Supplementary Data

Functional conservation in human and *Drosophila* of Metazoan ADAR2 involved in RNA editing: loss of ADAR1 in insects.

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Supplementary Table. Sequence of oligonucleotides used for RT and sequencing .

Rdl	
657R	AGCTGATCGATAGCACATACATGG
1381F	GCTCGTTATGGTCTTTGGCAGTC
1993F	TTCCAGCCCATCTTCTGCATCCGG
1042F	TCGGTGTCTCGAGTGAGGTTTCGC
1560R	CGCCCACATTTGCATTGGGATTGGG
540R	CGTCACCCAGCATGCTGCCACCGCC
1680R	TGTGGGCGTGGTGTCCATGCCCGTG
489F	CGGATCCATACAGTGCAAGCGG
1715R	CACTGTTTGCTTGGGCGCATGCGG
939R	GTAGATTCATCGGACACGATGCGG
960R	GGCGATCCATGGGGAAATATTGTAG
961F	AGCTGTGCCACATTGAAATCGAAAGC
541F	TAAACATATCCGCTATTCTCGACTCC
Ca alpha 1D	
1774F	GCTGGCCAAAGAGACAACAACGCCGT
2255R	CTTTAAAATTGAATTCAGCACAACCTG
2306R	GCAATGTGAAACAGTGGCACCATGGC
eag	
2674R	GACGAAATTTGGAGAAGATCTTCCGG
1741F	CCGGGCCAGAATTGGTTAATGGGCCG
2340R	CCACAACCTCGTCGTCCTGTATCAC
2700R	CCTTACTGCCCGCTTGGACCTG
1768F	CACGGAAAAGCATGTACGTGACGG
2417F	GCGTGCTCTGACCTATTGTGATTTG
nic 34E	
4241R	CAATTAACCACCTCGCCCATATGG
3433F	CGGGTGAAAAATTATCACTGGG
3391F	CATGGCCTTGCTCGGATTCACTCTGCC
4372R	CTTGGATGTATTATTTAGTTGATGG
4218R	CTACGAGACAATAATATGTGGTGCTG
3589F	TCAACGATTTTAATATTAAATTAT
3981F	GGTCACCCATCATACGTGCATCAC
3735R	GGTCGGGAACTCCAGGATCAGC

Supplementary Table 2. Invertebrate and human ADARs accession numbers used for the alignments/phylogenetic analysis.

Common Name	Scientific name	(NCBI Accession Number) or {JGI Name or ID}
Acorn worm	*Saccoglossus kowalevskii*	ADAR1 (Translated from NW_003134359) ADAR2 (XP_002740631)
Amphipod	*Parhyale hawaiensis*	ADAR2 {Translated from AFHB17469.g2, AFHB25923.g2}
Anemone	*Nematostella vectensis*	ADAR1 (XP_001642062) ADAR2 (XP_001629615)
Beetle	*Tribolium castaneum*	ADAR2 (XP_969823)
Coral	*Acropora millepora*	ADAR2 (Translated from EZ011992)
Fly	*Drosophila melanogaster*	ADAR2 (AAF45665)
Human	*Homo sapiens*	ADAR1 (NP_001102) ADAR2 (NP_001103)
Hydrozoan	*Hydra magnipapillata*	ADAR (XP_002166223)
Lancelet	*Branchiostoma floridae*	ADAR1 (XP_002605887)[1] ADAR2 (XP_002595015)
Leech	*Helobdella robusta*	ADAR1 {193655} ADAR2 {167455}
Louse	*Pediculus humanus*	ADAR2 (XP_002428026)
Mite	*Varroa destructor*	ADAR1 (Translated from ADDG01088043) ADAR2 (Translated from ADDG01007263)
Nematode	*Caenorhabditis elegans*	Adr-1 (NP_492154) Adr-2 (NP_498594)
Octopus	*Octopus vulgaris*	ADAR1[2] ADAR2[3]
Polychaete	*Capitella teleta*	ADAR1 {183692} ADAR2 {JGI 176450}
Slug	*Aplysia californica*	ADAR1 (Translated from AASC02013163)
Limpet	*Lottia gigantea*	ADAR1 {133644} ADAR2 {128560}
Sponge	*Amphipedon queenslandica*	No ADAR/ADAT found
Squid	*Loligo opalescens, Loligo pealei*	ADAR1[2] ADAR2 (ACO52475)
Tick	*Ixodes scapularis*	ADAR1 (XP_002403965) ADAR2 (XP_002434473)
Trematode	*Schistosoma mansoni*	No ADAR found
Trichoplax	*Trichoplax adhaerens*	ADAT1 {55696}
Tunicate	*Ciona intestinalis*	ADAR1 (XP_002122509) ADAR2 (XP_002128212)
Urchin	*Strongylocentrotus purpuratus*	ADAR1 (XP_001183590) ADAR2 (XP_781832)
Water flea	*Daphnia pulex*	ADAR2 {Translated from 211108}

