

Electroporation mixes (all per 700 µl of cuvette volume):

MymK CRISPR

140 µg MRF-2685/-1>CD4::GFP
25 µg MRF-2685/-1>H2B::mCherry
40 µg Mesp-1916/-1>Cas9
40 µg U6>MymK.2.6
40 µg U6>MymK.4.68

Negative Control (for MymK CRISPR)

140 µg MRF-2685/-1>CD4::GFP
25 µg MRF-2685/-1>H2B::mCherry
40 µg M-1>Cas9

MRF>MymK (larval tail overexpression)

70 µg MRF-2685/-1>Ciona robusta MymK
50 µg Tbx6-r.b-1249/+12>CD4::GFP
10 µg MRF-2685/-1>H2B::mCherry

Negative Control for larval tail overexpression

50 µg Tbx6-r.b-1249/+12>CD4::GFP
10 µg MRF-2685/-1>H2B::mCherry

MymK-509/-1>GFP

140 µg MymK-509/-1>Unc-76::GFP

Novel, unpublished sequences from this study:

sgRNA target sequences validated by TIDE (N19+**PAM**):

MymK.2.6: CAATGGAGTTGTGCAGAGG**TGG**
MymK.4.68: CAGCTCCAAGTGCAAACGA**TGG**

Peakshift assay negative control sgRNA sequence (N19+**PAM**):

Gsx.4: ACGACAGTGACGAAAGGTC**CGG**

sgRNAs rejected due to insufficient evidence of activity (N19+**PAM**):

MymK.2.22: AAAACAGAAGAGACAACA**TGG**
MymK.3.65: GTGGACCAGCATAAACACC**AGG**
MymK.3.97: CTGGTCCACTAATAACTGG**CGG**
MymK.4.24: CAAGAAATGTACCGATCCA**AGG**

>C. robusta MymK cDNA for riboprobes and overexpression **START STOP T7**
ATGGGTTTGGATTTTAGTGAAATTTCTACTCCCTGCAATTAGTAGTGGCGCTTTTTTTATACCAGGAATCT
TTGCTACGAAGAAAAGGCTTTTTCACGTTAGCATTTCTTTACATTTTCACTGCTTTCTTCCAAGTGT
CCACCTCTGCACAACCTCCATTGTTGTCTCTTCTGTTTTGCTTGATGGGAAAAAGCTTTTGCATTCTTC
TCCACGTATGGACTAGTGCTATCGATATACTCCACACTTACACAATTAACAAGATATAACCGATGATCGAA
AGCACTCAGCTGTGGTATGTGGTGGCTTGCTTATGGCGTGAGGATATCCAGGAAAATGAAGGACCTGG
TGTTTATGCTGGTCCACTAATAACTGGCGGCCTACTGCTTGCGATATCCTGGGGTCAAGAAATGTACCGA

TCCAAGGCTTTATATCCGGATAAAGAGAAATGGTTGAAGATAATTTTACCATCGTTTGCACCTGGAGCTG
TTTCTCTACTTCTACTTTGTGTTTTTCAAACAGTTGGAATTATGCTTTTGTTCATTCCATCCACCACTT
ATTAATGTCAGCTGCCATCACAATTATCCTTCGGCTCGTGGAAAGACGGAGAGAAGCAAGATAAATGTTGT
GGTTTATCCATTGCATGTTGTATATGTTGAgaattcCCTATAGTGAGTCGTATTA

>C. robusta MymK gene model, based on HT genome assembly¹

EXONS START STOP (UTRs not determined)

ATGGGTTTGATTTTAGTGAAATTTCTACTCCCTGCAATTAGTAGTGGCGCTTTTTTATAACCAGGAATCT
TTGCTACGAAGAAAAGGCTTTTACAGTTAGCATTCTTTACATTTTCACTGCTTCTTCCAACCTGtaag
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>MymK (-509/-1) promoter

TGCTCTGGAAAATTTACCAAGGGAAACTCCCTCACGTGGTAACATAGAAATAGTACAAAAATGCTTAAA
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AAAACAACCGAGAGTACCGTAGTACATTTGGTATGTCGCCTTTAGAGCATTTCATTGCATAGTGTTAAACC
GTAGATTAATCATAGTT

