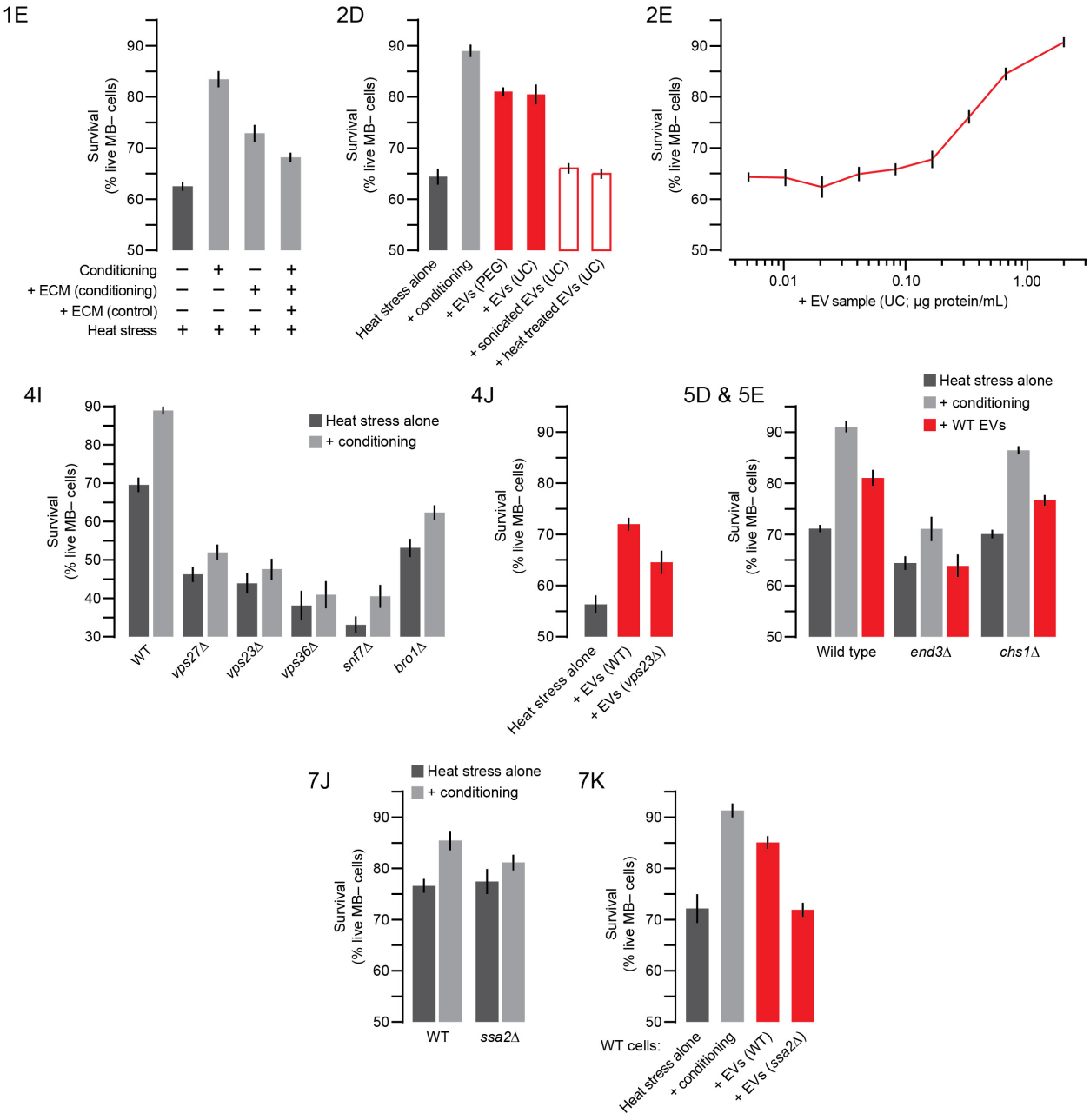
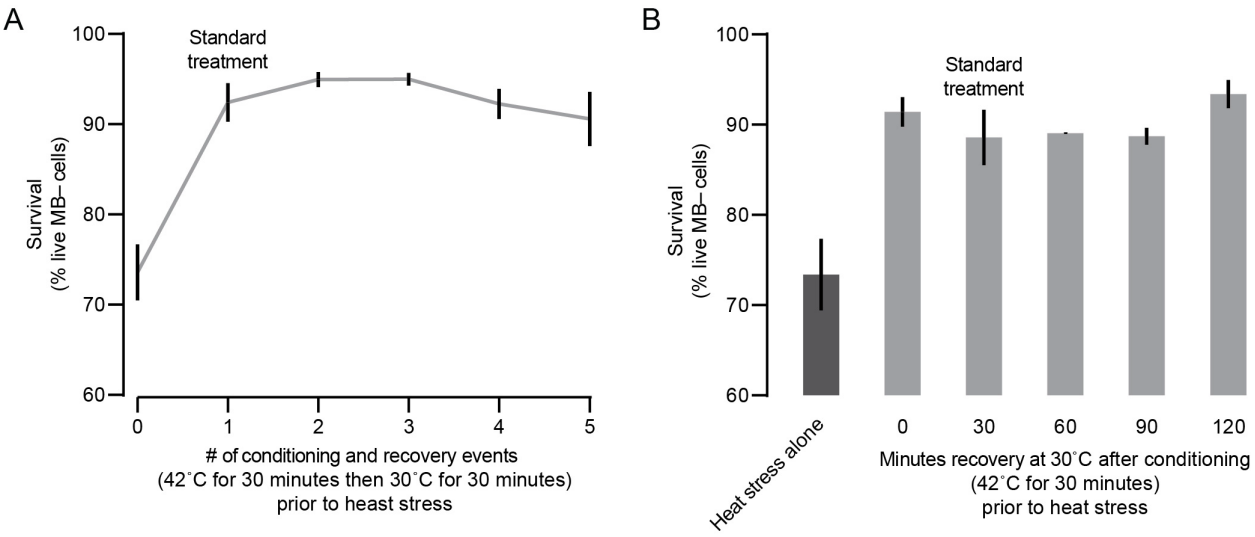
