Synergism between a simple sugar and a small intrinsically disordered protein mitigate the lethal stresses of severe water loss

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ABSTRACT

Anhydrobiotes are rare microbes, plants and animals that tolerate severe water loss. Understanding the molecular basis for their desiccation tolerance may provide novel insights into stress biology and critical tools for engineering drought-tolerant crops. Using the anhydrobiote, budding yeast, we show that trehalose and Hsp12, a small intrinsically disordered protein (sIDP) of the hydrophilin family, synergize to mitigate completely the inviability caused by the lethal stresses of desiccation. We show that these two molecules help to stabilize the activity and prevent aggregation of model proteins both *in vivo* and *in vitro*. We also identify a novel role for Hsp12 as a membrane remodeler, a protective feature not shared by another yeast hydrophilin, suggesting that sIDPs have distinct biological functions.

INTRODUCTION

In the near future, global food security will be challenged by the effect of climate change on crop yields (Schwalm et al., 2017; Tirado et al., 2013). Potential solutions to more frequent drought may come from the study of drought-tolerant organisms. Indeed, diverse organisms, collectively named anhydrobiotes, can survive extreme water loss. By identifying and understanding the molecular mechanisms underlying desiccation tolerance, we hope to provide key new insights into stress biology and suggest fruitful avenues for the engineering of drought-tolerant crops.

For almost a half a century, scientists have known that almost all anhydrobiotes have high levels of the disaccharide trehalose and hydrophilins, which are small, hydrophilic, and intrinsically disordered proteins (sIDPs) (Crowe et al., 1992; Crowe, 1971; Potts, 2001). Demonstrating a functional significance for this striking correlation eluded researches until very

34 recently. A requirement for trehalose production in desiccation tolerance was shown in genetic
35 studies of two anhydrobiotes, yeast and nematodes (Erkut et al., 2011; Tapia and Koshland,
36 2014). However, their requirement for trehalose was not identical; nematodes that were unable
37 to synthesize were sensitive to desiccation under all conditions while yeast that were unable to
38 synthesize trehalose were sensitive to long-term, but not short-term, desiccation (Erkut et al.,
39 2011; Tapia and Koshland, 2014).

40 Similarly, recent studies have also revealed disparate requirements for hydrophilins in
41 desiccation tolerance. In tardigrades, RNAi knock-down of individual hydrophilins caused
42 desiccation sensitivity (Boothby et al., 2017). In contrast, in yeast, mutants lacking a subset of
43 either individual or multiple hydrophilins display normal tolerance to desiccation (Calahan et al.,
44 2011). The different requirements of both hydrophilins and trehalose in different anhydrobiotes
45 suggest that overlapping activities, potentially of trehalose, hydrophilins, or additional, as yet
46 unidentified, stress effectors mitigate desiccation tolerance. Therefore, a major unanswered
47 question in the field, is what stress effectors are necessary and sufficient for desiccation
48 tolerance?

49 The identification of this set of stress effectors might provide a foundation to understand the
50 stresses imposed by desiccation. In principle, the lack of intracellular water could readily lead
51 to changes in proteostasis, membrane permeability, and/or osmolarity. Anyone or all of these
52 changes might cause lethality. Characterizing the molecular activities of the stress effectors
53 that promote desiccation tolerance could identify the critical/lethal stresses of desiccation.

54 Recent studies in budding yeast suggest that it is an excellent model anhydrobiote to
55 identify the stress effectors required for desiccation tolerance and to characterize their
56 molecular activities (Calahan et al., 2011; Tapia and Koshland, 2014; Tapia et al., 2015; Welch
57 et al., 2013). Importantly, budding yeast cells display conditional desiccation tolerance. In
58 stationary phase, 20-40% of cells survive desiccation. However, when dividing exponentially,
59 only approximately 0.001% of cells survive desiccation (Calahan et al., 2011). This conditional
60 tolerance provides a powerful genetic tool to assess the contributions of different factors to
61 desiccation tolerance.

62 The loss of desiccation tolerance, in mutant yeast cells grown to stationary-phase, has
63 been used to identify genes necessary for desiccation tolerance (Calahan et al., 2011).
64 Examination of the yeast, non-essential gene deletion collection, for mutants unable to tolerate
65 short-term desiccation tolerance, identified deletions in genes that block respiration (Calahan
66 et al., 2011). While identifying an essential process such as respiration as being important for

67 desiccation tolerance, this screen did not inform on potential stress effectors because changes
68 in respiration alters the expression of many hundreds of genes, including many potential stress
69 effectors. Strikingly, this genetic screen failed to identify deletions in non-regulatory genes. The
70 absence of these deletions supported the notion that stress effectors with overlapping
71 functions in desiccation tolerance must exist, precluding their identification by a single gene
72 deletion.

73 An increase in desiccation tolerance of exponentially dividing yeast cells provides a
74 measure of the sufficiency of individual factors to confer desiccation tolerance. Indeed,
75 elevated intracellular trehalose or expression of several individual tardigrade sIDPs increased
76 the survival of exponentially-dividing yeast after desiccation by 1000-fold (Boothby et al., 2017;
77 Tapia et al., 2015). These results showed that trehalose and sIDPs could act as potential
78 stress effectors in the same organism. However, the level of desiccation tolerance in cells with
79 elevated trehalose or the tardigrade sIDPs was still significantly less than the tolerance in
80 stationary-phase cells, further reinforcing the notion that tolerance likely requires multiple
81 effectors working in concert.

82 We sought to identify such effectors and to characterize their molecular functions. Here,
83 using our genetic assays with stationary-phase and exponentially growing yeast cells, we
84 demonstrate that trehalose and Hsp12, a yeast hydrophilin, synergize to completely alleviate
85 all of the lethal damage that occurs due to severe water loss. We provide evidence that these
86 two small molecules counteract the misfolding and aggregation of protein reporters that occurs
87 upon desiccation both *in vitro* and *in vivo*. We identify a novel role for Hsp12 as a membrane
88 remodeler, an activity likely required to provide desiccation tolerance. Finally, our data
89 suggests that despite sharing many of the same properties (high glycine content, small size,
90 high hydrophilicity, disordered secondary structure), yeast hydrophilins clearly have distinct
91 functions. We discuss the implications of these findings for both stress biology and potential
92 translational applications.

93

94 **RESULTS**

95 **Trehalose and Hsp12 are necessary and sufficient for desiccation tolerance**

96 We reasoned that the previous screen of the non-essential yeast deletion collection for
97 sensitivity to short-term desiccation sensitivity was unable to identify individual stress effectors
98 because likely more than one stress effector needed to be inactivated to compromise tolerance.
99 At least one of these factors was likely trehalose, given its role in long-term desiccation

100 tolerance. Therefore, we started with a base strain that was unable to synthesize trehalose
101 (*tps1*Δ) and introduced into it deletions for the remaining ~5,000 non-essential genes (Tong et
102 al., 2001). These 5000 double mutants were grown to stationary phase and assayed for
103 survival after short-term and long-term desiccation. We identified three groups of double
104 mutants with distinct phenotypes. The first group of strains were inviable when subjected to
105 short-term desiccation (Table S1A, S1-2). The second group of strains were inviable even in
106 the absence of stress (Table S1-1). The third group suppressed the long-term desiccation
107 sensitivity of a *tps1*Δ (Table S1-3). While these last two groups identify many potentially
108 interesting genes, we focused on the first group; the gene deletions in this group potentially
109 inactivated candidate stress effectors alongside trehalose that were needed for short-term
110 desiccation tolerance.

111 One candidate gene from our desiccation sensitive group was *HSP12*, which encodes one
112 of 12 previously identified yeast hydrophilins and is the most highly expressed member of the
113 family (Garay-Arroyo et al., 2010). To investigate the potential synergism between Hsp12 and
114 trehalose further, we compared quantitatively short- and long-term desiccation tolerance of
115 stationary cells that were wild type, *tps1*Δ*, hsp12*Δ, or *tps1*Δ *hsp12*Δ. After a 30-day
116 desiccation period, the viability of stationary wild type cells decreased only two-fold (Figure 1A).
117 Although the viability of *tps1*Δ cells was similar to that of wild type cells after 2 days of
118 desiccation, after the 30-day desiccation period, *tps1*Δ cell viability dropped more than 100-fold
119 (Figure 1A). This result corroborated our previous evidence demonstrating a requirement of
120 trehalose for long-term but not short-term desiccation tolerance (Tapia and Koshland, 2014).
121 While *hsp12*Δ cells exhibited wild type levels of desiccation tolerance after both short- and
122 long-term desiccation, the *tps1*Δ *hsp12*Δ cells displayed a 100-fold drop in viability after only 2
123 days of drying. Furthermore, after 30 days of desiccation, no viable *tps1*Δ*hsp12*Δ cells
124 remained (Figure 1A). These results revealed that trehalose and Hsp12 act cooperatively to
125 promote tolerance to both short- and long-term desiccation. Furthermore, although *tps1*Δ and
126 *hsp12*Δ had both been shown to confer elevated sensitivity to heat stress, the *tps1*Δ *hsp12*Δ
127 double mutant was no more heat-sensitive than either single mutant alone (Figure S1) (Gibney
128 et al., 2015; Welker et al., 2010). Thus, the protective synergism we observe between
129 trehalose and Hsp12 is specific to desiccation stress.

130 We had shown previously that exponentially growing cells with increased intracellular
131 trehalose showed a 1000-fold increase in desiccation tolerance (Tapia et al., 2015). Hence, we
132 tested whether exponentially dividing cells that expressed high-level expression of Hsp12

133 would also exhibit increased desiccation tolerance. Normally, Hsp12 is not highly expressed in
134 unstressed dividing cells. Therefore, we expressed Hsp12 under a strong constitutive promoter
135 to drive expression in dividing cells to a level similar to stationary cells. These dividing cells
136 exhibited a 1000-fold increase in the desiccation, comparable to the effect of increased
137 intracellular trehalose (Figure 1B). Thus, Hsp12, like trehalose, had a significant ability to
138 mitigate one or more of the lethal stresses imposed by desiccation.

139 Next, we looked for potential synergy between trehalose and Hsp12 by examining the
140 desiccation tolerance of exponentially dividing yeast cells containing high levels of both
141 protectants. The desiccation tolerance of these cells was ~60-fold greater than cells that
142 expressed only Hsp12 or trehalose. Furthermore, the absolute amount of tolerance (65-80%
143 survival) was even greater than the tolerance of wild type cells in stationary phase (20-40%
144 survival), the highest tolerance previously reported for yeast cells (Figure 1B) (Calahan et al.,
145 2011). Our findings suggest that the presence of these two stress effectors together are
146 sufficient to counter all the lethal effects of desiccation and subsequent rehydration on
147 exponentially dividing yeast.

148

149 **Trehalose and Hsp12 modulate proteostasis *in vivo* and *in vitro***

150 Having previously established that trehalose helped prevent desiccation-induced
151 proteotoxicity *in vivo* (Tapia and Koshland, 2014), we tested the *in vivo* role of Hsp12, with and
152 without trehalose to modulate proteostasis. We first utilized the inactivation of firefly luciferase
153 as a proxy for misfolding and aggregation *in vivo.* As a control, we expressed luciferase in
154 exponentially dividing wild-type cells that are expressing neither trehalose nor Hsp12. We also
155 expressed luciferase in exponentially dividing cells with high levels of Hsp12, trehalose or both
156 protectants together. After desiccation, luminescence in wild type cells was reduced one
157 hundred thousand-fold (Figure 2A). Thus, luciferase activity was extremely sensitive to
158 desiccation. Cells with elevated trehalose retained a four-fold increase in luminescence; while
159 cells with Hsp12 alone failed to retain any increase in luminescence after drying. However, the
160 luminescence of cells that contained both elevated trehalose and elevated Hsp12 was 14-fold
161 greater than wild type and 4-fold greater than cells with just elevated trehalose (Figure 2A).
162 These results demonstrate that Hsp12 can enhance the small, but reproducible, ability of
163 trehalose to prevent luciferase inactivation.

164 To complement the luminescence assay, we examined desiccation-induced luciferase
165 aggregation. Lysates were prepared from our panel of desiccated exponentially dividing cells

166 immediately after rehydration. The lysates were subjected to centrifugation to separate soluble
167 luciferase from insoluble luciferase that had aggregated with itself or other insoluble proteins.
168 For all strains tested, equivalent amounts of total luciferase were present in cell lysates (Figure
169 2B). In the wild type strain lacking trehalose and Hsp12, no luciferase was detected in the
170 soluble fraction (Figure 2B). Increased intracellular trehalose or Hsp12 significantly increased
171 luciferase solubility. Despite increasing solubility, Hsp12 failed to rescue any luciferase activity
172 (Figure 2A). Thus Hsp12 was more potent at blocking luciferase aggregation than it was at
173 preventing its denaturation. We conclude that desiccation, a naturally-occurring environmental
174 condition, potentiates denaturation and aggregation of luciferase, and these proteotoxic effects
175 can be mitigated *in vivo* by either trehalose, Hsp12, but more pronouncedly by a combination
176 of the two.

177 We next examined prion propagation as a second independent measure of the *in vivo* role
178 of trehalose and Hsp12 in proteostasis. Prion propagation provides two measures of
179 proteostasis. It requires stabilization of an altered protein state and a balance of
180 oligomerization, enough to transmit the altered protein state to other proteins but not too much
181 to prevent its transmission to daughter cells during division. Previously, we used [*PSI*$^+$], a
182 cytoplasmic prion or [*GAR*$^+$], a membrane-associated prion, to show that trehalose prevents
183 hyper-aggregation during desiccation of stationary yeast cells (Tapia and Koshland, 2014).
184 With this precedent, we constructed wild type, *tps1*Δ, *hsp12*Δ, or *tps1*Δ *hsp12*Δ strains with
185 either [*PSI*$^+$] or [*GAR*$^+$]. We grew aqueous cultures of these strains to stationary phase and
186 determined the percentage of cells that contained prions by growing on selective media
187 specific to the presence of the different prions. The cells in these cultures were also subjected
188 to two days of desiccation and then assessed for their ability to propagate the prions to their
189 rehydrated descendants.

190 Prior to desiccation, the percentage of stationary cells with [*PSI*$^+$] was statistically
191 indistinguishable in wild type, *hsp12*Δ, *tps1*Δ, or *tps1*Δ *hsp12*Δ cultures (Figure 2C). [*PSI*$^+$]
192 propagation was dramatically reduced in the descendants of desiccated *tps1*Δ cells (Figure
193 2C) as expected from our previous study (Tapia and Koshland, 2014). In contrast, the lack of
194 Hsp12 (*hsp12*Δ) alone did not affect the propagation of [*PSI*$^+$] after desiccation more than wild
195 type cells, nor did it exacerbate the loss of [*PSI*$^+$] propagation in cells unable to make trehalose
196 (*tps1*Δ *hsp12*Δ) (Figure 2C). These results suggest that trehalose is more effective than Hsp12
197 in preventing aggregation of this cytoplasmic prion.

198 In contrast, trehalose and Hsp12 exhibited a striking synergism in the propagation of
199 [*GAR*$^+$], a membrane prion. In *tps1*Δ cells, the percentage of [*GAR*$^+$] cells was ~10-fold lower
200 than wild type after desiccation (Figure 2D) as expected from our previous study (Tapia and
201 Koshland, 2014). The percentage [*GAR*$^+$] cells did not change significantly in *hsp12*Δ cultures
202 before or after transient desiccation (Figure 2D). In *tps1*Δ *hsp12*Δ cultures, the percentage of
203 [*GAR*$^+$] cells was undetectable after desiccation, as expected given that *tps1*Δ cells were
204 unable to propagate [*GAR*$^+$] through desiccation. However, even in the absence of desiccation,
205 the percentage of [*GAR*$^+$] cells in *tps1*Δ *hsp12*Δ cultures reduced almost 500-fold compared to
206 wild type cells (Figure 2D). Thus, these two small effectors act synergistically to modulate the
207 proteostasis of the [*GAR*$^+$] membrane prion even in the presence of water. This synergism also
208 may occur during desiccation, but was masked by the severe effect of the loss of trehalose
209 alone.

