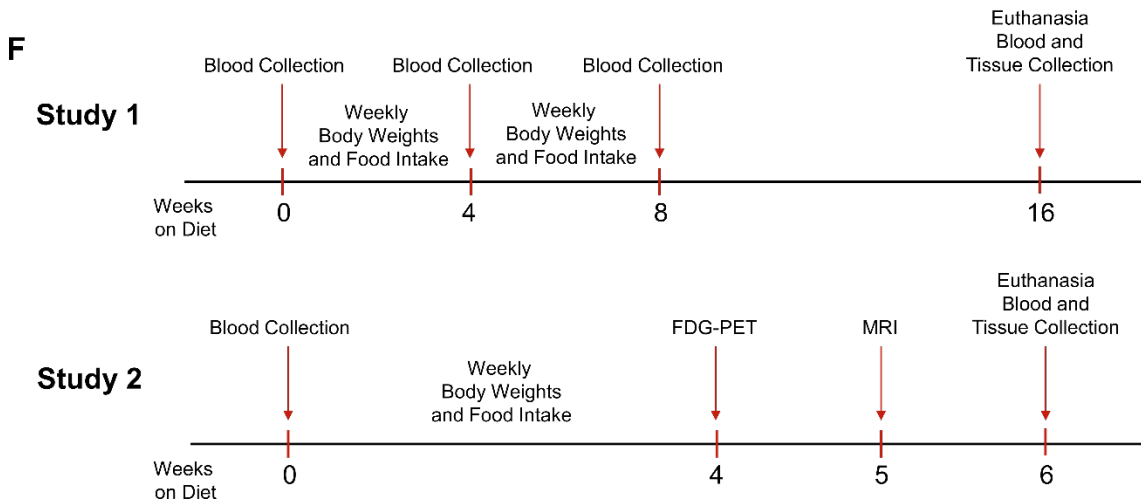
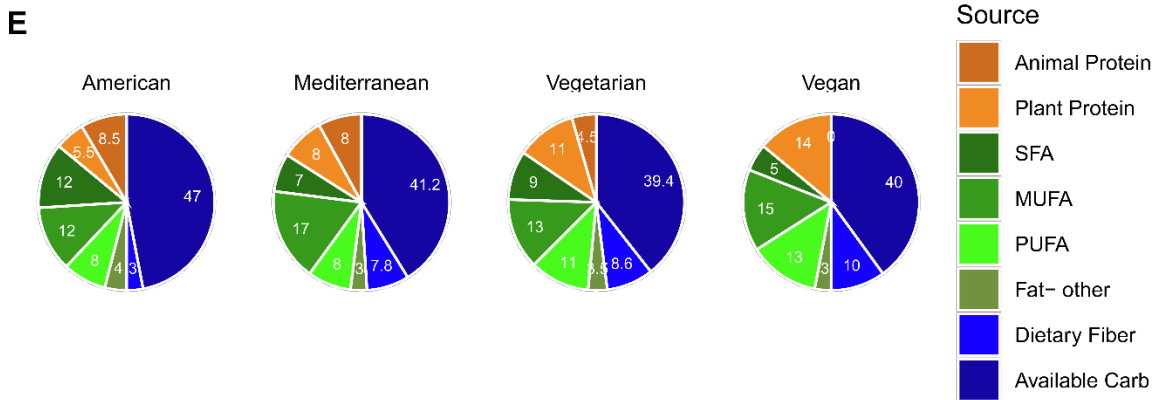


iScience, Volume 27

Supplemental information

Combined effects of genetic background and diet on mouse metabolism and gene expression

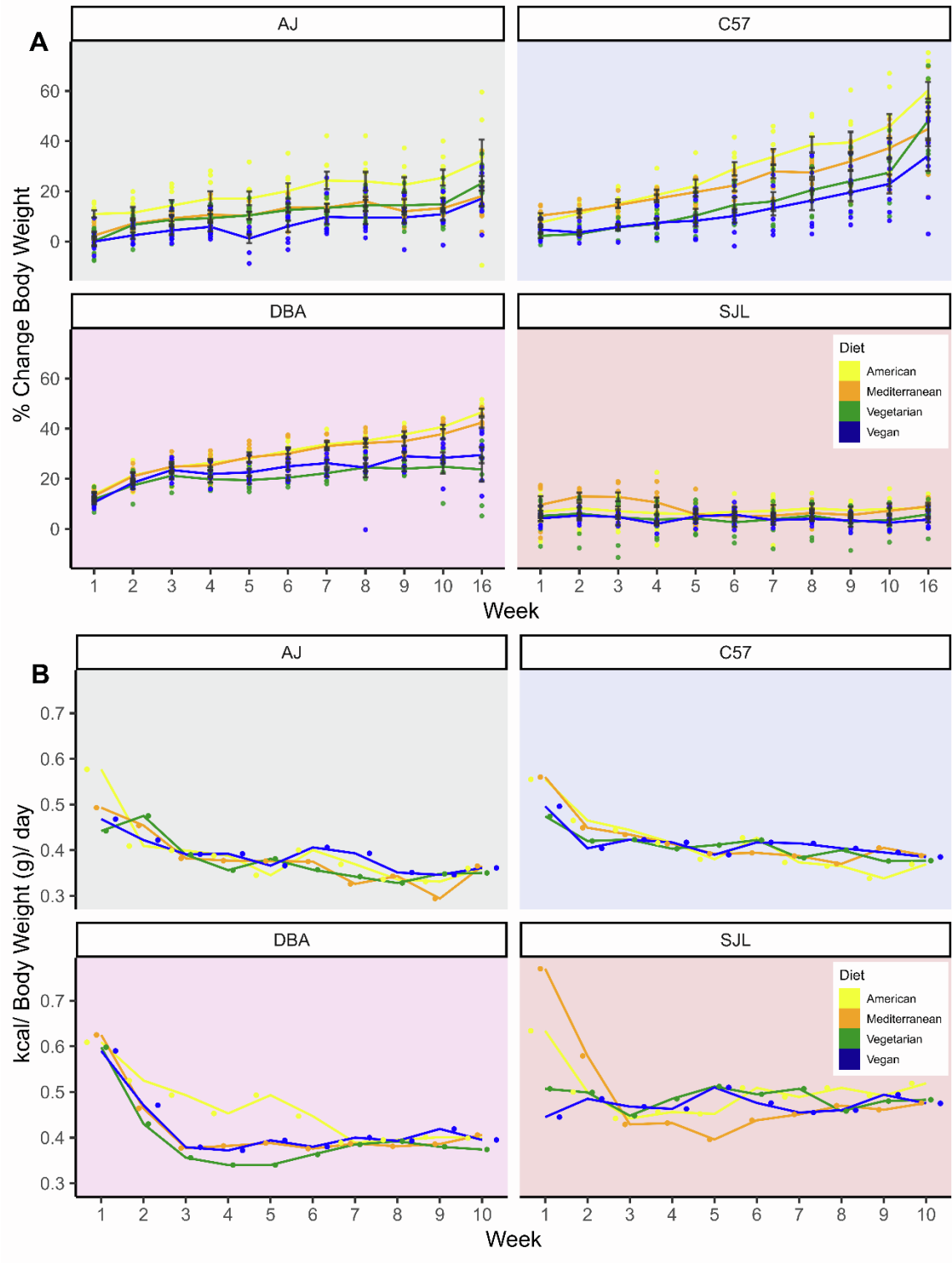
Jordan N. Reed, Faten Hasan, Abhishek Karkar, Dhanush Banka, Jameson Hinkle, Preeti Shastri, Navya Srivastava, Steven C. Scherping, Sarah E. Newkirk, Heather A. Ferris, Bijoy K. Kundu, Sibylle Kranz, Mete Civelek, and Susanna R. Keller



Supplemental Figure 1: Mouse Diets with Human Nutrient Sources and Study Design.

Related to STAR Methods.

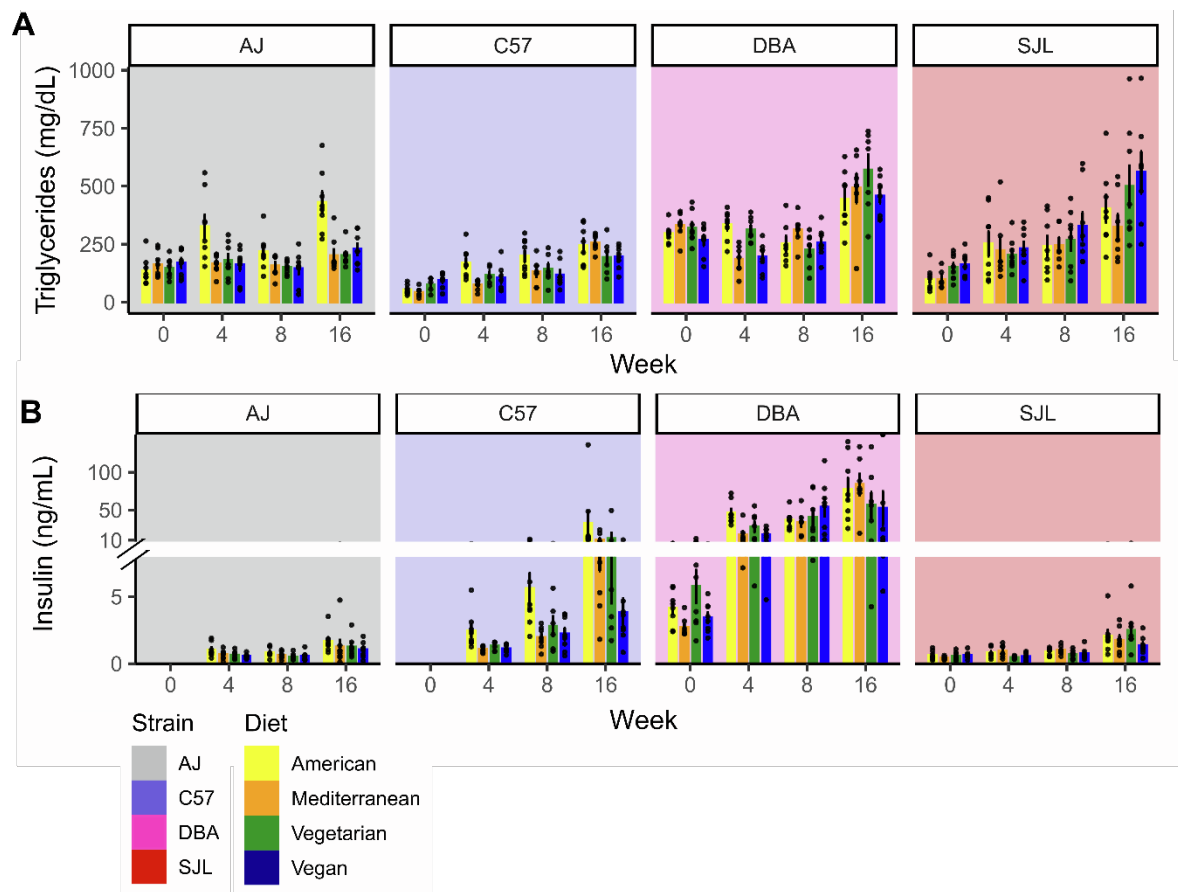
- A. Representative meal for human American diet and corresponding mouse chow
- B. Representative meal for human Mediterranean diet and corresponding mouse chow
- C. Representative meal for human Vegetarian diet and corresponding mouse chow
- D. Representative meal for human Vegan diet and corresponding mouse chow
- E. Major macronutrient sources for each of the 4 diets. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
- F. Experimental design for study 1 and 2. FDG PET, 2-[¹⁸F] fluoro-2-deoxy-D-glucose positron emission tomography; MRI, magnetic resonance imaging.



Supplemental Figure 2: Genetic background and diet interaction affect body weight gain in the presence of similar caloric intake. Related to Figure 1.

