Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Evidence of Cytolysin A Nanopore Incorporation in Mammalian Cells Assessed by a Graphical User Interface

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Supplementary Methods

Manual patch-clamp of GPMVs

Giant plasma membrane vesicles (GPMVs) were prepared fresh on the day of experimentation and stored on ice until transferred to the recording chamber of a manual patch-clamp rig.

GPMVs were isolated from naïve CHO-K1 cells grown in a T25 flask (70% confluence) by chemical vesiculation according to Sezgin et al 2012 (10.1038/nprot.2012.059). Briefly, cells were washed twice with 5 ml of ES. The last wash was carefully aspirated and replaced with 1 ml vesiculation buffer (25 mM PFA, 2 mM DTT in ES). The T25 flask was placed in the humidified incubators (37°C, 5% CO2). After 1 h, the presence of vesicles (dark free-floating spheres) was verified under an upright microscope (×20 magnification). The GPMV-rich supernatant was pipetted out and transferred to a microcentrifuge tube. The GPMV suspension was allowed to precipitate in situ for 30 min and the vesiculation buffer was carefully replaced with fresh ES and placed on ice.

Whole-GPMV recordings were obtained using a MultiClamp 700B amplifier controlled by the Clampex11/Digidata1440A acquisition system (Molecular Devices, San Jose, CA, United States) at room temperature (~23°C). The recording solutions were of the same compositions used in automated patch-clamp experiments. Ten microliters of fresh CHO-K1 GPMVs were added to the recording chamber filled with ES and allowed to settle in the bottom (~5-10 min). Fire-polished borosilicate pipettes (8–10 MΩ, Harvard Apparatus) were filled with IS and used to patch-clamp GPMVs in the whole-GPMV configuration. Currents were sampled at 10 kHz, low pass filtered at 1 kHz and series resistance was compensated (60–80%). Currents were elicited with a 1 s prepulse to +40mV followed by a 10 s pulse to –80 mV from a holding potential (Vh) of –40 mV repeated every 15 s. Five minutes of control traces were recorded before the addition of 10 µL of mClyA suspension to the bath. Single channel amplitudes were analyzed using Clampfit11 (Molecular Devices) and data plotted using IgorPro (Wave metrics).

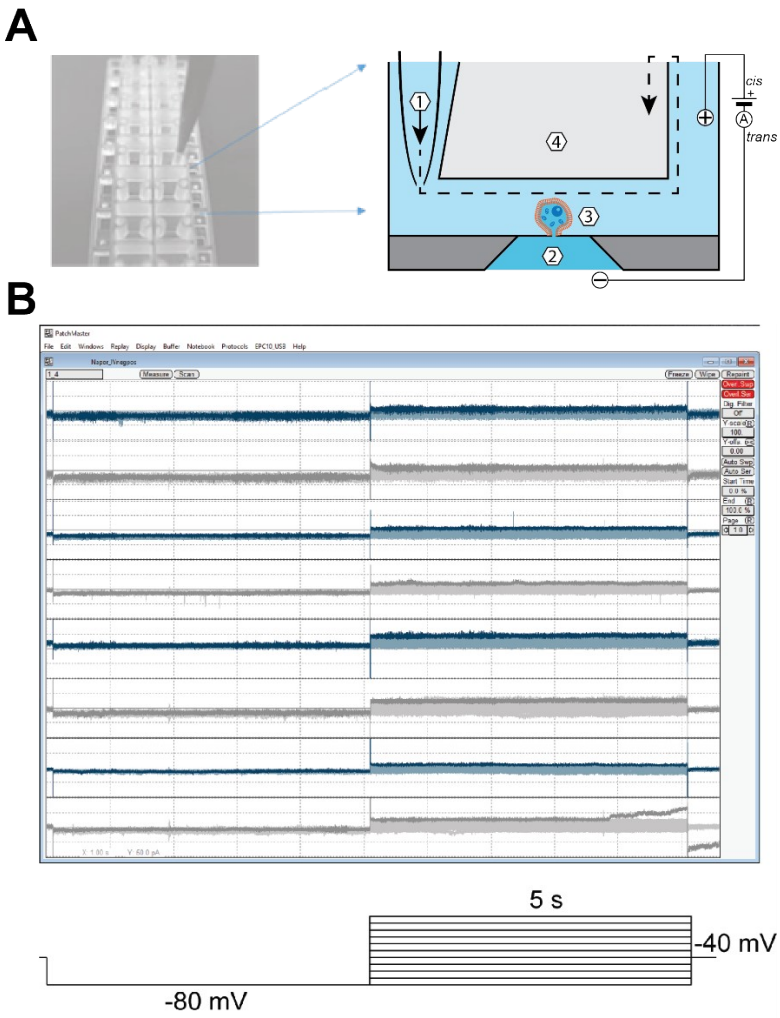

Fig. S1. Automated patch clamp using Patchmaster of naïve CHO-K1 cells.

