Supplementary Material

Supplementary Figure 1. FGF-dependent induction and early patterning of *Ciona* anterior neural tissue. (A-C) Schematics of neural plate development from 32- to 110-cell stages. Upper diagrams show simplified embryos with cells of interest highlighted. Lower diagrams show expression of key patterning genes within the future neural plate. Numbers indicate cell names using the nomenclature of Conklin. Only right-sided blastomeres are labeled, but names apply to their contralateral partners as well. (A) Anterior neural tissue is induced at the 32-cell stage by FGF9 from vegetal blastomeres. (B) At the 64-cell stage, *Dmrt* is induced in the six precursors of the anterior neural plate. (C) By the 112~~0~~-cell stage, the anterior neural boundary (ANB) has been established between the *ZicL*+ cells (anterior neural tube) and the *FoxC*+ cells (presumptive palps, OSP, neurogenic ectoderm).

Supplementary Figure 2. Sequential requirement for MEK in early neural plate patterning. (A-C) Embryos were electroporated with *ZicL>LacZ* and *Dmrt>H2B:YFP,* treated with DMSO (A) or U0126 at 76-cell (B) or 112-cell (C) stages, and fixed at mid-gastrula for *in situ* hybridization and immunohistochemistry. **(A)** In control embryos, *ZicL* and *Dmrt* reporters are co-expressed in rows III and IV. *Six3/6* is restricted to row IV. **(B)** U0126 treatment at 76-cell stage results in loss of *ZicL* reporter expression in rows III and IV and loss of *Six3/6* expression. **(C)** U0126 treatment at 112-cell stage results in normal expression of *ZicL* reporter and a poster expansion of *Six3/6* into row III.

Supplementary Figure 3. Expression of ETS family transcription factors in the neural plate. (A-C) Embryos were electroporated with *ZicL>LacZ* and *Dmrt>YFP-CaaX*, then fixed at mid-gastrula or neurula stages and assayed for expression of *Ets1/2* or *Elk1/3/4* by *in situ* hybridization with immunohistochemistry. Lower panels show *in situ* hybridization channel alone for clarity. (A,A') *Ets1/2* is expressed throughout the neural plate at mid-gastrula stage. (B,B') At early neurula stage, *Ets1/2* expression is more restricted, with strongest expression in the lateral a9.50 cells of row IV. (C,C') *Elk1/3/4* is expressed in rows I, III, and IV of the mid-gastrula neural plate, but excluded from row II.

Supplementary Figure 4. (A) Schematic of Ets1/2 and Elk1/3/4 proteins. Ets1/2 consists of a C-terminal ETS DNA-binding domain and a SAM/Pointed protein-protein interaction domain from residues 182-265. Elk1/3/4 has an N-terminal ETS DNA-binding domain. Domain predictions were made using SMART (~~http://smart.embl-heidelberg.de/ –~~Schultz et al., 1998; http://smart.embl-heidelberg.de/). Yellow and blue boxes indicate residues used in Ets1/2 and Elk1/3/4 fusion proteins, respectively. **(B)** Alignment of Ets1/2 and Elk1/3/4 proteins. Purple shading indicates identical residues, pink shading indicates similar residues, and unshaded residues are not conserved. Underlined residues indicate those included in fusion proteins. Red line indicates core ETS domain. Ets1/2 and Elk1/3/4 are ~65% identical within this region. Alignment was generated using T-Coffee Expresso (Armougom et al., 2006; http://www.tcoffee.org).

