

Up-regulation of voltage-gated sodium channels by peptides mimicking S4-S5 linkers reveals a variation
of the ligand-receptor mechanism

Olfat A. Malak^{1,2,§}, Fayal Abderemane-Ali^{1,3,§}, Yue Wei^{1,4}, Fabien C. Cohan¹, Gilyane Pontus¹, David Shaya⁵, Céline Marionneau¹, Gildas Loussouarn¹

1. *Université de Nantes, CNRS, INSERM, l'institut du thorax, F-44000 Nantes, France.*
2. *Present address: 8001 Redwood Blvd, Novato, California 9494, USA5*
3. *Present address: Cardiovascular Research Institute, University of California, San Francisco, California 941158-9001, USA*
4. *Present address: Department of Cardiology, Shanghai Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*
5. *Cardiovascular Research Institute, University of California, San Francisco, California 941158-9001, USA*

§ These authors contributed equally to this work.

Address correspondence to:

Dr. Gildas Loussouarn
L'institut du thorax
INSERM UMR 1087 / CNRS UMR 6291
IRS-UN, 8 Quai Moncousu BP 70721
44007 Nantes cedex 1, France
Tel: +33 (0)2 2808 0150
Fax: +33 (0)2 2808 0130
E-mail: gildas.loussouarn@inserm.fr

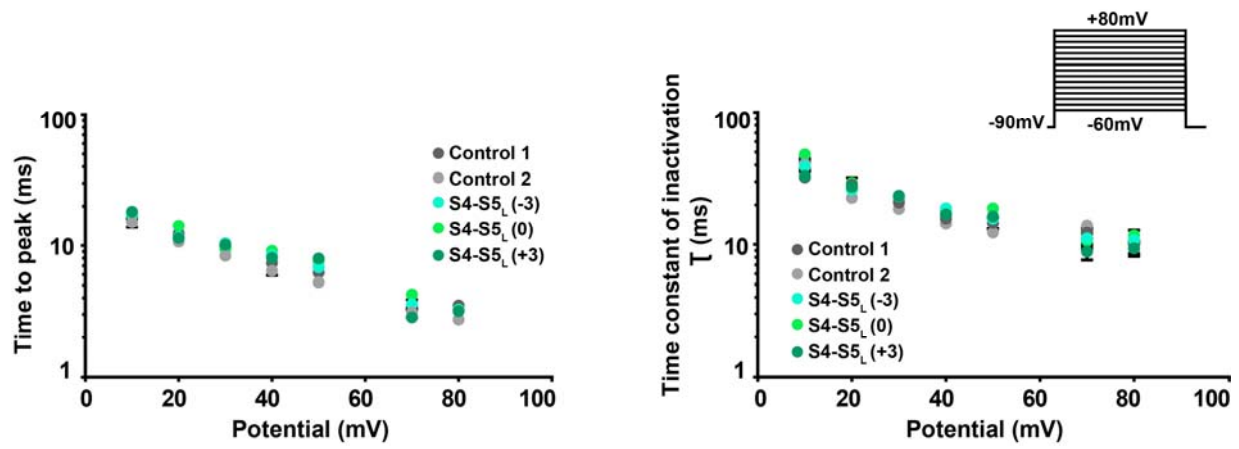
Keywords: Nav1.4 channel – NavSp1 channel – voltage dependence – S4-S5 linker – S6 C-terminus

	Control 1	Control 2	S4-S5 _L (-3) DI	S4-S5 _L (0) DI	S4-S5 _L (+3) DI
Current densities (n)	45	40	47	20	40
at 30mV (pA/pF)	-12.3 ± 2.8	-12.0 ± 4.0	-16.9 ± 3.2	-17.2 ± 4.9	-23.1 ± 6.2
P-values vs control 1	--	0.46	0.049	0.16	0.09
P-values vs control 2	0.46	--	0.03	0.12	0.06
Activation (n)	8	10	17	8	10
V _{0.5} Act (mV)	30.4 ± 1.9	29.3 ± 1.8	23.5 ± 1.8	26.4 ± 2	25.9 ± 2.7
P-values vs control 1	--	0.69	0.03	0.17	0.22
P-values vs control 2	0.69	--	0.04	0.31	0.32
K Act (mV)	11.1 ± 0.7	11.4 ± 0.9	9.9 ± 0.4	10.4 ± 0.6	9.6 ± 0.5
P-values vs control 1	--	0.79	0.11	0.09	0.34
P-values vs control 2	0.79	--	0.39	0.08	0.09