210 To test whether the impact of trehalose and Hsp12 on proteostasis was direct, we
211 examined their effect *in vitro* on the desiccation-induced aggregation of citrate synthase (CS).
212 We desiccated solutions of CS alone, or with trehalose, Hsp12 or both of the stress effectors
213 together. These samples were rehydrated, and CS proteostasis was assessed by assaying its
214 aggregation and its enzymatic activity.

215 Desiccation of CS caused a greater than 20-fold increase in its aggregation and a loss of
216 approximately 50% of its enzymatic activity (Figure 2E, S2A). The addition of increasing
217 amounts of trehalose or Hsp12 before desiccation decreased the desiccation-induced
218 aggregation of CS six to twelve-fold respectively. (Figure 2E). Both trehalose and Hsp12 also
219 restored nearly all CS enzymatic activity in a concentration-dependent manner (Figure S2A).
220 Limited synergism was observed between trehalose and Hsp12 against desiccation-induced
221 damage of this protein substrate (Figure S2B-C). The range of trehalose concentration that
222 impacted CS aggregation was very similar to the range of intracellular trehalose required to
223 promote desiccation tolerance *in vivo* (Tapia et al., 2015). Significant solubilization of CS
224 occurred at a 1:1 ratio of Hsp12 to CS (Figure 2E, 225 µg/ml). This equal stoichiometry
225 suggests that the proteostasis function of Hsp12 is not enzymatic, consistent with its high
226 expression during stress *in vivo*. These results suggest that both trehalose and Hsp12 have
227 independent proteostasis activities *in vitro* that may contribute to their protective *in vivo*
228 activities.

229

230 **Hsp12 remodels lipid vesicles**

231 The synergistic function of trehalose and Hsp12 in [*GAR*$^+$] propagation could be due to their
232 direct effect on membrane proteins and/or an indirect effect on overall membrane structure and
233 integrity. Intriguingly, prior studies indicate that trehalose stabilizes membranes *in vitro* and this
234 stabilization may prevent disruptive phase transitions that occur upon rehydration (Crowe et al.,
235 1984; 1987; Erkut et al., 2011; Leslie et al., 2002; Penkov et al., 2010; Tang et al., 2007).
236 Hsp12 has also been reported to increase membrane stability (Welker et al., 2010). A potential
237 membrane function for Hsp12 was also suggested by the fact that, although Hsp12 does not
238 exhibit any detectable secondary structure in solution, it acquires an alpha-helical conformation
239 in the presence of certain lipids (Welker et al., 2010). Secondary structure prediction and NMR
240 data identify four alpha-helical regions in Hsp12, with each of the three larger helices having
241 the features of amphipathic helices (Singarapu et al., 2011; Welker et al., 2010). Numerous
242 membrane-remodeling proteins use amphipathic helices, embedding the hydrophobic face
243 partway into the bilayer to shape membranes (Boucrot et al., 2012; Meinecke et al., 2013).
244 Given these considerations, we hypothesized that Hsp12 might have membrane remodeling
245 activities.
246 One readout of membrane remodeling activity is the capacity to vesiculate large liposomes
247 into nanovesicles (Boucrot et al., 2012). For example, proteins known to be involved in
248 endocytic membrane trafficking lead to the vesiculation of liposomes (Boucrot et al., 2012).
249 Therefore, we tested whether addition of Hsp12 to dimyristoylphosphatidylglycerol (DMPG)
250 liposomes would generate nanovesicles with bound Hsp12. DMPG liposomes incubated in the
251 absence of Hsp12 sedimented into the pellet fraction due to their large size (Figure 3A). By
252 contrast, when incubated in the presence of Hsp12, DMPG appeared in the supernatant
253 fraction (Figure 3A). To assess vesiculation directly, we examined these same samples by
254 electron microscopy. Prior to the addition of Hsp12, liposomes exhibited expected shapes and
255 sizes. After the addition of Hsp12, very few intact liposomes were observed, replaced by
256 membrane remnants (Figure 3B). Trehalose alone did not have any vesiculation activity nor
257 did it inhibit the effect of Hsp12 on the liposomes. These results show that Hsp12, but not
258 trehalose, has membrane-remodeling activities *in vitro*. Our findings suggest that their
259 synergistic impact on [*GAR*$^+$] propagation may result from different activities.
260
261 **Desiccation protection is not conserved among all hydrophilins**
262 To address whether the remarkable features of Hsp12 were common to other yeast
263 hydrophilins, we examined a second yeast hydrophilin, Stf2 (Garay-Arroyo et al., 2010).

264 Expressing high levels of Stf2 in exponentially dividing cells did not significantly improve their
265 desiccation tolerance and failed to provide any synergistic tolerance with trehalose (Figure 4A).
266 Additionally, deletion of Stf2 alone (*stf2*Δ), or in combination with a loss of trehalose synthesis
267 (*tps1*Δ *stf2*Δ) had no effect on the short-term desiccation tolerance of stationary phase yeast,
268 unlike the pronounced increase in desiccation sensitivity displayed by *tps1*Δ *hsp12*Δ cells
269 (Figure 1A and 4A). Unlike Hsp12, Stf2 also did not exhibit any detectable secondary structure
270 in the presence or absence of DMPG (Figure 4B). Additionally, the vesiculation activity of Stf2
271 varied greatly from what we observe with Hsp12. While Hsp12 clearly demonstrates
272 membrane vesiculation activity, Stf2 seems to lead to possible vesicle aggregation, even at
273 low concentrations (Figure 4C-D). These functional differences between Hsp12 and Stf2
274 suggest that hydrophilins likely carry out distinct biological and molecular functions, despite
275 sharing the general physical properties. Moreover, the biological and biochemical differences
276 between Sft2 and Hsp12 further support the view that the specific membrane remodeling
277 activity of Hsp12 contributes to its desiccation tolerance-promoting function.
278
279 **DISCUSSION**
280 We exploited the conditional desiccation tolerance of yeast to provide important new
281 insights into the stress effectors of desiccation tolerance. First, we demonstrated a dramatic
282 synthetic loss in desiccation tolerance in stationary cells that were deleted for *HSP12*, a
283 hydrophilin, *and TPS1*, a biosynthetic enzyme of trehalose. This synthetic sensitivity reveals a
284 function for this hydrophilin in desiccation tolerance that was missed because it was masked
285 by an overlapping function with trehalose. The function of Hsp12 as a stress effector of
286 desiccation was supported further by its ability, when highly expressed, to confer partial
287 desiccation tolerance to exponentially dividing cells. While Hsp12 has previously been reported
288 to mitigate lethality due to heat stress and osmolarity, we were unable to repeat those findings
289 (Figure S1) (Welker et al., 2010). Furthermore, the previous study used a temperature for heat
290 shock of 58°C, a temperature that budding yeast is unlikely to experience neither in the wild
291 nor fermenting vats (Welker et al., 2010). In contrast, desiccation occurs readily in nature, so
292 the desiccation tolerance conferred by Hsp12 is likely one of its true physiological functions.
293 These results, coupled with our previous demonstration for the importance of a subset of
294 tardigrade hydrophilins in desiccation tolerance, provide compelling evidence for the causal
295 roles of a hydrophilin in desiccation tolerance in most anhydrobiotes.

296 Hsp12's lipid-induced folding, its *in vitro* membrane remodeling activity, and its *in vivo* role
297 in desiccation tolerance are not shared with Stf2, another yeast hydrophilin. These features of
298 Hsp12 provide new insights into hydrophilins and stress biology. Like yeast, most organisms
299 have large families of hydrophilin-like genes. The distinct causal role of Hsp12 in desiccation
300 tolerance reveals that the generic properties of hydrophilins of charge, size and intrinsic
301 disorder, are not sufficient to mitigate the stresses of desiccation. Thus, the other hydrophilins
302 like Stf2 must have other unrelated functions. One simple idea is that these other functions
303 mitigate yet to be determined stresses. If so, hydrophilins may be a major new class of diverse
304 stress effectors.

305 A second major insight from this study is the demonstration that trehalose and Hsp12 can
306 synergize to counteract all the major stresses that yeast cells encounter upon severe water
307 loss. Exponentially dividing cells increased their desiccation tolerance 1000-fold when they
308 were engineered to have levels of Hsp12 or trehalose similar to levels in stationary cells. This
309 dramatic but partial desiccation tolerance was increased another 60-fold in exponentially
310 dividing cells expressing both trehalose and Hsp12, surpassing the tolerance seen in
311 stationary phase cells. The fact that this simple sugar and small protein together can confer
312 complete tolerance to sensitive cells speaks to their potency to mitigate major cellular stresses.
313 Importantly, given their small size and relatively simple structure, the molecular mechanisms
314 underlying their stress-effector activities are likely much simpler than the more conventional,
315 structurally and functionally complex stress effectors. The inferred simplicity of their molecular
316 activities may be important in conditions of desiccation where water and energy dependent
317 activities are limited.

318 However, we also show that compared to the *hsp12*Δ or *tps1*Δ single mutants, the *tps1*Δ
319 *hsp12*Δ double mutant was synergistically impaired in its ability to propagate a membrane
320 prion [GAR^+] in dividing aqueous cells. This result suggests that the molecular activities of
321 these two factors in membrane/membrane-protein stasis are not confined to the desiccation
322 stress. Rather, they can act together to modulate membrane or membrane-protein function in
323 aqueous conditions, perhaps providing a new clue for the reason why their regulation is linked
324 in response to numerous stresses.

325 We provide *in vivo* and *in vitro* evidence that both trehalose and Hsp12 can modulate
326 proteostasis in response to desiccation. We show that they prevent desiccation-induced
327 aggregation of luciferase *in vivo* and of citrate synthase *in vitro*. Trehalose and Hsp12 also
328 synergize, albeit weakly, to protect luciferase activity *in vivo*, and individually they protect

329 citrate synthase activity *in vitro*. Whether the stabilization of enzyme activity is different or just
330 another manifestation of their anti-aggregation activity is not clear. Interestingly, the
331 proteostasis functions of trehalose and Hsp12 are not identical as cells lacking only trehalose
332 are compromised for [*PSI*$^+$] and [*GAR*$^+$] propagation while lacking Hsp12 are not. These
333 differences likely indicate that trehalose and Hsp12 may modulate proteostasis by different
334 mechanisms.

335 In addition to Hsp12's proteostasis activities, we show that it has an *in vitro* activity of
336 membrane remodeling as evidenced by its vesiculation of lipid vesicles. This membrane
337 activity is consistent with several *in vivo* and *in vitro* observations. Hsp12 synergizes with
338 trehalose to allow propagation of the membrane prion [*GAR*$^+$] and also acquires secondary
339 structure upon binding to lipids. A previous study suggested that cells lacking Hsp12 have
340 altered membrane structure and fluidity (Welker et al., 2010).

341 Interestingly, trehalose prevents abnormal membrane vesiculation in desiccated
342 nematodes (Erkut et al., 2011). *In vitro* experiments have suggested that trehalose intercalates
343 into membranes to alter their melting properties (Crowe et al., 1984; 1987; Leslie et al., 2002;
344 Tang et al., 2007). Based upon these observations and our observations of Hsp12 in this study,
345 we propose that, alongside protecting against proteotoxicity, Hsp12 and trehalose cooperate in
346 membrane homeostasis. We propose that desiccation induces membrane damage. This
347 damage is prevented by the intercalation of trehalose into membranes. If damage occurs, it is
348 removed by Hsp12's membrane remodeling activity. These distinct activities explain the
349 synergistic requirement for trehalose and Hsp12 in both short- and long-term desiccation
350 tolerance. Additional genetic and biochemical experiment will be needed to test this model.

351 Regardless of their mode of function, the fact that just trehalose and Hsp12 are sufficient to
352 mitigate the major stresses of desiccation has major implications for engineering
353 desiccation/drought tolerance in other organisms. Water performs so many important functions
354 in biology; it was easy to imagine that the removal of water would generate many stresses that
355 require many stress effectors to combat. The need for a plethora of stress effectors would
356 make transmitting this trait to another organism too complex. The ability to generate
357 desiccation tolerance with only two factors makes this engineering eminently more feasible.
358 Attempts to engineer plants to synthesize more trehalose have been met with technical
359 difficulties because of the sugar's additional roles in metabolism. Our results provide new
360 impetus to overcome those hurdles. Furthermore, Hsp12 has no known metabolism side

361 effects. Given the sufficiency of Hsp12 alone to confer partial desiccation tolerance, it will be
362 very interesting to test whether Hsp12 by itself might confer drought tolerance in plants.
363

364 **MATERIALS AND METHODS**

365 **Strains and Growth Conditions**

366 Standard yeast propagation and transformation procedures were used. Yeast strains are
367 described in Supplemental Table 2. Strains were grown in nonselective (YP, 1% yeast extract
368 and 2% peptone) or selective (synthetic complete, SC) media containing 2% glucose. Cultures
369 were grown to saturation from a single colony by incubating cultures 5 days at 30°C for
370 stationary phase experiments. Cultures where grown to an OD – 0.5 for mid-log experiments.
371 All experiments were repeated at least two times on separate days with separate isolates
372 when appropriate. Hsp12 and Stf2 over-expression was driven from a plasmid under a
373 constitutively expressed promoter (p423-*TDH3pr-HSP12* (HTP126) or p426-*TDH3pr-STF2*
374 (HTP163)) or carry an empty vector plasmid (p423-*TDH3pr* (HTP64), p426-*TDH3pr* (HTP82)).

375 **Desiccation Tolerance Assay**

376 Saturated Cultures: Approximately 10^7 cells were withdrawn from liquid cultures and washed
377 twice in dilute water and then brought to a final volume of 1 ml. Undesiccated controls were
378 plated for colony counting. Two hundred microliter aliquots were then transferred to a 96-well
379 tissue culture plate (Becton Dickinson, 353075) and centrifugated, and water was removed
380 without disturbing the cell pellet. Cells were allowed to desiccate in a 23°C incubator with a
381 constant 60% relative humidity (RH), with the lid raised, for at least 48 hr. Long-term
382 desiccation experiments were kept for indicated time periods in a 96-well tissue culture plates
383 at 23°C, 60% RH. Samples were resuspended in assay buffer and plated for colony counting.

384 Logarithmic Samples: Cells were grown to mid-exponential phase (OD < 0.5) in different
385 selective media, depending on strain and plasmid necessities. Cells were then transferred to
386 inducible media: ± 2% trehalose, for 1 h. Following induction, $\sim 10^7$ cells were withdrawn from
387 liquid cultures and same parameters for drying where used as with saturated cultures. Data
388 were entered into a spreadsheet (Microsoft Excel 2008 for Mac version 12.3), and the number
389 of colony forming units per milliliter (cfu/mL) for each plate was computed. For each
390 experiment, number of colony forming units per milliliter for the two controls was averaged.

391 The relative viability of each of the two experimental samples was determined by dividing the
392 number of colony forming units per milliliter for that sample by the average number of colony
393 forming units per milliliter of the undesiccated control. These two relative viability values were
394 then averaged and their SD was computed using the STDEVP worksheet function.

