

Supplemental information

**Multimomics assessment
of dietary protein titration reveals
altered hepatic glucose utilization**

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	18%	14%	10%	6%	2%	0%
Component	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg
Research Diets D12450BSpx	405.3	405.3	405.3	405.3	405.3	405.3
Casein	89.0	69.2	49.4	29.7	9.9	0.0
Sucrose	0.0	19.8	39.5	59.3	79.1	89.0
Cystine	1.5	1.5	1.5	1.5	1.5	1.5
Agar	9.9	9.9	9.9	9.9	9.9	9.9
Water	494.3	494.3	494.3	494.3	494.3	494.3
Kcal/g	1.95	1.95	1.95	1.95	1.95	1.95
Percent energy protein	18	14	10	6	2	0
Percent energy carbohydrate	72	76	80	84	88	90
Percent energy fat	10	10	10	10	10	10

Supplemental table 1, related to Figure 1. Compositions of experimental diets.

	pct00 (N=6)	pct06 (N=5)	pct18 (N=6)	P-value
C Peptide				
Mean (SD)	240 (± 190)	1500 (± 460)	3400 (± 720)	<0.001
Ghrelin				
Mean (SD)	35 (± 2.9)	33 (± 2.5)	35 (± 3.2)	0.427
GIP				
Mean (SD)	410 (± 130)	600 (± 220)	580 (± 170)	0.177
GLP1				
Mean (SD)	44 (± 4.0)	47 (± 8.8)	48 (± 8.5)	0.625
IL6				
Mean (SD)	26 (± 2.5)	29 (± 4.1)	28 (± 3.9)	0.378
Glucagon				
Mean (SD)	96 (± 13)	98 (± 16)	110 (± 22)	0.224
Leptin				
Mean (SD)	15000 (± 2900)	20000 (± 1100)	21000 (± 880)	<0.001
MCP1				
Mean (SD)	37 (± 9.0)	38 (± 7.1)	42 (± 7.6)	0.601
PP				
Mean (SD)	75 (± 3.8)	70 (± 6.4)	76 (± 11)	0.398
PYY				
Mean (SD)	160 (± 18)	170 (± 26)	210 (± 35)	0.0196
TNF				
Mean (SD)	26 (± 3.7)	27 (± 3.4)	28 (± 3.4)	0.449
Amylin				
Mean (SD)	43 (± 5.2)	48 (± 3.7)	53 (± 8.9)	0.0586

Supplemental table 2, related to Figure 2: Serum Luminex parameter values of conventional donor mice in pg/mL with ANOVA omnibus P-value.

	pct00 (N=8)	pct06 (N=8)	pct18 (N=8)	P-value
C Peptide				
Mean (SD)	930 (± 330)	660 (± 250)	690 (± 210)	0.112
Ghrelin				
Mean (SD)	21 (± 4.9)	18 (± 1.1)	19 (± 6.0)	0.371
GIP				
Mean (SD)	400 (± 190)	360 (± 120)	420 (± 140)	0.736
GLP1				
Mean (SD)	43 (± 11)	38 (± 4.1)	36 (± 7.9)	0.195
IL6				
Mean (SD)	38 (± 30)	30 (± 7.0)	26 (± 8.1)	0.414
Glucagon				
Mean (SD)	63 (± 20)	49 (± 8.8)	43 (± 9.8)	0.0222
Leptin				
Mean (SD)	6900 (± 2600)	4900 (± 2000)	4100 (± 1700)	0.0443
MCP1				
Mean (SD)	48 (± 20)	40 (± 7.7)	39 (± 13)	0.435
PP				
Mean (SD)	49 (± 12)	41 (± 6.1)	42 (± 8.6)	0.232
PYY				
Mean (SD)	110 (± 52)	79 (± 27)	67 (± 14)	0.0409
TNF				
Mean (SD)	35 (± 5.4)	34 (± 8.5)	32 (± 10)	0.693
Amylin				
Mean (SD)	50 (± 15)	52 (± 7.8)	52 (± 15)	0.959

