Title: Convergent stress response repertoire to thermal challenges in a temperate aposymbiotic coral

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Abstract:
Corals are threatened worldwide due to rapidly warming oceans associated with anthropogenic climate change. A growing body of work has explored how corals respond to heat stress, however, responses of corals across the full range of their thermal breadth are rarely explored even though winter colds are also known to induce coral stress. Here, we leverage the temperate stony coral, *Astrangia poculata,* which naturally exhibits a facultative symbiosis with Symbiodiniaceae, to explicitly examine how thermal challenges influence coral hosts in isolation from their symbionts. Aposymbiotic *A. poculata* were collected from Woods Hole, MA, the northern range limit for this species. Corals were thermally challenged in two independent common garden experiments (Heat challenge: 31˚C, 10 days; Cold challenge: 6˚C, 16 days) to determine the effects of divergent thermal stressors. Behavioural responses to food stimuli were monitored throughout the thermal challenges and genome-wide gene expression profiling (TagSeq) was used to characterize molecular underpinnings of the coral's response to stress in its aposymbiotic state. Behaviourally, both thermal challenges induced polyp retraction and colonies failed to respond to food stimuli. Surprisingly, seven times as many genes were differentially expressed under cold challenge heat challenge. Despite a greater magnitude of response under cold challenge, significant similarities in gene expression were detected across the two thermal challenge experiments, with many of the most responsive genes having been previously implicated in coral heat stress response. Given that our data were generated from aposymbiotic colonies, we hypothesize that these genes previously linked to heat stress are more likely indicative of a generalized stress response. Overall this work highlights the unique insights facultatively symbiotic corals offer for deciphering the stress response of the coral host in the absence of bleaching.

31 ***Introduction***

32 Temperature is an important factor in determining species distribution patterns in ectothermic
33 organisms [1]. As sea surface temperatures continue to rise, understanding how this will affect
34 species distributions demands a broad understanding of an organisms' physiological sensitivities
35 to temperature across their native range. There is overwhelming evidence that temperature
36 increases associated with anthropogenic climate change are having widespread ecological
37 consequences on marine species distributions [2,3]. Coral reefs are particularly sensitive to these
38 thermal changes, which have been implicated in widespread reef declines [4]. Temperature
39 anomalies are the primary driver of the breakdown of the obligate symbiotic relationship between
40 corals and their endosymbiotic algae [5] in the family Symbiodiniaceae [6]. This breakdown
41 results in the expulsion of algae from coral host tissue in a process commonly referred to as coral
42 bleaching. Because symbiotic algae translocate carbon sugars to the coral host, losing these
43 symbionts results in significant energy loss and many corals are unable to survive extended periods
44 in a bleached state [7].

45

46 The majority of research on coral bleaching has focused on responses to elevated temperatures in
47 tropical reef-building corals [8]. However, tropical corals are known to bleach in response to a
48 variety of stressors, including high nutrients [9], ocean acidification [10], pathogens [11], low
49 salinity [12] and chemical exposures [13]. Coral responses to cold stress remain understudied, even
50 though these events can have substantial impacts on reefs. For example, in 2010 a cold water
51 bleaching event decimated inshore coral populations along the Florida reef tract [14], and cold
52 water has caused bleaching on the Great Barrier Reef [15]. While the main effect of climate change
53 on marine systems is a net increase in mean global sea surface temperatures, these cold thermal

challenges will still occur [16] and may disproportionately affect subtropical and temperate coral species.

Tropical reef-building corals live in oligotrophic waters and cannot survive long-term without their symbionts. Because energy deprivation in these corals results from any mechanism of symbiont loss [17], uncoupling a thermal stress response from a general stress response (i.e. energy deficit) is challenging. Given that many tropical corals exhibit an obligate symbiotic relationship, it is difficult to uncouple the host's stress response to extreme temperatures from that of the host's response to algal by-products released under stress (i.e. reactive oxygen species (ROS) [18]) and the resulting energy deprivation from the loss their algal symbiont induced by a bleached state. However, there are several species of subtropical and temperate reef-building corals that exhibit facultative symbioses. The Northern Star Coral, *Astrangia poculata,* exhibits a facultatively symbiotic relationship with *Breviolum psygmophilum* [19] and can be found naturally in sympatry across two symbiotic states that are visually distinguishable by colour. Symbiotic colonies appear brown due to high densities of *B. psygmophilum*, and much like a bleached coral, aposymbiotic colonies of *A. poculata* appear white due to very low algal densities [20]. Unlike obligately symbiotic corals, *A. poculata* can thrive in its "bleached" state by feeding autonomously via heterotrophy. Additionally, *A. poculata* experiences large seasonal variation in temperature at its northern range, making these populations ideal models for investigating how corals might withstand wide thermal ranges. Taken together, *A. poculata* provides a unique opportunity to leverage its aposymbiotic state to determine how broad thermal anomalies influence the coral host in isolation from its symbionts.

77 Characterising changes in gene expression profiles provides a snapshot into the current
78 physiological state of an organism, offering insights into the biological processes, molecular
79 functions, and cellular components that corals engage to withstand various stressors. Modern
80 transcriptomics have demonstrated that corals mount dynamic responses to pollutants [21,22], pH
81 [23,24] and bacterial challenges [25,26] and considerable efforts have been made to understand
82 how corals respond to heat challenges (for review see [8]). Interestingly, similar patterns of gene
83 expression emerge from these different stressors. Barshis *et al.* [27] made the connection that
84 corals demonstrate a widespread stress response across thousands of genes much like that of yeast
85 in what is termed the environmental stress response (ESR). The ESR in yeast is characterized by
86 a consistent response to diverse environmental stressors [28]. How corals respond to divergent
87 stressors remains understudied, and testing single stressors does not indicate whether genes being
88 expressed are a unique response to a particular stressor or a generalized ESR.
89
90 In order to disentangle how coral hosts respond to thermal challenges in isolation, we leverage the
91 facultative symbiosis offered by *A. poculata* to assess how thermal challenges influence the host's
92 stress response. We present two experiments that independently assess the behavioural and
93 molecular responses of aposymbiotic *A. poculata* to divergent thermal challenges. We then
94 contrast these stress responses to better understand this coral's ESR to a wide breadth of thermal
95 challenges to explore how conserved these response repertoires might be.

