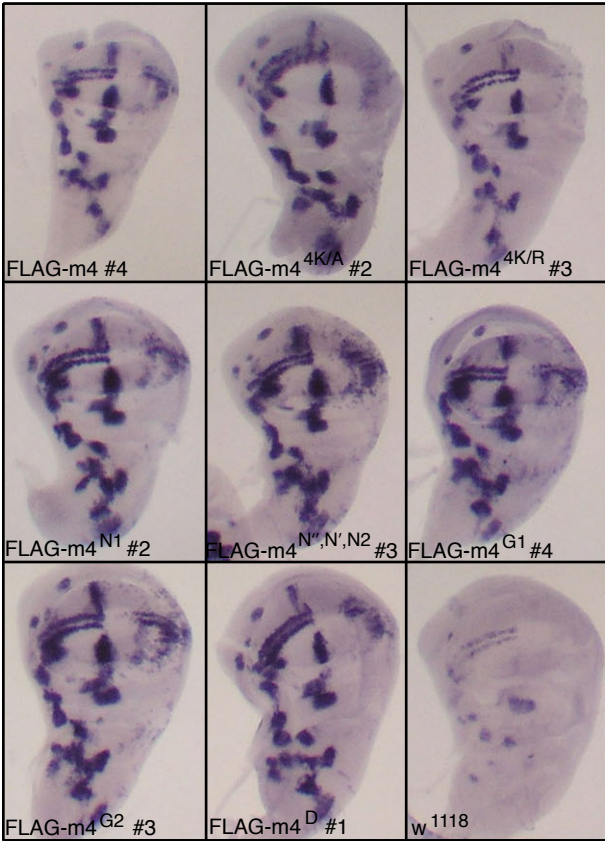


Figure S1

A



B

