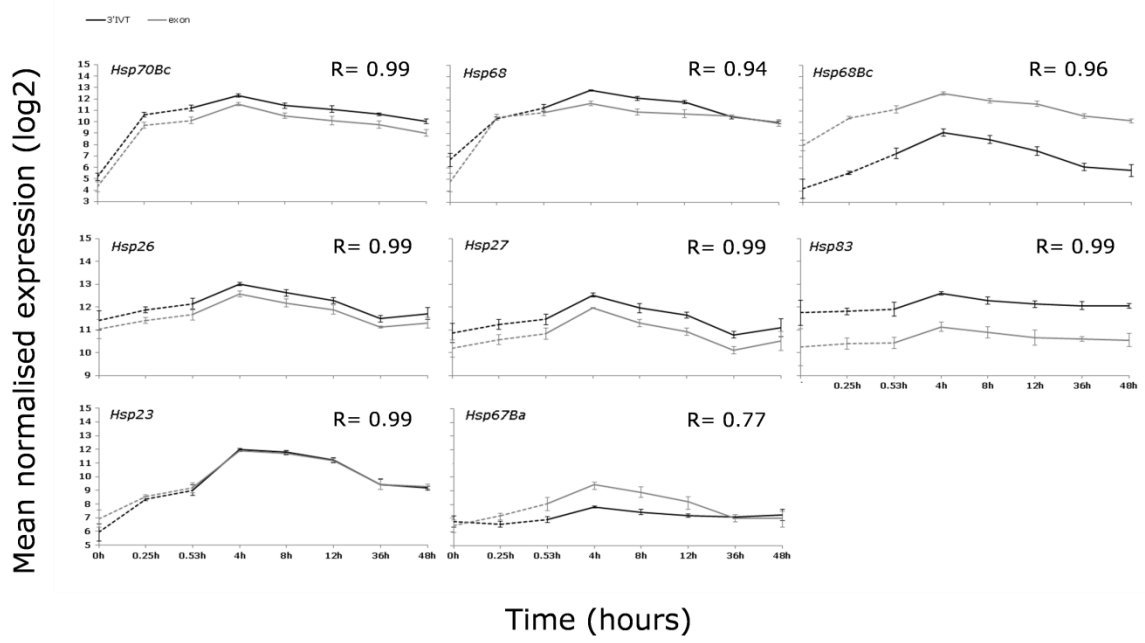

Figure S1 High signal comparability of the 3'IVT (black line) and exon modules (grey line) for eight thermally responsive *Hsps*. Dashed lines indicate thermal stress at 38.5º, solid lines indicate recovery up to 48 hours post stress at 25º. Signals are shown as average normalized expression on the Y-axis. Pearson's *R* is shown in the right hand corner of each graph.

