Supporting Information

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SI Materials and Methods

Gene Sequencing. There were two notable differences between the *Monosiga* reference sequence and our mRNA sequence (Fig. S1). First, the beginning of the ORF did not align with the reference. We took the first methionine to be the beginning of the ORF, but because we did not find any stop codons at the beginning of our sequence, we cannot exclude the possibility that there is an earlier methionine that we did not find, or that a later one begins the actual protein. Second, we found 125 bp of sequence that was not identified in our BLAST analysis. This sequence was in the Joint Genome Institute (JGI)'s *M. brevicollis* genome, but was classified as an intron. However, as we located this sequence in the mRNA, and because the translation remained in frame, we believe it is an exon that was misclassified by the BLAST analysis and is called the missing exon in this alignment. The rest of the alignment had very few differences, none of which threw the sequence out of frame.

Phylogenetic Methods. Besides the tree we report here, we also made maximum likelihood (ML) trees using Garli (1) with datasets that excluded the sponge and ctenophore sequences, which yielded completely consistent results. Analyses that further excluded two sodium channel genes (*Trichoplax* β and *Nematostella* β, which are relatively divergent), and a partially sequenced calcium channel (*Monosiga* Ca_v) using two different programs, Garli and SATe (2), yielded consistent results as well. We have used several different masking schemes throughout these analyses. These include masking with Guidance (3), as in the reported tree; removing the fast evolving cytoplasmic loops; and running the analyses without any masking. These different schemes can affect the exact placement of D/E/E/A channels within Bilateria and Cnidaria, indicating low phylogenetic signal in these channels, but does not affect the main conclusions of the paper. The *Monosiga* Na_v homolog always grouped with the Na_v family and ancestral state reconstruction was not affected.

Metazoan Phylogeny. A careful reanalysis of previous studies that had suggested nontraditional placements for basal groups finds that some of the inconsistencies regarding the basal placement of sponges can be resolved by removing genes of dubious orthology from alignments or increasing the taxon sampling (4). Doing so returns sponges to a basal position in the animal lineage and cnidarians to a sister group relation with bilaterians, a result which also agrees with the phylogenies in the *Trichoplax* and *Amphimedon* genome papers (5, 6). The revised phylogenies are not well supported, however, revealing low phylogenetic signal (4). The precise placement of ctenophores and placozoans is even less certain in these analyses, and one or the other group is left out of several studies (5–7). However, Philippe et al. (4) proposed that ctenophores should provisionally be placed in a group with cnidarians, with placozoans being sister to this group plus bilaterians. They call the group of animals with nervous systems "Eumetazoa," which contrasts with Srivastava et al. (6) who use this word to mean all animals with nervous systems plus *Trichoplax* (i.e., all animals except sponges). Knowing the precise placement of ctenophores and placozoans is critical because ctenophores have a fully developed nervous system, whereas *Trichoplax* has the simplest animal body plan. It is not possible to give a satisfying account of the evolution of the nervous system, or animal complexity in general, without knowing the phylogenetic positions of these groups.

PCR Primers. The following pairs of primers were used to amplify the *Monosiga brevicollis* gene segments during PCRs, with lower primers reported in their antisense form:

Segment 1 RT: 3′-GCGGAACCGGGGTCAAGGGC-5′. Primers: 5′-TTTTGTCGTCTTTATCATTTTTGGC-3′ and 3′-TCACTTTCTAGAAGATTGCACACGT-5′.
Segments 2 and 3 RT: 3′-GCGGAACCGGGGTCAAGGGC-5′.
Segment 2 primers: 5′-GAGTGGATTGAACTTCTATGGGAGA-3′ and 3′-TTGAGTCCGATACACCCTATGATAA-5′.
Segment 3 primers: 5′-ATGCATCCCTGCCCAAGCGCGC-3′, 3′-CTCGTTCAGTACAATGGGCGTAGA-5′.
Segment 4 RT: 3′-GATGATTTCAACGATGGACA-5′. Primers: 5′-TCGGAAGTTTGGTCAGACTGAGCCC-3′ and 3′-TCCACCCAGATGCAAAGTAGGAACG-5′.

The upper primer used in the third segment was designed from the putative beginning of the ORF, but actually bound further toward the 3′ end of the gene. It was later revealed that the part of the gene that this primer was designed from did not match the reference sequence, so the upper primer from the third segment should not be used.

