Genome-Wide Transcription Response of *Staphylococcus epidermidis* to Heat Shock and Medically Relevant Glucose Levels

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ABSTRACT

Skin serves as both barrier and interface between body and environment. Skin microbes are intermediaries evolved to respond, transduce, or act in response to changing environmental or physiological conditions. Here, we quantify genome-wide changes in gene expression levels for one abundant skin commensal, *Staphylococcus epidermidis*, in response to an internal physiological signal, glucose levels, and an external environmental signal, temperature. We find 85 of 2354 genes change up to ~34-fold in response to medically relevant changes in glucose concentration (0 mM to 17 mM; adj P value ≤ 0.05). We observed carbon catabolite repression in response to a range of glucose spikes, as well as upregulation of genes involved in glucose utilization in response to persistent glucose. We observed 366 differentially expressed genes in response to a physiologically relevant change in temperature (37°C to 45°C; adj P value ≤ 0.05) and an *S. epidermidis* heat-shock response that mostly resembles the heat-shock response of related staphylococcal species. DNA motif analysis also revealed CtsR and CIRCE operator sequences arranged in tandem upstream of *dnaK* and *groESL* operons. We further identified 38 glucose-responsive genes as candidate ON or OFF genes for use in controlling synthetic genetic systems. Such systems might be used to instrument the in-situ skin microbiome or help control microbes bioengineered to serve as embedded diagnostics, monitoring, or treatment platforms.

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Keywords: *Staphylococcus epidermidis*, skin, transcriptomics, glucose, diabetes, heat-shock, synthetic biology

48 **INTRODUCTION**

49

50 Skin serves as both a barrier to the external environment and home to diverse microbial
51 communities. Skin bacteria play a significant role in promoting and maintaining human health,
52 contributing to skin barrier homeostasis (Zheng et al., 2022), influencing our immune system
53 (Leech et al., 2019), and limiting pathogen invasion (Nakatsuji et al., 2017; Williams et al., 2019).
54 One abundant skin commensal is *Staphylococcus epidermidis,* a Gram-positive coagulase-negative
55 bacterium.

56

57 *S. epidermidis* has emerged as a promising microbial chassis to enable development of engineered
58 microbes with enhanced functionality. For example, Chin et al. engineered an *S. epidermidis* strain
59 to produce tumor-associated antigens unique to melanoma, an aggressive type of metastatic skin
60 cancer. When mice were colonized with the engineered *S. epidermidis* strain, a robust antitumor T
61 cell response against localized and metastatic melanoma was generated (Chin et al., 2023). As a
62 second example, Azitra, Inc. indicates they are engineering *S. epidermidis* strains to deliver
63 therapeutic proteins to treat skin diseases including Netherton Syndrome and to improve skin
64 appearance (Azitra, 2023).

65

66 Unfortunately, the tools and knowledge needed to study and reprogram *S. epidermidis* are quite
67 limited compared to those available for established model organisms such as *Escherichia coli* or
68 *Saccharomyces cerevisiae.* Introduction of new genes and predictable control of heterologous gene
69 expression remain considerable challenges in bioengineering *S. epidermidis.* The nascent *S.*
70 *epidermidis* knowledge base and toolkit contains methods for transformation (Monk et al., 2012;
71 Costa et al., 2017), methods for conjugation (Brophy et al., 2018), and a small number of

72 functionally validated promoters for control of gene expression: sarA-P1 (Bayer, Heinrichs and
73 Cheung, 1996), P_{pen} (Meredith, Swoboda and Walker, 2008), IPTG-inducible P_{spank} (Rokop,
74 Auchtung and Grossman, 2004), and xylose-inducible P_{xylR} (Franke et al., 2007). While successful
75 attempts have been made to identify and characterize constitutive promoters in related
76 staphylococcal species including *Staphylococcus aureus* (Liu et al., 2022), native transcription
77 control elements that can serve as starting points for endogenous and dynamic control of
78 bioengineered circuits have not yet been well characterized in *S. epidermidis.*
79
80 One application of bioengineered skin microbes could be to detect or respond to blood glucose
81 levels, which could help in the diagnosis or treatment of diabetes. Commensal skin microbes such
82 as *S. epidermidis* reside in subepidermal compartments of the skin with proximity to blood vessels,
83 such as the dermis and subcutaneous adipose tissue (Nakatsuji et al., 2013; Bay et al., 2020). Such
84 proximity could potentially facilitate the development of an engineered *S. epidermidis* strain that
85 can sense and respond to elevated blood glucose levels (i.e., > 7mM) as a therapeutic strategy for
86 diabetes, a chronic endocrine disorder characterized by elevated blood glucose levels and poor
87 glycemic control (World Health Organization, 2023). To make such work practical, one would
88 need to implement within *S. epidermidis* a transcription-based biosensor responsive to elevated
89 blood sugar levels that results in well-regulated and rapid production of single-chain insulin. Such
90 a use case supports the need for better characterization of glucose-inducible *S. epidermidis*
91 regulatory elements.
92
93 Another class of applications for bioengineered skin microbes could be in response to
94 environmental or physiological (e.g., exercise-induced) changes in temperature. With globally

95 increasing intensity, frequency, and duration of heat waves (Perkins-Kirkpatrick and Lewis, 2020),
96 there may be value in better understanding how commensal skin bacteria, including *S. epidermidis*,
97 adapt and respond to increases in temperature. While the heat-shock response has been well
98 characterized in related staphylococcal species and other prokaryotes, only three efforts have
99 investigated the *S. epidermidis* heat-shock response by using semi-quantitative protein assays
100 (Ooronfleh, Streips, and Wilkinson, 1990), focusing on only a small number of genes
101 (Vandecasteele et al., 2001) or using comparative genomics (Chastanet, Fert, and Msadek, 2003).
102 We thus chose to also quantitatively explore the genome-wide transcription response of *S.*
103 *epidermidis* to heat shock, both as a reference case for glucose response and, for its own merits.
104
105 We investigated genome-wide transcription response in the non-biofilm forming, nonpathogenic
106 *S. epidermidis* strain (ATCC 12228) to heat shock and medically relevant glucose concentrations.
107 We performed RNA sequencing on samples exposed to a sudden temperature increase and a
108 glucose challenge to investigate the ability of the organism to adapt and respond to changing
109 environmental conditions. We used differential expression analysis of samples taken during the
110 mid-exponential growth phase to identify candidate genes that are either upregulated or
111 downregulated in response to each condition. We further curated a subset of glucose-responsive
112 genes that might serve as templates for ON or OFF switches.
113
114
115
116

117 **MATERIALS AND METHODS**
118

119 **Bacterial Strain and Culture**

120 We started each *S. epidermidis* ATCC 12228 culture from a fresh colony plate (< 7 days old) using
121 a single colony. We used Tryptic Soy Broth (TSB) without Dextrose (BD 286220) as the culture
122 medium for all experiments.

123

124 **Heat-Shock Experiments**

125 We grew overnight broth cultures in fresh medium supplemented with 0.2% w/v glucose for 18 h
126 at 37°C with shaking. Cultures were then diluted 32-fold in fresh medium supplemented with 0.2%
127 w/v glucose and grown at 37°C with shaking. When cultures were in mid-exponential phase (OD_{600}
128 ~ 0.5), we transferred them to pre-warmed Erlenmeyer flasks followed by incubation at 45°C for
129 10 minutes. We then harvested cultures for RNA sequencing (below). Control cultures in mid-
130 exponential phase were not exposed to heat shock but instead were immediately harvested for
131 RNA sequencing. We performed our heat-shock experiments in triplicate to generate three
132 biological replicates.

133

134 **Glucose Challenge Experiments**

135 We grew overnight broth cultures in fresh medium supplemented with 13.9 mM glycerol for 25 h
136 at 37°C with shaking. We then diluted cultures 50-fold in fresh medium supplemented with 13.9
137 mM glycerol and continued growth at 37°C with shaking. When cultures were in mid-exponential
138 phase (OD_{600} ~ 0.5), we added glucose and measured the glucose concentration (2 mM, 5 mM, 10

139 mM, 17 mM, or 50 mM) of each culture using the Contour NEXT ONE Blood Glucose Monitoring
140 System. We added an equivalent volume of fresh medium lacking glucose to the control cultures.
141 We grew cultures at 37°C with shaking for an additional 20 minutes and then harvested for RNA
142 sequencing (below). We performed our glucose challenge experiments in triplicate to generate
143 three biological replicates.

144

145 **Step-down Experiments**

146 We grew overnight broth cultures in fresh medium supplemented with 13.9 mM glycerol for 25 h
147 at 37°C with shaking. We diluted cultures 50-fold in fresh medium supplemented with 13.9 mM
148 glycerol and continued growth at 37°C with shaking. When cultures were in mid-exponential phase
149 (OD_{600} ~ 0.5), we added glucose and measured the glucose concentration (10 mM) of each culture
150 using the Contour NEXT ONE Blood Glucose Monitoring System. Cultures were then grown at
151 37°C with shaking for 20 minutes and then pelleted at 5,000 xg for 10 minutes at 24°C. We then
152 resuspended the pellets in fresh medium supplemented with 2 mM glucose. We grew cultures at
153 37°C with shaking for an additional 20 minutes and harvested for RNA sequencing (below). We
154 used the 10 mM glucose challenge condition (above) as the control condition for our step-down
155 experiments. We performed our step-down experiments in triplicate to generate three biological
156 replicates.

157

158 **Batch Culture Experiments**

159 We grew overnight broth cultures in fresh medium supplemented with glucose (0.2% w/v or 1%
160 w/v) for 18 h at 37°C with shaking. We measured the glucose concentration of each culture using

161 the Contour NEXT ONE Blood Glucose Monitoring System. We diluted cultures 32-fold in fresh
162 medium supplemented with glucose (0.2% w/v or 1% w/v) and grew at 37°C with shaking. We
163 harvested mid-exponential phase cultures (OD_{600} ~ 0.5) for RNA sequencing (below). We
164 performed our batch culture experiments in duplicate to generate two biological replicates.

165

166 **RNA Stabilization and Extraction**

167 Immediately after each experiment, we pelleted samples by centrifugation at 5,000 x g for 10
168 minutes at 4°C and then resuspended the pellets in RNAlater (Invitrogen AM7021); samples were
169 incubated in RNAlater at 4°C for 24 h. After incubation, we pelleted samples by centrifugation at
170 5,000 x g for 10 minutes at 4°C and resuspended the pellets in 1µl of 100X TE Buffer, 50 µl of
171 lysostaphin (1 mg ml^{-1}), and 50 µl of mutanolysin (5KU ml^{-1}). We performed lysis for 25 minutes
172 at 37°C with vortexing at 5-minute intervals. We then treated samples with 25 µl of Proteinase K
173 (Qiagen 19131) and incubated for an additional 30 minutes at 37°C. We added 700 µl of Buffer
174 RLT (Qiagen 79216) to each sample and vortexed vigorously for 5 to 10 seconds. We transferred
175 the resulting suspension to a 2 ml Safe-Lock tube (Eppendorf 0030123620) and mechanically
176 disrupted the samples using a TissueLyser LT (Qiagen 85600) for 5 minutes at maximum speed
177 with intervals of 30 seconds of bead beating and 30 seconds of resting on ice. After bead beating,
178 we centrifuged the samples in an Eppendorf MiniSpin (022620100) for 15 seconds at maximum
179 speed (12,100 x g) and then transferred the supernatant to a new tube. We mixed the supernatant
180 well with an equal volume of 100% ethanol by pipetting. We applied this mixture to a RNeasy
181 Mini spin column and extracted RNA according to the manufacturer's instructions using a RNeasy
182 Mini Kit (Qiagen 74106). We performed on-column DNase digestion using the RNase-Free DNase
183 Set (Qiagen 79254). We eluted samples in RNase-free water according to the manufacturer's

184 instructions and stored recovered RNA at -80°C until library preparation. We used RNaseZap
185 RNase Decontamination Solution (Invitrogen AM9780) on all surfaces to prevent RNA
186 degradation. RNA quality was analyzed using an Agilent Bioanalyzer and quantified by a Qubit
187 fluorometer according to manufacturer's instructions. Our RNA integrity number (RIN) values
188 ranged from 8.0 to 10.

189

190 **Library Preparation and Sequencing**

191 We used Novogene Co., LTD (Beijing, China) to carry out our rRNA depletion, cDNA library
192 preparation, and sequencing as part of their Prokaryotic RNA Sequencing service. cDNA libraries
193 were sequenced on an Illumina NovaSeq 6000 Sequencing System with a 150 bp paired-end run
194 configuration to a depth of ~30 million reads.

195

196 **Raw Sequence Data Quality Control & Processing**

197 We processed raw reads (FASTQ files) using FastQC v0.12.1 (Andrews, 2010) with default
198 settings to assess initial read quality and then examined the results using MultiQC v1.14 (Ewels et
199 al., 2016). We processed FASTQ files using Trim Galore v0.6.10 (Krueger, 2012) with default
200 settings to trim low-quality (Phred score < 20) ends from reads and to trim auto-detected adapters.
201 Reads that became shorter than 20 bp because of either quality or adapter trimming were discarded.

202

203 **Reference Genome for Mapping**

204 We used the *Staphylococcus epidermidis* ATCC 12228 genome assembly ASM987345v1
205 (GenBank accession GCA_009873455.1, RefSeq accession GCF_009873455.1) from NCBI in the
206 FASTA format along with information on genes and other features in the GFF format. The genome
207 consists of a chromosome (GenBank accession CP043845.1, RefSeq accession NZ_CP043845.1)
208 of size 2,504,425 bp and a plasmid (GenBank accession CP043846.1, RefSeq accession
209 NZ_CP043846.1) of size 21,978 bp. We converted GFF features to GTF format by using the
210 *gffread* program in the Cufflinks v2.2.1 package (Trapnell et al., 2010) and to BED format by
211 using the AGAT v1.0.0 toolkit (Dainat, 2019) for use in downstream analysis.

212

213 **Mapping and Transcript Quantification**

214 We used Bowtie2 v2.5.1 (Langmead and Salzberg, 2012) to build a Bowtie index from the *S.*
215 *epidermidis* ATCC 12228 genome assembly ASM987345v1 before mapping the RNA-Seq reads
216 in the paired-end FASTQ files to this reference genome using default settings. The resulting BAM
217 files were coordinate-sorted and indexed; alignment summary statistics were reported using
218 SAMtools v1.17 (Danecek et al., 2021). We ran RSeQC v5.0.1 (Wang, Wang, and Li, 2012) on
219 the sorted BAM files to determine the strandedness of the reads for the strand-specific RNA-seq
220 data. We used the *featureCounts* in the Subread v2.0.6 package (Liao, Smyth, and Shi, 2013) to
221 count mapped reads at both the transcript and gene levels from sorted BAM files for genomic
222 features such as CDSs, based on previously determined read strandedness. We merged counts from
223 each sample at both the transcript and gene levels. We used the resulting merged count matrices
224 in subsequent differential expression analysis.