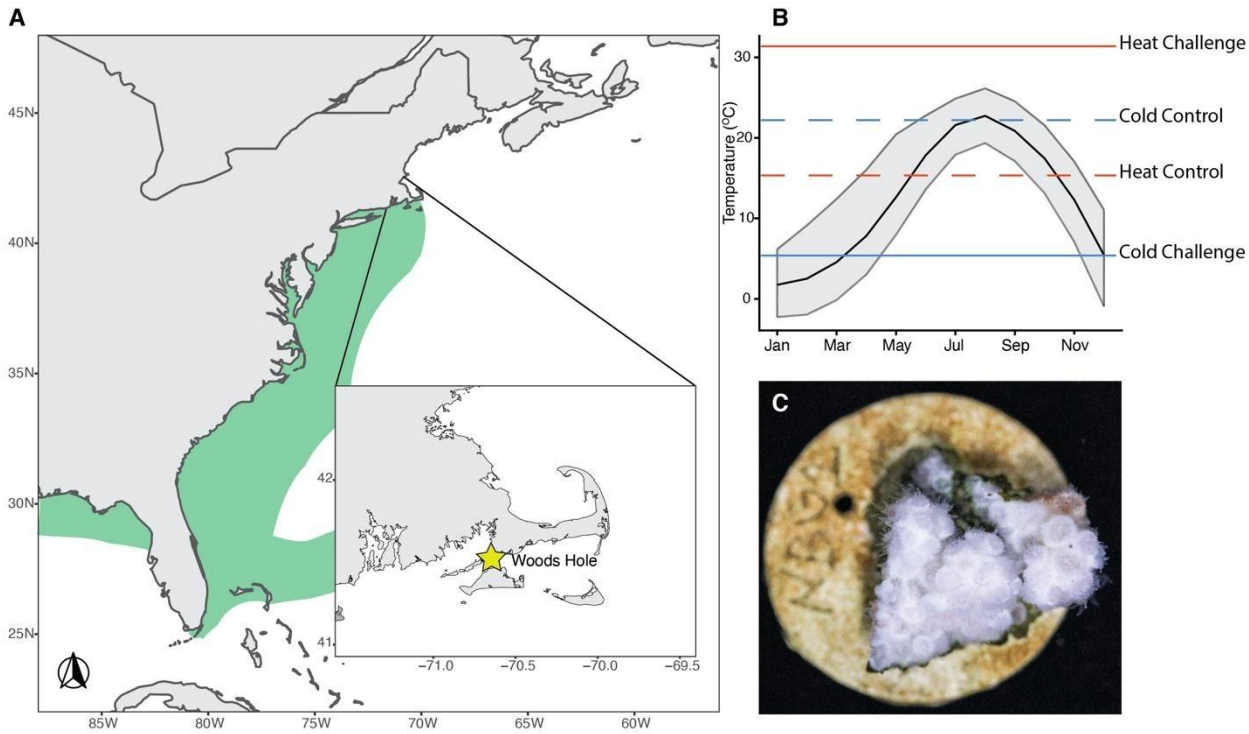

Figure 1 | A) Map of the eastern seaboard of the United States with a native range of *Astrangia poculata* in green. Inset shows the Woods Hole collection site denoted with a yellow star. Distributions are based on [29]. **B)** Seasonal temperature profile at Woods Hole averaged over ten years (2008-2018). The black solid line indicates mean monthly temperatures with mean monthly maximum and minimum temperatures in grey. Temperatures (˚C) of thermal challenge experimental controls (dashed lines) and treatments (solid lines) are superimposed with cold challenge treatments in blue and heat challenge treatments in red. Seasonal temperatures were mined from the National Oceanic and Atmospheric Administration weather buoy #BZBM3. **C)** Picture of an aposymbiotic *Astrangia poculata* colony fragment.

Methods

Thermal challenge common garden experiments

Aposymbiotic colonies of *Astrangia poculata* (n = 18; Figure 1C) were collected in Woods Hole, Massachusetts (41.54N, 70.64W; Figure 1A) in October 2017 and transported to the Marine Invertebrate Research Facility at Boston University. Colonies were acclimated to 16°C for three weeks. On November 17, 2017, colonies were fragmented, and each nubbin was assigned a unique ID based on colony ID and glued to a labelled dish. Corals were then further acclimated at 16°C under a 12 L:12 D photoperiod with light levels ranging from 6-12 µmol $m^{-2}s^{-1}$ and fed *Artemia* nauplii daily for 24 days.

Thermal challenge I: Cold experiment

Nine colonies were assigned to the cold challenge experiment. At least one nubbin (n = 43) from each colony was represented in one of three replicate tanks assigned to control conditions (22˚C) and one of three to the cold challenge treatment (22ºC that was incrementally lowered to 6ºC over 16 days). It is worth noting that 6ºC is above the minimum temperature *A. poculata* experience within their normal seasonal averages (Figure 1B), however, achieving lower temperatures was limited by the capacity of our chillers. In addition, the rate of chilling was much more rapid than what these corals experience naturally and given that these corals were collected in the summer, the rate of change and the temperature achieved is expected to represent a considerable thermal challenge.

Thermal challenge II: Heat experiment

An independent set of nine aposymbiotic colonies of *A. poculata* were fragmented (n = 43) and at least one nubbin from each colony were assigned to each treatment. There were three replicate tanks for control conditions (16ºC) as well as the heat challenge treatment (23ºC that was

132 incrementally ramped every three days until 31ºC was achieved; t_{total} = 10 days) and at least one
133 nubbin of each colony was assigned to each treatment (Figure 3B). Due to insufficient RNA
134 quality, some samples were not successfully represented in library preparations. As a result, four
135 colonies are only represented in one of the treatments. It is also worth noting that the final heat
136 challenge temperature tested here was well above the maximum temperature these corals typically
137 experience at their source location (Figure 1B).
138
139 *Coral polyp behaviour in response to food stimulus*
140 In the cold challenge experiment, corals were fed daily and subsequent feeding behaviours were
141 recorded. In contrast, in the heat challenge experiment, corals were offered food every third day.
142 Coral polyp behaviour in response to the food stimulus was quantified as the total coral surface
143 area that had observable polyp extension relative to retracted polyps. This was scored on a scale
144 of 1 to 5 based on the estimated percentage of active polyps within a fragment (1 = 0%, 2 = 25%,
145 3 = 50%, 4 = 75%, 5 = 100%) and the same researcher conducted all behavioural assays to control
146 for observer biases. An ordered logistic regression was performed to establish if temperature
147 influenced polyp extension rates using the *polr* function as part of the *MASS* package (version 7.3-
148 51.1) in R.
149
150 ***Global gene expression profiling***
151 Upon reaching maximum thermal differences between challenge and control treatments in both
152 experiments, several aposymbiotic polyps from all colonies were sampled using sterilized bone
153 cutters, immediately placed in 200 proof ethanol and stored at -80ºC. Total RNA was extracted
154 using an RNAqueous kit (Ambion by LifeTechnologies) following the manufacturer's

155 recommendations. An additional step using 0.5 mm glass beads (BioSpec), which were added to
156 the vial of lysis buffer and samples were homogenized using a bead beater for 1 min. RNA quantity
157 and integrity were determined using a DeNovix DS-11+ spectrophotometer and ribosomal RNA
158 bands were confirmed on 1% agarose gels. Trace DNA contamination was removed using a DNase
159 1 (Ambion) digestion at 37°C for 45 minutes, then libraries were created from 1500 ng of total
160 RNA following [30] and adapted for Illumina Hi-Seq sequencing [31,32]. In brief, RNA was heat-
161 sheared and transcribed into first-strand cDNA using a template-switching oligo and
162 SMARTScribe reverse transcriptase (Clontech). Complementary-DNA was then PCR- amplified,
163 individual libraries were normalized, and Illumina barcodes were incorporated using a secondary
164 PCR. Samples were pooled and size-selected prior to sequencing on Illumina Hiseq 2500 single-
165 end (SE) 50 basepair (bp) at Tufts University Core Facility (TUCF). Of the 43 samples within each
166 experiment, 23 and 27 libraries were prepared for the heat and cold challenge experiments,
167 respectively.
168
169 *Gene Expression Analyses*
170 A preliminary *A. poculata* holobiont transcriptome [33] was first separated into the host and
171 symbiont fractions (following [24]). In brief, the resulting host transcriptome was cleaned by
172 removing all genes that had high-level matches to the SILVA ribosomal small (16s/18s) and large
173 (34s/28s) subunit databases [34] using BLASTn [35]. Genes which had annotations associated
174 with chloroplasts were also removed as they are indicative of symbiont or turf algae contamination.
175 Raw reads across libraries ranged from 2.1-7.4 million SE 50 bp sequences. Illumina TruSeq
176 adapters and poly-A tails were removed using the *FASTX-Toolkit* (v 0.0.14,
177 http://hannonlab.cshl.edu/fastx_toolkit) and resulting sequences that were less than 20 bp in length

178 were removed. In addition, only those sequences with > 90% of bases having a quality score > 20
179 were retained. PCR duplicates were removed and resulting quality-filtered reads were mapped to
180 the cleaned *A. poculata* transcriptome using Bowtie2 [36]. Raw count data for each challenge
181 experiment were first tested for outliers using *arrayQualityMetrics* as part of DESeq [37] and no
182 outliers were detected for either experiment. DESeq2 [38] was then used to identify differentially
183 expressed genes (DEGs) associated with heat and cold thermal challenge relative to their
184 respective controls using a Wald's test. P-values were adjusted for multiple testing using the
185 Benjamini and Hochberg method (FDR < 0.05; [39]). Lastly, expression data for each experiment
186 were r-log normalized and these data were used as input for a principal component analysis. A
187 permutational multivariate analysis of variance was then used to determine if overall gene
188 expression patterns between thermal challenge treatments differed significantly from their controls
189 using the *adonis* function in Vegan v2.5-4 [40].

