Molecular processes of transgenerational acclimation to a warming ocean

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Some animals have the remarkable capacity to acclimate across generations to projected future climate change[1-4]; however, the underlying molecular processes are unknown. We sequenced and assembled *de novo* transcriptomes of adult tropical reef fish exposed developmentally or transgenerationally to projected future ocean temperatures and correlated the resulting expression profiles with acclimated metabolic traits from the same fish. We identified 69 contigs representing 53 key genes involved in thermal acclimation of aerobic capacity. Metabolic genes were among the most upregulated transgenerationally suggesting shifts in energy production for maintaining performance at elevated temperatures. Furthermore, immune and stress responsive genes were upregulated transgenerationally, indicating a new compliment of genes allowing the second generation of fish to better cope with elevated temperatures. Other differentially expressed genes were involved with tissue development and transcriptional regulation. Overall, we found a similar suite of differentially expressed genes among developmental and transgenerational treatments. Heat shock protein genes were surprisingly unresponsive, indicating that short-term heat stress responses may not be a good indicator of long-term acclimation capacity. Our results are the first to reveal the molecular processes that may enable marine fishes to adjust to a future warmer environment over multiple generations.

Over the next century, rising ocean temperatures due to climate change will pose a serious threat to the survival of many aquatic species. To persist, populations will either need to shift their geographic distributions[5] or adapt through genetic evolution or phenotypic plasticity[6-8]. Of particular concern for marine species, is that rising temperatures will reduce

the capacity for oxygen supply and delivery[9,10], limiting activities essential to survival and individual fitness. Reduced aerobic scope (the capacity for oxygen uptake above resting metabolic rate) at higher temperatures can affect vital functions such as growth, swimming performance, reproduction and competitive ability[10-14]. In reef fishes, aerobic scope declines at temperatures just a few degrees above the summer average, within the range projected to occur as a result of climate change[9,12,15]. However, aerobic capacity can be fully restored transgenerationally, when parents and their offspring both experience the same elevated temperatures (transgenerational acclimation)[1]. Understanding the molecular processes that make this transgenerational plasticity possible is important for assessing the performance of marine organisms and sustainability of their populations in a rapidly warming ocean.

We used a multi-generational rearing experiment to identify the molecular pathways associated with transgenerational thermal acclimation of metabolic traits in a common reef fish, *Acanthochromis polyacanthus*. Second generation fish were reared developmentally (from hatching to adulthood) and transgenerationally (two generations) at two elevated temperatures (+1.5 and +3.0 °C) and in control conditions (+0.0 °C; Fig. 1a). The full transcriptome of four to five adult fish from each of the five treatments (Fig. 1a) was sequenced and expression data correlated to standardised metabolic traits from the same fish: routine metabolic rate (RMR), maximum metabolic rate (MMR) and net aerobic scope (MMR - RMR; NAS; Supplementary Methods). As observed in previous studies[1], developmental exposure to elevated temperatures from just after hatching into adulthood led to a reduction in aerobic scope (Fig. 1b). However, when both parents and offspring were exposed to elevated temperatures, complete restoration of aerobic scope was achieved (Fig. 1b). Of 89,543 assembled contiguous sequences (contigs), 165 had significant

differential expression (adjusted $P < 0.05$) in at least one of the treatment comparisons (transgenerational and developmental treatments vs. control; transgenerational vs. developmental treatments). One hundred and sixty of the differentially expressed contigs had BLASTN and/or BLASTP[16] sequence matches with e-values less than 10^{-10}, of which 69 had expression that was significantly correlated to at least one of the standardised metabolic measures (RMR, MMR, and NAS). Comparing transgenerational and developmental treatments at the same temperatures enabled us to distinguish patterns of gene expression due to transgenerational effects, compared with effects of within-generation exposure to elevated temperatures.

