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Supplemental information

Tuning Hsp104 specificity

to selectively detoxify α-synuclein

Korrie L. Mack, Hanna Kim, Edward M. Barbieri, JiaBei Lin, Sylvanne Braganza, Meredith E. Jackrel, Jamie E. DeNizio, Xiaohui Yan, Edward Chuang, Amber Tariq, Ryan R. Cupo, Laura M. Castellano, Kim A. Caldwell, Guy A. Caldwell, and James Shorter

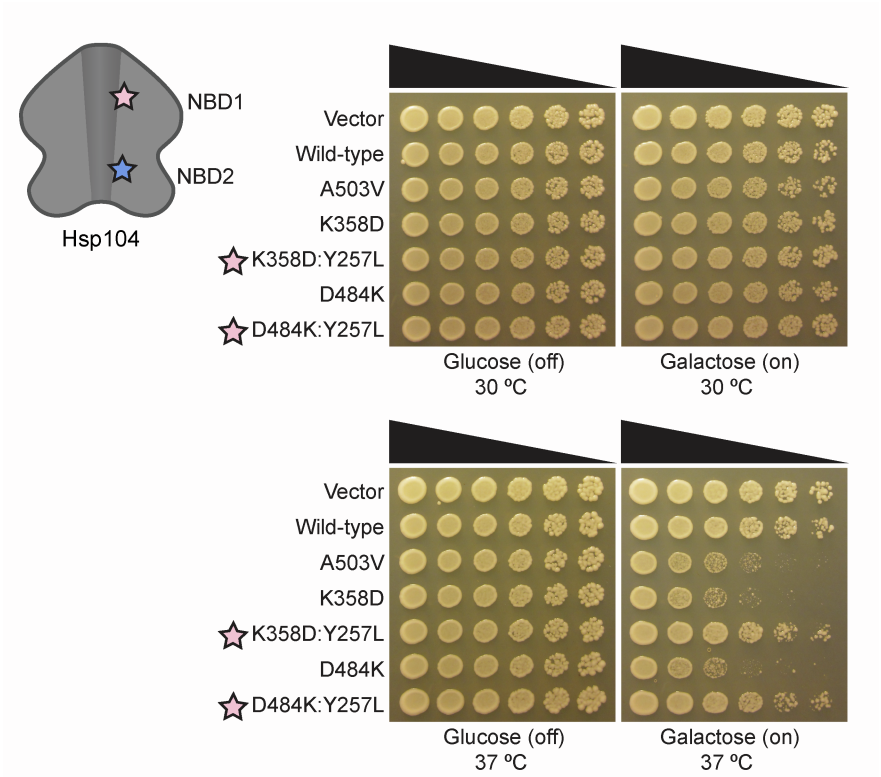

Figure S1. Hsp104^{K358D} and Hsp104^{D484K} are not toxic to yeast at 30°C. Δ*hsp104* yeast were transformed with galactose-inducible Hsp104 variants or an empty vector and spotted onto glucose (uninducing, off) and galactose (inducing, on) media in a five-fold serial dilution. Yeast were incubated at 30°C (top) or 37°C (bottom). Stars indicate substitution to pore loop in NBD1 (pink), NBD2 (purple).
Related to **Figure 2.**

