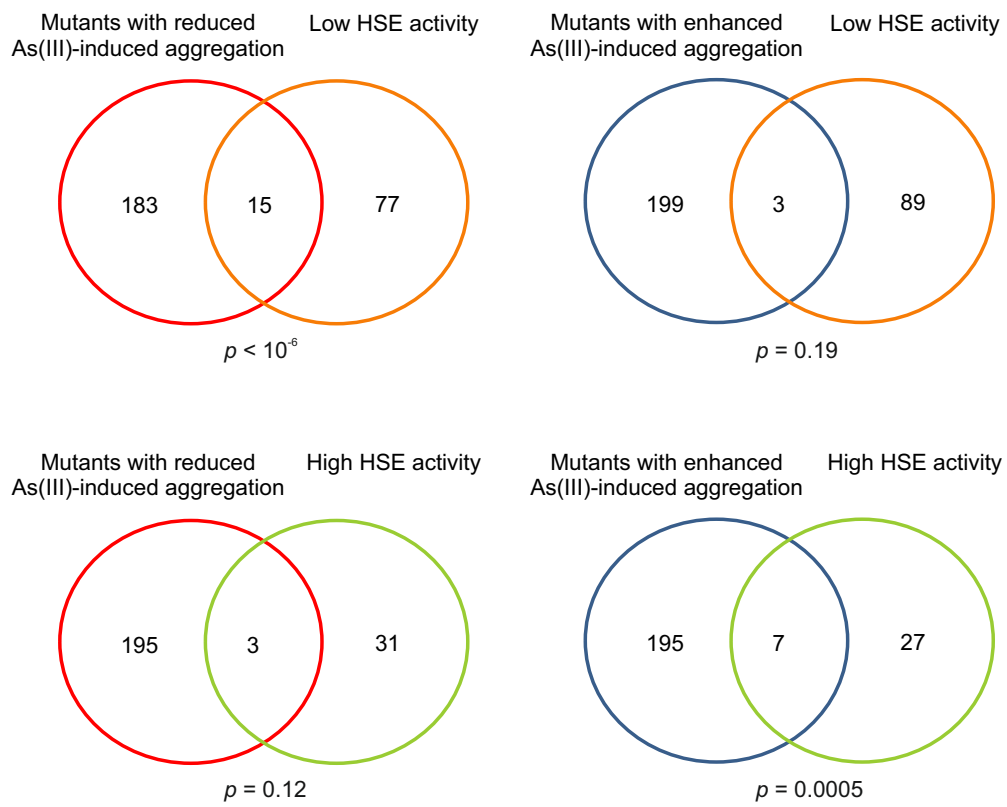
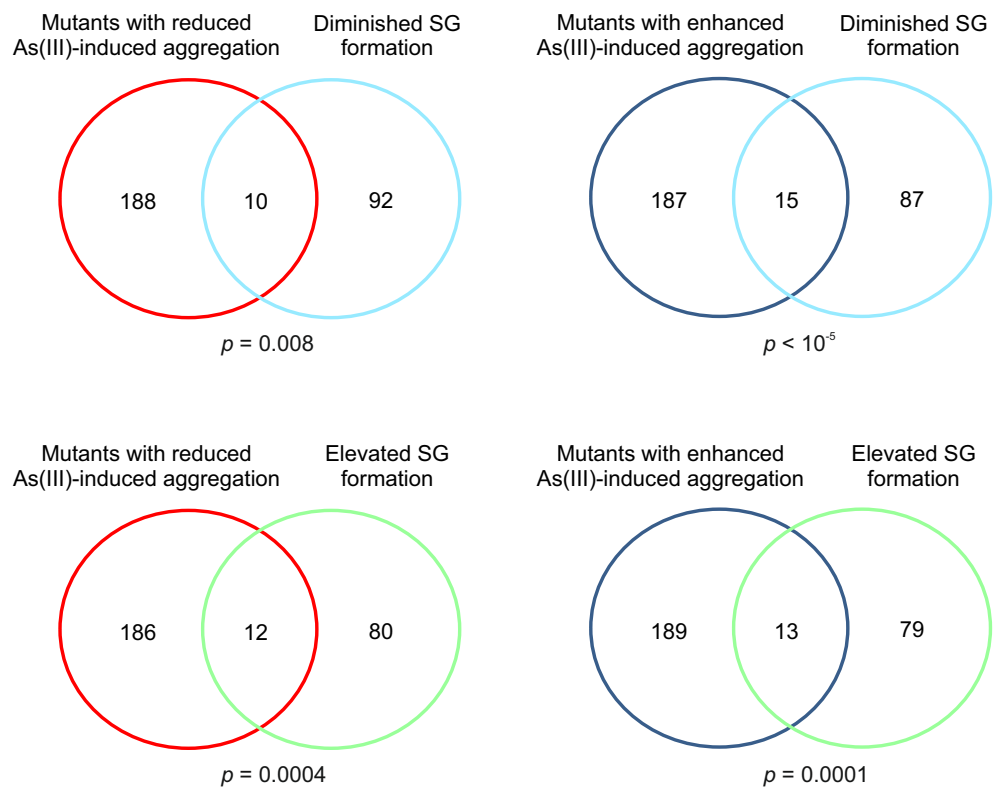


