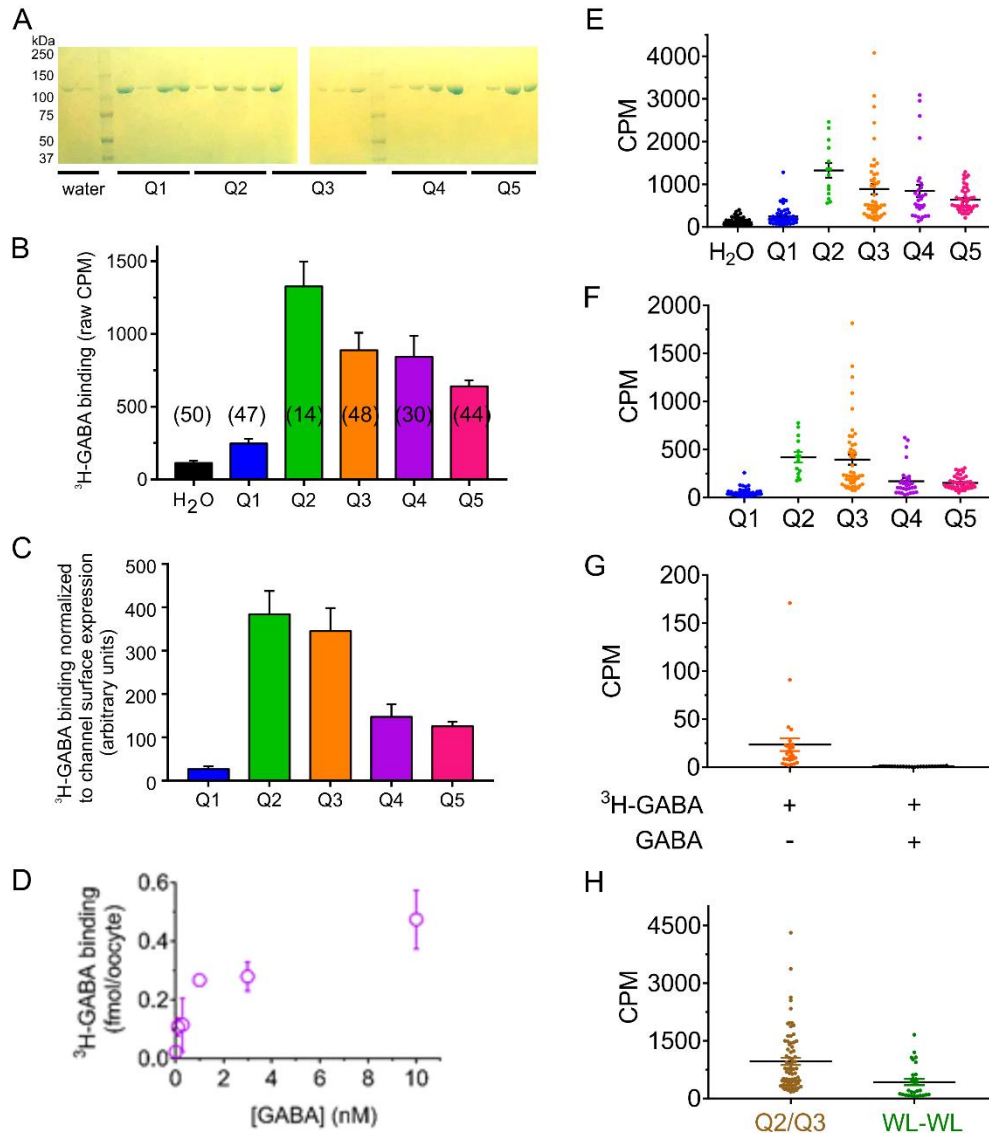


Direct neurotransmitter activation of voltage-gated potassium channels

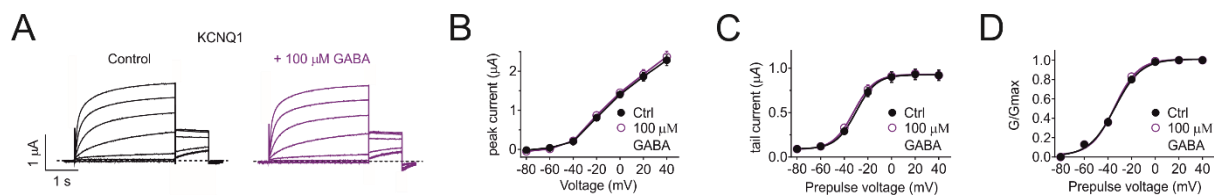
Manville *et al.*

Supplementary Figures and Tables



Supplementary Figure 1. Correction of ^3H -GABA binding for channel protein surface expression

- A. Representative Coomassie staining of surface-biotinylated proteins in water or KCNQ cRNA-injected oocytes ($n = 4$ lysates per group, each pooled from 5 oocytes). Duplicate gels showed the same pattern, ruling out gel-loading errors.
- B. Mean raw scintillation CPMs for oocytes injected with water or channel cRNA as indicated; n values for each group in parentheses. Error bars indicate SEM.
- C. Mean ^3H -GABA binding corrected for channel protein relative surface expression, and with mean water-injected CPM subtracted (n as in panel B). See Fig. 1 for statistical analysis. Error bars indicate SEM.
- D. Expansion of lower [GABA] range points for KCNQ3 mean GABA saturation binding curve plot, all details as for main plot (Fig. 1j). Error bars indicate SEM.
- E. Scatter plot corresponding to Supplementary Figure 1 B.
- F. Scatter plot corresponding to Fig 1h/Supplementary Figure 1C.
- G. Scatter plot corresponding to Figure 1i.
- H. Scatter plot corresponding to Figure 3j.



Supplementary Figure 2. Effects of GABA on KCNQ1 channels

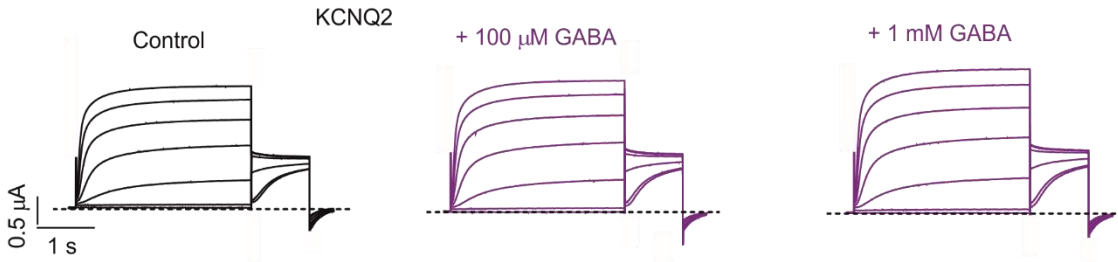
- A. Averaged KCNQ1 traces in the absence (black) and presence (purple) of GABA (100 μM), $n = 4$.
 B. Mean peak current-voltage relationship for recordings as in panel A, $n = 4$. Error bars indicate SEM.
 C. Mean tail current versus prepulse voltage relationships as in panel A, $n = 4$. Error bars indicate SEM.
 D. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 4$. Error bars indicate SEM.

Q1	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-34.1 ± 0.9 ($n = 4$)	-30.1 ± 2.2 ($n = 4$)	10.9 ± 0.9 ($n = 4$)
100 μM GABA	-34.2 ± 0.8 ($n = 4$)	-32.6 ± 1.9 ($n = 4$)	9.9 ± 0.7 ($n = 4$)

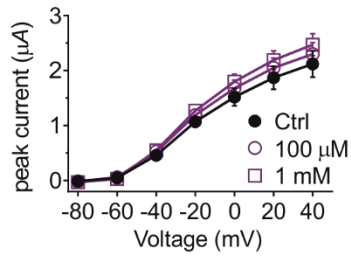
Supplementary Table 1. Summary of Effects of GABA on KCNQ1 channels.

Values indicate mean $\pm$ SEM.

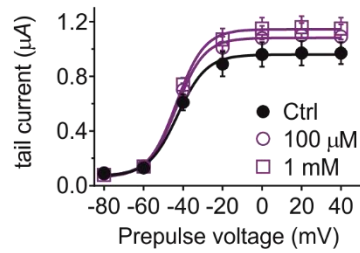
A



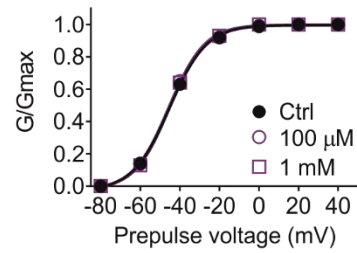
B



C



D



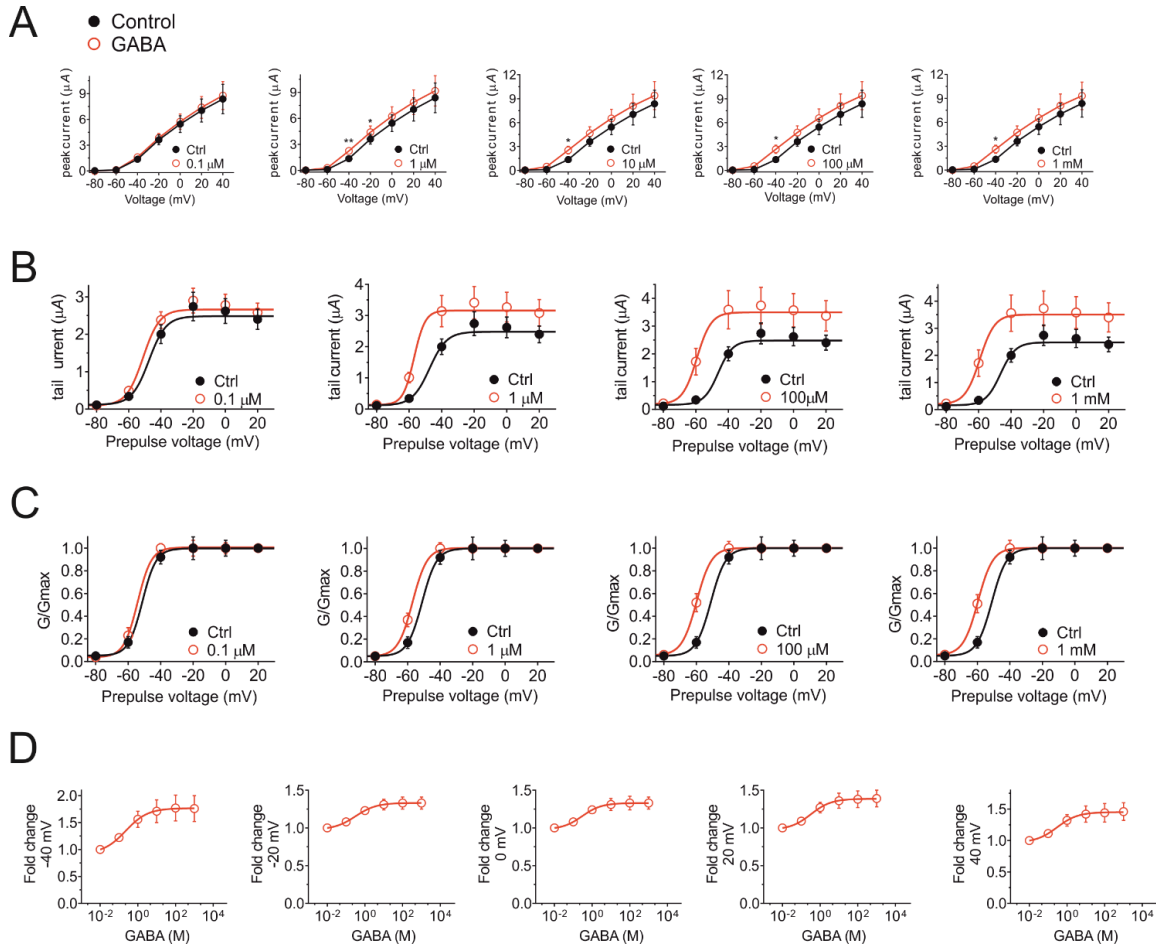
Supplementary Figure 3. KCNQ2 activity is relatively insensitive to GABA

- A. Averaged KCNQ2 traces in the absence (black) and presence (purple) of GABA (100 μ M; middle, 1 mM; right), $n = 6$.
 B. Mean peak current-voltage relationship for recordings as in panel A, $n = 6$. Error bars indicate SEM.
 C. Mean tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.
 D. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.

Q2	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-45.2 ± 0.6 ($n = 6$)	-42.8 ± 2.8 ($n = 6$)	9.3 ± 0.6 ($n = 6$)
100 μ M GABA	-45.8 ± 0.4 ($n = 6$)	-43.2 ± 2.5 ($n = 6$)	9.0 ± 0.3 ($n = 6$)
1 mM GABA	-45.1 ± 0.4 ($n = 6$)	-43.4 ± 2.3 ($n = 6$)	8.7 ± 0.3 ($n = 6$)

Supplementary Table 2. Summary of Effects of GABA on KCNQ2 channels.

Values indicate mean $\pm$ SEM.



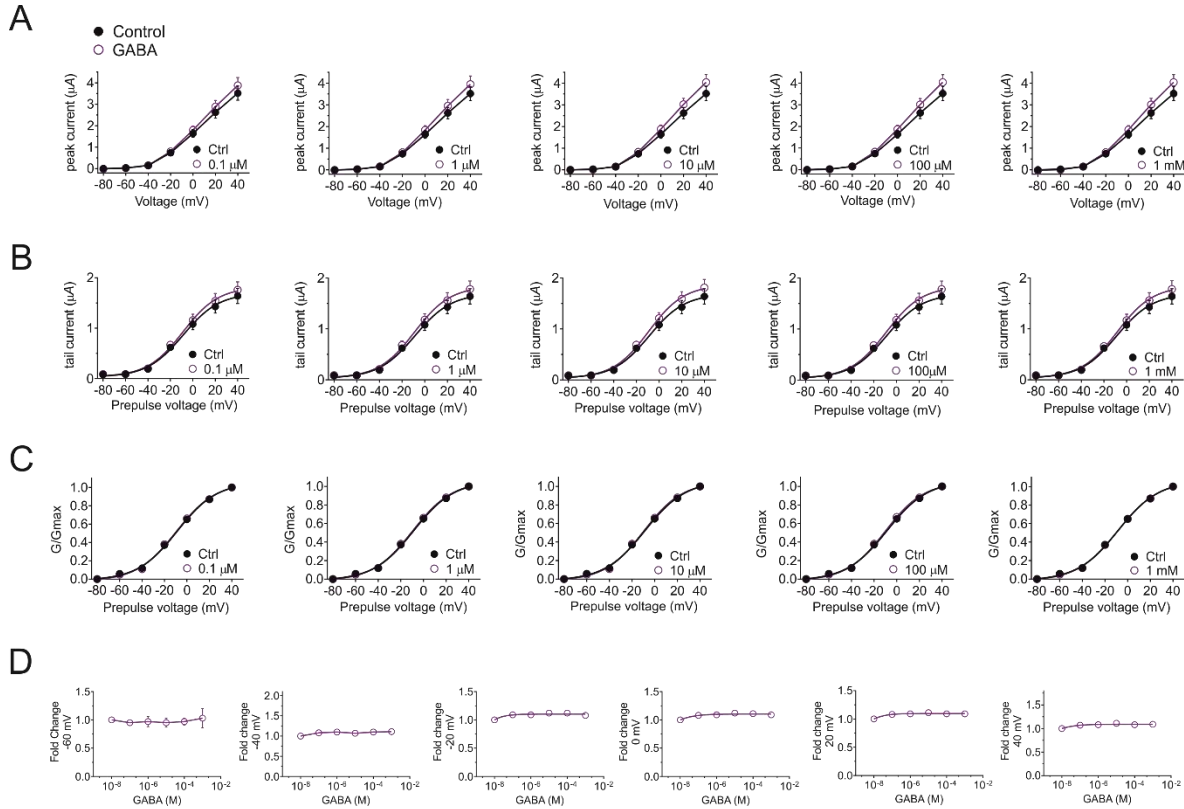
Supplementary Figure 4. Effects of GABA on KCNQ3*

- A. Mean peak current-voltage relationship for KCNQ3* channels in the absence (black) and presence (purple) of GABA, $n = 5$, ** $P=0.005$, * $P=0.05$ versus control. Error bars indicate SEM.
- B. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- C. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- D. Mean dose response of KCNQ3* channels between -40 and +40 mV, $n = 5$. Error bars indicate SEM.

