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Analysis of stress-induced hepatic gene expression in rainbow trout (*Oncorhynchus mykiss*) selected for high- and low-responsiveness to stress

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Abstract

The production and welfare of intensively reared fish would be improved by reducing stress responsiveness. One approach to achieving this goal is selective breeding utilizing stress-responsive genes as direct genetic markers of the desirable trait. As a first step in this process, microarray analysis has been carried out on liver tissues of rainbow trout selectively bred for high (HR) or low (LR) responsiveness to a stressor. Microarray hybridizations provided gene expression profiles for pooled samples of fish confined for 6 h, 24 h and 168 h and for individual fish (168 h only). 161 genes were shown to be differentially regulated in HR and LR fish during confinement exposure and eight of these gene expression profiles were validated by quantitative PCR. Genes of particular interest included intelectin-2 precursor which showed greater than 100-fold higher expression in HR fish compared to LR fish irrespective of whether the fish were confined or not; interferon inducible transmembrane protein 3 which was differentially stress-induced between the two lines; and hepatic pro-opiomelanocortin B (POMC B) which was upregulated during stress in HR fish but downregulated in LR fish. All these offer potential as direct markers of low stress responsiveness in a marker-assisted selection scheme.

Key words: cortisol, genetic markers, liver, microarray, qPCR, stress axis, selective breeding, time course

Abbreviations: POMC B, pro-opiomelanocortin B; qPCR, quantitative polymerase chain reaction;

1. Introduction

The vertebrate stress response is an adaptive suite of physiological and behavioural reactions to stimuli that present a threat or challenge to the organism. The response is initiated and coordinated by a neuroendocrine axis which in teleost fish comprises the hypothalamic-pituitary-interrenal system and the sympatho-chromaffin tissues (Wendelaar Bonga, 1997). The stress response deals most effectively with stressors that are brief in duration whereas during exposure to prolonged or intermittent stressors the costs associated with the response may outweigh its benefits (Romero et al., 2009). In particular, frequent or prolonged activation of the stress axis can result in immunosuppression, reproductive dysfunction and reduced growth.

The links between husbandry practices, stress, and the welfare and performance of farmed fish have received increasing attention in recent years (Huntingford et al., 2006). One strategy that has been considered as a means to reduce stress in fish within the aquaculture environment, and thus enhance production and improve welfare, is reduction of the responsiveness of aquacultured fish to unavoidable stressors by selective breeding (Pottinger, 2000).

The elevation of plasma cortisol in fish is widely employed as an index of the severity and duration of a stressor; cortisol is a causal factor in many adverse outcomes of stress, and the magnitude of the cortisol response to a stressor is a heritable trait (Pottinger et al., 1999). Therefore the stress axis is a logical locus upon which to apply selective pressure. To investigate the endocrine and behavioural consequences of modifying stress responsiveness in fish two lines of rainbow trout with divergent cortisol responsiveness to a stressor have been selectively bred (Øverli et al., 2007).

This study comprised one of several within the EU project Stressgenes (contract no. Q5RS-2001-002211) and focused on liver as a key target organ within the stress axis with important roles in stress-related processes and which was therefore considered likely to exhibit differences in gene expression profile linked with the HR/LR phenotypes. This attribute and the accessibility of the tissue to biopsy make liver a potentially useful tissue on which to base a marker-assisted selection scheme.

The present study follows a previous microarray-based investigation of hepatic gene expression during stress in unselected rainbow trout (Cairns et al., 2008) and was designed to address two questions: Does stress alter gene expression in the liver of high-responding and low-responding rainbow trout differently? If so, do any differentially expressed genes offer potential for use in a direct marker-assisted selective breeding programme?

2. Materials and methods

2.1. Selection of rainbow trout for magnitude of cortisol response to a stressor

The selection programme used to generate the high-responding (HR) and low-responding (LR) lines of rainbow trout (*Oncorhynchus mykiss*) has been described previously (Pottinger et al., 2001b). Individuals identified as either HR or LR via their corticosteroid response to a series of standardized confinement stressors were used to generate F1 lines comprising 15 and 14 unique full-sib families respectively of HR and LR progeny. An individual within family selection process was adopted to establish the F2 and subsequently the F3 generation which was used in the present

study. The studies were licensed by the UK Home Office and carried out in accordance with the Animals (Scientific Procedures Act) 1986.

2.2. Exposure of HR and LR trout to a standardized confinement stressor

A time-course study using HR and LR fish from the F3 generation in their second year (1+) was carried out during August and September 2004. During July, LR and HR fish (LR weight: 181.3 ± 4.4 g; length: 24.7 ± 0.21 cm; HR weight: 184.7 ± 4.9 g; length: 24.9 ± 0.2 cm) were distributed between twenty-two 1000 litre holding tanks (approx 110 fish/tank, 11 for each line). No mature females were present in the population and only four mature males were detected. These were not used in the gene expression analyses. Each tank was supplied with a constant flow of untreated Windermere lake water (20 litres min^{-1}) at ambient temperature (mean 16.7 ^{o}C). After a 2 week period of acclimation, LR fish from a single holding tank were transferred to twenty-two 50 litre confinement tanks, 5 fish per tank, each supplied with a constant flow of lake water. At intervals (0, 2, 4, 6, 8, 24, 48, 96, 168, 336, 504 h) a total of 6 fish (3 fish from each of two tanks) were sampled, with undisturbed LR fish in pairs of holding tanks acting as controls. At each time-point livers were collected and immediately frozen in liquid nitrogen for subsequent analysis. The confinement and sampling procedure was repeated 1 week later for the HR fish. Staggering the two time-series was necessary because of limits on manpower and tank space. Plasma cortisol was measured by a validated radioimmunoassay procedure (Pottinger et al., 2001b). Differences in plasma cortisol levels within and between lines were evaluated by analysis of variance (ANOVA, GenStat 10; VSN International Ltd). Where mean and variance did not vary independently, as indicated by a plot of residuals against fitted values, data were log-transformed to improve the homogeneity of variance.

123 Multiple comparison post-tests to assess significant differences between groups were
124 carried out using the estimated standard error of the differences between means
125 provided by the ANOVA output.
126

127 *2.3. RNA isolation and quantification*

128 Frozen liver samples from control and confined fish of both lines were used for total
129 RNA isolations. LR fish with the lowest intra-group cortisol levels (0.1 to 0.9 ng/mL
130 for controls, 0.0 to 152.1 ng/mL for confined) and HR fish with highest intra-group
131 cortisol levels (0.6 to 2.1 ng/mL for controls, 1.4 ng/mL to 446.9 ng/mL for confined)
132 from various time points were selected for RNA isolation. Total RNA was isolated
133 using the RNeasy Midi Kit (Qiagen) following the manufacturer's recommended
134 protocol after homogenising 0.2 - 0.3 mg of tissue in Qiazol reagent (Qiagen).
135 Contaminating genomic DNA was degraded using an on-column DNase I treatment
136 using RNase-free DNase Kit (Qiagen). The quality of RNA was checked using the
137 Agilent Bioanalyser 2100. RNA integrity numbers (RIN) were found to be 10 for
138 most of the samples. Total RNA was quantified by UV spectrophotometry at 260 nm
139 (Eppendorf Biophotometer).
140

141 *2.4. Microarray design*

142 The trout oligonucleotide array was designed and constructed as part of the
143 AQUAFIRST project (http://aquafirst.vitamib.com/). High density oligonucleotide
144 (50-mer) microarrays were used each containing 3343 gene-specific oligonucleotides
145 printed in triplicate. The array platform has been submitted to the Gene Expression
146 Omnibus (GEO) with accession number **GPL11206**. Two different design approaches

were used for microarray hybridizations. Due to the limiting number of microarray slides available, RNA from only 3 time points (6 h, 24 h and 168 h) was used for microarray analysis. A loop design (21 arrays, Fig. 1) was used for hybridizing pooled RNA from stressed fish (n = 5 per time point) against pooled RNA from control fish (n = 3 per time point). Across the six pools, approximately equivalent numbers of male and female fish were used. Subsequently a reference design was used for hybridizing individual HR stressed fish (n = 5) against individual LR stressed fish (n = 5) from the 168 h time point using pooled RNA from all samples as a common reference. Each fish was compared twice to the pooled reference using a dye-swap protocol (20 arrays).

2.5. cDNA synthesis and labelling

Five µg of total RNA was used to synthesize labelled cDNA using the ChipShot™ Direct Labelling and Clean-Up System (Promega) following the manufacturer's protocol. Labelled cDNA was quantified using a NanoDrop™ ND 1000 spectrophotometer (Thermo Scientific).

2.6. Microarray hybridization and washing

All chemicals were purchased from Sigma-Aldrich unless indicated otherwise. Cy3-labelled and Cy5-labelled cDNA were dried and resuspended in 22 µL of hybridization mix (5X Denhardt, 3.5X SSC, 0.3% SDS (Bio-Rad), 0.5 µg/µL yeast tRNA (Invitrogen), 0.5 µg/µL poly(A) RNA (Invitrogen) and 50 % (v/v) formamide). Slides were prehybridized with a solution containing 50 mM ethanolamine, 100 mM Tris base, 0.1% SDS, pH 9.0 at 50 ºC for 15 minutes followed by two washes in 18

171 MΩ water. Next, slides were incubated in a solution containing 4X SSC and 0.1%
172 SDS at 50 ºC for 15 minutes followed by one wash with 18 MΩ water and
173 centrifugation (260 *g*) for 3 minutes at room temperature. Slides were incubated in
174 prehybridization solution (3.5X SSC, 0.3% SDS, 1% BSA) at 42 ºC for one hour.
175 Slides were washed 5 times with 18 MΩ water and dried by centrifugation (260 *g*) for
176 3 minutes. Before hybridization, Cy3- and Cy5-labelled targets were pooled and
177 denatured at 100 ºC for 2 minutes. Targets were applied to the microarray slide and
178 covered with a coverslip (Erie Scientific). Hybridization of the slides was performed
179 in a hybridization chamber (Genetix) humidified with 3X SSC at an annealing
180 temperature of 42 ºC overnight followed by immersion of slides in 2X SSC, 0.1%
181 SDS for the removal of the coverslip. Slides were washed with 1X SSC for 2 minutes
182 and followed by 2 washes with 0.2X SSC for 2 minutes each. Slides were dried by
183 centrifugation (260 *g*) for 3 minutes at room temperature and immediately scanned
184 using the ScanArray Express HT Microarray Scanner (Perkin Elmer).
185

186 *2.7. Image and data analysis*

187 GenePix software (Molecular Devices, Corp) was used for feature acquisition from
188 the TIF images (GenePix Pro 6.0.1.25). Data from the time-course (loop) experiment
189 (Fig. 1) were analysed using separate channel analysis of two colour data using the
190 Limma package (available as part of the Bioconductor software project,
191 www.bioconductor.org). The raw data from this loop experiment was normalised
192 within slide using the “printtiploess” method and the “normexp” background
193 correction method (Smyth et al., 2003). Log2 fold changes were estimated using
194 linear models and empirical Bayes moderated F statistics for each gene: fold changes
195 in subsequent tables and figures were converted back from log2 values. Features were

196 only included where at least two out of three replicates were significant ($P<0.05$).
197 Final expression values for significant genes were obtained by averaging the replicate
198 spots.
199 Initial K-means clustering of a 531 gene stress-related gene list (see section 3.2)
200 identified a cluster of genes showing a sharp increase in expression between 24 h and
201 168 h in the LR line only, suggesting some anomalous behaviour in the LR 168 h
202 pool. A second set of microarray hybridizations (see section 3.5) performed on
203 individual animals sampled after 168 h confinement identified fish LS44 (LR,
204 confined) as atypical with respect to the other fish in this group. An ANOVA carried
205 out on the 531 genes between the HR and LR individuals in the presence or absence
206 of LS44 led to the removal of 44 genes that contributed a strong'LS44 effect'. These
207 genes were removed from all subsequent gene lists prior to clustering in order to
208 highlight only confinement-related effects (though a small effect caused by LS44 still
209 persisted).
210 Data from the indirect (reference design) microarray on individual fish (168 only)
211 were analysed using GeneSpring 7.3 software (Agilent Technologies). Loess
212 normalisation followed channel swapping of the appropriate dye-swapped samples.
213 The data series have been submitted to Gene Expression Omnibus with accession
214 number **GSE25584**.
215

216 *2.8. Quantitative RT-PCR analysis*

217 cDNA was synthesized from 5 µg of total RNA and 500 ng of poly(T) primer in a
218 reaction volume of 40 µL using SuperScript™ III reverse transcriptase (Invitrogen)
219 following the manufacturer's protocol. All cDNA samples used for qPCR were
220 derived from total RNA of individual fish and the same individual RNA preparations

were used for qPCR as were used for the microarray analysis. Primers were designed for qPCR for all eight candidate genes and one housekeeping gene (18S ribosomal RNA) using Vector NTI Advance™ software (Invitrogen). Size of the amplicons produced ranged between 100-150 base pairs. qPCR reactions were set up as follows: 10 µL of QuantiTect SYBR Green PCR Master Mix (Qiagen) containing 0.5 µM final concentration of primers, 5 µL of cDNA template (diluted 1:12.5) made up to a final volume of 20 µL with RNase-free water. The qPCR reactions were conducted in the Mx3000P QPCR System (Stratagene). The program used for qPCR was 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s, annealing at 51 °C-60 °C (depending on primers) for 30 s and extension at 72 °C for 30 s. Dissociation curves were checked at the end of PCR reactions for nonspecific amplification and dimerization of primers. Relative fold changes of candidate genes were calculated using the delta delta Ct method (Pfaffl, 2001). To assess PCR efficiency of each gene, a standard curve was created using serial dilutions of standard cDNA preparations. Differences in relative expression levels of each gene within and between lines were evaluated by ANOVA followed by Holm-Sidak pairwise multiple comparison tests (SigmaPlot for Windows version 11; Systat Software, Inc). Data were log-transformed prior to analysis to improve the homogeneity of variance.

3. **Results**

3.1. Cortisol levels during confinement

Plasma cortisol levels in confined fish of both lines were significantly elevated relative to control unconfined fish until between 48 h and 96 h after the onset of confinement, after which there was no significant difference between confined and

246 unconfined fish (Fig. 2). In addition to the overall effect of confinement on plasma
247 cortisol levels, there was a highly significant difference in plasma cortisol levels
248 between the confined HR and LR lines ($P<0.001$) with cortisol levels in the HR fish
249 significantly higher than those in LR fish from 2 h after the onset of confinement (HR:
250 142.5 ± 20.5 ng/mL; LR: 58.8 ± 12.0 ng/mL) to between 48 h and 96 h later (with the
251 exception of the sample at 6 h) after which there was no significant difference evident
252 between the lines. Plasma cortisol levels in the unconfined control fish of both lines
253 were statistically indistinguishable from each other (mean <5.0 ng/mL).
254
255 *3.2. Microarray analysis of stress-related effects on expression*
256 In order to investigate differences in gene expression between HR and LR trout during
257 stress, total RNA from liver tissue of confined HR and LR trout was converted to
258 cDNA and hybridized to microarray slides (Fig. 1). A time course of response to the
259 stressor in both lines was investigated using pooled RNA samples for each time point.
260 Analysis using the Limma package first identified genes whose expression was
261 significantly altered in either line by the confinement stressor by comparing the stress
262 and corresponding control values for the three time points. A second analysis
263 identified line-related differences by directly comparing corresponding samples from
264 the two different lines (section 3.3). The total numbers of genes differentially
265 regulated in response to the stressor were 139, 120 and 258 for HR and 50, 277 and
266 202 for LR at 6 h, 24 h and 168 h time points respectively. The overlap of these gene
267 lists between the LR and HR lines is summarized by Venn diagrams (Fig. S1). This
268 transcriptomic analysis showed that the number of hepatic genes that were
269 differentially regulated in <u>both</u> the HR and LR lines during stress increased from 6 at
270 6 h, and 21 at 24 h, to 50 at 168 h (Fig. S1). Only genes showing a 2-fold or greater

change in expression level in at least one of the fish lines are shown in Table 1. At 6 h confinement no gene showed upregulation of more than 2-fold. Genes that were downregulated by more than 2-fold after 6 h of confinement included mannose binding lectin and alpha-globin (Hemoglobin subunit alpha; Table 1). Mannose binding lectin was downregulated approximately 7-fold in HR and 9-fold in LR fish at 6 h. The only gene of the four in Table 1 to show a divergent pattern of expression at 6 h between the lines was POMC B (corticotropin-lipotropin B precursor) which was upregulated in the HR fish (1.3-fold) but downregulated in the LR fish (2-fold).

After 24 h confinement eight of the 21 genes whose expression changed in both HR and LR fish (Fig. S1) were upregulated and two were downregulated by more than 2-fold in fish from both lines compared to unconfined fish (Table 1). The most strongly upregulated gene after 24 h confinement was purine nucleoside phosphorylase, which was upregulated 8.1-fold in the HR line compared to 3.7-fold in the LR line. Only two genes showed changes at both 6 h and 24 h: consistent with the pattern of expression at 6 h, alpha-globin remained downregulated while POMC B continued to show modest downregulation in LR fish at 24 h (2.6-fold) and slight upregulation in HR fish (2-fold). EST **CT572011** and lamina-associated polypeptide 2 isoform beta showed a similar expression pattern to POMC B at 24 h.

Fifty genes were identified as being altered significantly in both HR and LR fish after 168 h of confinement (Fig. S1) with some very pronounced (up to 28-fold) and highly significant changes observed (Table 1). Both oligonucleotides representing ESTs **BX311643** and **CR944619** aligned with the Differentially Regulated in Trout Protein 1 (DRTP1) gene. This suggested strong upregulation of DRTP1 of between 12- to 28-

296 fold after 168 h confinement although no evidence of increased expression was
297 evident at the earlier sample times. Three genes which code for different heat shock
298 proteins - 78 kDa glucose-regulated protein precursor, 75 kDa glucose-regulated
299 protein precursor (stress-70 protein) and endoplasmin precursor (heat shock 108 kDa
300 protein) - behaved similarly with higher upregulation in HR than in LR fish after 168
301 h confinement. The difference in response between HR and LR lines was small for
302 stress-70 protein and endoplasmin precursor but for the 78 kDa glucose-regulated
303 protein precursor levels in HR fish were more than 16-fold higher than levels in LR
304 fish (Table 1). This heat shock protein was the only transcript of the three which also
305 exhibited significant upregulation after 24 h confinement. Insulin-like growth factor I
306 was downregulated at both 24 h and 168 h confinement. Complement factor H
307 precursor, a gene implicated in the innate immune system, (represented by ESTs
308 **CA357149** and **CA350548** on the array) was found to be upregulated approximately
309 2-fold in both HR and LR lines. The cDNA for leukocyte cell-derived chemotaxin 2
310 precursor was targeted on the oligonucleotide array by two different oligonucleotides
311 designed against the same contig. The upstream cDNA sequence-based
312 oligonucleotide was strongly upregulated in liver of both HR (14.5-fold) and LR
313 (18.8-fold) fish whereas the other oligonucleotide based on downstream cDNA
314 sequence showed higher expression in LR (11.3-fold) than in HR (2.9-fold) fish.
315 Genes encoding thymosin beta-11 and protein disulfide-isomerase A4 precursor both
316 showed a 5-6 fold upregulation in HR fish and a 2-3 fold upregulation in LR fish.
317 Two oligonucleotides corresponding to uncharacterized protein C6orf58 homolog
318 gave ambiguous results: the oligonucleotide corresponding to EST **CA342499**
319 suggested downregulation at 168 h (6.1-fold in HR fish and 2.1-fold in LR fish) while
320 the second oligonucleotide based on EST **CA357517** suggested modest upregulation

321 in both lines (approx. 2-fold). Although this could be an error in microarray
322 quantification, it could indicate differential splicing of the C6orf58 homolog message.
323
324 Table 1 details changes in gene expression that were significant in both fish lines.
325 However, genes that were significantly regulated in one selected fish line but not the
326 other were of equal interest and may offer greater utility as putative markers of stress
327 responsiveness. Further analyses were therefore carried out on all 326 stress-regulated
328 genes - the total number of genes (969 - see Fig. S1) was reduced by redundancy
329 across the time points, by removal of a cluster of genes subsequently shown to be
330 dominated by one fish (see section 2.7) and by only retaining genes showing an at
331 least 2-fold change in expression in either fish line. K-means clustering of this
332 modified stress-related gene list suggested six distinct temporal expression patterns
333 containing from 14 to 95 genes (Table S1): a seventh cluster was identified primarily
334 as a result of the influence of fish LS44.
335
336 *3.3. Line-related effects on expression*
337 In order to identify only those genes showing a line-related effect (as opposed to those
338 showing a stress-related effect that may or may not be line-related) a second contrast
339 matrix was set up in Limma for each time point. At time points 6 h, 24 h, and 168 h, a
340 total of 106, 170 and 177 genes respectively exhibited an expression profile that
341 differed between the HR and LR lines during confinement ($P<0.05$). The total number
342 of unique genes (364) was reduced to 161 after excluding genes that were either
343 absent from the stress-related list (see above; 153 genes) or displayed expression fold
344 differences of less than 2-fold (50 genes). K-means clustering split this gene list into 4
345 main clusters (see Table 2 for annotated genes; Table S2 for full list). Thirty of the

346 genes were clustered into Set 1 which was characterized by downregulation primarily
347 in HR fish. Set 2 (38 genes) mirrored Set 1 and was characterized by upregulation
348 primarily in HR fish. Set 3 (68 genes) showed downregulation in the LR fish with
349 minimal change in the HR fish. Set 4 (23 genes) showed upregulation in the LR line
350 but predominantly at 168 h suggesting an effect of fish LS44.
351
352 *3.4. Quantitative PCR*
353 Quantitative PCR was performed with eight selected candidate genes to validate the
354 microarray data. Primers were designed such that one primer was homologous to the
355 50-mer oligonucleotide sequence on the array while the other primer was homologous
356 to a sequence outside the 50-mer oligonucleotide (Table 3). The contigs used for
357 primer design were obtained from the AQUAFIRST trout contig database
358 (http://www.sigenae.org/aquafirst/). All cDNA samples used for qPCR were derived
359 from total RNA of individual fish. Expression values were calculated using the delta
360 delta Ct method (Pfaffl, 2001) with 6 h LR unstressed samples as control and the 18S
361 ribosomal gene as reference.
362
363 The expression profile of control samples from HR and LR fish remained at basal
364 level for all the genes at all three time points (6 h, 24 h and 168 h; Figure 3). The
365 microarray data shown in Figure 3 was taken from the time-course (loop) analysis and
366 is therefore derived from pooled RNAs; the qPCR data, on the other hand, represents
367 the mean expression level of individual fish. The magnitudes of expression changes
368 observed in confined fish obtained by qPCR were overall considerably higher than the
369 changes reported by microarray. Regardless of the mismatch in the magnitude of fold
370 changes, the expression profiles from microarray and qPCR were significantly

correlated. Correlation for the genes intelectin-2 precursor ($R^2 = 0.94$), GRP78 ($R^2 = 0.89$), interferon-induced transmembrane protein 3 ($R^2 = 0.82$), cytochrome b-245 heavy chain ($R^2 = 0.73$), **CR944591** ($R^2 = 0.85$) and purine nucleoside phosphorylase ($R^2 = 0.85$) were found to be significant at the $P < 0.01$ level. Correlation of microarray and qPCR data for thymosin beta-11 was not significant at the $P < 0.01$ level.

