

RAM Domain

DrosophilaNotch RAHGVTVYFPEGFRAPAAVMS--RRRRDFHGGEM--RNLNKKQVAMQSQSVGQPG-AHWSDDESIMPL
 MouseNotch RQHGGQLWFPECFKVSSEASK--KKRRRPLGEDS--VGLKPLKNASDGALMDDNQNEWGDE--DLET
 NvNotch RVYTKLWLFPECFVRRPVHQRRSLRRDFVCGEHSMSRSMKSSDLEEDAVGGDLTPPQEAR-DAKR

LNR Domains

DrosophilaNotch CDKRCCTEKQGNQICDSDCNTYACNFDGNDCSLGIN-FRANCTAN-----ECWKKF
 MouseNotch CELPECCQVDAGNKVCFNLQCNNEACGWDGGDCSLNYNDPMKKNCTQSL----CCWKYF
 NvNotch CPVPMCAKRFDDGKCNPKCNTHECNWDGTTCSLGIK-FNSNCTTVTKSGKACYQVF

DrosophilaNotch KNGKCNEECNNAACHYDGHDCERKLSKCDSLFDAYCQKHYGDGFCQYGCNNAECSW
 MouseNotch SDGHCDSCQNSAGCLFDGFDQQLTEGQCNPYDQYCKDHFSDGHCDQGCNSAECEW
 NvNotch ANGVCDRECNTEGGCLFDGFDCKPSVPRKG--ADKYCAARFANAECDAICNNVACQN

DrosophilaNotch DGLDCENKTQSPVLAE
 MouseNotch DGLDCAEHVP-ERLAA
 NvNotch DGLDCSFKXP--EIVE

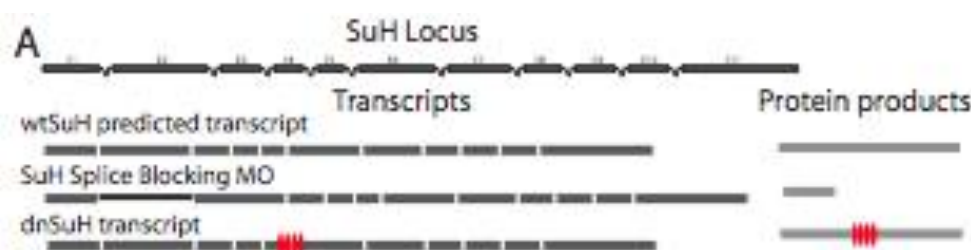
Ankyrin Repeats

DrosophilaNotch DKTGETSLHLAARPARADAARKRLLDAGADANCCDNTGRTPLHAAVAADAMGVFQILLRNR
 MouseNotch DRTGETALHLAAYSRSDRRKH-LEASADANIQNNMGRTPHAAVSAADAGGVFQILLRNR
 NvNotch DIEKETPLHLAARHSRADAARKRLLEAGADPNARDKLGRTPLHLAVGADAQGVFQILLRNR

DrosophilaNotch ATNINARMHDGTTPLILAARLAIEGMVEDLITADADINAAENSGRTALEWAAAVNNTAV
 MouseNotch ATDLDARMHGTTPLILAARLAVEGMLLEDLINSHADVNAVDDLCRSALEWAAAVNVDA
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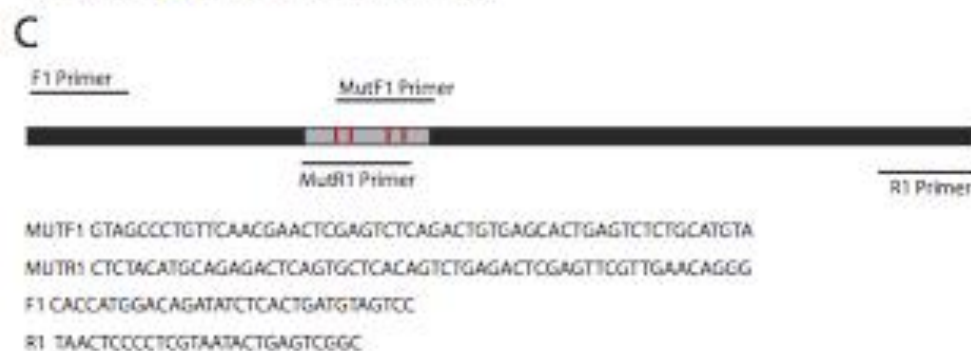
DrosophilaNotch NILLMHNANRDAQDDKDETPLFLAAREGSSYACKALLONTANR
 MouseNotch VVLLKNGANKDIENKKEETSLEPLSIRRESYETAKVLLDHFANR
 NvNotch SEICKNGAKKDAQDDKQGTPLFLGAREGSEAVRILLLSYANR

