Alternative splicing regulates the physiological adaptation of the mouse hind limb postural and phasic muscles to microgravity.

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Abstract

Muscle atrophy and fiber type alterations are well-characterized physiological adaptations to microgravity with both understood to be primarily regulated by differential gene expression (DGE). While microgravity-induced DGE has been extensively investigated, adaptations to microgravity due to alternative splicing (AS) have not been studied in a mammalian model. We sought to comprehensively elucidate the transcriptomic underpinnings of microgravity-induced muscle phenotypes in mice by evaluating both DGE and changes in AS due to extended spaceflight. Tissue sections and total RNA were isolated from the gastrocnemius and quadriceps, postural and phasic muscles of the hind limb, respectively, of 32-week-old female BALB/c mice exposed to microgravity or ground control conditions for nine weeks. Immunohistochemistry disclosed muscle type-specific physiological adaptations to microgravity that included i) a pronounced reduction in muscle fiber cross-sectional area in both muscles and ii) a prominent slow-to-fast fiber type transition in the gastrocnemius. RNA sequencing revealed that DGE and AS varied across postural and phasic muscle types with preferential employment of DGE in the gastrocnemius and AS in the quadriceps. Gene ontology analysis indicated that DGE and AS regulate distinct molecular processes. Various non-differentially expressed transcripts encoding musculoskeletal proteins (*Tnnt3*, *Tnnt1*, *Neb*, *Ryr1*, and *Ttn*) and muscle-specific RNA binding splicing regulators (*Mbnl1* and *Rbfox1*) were found to have significant changes in AS that altered critical functional domains of their protein products. In striking contrast, microgravity-induced differentially expressed genes were associated with lipid metabolism and mitochondrial function. Our work serves as the first comprehensive investigation of coordinate changes in DGE and AS in large limb muscles across spaceflight. We propose that substantial remodeling of pre-mRNA by AS is a major component of transcriptomic adaptation of skeletal muscle to microgravity. The alternatively spliced genes identified here could be targeted by small molecule splicing regulator therapies to address microgravity-induced changes in muscle during spaceflight.

Introduction

Prolonged disuse of skeletal muscle in microgravity environments, often referred to as mechanical unloading, precipitates acute muscle atrophy characterized by a loss of muscle mass and strength (Adams et al., 1985). Similar microgravity-associated muscle phenotypes have been identified in the limb muscles of mice (Sandonà et al., 2012), rats (Martin et al., 1985), and monkeys (Shenkman et al., 1994) exposed to microgravity as well as sedentary human populations such as the elderly (Suetta et al., 2012) and handicapped (Giangregorio et al., 2006). While countermeasures, chief among them being regular exercise, are beneficial (Gao et al., 2018), these interventions fail to completely prevent microgravity-induced atrophy (Buckley, 2006). Ultimately, extended spaceflight results in a 4.6 to 8.8% reduction in muscle size and a reciprocal 5.9 to 8.8% increase in fat content (Burkhart et al., 2019).

While aggressive muscle conditioning and rehabilitation on Earth eventually restore muscle mass and strength, this process can take anywhere from a few months to four years to occur (Burkhart et al., 2019), with differences in rehabilitation time attributed to functional distinctions between postural and phasic muscle types. Postural muscles are composed primarily of slow twitch muscle fibers and phasic muscles are composed primarily of fast twitch muscle fibers, a distinction that underlines their respective functions (Burkholder et al., 1994). Although both are required for locomotion, postural muscles (i.e. gastrocnemius) act primarily to support the body's posture in Earth's gravitational field, and phasic muscles (i.e. quadriceps) act primarily to support explosive movement (Charles et al., 2016). Attributable to their contrasting roles in motor function, postural and phasic muscles respond differently to extended spaceflight. Most importantly, exposure to microgravity significantly reduces the requirement for the postural component of motor function, a perturbation that precipitates an adaptive migration from a primarily slow twitch to a primarily fast twitch fiber type composition (Shenkman, 2016). The prominent slow-to-fast fiber type transition in postural muscles is speculated to account for their resistance to microgravity-induced atrophy (Fitts et al., 2001), as while phasic muscles undergo "typical" atrophic changes to microgravity, including loss of muscle mass and decreased strength of contraction (Roy et al., 1996; Recktenwald et al., 1999), postural muscles are not as affected by microgravity, displaying minimal loss of muscle mass and, in some cases, increased amplitude of muscle contraction (Roy et al., 1996; Recktenwald et al., 1999).

The development of high-throughput sequencing technologies has spurred extensive interest in elucidating the transcriptomic underpinnings of muscle type-specific microgravity-induced phenotypes. Most interest has been paid to the differential gene expression (DGE) of myosin heavy chain genes, long characterized as the canonical mechanism of fiber type transitions. There is evidence in humans (Trappe et al., 2009) as well as mice (Sandonà et al., 2012), rats (Martin et al., 1985), and monkeys (Shenkman et al., 1994) to support microgravity-induced downregulation of the myosin heavy chain gene *MYHC7*, which encodes the slow twitch muscle fiber protein MYHC I and upregulation of the myosin heavy chain genes *MYH2*, *MYH1*, and *MYH4* which encode fast twitch muscle fiber proteins MYHC IIA, MYHC IID/X, and MYHC IIB, respectively. In addition, microgravity-induced DGE has been shown to regulate gene networks related to protein, lipid, and carbohydrate metabolism as well as mitochondrial dysfunction in skeletal muscle (Chakraborty et al., 2020). However, a focus restricted to the level of gene expression fails to fully characterize the amplifying actions of alternative splicing (AS) on the host's transcriptome; for example, AS can affect a multi-fold increase in the diversity translatable mRNA isoforms over what can be accounted for by the 18,000 genes in the human genome. This additional complexity is attributable to the process of RNA binding protein (RBP)-mediated AS (Nilsen and Graveley, 2010). Approximately 90% of human genes have been found to exhibit alternatively spliced isoforms (Wang et al., 2008). While there are multiple types of AS events, the most prevalent and best characterized AS types are skipped exon (SE) and mutually exclusive exon (MXE) events (Park et al., 2018). Considering the well-annotated role of AS in musculoskeletal development (Nikanova and Kao, 2020; Nakka et al., 2018; Brinegar et al., 2017), muscle fiber type composition (Briggs Schachat, 1996; Tang et al., 2015; Lam et al., 2018), and atrophic musculoskeletal diseases (Kimura et al., 2005; Tang et al., 2015; Lam et al., 2018), there has been only a single examination of microgravity-induced AS conducted, and that study focused on *Arabidopsis thaliana*, a species of flowering plant (Beisel et al., 2019).

Here we set out to characterize, for the first time in an animal model, the regulatory role of AS in the physiological adaptation to extended spaceflight. Tissue sections and total RNA were isolated from the gastrocnemius and quadriceps muscles of 32-week-old female BALB/c mice exposed to either microgravity or ground control conditions for a total of nine weeks. Using immunohistochemistry and RNA sequencing (RNA-seq), we aimed to i) annotate muscle-type-specific patterns of muscle atrophy and fiber type changes as well as ii) characterize functionally significant AS changes in non-differentially expressed striated muscle genes that describe a previously uninvestigated means of modifying the transcriptomic response to microgravity.

Results

Fiber type composition and size of postural and phasic muscles are differentially perturbed by spaceflight.

Compared to ground controls, the overall abundance of slow-twitch fibers in the gastrocnemius decreased from 18% to 5%, with a reciprocal increase in the abundance of fast twitch fibers from 80% to 96% following nine weeks of microgravity (Figure 1A, 1C). In addition, slow and fast twitch myosin heavy chains were co-expressed at the level of individual muscle fibers (Figure 1B). By contrast, there was no evidence of a slow-to-fast fiber type transition in the quadriceps as a consequence of a 100% fast twitch fiber type composition (Figure 1A).

On average, the cross-sectional area of muscle fibers composing the gastrocnemius decreased from $1170 \pm 141 \text{ um}^2$ to $856 \pm 74 \text{ um}^2$, representing a 27% reduction in muscle fiber size as a result of extended exposure to microgravity. The average cross-sectional area of muscle fibers composing the quadriceps decreased from $1986 \pm 330 \text{ um}^2$ to $1046 \pm 201 \text{ um}^2$, representing a 47% reduction in muscle fiber size (Figure 1D). Therefore, the quadriceps underwent more significant microgravity-induced atrophy (approximately 1.8x more based on fiber area) than did the gastrocnemius.

DGE and AS are muscle-type-specific and functionally distinct mechanisms of microgravity-induced transcriptome regulation.

In response to extended spaceflight, the gastrocnemius and quadriceps of adult mice displayed evidence of change in both DGE and AS. However, the utilization of these two mechanisms of transcriptome regulation varied significantly between each of these muscle types. First, there was minimal overlap between microgravity-induced change in the transcriptomes of the gastrocnemius and quadriceps; only 9% of all differentially expressed genes and 6% of all alternatively spliced genes were held in common between these two muscles (Figure 2A). In addition, there was evidence of an inverse relationship between DGE and AS in the gastrocnemius and quadriceps; the ratio of differentially expressed genes to alternatively spliced genes approximated to 5:2 in the gastrocnemius and 2:5 in the quadriceps (Figure 2A).

