

1 **miRNA activity contributes to accurate RNA splicing in *C. elegans* intestine**  
2 **and body muscle tissues.**

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## ABSTRACT

45

46 MicroRNAs (miRNAs) are known to modulate gene expression, but their  
47 activity at the tissue-specific level remains largely uncharacterized. In order to  
48 study their contribution to tissue-specific gene expression, we developed novel  
49 tools to profile miRNA targets in the *C. elegans* intestine and body muscle.

50 We validated many previously described interactions, and identified  
51 ~3,500 novel targets. Many of the miRNA targets curated are known to modulate  
52 the functions of their respective tissues. Within our datasets we observed a  
53 disparity in the use of miRNA-based gene regulation between the intestine and  
54 body muscle. The intestine contained significantly more miRNA targets than the  
55 body muscle highlighting its transcriptional complexity. We detected an  
56 unexpected enrichment of RNA binding proteins targeted by miRNA in both  
57 tissues, with a notable abundance of RNA splicing factors.

58 We developed *in vivo* genetic tools to validate and further study three RNA  
59 splicing factors identified as miRNA targets in our study (*asd-2*, *hrp-2* and *smu-2*),  
60 and show that these factors indeed contain functional miRNA regulatory  
61 elements in their 3'UTRs that are able to repress their expression in the intestine.  
62 In addition, the alternative splicing pattern of their respective downstream targets  
63 (*unc-60*, *unc-52*, *lin-10* and *ret-1*) is dysregulated when the miRNA pathway is  
64 disrupted.

65 A re-annotation of the transcriptome data in *C. elegans* strains that are  
66 deficient in the miRNA pathway from past studies supports and expands on our  
67 results. This study highlights an unexpected role for miRNAs in modulating

68 tissue-specific gene isoforms, where post-transcriptional regulation of RNA  
69 splicing factors associates with tissue-specific alternative splicing.

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## INTRODUCTION

72 Multicellular organisms have evolved complex forms of gene regulation achieved  
73 at different stages throughout development, and equally executed at pre-, co-, and post-  
74 transcriptional levels. Alternative splicing, which leads to the production of different  
75 protein isoforms using single mRNA precursors, fine tune these regulatory networks,  
76 and contributes to the acquisition of tissue identity and function. In humans, more than  
77 95% of genes undergo alternative splicing (PAN *et al.* 2008; WANG *et al.* 2008), and this  
78 mechanism is required to ensure that each tissue possesses the correct gene  
79 expression pattern needed to thrive (BARALLE AND GIUDICE 2017). Many aberrant  
80 alternative splicing events are linked to diseases (SCOTTI AND SWANSON 2016; MONTES  
81 *et al.* 2019).

82 While several tissue-specific splicing factors are known to directly promote RNA  
83 splicing, most of the alternative splicing events are achieved through differential  
84 expression of particular classes of RNA binding proteins (RBPs), which in turn bind  
85 specific *cis*-acting elements located within exon/intron junctions in a combinatorial  
86 manner, promoting or inhibiting splicing. Serine Arginine (SR) proteins recognize exon  
87 splicing enhancers (ESEs) and are important in promoting constitutive and alternative  
88 pre-mRNA splicing, while heterogeneous nuclear ribonucleoproteins (hnRNPs) are a  
89 large class of nuclear RBPs that bind exon splicing silencers (ESSs) and usually  
90 promote exon retention (MATLIN *et al.* 2005). The relative expression levels of members



91 from these two classes of splicing factors vary between tissues, and this imbalance is  
92 believed to promote the outcome of tissue-specific alternative splicing events (CACERES  
93 *et al.* 1994; ZHU *et al.* 2001).

94 Tissue identity is also achieved through post-transcriptional gene regulation  
95 events, mostly occurring through 3' Untranslated Regions (3'UTRs), which are portions  
96 of genes located between the STOP codon and the poly(A) tail of mature eukaryotic  
97 mRNAs. 3'UTRs have been recently subjected to intense study as they were found to  
98 be targeted by a variety of factors, which recognize small regulatory elements in these  
99 regions and are able to modulate the dosage of gene output at the post-transcriptional  
100 level (MATOULKOVA *et al.* 2012; OIKONOMOU *et al.* 2014; MAYR 2017). While these  
101 regulatory mechanisms are still poorly characterized, and the majority of functional  
102 elements remain unknown, disorders in the 3' end processing of mRNAs have been  
103 found to play key roles in the loss of tissue identity and the establishment of major  
104 diseases, including neurodegenerative diseases, diabetes, and cancer (CONNE *et al.*  
105 2000; MAYR AND BARTEL 2009; DELAY *et al.* 2011; REHFELD *et al.* 2013).

106 3'UTRs are frequently targeted by a class of repressive molecules named  
107 microRNAs (miRNAs). miRNAs are short non-coding RNAs, ~22nt in length, that are  
108 incorporated into a large protein complex named the microRNA-induced silencing  
109 complex (miRISC), where they guide the interaction between the miRISC and the target  
110 mRNA by base pairing, primarily within the 3'UTR (BARTEL 2009). The final outcome  
111 miRNA targeting can be context-dependent, however mRNAs targeted by the miRISC  
112 are typically held in translational repression prior to degradation of the transcript  
113 (AMBROS AND RUVKUN 2018; BARTEL 2018). Initial studies showed that although

114 mismatches between miRNAs and their targets are common, many interactions make  
115 use of perfectly complementary at a small conserved heptametrical motif, located at  
116 position 2-7 at the 5' end of the miRNA (seed region), (AMBROS AND RUVKUN 2018;  
117 BARTEL 2018). Later findings showed that while important, the seed region may also  
118 contain one or more mismatches while pairing with its target mRNA, and that this  
119 element alone is not a sufficient predictor of miRNA targeting (HA *et al.* 1996; REINHART  
120 *et al.* 2000; DIDIANO AND HOBERT 2006; GRIMSON *et al.* 2007). Compensatory base  
121 pairing at the 3' end of the miRNA (nucleotides 10-13) can also play a role in target  
122 recognition (SHIN *et al.* 2010; CHI *et al.* 2012), and have been implicated in conferring  
123 target specificity to miRNAs that share the same seed regions (BROUGHTON *et al.* 2016;  
124 WOLTER *et al.* 2017).

125 miRNAs and their 3'UTR targets are frequently conserved and play a variety of  
126 roles in modulating fundamental biological processes across metazoans. Bioinformatic  
127 algorithms, such as miRanda (BETEL *et al.* 2008), TargetScan (LEWIS *et al.* 2005) and  
128 PicTar (LALL *et al.* 2006), use evolutionary conservation and thermodynamic principles  
129 to identify miRNA target sites, and are the preferred tools for miRNA target  
130 identification. Based on these algorithms it was initially predicted that each miRNA  
131 controls hundreds of gene products (CHEN AND RAJEWSKY 2007). Recent high-  
132 throughput wet bench approaches, have validated and expanded on these initial  
133 predictions, and provide further evidence that miRNAs can indeed target hundreds of  
134 genes, and regulate molecular pathways throughout development and in diseases  
135 (SELBACH *et al.* 2008; HELWAK *et al.* 2013; WOLTER *et al.* 2014; BROWN *et al.* 2017;  
136 WOLTER *et al.* 2017).

137           In the past few years, several groups produced tissue-specific miRNA  
138 localization data in mouse, rat, and human tissues (EISENBERG *et al.* 2007; LANDGRAF *et*  
139 *al.* 2007) and in cancer (JIMA *et al.* 2010). A previous low-throughput study has identified  
140 hundreds of *C. elegans* intestine and muscle specific miRNAs and their targets, which  
141 are mostly involved in the immune response to pathogens (KUDLOW *et al.* 2012). This  
142 study used a microarray-based approach, which unfortunately does not provide enough  
143 depth to fully understand miRNA function in a tissue-specific manner. In addition, this  
144 studies identified only a subset of miRNA targets, which rely on the scaffolding proteins  
145 AIN-1 and AIN-2, later found to be only present at specific developmental stages  
146 (KUDLOW *et al.* 2012; JANNOT *et al.* 2016).

147           In *C. elegans* there are three known Argonaute proteins that execute the miRNA  
148 pathway, and are named *alg-1*, *alg-2* and *alg-5*. A recent transcriptome analysis in  
149 strains deficient in each of these members show a remarkable difference in function,  
150 where *alg-1* and *alg-2* are mostly expressed in somatic tissue and are functionally  
151 redundant, and *alg-5* is expressed exclusively in the gonads, interacts with only a  
152 subset of miRNAs and is required for optimal fertility (BROWN *et al.* 2017). A more recent  
153 study used a novel methylation-dependent sequencing approach (mime-Seq), and  
154 identified high quality tissue-specific miRNAs in intestine and body muscle tissues  
155 (ALBERTI *et al.* 2018).

156           Taken together, these studies unequivocally show that there are indeed distinct  
157 functional miRNA populations in tissues, which are in turn capable of reshaping  
158 transcriptomes and contributing to the acquisition and maintenance of cell identity.

159 Since most miRNAs targets are only predicted, it is still unclear how these events are  
160 initiated and maintained.

161 Our group has pioneered the use of the round worm *C. elegans* to systematically  
162 study tissue-specific gene expression (BLAZIE *et al.* 2015; BLAZIE *et al.* 2017). In a  
163 previous study, we developed a method to isolate and sequence high quality tissue-  
164 specific mRNA from worms, and published several integrative analyses of gene  
165 expression in most of the *C. elegans* somatic tissues, including the intestine and body  
166 muscle (BLAZIE *et al.* 2015; BLAZIE *et al.* 2017). In these studies, we found an  
167 abundance of several tissue-specific RNA splicing factors, which could explain tissue-  
168 specific alternative splicing events. For example, we detected the RNA splicing factors  
169 *asd-2* and *sup-12*, previously shown to regulate splicing patterns of the *unc-60* gene in  
170 the *C. elegans* body muscle (OHNO *et al.* 2012), and *hrp-2*, a hnRNP known to induce  
171 alternative splicing isoforms of the three widely expressed genes; *unc-52* and *lin-10* and  
172 *ret-1* (KABAT *et al.* 2009; HEINTZ *et al.* 2017). The human orthologues of *hrp-2*, hnRNPQ  
173 and hnRNPR, have been shown to act in a dosage dependent manner to regulate the  
174 alternative splicing of the widely expressed gene PKM, demonstrating the importance of  
175 regulating the dosage of hnRNPs (CHEN AND CHENG 2012). Studies performed using  
176 human cell lines have revealed that miRNA-based regulation of splicing factor dosage  
177 can drive tissue development (MAKEYEV *et al.* 2007).

178 In order to better understand the tissue-specific contribution of miRNA-  
179 based regulation to, gene dosage, RBP's functions, and tissue identity, we  
180 performed RNA immunoprecipitation of the *C. elegans* Argonaute ortholog *alg-1*,  
181 isolated and sequenced the tissue-specific targets of miRNAs in *C. elegans*, and

182 used them to identify miRNA targets from two of its largest and most well  
183 characterized tissues, the intestine and body muscle.

184 As expected, we found that the number of genes regulated in each tissue  
185 correlates with its transcriptome size. However, there is a greater proportion of  
186 the transcriptome regulated in the intestine when compared to the body muscle,  
187 suggesting that the degree of regulation by miRNA in tissues is heterogeneous.  
188 In addition, a large number of identified targets possess RNA binding domains  
189 and include several mRNA splicing factors such as hnRNPs and SR proteins. We  
190 also detected and validated several tissue-specific miRNA-based regulatory  
191 networks involved in tissue-specific alternative splicing of genes. The analysis of  
192 splice junctions in transcriptomes from miRNA-deficient strains from past studies  
193 support and expand these observations, suggesting a potential role for miRNA in  
194 regulating mRNA biogenesis in addition to mRNA turnover.

195

## 196 MATERIALS AND METHODS

### 197 *Preparing MosSCI vectors for generating GFP::ALG-1 strains*

198 The strains used for the ALG-1 pull-down were prepared using a modified  
199 version of the previously published polyA-pull construct (BLAZIE *et al.* 2015;  
200 BLAZIE *et al.* 2017). We produced a second-position Entry Gateway vector  
201 containing the genomic sequence of *alg-1* tagged at its N-terminus with the GFP  
202 fluorochrome. Briefly, we designed primers flanking the coding sequence of *alg-1*  
203 and performed a Polymerase Chain Reaction (PCR) amplification to clone the  
204 *alg-1* locus from genomic DNA extracted from N2 *wt* worms (primer 1 and 2 in

205 Table S2). The resulting PCR product was analysed on a 1% agarose gel, which  
206 displayed a unique expected band at ~3,500 nucleotides. This band was then  
207 isolated using the QIAquick Gel Extraction Kit (QIAGEN, cat. 28704) according to  
208 the manufacturer's protocol. Upon recovery, we digested the purified PCR  
209 product with the restriction enzymes SacI and BamHI and then cloned it into the  
210 modified polyA-pull construct (BLAZIE *et al.* 2015; BLAZIE *et al.* 2017), replacing  
211 the gene *pab-1*. The ligation reaction was performed using the NEB Quick  
212 Ligation Kit (cat. MS2200S) according to the manufacturer's protocol. We used  
213 the QuikChange II Site-Directed Mutagenesis Kit (Agilent, cat. 200523) to  
214 remove the unnecessary C-terminal 3xFLAG tag from the polyA-pull vector  
215 (primers 3 and 4 in Table S2). We then cloned the previously described  
216 endogenous *alg-1* promoter (VASQUEZ-RIFO *et al.* 2012) by designing primers to  
217 add Gateway BP cloning elements, and then performed PCR using N2 *wt*  
218 genomic DNA as a template (primers 5 and 6 in Table S2). Using the resulting  
219 PCR product, we performed a Gateway BP cloning reaction into the pDONR  
220 P4P1R vector (Invitrogen) according to the manufacturers protocol. To assemble  
221 the final injection clones, we performed several Gateway LR Clonase II plus  
222 reactions (Invitrogen, cat. 12538-013) using the destination vector CFJ150  
223 (FROKJAER-JENSEN *et al.* 2012), the tissue-specific or endogenous promoters  
224 (*alg-1* for endogenous, *ges-1* for the intestine and *myo-3* for the body muscle),  
225 the *gfp* tagged *alg-1* coding sequence, and the *unc-54* 3'UTR as previously  
226 published(BLAZIE *et al.* 2017).

227

228 ***Microinjections and screening of transgenic C. elegans strains***

229 To prepare single copy integrated transgenic strains we used the *C. elegans*  
230 strain Eg6699 [ttTi5605 II; unc-119(ed3) III; oxEx1578](FROKJAER-JENSEN *et al.*  
231 2012), which is designed for MosI mediated single copy integration (MosSCI)  
232 insertion, using standard injection techniques. These strains were synchronized  
233 by bleaching(PORTA-DE-LA-RIVA *et al.* 2012), then grown at 20°C for 3 days to  
234 produce young adult (YA) worms. YA worms were then picked and subjected to  
235 microinjection using a plasmid mix containing; pCFJ601 (50ng/μl), pMA122  
236 (10ng/μl), pGH8 (10ng/μl), pCFJ90 (2.5ng/μl), pCFJ104 (5ng/μl) and the  
237 transgene (22.5ng/μl)(FROKJAER-JENSEN *et al.* 2008). Three injected worms were  
238 isolated and individually placed into single small nematode growth media (NGM)  
239 plates (USA Scientific, cat 8609-0160) seeded with OP50-1 and were allowed to  
240 grow and produce progeny until the worms had exhausted their food supply. The  
241 plates were then screened for progenies that exhibited wild type movement and  
242 proper GFP expression, and single worms exhibiting both markers were picked  
243 and placed onto separate plates to lay eggs overnight. In order to select for  
244 single copy integrated worms, an additional screen was performed to select for  
245 worms that lost the mCherry fluorochrome expression (extrachromosomal  
246 injection markers).

