

Table S1. Spearman’s rank correlations between replicates within treatments. *P*-values were corrected for multiple comparisons.

	RIL-D48				RIL-SH2				
	<i>Low density</i>		<i>High density</i>		<i>Low density</i>		<i>High density</i>		
	<i>HH</i>	<i>NHH</i>	<i>HH</i>	<i>NHH</i>	<i>HH</i>	<i>NHH</i>	<i>HH</i>	<i>NHH</i>	
<i>Females</i>									
R1 – R2	0.27	0.95*	0.20	0.32	0.26	-0.48	0.58	-0.09	
R1 – R3	0.42	0.83*	0.40	0.26	0.16	-0.44	0.18	0.25	
R2 – R3	0.29	0.88*	0.28	0.38	0.63	0.35	0.28	0.14	
<i>Males</i>									
R1 – R2	0.24	0.99*	0.30	0.09	-0.11	-0.18	-0.36	0.64	
R1 – R3	0.15	0.86*	0.30	0.37	0.38	-0.23	0.23	0.42	
R2 – R3	0.38	0.86*	-0.06	0.08	0.35	0.31	0.07	0.55	

**P* < 0.05.

Table S2. QTL for heat-stress survival identified by composite interval mapping in adults of *Drosophila melanogaster* with some of the candidates genes that are located within the QTL range.

Chrom.	QTL Range	Candidates genes
	1B8-3C6	<u>CG14629</u> , <u>CG11378</u> , period, CG14804, CG2694
X	4F1-10A2	CG3203, CG2186, <u>CG11700</u> , <u>CG14439</u> , Trxr-1, flapwing, <u>CG32687</u> , CG2887, <u>CG5966</u> , <u>CG1583</u> , <u>vermilion</u> , <u>CG2254</u> , <u>CG12116</u> , <u>Reg-3</u> , <u>CG3106</u>
	10A2-12E	CG1841, CG15737, Rbp1-like, hop, Hsc70-3, <u>CG15201</u> , Hsp60
	12D-16F6	RpS19, CG8289, Ucp4A, rut, <u>CG33173</u> , <u>CG9673</u> , <u>CG9675</u>
	21C3-26A	Lwr, dock, CG5156, CG3210, <u>Thor</u> , <u>CG3246</u> , TotM, Hsp60B, CG5001, <u>Iris</u> , <u>CG17224</u> , <u>CG3244</u> , <u>Cyp4ac2</u>
	28A1-30A6	Acer, <u>Glutactin</u> , <u>Uro</u> , <u>CG18585</u> , <u>CG7025</u> , <u>TepII</u> , <u>CG13095</u> , <u>CG9466</u> , <u>CG9468</u> , <u>Cyp4d21</u>
2	30A3-42A	cdc2, CG5384, CG6579, Idgf1, CG12288, Cyt-c-p, VhaSFD, CG15141, tj, CG13084, <u>CG7300</u> , <u>CG5945</u> , <u>CG9928</u> , <u>CG7916</u> , <u>CG8997</u> , <u>CG7953</u> , TotF, Dif, dl, TotE, <u>CG13086</u> , <u>CG10189</u> , <u>CG10680</u> , <u>CG16887</u> , <u>CG17108</u> , Hsp60D, DnaJ-H, ref(2)P, <u>CG10383</u> , <u>CG5390</u> , <u>CG16743</u> , <u>hgo</u> , <u>CG14935</u> , <u>CG6012</u> , <u>Irk3</u> , <u>CG10026</u> , <u>CG17124</u> , catsup, Trap1.
	42A-50C	CG3409, pnut, Ggamma1, Trap1, CG8235, Su(var)2-10, CG8520, CG13322, CG17724, <u>CG3270</u> , <u>CG1946</u> , <u>CG2065</u> , <u>CG12374</u> , <u>CG30359</u> , <u>LvpH</u> , <u>iotaTry</u> , <u>CG30502</u> , <u>CG7882</u> , <u>CG8693</u> , <u>lambdaTry</u> , <u>sug</u> , <u>CG30035</u> , <u>CG1809</u> , <u>Cyp6a13</u> , <u>LvpL</u> , <u>CG9080</u> , <u>CG8834</u>
	50C-56E6	Cyp6a22, Ric, Ef1beta, Ark, CG9646, EDTP, CG4989, betaTub56D, <u>CG18327</u> , <u>CG8317</u> , <u>CG6426</u> , Heat shock factor, <u>CG17522</u> , Hsc70-5, <u>GstE1</u> , <u>Gst3-2</u> , <u>Pepck</u> , <u>CG8093</u> , <u>CG8249</u> , <u>CG4847</u> , <u>CG6484</u> , <u>Jheh3</u> , <u>Obp56a</u> , <u>CG15065</u>
	56D11-59A2	CG4266, CG15675, CG11296, <u>CG16898</u> , CG16799, <u>Xbp1</u> , <u>CG13492</u> , <u>CG11200</u> , <u>Obp57c</u> , <u>CG16799</u>
	62A-63F1	Cdc37, Hsp90, CG12020, <u>Hsp83</u> , <u>CG16762</u> , <u>CG16985</u> , <u>CG16986</u> , <u>Cct1</u>
3	63D2-66E2	RpL14, DnaJ-1, <u>CG6602</u> , CG7409, CG7387, <u>CG6781</u> , <u>CG10592</u> , <u>CG5150</u> , <u>CG10477</u> , <u>CG10472</u> , <u>CG5804</u> , <u>CG13309</u> , <u>ImpL3</u> , <u>CG8562</u> , <u>CG8560</u>
	90B1-95C8	CG17752, CG10825, RpS3, TotA, TotB, TotC, TotX, TotZ, Hsr-omega, <u>CG5791</u> , <u>CG6660</u> , <u>CG31148</u> , <u>CG17836</u> , <u>CG3734</u> , <u>CG5023</u> , <u>CG3301</u> , <u>CG6726</u> , <u>CG6733</u> , <u>CG18493</u> , <u>CG3739</u> , <u>CG13833</u>
	97F-99D9	CG12881, Ef1gamma, CG7601, <u>Obp99b</u> , <u>Cyp6a18</u> , <u>CG14527</u> , <u>CG3348</u> , <u>CG14528</u> , <u>CG9682</u>

