Supplementary Information

Presynaptic Kv3 channels are required for fast and slow endocytosis of synaptic vesicles

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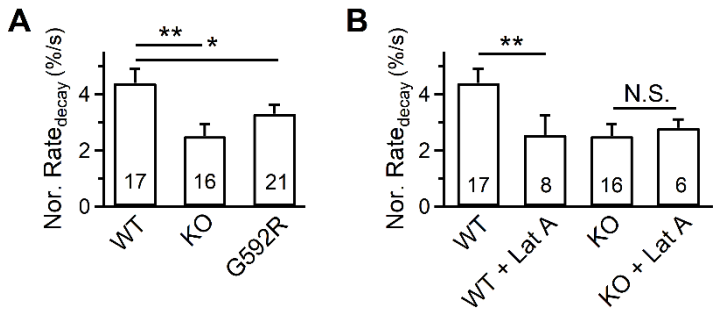

Figure S1. Kv3.3 KO or Kv3.3$_{G592R}$ KI reduced the normalized Rate$_{decay}$ at hippocampal synapses, related to Figure 4.

(A) The normalized Rate$_{decay}$ (Nor. Rate$_{decay}$) after 200 APs at 20 Hz in WT, Kv.3.3$^{-/-}$ (KO), or Kv3.3$_{G592R}$ boutons at 34-37°C. The normalized Rate$_{decay}$ was measured from F$_{SypH}$ with a different normalization method: ΔF, the F$_{SypH}$ increase induced by the 200-AP train, but not the F$_{SypH}$ baseline, was normalized as 100%. **: $p < 0.01$ (comparison between KO and WT); *: $p < 0.05$ (comparison between Kv3.3$_{G592R}$ and WT boutons); statistical test is t test. The experimental number is written inside the bar; each experiment contained 20-30 boutons; 1-3 experiments were taken from 1 culture; each culture was from 3-5 mice.

The normalization method used here removed the impact of ΔF on the measurement of the Rate$_{decay}$. The results shown in panel A are similar to those (See Figures 4E and 4F) without such normalization, indicating that the results drawn from Figures 4E and 4F are independent of the changes in ΔF.

(B) Similar to panel A, except from WT boutons, WT boutons in the presence of 20 μM latruculin A (WT + Lat A), Kv.3.3$^{-/-}$ boutons (KO), or KO boutons in the presence of 20 μM latruculin A (KO + Lat A). **: $p < 0.01$ (comparison between WT and WT+Lat A); N.S.: not significant, $p > 0.05$ (comparison between KO and KO + Lat A); statistical test is t test.

The results shown in panel B are similar to those (See Figures 4I and 4J) without such normalization, indicating that the results drawn from Figures 4I and 4J are independent of changes in ΔF.

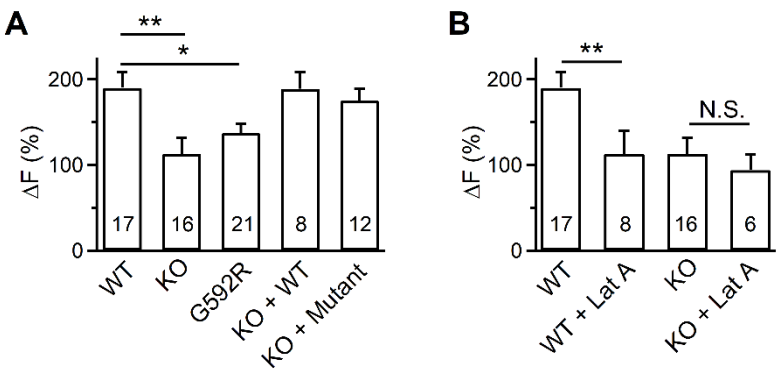

Figure S2. Peak SypH fluorescence increase induced by 200 APs at 20 Hz in various conditions, related to Figure 4.

(A) Peak SypH fluorescence increase induced by 200 APs at 20 Hz (ΔF, mean + s.e.m.) at hippocampal cultures in five conditions: 1) WT boutons, 2) Kv3.3$^{-/-}$ boutons (KO), 3) Kv3.3$_{G592R}$ boutons, 4) Kv.3.3$^{-/-}$ boutons overexpressed with WT Kv3.3 (KO + WT), 5) Kv.3.3$^{-/-}$ boutons overexpressed with W496F Kv3.3 mutant with no ionic conductance (KO + Mutant). **: $p < 0.01$ (comparison between WT and KO); *: $p < 0.05$ (comparison between WT and Kv3.3$_{G592R}$); statistical test is t test. The experimental number is written inside the bar; each experiment contained 20-30 boutons; 1-3 experiments were taken from 1 culture; each culture was from 3-5 mice.

