

1 Specificity and mechanism of 1,6 hexanediol-induced disruption of nuclear 2 transport

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12

13 **ABSTRACT**

14 Selective transport through the nuclear pore complex (NPC) depends on the dynamic binding of the
15 intrinsically disordered components of the NPC, the FG-nups, with each other and with nuclear
16 transport receptors (NTRs). Hydrophobic interactions with the phenylalanines of FG-nups are critical
17 for this dynamic binding. 1,6-hexanediol (1,6HD), is an aliphatic alcohol that interferes with
18 hydrophobic interactions. Here we assessed the specificity and mechanism by which 1,6HD disrupts
19 the permeability barrier of NPCs in live baker's yeast cells. Exposure to 1,6HD (10 min, 0-5%) leads to
20 gradual loss of the NPC permeability. This is likely a direct effect on the nuclear transport machinery
21 as cell viability, the pH and ATP levels in the cytosol, as well as the appearance of mitochondria, Golgi,
22 peroxisomes, ER, vacuoles, plasma membrane, nucleolus, secretory pathway and stress granules are
23 not notably changed. There are however effects on the cytoskeleton and Hsp104 to be noted. While
24 1,6HD treatment does not lead to dissociation or degradation of NPC subunits, a massive relocation
25 of multiple NTRs from NPCs does occur. This displacement quantitatively correlates with the increased
26 passive permeability of NPCs. The loss of NTRs and associated cargo will present a major change in the
27 macromolecular crowding and composition and hence the physicochemical properties of the central
28 channel. We conclude that 1,6HD provides a surprisingly specific intervention to temporarily
29 permeate NPCs and we present evidence that the mechanism includes release of NTRs from the NPCs.

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33 INTRODUCTION

34 The Nuclear Pore Complex (NPC) is the sole gate between the nucleus and cytosol. The central channel
35 of NPCs is lined with intrinsically disordered phenylalanine-glycine rich nucleoporins, the FG-nups, and
36 it hosts many nuclear transport receptors (NTRs) (Dultz et al. 2022; Hampoelz et al. 2019; Wing, Fung,
37 and Chook 2022; Fernandez-Martinez and Rout 2021). The NTRs bind their cargo and shuttle them
38 through the channel by transiently binding the FG-nups (Paci, Caria, and Lemke 2021; Wing, Fung, and
39 Chook 2022; Bayliss, Littlewood, and Stewart 2000). For the NTR Importin β it was shown that besides
40 a fraction that is shuttling cargo between the cytoplasm and nucleus, there is also a fraction that is
41 more stably associated with NPCs (Lowe et al. 2015; Kapinos et al. 2014). In addition to NTRs also
42 cargo and non-cargo are present in the NPC. In isolated yeast NPCs, 15,6 MDa worth of NTRs and 10,4
43 MDa worth of cargo add significantly to the 52,3 MDa mass of actual NPC subunits (Kim et al. 2018).
44 The central channel of the nuclear pore complex is thus a highly crowded and complex environment
45 where the joint presence of NTRs, FG-nups and cargo creates an environment that allows fast and
46 selective transport.

47 The exact structure of the central channel has remained elusive because experimentally probing its
48 behaviour in living cells is challenging. Our knowledge about the behaviour of the FG-nups and NTRs
49 is inferred from, amongst others, imaging detergent-perforated or live cells (Chowdhury, Sau, and
50 Musser 2022; Schnell, Tingey, and Yang 2022; Mattheyses et al. 2010; Yu et al. 2022), AFM
51 measurements on nuclear envelopes (Sakiyama et al. 2016), transport measurement in biomimetic
52 NPCs (Jovanovic-Talisman et al. 2009; Fisher et al. 2018; Kowalczyk et al. 2011), surface anchored FG-
53 nups (Kapinos et al. 2014) or from probing the structural conformation of purified FG-nups or FG-nup
54 fragment preparations (Frey, Richter, and Görlich 2006; Celetti et al. 2020; Ader et al. 2010; Hayama
55 et al. 2018; Sparks et al. 2018). These experimental studies, together with computational strategies
56 (Davis, Ford, and Hoogenboom 2022; Zheng and Zilman 2023; Isgro and Schulten 2007; Popken et al.
57 2015; Ghavami et al. 2014), have resulted in a number of models explaining the fast and selective
58 transport through the NPC (Dultz et al. 2022; Hampoelz et al. 2019; Wing, Fung, and Chook 2022;
59 Fernandez-Martinez and Rout 2021; Hoogenboom et al. 2021; Huang and Szleifer 2020). All models
60 agree that the phenylalanines of the FG-repeat regions that are engaging in hydrophobic interactions,
61 as well as the intrinsically disordered nature of the FG-nups, are key parameters. They enable the
62 highly dynamic intra- and inter-chain hydrophobic interactions between FG-repeat regions and with
63 the hydrophobic grooves on the surfaces of NTRs. In the Kap-centric models the slow exchanging pool
64 of NTRs are proposed to be important to create the proper barrier function (Kapinos et al. 2017; Kalita
65 et al. 2022; Fragasso et al. 2022).

66 Early experiments using aliphatic alcohols pointed to the importance of hydrophobic interactions for
67 import into nuclei of permeabilized cells (Ribbeck and Görlich 2002) and in live yeast cells (Shulga and
68 Goldfarb 2003). Early experiments in permeabilized HeLa cells showed that selective transport of
69 fluorescent reporters (MBP or IBB-MBP) was abrogated in the presence of hexane-1,2-diol but not by
70 the less hydrophobic hexane-1,2,3-triol (Ribbeck and Görlich 2002). In live yeast cells it was observed
71 that the nuclear accumulation of GFP fused to a classical nuclear localisation signal (NLS) was lost upon
72 addition of alcohols and the extend of equilibration was dependent on the hydrophobicity of the
73 alcohol (Shulga and Goldfarb 2003). Biochemical studies using purified FG-repeat fragments show that
74 some of them are cohesive and that their interactions are disrupted by 1,6HD (Patel et al. 2007;
75 Schmidt and Görlich 2015). Also, within the yeast cytosol such overexpressed fragments form foci that
76 are dispersed by 1,6HD (Patel et al. 2007). Lastly, 1,6 HD was shown to increases the diameter of NPCs
77 in *Xenopus* oocyte nuclear envelope preparations (Jäggi et al. 2003). Most dramatically, in the context
78 of mutant NPCs that lack the inner ring nucleoporins Nup170 or Nup188, 1,6HD can even lead to loss
79 of FG-nups from these NPCs (Shulga and Goldfarb 2003; Onischenko et al. 2017). The effect of
80 hexanediol in the above studies was attributed to a reversible disruption of inter-FG repeat cohesion.
81 However, as also the interactions between NTRs and FG-nups are based on hydrophobic interactions,
82 hexanediol will likely also take effect here. Illustrative for the high surface hydrophobicity of NTRs, is
83 their strong binding to a phenyl sepharose chromatography column yielding highly enriched fractions
84 from HeLa cell extracts (Ribbeck and Görlich 2002). Jointly these studies support the importance of
85 hydrophobic interaction for nuclear transport, and the potential of 1,6 HD to disrupt those.

86 Unrelated to nuclear transport, 1,6HD has also been widely used to dissolve liquid-liquid phase
87 separated compartments in cells and to dissolve condensates in *in vitro* studies. With aggregation-
88 prone peptides, the alcohol dissolves hydrogels (Molliex et al. 2015; Kroschwald, Maharana, and
89 Simon 2017; Shi et al. 2017) but not fibers (Lin et al. 2016; Van Lindt et al. 2022). In cells, the
90 interpretation of effects of 1,6HD are more difficult (Kroschwald, Maharana, and Simon 2017) and
91 depending on the cell type, growth condition and the concentration and length of treatment different
92 results may be obtained. There are many examples of discrepancies in the literature; only one example
93 is the organization of actin and tubulin. While some reports show that they are affected by 1,6HD
94 (Wheeler et al. 2016; Kroschwald, Maharana, and Simon 2017), others report that microtubules are
95 unaffected (Lin et al. 2016).