Underlined genes were heat up-regulated and doubly underlined were down-regulated in previous studies (Sørensen *et al.*, 2005).

References:

Sørensen, J. G., Nielsen, M. M., Kruhoffer, M., Justesen, J. and Loeschcke, V. (2005). Full genome gene expression analysis of the heat stress response in *Drosophila melanogaster*. Cell Stress Chaperones 10, 312-328.

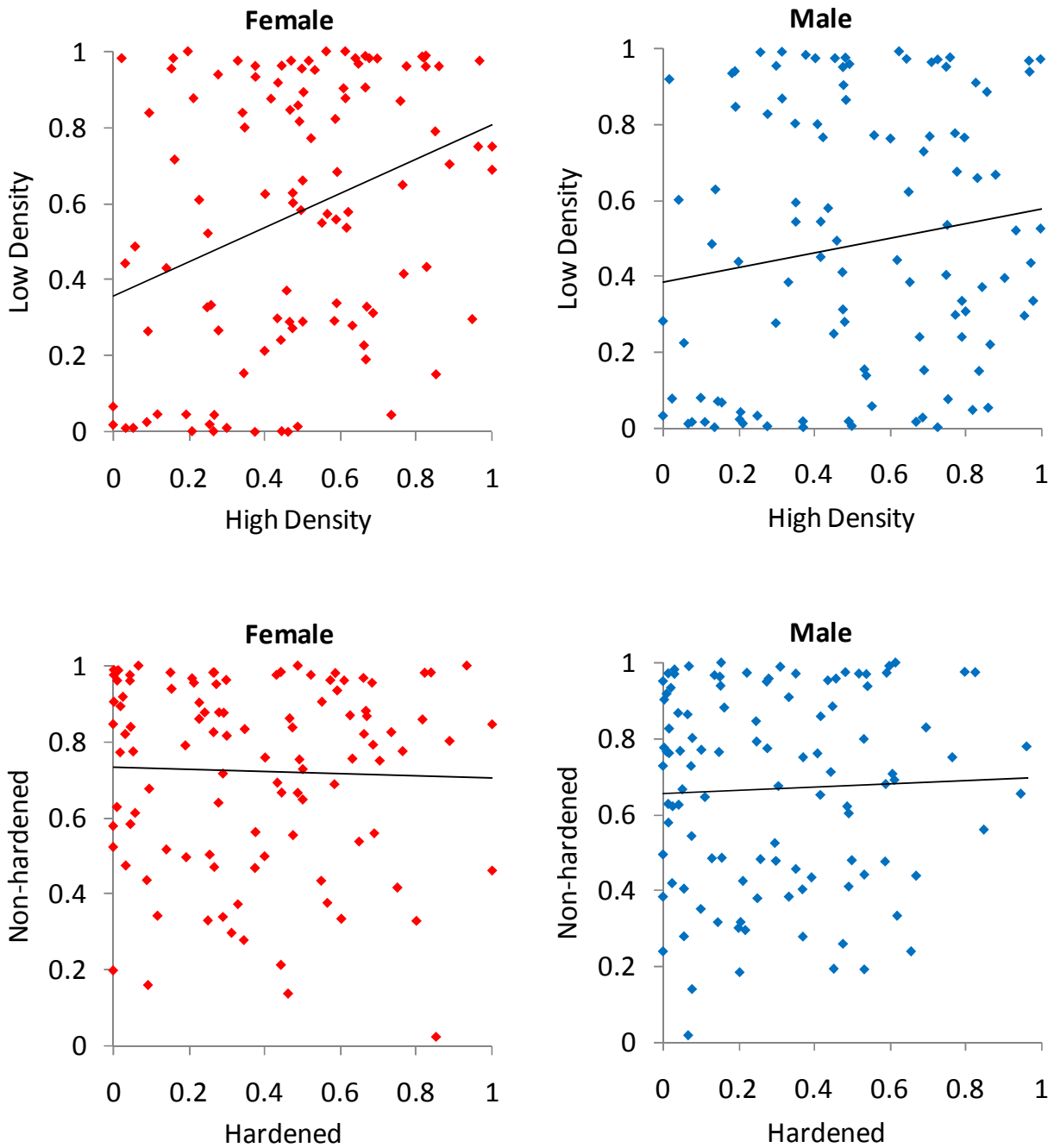
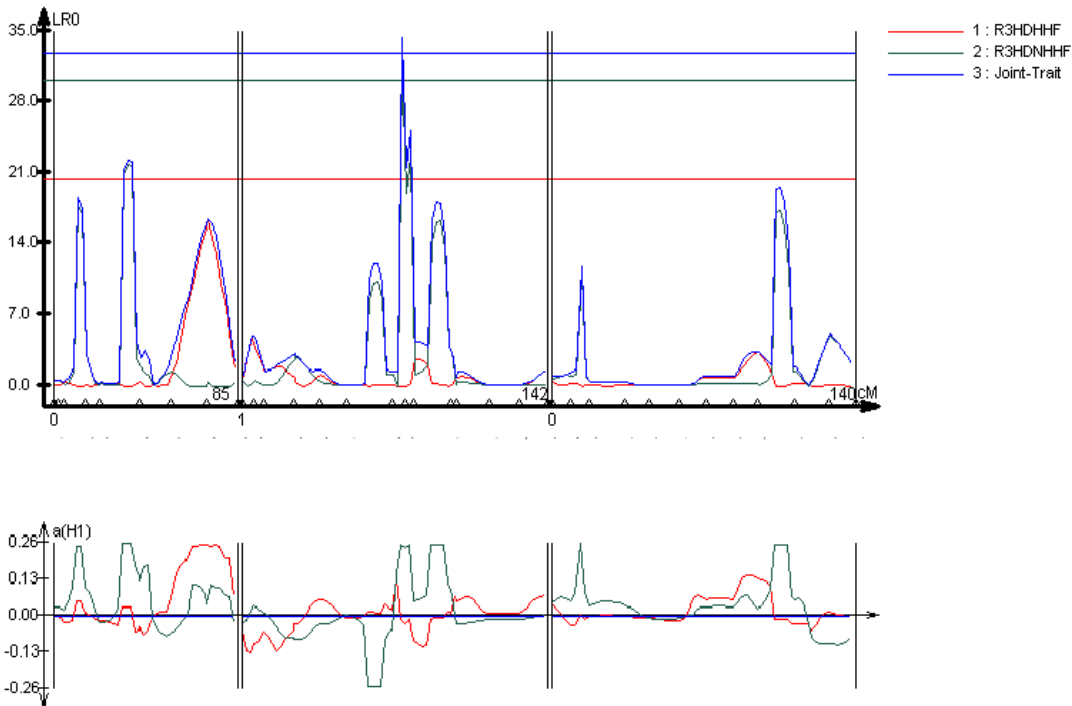


