Construction of $Na_v1.5$/ChR2 heteromers

To construct $Na_v1.5$-CC-1, we first removed the YFP stop codon in pEYFP-hH1 [1] by mutagenesis PCR. The corresponding primer was designed to incorporate triplet TTA instead of TAA as well as palindromic sequences for the restriction sites *Pac*I and *Not*I. Both sites in the respectively modified pEYFP-hH1 plasmid were used to insert the ChR2-ChR2 dimer, amplified by PCR from pGEM-β1-ChR2-ChR2 [2]. Forward and reverse primers, containing *Pac*I and *Not*I sequences, respectively, were designed to allow also for amplification of the flanking linker GGGS-GGGS-GGGS ($[GGGS]_3$), and the transmembrane region A_2 to N_{103} of the β subunit of the human H^+/K^+-ATPase ($h\beta HK_{A2-N103}$; accession number NM_000705). The resulting full-length $Na_v1.5$-CC-1 sequence was finally inserted in two separate cloning steps as *Bcl*I/*Xba*I and *Xba*I/*Hind*III fragments into the *BamH*I/*Hind*III sites of pGEMHEnew [3]. The resulting plasmid, pGEM-$Na_v1.5$-YCC, was expected to encode the following heteromeric channel protein: $Na_v1.5_{M1-V2016}$-$YFP_{M1-K239}$-[LINTGVQVAE]-$[GGGS]_3$-$h\beta HK_{A2-N103}$-$[GGGS]_3$-ChR2/T159$C_{N24-V309}$-GGGSR$[GGGS]_2$-$h\beta HK_{A2-N103}$-$[GGGS]_3$-ChR2/T159$C_{N24-V309}$ (see $Na_v1.5$-CC-1 in Fig. 1).
For the N-terminal linkage of the ChR2 dimer to human $Na_v1.5$, leading to CC-$Na_v1.5$, we first coupled EGFP in-frame to the $Na_v1.5$ start codon by recombinant PCR. The full-length EGFP-$Na_v1.5$ fragment was inserted into the *Hind*III/*Not*I sites of pcDNA3, resulting in plasmid pcDNA-GFP-$hNa_v1.5$. As the next step, the ChR2 sequence was amplified by PCR from plasmid pGEM-β1-ChR2-YFP [2]. The corresponding forward primer annealed in the upstream $h\beta HK_{A2-N103}$ sequence and contained the *Hind*III and *Pac*I sites for the subsequent insertion of the second ChR2 sequence. The reverse primer annealed in the 3'-sequence of the Golgi-to-plasma membrane trafficking signal (peptide [KSRITSEGEYIPLDQIDINVVDTSSR]) [4] and the adjacent first YFP triplets. The resulting 1.4 kb PCR fragment was fused in-frame by a recombinant PCR step to the start codon of EGFP in pcDNA-GFP-$hNa_v1.5$, leading to pcDNA-GFP-$hNa_v1.5$/PacC. In a third cloning step, the ChR2 sequence was first amplified by a single PCR step from pGEMHE-ChR2-T159C::YFP [5,6]. Forward and reverse primers contained *Hind*III and *Pac*I sites, respectively, so that the amplified ChR2 sequence could be coupled in-frame to the ChR2-GFP-$hNa_v1.5$ sequence of pcDNA-GFP-$hNa_v1.5$/PacC. The resulting plasmid pcDNA-ChR2-ChR2-GFP-$hNa_v1.5$ contained the full-length CC-$Na_v1.5$ cDNA sequence. For cRNA production, we had to insert this 11.4 kb fragment into the *Hind*III/*Not*I sites of pGEMHEnew. The CC-$Na_v1.5$ amino sequence in the resulting plasmid pGEM-ChR2-ChR2-GFP-$hNa_v1.5$ is as follows: ChR2/T159$C_{M1-V309}$-[LINGGS]-$h\beta HK_{A2-N103}$-$[GGGS]_3$-ChR2/T159$C_{D2-V309}$-[KSRITSEGEYIPLDQIDINVVDTSSR]-$EGFP_{M1-K239}$-$Na_v1.5_{M1-V2016}$ (see CC-$Na_v1.5$ in Fig. 1).