Q3*	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-51.4 ± 1.9 ($n = 5$)	-47.5 ± 4.4 ($n = 5$)	4.5 ± 0.9 ($n = 5$)
0.1 μ M GABA	-54.2 ± 1.5 ($n = 5$)	-51.1 ± 3.5 ($n = 5$)	4.6 ± 0.9 ($n = 5$)
1 μ M GABA	-56.9 ± 1.0 ($n = 5$) *	-56.9 ± 2.8 ($n = 5$)	4.3 ± 0.8 ($n = 5$)
10 μ M GABA	-58.9 ± 1.0 ($n = 5$) **	-58.4 ± 2.7 ($n = 5$)	4.3 ± 3.5 ($n = 5$)
100 μ M GABA	-59.8 ± 0.9 ($n = 5$) **	-59.4 ± 2.8 ($n = 5$)	4.8 ± 3.0 ($n = 5$)
1 mM GABA	-59.6 ± 0.9 ($n = 5$) **	-59.3 ± 2.5 ($n = 5$)	4.7 ± 3.3 ($n = 5$)

Supplementary Table 3. Summary of Effects of GABA on KCNQ3* channels.

Statistics versus same channel in absence of GABA: ** $p=0.004$, * $p=0.03$. Values indicate mean $\pm$ SEM.



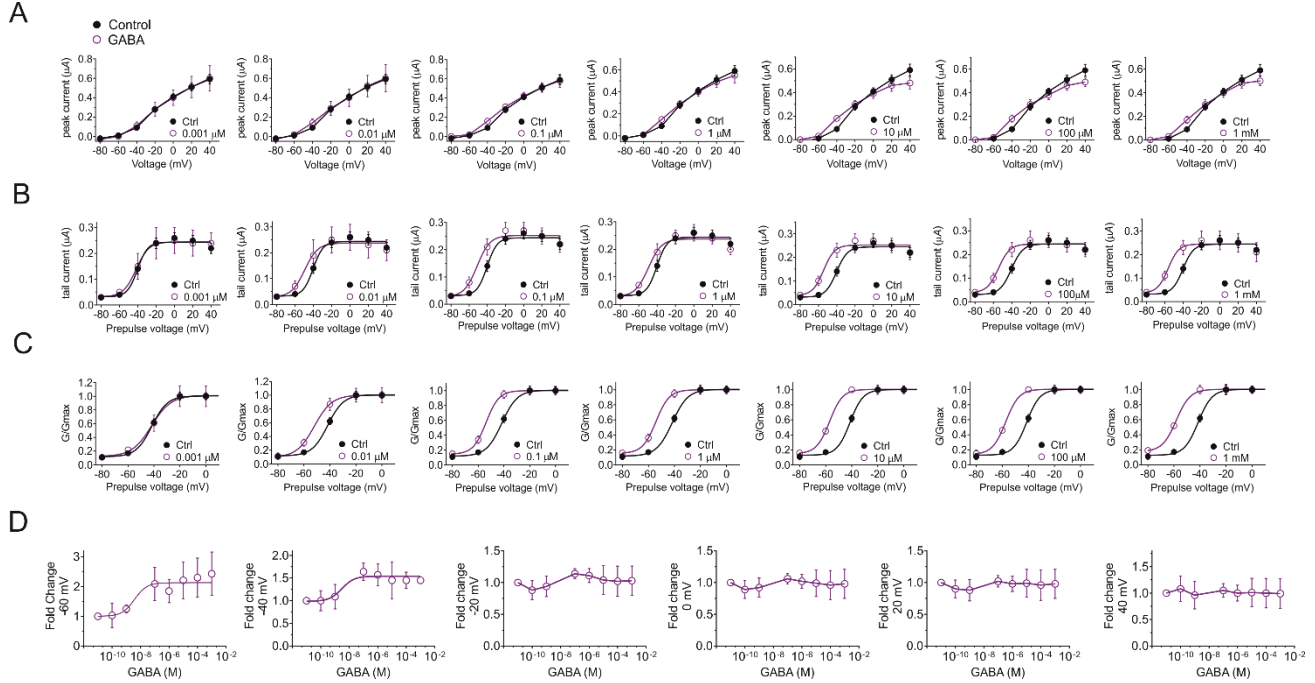
Supplementary Figure 5. Effects of GABA on KCNQ4

- A. Mean peak current-voltage relationship for KCNQ4 channels in the absence (black) and presence (purple) of GABA, $n = 5$. Error bars indicate SEM.
- B. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- C. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- D. Mean dose response of KCNQ4 channels between -40 and +40 mV, $n = 5$. Error bars indicate SEM.

Q4	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-8.7 ± 0.8 ($n = 5$)	-8.6 ± 4.3 ($n = 5$)	17.6 ± 0.5 ($n = 5$)
0.1 μM GABA	-9.6 ± 0.7 ($n = 5$)	-8.8 ± 4.3 ($n = 5$)	17.6 ± 0.5 ($n = 5$)
1 μM GABA	-9.6 ± 0.8 ($n = 5$)	-9.0 ± 4.3 ($n = 5$)	17.7 ± 0.6 ($n = 5$)
10 μM GABA	-9.8 ± 0.8 ($n = 5$)	-9.3 ± 3.7 ($n = 5$)	17.1 ± 0.8 ($n = 5$)
100 μM GABA	-10.3 ± 0.8 ($n = 5$)	-9.0 ± 3.8 ($n = 5$)	17.1 ± 0.9 ($n = 5$)
1 mM GABA	-8.8 ± 0.8 ($n = 5$)	-8.4 ± 3.4 ($n = 5$)	17.2 ± 0.8 ($n = 5$)

Supplementary Table 4. Summary of Effects of GABA on KCNQ4 channels.

Values indicate mean $\pm$ SEM.



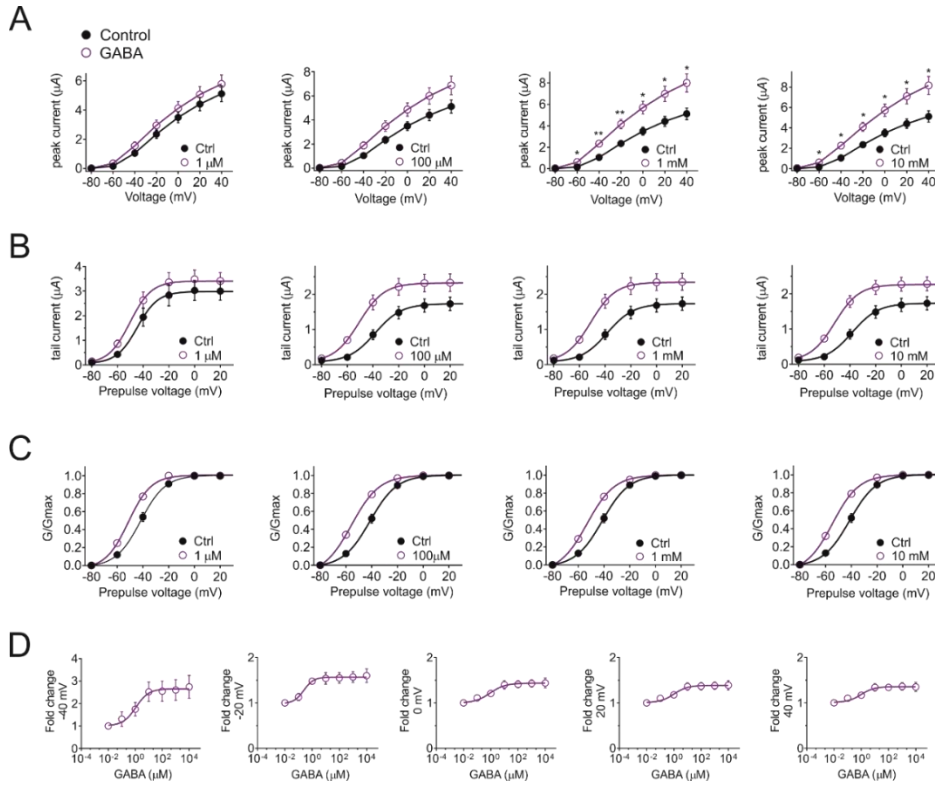
Supplementary Figure 6. Effects of GABA on KCNQ5

- A. Mean peak current-voltage relationship for KCNQ5 channels in the absence (black) and presence (purple) of GABA, $n = 4-8$. Error bars indicate SEM.
- B. Mean tail current versus prepulse voltage relationships as in panel A, $n = 4-8$. Error bars indicate SEM.
- C. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 4-8$. Error bars indicate SEM.
- D. Mean dose response of KCNQ5 channels between -40 and $+40$ mV, $n = 4-8$. Error bars indicate SEM.

Q5	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-41.7 ± 1.5 ($n = 8$)	-40.3 ± 2.1 ($n = 8$)	9.6 ± 1.9 ($n = 8$)
0.001 μM GABA	-41.9 ± 2.9 ($n = 4$)	-41.8 ± 2.8 ($n = 4$)	9.4 ± 4.3 ($n = 4$)
0.01 μM GABA	-50.1 ± 2.9 ($n = 4$) *	-49.5 ± 2.5 ($n = 4$) *	7.6 ± 2.4 ($n = 4$)
0.1 μM GABA	-53.9 ± 1.7 ($n = 8$) **	-51.3 ± 2.1 ($n = 8$) *	7.4 ± 1.2 ($n = 8$)
1 μM GABA	-54.2 ± 1.6 ($n = 8$) **	-51.2 ± 2.6 ($n = 8$) *	7.6 ± 1.2 ($n = 8$)
10 μM GABA	-57.0 ± 1.8 ($n = 4$) **	-54.5 ± 2.3 ($n = 4$) **	8.4 ± 1.4 ($n = 4$)
100 μM GABA	-57.4 ± 1.8 ($n = 4$) **	-54.5 ± 2.7 ($n = 4$) **	8.5 ± 1.5 ($n = 4$)
1 mM GABA	-58.3 ± 1.8 ($n = 4$) **	-56.4 ± 2.4 ($n = 4$) **	8.8 ± 1.5 ($n = 4$)

Supplementary Table 5. Summary of Effects of GABA on KCNQ5 channels.

Statistics versus same channel in absence of GABA: ** $p=0.003$, * $p=0.04$. Values indicate mean $\pm$ SEM.



Supplementary Figure 7. Effects of GABA on KCNQ2/3 channels

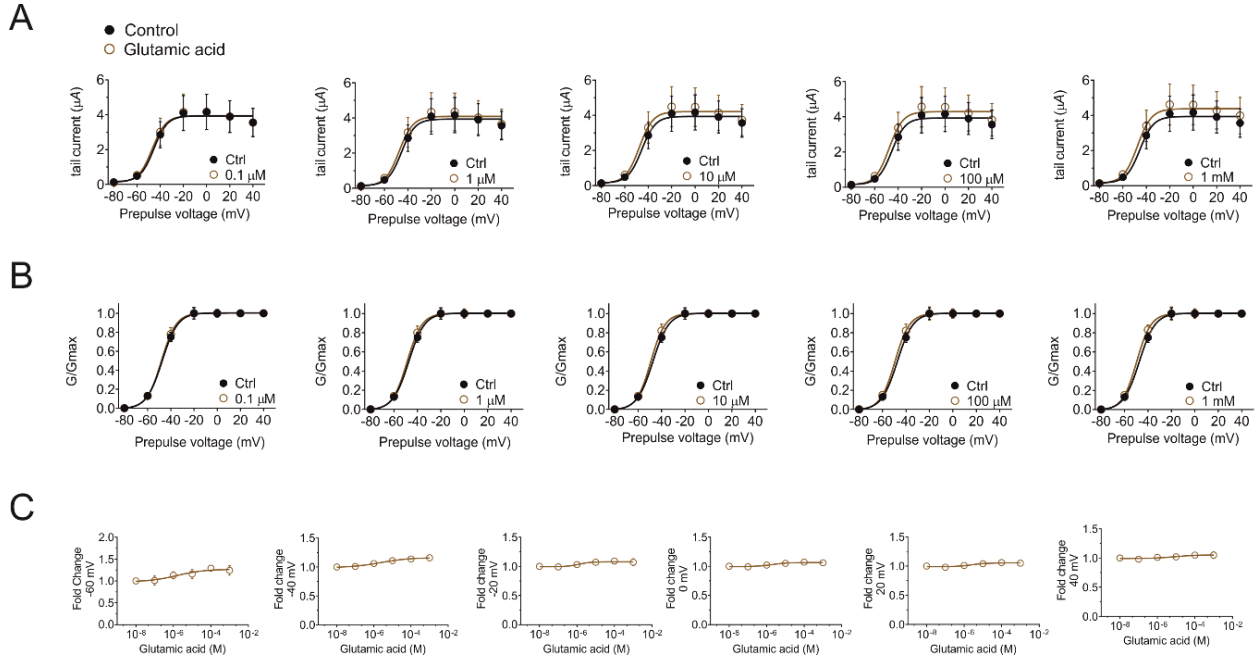
- A. Mean peak current-voltage relationship for KCNQ2/3 channels in the absence (black) and presence (purple) of GABA, $n = 10$, ** $P=0.007$, * $P=0.01$ versus control. Error bars indicate SEM.
- B. Mean tail current versus prepulse voltage relationships as in panel A, $n = 10$. Error bars indicate SEM.
- C. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 10$. Error bars indicate SEM.
- D. Mean dose response of KCNQ3* channels between -40 and +40 mV, $n = 10$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-39.1 ± 1.1 ($n=10$)	-38.2 ± 4.4 ($n=10$)	8.4 ± 1.0 ($n=10$)
0.1 μ M GABA	-39.8 ± 1.3 ($n=4$)	-38.4 ± 4.6 ($n=4$)	8.3 ± 0.9 ($n=4$)
1 μ M GABA	-51.3 ± 1.1 ($n=10$) ****	-51.2 ± 6.1 ($n=10$)	8.8 ± 0.8 ($n=4$)
10 μ M GABA	-53.0 ± 1.1 ($n=10$) ****	-51.0 ± 5.2 ($n=10$)	10.9 ± 0.8 ($n=10$)
100 μ M GABA	-53.0 ± 0.8 ($n=10$) ****	-51.0 ± 4.9 ($n=10$)	10.9 ± 0.6 ($n=10$)
1 mM GABA	-53.1 ± 0.7 ($n=10$) ****	-51.2 ± 4.2 ($n=10$) *	10.6 ± 0.6 ($n=10$)
10 mM GABA	-55 ± 0.7 ($n=10$) ****	-50.3 ± 4.3 ($n=10$) *	10.3 ± 0.6 ($n=10$)

Supplementary Table 6. Summary of Effects of GABA on KCNQ2/3 channels.