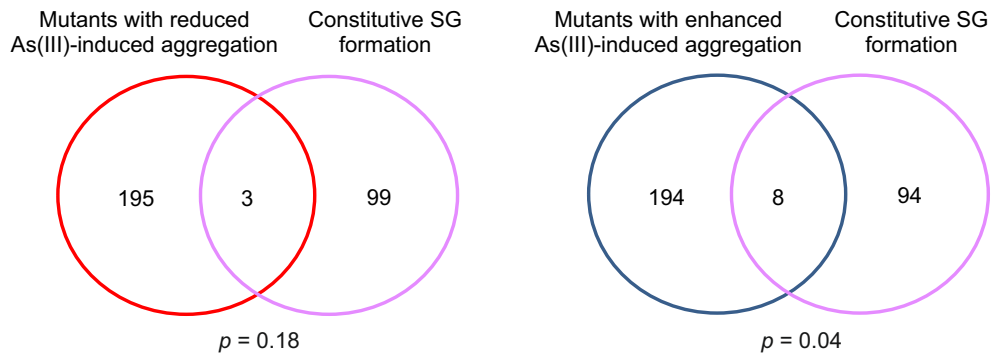
### S1A



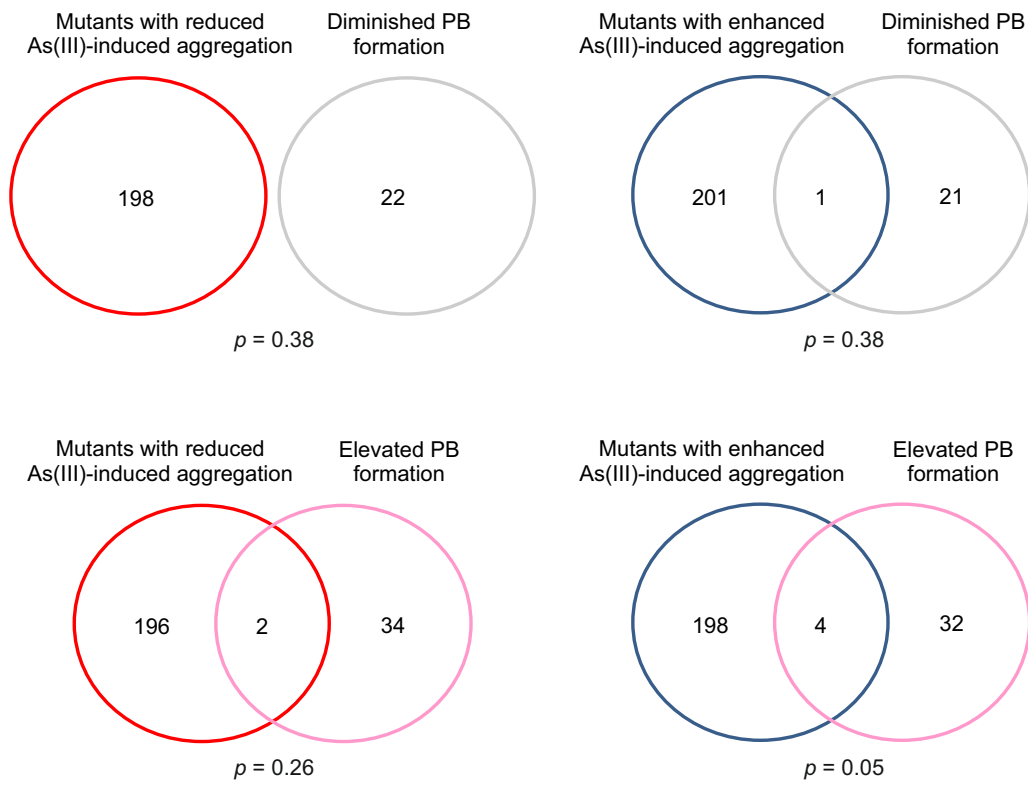
### S1B



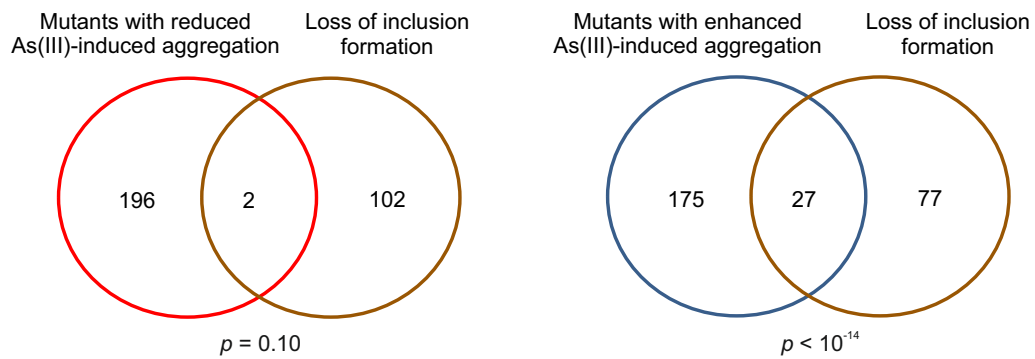
### S1C



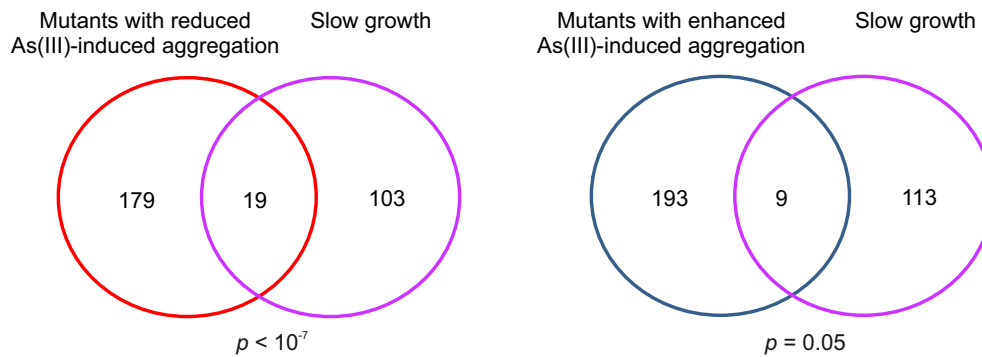
### S1D



### S1E



S1F



**Figure S1. Correlation between protein aggregation in the presence of As(III) and other genome-wide data-sets.**

**(A)** Correlation between protein aggregation in the presence of As(III) and HSE (Heat Shock Element) reporter activity. The Venn diagrams show the overlap between mutants with reduced or enhanced levels of protein aggregation and mutants having either high or low HSE activity (Brandman et al., 2012).

