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**Supplemental information**

**Single-molecule manipulation of macromolecules on GUV or SUV  
membranes using optical tweezers**

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Video 1 FRAP assay reveals free diffusion of VAMP2 in the GUV membrane. Real-time confocal fluorescence images of the Alexa Fluor 647 labeled VAMP2 in the GUV membrane after photobleaching.

Video 2 FRAP assay reveals immobilized VAMP2 in the lipid bilayer supported on the silica bead. Real-time confocal fluorescence images of the Alexa Fluor 647 labeled VAMP2 in the lipid bilayer coated on a silica bead 6  $\mu\text{m}$  in diameter after photobleaching.

Supplementary Figure 1 Optical trapped GUVs with high membrane tension do not significantly deform in response to high pulling force. (A) Bright-field images of the same optical trapped GUV and polystyrene bead in the presence of different pulling force. The GUV and bead were tethered by the DNA handle and hairpin as in Fig. 2C with 250 mM potassium chloride in the solution. The red circles mark the edges of the GUV and bead determined by computer software as illustrated in B. (B) Method to identify the edges of GUVs and beads based on their images. The edge pixels were selected by their intensities below a threshold and fit by a circle function in MATLAB (imfindcircles), which yielded the positions and diameters of the GUV (3.1  $\mu\text{m}$ ) and bead (2.1  $\mu\text{m}$ ).

### Supplementary Figure 1

