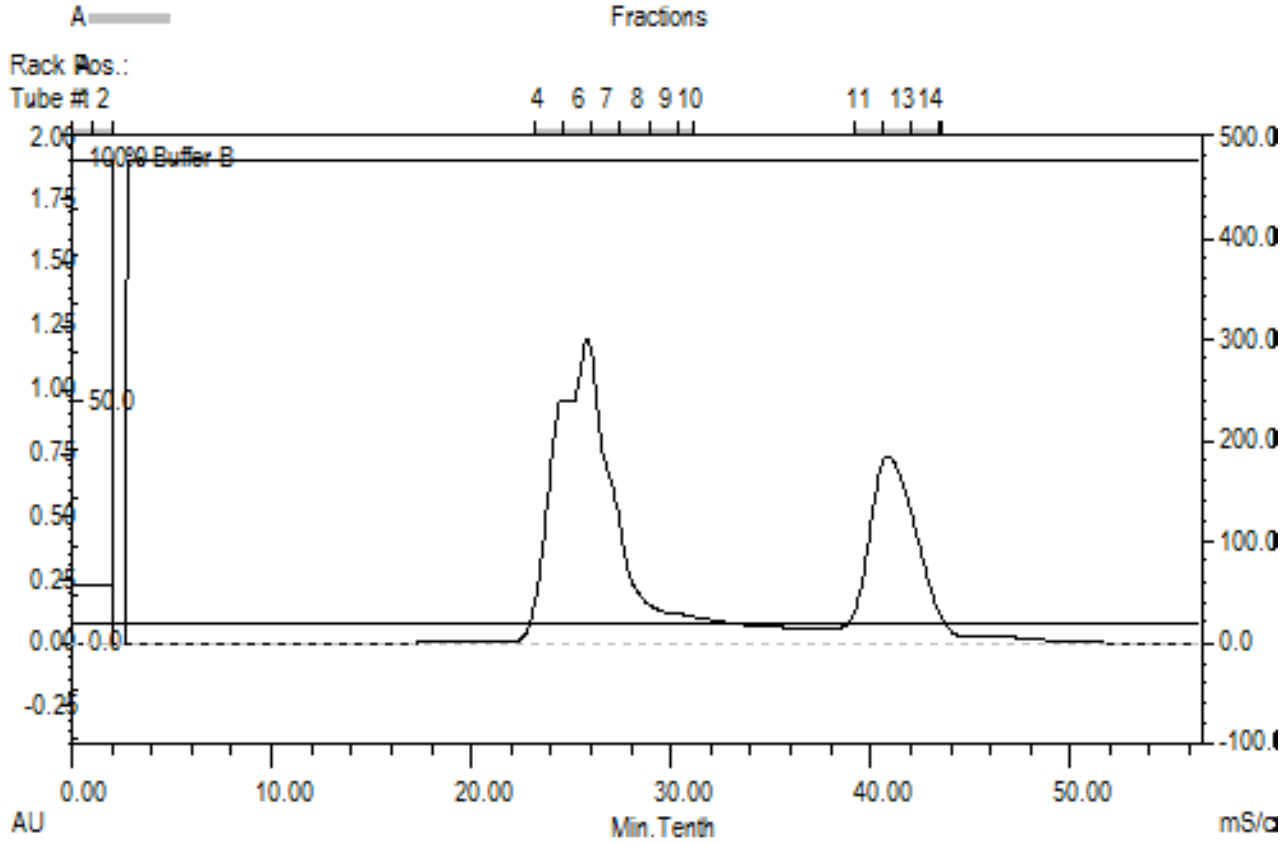
Supplementary Information

A



B

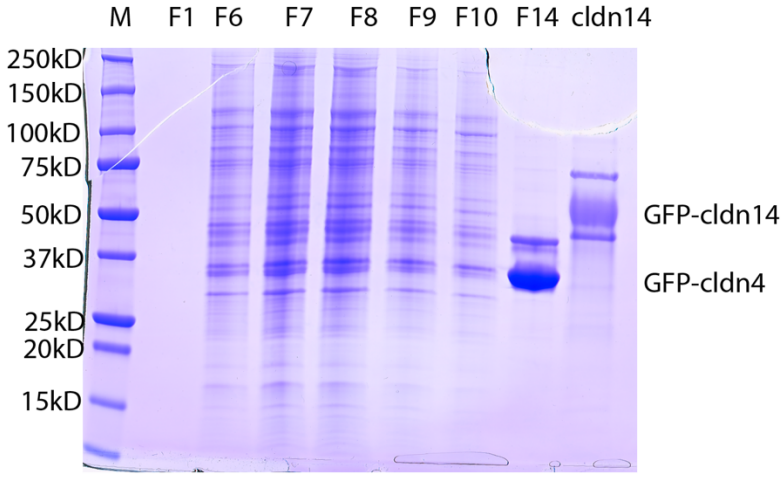


Fig. S1. Purification of recombinant GFP-Cldn4 from *Pichia* yeast cells. (**A**) Gel filtration chromatography showing the separation of GFP-Cldn4 proteins (fraction 11-14) from yeast cell endogenous proteins (fraction 4-10). (**B**) SDS-PAGE gel electrophoresis showing the enrichment of GFP-Cldn4 proteins in fraction 14 (F14). A positive control of purified GFP-CLDN14 is also shown (right).