3.5. Individual variation

A second set of microarray hybridizations were performed with total RNA taken from individual animals (n = 5 for both lines) sampled after 168 h confinement to investigate inter-individual variation. Genes coding for purine nucleoside phosphorylase, 78kDa glucose-regulated protein and interferon-induced transmembrane protein 3 were included in Set 2 of the cluster analysis of line-related genes (see Table 2) – these genes exhibited upregulation in both the HR and LR lines but with upregulation occurring both earlier and more strongly in the HR line. Gene expression in these individuals was also assessed with qPCR. As was the case for the time-course analysis, the expression profiles from microarray and qPCR of individual fish at 168 h were significantly ($P < 0.01$) correlated: intelectin-2 ($R^2 = 0.85$); GRP78 ($R^2 = 0.82$); interferon-induced transmembrane protein 3 ($R^2 = 0.72$); cytochrome b-245 heavy chain ($R^2 = 0.96$); **CR944591** ($R^2 = 0.42$); thymosin beta 11 ($R^2 = 0.82$); DRTP1 ($R^2 = 0.91$); and purine nucleoside phosphorylase ($R^2 = 0.75$). However, based on the more robust qPCR data (Fig. 4) it was evident that there was considerable inter-individual variation in relative expression levels within the lines. In particular, within the LR line fish LS43 and LS44 exhibited markedly lower relative expression patterns for five of the eight genes selected for qPCR (Fig. 4) than LS45, LS47 and

LS48. Fish LS44, already identified as anomalous, exhibited completely atypical expression patterns for cytochrome b-245 heavy chain and thymosin beta 11. Among the HR fish, expression levels in HS46 and HS47 were consistently lower than those in fish HS43, HS44 and HS45.

Principal Component Analysis (PCA) of the line-related list (n = 161) confirmed that fish LS44, and to a lesser extent HS47, behaved anomalously, though they did not behave like each other. K-means analysis of this list across the 10 individual fish under confinement at 168 h identified five clusters. Clusters 1 and 2 were comprised of 84 genes generally upregulated in LR fish whereas Clusters 3, 4 and 5 were composed of 77 genes generally downregulated in LR fish. Clusters 2 (48 genes) and 4 (33 genes) showed an LS44 effect driven by an upregulation (Cluster 2) or a downregulation (Cluster 4) in fish LS44. However, the LS44 effect was more dominant in Cluster 2. Therefore approximately 48 % of the genes showed a pattern of upregulation in the LR fish compared to the HR fish, with LS44 and HS47 not always following their group trends, and approximately 52 % of the genes showed a pattern of downregulation in the LR fish compared to the HR fish with HS47 not always following its group trend. There were five genes (all annotated) that consistently showed higher expression in the HR line than the LR line (interferon-induced transmembrane protein 3, ubiquitin-conjugating enzyme E2, carbamoyl phosphate synthase (CPS1), intelectin-2 and 2-hydroxyphytanoyl-CoA lyase) and six genes (only two of which were annotated) that consistently showed higher expression in the LR line than the HR line (enhancer of split groucho-like protein 2 and plasma retinol-binding protein 1). Only Clusters 1 and 3 (and only genes with annotations)

are shown in Figure S2 but these clusters include 10 of the 11 genes whose expression patterns were consistent across lines.

3.6. Gene Ontology

Although the number of genes was fewer than ideal for statistical analysis, it proved useful to look at the final modified stress-related and line-related lists in gene ontology (GO) terms. For this purpose all protein accession numbers (from multiple species) were converted into GenBank nucleotide accession numbers for *Danio rerio* by using tblastn against the zebrafish mRNA reference sequences. GO terms were obtained subsequently using OntoExpress (Khatri et al., 2002) and *P*-values are in relation to the Affymetrix whole genome array for zebrafish.

The modified stress-related gene list contained 487 genes (before introducing a 2-fold change filter) with 247 zebrafish equivalents - approximately half (123 genes) had GO annotation under Biological Process. In this list there were three GO categories (level 2, Biological Process) that were significantly enriched ($P < 0.1$): 'multi-organism process' ($P = 0.003$) – 4 genes, 'response to stimulus' ($P = 0.008$) - 16 genes and 'localisation' ($P < 0.016$) – 25 genes. When the 'response to stimulus' term was expanded another level the term 'response to biotic stimulus' was the most significant ($P = 0.007$) and, in fact, all four genes included were also those in the 'multi-organism process' ($P = 0.003$) term at level 2. These genes (serum amyloid A, leukocyte cell-derived chemotaxin 2, matrix metalloproteinase 9 and cyclic AMP-dependent transcription factor ATF-3) reflect the effect of a presumptive challenge to the immune system as exemplified by fish LS44. At level 3 other terms that became significant were 'metabolic process' (13 genes – $P = 0.0065$), 'cellular homeostasis'

445 (4 genes – $P = 0.0713$) and 'actin filament-based process' (5 genes – $P = 0.0035$). The
446 line-related gene list (see Table S2) contained 161 genes with 90 zebrafish equivalents
447 (after removing the 2-fold filter restriction and all of Set 4 which would bias the
448 analysis with pathogen effects). Approximately half (47 genes) have GO annotation
449 under Biological Process. GO analysis at level 3 identified the main categories as
450 'catabolic process' (5 genes, $P = 0.063$), 'oxidation reduction' (4 genes, $P = 0.073$),
451 'regulation of developmental process' (3 genes, $P = 0.094$) and 'negative regulation of
452 cellular process' (3 genes, $P = 0.065$).
453

454 **4. Discussion**

455 Microarray technology has previously been employed to investigate the effects of a
456 stressor on gene expression in the liver of rainbow trout (Momoda et al., 2007;
457 Wiseman et al., 2007; Cairns et al., 2008) and sea bream (*Sparus aurata*) (Calduch-
458 Giner et al., 2010). Overall, the findings of these studies tend to confirm that stress-
459 induced alterations in gene expression in the liver are extensive and complex although
460 between-study comparisons are made complicated by the variety of strategies adopted
461 to create the microarrays. This is the first study in which patterns of gene expression
462 in two lines of rainbow trout selected for a divergent HPI responsiveness to stressors
463 have been examined by this method. The selective breeding process by which the
464 lines were generated, and the endocrine and behavioural characteristics associated
465 with divergent responsiveness to stress are described elsewhere (Øverli et al., 2007).
466 The primary trait that distinguishes the two lines is the magnitude of the plasma
467 cortisol response to a stressor, with low-responding (LR) fish considered the
468 preferable phenotype for intensive rearing. Therefore interest was focused on genes
469 that were differentially expressed in the two lines during stress and where changes

were evident early in the confinement period. Liver was selected as one of several key target organs involved in stress-related processes and thus expected to exhibit differential gene expression patterns associated with the HR/LR phenotypes.

Exposure to a confinement stressor resulted in a marked elevation of plasma cortisol levels in both lines. Stress-induced plasma cortisol levels were higher in the HR fish than in the LR fish, a finding consistent with studies employing these selected lines of rainbow trout (Pottinger et al., 2001b). Unconfined fish from both lines exhibited levels of plasma cortisol characteristic of unstressed rainbow trout throughout with no significant differences evident between lines.

4.1. Downregulated genes

The patterns of gene expression in response to confinement that were seen in this study were similar to those previously reported for rainbow trout (Cairns et al., 2008) and sea bream (Calduch-Giner et al., 2010). Downregulation of gene expression during stress has been reported to occur as early as 2 h after the onset of the stressor and to persist and increase over the subsequent 168 h (Cairns et al., 2008). In this study, after K-means clustering of all 326 genes identified as being differentially regulated during confinement (Table S1), downregulated genes were apparent in Set 1 (in LR and HR fish), Set 3 (predominantly in LR fish), Set 4 (LR fish only) and Set 5 (HR fish only) and comprised approximately 59 % of all differentially expressed genes. In Set 4 (the largest set with 95 genes, approximately half of which were not annotated) the genes were only regulated (maximally at 24 h) in the LR line. The list of downregulated genes, and the implicit biological processes, was not dissimilar to that described for sea bream (Calduch-Giner et al., 2010) and reflects a reduction in

495 energy demands and subsequent reactive oxygen species (ROS) production. Processes
496 associated with downregulated genes included transcription/translation (28 %),
497 immune response (6 %), protein turnover (12 %), glycolysis (4 %), lipid and amino
498 acid metabolism (2.5 %), and cytoskeletal organization (9 %). The list of genes
499 included secreted phosphoprotein 24 precursor, apolipoprotein A-I-1, and 14-3-3
500 beta/alpha (though the latter two genes fall just below the 2-fold change threshold)
501 which have been shown previously to be downregulated in stressed trout (Cairns et
502 al., 2008).
503

504 *4.2. Upregulated genes*

505 Upregulation of expression was apparent in Set 2 (upregulated in HR fish), Set 6
506 (upregulated in both lines) and Set 7 (upregulated at 168 h in LR line) (see Table S1).
507 However, Set 7 (48 genes) was dominated by alterations in expression in one fish
508 (LS44, stressed, LR) which suggests an effect that was not specifically stress-related.
509 Set 6 (14 genes) included genes that were strongly upregulated at 24 h and 168 h –
510 stress-responsive genes that were similarly regulated in both the LR and HR lines.
511 Some of these genes (PDI, GRP78, GRP75, LECT2) exhibited very similar patterns
512 of expression in stressed sea bream (Calduch-Giner et al., 2010). Set 2 genes (n = 73)
513 were upregulated as early as 6 h in the HR line and showed sustained upregulation
514 during 168 h of confinement, however, these same genes remained relatively
515 unchanged in the LR line. A high proportion of these genes were linked with glucose
516 metabolism and the maintenance of ATP levels. In Set 7 there were many upregulated
517 cytoskeleton-associated genes. This response possibly relates to the disease status of
518 the individual fish LS44 (which was parasitized) and the requirement for tissue repair
519 or possibly phagocyte proliferation. Several other actin-related proteins were included

520 in Set 4 but these appeared to be actively downregulated in LR fish with little or no
521 change in HR fish. It is unclear why these genes might be downregulated. Some genes
522 that were previously identified as being upregulated in the liver during confinement
523 stress (e.g. haptoglobin, fibrinogens beta and gamma; (Cairns et al., 2008)) did not
524 exhibit similar patterns of expression in this study although Ca^{2+}-ATPase 2
525 (upregulated 1.4-fold and 1.9-fold in the LR and HR lines respectively at 168 h) did
526 conform to the earlier pattern. The highly inbred nature of the two selected lines may
527 have resulted in the loss or attenuation of some responses present in a more outbred
528 population, such as that employed in the earlier study, or it is possible that procedural,
529 environmental, or husbandry factors may have differed between the two studies
530 despite best efforts to standardise conditions.
531

532 *4.3. Immune system genes*

533 For ectotherms such as teleost fish, low temperatures can confound an effective
534 adaptive immune response and fish therefore must rely on innate immunity and the
535 acute phase response (APR) to provide a more immediate first line of defence (Russell
536 et al., 2006). There are many reports on the effect of elevated cortisol levels on non-
537 specific immune responses (Vazzana et al., 2002) and in the present study many of the
538 significant genes whose expression was altered during confinement stress were
539 associated with the innate immune response. Differentially regulated trout protein 1
540 (DRTP1) was the most upregulated gene during confinement in both HR and LR lines
541 at the 168 h time point (Fig. 3). Its strong expression has been reported in liver tissue
542 after activation of the acute phase response (Talbot et al., 2009a) but its function has
543 yet to be elucidated (Talbot et al., 2009b). A clear, though not strictly significant,
544 trend was evident for higher expression in HR fish during confinement ($P = 0.09$) but

545 there was considerable variation in DRTP1 expression between individual confined
546 fish at 168 h (Fig. 4). Four HR fish and three LR fish showed moderate to very high
547 levels of DRTP1 induction (8 to 150-fold) yet fish LS44, with a presumptive parasite
548 infection, exhibited an approximate 50-fold downregulation of DRTP1. This inter-
549 individual variation in DRTP1 expression has been noted previously in rainbow trout
550 in response to confinement stress (Talbot et al., 2009a). However, it has also been
551 shown that DRTP1 is induced in salmonids upon intra-peritoneal injection of
552 *Listonella anguillarum* bacterin (Gerwick et al., 2007) or a genetically attenuated
553 strain of *Aeromonas salmonicida* (Martin et al., 2006). Relatively low expression
554 levels of DRTP1 in fish LS44, identified as being infected with a cestode parasite
555 presumed to be *Diphyllobothrium* sp., suggests that DRTP1 responds specifically to
556 bacterial challenge rather than the presence of a cestode parasite or that this particular
557 fish possessed an atypical response to both confinement stress and infection. It is also
558 possible that the very low levels of expression of DRTP1 in fish LS44 were a
559 consequence, rather than cause of the parasite infection and relate to the fact that some
560 parasites secrete factors which alter host immune and inflammatory responses
561 ((Riffkin et al., 1996)). DRTP1 was also one of only seven hepatic genes (all immune
562 related) that were differentially expressed between three allopatric populations of
563 rainbow trout (Bayne et al., 2006). This suggests that DRTP1 can be naturally
564 selected for in trout populations in response to a persistent challenge and that
565 individuals within a population would benefit in the presence of such a challenge by
566 an increase or decrease in expression of DRTP1. Interestingly, 12 genes correlate with
567 the expression pattern of DRTP1 clone (> + 0.9) across the 10 individual fish (5 HR
568 and 5 LR confined at 168 h) and these include the two DRTP1 clones, LECT2 and
569 protein disulphide isomerase.

In the comparison of individual fish exposed to confinement stress for 168 h, HSP108 followed a microarray expression profile similar to DRTP1. HSP108 is a transferrin binding protein (Hayes et al., 1994) and transferrin binds free iron and facilitates its cellular uptake (Bullen, 1981). Scavenging iron is believed to be important in preventing the growth of iron-requiring bacteria therefore regulation of the iron concentration is likely to be part of the innate antimicrobial immune response in rainbow trout (Raida et al., 2009). Two other genes involved in iron homeostasis, intelectin-2 and ferritin, were found, according to microarray analysis, to be differentially expressed between HR and LR fish and clustered in Sets 1 and 2 respectively (Table 2). The constitutive expression of intelectin-2 transcripts was as much as 100-fold greater in the HR line than the LR line irrespective of stress, as confirmed by qPCR. The intelectins (or X-lectin family) are a relatively recently identified family of homologous proteins involved in innate immunity and development (Lee et al., 2004). Intelectin-1 is a Ca^{2+} dependent secretory glycoprotein involved in the innate immune response and is a mammalian lactoferrin receptor (Suzuki et al., 2001; Tsuji et al., 2001). By microarray analysis ferritin showed a heightened response to stress in the HR line compared to the LR line (especially at 168 h) but this was not confirmed by qPCR.

4.4. Heat shock protein genes

Genes coding for heat shock proteins GRP78, GRP75 and HSP108 (see above) were also over-expressed in HR and LR during confinement stress. In our experiment, although GRP78 (78kDa glucose-regulated protein: HSPA5) showed significantly higher expression in both HR ($P < 0.001$) and LR ($P = 0.01$) fish during confinement,

it exhibited a markedly different fold change in HR fish (~28-fold) compared to LR fish (~5.4-fold), and therefore it can be considered a potential marker for divergent stress responsiveness in fish. The gene encoding GRP78 has also been shown to increase in expression following heat shock in the rainbow trout cell line RTG-2 confirming its role as a heat shock protein (Ojima et al., 2005). However, in addition to its function as a molecular chaperone, it has been implicated in the immune response. The GRP78 gene is upregulated by bacterial infection in Atlantic salmon (Martin et al., 2006) and in the salmon SHK-1 cell line after treatment with recombinant IFN-γ (Martin et al., 2007).

Both GRP75 and HSP108 showed some minor difference in the magnitude of upregulation between the HR and LR lines, but neither showed as pronounced differences as GRP78. Upregulation of the gene coding for GRP75 (HSPA9) has previously been observed in sea bream during confinement (Calduch-Giner et al., 2010). Production of GRP75 protein is triggered by glucose deprivation, oxidative injury, ionizing radiation, calcium ionophores and hyperthyroidism (for reviews see (Wadhwa et al., 2002). It performs a broad spectrum of cellular functions ranging from involvement in the stress response to intracellular trafficking, antigen processing, and cell differentiation (Bermejo-Nogales et al., 2008). Another heat shock protein gene, an ortholog of HSP108 from chicken liver, showed a small but significant increase in expression after 168 h confinement in both HR and LR fish. As noted earlier, HSP108 followed a microarray expression profile similar to DRTP1 rather than GRP78 or GRP75. This suggests that although all three are heat shock proteins, HSP108 has a specific function in rainbow trout not related to those of GRP78 or GRP75.

620

621 *4.5. Atypical gene expression patterns in diseased fish*

622 Some of the HR and LR fish exposed to the confinement stressor exhibited minor
623 physical damage, (primarily to the tail), small lesions on the flank, and internal
624 evidence of parasite infestation from the 24 h time point onwards. One individual
625 (LS44) was parasitized, with cestode cysts (probably *Diphyllobothrium* sp.) evident in
626 the viscera. Confinement of rainbow trout in small groups can result in aggressive
627 interaction between individuals with consequent minor physical damage and the
628 possibility of pathogenic infection (Pottinger et al., 1992). Due to the limited number
629 of oligonucleotide array slides that were available for this study, hybridizations were
630 performed with pools of RNA from biological replicates in a direct design. A
631 limitation of this approach is that HR/LR trait-specific expression patterns may have
632 been confounded by strong individual responses to disease or physical damage during
633 confinement. In this study, complementary hybridizations with RNA from individual
634 animals from the HR and LR lines facilitated the differentiation of genes related to the
635 confinement stressor, to which all the fish were exposed, from those related to factors
636 that might affect individual fish only. The genes represented in K-means cluster Set 7
637 (48 genes, 35 with annotation) of the loop experiment (see Table S1) showed
638 significantly higher expression in confined LR fish than in HR at the 168h time point.
639 In the microarray analysis of individual fish however, the same genes were found to
640 be upregulated only in fish LS44 (LR, 168 h confinement), a fish identified at the time
641 of sampling as being parasitized, suggesting that this individual expression pattern for
642 LS44 was the result of infection. Over-expression of the cytochrome b-245 light chain
643 in LS44 which is a primary component of the microbicidal oxidase system of
644 phagocytes, strongly corroborates the involvement of cytochrome b-245 in an immune

response to infection. Genes coding for fish egg lectin, P-selectin glycoprotein ligand 1, matrix metalloproteinase-9 and lysozyme C II also followed a similar expression pattern to cytochrome b-245 and all are known to be involved in pathogen recognition and disease and inflammatory processes (Ewart et al., 2001; Johnson et al., 2004; Baïsse et al., 2007). These observations suggest it is extremely important to be aware of factors, such as incipient disease states, that might bias a gene expression profile and to augment the analysis of pooled RNA samples with analyses of individuals.

4.6. Candidate markers for low stress responsiveness

Comparison of the time-course pooled sample experiment and the individual fish experiment suggests that interferon-induced transmembrane protein 3 exhibited a pattern of expression under stress that differentiated the selected lines ($P = 0.041$). DRTP1 also showed an expression pattern that suggested differences between the selected lines ($P = 0.086$). However, in both cases, maximum divergence in expression occurred after a prolonged period of confinement, limiting the practical value of either gene as an accessible marker. Perhaps of greater significance was the observation that intelectin-2 transcripts were far more abundant (~ 100-fold) in the liver of HR fish, independent of stress state, than LR fish offering the possibility of a straightforward identifier of individuals with a tendency towards higher stress responsiveness and the prospect of eliminating them from breeding populations. Whether differences in constitutive expression of intelectin-2 are linked directly with selection for stress responsiveness, or relate to other aspects of the pedigree of these lines, will require further investigation. Genes such as carbamoyl phosphate synthase (CPS1), glucokinase, and somatotropin 2 (early upregulation in LR line) and other showing similar patterns of expression (see Table S2) offer possible utility as markers

of stress responsiveness but their usefulness in this context requires confirmation by qPCR.

4.7. Differential expression of POMC B

An interesting pattern of regulation was observed for hepatic corticotropin-lipotropin (pro-opiomelanocortin, POMC) B which was downregulated in LR fish but upregulated in HR fish at both 6 h and 24 h and thus appears in the list of genes regulated in both HR and LR lines. The identification of pro-opiomelanocortin transcripts in the liver of these fish was not unexpected (Leder et al., 2006) but changes in their abundance during stress, and in opposite directions in HR and LR fish, is of great interest and has not previously been reported. POMC is cleaved to yield a number of important regulatory peptides including adrenocorticotropin (ACTH), lipotropin (LPH), melanocyte-stimulating hormone (MSH) and endorphin (Bicknell, 2008). Although the anterior and intermediate lobes of the pituitary are the primary sites of expression of this gene POMC is expressed at sites other than the pituitary in mammals, birds and fish (DeBold et al., 1988; Leder et al., 2006; Bicknell, 2008). No clear functionality has yet been identified for POMC expression in the liver. The pattern of expression of POMC B observed in the present study was consistent across the 6 h and 24 h time points. At both times the expression of POMC B in livers of HR fish was greater than that in controls whereas in the livers of LR fish expression was reduced relative to controls. Given the important role played by POMC-derived peptides in regulating the stress response, and the divergent cortisol response to stress observed in the HR and LR lines, these changes in expression levels are suggestive of a functional involvement for hepatic POMC B in the stress response. However, stress-induced plasma ACTH levels in the HR and LR lines are reported not

to differ, with the difference in plasma cortisol levels during stress seemingly related to differences in interrenal sensitivity to ACTH (Pottinger et al., 2001a) and consistent with this, expression of brain POMC A following an acute stressor does not differ between the selected lines (Thomson et al., 2011). Thus the precise function of hepatic POMC B in this context remains unclear until regulatory influences, post-translational processing, and targets for the products of this gene can be identified. However, given the divergent nature of POMC B regulation in the two lines, and the relatively early appearance of differences in transcription following exposure to the stressor, this gene does offer some promise as a marker for reduced stress responsiveness in rainbow trout. Data from other genes in the present study whose expression was also quantified with qPCR suggests that the actual difference between lines in expression levels of POMC B may be greater than suggested by the relatively low fold-changes detected by the array.

