Supplemental Material

Intermittent fasting and caloric restriction interact with genetics to shape physiological health in mice

Authors

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Heritability analysis model details

We estimated heritability by fitting the Bayesian model $Y = X\beta + \epsilon$ where ϵ follows multivariate normal distribution with mean 0 and covariance matrix $\sigma^2(2h^2K + (1 - h^2)I)$ where σ^2 is the total phenotypic variance, h^2 is heritability, K is the kinship matrix and I is identity matrix. The prior information is as follows:

$$\sigma^2 \sim InverseGamma(1, 0.5)$$

$$h^2 \sim Uniform(0, 1)$$

$$\beta \sim MultivariateNormal(M, \Sigma)$$

where $M = [0, 0, 0, 0, 0]$ and $\Sigma = 2I_{5X5}$.

Genetic correlation analysis model details

Considering two traits Y_1 and Y_2, we estimated genetic correlation by fitting the Bayesian model: $\begin{bmatrix} Y_1 \\ Y_2 \end{bmatrix} = \begin{bmatrix} X\beta_1 \\ X\beta_2 \end{bmatrix} + \epsilon$, where ϵ follows multivariate normal distribution with mean 0 and covariance matrix $\begin{bmatrix} 2\sigma_{g1}^2 K + \sigma_{e1}^2 I & 2\gamma\sigma_{g1}\sigma_{g2}K + \lambda\sigma_{g1}\sigma_{g2I} \\ 2\gamma\sigma_{g1}\sigma_{g2}K + \lambda\sigma_{g1}\sigma_{g2I} & 2\sigma_{g2}^2 K + \sigma_{e2}^2 I \end{bmatrix}$ where K is the kinship matrix; I is the identity matrix; σ_{g1}^2 and σ_{e1}^2 are genetic and environmental variance for trait Y_1 respectively; σ_{g2}^2 and σ_{e2}^2 are genetic and environmental variance for trait Y_2 respectively; γ is genetic correlation and λ represents the correlation due to an individual's environment. The prior information is as follows:

$$\gamma, \lambda \sim Uniform(-1, 1)$$

$$\beta_1, \beta_2 \sim MultivariateNormal(M, \Sigma)$$

where $M = [0, 0, 0, 0, 0]$ and $\Sigma = 2I_{5X5}$. $\sigma_{g1}^2, \sigma_{g2}^2, \sigma_{e1}^2$ and σ_{e2}^2 are estimated by fitting each trait individually with diet as fix effect and kinship as random effect using maximum likelihood method.

Phenotyping Procedure	Trait	AL	1D	2D	20	40	Total
Rotarod (RR)	RR_FIRS	153	176	184	154	163	830
	RR_SLOP	153	176	184	154	163	830
	RR_TAVG	153	176	184	154	163	830
Grip strength (GS)	GS_APAW	185	176	184	184	178	907
	GS_FORE	182	176	184	183	178	903
Echocardiogram (EC)	EC_COUT	180	172	180	182	171	885
	EC_EFRA	180	172	180	182	171	885
	EC_HRAT	180	172	180	182	171	885
	EC_IVSD	180	172	180	182	169	883
	EC_IVSS	180	172	180	182	171	885
	EC_LVID	180	172	180	182	171	885
	EC_LVIS	180	172	180	182	171	885
	EC_LVMA	180	172	179	182	169	882
	EC_LVPD	180	172	179	182	170	883
	EC_LVPS	180	172	180	181	171	884
	EC_STRO	180	172	180	181	171	884
l-energy X-ray absorptiometry	DX_BARE	185	171	177	184	176	893
	DX_BODY	185	171	177	184	176	893
	DX_BOMC	185	171	177	184	176	893
	DX_BOMD	184	171	177	184	176	892
	DX_PFAT	185	171	177	184	176	893
	DX_SIZE	185	171	176	184	175	891
	DX_TARE	185	171	177	184	176	893
	DX_TTME	185	171	177	184	176	893
aze spontaneous alternation (	YM_DIST	174	149	154	172	167	816
	YM_ENTR	174	149	154	172	165	814
	YM_EPIS	175	149	154	172	167	817
	YM_MAXS	174	149	153	170	166	812
	YM_PALT	175	147	154	172	166	814
	YM_TIME	175	149	154	172	167	817
Acoustic startle (AS)	AS_SLOP	127	138	136	129	122	652
	AS_TAUC	138	143	148	135	142	706
	AS_XINF	132	141	141	134	131	679
	AS_YINF	133	141	140	134	131	679
Wheel running (WR)	WR_DDAY	178	170	173	178	167	866
	WR_DIST	182	173	176	183	174	888
	WR_DNIG	182	173	176	183	177	891
	WR_MDAY	181	172	177	179	175	884
	WR_MNIG	182	173	178	183	178	894
	WR_MSPE	181	171	178	182	176	888
	WR_TDAY	181	172	177	182	172	884
	WR_TNIG	182	174	178	183	178	895

Table S1 Total number of samples per trait and per diet after outlier removal.