395 **Luciferase Assay**

396 Yeast cells bearing 2μ plasmids that direct the expression of a temperature-sensitive firefly
397 luciferase protein from the constitutive glyceraldehyde-3-phosphate (*TDH3pr*) promoter
398 (HTP111 - p426-*TDH3pr*-FFL) or empty vector (HTP82 - p426-*TDH3pr*), were grown to mid-log
399 phase in SC -Ura-His. Luciferase activity was measured *in vivo* by addition of 0.5 mM D-
400 Luciferin (Sigma) to equal number of intact cells. Light emission was measured immediately
401 with a TD-20/20 Luminometer (Turner Designs, Sunnyvale CA). Desiccated samples were air
402 dried for two days followed by rehydration in SC-His - Ura + cycloheximide (10 μl/ml, to block
403 new FFL protein synthesis) and subjected to the same assay treatment after rehydration.
404 Measurements are reported as Relative Light Units. Using glass bead lysis, total cellular
405 protein was extracted. After removing unbroken cells by low-speed centrifugation (1,000g for 3
406 min), cleared lysates were spun at high speed (350,000g for 10 min, Beckman Coulter TLA-
407 100) to collect insoluble aggregates. Total protein from cleared lysates and high-speed
408 supernatants were then followed by SDS-PAGE and reacted with antiserum recognizing
409 luciferase (Promega). All experiments were repeated three times on separate days with
410 separate isolates.

411 **Citrate Synthase Assay**

412 Citrate Synthase from Porcine Heart ammonium sulfate suspension (C3260) was purchased
413 from Sigma Aldrich. Suspension was centrifugated at 14,000 rpm for 10 minutes at 4°C.
414 Supernatant was discarded and the pellet was resuspended in 750 μl MilliQ water. To remove
415 residual ammonium sulfate, citrate synthase was desalted in MilliQ water using a 5 ml HiTrap
416 Desalting Column (GE Healthcare). Fractions were evaluated for protein content via staining
417 30 μl of each fraction with 100 μl of Coomassie protein assay reagent (Sigma) diluted 1:5 in
418 MilliQ water. Fractions with detectable protein content were pooled and concentrated using an
419 Amicon Ultra 0.5 ml 10K centrifugal filter unit (Merck Millipore Ltd). Final concentration
420 determined via measuring absorbance at 280 nm on a Nanodrop 2000 spectrophotometer
421 (Thermo Scientific).

422 Citrate synthase aggregation and enzymatic activity assay:

423 Citrate synthase (CS) and protectants were added to MilliQ water to achieve final
424 concentration of 1.2 mg/ml CS, and varying concentrations of protectants in 100 µl. For
425 measuring enzymatic activity, 1 µl of each sample was diluted by a factor of 17 in MilliQ water.
426 2 µl of this dilution was then mixed with 198 µl of Enzymatic Activity Assay Solution [100mM
427 oxaloacetic acid (Sigma-Aldrich), 100mM 5,5'-Dithiobis(2-nitrobenzoic acid) (Sigma-Aldrich),
428 150mM Acetyl Coenzyme A (Sigma-Aldrich)] and absorbance at 412 nm was measured
429 immediately and after 120 seconds in a 50 µl Micro Cell cuvette (Beckman Coulter) with
430 measurements made with a 720 UV/Vis spectrophotomer (Beckman Coulter) equipped with a
431 50 µl Micro Cell adapter (Beckman Coulter). After measurement, samples were moved to 1.5
432 ml microcentrifuge tubes and set to dry in a Centrivap Concentrator (Labconco) set to 35°C
433 and 29 bar for 24 hours. Samples were then moved to a 23°C incubator and allowed to
434 incubate open to atmospheric pressure for an additional 5 hours. Samples were rehydrated in
435 100 µl MilliQ water, resuspended vigorously 5 minutes after addition of water. 2 µl of this
436 resuspension was added to 198 µl Enzymatic Activity Assay Solution, mixed and moved to a
437 cuvette, measuring absorbance at 412 nm immediately and after two minutes. Change in
438 absorbance across two minutes after drying compared to before drying to achieve relative
439 activity. For measuring aggregation, absorbance at 340 nm was measured for each sample
440 before and after drying. Drying and rehydration performed as described for enzymatic activity
441 assay. Measurements after drying subtracted from before drying measurements to achieve
442 change in aggregation after drying. Cuvette was washed with MilliQ water and 95% ethanol
443 between all measurements.

444 **Expression and purification of Hsp12p**

445 BL21 *E. coli* cells transformed with a pET-15B vector with HSP12 behind a galactose-inducible
446 promoter grown overnight in 30 ml cultures in LB with 100 ng/ml ampicillin. Transformants
447 were grown up overnight in 30 mL cultures in LB with 100 ng/ml ampicillin at 37° C. 20 mL of
448 this culture were added to 2L of LB with ampicillin and incubated at 37° C until culture reached
449 an OD600 of 0.5-0.9, as determined by spectrophotometry using a 720 UV/Vis
450 spectrophotomer (Beckman Coulter). HSP12 expression induced by addition of IPTG
451 (Isopropyl β-D-1-thiogalactopyranoside) to final concentration of 1 mM and incubation for an
452 additional 4 hours at 37°C. Cultures were then pelleted by centrifugation at 4,000 rpm for 30
453 minutes at 4°C. Discarded supernatant and resuspended cells in 50 mL of 1x PBS. Cells were
454 frozen with liquid nitrogen, and stored at -80°C until use. Pellets were isolated and
455 resuspended in lysis buffer (2mM EDTA, 2mM DTT, 20mM HEPES, pH 7.4) then sonicated

456 using a blunt tip sonicator set to 30% amplitude, 20 minutes, pulse 1 second on, 1 second off
457 at 4° C. Sonication-lysed cells were then centrifugated and supernatant was collected. This
458 sample was then boiled at 95° C for 15 mins and immediately moved to ice. A pale yellow
459 precipitate was separated away from the proteins that are still soluble after boiling by
460 centrifugation. Supernatant was then applied to a HiTrap Capto-Q ImpRes anion exchange
461 column (GE Healthcare Life Sciences), and washed with lysis buffer with increasing
462 concentrations of NaCl. Hsp12 was eventually eluted by buffer between 7.5 mM and 10 mM
463 NaCl. The protein was then concentrated using a MW3000 filtration unit, aliquoted, frozen in
464 liquid nitrogen, and stored at -80° C until use (Supple Fig. 2).

465 **Expression and purification of Stf2p**

466 STF2 fused to GST was cloned into a pGEX6 vector behind a galactose-inducible promoter
467 and transformed into BL21 cells. Transformants were grown overnight at 37° C in 30 mL of LB
468 liquid media, selecting for plasmid with 75 ng/ml ampicillin. 2 L LB cultures with 75 ng/ml
469 ampicillin were inoculated with 20ml of these overnight cultures and set to 37° C and 140 rpm
470 in an Innova 4330 incubator (New Brunswick Scientific). After 4 hours, expression of STF2
471 was induced with 4 mL of 0.1M IPTG and cultures were allowed to incubate overnight at 18° C.
472 Cells were harvested and lysed in lysis buffer 2 (150mM NaCl, 2mM EDTA, 2mM DTT, 20mM
473 HEPES, pH 7.4) using the same sonication method as above (see Purification of Hsp12). Then,
474 lysed cells were centrifugated and the supernatant was collected. 1.5 ml of Pierce Glutathione
475 Agarose beads from Thermo Fisher Scientific were added and incubated in the supernatant for
476 an hour at 4° C. Beads were washed in 3 washes in lysis buffer 2, 3 washes in lysis buffer 3
477 (500mM NaCl, 2mM EDTA, 2mM DTT, 20mM HEPES, pH 7.4), and 3 washes in lysis buffer 2,
478 and resuspended in lysis buffer 2. Prescission protease was added to washed beads and this
479 mixture was incubated at 4° C overnight. The mixture was then centrifugated and the
480 supernatant was collected, concentrated with an Amicon Ultra-4 Ultracel 3K centrifugal filter
481 unit from Millipore, aliquoted, frozen in liquid nitrogen, and stored at -80° C (Supple Fig. 2).

482 **Prion phenotypic assays**

483 To asses prion state, cells were plated on media lacking adenine (SC-ADE) for [*PSI*$^{+}$] strains,
484 or on YP media with 0.05% Glucosamine and 2% Glycerol for [*GAR*$^{+}$] growth was compared to
485 their ability to grow on rich YP-GAL media. To determine % Prion = 100 X CFU Desiccated
486 Cells (Prion Selective Media) / CFU Desiccated Cells (Non-Selective Rich Media).

487 **Liposome preparation and vesiculation**

488 DMPG liposomes (Avanti Polar Lipids (840445) in 100 mM NaCl, 20 mM Tris-HCl pH 7.4 were
489 sonicated for 2 minutes in a water bath at room temperature. Liposomes were incubated with
490 protein for 60 minutes and centrifugated at 250,000g for 15 min in a Beckman Coulter TLA-100
491 rotor. Resuspended pellets and supernatants were analyzed by SDS-PAGE. Gels were
492 stained with 0.1% Coomassie in 10% Acetic acid and destained in water. For EM, samples
493 were spread prior to centrifugation on glow-discharged electron microscopy grids and stained
494 using 2% uranyl acetate and viewed in a Joel 1200 EX Transmission Electron Microscope.

495 **Preparing liposomes for circular dichroism**

496 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) (DMPG) from Avanti Polar was stored in
497 chloroform at -80° C in glass tubes until used. DMPG aliquots were thawed and aspirated
498 using a stream of nitrogen gas to form a clear, dry film on the inside surface of the tube. Tubes
499 were then placed inside a vacuum desiccator and sealed. Vacuum was then applied to the
500 desiccator, and the samples were left at room temperature under vacuum overnight to remove
501 any remaining chloroform. Then, they were resuspended in 10mM sodium phosphate, pH 7.4
502 and moved to a 1.5 ml plastic microcentrifuge tube. Resuspended aliquots were then vortexed
503 until a homogenous, cloudy white solution was formed. Tubes were placed on ice in a 4° C
504 room, and sonicated using a probe tip sonicator equipped with a blunt tip and set to 20%
505 amplification, 7.5 minutes, with pulses of 2 seconds on and 2 seconds off. Lipids were then
506 spun down at 14,000 rpm at 4° C for 10 minutes to precipitate any metal shards from
507 sonication. Supernatant was then moved to a new 1.5 ml microcentrifuge tube and left on ice
508 until use in circular dichroism.

509 **Circular dichroism spectra**

510 Circular Dichroism (CD) spectra were obtained using an Aviv 2000 Circular Dichroism
511 Spectrometer Model 410 (Aviv Biomedical Inc.). CD signal was measured from 250 to 190 nm
512 at 25° C, averaging measurements at every nm for 10 seconds. Hydrophilins and DMPG were
513 kept on ice until added to samples. Once added, samples were mixed well by flicking and
514 incubated at room temperature for 10 minutes before spectra were measured. A 1M stock of
515 trehalose and 100mM stock of SDS were prepared in 10mM sodium phosphate, pH 7.4 and
516 added to achieve desired concentrations. Proteins were added to samples to achieve a
517 concentration of 0.32 mg/ml. For samples with DMPG, DMPG was added to achieve a
518 concentration of 1.32 mM (1.2 mg/ml. Samples were made at a volume of 400 µL and 300 µL
519 were pipetted into a 0.1 cm cuvette for CD measurements. The cuvette was washed with water
520 and ethanol between measurements, drying completely each time. Samples with protein were

521 blanked against those with the same buffer and concentration of DMPG without hydrophilins.
522 All spectra were converted to units of mean residue molar ellipticity before plotting on graph.

523 **SGA Strain Construction**

524 Strains were construction according to published SGA protocol from Tong et al. with minor
525 alterations (Tong et al., 2001). In brief, *tps1*Δ was grown overnight as well as every strain in
526 the ATCC deletion collection. Using a 48-density prong, *tps1*Δ was pinned onto solid YEP +
527 Galactose media and the deletion collection were pinned on top. This was allowed to grow
528 overnight at 30°C. Cells were then replica plated onto YEP + Galactose + G418 (100 mg/mL) +
529 Hygromycin (100 mg/mL) to select for diploids. These were grown at 30°C overnight. Diploids
530 were then replica plated onto sporulation media (10g Potassium Acetate, 1g Yeast Extract,
531 0.5g Galactose, 0.1g amino acid Histidine, Lysine, Leucine, and Uracil supplement, Zinc
532 Acetate, 0.2g Raffinose). Cells were sporulated for 5 days. After sporulation, we selected for
533 MATa haploids on SC + Galactose – Histidine – Arginine + Canavanine (50 mg/L) + Thialysine
534 (50mg/L). Then we selected for G418 resistance on SC + Galactose – Histidine – Arginine +
535 Canavanine (50 mg/L) + Thialysine (50 mg/L) + G418 (100 mg/mL). Finally, we selected for
536 Hygromycin resistance on SC + Galactose – Histidine – Arginine + Canavanine (50 mg/L) +
537 Thialysine (50 mg/L) + G418 (100 mg/mL) + Hygromycin (100 mg/mL).

538 **High Throughput Desiccation Tolerance Assay**

539 Single colonies of newly constructed double mutants are placed into 200 **µ**L of YEP + 2%
540 Galactose in wells of 96-well plates and allowed to grow to saturation at 30°C with agitation.
541 Strains were then pinned using a 48-density prong onto YEP + Galactose plates and allowed
542 to grow for 2 days as non-desiccated controls. 20 **µ**L of each strain was also transferred into
543 new 96-well plates to let air dry for 6 and 30 days at 23°C. Drying was done as previously
544 mentioned. After desiccation, strains are rehydrated in 200 **µ**L of YEP + Galactose and pinned
545 onto solid media as before. Desiccation tolerance is assayed by comparing growth of strains
546 after desiccation with non-desiccated controls.

547 **Heat Tolerance Assay**

548 Supplemental Figure 1 (**A**) Cells (wild type, *hsp12*Δ, *tps1*Δ, or *tps1*Δ *hsp12*Δ*)* were grown to
549 midexponential phase (OD < 0.5) in non-selective media (YP, 1% yeast extract and 2%
550 peptone) containing 2% glucose at 30C. Cells were plated and grown at either 30C or 37C, or
551 grown for one hour at 34C (pre-heat shock) before growing at either 30C or 37C. (**B**) Cells
552 (wild type, *hsp12*Δ, *tps1*Δ, or *tps1*Δ *hsp12*Δ*)* were grown to midexponential phase (OD < 0.5) in
553 non-selective media (YP, 1% yeast extract and 2% peptone) containing 2% glucose at 30C.