225

226 **BLASTP Homology Search**

227 The KEGG Pathway Database (Kanehisa and Goto, 2000) Genome Entry T00110 (Org code: sep)
228 lists genome assembly ASM764v1 (GenBank accession GCA_000007645.1, RefSeq accession
229 GCF_000007645.1) as the reference genome for *S. epidermidis* ATCC 12228. Genome assembly
230 ASM764v1 uses alternate gene designations compared to the genome assembly ASM987345v1
231 used in this study. To leverage KEGG pathway gene sets for Gene Set Enrichment Analysis
232 (GSEA), we conducted a BLASTP homology search between the two genome assemblies using
233 NCBI BLAST+ executable v2.14.0+ (Camacho et al., 2009) to find genes in genome assembly
234 ASM987345v1 with the highest degree of homology to genes in genome assembly ASM764v1
235 thereby enabling cross-mapping of the genes represented in KEGG Pathway Gene Sets.

236

237 **Differential Expression Analysis**

238 We used principal component analysis (PCA) to first visualize the expression data; we applied a
239 regularized log (rlog) transformation to all expression data. We then visualized sample-to-sample
240 distances via PCA and found that one replicate from the step-down experimental condition was
241 over 4-fold off on the second principal component against all other experimental samples, and over
242 10-fold off on the first principal component against the two other step-down samples (Figure S1).
243 We thus excluded the data from this one step-down replicate in all further analyses. We then
244 analyzed data from non-transformed count matrices using the DESeq2 R package (Love, Huber,
245 and Anders, 2014), which can evaluate differential expression on as few as two biological
246 replicates. We defined differentially expressed genes (DEGs) of significance using the following
247 criteria: |log2 fold change| (i.e., log2FC) ≥ 1.5 and adjusted P value ≤ 0.05. We applied the apeglm

248 (log fold change shrinkage) method (Zhu, Ibrahim, and Love, 2018) to the raw counts to stabilize
249 variability in log fold change calculations. We then constructed volcano plots using the
250 EnhancedVolcano R package (Blighe, Rana, and Lewis 2023) and further customized them using
251 ggplot2 (Wickham, 2016). We designed Circle plots using shinyCircos (Yu, Ouyang, and Yao,
252 2017). We also constructed the two scatter plots, visualizing the relationship between the heat-
253 shock and G17 experimental conditions and between the step-down and G2 experimental
254 conditions, using ggplot2.

255

256 **Pathway and Gene Identification**

257 We explored gene functions using the KEGG and GO pathways database and manually curated a
258 gene annotation table, drawing from the KEGG (organism code *sep*), BioCyc (GCF_000007645),
259 and UniProt databases. After determining gene-to-pathway annotations, we used the GSEA tool
260 (Subramanian et al., 2005; Mootha et al., 2003) and the fgsea R package (Korotkevich et al., 2021)
261 to conduct gene set enrichment analysis. We used Fisher's method to combine results that
262 overlapped across GSEA and fgsea, creating a single *P* value that reflected the two independent
263 adjusted *P* values. We reduced GO term redundancy using REVIGO (Supek et al., 2011), with
264 default parameters and a "small (0.5)" resulting list. Once KEGG and GO enriched pathways were
265 identified, we performed independent research to cross-validate the results and combined
266 pathways that were identified in both KEGG and GO databases.

267

268 **Switch Identification**

269 We identified switches using the DRomics package, a tool used for concentration-response (or
270 dose-response) characterization from -omics data (Marie Laure Delignette-Muller et al., 2023;
271 Floriane Larras et al., 2018). We modeled all genes with an absolute log fold change ≥ 2. We
272 performed a rlog transform on gene counts and then used DRomics to identify the appropriate best-
273 fit monophasic or biphasic model; genes that failed to model due to a slope near zero were deemed
274 dose-insensitive.

275

276 **Batch Culture Bioinformatics Analysis**

277 Novogene (Beijing, China) completed bioinformatics analyses for our batch culture experimental
278 condition as part of their Prokaryotic RNA Sequencing standard analysis. **Raw Sequence Data**
279 **Quality Control:** Novogene processed raw reads (FASTQ files) using Fastp (Chen et al., 2018).
280 Clean data for downstream analysis were obtained by removal of low-quality reads, adapters, and
281 poly-N sequences. **Reference Genome and Mapping:** Novogene obtained the reference genome
282 (GenBank accession GCA_009873455.1, RefSeq accession GCF_009873455.1) and gene model
283 annotation files from NCBI and aligned clean reads to the reference genome using Bowtie2
284 (Langmead and Salzberg, 2012). **Transcript Quantification:** Novogene used *FeatureCounts*
285 (Liao, Smyth, and Shi, 2013) to count reads mapped to each gene and then calculated the fragments
286 per kilobase of transcript per million fragments mapped (*FPKM*) of each gene based on gene length
287 and read counts mapped to the gene (Trapnell et al., 2010). **Differential Expression Analysis:**
288 Novogene performed differential expression analysis using the DeSeq2 R package (Love, Huber,
289 and Anders, 2014) and adjusted *P* values using the Benjamini and Hochberg method for controlling
290 the false discovery rate (Benjamini and Hochberg, 1995). Differentially expressed genes (DEGs)

291 of significance were defined using the following criteria: |log2 fold change| (i.e., log2FC) ≥ 1.5

292 and adjusted *P* value < 0.05.

293

294 **Data Deposition and Availability**

295 The original contributions presented in the study are publicly available. The data discussed in this

296 publication have been deposited in NCBI's Gene Expression Omnibus (Benjamin et al., 2024) and

297 are accessible through the GEO Series accession number GSE261664.

298 **RESULTS**

299

300 The heat-shock response (HSR), a transcription program observed in several eukaryotes and
301 prokaryotes, is a crucial strategy whereby cells adapt to a sudden temperature increase or other
302 environmental stresses (Cao et al., 1999). The HSR helps cells maintain protein homeostasis by
303 protection from heat-induced protein denaturation, misfolding, and aggregation. HSR has been
304 studied in detail in *Escherichia coli*, *Streptomyces* spp., and *Bacillus subtilis* (Lemaux et al., 1978;
305 Guglielmi et al., 1991; Schumann, 2003). While the HSR is highly conserved across prokaryotes,
306 the regulatory mechanisms that govern the expression of heat-shock genes exhibit great diversity
307 among bacterial species (Roncarati and Scarlato, 2017; Schumann, 2016). Prior studies of the HSR
308 in *S. aureus* (Chastanet, Fert, and Msadek, 2003; Anderson et al., 2006; Fleury et al., 2009) and
309 the gram-positive model organism *B. subtilis* provide a context from which to increase our
310 understanding of the HSR of *S. epidermidis* and other low-GC content gram-positive bacteria.

311

312 **Differential Gene Expression in *S. epidermidis* Under Heat Stress**

313

314 To identify differentially expressed genes in heat-shocked *S. epidermidis* ATCC 12228 cells, we
315 shifted mid-exponential phase cells from physiological growth (37°C) to heat-shock conditions
316 (45°C) for 10 minutes (Figure 1A). We used RNA sequencing to analyze gene expression profiles
317 and then compared the expression profiles of heat-shocked cells to those of unstressed cells.
318 Differentially-expressed genes (DEGs) of significance were defined using the following criteria:
319 |log2 fold change| (i.e., log2FC) ≥ 1.5 and adjusted *P* value ≤ 0.05. By these criteria, we identified
320 366 of 2354 genes (~15.5% of the genome) with log2FC values ≥ 1.5, among which 235 were

321 upregulated and 131 were downregulated (Table S1, Table S2). Downregulated and upregulated
322 genes were expressed over a -4 to +6 log2FC range (Figure 2A).
323
324 We observed increased expression of several heat-shock genes well-characterized in other
325 organisms (Anderson et al., 2006; Fleury et al., 2009; Schumann, 2003). For example, transcript
326 levels of the *dnaK* (*hrcA*, *grpE*, *dnaK*, *dnaJ*, *prmA*), *groESL* (*groES*, *groL*), and *clpC*
327 (F1613_RS04215 (*CtsR* family transcription regulator), F1613_RS04220 (UvrB/UvrC motif-
328 containing protein), F1613_RS04225 (protein arginine kinase), F1613_RS04230 (ATP-dependent
329 Clp protease ATP-binding subunit *clpC*)) operons, encoding the major cell chaperones and
330 proteases, were upregulated ~8-15, ~10-11, and ~42-53 absolute fold, respectively (Table S1).
331 Other known heat-shock genes including *clpB*, *clpP*, the Hsp33 family molecular chaperone *hslO*,
332 and MecA, an adaptor protein necessary for ClpC chaperone activity (Schlothauer et al., 2003)
333 were upregulated by 71-, 8.9-, 4.14-, and 3.84-fold, respectively (Table S1). Among the most
334 upregulated genes (~22-61-fold) were members of the *lac* operon (*lacA, lacB,* F1613_RS11920
335 (tagatose-6-phosphate kinase), *lacD*, F1613_RS11910 (PTS lactose/cellobiose transporter subunit
336 IIA), F1613_RS11905 (lactose-specific PTS transporter subunit EIIC), *lacG*), vraX,
337 F1613_RS03870 (ArgE/DapE family deacylase), cytochrome ubiquinol oxidase subunits I and II
338 (F1613_RS06745 and F1613_RS06750), F1613_RS01555 (MarR family transcription regulator),
339 F1613_RS12445 (hypothetical protein), F1613_RS01550 (NAD(P)/FAD-dependent
340 oxidoreductase), and F1613_RS03780 (MFS transporter) (Table S1).
341
342 We observed other upregulated genes of potential interest. For example, *BlaZ*, *blaI*, and *blaR1*,
343 components of the *bla* operon that encode for a β-lactamase (Llarrull, Prorok and Mobashery,

344 2010) were upregulated ~4.8-18.3-fold. Members of the urease operon (F1613_RS12320, *ureE*,
345 F1613_RS12330) along with two competence protein ComK orthologs (F1613_RS10000 and
346 F1613_RS06475) displayed increased transcript levels, consistent with previous observations of
347 genes induced by heat shock in *S. aureus* (Anderson et al., 2006; Fleury et al., 2009). Twenty-three
348 hypothetical proteins and 24 uncharacterized genes (47 total) were also upregulated under heat-
349 shock conditions.
350
351 Among the most downregulated genes (~10-21-fold) were F1613_RS05940 and *dltABCD*,
352 components of the *dlt* operon required for the d-alanylation of teichoic acids in gram-positive
353 bacterial cell walls (Kovacs et al., 2006) (Table S2). Several genes encoding ribosomal proteins
354 (*rplJ, rplL*, *rplT*, *rpmI*, *rpsF*, *rpsO*, *rpsR*) and tRNA-ligases (*ileS*, *thrS*, *serS*) were also
355 downregulated (~2.9-8.3-fold) (Table S2), consistent with the transient inhibition of protein
356 synthesis that occurs in response to heat shock in other organisms (Duncan and John W.B.
357 Hershey, 1989). Components of the *psm*β operon (F1613_RS07060, F1613_RS07065,
358 F1613_RS07070, F1613_RS07075) that encode for β-class phenol-soluble modulins (PSMs)
359 (Cheung et al., 2014; Wang et al., 2011), and the PSM transporter system (*pmtA*, *pmtB*, and *pmtC*)
360 (Chatterjee et al., 2013) were downregulated ~3-5-fold. In total, 24 genes involved in transport
361 were downregulated up to ~11-fold (Table S2), with more than half of them belonging to the ATP-
362 binding cassette (ABC) transporter superfamily. Two cold-shock genes (*cspA* and
363 F1613_RS05710) displayed decreased transcript levels, consistent with previous observations of
364 genes repressed by heat shock in *S. aureus* (Fleury et al., 2009). Two helix-turn-helix transcription
365 regulators (F1613_RS10440 and F1613_RS09035) were downregulated ~8.5 and ~3.5-fold,
366 respectively (Table S2). We also observed downregulation of other transcription regulators

367 including *rsp*, F1613_RS11065 (GntR family transcription regulator), and *pyrR* by 5.5-, 4.6-, and
368 4.1-fold respectively (Table S2). Sixteen hypothetical proteins and 23 uncharacterized genes (39
369 total) were also downregulated under heat-shock conditions.
370
371 **Functional Classification of Differentially Expressed Genes in *S. epidermidis* Under Heat**
372 **Stress**
373
374 The genome of *Staphylococcus epidermidis* ATCC 12228 contains 2354 protein-coding genes, of
375 which 207 are hypothetical and 71 are uncharacterized (278 total or ~12% of all genes), indicating
376 their biological functions are unknown or not yet established. We manually grouped 280 of 366
377 heat shock DEGs (~77%) into functional groups using GO and KEGG databases (Figure 2B); 23%
378 of heat shock DEGs had no assigned functions. We observed known functional classes that are
379 upregulated under heat-shock conditions in all domains of life (Richter, Haslbeck, and Buchner,
380 2010), namely Metabolism, Transport, Regulation, DNA/RNA Repair, Molecular Chaperones,
381 Protein Degradation, and Detoxification (Figure 2B). A significant proportion (85; ~36%) of
382 upregulated genes were involved in metabolism, including sugar, amino acid, and fatty acid
383 metabolism (Table S1; Figure S2). We also observed increased expression of genes in the
384 Virulence Factors, Secretion, and Stress Response functional classes (Figure 2B).
385 Ribosome/Translation, tRNA Biosynthesis, and Ribosome Biogenesis functional classes
386 accounted for a significant proportion (22; ~17%) of downregulated genes (Figure 2B; Table S2),
387 consistent with a transient inhibition of protein synthesis. Genes involved in Transport,
388 Metabolism, Cell Wall Structure, Regulation, DNA/RNA Repair, and Stress Response were also
389 downregulated under heat-shock conditions (Figure 2B). We assigned DEGs grouped into minor

