Text S1: Materials and Methods

Yeast strains and growth media.

The *S. cerevisiae* strains used in this study were derived from 74-D694 [MATa *ade1-14* (UGA) *trp1-289 leu2-3,112 his3*Δ*-200 ura3-52*]. When indicated, these strains carried strong ([*PSI*$^{+}$]^{S}) or weak ([*PSI*$^{+}$]^{W}) [*PSI*$^{+}$] prion variants, or no prion ([*psi*$^{-}$]). Strains SY87 ([*psi*$^{-}$]) and TRS331 ([*PSI*$^{+}$]) are 74-D694 strains expressing GFP-tagged Sup35p (1). Yeast cells were grown in YPDA medium (1% yeast extract, 2% peptones, 2% glucose, 0.002% adenine). Solid media contained 2% bacto-agar. Prion phenotypes were monitored on ¼-YPD medium (0.25% yeast extract, 2% peptones, 2% glucose) via a standard color-based phenotype assay, as described before (2, 3).

Isolation of extracellular vesicles

Isolation of yeast EV by ultracentrifugation of extracellular medium was performed based on a previously published procedure (4). The indicated yeast strains were grown overnight at 30°C with vigorous shaking in 200 mL YPDA. The next day, these cultures were diluted into 2 L of YPDA to an OD_{600nm} ~ 0.1 and allowed to grow for 24 h at 30°C with vigorous shaking (final OD_{600nm} ~ 5-6). Cells were eliminated by centrifugation at 4000 *g* for 10 min at 4°C and the supernatant recovered and filtered through a 0.2 µm filter sterilization device (Steritop, Millipore) to remove remaining cells and debris. The filtered medium was then centrifuged for 90 min at 100,000 *g* (30,000 r.p.m. on a 45 Ti rotor, Beckman; *k*-factor: 299) and at 4°C to collect EV. Multiple rounds of centrifugation steps were required to process 2 L of filtered medium. At each step, the supernatants were discarded and the EV pellets were resuspended in ~1-2 mL cold PBS and kept on ice. EV were pooled in a final volume of 14 mL of PBS and

pelleted by centrifugation for 70 min at 100,000 *g* (45,000 r.p.m. on a TLA100.3 rotor, Beckman; *k*-factor: 70) and at 4°C. EV pellets were resuspended and pooled in a final volume of ~250 µL of cold PBS. The EV suspension was centrifuged for 15 min at 10,000 *g* and at 4°C to remove remaining insoluble debris and aggregates that were formed during the procedure. The EV-containing supernatant was then aliquoted, snap-frozen in liquid nitrogen and stored at -80°C.

Quantification of extracellular vesicles

The total protein content of EV preparations was determined using Bradford's assay (Bio-Rad), and typical yields were in the range of 150-200 µg of EV protein per liter of filtered extracellular medium. We then obtained an approximate estimation of the number of vesicles produced by the yeast cultures in our experimental conditions based on the calculation method proposed by Sverdlov (5). For these calculations, we considered that the yeast cell volume is ~40 μm^3 and that the average EV size is ~60 nm (Fig. S1D), which corresponds to an average EV volume of ~1 x 10^{-4} μm^3. Therefore, an EV is ~4 x 10^5 times smaller than a yeast cell and should in principle contain ~4 x 10^5 times less protein. Yeast cells contain ~4 pg of protein (6, 7). Thus, the average mass of protein per EV is ~1 x 10^{-5} pg and 1 µg of purified EV corresponds to ~1 x 10^{11} vesicles. Our production and purification procedures therefore yields ~2 x 10^{13} EV per liter of extracellular medium, which corresponds to ~200 EV per yeast cell. It should be noted that for this calculation, the number of cells was determined from the final cell density of the culture. However, because it is unclear how and when EV are produced and released in the medium during growth, and because a fraction of the EV may be degraded (during cultivation or as a result of the purification procedure), the number of EV that can be produced by each individual cell may have been underestimated in our calculations.

