Interactions between lipids and voltage sensor paddles detected with tarantula toxins

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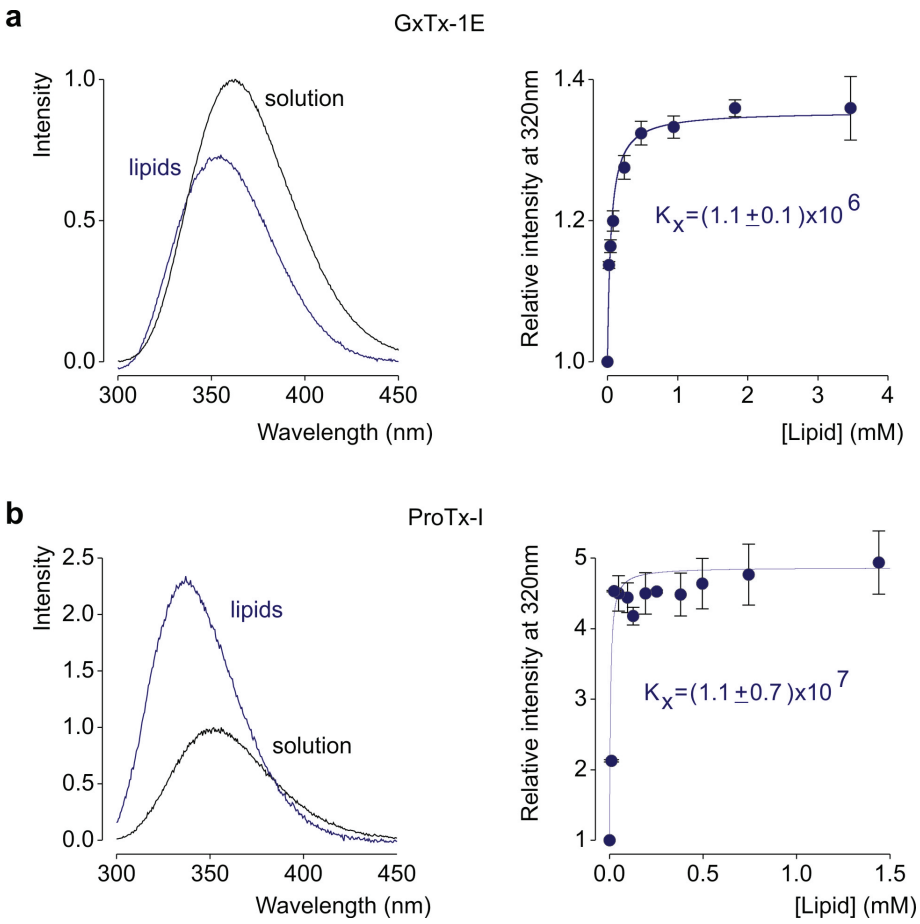
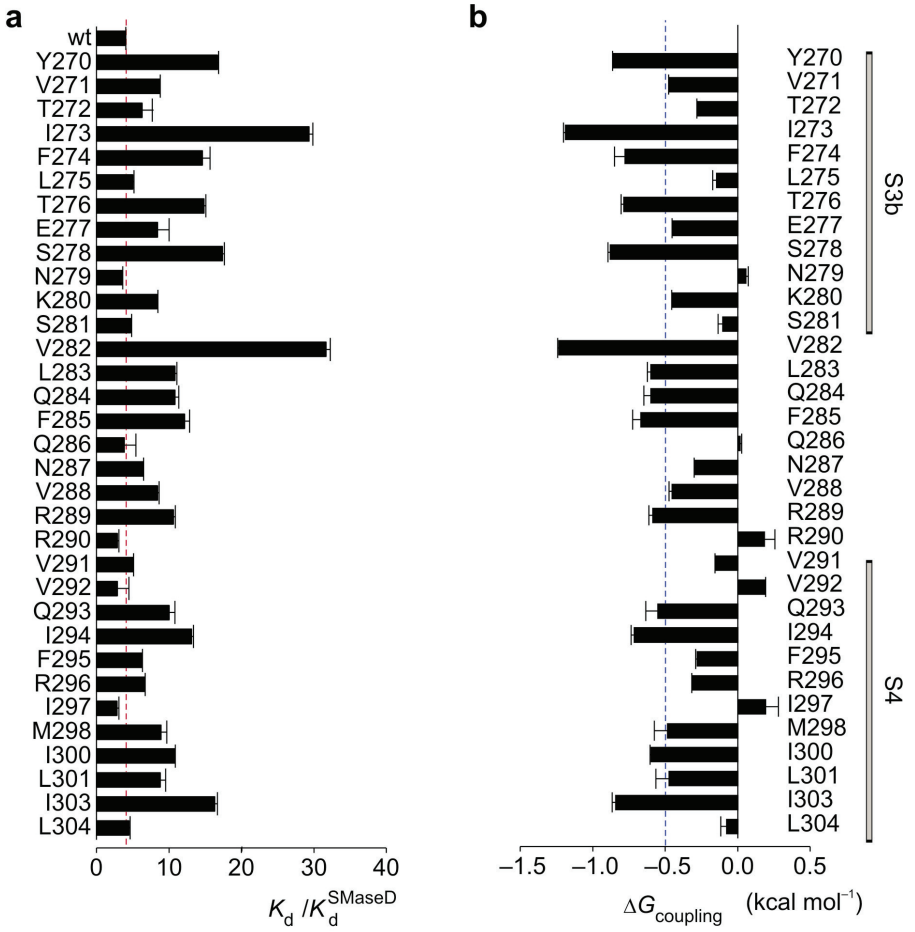
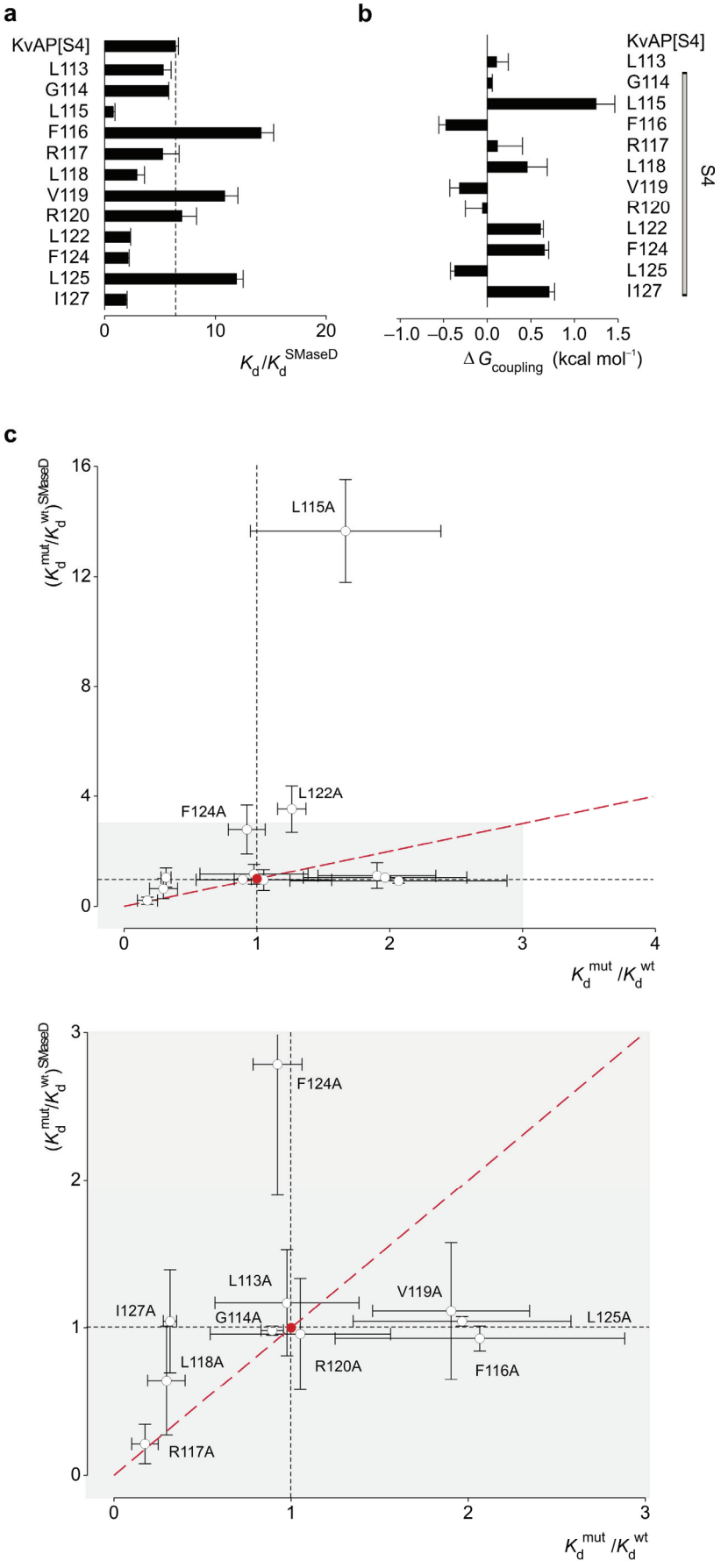