(B) Similar to A, except from different conditions: 1) WT boutons, 2) WT boutons in the presence of 20 μM latrunculin A (WT + Lat A), 3) Kv.3.3$^{-/-}$ boutons (KO), and 4) KO boutons in the presence of 20 μM latrunculin A (KO + Lat A). **: $p < 0.01$ (comparison between WT and WT + Lat A); N.S.: not significant, $p > 0.05$ (comparison between KO and KO + Lat A); statistical test is t test.

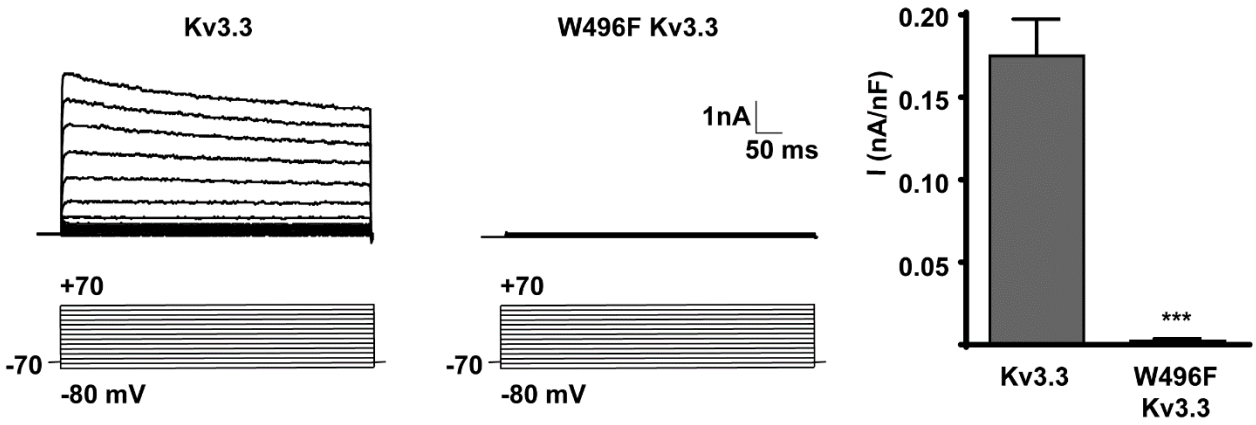

Figure S3. W496F Kv3.3 mutation abolishes potassium currents in CHO cells, related to Figure 4.

Left and Middle: representative traces of currents recorded in CHO cells expressing wild type Kv3.3 or W496F Kv3.3. Currents were evoked from a holding potential of -70 mV to test potentials between -80 mV and +70 mV in 10 mV increments.

Right: bar graph shows group data for current density recorded at +70 mV. Current density was calculated by dividing the peak current by cell capacitance. Values are shown as mean ± s.e.m. (n = 4, 4), and significance was tested using an unpaired two-tailed t- test, ***p=0.0002.

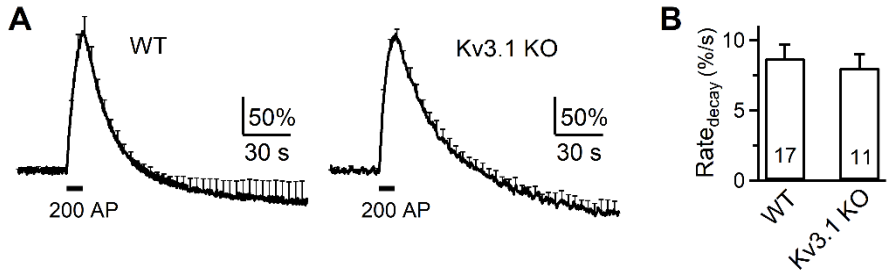

Figure S4. Kv3.1 knockout did not affect endocytosis at hippocampal synapses, related to Figure 4.

(A-B) F_{SypH} (mean + s.e.m., A) and $Rate_{decay}$ (mean + s.e.m., B) induced by 200 APs at 20 Hz in WT and Kv3.1$^{-/-}$ (Kv3.1 KO) boutons at 34-37ºC. F_{SypH} (A) was normalized to baseline; s.e.m. (A) is plotted every 4 s. The experimental number is written inside the bar of panel B, which also applies to panel A. $Rate_{decay}$ in panel B is not significantly different ($p > 0.05$, t test).