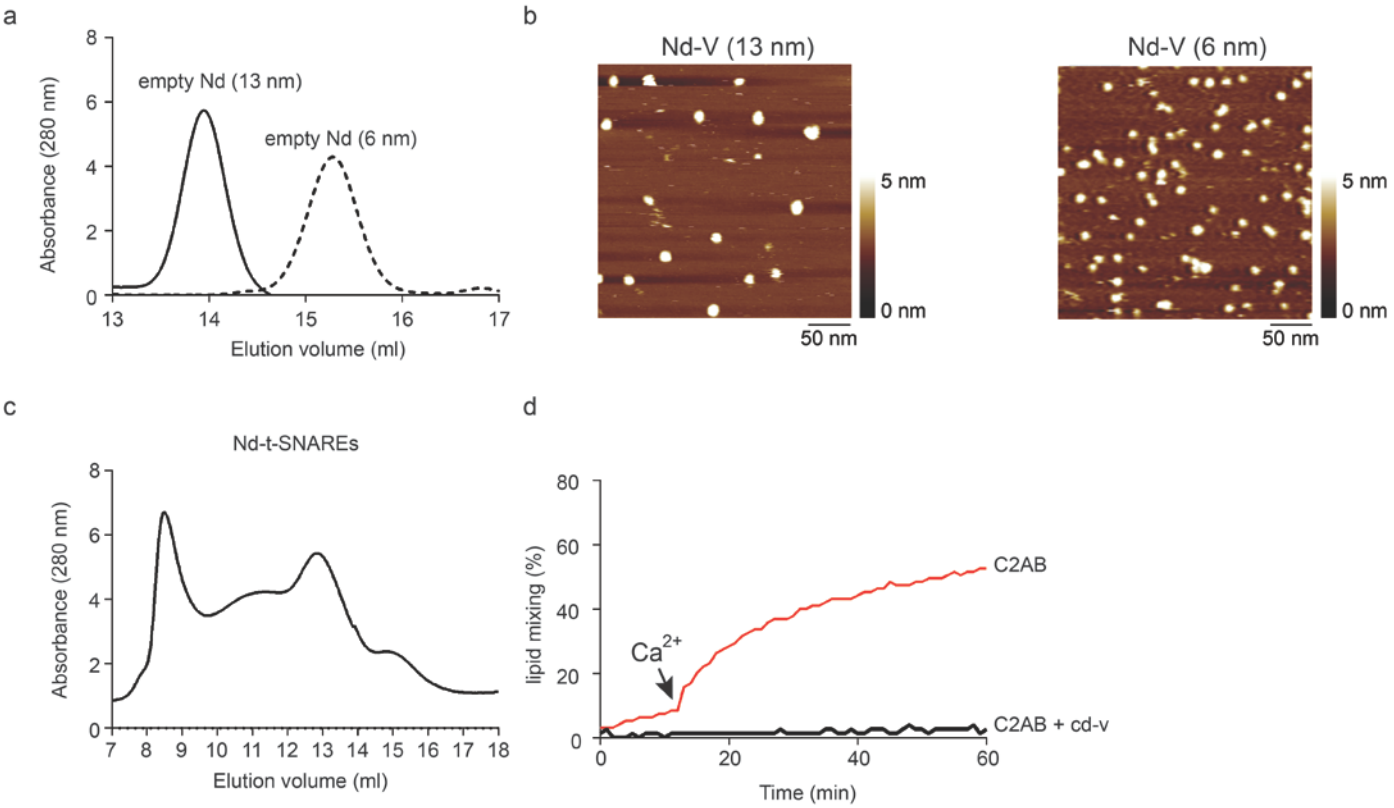
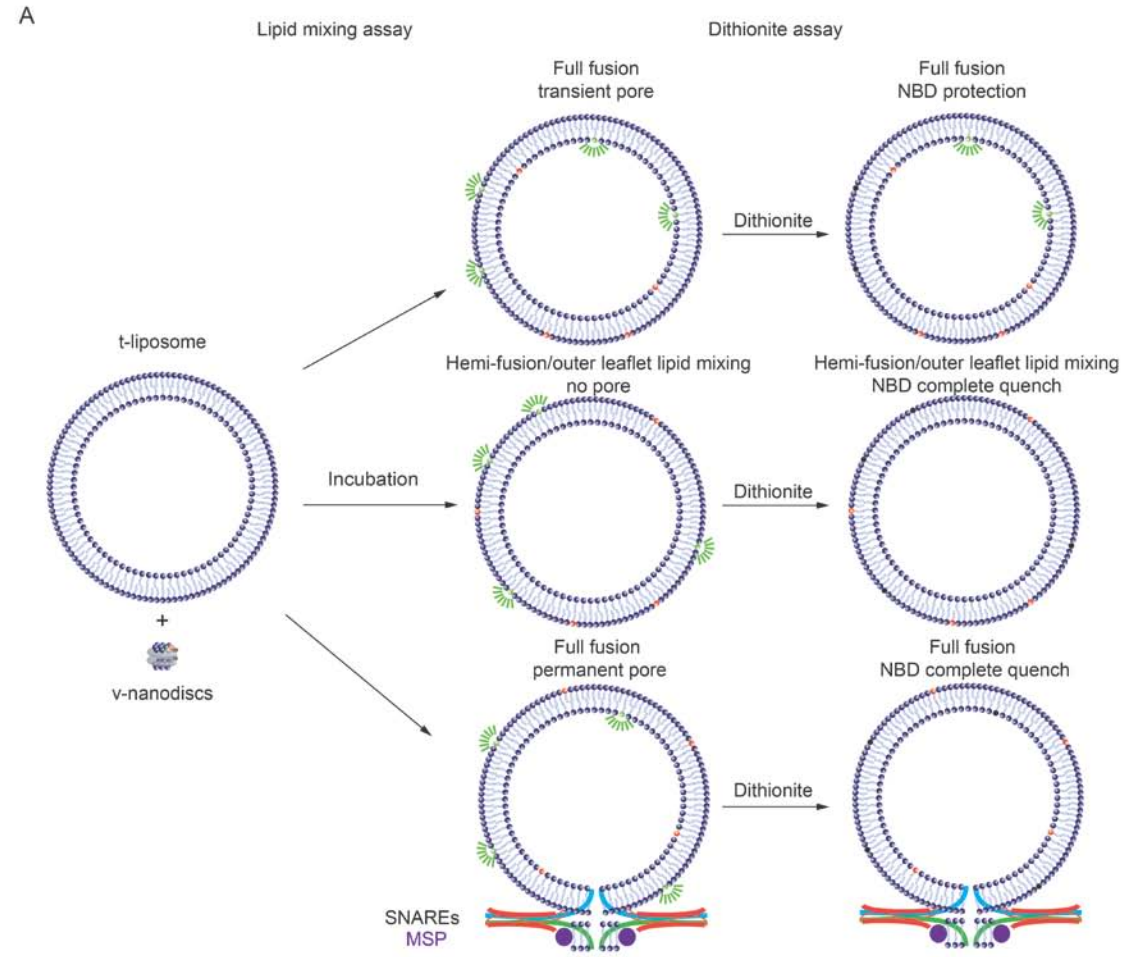
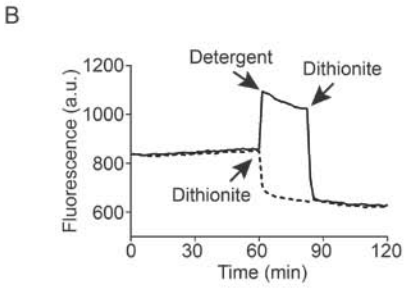