1. Predicted protein XP_002605887 had high similarity with ADAR1 consensus except for a poorly conserved region (residues 131-156). That specific region was blasted against *B. floridae* nucleotide database, and sequence ABEP02011549 appeared as a high score hit. Residues 131-266 were deleted from the predicted protein sequence and replaced by those translated from the nucleotide sequence. Residues 157-266 were >95% identical between both sequences, confirming that the replaced region is indeed homologous to ADAR1.

2. To be submitted to GenBank upon acceptance.

3. Kindly provided by L. Moroz (The Whitney Laboratory for Marine Bioscience, University of Florida) from an octopus EST library.

Supplementary Figure 1. *In vitro* poisoned primer extension assay of hADAR2 and hADAR (S/G).

A mutation was introduced in human ADAR2 to mimic the self-editing event found in exon 7 in *Drosophila* ADAR. A poisoned primer extension assay was performed with increasing amounts of purified recombinant proteins to measure RNA editing *in vitro* at the Q/R site in the *GluR2 B13* minigene transcript. The mutation in hADAR2 reduced editing activity eightfold which is the same reduction in activity that is observed with the *Drosophila* proteins.

Supplementary Figure 2. Alignment of invertebrate ADARs with human ADAR1 and ADAR2.

Only the core deaminase domain was used for the alignment. Positions that were defined as characteristic of vertebrate ADAR1 or ADAR2 by (1)are highlighted in red and blue, respectively. Residues the chelate zinc at the catalytic core are highlighted in black. All invertebrate ADAR sequences should be considered putative except for fruit fly and squid ADAR2.

Supplementary Figure 3. Phylogenetic tree of metazoan ADAR sequences.

Nodes include bootstrap values (n=1000). The tree was generated using the complete deaminase domain sequences and the *Trichoplax* ADAT sequence as an out group.

Reference

1. Keegan, L.P., Leroy, A., Sproul, D. and O'Connell, M.A. (2004) Adenosine deaminases acting on RNA (ADARs): RNA-editing enzymes. *Genome Biol*, **5**, 209.