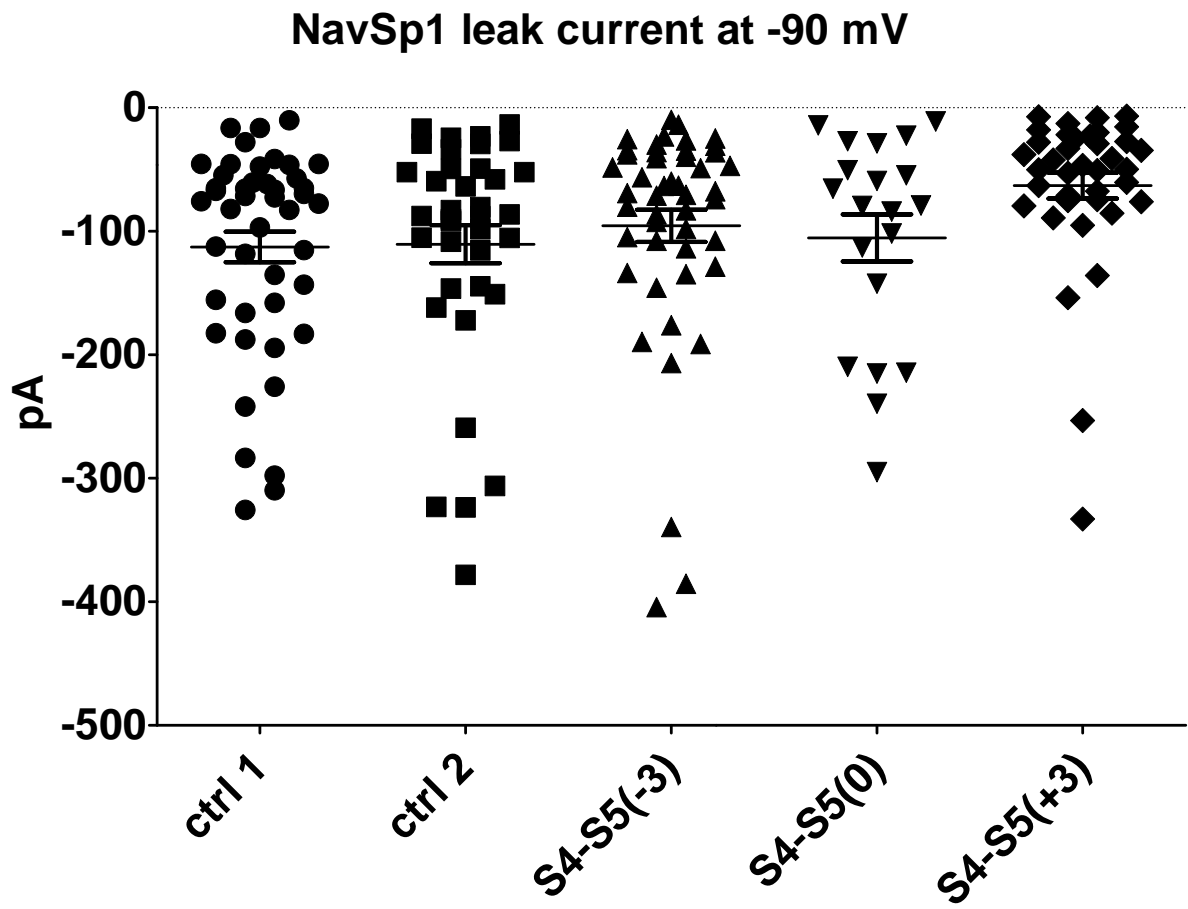
Supplemental Table 1: Effect of NavSp1 S4-S5_L mimicking peptides on NavSp1 current density and activation curve. Mean ± sem peak current densities recorded at +30 mV, in COS-7 cells co-transfected with NavSp1 and the indicated peptide. Mean ± sem half-activation potential and slope of the activation curves, obtained using the activation protocol shown in Figure 2. Mann-Whitney tests for current densities and Student's t-tests for activation parameters (V_{0.5} and K).

	Control 1	Control 2	S4-S5 _L (-3) DI	S4-S5 _L (0) DI	S4-S5 _L (+3) DI	S4-S5 _L (-3) DII	S4-S5 _L (0) DII	S4-S5 _L (+3) DII	S4-S5 _L (-3) DIII	S4-S5 _L (0) DIII	S4-S5 _L (+3) DIII	S4-S5 _L (-3) DIV	S4-S5 _L (0) DIV	S4-S5 _L (+3) DIV
Current densities (n)	119	110	18	24	21	20	25	22	20	21	25	31	19	22
at 0mV (pA/pF)	-67.4 ± 6.3	-60.5 ± 5.2	-84.4 ± 18.1	-70.9 ± 10.2	-102.9 ± 16.9	-93.1 ± 22.3	-76.2 ± 16.6	-99.1 ± 17.3	-69.9 ± 16.9	-90.1 ± 14.7	-58.7 ± 8.9	-80.4 ± 14.6	-63.9 ± 9.4	-97.9 ± 19.4
P-values vs control 1	--	0.38	0.21	0.16	0.01	0.13	0.42	0.03	0.41	0.04	0.47	0.28	0.26	0.06
P-values vs control 2	0.38	--	0.15	0.15	0.007	0.11	0.35	0.02	0.49	0.02	0.41	0.21	0.21	0.05
Activation (n)	58	49	6	12	10	9	5	11	9	13	12	17	13	10
V _{0.5} Act (mV)	-12.8 ± 0.6	-13.3 ± 0.6	-13.3 ± 1.6	-10.7 ± 0.7	-14.1 ± 1.5	-12.4 ± 1.8	-15.5 ± 2.4	-13.7 ± 1.1	18.1 ± 2.3	12.4 ± 0.9	-13.6 ± 1.9	-13 ± 1.1	-12.5 ± 1.4	12.2 ± 1.6
P-values vs control 1	--	0.59	0.81	0.15	0.41	0.83	0.25	0.53	0.005	0.79	0.62	0.84	0.88	0.76
P-values vs control 2	0.59	--	0.99	0.06	0.58	0.61	0.32	0.75	0.009	0.52	0.84	0.86	0.62	0.53
K Act (mV)	6.8 ± 0.1	6.7 ± 0.1	6.5 ± 0.4	6.9 ± 0.3	6.5 ± 0.1	6.5 ± 0.3	6.1 ± 0.4	6.6 ± 0.2	6.1 ± 0.3	6.5 ± 0.1	7.1 ± 0.3	6.8 ± 0.2	6.9 ± 0.2	6.4 ± 0.2
P-values vs control 1	--	0.69	0.43	0.67	0.38	0.34	0.13	0.6	0.049	0.27	0.37	0.90	0.64	0.22
P-values vs control 2	0.69	--	0.37	0.41	0.32	0.28	0.04	0.66	0.01	0.20	0.26	0.88	0.34	0.14
Inactivation (n)	28	22	11	12	10	12	9	13	9	12	13	14	14	11
V _{0.5} Inact (mV)	-60.3 ± 1.2	-62.1 ± 1.3	-59.3 ± 0.7	-62.5 ± 1.9	-57.7 ± 1.0	-61.5 ± 2.0	-61.3 ± 1.7	-59.2 ± 0.9	-59.1 ± 0.9	-58.1 ± 0.9	-61.7 ± 2.6	-60.8 ± 1.4	-61.2 ± 1.3	-58.2 ± 1.2
P-values vs control 1	--	0.33	0.59	0.33	0.22	0.59	0.67	0.56	0.57	0.25	0.58	0.80	0.65	0.31
P-values vs control 2	0.33	--	0.16	0.86	0.048	0.81	0.74	0.14	0.18	0.05	0.89	0.53	0.66	0.07
K Inact (mV)	5.1 ± 0.2	5.2 ± 0.1	5.1 ± 0.3	5.5 ± 0.3	6 ± 0.2	5.4 ± 0.2	5.7 ± 0.4	5.9 ± 0.4	5.5 ± 0.5	5.7 ± 0.4	6.2 ± 0.4	5.7 ± 0.3	5.6 ± 0.2	5.3 ± 0.4
P-values vs control 1	--	0.73	0.87	0.24	0.03	0.38	0.16	0.08	0.37	0.17	0.02	0.15	0.16	0.63
P-values vs control 2	0.73	--	0.92	0.22	0.007	0.35	0.12	0.07	0.40	0.16	0.01	0.15	0.13	0.75