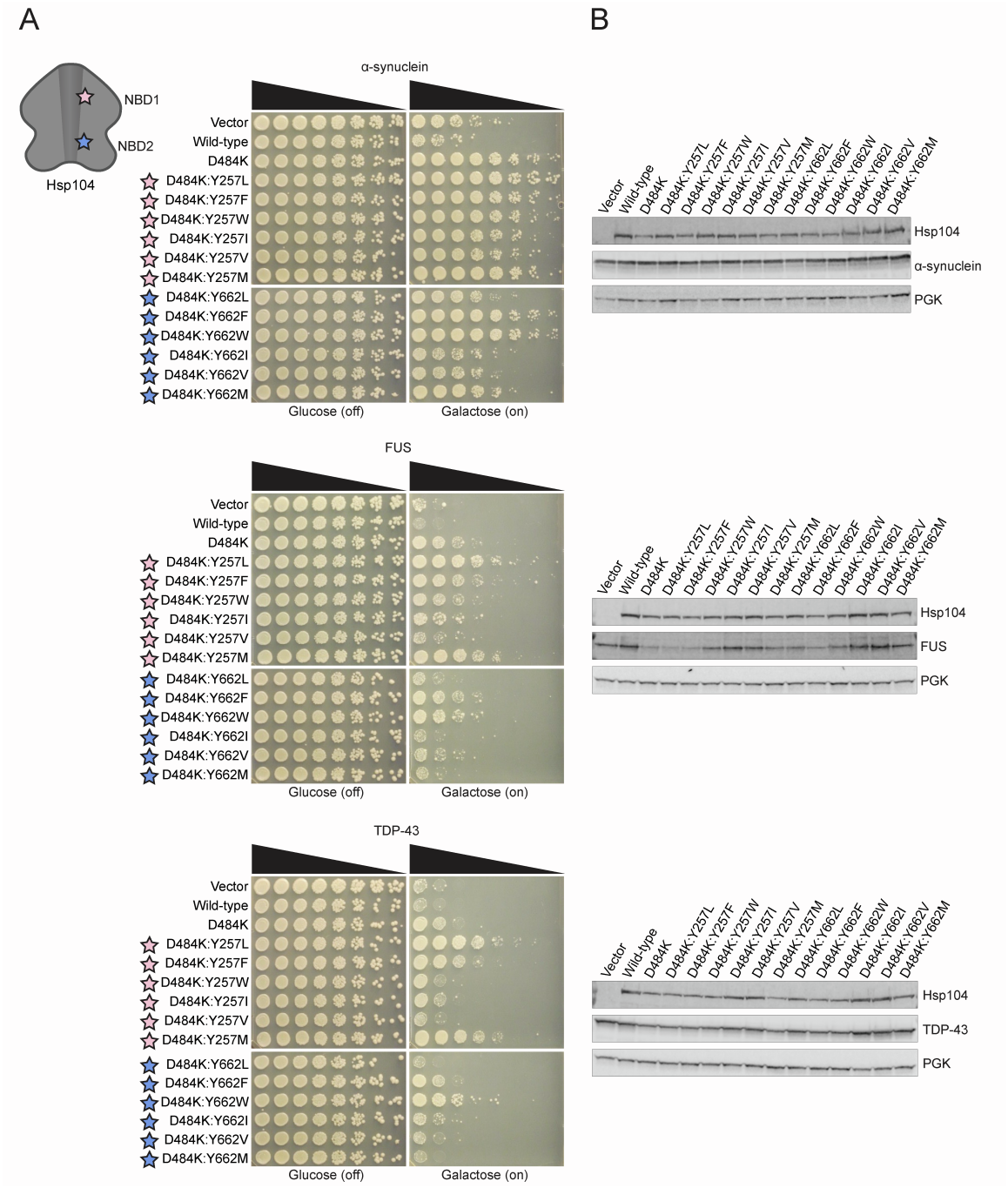

Figure S2. Hsp104^{D484K} pore loop variants have similar toxicity suppression profile to Hsp104^{K358D} variants. (A) Δ*hsp104* yeast integrated with α-syn-YFP (top), FUS (middle), or TDP-43 (bottom) on a galactose-inducible promoter were transformed with Hsp104 variants or an empty vector control. Yeast were spotted onto glucose (uninducing, off) and galactose (inducing, on) media in a five-fold serial dilution. Stars indicate substitution to pore loop in NBD1 (pink), NBD2 (purple). **(B)** Integrated strains from (A*)* were induced in the presence of Hsp104 variants or empty vector control for 5 hours (FUS, TDP-43) or 8 hours (α-syn). Yeast were lysed and lysates visualized via Western blot. 3-Phosphoglycerate kinase (PGK) is a loading control.

