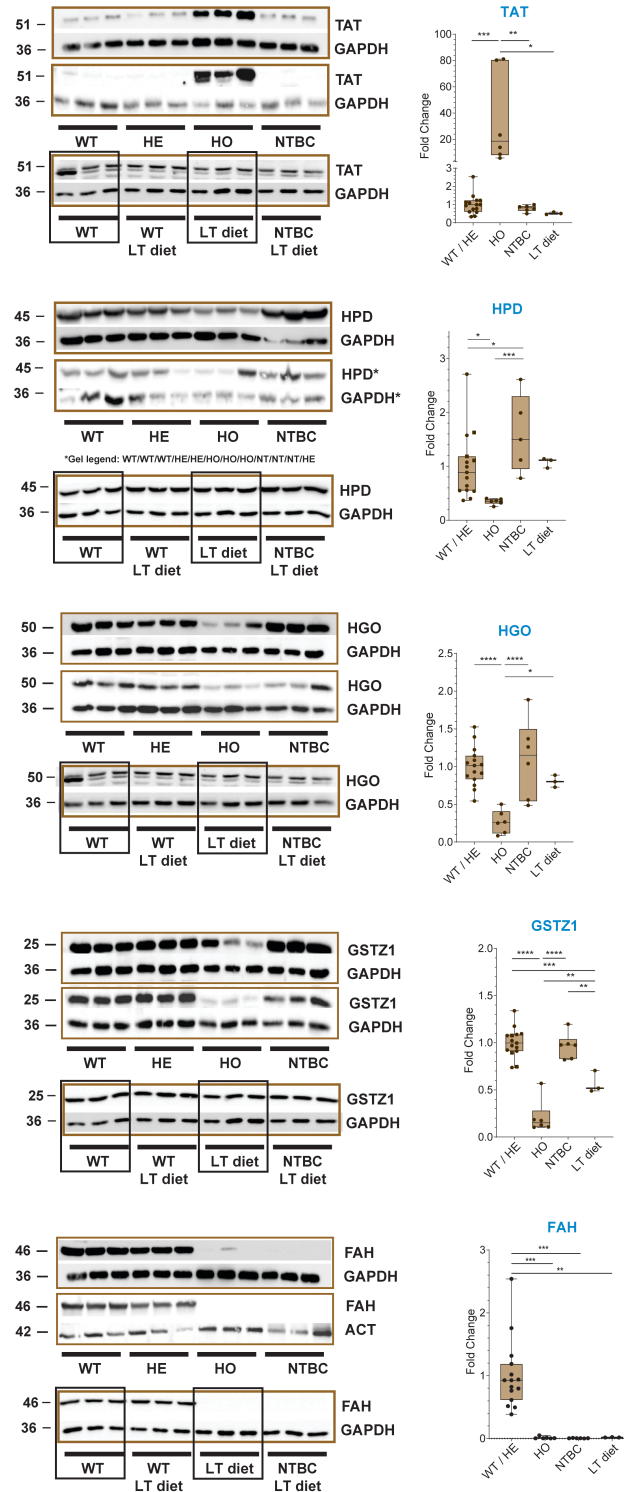


Figure S2. WB of the *LTYM* pathway in liver. Western blot strips for the specific enzymes and their corresponding housekeeping proteins, used to quantify the protein levels (bar plots). The specific antibodies used are described in Table S6. The different organs under consideration are color coded, as indicated in the legend. Legend: TAT, L-TYR aminotrasferase; HPD, 4-hydroxyphenylpyruvate dioxygenase; HGO, homogentisate 1,2-dioxygenase; GSTZ1, maleylacetoacetate isomerase; FAH, fumarylacetoacetate hydrolase; Legend for p-values: * < 0.05; ** < 0.01; *** < 0.001; **** < 0.0001.