- A. Body weight percent change from baseline over 16 weeks on diets separated by strain (n = 8, 14 weeks old at week 0, male)
- B. Food intake in kcal per gram body weight per day over 10 weeks on diets (n = 8, 14 weeks old at week 0, male, average per cage)

Error bars represent means \pm SEM



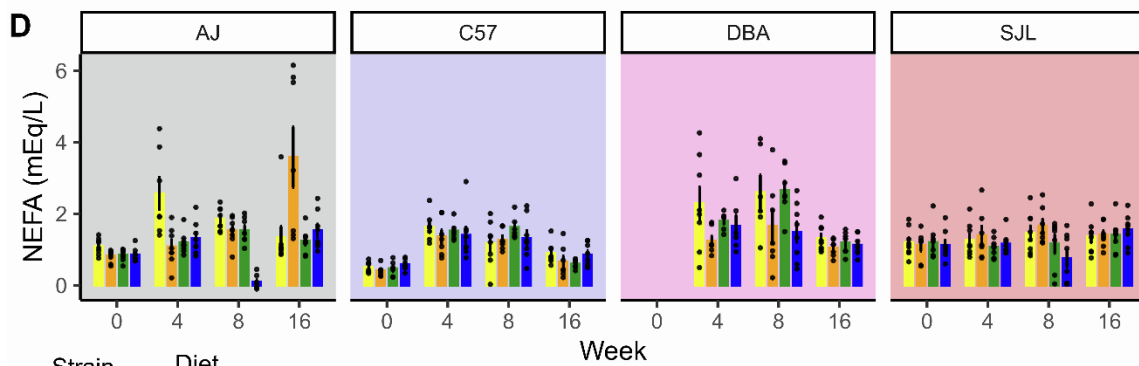
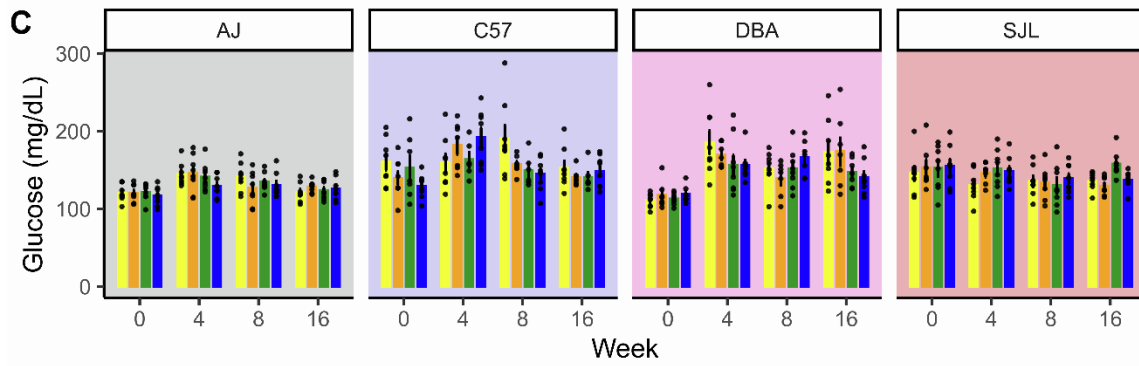
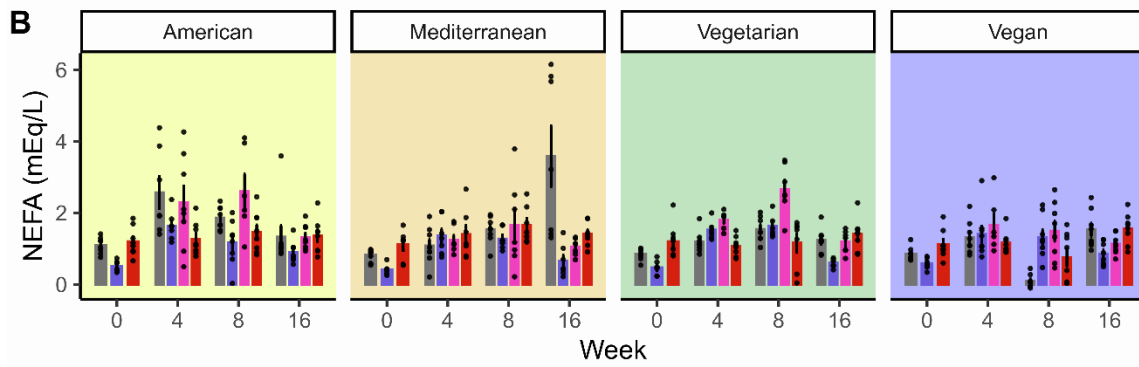
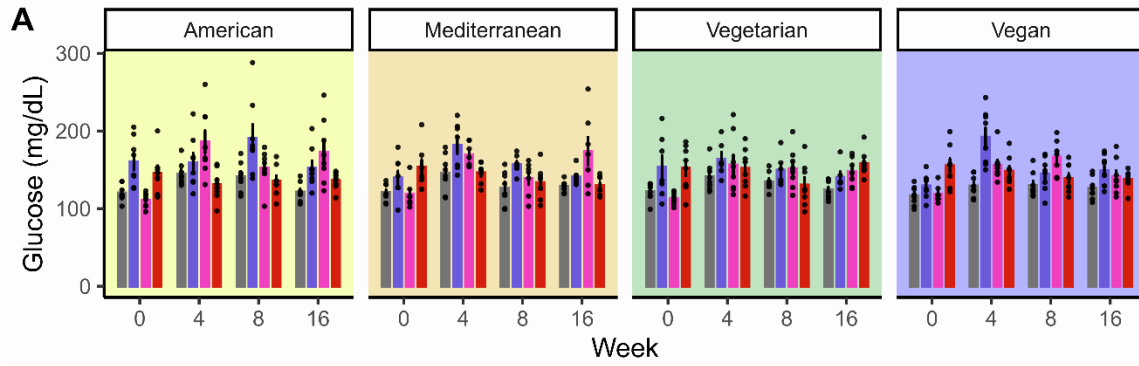
Supplemental Figure 3: Genetic background and diet impact metabolic parameters

(Study 1). Related to Figure 1

A. Plasma or serum triglycerides over 16 weeks on diets separated by strain (n = 8, 14 weeks old at week 0, male)

B. Plasma or serum insulin over 16 weeks on diets separated by strain (n = 8, 14 weeks old at week 0, male)

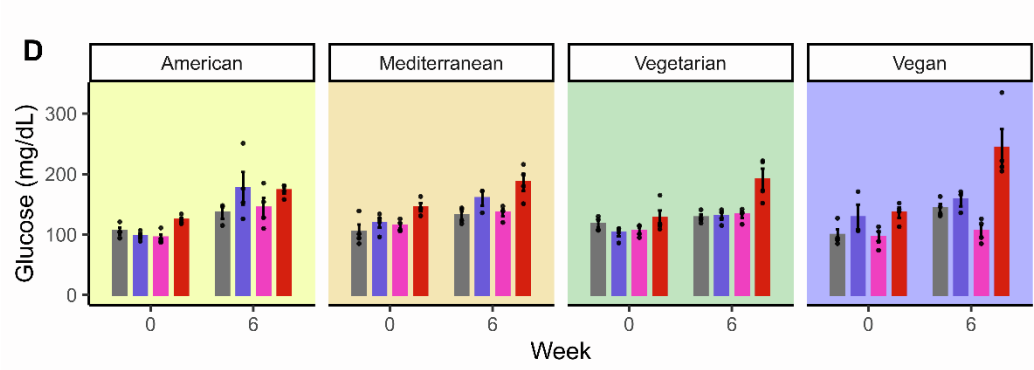
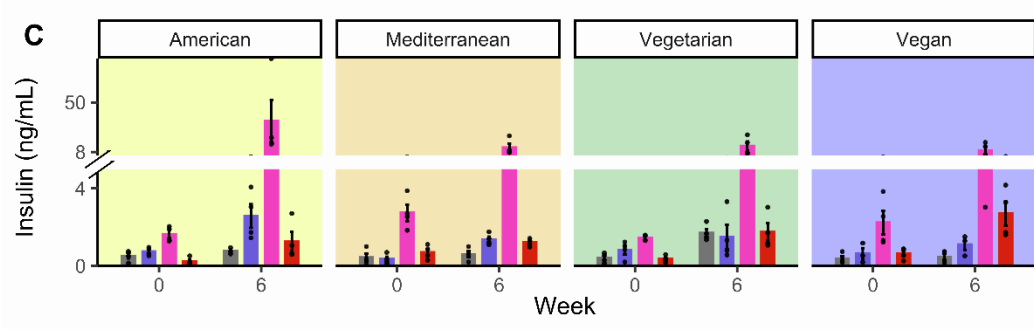
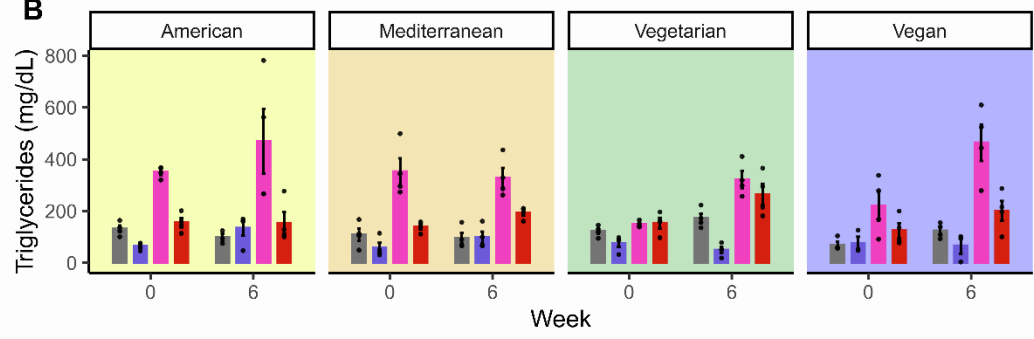
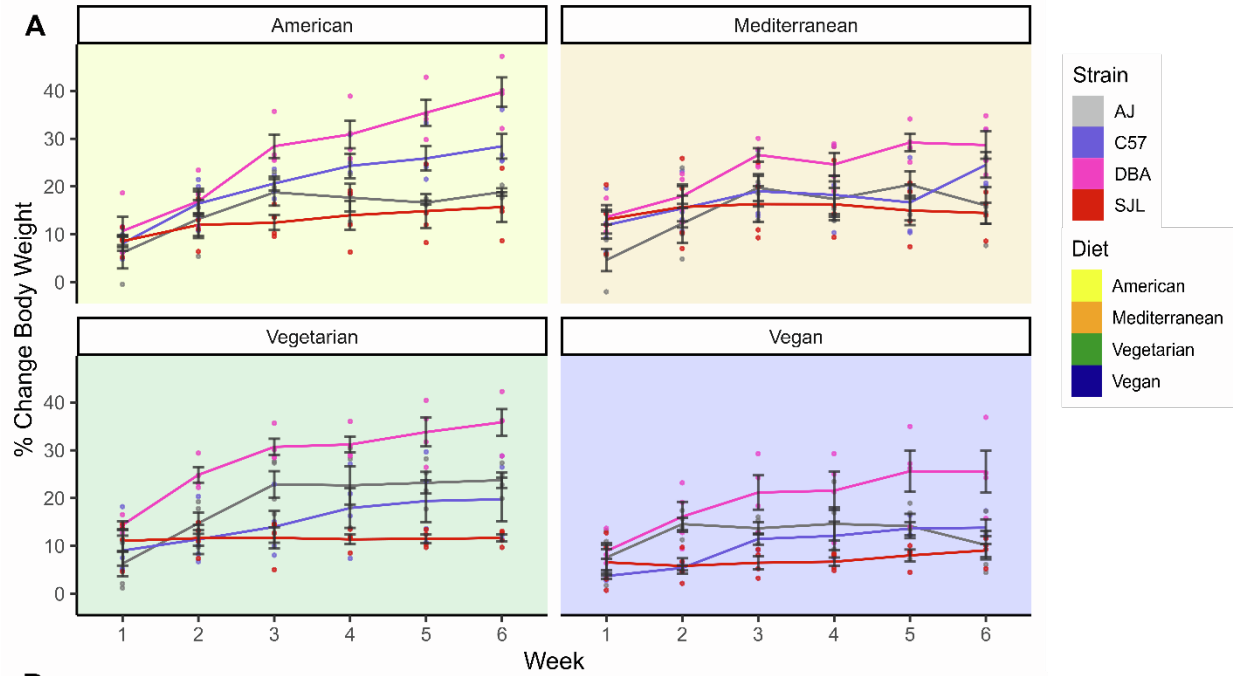
Error bars represent mean \pm SEM



Supplemental Figure 4: Genetic background impacts circulating glucose and non-esterified fatty acids (Study 1). Related to Figure 1 and Table 1.

