Supplementary Information

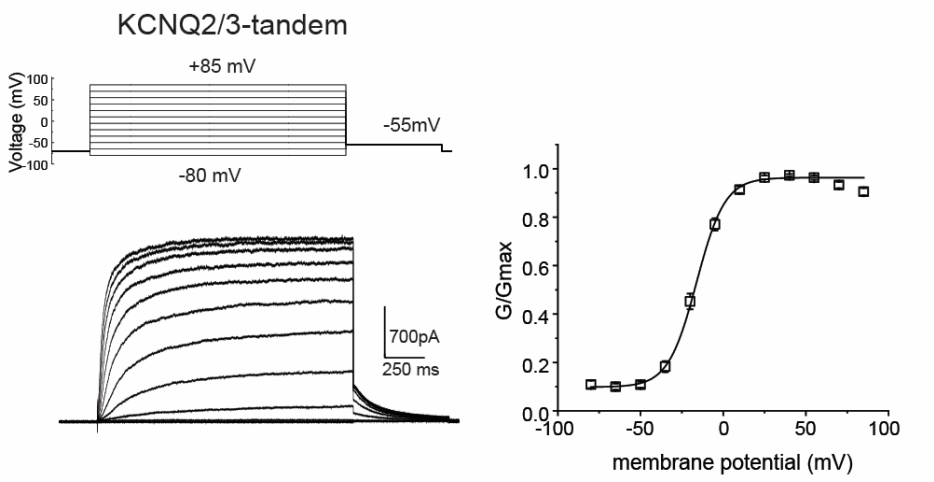

Figure S1. Trans-splicing of KCNQ2 and KCNQ3 leads to functional KCNQ2/3-tandem channels. Left, representative traces from HEK293T cells co-expressing KCNQ2-ER-n and KCNQ3-ER-c. KCNQ2/3-tandem refers to tandem KCNQ2/3 channels. Right, summary graph showing the KCNQ2/3-tandem conductance-to-voltage relationship. Data were fit using the Boltzmann equation ($V_{0.5}$ = -15.7 ± 1.4 mV, n=23). Data are displayed as mean ± s.e.m.