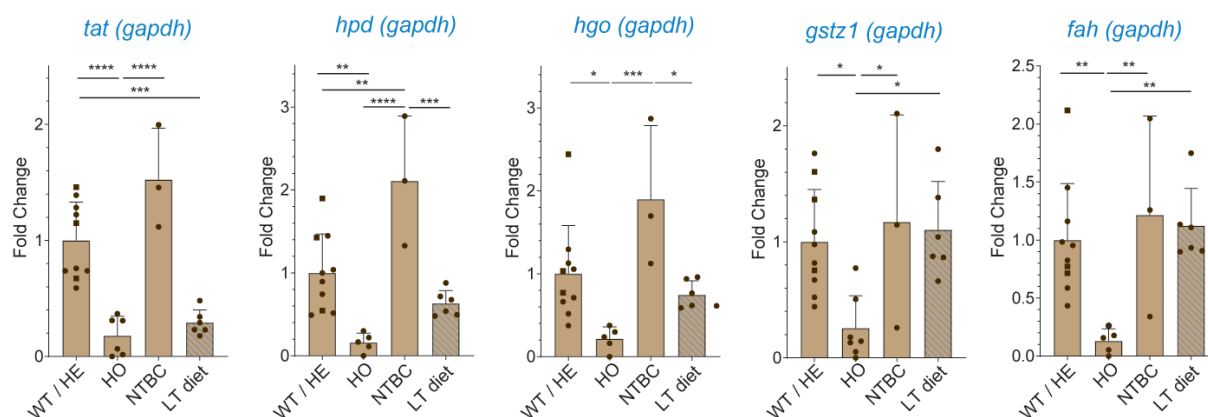


Figure S3. mRNA levels of the *LTYM* pathway in the liver. Fold changes of the mRNA for the specific enzymes as compared to their corresponding housekeeping genes (in brackets). The different organs under consideration are color coded, as indicated in the legend. Legend: fah, fumaryl acetoacetate hydrolase; gsta1, maleylacetoacetate isomerase; hgo, homogentisate 1,2-dioxygenase; hpd, 4-hydroxyphenylpyruvate dioxygenase; tat, L-TYR aminotransferase; gapdh, glyceraldehyde 3-phosphate dehydrogenase. Legend for p-values: * < 0.05; ** < 0.01; **** < 0.0001.

