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Supplementary Materials for

mTORC1 controls lysosomal Ca^{2+} release through the two-pore channel TPC2

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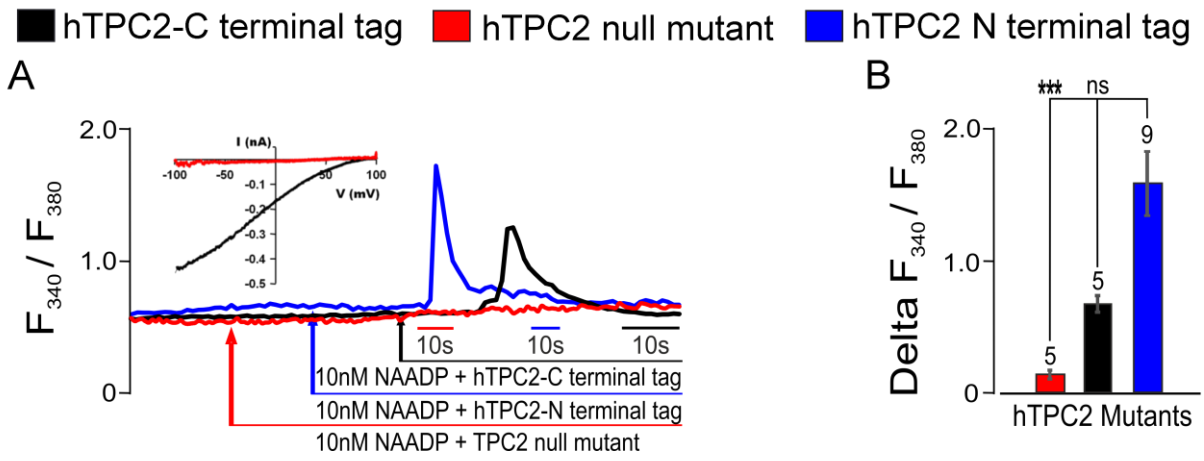

Fig. S1. Blind experiments on active and null hTPC2 constructs demonstrate robustness of intracellular dialysis technique. A, Records of F340 / F380 ratio against time during intracellular dialysis of 10 nmol/L NAADP into HEK 293 cells expressing either wild type hTPC2 with a C terminal EGFP tag (black), hTPC2 with an N terminal EGFP tag (blue) or an hTPC2 null mutant but lacking a $PI(3,5)P_2$-induced Na^+ current (red); arrows indicate the beginning of intracellular dialysis upon entering the whole-cell configuration. Inset shows whole-endolysosomal current evoked by 1 µmol / L $PI(3,5)P_2$ recorded from enlarged vacuoles isolated from HEK 293 cells that expressed C-terminal EGFP-tagged hTPC2 (black) or the hTPC2 null mutant (red). **B**, bar chart showing mean ± SEM for each construct tested. Number of experiments (n) indicated above bars $***P < 0.001$.

