

## Supplementary information

### Structural modeling and mutant cycle analysis predict pharmacoresponsiveness of a Na<sub>v</sub>1.7 mutant channel

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#### Supplementary Table S1

Data Summary for mutant cycle analysis and interpretation

	V <sub>1/2</sub>	Z	ΔG°	ΔΔG°	ΣΔG°
<b>WT</b>	-23.42	6.71	-3.61	-	-
<b>V400M</b>	-32.23	7.88	-5.84	-2.23	-
<b>S241T</b>	-37.67	7.01	-6.07	-2.46	-
<b>VM/ST</b>	-43.38	5.99	-5.98	-2.36	2.32
<b>F1449V</b>	-28.94	6.83	-4.54	-0.93	-
<b>VM/FV</b>	-41.67	7.46	-7.15	-3.54	-0.38

**Note:** F=0.023

#### Interpretation

##### VM/ST mutant pairs:

$$\Sigma\Delta G^\circ = (\Delta G^\circ_{WT} - \Delta G^\circ_{V400M}) - (\Delta G^\circ_{S241T} - \Delta G^\circ_{VM/ST}) = 2.32 \text{ (Kcal/mol)}$$

$$|2.32| > 1 \quad \text{energetically coupled}$$

##### VM/FV mutant pairs:

$$\Sigma\Delta G^\circ = (\Delta G^\circ_{WT} - \Delta G^\circ_{V400M}) - (\Delta G^\circ_{F1449V} - \Delta G^\circ_{VM/FV}) = -0.38 \text{ (Kcal/mol)}$$

$$|-0.38| < 1 \quad \text{energetically independent}$$

**Supplementary Table S2**

Effects of CBZ on channel properties

	activation $V_{1/2}$ (mV)	activation slope	fast inactivation $V_{1/2}$ (mV)	fast inactivation slope
<b>S241T DMSO</b>	-37.6±1.0 (n=10)	6.1±0.2	-79.9±1.7 (n=6)	6.2±0.2
<b>S241T CBZ</b>	-30.5±1.3 (n=13)**	6.7±0.5	-81.5±2.1 (n=6)	6.1±0.1
<b>F1449V DMSO</b>	-28.8±1.1 (n=9)	6.9±0.5	-71.6±1.5 (n=6)	6.5±0.2
<b>F1449V CBZ</b>	-27.1±1.5 (n=8)	7.1±0.4	-72.7±2.2 (n=8)	6.9±0.2

\*\*, P&lt;0.01, Student's t-test

## Supplementary Table S3

### Sequence alignment of NavAb and four transmembrane domains of hNav<sub>v</sub>1.7 by MUSTANG (A Multiple structural alignment)

