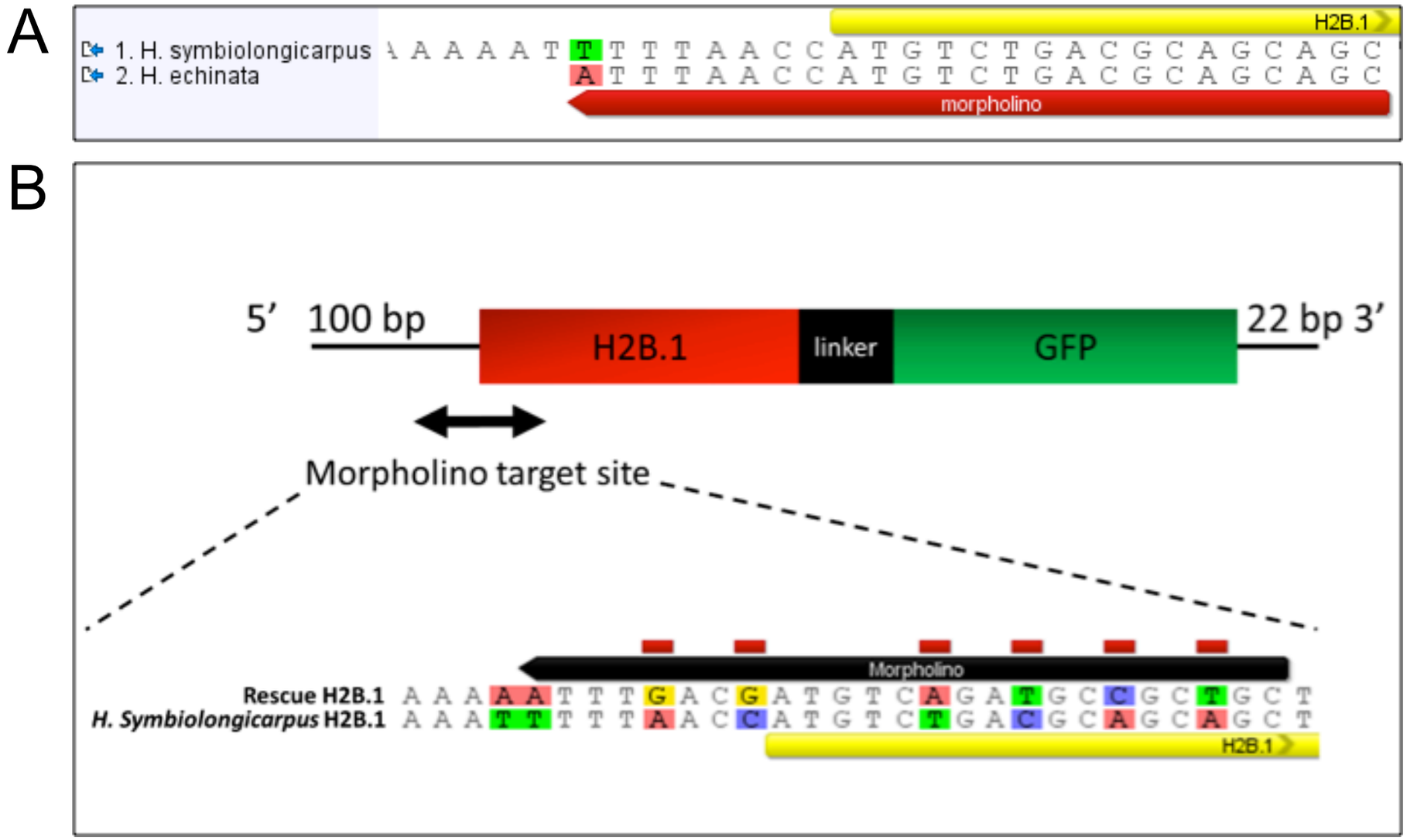

Fig. S1. Design of morpholinos. Alignment of the *H. echinata* H2B.1 morpholino with the H2B.1 sequence of *H. symbiolongicarpus* showing only 1 mismatch at 5’ position. B. Design of H2B.1 rescue mRNA with mutated morpholino target site.