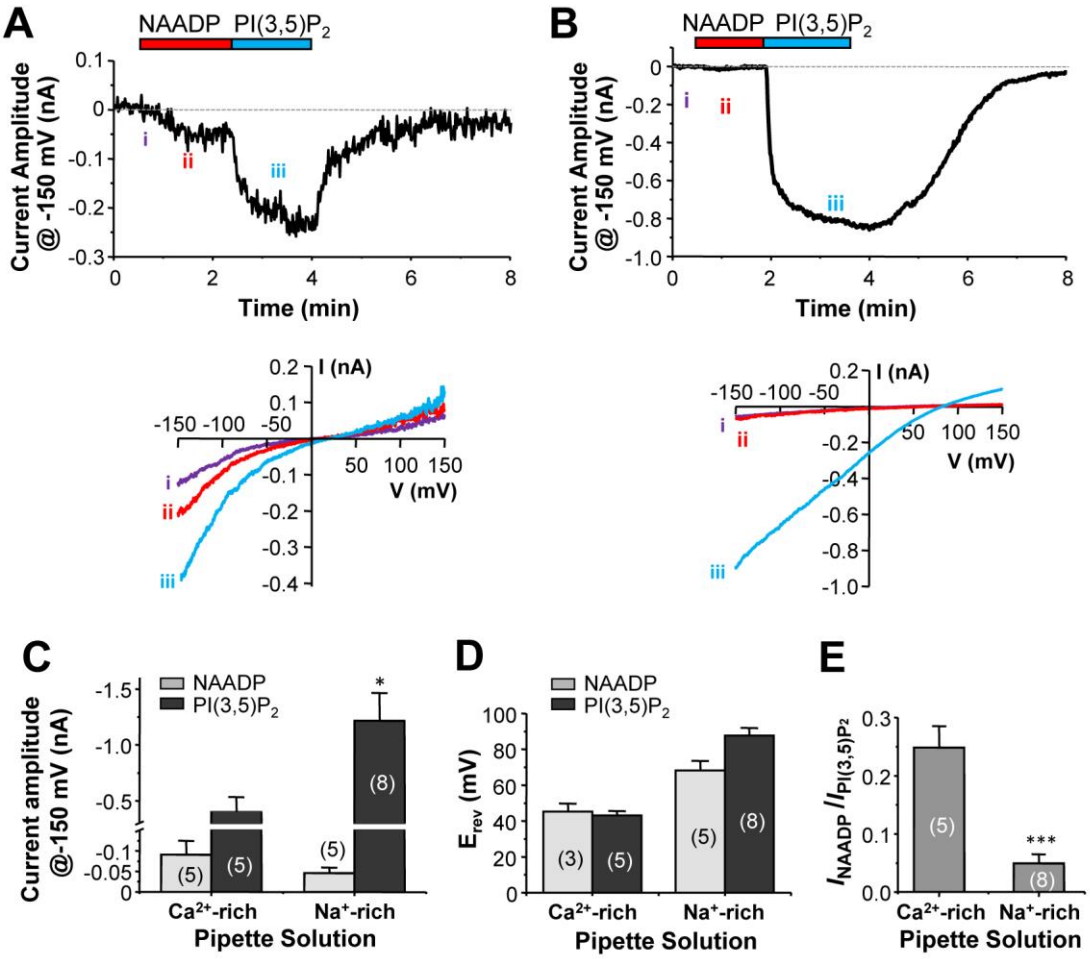

Fig. S2. Na^+ and Ca^{2+} currents mediated by endolysosomal TPC2 in response to NAADP and PI(3,5)P_2. A and **B**, representative traces of currents at -150 mV (*upper traces)* and current-voltage (I-V) curves at the indicated time points (*lower traces*). For A, the pipette solution was Ca^{2+}-rich (Na^+-free) and contained (in mmol / L) 70 K-methanesulfonate (MSA), 60 Ca-MSA, 1 $MgCl_2$, 10 Hepes (pH adjusted with MSA to 4.6 and mannitol used to adjust osmolarity); the bath solution contained (in mmol / L) 130 K-MSA, 0.2 mM Ca-MSA, 10 Hepes (pH adjusted with KOH to 7.2). For B, the pipette solution was Na^+-rich and contained (in mmol / L) 145 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, 10 MES, 10 glucose (pH adjusted with NaOH to 4.6); the bath solution contained (in mmol / L) 140 K-gluconate, 4 NaCl, 1 EGTA, 2 $MgCl_2$, 0.39 $CaCl_2$, 20 Hepes (pH adjusted with KOH to 7.2; free [Ca^{2+}] = 100 nM). NAADP (100 nmol / L) and PI(3,5)P_2 (1 μmol / L) were applied by perfusion to the cytoplasmic side of the endolysosomal vacuole as indicated by the bars above the current trace. **C-E**, bar charts show the mean ± SEM for the peak of current amplitude at -150 mV (C) and reversal potential (E_{rev}) of currents (D) evoked by 100 nmol / L NAADP and 1 μmol / L PI(3,5)P_2, as well as ratio of peak current evoked by NAADP to that by PI(3,5)P_2 (E) in the Ca^{2+}-rich or Na^+-rich pipette solution. Values were determined after subtracting basal current. The numbers of vacuoles recorded are indicated in parentheses. For some vacuoles, the E_{rev} was indiscernible because the evoked current was too small. $^*P$ <0.05, $^{***}P$<0.001 *vs.* Ca^{2+}-rich by unpaired *t* test.

