

Supplementary Information for

The Hippo pathway regulates axis formation and morphogenesis in *Hydra*

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Table S1

Figures S1 to S5

Table S1. DNA oligonucleotides used in the study. T7 promoter sequence is in red, gap sequence is in blue.

DNA oligo template for <i>shRNA</i> synthesis	
GFP sense	TAATACGACTCACTATAGAAGATGGAAACATTCTTGGATTCAAGAGATCCAA GAATGTTTCCATCTTCTT
GFP a-sense	AAGAAGATGGAAACATTCTTGGATCTCTTGAATCCAAGAATGTTTCCATCTT CTATAGTGAGTCGTATTA
HyYap1 sense	TAATACGACTCACTATAGACCTCCTCCGTCTCTAGATTCAAGAGAATCTAGA GACGGAGGAGGTCTT
HyYap1 a-sense	AAGACCTCCTCCGTCTCTAGATTCTCTTGAATCTAGAGACGGAGGAGGTCTA TAGTGAGTCGTATTA
HyYap2 sense	TAATACGACTCACTATACGACAAGACTCAGACACTGTTCAAGAGACAGTGTC TGAGTCTTGTCGTT
HyYap2 a-sense	AACGACAAGACTCAGACACTGTCTCTTGACAGTGTCTGAGTCTTGTCGTATA GTGAGTCGTATTA
HyYap1 scrambled sense	TAATACGACTCACTATAGAGCCCCTTAACGCTTCTCTCAAGAGAAGAGAAG CGTTAAGGGGCTCTT
HyYap1 scrambled a-sense	AAGAGCCCCTTAACGCTTCTCTCTCTTGAAGAGAAGCGTTAAGGGGCTCTA TAGTGAGTCGTATTA
HyYap2 scrambled sense	TAATACGACTCACTATAGGTCAACCGCTCATAACAATCAAGAGATTGTTATG AGCGGTTGACCTT
HyYap2 scrambled a-sense	AAGCGAAACCGCAACATGACTTCTCTTGAAGTCATGTTGCGGTTTCGCTATA GTGAGTCGTATTA
HyLATS1 sense	TAATACGACTCACTATAGGTGTCAAGCGTCTTACTTCAAGAGAAGTAAGA ACGCTTGACACCTT
HyLATS a-sense	AAGGTGTCAAGCGTCTTACTTCTCTTAAAAGTAAGAACGCTTGACACCTAT AGTGAGTCGTATTA
HyLATS2 sense	TAATACGACTCACTATAGACCAAGAGCAAATGAGAATCAAGAGATTCTCAT TTGCTCTTGGTCTT
HyLATS2 a-sense	AAGACCAAGAGCAAATGAGAATCTCTTGAATTCTCATTGCTCTTGGTCTAT AGTGAGTCGTATTA
HyWnt3 - 1 sense	TAATACGACTCACTATAGCAACGTAAGTGGAACTGTTCAAGAGAACAGTTCC ACTTACGTTGCTT

HyWnt3 – 1 a-sense	AAGCAACGTAAGTGGAAGTGTCTCTTGAACAGTTCCACTTACGTTGCTATA GTGAGTCGTATTA
HyWnt3 – 2 sense	TAATACGACTCACTATAAGTTGACGGTTGCCAACTTATCAAGAGATAAGTTCCG CAACCGTCAACTT
HyWnt3 – 2 a-sense	AAGTTGACGGTTGCCAACTTATCTCTTGATAAGTTCCGAACCGTCAACTATA GTGAGTCGTATTA
DNA oligos for qPCR analysis	
HyYap F	ATTTGCCAGAAGGTTGGGAACGTG
HyYap R	TGTTGACGATGACGCTTCTCTTGC
HyLATS F	GGTCCTGGAGCCTATGCGTT
HyLATS R	GCAGAACGCTTTGCAGGTGA


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% identity      1 | . . . . . : . . . . . 80
100.00 HyLats  MTEPSKKSSI I SSNQLLRNVFYNVLSHLHLHEHQPRHLDSAHIQSQSKFEDFLSQS --- YNIHALSQAAGDR---
43.88  DrLats1  MRRAEKP-----EGYRQMR-----PKTFPASNNSGNSQOMLQEIRESLRNLSPSSNASK---PEQSSGKG--
41.30  DrLats2  -----MR-----PKTFPATIYVGNTRQRLQDIKEGLKQPAKLVSQALHGGSSRSGEGCGRGGD
43.34  DmLats  -----MHP-AGEKRCGRP-----

81 | . . . . . : . . . . . 160
HyLats  -R-----SYPRASKAFTLEEIRKDLQPPFAWKNGFVASNLSSSKNNIKSSSINDLHQAAIEVNNNSVQSEQOKL
DrLats1  ---PPDESROQGRSTNPRNSHHHRAIQEIRKSLQPPFAHDSSSSGDNSSAD-SSK-----HTL
DrLats2  SKGKDQAARQQQLRPPQKFNQYQSALREIRKSLRFPYANDSGPGSSGHPGGEVSR-----OML
DmLats  ---NDKY--TAEALESIKODLTFEVCNNHRRN-----ONY-----TPL

161 | . . . . . 2 | . . . . . : . . . . . 240
HyLats  SOLVSYGYDEAIAADVLRNNSNKSIEVLVDII-----NSIIGCHKMKVPSVLTNHRVLGNN
DrLats1  Q-TQEPGFDELMVNRINFQEA-----VREQIPSARPVNTAIKQAGPPQIQOPL-----LRRQSWKGSK
DrLats2  QDLVNAQCQDEMAVRAIKQGTGRNIEAALEYISK--MSYLDPHTEQIVRVIKQTS PGKSGMPNLM-----DHRTAMEGSG
DmLats  RYTA-----TNGRNDALTPDYHHAQPMEPSPASAPADVVI PPPPAIVQPGAGSISVSGVGVGVVGVA