Statistics versus same channel in absence of GABA:

**** $p<0.0001$, * $p=0.02$. Values indicate mean $\pm$ SEM.



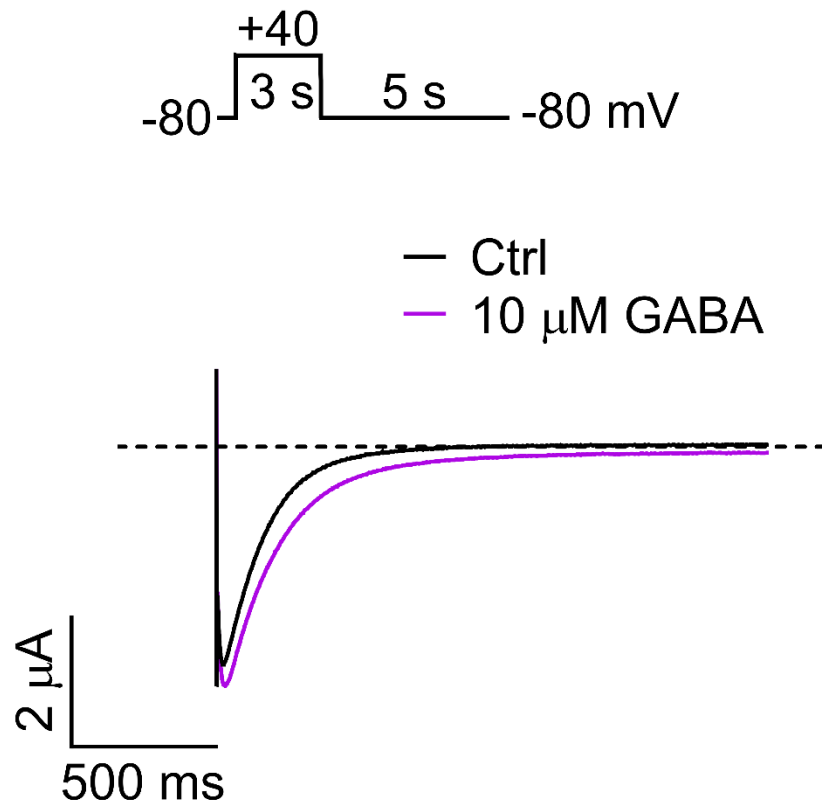
Supplementary Figure 8. Glutamate has no effect on KCNQ2/3 channels

- A. Mean tail current versus prepulse voltage relationships for KCNQ2/3 channels in the absence (black) and presence (brown) of glutamate, $n = 5$. Error bars indicate SEM.
- B. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- C. Mean dose response of KCNQ2/3 channels between -40 and $+40$ mV, $n = 5$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-47.4 ± 1.4 ($n = 5$)	-45.9 ± 8.4 ($n = 5$)	6.7 ± 0.9 ($n = 5$)
0.1 μ M Glutamate	-48.1 ± 1.6 ($n = 5$)	-46.9 ± 5.7 ($n = 5$)	6.4 ± 1.0 ($n = 5$)
1 μ M Glutamate	-48.8 ± 1.7 ($n = 5$)	-47.4 ± 5.5 ($n = 5$)	6.3 ± 1.7 ($n = 5$)
10 μ M Glutamate	-49.2 ± 1.6 ($n = 5$)	-47.6 ± 5.4 ($n = 5$)	6.1 ± 0.9 ($n = 5$)
100 μ M Glutamate	-49.5 ± 1.8 ($n = 5$)	-47.7 ± 5.3 ($n = 5$)	6.2 ± 1.0 ($n = 5$)
1 mM Glutamate	-49.7 ± 1.6 ($n = 5$)	-47.6 ± 5.6 ($n = 5$)	6.1 ± 0.9 ($n = 5$)

Supplementary Table 7. Summary of Effects of glutamate on KCNQ2/3 channels.

Values indicate mean $\pm$ SEM.



Supplementary Figure 9. Exemplar traces of effects of GABA on KCNQ2/3 deactivation

KCNQ2/3 deactivation at -80 mV after a 40 mV prepulse, using the voltage protocol (upper) in the absence or presence of GABA (10 μ M). See Figure 2k and Supplementary Table 7 for mean data.

KCNQ2/3	τ act, -40 mV (ms)	τ deact, -80 mV (ms)
Ctrl	1229 $\pm$ 153 (n=10)	156 $\pm$ 10 (n=10)
10 μ M GABA	701 $\pm$ 64 ** (n=10)	219 $\pm$ 11 *** (n=10)

Supplementary Table 8. Mean effects of GABA on KCNQ2/3 gating kinetics.

Statistics versus same channel in absence of GABA:

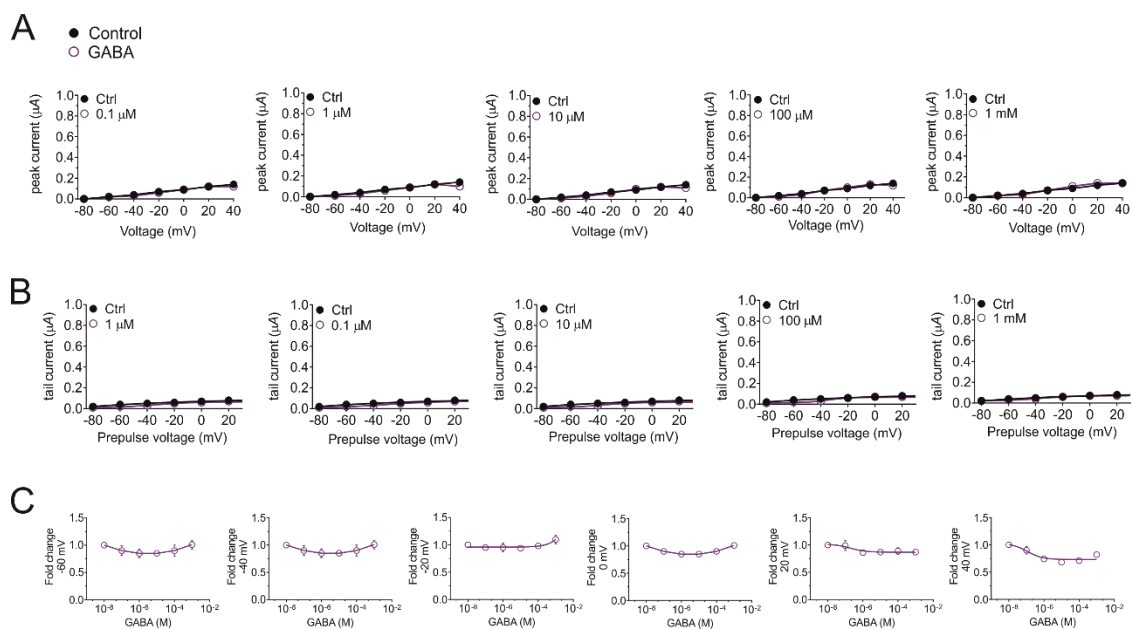
p<0.01; *p<0.001. Values indicate mean $\pm$ SEM.

GABA	EC₅₀ (μM)
Q2/Q3	0.85 ± 0.1 (n = 10)
Q2/Q3 Wort	1.2 ± 0.12 (n = 5)
Q3*	1.0 ± 0.1 (n = 5)
Q5	0.06 ± 0.01 (n = 4-8)

BHB	EC₅₀ (μM)
Q2/Q3	0.95 ± 0.13 (n = 5)

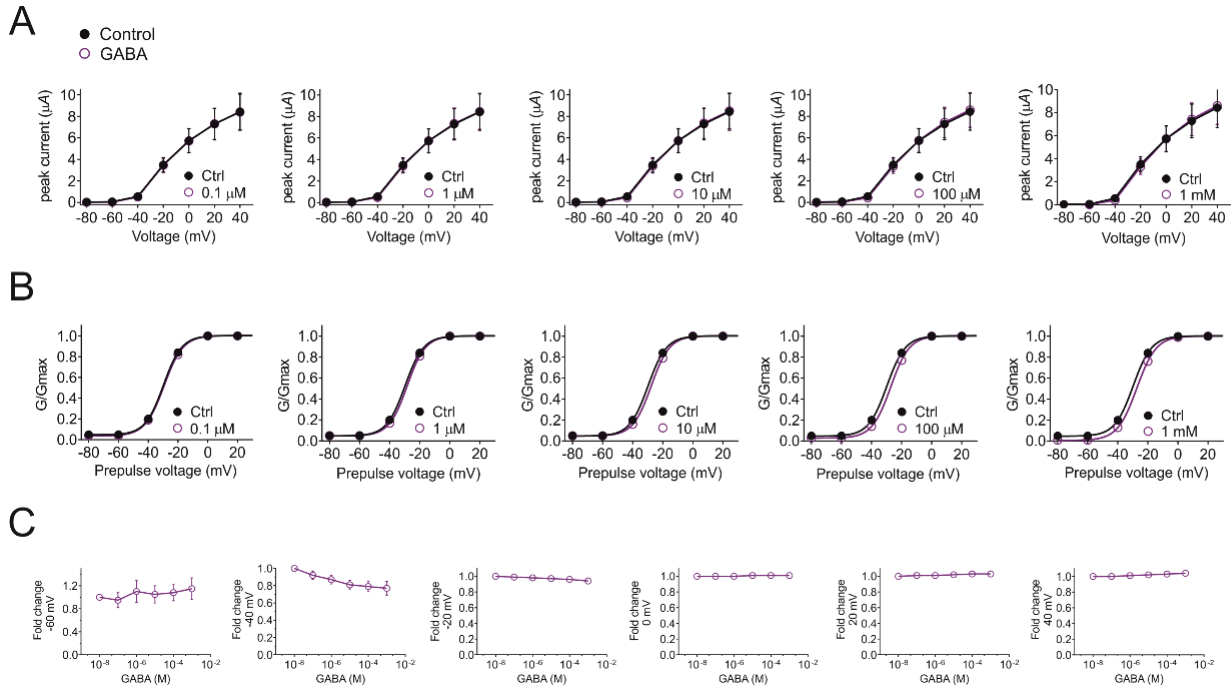
GABOB	EC₅₀ (μM)
Q2/Q3	0.1 ± 0.01 (n = 6)

Supplementary Table 9. EC₅₀ values calculated from dose responses at -60 mV.
Values indicate mean ± SEM.



Supplementary Figure 10. Lack of effects of GABA on H₂O-injected oocytes

- A. Mean peak current-voltage relationship for H₂O injected oocytes in the absence (black) and presence (purple) of GABA, $n = 4$. Error bars indicate SEM.
- B. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 4$. Error bars indicate SEM.
- C. Mean dose response of H₂O injected oocytes between -60 and +40 mV, $n = 4$. Error bars indicate SEM.



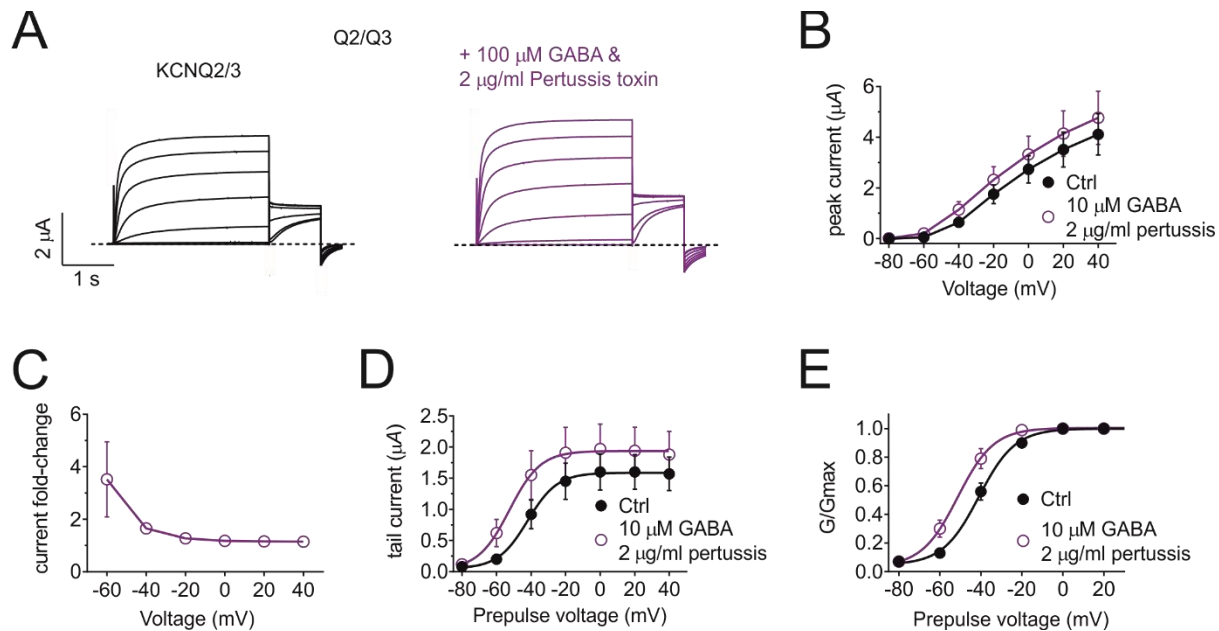
Supplementary Figure 11. Lack of effects of GABA on KCNA1

- A. Mean peak current-voltage relationship for KCNA1 channels in the absence (black) and presence (purple) of GABA, $n = 4$. Error bars indicate SEM.
- B. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 4$. Error bars indicate SEM.
- C. Mean dose response of KCNA1 channels between -60 and +40 mV, $n = 4$. Error bars indicate SEM.