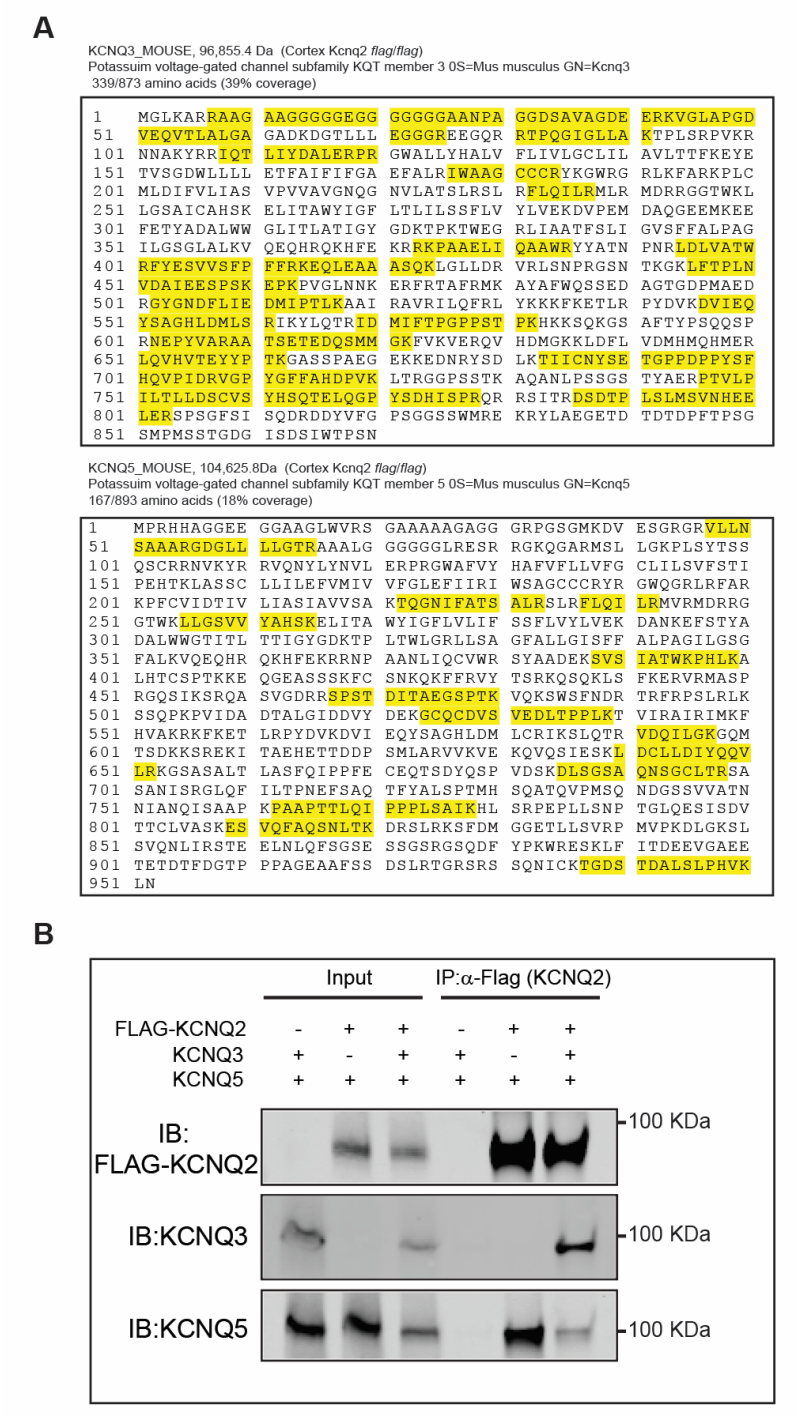

Figure S2. KCNQ3 and KCNQ5 forms a complex with KCNQ2 channels. (A) Representative KCNQ3 and KCNQ5 peptide coverage retrieved from anti-FLAG-KCNQ2 cortical IPs of *Kcnq2*$^{flag/flag}$ mice. Yellow indicates recovered peptides. **(B)** Immunoblot showing immunoprecipitated proteins from HEK293T cells expressing various 3X-FLAG KCNQ2, KCNQ3, and KCNQ5 subunit combinations. Immunoprecipitation was performed using an anti-FLAG M2 antibody.

Table S1. Biophysical properties of HEK293T cells expressing different combinations of KCNQ2-, KCNQ5-, and KCNQ3-containing channels.

	$V_{0.5}$ (mV) mean ± S.D.	**Slope (k) mean ± S.D.**	**Current density (G_{max}/pF) mean ± S.D.**
untransfected HEK293T	N.D.	N.D.	2.4 ± 1.0 (n=8)
KCNQ2-ER-n	N.D.	N.D.	2.6 ± 0.6 (n=7)
KCNQ3-ER-c	N.D.	N.D.	2.0 ± 0.2 (n=7)
KCNQ5-ER-c	N.D.	N.D.	2.4 ± 0.5 (n=7)
KCNQ2-ER-n + Calm	N.D.	N.D.	2.6 ± 1.0 (n=5)
KCNQ5-ER-c + Calm	N.D.	N.D.	4.2 ± 2.3 (n=5)
KCNQ2/Q5-tandem	-11.1 ± 9.3 (n=23)	13.4 ± 6.9 (n=23)	7.3 ± 17.7 (n=35)
KCNQ2/Q5-tandem + Calm	-8.6 ± 10.2 (n=22)	12.3 ± 4.2 (n=22)	13.5 ± 8.3 (n=32)[a] p=0.002
KCNQ2/Q5-tandem + Calm (30℃)	-15.8 ± 8.4 (n=9)	11.28 ± 2.9 (n=9)	17.3 ± 16.8 (n=14)[a] p=0.006
KCNQ2/Q5-tandem + PIP5K	-31.5 ± 6.2 (n=6)	15.5 ± 9.1 (n=6)	32.8 ± 15.2 (n=6)[a] $p=1.3x10^{-7}$
KCNQ3 WT	-21.7 ± 10.5 (n=11)	7.7 ± 1.3 (n=11)	41.0 ± 17.7 (n=10)
KCNQ2/Q5-tandem + KCNQ3	-14.4 ± 7.2 (n=18)	9.6 ± 3.4 (n=18)	78.4 ± 38.9 (n=19)[a,c] $p=1.8x10^{-14}$ p=0.008
KCNQ2/3	-29.6 ± 2.2 (n=3)	12.5 ± 1.5 (n=3)	197.1 ± 36.0 (n=3)[b] $p=7.2x10^{-8}$
KCNQ2/Q3-tandem	-15.7 ± 6.9 (n=23)	9.2 ± 2.8 (n=23)	28.7 ± 31.4 (n=18)[b]
KCNQ2/Q3-tandem + Calm	-19.0 ± 6.8 (n=19)	9.3 ± 2.8 (n=19)	54.4 ± 57.1 (n=20)[b] p=0.1

a, *t-test* versus KCNQ2/Q5-tandem
b, *t-test* versus KCNQ2/Q3-tandem
c, t-test versus KCNQ3 WT