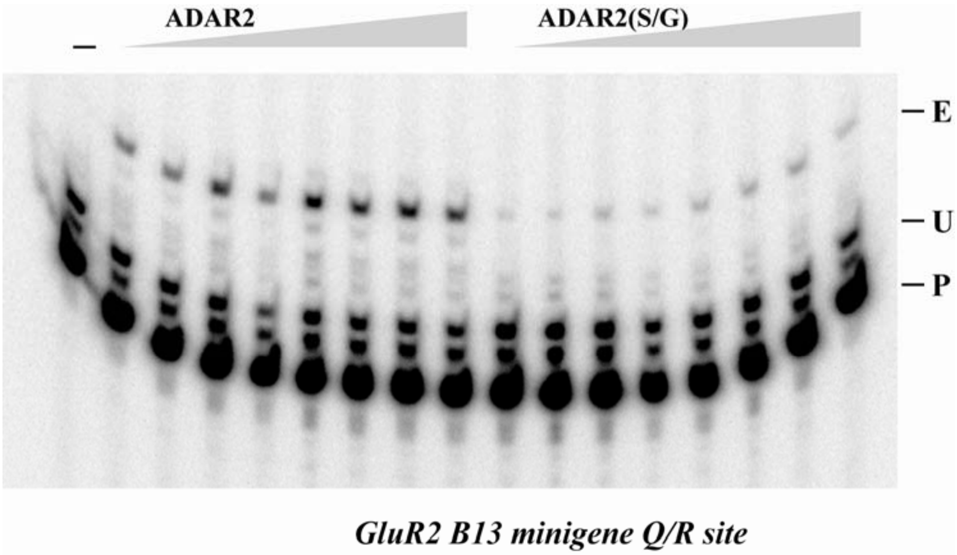


Figure S1

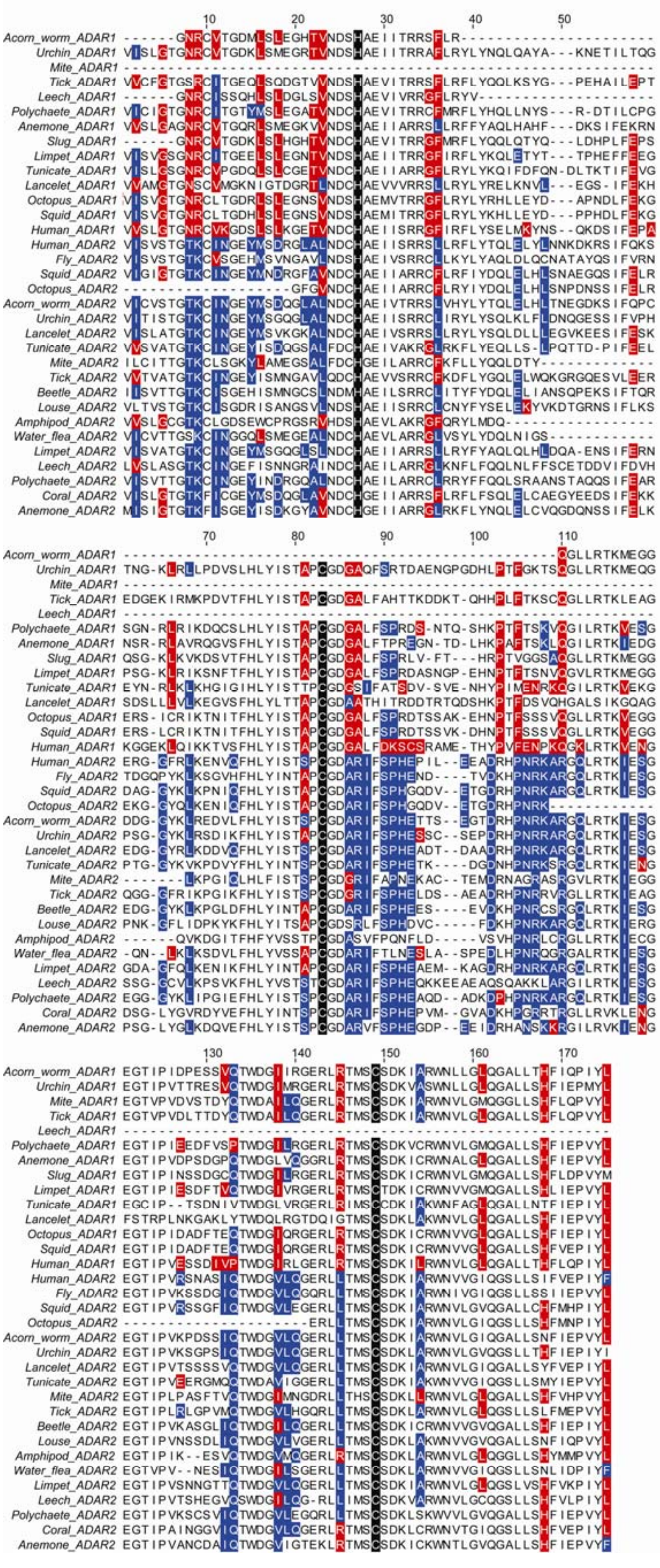
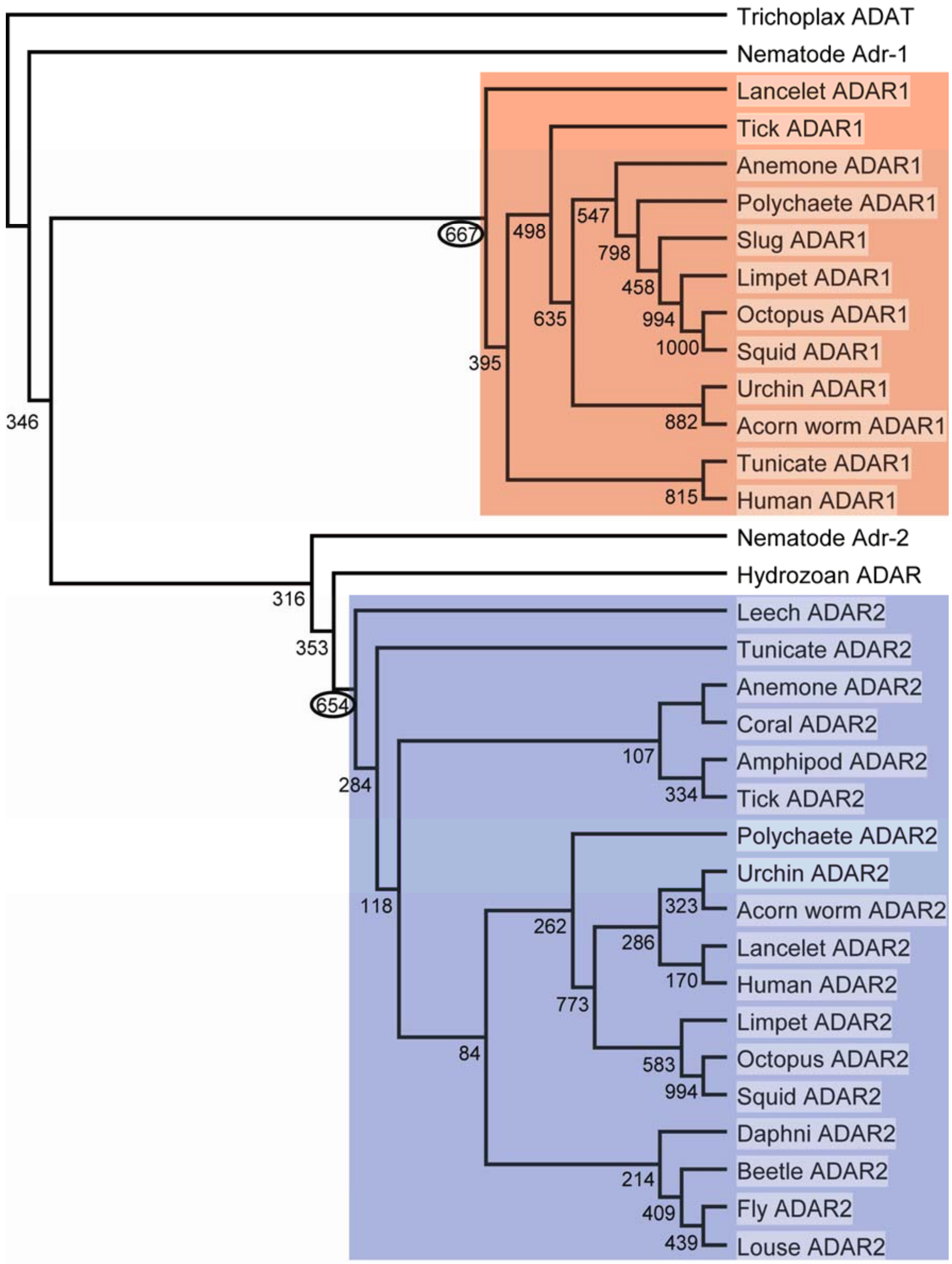