KCNA1	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-29.8 ± 0.69 ($n = 4$)	n/a	6.2 ± 0.4 ($n = 4$)
0.1 μ M GABA	-29.3 ± 0.88 ($n = 4$)	n/a	6.4 ± 0.5 ($n = 4$)
1 μ M GABA	-28.4 ± 0.89 ($n = 4$)	n/a	6.1 ± 0.5 ($n = 4$)
10 μ M GABA	-27.6 ± 0.82 ($n = 4$)	n/a	6.1 ± 0.5 ($n = 4$)
100 μ M GABA	-27.2 ± 0.67 ($n = 4$)	n/a	6.2 ± 0.4 ($n = 4$)
1 mM GABA	-27.3 ± 0.93 ($n = 4$)	n/a	6.4 ± 0.6 ($n = 4$)

Supplementary Table 10. Summary of effects of GABA on KCNA1 channels.

Values indicate mean $\pm$ SEM.



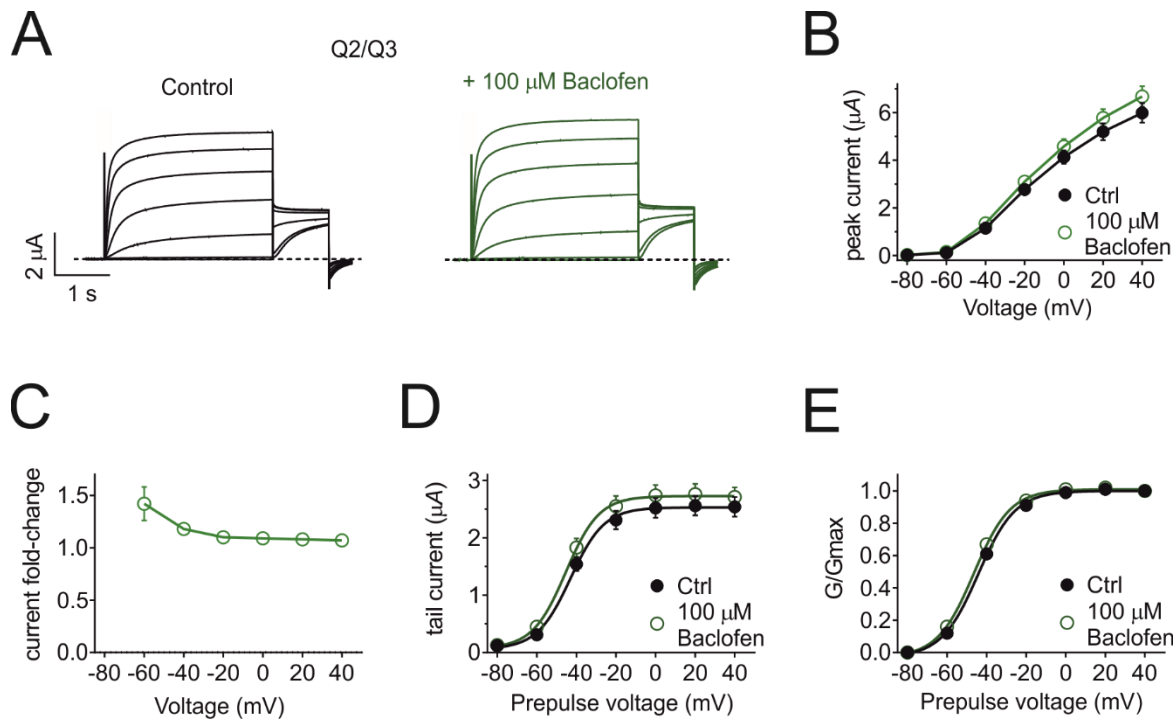
Supplementary Figure 12. Pertussis toxin has no effect on GABA sensitivity of KCNQ2/3 channels

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (purple) of GABA (10 μ M) and pertussis toxin (2 μ g/ml), $n = 7$.
 B. Mean peak current-voltage relationship for recordings as in panel A, $n = 7$. Error bars indicate SEM.
 C. Mean current fold-change versus voltage as in panel A, $n = 7$. Error bars indicate SEM.
 D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 7$. Error bars indicate SEM.
 E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 7$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-40.9 ± 1.4	-41.9 ± 6.3	8.8 ± 1.4
2 μ g/ml Pertussis toxin	($n = 7$)	($n = 7$)	($n = 7$)
100 μ M GABA	-51.3 ± 1.9	-52.6 ± 9.1	8.8 ± 1.4
2 μ g/ml Pertussis toxin	($n = 7$) **	($n = 7$)	($n = 7$)

Supplementary Table 11. Summary of Effects of pertussis toxin on GABA sensitivity of KCNQ2/3 channels. Statistics versus same

channel in absence of 100 μ M GABA: ** $p=0.001$. Values indicate mean $\pm$ SEM.



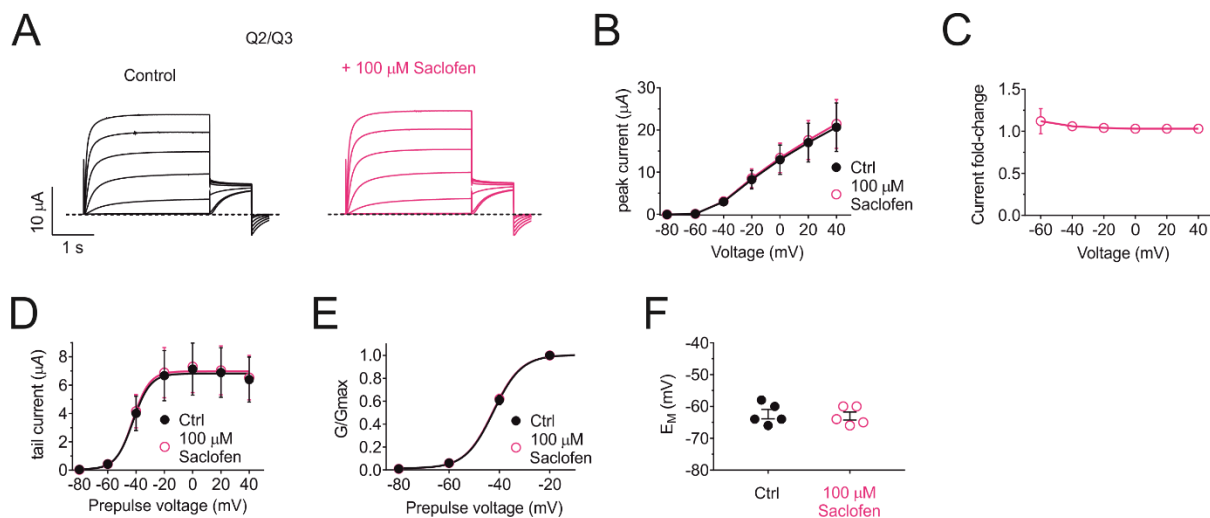
Supplementary Figure 13. Minimal effects of baclofen on KCNQ2/3 channels

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (green) of baclofen (100 μ M), $n = 5$.
 B. Mean peak current-voltage relationship as in panel A, $n = 5$. Error bars indicate SEM.
 C. Mean current fold-change versus voltage as in panel A, $n = 5$. Error bars indicate SEM.
 D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
 E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-44.2 ± 0.4 ($n = 5$)	-42.6 ± 2.3 ($n = 5$)	9.3 ± 0.3 ($n = 5$)
100 μ M Baclofen	-46.4 ± 0.6 ($n = 5$)	-45.4 ± 2.5 ($n = 5$)	9.2 ± 0.5 ($n = 5$)

Supplementary Table 12. Summary of Effects of baclofen on KCNQ2/3 channels.

Values indicate mean $\pm$ SEM.



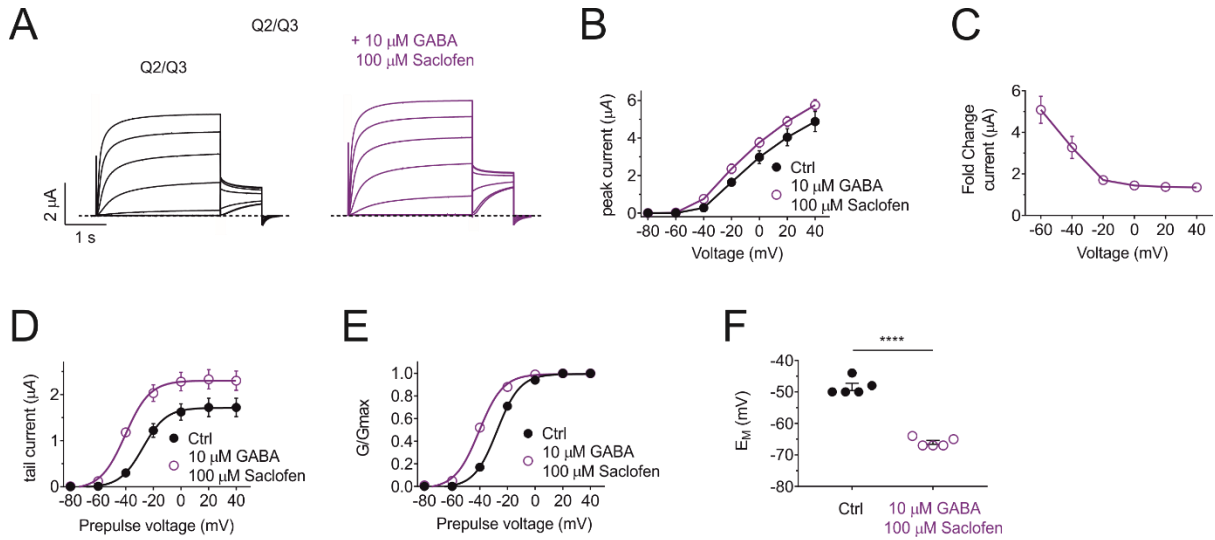
Supplementary Figure 14. Lack of effects of saclofen on KCNQ2/3 channels

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (pink) of saclofen (100 μ M), $n = 5$.
 B. Mean peak current-voltage relationship as in panel A, $n = 5$. Error bars indicate SEM.
 C. Mean current fold-change versus voltage as in panel A, $n = 5$. Error bars indicate SEM.
 D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
 E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
 F. Scatter plot of unclamped resting membrane potential (E_M) as in panel A, $n = 5$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-42.3 ± 0.8 ($n = 5$)	-41.1 ± 6.7 ($n = 5$)	5.6 ± 1.1 ($n = 5$)
100 μ M Saclofen	-42.6 ± 0.8 ($n = 5$)	-42.8 ± 6.6 ($n = 5$)	5.5 ± 0.8 ($n = 5$)

Supplementary Table 13. Summary of Effects of saclofen on KCNQ2/3 channels.

Values indicate mean $\pm$ SEM.



Supplementary Figure 15. Saclofen exhibits no effect on GABA sensitivity of KCNQ2/3 channels

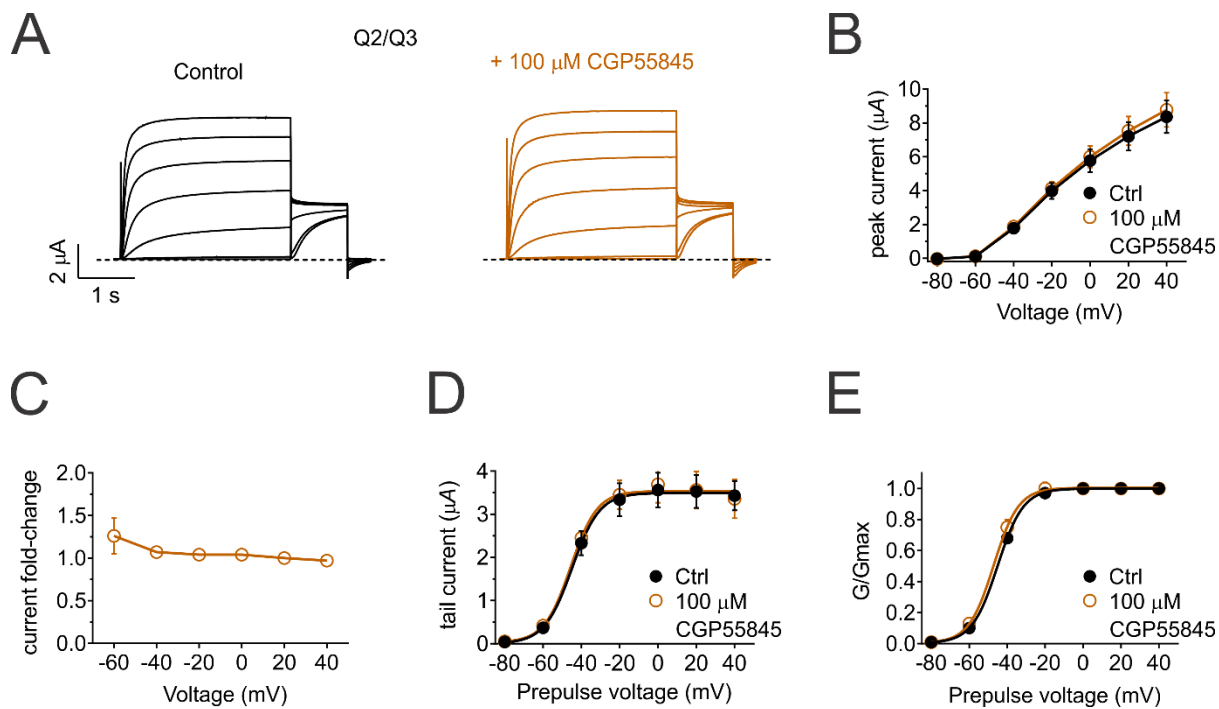
- A. Averaged KCNQ2/3 traces in the absence (black) and presence (purple) of GABA (10 μ M) and saclofen (100 μ M), $n = 6$.
- B. Mean peak current-voltage relationship for recordings as in panel A, $n = 6$. Error bars indicate SEM.
- C. Mean current fold-change versus voltage as in panel A, $n = 6$. Error bars indicate SEM.
- D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.
- E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.
- F. Scatter plot of unclamped resting membrane potential (E_M) for oocytes as in panel A, $n = 6$, **** $P < 0.0001$ versus control. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-27.5 ± 0.4 ($n = 6$)	-27.5 ± 3.2 ($n = 6$)	8.3 ± 0.4 ($n = 6$)
10 μ M GABA 100 μ M Saclofen	-40.5 ± 0.5 ($n = 6$) ****	-40.3 ± 2.7 ($n = 6$) *	8.7 ± 0.6 ($n = 6$)

Supplementary Table 14. Summary of Effects of saclofen on GABA sensitivity of KCNQ2/3 channels.

Statistics versus same channel in absence of 10 μ M GABA and 100 μ M saclofen: **** $p < 0.0001$, * $p = 0.01$.

Values indicate mean $\pm$ SEM.



