SUPPLEMENTAL DATA

AN NAADP-GATED TWO-PORE CHANNEL TARGETED TO THE PLASMA MEMBRANE UNCOUPLES TRIGGERING FROM AMPLIFYING Ca^{2+} SIGNALS

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Supplemental text

Detection of Single NAADP-Gated Channels in Excised Patches - Assuming that TPC2ΔN channels are randomly distributed in the plasma membrane, the number detected in a patch (*N*) is given by the Poisson distribution:

$$P_N = e^{-x}.\frac{x^N}{N!}$$

where P_N is the probability of detecting *N* channels, and x is the mean number of channels per patch. With ~60% of excised patches from cells expressing TPCΔN failing to respond to NAADP (36/61, see text), this suggests that the mean number of channels/patch is ~0.5, and only ~10% of patches would be expected to have more than one channel. This is consistent with our observations, where 5 from 61 recordings (8%) had more than one simultaneous opening.

Table S1. Sequences of the Primers Used. F and R denote forward and reverse primers.

Primer	Sequence
1F	5'-CACCATCGATATGGCTCTGACCTTGGATGAGGGTG-3'
1R	5'-CCTTGAATTCGAGGTAACGGTCTGGGAGCG-3'
2F	5'-CACCGAATTCATGGCGCTGACCACTTACCGCAGC-3'
2R	5'-AGGCTCGAGCCTGCACAGCCACAGGT-3'
3F	5'-CCTCGGATGTCACCAGGGCGGCGGAGACCCTCTCCCAGATG-3'
3R	5'-CGCCGCCCTGGTGACATCATCCGAGGA-3'
4F	5'-TTCCTGAAGGTTGCCGG-3'
5F	5'-CGGAGTCGGAGCCCGCGGCGGGCGGGGCCCGCGG-3'
5R	5'-CCGCGGGCCCCGCCCGCCGCGGGCTCCGACTCCG-3'
6F	5'-GTCTCTGACTTCCCCCCTGGTGCTGCTGA-3'
6R	5'-TCAGCAGCACCAGGGGGGAAGTCAGAGAC-3'

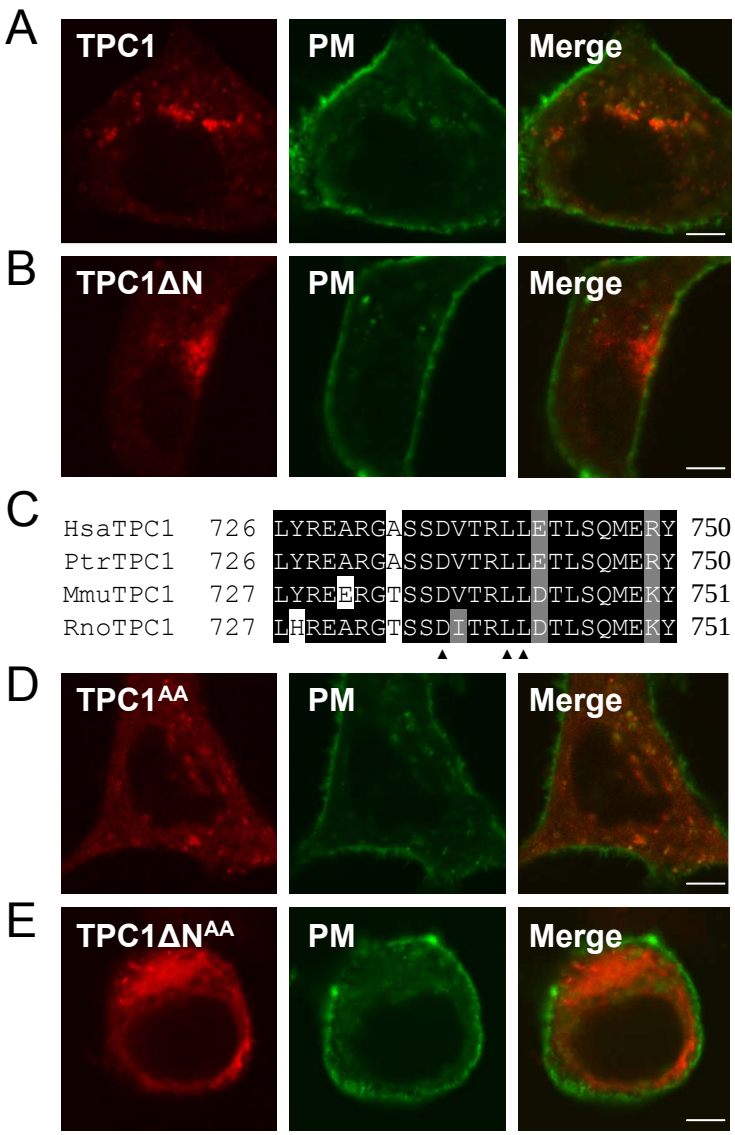
FIGURE S1. **Removal of di-leucine motifs does not cause TPC1 to be expressed at the plasma membrane.** *A-B*, Confocal fluorescence images of SKBR3 cells co-expressing GFP-tagged human reduced folate carrier to delineate the plasma membrane (PM) and either mRFP-tagged TPC1 (*A*) or TPC1 lacking residues 3-12 (TPC1ΔN, *B*). Merged images are overlays of the plasma membrane marker (green) and TPC constructs (red). *C*, Sequence alignment of human (Hsa), chimpanzee (Ptr), mouse (Mmu) and rat (Rno) TPC1 sequences highlighting a conserved di-leucine endo-lysosomal targeting motif within the C-terminal tail of TPC1 (arrows). (*D-E*) Images (similar to those shown in *A* and *B*) of cells expressing mRFP-tagged TPC1 in which only the C-terminal di-leucine motif was mutated ($TPC1^{AA}$, *D*) or in which this was combined with removal of the N-terminus containing its di-leucine motif ($TPC1\Delta N^{AA}$, *E*). Scale bars = 5 μm. Images are typical of those from 10-15 cells.

