1   **Muscleblind regulates *Drosophila Dscam2* cell-type-specific alternative splicing**

2   Joshua Shing Shun Li, Kevin Nzumbi Mutemi and S. Sean Millard.

3   School of Biomedical Sciences, The University of Queensland, Brisbane, 4072,

4   Australia.

5   Correspondence: s.millard@uq.edu.au

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7   **Summary**

8   **Alternative splicing of genes increases the number of distinct proteins in a cell.**

9   **In the brain it is highly prevalent, presumably because proteome diversity is**

10   **crucial for establishing the complex circuitry between trillions of neurons. To**

11   **provide individual cells with different repertoires of protein isoforms, however,**

12   **this process must be regulated. Previously, we found that the mutually exclusive**

13   **alternative splicing of *Drosophila Dscam2* exon 10A and 10B is tightly regulated**

14   **and crucial for maintaining axon terminal size, dendritic morphology and**

15   **synaptic numbers. Here, we show that *Drosophila muscleblind* (*mbl*), a**

16   **conserved splicing factor implicated in myotonic dystrophy, controls *Dscam2***

17   **alternative splicing. Removing *mbl* from cells that normally express isoform B**

18   **induces the expression of isoform A and eliminates the expression of B,**

19   **demonstrating that Mbl represses one alternative exon and selects the other.**

20   **Consistent with these observations, we show that *mbl* expression is cell-type-**

21   **specific and correlates with the expression of isoform B. Our study demonstrates**

22   **how cell-type-specific expression of a splicing factor can provide neurons with**

23   **unique protein isoforms.**

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26 **Introduction**

27 Alternative splicing occurs in approximately 95% of human genes and generates

28 proteome diversity much needed for brain wiring (Pan et al., 2008; Wang et al.,

29 2008). Specifying neuronal connections through alternative splicing would require

30 regulated expression of isoforms with unique functions in different cell types to carry

31 out distinct processes. Although there are some examples of neuronal cell-type-

32 specific isoform expression (Bell et al., 2004; Iijima et al., 2014; Lah et al., 2014;

33 Norris et al., 2014; Schreiner et al., 2014; Tomioka et al., 2016), the mechanisms

34 underlying these deterministic splicing events remain understudied. This is due, in

35 part, to the technical difficulties of assessing isoform expression at the single cell

36 level. Another obstacle is that most splicing regulators are proposed to be

37 ubiquitously expressed (Nilsen and Graveley, 2010), therefore it is not immediately

38 clear how cell-type specific expression would be achieved. For example, the broadly

39 expressed SR and heterogeneous nuclear ribonucleoproteins (hnRNPs) typically have

40 opposing activities, and the prevalence of splice site usage is thought to be controlled

41 by their relative abundances within the cell (Blanchette et al., 2009). There are many

42 notable examples where splicing regulators are expressed in a tissue-specific manner

43 (Calarco et al., 2009; Kuroyanagi et al., 2006; Markovtsov et al., 2000; Ohno et al.,

44 2008; Underwood et al., 2005; Warzecha et al., 2009), but tissues contain numerous

45 cell types and regulation at this level does not address how cell-type-specific

46 alternative splicing is achieved.

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48 In *Drosophila*, Dscam2 is a cell recognition molecule that mediates self- and cell-

49 type-specific avoidance (tiling) (Millard et al., 2007; Millard et al., 2010). Mutually

50 exclusive alternative splicing of exon 10A or 10B produces two isoforms with

51   biochemically unique extracellular domains (Millard et al., 2007). Previously, we

52   found that the splicing of *Dscam2* is cell-type-specific (Lah et al., 2014). This

53   deterministic splicing is crucial for the proper development of axon terminal size,

54   dendrite morphology and synaptic numbers (Kerwin et al., 2018; Lah et al., 2014; Li

55   et al., 2015). Although the functional consequences of disrupting regulated *Dscam2*

56   alternative splicing have been demonstrated, what regulates this process remained

57   unclear. Here, we conducted an RNAi screen and identified *muscleblind* (*mbl*) as a

58   regulator of *Dscam2* alternative splicing. Loss-of-function (LOF) and overexpression

59   (OE) studies suggest that Mbl acts both as a splicing repressor of *Dscam2* exon 10A

60   and as an activator of exon 10B (hereafter *Dscam2.10A* and *Dscam2.10B*). Consistent

61   with this finding, *mbl* expression is cell-type-specific and correlates with the

62   expression of *Dscam2.10B*. Driving mbl in mushroom body neurons that normally

63   select isoform A, induces the expression of isoform B and generates a phenotype

64   similar to that observed in animals that express a single isoform of *Dscam2*. Although

65   the *mbl* gene is itself alternatively spliced, we found that selection of *Dscam2.10B*

66   does not require a specific Mbl isoform and that human MBNL1 can also regulate

67   *Dscam2* alternative splicing. Our study demonstrates that mutually exclusive splicing

68   of *Dscam2* is regulated by the cell-type-specific expression of a highly conserved

69   RNA binding protein, Mbl.

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71

72 **Results**

73 **An RNAi screen identifies *mbl* as a repressor of *Dscam2* exon 10A selection**

74 We reasoned that the neuronal cell-type-specific alternative splicing of *Dscam2* is

75 likely regulated by RNA binding proteins, and that we could identify these regulators

76 by knocking them down in a genetic background containing an isoform reporter.   In

77 photoreceptors (R cells) of third instar larvae, *Dscam2.10B* is selected whereas the

78 splicing of *Dscam2.10A* is repressed (Lah et al., 2014; Tadros et al., 2016). Given that

79 quantifying a reduction in *Dscam2.10B* isoform reporter levels is challenging

80 compared to detecting the appearance of *Dscam2.10A* in cells where it is not normally

81 expressed, we performed a screen for repressors of isoform A in R cells.

82

83 To knock down RNA binding proteins, the *glass* multimer reporter (*GMR*)-*GAL4* was

84 used to drive RNAi transgenes selectively in R cells. Our genetic background

85 included *UAS-Dcr-2* to increase RNAi efficiency (Dietzl et al., 2007) and *GMR-GFP*

86 to mark the photoreceptors independent of the *Gal4/UAS* system (Brand and

87 Perrimon, 1993). Lastly, a *Dscam2.10A-LexA* reporter driving *LexAOp*-myristolated

88 tdTomato (hereafter *Dscam2.10A>tdTom*; Fig. 1A) was used to visualize isoform A

89 expression (Lai and Lee, 2006; Tadros et al., 2016). As expected,

90 *Dscam2.10B>tdTom* was detected in R cell projections in the lamina plexus as well as

91 in their cell bodies in the eye-disc, whereas *Dscam2.10A>tdTom* was not (Fig. 1C-

92 1D). Overexpression of Dcr-2 in R cells did not perturb the repression of

93 *Dscam2.10A* (Fig 1O). We knocked down ~160 genes using ~250 RNAi lines (Fig 1B

94 and Table S1) and identified two independent RNAi lines targeting *mbl* that caused

95 aberrant expression of *Dscam2.10A* in R cells where it is normally absent (Fig 1F,

96 1O). The penetrance increased when animals were reared at a higher temperature of

4

97   29°C, which is more favorable for Gal4 (Mondal et al., 2007; Ni et al., 2008) (Fig

98   1O).

99

100   Mbl-family proteins possess evolutionarily conserved tandem CCCH zinc-finger

101   domains through which they bind pre-mRNA. Vertebrate Mbl family members are

102   involved in tissue-specific splicing and have been implicated in myotonic dystrophy

103   (Pascual et al., 2006). Formerly known as *mindmelt*, *Drosophila mbl* was first

104   identified in a second chromosome *P*-element genetic screen for embryonic defects in

105   the peripheral nervous system (Kania et al., 1995). *Mbl* produces multiple isoforms

106   through alternative splicing (Begemann et al., 1997; Irion, 2012), and its function has

107   been most extensively characterized in fly muscles where both hypomorphic

108   mutations and sequestration of the protein by repeated CUG sequences within an

109   mRNA lead to muscle defects (Artero et al., 1998; Llamusi et al., 2013). To validate

110   the RNAi phenotype, we tested *Dscam2.10A>tdTom* expression in *mbl* loss-of-

111   function (LOF) mutants. Since *mbl* LOF results in lethality, we first conducted

112   complementation tests on six *mbl* mutant alleles to identify viable hypomorphic

113   combinations. These included two alleles created previously via imprecise *P*-element

114   excision (*mbl^{e127}* and *mbl^{e27}*; Begemann et al. 1997) two MiMIC splicing traps

115   (*mbl^{MI00976}* and *mbl^{MI04093}*; (Venken et al., 2011) and two 2nd chromosome deficiencies

116   (*Df(2R)BSC154* and *Df(2R)Exel6066*; Fig 1F-1G). Consistent with previous reports,

117   the complementation tests confirmed that the majority of the alleles were lethal over

118   one another (Fig 1G) (Kania et al., 1995). However, we identified two *mbl*

119   transheterozygous combinations that were partially viable and crossed these into a

120   *Dscam2.10A>tdTom* reporter background. Both *mbl^{e127}/mbl^{MI00976}* and

121   *mbl^{MI04093}/mbl^{MI00976}* animals presented aberrant *Dscam2.10A* expression in R cells

122   when compared to heterozygous and wild-type controls (Fig 1H-O). *Mbl* mutant

123   mosaic clones also exhibited aberrant *Dscam2.10A>tdTom* expression in R cells (Fig

124   S1A-S1F). The weakest allele, *mbl^{M00976}*, which removes only a proportion of the *mbl*

125   isoforms, was the only exception (Fig S1E-S1F).

126

127   One alternative explanation of how *Dscam2.10A>tdTom* expression could get

128   switched-on in *mbl* mutants, is through exon 10 skipping. Removing both alternative

129   exons simultaneously does not result in a frameshift mutation, and since the Gal4 in

130   our reporters is inserted directly downstream of the variable exons (in exon 11), it

131   would still be expressed. To test this possibility, we amplified *Dscam2* sequences

132   between exon 9 and 11 in *mbl^{e127}/mbl^{MI00976}* transheterozygous animals using RT-PCR.

133   In both control and *mbl* LOF mutants, we detected RT-PCR products (~690bp) that

134   corresponded to the inclusion of exon 10 (A or B) and failed to detect products

135   (~390bp) that would result from exon 10 skipping (Fig 1P). This suggested that Mbl

136   is not involved in the splicing fidelity of *Dscam2.10* but rather in the selective mutual

137   exclusion of its two isoforms. To assess whether the ratios of the two isoforms were

138   changing in the *mbl* hypomorphic mutants, we cut the exon 10 RT-PCR products with

139   the *ClaI* restriction enzyme that only recognizes exon 10A. Densitometric analysis

140   then allowed us to semi-quantitatively compare the relative levels of both isoforms.

