Nav1.1-1
Nav1.2-1 Nav1.3-1
Nav1.4-1
Nav1.5-1
Nav1.6-1
Nav1.7-1
Nav1.8-1 Nav1.9-1

Nav1.2-2
av1.3-2
Nav1.4-2
Nav1.5-2
Nav1.6-2
Nav1.7-2
Nav1.9-2

Nav1.1-3
Nav1.2-3
Nav1.3-3
Nav1.5-3
Nav1.5-3
Nav1.7-3
Nav1.8-3 Nav1.9-3

Nav1.2-4
Nav1.3-4
Nav1.4-4
Nav1.5-4 Nav1.7-4 Nav1.8-4 Nav1.9-4

KLSDVMILTVFCLSVFALIGLQLFMGNLPE-GYFDTFSWAFLSLFRLMTQDFW-ENLYQLTLRAAGKTYMIF-FVLVIFLGSFYLINLILAVVAMAY KLSDVMILTVFCLSVFALIGLQLFMGNLPE-GYFDTFSWAFLSLFRLMTQDFW-ENLYQLTLRAAGKTYMIF-FVLVIFLGSFYLINLILAVVAMAY KLSDVMILTVFCLSVFALIGLQLFMGNLPE-GYFDTFSWAFLSLFRLMTQDYW-ENLYQLTLRAAGKTYMIF-FVLVIFLGSFYLVNLILAVVAMAY KLSDVMILTVFCLSVFALVGLQLFMGNLPE-GYYDTFSWAFLALFRLMTQDYW-ENLFQLTLRAAGKTYMIF-FVVIIFLGSFYLINLILAVVAMAY KLADVMVLTVFCLSVFALIGLQLFMGNLPE-GYFDSFAWAFLALFRLMTQDCW-ERLYQQTLSAGKIYMIF-FMLVIFLGSFYLVNLILLAVVAMAY KLSDVMILTVFCLSVFALIGLQLFMGNLPE-GYFDTFSWAFLALFRLMTQDYW-ENLYQLTLRAAGKTYMIF-FVLVIFVGSFYLVNLILILAVVAMAY KLSDVMILTVFCLSVFALIGLQLFMGNLPE-GYFDTFSWAFLALFRLMTQDYW-ENLYQQTLRAAGKTYMIF-FVVVIFLGSFYLINLILAVVAMAY KLADVTILTIFCLSVFALVGLQLFKGNLKPDGYFDSFAWAFLSLFRLMTQDSW-ERLYQQTLRTSGKIYMIF-FVLVIFLGSFYLVNLILAVVTMAY KLVNVIILTFFCLSIFALVGQQLFMGSLNSIQYFDNFGWSFLAMFRLMTQDSW-GKLYQQTLRTTG-LYSVFFFIVVIFLGSFYLINLTLAVVTMAY

## P\|



IMNVLLVCLIFWLIFSIMGUNLFAGKFA-RWFDNVGLGYLSLLQVATFKGWMDIMYAAVKYEESN-LYMYLYFVIFIIFGSFFTLNLFIGVIIDNF SIMNVLLVCLIFWLIFSIMGVNLFAGKF-RWFDNVGAGYLALLQVATFKGWMDIMYAAVVYEEN-LYMYLYFVIFIIFGSFFTLNLFIGVIIDNF SIMNVLLVCLIFWLIFSIMGVNLFAGKF--RWYDNVGLGYLSLLQVATFKGWMDIMYAAVQYEVN-LYMYLYFVIFIIFGSFFTLNLFIGVI IDNF SIMNVLLVCLIFWLIFSIMGVNLFAGKF--YWFDNVGAGYLALLQVATFKGWMDIMYAAVQWEYN-LYMYIYFVIFIIFGSFFTLNLFIGVIIDNF SIMNVLLVCLIFWLIFSIMGVNLFAGKFV-RWFDNVGLGYLSLLQVATFKGWTIIMYAAVKYEYS-LYMYIYFVVFIIFGSFFTLNLFIGVIIDNF SIMNVLLVCLIFWLIFSIMGVNLFAGKFF-FWFDNVAMGYLALLQVATFKGWMDIMYAAVDKWEDNVYMYLYFVIFIIFGGFFTLNLFVGVIIIDNF


PIV
ALFNIGLLLFLVMFIYAIFGMSNF-----DMFFETFGNSMICLFQITTSAGWDGLLAPILDCGNPSVGIFFFVSYIIISFLLVVVNMYIAVILENF ALFNIGLLLFLVMFIYAIFGMSNF-----DMFFETFGNSMICLFQITTSAGWDGLLAPILDCGNPSVGIFFFVSYIIISFLVVVNMYIAVILENF ALFNIGLLLFLVMFIYAIFGMSNF-----DMFFETFGNSMICLFQITTSAGWDGLLAPILDCGNPSVGIFFFVSYIIISFLVVVNMYIAVILENF ALFNIGLLLFLVMFIYSIFGMSNF-----DMFFETFGNSIICLFEITTSAGWDGLLNPILDCGNPSIGICFFCSYIIISFLIVVNMYIAIILENF
 ALFNIGLLLFLVMFIFSIFGMSNF-----DMFFETFGNSMICLFQITTSAGWDGLLLPILDCGNPSVGIFFFVSYIIISFLIVVNMYIAIILENF ALFNIGLLLFLVMFIYAIFGMSNF-----DMFFETFGNSMICLFQITTSAGWDGLLAPILDCGNPSVGIFYFVSYIIISFLVVVNMYIAVILENF ALFNIGLLLFLVMFI

