

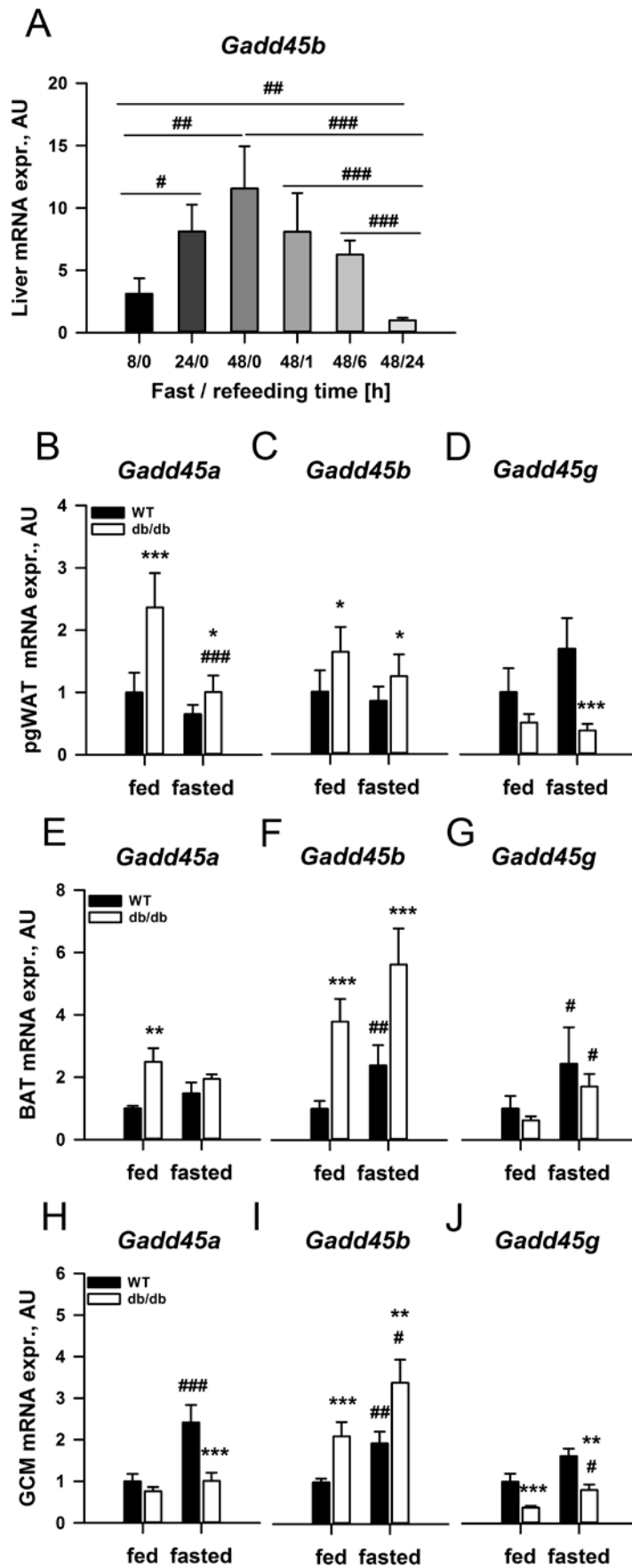
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