The 69 differentially expressed and correlated contigs represent 52 genes that are associated with transgenerational thermal acclimation. These genes are involved in a variety of cellular processes such as metabolism, transport, immune and stress responses, growth and development, cell cycle, cell organisation, and transcriptional regulation (Fig. 2; Supplementary Table 1). The expression profiles of these contigs separated into three distinct groups, with the first and largest group (Fig. 2a; 46 contigs) containing contigs with expression that primarily correlated to the acclimating phenotypic trait, NAS (78%). Metabolism is the major function associated with genes in this group (lipid, protein, and carbohydrate metabolism; nine, nine, and five contigs each, respectively), including 79% of the most highly upregulated contigs transgenerationally relative to controls (≥1.5 log2 fold change; Supplementary Table 1). During thermal stress, the composition of lipid membranes is altered (homeoviscous adaptation)[17] and there are changes in lipid use[18] and expression of the fatty acid pathway[19]. Of the nine contigs associated with lipid metabolism, six were strongly upregulated in transgenerational treatments (representing four genes:

acsl5, adtrp, apoEb, and pdzk1). ApoE has a major role in triglyceride and cholesterol homeostasis, suggesting that transgenerational upregulation of lipid metabolism may be critical for improved aerobic scope. ApoE and other apolipoproteins are also upregulated after short-term thermal challenge in fish[20,21], suggesting a link between short-term thermal stress and long-term thermal acclimation of aerobic capacity. Many of the metabolic genes in the first group (Fig. 2a) are involved in catabolism and digestion (Supplementary Table 1), suggesting their augmented expression provides increased energy for aerobic performance in transgenerational fish (Supplementary Table 1). Supporting this hypothesis, 11 contigs are involved in the cellular transport of ions, solutes, amino acids, lipids, and carbohydrates, possibly as a result of increased substrate digestion. Our results suggest that there is transgenerational regulation of lipid, protein, and carbohydrate metabolism and that each may be critical for increased energy use associated with acclimation of aerobic scope across generations.

In addition to metabolic responses, sixteen contigs with putative functions associated with immune responses and inflammation, apoptosis, homeostasis, and stress were significantly upregulated during transgenerational thermal acclimation (Fig. 2a). Immune responses can be maternally imprinted in fish[22], potentially by transferring maternal idiotypic networks to juveniles at a critical stage[23]. Such imprinting, we hypothesise, would then be augmented throughout development to establish an immune response better suited for survival under thermal stress. As chronic stress can suppress immune function and lead to increased susceptibility to disease and pathogens[24], the transgenerational augmentation of five putative immune-related contigs (gimap8, xpnpep2, mep1b, and natterin3) may represent

new baseline levels of immune-related genes to protect against elevated temperatures experienced across generations.

The second major group of genes (Fig. 2b) is comprised of 12 contigs, all of which had expression that was negatively correlated to standardised RMR. RMR was lower in fish exposed transgenerationally to +3.0 °C compared with controls (Supplementary Fig. 1). The high proportion of contigs in this group with putative function in organ development (two contigs; ppdpfa and ptf1a) and endothelial cell proliferation (four contigs; nlrp14 and timp2) suggests that lower metabolic costs enabled these cellular processes to function at a higher level in transgenerationally acclimated fish, which is consistent with acclimation of growth rates in fish exposed transgenerationally to elevated temperatures[3,4]. In addition, this group contains five contigs related to transcriptional regulation (three genes: rorb, ptf1a, and rps27), two of which enhance expression of genes involved in organogenesis (rorb, ptf1a). The third gene, rps27, is a nuclear protein induced upon DNA damage[25]. Therefore, increased transgenerational expression and negative correlation to standardised RMR suggest rps27 plays a role in maintaining DNA integrity after transgenerational exposure to elevated temperatures to restore routine metabolic function.

While the first two groups in the heatmap (Fig. 2a, Fig. 2b) contained contigs with expression that was significantly elevated transgenerationally, the third group (Fig. 2c) contained 11 contigs (16% of total) with expression that was downregulated transgenerationally. The majority of these contigs had expression that positively correlated to standardised RMR (64%; seven contigs) and largely matched genes with functions related to stress, homeostasis, and immune responses (Supplementary Table 1). As many other stress and immune-related genes were upregulated transgenerationally in the first two

groups, the downregulated genes with these functions in the final group suggests their expression was reduced in favour of other more beneficial genes for transgenerational acclimation.

