

**Cell Reports, Volume 27**

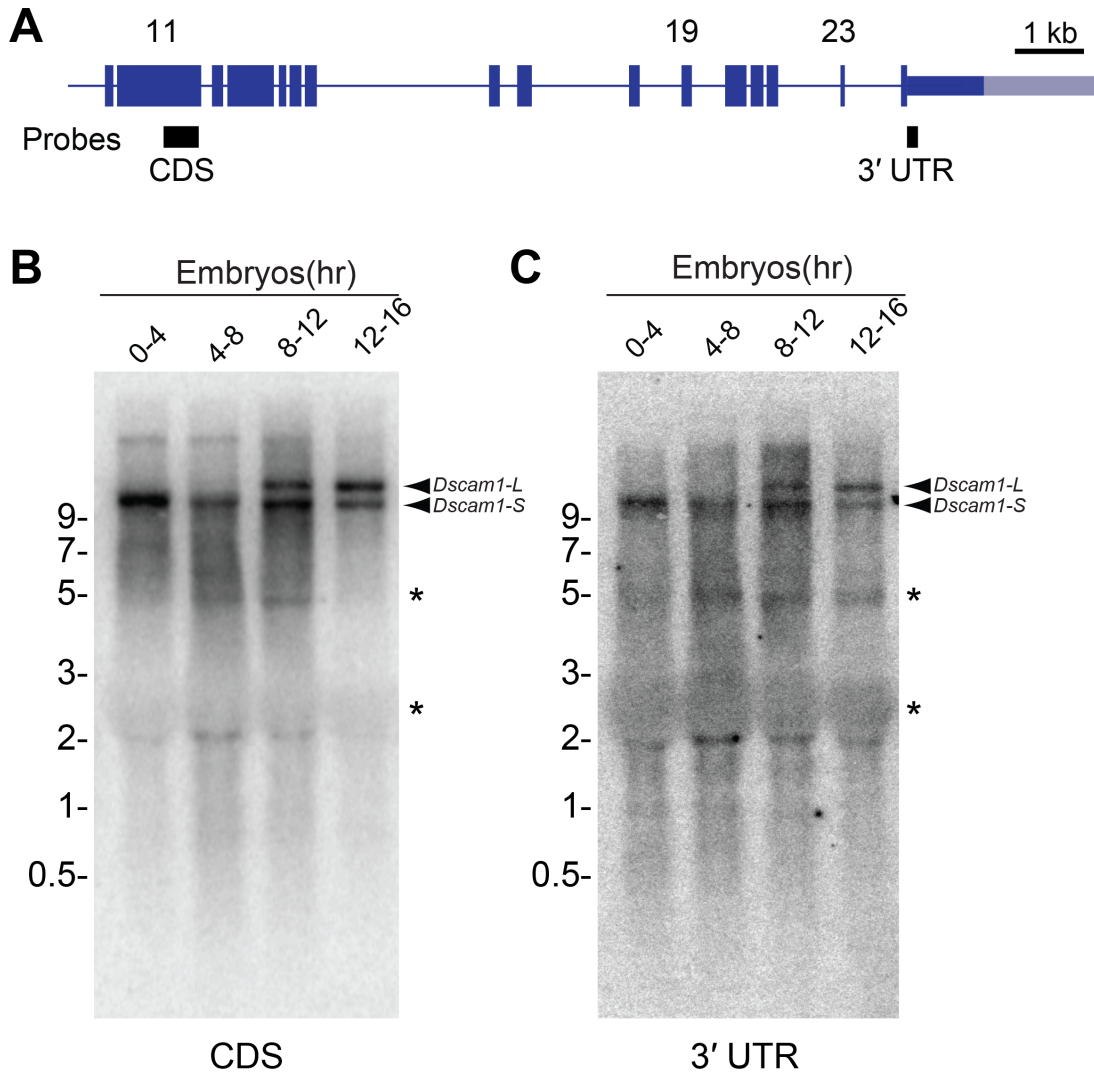
**Supplemental Information**

**Elav-Mediated