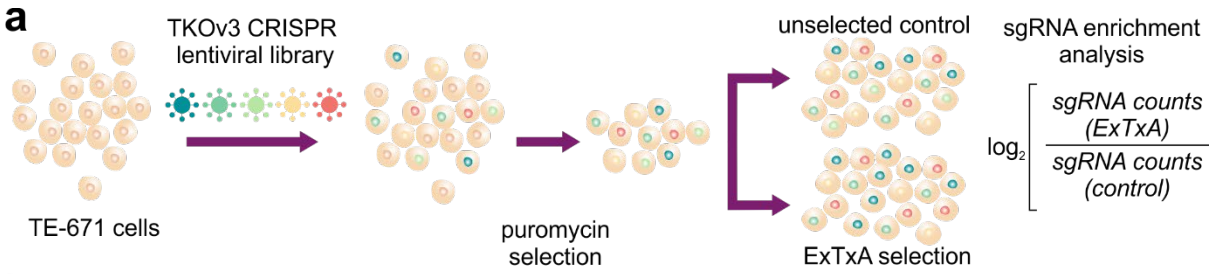
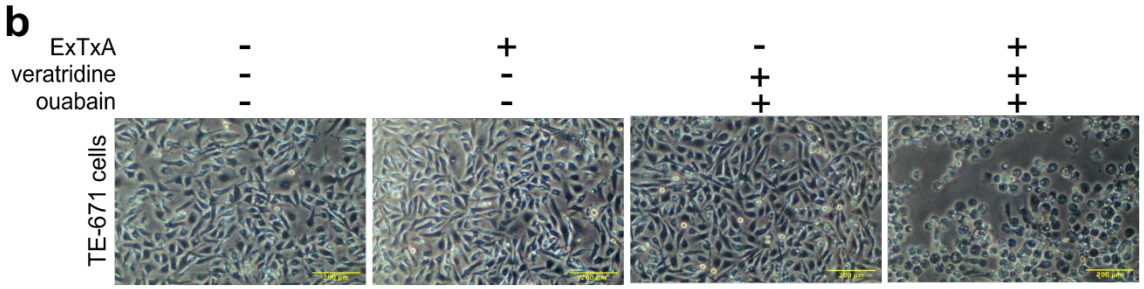
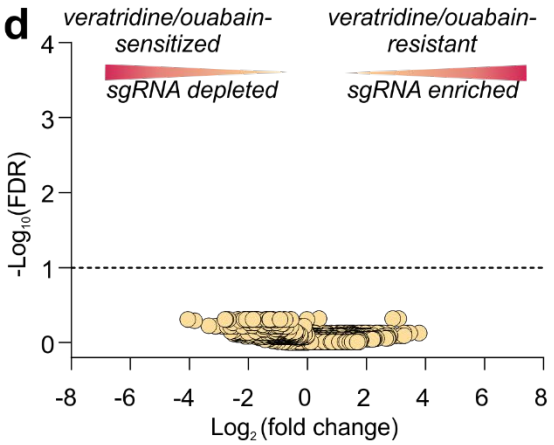
Supplementary information

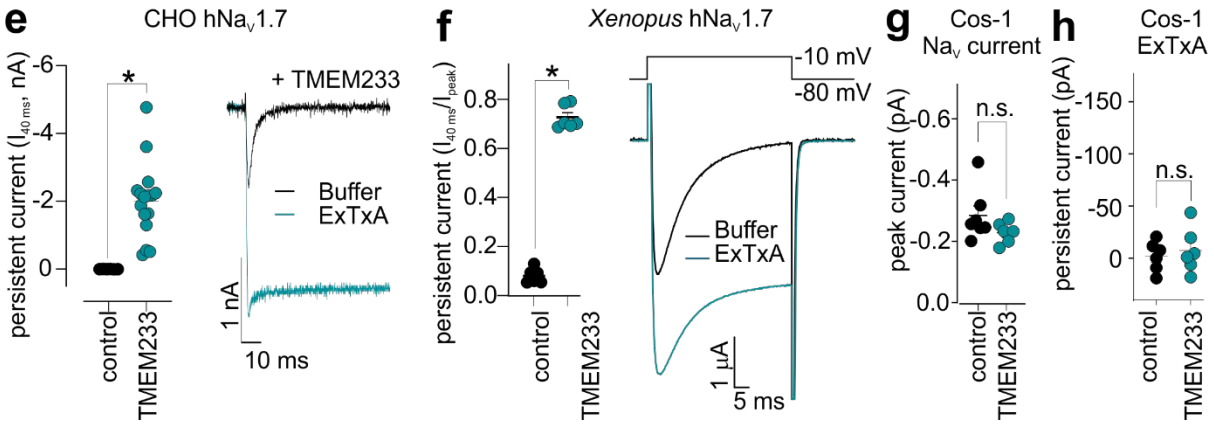




c

Gene	log2 (fold change)	log10 (FDR)
RNF121	7.9834	3.083546
GPAA1	6.9631	3.083546
PIGT	6.2353	3.083546
CRELD1	6.7265	3.083546
SCN9A	6.3493	3.083546
PIGK	6.1905	3.083546
STT3B	5.8503	2.731422
TMEM233	7.3975	2.731422
PIGS	4.7412	1.646815
MMGT1	4.5056	1.126377
LMAN2L	3.582	1.103705
SPAG5	-4.8357	1.04211
TMEM161B	-5.3599	1.04211
NEDD4L	-3.9677	1.460259

