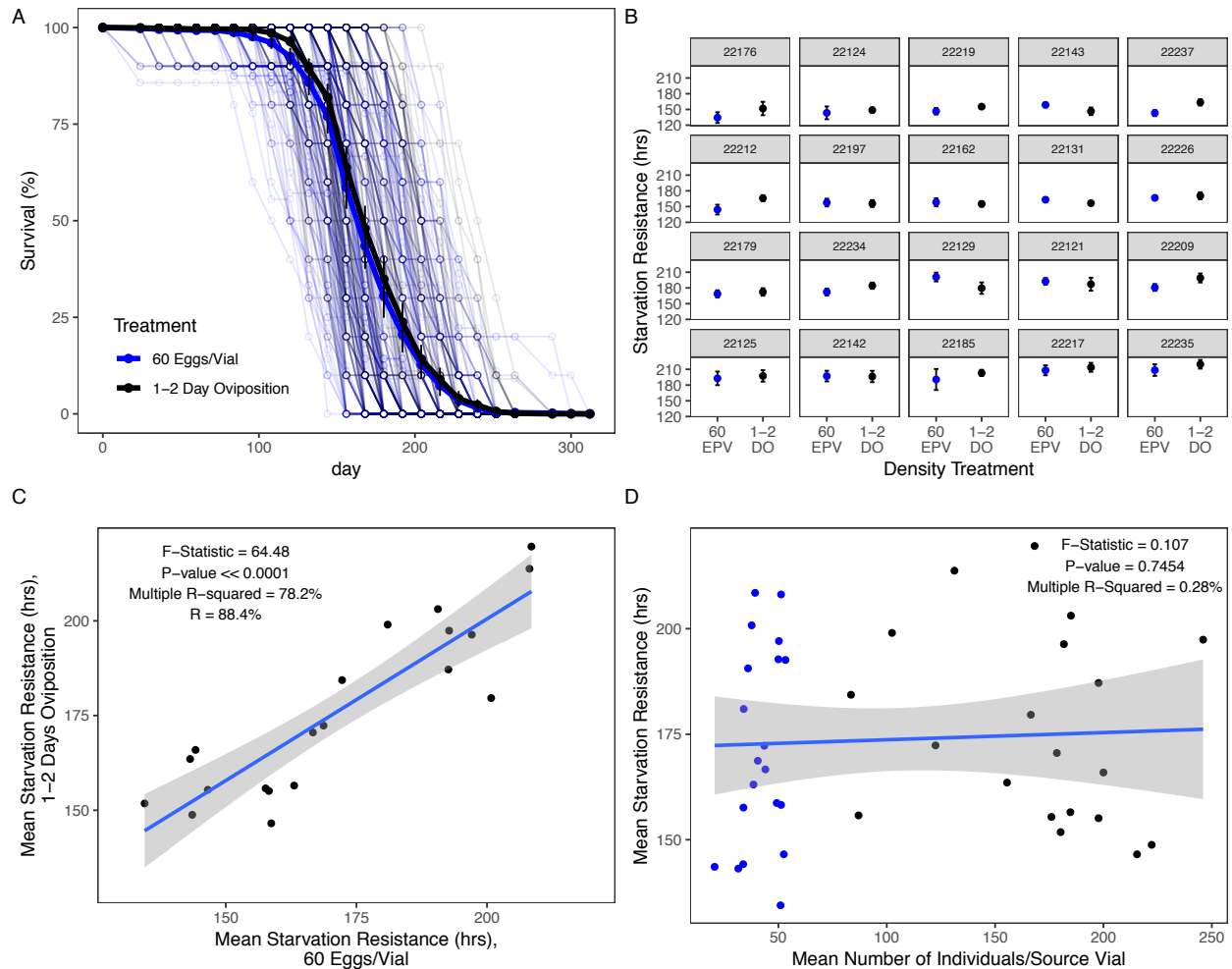# Supplemental Figures

Figure S1. *Egg density in vials used to generate experimental individuals has a limited influence on starvation resistance. This was tested in 20 randomly-selected DSPR RILs by rearing experimental individuals according to two treatments: with 60 eggs per vial (60 EPV) or with 1-2 days of oviposition (1-2 DO). The total number of adults emerging from each source vial was also counted. Starvation resistance of the experimental individuals from each density treatment was measured as described for the large-scale starvation screen. A. Percent survival per vial at each 12-hr assessment point was very similar throughout the course of this experiment regardless of rearing density. Bold lines and points indicate the overall mean ($\pm$95% CI) survival for each treatment group at each 12-hr assessment point. B. Mean ($\pm$95% CI) starvation resistance for each of the 20 randomly-selected DSPR RILs was rarely influenced by the density treatment. Overall, density treatment had a minor effect on the average lifespan of each DSPR RIL ($F_{1,19}$ = 18.15, P < 0.0001, % Variance Explained = 0.90%; see Table S1 for full breakdown of variance components). C. Mean starvation resistance by DSPR RIL was strongly correlated between the two density treatments. D. The mean number of individuals per source vial of experimental flies did not explain a significant amount of variation in starvation resistance. In A, B, and D, black corresponds to the 1-2 day oviposition treatment; blue corresponds to the 60 eggs per vial treatment. In C and D, grey shading represents the 95% CI of the regression.*
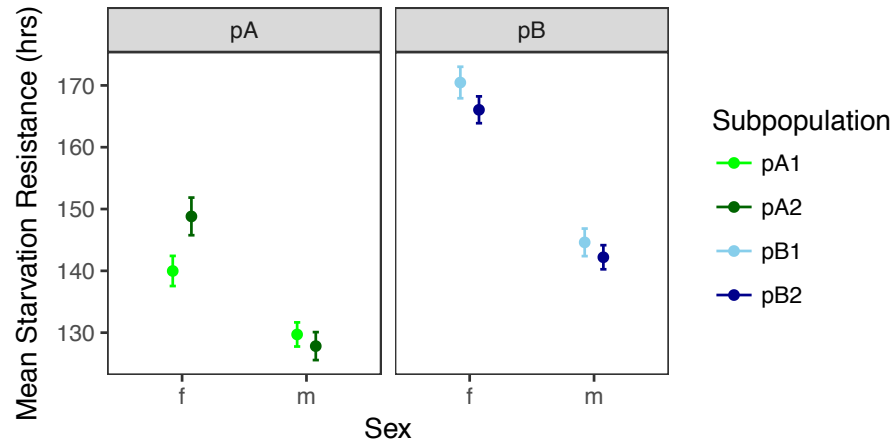
Figure S2. *Mean (± 95% CI) starvation resistance per subpopulation and sex. Sex and subpopulation interact to influence starvation resistance ($F_{3,3440} = 18.317$; $p < 0.0001$), though only females from the pA1 and pA2 subpopulations were significantly different within a panel (Tukey's HSD adj. $p < 0.0001$).*
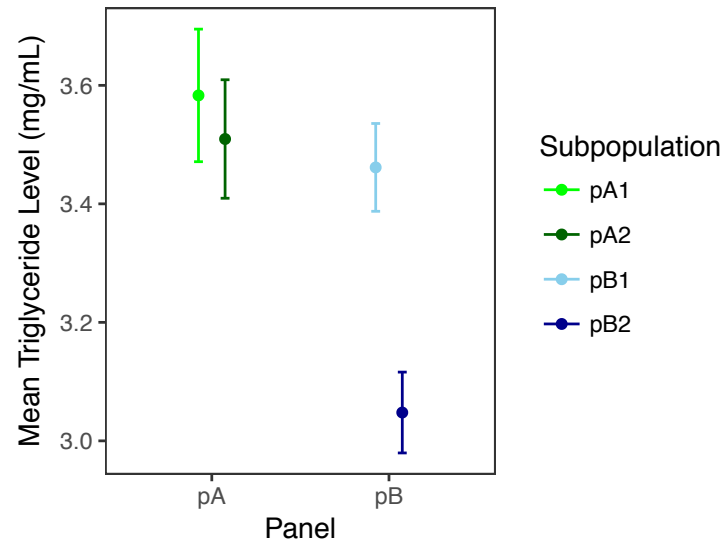
Figure S3. *Mean female triglyceride level per subpopulation (± 95% CI). Subpopulation influenced triglyceride level ($F_{3,935}$ = 37.099; p < 0.0001), though this was driven by differences between the pB subpopulations (Tukey's HSD adj. p < 0.0001)*
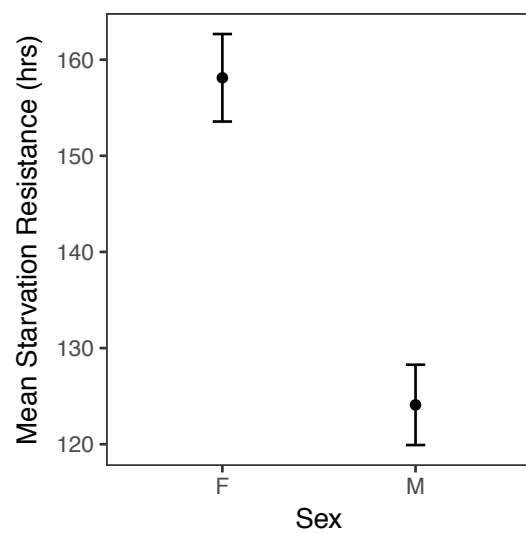
