Supplementary Information for

Tuning of the Na,K-ATPase by the beta subunit

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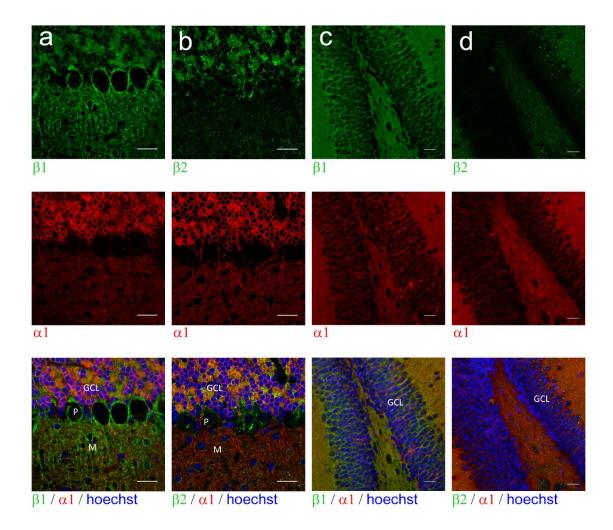
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**Supplementary Table S1:** Fit-Parameters of charge translocation curves, rate constants at -140 mV and tilt angle predictions from MD simulations. Data are represented as mean  $\pm$  SD, n.d.: not determined.

Construct	Midpoint / mV	Valence	Rate constant / s-1	Tilt angle (MD)
α1β1	$-82.8 \pm 0.6$	$0.796 \pm 0.01$	$208.4\pm2.1$	$32.7^\circ \pm 1.8^\circ$
$\alpha 1\beta 2$	$\textbf{-29.4} \pm 0.4$	$0.907 \pm 0.01$	$343.7\pm43.9$	$38.0^\circ\pm0.7^\circ$
α1β3	$-69.8 \pm 1.3$	$0.851\pm0.03$	$197.7\pm8.8$	$31.3^\circ\pm0.9^\circ$
α2β1	$\textbf{-18.1} \pm 0.9$	$0.842\pm0.03$	$400.1\pm25.1$	n.d.
$\alpha 2\beta 2$	$14.4 \pm 1.2$	$0.948 \pm 0.03$	$641.6\pm74.6$	n.d.
$\alpha 2\beta 3$	$-17.7 \pm 1.6$	$0.781 \pm 0.04$	$497.1\pm29.8$	n.d.
α3β1	$-59.7\pm0.9$	$0.735\pm0.02$	$210.6\pm38.9$	n.d.
$\alpha 3\beta 2$	$-28.7 \pm 1.1$	$0.869 \pm 0.03$	$385.8 \pm 12.4$	n.d.
α3β3	$-54.3 \pm 1.3$	$0.775\pm0.03$	$205.8\pm32.8$	n.d.
$\alpha 1\beta 1/\beta 2NT$	$-80.2 \pm 0.2$	$0.789 \pm 0.01$	$174.6 \pm 16$	n.d.
$\alpha 1\beta 1/\beta 2TM$	$-51.4 \pm 0.4$	$0.847 \pm 0.01$	$240.9\pm42$	$37.2^\circ\pm0.7^\circ$
α1β1/β2CT	$-70.4 \pm 0.3$	$0.837 \pm 0.01$	$203.5\pm95$	n.d.
α1β1/β2ΤΜC	$-86.2 \pm 1.4$	$0.756\pm0.03$	$220.3\pm56$	n.d.
$\alpha l\beta lAF$	-77.9 ±0.8	$0.852\pm0.02$	$152.5\pm31$	n.d.
α1β1ΤΑΜ	$-75.4 \pm 0.5$	$0.857\pm0.01$	$140.2 \pm 24$	n.d.
$\alpha 1\beta 1/3mut$	$-64.7 \pm 0.8$	$0.889 \pm 0.03$	$203.8\pm28.5$	$36.0^\circ\pm0.8^\circ$