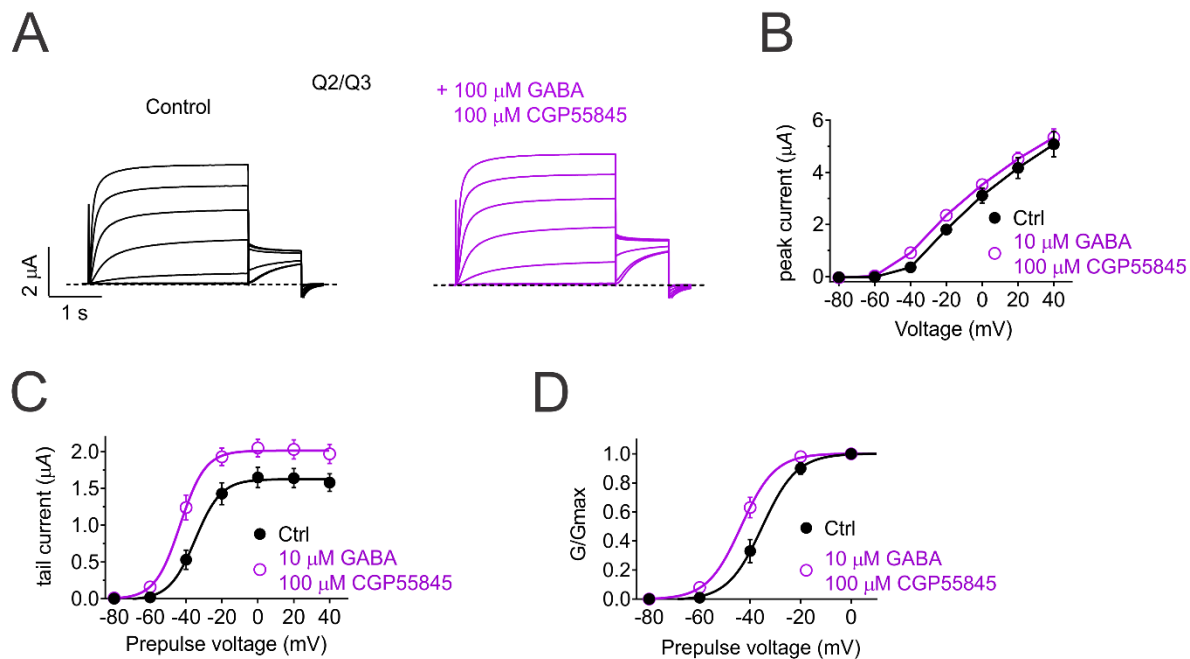
Supplementary Figure 16. Lack of effects of CGP55845 on KCNQ2/3 channels

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (brown) of CGP55845 (100 μ M), $n = 4$.
 B. Mean peak current-voltage relationship as in panel A, $n = 4$. Error bars indicate SEM.
 C. Mean current fold-change versus voltage as in panel A, $n = 4$. Error bars indicate SEM.
 D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 4$. Error bars indicate SEM.
 E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 4$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-45.1 ± 0.3 ($n = 4$)	-44.9 ± 3.5 ($n = 4$)	6.7 ± 0.3 ($n = 4$)
100 μ M CGP	-47.1 ± 1.1 ($n = 4$)	-45.6 ± 3.7 ($n = 4$)	6.6 ± 0.7 ($n = 4$)

Supplementary Table 15. Summary of Effects of CGP55485 on KCNQ2/3 channels.

Values indicate mean $\pm$ SEM.



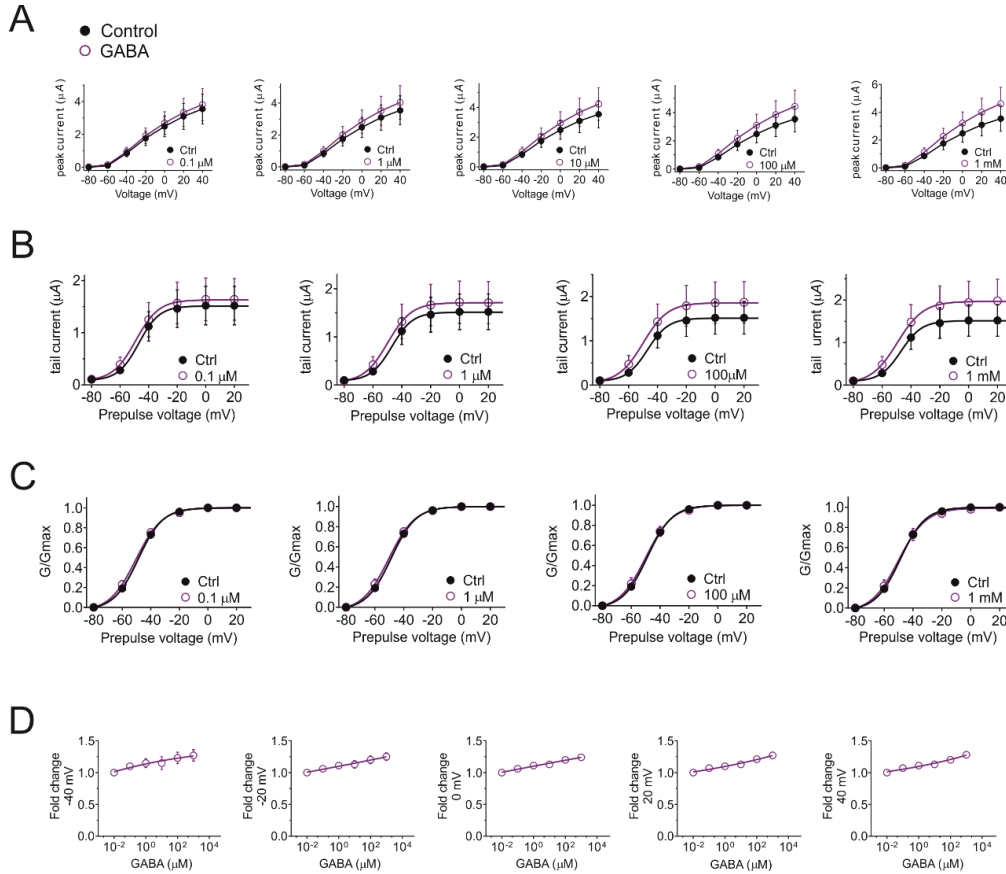
Supplementary Figure 17. CGP55845 exhibits no effect on GABA sensitivity of KCNQ2/3 channels

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (purple) of GABA (10 μ M) and CGP55845 (100 μ M), $n = 5$.
 B. Mean peak current-voltage relationship for recordings as in panel A, $n = 5$. Error bars indicate SEM.
 C. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
 D. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-35.3 ± 1.2 ($n = 5$)	-34.9 ± 25 ($n = 5$)	6.9 ± 1.1 ($n = 5$)
10 μ M GABA 100 μ M CGP	-43.6 ± 1.1 ($n = 5$) ***	-43.3 ± 2.1 ($n = 5$) *	6.6 ± 1.1 ($n = 5$)

Supplementary Table 16. Summary of Effects of CGP55845 on GABA sensitivity of KCNQ2/3 channels.

Statistics versus same channel in absence of 10 μ M GABA and 100 μ M CGP55845: *** $p=0.001$, * $p=0.03$. Values indicate mean $\pm$ SEM.



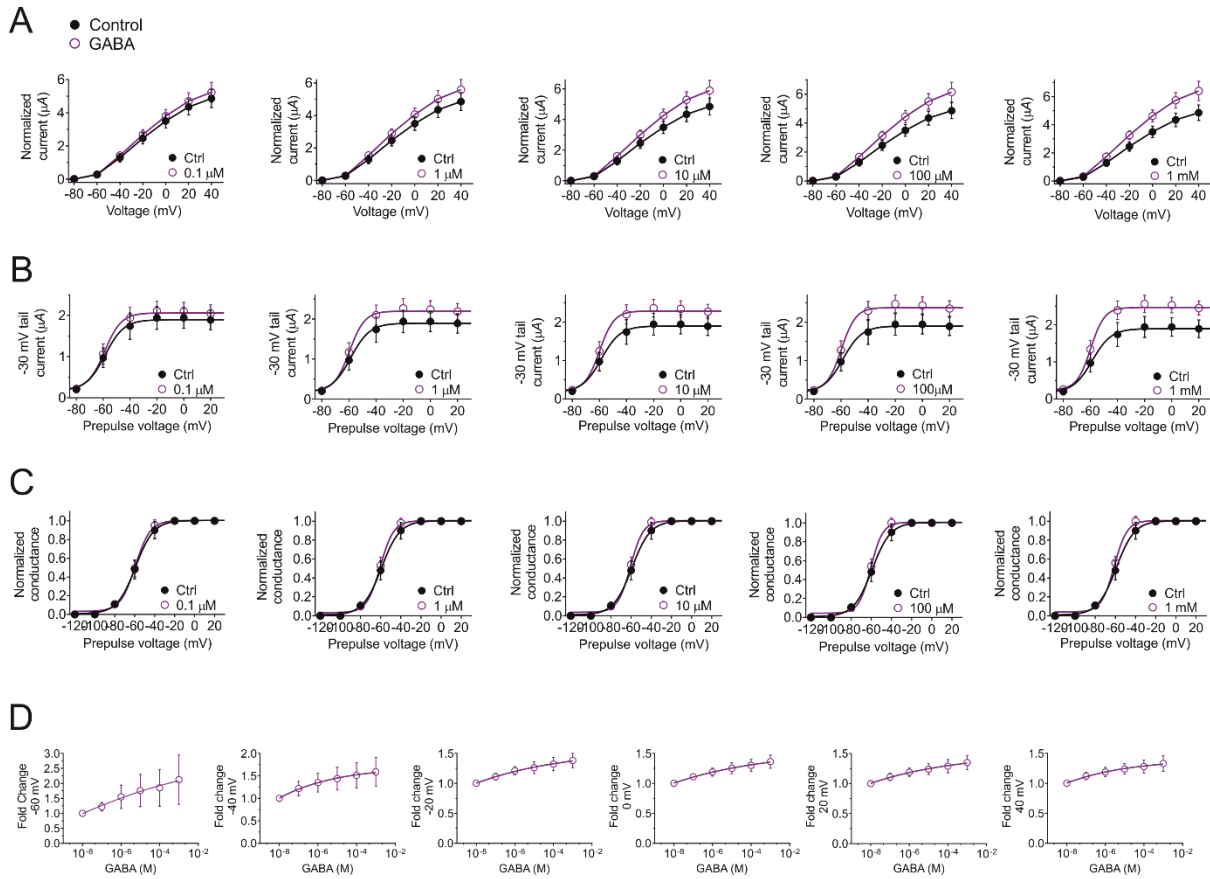
Supplementary Figure 18. Q2/Q3-W265L has diminished sensitivity to GABA

- A. Mean peak current-voltage relationship for W265L-Q3/Q2 channels in the absence (black) and presence (purple) of GABA, $n = 5$. Error bars indicate SEM.
- B. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- C. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- D. Mean dose response of W265L-Q3/Q2 channels between -40 and +40 mV, $n = 5$. Error bars indicate SEM.

Q2/W265L-Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-48.8 ± 0.6 ($n = 5$)	-46.6 ± 8.7 ($n = 5$)	8.6 ± 0.4 ($n = 5$)
0.1 μ M GABA	-50.5 ± 1.2 ($n = 5$)	-49.0 ± 9.7 ($n = 5$)	9.2 ± 0.9 ($n = 5$)
1 μ M GABA	-50.4 ± 1.3 ($n = 5$)	-49.7 ± 10 ($n = 5$)	9.1 ± 0.9 ($n = 5$)
10 μ M GABA	-49.9 ± 1.5 ($n = 5$)	-49.9 ± 10.4 ($n = 5$)	9.1 ± 1.1 ($n = 5$)
100 μ M GABA	-49.9 ± 1.5 ($n = 5$)	-49.9 ± 10.2 ($n = 5$)	9.2 ± 1.4 ($n = 5$)
1 mM GABA	-49.9 ± 1.9 ($n = 5$)	-49.4 ± 10.7 ($n = 5$)	9.4 ± 1.5 ($n = 5$)

Supplementary Table 17. Summary of Effects of GABA on Q2-W265L-Q3 channels.

Values indicate mean $\pm$ SEM.



Supplementary Figure 19. W236L-Q2/W265L-Q3 heteromers show reduced GABA sensitivity

- A. Mean peak current-voltage relationship for W236L-Q2/W265L-Q3 channels in the absence (black) and presence (purple) of GABA, $n = 5$. Error bars indicate SEM.
- B. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- C. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- D. Mean dose response of W236L-Q2/W265L-Q3 channels between -40 and +40 mV, $n = 5$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-2.9 ± 2.7 (n = 6)	14.3 ± 1.6 (n = 6)
100 μ M GABA	-17.5 ± 3.7 (n = 6)	16.2 ± 3.0 (n = 6)

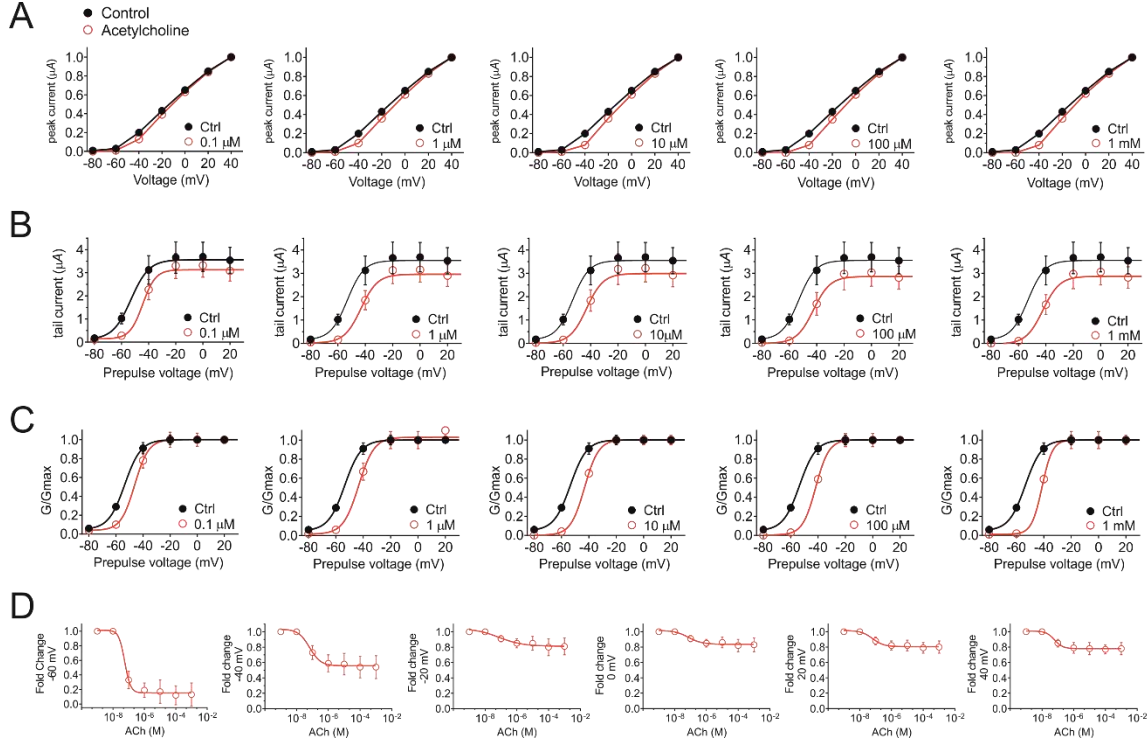
Supplementary Table 18. Summary of effects of 100 μ M GABA on KCNQ2/3 expressed in CHO cells.