Figure S1. Heat-stress survival (HSS) is plotted for low versus high density. Spearman’s rank correlations between low and high density are: $r_s = 0.32^{**}$ and 0.1515 for females and males, respectively. Spearman’s rank correlations between heat-hardened and non-hardened SHS are: $r_s = 0.0505$ and 0.0303 for females and males, respectively.

a) Females from RIL-D48 at high density



b) Males from RIL-D48 at high density

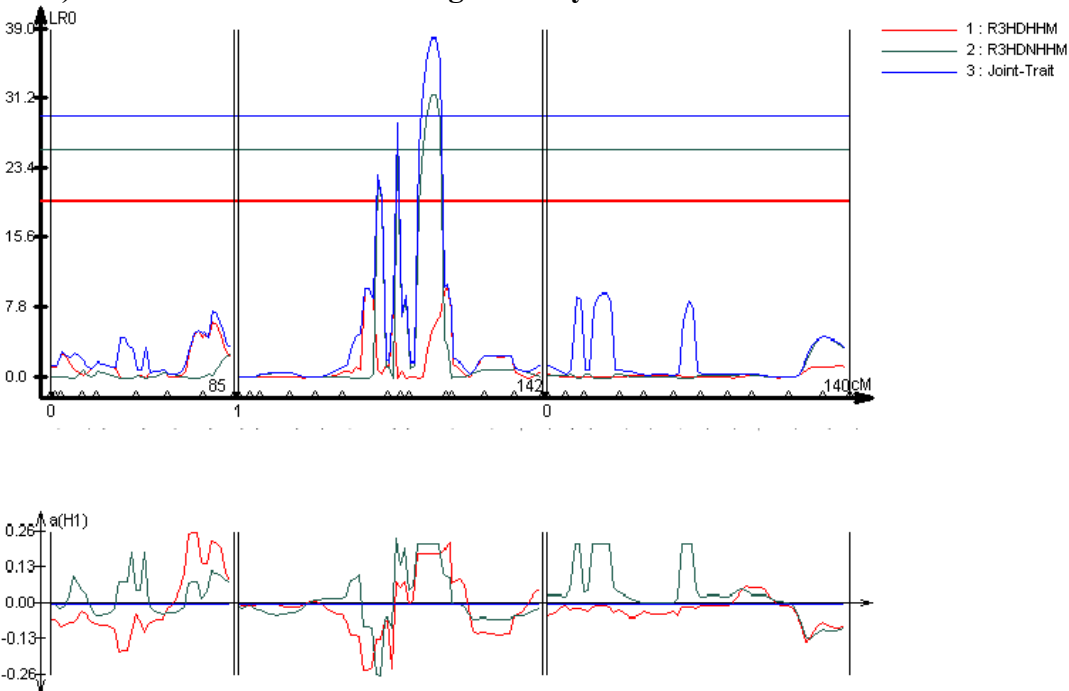


Figure S2. Plots of likelihood ratio (LR) scores against map position (in cM) from multi-trait composite interval mapping (MCIM) are shown for survival to heat stress in all cases where MCIM indicated significant QTL for heat-hardened versus non-hardened flies. Results were significant only for replicate 3 in females (a) and males (b) from RIL-D48 at high density. Significance thresholds were determined by 1000 random permutations. Triangles on the x-axis correspond to the location of markers used in MCIM. Additive values [a(H1)] are shown as profiles below the panel for LR profiles.