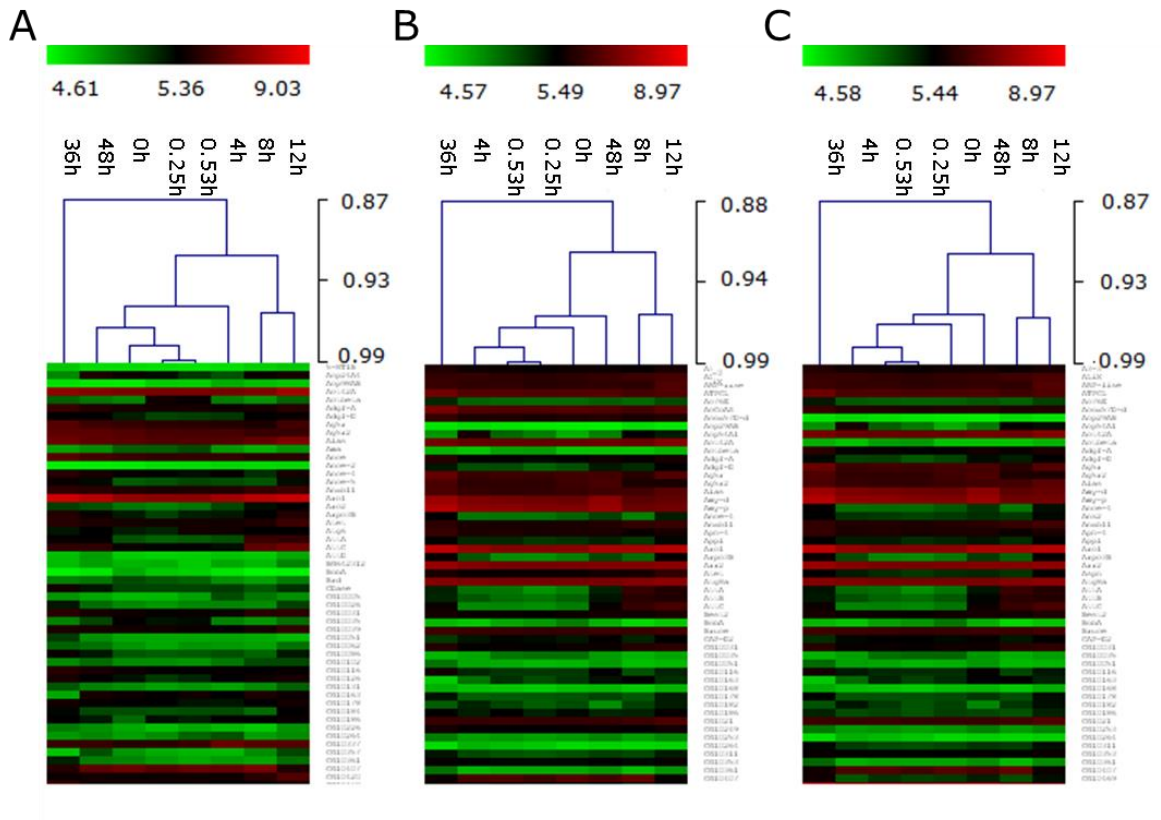

Figure S2 Average linkage hierarchical clustering of the eight point time-series across three replicates for the significant time-point term (FDR 0.2). A) 3'IVT module (*n* =1114 genes), B) constitutive probes from the exon module (*n* =1282 genes), and C) for all probes in the exon module (*n* =1171 genes). Similar temporal expression patterns are seen between the module and probe comparisons where the stress and early recovery period cluster closely together and are grouped with time zero and 48 hours recovery. The transcriptional response to thermal stress appears closer to basal levels by 48 hours post stress.