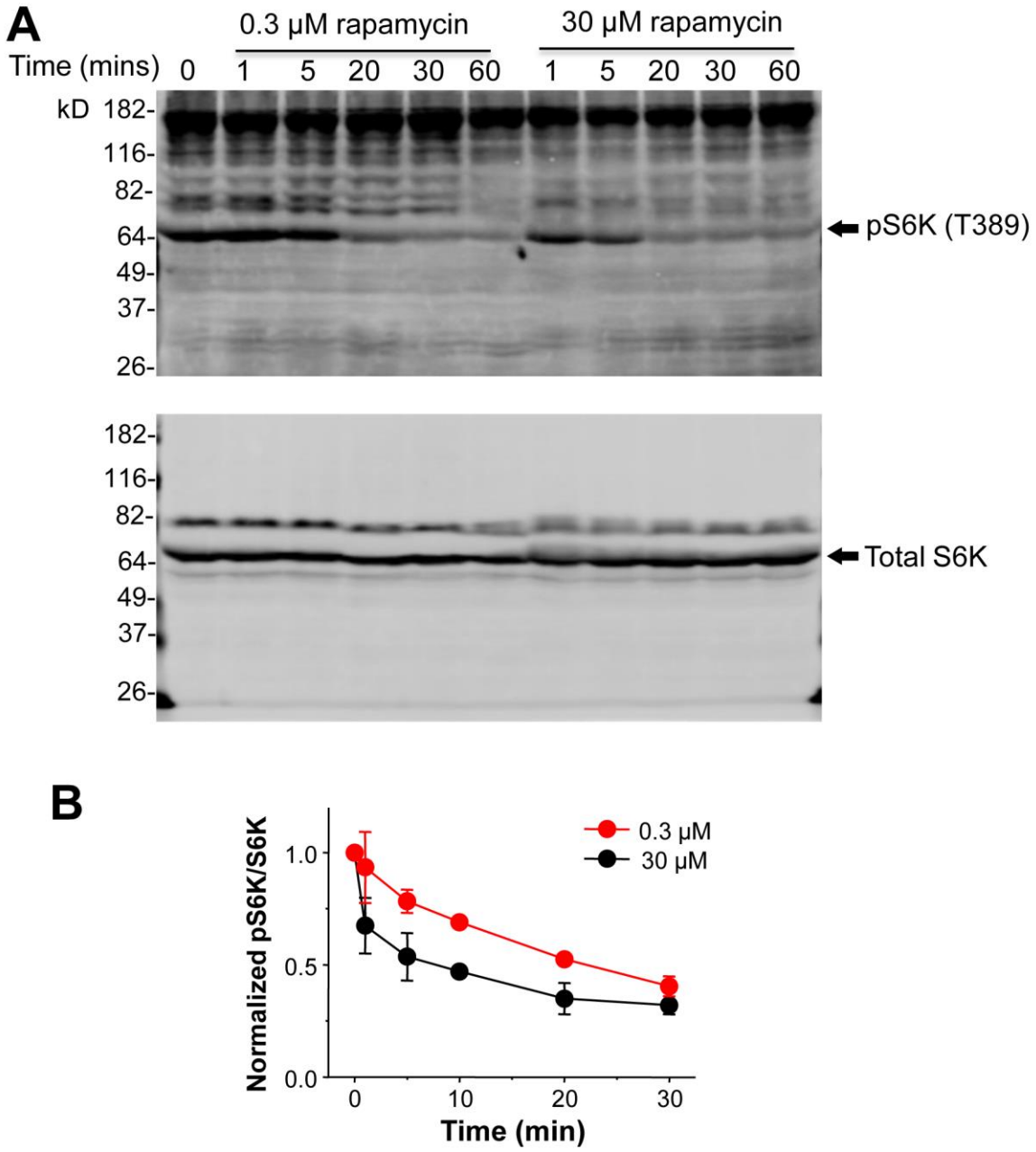

Fig. S3. High and low concentrations of rapamycin suppress mTORC1 activities in HEK293 cells at different rates. A, Upper panel shows full immune-blot of phospho-S6K (pS6K) versus time as an index of mTORC1 activity for HEK 293 cells treated with either 0.3 or 30 μmol / L rapamycin for the time periods indicated; at each time point cells were lysed immediately. Lower panel shows full immunoblot for total S6K at each time point. **B**, summary data for pS6K / total S6K ratios (normalized to untreated cells) compared to time. Each data point represents results from 2 to 4 experiments expressed as mean ± SD or range.