390 functional classes that contained only a small number of genes to the "Others" category in each
391 pie chart (Figure 2B). Fourteen upregulated genes and 10 downregulated genes were assigned to
392 the "Others" category and their functions are detailed in the supplementary material (Table S1;
393 Table S2).
394
395 **Transcription Responses to Glucose in *S. epidermidis***
396
397 Six-carbon sugars (hexoses) such as glucose are the preferred carbon and energy sources for many
398 prokaryotes including *S. epidermidis*. Prior studies in staphylococcal species demonstrated that
399 glucose utilization supports faster growth and higher metabolic rates (Halsey et al., 2017). The
400 presence of glucose also inhibits the expression of genes required for uptake and utilization of
401 alternative carbon sources, an adaptive regulatory mechanism called carbon catabolite repression
402 (CCR) (Görke and Stülke, 2008). We performed RNA sequencing on cultures exposed to 20-
403 minute glucose spikes across a range of concentrations and to persistent glucose to better
404 understand the ability of *S. epidermidis* to adapt and respond to glucose. Our underlying goal was
405 to support development of commensal microbes bioengineered to diagnose, monitor, or treat
406 diabetes.
407
408 **Identifying Genes that Might be Useful Starting Points for Controlling Bioengineered**
409 **Bacteria in Treating Diabetes**
410
411 We challenged mid-exponential phase cells by subjecting them to 2 mM, 5 mM, 10 mM, 17 mM,
412 or 50 mM glucose spikes for 20 minutes (Figure 1B). We used RNA sequencing to analyze gene

413 expression profiles and compared the resulting expression profiles of glucose-challenged cells to
414 those of unchallenged cells (Figure 3A). Differentially expressed genes (DEGs) of significance
415 were identified using the following criteria: |log2 fold change| (i.e., log2FC) ≥ 1.5 and adjusted *P*
416 value ≤ 0.05 (Table S3-S7). We examined rlog transformed counts data from the medically
417 relevant (G2-G17) glucose concentrations, searching for candidate transcripts that might be
418 potential starting points for glucose-responsive switches. We found 38 potential switches by
419 modeling all genes with absolute log2 fold change values ≥ 2 in at least one medically relevant
420 glucose challenge experimental condition (Figure S3).
421
422 We selected twenty genes as representative candidates with potentially interesting glucose-
423 responsive switch properties (Figure 3B). Among the potential switches that exhibited an OFF-to-
424 ON transition were two DUF2871 domain-containing proteins (F1613_RS03065 and
425 F1613_RS02965), F1613_RS00340 (ABC transporter ATP-binding protein), F1613_RS00345
426 (ABC transporter permease), *pyrR* (bifunctional pyr operon transcriptional regulator), *ffs (*signal
427 recognition particle sRNA), and four tRNA genes. We also identified genes likely subject to
428 carbon catabolite repression (CCR) that might serve as potential ON-to-OFF switches, including
429 F1613_RS01060 (PTS sugar transporter subunit IIC), *lacA*, *pfkB*, and F1613_RS09950 (proline
430 dehydrogenase) (Görke and Stülke, 2008; Nuxoll et al., 2012). Other promising ON-to-OFF switch
431 candidates include *pflB* (formate C-acetyltransferase), *raiA* (ribosome-associated translation
432 inhibitor), *mqo* (malate dehydrogenase (quinone)), F1613_RS05750 (hypothetical protein),
433 F1613_RS07845 (homoserine dehydrogenase), and F1613_RS06465 (IDEAL domain-containing
434 protein) (Figure 3B). We examined counts data from the medically relevant (G2-G17) glucose
435 concentrations and also noted a class of genes whose expression did not change in response to a

436 glucose spike compared to an unchallenged (0 mM) control. These glucose-independent genes
437 included *lqo* (L-lactate dehydrogenase (quinone)), F1613_RS08490 (transglycosylase domain-
438 containing protein), *typA* (translational GTPase TypA), *rnr* (ribonuclease R), and *noc* (nucleoid
439 occlusion protein).
440
441 **Genes Repressed in Response to 20-minute Glucose Spikes**
442
443 We observed 18 genes that were downregulated across all five glucose spike conditions and an
444 additional ten genes that were downregulated across the top four glucose spike conditions (Figure
445 4B; Figure S4). For example, genes involved in lactose metabolism (F1613_RS11920 (tagatose-
446 6-phosphate kinase), *lacB*, and *lacA*), ribose transport (*rbsU*, *rbsD*), fructose utilization
447 (F1613_RS05160 (PTS fructose transporter subunit IIABC), *pfkB*, and F1613_RS05150
448 (DeoR/GlpR family DNA-binding transcription regulator)), proline catabolism (F1613_RS09950
449 (proline dehydrogenase)), the glyoxalase pathway (F1613_RS05685 (glyoxalase)), the succinate
450 dehydrogenase complex (F1613_RS07025 (succinate dehydrogenase cytochrome b558 subunit)),
451 and ethanol degradation (*adhP*) were downregulated, consistent with previous observations of
452 gene expression changes that occur during CCR (Gutierrez-Ríos et al., 2007; Penninckx, Jaspers,
453 and Legrain, 1983; Nam, 2005; Arndt and Eikmanns, 2007; Görke and Stülke, 2008; Nuxoll et al.,
454 2012; Halsey et al., 2017) (Table S3-S7). We also observed decreased expression of *sdaAB* (L-
455 serine ammonia-lyase iron-sulfur-dependent subunit beta), *raiA*, F1613_RS03360 (universal stress
456 protein), F1613_RS00870 (GntR family transcription regulator), F1613_RS06465 (IDEAL
457 domain-containing protein), F1613_RS10135 (AAA family ATPase), F1613_RS07845
458 (homoserine dehydrogenase), F1613_RS10140 (DUF4238 domain-containing protein),

459 F1613_RS06500 (fatty acid desaturase), and genes involved in formate metabolism (*pflA* and *pflB)*
460 across at least four glucose spike conditions. Four hypothetical proteins and one uncharacterized
461 gene (five total) were also downregulated across at least four glucose spike conditions (Table S3-
462 S7).
463
464 ***S. epidermidis* Transcription Response to a 20-minute 17 mM Glucose Spike**
465
466 We identified 85 of 2354 genes (~4% of the genome) that change in response to a 17 mM glucose
467 spike with log2FC values ≥ 1.5, among which 43 were upregulated and 42 were downregulated
468 (Table S6). Downregulated and upregulated genes were expressed over a -5 to +5 log2FC range
469 (Figure 4A). While gene expression changes are similar across all glucose levels, we observed a
470 more robust change (i.e., -5 to +5 log2FC), a higher number of upregulated genes, and a higher
471 total number of DEGs in the 17 mM glucose condition (Table S3-S5; Table S7).
472
473 Among the most downregulated genes (~6-34-fold) in the 17 mM glucose spike condition were
474 *pflB* and members of the *glpR-pfkB* operon, which plays an essential role in the utilization of
475 fructose, (*pfkB*, F1613_RS05150 (DeoR/GlpR family DNA-binding transcription regulator), and
476 F1613_RS05160 (PTS fructose transporter subunit IIABC)) (Ge et al., 2024) (Table S6). We found
477 that L-serine ammonia-lyase iron-sulfur-dependent subunits alpha and beta (*sdaAA* and *sdaAB*),
478 *raiA*, F1613_RS01060 (PTS sugar transporter subunit IIC), and F1613_RS00520 (nitrate reductase
479 subunit alpha) were also downregulated (~8-10-fold) (Table S6). Six hypothetical proteins and one
480 uncharacterized gene (seven total) were downregulated in the 17 mM glucose spike condition.
481 tRNA genes accounted for almost 60% (24 of 43) of the upregulated genes in the 17 mM glucose

482 spike condition, consistent with increased protein synthesis and faster growth rates in the presence
483 of glucose (Halsey et al., 2017). F1613_RS07200 (NCS2 family nucleobase: cation symporter)
484 and *ffs* were among the most upregulated genes (~7 to 11-fold) in the 17 mM glucose spike
485 condition. Two hypothetical proteins and three uncharacterized genes (five total) were also
486 upregulated.
487
488 **Functional Classification of Downregulated Genes in *S. epidermidis* in Response to a 17 mM**
489 **Glucose Spike**
490
491 To further understand the functions of significantly downregulated genes we used the data from
492 the 17 mM glucose spike condition to assign functional pathways against the GO and KEGG
493 databases. We ordered pathways based on increasing significance level (*P* value) (Figure 4C).
494 Functional pathways with decreased expression include Carbohydrate Metabolism, Butanoate
495 Metabolism, TCA Cycle, Propanoate Metabolism, Lipoic Acid Metabolism, Carbohydrate
496 Transport, Hexose Metabolism, Oxidative Phosphorylation, Phosphoenolpyruvate-Dependent
497 Sugar Phosphotransferase system (PTS), and Amino Acid Metabolism (Figure 4C; Table S6). We
498 observed several downregulated pathways likely consistent with carbon catabolite repression
499 (CCR) (Görke and Stülke, 2008).
500
501 ***S. epidermidis* Transcription Response to Persistent Glucose via Batch Culture**
502
503 To identify differentially expressed genes in *S. epidermidis* exposed to persistent glucose via batch
504 culture, we grew cells overnight in medium containing 0.2% w/v or 1% w/v glucose. We used

505 RNA sequencing to analyze gene expression profiles and compared the expression profiles of cells
506 exposed to 1% w/v glucose against cells exposed to 0.2% w/v glucose. Differentially expressed
507 genes (DEGs) of significance were defined using the following criteria: |log2 fold change| (i.e.,
508 log2FC) ≥ 1.5 and adjusted *P* value < 0.05. By these criteria, we identified 195 of 2354 genes
509 (~8% of the genome) with log2FC values ≥ 1.5, among which 133 were upregulated and 62 were
510 downregulated (Table S8). We observed more upregulated genes, a higher total number DEGs,
511 and unique gene expression changes in the persistent glucose via batch culture experimental
512 condition compared to the 20-minute glucose spike experimental condition (Table S3-S7; Table
513 S8).
514
515 Among the most upregulated genes (~13-30-fold) in the persistent glucose condition were
516 members of the *nrdDG* operon (*nrdD* and *nrdG*), which encodes for an oxygen-independent
517 ribonucleotide reductase (Masalha et al., 2001), and the *dha* operon (F1613_RS03960 (glycerol
518 dehydrogenase), *dhaK*, *dhaL*, *dhaM*)), which encodes for components of the glycerol
519 dehydrogenase- and PTS-dependent dihydroxyacetone kinase system (Céline Monniot et al., 2012)
520 (Table S8). Genes involved in nitrate/nitrite reduction (*narGHJI*, *nirBD*, *nreABC, and*
521 F1613_RS00485 (NarK/NasA family nitrate transporter)) were also upregulated (~4.8-11.9-fold)
522 (Kamps et al., 2004) (Table S8). Sixteen genes involved in glycolysis, gluconeogenesis, and the
523 TCA cycle including the glycolytic *gapA* operon (*gap*, F1613_RS05590 (phosphoglycerate
524 kinase), *tpiA*, *gpmI*, and *eno*), the *alsS/budA* operon, F1613_RS00620 (2,3-diphosphoglycerate-
525 dependent phosphoglycerate mutase), F1613_RS01410 (fructose bisphosphate aldolase), *fdaB*,
526 F1613_RS01355 (L-lactate dehydrogenase), *sdaAA*, *pyk*, *ilvB*, F1613_RS06110 (glucose-6-
527 phosphate isomerase) and *sdhB* were slightly upregulated (~3-8 fold) in the persistent glucose

528 condition, consistent with previous observations of glucose-responsive genes in *S. aureus* (Seidl
529 et al., 2009). Seven hypothetical proteins were also upregulated (Table S8).
530
531 We observed downregulation (up to ~7 fold) of the energy-coupling factor (ECF) transporter
532 module components (F1613_RS11970 (energy-coupling factor transporter ATPase),
533 F1613_RS11965 (energy-coupling factor transporter ATPase), F1613_RS11960 (energy-coupling
534 factor transporter transmembrane protein EcfT)) (Slotboom, 2013), F1613_RS03610
535 (isoprenylcysteine carboxyl methyltransferase family protein), and *ugpC* (Table S8).
536 F1613_RS05940, *dltC*, and *dltD*, components of the *dlt* operon required for the d-alanylation of
537 teichoic acids in gram-positive bacterial cell walls (Kovacs et al., 2006), were also downregulated
538 (~3-4 fold). We observed downregulation of four transcription regulators including *rsp*,
539 F1613_RS01465 (GbsR/MarR family transcription regulator), F1613_RS08735 (AraC family
540 transcription regulator), and F1613_RS10440 (helix-turn-helix transcription regulator) by 3.3-,
541 3.5-, 3.7-, and 4.2-fold, respectively (Table S8). Two hypothetical proteins were also
542 downregulated in the persistent glucose condition (Table S8).
543
544 ***S. epidermidis* Transcription Response to a Step Down in Glucose Concentration from 10**
545 **mM to 2 mM**
546
547 To identify differentially expressed genes in *S. epidermidis* exposed to a step down in glucose
548 concentration, we challenged mid-exponential phase cells by subjecting them to a 10 mM glucose
549 spike for 20 minutes immediately followed by a 2 mM glucose spike for 20 minutes (Figure 1B).
550 We used RNA sequencing to analyze gene expression profiles and compared the expression

551 profiles of cells exposed to a step down in glucose concentration against cells exposed to a 10 mM
552 glucose spike only. Differentially expressed genes (DEGs) of significance were defined using the
553 following criteria: |log2 fold change| (i.e., log2FC) ≥ 1.5 and adjusted *P* value ≤ 0.05. By these
554 criteria, we identified 43 of 2354 genes (~1.8% of the genome) with log2FC values ≥ 1.5, among
555 which 10 were upregulated and 33 were downregulated (Table S9; Figure S5). Downregulated
556 and upregulated genes were expressed over a -6 to +3 log2FC range (Figure 5A).
557
558 We observed upregulation (~3-6-fold) of F1613_RS03760 ((NAD(P)-binding domain-containing
559 protein), *betB*, *betA*, F1613_RS03755 (ferrous iron transporter B), *rpsN*, F1613_RS06020
560 (NAD(P)-binding domain-containing protein), F1613_RS00615 (putative metal homeostasis
561 protein), F1613_RS02245 (putative sulfate exporter family transporter), F1613_RS03765 (zinc
562 ABC transporter substrate-binding protein), and F1613_RS01245 (aminotransferase class I/II-fold
563 pyridoxal phosphate-dependent enzyme) in the step-down experimental condition. Among the
564 most downregulated genes (~5-50-fold) were members of the purine biosynthetic operon
565 (*purEKCSQLFMNHD*), which encodes for 11 enzymes that convert phosphoribosyl
566 pyrophosphate (PRPP) to inosine-5′-monophosphate (IMP) (Goncheva et al., 2019), purine
567 biosynthesis-associated gene *purB*, and glycine cleavage system genes (*gcvT*, *gcvPA*, *gcvPB*). One
568 hypothetical protein was also downregulated in the step-down experimental condition (Table S9).
569
570 **Functional Classification of Differential Expressed Genes in *S. epidermidis* in Response to a**
571 **Step Down in Glucose Concentration from 10 to 2 mM**
572