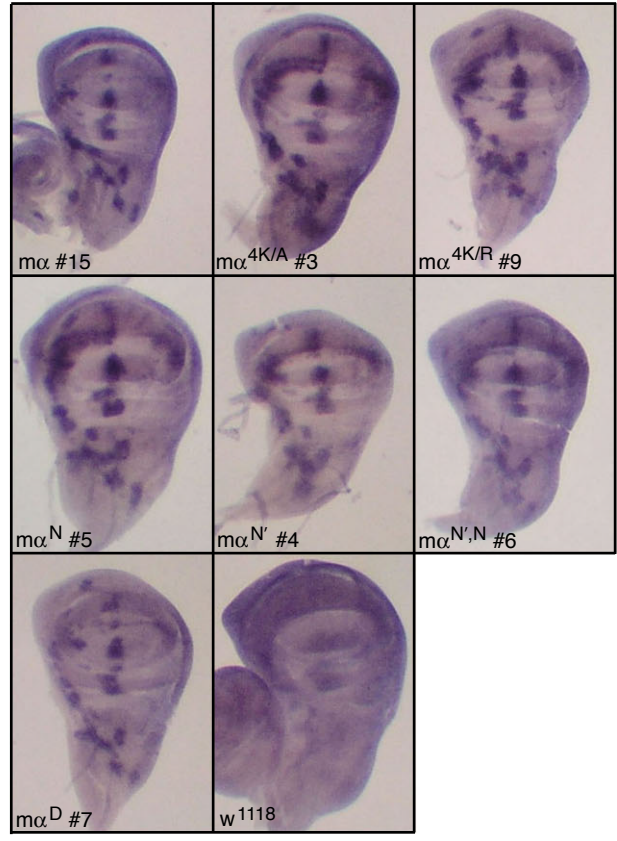
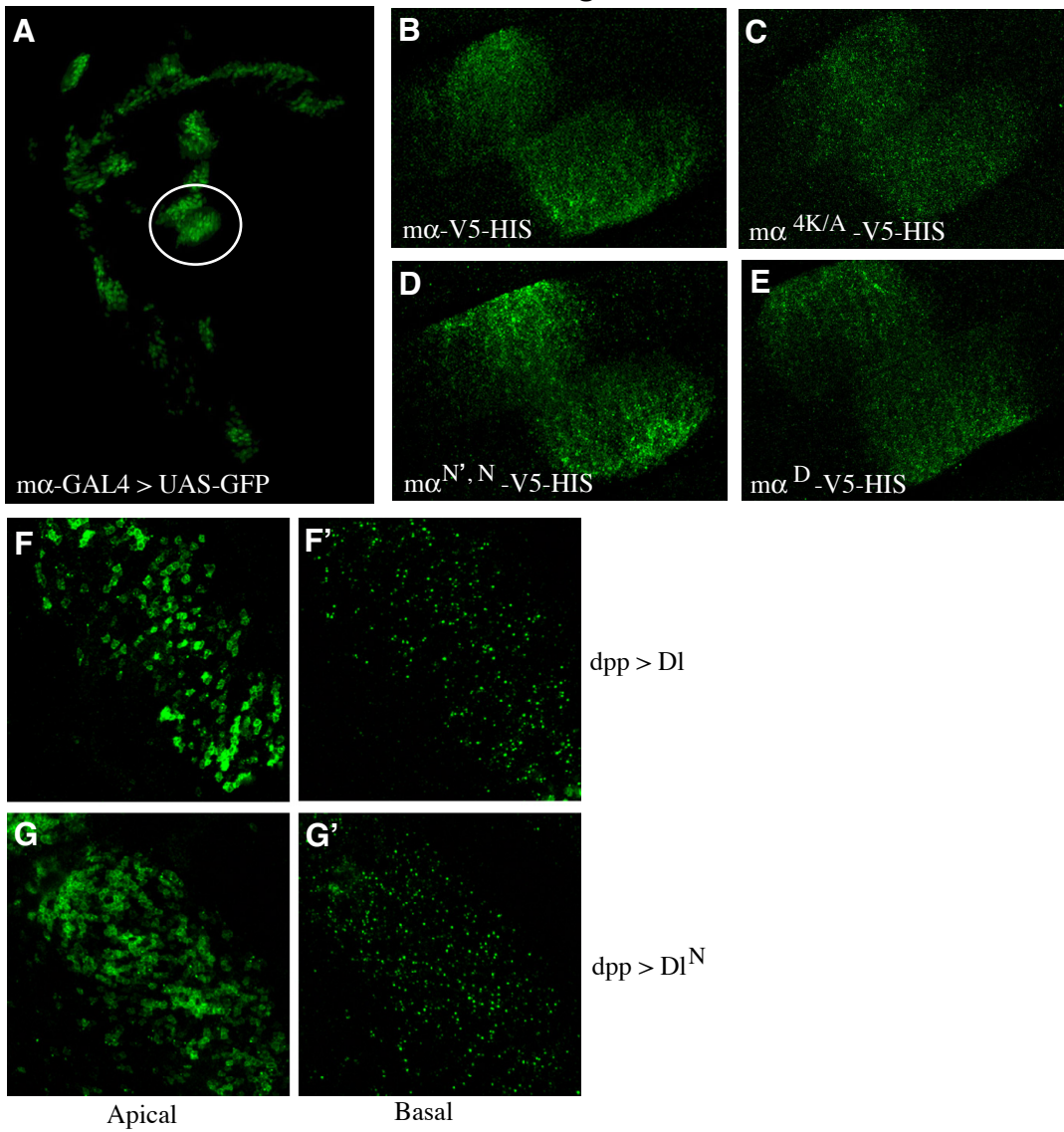