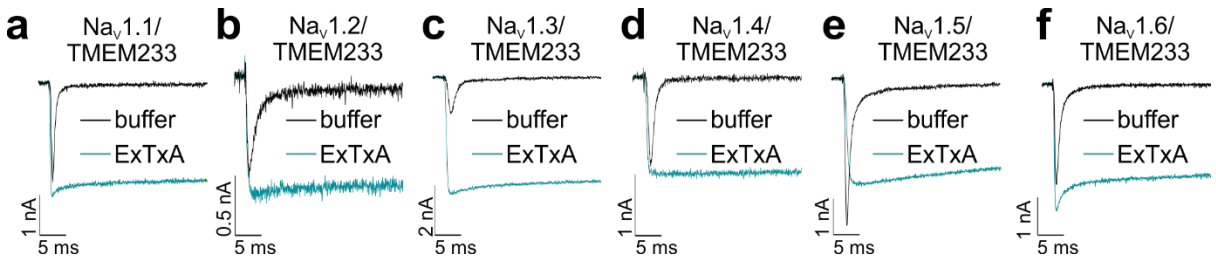




Supplementary Fig. S1. A CRISPR knockdown screen in TE-671 cells identifies TMEM233 as the molecular target of ExTxA.

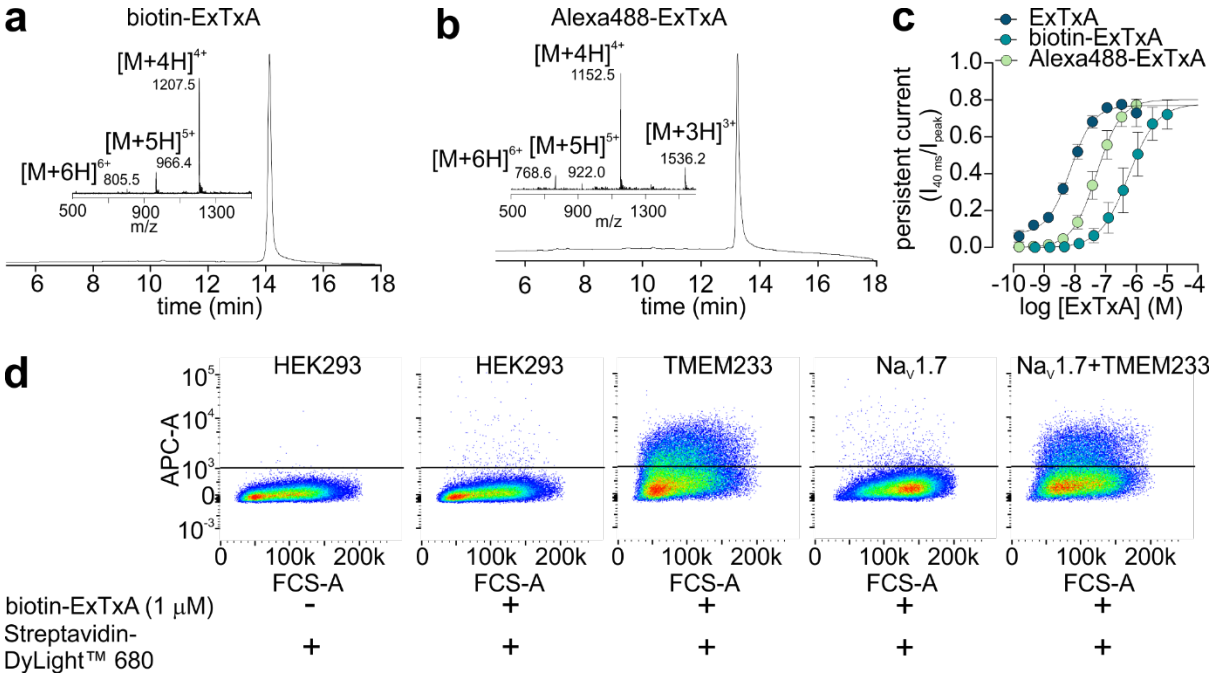
a) *Schematic illustrating the LentiCRISPR-Cas9 TKOv3 screen used to identify the genes required for ExTxA-induced cytotoxicity.* ***b)*** *Representative light microscope images showing*