573 We used the data from the step-down experimental condition to assign functional pathways against
574 the GO and KEGG databases. We ordered pathways based on increasing significance levels (*P*
575 value) (Figure 5C). Functional pathways with decreased expression include Purine Metabolism,
576 Nucleotide Biosynthesis, Amino Acid Metabolism, Nitrogen Compound Metabolism, Vitamin
577 Metabolism, Lipid Acid Metabolism, Organic Compound Biosynthesis, and Sulphur Compound
578 Metabolism (Figure 5C; Table S9). Among upregulated pathways Protein Transport scored the
579 highest significance, according to both GO and KEGG pathway enrichment analysis, under the
580 step-down experimental condition (Figure 5B).
581
582 We constructed a Venn diagram to understand the relationship between our step-down, 10 mM
583 glucose spike (G10), and 2 mM glucose spike (G2) data sets (Figure S5); we observed no shared
584 differentially expressed genes (DEGs) in common among the step-down condition (from 10 to 2
585 mM glucose) and G10 (from 0 to 10 mM glucose). There were also no shared differentially
586 expressed genes among the step-down (from 10 to 2 mM glucose) and G2 (from 0 to 2 mM
587 glucose) experimental conditions either, indicating potentially unique gene expression changes as
588 a function of increasing versus decreasing glucose concentrations (Figure S5; Table S3; Table S9).
589
590 We sought to further understand if and how genes might be differentially expressed at an
591 intermediate glucose concentration (2 mM glucose) as a function of whether cells had been
592 previously exposed to a lower (0 mM) or higher (10 mM) glucose concentration. If prior glucose
593 concentrations do not matter, we would expect no such differences. We performed scatter plot
594 analysis of expression levels for all genes at 2 mM glucose as a function of prior glucose
595 concentration (Figure 6). Most genes differentially expressed under a 0 to 2 mM glucose spike

596 were similarly expressed under a 10 to 2 mM glucose step down (Figure 6 blue dots). Over 14
597 genes differentially expressed under a 10 to 2 mM glucose step down were not similarly expressed
598 under a 0 to 2 mM glucose spike (Figure 6 red dots; Discussion). Further analysis indicated these
599 genes are primarily involved in purine metabolism (above; Table S9).
600
601 **Discriminating Between Glucose and Heat Shock Conditions**
602 Differential gene expression analysis of and within the skin microbiome might be useful as a
603 potential platform for clinical diagnosis. To explore this idea, we compared gene expression levels
604 during heat shock to those observed during high (17 mM) glucose levels. Most (~93.6%) genes
605 are similarly expressed (95% c.i.) under both conditions (Figure 7). However, 341 and 60 genes
606 are differentially expressed under heat shock or high glucose, but not both conditions, respectively.
607 Such genes may offer a starting point for developing nucleic acid amplification-based methods for
608 determining the current or prior physical experience of microbes on patients.
609
610 **DISCUSSION**
611 To support bioengineering of skin microbes to diagnose, monitor, or treat disease, we sought to
612 understand how *S. epidermidis* responds to environmental perturbations including heat shock and
613 medically relevant glucose levels. We used RNA sequencing to investigate differential gene
614 expression followed by gene set enrichment analysis (GSEA) to understand the functions of
615 differentially expressed genes. We observed an *S. epidermidis* heat-shock response that mostly
616 resembles the heat-shock response of related staphylococcal species and other gram-positive
617 bacteria (below). We observed carbon catabolite repression in response to a range of glucose
618 spikes, upregulation of genes involved in glycolysis, gluconeogenesis, and the TCA cycle in

619 response to persistent glucose via batch culture, as well as a potentially unique gene expression
620 signature in response to a step down in glucose concentration from 10 to 2 mM. Building upon our
621 analyses we curated a subset of glucose-responsive genes that might serve as starting points for
622 engineering endogenous dynamic control of circuits in *S. epidermidis*.
623
624 We observed contrasting patterns of gene expression depending on whether cells were exposed to
625 a spike or persistent level of glucose. For example, we observed downregulation (up to ~34 fold)
626 across all five glucose spike conditions for genes involved in lactose metabolism, ribose transport,
627 fructose utilization, proline catabolism, the glyoxalase pathway, the succinate dehydrogenase
628 complex, and ethanol degradation (Table S3-S7). We believe this repression of genes involved in
629 secondary carbon source utilization to be convincing evidence of carbon catabolite repression
630 (CCR) in our glucose spike data (Görke and Stülke, 2008). By contrast, we found no evidence of
631 CCR in our persistent glucose via batch culture data. (Table S8). As a second example, while we
632 observed the induction (~3-8 fold) of several essential glycolytic genes, the *dha* operon,
633 gluconeogenesis genes, and TCA cycle genes in our persistent glucose via batch culture samples
634 (Table S8), we did not observe such gene expression patterns among the upregulated genes in our
635 glucose spike data. Instead, tRNA genes accounted for most of the upregulated genes in our
636 glucose spike data (Table S3-S8). One explanation could be that *S. epidermidis* first adapts to
637 glucose exposure by preferentially downregulating genes involved in secondary carbon source
638 utilization to avoid the production of proteins that are not useful in the presence of glucose; only
639 following sufficiently prolonged exposure to glucose does *S. epidermidis* adjust its transcriptome
640 to upregulate genes involved in glucose utilization. We note that Seidl et al. found in *S. aureus* that
641 a 30-minute exposure to 10 mM glucose was sufficient to realize gene expression changes similar

642 to our prolonged exposure conditions, suggesting that between 20 to 30 minutes could be sufficient
643 to fully transition to a persistent glucose transcriptome in *S. epidermidis* (Seidl et al., 2009).
644
645 Under heat shock conditions we found patterns of gene expression similar to other *Staphylococcus*
646 species. For example, at 45°C, we observed upregulation of F1613_RS04215 (*CtsR* family
647 transcription regulator) and *hrcA* (Table S1), known heat-shock gene expression regulators in
648 *Staphylococcus aureus, Bacillus subtilis*, and other firmicutes (Derre, Rapoport, and Msadek,
649 1999; Chastanet, Fert, and Msadek, 2003; Schumann, 2003). We also observed rapid induction of
650 *clpB*, *clpP*, and the *dnaK*, *groESL*, and *clpC* operons (Table S1). Our data also provides evidence
651 of an *S. epidermidis* heat-shock regulatory network that utilizes both the *hrcA*- and *ctsR*-encoded
652 repressors. For example, we carried out DNA motif analysis and found CtsR (GGTCAAA/T) and
653 CIRCE (controlling inverted repeat of chaperone expression) operator sequences arranged in
654 tandem upstream of the *dnaK* and *groESL* operons consistent with previous observations of dual
655 heat-shock regulation by *hrcA* and *CtsR* in *S. aureus* and *S. epidermidis* (Derre, Rapoport and
656 Msadek, 1999; Chastanet, Fert, and Msadek, 2003) (Figure S6). We also found CtsR recognition
657 sequences upstream of *clpB*, *clpP*, and the *clpC* operon also consistent with previous observations
658 of CtsR regulons in *B. subtilis* and *Streptococcus pneumoniae* (Derre et al., 1999; Chastanet et al.,
659 2001) (Figure S6).
660
661 While we observed upregulation of universal stress proteins (F1613_RS09680 and
662 F1613_RS09700), we did not detect upregulation of the general stress-responsive alternative
663 sigma factor *sigB,* which is a component of the heat-shock regulon in *S. aureus, B. subtilis*, and
664 *Listeria monocytogenes* (Kullik and Giachino, 1997; Schumann, 2003; Ferreira, O'Byrne, and

665 Boor, 2001). By contrast, we did observe upregulation (~5 fold) of F1613_RS09995, another
666 sigma-70 family RNA polymerase sigma factor (Table S1). This difference suggests that the *S.*
667 *epidermidis* heat-shock regulatory network may differ slightly from that of *S. aureus* and other
668 gram-positive bacteria.
669
670 We compared the genome-wide *S. epidermidis* heat-shock response to the 17 mM glucose spike
671 (G17) and step-down responses (Figure 8). We observed a more robust increase in gene expression
672 in response to heat shock (i.e., -4 to +6 log2FC range) compared to G17 (i.e., -5 to +5 log2FC) and
673 step down (i.e., -6 to +3 log2FC range) and detected more differentially expressed genes (DEGs)
674 in the heat-shock condition (366 genes) compared to G17 (85 genes) and step-down conditions (43
675 genes) (Figure 2A; Figure 4A; Figure 5A). In response to acute heat stress and subsequent loss of
676 protein homeostasis (e.g., due to heat-induced protein denaturation, misfolding, and aggregation),
677 we observed a rapid and global reprogramming of gene expression, unlike the transcription
678 changes observed when *S. epidermidis* adapts to a preferred carbon source (e.g., glucose) at non-
679 toxic concentrations (Figure 8; Figure 3A). We believe these disparate gene expression profiles
680 could be of limited clinical utility; more specifically, DEGs unique to heat shock (341 genes) or
681 high glucose (60 genes) may be a promising starting point for the development of simple nucleic
682 acid-based tools for the diagnosis and monitoring of disease (Figure 7).
683
684 We observed downregulation (up to ~50-fold) of genes involved in purine biosynthesis
685 (*purEKCSQLFMNHD*) in response to a step down in glucose concentration from 10 to 2 mM
686 (Table S9). We did not observe such downregulation in the G2 (from 0 to 2 mM glucose) or G10
687 (from 0 to 10 mM glucose) glucose spike conditions (Table S3; Table S5). Further, we found no

688 differentially expressed genes (DEGs) in common among the step-down and G10 conditions and
689 the step-down and G2 conditions (Figure S5). Taken together we wondered if there is a unique
690 step-down gene expression signature that does not resemble that of G2 or G10. We performed
691 scatter plot analysis to visualize the relationship between the step-down and G2 conditions (Figure
692 6). We noticed that, while most genes are similarly expressed under both conditions, over 14 genes
693 differentially expressed under step-down conditions were not similarly expressed under G2
694 conditions (Figure 6, red dots). Further analysis revealed that these genes were mainly involved in
695 purine biosynthesis. We note that our step-down samples underwent two rounds of centrifugation
696 while our G10 samples underwent a single round of centrifugation prior to RNA harvesting
697 (Methods); this methodological difference may account for the unique step-down gene expression
698 signature observed here.
699
700 Finally, we sought to identify glucose-responsive promoters that might eventually be used to
701 control the expression of an insulin gene in a bioengineered *S. epidermidis* strain developed to aid
702 in treating diabetes. To this end, we constructed glucose concentration-response curves across
703 medically relevant (G2-G17) glucose levels. We identified 38 glucose-responsive genes that might
704 serve as ON or OFF switches for controlling synthetic genetic systems (Figure S3; Figure 3B).
705 Most (~70%) of the potential switches that exhibited an OFF-to-ON transition were tRNA genes
706 (Figure S3). We suspect these switches are not specific to glucose given that increased tRNA
707 expression might also occur in response to various other carbon sources (Dong, Nilsson and
708 Kurland, 1996). We also observed 19 potential ON-to-OFF switches (Figure S3). Each glucose-
709 responsive gene reported here is a starting point requiring additional characterization (e.g.,

710 response specificity) to identify those most appropriate for any given application (e.g., controlling
711 expression of insulin in a glucose-dependent manner).
712
713 The human skin microbiome is a diverse and dynamic microbial community that plays an essential
714 role in maintaining our health and well-being. A more intimate understanding of how our skin
715 microbes adapt to environmental perturbations (e.g., stress or increased glucose levels) is required
716 to ultimately enable development of bioengineered skin microbes that can help diagnose and treat
717 disease. We hope our investigation of the genome-wide transcription response in *S. epidermidis* to
718 heat shock and medically relevant glucose concentrations helps further motivate ongoing work.
719 We are excited to imagine a future in which the bioengineering of skin microbes has been made
720 routine, helping doctors and patients to realize healthier lives and better clinical outcomes.
721

722 **AUTHOR CONTRIBUTIONS**

723 KNB: conceptualization, design and execution of the experiments, data interpretation, funding
724 acquisition and writing (original draft, review, and editing). AG: data analysis and interpretation,
725 data visualization, writing (part of the methods section, and review). RN: data preprocessing
726 pipeline development and writing (part of the methods section, and review). DE: supervision, data
727 interpretation, funding acquisition, and writing (review, and editing). All authors contributed to
728 the article and approved the final manuscript.
729