Supplemental Table 2: Effect of Nav1.4 S4-S5_L mimicking peptides on Nav1.4 current density and activation/inactivation curves. Mean ± sem tail current densities recorded at 0 mV, in COS-7 cells co-transfected with hNav1.4, hNavβ1, and the indicated peptide. Half-activation/inactivation potentials and slopes of the activation/inactivation curves, obtained from tail currents, using the protocols shown in figures 5,6,7,8 and suppl. figures 6,7,8,9. Mann-Whitney tests for current densities and Student's t-tests for activation parameters (V_{0.5} and K).



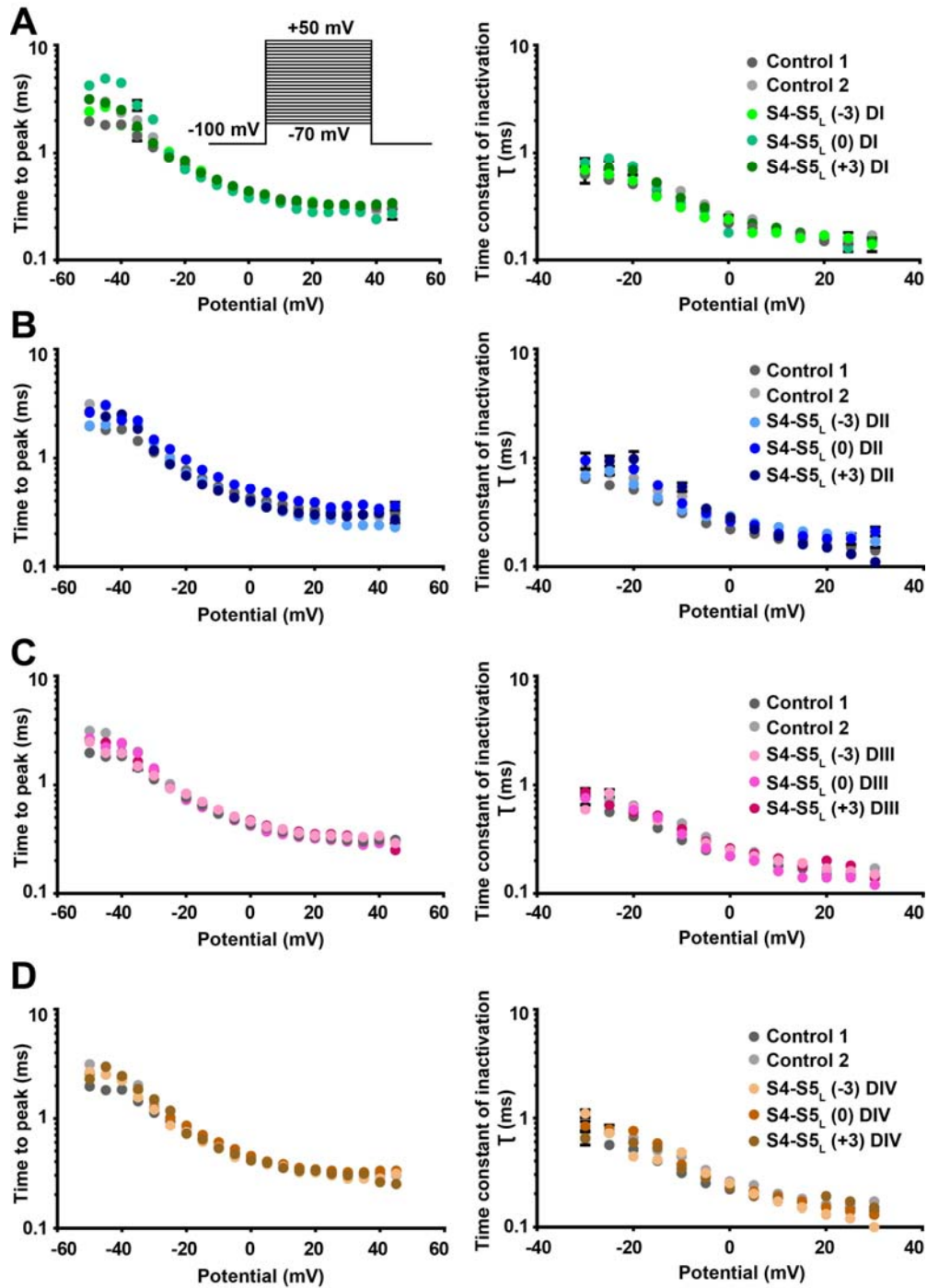
Supplemental Figure 1: Effect of NavSp1 S4-S5_L mimicking peptides on NavSp1 activation and inactivation kinetics. Left: mean \pm sem time to peak of the sodium current measured at various potentials. Right: mean \pm sem NavSp1 inactivation time constant, obtained from a monoexponential fit of the inactivating current (n=7-10).