Figure S4. *Mean starvation resistance ($\pm$ 95% CI) for males and females in the DGRP ($F_{1,334}$ = 118.21, p < 0.0001).*
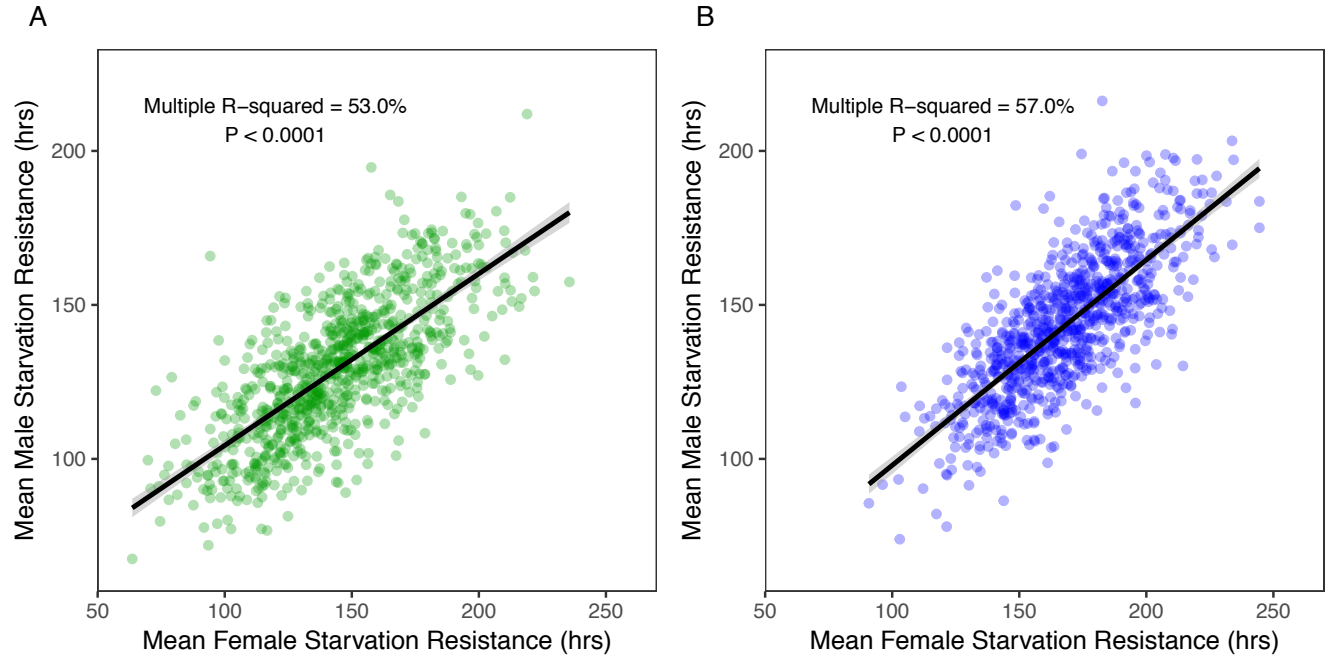
Figure S5. *Correlation between sex-specific mean starvation resistance for the DSPR pA mapping panel (A) and pB mapping panel (B). Sex-specific responses were significantly correlated for both panels (pA: $F_{1,859}$ = 968.5, P < 0.0001; pB: $F_{1,860}$ = 1138, p < 0.0001). Grey shading around the regression line in both plots indicates the 95% confidence interval.*
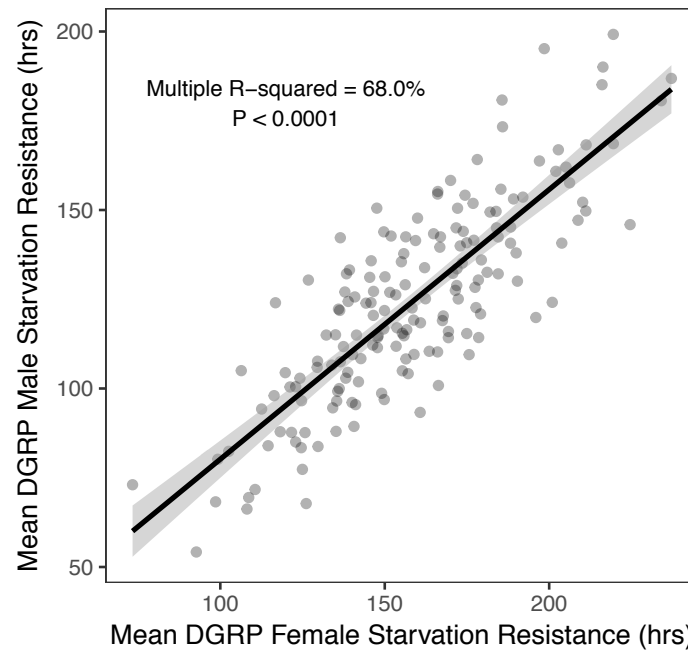
Figure S6. Male and female mean starvation resistance was significantly correlated in the DGRP ($F_{1,334}$ = 118.21, $P < 0.0001$). Grey shading around the regression line indicates the 95% confidence interval.
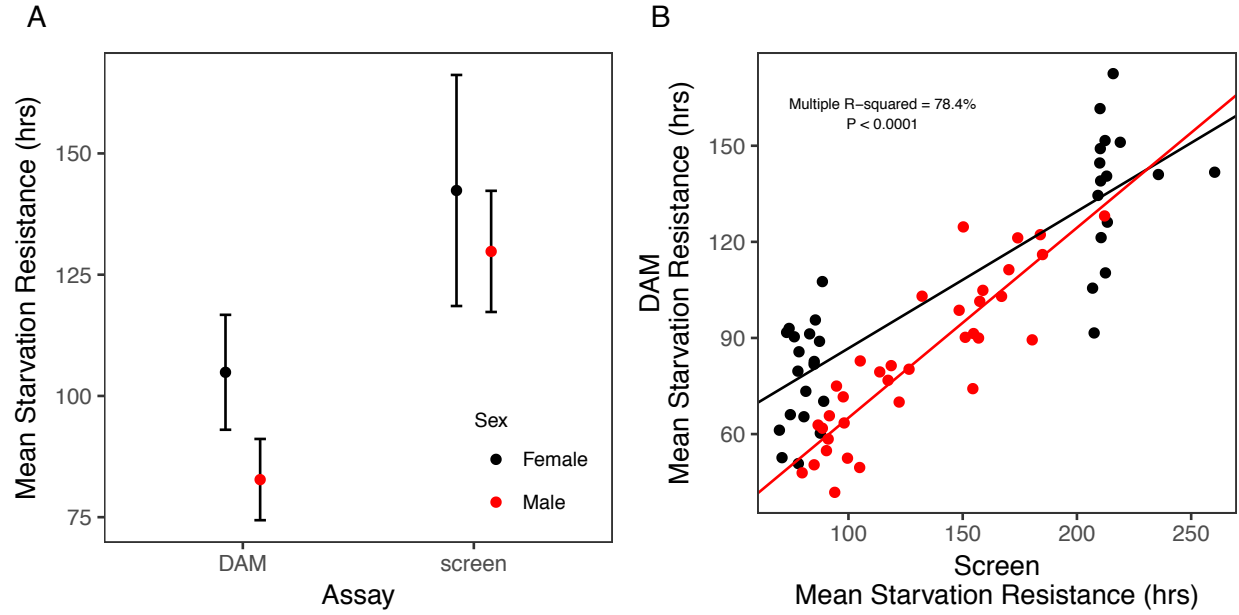
Figure S7. *A. Starvation resistance was significantly higher in the large-scale starvation screen of all DSPR RILs compared to the DAM (Drosophila Activity Monitor) starvation assay for the selected subset of RILs (Assay: $F_{1,136}$ = 31.60, p < 0.0001). Mean starvation resistance across RIL means is presented ($\pm$ 95% CI). B. Mean starvation resistance measured in the large-scale starvation resistance screen (x-axis) was correlated with mean starvation resistance measured in the DAM assay (y-axis) in the DSPR (Females: β = 0.43 $\pm$ 0.04, t = 9.7, p < 0.0001, R^2 = 73.9%; Males: β = 0.59 $\pm$ 0.05, t = 10.9, p < 0.0001, R^2 = 78.3%). The multiple R^2 value in the plot includes the interaction between starvation resistance measured under different assay conditions with sex.*
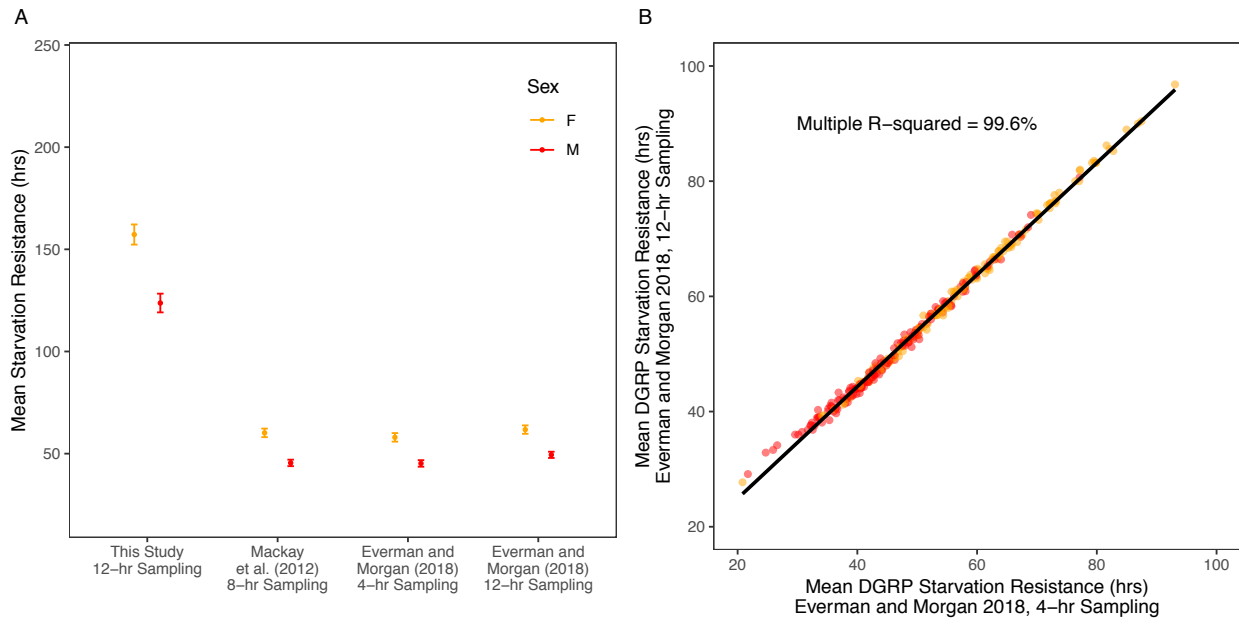
Figure S8. *A. Mean starvation resistance ($\pm$ 95% CI) was significantly higher in this study compared to Mackay et al. (2012) and Everman and Morgan (2018) ($F_{2.532}$ = 1457.5, P < 0.0001). The increased mean and variation in starvation resistance observed in this study was not driven by differences in the frequency at which survival was assessed, since a re-analysis of data from Everman and Morgan (2018) with a longer interval between fly counting events matching the interval from the present study, revealed essentially no difference in the phenotypes assayed. B. Mean starvation resistance by line and sex measured according to the 4-hr sampling interval was highly correlated to our re-analysis of the Everman and Morgan (2018) data using a 12-hr sampling interval.*