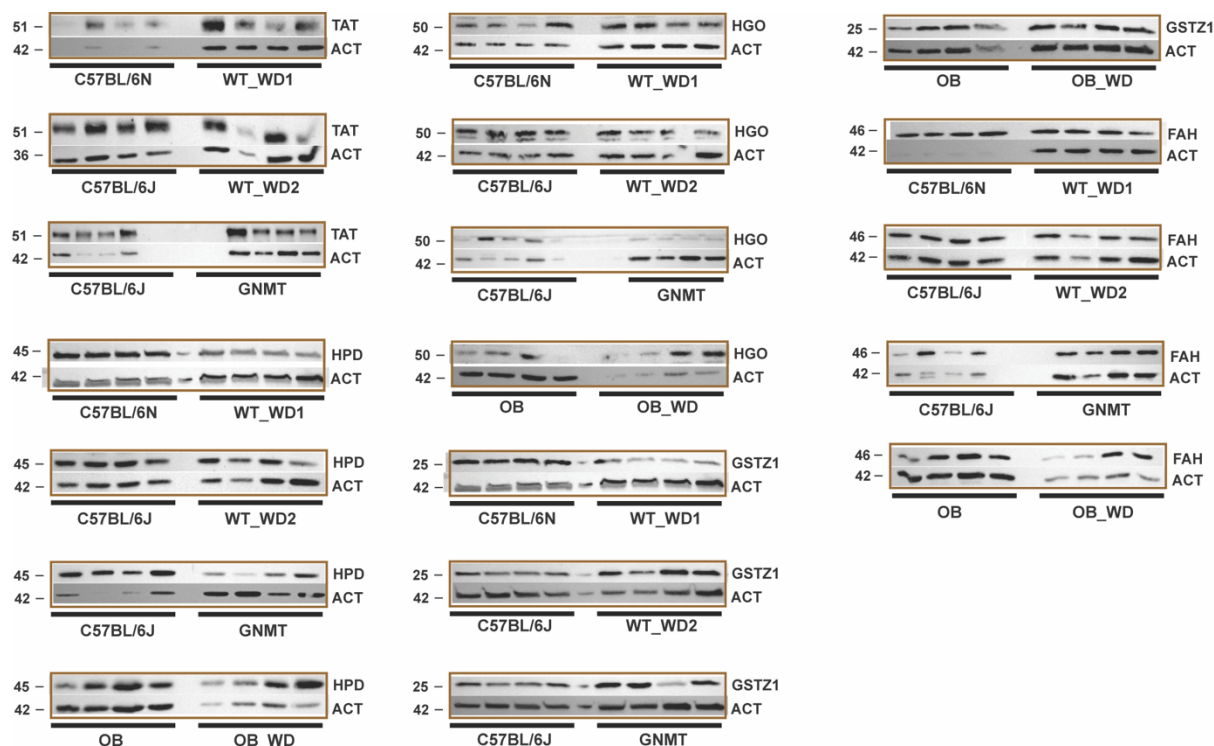


Figure S4. WB of the liver disease mouse models. Western blot strips for the specific enzymes and their corresponding housekeeping proteins, used to quantify the protein levels (bar plots). The specific antibodies used are described in Table S6. Legend: TAT, L-TYR aminotrasferase; HPD, 4-hydroxyphenylpyruvate dioxygenase; HGO, homogentisate 1,2-dioxygenase; GSTZ1, maleylacetoacetate isomerase; FAH, fumarylacetoacetate hydrolase.

