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Supplemental Information

Mining Disaggregase Sequence Space to Safely Counter TDP-43, FUS, and α-Synuclein Proteotoxicity

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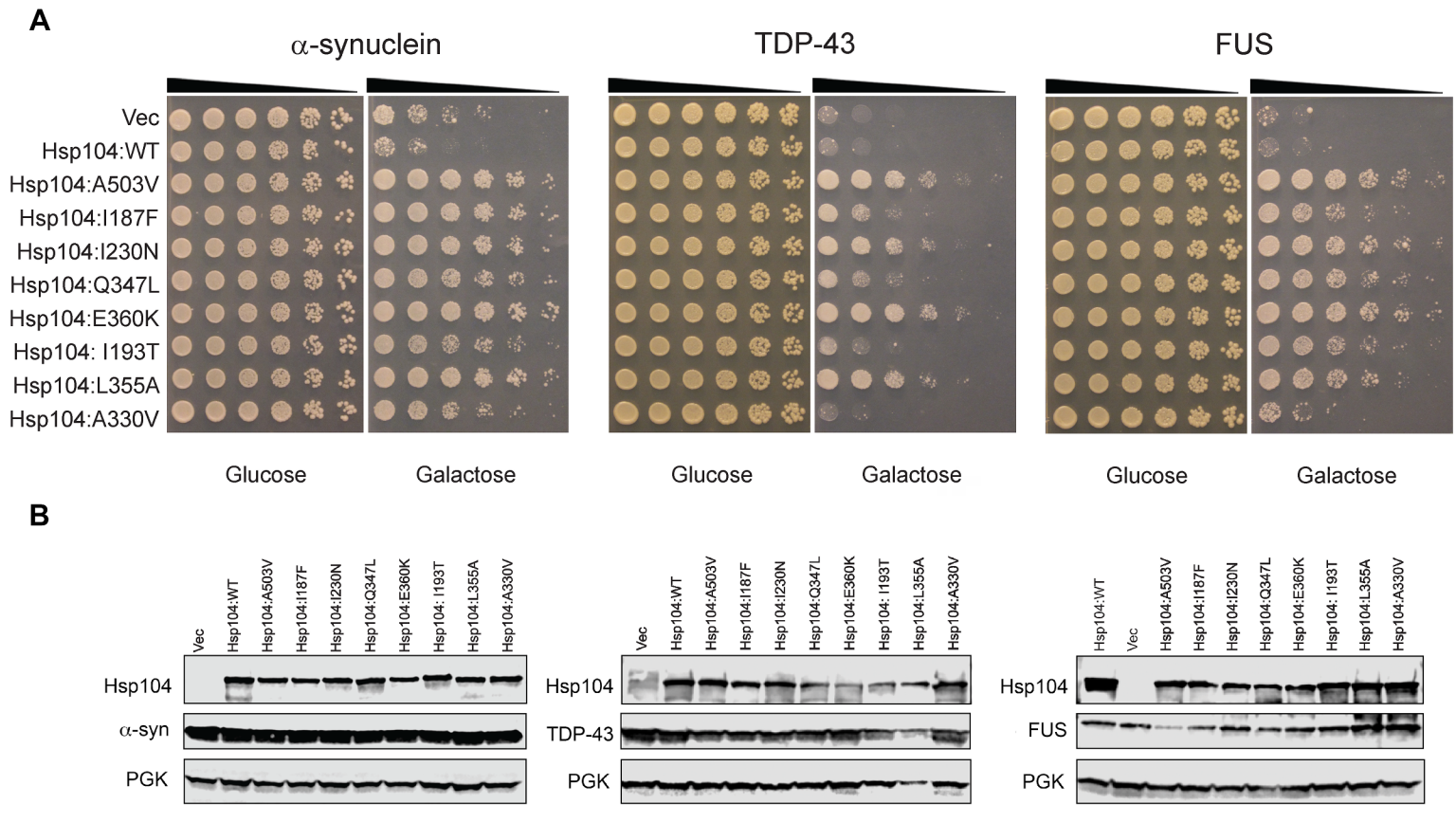