Cluster size	Threshold
2	0.55
3	0.59
4	0.64
5	0.66
6	0.68
7	0.71
8	0.71
9	0.75
10	0.73
11	0.75
12	0.75
13	0.76
14	0.77
15	0.79

Table S2 Significance threshold for unsupervised hierarchical clustering analysis.

Cluster	Trait (s)	Description	PC Trait Abbreviation
1	EC_LVPD, EC_LVPS	ECHO 1.- LV posterior wall thickness	PC_ECL
2	EC_IVSD, EC_IVSS	ECHO 2.- IV septum thickness	PC_ECI
3	EC_EFRA, EC_LVIS	ECHO 3.- ejection fraction	PC_ECE
4	RR_FIRS, RR_SLOP, RR_TAVG	rotarod	PC_ROR
5	DX_BARE, DX_BOMC, DX_BOMD	DEXA i.- bone composition	PC_DXB
6	AS_TAUC, AS_YINF	Hearing	PC_DXB
7	EC_COUT, EC_LVID, EC_LVMA, EC_STRO	ECHO 4.- heart output	PC_ECC
8	WR_DDAY, WR_MDAY, WR_TDAY	Wheel Run 1.-day	PC_WRD
9	DX_BODY, DX_PFAT, DX_TARE, DX_TTME	DEXA ii.- body composition	PC_DXS
10	WR_DIST, WR_DNIG, WR_MNIG, WR_MSPE, WR_TNI	Wheel Run 2. - night	PC_WRN

Singletons

1	EC_HRAT	ECHO - heart rate
2	AS_XINF	Hearing - model fit, x intercept
3	GS_APAW	Grip Strength - All Paw
4	GS_FORE	Grip Strength - Fore Paw
5	AS_SLOP	Hearing - model fit, slope
6	DX_SIZE	DEXA - body length

Table S3 Principal component derived trait descriptions. For trait groups identified in hierarchical clustering analysis, we list the directly measured and the principal component derived traits.

	Diet	Model	LOD_model_I − LOD_model_II
PC_ECE2 Chr16: UNC26651633	1D	Model I: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet20} * G + \text{Diet2D} * G + \text{Diet40} * G + \text{Kinship} + E$ Model II: $Y = \text{Diet} + G + \text{Diet2D} * G + \text{Diet20} * G + \text{Diet40} * G + \text{Kinship} + E$	3.4
	20	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet2D} * G + \text{Diet40} * G + \text{Kinship} + E$	7.2
	2D	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet20} * G + \text{Diet40} * G + \text{Kinship} + E$	7.7
	40	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet20} * G + \text{Diet2D} * G + \text{Kinship} + E$	1.8
	1D/20	Model II: $Y = \text{Diet} + G + \text{Diet2D} * G + \text{Diet40} * G + \text{Kinship} + E$	8.1
	20/2D	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet40} * G + \text{Kinship} + E$	11.6
	20/40	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet2D} * G + \text{Kinship} + E$	8.9
AS_YINF Chr9: UNC16962149	1D	Model I: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet20} * G + \text{Diet2D} * G + \text{Diet40} * G + \text{Kinship} + E$ Model II: $Y = \text{Diet} + G + \text{Diet2D} * G + \text{Diet20} * G + \text{Diet40} * G + \text{Kinship} + E$	3.6
	20	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet2D} * G + \text{Diet40} * G + \text{Kinship} + E$	0.7
	2D	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet20} * G + \text{Diet40} * G + \text{Kinship} + E$	3.9
	40	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet20} * G + \text{Diet2D} * G + \text{Kinship} + E$	4.8
	1D/40	Model II: $Y = \text{Diet} + G + \text{Diet20} * G + \text{Diet2D} * G + \text{Kinship} + E$	8.9
	20/40	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet2D} * G + \text{Kinship} + E$	7.3
	2D/40	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet20} * G + \text{Kinship} + E$	7.2
PC_ECL1* Chr2: JAX00486864	1D	Model I: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet20} * G + \text{Diet2D} * G + \text{Diet40} * G + \text{Kinship} + E$ Model II: $Y = \text{Diet} + G + \text{Diet2D} * G + \text{Diet20} * G + \text{Diet40} * G + \text{Kinship} + E$	6.2
	20	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet2D} * G + \text{Diet40} * G + \text{Kinship} + E$	5.6
	2D	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet20} * G + \text{Diet40} * G + \text{Kinship} + E$	1.8
	40	Model II: $Y = \text{Diet} + G + \text{Diet1D} * G + \text{Diet20} * G + \text{Diet2D} * G + \text{Kinship} + E$	1.8
	1D/20	Model II: $Y = \text{Diet} + G + \text{Diet2D} * G + \text{Diet40} * G + \text{Kinship} + E$	11.9
	1D/2D	Model II: $Y = \text{Diet} + G + \text{Diet20} * G + \text{Diet40} * G + \text{Kinship} + E$	9.4
	1D/40	Model II: $Y = \text{Diet} + G + \text{Diet20} * G + \text{Diet2D} * G + \text{Kinship} + E$	8.0

