

## Supplementary Materials

### **Disparate mutations confer therapeutic gain of Hsp104 function**

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**Supplementary Figure 1. Hsp110 is not required for Hsp104-mediated suppression of TDP-43 toxicity.** BY4741 WT,  $\Delta$ *sse1*, and  $\Delta$ *sse2* yeast strains were transformed with 413GAL TDP-43 and the indicated Hsp104 missense mutants or control. Strains were serially diluted fivefold and spotted on glucose (off) or galactose (on) media.

**Supplementary Figure 2. Deletion of MD motif 2, helix 3, or helix 4 potentiates Hsp104.** W303 $\Delta$ *hsp104* yeast integrated with pAG303GAL-FUS (left) or pAG303GAL- $\alpha$ -syn + pAG304GAL- $\alpha$ -syn (right) was transformed with the indicated 416GAL-Hsp104 deletion construct, empty plasmid, Hsp104<sup>WT</sup>, or Hsp104<sup>A503V</sup>. Strains were serially diluted fivefold and spotted on glucose (off) or galactose (on) media.

**Supplementary Table 1. Potentiating Hsp104 mutations.**

The mutations that potentiate Hsp104 activity, their location, and their identification are listed.

<b>Potentiating Mutations</b>	<b>Middle domain helix</b>	<b>Source</b>
V426L/G	1	Jackrel et al., 2014
A437W	Distal loop	Jackrel et al., 2014
K451E	2	This paper
R465G	2	This paper
Y466S	2	This paper
E469D	2	This paper
K470Q	2	This paper
E474V	2	This paper
K480E	2	This paper
L483S	2	This paper
A493T	2	This paper
D498V	3	Jackrel et al., 2014
A503X*	3	Jackrel et al., 2014
D504V	3	Jackrel et al., 2014
Y507A/C/D/V	3	Jackrel et al., 2014
P511A	4	This paper
I513F	4	This paper
G532S	4	This paper
M536K	4	This paper
N539L/E/D/G/K	Small domain NBD1	Jackrel et al., 2014
A430V-K514E	Distal loop and 4	This paper
A430V-N534I	Distal loop and 4	This paper
A430V-K514E-N534I	Distal loop and 4	This paper

\*X = any amino acid except A or P

**Supplementary Table 2, Mutations in the Hsp104 MD that do not potentiate activity.**

The MD mutations do not that potentiate Hsp104 activity, their location, and their identification are listed.

<b>Non-potentiating Mutations</b>	<b>Middle domain helix</b>	<b>Source</b>
Q425R	1	This paper
E427K	1	This paper
A430V	Distal loop	This paper
E432D	Distal loop	This paper
S439T	Distal loop	This paper
L447I	2	This paper
L455S	2	This paper
E457K	2	This paper
L459S	2	This paper
R463K	2	This paper
K470R	2	This paper
K481R	2	This paper
D484G	2	This paper
N488I	2	This paper
T499V	3	Jackrel et al., 2014
A500V	3	Jackrel et al., 2014
T501V	3	Jackrel et al., 2014
A502V	3	Jackrel et al., 2014
L505V	3	Jackrel et al., 2014
R506V	3	Jackrel et al., 2014
K514E	4	This paper
Q523R	4	This paper
E528G	4	This paper
A531V	4	This paper
N534I	4	This paper

Fig. S1. Jackrel et al.

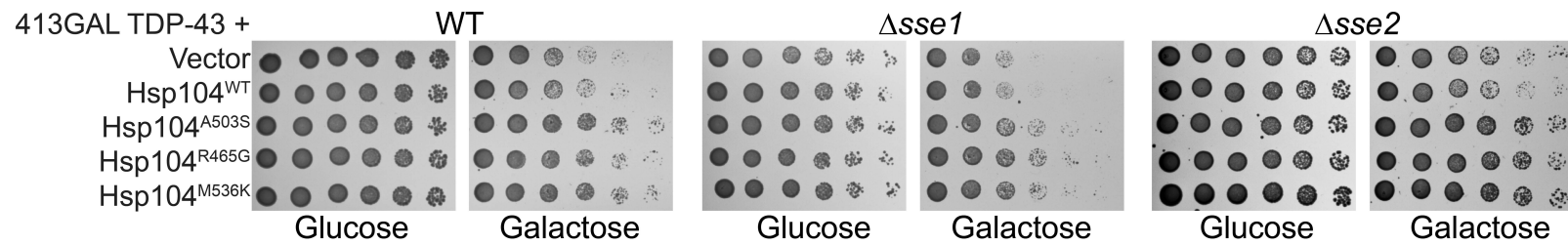


Fig. S2. Jackrel et al.

