Supplemental Materials

RNA interference can be used to disrupt gene function in tardigrades

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Online Resource 1: *D. melanogaster* and *C. elegans* homologs of *H. dujardini* predicted genes

***H.d.* Gene name**	**Homology[a]**				
	Gene[b]	**% identity**	**% similarity**	**# AA[c]**	**E value**
Hd-act-1	*Dm-Act5C*	99	100	196	3.6e-111
	Ce-act-1,2,3,4	98	100	196	1e-111
Hd-dfd-1	*Dm-Dfd*	49	52	109	7.7e-17
Hd-dlc-1	*Dm-Cdlc2*	97	99	89	1.5e-45
	Ce-dlc-1	92	97	87	3e-44
Hd-ftt-1	*Dm-14-3-3zeta*	87	96	143	9.7e-70
	Ce-par-5	82	88	140	5e-49
	Ce-ftt-2	87	92	141	6e-57
Hd-mag-1	*Dm-Mago*	80	89	147	1.5e-67
	Ce-mag-1	79	89	142	3e-64
Hd-myo-1	*Dm-Mhc*	65	82	156	1.1e-50
	Ce-unc-54	59	72	156	3e-29

[a] Table lists best *D. melanogaster* and *C. elegans* hits after BLAST with *H. dujardini* genes.
[b] Abbreviations: Ce = Caenorhabditis elegans; Dm = Drosophila melanogaster; Hd = Hypsibius dujardini
[c] AA = amino acids

Online Resource 2 *H. dujardini* sequence alignments. The predicted protein encoded by each of six *H. dujardini* (Hd) genes included in this study was aligned with their *C. elegans* (Ce) and *D. melanogaster* (Dm) homologs. Identical residues shared with *H. dujardini* sequences are highlighted in gray. Putative full-length sequences are noted. For Deformed/HoxB4, no *C. elegans* homolog was identified

Actin (partial sequence):

```
Hd-act-1        M-EDEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQ
Dm-Act5C        MCDEEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQ
Ce-act-1        MCDDEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEAQ
                * ::************************************************************

Hd-act-1        SKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM
Dm-Act5C        SKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM
Ce-act-1        SKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKM
                ************************************************************

Hd-act-1        TQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLD
Dm-Act5C        TQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRLD
Ce-act-1        TQIMFETFNTPAMYVAIQAVLSLYASGRTTGVVLDSGDGVTHTVPIYEGYALPHAILRLD
                ********************************:********:*******************

Hd-act-1        LAGRDLTDYLMKILTERG
Dm-Act5C        LAGRDLTDYLMKILTERG
Ce-act-1        LAGRDLTDYLMKILTERG
                ******************
```

Cytoplasmic dynein light chain (predicted complete sequence):

```
Hd-dlc-1        MSERKAVIKNADMSEEMQQDAVDIATQALEKYNIEKDIAAFIKKEFDKKYNPTWHCIVGR
Dm-Cdlc2        MSDRKAVIKNADMSEEMQQDAVDCATQALEKYNIEKDIAAFIKKEFDKKYNPTWHCIVGR
Ce-dlc-1        MVDRKAVIKNADMSDDMQQDAIDCATQALEKYNIEKDIAAYIKKEFDKKYNPTWHCIVGR
                * :***********::*****:* ****************:*******************

Hd-dlc-1        NFGSYVTHETKHFIYFYLGQVAILLFKSG•
Dm-Cdlc2        NFGSYVTHETRHFIYFYLGQVAILLFKSG•
Ce-dlc-1        NFGSYVTHETKHFIYFYLGQVAILLFKSG•
                **********:*******************
```