Not only were DGE and AS differentially employed in each muscle investigated, these two mechanisms of transcriptome regulation were also enriched in functionally distinct gene networks. All alternatively spliced genes were non-differentially expressed (Figure 2C), indicating that AS and DGE are differentially deployed to affect different transcripts. In addition, gene ontology analyses (Supplemental Tables 1, 2, and 3) of all differentially expressed (Supplemental Table 4) and alternatively spliced (Supplemental Tables 5 and 6) genes suggest that AS and DGE regulate unique molecular processes. Upregulated genes in the gastrocnemius quadriceps displayed insignificant and nonspecific gene network enrichment, respectively. However, downregulation of gene networks involved in mitochondrial function and lipid metabolism were found in the gastrocnemius and quadriceps, respectively. While there was some evidence to support downregulation of musculoskeletal genes in the gastrocnemius, alternatively spliced genes in both the gastrocnemius and quadriceps were overwhelmingly enriched for processes relating to the structure and function of the muscle sarcomere.

Troponin T3 (*Tnnt3*) and Troponin T1 (*Tnnt1*) are alternatively spliced in favor of isoforms that enhance fast twitch muscle fiber function.

Despite evidence at the level of immunostaining of a slow-to-fast fiber type transition in the gastrocnemius of adult mice exposed to spaceflight, there was no evidence at the level of RNA sequencing of differential expression of myosin heavy chain genes. In fact, the only differentially expressed genes with annotated roles in muscle fiber composition were those encoding small, regulatory proteins in the muscle sarcomere (*Tnni1*, *Tnnc1*, and *Myl*, Supplemental Table 4). Interestingly, *Troponin T*, which encodes a subunit of the troponin complex that facilitates muscle contraction via functional interaction with two of these differentially expressed genes (*Tnni1* and *Tnnc1*; Farah and Reinach, 1995),

was not differentially expressed in either the gastrocnemius or the quadriceps (Supplemental Table 4). Instead, *Tnnt3* and *Tnnt1*, the fast- and slow-twitch isoforms of *Troponin T*, respectively, were alternatively spliced (Figure 3, 4) in favor of transcripts encoding protein isoforms that functionally support fast-twitch fiber environments.

The 3' region of *Tnnt3* encodes the C-terminal binding domains for troponin I and tropomyosin (Stefancsik et al., 2003); therefore, the generation of two distinct *Tnnt3* isoforms via the mutual exclusion of two 3' exons (Figure 3A, 3B) influences the affinity of troponin T3 for its binding partners. Previous research suggests that exon 16 and exon 17 of *Tnnt3* vary in their sequence similarity to the functionally equivalent exon in *Tnnt1*. Specifically, exon 17 shows a much higher degree of similarity (61%) than does exon 16 (32%). As a consequence the binding affinity of the exon 17-included troponin T3 isoform is higher for the slow-twitch isoforms of troponin I and tropomyosin and, reciprocally, the binding affinity of the exon 16-included Troponin T3 isoform is higher for the fast-twitch isoforms of troponin I and tropomyosin (Wang and Jin, 1997). In the quadriceps of mice exposed to spaceflight, the exon 16-included *Tnnt3* isoform was 9.1% more abundant, while the exon 17-included *Tnnt3* isoform was reciprocally 9.1% less abundant (Figure 3C). In the gastrocnemius of mice exposed to spaceflight, exon 16-included *Tnnt3* isoform was 4.1% more abundant while the exon 17-included *Tnnt3* isoform was reciprocally 4.1% less abundant, just failing to meet statistical significance (Figure 3C). Together, these results support an exon 17-to-16 transition in the quadriceps during extended spaceflight.

While the N-terminal variable region of troponin T3 has no known binding partners in the thin filament regulatory system, alternative splicing within the 5' variable region of *Tnnt3* (Figure 4A) generates various isoforms that fall into either acidic residue-enriched or basic residue-enriched isoform classes (Figure 4B). Changes in the charge of the 5' variable region alters the three-dimensional structure of the resulting protein (Biesiadecki et al., 2007; Wei and Jin, 2011) resulting in changes in calcium (Ca^{2+}) sensitivity of the troponin complex. Specifically, basic isoforms tend to have a greater Ca^{2+} sensitivity to contraction that contributes to their preferential utilization in fast-twitch skeletal muscle fibers (Briggs et al., 1996; Ogut et al., 1999). In comparison to acidic isoforms, basic isoforms preferentially exclude exons 4, 8, and F (Figure 4B) (Wang and Jin, 1997). In the quadriceps there was significant exclusion of exons 4 (-8.3%) and 8 (-7.3%). While exon F was included 3.0% less in spaceflight conditions, this decrease was insufficient to meet statistical significance. Likewise, in the gastrocnemius there was significant exclusion of two of the exons, 8 (-11.9%) and F (-5.3%), while exon 4 was included 2.7% less in spaceflight conditions, just failing to meet statistical significance (Figure 4C). Together, these results provide evidence of an acidic-to-basic transition of the 5' variable region of *Tnnt3* in both the quadriceps and gastrocnemius during extended spaceflight.

Similar to *Tnnt3*, *Tnnt1* undergoes AS within its 5' variable region (Figure 4D). While the resulting isoforms do not differ in their acid or basic nature, the functional consequences of AS are comparable. The exclusion or inclusion of *Tnnt1* exon 5 creates lower and higher molecular weight protein isoforms, respectively (Figure 4E). Changes in the molecular weight of the 5' variable region alters the three-dimensional structure of the resulting protein (Wei and Jin, 2011), similarly influencing the calcium (Ca^{2+}) sensitivity of the troponin complex. Specifically, the higher molecular weight isoform (exon 5-included), tends to have a greater sensitivity to Ca^{2+} , a change that contributes to its preferential utilization in fast-twitch skeletal muscle fibers (Briggs et al., 1996; Ogut et al., 1999). In the quadriceps of mice exposed to spaceflight, exon 5 was significantly more abundant (10%). Whereas in the gastrocnemius of mice exposed to spaceflight, exon 5 was 4% less abundant, just failing to reach significance (Figure 4F). Therefore, our results support a lower-to-higher molecular weight *Tnnt1* isoform transition in the quadriceps during extended spaceflight.

The transition between distinct, developmentally immature and mature isoforms of the *nebulin* (*Neb*) and *ryanodine receptor 1* (*Ryr1*) genes during spaceflight.

There is extensive evidence to suggest that developmental transitions in muscle phenotype are under the control of AS. At a transcriptomic level, the employment of AS fluctuates across development, with a surge in AS events immediately after birth and a resurgence of AS in adulthood (Brinegar et al., 2017). At an individual transcript level, certain exons are included to a greater degree in younger individuals while others to a greater degree in older individuals. This is the case of *Neb* and *Ryr1* (Donner et al., 2006; Tang et al., 2015). The *Neb* gene encodes nebulin, an actin-binding protein that is localized to the thin filament of the sarcomeres in skeletal muscle. Nebulin is a very large protein (600–900 kDa) that binds as many as 200 actin monomers during muscle contraction (Chu et al., 2016). *Neb* possesses two mutually exclusive exons (127 and 128), with the exon 127-included isoform being more abundant in developmentally immature mice and the exon 128-included isoform being more abundant in developmentally mature mice (Figure 5A) (Donner et al., 2006). Appropriately, these two isoforms are labeled as the fetal and adult *Neb* isoforms, respectively (Figure 5B). The fetal isoform is significantly more abundant in the quadriceps (38 %). Whereas in the gastrocnemius of mice exposed to spaceflight, the fetal isoform was 4% less abundant, just failing to reach significance (Figure 5C). Therefore, spaceflight-induced AS seems to induce a reversion towards a more developmentally primitive isoform of the mRNA and encoded protein in the quadriceps.

A similar phenomenon is seen in the context of *Ryr1*, a gene that encodes a calcium release channel in skeletal muscle (Hernandez-Ochoa et al., 2016). *Ryr1* is alternatively spliced in an exon skipping manner to generate two isoforms, with the exon 70-excluded isoform being more abundant in developmentally immature mice and the exon 70-included isoform being more abundant in developmentally mature mice (Figure 5D) (Tang et al., 2015). Appropriately, these two isoforms are labeled as the fetal and adult *Ryr1* isoforms, respectively (Figure 5E). The adult isoform is significantly more abundant in both the quadriceps (9.3%) and gastrocnemius (8.8%) of adult mice exposed to spaceflight (Figure 5F). Therefore, in contrast to *Neb*, spaceflight-induced AS of *Ryr1* seems to induce a progression towards a more developmentally mature isoform of the mRNA and encoded protein in both muscles.

Microgravity-induced AS of large musculoskeletal genes, *titin* (*Ttn*) and *nebulin* (*Neb*), precipitates atrophic phenotypes.

The splicing changes discussed so far have been distinct AS events involving exons with likely functional consequence in the encoded protein. However, numerous AS events may also act concomitantly to impact the structure and function of large musculoskeletal with significant alterations to the size of these proteins possibly precipitating the development of microgravity-induced atrophic muscle phenotypes. This appears to be the case with *Ttn* and *Neb*.

Titin, which is encoded by the gene *Ttn*, regulates the elasticity and contractile strength of the sarcomere via the length of its PEVK “spring” domain; the longer the length of the PEVK domain, the weaker the contractile strength of the sarcomere (Ottenheijm et al., 2009). In the quadriceps of mice exposed to microgravity, there was evidence of extensive AS within the PEVK domain (Figure 6A). The average Δ PSI value across all significant AS events was 8.10% (Figure 6B). When taking into account the size of these exons in relation to the domain as a whole, AS may account for up to a 2.0% increase in the size of the PEVK domain in the quadriceps. Considering that a longer PEVK domain correlates with weaker contractile strength, spaceflight-induced extension of the PEVK domain might weaken the overall contractile strength of this muscle. In the gastrocnemius of mice exposed to microgravity, there was a relatively equal distribution of positive and negative Δ PSI events among the 10 significant AS events within the PEVK domain. As such, the average Δ PSI value across all AS events was -1.97% (Figure 6C). Therefore, a lack of spaceflight-induced alterations to the PEVK domain might maintain the overall contractile strength of this muscle.