Figure S2



# Figure S3

**A**

Drosophilid BFM5									
N' motifs									
melanogaster	C E N E A N E R L L S	D E N A A N E K L A	C D N M A N E E L E	M D N M A N E H L E	A Q N Q A N E R M L	L Q N S Q N E Q L E	H Q N W L N E Q L E		
simulans	C E N E A N E R L L S	D E N A A N E K L A	C D N M A N E E L E	M D N M A N E H L E	A Q N Q A N E R M L	L Q N S Q N E Q L E	H Q N W L N E Q L E		
sechellia	C E N E A N E R L L S	D E N A A N E K L A	C D N M A N E E L E	M D N M A N E H L E	A Q N Q A N E R M L	L Q N S Q N E Q L E	H Q N W L N E Q L E		
yakuba	C E N E A N E R L L S	D E N A A N E K L A	C D N M A N E E L E	M D N M A N E H L E	A Q N Q A N E R M L	L Q N S Q N E Q L E	H Q N W L N E Q L E		
erecta	C E N E A N E R L L S	D E N A A N E K L A	C D N M A N E E L E	M D N M A N E H L E	A Q N Q A N E R M L	L Q N S Q N E Q L E	H Q N W L N E Q L E		
ananasae	C E N E A N E R L L S	D E N A A N E K L A	C D N M A N E E L E	M D N M A N E H L E	A Q N Q A N E R M L	L Q N S Q N E Q L E	H Q N W L N E Q L E		
pseudobscura	Y E N E A N E R L L S	D E N A A N E K L A	C D N M A N E E Q L E	M D N M A N E H L E	A Q N Q A N E R M L	L Q N S Q N E E L E	H Q N W L N E Q W E		
persimilis	Y E N E A N E R L L S	D E N A A N E K L A	C D N M A N E E Q L E	M D N M A N E H L E	A Q N Q A N E R M L	L Q N S Q N E E L E	H Q N W L N E Q W E		
willistoni	F E N E A N E R L L S	D E N A A N E K L A	C D N T A N E L L E	M D N M S N E H L E	A Q N Q A N E R M L	L Q N S Q N E E L E	L Q N W L N E Q I E		
mojavensis	F E N E A N E R L L S	D E N A A N E K L A	C D N T A N E L L E	M D N M S N E H L E	A Q N Q A N E R M L	L Q N S Q N E E L E	L Q N W L N E Q I E		
virilis	Y E N E A N E R L L S	D E N A A N E K L A	C D N M A N E Q L E	M D N M S N E H L E	A Q N Q A N E R M L	L Q N S Q N E E L E	L Q N W L N E Q L E		
grimshawi	Y E N E A N E R L L S	D E N A A N E K L A	C D N M A N E Q L E	M D N M S N E H L E	A Q N Q A N E R M L	L Q N S Q N E E L E	L Q N W L N E Q L E		
Consensus	C F E N E A N E R L L S Y	D E N A A N E K L A	C D N M T A N E L L E E Q	M D N M A N S E H Q L E	A Q N Q A N E R M L	L Q N A L S Q N E X L E	H Q N W L N E Q L E I W	D X E N X X N E X I Q O L M W	

Drosophilid BFM6									
N' motifs									
melanogaster	M E N S R N A D L E	V D N L R N A Q V E	N Q N A V N A S L E						
simulans	M E N S R N A D L E	V D N L R N A Q V E	N Q N A V N A S L E						
sechellia	M E N S R N A D L E	V D N L R N A Q V E	N Q N A V N A S L E						
yakuba	M E N S R N A D L E	V D N L R N A Q V E	N Q N A V N A S L E						
erecta	M E N S R N A D L E	V D N L R N A Q V E	N Q N A V N A S L E						
ananasae	I E N S R N A D L E	V D N L R N A Q V E	A Q N A V N A S L E						
pseudobscura	I E N S R N A D L E	N D N L R N A Q V E	N O N D I N A S L E						
persimilis	I E N S R N A D L E	N D N L R N A Q V E	N O N D I N A S L E						
willistoni	I E N S R N A D L E	V E N L R N A Q V E	T O N N I N A S L E						
mojavensis	M E N S R N A D L E	V D N L R N A E V E	S O N E I N S S L E						
virilis	I E N S R N A D L E	V D N L R N T E V E	S O N E I N S S L E						
grimshawi	I E N S R N A D L E	V D N L R N T E V E	S O N E I N S S L E						
Consensus	I M E N S R N A D L E	N D N L R N A E T Q V E	X Q N X I A S L E V S						D I A X L E Q V T V

Drosophilid BFM7									
N' motifs									
melanogaster	E Q N L K N A L K A								
simulans	E Q N L K N A L K A								
sechellia	E Q N L K N A L K A								
yakuba	E Q N L K N A L K A								
erecta	E Q N L K N A L K A								
ananasae	E Q N L K N T I K A								
pseudobscura	E Q N L K N T L K A								
persimilis	E Q N L K N T L K A								
willistoni	Q Q N Y I N S K K A								
mojavensis	Q Q N M Q N T H K A								
virilis	Q Q N M Q N T H K A								
grimshawi	Q Q N M Q N T H K A								
Consensus	E Q N M K N S X K A Y Q T								E L I A X Q N M K N S X K A Y Q T