14-3-3 protein (partial sequence):

```
Hd-ftt-1            ---MEKDELVQRAKLAEQAERYDDMATAMKNVTELGVELSNEERNLLSVAYKNVVGARRS
Dm-14-3-3zeta       MSTVDKEELVQKAKLAEQSERYDDMAQAMKSVTETGVELSNEERNLLSVAYKNVVGARRS
Ce-ftt-2            -MSDGKEELVNRAKLAEQAERYDDMAASMKKVTELGAELSNEERNLLSVAYKNVVGARRS
Ce-par-5            -MSDTVEELVQRAKLAEQAERYDDMAAAMKKVTEQGQELSNEERNLLSVAYKNVVGARRS
                          :***::******:******* :**.*** * **********************

Hd-ftt-1            SWRVISSIEQKTEGSEKKQQMAKEYREKVEKELREICNDVLGLLDKYLIPKASNPESKVF
Dm-14-3-3zeta       SWRVISSIEQKTEASARKQQLAREYRERVEKELREICYEVLGLLDKYLIPKASNPESKVF
Ce-ftt-2            SWRVISSIEQKTEGSEKKQQMAKEYREKVEKELRDICQDVLNLLDKFLIPKAGAAESKVF
Ce-par-5            SWRVISSIEQKTEGSEKKQQLAKEYRVKVEQELNDICQDVLKLLDEFLIVKAGAAESKVF
                    *************.* :***:*:*** :**:**.:** :** ***::** **. .*****

Hd-ftt-1            YLKMKGDYYRYLAEVATGDTRNTIVET
Dm-14-3-3zeta       YLKMKGDYYRYLAEVATGDARNTVVDD
Ce-ftt-2            YLKMKGDYYRYLAEVASGDDRNSVVEK
Ce-par-5            YLKMKGDYYRYLAEVAS-EDRAAVVEK
                    ****************: : * ::*:
```

Deformed/HoxB4 (partial sequence):

```
Hd-dfd-1        VIYPWMKKAYTGIHHGEAGYRNGQSTGTYNECGSPNGSVNGSGNGLVNSTCGSMGPGHST
Dm-Dfd          IIYPWMKKIHV-----------------------------------------AGVANGS
                :******* :.                                          * .:.:

Hd-dfd-1        ASHLQETKRQRTAYTRHQILELEKEFYYNRYLTRRRRIEIAHVLQLTER
Dm-Dfd          YQPGMEPKRQRTAYTRHQILELEKEFHYNRYLTRRRRIEIAHTLVLSER
                 .   *.******************:***************.* *:**
```

Mago nashi (predicted complete sequence):

```
Hd-mag-1        -----MTEDRFYVRYYVGHKGKFGHEFLEFEFRPDGRLRYANNSNYKNDTM
Dm-mago         -----MSTEDFYLRYYVGHKGKFGHEFLEFEFRPDGKLRYANNSNYKNDTM
Ce-mag-1        MSGEEEKAADFYVRYYVGHKGKFGHEFLEFEFRPNGSLRYANNSNYKNDTM
                         **:********************:* **************

Hd-mag-1        IRKEVVVHPAILEEVKRIIQDSEILRENDAKWPQPDRVGRQELEILLDDEH
Dm-mago         IRKEAFVHQSVMEELKRIIIDSEIMQEDDLPWPPPDRVGRQELEIVIGDEH
Ce-mag-1        IRKEATVSESVLSELKRIIEDSEIMQEDDDNWPEPDKIGRQELEILYKNEH
                ****. *  ::::.*:**** ****::*:*  ** **::*******:  :**

Hd-mag-1        ISFNTGKIGSLMDVNNSPDPEGLRCFYYLVQDLKCLVFSLIALHFKIKPI•
Dm-mago         ISFTTSKTGSLVDVNRSKDPEGLRCFYYLVQDLKCLVFSLIGLHFKIKPI•
Ce-mag-1        ISFTTGKIGALADVNNSKDPDGLRSFYYLVQDLKCLVFSLIGLHFKIKPI•
                ***.*.* *:* ***.* **:***.***************.*********
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Myosin heavy chain (partial sequence):

```
Hd-myo-1        EKEEEFETTRKNHQRSLDSMQATLEAESRGRAEALRMKKKLESDINELEIALDHANKANA
Dm-Mhc          EKEEEFENTRKNHQRALDSMQASLEAEAKGKAEALRMKKKLEADINELEIALDHANKANA
Ce-unc-54       EKEEEFENTRKNHARALESMQASLETEAKGKAELLRIKKKLEGDINELEIALDHANKANA
                *******.***** *:*:****:**:*::*:** **:*****.****************

Hd-myo-1        EAQKNVKKYQEQIKTLQTQVEEEQRARDDAREQMNSAERRANSLQSEVDELRNTLEQAER
Dm-Mhc          EAQKNIKRYQQQLKDIQTALEEEQRARDDAREQLGISERRANALQNELEESRTLLEQADR
Ce-unc-54       DAQKNLKRYQEQVRELQLQVEEEQRNGADTREQFFNAEKRATLLQSEKEELLVANEAAER
                :****:*:**:*:: :*  :*****   *:***:  :*:**. **.* :*     * *:*