247

248 ***Genotyping of transgenic C. elegans strains***

249 Single adult worms were isolated and allowed to lay eggs overnight and then  
250 genotyped for single copy integration of the transgene by single worm PCR as

251 previously described (BROUGHTON *et al.* 2016) (primers 7-9 in Table S2). Progeny  
252 from worms that contained the single copy integrations were propagated and  
253 used for this study. A complete list of worm strains produced in this study is  
254 shown in Table S3.

255

### 256 ***Validating expression of the transgenic construct***

257 To validate the expression of our transgenic construct, and to evaluate our ability  
258 to immunoprecipitate GFP tagged ALG-1, we performed an immunoprecipitation  
259 (as described below) followed by a western blot. For the western blot we used a  
260 primary anti-GFP antibody (Novus, NB600-303) (1:2000) and a fluorescent  
261 secondary antibody (LI-COR, 925-32211)(1:5000), followed by imaging using the  
262 ODYSSEY CLX system (LI-COR Biosciences, NE) (Figure S1).

263

### 264 ***In vivo validation of GFP::*ALG-1* functionality by brood size assay***

265 In order to validate the *in vivo* functionality of our transgenic GFP tagged ALG-1,  
266 we used a genetic approach. It was previously shown that the knock out *alg-1*  
267 strain RF54 [*alg-1(gk214) X*] lead to a decrease in fertility (BUKHARI *et al.* 2012).  
268 We rescued this decrease in fertility in the *alg-1* knockout strain RF54[*alg-*  
269 *1(gk214) X*] by crossing it into our strain MMA17 (Table S3), which expresses our  
270 GFP tagged transgenic ALG-1, driven by the endogenous *alg-1* promoter. The  
271 resulting strain MMA20 [*alg-1(gk214)X; alg-1p::gfp::alg-1::unc-54 II*] only  
272 expresses our cloned *alg-1* gene tagged with the GFP fluorochrome. We  
273 validated the genotype of MMA20 using single worm PCRs as previously



274 described(BROUGHTON *et al.* 2016) (primers 10 and 11 in Table S2 and Figure  
275 S2). The brood size assay was used to evaluate the ability of our transgenic GFP  
276 tagged ALG-1 construct to rescue the loss in fertility seen in the *alg-1* knockout  
277 strain (RF54). The brood size assay was performed by first synchronizing N2 (*wt*),  
278 RF54 and MMA20 strains to arrested L1 larvae, through bleaching followed by  
279 starvation overnight in M9 solution. We then plated the L1 arrested worms on  
280 NGM plates seeded with OP50-1 and allowed the worms to develop to the adult  
281 stage for 48 hours after which single worms were isolated onto OP50-1 seeded  
282 plates. The adult worms were left to lay eggs overnight (16 hours) after which the  
283 adult worms were removed. The eggs were allowed to hatch and develop for 24  
284 hours and the number of larvae in each plate was counted.

285

### 286 ***Sample preparation and crosslinking***

287 0.5ml of mixed stage *C. elegans* of each strain was grown on five large 20 cm  
288 plates (USA Scientific, cat 8609-0215) and harvested by centrifugation at 400rcf  
289 for 3 minutes. The pellets were initially washed in 15ml dH<sub>2</sub>O water and spun  
290 down at 400 rcf for 3 minutes and then resuspended in 10ml of  
291 M9+0.1%Tween20 and then cross-linked 3 times on ice, with energy setting:  
292 3000 x 100  $\mu\text{J}/\text{cm}^2$  (3kJ/m<sup>2</sup>) (Stratalinker 2400, Stratagene)(MOORE *et al.* 2014).  
293 After the crosslinking, each *C. elegans* strain was recovered by centrifugation at  
294 400 rcf for 3 minutes, and resuspended in two volumes (1ml) of lysis buffer  
295 (150mM NaCl, 25mM HEPES(NaOH) pH 7.4, 0.2mM DTT, 10% Glycerol, 25  
296 units/ml of RNasin® Ribonuclease Inhibitor (Promega, cat N2611), 1% Triton X-

297 100 and 1 tablet of protease inhibitor for every 10ml of lysis buffer (Roche  
298 cOmplete ULTRA Tablets, Sigma, cat 5892791001). The lysed samples were  
299 subjected to sonication using the following settings: amplitude 40%; 5x with  
300 10sec pulses; 50sec rest between pulses (Q55 Sonicator, Qsonica). After the  
301 sonication, the cell lysate was cleared of debris by centrifugation at 21,000rcf at  
302 4°C for 15 min and the supernatants were then transferred to new tubes.

303

#### 304 ***GFP-TRAP bead preparation and immunoprecipitation***

305 25µl of GFP-TRAP beads (Chromotek, gtma-10) (total binding capacity 7.5µg)  
306 per immunoprecipitation were resuspended by gently vortexing for 30 seconds,  
307 and washed three times with 500µl of cold Dilution/Wash buffer (10 mM Tris/Cl  
308 pH 7.5; 150 mM NaCl; 0.5 mM EDTA). The beads were then resuspended in  
309 100µl/per IP of Dilution/Wash buffer. 100µl of resuspended beads were then  
310 incubated with 0.5ml of lysate for 1 hour on the rotisserie at 4°C. At the  
311 completion of the incubation step, the beads were collected using magnets. The  
312 unbound lysate was saved for PAGE analysis. The beads containing the  
313 immunoprecipitated *alg-1* associated to the target mRNAs were then washed  
314 three times in 200µl of Dilution/Wash buffer (10 mM Tris/Cl pH 7.5; 150 mM NaCl;  
315 0.5 mM EDTA), and then the RNA/protein complex was eluted using 200µl of  
316 Trizol (Invitrogen, cat 15596026) and incubated for 10 minutes at room  
317 temperature.

318

#### 319 ***Trizol/Driectzol RNA purification***

320 The RNA purification was performed using the RNA MiniPrep kit (Zymo  
321 Research, cat ZR2070) as per the manufacturers protocol. All centrifugation  
322 steps were performed at 21,000g for 30 seconds. We added an equal volume  
323 ethanol (95-100%) to each sample in Trizol and mixed thoroughly by vortexing (5  
324 seconds, level 10). The samples were then centrifuged, recovered using a  
325 magnet, and the supernatant was transferred into a Zymo-Spin IIC Column in a  
326 Collection Tube and centrifuged. The columns were then transferred into a new  
327 collection tube and the flow through were discarded. 400 µl of RNA wash buffer  
328 was added into each column and centrifuged. In a separate RNase-free tube, we  
329 added 5 µl DNase I (6 U/µl) and 75 µl DNA Digestion Buffer, mixed and  
330 incubated at room temperature (20-30°C) for 15 minutes. 400 µl of Direct-zol  
331 RNA PreWash (Zymo Research, cat ZR2070) was added to each sample and  
332 centrifuged twice. The flow-through was discarded in each step. 700 µl of RNA  
333 wash buffer was then added to each column and centrifuged for 2 minutes to  
334 ensure complete removal of the wash buffer. The columns were then transferred  
335 into RNase-free tubes, and the RNAs were eluted with 30 µl of DNase/RNase-  
336 Free Water added directly to the column matrix and centrifuging.

337

### 338 ***cDNA library preparation and sequencing***

339 Each cDNA library was prepared using a minimum of 500pg of  
340 immunoprecipitated RNA from each tissue. The total RNA was reverse  
341 transcribed using the IntegenX's (Pleasanton, CA) automated Apollo 324 robotic  
342 preparation system using previously optimized conditions(BLAZIE *et al.* 2015).

343 The cDNA synthesis was performed using a SPIA (Single Primer Isothermal  
344 Amplification) kit (IntegenX and NuGEN, San Carlos, CA)(KURN *et al.* 2005). The  
345 cDNA was then sheared to approximately 300 bp fragments using the Covaris  
346 S220 system (Covaris, Woburn, MA). We used the Agilent 4200 TapeStation  
347 instrument (Agilent, Santa Clara, CA) to quantify the abundance of cDNAs and  
348 calculate the appropriate amount of cDNA necessary for library construction.  
349 Tissue-specific barcodes were then added to each cDNA library, and the  
350 finalized samples were pooled and sequenced using the HiSeq platform (Illumina,  
351 San Diego, CA) with a 1x75bp HiSeq run.

352

### 353 ***Data analysis***

354 We obtained ~15M unique reads per sample (~130M reads total). The software  
355 Bowtie 2 (LANGMEAD *et al.* 2009) run using default parameters was used to  
356 perform the alignments to the *C. elegans* genome WS250. We used custom Perl  
357 scripts and Cufflinks (TRAPNELL *et al.* 2010) algorithm to study the differential  
358 gene expression between our samples. A summary of the results is shown in  
359 (Figure S3). Mapped reads were further converted into a bam format and sorted  
360 using SAMtools software run with generic parameters (LI *et al.* 2009), and used  
361 to calculate Fragments Per Kilobase Million (FPKM) values, as an estimate of the  
362 abundance of each gene per sample. We used an FPKM  $\geq 1$  on the median from  
363 each replicate as a threshold for identifying positive hits. This stringent approach  
364 discarded ~50-75% of mapped reads for each sample (Figure S3B). The quality

365 of our finalized list of target genes was tested using a principle component  
366 analysis versus our N2 *wt* negative control (Supplemental Fig S3C).

367

### 368 ***Molecular cloning and assembly of the expression constructs***

369 The promoters of candidate genes were extracted from genomic DNA using  
370 genomic PCR and cloned into Gateway-compatible entry vectors (Invitrogen).

371 We designed Gateway-compatible primers (primers 12-19 in Table S2) targeting  
372 2,000 bp upstream of a given transcription start site, or up to the closest gene.

373 Using these DNA primers, we performed PCRs on *C. elegans* genomic DNA,  
374 amplified these regions, and analysed the PCR products by gel electrophoresis.

375 Successful DNA amplicons were then recombined into the Gateway entry vector

376 pDONR P4P1R using Gateway BP Clonase reactions (Invitrogen). The reporter

377 construct pAPArege has been previously described in Blazie et al., 2017 (BLAZIE *et*

378 *al.* 2017). The coding region of this construct was prepared by joining the coding

379 sequence of the mCherry fluorochrome to the SL2 splicing element found

380 between the *gpd-2* and *gpd-3* genes, and to the coding sequence of the GFP

381 gene. The entire cassette was then PCR amplified with Gateway-compatible

382 primers and cloned into pDONR P221 by Gateway BP Clonase reactions

383 (Invitrogen).

384 The 3'UTRs of the genes in this study were cloned by anchoring the

385 Gateway-compatible primers at the translation STOP codon of each gene, to the

386 longest annotated 3'UTR. We have included 50 base pairs downstream of the

387 annotated PAS site to include 3'UTR end processing elements (primers 20-27 in

388 Table S2). The PCR products were analysed using gel electrophoresis analysis  
389 and used to perform Gateway BP Clonase reactions (Invitrogen, cat. 11789020)  
390 into pDONR P2RP3 as per the manufacturers protocol. The *unc-54* 3'UTR used  
391 in this study was previously described in Blazie et al., 2017. The constructs  
392 injected were assembled by performing Gateway LR reactions (Invitrogen) with  
393 each promoter, reporter, and 3'UTR construct per the manufacturers protocol  
394 into the MosSCI compatible destination vector CFJ150. We then microinjected  
395 each reporter construct (100ng/μl) with CFJ601 (100ng/μl) into MosSCI  
396 compatible *C. elegans* strains using standard microinjection techniques (EVANS  
397 (ED.)).

398

### 399 ***Fluorescent imaging and analysis of nematodes***

400 Confocal images used in Figure 4 were acquired in the Biodesign Imaging Core,  
401 Division of the Arizona State University Bioimaging Facility. Transgenic strains  
402 were grown at room temperature on NGM plates seeded with OP50-1. The  
403 mixed stage worms were washed twice with M9 and resuspended in 1mM of  
404 levamisole before imaging using a Nikon C1 Ti-E microscope with 488 nm and  
405 561 nm lasers, 0.75 numerical aperture, 90 μM pinhole microscope with a 40x  
406 magnification objective lens. We acquired 10 images for each transgenic strain  
407 (total 40 images) using the same microscope settings. The fluorescence of GFP  
408 and mCherry fluorochromes from the acquired images were individually  
409 quantified using the integrated density (ID) function of the ImageJ software  
410 (SCHNEIDER *et al.* 2012). Fluorescence ratios were then calculated for each worm

411 (n=10, total 40 images) by dividing the ID for GFP by the ID for mCherry. The  
412 finalized result for each strain is the averaged fluorescence ratio calculated  
413 across all 10 imaged worms. We performed a two tailed student t-test to compare  
414 the mean fluorescence ratios for each strain with a p-value cut off <0.05 to  
415 establish the presence of post-transcriptional gene regulation.

416

### 417 ***Bioinformatic analysis of tissue-specific miRNA targeting biases***

418 The tissue-specific miRNA studies were performed in two steps. First, we utilized  
419 custom-made Perl scripts to scan across the longest 3'UTR of each *C. elegans* protein  
420 coding gene (WS250) in our datasets, searching for perfect sequence complementarity  
421 to the seed regions of all *C. elegans* miRNAs present in the miRBase database (release  
422 21) (GRIFFITHS-JONES 2004; GRIFFITHS-JONES *et al.* 2006; GRIFFITHS-JONES *et al.* 2008;  
423 KOZOMARA AND GRIFFITHS-JONES 2011; KOZOMARA AND GRIFFITHS-JONES 2014). This  
424 result was then used to calculate the percentage of seed presence in the intestine and  
425 body muscle datasets. To calculate the percentage of predicted targets, we extracted  
426 both predicted target genes, and their target miRNA name from the miRanda database  
427 (BETEL *et al.* 2008) and compared the results with our study. A complete list of miRNA  
428 predictions for each tissue profiled is shown in Table S1.

429

### 430 ***Comparison with other datasets***

431 We extracted the WormBase IDs of genes in the intestine and body muscle  
432 transcriptomes previously published by our group (BLAZIE *et al.* 2017), and most  
433 abundant miRNA targets (transcript names) identified by Kudlow *et al.*, 2012 in these

434 tissues (KUDLOW *et al.* 2012). We then translated the transcript names from Kudlow et  
435 al., 2012 into WormBase IDs using custom Perl scripts, and compared how many genes  
436 in each of these groups overlap with our ALG-1 pull-downs. The results are shown in  
437 Figure S4. For the analysis shown in Figure S6 we extracted the names of the miRNAs  
438 previously identified by Alberti et al., 2018 in the *C. elegans* intestine and body muscle  
439 tissues (ALBERTI *et al.* 2018). We then used custom Perl scripts to search for the  
440 presence of the seed regions of these miRNAs in the 3'UTRs of the genes identified in  
441 this study (Figure S6).