96 From the above, the question arises how specific the effects of 1,6HD on nuclear transport are and,
97 whether they are based on a loss of cohesion between the FG-repeat regions, or between FG-nups
98 and NTRs, or both. Here, we probe the impact of 1,6HD on nuclear transport by measuring the effects
99 on passive transport, on NTR-facilitated import and export, and on the cellular localisation of Nups

100 and NTRs. We also assess a large number of possible indirect effects of 1,6HD, namely cell viability,
101 the pH and ATP levels in the cytosol, and the appearance of mitochondria, Golgi, peroxisomes, ER,
102 vacuoles, plasma membrane, nucleolus, secretory pathway, stress granules, the cytoskeleton and
103 Hsp104 foci. Our data support that 1,6HD provides a surprisingly specific intervention to temporarily
104 increase the passive permeability of NPCs by the release of NTRs from the NPC.

105

106 **RESULTS**

107 **Disruption of the permeability barrier of NPCs by 1,6 hexanediol**

108 Previous reports already showed that 1,6HD disrupts the permeability barrier of NPCs in yeast cells
109 (Shulga and Goldfarb 2003; Patel et al. 2007). We add to this work and provide a quantitative
110 assessment of the impact of 1,6HD on passive nuclear entry of large reporters and NTR-mediated
111 transport of GFP-NLS and GFP-NES reporters in yeast. To assess passive nuclear entry, the MG5
112 reporter, composed of a Maltose Binding Protein and 5 GFPs is used. MG5 has a molecular weight of
113 177 kDa and is excluded from the nucleus in wild type cells (Popken et al. 2015). Mid exponential
114 growing cells were exposed for 10 minutes to zero, 0.625, 1.25, 2.5 or 5% 1,6HD, or to the less
115 hydrophobic alcohol 2,5 hexanediol (2,5HD). The steady state distribution of the GFP-reporters was
116 calculated by taking the ratio of the fluorescence measured in the nucleus and the cytosol (the N/C
117 ratio). The permeability of the NPCs for entry of MG5 increased gradually with increasing
118 concentrations of 1,6HD (Fig 1A) indicating that NPCs became more permeable for this large protein.
119 1,6HD had a stronger effect on the passive permeability of NPCs than 2,5HD, as MG5 remains properly
120 excluded from the nucleus, even at a concentration of 5% 2,5HD (Fig 1A).

121 To assess active import and export, GFP with a classical NLS (GFP-cNLS) and GFP-NES reporters are
122 used. The balance between Kap60/Kap95-facilitated import of GFP-cNLS and its passive efflux leads
123 to nuclear accumulation. Similarly, the balance of CRM1-facilitated export of GFP-NES and its passive
124 influx leads to a steady-state nuclear exclusion. The import and export reporters showed a gradual
125 decline in nuclear accumulation and exclusion, respectively, with increasing 1,6HD concentrations (Fig
126 1B,C). This loss of nuclear compartmentalisation could solely be the consequence of the increased
127 passive permeability (Fig 1A), but could additionally be the result of a decrease in the active transport
128 rates. As for passive transport 1,6HD had a stronger effect for active transport than 2,5HD, as higher
129 concentrations of 2,5HD were needed to decrease the compartmentalisation of GFP-NLS and GFP-NES
130 (Fig 1B,C). From this we conclude that exposure of live yeast cells to 1,6HD (10 min, 0-5%) leads to a
131 gradual loss of the permeability barrier of NPCs.

132

133 **On the specificity of 1,6HD towards disrupting nuclear transport**

134 The question if the increased NPC permeability after exposure to 1,6HD is a direct consequence of an
135 altered nuclear transport system, or rather a consequence of indirect effects on the cell's physiology,
136 is pertinent. Indeed, depending on the exposure time and concentration 1,6HD may well have
137 pleiotropic effects in cells, as also previously discussed (Kroschwald, Maharana, and Simon 2017).
138 Using the set concentration of 5% 1,6HD, we assessed all aspects of cell physiology that we deemed
139 relevant and could assess. First, we treat the cells for 10 or 30 min with 5% 1,6HD or 2,5HD and
140 observed no effects on cell viability (Fig 2A). Then, we assessed if 10 min exposure to 5% 1,6HD leads
141 to changes in free ATP levels or cytosolic pH, using fluorescence-based sensors (Imamura et al. 2009;
142 Miesenböck, De Angelis, and Rothman 1998). Our rationale for testing these was that ATP and pH
143 levels could change when cells are experiencing metabolic stresses. We find, however, that the levels
144 of free ATP are unchanged after 1,6HD treatment. As a control, sodium azide (NaN₃) and 2-deoxy-
145 glucose (2DG) were used, which both depleted the cell of energy (Fig 2B). The cytosolic pH values,
146 calibrated as described in (Mouton et al. 2020), decrease mildly from 7.2 to 6,8 or 6,7 after exposure
147 to 1,6HD and 2,5HD respectively, and therefore remain in the physiological range (Fig 2C).

148 Next, we looked at the morphology and localization of different subcellular structures using GFP- or
149 RFP-tagged proteins marking the mitochondria, Golgi, peroxisome, ER, vacuole, plasma membrane,
150 nucleolus, secretory pathway, and ESCRT machinery. From visual inspection we conclude there are no
151 obvious changes in their appearance after 10 min exposure to 5% 1,6HD (Fig 2D). In contrast, the
152 appearance of microtubules and actin filaments does change after treatment with 1,6HD, which aligns
153 with some previous literature (Wheeler et al. 2016; Kroschwald, Maharana, and Simon 2017). Hsp104,
154 a disaggregase that can refold and reactivate previously aggregated proteins and responds to alcohol-
155 stress (Bösl, Grimminger, and Walter 2006; Sanchez and Lindquist 1990; Glover and Lindquist 1998;
156 Harari et al. 2022), forms foci upon exposure to 1,6HD, similar to when cells are exposed to either
157 nitrogen starvation, energy depletion or heat shock (Fig 2E), suggesting that 1,6HD induces some level
158 of protein stress. Finally, 1,6HD does not induce the formation of p-bodies (Fig 2F) or stress granules
159 (Fig 2G).

160 Taking the above together, under the conditions where mid exponentially growing cells are exposed
161 to 5% 1,6HD for 10 min, there are effects on the cytoskeleton and Hsp104 to be noted, but cell viability,
162 the pH and ATP levels in the cytosol, and the appearance of mitochondria, Golgi, peroxisomes, ER,
163 vacuoles, plasma membrane, nucleolus, the secretory and ESCRT pathways and stress granules are
164 not notably changed. While this is not an absolute proof of absence of indirect effects on nuclear

165 transport, the data strongly suggest that the 1,6HD-dependent effects on NPC permeability shown in
166 Fig 1 is due to direct effects on the nuclear transport machinery. Exposure to 10 min 5% 1,6HD thus
167 permeabilizes NPCs with surprising specificity.

168

169 **1,6HD induced loss of NTRs from the NPCs disrupts the permeability barrier**

170 Previous work proposed that the effects of 1,6HD are related to the alcohol-sensitive hydrophobic
171 interactions between the FG-nups that maintain the permeability barrier (Patel et al. 2007; Ribbeck
172 and Görlich 2002; Schmidt and Görlich 2015). Indeed, when the FG-domains of Nup100 (Nup100FG)
173 in preformed condensates are exposed to the concentrations of 1,6HD that were also used in life cells
174 (0-5%), partial solubilisation of the condensates is observed (Sup fig 1). While disruption of FG-nup
175 interactions by 1,6HD is indeed a scenario that is supported by *in-vitro* data, it is also one that is not
176 easy to proof or disproof in *in vivo* experiments. Alternative or additional explanations for the
177 increased permeability of NPCs in 1,6HD treated cells that can be experimentally addressed, relate to
178 the composition of the NPCs and to the NTRs. We explore them both.

179 Previous work (Shulga and Goldfarb 2003) showed that 1,6HD did not lead to release of NPC
180 components in wild type W303 cells, but it did in a mutant lacking Nup170. We noticed that even in
181 wild type cells the exposure to 10% 1,6HD lead to release of NPC components (data not shown).
182 Therefore, we repeated the analysis of nup localisation, and expanded on it with an analysis of
183 proteins levels. We assessed the effects of 5% 1,6HD on the protein levels and NPC-association of nine
184 representative endogenously tagged nups. The five tested FG-nups (Nsp1, Nup49, Nup159, Nup100,
185 Nup116), two of the scaffold nups (Nup133 and Nup170) and two basket nups (Nup60 and Nup2) did
186 not show changes in expression levels by western blot (Fig 3B). Also, their localization to the nuclear
187 envelope was unchanged, consistent with (Shulga and Goldfarb 2003) (Fig 3B). We conclude that the
188 10 minutes 1,6HD treatment did not lead to dissociation or degradation of the tested NPC
189 components, and hence it is unlikely that the increased permeability is a result of changes to the Nup-
190 composition of the NPCs.

