Supplementary information, Data S1 Materials and Methods

Sample preparation for MudPIT

SYP61-containing vesicles were immunoprecipitated on-beads and subjected to trypsin digestion and MudPIT analyses. Due to the low quantity of the protein contents, the common in-gel digestion approach was omitted and instead, a gel-free strategy was employed. The SYP61-containing vesicles on beads were digested over night with trypsin (1 µg in 100 µl, 50 mM ammonium bicarbonate/10% acetonitrile) while vortexing at 37 ºC. After on-beads digestion and brief centrifugation, the supernatant was transferred to a new tube and beads were washed by vortexing for 10 min with 100 µl 50% acetonitrile, 5% acetic acid. Following a brief centrifugation, the supernatant was collected and pooled with the previous supernatant. Samples were pelleted by speedvac. All tryptic-peptide pellets were redissolved in 20 µl 0.1% TFA (trifluoroacetic acid) and were used in MudPIT experiments.