SUPPLEMENTARY DATA

Western blot and gel analysis

To confirm presence of HA-HsTPC2 in the eluted protein complexes western blot analysis with an anti-HA antibody was carried as in Calcraft et al. (2009) (1). Briefly, samples were prepared using 4x Laemmli buffer and 200 μM DTT. To minimize heat-induced aggregation, samples were not boiled. Proteins were resolved by SDS-PAGE with 7 % acrylamide gels and were transferred to PVDF membranes (GE Healthcare Life Sciences, Buckinghamshire, UK). Blocked membranes were incubated with 25 ng/ml anti-HA antibody conjugated directly to HRP (Roche clone 3F10-HRP) diluted in PBS, 0.5 % Tween-20, and blocking agent. Immunoreactive bands were detected by chemiluminescence using ECL reagents (GE Healthcare Life Sciences, Buckinghamshire, UK). Gel analysis and coomassie staining was carried out but amounts of protein loaded were insufficient to allow identification of HA-HsTPC2 by direct means or determine whether any proteins contribute to the properties of the TPC2 complex.

Ion-channel reconstitution

TPC2 always appeared to incorporate into the bilayer in a fixed orientation such that the *cis* chamber always corresponded to the side of the channel that was responsive to NAADP, whereas *trans* NAADP had no effect. We are not certain why this should occur but a similar situation has been reported with other purified ion-channels including RyR2 (2) and TRIC-A channels (3).

Supplementary Fig 1. Mock IP preparations do not respond to NAADP. No single-channel currents were observed after addition of control IP preparations using non-immune rabbit serum (see Fig. 1A) to the *cis* chamber (top trace; n=20). In symmetrical 210 mM KCl solutions, sequential addition of NAADP (10 μM) to the *cis* chamber also did not induce any channel-like events (n=20). The dotted line denotes the zero current level.

Supplementary Fig 2. The effect of high [NAADP] on TPC2 single-channel function. The top trace shows a typical experiment where constitutive, brief TPC2 single-channel openings can be observed in the absence of NAADP. K^+ is the permeant ion and the luminal [Ca^{2+}] is 200 μM, pH 7.2. Po=0.0003±0.0002 (s.d; n=5). Subsequent addition of a high concentration of NAADP (1 mM) completely abolishes channel openings (Po=0; n=5).

Supplementary Fig 3. The effect of high [Ned-19] on TPC2 channel gating. A typical TPC2 control recording in the absence of NAADP. K^+ is the permeant ion and the luminal [Ca^{2+}] is 200 μM, pH 7.2. Occasional, constitutive TPC2 channel openings occur (Po=0.0004±0.0004 (s.d.; n=3) in the absence of NAADP but following the cytosolic addition of 1 μM Ned-19, no further brief openings are observed (Po=0, n=3) showing that Ned-19 (1 μM) closes TPC2 channels.

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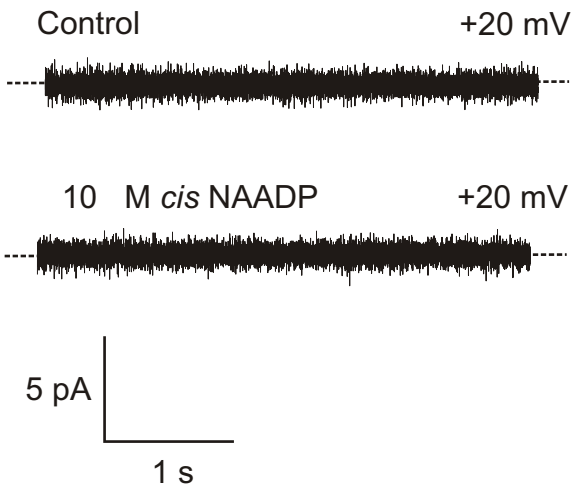
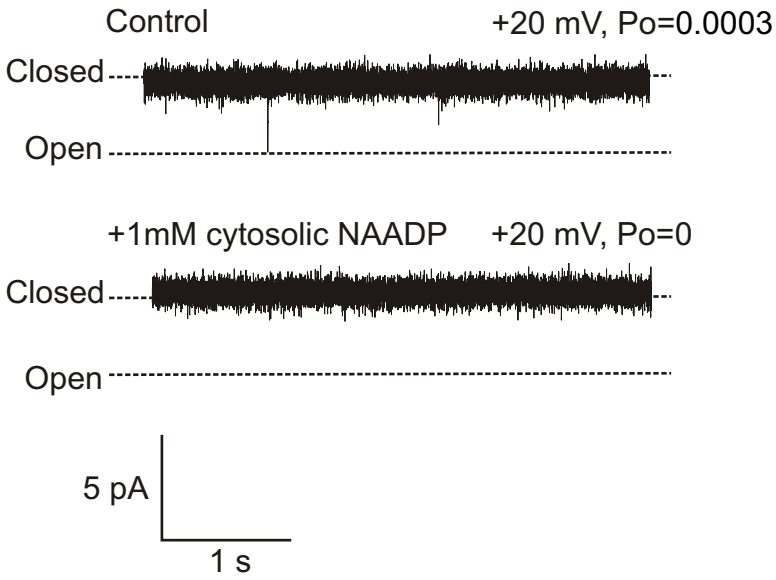
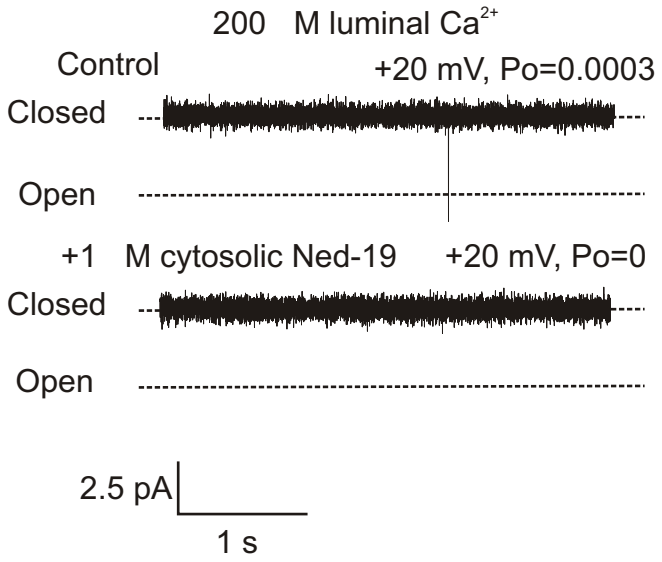


Supplementary Fig 1.



Supplementary Fig 2.



Supplementary Fig 3.