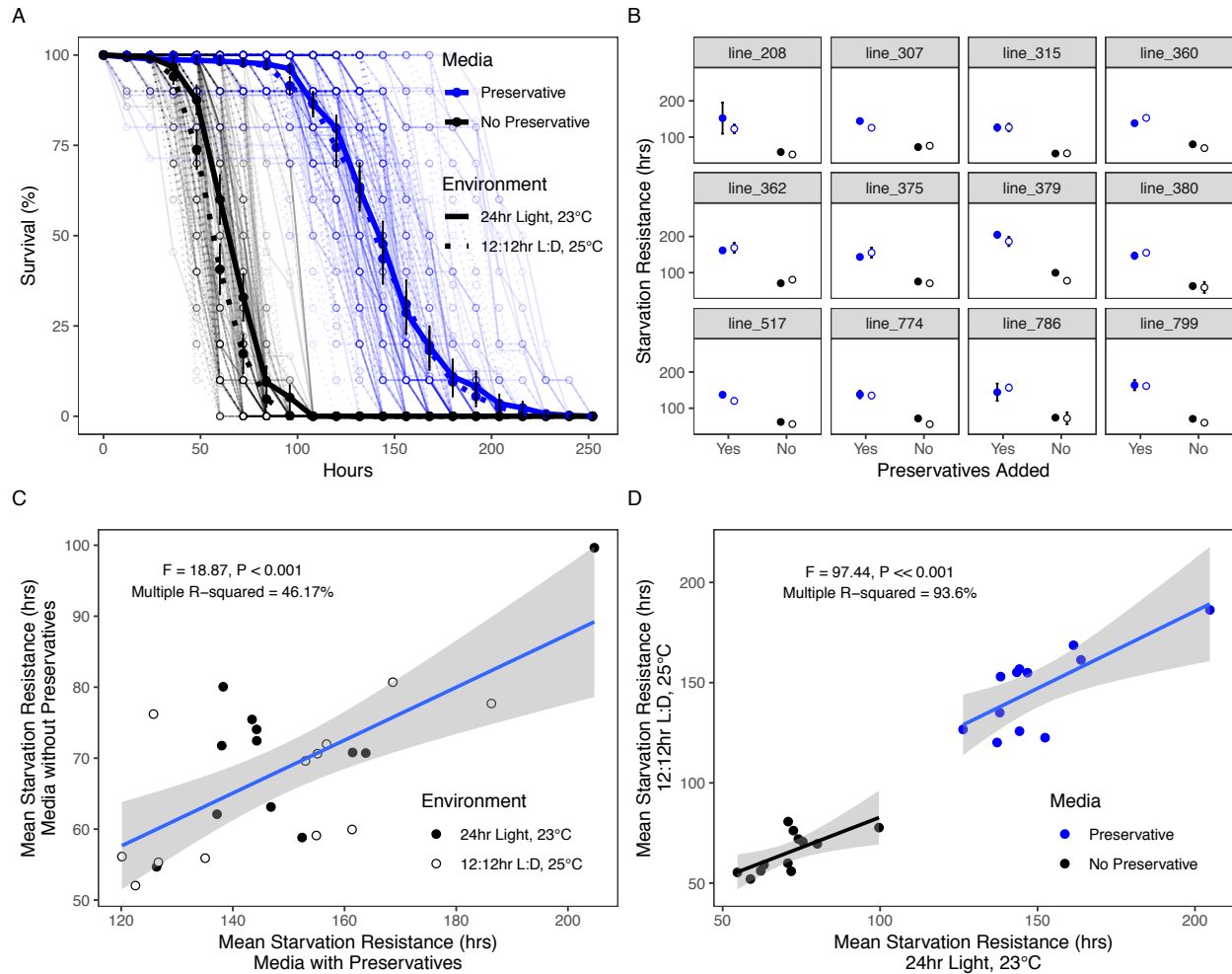***Figure S9.*** *Flies maintained on starvation media with preservatives lived much longer than flies on starvation media without preservatives, regardless of environmental conditions (24-hr light, 23°C as used in the large-scale starvation screen vs. 12:12hr L:D, 25°C as used in Mackay* et al. *(2012) and Everman and Morgan (2018)). This was tested in 12 randomly-selected DGRP lines. A. Percent survival per vial was different between the two media treatments, and differed slightly due to environment, but only when media did not contain preservatives. Black lines and points indicate media with no preservatives; blue lines and points indicate media with preservatives; solid lines indicate the 24hr Light, 23°C environment; dashed lines indicate the 12:12hr L:D, 25°C treatment. The bold points and lines for each treatment indicate the overall mean (±95% CI) survival of each treatment group at each 12-hr assessment point. B. Mean (±95% CI) starvation resistance for each of the 12 randomly selected DGRP lines was rarely influenced by the environment treatment (closed symbols = 24hr Light, 23°C; open symbols = 12:12hr L:D, 25°C), but media preservatives consistently resulted in higher survival for each DGRP line. C. Mean starvation resistance by DGRP line was significantly correlated between the two media treatments. D. DGRP line, preservatives in the media, and environmental conditions together explained nearly all phenotypic variation in starvation resistance. The full reporting of variance components is presented in Table S6. In C and D, grey shading represents the 95% CI of the regression.*
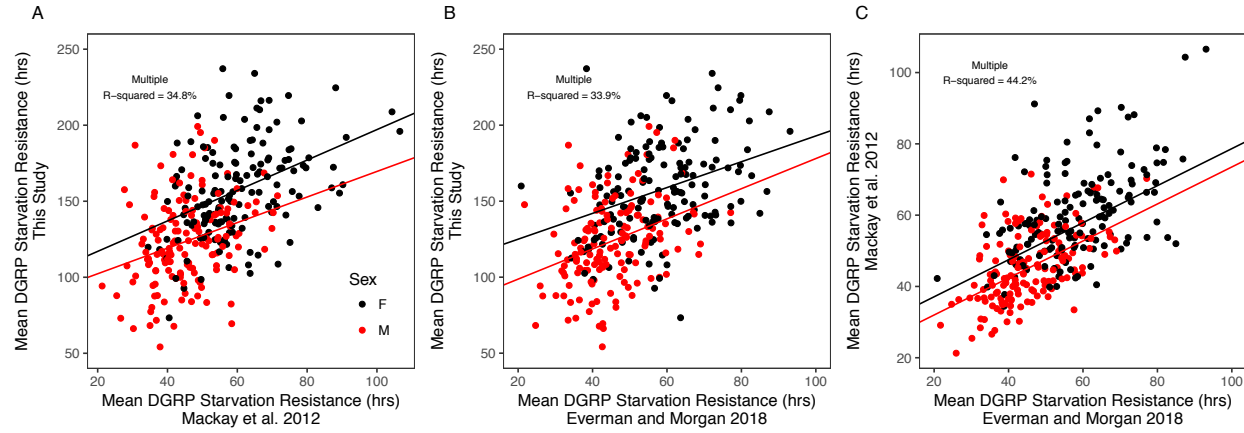
Figure S10. *Correlation between sex-specific mean starvation resistance in the DGRP panel for 150 lines that overlap between this study, Mackay* et al. *2012, and Everman and Morgan 2018. Red points indicate males and black points indicate females. All comparisons showed that the three independent measures of starvation resistance were significantly correlated (A: $F_{3,296}$ = 52.69, p < 0.0001; B: $F_{3, 296}$ = 50.66, p < 0.0001; C: $F_{3.296}$ = 78.18, p < 0.0001).*