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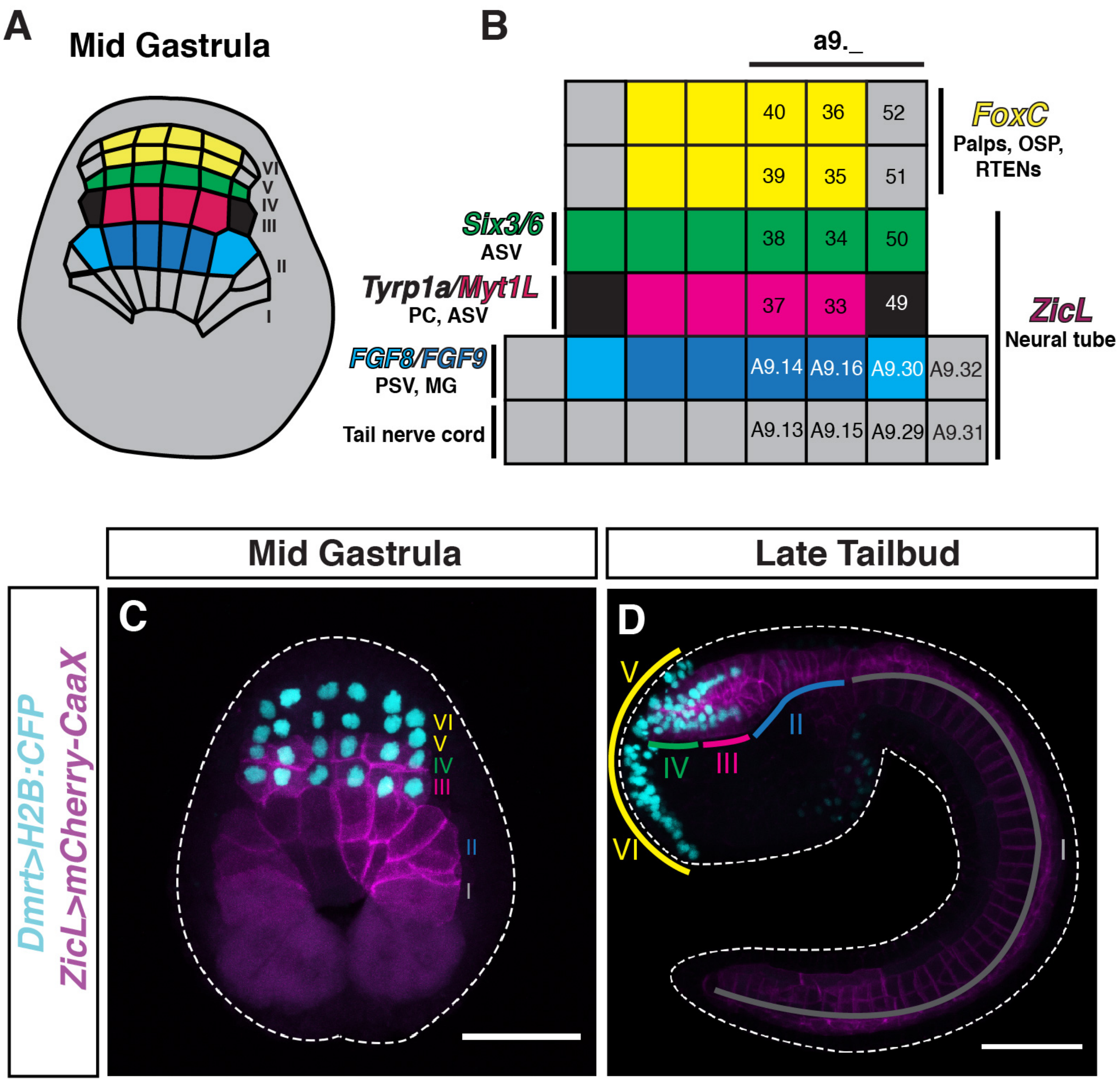