- A. Blood glucose over 16 weeks on diets (n = 8, 12 weeks old at week 0, male mice)
- B. Serum non-esterified fatty acids (NEFA) over 16 weeks on diets (n = 8, 12 weeks old at week 0, male mice)
- C. Blood glucose over 16 weeks on diets separated by strain (n = 8, 14 weeks old at week 0, male mice) .
- D. Serum non-esterified fatty acids (NEFA) over 16 weeks on diets separated by strain (n = 8, 14 weeks old at week 0, male mice). Data for DBA/2J at time 0 are missing due to assay failure and lack of additional blood samples for a repeat measurement.

Error bars represent mean \pm SEM

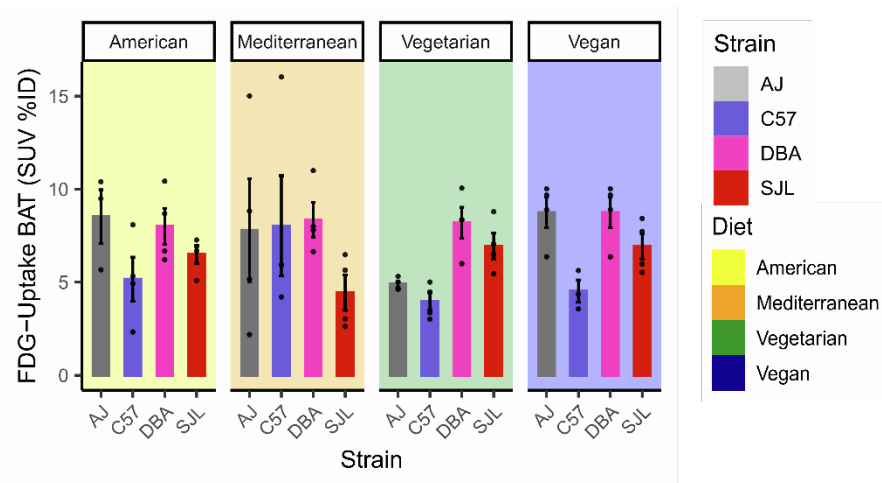


Supplemental Figure 5: Genetic background and diet impact metabolic parameters

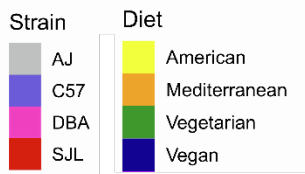
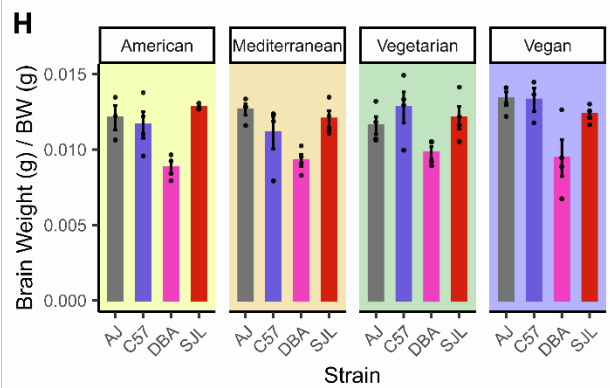
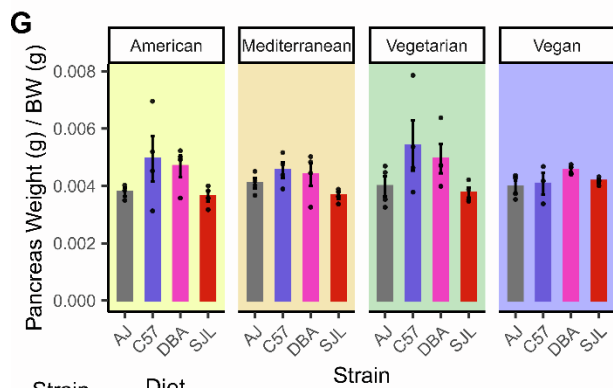
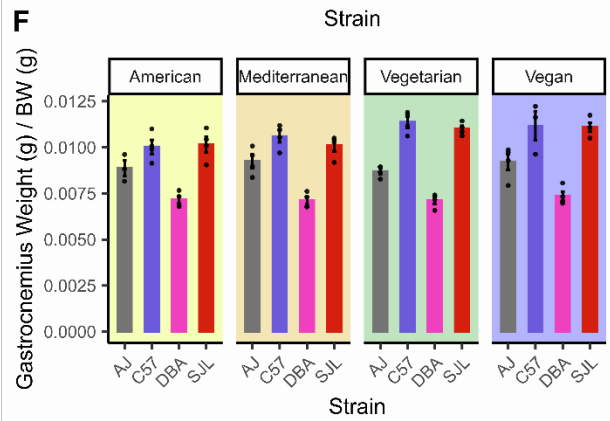
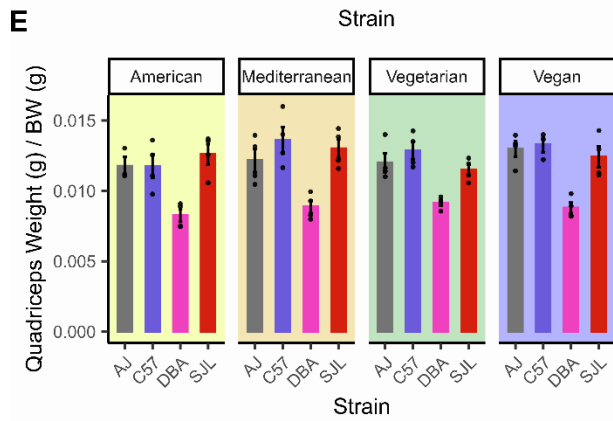
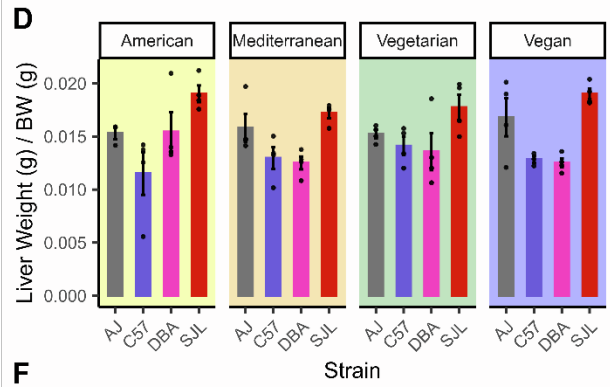
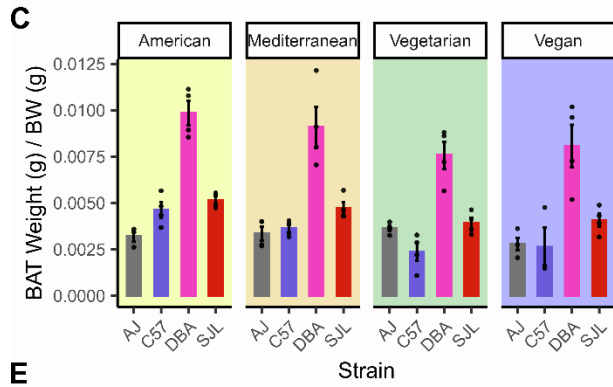
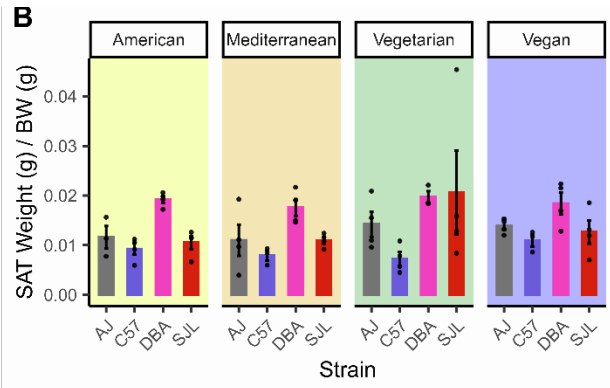
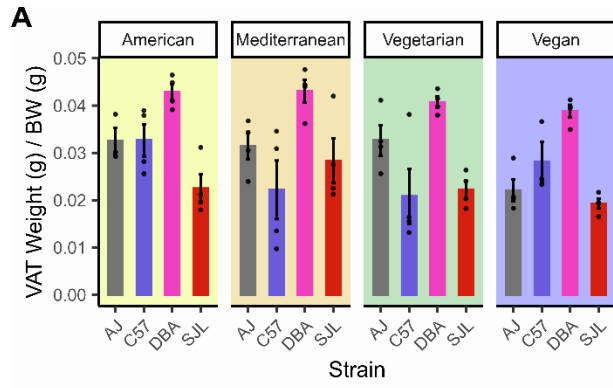
(Study 2). Related to Figure 2 and Table 2.

- A. Body weight percent change from baseline over 6 weeks on diets (n = 4, 12 weeks old at week 0, male)
- B. Plasma or serum triglycerides before and after 6 weeks on diets (n = 4, 12 weeks old at week 0, male)
- C. Plasma or serum insulin before and after 6 weeks on diets (n = 4, 12 weeks old at week 0, male)
- D. Blood glucose before and after 6 weeks on diets (n = 4, 12 weeks old at week 0, male)

Error bars represent mean \pm SEM



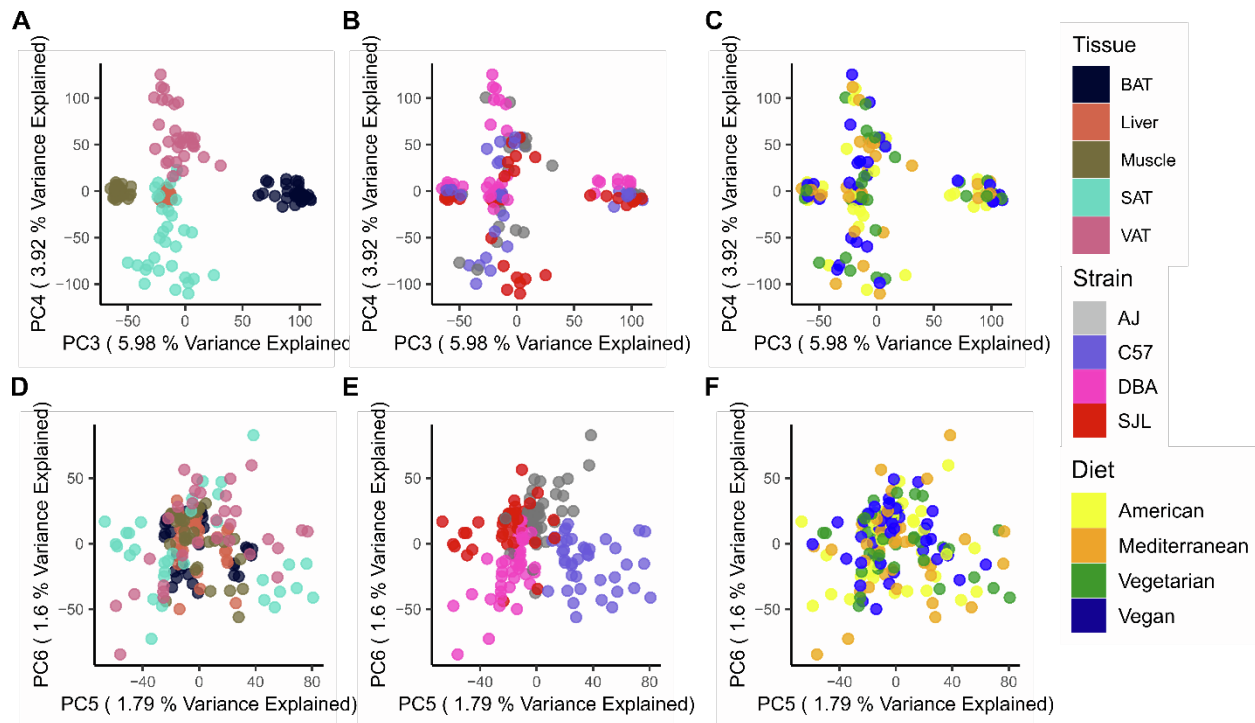
Supplemental Figure 6: Glucose uptake in brown adipose tissue (BAT) measured with 2-[¹⁸F] fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) imaging after 4 weeks on diets (n = 4, 16 weeks old, male). SUV%ID, standardized uptake values (SUV) normalized to injected dose per gram body weight. Related to Figure 2 and Table 2.



Supplemental Figure 7: Genetic background and diet impact tissue weights. Related to Figure 2 and Table 2.