The following primers were used to sequence ~1,000 bp from both *Trichoplax* genes:

Trichoplax α RT: 3′-CAACTAATGCTTCTAAAACG-5′. Primers: 5′-TTGGATCTTTTTTCTCATTAAACCT-3′ and 3′-CATGAAAATGCTGTCGCTGAGTTAT-5′.
Trichoplax β RT: 3′-GAGAGTAAAAAAGGTGCCAA-5′. Primers: 5′-ATCAGTCTTCAAGGCCACGACTTAC-3′ and 3′-TGCCATGTTAAGCCATTATCTAAAC-5′.

1. Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. PhD dissertation (University of Texas, Austin, TX).
2. Liu K, Raghavan S, Nelesen S, Linder CR, Warnow T (2009) Rapid and accurate large-scale coestimation of sequence alignments and phylogenetic trees. *Science* 324: 1561–1564.
3. Penn O, et al. (2010) Guidance: A web server for assessing alignment confidence scores. *Nucleic Acids Res* 38:W23–W28.
4. Philippe H, et al. (2011) Resolving difficult phylogenetic questions: Why more sequences are not enough. *PLoS Biol* 9:e1000602.
5. Srivastava M, et al. (2008) The Trichoplax genome and the nature of placozoans. *Nature* 454:955–960.
6. Srivastava M, et al. (2010) The *Amphimedon queenslandica* genome and the evolution of animal complexity. *Nature* 466:720–726.
7. Dunn CW, et al. (2008) Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* 452:745–749.

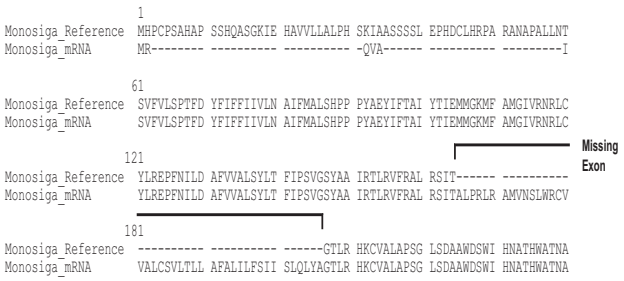

Fig. S1. Alignment of the beginning of the sequenced ORF from *Monosiga* with the reference obtained from JGI.