the effect of ExTxA (1 μM), veratridine (5 μM) and ouabain (20 nM) on viability of TE-671 cells after 24 h. Scale bars, 200 μm. ***c)*** *Gene names, log 2 (fold change) and log10 FDR (false discovery rate) of hits from ExTxA-selection in TE-671 cells.* ***d)*** *LentiCRISPR-Cas9 TKOv3 screen showing no depletion or enrichment of sgRNAs in control (untreated) and veratridine/ouabain-treated (veratridine, 5 μM; ouabain, 20 nM) TE-671 cells.* ***e)*** *ExTxA (1 μM)-induced persistent current ($I_{40\ ms}$, nA) in CHO cells expressing $hNa_V1.7$ alone (control) or co-expressing $hNa_V1.7$ and TMEM233 (TMEM233). Right; representative current traces; depolarization to -20 mV from -90 mV holding potential.* ***f)*** *ExTxA (1 μM)-induced normalized persistent current ($I_{40\ ms}/I_{peak}$) in* Xenopus *oocytes expressing $hNa_V1.7$ alone (control) or co-expressing $hNa_V1.7$ and TMEM233 (TMEM233). Right; representative current traces; depolarization to -10 mV from -80 mV holding potential.* ***g)*** *Depolarization-induced (holding potential -90 mV, 50 ms step to -20 mV) peak current (pA) in untransfected Cos-1 cells (control) and Cos-1 cells transfected with TMEM233 only (TMEM233).* ***h)*** *ExTxA (1 μM)-induced persistent current (40 ms; pA) in untransfected Cos-1 cells (control) and Cos-1 cells transfected with TMEM233 only (TMEM233) during depolarization to -20 mV from a holding potential of -90 mV. Data are shown as mean ± SEM; n.s., not significant; *, $p < 0.05$. n values and statistical information are detailed in Supplementary data Table 1. Source data are provided as a Source Data file.*



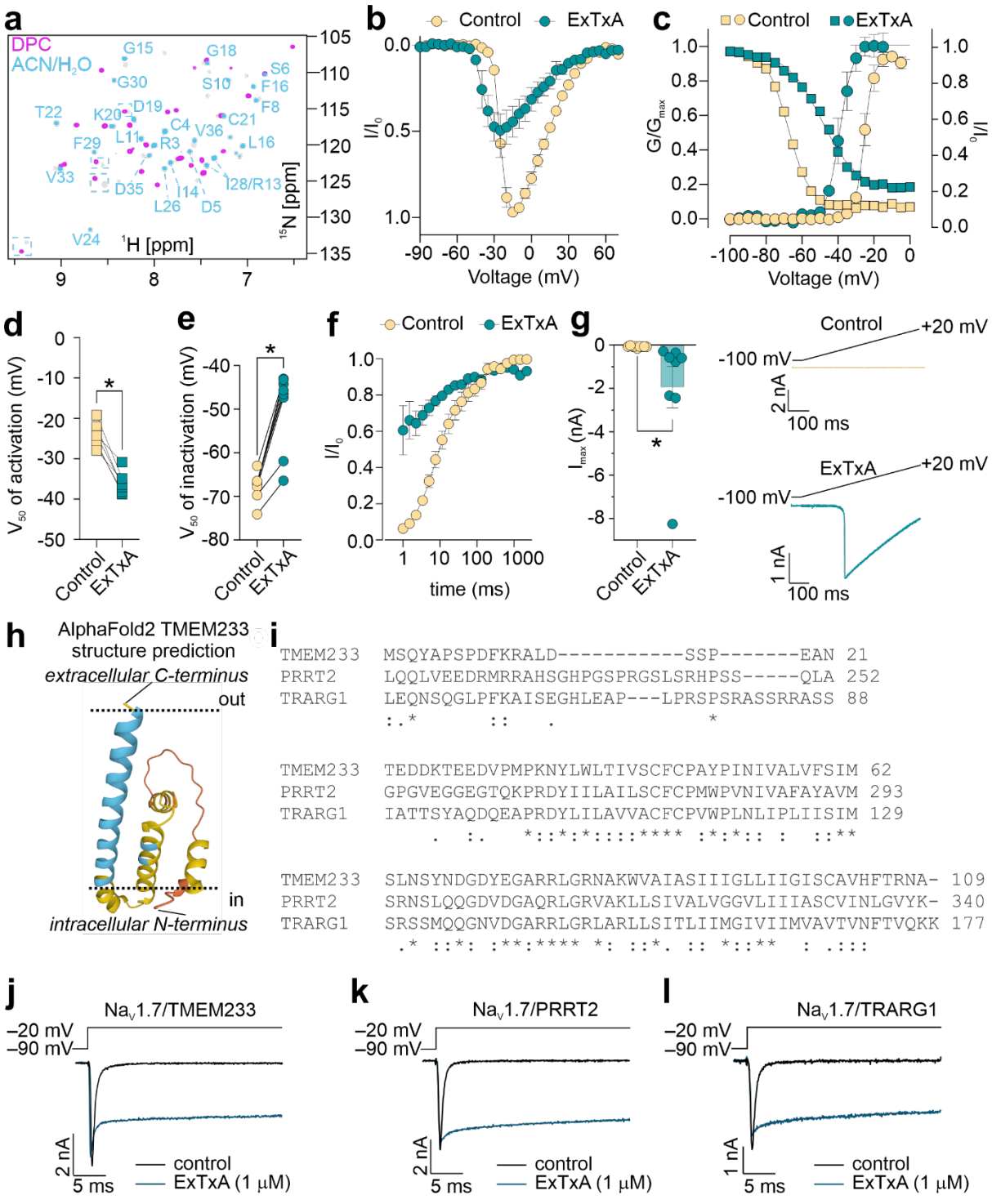
Supplementary Fig. S2. Co-expression of TMEM233 confers ExTxA sensitivity to Na$_V$1.1-1.6.