To obtain Na_v1.5-CC-5, we first removed the ChR2 stop codon in pTSV40G-hNa_v1.5-ChR2, encoding fusion channel hNa_v1.5-ChR2$_{D2-V309}$, by mutagenesis PCR (for pTSV40G-hNa_v1.5-ChR2, encoding the similar variant hNa_v1.5-ChR2$_{N24-V309}$, see [2]). The corresponding forward primer annealed in the hNa_v1.5 sequence upstream to the unique *Tth111*I site. The reverse primer was designed to incorporate triplet TTA instead of TAA as well as sequences for the restriction sites *Pac*I and *Not*I. The 1.7 bp PCR fragment was inserted into the *Tth111*I/*Not*I sites of pTSV40G-hNa_v1.5-ChR2, resulting in pTSV40G-hNa_v1.5-ChR2/Pac. Next, we amplified by PCR the coding region for VQVAE-[GGGS]$_3$-hβHK$_{A2-N103}$-[GGGS]$_3$-ChR2/T159C$_{N24-V309}$-[KSRITSEGEYIPLDQIDINVVDTSSR]-YFP$_{M1-K239}$-[SRFCYENEV] from pGEM-β1-ChR2-YFP [2]. The Golgi-to-plasma membrane trafficking signal peptide [KSRITSEGEYIPLDQIDINVVDTSSR] [4] and the ER export motif [SRFCYENEV] [7] were previously incorporated into the β1-ChR2-YFP construct in order to increase the channel number in the plasma membrane [2]. The corresponding forward primer contained a *Pac*I site; the reverse primer annealed in the 3'-non-coding region of the β-globin sequence of pGEMHEnew and contained a *Not*I site. The resulting 2.3 kb PCR fragment was digested with *Pac*I and *Not*I, and inserted into the same sites of pTSV40G-hNa_v1.5-ChR2/Pac. The resulting plasmid, pTSV40G-hNa_v1.5-ChR2-ChR2-YFP, was used to produce the Na_v1.5-CC-5 cRNA, and finally the following channel protein: Na_v1.5$_{M1-V2016}$-[GGGS]$_3$-hβHK$_{A2-N103}$-[GGGS]$_3$-ChR2/T159C$_{D2-V309}$-[LINVQVAE]-[GGGS]$_3$- hβHK$_{A2-N103}$-[GGGS]$_3$-ChR2/T159C$_{N24-V309}$-[KSRITSEGEYIPLDQIDINVVDTSSR]-YFP$_{M1-K239}$-[SRFCYENEV] (see Na_v1.5-CC-5 in Fig. 1).

For the construction of Na_v1.5-CC-4, the ER export motif [SRFCYENEV] of Na_v1.5-CC-5 was deleted in pTSV40G-hNa_v1.5-ChR2-ChR2-YFP by recombinant PCR. The amino sequence, encoded by the resulting plasmid pTSV40G-hNa_v1.5-ChR2-ChR2-YFP0, is as follows: Na_v1.5$_{M1-V2016}$-[GGGS]$_3$-hβHK$_{A2-N103}$-[GGGS]$_3$-ChR2/T159C$_{D2-V309}$-[LINVQVAE]-[GGGS]$_3$-hβHK$_{A2-N103}$-[GGGS]$_3$-ChR2/T159C$_{N24-V309}$-[KSRITSEGEYIPLDQIDINVVDTSSR]-YFP$_{M1-K239}$ (see Na_v1.5-CC-4 in Fig. 1).

To obtain Na_v1.5-CC-3, we used plasmid pTSV40G-hNa_v1.5-ChR2-ChR2-YFP as a PCR template to amplify a 1.4 kb fragment, encoding the following protein sequence: TGVQVAE-[GGGS]$_3$- hβHK$_{A2-N103}$-[GGGS]$_3$-ChR2/T159C$_{N24-V309}$-[KSRITSEGEYIPLDQIDINVVDTSSR]-MVSKG. Forward and reverse primers contained restriction sites *Pac*I and *Xba*I, respectively, so that the resulting PCR product could be inserted in-frame into the same sites of pGEM-Na_v1.5-YCC (see cloning for Na_v1.5-CC-1 above). The resulting plasmid, pGEM-Na_v1.5-YC-ts-C, allowed for the production of the Na_v1.5-CC-3 cRNA, encoding the following fusion channel: Na_v1.5$_{M1-V2016}$-YFP$_{M1-K239}$-[LINTGVQVAE]-[GGGS]$_3$-hβHK$_{A2-N103}$-[GGGS]$_3$-ChR2/T159C$_{N24-V309}$-[KSRITSEGEYIPLDQIDINVVDTSSR]-[GGGS]$_2$-hβHK$_{A2-N103}$-[GGGS]$_3$-ChR2/T159C$_{N24-V309}$ (see Na_v1.5-CC-3 in Fig. 1).