Exon Skipping and Alternative**

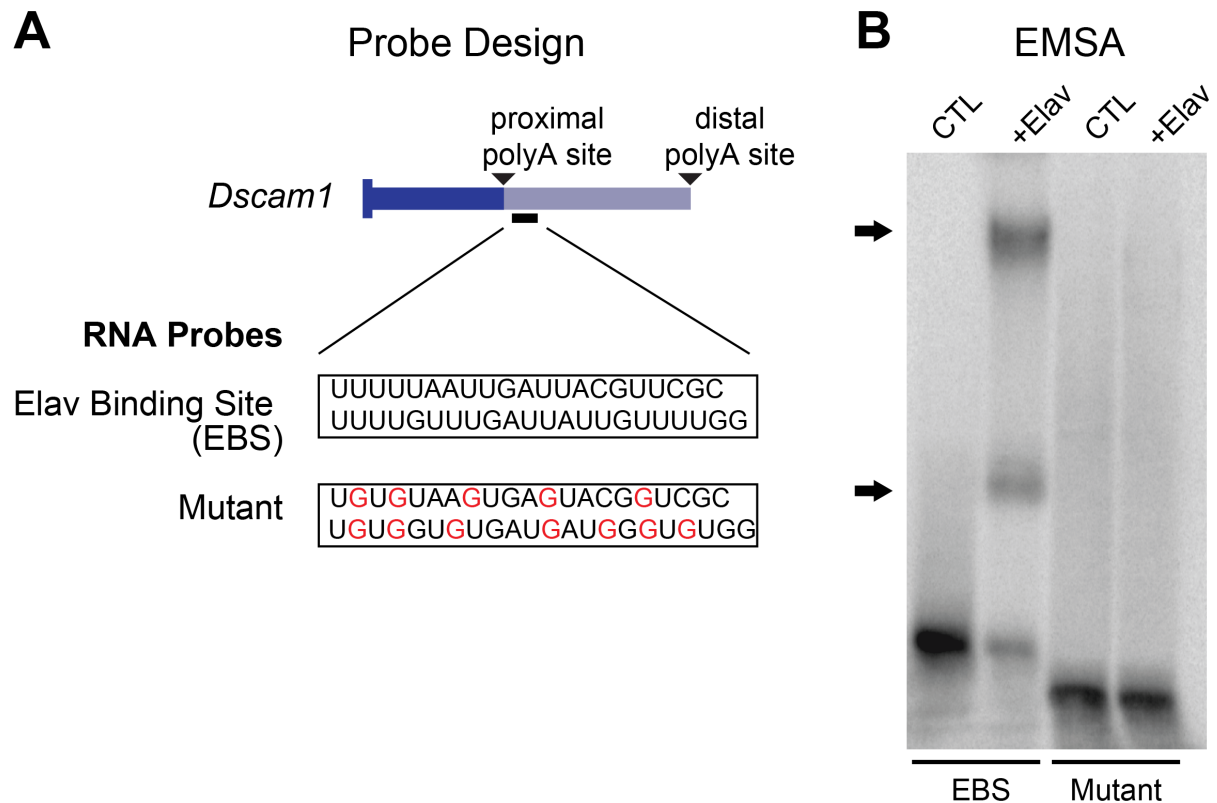
**Polyadenylation of the *Dscam1* Gene**