>MRF (-2685/-1) promoter

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ttacaaccataagttgcaagatattaagattatgtattgacctatataatttgtatttcagaaatctag
ccggtagtttgacatatttatacg

Previously published sequences (hypothetical sequences given, not actual verified sequences):

>CD4::GFP (Tolkin et al. 2016)² Homo sapiens CD4 GFP
ATGAACCGGGGAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
AGGGAAAGAAAGTGGTGTGGGCAAAAAGGGGATACAGTGGAAGTACCTGTACAGCTTCCCAGAAGAA
GAGCATAACAATTCCACTGGAAAACTCCAACCAGATAAAGATTCTGGGAAATCAGGGCTCCTTCTTA
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GGCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCCGACAAGC
AGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGA
CCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACC
CAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCCG
CCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA

>H2B::mCherry (Stolfi et al. 2010)³ Helobdella (leech) H2B mCherry
ATGCCACCAAAGCCTGCCAGCAAGGGAGCTAAGAAGGCCGCCAGCAAGGCGAAAGCTGCTCGCAGCACGG
ACAAGAAGCACAAAGAGAAGGCGAAAGGAAAGCTACTTTATATACATATACAAAGTGCTGAAGCAGGTTCA
CCCGGACACGGGCATCAGCGGCAAAGCCATGTCAATAATGAACTCGTTCGTCAATGACATCTTTGAACGA
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CAAGTACACCAGCTCAAAGgtcgcacaggccaatctggccgcggggtcgacggtaccgcgggcccgggatcc
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TGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGA
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GGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCTCCGAC
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CACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACC
TCCCACAACGAGGACTACACCATCGTGGAAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCA
TGGACGAGCTGTACAAGTAA

>Unc-76::GFP (Imai et al. 2009)⁴ C. elegans Unc-76 tag GFP
ATGGCGGATCTGCGAGTACCGGACATTCGGCTCGCCTCGTGTGATGATGATGATATCGATAGTAATAAGA
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CCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGGAGTTCGTGACCGCC
GCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA

>Cas9 (Stolfi et al. 2014)⁵ START NLS STOP
ATGGCTAGCCCCAAAAGAAGAGGAAAGTGACAAAGAAGTATTCTATCGGACTGGACATCGGGACTAATA
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ATAAAGTGATGAAGCAACTTAAACGGAGAAGATATACCGGATGGGGACGCCTTAGCCGCAAACCTCATCAA
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CCGCCCTGATCAAGAAGTACCCAAAGCTGGAAAGCGAGTTCGTGTACGGAGACTACAAGGTCTACGACGT
GCGCAAGATGATTGCCAAATCTGAGCAGGAGATCGGAAAGGCCACCGCAAAGTACTTCTTCTACAGCAAC
ATCATGAATTTCTTCAAGACCGAAATCACCCCTTGCAAACGGTGAGATCCGGAAGAGGGCCGCTCATCGAGA
CTAATGGGGAGACTGGCGAAATCGTGTGGGACAAGGGCAGAGATTTTCGCTACCGTGCGCAAAGTGTCTTC
TATGCCCTCAAGTGAACATCGTGAAGAAAACCGAGGTGCAAACCGGAGGCTTTTCTAAGGAATCAATCCTC
CCCAAGCGCAACTCCGACAAGCTCATTTGCAAGGAAGAAGGATTTGGACCCCTAAGAAGTACGGCGGATTTCG
ATTCACCAACTGTGGCTTATTCTGTCTGCTGGTCAAGGTGGAAAAAGGAAAGTCTAAGAAGCTCAA
GAGCGTGAAGGAACTGCTGGGTATCACCATTATGGAGCGCAGCTCCTTCGAGAAGAACCAATTGACTTT
CTCGAAGCCAAAGGTTACAAGGAAGTCAAGAAGGACCTTATCATCAAGCTCCCAAAGTATAGCCTGTTTCG
AACTGGAGAATGGGCGGAAGCGGATGCTCGCCTCCGCTGGCGAACTTCAGAAGGGTAATGAGCTGGCTCT
CCCTCCAAGTACGTGAATTTCTCTACCTTGCAAGCCATTACGAGAAGCTGAAGGGGAGCCCCGAGGAC