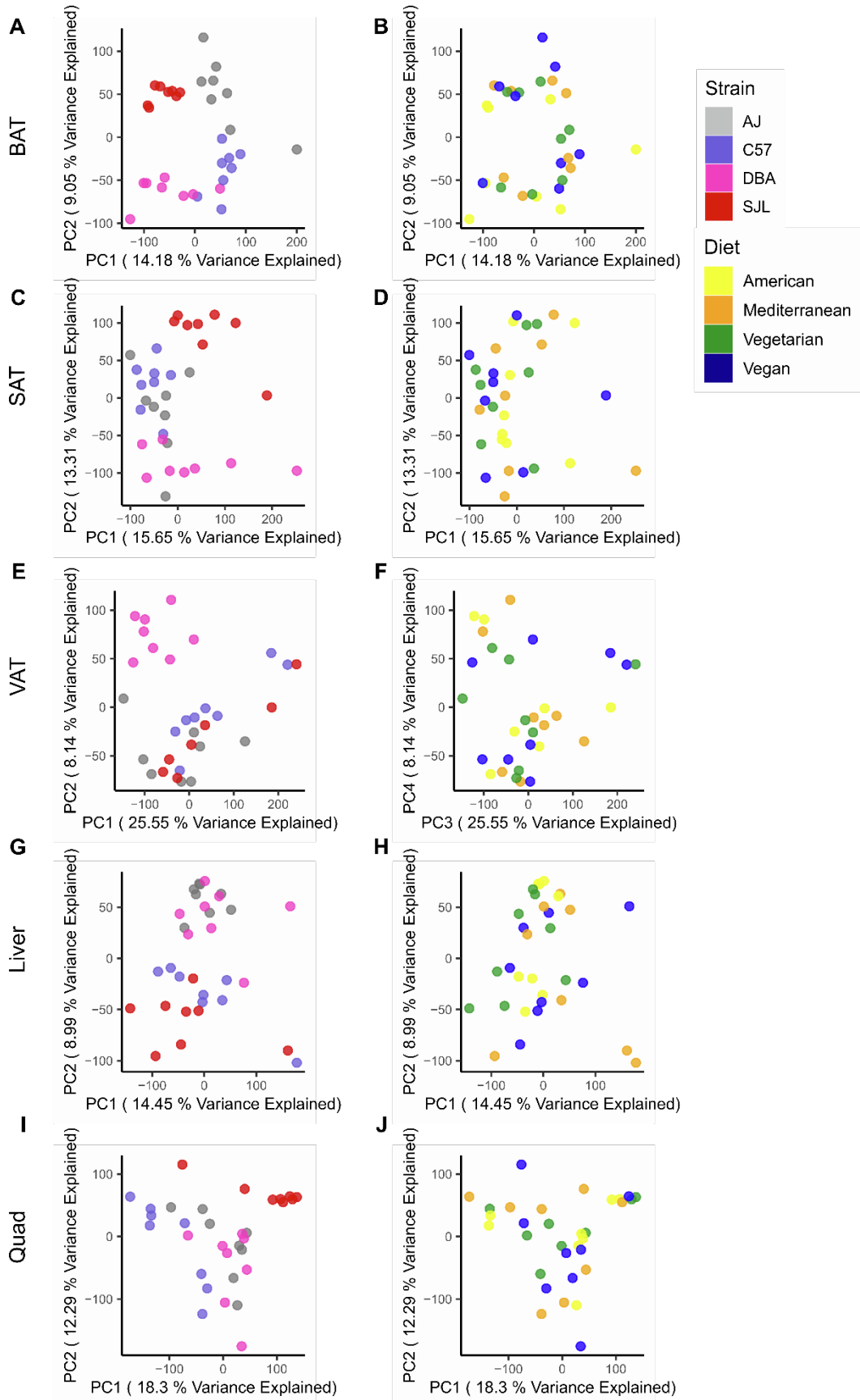
- A. Visceral adipose tissue (VAT) weight (g) per gram body weight after 6 weeks on diets (n = 4, 18 weeks old, male)
- B. Subcutaneous adipose tissue (SAT) weight (g) per gram body weight after 6 weeks on diets (n = 4, 18 weeks old, male)
- C. Brown adipose tissue (BAT) weight (g) per gram body weight after 6 weeks on diets (n = 4, 18 weeks old, male)
- D. Liver weight (g) per gram body weight after 6 weeks on diet (n = 4, 18 weeks old, male)
- E. Quadriceps muscle weight (g) per gram body weight after 6 weeks on diet (n = 4, 18 weeks old, male)
- F. Gastrocnemius muscle weight (g) per gram body weight after 6 weeks on diet (n = 4, 18 weeks old, male)
- G. Brain weight (g) per gram body weight after 6 weeks on diet (n = 4, 18 weeks old, male)
- H. Pancreas weight (g) per gram body weight after 6 weeks on diet (n = 4, 18 weeks old, male)

Error bars represent mean \pm SEM



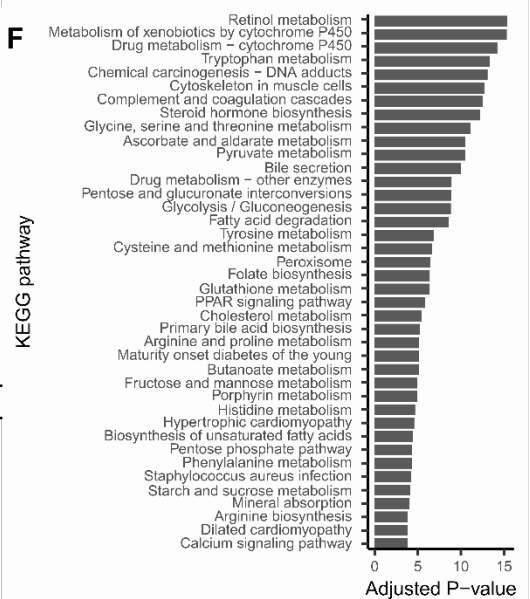
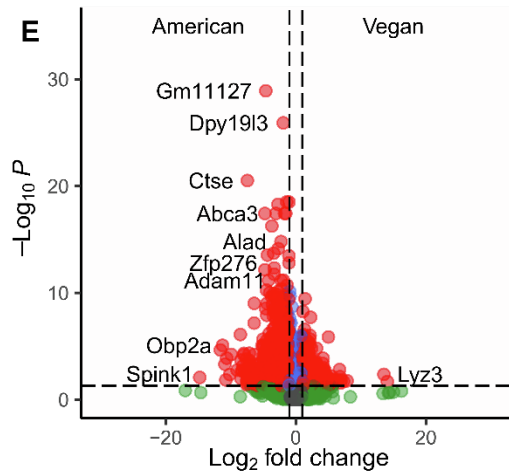
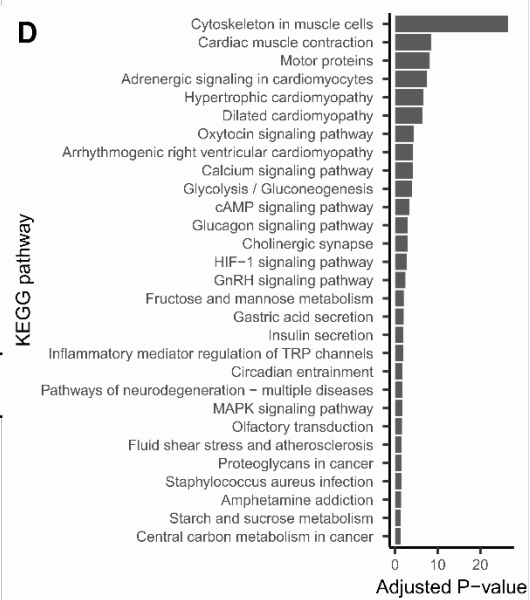
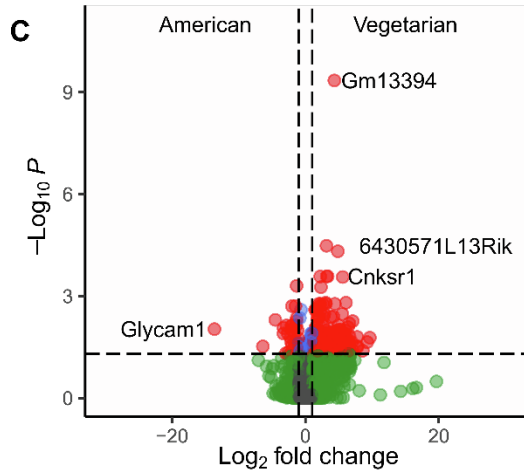
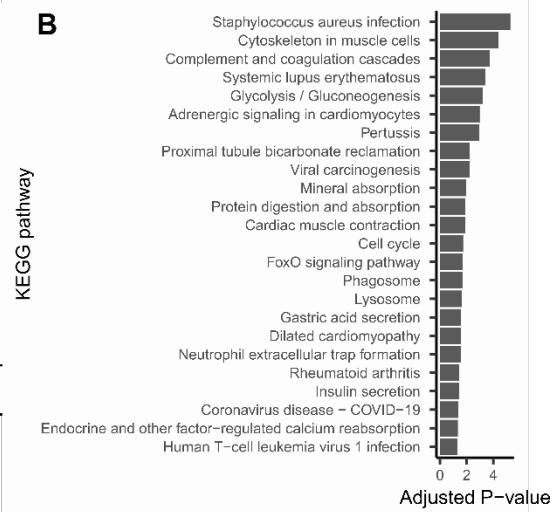
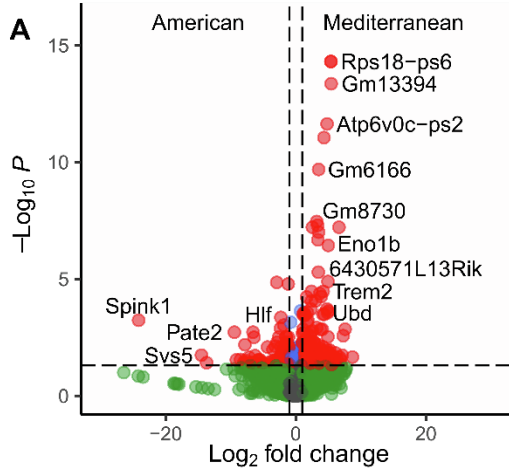
Supplemental Figure 8: RNA expression separates samples by tissue and genetic background. Related to Figure 3.

- A. Principal components 3 and 4 (PC3 and PC4), with samples colored by tissue
- B. PC3 and PC4, with samples colored by genetic background
- C. PC3 and PC4, with samples colored by diet
- D. PC5 and PC6, with samples colored by tissue
- E. PC5 and PC6, with samples colored by genetic background
- F. PC5 and PC6, with samples colored by diet



Supplemental Figure 9: RNA expression separates samples by genetic background
when considering each tissue individually. Related to Figure 3.