**Are Required for Axon Outgrowth**

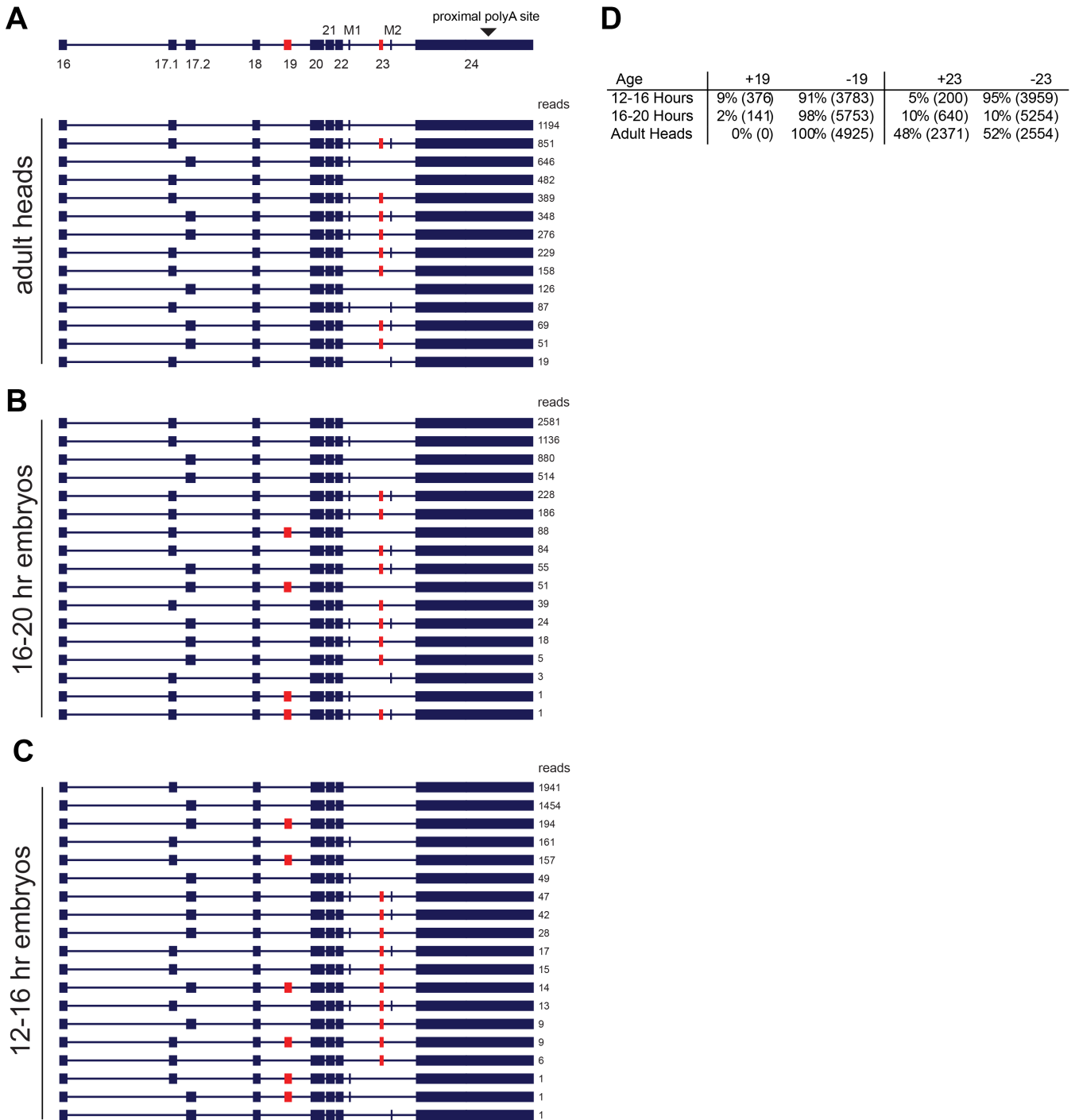
**Zhiping Zhang, Kevin So, Ryan Peterson, Matthew Bauer, Henry Ng, Yong Zhang, Jung Hwan Kim, Thomas Kidd, and Pedro Miura**



**Figure S1. Northern analysis of *Dscam1* during embryonic development. Related to Figure 1. (A)** Schematic of Northern probe locations. **(B)** Non-cropped northern blot using a probe to *Dscam1* exon 11 which is expressed in all *Dscam1* isoforms (CDS). **(C)** Stripped and re-probed blot using probe targeting the proximal 3' UTR (3' UTR). \*, denotes common background bands often observed with these *Drosophila* northern blots.