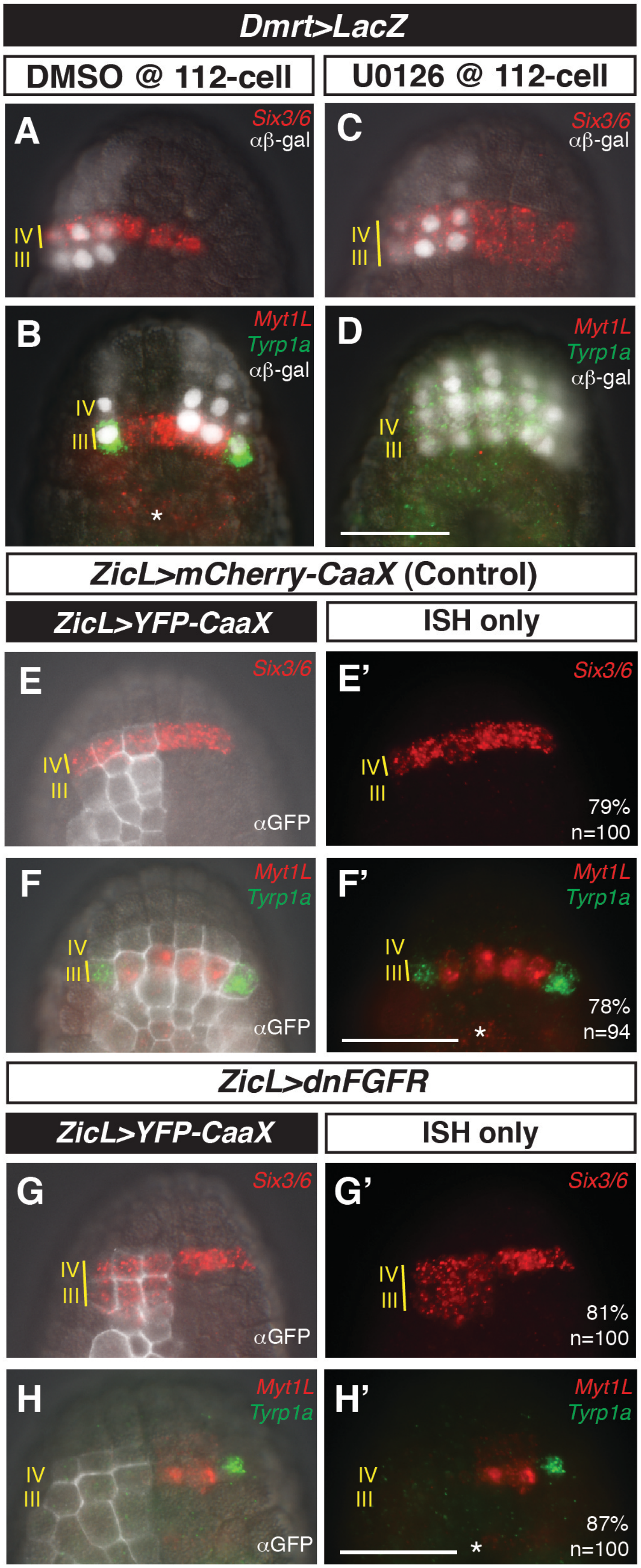
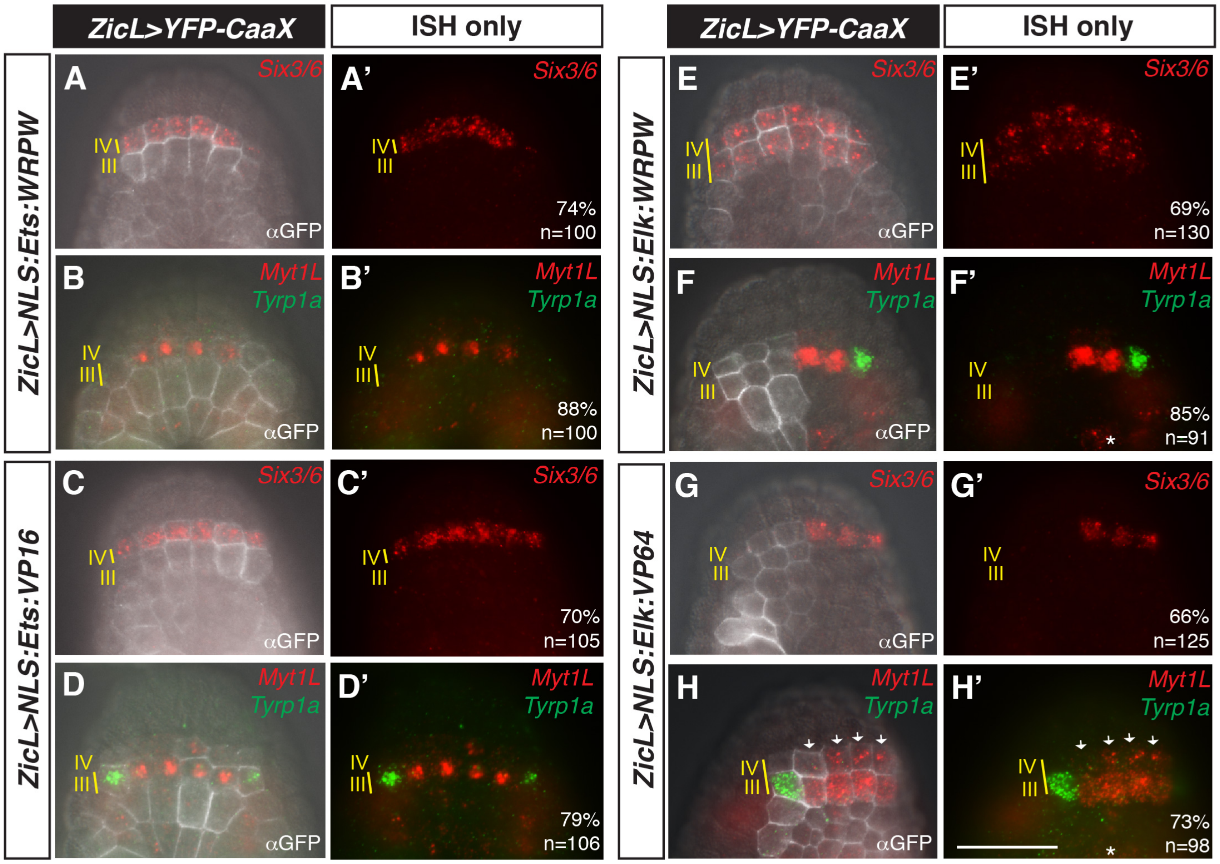