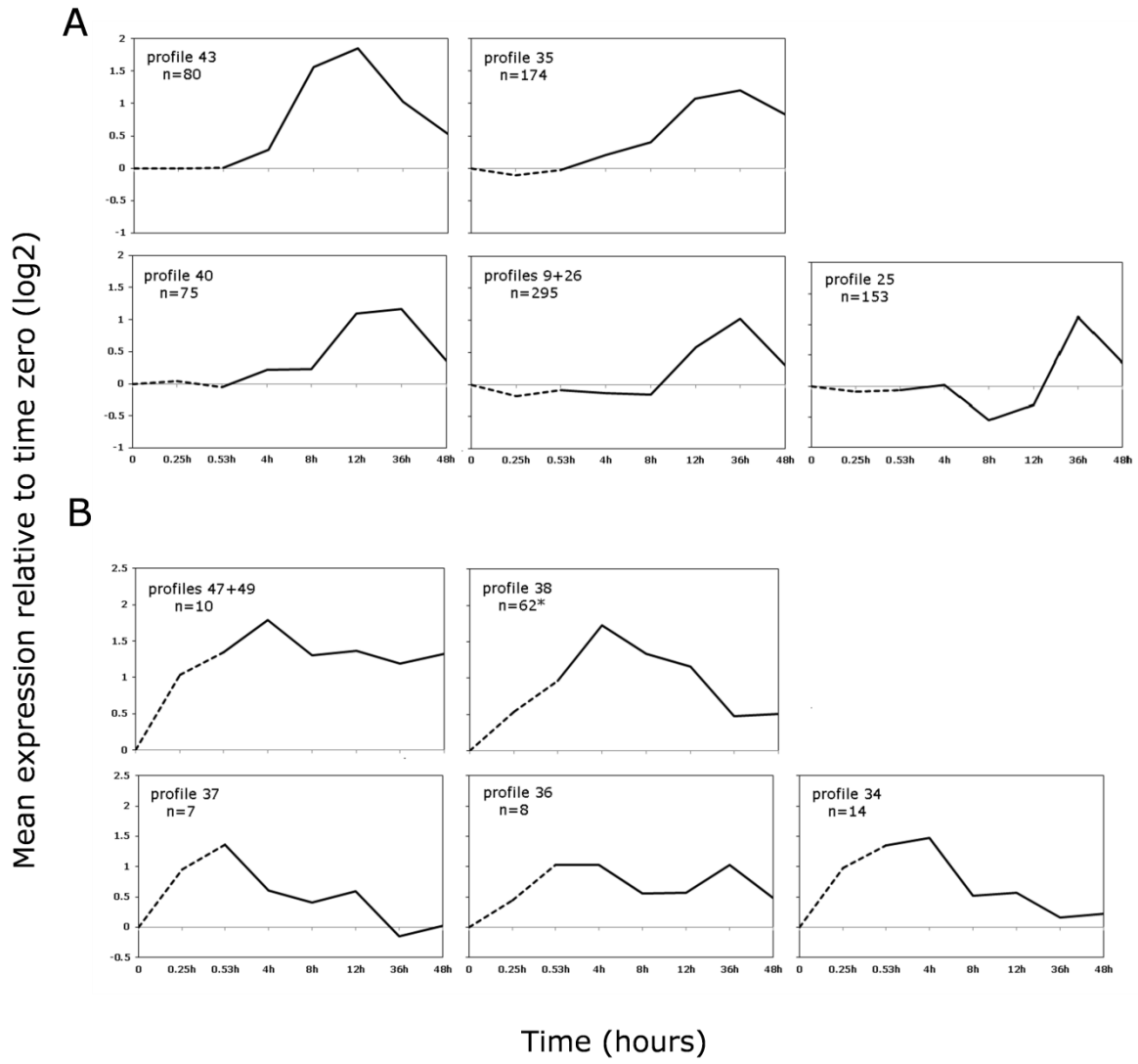

Figure S3 Short time-series expression miner (STEM) profiles of 1078 genes identified from the 3'IVT probe module with ANOVA. Average normalized (relative to time zero) signals of each profile/profile cluster are shown on the Y-axis, time in hours is shown on the X-axis. The dashed line indicates thermal stress sampled at 15 and 31.5 minutes at 38.5º, solid line indicates recovery up to 48 hours post stress at 25º. (A) Profiles enriched for mid-late recovery expressed genes with peak expression at 12 or 36 hours. Rapidly heat responsive genes (B) are under enriched with peak at either at late stress/early recovery.

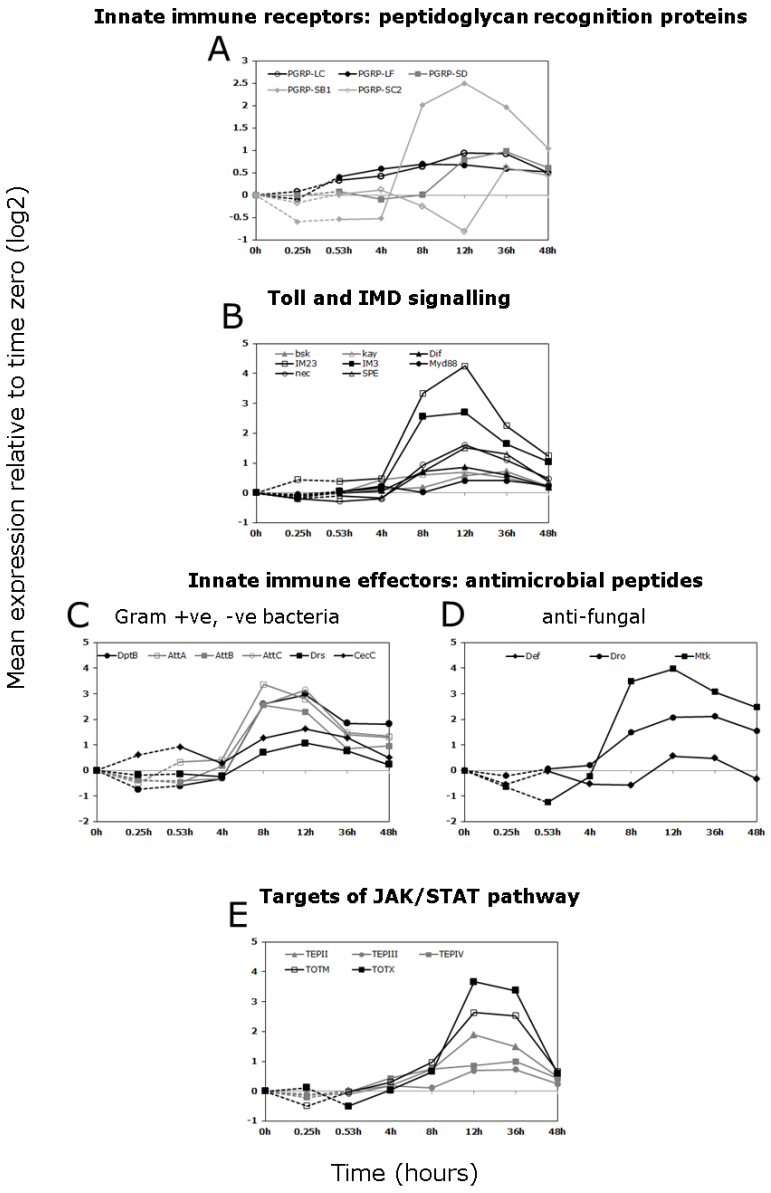

Figure S4 Up-regulation of genes spanning the repertoire of Drosophila innate immune response. Dashed lines indicate thermal stress at 38.5º, solid lines indicate recovery up to 48 hours post stress at 25º. Exon profiles from the microarray analyses (constitutive exon set) are shown as average normalized expression relative to time zero on the Y-axis (log2). A) Expression profiles of the PGRPs that activate the immune pathways to regulate the expression of antimicrobial peptides; PGRP-LC and LF (black circles) are required to activate the IMD pathway, PGRP-SD (dark grey squares) is required to activate the Toll pathway, other non-activating PGRPs SB1 and SC2 (light grey triangles). B) IMD (grey) and Toll (black) signaling components. C) Bacterial (gram –ve (IMD) and +ve (Toll)) and D) fungal (Toll) AMPs activated by the humoral immune pathways. E) AMPs and other genes regulated by the JAK/STAT pathway (IMD).