554 Cells were then heat-shocked for 30 min at temperatures ranging from 42-60C, followed by
555 plating and growing at 30C. Cells were also grown at 34C prior to heat shock.
556

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563 12) to G.Ç..
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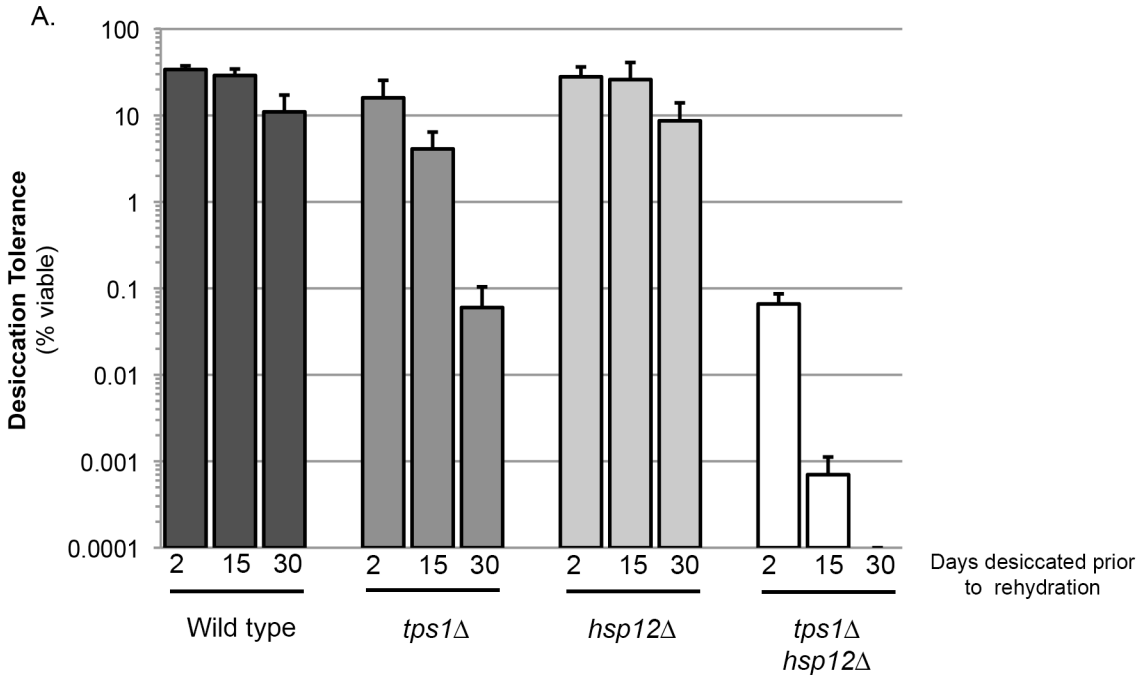
626 **Figure 1.**



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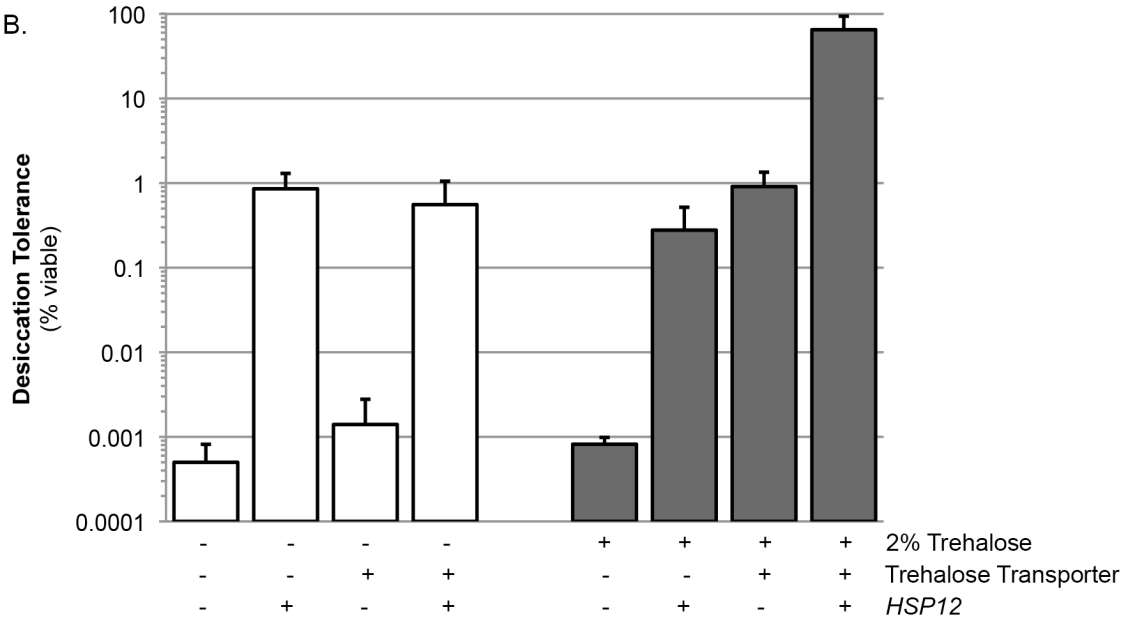

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629 **Figure 1. Trehalose and Hsp12 are necessary and sufficient for desiccation tolerance.**

630 (**A**) Yeast cells were grown to saturation (5 days), air-dried for 2, 15, or 30 days at 23 °C, 60%
631 relative humidity (RH), then rehydrated and assessed for viability by counting colony forming

632 units (CFU). Desiccation tolerance of wild type (BY4742), HTY104 (*tps1*Δ), HTY11 (*hsp12*Δ) or
633 HTY176 (*tps1*Δ*hsp12*Δ) cells. (**B**) Yeast cells were grown to mid-exponential phase (OD < 0.5)
634 in selective media (SC-His+Dextrose). Cells were then transferred to either selective media
635 (SC-His+Dextrose, 0% trehalose) or selective media with trehalose (SC-His+Dextrose, 2%
636 trehalose) for 1 h. Cells were collected, washed, and air dried for 2 d at 23 °C, 60% relative
637 humidity (RH), then rehydrated and assessed for viability by counting colony forming units
638 (CFU). Log-phase experiments performed in strain HTY121 (*nth1*Δ (trehalase delete)),
639 additionally: +/- Trehalose transporter (*AGT1* expressed constitutively (*TDH3pr-AGT1)*) and +/-
640 *HSP12* (Hsp12 expressed constitutively from a plasmid (HTP126), strains not over-expressing
641 *HSP12* carry an Empty Vector plasmid (HTP64).
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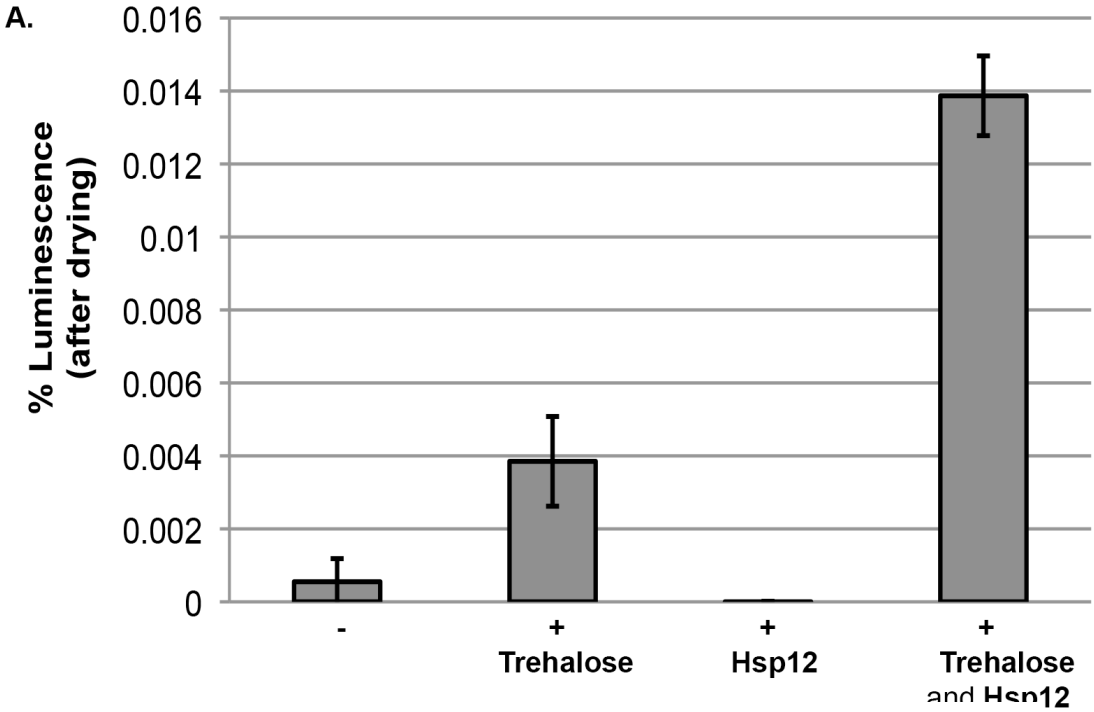
665 **Figure 2.**



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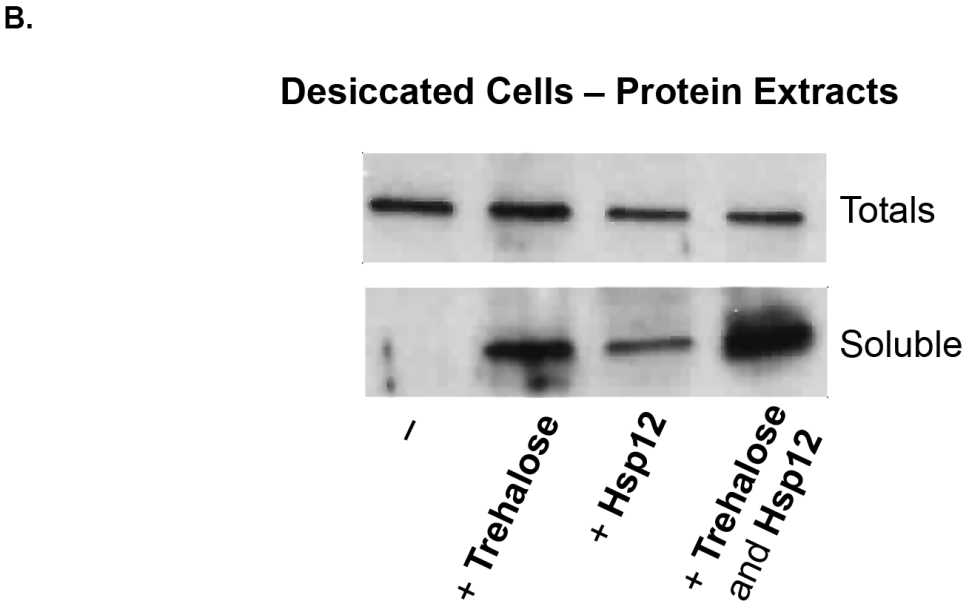

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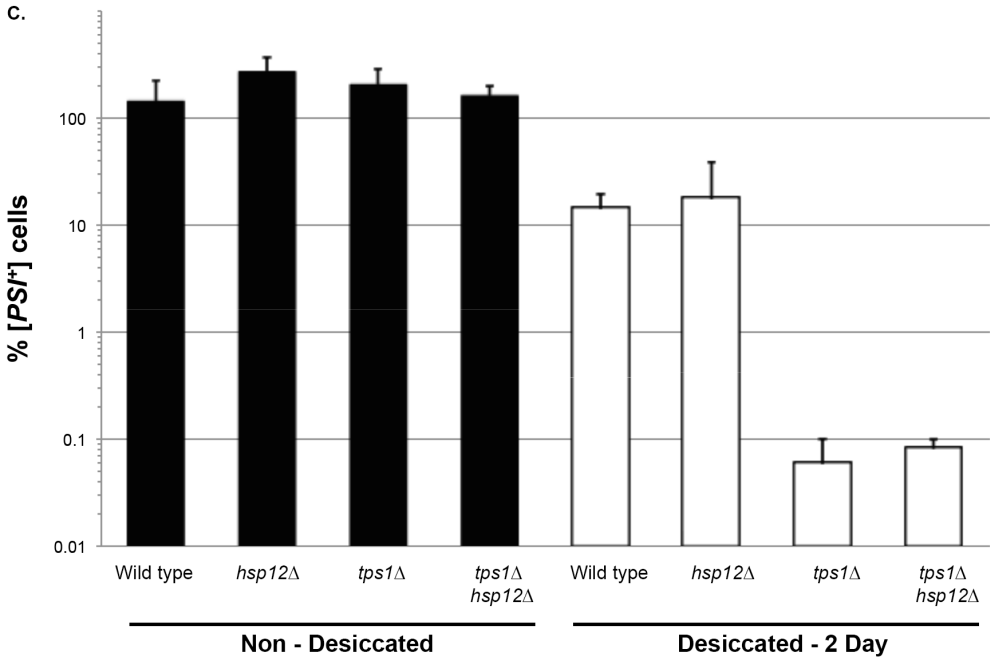
C.
% [PSI+] cells
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Wild type
hsp12Δ
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tps1Δ hsp12Δ
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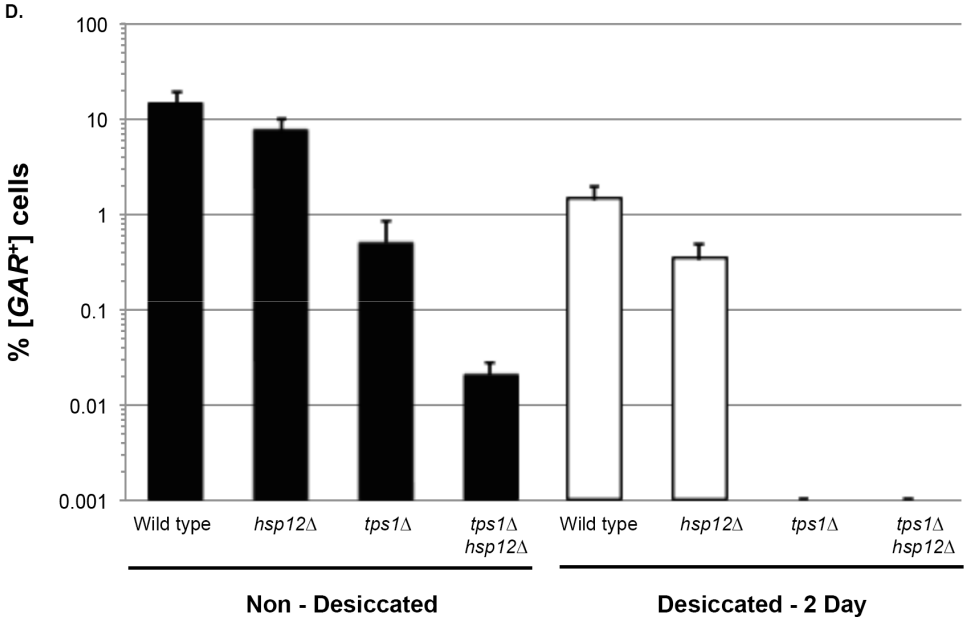
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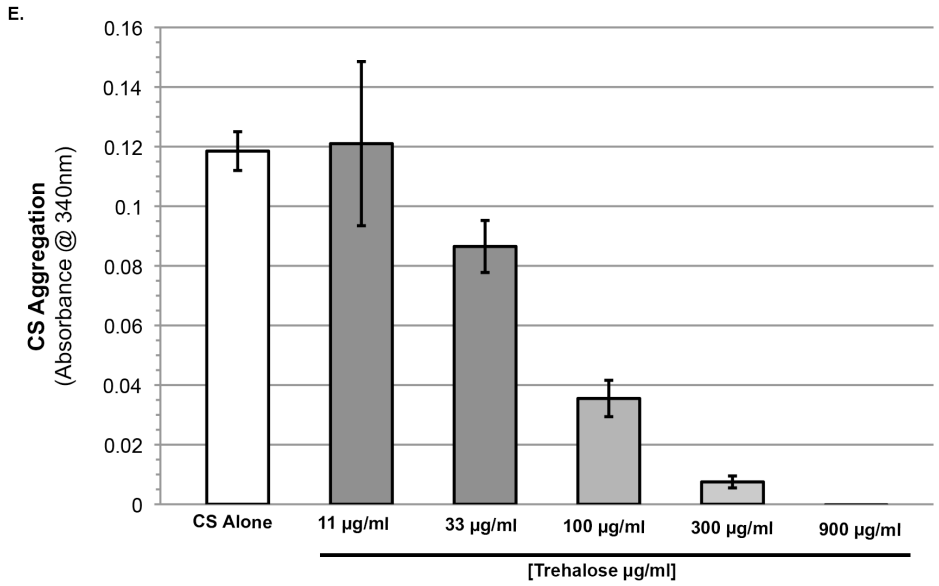