FIGURE S2. **Removal of the N-terminus redirects TPC2 from lysosomes to the plasma membrane.** *A*, Western blot analysis of HEK cell extracts expressing GFP-tagged TPC2 or TPC2ΔN. Samples (10 μg/lane) were incubated in the absence (-) or presence (+) of PNGaseF prior to analysis. Arrow heads mark the positions of fully glycosylated (►), core glycosylated (►) and de-glycosylated (▷) TPC2 or TPC2ΔN. Results are representative of 3 experiments. *B*, Confocal fluorescence images of HEK cells co-expressing GFP-tagged human reduced folate carrier to delineate the plasma membrane (PM) and either mRFP-tagged TPC2 (top) or mRFP-tagged TPC2 lacking residues 3-25 (TPC2ΔN; bottom). Merged images are overlays of the plasma membrane marker (green) and TPC constructs (red). Transect analyses of red and green fluorescence intensities across the regions marked by dotted lines are shown to the right. *C*, Confocal fluorescence images (left) and transect analysis (right) of the indicated cell type expressing GFP-tagged TPC2 in which Leu11 and Leu12 were replaced with Ala ($TPC2^{AA}$). *D*, Epifluorescence (Epi.) and TIRF images of SKBR3 cells expressing C-terminally GFP-tagged TPC2, TPC2ΔN or $TPC2^{AA}$. *E*, Confocal fluorescence images (left) and transect analysis (right) of HEK cells expressing GFP-tagged TPC2 (top) or TPC2ΔN (bottom) in which Leu265 was replaced with Pro ($TPC2^{L265P}$ and $TPC2\Delta N^{L265P}$). Images (*B-E*) are typical examples from samples of 6-20 cells. All scale bars = 5 μm.

FIGURE S3. **Variation in single-cell responses to NAADP injection.** *A-B*, Cytosolic Ca^{2+} responses of individual fura-2-loaded SKBR3 cells expressing GFP-tagged TPC2 (*A*, n = 10) or TPC2ΔN (*B*, n = 16) and microinjected with 10 nM NAADP (arrowheads).

FIGURE S4. **TPC2 targeted to the plasma membrane is uncoupled from ryanodine receptors.** *A-C*, Summary of data from Fig. 2 quantifying amplitudes of the Ca^{2+} signals in individual cells (mean ± s.e.m) expressing TPC2 (black) or TPC2ΔN (red). *A-B*, Responses to NAADP injection (10 nM) from untreated cells (-) or cells incubated with ryanodine (10 μM, 15 min), bafilomycin-A_1 (1 μM, 60 min) or NED19 (100 nM, 15 min). *C*, Responses to NAADP (10 nM) in Ca^{2+}-free medium or cyclic ADP-ribose (500 nM, in Ca^{2+}-containing medium). *D*, Summary data quantifying the times to peak of the Ca^{2+} signals in individual cells (mean ± s.e.m) expressing TPC2 (black, n= 7) or TPC2ΔN (red, n = 13).

