Supplementary Information

A single vesicle content mixing assay for SNARE-mediated membrane fusion

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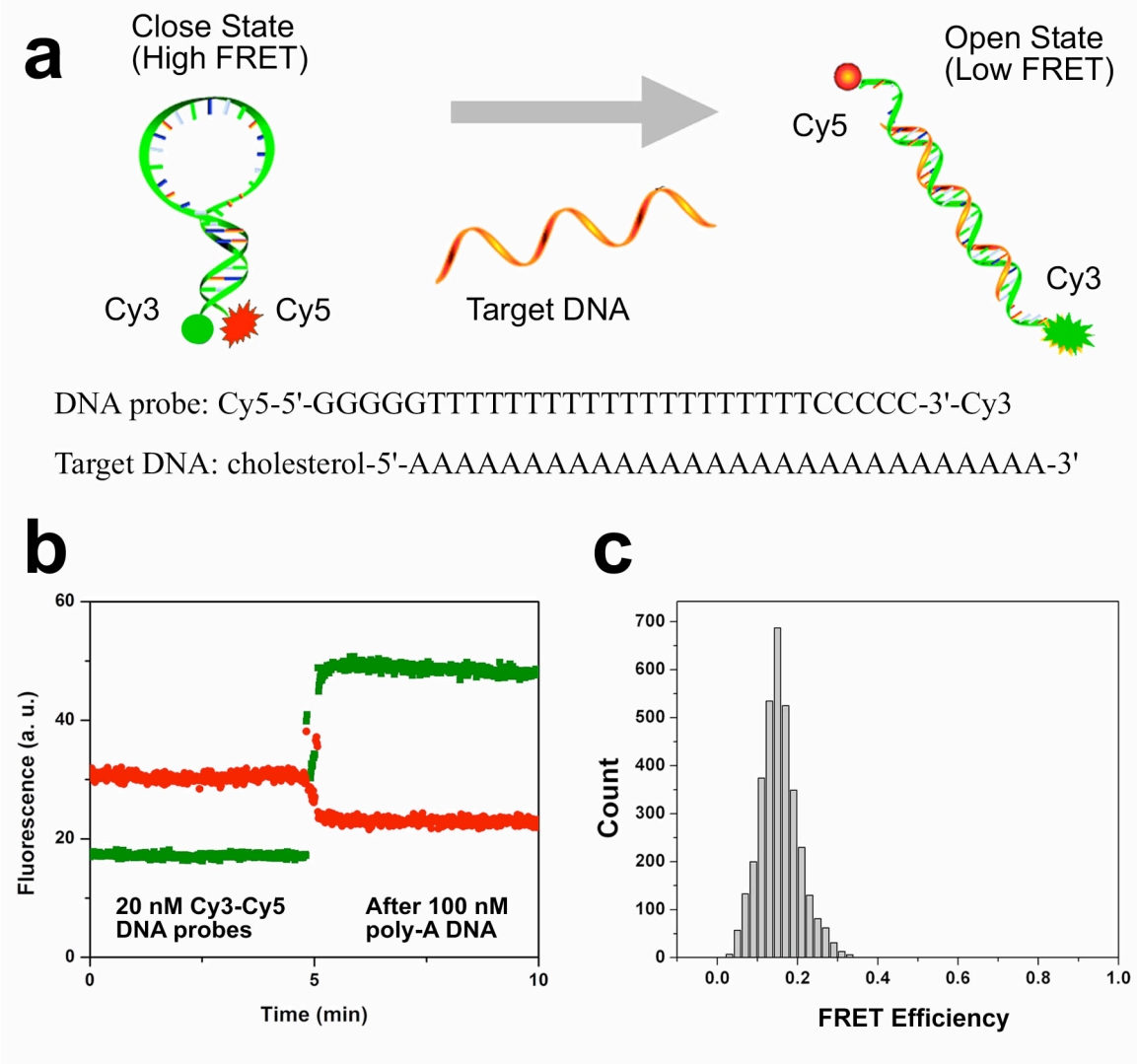
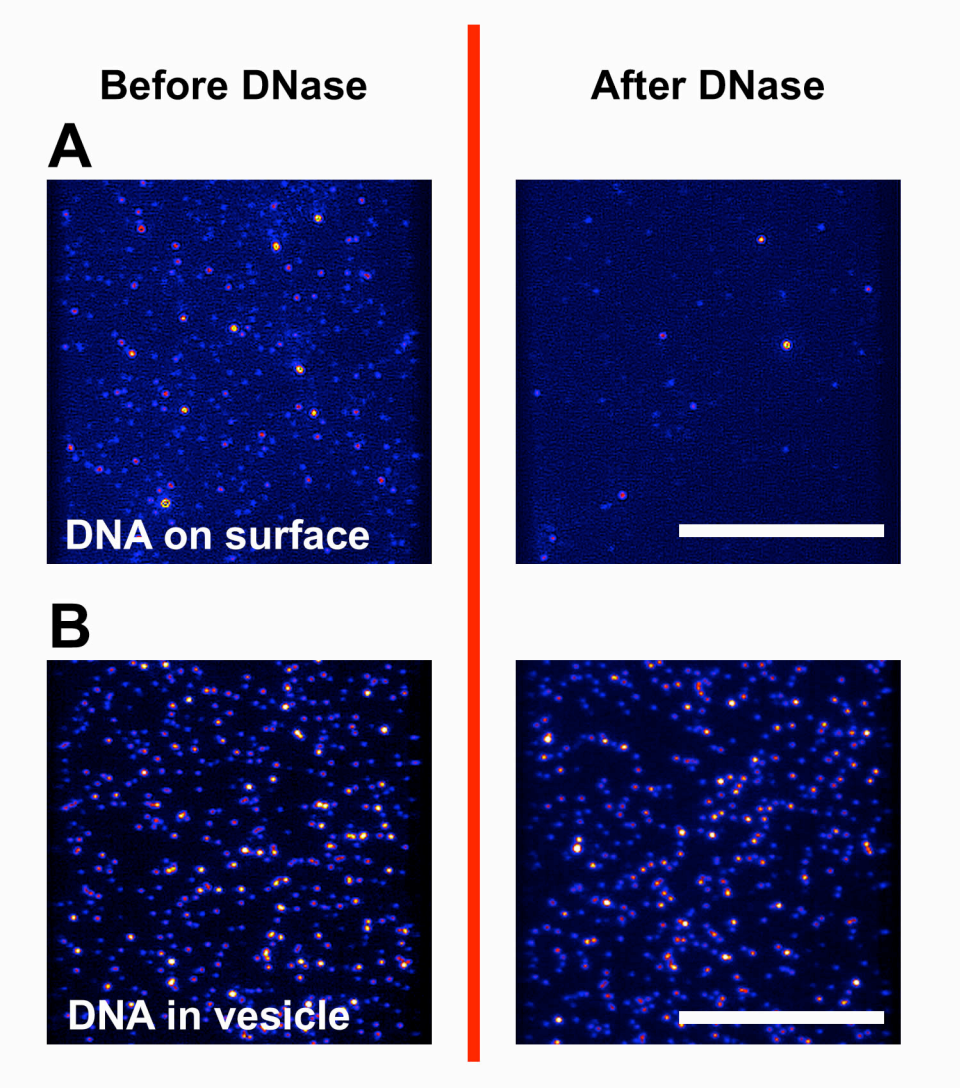
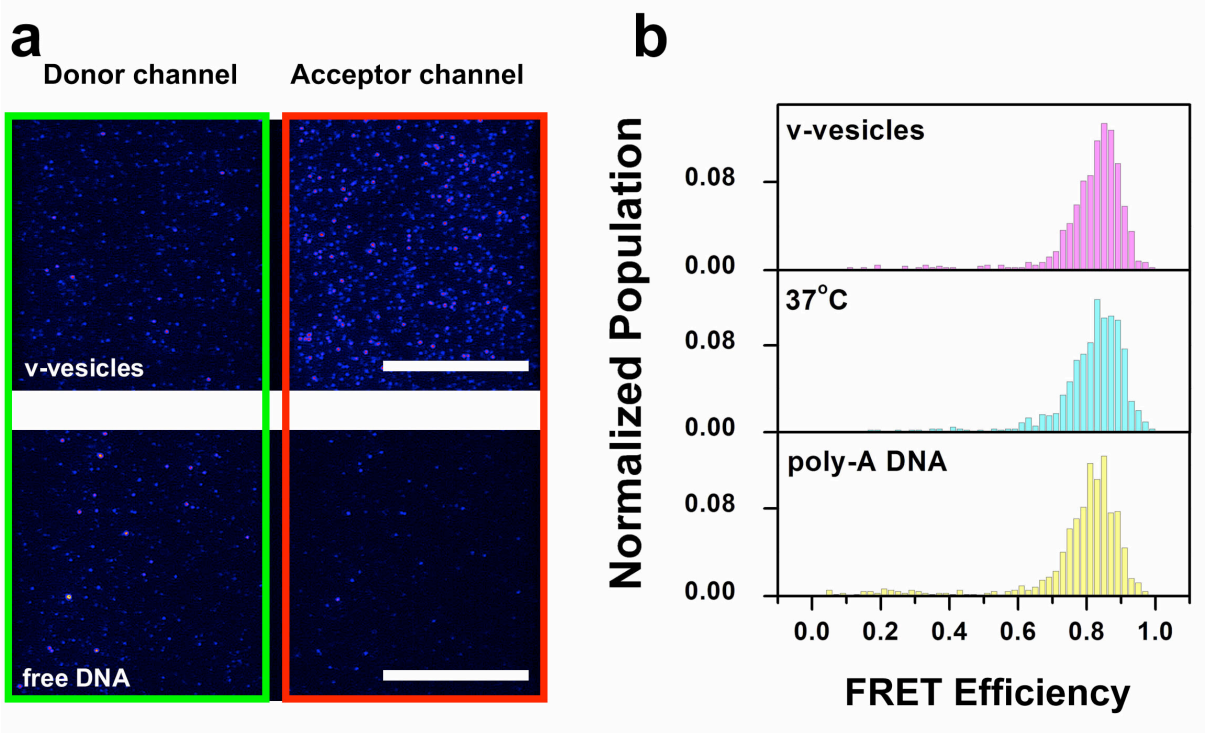