The heatmap indicates that many contigs had higher differential expression in transgenerational compared with developmental treatments (Fig. 2); however, only three were statistically significant: cytochrome p450 2j2 (cyp2j2), ribosomal protein large P1 (rplp1), and an uncharacterised gene (Supplementary Table 1). Cyp2j2 is associated with epoxidation of arachidonic acid[26], of which the primary products formed, epoxyeicosatrienoic acids, are involved in a variety of processes such as vasodilation, anti-inflammation, and cytoprotection. For example, cyp2j2 appears to play a cytoprotective role in animals exposed to hypoxia[27] and high-fat diets[28]. Thus, we hypothesize that increased transgenerational cyp2j2 expression may play an important cytoprotective role, allowing proper cellular function after transgenerational but not developmental exposure to elevated temperatures. Rplp1 plays a key role in the elongation step of protein synthesis. Therefore, rplp1 may be required in developmental treatments to increase protein translation due to a higher rate of protein degradation during thermal stress, but is no longer required transgenerationally due to the aforementioned increases in cytoprotective gene expression. Importantly, there was only one contig (btn1a1) that was significantly differentially expressed in developmental but not transgenerational treatments (Supplementary Table 1). This suggests that there is not a different suite of genes and cellular processed engaged during developmental exposure to elevated temperatures compared with transgenerational acclimation.

A commonly used molecular measure of thermal stress has been to examine molecular chaperone expression, specifically heat shock proteins (HSPs). Some HSPs are constitutively expressed and are involved in nascent polypeptide folding, while others are expressed to help refold proteins that unfolded due to various stressors[29]. We found no HSP genes with significantly altered expression in developmental or transgenerational *A. polyacanthus*. Of all 160 significantly differentially expressed contigs identified in this study, including contigs with expression that did and did not correlate to metabolic traits (Supplementary Fig. 2), only one matched a gene with putative chaperone function: eukaryotic translation elongation factor 1a (eef1a). This gene has been shown to protect aminoacyl-tRNA synthetases from denaturation in mammals[30], and may therefore have a more specific role in maintaining the integrity of transgenerational protein synthesis in our study. While contigs with matches for many HSPs were found within the *A. polyacanthus* transcriptome, none were significantly differentially expressed among the five treatments (Fig. 3; adjusted $P > 0.7$). Therefore, the lack of differential HSP gene expression and limited chaperone activity suggests that other genes outlined in this study are better indicators of transgenerational thermal acclimation, at least in *A. polyacanthus*. While HSPs may be good indicators of acute thermal stress[29,31], our results suggest they may not be good indicators of the capacity for long-term thermal acclimation to predicted temperatures under climate change.

Acclimation of aerobic scope within two generations[1] suggests epigenetic inheritance is involved. Future research into epigenetic mechanisms and their effect on genes identified in this study will be useful to improve our understanding of adaptive responses to rapid environmental change. In this study we identified key genes and processes involved in

transgenerational thermal acclimation, including genes involved in enhanced fatty acid oxidation, protein and carbohydrate metabolism, and changes in genes involved in cytoprotection, immunity, organogenesis, and cellular organisation. The plasticity of these genes and their strong correlation to known acclimating phenotypic traits suggests they may be critical in aiding reef fishes, and possibly other marine organisms to survive in a warmer future environment.

Methods Summary

Two generations of *A. polyacanthus* were reared as per Donelson *et al*.[1] and livers were dissected from control, developmental, and transgenerational temperature treated adults. mRNA was extracted and cDNA libraries constructed for transcriptome sequencing on an Illumina HiSeq 2000 platform analyser. Reads were assembled *de novo* using Trans-ABySS[32]. Normalised transcriptome expression data was validated by quantitative real-time PCR for eight genes, consisting of both up and downregulated genes (Supplementary Fig. 3, Supplementary Table 2). Standardised metabolic measurements (RMR, MMR and NAS; Supplementary Methods) for each individual were correlated to the normalised and variance stabilised expression data for each contig, of which only those with a significant ($P \leq 0.05$) correlation were selected.