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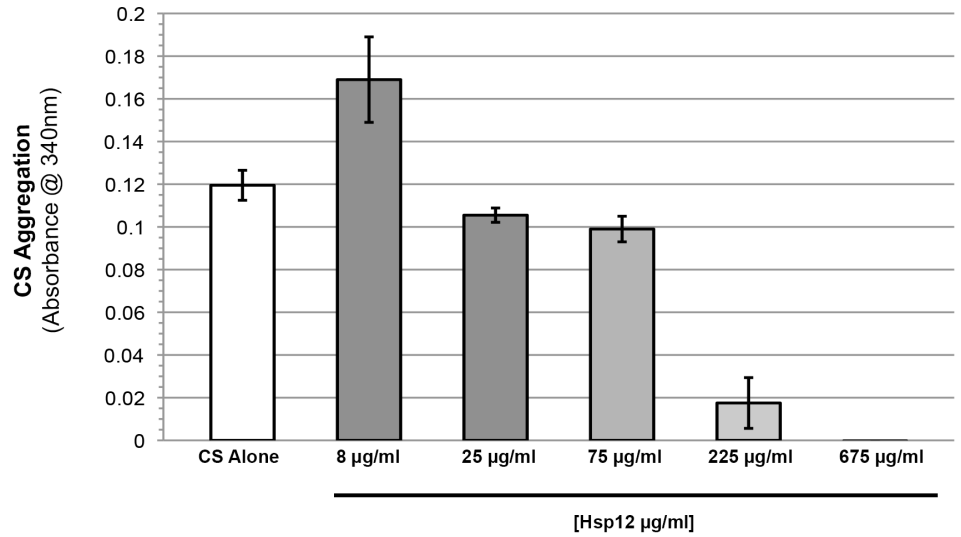

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673 **Figure 2. Trehalose and Hsp12 stabilize activity and prevent aggregation of *in vivo* and**

674 ***in vivo* model substrates.**

675 (**A-B**) Luciferase activity and aggregation as a reading of proteostasis of logarithmically
676 growing and desiccated cells. (**A**) Yeast cells were transformed with a temperature-sensitive
677 firefly luciferase protein expressed from the constitutive (GPD) promoter (HTP111) or empty
678 vector (HTP82). Strains were grown to mid-log phase in selective media (SC-Ura-
679 His+Dextrose) and then transferred to selective media with trehalose (SC-Ura-His+Dextrose,
680 2% trehalose) for 1 h. Desiccated samples were air-dried for 2 days followed by rehydration in
681 SC-Ura-His + cycloheximide (10 µl/ml, to block new FFL protein synthesis). Luciferase activity
682 was measured in *vivo* by addition of 0.5 mM D-Luciferin to equal number of intact cells. Light
683 emission was measured immediately with a luminometer. Percentage of activity was
684 normalized to WT saturated ''wet'' sample. Luciferase experiments performed in strain HTY163
685 (*nth1*Δ (trehalase delete)), additionally: +/- Trehalose transporter (*TDH3pr-AGT1)* and +/-
686 Hps12 (HSP*12* expressed constitutively from a plasmid (HTP126), strains not over-expressing
687 *HSP12* carry an Empty Vector plasmid (HTP64). (**B**) Cells were prepared as in A. Total cellular
688 protein was extracted, and aggregated proteins were sedimented by high-speed centrifugation.
689 The amounts of luciferase in the total protein fraction (total) and in high-speed supernatants
690 (soluble) were determined by SDS-PAGE and followed by immunoblotting with antiserum
691 recognizing luciferase. (**C-D)** Prion propagation as a measure of *in vivo* protein propagation
692 after desiccation. (**C**) [*PSI*$^+$] prion propagation before, and after desiccation. To assess [*PSI*$^+$]
693 prion state, cells were plated on media lacking adenine (SC-ADE) compared to non-dried
694 controls. (**D**) [*GAR*$^+$] prion propagation before, and after desiccation. To assess [*GAR*$^+$] prion
695 state, cells were plated on YP media with 2% glycerol and 0.05% glucosamine compared to
696 non-dried controls. Strain information available on strain Table S2. (**E**) Desiccation-induced
697 aggregation of citrate synthase in the presence of varying concentrations of trehalose or
698 Hsp12. Absorbance at 340 nm. Citrate Synthase (0.12 mg) and increasing concentrations of
699 trehalose, or Hsp12. Absorbance measured after drying at 36°C for 24 hours followed by 5
700 hours at 23°C, then rehydration in water. Measurements after drying compared to
701 measurements before drying.
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707

708 **Figure 3.**

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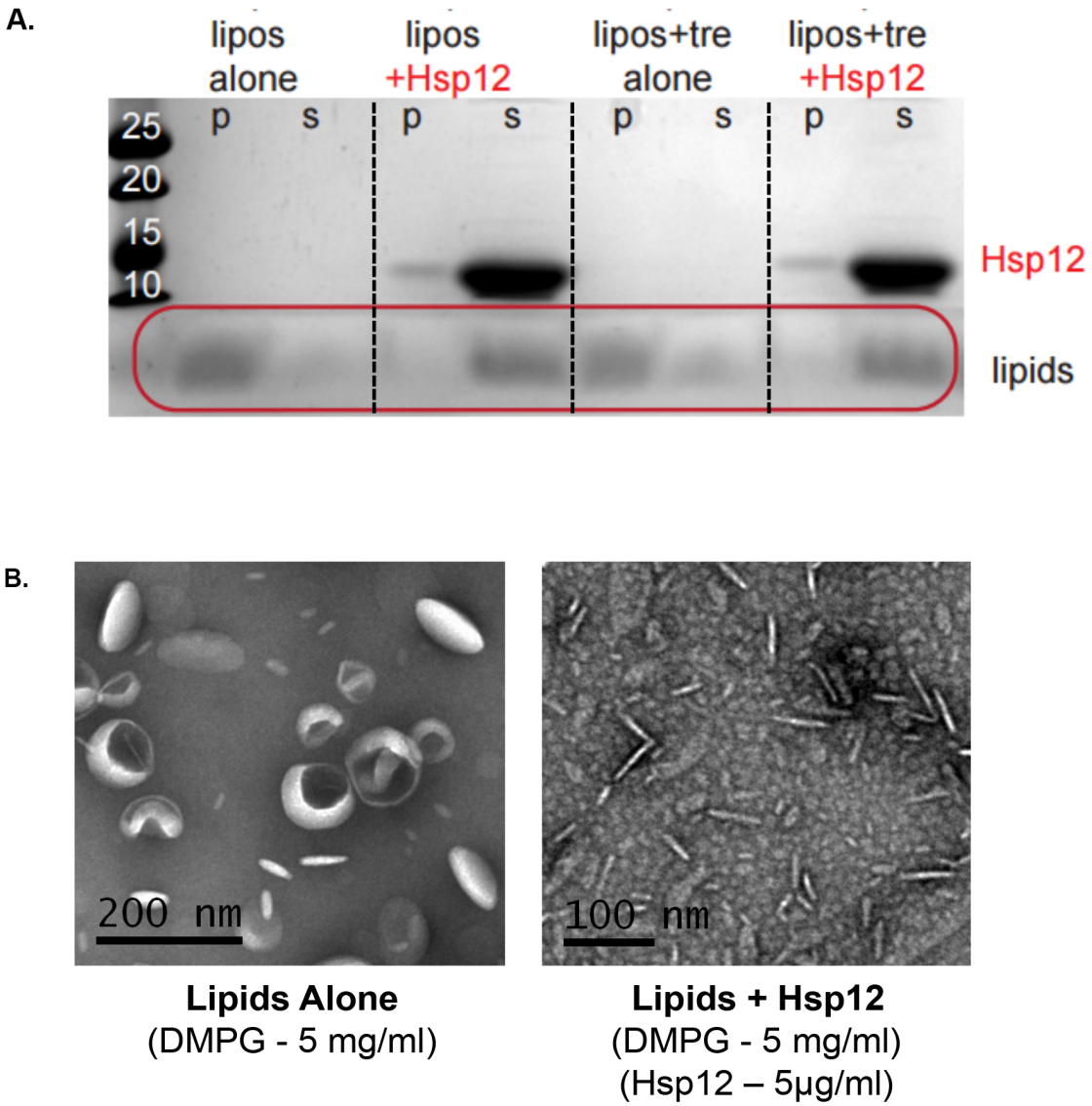

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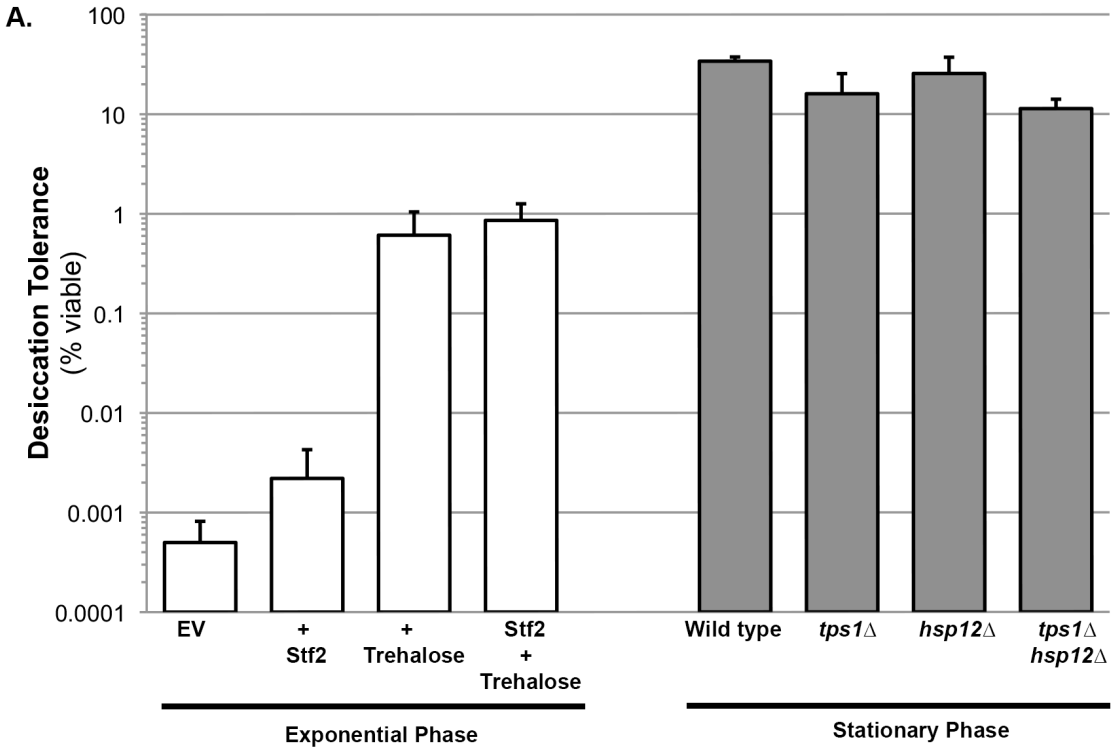
712 **Figure 3. Hsp12 causes membrane remodeling.**

713 (**A**) DMPG liposomes (5 mg/ml) where incubated for 1 hour at room temperature in the
714 presence or absence of Hsp12 (25 ug/ml) and/or in the presence or absence of trehalose (2%
715 final). Pellet (p) and supernatant (s) fractions where separated by high-speed centrifugation,
716 and lipid distribution was assessed by SDS-PAGE followed by altered Coomassie staining
717 (10% acetic acid only). (**B**) Samples for electron microscopy were prepared the same as in A,
718 with Hsp12 at 5 ug/ml. Images where taken prior to centrifugation. Samples where spread on
719 glow-discharged EM grids and stained using 2% uranyl acetate.

720 **Figure 4.**



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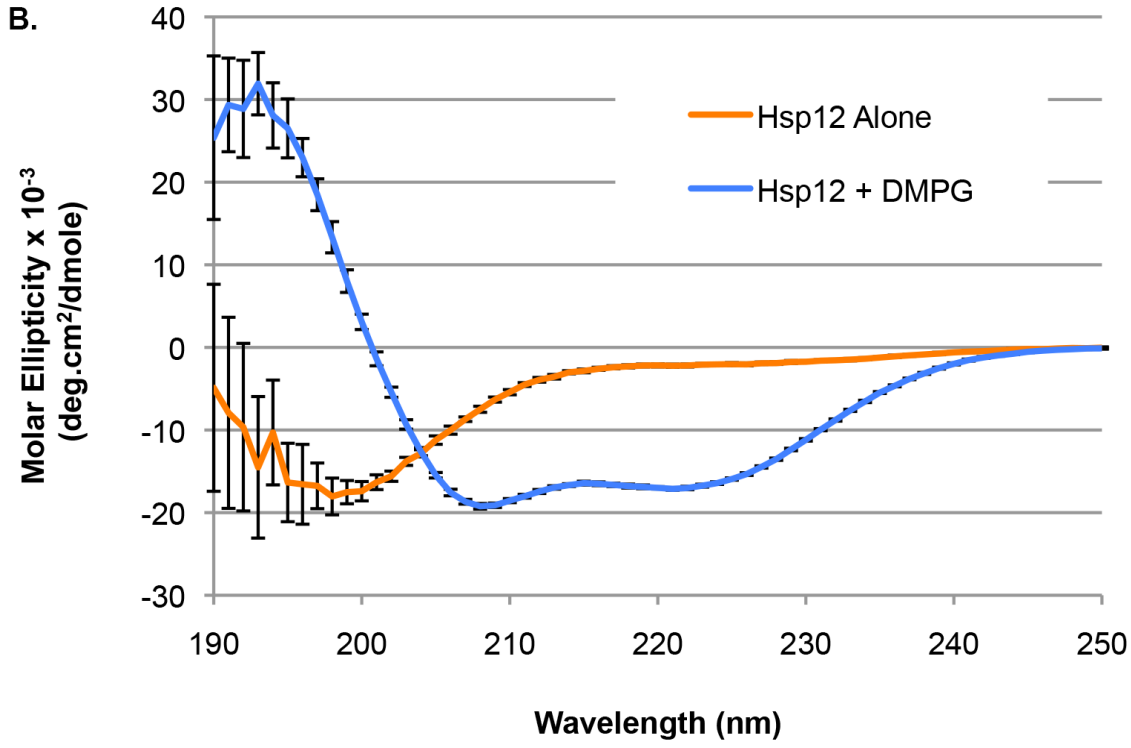
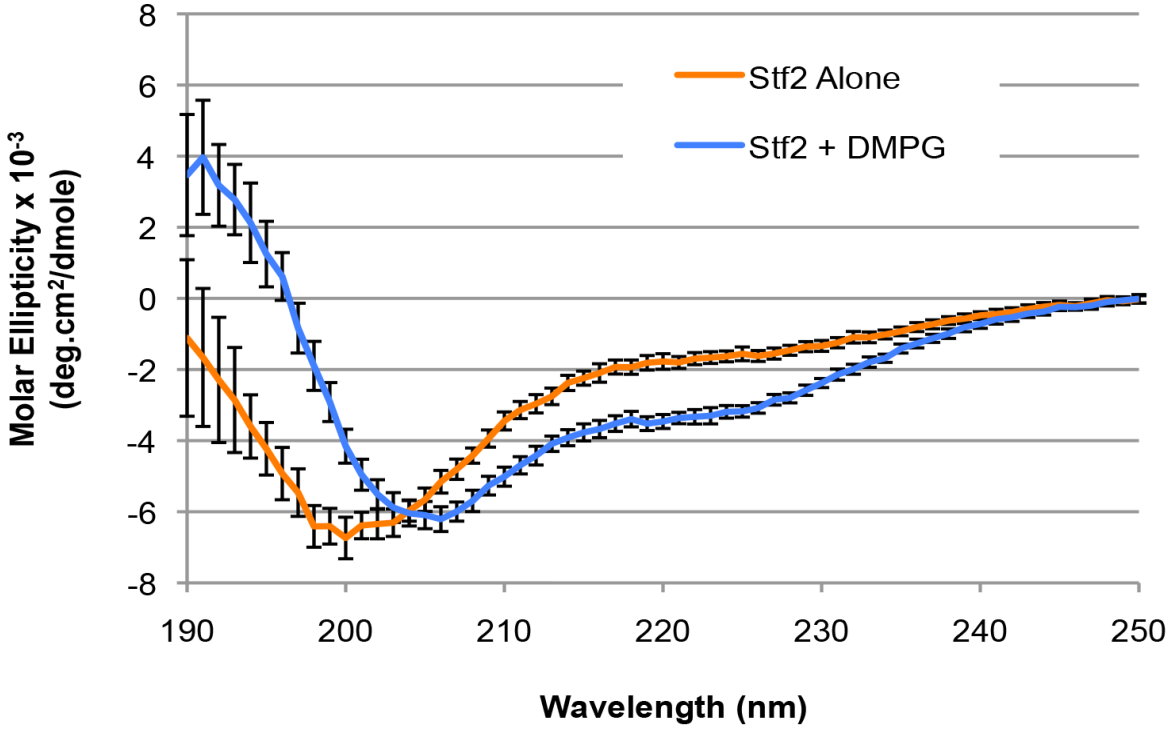