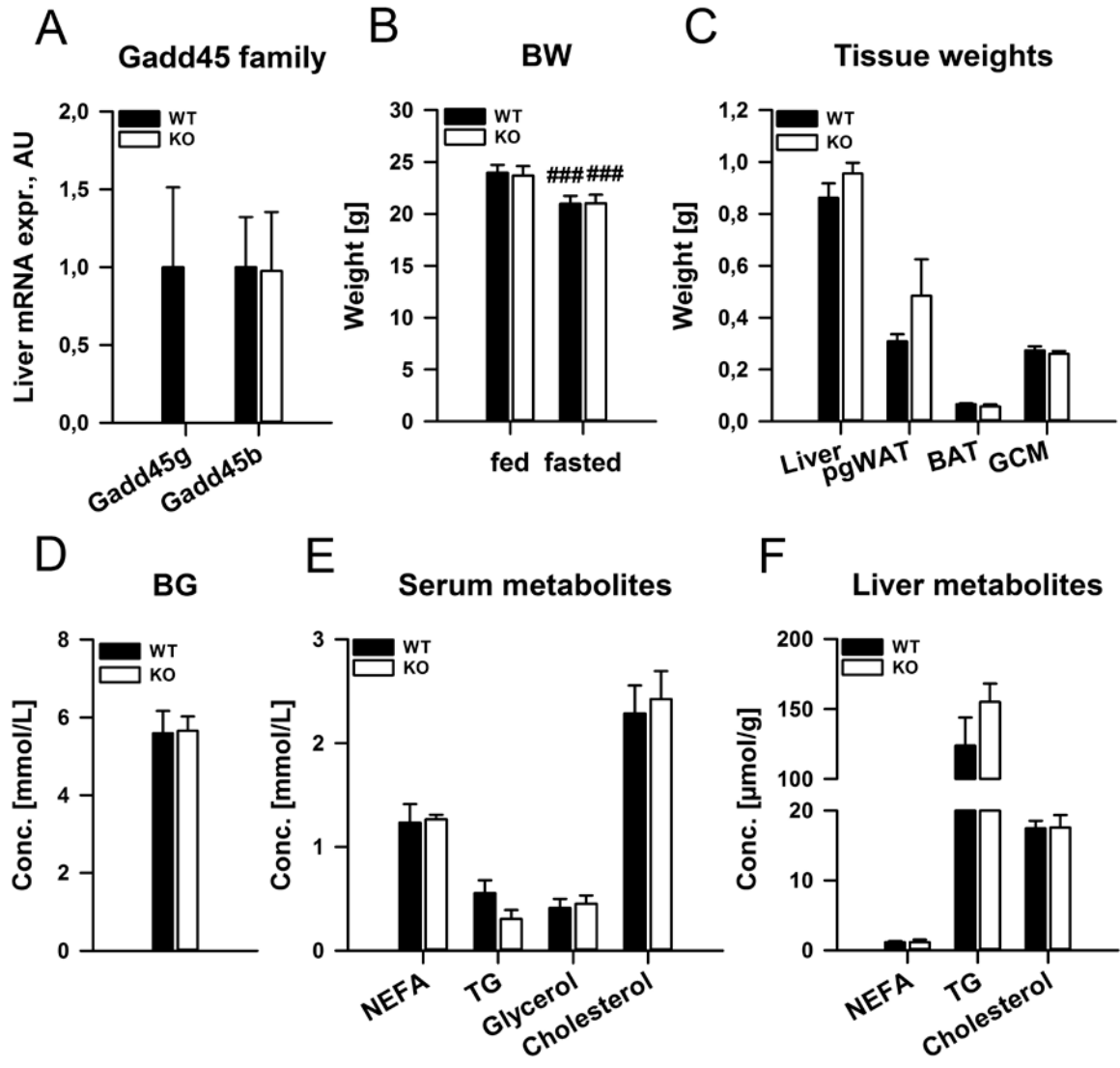
Appendix Figure S2