Online Content

Methods, Supplementary tables, and additional Supplementary display items are available in the online version of the paper; references unique to these sections appear only in the online paper.

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210 **Author contributions**

211 J.M.D. and P.L.M. designed and managed the fish rearing experiments. J.M.D. performed
212 metabolism experiments. H.D.V. prepared samples for sequencing. T.Ry. assembled
213 transcriptome. T.Ry., T. Ra., L.S., and Y.G. analysed expression and assessed assembly
214 quality. H.D.V. performed qRT-PCR expression validation. H.D.V. analysed the data. H.D.V.,
215 P.L.M., T.Ry., J.M.D., L.v.H., M.L.B., W.L., and T.Ra. wrote the paper and all authors read and
216 approved the manuscript.

217 **Additional information**

218 RNA-seq transcriptome sequences have been deposited in GenBank under BioProject ID
219 PRJNA255544. The authors declare no competing financial interests. Supplementary
220 information accompanies this paper on http://www.nature.com/nature. Reprints and
221 permissions information is available online at http://www.nature.com/reprints.
222 Correspondence and request for material should be addressed to P.L.M and T.Ra.

223 **Figure Legends**

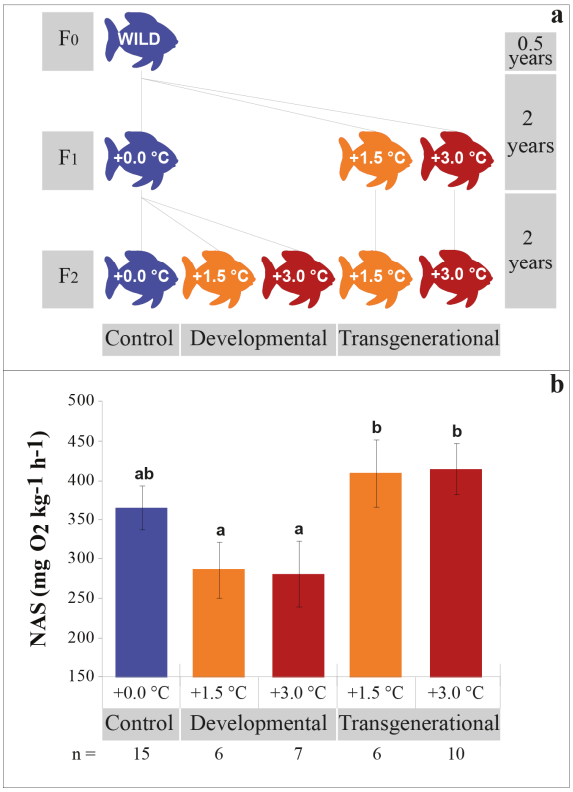
224 **Figure 1 | Transgenerational experimental design and corresponding net aerobic scope**
225 **measures. a,** Experimental design tree showing the three temperature treatments (+0.0 °C,
226 +1.5 °C and +3.0 °C) at which three generations (F_0, F_1 and F_2) of *Acanthochromis*
227 *polyacanthus* were reared. Temperature treatments are colour coded and experimental
228 duration for each generation is shown in the vertical grey bars to the right. Fish in the F_2
229 generation representing control, developmental and transgenerational temperature

230 treatments are indicated by horizontal grey bars. **b**, Net aerobic scope (NAS) of fish in
231 control, developmental and transgenerational F_2 treatments (mean ± s.e.m.). Lower case
232 letters above bars indicate significant differences ($P < 0.05$) among treatments. Number of
233 fish used to measure NAS for each treatment is shown beneath the grey bars.