442

443 ***Re-annotation of alg-1 and alg-2 knockout transcriptome datasets and splice***  
444 ***junction identification***

445 We downloaded from the GEO database the following transcriptome datasets  
446 published by Brown et al., 2017 (BROWN *et al.* 2017): Project number GSE98935, Wild  
447 type Rep 1-3 (GSM2628055, GSM2628056, GSM2628057); *alg-1(gk214)* Rep 1-3  
448 (GSM2628061, GSM2628062, GSM2628063); *alg-2(ok304)* Rep 1-3 (GSM2628064,  
449 GSM2628065, GSM2628066). We used in-house Perl scripts to prepare the reads for  
450 mapping, and then these reads as input to the TopHat algorithm (TRAPNELL *et al.* 2012)  
451 to map splice junctions in all nine datasets independently. The TopHat algorithm  
452 mapped between 30-56M reads to splice junctions in each sample. *wt\_rep1*;  
453 43,721,355 mapped reads (64% of total input reads), *wt\_rep2*; 44,440,441 (64%),  
454 *wt\_rep3*; 37,248,408(62.7%), *alg-1\_rep1*; 30,808,645 (62.3%), *alg-1\_rep2*; 35,914,514  
455 (63.2%), *alg-1\_rep3*; 43,721,355(63.9%), *alg-2\_rep1*; 54,471,761(63.2%), *alg-2\_rep2*;  
456 56,000,173 (66.8%), *alg-2\_rep3*; 46,638,369 (63.9%). We then combined the mapped



457 reads obtained in the three replicates for each strain and used the open source software  
458 regtools (Griffith Lab, McDonnell Genome Institute) to annotate these splice junctions  
459 using the following command 'regtools junctions annotate junctions.bed WS250.fa  
460 WS250.gtf'. The software produced ~41.8k splice junctions supported by at least 10  
461 reads for the combined N2 *wt* dataset, ~42.3k splice junctions for the *alg-1* dataset and  
462 46.3k for the *alg-2* dataset. We analysed the three resulting cumulative datasets  
463 normalized by dividing each score by the total number of mapped reads within each  
464 sample. This approach produced 36.7k high quality splice junction for the combine N2  
465 *wt* dataset, ~37k for the *alg-1* combined dataset and ~38k for the combined *alg-2*  
466 dataset. The analysis in Figure 6A was performed using splice junctions that are  
467 present in all three datasets (30,115 total). To calculate the fold-change for each splice  
468 junction, we divided the normalized scores of each splice junction in the *alg-1* and *alg-2*  
469 combined datasets by the corresponding scores in the wild type combined dataset. The  
470 fold change of each splice junction was then plotted on a  $\log_2$  scale shown in Figure 6.

471

## 472 ***RNAi experiments***

473 The RNAi experiments shown in Figure 5 and Figure S7 were performed as follows. N2  
474 worms were synchronized by bleaching and starving overnight in M9 buffer until they  
475 reached the L1/dauer stage and then transferred to agar plates containing OP50-1  
476 bacteria, HT115 bacteria with pL4440 *hrp-2* RNAi or pL4440 *asd-2* RNAi (KAMATH AND  
477 AHRINGER 2003). We used *par-2* RNAi as a positive control for the experiments, which  
478 results in 100% embryonic lethality. To measure the brood size, individual synchronized  
479 young adult worms were left overnight (16 hours) to lay eggs. Hatched larvae were

480 counted 24 hours later. Total RNA was extracted from N2 worms treated with either *hrp-*  
481 *2* or *asd-2* RNAi at the adult stage in triplicates.

482

### 483 ***RNA extraction for detection of intestine specific splicing variants***

484 We extracted total RNA using the Direct-zol™ RNA MiniPrep Plus kit (Zymo Research,  
485 cat ZR2070) from (1) N2 *wt* worms, (2) RF54 (*alg-1(gk214)* X) strain, (3) WM53 (*alg-*  
486 *2(ok304)* II) strain, (4) N2 strain subjected to RNAi as previously described(AHRINGER  
487 (ED.)) for *asd-2* and *hrp-2*, and (5) transgenic worms overexpressing the *asd-2* 3'UTR or  
488 the *hrp-2* 3'UTR under control of an intestinal promoter (*ges-1p::pAPArege::3'UTR* ).

489 Each strain was synchronized by growing in M9 media to L1/dauer stage then  
490 transferred to plates containing HT115. We extracted RNA 48 hours later from adult  
491 worms in triplicate for each condition.

492

### 493 ***cDNA preparation, image acquisition and splicing isoform analysis***

494 At the completion of the RNA extractions, the cDNA was synthesized from each sample  
495 using SuperScript III RT (Life Technologies, cat 18080093) according to the  
496 manufacturers protocol. Briefly, 200ng of each RNA sample was incubated with 1  $\mu$ L of  
497 50mM poly dT anchor, 1  $\mu$ L of 10mM dNTP mix and brought to a total volume of 14  $\mu$ L  
498 with nuclease free H<sub>2</sub>O and incubated for 5 minutes at 60°C then iced for 1 minute. 4  $\mu$ L  
499 of 5x first strand buffer, 1  $\mu$ L of 0.1M DTT and 1  $\mu$ L (200 units) of SuperScript III reverse  
500 transcriptase were added to each sample and incubated at 50°C for 60 minutes then  
501 heat inactivated at 70°C for 15 minutes. 200ng of cDNA from each sample was used in  
502 PCRs consisting of 34 cycles using HiFi Taq Polymerase (Invitrogen, cat 11304011)

503 according to manufacturer protocols. Primers used to test alternative splicing of *unc-60*,  
504 *unc-52*, *lin-10*, and *ret-1* were designed to flank the alternatively spliced exons and were  
505 adapted from previous studies (KABAT *et al.* 2009; OHNO *et al.* 2012; HEINTZ *et al.*  
506 2017)(Table S2 primers 28-36). We then acquired images of the PCR amplicons (5  $\mu$ L)  
507 separated by agarose gel electrophoresis and assign the alternatively spliced isoforms  
508 using the ImageJ software package (SCHNEIDER *et al.* 2012). We used the integrated  
509 density function of ImageJ by defining equally sized regions of interest around each  
510 band in the images and compared the integrated density values by normalizing the  
511 smaller bands to the larger bands. The resulting isoform ratios are displayed in Figure  
512 S8. Each strain was quantified in triplicate and subjected to a two-tailed student t-test.  
513 Statistical significance was assigned for p-values <0.05.

514

#### 515 **Data Access**

516 Raw reads were submitted to the NCBI Sequence Read Archive  
517 (<http://trace.ncbi.nlm.nih.gov/Traces/sra/>). The results of our analyses are available in  
518 Excel format as Supplementary Table S1, and in our APA-centric website  
519 [www.APAome.org](http://www.APAome.org).

520

## 521 **RESULTS**

### 522 **A method for the identification of tissue-specific miRNA targets**

523 In order to study the contribution of miRNA activity in producing and  
524 maintaining tissue identity, we performed RNA immunoprecipitations of miRNA  
525 target genes in two of the largest, morphologically different, and most well

526 characterized tissues in *C. elegans*: the intestine (MCGHEE) and body muscle  
527 (GIESELER *et al.*) (Figure 1A). We took advantage of the ability of the Argonaute  
528 protein to bind miRNA target genes, and cloned *alg-1*, one of the worm orthologs  
529 of the human Argonaute 2 protein, downstream of the green fluorescent protein  
530 (GFP). The expression of this construct was then driven by the endogenous  
531 promoter (*alg-1p*), or restricted to the intestine (*ges-1p*) or body muscle (*myo-3p*)  
532 using tissue-specific (TS) promoters (Figure 1B).

533 We produced transgenic strains for each construct (Figure 1C) using  
534 single copy integration technology (MosSCI) (FROKJAER-JENSEN *et al.* 2012;  
535 FROKJAER-JENSEN *et al.* 2014) to minimize the expression mosaics produced by  
536 repetitive extrachromosomal arrays. The strains were validated for integration  
537 using genomic PCRs and Western blots (Figure S1).

538 We then examined the functionality of our cloned *alg-1* in rescue  
539 experiments using the *alg-1*<sup>-/-</sup> strain RF54(*gk214*). This strain has a decrease in  
540 fertility caused by the loss of functional *alg-1* (BUKHARI *et al.* 2012), which was  
541 fully rescued by our cloned *alg-1* construct in a brood size assay (Figure S2),  
542 suggesting that our cloned *alg-1* is functional and able to fully mimic endogenous  
543 *alg-1*.

544 We then used our strains to perform tissue-specific RNA  
545 immunoprecipitations. Each tissue-specific ALG-1 IP and control IPs were  
546 performed in duplicate using biological replicates (total 6 sequencing runs). We  
547 obtained ~25M reads on average for each tissue, of which ~80% were  
548 successfully mapped to the *C. elegans* genome (WS250) (Figure S3). In order to

549 maximize our success we used very stringent filters to determine gene presence,  
550 using only the top 25-50% of genes mapped in each dataset (Figure S3B-C)  
551 (Materials and Methods) (BLAZIE *et al.* 2015; BLAZIE *et al.* 2017). Our analysis  
552 resulted in 3,681 different protein-coding genes specifically targeted by the  
553 miRISC using the endogenous *alg-1* promoter or in the intestine or body muscle.  
554 The complete list of genes detected in this study is shown in Table S1.

555         There are only 27 validated *C. elegans* miRNA-target interactions with  
556 strong evidence reported in the miRNA target repository miR-TarBase v7, and  
557 our study confirmed 16 of these interactions (59%), which is threefold enrichment  
558 when compared to a random dataset of similar size ( $p < 0.05$ , chi square test)  
559 (Figure 2A left panel). When compared to genes present in the *C. elegans*  
560 intestine and body muscle transcriptomes (BLAZIE *et al.* 2017), 81% of the  
561 intestine and 56% of the body muscle targets identified in this study match with  
562 their respective tissues (Figure 2A right panel). A comparison between our hits  
563 and a previously published ALG-1 IP dataset in all tissues also support our  
564 results (Figure S4) (ZISOULIS *et al.* 2010).

565         In order to further validate the quality of our hits, we used GFP-based  
566 approaches to confirm the tissue localization of a few tissue-specific genes  
567 identified in our study, and found with the exception of one, their observed  
568 localization match the expected tissue (Figure S5). In addition, to further test the  
569 quality of our data, we compared our results with the intestine and body muscle  
570 specific miRNA localization data from past studies (ALBERTI *et al.* 2018) (Figure  
571 S6). We found that more than 84% of the genes identified in our study possess

572 predicted binding sites in their 3'UTRs for miRNAs detected in each tissue,  
573 suggesting strong correlation between our results and Alberti et al., 2018  
574 (ALBERTI *et al.* 2018) (Figure S6).

575

### 576 **ALG-1 targets in the intestine regulate key metabolic enzymes**

577 The *C. elegans* intestine is composed of 20 cells that begin differentiation  
578 early in embryogenesis and derive from a single blastomere at the 8-cell stage  
579 (MCGHEE). As the primary role of the intestine is to facilitate the digestion and the  
580 absorption of nutrients, many highly expressed genes in this tissue are digestive  
581 enzymes, ion transport channels and regulators of vesicle transport (MCGHEE).

582 In our intestinal ALG-1 pull-down we identified 3,089 protein-coding genes  
583 targeted by miRNAs. 2,367 of these genes were uniquely targeted by miRNAs in  
584 this tissue (Figure 2B). As expected, and consistent with the function of the  
585 intestine, we find a number of enzymes involved with glucose metabolism, such  
586 as *enol-1* an enolase, *ipgm-1* a phosphoglycerate mutase, and 3 out of 4  
587 glyceraldehyde-3-phosphate dehydrogenases (*gpd-1*, *gpd-2* and *gpd-4*). The  
588 human orthologue of the *C. elegans* gene *enol-1*, *eno1* has been previously  
589 identified as a target of *miR-22* in the context of human gastric cancer (QIAN *et al.*  
590 2017). In addition, some of our top hits are the fatty acid desaturase enzymes *fat-*  
591 *1*, *fat-2*, *fat-4* and *fat-6*, which are all involved with fatty acid metabolism,  
592 suggesting that these metabolic pathways are subjected to a high degree of  
593 regulation in the intestine. All of these genes contain seed elements in their 3'  
594 UTRs (Table S1). Additionally, we find 5 out of 6 vitellogenin genes (*vit-1*, *vit-2*,

595 *vit-3*, *vit-5* and *vit-6*) strongly targeted by miRNAs, with *vit-2* and *vit-6* being the  
596 most abundant transcripts in our immunoprecipitation (Table S1). *vit-2* was  
597 shown to be targeted by ALG-1 in a previous study (KUDLOW *et al.* 2012), and  
598 both possess MiRanda (BETEL *et al.* 2008; BETEL *et al.* 2010) and/or PicTar (LALL  
599 *et al.* 2006) predicted binding sites (Table S1). These vitellogenin genes produce  
600 yolk proteins and are energy carrier molecules synthesized in the intestine.  
601 These yolk proteins are then transported to the gonads and into the oocytes to  
602 act as an energy source for the developing embryos (DEPINA *et al.* 2011).  
603 Accordingly, we also find a number of RAB family proteins that are responsible  
604 for intracellular vesicular transport (*rab-1*, *rab-6.1*, *rab-7*, *rab-8*, *rab-21*, *rab-35*  
605 and *rab-39*).

606 Several transcription factors were also identified as a miRNA targets in the  
607 intestine. *skn-1* is a bZip transcription factor that is initially required for the  
608 specification of cell identity in early embryogenesis, and then later plays a role in  
609 modulating insulin response in the intestine of adult worms (BLACKWELL *et al.*  
610 2015). This gene has already been found to be targeted by miRNA in many past  
611 studies (ZISOULIS *et al.* 2010; KUDLOW *et al.* 2012) and contains many predicted  
612 miRNA binding sites and seed regions from both MiRanda (BETEL *et al.* 2008;  
613 BETEL *et al.* 2010) and PicTar (LALL *et al.* 2006) prediction software (Table S1). A  
614 second transcription factor *pha-4* is expressed in the intestine, where it has an  
615 effect on dietary restriction mediated longevity (SMITH-VIKOS *et al.* 2014). *pha-4*  
616 is a validated target of *let-7* in the intestine (GROSSHANS *et al.* 2005), and along

617 with *skn-1*, is also targeted by *miR-228* (SMITH-VIKOS *et al.* 2014). Additionally,  
618 *pha-4* is targeted by *miR-71* (SMITH-VIKOS *et al.* 2014).

619 We also find as a target of miRNA, *die-1* a gene which associated with the  
620 attachment of the intestine to the pharynx and the rectum (HEID *et al.* 2001), and  
621 the chromatin remodeling factor *Iss-4* (let seven suppressor), which is able to  
622 prevent the lethal phenotype induced by knocking out the miRNA *let-7*  
623 (GROSSHANS *et al.* 2005). These two genes were also validated by others as  
624 miRNA targets (GROSSHANS *et al.* 2005).

625 The intestine plays an important role in producing an innate immune  
626 response to pathogens. The genes *atf-7*, *pmk-1* and *sek-1* were all identified as  
627 targets of miRNAs in this tissue. These three genes act together to produce a  
628 transcriptional innate immune response where the transcription factor *atf-7* is  
629 activated through phosphorylation by kinases *pmk-1* and *sek-1*. Consistent with  
630 our findings, the role of miRNAs in regulating the innate immune response  
631 through the intestine and these genes has been reported in multiple studies  
632 (DING *et al.* 2008; KUDLOW *et al.* 2012; SUN *et al.* 2016).

633

### 634 **Muscle ALG-1 targets modulate locomotion and cellular architecture**

635 *C. elegans* possess 95 striated body wall muscle cells, which are essential  
636 for locomotion (GIESELER *et al.*). Its sarcomeres are composed of thick filaments  
637 containing myosin associated with an M-line, and thin filaments containing actin  
638 associated with the dense body. The pulling of actin filaments by myosin heads  
639 generates force that produces locomotion (MOERMAN AND WILLIAMS).



640 Our ALG-1 pull-down identified 1,047 protein-coding genes targeted by  
641 miRNAs in the body muscle tissue (Table S1). Within this group, 348 genes were  
642 not present in our intestine dataset, and are specifically restricted to the body  
643 muscle tissue (Table S1). Our top hits include genes involved in locomotion, and  
644 general DNA maintenance (*grd-5*, *gcc-1*, *gop-2*, etc.) and several with unknown  
645 function. Consistent with muscle functions, we detected *mup-2*, which encodes  
646 the muscle contractile protein troponin T, *myo-3*, which encodes an isoform of  
647 the myosin heavy chain, *dlc-1*, which encodes dynein light chain 1 and F22B5.10,  
648 a poorly characterized gene involved in striated muscle myosin thick filament  
649 assembly. *mup-2*, *myo-3* and *dlc-1* were all found to be targeted by ALG-1 in  
650 previous studies (ZISOULIS *et al.* 2010; KUDLOW *et al.* 2012). Consistent with  
651 muscle function, a GO term analysis of this dataset highlights an enrichment of  
652 genes involved in locomotion (Table S1), suggesting a potential role for miRNAs  
653 in this biological process.