Appendix Figure S3

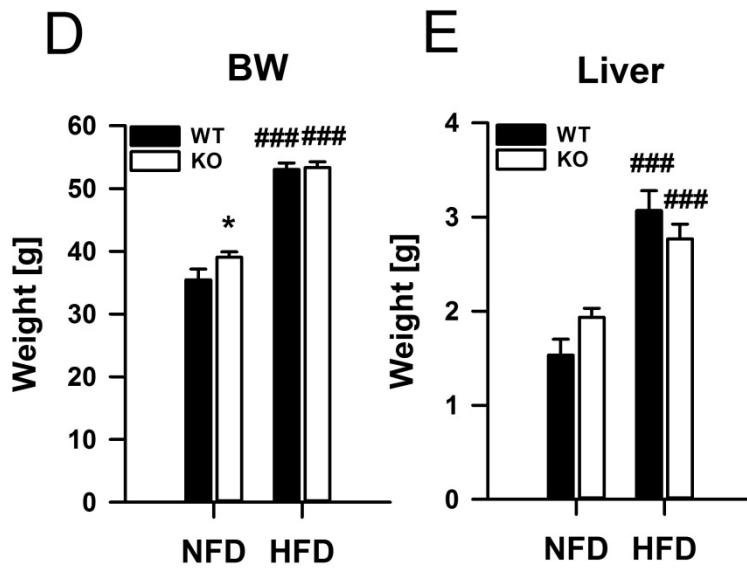
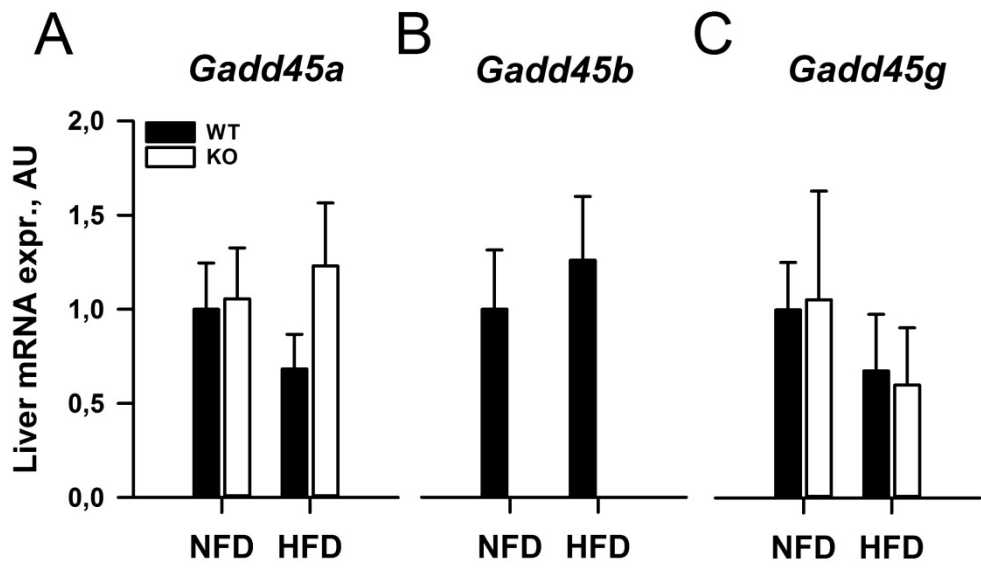
Appendix Table 1



**Figure S1. Liver hepatocyte GADD45b expression is consistently dysregulated upon starvation in several mouse models of metabolic dysfunction.** Male, 12wk old C57Bl/6J mice were fasted for 8, 24, or 48h or fasted for 48h and subsequently refed for 1, 6, or 24h. Liver *Gadd45b* mRNA expression was measured (A) (n = 4/group). Male, 12wk wildtype (WT; C57bl/6J) or obese/diabetic (*db/db*; BKS.Cg-m+/+ Lepr DB/J) were fed *ad libitum* (fed) or fasted for 24h (fasted) (n=4/group). mRNA expression of Growth Arrest and DNA Damage inducible 45 alpha (*Gadd45a*; B, E, H), beta (*Gadd45b*; C, F I) and gamma (*Gadd45g*; D, G, J) was measured from perigonadal white adipose (B-D), intrascapular brown adipose (E-G) and gastrocnemius complex muscle (H-J). Data are mean  $\pm$  SEM. Effect of genotype/age/fraction, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001. Effect of nutritional state: # p < 0.05, ## p < 0.01, ### p < 0.001. The statistical test used and respective p-value outputs can be found in Appendix Table S1.