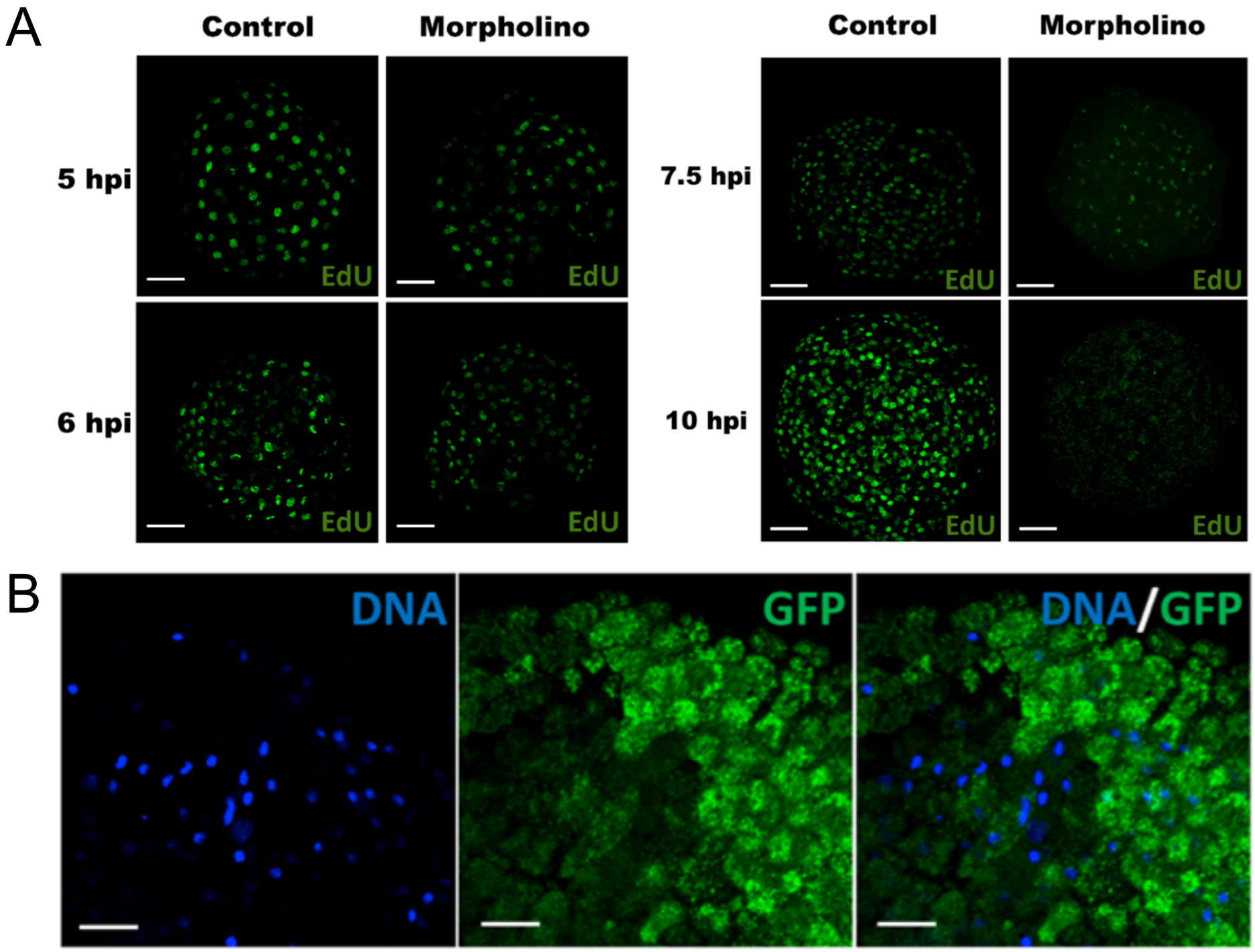

Fig. S2. Analysis of S phase cells after H2B.1 morpholino injection. A. Comparison of EdU incorporation at indicated hours post injection (hpi) with either non-specific control of H2B.1-specific morpholino. Scale bars 50 μm. B. GFP control expression in the cytoplasm during embryo development. Scale bars 25 μm.