*Note: EC_LVPD (Chr2: UNCHS004526) has the same pattern as PC_ECL1.

Table S4 Reduced genotype x diet association model test. For each lead marker at a GxD interaction QTL, we compare the LOD scores of full (Model I) and reduced (Model II) genetic association models. Reduced models test the effect of four, non AL diets in isolation, and for the single diet with the maximum difference between Model I and Model II LOD score, the three possible two diet combinations are also tested.
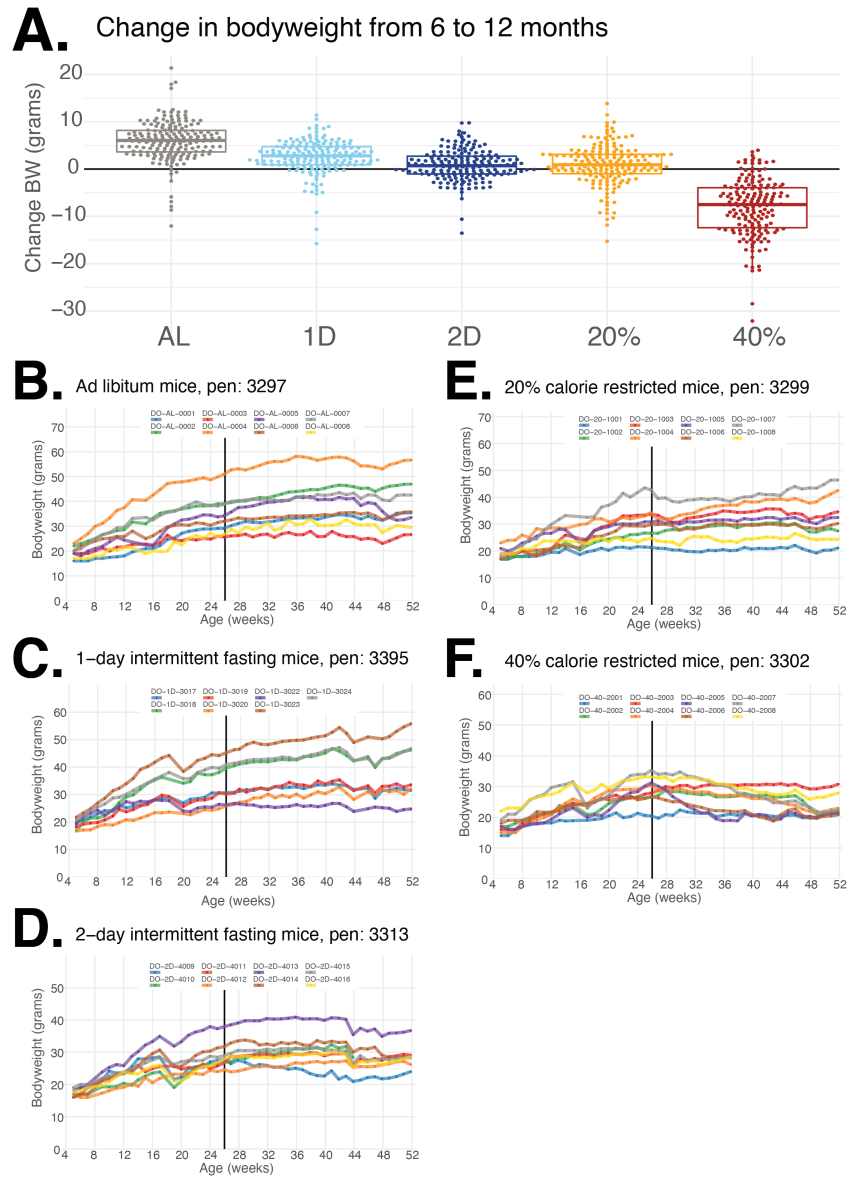
Figure S1 Change in body weight following dietary intervention. A. Diet-specific change in body weight after six