191 NPCs constitute a significant amount of NTRs at any point in time and their presence critically shapes
192 the permeability barrier (Jovanovic-Taliman et al. 2009; Kalita, Kapinos, and Lim 2021; Lowe et al.
193 2015; Kim et al. 2018). Therefore, we addressed the localisation and abundance of endogenously GFP-
194 tagged NTRs after treatment with 1,6HD. The interaction between the FG-nups and NTRs are based
195 on dynamic multivalent binding with the phenylalanine's of the FG-nups (Hoogenboom et al. 2021;
196 Hough et al. 2015; Milles et al. 2015; Hayama et al. 2018; Sparks et al. 2018; Wing, Fung, and Chook

2022) and will thus also be sensitive to interventions disrupting hydrophobic interaction. We
evaluated the localisation of endogenously GFP-tagged NTRs. Under normal conditions most NTRs are
enriched at the nuclear envelope (NE) showing a punctate rim staining, e.g., Kap109, and few are
enriched in the nucleus, e.g. Kap104 (Fig 4A). Strikingly, the exposure to 1,6HD led to a clear
relocalisation of NTRs (Fig 4A). Kap104, Sxm1 (Kap108), Kap114, Nmd5 (Kap119), Pse1 (Kap121),
Kap122 and Kap123 lose their accumulation at the NE or nucleus upon exposure to 1,6HD and
distribute over the cytosol and nucleus (Fig 4A). Cse1 (Kap109), Kap120, Crm1 (Kap124) and Msn5
(Kap142) which are normally enriched at the NE, partly relocate. Kap60 and Kap95 were not visibly
affected by the treatment probably related to the previously described immobile pool of Kap95 at
NPCs (Lowe et al. 2015). Kap60 and Kap95 remain at NPCs while GFP-cNLS, whose active import is
driven by Kap60-Kap95, loses nuclear accumulation (Fig 1B), suggesting that 1,6HD treatment
increases passive permeability. When the less hydrophobic alcohol 2,5HD was used, it led to some
NTRs losing their accumulation at the NE or nucleus, but always to a lesser extent compared to 1,6HD
(Sup Fig 2). We conclude that the massive relocation of NTRs from NPCs may mechanistically explain
the 1,6HD induced increase in the permeability of NPCs.

To further strengthen this interpretation, we sought to quantitatively correlate the concentration
dependent NTR relocalisation, with the 1,6HD concentration dependent entry of the reporters used
before: MG5 (Fig 1A), GFP-NLS (Fig 1B) and GFP-NES (Fig 1C). We chose Kap122 for this analysis as
Kap122 clearly loses its accumulation at the NE and distributes over the cytosol and nucleus (Fig 4A).
The localisation of endogenously tagged Kap122-GFP in the nucleus and NE was assessed in a strain
co-expressing endogenously tagged Nup133-mCherry to mark the NE. The average nuclear
accumulation of Kap122 gradually decreased from 4,3 to 3,8 to 3,1 to 2,6 to 1,6 upon exposure to
zero, 0.625, 1.25, 2.5 or 5% 1,6HD. Moreover, we could correlate Kap122 relocalisation from the
nuclear envelope (NE) under these conditions with the measured passive permeability of NPCs for
MG5 (Fig 4C), GFP-NLS (Fig 4C) and GFP-NES (Fig 4D) with a Pearson correlation coefficient of 0.9, 0.8
and 0.9 respectively. These correlations support that 1,6HD perturbs the NPC permeability barrier by
releasing the NTRs.

224

225 **DISCUSSION**

226 Here we assessed the specificity and mechanism by which 1,6-hexanediol (1,6HD), an aliphatic alcohol
227 that interferes with hydrophobic interactions, disrupts the permeability barrier of NPCs in live baker's
228 yeast cells. Exposure of live yeast cells to 1,6HD (10 min, 0-5%) leads to a gradual loss of the
229 permeability barrier of NPCs. We conclude this is likely a direct effect on the nuclear transport

230 machinery as cell viability, the pH and ATP levels in the cytosol, and the appearance of mitochondria,
231 Golgi, peroxisomes, ER, vacuoles, plasma membrane, nucleolus, secretory pathway and stress
232 granules were not notably changed. There were effects on the cytoskeleton and protein homeostasis
233 (Hsp104 foci) to be noted and we cannot exclude that 1,6 HD impacts the cell's physiology in ways
234 that we did not monitor. Mechanistically we propose that the displacement of NTRs from the NPC
235 underlies the loss of NPC function because 1,6HD treatment induced a massive relocation of multiple
236 NTRs from NPCs. This displacement from the nuclear envelope quantitatively correlated with the
237 passive permeability of NPCs.

238 Our studies align well with previous reports that showed that the selective properties of the FG-nups
239 rely on the physical presence of NTRs within the NPC. The earliest study is one showing that the
240 presence of transport factor enhances the selectivity of FG-nucleoporin-coated membranes
241 (Jovanovic-Taliman et al. 2009). The most recent reports on detergent-permeabilized human cells
242 show that the enrichment of NTRs at the NPCs is important for the permeability barrier by preventing
243 passive permeability (Kalita et al. 2022). Our work adds to this by showing the importance of NTRs in
244 live cells. The benefit being that in live cells there is a constant and large flux of transport and
245 therefore, together with the loss of the estimated 15,6 MDa of NTRs from the central channel also
246 10,4 MDa worth of cargo is being lost (Kim et al. 2018). This joint loss of NTRs *and* cargo from the NPC
247 central channel will present a major change in the macromolecular crowding and composition, and
248 hence its physicochemical properties. How this alters the structural dynamics of the FG-nups, and if
249 this poses a risk for NPC function would be interesting questions for the future.

250 Extrapolating from studies using purified FG-nup fragments that proposed that the effects of 1,6HD is
251 related to the alcohol-sensitive hydrophobic interactions between the FG-nups (Patel et al. 2007;
252 Ribbeck and Görlich 2002; Schmidt and Görlich 2015) one may expect that 1,6HD also alters the
253 interactions between the FG-nups in our assays using live cells. This is, however, difficult to address in
254 live cells. Hence it remains unclear if the NTRs are released from the NPCs as a consequence of a
255 lowered binding affinity between FG-nups, or because 1,6HD directly lowered the binding affinity of
256 NTRs for the FG-repeat regions. If one considers that the functional composition of central channel is
257 a system composed of NTRs *and* FG-nups in close collaboration, then the discrimination between
258 these scenarios becomes less important.

259 An unanswered question in the field is if NPCs that are dysfunctional can be detected and removed.
260 To assess this question, one needs to be able to inducibly damage NPCs. NPC permeabilization is
261 expected to be an intervention that triggers quality control similar to when assembly fails (Thaller et

262 al. 2019; Webster et al. 2016; Thaller et al. 2021). The here described method could provide a tool to
263 study the recruitment of quality control factors and to follow the repair or degradation.

264 Lastly, our study may serve as a warning that the effects of 1,6HD on liquid-liquid phase separation of
265 diverse cellular macromolecular complexes may actually be the consequence of to 1,6HD's prime
266 effect on the NPC and cognate NTRs. We speculate that the hydrophobic and highly acidic nature of
267 NTRs may readily compromise their stability above a critical concentration. Consistent with this is that
268 overexpression of Sxm1, Kap95, and Kap114 is toxic to cells (Simmelink et al. 2022). In any case, a
269 major misplacement of NTRs and associated cargo will dramatically change the nuclear and
270 cytoplasmic proteomes and this may generally compromise their stability. The increase in the number
271 of Hsp104 foci that we observe may indeed reflect such loss of protein homeostasis.

272 Altogether, this paper puts hydrophobic interactions between NTRs and FG-Nups centre stage in the
273 explanation of the selective properties of NPCs supporting the Kap-centric model for nuclear transport
274 proposed by the Lim laboratory (Springhower, Rosen, and Chook 2020).

275

276 **MATERIAL AND METHODS**

277 **Strains and Growth conditions**

278 All *Saccharomyces cerevisiae* strains used in this study have the BY4741 background, except yER016,
279 which were created in the W303 background. Strains are listed in Table 2. yER016, yER020 and yER023
280 were created as described in (Janke et al. 2004). GFP-tagged strains were taken from the 4000-GFP
281 yeast library (Thermofisher), RFP-tagged strains were taken from the localization database collection
282 (Huh et al. 2003).