FIGURE S5. **NAADP stimulates Ca^{2+} currents mediated by TPC2ΔN.** An excised patch-clamp recording (similar to that shown in Fig. 4*F*) from a patch held at -100 mV, stimulated with 500 nM NAADP and with Ca^{2+} as charge-carrier. The three traces show a continuous recording lasting ~30 s. C denotes the closed state.


A
TPC1
PM
Merge
B
TPC1ΔN
PM
Merge
C
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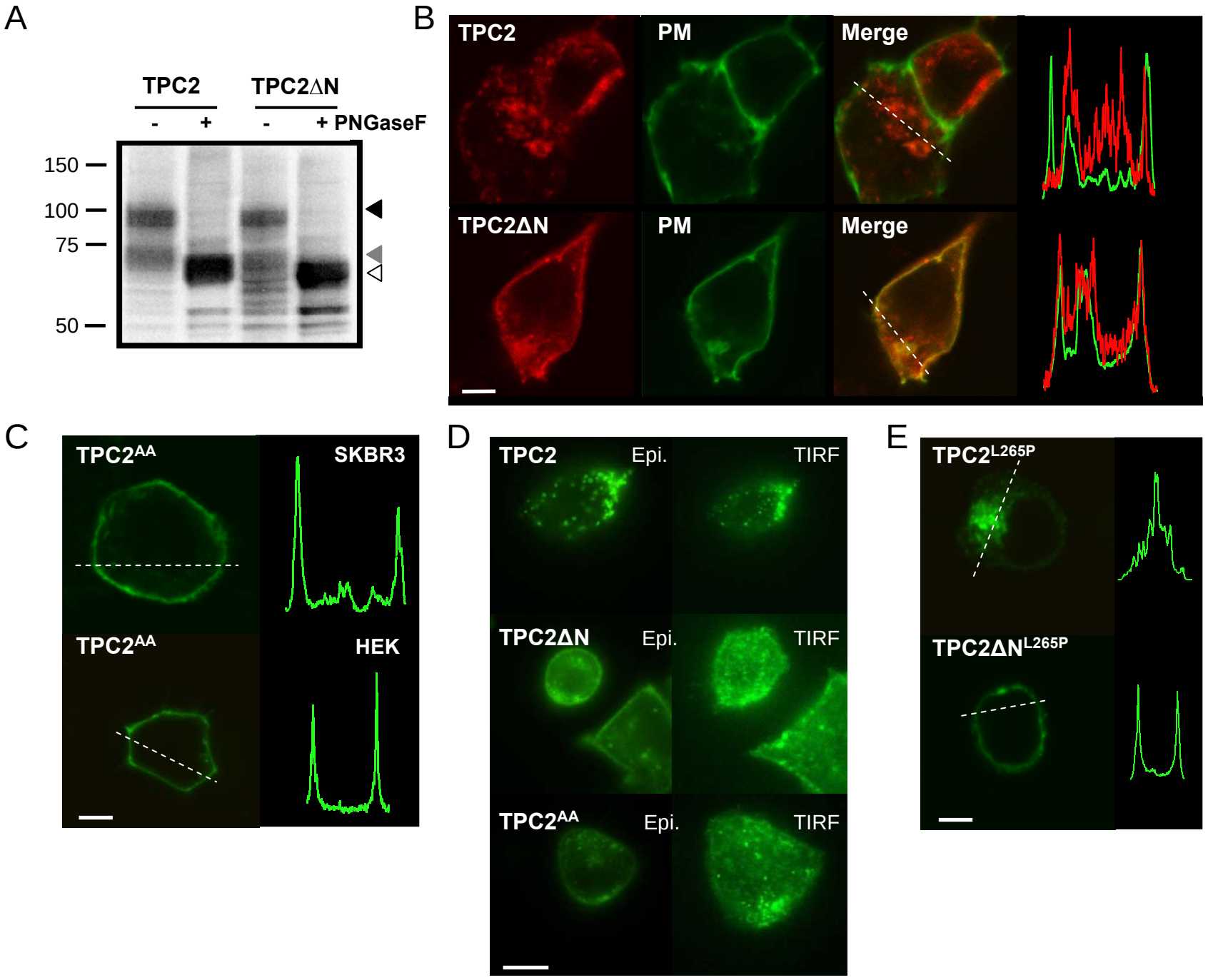
PtrTPC1 726 LYREARGASSDVTRLLETLSQMERY 750
MmuTPC1 727 LYREERGTSSDVTRLLDTLSQMEKY 751
RnoTPC1 727 LHREARGTSSDITRLLDTLSQMEKY 751
D
TPC1AA
PM
Merge
E
TPC1ΔNAA
PM
Merge