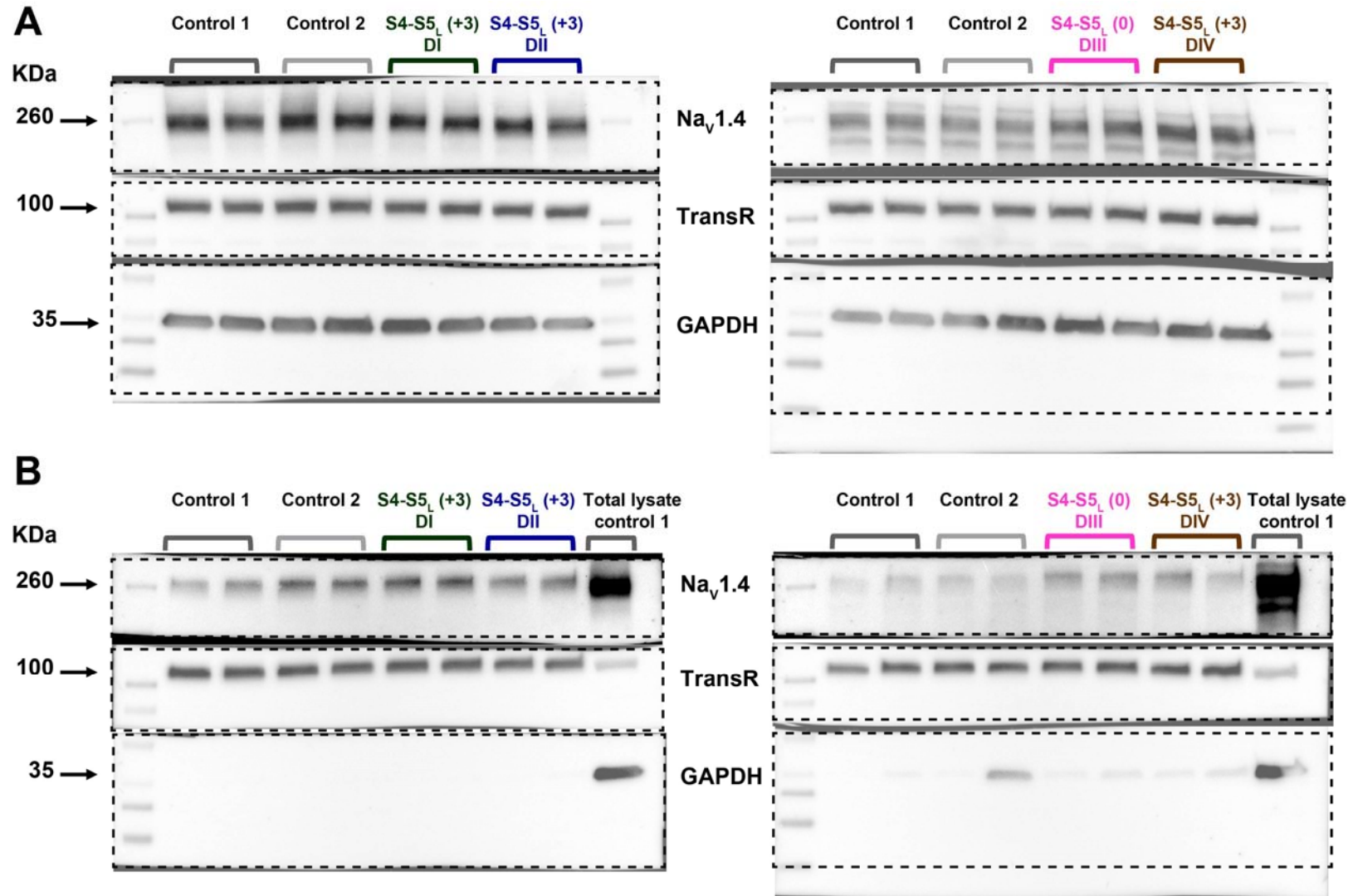
Supplemental Figure 2: Effect of NavSp1 S4-S5_L mimicking peptides on NavSp1 current measured at -90 mV.

	S4-S5	S6
Na_vSp1 :	-- PRLRRVVEGFITALP	---- YAWLFFVVPFIMITTFVAVNLLVGLIVNSMQDAHHAEDGERTD
	↓ ↓ ↓	↓
Na_vMs :	-- PTMR R VV Q GMLLALP	---- NAWVFFIPFIMLTTFVTLNLFIGIIVDAMA I TKEQ E E E AKTG
Na_vAb :	-- PQMRK I VSA I ISV I P	---- YAWVFFIPFIFVVTFVMI N LVVA I IVDAMAILNQKEEQHIID