**(B,C)** Correlation between protein aggregation in the presence of As(III) and SG (stress granule) formation. The Venn diagrams show the overlap between mutants with reduced or enhanced levels of protein aggregation and mutants having high or low SG formation **(B)** or constitutive SG formation **(C)** (Buchan et al., 2013; Yang et al., 2014).

**(D)** Correlation between protein aggregation in the presence of As(III) and PB (Processing Body) formation. The Venn diagrams show the overlap between mutants with reduced or enhanced levels of protein aggregation and mutants with either elevated or diminished PB formation (Buchan et al., 2013).

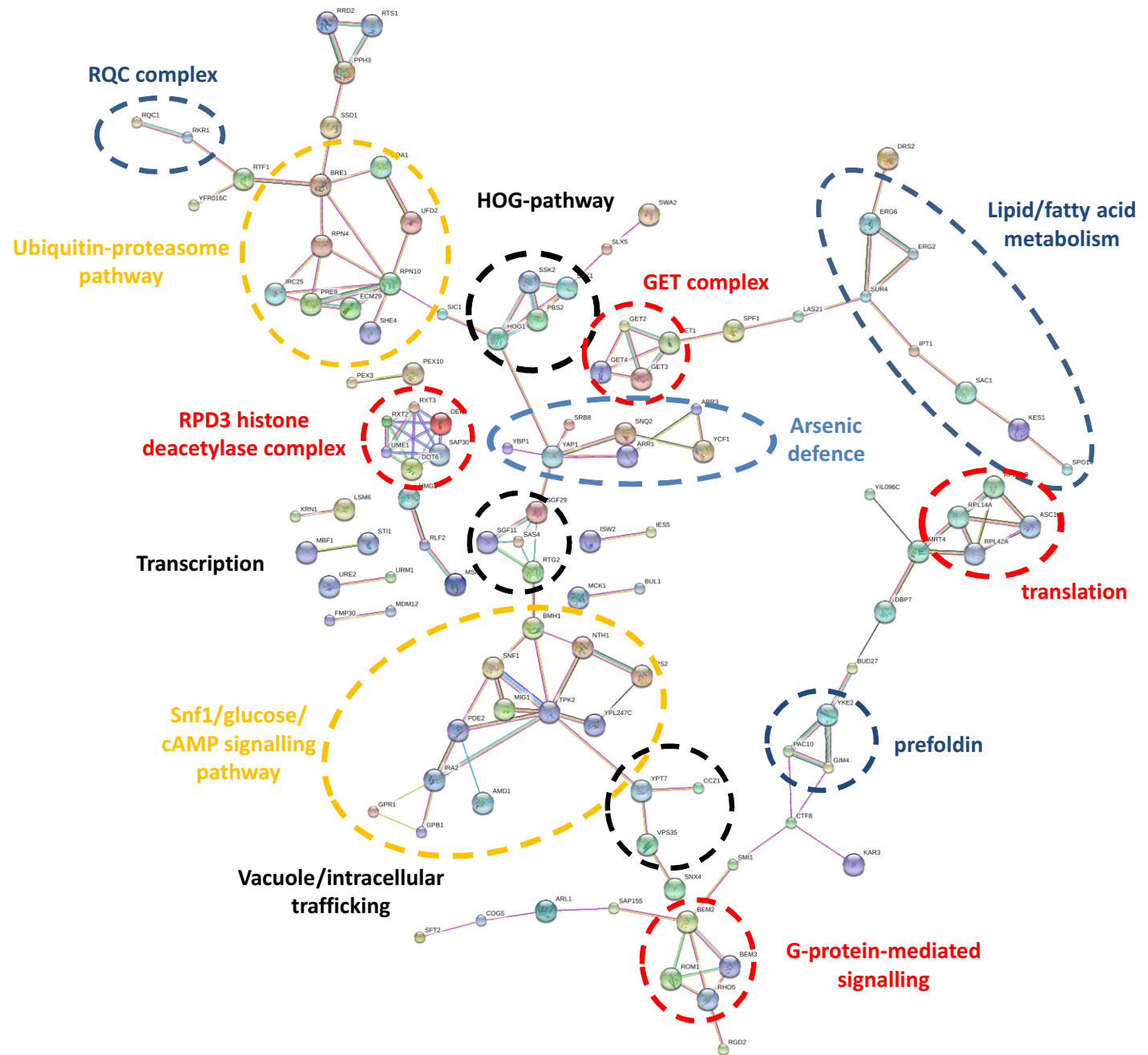
**(E)** Correlation between protein aggregation in the presence of As(III) and mutants with defects in assembly of large inclusions during heat stress. The Venn diagrams show the overlap between mutants with reduced or enhanced levels of protein aggregation and of mutants with defects in assembly of large inclusions during heat stress (Babazadeh et al., 2019).