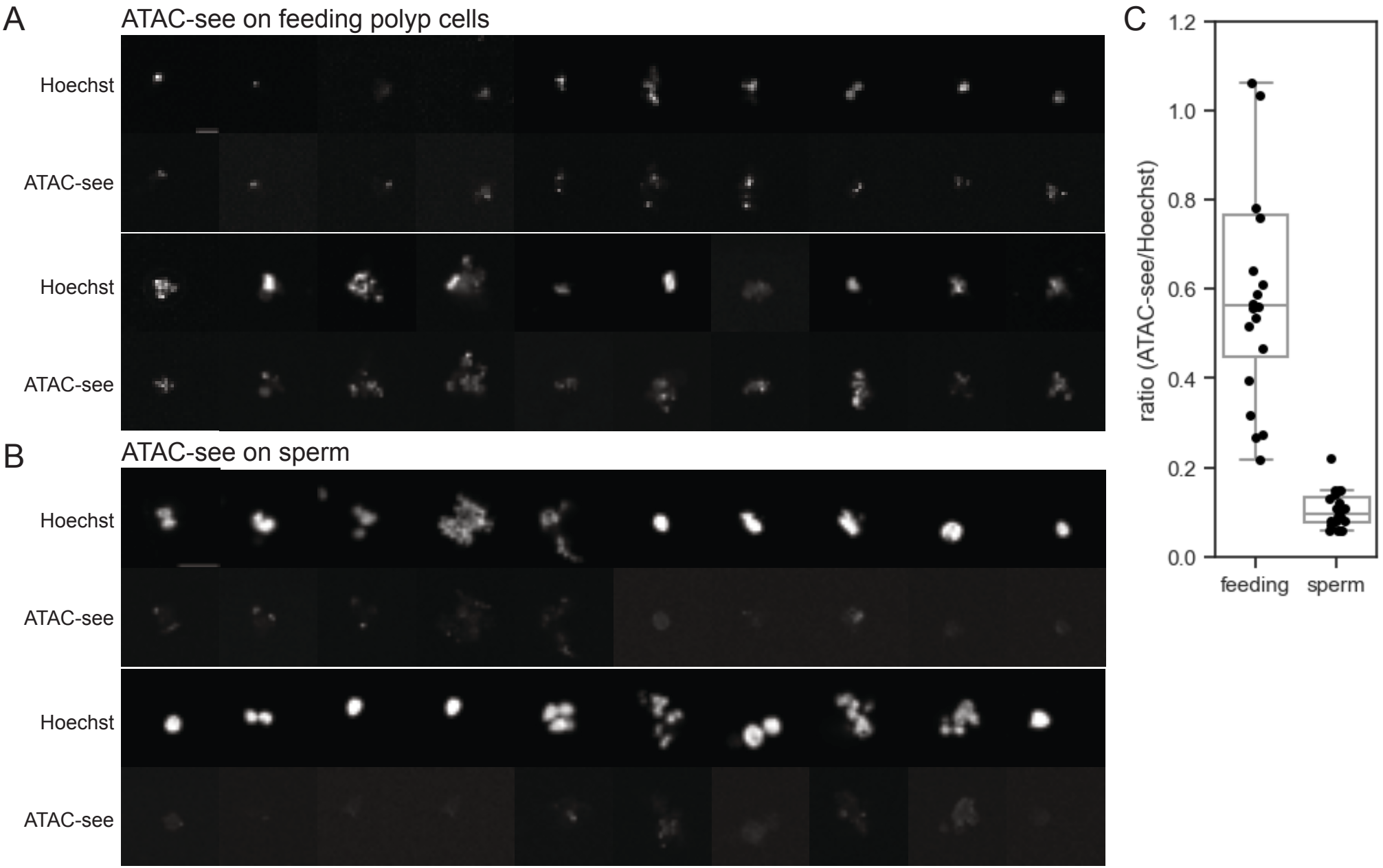

Fig. S3. ATAC-see of feeding polyp and sperm cells. A. Feeding polyp cells imaged for Hoechst 33258 staining of DNA (upper row) and ATAC-see (lower row) for 20 representative images at 40x magnification. Scale bar 10 μm. B. Imaging for sperm cells as in A at 100x magnification. C. Quantitation of ratio of total ATAC-see to Hoechst signal in images showing reduced ATAC-see for sperm under equivalent reaction conditions.