190 Gene ontology (GO) enrichment analysis was performed using adaptive clustering of GO
191 categories and Mann–Whitney U tests (GO-MWU) based on the ranking of signed log p-values
192 [41], which is particularly suitable for non-model organisms [42]. Results were visualized in
193 dendrograms tracing the level of gene sharing between significant categories and direction of
194 change in treatment temperatures compared to their respective controls. All data for the two
195 thermal challenge experiments were analyzed separately.

196

197 *Testing for a convergent response to thermal challenge*

198 Lists of DEGs (FDR < 0.05) between the two thermal challenge experiments were compared and
199 visualized using a Venn Diagram and significant enrichment of genes at the intersection between
200 experiments was tested for using a hypergeometric test. The DEGs at the intersection between

201 experiments (common DEGs) were visualized based on log fold change for each experiment and
202 the most highly up- and down-regulated genes were highlighted and defined as convergently
203 responding genes (CRG). GO categories that were independently identified as enriched (FDR <
204 0.05) in both experiments were visualized by their respective delta-ranks of enrichment to
205 demonstrate the conservation of GO function across the thermal challenges (for details, see [42]).
206

207 ***Results***

208 Astrangia poculata *response to cold challenge*

209 Throughout the experiment, *Astrangia poculata* behavioural responses to food stimulus under
210 control conditions varied, however, nearly all colonies exhibited some polyp extension (Figure
211 2A). This contrasts with behaviours observed under cold challenge, where rapid declines in polyp
212 activity were observed by day eight (12˚C) and most polyps remained inactive across cooler
213 temperatures (10˚C-6˚C, Figure 2A). Overall, *A. poculata* polyp activity was significantly reduced
214 under cold challenge ($p < 0.01$). Transcriptomes of *A. poculata* were also significantly influenced
215 by cold challenge and a strong treatment effect on overall gene expression was observed (*Adonis*
216 $p_{treatment} < 0.001$, Figure 2C), with cold challenge resulting in 3,428 (7.8%) DEGs (FDR < 0.05;
217 1,468 (3.3%) up-regulated; 1,960 (4.5%) down-regulated). There were also many GO terms
218 enriched between cold challenge and control conditions (FDR < 0.10; BP = 419, MF = 106, CC =
219 146; Supplemental Figure 1). Of these, notable GO terms include: SNAP receptor activity
220 (GO:0005486), tumour necrosis factor-activated receptor (GO:0005031), apoptotic signalling
221 (GO:0097190) and cell death (GO:0008219).
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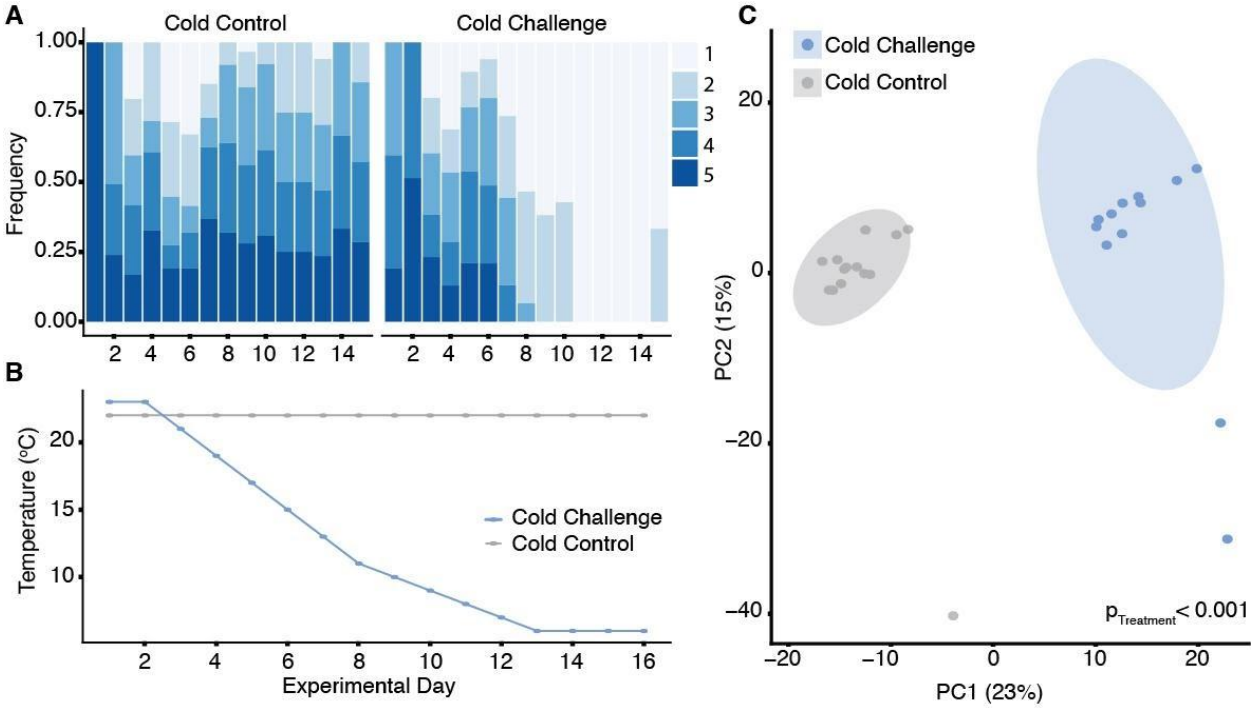

Figure 2 | *Astrangia poculata* behavioural and transcriptomic response to cold challenge. A) Frequency of behavioural scores (proportion of polyps extended per fragment, 1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100%) in response to food stimulus throughout the 16-day experiment (Left: Cold Control, Right: Cold Challenge, $p < 0.01$). B) Temperature profile of experimental tanks throughout the 16-day experiment, where blue represents cold challenge and grey represents control conditions. C) Principal component analysis of overall gene expression with blue symbols representing gene expression of coral fragments from cold challenge conditions and grey symbols representing gene expression of coral fragments under control conditions. Percentages represent the total amount of variance explained by each axis and shaded areas are 95% confidence ellipses. P-value indicates the significance of treatment using a permutational multivariate analysis of variance.

237 *Astrangia poculata responses to heat challenge*

238 Throughout the experiment, *Astrangia poculata* behavioural responses to food stimulus under
239 control conditions were consistent and coral polyps remained fully extended (Figure 3A). This
240 contrasts with behavioural responses under heat challenge, where corals exhibited less polyp
241 activity in response to food stimulus as temperatures increased. By the end of the experiment (day
242 10), only one colony under heat challenge was observed to have 100% polyp extension and half
243 of the colonies had less than 25% of their polyps extended (Figure 3A). Overall, *A. poculata* polyp
244 activity was significantly reduced under heat challenge ($p < 0.01$). Transcriptomes of *A. poculata*
245 were also significantly influenced by heat challenge and a significant effect of treatment on overall
246 gene expression was observed (*Adonis* $p_{treatment} < 0.001$, Figure 3C) with 485 (1.1%) DEGs (FDR
247 < 0.05; 230 (0.53%) up-regulated; 255 (0.58%) down-regulated) observed. Many GO terms were
248 found to be significantly enriched under heat challenge relative to control conditions (FDR < 0.10;
249 BP = 179, MF = 91, CC = 93; Supplemental Figure 1) and notable GO terms include: NF-kappaB
250 complex (GO:0033256), mitochondrial parts (GO:0005739), catabolic process (GO:0009894), and
251 regulation of proteolysis (GO:0030162).