**(F)** Correlation between protein aggregation in the presence of As(III) and slow growth in minimal synthetic complete growth medium. The Venn diagrams show the overlap between mutants with reduced or enhanced levels of protein aggregation and mutants that grow slowly (Zackrisson et al., 2016).

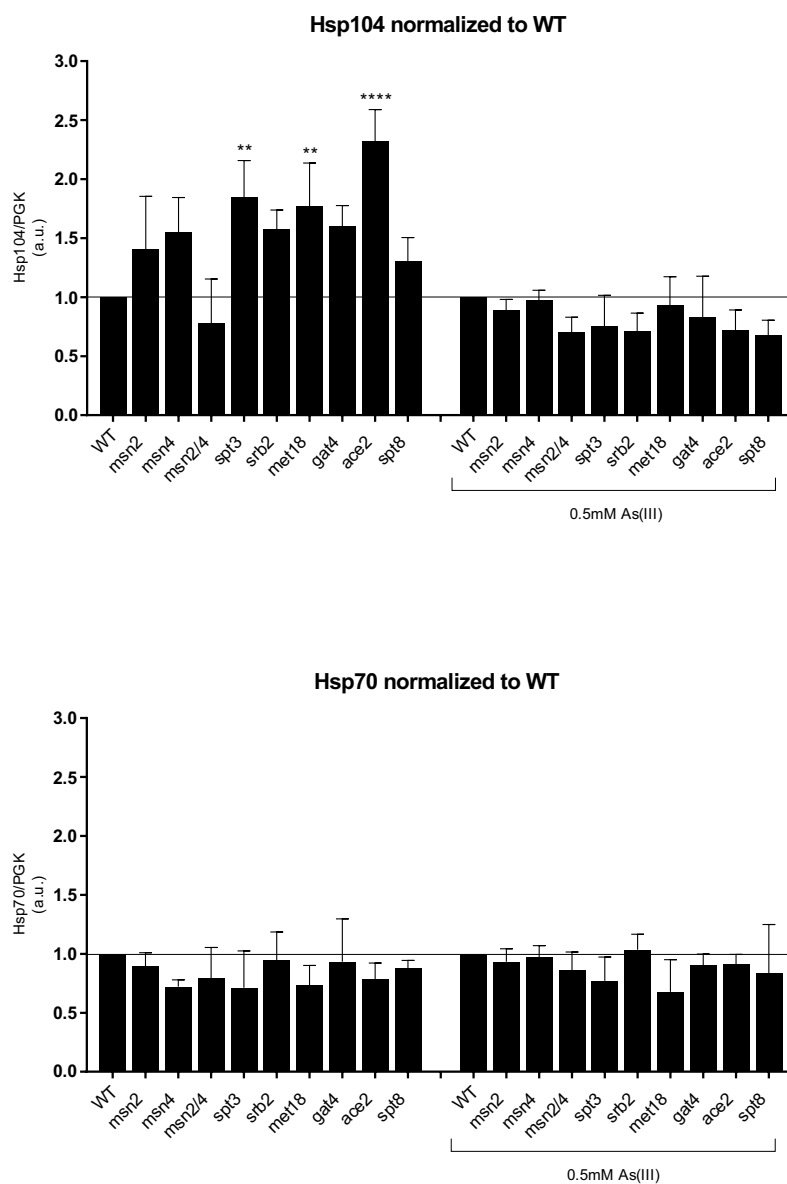
Significance of the overlaps was calculated by the hyper-geometric test and the corresponding  $p$ -values are indicated.