A
α-synuclein
TDP-43
FUS
Vec
Hsp104:WT
Hsp104:A503V
Hsp104:I187F
Hsp104:I230N
Hsp104:Q347L
Hsp104:E360K
Hsp104: I193T
Hsp104:L355A
Hsp104:A330V
Glucose
Galactose
Glucose
Galactose
Glucose
Galactose
B
Vec
Hsp104:WT
Hsp104:A503V
Hsp104:I187F
Hsp104:I230N
Hsp104:Q347L
Hsp104:E360K
Hsp104: I193T
Hsp104:L355A
Hsp104:A330V
Hsp104
α-syn
PGK
Vec
Hsp104:WT
Hsp104:A503V
Hsp104:I187F
Hsp104:I230N
Hsp104:Q347L
Hsp104:E360K
Hsp104: I193T
Hsp104:L355A
Hsp104:A330V
Hsp104
TDP-43
PGK
Hsp104:WT
Vec
Hsp104:A503V
Hsp104:I187F
Hsp104:I230N
Hsp104:Q347L
Hsp104:E360K
Hsp104: I193T
Hsp104:L355A
Hsp104:A330V
Hsp104
FUS
PGK

Figure S1. Potentiated Hsp104 NBD1 variants suppress α-syn, TDP-43, and FUS toxicity. Related to Figure 1, 2, and 3. (A) NBD1 variants suppress α-syn, TDP-43, and FUS toxicity in yeast. The indicated Hsp104 variants and relevant controls were transformed in yeast harboring α-syn (left), TDP-43 (center), or FUS (right) genes. The strains were serially diluted five-fold and spotted in duplicate onto glucose (non-inducing) or galactose (inducing) media. **(B)** NBD1 variants do not grossly reduce α-syn, TDP-43, or FUS expression in yeast. Strains in (A) were induced for 5h (FUS and TDP-43) or 8h (α-syn), lysed, and immunoblotted. PGK serves as a loading control.