Table S1. Sequences of Hydractinia histone genes, proteins and promoter region.

>HeH2A gene
ATGTCTGGACGTGGAAAAGGTGGAAAAGCTAAGGCTAAAGCCAAGACAAGATCCTCAAGAGCTGGACTTCAAT
TTCCAGTCGGTAGAGTGCATAGATTCCTTCGTAGAGGGCACTATGCTAACCGAATTGGATCTGGAGCACCAGT
ATACTTAGCAGCCGTCTTGGAATATTTATCTGCTGAGATATTGGAGTTGGCTGGTAACGCAGCAAGAGACAAC
AAAAAAGCTAGAATTATCCCAAGACATTTACAATTGGCTGTTCGTAATGATGAAGAATTAAACAAACTTTTGA
GCGGTGTAACCATTGCAGCTGGTGGTGTTTTGCCAAACATTCAAGCTGTCTTACTTCCAAAGAAGAACGACAA
AGGACAGAAGAAGTAA

>HeH2B.1 gene
ATGTCTGACGCAGCAGCTAAAGGAGGAAAACAGGCACCTAAAGTAGCCAAGAAAGGTGAAAAGAGAGCCGGCA
AAAAAGGAGGAAAGATTGGTGGAACTGGTGAAAAGAAACGCAAGAAGAAGAGAAAGGAAAGTTATGCCATTTA
CATCTACAACGTTTTGAAACAAGTTCACCCAGATGTCGGAGTTTCTAGCAAAGCTATGAGCATCATGAACTCA
TTTGTCAACGACATCTTTGAGCGCATTGCTTCTGAAGCTTCGCGTTTGGCTCTTCAAAACAAAAAGTCGACCA
TCTCTTCTCGTGAAATTCAAACCGCAGTACGTCTTCTCTTGCCTGGAGAACTTGCAAAACACGCAGTCAGTGA
AGGAACAAAAGCCGTCACAAAATACACAAGCAGCAAGTAA

>HeH2B.3 gene
ATGGCTGGAAGTCCAAGAAAAGGAAGCCCAAAGAAAGCATCCTCTAGAGCTGCAAGCCCTAAAAGAGCTGCGA
GTCCTAAAAGAGGTGGAAGTCCAAAAAGAGGTGGAAGCCCAGCGAAAAAGGGAAAGGCGATTAAGAAAGCTGG
CAAGAGAAAAACAAACAAGAAAGCCACAACCAAGAGGCGAAGAAGTCGAAGGGAGAGTTATGGCATGTATATC
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TCAACGACATCTTTGAAAGATTAGCAGGCGAAGCTTCCAAACTTGCTCATCACAACAAACTACGAACCATCTC
GTCTCGTGAGGTACAAACTTCAGTTCGCTTGTTGTTGCCTGGTGAACTTGCGAAACACGCTGTGAGTGAAGGA
ACGAAAGCTGTTACCAAGTACACAAGCTCCAGATAA

>HeH2B.6 gene
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CTGCTAGTCCGAAAAGGGGAAGCCCAAAGAAAGGGAAAGGCATGGCAGCCAAGAAAGGAGGTGTGAGAAAGGG
TGCAAAGAAAAATGCAACAAAGAGACGAAGAAGCCGAAGAGAAAGTTATGGTATCTATATTTACAAAGTTTTG
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TCGAAAGATTGGCTGGCGAGGCGTCGAGACTTGCTCATCACAACAAGAAACAAACCATCGCTTCTCGTGAAGT
CCAAACTTCAGTACGTTTGTTGCTTCCTGGTGAACTTGCAAAGCATGCAGTGAGCGAGGGAACAAAGGCTGTG
ACCAAATACACAAGTTCCAAGTAA

>HeH3 gene
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CAGAGAAATCAGAAGATACCAGAAGTCAACCGAGCTCTTGATCCGCAAGTTGCCTTTCCAGCGTCTTGTGCGA
GAAATTGCTCAGGACTTCAAAACAGATCTGCGATTCCAGAGCACAGCCGTTATGGCTCTGCAGGAGGCTTCTG
AAGCGTACCTTGTTGGCTTATTCGAAGATACTAACTTGTGTGCCATTCACGCAAAACGAGTTACTATCATGCC
TAAAGACATCCAGTTGGCAAGAAGAATTCGTGGTGAACGAGCATAA