283 Cells were grown at 30°C, with shaking at 200 RPM on Synthetic Complete (SD) medium supplemented
284 with 2% (w/v) glucose. Cells from an overnight culture were diluted 1:10 during the day and then again
285 for an overnight culture in SD- 2% glucose. Cells were diluted again on the day of the experiment, and
286 grown for several hours to obtain cultures in exponential growth phase (OD_{600} 0.6-0.8) before each
287 experiment.

288 **Spot assay**

289 On the day of the experiment, exponentially growing cells were treated with 5% 1,6HD or 5% 2,5HD
290 for 10 or 30 minutes, as indicated in Fig. 2A, and diluted in sterilized milliQ water to obtain 10^6 cells/ml,
291 and further serial diluted in milliQ water. 5 μ l of each dilution was spotted on YPD plates and the plates
292 were imaged after 48H growth at 30°C.

293 **Microscopy**

294 All *in vivo* experiments were performed at 30°C. Images were acquired using a DeltaVision Elite
295 imaging system (Cytiva) composed of an inverted microscope (IX-71; Olympus) equipped with a
296 UPlanSApo 100x (1.4 NA) oil immersion objective, InsightSSI solid-state illumination, and an EDGE
297 sCMOS 5.5 camera. For all experiments, stacks of 30 images with 0.2µm spacing were taken.

298 **Protein lysate and Western Blot**

299 20 ml of yeast culture was grown to an OD₆₀₀ 0.8-1.2. Cells were subsequently treated with 5% 1,6HD
300 for 10 min at 30°C, with shaking at 200 RPM. After the treatment, cells were centrifuged, and all the
301 following steps were performed at 4°C. The cell lysate was resuspended in 0.25ml of lysis buffer
302 (50mM HEPES, 200mM sodium acetate, 1mM EDTA, 5mM magnesium acetate, 5% glycerol, 1% triton
303 x-100, 10mM β-mercaptoethanol, protease inhibitor without EDTA) and lysed in two rounds of bead-
304 beating in a Fastprep device (MP biomedical). Lysates were cleared by consecutive centrifugations at
305 6000 x g for 5 min, followed by centrifugation of the supernatant at 17700 x g for 5 min. The resulting
306 supernatant was centrifuged once more at 17700 x g.

307 Western blots were performed as follows: whole cell lysates were separated by SDS-PAGE. The
308 proteins were subsequently transferred to PVDF membranes. After blocking with 5% skim milk in TBS-
309 T, GFP-tagged proteins were detected with anti-GFP (Santa Cruz sc-9996 HRP) was used, followed by
310 HRP-conjugated mouse IgG kappa binding protein (Santa Cruz sc-516102, m-igGκ BP-HRP).

311 **Expression and purification of nucleoporin FG-domains**

312 Nup100FG domains were expressed and purified as described in (Kuiper et al. 2022). In short: FG-
313 domains proteins with an N terminal His-tag and a unique C-terminal cysteine were expressed in
314 *Escherichia coli*, by induction with 0.5mM IPTG and purified from cell extracts on a Nickel-Sepharose
315 column under denaturing conditions (2M GuHCl, 100mM Tris-HCl pH 8). The C-terminal cysteine was
316 reduced with DTT and blocked by modification with Iodoacetamide. Protein purity was checked with
317 SDS-PAGE and subsequent Brilliant Blue staining.

318 **Spin Assay**

319 A concentrated stock of 100µM Nup100FG domains in 2M GuHCl, 100mM Tris-HCl pH 8, was diluted
320 to 3µM into TBS (50mM Tris-HCl, 150mM NaCl pH 8). The protein was left to self-assemble into
321 particles for 1h at RT, and then the protein was treated for 10 min with different concentrations of
322 1,6HD. Samples were centrifuged (17.700 x g for 10 min at RT), and soluble and insoluble fractions
323 were run separately on SDS PAA gels. Gels were stained with Brilliant Blue G (Sigma-Aldrich, G-250)

324 and imaged using a BioRad chemidoc (BioRad). Band intensities were determined using Fiji (Image J,
325 National Institute of Health).

326 **Determining the intracellular pH with the pFluorin sensor**

327 pFluorin ratios were calibrated in live cells in buffers with a pH of 5, 5.5, 6, 6.5, 7, 7.5, and 8, as
328 described in (Mouton et al. 2020). The FRET/CFP and FRET/mEGFP (F390/F475) ratios were
329 determined from cells on a glass slide. Cells were then treated with 1,6HD as described in Fig 2, and a
330 calibration curve was used to determine the pH change after treatment.

331 **ATP sensor values and free ATP levels**

332 Cells expressing a FRET-based ATP sensor (Semmelink et al. 2022), were used to determine free ATP
333 levels as described in (Semmelink et al. 2022). Cells were treated as described in Fig 2, imaged, and
334 the FRET over GFP ratio was calculated using Fiji (see below).

335 **Image Analysis**

336 All images were processed using Fiji (Image J, National Institute of Health). For each image, the z-stack
337 with the best focus was selected. For GFP-tagged reporters, we determined the fluorescence around
338 the nuclear envelope and subtracted the background from outside the cell. For pFluorin and the ATP
339 sensor, we determined the fluorescence in each channel for each cell and took the fluorescence of the
340 entire cell and subtracted the background from a region outside the cell for each channel. The
341 respective ratios were subsequently calculated. To quantify the nuclear localization (N/C ratio) of the
342 GFP-based reporters and Kap122, the average fluorescent intensity of the nucleus and the cytosol was
343 measured. The nucleus area was determined using either the mCherry-TM reporter (pACM063) that
344 indicated the nuclear envelope (Fig 1) or Nup133-mCherry (Fig 4B). A section of the cytosol excluding
345 the vacuole was selected to measure the fluorescence in the cytosol.

346 **Statistical Analysis**

347 Statistical parameters, including the number of cells analyzed, are reported in figure legends. All
348 regressions and correlations leading to the sigmoidal curve equation, R^2 , and all Pearson's correlation
349 statistics were done in GraphPad Prism.

350

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355 assistance.

356 **AUTHOR CONTRIBUTIONS**

357 ERB and LMV conceived the project. ERB designed, performed and analysed all experiments with help
358 from SNM (Fig. 2BC) and TO (Supfig1). The manuscript was written by ERB and LMV with input of all
359 authors.