Supplemental table 3, related to Figure 2: Serum Luminex parameter values of gnotobiotic recipient mice in pg/mL with ANOVA omnibus P-value.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Mouse <i>Atf4</i> flox genotyping F: GCAGACGTTCTGGGTTAGA	(Ebert, Dyle et al. 2012)	N/A
Mouse <i>Atf4</i> flox genotyping R: GCTTCCTGCCTACATTGCTC	(Ebert, Dyle et al. 2012)	N/A
Mouse universal Cre genotyping F: GCCAGCTAAACATGCTTCATC	This paper	N/A
Mouse universal Cre genotyping R: ATTGCCCTGTTTCACTATCC	This paper	N/A
Mouse Cre control genotyping F: TTACGTCCATCGTGGACAGC	This paper	N/A
Mouse Cre control genotyping R: TGGGCTGGGTGTTAGCCTTA	This paper	N/A

Supplemental table 4, related to Key Resource Table: Genotyping primers for mouse strains used in this paper.

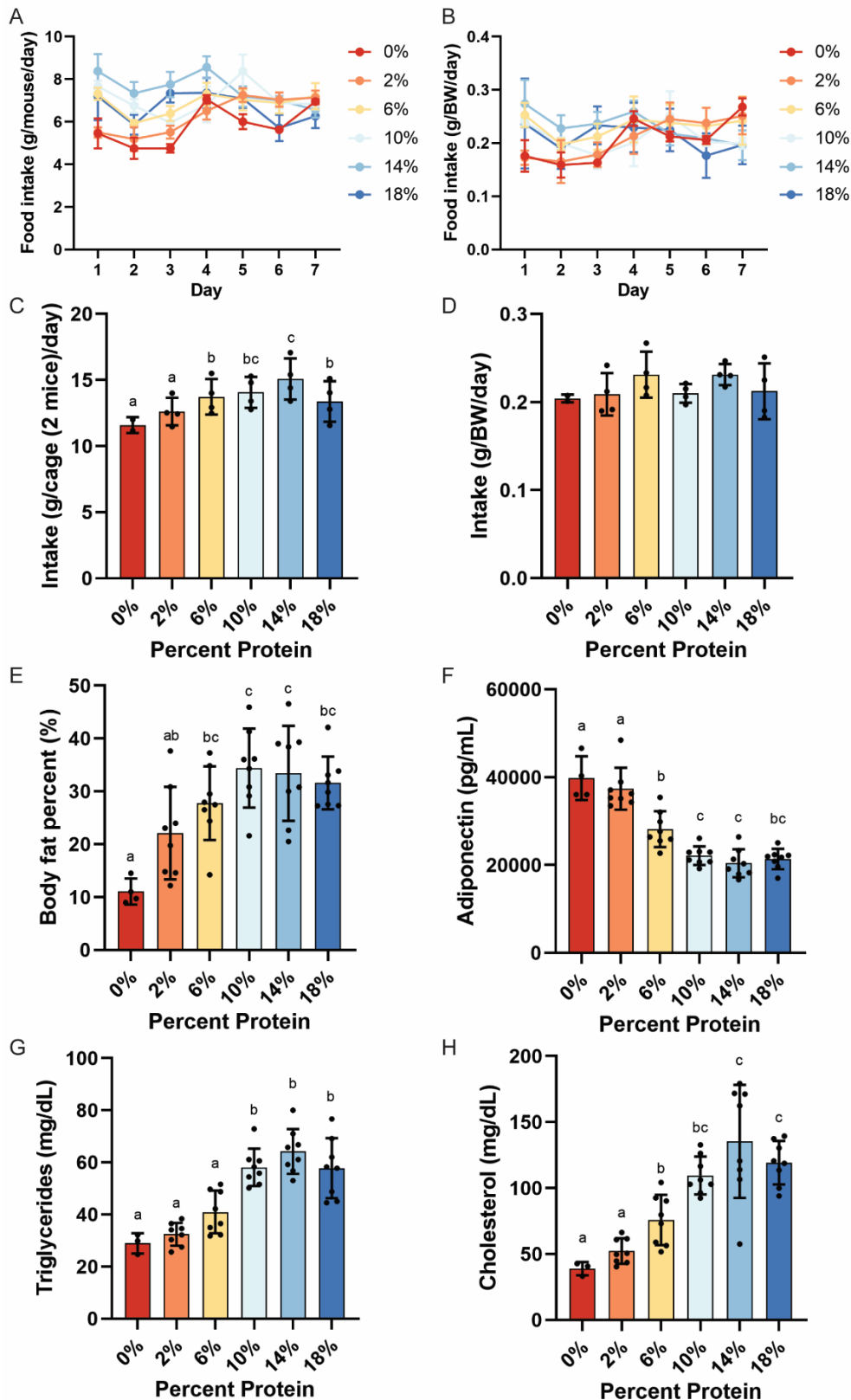


Figure S1. Phenotypic effects of dietary amino acid titration, related to Figure 1. Adult male B6D2F1 mice (4/group) were fed ad libitum for one week on an isocaloric semi-purified diet of the indicated protein calorie percentage. (A-D) Food intake per day (A,B) or daily average (C,D) expressed as gram/mouse (A,C) or corrected for body weight (B,D). (E) Percent body fat after one week on the indicated diet. (F-H) Values from blood serum of fasted mice after one week on the indicated diet of adiponectin (F), triglyceride (G) and cholesterol (H). Error bars are standard deviations, different letters indicate $p < 0.05$ in Tukey post-hoc test. Related to Figure 1.