Western blot and semi-denaturant detergent agarose gel electrophoresis (SDD-AGE) analyses

Total protein extracts were prepared and analyzed by Western blot as described previously (2, 3). For SDD-AGE analyses, yeast cell pellets were washed with ice-cold water and lysis buffer (100 mM Tris.Cl pH 7.5, 50 mM KCl, 10 mM β-mercaptoethanol), before being resuspended into smooth slurry in a residual volume of lysis buffer. The cell slurry was then dripped into liquid nitrogen and ground to a fine powder using a mortar and pestle. The cell powder was thawed in lysis buffer and the crude lysate was cleared by centrifugation at 4000 *g* for 5 min at 4°C. Crude cell lysates and EV were mixed with an equal volume of 2X sample buffer [1X Tris-Acetate-EDTA (TAE), 10% glycerol, 4% SDS, 0.025% bromophenol blue], incubated for 10 min at room temperature and then analyzed by SDD-AGE as described before (2, 3).

Proteinase K protection assays

EV (10 µg proteins) were incubated in PBS with or without 2% Triton X-100 for 30 min on ice, before Proteinase K (Roche Diagnostics) was added at a final concentration of 0.01 $mg.ml^{-1}$. Reactions were further incubated for 15 min on ice and stopped with the addition of PMSF at a final concentration of 2 mM. An untreated control reaction (without Triton X-100 and without Proteinase K) was run in parallel in identical conditions. Reactions were then analyzed by Western blot using the indicated antibodies.

Discontinuous sucrose gradients

EV (80 µg protein) were incubated with or without 1% Triton X-100 for 30 min at 4°C. EV suspensions (~100 µL) were then mixed with 1.3 mL of a 60% (w/w) sucrose solution in 20 mM Hepes-NaOH pH 7.5 and poured into centrifugation tubes. Two solutions (1.3 mL) of 40% (w/w) and 20% (w/w) sucrose in 20 mM Hepes-OH pH 7.5 were successively layered on top of the EV suspensions. The tubes were centrifuged for ~17 h at 150,000 *g* (34,000 r.p.m. in a SW60 rotor, Beckman; *k*-factor: 140) and at 4°C. Ten fractions of 400 µL were subsequently collected from the top and then precipitated with TCA and acetone for Western blot analyses.

Electron microscopy and immunogold labeling of EV

Electron microscopy of isolated EV was performed essentially as described (4), with minor modifications. Briefly, EV preparations were thawed, mixed with an equal volume of 4% paraformaldehyde (PFA), and incubated for 20 min at 4°C. Fixed EV were then applied to carbon coated EM grids and allowed to adsorb for 20 min at room temperature. EM grids were then washed by sequential transfers on drops of PBS, incubated for 5 min at room temperature in 1% glutaraldehyde, and then washed by sequential transfers on drops of distilled water. For immunogold labelling, fixed EV adsorbed on EM grids were permeabilized in blocking buffer (PBS, 5% BSA) containing 0.02% Triton X-100 for 10 min at room temperature. EM grids were transferred to a drop of blocking buffer for 20 min at room temperature, and then incubated for one hour in PBS containing 1% BSA, with or without anti-Sup35p rabbit polyclonal antibodies (at a 1:100 dilution). EM grids were then washed in multiple drops of PBS containing 0.5% BSA and then incubated for 30 min at room temperature in the same buffer containing 10 nm gold-conjugated anti-rabbit antibodies (at a 1:50 dilution; BBI Solutions). EM grids were then washed in drops of PBS, fixed with

1% glutaraldehyde for 5 min at room temperature, and then further washed in multiple drops of distilled water. Following negative-staining with 1% uranyl acetate for 10 min at room temperature, samples were imaged in a Jeol 1400 transmission electron microscope. Images were recorded with a Gatan Orius CCD camera (Gatan, Pleasanton, CA) and processed with the ImageJ software (NIH).

Fluorescence microscopy

Drops of EV prepared from the indicated strains were directly mounted between microscope slides and glass coverslips. Images were acquired on an AxioObserver Z1 epifluorescence microscope (Carl Zeiss) equipped with an Orca-R2 camera (Hamamatsu).

Induction of [PSI$^+$] by transformation of yeast spheroplasts with EV preparations

Yeast 74-D694 [*psi*$^-$] cells were converted to spheroplasts and transformed as described previously (2). Transformation mixtures contained 50 µl of spheroplasts, 100 µg.ml^{-1} salmon sperm DNA, 20 µg.ml^{-1} of the *URA3*-based pRS316 plasmid, and the indicated cell extracts or EV (~1-10 µg protein). Mock transformation reactions lacking spheroplasts and/or pRS316 ensured the absence of viable cells in the extracts and EV preparations. It should be noted here that intact EV were used in the transformation experiments, although we cannot ascertain they remained so during the transformation procedure or upon delivery inside recipient cells.

References

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