241 | . . . . . 3 | . . . . . : . . . . . 320
HyLats  NHPQPSFLOTLONKYA-----S-----NSRYYPSN
DrLats1  ESLAPQRHSAINDVLIYRPSSPGQSDLSR PATFPQN LASASSGQRVNAPLSRQVRSITPPPSSWDSNPS--TKRYSGN-
DrLats2  DGALPPYHOM---GAPLYEGASYGAE GEM-----ARAYMGGP
DmLats  NGRVPKMMTALMPNKLI RKPSIERDTAS--SHYLRCSPALDSGAGSSRSDSPHSHHTH---QPSRRTVGNPGGNGGFSPS

321 | . . . . . : . . . . . 4 | 400
HyLats  DLTRKGMQYKD-----LSPTNFTNN-----LPFEHFSNVQSCK---YPRETDHAQRYQSSVFSAHNLYHQDMPDD
DrLats1  ---LDYL---VPRISP---V---PQGPRGDGYINPQS-----QNORGLSPVIVGRQP---I---
DrLats2  ---FVNNYM---PPPANPQGSQ---PSGGGGAHYDYRSH-----MME--NSSYGVKRSASFQNK---
DmLats  ---SGFSEVAPPAPPRNPTASSAATPPPVPPTSQAYVKRRSPALNNRPPAIAPPTORGNSPVITQ---NGLKNP---QQ

401 | . . . . . : . . . . . : . . . . . 480
HyLats  SYLQNLGAENFGRPSVV TMRQKKTDKQFNPNRWSADVLO-----TYEIE---NVPNKPPPPYPL
DrLats1  ---IMONSNSNKNFPPSPW---FONGSMQNEYMGMN SGARQPPPPY SMHQTNRQSP TAAQQMS
DrLats2  ---MPOLTENYVMPSKAAMGQAGGYAPNLLPPHSHPRQASFTSHQVHMMORPPGVAPG---
DmLats  QLTQQL-----KSLNLYPGGGSGAVVEPPPYLIQGGAGG-----AAPP P P P P S Y T A S M Q S R Q S P T Q S Q Q S D

481 | . . . . . 5 | . . . . . : . . . . . : . . . . . 560
HyLats  -----LKRPSLPDNF SVTN-----
DrLats1  SALTAPPNGASLPQSM LVPNRNSHNDMYNLGVIRPPVQQAQTPPGQDTSSSW--THSVFGRSNTSAPNRR-----
DrLats2  ---AMGGEFSDVPQGLLTPSRASLNLDLIEHHWPAQAGP-EGTPPARQPGPFGRGEVVRVSRNNSFNHQQKVTVRQTLPP
DmLats  Y-----RKS P S S G I Y S A T S A G S P S P I T V-----SLPP

561 | . . . . . 6 | . . . . . : . . . . . : . . . . . 640
HyLats  -----ISDALENV-----RT---GVDLPTYNG-----
DrLats1  ---RQCPNS-QLSATTVTTVTQAPILQPVKSMRVQKPELHTAVAPAHFWLQ-----
DrLats2  TASVAGKPESSLGPPNTITAVTSPPIQPPVKSIRVMRPEPKAVCPCHSGWLS-----
DmLats  APLAKP-----QPRVYQARSQPIIMQSVKSTQVQKPVLOAVARQSSASASNSPVHVLAA P P S Y P Q K S A A V V Q Q Q Q

641 | . . . . . : . . . . . 7 | . . . . . : . . . . . 720
HyLats  -----YTKNSNHLST-----TQGR-----NIINSPHN
DrLats1  -----
DrLats2  -----
DmLats  QAAAAHQQQQHQQSKPPTPTPPLVGLNPKNCLPEPPSYAKSMQAKAATVVQQQQQQQQQQVQVQQQQVQQQQQQQQQ

721 | . . . . . : . . . . . : . . . . . 8 | 800
HyLats  IQAVHVFGTPT---KMSDN-FS-----PIVESVIPRRSPPPYSAGGSQR---SSKCNLSNIEELENLLYTFSSLLDRN
DrLats1  ---PPPTAYQEPPTPPA-P---AAVPSYQGGPPYPKHLLOQQQ-PPPGYDSSPALK-----
DrLats2  ---AQSPAQDSLGYIPD-ENFTLEPSQERRCPPPYPKTL LVSGS-GPEPASLEPVAM-----SG
DmLats  LQALRVLQAQAQRERDRERERDQKLANGNPGRQMLPPPYSNNNNNSEIKPPSCNNNNIQIS-NSNATPPPIPPAK

801 | . . . . . : . . . . . : . . . . . : . . . . . 880
HyLats  FNKEDVESNLGDSSTFLSNSDNTERFDGVKRS---YSPLPECSNPHY-VTMIVRKDHEKESAIEENENQHENASLR LKNY
DrLats1  ---PCSKEGDAAD-----NENDTSTCSSERAEGSEBEKKQITTSVPVVRH-----KRDEERKGESRIPMY
DrLats2  LQ---DVSATGRTIQSSSVGQEEPIFRDKVKMGKEKAVDKKQIQTSVPVVRN-----ARDEE-KRESRTKSY
DmLats  YNN--NSSNIGANS SGGNGS-----TGTTASSTSSCKKIKHASPIPERK-----ISKEKEBERKEFRIQY