654 We also identified numerous actin gene isoforms (*act-1*, *act-2*, *act-3* and  
655 *act-4*), which are required for maintenance of cellular architecture within the body  
656 wall muscle, and the Rho GTPase *rho-1*, which is required for regulation of actin  
657 filament-based processes including embryonic polarity, cell migration, cell shape  
658 changes, and muscle contraction (Table S1). Small GTPase are a gene class  
659 heavily targeted by miRNAs (ENRIGHT *et al.* 2003; LIU *et al.* 2012). The human  
660 ortholog of *rho-1* is a known target for *miR-31*, *miR-133*, *miR-155* and *miR-185*  
661 (LIU *et al.* 2012).

662           Importantly, we also found several muscle-specific transcription factors  
663 including *mxl-3*, a basic helix-loop-helix transcription factor and K08D12.3, an  
664 ortholog of the human gene *ZNF9*. These genes are known to regulate proper  
665 muscle formation and cell growth. *mxl-3* is targeted by *miR-34* in the context of  
666 stress response (CHEN *et al.* 2015). Both genes have been detected in past ALG-  
667 1 immunoprecipitation studies (ZISOULIS *et al.* 2010).

668           Our top hit in this tissue is the zinc finger CCCH-type antiviral gene *pos-1*,  
669 a maternally inherited gene necessary for proper fate specification of germ cells,  
670 intestine, pharynx, and hypodermis (FARLEY *et al.* 2008). *pos-1* contains several  
671 predicted miRNA binding sites in its 3' UTR (Table S1), and based on our GFP  
672 reporter validation study is strongly expressed in the body muscle (Figure S4).  
673 We also find the KH domain containing protein *gld-1*, the homolog of the human  
674 gene *QKI*, which is targeted by *miR-214* (WU *et al.* 2017), *miR-200c* and *miR-375*  
675 (PILLMAN *et al.* 2018).

676

677 **miRNA targeting is more extensive in the intestine than it is in the body**  
678 **muscle**

679           By comparing the percentage of tissue-specific miRNA targets identified in  
680 our study to the previously published intestine and body muscle transcriptomes  
681 (BLAZIE *et al.* 2015; BLAZIE *et al.* 2017), we found that the hits in the intestine are  
682 almost twice the number of hits we obtained in the body muscle tissue (30.3% vs  
683 18.2%) (Figure 2B). The length of the 3' UTRs of genes identified as miRNA  
684 targets in the intestine and the body muscle tissues are similar when comparing

685 the two tissues, but are on average longer and have more predicted miRNA  
686 binding sites than the overall *C. elegans* transcriptome (Figure 2C, top panel).  
687 Our results indicate that despite similarity in average 3'UTR length in tissues, the  
688 extent of miRNA-based regulatory networks are not similar across tissues. In  
689 this specific case, we found that the intestine utilizes miRNA-based gene  
690 regulation to a greater extent, when compared with the body muscle.

691

692 **MiRNA targets in the intestine and body muscle are enriched for *miR-355***  
693 **and *miR-85* binding sites**

694 A bioinformatic analysis of the longest 3'UTR isoforms of the targeted  
695 genes showed there was no specific requirement for the seed regions in either  
696 tissue (Figure 2C bottom left panel). However, the use of predictive software  
697 showed that in addition to others, there is an intestine-specific bias for *miR-355*  
698 targets (Figure 2C, right panel, green mark). This miRNA is involved in the insulin  
699 signaling and innate immunity (ZHI *et al.* 2017), which in *C. elegans* are both  
700 mediated through the intestine.

701 In contrast, we observed an enrichment of targets for the poorly  
702 characterized *miR-85* in the body muscle dataset (Figure 2C, right panel, blue  
703 mark). These two miRNAs are uniquely expressed in the respective tissues  
704 (MARTINEZ *et al.* 2008).

705 While *miR-85* and *miR-355* were the most abundant and tissue-restricted  
706 miRNAs identified in this study, several other miRNAs, including *miR-71*, *miR-86*,

707 *miR-785* and *miR-792* were also found highly expressed but less spatially  
708 restricted.

709

### 710 **Intestine and body muscle miRNAs target RNA binding proteins**

711       Upon further analysis, we observed an unexpected enrichment of genes  
712 containing RNA binding domains in both datasets (Supplemental Table 1). RNA  
713 binding proteins are known to play an important role in producing tissue-specific  
714 gene regulation by controlling gene expression at both the co- and post-  
715 transcriptional levels (TAMBURINO *et al.* 2013), and out of the ~887 RNA binding  
716 proteins (RBPs) defined in *C. elegans* (TAMBURINO *et al.* 2013), we identified  
717 almost half as targets of miRNAs across both tissues (45%).

718       We found that out of the 599 known RBPs present in the intestine  
719 transcriptome (BLAZIE *et al.* 2015; BLAZIE *et al.* 2017), 380 (64%) were present in  
720 our intestine dataset as targets of miRNAs (Figure 3A). This is a notable  
721 enrichment when compared to transcription factors and non-RBP genes found in  
722 these tissues by Blazie *et al.* 2017 (BLAZIE *et al.* 2017), of which only a fraction  
723 were identified in our study as miRNA targets (Figure 3A left panel). A similar  
724 trend is also present in the body muscle, with 170 (54%) of RBPs identified as  
725 miRNA targets (Figure 3A left panel). Importantly, the largest pool of targeted  
726 RBPs in both tissues was composed of general factors (GF), such as translation  
727 factors, tRNA interacting proteins, ribosomal proteins, and ribonucleases (Figure  
728 3A right panel), suggesting extensive miRNA regulatory networks are in place in  
729 this tissue.

730

### 731 **miRNAs target RNA splicing factors**

732 A further analysis of the RNA binding proteins targeted in each tissue  
733 revealed that one of the most abundant class of RBPs detected in our ALG-1  
734 pull-down in intestine and body muscle datasets was RNA splicing factors  
735 (Figure 3B). The *C. elegans* transcriptome contains at least 78 known RNA  
736 splicing factors involved in both constitutive and alternative splicing (TAMBURINO  
737 *et al.* 2013). 64 RNA splicing factors (82%) have been previously assigned by our  
738 group in the intestine (BLAZIE *et al.* 2015; BLAZIE *et al.* 2017) and presumably are  
739 responsible for tissue-specific RNA splicing. 31 RNA splicing factors (40%) were  
740 also previously assigned by our group to the body muscle tissue (BLAZIE *et al.*  
741 2015; BLAZIE *et al.* 2017).

742 Our tissue-specific ALG-1 pull-down identified 37 RNA splicing factors as  
743 miRNA targets in the intestine (~47%) (Figure 3B), and 34 of these were also  
744 previously identified by our group as being expressed in this tissue (BLAZIE *et al.*  
745 2015; BLAZIE *et al.* 2017). In contrast, we have detected only nine RNA splicing  
746 factors targeted by miRNAs in our body muscle tissue ALG-1 pull-down, five of  
747 which previously assigned by our group in the body muscle transcriptome (BLAZIE  
748 *et al.* 2015; BLAZIE *et al.* 2017) (Figure 3B).

749 The difference in RNA splicing factors targeted by miRNA in these two  
750 tissues is significant as with the intestine contains three orders of magnitude  
751 more miRNA targeted RNA splicing factors than the body muscle. Of note, many

752 different sub-types of RNA splicing factors identified in this study have human  
753 homologs, such as well-known snRNPs, hnRNPs and SR proteins (Figure 3B).

754

755 **Expression of the RNA splicing factors *asd-2*, *hrp-2* and *smu-2* is**  
756 **modulated through their 3'UTRs**

757 In order to validate that RNA splicing factors found in our ALG-1 pull-down  
758 IPs are targeted by miRNAs in the intestine, we used the pAPAre dual  
759 fluorochrome vector we developed in a past study (BLAZIE *et al.* 2017) (Figure  
760 4A). This vector uses a single promoter to drive the transcription of a  
761 polycistronic pre-mRNA where the coding sequence of the mCherry  
762 fluorochrome is separated from the coding sequence of GFP by a SL2 trans-  
763 splicing element (SE) (BLAZIE *et al.* 2017). The test 3'UTR is cloned downstream  
764 of the GFP gene. Since the mCherry transcript is trans-spliced, it reports  
765 transcription activation. The GFP gene instead reports translational activity; since  
766 its expression is dictated by the downstream tested 3'UTR. If a given miRNA  
767 targets the test 3'UTR, the GFP intensity decreases when compared with an  
768 untargeted 3'UTRs (*ges-1*). By comparing the ratio of the mCherry (indicating  
769 transcription) to the GFP (indicating translation) fluorochromes, we are able to  
770 define the occurrence of post-transcriptional silencing triggered by the tested 3'  
771 UTR (Figure 4A) (BLAZIE *et al.* 2017).

772 We selected three representative RNA splicing factors identified in our  
773 study in the intestine (*asd-2*, *hrp-2* and *smu-2*) (Table 1) and prepared transgenic  
774 strains to validate their expression and regulation (Figure 4B). We used the *ges-1*  
775 3'UTR as a negative control for miRNA targeting, as it is strongly transcribed  
776 and translated in the intestine, with no predicted miRNA binding sites (PicTar),  
777 and poorly conserved seed regions (TargetScan), suggesting minimal post-  
778 transcriptional gene regulation (EGAN *et al.* 1995; MARSHALL AND MCGHEE 2001).  
779 *ges-1* was not significantly abundant in our intestine ALG-1 pull-down (Table S1).  
780 The presence of the *ges-1* 3'UTR in the pAPAre vector led to the expression of  
781 both mCherry and GFP fluorochromes, indicating robust transcription and  
782 translation of the construct as expected (Figure 4B).

783 We then cloned *asd-2*, *hrp-2* and *smu-2* 3'UTRs downstream of the GFP  
784 fluorochrome in our pAPAre vector, prepared transgenic worms expressing  
785 these constructs, and studied the fluctuation of the expression level of the GFP  
786 fluorochrome in these transgenic strains. All three 3'UTRs were able to  
787 significantly lower GFP expression when compared to the control strain with the  
788 *ges-1* 3'UTR, with ~40% repression, while the mCherry signal was similar in all  
789 strains (Figure 4B). These results suggest that these three RNA binding proteins  
790 contain regulatory binding sites within their 3'UTRs potentially able to repress  
791 their expression.

792

793 **MiRNAs target intestine RNA splicing factors promoting tissue-specific**  
794 **alternative splicing**

795 We then tested changes to tissue-specific alternative splicing in the  
796 intestine caused by the STAR protein family member *asd-2*, which regulates the  
797 alternative splicing pattern of the gene *unc-60*. *unc-60* is expressed as two  
798 alternatively spliced isoforms in a tissue-specific manner (OHNO *et al.* 2012)  
799 (Figure 5A); *unc-60a* is expressed predominantly in the body muscle while *unc-*  
800 *60b* is expressed in many other tissues including the intestine (OHNO *et al.* 2012).

801 We first tested the *unc-60* RNA isoform ratio in *wt* N2 worms. We  
802 extracted total RNA from N2 worms in triplicate and performed RT-PCR  
803 experiments using primers flanking the two *unc-60* isoforms (Figure 5A). As  
804 expected, we found that the *unc-60a* longer isoform was more abundantly  
805 expressed in *wt* worms (62%) (Figure 5A).

806 We then investigated if the miRNA pathway has a role in regulating these  
807 splicing events, by testing changes in *unc-60* isoform abundance in the *alg-1* and  
808 *alg-2* knockout strains (RF54 (*alg-1(gk214)* X) and WM53(*alg-2(ok304)* II). These  
809 strains are deficient in miRNA-based gene regulation. We found that loss of  
810 these miRNA effectors lead to a 10-20% shift in the expression of the two *unc-60*  
811 isoforms (Figure 5A), indicating the importance of the miRNA pathway in  
812 regulating alternative splicing of this gene.

813 We then used a genetic approach to test the alternative splicing of this  
814 gene in the context of miRNA regulation. We reasoned that if ALG-1 targets the  
815 *asd-2* 3'UTR in the intestine lowering the expression of *asd-2*, which in turn  
816 causes *unc-60* alternative splicing pattern, we should be able to interfere with this



817 mechanism by overexpressing the *asd-2* 3'UTR in this tissue and in turn test the  
818 role of the miRNA pathway in this process.

819 As expected, the overexpression of the *asd-2* 3'UTR in the intestine led  
820 to changes in the *unc-60* alternative splicing pattern, indicating that post-  
821 transcriptional regulation of *asd-2* through its 3'UTR is important for the  
822 alternative splicing pattern of *unc-60* in the intestine (Figure 5A). Conversely,  
823 *asd-2* RNAi did not induce changes in *unc-60* alternative splicing pattern (Figure  
824 5A). We validated the efficiency of our RNAi experiments by performing a brood  
825 size assay, which indicated strong RNAi activity (Figure S7). Similar results were  
826 observed by testing a second splicing factors (*hrp-2*) known to direct alternative  
827 splicing of the genes *ret-1*, *lin-10* and *unc-52* (KABAT *et al.* 2009; HEINTZ *et al.*  
828 2017) (Figure S8).

829

### 830 **Loss of miRNA function lead to dispersed changes in splice junction usage**

831 Since our data support a role for the miRNA pathway in modulating mRNA  
832 biogenesis, we were interested in testing the extent of these effects at  
833 the transcriptome level. We decided to download and mapped splicing  
834 junctions in genes from *alg-1* and *alg-2* knockout strains previously published by  
835 Brown *et al.*, 2017 (BROWN *et al.* 2017). These worm strains are viable but are  
836 severely impaired. We reasoned that if the miRNA pathway contributes at some  
837 level to mRNA biogenesis, we should be able to see widespread changes in the  
838 usage of splice junctions in these datasets. To test this hypothesis, we  
839 downloaded the *alg-1* and *alg-2* datasets (three replicates for each strain plus *wt*

840 N2 control), and extracted splice junction information. We first tested if the effects  
841 we observed in *unc-60* with our biochemical and genetic approaches (Figure 5A)  
842 could be also detected in these datasets. In the case of *unc-60*, there is a 6-10%  
843 change in splice junction usage between isoforms consistently in all re-annotated  
844 replicates, in both *alg-1* and *alg-2* knockout strains (Figure 5B). This result is in  
845 line with our analysis in Figure 5A. A similar and more striking aberrant splice  
846 junction usage is observed in the case of *lin-10*, and *unc-52*, and with a less  
847 pronounced effect in *ret-1* (Figure S9). These results are also in agreement with  
848 our study in Figure S8.

849 We then expanded this analysis to all splicing junctions we were able to  
850 map using these transcriptomes. From a total of 30,115 high quality known splice  
851 junctions present in all three datasets (Table S4), we identified ~3,946 of them in  
852 ~2,915 protein coding genes that were affected by more than 2-fold change in  
853 usage in both *alg-1* and *alg-2* knockout datasets (~13.2% of the total mapped  
854 splice junctions) (Figure 6A). In addition, we detect several cases of exon  
855 inclusion, skipping and aberrant splicing events that occur exclusively in the *alg-1*  
856 and/or *alg-2* mutant strains (Figure 6B).

857

858

## DISCUSSION

859 In this manuscript we have developed tools and techniques to identify  
860 tissue-specific miRNA targets and applied them to uniquely define the genes  
861 targeted by miRNAs in the *C. elegans* intestine and body muscle. We validated

862 previous findings and mapped ~3,000 of novel tissue-specific interactions (Figure  
863 2 and Table S1).

