Supplemental Figure Legends

Figure S1. Dynamics of RBP expression in muscle. **(A)** Venn diagram illustrating the overlap between 4 different annotations of RBPs or spliceosome components, including "RNA binding" and "mRNA binding" terms from AmiGO (purple), RBPs in the GLAD database (cyan), RBPs identified by RNA interactome capture (RIC, green), and spliceosome components in Spliceosome Database (yellow). **(B)** Bar plot of the number of RBP genes in each annotation that are expressed (yellow, DESeq2 normalized counts > 100) in mRNA-Seq data from *Drosophila* muscle. **(C)** Venn diagram comparing expression of 568 muscle RBPs (expressed in muscle and identified in at least two annotations) between 1 d adult muscle fiber types (fibrillar IFM, purple; tubular TDT or jump muscle, yellow; tubular leg, cyan). Most RBPs are expressed in all muscle types. **(D)** Hierarchical clustering and heatmap of RBP expression (DESeq2 normalized counts) in IFM, leg and TDT as well as *bru1-IR* IFM and tubular-converted $salm^{-/-}$ IFM. Note that RBP expression levels vary widely and can be higher or lower in different fiber types. **(E)** Mfuzz clustering of RBP temporal expression profiles in IFM. DESeq2 normalized count data from mRNA-Seq at 8 timepoints in IFM development were standard normalized and clustered into 9 distinct clusters. Traces in red represent genes with high cluster membership values (alpha > 0.9), while those in other colors reflect lower cluster membership values (alpha 0.5 – 0.9). RBPs display dynamic expression patterns during IFM development. **(F)** Venn diagram comparison of the number of differentially expressed (DE) RBP genes (purple) to the number of RBP genes with differential exon use (yellow) between fibrillar IFM and either tubular leg or TDT.

Figure S2. RBP phenotypes with Mef2-Gal4 mediated knockdown in *Drosophila* muscle. **(A)** Sunburst plot of all* RBP phenotypes in muscle from a published genome-wide RNAi screen (Schnorrer et al., 2010). * = "All" RBPs in this plot is defined as the 1924 RBP genes

expressed in mRNA-Seq data from *Drosophila* muscle and annotated in any of the four annotations (AmiGO, GLAD, RIC or Spliceosome Database), and thus reflects a less conservative estimate than the 568 muscle RBPs defined in Figure 1 A. The inner ring summarizes lethality and flight phenotypes. The second ring depicts the stage of lethality. The third and fourth rings summarize myofiber (magenta) and sarcomere (yellow) phenotypes, respectively. **(B)** Venn diagram of the 35 RBPs included in the candidate screen (green) and their overlap with muscle RBPs as defined in Figure 1 A (purple) versus all* RBPs from Figure S2 A (cyan). 5 of the RBPs we tested were not included in the original genome-wide screen (yellow). **(C)** Pie chart summary of RBP phenotypes for individual hairpins from our candidate screen when crossed to Mef2-Gal4. **(D)** Pie chart summary of the phenotypic strength of RNAi hairpins tested in this screen. Only 76 hairpins where more than one hairpin targeted the same gene were included. "Strength" was defined as "strong" if a hairpin line resulted in complete loss of flight or lethality, "medium" if > 50% of flies were flightless, "weak" if 20-50% of the flies were flightless and "no phenotype" if the line had wildtype flight ability.

Figure S3. Act88F-Gal4 is expressed in a subset of tubular muscles. **(A)** Pie chart summary of RBP phenotypes for individual hairpins when crossed to Act88F-Gal4. **(B-G)** Confocal images from young adult flies of Act88F-Gal4 expression in different muscle types as detected by a nuclear localized UAS-Unc84-GFP or UAS-nuc-GFP reporter. High levels of GFP expression are restricted to IFM (B1-C2), but weaker levels of GFP expression can be observed in nuclei from jump muscle (TDT, D1-2), in specific legs muscles, typically in the upper leg segment (E1-2) and sporadically in gut muscle (F1-2). GFP was never observed in abdominal muscle (Abd-M, G1-2). As nuclear-localized GFP is very stable, we cannot

distinguish if this is transient, developmental expression or continuous expression of the driver line.

