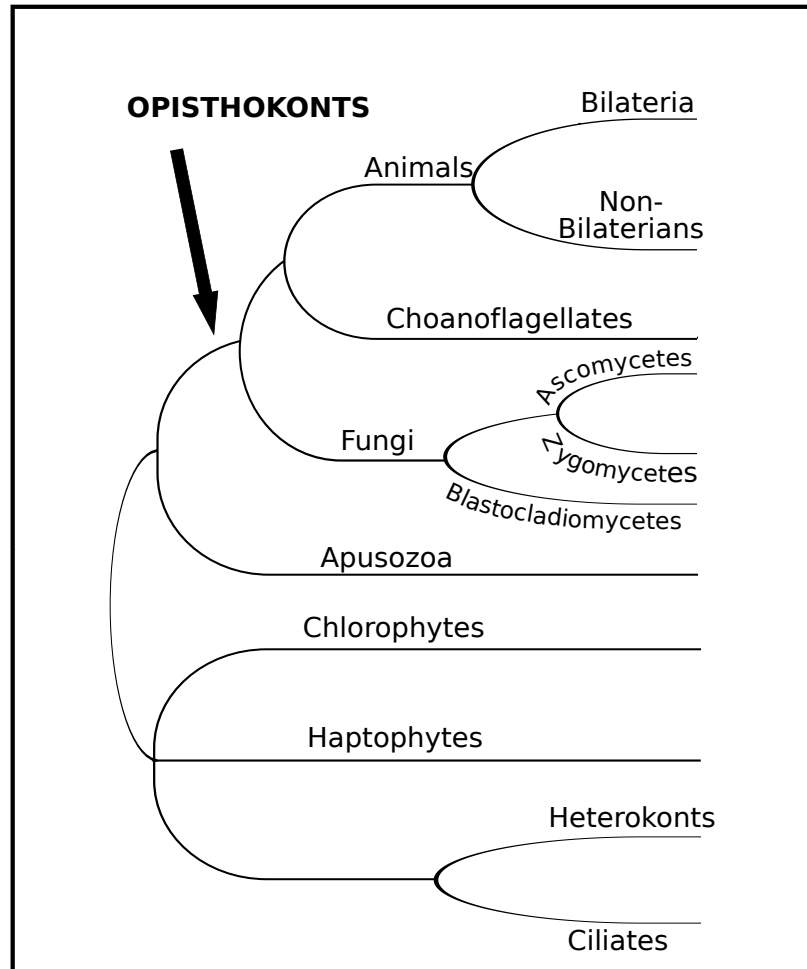


## Supplemental Figures



**Supplemental Figure 1.** Species tree of taxa used in this study, after (Parfrey et al. 2011) and (Porter et al. 2011). Soft polytomies added to convey grouping that are not agreed upon in the literature.