SupplementalFigure1: Conserved domains of Nv Notch in comparison to Mouse and Drosophila. Invariant residues shared by all three species are pink while those shared between Nematostella and one of the two species are blue.



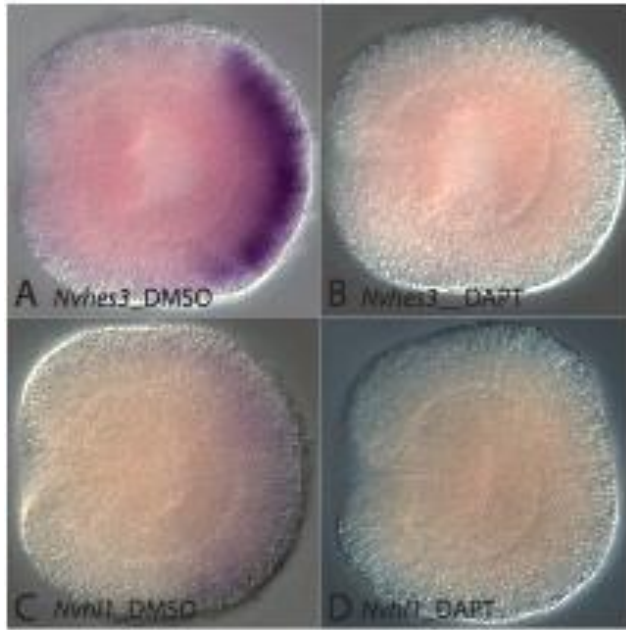
B

DeSuH	EEKRLTSEAMNRYLKERGGQTVLLILBAKVAGKSYGNERKRFPCPPCVYLMGGGKRRKRD	[60]
DeSuH	QPKRLTSEAMNRYLKERGGQTVLLILBAKVAGKSYGNERKRFPCPPCVYLMGGGKRRKRD	[60]
HaSuH	PPKRLTSEAMNRYLKERGGQTVLLILBAKVAGKSYGNERKRFPCPPCVYLMGGGKRRKRD	[60]
NvSuH	MDATLTIDVVMN-----GLQTVVIFSAKVAGKSYGNERKRFPCPPCVYLMGGGKRRKRD	[55]
XenopusSuH	QPKRLTSEAMNRYLKERGGQTVLLILBAKVAGKSYGNERKRFPCPPCVYLMGGGKRRKRD	[60]
<hr/>		
DeSuH	MLQGGDSE-QGAGLCAFTIGIGRS-DQHQQLDLNGQYCAARTLFIISDSEKRRHPLSVK	[118]
DeSuH	MEKQDCE-DESDPCAFIGIGRS-DQHQQLDLNGQYCAARTLFIISDSEKRRHPLSVK	[118]
HaSuH	NERQDCE-QSDPCAFIGIGRS-DQHQQLDLNGQYCAARTLFIISDSEKRRHPLSVK	[118]
NvSuH	SDQAGSDGQDQVAFVAFIGIGRSQDQDGLIIEKQYGAARTLFIISDSEKRRHPLSVK	[115]
XenopusSuH	NERQDCE-QSDPCAFIGIGRS-DQHQQLDLNGQYCAARTLFIISDSEKRRHPLSVK	[118]
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DeSuH	MFYGNAGDIQVFLERRIRVIEKPSKRRQSLKNAQLCIASQTVVALFNLRSGQVSTRYLE	[178]
DeSuH	MFYGNAGDIQVFLERRIRVIEKPSKRRQSLKNAQLCIASQTVVALFNLRSGQVSTRYLE	[178]
HaSuH	MFYGNAGDIQVFLERRIRVIEKPSKRRQSLKNAQLCIASQTVVALFNLRSGQVSTRYLE	[178]
NvSuH	LLYFGQDVGIFNERRIRVIEKPSKRRQSLKNAQLCIASQTVVALFNLRSGQVSTRYLE	[175]
XenopusSuH	MFYGNAGDIQVFLERRIRVIEKPSKRRQSLKNAQLCIASQTVVALFNLRSGQVSTRYLE	[178]
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DeSuH	VSDGNFASDQNGAFYIILLDDESESEKFTVSDGYIHYGQTVKLVCSVTGMALPRLII	[238]
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HaSuH	VSDGNFASDQNGAFYIILLDDESESEKFTVSDGYIHYGQTVKLVCSVTGMALPRLII	[238]