Figure S4. Knockdown of spliceosome components produces muscle-specific phenotypes. **(A)** Schematic of spliceosome components included in the screen and their association to described spliceosome complexes including the A complex (yellow), U1 complex (brown), U2 complex (orange), U2 associated factors (light orange), Prp19 complex (light purple) and tri-snRNP complex (purple) as annotated in the Spliceosome Database (Cvitkovic and Jurica, 2013). **(B)** Overview schematic of the splicing reaction. The upstream exon (pink) ends with the 5'-SS (splice site), also referred to as the splice donor (SD), while the downstream exon (light pink) begins with the 3'-SS, also referred to as the splice acceptor (SA). U1 associates at the 5'-SS while A-complex component SF1 binds the branch point (BP) sequence and U2AF together with other factors, including the hnRNP-A family RBP Hrb87F, bind at the 3'-SS to form the E complex. U2 assembles on the 3'-SS and U1 and U2 associate forming the A-complex. The Prp19 and tri-snRNP complexes assemble with U1 and U2 forming the B complex, which is activated as U1 and U4 are released. The two-step transesterification reaction to remove the intron and join the exons is accomplished as the catalytic core transitions through the C complex and ultimately dissociates. **(C)** Heatmap-style plot of lethality, flight ability and confocal phenotype for all spliceosome genes and hairpins tested in the screen. Spliceosome association of each gene is listed and colored as in Figure 2 A. For all surviving adults, RNAi lines were scored as wildtype (WT, light yellow), weak flier (purple) or flightless (magenta). For lines with no surviving adults, lethality stage was scored as pre-pupal, pupal, late pupal, pharate or undetermined (shades of blue). Muscle phenotypes were evaluated by confocal microscopy in young adults or 90 h APF pupae. Lethal lines where pupae died before 90 h APF and could not be imaged are marked with an "X". Lines

where data is unavailable are colored light gray. Confocal phenotypes are summarized from weakest to strongest as Zebra bodies or actin blobs (light green), thin myofibrils (green), stretched myofibers or split or frayed myofibrils (medium green) and missing fibers or degenerate myofibrils and sarcomeres (dark green). Note that phenotypes for B and C complex components tend to be stronger and that phenotypic severity for different hairpins targeting the same gene can vary, likely reflecting differences in knockdown efficiency.

Figure S5. *SF1-IR* and *Hrb87F-IR* cause changes in gene expression and exon use in z-disc components. **(A)** Violin plot of log_2FC values for all sarcomere genes (blue) and sarcomere gene exons (green) in *SF1-IR*, *Hrb87F-IR*, and *bru1-IR*. Gray shaded region denotes decreased expression in the knockdown condition. Fold change values have a broader distribution at the level of exon use, reflecting the function of SF1, Hrb87F and Bru1 in splicing. **(B)** Heatmap of gene and exon log_2FC values in *SF1-IR*, *Hrb87F-IR*, and *bru1-IR* knockdown IFM at 72 h APF for z-disc components that directly bind or influence the localization of Kettin, including *bt* (which encodes the Titin-like protein Projectin), *sls* (which encodes the Titin-related Sallimus and Kettin protein isoforms), *Zasp52*, *Zasp66*, *Zasp67* (Zasp proteins contribute to z-disc integrity and regulate myofibril width), *cher* (an F-actin binding filamin family protein) and *zormin*. The PEVK region of *bt* that is differentially spliced between fiber-types and implicated in passive stiffness is outlined in green, and Kettin encoding exons in *sls* are outlined in magenta. A dot denotes genes with a DESeq2 adjusted p-value < 0.01 and exons with a DEXSeq p-value < 0.01.