Representative current traces showing ExTxA-induced persistent currents in HEK293 cells expressing ***a)*** *Na$_V$1.1 + TMEM233,* ***b)*** *Na$_V$1.2 + TMEM233,* ***c)*** *Na$_V$1.3 + TMEM233,* ***d)*** *Na$_V$1.4 + TMEM233,* ***e)*** *Na$_V$1.5 + TMEM233, and* ***f)*** *Na$_V$1.6 + TMEM233.*



Supplementary Fig. S3. Biotin- and Alexa488-labelled ExTxA as probes to determine TMEM233 binding.

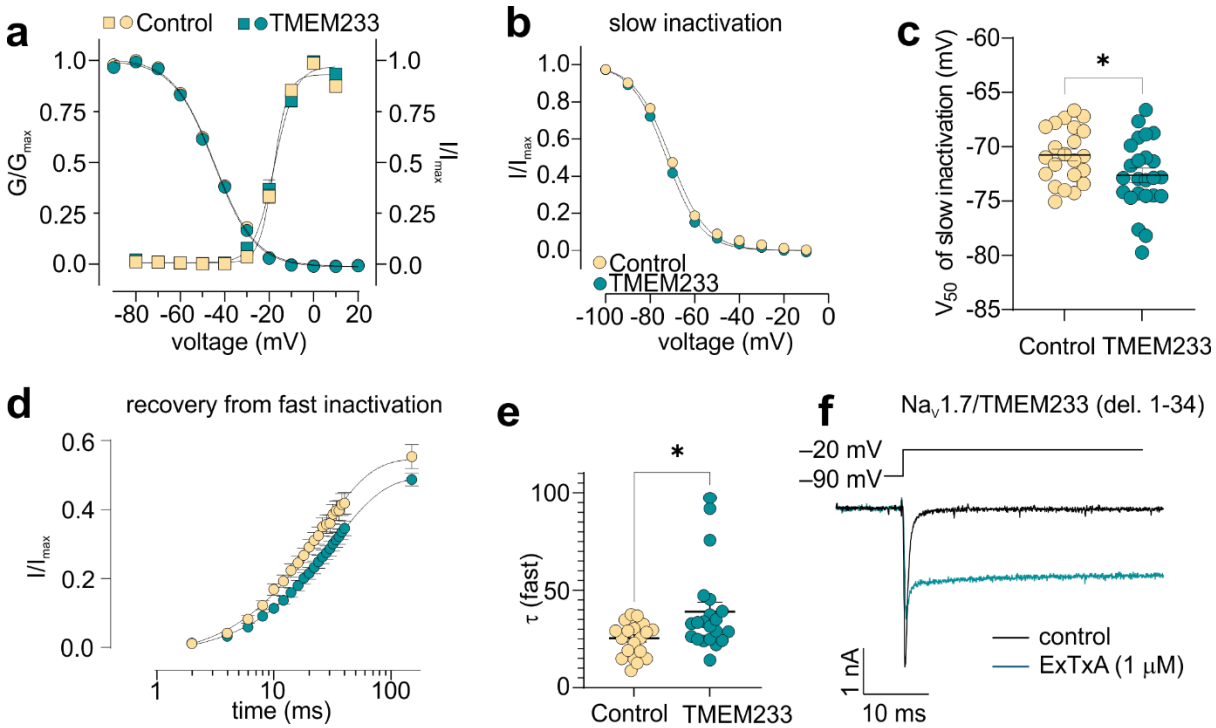
UPLC and mass spectrometry profile of ***a)*** *N-terminally biotinylated ExTxA and* ***b)*** *N-terminally Alexa488-tagged ExTxA.* ***c)*** *Concentration-response of native, biotinylated and Alexa488-tagged ExTxA in HEK293-Na$_V$1.7 cells co-transfected with TMEM233.* ***d)*** *Representative flow cytometry analysis of untransfected (HEK293) and TMEM233-transfected HEK293 cells, as well as HEK293 cells expressing hNa$_V$1.7 alone (Na$_V$1.7) or co-expressing hNa$_V$1.7 and TMEM233 (Na$_V$1.7+TMEM233) stained with biotinylated ExTxA and DyLight^{TM}-680-conjugated streptavidin. Solid line indicates cut-off for signal-positive cells. Data in c) are shown as mean ± SEM. n values and statistical information are detailed in Supplementary data Table 1.*