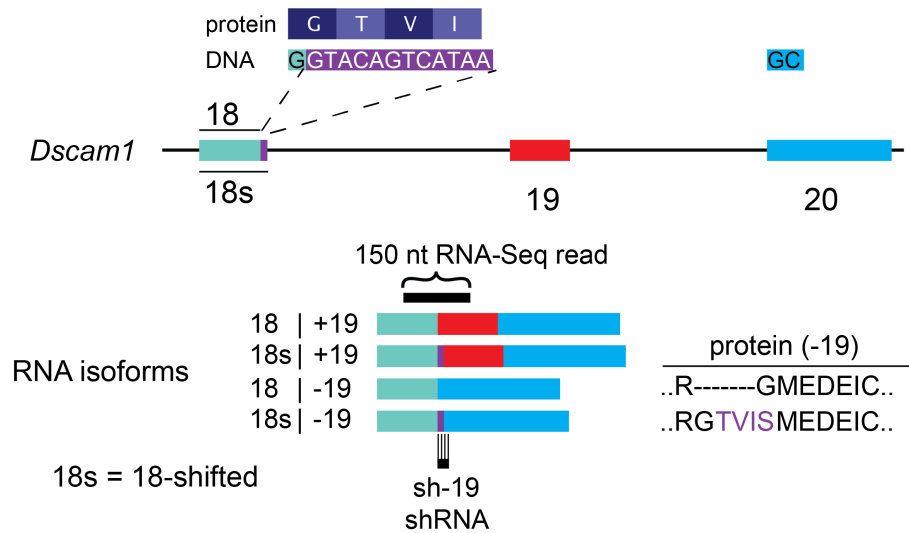


**Figure S2. EMSA demonstrates Elav binds a U-rich region downstream of the *Dscam1* proximal polyA site. Related to Figure 1. (A)** Schematic showing probe design with location of proximal 3' UTR Elav binding site (EBS) probe used in RNA electrophoretic mobility shift assays (EMSA). Mutant probe has disrupted integrity of U-rich stretches generated by U to G mutations (indicated in red text). **(B)** EMSA reveals that upon the addition of recombinant purified Elav, an upward shift occurs, indicating Elav binding. Elav has been shown to bind as a multimer (Soller and White, 2005) which might explain the higher molecular weight supershift. Mutant probe was incapable of Elav binding, as evidenced by the lack of shift.

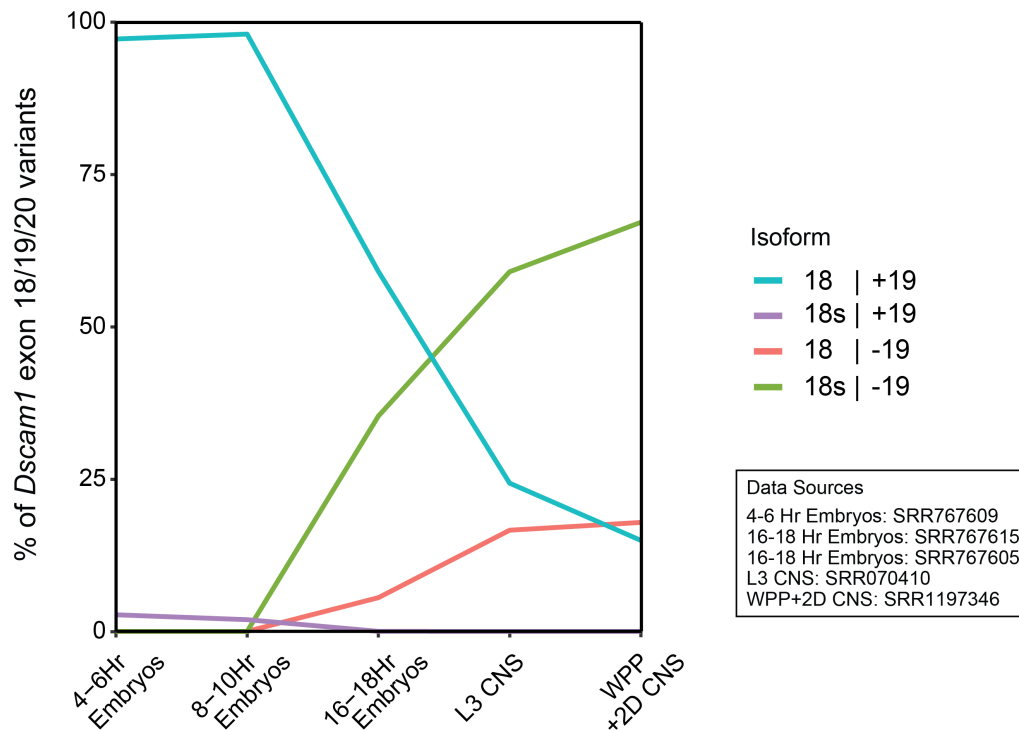


**Figure S3. Frequency of *Dscam1* transcript isoforms connected to extended 3' UTR determined by Nanopore sequencing of *Dscam1*. Related to Figure 3. (A) Adult heads. (B) 16-20 hr embryos. (C) 12-16 hr embryos. (D) Summary table showing the frequencies of exons 19 and 23 in transcripts including the extended 3' UTR. M1- microexon 1, M2- microexon 2.**

**A**



**B**



**Figure S4. Exon 18/18s/19/20 usage determined from short read RNA-Seq data. Related to Figures 3 & 4. (A)** Schematic of *Dscam1* exon usage from exons 18 to 20. An exon 18 variant that is 12 nt longer at the 3' end is named 18-shifted (18s) (highlighted in purple). Compared to the 18 | -19 isoform, the 18s | -19 isoform gains the amino acid sequence T-V-I-S. Note the location of the sh-19 shRNA that uniquely knocks down 18s | -19 isoforms. RNA-Seq short-reads (150 nt) had sufficient length to resolve the connectivity between exons 18, 18s, 19, and 20. **(B)** Exon usage during embryonic development, L3 CNS, and WPP +2D CNS as analyzed from short read RNA-Seq data.