**B**

non-Drosophilid BFM5									
Canonical or primary BFM5									
Ceratitis capitata Ocho	M D N R A N E D L E								
Ceratitis capitata Tom	D D N L A N E A L E	Q Q N D Y N S S L E	I E N F E N T K K A						
Ceratitis capitata m4	T E N D A N E R L A	L E N S R N A D L E							
Haematobia irritans irritans Ocho	M E N M A N E H L E								
Haematobia irritans irritans Tom	Q D N V A N E A L E	Q Q N T Y N S S L E							
Culex pipiens	Y D N S S N E L L E								
Aedes aegypti	Y D N D A N E R L E								
Anopheles gambiae	Y D N S A N E R L E								
Bombyx mori BFM1	A Q N S S N E L L E								
Plodia interpunctella BFM1	E Q N S S N E L L E								
Antheraea assama	E Q N S S N E M L E								
Tribolium castaneum BFM1	E D N L A N E L L E								
Apis mellifera BFM1	N O N N A N E A L E								
Acyrtosiphon pisum	L E N L Q N E Y I E	A D N A I N E R L E							
Pediculus humanis corporis	L E N E K N E K L E								
Artemia franciscana	N E N T A N E L L E								
Daphnia pulex	D E N A L N E A L E								
Callinectes sapidus	E D N L A N E A L E								
Consensus	D X E N X X N E X I A Q O L E	A D I A D L E L E N X R N E R L E Q Q Y S S	I E N F E N T K K A	D X E N X X N E X I A Q O L E					

non-Drosophilid BFM6									
Secondary (non-canonical) BFM6									
Bombyx mori BFM2	D E N M A N E V R E	N O N I L N S N R F	N D N A E N Q N I L						
Plodia interpunctella BFM2	V D N M A N E V R E	N O N I L N S T R F	N D N A E N Q N I L						
Manduca sexta BFM2	N D N M A N E V R E	N O N I V N T R F	N D N A E N Q N I V						
Samia cynthia ricini BFM2	N D N M A N E V R E	N O N I V N S N R F	N D N A E N Q N I V						
Heliconius erato BFM2	S D N M A N E V R E	N O N I L N S N R F	N D N A E N Q N I L						
Tribolium castaneum BFM2	N E N L A N E L K E	L D N E I N E N L A							
Apis mellifera BFM2	N E N L E N E L K E								
Consensus	X D E N L A N E L K E E M E N E V R E	L D N I L N S T R F N Q N E V T L A	N D N A E N Q N I L V	D X E N X X N E X I L Q O T R					

# Figure S4

## A Protostome DSLs

*Xiphinema index* Delta RKTKRQSNCSALTVDTASDLEACRKNERNERNERERRLMECQMCNNAVNPPIRIAKTDN  
*Capitella sp. I* Delta RRRSRL-RESEQK--DNVQNEHRSMNNKLSSIESPPPPHAAPPVSSSSS  
*Euprymna scolopes* Delta RHRQHYFRENMQKEGEONKINSKC---IETDIFTTIPASASDKITKDEL  
*Lottia gigantea* Delta RRKNNLMRDNMEKEREQONIVNNINMKKIDSKIFTTPTNTNPVASASIKIN  
  