5. Conclusions

A trout oligonucleotide microarray has been used to compare hepatic gene expression during exposure to a prolonged stressor between two lines of rainbow trout selectively bred for high or low stress responsiveness. One hundred and sixty one genes were found to be differentially regulated between the HR and LR fish during confinement exposure. Expression profiles of eight genes from the microarray analysis were validated by quantitative PCR. Hepatic POMC B was differentially regulated with increased expression during stress in HR fish but reduced expression relative to controls in LR fish. Intelectin-2 precursor showed greater than 100-fold higher expression in HR fish compared to LR fish irrespective of whether the fish were confined or not. These genes, together with interferon-inducible transmembrane

720 protein 3, DRTP1 and several other genes not yet confirmed by qPCR have potential
721 to be developed as direct markers of stress responsiveness for use in marker-assisted
722 selective breeding of rainbow trout exhibiting an attenuated responsiveness to
723 stressors.
724
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737

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Figure 1: Loop design for microarray hybridizations

Loop design used for microarray hybridizations (LC = low responders control fish (n=3); LS = low responders stressed fish (n=5); HC = high responders control fish (n=3); HS = high responders stressed fish (n=5); 6, 24, 168 represents fish exposed to confinement stress for 6 h, 24 h and 168 h.

Figure 2: Plasma cortisol levels in stressed and control rainbow trout

Plasma cortisol levels in high-responding (HR) and low-responding (LR) rainbow trout sampled at intervals during a 21-day (504 h) period. Fish were stressed by confinement (HR: ○; LR: ●). Cortisol levels in unconfined controls are also depicted (HR: □; LR: ■). Each point represents the mean ± SEM, n = 6. Significant differences in mean plasma cortisol levels between confined HR and LR fish at each time point are denoted by asterisks: *: $P<0.05$; **: $P<0.01$;***: $P<0.001$.

Figure 3: Expression profiles by qPCR of candidate genes which have shown differential expression between high and low responders upon confinement

qPCR was carried out in triplicate on individual animals (for stress fish, n = 5, for control fish, n = 3 for each time point and genetic line), and relative expression was calculated using the low responder control fish as reference and the 18S ribosomal gene for normalisation (Pfaffl, 2001). For each time point average relative expression and standard errors were calculated for each gene. For clarity in the LR line log10

values were plotted for intelectin-2. The microarray data was obtained from analysis of the direct design (loop) microarray experiment using Limma software and uses the low responder 6 h control fish as reference. For primer sequences see Table 3. The HR fish data is indicated by dark grey columns and the LR fish data by light grey columns.

Figure 4: Expression levels across individual fish of eight candidate genes which have shown differential expression between high and low responders upon confinement

qPCR was performed on individual animals (n = 5) from both HR and LR lines after 168 h confinement stress fish. qPCR was carried out as described in the legend to figure 3. The HR fish data are indicated by dark grey columns and the LR fish data by light grey columns. Where the scale would be distorted by a high value (cytochrome b-245, LS44) or a low value (**CR944591** and DRTP1, LS44) the value is given (127.5, 0.017 and 0.020 respectively).

Tables

Table 1: Hepatic genes altered in expression after confinement in both LR and HR fish lines

Table 2: K-means clusters of all annotated genes reflecting significant differences in gene expression behaviour between the LR and HR lines

Table 3: Primers used for qPCR

901 Table 1: Hepatic genes altered in expression after confinement in both LR and HR

902 fish lines.

ID	Gene Description	LR	HR
		Fold change[a]	
	6 h		
BX072864.1.a.om.1	COLI2_ONCMY (Q04618) Corticotropin-lipotropin B precursor	-2.0	1.3
CA344467.1.a.om.1	TRHY_RABIT (P37709) Trichohyalin	-2.0	-2.1
CA358005.1.a.om.1	HBA_SALSA (P11251) Hemoglobin subunit alpha	-2.1	-2.3
X_CU070025.1.a.om.1	CU070025 [Mannose Binding Lectin-1][b]	-8.8	-6.8
	24 h		
CA355465.1.a.om.1	PNPH_BOVIN (P55859) Purine nucleoside phosphorylase	3.7	8.1
CR944338.1.a.om.1	CR944338	5.1	4.1
BX866751.1.a.om.1	CP24A_HUMAN (Q07973) Cytochrome P450 24A1	2.5	3.9
X_CA368961.1.a.om.1	GRP78_RAT (P06761) 78 kDa glucose-regulated protein precursor (GRP 78)	2.0	3.7
CA374294.1.a.om.1	ONCMY (P68503) Metallothionein A (MT-A), complete	2.2	3.4
CA342330.1.a.om.1	CPNS1_RAT (Q64537) Calpain small subunit 1 (CSS1)	3.1	3.3
CA371796.1.a.om.1	CA371796	2.8	2.9
X_CA372046.1.a.om.1	METK2_PONPY (Q5R5H1) S-adenosylmethionine synthetase isoform type-2	1.9	2.5
CR944591.1.a.om.1	CR944591	2.2	2.2
CA382759.1.a.om.1	CSN8_BRARE (Q7ZUZ0) COP9 signalosome complex subunit 8	2.0	2.1
X_CA358932.1.a.om.1	TYB11_ONCMY (P26351) Thymosin beta-11	2.2	2.1
CA370601.1.a.om.1	RED_MOUSE (Q9Z1M8) Protein Red (Protein RER) (IK factor) (Cytokine IK)	1.3	2.0
CB492564.1.a.om.1	BETA3_MESAU (O09029) Protein BETA3	-2.5	-1.8
rtay11a08d06r1.1.a.om.1	rtay11a08d06r1	1.0	-2.0
X_CA344596.1.a.om.1	NXT2_HUMAN (Q9NPJ8) NTF2-related export protein 2 (p15-2 protein)	-1.8	-2.3
CA381456.1.a.om.1	IGF1_ONCMY (Q02815) Insulin-like growth factor I precursor (IGF-I) (Somatomedin)	-2.1	-2.5
CA358005.1.a.om.1	HBA_SALSA (P11251) Hemoglobin subunit	-2.3	-2.6

	alpha		
CT572011.1.a.om.1	CT572011	-2.8	2.5
CA343235.1.a.om.1	LAP2_RAT (Q62733) Lamina-associated polypeptide 2 isoform beta	-1.2	2.1
BX072864.1.a.om.1	COLI2_ONCMY (Q04618) Corticotropin-lipotropin B precursor (POMC B)	-2.6	2.0
	168 h		
BX311643.1.a.om.1	BX311643 [DRTP1]	28.1	22.6
X_CA368961.1.a.om.1	GRP78_RAT (P06761) 78 kDa glucose-regulated protein precursor	3.5	20.1
CA354409.1.a.om.1_1	LECT2_MOUSE (O88803) Leukocyte cell-derived chemotaxin 2 precursor	18.8	14.5
CR944619.1.a.om.1	CR944619 [DRTP1]	14.8	12.6
X_CA358932.1.a.om.1	TYB11_ONCMY (P26351) Thymosin beta-11	2.3	5.9
tcad0003a.f.24_5.1.s.om.8	[BRBH] PDIA4_HUMAN (P13667) Protein disulfide-isomerase A4 precursor	2.5	5.6
BX886962.1.a.om.1	BX886962	2.8	3.9
CA358247.1.a.om.1	GRN_HUMAN (P28799) Granulins precursor	2.7	3.6
CA348401.1.a.om.1	GRP75_HUMAN (P38646) Stress-70 protein	2.2	3.4
BX888472.1.a.om.1	BX888472	2.2	3.4
X_CU070025.1.a.om.1	CU070025 [Mannose Binding Lectin-1]	-4.0	3.1
CA367141.1.a.om.1	CA367141	2.0	3.0
CA354409.1.a.om.1_2	LECT2_MOUSE (O88803) Leukocyte cell-derived chemotaxin 2 precursor	11.3	2.9
tcay0002b.a.20_5.1.s.om.8	ENPL_CHICK (P08110) Endoplasmin precursor (Heat shock 108 kDa protein) HSP108	2.1	2.9
BX304351.1.a.om.1	EI2BG_MACFA (Q4R6T3) Translation initiation factor eIF-2B subunit gamma	1.9	2.8
CA365789.1.a.om.1	COF2_MOUSE (P45591) Cofilin-2	5.2	2.8
CA363035.1.a.om.1	TMEDA_FUGRU (Q90515) Transmembrane emp24 domain-containing protein 10 precursor	2.1	2.6
CA360106.1.a.om.1	ROA0_HUMAN (Q13151) Heterogeneous nuclear ribonucleoprotein A0 (hnRNP A0)	3.6	2.6
CA342819.1.a.om.1	HM13_MOUSE (Q9D8V0) Minor histocompatibility antigen H13 (EC 3.4.99.-)	1.8	2.5
CA342689.1.a.om.1	ETFA_BOVIN (Q2KJE4) Electron transfer flavoprotein subunit alpha	2.1	2.4
X_CA357149.1.a.om.1	CFAH_MOUSE (P06909) Complement factor H precursor (Protein beta-1-H)	2.8	2.3
CT567292.1.a.om.1	CT567292	2.0	2.3
CA344758.1.a.om.1	RAB5C_MOUSE (P35278) Ras-related protein Rab-5C	2.1	2.3

CA357517.1.a.om.1	CF058_ONCMY (Q5QT17) Uncharacterized protein C6orf58 homolog precursor	2.1	2.2
CA348751.1.a.om.1	CA348751	2.6	2.1
CA350548.1.a.om.1_1	CFAH_BOVIN (Q28085) Complement factor H precursor (H factor 1)	1.7	2.0
CF752535.1.a.om.1	PI3R6_MOUSE (Q3U6Q4) Phosphoinositide 3-kinase regulatory subunit 6	2.0	2.0
CA354597.1.a.om.1	SH3L3_HUMAN (Q9H299) SH3 domain-binding glutamic acid-rich-like protein 3	2.4	1.8
CA352504.1.a.om.1	SELPL_MOUSE (Q62170) P-selectin glycoprotein ligand 1 precursor (	3.0	-1.9
CT962822.1.a.om.1	CT962822	2.0	-1.9
CB492286.1.a.om.1	ABD12_MOUSE (Q99LR1) Abhydrolase domain-containing protein 12	-2.3	-2.0
BX866014.1.a.om.1	CRIM1_CHICK (Q8AWW5) Cysteine-rich motor neuron 1 protein precursor (CRIM-1)	-2.3	-2.0
X_CA342046.1.a.om.1	RS4X_BRARE (Q642H9) 40S ribosomal protein S4	-2.3	-2.0
tcay0025b.n.05_5.1.s.om.8	HCC1_PONPY (Q5R4V4) Nuclear protein Hcc-1	-1.9	-2.2
X_CA367581.1.a.om.1	LECA_PLEWA (Q02988) Lectin precursor	-2.1	-2.3
BU993928.1.a.om.1	TPM1_LIZAU (P84335) Tropomyosin 1 alpha chain (Alpha-tropomyosin)	2.2	-2.3
CA349504.1.a.om.1	SAHHB_XENLA (O93477) Adenosylhomocysteinase B	-3.0	-2.3
CA375714.1.a.om.1	CA375714.1.a.om.1	-1.8	-2.3
X_CA348235.1.a.om.1	CP2K1_ONCMY (Q92090) Cytochrome P450 2K1 (EC 1.14.14.1) (CYPIIK1) (P450 LMC2)	-2.8	-2.4
CA358169.1.a.om.1	ODBB_HUMAN (P21953) 2-oxoisovalerate dehydrogenase subunit beta	2.8	-2.5
CA351175.1.a.om.1	CA351175	-1.9	-2.5
CA381456.1.a.om.1	IGF1_ONCMY (Q02815) Insulin-like growth factor I precursor (IGF-I) (Somatomedin)	-1.9	-2.6
X_CA341853.1.a.om.1	CA341853	2.8	-2.8
CR943512.1.a.om.1	CR943512	-1.8	-3.0
CA365719.1.a.om.1	CA365719	-1.9	-3.1
rtay12a02h11f1.1.a.om.1	IMB1_RAT (P52296) Importin beta-1 subunit (Karyopherin beta-1 subunit) (Nuclear factor P97)	-2.2	-4.2
CA342499.1.a.om.1	CF058_ONCMY (Q5QT17) Uncharacterized protein C6orf58 homolog precursor	-2.1	-6.1

[a]Fold change values are stress values relative to the corresponding control values. Negative values indicate downregulation. Only genes showing fold changes greater than or equal to 2 in either fish

905 line are listed. [b]Any identifications within square brackets [ ] were made manually after automatic
906 annotation of the array.

Table 2: K-means clusters of all annotated genes reflecting significant differences in gene expression behaviour between the LR and HR lines

ID	Gene Description	LR	HR	Functions
		Fold change		
		6h, 24h, 168h	6h, 24h, 168h	
SET 1: Downregulation primarily in the HR line				
CA352504.1.a.om.1	P-selectin glycoprotein ligand 1	1.2, -1.1, 3.0	-1.7, -1.6, -1.8	adhesion
CA358169.1.a.om.1	2-oxoisovalerate dehydrogenase subunit beta	-1.4, 2.1, 2.8	-3.6, 2.2, -2.5	amino acid metabolism, response to glucocorticoid
X_CT572262.1.a.om.1	ATP synthase a chain	1.3, 1.0, -1.2	-1.2, -3.1, -2.2	ATP maintenance, oxidation reduction
BU993928.1.a.om.1	Tropomyosin 1 alpha chain	-1.1, -1.3, 2.2	-1.3, -1.3, -2.3	cytoskeletal organisation
CA359238.1.a.om.1	F-actin capping protein subunit beta	1.0, -1.1, 2.0	-1.5, -1.4, -1.1	cytoskeletal organisation
CA344467.1.a.om.1	Trichohyalin	-2.0, -1.6, -1.1	-2.1, -3.6, -1.5	cytoskeletal organisation
X_CU072064.1.a.om.1	Glucokinase	2.5, 1.0, -1.1	-1.4, 1.7, -1.8	gluconeogenesis, glucose maintenance
CA368454.1.a.om.1	Carbonic anhydrase 5B	-1.2, 2.2, -1.3	-1.1, -1.1, -1.4	gluconeogenesis, lipogenesis
CA343347.1.a.om.1	5-aminolevulinate synthase	-1.1, 1.0, -1.4	-2.1, -1.5, -2.1	haem biosynthesis
BX074651.1.a.om.1	Somatotropin 2	2.3, 1.1, 1.1	-1.4, -1.2, 1.6	hormone signalling
CA342499.1.a.om.1	Uncharacterized protein C6orf58 homolog	1.0, -1.1, -2.1	-1.1, -1.2, -6.3	hypothetical protein
CA387966.1.a.om.1	Liver-expressed antimicrobial peptide 2	1.9, 1.0, -1.2	-3.1, -2.3, -5.3	immune response
CA347369.1.a.om.1	Intelectin-2	6.5, 1.5, 1.8	-1.5, -1.5, 1.1	immune response
rtay12a02h11f1.1.a.om.1	Importin beta-1 subunit	1.6, 1.0, -2.1	-2.6, -1.9, -4.2	protein localisation
CA365301.1.a.om.1	Dipeptidyl-peptidase 3	-1.2, -1.5, -1.2	-3.6, -2.8, -2.4	proteolysis
U65893.1.a.om.1	Carbamoyl-phosphate synthase [ammonia], mitochondrial (CPS1)	3.1, 1.1, 1.6	1.1, 1.1, -1.4	response to stimulus, response to glucocorticoid
CA348176.1.a.om.1	14-3-3 protein beta/alpha-1	1.5, -1.1, 1.8	-2.3, -1.6, -1.8	signal transduction
CX262061.1.a.om.1	Enhancer of split groucho-like protein 2	-1.2, -1.2, -1.3	-2.0, -2.1, -3.6	transcription
CT567216.1.a.om.1	Protein FAM57B	-1.2, 3.2, -1.5	1.1, 1.0, -1.7	unknown
CA342509.1.a.om.1	Plasma retinol-binding protein I	1.2, -1.4, -1.2	1.0, -1.7, -3.0	Vit A transport, fat metabolism

SET 2: Upregulation primarily in the HR line				
X_CA372046.1.a.om.1	S-adenosylmethionine synthetase isoform type-2	1.2, 1.7, 1.2	2.3, 2.5, 2.9	amino acid metabolism
tcbk0031c.o.22_5.1.s.om.8	Cationic amino acid transporter 3	-1.2, -1.1, 1.0	2.2, 1.4, 1.9	amino acid metabolism
X_CA347249.1.a.om.1	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	1.5, 1.1, 1.2	3.3, 1.6, 1.0	ATP maintenance
CA348766.1.a.om.1	Protein disulfide-isomerase	1.3, 1.1, 1.7	1.0, -1.1, 5.3	cell redox homeostasis
X_CA358932.1.a.om.1	Thymosin beta-11	1.1, 2.2, 2.3	1.3, 1.9, 6.0	cytoskeletal organisation
CA382759.1.a.om.1	COP9 signalosome complex subunit 8	1.1, 1.9, 1.0	2.6, 2.1, 2.2	developmental
tcbk0024c.i.05_5.1.s.om.8	Carnitine O-palmitoyltransferase I	-1.5, -1.2, -1.2	2.0, 1.8, 2.0	fatty acid metabolism, glucose maintenance
X_CA343987.1.a.om.1	Interferon-induced transmembrane protein 3	1.0, -1.3, 1.5	1.7, 2.7, 6.7	immune response
X_CA351045.1.a.om.1	Proto-oncogene tyrosine-protein kinase LCK	-1.2, 1.0, 1.7	-1.1, 1.2, 3.9	immune response
CA347059.1.a.om.1	Ferritin	1.9, 1.7, -1.2	-1.1, 1.2, 2.1	iron homeostasis
CA352463.1.a.om.1	2-hydroxyphytanoyl-CoA lyase	1.1, -1.2, -1.2	1.4, 1.6, 2.1	lipid metabolism
X_BX308005.1.a.om.1	Patatin-like phospholipase domain-containing protein 4	1.1, -1.2, -1.1	2.9, 2.1, -1.4	lipid metabolism
CA355465.1.a.om.1	Purine nucleoside phosphorylase	1.2, 3.0, 1.1	4.5, 8.1, 3.8	nucleotide metabolism
CA351531.1.a.om.1	Peroxiredoxin-5	-1.3, -1.1, 1.5	1.0, 2.0, 2.1	oxidation reduction
X_CA368961.1.a.om.1	78 kDa glucose-regulated protein precursor (GRP 78)	1.0, 2.0, 3.5	1.6, 3.7, 20.2	proteolysis, apoptosis, ER stress response
BX299056.1.a.om.1	Retinitis pigmentosa 9 protein homolog	-1.1, 1.4, 1.1	1.0, 1.4, 2.3	RNA splicing
CA370303.1.a.om.1	Tetraspanin-3	1.0, -1.1, 1.2	1.2, 2.2, 2.0	signal transduction
BX866751.1.a.om.1	Cytochrome P450 24A1	-1.2, 2.3, -1.8	1.7, 3.9, 1.4	steroid metabolism, oxidation reduction, vitamin metabolism
X_CA342458.1.a.om.1	CCAAT/enhancer-binding protein delta	1.0, 1.0, -1.7	2.8, 3.7, 1.2	transcription
BX863020.1.a.om.1	Circumsporozoite protein	1.0, 1.6, 1.0	2.2, 3.1, 3.6	unknown
SET 3: Downregulation in the LR line (minimal changes in HR line)				
CA361557.1.a.om.1	Claudin-4	-1.4, -2.5, -1.5	1.1, 1.0, 1.5	adhesion
CA342924.1.a.om.1	p53 apoptosis effector related to PMP-22	-2.0, -2.9, -2.1	1.5, 1.3, 1.7	apoptosis

X_BX072690.1.a.om.1	Beta actin-like	-1.6, -3.3, -1.3	1.0, 1.4, 1.3	cytoskeletal organisation
CB494094.1.a.om.1	Kinetochore protein Hec1 homolog	-1.4, -2.6, -1.4	1.1, 1.3, 1.1	cytoskeletal organisation
CA357969.1.a.om.1	Actin-related protein 2/3 complex subunit 4	-1.4, -2.4, 1.0	1.0, 1.0, -1.1	cytoskeletal organisation
CA368043.1.a.om.1	cAMP-regulated phosphoprotein 19	-1.7, -2.9, -1.8	1.0, -1.1, -1.1	gluconeogenesis
CA342950.1.a.om.1	Alcohol dehydrogenase [NADP+]	-1.5, -3.0, -1.9	-1.2, -1.2, -1.1	glucuronate metabolism, oxidation reduction
AF438178.1.a.om.1	Growth hormone receptor binding protein) (GHBP) (Serum-binding protein)]	-2.8, -1.5, -1.8	1.5, 1.1, 1.0	hormone signalling
CA351682.1.a.om.1	Ig kappa chain V-III region MOPC 63	-1.8, -2.7, 1.3	-1.1, 1.4, 2.0	immune response
BX298925.1.a.om.1	Beta-catenin-like protein 1	-1.9, -2.5, -2.3	1.8, 1.4, 1.3	immune response, apoptosis
CA359082.1.a.om.1	Glucosidase 2 subunit beta	-1.5, -2.1, -1.3	1.5, 1.3, 1.6	N-linked glycan processing
X_CA344701.1.a.om.1	Cytochrome P450 2J2	-1.4, -1.4, -2.4	1.5, 1.2, -1.1	oxidation reduction, fatty acid metabolism
BX305470.1.a.om.1	Cytochrome P450 11A1	-2.2, -1.7, -2.4	1.5, 1.0, 1.5	oxidoreductase activity, xenobiotic metabolism
CA343197.1.a.om.1	Conserved oligomeric Golgi complex component 5	-1.2, -2.6, -1.1	-1.1, 1.1, 1.3	protein localisation
CA386664.1.a.om.1	Ubiquitin-conjugating enzyme E2 variant 1	-1.6, -2.9, -1.5	1.1, 1.3, 1.4	proteolysis
CA363489.1.a.om.1	Carboxypeptidase A1	-1.2, -2.9, -1.2	-1.4, 1.0, 1.8	proteolysis
tcav0003c.f.14_3.1.s.om.8	Zona pellucida sperm-binding protein 3 precursor	-1.5, -2.1, -1.2	-1.2, 1.0, 1.0	reproduction
X_AJ347728.1.a.om.1	D(2)-like dopamine receptor	1.1, -1.5, -2.3	1.1, -1.2, -1.1	signal transduction
CA348507.1.a.om.1	Annexin A4	-1.1, -2.3, -1.3	1.4, -1.1, 1.2	signal transduction
X_BX859379.1.a.om.1	Adenylyl cyclase type 6	-1.2, -2.1, -1.2	1.3, 1.3, 1.2	signal transduction
CA377055.1.a.om.1	SLIT-ROBO Rho GTPase-activating protein 3	-2.1, -1.9, -1.7	1.3, 2.5, 2.3	signal transduction
CA378890.1.a.om.1	Solute carrier family 2	-2.6, -2.0, -1.5	1.5, -1.1, -1.4	sugar transport, glucose maintenance
rtay12a01d09r1.1.a.om.1	NFX1-type zinc finger-containing protein 1	-1.5, -2.5, -1.4	1.2, 1.1, 1.1	transcription
CA375698.1.a.om.1	Transcription intermediary factor 1-alpha	-1.2, -2.6, -1.4	1.0, 1.0, 1.3	transcription
CA353784.1.a.om.1	Tob1 protein (Transducer of erbB-2 1)	-1.6, -2.4, -1.2	1.2, 1.1, -1.1	transcription