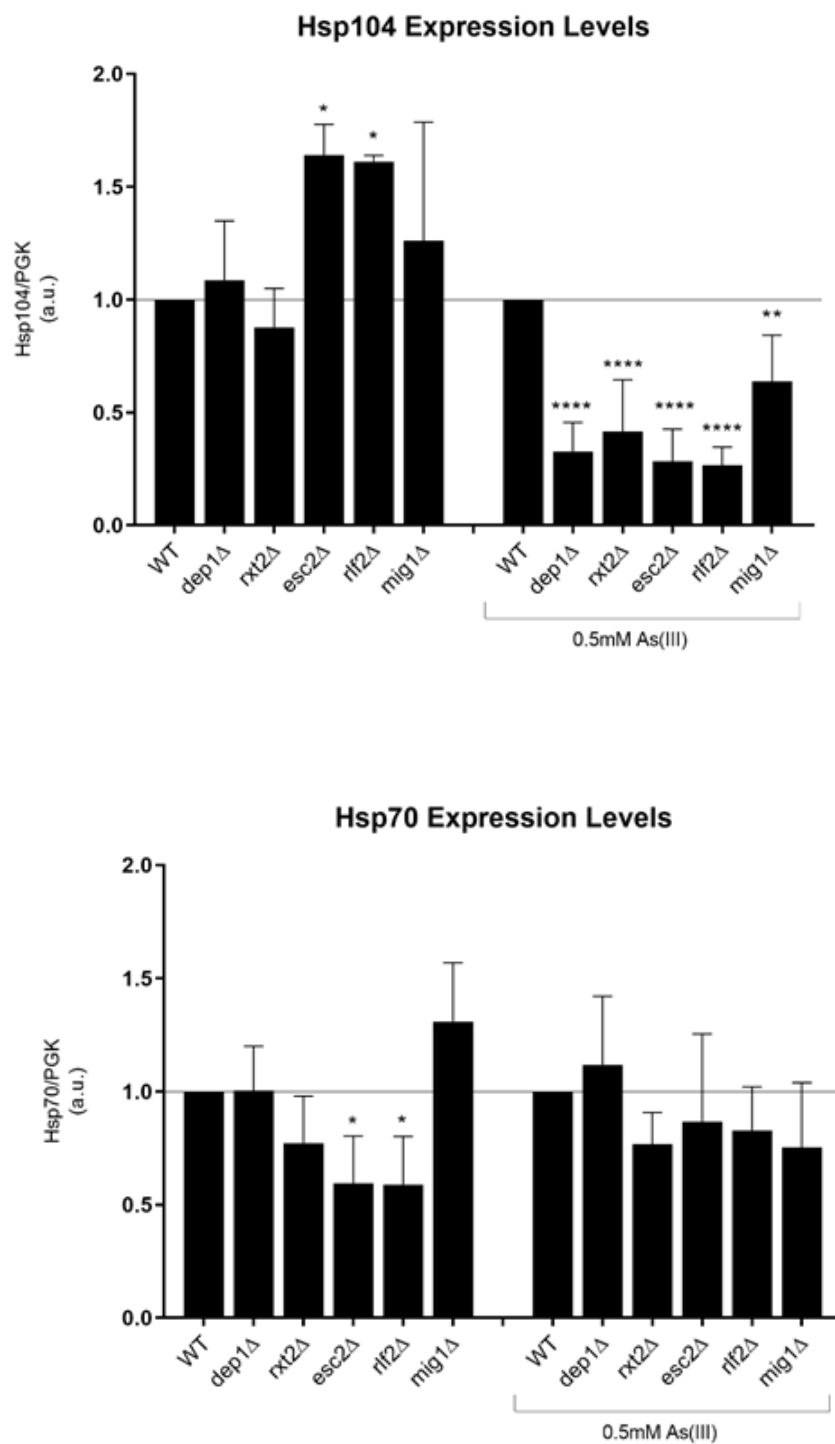




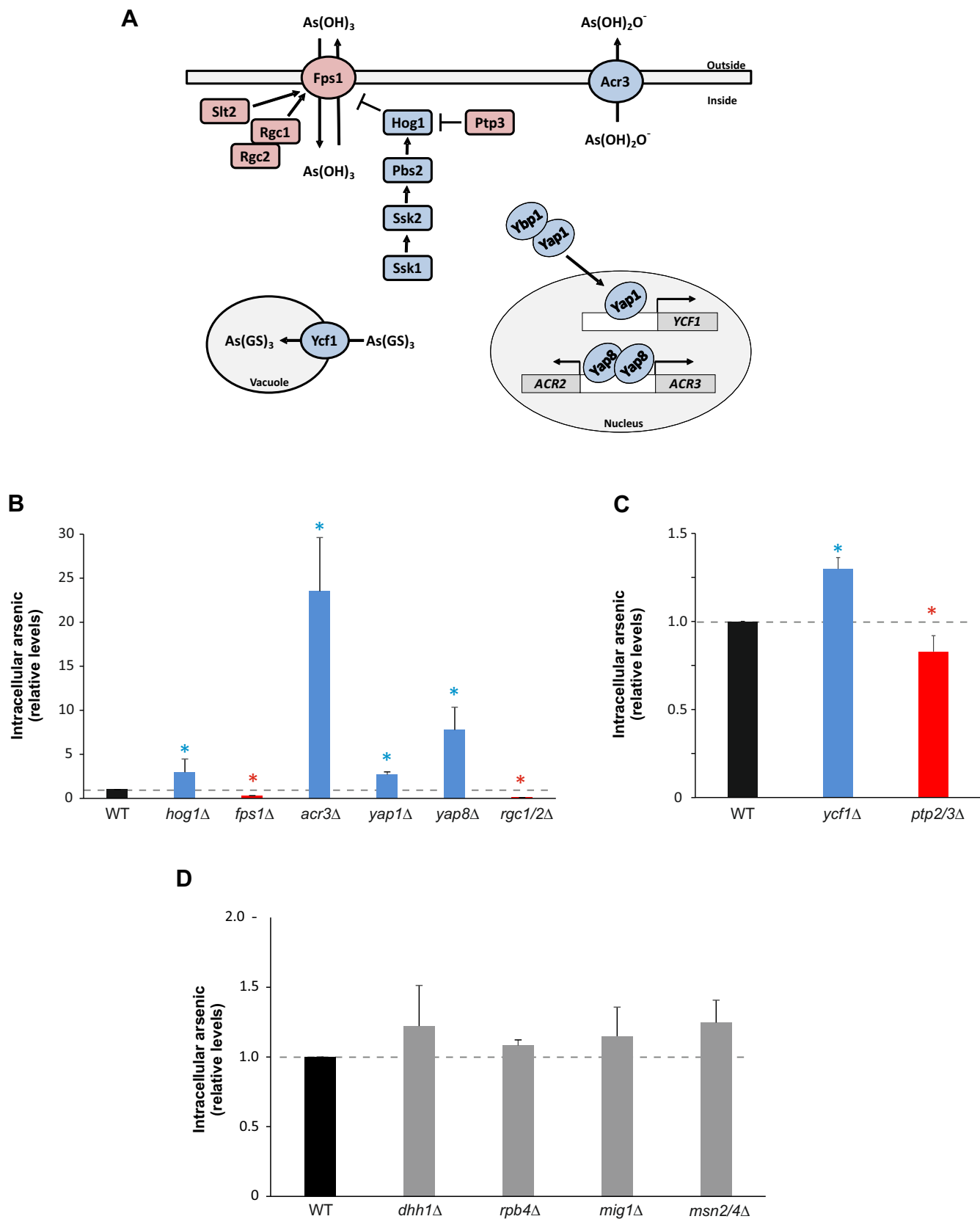
**Figure S3. Protein-protein interaction networks among hits with enhanced aggregation levels.** Protein-protein interaction networks were constructed and visualized using the STRING database (Snel et al., 2000; Szklarczyk et al., 2017) (evidence view, confidence score set to 0.9 (highest confidence)). Only connected proteins are shown.