Supplementary Fig. S1. Dual-labeled DNA hairpin probes are single-stranded oligonucleotide hybridization probes that form a stem-and-loop hairpin structure. The loop contains a probe sequence (poly-T, 20mer long) that is complementary to a target sequence (poly-A, 30mer), and the 5 base pair stem is formed by the annealing of complementary arm sequences that are located on either side of the probe sequence (GGGGG & CCCCC). A Cy3 donor is covalently linked to the end of one arm and a Cy5 acceptor is covalently linked to the end of the other arm. **(a)** Illustration of the configuration change for dual-labeled DNA probes. **(b)** Ensemble fluorescent trace for Cy3 (green) and Cy5 (red) channels of dual-label DNA probes before and after hybridization obtained in a cuvette via manual addition of the probe

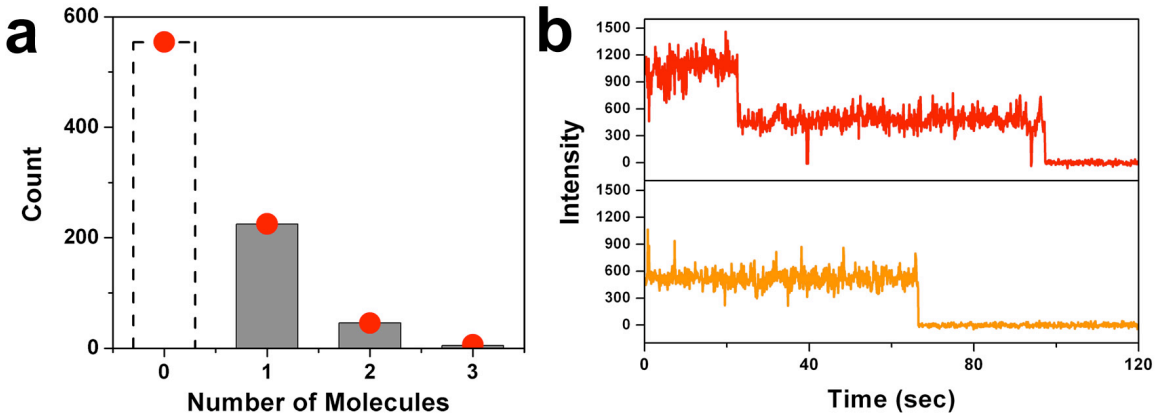
strand at 5 minute. **(c)** The FRET efficiency, *E* distribution obtained outside vesicles with poly-A targets inserted into the membrane via the cholesterol link and externally added dual-labeled DNA hairpin probes. The peak at *E* = 0.15 corresponds to the DNA hairpin annealed to the target DNA.



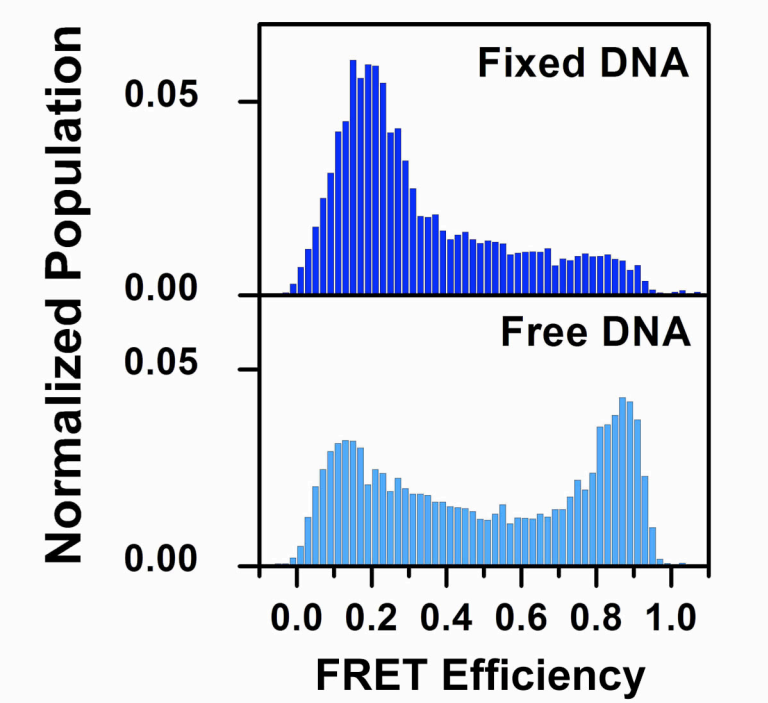
Supplementary Fig. S2. 633 nm laser excited images of acceptor channels for surface immobilized **(a)** and vesicle encapsulated **(b)** DNA probes before and after a DNase treatment. Scale bars are for 25 μm.



