

Supplementary Information

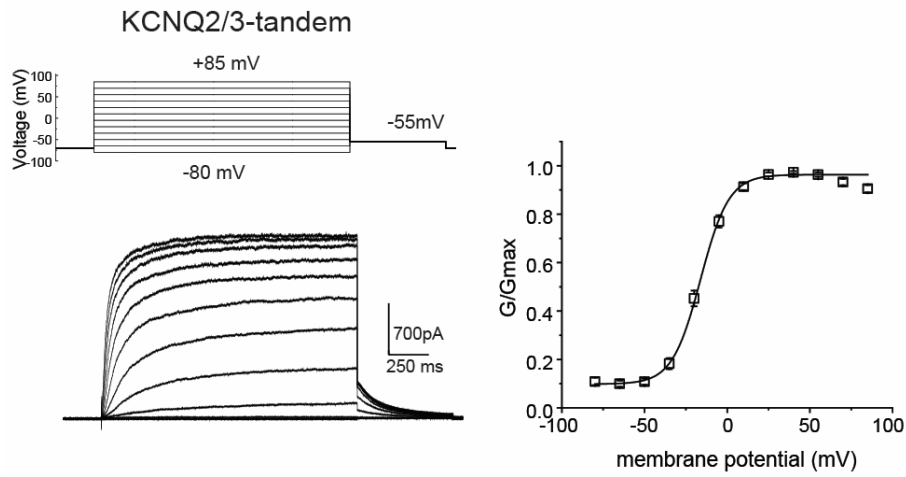


Figure S1. Trans-splicing of KCNQ2 and KCNQ3 leads to functional KCNQ2/3-tandem channels. Left, representative traces from HEK293T cells co-expressing KCNQ2-ER-n and KCNQ3-ER-c. KCNQ2/3-tandem refers to tandem KCNQ2/3 channels. Right, summary graph showing the KCNQ2/3-tandem conductance-to-voltage relationship.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

A

KCNQ3_MOUSE, 96,855.4 Da (Cortex Kcnq2 flag/flag)
Potassium voltage-gated channel subfamily KQT member 3 OS=Mus musculus GN=Kcnq3
339/873 amino acids (39% coverage)

1	MGLKARRAAG	AAGGGGEGG	GGGGGAANPA	GGDSAVAGDE	ERKVG LAPGD
51	VEQVTLALGA	GADKDG TLL	EGGREGGQR	RTPQGIGLLA	KTPLSRPVKR
101	NNAKYRRIQT	LIYDALERPR	GWALLYHALV	FLIVLGC LIL	AVLTTFK EYE
151	TVSGDWL LLL	ETFAIFIFGA	EFALR IWAAG	CCCR YKGRWG	RLKFARKPLC
201	MGDIFVLIAS	VPVVAVGNQG	NVLATSLRSL	RFLQILRLR	MDRRGGTWKL
251	LGS AICAHSK	ELITAWYIGF	LTLILSSFLV	YLVEKDVPEM	DAQGEEMKEE
301	FETYADALW	GLITLATIGY	GDKTPKTWEG	RLIAATFSLI	GVSFFALPAG
351	ILGSG LALKV	QEQRQKHFE	KRKKPAELI	QAAR Y YATN	PNRLDLVATW
401	RFYESVVSFP	FFRKEQLEAA	ASQKLG LLD	VRLSNPRGSN	TKGK LFTPLN
451	YDAIEESPSK	EPKPVGLNKK	ERFR TAFR MK	AYAFWQSS ED	AGTGDPMAED
501	RGYGNDFLIE	DMIPTLWAAI	RAVRILQFRL	YKKKFKETLR	PYDVK DVIEQ
551	YSAGHLMLS	RIKYLQTRID	MIPTGPPST	PKHKKSQKGS	AFTYPSQQSP
601	RNEPYVARAA	TSETEDQSM	GK FVKVERQV	HDMGK LDFL	VDMHMHMER
651	LQVHVTEYYP	TKGASSPAEG	EKKEDNRYSD	LKTIICNYSE	TGPPDP PYSF
701	HQVPIDRVGP	YGFFAHDPVK	LTRGGPSSTK	AQANLPSSGS	TYAERPTVLP
751	LLTLLDSCVS	YHSQTE LQGP	YSDHISFRQR	RSITRDS DTF	LSLMSVNH EE
801	LERSPSFGFSI	SQRDDYVFG	PSGGSSWMRE	KRYLAEGETD	TDTD PFTPSG
851	SMPMSSTGDG	ISDSIWTPSN			