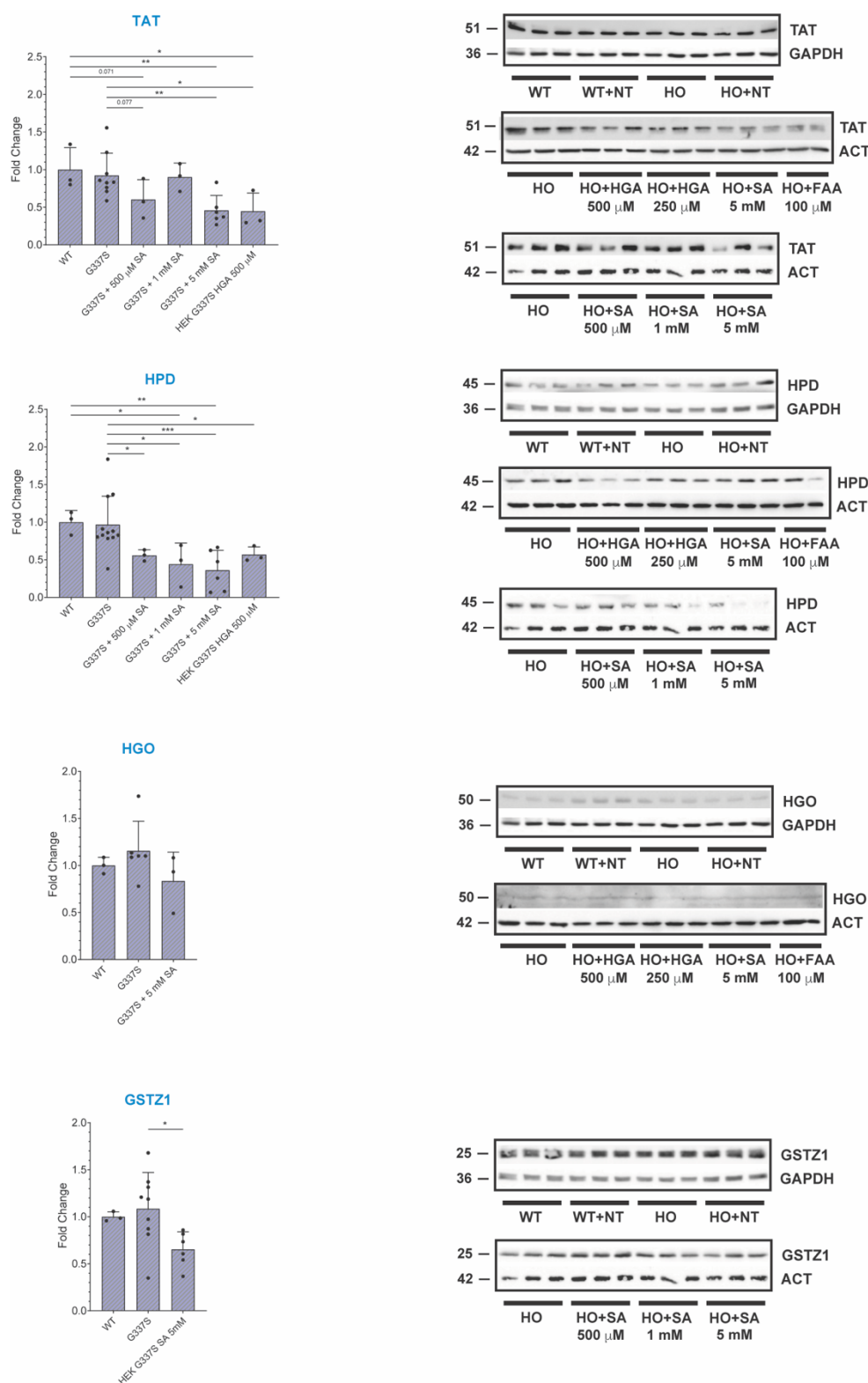
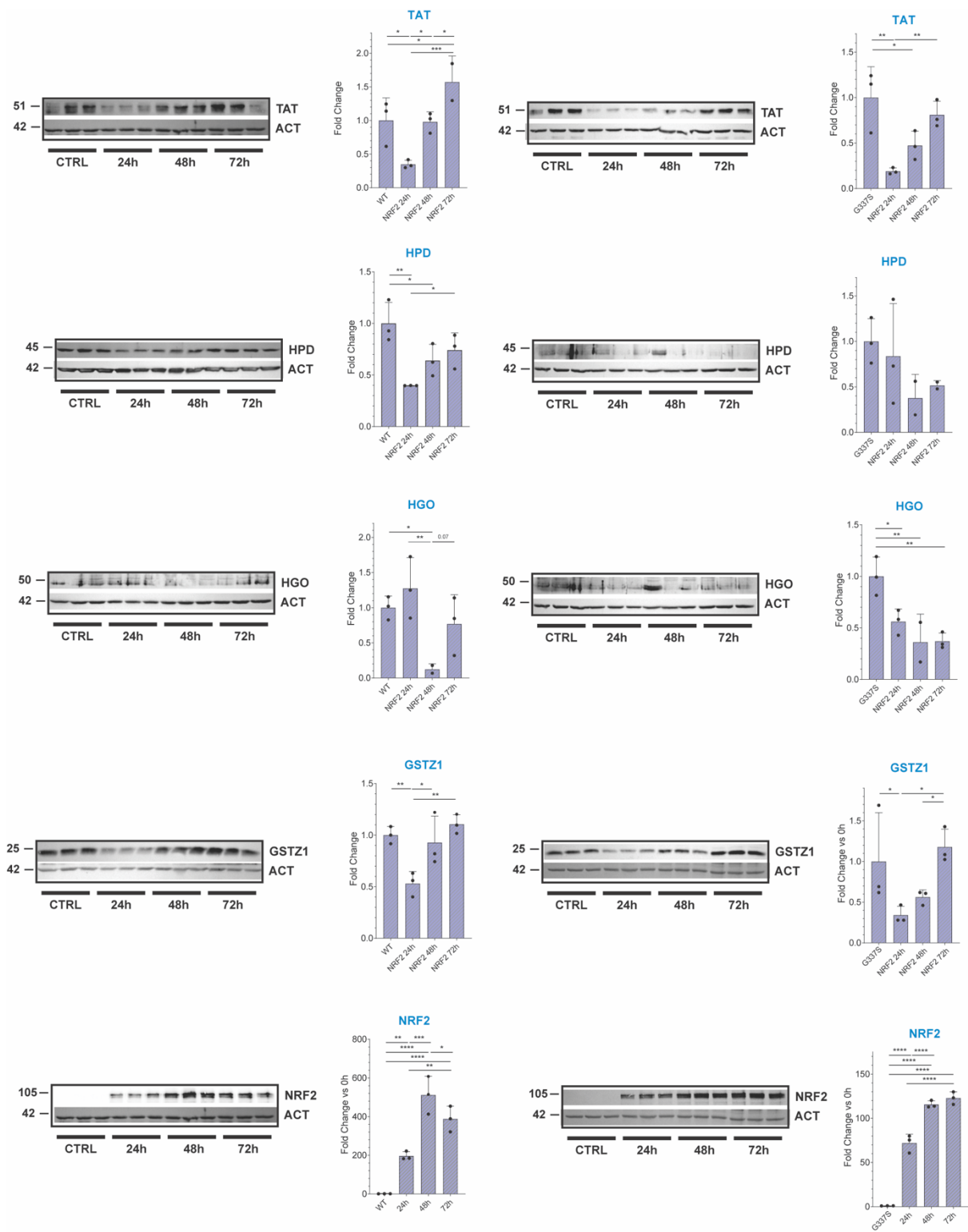