**Figure S2. Systemic GADD45 $\gamma$  deletion does not affect metabolic homeostasis during fasting.** Female, GADD45 $\gamma$ <sup>+/+</sup> (WT) or GADD45 $\gamma$ <sup>-/-</sup> (KO) were fasted for 24h (fasted) (n = 5/group) with blood samples taken before and during fasting. Liver mRNA expression of *Gadd45* family members (A). Body weight (B) before and after fasting and fasted tissue weights (C) including perigonadal white adipose, brown adipose and gastrocnemius complex muscle tissues. Blood glucose (D) as well as serum (E) and liver (F) metabolite concentrations, including non-esterified fatty acids, triglycerides, glycerol and cholesterol. Data are mean  $\pm$  SEM. Effect of genotype, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001. Effect of nutritional state: # p < 0.05, ## p < 0.01, ### p < 0.001. The statistical test used and respective p-value outputs can be found in Appendix Table S1.



**Figure S3. Effects of chronic dietary treatment on metabolic homeostasis in mice with systemic GADD45 $\beta$  deletion.** GADD45 $\beta$   $+/+$  (WT) or GADD45 $\beta$   $-/-$  (KO) were chronically fed a normal- (NFD) or high (HFD) fat diet (n= 6-9/group) and sacrificed in the *ad libitum* fed state. Liver mRNA expression of *Gadd45* family members (A-C). Body weight (D), and liver mass (F). Data are mean  $\pm$  SEM. Effect of genotype, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ . Effect of nutritional state: #  $p < 0.05$ , ##  $p < 0.01$ , ###  $p < 0.001$ . The statistical test used and respective p-value outputs can be found in Appendix Table S1.

Appendix Table S1. P values from statistical tests. NP: not performed.

Figure 1	Main effects			Interactions				Post-hoc testing				test performed
	nutrition	Genotype/strain/age		fed		fasted		wt/NZB/young		db/NZO/aged		
				wt/NZB/ young vs db/NZO/aged	wt/NZB/ young vs db/NZO/aged	wt/NZB/ young vs db/NZO/aged	wt/NZB/ young vs db/NZO/aged	fed vs fasted	fed vs fasted	wt/NZB/ young vs db/NZO/aged	wt/NZB/ young vs db/NZO/aged	
A	0.004	0.043	0.033	0.233	0.033	0.014	0.031					
B	0.158	0.243	0.012	0.042	0.016	0.038	0.025					
C	>0.001	0.078	0.046	0.672	0.043	>0.001	>0.001					
D	0.077	0.011	0.003	0.088	>0.001	>0.001	0.342					
E	0.006	0.044	>0.001	0.279	>0.001	>0.001	0.149					
F	0.047	0.027	0.044	0.389	0.033	0.023	0.231					
G	0.090	0.113	0.009	0.044	0.028	0.031	0.428					
H	>0.001	0.222	0.031	0.385	0.024	>0.001	0.008					
I	0.088	0.197	0.027	0.179	0.088	0.009	0.527					
J	0.012	0.333	0.021	0.428	>0.001	>0.001	0.200					
K	0.009	0.198	0.012	0.371	>0.001	>0.001	>0.001					
L	0.021	0.033	0.028	>0.001	>0.001	0.008	0.006					
M	>0.001	0.081	0.009	0.287	>0.001	>0.001	>0.001					
N	0.031	0.019	0.003	0.471	>0.001	0.006	0.197					
O	0.002	0.208	0.019	0.309	>0.001	>0.001	0.06					
P	0.005	0.081	0.044	0.618	0.007	0.021	0.009					
Q	0.002	0.194	0.049	0.777	0.418	0.033	0.028					
R	0.018	0.090	0.037	0.447	>0.001	0.088	0.006					
	NEFA	Genotype		BSA	BSA-NEFA	wt	db/db					
				wt vs db/db	wt vs db/db	BSA vs NEFA	BSA vs NEFA					
S	0.033	0.011	0.032	0.009	>0.001	0.038	0.008	Two-way ANOVA, Holm-Sidak post-hoc test				