KCNQ5_MOUSE, 104,625.8Da (Cortex Kcnq2 flag/flag)
Potassium voltage-gated channel subfamily KQT member 5 OS=Mus musculus GN=Kcnq5
167/893 amino acids (18% coverage)

1	MPRHAGGEE	GGAAGLWVRS	GAAAAAGAGG	GRPGSGMKDV	ESGRGRVLLN
51	SAAARGDGLL	LLGTRAAALG	GGGGGLRESR	RGKQGARMSL	LGKPLSYTSS
101	QSCRRNVKYR	RVQNYLYNVL	ERPRGWAFVY	HAFVFLLVFG	CLILSVFSTI
151	PEHTKLASSC	LLILEFVMIV	VFGLEFIIRI	WSAGCCCRYR	GWQGR LRFAR
201	KPFCVIDTIV	LIASIAV VSA	KFOGNIFATS	ALRSLRFLQI	LRMVRMDRRG
251	GTWKL LGSVV	YAHSKELITA	WYIGFLV LIF	SSFLVYLV EK	DANKEFSTYA
301	DALWWTITL	TTIGYGDKTP	LTWLGRLLSA	GFALLGISFF	ALPAGILGSG
351	FALKVQEQHR	QKHFEKRRNP	AANLIQC VWR	SYAADEKSVS	IATWKPHLKA
401	LHTCSPTKKE	QGEASSSKFC	SNKQKFFRVY	TSRQSQSKLS	FKERV RMASP
451	RGQSIKSRQA	SVGDRRSPST	DITAE GSPTK	VQKSWSFNDR	TRFRSLRLK
501	SSQPKPVIDA	D TALGIDDDVY	DEKGCQCDVS	VEDLTPPLKT	VIRAIRIMKF
551	HVAKRKFKET	LRPYDVKDVI	EQYSAGHLDM	LCRIKSLQTR	VDQILGK GQM
601	TSDKKSREKI	TAEHETDDP	SMLARVVKVE	KQVQSIESKL	DCLLDIYQQV
651	LRKGSASALT	LASFQIP PFE	CEQTS DYQSP	VDSK DLSGSA	QNSGCLTRSA
701	SANISRGLQF	ILTPNEFSAQ	TFYALSPTMH	SQATQVPMSQ	NDGSSVVATN
751	NIANQISAAP	KPAAPTTLQI	PPPLSAIKHL	SRPEPLSNP	TGLQESISDV
801	TTCLVASKES	VQFAQSNLTK	DRSLRKS FDM	GGETLLSVRP	MVPKDLGKSL
851	SVQNLIRSTE	ELNLQFSGSE	SSGSRGSQDF	YPKWRESKLF	ITDEEVGAEE
901	TETD TDFDGT	PPAGEAAFSS	DSLRTGRSRS	SQNICK TGD	TDALSLPHVK
951	LN				

B

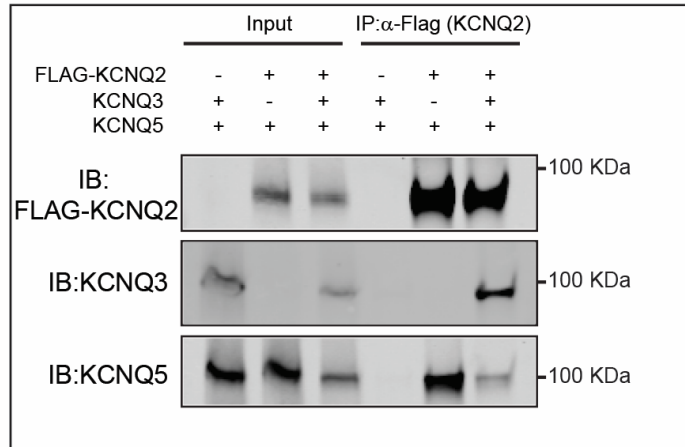


Figure S2. KCNQ3 and KCNQ5 forms a complex with KCNQ2 channels. (A) Representative KCNQ3 and KCNQ5 peptide coverage retrieved from anti-FLAG-KCNQ2 cortical IPs of *Kcnq2^{flag/flag}* mice. Yellow indicates recovered peptides. (B) Immunoblot showing immunoprecipitated proteins from HEK293T cells expressing 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°C)	-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