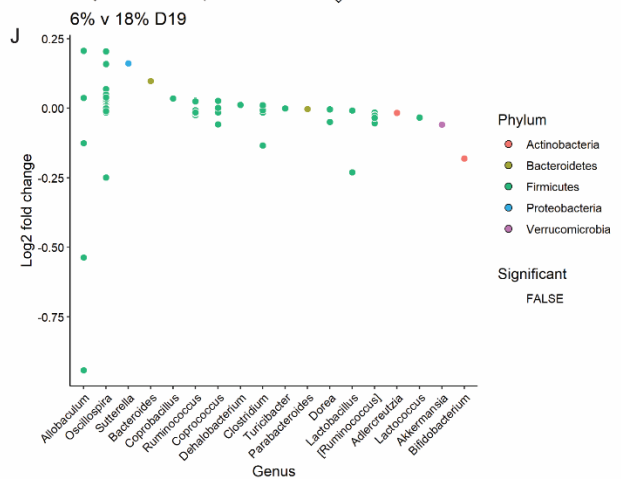
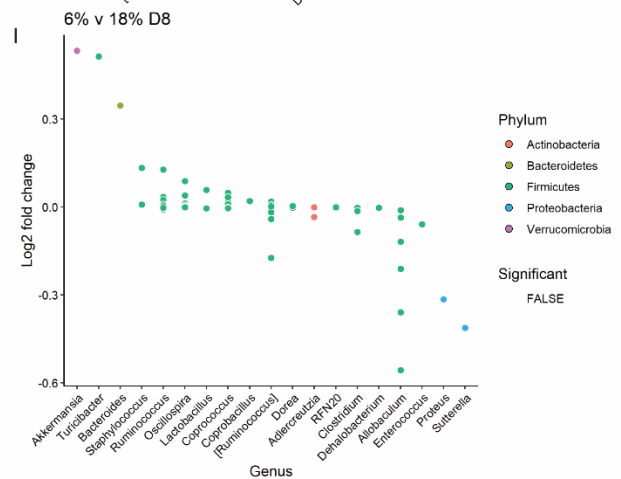
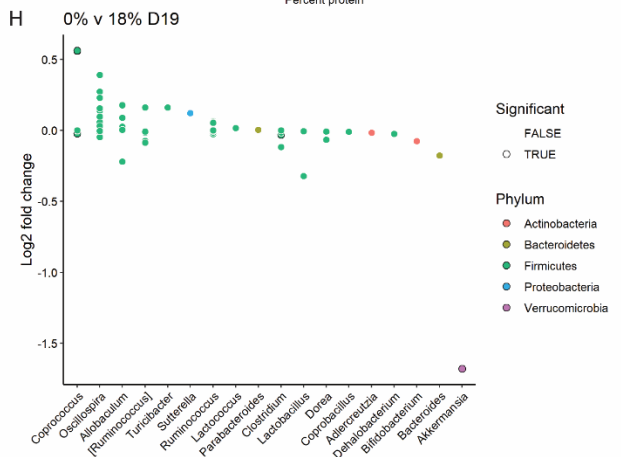