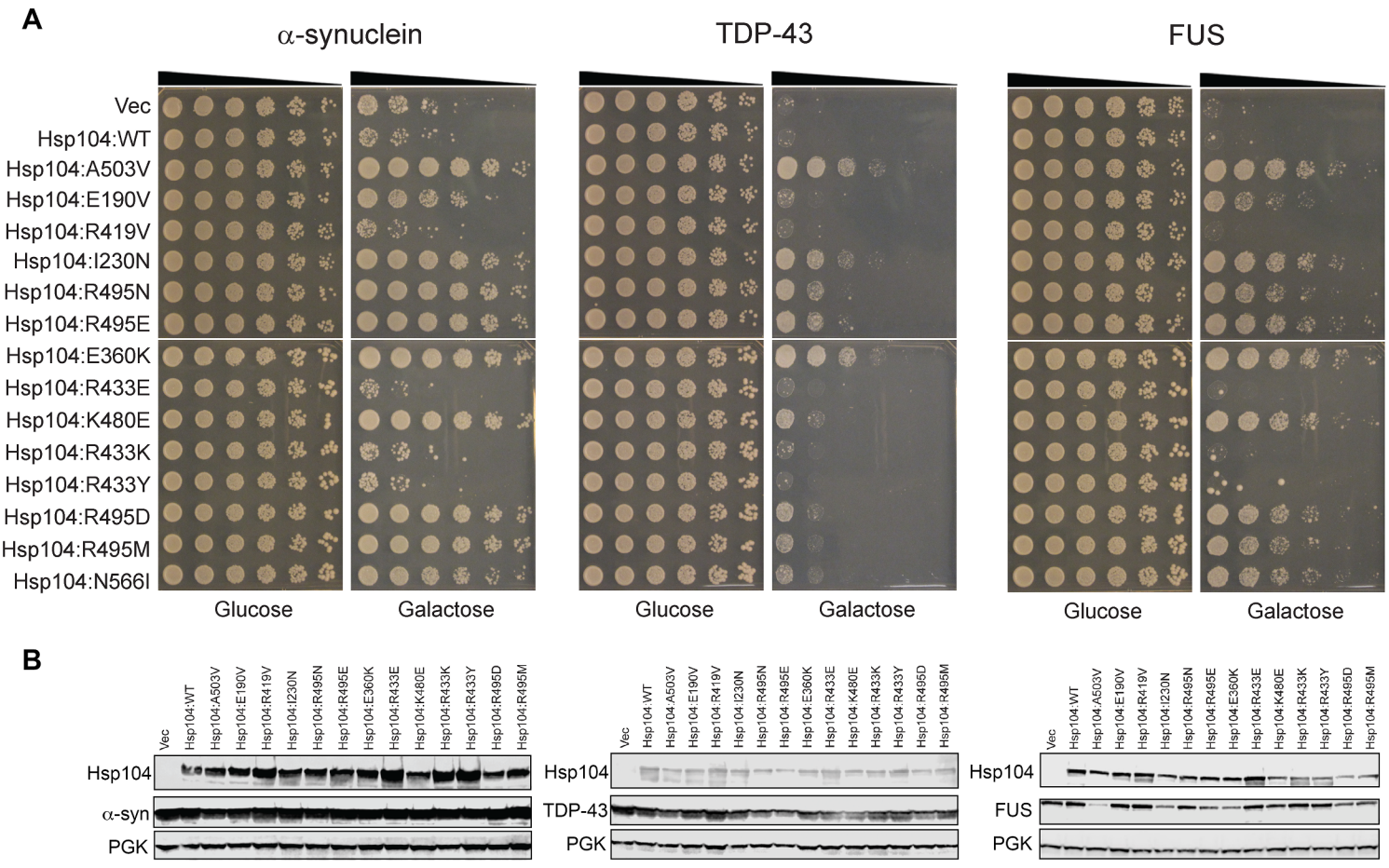

A
α-synuclein
TDP-43
FUS
Vec
Hsp104:WT
Hsp104:A503V
Hsp104:E190V
Hsp104:R419V
Hsp104:I230N
Hsp104:R495N
Hsp104:R495E
Hsp104:E360K
Hsp104:R433E
Hsp104:K480E
Hsp104:R433K
Hsp104:R433Y
Hsp104:R495D
Hsp104:R495M
Hsp104:N566I
Glucose
Galactose
Glucose
Galactose
Glucose
Galactose
B
Vec
Hsp104:WT
Hsp104:A503V
Hsp104:E190V
Hsp104:R419V
Hsp104:I230N
Hsp104:R495N
Hsp104:R495E
Hsp104:E360K
Hsp104:R433E
Hsp104:K480E
Hsp104:R433K
Hsp104:R433Y
Hsp104:R495D
Hsp104:R495M
Hsp104
α-syn
PGK
Hsp104
TDP-43
PGK
Hsp104
FUS
PGK

Figure S2. Potentiated Hsp104 NBD1, NBD2, and MD variants suppress α-syn, TDP-43, and FUS toxicity. Related to Figure 1, 2, and 3. (A) Specific NBD1, MD, and NDB2 variants suppress α-syn, TDP-43, and FUS toxicity in yeast. The indicated Hsp104 variants and relevant controls were transformed in yeast harboring α-syn (left), TDP-43 (center), or FUS (right) genes. The strains were serially diluted five-fold and spotted in duplicate onto glucose (non-inducing) or galactose (inducing) media. Note that R419V, R433E, R433K, and R433Y are unable to rescue α-syn, TDP-43, or FUS toxicity. **(B)** Strains in (A) were induced for 5h (FUS and TDP-43) or 8h (α-syn), lysed, and immunoblotted. PGK serves as a loading control.