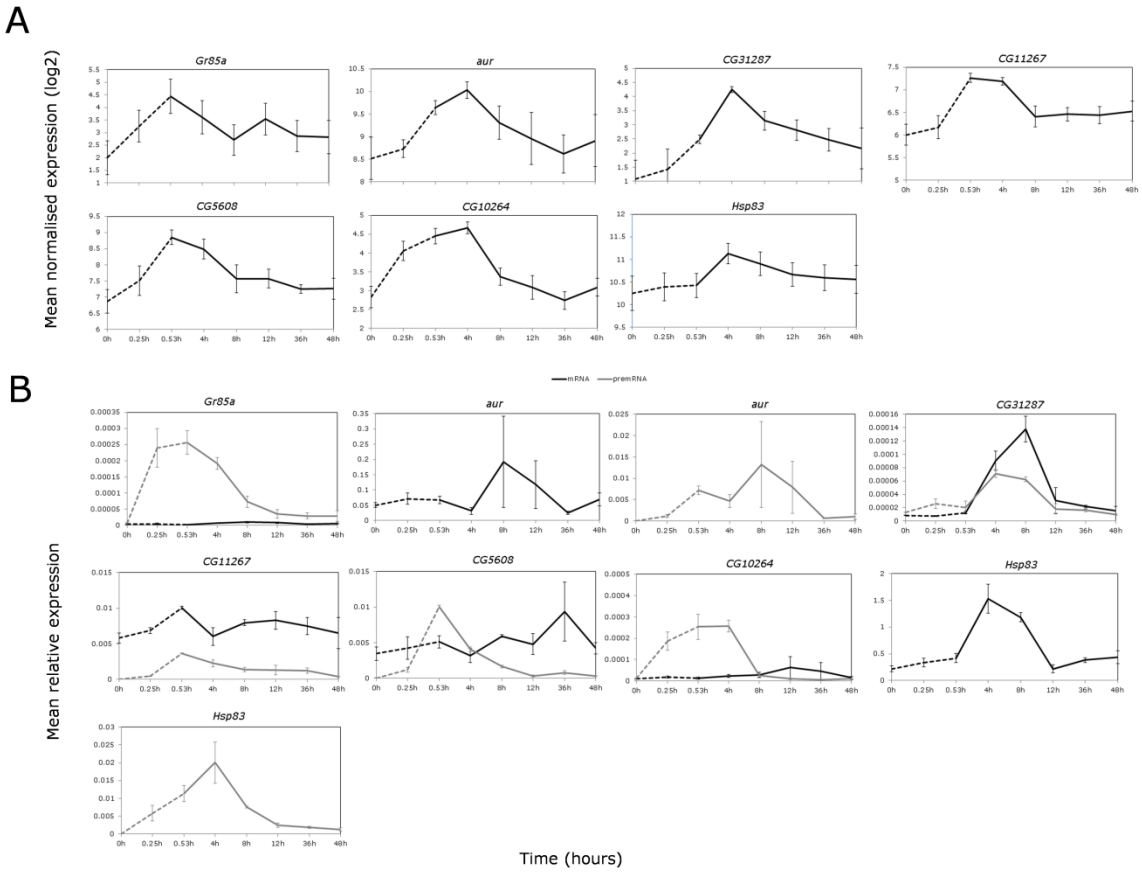

Figure S5 Detailed transcript analyses of early responding genes confirm RNA splicing inhibition during heat shock. Dashed lines indicate thermal stress at 38.5º, solid lines indicate recovery up to 48 hours post stress at 25º. Exon profiles from the microarray analyses (constitutive exon set), shown as average normalized expression on the Y-axis (log2). B) Real-time PCR profiling using exon-junction primers to target mature mRNA levels (black line) and intron/exon primers to target pre-mRNA levels (grey line) relative to *RpL11* (Y-axis). Negligible *Gr85a* and *CG10264* mRNA suggests that these genes may be transcriptionally active during heat shock. *Hsp83* is shown in the last panels of A and B to confirm nascent transcript accumulation during heat stress. Error bars are the ± SE of the mean.