Values indicate mean $\pm$ SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl (extracellular solution only)	-9.2 ± 4.9	20.3 ± 4.5
100 μ M picrotoxin 10 μ M CGP55845	-7.1 ± 7.6	17.4 ± 3.0
100 μ M picrotoxin 10 μ M CGP55845 100 μ M GABA	-20.6 ± 4.9	16.0 ± 3.2
100 μ M picrotoxin 10 μ M CGP55845 10 μ M XE991	-4.9 ± 4.3	18.4 ± 2.2

Supplementary Table 19. Summary of effects of 100 μ M GABA on native M-current in PC12 cells.

Recordings performed for each condition, in order, on each cell (top to bottom in table); $n = 7$ cells. Values indicate mean $\pm$ SEM.



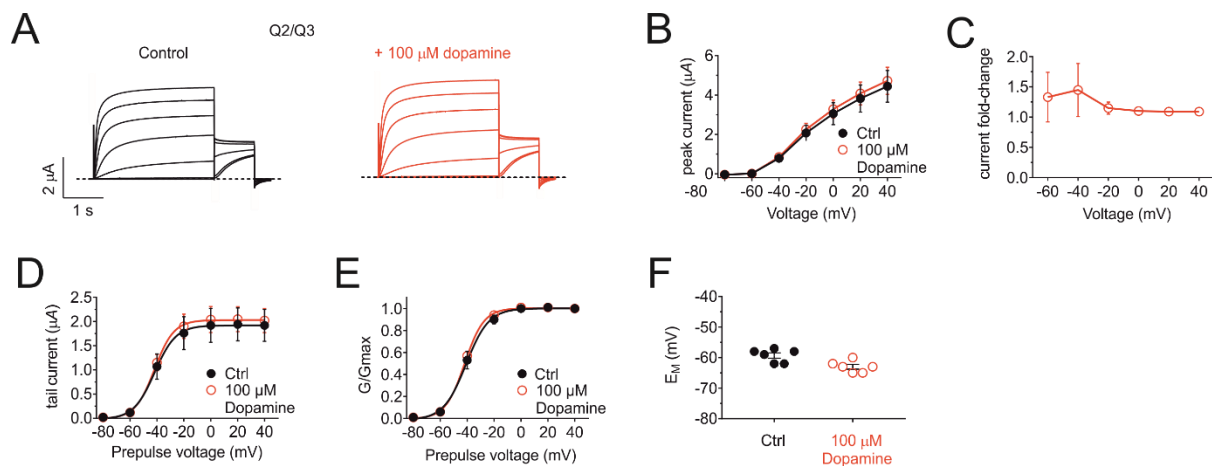
Supplementary Figure 20. Acetylcholine effects on KCNQ2/3 channels

- A. Mean normalized peak current-voltage relationship for KCNQ2/Q3 channels in the absence (black) and presence (red) of Acetylcholine, $n = 5$. Error bars indicate SEM.
- B. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- C. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- D. Mean dose response of KCNQ2/3 channels between -60 and +40 mV, $n = 5$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-53.5 ± 1.6 ($n = 5$)	-53.1 ± 5.8 ($n = 5$)	6.0 ± 0.9 ($n = 5$)
0.1 μM ACh	-46.2 ± 2.5 ($n = 5$) *	-44.4 ± 6.3 ($n = 5$)	5.1 ± 2.5 ($n = 5$)
1 μM ACh	-43.4 ± 2.4 ($n = 5$) *	-43.2 ± 4.7 ($n = 5$)	5.6 ± 2.7 ($n = 5$)
10 μM ACh	-43.1 ± 1.5 ($n = 5$) **	-42.9 ± 4.7 ($n = 5$)	5.1 ± 1.9 ($n = 5$)
100 μM ACh	-41.7 ± 1.4 ($n = 5$) ***	-42.4 ± 4.8 ($n = 5$)	4.8 ± 2.7 ($n = 5$)
1 mM ACh	-41.3 ± 2.5 ($n = 5$) **	-42.2 ± 4.6 ($n = 5$)	3.8 ± 6.5 ($n = 5$)

Supplementary Table 20. Summary of effects of acetylcholine on KCNQ2/3 channels.

Statistics versus same channel in absence of acetylcholine: *** $p=0.006$, ** $p=0.001$, * $p=0.04$. Values indicate mean $\pm$ SEM.



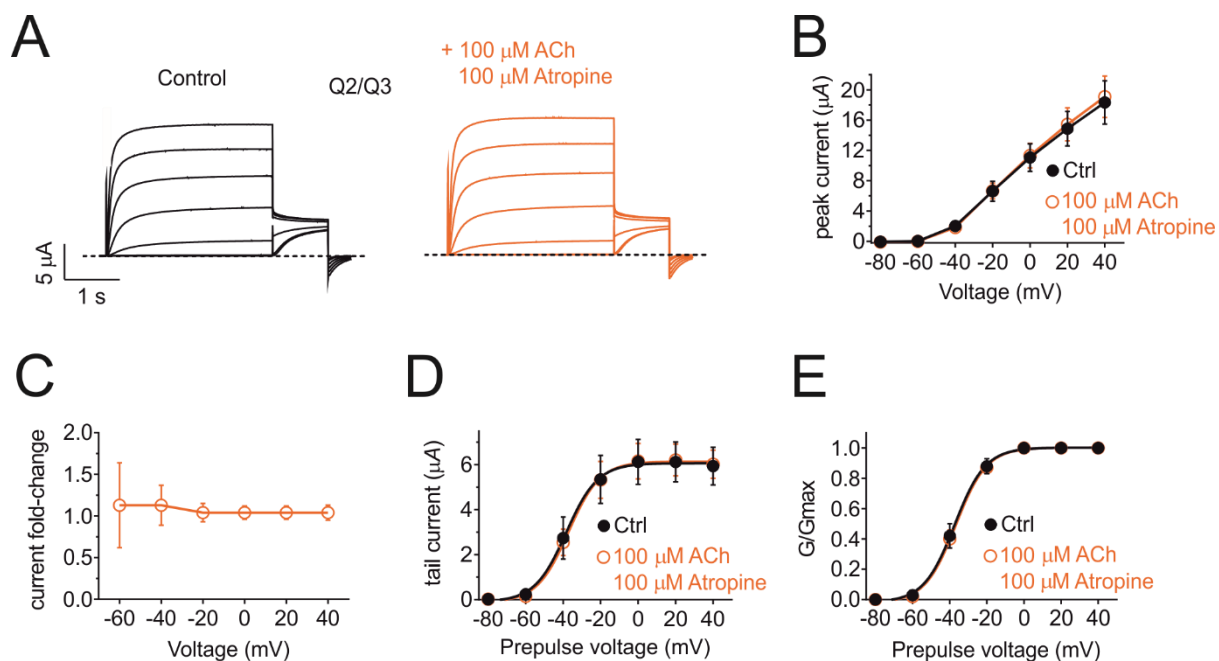
Supplementary Figure 21. KCNQ2/3 channels are insensitive to dopamine

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (red) of dopamine (100 μ M), $n = 6$.
 B. Mean peak current-voltage relationship for recordings as in panel A, $n = 6$. Error bars indicate SEM.
 C. Mean current fold-change versus voltage as in panel A, $n = 6$. Error bars indicate SEM.
 D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.
 E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.
 F. Scatter plot of resting membrane potential (E_M) as in panel A, $n = 6$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-40.8 ± 1.4 ($n = 6$)	-41.6 ± 5.3 ($n = 6$)	8.4 ± 1.5 ($n = 6$)
100 μ M Dopamine	-41.9 ± 0.6 ($n = 6$)	-41.8 ± 3.6 ($n = 6$)	7.2 ± 0.6 ($n = 6$)

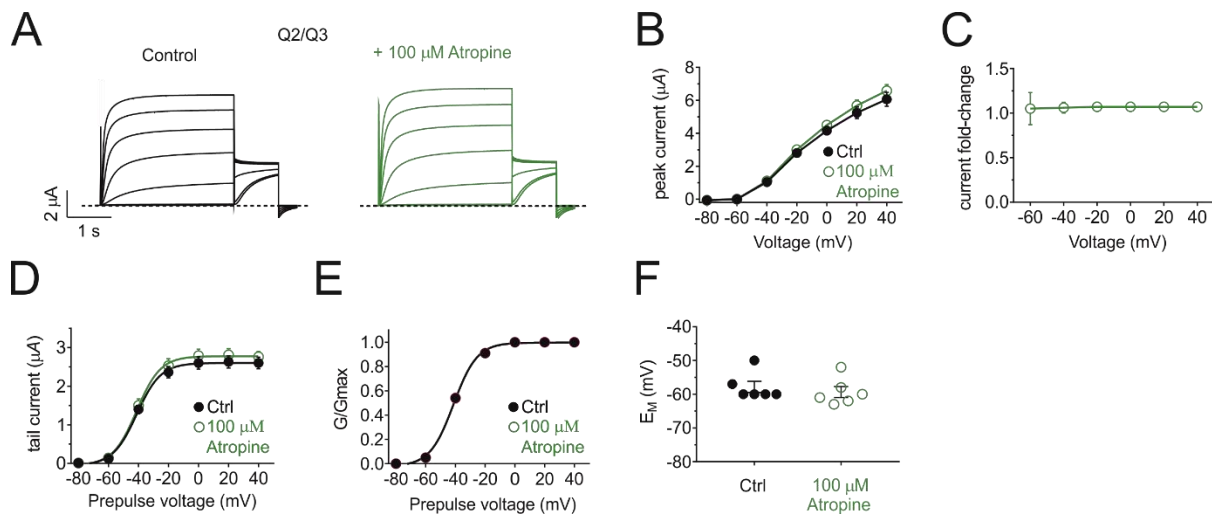
Supplementary Table 21. Summary of Effects of dopamine on KCNQ2/3 channels.

Values indicate mean $\pm$ SEM.



Supplementary Figure 22. Atropine prevents acetylcholine-mediated inhibition of KCNQ2/3 channels

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (orange) of Acetylcholine (10 μ M) and atropine (100 μ M), $n = 5$.
- B. Mean peak current-voltage relationship as in panel A, $n = 5$. Error bars indicate SEM.
- C. Mean current fold-change versus voltage as in panel A, $n = 5$. Error bars indicate SEM.
- D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.



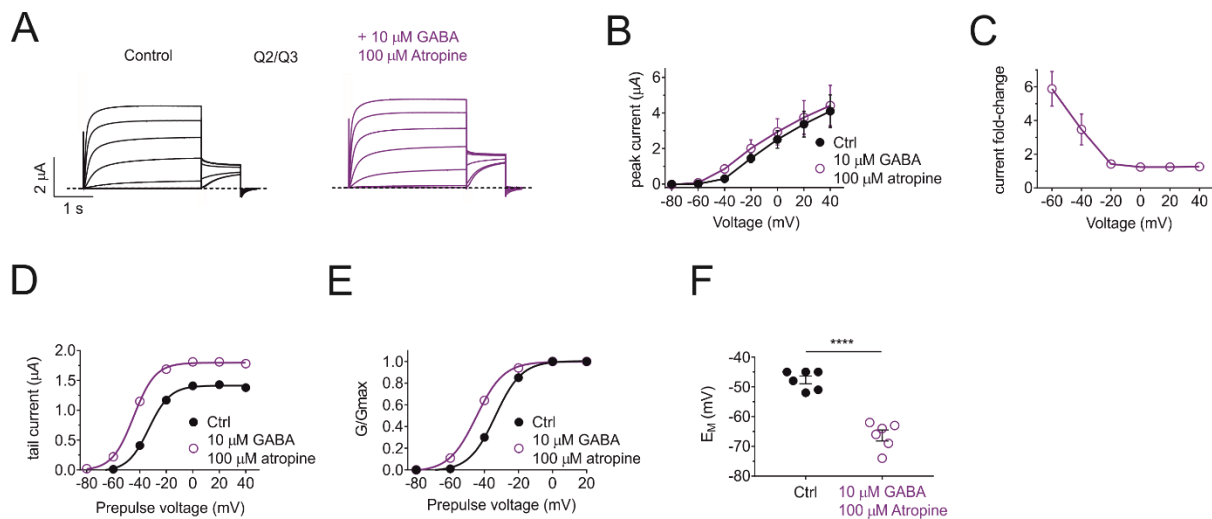
Supplementary Figure 23. Lack of effects of atropine on KCNQ2/3 channels

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (green) of atropine (100 μ M), $n = 5$.
 B. Mean peak current-voltage relationship as in panel A, $n = 5$. Error bars indicate SEM.
 C. Mean current fold-change versus voltage as in panel A, $n = 5$. Error bars indicate SEM.
 D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
 E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
 F. Scatter plot of resting membrane potential (E_M) from oocytes as in panel A, $n = 5$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-41.3 ± 0.4 ($n = 5$)	-41.1 ± 1.8 ($n = 5$)	7.9 ± 0.5 ($n = 5$)
100 μ M Atropine	-41.3 ± 0.7 ($n = 5$)	-41.2 ± 1.9 ($n = 5$)	7.9 ± 0.8 ($n = 5$)

Supplementary Table 22. Summary of Effects of atropine on KCNQ2/3 channels.

Values indicate mean $\pm$ SEM.