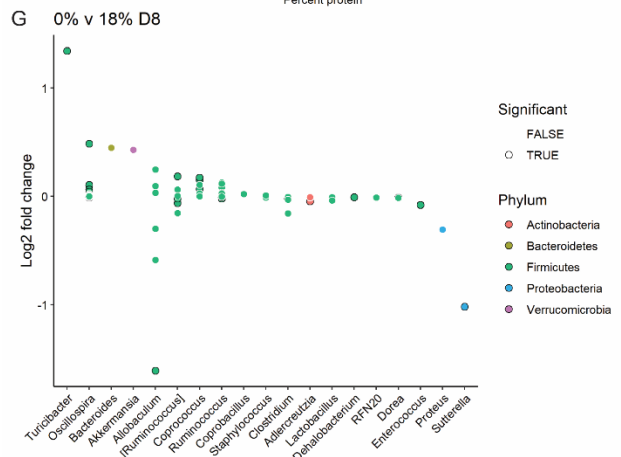
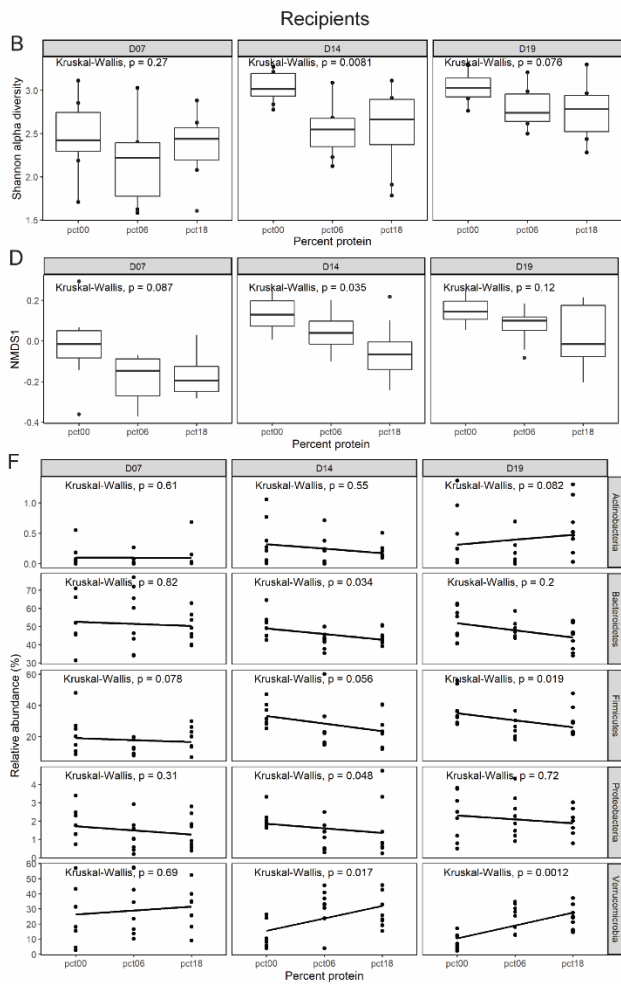
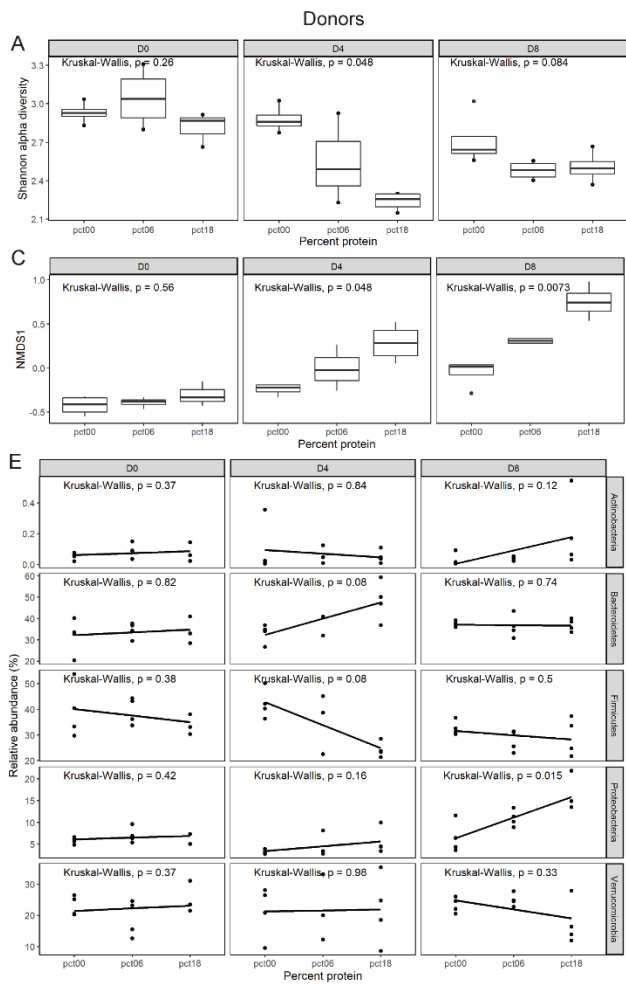