881 | . . . . . 9 | . . . . . : . . . . . : . . . . . 960
HyLats  TPQACKFYLEQHFENLLKROEQREVRKQLEDEM SVGLPQDQEQMRKLLROKESNYIRLRAKMDKTMFTKIKIIGIG
DrLats1  SPQAFKPFMEQHVENILKNHQORRRKQLESEMORVGLSGDAQEQMRMLLQKESNYIRLRAKMDKCMFEKIKTLGIG
DrLats2  SPFAFKFYMEQHVENVKTYQKLNRRQLEQEMSKAGLSEAEQEQMRMLLQKESNYIRLRAKMDKSMFVKIKTLGIG
DmLats  SPQAFKPFMEQHIENVIKSYRQRTYRKNQLEKEMKVGLPDQIQIEMRMLNOKESNYIRLRAKMDKSMFVKIKPIGVG

961 | . . . . . 0 | . . . . . : . . . . . 1040
HyLats  AFGEVSLVRKNGTE-AYYAMKTLRKSEVVRNQAHVHKAERDILAEADNEWVVKLYSFDQDNNLYFVMDYVPGGDLMLAL
DrLats1  AFGEVCLARRVDTG-ALYAMKTLRKDVLLRNQVAHVHKAERDILAEADNEWVRLYYSFDQDNNLYFVMDYVPGGDMMSL
DrLats2  AFGEVCLARRVDTG-ALYAMKTLRKDVLLRNQVAHVHKAERDILAEADNEWVRLYYSFDQDNNLYFVMDYVPGGDMMSL
DmLats  AFGEVTLVSKIDTSNHLVAMKTLRKADVLRNQAHVHKAERDILAEADNEWVVKLYSFDQDNNLYFVMDYVPGGDLMSL

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STKc-LATS


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1041      . . . . . 1 . . . . . 1120
HyLats  LIKRGIFEDNLARFYIGELVLAIESVHKLGFIHRDIKPDNVLIDRYGHIKLTDGFLCTGFHWTHDSKYYQPEQALENHSR
DrLats1  LIRMGIFQEDLAQFYIAELICAVESVHKMGFIHRDIKPDNMLIDRDGHIKLTDGFLCTGFRWTHDSKYYQS---GDHVR
DrLats2  LIRMGVFPEVLARFYVAELTLAIESVHKMGFIHRDIKPDNMLIDLDGHIKLTDGFLCTGFRWTHDSKYYQK---GNHIR
DmLats   LIKRGIFEEELARFYIAEVTCAVDSVHKMGFIHRDIKPDNMLIDRDGHIKLTDGFLCTGFRWTHDSKYYQEN---GNHSR

1121      . . . . . 2 1200
HyLats  QFSMEPEGGWDSLVEEGDCGCKSSERLDNDLYEPLORRNMRRHMRCAHSLVGTPNYIAPEVLMRIPYSQOCDWWSVGVV
DrLats1  QDSMDFSMEWEDS---ANCRCG-----DRLKPLERRAAROHORCLAHSLVGTPNYIAPEVLLRTGYTOLCDWWSVGVV
DrLats2  QDSMEPSDSWDDV---SNCRCG-----DRLMTLEQRATROHORCLAHSLVGTPNYIAPEVLLRKGYTOLCDWWSVGVV
DmLats   QDSMEPWEEYSE-----NG-----PKPTVLERRRMRDEHORVLAHSLVGTPNYIAPEVLERSGYTOLCDWWSVGVV

                                STKc-LATS

1201      . . . . . 1280
HyLats  LYEMLIGHPPFMARTPAEIQLKIIINWKETLIR--KKLPRHSENLIQLCSAPENRIGRNGAHEIKNHPIYFDNFNFLSIH
DrLats1  LYEMVVGOPPFDAITPLETOMKVIIRWQTSLEIPLQAKLSPEATDLILKLCRGPDDRLGRNGADEIKAOPIFFRTIDFSKDL
DrLats2  LFEMLVGOPPFDAITPTEAOLKVINWENTLQVPPQVKLSPEAVDIIGQLCSAERLGGNGAGEIKAHPIFFSEVDFSSNL
DmLats   LYEMLVGOPPFDAITPLEAOKVINWETLEIIPPOAELSRREATDLIRRLCASADKRLCK-SVDEVKSHDFFKGIDFADMR

1281      . . . . . 3 . . . . . 1360
HyLats  E--EKAPFVPIINHPTDTSNFDVPVPEKSNEFDIGDVSSQDV-----DDKLGPGAYAFYEFTFRHFFDDGGFANPVAKK
DrLats1  RQQHQAPYIIPKITHSTDTSNFDVDPD---KLWSEADAGNHNDTLTRWFKNGKHEHAFYEFTFRFFDDNGHPYSCPYP
DrLats2  RTQ--PAPYRPKIAHFMOTSNFDVPEEEGGPGAWSDSGDSSTRT-WDTLCSPHGKHPEHAFYEFTFRFFDDNGCPFRYPKP
DmLats   K--CKAPYIPEIKHPTDTSNFDVDPKELRSNDSTMSGDD-----VDQNDRTFHGFFFEFTFRFFDDKQPPDMTDD-

1361      . . . . . 4 ] 1406
HyLats  IEQNIKPNVTVSPAKRSATTTNSISIPNKVSVPNSDANKSKLPVVF
DrLats1  IESEEEDEDEDYVEE-----DEP-----SQRPMESRDLVYV
DrLats2  LEATQSLSSSDGAGS-----LKPELEEEEAHEEEEQGEGCEPVYV
DmLats   -----QAPVYV