360

361 **COMPETING INTERESTS**

362 The authors declare no competing interests.

363

364 **DATA AND REAGENT AVAILABILITY**

365 All data and reagents are available upon request.

366

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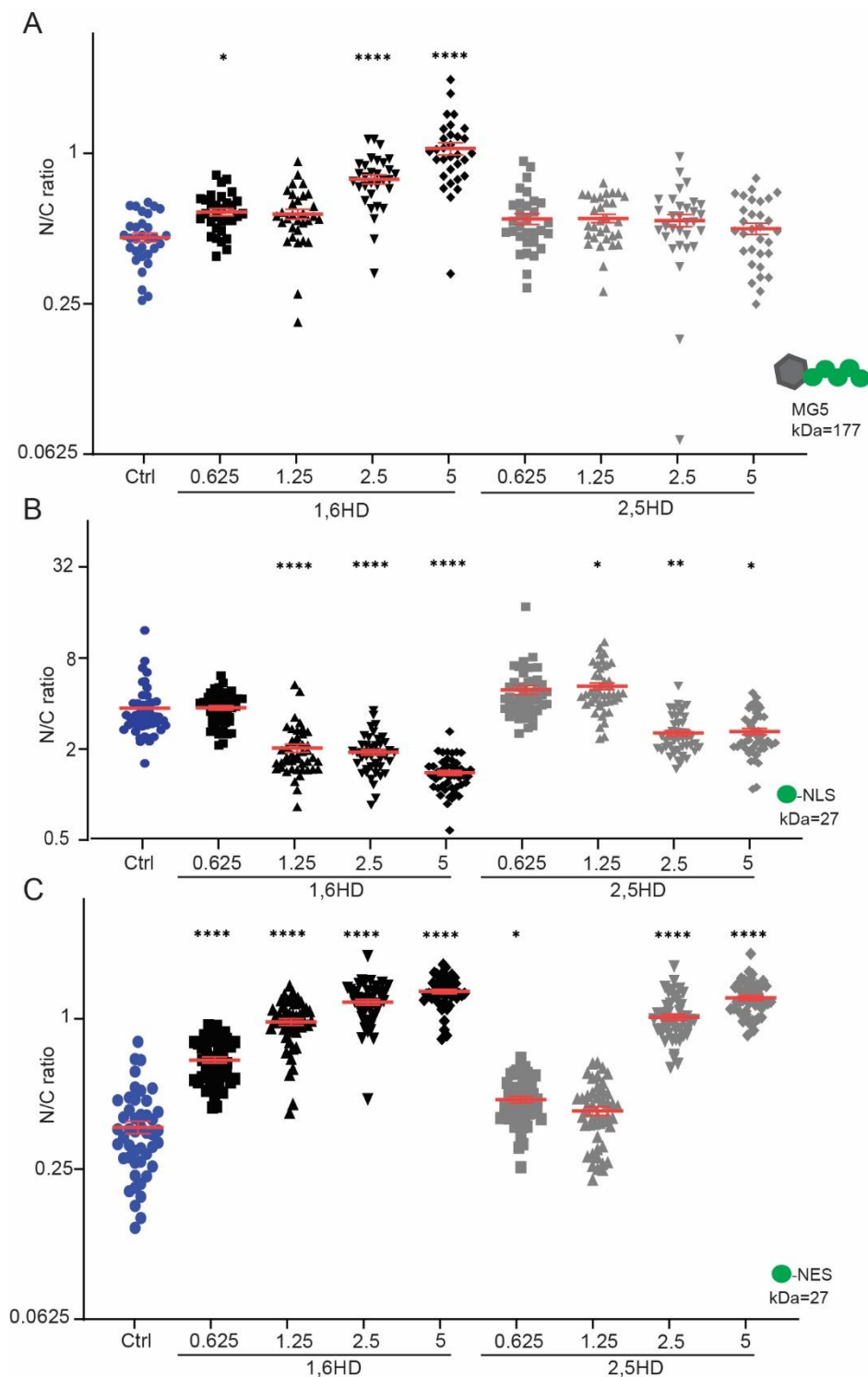
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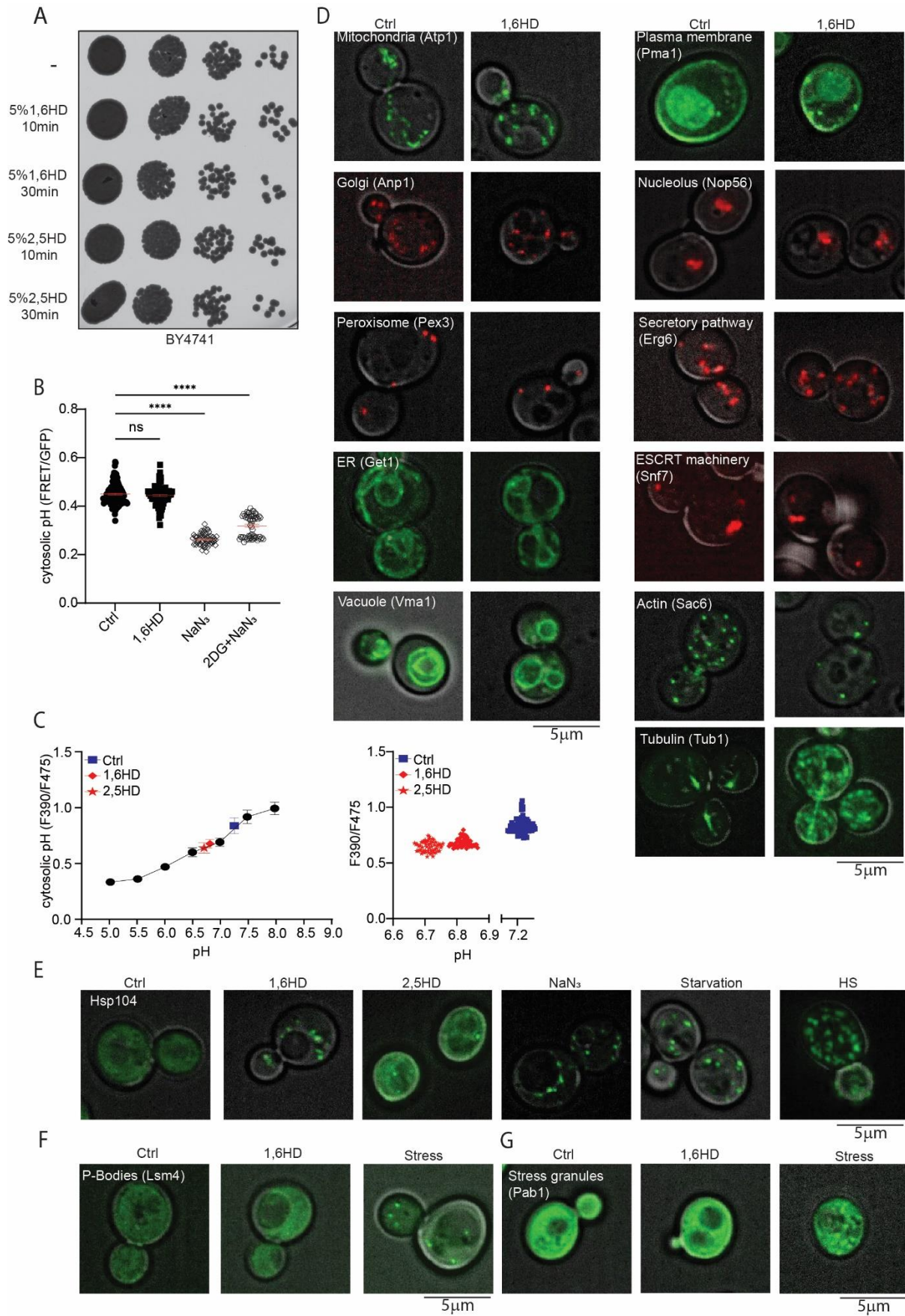
590 **FIGURES**



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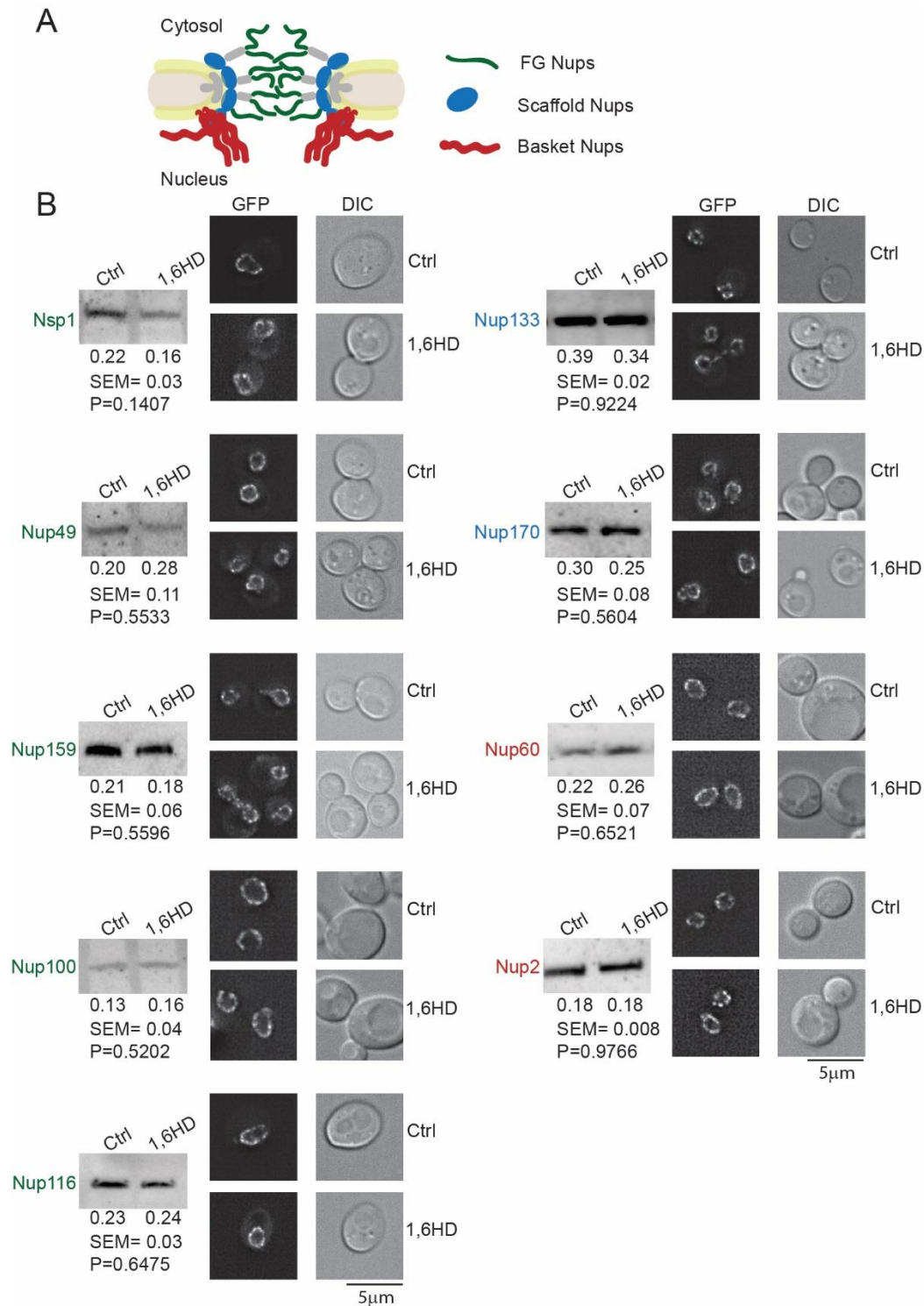
592 **Figure 1: Disruption of NPC permeability barrier by 1,6HD.** (A-C) Nuclear compartmentalization of
593 GFP-based reporter proteins (MG5, GFP-NES, GFP-NLS) in yeast cells exposed for 10 min with the
594 indicated concentrations of 1,6HD or 2,5HD. MG5 is a fusion of Maltose Binding Protein and 5 GFPs;
595 GFP-NLS features the classical Simian Virus 40 NLS and GFP-NES the Stress-Seventy subfamily B1 NES.
596 The N/C ratio is the ratio of the average fluorescence in the nucleus (N) over that in the cytoplasm (C).