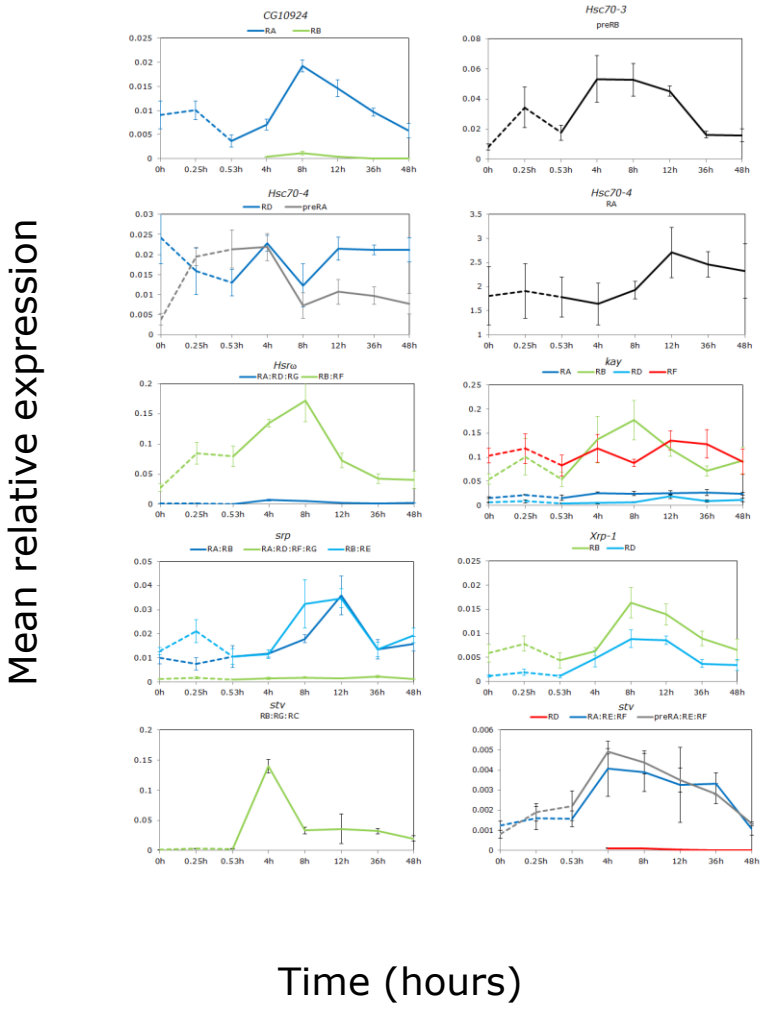

Figure S6 Raw data for PCR data for multi-transcript genes using exon-junction primers to target mature mRNA levels and intron/exon primers to target pre-mRNA levels relative to *RpL11* (Y-axis). Error bars are the ± SE of the mean.

File S1

Supplementary results

Array hybridization quality was evaluated for individual probe signals by examining kernel density distributions across modules and detection above background (DABG) within modules. Similar kernel density distributions were observed for all hybridizations, although the signal intensities for time-point zero, replicate one were slightly dimmer. This was reflected in the proportion of probes detected above the median of the GC band control signals, where DABG ranged from 80.5-86.3% in the 3'IVT module and 84.1-89.3% in the exon module, and 78.8% and 82.3% in the 3'IVT and exon modules respectively for the dimmer slide. All hybridizations were well in the expected range for *D. melanogaster* given that an average of 72% DAGB was reported for heterologous hybridizations in *D. simulans* (Yang, Graze et al. 2011). Despite the dimmer slide, Kappa statistics indicated good agreement between biological replicates at the individual probe level, and excellent agreement at the probeset and gene level when modules were considered separately (Table 1.). Within a slide, signals for probesets corresponding to the same gene in the 3'IVT and exon modules were in good agreement indicating inter-slide reliability of gene expression between modules (Table 1.). Normalised signal agreement between the *heat shock* genes (*Hsps)* was excellent within and between modules (Table 1.), in addition average signal intensities of seven early responding *Hsps* correlate strongly **(**R 0.77-0.99, P <0.05- 0.0001, Fig S1).

Overall expression patterns at the level of sampling temperature and time-point were visualised using hierarchical clustering. Average linkage clustering was applied to the average normalised signals of 1114, 1285, and 1173 differentially expressed genes from the 3'IVT, exon constitutive and exon modules respectively (Fig S2). The modules clustered similarly, with the dendograms revealing two main expression clusters; 1) the early time points plus 48 hours, and 2) the mid time-points (Fig S2). For cluster 1, the high temperature points clustered closely with time zero in the 3'IVT module (Fig S2a), while these were more similar to 4 hours recovery in the exon module (Fig S2b,c). In all cases, expression tended to return to basal levels by 48 hours recovery indicated by the grouping of time zero and 48 hours. Cluster 2 comprised the 8 and 12 hour recovery points, while 36 hours recovery grouped independently (Figure S2).