B.
Hsp12 Alone
Hsp12 + DMPG
Molar Ellipticity x 10-3 (deg.cm2/dmole)
Wavelength (nm)
40
30
20
10
0
-10
-20
-30
190
200
210
220
230
240
250

722


Stf2 Alone
Stf2 + DMPG
Molar Ellipticity x 10-3 (deg.cm2/dmole)
Wavelength (nm)
8
6
4
2
0
-2
-4
-6
-8
190
200
210
220
230
240
250

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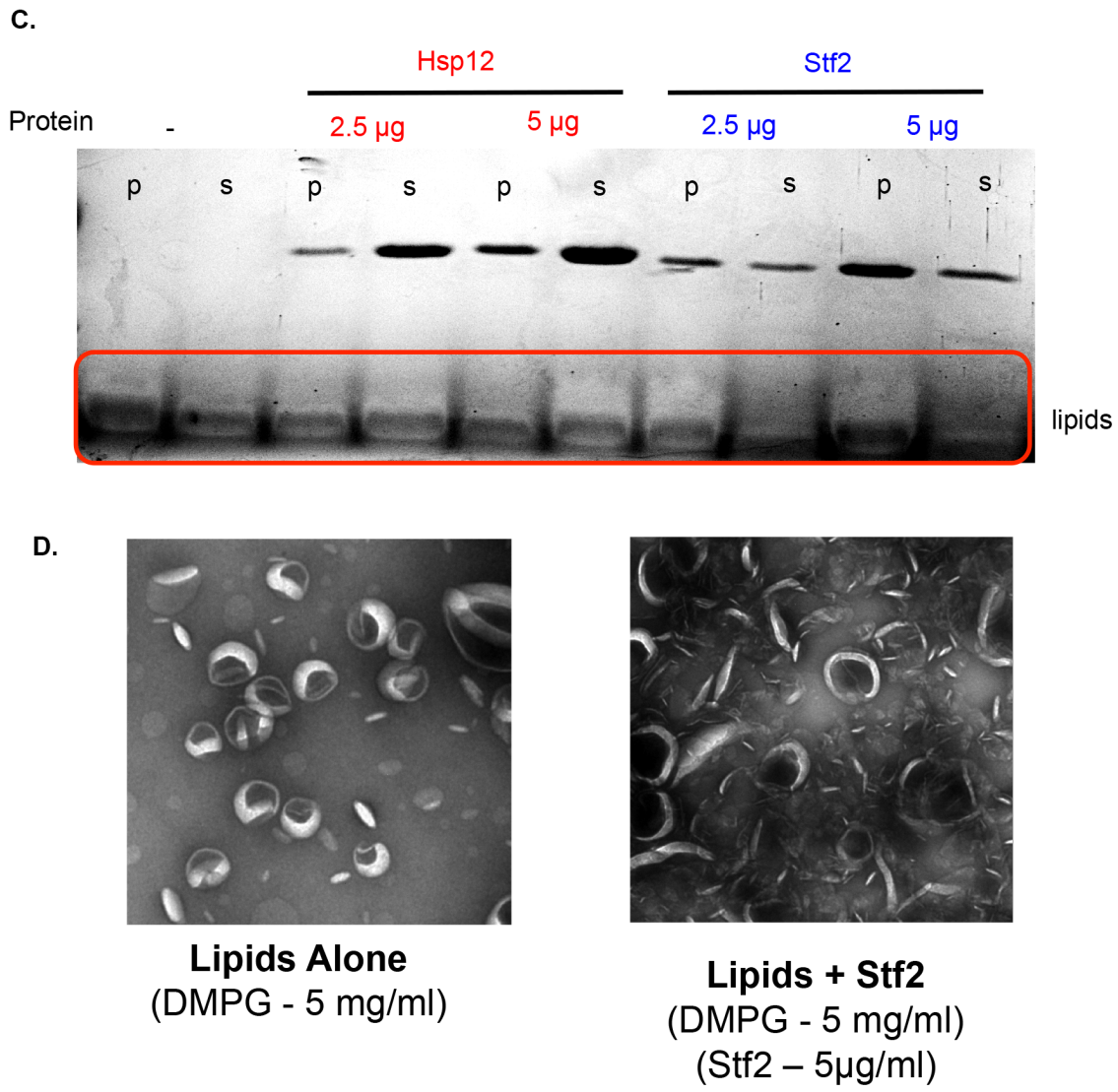

Figure 4. Desiccation protection not a common hydrophilin feature

(**A**) Exponential Phase. Yeast cells were grown to mid-exponential phase (OD < 0.5) in selective media (SC-Ura+Dextrose). Cells were then transferred to selective media with trehalose (SC-Ura+Dextrose, 2% trehalose) for 1 h. Cells were collected, washed, and air dried for 2 d at 23 °C, 60% relative humidity (RH), then rehydrated and assessed for viability by counting colony forming units (CFU). Yeast cells used for exponential-phase experiments performed in strain HTY163 (*nth1*Δ (trehalase delete)), additionally: + EV (HTP82 - p426-*TDH3pr),* + Stf2 (HTP163*),* + Trehalose Transporter (*TDH3pr-AGT1*), or both Stf2 and Trehalose Transporter (HTP163 and *TDH3pr-AGT1*). Stationary Phase. Yeast cells were grown to saturation (5 days), air-dried for 2 days at 23 °C, 60% relative humidity (RH), then rehydrated and assessed for viability by counting colony forming units (CFU). Desiccation

737 tolerance of wild type (BY4742), HTY104 (*tps1*Δ), HTY14 (*stf2*Δ) or HTY179 (*tps1*Δ*stf2*Δ) cells
738 (**B**) Circular dichroism spectroscopy performed on 0.32 mg/ml Hsp12 or 0.32 mg/ml of Stf2 in
739 the presence or absence of 1.2 mg/ml DMPG small unilamellar vesicles (SUVs), measuring
740 from 250 to 190 nm. Units converted to Mean Molar Residue Ellipticity, accounting for
741 concentration and protein size. (**C**) DMPG liposomes (5 mg/ml) where incubated for 1 hour at
742 room temperature in the presence or absence of Hsp12 (2.5-5 µg/ml) or in the presence of Stf2
743 (2.5-5 µg/ml). Pellet (p) and supernatant (s) fractions where separated by high-speed
744 centrifugation, lipid distribution was assessed by SDS-PAGE followed by altered Coomassie
745 staining (10% acetic acid only, deeper stain used to visualize lipids in cleared stain). (**D**)
746 Samples for electron microscopy where the same as in A, with Stf2 at 5 µg/ml. Images where
747 taken prior to centrifugation. Samples where spread on glow-discharged EM grids and stained
748 using 2% uranyl acetate.
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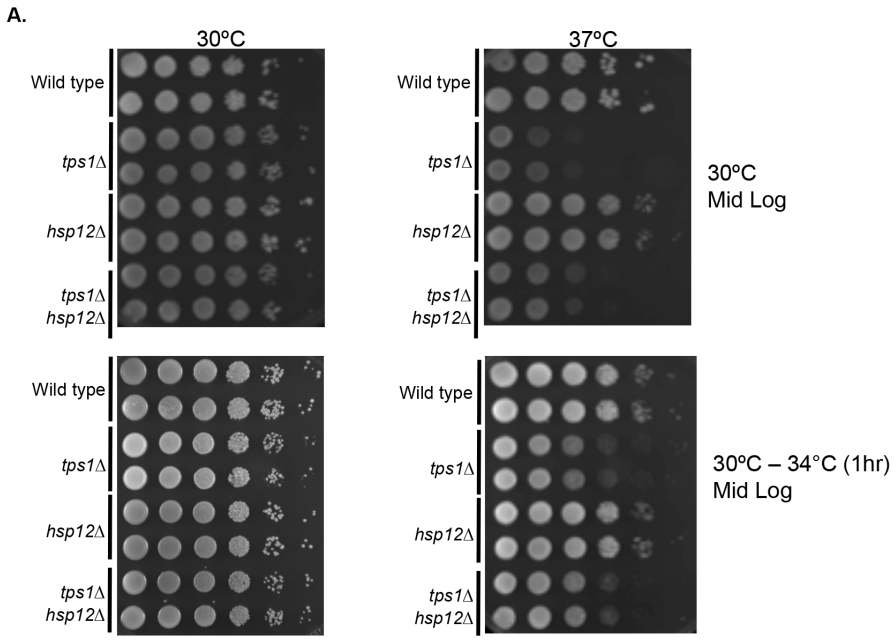
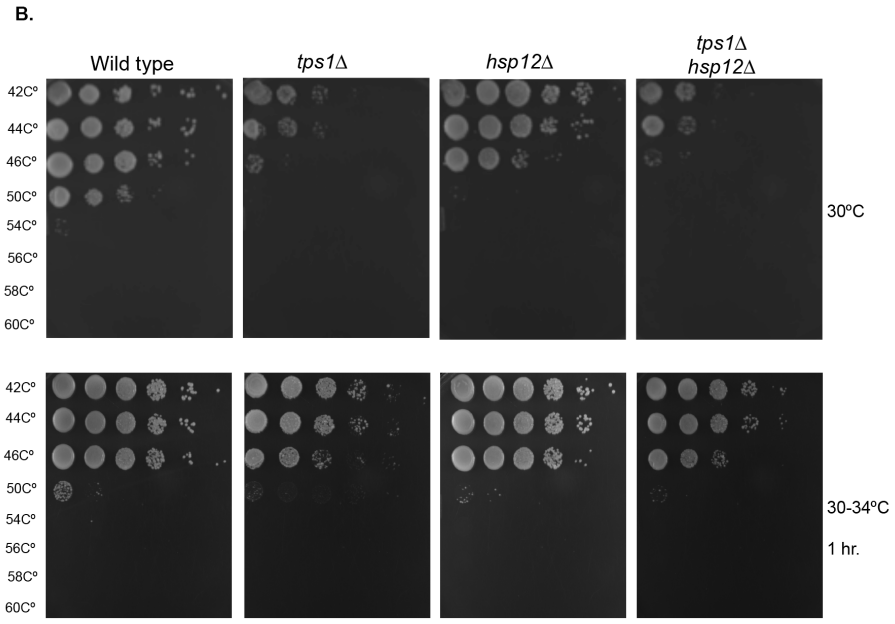
770 **SUPPLEMENTAL DATA**

771 **Figure S1.**



772



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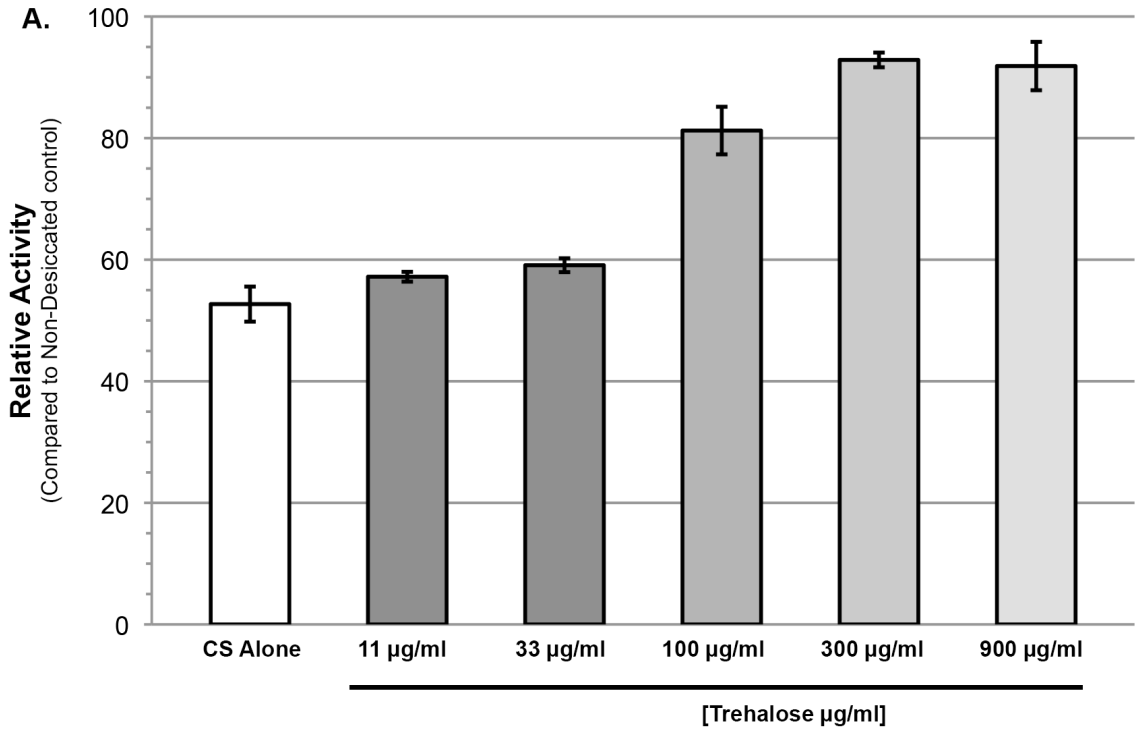
774