864 In order to perform these experiments, we have prepared worm strains  
865 expressing ALG-1 fused to GFP and expressed this cassette in the intestine and  
866 body muscle using tissue-specific promoters. We validated the ALG-1 expression  
867 (Figure S1), and the viability of our ALG-1 construct in *in vivo* studies (Figure S2).  
868 We have then performed ALG-1 immunoprecipitations in duplicate, separated the  
869 miRNA complex from their targets, and sequenced the resultant RNA using  
870 Illumina sequencing (Figure 1, Supplementary Figure S3). To confirm our results  
871 we validated few selected hits with expression localization studies in both tissues  
872 (Figure S5). Importantly, our ALG-1 pull-down results are in agreement with  
873 previous studies (Figure 2A, Figure S4, and Supplementary Figure S6), and are  
874 significantly enriched with predicted miRNA targets (Figure 2C).

875 The genes identified in this study overall match with the intestine  
876 transcriptome previously published by our group (81%) (BLAZIE *et al.* 2015;  
877 BLAZIE *et al.* 2017). Of note, only 56% of genes identified as miRNA targets in the  
878 body muscle match the body muscle transcriptome (Figure 2B). Perhaps, the  
879 remaining targets are genes strongly down-regulated by miRNAs in this tissue,  
880 leading to rapid deadenylation and mRNA degradation that make them  
881 undetectable using our PAB-1-based pull-down approach. Given the fact the  
882 body muscle transcriptome is significantly smaller than the intestine  
883 transcriptome, it may also be subjected to less regulation through miRNA.  
884 However, if we normalize the number of genes expressed in each tissue and

885 study the proportion of the transcriptome targeted by miRNA, we still find  
886 significantly more regulation in the intestine (Figure 2A), suggesting that this  
887 tissue may indeed employ miRNA-based gene regulation to a greater extent.

888 We found a disparity in the proportion of each tissue-specific  
889 transcriptome targeted by miRNAs, with a notably larger proportion of genes  
890 targetted in the intestine. The majority of the targeted genes in our intestine pull-  
891 down IP are unique to the intestine and share only a handful of genes with our  
892 body muscle dataset (725 genes, 23% of the total intestine dataset) (Figure 2B).  
893 Conversely, very few genes are unique in the body muscle pull-down IP. The  
894 small pool of shared genes includes housekeeping genes that are most likely  
895 regulated similarly in both tissues. Of note, this minimal overlap between our  
896 tissue-specific datasets indicates that our ALG-1 pull-down is indeed tissue-  
897 specific with marginal cross-contamination.

898 Intriguingly, when we look at the miRNA population predicted to target the  
899 genes in our datasets as from MiRanda (Figure 2C Bottom Panels), we found an  
900 enrichment of known tissue-specific miRNA targets (BETEL *et al.* 2010), which is  
901 in agreement with miRNA localization datasets (ALBERTI *et al.* 2018) (Figure S6).  
902 This in turn indicates that there is a tissue-specific miRNA targeting bias in *C.*  
903 *elegans*, with unique tissue-specific miRNAs targeting unique populations of  
904 genes.

905 Our experimental approach was designed for tissue-specific mRNA target  
906 identification, and unfortunately did not provide miRNA data. We assign tissue-  
907 specific targets to miRNAs relying primarily on prediction software and correlation

908 to past-published datasets. These comparative approaches required conversion  
909 between genomic releases and data consolidation across different  
910 developmental stages and conditions, which may have add unwanted variability  
911 to our comparative analysis.

912 One of the most surprising findings of this study is that a large number of  
913 targets obtained with our tissue-specific ALG-1 pull-down are RBPs. 64% of the  
914 intestinal RBPs were found in our intestine ALG-1 pull-down, while 54% of the  
915 muscle RBPs were in our muscle ALG-1 pull-down. This result was unexpected  
916 given the small number of RNA binding proteins previously identified in the *C.*  
917 *elegans* genome (n = 887) (TAMBURINO *et al.* 2013), which amounted to only 4%  
918 of the total *C. elegans* protein coding genes. However, previous studies have  
919 hinted at a strong regulatory network between miRNAs and RBPs, as the 3'  
920 UTRs of RBPs were found to contain on average more predicted miRNA binding  
921 sites than other gene classes (TAMBURINO *et al.* 2013).

922 Some of these RBPs in our top hits are well-characterized factors involved  
923 in the *C. elegans* fertilization and early embryogenesis but are not well  
924 documented in somatic tissues. For example, within our top 100 hits we obtained  
925 the genes *pos-1* and *mex-5* in the body muscle, and *gld-1* and *oma-2* in the  
926 intestine. We were surprised by these results, but at least in the case of *pos-1*,  
927 which is our top hit in our body muscle dataset, we validated its presence in the  
928 body muscle (Figure S5), suggesting a potential role for this and other RBPs  
929 outside the gonads.

930 RNA binding domain containing proteins are involved in many biological  
931 processes, and their role is not limited to RNA biogenesis (TAMBURINO *et al.*  
932 2013). RBPs can bind single or double strand RNAs, and associate with proteins  
933 forming ribonucleoprotein complexes (RNPs). Longevity, fat metabolism, and  
934 development are all processes controlled by RNPs (LEE AND SCHEDL ; MASUDA *et*  
935 *al.* 2009; ARYAL *et al.* 2017), and in the context of miRNA regulation, the ability of  
936 miRNAs to control RBPs abundance and function allow for an increased control  
937 of fundamental cellular core processes. 234 RBPs uniquely detected as miRNA  
938 targets in the intestine, while 147 RBPs are shared between both datasets.

939 Within this intestinal dataset we mapped a surprising number of RBPs  
940 involved in RNA splicing (Figure 3B). We performed a literature search for known  
941 RNA splicing factors in *C. elegans*; out of the 72 total protein identified, 37 of  
942 them were detected at different level of strength in our intestine ALG-1 pull-down.  
943 In contrast, we do not observe this level of complexity in the body muscle, with  
944 only 9 RNA splicing factors identified in this dataset (Figure 3B).

945 *asd-2* and *smu-2* are well-known RNA splicing factors that induce exon  
946 retention in a dosage dependent manner (SPARTZ *et al.* 2004; OHNO *et al.* 2012),  
947 while *hrp-2* abundance lead to exon skipping (KABAT *et al.* 2009). Here we show  
948 that all three RNA splicing factors possess regulatory targets within their 3'  
949 UTRs (Figure 4) that amount to ~40% silencing activity in the intestine (Figure 4).  
950 Although we do not know which miRNAs target the *asd-2* and *hrp-2* 3'UTRs, in  
951 Figure 5A and Figure S8 we show that the miRNA pathway influences splice  
952 junction usage by regulating these genes, and the depletion of miRNAs which

953 target these RNA splicing factors by using sponge approaches led to defects in  
954 the alternative splicing pattern of downstream genes regulated by *asd-2* and *hrp-*  
955 *2*.

956 Interestingly, the miRNAs predicted to target most splicing factors were  
957 not found highly expressed in this study. *miR-85* and *miR-355*, the most  
958 abundant and tissue-restricted miRNAs identified, are only predicted to target  
959 less than 10% of all the RBPs found. This suggests that since miRNAs are  
960 highly reactive, the abundance of those involved in RNA alternative splicing may  
961 be tightly regulated in tissues, to make sure splicing events are properly  
962 executed.

963 Our genome-wide splice junction mapping effort in miRNA deficient  
964 strains shows similar trends of aberrant splicing of *unc-60*, *unc-52*, *lin-10* and *ret-*  
965 *1* (Figure 5B and Figure S9), and display an overall disruption of splicing events  
966 (~13.2% of all splice junctions mapped) (Figure 6A-B). Most of these defects are  
967 in known donor-acceptor splicing events, perhaps because RNA surveillance  
968 mechanisms may hide more severe disruptions.

969 Unfortunately, our *in vivo* approach does not reach the resolution needed  
970 to conclusively pinpoint the extent of the miRNA pathway in this process. In order  
971 to perform *in vivo* experiments, we used total RNA extracted from transgenic  
972 worms, and studied change in exon abundance occurring in a single tissue within  
973 a whole animal, which prevented us from reaching the same resolution  
974 obtainable with *in vitro* splicing experiments and mini-genes. In addition, the  
975 effects we observe are ameliorated by the presence of at least one functional

976 Argonaute protein, which is able to compensate for the loss of the other.  
977 Knockout of the entire miRNA pathway is lethal in *C. elegans*, and while aberrant  
978 splicing may play a role in producing this phenotype, these activities are  
979 challenging to detect *in vivo*.

980       Taken together, our results support a role for miRNAs in regulating  
981 alternative splicing in the intestine, where their presence in a tissue-specific  
982 manner may lead to alteration of the dosage balance of RNA splicing factor,  
983 leading to tissue-specific alternative splicing (Figure 6C). MiRNAs are known to  
984 alter gene expression dosage, rather than induce complete loss of protein  
985 function (WOLTER *et al.* 2017; BARTEL 2018). On the other hand, many RNA  
986 splicing factors involved with constitutive and alternative splicing are ubiquitously  
987 expressed (SHIN AND MANLEY 2004), but are somehow able to induce tissue-  
988 specific alternative splicing in a dosage dependent manner. In this context, it is  
989 feasible that miRNAs may alter the dosage of RNA splicing factors, leading to  
990 tissue-specific alternative splicing (Figure 6C).

991       We have uploaded our intestine and body muscle miRNA target datasets  
992 into the 3'UTRome database ([www.UTRome.org](http://www.UTRome.org)), which is the publicly available  
993 resource for the *C. elegans* community interested in 3'UTR biology (MANGONE *et*  
994 *al.* 2008; MANGONE *et al.* 2010). In order to provide a more comprehensive  
995 overview, we have also manually curated and included results from several  
996 available datasets including PicTar (LALL *et al.* 2006) and TargetScan (LEWIS *et*  
997 *al.* 2005) miRNA target predictions, experimentally validated ALG-1 interaction  
998 (ZISOULIS *et al.* 2010; KUDLOW *et al.* 2012), tissue-specific gene expression and



999 expanded 3'UTR isoform annotation data (JAN *et al.* 2011; BLAZIE *et al.* 2015;  
1000 BLAZIE *et al.* 2017).

1001

## 1002 **Author Contribution**

1003 MM and KK designed the experiments. KK executed the experiments.  
1004 ALS executed a portion of the experiments. HSS assisted with the experiments  
1005 and the imaging of the *C. elegans* transgenic lines and performed the analysis in  
1006 Figure S4. MM and KK performed the bioinformatics analysis and uploaded the  
1007 results to the UTRome.org database. MM and KK led the analysis and  
1008 interpretation of the data, assembled the Figures, and wrote the manuscript. All  
1009 authors read and approved the final manuscript.

1010

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1014

## 1015 **Conflict of Interest**

1016 The authors declare that they have no competing interests.

1017

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TABLES

Gene	Transcriptome		miRNAome		miRNA predictions		Known targets
	Body muscle	Intestine	Body muscle	Intestine	miRanda	TargetScan	
<i>asd-2</i>	Y	Y	—	Y	<i>miR-86, miR-255, miR-259, miR-785</i>	<i>miR-1/796, miR-46/47</i>	<i>let-2, unc-60</i>
<i>hrp-2</i>	Y	Y	—	Y	<i>miR-58, miR-62, miR-80, miR-81, miR-82, miR-83, miR-84, miR-85, miR-86, miR-90, miR-232, miR-244, miR-259, miR-357, miR-785</i>	<i>miR-1018, miR-1821, miR-4809</i>	<i>ret-1, unc-52, lin-10</i>
<i>smu-2</i>	—	Y	—	Y	—	<i>miR-60-3p, miR-234, miR-230, miR-392, miR-789, miR-792, miR-1020, miR-1818, miR-1828</i>	<i>unc-52, unc-73</i>

1249

1250 **Table 1: Summary of expression pattern, miRNA targets and predicted**  
 1251 **miRNA binding sites for *asd-2*, *hrp-2* and *smu-2*.**

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## FIGURE LEGENDS

1256

1257 **Figure 1:** Identification of miRNA targets by tissue-specific immunoprecipitation  
1258 and sequencing. (A) The anatomical location of the two somatic tissues used in  
1259 this study. (B) Workflow for the identification of tissue-specific miRNA targets. We  
1260 cloned the *C. elegans* Argonaute 2 ortholog *alg-1* and fused with the GFP  
1261 fluorochrome and the unspecific *unc-54* 3'UTR. The expression of this cassette  
1262 was driven in the intestine and body muscle by using tissue-specific (TS)  
1263 promoters. These constructs were microinjected into MosSCI-compatible *C.*  
1264 *elegans* strains to produce single-copy integrated transgenic animals. These  
1265 strains were then subjected to UV crosslinking and lysed by sonication. The  
1266 resulting lysate was subjected to RNA immunoprecipitations with  $\alpha$ -GFP  
1267 antibodies. The resultant tissue-specific miRNA target transcripts were purified,  
1268 the cDNA libraries were made and sequenced using Illumina HiSeq. (C)  
1269 Representative images of *C. elegans* single copy integrated strains showing the  
1270 expression of GFP tagged *alg-1* in endogenous (*alg-1p*), intestine (*ges-1p*), body  
1271 muscle (*myo-3p*) tissue. Yellow box indicated magnified regions, yellow arrows  
1272 mark intestine cells, and red arrows mark body muscle cells.

1273

1274 **Figure 2:** A comparative analysis of ALG-1 immunoprecipitations to identify  
1275 miRNA targets in the intestine and body muscle. (A) Comparison of miRNA  
1276 targets identified in this study to other previously published datasets. The  
1277 numbers indicate protein-coding genes targeted by miRNA in each tissue. Left



1278 Panel: Venn diagram showing the comparison of the genes identified in this  
1279 study to miR-TarBase v7, a compendium of all experimentally validated miRNA  
1280 targets. 59% of the genes in this database match those identified in this study.  
1281 Right Panel: Venn diagram showing the comparison of the genes identified in this  
1282 study to previously published tissue-specific intestine and body muscle  
1283 transcriptomes. The majority of the targets in both datasets were previously  
1284 assigned to each tissue. Green – Intestine (*ges-1p*). Blue – Body muscle (*myo-*  
1285 *3p*). (B) Pie chart showing the proportion of miRNA targets detected in this study  
1286 compared to tissue-specific transcriptomes previously characterized by Blazie  
1287 et.al 2017. The Venn diagram in the center shows the number of protein-coding  
1288 genes identified in this study as miRNA targets between the intestine and body  
1289 muscle. The tables show a Gene Ontology analysis for pathway enrichment  
1290 using the top 100 genes from each dataset used in this study. Green – Intestine  
1291 (*ges-1p*). Blue – Body muscle (*myo-3p*). (C) Top Left panel: The length of 3'  
1292 UTRs from protein coding genes as from the 3'UTRome v1 (MANGONE *et al.* 2010)  
1293 compared to the intestine and body muscle targets identified in this study. The  
1294 arrows indicate the median 3'UTR length. Genes targeted in the intestine and  
1295 body muscle have longer 3'UTRs on average than those published in the C.  
1296 *elegans* 3'UTRome v1. Top Right panel: Proportion of 3'UTRs with predicted  
1297 miRNA binding sites (miRanda and PicTar) (LALL *et al.* 2006; BETEL *et al.* 2008)  
1298 in the 3'UTRome (gray), and in 3'UTRs of genes identified in this study. Green –  
1299 Intestine; Blue – Body muscle. The genes identified in this study in the intestine

1300 and body muscle are enriched for predicted miRNA binding sites. Bottom panel:  
1301 Analysis of miRNA target sites identified in this study. The two axis show the  
1302 proportion of 3'UTRs with perfect seeds or with predicted target sites (miRanda)  
1303 (BETEL *et al.* 2008), normalized to the total number of genes targeted in each  
1304 tissue for each miRNA. miRNAs that target more than 2% of the genes are listed.  
1305 The blue mark denotes *miR-85*, a body muscle specific miRNA. The green mark  
1306 denotes *miR-355*, an intestine specific miRNA.