AACGAGCAAAGCAACTGTTTGTGGAGCAGCATAAGCATTATCTGGACGAGATCATTGAGCAGATTTCCG
AGTTTTCTAAACGCGTCATTCTCGCTGATGCCAACCTCGATAAAGTCCTTAGCGCATAACAATAAGCACAG
AGACAAACCAATTCGGGAGCAGGCTGAGAATATCATCCACCTGTTACCCTCACCAATCTTGGTGCCCT
GCCGCATTCAAGTACTTCGACACCACCATCGACCGGAAACGCTATACCTCCACCAAAGAAGTGCTGGACG
CCACCTCATCCACCAGAGCATCACCGGACTTTACGAAACTCGGATTGACCTCTCACAGCTCGGAGGGGA
TGAGGGAGCTCCC**AAGAAAAGCGCAAGGTAGGTTAATGA**

>U6 promoter (Nishiyama et al. 2008)⁶

TGGCGGTGTATTAAACCACTAAACAACAATTGCCCAAGCTCTCTCACAATTATAAACACTATAATG
TTTGGACAAGAGATTAGCGTGGCTGTGACGAGAACTCTCAAAGGCTTGGTGTAATTGATATTTTATAAGA
AGCAGATTAAACTTCAATACAGTTTACACCTCATTACAAAAAATTGGCTGCCAAAATCGCTAATTAACA
CATATTTAAAACAATTTCTACAGATATACACAGTATAGTATGATTACTAACTGCATAATAAACAAACATA
TCCAACAGACACTCACTAATCTGCCATAACAAGCTTCAAAAACCTAAACTCGAAAATTTAGTGAATCTTT
TTTTTTAAATGAAGATTTTATTTAAAAAGTTAAAAATATTACAGTTCAGGTATAGGTTTACACCTAATCT
TTAATAATCCGAACTAAATTTTAACTATTTAGAAAATTTTTCAACCAAAGTTTAAAAAATAGATTCTTC
GCACGCTAAAACCTATCATTTACACAAAAAATGCAACAAAATGCAGAAAAAATACATTAGAGTTTAGG
TTAGTTACCTGCTAATCAATATAAACTAACTTCCCGCATAATATTCATCTAAAATTAGCAATAATCACGT
TTTACGCTAAAATTTGTGTAAAACCTAACTTCGTCTTTGTCAAGGAGAAAATTTGACTCAAAAAGCTGCG
CGCGCAGGGGAGATCCCCAAGCGAGTGTGTTTACATCATAATCATGTGAAAAATCCCCTAATAAGTAA
AAATACATATTTTTTAATTTTGGGGCAAATAAACCGCTTTTTTATGTCTAAAACGCCAAAATGGATCG
CGGAGCCCCAAAACGCACAAATAACGTACAGACAGTGTCTCTGCGTACACAGACGGTATTTCCCTTTA
AATTGAGAACTAGACTTAAGCACGCTTATAAGTCTGGAAGGCATCCGATGGTATAGAT