NvSuH	VSDGNFASDQNGAFYIILLDDESESEKFTVSDGYIHYGQTVKLVCSVTGMALPRLII	[235]
XenopusSuH	VSDGNFASDQNGAFYIILLDDESESEKFTVSDGYIHYGQTVKLVCSVTGMALPRLII	[238]
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DeSuH	KKYDQDALLDADDVPSQLKCAFYLRKTERMYLCLC-QERLIQFQATPCPKKFNKEMIN	[298]
HaSuH	KKYDQDALLDADDVPSQLKCAFYLRKTERMYLCLC-QERLIQFQATPCPKKFNKEMIN	[297]
NvSuH	KKYDQDALLDADDVPSQLKCAFYLRKTERMYLCLC-QERLIQFQATPCPKKFNKEMIN	[294]
XenopusSuH	KKYDQDALLDADDVPSQLKCAFYLRKTERMYLCLC-QERLIQFQATPCPKKFNKEMIN	[297]
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DeSuH	DGASWTIISTDKASTTYTEGNGFVPSVPTVFP-VVSEQLMGDDGVANLELTQGNPTFNL	[356]
DeSuH	DGASWTIISTDKASTTYTEGNGFVPSVPTVFP-VVSEQLMGDDGVANLELTQGNPTFNL	[358]
HaSuH	DGASWTIISTDKASTTYTEGNGFVPSVPTVFP-VVSEQLMGDDGVANLELTQGNPTFNL	[356]
NvSuH	DGASWTIISTDKASTTYTEGNGFVPSVPTVFP-VVSEQLMGDDGVANLELTQGNPTFNL	[353]
XenopusSuH	DGASWTIISTDKASTTYTEGNGFVPSVPTVFP-VVSEQLMGDDGVANLELTQGNPTFNL	[356]
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DeSuH	QVWFQDVAETMYRCDSNLCVFPDLSAFREGHRNVKQVQVPTLVRRDGIYVSTGLTF	[416]
DeSuH	QVWFQDVAETMYRCDSNLCVFPDLSAFREGHRNVKQVQVPTLVRRDGIYVSTGLTF	[418]
HaSuH	QVWFQDVAETMYRCDSNLCVFPDLSAFREGHRNVKQVQVPTLVRRDGIYVSTGLTF	[416]
NvSuH	QVWFQDVAETMYRCDSNLCVFPDLSAFREGHRNVKQVQVPTLVRRDGIYVSTGLTF	[413]
XenopusSuH	QVWFQDVAETMYRCDSNLCVFPDLSAFREGHRNVKQVQVPTLVRRDGIYVSTGLTF	[416]
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DeSuH	TYTFEPGPRPICS-AAGATLRAN	[439]
DeSuH	TYTFEPGPRPICS-AAGATLRAN	[440]
HaSuH	TYTFEPGPRPICS-AAGATLRAN	[438]
NvSuH	TYTFEPVNPALS-VADSVLRGE	[435]
XenopusSuH	TYTFEPGPRPICS-AAGATLRAN	[438]