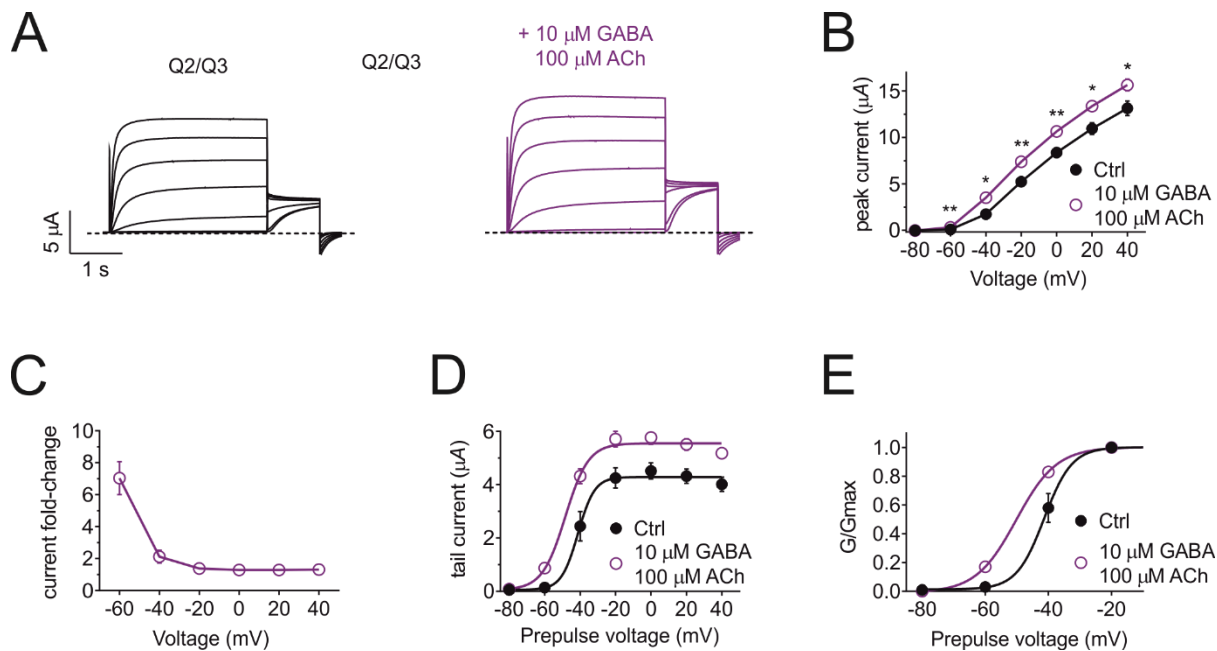
Supplementary Figure 24. Atropine exhibits no effect on GABA sensitivity of KCNQ2/3 channels

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (purple) of GABA (10 μ M) and atropine (100 μ M), $n = 5$.
 B. Mean peak current-voltage relationship as in panel A, $n = 5$. Error bars indicate SEM.
 C. Mean current fold-change versus voltage as in panel A, $n = 5$. Error bars indicate SEM.
 D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
 E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
 F. Scatter plot of unclamped resting membrane potential (E_M) for oocytes as in panel A, $n = 5$, **** $P < 0.0001$ versus control. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-33.6 ± 0.9 ($n = 5$)	-33.1 ± 4.9 ($n = 5$)	7.7 ± 0.8 ($n = 5$)
10 μ M GABA 100 μ M Atropine	-47.7 ± 1.1 ($n = 5$) ****	-44.6 ± 8.4 ($n = 5$)	8.0 ± 0.9 ($n = 5$)

Supplementary Table 23. Summary of effects of atropine on GABA sensitivity of KCNQ2/3 channels.

Statistics versus same channel in absence of 10 μ M GABA and 100 μ M atropine: **** $p < 0.0001$. Values indicate mean $\pm$ SEM.



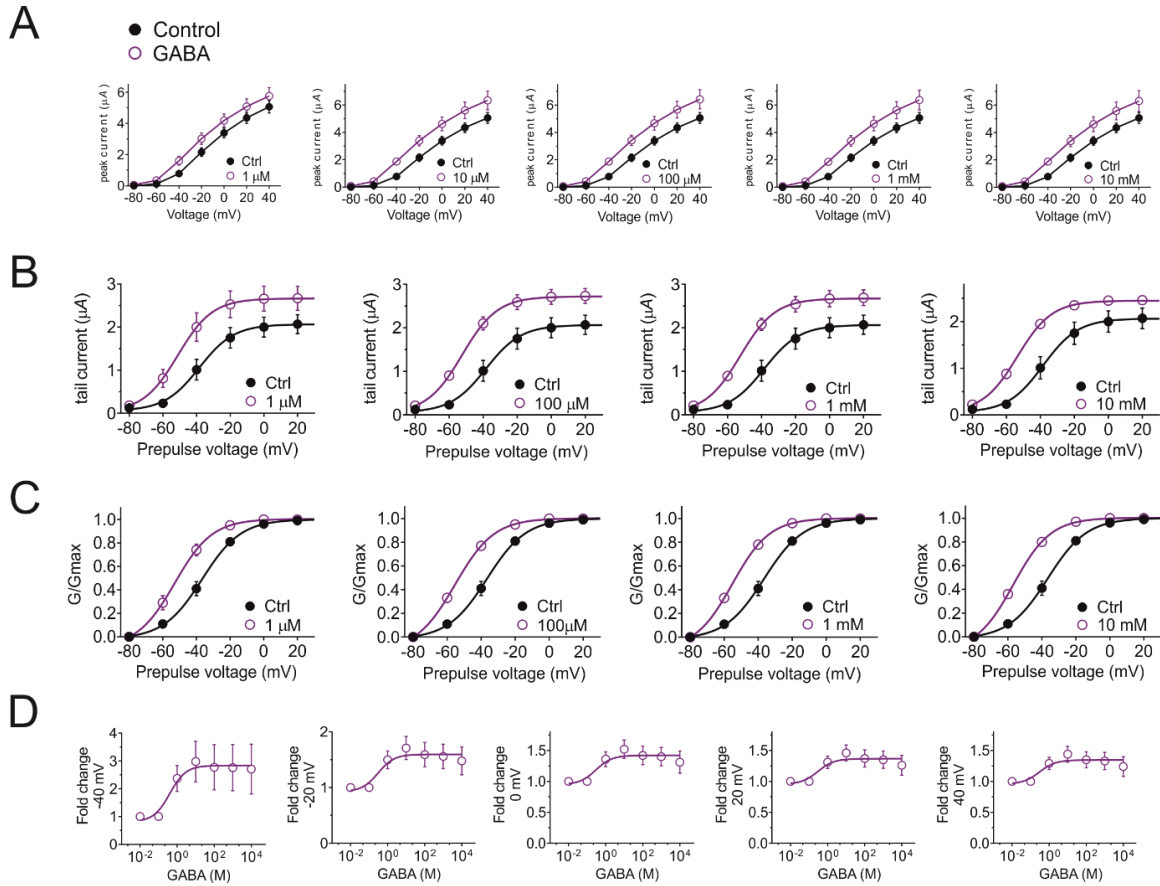
Supplementary Figure 25. GABA overcomes acetylcholine inhibition of KCNQ2/3 channels

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (purple) of GABA (10 μ M) and ACh (100 μ M), $n = 5$.
 B. Mean peak current-voltage relationship for recordings as in panel A, $n = 5$. * $P < 0.05$; ** $P < 0.01$, versus same-voltage control. Error bars indicate SEM.
 C. Mean current fold-change versus voltage as in panel A, $n = 6$. Error bars indicate SEM.
 D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.
 E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-41.3 ± 1.3 ($n = 6$)	-41.9 ± 2.1 ($n = 6$)	4.4 ± 3.2 ($n = 6$)
10 μ M GABA 100 μ M ACh	-50.2 ± 0.6 ($n = 6$) ***	-48.3 ± 1.7 ($n = 6$) *	6.3 ± 0.4 ($n = 6$)

Supplementary Table 24. Summary of Effects of acetylcholine on GABA sensitivity of KCNQ2/3 channels.

Statistics versus same channel in absence of 10 μ M GABA and 100 μ M acetylcholine: *** $p = 0.0004$, * $p = 0.04$. Values indicate mean $\pm$ SEM.



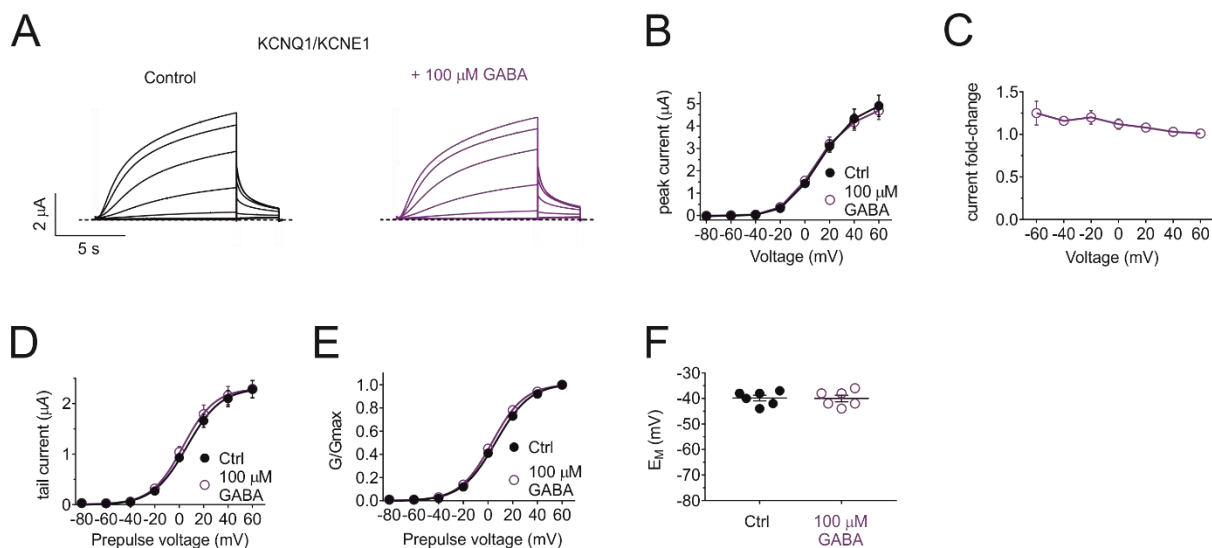
Supplementary Figure 26. GABA effects on KCNQ2/3 after pre-incubation with wortmannin

- A. Mean peak current-voltage relationship for KCNQ2/Q3 channels in the absence (black) and presence (purple) of GABA, $n = 5$. Error bars indicate SEM.
- B. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- C. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- D. Mean dose response of KCNQ2/3 channels between -40 and +40 mV, $n = 5$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-34.9 ± 1.4 ($n = 5$)	-34.3 ± 4.6 ($n = 5$)	11.5 ± 1.3 ($n = 5$)
1 μ M GABA	-52.9 ± 1.9 ($n = 5$) ***	-54.7 ± 6.4 ($n = 5$)	11.2 ± 1.6 ($n = 5$)
10 μ M GABA	-57.6 ± 1.3 ($n = 5$) ****	-57.8 ± 5.4 ($n = 5$)	10.8 ± 0.9 ($n = 5$)
100 μ M GABA	-55.4 ± 0.9 ($n = 5$) ****	-55.4 ± 3.3 ($n = 5$) *	11.5 ± 0.7 ($n = 5$)
1 mM GABA	-55.1 ± 0.8 ($n = 5$) ****	-55.5 ± 3.5 ($n = 5$) *	10.9 ± 0.6 ($n = 5$)
10 mM GABA	-56.8 ± 0.9 ($n = 5$) ****	-56.9 ± 1.6 ($n = 5$) *	10.9 ± 0.7 ($n = 5$)

Supplementary Table 25. Summary of Effects of wortmannin on GABA sensitivity of KCNQ2/3 channels.

Statistics versus same channel in absence of GABA: **** $p < 0.0001$, *** $p = 0.002$, * $p = 0.01$. Values indicate mean $\pm$ SEM.



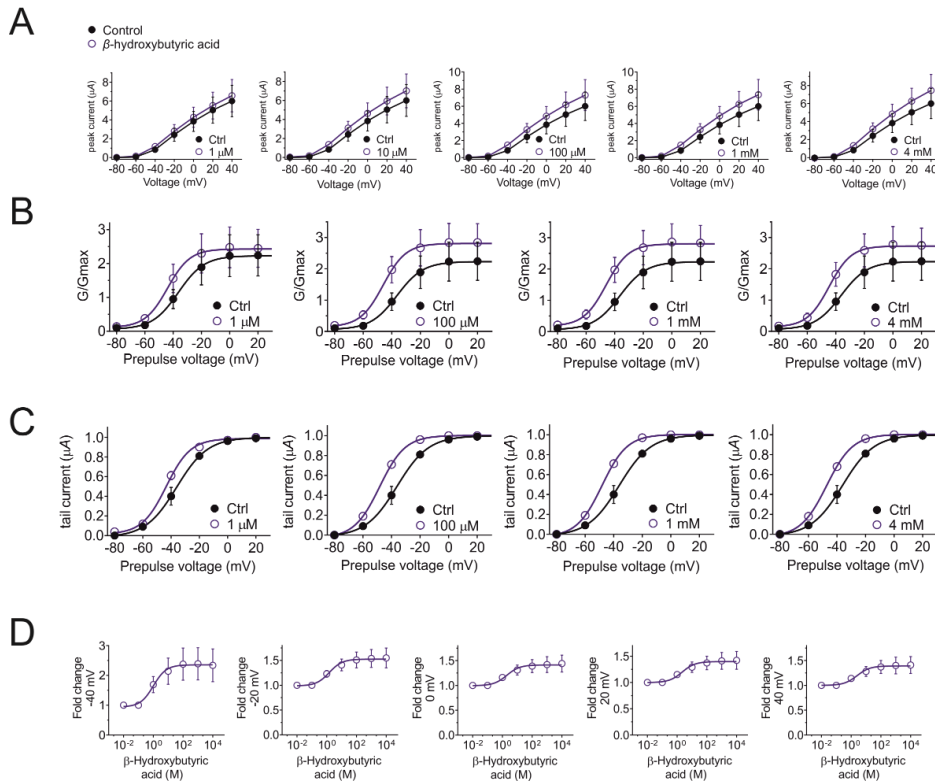
Supplementary Figure 27. Effects of GABA on KCNQ1-KCNE1 channels

- A. Averaged KCNQ1/KCNE1 traces in the absence (black) and presence (purple) of GABA (100 μ M), $n = 6$.
- B. Mean peak current-voltage relationship as in panel A, $n = 6$. Error bars indicate SEM.
- C. Mean current fold-change versus voltage as in panel A, $n = 6$. Error bars indicate SEM.
- D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.
- E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.
- F. Scatter plot of resting membrane potential (E_m) as in panel A, $n = 6$. Error bars indicate SEM.

Q1/E1	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	6.1 ± 0.8 ($n = 6$)	6.3 ± 2.6 ($n = 6$)	14 ± 0.7 ($n = 6$)
100 μ M GABA	3.3 ± 1.2 ($n = 6$)	3.1 ± 2.7 ($n = 6$)	13.2 ± 0.9 ($n = 6$)

Supplementary Table 26. Summary of Effects of GABA on KCNQ1/KCNE1 channels.

Values indicate mean $\pm$ SEM.