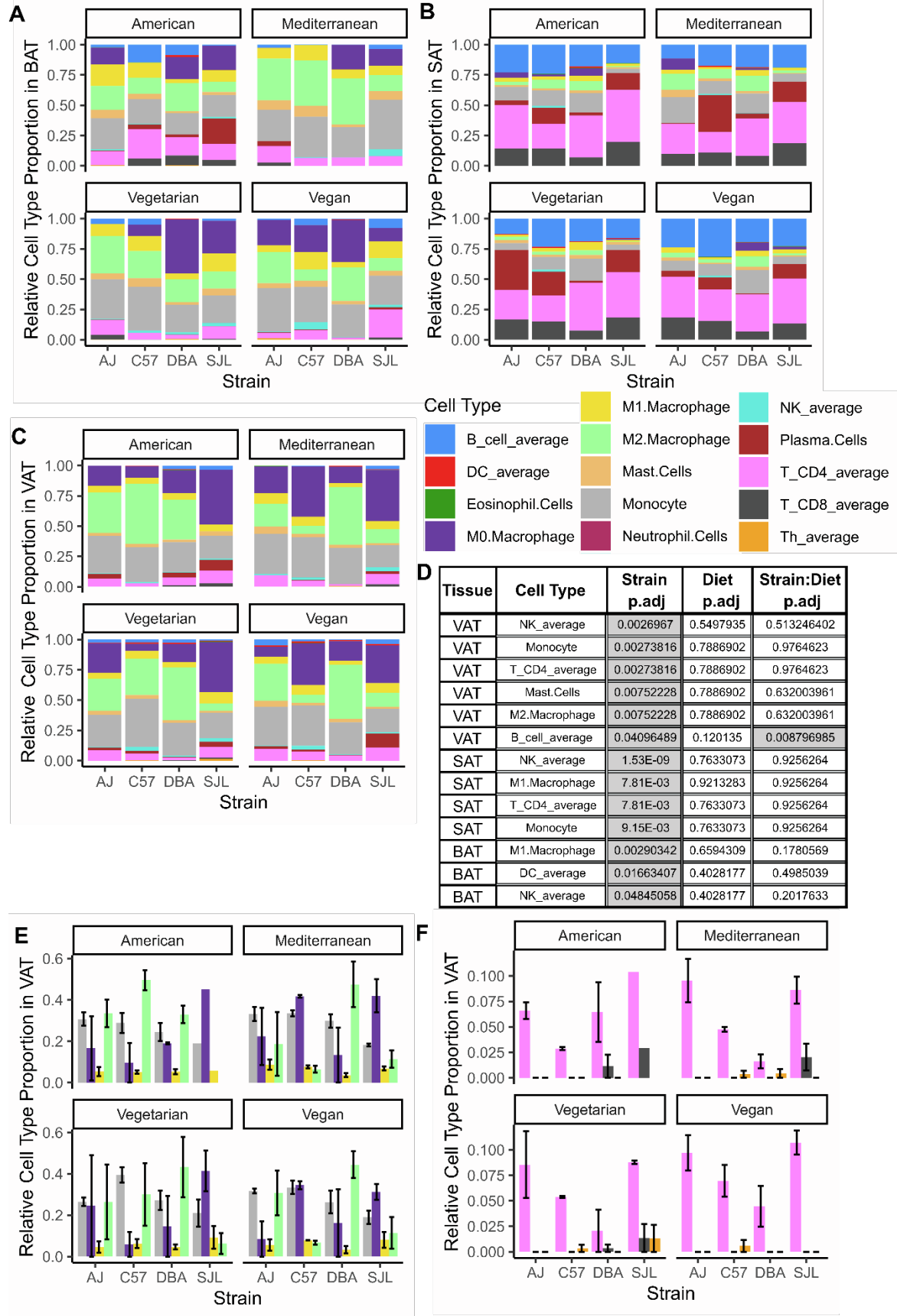
- A. Brown adipose tissue (BAT) principal components 1 and 2 (PC1 and PC2), with samples colored by genetic background
- B. Brown adipose tissue PC1 and PC2, with samples colored by diet
- C. Subcutaneous adipose tissue (SAT) PC1 and PC2, with samples colored by genetic background
- D. Subcutaneous adipose tissue PC1 and PC2, with samples colored by diet
- E. Visceral adipose tissue (VAT) PC1 and PC2, with samples colored by genetic background
- F. Visceral adipose tissue PC1 and PC2, with samples colored by diet
- G. Liver principal PC1 and PC2, with samples colored by genetic background
- H. Liver PC1 and PC2, with samples colored by diet
- I. Quadriceps (Quad) muscle PC1 and PC2, with samples colored by genetic background
- J. Quadriceps muscle PC1 and PC2, with samples colored by diet



Supplemental Figure 10: Differential expression of genes between the American diet and the three other diets in visceral adipose tissue. Related to Figure 4.

- A) Volcano plot showing genes differentially expressed between American and Mediterranean diets. Using cutoffs $\text{abs}(\log_2 \text{ fold change}) > 1$ and adjusted p-value < 0.05 , 248 genes were upregulated on Mediterranean diet and 101 genes on American diet.
- B) KEGG pathways enriched with genes differentially expressed between American and Mediterranean diets.
- C) Volcano plot showing genes differentially expressed between American and vegetarian diets. Using cutoffs $\text{abs}(\log_2 \text{ fold change}) > 1$ and adjusted p-value < 0.05 , 251 genes were upregulated on vegetarian diet and 35 genes on American diet.
- D) KEGG pathways enriched with genes differentially expressed between American and vegetarian diets.
- E) Volcano plot showing genes differentially expressed between American and vegan diets. Using cutoffs $\text{abs}(\log_2 \text{ fold change}) > 1$ and adjusted p-value < 0.05 , 750 genes were upregulated on vegan diet and 1762 genes on American diet.
- F) KEGG pathways enriched with genes differentially expressed between American and vegan diets.

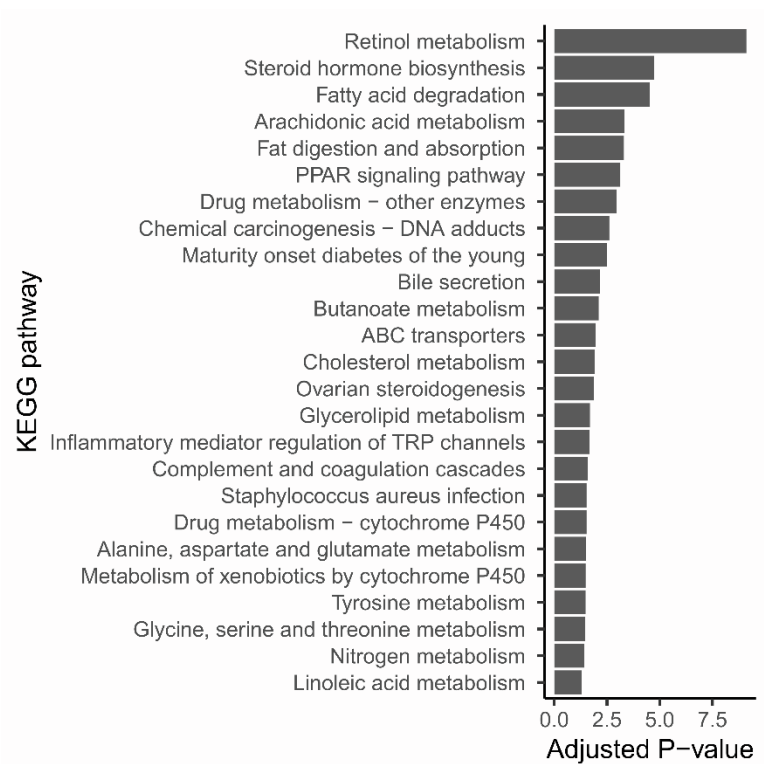
Green colored dots in A, C, and E have $\text{abs}(\log_2 \text{ fold change}) > 1$, blue colored dots have adjusted p-value < 0.05 , and red colored dots fit both criteria.



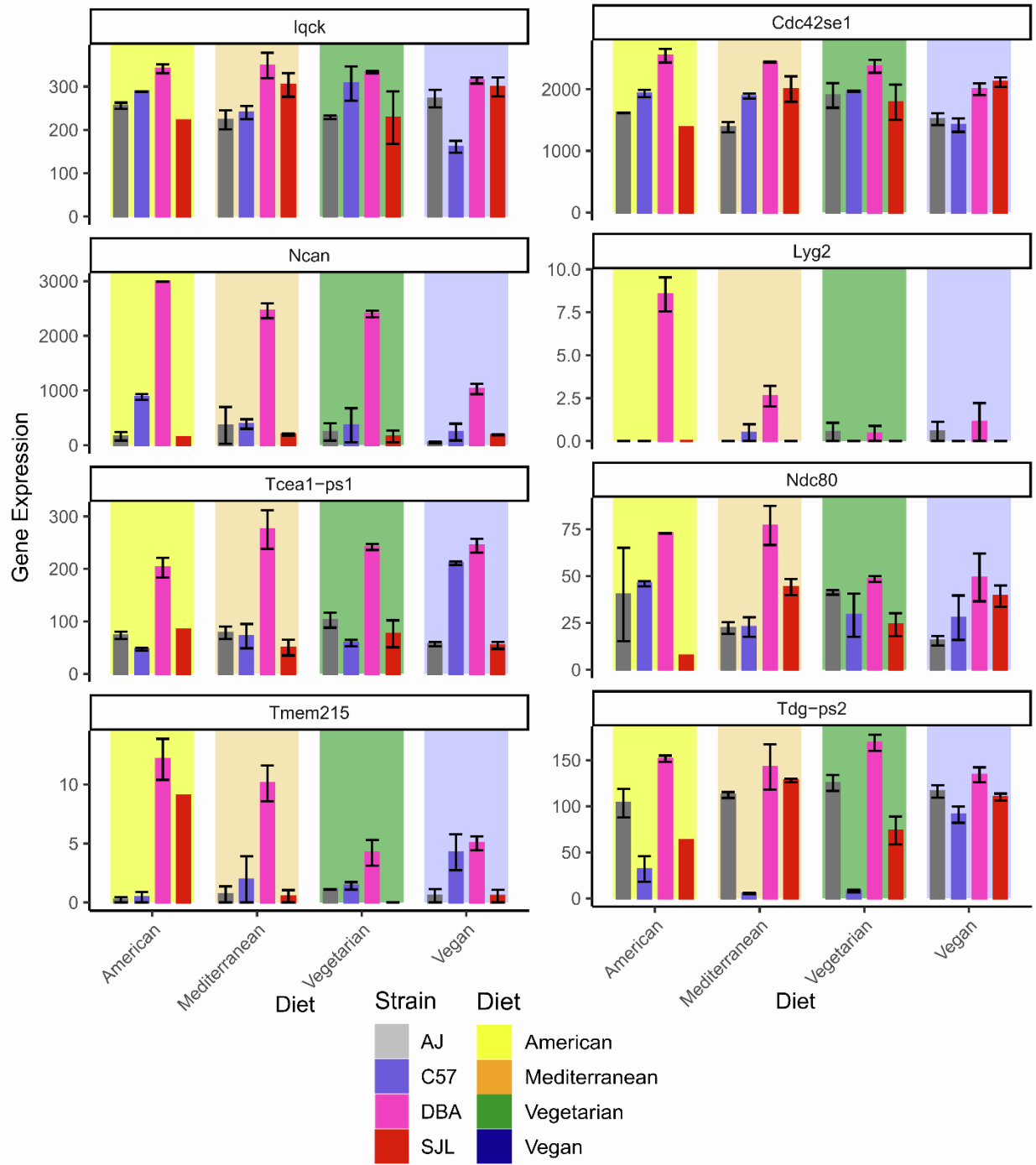
Supplemental Figure 11: Immune cell type proportions in adipose tissues with the different diets. Related to Figure 4.

- A) Relative proportions of immune cells in brown adipose tissue (BAT)
- B) Relative proportions of immune cells in subcutaneous adipose tissue (SAT)
- C) Relative proportions of immune cells in visceral adipose tissue (VAT)
- D) Significant results of 2-way ANOVA predicting immune cell proportions by strain and diet
- E) Relative proportions of macrophage subtypes in visceral adipose tissue (VAT)
- F) Relative proportions of T cell subtypes in visceral adipose tissue (VAT)