Figure S2: Dietary protein titration alters fecal microbiome composition, related to Figure 2.

A) Shannon alpha-diversity in fecal samples of donor mice after 0, 4 and 8 days of diet and **(B)** in recipient mice 7, 14 and 19 days after inoculation. **C)** NMDS1 in donor mice after 0, 4 and 8 days of diet and **(D)** in recipient mice 7,14 and 19 days after inoculation. **E)** relative abundances of the 5 major phyla present in fecal samples from donor mice and **(F)** recipient mice across the timecourses. **G)** Differential abundance analysis of species in the 0% vs 18% protein donor mice after 8 days of diet and **(H)** in recipient mice after 19 days of diet. **I)** Differential abundance analysis of species in the 6% vs 18% protein donor mice after 8 days of diet and **(J)** in recipient mice after 19 days of diet. Colors represent phyla. Species which are significantly differentially abundant (FDR adjusted $p < 0.05$) are outlined with a black circle.

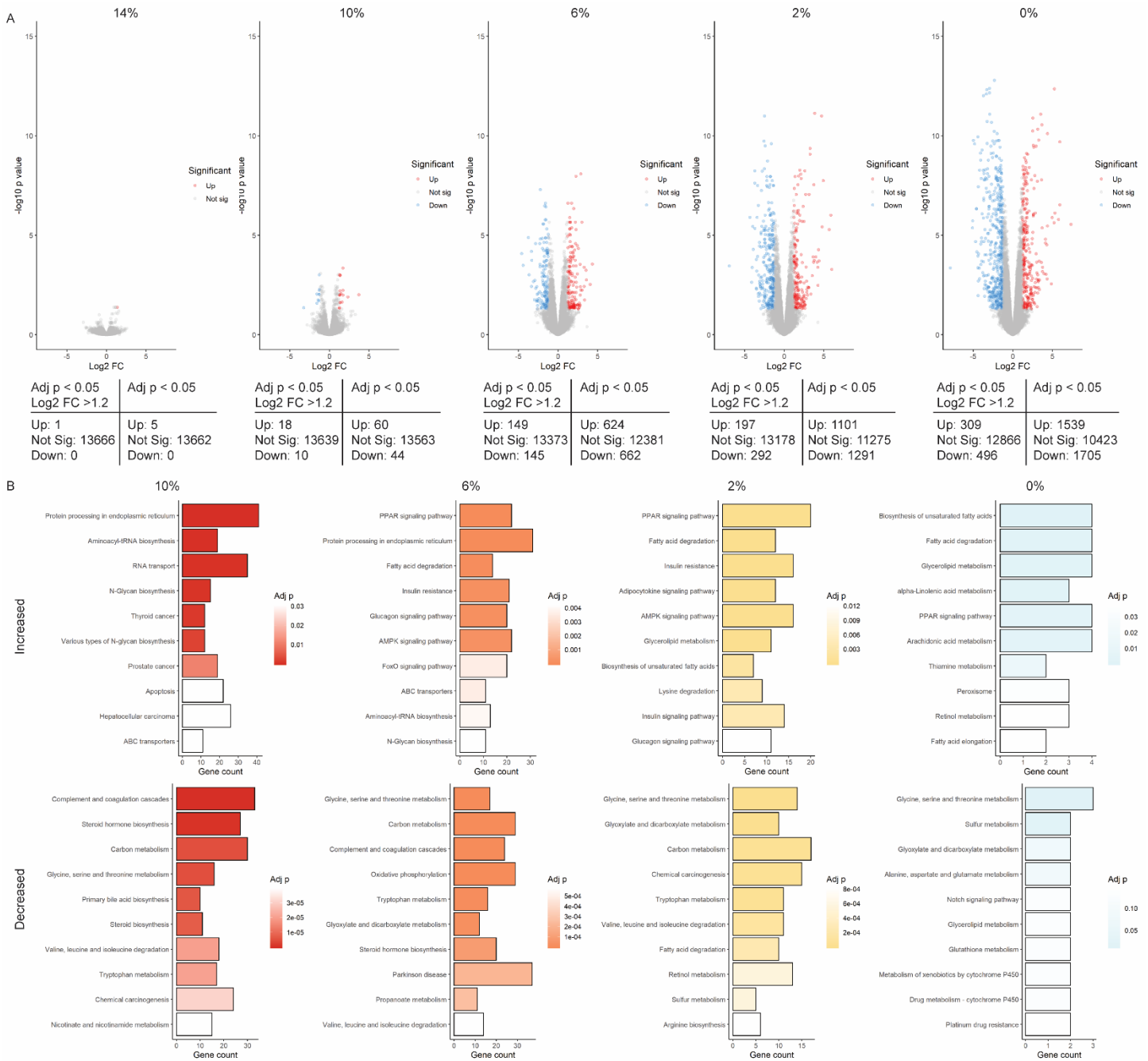


Figure S3: Effects of dietary protein titration on hepatic transcriptome, related to Figure 3. A) Differentially expressed genes in the indicated diet group compared to 18% protein intake. **B)** Pathway enrichment in significantly up and downregulated gene sets at the indicated dietary protein level versus 18% protein control group.