Figure S11. *DSPR RILs with low starvation resistance ranged from low to high for other measures of fitness.*
A. Distribution of mean female lifespan in DSPR pB RILs (Highfill et al. *(2016). RILs with low female*
starvation resistance (in the bottom 25% of the distribution) are shown in solid black symbols, while other
RILs are shown in gray. B. The correlation between mean female starvation resistance and mean female
lifespan is minimal, suggesting there is no association between low starvation resistance and reduced
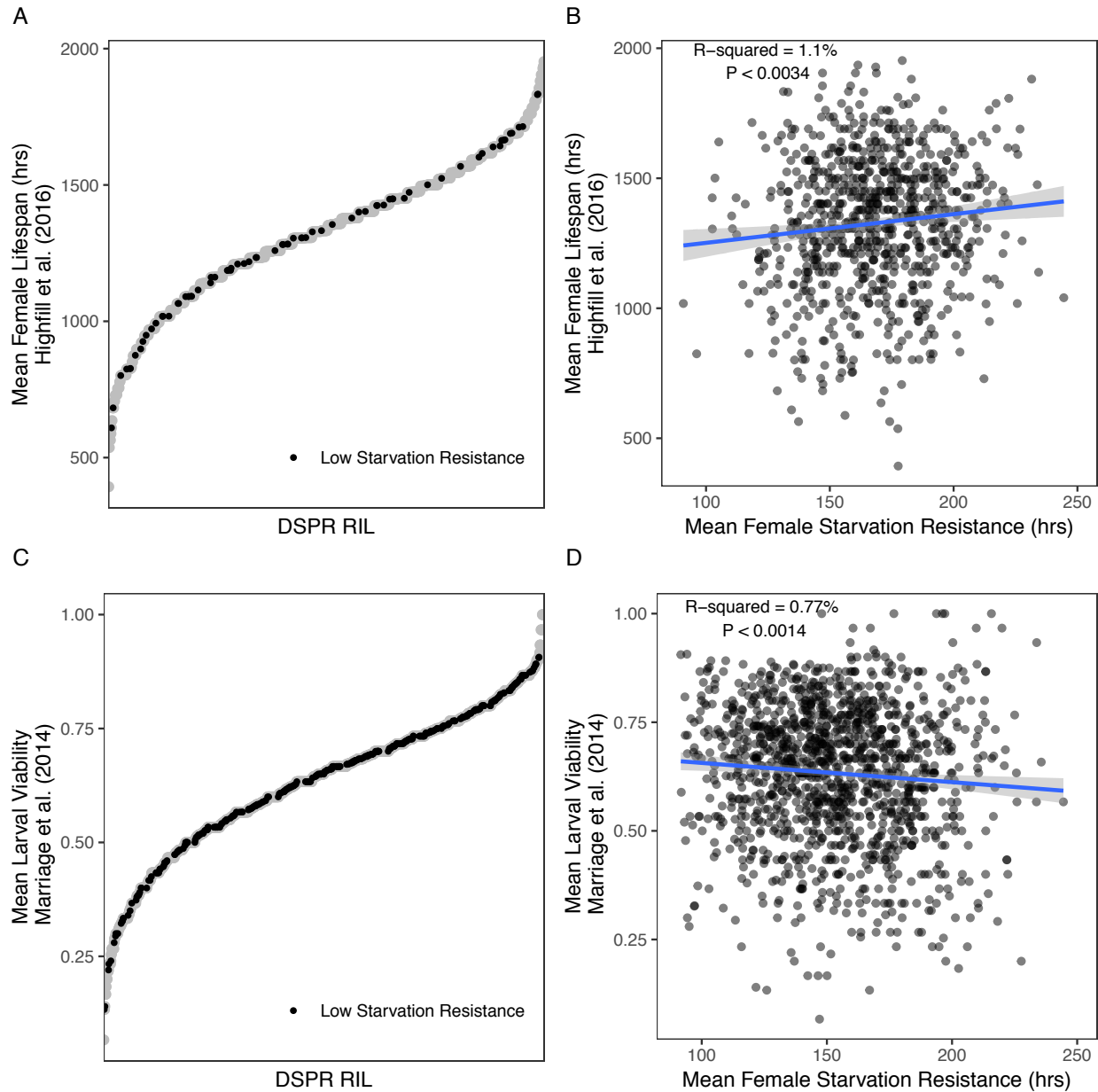*lifespan. C. Distribution of mean larval viability measured as the proportion of 1*[st] *instar larvae reared under*
control conditions that emerged as adults (Marriage et al. *(2014). RILs with low female starvation*
resistance (in the bottom 25% of the distribution) are shown in solid black symbols, while other RILs are
shown in gray. D. The weak correlation between mean female starvation resistance and mean larval
viability again suggests that there is no association between low starvation resistance and low larval
viability. We also fail to find strong associations between starvation resistance and other measures of
fitness in the DGRP (Fig S12).
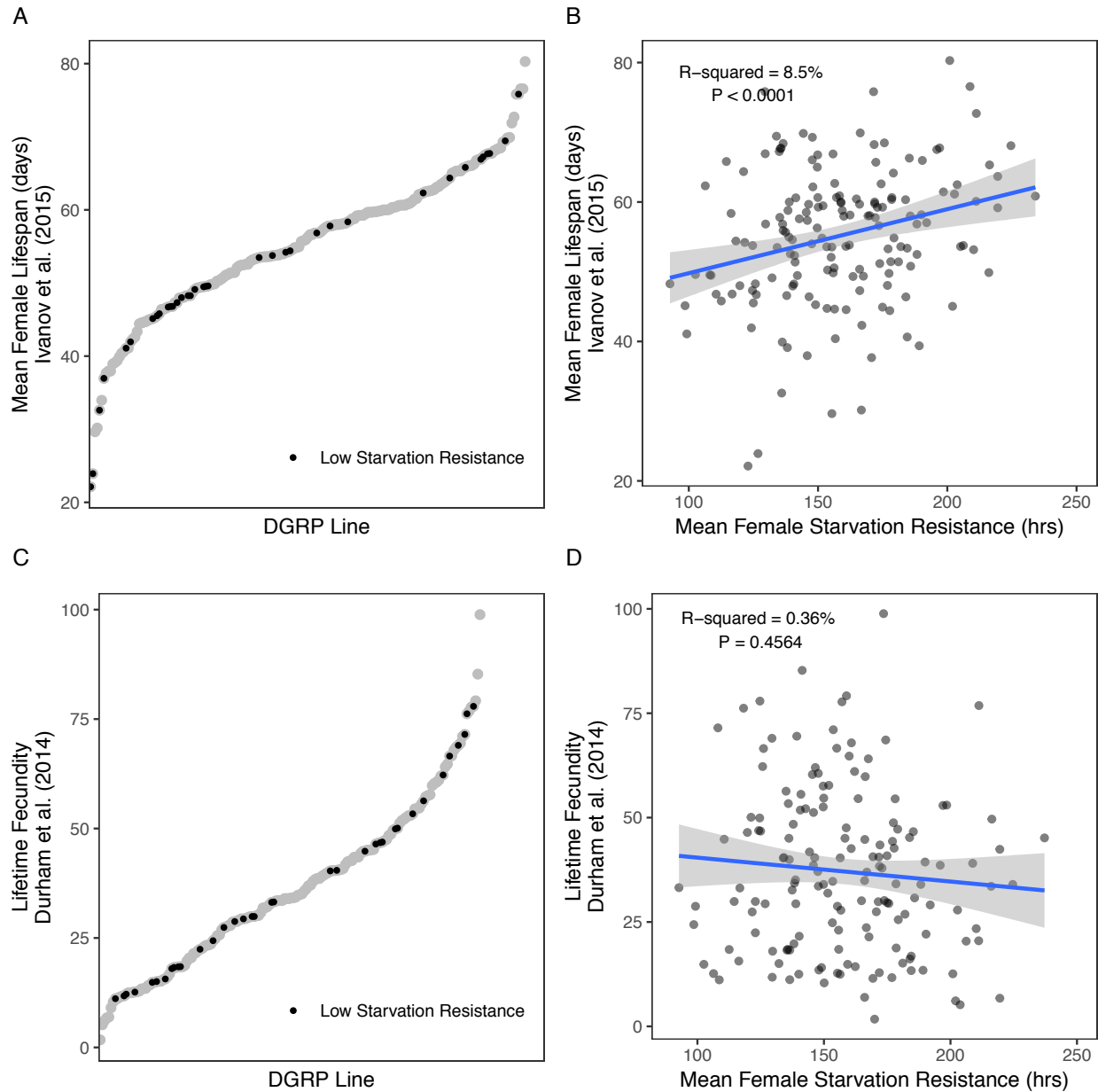
Figure S12. *DGRP Lines with low starvation resistance ranged from low to high for other measures of fitness. A. Distribution of mean female lifespan in the DGRP (Ivanov* et al. *(2015). Lines with low female starvation resistance (in the bottom 25% of the distribution) are shown in solid black symbols, while other lines are shown in gray. B. The correlation between mean female starvation resistance and mean female lifespan is minimal, suggesting there is little association between low starvation resistance and reduced lifespan. C. Distribution of lifetime fecundity (Durham* et al. *(2014). Lines with low female starvation resistance (in the bottom 25% of the distribution) are shown in solid black symbols, while other lines are shown in gray. D. The weak correlation between mean female starvation resistance and fecundity suggests that there is no association between low starvation resistance and this measure of fitness.*