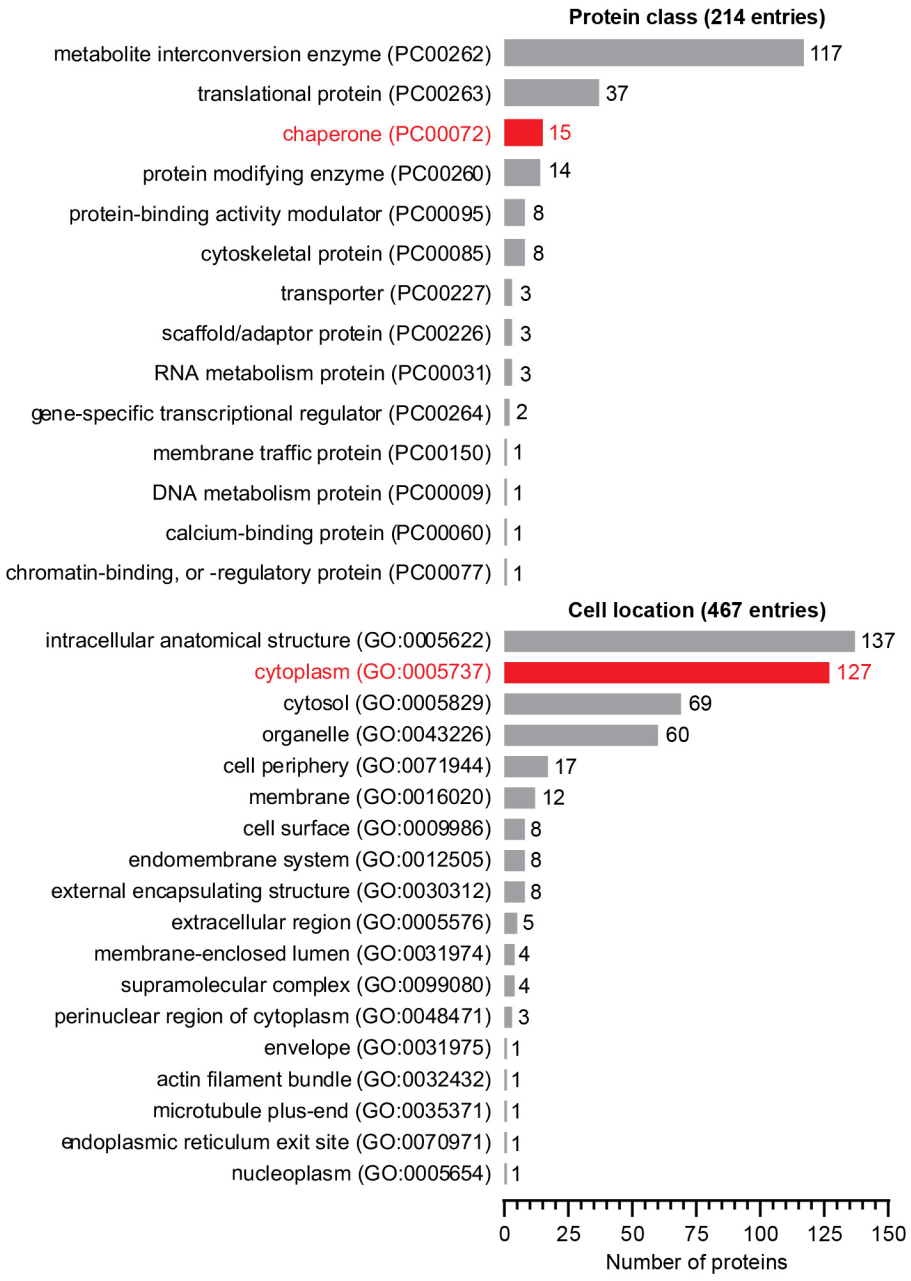
SUPPLEMENTARY INFORMATION