Figure EV1	nutrition	Genotype/strain/age		fed		fasted		wt/NZB/young		db/NZO/aged		test performed
				wt/NZB/ young vs db/NZO/aged		wt/NZB/ young vs db/NZO/aged		fed vs fasted		fed vs fasted		
A	0.001	0.005	0.008	0.011	0.023	0.021	0.018	Two-way ANOVA, Holm-Sidak post-hoc test				
B	0.009	0.222	0.014	0.398	0.121	>0.001	>0.001	Two-way ANOVA, Holm-Sidak post-hoc test				
C	0.030	0.281	0.039	0.444	0.319	0.007	0.018	Two-way ANOVA, Holm-Sidak post-hoc test				
D	0.039	0.315	0.049	0.199	0.551	0.051	0.009	Two-way ANOVA, Holm-Sidak post-hoc test				
E	0.009	0.008	0.007	0.040	0.044	0.033	0.028	Two-way ANOVA, Holm-Sidak post-hoc test				
F	0.033	0.071	0.088	NP	NP	NP	NP	Two-way ANOVA, Holm-Sidak post-hoc test				
G	0.055	0.071	0.033	0.388	0.021	0.081	0.050	Two-way ANOVA, Holm-Sidak post-hoc test				
H	0.221	0.038	0.111	NP	NP	NP	NP	Two-way ANOVA, Holm-Sidak post-hoc test				
I	0.002	0.091	0.021	0.424	>0.001	>0.001	0.033	Two-way ANOVA, Holm-Sidak post-hoc test				
J	0.379	0.559	0.394	NP	NP	NP	NP	Two-way ANOVA, Holm-Sidak post-hoc test				
K	0.021	0.189	0.172	NP	NP	NP	NP	Two-way ANOVA, Holm-Sidak post-hoc test				
L	0.465	0.444	0.451	NP	NP	NP	NP	Two-way ANOVA, Holm-Sidak post-hoc test				

Figure 2	nutrition	Genotype/strain/age		fed		fasted		wt/NZB/young		db/NZO/aged		test performed
				wt/NZB/ young vs db/NZO/aged		wt/NZB/ young vs db/NZO/aged		fed vs fasted		fed vs fasted		
A	0.033	0.066	0.031	0.111	>0.001	0.008	0.189	Two-way ANOVA, Holm-Sidak post-hoc test				
B	0.003	0.004	0.008	0.089	>0.001	>0.001	0.009	Two-way ANOVA, Holm-Sidak post-hoc test				
C	0.069	0.077	0.002	0.491	>0.001	>0.001	0.321	Two-way ANOVA, Holm-Sidak post-hoc test				
D	0.444	0.111	0.044	0.099	0.048	0.122	0.366	Two-way ANOVA, Holm-Sidak post-hoc test				
E	0.033	0.008	0.021	0.088	0.033	0.007	0.086	Two-way ANOVA, Holm-Sidak post-hoc test				
F	0.005	0.199	0.033	0.077	0.336	0.021	0.009	Two-way ANOVA, Holm-Sidak post-hoc test				
G	>0.001	0.111	0.028	0.217	>0.001	>0.001	>0.001	Two-way ANOVA, Holm-Sidak post-hoc test				
H	0.033	0.041	0.015	0.039	>0.001	>0.001	0.199	Two-way ANOVA, Holm-Sidak post-hoc test				
I	0.044	0.056	0.027	0.035	0.009	>0.001	0.289	Two-way ANOVA, Holm-Sidak post-hoc test				
J	0.098	0.181	>0.001	0.389	>0.001	>0.001	0.225	Two-way ANOVA, Holm-Sidak post-hoc test				
K	0.153	0.210	>0.001	0.255	0.008	0.005	0.351	Two-way ANOVA, Holm-Sidak post-hoc test				
L	0.073	0.222	>0.001	0.400	0.007	0.006	0.492	Two-way ANOVA, Holm-Sidak post-hoc test				