Figure S13. *Mean activity levels for females (top panel, black points) and males (bottom panel, red points) (± 95% CI) across the 12-hr daily light or dark periods during starvation in the DAM (Drosophila Activity Monitor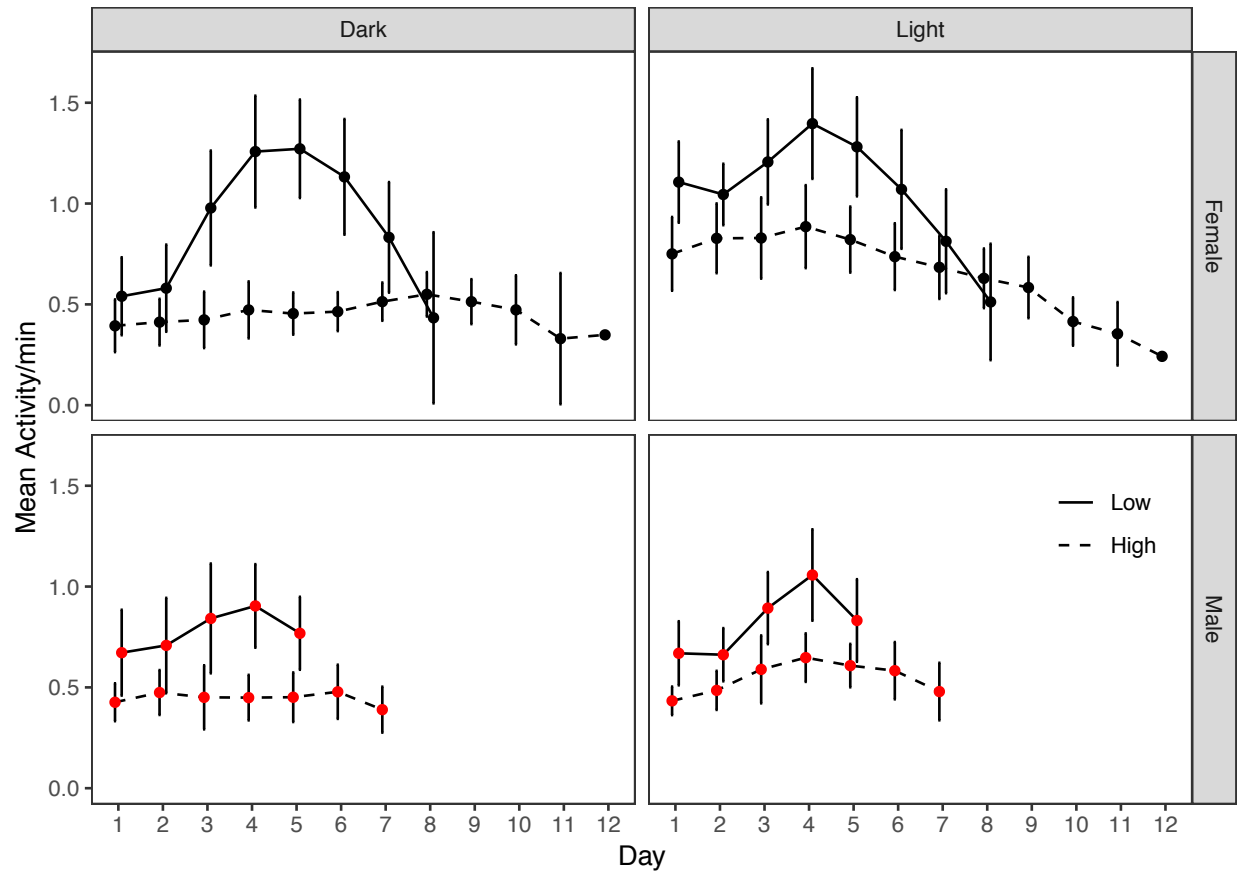) assay until death. Low starvation resistance RILs (solid line) tended to be more active during starvation compared to high starvation resistance RILs (dashed line) under both light and dark conditions. Light status (dark versus light) influenced the overall activity level of females but did not influence male activity. Data were analyzed with a repeated measures ANOVA; results are presented in Table S8. Similar to the pre-starvation period (Fig 4), waking activity levels of individuals during the DAM starvation experiment were primarily driven by starvation resistance rank in both sexes (females: $F_{1,8} = 14.87$, $p < 0.01$; males: $F_{1,6} = 13.87$, $p < 0.01$; Table S8). Light status did not influence activity between days for either sex (females: $F_{1,8} = 0.84$, $p = 0.39$; males: $F_{1,6} = 0.23$, $p = 0.65$; Table S8). However, light status did significantly influence activity in females within each day (females: $F_{1,8} = 43.09$, $p < 0.0001$; Table S8), indicating that female activity in both high and low starvation resistance RILs was consistently higher during times when lights were on. Male activity of high and low starvation resistance RILs tended to remain constant under different light conditions. The larger influence of light status on females is consistent with patterns observed in activity during the pre-starvation period (Fig 4).*
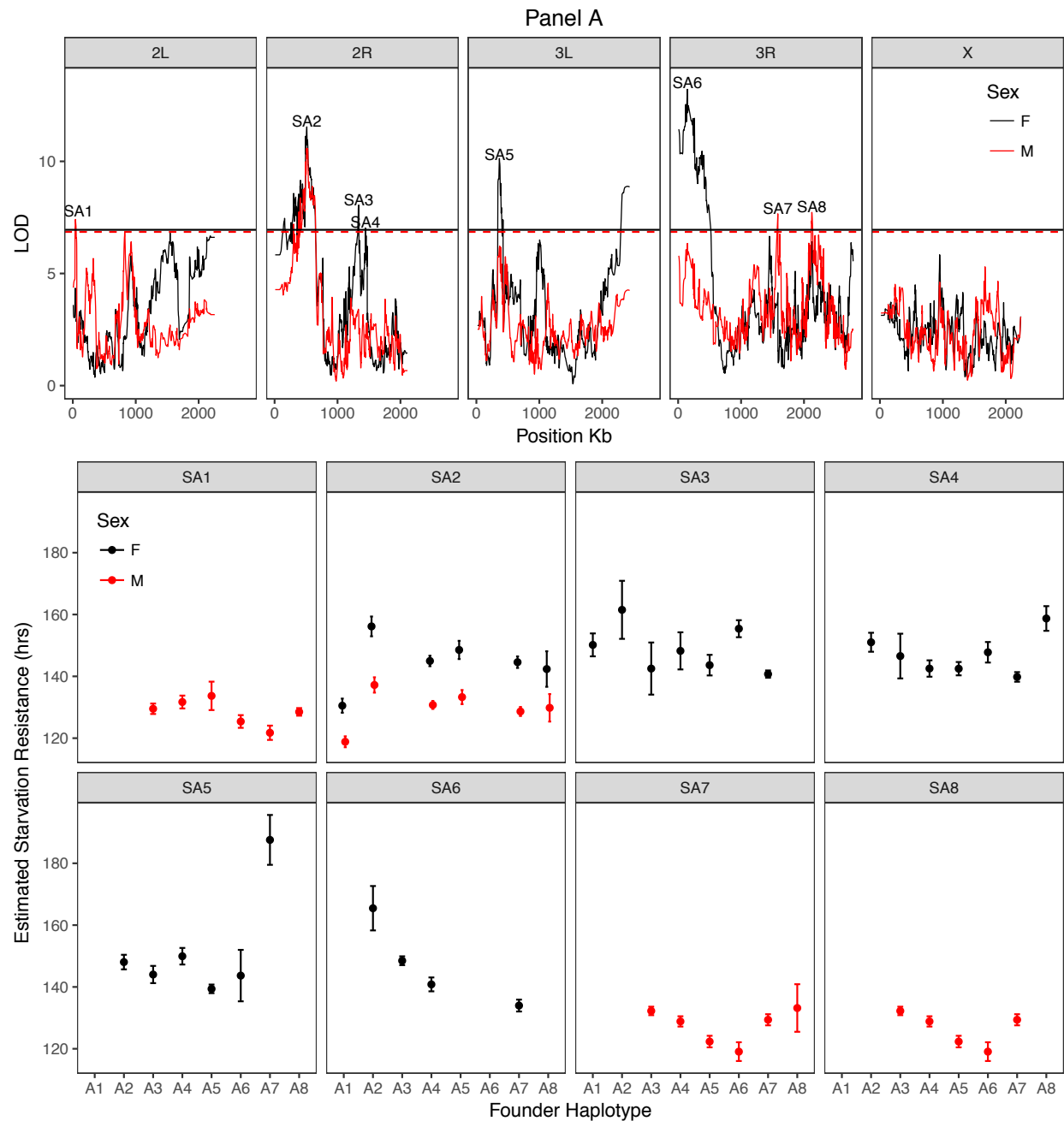
Figure S14. *Starvation resistance QTL and estimated allele effects at each QTL. Data are presented as RIL means (±SE) for estimated starvation resistance when the founder haplotype was present in more than 5 RILs. As has been seen in a number of studies using the DSPR and other multiparental populations (King et al. 2012b; Giraud et al. 2014; Najarro et al. 2015), the estimated phenotypic effects of each founder haplotype suggest that multiple alleles may be present at our starvation QTL, since the effects do not fall into two clear "high" and "low" classes.*

Panel B
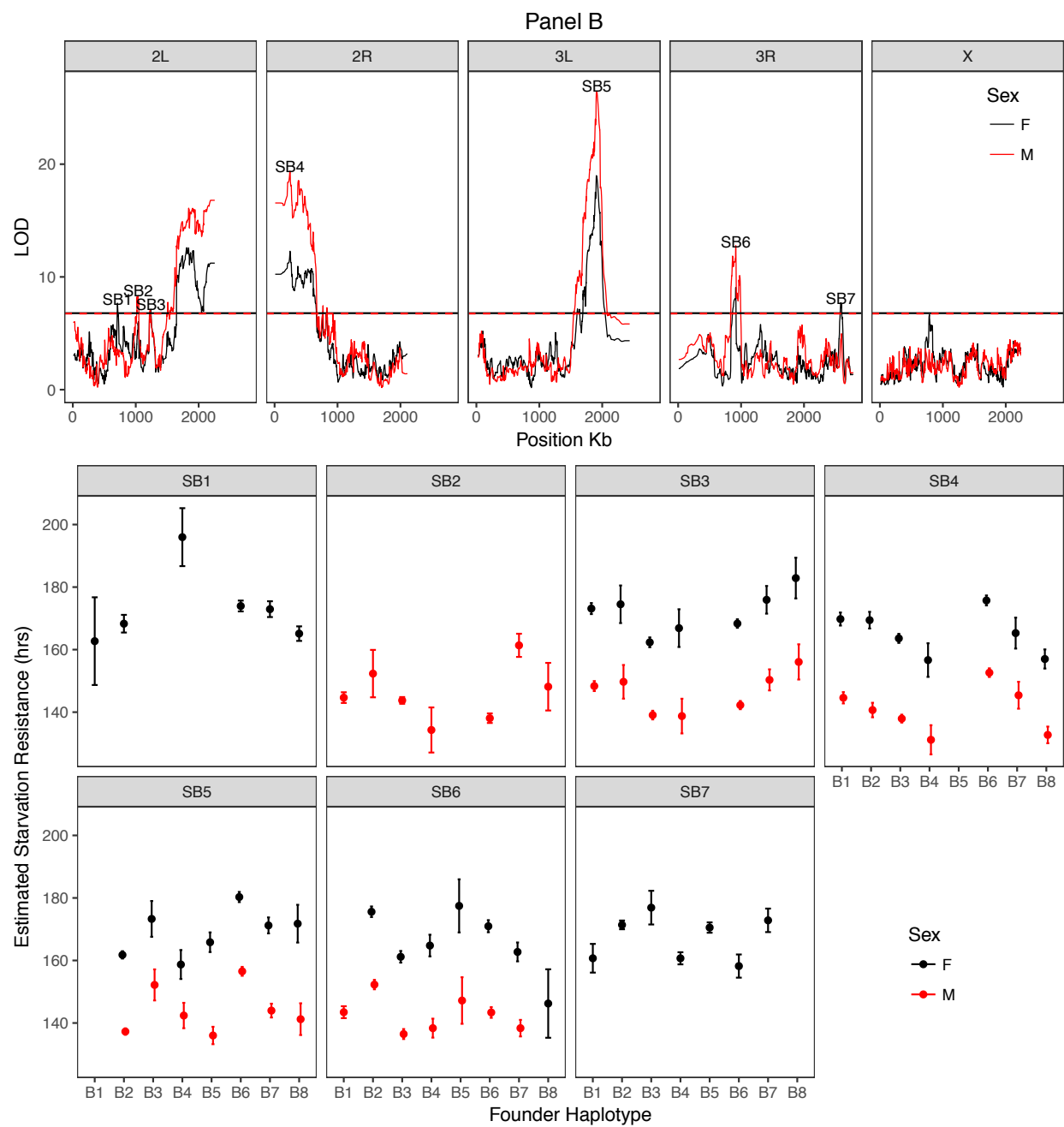
Figure S14 continued.

