Figure S1

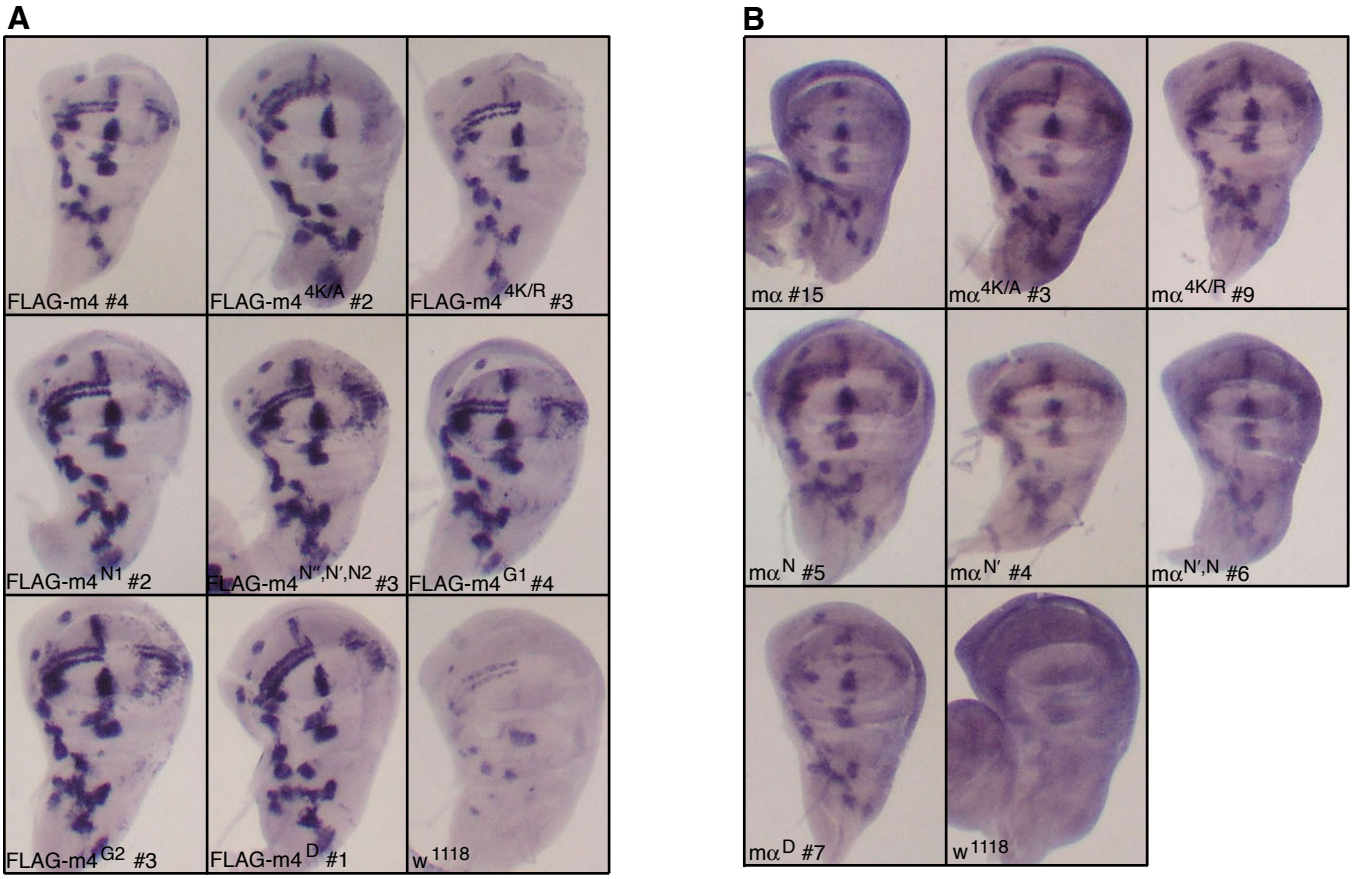
A
FLAG-m4 #4
FLAG-m4 4K/A #2
FLAG-m4 4K/R #3
FLAG-m4 N1 #2
FLAG-m4 N'',N',N2 #3
FLAG-m4 G1 #4
FLAG-m4 G2 #3
FLAG-m4 D #1
w 1118
B
mα #15
mα 4K/A #3
mα 4K/R #9
mα N #5
mα N' #4
mα N',N #6
mα D #7
w 1118