**Supplementary Table S2:** Primers used to construct the  $\beta$ 1- $\beta$ 2 chimeras and  $\beta$ 1 mutants.

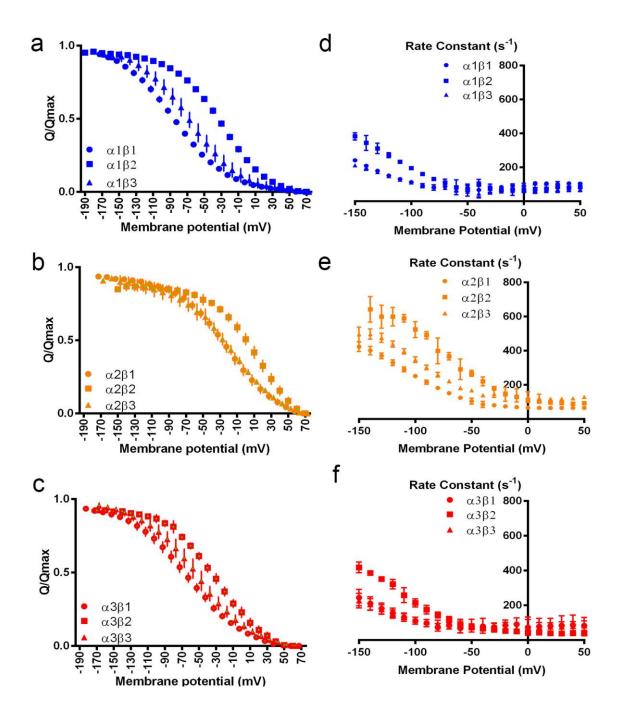
Primer	Sequence
β2 fwd	GTGCAAGGATCCAACGAATTCGCCCTT
β2 rev	GAATTGAATTTAGCGGAAGCTTGATGCC
$\beta 1/\beta 2NT$ fwd	CAGCTGCTGACCCGCCATCGCCATGGTCATCCAGAAAGA
$\beta 1/\beta 2NT$ rev	AGAAGGATCTTAAACCAACTGCCACCGGTGCGGCCCATAAACTG
$\beta 1/\beta 2TM$ fwd	AGGAGTTTCTGGGCAGGACCGGTGGCAGCTGGGCCTTTATCCTC
$\beta 1/\beta 2TM$ rev	ATCCTGGCGGGGCCACTCGGTCCTGATATGTGGGGGGTATGGTCGGA
$\beta 1/\beta 2CT$ fwd	GCCCGGACTCGCCCCGCGCCACCAAGATGGCCCGCGGGAAAGCCAAG
$\beta 1/\beta 2CT$ rev	GGCCAGTCGGTCCTGGTACTTGGGCTTAAATTCACTATGGTGA
$\beta 1/\beta 2TMC$ fwd	TATGGCTGCCTGGCTGGCATCTTCACCCTCACCATGTGGGT
β1 VSD fwd	CTGATATGTGGGCTTAAAATCACTGACGGTGAGCAGCATCACTTG
β1 VSD rev	CAAGTGATGCTGCTCACCGTCAGTGATTTTAAGCCCACATATCAG
β1 AF fwd	AAAATATTACGTAGAATAGAAGGATGAAAGCCCAACTGCCACCGGTCCTGCCCAG
βl AF rev	CTGGGCAGGACCGGTGGCAGTTGGGCTTTCATCCTTCTATTCTACGTAATATTTT
β1 TAM fwd	TGGCTGCCTGACTGCCATGTTCATCGGAACCATCCA
β1 TAM rev	TGGTTCCGATGAACATGGCAGTCAGGCAGCCATAAAATAT



## Supplementary Figure S1: Localization of $\alpha 1$ , $\beta 1$ and $\beta 2$ subunit isoforms in cerebellum and dentate gyrus in brain slices from three month old mice.

a-b) Fluorescence immunohistochemistry of  $\beta 1$  and  $\beta 2$  in cerebellum co-stained with  $\alpha 1$ .

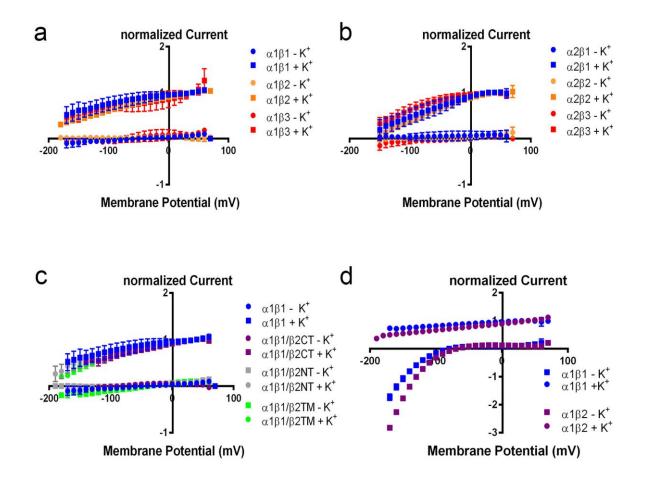
c-d)  $\beta 1$  and  $\beta 2$  in the dentate gyrus of hippocampus co-stained with  $\alpha 1$ . GCL: granule cell layer. P: Purkinje cell layer. ML: molecular layer. Scale bars represent 20  $\mu$ m.



Supplementary Figure S2: Voltage-dependence of charge translocation and rate constants of nine human  $\alpha\beta$  combinations.