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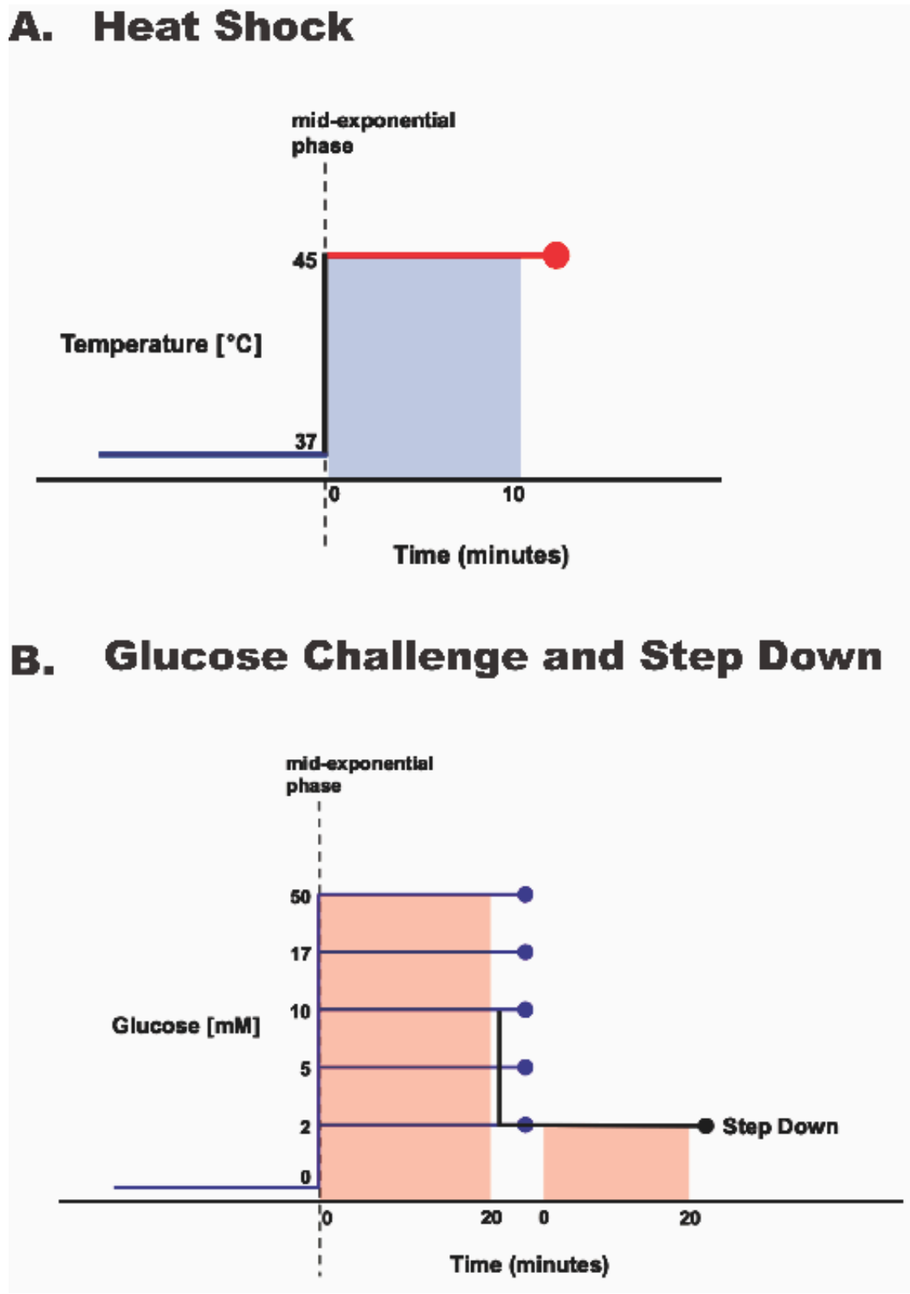


Figure 1 | Environmental Perturbation of *Staphylococcus epidermidis*. Log-phase cultures were exposed to **(A)** a 10-minute increase in temperature from 37°C to 45°C or **(B)** a range of 20-minute glucose spikes (concentrations as noted) and a 10 mM spike followed by a step down to 2 mM.

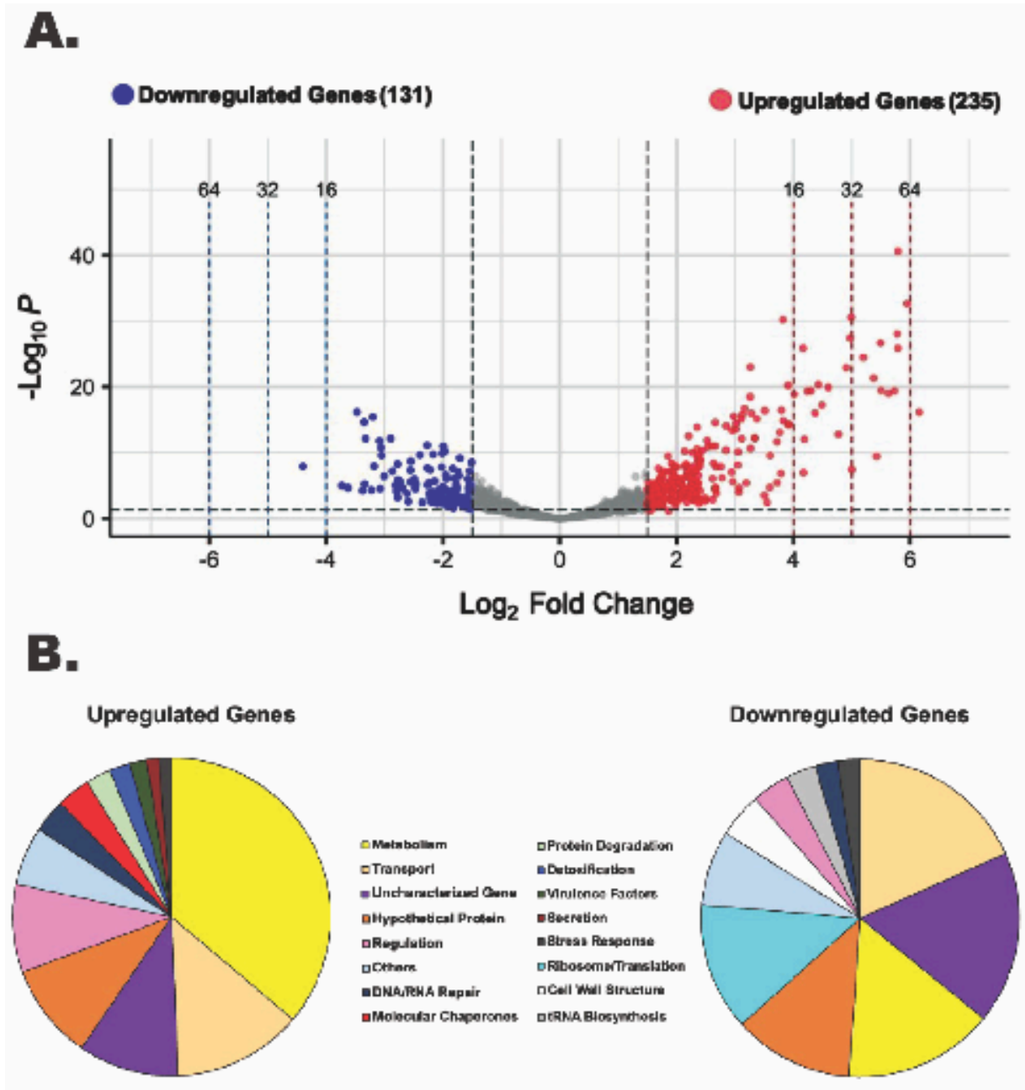


Figure 2 | A Sudden Temperature Increase Causes Transcript Levels to Change up to ~71-fold. (A) Volcano plot showing the differentially expressed genes (DEGs) for the heat-shock experimental condition with |log2 FC|≥ 1.5 and adjusted *P* value ≤ 0.05 as the threshold. The red dots represent 235 significantly upregulated genes, and the blue dots represent 131 significantly downregulated genes**. (B)** Summary of the significantly upregulated and downregulated genes during the heat-shock response in *S. epidermidis* assigned to functional groups according to GO and KEGG pathways (in %).

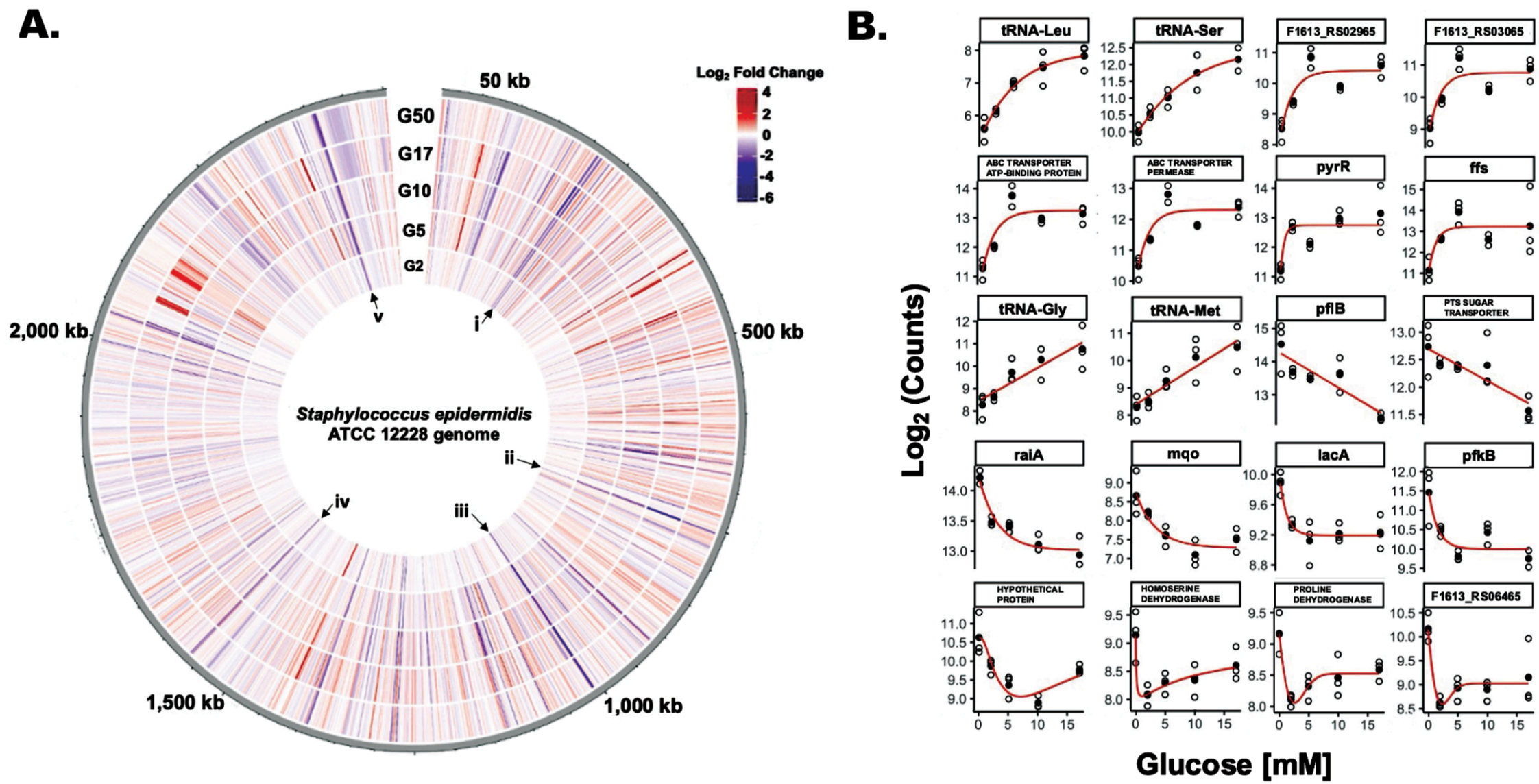


Figure 3 | Eighty-five *S. epidermidis* Genes Change Expressions Levels in Response to Glucose. (A) Circular transcriptome map showing normalized gene expression levels in the *S. epidermidis* genome in response to glucose. Log2 fold change relative to control for cells exposed to 2 mM (G2), 5 mM (G5), 10 mM (G10), 17 mM (G17), or 50 mM (G50) glucose spikes. Each bar denotes a single gene; red bars represent significantly upregulated genes and blue bars represent significantly downregulated genes. Roman numerals **i** (*sdaAB, rbsU*), **ii** (*pflB*), **iii** (*glpR-pfkB* operon), **iv** (F1613_RS07845 (homoserine dehydrogenase), and **v** (members of the *lac* operon) correspond to select groups of genes that are downregulated across all five glucose spike conditions. **(B)** Glucose concentration-response curves for a representative subset of genes that have potentially interesting glucose-responsive switch properties.

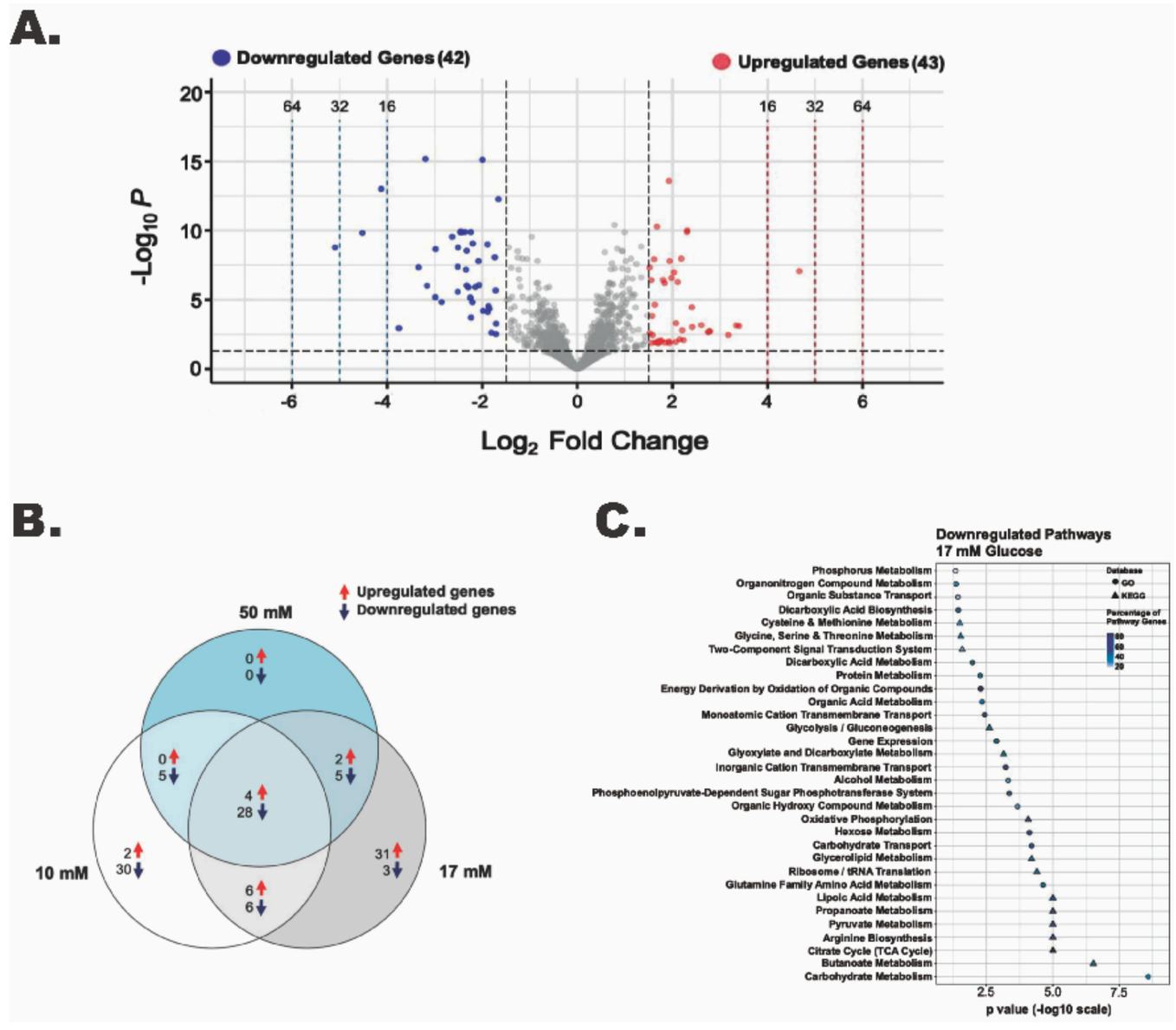


Figure 4 | A 17 mM Glucose Spike Causes Transcript Levels to Change up to ~34-fold. (A) Volcano plot showing the differentially expressed genes (DEGs) for the 17 mM glucose spike experimental condition with $|\log2\ FC| \geq 1.5$ and adjusted P value ≤ 0.05 as the threshold. The red dots represent 43 significantly upregulated genes, and the blue dots represent 42 significantly downregulated genes. **(B)** Venn diagram illustrating the number of unique and shared DEGs from the 10 mM, 17 mM, and 50 mM glucose challenge experimental conditions. **(C)** Summary of the significantly downregulated genes for the 17 mM glucose spike experimental condition assigned to functional classes according to GO and KEGG pathways.