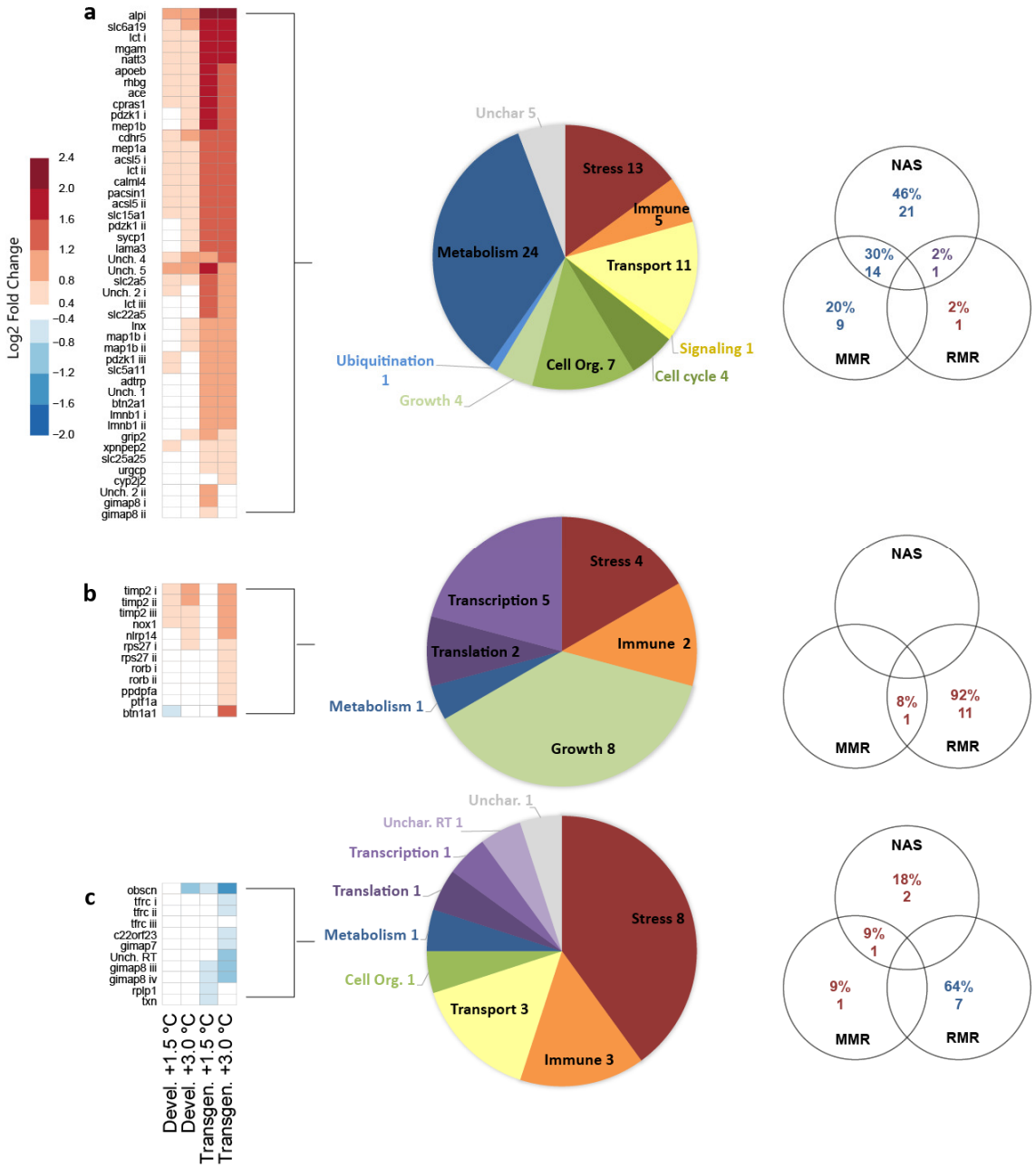
234 **Figure 2 | Differentially expressed contigs, correlations to metabolic performance, and**
235 **putative cellular function.** Heatmap (left) of differentially expressed contigs (adjusted $P <$
236 0.05) from *Acanthochromis polyacanthus*, comparing +1.5 °C and +3.0 °C developmental
237 (devel.) and transgenerational (transgen.) treatments to control (+0.0 °C). Based on
238 expression patterns, contigs were separated into three groups (a, b, and c). The associated
239 cellular functions for each group are presented as pie charts (middle), with each contig
240 represented by two functions with the exception of those that were uncharacterised.
241 Numbers within pie chart sections represent the total number of contigs that correspond to
242 that function. Venn diagrams (right) indicate the proportion of contigs with expression that
243 positively (blue) or negatively (red) correlated to metabolic data (NAS - net aerobic scope,
244 MMR - maximum metabolic rate, and RMR - routine metabolic rate). Purple text indicates
245 negative NAS and positive RMR.