Three human  $\alpha$  isoforms were paired with the three  $\beta$  isoforms. The difference curves in K<sup>+</sup>-free buffers with and without 10 mM ouabain were fitted with single exponentials and analyzed as described in Fig.1 yielding the charge translocation curves a), b), c) and on-rate constants d), e) and f) for  $\alpha$ 1,  $\alpha$ 2 and  $\alpha$ 3 respectively.

N = 3-8 with oocytes from at least two *Xenopus laevis* females. Data are represented as mean  $\pm$  SD.



## Supplementary Figure S3: Voltage dependence of steady-state currents for six human $\alpha\beta$ combinations and three chimeras.

The forward ouabain-sensitive current induced by 15 mM K<sup>+</sup> is similar for a)  $\alpha$ 1, b)  $\alpha$ 2 and c) the chimeras.

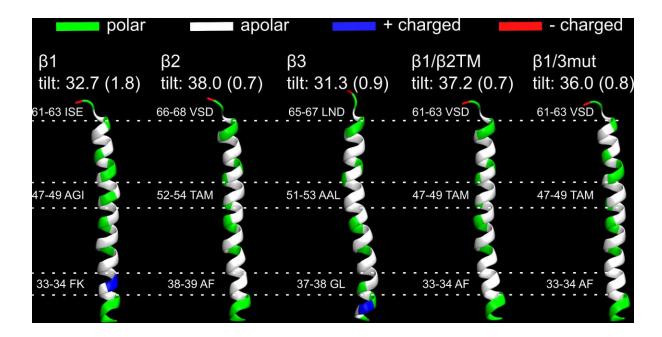
d) In the absence of extracellular Na<sup>+</sup>, the forward K<sup>+</sup>-induced, ouabain-sensitive currents are similar with  $\alpha 1\beta 1$  and  $\alpha 1\beta 2$ . In the absence of K<sup>+</sup> and Na<sup>+</sup>, both combinations have inwardly rectifying currents <sup>20</sup>, slightly more pronounced for  $\beta 2$ .

N = 3 with oocytes from at least 2 *Xenopus laevis* females. Data are represented as mean  $\pm$  SD.

10
ATP1B1 MARGK AKEEGSWKKF IWNSEKKEFL GRTGGSWFKI LLFYVIFYGC
ATP1B2 MVIQKEKKSC GQVVEEWKEF VWNPRTHQFM GRTGTSWAFI LLFYLVFYGF
ATP1B3 MT-KNEKKSL NOSLAEWKLF IYNPTTGEFL GRTAKSWGLI LLFYLVFYGF
Consistency * 401133762 373336 * * 3 * 96 * 53527 * 8 * * * 62 * * 22 * * * * * 79 * * * 4
$\overbrace{}$
ATP1B1 LAGIFIGTTQ VMLLTISEFK PTYQDRVAPP GLTQIPQI-Q KTEISFRPND
ATP1B2 LTAMFTL <mark>TMW VML</mark> QTVSDHT PKYQDRLATP GLMIRPKT-E NLDVIVNVSD
ATP1B3 LAALFSF <mark>T</mark> MW VML <mark>QTLNDEV PKYRDQIPSP GL</mark> MVFPKPVT ALEYTFSRSD
Consistency * 6 6 6 * 3 1 * 5 4 * * * 4 * 7 7 7 2 3 * 5 * 7 * 7 7 5 4 * * * 5 4 2 * 7 2 0 4 3 5 7 5 3 5 4 1 7 *
$\dots \dots $
ATP1B1 PKSYEAYVIN IVRFLEKYKD SAQRDDMIFE DCGDVPSEPK ERGDFNHERG
ATP1B2 TESWDQHVQK LNKFLEPYND SIQAQKND VCRPGRYY EQPDNGVLNY
ATP1B3 PTSYAGYIED LKKFLKPYTL EEQKNLTVCPDGALF EQKGP
Consistency <b>54 * 6 4 3 6 9 3 4 8 2 7 * * 7 5 * 3 3 6 2 * 4 4 1 3 3 0 0 0 * 0 0 1 5 6 3 1 3 * 7 0 3 0 1 0 3 3 1</b>
170 180 100 200
ATP1B1 ERKVCREKLE WLGNCSGLNDET-YGYKEGK PCIIIKLNRV LGFKPKPPKN
ATP1B1 ERKVCREKLE WLGNCSGLND ET-YGYKEGK PCIIIKLNRV LGFKPKPPKN ATP1B2 PKRACOFNRT QLGNCSGIGD STHYGYSTGO PCVFIKMNRV INFYAGA
ATP1BZ PKRACQENKT QIGNESGIGD STHIGISTGQ PCVFIKMNRV INFIAGA ATP1B3 VYVACQEPIS LLQACSGMND PD-FGYSQGN PCILVKMNRI IGLKPE
Consistency 2 3 3 6 * 7 * 3 3 4 1 * 4 4 * * * 6 6 * 350 7 * * 6 4 * 4 * * 9 5 9 * 8 * * 9 8 6 6 4 5 3 1 0 0 0
ATP1B1 ESLETYPVMK YNPNVLP <mark>VQC</mark> TGKRDEDKDK VGNVEYFGLG NSPGFPLQYY
ATP1B2
ATP1B3 GVPRIDC VSKNED IPNVAVYPHN GMIDLKYF
Consistency 00000000000 000 3532 93 4 46 4677 3121 74 534742 6 6300 65 4 4 7
ATP1B1 PYYGK <mark>LLQPK YLQPLLAVQF TNLTMDTE IRIECKAYGE NIGYSEKD</mark>
ATP1B2 PYYGK <mark>KFHVN YTQPLVAVKF LNVTPNVE VNVECRINAA NIAT-D</mark> DERD
ATP1B3 PYYGK <mark>KLHVG YLQPLVAVQV SFAPNNTGKE VTVECKIDGS ANLKSQ</mark> DDRD
Consistency * * * * * <mark>4 6 5 4 3 * 5 * * * 7 * * 7 5 3 4 5 5 2 6 0 0 3</mark> * 9 3 9 * * 7 5 <mark>2 6 4 4 4 2 2 0 1</mark> 6 7 7 *
210
ATP1B1 RFQGRFDVKI EVKS- ATP1B2 KFAGRVAFKL RINKT
ATP1B2 KFAGRVAFKL KINKT ATP1B3 KFLGRVMFKI TARA-
Consistency 7 * 3 * * 5 2 5 * 8 3 5 5 4 0