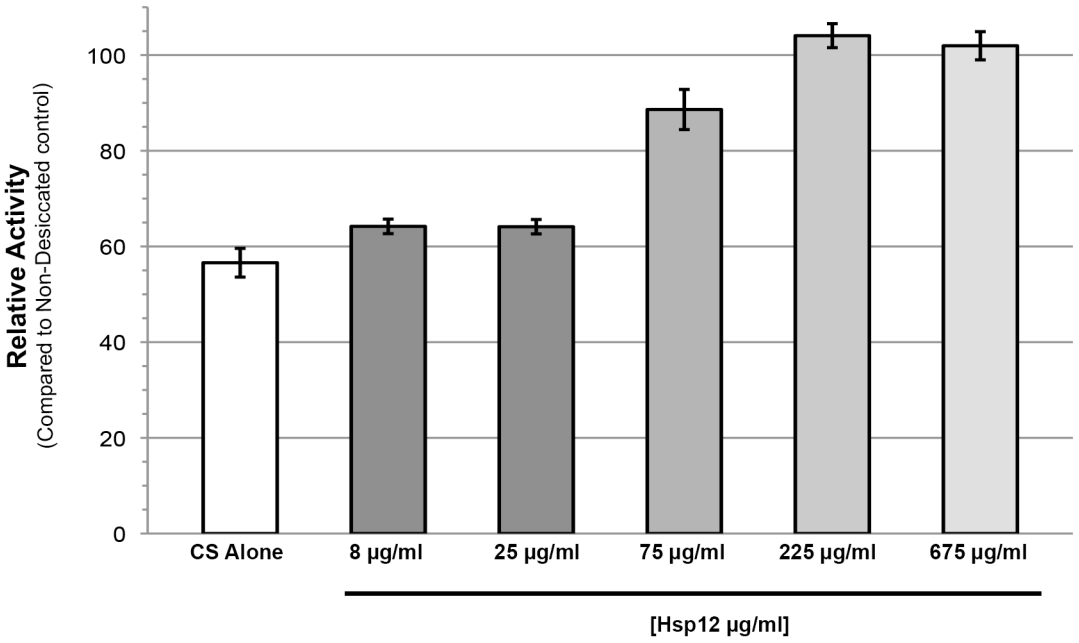
775 **Figure S1. Trehalose and Hsp12 heat tolerance**

776 (**A**) Cells (wild type, *hsp12*Δ, *tps1*Δ, or *tps1*Δ *hsp12*Δ) were grown to midexponential phase

777 (OD < 0.5) in non-selective media (YP, 1% yeast extract and 2% peptone) containing 2%
778 glucose at 30C. Cells were plated and grown at either 30C or 37C, or grown for one hour at
779 34C (pre-heat shock) before growing at either 30C or 37C. (**B**) Cells (wild type, *hsp12*Δ, *tps1*Δ,
780 or *tps1*Δ *hsp12*Δ) were grown to mid-exponential phase (OD < 0.5) in non-selective media (YP,
781 1% yeast extract and 2% peptone) containing 2% glucose at 30C. Cells were then heat-
782 shocked for 30 min at temperatures ranging from 42-60C, followed by plating and growing at
783 30C. Cells were also grown at 34C prior to heat shock.
784
785 **Figure S2.**
786



787


Relative Activity
(Compared to Non-Desiccated control)
100
80
60
40
20
0
CS Alone
8 µg/ml
25 µg/ml
75 µg/ml
225 µg/ml
675 µg/ml
[Hsp12 µg/ml]

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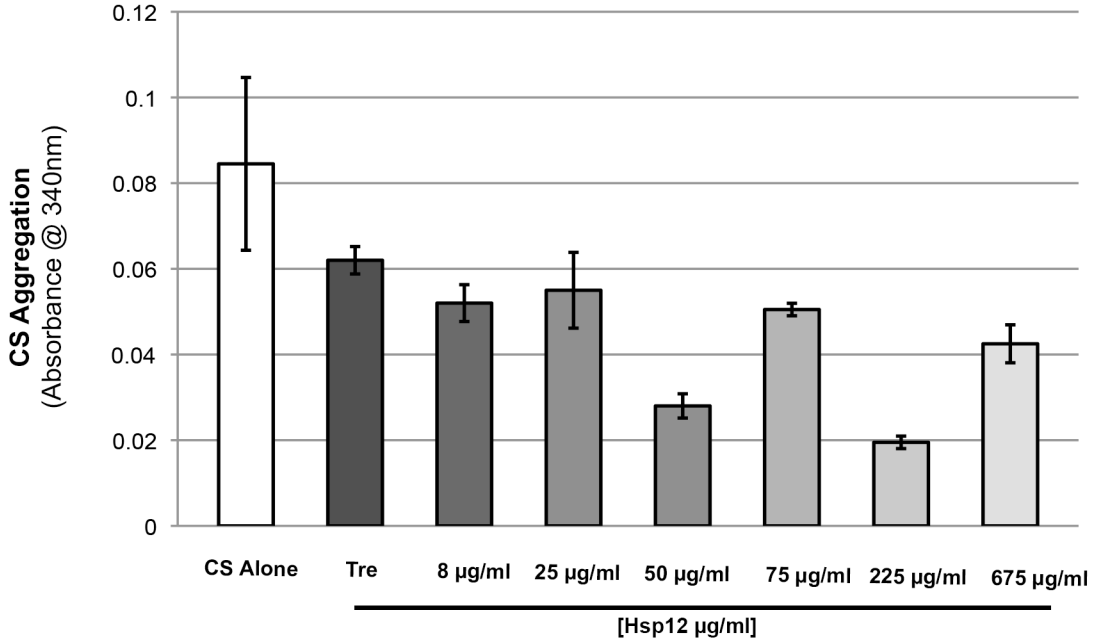
B.


CS Aggregation
(Absorbance @ 340nm)
0.12
0.1
0.08
0.06
0.04
0.02
0
CS Alone
Tre
8 µg/ml
25 µg/ml
50 µg/ml
75 µg/ml
225 µg/ml
675 µg/ml
[Hsp12 µg/ml]

789

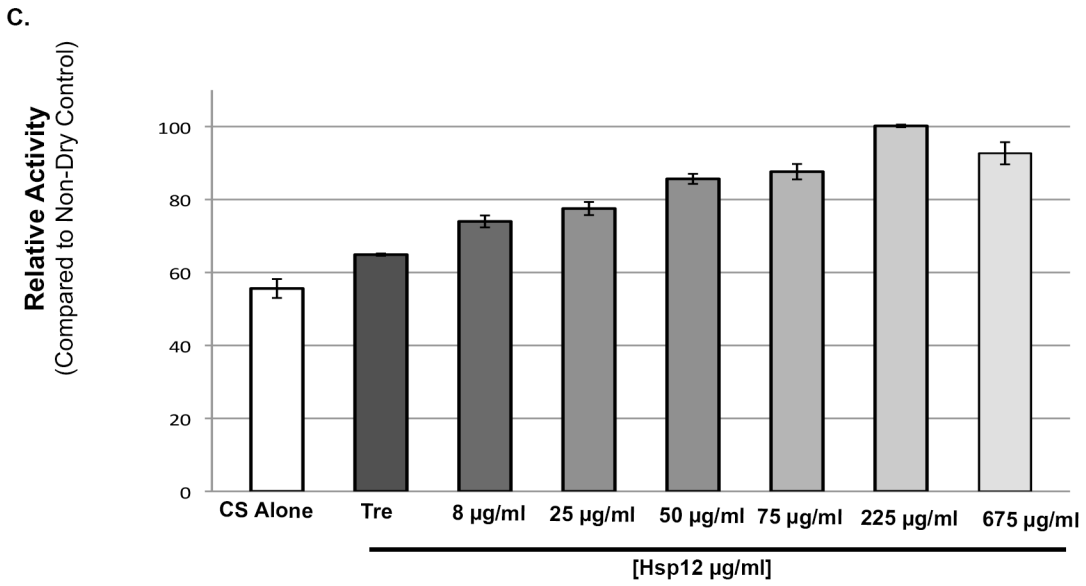
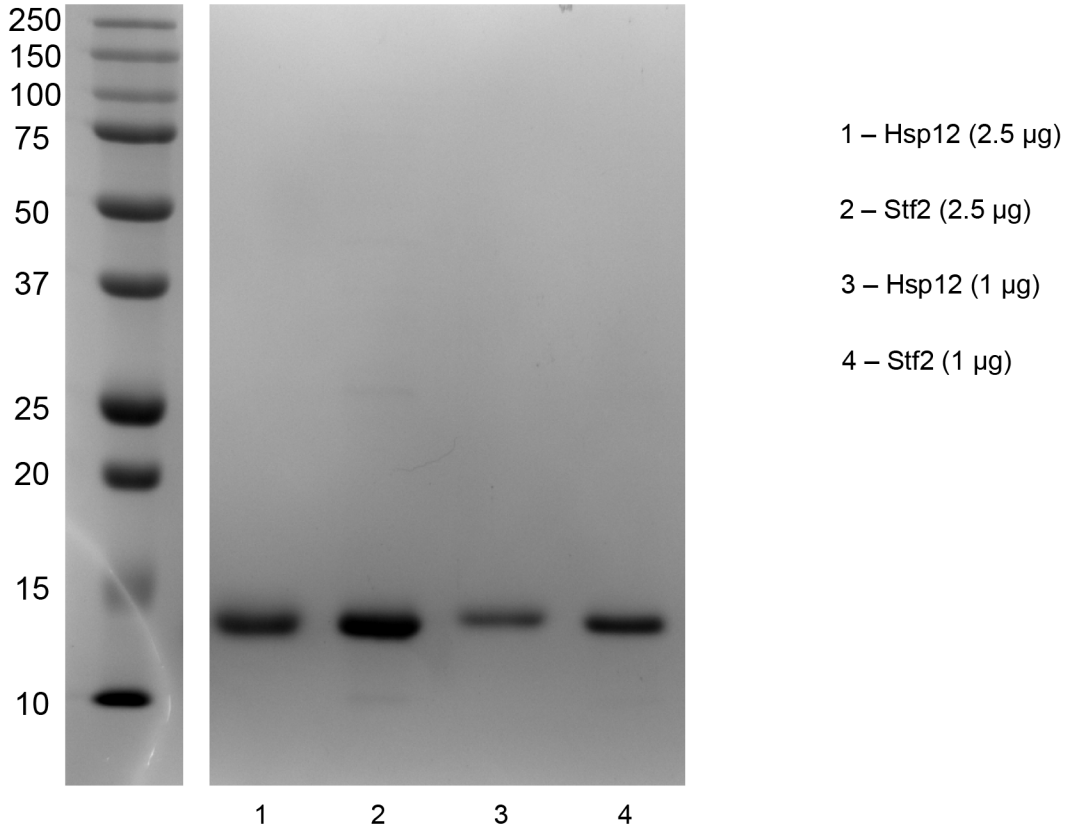


Figure S2. Citrate synthase activity and synergistic protection. (A) Enzymatic activity after drying of CS in presence of varying concentrations of trehalose or Hsp12. CS enzymatic was measured by examining activity-induced changes in absorbance at 412 nm after drying compared to measurements before drying. (B). Citrate synthase desiccation-induced aggregation in the presence of constant concentrations of trehalose and varying concentrations of Hsp12. Absorbance at 340 nm. Citrate Synthase (0.12 mg) and trehalose (33ug/ml). Absorbance measured after drying at 36°C for 24 hours followed by 5 hours at 23°C, then rehydration in water. Measurements after drying compared to measurements before drying. (C) Assay as in S2B, but tested for CS activity.

807 **Figure S3.**

808



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812 **Figure S3. Hydrophilin protein purifaction.** Hsp12 and Stf2 are bacterial derived protein

813 preps. Gel stained with Coomassie Blue demonstrates purity of protein preps.

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824 **Table S1**

825 **A.**

	Number of Hits	Total Genes
Synthetic Lethal with *tps1*Δ	**178**	**4700**
Desiccation Sensitive with *tps1*Δ	**174**	**4700**
Desiccation Tolerant suppressors of *tps1*Δ after 30 Day Dry	**121**	**4700**

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828

1. Synthetic Lethality with *tps1*Δ - SGA

Strain	*Standard Name*	Strain	*Standard Name*	Strain	*Standard Name*	Strain	*Standard Name*
YBR278W	*DPB3*	YMR154C	RIM13	YML073C	YL16A	YDR419W	*RAD30*
YBR279W	*PAF1*	YBR191W	RPL21A	YNL055C	POR1	YEL024W	*RIP1*
YBR282W	*MRPL27*	YLR370C	ARC18	YNL284C	MRPL10	YEL027W	*VMA3*
YCR020W-B	*HTL1*	YLR382C	NAM2	YPR133W-A	TOM5	YEL029C	*BUD16*
YCR024C	*SLM5*	YJR118C	ILM1	YAL044C	GCV3	YEL043W	
YCR044C	*PER1*	YJR139C	HOM6	YAL039C	CYC3	YEL044W	*IES6*
YCR063W	*BUD31*	YJL006C	CTK2	YAL013W	DEP1	YEL051W	*VMA8*
YCR071C	*IMG2*	YJR030C	RBH2	YAL002W	VPS8	YER050C	*RSM18*
YCR081W	*SRB8*	YJR033C	RAV1	YBL016W	*FUS3*	YER044C	*BUD18*
YDR363W-A	*SEM1*	YJR035W	RAD26	YBL038W	*MRPL16*	YFL036W	*RPO41*
YFR040W	*SAP155*	YJR036C	HUL4	YBL063W	*KIP1*	YFL053W	*DAK2*
YGR219W		YJR040W	GEF1	YBL066C	*SEF1*	YFR001W	*LOC1*
YGR229C	*SMI1*	YJR060W	CBF1	YBL101C	*ECM21*	YFR010W	*UBP6*
YGR237C		YJR066W	TOR1	YBR015C	*TTP1*	YGL016W	*PDR6*
YGR270W	*YTA7*	YJL136C	RPS25B	YBR042C	*CST26*	YGL025C	*PGD1*
YGR275W	*RTT102*	YJL165C	HAL5	YBR065C	*ECM2*	YGL058W	*RAD6*
YGR285C	*ZUO1*	YJL175W		YBR094W	*PBY1*	YGL124C	*MON1*
YHR039C-B	*VMA10*	YJL184W	GON7	YBR098W	*MMS4*	YGL136C	*MRM2*
YKR035W-A	*DID2*	YJL189W	RPL39	YBR188C	*NTC20*	YGL151W	*NUT1*
YKR082W	NUP133	YFR025C	HIS2	YBR221C	*PDB1*	YGL244W	*RTF1*
YLL018C-A	COX19	YJL101C	GSH1	YBR262C	*AIM5*	YGR003W	*CUL3*
YNR050C	LYS9	YKR085C	MRPL20	YCL032W	*STE50*	YGR020C	*VMA7*
YNR068C	BUL3	YLR439W	MRPL4	YCL037C	*SRO9*	YGR055W	*MUP1*
YER106W	MAM1	YLR442C	SIR3	YCR007C		YGR101W	*PCP1*
YER108C	FLO8	YNR052C	POP2	YCL039W	*GID7*	YGR104C	*SRB5*
YER141W	COX15	YPL268W	PLC1	YCL063W	*VAC17*	YGR122W	
YER145C	FTR1	YPR067W	ISA2	YDL033C	*SLM3*	YGR171C	*MSM1*
YER151C	UBP3	YBR131W	CCZ1	YDL045W-A	*MRP10*	YGR215W	*RSM27*
YER154W	OXA1	YBR289W	SNF5	YDL069C	*CBS1*	YGR217W	*CCH1*
YER161C	SPT2	YCR028C-A	RIM1	YDL072C	*YET3*	YHL027W	*RIM101*
YER177W	BMH1	YCR046C	IMG1	YDL159W	*STE7*	YHL007C	*STE20*
YMR058W	FET3	YCR047C	BUD23	YDL185W	*VMA1*	YHR121W	*LSM12*
YMR066W	*SOV1*	YCR084C	TUP1	YDR027C	*VPS54*	YHR132C	*ECM14*
YMR077C	*VSP20*	YNL052W	COX5A	YDR123C	*INO2*	YIL040W	*APQ12*
YOL086C	*ADH1*	YNL073W	MSK1	YDR175C	*RSM24*	YIL148W	*RPL40A*
YOL115W	TRF4	YNL107W	YAF9	YDR226W	*ADK1*	YIL149C	*MLP2*
YOL129W	*VPS64*	YDR512C	EMI1	YDR227W	*SIR4*	YIL153W	*RRD1*
YER092W	IES5	YFL001W	DEG1	YDR237W	*MRPL7*		
YHL025W	SNF6	YFL013C	IES1	YDR279W	*RNH202*		
YHR064C	SSZ1	YGR252W	GCN5	YDR293C	*SSD1*		
YHR168W	MTG2	YGR255C	COQ6	YDR295C	*HDA2*		
YOR096W	RPS7A	YGR257C	MTM1	YDR296W	*MHR1*		
YMR097C	MTG1	YIR023W	DAL81	YDR332W	*IRC3*		
YMR135C	GID8	YJR055W	HIT1	YDR335W	*MSN5*		
YHR005C	GPA1	YNL138W	SRV2	YDR388W	*RVS167*		
YHR041C	SRB2	YOR270C	VPH1	YDR389W	*SAC7*		
YHR067W	HTD2	YPL078C	ATP4	YDR405W	*MRP20*		