141   There was ~25% increase in the level of exon 10A inclusion in *mbl^{e127}/mbl^{MI00976}*

142   animals compared to controls (Fig 1P), consistent with the derepression we observed

143   in our 10A reporter lines. To determine whether Mbl was specifically regulating

144   *Dscam2* exon 10 mutually exclusive splicing, we assessed other *Dscam2* alternative

145   splicing events. These included an alternative 5' splice site selection of *Dscam2* exon

146   19 and the alternative last exon (ALE) selection of exon 20 (Fig S2A). The expression

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147   of these different isoforms was unchanged in *mbl* hypomorphic mutants (Fig S2B).

148   Together, our results indicate that Mbl is an essential splicing factor that specifically

149   represses *Dscam2.10A*.

150

151   **_Mbl_ is necessary for the selection of _Dscam2_ exon 10B**

152   Since *Dscam2* exon 10 isoforms are mutually exclusively spliced, we predicted that

153   selection of exon 10A would lead to the loss of exon 10B selection. To test this, we

154   conducted mosaic analysis with a repressible cell marker (MARCM) (Lee and Luo,

155   1999) to analyse *Dscam2.10B* expression in *mbl* mutant clones. In late third instar

156   brains, clones homozygous (GFP-positive) for *mbl^E127* and *mbl^E27* exhibited a

157   dramatic reduction in *Dscam2.10B>tdTom* expression in R cell axons projecting to

158   the lamina plexus compared to controls (Fig 2B, C, E). The absence of

159   *Dscam2.10B>tdTom* in *mbl* mutant clones was more striking during pupal stages (Fig

160   2D), suggesting that perdurance of Mbl could explain the residual signal observed in

161   third instar animals. These results reveal that *mbl* is cell-autonomously required for

162   the selection of the *Dscam2.10B* isoform.

163

164   **Cell-type-specific _mbl_ expression is transcriptionally regulated**

165   Previous studies have reported that *mbl* is expressed in third instar eye-discs and

166   muscles (Artero et al., 1998; Brouwer et al., 1997). Since *mbl* LOF results in both the

167   production of *Dscam2.10A* and the loss of *Dscam2.10B*, we predicted that *mbl*

168   expression would correlate with the presence of isoform B. To test this, we

169   characterized several *mbl* reporters (Fig S3A). We analyzed three enhancer trap

170   strains (transcriptional reporters) inserted near the beginning of the *mbl* gene

171   (*mbl^k01212-LacZ*, *mbl^NP1161-Gal4* and *mbl^NP0420-Gal4*), as well as a splicing trap line

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172 generated by the Trojan-mediated conversion of a *mbl* MiMIC (Minos Mediated

173 Integration Cassette) insertion (Fig S2A, *mbl^{MiMIC00139}-Gal4;* (Diao et al., 2015). The

174 splicing trap reporter consists of a splice acceptor site and an in-frame *T2A-Gal4*

175 sequence inserted in an intron between two coding exons. This *Gal4* cassette gets

176 incorporated into *mbl* mRNA during splicing and therefore Gal4 is only present when

177 *mbl* is translated. Consistent with previous studies, and its role in repressing the

178 production of *Dscam2.10A*, all four *mbl* reporters were expressed in the third instar

179 photoreceptors (Fig 3A, S3A-S3B and data not shown). We next did a more

180 extensive characterization of *mbl* expression by driving nuclear localized GFP

181 (*GFP.nls*) with one transcriptional (*mbl^{NP0420}-Gal4)* and one translational

182 (*mbl^{MiMIC00139}-Gal4)* reporter. In the brain, we found that *mbl* was expressed

183 predominantly in postmitotic neurons with some expression detected in glial cells (Fig

184 S3C-S3F and S3H-S3K). Interestingly, we detected the translational but not the

185 transcriptional reporter in third instar muscles (Fig S3G and S3L). The absence of

186 expression is likely due to the insertion of the *P*-element into a neural-specific

187 enhancer, as previously described (Bargiela et al., 2014). To assess the expression of

188 *mbl* in the five lamina neurons L1- L5, all of which express *Dscam2* (Lah et al., 2014;

189 Tadros et al., 2016), we implemented an intersectional strategy using a

190 *UAS>stop>epitope* reporter (Nern et al., 2015) that is dependent on both *FLP* and

191 *Gal4*. The *FLP* source (*Dac-FLP*) was expressed in lamina neurons and able to

192 remove the transcriptional stop motif in the reporter transgene. The overlap between

193 *mbl-Gal4* and *Dac-FLP* allowed us to visualize *mbl* expression in lamina neurons at

194 single-cell resolution (Fig 3B). As a proof of principle, we first did an intersectional

195 analysis with a pan-neuronal reporter, *elav-Gal4* (Fig 3C$_1$). We detected many clones

196 encompassing various neuronal-cell-types including the axons of L1-L5 and R7-R8

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197   (Fig 3C-3D). This confirmed that all lamina neurons could be detected using this

198   strategy. Using *mbl-Gal4* reporters we found that L1, which expresses *Dscam2.10B*,

199   was the primary neuron labelled. A few L4 cells were also identified, which is

200   consistent with this neuron expressing *Dscam2.10B* early in development and

201   *Dscam2.10A* at later stages (Tadros et al., 2016). To confirm this finding, we

202   dissected the expression of *mbl* in lamina neurons during development. Using the

203   same intersectional strategy, we detected a high number of L4 clones at 48hr apf

204   (30%, n=10). This was followed by a decline at 60hr apf (26.7%, n = 30) and 72hr apf

205   (11.8%, n = 85) reaching the lowest at eclosion (Fig S4A and S4B;1.7%, n=242).

206   Thus, *mbl* expression in L4 neurons mirrors the expression of *Dscam2.10B*.

207   Consistent with this, L2, L3 and L5, were all detected using the intersectional strategy

208   with *Dscam2.10A-Gal4* but were not labelled using *mbl-Gal4* (Fig 3E). Together,

209   these results show that cell-type-specific *mbl* expression is transcriptionally regulated

210   and correlates with the cell-type-specific alternative splicing of *Dscam2*. Cells that

211   select *Dscam2.10B* and repress *Dscam2.10A* express *mbl*. In contrast, *mbl* was not

212   detected in cells that repress *Dscam2.10B* and select *Dscam2.10A*.

213

214   **Ectopic expression of multiple *mbl* isoforms are sufficient to promote the**

215   **selection of *Dscam2* exon 10B**

216   Our analysis in the visual system demonstrated that *mbl* is necessary for the selection

217   of *Dscam2.10B*, but we wondered whether it was sufficient to promote exon 10B

218   selection in cell types that normally repress this isoform. To test this possibility, we

219   overexpressed *mbl* ubiquitously and monitored isoform B expression using

220   *Dscam2.10B>tdTom*. We focussed on the mushroom body (MB), as this tissue

221   expresses isoform A specifically in α'β' neurons at 24hr apf where mbl is not detected

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222   (Fig 3G-3H, 4A-4C). Consistent with *mbl* being sufficient for isoform B selection,

223   ubiquitous expression of *mbl* using an enhancer trap containing a *UAS* insertion at the

224   5' end of the gene (*Act5c>mbl^{B2-E1}*), switched on *Dscam2.10B* in α'β' MB neurons,

225   where it is normally absent (Fig 4D). Ectopic *mbl* expression in MB neurons with

226   *OK107-Gal4* also led to selection of *Dscam2.10B* expression specifically in α'β'

227   neurons at 24-36hr apf. Although our two *Gal4* drivers expressed *mbl* in all MB

228   neurons, *Dscam2.10B* was only observed in α'β' neurons, demonstrating that

229   transcription of *Dscam2* is a pre-requisite for this splicing modulation. Previous

230   studies have suggested that the *mbl* gene is capable of generating different isoforms

231   with unique functions depending on their subcellular localization (Vicente et al.,

232   2007). This also includes the production of a highly abundant circular RNA that can

233   sequester the Mbl protein (Ashwal-Fluss et al., 2014; Houseley et al., 2006). To

234   assess whether *Dscam2* exon 10B selection is dependent on a specific alternative

235   variant of Mbl, we overexpressed the cDNAs of fly *mbl* isoforms (*mblA*, *mblB* and

236   *mblC*; (Begemann et al., 1997; Juni and Yamamoto, 2009) as well as an isoform of

237   the human *MBNL1* that lacks the linker region optimal for CUG repeat binding

238   (*MBNL1$_{35}$*; (Kino et al., 2004; Li et al., 2008) with either *Act5c-Gal4* or *OK107-Gal4*.

239   These constructs all possess the tandem N-terminal CCCH motif that binds to YCGY

240   sequences and lack the ability to produce *mbl* circRNA. In all cases, overexpression

241   resulted in the misexpression of *Dscam2.10B* in α'β' MBs (with the exception

242   *Act5C>mblC*, which resulted in lethality; Fig 4D-4E). Using semi-quantitative RT-

243   PCR from the *Act5C>mbl* flies, we demonstrated that overexpression of *mbl* did not

244   lead to exon 10 skipping and that it increased exon 10B selection by 8-24% (Fig 4F),

245   depending on the *mbl* isoform used. The inability of Mbl to completely inhibit exon

246   10A selection suggests that other factors or mechanisms may also contribute to cell-

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247   specific *Dscam2* isoform expression (see Discussion). These results suggest that Mbl

248   protein isoforms are all capable of *Dscam2.10B* selection and independent of *mbl*

249   circRNA. The ability of human MBNL1 to promote the selection of exon 10B

250   suggests that the regulatory logic for *Dscam2* splicing is likely conserved in other

251   mutually-exclusive cassettes in higher organisms.

252

253   Finally, we observed a phenotype in MB neurons overexpressing *mbl* where the β

254   lobe neurons inappropriately crossed the midline (Fig 4G-4I). Interestingly, a similar

255   phenotype was observed in flies expressing a single isoform of *Dscam2* that we

256   previously generated using recombinase-mediated cassette exchange (Lah et al.,

257   2014). These flies express a single isoform in all *Dscam2* positive cells. We

258   quantified this phenotype and found that the *Dscam2A*, but not the *Dscam2B*, single

259   isoform line generated a MB fusion phenotype that was significantly different from

260   controls. All of the *UAS-mbl* constructs, except human *MBNL1*, generated this

261   phenotype at a penetrance that was equal to or greater than *Dscam2A* single isoform

262   lines (Fig 4I). The lack of a phenotype with the human transgene is consistent with

263   this modified isoform having a reduced CUG-binding capacity due to its missing

264   linker domain (Kino et al., 2004; Li et al., 2008). These data demonstrate that MB

265   phenotypes generated in animals overexpressing *mbl*, phenocopy *Dscam2* single

266   isoform mutants. While the origin of this non-autonomous phenotype is not known, it

267   correlates with the misregulation of *Dscam2* alternative isoform expression.