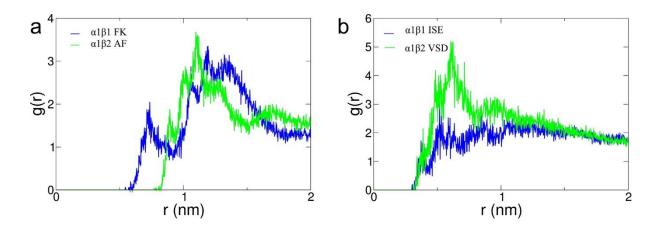
## Supplementary Figure S4: Sequence comparison of the three human β isoforms.

The dark blue bar above the numbering denotes the area of  $\beta$ 1 that was exchanged for the equivalent area of  $\beta$ 2 to yield  $\beta$ 1/ $\beta$ 2NT, the green bar shows the area of  $\beta$ 1 that was exchanged for that of  $\beta$ 2 to yield  $\beta$ 1/ $\beta$ 2TM, and the yellow bar denotes the area swapped to yield  $\beta$ 1/ $\beta$ 2TMC. The unmarked area is the C-terminal domain, where the  $\beta$ 1 sequence was swapped with that of  $\beta$ 2 to yield  $\beta$ 1/ $\beta$ 2CT. The FK, AGI and VSD pockets are denoted by arrows. Fully conserved residues are shown in red. The lower the consistency score, the less conserved the residues. Figure was made using the PRALINE software server.



## Supplementary Figure S5: Comparison of the $\beta$ transmembrane helices in MD simulations of $\alpha 1\beta 1$ , $\alpha 1\beta 2$ , $\alpha 1\beta 3$ , $\alpha 1\beta 1/\beta 2TM$ and $\alpha 1\beta 1/3mut$ .

The  $\beta$  transmembrane helices alone are shown from MD simulations of the indicated full-length  $\beta$  in complex with  $\alpha 1$  and the  $\gamma$  subunit in a POPC lipid membrane. The 33-34 and 61-63 residues located at or near the membrane interfaces as well as the central 47-49 residues are indicated with dotted lines ( $\beta 1$  numbering, corresponding residues in  $\beta 2$  and  $\beta 3$  are labelled similarly). The helix tilt is given for each  $\beta$  helix, defined as the angle between the helix axis and the z-axis perpendicular to the membrane surface. Presented values are the averages from the last 40 ns of simulations, with error estimations obtained with block averaging. Polar, apolar and charged residues are color coded as indicated.



Supplementary Figure S6: Radial distribution functions (RDFs) for the residues that anchor the transmembrane helix of the β1 and β2 subunits in the lipid bilayer and the lipid headgroups.

a) Interactions between the POPC headgroups and the N-terminus of the  $\beta$  transmembrane helix (residues 33-34,  $\beta$ 1 numbering). The interactions of residues from  $\beta$ 1 are plotted in blue, from  $\beta$ 2 in green. The last 40 ns of the simulations were used to calculate RDFs.

b) Interactions between the POPC headgroups and the C-terminus of the  $\beta$  transmembrane helix (residues 61-63,  $\beta$ 1 numbering).