CA353468.1.a.om.1	Splicing factor	-2.8, -2.6, -1.8	1.1, 1.1, 1.0	transcription
BX077950.1.a.om.1	Zinc finger protein Xfin	-1.1, -2.0, -1.4	1.1, -1.2, 1.0	transcription
BX076522.1.a.om.1	Forkhead box protein D3	-1.4, -2.5, -1.8	1.3, 1.5, 1.4	transcription
CR943405.1.a.om.1	Retinoblastoma-like protein 2	-2.0, -2.1, -1.6	2.2, 1.8, 2.0	transcription, cell cycle
CA384952.1.a.om.1	Splicing factor	-1.5, -2.2, -2.0	1.3, -1.2, 1.1	transcription, DNA repair
rtay11a03c02f1.1.a.om.1	RNA polymerase II elongation factor ELL	-1.2, -2.2, -1.1	1.2, -1.1, -1.1	transcription/translation
CA343180.1.a.om.1	60S ribosomal protein L32	-1.8, -2.4, -1.1	-1.3, 1.2, 1.8	translation
tcbi0028c.b.02_5.1.s.om.8	60S ribosomal protein L14	-1.3, -3.0, -1.2	1.1, 1.2, 1.1	translation
tcaa0001c.e.22_5.1.s.om.8	60S ribosomal protein L4-A	1.1, -1.7, 1.0	1.5, -1.1, 2.4	translation
CA363996.1.a.om.1	Eukaryotic translation initiation factor 4B	-1.5, -1.9, -2.3	-1.3, -1.1, 1.2	translation
SET 4: Upregulation in the LR line –predominantly an LS44 effect				
CA349582.1.a.om.1	Fish-egg lectin	1.1, -1.2, 3.9	-1.1, -1.3, 1.1	adhesion
CA345645.1.a.om.1	Thymosin beta-11	-1.1, -1.5, 3.0	1.0, -1.1, 1.1	cytoskeletal organisation
X_CA371947.1.a.om.1	Glyceraldehyde-3-phosphate dehydrogenase	-1.1, -1.5, 5.8	1.3, 1.4, 1.9	glucose metabolism, oxidation reduction, glycolysis
CA354409.1.a.om.1_2	Leukocyte cell-derived chemotaxin 2	-1.1, 1.5, 11.3	-3.1, 2.3, 3.0	immune response
CA354409.1.a.om.1_1	Leukocyte cell-derived chemotaxin 2	-1.1, 1.3, 18.7	-1.5, 8.6, 14.6	immune response
CA343278.1.a.om.1	Tyrosine-protein kinase HCK	-1.1, 1.0, 2.5	1.0, 1.1, 1.0	immune response
CA343672.1.a.om.1	Leukocyte common antigen precursor (CD45 antigen)	-1.4, -1.7, 2.6	1.0, 1.0, -1.2	immune response
CA350548.1.a.om.1_2	Complement factor H precursor	1.3, 1.1, 3.0	1.0, 1.7, 1.3	immune response
CA342316.1.a.om.1	Cytochrome b-245 light chain	-1.2, -1.4, 4.7	-1.4, -1.7, 1.1	immune response, oxidation reduction
CA348097.1.a.om.1	Neutrophil cytosol factor 1	-1.5, -1.9, 2.7	1.1, -1.3, 1.0	immune response, oxidation reduction
CA345431.1.a.om.1	Rhesus blood group-associated glycoprotein (CD241 antigen)	1.2, -1.2, 3.1	1.0, -1.1, 1.2	ion homeostasis
CA347328.1.a.om.1	Talin-2	-1.1, 1.2, 2.4	1.0, 1.2, -1.2	protein localisation
CA346484.1.a.om.1	Collagenase 3 (MMP-13)	-1.8, -1.9, 3.9	1.0, 1.1, -1.1	proteolysis
X_CA342769.1.a.om.1	Matrix metalloproteinase-9	-1.1, -1.5, 5.3	1.1, -1.2, -1.1	proteolysis, ECM organisation
CA351742.1.a.om.1	Lysozyme C II	1.0, 1.1, 4.1	-1.2, 1.3, 1.1	proteolysis, immune response

CA346948.1.a.om.1	Leukotriene A-4 hydrolase	-1.1, -2.0, 2.3	1.1, 1.5, -1.4	proteolysis, inflammatory response
CA353080.1.a.om.1	High mobility group protein B2	1.0, -1.2, 4.4	-1.2, -1.3, -1.1	transcription, DNA repair
CA355051.1.a.om.1	Tetratricopeptide repeat protein KIAA0103	-1.1, -1.1, 3.0	1.1, 1.3, 1.2	unknown

All genes show significant differential expression between the two fish lines ($P < 0.05$) in at least 2 of 3 replicate spots/features. See also footnotes to Table 1.

Table 3: Primers used for qPCR

Gene	Accession	Forward primer (5' to 3')	Reverse primer (5' to 3')
Intelectin-2 precursor	**CA347369.1**	5'CTCCAGGAGCCACAACAGTCTTGA3'	5'GTCATGTCGCAGAAGGTCTGGTAGA3'
Differentially regulated trout protein1(DRTP1)	**BX311643.1**	5'TTGGAACGACAGATCGAAAGACTCC3'	5'TCATGGTCAACACGGCAGGTCT3'
Purine nucleoside phosphorylase	**CA355465.1**	5'GCTTTTGAGAGGATTCCAGTCGC3'	5'GTCCTCCCATTCCTGAACCACAC3'
78 kDa glucose-regulated protein	**CA368961.1**	5'GGACAAGGAAGCCATAGAGAAAG3'	5'AGCTTGCTGATGATGGGCTG3'
Interferon induced transmembrane protein 3	**CA343987.1**	5'TATGGTTGGAGACTTGGAGGGAG3'	5'CACCTGCCAAGACTTCAAATGAACA3'
Cytochrome b-245 heavy chain	**CA349240.1**	5'GCCTGCGGCTTAATTTGACT3'	5'TCATTGTCCCAGTTAGGTTTCCCAT3'
CR944591.1	**CR944591.1**	5'ACATCATGTGTCATGTTTGTGAA3'	5'ACCTGCGTTGTTGGTTAAGG3'
Thymosin beta 11	**CA358932.1**	5'CTCGTGTGGATTTTATTTACATTGATG3'	5'GGCAGATTAACATGAGTTGGGACTTTA3'
18S ribosomal RNA	**AF309412**	5'ACCACCCACAGAATCGAGAAA3'	5'GCCTGCGGCTTAATTTGACT3'

Additional files

Additional file 1 –

Microsoft Word format

Table S1: K-means clusters of the full modified stress-related gene list

Additional file 2 –

Microsoft Word format

Table S2: K-means clusters of the line-related gene list

This is an expanded version of Table 2 in the manuscript. It includes all microarray features for which there are sequences but no additional annotation.

Additional file 3 –

Microsoft Word format

Figure S1: Differentially expressed genes between high and low responders upon exposure to confinement stress

Venn diagrams of significant differentially expressed genes ($P<0.05$) between high and low responders upon exposure to confinement stress for 6 h, 24 h, and 168 h

Additional file 4 –

Microsoft Word format

Figure S2: Two K-means gene clusters showing significant differences between the HR and LR lines under confinement stress.

The heat map compares expression levels after 168 h confinement stress in five individuals from each of the high and low responder lines as analysed using GeneSpring software. Only clusters 1 and 3 are presented.

Figure 1.

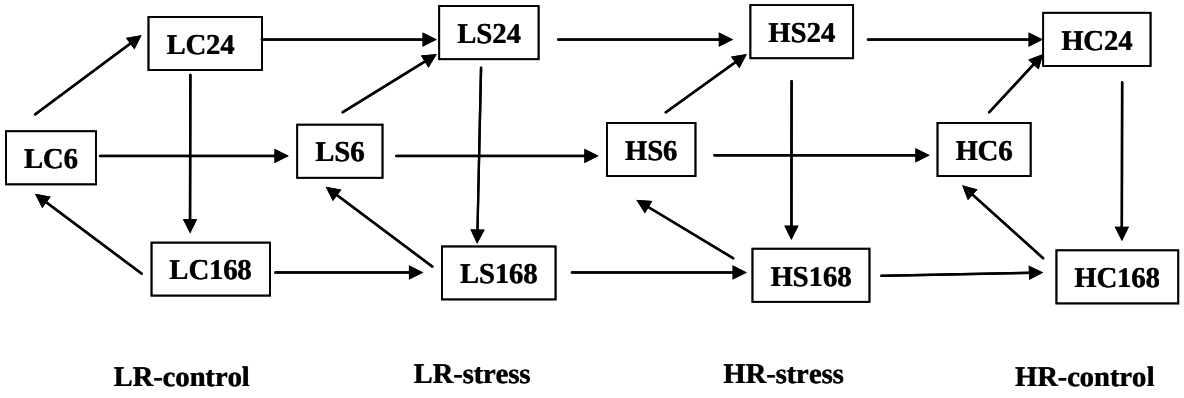

LC24
LS24
HS24
HC24
LC6
LS6
HS6
HC6
LC168
LS168
HS168
HC168
LR-control
LR-stress
HR-stress
HR-control

Figure 2:

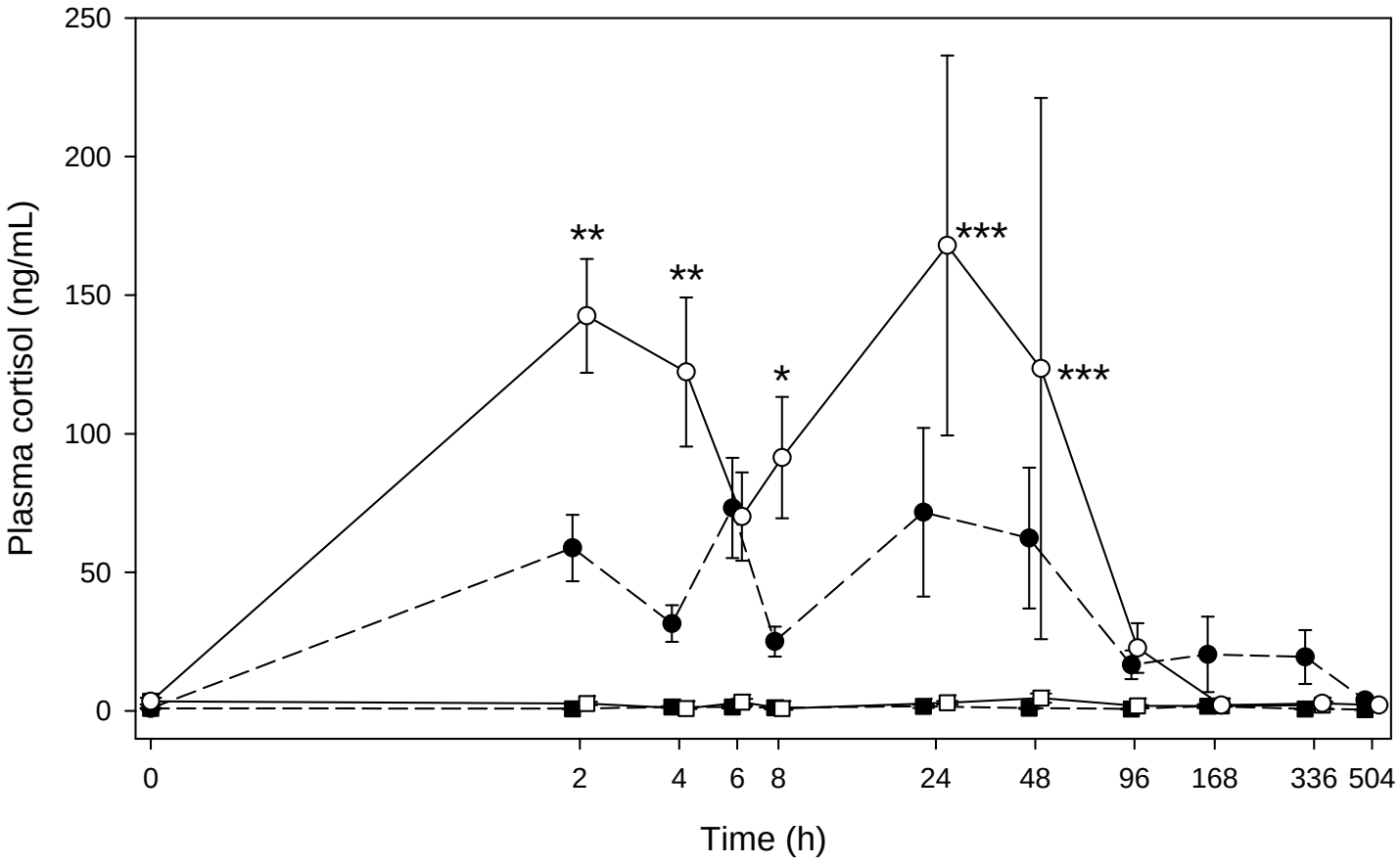
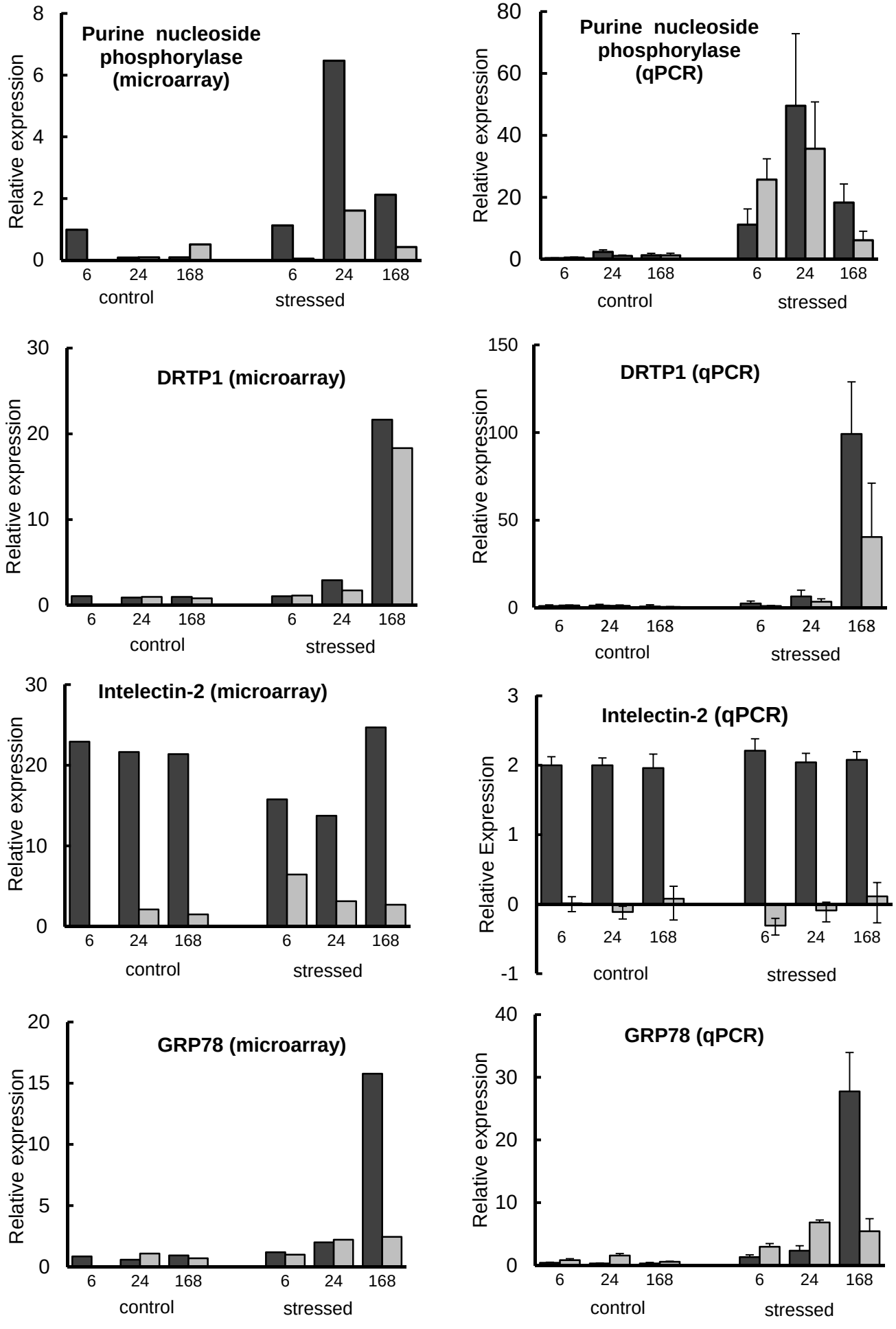
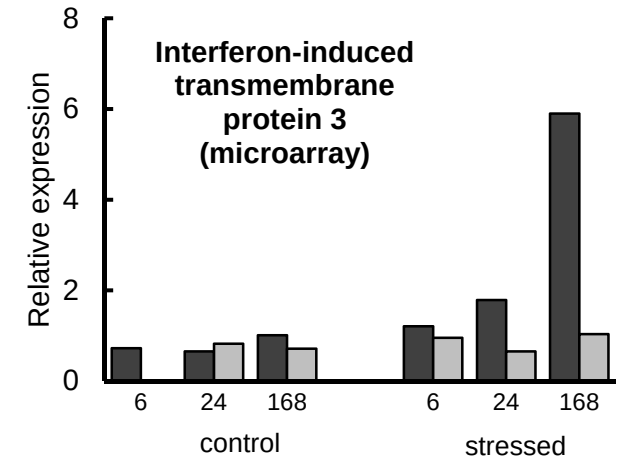
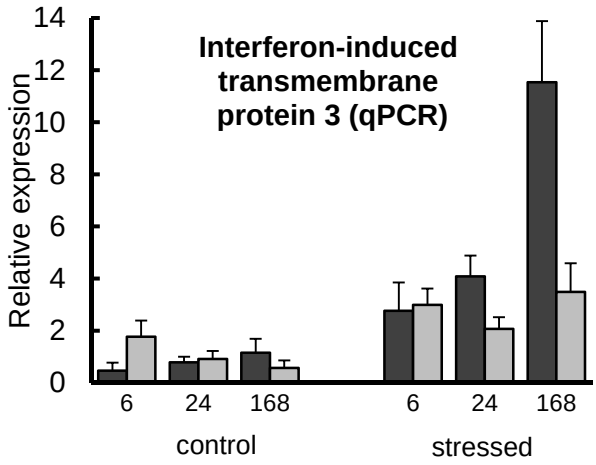
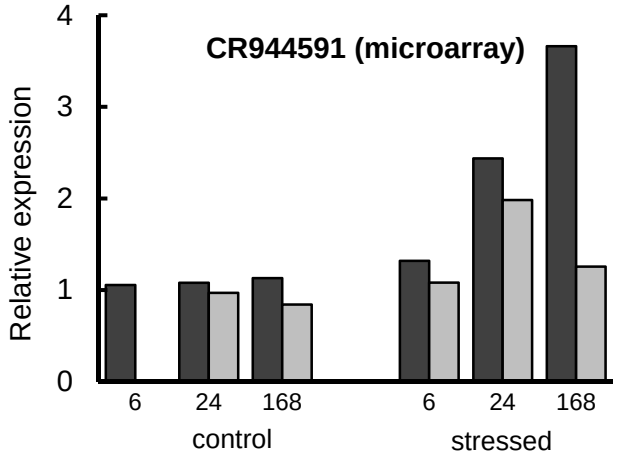

250
200
150
100
50
0
Plasma cortisol (ng/mL)
**
**
*
***
***
0
2
4
6
8
24
48
96
168
336
504
Time (h)

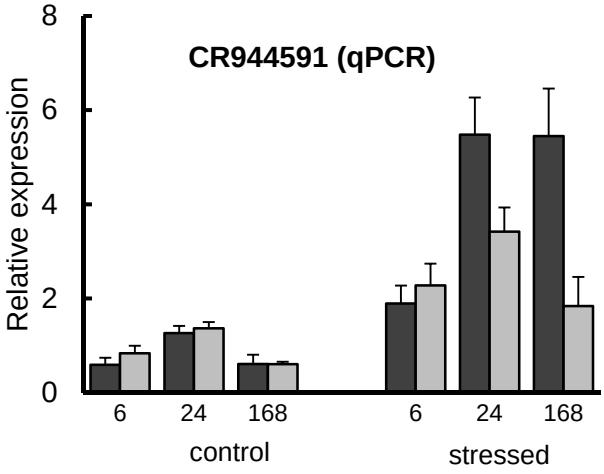
Figure 3.