1307

1308 **Figure 3:** An enrichment of RBPs, and RNA splicing factors targeted by miRNAs  
1309 in the intestine and body muscle. (A) Left panel: Proportion of RBPs targeted by  
1310 miRNAs in each tissue. There is an enrichment of RBPs targeted in the intestine  
1311 (green 63.6%) and the body muscle (blue 53.5%). 'TFs' represents genes  
1312 annotated as transcription factors while 'Other' represents protein-coding genes  
1313 that are not RBPs. Right panel: Subtypes of RBPs targeted by miRNAs in the  
1314 intestine and body muscle. GF - General Factors, including translation factors,  
1315 tRNA proteins, ribosomal proteins and ribonucleases; ZF - Zinc finger; RRM -  
1316 RNA recognition motif; HEL - RNA Helicase; PAZ - PIWI PAZ, PIWI, Argonautes.  
1317 The majority of the targeted RBPs are general and zinc finger-containing factors.  
1318 (B) RNA Splicing factors identified as miRNA targets in the intestine (green) and  
1319 body muscle (blue) tissues. More than half of the RNA splicing factors examined  
1320 are targeted by miRNAs in the intestine as compared to body muscle.

1321

1322 **Figure 4:** *asd-2*, *hrp-2* and *smu-2* 3'UTRs regulate post-transcriptional gene  
1323 expression in the intestine. (A) Diagram of the construct used in these  
1324 experiments (pAPAreg). An intestine-specific promoter drives the expression of a  
1325 bi-cistronic dual fluorochrome vector in the intestine. The mCherry fluorochrome  
1326 reports transcription activity of the construct, while the GFP reports post-  
1327 transcriptional activity through the test 3'UTR cloned downstream of the GFP  
1328 reporter sequence. If the test 3'UTR is targeted by repressive regulatory factors,  
1329 such as miRNAs, the GFP fluorochrome lowers in its expression. SE: trans-  
1330 splicing element extracted from the intergenic region located between the genes  
1331 *gpd-2* and *gpd-3*. (B) Representative images of *C. elegans* strains generated with  
1332 pAPAreg constructs expressing one of the following 3'UTRs: *ges-1*, *asd-2*, *hrp-2*  
1333 or *smu-2* downstream of the GFP fluorochrome. Yellow boxes indicate magnified  
1334 regions. White dotted lines indicate the intestine. (C) The bar graphs show the  
1335 quantified and normalized mean fluorescence ratio between the GFP and the  
1336 mCherry fluorochromes. The mean fluorescence ratio is calculated from 10  
1337 worms per strain. The error bars indicate the standard error of the mean. \* $p < 0.05$ .  
1338 We observed ~40% reduction in normalized GFP intensity modulated by *asd-2*,  
1339 *hrp-2*, and *smu-2* 3'UTRs.

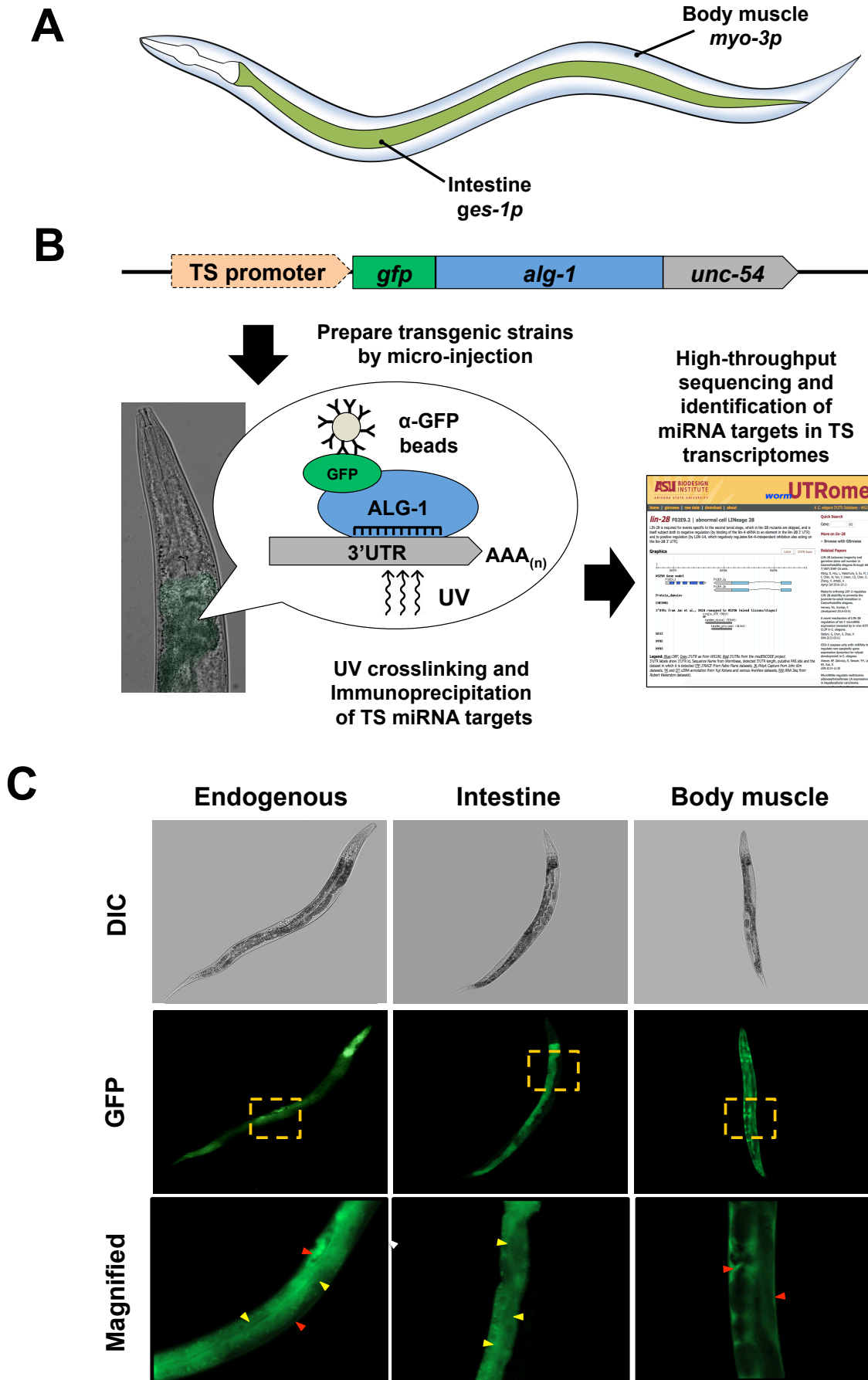
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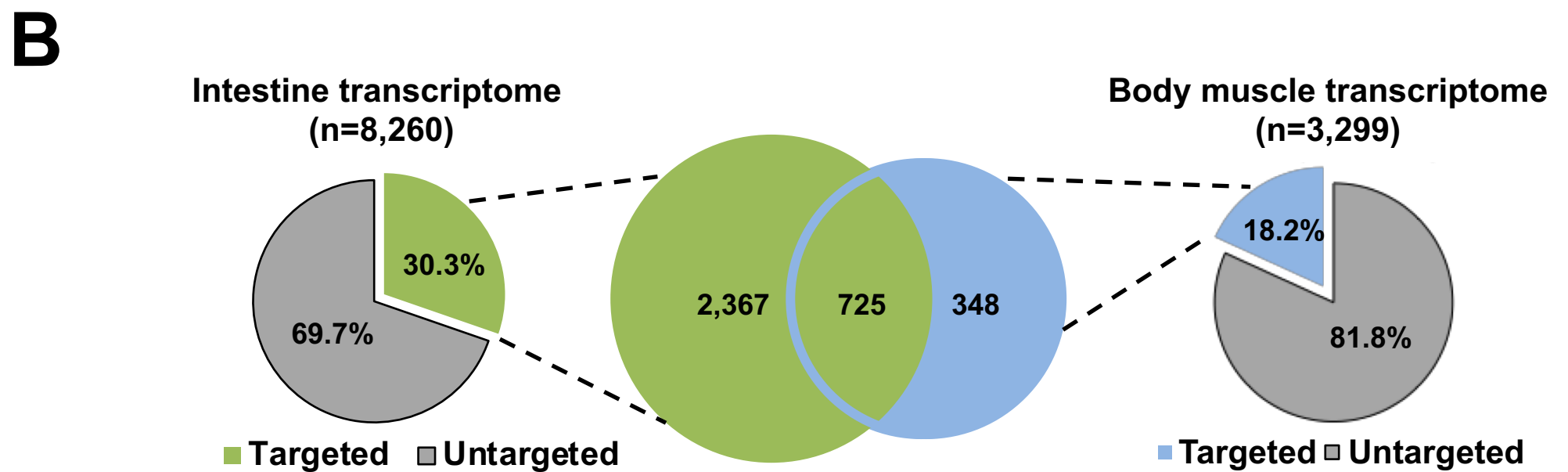
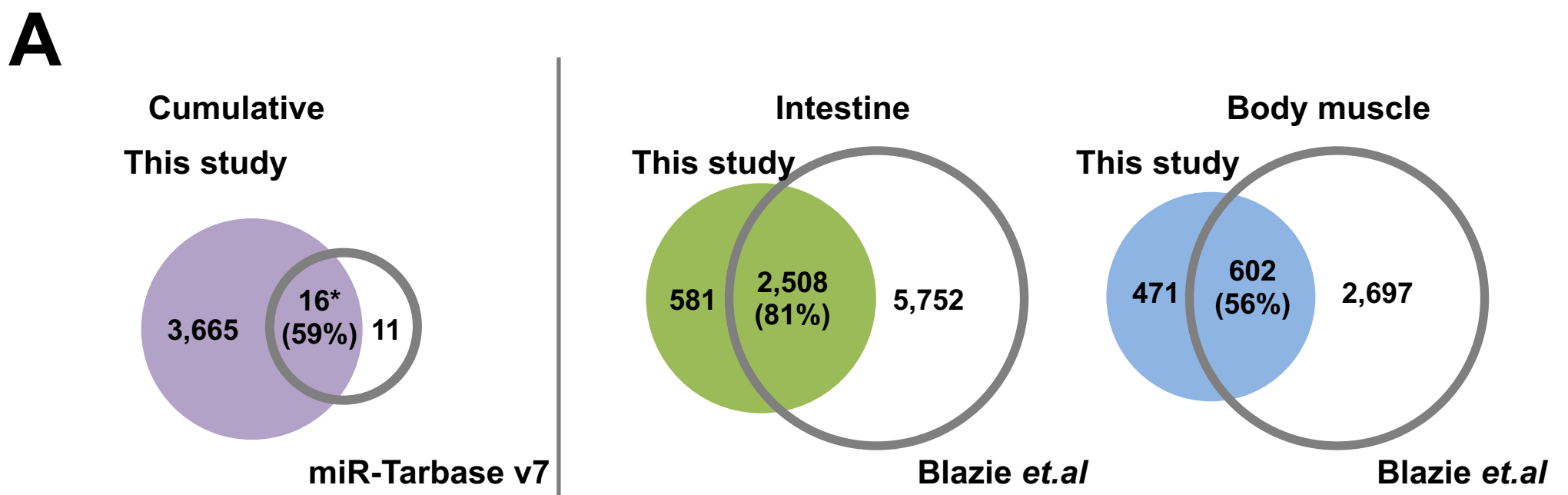
1341 **Figure 5:** The splicing pattern of *unc-60* is modulated by miRNA activity in the  
1342 intestine. (A) Top Panel: Schematic of the genomic locus of *unc-60*. This gene is

1343 expressed as a longer *unc-60a* isoform, and a shorter *unc-60b* isoform. Arrows  
1344 mark the binding sites of the primers used to detect the two isoforms. Middle  
1345 Panel: RT-PCR performed from total RNA extracted from biological replicates in  
1346 triplicate and visualized in 1% agarose gel. 1) N2: *wt* worms. 2) *alg-1* k/o:  
1347 RF54[*alg-1(gk214)* X], 3) *alg-2* k/o: WM53[*alg-2(ok304)* II], 4) *asd-2* RNAi: N2  
1348 worms subjected to *asd-2* RNAi, 5) Over expression of *asd-2* 3'UTR in the  
1349 intestine. The pie charts below each gel shows a quantification of each of the  
1350 occurrence of the two isoforms. The percentage below the pie chart is the  
1351 increase in *unc-60* isoform b abundance when compared to N2(*wt*). The bar  
1352 chart shows the change in isoform ratio between strains. The y-axis shows the  
1353 abundance ratio (shorter isoform/longer isoform) of the two alternatively spliced  
1354 isoforms examined. Exon skipping increases in *alg-1* and *alg-2* k/o strains, and in  
1355 *asd-2* 3'UTR overexpression strains. Error bars indicate standard error of the  
1356 mean. Student t-test \* $p < 0.05$  \*\* $p < 0.01$ . (B) A comparison of the splice junction  
1357 usage in *unc-60* as observed in transcriptome data for *alg-1* and *alg-2* knockout  
1358 strains (BROWN *et al.* 2017). The numbers above each splice junction indicates the  
1359 number of reads mapped to that splice junction. The total reads for each isoform  
1360 are indicated next to the gene model. The isoform ratios indicated next to the  
1361 gene models are calculated by dividing the total reads for each isoform. There is  
1362 a ~6-10% increase in the expression of the shorter *unc-60b* isoform in the miRNA  
1363 deficient strains. Blue: reads corresponding to *unc-60a*. Orange: reads  
1364 corresponding to *unc-60b*  
1365

1366 **Figure 6:** (A) Genome wide changes in splice junction usage in *C. elegans*  
1367 strains deficient in the miRNA pathway. Analysis of splice junction (SJ) usage in  
1368 miRNA deficient strains (*alg-1(gk214)* or *alg-2(ok304)*) re-annotated from Brown  
1369 et al. 2017 (BROWN *et al.* 2017). The graphs illustrate the changes in splice  
1370 junction abundance for different types of splicing events. The x-axis represents  
1371 the fold-change of the normalized number of reads for each splice junction,  
1372 comparing the *alg-1(gk214)* strain to *wt*, while the y-axis represent the fold  
1373 change obtained when comparing *alg-2(ok304)* to *wt*. Splice junctions with more  
1374 than 2-fold enrichment in both strains are highlighted in blue, while splice  
1375 junctions with 2-fold depletion in both strains are highlighted in red. The number  
1376 of genes (G) with enriched or depleted splice junctions are indicated next to the  
1377 graphs. There is a 2-fold change in ~13% of all the splicing events mapped in the  
1378 knockout strains, effecting 3,301 genes, when compared to the N2 *wt* control.  
1379 (B) The table summarizes the number of novel splicing events seen in *alg-1* and  
1380 *alg-2* datasets. These are splicing events not observed in the N2 *wt* control, and  
1381 indicate an increase in novel and aberrant splicing events in miRNA deficient  
1382 strains. (C) A proposed role for miRNAs in the modulation of tissue-specific  
1383 alternative splicing. The abundance of RNA splicing factors (yellow circles)  
1384 dictates the splicing events in a given tissue A. The presence of a miRNA in  
1385 Tissue B may lower the dosage of splicing factors resulting in tissue-specific  
1386 alternative splicing.

