

## Images of pellets and supernatants obtained during SV purification.

Flow diagram of synaptic vesicle purification, illustrating pellets and supernatants formed at each stage. Careful handling of pellets and supernatants is essential to avoid contamination in the final synaptic vesicle fraction. When removing the S1 supernatant, do not disturb any of the white material surrounding the P1 pellet (red arrowhead). Likewise, when resuspending the P2 (synaptosomes) avoid the brown material in the middle of the pellet (red arrowhead), which is enriched in mitochondria. After centrifugation of the sucrose cushion, proteasome contamination will be enriched on the top of the sucrose layer and should be avoided (red boxed region in both low magnification (left) and high magnification (right) images), while a vesicle rich pellet will be formed at the bottom of the tube (red circles).

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