

Supporting Information

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A

PhUbx-I

CTTTAGTGGAGTGTGAGAAGAGTGAGGGTGTGGGAAAGATTCTGCTGCCGTTTGTGTGAAACTTGC
TAAGCCTGTGCATCTTGTATCGGTGTTTGAAGCAAACGCTTAAGAATCACAGCGGTTTAATAACGTA
CGCCGAGCTGCTCTTCTGCTGCAAACGAAGGCGAAAGAACGTTAAACGTTATGAGCTCATCTCGAC
M S S S S T

GATGGACAATTTTCGAGCCCTTCTCAGGCGGGTTTTACGGAGGCGGTTTCAGGAGGAGATCAAGCTTAT
M D N F R A L L Q G G F Y G G G S G G D Q A Y

CGTTCCCTCCACTTGGACTCAGCGTTGGGCACTACGGGCAGCCTGCACCACGACAAGATGCTTATGACC
R F P P L G L S V G H Y G Q P A P R Q D A Y D

CCAGTGCTTATGTAACCTTTACCCGACCCACAAGACCACATGACTCCAAAATCTTTCAAAGTAGACTG
P S A S C K L Y P T H Q D H M T P N P F K V D C

CAGTGGCGGCAAGACCAGAATGGATACGGTGCCAAAGATATGTCGGTTGGATGGGGCCAGTCAGCACGG
S G G K D Q N G Y G A K D M S V G W G Q S A R

CCTGTCTGCACTCCGGATCCAATTTCTGGTCGAGGGTATCCTCCAGACCCTAGTACGTCACCCAGAGATA
P V C T P D P I S G R G Y P P D P S T S P R D

GGGCCATGCCGGGCTGGATGGAATACATGCGGTATGACAGCCGTCACCAACAACACCACCAACA
R A H A G A G W N T C G M T A V Q H Q Q H H Q Q

ACAACCACAACAAATCCTGGACAAATGGGACAGGGACAAAATACCACAACCTTTCTATCCGTGGATGGCA
Q P Q Q N P G Q M G Q G Q N T T T F Y P W M A

TTAGCAGGTGCAAAATGGATTACGGCGCAGGAGCGCCAAACGTATACTCGATATCAGACTTTGGAATTGG
L A G A N G L R R R G R Q T Y T R Y Q T L E L

AGAAAGAATTCATACAAATCACTACTTGACCCGTAGGAGAAGAATAGAAATGGCGCACGCTCTTTGTCT
E K E F H T N H Y L T R R R R I E M A H A L C L

CACTGAGCGACAAATCAAAATCTGGTTCGAGAACAGGCGGATGAAGCTGAAGAAGGAAATCAAGCAATC
T E R Q I K I W F Q N R R M K L K K E I Q A I

AAAGAATTGAATGAACAGGAAAAGGTAGCTCAAAATCAGAAATTAATGCAGCAACAGCAACAGCAGCAGC
K E L N E Q E K V A Q N Q K L M Q Q Q Q Q Q Q

CGCAGCAGCTGCACCCCAACAGACGCAACAGACTCCCACAGTTCGGCCCAACATGACACCAAGTCAAGC
P Q Q P A P Q Q T Q Q T P T V P P N M T P S Q A

TGGTGGTGGAGTCCAGACCATACGGGGCAAAACCCCGGACAAAACCTACTCTTCGGCTCATCTTTAAG
G G G V P D H T G Q N P G Q N *

TTAGAATAAGGTATGTTGGCTGTATCTAAGGCTCGATTCCATAGTTTCGGAGTTTGTGCGAGTGAAGTATGATATTA
TATAACAGTGGCTGTGAATCTACGTATGTCTGTTAATAACAACAAAATATGTTACCTCGGAACATATGGGAA

Fig. S1. *PhUbx* sequence and alignments with Ubx proteins from other species. (A) cDNA sequences for all of *PhUbx-I* and for the region unique to *PhUbx-II*; both transcripts are identical following a shared splice junction ("AGG" in blue). The 2 have unique translation start sites ("ATG" in green), but share the same stop codon ("TAA" in red); corresponding amino acids are displayed immediately underneath the nucleotide sequence. Nucleotides that encode the homeodomain are underlined and sequences corresponding to the 3 *PhUbx* siRNA oligos are highlighted in yellow. (B) N-termini of *Parhyale* and *Drosophila* Ubx proteins. Amino acids conserved across species are highlighted in black. Sequences shared by both *PhUbx-I* and *PhUbx-II* are in red. (C) Alignment of the hexapeptide, homeodomain and UBDA domains of Ubx proteins from *Parhyale hawaiiensis* (*Ph*), *Porcellio scaber* (*Ps*), *Artemia franciscana* (*Af*), *Strigamia maritima* (*Sm*), and *Drosophila melanogaster* (*Dm*), along with homologous regions of *D. melanogaster* Antennapedia (*Dm Antp*) and abdominal-A (*Dm abd-A*) proteins. The conserved hexapeptide domain is marked with asterisks and the homeodomain is underlined; the UBDA domain immediately follows the homeodomain. Gaps in the alignment are represented by hyphens. Amino acid residues that are identical to *PhUbx* are shown in black; those that are not identical are in red. (D) Alignment of the C-terminal-most regions of Ubx proteins from *P. hawaiiensis* (*Ph*), *A. franciscana* (*Af*), *D. melanogaster* (*Dm*) and the Onychophoran species *Akanthokara kaputensis* (*Ak*). C-termini are denoted by asterisks and sequences that match the consensus CK2 phosphorylation site (S/T-X-X-D/E) are underlined.