Table S1 Primer sequences for real-time PCR

Gene	Isoform/s	Forward	Reverse	Transcript type
RPL11	RA	CGA TCC CTC CAT CGG TAT CT	AAC CAC TTC ATG GCA TCC TC	mRNA
Hsrω	RB:RF	TCC GCA TTT ATT TTT CTC CAC	GTG TAT AGA ATT TGG GAC CTC CA	nCRNA
	RA:RD:RG	TAG GAA GCC AGT GGG	CCG AGT GCG TTT TCA GCA	nCRNA
Gr85a	RA	TGG AAC GAA GTA TCG AAT GGC T	CAC CAT GTA GAG CAC GTG GA	mRNA
	RA	TGT ATC CAA CCA TTG ATG CTC T	GGA TTG GAA CGC CAG GAT AC	pre-mRNA
Aur	RA	ACC AAG ACT GAA ACC CAG CC	TTT CCC GCG CCA AAT AAA CG	mRNA
	primary	GAA AAT GCT CCG CAC AGA A	TTT CAG CTG CAC TCC AGA GA	pre-mRNA
CG31287	RA	TGC CAA AAA TGC ACT TCC CA	ACT CGG ATA GCT CTG CTC CA	mRNA
	primary	AGA TCC GTC GAC ATT CCT GT	TGC CTA TGG CCA TTG AGT CT	pre-mRNA
CG5608	RA	CGC AGG AGA TCG AGA AAA TGG	CAG ACC GAT AAG CGC TCC TT	mRNA
	primary	TCA CCT GGA TAC GAG AGT TTG	GGA ATT AAA CGA GCG CTT TC	pre-mRNA
CG12267	RA	TCA AGT TCA GGC TGG TGG AC	ACC AGG TGA ACG TAA CGA GG	mRNA
	primary	CCA GGA ACA GTT TAT TCA TGT CA	TTT CCT CGA CCA CAC TCA CA	pre-mRNA
CG10264	RA	GAG AGG CCC TCG TGG CT	CGG AAA GCA TCC CTC GAA GA	mRNA
	primary	CGG CAA CCT GGT GCT ATC	TTT CCT CGA CCA CAC TCA CA	pre-mRNA
Hsp83	RA	CAT ACA AGA TGC CAG AAG AAG C	TGG GGT CAG TAA GGG ACT CA	mRNA
	primary	TGA GGC ATG TGC AAA AGA GA	AGC CTG GAA TGC AAA GGT C	pre-mRNA
Stv	primary	CCC AAA ACG CTT ACG GAT CG	GGG GGC CAC TCA CCT GAA AA	pre-mRNA
	RA:RE:RF	CAC AGT TCC ACA CTC CCC AA	GAA TCC AAA GGT CGG CTG AA	mRNA
	RB:RC:RG	GTC ACC AAG CGG AAA AGC AT	CAA AGG TCG GCT TTT GCC TG	mRNA
	RD	ACA TAG TTG ATG TGA AAC AGC G	CCA AAG GTC GGC TGT TTT ATA ATT T	mRNA
Hsc70-4	primary	CAG TTT GAT CGA AGG TGC GG	ACT TAA TCG AGG TGG TCG CA	pre-mRNA
	RA	CAG TTT GAT CGA AGG TGC G	CAG GAG CTT TAG ACA TCT TGT G	mRNA
	RD	CGT AAT TGA TGT CTA AAG CTC C	GAG TGG TAC GAT TAC CCT G	mRNA
Hsc70-3	primary	GGG CAC AGT GAT CGG CAT T	GGG TTT TAG AGC CGA AGG ACG	pre-mRNA
Srp	RA:RD:RF:RG	AGG AAG AGA GGA GCC AAA GAG AGG	CAA CGA GCC AGC ATA AAC AGA GTC	mRNA
	RA:RB	CAG AGC TTC ACC CAG CTG AC	AAC AGA GCT GTT CTG CAA GC	mRNA
	RB:RE	CGG GAC ACT ATT TGT GCA ATG CC	CGC TTT GAG GCG CTC AAT CTT C	mRNA
Xrp1	RB	GTC GCC GCA CTT TCT TTT GA	ACA AGT TCC CCT TAA ACC TCC A	mRNA
	RD	CGG AAC CGC TTA AAA GAC AGC	TTC CGT TTT CGC TGT TGC AC	mRNA
CG10924	RA	CCA AGA GTA TTA GCG GGC GA	TGT GGT GAG ACC AAT CCG C	mRNA
	RB	TGC TCG TTT CGG TTA GTC GG	CGG TGT GGT GAG ACC AAT CTT T	mRNA
Kay	RA	ACT TTC TGC CCG CCG ATC TAA G	GGT CTC AAA GTT GCC GAG GAT AAG	mRNA
	RB	TCG GTG TGC GGA ATA CAA AGG C	TCG TAT GGC CGC ACA AAG TCT G	mRNA
	RD	ACA GCA TCA GCG ACA GGA TTA TGC	CGG TCT CAA AGT TGC CGA GTT G	mRNA
	RF	CTT TGC AAT GGA CGC CAG TGA G	AAA GTT GCC GAG CTG CTG TAG G	mRNA

Table S2 Kappa statistics for signal intensity agreement within modules at the individual (raw) probe, normalised probeset, and gene levels. Signal agreement between the 3'IVT and exons modules was comparable at the gene level only.