Related to **Figure 2.**

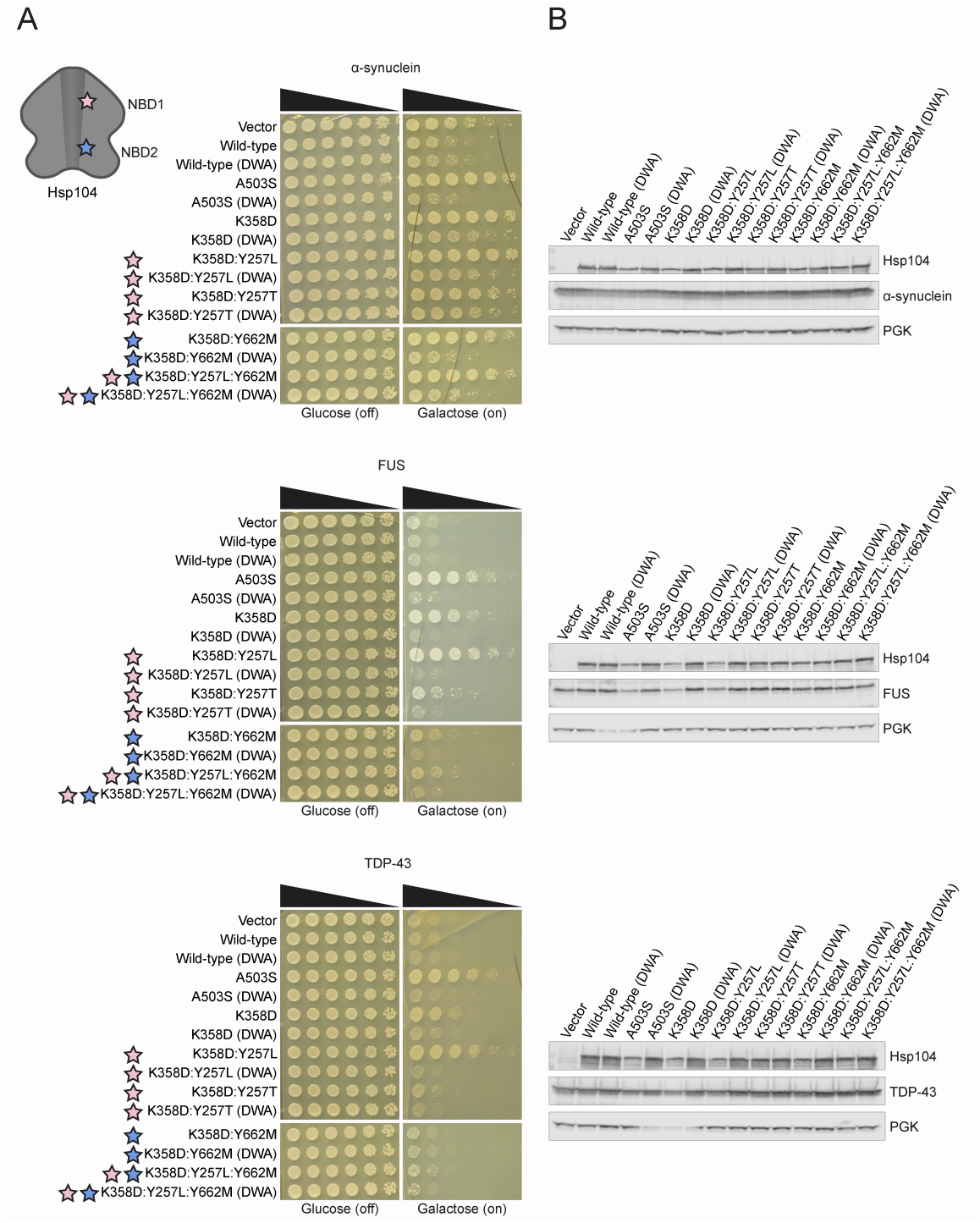

Figure S3. Hsp104^{DWA} pore-loop variants have diminished substrate toxicity suppression. (A) Δ*hsp104* yeast integrated with α-syn-YFP (top), FUS (middle), or TDP-43 (bottom) on a galactose-inducible promoter were transformed with Hsp104 variants or an empty vector control. Yeast were spotted onto glucose (uninducing, off) and galactose (inducing, on) media in a five-fold serial dilution. Stars indicate substitution to pore loop in NBD1 (pink), NBD2 (purple). **(B)** Integrated strains from (A) were induced in the presence of Hsp104 variants or empty vector control for 5 hours