15
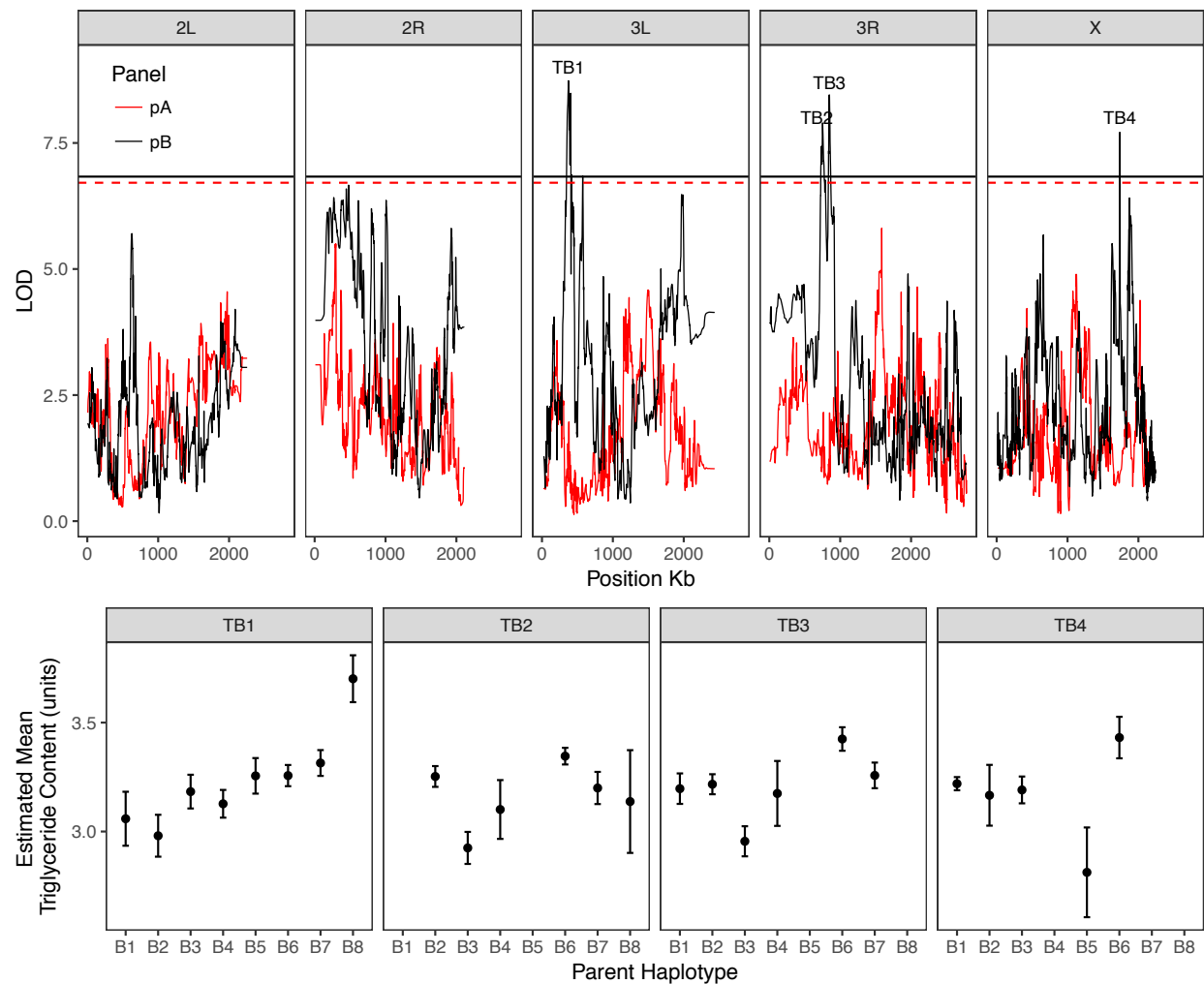
Figure S15. *Triglyceride level QTL and estimated allele effects of founders at each QTL. Data are presented as RIL means (±SE) for estimated triglyceride level when the founder haplotype was present in more than 5 RILs. Similar to starvation resistance, the estimated phenotypic effects of each founder haplotype suggest that multiple alleles may be present at our triglyceride QTL.*
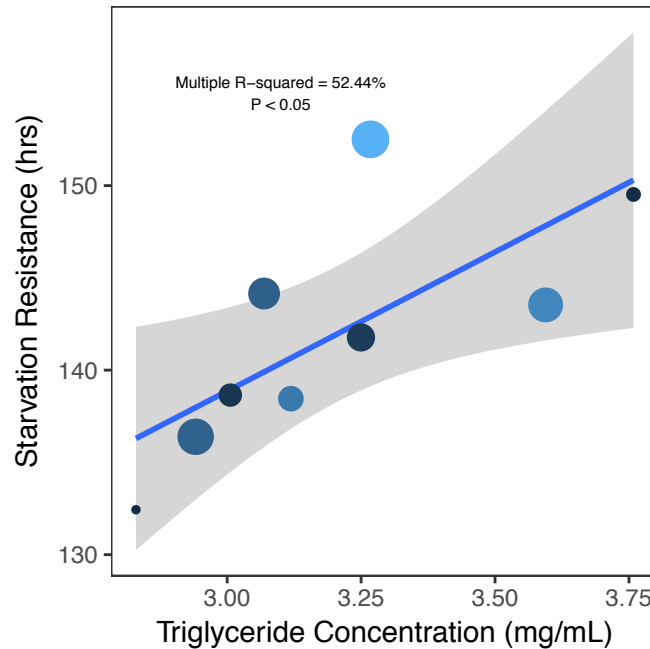
Figure S16. *Triglyceride level and starvation resistance were correlated after accounting for variation due to founder haplotype at the overlapping peaks TB3 and SB6 ($F_{1,7}$ = 7.72, P < 0.05). Data presented are averages for each founder haplotype in the pB panel, including "NA" for RILs that could not be assigned with confidence to a known haplotype. Point size relates the number of RILs per haplotype for the starvation resistance peak (smallest = 1 RIL; largest = 193 RILs); point color relates the number of RILs per haplotype for the triglyceride level peak (black = 1 RIL; lightest blue = 181 RILs). Grey shading around the regression line indicates the 95% confidence interval.*

A.