Hd-myo-1        LRKQAENELNDANERLAELTATNTSLSGAKRKMEAE
Dm-Mhc          GRRQAEQELADAHEQLNEVSAQNASISAAKRKLESE
Ce-unc-54       ARKQAEYEAADARDQANEANAQVSSLTSAKRKLEGE
                 *:*** *  **.::  * .*  :*::.****:*.*
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Online Resource 3: *H. dujardini* primer sequences designed for this study

Gene name	Sequence	Template	Product size (bp)
gfp	5′-GAGTGAGCTGATACCGC-3′ 5′-GTGCTGCAAGGCGATTAAGTTG-3′	plasmid pJT06	897
Hd-act-1	First round: 5′-GTTTCGCGTAATCCCTCAGAAC-3′ 5′-AGCGTTGTGACGAAGCTGTA-3′ Second round: 5′-GATAATACGACTCACTATAGGGCCTCAGAACAGTCGCAATGG-3′ 5′-GATAATACGACTCACTATAGGGCCAGAGTCCAGCACGATAC-3′	gDNA cDNA gDNA cDNA	1070 640 957 527
Hd-dfd-1	First round: 5′-GATTCAGCCTCCTCACAACAAG-3′ 5′-GCGAGTGAGGTAGCGATTGTAA-3′ Second round: 5′-GATAATACGACTCACTATAGGGGATTCAGCCTCCTCACAACAAG-3′ 5′-GATAATACGACTCACTATAGGGTTCGAGAATTTGATGACGAGTG-3′	gDNA	1063 1107
Hd-dlc-1	First round: 5′-TCTCCCAGCCCACTTTACATTC-3′ 5′-TGGGACAGAATACGCCGTTC-3′ Second round: 5′-GATAATACGACTCACTATAGGGGGACAGAATACGCCGTTC-3′ 5′-GATAATACGACTCACTATAGGGCCAGCCCACTTTACATTC-3′	gDNA cDNA gDNA cDNA	1025 628 1099 668

Gene name	Sequence	Template	Product size (bp)
Hd-ftt-1	First round: 5′-CTGACTATCACCAGTGGAGGAG-3′ 5′-CTGCATCTCGCTCTTGGCAATG-3′ Second round: 5′-GATAATACGACTCACTATAGGGCCCGTGTGGAATTGTGTCTTC-3′ 5′-GATAATACGACTCACTATAGGGGGTTTCCACGATGGTGTTGCG-3′	cDNA	619 590
Hd-mag-1 (FL)	5′-CGTTTCTACGTGCGATACTAC-3′ 5′-GAGGGTGCTAGTGCAATCTTC-3′	cDNA	480
Hd-mag-1 (399bp)	5′-GTGCGATACTACGTCGGCC-3′ 5′-CAGCGCAATGAGGGAGAAG-3′	*Hd-mag-1(FL)*	399
Hd-mag-1 (5'L)	5′-GATAATACGACTCACTATAGGGCGTGCGATACTACGTCGGC-3′ 5′-GATAATACGACTCACTATAGGGCAGAATCTCTAACTCCTGC-3′	*Hd-mag-1(FL)*	300
Hd-mag-1 (5'S)	5′-GATAATACGACTCACTATAGGGCCATAAAGGCAAGTTCG-3′ 5′-GATAATACGACTCACTATAGGGGCTGCGGCCACTTG-3′	*Hd-mag-1(FL)*	246
Hd-mag-1 (3'L)	5′-GATAATACGACTCACTATAGGGGAGAACGACGCCAAGTGGC-3′ 5′-GATAATACGACTCACTATAGGGCAGCGCAATGAGGGAGAAG-3′	*Hd-mag-1(FL)*	248
Hd-mag-1 (3'S)	5′-GATAATACGACTCACTATAGGGCAGGAGTTAGAGATTCTG-3′ 5′-GATAATACGACTCACTATAGGGCGCTTGATTGGTTGATTAC-3′	*Hd-mag-1(FL)*	255