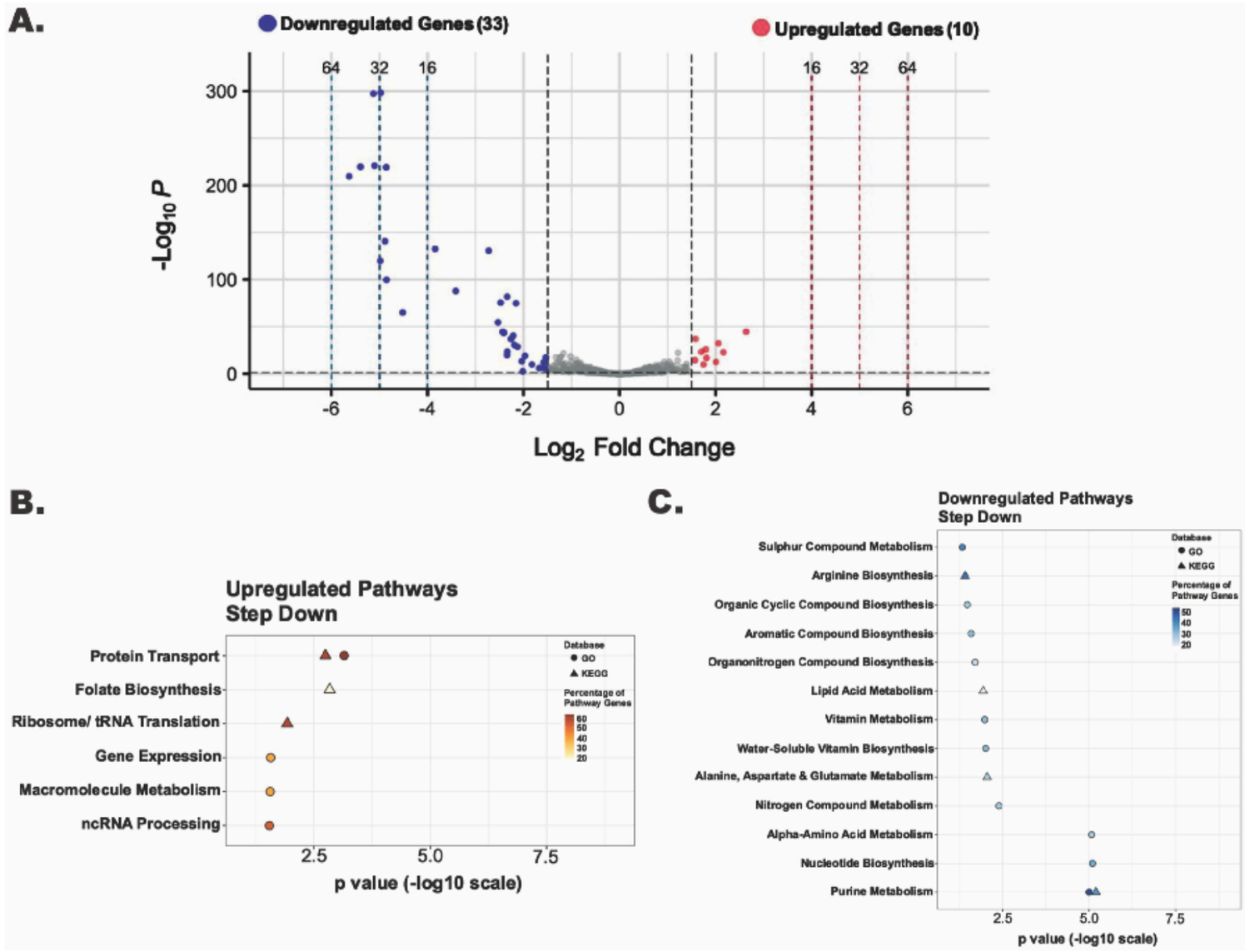


Figure 5 | Genes Involved in Purine Metabolism Are Significantly Downregulated in Response to a Step Down in Glucose Concentration From 10 mM to 2 mM. (A) Volcano plot showing the differentially expressed genes (DEGs) for the Step-down experimental condition with $|\log2\ FC| \geq 1.5$ and adjusted *P* value ≤ 0.05 as the threshold. The red dots represent 10 significantly upregulated genes, and the blue dots represent 33 significantly downregulated genes. Summary of the significantly upregulated **(B)** and downregulated **(C)** genes for the Step-down experimental condition assigned to functional classes according to GO and KEGG pathways.

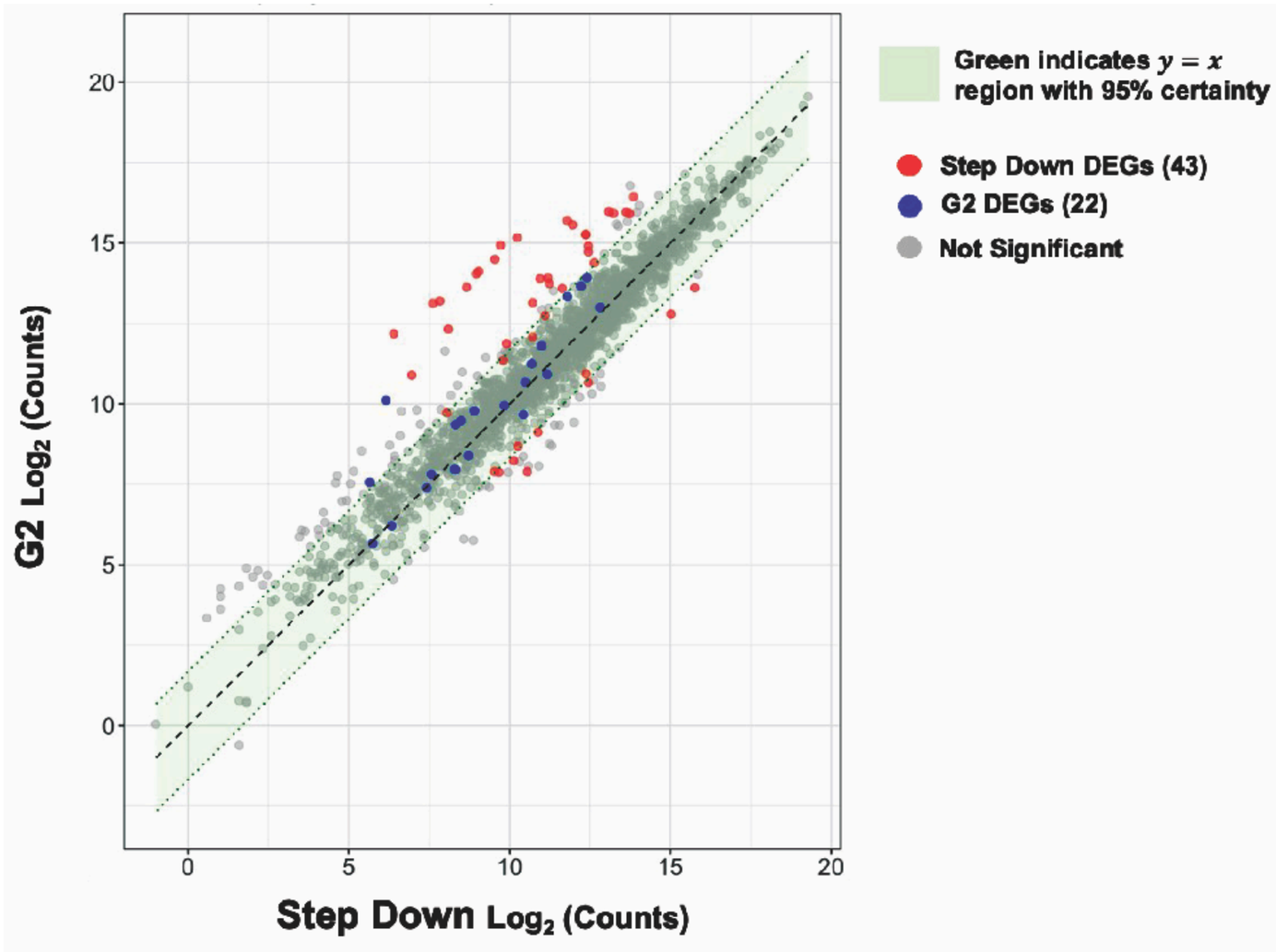


Figure 6| Expression levels of purine biosynthesis genes at intermediate glucose levels are sensitive to prior glucose levels. Scatter plot visualizing the relationship between the step-down and 2 mM glucose spike (G2) experimental conditions. Each dot denotes a single gene. The red and blue dots represent step-down, and G2 differentially expressed genes (DEGs) respectively. The gray dots represent genes with no significant change. A 95% confidence interval was calculated around the residuals of gene expression differences between the two experimental groups. Genes that fall within the green highlighted region are predicted to have near identical average expression levels with 95% certainty.

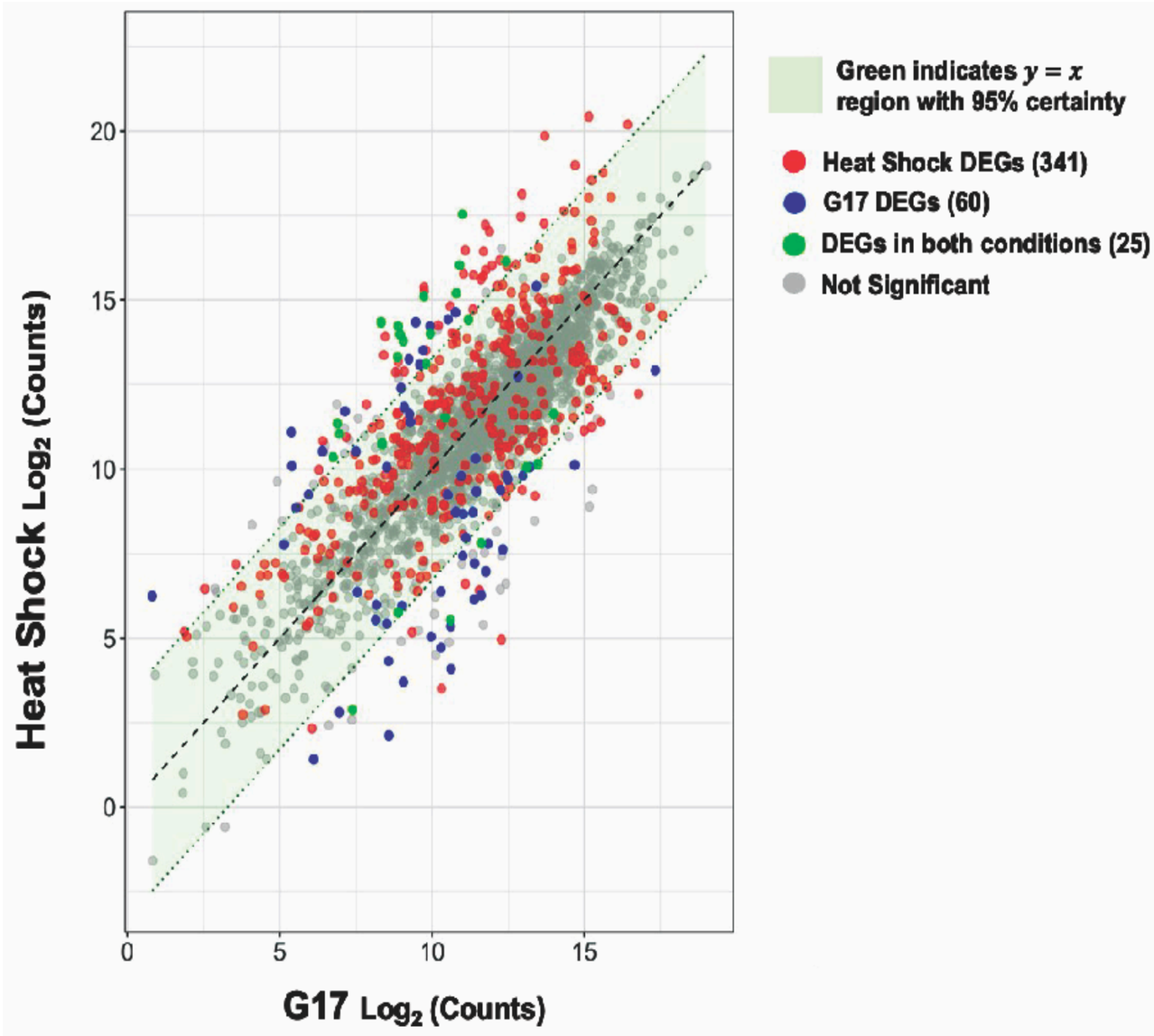


Figure 7| Heat shock and glucose spikes create statistically unique signatures. Scatter plot visualizing the relationship between the heat-shock and 17 mM glucose spike (G17) experimental conditions. Each dot denotes a single gene. The red and blue dots represent heat-shock, and G17 differentially expressed genes (DEGs) respectively. The green dots represent DEGs found in both conditions and the gray dots represent genes with no significant change. A 95% confidence interval was calculated around the residuals of gene expression differences between the two experimental groups. Genes that fall within the green highlighted region are predicted to have near identical average expression levels with 95% certainty.