Nebulin, which is encoded by the gene *Neb*, regulates thin filament length. In contrast to titin, a shorter length correlates with a weaker contractile strength of the sarcomere (Bang et al., 2006). In both the quadriceps and gastrocnemius of mice exposed to microgravity, there was extensive AS within the 3' region of the *Neb* transcript, with involved exons encoding the actin, tropomyosin, or desmin binding regions of nebulin (Figure 6D). The average Δ PSI value across all AS events was -6.06% and -16.09% in the quadriceps and gastrocnemius, respectively (Figure 6E, 6F). When taking into account the size of these exons in relation to the size of the 3' variable region, AS may account for approximately 1.0% and 3.5% decreases in the overall size of nebulin in the quadriceps and the gastrocnemius, respectively. Considering that a shorter length correlates with weaker contractile strength, spaceflight-induced shortening of nebulin might weaken the overall contractile strength of these two muscles.

Musculoskeletal splicing regulators, *muscleblind-like splicing regulator 1 (Mbnl1)* and *RNA binding Fox-1 homolog 1 (Rbfox1)* are differentially spliced but non-differentially expressed across spaceflight.

AS is under the regulatory control of RNA binding proteins (RBPs) (Nilsen and Graveley, 2010). As such, it has long been hypothesized that DGE of these RBPs invokes downstream changes in AS. However, despite identifying numerous spaceflight-induced AS events, there was no evidence of spaceflight-induced DGE of RBPs (Supplemental Table 4). Surprisingly, instead, we found evidence of functionally significant AS of RBPs themselves.

Mbnl1 is an RBP with two tandem RNA binding domains (ZF1-2 and ZF 3-4) that acts as a splicing regulator of various musculoskeletal genes. *Mbnl1* undergoes AS of exon 2 that results in the generation of two isoforms, each with a different translational start codon (Figure 7A). In the exon 2-included isoform, the start codon resides in exon 2 and the final protein isoform contains both tandem binding domains. However, in the exon 2-excluded isoform, the start codon resides in exon 3 and the final protein isoform contains only the ZF 3-4 tandem RNA binding domain (Figure 7B). Importantly, these tandem RNA binding domains are not functionally equivalent; the ZF1-2 domain exhibits more motif specificity and five times more activity than the ZF3-4 domain (Lee, 2013). Therefore, it is interesting to note that the exon 2-included isoform, which contains both the ZF1-2 and ZF 3-4 domains, is 13% less abundant in the quadriceps of adult mice exposed to microgravity. In the gastrocnemius of mice exposed to spaceflight, the exon 2 included isoform was 3% less abundant (Figure 7C), a trend that did not reach statistical significance.

Rbfox1 is an RBP that acts as a splicing regulator in both muscle and brain tissue, with the tissue-specific function of this protein being regulated by a mutual exon exclusion (MXE) event involving B40 (brain-specific exon consisting of 40 base pairs) and M43 (muscle-specific exon consisting of 43 base pairs) (Figure 7D, 7E) (Conboy, 2017). While this has previously been annotated as a mutually exclusive event, we observed a significantly increased inclusion of B40 in both the quadriceps (8.9%) and gastrocnemius (8.1%) without a significant, reciprocal decrease in the inclusion of M43 in either muscle (Figure 7F).

Discussion

While we set out first and foremost to characterize the role of AS in the physiological adaptation of the mouse hind limb postural and phasic muscles to microgravity, the work presented here has also provided novel insights more broadly into the reciprocal, tissue-specific relationship between DGE and AS. Co-transcriptional splicing was first documented as long as 30 years ago (Beyer and Osheim, 1988), but the molecular mechanisms underlying the coupling of DGE and AS have only recently been elucidated, including regulation of splicing by transcriptional elongation rate (Kadener, 2001) and modulation of splicing factor recruitment by nucleosome positioning and histone modifications (Spies, et al., 2009; Schwartz et al., 2009). In addition, more recent research has characterized a direct physical connection between RNA polymerase II and the spliceosome at the point of emergence of the pre-mRNA from the

transcriptional machinery (Zhang et al., 2021). Together, this evidence suggests that these distinct mechanisms of transcriptome regulation (transcription and splicing) are intricately related. While it is believed that this intricate relationship is regulated by tissue-specific regulatory factors, DGE and AS have either been investigated together in a single biological system (cell type, tissue type, disease state, etc.) (Ullah et al., 2020; Brinegar et al., 2017) or separately across multiple biological systems (Shen-Orr et al., 2010; Yeo et al., 2004), without any comprehensive investigations of DGE and AS together across multiple biological systems. As such, the experiments here, documenting an equal but opposite propensity for the quadriceps and gastrocnemius to adapt their transcriptomes to microgravity via DGE or AS, respectively (see Figure 2A, 2B), provides the first evidence of a possible reciprocal, muscle-type-specific relationship between DGE and AS, such that each muscle preferentially employs one mechanism of transcriptome regulation at the other mechanism's expense.

We hypothesize that the muscle-type-specific regulatory biases we identified are indicative of differences in energy availability across each muscle. While it has long been proposed that energy availability influences patterns of transcriptome regulation, only recently has it been shown that patterns of both DGE and AS vary across high and low ATP environments (Guantes et al., 2015) as a result of diminished supply of mitochondrial adenine nucleotides (AdNs: ATP, ADP, AMP) in atrophic skeletal muscle (Ji et al., 2019; Miller et al., 2020). In accordance with previous research (Roy et al., 1996; Recktenwald et al., 1999), at the level of muscle fiber immunostaining we observed more significant microgravity-induced atrophy in the quadriceps compared to the gastrocnemius (see Figure 1C), with the resistance to microgravity-induced atrophy in the gastrocnemius attributable to its more prominent slow-to-fast fiber type transition (see Figure 1A, 1B and 1D) (Fitts et al., 2001). Diminished adenine nucleotide supply in the more atrophied quadriceps may direct the use of AS, a less energy-dependent mode of gene expression, over DGE, a more-energy dependent mode of gene expression (Lynch and Marinov, 2015; Lane and Martin, 2010). In contrast, adequate supply of adenine nucleotides in the less atrophied gastrocnemius favors DGE over AS. While our results are restricted to a perturbation in gravity, a similar transcriptomic bias towards DGE over AS during development has been observed in the mouse gastrocnemius in utero (Brinegar et al., 2017), suggesting that our findings in the context of microgravity may reflect those of a “floating” amniotic environment.

Regardless of the mechanism of transcriptome regulation was preferentially employed in each muscle, we found a more significant role for AS than DGE in supporting microgravity-induced fiber type transitions. For example, in the absence of considerable DGE of genes encoding structural proteins in the muscle sarcomere, the increased reliance on fast twitch fiber function (whether in the 100% fast twitch quadriceps or the markedly slow-to-fast twitch transitioned gastrocnemius) in a microgravity environment was principally supported by extensive changes in AS of *Troponin T* (*Tnnt*). Our results provide evidence of three concomitant microgravity-induced isoform transitions, i) a move from *Tnnt3* pre-mRNA exon 17-to-16 inclusion (see Figure 3), ii) a *Tnnt3* acidic-to-basic residue change (see Figure 4), and iii) a *Tnnt1* lower-to-higher molecular weight change (see Figure 4). Taken in the context of previous research (Wang and Jin, 1997; Biesiadecki et al., 2007; Wei and Jin, 2011; Briggs et al., 1996; Ogut et al., 1999), when translated such isoforms would possess N- and C-terminal alterations that, together, contribute in the three dimensional protein structures that strongly favor fast-twitch troponin I and tropomyosin binding as well as possess increased calcium sensitivity to contraction required by those fast-twitch fibers; that being said, it is unlikely that the aberrant splicing of *Troponin T* alone significantly enhanced the fast twitch function of either the quadriceps or gastrocnemius. In addition to the annotated events in *Tnnt3* and *Tnnt1*, there is evidence to suggest that AS of *Neb* and *Ryr1* may contribute to the function of fast twitch muscle. Specifically, the human homologs of *Neb* exon 127 (*NEB* exon 143) and *Ryr1* exon 70 (*RYR1* exon 70) are known to be preferentially included in the mRNA of fast twitch muscles and reciprocally excluded in slow twitch muscles (Tang et al., 2015; Lam et al., 2018). While the mechanism that contributes to increased use of these isoforms in fast-twitch muscle remains unknown, the microgravity-

induced inclusion of *Neb* exon 127 and *Ryr1* exon 70 can be taken as further evidence of splicing-supported fiber type transitions in response to extended spaceflight.