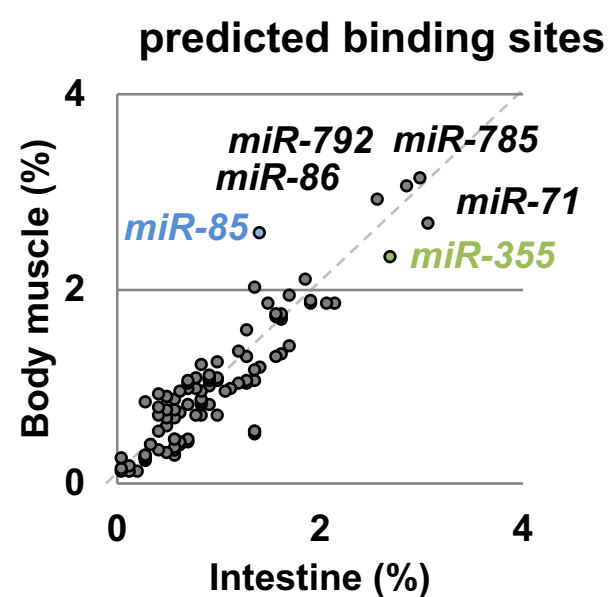
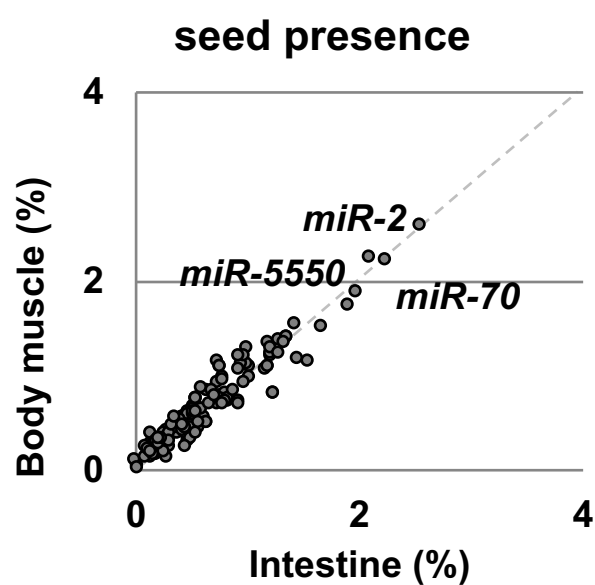
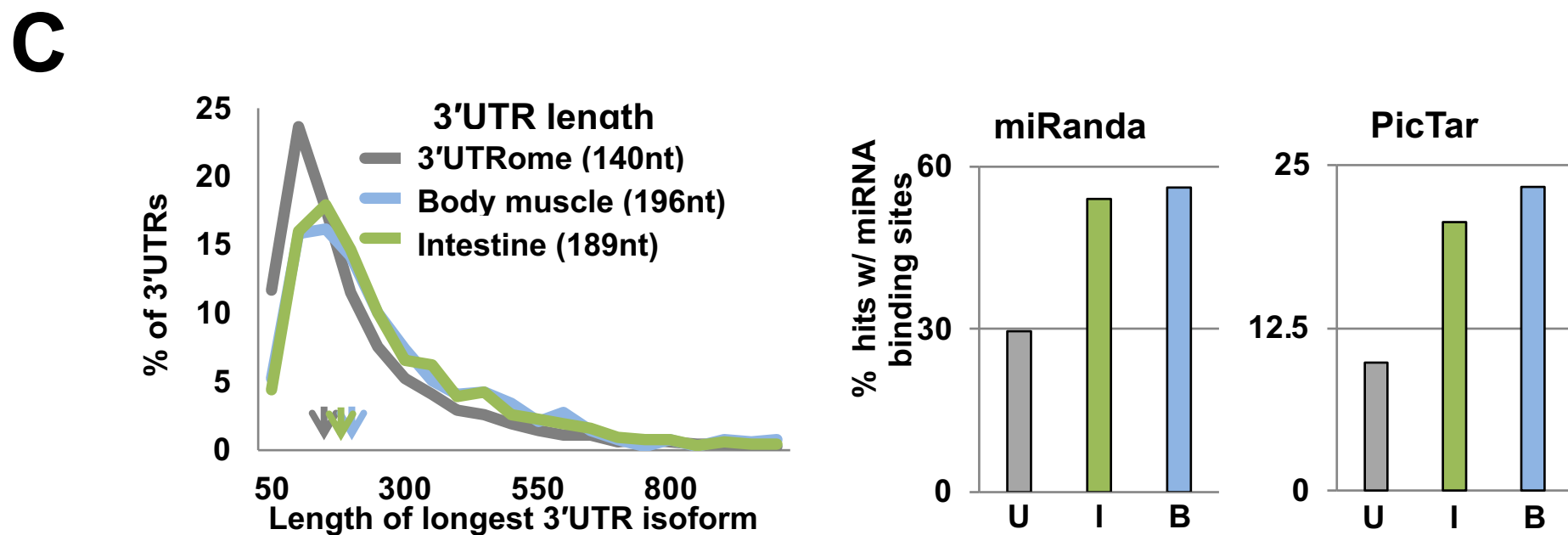
Figure 1





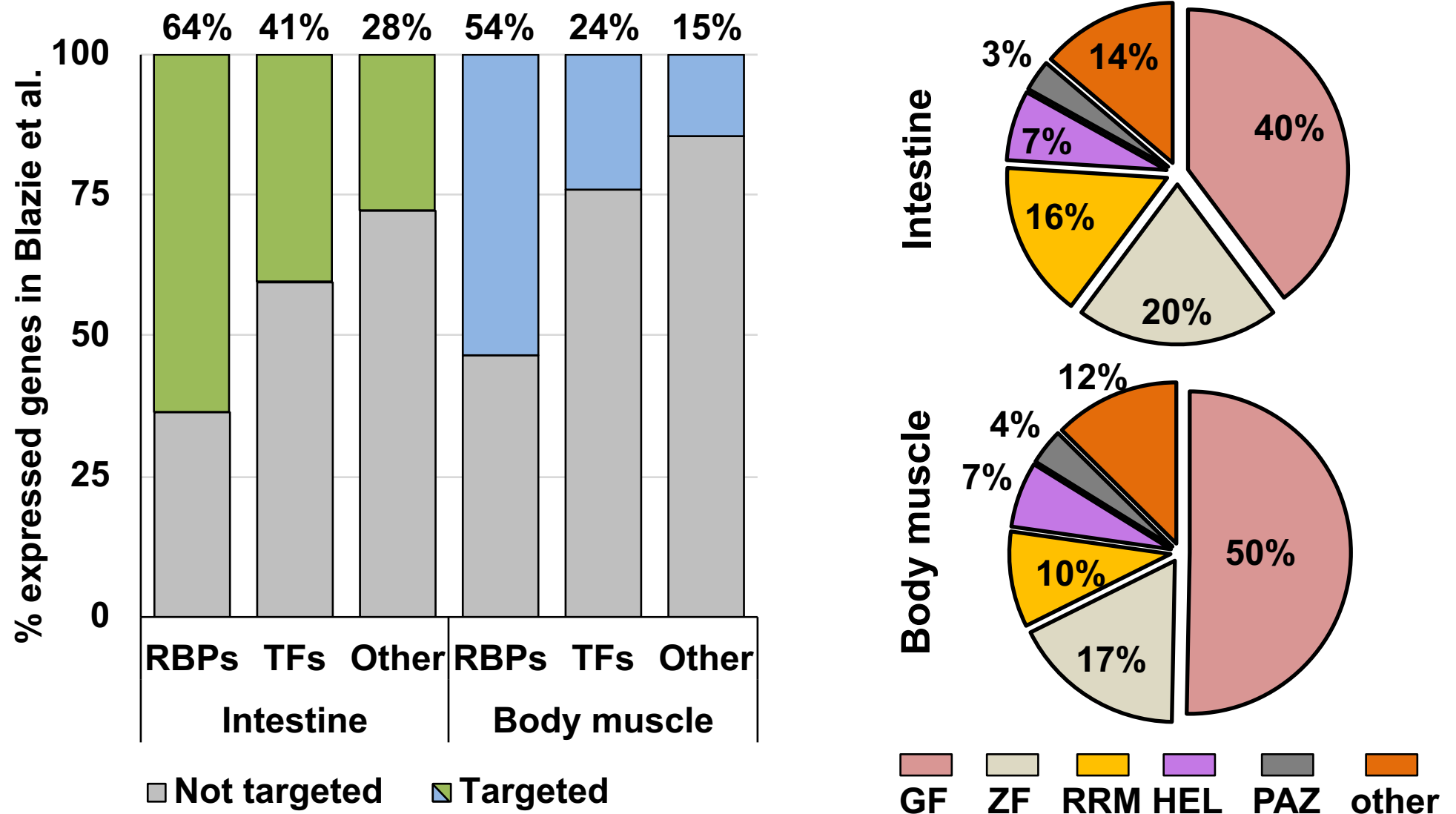
Biological Process	FE	p-value
cytoskeleton organization	11.54	6.98E-07
chromatin organization	6.92	9.11E-04
organelle organization	5.28	7.09E-08

Biological Process	FE	p-value
cell growth	46.75	6.14E-05
locomotion	14.07	3.86E-05
cellular component movement	7.69	1.59E-04





A



B

#	Splicing factor	Human homologue
1	<i>prp-17</i>	CDC40
2	M03F8.3	CRNKL1
3	<i>fubp-3.2</i>	FUBP3
4	<i>hrpf-1</i>	HNRNPF
5	<i>hrpf-2</i>	HNRNPH3
6	<i>hrp-2</i>	HNRNPR
7	<i>hrp-1</i>	HNRPA0
8	<i>smu-2</i>	IK
9	F53B7.3	ISY1
10	<i>prp-19</i>	PRPF19
11	<i>prp-3</i>	PRPF3
12	<i>prp-31</i>	PRPF31
13	<i>prp-4</i>	PRPF4
14	<i>rnp-6</i>	PUF60
15	<i>asd-2</i>	QKI
16	<i>fox-1</i>	RBFOX

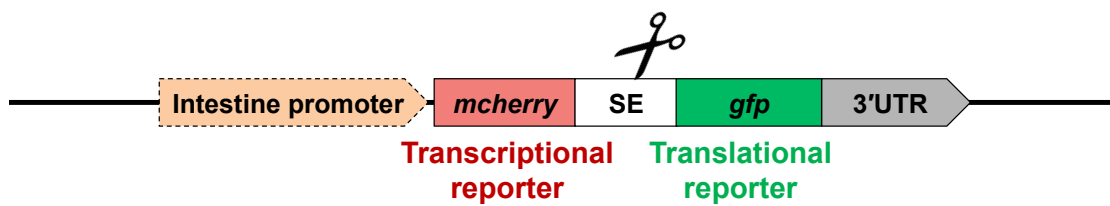
#	Splicing factor	Human homologue
17	<i>sfa-1</i>	SF1
18	T13H5.4	SF3A3
19	T08A11.2	SF3B1
20	W03F9.10	SF3B2
21	<i>sap-49</i>	SF3B4
22	<i>rsp-2</i>	SFRS4
23	<i>swp-1</i>	SFSWAP
24	K07C5.6	SLU7
25	<i>snr-3</i>	SNRPD1
26	<i>snr-4</i>	SNRPD2
27	<i>snr-6</i>	SNRPE
28	<i>snr-5</i>	SNRPF
29	<i>rsp-7</i>	SREK1
30	<i>spk-1</i>	SRPK1
31	<i>rsp-3</i>	SRSF1
32	<i>rsp-4</i>	SRSF2

#	Splicing factor	Human homologue
33	<i>rsp-5</i>	SRSF4
34	<i>rsp-1</i>	SRSF6
35	<i>rsp-6</i>	SRSF7
36	<i>rsr-2</i>	U2SURP
37	F56G4.4	WBP4

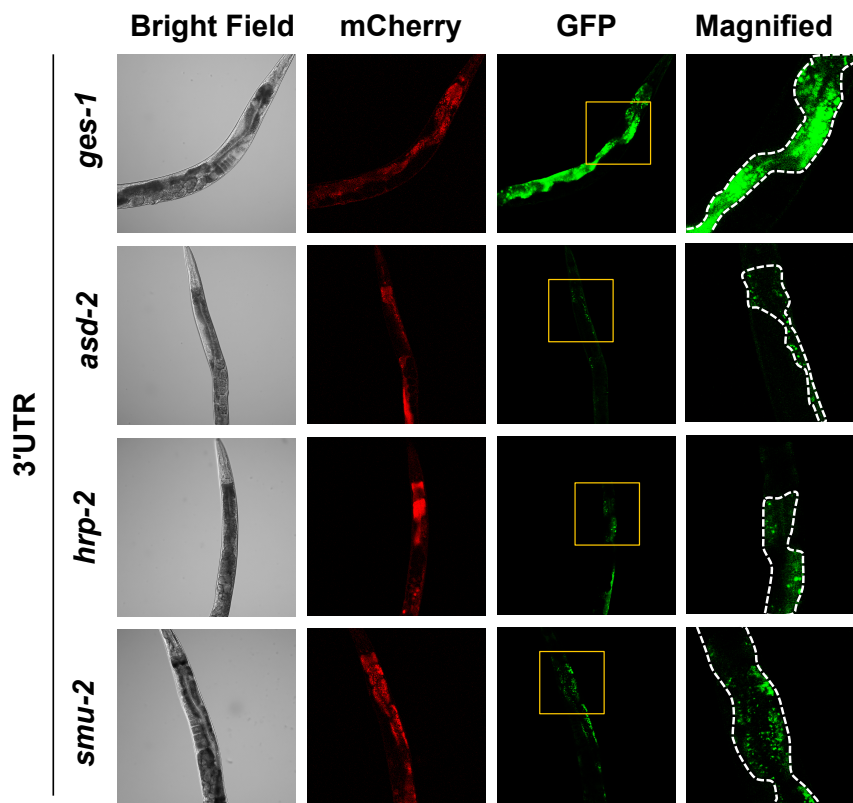
#	Splicing factor	Human homologue
1	F56G4.4	WBP4
2	<i>prp-3</i>	PRPF3
3	<i>rsp-2</i>	SFRS4
4	<i>rsp-3</i>	SRSF1
5	<i>rsp-5</i>	SRSF4
6	<i>swp-1</i>	SFSWAP
7	<i>prp-38</i>	PRPF38A
8	<i>rsr-1</i>	SRRM1
9	T10C6.5	CWC15



