

## Supplementary material legends

**Figure S1.** *Caspase3/7a mRNA* resistant to shRNA due to silent mutations on target site.

**Figure S2.** The inhibition of apoptosis, in addition to induce head morphogenesis disruption, resulted in a significant reduction of lectin<sup>+</sup> cells in the body column (magenta area). Scale bar = 200µm. \* in polyps images: oral pole. \* in graph: p<0.05.

**Figure S3.** 3 days old larvae injected with the *Caspase3/7a* shRNA. *Caspase3/7a* loss of function did not impair nerve net and neurons in larvae before metamorphosis. Scale bar = 100µm. \* in polyps images: oral pole. \* in graph: p<0.05.

**Figure S4.** Results of genes ontology enrichments from genes up- and down-regulated by apoptosis at 5, 10, and 20 hpi.

**Figure S5.** The expression of *Nco1* is significantly downregulated at 20 hpi in larvae electroporated with the *Caspase3/7a* shRNA. Hpi: hours post-induction.

**Figure S6:** Loss of function of NFκB, Wnt11b, and Frizzled1 to 3 did not affect metamorphosis. \*: oral pole. Scale bar = 200µm.

**Figure S7.** Expression of *Hydractinia Wnt* genes during metamorphosis of larvae electroporated with the *Caspase3/7a* shRNA. Among the expression profile, Wnt11b is downregulated at 20 hpi. Conversely, both Wnt1 and Wnt3 are up-regulated at 20 hpi. Hpi: hours post-induction.

571 **Table S1:** List of *Hydractinia symbiolongicarpus* Caspases with identification number from both  
572 available genome assemblies.

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|             | <a href="https://research.nhgri.nih.gov/hydractinia/">https://research.nhgri.nih.gov/hydractinia/</a> | Kon-Nanjo <i>et al</i> , 2023                    |
| Caspase3/7a | HyS0031.82 ; HyS0253.8                                                                                | HSymV2.0_g03.04853_t1                            |
| Caspase3/7b | HyS0003.486                                                                                           | HSymV2.0_g09.17038_t1                            |
| Caspase3/7c | HyS0034.180                                                                                           | HSymV2.0_g14.24224_t1                            |
| Caspase3/7d | HyS0074.45                                                                                            | HSymV2.0_g03.05227_t1                            |
| Caspase3/7e | HyS0012.28                                                                                            | HSymV2.0_g15.24941_t1                            |
| Caspase1    | HyS0118.8                                                                                             | HSymV2.0_g02.03211_t1                            |
| Caspase2    | HyS0023.328                                                                                           | HSymV2.0_g13.23640_t1                            |
| Caspase8a   | HyS0046.99                                                                                            | HSymV2.0_g10.17616_t1 ;<br>HSymV2.0_g10.19163_t1 |
| Caspase8b   | HyS0024.235                                                                                           | HSymV2.0_g10.18231_t1                            |

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| Genes                        | shRNA                                                                                                                                                                                                                                                                                                                                                                                     |
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| Caspase3/7a<br>(HyS0253.8)   | Fw: TAATACGACTCACTATAGAAAATAATTATCATTCAAATTTACTTTGAATGATAAATATTTTCTT<br>Rv: AAGAAAATATTTATCATTCAAAGTAAATTTGAATGATAATTATTTTCTATAGTGAGTCGTATTA                                                                                                                                                                                                                                              |
| Caspase3/7b<br>(HyS0003.486) | Fw: TAATACGACTCACTATAGGCAAAGCAATACGATTACCTTATTTACTAGGTAATCGTTTTGCTTTGCTTGCCTT<br>Rv: AAGGCAAAGCAAAACGATTACCTAGTAAATAAGGTAATCGTATTGCTTTGCCTATAGTGAGTCGTATTA                                                                                                                                                                                                                                |
| Caspase3/7c<br>(HyS0034.180) | Fw: TAATACGACTCACTATAGCTTGTAGACGGAGAAAGTATATTTACTATACTTTCTCCCTCTACAAGCTT<br>Rv: AAGCTTGTAGAGGGAGAAAGTATAGTAAATATACTTTCTCCGTCTACAAGCTATAGTGAGTCGTATTA                                                                                                                                                                                                                                      |
| Caspase3/7d<br>(HyS0074.45)  | Fw: TAATACGACTCACTATAGGTCCTGGTGGTCATCTCATTATTTACTAATGAGATGACGACCAGGACCTT<br>Rv: AAGGTCCTGGTCGTCTCATTAGTAAATAATGAGATGACCACCAGGACCTATAGTGAGTCGTATTA                                                                                                                                                                                                                                         |
| Caspase1<br>(HyS0118.8)      | Fw: TAATACGACTCACTATAGGTTCCCATCATGGCGTATTAATTTACTTAATACGCCATGTTGGGAACCTT<br>Rv: AAGGTTCCCAACATGGCGTATTAAGTAAATTAATACGCCATGATGGGAACCTATAGTGAGTCGTATTA                                                                                                                                                                                                                                      |
| Traf1<br>(HyS0014.170)       | Fw: TAATACGACTCACTATAGGAAGGAAGAATACCTTTATAATTTACTTATAAAGCTATTGTTCTTCCCTT<br>Rv: AAGGAAGGAACAATAGCTTTATAAGTAAATTATAAAGGTATTCTTCTTCTCCTATAGTGAGTCGTATTA                                                                                                                                                                                                                                     |
| Traf2<br>(HyS0003.508)       | Fw: TAATACGACTCACTATAGGAGTGGCCTACATTGTGATTATTTACTAATCACAATGTACGCCACTCTT<br>Rv: AAGGAGTGGCGTACATTGTGATTAGTAAATAATCACAATGTAGGCCACTCCTATAGTGAGTCGTATTA                                                                                                                                                                                                                                       |
| eGFP                         | Fw: TAATACGACTCACTATAGTGAAGGTGATGCAACATATTCAAGAGATATGTTGCATCACCTTCACTT<br>Rv: AAGTGAAGGTGATGCAACATATCTCTTGAATATGTTGCATCACCTTCACTATAGTGAGTCGTATTAA                                                                                                                                                                                                                                         |
| Wnt11b<br>(HyS0031.57)       | Fw1: TAATACGACTCACTATAGGGAAGTTAAACGAGGTGAATATTTACTATTACCTCGATTAACTTCCCTT<br>Rv1: AAGGGAAGTTAATCGAGGTGAATAGTAAATATTACCTCGTTTAACTTCCCTATAGTGAGTCGTATTA<br>Fw2: TAATACGACTCACTATAGCGCTTGCTACTGGTGAAGAAATTTACTTTCTTACCAGTACCAAGCGCTT<br>Rv2: AAGCGCTTGGTACTGGTGAAGAAAGTAAATTTCTTACCAGTAGCAAGCGCTATAGTGAGTCGTATTA<br>Fw3: TAATACGACTCACTATAGGGCAAATTCGAGATTGTTCAATTTACTTGAACAATCTGGAATTTGCCCTT |