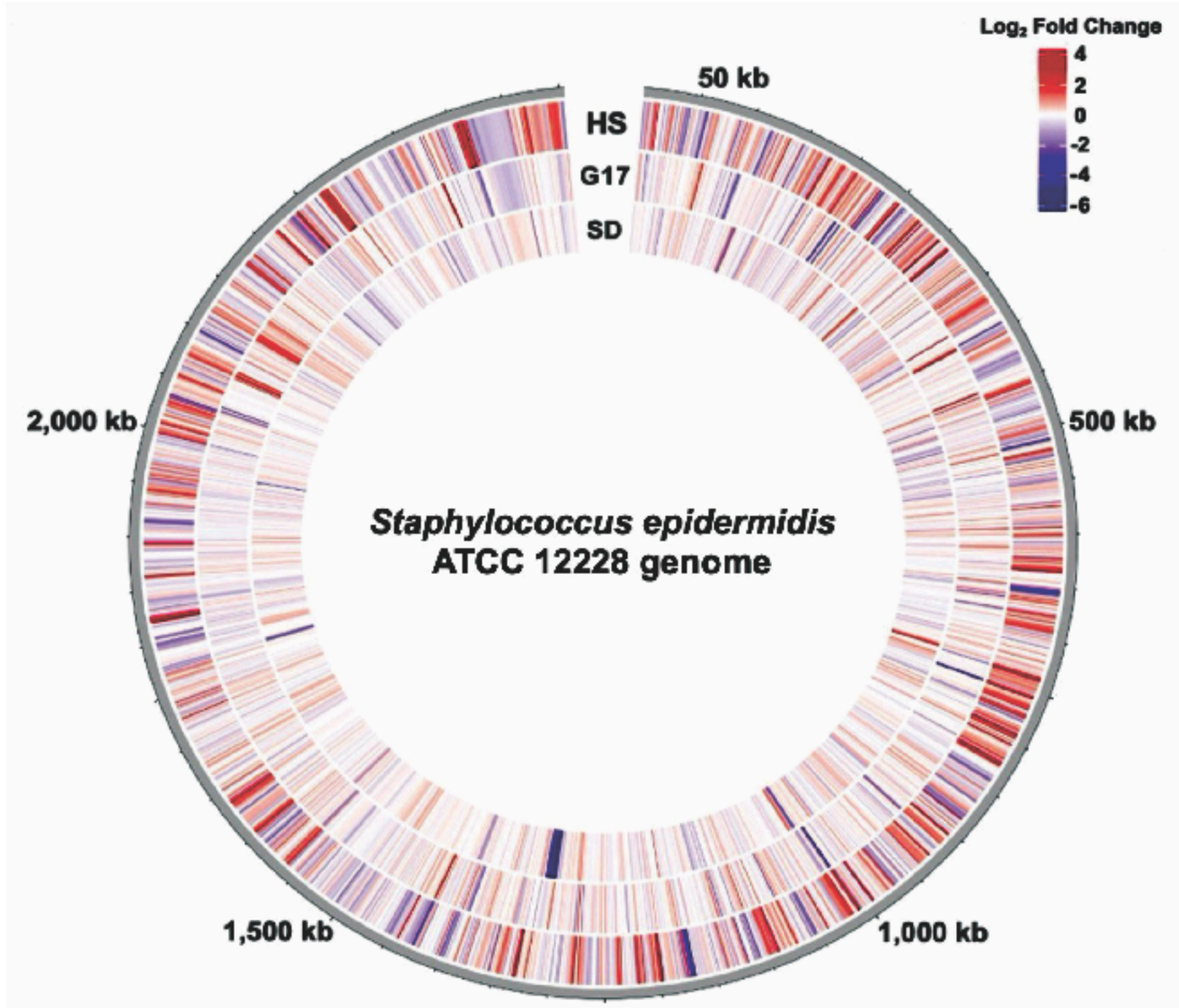


Figure 8| The Genome-wide Transcription Response of *Staphylococcus epidermidis* to Perturbations. Circular transcriptome map showing normalized gene expression levels in the *Staphylococcus epidermidis* genome. Log2-fold change relative to control for cells exposed to Heat Shock (HS), a 17 mM glucose spike (G17), or Step Down (SD) experimental conditions. Each bar denotes a single gene. The red bars represent significantly upregulated genes, and the blue bars represent significantly downregulated genes.

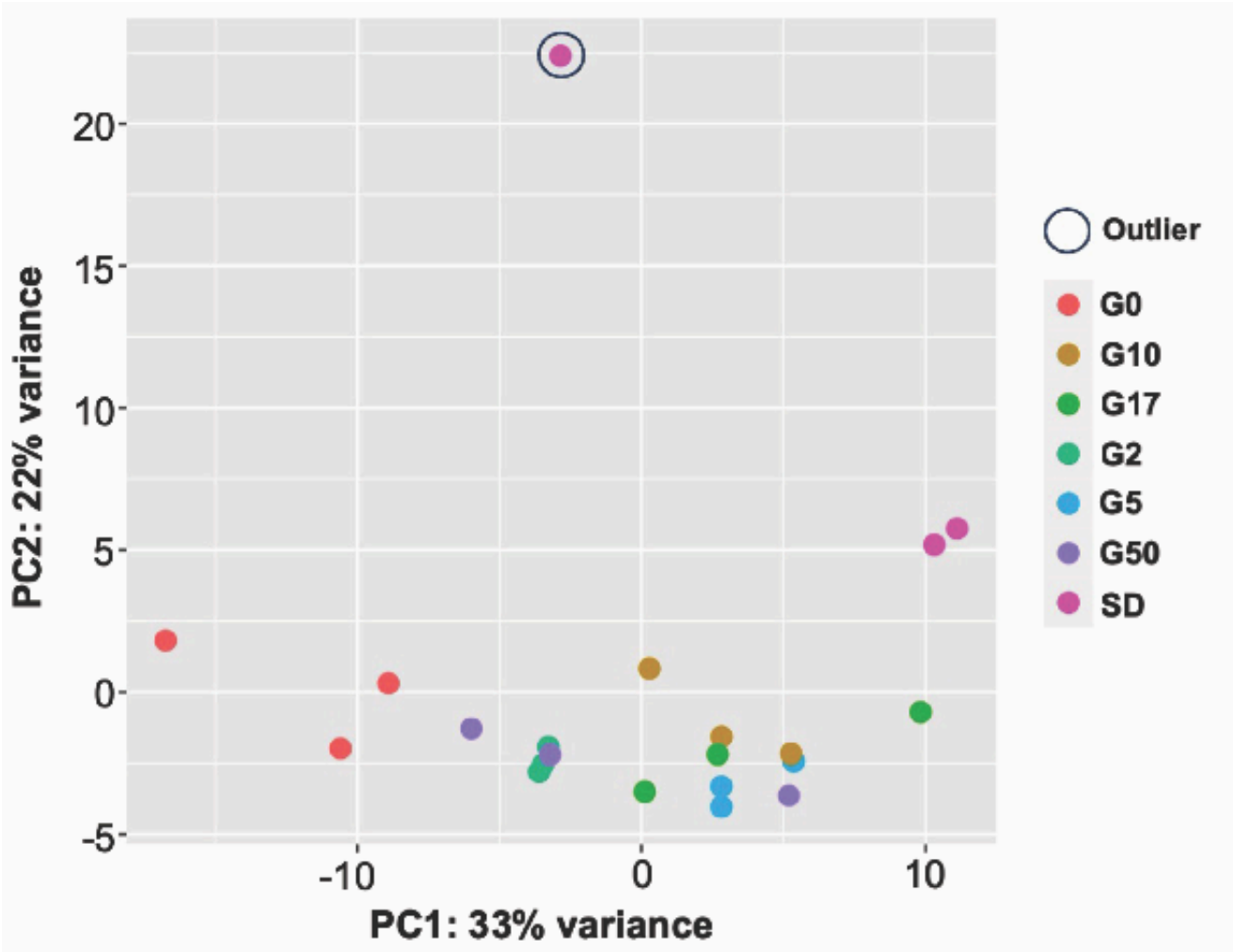


Figure S1 | Principal Component Analysis (PCA) Reveals One Step-Down Replicate is an Outlier. PCA plot of RNA-seq data for three biological replicates for the Glucose Challenge and Step-Down experimental conditions.

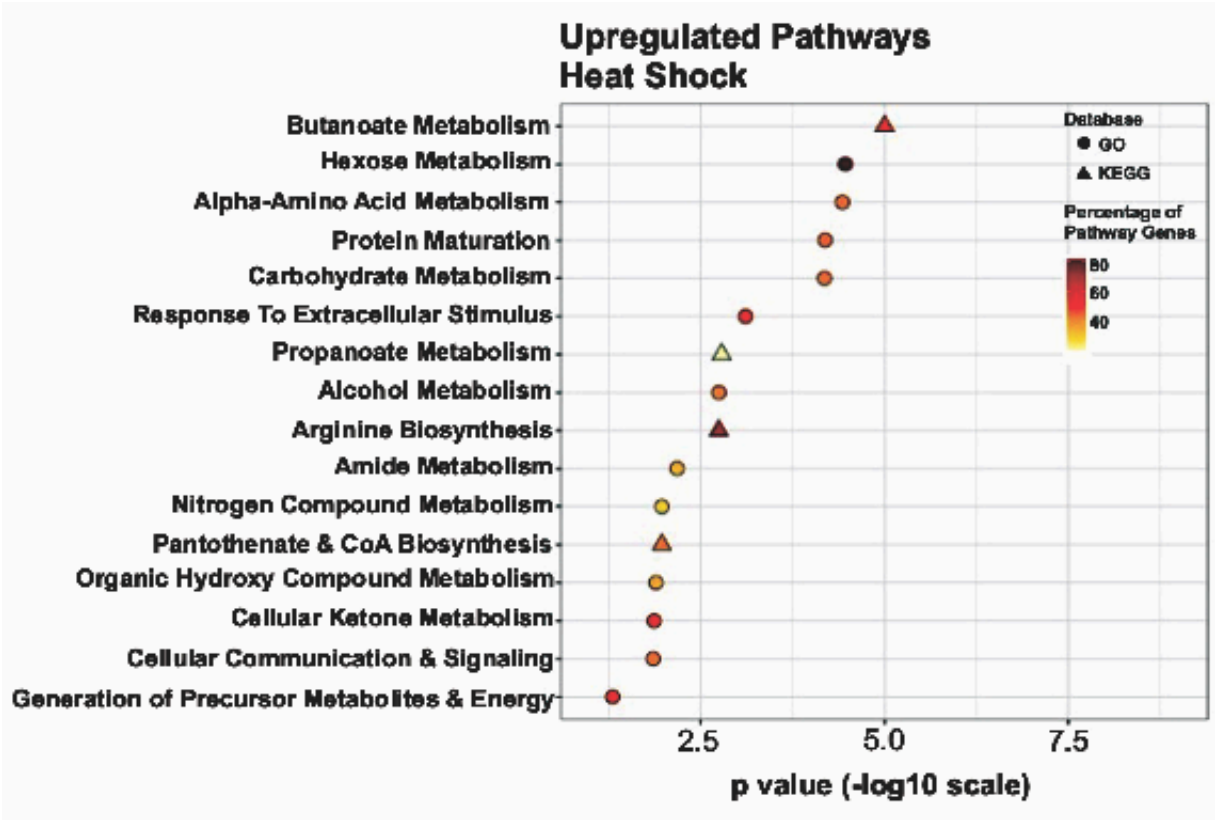


Figure S2 | Genes Upregulated During Heat-shock Span Metabolism and Cell Signaling. Summary of the significantly upregulated genes for the heat-shock experimental condition assigned to functional classes according to GO and KEGG pathways.

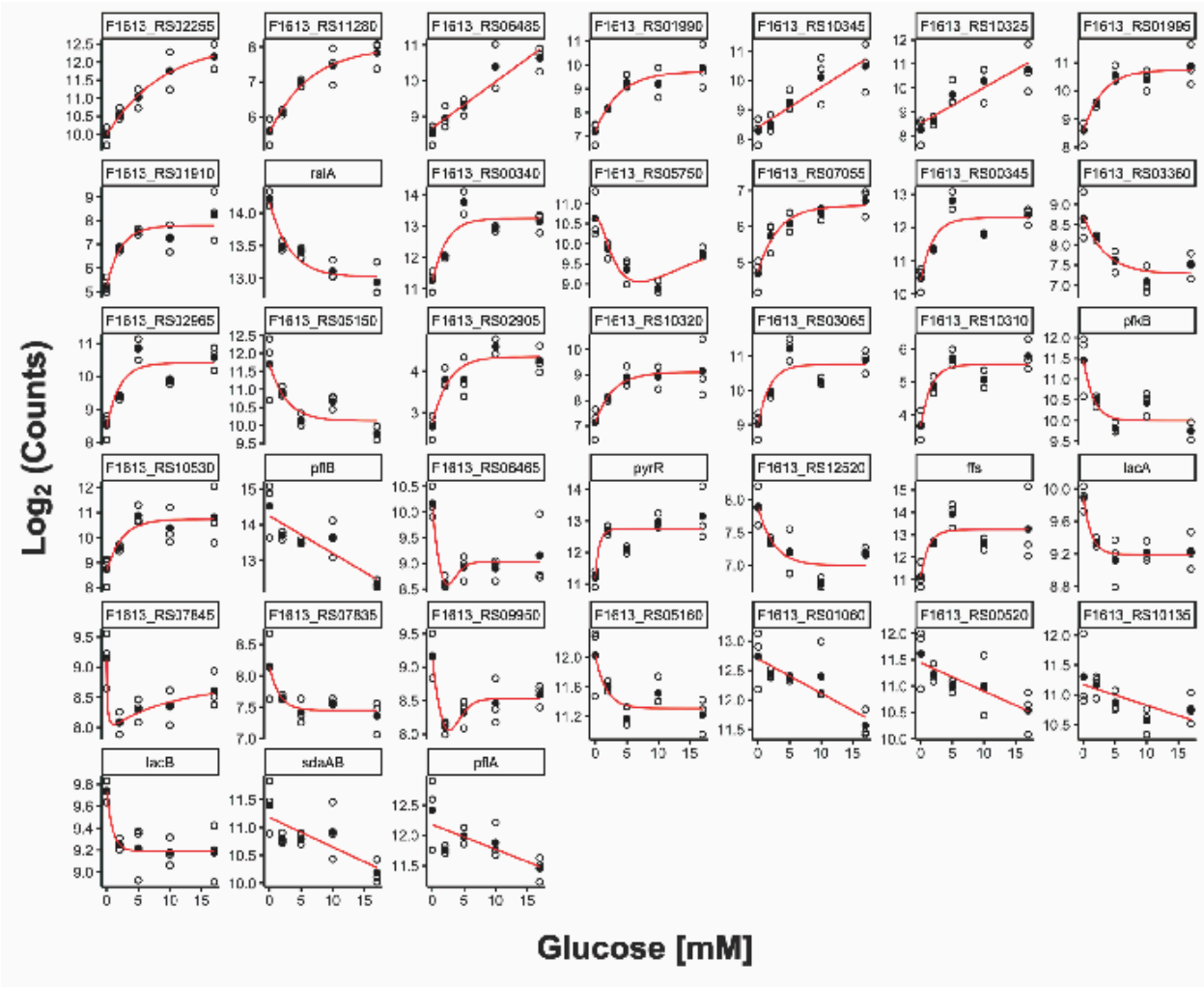


Figure S3 | Thirty-eight Candidate Genes that Have Potentially Interesting Glucose-Responsive ON or OFF Switch Properties. Concentration-response curves generated for genes with absolute log2 fold change values ≥ 2 in at least one medically relevant (G2-G17) glucose challenge experimental condition.