268

269   **Discussion**

270   In this study, we identify Mbl as a regulator of *Dscam2* alternative splicing. We

271   demonstrate that removing *mbl* in a *mbl*-positive cell-type results in a switch from

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272   *Dscam2.10B* to *Dscam2.10A* selection. Ectopic expression of a variety of Mbl protein

273   isoforms in a normally *mbl*-negative neuronal cell-type is sufficient to trigger the

274   selection of *Dscam2.10B*. Consistent with this, transcriptional reporters demonstrate

275   that *mbl* is expressed in a cell-type-specific manner, which tightly correlates with

276   *Dscam2.10B*. Lastly, misexpression of *mbl* leads to a MB phenotype that is also

277   observed in flies that express a single *Dscam2* isoform.

278

279   One surprising finding in this study was that *mbl* expression itself is regulated in a

280   cell-specific manner. *Mbl* was present in all cells tested that express *Dscam2.10B* and

281   absent from *Dscam2.10A* cells. Mbl appears to be regulated at the transcriptional level

282   since enhancer-trap as well as splicing-trap reporters exhibit similar expression

283   patterns (Fig 3). This was unexpected as 1) examples of cell-specific expression of

284   splicing factors are rare in the literature and 2) *mbl* encodes numerous alternative

285   isoforms that could be individually post-transcriptionally regulated, thus bypassing

286   the need for transcriptional control of the gene. It will be interesting to explore the *in*

287   *vivo* expression patterns of other splicing factors to determine whether cell-specific

288   expression of a subset of splicing factors is a common mechanism for regulating

289   alternative splicing in the brain.

290

291   Given that Mbl can repress exon 10A and select exon 10B (Fig 4J), it is possible that

292   this single splicing factor and its associated co-factors are sufficient to regulate

293   *Dscam2* cell-specific isoform expression. It could be that *Dscam2.10A* is the default

294   exon selected when the Mbl complex is not present. In this way, cells that express

295   *Dscam2* would be '10A' positive if they did not express *mbl* and '10B' positive if

296   they did. The observation that *Dscam2* is not expressed in all neurons and our RT-

297 PCR data, however, argue that *Dscam2* mutually exclusive alternative splicing may

298 be more complicated than this model. In MB α'β' neurons, which select exon 10A,

299 ectopic expression of *mbl* using *Act5C-Gal4* can switch on a *Dscam2.10B>tdTom*

300 reporter, but the change in isoform expression in the whole brain as measured by RT-

301 PCR is only 8-24% (see Fig 4F). One might expect a much more dramatic shift to

302 isoform B if Mbl were the only regulator/mechanism involved. In addition, if

303 *Dscam2.10A* were expressed by default in the absence of *mbl*, we would expect all

304 MB neurons to express this isoform, but this is not the case. Further studies, including

305 screens for repressors or activators of exon 10B, will be required to resolve this issue.

306

307 The MB midline crossing phenotype that is generated through both the ectopic

308 expression of *mbl* and *Dscam2A* single isoform lines supports the idea that this

309 phenotype arises from a disruption in *Dscam2* cell-specific isoform expression.

310 However, since both single isoform lines have identical expression patterns

311 (expressed in all *Dscam2*-positive cells), one would expect both lines to exhibit the

312 midline crossing phenotype if it is caused by inappropriate homophilic interactions

313 between cells that normally express different isoforms. Although there is a trend

314 towards increased fusion in animals expressing only Dscam2B (Fig 4I), it did not

315 reach statistical significance. This issue may have to do with innate differences

316 between isoform A and isoform B that are not completely understood. It is possible

317 that isoform A and B are not identical in terms of signalling due to either differences

318 in homophilic binding or differences in co-factors associated with specific isoforms.

319 Consistent with this notion, we previously reported that *Dscam2A* lines produce

320 stronger phenotypes at photoreceptor synapses compared to *Dscam2B*. Another

321 perplexing aspect about the MB phenotype is that it occurs in neurons that either do

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322   not express *Dscam2* (β lobe neurons) or express it at such low levels that it is not

323   detectable with our reporters. Thus, the phenotype must arise indirectly. This could

324   occur through inappropriate interactions between α'β' neurons and another non-MB

325   cell type within this brain region that expresses *Dscam2*. Alternatively, this phenotype

326   could be independent of Dscam2 homophilic binding and instead reflect differences in

327   isoform complexes that form in different neurons.

328

329   How does Mbl repress *Dscam2.10*A and select *Dscam2.10B* at the level of pre-

330   mRNA? The vertebrate orthologue of Mbl, MBNL1 binds to YCGY (where Y is a

331   pyrimidine) in pre-mRNAs and untranslated regions using its tandem zinc-finger

332   domains, but it is quite promiscuous (Wang et al., 2012). The best-characterised

333   alternative splicing events regulated by MBNL1 are exon skipping or inclusion

334   events. In general, an exon that contains MBNL1 binding sites upstream or within the

335   coding sequence is subject to skipping, whereas downstream binding sites more often

336   promote inclusion (reviewed in Konieczny et al 2014). The mechanisms used by fly

337   Mbl to regulate splicing have not been characterised in detail, but given that human

338   MBNL1 can rescue fly *mbl* lethality and promote the endogenous expression of

339   *Dscam2* exon 10B in MBs, presumably the mechanisms are conserved. A simple

340   explanation for how Mbl regulates *Dscam2* mutually exclusive splicing would be that

341   it binds upstream of exon 10A to repress exon inclusion and downstream of exon 10B

342   to promote inclusion. Although there are many potential binding sites for Mbl

343   upstream, downstream and within the alternative exons, an obvious correlation

344   between location and repression vs inclusion is not observed. There is also a large

345   (3kb) intron downstream of exon 10B that could contain *cis* regulatory elements.

346   Identification of the sequences required for regulation by Mbl will therefore require

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347   extensive mapping and ultimately validation using a technique like cross-linking

348   followed by immunoprecipitation (CLIP).

349

350   Together, our results demonstrate that selective expression of a splicing factor can

351   drive neuronal cell-type specific alternative splicing. These data provide clues into

352   how the brain can diversify its repertoire of proteins that promote neural connectivity.

353   It is likely that Mbl is regulating the alternative splicing of other developmental genes

354   in concert with *Dscam2* and therefore regulated splicing factors such as Mbl may

355   represent hubs of neurodevelopment.

356

357   **Experimental procedures**

358   **Fly strains**

359   *Dscam2.10A-LexA* and *Dscam2.10B-LexA* (Tadros et al., 2016), *UAS-Dcr2* and *UAS-*

360   *mbl-RNAi^{VDRC28732}* (Dietzl et al., 2007), *LexAop-myr-tdTomato* (attP2, (Chen et al.,

361   2014), *UAS-Srp54-RNAi$^{TRiP.HMS03941}$*, *CadN-RNAi$^{TRiP.HMS02380}$* and *UAS-mbl-*

362   *RNAi$^{TRiP.JF03264}$*(Ni et al., 2008), *UAS-mCD8-GFP* (Lee and Luo, 1999), *FRT42D* (Xu

363   and Rubin, 1993), *mbl^{e127}* and *mbl^{e27}*(Begemann et al., 1997), *mbl^{MI00976}* and

364   *mbl^{MI04093}* (Venken et al., 2011), *Df(2R)BSC154* (Cook et al., 2012), *Df(2R)Exel6066*

365   (Parks et al., 2004), *ey-FLP* (Chr.1, (Newsome et al., 2000), *GMR-myr-GFP*,

366   *mbl^{NP0420}-Gal4* and *mbl^{NP1161}-Gal4* (Hayashi et al., 2002), *mbl^{k01212}-LacZ* (Spradling

367   et al., 1999), *mbl^{MiMIC00139}-Gal4* (H. Bellen Lab), *Dac-FLP* (Chr.3, (Millard et al.,

368   2007), *UAS>stop>myr::smGdP-V5-THS-UAS>stop>myr::smGdP-cMyc* (attP5,

369   (Nern et al., 2015), *Dscam2.10A-Gal4* and *Dscam2.10B-Gal4* (Lah et al., 2014)

370   *Act5C-Gal4* (Chr.3, from Yash Hiromi), *OK107-Gal4* (Connolly et al., 1996), *UAS-*

15

371   *mblA*, *UAS-mblB* and *UAS-mblC* (D. Yamamoto Lab)*, P{EP}mbl^{B2-E1}*, *UAS-mblA-*

372   *FLAG* and *UAS-MBNL1_{35}* (Li et al., 2008).

373

374   **RNAi screening**

375   The RNAi screen line was generated as follows: *GMR-Gal4* was recombined with

376   *GMR-GFP* on the second chromosome. *Dscam2.10A-LexA* (Tadros et al. 2016) was

377   recombined with *LexAop-myr-tdTomato* on the third chromosome. These flies were

378   crossed together with *UAS-Dcr-2* (X) to make a stable RNAi screen stock. Virgin

379   females were collected from this RNAi screen stock, crossed to UAS-RNAi males

380   and reared at 25°C. Wandering third instar larvae were dissected and fixed. We tested

381   between one and three independent RNAi lines per gene. Brains were imaged without

382   antibodies using confocal microscopy. RNAi lines tested are listed in Table S1.

383

384   **Semiquantitative RT-PCR**

385   Total RNA was isolated using TRIzol (Ambion) following the manufacturer's

386   protocol. Reverse transcription was performed on each RNA sample with random

387   primer mix using 200 units of M-MULV (NEB) and 2 μ g of RNA in a 20 μ l

388   reaction, at 42°C for 1 hr. PCR reactions were set up with specific primers to analyse

389   alternative splicing of various regions of *Dscam2*. Where possible, semi-quantitative

390   PCR was performed to generate multiple isoforms in a single reaction and relative

391   levels were compared by electrophoresis.