Figure S5. WB of HEK cells. Western blot strips for the HEK293 G337S specific enzymes and their corresponding housekeeping proteins, used to quantify the protein levels (barplots). The specific antibodies used are described in Table S6. Legend: TAT, L-TYR aminotransferase; HPD, 4-hydroxyphenylpyruvate dioxygenase; HGO, homogentisate 1,2-dioxygenase; GSTZ1, maleylacetoacetate isomerase; HGA homogentisic acid. Legend for p-values: * < 0.05; ** < 0.01; *** < 0.001; **** < 0.0001.



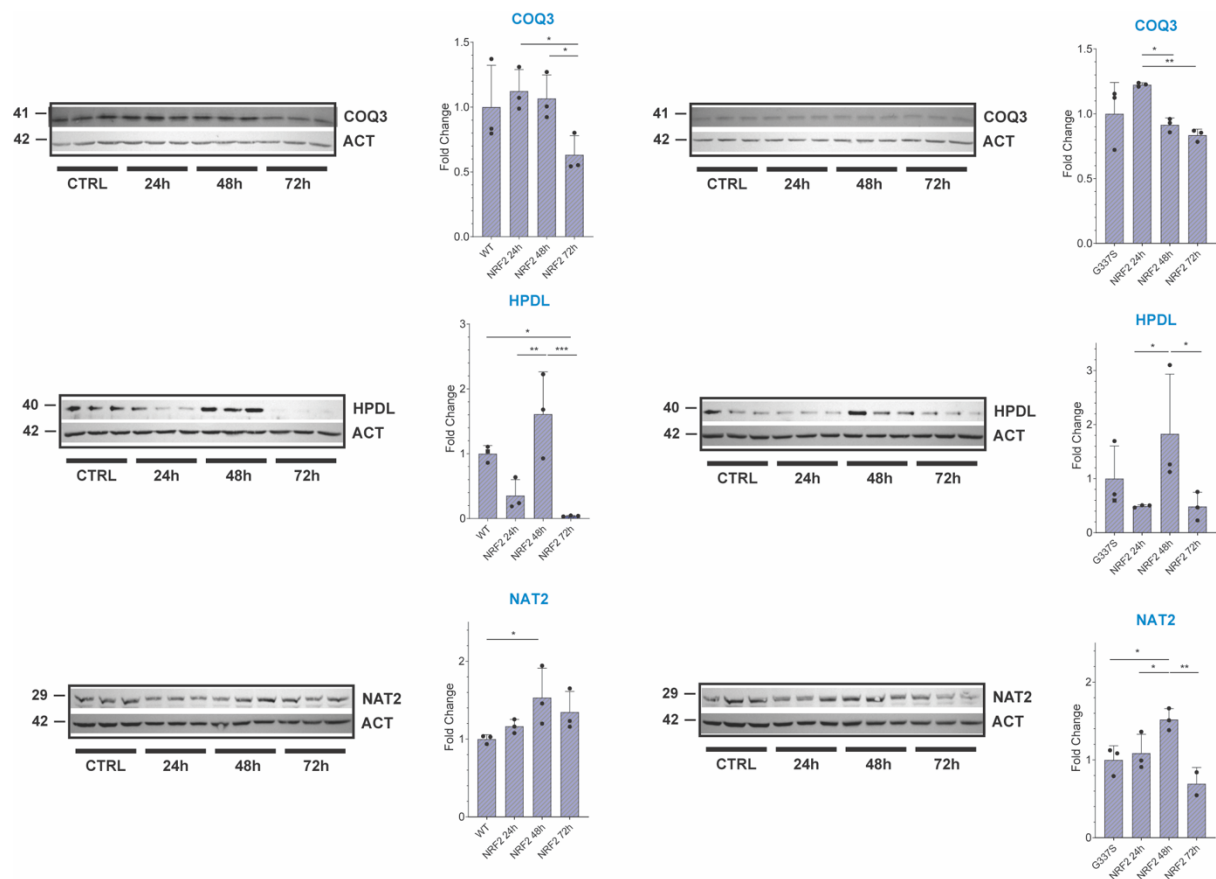


Figure S6. WB of HEK cells (II). Western blot strips for the specific enzymes and their corresponding housekeeping proteins, used to quantify the protein levels (bar plots). The specific antibodies used are described in Table S6. The different organs under consideration are color coded, as indicated in the legend. Legend: TAT, L-TYR aminotransferase; HPD, 4-hydroxyphenylpyruvate dioxygenase; HGO, homogentisate 1,2-dioxygenase; GSTZ1, maleylacetoacetate isomerase; FAH, fumarylacetoacetate hydrolase; NAT2, N-acetyltransferase 