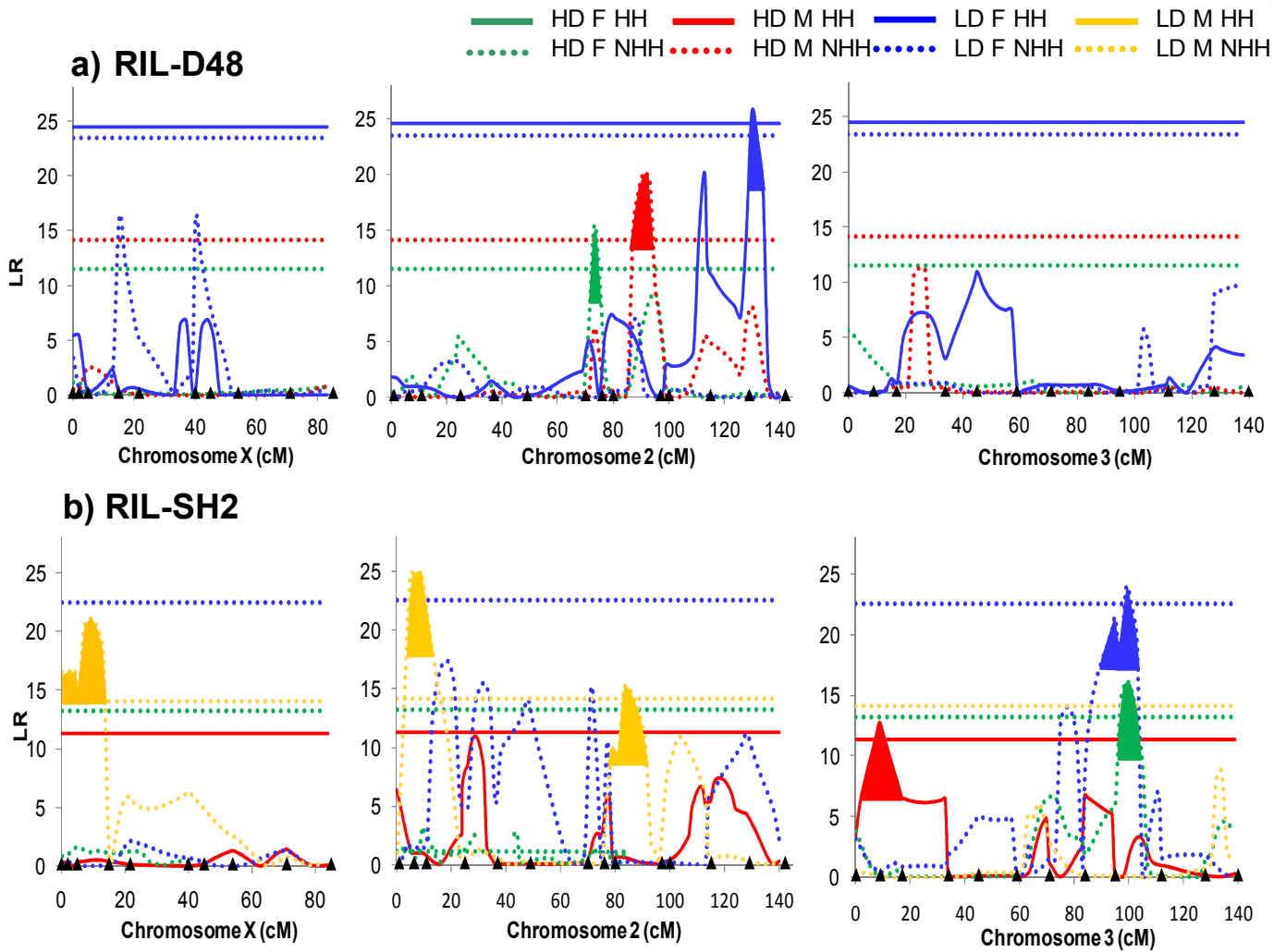


Figure S3. Plot of likelihood ratio (LR) scores against map position (in cM) from composite interval mapping for survival to heat stress is shown for an analysis pooling all replicates (R1, R2, R3) in RIL-D48 (a) and RIL-SH2 (b). The analyses were performed for heat-hardened (HH) and non-hardened (NHH) flies at either high (HD) and low (LD) density in both females (F) and males (M). Significance thresholds were determined by 1000 random permutations (horizontal lines). To avoid overlapping of lines of LR scores, only cases that were significant are shown.