392

393   **Immunohistochemistry**

394   Immunostaining were conducted as previously described (Lah et al. 2014). Antibody

395   dilutions used were as follows: mouse mAb24B10 (1:20; DSHB), mouse anti-Repo

16

396   (1:20; DSHB), mout anti-DAC (1:20; DSHB), mouse anti-Fas2 (1:20; DSHB) rat

397   anti-ELAV (1:200), V5-tag:DyLight anti-mouse 550 (1:500; AbD Serotec), V5-

398   tag:DyLight anti-mouse 405 (1:200; AbD Serotec), myc-tag;DyLight anti-mouse 549

399   (1:200; AbD Serotec), Phalloidin:Alexa Fluor 568 (1:200; Molecular Probes),

400   DyLight anti-mouse 647 (1:2000; Jackson Laboratory), DyLight Cy3 anti-rat (1:2000;

401   Jackson Laboratory).

402

403   **Image acquisition**

404   Imaging was performed at the School of Biomedical Sciences Imaging Facility.

405   Images were taken on a Leica SP8 laser scanning confocal system with a 63X

406   Glycerol NA 1.3.

407

408   **Fly genotypes**

409   R cell RNAi experiments (Figure 1)

410   *w; GMR-GFP, GMR-Gal4/CyO; Dscam2.10B-LexA, LexAop-myr-tdTomato/TM6B*

411   *w; GMR-GFP, GMR-Gal4/CyO; Dscam2.10A-LexA, LexAop-myr-tdTomato/TM6B*

412   *w, UAS-Dcr-2; GMR-GFP, GMR-Gal4/CyO; Dscam2.10A-LexA, LexAop-myr-*

413   *tdTomato/TM6B*

414   *w, UAS-Dcr-2; GMR-GFP, GMR-Gal4/UAS-mCD8-RFP; Dscam2.10A-LexA,*

415   *LexAop-myr-tdTomato/+*

416   *w, UAS-Dcr-2; GMR-GFP, GMR-Gal4/UAS-mbl-RNAi(v28732); Dscam2.10A-LexA,*

417   *LexAop-myr-tdTomato/+*

418   *w, UAS-Dcr-2; GMR-GFP, GMR-Gal4/+; Dscam2.10A-LexA, LexAop-myr-*

419   *tdTomato/UAS-mbl-RNAi(TRiP.JF03264)*

420

17

421   <u>mbl whole animal experiments (Figure 1)</u>

422   *w; +; Dscam2.10B-LexA, LexAop-myr-tdTomato/TM6B*

423   *w; +; Dscam2.10A-LexA, LexAop-myr-tdTomato/TM6B*

424   *w; mbl^{e127}/CyO,GFP; Dscam2.10A-LexA, LexAop-myr-tdTomato/TM6B*

425   *w; mbl^{MI00976}/CyO,GFP; Dscam2.10A-LexA, LexAop-myr-tdTomato/TM6B*

426   *w; mbl^{MI04093}/CyO,GFP; Dscam2.10A-LexA, LexAop-myr-tdTomato/TM6B*

427   *w; mbl^{e127}/ mbl^{MI00976}; Dscam2.10A-LexA, LexAop-myr-tdTomato/+*

428   *w; mbl^{MI04093}/ mbl^{MI00976}; Dscam2.10A-LexA, LexAop-myr-tdTomato/+*

429

430   <u>mbl ey-FLP mosaic experiments (Figure 1)</u>

431   *w, ey-FLP; FRT42D, GMR-myr-GFP/FRT42D; Dscam2.10B-LexA, LexAop-myr-*

432   *tdTomato, UAS-mCD8-GFP/+*

433   *w, ey-FLP; FRT42D, GMR-myr-GFP/FRT42D; Dscam2.10A-LexA, LexAop-myr-*

434   *tdTomato, UAS-mCD8-GFP/+*

435   *w, ey-FLP; FRT42D, GMR-myr-GFP/FRT42D, Df(2R)154 ; Dscam2.10A-LexA,*

436   *LexAop-myr-tdTomato, UAS-mCD8-GFP/+*

437   *w, ey-FLP; FRT42D, GMR-myr-GFP/FRT42D, mbl^{e27}; Dscam2.10A-LexA, LexAop-*

438   *myr-tdTomato, UAS-mCD8-GFP/+*

439   *w, ey-FLP; FRT42D, GMR-myr-GFP/FRT42D, mbl^{MI00976}; Dscam2.10A-LexA,*

440   *LexAop-myr-tdTomato, UAS-mCD8-GFP/+*

441

442   <u>mbl ey-FLP MARCM experiments (Figure 3)</u>

443   *w, ey-FLP; FRT42D, Tub-Gal80/FRT42D; Dscam2.10A-LexA, LexAop-myr-*

444   *tdTomato, Act5c-Gal4, UAS-mCD8-GFP/+*

18

445   *w, ey-FLP; FRT42D, Tub-Gal80/FRT42D, mbl^{e27}; Dscam2.10A-LexA, LexAop-myr-*

446   *tdTomato, Act5c-Gal4, UAS-mCD8-GFP/+*

447   *w, ey-FLP; FRT42D, Tub-Gal80/FRT42D, mbl^{e127}; Dscam2.10A-LexA, LexAop-myr-*

448   *tdTomato, Act5c-Gal4, UAS-mCD8-GFP/+*

449

450   mbl expression experiments (Figure 3)

451   *w; UAS-mCD8-GFP/+; mbl^{NP0420}-Gal4/+*

452   *w; UAS-mCD8-GFP/+; mbl^{MI00139}-Gal4/+*

453   *w; Dac-FLP/+; elav-Gal4/ UAS>stop>myr::smGdP-V5-THS-*

454   *UAS>stop>myr::smGdP-cMyc*

455   *w; Dac-FLP/+; mbl^{NP0420}-Gal4/ UAS>stop>myr::smGdP-V5-THS-*

456   *UAS>stop>myr::smGdP-cMyc*

457   *w; Dac-FLP/+; mbl^{MI00139}-Gal4/ UAS>stop>myr::smGdP-V5-THS-*

458   *UAS>stop>myr::smGdP-cMyc*

459   *w; Dac-FLP/+; Dscam2.10A-Gal4/ UAS>stop>myr::smGdP-V5-THS-*

460   *UAS>stop>myr::smGdP-cMyc*

461   *w; Dac-FLP/+; Dscam2.10B-Gal4/ UAS>stop>myr::smGdP-V5-THS-*

462   *UAS>stop>myr::smGdP-cMyc*

463   *w; +; mbl^{NP0420}-Gal4/UAS-GFP.nls*

464   *w; +; mbl^{MI00139}-Gal4/UAS-GFP.nls*

465

466   mbl ectopic expression in MBs (Figure 4)

467   *w; +; Dscam2.10A-LexA, LexAop-myr-tdTomato, Act5c-Gal4, UAS-mCD8-GFP/+*

468   *w; +; Dscam2.10B-LexA, LexAop-myr-tdTomato, Act5c-Gal4, UAS-mCD8-GFP/+*

19

469 *w; P{EP}mbl^{B2-E1}/+; Dscam2.10B-LexA, LexAop-myr-tdTomato, Act5c-Gal4, UAS-*

470 *mCD8-GFP/+*

471 *w; +; Dscam2.10B-LexA, LexAop-myr-tdTomato, Act5c-Gal4, UAS-mCD8-*

472 *GFP/UAS-mblA*

473 *w; +; Dscam2.10B-LexA, LexAop-myr-tdTomato, Act5c-Gal4, UAS-mCD8-*

474 *GFP/UAS-mblB*

475 *w; +; Dscam2.10B-LexA, LexAop-myr-tdTomato, Act5c-Gal4, UAS-mCD8-*

476 *GFP/UAS-mblC*

477 *w; +; Dscam2.10B-LexA, LexAop-myr-tdTomato, Act5c-Gal4,UAS-mCD8-GFP/UAS-*

478 *MBNL1$_{35}$*

479 *w; +; Dscam2.10B-LexA, LexAop-myr-tdTomato, UAS-mCD8-GFP/UAS-mblA;*

480 *OK107-Gal4/+*

481 *w; +; Dscam2.10B-LexA, LexAop-myr-tdTomato, UAS-mCD8-GFP/UAS-mblB;*

482 *OK107-Gal4/+*

483 *w; +; Dscam2.10B-LexA, LexAop-myr-tdTomato, UAS-mCD8-GFP/UAS-mblC;*

484 *OK107-Gal4/+*

485 *w; +; Dscam2.10B-LexA, LexAop-myr-tdTomato, UAS-mCD8-GFP/UAS-MBNL1$_{35}$;*

486 *OK107-Gal4/+*

487

488

489 **Author contribution**

490 J.S.S.L designed and performed all experiments. K.N.M characterized Dscam2

491 isoform expression in mushroom bodies and midline crossing defects in Dscam2

492 single isoform mutant animals. S.S.M supervised the project. J.S.S.L and S.S.M wrote

493 the manuscript.

494

508

509 **Figure legends**

510 **Figure 1.** *Drosophila mbl* is required for the repression of *Dscam2* exon 10A in R

511 cells. (A) Schematic showing the region of *Dscam2* exon 10 that undergoes mutually

512 exclusive alternative splicing and the LexA isoform-specific reporter lines. Frame-

513 shift mutations in the exon not reported are shown. (B) Schematic RNAi screen

514 design for identifying repressors of *Dscam2* exon 10A selection. R cells normally

515 select exon 10B and repress exon 10A. We knocked-down RNA binding proteins in R

516 cells while monitoring 10A expression.

517 (C-E) *Dscam2* exon 10A is derepressed in R cells when mbl is knocked-down. (C_1-

518 C_3) *Dscam2.10B* control. R cells (green) normally select exon 10B (red). R cell

519 terminals can be observed in the lamina plexus (angle brackets). *Dscam2.10B* is also

520   expressed in the developing optic lobe (arrowhead). (D$_1$-D$_3$) *Dscam2.10A* is not

521   expressed in R cells (green) but is expressed in the developing optic lobe (arrowhead).

522   (E$_1$-E$_3$) RNAi lines targeting *mbl* in R cells results in the aberrant expression of

523   *Dscam2.10A* in R cells.

524   (F) Schematic of the *mbl* gene showing the location of two small deletions (*E27* and

525   *E127*), two MiMIC insertions (*MI04093* and *MI00976*) and two deficiencies

526   (*Df(2R)Exel6066 and Df(2R)BSC154*) used in this study. Non-coding exons are in

527   gray, coding exons are black.

528   (G) Complementation test of *mbl* loss-of-function (LOF) alleles. Numbers in the table

529   represent the number of non-*CyO* offspring over the total. Most transheterygote

530   combinations were lethal with the exception of *mbl^{MI00976}/mbl^{e27}* and

531   *mbl^{MI00976}/mbl^{MI04093}* (green).