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% identity      1 [ . . . . . : . . . . . 80
100.0% HyMST   -----MSRSLKKLSEESLNRPAAEVFDLLAKLGEAGYGSVYKAMHKESGEVLAIKQVPVDTDLQ
56.20% DmHippo MSEPEVTSVVDMKSPNIISSSCSFFFLKKLSEESLQPPKRVFDIMYKLGEGSYGSVYKAVHKESSSIVAIKLVVPESDLH
60.00% HsMst     -----METVQLRNPPRRQLKKLDEDSLTKQPEEVFDVLEKLGEGSYGSVYKAIHKETGQIVA IKQVPVDESLO
60.95% DrMST     -----MEHSVPKNKLKKLSEDSLTKQPEEVFDVLEKLGEGSYGSVFKAIHKESGQVVAIKQVPVDESLO

81 . . . . . : . . . . . 160
HyMST   EIIKEISIMQQCDSPYVVKYFGSYFKNTDLWIVMEYCGAGSVSDCMLRNKTLTEDEIACICRDTLKGLEYLHLRRKIHR
DmHippo EIIKEISIMQQCDSPYVVKYFGSYFKQYDLWICMEYCGAGSVSDIMRLRNKTLTEDEIATILSDTLQGLVYLHLRRKIHR
HsMst   EIIKEISIMQQCDSFHVVKYVGSYFKNTDLWIVMEYCGAGSVSDIIRLRNKTLEDEIATILQSTLKGLEYLHFMRKIHR
DrMST   EIIKEISIMQQCDSPYVVKYVGSYFKNTDLWIVMEYCGAGSVSDIIRLRNKTLEDEIATVLKSTLKGLEYLHFMRKIHR

161 . . . . . 2 . . . . . 240
HyMST   DIKAGNILLNTEGHSKLADFGVAGQLTDTMAKRNTVIGTPFFWMAPEVIOEIGYDCKADIWSLGITLLEMAEGKPPHADIH
DmHippo DIKAGNILLNTEGYAKLADFGVAGQLTDTMAKRNTVIGTPFFWMAPEVIEEIGYDCVADIWSLGITLEMAEGKPPYGEIH
HsMst   DIKAGNILLNTEGHAKLADFGVAGQLTDTMAKRNTVIGTPFFWMAPEVIOEIGYNCVADIWSLGITALEMAEGKPPYADIH
DrMST   DIKAGNILLNTEGHAKLADFGVAGQLTDTMAKRNTVIGTPFFWMAPEVIOEIGYNCVADIWSLGITSIEMAEAGKPPYADIH

241 . . . . . 3 . . . . . 320
HyMST   PMRAIFMIPKPPPTFKHPEKWSKDMIDFVSKCLVKNPDDRLSATALLQHPFIKNSKPVEVLAQMLSDAMRIRREEVEKN
DmHippo PMRAIFMIPKPPPSFREPDWSTEFIDFVSKCLVKEPDDRATATLLEEFIRNAKHSRSLKPMLEETCAIREQQRANR
HsMst   PMRAIFMIPNPPPTFRKPELWSDNFDVFKQCLVKSPEQRATATLLOHPFVRSAGVSIIRDLINEAMDVKLKROESQ
DrMST   PMRAIFMIPNPPPTFRKPEHWSDFDFVFKCLVKNPEQRATATLLOHPFIIVGAKPVSIIIRDLITEAMDMAKAKROQEQ

321 . . . . . : . . . . . 4 400
HyMST   NGN--D-----DEGDIDN--DQDIFV--TAHTDTIM-----ANQDMGMTILNNDIDSNDGMTI-----
DmHippo SFGGVLAAASQAKSLATQENCMQQHITDNAFMEDPGTLVPEKFGYEQSSASDATMIAHAEQGVDECTLGPGGLRNLSKAA
HsMst   QRE--M-----DQDDEE--N--SEEDM--DSGTMVRA-----VGDEMGTVRVASTMTDGA-----
DrMST   QRE--L-----EDDEN--S--EEEVEV--DSHTMVKS-----GSESAGTVRATGTMDSGA-----

401 . . . . . : . . . . . 480
HyMST   ---INSCTMIGHEMDTMITINSCTMIEENMGTMVINDDGDDCTMKSLE---SSTGKSTQYRPAYLEHFKEKLELQNRNI---
DmHippo APAAAASAASPLDMPAVDSGTMVELESNLGTMVINSDSDSTAKN---NDDQKPRNRYRPFLEHEDRKNAGDGRGDEKP
HsMst   -----NTMIEHDDT-LPSQLGTMVINAEDEEEE---GTMKRRETMQPAKPSFLEYFEQEKENQINS---
DrMST   -----CTMIEHGSITMLESNLGTMVINSDDEEEEDIGSMRRNPTSQOIRPSPFMDYIDKQDSNKAQEG---

481 . . . . . 5 . . . . . : . . . . . 560
HyMST   -----VGNK--R-----
DmHippo IATEYSPAAAEQQQQQQQQQQQQQDEQHLASGANDLNNWEHNMEMQFQQISAINOYGLQQHQQQQVLMAYPLMNEQLI
HsMst   -----FCKS--V-----
DrMST   -----FNHN--Q-----

561 . . . . . 6 . . . . . 640
HyMST   ---VNEENML--KGS-----DPNVPGKLLGPIDFEFLKNLSFEELQORMSSLDTDMEREIEDLR
DmHippo ALNNQPNLLSNAAPMGQGI PAAAPAQPPPAYQNQHMTQSHAYVEGEFEFLKFLTFDDLNRCLNIDHEMEDIENLN
HsMst   ---PGP---L-----KNSSDWKIPQDGDVEFLKSWTVEDLCKRLLALDPMMEQIEIEIR
DrMST   ---QDPLCI--SKT-----AFPDNWIKVPQDGDVEFLKNLDFEELQMLTALDPMEREIEBLR