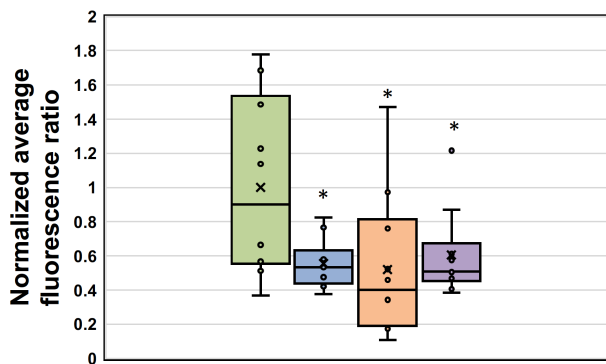
A



B

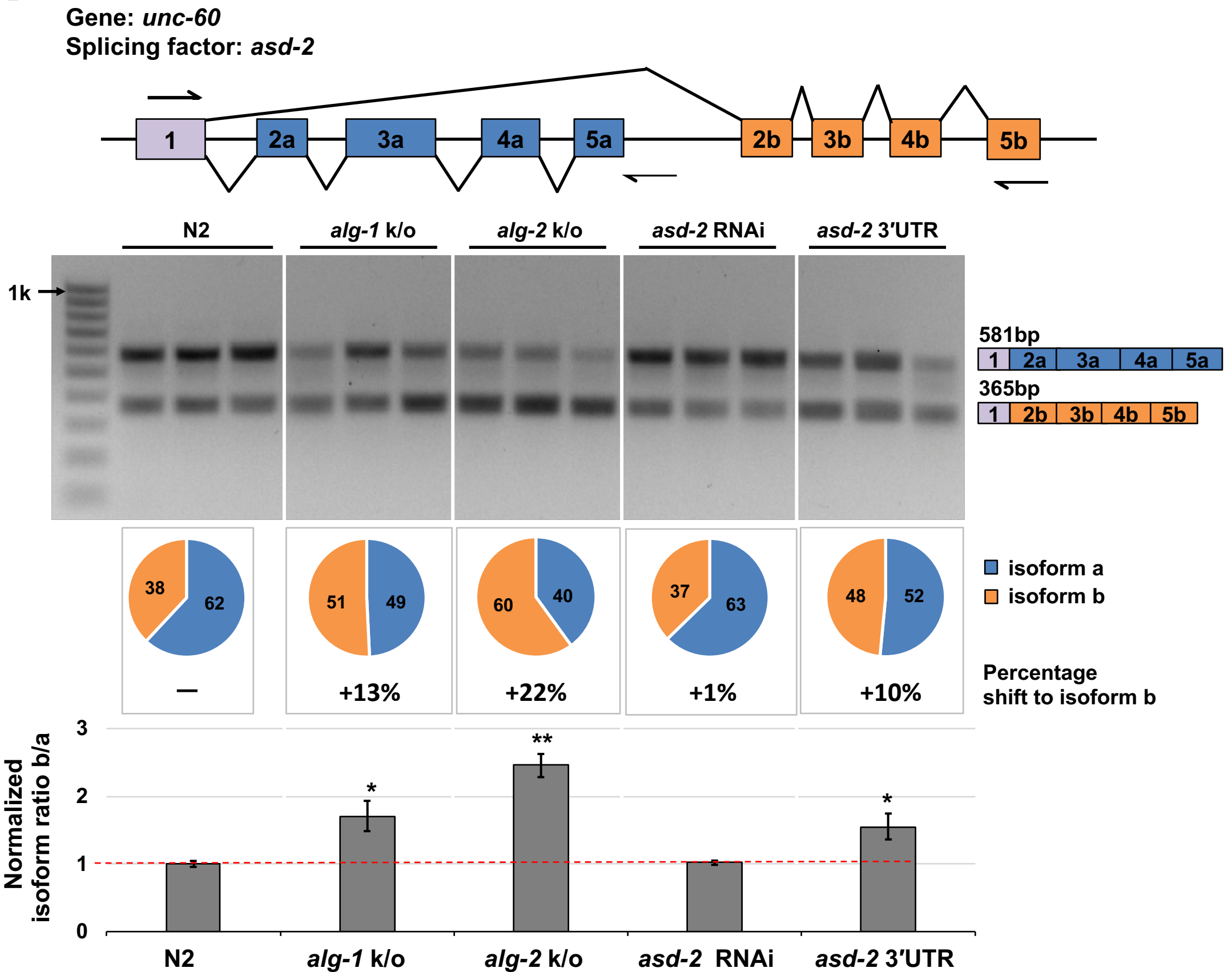


C

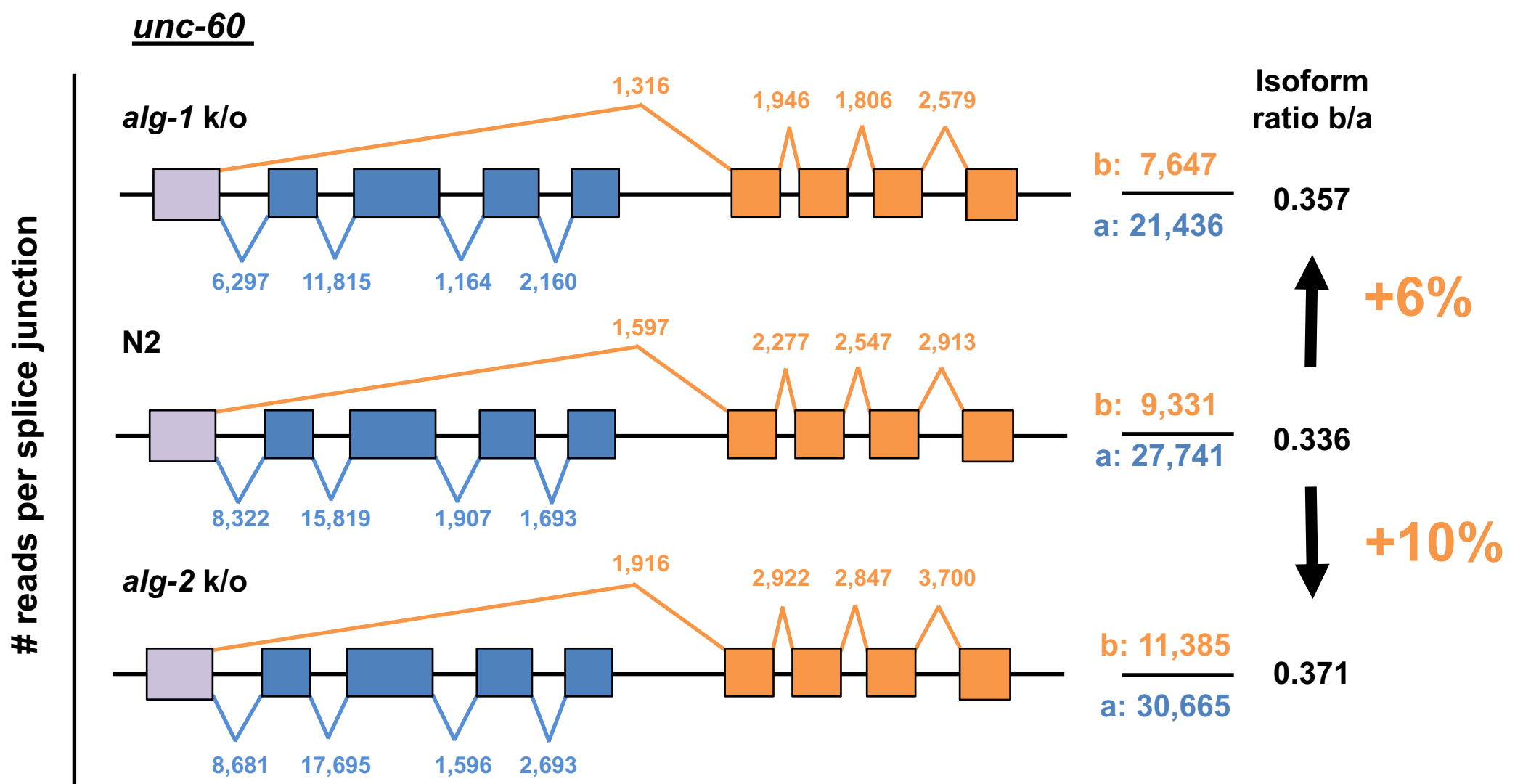


3'UTR	Normalized average fluorescence ratio (n=10)
<i>ges-1</i>	1.00
<i>asd-2</i>	0.55
<i>hrp-2</i>	0.52
<i>smu-2</i>	0.61

A

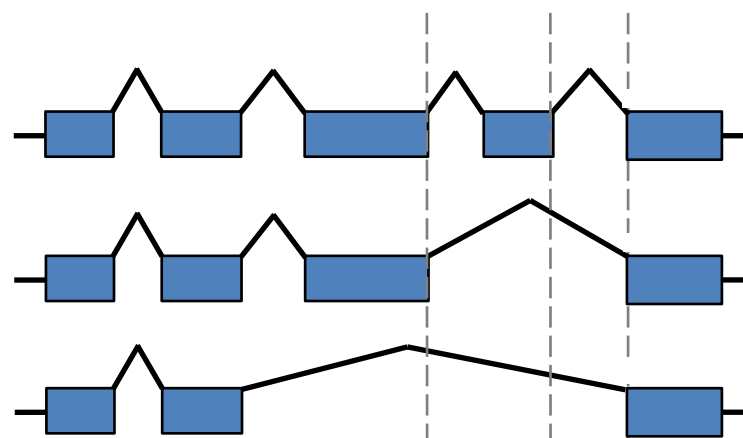


B

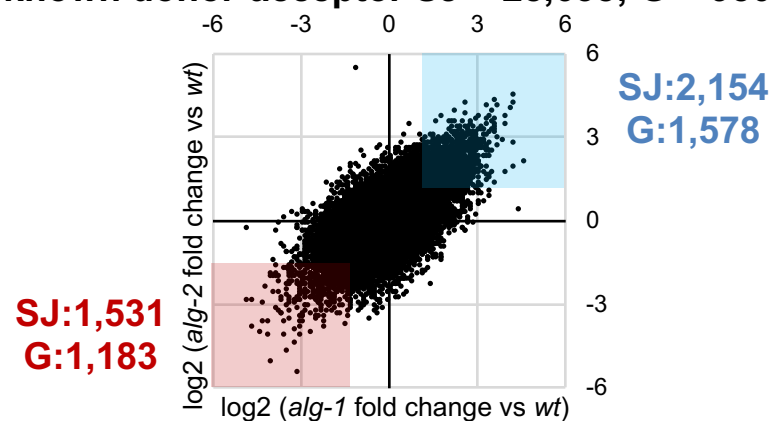


A

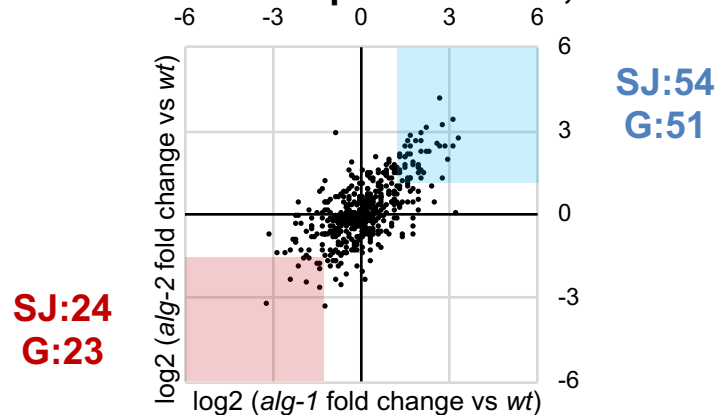
**SJ: Splice junctions**  
 n = 30,115  
**G: Genes = 10,265**  
**2-fold Enrichment**  
 SJ: 2,323 (7.8%)  
 G: 1,678 (16.3%)  
**2-fold Depletion**  
 SJ: 1,623 (5.4%)  
 G: 1,237 (12%)



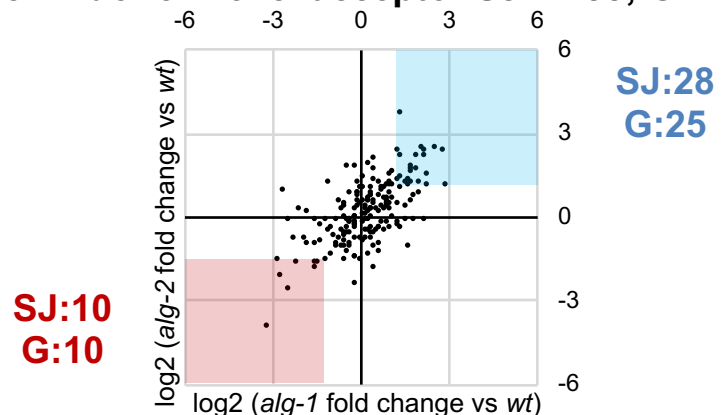
**known donor-acceptor SJ = 28,638; G = 9508**



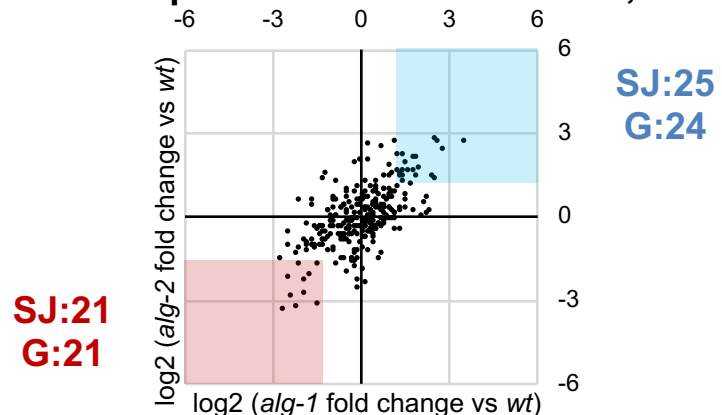
**novel donor-acceptor SJ = 510; G = 310**



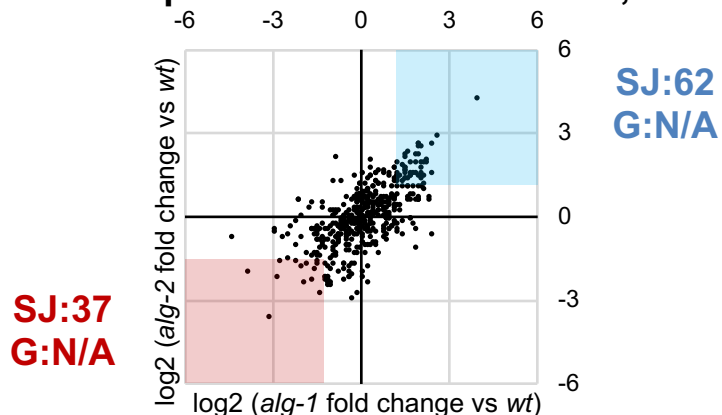
**known donor novel acceptor SJ = 188; G = 172**



**known acceptor novel donor SJ = 287; G = 275**



**novel acceptor novel donor SJ = 492; G = N/A**



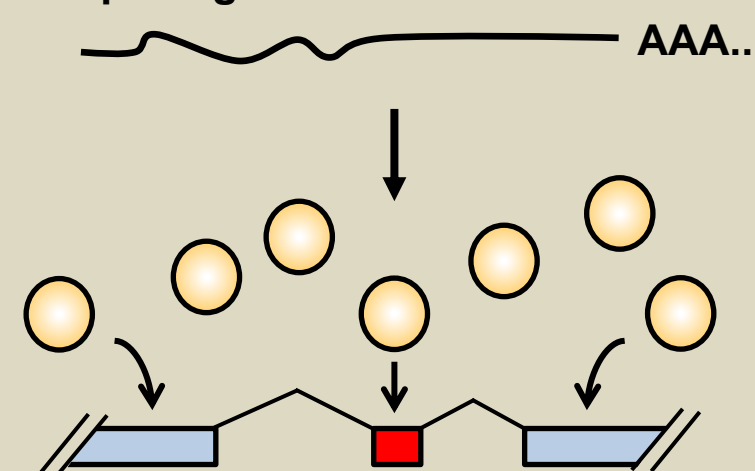
B

	# of novel splice junction		
	exon		aberrant
	inclusion	skipping	
<i>alg-1</i>	1,622	1,840	409
<i>alg-2</i>	1,897	1,231	456
both	2,526	1,766	153
either	6,045	4,837	1,018

C

## Tissue A

Splicing factor 1



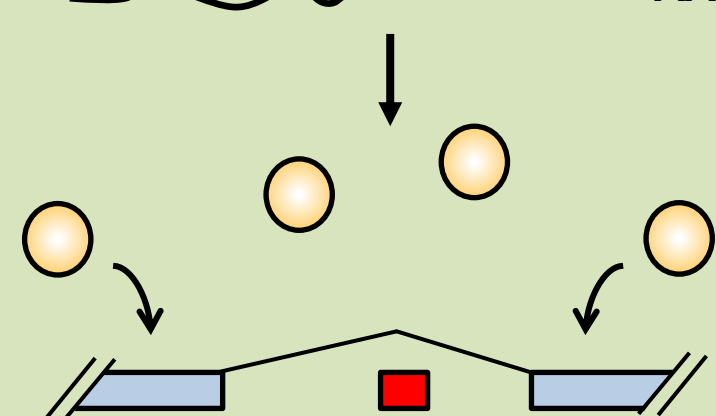
exon inclusion

Isoform A

## Tissue B

miRNA

Splicing factor 1



exon skipping

Isoform B