Figure S1	8/0 vs 24/0			8/0 vs 48/0		8/0 vs 48/24		48/24 vs 48/0		48/24 vs 48/1		48/24 vs 48/6		test performed
A	0.039	0.007	0.002	>0.001	>0.001	>0.001	>0.001	One-way ANOVA, Holm-Sidak post-hoc test						
	nutrition	Genotype/strain/age		fed	fasted	wt/NZB/young	db/NZO/aged							
				wt/NZB/ young vs db/NZO/aged	wt/NZB/ young vs db/NZO/aged	fed vs fasted	fed vs fasted							
B	0.033	0.008	0.021	>0.001	0.038	0.222	>0.001	Two-way ANOVA, Holm-Sidak post-hoc test						
C	0.111	0.012	0.049	0.038	0.044	0.219	0.200	Two-way ANOVA, Holm-Sidak post-hoc test						
D	0.288	0.231	0.046	0.051	>0.001	0.191	0.099	Two-way ANOVA, Holm-Sidak post-hoc test						
E	0.121	0.037	0.039	0.009	0.318	0.298	0.194	Two-way ANOVA, Holm-Sidak post-hoc test						
F	0.030	>0.001	0.022	>0.001	>0.001	0.008	0.099	Two-way ANOVA, Holm-Sidak post-hoc test						
G	0.007	0.055	0.044	0.111	0.192	0.035	0.032	Two-way ANOVA, Holm-Sidak post-hoc test						
H	0.288	0.391	0.014	0.399	>0.001	>0.001	0.404	Two-way ANOVA, Holm-Sidak post-hoc test						
I	0.010	0.009	0.009	>0.001	0.008	0.007	0.020	Two-way ANOVA, Holm-Sidak post-hoc test						
J	0.066	0.004	0.017	>0.001	0.007	0.097	0.046	Two-way ANOVA, Holm-Sidak post-hoc test						



Figure 3										
	nutrition	genotype		pre-fast vs fast	pre-fast vs. refed	fast vs refed				
A	>0.001	0.398	0.021	>0.001	0.044	>0.001				Two-way RM ANOVA, Holm-Sidak post-hoc test
B	>0.001	0.289	0.012	>0.001	0.181	>0.001				Two-way RM ANOVA, Holm-Sidak post-hoc test
C	>0.001	0.455	0.007	>0.001	0.233	>0.001				Two-way RM ANOVA, Holm-Sidak post-hoc test
	nutrition	genotype		fed	fasted	genotype	genotype			
				genotype	genotype	fed vs fasted	fed vs fasted			
D	>0.001	0.033	0.044	0.321	0.008	>0.001	>0.001			Two-way ANOVA, Holm-Sidak post-hoc test
E	>0.001	0.358	0.007	0.243	0.198	>0.001	>0.001			Two-way ANOVA, Holm-Sidak post-hoc test
F	0.055	0.256	0.169	NP	NP	NP	NP			Two-way ANOVA, Holm-Sidak post-hoc test
G		0.009								
	time	genotype		0	90	180	270			
				genotype	genotype	genotype	genotype			
H	>0.001	0.007	>0.001	0.288	>0.001	0.098	0.213			Two-way RM ANOVA, Holm-Sidak post-hoc test
I	0.009	0.032	>0.001	0.555	0.008	>0.001	0.121			Two-way RM ANOVA, Holm-Sidak post-hoc test
J	>0.001	0.013	>0.001	0.423	>0.001	0.009	0.333			Two-way RM ANOVA, Holm-Sidak post-hoc test
	time	genotype-diet		0 (WT vs KO HFD)	20 (WT vs KO HFD)	40 (WT vs KO HFD)	60 (WT vs KO HFD)			
K	>0.001	0.041	0.023	0.099	0.008	0.021	0.056			Two-way RM ANOVA, Holm-Sidak post-hoc test
	nutrition	genotype		NFD	HFD	genotype	genotype			
				genotype	genotype	NFD vs HFD	NFD vs HFD			
L	>0.001	0.121	0.040	0.222	0.087	0.021	>0.001			Two-way ANOVA, Holm-Sidak post-hoc test
M	>0.001	0.044	0.012	0.121	0.032	0.008	>0.001			Two-way ANOVA, Holm-Sidak post-hoc test
N	>0.001	0.031	0.009	0.257	0.006	>0.001	>0.001			Two-way ANOVA, Holm-Sidak post-hoc test