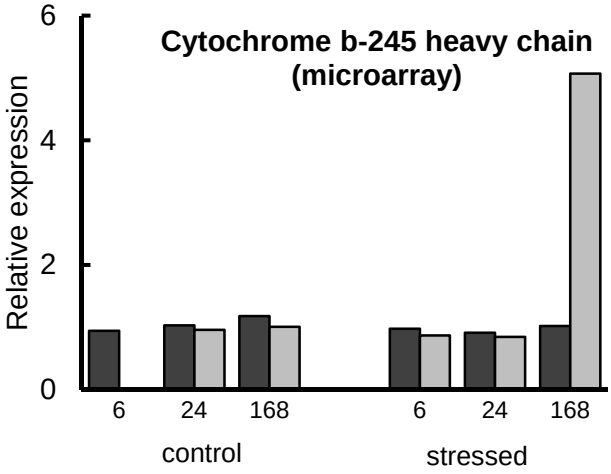

Purine nucleoside phosphorylase (microarray)
Relative expression
8
6
4
2
0
6
24
168
6
24
168
control
stressed
Purine nucleoside phosphorylase (qPCR)
Relative expression
80
60
40
20
0
6
24
168
6
24
168
control
stressed
DRTP1 (microarray)
Relative expression
30
20
10
0
6
24
168
6
24
168
control
stressed
DRTP1 (qPCR)
Relative expression
150
100
50
0
6
24
168
6
24
168
control
stressed
Intelectin-2 (microarray)
Relative expression
30
20
10
0
6
24
168
6
24
168
control
stressed
Intelectin-2 (qPCR)
Relative Expression
3
2
1
0
-1
6
24
168
6
24
168
control
stressed
GRP78 (microarray)
Relative expression
20
15
10
5
0
6
24
168
6
24
168
control
stressed
GRP78 (qPCR)
Relative expression
40
30
20
10
0
6
24
168
6
24
168
control
stressed

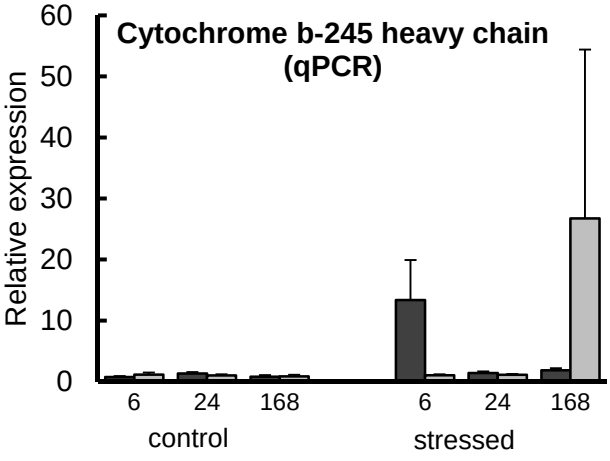

Interferon-induced transmembrane protein 3 (microarray)
Relative expression
0
2
4
6
8
6
24
168
6
24
168
control
stressed

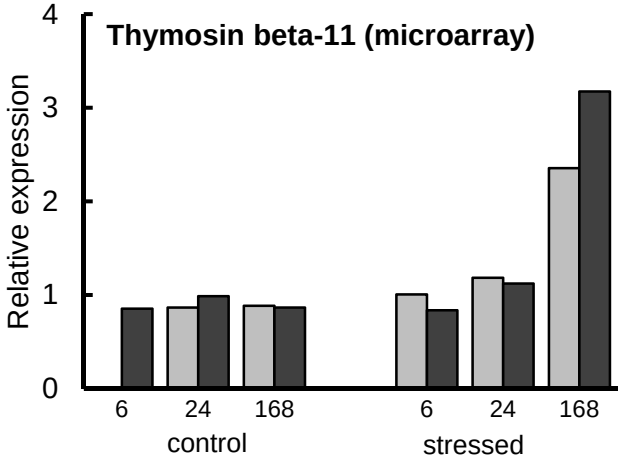

Interferon-induced transmembrane protein 3 (qPCR)
Relative expression
0
2
4
6
8
10
12
14
6
24
168
6
24
168
control
stressed


CR944591 (microarray)
Relative expression
0
1
2
3
4
6
24
168
6
24
168
control
stressed


CR944591 (qPCR)
Relative expression
0
2
4
6
8
6
24
168
6
24
168
control
stressed


Cytochrome b-245 heavy chain (microarray)
Relative expression
0
2
4
6
6
24
168
6
24
168
control
stressed


Cytochrome b-245 heavy chain (qPCR)
Relative expression
0
10
20
30
40
50
60
6
24
168
6
24
168
control
stressed


Thymosin beta-11 (microarray)
Relative expression
0
1
2
3
4
6
24
168
6
24
168
control
stressed

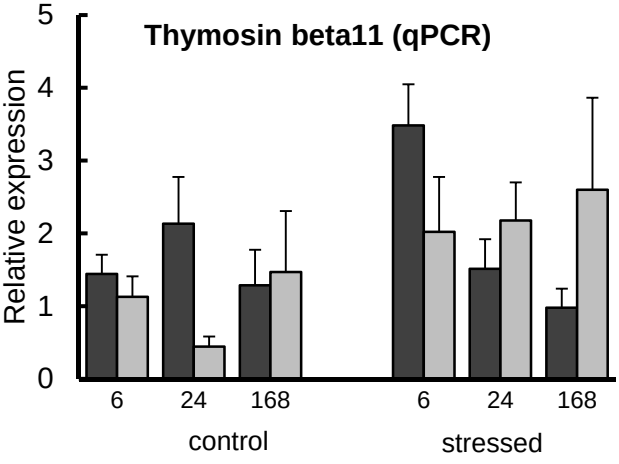

Thymosin beta11 (qPCR)
Relative expression
0
1
2
3
4
5
6
24
168
6
24
168
control
stressed

Figure 4.

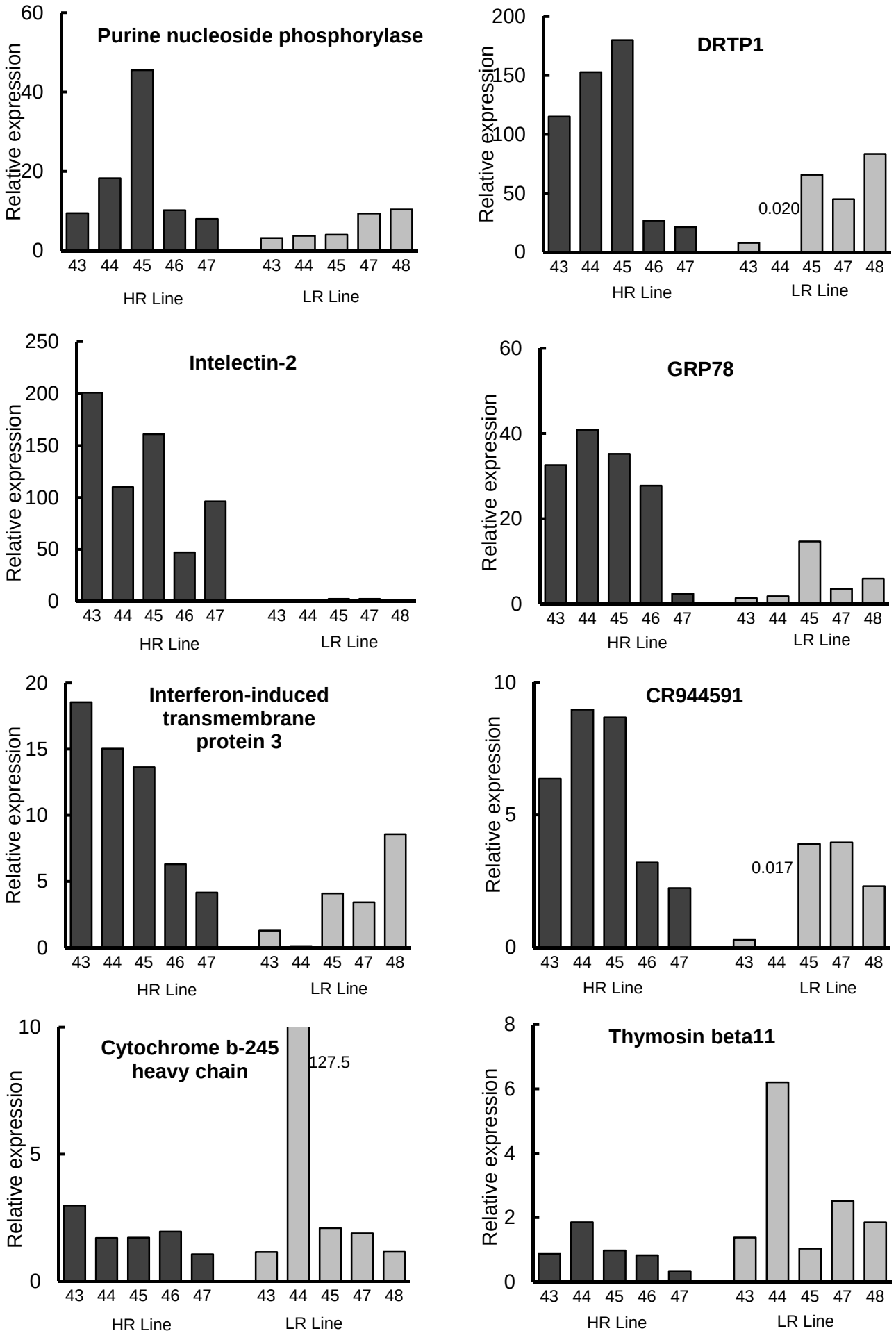

Purine nucleoside phosphorylase
DRTP1
0.020
Intelectin-2
GRP78
Interferon-induced transmembrane protein 3
CR944591
0.017
Cytochrome b-245 heavy chain
127.5
Thymosin beta11
Relative expression
HR Line
LR Line
43 44 45 46 47 43 44 45 47 48

Figure S1.

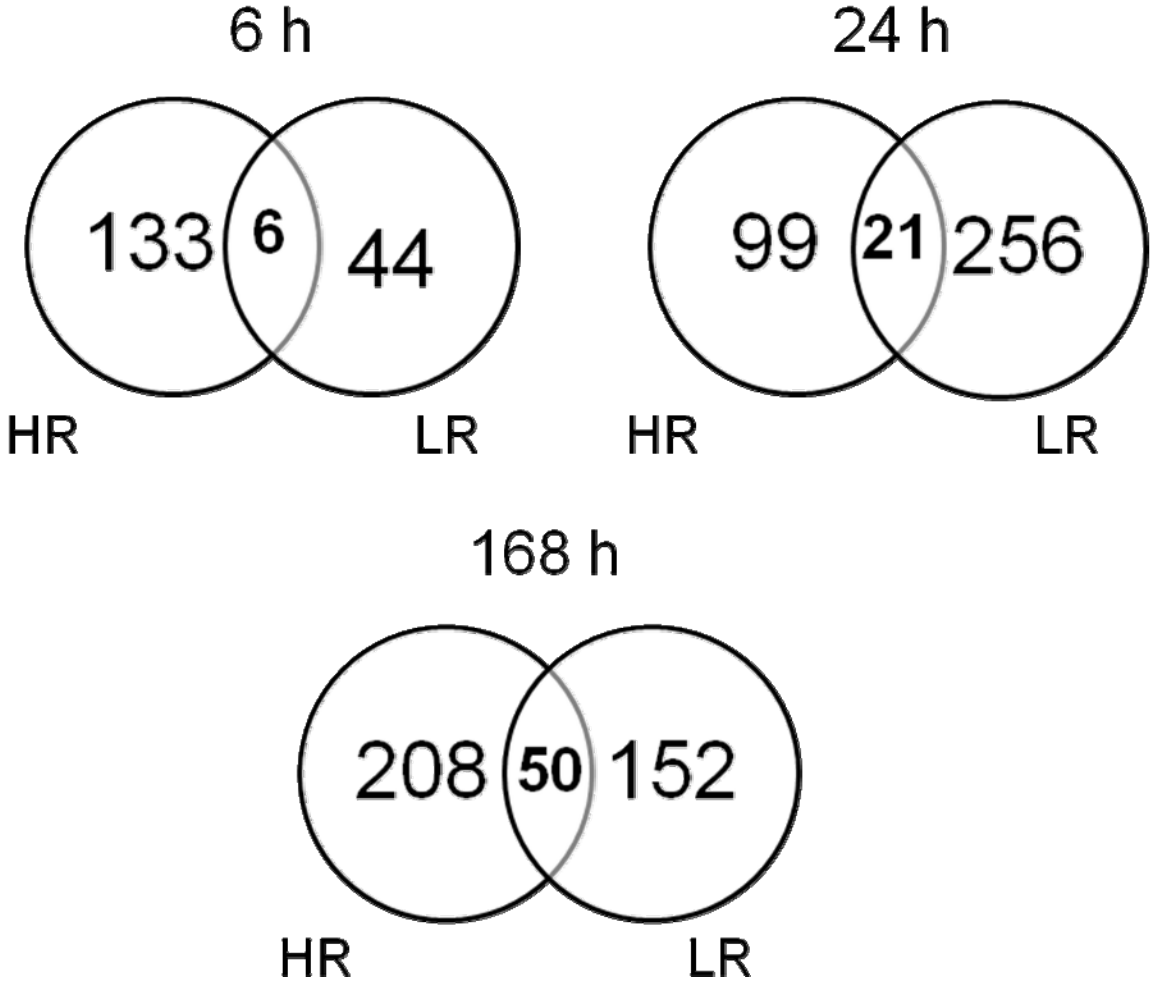
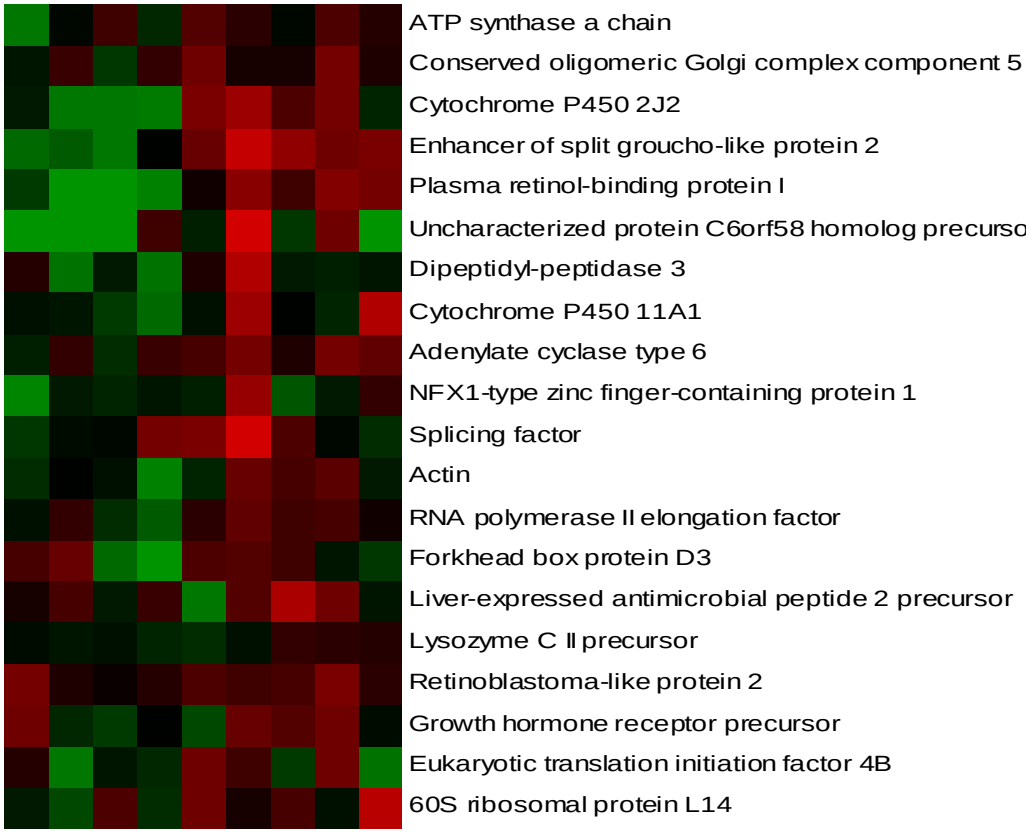


Figure S2

Cluster 1



Cluster 3

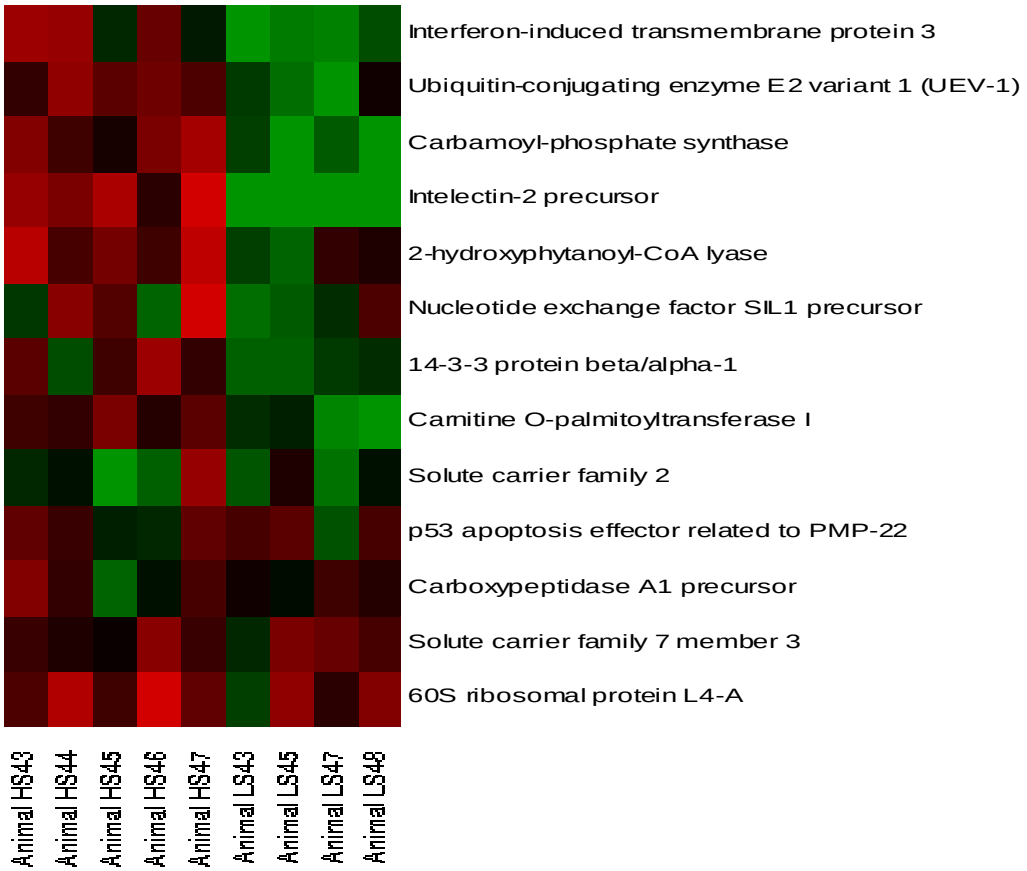


Table S1: K-means clusters of the full modified stress-related gene list

ID	Gene Description	LR 6h, 24h, 168h	HR 6h, 24h, 168h	Functions
SET 1: Down-regulation in LR and HR fish				
X_CA350479.1.a.om.1	CA350479	-1.3, -1.5, -1.2	-1.1, -1.3, -3.4	
CR944250.1.a.om.1	CR944250	-1.2, -1.7, -1.4	-1.8, -1.1, -2.0	
CA365719.1.a.om.1	CA365719	1.1, -1.2, -1.7	-1.2, -1.6, -3.1	
CA351175.1.a.om.1	CA351175	-1.2, -1.3, -1.8	-1.2, 1.0, -2.5	
rtay12a04d11f1.1.a.om.1	rtay12a04d11f1	1.0, -1.3, -1.3	-1.6, -2.7, -3.0	
rtay11a07e12f1.1.a.om.1	rtay11a07e12f1	-1.1, -1.1, -1.4	-1.3, -1.2, -2.0	
rtay11a04a02r1.1.a.om.1	rtay11a04a02r1	1.1, -1.2, -1.2	-1.1, -1.2, -2.0	
CT571404.1.a.om.1	CT571404	1.1, -1.1, -1.2	-1.3, -1.2, -2.0	
CT563636.1.a.om.1	CT563636	-1.1, -1.5, -1.3	-1.1, -1.4, -2.0	
CR943512.1.a.om.1	CR943512	1.1, 1.2, -1.7	-1.1, -1.1, -3.0	
CA375714.1.a.om.1	CA375714	1.1, 1.1, -1.6	-1.2, -1.5, -2.3	
CA373463.1.a.om.1	CA373463	-1.1, -1.2, -1.3	-1.5, -1.2, -2.4	
CA342327.1.a.om.1	CA342327	-1.1, -1.3, -1.1	-2.0, -1.2, -2.0	
BX862864.1.a.om.1	BX862864	1.3, 1.1, -1.2	-1.7, -1.4, -2.4	
BX086863.1.a.om.1	BX086863	1.0, 1.0, 1.0	-1.1, -1.6, -2.2	
BX077866.1.a.om.1	BX077866	1.3, -1.4, -1.6	1.2, -1.7, -2.6	
X_CT572262.1.a.om.1	ATP synthase a chain	1.3, 1.0, -1.2	-1.2, -3.1, -2.2	ATP maintenance, oxidation reduction
CA349504.1.a.om.1	Adenosylhomocysteinase B	1.2, 1.0, -2.9	-1.1, 1.0, -2.3	developmental
CB492286.1.a.om.1	Abhydrolase domain-containing protein 12	-1.1, -1.5, -2.3	1.1, -1.4, -1.9	fat metabolism
CA343347.1.a.om.1	5-aminolevulinate synthase	-1.1, 1.0, -1.4	-2.1, -1.5, -2.1	haem biosynthesis
CA342499.1.a.om.1	Uncharacterized protein C6orf58 homolog	1.0, -1.1, -2.1	-1.1, -1.2, -6.3	hypothetical protein
CA387966.1.a.om.1	Liver-expressed antimicrobial peptide 2	1.9, 1.0, -1.2	-3.1, -2.3, -5.3	immune response
CR942994.1.a.om.1	Nucleoside diphosphate kinase A	-1.2, -1.6, -1.3	-1.3, -1.9, -2.6	nucleotide metabolism
X_AF059711.1.a.om.1	Cytochrome P450 1A3	1.0, -2.1, -1.1	-1.1, -1.3, -2.0	oxidation reduction
CA359096.1.a.om.1	Dimethylaniline monooxygenase	1.0, -1.4, -1.5	1.1, -1.4, -2.2	oxidation reduction
CA347323.1.a.om.1	NADP-dependent malic enzyme	1.7, -1.4, -1.5	-1.1, -1.6, -3.2	oxidation reduction
AF046012.1.a.om.1	Cytochrome P450 4V2	1.1, -1.2, -1.6	-1.1, -1.5, -2.1	oxidation reduction, perception
X_CA348235.1.a.om.1	Cytochrome P450 2K1	1.1, -1.4, -2.3	1.2, -1.4, -2.0	oxidoreductase activity, xenobiotic metabolism
CA358005.1.a.om.1	Hemoglobin subunit alpha	-2.1, -2.0, -1.6	-2.3, -2.6, -1.9	oxygen transport
rtay12a02h11f1.1.a.om.1	Importin beta-1 subunit	1.6, 1.0, -2.1	-2.6, -1.9, -4.2	protein localisation
CA365301.1.a.om.1	Dipeptidyl-peptidase 3	-1.2, -1.5, -1.2	-3.6, -2.8, -2.4	proteolysis
CA348733.1.a.om.1	Cathepsin F	-1.1, -2.0, -1.4	-1.5, -1.6, -2.3	proteolysis
tcay0001b.j.03_3.1.s.om.8	Secreted phosphoprotein 24 precursor	1.1, 1.0, -1.5	-1.3, -1.2, -2.1	proteolysis, inhibitor
BX866014.1.a.om.1	Cysteine-rich motor neuron 1 protein	1.4, -1.5, -2.3	-1.5, 1.0, -2.0	proteolysis, inhibitor
BX879299.1.a.om.1	CD81 antigen	1.1, -1.1, -1.7	-1.9, -1.6, -2.4	signal transduction