(FUS, TDP-43) or 8 hours (α-syn). Yeast were lysed and lysates visualized via Western blot. 3-Phosphoglycerate kinase (PGK) is a loading control.
Related to **Figures 2, 3, and 4.**

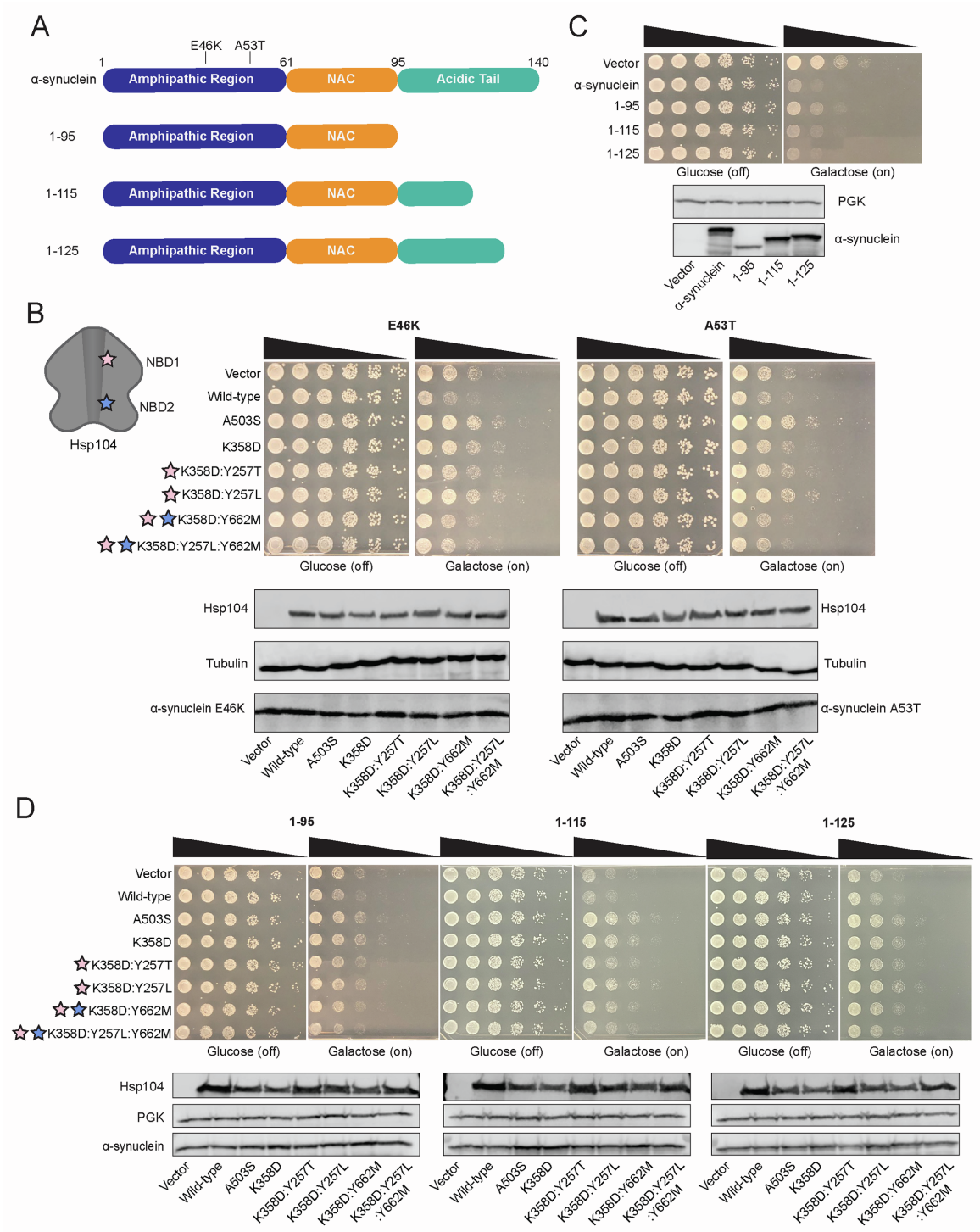

Figure S4. α-Syn-specific Hsp104 variants do not mitigate toxicity of α-syn^{E46K}, α-syn^{A53T}, or α-syn$^{1-95}$. (A) Domain architecture of α-syn showing the location of the amphipathic region (blue), non-amyloid component (NAC, orange), and acidic region (green). The PD-linked E46K and A53T mutations are in the amphipathic region. The C-terminal truncations α-syn$^{1-95}$, α-syn$^{1-115}$, and α-syn$^{1-125}$ are also shown. **(B)** Top, Δ*hsp104* yeast transformed with α-syn^{E46K} (left) or α-syn^{A53T} (right) on a galactose-

inducible promoter were transformed with Hsp104 variants or an empty vector control. Yeast were spotted onto glucose (uninducing, off) and galactose (inducing, on) media in a five-fold serial dilution. Stars indicate substitution to pore loop in NBD1 (pink), NBD2 (purple). Bottom, yeast strains were induced in the presence of Hsp104 variants or empty vector control for 8 hours. Yeast lysates were generated and