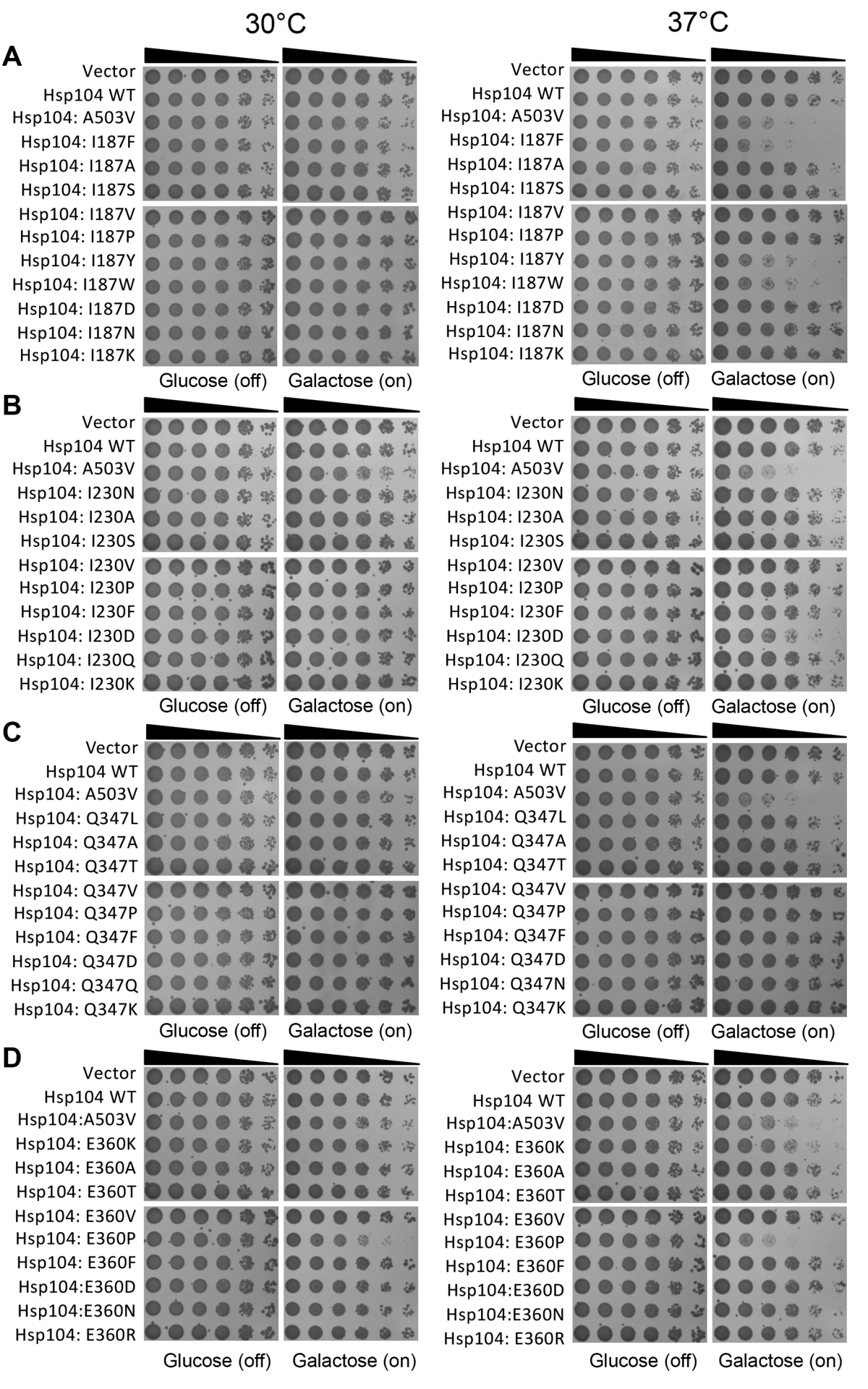

30°C
37°C
A
Vector
Hsp104 WT
Hsp104: A503V
Hsp104: I187F
Hsp104: I187A
Hsp104: I187S
Hsp104: I187V
Hsp104: I187P
Hsp104: I187Y
Hsp104: I187W
Hsp104: I187D
Hsp104: I187N
Hsp104: I187K
Glucose (off)
Galactose (on)
B
Vector
Hsp104 WT
Hsp104: A503V
Hsp104: I230N
Hsp104: I230A
Hsp104: I230S
Hsp104: I230V
Hsp104: I230P
Hsp104: I230F
Hsp104: I230D
Hsp104: I230Q
Hsp104: I230K
Glucose (off)
Galactose (on)
C
Vector
Hsp104 WT
Hsp104: A503V
Hsp104: Q347L
Hsp104: Q347A
Hsp104: Q347T
Hsp104: Q347V
Hsp104: Q347P
Hsp104: Q347F
Hsp104: Q347D
Hsp104: Q347N
Hsp104: Q347K
Glucose (off)
Galactose (on)
D
Vector
Hsp104 WT
Hsp104:A503V
Hsp104: E360K
Hsp104: E360A
Hsp104: E360T
Hsp104: E360V
Hsp104: E360P
Hsp104: E360F
Hsp104:E360D
Hsp104:E360N
Hsp104: E360R
Glucose (off)
Galactose (on)