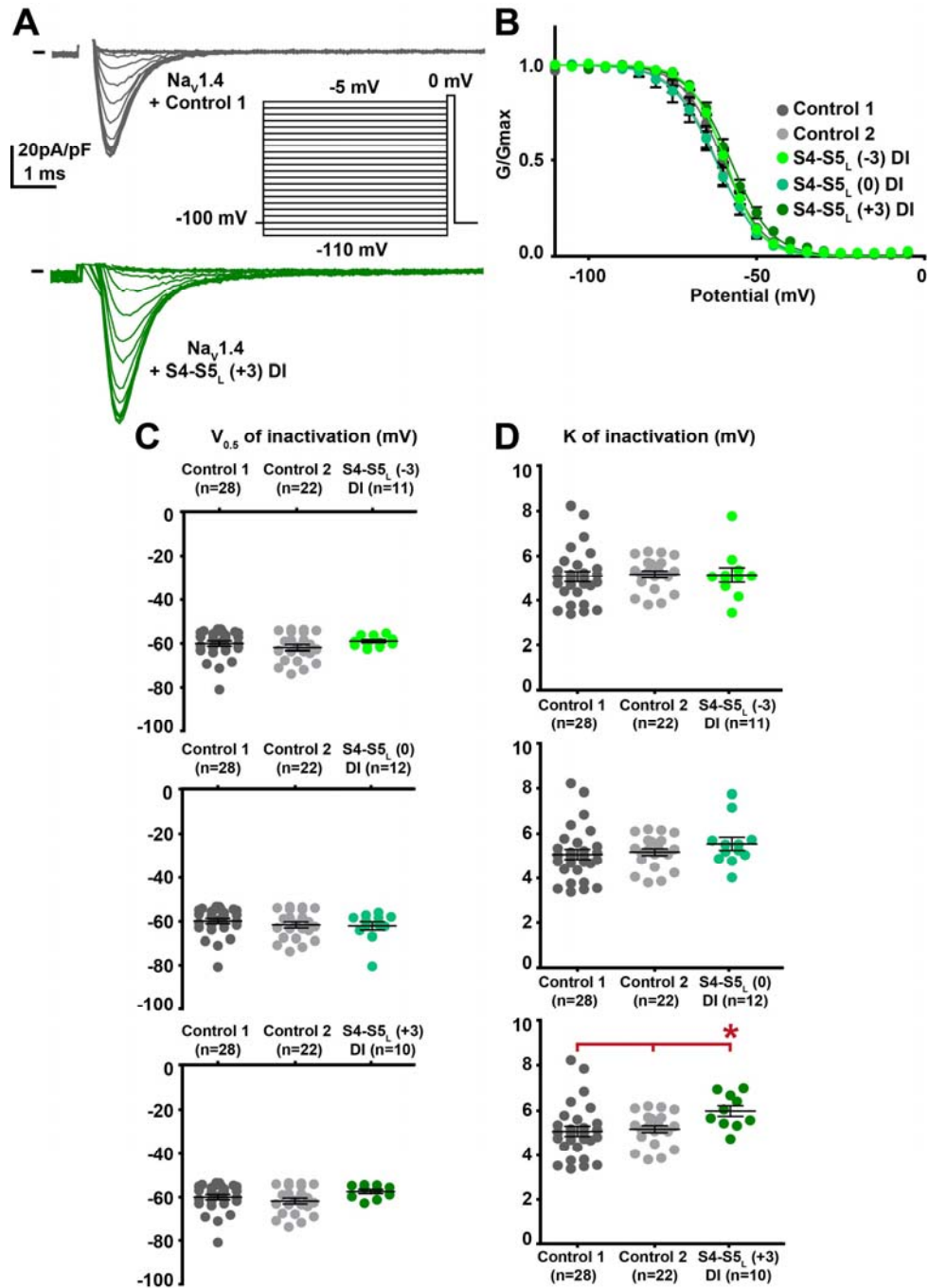
Supplemental Figure 3. Alignment of NavSp1, NavMs and NavAb channels indicating the interacting S4-S5_L (blue) and S6_T (yellow) amino-acids in the NavMs open state and the interacting S4-S5_L (magenta) and S6_T (orange) amino-acids in the NavAb closed state. Figure extracted from ref. 16 and modified. Arrows indicate residues that were selected for mutagenesis experiments presented in figures 3 and 4.



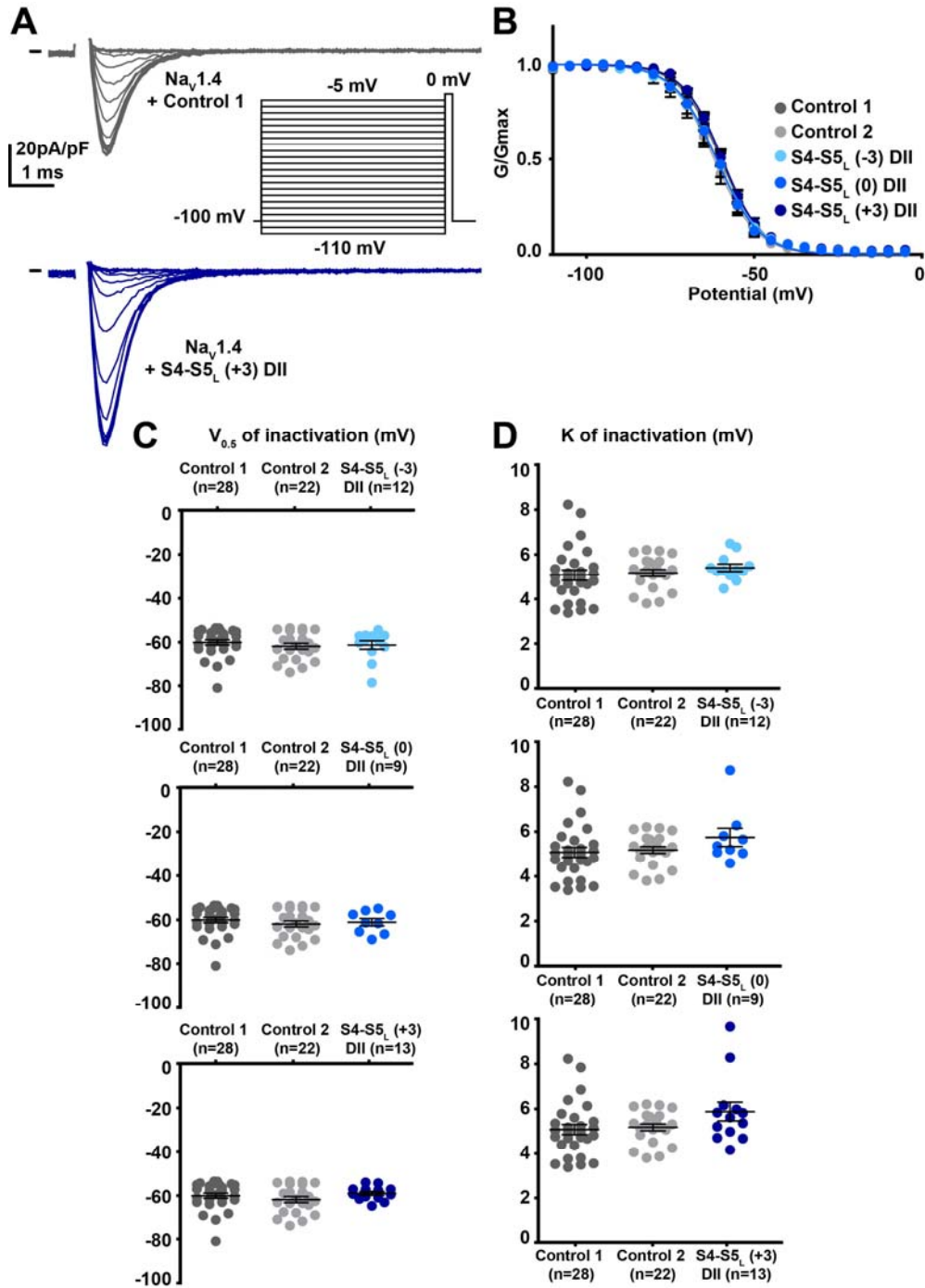
Supplemental Figure 4: Effect of domain I, II, III and IV (A, B, C and D) S4-S5_L mimicking peptides on Nav1.4 activation and inactivation kinetics. Left: mean \pm sem time to peak of the sodium current measured at various potentials. Right: mean \pm sem Nav1.4 inactivation time constant, obtained from a monoexponential fit of the inactivating current (n=6-43).