DC: Dendritic cells, NK: Natural Killer cells, Th: helper T cells

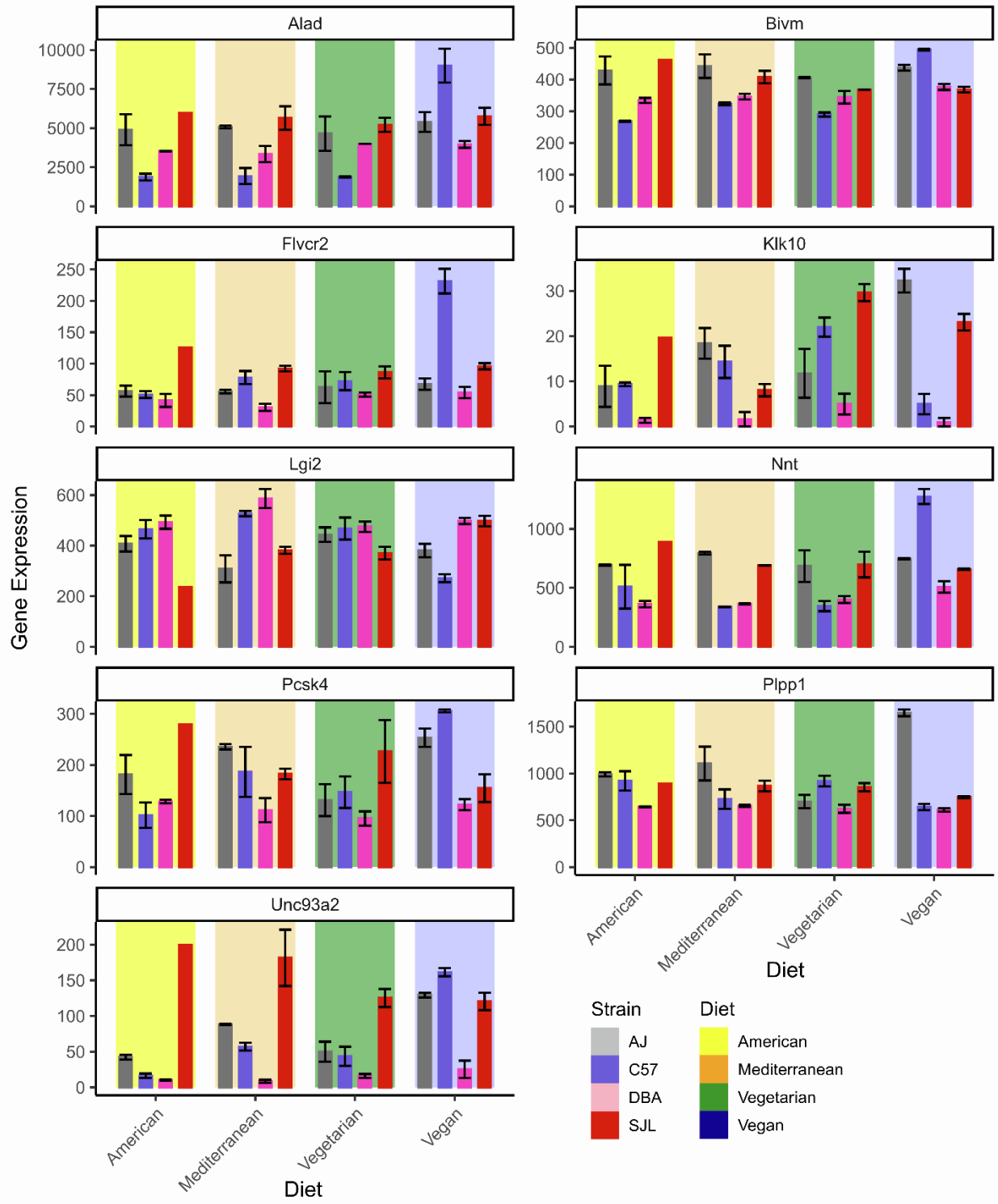


Supplemental Figure 12: KEGG pathways that are enriched within the set of 421 VAT strain:diet dependent genes. Related to Figure 4.



Supplemental Figure 13: Expression of 8 genes in visceral adipose tissue with significant positive correlations with several parameters including body weight, relative

adipose tissue weights, and/or circulating insulin or triglyceride levels (Pearson, p-value adjusted with FDR correction for $n = 421 \text{ genes} * 28 \text{ traits}$) and significant strain:diet-dependent interactions effects (2-way ANOVA, p-value adjusted with FDR correction for $n = \sim 31,000 \text{ genes}$). Gene expression represents normalized counts. Related to Figure 4.



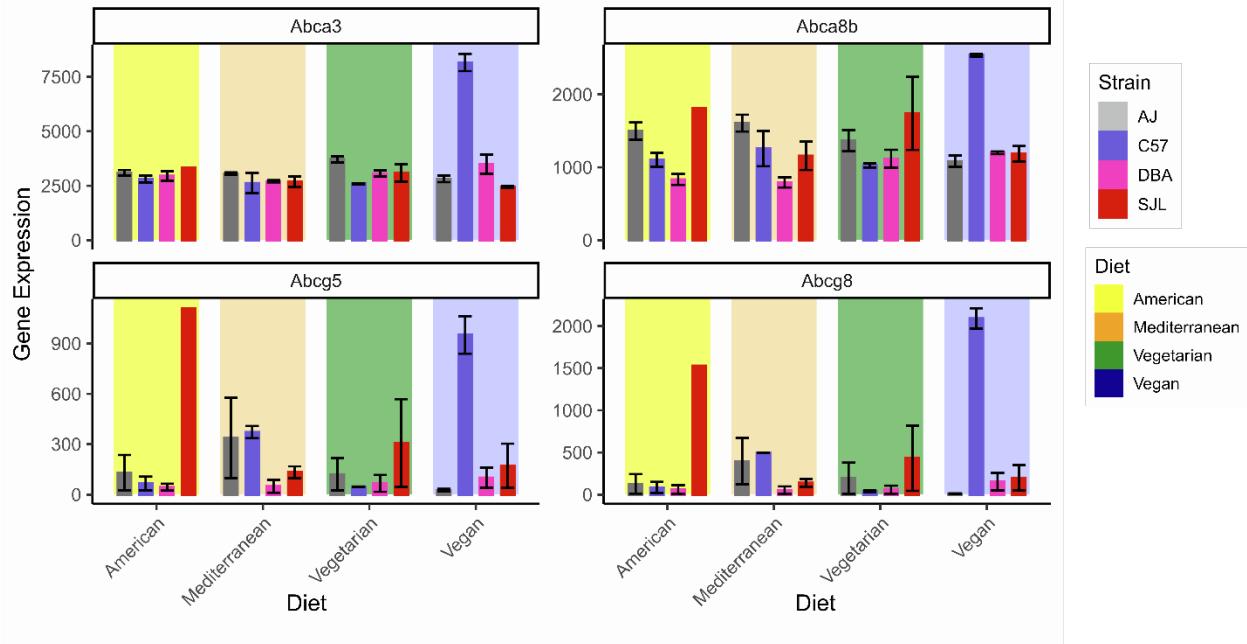
Supplemental Figure 14: Expression of 9 genes in visceral adipose tissue with significant negative correlations with percent change in body weight and/or relative

adipose tissue weights (Pearson, p-value adjusted with FDR correction for $n = 421$ genes * 28 traits) and significant strain:diet-dependent interactions effects (2-way ANOVA, p-value adjusted with FDR correction for $n = \sim 31,000$ genes). Gene expression plots show normalized counts. Related to Figure 4.

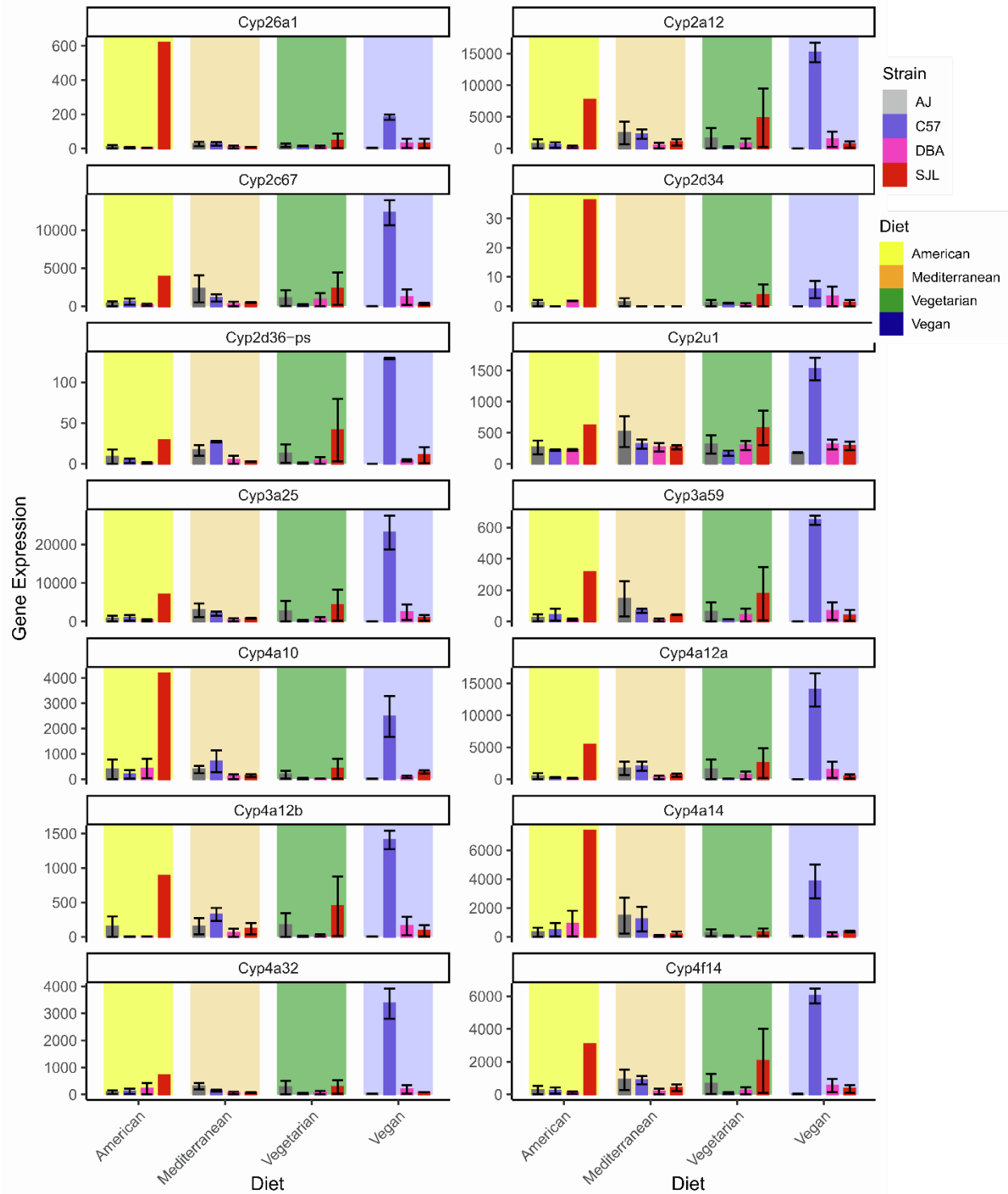


Supplemental Figure 15: Expression of metabolically relevant transporter genes that exhibit C57BL/6J-vegan interaction effects among the 421 visceral adipose tissue genes with significant strain:diet-dependent effects, (2-way ANOVA, p-value adjusted

with FDR correction for $n = \sim 31,000$ genes). Gene expression plots show normalized counts. Related to Figure 4.

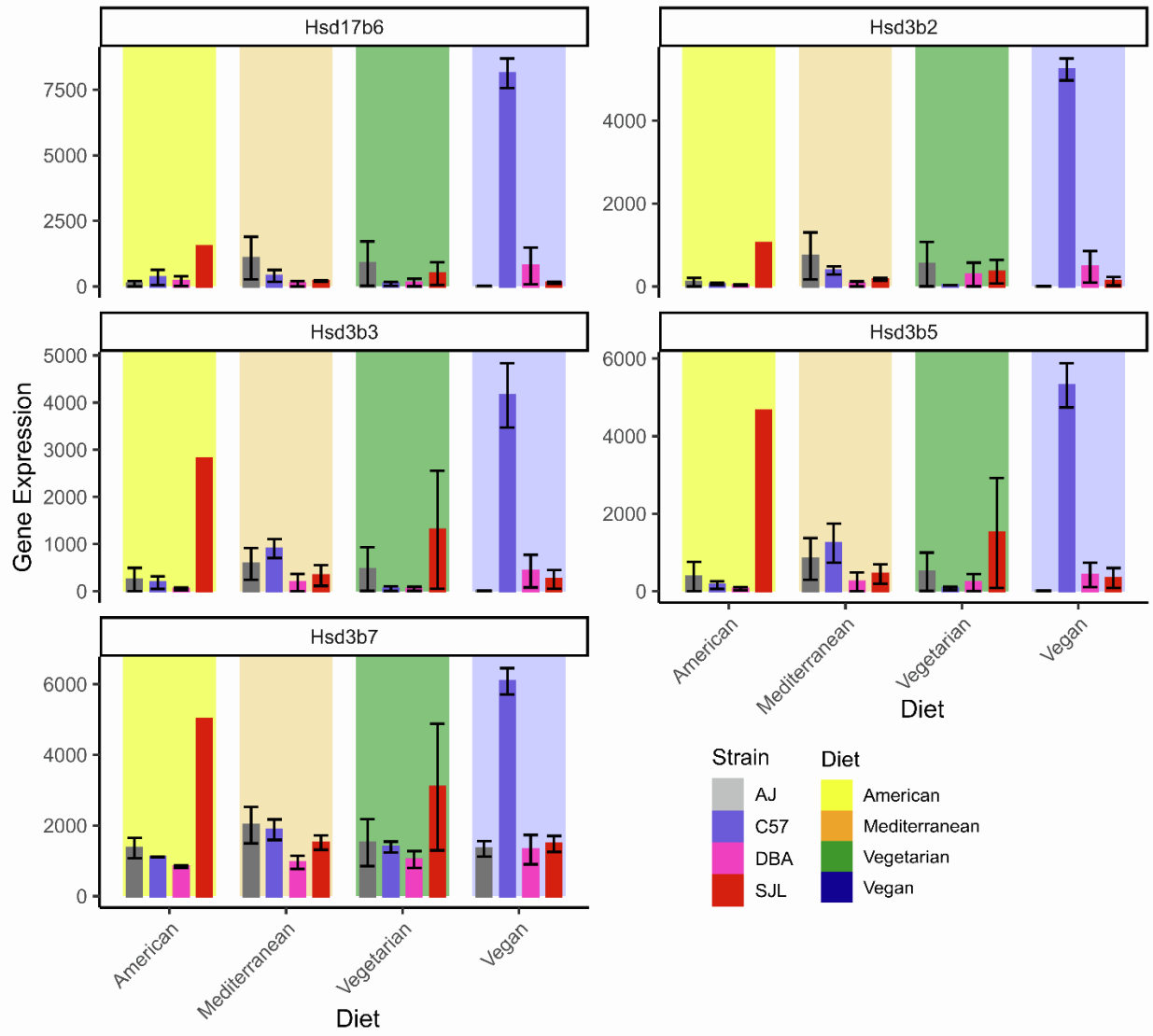


Supplemental Figure 16: Expression of ATP-binding cassette (ABC) transporter genes that exhibit C57BL/6J-vegan effects among the 421 visceral adipose tissue genes with significant strain:diet-dependent effects (2-way ANOVA, p-value adjusted with FDR correction for $n = \sim 31,000$ genes). Gene expression plots show normalized counts. Related to Figure 4.