D

Ph Ubx AIKELNEQEKVAQNOKLMQOQQQQQQPQQPAPQQTQQTPTVPPNMTPSQAGGGVPDHTGQNPQN*
Af Ubx AIKELNEQDKRITPSKLSNCSSPTGDISDDEKDEKL*
Dm Ubx AIKELNEQEKQAQAQKAAAAAAAAAAVQGGHLDQ*
Ak Ubx TIKDLNEQEKKQRDTSLTV*

Fig. 51. Continued.

A

PhDII-e

TTTTGTCAGGGATCTGCCATTAACATTCTAAGAGGAGCCTAATTGATGTTGATAGGGAATGGTGAAGGAAGAGCGTT
GCTCATAGTGCAAGTTGCCGAGGAAGATGCTAGTGCTGGCGCAGTAGCTTCTCTGCCTGGCGCTCTCAAACCTTTGCA
CACACGCGTCCAATTTTGCCGACTTGCTCAGTGAAGTTAGCCTCTGATTCTTATAAGTGTACTGGCGCAGTCTCT
CAAACCGTCCGGACTCTTTGCTGCCAGTGTGTGTGTGATACAATTGTTGCTGTGCGCGAGTTATTCATCTCCGGAAT
CCTTGTGGTTTTAGCAACAGTGAAGTGATTTGCAAAATTAATTCAAATGCTTGACCAAGAGTTGTTACCAAAGAA

M L D Q E L L P K N

CGGCTTGGGTGATGTCCAACAGCCACCGCCTCACCTCGCCTCCTATTCACAGTTCCAACAGTACCAACAGACTATGG
G L G D V Q Q P P P H L A S Y S Q F Q Q Y Q Q T M

CGGGGTACAGTAACATGGGGTACGGCTTCCCCGCCGCGCATGTACGGACAGAACGGTTACGGGTACTCCTTGGCAGGG
A G Y S N M G Y G F P A A M Y G Q N G Y G Y S L A G

TACCCTCACGCTCCCAGTCCGTCATCTGATGTCACAGAGAAGCCGGAGGGCGGAGAAGTCCGCGTAACAGCGAAGGG
Y P H A P S P S S D V T E K P E G G E V R V T A K G

CAAGAAGATCCGCAAGCCCCGACCATATACTCGAGTCTGCAGCTGCAGCAGCTCAACAAAATGTTCCAACGGACGC
K K I R K P R T I Y S S L Q L Q Q L N K M F Q R T

AATATTTGGCGCTACCAGAGCGAGCCGAGCTCGCGGCTAAGCTGGGCCTCACGCAGACGCAGGTGAAGATTTGGTTT
Q Y L A L P E R A E L A A K L G L T Q T Q V K I W F

CAGAACAAGCGGTCCAAGTTCAAGAAGATATAACAAGACACAGGGTGCCGGAGCACAGCTGGCGGTGGACGCAGAACT
Q N K R S K F K K I Y K T Q G A G A Q L A V D A E L

TGCCGGCGAGATGCAGAACAAATCTTGGCGTGTGGTGAACGGGCCGGAGTCTCCTGCCTCGCCAGCGTCCACCATTG
A G E M Q N N L A L M V N G P E S P A S P A S T I

ACCACAGCATAGACTCCCACCCGACCTCAGGTCATGCAGGGTCTCCTCTTCCAGAGGTCTCCTCCAGCTCCCACT
D H S I D S H P T S G H A G S S S S R G P P P A P T

CCAGAGCCCCACGGTCACTCCTCCCCTCTTCCCCTGCAGCCTCTGGGTCCAGTTCAACCTCTCGGTCGTCAGGCTC
P E P H G H S S H S S P A A S G S S S T S R S S G S

ACAGCAACACATGCACCACCCGAGCACCCACAGCACCCACAGCAGCAACAGCACCAGACAGACAGACCGCACTCCC
Q Q H M H H P Q H P Q H P Q Q Q Q H Q T D R P H S