CA383629.1.a.om.1	Estrogen receptor	1.2, -1.5, -1.3	-1.4, -1.4, -2.1	transcription
CX262061.1.a.om.1	Enhancer of split groucho-like protein 2	-1.2, -1.2, -1.3	-2.0, -2.1, -3.6	transcription
CA347196.1.a.om.1	Thioredoxin-interacting protein	1.0, -1.9, -1.4	-1.4, 1.1, -2.6	transcription,
tcay0025b.n.05_5.1.s.om.8	Nuclear protein Hcc-1	1.0, -1.1, -1.8	1.0, -1.3, -2.2	transcription/translation
X_CA342046.1.a.om.1	40S ribosomal protein S4	-1.2, -1.6, -2.3	-1.2, -2.4, -2.0	translation
CA356000.1.a.om.1	Motile sperm domain-containing protein 1	1.2, 1.0, -1.5	-1.1, -1.7, -2.4	unknown
CA342509.1.a.om.1	Plasma retinol-binding protein I	1.2, -1.4, -1.2	1.0, -1.7, -3.0	Vit A transport, fat metabolism
	SET 2: Up-regulation in HR fish			
CA344519.1.a.om.1	CA344519	-1.2, -1.5, 1.2	1.4, 1.2, 3.3	
BX076478.1.a.om.1	BX076478	1.2, -1.2, 1.3	2.1, 1.4, 1.6	
rtay11a02e05r1.1.a.om.1	rtay11a02e05r1	1.0, -1.4, 1.1	2.2, 1.5, 1.4	
CX030984.1.a.om.1	CX030984	1.0, -1.3, -1.2	1.4, 1.3, 2.1	
CA344960.1.a.om.1	CA344960	-1.1, -1.2, -1.4	1.2, 2.0, 2.4	
BX856121.1.a.om.1	BX856121	1.2, 1.0, -1.1	2.3, 1.4, 1.9	
BX082276.1.a.om.1	BX082276	-1.1, -1.4, -1.2	1.2, 1.6, 2.1	
BX074470.1.a.om.1_1	BX074470	-1.1, -1.6, -1.3	1.6, 1.5, 2.9	
X_CA356842.1.a.om.1	CA356842	1.0, -1.4, 1.3	1.3, 1.6, 2.1	
X_CA350158.1.a.om.1	CA350158	-1.4, -2.0, -1.6	2.3, 1.9, 2.7	
rtay12a06e10r1.1.a.om.1	rtay12a06e10r1	-1.3, -1.3, 1.1	2.4, 1.7, 2.0	
CX720452.1.a.om.1	CX720452	-1.4, -1.4, -1.3	1.6, 1.4, 2.1	
CT572011.1.a.om.1	CT572011	-1.2, -2.2, -1.3	2.0, 2.1, 2.2	
CT571312.1.a.om.1	CT571312	1.1, -1.2, -1.1	1.6, 1.9, 3.2	
CT564232.1.a.om.1	CT564232	-1.2, -2.2, -1.3	2.1, 2.0, 2.2	
CA382773.1.a.om.1	CA382773	-1.2, 1.1, 1.0	1.3, 1.3, 3.1	
CA372503.1.a.om.1	CA372503	1.0, 1.1, -1.1	1.1, -1.1, 3.2	
CA364091.1.a.om.1	CA364091	1.2, 1.4, -1.1	2.3, 1.7, -1.1	
CA357750.1.a.om.1	CA357750	-1.2, 1.0, 1.2	1.1, 1.4, 2.5	
BX873929.1.a.om.1	BX873929	-1.1, 1.1, 1.4	-1.3, 1.2, 2.1	
tcad0004a.l.24_3.1.s.om.8	tcad0004a.l.24_3.1.s.om.8	-1.2, -1.2, 1.6	1.6, 1.3, 3.2	
rtay13a02c05f1.1.a.om.1	rtay13a02c05f1	-1.2, 1.3, 1.4	-1.1, 1.2, 2.5	
CR944563.1.a.om.1	CR944563	1.4, -1.2, 1.3	1.4, 2.2, 1.7	
CA349038.1.a.om.1	CA349038	1.0, 1.2, 1.4	1.9, 1.9, 2.3	
X_CA351975.1.a.om.1	CA351975	-1.1, 1.3, 1.6	1.5, 2.0, 2.2	
X_CA349870.1.a.om.1	CA349870	1.2, 1.5, 1.9	1.1, 1.0, 2.0	
CA374294.1.a.om.1	CA374294	1.7, 2.2, -1.1	-1.2, 3.4, 1.7	
CA352042.1.a.om.1	CA352042	-1.1, 1.2, 2.2	1.0, 2.1, 1.6	
CA367141.1.a.om.1	CA367141	1.2, 1.2, 2.0	-1.2, 1.6, 3.0	
CR944591.1.a.om.1	CR944591	1.1, 2.1, 1.5	1.3, 2.2, 2.9	
CA371796.1.a.om.1	CA371796	1.7, 2.2, 1.4	3.3, 3.0, 2.4	
CA384724.1.a.om.1	CA384724	-1.8, -2.6, 1.1	1.6, 1.9, 3.4	
tcbk0031c.o.22_5.1.s.om.8	Cationic amino acid transporter 3	-1.2, -1.1, 1.0	2.2, 1.4, 1.9	amino acid metabolism
X_CA372046.1.a.om.1	S-adenosylmethionine synthetase isoform type-2	1.2, 1.7, 1.2	2.3, 2.5, 2.9	amino acid metabolism
X_CA347249.1.a.om.1	Dihydrolipoyllysine-residue succinyl-transferase	1.5, 1.1, 1.2	3.3, 1.6, 1.0	ATP maintenance
BX878991.1.a.om.1	Cytochrome c oxidase subunit 4 isoform 1	1.2, -2.6, 1.4	1.7, 2.5, 4.4	ATP maintenance, oxidation reduction

X_CA370364.1.a.om.1	NADH-ubiquinone oxidoreductase chain 3	-1.1, 1.7, 1.3	-1.4, 1.5, 2.0	ATP maintenance, oxidation reduction
CA348766.1.a.om.1	Protein disulfide-isomerase	1.3, 1.1, 1.7	1.0, -1.1, 5.3	cell redox homeostasis
BX301625.1.a.om.1	Adseverin (Scinderin)	-1.2, 1.6, 1.0	1.1, 2.2, 1.1	cytoskeletal organisation
CA382759.1.a.om.1	COP9 signalosome complex s/u 8	1.1, 1.9, 1.0	2.6, 2.1, 2.2	developmental
tcbk0024c.i.05_5.1.s.om.8	Carnitine O-palmitoyltransferase I	-1.5, -1.2, -1.2	2.0, 1.8, 2.0	fatty acid metabolism, glucose maintenance
tcac0001c.a.17_3.1.s.om.8	Fructose-bisphosphate aldolase A	1.0, 1.4, 1.4	1.0, 1.4, 2.0	glucose metabolism
CA370601.1.a.om.1	Protein Red	1.4, 1.3, 1.4	1.9, 2.0, 1.8	immune response
tcag0003b.k.07_5.1.s.om.8	Interferon-inducible protein	1.4, 1.8, 1.6	1.7, 1.6, 2.1	immune response
X_CA351045.1.a.om.1	Proto-oncogene tyrosine-protein kinase LCK	-1.2, 1.0, 1.7	-1.1, 1.2, 3.9	immune response
BX884287.1.a.om.1	High-affinity copper uptake protein 1	1.0, 1.6, 1.6	1.6, 2.1, 3.7	ion homeostasis
CA347059.1.a.om.1	Ferritin	1.9, 1.7, -1.2	-1.1, 1.2, 2.1	iron homeostasis
CA352463.1.a.om.1	2-hydroxyphytanoyl-CoA lyase	1.1, -1.2, -1.2	1.4, 1.6, 2.1	lipid metabolism
BX077158.1.a.om.1	Very low-density lipoprotein receptor	-1.2, 1.3, 1.4	1.2, 2.0, 1.3	lipid metabolism
CT567315.1.a.om.1	Lanosterol synthase	-1.1, -1.7, 1.3	-1.3, 1.2, 2.0	lipid/steroid metabolism
CA351531.1.a.om.1	Peroxiredoxin-5	-1.3, -1.1, 1.5	1.0, 2.0, 2.1	oxidation reduction
CA342689.1.a.om.1	Electron transfer flavoprotein subunit alpha	1.2, 1.5, 1.9	1.1, 1.6, 2.4	oxidation reduction
CA342560.1.a.om.1	Ser/Thr-protein phos-phatase 2A cat. s/u beta	-1.1, 1.3, 1.3	1.2, 1.6, 2.1	oxidation reduction, apoptosis
CT568331.1.a.om.1	Ribophorin II	1.0, 1.2, 1.3	1.2, 1.2, 2.1	protein glycosylation
CA348463.1.a.om.1	ER lumen protein retaining receptor 2	-1.1, -1.2, 1.4	1.2, 1.1, 2.1	protein localisation
CA342236.1.a.om.1	Nascent polypeptide-associated complex subunit alpha	-1.1, -1.2, -1.1	1.9, 1.3, 2.0	protein localisation
CA349863.1.a.om.1	Transmembrane emp24 domain-contain protein 2	1.2, 1.0, 1.5	-1.1, -1.1, 2.0	protein localisation, vesicule targetting
CA359265.1.a.om.1	Elastase-2A	1.2, -1.4, 1.0	1.3, 1.0, 2.4	proteolysis
CA342819.1.a.om.1	Minor histocompatibility antigen H13	1.2, -1.1, 1.6	1.1, 1.3, 2.2	proteolysis
CA342330.1.a.om.1	Calpain small subunit 1	1.2, 2.4, 1.0	2.6, 2.6, 1.5	proteolysis
BX875761.1.a.om.1	Homocysteine-responsive ER-resident ubiquitin-like domain member 1 protein	-1.3, -1.5, 1.1	1.2, 1.1, 2.1	proteolysis, inhibitor, ER stress response
CB491185.1.a.om.1	Protein ZNF403	1.1, 1.8, 1.0	2.0, 2.9, 1.0	reproduction
BX299056.1.a.om.1	Retinitis pigmentosa 9 protein homolog	-1.1, 1.4, 1.1	1.0, 1.4, 2.3	RNA splicing
CA370303.1.a.om.1	Tetraspanin-3	1.0, -1.1, 1.2	1.2, 2.2, 2.0	signal transduction
CA347907.1.a.om.1	Guanine nucleotide-binding protein s/u beta 1	1.1, 2.0, 1.5	-1.1, 1.4, 1.3	signal transduction
BX866751.1.a.om.1	Cytochrome P450 24A1	-1.2, 2.3, -1.8	1.7, 3.9, 1.4	steroid metabolism, oxidation reduction, vitamin metabolism
X_CA342458.1.a.om.1	CCAAT/enhancer-binding protein delta	1.0, 1.0, -1.7	2.8, 3.7, 1.2	transcription
tcaa0001c.e.22_5.1.s.om.8	60S ribosomal protein L4-A	1.1, -1.7, 1.0	1.5, -1.1, 2.4	translation
CA342929.1.a.om.1	60S Rpl37a	-1.1, 1.1, 1.5	1.2, 1.1, 2.1	translation

BX304351.1.a.om.1	Translation initiation factor eIF-2B subunit gamma	-2.0, 1.1, 1.6	1.1, 1.1, 2.8	translation
tcav0001c.n.20_3.1.s.om.8	Translation initiation factor eIF-2B alpha subunit	1.0, -1.4, -1.1	-1.2, 1.1, 2.7	translation, response to glucose and various stimulants
BX863020.1.a.om.1	Circumsporozoite protein	1.0, 1.6, 1.0	2.2, 3.1, 3.6	unknown
CA363035.1.a.om.1	Transmembrane emp24 domain-containing protein 10	1.1, 1.3, 1.9	1.1, 1.4, 2.6	vesicle trafficking
SET 3: Down-regulation, predominantly in LR fish				
X_CA343113.1.a.om.1	CA343113	-1.3, -1.4, -1.8	1.1, 2.1, -1.9	
rtay12a06c09f1.1.a.om.1	rtay12a06c09f1	1.2, -1.7, -1.3	2.6, 1.3, -1.7	
rtay12a04d10r1.1.a.om.1	rtay12a04d10r1	-1.1, 1.0, -2.0	1.6, 1.5, -1.3	
CT567619.1.a.om.1	CT567619	1.3, 1.1, 1.1	2.3, 1.4, -1.4	
CA377220.1.a.om.1	CA377220	1.1, 1.0, -1.8	2.2, 2.3, -1.2	
X_CA384133.1.a.om.1	CA384133	-2.0, -1.1, #N/A	1.4, -1.1, 1.1	
rtay12a07c10f1.1.a.om.1	rtay12a07c10f1	-2.0, -1.3, -2.1	-1.4, 1.0, 1.0	
BX871567.1.a.om.1	BX871567	-2.0, 1.8, -1.4	-1.1, 1.1, 1.1	
tcbw0006a.f.09_5.1.s.om.8	tcbw0006a.f.09_5.1.s.om.8	-1.7, -1.6, -2.3	1.6, 1.1, 1.2	
X_CU070025.1.a.om.1	CU070025	-9.1, 2.5, -4.0	-4.8, 2.8, 3.1	
BX318614.1.a.om.1	SET and MYND domain-containing protein 3	-1.4, -1.3, -2.5	1.5, 1.1, 1.1	chromatin organisation, transcription
CA373655.1.a.om.1	Roundabout homolog 1	-1.3, -1.4, -2.0	1.1, 1.1, -1.1	developmental
X_CA355197.1.a.om.1	Fatty acyl-CoA hydrolase	-1.2, -1.3, -2.4	1.0, -1.1, -1.2	fatty acid metabolism
CA368454.1.a.om.1	Carbonic anhydrase 5B	-1.2, 2.2, -1.3	-1.1, -1.1, -1.4	gluconeogenesis, lipogenesis
CA363783.1.a.om.1	C-reactive protein precursor	1.2, -1.3, -2.9	1.2, -1.4, -1.5	immune response
X_CB492852.1.a.om.1	Nattectin	-1.9, 1.7, -2.0	-1.4, 1.5, 1.2	immune response
CA354569.1.a.om.1	Alpha-2-HS-glycoprotein precursor	-1.4, -1.6, -3.3	1.3, -1.3, 1.0	immune response
CA369692.1.a.om.1	Sequestosome-1	-2.0, -1.3, -1.7	1.1, -1.1, -1.7	immune response, apoptosis
CA363325.1.a.om.1	Very-long-chain acyl-CoA synthetase	1.2, 2.0, -1.6	1.1, 1.6, -1.1	lipid metabolism
X_BX308005.1.a.om.1	Patatin-like phospholipase domain-containing protein 4	1.1, -1.2, -1.1	2.9, 2.1, -1.4	lipid metabolism
X_CA344701.1.a.om.1	Cytochrome P450 2J2	-1.4, -1.4, -2.4	1.5, 1.2, -1.1	oxidation reduction, fatty acid metabolism
BX077021.1.a.om.1	Heat shock protein 30	-1.1, -1.1, -2.0	-1.2, -1.1, -1.3	response to stress
X_AJ347728.1.a.om.1	D(2)-like dopamine receptor	1.1, -1.5, -2.3	1.1, -1.2, -1.1	signal transduction
CT567216.1.a.om.1	Protein FAM57B	-1.2, 3.2, -1.5	1.1, 1.0, -1.7	unknown
CB494028.1.a.om.1	Retinol-binding protein II	-2.0, -1.2, -1.6	1.1, 1.2, 1.1	vitamin metabolism, fatty acid metabolism
SET 4: Down-regulation in LR only				
rtlm25p23_3.1_a.1.s.om.8	rtlm25p23_3.1_a.1.s.om.8	-1.7, -2.4, 1.1	1.1, 1.5, 1.3	
rtay12a02b10f1.1.a.om.1	rtay12a02b10f1	-1.7, -2.1, -1.3	1.3, 1.1, 1.2	
CU067093.1.a.om.1	CU067093	-1.2, -3.1, -1.3	1.2, 1.2, 1.3	
CT572122.1.a.om.1	CT572122	-1.6, -2.7, -1.9	-1.2, -1.1, -1.3	
CR944058.1.a.om.1	CR944058	-1.7, -2.0, -1.9	-1.1, 1.1, 2.5	
CR944035.1.a.om.1	CR944035	-1.1, -2.8, -1.2	1.4, 1.4, 1.1	
CR943872.1.a.om.1	CR943872	-1.6, -2.3, -1.4	1.3, 1.1, 1.1	
CR943749.1.a.om.1	CR943749	-1.5, -2.2, -1.4	1.0, -1.3, 1.4	
CA354386.1.a.om.1	CA354386	-1.6, -3.1, -1.4	1.0, 1.1, 1.0	

BX310423.1.a.om.1	BX310423	-3.3, -4.8, -1.6	1.7, 1.9, 8.4	
TCBM_O16.SEQ	TCBM_O16.SEQ	-1.6, -2.5, -1.5	1.1, -1.1, 1.4	
rtgl23d03_5.1_a.1.s.om.8	rtgl23d03_5.1_a.1.s.om.8	-1.4, -2.9, -1.8	1.4, -1.1, 1.2	
rtay13a04b04f1.1.a.om.1	rtay13a04b04f1	-1.4, -2.2, -1.2	-1.1, 1.0, 1.0	
rtay12a02e04f1.1.a.om.1	rtay12a02e04f1	1.0, -2.0, -1.4	1.4, 1.1, 1.6	
rtay12a01e12r1.1.a.om.1	rtay12a01e12r1	-1.7, -2.6, -2.0	1.2, 1.2, 1.5	
rtay11a08g07r1.1.a.om.1	rtay11a08g07r1	-1.5, -2.3, -1.5	1.6, 1.4, 1.9	
rtay11a05d05r1.1.a.om.1	rtay11a05d05r1	-1.3, -2.0, -1.2	1.6, 1.4, 1.3	
rtay11a04a05f1.1.a.om.1	rtay11a04a05f1	-1.9, -2.3, -2.2	1.3, 2.0, 1.8	
rtay11a03h02f1.1.a.om.1	rtay11a03h02f1	-1.9, -2.9, -2.0	1.5, 1.3, 2.0	
CT572635.1.a.om.1	CT572635	-1.1, -2.2, -1.7	1.1, 1.5, 1.3	
CT572176.1.a.om.1	CT572176	-1.8, -3.1, -1.7	1.2, 1.7, 1.2	
CT566501.1.a.om.1	CT566501	-3.2, -2.1, -1.3	1.4, 1.2, 1.4	
CT565138.1.a.om.1	CT565138	-1.4, -2.9, 1.2	1.1, -1.1, 1.2	
CR943993.1.a.om.1	CR943993	-1.2, -2.5, -1.1	1.0, -1.1, 1.1	
CR943926.1.a.om.1	CR943926	-1.2, -2.1, -1.1	1.2, 1.0, 1.1	
CR943810.1.a.om.1	CR943810	-1.4, -2.0, -1.6	-1.4, 1.2, 1.0	
CR943258.1.a.om.1	CR943258	-1.3, -2.0, 1.1	1.1, 1.0, 1.1	
CR942918.1.a.om.1	CR942918	-1.3, -2.0, -1.5	1.5, 1.5, 1.1	
CA385505.1.a.om.1	CA385505	-2.9, -3.8, -2.9	1.4, 1.5, 1.6	
CA384955.1.a.om.1	CA384955	-1.8, -2.6, 1.0	1.2, 1.2, 1.5	
CA366603.1.a.om.1	CA366603	-1.6, -2.3, -1.7	1.3, 1.0, 1.3	
CA364858.1.a.om.1	CA364858	-1.6, -2.1, -1.3	1.1, 1.1, 1.2	
CA359373.1.a.om.1	CA359373	-1.4, -2.0, -1.6	1.1, 1.1, 1.1	
CA351730.1.a.om.1	CA351730	-1.2, -2.1, -1.2	1.0, 1.0, 1.2	
CA349873.1.a.om.1	CA349873	-1.4, -2.2, -2.1	1.3, 1.1, 1.1	
CA343052.1.a.om.1	CA343052	-1.4, -2.9, -1.3	-1.2, -1.3, -1.1	
BX889673.1.a.om.1	BX889673	-2.6, -2.9, -1.1	1.1, 1.6, 1.5	
BX881151.1.a.om.1	BX881151	-1.6, -2.1, -1.3	1.0, 1.1, 1.2	
BX320570.1.a.om.1	BX320570	-1.5, -2.3, 1.0	1.2, 1.0, 1.2	
BX086467.1.a.om.1	BX086467	-1.4, -2.1, -1.6	1.3, -1.1, 1.5	
BX075906.1.a.om.1	BX075906	1.0, -2.0, -1.2	1.4, 1.1, 1.3	
CA342273.1.a.om.1	CA342273	-1.2, -2.0, -1.2	-1.1, 1.0, 1.0	
X_CT568325.1.a.om.1	CT568325	-1.2, -2.2, -1.2	-1.3, 1.1, -1.1	
TCBN_1B172K9.CON	TCBN_1B172K9.CON	-1.2, -2.1, -1.3	1.0, -1.5, 1.1	
rtay12a01f03r1.1.a.om.1	rtay12a01f03r1	-1.9, -1.6, 1.1	1.1, -1.1, 2.4	
tcba0001c.m.02_5.1.s.om.8	tcba0001c.m.02_5.1.s.om.8	-1.1, -2.0, -1.3	1.0, 1.1, 1.0	
rtrs05b02c12f1_t7.1.a.om.1	rtrs05b02c12f1_t7	-1.3, -2.2, -1.7	1.1, -1.1, -1.2	
CA347413.1.a.om.1	CA347413	1.6, -2.1, -1.1	1.2, 1.1, 1.0	
CA361557.1.a.om.1	Claudin-4	-1.4, -2.5, -1.5	1.1, 1.0, 1.5	adhesion
CX258654.1.a.om.1	Alpha-tectorin	-1.2, -2.2, -1.3	1.1, -1.2, 1.1	adhesion
BX075633.1.a.om.1	Dromaiocalcin-1	-1.6, -2.8, 1.1	-1.1, 1.0, 1.4	antifreeze lectin
CA342924.1.a.om.1	p53 apoptosis effector related to PMP-22	-2.0, -2.9, -2.1	1.5, 1.3, 1.7	apoptosis
BX310686.1.a.om.1	Serine-protein kinase ATM	1.2, -2.9, 1.3	1.1, 1.2, 1.6	apoptosis
X_BX072690.1.a.om.1	Beta actin-like	-1.6, -3.3, -1.3	1.0, 1.4, 1.3	cytoskeletal organisation
CB494094.1.a.om.1	Kinetochore protein Hec1 homolog	-1.4, -2.6, -1.4	1.1, 1.3, 1.1	cytoskeletal organisation
CA357969.1.a.om.1	Actin-related protein 2/3 complex subunit 4	-1.4, -2.4, 1.0	1.0, 1.0, -1.1	cytoskeletal organisation