Supplemental Figure 2: *Nematostella* Su(H) and experimentally modified forms

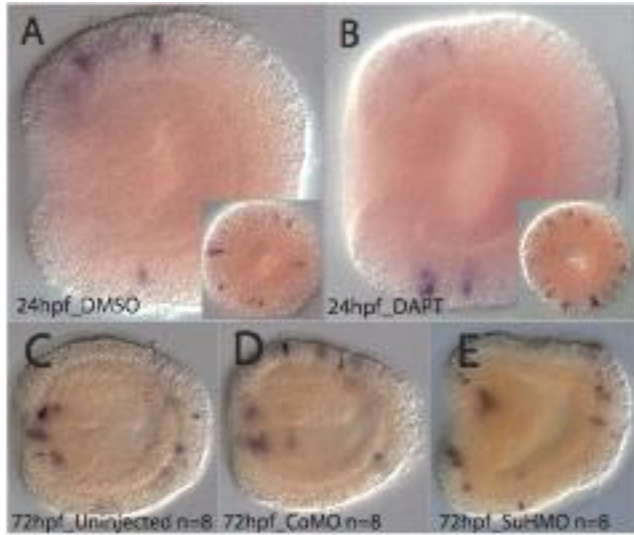
A, The *SuH* locus consists of eleven exons (E) and ten introns (solid line). Predicted transcript structures in wildtype (wt) transcripts, those generated by splice-blocking morpholin directed against *SuH* and that generated for dominant negative overexpression (*dnSu(H)*). Red ovals indicate the position of introduced mutations in the DNA binding site. The size of predicted protein products resulting from the transcripts are shown in light gray substitute residues in the DNA-binding site depicted in red. B, Suppressor of Hairless SuH/RBP-Jkappa is highly conserved between *Nematostella* and Bilateria taxa and is invariant in the majority of residues in fish (Dr), frog (*Xenopus*), human (Hs), flies (Dm) and *Nematostella* (Nv). The conserved DNA binding domains, LAG1 (blue), beta-trefoil (yellow), and TIG (green) are highlighted. A portion of the conserved beta-trefoil DNA-binding site is shaded in gray and residues substituted for dominant negative functional studies are shown in red. C, Construction of the dominant negative SuH, *dnSuH*, construct through introduction of point mutations by PCR with the specified primers. Point mutations in the DNA binding domain are shown in red.



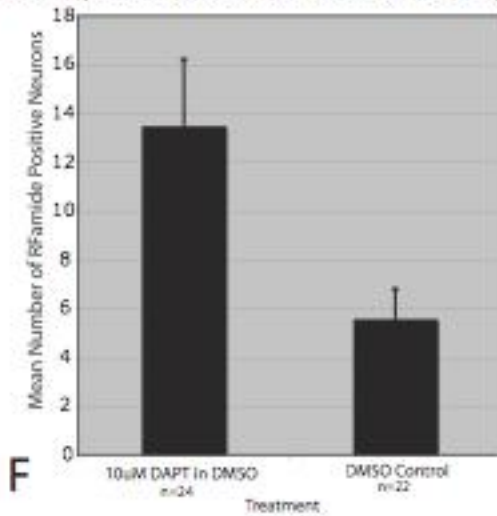
Supplemental Figure 3: DAPT treatment in 24 hpf embryos results in a change in *hes* and *hes*

-

***like* gene expression.** A, 24 hpf control DMSO treated embryo showing a wild type expression of *Nvhes3* in the aboral ectoderm. B, Expression of *Nvhes3* is completely lost in DAPT-treated 24 hpf embryos. C, *Nvhl* expression in aboral ectoderm of a control DMSO treated embryo. D, *Nvhl* expression is completely absent in a DAPT treated embryo.