Figure S2

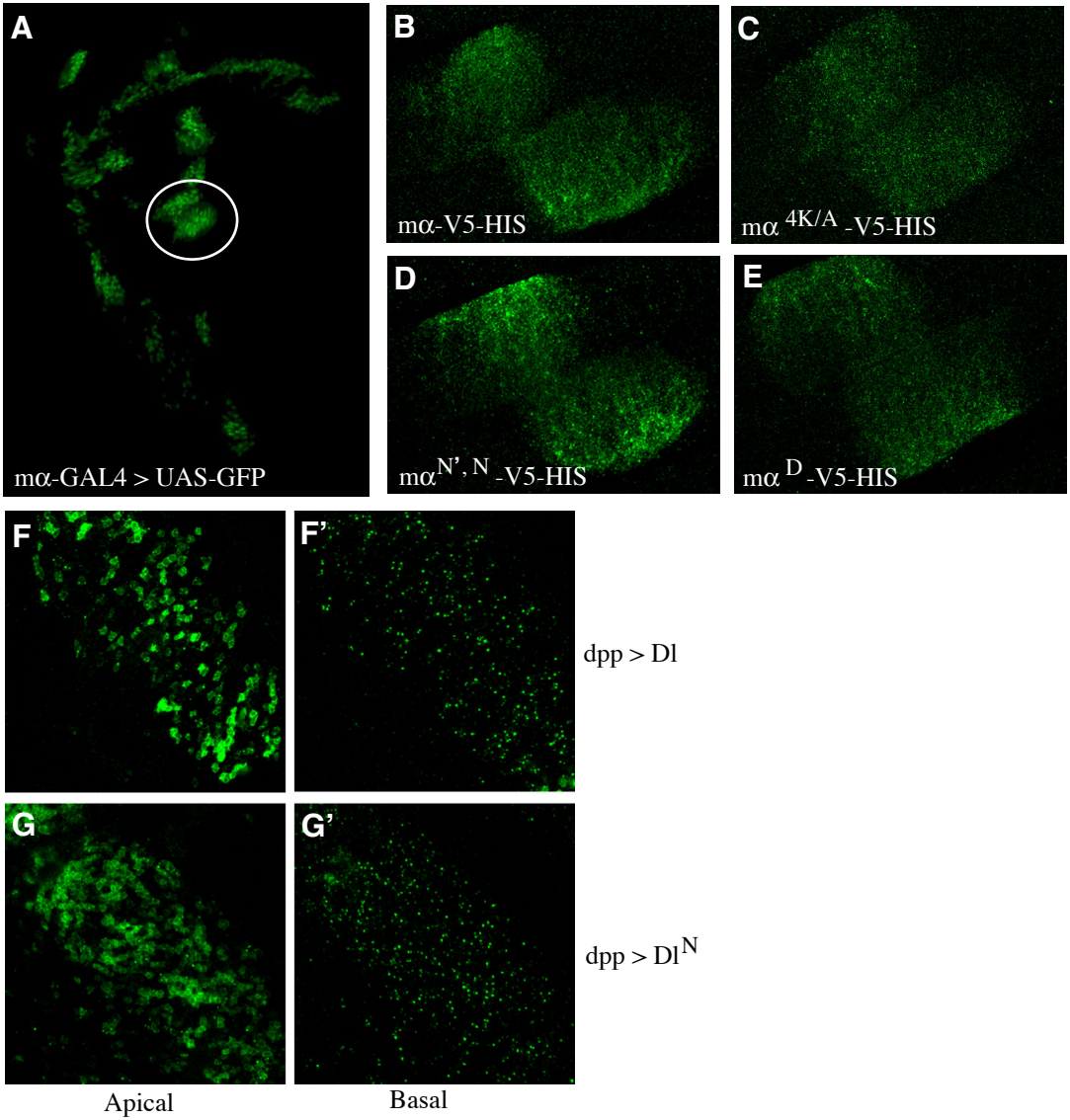
A
mα-GAL4 > UAS-GFP
B
mα-V5-HIS
C
mα 4K/A -V5-HIS
D
mαN', N -V5-HIS
E
mα D -V5-HIS
F
F'
dpp > Dl
G
G'
dpp > DlN
Apical
Basal