597 One-way ANOVA with Dunnett's multiple comparison test comparing treatment to control was used
598 to calculate the statistical significance of (A) MG5 and (C) GFP-NES, while the non-parametrical
599 Kruskal-Wallis with Dunn's multiple comparison test comparing treatment to control was used to
600 calculate the statistical significance of (B) GFP-NLS. Error bars reflect SEM from the mean of three
601 independent experiments. At least 30 cells per condition were analysed. P-values* $<0,05$ ** $<0,01$
602 **** $<0,0001$.



603

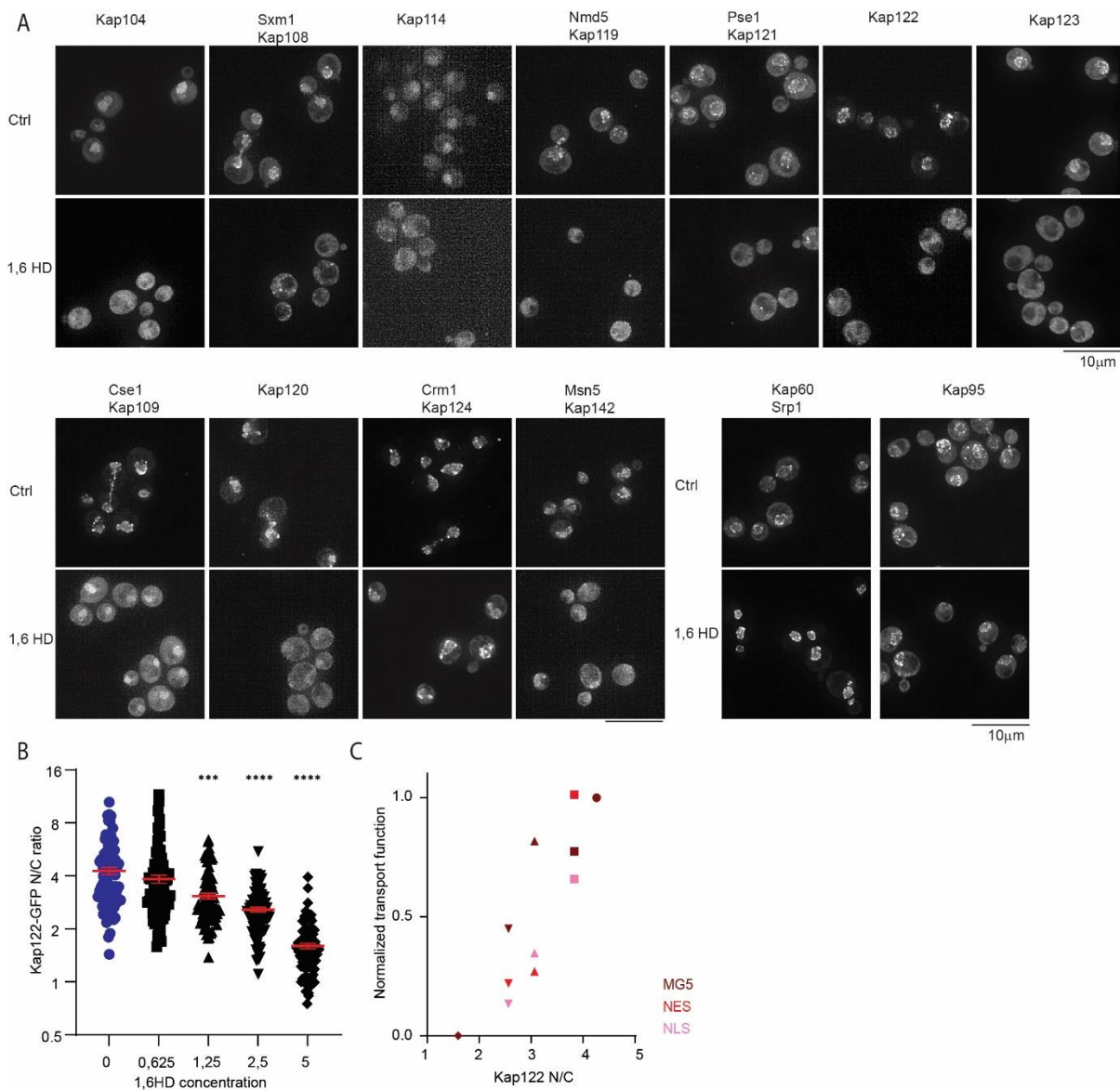
604 **Figure 2: Impact of 1,6HD on cell survival, physiology and subcellular structures.** (A) Growth assay
605 showing serial dilutions of cultures exposed to 5% 1,6HD or 2,5HD for the indicated times. (B) Free
606 ATP levels in cells measured using a FRET-based ATP-sensor; lower FRET/GFP ratio indicates lower free
607 ATP. Cells were untreated (ctrl), exposed to 5% 1,6HD for 10 min, or exposed for 30 min to metabolic
608 poisons azide (NaN_3) or to NaN_3 plus deoxyglucose ($\text{NaN}_3 + 2\text{DG}$). The error bar of the scatter plot
609 reflects SEM from the mean of three independent experiments. At least 60 cells per condition were
610 analysed. Non parametric Mann-Whitney test was used to calculate statistical significance in
611 FRET/GFP ratios comparing treatment to control. (C) Calibration curve for cytosolic pH values of the
612 pH sensor pHluorin (F390/F475) in cells (black circles). The pH before (ctrl, blue squares) and after 10
613 min exposure to 1,6HD (red diamonds) or 2,5 HD (red stars) are indicated. Each point represents data
614 from 60 cells (left graph), individual measurements are shown (right graph). (D) Fluorescence images
615 of different cellular structures endogenously tagged with either GFP or mCherry, before and after 10
616 min exposure to 5% 1,6HD. (E) Fluorescence images showing localization of endogenously tagged
617 Hsp104-GFP after 10 min exposure to 5% 1,6HD or 5% 2,5HD and under indicated stress conditions.
618 (F,G) Fluorescence images showing localization of endogenously tagged Lsm4 (P-bodies, F) or Pab1
619 (Stress granules, G) with GFP after 10 min exposure with 5% 1,6HD and after induction of stress.
620 Representative images of three independent replicates. The scales bars are $5\mu\text{m}$.



621

622 **Figure 3: Impact of 1,6HD on the abundance and localization of NPC components.** (A) Cartoon
 623 representation of NPC indicating the position of the nups analyzed in B. (B) Western blot of
 624 endogenous Nup-GFP protein levels before and after 10 min exposure to 5% 1,6HD; quantification
 625 gives mean, SEM and P values from at least three independent replicates. Fluorescence images of
 626 endogenously GFP-tagged nups after 10 min exposure with 5% 1,6HD. Representative images of three
 627 independent replicates. The scale bar is 5 μ m.