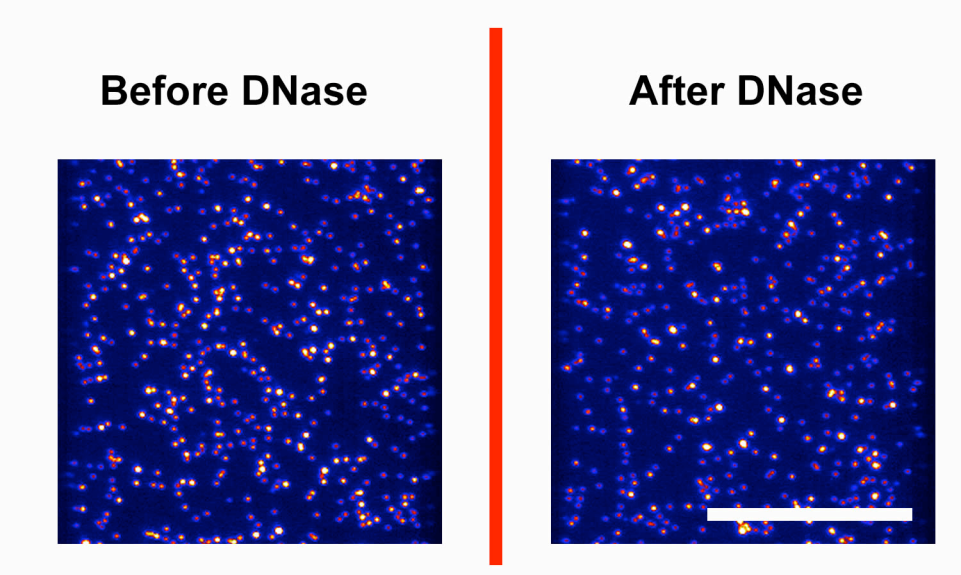
Supplementary Fig. S3. (a) 532 nm laser excited images of experimental channels after 160 pM v-vesicles with DNA probes or 2 nM free DNA probes. Lower panel only shows dim spots in the donor and acceptor channels, demonstrating that the nonspecific adhesion of the free DNA probes to the surface is minimal. **(b)** *E* distributions of v-vesicles at room temperature, at 37°C, and after incubating with 2 μM poly-A DNA at 37°C for 30 minutes. Vesicle encapsulated DNA probes are not sensitive to either temperature or free target DNA in solution. Scale bars are for 25 μm.



Supplementary Fig. S4. Single-molecule analysis of encapsulated DNA after protein reconstitution. **(a)** The distribution of the number of molecules inside a vesicle. Solid bars are experimental results. Red dots indicate the Poisson fitting, by which we obtain the bar represents 0 molecule. Based on that, we estimate that the average number of DNA probes inside a vesicle is ~0.4. **(b)** Representative time records for two vesicles. The number of encapsulated DNA molecules can be determined by counting the digital photobleaching events. In the upper trace, the Cy5 intensity reduces to its background level in two discrete steps, which implies that there are two molecules entrapped within this vesicle, whereas in the lower trace photobleaching occurs in a single step, indicating occupancy by a single DNA molecule.



Supplementary Fig. S5. Yeast SNARE content mixing: *E* distributions of yeast SNARE-mediated fusion products for t-vesicles containing free poly-A DNA and fixed poly-A DNA through a cholesterol molecule. The distinction of low FRET peak change is due to the difference on DNA encapsulation rate. Cholesterol linked target DNA dramatically increases the degree of content mixing.



Supplementary Fig. S6. 633 nm laser excited images of acceptor channels for fusion products before and after a DNase treatment. The number of florescent spots did not change significantly, which confirms that the DNA hybridization is inside vesicles. Scale bar is for 25 μm.