Supplementary Figure 1 |
Partitioning of GxTx-1E and ProTx-I into lipid membranes.

Partitioning of GxTx-1E and ProTx-I into membranes was determined using tryptophan fluorescence as previously described (ref 9). Fluorescence spectra for 10 μM toxins in 10 mM HEPES, 1 mM EDTA, pH 7.6 were recorded between 300 and 400 nm (5 nm band pass, 0^o polarizer) using an excitation wavelength of 280 nm (5 nm band pass, 90^o polarizer) (SPEX FluoroMax 3 spectrofluorometer) and corrected for vesicle scattering. Left Panels: Fluorescence emission spectra of GxTx-1E **(a)** and ProTx-I **(b)** in the absence (black) or presence (blue) of 3.4 mM and 1.5 mM, respectively, lipid vesicles (1:1 mix of POPC:POPG) (blue). Right Panels: Fluorescence intensity at 320 nm plotted as a function of available lipid concentration (60% of total lipids). Smooth curves correspond to fits of $F/F_0(L)=1+(F/F_0^{max}-1)K_x[L]/([W]+K_x[L])$ to the data, where $F/F_0(L)$ is the relative increase in fluorescence intensity at 320 nm for a given concentration of lipid, F/F_0^{max} is the maximum fluorescence increase at high lipid concentrations, [L] is the average available lipid concentration (60% of total lipid concentration), [W] is the molar concentration of water (55.3 M) and K_x is the mole-fraction partitioning coefficient. $K_x=(1.1\pm0.1)x10^6$ and $F/F_0^{max}=1.3\pm0.01$ for GxTx-1E and $K_x=(1.1\pm0.7)x10^7$ and $F/F_0^{max}=4.8\pm0.01$ for ProTx-I. n=3, error bars are s.e.m.



Supplementary Figure 2 | Coupling between membrane modification and paddle mutations in the Kv2.1 channels. a) Paddle mutations influence the effects of SMaseD on the apparent affinity of GxTx-1E for Kv2.1 channels. Each mutant was examined initially using a concentration of toxin near the K_d for the wild type; mutants with different K_d were further examined using a wider range of concentrations. **b)** Plot of coupling energies for interactions between paddle mutations and lipid modification. $\Delta G = RT\ln\Omega$, where the coupling coefficient $\Omega = (K_d^{wt} . K_d^{wtSMaseD}) /(K_d^{mut} . K_d^{mtSMaseD})$. n=3, error bars are s.e.m.