641 . . . . . .] 671
HyMST   RRYQVKKRHPILAEAMEVKKRRQANF-----
DmHippo KRYNAKRQPIVDAMNAKRRQONINNNLIKI
HsMst   QKYQSKRQPIILDAMDAKKRRQONF-----
DrMST   QRYTAKRQPIILDAMDAKKRRQONF-----

MST1_SARAH

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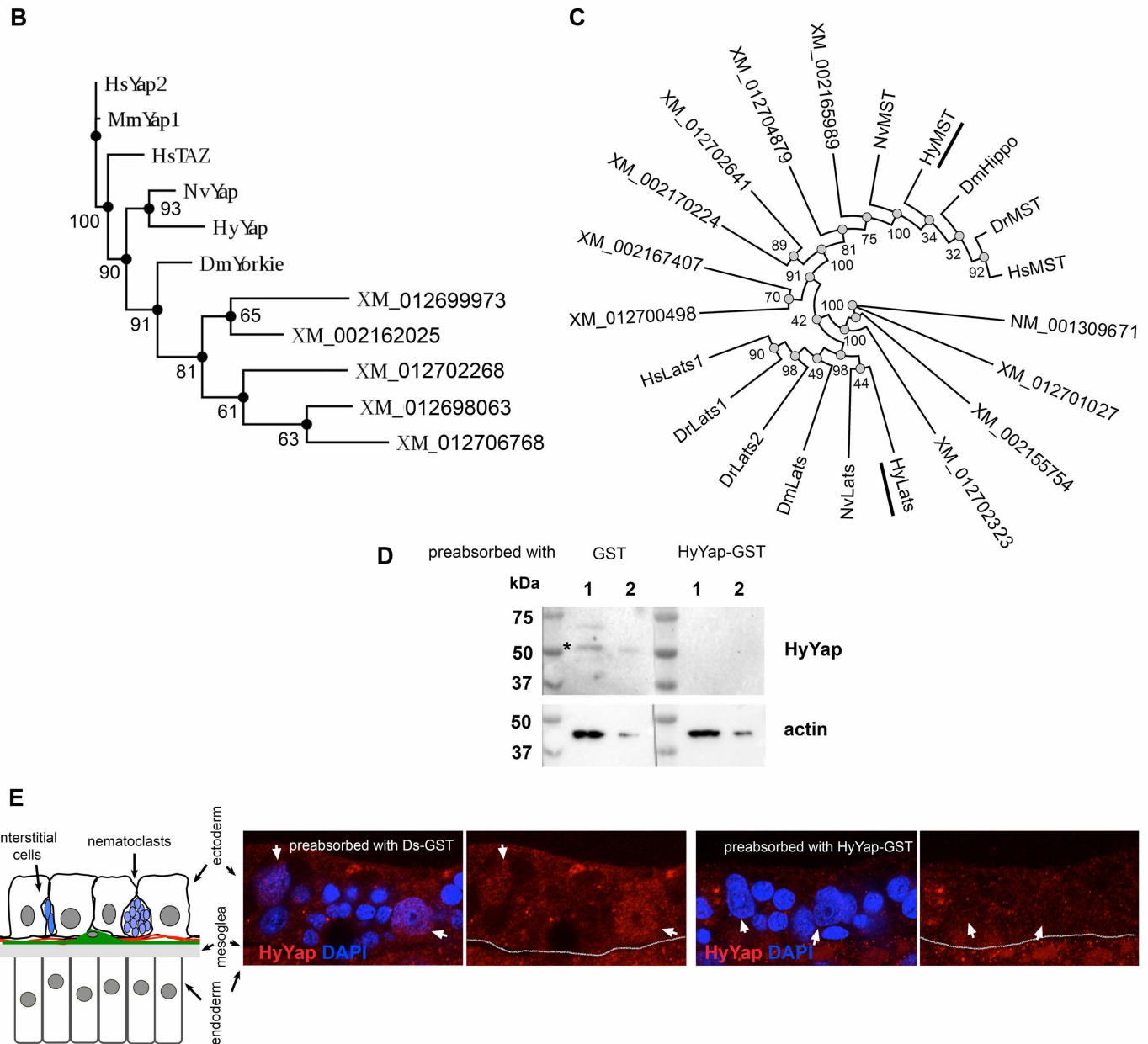


Fig. S1. (A) Alignments of predicted *Hydra* Yap, LATS and MST proteins with *Drosophila* and vertebrate homologues. Characteristic domains of proteins are underlined: WW – tryptophan containing domain that binds specific proline-rich sequences, STKc-LATS – catalytic domain of LATS family of serine/threonine kinases, STKc-MST1/2 catalytic domain of MST family of serine/threonine kinases, MST1-SARAH – apoptosis-mediating SARAH domain of MST1 proteins.