Northern blot probes					
Gene name	probe name	5' primer	3' primer	probe coordinates	Figure
GFP	GFP	TAAACGGCCACAAGTTCAGC	CTGTACAGCTCGTCCATGC		1G
Dscam1	Dscam1 coding	GGTCTTGATCACTCGTTGG	CACCTACAACATTCGCATCG	chr2R:7331013-7331523	1B, 1D, 2A, 4A, 5B, S1
Gapdh2	Gapdh2	GGCATCCACTCACTTGAAGG	TCAGCTTCACGAACCTGTCCG	chrX:15868647-15869257	1D, 1G, 2A, 5B
Dscam1	Dscam1 uni 3'UTR	ATTTGTAAGCGCCCTCTGC	ACTCCCTCTCTTCTCTATCTCT	chr2R:7320524-7320665	S1
PCR primers					
Parent gene	Amplicon name	5' primer	3' primer	product coordinates	Figure
Dscam1	Dscam1-uni	TCCGGAGTACAGGCTACCG	GGACAGTCCCTCAATCTACACG	chr2R:7322909-7323093	1F
Dscam1	Dscam1-ext	GCGTTTTAAACTGCCTGTCC	CAACTTCAACGCACATCAGG	chr2R:7318329-7318425	1F
Dscam1	exon19	CTGGAAGTTCATGGCCTTGG	TGTGACCGGATTAAAGCGAGG	chr2R:7323244-7324600	3B, 4B, 5F, 6A, 6B
Dscam1	exon23	TGTTGGAGTTGGAGTTGTTTTGG	GAGAGTAATGAGATCTCAGAGGC	chr2R:7320729-7322623	3B
Rpl32	Rpl32	ACGTTGTGCACCAGGAACCT	CCGCCTCAAGGGACAGTATC	chr3R:3004576-30045580	4B, 5F, 6B, 6D, 6F
Dscam1	Nanopore 16-24	ACTTGCCTGTGCTCTATCTTCTGTAGCTCCATTGCATCG	TTTCTGTTGGTGTGATATTGCCGAATACGACTTTGCCACCT	chr2R:7319384-7329325	3C, 3D, S3
Dscam1	Flag-exon20	CAAGGATGACGATGACAAGG	ATCATCGTATTCGGGAGCTG		6D, 6F
qPCR primers					
Parent gene	Amplicon name	5' primer	3' primer	product coordinates	Figure
Dscam1	Dscam1-uni	TCCGGAGTACAGGCTACCG	GGACAGTCCCTCAATCTACACG	chr2R:7322909-7323093	1C
Dscam1	Dscam1-ext	GCGTTTTAAACTGCCTGTCC	CAACTTCAACGCACATCAGG	chr2R:7318329-7318425	1C
Rpl32	Rpl32	ACGTTGTGCACCAGGAACCT	CCGCCTCAAGGGACAGTATC	chr3R:3004576-30045580	1C
Dscam1	Dscam1-CDS	GGCTGTTCTTTCGCTGGTAT	GCACATATGCTTCCACCAGGA	chr2R:7323099-7323194	1E
Dscam1	Dscam1-EBS	TGATTACGTTTCGCTTTTGTGG	TGTTGGTGGTGTCTCCACAG	chr2R:7319479-7319540	1E
Elav	Elav-CDS	TCCGAGCAATAACACATCG	CTCCITTCGTCTGCATATCG	chrX:513912-513983	1E
Elav	Elav-EBS	CTGTACGATTTTCGATTAAACAACCA	AGAAAGATAGACAGAGAGGGAACAAGTA	chrX:513912-513983	1E

**Table S1: Oligonucleotide sequences. Related to Figures 1, 2, 3, 4, 5, 6.** Coordinates correspond to *D. Melanogaster* dm6 genome.

## **Supplemental Multimedia Files**

**Video #1. Related to Figures 2 and 4.** *Elav-GAL4/+; UAS-Dicer2/UAS-shExt* flies display impaired locomotion and could not fly. Flies were placed on pad without anesthesia.

**Video #2. Related to Figure 4.** *Elav-GAL4/+; UAS-Dicer2/UAS-sh-19* flies display impaired locomotion and could not fly. Flies were placed on pad without anesthesia.