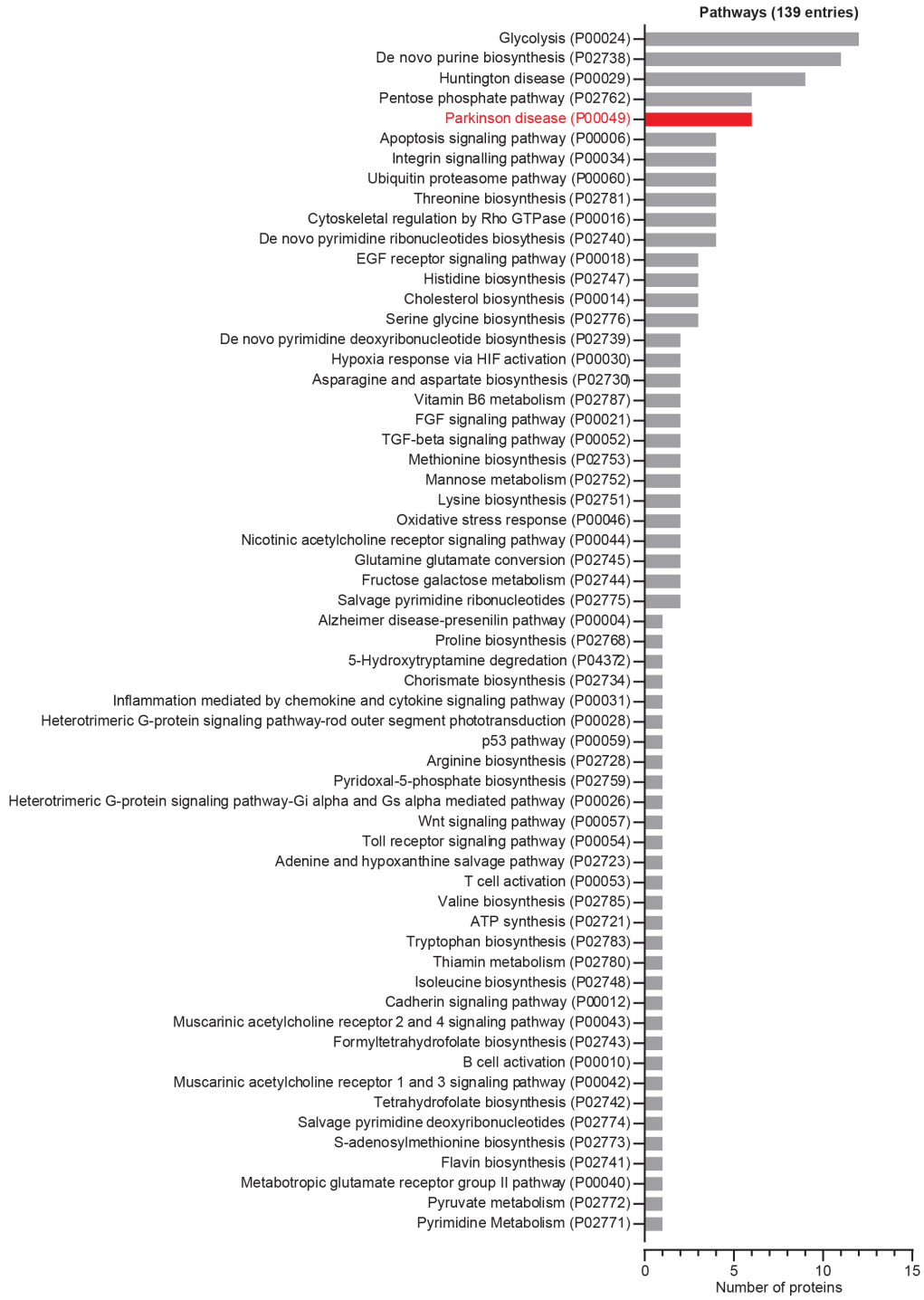
Supplementary Figure 1. Yeast cell survival data used to calculate thermotolerance
Methylene blue staining was used to measure cell survival when yeast cultures were subjected to heat stress alone (dark grey), conditioning then heat stress (light grey) or EV addition then heat stress (red). Panel labels indicate Figures showing related thermotolerance data. Means ± S.E.M. are indicated. Survival was > 95% when cells were untreated (no stress) or subjected to conditioning alone (data not shown).