Dm – *Drosophila melanogaster*, Mm – *Mus musculus*, Hs – *Homo sapiens*, Dr – *Danio rerio*. (B) Phylogenetic tree of Yap family proteins based on the complete protein sequence, Maximum Likelihood analysis (100 bootstrap replicates, bootstrap values are indicated for each node); predicted *Hydra* homologue of Yap is underlined; Hs – *Homo sapiens*, Mm – *Mus musculus*, Nv – *Nematostella vectensis*, Hy – *Hydra vulgaris*, Dm – *Drosophila melanogaster*; XM_012699973, XM_002162025, XM_012702268, XM_012698063, XM_012706768 - sequences that came at the top of BLAST search of *Hydra* nucleotide databases for Yap along with NP_001296578 (HyYap) and encode homologues of WW domain containing proteins; sequences were aligned with Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) and analyzed using Akaike Information Criterion (<http://www.atgc-montpellier.fr>). (C) Phylogenetic tree of LATS and MST families based on the complete protein sequence, Maximum Likelihood analysis (100 bootstrap replicates, bootstrap values are indicated for each node); predicted *Hydra* homologues LATS and MST proteins are underlined; XM_012702323, NM_001309671, XM_012701027, XM_002155754 – sequences that came at the top of BLAST search of *Hydra* nucleotide databases for LATS along with MW650881 (HyLATS) and encode homologues of serine/threonine protein kinases; XM_002167407, XM_012700498, XM_002165989, XM_012704879, XM_002170224, XM_012702641 - sequences that came at the top of BLAST search of *Hydra* nucleotide databases for MST along with MW650879 (HyMST) and encode serine/threonine protein kinases (S6, MINK1, Pak, PKA); sequences were aligned with Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) and analyzed using Akaike Information Criterion (<http://www.atgc-montpellier.fr>). (D) Western blot analysis of total *Hydra* lysates with anti-HyYap serum preabsorbed with either GST or HyYap-GST. Lysates are made from either 3 animals (lanes 1) or 1 animal (lanes 2); asterisk indicates a 52kDa band corresponding to the predicted full length HyYap protein (46 kDa, accession number NP_001296578), the differences in the molecular weight may be due to phosphorylation. For the loading control the same blot was immunostained with actin antibodies. (E) Lateral view of *Hydra* ectoderm immunostained with anti-HyYap serum preabsorbed with HyDs-GST or with HyYap-GST antigens. Arrows point to the nuclei of ectodermal epithelial cells; schematic drawing of the lateral view of *Hydra* ecto- and endoderm is shown on the left.