Gene name	Sequence	Template	Product size (bp)
Hd-myo-1	First round: 5′-GGAGAAGGAAGAGGAGTTCG-3′ 5′-GTTCGGCCTCCATCTTTCGC-3′ Second round: 5′-GATAATACGACTCACTATAGGGGAAGAGGAGTTCGAGACCAC-3′ 5′-GATAATACGACTCACTATAGGGGCCTCCATCTTTCGCTTAGC-3′	cDNA	470 502
Hd-tbb-1	5′-CCAACTGAACGCCGATCTCCGCAAG-3′ 5′-GGTACTCCGAGATGAGATCG-3′	cDNA	535

Online Resource 4 Characterization of full-length gene sequences. Genomic DNA and cDNA were amplified for **a** *Hd-dlc-1/dynein light chain* and **b** *Hd-mag-1/mago nashi*, and sequences were aligned by ClustalW. The predicted protein sequence is shown below the DNA sequence. Exons are shown in capital letters and introns are in lower-case letters.

a *Hd-dlc-1/dynein light chain*

```
dlc(gDNA)      ATGTCGGAACGAAAGGCCGTTATTAAAAACGCAGACATGAGCGAGGAGATGCAGCAGGAT
dlc(cDNA)      ATGTCGGAACGAAAGGCCGTTATTAAAAACGCAGACATGAGCGAGGAGATGCAGCAGGAT
            1   M  S  E  R  K  A  V  I  K  N  A  D  M  S  E  E  M  Q  Q  D

dlc(gDNA)      GCTGTTGATATCGCGACGCAAGCCCTTGAGAAATATAACATCGAGAAGGATATTGCGGCC
dlc(cDNA)      GCTGTTGATATCGCGACGCAAGCCCTTGAGAAATATAACATCGAGAAGGATATTGCGGCC
           21   A  V  D  I  A  T  Q  A  L  E  K  Y  N  I  E  K  D  I  A  A

dlc(gDNA)      TTCATCAAGAAGGAGTTCGACAAAAAGTACAATCCTACTTGGCATTGTATCGTGGGCCGA
dlc(cDNA)      TTCATCAAGAAGGAGTTCGACAAAAAGTACAATCCTACTTGGCATTGTATCGTGGGCCGA
           41   F  I  K  K  E  F  D  K  K  Y  N  P  T  W  H  C  I  V  G  R

dlc(gDNA)      AACTTTGGCAGCTATGtaagttgattaatgcattcggacagggaccggctgatttattgc
dlc(cDNA)      AACTTTGGCAGCTATG-------------------------------------------
           61   N  F  G  S  Y

dlc(gDNA)      cggtgtgcggtctcccgaccagttgcttaagccgtactcggtgggctaagccggtctgct
dlc(cDNA)      ------------------------------------------------------------

dlc(gDNA)      ggaccaatcatgagcctgtttttaatcgacgaatgaaatatcaaatcaagtattgcaatc
dlc(cDNA)      ------------------------------------------------------------

dlc(gDNA)      aatcaatcggtcaatcaaaattttcagtgcacaatcgatgaaaacaaatcactcttccaa
dlc(cDNA)      ------------------------------------------------------------

dlc(gDNA)      atacggtttttcatttcgatttttttgctgatttcaggTGACTCACGAGACCAAACACTT
dlc(cDNA)      -------------------------------------TGACTCACGAGACCAAACACTT
           66                                         V  T  H  E  T  K  H  F

dlc(gDNA)      CATATACTTCTATCTCGGGCAAGTTGCGATCCTGTTGTTCAAGTCTGGATGAGCAGAGAC
dlc(cDNA)      CATATACTTCTATCTCGGGCAAGTTGCGATCCTGTTGTTCAAATCTGGATGAGCAAAGAC
           74   I  Y  F  Y  L  G  Q  V  A  I  L  L  F  K  S  G  *
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b *Hd-mag-1/mago nashi*

```
mago(gDNA)      ATGACGGAGGACCGTTTCTACGTGCGATACTACGTCGGCCATAAAGGCAAGTTCGGGCAT
mago(cDNA)      ATGACGGAGGACCGTTTCTACGTGCGATACTACGTCGGCCATAAAGGCAAGTTCGGGCAT
            1    M  T  E  D  R  F  Y  V  R  Y  Y  V  G  H  K  G  K  F  G  H

mago(gDNA)      GAATTTTTGGAATTCGAATTTCGCCCGGACGGTCGCCTGCGTTACGCCAACAATTCCAAC
mago(cDNA)      GAATTTTTGGAATTCGAATTTCGCCCGGACGGTCGCCTGCGTTACGCCAACAATTCCAAC
            21   E  F  L  E  F  E  F  R  P  D  G  R  L  R  Y  A  N  N  S  N