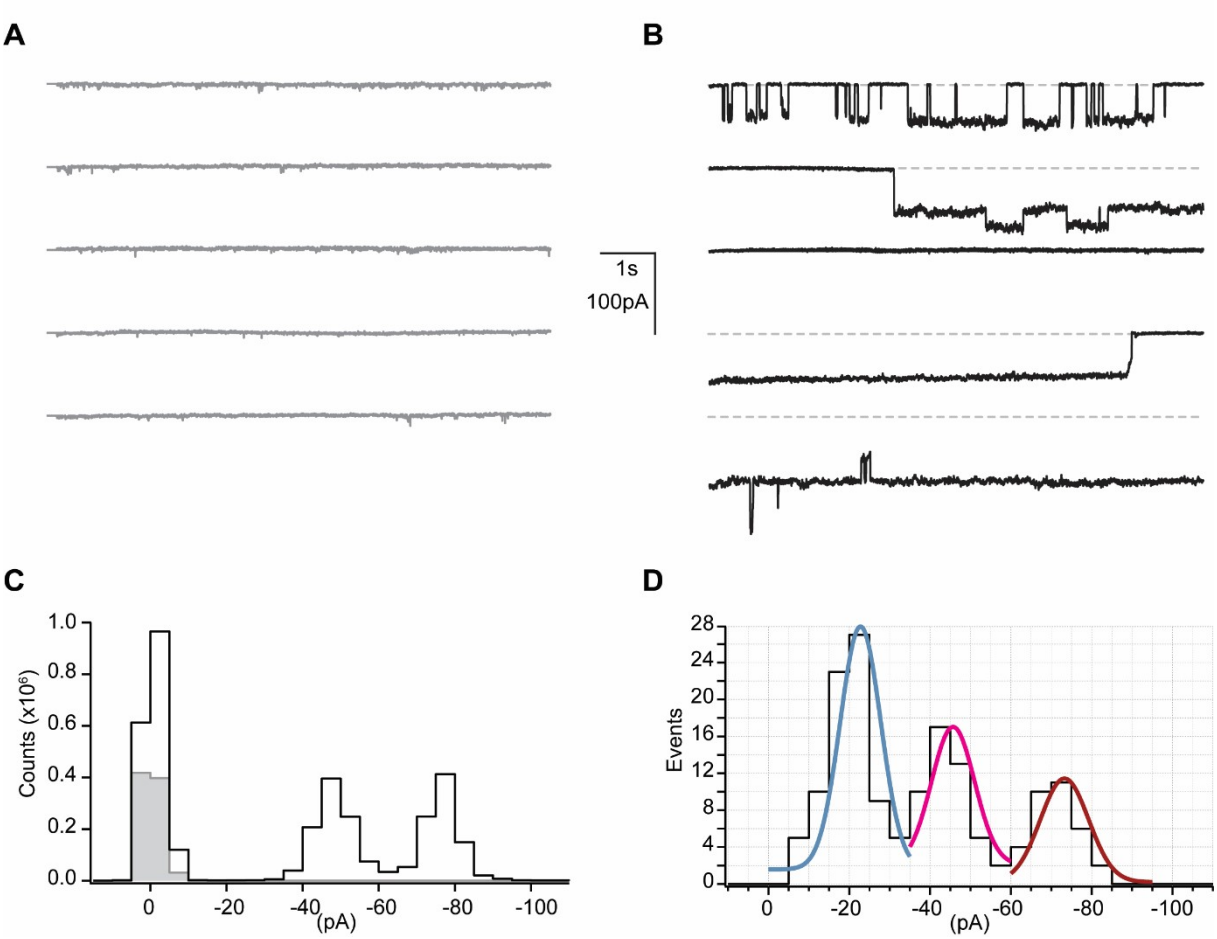

Figure S2. ClyA nanopore mediated currents incorporated into CHO-K1 giant plasma membrane vesicles. A. Whole cell recording of CHO-K1 GPMV before addition of ClyA (Vh= -80 mV, 9.5 s). B. Representative traces from GPMV after addition of ClyA. C. All points histogram (Control, grey, and ClyA,black). D. Histogram of the single channel current in the presence of ClyA highlighting three amplitude levels. 22.8 ± 0.5 pA (teal), 45.7 ± 0.3 pA (magenta), and 73.3 ± 0.3 pA (maroon).