Similar to microgravity-induced fiber type transitions, we found a more significant role for AS than DGE in supporting microgravity-induced muscle atrophy. Unlike the role of AS in the slow-to-fast fiber transition, the precipitation of muscle atrophy via AS is not an entirely novel concept; most support for this phenomenon comes in the context of atrophic musculoskeletal diseases. For example, the aberrant splicing of *NEB* exon 143 and *RYR1* exon 70, have been shown to be required for the development of atrophic musculoskeletal diseases in humans (Garg et al., 2017; Kimura et al., 2005). While we observed aberrant splicing of both *Neb* exon 127 and *Ryr1* exon 70 in the muscle transcriptome of our mice, those changes opposed the AS trends that would generate atrophy. As such, we found no evidence to support a “canonical” role of AS in precipitating muscle atrophy. Despite this, we were successful in identifying a relatively novel mechanism of AS-induced atrophy: the regulation of large musculoskeletal gene transcript length. The unique composition of musculoskeletal tissue in sarcomere units necessitates the employment of some of the largest proteins in nature. This includes titin and nebulin (see Figure 6). Owing to their size (350 exons and 166 exons, respectively) and complexity, the pre-mRNAs of these genes lend themselves to extensive regulation via AS. In fact, AS is well-characterized in regulating developmental changes in the size of these proteins; for example, on average, the size of titin decreases by approximately 7.0% during musculoskeletal development as a result of extensive AS within the PEVK domain (Ottenheijm et al., 2009). In contrast to the typical developmental shortening of the *Ttn* transcript, we characterized AS-directed extension of the *Ttn* transcript as a result of extended exposure to microgravity, which, together with the AS-directed shortening of the *Neb* transcript, serves to diminish the overall contractile strength of the muscle sarcomere. In addition, differences in these AS events across the quadriceps and gastrocnemius may help to further explain the resistance of postural muscles to microgravity-induced atrophy, as the gastrocnemius displayed AS-directed shortening of the *Neb* transcript but no AS-directed extension of the *Ttn* transcript.

In the absence of significant differential expression of RBPs in either the of the postural or phasic limb muscles studied here, we discovered functionally significant spaceflight-induced AS of RBPs themselves, a likely upstream provocation that can account for changes in AS of downstream gene targets. For example, *Mbnl1* was found to be significantly alternatively spliced in the quadriceps in microgravity and *Rbfox1* significantly alternatively spliced in both the quadriceps and gastrocnemius in microgravity (see Figure 7). *Mbnl1* regulates the splicing patterns of various musculoskeletal genes, including *Tnnt1*, *Tnnt3*, *Ttn*, and *Ryr1*. Specifically, *Mbnl1* has been motif-mapped to the 3' variable region of *Tnnt3*, exon 5 of *Tnnt1*, the PEVK domain of *Ttn*, and exon 70 of *Ryr1* (Lee, 2013), all events that were found in the work presented here to be significantly alternatively spliced as a result of exposure to microgravity (see Figure 3, 4, 5, 6). Therefore, AS of *Tnnt1*, *Tnnt3*, *Ttn*, and *Ryr1* may be the result of spaceflight-induced exclusion of the functionally superior ZF1-2 domain of *Mbnl1*. Similar to *Mbnl1*, *Rbfox1* regulates the splicing patterns of various musculoskeletal genes, including *Ryr1* and *Mybp1*. *Rbfox1* has been motif-mapped to exon 70 of *Ryr1* and exon 3 of *Mybp1* (Pedrott et al., 2015), both events that were found in the work presented here to be significantly alternatively spliced as a result of exposure to microgravity (see Figure 5, Supplemental Tables 5 and 6). Therefore, AS of *Ryr1* and *Mybp1* may be the result of spaceflight-induced inclusion of the aberrant brain-specific exon in place of the muscle-specific exon of *Rbfox1* mRNA. Taken together, these data suggest that pre-mRNA targets for differential AS are subject to control by AS of the RBP splicing factors itself that ultimately directs AS of that target pre-mRNA.

While our experiments employed adult mice (32 weeks), it is unlikely that age alone can account for our findings. Previous characterizations of the musculoskeletal adaptation to spaceflight have occurred in the context of mice ranging from 8-20 weeks at the time of spaceflight (Harrison et al., 2003; Gambari et al., 2017; Camerino et al., 2013; Chakraborty et al., 2020), requiring inquiry as to the role age may have played in generating the phenotypes we observed and the transcriptomic alterations we characterized.

First, there is evidence in humans to suggest that a fast-to-slow fiber type transition accompanies aging (Ohlendiek, 2011). Therefore, the presence of a microgravity-induced slow-to-fast fiber transition suggests that, despite the opposing force of aging on fiber type composition, the slow-to-fast fiber plasticity of skeletal muscle fibers is maintained even at an increased age. In contrast to fiber transitions, aging mirrors the effect of microgravity on muscle atrophy (Ohlendiek, 2011); it is possible that the advanced age of our mice compared to early age mice in other studies (Harrison et al., 2003; Gambari et al., 2017; Camerino et al., 2013; Chakraborty et al., 2020) amplified the microgravity-induced atrophy that was observed here. Most important to our work, however, is the relationship between modes of transcriptomic regulation and age. While relative employment of DGE and AS fluctuates with age and organismal development, most of this fluctuation occurs immediately preceding, at, and immediately following birth (until approximately post-natal day 28; Brinegar et al., 2017). In addition, there is evidence in both mice and humans to suggest relative stability of the striated muscle transcriptome across adult years and even into later life (Balliu et al., 2019). Therefore, it is unlikely that the transcriptome alterations we characterized are simply a product of the age of the mice employed. In fact, investigations of microgravity-induced differential gene expression in younger mice (Gambara et al., 2017; Chakraborty et al., 2020) showed downregulation of metabolic and mitochondrial pathways similar to what we identified in our older mice. Therefore, while AS has not been adequately investigated in the context of spaceflight, it can be speculated that extensive microgravity-induced AS would be identified regardless of age.

In summary, we have shown that 1) AS and DGE vary in a reciprocal manner, possibly in response to tissue-specific energy availability; 2) AS plays a more functionally significant role than DGE in supporting microgravity-induced phenotypes; 3) AS augments fast twitch fiber function via aberrant splicing of *Tnnt3*, *Tnnt1*, *Neb*, and *Ryr1*; 4) AS contributes to muscle atrophy via splicing-directed shortening of *Neb* and extension of the *Tm*; and 5) RBPs, transregulators of AS, are themselves differentially spliced while being non-differentially expressed. Together, the results of our work emphasize the importance of AS in determining the plasticity and functional status of the skeletal muscle transcriptome. Considering the current development of novel treatment methods for atrophic musculoskeletal diseases using small-molecule splicing modulators (Jarecki et al., 2005; Cheung et al., 2018; Ando et al., 2020), it is possible that further characterization of microgravity-induced AS targets will allow for the development of small-molecule splicing modulator-based therapies to prevent microgravity-induced muscle atrophy and other detrimental physiologic adaptations to spaceflight.

Materials and Methods

Animals

All the animal procedures were performed according to the guidelines of the Chancellor's Animal Research Committee at UCLA (Protocol # 2009-127) and NASA Institutional Animal Care and Use Committees. Twenty 32-week-old female BALB/c mice (Taconic Biosciences, NY) were used in this study. All mice were housed at Kennedy Space Center (KSC) for 4 weeks before rocket launch and were randomly divided into ground control group and flight groups (n=10 per group). On June 3rd, 2017, mice of in the flight group were transported to the International Space Station (ISS) as part of SpaceX CRS-11 and kept at ISS for the full 9 weeks of experimentation, while mice of ground control group were kept at KSC for the same duration. The mice housed at KSC were in the NASA's Rodent Research Hardware System and were fed food bar diet ad libitum. The food bar essentially has added moisture in the standard rodent chow to form a bar form and prevent it from floating in microgravity.

Sample preparation

At the end of the study, all mice were euthanized at ISS or KSC by our NASA partners on site. Mice were then deeply frozen in situ under -80°C or colder until later transportation to UCLA on dry ice. Frozen carcasses were thawed on wet ice before tissue dissection. Mice skeletal muscles were dissected separately and either fixed in 10% neutral buffered formalin or preserved in *RNAlater* solution.

Immunohistochemistry and morphological analysis

Formalin-fixed paraffin-embedded sections of mouse muscle (5μm thickness) from gastrocnemius and quadriceps were cut with a microtome and mounted on charged slides. Sections were either subjected to standard hematoxylin-eosin (H&E) staining for overview, or immunolabeled with the following anti-myosin heavy chain (MyHC) isoform antibodies: type I MyHC isoform (Abcam, Cat# ab11083); type II MyHC isoform (Abcam, Cat# ab51263). The sections were co-stained with an anti-laminin antibody (Abcam, Cat# ab11575) to allow measurement of fiber size. In all protocols, donkey anti rabbit Alexa-488 conjugated secondary antibody was used for laminin staining, donkey anti-mouse Alexa-594 conjugated secondary antibody for type I MyHC antigen staining, and anti-mouse Alexa-488 conjugated secondary antibody for type II MyHC antigen staining. Photomicrographs were acquired using Olympus BX 51 and IX 71 microscopes equipped with Cell Sense digital imaging system (Olympus, Japan). To assess the cross-sectional area (CSA) of the different myofiber types, digitized photographs were acquired and the myofiber CSA was automatically measured by means of ImageJ 1.45 g (NIH, freeware imaging software).