To obtain Na_v1.5-CC-2, we inserted by a recombinant PCR approach the sequence for the Golgi-to-plasma membrane trafficking peptide (see above) in-frame between the Na_v1.5 and YFP sequences in plasmid pGEM-Na_v1.5-YCC (see cloning for Na_v1.5-CC-1). The Na_v1.5-CC-2 amino sequence in the resulting plasmid pGEM-hNa_v1.5-ts-YCC is as follows: $Na_v1.5_{M1-V2016}$-[KSRITSEGEYIPLDQIDINVVDTSSR]-$YFP_{M1-K239}$-[LINTGVQVAE]-$[GGGS]_3$-h$\beta HK_{A2-N103}$-$[GGGS]_3$-ChR2/T159$C_{N24-V309}$-GGGSR$[GGGS]_2$-h$\beta HK_{A2-N103}$-$[GGGS]_3$-ChR2/T159$C_{N24-V309}$ (see Na_v1.5-CC-1 in Fig. 1).

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Table S1. Supplementary statistical AP and oocyte parameters. Oocytes with sub-threshold depolarization were excluded.

Channel constructs	Peak Na^+ current (µA)	Peak photocurrent (µA)	K^+ current at 10 mV (µA)	K^+ current at 30 mV (µA)	Oocyte features: Resting potential (mV)	Oocyte features: Membrane resistance (MΩ)	Number of oocyte batches	Number of measurements
$Na_v1.5$-CC-2	15.58±0.66	0.37±0.03	-	-	-20.58±1.02	0.96±0.07	8	12
$Na_v1.5$-CC-5	18.02±0.53*	0.22±0.02*	-	-	-25.67±0.40	1.03±0.09	4	12
$Na_v1.5$-CC-2 + $K_v1.2$	15.99±0.95	0.62±0.04*	3.00±0.25	4.25±0.39	-30.80±0.53*	0.79±0.04*	5	15
$Na_v1.5$-CC-2 + hERG	13.49±0.47*	0.47±0.02*	1.97±0.13	2.04±0.13	-49.94±0.62*	0.52±0.02*	5	16
$Na_v1.5$-CC-ΔKPQ	11.27±0.53*	0.51±0.03*	-	-	-16.17±0.74*	0.94±0.05	6	18

* indicates $p<0.05$ *versus* $Na_v1.5$-CC-2

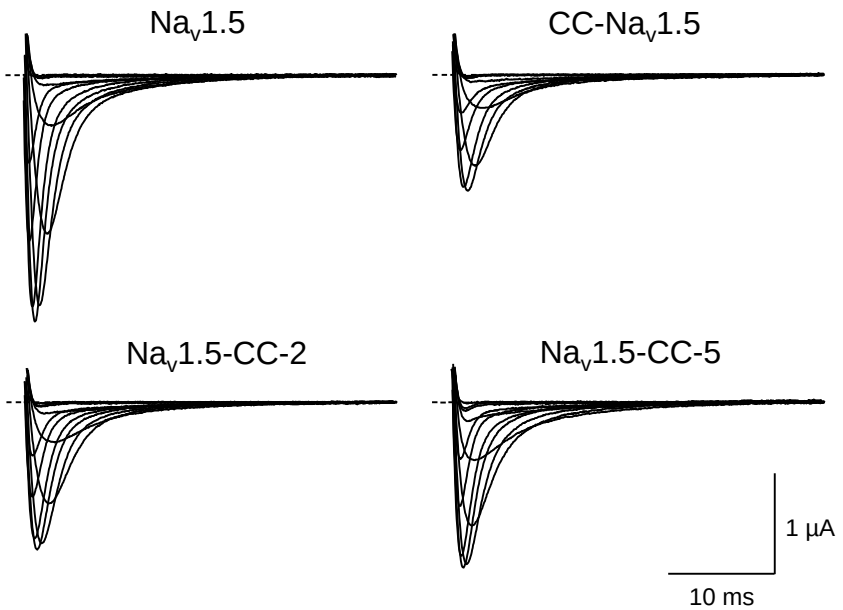

Figure S1. Representative Na^+ current families of wild-type Na_v1.5 and three fusion channels. Whole-cell currents were recorded three days after cRNA injection at a test potential between -80 and -45 mV (holding potential -100 mV). For the injected cRNA amount and detailed statistics on Na^+ channel kinetics see Table 2.