Figure S3

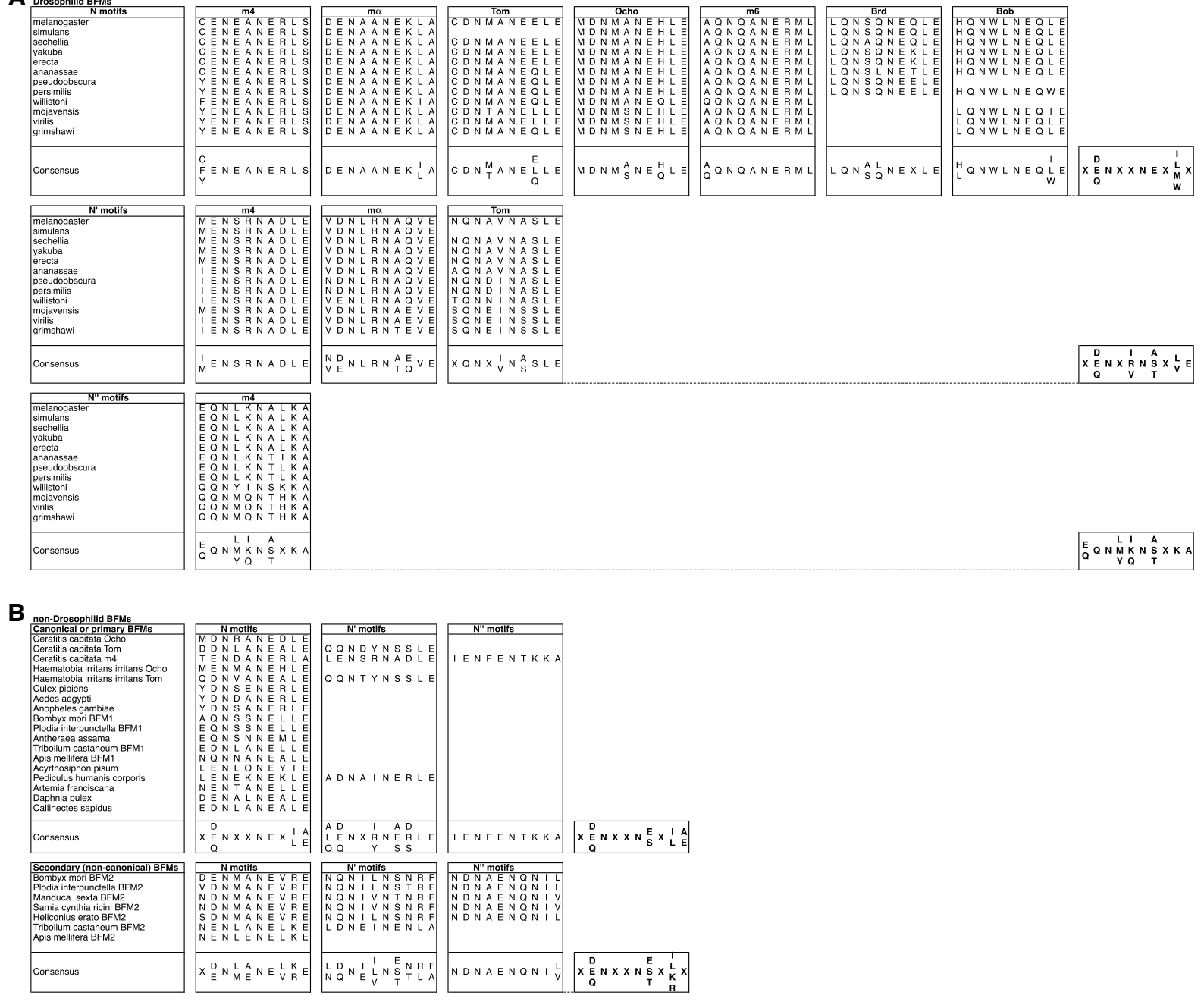
A
Drosophilid BFMs
N motifs
m4
mα
Tom
Ocho
m6
Brd
Bob
melanogaster
simulans
sechellia
yakuba
erecta
ananassae
pseudoobscura
persimilis
willistoni
mojavensis
virilis
grimshawi
Consensus
N' motifs
N'' motifs
B
non-Drosophilid BFMs
Canonical or primary BFMs
Ceratitis capitata Ocho
Ceratitis capitata Tom
Ceratitis capitata m4
Haematobia irritans irritans Ocho
Haematobia irritans irritans Tom
Culex pipiens
Aedes aegypti
Anopheles gambiae
Bombyx mori BFM1
Plodia interpunctella BFM1
Antheraea assama
Tribolium castaneum BFM1
Apis mellifera BFM1
Acyrthosiphon pisum
Pediculus humanis corporis
Artemia franciscana
Daphnia pulex
Callinectes sapidus
Secondary (non-canonical) BFMs
Bombyx mori BFM2
Plodia interpunctella BFM2
Manduca sexta BFM2
Samia cynthia ricini BFM2
Heliconius erato BFM2
Tribolium castaneum BFM2
Apis mellifera BFM2