Table S1-1

2. Desiccation Sensitive with *tps1*Δ - 6 Day Dry - SGA

Strain	*Standard Name*	Strain	*Standard Name*	Strain	*Standard Name*	Strain	*Standard Name*
YFL014W	***HSP12***	YJR052W	*RAD7*	YKL137W	*CMC1*	YNR002C	*ATO2*
YBR295W	*PCA1*	YJR061W		YKL150W	*MCR1*	YNR039C	*ZRG17*
YBR297W	*MAL33*	YFL033C	*RIM15*	YKL159C	*RCN1*	YNR047W	*FPK1*
YCR026C	*NPP1*	YAL008W	*FUN14*	YKL161C	*KDX1*	YOR061W	*CKA2*
YCR027C	*RHB1*	YBL001C	*ECM15*	YKL170W	*MRPL38*	YOR065W	*CYT1*
YGR256W	*GND2*	YBR019C	*GAL10*	YKL171W	*NNK1*	YOR086C	*TCB1*
YIR021W	*MRS1*	YBR037C	*SCO1*	YKL175W	*ZRT3*	YOR107W	RGS2
YIR023W	*DAL81*	YBR203W	*COS111*	YKL207W	*EMC3*	YOR115C	TRS33
YIR031C	*DAL7*	YBR215W	*HPC2*	YKR003W	*OSH6*	YOR118W	RTC5
YIR033W	*MGA2*	YBR227C	*MCX1*	YKR055W	*RHO4*	YOR149C	*SMP3*
YIR038C	*GTT1*	YCR009C	*RVS161*	YKR059W	*TIF1*	YOR161C	PNS1
YLR423C	*ATG17*	YDL107W	*MSS2*	YLL006W	*MMM1*	YOR166C	SWT1
YLR425W	*TUS1/SOP10*	YDR148C	*KGD2*	YLL014W	*EMC6*	YOR178C	*GAC1*
YLR447C	*VMA6*	YDR194C	*MSS116*	YLL019C	*KNS1*	YOR182C	*RPS30B*
YER118C	*SSU81*	YDR197W	*CBS2*	YLL026W	*HSP104*	YOR191W	ULS1
YER128W	*VFA1*	YDR204W	*COQ4*	YLL040C	*VPS13*	YOR231W	*MKK1*
YER153C	*PET122*	YDR350C	*TCM10*	YLL043W	*FPS1*	YOL015W	*IRC10*
YER155C	*BEM2*	YDR375C	*BCS1*	YLL051C	*FRE6*	YOL083W	*ATG34*
YER167W	*BCK2*	YER030W	*CHZ1*	YLR025W	*SNF7*	YOL084W	*PHM7*
YMR070W	*MOT3*	YFL016C	*MDJ1*	YLR006C	*SSK1*	YPL274W	*SAM3*
YOL095C	*HMI1*	YGL107C	*RMD9*	YLR064W	*PER33*	YPL273W	*SAM4*
YOL096C	*COQ3*	YGL115W	*SNF4*	YLR079W	*SIC1*	YPL249C	*GYP5*
YER078C	*ICP55*	YGL135W	*RPL1B*	YLR080W	*EMP46*	YPL193W	*RSA1*
YER008C	*SEC3*	YGL203C	*KEX1*	YLR083C	*EMP70*	YPL178W	*CBC2*
YDR424C	*DYN2*	YGL213C	*SKI8*	YLR093C	*NYV1*	YPL105C	*SYH1*
YER090W	*TRP2*	YGL234W	*ADE5,7*	YLR114C	*AVL9*	YPL096W	*PNG1*
YEL011W	*GLC3*	YGR078C	*PAC10*	YLR134W	*PDC5*	YPL089C	*RLM1*
YLR034C	*SMF3*	YGR105W	*VMA21*	YLR375W	*STP3*	YPL057C	*SUR1*
YMR315W		YGR183C	*QCR9*	YMR035W	*IMP2*	YPL049C	*DIG1*
YMR316C-A		YHL038C	*CBP2*	YMR256C	*COX7*	YPR003C	
YML095C	*RAD10*	YHL023C	*NPR3*	YNL339C	*YRF1-6*	YPR004C	*AIM25*
YML108C		YHR010W	*RPL27A*	YNL330C	*RPD3*	YPR124W	*CTR1*
YML110C	*COQ5*	YHR011W	*DIA4*	YNL329C	*PEX6*	YPR194C	*OPT2*
YMR099C		YHR016C	*YSC84*	YNL233W	*BN14*	YJR004C	*SAG1*
YER063W	*THO1*	YHR021C	*RPS27B*	YNL229C	*URE2*		
YER066W	*RRT13*	YIL013C	*PDR11*	YNL225C	*CNM67*		
YLR246W	*ERF2*	YIL162W	*SUC2*	YNL218W	*MGS1*		
YLR346C		YJL168C	*EZL1*	YNL162W	*RPL41A*		
YPL217C	*BMS1*	YJL046W	*AIM22*	YNL145W	*MFA2*		
YPL224C	*MMT2*	YKL002W	DID4	YNL139C	*THO2*		
YCL023C		*YKL037W*	*AIM26*	YNL144C			
YJR087W		YKL048C	*ELM1*	YNL098C	*RAS2*		
YJR134C	*SGM1*	YKL055C	*OAR1*	YNL091W	NST1		
YML072C	*TCB3*	YKL101W	*HSL1*	YNL077W	APJ1		
YML085C	*TUB1*	YKL134C	*OCT1.*	YNL054W	*VAC7*		
YJL005W	*CYR1*	YKL146W	*AVT3*	YNL001W	*DOM34*		
YJL028W		YKL148C	*SDH1*	YNR001C	*CIT1*		

Table S1-2

3. Desiccation Tolerant Suppressors of *tps1*Δ - 30 Day Dry - SGA

Strain	*Standard Name*	Strain	*Standard Name*	Strain	*Standard Name*
YER093C-A	*AIM11*	YDR108W	*GSG1*	YIR013C	*GAT4*
YMR142C	*RPL14B*	YDR159W	*SAC3*	YJL170C	*ASG7*
YMR319C	*FET4*	YDR162C	*NBP2*	YJL165C	*HAL5*
YML094W	*GIM5*	YDR173C	*ARG82*	YJL079C	*PRY1*
YMR106C	*YKU80*	YDR207C	*UME6*	YJL038C	*LOH1*
YMR116C	*ASC1*	YDR231C	*COX2*	YJR092W	*BUD4*
YER031C	*YPT8*	YDR264C	*AKR1*	YKL096W	*CWP1*
YER046W	*SPO73*	YDR320C	*SWA2*	YKL103C	*APE1*
YGR204W	*ADE3*	YDR360W	*OPI7*	YKL114C	*APN1*
YHR119W	*YTX1*	YDR369C	*XRS2*	YKL119C	VPH2
YJR121W	*ATP2*	YDR432W	*NPL3*	YKL126W	YPK1
YJR140C	*HIR3*	YDR441C	*APT2*	YKL127W	PGM1
YAL054C	*ACS1*	YDR457W	*TOM1*	YKL185W	ASH1
YJR010W	*MET4*	YDR511W	*ACN9*	YKL187C	FAT3
YJR063W	*RPA12*	YDR524C	*AGE1*	YKL197C	PEX1
YJR066W	*TOR1*	YEL007W	*MIT1*	YKR007W	MEH1
YGR239C	*PEX21*	YEL052W	*AFG1*	YLL001W	DNM1
YJL137C	*GLG2*	YER005W	*YND1*	YLL021W	SPA2
YBR083W	*TEC1*	YFL011W	*HXT10*	YLL052C	AQY2
YBR084C-A	*RPL19A*	YFL030W	*AGX1*	YLR068W	FYV7
YBR125C	*PTC4*	YFL055W	*AGP3*	YLR070C	XYL2
YBR168W	*PEX32*	YFL008W	*SMC1*	YLR074C	BUD20
YNL079C	*TPM1*	YGL006W	*PMC1*	YLR121C	YPS3
YNL080C	*EOS1*	YGL028C	*SCW11*	YLR172C	DPH5
YOR265W	*RBL2*	YGL110C	*CUE3*	YLR191W	PEX13
YNL059C	*ARP5*	YGL153W	*PEX14*	YLR206W	ENT2
YOL148C	*SPT20*	YGL216W	*KIP3*	YLR216C	CPR6
YAL059W	*SIM1*	YGL227W	*VID30*	YLR248W	RCK2
YAL035W	*FUN12*	YGL229C	*SAP4*		
YAL011W	*SWC3*	YGL236C	*MTO1*		
YBL027W	*RPL19B*	YGR007C	*MUQ1*		
YBL006C	*LDB7*	YGR016W			
YBL021C	*HAP3*	YGR025W			
YBL045C	*COR1*	YGR059W	*SPR3*		
YBL080C	*PET112*	YGR141W	*VPS62*		
YBL089W	*AVT5*	YGR144W	*THI4*		
YBR136W	*ESR1*	YGR157W	*CHO2*		
YBR229C	*ROT2*	YHL044W			
YDL009C		YHR109W	*CTM1*		
YDL021W	*GPM2*	YHR113W	*APE4*		
YDL038C		YHR115C	*DMA1*		
YDL116W	*NUP84*	YHR132C	*ECM14*		
YDL137W	*ARF2*	YIL011W	*TIR3*		
YDL191W	*RPL35A*	YIL039W	*TED1*		
YDL215C	*GDH2*	YIL088C	*AVT7*		
YDR024W	*FYV1*	YIL090W	*ICE2*		
YDR025W	*RPS11A*	YIL095W	*PRK1*		

Table S1-3

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844 **B.**

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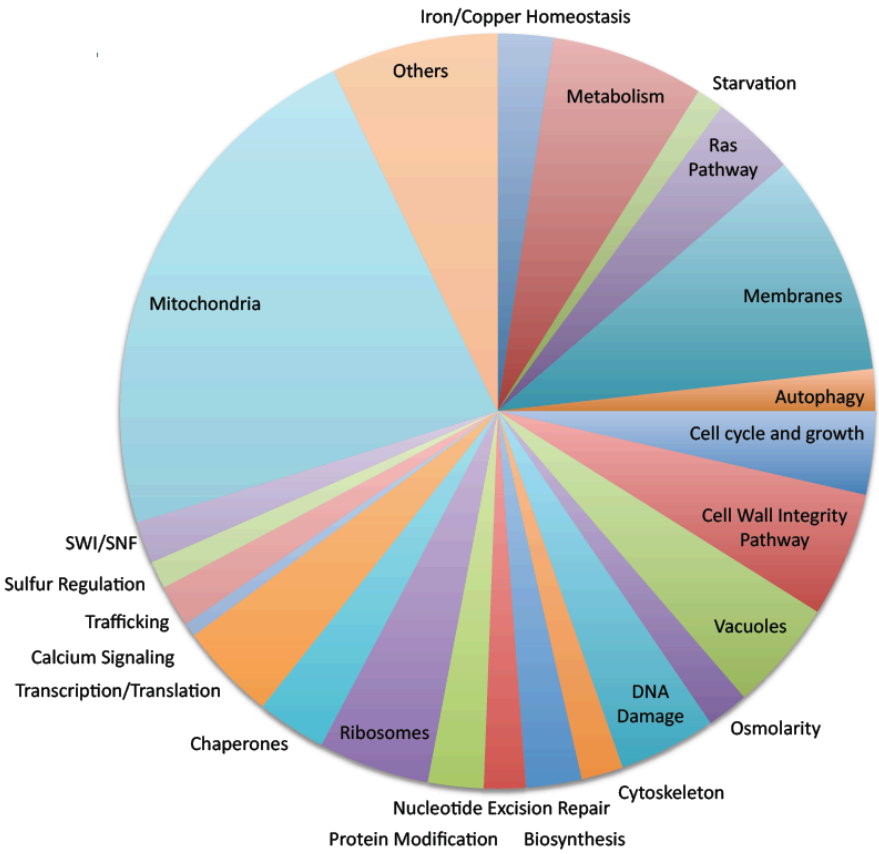

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847 **Table 1. Synthetic Genetic Array Desiccation Screen – *tps1*Δ**

848 (**A**) Three different groups where identified from our SGA - *tps1*Δ screen. (**1)** gene deletions
849 that were synthetic lethal with *tps1*Δ: failed to grow completely. (**2**) gene deletions that lead to
850 desiccation sensitivity with *tps1*Δ, and (**3**) gene deletions that allowed *tps1*Δ to grow after 30
851 days of drying: suppressors. List of genes attached as 1-3.

852 (**B**) Breakdown of desiccation sensitive *tps1Δ* double mutants into categories based on cellular
853 function. Each desiccation sensitive double mutant was placed into a category based on their
854 cellular function (GO).

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861 **Table S2.**

Stain List		
BY4742	Wild type	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0
HTY104	*tps1*Δ	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 tps1::G418
HTY11	*hsp12*Δ	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 hsp12::G418
HTY176	*tps1*Δ*hsp12*Δ	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 tps1::HYG hsp12::G418
HTY14	*stf2*Δ	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 stf2::G418
HTY179	*tps1*Δ*stf2*Δ	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 tps1::HYG stf2::G418
Transporter Strains		
HTY121	*nth1*Δ	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 nth1::G418
HTY164	*nth1*Δ *AGT1*	MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 nth1::G418 can1::AGT1
Prion Strains		
HTY92	[*PSI*+]	Mat α ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112 (ADE+)
HTY187	[*PSI*+] *hsp12*Δ	Mat α ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112 (ADE+) hsp12::HYG
HTY120	[*PSI*+] *tps1*Δ	Mat α ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112 (ADE+) tps1::G418
HTY188	[*PSI*+] *tps1*Δ *hsp12*Δ	Mat α ade1-14 trp1-289 his3Δ-200 ura3-52 leu2-3,112 (ADE+) tps1::G418 hsp12::HYG
HTY82	[*GAR*+]	Mat a can1-100 his3-11,15 leu2-2,112 trp1-1 ura3-1, ade2-1 (GlucosamineR)
HTY189	[*GAR*+] *hsp12*Δ	Mat a can1-100 his3-11,15 leu2-2,112 trp1-1 ura3-1, ade2-1 (GlucosamineR) hsp12::HYG
HTY115	[*GAR*+] tps1Δ	Mat a can1-100 his3-11,15 leu2-2,112 trp1-1 ura3-1, ade2-1 (GlucosamineR) tps1::G418
HTY190	[*GAR*+] *tps1*Δ*hsp12*Δ	Mat a can1-100 his3-11,15 leu2-2,112 trp1-1 ura3-1, ade2-1 (GlucosamineR) tps1::NAT hsp12::HYG

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864 **Table 2. Strain List.**

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