Figure S3. Hsp104 NBD1 variants typically do not exhibit off-target toxicity. Related to Figure 5. (A-D) Hsp104 variants at the I187 position **(A)**, I230 position **(B)**, Q347 position **(C)**, or E360 position **(D)** were expressed in the 416GAL vector in Δ*hsp104* yeast in the absence of any disease protein. Empty vector, Hsp104, and $Hsp104^{A503V}$ serve as controls. The strains were serially diluted five-fold and spotted in duplicate onto glucose (non-inducing) and galactose (inducing) media and analyzed at both 30°C (left column) and 37°C (right column).

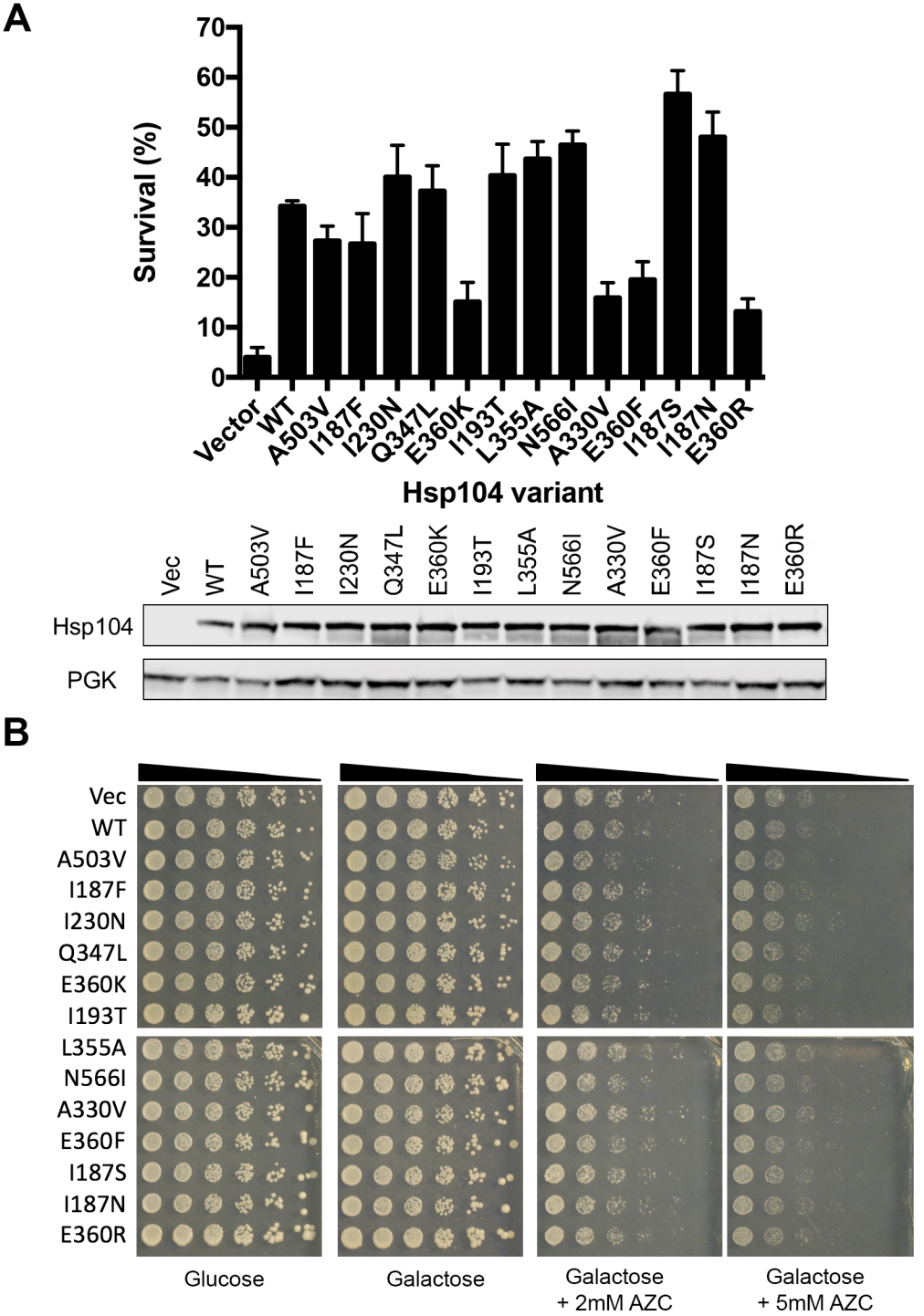
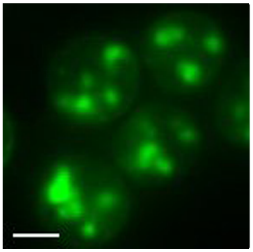
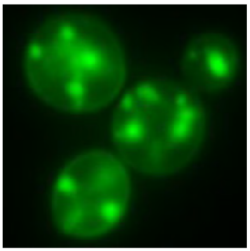