532   (H-N) *Mbl* transheterozygotes express *Dscam2.10A* in R cells. (H) *Dscam2.10B*

533   control showing expression in the lamina plexus (angle brackets). (I) *Dscam2.10A*

534   control showing no expression of this isoform in R cells. (J-L) Heterozygous animals

535   for *mbl* LOF alleles are comparable to control. (M-N) Two different *mbl*

536   transheterozygote combinations exhibit de-repression of *Dscam2.10A* in R cells.

537   (O) Quantification of *Dscam2.10>tdTom* expression in third instar R cells with

538   various *mbl* manipulations; including RNAi knockdown (black bars) and whole

539   animal transheterozygotes (white). Y-axis represents the number of optic lobes with R

540   cells positive for tdTom over total quantified as a percentage. On the x-axis, the

541   presence of a transgene is indicated with a blue box and the temperature at which the

542   crosses were reared (25°C or 29°C) is indicated.

543   (P) *Dscam2* exon 10A inclusion is increased in *mbl* transheterozygotes. (Top)

544   Semiquantitative RT-PCR from different genotypes indicated. Primers amplified the

545   variable region that includes exon 10. A smaller product that would result from exon

546   10 skipping is not observed. (Bottom) Exon 10A-specific cleavage with restriction

547   enzyme ClaI shows an increase in exon 10A inclusion in mbl transheterozygotes.

548   Percentage of exon 10A inclusion was calculated by dividing 10A by 10A+10B bands

549   following restriction digest. See also Figures S1 and S2.

550

551   **Figure 2.** *Drosophila* Mbl is necessary for the selection of *Dscam2* exon 10B in R

552   cells.

553   (A) Schematic of our predicted *mbl* MARCM results using *ey-FLP*. WT R cell clones

554   will be GFP(+) and *Dscam2.10B>tdTom*(+) (yellow), whereas *mbl* mutant clones will

555   be *Dscam2.10B>tdTom*(-) (green). (B$_1$-B$_3$) Control MARCM clones (green) in 3rd

556   instar R cells (angle brackets) are positive for *Dscam2.10B>tdTom* (arrowhead). (C$_1$-

557   C$_3$) In *mbl^{e27}* clones, *Dscam2.10B* labelling in the lamina plexus is discontinuous and

558   its absence correlates with the loss of Mbl (arrowhead). (D$_1$-D$_2$) *Mbl* MARCM clones

559   from midpupal optic lobes lack *Dscam2.10B*>tdTom. (E$_1$-E$_3$) A different allele

560   (*mbl^{e127}*) exhibits a similar phenotype in third instar brains.

561

562   **Figure 3. *Mbl* is expressed in a cell-specific manner that correlates with**

563   *Dscam2.10B*

564   (A) A *mbl* Gal4 reporter (green) is expressed in third instar R cells but not in lamina

565   neuron precursor cells labelled with an antibody against Dacshund (DAC, red).

566   (B) Schematic of MultiColor FlpOut (MCFO) approach to characterize *mbl* reporter

567   expression in lamina neurons at adult stages. The UAS FlpOut construct produces an

568   epitope-tagged version of a non-fluorescent GFP (smGFP,(Nern et al., 2015))

569 (C_1-C_4) *Mbl* can be detected in all lamina neurons using a MCFO strategy with a pan-

570 neuronal reporter (*elav-Gal4*). Lamina neurons were identified based on their unique

571 axon morphologies. (D_1-D_4) An intersectional strategy using *mbl-Gal4* labels

572 primarily L1 lamina neurons. (E) Quantification of lamina neurons and R7-R8

573 neurons observed using the intersectional strategy. Dark blue and light blue boxes

574 represent high and low numbers of labelled neurons, respectively. (F-H) *Mbl* is not

575 expressed in mushroom body (MB) neurons that express *Dscam2.10A* at 24hr apf.

576 (F_1-F_2) *Dscam2.10A* is expressed in α'β' MB neurons that are not labelled by Fas2.

577 Fas2 labels the αβ and γ subsets of MB neurons. (G-H) Neither *Dscam2.10B* (G_1-G_2)

578 nor *mbl* (H_1-H_2) are detected in MB neurons. See also Figures S3 and S4.

579

580 **Figure 4.** All fly *mbl* isoforms can select *Dscam2* exon 10B and promote a midline

581 crossing phenotype in MBs.

582 (A) Schematic showing that *mbl* is sufficient to drive *Dscam2.10B* selection in

583 α'β'neurons.

584 (B) Control showing that *Dscam2.10A* (red) is expressed in α'β' neurons at 24hr apf.

585 (C) *Dscam2.10B* is normally repressed in α'β' neurons. (D) Overexpression of *mbl*

586 activates *Dscam2.10B* selection (red) in α'β' neurons. (E) Quantification of

587 *Dscam2.10* expression in α'β' neurons at 24-36hr apf with and without *mbl* OE.

588 Ubiquitous driver (*Act5c-Gal4*, black bars) and pan-mushroom body neuron driver

589 (*OK107-Gal4*, white bars). Y-axis represents the number of tdTom positive (+) α'β'

590 over the total, expressed as a percentage. Ratio of tdTom(+)/total is shown in each

591 bar. (F) *Mbl* OE increases *Dscam2* exon 10B inclusion. Semiquantitative RT-PCR as

592 in Figure 1. Exon 10A-specific cleavage with restriction enzyme ClaI shows an

593 increase in exon 10B inclusion in *mbl* OE animals, without exon 10 skipping.

594   (G) A representative confocal image of control adult αβ lobes (red) with clear

595   separation between the two β-lobes at the midline. (H) A representative confocal

596   image of adult αβ lobes from an animal overexpressing *mblA*. β-lobe axons

597   inappropriately cross the midline (arrowhead). (I) Quantification of β-lobe axon

598   midline crossing defects. Numbers in parentheses represent total number of

599   mushroom bodies quantified. Fishers exact test was used to compare genotypes to

600   their corresponding controls (white bars). n.s (not significant) $P>0.05$, * $P<0.05$ and

601   **** $P<0.0001$. (J) Model illustrating that Mbl represses *Dscam2.10A* and selects

602   *Dscam2.10B*.

603

604

605

606

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A Dscam2 gene / Dscam2 10A LexA reporters / Dscam2 10B LexA reporters

WT: CAAAGGTAGC → 10B*: C-AAGGTAGC

WT: CAATGGTAAA → 10A*: C-ATGGTAAA

800bp · 2A viral peptide

B RNAi screen: Identifying repressors of Dscam2 exon 10A

Dscam2.10A>reporter

R cells are Dscam2.10A(−) → R cells becomes Dscam2.10A(+)

knockdown of ~160 proteins related to RNA processing with ~250 RNAi lines in R cells

C–E Dscam2.10B>tdTom / Dscam2.10A>tdTom / mbl-RNAi knockdown

R cells / tdTom / merge

F *muscleblind (mbl)* gene — not to scale

E27 / E127 / MI04093 / MI00976 / Df(2R)Exel6066 / Df(2R)BSC154

G Complementation test: *mbl* alleles

	mbl^{e27}/CyO	mbl^{e127}/CyO	$mbl^{MI00976}$/CyO	$mbl^{MI04093}$/CyO	Df(2R)BSC154/CyO	Df(2R)Exel6066/CyO	
	0/256	0/20	0/39	1/285	0/224	0/26	mbl^{e27}/CyO
	0/118	47/246	0/64	0/309	0/223		mbl^{e127}/CyO
		0/271	24/173	8/334	4/116		$mbl^{MI00976}$/CyO
			1/313	0/313	0/135		$mbl^{MI04093}$/CyO
				0/226	0/92		Df(2R)BSC154/CyO
					0/110		Df(2R)Exel6066/CyO

no of non-CyO offspring / total offspring

H–N Dscam2.10B>tdTom / Dscam2.10A>tdTom

+/+ / +/+ / $mbl^{MI00976}$/+ / mbl^{e127}/+ / $mbl^{MI00976}$/+ / mbl^{e127}/$mbl^{MI00976}$ / $mbl^{MI04093}$/$mbl^{MI00976}$

O

R-cell knockdown / Whole animals

25°C / 29°C

% hemispheres with R cellspositive for tdtomato

38/43 · 1/33 · 0/44 · 19/33 · 17/26 · 0/48 · 11/12 · 16/16 · 15/15 · 0/22 · 0/29 · 0/19 · 0/15 · 26/26 · 27/28

Dscam2.10B>tdTom / Dscam2.10A>tdTom / UAS-Dcr-2

+ · UAS-mbl-RNAi #1 #2 · UAS-mbl-RNAi #1 #2 · + · mbl^{e127}/+ · $mbl^{MI00976}$/+ · $mbl^{MI04093}$/+ · mbl^{e127}/$mbl^{MI00976}$ · $mbl^{MI04093}$/$mbl^{MI00976}$

transgene absent / transgene present

P

control / mbl^{e127}/+ / $mbl^{MI00976}$/+ / mbl^{e127}/$mbl^{MI00976}$

clal −ve : 9 10A 11 + 9 10B 11 / 9 11

clal +ve : 9 10B 11 / 9 10A 11 (clal)

44.5 · 49.0 · 49.1 · 55.3 · % exon 10A

Figure 2

Li et al. Figure 2.

A ey-FLP MARCM: Mitotic recombination generates clones of mbl LOF R cell

os

Dscam2.10B>reporter

la

ol

me

WT R cells are Dscam2.10B(+)

mbl LOF R cells are Dscam2.10(-)

merge   MARCM clones   Dscam2.10B>tdTom

FRT42D

B₁   la   me   B₂   B₃

mbl^e27, FRT42D

C₁   C₂   C₃

D₁   D₂

mbl^e127, FRT42D

E₁   E₂   E₃

Li et al. Figure 3.