Supplementary Fig. S4. Effects of ExTxA on $Na_V1.7$ co-expressed with the dispanins.

a) *^{1}H-^{15}N transverse relaxation optimized spectroscopy (TROSY) spectra of ^{15}N labelled recombinant ExTxA in acetonitrile/H_2O and dodecylphosphocholine micelles (DPC) showing distinctly different chemical shifts in a lipid membrane-like environment.* ***b-g)*** *Electrophysiological characterization of ExTxA effects on $Na_V1.7$ co-expressed with*

TMEM233. ***b)*** *Current-voltage (IV) relationship,* ***c)*** *conductance-voltage (GV, circles) and steady-state inactivation (squares) curves,* ***d)*** V_{50} *of activation and* ***e)*** V_{50} *of steady-state fast inactivation as well as* ***f)*** *time-dependence of recovery from fast inactivation and* ***g)*** *(left) peak ramp currents and (right) representative ramp (0.2 mV/s) current traces from HEK293 cells co-expressing* $Na_V1.7$ *and TMEM233 after the addition of 0.1% BSA (yellow, control) and ExTxA (teal, 1 μM in 0.1% BSA).* ***h)*** *AlphaFold2 TMEM233 (UniProt ID B4DJY2) structure prediction. Dotted lines indicate approximate locations of plasma membrane.* ***i)*** *Sequence alignment of the human dispanins TMEM233 (DSPB2; UniProt ID B4DJY2), PRRT2 (DSPB3; UniProt ID Q7Z6L0), and TRARG1 (DSPB1; UniProt ID Q8IXB3). The large N-terminal domains of PRRT2 and TRARG1 are truncated for clarity.* ***j)*** *Representative current traces from HEK293-*$Na_V1.7$ *cells co-transfected with TMEM233,* ***k)*** *PRRT2 or* ***l)*** *TRARG1 showing ExTxA (1 μM)-induced inhibition of inactivation. Shown is a 50 ms depolarization to -20 mV from a holding potential of-90 mV. Data are shown as mean ± SEM; *,* $p < 0.05$*. n values and statistical information are detailed in Supplementary data Table 1.*



Supplementary Fig. S5. Effect of TMEM233 co-expression on $Na_V1.7$ expressed in* Xenopus *oocytes.

a) *Superimposed conductance–voltage (GV, squares) and steady-state fast inactivation (circles) curves of* Xenopus *oocytes expressing $hNa_V1.7$ alone (control, yellow) or co-expressing $hNa_V1.7$ and TMEM233-transfected (TMEM233, teal).* ***b)*** *Voltage-dependence and* ***c)*** *V_{50} of slow inactivation of $hNa_V1.7$ alone (control, yellow) or $hNa_V1.7$ co-expressed with TMEM233 (TMEM233) in* Xenopus *oocytes.* ***d)*** *Time-dependence and* ***e)*** *time constant τ of recovery from fast inactivation of $hNa_V1.7$ alone (control, yellow) or $hNa_V1.7$ co-expressed with TMEM233 (TMEM233) in* Xenopus *oocytes.* ***f)*** *Representative current trace of ExTxA (1 μM)-induced persistent current in HEK293-$hNa_V1.7$ cells co-expressing N-terminally truncated TMEM233 (del. 1-34). Data are shown as mean ± SEM; *, $p < 0.05$. n values and statistical information are detailed in Supplementary data Table 1.*

Supplementary Table 1.

