Supplemental Data for *Molecular Pharmacology* article:

Negative Gating Modulation by (*R*)-*N*-(Benzimidazol-2-yl)-tetrahydro-1-naphtylamine (NS8593) Depends on Residues in the Inner Pore Vestibule: Pharmacological Evidence of Deep-Pore Gating of $K_{Ca}2$ Channels

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This file contains:

1 Supplemental Table with information on additional chimeras.

Supplemental Table 1: Additional Chimeras

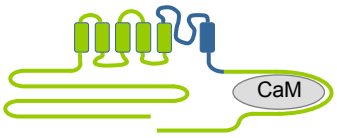
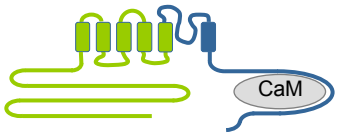
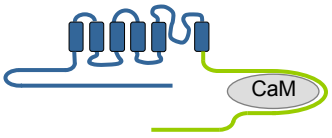
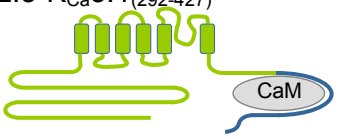
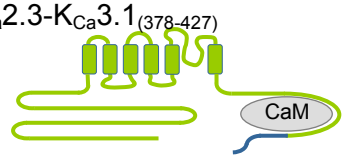
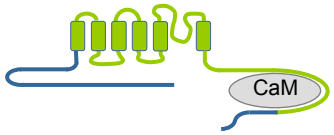
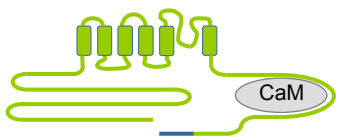
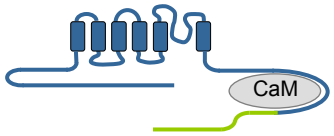
	NS8593 IC_{50} [nM]	Fold current increase by NS309	% current inhibition by BMB	Comments
Non-functional or pharmacologically "oddly"-behaving chimeras				
$K_{Ca}2.3$-$K_{Ca}3.1_{(233-281)}$-$K_{Ca}2.3_{(490-731)}$ 	Not tested	No current increase (n = 5 cells)	Not tested	< 200 pA current (n = 5 cells)
$K_{Ca}2.3$-$K_{Ca}3.1_{(233-427)}$ 	No inhibition at 0.3 µM (n=1) or 10 µM (n=2).	No current increase at 0.03 µM (n=2) and only small increase at 10 µM (n=2)	Not tested	Inhibition by ChTX (n = 1) and TRAM-34 (n=3)
$K_{Ca}3.1$-$K_{Ca}2.3_{(539-731)}$ 	Not tested	Not modulated at 0.3 µM (n = 2)	Not tested	No current (n = 10 cells)
Functionally "redundant" chimeras				
$K_{Ca}2.3$-$K_{Ca}3.1_{(292-427)}$ 	250 ± 60 (4)	126 ± 2 (2)	89 ± 5 (4)	Increased Ca^{2+}-sensitivity in inside-out experiments
$K_{Ca}2.3$-$K_{Ca}3.1_{(378-427)}$ 	107 ± 11 (2)	199 ± 15 (3)	90 ± 6 (3)	
$K_{Ca}3.1$-$K_{Ca}2.3_{(294-642)}$-$K_{Ca}3.1$ 	457 (1)	130 ± 11 (2)	91 ± 13 (2)	
$K_{Ca}2.3$-$K_{Ca}3.1_{(404-427)}$ 	52 (1)	209 (1)	100 (1)	
$K_{Ca}3.1$-$K_{Ca}2.3_{(644-731)}$ 	>10 µM (2)	400 ± 0 (2)	0 (2)	

Table 1: List of chimeras not included in the main article because they were either found to be non-functional/low expressing (1-3) or because they were considered to yield only supportive but not additional information with respect to defining the site- or mode-of-action of NS8593 (4-8). The cartoons are color coded with the $K_{Ca}2.3$ derived sequence in green and the $K_{Ca}3.1$ sequence in blue. Data are given as n (the number of independent experiments) ± SEM (standard error of the mean). All chimaeras were constructed, expressed and evaluated as detailed in the *Materials and Methods* section of the article. The concentrations used of NS309 (6,7-dichloro-1*H*-indole-2,3-dione 3-oxime) and BMB (bicuculline methobromid) were 30 nM and 100 µM, respectively.