A — $mbl^{INP0420}>mCD8-GFP$ DAC — 3rd instar

B — MultiColor FlpOut (MCFO) approach to visualize mbl expression

medulla

C_1 — Dac-FLP / elav-Gal4

C_2 — elav-Gal4 L1 L2

C_3 — elav-Gal4 L3 L3 L5

C_4 — elav-Gal4 L4

D_1 — Dac-FLP / mbl-Gal4

D_2 — $mbl^{INP0420}$-Gal4 L1 L1

D_3 — $mbl^{MI00139}$-Gal4 L1 L1

adult

E

Genotype	L1	L2	L3	L4	L5	R7	R8	Total	no. of OLs
elav-Gal4	71	125	69	39	93	12	28	437	37
$mbl^{INP0420}$-Gal4	238	0	0	4	0	76	146	464	48
$mbl^{MI00139}$-Gal4	95	0	0	0	0	111	210	416	61
$Dscam2^{10A}$-Gal4	0	119	115	58	145	0	0	437	32
$Dscam2^{10B}$-Gal4	251	0	0	138	0	16	55	460	21

(row label: Dac-FLP(III), UAS>stop>epitope)

■ Many clones detected
■ Few clones detected
□ No clones detected

Mushroom bodies — 24apf

$Dscam2^{10A}>mCD8-GFP$

$Dscam2^{10B}>mCD8-GFP$

$mbl^{MI00139}>mCD8-GFP$

F_1 **F_2** Fas2 **G_1** **G_2** **H_1** **H_2**
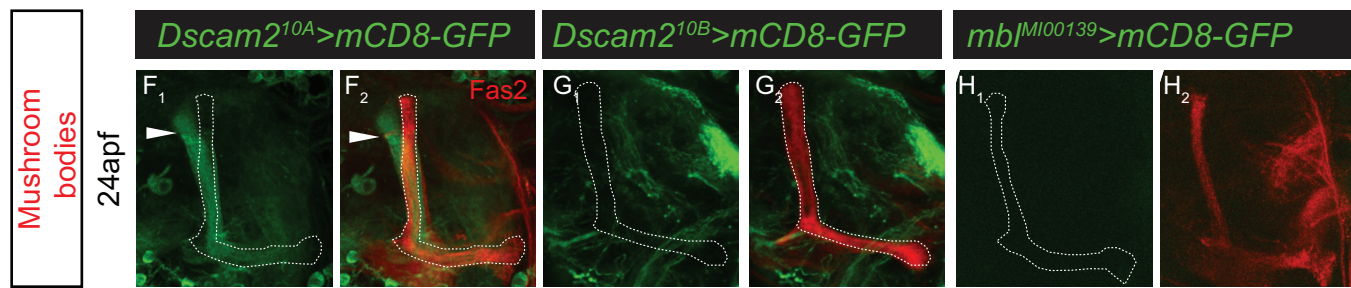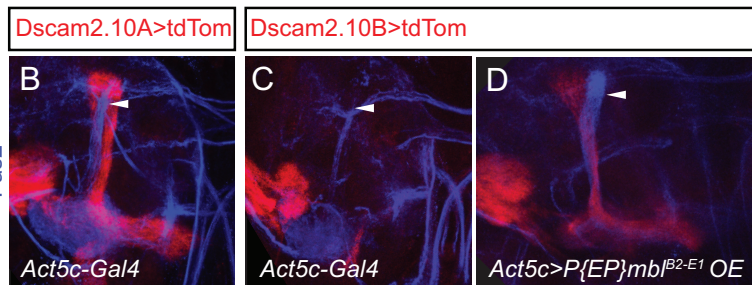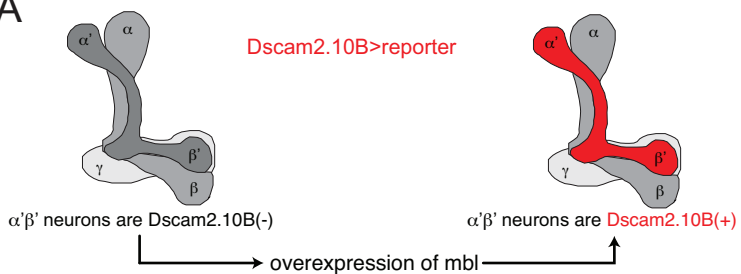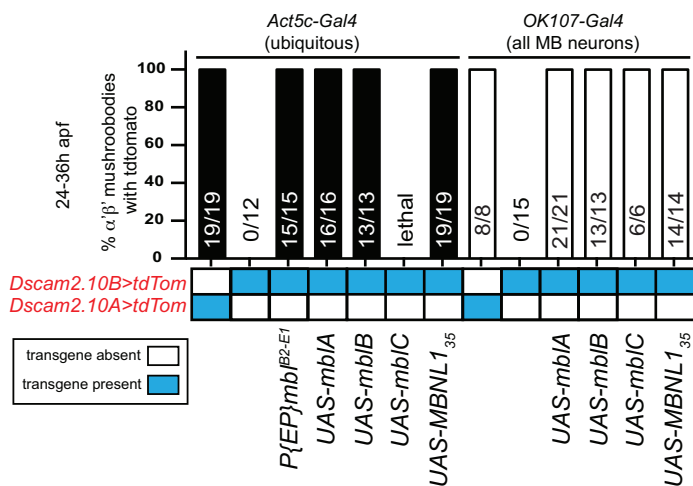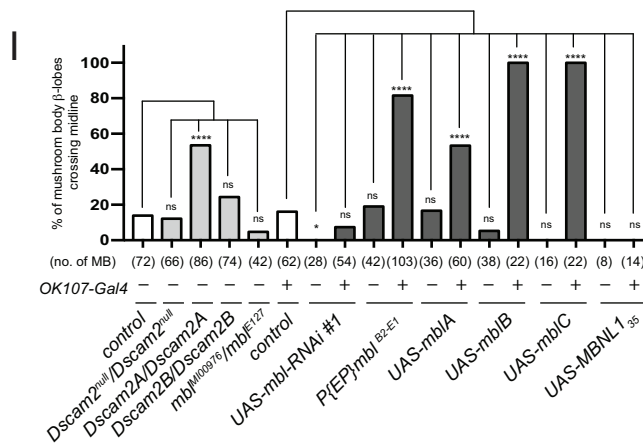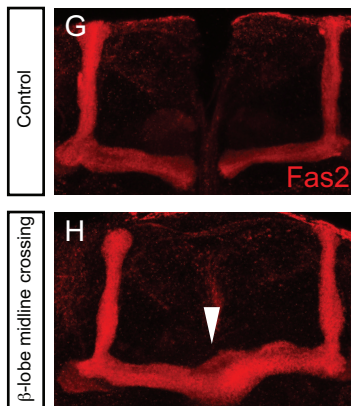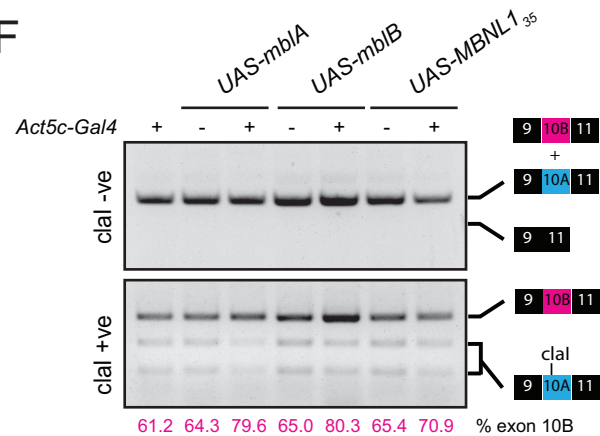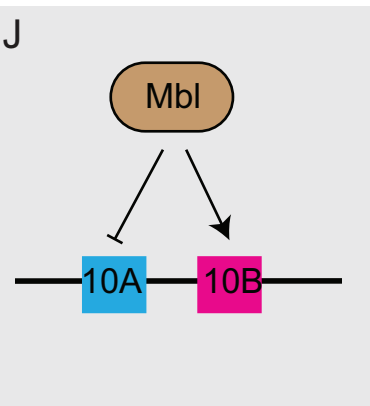
Figure S1. Related to Figure 1. *Mbl* LOF results in aberrant *Dscam2*.10A reporter expression in eye mosaic clones.

(A-F) Eye mosaics of *mbl* LOF alleles cause derepression of *Dscam2.10A>tdTom* in R cells. *WT* mosaic clones (GFP-negative) express *Dscam2.10B>tdTom* (A_1-A_4) but not *Dscam2.10A>tdTom* (B_1-B_4). *Mbl* mutant (GFP-negative) clones, *Df(2R)BSC154* show aberrant *Dscam2.10A* expression in R cells (C_1-C_4). (D) mbl^{e27} eye clones exhibit derepression of Dscam2.10A (red). (E) Clones of a *mbl* allele that deleted only a portion of all mbl isoforms (*mbl^{MI00976}*) do not exhibit derepression of Dscam2.10A. (F) Quantification of *Dscam2.10>tdTom* expression in third instar R cells with *mbl* LOF eye mosaic clones. Y-axis represents the number of optic lobes with R cells positive for tdTom over total number of optic lobes quantified as a percentage. On the x-axis, the presence of a transgene is indicated with a blue box.

Figure S2. Related to Figure 1. *Mbl* LOF does not affect other *Dscam2* splicing events.

(A) *Mbl* LOF does not affect other *Dscam2* splicing events. Semiquantitative RT-PCR from different genotypes indicated. Primers amplified the variable region that includes exon 19S/19L or three alternative last exons (ALE). Percentage of 19L inclusion was calculated by dividing the 19L band by 19L+19S. Percentage of ALE 21A and ALE 21BL inclusion was calculated by dividing respectively the 21A and 21BL band by 21A+21BL+21BS (total). (B) Graphs of RT-PCR data from L. Plots show minimum (bottom line), mean (middle line) and maximum (top line) points, where individual points depict biological replicates. Dashed line represents mean of control.

Figure S3. Related to Figure 3. *Mbl* is expressed in R cells, neurons and glia

(A) Schematic showing the insertion locations of different *mbl* reporters. Translated

regions (black) and non-translated regions (grey) are shown.

(B) *Mbl* is expressed in R cells (red) in third instar eye-discs (ed). The *mbl* splicing

trap reporter (green*)* overlapped with a marker of R cells (24B10).

(C-G) $mbl^{MI00139}$>GFP.nls is expressed in neurons and muscles. (C_1-C_2)

Representative confocal image of a $mbl^{MI00139}$>GFP.nls (green) adult brain co-

labelled with an ELAV antibody (red). Dashed lines demarcate GFP(+) cells. Yellow

solid arrowheads show GFP(+) cells that are ELAV(-). (D) Quantification of *mbl* in

third instar and adult brains where ~90-100% of GFP(+) cells are also ELAV(+)

(black bars). Y-axis represents the number of GFP(+) cells positive for ELAV

quantified as a percentage. (E_1-E_2) Representative confocal image of a

$mbl^{MI00139}$>GFP.nls adult brain labelled with a Repo antibody (red). Dashed lines

demarcate GFP(+) cells. White solid arrowheads show GFP(+) cells that are positive

for Repo. (F) Quantification of $mbl^{MI00139}$>GFP.nls where ~0-10% of

$mbl^{MI00139}$>GFP.nls (+) cells are also Repo(+).Y-axis represents the number of

GFP(+) cells positive for Repo quantified as a percentage. (G_1-G_2)

$mbl^{MI00139}$>GFP.nls expression is also found in third instar muscles m4-m8, m12 and

m13 (Phalloidin, red).