Gainous et al., Figure 1



Gainous et al., Figure 2



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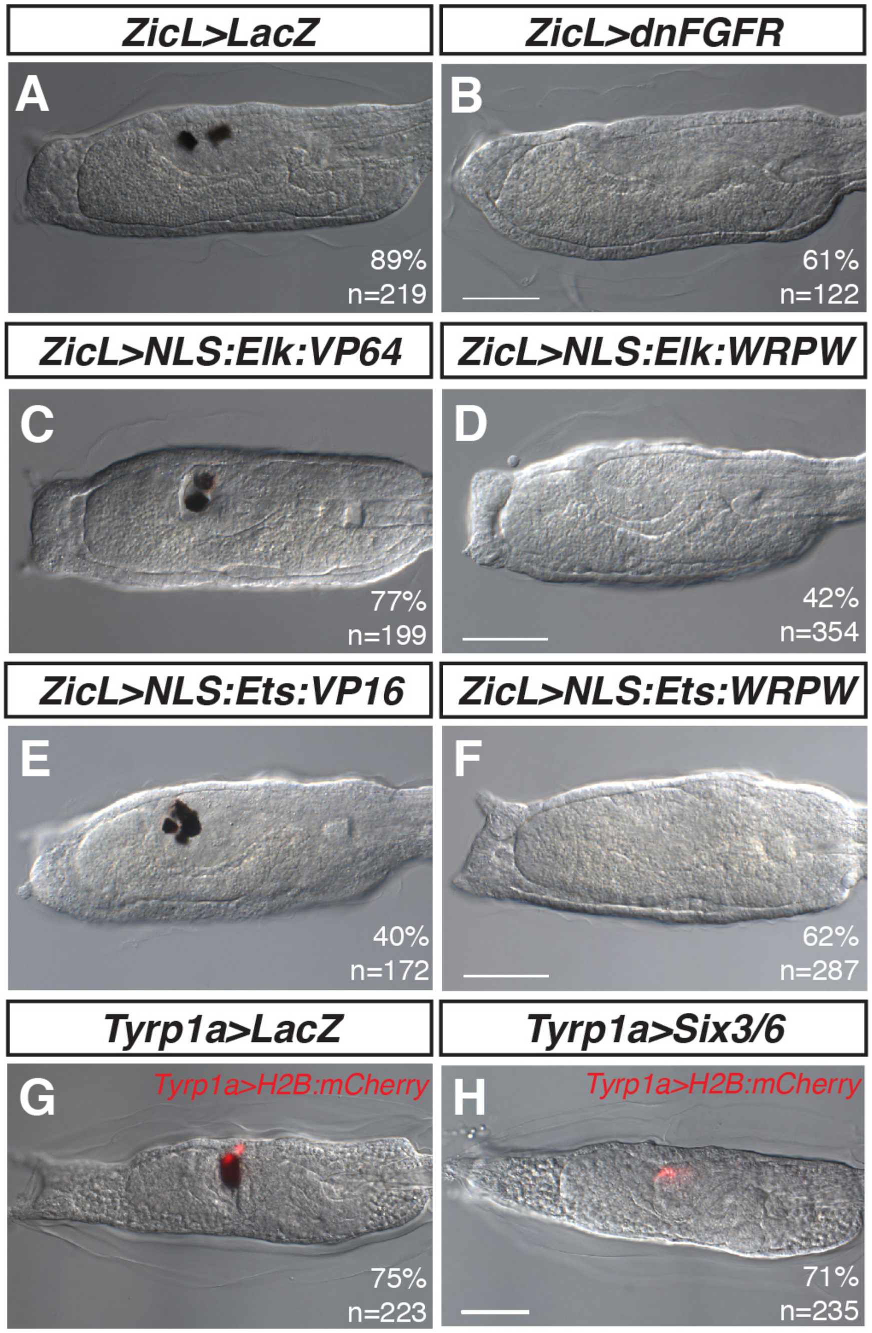

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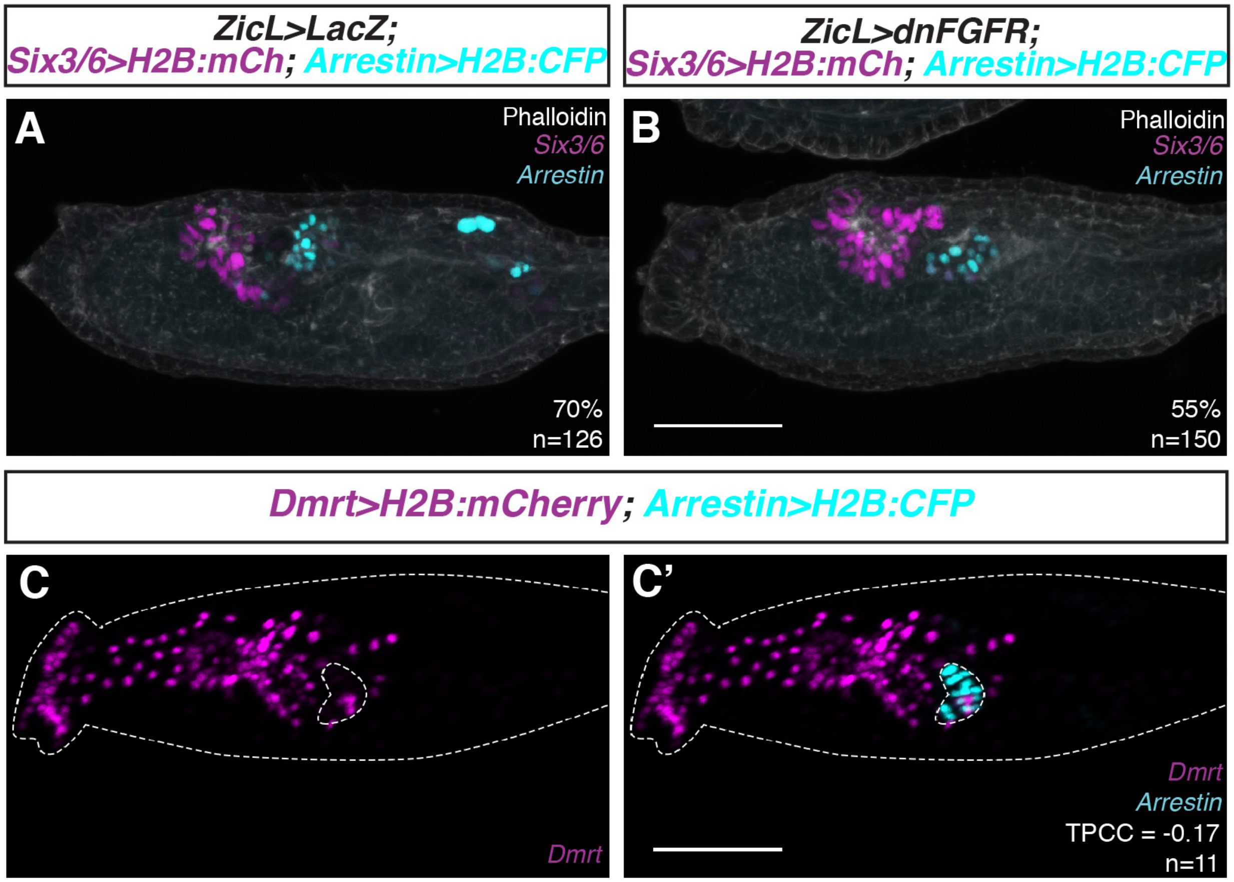

