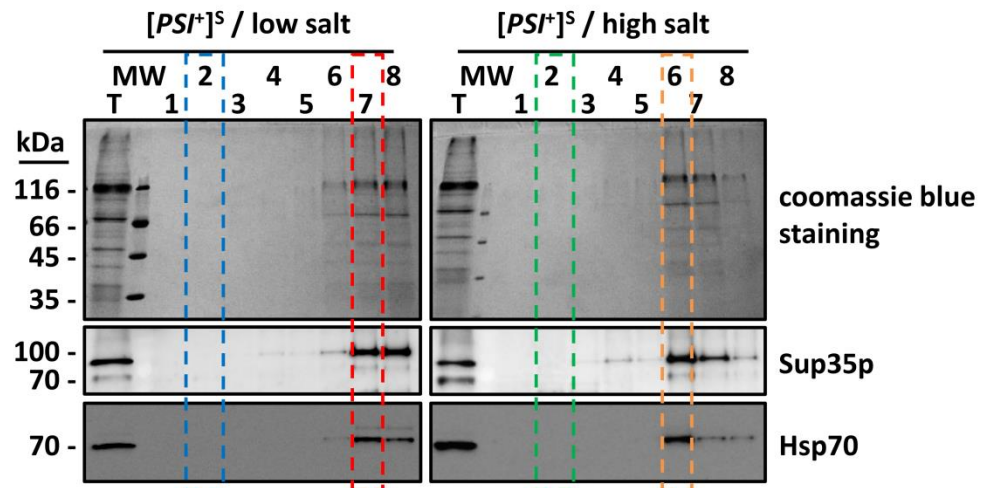
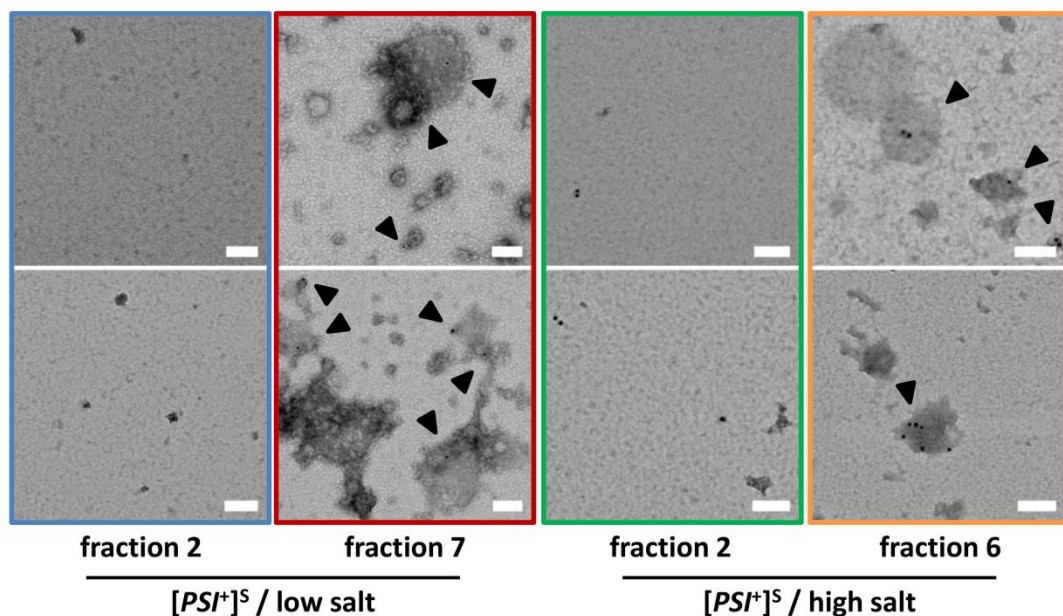


**A****B**

**Figure S5. Sup35p and Hsp70 co-localize with  $[PSI^+]^S$  EV in discontinuous sucrose gradients under low or high salt conditions.** (A) Purified EV from the  $[PSI^+]^S$  strain were adjusted to 1.75 M sucrose in PBS (low salt condition) or PBS supplemented with 400mM NaCl (high salt condition) and deposited at the bottom of ultracentrifugation tubes. Equal volumes of 1.17 M and 0.58 M sucrose solutions in PBS (low salt condition) or PBS supplemented with 400 mM NaCl (high salt condition) were layered on top of the EV suspensions. Following centrifugation for 17 h at 150,000 g and at 4°C, eight fractions were collected from the top of the gradients and analyzed by SDS-PAGE and coomassie blue staining (upper panel) and Western blot (middle and lower panels) using the indicated

antibodies (*T* stands for *total* and represents the input material). **(B)** The fractions highlighted by colored dashed-line boxes from the sucrose gradients described in (A) were concentrated by centrifugation for 1 h at 100,000 g and at 4°C, washed in PBS, and then analyzed by immunogold labeling of Sup35p and negative-stained electron microscopy (scale bars: 100 nm; arrowheads point to gold-labeled Sup35p-positive vesicles).