(H_1-H_2) Representative confocal image of a mbl^{NP0420}>GFP.nls (green) adult brain

co-labelled with an ELAV antibody (red). Dashed lines demarcate GFP(+) cells. (I)

Quantification of mbl^{NP0420}>GFP.nls in third instar and adult brains where ~80-90%

of GFP(+) cells are also ELAV(+). (J-K) In third instar and adult brains,

mbl^{NP0420}>GFP.nls overlaps minimally with Repo (red). (J_1-J_2) Representative

confocal image of a mbl^{NP0420}>GFP.nls adult brain labelled with Repo. Dashed lines

demarcate GFP(+) cells. White solid arrowheads show GFP(+) cells that are positive for Repo. (K) Quantification of mbl^{NP0420}>GFP.nls in third instar and adult brains where ~10-15% of GFP (+) cells are also Repo(+). (L$_1$-L$_2$) mbl^{NP0420}>GFP.nls expression is not detected in third instar muscles m4-m8, m12 and m13 (Phalloidin, red).

Figure S4. Related to Figure 3. *Mbl* expression is cell-type-specific and correlates with *Dscam2.10B*.

(A) Quantification of lamina neurons and R7-R8 neurons observed using the intersectional strategy during development. Two different *mbl* reporters were used. The transcriptional reporter labelled L4 cells early in development whereas the splicing trap reporter did not. This is most likely due to the lower efficiency of the splicing trap given that it produced 5X fewer L1 clones at 72hr compared to the transcriptional reporter. Blue boxes represent detection of reporter expression at different hours after pupal formation (apf). (B) A plot of the percentage of L4 lamina neurons over total lamina neurons during development (data from the *mbl* transcriptional reporter).

(C-E) *Mbl* is not detected in MB neurons that express *Dscam2.10A* in adults. (C$_1$-C$_2$) *Dscam2.10A* is expressed in α'β' mushroom body neurons (asterisks) but not the αβ and γ subsets of MB neurons labelled by Fas2 (red). Neither *Dscam2.10B* (D$_1$-D$_2$) nor *mbl* (E$_1$-E$_2$) are expressed in MB neurons. Neurons in the midline express both *Dscam2.10B* and *mbl* (white arrowhead).

3rd instar
Eye mosaics (ey-FLP)

| Dscam2.10B>tdTom | Dscam2.10A>tdTom |

FRT42D | *Df(2R)BSC154, FRT42D*

mbl^{e27},FRT42D | *mbl^{MI00976},FRT42D*

F

A — *mbl* gene

mbl^{k01212}-LacZ (enhancer trap)

mbl^{NP0420}-Gal4 (transcriptional reporter)

not to scale

1 2 3 4

mbl^{MI00139}-Gal4 (splicing trap)

mbl^{NP1161}-Gal4 (transcriptional reporter)

B mbl^{MI00139}>mCD8-GFP

mbl^{MI00139}>GFP.nls

ELAV

C_1 adult
C_2

D % of GFP(+) cells that are ELAV(+)
791/793 604/656
3rd instar adult

Repo

E_1 adult
E_2

F % of GFP(+) cells that are repo(+)
1/779 107/1098
3rd instar adult

Phalloidin

G_1 3rd instar   m7 m6 m13 m12 m5 m4 m8
G_2

mbl^{NP0420}>GFP.nls

ELAV

H_1 adult
H_2

I % of GFP(+) cells that are ELAV(+)
240/282 808/854
3rd instar adult

Repo

J_1 adult
J_2

K % of GFP(+) cells that are repo(+)
60/434 108/1174
3rd instar adult

Phalloidin

L_1 3rd instar   m8 m7 m6 m13 m12 m5
L_2

Figure S4

A

	Genotype	L1	L2	L3	L4	L5	R7	R8	Total	no. of OLs
Dac-FLP(III), UAS>stop>epitope	*mbl^{NP0420}-Gal4* 72 apf	75	0	0	10	0	10	38	133	8
	60 apf	22	0	0	8	0	15	29	74	8
	48 apf	7	0	0	3	0	1	9	20	2
	mbl^{MI00139}-Gal4 72 apf	15	0	0	0	0	6	15	36	8
	48 apf	12	0	0	0	0	4	24	40	8

Clones detected
No clones detected

B

mbl^{NP0420}-Gal4

% of LN cells that are L4

48 apf 60 apf 72 apf adult

Mushroom bodies — adult

$Dscam2^{10A}>CD8\text{-}GFP$ — C₁ / C₂ (Fas2)

$Dscam2^{10B}>CD8\text{-}GFP$ — D₁ / D₂

$mbl^{MI00139}>CD8\text{-}GFP$ — E₁ / E₂

Table S1

Table S1. List of tested RNAi that did not de-repress Dscam2 exon 10A in R cells

Flybase Number	CG Number	Gene Name	RNAi ID	no. of ol/ed	no. of animals
FBgn0052062	CG32062	A2bp1	27286	12	6
FBgn026239	CG6671	AGO1	33727	3	2
FBgn0000114	CG31762	aret	44483	18	9
FBgn0004587	CG10851	B52	v38862	16	8
FBgn0004587	CG10851	B52	v38860	4	2
FBgn0037660	CG18005	beag	v103832	8	4
FBgn0015907	CG13425	bl	v2912	10	6
FBgn0015907	CG13425	bl	v105271	9	5
FBgn0262475	CG6319	bru-2	50631	13	7
FBgn0264001	CG43744	Bru-3	50734	8	4
FBgn0031883	CG11266	Caper	55742	10	6
FBgn0031883	CG11266	Caper	55742	8	4
FBgn0022942	CG7035	Cbp80	v22331	12	8
FBgn0035136	CG6905	Cdc5	v13492	2	1
FBgn0035136	CG6905	Cdc5	v109369	10	5
FBgn0032690	CG10333	CG10333	v18132	12	8
FBgn0032690	CG10333	CG10333	v18133	4	2
FBgn0036277	CG10418	CG10418	v105940	11	6
FBgn0037531	CG10445	CG10445	v104753	14	7
FBgn0036314	CG10754	CG10754	v31346	11	8
FBgn0039920	CG11360	CG11360	v38491	15	9
FBgn0039920	CG11360	CG11360	v38492	11	6
FBgn0035692	CG13298	CG13298	55257	8	4
FBgn0035162	CG13900	CG13900	v18955	9	6
FBgn0035163	CG13900	CG13900	v108248	16	8
FBgn0037220	CG14641	CG14641	v110507/CyOtb	11	6
FBgn0038464	CG16941	CG16941	v20338	1	1
FBgn0033089	CG17266	CG17266	v25243	10	5
FBgn0033089	CG17266	CG17266	v25244	2	1
FBgn0029751	CG17764	CG17764	v20541	12	7
FBgn0029751	CG17764	CG17764	v101894	10	5
FBgn0035271	CG2021	CG2021	28579	8	5
FBgn0031266	CG2807	CG2807	v25162	8	5
FBgn0037344	CG2926	CG2926	v33589	11	5
FBgn050122	CG30122	CG30122	55209	6	3
FBgn0031631	CG3225	CG3225	v24725	9	5
FBgn0052533	CG32533	CG32533	v38634	1	1
FBgn0052533	CG32533	CG32533	v51785	11	6
FBgn0031628	CG3294	CG3294	v26111/TM6B	12	6
FBgn0031628	CG3294	CG3294	v26111/TM6B	12	6
FBgn0053108	CG33108	CG33108	v24996	9	5
FBgn0031229	CG3436	CG3436	55207/CyOtb	4	2
FBgn0031492	CG3542	CG3542	v26227	10	5
FBgn0031492	CG3542	CG3542	v26229	4	2
FBgn0031493	CG3605	CG3605	v26250	12	7
FBgn0031493	CG3605	CG3605	v26252	8	5
FBgn0035987	CG3689	CG3689	v45278	10	5
FBgn0028474	CG4119	CG4119	v26395	9	5
FBgn0028474	CG4119	CG4119	v106696/CyOtb	10	6
FBgn0034598	CG4266	CG4266	v26472	14	7
FBgn0034598	CG4266	CG4266	v26475	4	2
FBgn0031287	CG4291	CG4291	v21819/TM6B	11	6
FBgn0035016	CG4612	CG4612	v52497	9	5
FBgn0039566	CG4849	CG4849	v21962	9	5
FBgn0032194	CG4901	CG4901	v34904	11	6
FBgn0038344	CG5205	CG5205	v107282	9	5
FBgn0039182	CG5728	CG5728	v24697	14	7
FBgn0038927	CG6015	CG6015	34565	lethal	
FBgn0030631	CG6227	CG6227	v40351	11	8
FBgn0030632	CG6227	CG6227	v40352	12	6
FBgn0004903	CG6354	CG6354	31333	12	9
FBgn0004903	CG6354	CG6354	55662	8	4
FBgn0035675	CG6610	CG6610	v106830	10	6
FBgn0035675	CG6610	CG6610	31870	10	6
FBgn0036828	CG6841	CG6841	v34253/CyOtb	10	5
FBgn0030085	CG6999	CG6999	v110143	11	7
FBgn0030085	CG6999	CG6999	55157	12	6
FBgn0035872	CG7185	CG7185	v107147	5	3
FBgn0035872	CG7185	CG7185	34804	14	7
FBgn0036734	CG7564	CG7564	v100562	10	5
FBgn0035235	CG7879	CG7879	56930	10	5
FBgn0038887	CG7907	CG7907	55370	6	3
FBgn0035253	CG7971	CG7971	v101384	10	7
FBgn0027567	CG8108	CG8108	v35344	12	7
FBgn0030697	CG8565	CG8565	v100449	10	7
FBgn0030697	CG8565	CG8565	55368	11	6
FBgn0032883	CG9323	CG9323	v44984	12	8
FBgn0032883	CG9323	CG9323	v110410	8	4
FBgn0015621	CG3642	Clp	v26259	12	7
FBgn0015621	CG3642	Clp	v26261	13	7
FBgn0263995	CG43738	cpo	28360	8	4
FBgn0027873	CG1957	Cpsf100	50893/TM6B	8	5
FBgn0027873	CG1957	Cpsf100	50893/TM6B	8	5