Module/s	Weighted Kappa Coefficient (range)			
	Individual probes	Probesets	Genes (all)	Genes (Hsps) [c]
3'IVT[a]	0.69-0.77	0.88-0.93	0.89-0.93	0.80-1
Exon[a]	0.74-0.8	0.87-0.92	0.89-0.94	0.86-1
3'IVT vs exon[b]	N/A	N/A	0.61-0.68	0.66-1

[a]Kappa statistics were calculated for each module between replicate slides

[b]Kappa statistics were calculated between modules on the same slide

[c]Agree statistics were calculated only for comparisons where the number of rows and columns were equal

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Table S3 Gene level average log background corrected signals from Model I analyses (fixed term of time-point).

Table S4 STEM profiling for significant genes (time-point term) from the constitutive exon analysis.

Table S5 DAVID functional annotation clustering analysis of the genes from the enriched recovery STEM profiles as well as the 'early up' profiles from the gene-level analyses (FDR 0.05).

Table S6 Table of genes spanning the repertoire of the Drosophila innate immune response from the gene-level (constitutive probeset) analyses.

Table S7 Results for two-way ANOVAs testing for expression changes over time following heat stress (time term), transcript-type (transcript, mRNA or pre-mRNA) and interaction term. Significant terms are bolded.

Gene	Effect	DF	SS	MS	Fvalue	*Pvalue*
CG10264	time	7	64.09	9.150	6.77	**0.0001**
CG10264	Transcript	1	11.36	11.36	8.40	**0.0067**
CG10264	time*Transcript	7	63.91	9.13	6.75	**0.0001**
CG12267	time	7	61.93	8.84	18.16	**0.0000**
CG12267	Transcript	1	138.09	138.09	283.38	**0.0000**
CG12267	time*Transcript	7	44.47	6.35	13.04	**0.0000**
CG32187	time	7	55.38	7.91	17.91	**0.0000**
CG32187	Transcript	1	0.020	0.02	0.05	0.8294
CG32187	time*Transcript	7	7.105	1.01	2.30	0.0532
CG5608	time	7	69.16	9.88	20.53	**0.0000**
CG5608	Transcript	1	69.17	69.17	143.75	**0.0000**
CG5608	time*Transcript	7	58.90	8.41	17.49	**0.0000**
Hsp83	time	7	133.96	19.13	38.00	**0.0000**
Hsp83	Transcript	1	649.16	649.16	1288.98	**0.0000**
Hsp83	time*Transcript	7	56.23	8.03	15.95	**0.0000**
Aur	time	7	131.02	18.71	12.20	**0.0000**
Aur	Transcript	1	374.94	374.94	244.35	**0.0000**
Aur	time*Transcript	7	109.20	15.60	10.17	**0.0000**
gr85A	time	7	54.437	7.776	10.16	**0.0000**
gr85A	Transcript	1	142.06	142.06	185.55	**0.0000**
gr85A	time*Transcript	7	38.76	5.53	7.23	**0.0000**

Table S8 Results for one-way ANOVAs with Dunnett's tests comparing pre-stress relative transcript abundances with high temperature (38.5°) and recovery (25C°). LSMean= least squares mean, significant terms are bolded.

Gene	Transcript	time	LSMean	*P*value
CG10264	**mRNA**	0	-16.7583361	
		0.25	-15.9483352	0.9714
		0.53	-16.3533329	0.9995
		4.315	-15.5166648	0.8255
		8.315	-15.4900004	0.8124
		12.315	-15.0916692	0.5932
		36.315	-15.9966668	0.9791
		48.315	-16.2116654	0.9967
	Pre-mRNA	0	-16.4033326	
		0.25	-12.4600007	**0.0001**
		0.53	-12.0166658	**0.0000**
		4.315	-11.9433335	**0.0000**
		8.315	-15.4550002	0.5240
		12.315	-16.8533346	0.9638
		36.315	-17.7083332	0.2303
		48.315	-16.7416690	0.9918
CG12267	**mRNA**	0	-7.4566667	
		0.25	-7.1916666	0.9822
		0.53	-6.6366666	0.2932
		4.315	-7.4450001	1.0000
		8.315	-6.9816666	0.7843
		12.315	-6.9500000	0.7369
		36.315	-7.0916666	0.9188
		48.315	-7.5283333	1.0000
	pre-mRNA	0	-15.3516682	
		0.25	-11.1433333	**0.0001**
		0.53	-8.1049998	**0.0000**
		4.315	-8.8616665	**0.0000**
		8.315	-9.5366668	**0.0000**
		12.315	-10.0699998	**0.0000**
		36.315	-9.8750003	**0.0000**
		48.315	-11.4766660	**0.0002**
	mRNA	0	-16.8366663	
		0.25	-16.9783335	0.9999
CG32187		0.53	-16.3133328	0.8981
		4.315	-13.4783311	**0.0002**
		8.315	-12.8400027	**0.0001**
		12.315	-15.5800008	0.1967
		36.315	-15.5066658	0.1585
		48.315	-16.1916670	0.7838
	pre-mRNA	0	-16.2349999	
		0.25	-15.3650017	0.4116
		0.53	-15.8116684	0.9310
		4.315	-13.7866662	**0.0014**
		8.315	-13.9674991	**0.0068**
		12.315	-16.0000006	0.9968
		36.315	-15.9383329	0.9879
		48.315	-16.6700025	0.9225