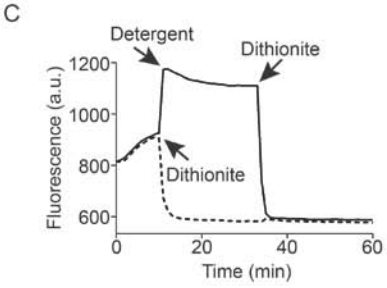
Supplementary Figure 1

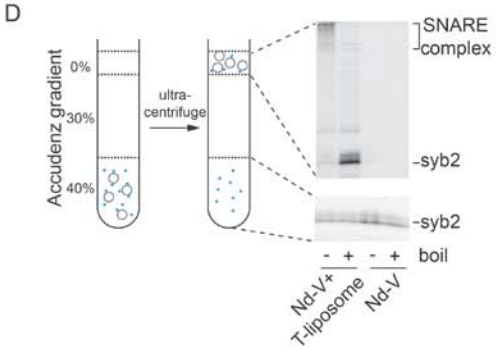
Characterization of nanodiscs.

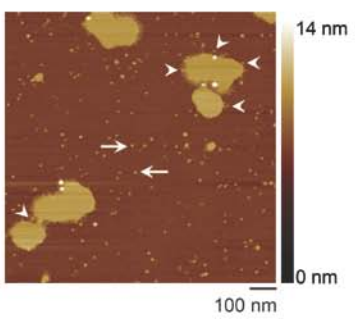
(a) Empty nanodiscs were analyzed using a Superdex 200 10/300 GL column equilibrated in 25 mM HEPES pH 7.5, 100 mM KCl, 1mM DTT and 5% glycerol. (b) AFM images of v-SNARE nanodiscs. (c) t-SNARE nanodiscs were analyzed using a Superdex 200 10/300 column as described in *panel A*. (d) Lipid mixing between t -SNARE nanodiscs and v-SNARE vesicles was monitored in the presence of Ca^{2+} (1 mM) and C2AB, with and without cd-v.


A
Lipid mixing assay
Dithionite assay
Full fusion
transient pore
Full fusion
NBD protection
Dithionite
t-liposome
Hemi-fusion/outer leaflet lipid mixing
no pore
Hemi-fusion/outer leaflet lipid mixing
NBD complete quench
Incubation
Dithionite
+
v-nanodiscs
Full fusion
permanent pore
Full fusion
NBD complete quench
Dithionite
SNAREs
MSP


B
Detergent
Dithionite
Dithionite
Fluorescence (a.u.)
1200
1000
800
600
0
30
60
90
120
Time (min)


C
Detergent
Dithionite
Dithionite
Fluorescence (a.u.)
1200
1000
800
600
0
20
40
60
Time (min)


D
Accudenz gradient
0%
30%
40%
ultra-
centrifuge
SNARE
complex
syb2
syb2
- + - + boil
Nd-V+
T-liposome
Nd-V

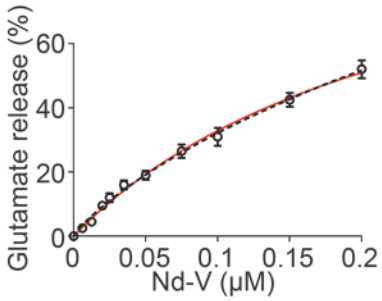

E
14 nm
0 nm
100 nm

Supplementary Figure 2

Fusion pores close after fusion.

(a) Illustration of lipid mixing and dithionite quenching experiments. (b-c) Protection of NBD fluorescence is not observed using empty nanodiscs (b) or when dithionite is added at the beginning of the fusion reaction (c). (d) Left Panel: illustration of the co-flotation assay. Samples containing syb2 nanodiscs and t-SNARE liposomes were mixed with an equal volume of 80% Accudenz, and then layered with 30% and 0% Accudenze. Ultra-centrifugation was carried out at 55,000 rpm at 4 °C for 2 h. Samples were collected at the 40% Accudenze layer and at the 30%-0% interface. Right panel: DY650-labeled syb2 nanodiscs (Nd-V) were incubated with or without t-SNARE liposomes (T-liposome) at 37 °C for 1 h in the presence of Ca^{2+} (1 mM) and C2AB (1 μM). Samples were analyzed by co-flotation, and the top and bottom fractions were subjected to SDS-PAGE and fluorescence scanning. (e) Syb2 nanodiscs were incubated with t-SNARE liposomes at 37 °C for 1 h, followed by AFM analysis. Arrows indicate free nanodiscs; arrowheads indicate liposome-associated nanodiscs. Scale bar: 100 nm.