>HeH4 gene
ATGTCTGGTCGTGGTAAAGGTGGCAAAGGTCTGGGTAAAGGTGGTGCGAAACGTCATCGTAAAATCCTGCGCG
ATAACATTCAGGGCATTACCAAACCAGCCATCCGTCGTTTGGCTCGCCGCGGCGGCGTTAAGCGTATCTCGGG
CCTGATCTATGAAGAGACTCGCGGCGTACTGAAGGTGTTCCTTGAAAATGTAATCCGTGACGCGGTTACCTAT
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TGTACGGGTTCGGTGGTTAA

>HeH2A protein
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>H2B.1 protein
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FVNDIFERIASEASRLALQNKKSTISSREIQTAVRLLLPGELAKHAVSEGTKAVTKYTSSK

>HeH2B.3 protein
MAGSPRKGSPKKASSRAASPKRAASPKRGGSPKRGGSPAKKGKAIKKAGKRKTNKKATTKRRRSRRESYGMYI
YKVLKQVHPDVGISSKAMSIMNSFVNDIFERLAGEASKLAHHNKLRTISSREVQTSVRLLLPGELAKHAVSEG
TKAVTKYTSSR

>HeH2B.6 protein
MASPRKGSPRKGSPKKGSPKKTSRAASPKRGSPKKGKGMAAKKGGVRKGAKKNATKRRRSRRESYGIYIYKVL
KQVHPDVGISSKAMNIMNSFVNDIFERLAGEASRLAHHNKKQTIASREVQTSVRLLLPGELAKHAVSEGTKAV
TKYTSSK

>HeH3 protein
MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPFQRLVR
EIAQDFKTDLRFQSTAVMALQEASEAYLVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA