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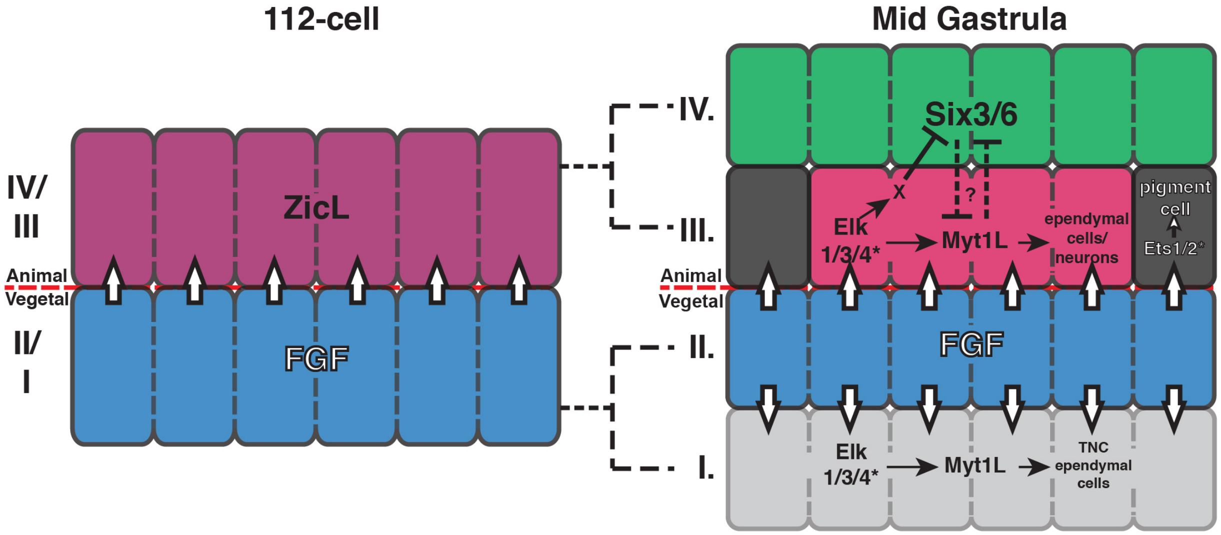

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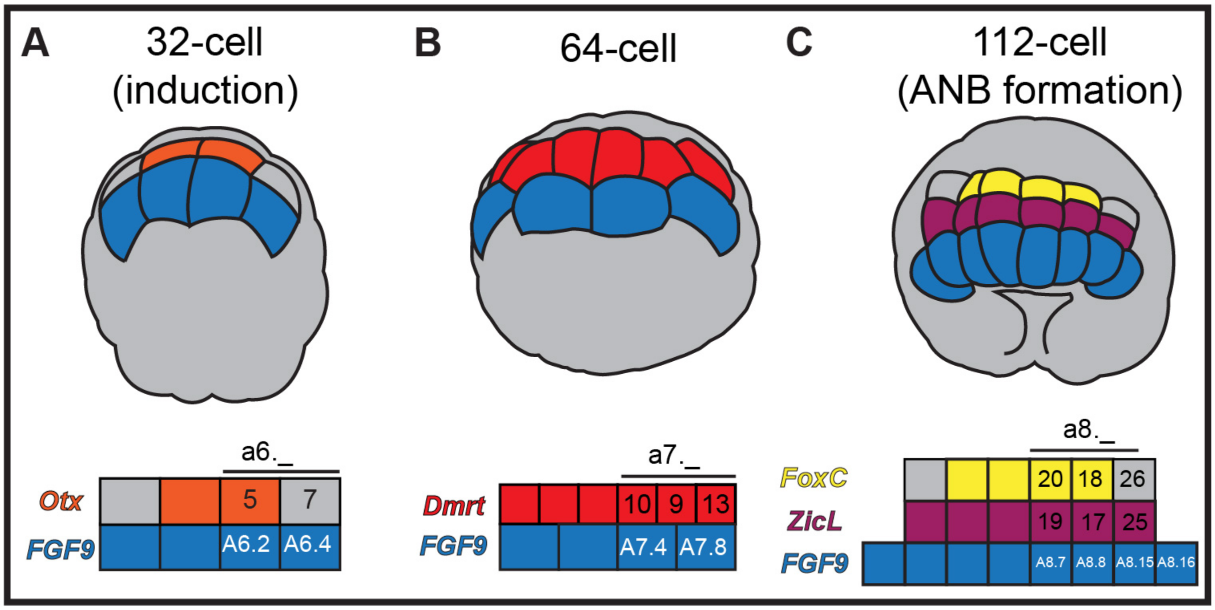