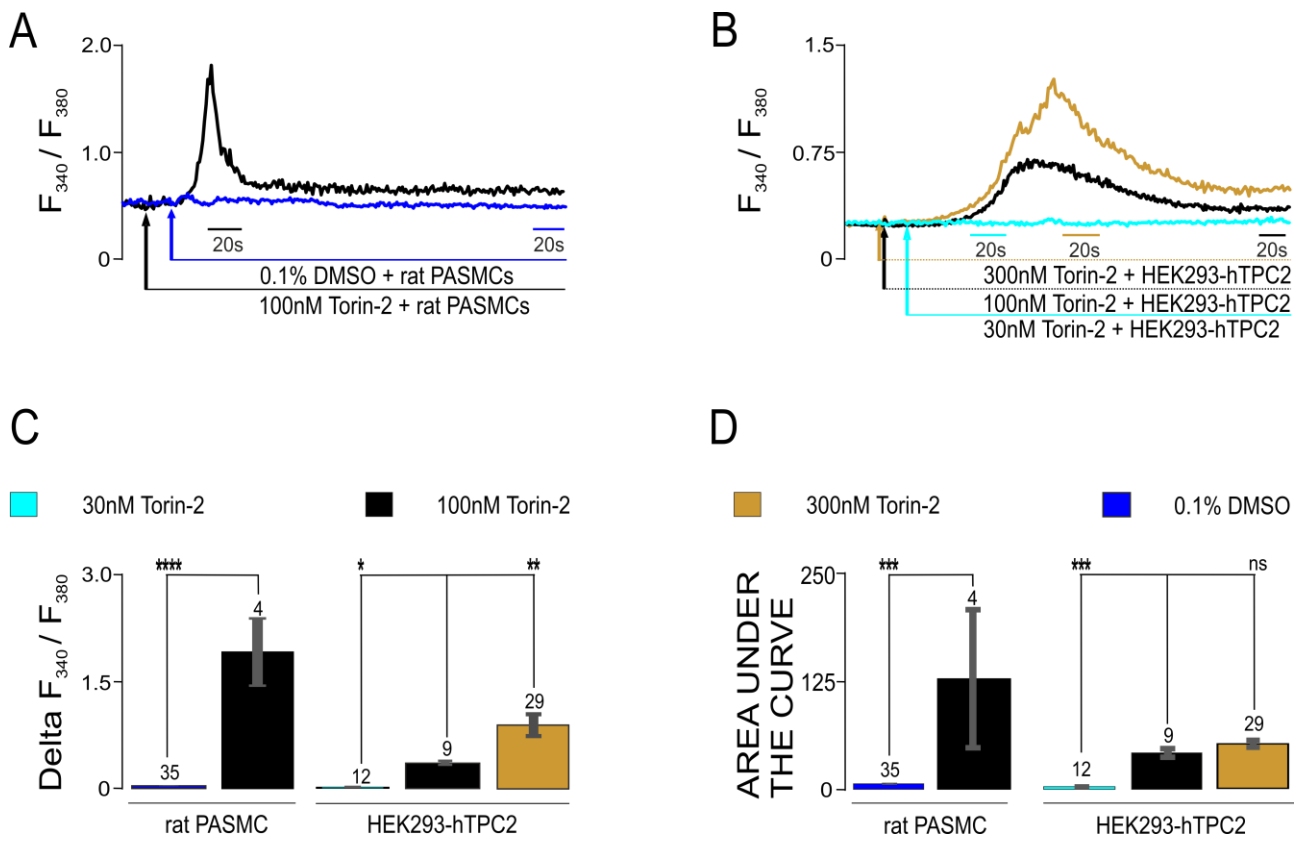

Fig. S4. Torin-2 induces increases in intracellular Ca^{2+} in HEK293 cells stably overexpressing hTPC2 and in rat pulmonary arterial myocytes. A, Records of F340 / F380 ratio against time during extracellular application of 100 nmol / L torin-2 (black) and vesicle (0.1% DMSO, blue) on to a rat pulmonary arterial myocyte. **B**, Records of F340 / F380 ratio against time during extracellular application of 30 (cyan), 100 (black) and 300 (brown) nmol / L torin-2 on to HEK 293 cells stably overexpressing hTPC2. **C** and **D**, bar charts showing mean ± SEM for each cell type and treatment for the peak change in Fura-2 fluorescence ratio (Delta F340 / F380; C) and area under the curve (D). Number of cells (n) indicated above bars. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