Figure S4

A **Protostome DSLs**

```
Xiphinema index Delta      RKTKRQSNSCSALTVDTASDLEACRKQNERNERNERERRLMECQMCNNAVNPIRIAKTDN
Capitella sp. I Delta            RRRSRL-RESEQK---DNVQNEHRSMNNKLSSIESPPPPHAAPPVSSSSS
Euprymna scolopes Delta          RHRQHYFRENMQKEGEQNKINSKC---IETDIFTTIPSASASDKITKDEL
Lottia gigantea Delta            RRKNNLMRDNMEKEREQNIVNNINNKKIDSKIFTTPTNTNPVASASIKIN

Lottia gigantea Serrate            KRKKKLRLRRDSYLTDNRTNNETEETIRRYRNPLFSHDKSRPTSGASGGC
```

B **Vertebrate Delta-like1**

```
Homo sapiens               568   ----CVRLRLQK--HRPPADPCRGETETMNNLAN-CQREKDISVSIIGAT
Mus musculus                     ----CVRLKLQK--HQPPPEPCGGETETMNNLAN-CQREKDVSVSIIGAT
Gallus gallus                    ----CVRLKVQK--RHHQPEACRSETETMNNLAN-CQREKDISISVIGAT
Anolis carolinensis              ----CFRVKMQK--RQHQPDACRSETETMNNLAN-CQREKDISISVIGAT
Xenopus tropicalis               ----CVRVRVQK--RRHQPEACRGETKTMNNLAN-CQRDKDISVSIIGTT
Cynops pyrrhogaster              ----CFRLKMHKQ-RQRDSDSYRGESETMNNLAN-CRREKDISVSVIGAT
Tetraodon nigroviridis           -----CMVGLALRHIHRQAQRERAETETMNNLSN-IQRD-----NLIPAS
Danio rerio DeltaA               ----CVRSKVQQRRRDREDEVANGENETINNLTNNCHRDKDLAVSVVGVA
Danio rerio DeltaD               VFVIYIRLKLQQRSQQIDS---HSEIETMNNLTNNRSREKDLSVSIIGAT
                                           :             .* :*:***:*   *:     .:: .:
                                                          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)mα* variant *UAS* transgenes. (A,B) In situ hybridizations using (A) *E(spl)m4* probe or (B) *E(spl)mα* 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^{1118}).

Fig. S2. Localization and/or levels of accumulation displayed by variants of E(spl)mα and Dl proteins when misexpressed in imaginal disc tissue. (A) Expression of GFP driven by *mα-GAL4* in the late third-instar wing disc. The region magnified in B-E is circled. (B-E) 15-20-μm confocal stack images of wing disc tissue expressing (*mα-GAL4* driver) the indicated tagged E(spl)mα protein variants stained with anti-V5 antibody. (F,G) Comparison of the accumulation and subcellular localization of Dl and Dl^N. Shown are 1-μm confocal slice images of anti-Dl antibody stains of wing imaginal disc tissue expressing Dl (F, apical level; F′, basal level) or Dl^N (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 BFMs in (A) the 12 fully sequenced *Drosophila* species and (B) other arthropods are aligned. Combining the original NXXN motifs recognized in *Drosophila* BFMs (A, upper row) with the NXXN motifs in the canonical or primary BFMs 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.