**Figure S4. Hsp104 and Hsp70 protein levels in wild type cells and deletion mutants lacking positive regulators of transcription.** Hsp104 and Hsp70 protein levels in wild type cells and deletion mutants lacking positive regulators of transcription before and after 1 h of 0.5 mM As(III) treatment. Hsp104 and Hsp70 levels were normalized to the levels of the loading control Pgk1. Protein levels were normalized to the wild type indicated by the horizontal line. Error bars represent standard deviations (S.D.) from four independent biological replicates (n=4). Statistical analyses were performed by One-Way ANOVA with Dunnett Test (\*\* $p < 0.005$ , \*\*\*\* $p < 0.00005$ )



**Figure S5. Hsp104 and Hsp70 protein levels in wild type cells and deletion mutants lacking negative regulators of transcription.** Hsp104 and Hsp70 protein levels in wild type cells and deletion mutants lacking negative regulators of transcription before and after 1 h of 0.5 mM As(III) treatment. Hsp104 and Hsp70 levels were normalized to the levels of the loading control Pgk1. Protein levels were normalized to the wild type indicated by the horizontal line. Error bars represent standard deviations (S.D.) from six independent biological replicates (n=6). Statistical analyses were performed by One-Way ANOVA with Dunnett Test (\*\* $p < 0.005$ , \*\*\*\* $p < 0.00005$ )



**Figure S6. Correlation intracellular arsenic and protein aggregation. (A)** Cartoon showing proteins that regulate arsenic transport and intracellular arsenic levels. Loss of proteins coloured red reduces protein aggregation whilst loss of blue-coloured proteins enhances protein aggregation levels during As(III) exposure. **(B,C,D)** Intracellular arsenic. Cells were exposed to As(III) for 1 h, and intracellular arsenic levels were determined. The data shown represents the average of at least two independent biological replicates ( $n=2$ ) and the error bars represent the S.D. Red bars represent mutants with less intracellular arsenic whilst blue bars represent mutants with more intracellular arsenic compared to wild type cells. Grey bars represent mutants with similar intracellular arsenic as the wild type. Relative values are shown to enable comparison between strain backgrounds. Statistical analyses were performed by Student's t-test and significant differences are indicated by \* ( $p<0.05$ ).

### **Supplemental Tables**

#### **Table S1. Hits mutants with reduced aggregation**

[Click here to download Table S1](#)

#### **Table S2. Hits mutants with enhanced aggregation**

[Click here to download Table S2](#)

#### **Table S3. Datasets comparisons**

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#### **Table S4. Yeast strains**

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