RNA extraction and purification

Total RNA was isolated from mouse gastrocnemius (n=3) and quadriceps (n=3) of each experimental group (flight and ground control) using the acid guanidinium thiocyanate-phenol-chloroform extraction followed by silica-membrane purification. Briefly, frozen tissue samples were minced into small pieces (1mm x 1mm x 1mm). A homogeneous lysate was achieved by adding lysing buffer and gentle ultrasound vibration on ice. Tissue lysate was centrifuged and the supernatant was used for RNA phenol/chloroform extraction. After phase separation, the aqueous layer was transferred and mixed with an equal volume of 70% ethanol. Total RNA was then extracted using Rneasy spin columns from the Rneasy micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

RNA seq analysis

For each replicate RNA sample, a cDNA library was prepared and sequenced by the UCLA Technology Center for Genomics and Bioinformatics using an Illumina sequencer, generating an average of 41.5 million single-end 50bp reads. The resulting RNASeq reads were aligned to the mouse genome (mm10) reference using STAR software (Dobin et al., 2013). Quality of the RNA-seq dataset was confirmed by read depth and mapping statistics (Supplemental Figure 1A). Read depth for all samples was at or above 30 million uniquely mapped reads (the desired read depth for differential splicing analyses), with the exception of one quadricep ground sample with 27.4 million uniquely mapped reads. The uniquely mapped read percentage for all samples was above 80%, the desired mapping percentage for differential splicing analyses. Transcript abundance was measured directly from FASTQ files as TPM (Transcripts Per Million) using kallisto (Bray et al., 2016) and summarized into gene expression matrix by R package 'tximport' (Soneson et al., 2015). Lowly expressed genes (TPM $\leq$ 5 in all samples) were filtered out before conducting differential analysis with DeSeq2 (Love et al., 2014). For each comparison, genes with an absolute log2 fold change $> \log_2(1.5)$ and an FDR (false discovery rate)-adjusted p-value < 0.01 were assumed to be differentially expressed genes.

Alternative splicing events were detected and quantified as Percent Spliced In (PSI) values by rMATS-turbo (Shen et al., 2014) using junction reads (reads spanning the splicing junctions). AS events with low junction read support (≤ 10 average junction reads, ≤ 10 total inclusion junction reads or ≤ 10 total skipping junction reads over all 12 samples), or with extreme PSI value ranges ($PSI \leq 0.05$ or $PSI \geq 0.95$ in all 12 samples) were excluded from downstream analysis. Differential splicing analysis was then performed using rMATS-turbo by comparing the replicate ground samples and flight samples in each tissue. Differential splicing events were identified by the following criteria: 1) >10 average junction reads (inclusion and skipping junction reads) in both groups; 2) no extreme PSI values ($PSI \leq 0.05$ or $PSI \geq$

0.95 for all 6 samples in the comparison); 3) FDR < 0.01; and 4) absolute change in PSI ($|\Delta\text{PSI}|$) > 0.05. Five AS event types were annotated (Supplemental Figure 1B), including skipped exon (SE), alternative 5' splice site (A5SS), alternative 3' splice site (A3SS), mutually exclusive exon (MXE), and retained intron (RI). SE and MXE, AS event types focused on in this work, composed approximately 50% and 20% of all AS events, respectively. Gene ontology (GO) analysis was performed to reveal enriched functional pathways affected by significant gene expression changes as well as alternative splicing changes using EnrichR (Chen et al., 2013; Kuleshov et al., 2016; Xie et al., 2021). The top enriched GO terms with smallest adjusted p values were visualized as bar plots.

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Figure 1. (A) Representative immunofluorescence images of gastrocnemius and quadriceps stained for laminin and MyHC isoforms (MyHC I: red, II: green) in flight and ground groups. (B) Some myofibers (yellow arrows) were co-stained by both MyHC isoforms (MyHC I: red, II: green). (C) Quantification of the muscle fiber type distribution of gastrocnemius (n = 10). (D) Quantification of the myofiber cross sectional area (CSA) in both gastrocnemius and quadriceps (n = 10 per group).

Figure 2. (A) Venn diagrams comparing differentially expressed (left) and alternatively spliced (right) genes in the gastrocnemius and quadriceps. The size of each circle is representative of the number of genes that were differentially expressed or alternatively spliced in each muscle, with blue and red circles representing such genes from the gastrocnemius and quadriceps, respectively. Overlapping regions depict genes that were held in common between the two muscles. Scatter plots comparing gene expression in ground vs. flight in the gastrocnemius **(B)** and quadriceps **(C)**. Red and blue dots represent up-regulated and down-regulated genes, respectively. Genes with significant AS changes (denoted as AS genes) are depicted in yellow. Background genes, e.g. those with no differential gene expression and no exon skipping changes, are shown in grey.

Figure 3. (A) Gene structure representation of the *Tnnt3* gene. Bars and dashed lines represent exons and introns, respectively, with exon numbering below. Untranslated regions (UTR) are denoted in black. Solid lines connecting exons 16 (depicted in red and labeled the fast twitch exon) and exon 17 (depicted in blue and labeled the slow twitch exon) to nearby splice junctions represent a mutually exclusive splicing event in the 3' region of the transcript that encodes for the tropomyosin and troponin I binding sites of the resulting troponin T protein. (B) Protein structure representation of the two *Tnnt3* isoforms. The fast twitch *Tnnt3* isoform has exon 16 included and exon 17 excluded while the slow twitch *Tnnt3* isoform has exon 16 excluded and exon 17 included. (C) Bar plot depicting the PSI (percent spliced in) value of the exon 16/17 mutually exclusive exon (MXE) event in ground and flight for quadriceps (top) and gastrocnemius (bottom). The delta PSI value indicates the difference in percent inclusion of exon 16/17 between flight and ground. Asterisks represent significance by two-tailed t-test.

Figure 4. (A) Gene structure representation of the 5' variable region (exons 1-9) of *Tnnt3* gene. Bars and dashed lines represent exons and introns, respectively, with exon numbering below. Untranslated regions (UTR) are denoted in black. Exon 4 (orange-red), 8 (mint), and F (pink-purple) are all alternatively spliced in an exon skipping manner with solid lines of corresponding color connecting these exons to their respective splice junctions. (B) Protein structure representation of two 5' *Tnnt3* isoforms. The basic *Tnnt3* isoform has exon 4, 8, and F all excluded while the acid *Tnnt3* isoform has exon 4, 8, and F all included. (C) Bar plot depicting the PSI (percent spliced in) value for each exon (4, 8, and F) in ground and flight for quadriceps (top) and gastrocnemius (bottom). The delta PSI value indicates the difference in percent inclusion of each exon between flight and ground. Asterisks represent significance by two-tailed t-test. (D) Gene structure representation of the 5' variable region (exons 1-6) of *Tnnt1* gene. Bars and dashed lines represent exons and introns, respectively, with exon numbering below. Untranslated regions (UTR) are denoted in black. Exon 5 (orange) is alternatively spliced in an exon skipping manner with solid lines connecting this exon to its respective splice junctions. (E) Protein structure representation of two 5' *Tnnt1* isoforms. The high molecular weight *Tnnt1* isoform has exon 5 included while the low molecular weight *Tnnt1* isoform has exon 5 excluded. (F) Bar plot depicting the PSI (percent spliced in) value for exon 5 in ground and flight for quadriceps (top) and gastrocnemius (bottom). The delta PSI value indicates the difference in percent inclusion of exon 5 between flight and ground. Asterisks represent significance by two-tailed t-test.

Figure 5. (A) Gene structure representation of exons 126-129 of *Neb* gene. Bars and dashed lines represent exons and introns, respectively, with exon numbering below. Solid lines connecting exons 127 (depicted in light blue and labeled the fetal exon) and exon 128 (depicted in peach and labeled the adult exon) to nearby splice junctions represent a mutually exclusive splicing. **(B)** Protein structure representation of the two *Neb* isoforms. The fetal *Neb* isoform has exon 127 included and exon 128 excluded while the adult *Neb* isoform has exon 127 excluded and exon 128 included. **(C)** Bar plot depicting the PSI (percent spliced in) value of the exon 127/128 mutually exclusive exon (MXE) event in ground and flight for quadriceps (top) and gastrocnemius (bottom). The delta PSI value indicates the difference in percent inclusion of exon 127/128 between flight and ground. Asterisks represent significance by two-tailed t-test. **(D)** Gene structure representation of exons 69-71 of *Ryr1* gene. Bars and dashed lines represent exons and introns, respectively, with exon numbering below. Exon 70 (green) is alternatively spliced in an exon skipping manner with solid lines connecting this exons to its respective splice junctions. **(E)** Protein structure representation of two *Ryr1* isoforms. The fetal *Ryr1* isoform has exon 70 excluded while the adult *Ryr1* isoform has exon 70 included. **(F)** Bar plot depicting the PSI (percent spliced in) value for exon 70 in ground and flight for quadriceps (top) and gastrocnemius (bottom). The delta PSI value indicates the difference in percent inclusion of exon 70 between flight and ground. Asterisks represent significance by two-tailed t-test.

Figure 6. (A) Protein structure representation of *Ttn* gene. Colored regions represent protein domains with pink, dark blue, yellow, green, and light blue regions corresponding to Z-disk, I-band, PEVK, A-band, and M-band regions of *Ttn* respectively. Solid numbers represent the number of significant AS events within the corresponding region of interest in the quadriceps (top) and gastrocnemius (bottom). Line graphs displaying delta PSI values for exons within the *Ttn* PEVK domain (exons 122-202) that underwent significant AS between ground and flight groups in **(B)** quadriceps and **(C)** gastrocnemius. Positive delta PSI value events represent exons that were included more in the flight group while negative delta PSI value events represent exons that were included less in the flight group. Non-alternatively spliced and non-significantly alternatively spliced exons were provided a delta PSI value of 0. **(D)** Protein structure representation of *Neb* gene. Colored regions represent protein domains with blue, pink, yellow, and green regions corresponding to the actin and tropomyosin binding region (which spans the entire protein length), tropomodulin binding region, desmin binding region, and titin and myopalladin binding regions, respectively, in the quadriceps (top) and gastrocnemius (bottom). Line graphs displaying delta PSI values for exons within the variable region of *Neb* (exons 127-159) that underwent significant AS between ground and flight groups in **(E)** quadriceps and **(F)** gastrocnemius. Positive delta PSI value events represent exons that were included more in the flight group while negative delta PSI value events represent exons that were included less in the flight group. Non-alternatively spliced and non-significantly alternatively spliced exons were provided a delta PSI value of 0.