processed for Western blot. 3-Phosphoglycerate kinase (PGK) is a loading control. **(C)** Top, Δ*hsp104* yeast transformed with empty vector, or galactose-inducible α-syn, α-syn$^{1-95}$, α-syn$^{1-115}$, or α-syn$^{1-125}$. Yeast were spotted onto glucose (uninducing, off) and galactose (inducing, on) media in a five-fold serial dilution. Bottom, yeast strains were induced for 8 hours. Yeast lysates were generated and processed for Western blot. 3-Phosphoglycerate kinase (PGK) is a loading control. **(D)** Top, Δ*hsp104* yeast transformed with α-syn$^{1-95}$ (left), α-syn$^{1-115}$ (middle), or α-syn$^{1-125}$ (right) on a galactose-inducible promoter were transformed with Hsp104 variants or an empty vector control. Yeast were spotted onto glucose (uninducing, off) and galactose (inducing, on) media in a five-fold serial dilution. Stars indicate substitution to pore loop in NBD1 (pink), NBD2 (purple). Bottom, yeast strains were induced in the presence of Hsp104 variants or empty vector control for 8 hours. Yeast lysates were generated and processed for Western blot. 3-Phosphoglycerate kinase (PGK) is a loading control.
Related to **Figures 2**, **3, and 4.**

