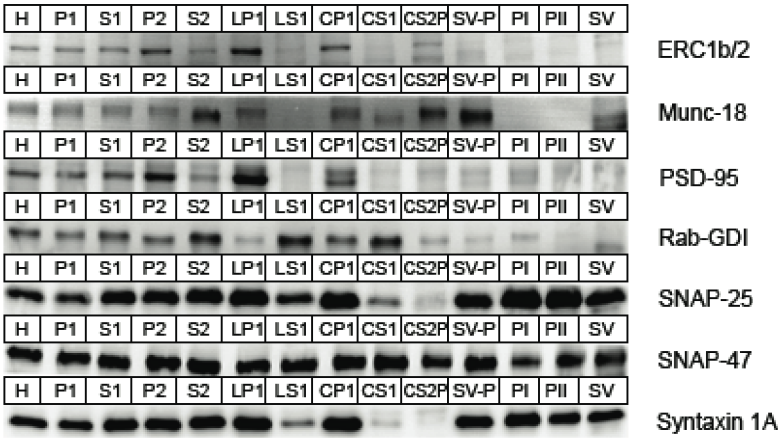

Monitoring vesicle purity by immunoblotting of subcellular fractions.
Subfractions taken during the isolation of synaptic vesicles were separated by SDS-PAGE and immunoblotted to determine the distribution profiles of various marker proteins (additional to those shown in Figure 4B). Some markers ERC1b/2 (active zone protein), PSD-95 (post-synaptic scaffolding protein) and Rab-GDI (regulator of Rab protein activity) should be absent from the purified vesicle fraction. The remaining protein profiles are for known residents of the plasma membrane (syntaxin 1A and SNAP-25, or for known interacting partners, such as Munc-18). While the degree of plasma membrane contamination within the synaptic vesicle fraction is known to be low (as judged by Na^+/K^+-ATPase immunoreactivity), it is conceivable that some plasma membrane proteins may use synaptic vesicles as part of their recycling pathway. This is especially true for syntaxin 1 and SNAP-25, that are both members of the synaptic core-complex essential for vesicle fusion, and which may be recycled to some degree with synaptic vesicles[1].

1. Walch-Solimena, C. et al. The t-SNAREs syntaxin 1 and SNAP-25 are present on organelles that participate in synaptic vesicle recycling. *J. Cell Biol.* **128**, 637-645 (1995).