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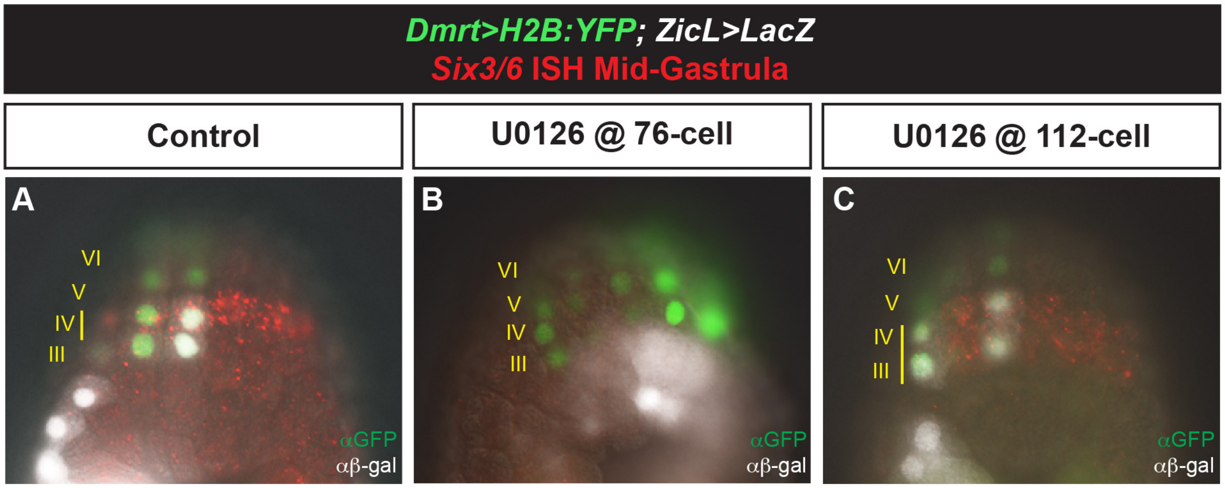