CA351928.1.a.om.1	Actin-related protein 6	-1.4, -2.1, -1.5	1.0, 1.0, -1.1	cytoskeletal organisation
CA368043.1.a.om.1	cAMP-regulated phosphoprotein 19	-1.7, -2.9, -1.8	1.0, -1.1, -1.1	gluconeogenesis
CA342950.1.a.om.1	Alcohol dehydrogenase [NADP+]	-1.5, -3.0, -1.9	-1.2, -1.2, -1.1	glucuronate metabolism, oxidation reduction
AF438178.1.a.om.1	Growth hormone receptor	-2.8, -1.5, -1.8	1.5, 1.1, 1.0	hormone signalling
CA351682.1.a.om.1	Ig kappa chain V-III region MOPC 63	-1.8, -2.7, 1.3	-1.1, 1.4, 2.0	immune response
BX298925.1.a.om.1	Beta-catenin-like protein 1	-1.9, -2.5, -2.3	1.8, 1.4, 1.3	immune response, apoptosis
CA343019.1.a.om.1	Fatty acid-binding protein	-1.1, -2.3, -1.2	1.2, 1.0, 1.0	lipid metabolism
CA359082.1.a.om.1	Glucosidase 2 subunit beta	-1.5, -2.1, -1.3	1.5, 1.3, 1.6	N-linked glycan processing
BX305470.1.a.om.1	Cytochrome P450 11A1	-2.2, -1.7, -2.4	1.5, 1.0, 1.5	oxidoreductase activity, xenobiotic metabolism
CA343197.1.a.om.1	Conserved oligomeric Golgi complex cpt. 5	-1.2, -2.6, -1.1	-1.1, 1.1, 1.3	protein localisation
CB488633.1.a.om.1	Nucleotide exchange factor SIL1 precursor	-1.1, -1.4, -1.1	-2.7, 2.4, 1.3	protein localisation
CA386664.1.a.om.1	Ubiquitin-conjugating enzyme E2 variant 1	-1.6, -2.9, -1.5	1.1, 1.3, 1.4	proteolysis
CA363489.1.a.om.1	Carboxypeptidase A1	-1.2, -2.9, -1.2	-1.4, 1.0, 1.8	proteolysis
CB493045.1.a.om.1	NEDD8-activating enzyme E1 catalytic subunit	-1.6, -2.0, -1.4	-1.6, -1.1, -1.2	proteolysis
CB491979.1.a.om.1	Ubl carboxyl-terminal hydrolase 18	-1.3, -2.2, -1.3	-1.1, -1.4, -1.1	proteolysis
CA345836.1.a.om.1	Cystatin	-1.5, -2.2, -1.6	1.2, 1.1, 1.4	proteolysis, inhibitor
BX860632.1.a.om.1	Peroxisomal membrane protein 11C	-1.2, -2.0, 1.1	-1.3, -1.1, 1.2	redox homeostasis, lipid homeostasis
tcav0003c.f.14_3.1.s.om.8	Zona pellucida sperm-binding protein 3 precursor	-1.5, -2.1, -1.2	-1.2, 1.0, 1.0	reproduction
BX866783.1.a.om.1	Hypothetical RNA-binding protein C23E6.01c	-1.1, -2.6, -1.1	1.1, -1.2, -1.2	RNA-binding
CA381852.1.a.om.1	Nucleolar protein of 40 kDa	-1.1, -2.0, -1.3	1.2, 1.5, 1.0	RNA-binding
X_BX859379.1.a.om.1	Adenylyl cyclase type 6	-1.2, -2.1, -1.2	1.3, 1.3, 1.2	signal transduction
CA377055.1.a.om.1	SLIT-ROBO Rho GTPase-activating protein 3	-2.1, -1.9, -1.7	1.3, 2.5, 2.3	signal transduction
CA348507.1.a.om.1	Annexin A4	-1.1, -2.3, -1.3	1.4, -1.1, 1.2	signal transduction
CA378890.1.a.om.1	Solute carrier family 2	-2.6, -2.0, -1.5	1.5, -1.1, -1.4	sugar transport, glucose maintenance
BX876961.1.a.om.1	Solute carrier family 2	-1.3, -2.0, -1.8	1.2, -1.1, -1.1	sugar transport, glucose maintenance
rtay12a01d09r1.1.a.om.1	NFX1-type zinc finger-containing protein 1	-1.5, -2.5, -1.4	1.2, 1.1, 1.1	transcription
CA375698.1.a.om.1	Transcription intermediary factor 1-alpha	-1.2, -2.6, -1.4	1.0, 1.0, 1.3	transcription
CA353784.1.a.om.1	Tob1 protein (Transducer of erbB-2 1)	-1.6, -2.4, -1.2	1.2, 1.1, -1.1	transcription
CA353468.1.a.om.1	Splicing factor	-2.8, -2.6, -1.8	1.1, 1.1, 1.0	transcription
BX076522.1.a.om.1	Forkhead box protein D3	-1.4, -2.5, -1.8	1.3, 1.5, 1.4	transcription
tcba0017c.d.18_5.1.s.om.8	Nuclear transcription factor Y subunit beta	-1.3, -2.3, -1.1	1.0, -1.4, -1.1	transcription
BX077950.1.a.om.1	Zinc finger protein Xfin	-1.1, -2.0, -1.4	1.1, -1.2, 1.0	transcription

CR943405.1.a.om.1	Retinoblastoma-like protein 2	-2.0, -2.1, -1.6	2.2, 1.8, 2.0	transcription, cell cycle
CA384952.1.a.om.1	Splicing factor	-1.5, -2.2, -2.0	1.3, -1.2, 1.1	transcription, DNA repair
rtay11a03c02f1.1.a.om.1	RNA polymerase II elongation factor ELL	-1.2, -2.2, -1.1	1.2, -1.1, -1.1	transcription/translation
tcbi0035c.n.23_5.1.s.om.8	39S ribosomal protein L32	-1.3, -2.1, -1.7	1.3, 1.2, 1.2	translation
tcbi0028c.b.02_5.1.s.om.8	60S ribosomal protein L14	-1.3, -3.0, -1.2	1.1, 1.2, 1.1	translation
CA363996.1.a.om.1	Eukaryotic translation initiation factor 4B	-1.5, -1.9, -2.3	-1.3, -1.1, 1.2	translation
CA343180.1.a.om.1	60S ribosomal protein L32	-1.8, -2.4, -1.1	-1.3, 1.2, 1.8	translation
tcba0022c.f.11_5.1.s.om.8	Hypothetical protein ycbX	-1.6, -2.9, -1.5	1.1, 1.0, 1.2	unknown
CA376533.1.a.om.1	EGF-containing fibulin-like extracell. matrix protein 2	-1.1, -2.0, #N/A	1.2, -1.4, -1.1	wound healing
SET 5: Down-regulation (or lower expression) in HR fish				
CT572198.1.a.om.1	CT572198	1.0, -1.1, -1.1	-1.3, -2.0, 1.5	
BX309429.1.a.om.1	BX309429	1.0, -1.3, -1.1	-1.2, -2.1, 1.1	
BX075599.1.a.om.1_2	BX075599	1.2, 1.0, 1.2	-2.1, -1.4, -1.1	
X_rtrs05b02j09f1_t7.1.a.om.1	rtrs05b02j09f1_t7	1.4, 1.1, 1.2	-2.2, -1.7, 1.0	
X_CT566301.1.a.om.1	CT566301	-1.1, 2.6, -1.2	-1.6, 1.0, -1.2	
CA377868.1.a.om.1	CA377868	1.2, -1.2, 1.6	-1.7, -2.0, #N/A	
CA348877.1.a.om.1	CA348877	-1.1, 1.0, 1.1	-3.1, -1.2, -1.6	
CR376481.1.a.om.1	CR376481	-1.1, -1.1, 1.0	-1.6, -2.2, -1.4	
CT568598.1.a.om.1	CT568598	-1.3, -1.5, -1.3	-1.4, -2.1, -1.3	
X_CA341853.1.a.om.1	CA341853	-1.6, -1.9, 2.4	-1.5, -1.8, -2.8	
rtay13a04e03f1.1.a.om.1	rtay13a04e03f1	1.1, -2.0, -1.1	-2.0, -1.3, -1.6	
CA351549.1.a.om.1	CA351549	-1.1, -1.2, -1.5	-2.4, -1.6, -1.4	
CX256225.1.a.om.1	CX256225	1.0, 1.1, -1.2	-1.6, -2.1, -1.7	
rtay11a06b10f1.1.a.om.1	Tubulin alpha chain	-1.3, -1.2, 1.1	-2.0, -1.6, 1.0	cytoskeletal organisation
BU993928.1.a.om.1	Tropomyosin 1 alpha chain	-1.1, -1.3, 2.2	-1.3, -1.3, -2.3	cytoskeletal organisation
CA344467.1.a.om.1	Trichohyalin	-2.0, -1.6, -1.1	-2.1, -3.6, -1.5	cytoskeletal organisation
X_CU072064.1.a.om.1	Glucokinase	2.5, 1.0, -1.1	-1.4, 1.7, -1.8	gluconeogenesis, glucose maintenance
BX074651.1.a.om.1	Somatotropin 2	2.3, 1.1, 1.1	-1.4, -1.2, 1.6	hormone signalling
CA347369.1.a.om.1	Intelectin-2	6.5, 1.5, 1.8	-1.5, -1.5, 1.1	immune response
CA376934.1.a.om.1	Sodium/calcium exchanger 3	1.0, -1.1, 1.1	-1.6, -2.0, -1.2	ion homeostasis
CT566515.1.a.om.1	Protein kinase C and casein kinase II substrate protein 3	-1.2, -1.5, -1.3	-1.4, -2.0, -1.3	membrane organisation
CA342676.1.a.om.1	Thioredoxin reductase 1	1.2, 2.1, 1.2	-1.3, 1.1, 1.0	oxidation reduction
X_CA344596.1.a.om.1	NTF2-related export protein 2	1.0, -1.7, 1.0	1.0, -2.3, -1.1	protein localisation
CB490062.1.a.om.1	Protein TMED8	-1.4, -1.6, -1.3	-1.6, -2.0, -1.1	protein localisation, vesicule targetting
U65893.1.a.om.1	Carbamoyl-phosphate synthase [ammonia], mitochondrial (CPS1)	3.1, 1.1, 1.6	1.1, 1.1, -1.4	response to stimulus, response to glucocorticoid
CA348176.1.a.om.1	14-3-3 protein beta/alpha-1	1.5, -1.1, 1.8	-2.3, -1.6, -1.8	signal transduction
CA380214.1.a.om.1	Transcription factor HES-1	1.1, -1.1, 1.1	-1.2, -2.0, -1.2	transcription
X_CA345412.1.a.om.1	Methylglutaconyl-CoA hydratase	1.0, -1.4, 1.1	-2.0, -1.3, -1.6	transcription/translation

X_CA357991.1.a.om.1	Eukaryotic translation initiation factor 1b	-1.4, -1.8, 1.0	-2.1, -1.4, -1.7	translation
SET 6: Strong up-regulation in both fish lines				
CR944619.1.a.om.1	CR944619	1.1, 1.7, 14.8	1.1, 3.2, 12.6	
CA359724.1.a.om.1	CA359724	-1.1, 2.0, 2.3	2.3, 3.6, 9.2	
BX311643.1.a.om.1	BX311643	1.2, 1.8, 28.0	1.0, 3.1, 22.6	
CR944338.1.a.om.1	CR944338	1.1, 5.1, 1.3	3.0, 4.1, 4.4	
BX886962.1.a.om.1	BX886962	-1.1, 1.0, 2.8	-1.3, 1.9, 3.9	
X_CA358932.1.a.om.1	Thymosin beta-11	1.1, 2.2, 2.3	1.3, 1.9, 6.0	cytoskeletal organisation
CA358247.1.a.om.1	Granulins	1.0, -1.1, 2.7	1.1, 1.8, 3.6	growth factor
X_CA343987.1.a.om.1	Interferon-induced transmembrane protein 3	1.0, -1.3, 1.5	1.7, 2.7, 6.7	immune response
CA354409.1.a.om.1_2	Leukocyte cell-derived chemotaxin 2	-1.1, 1.5, 11.3	-3.1, 2.3, 3.0	immune response
CA354409.1.a.om.1_1	Leukocyte cell-derived chemotaxin 2	-1.1, 1.3, 18.7	-1.5, 8.6, 14.6	immune response
CA355465.1.a.om.1	Purine nucleoside phosphorylase	1.2, 3.0, 1.1	4.5, 8.1, 3.8	nucleotide metabolism
CA348401.1.a.om.1	Stress-70 protein (GRP75)	-1.1, 1.7, 2.2	1.6, 2.0, 3.4	protein localisation, apoptosis
tcad0003a.f.24_5.1.s.om.8	Protein disulfide-isomerase A4	1.3, -1.5, 2.2	1.0, 1.8, 5.6	protein localisation, cell redox homeostasis, oxidation/reduction
X_CA368961.1.a.om.1	78 kDa glucose-regulated protein precursor (GRP 78)	1.0, 2.0, 3.5	1.6, 3.7, 20.2	proteolysis, apoptosis, ER stress response
SET 7: Strong up-regulation at 168 h in LR line (LS44 effect)				
CT572491.1.a.om.1	CT572491	-1.1, 1.0, 2.3	1.0, 1.0, -1.2	
BX087755.1.a.om.1	BX087755	1.1, 1.1, 2.5	-1.2, 1.1, 1.2	
rtrs05b02c16f1_t7.1.a.om.1	rtrs05b02c16f1_t7	1.1, -1.5, 2.1	1.2, -1.2, -1.1	
CA350055.1.a.om.1	CA350055	-1.1, -1.2, 3.8	-1.5, -1.4, -1.3	
CA343218.1.a.om.1_2	CA343218	-1.2, -1.7, 2.1	1.1, -1.3, 1.0	
X_CT572430.1.a.om.1	CT572430	-1.2, -1.1, 2.0	-1.1, 1.0, 1.1	
X_CA344317.1.a.om.1	CA344317	-1.3, -1.6, 18.3	-1.1, -1.2, 1.9	
rtay12a06c04f1.1.a.om.1	rtay12a06c04f1	1.4, 1.6, 2.8	1.1, 1.0, 1.1	
rtay11a04g10f1.1.a.om.1	rtay11a04g10f1	1.0, -1.1, 2.0	1.1, 1.0, 1.5	
CT572048.1.a.om.1	CT572048	-1.2, 1.2, 2.2	1.1, -1.4, 1.3	
CA384581.1.a.om.1	CA384581	-1.3, -1.4, 2.9	1.2, 1.1, 1.2	
CA348751.1.a.om.1	CA348751	-1.2, 1.1, 2.7	-1.2, 1.0, 2.1	
BX888472.1.a.om.1	BX888472	-1.9, -1.8, 2.2	-1.1, -1.4, 3.4	
CA349582.1.a.om.1	Fish-egg lectin	1.1, -1.2, 3.9	-1.1, -1.3, 1.1	adhesion
CA352504.1.a.om.1	P-selectin glycoprotein ligand 1	1.2, -1.1, 3.0	-1.7, -1.6, -1.8	adhesion
CA358169.1.a.om.1	2-oxoisovalerate dehydrogenase subunit beta	-1.4, 2.1, 2.8	-3.6, 2.2, -2.5	amino acid metabolism, response to glucocorticoid
CA344058.1.a.om.1	Zinc-binding protein A33	1.0, 1.0, 2.6	-1.2, 1.5, 1.4	chromatin organisation
CX244910.1.a.om.1	Myosin-2 heavy chain	1.0, 1.0, 2.0	-1.5, 1.0, -1.1	cytoskeletal organisation
CA359238.1.a.om.1	F-actin capping protein subunit beta	1.0, -1.1, 2.0	-1.5, -1.4, -1.1	cytoskeletal organisation
CA345645.1.a.om.1	Thymosin beta-11	-1.1, -1.5, 3.0	1.0, -1.1, 1.1	cytoskeletal organisation

CA343335.1.a.om.1	Actin-related protein 2/3 complex subunit 5	-1.3, -2.3, 1.8	-1.1, -1.1, -1.1	cytoskeletal organisation
X_CA344875.1.a.om.1	Tubulin alpha chain	1.2, -1.2, 2.2	1.2, 1.0, 1.6	cytoskeletal organisation
CA365789.1.a.om.1	Cofilin-2	1.0, -1.1, 5.2	-1.1, 1.3, 2.8	cytoskeletal organisation
X_CA371947.1.a.om.1	Glyceraldehyde-3-phosphate dehydrogenase	-1.1, -1.5, 5.8	1.3, 1.4, 1.9	glucose metabolism, oxidation reduction, glycolysis
rtay12a05g10f1.1.a.om.1	Transforming growth factor beta-1	1.0, 1.5, 2.0	-1.3, -1.2, -1.2	growth factor activity
CA357517.1.a.om.1	Uncharacterized protein C6orf58 homolog	1.4, -1.2, 2.1	1.2, -1.1, 2.2	hypothetical protein
CA343672.1.a.om.1	Leukocyte common antigen precursor (CD45 antigen)	-1.4, -1.7, 2.6	1.0, 1.0, -1.2	immune response
CA344819.1.a.om.1	High affinity immunoglobulin gamma Fc receptor I	-1.2, -1.4, 3.0	-1.2, 1.4, 1.5	immune response
CA343278.1.a.om.1	Tyrosine-protein kinase HCK	-1.1, 1.0, 2.5	1.0, 1.1, 1.0	immune response
X_CA375508.1.a.om.1	Serum amyloid A-1 protein	1.0, 1.0, 2.0	1.0, 1.2, 1.5	immune response
CA350548.1.a.om.1_2	Complement factor H precursor	1.3, 1.1, 3.0	1.0, 1.7, 1.3	immune response
CA348097.1.a.om.1	Neutrophil cytosol factor 1	-1.5, -1.9, 2.7	1.1, -1.3, 1.0	immune response, oxidation reduction
CA342316.1.a.om.1	Cytochrome b-245 light chain	-1.2, -1.4, 4.7	-1.4, -1.7, 1.1	immune response, oxidation reduction
BX873960.1.a.om.1	C-type lectin domain family 4 member M	-1.2, -1.9, 2.2	1.1, -1.3, 1.1	immune system, pathogen recognition
CA345431.1.a.om.1	Rhesus blood group-associated glycoprotein (CD241 antigen)	1.2, -1.2, 3.1	1.0, -1.1, 1.2	ion homeostasis
X_CB492492.1.a.om.1	Apolipoprotein A-IV	-1.1, -1.7, 1.5	1.0, -1.2, 2.0	lipid metabolism, oxidation reduction, cholesterol metabolism
CA354597.1.a.om.1	SH3 domain-binding glutamic acid-rich-like protein 3	-1.1, -1.3, 2.4	1.1, 1.0, 1.6	oxidation reduction
CT571596.1.a.om.1	Acyl-CoA desaturase	2.1, -1.5, 1.6	1.0, 1.6, -1.2	oxidoreductase activity, fatty acid metabloism
CA347328.1.a.om.1	Talin-2	-1.1, 1.2, 2.4	1.0, 1.2, -1.2	protein localisation
CA345241.1.a.om.1	Secretory granule proteoglycan core protein	-2.0, -1.5, 2.5	1.0, 1.1, 1.3	protein localisation
CA346484.1.a.om.1	Collagenase 3 (MMP-13)	-1.8, -1.9, 3.9	1.0, 1.1, -1.1	proteolysis
X_CA342769.1.a.om.1	Matrix metalloproteinase-9	-1.1, -1.5, 5.3	1.1, -1.2, -1.1	proteolysis, ECM organisation
CA351742.1.a.om.1	Lysozyme C II	1.0, 1.1, 4.1	-1.2, 1.3, 1.1	proteolysis, immune response
CA346948.1.a.om.1	Leukotriene A-4 hydrolase	-1.1, -2.0, 2.3	1.1, 1.5, -1.4	proteolysis, inflammatory response
CA363566.1.a.om.1	Antho-RFamide neuropeptides type 1	1.0, -1.1, 2.1	1.0, -1.2, 1.3	signal transduction
CA360106.1.a.om.1	Heterogeneous nuclear ribonucleoprotein A0	1.1, 1.0, 3.6	1.5, 1.2, 2.6	transcription
CA353080.1.a.om.1	High mobility group protein B2	1.0, -1.2, 4.4	-1.2, -1.3, -1.1	transcription, DNA repair
CA355051.1.a.om.1	Tetratricopeptide repeat protein KIAA0103	-1.1, -1.1, 3.0	1.1, 1.3, 1.2	unknown

