**Supplementary Information**

**Supplementary Methods:**

*Standard yeast methods:*

Paraformaldehyd (PFA) fixation of yeast cells was performed by adding equal volumes of a solution of 8% PFA in PBS to a yeast culture (final PFA concentration 4%) and incubation for 10 min at room temperature, followed by washing with PBS.

Calcofluor White staining was performed according to standard protocols using a final concentration of 60 µM of Calcofluor White M2R (Sigma) in PBS for 15 min at RT followed by one washing step with PBS.

*Yeast deletion library Screening using SGA.*

The Systematic Gene Analysis (SGA) strategy ([32](#_ENREF_32)) was followed for the screening approach to test in a proof-of-principle approach the effects of the 94 gene deletions present in the first plate of the yeast deletion library (Invitrogen) for differences of PrD-GFP aggregation patterns. This was achieved by mating of the Y8205 ΔNM(SUP35)-GPD-PrD-GFP strain (see above) to the first deletion library plate in 96-well format followed by several selection steps according to SGA. After the final step of haploid MatA progeny selection ([28](#_ENREF_28),[32](#_ENREF_32)), cells were inoculated into 200 µl of haploid selective media in 96-well plates and grown to an OD600 of ~ 0.3 to 0.8 at 30 °C on a shaker. Cells were then subjected directly to flow cytometry in 96-well format without fixation.

*Fluorescence Microscopy.*

Cells were examined with an Olympus IX81 inverted microscope with a 100x/1.45 oil objective and narrow band-pass filters. Images were taken with a Hamamatsu ORCA-R2 camera. All displayed fluorescence microscopy images represent a merged image (maximum intensity projection) from single images from z-stacks of cells with a step width of 0,2 µm. Images were analyzed with the ImageJ software and brightness and contrast were linearly adjusted. Single particle analysis was performed in ImageJ using the threshold “otsu” and the following settings: size: 0 – infinity, circularity: 0,00 – 1,00, exclude on edges enabled.

**Supplementary tables**

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| **Pulse Shape Parameter** | **Phenotype** | | |
| **Single Aggregate** | **Cured** | **hsp104** |
| Mean | 65.5 ± 3.549 | 75.47 ± 2.769\*\* | 79.00 ± 1.697°° |
| SD | 7.008 ± 1.376 | 11.54 ± 0.554\* | 16.35 ± 1.865°°°/+ |
| Amplitude | 47.86 ± 9.159 | 23.08 ± 5.482\*\* | 21.27 ± 3.204°° |

**Table S1. Parameters for the pulse shape analysis of PrD-GFP Aggregation Phenotypes** Data represents mean ± SD (*N* = 3); \*\*P<0.001, \*P<0.05 Cured vs. single aggregate; °°°P<0.0001, °°P < 0.001 Hsp70 vs. single aggregate; +P<0.05 Cured vs.Hsp70

**Supplementary Figure Legends**

**Figure S1. Optical and fluorescence parameters measured by flow cytometry.**

FCS: forward scatter, SSC: side-scatter, GFP-A: area under the signal pulse, corresponds to the amount of GFP fluorescence/event (cell), GFP-W: GFP-width of the signal pulse, GFP-H: GFP-hight of the signal pulse; for detailed description, see text.

**Figure S2: Comparison of the growth curves of the PrD-GFP in the aggregated state (WT) and in the diffuse, non-aggregated state (WT cured and Δhsp104) strains.**

Graph and table displaying the OD600 of 3 PrD-GFP expressing cultures, no observable differences between the culture exhibiting one single aggregate (WT) and those displaying diffuse fluorescence (WT cured and Δhsp104).

**Figure S3. Additional hits from a screen for aggregate load and distribution of PrD-GFP of the first plate of the yeast deletion library.**

Pseudocolor scatter plots of SSC-A vs. FSC-A **(left panels)** or GFP-H vs. GFP-W of the GFP+ population **(middle panels)** and the corresponding fluorescence microscopy images **(right panels)** of different yeast deletion strains as indicated, generated through systematic gene analysis (SGA) of the first plate of the yeast deletion library and a yeast strain constitutively expressing PrD-GFP in the prion state (1 single aggregate/cell). Given is also the average GFP fluorescence intensity per cell/event in arbitrary units (GFP-A mean), measured by flow cytometry. Percentage numbers displayed within the fluorescence image correspond to the frequency of the phenotype shown. For the Δpmt2 and Δkin3 deletion strains, in comparison to the wild type strain, the average “single aggregate” size was determined by single particle analysis using the ImageJ software; “n” gives the number of cells used for the analysis. All strains were analyzed directly after SGA as non-fixed cells. The dashed lines represent the borders of the cells and the scale bar 2μm. Each scatter plot represents 10.000 events.