2; HPDL, 4-hydroxyphenylpyruvate dioxygenase-like protein; COQ3, ubiquinone biosynthesis O-methyltransferase. Legend for p-values: * < 0.05; ** < 0.01; *** < 0.001; **** < 0.0001.

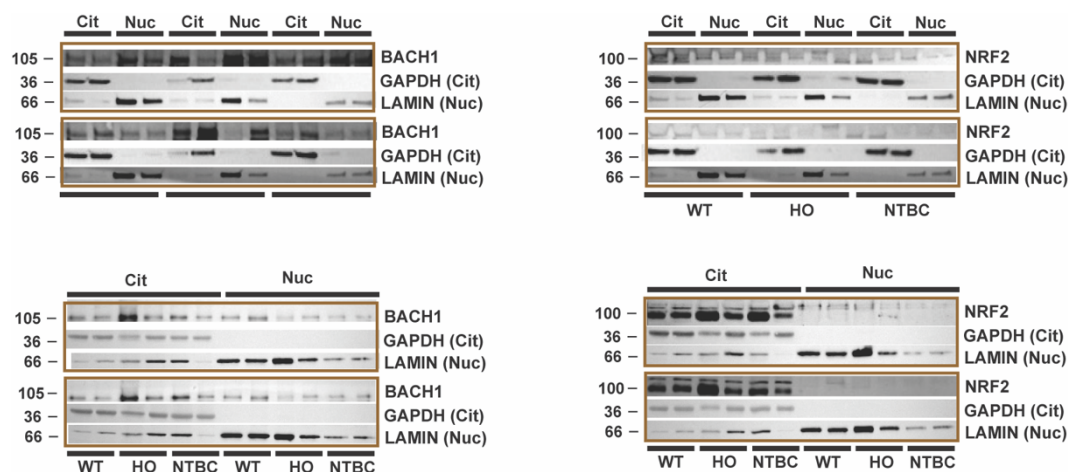


Figure S7. WB of NRF2 and BACH1. Western blot strips for the specific enzymes and their corresponding housekeeping proteins, separated by nucleus and cytosol and used to quantify the protein levels. The specific antibodies used are described in Table S6.

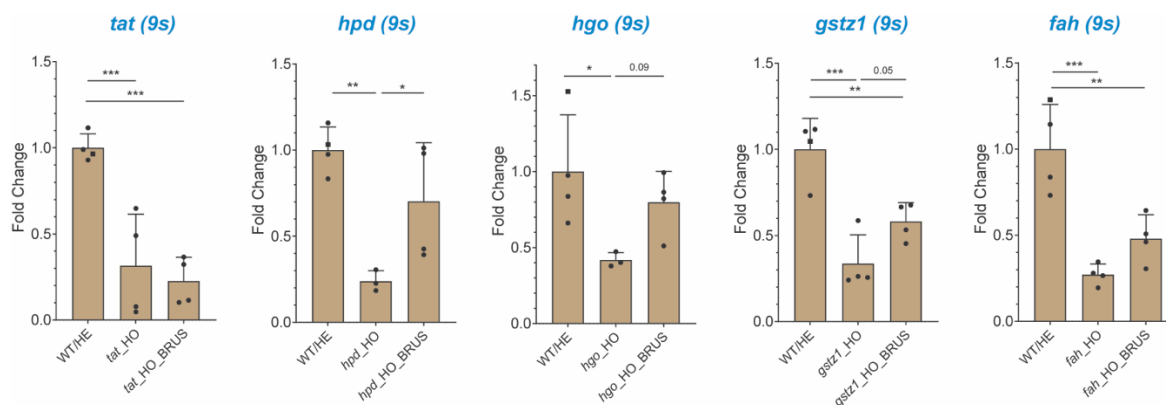


Figure S8. mRNA levels of brusatol treated mice. Fold changes of the mRNA for the specific enzymes as compared to their corresponding housekeeping genes (in brackets). The different organs under consideration are color coded, as indicated in the legend. Legend: fah, fumaryl acetoacetate hydrolase; gstz1, maleylacetoacetate isomerase; hgo, homogentisate 1,2-dioxygenase; hpd, 4-hydroxyphenylpyruvate dioxygenase; tat, L-TYR aminotransferase; 9s, ribosomal protein. Legend for p-values: * < 0.05; ** < 0.01; *** < 0.001; **** < 0.0001.