months of intervention. Dots show values for individual mice. Box plots show median, 25% and 75% quantiles. B-F. Weekly body weight values for group housed mice in a single representative pen for each dietary intervention.
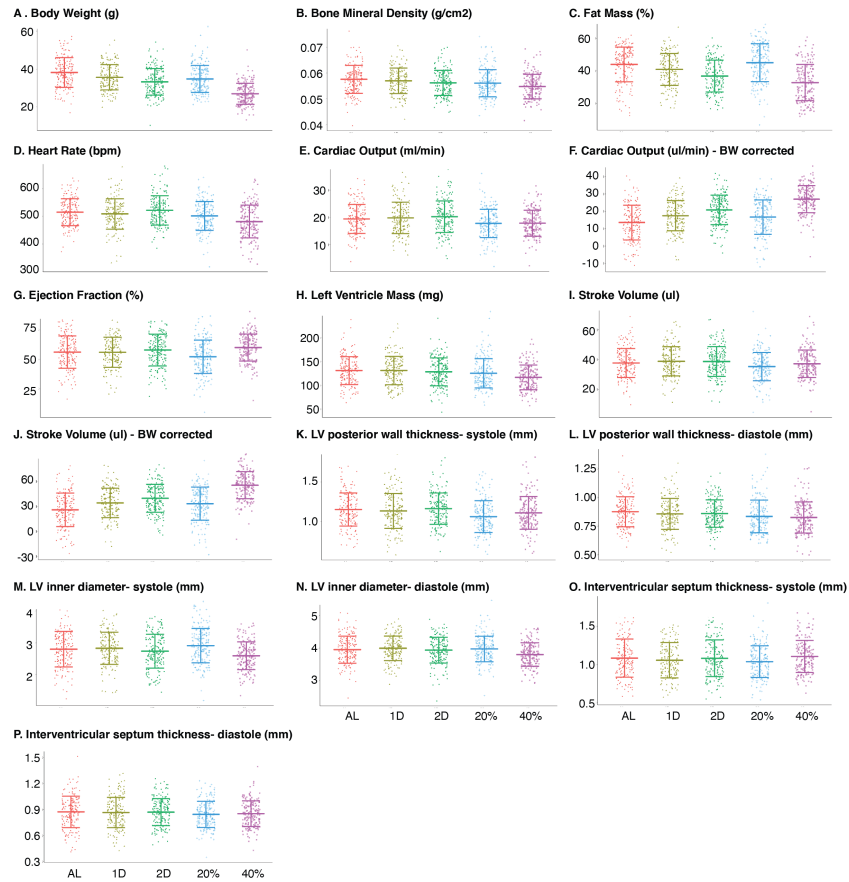
Figure S2 Diet specific trait values for DEXA and echocardiogram assays. Horizontal bars display Mean +/- SD. For cardiac output (EC_COUT) and stroke volume (EC_STRO) we present the raw values and body weight corrected values (calculated following the grip strength and rotarod specific analyses described in Methods.)
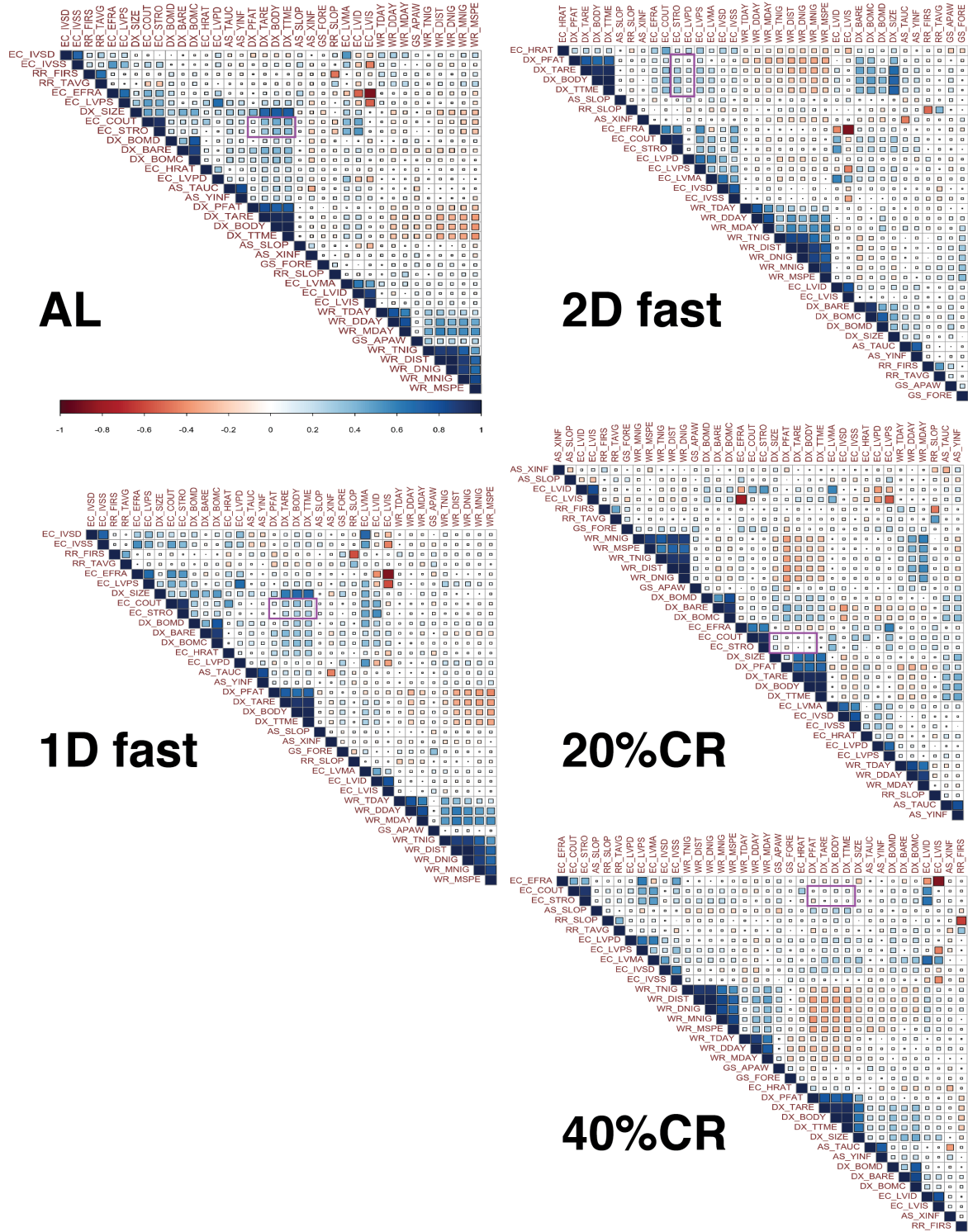