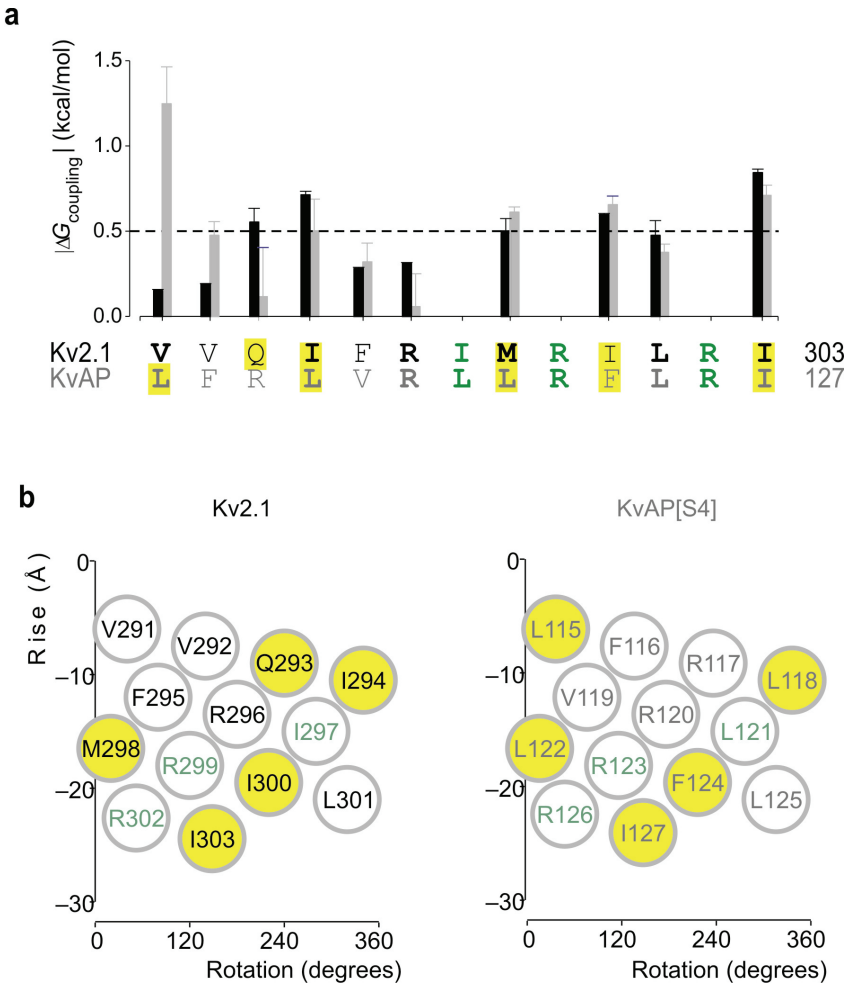


Supplementary Figure 3| Coupling between membrane modification and paddle mutations in the KvAP[S4] chimaera.

a) Ala mutations within S4 influence the effects of SMaseD on the apparent affinity of VSTx for the KvAP[S4] chimaera. n=3, error bars are s.e.m.