Figure EV2										
	nutrition	genotype		pre-fast vs fast	pre-fast vs. refed	fast vs refed				
A	>0.001	0.350	0.011	>0.001	0.033	0.222				Two-way RM ANOVA, Holm-Sidak post-hoc test
B	>0.001	0.189	0.008	>0.001	0.027	0.005				Two-way RM ANOVA, Holm-Sidak post-hoc test
C	>0.001	0.675	0.023	0.007	0.009	0.229				Two-way RM ANOVA, Holm-Sidak post-hoc test
				Gadd45a	Gadd45b	Gadd45g				
D				0.448	>0.001	0.664				t-test
	nutrition	genotype		fed	fasted	WT	KO			
				WT vs KO	WT vs KO	fed vs fasted	fed vs fasted			
E	>0.001	0.385	0.012	0.444	0.398	>0.001	>0.001			Two-way ANOVA, Holm-Sidak post-hoc test
H	>0.001	0.251	0.009	0.210	0.206	>0.001	>0.001			Two-way ANOVA, Holm-Sidak post-hoc test
I	0.077	0.222	0.438	NP	NP	NP	NP			Two-way ANOVA, Holm-Sidak post-hoc test
				Liver	Fat	GCM				
F				0.329	0.287	0.401				t-test
				NEFA	Cholesterol	Glycogen				
G				0.201	0.363	0.191				t-test
				C0	C2	SCAC	MCAC	LCAC		
J				0.298	0.329	0.189	0.210	0.427		t-test

Figure S2										
	nutrition	genotype		Gadd45g	Gadd45b					
A				>0.001	0.456					t-test
	nutrition	genotype		fed	fasted	genotype	genotype			
				genotype	genotype	fed vs fasted	fed vs fasted			
B	>0.001	0.284	0.007	0.429	0.321	>0.001	>0.001			Two-way ANOVA, Holm-Sidak post-hoc test
				Liver	pgWAT	BAT	GCM			
C				0.222	0.198	0.267	0.335			t-test
				BG						
D				0.333						t-test
				NEFA	TG	Glycerol	Cholesterol			
E				0.222	0.397	0.442	0.597			t-test
				NEFA	TG	Cholesterol				
F				0.189	0.398	0.523				t-test

Figure S3										
	nutrition	genotype		NFD	HFD	genotype	genotype			
				genotype	genotype	NFD vs HFD	NFD vs HFD			
A	0.345	0.231	0.667	NP	NP	NP	NP			Two-way ANOVA, Holm-Sidak post-hoc test
B	0.287	>0.001	>0.001	>0.001	>0.001	0.324	0.920			Two-way ANOVA, Holm-Sidak post-hoc test
C	0.138	0.409	0.567	NP	NP	NP	NP			Two-way ANOVA, Holm-Sidak post-hoc test
D	>0.001	0.125	>0.001	0.033	0.230	>0.001	>0.001			Two-way ANOVA, Holm-Sidak post-hoc test
E	>0.001	0.238	>0.001	0.099	0.239	>0.001	>0.001			Two-way ANOVA, Holm-Sidak post-hoc test

Figure 4									
	nutrition	genotype		fed/BSA WT vs KO	fasted/BSA-NEFA WT vs KO	WT fed vs fasted/BSA vs NEFA	KO fed vs fasted/BSA vs NEFA		
A	>0.001	0.087	>0.001	0.234	>0.001	0.022	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
B	>0.001	0.543	>0.001	0.309	0.290	>0.001	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
C	0.043	0.285	>0.001	0.444	>0.001	0.127	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
D	>0.001	0.105	>0.001	0.287	>0.001	>0.001	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
	nutrition	fraction		Hepas fasted vs fed	NP fasted vs fed	fed Hepas vs NP	fed Hepas vs NP		
E	>0.001	0.106	0.025	0.030	0.179	0.088	0.197		Two-way ANOVA, Holm-Sidak post-hoc test
	nutrition	miR		fed NC miR vs G45b miR	fasted NC miR vs G45b miR	NC miR fed vs fasted	G45b miR fed vs fasted		
F	>0.001	0.174	>0.001	0.418	0.026	>0.001	0.005		Two-way ANOVA, Holm-Sidak post-hoc test
G	>0.001	0.201	>0.001	0.317	0.011	0.007	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
	AD	genotype		NC WT vs KO	G45b OE WT vs KO	WT NC vs G45b	KO NC vs G45b		
H	0.031	0.055	0.012	0.068	0.266	0.087	0.008		Two-way ANOVA, Holm-Sidak post-hoc test
	nutrition	miR		NFD NC miR vs G45b miR	HFD NC miR vs G45b miR	NC miR NFD vs HFD	G45b miR NFD vs HFD		
I	>0.001	0.197	0.023	0.314	0.451	0.005	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
J	>0.001	0.182	>0.001	0.202	0.028	>0.001	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
K	>0.001	0.213	>0.001	0.438	0.024	>0.001	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test