Figure S3 Diet-specific pairwise phenotypic correlation values. Size and color of squares represent the positive (blue) or negative (red) correlation values. Purple box highlights pairwise correlations between cardiac output and stroke volume (EC_COUT, EC_STRO) and multiple body composition traits (DX_PFAT, DX_TARE, DX_BODY, and DX_TTME).
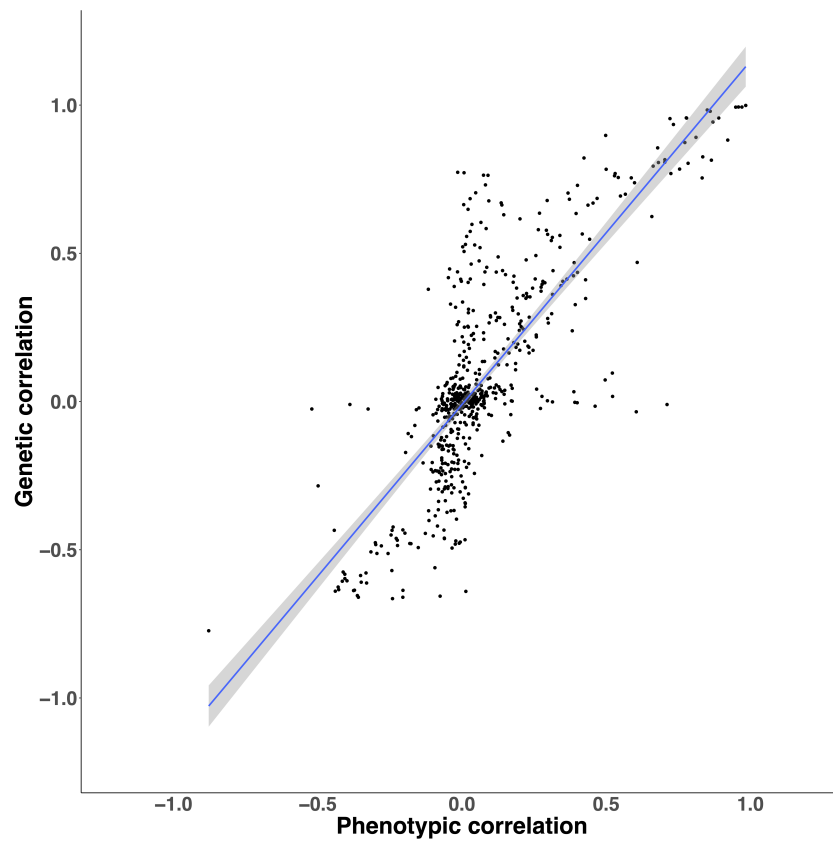
Figure S4 Scatterplot of phenotypic versus genetic correlations. Grey line depicts linear correlation with 95% CI in shaded area.
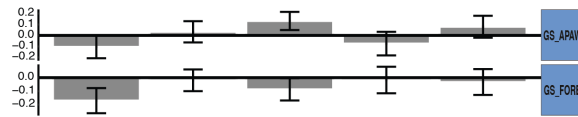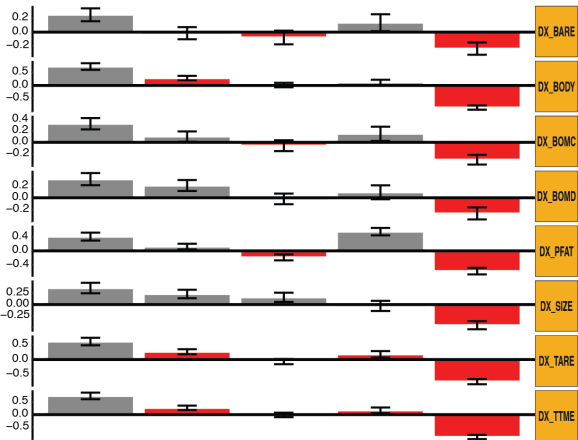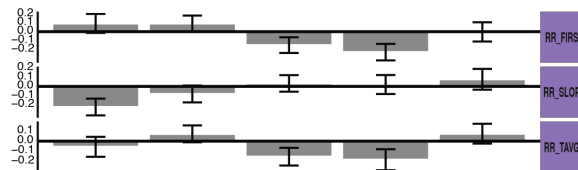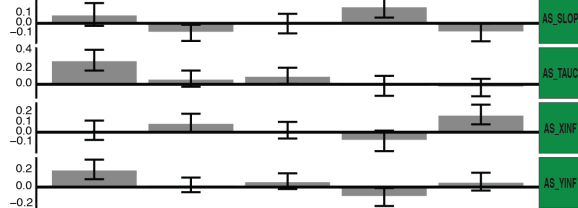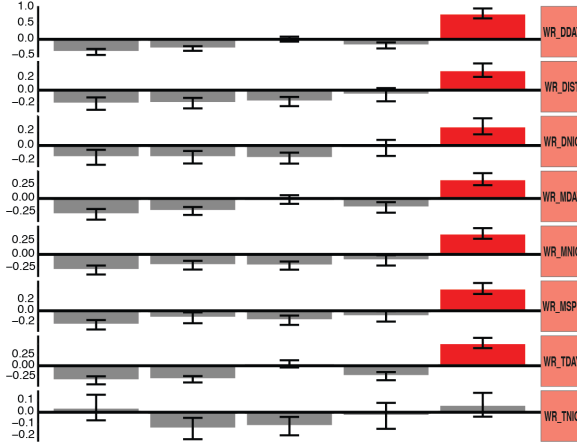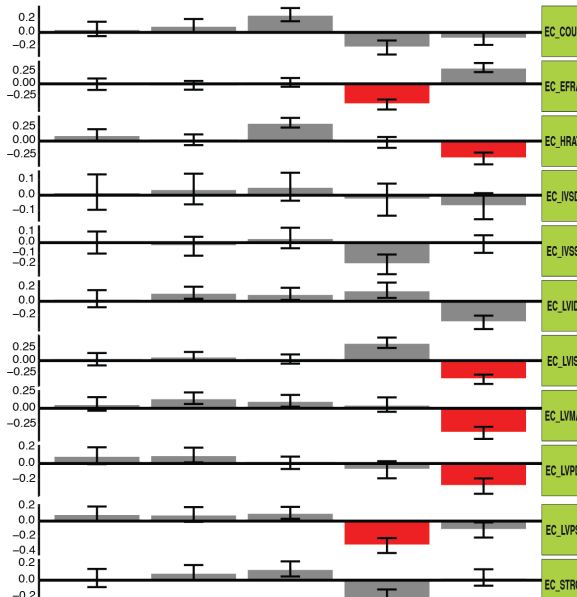