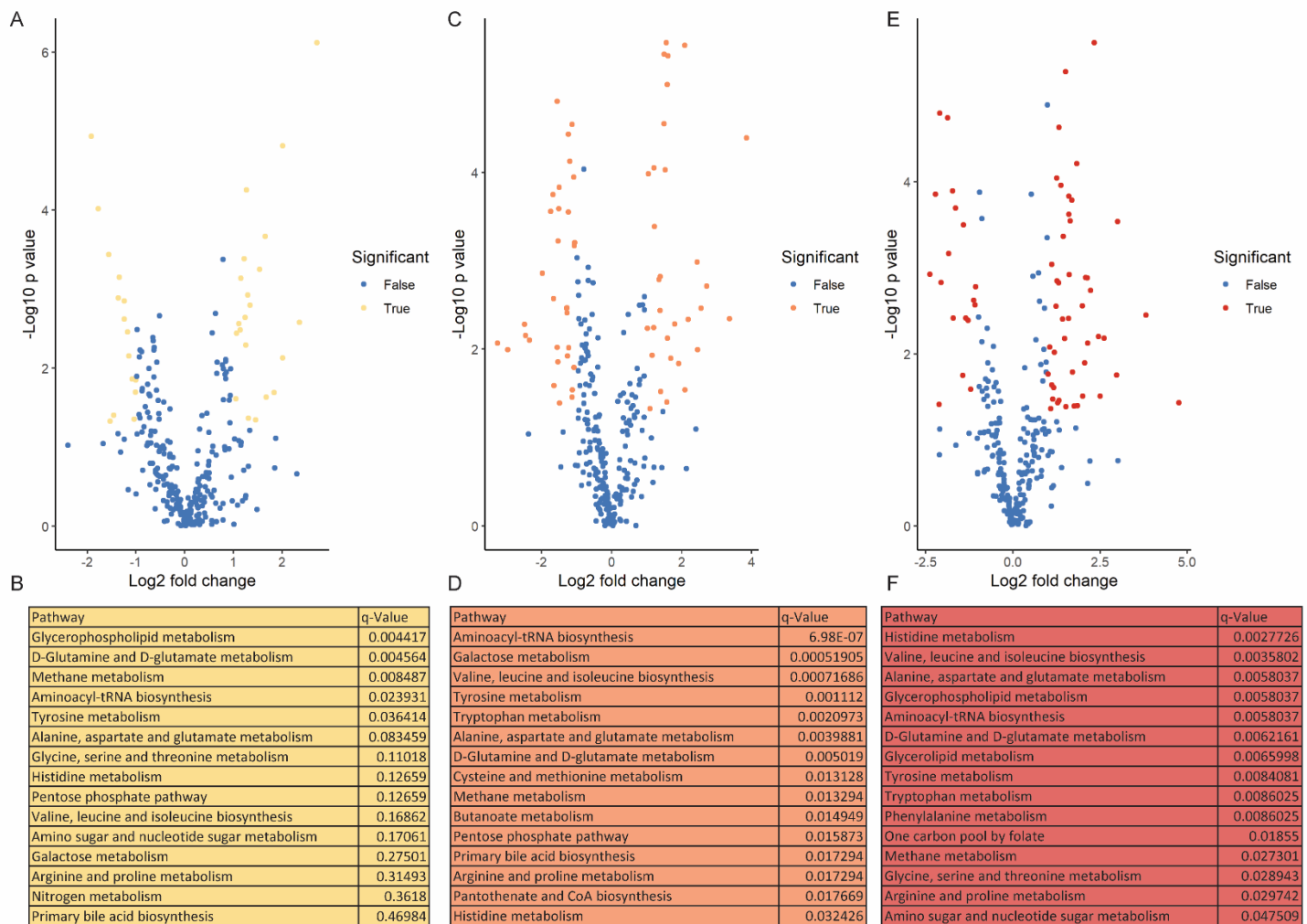


Figure S4: Effects of dietary protein titration on hepatic metabolome, related to Figure 4. Changes in individual metabolites (**A,C,E**) and associated pathway enrichment analysis (**B,D,F**) in the 6% (**A,B**), 2% (**C,D**) and 0% (**E,F**) protein vs. 18% protein control group.

