

Supplemental Table 1

Organism	Phylum/group	Protein	Accession
<i>Acropora millepora</i> (stony coral)	Cnidaria	AmiCa _v 1	JR974719
		AmiCa _v 2a	JT013764
		AmiCa _v 2b	JT007682
		AmiCa _v 2c	JR989836
		AmiCa _v 3a	JT013008
		AmiCa _v 3b	JR996966
		Ami $\alpha_2\delta$	JR983982
		Ami β	JT018560
<i>Amphimedon queenslandica</i> (demosponge)	Porifera	AquCa _v 1	XP_003383036
<i>Caenorhabditis elegans</i> (round worm)	Nematoda	CelCa _v 1	NP_001023079
		CelCa _v 2	NP_741734
		CelCa _v 3	AAP79881
		Cel $\alpha_2\delta$	P34374
		Cel β	NP_491193
<i>Capitella teleta</i> (polychete worm)	Annelida	Cte $\alpha_2\delta$	ELU02468
		Cte β	ELU02207
		Cte γ	ELT88457
<i>Ciona intestinalis</i> (Ascidian)	Chordata	CinCa _v 1	XP_002123864
		CinCa _v 2	XP_002123971
		CinCa _v 3	XP_002122425
		Cin $\alpha_2\delta$	XP_002123859
		CinCa _v β	XP_002129248
<i>Cyanea capillata</i> (lion mane's jellyfish)	Cnidaria	CcaCa _v 1	AAC63050
		Cca β	AAB87751
<i>Drosophila melanogaster</i> (fruit fly)	Arthropoda	DmeCa _v 1	Q24270
		DmeCa _v 2	NP_001245639
		DmeCa _v 3	NP_001245544

<i>Drosophila melanogaster</i> (fruit fly)		Dme $\alpha_2\delta a$	NP_610902
		Dme $\alpha_2\delta b$	NP_001097164
		Dme $\alpha_2\delta c$	NP_723955
		DmeWVFAC1	NP_611469
		Dme β	NP_001260377
		DmNa _v 1	AAB59195
		DmNa _v 2	Q9W0Y8
<i>Helobdella robusta</i> (leech)	Annelida	Hroy	ESO09520
<i>Homo sapiens</i> (human)	Chordata	HsaCa _v 1.1	NP_000060
		HsaCa _v 1.2	CAA12174
		HsaCa _v 1.3	NP_001122311
		HsaCa _v 1.4	NP_001243718
		HsaCa _v 2.1	NP_001120693
		HsaCa _v 2.2	NP_000709
		HsaCa _v 2.3	NP_001192222
		HsaCa _v 3.1	NP_001243260
		HsaCa _v 3.2	CAD12646
		HsaCa _v 3.3	AAM67414
		Hsa $\alpha_2\delta 1$	NP_000713
		Hsa $\alpha_2\delta 2$	NP_001278030
		Hsa $\alpha_2\delta 3$	CAB75962
		Hsa $\alpha_2\delta 4$	AAN06672
		Hsa $\beta 1$	AAA36168
		Hsa $\beta 2$	NP_963864
		Hsa $\beta 3$	NP_001193846
		Hsa $\beta 4$	NP_001139270
		HsaNa _v 1.2	Q99250
		HsaNa _v 1.4	AAO83647
HsaVWFAC1	NP_065976		
<i>Hydra magnipapillata</i>	Cnidaria	HmaCa _v 1	GAOL01025755
		HmaCa _v 2	GAOL01023479

<i>Hydra magnipapillata</i>	Cnidaria	HmaCa _v 3	GAOL01025477
		Hma $\alpha_2\delta$ a	GAOL01024861
		Hma $\alpha_2\delta$ b	GAOL01020296
		Hma $\alpha_2\delta$ c	GAOL01025603
		Hma β	XP_002156135
		HmaNa _v 2.1	JQ066819
		HmaNa _v 2.5	JQ066820
<i>Lymnaea stagnalis</i> (pond snail)	Mollusca	LstCa _v 1	AAO83840
		LstCa _v 2	AAO83842
		LstCa _v 3	AFN89594
		Lst β	AEE65419
<i>Lottia gigantea</i> (owl limpet)	Mollusca	Lgia $\alpha_2\delta$	ESP00317
<i>Mnemiopsis leidyi</i> (comb jelly)	Ctenophora	MleCa _v 2	AEF59084
<i>Monosiga brevicollis</i>	Choanozoa	Mbr β	XP_001748252
<i>Nematostella vectensis</i> (starlet sea anemone)	Cnidaria	NveCa _v 1	XP_001639054
		NveCa _v 2a	gw.15.189.1
		NveCa _v 2b	gw.49.149.1
		NveCa _v 2c	gw.11.380.1
		NveCa _v 3a	XP_001628907
		NveCa _v 3b	XP_001627598
		Nve $\alpha_2\delta$	XP_001636666
		Nve β	XP_001626057
		NveVWFAC	XP_001639042
<i>Physalia physalis</i> (hydrozoan)	Cnidaria	Pph β	ABD59026
<i>Salpingoeca rosetta</i>	Choanozoa	SroCa _v 1/2	XP_004989719
		SroCa _v 3	XP_004995501
		Sro β	XP_004990138
<i>Stylophora pistillata</i> (stony coral)	Cnidaria	SpiCa _v 1	AAD11470

<i>Saccoglossus kowalevskii</i>	<i>Hemichordata</i>	Skoy	XP_006816113
<i>Thalassiosira pseudonana</i> (diatom)	Bacillariophyta	TpCa _v	XP_002289136
<i>Thecamonas trahens</i>	Apusozoa	TtrNa _v 2a	AMSG_03287.2
		TtrNa _v 2b	AMSG_06300.2
<i>Trichoplax adhaerens</i>	Placozoa	TadCa _v 1	XP_002108930
		TadCa _v 2	XP_002109775
		TadCa _v 3	XP_002109544
		Tadβ	XP_002110341
		Tadα ₂ δ	XP_002112661

Table S1. Ion channels used in the phylogenetic analysis. Most channels are derived from data deposited in Genbank. Some of the channels from *Nematostella vectensis* are from the genome sequencing project performed by the Joint Genome Institute (JGI) available at <http://genome.jgi-psf.org/Nemve1/Nemve1.home.html>. The channel protein model of *Thecamonas trahens* is from the Origins of Multicellularity project by the Broad Institute available at http://broadinstitute.org/annotation/genome/multicellularity_project.

Supplementary Table 2

Gene	Forward primer	Reverse primer
NveCa _v 1	GTAAAAGCTCTCAGAGCCTTC CGAGTTC	CCAGATTGTCCACAGCAATG GCCAAGA
NveCa _v 2a	GAAGCAGTGCTCAAGATTAT AGCGCTG	ATCCTGAGGGCACGAAGCAC GGATATG
NveCa _v 2b	TATGCTCAACTTAGTTCTTGG AGTCC	GACGACGATGGCATCGATGA TGTTCC
NveCa _v 2c	GGAACATCTTAGATTTTGCAG TCGTCC	GATTAAACTCAAGATTGAGC GCATGCT
NveCa _v 3a	GCTCCTATTGGACACTCTCCC TATGCT	CTCGACTAAGATAGCGACGA GCAGGT
NveCa _v 3b	ATGCTGGTCTTCACCATCATA TTCACA	GTACCGAAATCCCTGAAGTG GGCGTG

Table S2. Primers used to amplify Ca_v fragments for *Nematostella vectensis* in situ hybridization. Primers are shown in a 5' to 3' orientation.