Blocking Notch Causes an Increase in Neural Cell Types



SupplementalFigure4:ThenumberofNvanthoRFamideexpressingneuronsincreasesin

DAPTtreatedembryosA-

B,EmbryostreatedwithDAPThavemoreneurons(B)comparedwithcontrolstreatedonlywith DMSO(A)at24hours.C-

E,EmbryosinjectedwithaNvSu(H)morpholinodotshowanincreaseinthenumerofofNvantho RFamideexpressingcellswhencomparedwithcontrolembrjos.F,Thenumberofneuronsinem

bryostreatedwithDAPTandDMSOat20hpf.

PublicationName	Simionatoet.al	JGIproteinID	GenBankID
NvNotch			JN982705
NvHes1	Nem50	28948	JN982707
NvHes2	Nem53	98450	JN982708
NvHes3	Nem60	241656	JN982709
NvHL1	Nem51	47054	JN982710
NvHes4	Nem52	67328	JN982711
NvHL2	Nem56	241655	JN982712
NvHL3	Nem62	242118	JN982713
NvRDSL1		60816	JN982715
NvRDSL2	11703	130979	JN982714

Supplemental Table 1 Names in red are names used in manuscript

>NvNotch/Notch Translation

SCPKNFTGARCEIDVDECTTLSQPCQNGGTC SNVYGGYMCRCITGWDGADCSVNIDECKQNDPFPRCQHGGTCVDKIGSYT
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 DKGQTPFLGAREGSLEAVRILLLSYANRMIADNMDKTPEEVARQRAHNDIVELLSDWSIGCNSPKAAPAPTSPPDQRSPLN
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 GYPSNQSSIQACKLSNRLEEHVMMETTLHHNNQSMCVPEVESGMFREKHHTPPSAHSYGTSSYDSSPQKLPISYLTSPSPESPKD
 WSSSPSSHSDWSN

>Corrected Notch Transcript

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>NvDeltaCoding

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GTTACTGGCAAGAAGTGGAAAGTGGAAATATAA

>Delta Translation

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GVSSNFLSFRMVCNPNQYGDSCSKFCEPRDDKFGHMTCDANGTHVCLPGWQGLPYCTTPVCSKSCRPNGNCSVPDRCSQ
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>NvHes1

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GACTTGTGCCGACAGCTTTGGAGAAGGAGGGCTCCTCGAAGCTCGAGAAAGCTGAGATCTTGCACTTGACAGTGGAGC
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>NvHes1 Translation

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PVMPMGWPLGLATPVPIPLPTNPSQAQVKKDPSDEGEIPADADGSLKTRPGFEGGGVQGLQGVLDRAVHLVRFHGPVV
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>NvHes2

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CCTCCCACCACCATCACCCTCAGCTCTCGCCACCGATATCCCACTGAGCTCGCATGTCTCGTCTCCGCCACAAACC
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TGA

>NvHes2 Translation

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>NvHes3

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GAACACCACTCTCAGTCGACAGCCCTGGTAGCTCCGAGCTTTTCTCCGGCACCATGTGGGTTCCATACCCCTCTCCG
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CCCGTGCAGCATCGCCGGCGCAGACGACTATCGTCAGCACAAACGCGAAAAACAACAGAAAGGAGAACCAGCACTGT
GGCGCCCATGGTGA

>NvHes3 Translation

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RTQGSTPPSDFRAGFNACAVEVSNRLSPADANTDNLRETLTSLHLVGTGCHGNTTPQSQTLVAPSFSSGTMWVPYPPSPINQ
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>NvHI1

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GTTCTAA

>NvHI1 Translation

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>NvHes4

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>NvHes4 Translation

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>NvHI2

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>NvHI2 Translation

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>NvHI3

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>NvHl3Translation

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>NvRds12

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CGC

>NvRds12Translation

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>NvRds11

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>NvRds11translation

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qt_Nvhes3R1	CGTAGGTTATCAGTATTCGCATCC
qt_Nvhl1F1	AGCAGCCGATGATTTCTGTC
qt_Nvhl1R1	CACGGAAATCCTTCGTTTGG
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qt_NvashA_R1	TTGCACTGCGAAGTGTCTTAAC

SupplementalTable2QuantitativePCRprimersusedinthisstudy