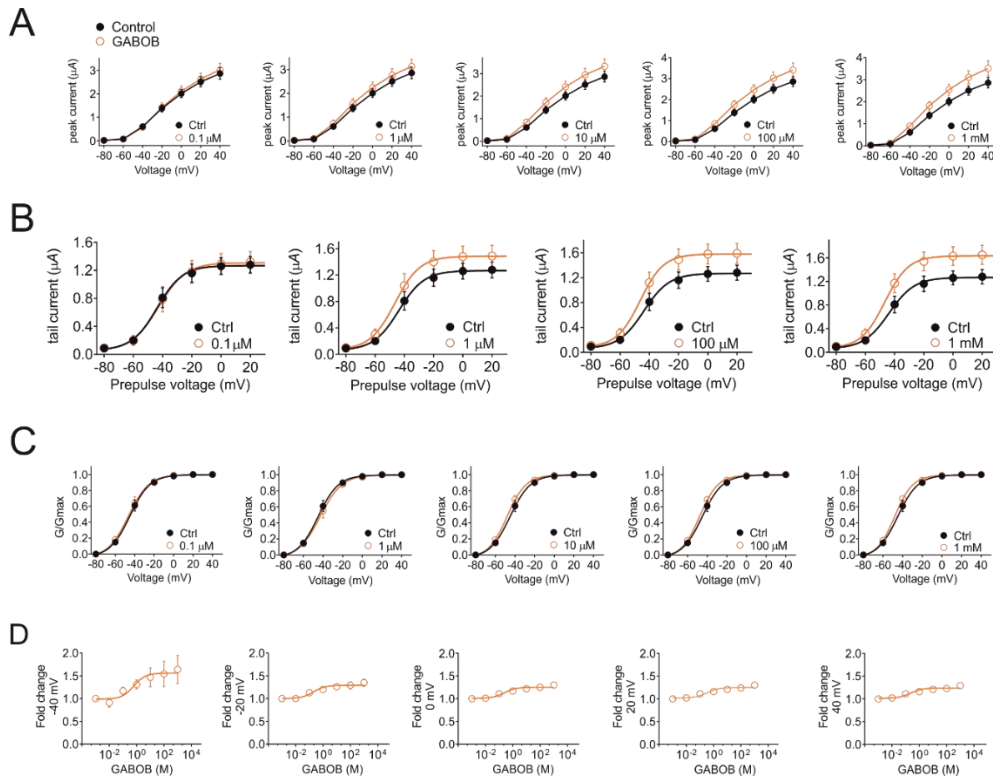
Supplementary Figure 28. Effects of β -Hydroxybutyric acid on KCNQ2/3 channels

- A. Mean peak current-voltage relationship for KCNQ2/3 channels in the absence (black) and presence (blue) of β -Hydroxybutyric acid, $n = 5$. Error bars indicate SEM.
- B. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- C. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
- D. Mean dose response of KCNQ2/3 channels between -40 and $+40$ mV, $n = 5$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-36.1 ± 1.8 ($n = 5$)	-36.4 ± 8.7 ($n = 5$)	6.2 ± 0.8 ($n = 5$)
1 μ M β -Hydrox	-46.1 ± 0.6 ($n = 5$) **	-43.9 ± 8.2 ($n = 5$)	5.9 ± 0.4 ($n = 5$)
10 μ M β -Hydrox	-48.1 ± 0.4 ($n = 5$) **	-45.5 ± 7.9 ($n = 5$)	5.9 ± 0.4 ($n = 5$)
100 μ M β -Hydrox	-50.2 ± 0.3 ($n = 5$) **	-46.0 ± 7.9 ($n = 5$)	5.9 ± 0.3 ($n = 5$)
1 mM β -Hydrox	-46.5 ± 0.9 ($n = 5$) **	-46.0 ± 7.4 ($n = 5$)	5.7 ± 0.6 ($n = 5$)
4 mM β -Hydrox	-44.9 ± 0.8 ($n = 5$) **	-44.7 ± 7.1 ($n = 5$)	4.9 ± 0.6 ($n = 5$)

Supplementary Table 27. Summary of Effects of β -Hydroxybutyric acid on KCNQ2/3 channels.

Statistics versus same channel in absence of β -Hydroxybutyric acid: ** $p=0.001$. Values indicate mean $\pm$ SEM.



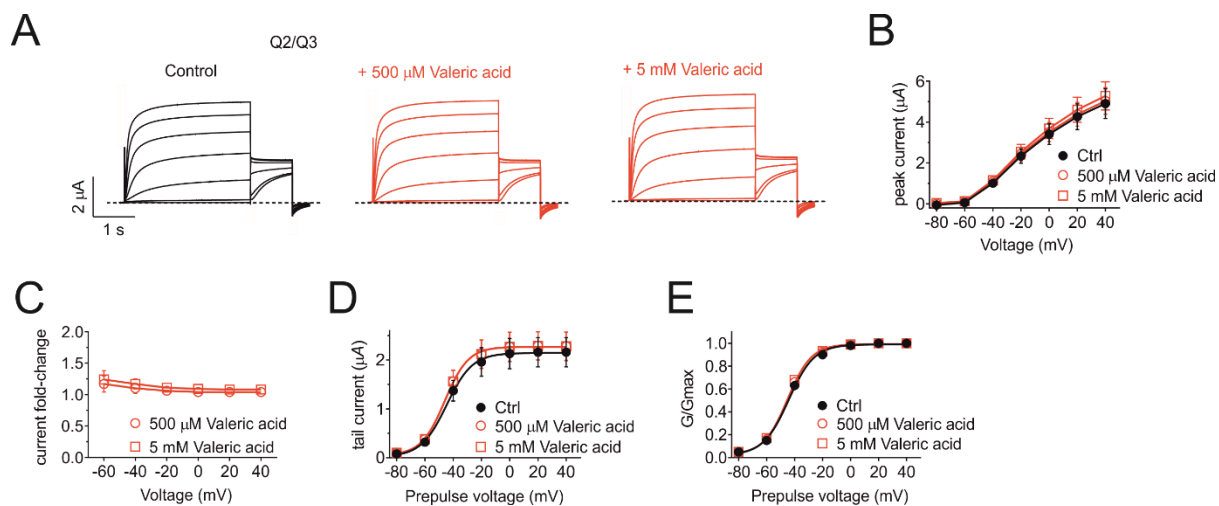
Supplementary Figure 29. Effects of GABOB on KCNQ2/3 channels

- A. Mean peak current-voltage relationship for KCNQ2/3 channels in the absence (black) and presence (orange) of GABOB, $n = 6$. Error bars indicate SEM.
- B. Mean tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.
- C. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.
- D. Mean dose response of KCNQ2/3 channels between -40 and +40 mV, $n = 6$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-44.9 ± 1.5 ($n = 6$)	-43.8 ± 3.9 ($n = 6$)	10.3 ± 1.3 ($n = 6$)
0.01 μ M GABOB	-42.8 ± 2.0 ($n = 6$)	-42.7 ± 5.0 ($n = 6$)	11.1 ± 1.8 ($n = 6$)
0.1 μ M GABOB	-46.6 ± 1.9 ($n = 6$)	-45.8 ± 5.1 ($n = 6$)	10.6 ± 1.6 ($n = 6$)
1 μ M GABOB	-47.1 ± 1.4 ($n = 6$)	-46.4 ± 4.7 ($n = 6$)	10.1 ± 1.2 ($n = 6$)
10 μ M GABOB	-48.1 ± 1.2 ($n = 6$)	-46.8 ± 4.5 ($n = 6$)	9.6 ± 0.9 ($n = 6$)
100 μ M GABOB	-47.8 ± 0.9 ($n = 6$)	-46.3 ± 4.2 ($n = 6$)	9.3 ± 0.8 ($n = 6$)
1 mM GABOB	-48.0 ± 0.9 ($n = 6$)	-46.7 ± 4.0 ($n = 6$)	9.2 ± 0.7 ($n = 6$)

Supplementary Table 28. Summary of Effects of GABOB on KCNQ2/3 channels.

Values indicate mean $\pm$ SEM.



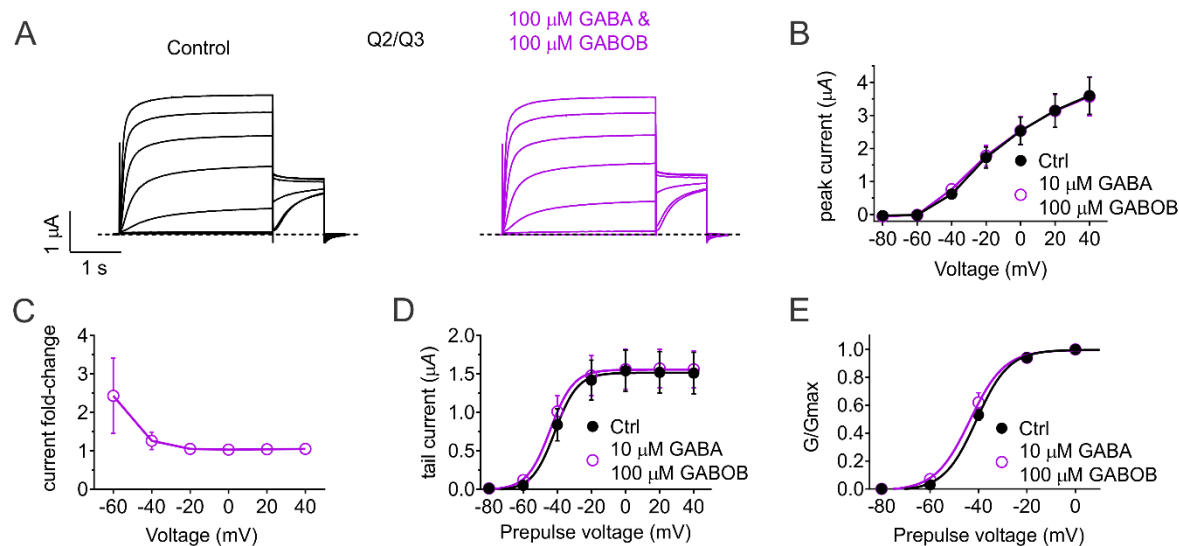
Supplementary Figure 30. Lack of effects of valeric acid on KCNQ2/3 channels

- A. Mean peak current-voltage relationship for KCNQ2/3 channels in the absence (black) and presence (red) of valeric acid (500 μ M; middle, 5 mM; right), $n = 6$.
- B. Mean peak current-voltage relationship as in panel A, $n = 6$. Error bars indicate SEM.
- C. Mean current fold-change versus voltage as in panel A, $n = 6$. Error bars indicate SEM.
- D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.
- E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 6$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-44.2 ± 0.6 ($n = 6$)	-44.5 ± 5.4 ($n = 6$)	9.1 ± 0.5 ($n = 6$)
500 μ M Valeric acid	-45.1 ± 0.6 ($n = 6$)	-45.6 ± 4.9 ($n = 6$)	8.5 ± 0.6 ($n = 6$)
500 mM Valeric acid	-45.1 ± 0.6 ($n = 6$)	-45.9 ± 4.9 ($n = 6$)	8.5 ± 0.6 ($n = 6$)

Supplementary Table 29. Summary of Effects of valeric acid on KCNQ2/3 channels.

Values indicate mean $\pm$ SEM.



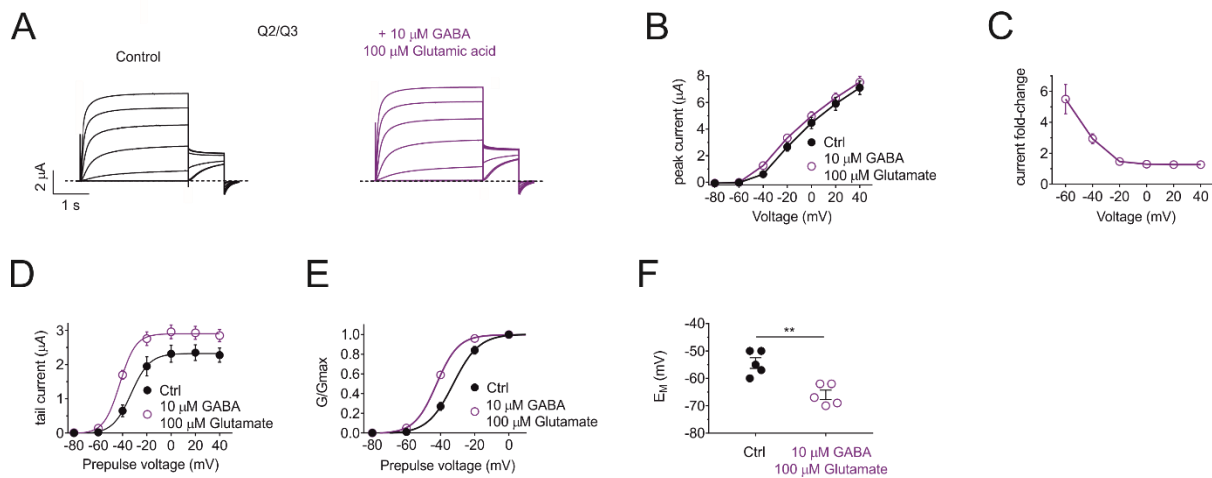
Supplementary Figure 31. GABOB reduces GABA sensitivity in KCNQ2/3 channels

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (purple) of GABA (10 μ M) and GABOB (100 μ M), $n = 4$.
 B. Mean peak current-voltage relationship for recordings as in panel A, $n = 4$. Error bars indicate SEM.
 C. Mean current fold-change versus voltage as in panel A, $n = 4$. Error bars indicate SEM.
 D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 4$. Error bars indicate SEM.
 E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 4$. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-48.1 ± 0.7 ($n = 4$)	-47.0 ± 2.4 ($n = 4$)	6.7 ± 0.5 ($n = 4$)
10 μ M GABA 100 μ M GABOB	-51.0 ± 0.7 ($n = 4$)	-49.6 ± 2.0 ($n = 4$)	7.2 ± 0.4 ($n = 4$)

Supplementary Table 30. Summary of effects of GABOB on GABA sensitivity of KCNQ2/3 channels.

Values indicate mean $\pm$ SEM.



Supplementary Figure 32. Glutamate has no effect on GABA activation of KCNQ2/3 channels

- A. Averaged KCNQ2/3 traces in the absence (black) and presence (purple) of GABA (10 μ M) and glutamate (100 μ M), $n = 5$.
 B. Mean peak current-voltage relationship as in panel A, $n = 5$. Error bars indicate SEM.
 C. Mean current fold-change versus voltage as in panel A, $n = 5$. Error bars indicate SEM.
 D. Mean tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
 E. Mean normalized tail current versus prepulse voltage relationships as in panel A, $n = 5$. Error bars indicate SEM.
 F. Scatter plot of resting membrane potential (E_M) as in panel A, $n = 5$, ** $P < 0.002$ versus control. Error bars indicate SEM.

Q2/Q3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	-32.5 ± 0.8 ($n = 5$)	-32.9 ± 3.1 ($n = 5$)	7.5 ± 0.6 ($n = 5$)
10 μ M GABA 100 μ M Glutamate	-42.4 ± 0.2 ($n = 5$) ***	-42.4 ± 2.2 ($n = 5$) *	6.4 ± 0.2 ($n = 5$)

Supplementary Table 31. Summary of Effects of glutamate on GABA sensitivity of KCNQ2/3 channels.

Statistics versus same channel in absence of 10 μ M GABA and 100 μ M glutamate: *** $p = 0.0001$, * $p = 0.03$. Values indicate mean $\pm$ SEM.