B.
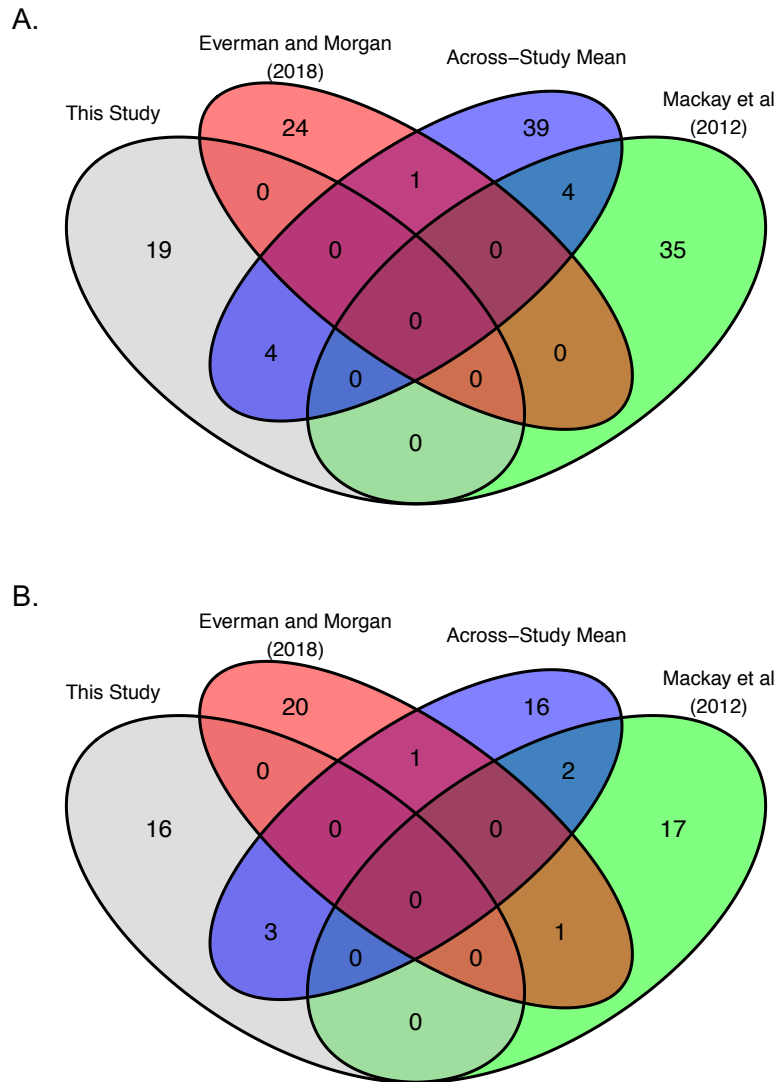
Figure S17. *Overlap in SNPs associated with starvation resistance for each DGRP dataset using the P < 10^-5 significance threshold. Overlap between data sets was minimal. Plot A presents results for females; plot B presents results for males.*
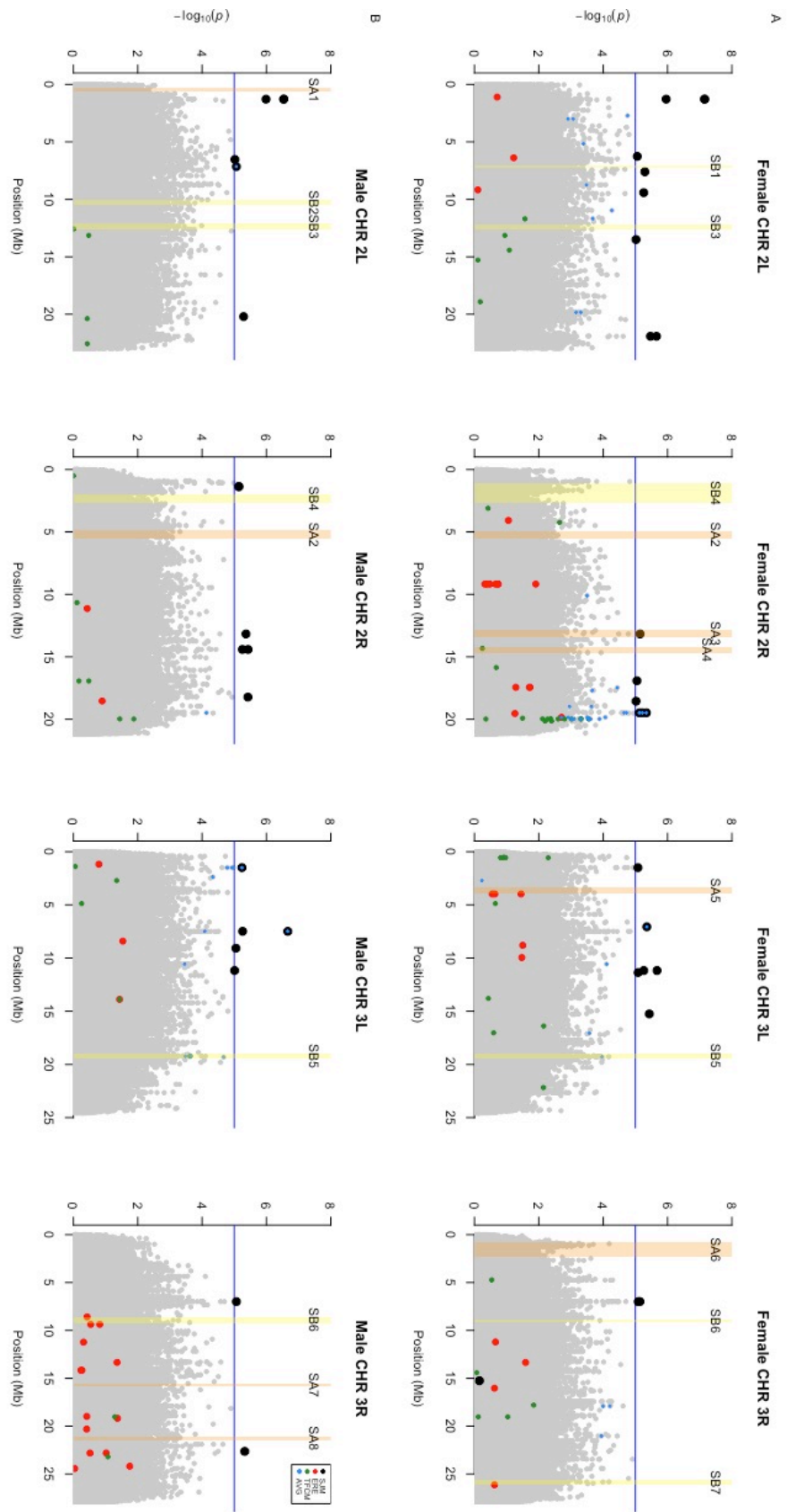
Figure S18. *Manhattan plots of mean starvation resistance in the DGRP with SNPs that were associated with starvation resistance in previous studies and intervals of sex-specific QTL identified for starvation resistance in the DSPR highlighted. Plots are broken up by chromosome arm in A for females and in B for males. In all plots, points highlighted in black indicate SNPs that are associated with starvation resistance in the DGRP from data obtained in this study; red points indicate SNPs associated with starvation resistance in Everman and Morgan 2018; green points indicate SNPs associated with starvation resistance in Mackay et al. 2012; blue points indicate SNPs associated with starvation resistance averaged across the three datasets. A genomewide significance threshold of $P < 10^{-5}$ is shown with the blue line. Yellow shaded boxes and labels correspond to QTL intervals around peaks mapped in the pB DSPR panel; orange shaded boxes correspond to QTL intervals around peaks mapped in the pA DSPR panel.*
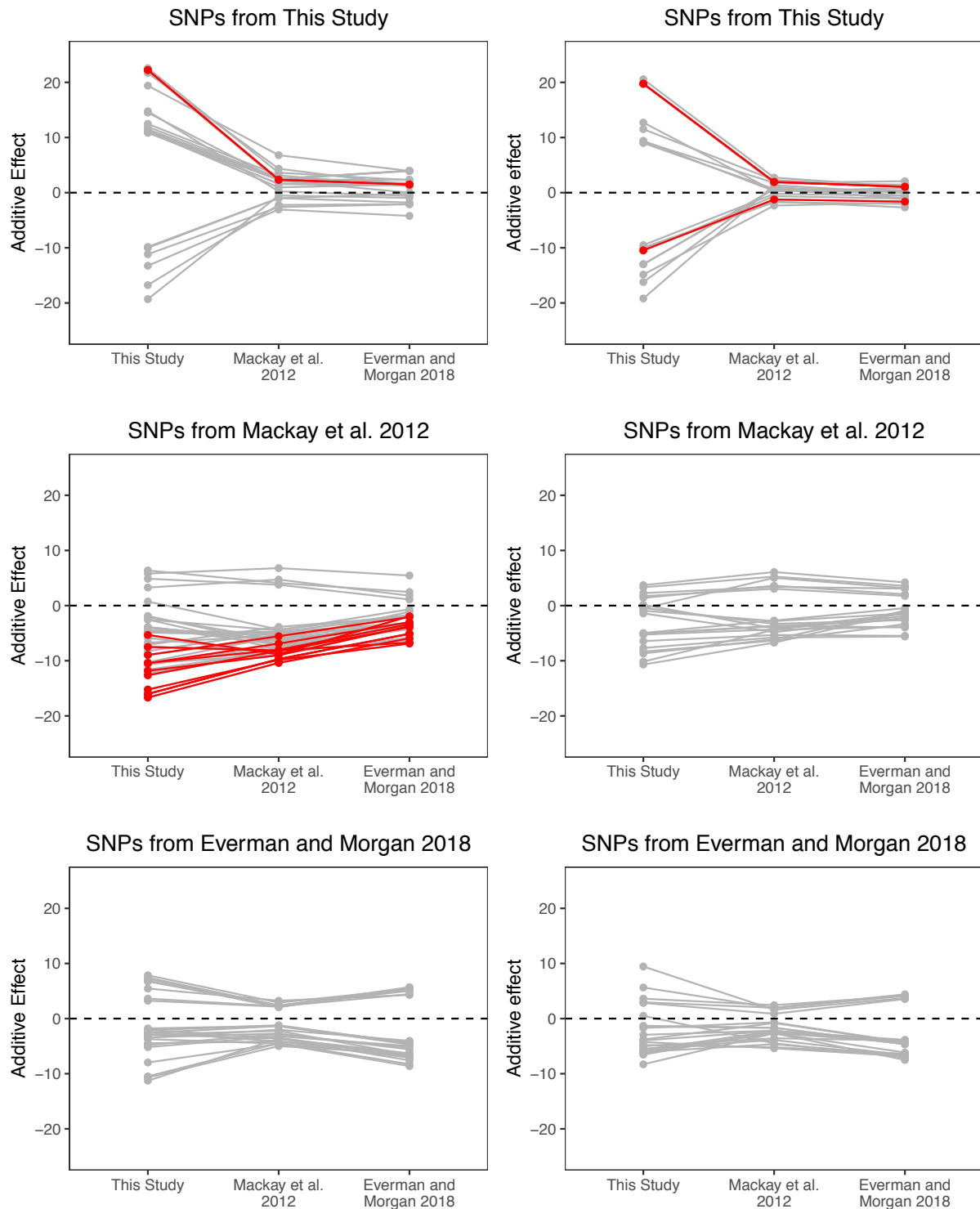
Figure S19. *Additive effects of SNPs associated with starvation resistance (at P < 10^{-5}) in each study, along with their additive effects estimated in the other two studies. Female data is presented in the left column of plots; male data is presented in the right column of plots. SNPs that passed the FDR threshold of 0.2 are highlighted in red. Generally, SNPs had similar effects (of the same +/- sign) on starvation resistance in all three experiments.*

Supplemental Tables

Table S1. Analysis of variance of the effect of DSPR rearing density on starvation resistance.