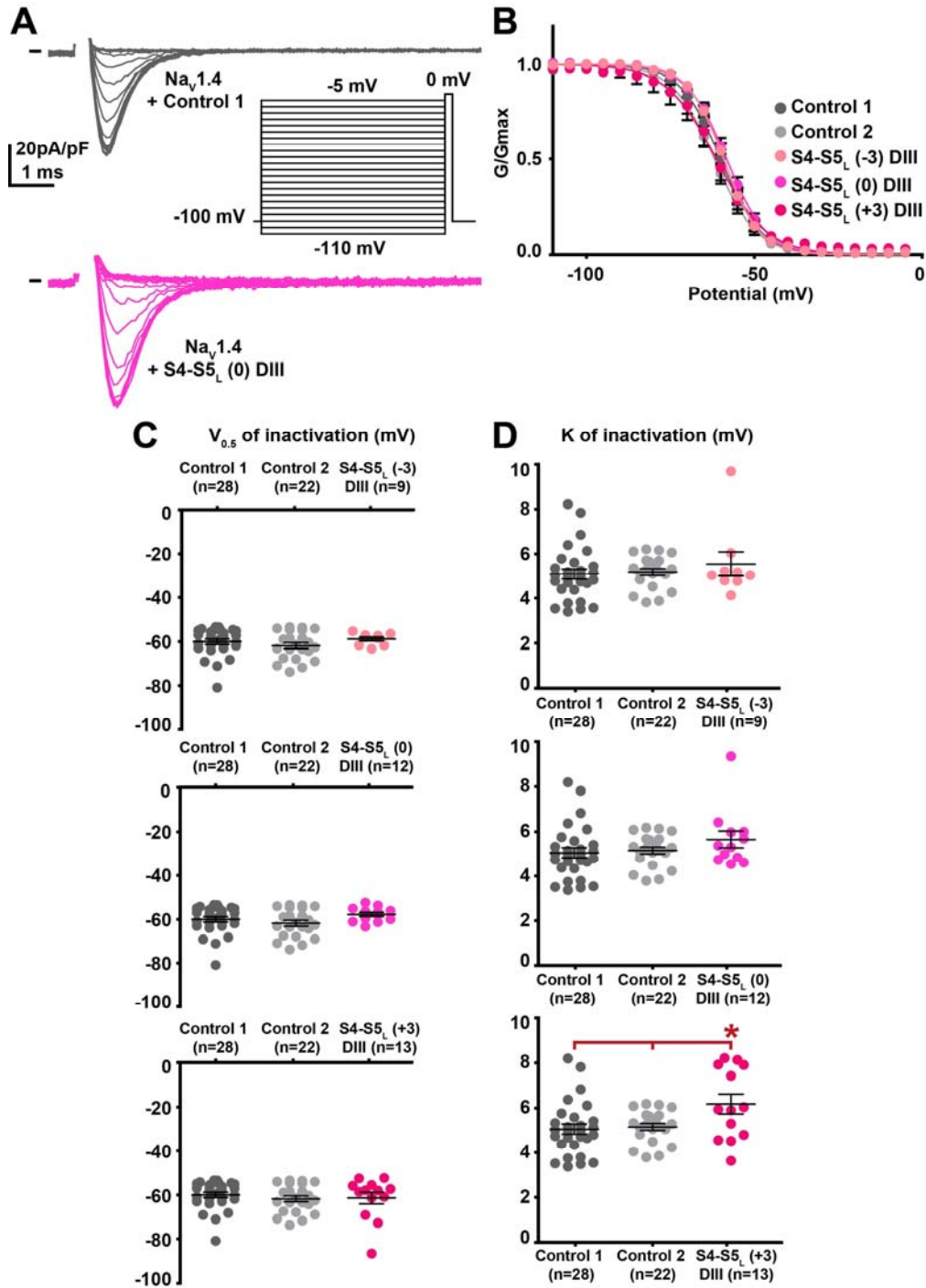
Supplemental Figure 5: Original Western blot panels corresponding to Figure 9. Nav1.4, TransR and GAPDH proteins were detected on the same corresponding membranes. The membranes were cut in three pieces directly after the transfer. The boxes represent the bands shown in figure 9.