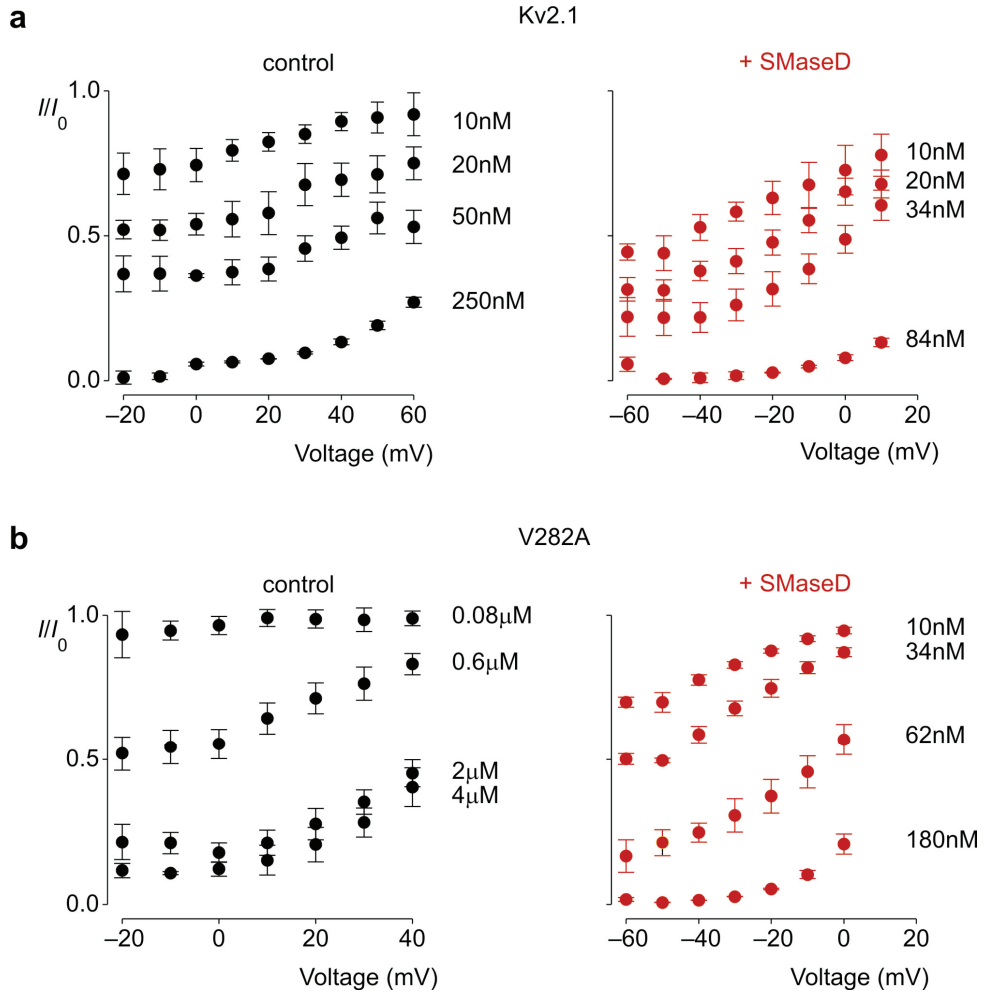
b) Plot of coupling energies for interactions between channel mutations and lipid modification. Same analysis as in Fig 3.

c,d) Comparison of the effects of KvAP[S4] mutations on VSTx affinity before and after membrane modification with SMaseD. Data from a) and Table 3. The red dotted line corresponds to the values for which no coupling is observed ($\Omega = 1$).



Supplementary Figure 4 |
Comparison of coupling energies for S4 helices from Kv2.1 and KvAP.

a) Comparison of coupling energies for S4 Ala mutations and lipid modification for GxTx-1E interaction with Kv2.1 and VSTx1 interaction with KvAP. Data are from Fig 3 and Supplementary Fig.3. **b)** Distribution of coupling energies within S4 helices from Kv2.1 and KvAP. Yellow highlighting indicates positions where $\Delta G_{coupling}$ > 0.5 kcal mol^{-1}. Mutations at positions indicated with green text cause large shifts in channel activation to negative voltages (>60 mV), making it difficult to measure voltage-activation relationships or toxin affinities after SMaseD treatment. Only in the case of I297A in Kv2.1 (the least perturbed of these), could we obtain reliable measurements.



Supplementary Figure 5 |
Estimating the concentration-dependence for GxTx-1E occupancy of Kv channels.

a) Fractional inhibition of Kv2.1 tail currents (I/I_0) elicited following depolarization to the indicated voltages. The values of I/I_0 measured in the plateau phase at negative voltages where toxin-bound channels do not open were taken as the fraction unbound (Fu). Data for control membranes is shown in black symbols (left), while that following treatment with SMaseD is shown in red (right). Holding voltage is -100 mV, and tail voltage is -60 mV for control and -80 mV for SMaseD. **b)** Fractional inhibition of Kv2.1 V282A tail currents elicited following depolarization to the indicated voltages for control (black) and SMaseD-treated (red) membranes. Same voltage protocols as in a. n=3-5 and errors bars are s.e.m..