Source	df	SS	MS	F	P	% Var. Exp.
Density	1	1758.00	1758.20	18.15	< 0.0001	0.90
DSPR RIL	19	148747	7828.80	80.83	<< 0.0001	80.2
Density x DSPR RIL	19	8905	468.70	4.84	< 0.0001	4.80
Residual	269	26054	96.90			

Table S2. Analysis of variance of mean starvation resistance in the DSPR.

Source	df	SS	MS	F	P
Subpopulation[a]	3	330782	110261	183.702	< 0.0001
Sex	1	342197	342197	570.121	< 0.0001
Subpopulation x Sex	3	32982	10994	18.317	< 0.0001
Residual	3440	2064747	600		

[a] We note that since most RILs from a given subpopulation were tested in the same batch, batch effects may contribute to some of the subpopulation-to-subpopulation differences we report.

Table S3. Analysis of variance of mean triglyceride level in the DSPR.

Source	df	SS	MS	F	P
Subpopulation	3	46.81	15.6020	37.099	< 0.0001
Residual	935	393.22	0.4206		

Table S4. Stratification of the top 50 SNPs associated with starvation resistance in the DGRP across five frequency bins.

Study	Sex	Allele Frequency Bin	No. SNPs
This Study	F	0.05 - 0.1	14
This Study	F	> 0.1 - 0.2	9
This Study	F	> 0.2 - 0.3	13
This Study	F	> 0.3 - 0.4	6
This Study	F	> 0.4 - 0.5	8
This Study	M	0.05 - 0.1	11
This Study	M	> 0.1 - 0.2	12
This Study	M	> 0.2 - 0.3	13
This Study	M	> 0.3 - 0.4	3
This Study	M	> 0.4 - 0.5	11
Mackay *et al.* 2012	F	0.05 - 0.1	28
Mackay *et al.* 2012	F	> 0.1 - 0.2	11
Mackay *et al.* 2012	F	> 0.2 - 0.3	5
Mackay *et al.* 2012	F	> 0.3 - 0.4	2
Mackay *et al.* 2012	F	> 0.4 - 0.5	4
Mackay *et al.* 2012	M	0.05 - 0.1	21
Mackay *et al.* 2012	M	> 0.1 - 0.2	13
Mackay *et al.* 2012	M	> 0.2 - 0.3	7
Mackay *et al.* 2012	M	> 0.3 - 0.4	2
Mackay *et al.* 2012	M	> 0.4 - 0.5	7
Everman and Morgan 2018	F	0.05 - 0.1	11
Everman and Morgan 2018	F	> 0.1 - 0.2	13
Everman and Morgan 2018	F	> 0.2 - 0.3	10
Everman and Morgan 2018	F	> 0.3 - 0.4	8
Everman and Morgan 2018	F	> 0.4 - 0.5	8
Everman and Morgan 2018	M	0.05 - 0.1	18
Everman and Morgan 2018	M	> 0.1 - 0.2	13
Everman and Morgan 2018	M	> 0.2 - 0.3	12
Everman and Morgan 2018	M	> 0.3 - 0.4	4
Everman and Morgan 2018	M	> 0.4 - 0.5	3

Table S5. Analysis of variance of mean starvation resistance in the DGRP.

Source	df	SS	MS	F	P
Sex	1	97213	97213	118.21	< 0.0001
Residual	334	274680	822		

Table S6. Analysis of variance of the effect of preservatives and environment on starvation resistance in the DGRP.

Source	df	SS	MS	F value	P	% Var. Exp.
Environment	1	2400	2400	16.71	< 0.0001	0.30
Preservatives	1	604184	604184	4205.70	< 0.0001	81.17
DGRP Line	11	63653	5787	40.28	< 0.0001	8.55
Environment x Preservatives	1	155	155	1.08	0.30	0.02
Environment x DGRP Line	11	7289	663	4.61	< 0.0001	0.98
Preservatives x DGRP Line	11	14718	1338	9.31	< 0.0001	1.98
Environment x Preservatives x DGRP Line	11	4961	451	3.14	< 0.001	0.67
Residual	327	46976	144			

Table S7. Analysis of variance of activity during the 24-hour period prior to the DAM (Drosophila Activity Monitor) starvation assay.

Source	df	SS	MS	F value	P	Effect Size
Starvation Resistance Rank (High vs. Low)	1	1.37	1.37	12.48	< 0.001	0.31
Sex	1	0.61	0.61	5.57	< 0.05	0.21
Lights On/Off	1	1.55	1.55	14.11	< 0.001	0.33
Starvation Resistance Rank x Sex	1	0.00	0.00	0.01	0.92	0.01
Starvation Resistance Rank x Lights On/Off	1	0.00	0.00	0.00	0.98	0.00
Sex x Lights On/Off	1	1.85	1.85	16.87	< 0.0001	0.36
Starvation Resistance Rank x Sex x Lights On/Off	1	0.02	0.02	0.21	0.64	0.04
Residuals	132	14.49	0.11	0.00		

Table S8. Repeated measures analysis of variance across days for activity during the DAM (Drosophila Activity Monitor) starvation assay for males and females.

Female Activity During Starvation
Between Days

Source	df	SS	MS	F value	P
Starvation Resistance Rank (High vs. Low)	1	11.15	11.15	14.19	< 0.01
Lights On/Off	1	0.66	0.66	0.84	0.39
Starvation Resistance Rank (High vs. Low) x Lights On/Off	1	0.48	0.48	0.61	0.46
Residuals	8	6.29	0.79		

Within Day

Source	df	SS	MS	F value	P
Starvation Resistance Rank (High vs. Low)	1	19.06	19.06	115.58	< 0.0001
Lights On/Off	1	7.10	7.11	43.09	< 0.0001
Starvation Resistance Rank (High vs. Low) x Lights On/Off	1	0.16	0.16	0.99	0.32
Residuals	575	94.81	0.17		

Male Activity During Starvation
Between Days

Source	df	SS	MS	F value	P
Starvation Resistance Rank (High vs. Low)	1	5.47	5.47	13.79	< 0.01
Lights On/Off	1	0.09	0.09	0.23	0.65
Starvation Resistance Rank (High vs. Low) x Lights On/Off	1	0.67	0.67	1.69	0.24
Residuals	6	2.38	0.40		

Within Day

Source	df	SS	MS	F value	P
Starvation Resistance Rank (High vs. Low)	1	5.03	5.04	46.70	< 0.0001
Lights On/Off	1	0.32	0.32	2.93	0.09
Starvation Resistance Rank (High vs. Low) x Lights On/Off	1	0.12	0.12	1.14	0.29
Residuals	471	50.78	0.11		

Table S9. Data from genes mapped to the region under QTL intervals for starvation resistance in the pA and pB DSPR mapping panels and triglyceride level in the pB DSPR mapping panel based on Flybase release version FB2018_1. *Highlighted genes indicate those previously identified in QTL mapping studies of starvation resistance.*

See independent "Table_S9.xlsx" Excel file.

Table S10. Gene ontology analysis of genes that are included within QTL intervals for starvation resistance and triglyceride level.

Starvation Resistance GO Analysis: pA DSPR Panel

Category	Fold Enrichment	FDR
glutathione metabolic process (GO:0006749)	6.91	1.46E-04
cellular modified amino acid metabolic process (GO:0006575)	3.85	1.79E-02

Triglyceride Level GO Analysis: pB DSPR Panel

Category	Fold Enrichment	FDR
heat shock-mediated polytene chromosome puffing (GO:0035080)	42.66	4.25E-04
polytene chromosome puffing (GO:0035079)	38.39	3.35E-04
sensory perception of sweet taste (GO:0050916)	27.42	7.71E-04
detection of chemical stimulus involved in sensory perception of taste (GO:0050912)	19.47	7.16E-04
chaperone cofactor-dependent protein refolding (GO:0051085)	16	5.94E-03
'de novo' posttranslational protein folding (GO:0051084)	16	5.19E-03
protein refolding (GO:0042026)	15.36	5.65E-03
'de novo' protein folding (GO:0006458)	13.24	9.68E-03
cellular response to unfolded protein (GO:0034620)	12.8	4.87E-03
response to unfolded protein (GO:0006986)	12.44	4.78E-03
cellular response to topologically incorrect protein (GO:0035967)	9.95	1.06E-02
response to topologically incorrect protein (GO:0035966)	9.74	1.00E-02
cellular response to heat (GO:0034605)	8.93	5.11E-02
chaperone-mediated protein folding (GO:0061077)	8.93	4.79E-02
response to hypoxia (GO:0001666)	7.22	4.89E-02
sensory perception of taste (GO:0050909)	6.65	2.84E-02

Gene lists used in each analysis included all genes unique to male and female analyses for each trait. GO analysis was performed using the PANTHER Overrepresentation Analysis (Released 2018-05-21) with Fisher's Exact test with FDR multiple test correction. We found no GO enrichment among the genes implicated by QTL mapped for starvation resistance in the pB panel or genes associated with SNPs implicated in the DGRP.

Table S11. Data from GWA, generated from the DGRP Freeze 2.0 pipeline, based on Flybase release version FB2018_1. *All SNPs shown passed the $P < 10^{-5}$ significance threshold; highlighted SNPs passed the FDR threshold of 0.2.*

See independent "Table_S11.xlsx" Excel file.

Supplemental Text

Text S1. Starvation media recipe.

    1000mL water

    15g agar

    12mL of acid mix (330mL water/259mL Proprionic Acid/31mL Phosphoric Acid)

    2g of Tegosept dissolved in 20mL 95% ethanol

Bring agar in water to a boil, reduce heat and simmer 20 minutes. Stir in acid mix and Tegosept off heat.

Information on Supplemental Files

File S1. All code associated with the bootstrapping analysis of SNPs associated with starvation resistance measured in the DGRP in this study, Mackay *et al.* (2012), and Everman and Morgan (2018).

See independent "File_S1.txt" simple text file.

File S2. Description of each dataset associated with this study.

See independent "File_S2.pdf" PDF. The data itself is presented in the compressed archive "EvermanData.tar.gz".