Figure 7. (A) Gene structure representation of the *Mbnl1* gene. Bars and dashed lines represent exons and introns, respectively, with exon numbering below. Untranslated regions (UTR) are denoted in black. Solid lines connect exon 2 to nearby splice junctions. Two start codons residing in exons 2 and 3 are depicted in green. Four zinc-finger domains (ZF1-4) residing in exons 2, 3, 6, and 7 are depicted in yellow. **(B)** Protein structure representation of the two *Mbnl1* isoforms. The exon 2 included *Mbnl1* isoform has exon 2 included and all four zinc finger domains (ZF1-4) are translated. The exon 2 excluded *Mbnl1* isoform has exon 2 excluded and only two zinc finger domains (ZF3-4) are translated. **(C)** Bar plot depicting the PSI (percent spliced in) value for exon 2 in ground and flight for quadriceps (top) and gastrocnemius (bottom). The delta PSI value indicates the difference in percent inclusion of exon 2 between flight and ground. Asterisks represent significance by two-tailed t-test **(D)** Gene structure representation of the *Rbfox1* gene. Bars and dashed lines represent exons and introns, respectively, with exon numbering below. Untranslated regions (UTR) are denoted in black. Solid lines connecting exons B40 (depicted in light blue) and exon M43 (depicted in red) to nearby splice junctions represent a mutually exclusive splicing. The RRM protein domain extends from exon 2 to 5 as depicted by the double sided arrow labeled as such. **(E)** Protein structure representation of the two *Rbfox1* isoforms. The brain-specific *Rbfox1* isoform has exon B40 included and exon M43 excluded while the muscle-specific *Rbfox1* isoform has exon B40 excluded and exon M43 included. **(F)** Bar plot depicting the PSI (percent spliced in) value of the exon B40/M43 mutually exclusive exon (MXE) event in ground and flight for quadriceps (top) and gastrocnemius (bottom). The delta PSI value indicates the difference in percent inclusion of exon B40/M43 between flight and ground. Asterisks represent significance by two-tailed t-test.

Supplemental Figure 1. (A) Summary of read depth and mapping statistics for RNA-seq dataset. **(B)** Summary table of AS events detected by rMATS-turbo after filtering by read coverage and PSI value range. SE, skipped exon; A5SS, alternative 5' splice site; A3SS, alternative 3' splice site; MXE, mutually exclusive exon; RI, retained intron. Representative images below depict examples of the above listed alternative splicing events.

	<i>Gastrocnemius</i>			<i>Quadriceps</i>		
	GO Term	GO category	Adjusted P-value	GO Term	GO category	Adjusted P-value
Upregulated Genes	Spinal cord motor neuron differentiation	Biological Process	0.0821	Synaptic transmission, glycinergic	Biological Process	0.03089
	Cardiac ventricle formation		0.0821	Inner ear receptor differentiation		0.03089
	Lymphoid progenitor cell		0.0821	Response to catecholamine		0.03089
	Dendrite extension		0.0821	Cellular response to monoamine stimulus		0.03089
	Cell differentiation in spinal cord		0.0821	Positive regulation of myeloid leukocyte cytokine production involved in immune response		0.03089
	Peroxisome proliferator activated receptor binding	Molecular Function	0.08081	Glucocorticoid receptor binding	Molecular Function	0.08182
	AT DNA binding		0.08081	Protein phosphatase 1 binding		0.08182
	Retinoid X receptor binding		0.08081	Glycine binding		0.08182
	Filamin binding		0.08081	Inhibitory extracellular ligand-gated ion channel activity		0.08182
	Sequence-specific double-stranded DNA binding		0.08081	Ligand-gated anion channel activity		0.08182
	Synaptonemal structure	Cellular Component	0.175	Cytoplasmic microtubule	Cellular Component	0.02628
	Synaptonemal complex		0.175	Microtubule		0.1374
	Cytoplasmic microtubule		0.175	Polymeric cytoskeletal fiber		0.1441
	Contractile actin filament binding		0.175	Microtubule cytoskeleton		0.2399
	Stress fiber		0.175	Integral component of plasma membrane		0.2733

Supplemental Table 1. Enrichment by gene ontology of upregulated genes in gastrocnemius and quadriceps. Reported here are the GO term (top five for each category), GO category, and adjusted p-value.

	<i>Gastrocnemius</i>			<i>Quadriceps</i>		
	GO Term	GO category	Adjusted P-value	GO Term	GO category	Adjusted P-value
Downregulated Genes	SRP-dependent protein targeting to membrane	Biological Process	5.29E-11	Long-chain fatty acid transport	Biological Process	0.001642
	Contraslational protein targeting to membrane		5.29E-11	Fatty-acy-CoA biosynthetic process		0.001642
	Protein targeting to ER		5.40E-11	Long chain fatty acid import		0.004286
	Viral gene expression		1.29E-10	Cholesterol homeostasis		0.004286
	Nuclear-transcribed mRNA catabolic process, nonsense-mediated decay		1.29E-10			
	RNA binding	Molecular Function	0.001204	Sterol homeostasis	Molecular Function	0.009229
	acetyl-CoA C-acyltransferase activity		0.004221	Protein-disulfide reductase activity		0.1134
	cation-transporting ATPase activity		0.004221	Long-chain fatty acid binding		0.1134
	myosin heavy chain binding		0.004424	Ether hydrolase activity		0.1134
	proton-transporting ATP synthase activity, rotational mechanism		0.007851	Oxidoreductase activity, acting on a sulfur group of donors, NAD(P) as acceptor		0.1134
	Cytosolic ribosome	Cellular Component	6.10E-12	RNA 7-methylguanosine cap binding		0.1134
	Cytosolic part		4.78E-11			
	Cytosolic small ribosome subunit		1.20E-07	Lipid droplet	Cellular Component	0.000108
				Cytosolic large ribosome subunit		0.004484
	Small ribosomal subunit		1.83E-07	Large ribosomal subunit		0.004873
	Cytosolic large ribosomal subunit		2.46E-05	Mitochondrial proton-transporting ATP synthase complex, coupling factor F ₀		0.01011
				mitochondrial outer membrane		0.01202

Supplemental Table 2. Enrichment by gene ontology of downregulated genes in gastrocnemius and quadriceps. Reported here are the GO term (top five for each category), GO category, and adjusted p-value.

	<i>Gastrocnemius</i>			<i>Quadriceps</i>		
	GO Term	GO category	Adjusted P-value	GO Term	GO category	Adjusted P-value
Alternatively Spliced Genes	Actomyosin structure organization	Biological Process	0.001442	Sarcomere organization	Biological Process	9.1E-06
	Muscle contraction		0.001442	Myofibril assembly		0.000062
	Muscle fiber development		0.002096	Actin-myosin filament sliding		0.0003
	Sarcomere organization		0.002405	Dynamin family protein polymerization involved in membrane fission		0.0003
	Muscle filament sliding		0.0027	Sarcoplasmic reticulum calcium ion transport		0.05247
	Actin binding	Molecular Function	0.005557	Titin binding	Molecular Function	0.006467
	Protease binding		0.0504	Spectrin binding		0.007605
	Potassium channel regulator activity		0.0824	Microtubule plus-end binding		0.04369
	Telethonin binding		0.1036	Tropomyosin binding		0.04369
	AT DNA binding		0.1036	1-phosphatidylinositol binding		0.04369
	Contractile actin filament bundle	Cellular Component	0.02543	Microtubule	Cellular Component	0.009541
	Stress fiber		0.02543	Microtubule cytoskeleton		0.009541
	Cytoplasmic stress granule		0.02543	Contractile fiber		0.009908
	Actin cytoskeleton		0.02543	Myofibril		0.009908
	Cytoskeleton		0.02543	Microtubule organization center		0.02633

Supplemental Table 3. Enrichment by gene ontology of alternatively spliced genes in gastrocnemius and quadriceps. Reported here are the GO term (top five for each category), GO category, and adjusted p-value.