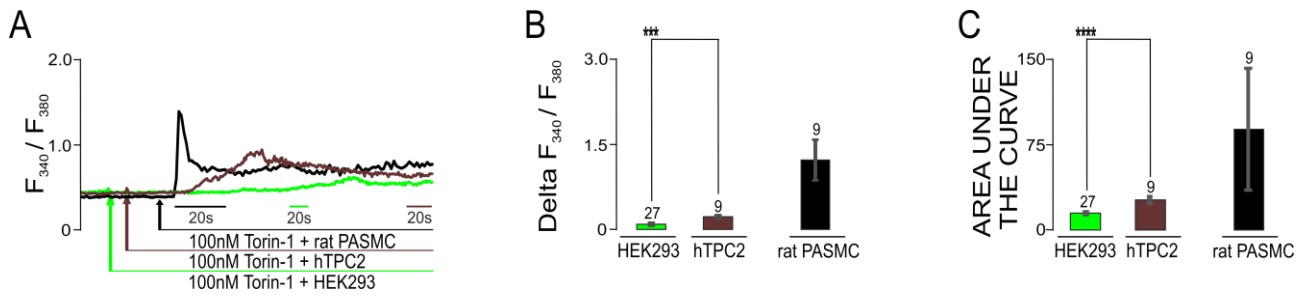

Fig. S5. Torin-1 induces low-magnitude, sustained increases in intracellular Ca^{2+} in HEK293 cells stably overexpressing hTPC2 and in rat pulmonary arterial myocytes. A, Records of F340 / F380 ratio against time during extracellular application of 100 nmol / L torin-1 on to a wild type HEK 293 cell (green), a HEK 293 cell stably overexpressing hTPC2 (brown) and a pulmonary arterial myocyte (black). Note, the fluorescence ratio did not return to baseline at the end of the recording. **B**, bar charts showing mean ± SEM for each cell type for the peak change in Fura-2 fluorescence ratio (Delta F340 / F380; B) and area under the curve (**C**). Number of cells (n) indicated above bars. ***$P < 0.001$, ****$P < 0.0001$.