628



629

630 **Figure 4: Impact of 1,6HD on NTRs.** (A) Fluorescence images of endogenously GFP-tagged NTRs after

631 10 min exposure with 5% 1,6HD. Representative images of three independent replicates. The scale

632 bar is 10 μ m. (B) Nuclear accumulation of Kap122-GFP in yeast cells exposed for 10 min with the

633 indicated concentrations of 1,6HD. Non-parametrical Kruskal-Wallis with Dunn's multiple comparison

634 test was used to calculate statistical significance, comparing treatment to control. Error bars reflect

635 SEM from the mean of three independent experiments. 90 cells per condition were analyzed. P-values

636 ***<0,0005 ****<0,0001. (C) Average transport function measured with MG5 (dark red, normalized

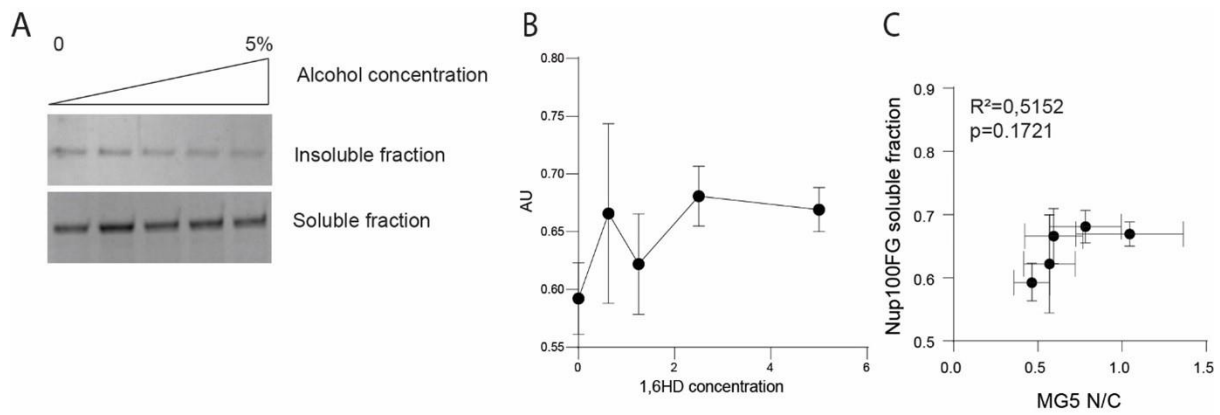
637 N/C from Fig1A), GFP-NLS (pink, normalized N/C from Fig1B) and GFP-NES (red, normalized N/C from

638 Fig 1C) as a function of Kap122-GFP location at the NE and nucleus (from Fig 4B) under control

639 conditions and increasing concentrations of 1,6HD (symbols as in 4B: 0% circles; 0,625% squares;

640 1,25% triangles up; 2,5% triangles down; 5% diamonds).

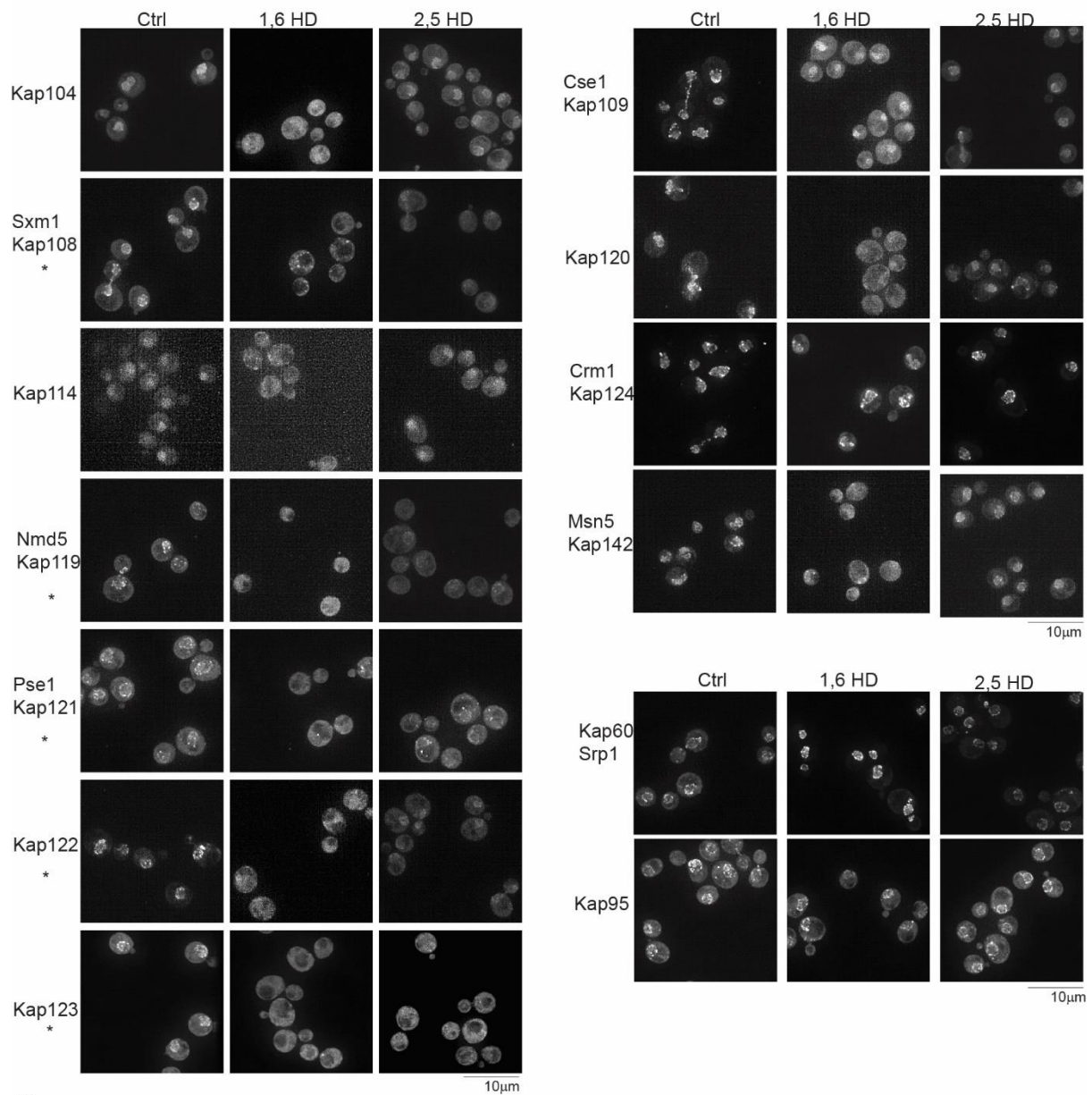
25



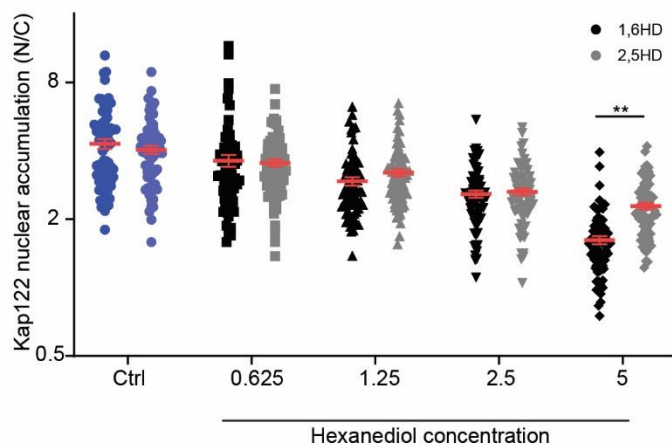
641

642 **Supplementary Figure 1.** (A) Purified Nup100FG domains were left to form condensates for 1 hour
643 and subsequently treated for 10min with 0, 0.625, 1.25, 2.5 or 5% 1,6HD. Soluble and insoluble
644 fractions were obtained by centrifugation, separated by SDS-PAGE and visualized by Brilliant Blue
645 staining. Representative image of three independent experiments. (B) Quantification of the soluble
646 fractions in (A) Error bars reflect SEM of three independent experiments. (C) Pearson correlation
647 coefficient and two-tailed P values were calculated for the N/C ratio of reporter MG5 against the
648 soluble fraction of Nup100FG domain after different concentrations of 1,6HD. Error bars reflect SEM
649 from the mean of three independent experiments.