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|---------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                 | Rv3:<br>AAGGGCAAATTCCAGATTGTTCAAGTAAATTGAACAATCTCGAATTTGCCCTATAGTGAGTCGTA<br>TTA                                                                                   |
| Frizzled1<br>(HyS0011.93)       | Fw:<br>TAATACGACTCACTATAGGAAGTTAACGCGAGGTCAAAATTTACTTTTGACCTGGCGTTTACTTC<br>CTT<br>Rv:<br>AAGGAAGTAAACGCCAGGTCAAAAGTAAATTTTGACCTCGCGTTAACTTCCTATAGTGAGTCGT<br>ATTA |
| Frizzled2<br>(HyS0030.17<br>5)  | Fw:<br>TAATACGACTCACTATAGGAGCAGGAAAAGAACTGAAAATTTACTTTTCAGTTCTATTCCTGCTC<br>CTT<br>Rv:<br>AAGGAGCAGGAATAGAACTGAAAAGTAAATTTTCAGTTCTTTTCCTGCTCCTATAGTGAGTCGTA<br>TTA |
| Frizzled3<br>(HyS0072.42)       | Fw:<br>TAATACGACTCACTATAGGCTTAATCCGACCGATCAAAATTTACTTTTGAACGGTCGGAATAAGC<br>CTT<br>Rv:<br>AAGGCTTATTCCGACCGTTCAAAAGTAAATTTTGATCGGTCGGATTAAGCCTATAGTGAGTCGTA<br>TTA |
| NF-Kappa-B<br>(HyS0012.16<br>9) | Fw:<br>TAATACGACTCACTATAGGCGAGTCCTCTTTAGAACTTATTTACTAAGTTCTAAAGACGACTCGC<br>CTT<br>Rv:<br>AAGGCGAGTCGTCTTTAGAACTTAGTAAATAAGTTCTAAAGAGGACTCGCCTATAGTGAGTCGT<br>ATTA |

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582 **Table S3:** list of real-time PCR primers and probes.

| Genes                      | Real-time PCR primers                                                                                       |
|----------------------------|-------------------------------------------------------------------------------------------------------------|
| Caspase3/7a<br>(HyS0253.8) | Fw: CCTCGAGAAGGTACCGAAATAG<br>Rv: TCGTAAGAAGCCATCGTCATAA<br>Probe: [6FAM] ACGCAAGGGAATTGACTCGGTTGT [TAMRA]  |
| Traf1<br>(HyS0014.170)     | Fw: GAGAAGTAACAGCGCTCCATAG<br>Rv: CCCATAACCATCGCCGAATAG<br>Probe: [6FAM] TCGCTATGGTTACAAATTCTGCGCGA [TAMRA] |
| Traf2<br>(HyS0003.508)     | Fw: CGGATGTTGATCGGAGATTGA<br>Rv: CTACCAGTGGTCGCATCTTT<br>Probe: [6FAM] TTAGAACGTGACGGTTTGCCGGAA [TAMRA]     |
| GAPDH<br>(HyS0002.485)     | Fw: CAAAGGCTGTTGGAAAGGTTATC<br>Rv: TCTTCAAACGCACTGTCAAATC<br>Probe: [6FAM] AGTGCCAGTTCCTGATGTTTCGGT [TAMRA] |

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585 **Files S1.** List of up and downregulated genes during metamorphosis between control larvae and  
586 apoptosis disrupted larvae at 5, 10, and 15 hours post-fertilization.