Figure S1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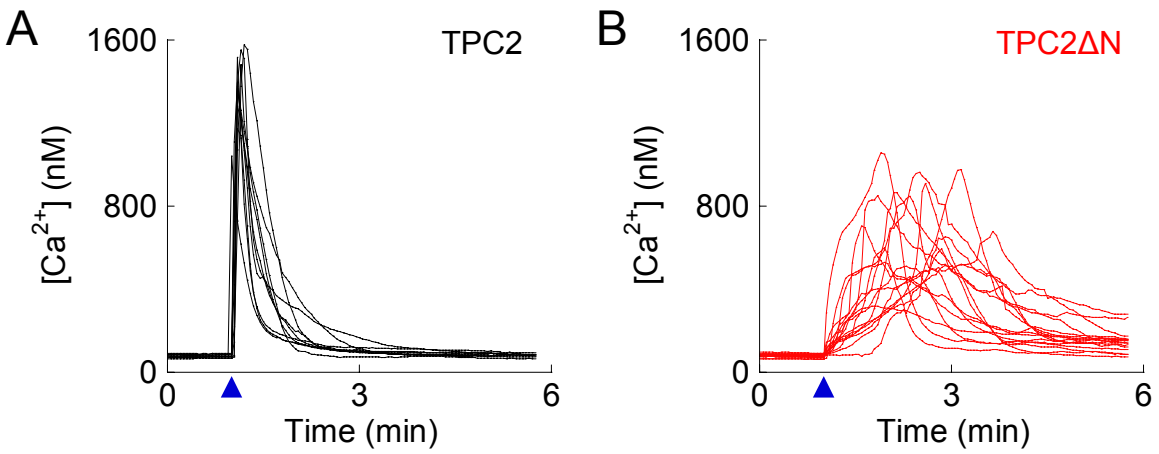
TPC2
TPC2ΔN
- + - + PNGaseF
150
100
75
50
B
TPC2
PM
Merge
TPC2ΔN
PM
Merge
C
TPC2AA
SKBR3
TPC2AA
HEK
D
TPC2
Epi.
TIRF
TPC2ΔN
Epi.
TIRF
TPC2AA
Epi.
TIRF
E
TPC2L265P
TPC2ΔNL265P

Figure S2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TPC2
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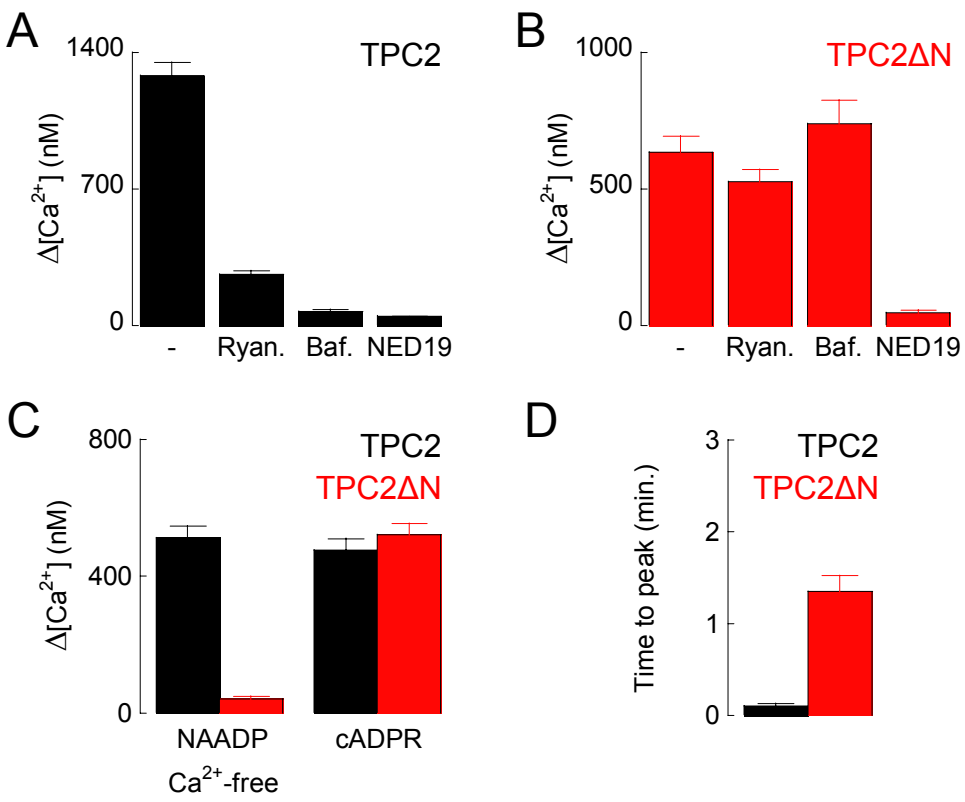
800
0
[Ca2+] (nM)
0
3
6
Time (min)
B
TPC2ΔN
1600
800
0
[Ca2+] (nM)
0
3
6
Time (min)