Figure S2

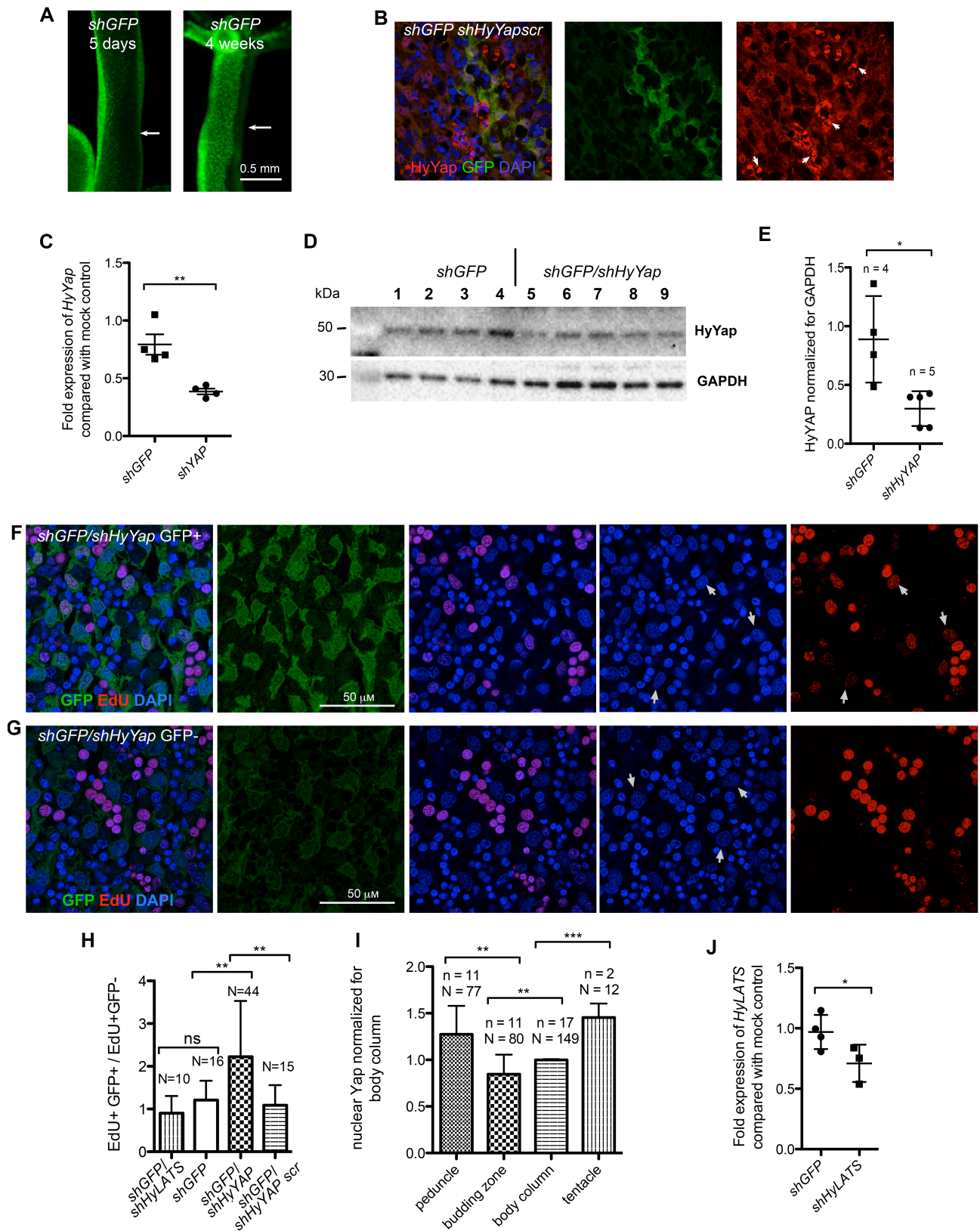


Fig. S2. (A) *GFP* hydras electroporated with *shGFP* 5 days and 4 weeks after electroporation. Arrows point to the areas of GFP knockdown. Different animals are shown in two panels. Reduced expression of GFP is still visible 4 weeks after electroporation. (B) Apical view of ectoderm of *GFP* hydras electroporated with *shGFP/shHyYapscr* hairpin and immunostained with anti-GFP and anti-HyYap antibodies. Arrows point to a non-specific staining of nematocyte capsules that is observed for a variety of antibodies when used *Hydra* immunostaining protocol. (C) qPCR analysis of *HyYap* in *GFP* hydras electroporated with either *shGFP* or *shGFP/shHyYap*. 4 animals for each condition; two-tailed unpaired t-test. (D, E) Western blot analysis of total lysate from *GFP* hydras electroporated with either *shGFP* alone or *shGFP/shHyYap*, 1 animal per lane; n – number of animals. For the loading control the same blot was immunostained with GAPDH antibodies; two-tailed unpaired t-test. (F, G) Apical view of *Hydra* ectoderm electroporated with *shGFP/shHyYap*, pulse labeled with EdU 6 days after electroporation and immunostained for GFP and EdU; (F) – GFP⁺ area, (G) – GFP⁻ area; arrows point to epithelial cells; images are taken in the body column areas. Only ectodermal epithelial cells express GFP in *GFP Hydra*, therefore not all cells are GFP⁺ in (F). (H) Graph shows ratio between numbers of EdU⁺ ectodermal epithelial cells in GFP⁺ and GFP⁻ areas in *GFP* hydras electroporated with either *shGFP* alone, *shGFP/shHyYap* or *shGFP/shHyYap scr* and pulse-labeled with EdU 6 – 7 days after electroporation; EdU⁺GFP⁺/EdU⁺GFP⁻ ratio was calculated for each individual animal; the total of 60 – 200 cell was used in analysis of each animal; n – number of animals; two-tailed unpaired t-test. (I) Graph shows the intensities of HyYap immunostaining in nuclei of tentacles, body column, budding zone and a peduncle normalized for the projection area of nuclei and the intensity in the body column; n – number of animals, N – number of nuclei; two-tailed unpaired t-test. (J) qPCR analysis of *HyLATS* in *GFP* hydras electroporated with either *shGFP* or *shGFP/shHyLATS*. Each point represents an individual animal; one-tailed unpaired t-test.