Table S2: K-means clusters of the line-related gene list

ID	Gene Description	LR 6h 24h, 168h	HR 6h 24h, 168h	Functions
SET 1: Early and sustained down-regulation in HR fish				
CA344467.1.a.om.1	Trichohyalin	-2.0, -1.6, -1.1	-2.1, -3.6, -1.5	cytoskeletal organisation
CA343347.1.a.om.1	5-aminolevulinate synthase	-1.1, 1.0, -1.4	-2.1, -1.5, -2.1	haem biosynthesis
CA342509.1.a.om.1	Plasma retinol-binding protein I	1.2, -1.4, -1.2	1.0, -1.7, -3.0	VitA transport, fat metabolism
CA348176.1.a.om.1	14-3-3 protein beta/alpha-1	1.5, -1.1, 1.8	-2.3, -1.6, -1.8	signal transduction
CA368454.1.a.om.1	Carbonic anhydrase 5B	-1.2, 2.2, -1.3	-1.1, -1.1, -1.4	gluconeogenesis, lipogenesis
CA351549.1.a.om.1	CA351549	-1.1, -1.2, -1.5	-2.4, -1.6, -1.4	N/A
CA348877.1.a.om.1	CA348877	-1.1, 1.0, 1.1	-3.1, -1.2, -1.6	N/A
BX862864.1.a.om.1	BX862864	1.3, 1.1, -1.2	-1.7, -1.4, -2.4	N/A
CA365301.1.a.om.1	Dipeptidyl-peptidase 3	-1.2, -1.5, -1.2	-3.6, -2.8, -2.4	proteolysis
CA342499.1.a.om.1	Uncharacterized protein C6orf58 homolog	1.0, -1.1, -2.1	-1.1, -1.2, -6.3	hypothetical protein
BX075599.1.a.om.1_2	BX075599	1.2, 1.0, 1.2	-2.1, -1.4, -1.1	N/A
CA387966.1.a.om.1	Liver-expressed antimicrobial peptide 2	1.9, 1.0, -1.2	-3.1, -2.3, -5.3	immune response
BX074651.1.a.om.1	Somatotropin 2	2.3, 1.1, 1.1	-1.4, -1.2, 1.6	hormone signalling
CA347369.1.a.om.1	Intelectin-2	6.5, 1.5, 1.8	-1.5, -1.5, 1.1	immune response
rtay12a04d11f1.1.a.om.1	rtay12a04d11f1	1.0, -1.3, -1.3	-1.6, -2.7, -3.0	N/A
CA352504.1.a.om.1	P-selectin glycoprotein ligand 1	1.2, -1.1, 3.0	-1.7, -1.6, -1.8	adhesion
BU993928.1.a.om.1	Tropomyosin 1 alpha chain	-1.1, -1.3, 2.2	-1.3, -1.3, -2.3	cytoskeletal organisation
X_rtrs05b02j09f1_t7.1.a.om.1	rtrs05b02j09f1_t7	1.4, 1.1, 1.2	-2.2, -1.7, 1.0	N/A
CA358169.1.a.om.1	2-oxoisovalerate dehydrogenase subunit beta	-1.4, 2.1, 2.8	-3.6, 2.2, -2.5	amino acid metabolism, response to glucocorticoid
X_CA341853.1.a.om.1	CA341853	-1.6, -1.9, 2.4	-1.5, -1.8, -2.8	N/A
U65893.1.a.om.1	Carbamoyl-phosphate synthase [ammonia], mitochondrial (CPS1)	3.1, 1.1, 1.6	1.1, 1.1, -1.4	response to stimulus, response to glucocorticoid
CA359238.1.a.om.1	F-actin capping protein subunit beta	1.0, -1.1, 2.0	-1.5, -1.4, -1.1	cytoskeletal organisation
X_CU072064.1.a.om.1	Glucokinase	2.5, 1.0, -1.1	-1.4, 1.7, -1.8	gluconeogenesis, glucose maintenance
CX262061.1.a.om.1	Enhancer of split groucho-like protein 2	-1.2, -1.2, -1.3	-2.0, -2.1, -3.6	transcription

CT568598.1.a.om.1	CT568598	-1.3, -1.5, -1.3	-1.4, -2.1, -1.3	N/A
CT567216.1.a.om.1	Protein FAM57B	-1.2, 3.2, -1.5	1.1, 1.0, -1.7	unknown
rtay12a02h11f1.1.a.om.1	Importin beta-1 subunit	1.6, 1.0, -2.1	-2.6, -1.9, -4.2	protein localisation
X_CT572262.1.a.om.1	ATP synthase a chain	1.3, 1.0, -1.2	-1.2, -3.1, -2.2	ATP maintenance, oxidation reduction
X_CT566301.1.a.om.1	CT566301	-1.1, 2.6, -1.2	-1.6, 1.0, -1.2	N/A
CA350479.1.a.om.1	CA350479	-1.3, -1.5, -1.2	-1.1, -1.3, -3.4	N/A
SET 2: Early and sustained up-regulation in HR fish				
BX074470.1.a.om.1_1	BX074470	-1.1, -1.6, -1.3	1.6, 1.5, 2.9	N/A
BX863020.1.a.om.1	Circumsporozoite protein	1.0, 1.6, 1.0	2.2, 3.1, 3.6	unknown
CR944563.1.a.om.1	CR944563	1.4, -1.2, 1.3	1.4, 2.2, 1.7	N/A
CX030984.1.a.om.1	CX030984	1.0, -1.3, -1.2	1.4, 1.3, 2.1	N/A
rtay12a06e10r1.1.a.om.1	rtay12a06e10r1	-1.3, -1.3, 1.1	2.4, 1.7, 2.0	N/A
CA342458.1.a.om.1	CCAAT/enhancer-binding protein delta	1.0, 1.0, -1.7	2.8, 3.7, 1.2	transcription
CA356842.1.a.om.1	CA356842	1.0, -1.4, 1.3	1.3, 1.6, 2.1	N/A
CA372046.1.a.om.1	S-adenosylmethionine synthetase type-2	1.2, 1.7, 1.2	2.3, 2.5, 2.9	amino acid metabolism
CU070025.1.a.om.1	CU070025	-9.1, 2.5, -4.0	-4.8, 2.8, 3.1	N/A
BX082276.1.a.om.1	BX082276	-1.1, -1.4, -1.2	1.2, 1.6, 2.1	N/A
BX299056.1.a.om.1	Retinitis pigmentosa 9 protein homolog	-1.1, 1.4, 1.1	1.0, 1.4, 2.3	RNA splicing
BX866751.1.a.om.1	Cytochrome P450 24A1	-1.2, 2.3, -1.8	1.7, 3.9, 1.4	steroid metabolism, oxidation reduction, vitamin metabloism
CA344960.1.a.om.1	CA344960	-1.1, -1.2, -1.4	1.2, 2.0, 2.4	N/A
CA347059.1.a.om.1	Ferritin	1.9, 1.7, -1.2	-1.1, 1.2, 2.1	iron homeostasis
CA352463.1.a.om.1	2-hydroxyphytanoyl-CoA lyase	1.1, -1.2, -1.2	1.4, 1.6, 2.1	lipid metabolism
CA355465.1.a.om.1	Purine nucleoside phosphorylase	1.2, 3.0, 1.1	4.5, 8.1, 3.8	nucleotide metabolism
CA370303.1.a.om.1	Tetraspanin-3	1.0, -1.1, 1.2	1.2, 2.2, 2.0	signal transduction
CA377220.1.a.om.1	CA377220	1.1, 1.0, -1.8	2.2, 2.3, -1.2	N/A
CA382759.1.a.om.1	COP9 signalosome complex subunit 8	1.1, 1.9, 1.0	2.6, 2.1, 2.2	developmental
CR944338.1.a.om.1	CR944338	1.1, 5.1, 1.3	3.0, 4.1, 4.4	N/A
CX720452.1.a.om.1	CX720452	-1.4, -1.4, -1.3	1.6, 1.4, 2.1	N/A

rtay11a02e05r1.1.a.om.1	rtay11a02e05r1	1.0, -1.4, 1.1	2.2, 1.5, 1.4	N/A
tcbk0024c.i.05_5.1.s.om.8	Carnitine O-palmitoyltransferase I	-1.5, -1.2, -1.2	2.0, 1.8, 2.0	fatty acid metabolism, glucose maintenance
tcbk0031c.o.22_5.1.s.om.8	Cationic amino acid transporter 3	-1.2, -1.1, 1.0	2.2, 1.4, 1.9	amino acid metabolism
BX308005.1.a.om.1	Patatin-like phospholipase domain-containing protein 4	1.1, -1.2, -1.1	2.9, 2.1, -1.4	lipid metabolism
CA343987.1.a.om.1	Interferon-induced transmembrane protein 3	1.0, -1.3, 1.5	1.7, 2.7, 6.7	immune response
CA347249.1.a.om.1	Dihydrolipoyllysine-residue succinyl-transferase	1.5, 1.1, 1.2	3.3, 1.6, 1.0	ATP maintenance
CA351531.1.a.om.1	Peroxiredoxin-5	-1.3, -1.1, 1.5	1.0, 2.0, 2.1	oxidation reduction
CA357750.1.a.om.1	CA357750	-1.2, 1.0, 1.2	1.1, 1.4, 2.5	N/A
CA359724.1.a.om.1	CA359724	-1.1, 2.0, 2.3	2.3, 3.6, 9.2	N/A
CA372503.1.a.om.1	CA372503	1.0, 1.1, -1.1	1.1, -1.1, 3.2	N/A
CA382773.1.a.om.1	CA382773	-1.2, 1.1, 1.0	1.3, 1.3, 3.1	N/A
tcad0004a.l.24_3.1.s.om.8	tcad0004a.l.24_3.1.s.om.8	-1.2, -1.2, 1.6	1.6, 1.3, 3.2	N/A
CA350158.1.a.om.1	CA350158	-1.4, -2.0, -1.6	2.3, 1.9, 2.7	N/A
CA351045.1.a.om.1	Proto-oncogene tyrosine-protein kinase LCK	-1.2, 1.0, 1.7	-1.1, 1.2, 3.9	immune response
CA358932.1.a.om.1	Thymosin beta-11	1.1, 2.2, 2.3	1.3, 1.9, 6.0	cytoskeletal organisation
CA368961.1.a.om.1	78 kDa glucose-regulated protein precursor (GRP 78)	1.0, 2.0, 3.5	1.6, 3.7, 20.2	proteolysis, apoptosis, ER stress response
CA348766.1.a.om.1	Protein disulfide-isomerase	1.3, 1.1, 1.7	1.0, -1.1, 5.3	cell redox homeostasis
SET 3: Early and sustained down-regulation in LR fish				
BX298925.1.a.om.1	Beta-catenin-like protein 1	-1.9, -2.5, -2.3	1.8, 1.4, 1.3	immune response, apoptosis
BX305470.1.a.om.1	Cytochrome P450 11A1	-2.2, -1.7, -2.4	1.5, 1.0, 1.5	oxidoreductase activity, xenobiotic metabolism
CA342924.1.a.om.1	p53 apoptosis effector related to PMP-22	-2.0, -2.9, -2.1	1.5, 1.3, 1.7	apoptosis
CA348507.1.a.om.1	Annexin A4	-1.1, -2.3, -1.3	1.4, -1.1, 1.2	signal transduction
CA351682.1.a.om.1	Ig kappa chain V-III region MOPC 63	-1.8, -2.7, 1.3	-1.1, 1.4, 2.0	immune response
CA353784.1.a.om.1	Tob1 protein (Transducer of erbB-2 1)	-1.6, -2.4, -1.2	1.2, 1.1, -1.1	transcription
CA354386.1.a.om.1	CA354386	-1.6, -3.1, -1.4	1.0, 1.1, 1.0	N/A
CA357969.1.a.om.1	Actin-related protein 2/3 complex subunit 4	-1.4, -2.4, 1.0	1.0, 1.0, -1.1	cytoskeletal organisation
CA359082.1.a.om.1	Glucosidase 2 subunit beta	-1.5, -2.1, -1.3	1.5, 1.3, 1.6	N-linked glycan processing

CA359373.1.a.om.1	CA359373	-1.4, -2.0, -1.6	1.1, 1.1, 1.1	N/A
CA384952.1.a.om.1	Splicing factor	-1.5, -2.2, -2.0	1.3, -1.2, 1.1	transcription, DNA repair
CR943405.1.a.om.1	Retinoblastoma-like protein 2	-2.0, -2.1, -1.6	2.2, 1.8, 2.0	transcription, cell cycle
CR944058.1.a.om.1	CR944058	-1.7, -2.0, -1.9	-1.1, 1.1, 2.5	N/A
CT572011.1.a.om.1	CT572011	-1.2, -2.2, -1.3	2.0, 2.1, 2.2	N/A
CT572122.1.a.om.1	CT572122	-1.6, -2.7, -1.9	-1.2, -1.1, -1.3	N/A
rtay12a01e12r1.1.a.om.1	rtay12a01e12r1	-1.7, -2.6, -2.0	1.2, 1.2, 1.5	N/A
tcav0003c.f.14_3.1.s.om.8	Zona pellucida sperm-binding protein 3 precursor	-1.5, -2.1, -1.2	-1.2, 1.0, 1.0	reproduction
tcbi0028c.b.02_5.1.s.om.8	60S ribosomal protein L14	-1.3, -3.0, -1.2	1.1, 1.2, 1.1	translation
CA344701.1.a.om.1	Cytochrome P450 2J2	-1.4, -1.4, -2.4	1.5, 1.2, -1.1	oxidation reduction, fatty acid metabolism
AF438178.1.a.om.1	Growth hormone receptor binding protein) (GHBP) (Serum-binding protein)]	-2.8, -1.5, -1.8	1.5, 1.1, 1.0	hormone signalling
BX075906.1.a.om.1	BX075906	1.0, -2.0, -1.2	1.4, 1.1, 1.3	N/A
BX076522.1.a.om.1	Forkhead box protein D3	-1.4, -2.5, -1.8	1.3, 1.5, 1.4	transcription
BX310423.1.a.om.1	BX310423	-3.3, -4.8, -1.6	1.7, 1.9, 8.4	N/A
BX881151.1.a.om.1	BX881151	-1.6, -2.1, -1.3	1.0, 1.1, 1.2	N/A
CA342950.1.a.om.1	Alcohol dehydrogenase [NADP+]	-1.5, -3.0, -1.9	-1.2, -1.2, -1.1	glucuronate metabolism, oxidation reduction
CA343180.1.a.om.1	60S ribosomal protein L32	-1.8, -2.4, -1.1	-1.3, 1.2, 1.8	translation
CA343197.1.a.om.1	Conserved oligomeric Golgi complex component 5	-1.2, -2.6, -1.1	-1.1, 1.1, 1.3	protein localisation
CA349873.1.a.om.1	CA349873	-1.4, -2.2, -2.1	1.3, 1.1, 1.1	N/A
CA351730.1.a.om.1	CA351730	-1.2, -2.1, -1.2	1.0, 1.0, 1.2	N/A
CA353468.1.a.om.1	Splicing factor	-2.8, -2.6, -1.8	1.1, 1.1, 1.0	transcription
CA361557.1.a.om.1	Claudin-4	-1.4, -2.5, -1.5	1.1, 1.0, 1.5	adhesion
CA363489.1.a.om.1	Carboxypeptidase A1	-1.2, -2.9, -1.2	-1.4, 1.0, 1.8	proteolysis
CA363996.1.a.om.1	Eukaryotic translation initiation factor 4B	-1.5, -1.9, -2.3	-1.3, -1.1, 1.2	translation
CA368043.1.a.om.1	cAMP-regulated phosphoprotein 19	-1.7, -2.9, -1.8	1.0, -1.1, -1.1	gluconeogenesis
CA375698.1.a.om.1	Transcription intermediary factor 1-alpha	-1.2, -2.6, -1.4	1.0, 1.0, 1.3	transcription
CA377055.1.a.om.1	SLIT-ROBO Rho GTPase-activating protein 3	-2.1, -1.9, -1.7	1.3, 2.5, 2.3	signal transduction

CA378890.1.a.om.1	Solute carrier family 2	-2.6, -2.0, -1.5	1.5, -1.1, -1.4	sugar transport, glucose maintenance
CA384724.1.a.om.1	CA384724	-1.8, -2.6, 1.1	1.6, 1.9, 3.4	N/A
CA385505.1.a.om.1	CA385505	-2.9, -3.8, -2.9	1.4, 1.5, 1.6	N/A
CA386664.1.a.om.1	Ubiquitin-conjugating enzyme E2 variant 1	-1.6, -2.9, -1.5	1.1, 1.3, 1.4	proteolysis
CB494094.1.a.om.1	Kinetochore protein Hec1 homolog	-1.4, -2.6, -1.4	1.1, 1.3, 1.1	cytoskeletal organisation
CR942918.1.a.om.1	CR942918	-1.3, -2.0, -1.5	1.5, 1.5, 1.1	N/A
CR943749.1.a.om.1	CR943749	-1.5, -2.2, -1.4	1.0, -1.3, 1.4	N/A
CR943810.1.a.om.1	CR943810	-1.4, -2.0, -1.6	-1.4, 1.2, 1.0	N/A
CR944035.1.a.om.1	CR944035	-1.1, -2.8, -1.2	1.4, 1.4, 1.1	N/A
CT564232.1.a.om.1	CT564232	-1.2, -2.2, -1.3	2.1, 2.0, 2.2	N/A
CT565138.1.a.om.1	CT565138	-1.4, -2.9, 1.2	1.1, -1.1, 1.2	N/A
CT566501.1.a.om.1	CT566501	-3.2, -2.1, -1.3	1.4, 1.2, 1.4	N/A
CT572176.1.a.om.1	CT572176	-1.8, -3.1, -1.7	1.2, 1.7, 1.2	N/A
CT572635.1.a.om.1	CT572635	-1.1, -2.2, -1.7	1.1, 1.5, 1.3	N/A
CU067093.1.a.om.1	CU067093	-1.2, -3.1, -1.3	1.2, 1.2, 1.3	N/A
rtay11a03c02f1.1.a.om.1	RNA polymerase II elongation factor ELL	-1.2, -2.2, -1.1	1.2, -1.1, -1.1	transcription/translation
rtay11a03h02f1.1.a.om.1	rtay11a03h02f1	-1.9, -2.9, -2.0	1.5, 1.3, 2.0	N/A
rtay11a04a05f1.1.a.om.1	rtay11a04a05f1	-1.9, -2.3, -2.2	1.3, 2.0, 1.8	N/A
rtay11a08g07r1.1.a.om.1	rtay11a08g07r1	-1.5, -2.3, -1.5	1.6, 1.4, 1.9	N/A
rtay12a01d09r1.1.a.om.1	NFX1-type zinc finger-containing protein 1	-1.5, -2.5, -1.4	1.2, 1.1, 1.1	transcription
rtay13a04b04f1.1.a.om.1	rtay13a04b04f1	-1.4, -2.2, -1.2	-1.1, 1.0, 1.0	N/A
rtgl23d03_5.1_a.1.s.om.8	rtgl23d03_5.1_a.1.s.om.8	-1.4, -2.9, -1.8	1.4, -1.1, 1.2	N/A
rtlm25p23_3.1_a.1.s.om.8	rtlm25p23_3.1_a.1.s.om.8	-1.7, -2.4, 1.1	1.1, 1.5, 1.3	N/A
tcaa0001c.e.22_5.1.s.om.8	60S ribosomal protein L4-A	1.1, -1.7, 1.0	1.5, -1.1, 2.4	translation
TCBM_O16.SEQ	TCBM_O16.SEQ	-1.6, -2.5, -1.5	1.1, -1.1, 1.4	N/A
tcbw0006a.f.09_5.1.s.om.8	tcbw0006a.f.09_5.1.s.om.8	-1.7, -1.6, -2.3	1.6, 1.1, 1.2	N/A
AJ347728.1.a.om.1	D(2)-like dopamine receptor	1.1, -1.5, -2.3	1.1, -1.2, -1.1	signal transduction
BX072690.1.a.om.1	Beta actin-like	-1.6, -3.3, -1.3	1.0, 1.4, 1.3	cytoskeletal organisation

BX859379.1.a.om.1	Adenylyl cyclase type 6	-1.2, -2.1, -1.2	1.3, 1.3, 1.2	signal transduction
CA343113.1.a.om.1	CA343113	-1.3, -1.4, -1.8	1.1, 2.1, -1.9	N/A
BX077950.1.a.om.1	Zinc finger protein Xfin	-1.1, -2.0, -1.4	1.1, -1.2, 1.0	transcription
CA343052.1.a.om.1	CA343052	-1.4, -2.9, -1.3	-1.2, -1.3, -1.1	N/A
SET 4: Up-regulation in LR fish but predominantly at 168 h (LS44 effect)				
CA354409.1.a.om.1_2	Leukocyte cell-derived chemotaxin 2	-1.1, 1.5, 11.3	-3.1, 2.3, 3.0	immune response
CA344317.1.a.om.1	CA344317	-1.3, -1.6, 18.3	-1.1, -1.2, 1.9	N/A
CA342316.1.a.om.1	Cytochrome b-245 light chain	-1.2, -1.4, 4.7	-1.4, -1.7, 1.1	immune response, oxidation reduction
CA346484.1.a.om.1	Collagenase 3 (MMP-13)	-1.8, -1.9, 3.9	1.0, 1.1, -1.1	proteolysis
CA346948.1.a.om.1	Leukotriene A-4 hydrolase	-1.1, -2.0, 2.3	1.1, 1.5, -1.4	proteolysis, inflammatory response
CA347328.1.a.om.1	Talin-2	-1.1, 1.2, 2.4	1.0, 1.2, -1.2	protein localisation
CA349582.1.a.om.1	Fish-egg lectin	1.1, -1.2, 3.9	-1.1, -1.3, 1.1	adhesion
CA350055.1.a.om.1	CA350055	-1.1, -1.2, 3.8	-1.5, -1.4, -1.3	N/A
CA354409.1.a.om.1_1	Leukocyte cell-derived chemotaxin 2	-1.1, 1.3, 18.7	-1.5, 8.6, 14.6	immune response
CA343278.1.a.om.1	Tyrosine-protein kinase HCK	-1.1, 1.0, 2.5	1.0, 1.1, 1.0	immune response
CA343672.1.a.om.1	Leukocyte common antigen precursor (CD45 antigen)	-1.4, -1.7, 2.6	1.0, 1.0, -1.2	immune response
CA345431.1.a.om.1	Rhesus blood group-associated glycoprotein (CD241 antigen)	1.2, -1.2, 3.1	1.0, -1.1, 1.2	ion homeostasis
CA345645.1.a.om.1	Thymosin beta-11	-1.1, -1.5, 3.0	1.0, -1.1, 1.1	cytoskeletal organisation
CA348097.1.a.om.1	Neutrophil cytosol factor 1	-1.5, -1.9, 2.7	1.1, -1.3, 1.0	immune response, oxidation reduction
CA350548.1.a.om.1_2	Complement factor H precursor	1.3, 1.1, 3.0	1.0, 1.7, 1.3	immune response
CA351742.1.a.om.1	Lysozyme C II	1.0, 1.1, 4.1	-1.2, 1.3, 1.1	proteolysis, immune response
CA353080.1.a.om.1	High mobility group protein B2	1.0, -1.2, 4.4	-1.2, -1.3, -1.1	transcription, DNA repair
CA355051.1.a.om.1	Tetratricopeptide repeat protein KIAA0103	-1.1, -1.1, 3.0	1.1, 1.3, 1.2	unknown
CT572491.1.a.om.1	CT572491	-1.1, 1.0, 2.3	1.0, 1.0, -1.2	N/A
rtay12a06c04f1.1.a.om.1	rtay12a06c04f1	1.4, 1.6, 2.8	1.1, 1.0, 1.1	N/A
CA342769.1.a.om.1	Matrix metalloproteinase-9	-1.1, -1.5, 5.3	1.1, -1.2, -1.1	proteolysis, ECM organisation
CA371947.1.a.om.1	Glyceraldehyde-3-phosphate dehydrogenase	-1.1, -1.5, 5.8	1.3, 1.4, 1.9	glucose metabolism, oxidation reduction, glycolysis

CA343218.1.a.om.1_2	CA343218	-1.2, -1.7, 2.1	1.1, -1.3, 1.0	N/A