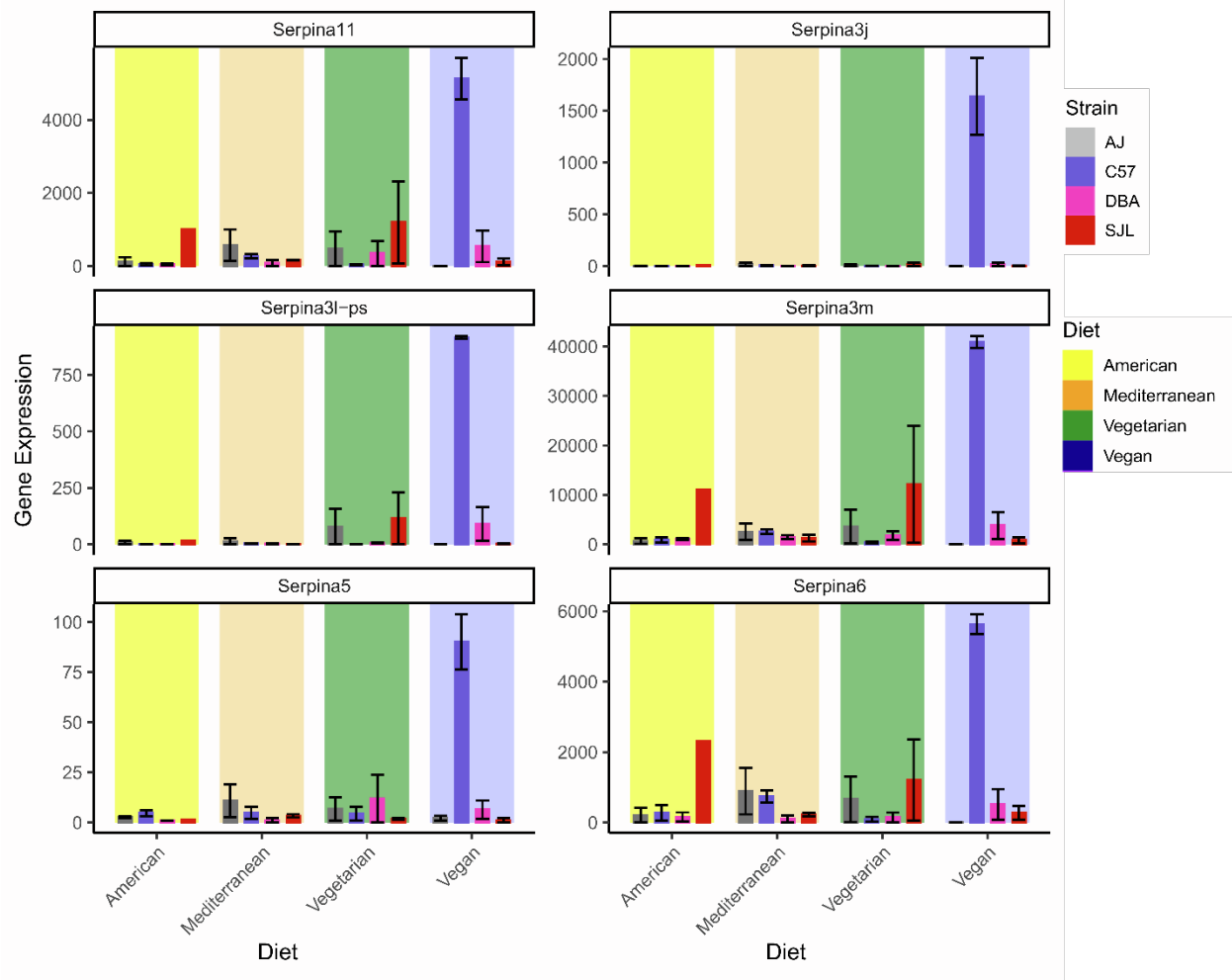


Supplemental Figure 17: Expression of cytochrome P450 genes that exhibit C57BL/6J-vegan effects among the 421 visceral adipose tissue genes with significant strain:diet-

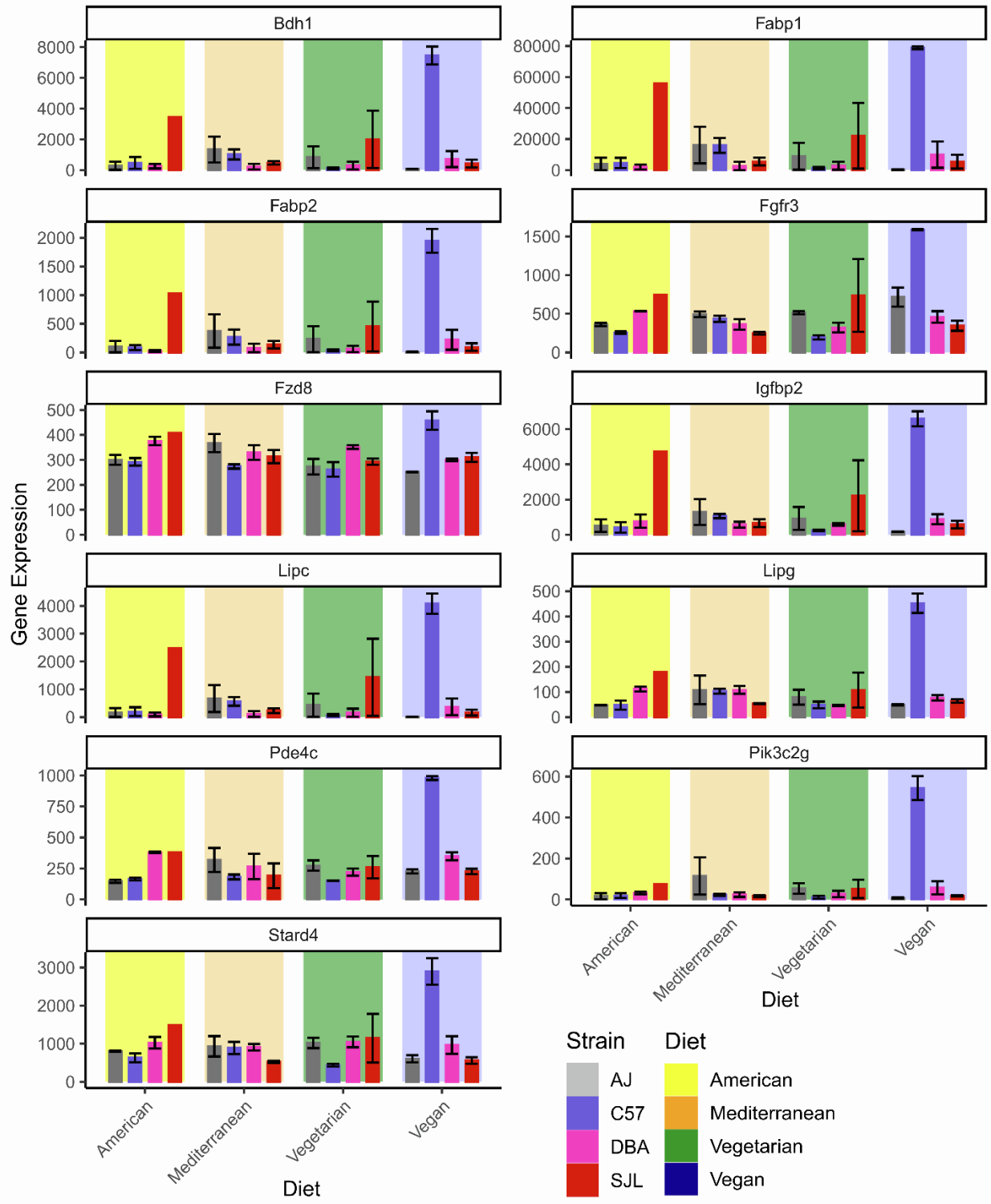
dependent effects (2-way ANOVA, p-value adjusted with FDR correction for $n =$
~31,000 genes). Gene expression plots show normalized counts. Related to Figure 4.



Supplemental Figure 18: Expression of hydroxysteroid dehydrogenase genes that exhibit C57BL/6J-vegan effects among the 421 visceral adipose tissue genes with significant strain:diet-dependent effects (2-way ANOVA, p-value adjusted with FDR correction for n = ~31,000 genes). Gene expression plots show normalized counts. Related to Figure 4.



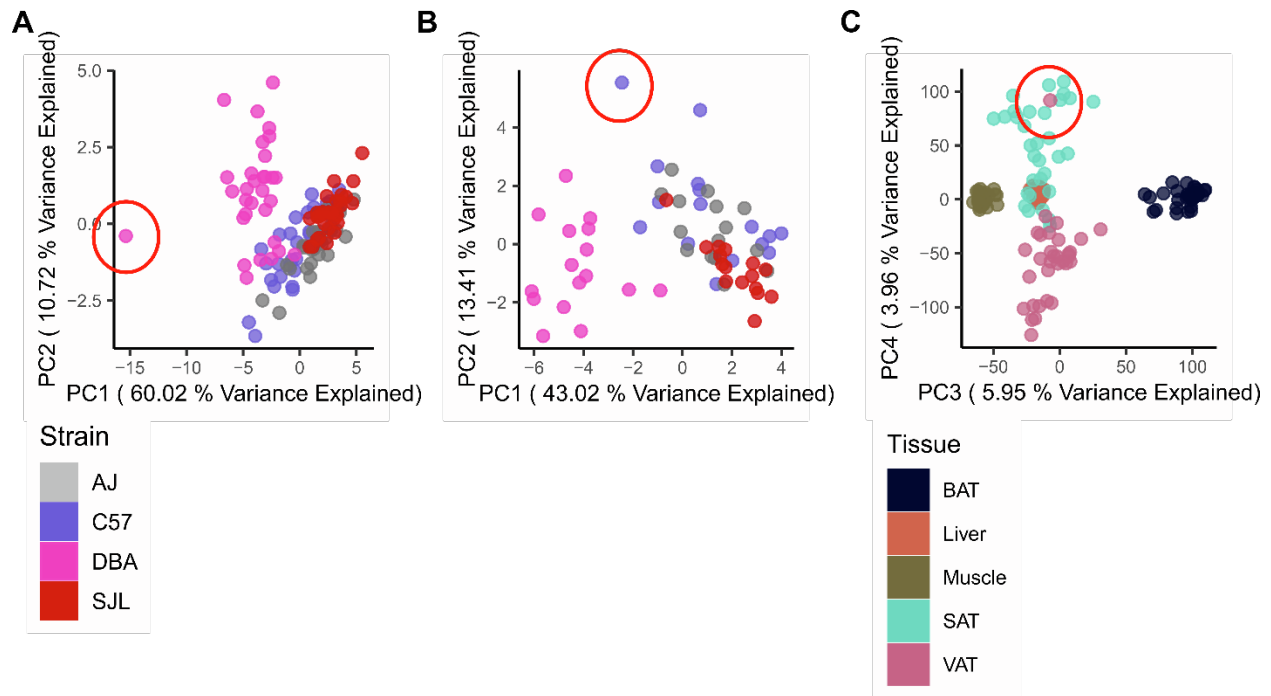
Supplemental Figure 19: Expression of serine protease inhibitor genes that exhibit C57BL/6J-vegan effects among the 421 visceral adipose tissue genes with significant strain:diet-dependent effects (2-way ANOVA, p-value adjusted with FDR correction for $n = \sim 31,000$ genes). Gene expression plots show normalized counts. Related to Figure 4.