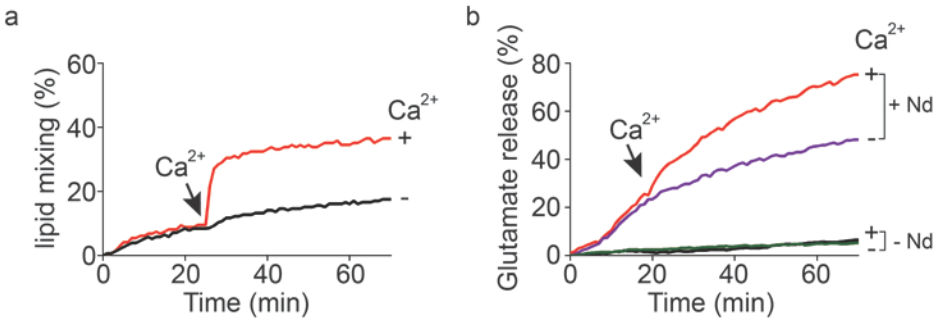
Supplementary Figure 3



Supplementary Figure 3

Glutamate release during SUV-nanodisc fusion as a function of 6-nm Nd-V concentration.
Data were fitted to the Hill (black dotted line) and Michaelis-Menten equations (red line). Data points are presented as means ± s.d. from three independent trials.