mago(gDNA)      TACAAGAACGACACGATGATCCGCAAGGAGGTGGTCGTCCATCCGGCTATCTTGGAAGAG
mago(cDNA)      TACAAGAACGACACGATGATCCGCAAGGAGGTGGTCGTCCATCCGGCTATCTTGGAAGAG
            41   Y  K  N  D  T  M  I  R  K  E  V  V  V  H  P  A  I  L  E  E

mago(gDNA)      GTGAAGCGCATCATTCAGGACAGTGAGATTCTCCGGGAGAACGACGCCAAGTGGCCGCAG
mago(cDNA)      GTGAAGCGCATCATTCAGGACAGTGAGATTCTCCGGGAGAACGACGCCAAGTGGCCGCAG
            61   V  K  R  I  I  Q  D  S  E  I  L  R  E  N  D  A  K  W  P  Q

mago(gDNA)      CCGGACCGCGTGGGCCGGCAGGAGTTAGAGATTCTGCTGGACGACGAGCACATCTCCTTC
mago(cDNA)      CCGGACCGCGTGGGCCGGCAGGAGTTAGAGATTCTGCTGGACGACGAGCACATCTCCTTC
            81   P  D  R  V  G  R  Q  E  L  E  I  L  L  D  D  E  H  I  S  F

mago(gDNA)      AACACGGGAAAGATCGGCTCGCTGATGGACGTCAACAACAGCCCCGATCCAGAAGGTCTG
mago(cDNA)      AACACGGGAAAGATCGGCTCGCTGATGGACGTCAACAACAGCCCCGATCCAGAAGGTCTG
            101  N  T  G  K  I  G  S  L  M  D  V  N  N  S  P  D  P  E  G  L

mago(gDNA)      CGTTGCTTCTACTACCTCGTGCAGGATCTCAAGTGTCTCGTCTTCTCCCTCATTGCGCTG
mago(cDNA)      CGTTGCTTCTACTACCTCGTGCAGGATCTCAAGTGTCTCGTCTTCTCCCTCATTGCGCTG
            121  R  C  F  Y  Y  L  V  Q  D  L  K  C  L  V  F  S  L  I  A  L