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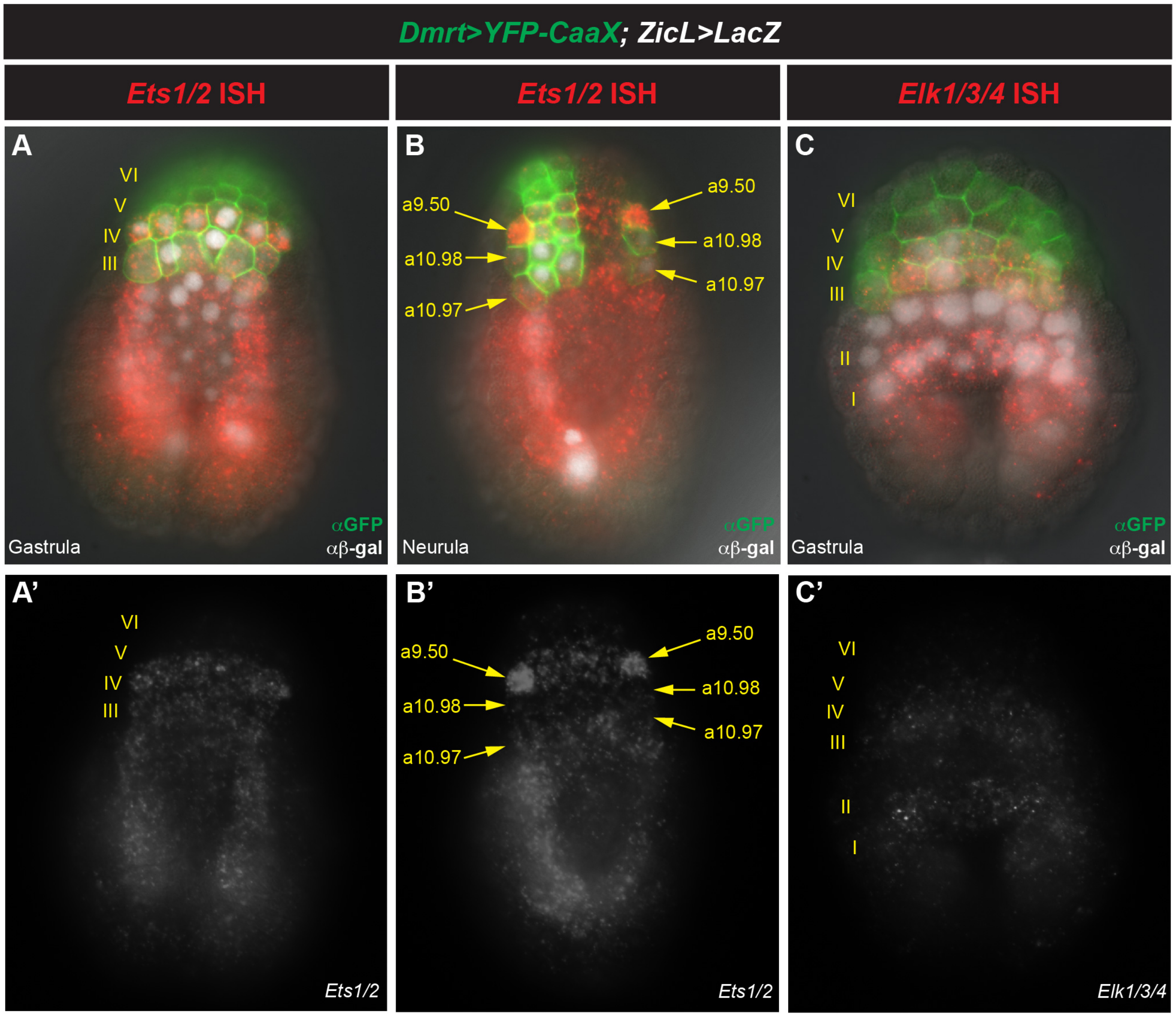

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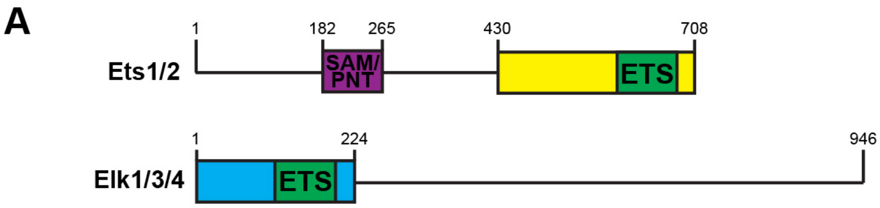

B

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Ci-Ets1/2      1 MVDQHVVPSSFDINIPSINIKPDDDFFDNDLDFDVKLSNEDFSSPEDFVSTPVLPDDFLP
Ci-Elk1/3/4    1 MMTLKIDTADFDR--SSIEQQPQQDG----------------------------------
consensus      1 *.  ..    ** .. **   *  * ..................................

Ci-Ets1/2     61 DLNHCSIKQSSFDKQIAYENLSAHDASHIQCNESTEYSSSYNGKHEVCTPVKNYPGKGKD
Ci-Elk1/3/4   25 -----------------------------KCNNSGHKS--------------------TE
consensus     61 ............................. ** *   *.................... .

Ci-Ets1/2    121 ICNITPNVTPRSAQKSMPGTPLEELTGLPEKLAPEDVPKTPLFPLITPGTGAKMNDAITQ
Ci-Elk1/3/4   36 TR----NVTD----------------------------------------SAVENDRIKS
consensus    121   ....*** ........................................ *  ** *

Ci-Ets1/2    181 SFSSFRNIMEANSMSKDPKLWSAPQVKTWARWIAQEFSIPSLDESNFCISGSMMCSLRKE
Ci-Elk1/3/4   52 SD---------------------PEKGLLD---RENDGSPSLDERSSMVNM---------
consensus    181 * .....................*      ...      *****    .  .........

Ci-Ets1/2    241 SFLHLCPPFVGEILWEHLDRLQSECGNDTRIPECSNLNQNNDKISSSTTPQNPPSCTTNP
Ci-Elk1/3/4   79 -----------------------------------------EEIDM--------------
consensus    241 .......................................... *  ..............

Ci-Ets1/2    301 PYPTRPAPPYTKNPSFNQPIQNHFSLDQPPQFKEERYRPQHLDTPSHFNPTDAPPVDMS-
Ci-Elk1/3/4   84 ---------------------------------------------------QAPPSDKRL
consensus    301 ................................................... *** *  .

Ci-Ets1/2    360 CVKREQLPHYPSHAFPVNTNRRNFSFEHSMNIKPEPMSHINAFDMHRQMSHPHKPNQRFG
Ci-Elk1/3/4   93 CL---------------------------------------------------ANMQQLG
consensus    361 *....................................................   *  *

Ci-Ets1/2    420 MHMENRRSSEPILTPLKPFYRHRPLSQQMSHPFPTPTTPNAPELPGHPMHLPPHEMGGQQ
Ci-Elk1/3/4  102 AHLPR-------------------------------------------------------
consensus    421  *.  .......................................................