ACCAGTACCCGCGCTACACCCGACCTACAACAGCCCATGAAGTGTGAAGTCATGAATTCACCCGGAGACCTCATG
H Q S P A L H P D L Q Q P M K C E V M N S P G D L M

GCTCATCAACAGAGGGATCACATGGCTTCAACCAGAGACATGATGACATCTCCACCCATGCCGTCACCCAAGGATAT
A H Q Q R D H M A S P R D M M T S P P M P S P K D M

Fig. S2. *PhDII* sequences and alignments with DII proteins from other species. (A) cDNA sequence for *PhDII-e*; the start codon is green and the stop codon is red. Corresponding amino acids are displayed immediately underneath the nucleotide sequence. Nucleotides that encode the homeodomain are underlined and the sequence corresponding to the *PhDII-e* siRNA is highlighted in yellow. (B) Alignment of the homeodomain of DII proteins from *Parhyale hawaiensis* (*Ph*), *Drosophila melanogaster* (*Dm*), *Apis mellifera* (*Am*), *Manduca sexta* (*M*), *Cupiennius salei* (*Cs*), and *Homo sapiens* (*Hs*). Amino acid residues that are identical to *PhDII-e* are shown in black; those that are not identical are in red.

GATTCCAGCAGCCATGGCAGCGAATCCTGCCGTGCAAGGAGGACCAATGGACCCCGCAGGATGGCAATGGATGCGG
 I P A A M A A N P A V Q G G P M D P R R M A M D A

CTGGGCACATGGCTGGCTATCCGATCCCTCATCCACATGCGGGACACCCGGGACATCCCCAGTGGGATCCTGCGTCT
 A G H M A G Y P I P H P H A G H P G H P Q W D P A S

ACCTACATGTACTGGAACCACTACGGGGACATGGCGGCAGCGCATCAGATCAATCAACAGATTATGACGTAAGACGG
 T Y M Y W N H Y G D M A A A H Q I N Q Q I M T *

GCACTGTCAAACGTAAGACCGTTGCCGCTGAACATTATATAATGTTAAACATTACATAACGTAAGACGGGCGCTGTT
 AAACACTACATACCTCGAACGTAGGACGGGCGCCAATAAACATAATATGACGTAATAATTGTTAACGTGGGTACCACGCC
 ACGGTATTCCTTCCTTCCTGCCGAGCAGGCGTACAACACAGGGGCAGCTTCCCTATATGCTTTGGAGGACGATGTCT
 ACCTTAGGCATATGGCTGCTGACGCCATGTCACAGCCCCAATGCTAGACTTGATGCTTTAGGTAACAACCGTCAGCC
 GCTAGCTCTAGCCCCAGCC

B

<i>Ph</i> D11-e	IRKPRTIYSSLQLQQLNKMFRQTQYLALPERAELAAKLGLTQTQVKIWFQNKRSKFKKIYK
<i>Ph</i> D11-11	MRKPRTIYSSLQLQQLNKIFQRTQYLSLPERAELAAKLGLTQTQVKIWFQNRRSKYKKLVK
<i>Ph</i> D11-12	MRKPRTIYSSLQLQHNLNKIFQRTQYLSLPERAELAA SLGLTQTQIKIWFQNRRSKYKKLMK
<i>Dm</i> D11	MRKPRTIYSSLQLQQLNRRFQRTQYLALPERAELAA SLGLTQTQVKIWFQNRRSKYKKMMK
<i>Am</i> D11	MRKPRTIYSSLQLQQLNRRFQRTQYLALPERAELAA SLGLTQTQVKIWFQNRRSKYKKMMK
<i>Ms</i> D11	MRKPRTIYSSLQLQQLNRRFQRTQYLALPERAELAA SLGLTQTQVKIWFQNRRSKYKKMMK
<i>Cs</i> D11	MRKPRTIYSSLQLQQLNRRFQRTQYLALPERAELAA SLGLTQTQVKIWFQNRRSKYKKMLK
<i>Hs</i> D1x2	VRKPRTIYSSFQLAALQRRFQKTQYLALPERAELAA SLGLTQTQVKIWFQNRRSKFKKMWK

Fig. S2. Continued.