mago(gDNA)      CACTTTAAAATCAAGCCGATTTGAACGCTTGTAATCAACCAATCAAGCGCTAAGATTTGC
mago(cDNA)      CACTTTAAAATCAAGCCGATTTGAACGCTTGTAATCAACCAATCAAGCGCGAAGATTTGC
            141  H  F  K  I  K  P  I  *
```

Online Resource 5: Summary of RNAi experiments

A. All progeny

Treatment	# adults injected	# broods	# embryos	% lethality	Avg. # broods (± SD)[a]
uninjected	16	47	98	0.0	2.9 ±2.3
water	38	128	213	1.9	3.4 ±2.1
gfp	22	51	63	1.6	2.3 ±1.2
Hd-act-1	14	14	22	86.4	1.0 ±0.0
Hd-dfd-1	12	13	20	0.0	1.9 ±0.9
Hd-dlc-1	16	8	13	23.1	1.3 ±0.5
Hd-ftt-1	28	39	43	25.6	1.4 ±0.9
Hd-mag-1(399bp)	24	117	207	16.4	4.9 ±2.1
Hd-mag-1(5'L)	20	43	59	25.4	2.7 ±0.6
Hd-mag-1(5'S)	20	36	94	27.7	2.6 ±0.3
Hd-mag-1(3'L)	20	41	65	15.4	3.2 ±0.2
Hd-mag-1(3'S)	56	164	280	15.0	3.2 ±0.6
Hd-myo-1	16	40	60	16.7	2.5 ±1.6

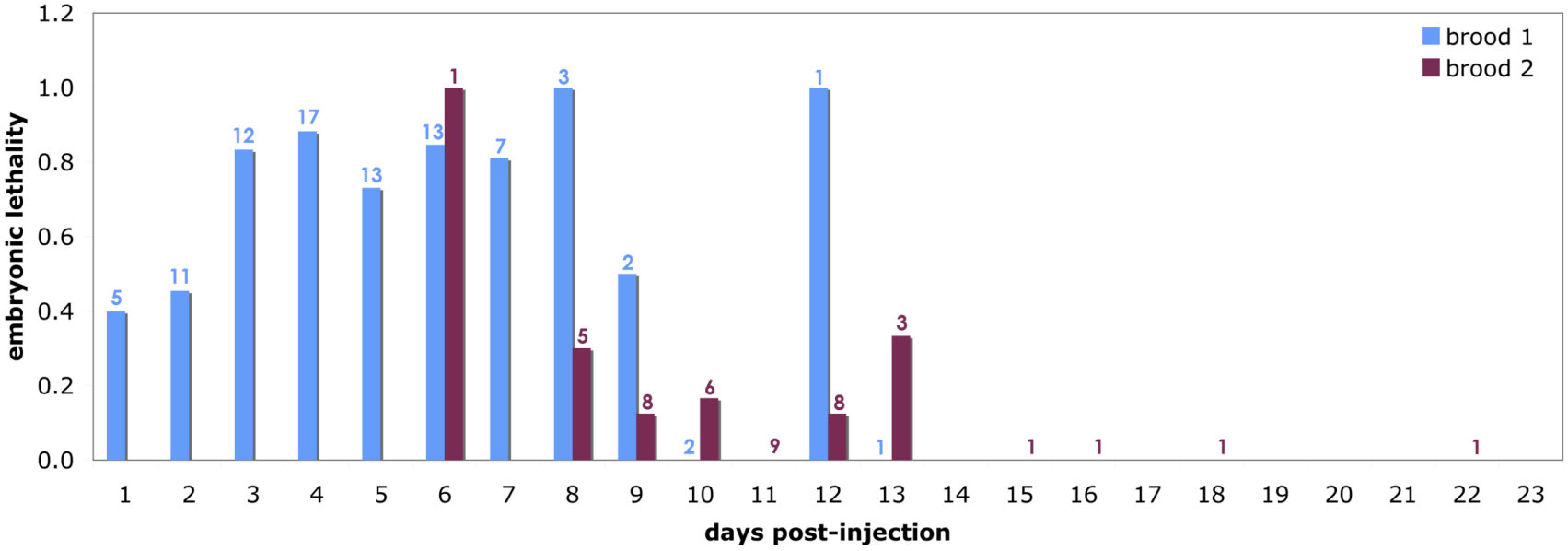
[a] SD – standard deviation

B. First brood only

Treatment	# embryos	% lethality	Phenotypes observed[b]
uninjected	19	0.0	NA[c]
water	56	3.6	ND[d]
gfp	32	0.0	NA
Hd-act-1	22	86.4	Embryos arrest early, with multi-nucleated cells (12/24) Multicellular embryos that arrest prior to elongation and morphogenesis (7/24)
Hd-dfd-1	11	0.0	NA
Hd-dlc-1	11	27.3	Embryos initiate morphogenesis but fail to hatch; partially developed pharynx, intestine that lacks gut granules (3/3)
Hd-ftt-1	26	30.8	Multiple phenotypes observed
Hd-mag-1(399bp)	39	59.0	Failure to elongate along A/P axis (17/23)
Hd-mag-1(5'L)	15	73.3	Failure to elongate along A/P axis (6/10)
Hd-mag-1(5'S)	28	53.6	Failure to elongate along A/P axis (4/6)
Hd-mag-1(3'L)	19	47.4	Failure to elongate along A/P axis (6/8)
Hd-mag-1(3'S)	69	49.9	ND
Hd-myo-1	20	40.0	Multiple phenotypes observed

[b] Numbers in parentheses indicate number of embryos with each phenotype, out of total number of embryos examined
[c] NA – not applicable
[d] ND – not determined

Online Resource 6 Penetrance of *Hd-mag-1(RNAi)* is time-sensitive. For all progeny of *Hd-mag-1* dsRNA-injected females laid on the indicated day post-injection, the rate of embryonic lethality was calculated as the fraction of embryos that failed to hatch (a rate of 1 indicates that all embryos laid failed to hatch). Progeny laid during the first brood or second brood are distinguished from each other by colored bars (blue and purple, respectively). Numbers above each bar indicate the total number of embryos examined for each time point.



Online Resource 7 Time-lapse video recording of the first 12 hours of embryogenesis in untreated (left) and *actin(RNAi)* (right) embryos.