Ci-Ets1/2    480 NLQDLYYINIASLRHHQQIETMKRHEMAKGKIQQTITNRSFGDQQVNQHHMLSRSDSVGW
Ci-Elk1/3/4  107 ------------------------------------------------------------
consensus    481 ............................................................

Ci-Ets1/2    540 NGNKGNINGLLTPEPEDFEGGFMQHSRMNSCMTQNTTAEFPSNPVIPGAFLTGYNGSGPI
Ci-Elk1/3/4  107 --------------VL----------------------------------------DMNV
consensus    541 ..............  ........................................   .

Ci-Ets1/2    600 QLWQFLIELLTDRS-CQHFVTWTGDGWEFKMIDPDEVARRWGRRKNKPKMNYEKLSRGLR
Ci-Elk1/3/4  113 TLWQFLLELLMDPSSNSHLISWTSADGEFKLHNSEEVARLWGLRKNKTNMNYDKLSRALR
consensus    601  *****.*** * *.  * ..**    ***.   .**** ** ****  ***.****.**

Ci-Ets1/2    659 YYYDKNIIQKTAGRRYVYRFVCDLQS----------------------------------
Ci-Elk1/3/4  173 YYYDKNIIKKVNGQKFVYKFVSFPEIIKTETKIPFRVKMERLTQNENGQGGIDDDDEGDP
consensus    661 ******** *  * ..**.**     ..................................

Ci-Ets1/2    685 ------------------------------------------------------------
Ci-Elk1/3/4  233 PSPTPSSSPPSMVVGRSDVTEEDYRYFIQRQQEAKELEQAEKRKKLSHQNEQRQEQRRQL
consensus    721 ............................................................

Ci-Ets1/2    685 ----LLGYSPTE------------------------------------------------
Ci-Elk1/3/4  293 KQLNYSNYSPTQSPADNWRHREMAEESHHATGSLQHATRSVEDVARMSLGAASSAAAAAA
consensus    781 ....   **** ................................................

Ci-Ets1/2    693 ------------------------------------------------------------
Ci-Elk1/3/4  353 AAALSATQEERARNAAATAAFWAGFRQAAAAAVVASTSKPEDPLLTQRYLQAGERQNSGR
consensus    841 ............................................................

Ci-Ets1/2    693 ---------------------------------------------------LHSMLDVKP
Ci-Elk1/3/4  413 KYHTESPPINPRGWDRHNSNNNHRSNTHSYRQQQHNDKYKHDRLSPPYPTQFHKSRKSPP
consensus    901 ................................................... *      *
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Ci-Ets1/2    702 EDRLSD------------------------------------------------------
Ci-Elk1/3/4  473 KDTVSQYQQYLQQYEKDYSLSMSHHRFKEQPEHTPEELINHNKKALAEAFMARAPQINKP
consensus    961  * .* ......................................................

Ci-Ets1/2    708 ------------------------------------------------------------
Ci-Elk1/3/4  533 RPRSPYSSGQKRRYPGEDDVMDVRRRDPSLFCSRLEQDDIPASSNRSSIELKRPKMECSD
consensus   1021 ............................................................

Ci-Ets1/2    708 ------------------------------------------------------------
Ci-Elk1/3/4  593 ISSNAFPFADNFSSYSRELVGKSPPLPRPFDERNEHAESTLAMLMNGHNSAPIENTSNTN
consensus   1081 ............................................................

Ci-Ets1/2    708 ------------------------------------------------------------
Ci-Elk1/3/4  653 TLKRNSLNEAPLDLCTSPKASNSPPGSPRALSPMLKHSPPVSSMDGDTSRTEEKRKSPEL
consensus   1141 ............................................................

Ci-Ets1/2    708 ------------------------------------------------------------
Ci-Elk1/3/4  713 LPAPEQREDEELEKTKQDSGPVTPQKRRLTGKKSIDRKPSPIDLSKPTRTEADDYMDGIS
consensus   1201 ............................................................

Ci-Ets1/2    708 ------------------------------------------------------------
Ci-Elk1/3/4  773 SALYAQFPGIRGSSLLSHDGKSINTPDAVSRKTPSDTNATFFPTSVIMTPSPMVIPSITF
consensus   1261 ............................................................

Ci-Ets1/2    708 ------------------------------------------------------------
Ci-Elk1/3/4  833 WSTLSPMPPHGLGKTDLLAGVREGCSSKSTNNDSKDGEGEKDQKAGSVFQFPTVVNGQMT
consensus   1321 ............................................................

Ci-Ets1/2    708 -----------------------------------------------------D
Ci-Elk1/3/4  893 FAGVPVRPIAAALGQPIPANQVSSVASQLTSHMTGVASPLNLVSSSAVTTLTSS
consensus   1381 .....................................................
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Gainous et al., Supplementary Figure 4

References

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