*Lottia gigantea* Serrate KRKKKLRRLRRDSYLTDNRTNNETEETIRRYRNPLFSHDKSRPTSGASGGC

## B Vertebrate Delta-like1

*Homo sapiens* 568 ----CVRLRLQK--HRPPADPCRGETETMNNLAN-CQEKDISVSIIGAT  
*Mus musculus* ----CVRLKLQK--HQPPPEPCGGETETMNNLAN-CQEKDVSVSIIGAT  
*Gallus gallus* ----CVRLKVQK--RHHQPEACRSETETMNNLAN-CQEKDISISVIGAT  
*Anolis carolinensis* ----CFRVKMQK--RQHQPDACRSETETMNNLAN-CQEKDISISVIGAT  
*Xenopus tropicalis* ----CVRVRVQK--RRHQPEACRGETKTMNNLAN-CQDKDISVSIIGTT  
*Cynops pyrrhogaster* ----CFRLKMHKQ-RQRSDSYRGESETMNNLAN-CRREKDISVSVIGAT  
*Tetraodon nigroviridis* ----CMVGLALRHIHRQAQRERAETETMNNLSN-IQRD-----NLIPAS  
*Danio rerio* DeltaA ----CVRSKVQQRDRREDEVANGENETINNLTNNCHRDKDLAVSVVGVA  
*Danio rerio* DeltaD VFVIYIRLKLQQRSQQIDS--HSEIETMNNLTNNRSREKDLVSVSIIGAT  
  
: . \* : \* : \* \* : \* : : : :  
E T NNL N R

## C Vertebrate Jagged1

*Homo sapiens* 1099 PGSH-----THSASEDNTTNNVREQLNQIKNPIEKHGAN-TVPIK--  
*Mus musculus* PSSH-----THSAPEDNTTNNVREQLNQIKNPIEKHGAN-TVPIK--  
*Gallus gallus* QSSH-----THTASDDNTTNNVREQLNQIKNPIEKHGAN-TVPIK--  
*Xenopus laevis* QSSH-----SHTASEDNTTNNVREQLNQIKNPIEKHGAN-TVPIK--  
*Tetraodon nigroviridis* QSNHN-----GASATGSEDNTTNNVREQLNQIKNPIEKHVGL-TVAIK--  
*Danio rerio* QSSSATAINPTSPFSTPEENTANNAREHLNQIKNHIEKNASNGSLPGKEL  
  
.. : : : \* : \* : \* : \* \* : \* \* : . : : \*  
NT NN RE LNQIKN IEK K

## SUPPLEMENTARY MATERIAL

### Supplementary figure legends

**Fig. S1.** Transcript accumulation from *FLAG-m4* and *E(spl)m $\alpha$*  variant *UAS* transgenes.

(A,B) In situ hybridizations using (A) *E(spl)m4* probe or (B) *E(spl)m $\alpha$*  probe, showing levels of transcript accumulation in late third-instar wing discs from representative lines carrying the indicated *UAS* transgenes, expressed under the control of *sca-GAL4*, compared to wild type (*w<sup>1118</sup>*).

**Fig. S2.** Localization and/or levels of accumulation displayed by variants of *E(spl)m $\alpha$*

and *Dl* proteins when misexpressed in imaginal disc tissue. (A) Expression of GFP driven by *m $\alpha$ -GAL4* in the late third-instar wing disc. The region magnified in B-E is circled. (B-E) 15-20- $\mu$ m confocal stack images of wing disc tissue expressing (*m $\alpha$ -GAL4* driver) the indicated tagged *E(spl)m $\alpha$*  protein variants stained with anti-V5 antibody.

(F,G) Comparison of the accumulation and subcellular localization of *Dl* and *Dl<sup>N</sup>*.

Shown are 1- $\mu$ m confocal slice images of anti-*Dl* antibody stains of wing imaginal disc tissue expressing *Dl* (F, apical level; F', basal level) or *Dl<sup>N</sup>* (G, apical level; G', basal level) under the control of the *dpp-GAL4* driver.

**Fig. S3.** Deriving consensus sequences for NXXN motifs in Brd family proteins. The

NXXN motifs of known BFM s in (A) the 12 fully sequenced *Drosophila* species and (B) other arthropods are aligned. Combining the original NXXN motifs recognized in *Drosophila* BFM s (A, upper row) with the NXXN motifs in the canonical or primary BFM s of other species (B, upper row) yields the eight-residue consensus

(D/E/Q)NXXNEX(I/L/M). Every species listed for which whole-genome sequence data are available possesses a Brd family protein with an NXXN motif fitting this consensus.

**Fig. S4.** Alignment of putative NXXN motifs in the intracellular domains of Notch ligands. Shown are the portions of the intracellular domains immediately adjacent to the transmembrane domains of (A) Dl and Ser orthologs of various non-arthropod protostomes, (B) Delta-like 1 orthologs of representative vertebrate species, and (C) vertebrate Jagged1 orthologs. Putative NXXN motifs are boxed, and fully conserved residues indicated.