		S1N	S1		
NavAb (3rvyA)	1	----- <u>MYLRITNIVESSFFTKFIYLVLVNGITMGLETS</u> -KTFM-QSFGVYTT			46
Nav1.7 DI	1	----LSP--FSPLRRISIKILVHSLFSMLIMCTILTNCIFMTMNP-----PDWTK			45
Nav1.7 DII	1	---P--Y--WIKFKKCIYFIVMDPFVDLAIITICIVLNTLFMAMEHH-PM-T-EEFKNVLA			50
Nav1.7 DIII	1	-----KIWWNIRKTCYKIVEHSWFESFIVLMILLSSGALAFEDIYIE-RKTKIKIILE			52
Nav1.7 DIV	1	PIPR--P--GNKIQGCIFDLVTNQAFDISIMVLICLNMTMMVEKE-GQ-S-QHMTVELY			53
		S2	S3		
NavAb (3rvyA)	47	<u>LFNQIVITIFTIEIILRIYVH</u> -----RISFFKDPWSLFDFFVVAISLV---PT-----			91
Nav1.7 DI	46	NVEYTFGTGIYTFESLVKILARGFCVGEFTFLRDPWNWLDVWVIVFAYLTFEYFN-----			98
Nav1.7 DII	51	IGNLVFTGIFAAEMVLKLIAMD----PYEYFQVGWNI FDSLIVTTLSELVEFLA-----D-			100
Nav1.7 DIII	53	YADKIPTYIFILEMLLKWIAYG----YKTYFTNAWCWLDFLIVDVS LVTLVANTLGYS-			107
Nav1.7 DIV	54	WINVVFII LFTGECVLKLI SL-----RHYYFTVGNWIFDFVWVVIISIVGMFLAD-LIETY			107
		S4	S4-S5 linker	S5	
NavAb (3rvyA)	92	---SSGFEILRVLRLRFLRLVTAVPQMRKIVSALISVIP <u>GMLSVIAIMTLFFYIFAIMA</u>			148
Nav1.7 DI	99	---LGNVSALRTFRVLRALKTISVIPGLKTIVGALIQSVK <sup>S</sup> KLSDVMILTVFCLSVFALIG			155
Nav1.7 DII	101	---VEGLSVLRSFRLRLRVFKLAKSWPTLNMLIKIIGNSVGALGNLTLVLAIVFIFAVVG			157
Nav1.7 DIII	108	---LGP <sup>S</sup> IKSLRTLRLALRPLRALS RFEGMRVVVNALIGAI <sup>S</sup> PSIMNVLLVCLIFWLI <sup>S</sup> FSIMG			164
Nav1.7 DIV	108	FVSP <sup>S</sup> TLFRVIRLARIGRILRLVKGAKGIRTL <sup>S</sup> LFALMMSLPALFNIGLLLFLVMFIYAIFG			167
NavAb (3rvyA)	149	<u>TQLFGERFP</u> -----			157
Nav1.7 DI	156	LQLFMGNLK-----H-----KCFRNS-----LENNETLESIMNTLESEEDFRKYFYFL			198
Nav1.7 DII	158	MQLFGKSYK-----ECVCKIN---DDC-----			176
Nav1.7 DIII	165	VNLFAGKF-Y-----E-----CINTTDGSRFP-----ASQVP-N-RS---ECFA--			197
Nav1.7 DIV	168	MSNFAYVKK-EDGIN-----			181
NavAb (3rvyA)	158	-----			179
Nav1.7 DI	199	EGSKDALLCGFS---TDSGQCEGYTCVKIGRNPDYGYTSFDTFSWAFALFLRMTQDYW			255
Nav1.7 DII	177	-----TLP-----RWHMNDFFHSFLIVFRVLCGE-W			201
Nav1.7 DIII	198	-----LMNVSQNV-----RWKNLKVNF <sup>S</sup> DNVGLGYSLLQVATFKGW			233
Nav1.7 DIV	182	-----DMFN <sup>S</sup> FETFGNSMICLFQITTSAGW			205
				S6	
NavAb (3rvyA)	180	SMGIVRPLMEVYP----- <u>YAWVFIFPFI<sup>S</sup>FVVFV</u>			208
Nav1.7 DI	256	ENLYQQTLRAAGK-----TYMIFV <sup>S</sup> VVIFLGSFY			284
Nav1.7 DII	202	IETMWD <sup>S</sup> CMEVAGQ-----A-----MCLIVYMMVMVIGNLV			231
Nav1.7 DIII	234	TIIMYAAVDSVNV-----DKQPKYEYSLYMYIYFVVFII <sup>S</sup> FGSFF			272
Nav1.7 DIV	206	DGLLAPILNSKPPDCD <sup>S</sup> PKKVHPGSSVEGDCGNPS-----VGIFVFSYIIISFLV			255
NavAb (3rvyA)	209	<u>MINLVVAICVDAM</u> -----	221		
Nav1.7 DI	285	LINLILAVVAMAYEEQNQANIEEAK---	309		
Nav1.7 DII	232	VLNLFALLLLSSFS <sup>S</sup> SDNLTAEEDP---	256		
Nav1.7 DIII	273	TLNLFIVGVIDN <sup>S</sup> FNQKKK-LGG--QDI	297		
Nav1.7 DIV	256	VVNM <sup>S</sup> YIAVILENFSVATEESTEP-L---	279		

Note:

S234, V400 and F1449 are highlighted in yellow.

Underlines were made in NavAb sequence. Single underlines indicate S1 to S4 helix; wave underline indicates S4-S5 linker; double underlines indicate S5 and S6 helix.