A
Survival (%)
70
60
50
40
30
20
10
0
Vector
WT
A503V
I187F
I230N
Q347L
E360K
I193T
L355A
N566I
A330V
E360F
I187S
I187N
E360R
Hsp104 variant
Vec
WT
A503V
I187F
I230N
Q347L
E360K
I193T
L355A
N566I
A330V
E360F
I187S
I187N
E360R
Hsp104
PGK
B
Vec
WT
A503V
I187F
I230N
Q347L
E360K
I193T
L355A
N566I
A330V
E360F
I187S
I187N
E360R
Glucose
Galactose
Galactose
+ 2mM AZC
Galactose
+ 5mM AZC

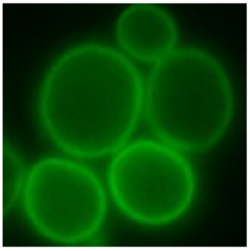
Figure S4. Potentiated Hsp104 NBD1, NBD2, and MD variants confer thermotolerance but do not rescue AZC toxicity. Related to Figure 6. (A) W303aΔ*hsp104* yeast were transformed with the indicated Hsp104 variant or empty vector control. After incubation at 37°C for 30 min to induce Hsp104 expression, cells were heat shocked for 20 min at 50°C, immediately transferred to ice for 2 min, plated, and after a 2-day incubation at 30°C colonies were counted using an aCOLyte automated colony counter. Values represent means ± S.E.M. (n=4). Hsp104 expression was confirmed by immunoblot. PGK serves as a loading control. **(B)** W303aΔ*hsp104* yeast were transformed with the indicated Hsp104 variant or empty vector control. Hsp104 expression was induced in SG-Ura liquid for 6h at 30°C. The strains were serially diluted five-fold and spotted onto glucose (non-inducing), galactose (inducing), or galactose plus AZC (2mM or 5mM) media and analyzed after 3 days at 30°C.



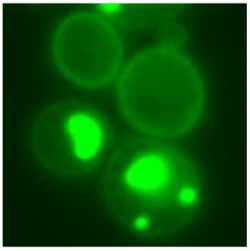
Vec



WT



A503V



N566I

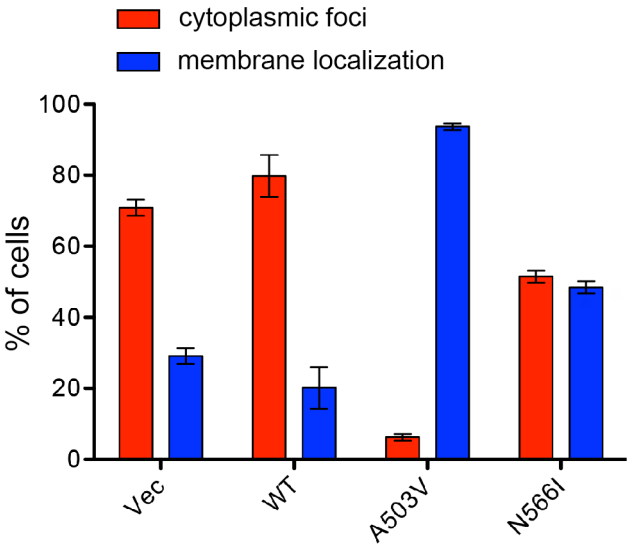
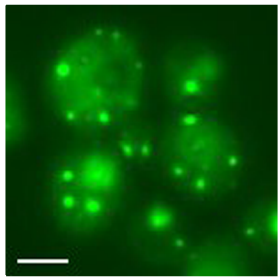
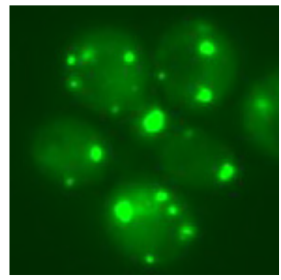