Figure S1. Sequence alignment between the four PIIIA binding domains of the varied Nav
subtypes. Loops with low sequence identify as well as the voltage sensing domain (VSD) were
truncated from the sequence alignment. The P loop from the four domains were labelled as PI to


Figure S2. The top 10 conformations of PIIIA at the binding site of Nav1.4 from docking in

AutoDock. A) and B) show the top 10 conformations of PIIIA together at the binding site of Nav1.4.

Obviously, the top 10 conformations of PIIIA at the binding site are in varied orientations. C) -K )
show the orientation of the top 10 conformations of PIIIA at the binding site of $\mathrm{Na}_{\mathrm{v}} 1.4$, respectively.


Figure S3. Binding modes of the top 10 conformations refined using MD. A) - J), Comparison of the MD refined 10 conformations of PIIIA bound with $\mathrm{Na}_{\mathrm{v}} 1.4$ (in varied colors) with conformations prior to MD (in gray). MD refinement could result in significant conformation deviation from that determined using docking.

A


Figure S4. Surface electrostatic potential of PIIIA and Nav channels. A) shows the structure of

PIIIA and five key residues on its surface; B) shows the electrostatic potential of PIIIA; C) -K ),
show the surface electrostatic potential of the pore domain/ or PIIIA binding site of Nav subtypes.


Figure S5. Conformation of the Nav1.4 embedded in membrane in MD simulations. Root mean square deviation (RMSD) for the backbone of Nav1.4 embedded in membrane in 100 ns nonrestraint MD simulations. A),B) show the backbone RMSD of Nav 1.4 in apo and bound with PIIIA, respectively. C,D) show the binding mode of PIIIA to Nav1.4. Residues at the PIIIA were labelled in italic.

Table S1. Energetic contribution for the side chain of the key residues at the binding site of

Nav subtypes.

| $\mathbf{N a v}$ | $\mathbf{4 0 1}^{\mathbf{a}}$ | $\mathbf{4 0 4}^{\mathbf{a}}$ | $\mathbf{4 0 8}^{\mathbf{a}}$ | $\mathbf{7 5 5}^{\mathbf{a}}$ | $\mathbf{7 5 8}^{\mathbf{a}}$ | $\mathbf{7 6 1}^{\mathbf{a}}$ | $\mathbf{1 2 4 1}^{\mathbf{a}}$ | $\mathbf{1 5 3 2}^{\mathbf{a}}$ | $\mathbf{1 5 3 6}^{\mathbf{a}}$ | $\mathbf{1 5 4 1}^{\mathbf{a}}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| $\mathrm{Na}_{\mathrm{v}} 1.1$ | -2.07 | -6.18 | -2.29 | -5.29 | -2.64 | -0.90 | -0.58 | 0.67 | -0.92 | -3.31 |
| $\mathrm{Na}_{\mathrm{v}} 1.2$ | -1.91 | -6.32 | -2.23 | -6.40 | -2.32 | -1.44 | -1.05 | 0.47 | -1.00 | -2.28 |
| $\mathrm{Na}_{\mathrm{v}} 1.3$ | -1.69 | -4.52 | -1.90 | -6.50 | -1.80 | -1.69 | -0.80 | -0.89 | -0.59 | -1.32 |
| $\mathrm{Na}_{\mathrm{v}} 1.4$ | -2.07 | -5.17 | -1.68 | -6.72 | -3.34 | -1.20 | -1.58 | 0.14 | -1.67 | -1.75 |
| $\mathrm{Na}_{\mathrm{v}} 1.5$ | -0.21 | -1.71 | -1.95 | -3.82 | -2.46 | -1.21 | -0.51 | -0.27 | -0.83 | -1.46 |
| $\mathrm{Na}_{\mathrm{v}} 1.6$ | -1.91 | -6.48 | -2.03 | -6.19 | -2.42 | -1.43 | -0.95 | 0.35 | -2.28 | -1.97 |
| $\mathrm{Na}_{\mathrm{v}} 1.7$ | -1.43 | -5.61 | -0.99 | -5.90 | -2.35 | -0.51 | -0.57 | -0.07 | -0.94 | -2.71 |
| $\mathrm{Na}_{\mathrm{v}} 1.8$ | -0.50 | -1.12 | -1.15 | -4.31 | -0.83 | -0.80 | -0.77 | 0.06 | -1.90 | -2.05 |
| $\mathrm{Na}_{\mathrm{v}} 1.9$ | -0.13 | -0.80 | -0.87 | -3.67 | -1.94 | -1.19 | -0.10 | -0.98 | -0.11 | -0.01 |

${ }^{a}$ Side chain energetic contribution was calculated based on binding energy decomposition using

MMGB/SA method.