Figure	Parameter (units)	Group	mean ± SEM	n	Statistical test
Fig. 1a,b	DRG total persistent Na_V current ($I_{40\ ms}/I_{peak}$)	Control	0.027 ± 0.01	6	* p< 0.05 by repeated measures one-way ANOVA with Tukey's multiple comparison test
		ExTxA (100 nM)	0.513 ± 0.10		
		ExTxA (100 nM) + TTX (1 μM)	0.001 ± 0.004		
Fig. 1c,d	DRG $Na_V1.8$ Δ persistent current ($I_{40\ ms}/I_{peak}$):	Control	0.009 ± 0.005	13	p = 0.024 by two-sided Mann-Whitney test
		ExTxA (100 nM)	0.050 ± 0.015	13	
Fig. 1e,f	DRG $Na_V1.9$ Δ persistent current ($I_{90\ ms}/I_{peak}$):	Control	-0.034 ± 0.015	9	p = 0.447 by two-sided t-test
		ExTxA (1 μM)	-0.057 ± 0.028	7	
Fig. 1g,h	hiPSC-derived sensory neurons persistent current ($I_{40\ ms}/I_{peak}$):	Buffer control (0.1% BSA)	0.025 ± 0.009	5	*p = 0.0002 (control vs ExTxA); #, p = 0.0123 (ExTxA vs ExTxA + Pn3a) by Repeated measures one-way ANOVA with Tukey's multiple comparison test
		ExTxA (100 nM)	0.151 ± 0.024		
		ExTxA (100 nM) + Pn3a (100 nM)	0.069 ± 0.012		
		ExTxA (100 nM) + Pn3a (100 nM) + TTX (1 μM)	0.003 ± 0.001		
Fig. 1i,j	TE-671 persistent current ($I_{40\ ms}/I_{peak}$)	Buffer control	0.021 ± 0.009	8	*,#p < 0.001 by Repeated measures one-way ANOVA with Tukey's multiple comparison test
		ExTxA (100 nM)	0.272 ± 0.018		
		ExTxA +Pn3a (1 μM)	0.068 ± 0.011		
		ExTxA +Pn3a + TTX (1 μM)	0.025 ± 0.008		
Fig. 1k	cumulative flinches (60 min) following i.pl. ExTxA (10 nM)	wt	319.3 ± 21.9	4	*p <0.0001 by two-tailed student's t-test
		$Na_V1.7^{Advill}$	40.0 ± 6.5	4	
Fig. 2a,b	Δ persistent current ($I_{40\ ms}/I_{peak}$ buffer *cf.* ExTxA)	HEK293-$Na_V1.1$	-0.003 ± 0.0040	11	
		HEK293-$Na_V1.2$	-0.002 ± 0.0053	11	

		HEK293-$Na_V1.3$	0.0265 ± 0.0049	11	*p<0.0001 by one-way ANOVA with Tukey's multiple comparison test
		HEK293-$Na_V1.4$	7.89E-07 ± 0.0005	24	
		HEK293-$Na_V1.5$	0.0004 ± 0.0005	9	
		HEK293-$Na_V1.6$	-0.008 ± 0.0103	12	
		HEK293-$Na_V1.7$	-0.0004 ± 0.0008	8	
		HEK293-$Na_V1.8$	0.027 ± 0.0057	11	
		ND7/23	-0.007 ± 0.0102	6	
		F11	0.0202 ± 0.0041	11	
		SH-SY5Y	0.006 ± 0.0175	6	
		h$Na_V1.7$ Xenopus oocytes	0.0381 ± 0.0074	5	
		TE-671	0.719 ± 0.0230	11	
Fig. 2c,d	Δ persistent current ($I_{40\ ms}/I_{peak}$) in CHO h$Na_V1.7$	no β subunit	0.001 ± 0.004	12	p > 0.05 by one-way ANOVA
		β1	0.0001 ± 0.001	11	
		β2	-0.002 ± 0.003	14	
		β3	-0.001 ± 0.002	15	
		β4	-0.004 ± 0.004	14	
Fig. 3b	Δ F/F in $Na_V1.7$ HEK293 (ExTxA 1 μM)	Control	0.016 ± 0.013	3	*p < 0.0001 by one-way ANOVA with Tukey's multiple comparison test
		Creld1	0.001 ± 0.0079	3	
		Lman2L	0.014 ± 0.0048	3	
		Mmgt1	0.0059 ± 0.0015	3	
		RNF121	0.007 ± 0.0074	3	
		Stt3b	0.013 ± 0.00098	3	
		GPI complex	0.0067 ± 0.0034	3	
		TMEM233	0.29 ± 0.0075	3	
Fig. 3c	ExTxA-induced persistent current ($I_{40\ ms}$; pA) in HEK293-h$Na_V1.7$	$Na_V1.7$ + ExTxA (1 μM)	-5.2 ± 3.7	11	*p < 0.0001 by two-tailed student's t-test
		$Na_V1.7$/ TMEM233 + ExTxA (1 μM)	1,473 ± 407.8	4	

Fig. 3d	ExTxA-induced persistent current ($I_{40\ ms}/I_{peak}$; nA) in TE-671 cells	Wildtype + ExTxA (1 μM)	0.81 ± 0.015	7	*p < 0.0001 by two-tailed student's t-test
		TMEM233 KO + ExTxA (1 μM)	0.047 ± 0.012	6	
Fig. 3e	ExTxA-induced persistent current (I_{40ms}/I_{peak}; nA) in DRG neurons	Wildtype + ExTxA (30 nM)	0.573 ± 0.086	6	*p < 0.0001 by two-way ANOVA
		$Tmem233^{Cre}$ KO + ExTxA (30 nM)	0.114 ± 0.04	7	
Fig. 3f	ExTxA (10 nM)-induced DRG Ca^{2+} responses (% neurons activated)	wt	53.20 ± 1.789	10 wells	*p <0.0001 by two-tailed student's t-test
		$Tmem233^{Cre}$ KO	22.33 ± 1.942	7 wells	
Fig. 3g	ExTxA (10 nM)-induced pain behaviours	wt	234.2 ± 27.7	17	*p = 0.037 by two-tailed student's t-test
		$Tmem233^{Cre}$ KO	115.9 ± 25.3	16	
Fig. 4a	% positive signal (flow cytometry)	HEK293 + biotin-ExTxA (1 μM):	0.91 ± 0.51	3	*p < 0.05 (HEK293 vs. TMEM233, p = 0.002; HEK293 vs. $Na_V1.7$/TMEM233, p = 0.0015) by one-way ANOVA with Tukey's multiple comparison test
		TMEM233 + biotin-ExTxA (1 μM)	23.00 ± 2.66	3	
		$Na_V1.7$ + biotin-ExTxA (1 μM):	1.23 ± 0.28	3	
		$Na_V1.7$/TMEM233 + biotin-ExTxA (1 μM):	17.87 ± 2.93	3	
Fig. 4b	Max fluorescence (AFU)	HEK293 + Alexa488-ExTxA (1 μM):	15,740 ± 2133	3	