10
20
30
40
50
70
80
90
100
110
130
140
150
160
170
Acorn_worm_ADAR1
Urchin_ADAR1
Mite_ADAR1
Tick_ADAR1
Leech_ADAR1
Polychaete_ADAR1
Anemone_ADAR1
Slug_ADAR1
Limpet_ADAR1
Tunicate_ADAR1
Lancelet_ADAR1
Octopus_ADAR1
Squid_ADAR1
Human_ADAR1
Human_ADAR2
Fly_ADAR2
Squid_ADAR2
Octopus_ADAR2
Acorn_worm_ADAR2
Urchin_ADAR2
Lancelet_ADAR2
Tunicate_ADAR2
Mite_ADAR2
Tick_ADAR2
Beetle_ADAR2
Louse_ADAR2
Amphipod_ADAR2
Water_flea_ADAR2
Limpet_ADAR2
Leech_ADAR2
Polychaete_ADAR2
Coral_ADAR2
Anemone_ADAR2

Figure S2



Trichoplax ADAT
Nematode Adr-1
Lancelet ADAR1
Tick ADAR1
Anemone ADAR1
Polychaete ADAR1
Slug ADAR1
Limpet ADAR1
Octopus ADAR1
Squid ADAR1
Urchin ADAR1
Acorn worm ADAR1
Tunicate ADAR1
Human ADAR1
Nematode Adr-2
Hydrozoan ADAR
Leech ADAR2
Tunicate ADAR2
Anemone ADAR2
Coral ADAR2
Amphipod ADAR2
Tick ADAR2
Polychaete ADAR2
Urchin ADAR2
Acorn worm ADAR2
Lancelet ADAR2
Human ADAR2
Limpet ADAR2
Octopus ADAR2
Squid ADAR2
Daphni ADAR2
Beetle ADAR2
Fly ADAR2
Louse ADAR2
346
667
498
547
798
458
635
994
1000
395
882
815
316
353
654
284
107
334
118
262
323
286
170
773
583
994
84
214
409
439

Figure S3