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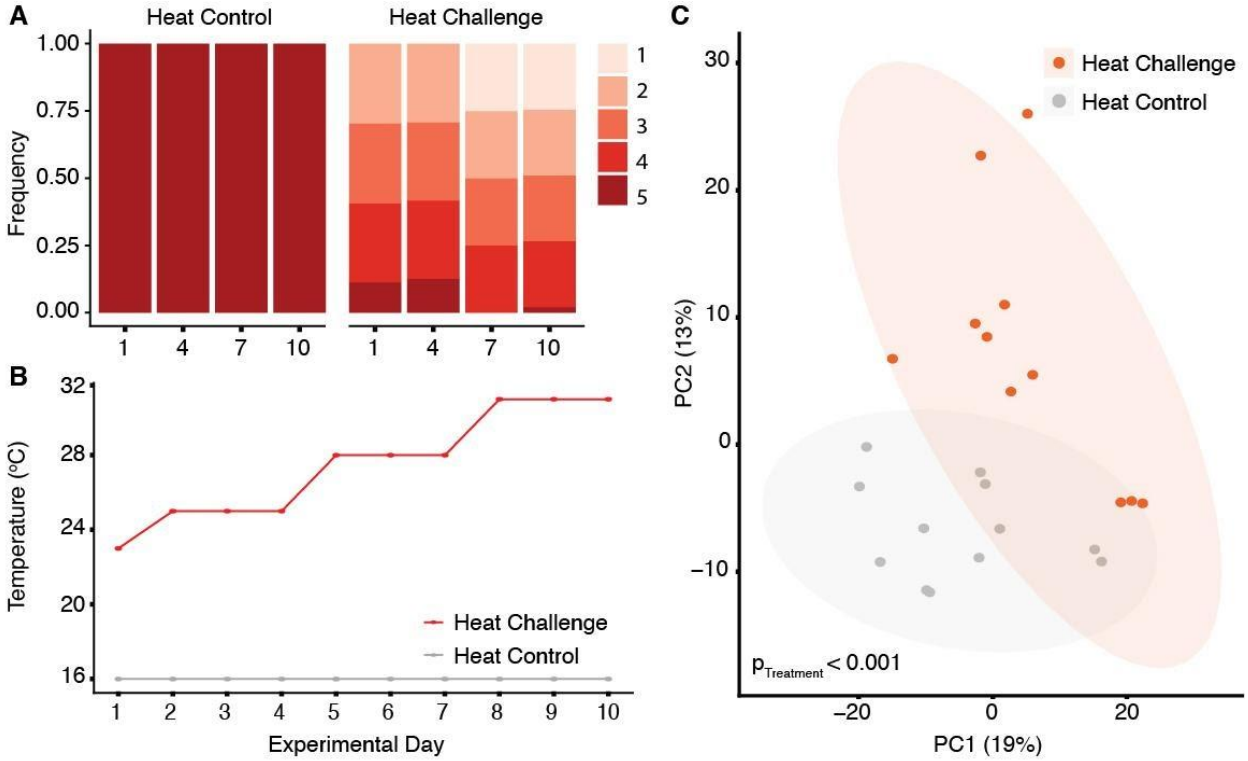

Figure 3 | *Astrangia poculata* behavioural and transcriptomic response to heat challenge. A) Frequency of behavioural scores (proportion of polyps extended per fragment, 1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100%) in response to food stimulus throughout the 10-day experiment (Left: Heat Control, Right: Heat Challenge, $p < 0.01$). B) Temperature profile of experimental tanks throughout the 16-day experiment, where red represents heat challenge and grey represents control conditions. C) Principal component analysis of overall gene expression with red symbols representing gene expression of coral fragments from heat challenge conditions and grey symbols representing gene expression of coral fragments under control conditions. Percentages represent the total amount of variance explained by each axis and shaded boxes are 95% confidence ellipses. P-value indicates the significance of treatment using a permutational multivariate analysis of variance.

266

267 *Convergent response repertoires to heat and cold challenge in* Astrangia poculata

268 Both sets of thermal challenges induced a reduction in polyp activity in response to food stimulus
269 (Figure 2A, 3A), however, there was a more pronounced reduction in behaviour to cold challenge
270 where nearly all polyps were retracted. There were seven times more genes that were differentially
271 expressed under cold challenge compared to heat challenge (Figure 4). More than half (267 out of
272 485) of DEGs in the heat challenge experiment were also differentially expressed in the cold
273 challenge experiment, which is significantly more genes shared between experiments than would
274 be expected by chance (hypergeometric test, $p < 0.05$). Genes that were up- or down-regulated
275 under both thermal challenges were visualized and the most notable highly up- or down-regulated
276 genes are highlighted in Figure 4B. These genes include *tumour necrosis receptor 3* (TRAF3),
277 *malignant in brain tumours 1* (DMBT1), *universal stress protein A* (USPA), *trypsin,* and
278 *oncoprotein-induced transcript 3* (OIT). GO terms enriched in both experiments were also plotted
279 by heat and cold experimental delta-ranks of enrichment for each comparison (FDR < 0.10; BP =
280 113, CC = 49, MF = 24, supplemental figures 2-4) and the top three similarly enriched GO terms
281 for each GO category have been highlighted (Table 1).

282

283 **Table 1 |** Top enriched GO terms conserved between cold and heat challenge experiments.

Biological Process	Molecular function	Cellular Component
Regulation of glucose import in response to insulin stimulus (GO:2001273)	Oxidoreductase activity, acting on a sulfur group of donors, quinone or similar compound as acceptor (GO:0016672)	Methylosome (GO:0034709)
Spliceosomal snRNP assembly (GO:0000387)	Death receptor activity (GO:0005035)	Azurophil granule membrane (GO:0035577)

Mitochondrial transmembrane transport (GO:1990542)	Prostaglandin-D synthase activity (GO:0004667)	Amphisome (GO:0044753)