Construct	K_d , nM	K_d^{SMaseD}, nM
wt	203 ± 38	52 ± 8
Y270A	560 ± 68	33 ± 1
V271A	394 ± 32	45 ± 1
T272A	3900 ± 275	620 ± 34
I273A	4400 ± 427	150 ± 21
F274A	16800 ± 1300	1148 ± 425
L275A	121 ± 32	30 ± 3
T276A	20000 ± 2600	1350 ± 200
E277A	30000 ± 1500	3570 ± 150
S278A	5798 ± 879	334 ± 36
N279A	237 ± 22	66 ± 10
K280A	401 ± 32	48 ± 4
S281A	6200 ± 1600	1322 ± 50
V282A	3800 ± 400	120 ± 9
L283A	6600 ± 1200	609 ± 74
Q284A	3280 ± 313	303 ± 88
F285A	3700 ± 1200	305 ± 12
Q286A	1236 ± 154	324 ± 16
N287A	469 ± 56	73 ± 5
V288A	484 ± 68	57 ± 9
R289A	2182 ± 482	206 ± 12
R290A	1750 ± 566	607 ± 124
V291A	543 ± 50	107 ± 8
V292A	120 ± 6	42 ± 2
Q293A	480 ± 72	48 ± 18
I294A	942 ± 151	72 ± 10
F295A	1250 ± 160	198 ± 6
R296A	10000 ± 563	1500 ± 160
I297A	534 ± 158	188 ± 56
M298A	321 ± 84	36 ± 12
I300A	656 ± 56	61 ± 5
L301A	1461 ± 589	166 ± 20
I303A	1337 ± 243	82 ± 8

Table 1|
Apparent K_d values for GxTx-1E interaction with Kv2.1 Ala mutants in native or SMaseD modified membranes.

n=3, errors are s.e.m.

Construct	- SMaseD		+ SMaseD	
	$V_{1/2}$, mV	z	$V_{1/2}$, mV	z
wt	-4 ± 1	2.6 ± 0.2	-42 ± 1	3.3 ± 0.3
Y270A	16 ± 3	1.8 ± 0.5	-26 ± 1	2.1 ± 0.2
V271A	-9 ± 1	3.2 ± 0.5	-50 ± 1	3.3 ± 0.5
T272A	-2 ± 1	2.5 ± 0.3	-32 ± 1	2.8 ± 0.3
I273A	7 ± 1	3.6 ± 0.4	-32 ± 2	2.2 ± 0.3
F274A	-18 ± 1	2.2 ± 0.2	-46 ± 1	2.9 ± 0.3
L275A	9 ± 1	2.8 ± 0.3	-32 ± 1	2.2 ± 0.3
T276A	5 ± 1	3 ± 0.2	-31 ± 2	2.7 ± 0.4
E277A	6 ± 1	2 ± 0.7	-34 ± 2	2 ± 0.3
S278A	-16 ± 1	3 ± 0.4	-50 ± 1	3 ± 0.4
N279A	-11 ± 1	2.5 ± 0.4	-50 ± 1	2 ± 0.3
K280A	-5 ± 2	2 ± 0.3	-40 ± 2	2 ± 0.2
S281A	-1 ± 1	2.7 ± 0.4	-30 ± 1	3 ± 0.4
V282A	-7 ± 2	2 ± 0.5	-38 ± 2	1.8 ± 0.3
L283A	-5 ± 2	2.7 ± 0.7	-41 ± 1	3.1 ± 0.4
Q284A	4 ± 1	2.6 ± 0.1	-25 ± 1	2.5 ± 0.3
F285A	-19 ± 1	2.5 ± 0.3	-58 ± 1	2.9 ± 0.5
Q286A	-13 ± 1	2 ± 0.3	-47 ± 7	1 ± 0.2
N287A	-9 ± 2	2 ± 0.3	-47 ± 2	2.5 ± 0.5
V288A	6 ± 1	2.6 ± 1.6	-33 ± 2	3 ± 0.6
R289A	5 ± 2	2.5 ± 0.6	-27 ± 2	2.4 ± 0.4
R290A	-7 ± 1	2.5 ± 0.1	-33 ± 1	2.3 ± 0.1
V291A	-9 ± 1	3.5 ± 0.6	-50 ± 1	5.6 ± 0.7
V292A	22 ± 2	3.7 ± 1	-8 ± 2	1.6 ± 0.3
Q293A	7 ± 1	1.6 ± 0.1	-38 ± 1	2.9 ± 0.2
I294A	-11 ± 2	1.5 ± 0.3	-33 ± 2	1.7 ± 0.8
F295A	-12 ± 2	1.9 ± 0.7	-50 ± 4	1.7 ± 0.3
R296A	-1 ± 1	1.9 ± 0.1	-33 ± 2	1.3 ± 0.2
I297A	-57 ± 1	4 ± 0.5	-70 ± 1	4.8 ± 0.6
M298A	-5 ± 1	2.6 ± 0.6	-39 ± 2	2.5 ± 0.3
I300A	-5 ± 2	1.6 ± 0.3	-28 ± 2	1.6 ± 0.2
L301A	9 ± 5	2.3 ± 0.2	-30 ± 1	3.3 ± 0.4
I303A	25 ± 1	3 ± 0.2	-7 ± 1	2.6 ± 0.3