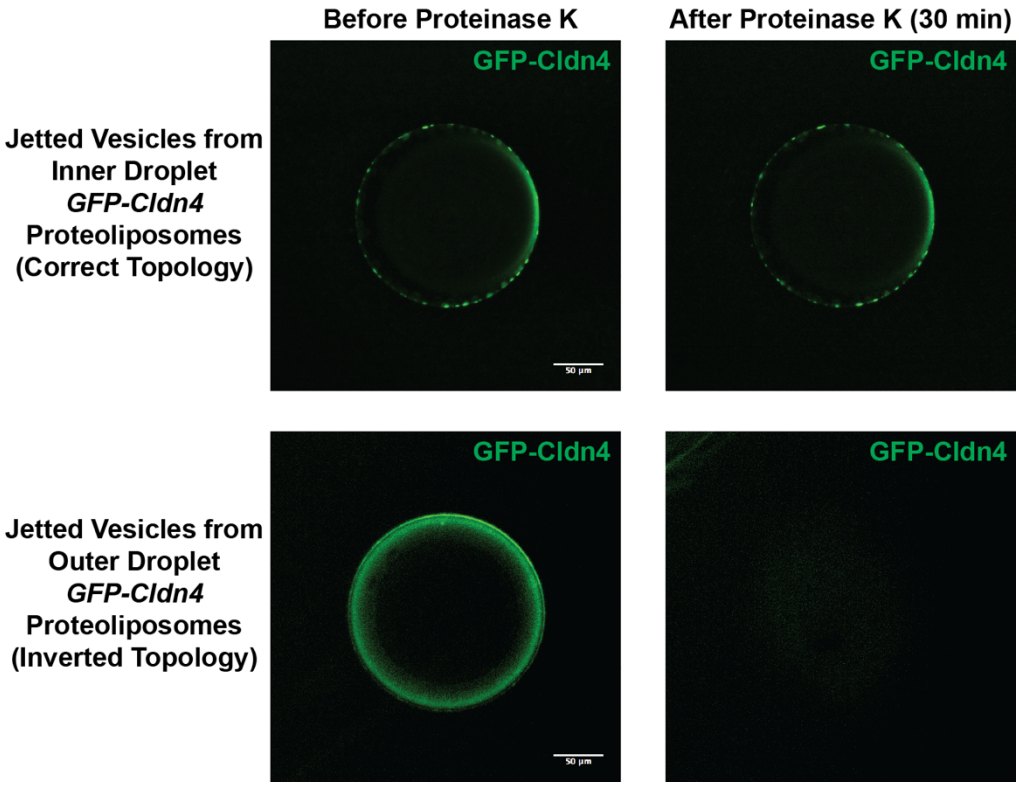


Fig. S2. Proteinase K treatment of GFP-Cldn4 GUVs. GFP-Cldn4 GUVs (left) were jetted from black lipid membranes prepared by incubating either the inner droplet with GFP-Cldn4 proteoliposomes (top panel), leading to GFP in the lumen of the vesicle, or the outer droplet with GFP-Cldn4 proteoliposomes (bottom panel), leading to GFP on the exterior of the vesicle. Fluorescence micrographs of the GFP-Cldn4 GUVs show that only the GFP-Cldn4 incubated in the outer droplet is digested by Proteinase K (right), leading to diminished fluorescence.

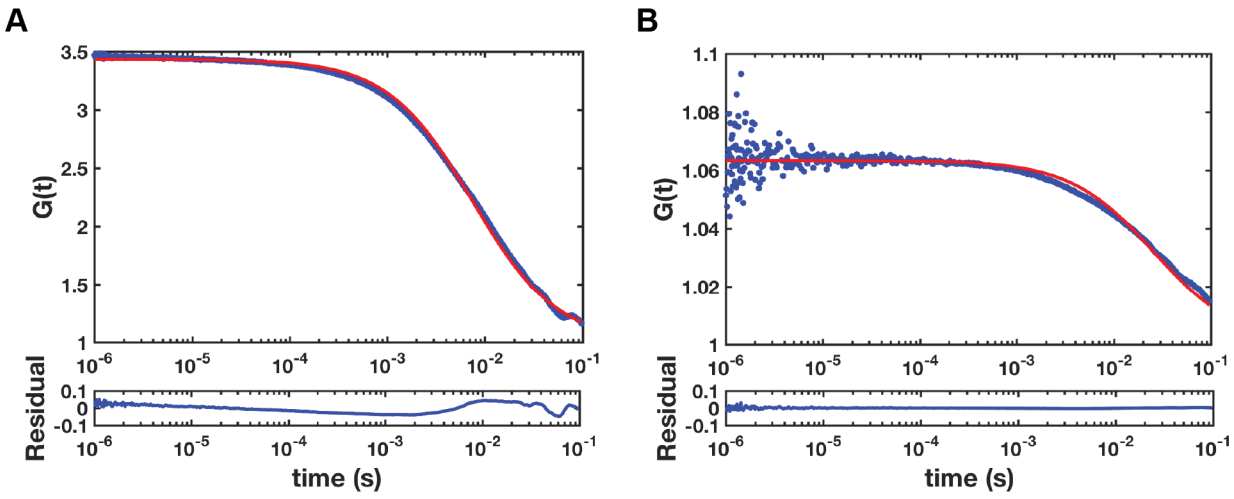


Fig. S3. FCS of GFP-Cldn4 in jetted GUVs. (A) Autocorrelation curve and fit for GFP-Cldn4 at free membrane regions. (B) Autocorrelation curve and fit for GFP-Cldn4 at membrane interfaces. Repeated from Figure 2B for comparative purposes. The diffusion time for GFP-Cldn4 is reduced by over a factor of 3 at membrane interfaces compared to free membrane regions.