Figure S3

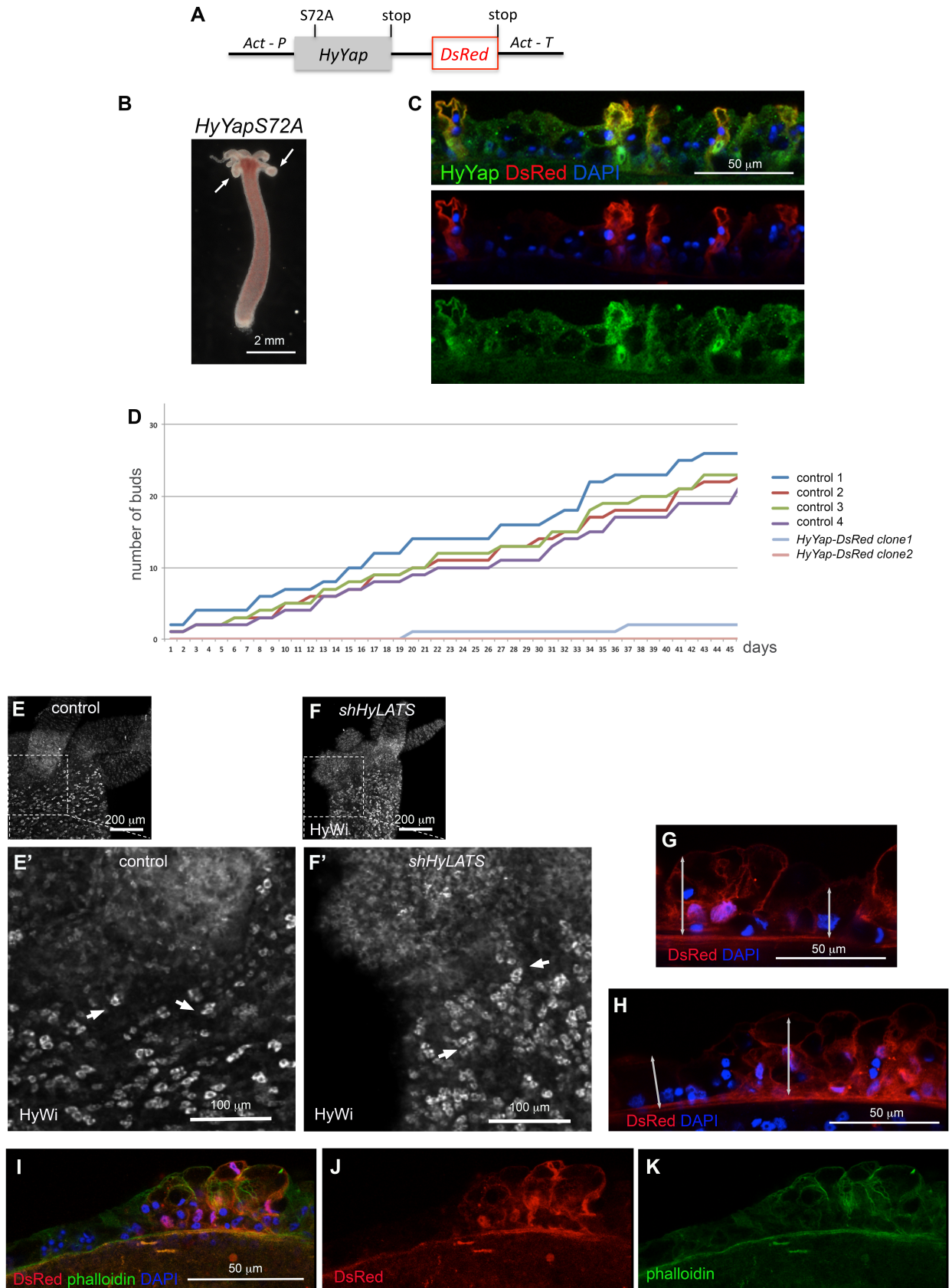


Fig. S3. (A) Schematic drawing of the construct used to generate *HyYap-DsRed* transgenic *Hydra*. (B) Photos of live *HyYap-DsRed* hydras. Arrows point to the thick tentacles. (C) Lateral view of the ectoderm of *HyYap-DsRed Hydra* immunostained for HyYap and DsRed. (D) Budding rate of control and *HyYap-DsRed* hydras shown as the number of buds produced by a single polyp over time. (E – F') The stem/progenitor marker HyWi is not detected in either control (E, E') or thick (F, F') tentacles, arrows point to undifferentiated interstitial cells expressing HyWi. (G) Lateral view of the ectoderm of *HyYap-DsRed Hydra* immunostained for DsRed. Double-headed arrows indicate the apico-basal dimension of transgenic and wild type cells. (H) Lateral view of ectoderm of *HyYap-DsRed Hydra* immunostained for DsRed. Double-headed arrows indicated the apico-basal dimension of transgenic and wild type cells. (I - K) Lateral view of the ectoderm of *HyYap-DsRed Hydra* immunostained for DsRed and phalloidin.

Figure S4

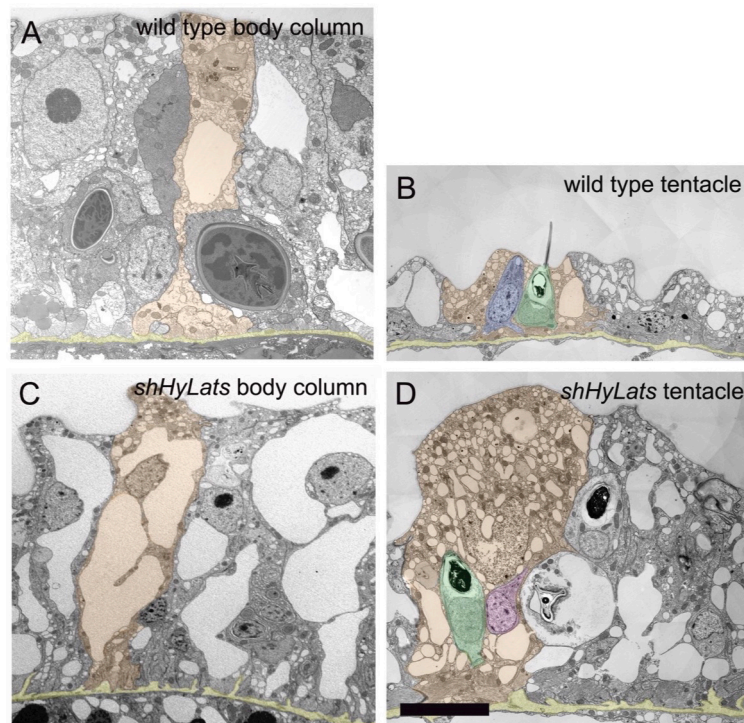


Fig. S4. Low magnification transmission electron microscopy images of wild type and *shHyLats* ectoderm. (A,B) Images of body column and tentacle tissue in wild type polyps; (C,D) Corresponding images of *shHyLats* polyps. One representative ectodermal epithelial cell is colored in orange. The mesoglea is colored in yellow. *shHyLATS* thick tentacles show a dramatic expansion of ectodermal epithelial cells in along their apical-basal axis. They generally exhibit a higher volume of intracellular vacuoles indicating that their osmoregulation is affected. (B) Wild type epithelial cells in the tentacles (called battery cells) incorporate differentiated nematocytes (stinging cells; green) mounted at their apical membrane and a sensory nerve cell (blue). (D) Tentacle epithelial cells in *shHyLATS* thick tentacles also exhibit incorporated and fully differentiated nematocytes, but these are usually not mounted at the apical membrane. In addition, these cells show interstitial precursor cells (violet), which are not occurring in the tentacles of wild type polyps. Furthermore, *shHyLATS* polyps exhibit major disruptions of ectodermal basal muscle fibers and mesoglea structure as discussed in detail using high magnification images in the main paper (see Fig. 4). Size bar: 20 μm .

Figure S5

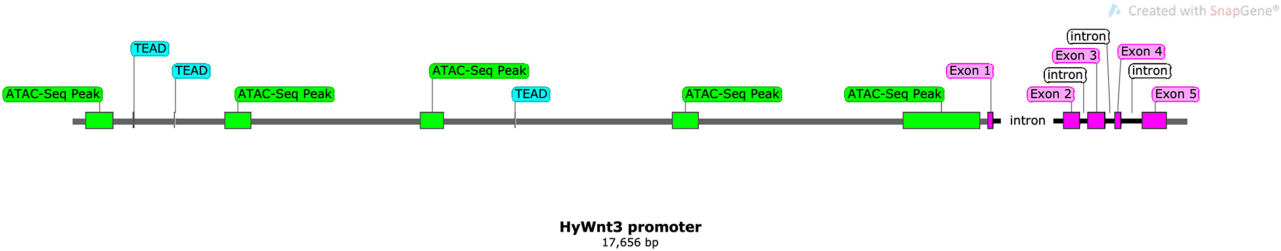


Fig. S5. Map of the HyWnt3 promoter region indicating TEAD binding sites.