Figure S3


A
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700
0
Δ[Ca2+] (nM)
TPC2
-
Ryan.
Baf.
NED19
B
1000
500
0
Δ[Ca2+] (nM)
TPC2ΔN
-
Ryan.
Baf.
NED19
C
800
400
0
Δ[Ca2+] (nM)
TPC2
TPC2ΔN
NAADP
cADPR
Ca2+-free
D
3
2
1
0
Time to peak (min.)
TPC2
TPC2ΔN

Figure S4

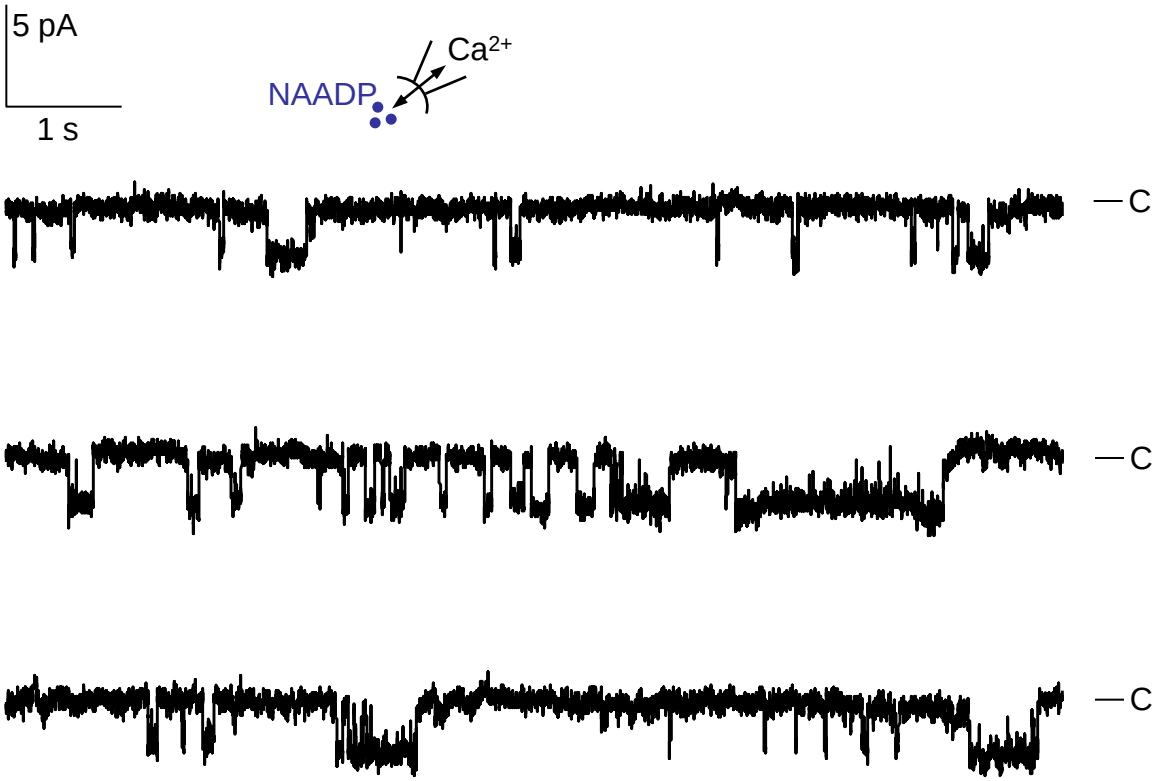

Figure S5