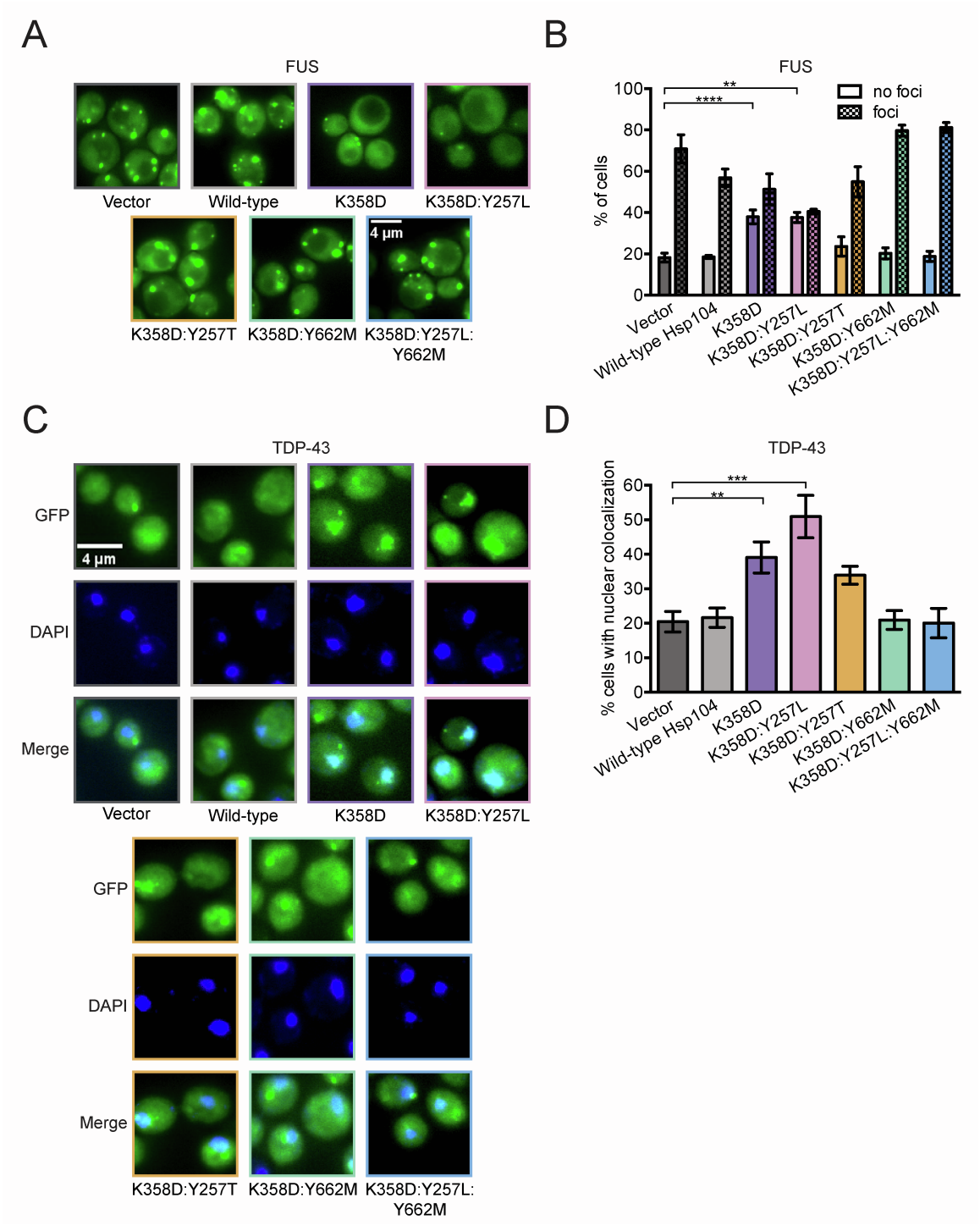

Figure S5. α-Syn-specific Hsp104 variants do not significantly act on FUS or TDP-43 aggregates. (A) Representative fluorescence microscopy images of Δ*hsp104* yeast integrated with FUS-GFP and transformed with Hsp104 variants or an empty vector control. Bar, 4µm. **(B)** FUS-GFP aggregation in yeast was quantified by counting the number of cells with FUS-GFP foci or no FUS-GFP foci. Values represent means ± SEM (n = 3-6). ***P*<0.01, *****P*<0.0001; One-way ANOVA with Dunnett's *post-hoc* test. **(C)** Representative fluorescence microscopy images of Δ*hsp104* yeast integrated with TDP-

43-GFPS11 and transformed with Hsp104 variants or an empty vector control. Bar, 4μm. **(D)** TDP-43-GFPS11 localization in yeast was quantified by counting the number of cells with nuclear-localized TDP-43-GFPS11. Values represent means ± SEM (n = 3-6). ***P*<0.01, ****P*<0.001; One-way ANOVA with Dunnett's *post-hoc* test.
Related to **Figure 5**.