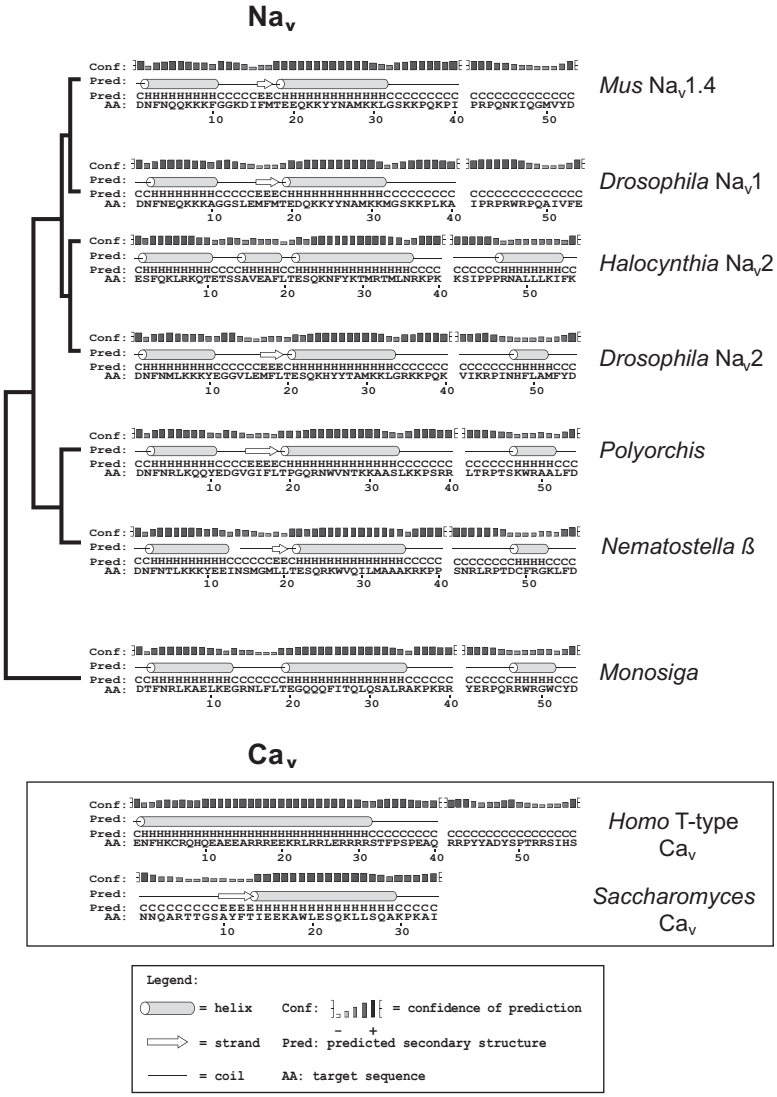

Fig. S2. Representative secondary structure predictions for the Na$_v$ inactivation gate mapped onto a simplified phylogeny. The two major helices are present in Na$_v$ genes but absent in the Ca$_v$ genes.

Table S1. Accession numbers for the genes used in the phylogenetic analysis

	Channel	Accession number/protein ID	Database
T type			
	Homo	O95180.4	Swiss-Prot
	Ciona	269719	JGI-Genome Portal
	Helobdella	66349	JGI-Genome Portal
	Drosophila	NM_001103419.1	RefSeq
	Strongylocentrotus	GLEAN3_25833	SpBase
	Lymnea	AAO83843.1	GenBank
	Caenorhabditis	WP:CE36117	WormBase
	Nematostella	170705	JGI-Genome Portal
	Trichoplax	21513	JGI-Genome Portal
N/P/Q			
	Homo P/Q	O00555.2	Swiss-Prot
	Homo N	O55017.1	Swiss-Prot
	Strongylocentrotus	GLEAN3_11692	SpBase
	Schistosoma	AAK84313.1	GenBank
	Caenorhabditis	WP:CE31225	WormBase
	Nematostella	59997	JGI-Genome Portal
	Trichoplax	53006	JGI-Genome Portal
	Mnemiopsis	JF905561	GenBank
L type			
	Oryctolagus	P15381.1	Swiss-Prot
	Ciona	239620	JGI-Genome Portal
	Helobdella	128998	JGI-Genome Portal
	Caenorhabditis	WP:CE31165	WormBase
	Drosophila	NM_080365.2	RefSeq
	Strongylocentrotus	GLEAN3_07770	SpBase
	Nematostella	88037	JGI-Genome Portal
	Stylophora	AAD11470.1	GenBank
	Cyanea	AAC63050.1	GenBank
	Trichoplax	18642	JGI-Genome Portal
Other			
	Monosiga	23875	JGI-Genome Portal
	Amphimedon	228755	Metazome
Na_v			
	Mus 1.4 Na_v1	NM_133199.2	RefSeq
	Xenopus 1.6 Na_v1	464193	JGI-Genome Portal
	Homo 1.6 Na_v1	NM_014191.2	RefSeq
	Ciona Na_v1	249763	JGI-Genome Portal
	Halocynthia Na_v1	662385	NCBI
	Lottia Na_v1	177540	JGI-Genome Portal
	Helobdella Na_v1	109965	JGI-Genome Portal
	Drosophila Na_v1	150421666	NCBI
	Daphnia Na_v1	50283	JGI-Genome Portal
	Halocynthia Na_v2	8096345	NCBI
	Ciona Na_v2	259743	JGI-Genome Portal
	Strongylocentrotus	GLEAN3_25997	SpBase
	Lottia Na_v2	161240	JGI-Genome Portal
	Daphnia Na_v2	40660	JGI-Genome Portal
	Drosophila Na_v2	166215092	NCBI
	Polyorchus	3005564	NCBI
	Cyanea	994814	NCBI
	Nematostella α	122010	JGI-Genome Portal
	Aiptasia	2791840	NCBI
	Nematostella β	88459	JGI-Genome Portal
	Trichoplax β	54699	JGI-Genome Portal
	Trichoplax α	23340	JGI-Genome Portal
	Mnemiopsis α	JF905562	GenBank
	Mnemiopsis β	JF905563	GenBank
	Monosiga	JF827087	GenBank
Fungi			
	Saccharomyces	1323391	NCBI
	Aspergillus	55835	JGI-Genome Portal