



SI Figure 1

Supplementary Figure 1. Snapshots from a typical movie acquired using TIRFM (256 pixels by 402 pixels, 57 frames/s). Top panel: frame 1, middle panel: frame 1322, bottom panel: frame 1419. Time between consecutive frames is ~17 ms. *Filled green arrowheads*: remnant fluorescent single LR-PE molecules from recently fused v-SUVs. *Yellow arrows*: docked vesicles that have not undergone fusion within 1419 frames (the two vesicles on the right dimmed to become indistinguishable from the background at about frame 2200 due to continuous bleaching. The vesicle on the left also dimmed considerably, but was still discernible at the end of the movie, fr. 3393). *Blue open arrows*: two background particles that are most likely single LR-PE molecules. Such particles may be survivors from previous fusion events (see green arrowheads) or may be contaminants. *Red open arrows* indicate a freshly docked vesicle (middle panel) that undergoes fusion (bottom panel). The display range is adjusted here such that single LR-PE molecules are clearly visible. Freshly docked vesicles appear saturated with these display settings, but their actual pixel values are not saturated. When the display range is adjusted such that the vesicles are visualized without apparent saturation, the single molecules become too dim to be distinct from the background.