A



B



650

27

651 **Supplementary Figure 2** (A) Fluorescence images of endogenously GFP-tagged NTRs after 10 min
652 exposure with either 5% 1,6HD (middle, as in Fig 4A) or 5% 2,5HD (right). Representative images of
653 three independent replicates. The scale bar is 10 μ m. (B) Nuclear accumulation of Kap122-GFP in yeast
654 cells exposed for 10 min to the indicated concentrations of either 1,6HD (as in Fig 4B) or 2,5HD. Non-
655 parametrical Kruskal-Wallis with Dunn's multiple comparison test comparing treatment to control was
656 used to calculate statistical significance. Error bars reflect SEM from the mean of three independent
657 experiments. 70 cells per condition were analyzed. P-values **<0,005.

658

659 **Table 1: Key resources table**

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Gene (<i>S. cerevisiae</i>)	See table 2			
strain, strain background (<i>S. cerevisiae</i>)	BY4741	Invitrogen		
strain, strain background (<i>S. cerevisiae</i>)	BY4742	Invitrogen		
strain, strain background (<i>S. cerevisiae</i>)	W303	Invitrogen		
Genetic reagent (<i>S. cerevisiae</i>)	See table 2			
Antibody	Monoclonal antibody mouse anti-GFP	Santa Cruz	sc-9996	(1:500)
Antibody	Mouse IgG kappa binding protein conjugated to HRP; m-IgGκ-BP-HRP	Santa Cruz	sc-516102	(1:10000)
Recombinant DNA reagent	See table 3			
Sequenced-based reagent	Nup60_F	This paper	PCR primers GTTGATGAAAATAAAGTTGAGGC TTTCAAGTCCCTATATACCTTTTCG TACGCTGCAGGTCGAC	
Sequenced-based reagent	Nup60_R	This paper	PCR primers TTGGGCTATACGGTAATTATGTC ACGGCTAAAATTTTCATTATCAAT CGATGAATTCCGAGCTCG	

Sequenced-based reagent	Nup133_F	This paper	PCR primers GAAAAAACTATACCATCAACTA TGAAACCAACTGTAGAATACG GTGACGGTGCTGG	
Sequenced-based reagent	Nup133_R	This paper	PCR primers CAGTAAAGTTTATTATATATATGT AAAATTGTATTATAGATATTATCG ATGAATTCGAGCTCG	
Sequenced-based reagent	Pab1_F	This paper	PCR primers GTCTTTCAAAAAGGAGCAAGAAC AACAACTGAGCAAGCTCGTACG CTGCAGGTCGAC	
Sequenced-based reagent	Pab1_R	This paper	PCR primers GTTTGTTGAGTAGGGAAGTAGGT GATTACATAGAGCATTAAATCGAT GAATTCGAGCTCG	
chemical compound, drug	Yeast extract	BD	291946	
chemical compound, drug	Complete supplement mixture complete	Formedium	DCS0019	
chemical compound, drug	D-Glucose anhydrous	Fisher Chemical™	10141520	
chemical compound, drug	D-Raffinose pentahydrate	Thermo Scientific	195675000	
chemical compound, drug	D-Galactose	Acros Organics	150610010	
chemical compound, drug	Phosphatase buffered saline	Sigma-Aldrich	P4417	
chemical compound, drug	Tris base	Fisher Scientific™	BP152-1	
chemical compound, drug	HEPES	Fisher Scientific™	BP310-500	
chemical compound, drug	Sodium dodecyl sulfate (SDS) solution, 20%	SERVA	20767.03	

chemical compound, drug	EDTA	Sigma-Aldrich	ED2P-500	
chemical compound, drug	Triton X-100	Acros Organics	215682500	
chemical compound, drug	2-mercaptoethanol	Sigma-Aldrich	M6250-100	
chemical compound, drug	Sodium chloride	Acros Organics	207790010	
chemical compound, drug	Tween20	MP Biomedicals	TWEEN201	
chemical compound, drug	Magnesium chloride hexahydrate	Sigma-Aldrich	M2393	
chemical compound, drug	Sodium acetate anhydrous	Fisher Chemical™	S2080/53	
chemical compound, drug	Magnesium acetate tetrahydrate	Fisher Scientific™	BP215	
chemical compound, drug	Glycerol	Sigma-Aldrich	G5516	
chemical compound, drug	Phenylmethanesulfonyl fluoride (PMSF)	Sigma-Aldrich	P7626	
chemical compound, drug	cOmplete ULTRA tablets, Mini EDTA-free	Roche	05892791001	
chemical compound, drug	Albumine bovine serum (BSA)	Acros Organics	268131000	
chemical compound, drug	Glass beads	BioSpec Products	11079105	
chemical compound, drug	Pierce™ BCA Protein Assay Kit	Fisher Scientific™	23225	

chemical compound, drug	ECL Prime Western Blotting Detection Reagent	Amersham	RPN2232	
chemical compound, drug	GX Stain-Free™ FastCast™ Acrylamide Kit, 10%	BioRad	1610183	
chemical compound, drug	PVDF Transfer Membrane	Thermo Scientific	88518	
chemical compound, drug	Methanol Technical	VWR	20903.368	
chemical compound, drug	IPTG	Sigma-Aldrich	10724815001	
chemical compound, drug	Ni sepharose	Cytiva	17531802	
chemical compound, drug	Guanidine hydrochloride	Thermo Scientific	24110	
chemical compound, drug	Brilliant blue G	Sigma-Aldrich	G-250	
chemical compound, drug	1,6 hexandiol	Sigma-Aldrich	240117-50	
chemical compound, drug	2,5 hexandiol	Sigma-Aldrich	H11904-50	
chemical compound, drug	Sodium azide	Sigma-Aldrich	S2002-100	
chemical compound, drug	2-deoxy-d-glucose	Sigma-Aldrich	D8375-1	
software, algorithm	Fiji	(Schindelin et al. 2012)		
software, algorithm	Resolve3D SoftWoRx	Cytiva		

660

661 Table 2 Yeast strains used in this publication

Strain BY4741 ¹	Genotype	Source
yPP008; GFP-tcNLS	Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 GFP-tcNLS(pGal1)::His Nup49-mCh::URA	(Rempel et al. 2019)
yPP011; GFP-NES	Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 GFP-NES(pGal1)::His Nup49- mCh::URA	(Rempel et al. 2019)
GFP collection ²⁾	Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 XX -GFP::HIS3M X 6	ThermoFisher
Nup116-GFPboundary	Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	(Rempel et al. 2019)
yER016; Nup60-GFP ¹⁾	Mata leu2-3, 112 trp1-1 can1-100 ura3-1 ade 2-1 his3-11, 15 Nup60- GFP::KanMX4	This paper
yIS010; Nup2-GFP Nup49mCherry	Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Nup2-GFP::His3MX6 Nup49- mCherry::URA	(Rempel et al. 2019)
yER020; Pab1-GFP	Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Pab1-GFP::HIS3M X 6	This paper
RFP localization database ³⁾	Mata his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 YY -RFP::KanMX6	(Huh et al. 2003)
SMY15	Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pTEF1-pHluorin::His3M X 6	(Mouton et al. 2020)
SMY16	Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ATP sensor pTEF1-his6- ymEGFP Δ11-B.subtilis ε- ymScarletl::HIS3M X 6	(Semmelink et al. 2022)
yER023; Kap122-GFP Nup133mCherry	Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Kap122-GFP::HIS3M X 6 Nup133-mCherry::URA	This paper
<p>1) yER016 is in W303 background</p> <p>2) XX is: NSP1, Nup49, Nup100, Nup133, Nup159, Nup170, LSM4, Hsp104, ATP1, Get1, Vma1, Pma1, Tub1, Kap124, Kap95, Kap60, Kap122, Kap104, Kap142, Kap119, Kap121, Kap108, Kap109, Kap114, Kap120, Kap123.</p> <p>3) YY is: Anp1, Pex3, Nop56, Erg6, Snf7.</p>		

662

663 Table 3 Plasmids used in this publication.

Plasmid number	Genotype	Source
pPP008; MG5	pUG34-Gal1-MBP-5XGFP-His	(Popken et al. 2015)
pACM063; mCh-L-TM	pUG36-Gal-mCherry linker-TM-URA	(Meinema, Poolman, and Veenhoff 2013)
pYM28	pAgTEF-SpHIS5-tAgTEF	Euroscarf, Janke et al 2004
pYM30	pAgTEF-kanMX-tAgTEF	Euroscarf, Janke et al 2004
pPP014	mCherry-Ura cassette	(Rempel et al. 2019)

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