		TMEM233 + Alexa488-ExTxA (1 μM)	38,681 ± 951.2	3	*p < 0.001 by one-way ANOVA with Tukey's multiple comparison test
		$Na_V1.7$ + Alexa488-ExTxA (1 μM):	20,583 ± 3970	3	
		$Na_V1.7$/TMEM233 + Alexa488-ExTxA (1 μM):	38,799 ± 2471	3	
Fig. 4d	persistent current ($I_{40\ ms}/I_{peak}$)	$Na_V1.7$ + ExTxA (100 nM) in ECS	0.04 ± 0.014	10	*p < 0.0001 by two-way ANOVA with Šídák's multiple comparisons test
		$Na_V1.7$ + ExTxA (100 nM) in ICS	-0.002 ± 0.004	10	
		$Na_V1.7$/TMEM233 + ExTxA (100 nM) in ECS	0.543 ± 0.061	6	
		$Na_V1.7$/TMEM233 + ExTxA (100 nM) in ICS	0.003 ± 0.023	6	
Fig. 4e,f	% positive signal (flow cytometry)	C-terminal HA-tagged TMEM233, permeabilized	93.93 ± 3.526	3	*p < 0.0001 by one-way ANOVA with Tukey's multiple comparison test
		N-terminal HA-tagged TMEM233, permeabilized	93.17 ± 2.696	3	
		C-terminal HA-tagged TMEM233, surface	97.00 ± 0.1732	3	
		N-terminal HA-tagged TMEM233, surface	15.33 ± 1.534	3	
Fig. 4g	ExTxA EC_{50}	$Na_V1.7$/TMEM233	4.3 – 7.9 nM (95% CI)	7	p < 0.05 by one-way ANOVA with Tukey's multiple comparison test
		$Na_V1.7$/PRRT2	128.2 – 611.0 nM (95% CI)	10	
		$Na_V1.7$/TRARG1	308.6 – 6570.0 nM (95% CI)	7	

Fig. 5e	Proximity ligation assay (particles/cell)	HEK293 β1/β2 control	2.94 ± 0.21	3	*p < 0.0001 by one-way ANOVA with Tukey's multiple comparison test
		FLAG-tagged $Na_V1.7$	5.25 ± 1.68	3	
		N-terminal HA-tagged TMEM233	7.49 ± 1.82	3	
		FLAG-tagged $Na_V1.7$ + N-terminal HA-tagged TMEM233	36.72 ± 5.22	3	
Fig. 6c,d,e	V_{50} of activation (mV)	$Na_V1.7$	-26.11 ± 1.74	6	p > 0.05 by two-sided student's t-test
		$Na_V1.7$/TMEM233	-26.55 ± 1.40	11	
	V_{50} of inactivation (mV)	$Na_V1.7$	-64.95 ± 0.76	6	*p = 0.0045 by two-sided student's t-test
		$Na_V1.7$/TMEM233	-68.89 ± 0.76	11	
Fig. 6f	Slow inactivation best-fit parameters	$Na_V1.7$	95% CI Bottom: 0.36 to 0.41 Top: 0.97 to 1.02 V_{50}: -60.99 to -57.13 Slope: -9.57 to -4.03	11	
		$Na_V1.7$/TMEM233	95% CI Bottom: 0.19 to 0.26 Top: 0.99 to 1.07 V_{50}: -67.99 to -63.27 Slope: -9.11 to -5.16	13	
Fig. 6g	V_{50} of slow inactivation (mV)	$Na_V1.7$	-59.66 ± 0.61	11	*p = 0.0108 by two-tailed student's t-test
		$Na_V1.7$/TMEM233	-65.18 ± 1.74	13	