Figure S5 Diet specific trait values for the subset of animals with no missing data. Diet specific mean (SE) trait values for all experimental procedures for the subset of animals ($N = 525$) with no missing data across any trait. All trait values were z-score transformed following batch and generation correction. Red bars denote traits that were significantly different from AL diet at a Westfall-Young multiple tests adjusted p-value threshold of 0.05.
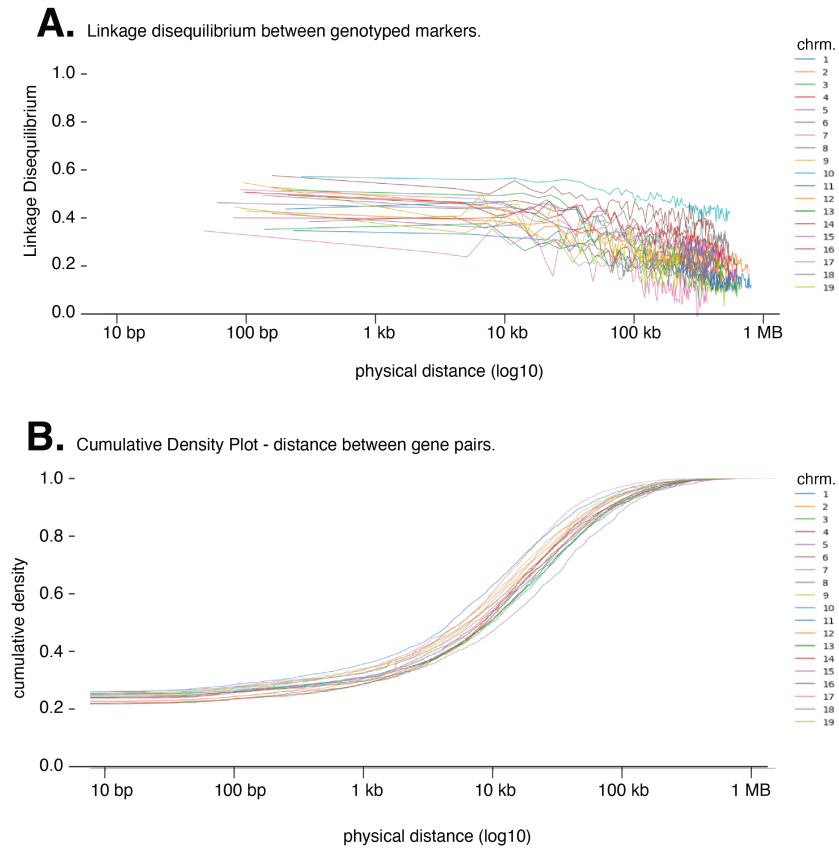
A. Linkage disequilibrium between genotyped markers.