>HeH4 protein
MSGRGKGGKGLGKGGAKRHRKILRDNIQGITKPAIRRLARRGGVKRISGLIYEETRGVLKVFLENVIRDAVTY
TEHAKRKTVTAMDVVYALKRQGRTLYGFGG

>Hydractinia Piwi promoter 2080bp
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TGCATGGCAGGCAAAAAGATGCGGGTGCTAGGTAAAGTGAGGTCCTTTTTAGTCTGTTTTATTATTACCACTT
TATATGAATATATGTCAATCAAAAGTTATCTATCTACACTCGATAATAGTTTAAAGCCTCAAAAATGTTTCCG
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GGTTGGCAATAGAATGAATATATATATAAGGAATAATGAAATTTTCCCCCATGTATTATTTAATATGTCTAGG
ACAAAAAAGTCATGTGACTTGGCTTTTAGCCAATAAGATTGTTGTAATTTTTAGGGGGAAAACACTGTGGCAA
AATGTTCCTATAACACTTTTATATGCAACTCATATCATAAATTCAGGAGAGTGTGTATTGTTTGCCCCAAATG
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AATAATTTTTTTGTGAAATATCTTGAAAAAAAAAGTTCAAATTCCTTCACATTGTTTTAAGTAGGGTTACTGC
CAATCTATGAAGATTGGTCAGTGGTCCCTCGCTTTACATGAAAAAATAGCCAGACTTCAACGCCAAGTATAAA
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TGGTGGGCCGTTGGATGTTGCAGGGAGTTGTCTGGCAGAGCGCTGACCCTAATTGAGTTTACGTAGCTCAAAG
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GTGAGGCTAGCCATGTAAAATATGCACATCTATCTATCTATTACATGATAACTGGTCTGGTCTTGGTACACAT
GCATCACCACTTTCCCTACCATGTTTTATTTTGTGATGCTGCGCCAAAGTATATCACAATGGTTGTATTTGTT
CAAATTCAGCGCCCCTTTAGCTTACGGTGGATTTTCACTTTGATTTCAGTGAGGAACAGAAATGTGCAAGGTT
ACCGCGTTATATGATATTTATTTTTTATTCATTTTTTTTATCCCATCCTTTTTTAACTTCTAGCACAAAATAA
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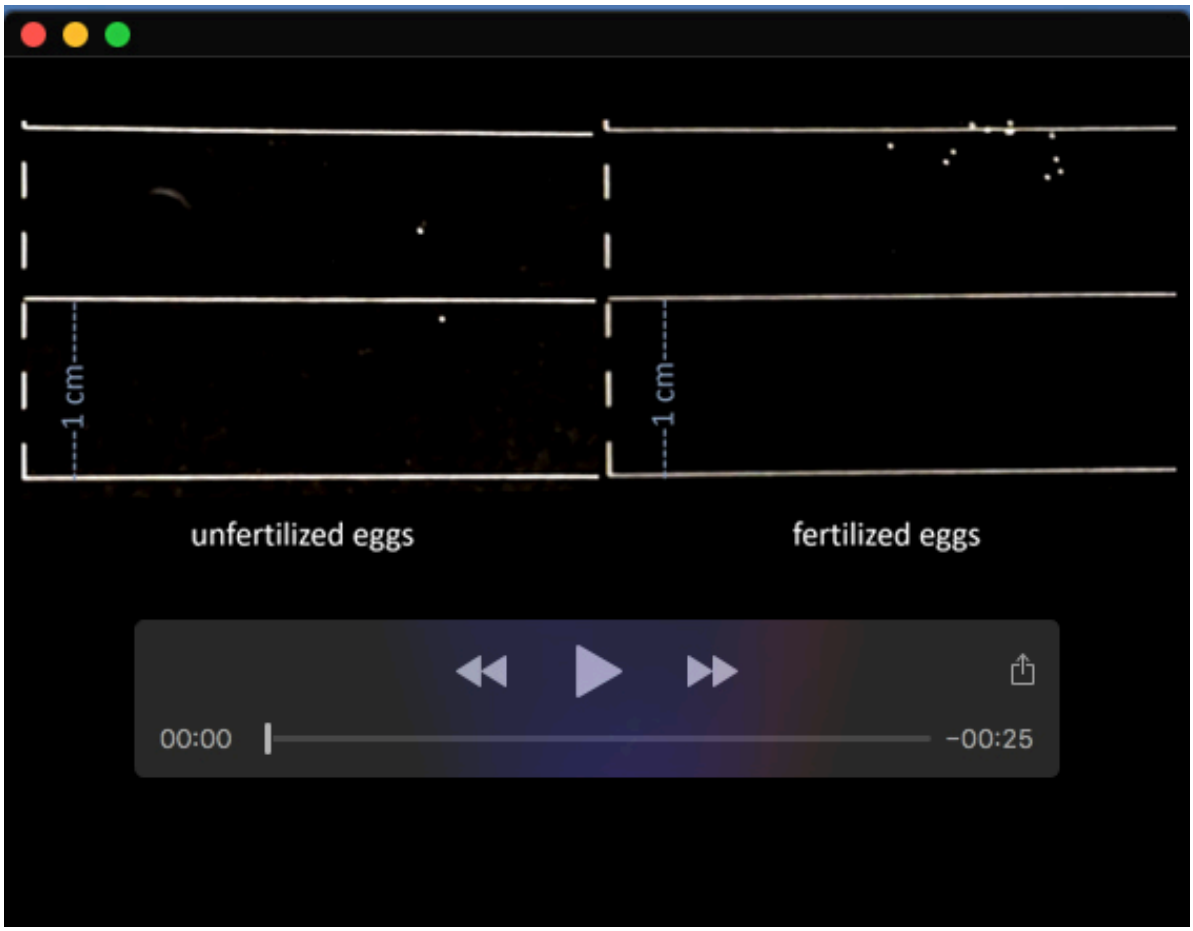
TTCTGTTTGGTGCATTATTAGACCATCACTGAACTTCTTCTCAACATTTATGAATAAAATAACGTATTTTGAG
TCATATTTAATAATTACGAAAATTTTCATTACATTT

Movie 1. Spawning of Hydractinia female colony. Orange arrow indicates female sexual polyp suspended on vertical surface. Orange circle highlights release and slow sedimentation of unfertilised eggs in real time. Width of region in movie frame is 1.5 cm



Movie 2. Hydractinia eggs are more buoyant than zygotes. Unfertilised egg (left) and fertilised zygote (right) sedimentation in real time through a 1 cm window.