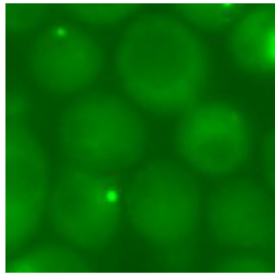
Figure S5. Hsp104^{N566I} rescues α-syn aggregation in yeast and restores α-syn to the plasma membrane. Related to Figure 7. Fluorescence microscopy of W303aΔ*hsp104* yeast cells coexpressing α-syn-YFP and the indicated Hsp104 variant or vector control. α-Syn-YFP and Hsp104 expression were induced for 8h and prepared for fluorescence microscopy. Scale bar, 2.5µm. α-Syn aggregation and localization were quantified by calculating the proportion of cells exhibiting either cytoplasmic aggregates or plasma membrane localization. Values represent means ± SEM (n=3).



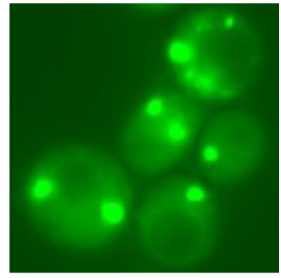
Vec



WT



A503V



N566I

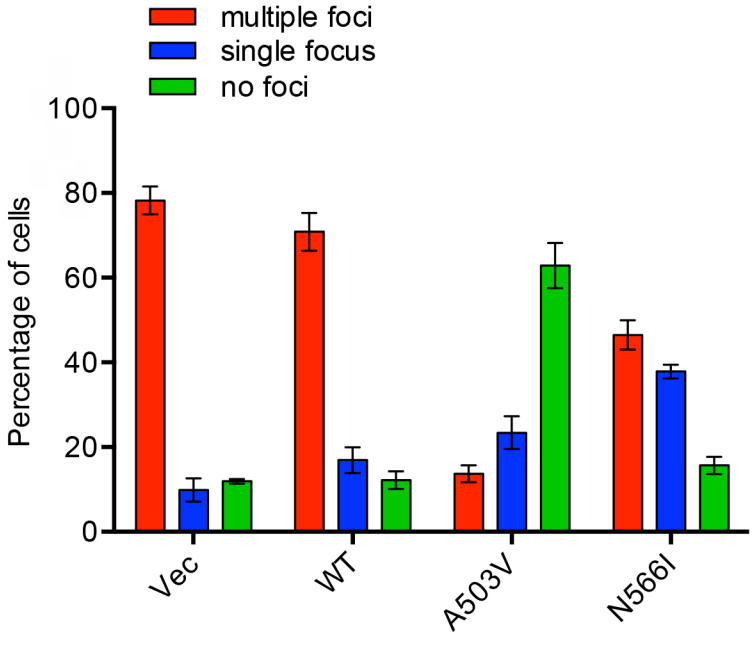


Figure S6. Hsp104^{N566I} rescues FUS aggregation in yeast. Related to Figure 7.
Fluorescence microscopy of W303aΔ*hsp104* yeast cells coexpressing FUS-GFP and the indicated Hsp104 variant or vector control. FUS-GFP and Hsp104 expression were induced for 5h and prepared for fluorescence microscopy. Scale bar, 2.5µm. FUS aggregation was quantified by calculating the proportion of cells exhibiting cytoplasmic aggregates. Values represent means ± SEM (n=3).