B. Cumulative Density Plot - distance between gene pairs.

Figure S6 Linkage disequilibrium per chromosome A. For each chromosome, we measured linkage disequilibrium (LD) between all genotyped markers. LD was measured as the Pearson correlation value between allele counts (e.g. 0, 1 or 2) at each marker pair. Line plot shows the median LD for all marker pairs binned by physical distance percentiles, interval size 1% B. Cumulative density plot of median pairwise distance between nearest neighbor gene pairs. Pairs binned by physical distance percentiles, interval size 1%.
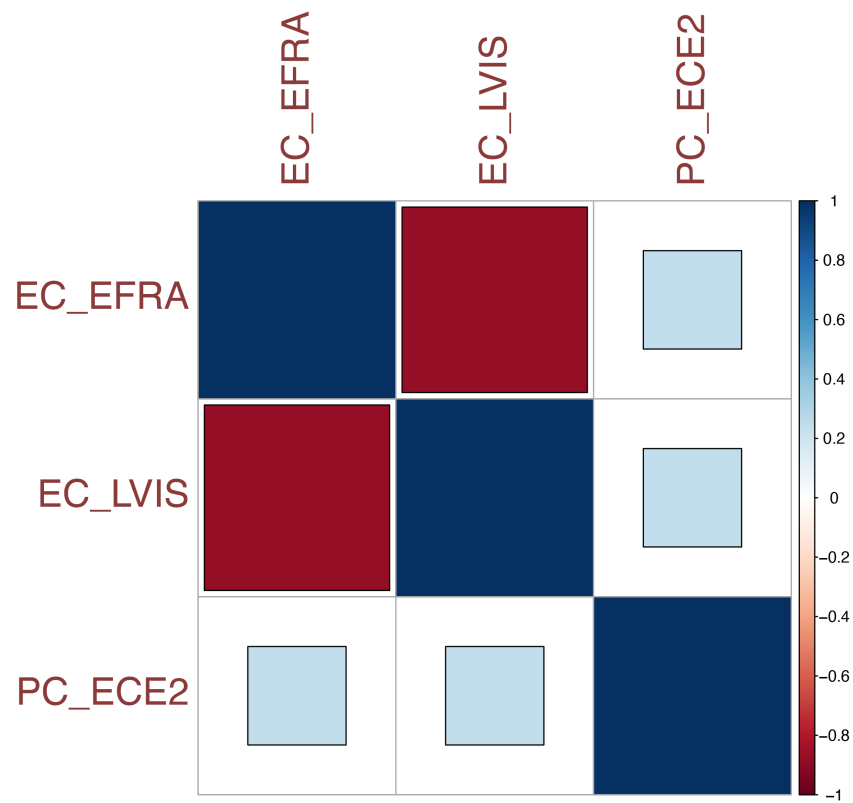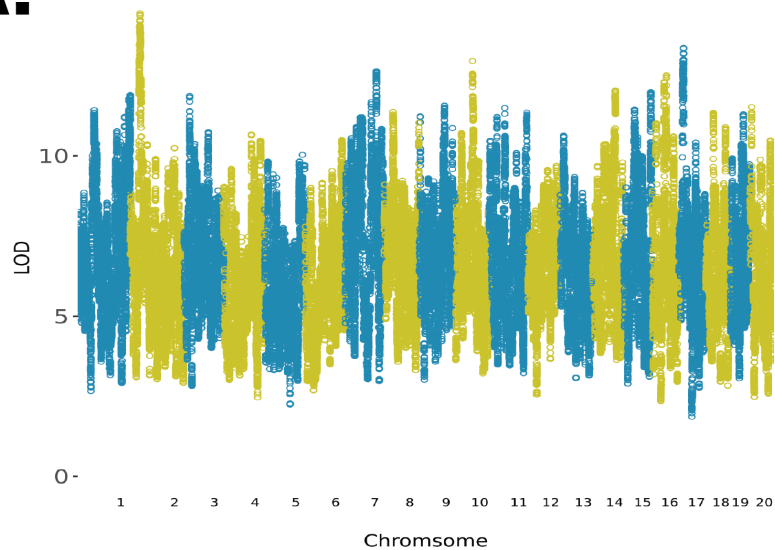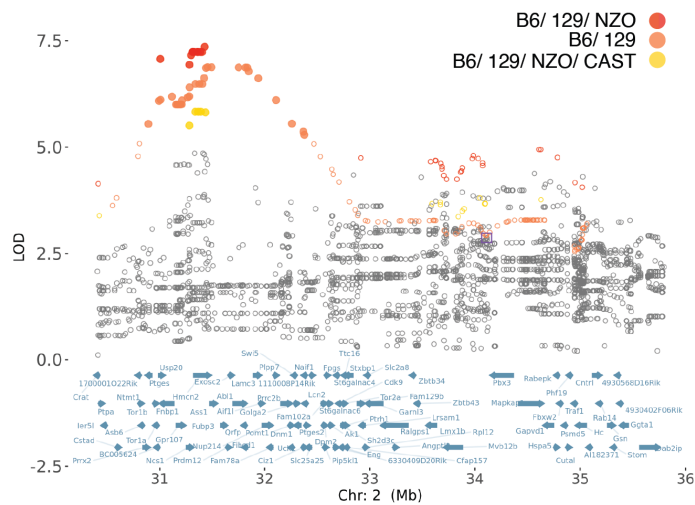
Figure S7 Pairwise Pearson correlation values. Correlation between PC_ECE2 and the two directly measured traits used to calculate this principle component analysis trait: EC_EFRA and EC_LVIS.
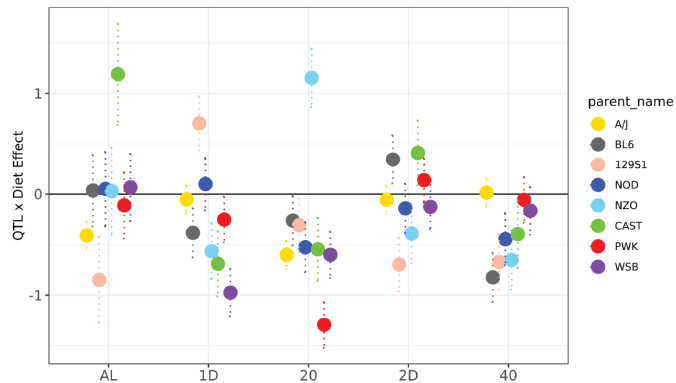
Figure S8 QTL Mapping for EC_LVIS. A. Manhattan plot of diet-dependent genome-wide linkage mapping results for EC_LVIS. B. Fine-mapping of chromosome 2 locus. Rank 1, 2, and 3 FAP variants shown in red, orange, and yellow circles. C. Diet-specific effect of lead genotyped variant for each of the eight founder variants.