Figure S6. SF1 and Hrb87F do not regulate fiber-type specific gene expression or exon use. **(A)** Plot of log_2FC values for all 229 significantly differentially expressed (DE) genes (DESeq2 adjusted p-value < 0.01) in *SF1-IR* (orange) or *Hrb87F-IR* (purple). **(B)** Plot of

100 log_2FC values for 492 fiber-type specific genes significantly differentially expressed
101 (DESeq2 adjusted p-value < 0.01) in either *bru1-IR* (green), *SF1-IR* (orange) or *Hrb87F-IR*
102 (purple). Fiber-type specific genes are significantly regulated (DESeq2 adjusted p-value <
103 .01, abs(log_2FC) > 1.5) in the same direction in 1 d adult *salm*$^{-/-}$ IFM, leg and jump muscle
104 (TDT) samples as compared to WT IFM (Spletter et al., 2015). As compared to Bru1, SF1
105 and Hrb87F do not impact fiber-type specific gene expression. **(C)** Plot of log_2FC values for
106 all 1049 significantly differentially expressed (DE) exons (DEXSeq p-value < 0.01) in *SF1-*
107 *IR* (orange) or *Hrb87F-IR* (purple). **(D)** Plot of log_2FC values for 68 significantly DE fiber-
108 type specific exons (DEXSeq p-value < 0.01) (*bru-IR* exons, green; *Hrb87F-IR* exons,
109 purple; *SF1-IR* exons, orange). The number of exons is noted in the top left of each plot
110 region. Fiber-type specific exons are significantly regulated (DEXSeq p-value < 0.01) in the
111 same direction in 1 d adult *salm*$^{-/-}$ IFM, leg and jump muscle (TDT) samples as compared to
112 WT IFM (Spletter et al., 2015). Note the distinct regulatory dynamics for individual exons
113 between *bru1-IR* and *SF1-IR* or *Hrb87F-IR*, as well as the 148 exons that are not
114 significantly regulated by Bru1, SF1 or Hrb87F. In all plots, dotted lines mark a log_2FC value
115 of 1 and -1. Gray shaded region denotes genes downregulated in the knockdown condition.
116
117 **Supplemental Table Legends**
118 **Table S1.** Key Resources Table. This table includes a list of the sources and identifiers for
119 genetic and chemical reagents used in this manuscript, as well as the references and sources
120 for bioinformatic packages used in mRNA-Seq data analysis.
121
122 **Table S2.** Expression data of muscle RBPs. This spreadsheet contains the data used to
123 generate plots in Figures 1, S1, 2 and S2. It includes a list of all RBPs contained in different
124 annotations, those RBPs we categorize as “muscle RBPs” and a list of expression values for

those genes in muscle. Standard normalized expression values used for Mfuzz clustering as well as cluster membership and core cluster expression profiles are provided. A list of both differentially expression RBPs and RBP exons used for hierarchical clustering and heatmaps is included.

Table S3. Data from candidate RBP screen. This table includes data used to generate the plots in Figures 2-5 and summarizing results from the various assays included in our RBP screen. It includes a list of all 35 genes tested, summarized data from lethality, flight and confocal assays and raw flight test data including number of flies tested.

Table S4. mRNA-Seq data from *SF1-IR* and *Hrb87F-IR*. This table lists data related to the bioinformatic analysis of mRNA-Seq data from *SF1-IR* and *Hrb87F-IR* and *bru1-IR* in IFM dissected from 72 h APF pupae. Full data tables of DESeq2 and DEXSeq analysis are provided, as well as expression data for select z-disc proteins. Lists of gene symbols used to perform GO analysis of biological process terms as well as the resulting lists of terms are included. Lists of genes and exons differentially regulated between tubular and fibrillar muscle types as well as their log2FC values in *SF1-IR* and *Hrb87F-IR* and *bru1-IR* are also provided.