CG5608	**mRNA**	0	-8.3016668	
		0.25	-8.0700000	0.9986
		0.53	-7.6583333	0.7849
		4.315	-8.4150002	1.0000
		8.315	-7.4016666	0.4928
		12.315	-7.8383334	0.9388
		36.315	-7.0016667	0.1721
		48.315	-7.9349999	0.9802
	pre-mRNA	0	-14.3666667	
		0.25	-9.8333336	**0.0000**
		0.53	-6.6416667	**0.0000**
		4.315	-7.9500001	**0.0000**
		8.315	-9.2449999	**0.0000**
		12.315	-11.7933334	**0.0019**
		36.315	-10.4633332	**0.0000**
		48.315	-11.6633344	**0.0012**
Hsp83	**mRNA**	0	-2.2916667	
		0.25	-1.6900000	0.7346
		0.53	-1.3366667	0.3080
		4.315	0.5733333	**0.0002**
		8.315	0.2375000	**0.0024**
		12.315	-2.4100000	1.0000
		36.315	-1.4116667	0.3833
		48.315	-1.3033333	0.2782
	pre-mRNA	0	-14.8999997	
		0.25	-7.6866666	**0.0000**
		0.53	-6.5233334	**0.0000**
		4.315	-5.7883333	**0.0000**
		8.315	-7.0400001	**0.0000**
		12.315	-8.7466664	**0.0000**
		36.315	-9.1483334	**0.0000**
		48.315	-9.8266670	**0.0000**
aur	**mRNA**	0	-4.3550000	
		0.25	-3.8966667	0.9939
		0.53	-3.9383333	0.9965
		4.315	-5.2000000	0.8809
		8.315	-3.3883333	0.8069
		12.315	-3.7900000	0.9810
		36.315	-5.3866667	0.7621
		48.315	-3.9850000	0.9983
	pre-mRNA	0	-17.2433333	
		0.25	-9.8766670	**0.0000**
		0.53	-7.1400001	**0.0000**
		4.315	-7.9633332	**0.0000**
		8.315	-7.1616666	**0.0000**
		12.315	-8.1666669	**0.0000**
		36.315	-10.6350007	**0.0001**
		48.315	-10.4716673	**0.0001**
Gr85a	**mRNA**	0	-18.4216659	
		0.25	-18.1483327	0.9992
		0.53	-18.4624992	1.0000
		4.315	-17.1700001	0.4185
		8.315	-16.7466676	0.1727
		12.315	-17.0500007	0.3321

	36.315	-18.2216673	0.9999
	48.315	-17.7816679	0.9174
pre-mRNA	0	-17.4133322	
	0.25	-12.1449989	**0.0000**
	0.53	-11.9566662	**0.0000**
	4.315	-12.3683326	**0.0000**
	8.315	-13.8200010	**0.0005**
	12.315	-15.0516677	**0.0189**
	36.315	-15.2083341	**0.0296**
	48.315	-15.5583342	0.0789

Table S9 ANOVA results for model III fit with the constitutive exons as a covariate for 1,094 genes with constitutive exons and at least two alternative exons. The main effects of constitutive exon, alternative exon, time-point and alternative exon-by-time-point interactions are shown. Results are based on type III SS, significance threshold <0.2.

FDR level	Constitutive exon	Alternative exons	Time-Point	Alternative exon-by-time-point interaction
<0.05	1408	1031	29	56
<0.1	88	12	8	20
<0.2	120	8	27	24
>0.2	295	43	1030	994

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Table S10 Isoform/isoform subset level average log background corrected signals from Model II and III analyses.

Table S11 STEM profiling for significant multi-transcript genes from the master list (time-point term, time-point-by-exon-type interactions.

Table S12 DAVID functional annotation clustering analysis of the isoforms/subsets from the enriched recovery STEM profiles as well as the 'early up' profiles from the significant genes from the master list from models II and III.