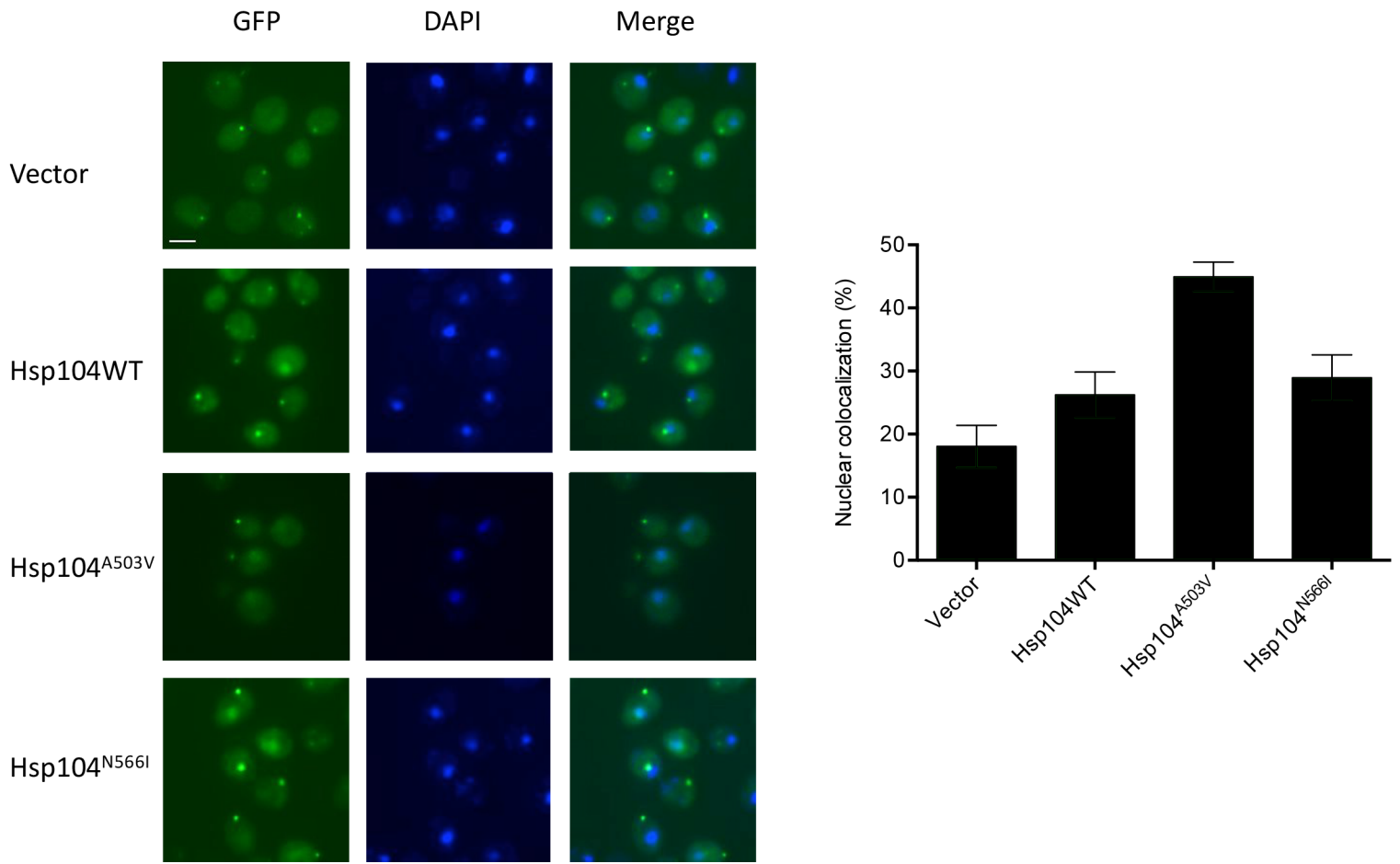

GFP
DAPI
Merge
Vector
Hsp104WT
Hsp104A503V
Hsp104N566I
Nuclear colocalization (%)
0
10
20
30
40
50
Vector
Hsp104WT
Hsp104A503V
Hsp104N566I

Figure S7. Hsp104^{N566I} does not suppress TDP-43 aggregation or restore TDP-43 to the nucleus in yeast. Related to Figure 7. Fluorescence microscopy of W303aΔ*hsp104* yeast cells coexpressing fluorescently tagged TDP-43 and the indicated Hsp104 variants or vector control. Strains were induced for 5h in galactose, fixed, and stained with DAPI (blue) to visualize nuclei. Scale bar, 2.5µm. TDP-43 localization was quantified by calculating the proportion of cells containing colocalized nuclear staining. Values represent means ± SEM (n=3).