246 **Figure 3 | Heat shock protein (HSP) contig expression pattern.** Heatmap of HSP expression
247 from *Acanthochromis polyacanthus*, comparing +1.5 °C and +3.0 °C developmental (devel.)
248 and transgenerational (transgen.) treatments to control (+0.0 °C). There were no significant
249 differences in expression (adjusted $P < 0.05$). Expression values correspond to the contig
250 with the best match (E-value < 10^{-27}) to HSP genes within our transcriptome.

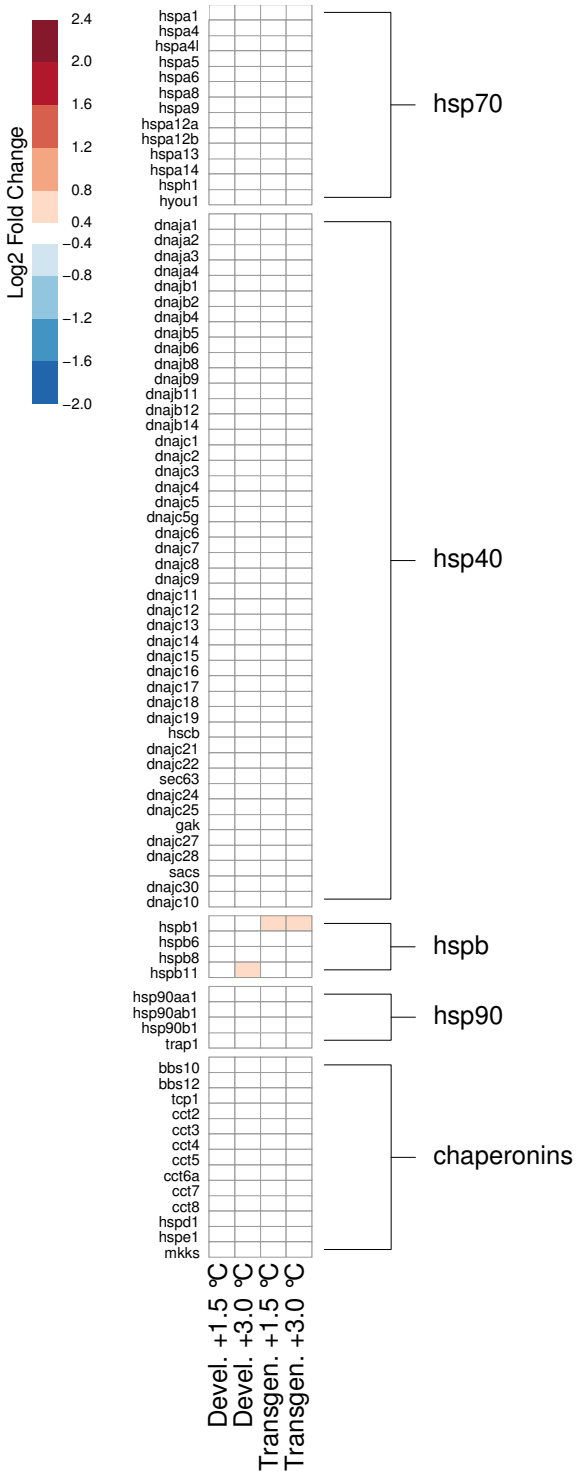
251 **Figure 1.**


a
F0
WILD
F1
+0.0 °C
+1.5 °C
+3.0 °C
F2
+0.0 °C
+1.5 °C
+3.0 °C
+1.5 °C
+3.0 °C
0.5 years
2 years
2 years
Control
Developmental
Transgenerational
b
NAS (mg O2 kg-1 h-1)
500
450
400
350
300
250
200
150
ab
a
a
b
b
+0.0 °C
+1.5 °C
+3.0 °C
+1.5 °C
+3.0 °C
Control
Developmental
Transgenerational
n =
15
6
7
6
10