Table 2|
Gating properties of Kv2.1 Ala mutants in native or SMaseD modified membranes.

Midpoint ($V_{1/2}$) and slope (z) values were calculated by fitting a single Boltzmann function to voltage-activation relations, obtained from tail current measurements. n=3, errors are s.e.m.

Construct	- SMaseD			+ SMaseD		
	K_d, μM	$V_{1/2}$, mV	z	K_d, μM	$V_{1/2}$, mV	z
KvAP[S4]	10.0 ± 2.5	-12 ± 1	2.2 ± 0.2	1.6 ± 0.3	-33 ± 3	2.1 ± 0.2
L113	9.8 ± 4.1	-29 ± 3	2.3 ± 0.2	1.8 ± 0.6	-37 ± 1	2.1 ± 0.1
G114	8.9 ± 0.6	-12 ± 2	2.2 ± 0.2	1.6 ± 0.1	-42 ± 1	2.7 ± 0.3
L115	16.7 ± 7.2	22 ± 2	1.6 ± 0.1	21.6 ± 10.8	-20 ± 1	1.9 ± 0.1
F116	20.7 ± 8.2	-30 ± 3	2.4 ± 0.3	1.5 ± 0.1	-55 ± 1	2.8 ± 0.5
R117	1.7 ± 0.8	-14 ± 2	2.3 ± 0.2	0.3 ± 0.2	-43 ± 1	3.2 ± 0.3
L118	3.0 ± 1.1	-10 ± 3	2.4 ± 0.2	1.0 ± 0.6	-37 ± 1	2.1 ± 0.2
V119	19.0 ± 4.4	-24 ± 2	2.2 ± 0.2	1.8 ± 0.7	-50 ± 2	1.8 ± 0.2
R120	10.5 ± 5.1	11 ± 2	2.4 ± 0.1	1.5 ± 0.6	-30 ± 1	2.2 ± 0.2
L122	12.6 ± 1.1	-13 ± 1	2.7 ± 0.2	5.6 ± 1.4	-35 ± 1	1.8 ± 0.2
F124	9.2 ± 0.4	-50 ± 3	2.3 ± 0.3	4.4 ± 1.4	-77 ± 2	2.3 ± 0.4
L125	19.6 ± 6.1	-9 ± 1	2.2 ± 0.2	1.7 ± 0.1	-37 ± 3	1.6 ± 0.2
I127	3.2 ± 0.4	-8 ± 1	2.4 ± 0.2	1.7 ± 0.5	-34 ± 1	2.8 ± 0.2

Table 3|
Apparent K_d values for VSTx and gating properties of KvAP[S4] Ala mutants in native or SMaseD modified membranes.

Midpoint ($V_{1/2}$) and slope (z) values were calculated by fitting a single Boltzmann function to voltage-activation relations, obtained from tail current measurements. n=3, errors are s.e.m.