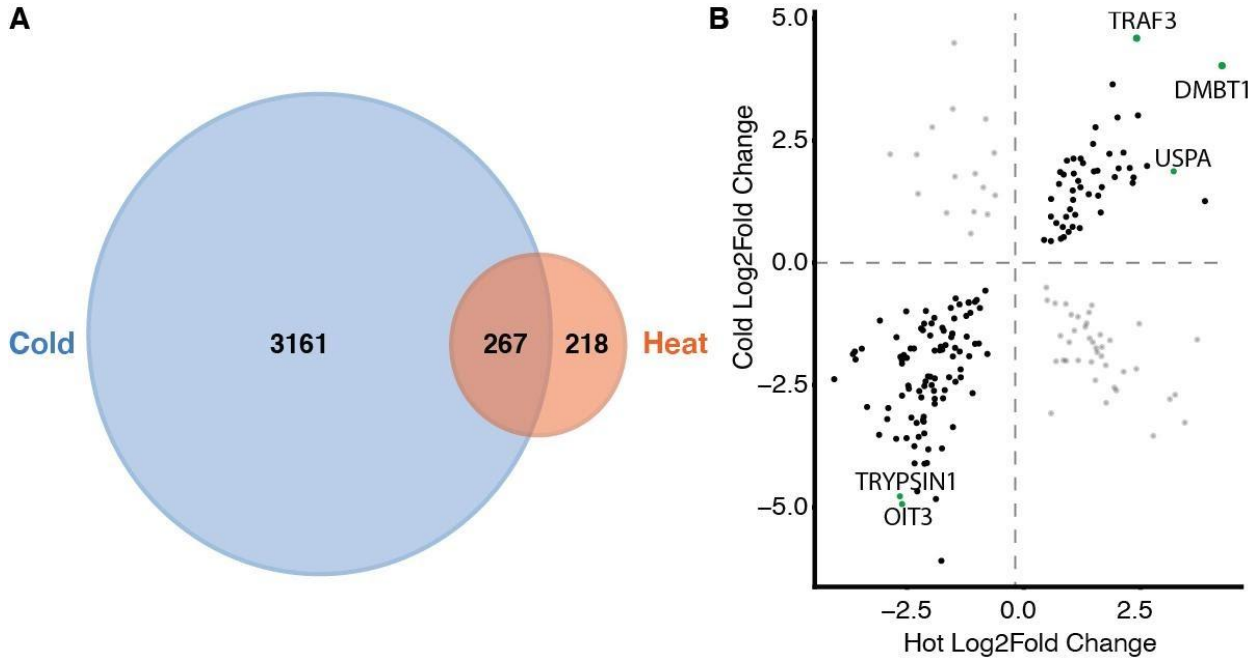

Figure 4 | *Astrangia poculata* transcriptomic response to heat and cold thermal challenge. **A)** Venn diagram of differentially expressed genes that are shared (intersection) between cold (blue) and heat (red) thermal challenges. These genes were designated as convergently responding genes (CRG). **B)** CRG (n = 267) under heat and cold thermal challenge with their respective log-2fold changes. Annotated CRG of interest that are consistently up- or down-regulated are highlighted and labelled with gene symbols. Highlighted up-regulated CRG include *tumour necrosis receptor 3* (TRAF3), *deleted in malignant brain tumours 1* (DMBT1), *universal stress protein A* (USPA). Highlighted down-regulated CRG include *trypsin1,* and *oncoprotein-induced transcript 3* (OIT).

297 ***Discussion***

298 Astrangia poculata *exhibit strong response to cold challenge*

299 *Astrangia poculata* from Woods Hole represents one of the most northern populations of the
300 species and these corals experience a wide range of temperatures throughout the year (Figure 1B).
301 Given this temperature range, it was surprising that such strong behavioural and transcriptomic
302 responses were observed under cold challenge (Figure 2A; 2C). This reduction in polyp activity
303 under cold temperatures is consistent with field observations during winter months, when corals
304 fail to respond to stimulus in the field [43]. This dormant polyp behaviour is possibly a quiescent
305 response to cope with long winter cold periods. However, very little is known about quiescence in
306 coral, and even less is known about what a transcriptomic signature of quiescence might look like.
307 Previous gene expression work described larval transcriptomic profiles associated with diapause
308 [44], which may have signatures similar to quiescence. Expression profiles associated with
309 diapause included cell cycle arrest, reduced transcription, increased ribosome production and
310 upregulation of oxidative stress defences [44]. Here, we observed strong overall transcriptomic
311 modifications associated with the cold challenge (Figure 2C), however, these gene expression
312 patterns were not consistent with diapause. Instead, we found GO enrichment that is consistent
313 with stress signatures in previous coral studies. SNAP receptor activity (GO:0005486) was highly
314 enriched under cold challenge, which has been implicated in stress from high solar irradiance and
315 is thought to be involved in coral bleaching [45]. Tumour necrosis factor–activated receptor
316 (GO:0005031), which is involved in regulating death receptor activity, was enriched and these
317 receptors have been heavily implicated in responses to heat-related stressors [27,46,47]. This stress
318 signature is further corroborated by enrichment of regulation of apoptotic signalling pathways
319 (GO:0097190) and cell death (GO:0008219), which were enriched under cold challenge (Fig S1).

320 Apoptosis is a universal response of severely stressed cells [48] and its upregulation suggests that
321 the cold challenge performed here elicited a strong stress response from *A. poculata*. This stress
322 response is intriguing considering that the temperatures achieved in this experiment were well
323 within the thermal range that these corals experience in their natural environment (Figure 1B).
324 However, we also acknowledge that the rate of temperature change is important and that in their
325 native environments these corals would experience these temperature shifts across seasons. We,
326 therefore, suspect that this rate of change contributed to the strong stress signature observed here.
327 We propose that future work would benefit from a gradual decrease in temperature to mirror the
328 natural temporal progression of the field more closely. In addition, field-collected samples across
329 seasons may be more likely to capture the gene expression signature of quiescence.
330

331 Astrangia poculata *exhibit subtle responses to extreme heat challenge*

332 Natural summer temperatures reached at Woods Hole over the last 10 years was much lower than
333 the heat challenge reached in our experiment (Figure 1B). It is therefore surprising that *A. poculata*
334 from this site did not exhibit extreme behavioural and transcriptomic responses under this heat
335 challenge given that the temperature was considerably higher than temperatures they ever
336 experience *in situ* (Figure 3A; 3C). Corals did exhibit reductions in polyp activity in response to
337 food stimulus under heat challenge, however, unlike observed polyp inactivity during winter
338 months [43], there is no prior evidence for a decrease in polyp activity due to warm temperatures
339 in this species. In tropical corals, polyp contraction can occur as a response to heat stress and often
340 precedes tissue necrosis [49]. We speculate that similar to the response to the cold challenge, this
341 reduced behaviour was most likely due to stress.
342

343 A coral's molecular response to heat stress has been meticulously explored in tropical reef-building
344 corals (as reviewed in [8]), however, this is one of the first studies to explore the molecular
345 response of an aposymbiotic reef-building coral to heat challenge in the absence of the algal
346 symbiont. Much like we observed in response to cold challenge, heat challenge elicited a stress
347 signature often observed in coral. For example, NF-kappaB complex (GO:0033256) which is
348 important to coral innate immunity [50] was enriched under heat challenge (Figure S1) and this is
349 consistent with other coral heat stress studies [51–53]. We also observed an enrichment of many
350 terms related to mitochondria, suggesting that *A. poculata* were exhibiting increased respiration
351 under heat challenge. Enrichment of respiration under high temperatures is not surprising given
352 that these patterns have been demonstrated in heat-tolerant coral larvae [42]. Interestingly,
353 enrichment of genes associated with respiration was also previously found under extreme pCO_2
354 stress in *Siderastrea siderea*, highlighting the potential for greater energetic demands associated
355 with the putative coral ESR [24]. Lastly, we observed an enrichment of regulation of catabolic
356 processes (GO:0009894), specifically the regulation of proteolysis (GO:0030162). Protein
357 catabolism is typical during starvation [54], as it's indicative of depleted stores of fats and
358 carbohydrates following the cessation of heterotrophy (i.e. reduced polyp extension), which would
359 be particularly detrimental to aposymbiotic corals. Overall, it appears that aposymbiotic *A.*
360 *poculata* mirror the hallmarks of heat stress responses that have been previously described in their
361 symbiotic tropical relatives. It would be interesting to pursue work contrasting how aposymbiotic
362 and symbiotic *A. poculata* respond to heat stress to see how symbiotic state modulates the heat
363 stress response.
364
365 *Cold challenge elicits a much stronger response than heat challenge in* A. poculata

366 Our data demonstrate that *A. poculata* exhibits greater behavioural and transcriptomic responses
367 to the cold challenge applied here relative to heat challenge, which is surprising considering that
368 the cold challenge temperatures achieved were within the ecological bounds of *A. poculata*'s
369 normal environmental temperature range (Figure 1B). This contrasts with heat challenge
370 temperatures, which far exceeded any temperature experienced within their native environment
371 and very likely reached their thermal maximum in their southern range. While few studies have
372 directly compared thermal extremes in corals, similar studies that have contrasted heat and cold
373 challenges in marine invertebrates have demonstrated mixed results. For example, Roth & Deheyn
374 found that acute cold stress was more detrimental to the tropical coral *Acropora yongei* than heat
375 stress, however, they did suggest that heat stress may be more detrimental over long term
376 timescales [55]. This contrasts with Nielsen *et al.* who found that *Acropora millepora* improved
377 its physiological condition under cold temperatures compared with ambient or heat conditions
378 [56]. Bellis & Denver found that heat stress caused greater bleaching in the sea anemone *Aiptasia*
379 than did cold stress [57] and Zhu *et al.* observed similar transcriptional responses of oysters to both
380 heat and cold stress [58]. While there is no clear consensus, it is widely accepted that the specific
381 temperatures reached in each stress treatment and the rate at which those temperatures are reached
382 are both important factors and these responses are dependent on the species-specific thermal niche
383 breadth [59]. For example, the heat challenge exposure in our study may have elicited a more
384 muted response because *A. poculata* were collected in the summer, so perhaps they were already
385 acclimated to warmer conditions. Future experimental designs should, therefore, focus on
386 assessing the influence of thermal challenges across seasons to better understand the influence of
387 these divergent thermal stressors.
388