Figure EV3									
	nutrition	fraction		Hepas fasted vs fed	NP fasted vs fed	fed Hepas vs NP	fed Hepas vs NP		
A	0.444	0.014	0.105	NP	NP	NP	NP		Two-way ANOVA, Holm-Sidak post-hoc test
B	0.600	>0.001	0.104	NP	NP	NP	NP		Two-way ANOVA, Holm-Sidak post-hoc test
C	0.537	>0.001	0.283	NP	NP	NP	NP		Two-way ANOVA, Holm-Sidak post-hoc test
D	0.329	>0.001	0.406	NP	NP	NP	NP		Two-way ANOVA, Holm-Sidak post-hoc test
	nutrition	miR		fed NC miR vs G45b miR	fasted NC miR vs G45b miR	NC miR fed vs fasted	G45b miR fed vs fasted		
E	>0.001	>0.001	>0.001	0.003	>0.001	>0.001	0.037		Two-way ANOVA, Holm-Sidak post-hoc test
	AD	genotype		NC WT vs KO	G45b OE WT vs KO	WT NC vs G45b	KO NC vs G45b		
F	>0.001	0.105	>0.001	>0.001	0.211	>0.001	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
G	0.229	0.381	0.449	NP	NP	NP	NP		Two-way ANOVA, Holm-Sidak post-hoc test
H	0.294	0.087	0.009	0.223	>0.001	0.276	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
	nutrition	miR		NFD NC miR vs G45b miR	HFD NC miR vs G45b miR	NC miR NFD vs HFD	G45b miR NFD vs HFD		
I	>0.001	0.041	>0.001	0.319	0.033	>0.001	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
J	>0.001	0.143	>0.001	0.252	0.128	>0.001	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
K	>0.001	0.261	>0.001	0.278	0.124	>0.001	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test

Figure 5									
	AD	genotype		NC WT vs db/db	G45b OE WT vs db/db	WT NC vs G45b	db/db NC vs G45b		
A	0.030	>0.001	>0.001	>0.001	0.038	0.081	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
B	0.201	>0.001	>0.001	>0.001	0.061	0.286	0.028		Two-way ANOVA, Holm-Sidak post-hoc test
C	0.044	>0.001	>0.001	>0.001	0.092	0.099	>0.001		Two-way ANOVA, Holm-Sidak post-hoc test
D		NGT vs T2D							Mann-Whitney Rank Sum Test

Figure EV4									
	AD	genotype		NC WT vs db/db	G45b OE WT vs db/db	WT NC vs G45b	db/db NC vs G45b		
A	0.005	0.150	0.065	NP	NP	NP	NP		Two-way ANOVA, Holm-Sidak post-hoc test
B	0.122	>0.001	>0.001	>0.001	>0.001	0.124	0.048		Two-way ANOVA, Holm-Sidak post-hoc test
C	0.228	0.356	0.437	NP	NP	NP	NP		Two-way ANOVA, Holm-Sidak post-hoc test
D	0.264	>0.001	0.008	>0.001	>0.001	0.187	0.235		Two-way ANOVA, Holm-Sidak post-hoc test