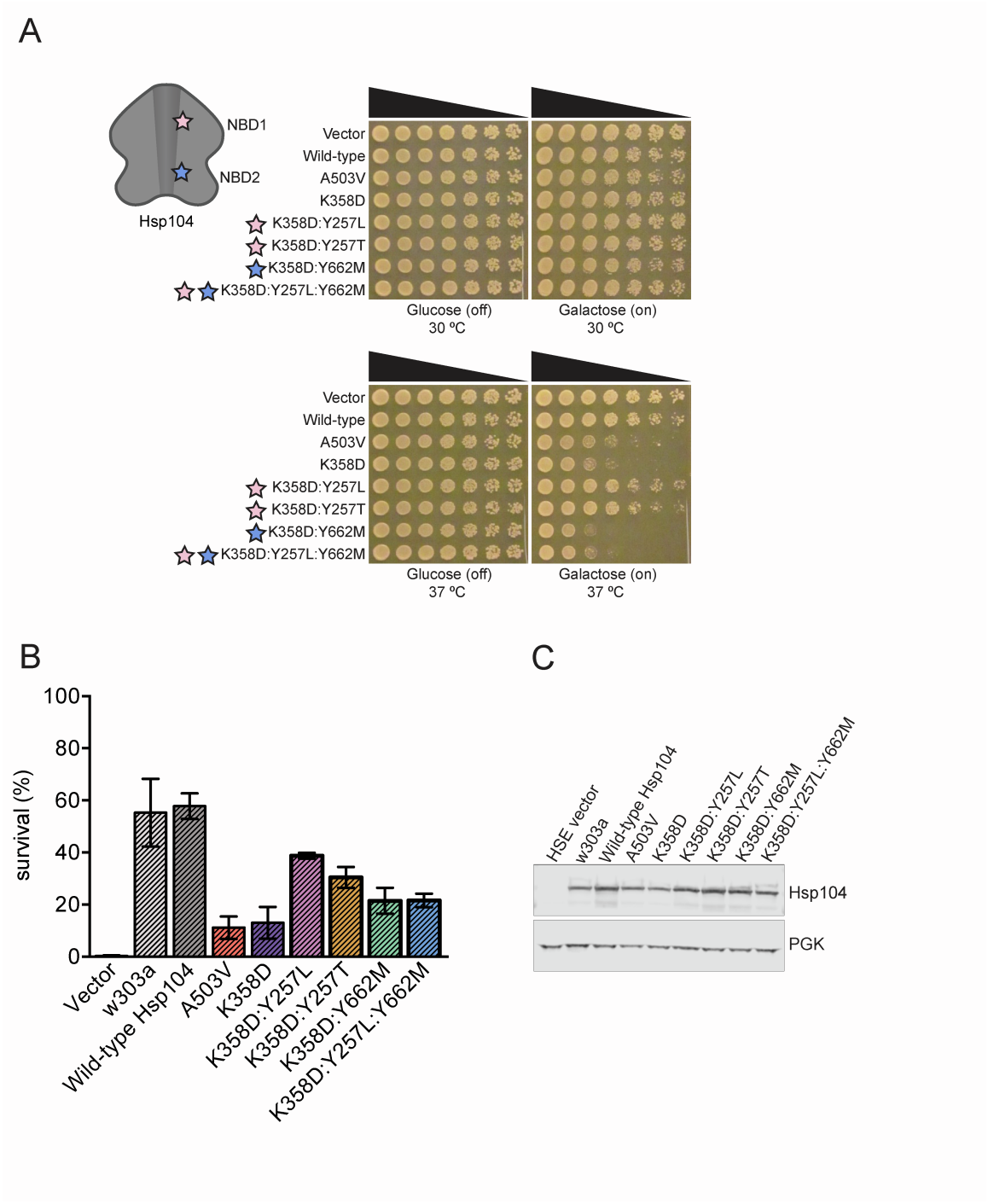

Figure S6. α-Syn-specific Hsp104 variants have inherently different toxicity in yeast and confer thermotolerance to different extents. (A) Δ*hsp104* yeast were transformed with galactose-inducible Hsp104 variants or an empty vector and spotted onto glucose (uninducing, off) and galactose (inducing, on) media in a five-fold serial dilution. Yeast were incubated at 30°C (top) or 37°C (bottom). Stars indicate substitution to pore loop in NBD1 (pink), NBD2 (purple). **(B)** Δ*hsp104* yeast were transformed with Hsp104 variants or an empty vector under the HSE promoter. Yeast were grown in selective media for 4 hours, and cultures were normalized and incubated at 37°C for 30

minutes to induce Hsp104 variant expression. Cultures were then heat-shocked for 0 or 30 minutes and plated on selective media. Plates were incubated at 30°C for ~ 2 days and colonies counted. Wild-type w303a yeast expressing endogenous Hsp104 was included as a control. Values represent means ± SEM (n=3). **(C)** Yeast strains from (B) were grown in selective media for 4 hours. Cultures were normalized and grown for 30 minutes at 37°C to induce Hsp104 variant expression. Yeast were lysed, and lysates visualized via Western blot. 3-Phosphoglycerate kinase (PGK) is a loading control. Related to **Figure 6**.