Supplementary Figure 2. A single conditioning treatment is sufficient for thermotolerance (A) Methylene blue staining was used to measure cell survival when wild type yeast cultures were subjected to conditioning (42˚C for minutes followed by 30 minutes recovery at 30˚C) up to 5 consecutive times prior to challenging them with heat stress. No conditioning (0) is shown as a negative control. Mean ± S.E.M. shown are from 4 biological replicates. (B) Methylene blue staining was used to measure cell survival when wild type yeast cultures were subjected to 42˚C for 30 minutes followed by recovery at 30˚C for 0, 30, 60, 90 or 120 minutes prior to heat stress. Heat stress alone (no pretreatment) is shown a negative control. Mean ± S.E.M. shown are from 2 biological replicates. A single treatment of 42˚C for 30 minutes then 30 minutes recovery at 30˚C was used as a standard to condition cells in all other thermotolerance experiments conducted.



Supplementary Figure 3. Protein class and cell location of proteins identified in EV samples Results from gene ontology analysis of 266 proteins identified in EV samples collected from conditioned wild type cells. Only categories with at least one protein are shown for protein class (top) and cell location (bottom). Unclassified proteins are not shown. Categories shown in red include the Hsp70 ortholog Ssa2. Protein identities are shown in Supplementary Data.



Supplementary Figure 4. Pathways of proteins identified in EV samples

Results from gene ontology analysis of 266 proteins identified in EV samples collected from conditioned wild type cells. Only categories with at least one protein are shown. Unclassified proteins are not shown. Categories shown in red include the Hsp70 ortholog Ssa2. Protein identities are shown in Supplementary Data.

Supplementary Data. Proteomics data and gene ontology analysis

The attached spreadsheet includes names and descriptions of the 266 proteins identified in EV samples collected by ultracentrifugation from conditioned wild type *S. cerevisiae* (2 biological replicates are shown). Results from gene ontology analysis by PANTHER 18.0 are included.