Supplemental Figure 20: Expression of other genes that exhibit C57BL/6J-vegan effects among the 421 visceral adipose tissue genes with significant strain:diet-dependent

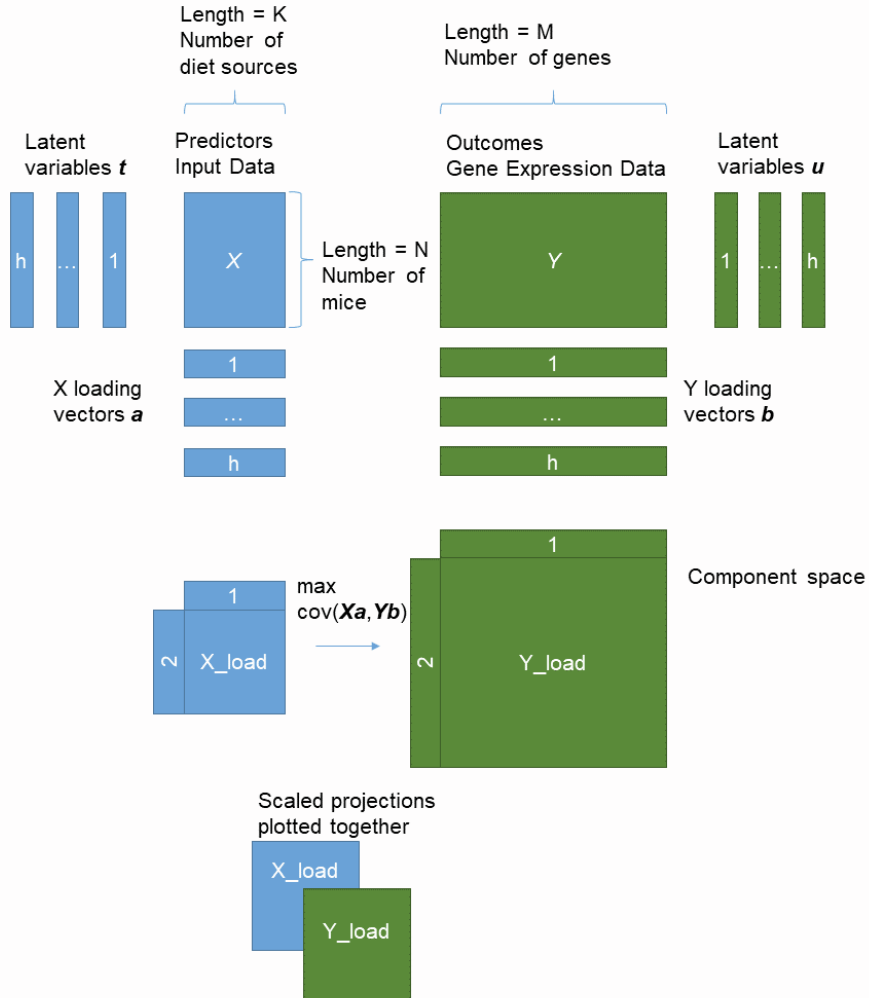
effects (2-way ANOVA, p-value adjusted with FDR correction for $n = \sim 31,000$ genes).

Gene expression plots show normalized counts. Related to Figure 4.



Supplemental Figure 21: Principal components analysis to remove outliers. Related to STAR Methods.

- A) Study 1 phenotypic data plotted on principal components 1 and 2 (PC1 and PC2). An outlier, from a DBA mouse on Mediterranean diet, was identified (circled in red) and removed from subsequent analyses.
- B) Study 2 phenotypic data plotted on PC1 and PC2. An outlier, a VAT RNA sample from a C57 mouse on Vegan diet, was identified (circled in red) and removed from subsequent analyses. This sample was not chosen for RNA sequencing.
- C) Study 2 RNA expression data plotted on PC3 and PC4. An outlier, a VAT RNA sample from an SJL mouse on American diet, was identified (pink dot in the red circle) and removed from subsequent analyses. This outlier is likely a mislabeled SAT sample.



Supplemental Figure 22: Partial least squares regression spaces. Related to STAR

Methods. Predictor and outcome data matrices X and Y were decomposed into loading vectors and latent components, such that the covariance of the representations of X and Y in component space (Xa, Yb) is maximized (Methods). The new axes represent scaled, centered versions of X - and Y - loading vectors, called components, which are linear combinations of X and Y input variables, respectively. The position of X input variables and Y output variables on the new component axes are defined as the calculated X - or Y - loading for each variable.

Supplemental Table 1: Humanized Mouse Diet Ingredients. Related to STAR Methods and Figure 5.

Product #	D19062002	D19062003	D19062004	D19062005
	American	Mediterranean	Vegetarian	Vegan
Ingredient	[g]	[g]	[g]	[g]
Beef, Cooked, Powdered, 5013	39.85	19	0	0
Casein	37.62	34.07	32.81	0
Chicken, Powdered (ChickPRO)	23.4	16.54	0	0
Egg White	20.38	7.47	19.51	0
Fish Protein Isolate	0	16.53	0	0
Peanut Flour	15.3	30.22	51.41	50.89
Wheat Gluten (Sigma G5004)	27.08	29.8	41.71	52.93
Pea Protein, 85% - Pure Bulk	25.26	31.46	24.43	45.36
Soy Protein Isolate, Supro 661	0	0	19.56	30
L-Cystine	0.555	0.513	0.492	1
Corn Starch	54.65	0	19.72	23.87
Maltodextrin	0	0	0	0
Wheat Starch	197.14	218.34	208.03	185.29
Rice Starch	11.12	50.72	19.99	44.6
Potato Starch	40.64	75.87	49.71	43.82
Fructose	39.18	62.95	41.25	44.81
Glucose (Dextrose)	54.31	61.88	47.14	54.8
Lactose	11.85	8.26	41.83	0
Sucrose	84.37	28.24	46.3	53.98
Cellulose, BW200	13.94	53.62	75.38	81.96
Inulin	0	0	0	0
Pectin, Tic Gums 1400	4.04	14.54	19.17	20.87
Psyllium Husk Powder, NOW Foods	5.5	5.5	5.5	5.5
Beta Glucan (Nutrim Oat Bran, 14.1% b glucan)	44.12	30.58	51.4	86.47
Beef Fat, Bunge	13.72	4.76	0	0
Butter, Anhydrous	46.35	12.86	36.58	0
Canola Oil	0	0	15	10.2
Cocoa Butter	0	0	3.16	2.04
Corn Oil	42.1	0	6.16	8.66
Corn Oil, Partially Hydrogenated	9.8	3.1	4	0.69
Flaxseed Oil	4.5	1.84	3.25	6.12
Menhaden Oil (200 ppm tBHQ)	0	13.42	0	0

Olive Oil	15.45	80.9	6.23	35.01
Peanut Oil	0	0	25.83	29.9
Safflower Oil	0	20.7	10.67	16.01
Soybean Oil)	0	0	31.63	29.9
Mineral Mix S10026A	5	5	5	5
Dicalcium Phosphate	13	13	13	13
Calcium Carbonate	5.5	5.5	5.5	5.5
Potassium Citrate, 1 H2O	16.5	16.5	16.5	16.5
Vitamin Mix V10001	10	10	10	10
Beta Carotene (1%, CWS, Powder)	0.241	2.128	1.925	1.96
Biotin (1%)	0.1	0.1	0.1	0
Choline Bitartrate	2	2	2	2
Cholesterol	0.218	0.263	0.08	0
Indole-3-Carbinol (98%, Toronto Research Chemicals I578000)	0	0.0181	0.0185	0.0186
Sulforaphane (95%, Toronto Research Chemicals S699115)	0	0.0053	0.0054	0.0055
Total	934.784	988.1974	1011.9809	1018.6641

Supplemental Table 2: Nutrient Composition of Humanized Mouse Diets. Related to STAR Methods.

Product #	D19062002	D19062003	D19062004	D19062005
	American	Mediterranean	Vegetarian	Vegan
Nutrient	[g]	[g]	[g]	[g]
Protein (including suppl Cystine)	152.7	152.6	152.6	153.1
Supplemental Cystine	0.6	0.5	0.5	1.0
Arginine (from ingredients)	9.0	9.9	10.2	11.2
Histidine (from ingredients)	3.7	3.8	3.7	3.6
Isoleucine (from ingredients)	6.7	6.9	6.9	6.7
Leucine (from ingredients)	11.9	12.2	12.0	11.3
Valine (from ingredients)	7.7	7.6	7.6	7.1
Threonine (from ingredients)	5.7	5.9	5.4	4.9
Lysine (from ingredients)	9.6	9.7	8.0	7.3
Methionine (from ingredients)	3.3	3.2	2.9	2.1
Cystine (from ingredients and suppl Cystine)	2.4	2.3	2.6	3.0
Phenylalanine (from ingredients)	7.3	7.5	7.9	7.8
Tryptophan (from ingredients)	1.6	1.7	1.7	1.6
Protein (not including suppl Amino Acids)	152.1	152.1	152.1	152.1
Animal Protein	92.3	76.5	44.4	0.0
Vegetable Protein (Plant Protein)	59.9	75.6	107.8	152.1
Carbohydrate (Caloric)	507.1	507.1	507.1	507.1
Total Sugars	199.7	171.4	186.6	163.6
Fructose	39.2	63.0	41.3	44.8
Glucose (Dextrose)	54.3	61.9	47.1	54.8
Lactose	11.9	8.3	41.9	0.0
Sucrose	94.4	38.2	56.3	64.0
Starch	263.3	293.0	256.3	254.7
Fat	157.8	157.8	157.8	157.8
Saturated Fatty Acids (SFA)	53.8	33.5	40.7	23.0
Monounsaturated Fatty Acids (MUFA)	56.0	78.1	58.9	69.2
Polyunsaturated Fatty Acids (PUFA)	36.8	38.0	49.7	59.4
Trans	3.5	1.1	1.4	0.2
n-6 Fatty Acids	29.7	14.9	31.8	36.2
n-3 Fatty Acids	3.9	6.1	6.5	7.1
20:5 Eicosapentaenoic Acid (EPA), gm	0.0	1.9	0.0	0.0
22:6 Docosahexaenoic Acid (DHA), gm	0.0	1.4	0.0	0.0
Cholesterol	0.470	0.425	0.180	0.0

Fiber	32.1	81.0	111.7	125.2
Insoluble Fiber	20.8	62.7	86.8	94.0
Soluble Fiber	10.6	17.7	24.8	31.2
Pectins	3.2	11.6	15.3	16.7
Calcium	6.6	6.7	6.7	6.8
Phosphorus	3.4	3.4	3.5	3.3
Sodium	0.9	0.7	0.9	1.0
Potassium	6.6	6.4	6.4	6.7
Choline (Choline Bitartrate)	2.0	2.0	2.0	2.0
Vitamin A (IU)	4007	4006	4006	4000
Beta Carotene (mcg)	2410	21280	19250	19600
Indole-3-Carbinol (mg/kg diet)	0	17.9	17.9	17.9
Sulforaphane (mg/kg diet)	0	5.1	5.1	5.1
g%				
Protein	16.3	15.4	15.0	14.9
Carbohydrate	54.2	51.3	50.1	49.8
Fat	16.9	16.0	15.6	15.5
Cholesterol	0.050	0.043	0.018	0.000
Fiber	3.4	8.2	11.0	12.3
Insoluble Fiber	2.2	6.3	8.6	9.2
Soluble Fiber	1.1	1.8	2.5	3.1
kcal				
Protein	609	609	609	609
Carbohydrate	2028	2028	2028	2028
Fat	1420	1420	1420	1420
Total	4057	4057	4057	4057
kcal/gm	4.34	4.11	4.01	3.98
kcal%				
Protein	15	15	15	15
Carbohydrate	50	50	50	50
Fat	35	35	35	35
Saturated Fatty Acids (SFA)	12	7	9	5
Monounsaturated Fatty Acids (MUFA)	12	17	13	15
Polyunsaturated Fatty Acids (PUFA)	8	8	11	13