389 *Convergent stress response repertoire to cold and heat challenge*

390 Our behavioural results align with field observations of *A. poculata* where polyp extension
391 diminishes during winter months [43]. This lack of polyp activity could be indicative of corals
392 entering a quiescent state, where they lower metabolic activity as an adaptation to cooler
393 temperatures. However, our behavioural data also suggest that this reduction in polyp activity is
394 convergent across thermal challenges (Fig 2A, 3A). Interestingly, our transcriptomic responses
395 under both thermal challenges show up-regulation of genes associated with catabolic activity,
396 particularly protein catabolism (Fig S2-3). Large scale protein catabolism often occurs during
397 starvation after an organism has utilized most of its carbohydrate and lipid stores [54], which is
398 consistent with reductions in polyp activities observed here under both thermal challenges. Given
399 that quiescence is associated with reduced metabolism, we suggest that even though our cold-
400 challenged corals were exhibiting quiescence phenotypes, increases in catabolic-related genes
401 point instead to high energetic demands associated with stress-related cell functions at both thermal
402 extremes [48].

403

404 In addition to *A. poculata* exhibiting similar behavioural responses to both heat and cold thermal
405 challenges, we also found a set of convergently responsive genes (CRG) that were consistently
406 regulated across thermal challenge experiments (Figure 4B). These CRG included upregulation of
407 *Deleted in malignant brain tumours 1* (DMBT1) under both thermal challenges. DMBT1 was
408 previously found highly up-regulated in corals that survived bacterial [26] and lipopolysaccharide
409 challenges [60]. Taken together, these data suggest that DMBT1 plays a role in both microbial and
410 broad thermal stress responses. Furthermore, *Tumour necrosis factor receptor 3* (TRAF3) was also
411 a highly up-regulated CRG under both thermal stressors. TRAF3 is an intracellular signalling

412 molecule that regulates mitogen-activated protein kinase activity and nuclear factor-κB (Nf-κB)
413 signalling [61], which has been shown to be upregulated during stress-induced bleaching in
414 *Aiptasia* [50]. TRAF3 has also been found to be constitutively up-regulated or "front-loaded" in
415 corals tolerant to heat stress [27,47,51] and has been found upregulated under low magnesium
416 [62], white band disease [63], and high carbon dioxide treatments [64]. Our results provide
417 evidence that TRAF3 may be a CRG that is consistently up-regulated in response to all stressors,
418 not just high temperatures.

419 *Universal stress protein A* (USPA) was also an up-regulated CRG under heat and cold
420 challenge (Fig 5B). USPA was previously found to be up-regulated under light stress [65] and, as
421 the name would suggest, USPA is produced in response to a large number of different
422 environmental stressors and is a highly conserved stress response gene in diverse taxa [66]. In
423 addition to up-regulated CRGs, a number of CRGs are similarly down-regulated in *A. poculata*
424 under both heat and cold challenge. These genes include *oncoprotein-induced transcript 3* (OIT),
425 which - like TRAF3 - was previously found to be frontloaded in thermally tolerant corals [27].
426 OIT3 has been found to be up-regulated under toxic stressors from a crude oil treatment [22] and
427 downregulated in response to low magnesium [62], providing evidence that this gene may serve a
428 fundamental role in stress responses more generally. *Trypsin* was also found to be a CRG, however,
429 it is not well established for its role in stress response in coral. Trypsin is involved in protein
430 catabolism [67] and it is therefore consistent with our findings that protein catabolism is being
431 regulated by both heat and cold challenges. Taken together, these CRG highlighted here appear to
432 be regulated in response to a wide range of stressors and may represent a core set of genes
433 comprising a generalized stress response.

434 In addition to CRG, we also found consistently enriched GO categories under both heat
435 and cold challenges that corroborate the biological functions of the CRGs highlighted above. For
436 example, regulation of glucose import in response to insulin stimulus (GO:2001273) was enriched
437 under both challenges (Table 1). While there is no direct evidence of insulin in corals, insulin-like
438 signalling has been reported to antagonize the transforming growth factor β pathway in sea sponges
439 (TGFβ) [68]. TGFβ signalling has been widely implicated in coral immunity and stress responses
440 including signalling apoptosis [69]. Apoptosis is a universal response of severely stressed cells
441 [48] and is associated with death receptor activity (GO:0008219), which was also enriched under
442 both heat and cold challenges. Corals that are sensitive to disease have also been observed to highly
443 upregulate apoptotic pathways, which have been suggested as clear indications of stress [25].
444 Overall, the enrichment of apoptosis-associated GO terms observed here under thermal challenges
445 likely indicate extreme stress and cell death and further confirm that the thermal challenges applied
446 here were likely achieved at too rapid a pace to allow for acclimation.
447

448 ***Conclusions***

449 While the stress response repertoire in tropical reef-building corals has been widely studied,
450 especially in response to hot thermal extremes, this study represents the first to characterize the
451 stress response of a naturally aposymbiotic coral to divergent thermal challenges. Our results
452 demonstrate that divergent thermal challenges elicit a convergent stress response in aposymbiotic
453 corals in the absence of symbiont-associated ROS. The CRG highlighted here will provide the
454 foundation for future research into how the added complexity of symbiosis influences the stress
455 response of coral. Overall, this work effectively highlights the benefits to studying facultatively
456 symbiotic corals to disentangle stress responses of the coral host from their algal symbionts and

457 future work leveraging this facultative relationship may lead to a stronger mechanistic
458 understanding of why coral dysbiosis is increasing in frequency in corals worldwide.
459

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470

471 *Data Availability*
472 All sequences are available from the NCBI SRI under accession PRJNA595158. Code for all
473 analyses are available by request or can be found at wuitchik.weebly.com/bioinformatics.
474

475 *Author Contributions*
476 S.W.D designed the experiment. A.A., S.A.B., J.D.C., M.B.L., J.L.R., M.K.S., and I.F.T.
477 conducted the experiment. B.E.B. and C.L.R. completed all molecular work and TagSeq library
478 preparations. D.M.W. performed all statistical and bioinformatic analyses and drafted the

manuscript. S.W.D. supervised the experiment, analyses and co-authored the manuscript. All authors edited and approved the manuscript.