Supplementary Figure 4

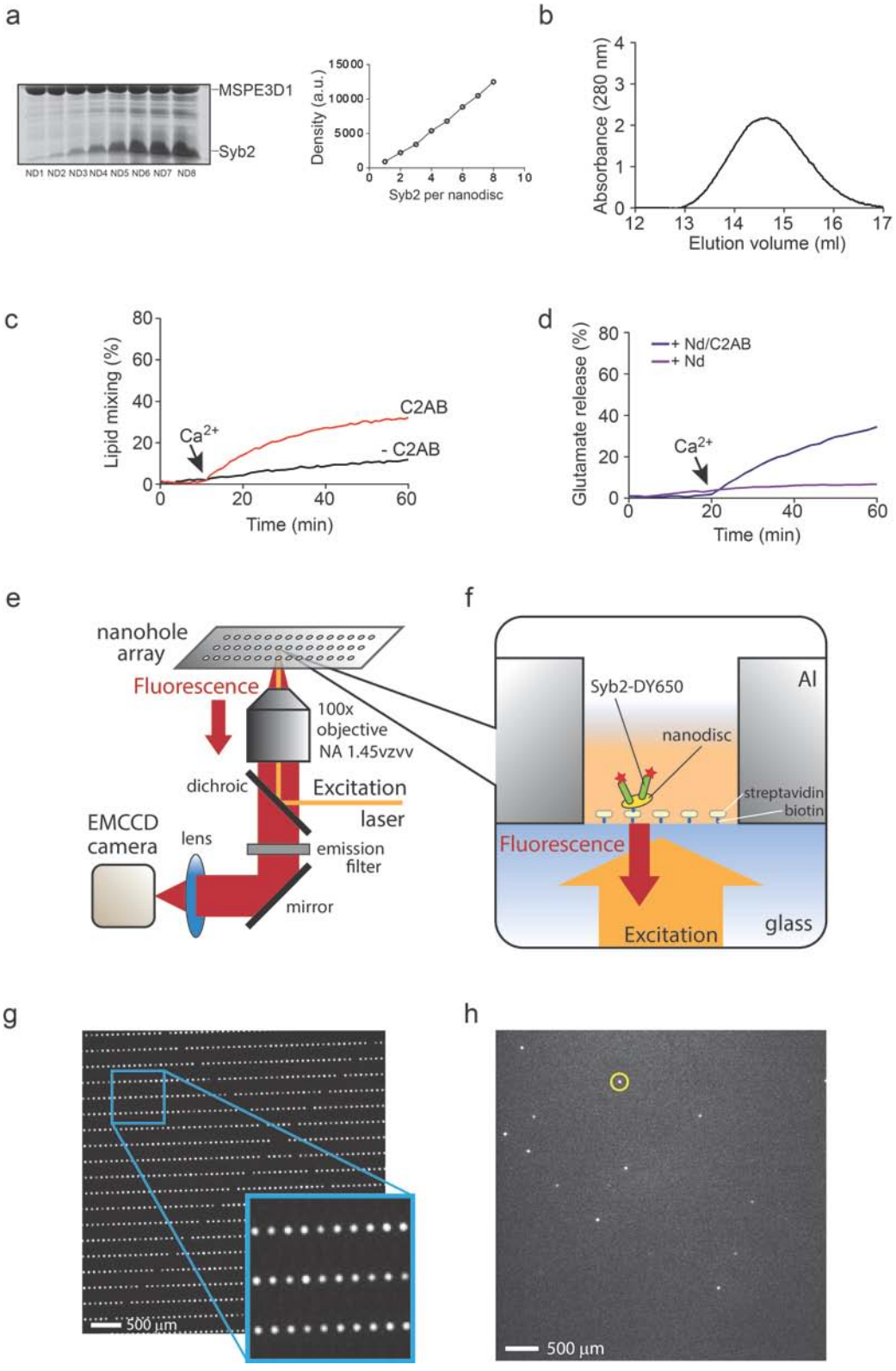


Supplementary Figure 4

Reconstitution of Ca^{2+}-triggered lipid mixing and glutamate release with full-length Syt1.

(a) Lipid mixing between t-SNARE vesicles and nanodiscs harboring syb2 and full-length syt1. (b) Glutamate release from t-SNARE vesicles in the presence and absence of nanodiscs harboring syb2 and full-length syt1. Arrow indicates the addition of Ca^{2+} (1 mM final concentration).

Supplementary Figure 5



Supplementary Figure 5

Efficient Ca^{2+}-triggered bilayer fusion and glutamate release occurs with two v-SNAREs in 6-nm nanodiscs.
(a) Left panel: SDS-PAGE of nanodiscs (ND1, ND2, ND3, ND4, ND5, ND6, ND7 and ND8). Right panel: densitometry of the syb2 protein bands from the gel in panel (a) were plotted versus the syb2 copy number per nanodisc. (b) Size exclusion chromatography of 6-nm ND2. (c) Lipid mixing between 6-nm ND2 and t-SNARE vesicles, in the presence and absence of C2AB; addition of Ca^{2+} (1 mM final concentration) is indicated by an arrow. (d) Glutamate release from t-SNARE vesicles in the presence of 6-nm ND2 and C2AB; the arrow indicates the addition of Ca^{2+} (1 mM final concentration). (e) Experimental setup for imaging bleaching steps from arrays of zero-mode waveguide nanoholes containing single nanodiscs that bear fluorescently labeled syb2. (f) Illustration of an individual zero-mode waveguide nanohole with a nanodisc containing two syb2-DY650 molecules immobilized on the glass surface via biotin/streptavidin. Fluorescence from DY650 was measured upon epi laser illumination, with the effective excitation volume limited to attoliters. (g) Brightfield image of an array of zero-mode waveguide nanoholes with diameters of ~ 200 nm. (h) Fluorescence image of an array as in (g) after sparse labeling with syb2-DY650 containing nanodiscs such that only a few nanoholes contained a single nanodisc (one of these is indicated with a yellow circle).