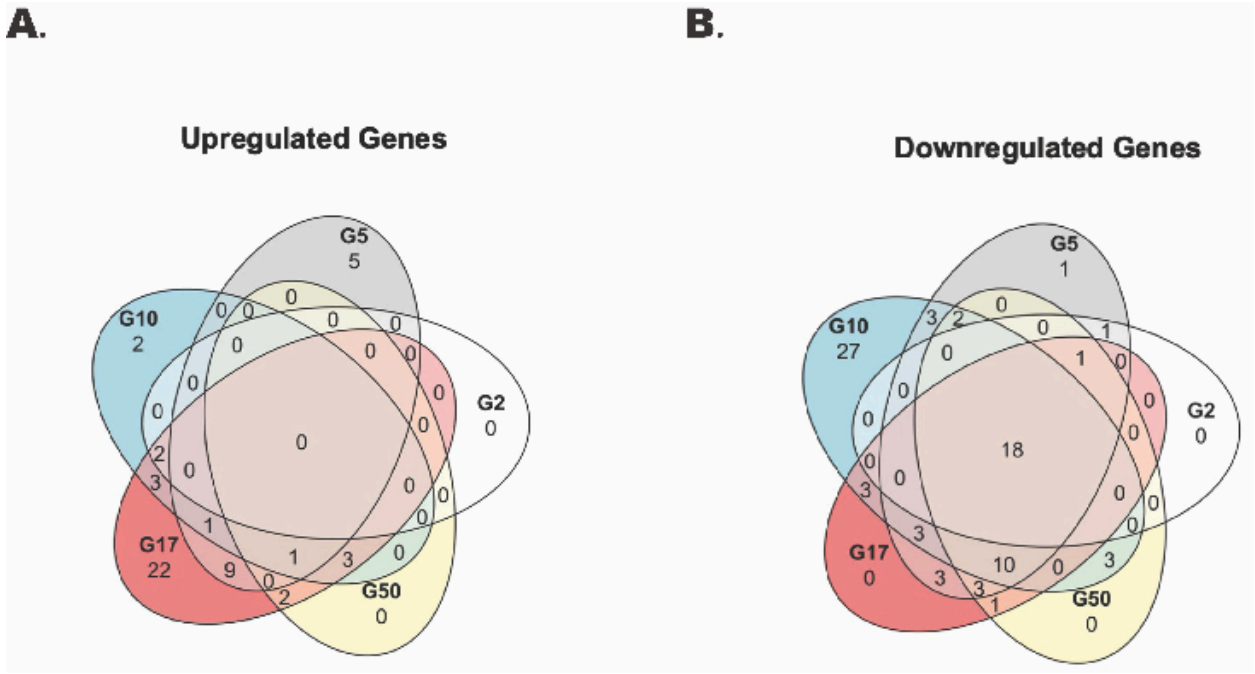


Figure S4 | Twenty-eight Genes are Downregulated in Common in Response to Increasing Glucose Spike Concentrations. Venn diagrams illustrating the number of unique and shared upregulated **(A)** and downregulated genes **(B)** from the 2 mM (G2), 5 mM (G5), 10 mM (G10), 17 mM (G17), and 50 mM (G50) glucose challenge experimental conditions.

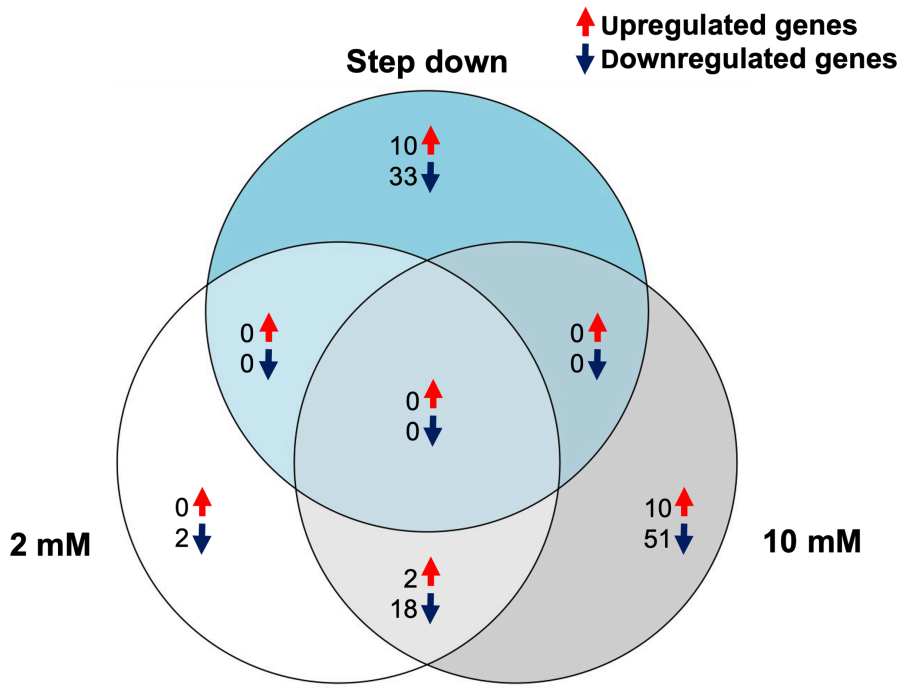


Figure S5 | Step-down Samples Have a Unique Gene Expression Profile Distinct from the 2 mM and 10 mM Glucose Spike Profiles. Venn diagram illustrating the number of unique and shared DEGs from the 2 mM, 10 mM, and step-down experimental conditions.

dnaK operon

CtsR (GGTCAAA/T) and CIRCE operator sequences arranged in tandem upstream of the *dnaK* operon.

*** CIRCE operator sequence is comprised of two 8-pb inverted repeats and a 11-bp variable middle spacer. (TTAGCACT-N11-AGTGCTAA)**

groESL operon

CtsR (GGTCAAA/T) and CIRCE operator sequences arranged in tandem upstream of the *groESL* operon.

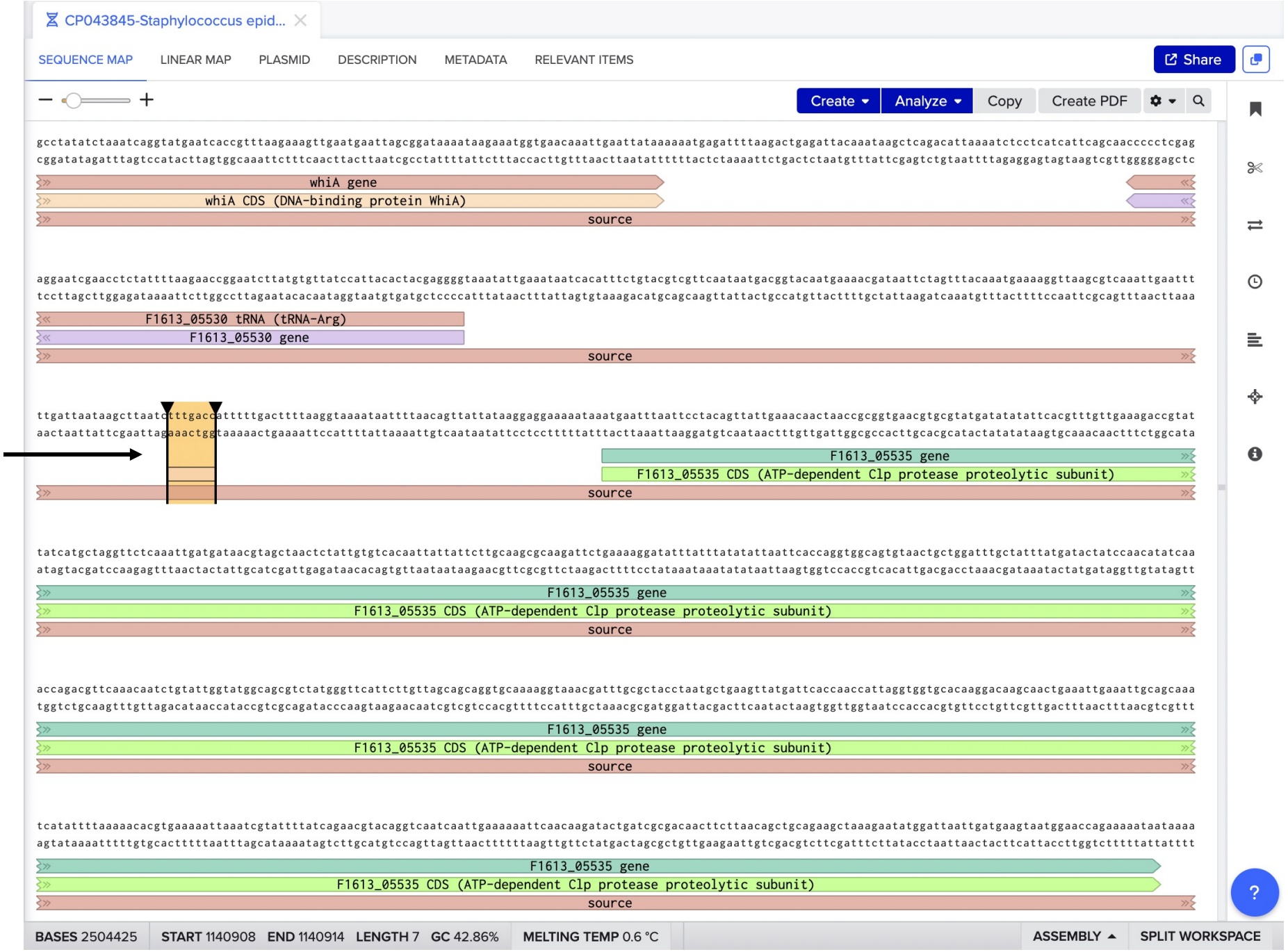
*** CIRCE operator sequence is comprised of two 8-pb inverted repeats and a 11-bp variable middle spacer. (TTAGCACT-N11-AGTGCTAA)**

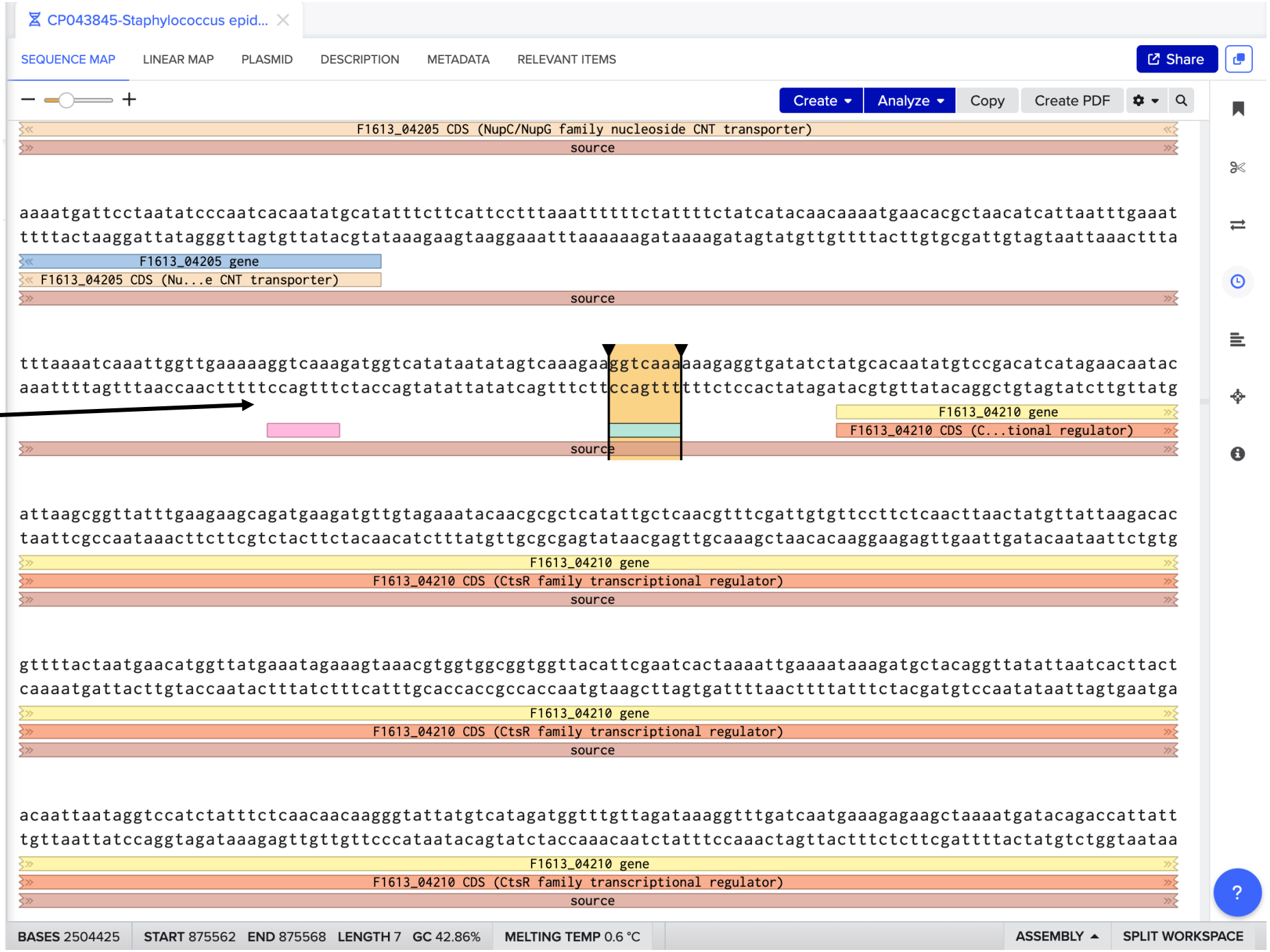
clpB

CtsR (GGTCAAA/T) operator sequence

clpP

CtsR (GGTCAAA/T) operator sequence



clpC operon

Two CtsR (GGTCAAA/T) operator sequences (annotated in pink and green)