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FBgn0024698	CG10110	Cpsf160	v18009	11	6
FBgn0024698	CG10110	Cpsf160	v110571	9	6
FBgn0261065	CG7698	Cpsf73	v39558	9	5
FBgn0000377	CG3193	crn	v25919	lethal	
FBgn0039867	CG2261	CstF-50	v43716	10	5
FBgn0039867	CG2261	CstF-50	v109583	8	4
FBgn0027841	CG7697	CstF-64	v21045/CyOtb	10	6
FBgn0010220	CG12759	Dbp45A	v17306	6	3
FBgn0010221	CG12760	Dbp45A	v104183	13	7
FBgn0033160	CG11107	Dhx15	v44119/CyOtb	10	6
FBgn0031601	CG3058	Dim1	v21258	10	5
FBgn0259220	CG42320	Doa	v19066	9	5
FBgn0020306	CG696	dom	v7787	2	1
FBgn0000562	CG4051	egl	28969	8	4
FBgn0001942	CG9075	eIF-4a	v42202	lethal	
FBgn0034237	CG4878	eIF3-S9	32880	lethal	
FBgn0260400	CG4262	elav	28371	2	1
FBgn0033859	CG6197	fand	v104186	10	5
FBgn0036850	CG10419	Gem2	v47372	13	8
FBgn0036850	CG10419	Gem2	v47374	10	7
FBgn0259139	CG6946	glo	33668	9	6
FBgn0259139	CG6946	glo	v27752	12	6
FBgn0001179	CG8019	hay	v41023	12	8
FBgn0014189	CG7269	Hel25E	v22557	9	5
FBgn0011224	CG31000	heph	v33735	10	6
FBgn0011224	CG31000	heph	v110749	18	10
FBgn0264491	CG10293	how	v13756	13	7
FBgn0264491	CG10293	how	v100775	10	5
FBgn0004838	CG10377	Hrb27c, Hrp48	v16040	12	7
FBgn0004838	CG10377	Hrb27c, Hrp48	31685	6	3
FBgn0004838	CG10377	Hrb27c, Hrp48	33716	8	4
FBgn0004237	CG12749	Hrb87F, hrp36	v51759	9	6
FBgn0004237	CG12749	Hrb87F, hrp36	52937	11	6
FBgn0004237	CG12749	Hrb87F, hrp36	31244	14	8
FBgn0001215	CG9983	Hrb98DE, hrp38	31303	10	7
FBgn0001215	CG9983	Hrb98DE, hrp38	32351	13	8
FBgn0015949	CG9854	hrg	v42283	12	6
FBgn0002431	CG9484	hyd	v44675	12	6
FBgn0039691	CG1972	IntS11	v33450	7	5
FBgn0039691	CG1972	IntS11	v109408	8	5
FBgn0036570	CG5222	IntS9	v110367	10	5
FBgn0026713	CG32604	l(1)G0007	v31908	15	8
FBgn0026714	CG32605	l(1)G0008	v31909	4	2
FBgn0086444	CG10689	l(2)37Cb	v31324	9	6
FBgn0263599	CG5931	l(3)72Ab	v43962	5	3
FBgn0263600	CG5932	l(3)72Ab	v110666	6	3
FBgn0035838	CG7942	ldbr	v110582	8	5
FBgn0035838	CG7942	ldbr	55661	8	6
FBgn0034834	CG3162	LS2	v21379	11	7
FBgn0034834	CG3162	LS2	v21380	14	7
FBgn0261067	CG4279	LSm1	v28793	11	6
FBgn0261067	CG4279	LSm1	v50653	10	5
FBgn0033450	CG12924	Lsm11	v108336	12	6
FBgn0051184	CG31184	LSm3	56892	4	2
FBgn0261068	CG13277	Lsm7	v23862	10	6
FBgn0011666	CG5099	msi	55152	10	5
FBgn0262737	CG7437	mub	v28024	16	9
FBgn0014366	CG2925	noi	v20943	9	5
FBgn0015520	CG10328	nonA-l	v101567	7	4
FBgn0015520	CG10328	nonA-l	52934	3	2
FBgn0261619	CG5119	pAbp	v22007	9	5
FBgn0005648	CG2163	Pabp2	v106466	10	5
FBgn0086895	CG8241	pea	v47782	9	5
FBgn0027784	CG6011	Prp18	v13760	10	5
FBgn0027784	CG6011	Prp18	v100287	2	1
FBgn0261119	CG5519	Prp19	v108575	11	6
FBgn0261119	CG5519	Prp19	v41438	3	2
FBgn0036915	CG7757	Prp3	v25548	9	6
FBgn0036487	CG6876	Prp31	v35131	3	2
FBgn0036487	CG6876	Prp31	v103721	6	3
FBgn0033688	CG8877	Prp8	v18565	12	7
FBgn0261552	CG42670	Ps	v44710	18	9
FBgn0261552	CG42670	Ps	v24214	10	5
FBgn0014870	CG8912	Psi	v28989	16	8
FBgn0014870	CG8912	Psi	v28990	10	5
FBgn0014870	CG8912	Psi	v105135	10	7
FBgn0028577	CG12085	pUf68	v109796	8	4
FBgn0003165	CG9755	pum	36676	12	7
FBgn0022987	CG4816	qkr54B	34896	10	5
FBgn0022986	CG3613	qkr58E-1	56159	10	5
FBgn0022985	CG5821	qkr58E-2	55279/CyOtb	6	3
FBgn0022984	CG3584	qkr58E-3	55922	10	5
FBgn0260944	CG17136	Rbp1	v21083/TM6B	12	6

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FBgn0260944	CG17136	Rbp1	v110008	11	6
FBgn0030479	CG1987	Rbp1-like	v105883	10	6
FBgn0030479	CG1987	Rbp1-like	44100	4	2
FBgn0260943	CG32169	rbp6	61324/CyOtb	8	4
FBgn0015778	CG9412	rin	33392/TM6B	12	7
FBgn0003261	CG10279	Rm62	v46908/TM6B	12	6
FBgn0037707	CG16788	RnpS1	56910	10	5
FBgn0037707	CG16788	RnpS1	36580	6	3
FBgn0005649	CG5422	Rox8	v100563	10	5
FBgn0005649	CG5422	Rox8	v41439	12	6
FBgn0011305	CG5655	Rsf1	v22186/TM3	15	10
FBgn0267790	CG9373	rump	42665/CyOtb	6	3
FBgn0039229	CG6995	Saf-B	51759		5
FBgn0265298	CG5442	SC35	v40590	5	3
FBgn0265298	CG5442	SC35	v104978	6	3
FBgn0025571	CG5836	SF1	v13426	3	2
FBgn0040284	CG6987	SF2	v27775/TM3	13	7
FBgn0040284	CG6987	SF2	v27776/TM6B	6	4
FBgn0052423	CG32423	shep	43545	4	3
FBgn0002354	CG1420	Slu7	v103587	5	3
FBgn0262601	CG5352	SmB	v40587	3	2
FBgn0262601	CG5352	SmB	v110713	12	6
FBgn0261933	CG10753	SmD1	v31343/TM6B	8	4
FBgn0261933	CG10753	SmD1	v31342	7	4
FBgn0261789	CG1249	SmD2	v31947	4	2
FBgn0261789	CG1249	SmD2	v31946	8	4
FBgn0261789	CG1249	SmD2	v100690	4	2
FBgn0023167	CG8427	SmD3	v35933	8	5
FBgn0261790	CG18591	SmE	v23569	4	2
FBgn0261790	CG18591	SmE	v23570/TM6B	10	5
FBgn0000426	CG16792	SmF	v107644/CyOtb	lethal	
FBgn0000426	CG16792	SmF	26734	12	6
FBgn0036641	CG16725	Smn	v100392	7	4
FBgn0003449	CG4528	snf	51459	16	8
FBgn0003449	CG4528	snf	55914	9	5
FBgn0016978	CG8749	snRNP-U1-70K	v23150	11	8
FBgn0016978	CG8749	snRNP-U1-70K	v23151	10	6
FBgn0261792	CG5454	snRNP-U1-C	v22132	11	6
FBgn0261792	CG5454	snRNP-U1-C	v22133	10	5
FBgn0261791	CG9742	SNRPG	v39256	10	5
FBgn0015818	CG3780	Spx	v40471	9	5
FBgn0015818	CG3780	Spx	v40472	9	5
FBgn0263396	CG16901	sqd, hrp40	v32395	12	6
FBgn0263396	CG16901	sqd, hrp40	31302	20	10
FBgn0036340	CG11274	SRm160	v6439	9	5
FBgn0036340	CG11274	SRm160	v100751	8	4
FBgn0015787	CG4457	Srp19	51160	lethal	
FBgn0024285	CG4602	Srp54	v51088	8	6
FBgn0024285	CG4602	Srp54	55254	9	5
FBgn0026370	CG8174	SRPK	v103416	9	6
FBgn0025702	CG11489	srpk79D	v47544	8	5
FBgn0025702	CG11489	srpk79D	v47545	10	5
FBgn0003520	CG5753	stau	31247	9	5
FBgn0003559	CG17170	su(f)	v110125	6	3
FBgn0003638	CG3019	su(wa)	v25597	12	9
FBgn0003638	CG3019	su(wa)	v104716	10	5
FBgn0264270	CG43770	Sxl	34393	10	5
FBgn0037371	CG2097	Sym	v33470	9	5
FBgn0038826	CG17838	syp	56972	10	5
FBgn0038826	CG17838	syp	v33012	15	9
FBgn0025790	CG10327	TBPH	v38377	7	4
FBgn0025790	CG10327	TBPH	v38379	10	5
FBgn0003741	CG16724	tra	2560	12	6
FBgn0003742	CG10128	tra2	v8868	9	5
FBgn0039117	CG10210	tst	v38356	8	4
FBgn0039117	CG10210	tst	v108216	12	6
FBgn0033378	CG8781	tsu	55367	11	6
FBgn0033378	CG8781	tsu	28955	9	5
FBgn0033210	CG1406	U2A	v17358/TM6B	9	5
FBgn0033210	CG1406	U2A	v109815	11	6
FBgn0017457	CG3582	U2af38	v110075	9	5
FBgn0017457	CG3582	U2af38	29304	13	7
FBgn0005411	CG9998	U2af50	v24176	11	6
FBgn0005411	CG9998	U2af50	v24177	10	6
FBgn0036733	CG6322	U4-U6-60K	v34242	8	6
FBgn0036733	CG6322	U4-U6-60K	v110393	10	6
FBgn0030354	CG1559	Upf1	43144	12	7
FBgn0028554	CG10203	xl6	v31203	14	7
FBgn0028555	CG10204	xl6	v100226	11	7
FBgn0021895	CG18426	ytr	55704	4	2