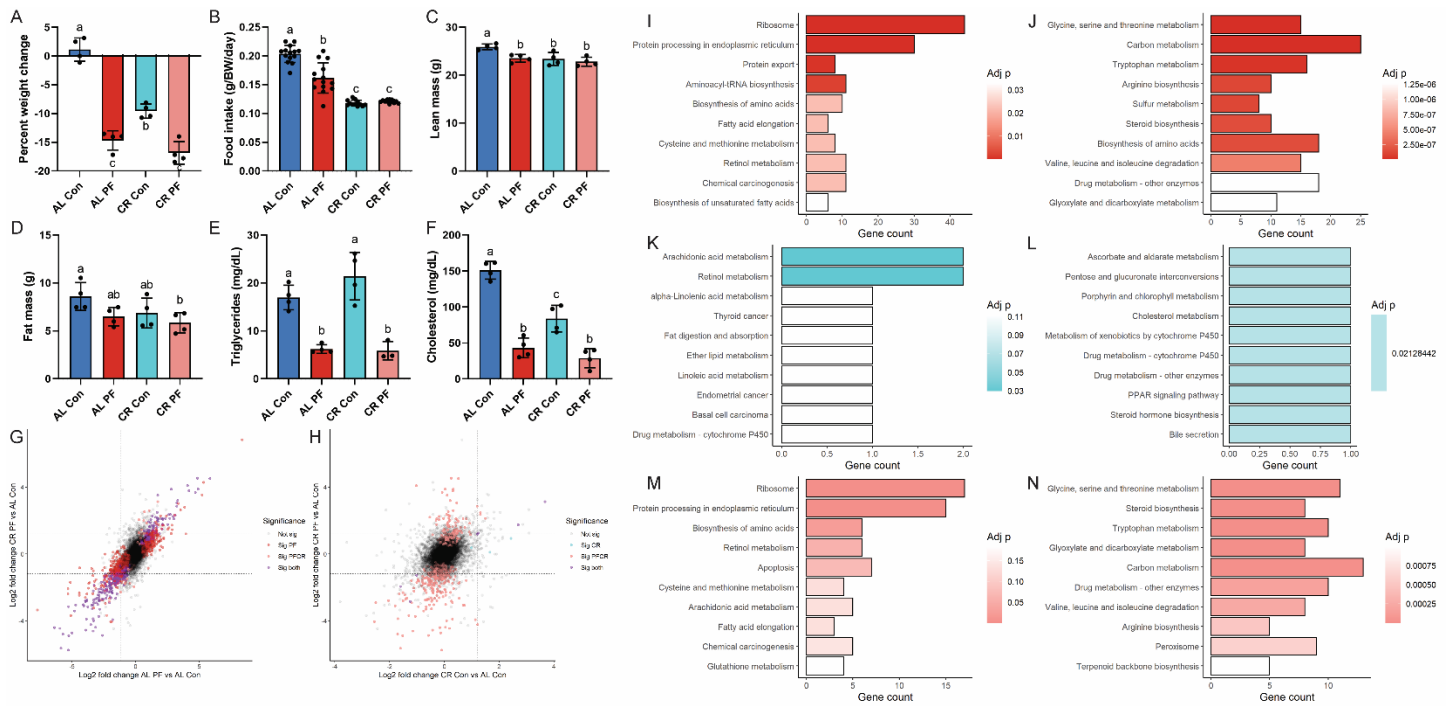


Figure S5: Phenotypic effects of dietary protein and calorie restriction, related to Figure 5. Adult male B6D2F1 mice ($n=4/\text{group}$) were fed ad libitum (AL) or 40% calorie restricted (CR) on a control 18% protein (Con) or 0% protein free (PF) diet for one week. **A)** Body weight change expressed as percent starting weight. **B)** Average daily food intake expressed as gram food intake per gram of mouse body weight. **C-F)** Lean body mass (**C**), fat mass (**D**), serum triglycerides (**E**) and serum cholesterol (**F**) in the fasted state after one week on the indicated diet. **G-H)** Scatterplots of log₂ fold changes of hepatic transcripts for AL PF and CR PF vs AL Con, $r = 0.76$, $p < 0.001$ and for CR Con and CR PF vs AL Con, $r = 0.36$, $p < 0.001$. **I-N)** Pathway enrichment in upregulated (left) and downregulated (right) gene sets of protein restriction (AL PF vs. AL Con, **I**); 40% calorie restriction on a control diet (AL Con vs. CR Con, **J**); and 40% calorie restriction on a protein free diet (AL PF vs. CR PF, **K**); different letters indicate $p < 0.05$ in Tukey post-hoc test. Error bars are standard deviations.