<i>Gastrocnemius</i>					<i>Quadriceps</i>				
Gene	log2fc	lfcSE	pvalue	padj	Gene	log2FC	lfcSE	pvalue	padj
<i>Gm12254</i>	2.8650146	0.3421034	5.54E-17	1.07E-13	<i>Rpl31-ps8</i>	9.736247	1.2735564	2.09E-14	2.64E-10
<i>Sox4</i>	1.5392035	0.3156536	1.08E-06	0.0001866	<i>Sbk3</i>	2.1905462	0.4713823	3.37E-06	0.0015661
<i>Gm48583</i>	0.976333	0.2544155	0.0001243	0.008059	<i>Grem2</i>	1.9153984	0.3229391	3.01E-09	4.82E-06
<i>Vcp-rs</i>	1.1369977	0.2523758	6.63E-06	0.0008216	<i>Glr3</i>	1.0202335	0.2425734	2.60E-05	0.0062743
<i>Gm28635</i>	1.0958251	0.2359258	3.40E-06	0.0004767	<i>Mid1</i>	1.4161726	0.2271019	4.49E-10	1.13E-06
<i>Gm10563</i>	0.906756	0.2263088	6.16E-05	0.0050546	<i>Gm15501</i>	1.2305243	0.2244627	4.20E-08	4.08E-05
<i>Hmga1</i>	1.1598402	0.2257119	2.77E-07	6.86E-05	<i>Nr4a3</i>	1.2853187	0.2239407	9.49E-09	1.09E-05
<i>Gm13772</i>	0.8550244	0.2196276	9.90E-05	0.0069899	<i>Pdzd7</i>	0.9072751	0.2231495	4.79E-05	0.0096641
<i>Gm7329</i>	0.9593701	0.2087775	4.32E-06	0.0005969	<i>Peg3</i>	1.0458756	0.1632135	1.47E-10	4.65E-07
<i>Snx30</i>	0.916164	0.208642	1.13E-05	0.0012673	<i>Sh3rf2</i>	0.622775	0.1534475	4.94E-05	0.0096641
<i>Gm21596</i>	0.8489464	0.2038213	3.11E-05	0.002919	<i>Agbl1</i>	0.6086136	0.1496037	4.74E-05	0.0096641
<i>Fblim1</i>	0.9987318	0.2032556	8.94E-07	0.000163	<i>Mpp3</i>	0.7209741	0.1375563	1.59E-07	0.0001257
<i>Gm5898</i>	0.8187563	0.2001244	4.29E-05	0.0037693	<i>Nav2</i>	0.6255754	0.1236282	4.19E-07	0.0002935
<i>Peg3</i>	0.9219438	0.1882508	9.71E-07	0.0001738	<i>Gm5537</i>	0.6944585	0.0959539	4.57E-13	2.88E-09
<i>Auts2</i>	0.6856395	0.1805116	0.0001457	0.0092009	<i>Pgk1</i>	-0.628311	-0.10633	3.44E-09	4.82E-06
<i>Hmcn1</i>	0.8881328	0.1681027	1.27E-07	3.50E-05	<i>Acs1</i>	-0.596456	-0.110605	6.94E-08	5.84E-05
<i>Col24a1</i>	0.6767221	0.1555463	1.36E-05	0.0014907	<i>Rpl37a</i>	-0.66853	-0.120601	2.97E-08	3.12E-05
<i>Ccnb1ip1</i>	0.8521407	0.1549927	3.84E-08	1.28E-05	<i>Ephx2</i>	-0.630345	-0.138817	5.60E-06	0.0021412
<i>Gm48417</i>	0.8832073	0.1379735	1.54E-10	1.06E-07	<i>Fabp3</i>	-0.677114	-0.146104	3.58E-06	0.0015661
<i>Agbl1</i>	0.6302463	0.1378048	4.80E-06	0.0006438	<i>Cnih4</i>	-0.702254	-0.151572	3.60E-06	0.0015661
<i>Ccdc88c</i>	0.712383	0.1244774	1.05E-08	4.21E-06	<i>Rpl24</i>	-0.735119	-0.15532	2.21E-06	0.0012114
<i>Mid1</i>	1.2538014	0.1172607	1.10E-26	1.07E-22	<i>Fabp4</i>	-0.766511	-0.176893	1.47E-05	0.0047521
<i>Nav2</i>	0.5913388	0.1169067	4.23E-07	9.51E-05	<i>Hspb7</i>	-0.862529	-0.184869	3.08E-06	0.0015521
<i>AC149090.1</i>	0.5916471	0.1154075	2.95E-07	6.95E-05	<i>Cyfp2</i>	-1.176927	-0.198667	3.14E-09	4.82E-06
<i>Myo5c</i>	0.6014949	0.1089276	3.35E-08	1.16E-05	<i>Atp5g2</i>	-1.365511	-0.20493	2.68E-11	1.13E-07
<i>Ech1</i>	-0.65255	-0.090052	4.28E-13	4.60E-10	<i>Acaca</i>	-0.867851	-0.206268	2.58E-05	0.0062743
<i>Idh2</i>	-0.669761	-0.091291	2.19E-13	2.65E-10	<i>Dbn2d2</i>	-0.93302	-0.222002	2.64E-05	0.0062743
<i>Acaa2</i>	-0.722474	-0.09504	2.92E-14	4.03E-11	<i>Insig1</i>	-1.129597	-0.238898	2.26E-06	0.0012114
<i>Ldhd</i>	-0.68086	-0.097417	2.77E-12	2.67E-09	<i>Lgl2</i>	-1.04756	-0.256912	4.55E-05	0.0096641
<i>Atp2a2</i>	-0.916248	-0.105918	5.12E-18	1.65E-14	<i>Mgst1</i>	-1.319422	-0.262331	4.91E-07	0.0003262
<i>Rpl9</i>	-0.602521	-0.108222	2.58E-08	9.25E-06	<i>Cfd</i>	-1.169841	-0.264566	9.79E-06	0.0033372
<i>Chchd2</i>	-0.677631	-0.108475	4.19E-10	2.53E-07	<i>Cidec</i>	-1.565628	-0.27007	6.75E-09	8.51E-06
<i>Hadhb</i>	-0.585745	-0.108977	7.66E-08	2.31E-05	<i>Agpat2</i>	-1.72734	-0.280172	7.04E-10	1.48E-06
<i>Myf3</i>	-0.937661	-0.109681	1.24E-17	3.00E-14	<i>Cyp2e1</i>	-1.200394	-0.284709	2.48E-05	0.0062743
<i>Tnnc1</i>	-0.594927	-0.116808	3.52E-07	8.10E-05	<i>Plin1</i>	-1.324554	-0.31163	2.13E-05	0.0057253
<i>Rps27a</i>	-0.772483	-0.117212	4.39E-11	3.26E-08	<i>Rps15a-ps5</i>	-1.762974	-0.326341	6.58E-08	5.84E-05
<i>Fabp3</i>	-0.918917	-0.120771	2.77E-14	4.03E-11	<i>Ankrd1</i>	-1.551984	-0.336777	4.06E-06	0.0017063
<i>Slc16a1</i>	-0.596167	-0.121991	1.02E-06	0.0001799	<i>Slc7a10</i>	-1.384167	-0.337066	4.02E-05	0.0089603
<i>Uqcrfs1</i>	-0.59029	-0.126506	3.07E-06	0.0004564	<i>Retn</i>	-1.623341	-0.343591	2.31E-06	0.0012114
<i>Rps20</i>	-0.608527	-0.129411	2.57E-06	0.0003884	<i>Slc25a1</i>	-1.486232	-0.35483	2.81E-05	0.0065555
<i>Ugp2</i>	-0.633086	-0.133028	1.95E-06	0.0003133	<i>Cebpa</i>	-1.551165	-0.358888	1.55E-05	0.0047817
<i>Rps8</i>	-0.768613	-0.135459	1.39E-08	5.18E-06	<i>Nat8l</i>	-2.376416	-0.52847	6.90E-06	0.002521
<i>Actn2</i>	-0.608757	-0.135933	7.52E-06	0.0009085	<i>Arx2</i>	-2.642494	-0.531556	6.65E-07	0.0003995

<i>Eif1</i>	-0.588172	-0.136217	1.58E-05	0.0016545
<i>Rps15a</i>	-0.71576	-0.138147	2.21E-07	5.61E-05
<i>Tnni1</i>	-0.82943	-0.140094	3.21E-09	1.63E-06
<i>Myl2</i>	-1.371081	-0.141912	4.40E-22	2.12E-18
<i>Nipa2</i>	-0.675467	-0.143345	2.45E-06	0.0003759
<i>Prdx2</i>	-0.711187	-0.143792	7.58E-07	0.0001498
<i>Cryab</i>	-0.823312	-0.144123	1.11E-08	4.30E-06
<i>Pla2g12a</i>	-0.588127	-0.147176	6.44E-05	0.0051451
<i>Rps6</i>	-0.642959	-0.148693	1.53E-05	0.0016265
<i>Myoz2</i>	-0.802693	-0.148798	6.87E-08	2.14E-05
<i>Hspa1l</i>	-0.687876	-0.152593	6.55E-06	0.0008216
<i>Rpl37a</i>	-0.678601	-0.153338	9.62E-06	0.0011067
<i>Atp5l</i>	-1.055952	-0.153602	6.22E-12	5.46E-09
<i>Fndc5</i>	-0.664844	-0.154587	1.70E-05	0.0017685
<i>Zfp503</i>	-0.608657	-0.158268	0.0001202	0.0079654
<i>Med28</i>	-0.628229	-0.160181	8.78E-05	0.0063805
<i>Nudt7</i>	-0.65168	-0.163084	6.44E-05	0.0051451
<i>Armcx4</i>	-0.66341	-0.165817	6.31E-05	0.0051252
<i>Cyfp2</i>	-1.064797	-0.168055	2.36E-10	1.52E-07
<i>Hspb7</i>	-0.994988	-0.171608	6.71E-09	2.95E-06
<i>Myl12a</i>	-0.82019	-0.172608	2.02E-06	0.0003195
<i>Pgk1</i>	-0.817645	-0.175736	3.28E-06	0.0004749
<i>Homer2</i>	-0.673125	-0.176269	0.0001341	0.0085841
<i>Abhd18</i>	-0.683709	-0.178011	0.0001226	0.008059
<i>Gm5421</i>	-0.718665	-0.179367	6.16E-05	0.0050546
<i>Myom3</i>	-0.926656	-0.180608	2.89E-07	6.95E-05
<i>Dusp7</i>	-0.908798	-0.182102	6.02E-07	0.0001292
<i>Gm10053</i>	-0.712211	-0.183333	0.0001024	0.0070691
<i>Slc25a34</i>	-0.723514	-0.188638	0.0001253	0.0080736
<i>Rpl24</i>	-0.945604	-0.195794	1.37E-06	0.0002241
<i>Hist1h2be</i>	-0.941778	-0.199097	2.24E-06	0.0003495
<i>Pcp4l1</i>	-1.064497	-0.203383	1.66E-07	4.45E-05
<i>Tcta</i>	-0.833162	-0.20554	5.04E-05	0.0043526
<i>Dusp18</i>	-1.372959	-0.206339	2.85E-11	2.30E-08
<i>Gm3272</i>	-0.965299	-0.211324	4.93E-06	0.0006522
<i>H2afz</i>	-1.05661	-0.213319	7.30E-07	0.0001498
<i>Rps13</i>	-1.291216	-0.217204	2.77E-09	1.50E-06
<i>Zdhhc23</i>	-1.203908	-0.2217	5.62E-08	1.81E-05
<i>Rpl22l1</i>	-0.936644	-0.226155	3.45E-05	0.003144
<i>Rpl27</i>	-0.950244	-0.233182	4.60E-05	0.0040037
<i>Nipal1</i>	-1.14618	-0.288314	7.02E-05	0.0054738
<i>Bdh1</i>	-2.11327	-0.355585	2.80E-09	1.50E-06
<i>Atp5g2</i>	-1.861167	-0.356201	1.74E-07	4.55E-05