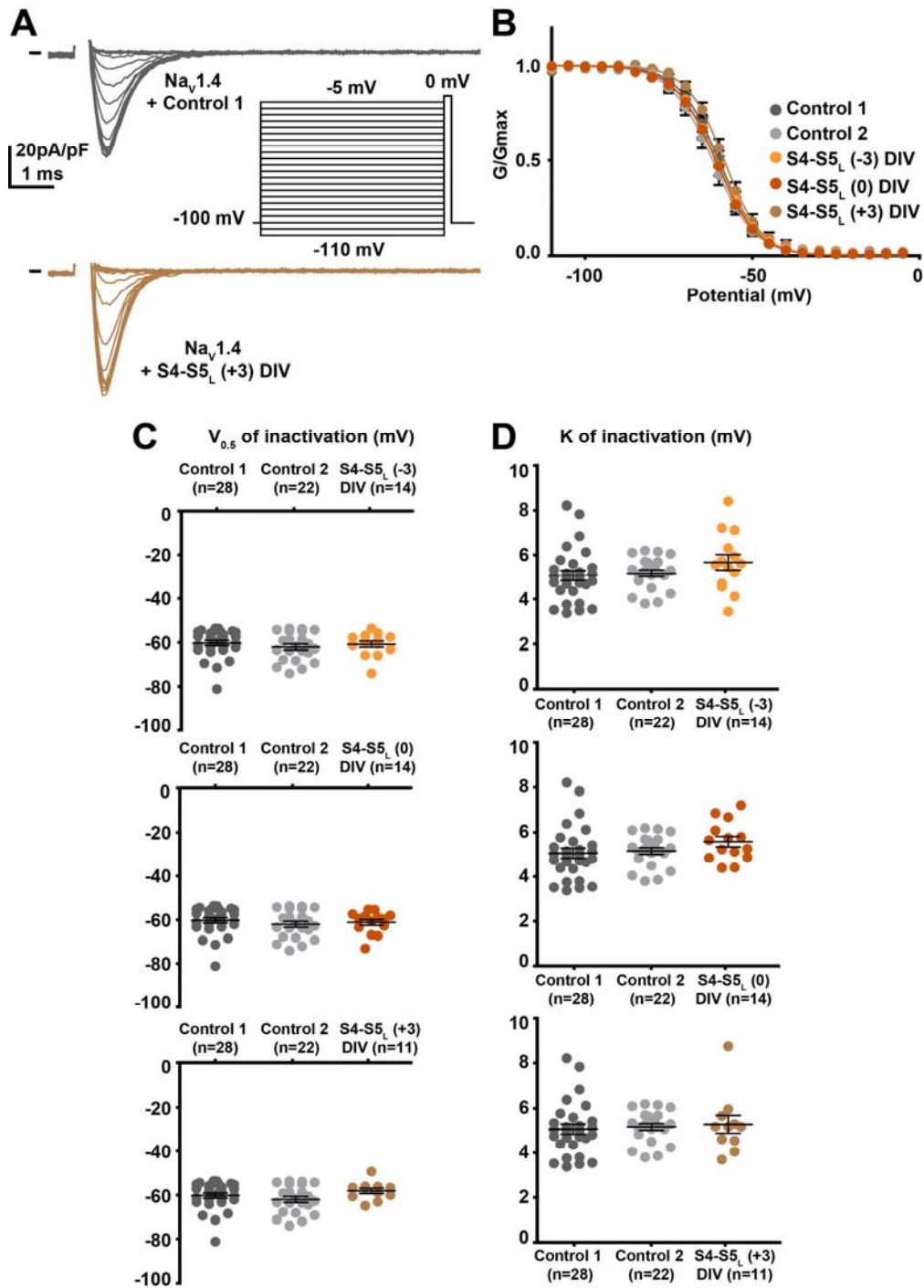
Supplemental Figure 6. Effect of S4-S5_L mimicking peptides of domain I on Na_v1.4 inactivation curve. **A:** representative, superimposed recordings of the Na_v1.4 current in presence or absence of domain I S4-S5_L(+3). **Inset:** voltage protocol used (pre-pulse: 500 msec, test-pulse 20 msec to 0 mV, one sweep every 4 s). **B:** Steady-state channel availability curves for Nav1.4 channels. Data are mean normalized peak current (I/I_{max}) measured at 0 mV versus pre-pulse voltage. **C & D:** Mean ± sem of Na_v1.4 half-inactivation potential (V_{0.5}; **C**) and inactivation slope (K; **D**), in COS-7 cells co-transfected with Na_v1.4, Na_vβ1, and the indicated peptide. *, p < 0.05 vs. both controls.