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A
Cellular Components
49/162 catalytic step 2 spliceosome
129/433 spliceosomal complex
475/1596 ribonucleoprotein complex
87/274 P–body
55/192 large ribosomal subunit
25/103 mitochondrial large ribosomal subunit
196/746 organelle inner membrane
546/2194 mitochondrion
446/1700 mitochondrial part
27/70 NADH dehydrogenase complex
87/273 mitochondrial membrane part
69/178 inner mitochondrial membrane protein complex
47/119 respiratory chain complex
125/397 mitochondrial protein complex
70/187 oxidoreductase complex
635/2377 catalytic complex
337/1270 nucleoplasm part
774/2864 nucleoplasm
331/1379 transferase complex
304/1122 chromosomal part
9/19 I–kappaB/NF–kappaB complex
420/1564 extracellular space
478/1754 extracellular region
172/602 dendrite
418/1555 intrinsic component of plasma membrane
419/1658 plasma membrane region
116/377 transporter complex
B
Cellular Components
59/190 Sm–like protein family complex
25/62 U2 snRNP
11/25 U4 snRNP
148/448 spliceosomal complex
487/1626 ribonucleoprotein complex
30/84 precatalytic spliceosome
13/21 methylosome
10/20 post–mRNA release spliceosomal complex
63/166 catalytic step 2 spliceosome
424/1274 nucleoplasm part
15/31 proteasome core complex, alpha–subunit complex
30/71 proteasome core complex
930/2906 nucleoplasm
799/2389 catalytic complex
38/107 endopeptidase complex
53/144 peptidase complex
54/170 preribosome
26/56 phagophore assembly site
129/313 spindle pole
60/161 extracellular matrix component
611/1706 extracellular region
10/23 fibrillar collagen trimer
149/379 collagen trimer
195/545 extracellular matrix
110/283 collagen–containing extracellular matrix
112/306 endoplasmic reticulum lumen
11/14 interleukin–5 receptor complex
496/1560 extracellular space
128/343 cell–cell adherens junction
6/7 H zone
38/87 actin filament bundle
22/39 myosin II complex
7/20 nuclear pore cytoplasmic filaments
104/253 cation channel complex
61/131 potassium channel complex
79/247 intrinsic component of endoplasmic reticulum membrane
p < 1e–05
p < 1e–04
p < 0.001
C
Molecular Functions
338/1391 molecular transducer
86/352 transmembrane receptor protein kinase
123/532 protein tyrosine kinase
26/103 peptide receptor
143/509 G protein–coupled receptor
501/1847 calcium ion binding
7/9 prostaglandin–D synthase
93/353 metallopeptidase
297/1108 endopeptidase
462/1767 peptidase
165/655 metallopeptidase
123/468 serine hydrolase
85/300 symporter
126/483 secondary active transmembrane transporter
41/161 solute:cation symporter
197/749 active transmembrane transporter
23/71 neurotransmitter:sodium symporter
35/101 neurotransmitter transporter
364/1376 ion transmembrane transporter
275/957 cation transmembrane transporter
20/60 organic acid:sodium symporter
145/468 monovalent inorganic cation transmembrane transporter
53/186 sodium ion transmembrane transporter
30/100 solute:sodium symporter
39/90 voltage–gated potassium channel
166/603 passive transmembrane transporter
20/44 delayed rectifier potassium channel
137/439 cation channel
556/2103 transporter
119/422 gated channel
13/21 outward rectifier potassium channel
54/129 potassium ion transmembrane transporter
190/638 metal ion transmembrane transporter
317/1240 ubiquitin–like protein transferase
20/119 p53 binding
142/487 ubiquitin–like protein ligase binding
6/24 cullin family protein binding
41/151 ubiquitin–like protein binding
26/84 unfolded protein binding
56/190 oxidoreductase, acting on NAD(P)H
24/68 NADH dehydrogenase
29/103 oxidoreductase, acting on NAD(P)H, quinone or similar
587/2010 RNA binding
D
Molecular Functions
63/224 Rab GTPase binding
249/791 GTPase binding
903/2581 enzyme binding
7/15 receptor–receptor interaction
7/10 tumor necrosis factor–activated receptor
260/761 kinase binding
21/54 ubiquitin–like protein conjugating enzyme binding
63/155 ubiquitin–like protein binding
31/80 cytoskeletal adaptor
11/14 interleukin–5 receptor binding
94/292 molecular adaptor
15/28 syndecan binding
102/273 cadherin binding
24/76 growth factor receptor binding
35/71 protein N–terminus binding
145/439 cysteine–type peptidase
66/189 ubiquitin–like protein–specific protease
12/26 Lys63–specific deubiquitinase
19/32 channel inhibitor
31/79 SNAP receptor
8/23 BH domain binding
9/13 ATP–dependent peptidase
32/104 dipeptidase
107/397 exopeptidase
30/73 L–ascorbic acid binding
170/453 carbohydrate binding
111/306 organic acid binding
646/1840 calcium ion binding
7/8 prostaglandin–D synthase
104/202 extracellular matrix structural constituent
23/72 solute:cation antiporter
14/30 galactosidase
125/347 hydrolase, acting on glycosyl bonds
162/524 oxidoreductase, acting on CH–OH group of donors
12/22 carbonate dehydratase
32/73 threonine–type endopeptidase
6/8 glutathione disulfide oxidoreductase
p < 1e–04
p < 0.001
p < 0.01
E
Biological Processes
91/350 organic acid transport
147/602 anion transport
108/370 divalent inorganic cation transport
440/1632 ion transport
266/874 cation transport
176/652 sensory perception
13/52 glyoxylate metabolic process
42/134 G protein–coupled receptor signaling pathway, coupled to cyclic
148/535 G protein–coupled receptor signaling pathway
143/547 positive regulation of catabolic process
296/1093 regulation of catabolic process
225/847 regulation of proteolysis
304/1102 proteolysis involved in cellular protein catabolic process
598/2374 establishment of localization in cell
270/1101 intracellular protein transport
185/774 protein localization to organelle
67/240 endoplasmic reticulum to Golgi vesicle–mediated transport
340/1312 anatomical structure formation involved in morphogenesis
25/92 neural crest cell migration
211/773 tissue development
223/842 enzyme linked receptor protein signaling pathway
615/2400 cell surface receptor signaling pathway
400/1428 biological adhesion
72/212 positive regulation of epithelial cell migration
38/119 collagen metabolic process
397/1459 RNA processing
102/313 RNA splicing, via transesterification reactions
176/583 RNA splicing
281/973 mRNA metabolic process
292/1035 cellular protein–containing complex assembly
F
Biological Processes
22/43 necroptotic process
829/2402 positive regulation of response to stimulus
689/2087 regulation of intracellular signal transduction
168/490 regulation of apoptotic signaling pathway
642/1919 regulation of cell death
20/53 positive regulation of lipase
808/2281 regulation of protein modification process
776/2327 intracellular signal transduction
378/1241 cell death
26/64 response to ischemia
214/610 regulation of cellular protein localization
389/1185 regulation of protein localization
327/1002 regulation of cellular localization
149/373 negative regulation of transport
333/1055 positive regulation of transcription by RNA polymerase II
982/2946 positive regulation of gene expression
777/2409 establishment of localization in cell
17/23 regulation of extracellular exosome assembly
808/2510 establishment of protein localization
375/1112 intracellular protein transport
24/36 negative regulation of ubiquitin–dependent protein catabolic process
404/1103 regulation of catabolic process
192/513 regulation of protein catabolic process
158/432 regulation of cellular protein catabolic process
39/86 negative regulation of cellular protein catabolic process
407/1125 proteolysis involved in cellular protein catabolic process
523/1548 macromolecule catabolic process
66/191 protein modification by small protein removal
552/1436 biological adhesion
452/1492 RNA processing
118/331 RNA splicing, via transesterification reactions
195/601 RNA splicing
312/1005 mRNA metabolic process
104/387 ribonucleoprotein complex subunit organization
p < 1e–07
p < 1e–06
p < 1e–05

Figure S1 | Gene ontology categories significantly enrichment (red: heat challenge, blue: cold challenge). Font identifies adjusted-value from Mann-Whitney U test. Dendrogram clustering similar GO categories.