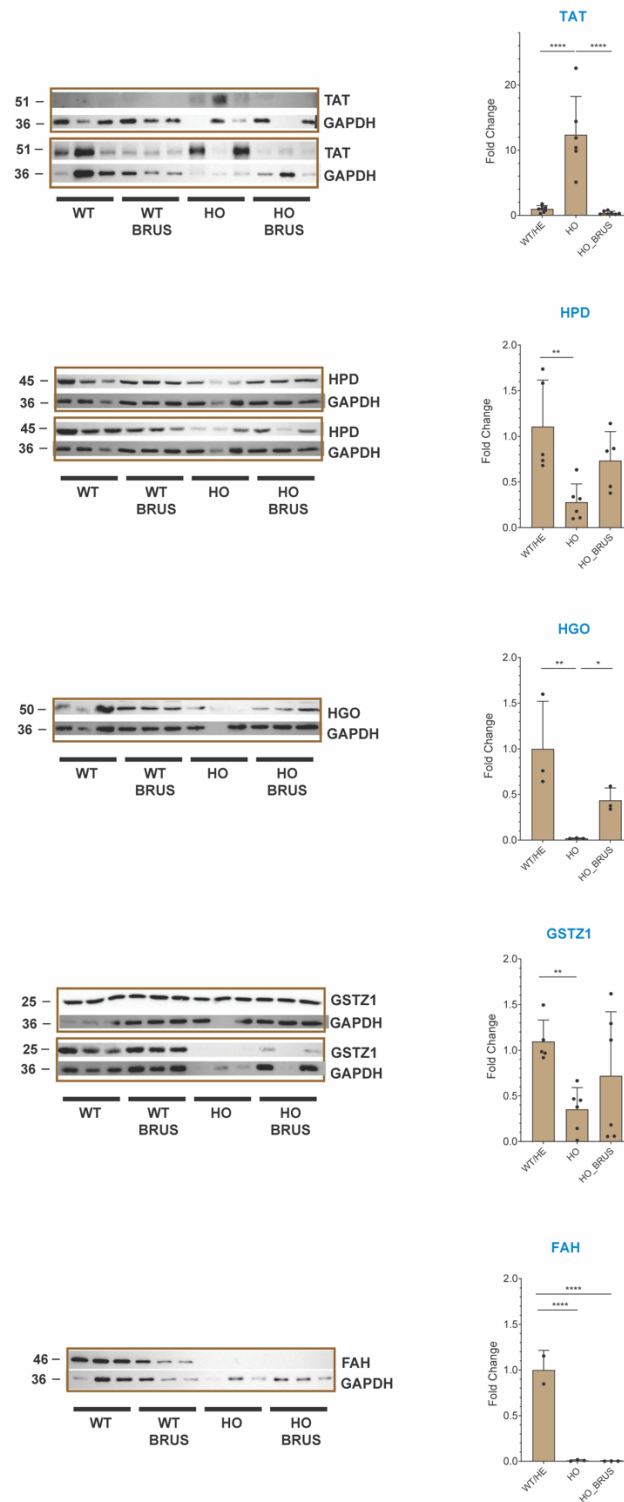


Figure S9. WB of brusatol treated mice. Western blot strips for the specific enzymes and their corresponding housekeeping proteins, used to quantify the protein levels (bar plots). The specific antibodies used are described in Table S6. The different organs under consideration are color coded, as indicated in the legend. Legend: TAT, L-TYR aminotrasferase; HPD, 4-hydroxyphenylpyruvate dioxygenase; HGO, homogentisate 1,2-dioxygenase; GSTZ1, maleylacetoacetate isomerase; FAH, fumarylacetoacetate hydrolase. Legend for p-values: * < 0.05; ** < 0.01; *** < 0.001; **** < 0.0001.