Fig. 6h,i	τ of fast inactivation	$Na_V1.7$	6.70 ± 0.75	8	*p = 0.0236 by two-tailed student's t-test
		$Na_V1.7$/TMEM233	9.64 ± 0.73	18	
Fig. 6j	Ramp currents (1 mV/ms) (nA)	$Na_V1.7$	-0.18 ± 0.04	11	p > 0.05 by two-tailed student's t-test
		$Na_V1.7$/TMEM233	-0.12 ± 0.01	9	
Fig. 6l	Use-dependence (I_{first}/I_{last} @ 20 mHz)	$Na_V1.7$	0.80 ± 0.03	8	*p = 0.016 by two-way ANOVA with Šídák's multiple comparisons test
		$Na_V1.7$/TMEM233	0.68 ± 0.07	6	
Fig. 6m	τ of fast inactivation	$Na_V1.7$	6.05 ± 0.52	16	*p < 0.05 by one-way ANOVA with Tukey's multiple comparison test
		$Na_V1.7$/TMEM233	10.53 ± 1.07	7	
		$Na_V1.7$/del.1-34 TMEM233	6.29 ± 0.49	7	
		Buffer control	6.91 ± 0.39	9	
		N-terminal TMEM233 peptide	10.18 ± 1.30	9	
Supplementary figures					
Fig. 1e	CHO cells persistent current ($I_{40\ ms}$, nA)	$hNa_V1.7$ + ExTxA (1 μM)	0.000 ± 0.002	6	*p = 0.0003 by two-tailed student's t-test
		$hNa_V1.7$/ TMEM233 + ExTxA (1 μM)	-2.010 ± 0.277	16	
Fig. 1f	*Xenopus oocyte* persistent current ($I_{40\ ms}/I_{peak}$)	$hNa_V1.7$ + ExTxA (1 μM)	0.081 ± 0.012	6	*p < 0.0001 by two-tailed student's t-test
		$hNa_V1.7$/ TMEM233 + ExTxA (1 μM)	0.727 ± 0.020	6	
Fig. 1g	Cos-1 cells Peak current (pA)	control	-284.6 ± 31.92	7	p >0.05 by two-tailed student's t-test
		TMEM233	-228.9 ± 14.15	6	
Fig. 1h	Cos-1 cells	Cos-1 + ExTxA (1 μM)	-2.019 ± 5.908	6	p >0.05 by two-tailed

	ExTxA (1 μM)-induced persistent current (pA)	Cos-1/TMEM233 + ExTxA (1 μM)	-7.493 ± 8.833	6	student's t-test
Fig. 3c	Best-fit parameters	ExTxA	Bottom: 0.0729 Top: 0.7692 $logEC_{50}$: -8.146 Hill slope: 1.156	7	
		Biotin-ExTxA	Bottom: -0.0014 Top: 0.7788 $logEC_{50}$: -6.256 Hill slope: 0.9303	6	
		Alexa488-ExTxA	Bottom: 0.00002 Top: 0.8013 $logEC_{50}$: -7.290 Hill slope: 1.084	7	
Fig. 4b,c,d	V_{50} of activation (mV)	$Na_V1.7$/TMEM233	-24.31 ± 1.11	7	*p < 0.0001 by two-tailed student's t-test
		$Na_V1.7$/TMEM233 + ExTxA (1 μM)	-35.92 ± 1.00	7	
Fig. 4c,e	V_{50} of inactivation (mV)	$Na_V1.7$/TMEM233	-67.77 ± 1.12	8	*p < 0.0001 by two-tailed Student's t-test
		$Na_V1.7$/TMEM233 + ExTxA (1 μM)	-49.80 ± 3.20	8	*p < 0.0001 by two-tailed student's t-test
Fig. 4f	τ_1 (recovery from fast inactivation)	$Na_V1.7$/TMEM233	7.23 ± 0.41	9	*p < 0.0001 by two-tailed student's t-test
		$Na_V1.7$/TMEM233 + ExTxA (1 μM)	1.17 ± 0.52	9	
Fig. 4g	Ramp current (0.2 mV/s) (nA)	$Na_V1.7$/TMEM233	-0.075 ± 0.012	9	*p < 0.0001 by two-tailed student's t-test
		$Na_V1.7$/TMEM233 + ExTxA (1 μM)	-1.952 ± 0.950	8	

Fig. 5a	V_{50} of activation (mV)	$Na_V1.7$	-17.53 ± 0.69	27	p > 0.05 by two-tailed student's t-test
		$Na_V1.7$/TMEM233	-17.38 ± 1.05	27	
	V_{50} of inactivation (mV)	$Na_V1.7$	-44.82 ± 0.54	22	p > 0.05 by two-tailed student's t-test
		$Na_V1.7$/TMEM233	-44.99 ± 0.72	24	
Fig. 5b,c	V_{50} of slow inactivation (mV)	$Na_V1.7$	-70.76 ± 0.54	22	p = 0.036 by two-tailed student's t-test
		$Na_V1.7$/TMEM233	-72.63 ± 0.66	24	
Fig. 5d,e	τ_1 (recovery from fast inactivation)	$Na_V1.7$	25.35 ± 1.89	21	*p = 0.0125 by two-tailed student's t-test
		$Na_V1.7$/TMEM233	39.01 ± 4.86	21	