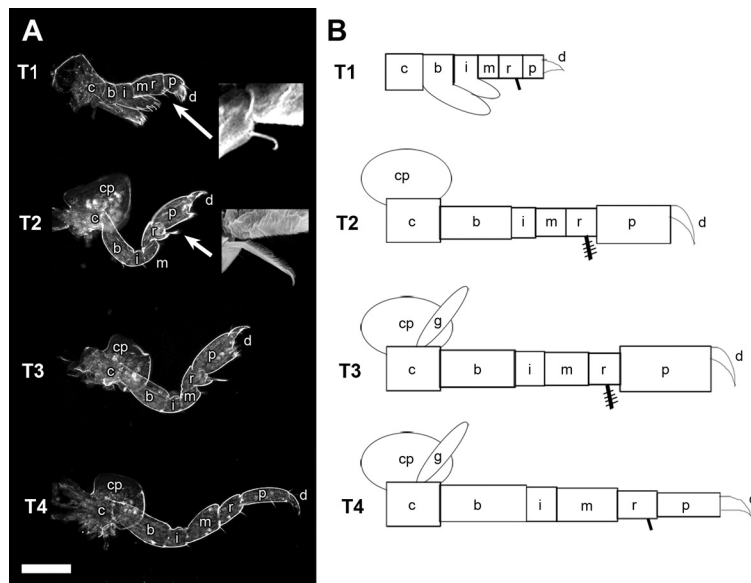


Fig. S3. Morphology of *Parhyale* thoracic appendages. (A) Darkfield images of hatchling thoracic appendages. (B) Schematics illustrating appendage segments in each of the appendages shown in (A). Appendage images and corresponding schematics are oriented with proximal (animal midline) to the left, distal to the right, anterior to the top. The segments are labeled in both (A) and (B) using the same abbreviations: c, coxa; cp, coxal plate; g, gill; b, basis; i, ischium; m, merus; r, carpus; p, propodus; and d, dactyl. Legs are labeled by their thoracic segment (i.e., T1 is the appendage on the first thoracic segment). T1 is the maxilliped, T2–T3 are gnathopods, and T4 is a walking appendage. Insets are SEM images that show the distinction between the straight bristle on T1 (long arrow) versus the comb-like bristle on T2 (short arrow, comb-like bristle is also on T3). (Scale bar, 100 μm .)

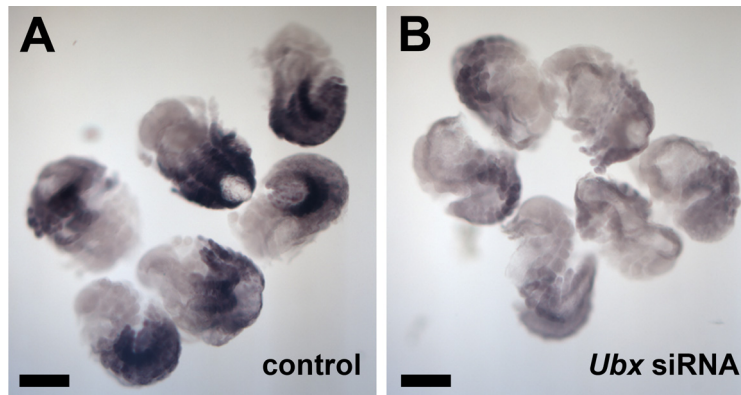


Fig. S4. siRNAs reduce *Parhyale* Ubx protein expression during development. (A) Uninjected embryos show wild-type levels of staining. Note the especially intense staining for Ubx protein in the thoracic segments. (B) *PhUbx* siRNA injected embryos that were stained in parallel with the controls in A. The embryos show a prominent reduction in staining intensity, but while thoracic expression of PhUbx protein is significantly diminished, it is certainly still present. (Scale bar, 200 μ m.)

Table S1. *PhUbx* siRNA injections

Conc., μ M	No. inj.	No. hatch.	No. phenotype (% of hatchlings)
<i>PhUbx</i> siRNA mix			
20	n.a.	56	14 (25%)
67	95	54	13 (24.1%)
<i>PhUbx-A</i> siRNA			
67	52	11	3 (27.3%)
200	76	55	11 (20%)
<i>PhUbx-B</i> siRNA			
67	81	53	25 (47.2%)
200	52	19	5 (26.3%)
<i>PhUbx-C</i> siRNA			
67	47	26	2 (7.7%)
200	35	18	1 (5.6%)
<i>DsRed</i> siRNA mix			
67	110	85	0 (0%)

Injection of *PhUbx* siRNAs creates homeotic transformations of T2 and T3 towards T1. Embryos were injected at the 1-cell stage, or in both cells at the 2-cell stage. See *Methods* and [Fig. S1](#) for the siRNA sequences. "Conc." refers to the siRNA concentration; for *PhUbx* mix and *DsRed* mix, the value represents the concentration of each individual siRNA present in the mix. "No. inj." indicates the number of embryos injected (n.a., not recorded for the 20 μ M *PhUbx* mix experiment); "No. hatch." indicates the number of injected embryos that hatched; "No. phenotype" indicates the number of injected embryos that hatched with a phenotype, as defined by the addition of branches on T2 or T3 appendages. No other malformations of T2 and T3 were observed. Approximately an additional 50 animals were generated with limb transformations from *PhUbx* knockdown with *PhUbx-B* siRNA by other lab members, but were not included in the table because they were not part of experiments where hatching and phenotype percentages were recorded.