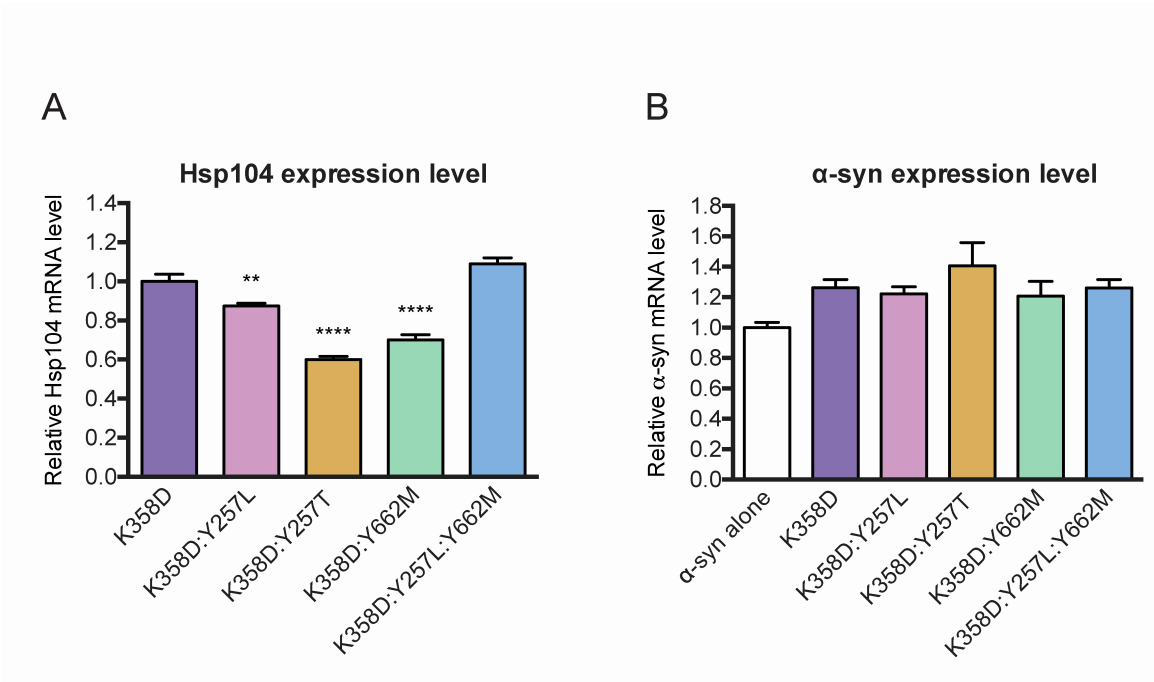

Figure S7. Confirmation of transcription levels of HSP104 variants and α-syn by qRT-PCR. (A and B) Transgenic *C. elegans* co-overexpressing Hsp104 variants + α-syn within DA neurons were subjected to qRT-PCR. **(A)** Hsp104 transgenic lines exhibit variable transcription levels. In this graph, all transgenic lines are displayed with their HSP104 mRNA levels relative to $Hsp104^{K358D}$. Although Hsp104 variants $Hsp104^{K258D:Y257T}$ and $Hsp104^{K358D:Y662M}$ have lower expression levels than $Hsp104^{K358D}$, they show greater protective activity against α-syn toxicity in Figure 7. Values represent means ± SEM. ***P*<0.01, *****P*<0.0001; One-way ANOVA with Dunnett's *post-hoc* test (compared to $Hsp104^{K358D}$ control). **(B)** In this graph, all transgenic lines are displayed with their α-syn mRNA levels relative to *C. elegans* expressing α-syn without Hsp104. The overexpression of Hsp104 variants does not affect the transcription of α-syn, indicating that the observed neuroprotection is not caused by reduced α-syn expression. Values represent means ± SEM. ***P*<0.01, *****P*<0.0001; One-way ANOVA with Dunnett's *post-hoc* test (compared to a-syn alone control).
Related to **Figure 7**.