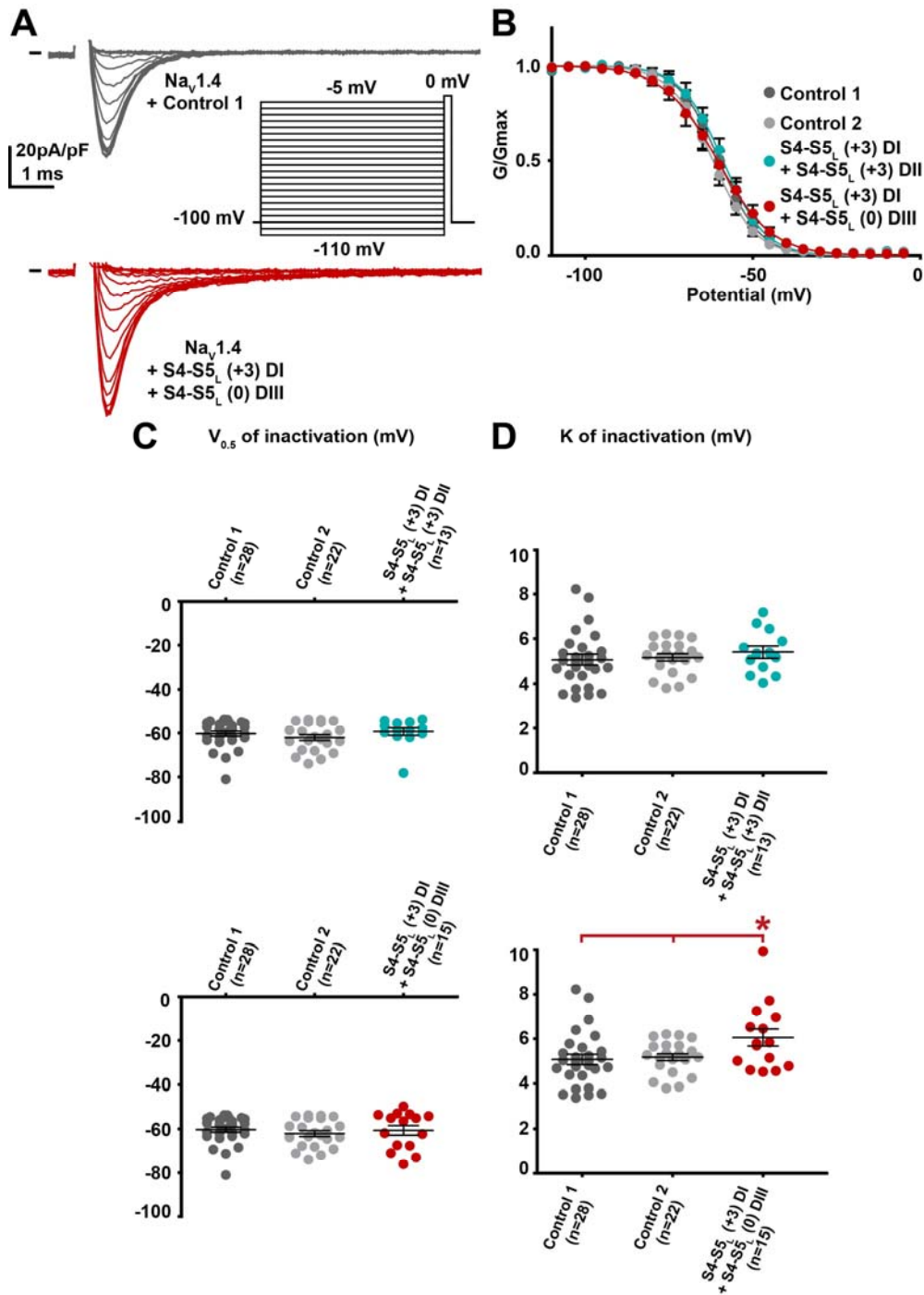
Supplemental Figure 7. Effect of S4-S5_L mimicking peptides of domain II on Nav1.4 inactivation curve. **A:** representative, superimposed recordings of the Nav1.4 current in presence or absence of domain II S4-S5_L(+3). **Inset:** voltage protocol used (pre-pulse: 500 msec, test-pulse 20 msec to 0 mV, one sweep every 4 s). **B:** Steady-state channel availability curves for Nav1.4 channels. Data are mean normalized peak current (I/I_{max}) measured at 0 mV versus pre-pulse voltage. **C & D:** Mean ± sem of Nav1.4 half-inactivation potential (V_{0.5}; **C**) and inactivation slope (K; **D**), in COS-7 cells co-transfected with Nav1.4, Navβ1, and the indicated peptide.



Supplemental Figure 8. Effect of S4-S5_L mimicking peptides of domain III on Nav1.4 inactivation curve. **A:** representative, superimposed recordings of the Nav1.4 current in presence or absence of domain III S4-S5_L(0). **Inset:** voltage protocol used (pre-pulse: 500 msec, test-pulse 20 msec to 0 mV, one sweep every 4 s). **B:** Steady-state channel availability curves for Nav1.4 channels. Data are mean normalized peak current (I/I_{max}) measured at 0 mV versus pre-pulse voltage. **C & D:** Mean ± sem of Nav1.4 half-inactivation potential (V_{0.5}; **C**) and inactivation slope (K; **D**), in COS-7 cells co-transfected with Nav1.4, Navβ1, and the indicated peptide. *, p < 0.05 vs. both controls.



Supplemental Figure 9. Effect of S4-S5_L mimicking peptides of domain IV on Nav1.4 inactivation curve. **A:** representative, superimposed recordings of the Nav1.4 current in presence or absence of domain IV S4-S5_L(+3). **Inset:** voltage protocol used (pre-pulse: 500 msec, test-pulse 20 msec to 0 mV, one sweep every 4 s). **B:** Steady-state channel availability curves for Nav1.4 channels. Data are mean normalized peak current (I/I_{max}) measured at 0 mV versus pre-pulse voltage. **C & D:** Mean ± sem of Nav1.4 half-inactivation potential (V_{0.5}; **C**) and inactivation slope (K; **D**), in COS-7 cells co-transfected with Nav1.4, Navβ1, and the indicated peptide.



Supplemental Figure 10. Effect of the combination of two activating S4-S5_L mimicking peptides on Nav1.4 inactivation curve. **A:** representative, superimposed recordings of the Nav1.4 current in presence or absence of the indicated peptides. **Inset:** voltage protocol used (pre-pulse: 500 msec, test-pulse 20 msec to 0 mV, one sweep every 4 s). **B:** Steady-state channel availability curves for Nav1.4 channels. Data are mean normalized peak current (I/I_{max}) measured at 0 mV versus pre-pulse voltage. **C & D:** Mean ± sem of Nav1.4 half-inactivation potential (V_{0.5}; **C**) and inactivation slope (K; **D**), in COS-7 cells co-transfected with Nav1.4, Navβ1, and the indicated peptides. *, p < 0.05 vs. both controls.