>Tbx6-r.b promoter (-1249/+12, Christiaen et al. 2009)⁷

caacggagtacgctgtcaagtttaatggcgtaattaccgaacaactggtgataagtaatgaggaccccg
ctgcgtaacctttcgaattgctggttgcaagcgggtggtggtgataataaggaaaagcggtaagcgt
tattttttgatccaccacaaagaccaaacctataattgatacactaaactaaatcaaattcgaacctatcc
attaaagtgcgaaatatacatagaatgaacaataatccgagctatattatgggcaatattttggctagtt
ttctgcttacaataatacataagaggcctacactaaatacggcggtatataaaaactatgcaagacatacc
catatattagtttttaatacaattgattttaaataatttttaaaactattaggcaactttgagtagaataag
ggtttaacctaacgtagtggttttagccatcgatggaaacggttttacaattatcgttacttttttgaa
gaccttttcgttctatttttaagaagaacattcaaagaaatggaaaaccgttttcttacgaatcctatat
accggtgtaattggttaaaaacacgataaggatattatggtctgaatggtgccatcttaccacacagc
actatataataaaaattttagttttgtgacagacttatgtcgcatctttaaaccgatgatccccaactgg
ttaacaacgtaacaagccttgcaaaagcgaaggtgacattggtaacaacgtacgataactattgtctacta
ataacaatagacttaacacataacatatttaggaaatggcttcatatggcggactgtcaacttagttgtc
aaaattaccttttaaatctctataaacgaagctgtttaataaaaaaaactaacaaggctattctacatcaa
accataataatgaaattatcaaacacatttttaagcgttttcatcaaaccaacgcgccacatgcaagac
ggtagcgtcacactgagttttggagtggtctgcatgctgacttgaatcagcaggagagttcggagg
cttatcaggaagcagttgtccttgtaaatgactcgtaagatcaaagtggcatcgaacacgagtcctcgt
ataaaacgggttttagtttcacagtacctcattccgctttctgttctcattggatatacaccaaactgaaa
gtacgagttaaatcgaagagagaggaaattgtaagttttattggaccagacaagactatggcgaatag

>Mesp promoter (-1916/-1, Davidson et al. 2005)⁸

cggttcaacgtgacgtcccatgccgatatcgtaacctccggaacctctgatgctttttcaatatcatct
tttttgaaatccttcattttcgtttcatcgctcatttttgaaagccgggttctcactactcgtttttgggtgc
tgaaccggttttctgacattttttatctcatccaagtgcgaaccacttcaaagatggatagatacaccaga
ttttattacaacaattagcaattcacgaaagttaaaaaacgcataaaaactataaaaataaaattatac

ttattaaatgaagaaaaatatgcatttttaacattctattgcaacaaatcggatatttttcgtattct
cttatacgaagattgcatacaagcttaacgtttcatctgtttccattggaataaaatagaaaaacgtccca
ccgcctgccctaactttttataagcttcacaaattatcaaagttaccggtgaactcctgataaatgatt
attcagctattttaaacccaacttcgtttataagtgatgccgtctccttcttctgtagaccccgctctac
taaatacatgtttgcacactgatcctaatagaagaccgcacctaaacctttacatagccgatgcattcaca
tataatgttcttggtgcaaccgaagtcgtatttgagcaaaccaaatatccaacttatataagtagacctt
taaaaatggacgggtaacaaaactttgcaagtcataaattgaaggtttatttagctctcctattgta
gggaaagatcctatggatgctccaaaacgataaacccgacttacgaaaacgcggcggtcgtagaccat
gcttattgataaacacatacatacaaaacttttagcgcaaacctcggtatatactttacaacattagacaaa
ttctctttcatgtattttcatgggattataaagtgtctatttttaggtaacaagactttaataaaaatc
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agtaatgagtatacagcagcagacagaaagttgtcgggggtatcctatgattgtacatcatgtgagca
aataacttcaactgctagtgaataatatttgatatacgcacaagtcgtacaaatacagcactacaata
agtcatacttgtcgaagtttgtattcccctgtttctgatcaatcttatcagccacaaaaaatggaaaaat
tccaaaaacgtagacacccacaaagtaacatacatggtaactcgttaagtaatacagagcaccggtgta
aaacgactgtcgtttccggccacgcgagaataaagtaagttacattgattcattcactgacagtttccc
acaaaaactgaagtattaatcaattatgaatcggcagtgctttcagtggggaagatttcggaaaatgtggg
tctaatgtcacagtaaacctttgtggtttgttttaaaaacaatcatcgttaaatcaacatcgcaattctgtc
aatcactccacaataaactatatggctaattggaaacaggctggttgggttatatacataaaataacttgcg
tcgtattatctgaccaacaaaagcgttacaggaacgggtccagcttcaaaatttgctgatgcatcagatt
ttacatttgaaatgtgattaattacgaaaatccagcgaatagaattgtcacaacaagtcattagcgcg
atatttcgcctttgaaacttaaaggcgataatgactttgcccggttcatgcccgcgataaacgaaactaatt
agacacctctacagatataatggtaattcagaatcgtgtgggtatgtaattcacaaaaacattttaaca
aaacaggttgattgaaacttgatt

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