252 **Figure 2.**


a
Log2 Fold Change
2.4
2.0
1.6
1.2
0.8
0.4
−0.4
−0.8
−1.2
−1.6
−2.0
alpi
slc6a19
lct i
mgam
natt3
apoeb
rhbg
ace
cpras1
pdzk1 i
mep1b
cdhr5
mep1a
acsl5 i
lct ii
calml4
pacsin1
acsl5 ii
slc15a1
pdzk1 ii
sycp1
lama3
Unch. 4
Unch. 5
slc2a5
Unch. 2 i
lct iii
slc22a5
lnx
map1b i
map1b ii
pdzk1 iii
slc5a11
adtrp
Unch. 1
btn2a1
lmnb1 i
lmnb1 ii
grip2
xpnpep2
slc25a25
urgcp
cyp2j2
Unch. 2 ii
gimap8 i
gimap8 ii
Unchar 5
Stress 13
Immune 5
Transport 11
Signaling 1
Cell cycle 4
Cell Org. 7
Growth 4
Ubiquitination 1
Metabolism 24
NAS
46%
21
30%
14
2%
1
20%
9
2%
1
MMR
RMR
b
timp2 i
timp2 ii
timp2 iii
nox1
nlrp14
rps27 i
rps27 ii
rorb i
rorb ii
ppdpfa
ptf1a
btn1a1
Stress 4
Transcription 5
Translation 2
Immune 2
Metabolism 1
Growth 8
NAS
8%
1
92%
11
MMR
RMR
c
obscn
tfrc i
tfrc ii
tfrc iii
c22orf23
gimap7
Unch. RT
gimap8 iii
gimap8 iv
rplp1
txn
Devel. +1.5 °C
Devel. +3.0 °C
Transgen. +1.5 °C
Transgen. +3.0 °C
Unchar. 1
Unchar. RT 1
Transcription 1
Translation 1
Metabolism 1
Cell Org. 1
Transport 3
Immune 3
Stress 8
NAS
18%
2
9%
1
9%
1
64%
7
MMR
RMR

253 **Figure 3.**


Log2 Fold Change
2.4
2.0
1.6
1.2
0.8
0.4
−0.4
−0.8
−1.2
−1.6
−2.0
hspa1
hspa4
hspa4l
hspa5
hspa6
hspa8
hspa9
hspa12a
hspa12b
hspa13
hspa14
hsph1
hyou1
hsp70
dnaja1
dnaja2
dnaja3
dnaja4
dnajb1
dnajb2
dnajb4
dnajb5
dnajb6
dnajb8
dnajb9
dnajb11
dnajb12
dnajb14
dnajc1
dnajc2
dnajc3
dnajc4
dnajc5
dnajc5g
dnajc6
dnajc7
dnajc8
dnajc9
dnajc11
dnajc12
dnajc13
dnajc14
dnajc15
dnajc16
dnajc17
dnajc18
dnajc19
hscb
dnajc21
dnajc22
sec63
dnajc24
dnajc25
gak
dnajc27
dnajc28
sacs
dnajc30
dnajc10
hsp40
hspb1
hspb6
hspb8
hspb11
hspb
hsp90aa1
hsp90ab1
hsp90b1
trap1
hsp90
bbs10
bbs12
tcp1
cct2
cct3
cct4
cct5
cct6a
cct7
cct8
hspd1
hspe1
mkks
chaperonins
Devel. +1.5 °C
Devel. +3.0 °C
Transgen. +1.5 °C
Transgen. +3.0 °C