		D1	D2	
N A L C N	<i>Mus Na<sub>v</sub>1.4</i>	<b>R</b> TFRVLR <b>A</b> LKTIT	<b>R</b> SFRLLR <b>V</b> FKLAK	
	<i>Homo P/Q (Ca<sub>v</sub>2.1)</i>	<b>R</b> TLRAVR <b>V</b> LRPLK	<b>R</b> ALRLL <b>R</b> IFK <b>V</b> TK	
	<i>Homo</i>	<b>R</b> IPRPLIM <b>I</b> RAFR	TYFQ <b>V</b> LR <b>V</b> RLIK	
	<i>Caenorhabditis</i>	<b>R</b> SIRPFII <b>I</b> RLIP	TYFQ <b>T</b> FRL <b>L</b> RLIK	
	<i>Drosophila</i>	<b>R</b> APRPLIM <b>I</b> RFLR	TYFQ <b>V</b> LR <b>V</b> RLIK	
	<i>Nematostella</i>	<b>R</b> APRALIM <b>V</b> RVFK	AIFH <b>V</b> M <b>R</b> VLRLIG	
	<i>Trichoplax</i>	-----	TVFA <b>V</b> LR <b>I</b> L <b>R</b> IVR	
	<i>Amphimedon</i>	SVTSAA <b>K</b> LFIPLK	VVFQ <b>A</b> L <b>R</b> LPRLIR	
	F U N G I	<i>Saccharomyces</i>	<b>K</b> PLAIL <b>R</b> ILRLVN	SIFH <b>I</b> S <b>R</b> FYRVII
		<i>Aspergillus</i>	SML <b>S</b> CL <b>R</b> ILRLLN	TLFQ <b>I</b> L <b>R</b> VYRVVL
	<i>Phycomyces</i>	<b>K</b> MLSTL <b>I</b> LLRLLN	TGFQ <b>V</b> L <b>R</b> IYRVVV	
	<i>Mucor</i>	<b>K</b> MLSAL <b>I</b> LLRLLN	TGFQ <b>V</b> L <b>R</b> IYRLVV	
	<i>Allomyces</i>	<b>R</b> GMAAL <b>R</b> VFRLLS	TGFQ <b>L</b> ART <b>N</b> KLVT	
	<i>Thecamonas3</i>	<b>R</b> AF <b>R</b> AL <b>R</b> PMRALK	<b>R</b> VFR <b>V</b> L <b>R</b> ITRLLV	
		D3	D4	
N A L C N	<i>Mus Na<sub>v</sub>1.4</i>	<b>K</b> SLRTL <b>R</b> ALRPLR	<b>R</b> LARIG <b>R</b> VLRLIR	
	<i>Homo P/Q (Ca<sub>v</sub>2.1)</i>	<b>K</b> SLRVLR <b>V</b> LRPLK	<b>R</b> LFRAAR <b>L</b> IKLLR	
	<i>Homo</i>	--LM <b>V</b> L <b>R</b> CLRPLR	ACV <b>I</b> VF <b>R</b> FFSICG	
	<i>Caenorhabditis</i>	--LM <b>I</b> CRAMRPLR	YLV <b>V</b> IL <b>R</b> FFTIAS	
	<i>Drosophila</i>	--LM <b>I</b> LRCV <b>R</b> PLR	FM <b>V</b> IL <b>R</b> FFTITG	
	<i>Nematostella</i>	--LM <b>I</b> FRCLRPLR	V <b>V</b> IIIF <b>R</b> FLTLSG	
	<i>Trichoplax</i>	--LAV <b>L</b> RCLRPLR	-----	
	<i>Amphimedon</i>	IVLMG <b>V</b> RALRPLH	----I <b>L</b> K <b>C</b> L <b>K</b> AML	
	F U N G I	<i>Saccharomyces</i>	<b>R</b> IF <b>K</b> GLTALR <b>A</b> L <b>R</b>	GF <b>F</b> LL <b>V</b> IF <b>L</b> FIIP
		<i>Aspergillus</i>	<b>R</b> AIGAF <b>K</b> ALR <b>A</b> L <b>R</b>	<b>K</b> LFL <b>V</b> SITLLIIP
	<i>Phycomyces</i>	<b>R</b> VFRA <b>F</b> KALR <b>A</b> L <b>R</b>	<b>K</b> LFMTAL <b>C</b> F <b>K</b> LVQ	
	<i>Mucor</i>	<b>R</b> GFRA <b>F</b> KALR <b>A</b> L <b>R</b>	<b>K</b> LFMTAL <b>C</b> F <b>K</b> LVQ	
	<i>Allomyces</i>	G <b>V</b> LRL <b>M</b> RS <b>L</b> RPLR	<b>K</b> LVLIG <b>Y</b> AL <b>R</b> IAR	
	<i>Thecamonas3</i>	<b>R</b> LV <b>R</b> Y <b>F</b> RALRPLR	<b>R</b> FF <b>R</b> IAR <b>I</b> F <b>R</b> L <b>V</b> R	

**Supplemental Figure 2.** Alignment of the voltage sensing S4 segments from Na<sub>v</sub>, Ca<sub>v</sub>, NALCN, fungal calcium channels and an apusozoan outgroup (*Thecamonas 3*). D1-4 are the constituent domains and the voltage sensing residues, arginine (R) or lysine (K), are in bold. NALCN and fungal channels have reduced numbers of sensors relative to animal Na<sub>v</sub>, Ca<sub>v</sub>, and apusozoan channels



	<b>Pore State</b>	<b>Inactivation Particle</b>
<i>Mus</i> Na <sub>v</sub> 1.4	D/E/K/A	IFMTEEQ
<i>Homo</i> Na <sub>v</sub> 1.6	D/E/K/A	IFMTEEQ
<i>Drosophila</i> Na <sub>v</sub> 1	D/E/K/A	MFMTEDQ
<i>Micromonas</i>	E/E/E/E	LFVTEEQ *
<i>Chlamydomonas</i>	E/E/E/E	VFVTPQQ
<i>Volvox</i>	S/E/S/E	VDLTPAQ
<i>Ectocarpus</i>	E/E/E/E	VLVTEEQ *
<i>Phytophthora</i> 2	E/E/E/E	YLLSHIQ
<i>Thalassiosira</i>	E/E/E/E	GLMTQAAQ
<i>Paramecium</i>	E/E/E/E	FYMTLEQ
<i>Ostreococcus</i> 2	E/E/E/E	TIMSESQ
<i>Emiliana</i>	E/E/E/E	AMMTDAQ
<i>Ostreococcus</i> 1	E/E/D/E	KLLTERQ
<i>Phytophthora</i> 1	D/D/K/D	EILTESQ
<i>Homo</i> P/Q Ca <sub>v</sub>	E/E/E/E	YSLEKNE

**Supplemental Figure 4.** Pore motifs and inactivation particle motifs of non-opisthokont channels. Well characterized Na<sub>v</sub> channels from *Mus*, *Homo*, and *Drosophila* and *Homo* P/Q-type Ca<sub>v</sub> are given for comparison. Several non-opisthokont channels have Cav-like pore motifs and Nav-like motifs at the region that is putatively homologous to the Nav inactivation particle. The channels from the green alga *Micromonas* and the brown alga *Ectocarpus* (stars) are the most similar to bilaterian Nav channels in the inactivation particle region.