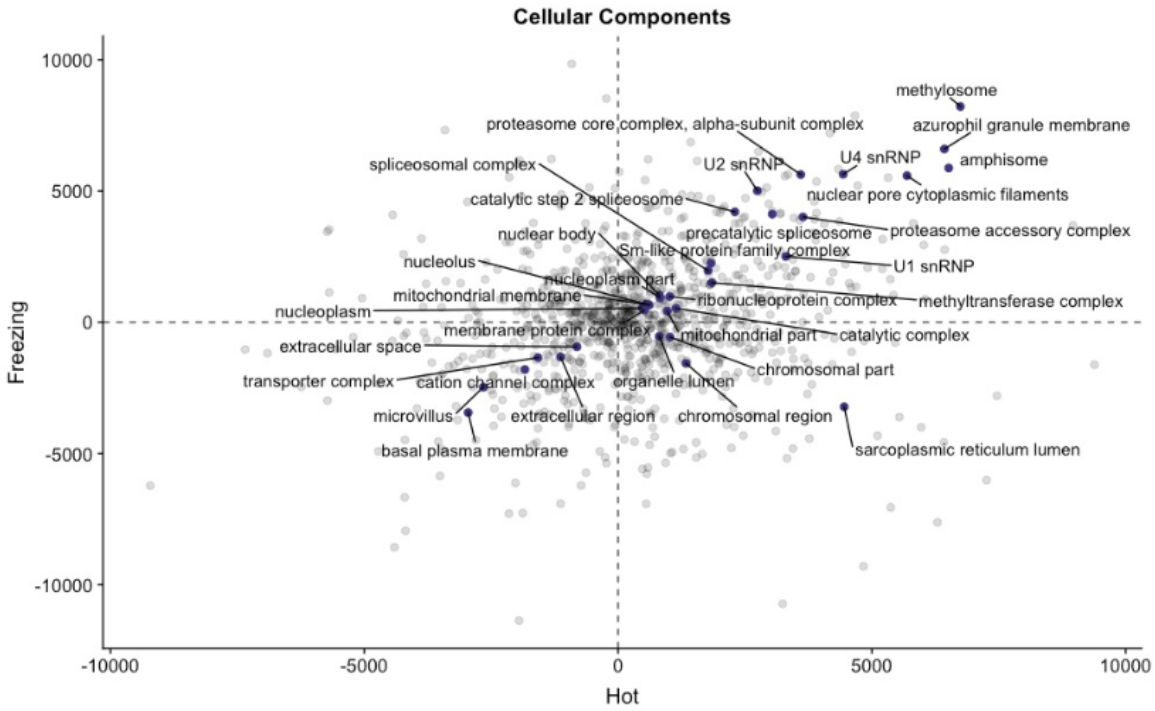

Figure S2| Delta-ranks of enriched cellular components between cold and heat challenge experiments.

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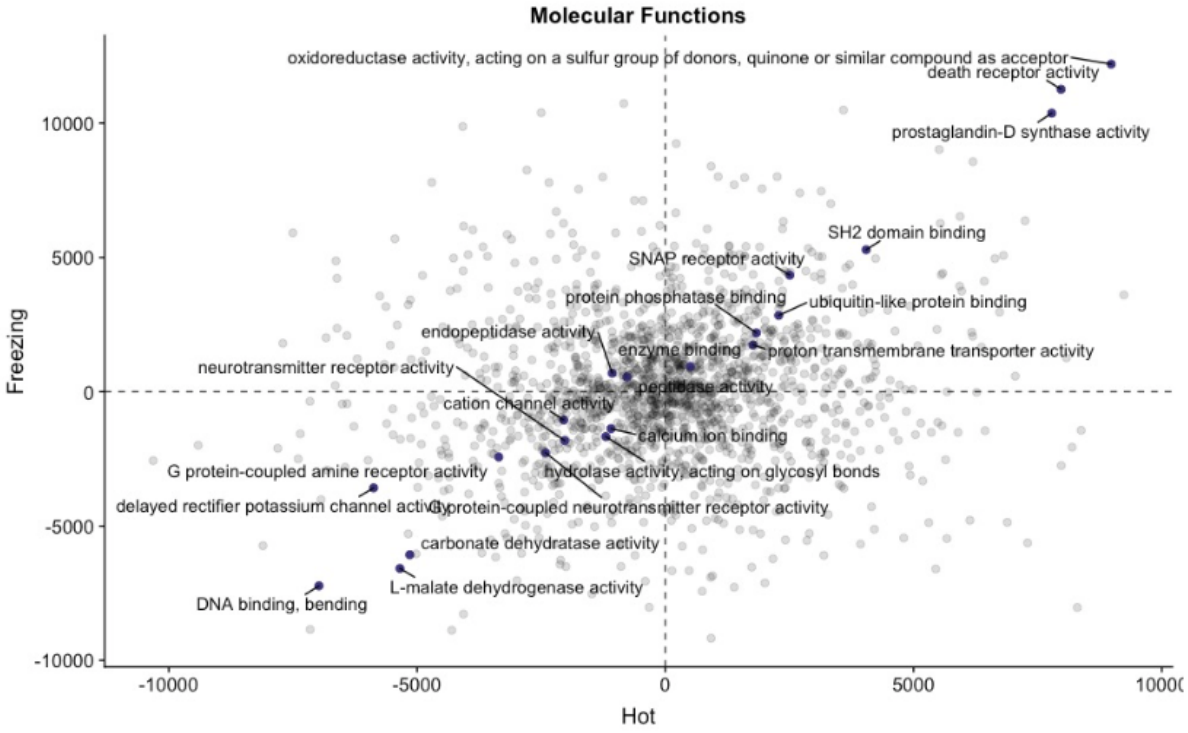

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677 **Figure S3|** Delta-ranks of enriched molecular functions between cold and heat challenge

678 experiments.

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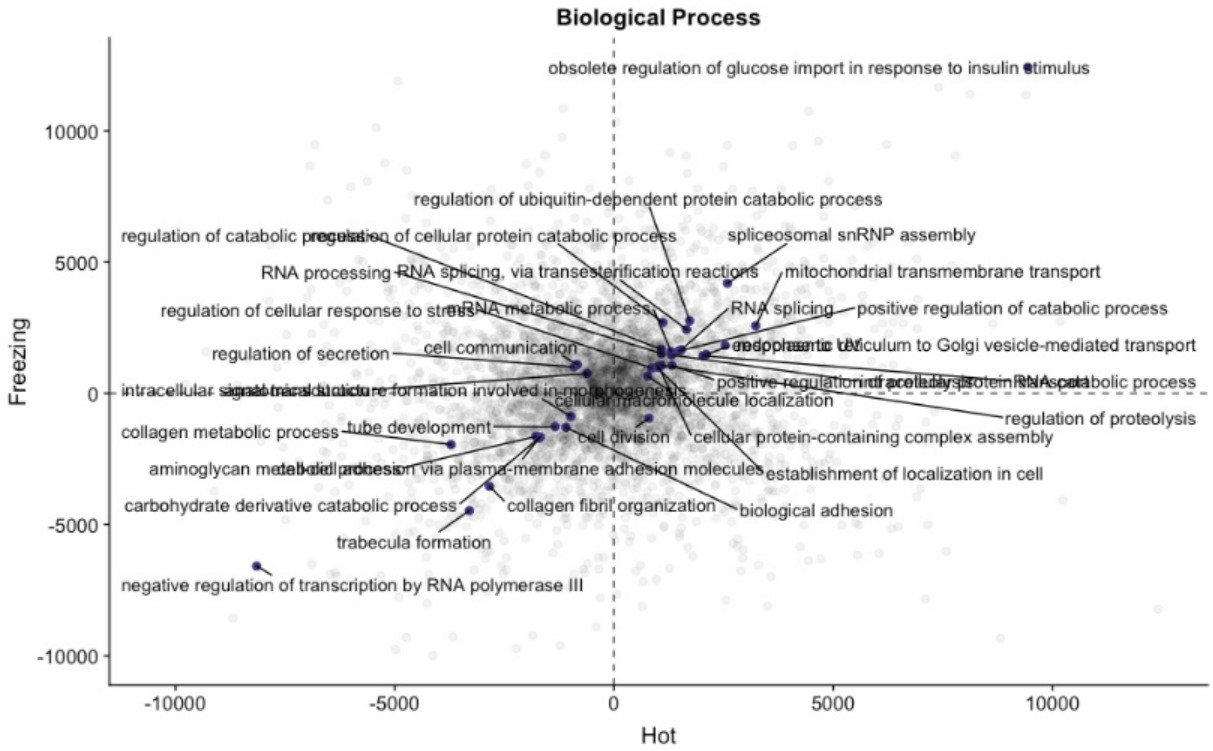

Figure S4| Delta-ranks of the enriched biological process between cold and heat challenge experiments.