Supplemental Table 4. 86 and 43 genes were significantly differentially expressed across spaceflight in the gastrocnemius and quadriceps, respectively. Reported here are the gene symbol, log2FC, p value, and FDR.

<i>Gastrocnemius</i>					
Gene	Event Type	Δ PSI	PValue	FDR	Coorindates
<i>Abcc9</i>	SE	0.156655	0.0017505	0.0426415	chr6:142593966-142594142 - 142590503 142594708
<i>Akap9</i>	SE	-0.240186	6.11E-05	0.0042347	chr5:4044798-4044978 + 4044070 4045897
<i>Ank2</i>	SE	-0.144887	0.0003202	0.0139277	chr3:126926802-126926894 - 126923971 126928664
<i>Azi2</i>	SE	-0.216479	1.60E-05	0.0015024	chr9:118049896-118049996 + 118049435 118051353
<i>Bag6</i>	SE	0.176141	0.0007414	0.0237629	chr17:35142497-35142605 + 35142384 35142726
<i>Bin1</i>	SE	0.1897148	6.03E-05	0.0042347	chr18:32426223-32426352 + 32424967 32431982
<i>Bin1</i>	MXE	0.133	0.0004084	0.0059358	chr18:32426223-32426352:32431670-32431742 + 32424967 32431982
<i>Btrc</i>	MXE	0.156	0.0002645	0.004324	chr19:45456532-45456610:45471014-45471104 + 45423240 45502931
<i>Chd2</i>	SE	-0.116613	0.0002847	0.0128427	chr7:73507805-73507913 - 73505128 73514089
<i>Hexa</i>	SE	0.1091618	0.0006044	0.0211212	chr9:59562295-59562368 + 59562026 59562866
<i>Ktn1</i>	SE	-0.114317	0.0008391	0.0255509	chr14:47734154-47734264 + 47733322 47736099
<i>Mbnl1</i>	SE	0.188131	3.74E-05	0.003033	chr3:60621422-60621458 + 60615773 60622145
<i>Mef2c</i>	SE	0.0706213	0.0003009	0.0133257	chr13:83625264-83625408 + 83592981 83633054
<i>Mtdh</i>	SE	0.0863858	0.0016258	0.0408887	chr15:34116310-34116376 + 34114975 34117879
<i>Mycbp2</i>	SE	0.1352985	0.0009825	0.0281576	chr14:103269357-103269465 - 103262821 103275788
<i>Nbr1</i>	SE	-0.094603	5.60E-05	0.0042135	chr11:101577931-101578037 + 101576409 101580584
<i>Nbr1</i>	SE	-0.186696	2.22E-05	0.0019324	chr11:101577193-101577251 + 101576409 101580584
<i>Neb</i>	SE	-0.071405	0.0004433	0.0171424	chr2:52163936-52164050 - 52163009 52164591
<i>Neb</i>	SE	-0.08903	0.0016449	0.0408887	chr2:52165166-52165277 - 52164702 52167559
<i>Neb</i>	SE	-0.093407	5.71E-05	0.0042135	chr2:52153055-52153148 - 52151456 52159476
<i>Neb</i>	SE	-0.119917	1.30E-08	3.17E-06	chr2:52161370-52161475 - 52160748 52162067
<i>Neb</i>	SE	-0.146734	1.52E-05	0.0014795	chr2:52150966-52151059 - 52149697 52151363
<i>Neb</i>	SE	-0.191755	1.33E-05	0.0013988	chr2:52185270-52185375 - 52183836 52186011
<i>Neb</i>	SE	-0.22217	0.0002768	0.0127221	chr2:52153682-52153775 - 52153148 52157831
<i>Neb</i>	SE	-0.352551	1.55E-08	3.44E-06	chr2:52153682-52153775 - 52153148 52157290
<i>Neb</i>	MXE	0.207	3.10E-08	1.07E-06	chr2:52153682-52153775:52157290-52157383 - 52153148 52157831
<i>Neb</i>	MXE	0.199	1.33E-06	3.79E-05	chr2:52156354-52156447:52157831-52157924 - 52154662 52159476
<i>Neb</i>	MXE	0.144	0.0003272	0.0049763	chr2:52156354-52156447:52157290-52157383 - 52154662 52157831
<i>Neb</i>	MXE	0.086	4.22E-05	0.0009194	chr2:52292800-52292905:52293627-52293726 - 52292720 52295290
<i>Neb</i>	MXE	0.077	8.44E-05	0.0015763	chr2:52151363-52151456:52153055-52153148 - 52151059 52157290
<i>Neb</i>	MXE	0.066	2.20E-11	1.20E-09	chr2:52188682-52188787:52189598-52189703 - 52187515 52189853
<i>Neb</i>	MXE	0.064	2.43E-11	1.22E-09	chr2:52185270-52185375:52186011-52186116 - 52183836 52187410
<i>Neb</i>	MXE	-0.107	0.0001582	0.0027967	chr2:52150536-52150629:52153682-52153775 - 52149697 52154569

<i>Neb</i>	MXE	-0.115	8.46E-06	0.0002128	chr2:52150536-52150629:52150966-52151059 - 52149697 52151363
<i>Neb</i>	MXE	-0.132	1.52E-08	5.52E-07	chr2:52160655-52160748:52161370-52161475 - 52149697 52162067
<i>Neb</i>	MXE	-0.167	5.14E-12	3.36E-10	chr2:52151363-52151456:52154569-52154662 - 52150629 52156354
<i>Obscn</i>	SE	0.1203594	1.13E-05	0.0012459	chr11:59015435-59015478 - 59010536 59020456
<i>Obscn</i>	SE	0.1036586	0.001823	0.0437493	chr11:59062030-59062294 - 59061687 59062646
<i>Pabpc4</i>	SE	-0.098167	0.0003916	0.0156372	chr4:123295749-123295836 + 123295321 123295929
<i>Pdlim7</i>	SE	0.0723805	0.0009993	0.0283044	chr13:55508207-55508326 - 55507880 55508779
<i>Pdlim7</i>	MXE	0.09	3.54E-06	9.26E-05	chr13:55507863-55507880:55508207-55508326 - 55507624 55508779
<i>Phka1</i>	SE	0.1516006	0.0003639	0.0147806	chrX:102557095-102557272 - 102555617 102559564
<i>Ppp6r2</i>	SE	-0.111111	0.0005987	0.0211212	chr15:89246257-89246340 + 89243459 89253018
<i>Pxl2c</i>	SE	-0.302741	9.43E-05	0.0057442	chr13:64309092-64309146 - 64308927 64312266
<i>Rbfox1</i>	SE	0.0824947	0.0015641	0.0406894	chr16:7363864-7363904 + 7353064 7369800
<i>Ryr1</i>	SE	0.0870378	2.20E-06	0.0003156	chr7:29056233-29056248 - 29055059 29056923
<i>Ski</i>	SE	-0.184883	0.0002525	0.0121937	chr4:155160833-155160956 - 155160695 155221551
<i>Sorbs1</i>	SE	-0.347899	0.0001788	0.0096811	chr19:40340044-40340146 - 40337035 40349898
<i>Sorbs1</i>	SE	-0.354875	8.58E-05	0.0053889	chr19:40340684-40340750 - 40337035 40344355
<i>Sorbs1</i>	SE	-0.384114	5.01E-05	0.0039378	chr19:40340044-40340146 - 40337035 40344355
<i>Tacc2</i>	SE	-0.077082	0.0002584	0.0121937	chr7:130663015-130663141 + 130627499 130674601
<i>Tbc1d1</i>	SE	0.1299651	0.0018498	0.0437493	chr5:64283966-64284125 + 64282045 64285944
<i>Tnnt3</i>	SE	-0.052947	9.45E-06	0.0011506	chr7:142507749-142507788 + 142504065 142508334
<i>Tnnt3</i>	SE	-0.063194	6.26E-05	0.0042347	chr7:142514325-142514366 + 142513705 142515775
<i>Tnnt3</i>	SE	-0.083328	0.0004092	0.0160758	chr7:142504249-142504264 + 142504065 142508334
<i>Tnnt3</i>	SE	-0.099384	0.0001096	0.0062105	chr7:142505287-142505299 + 142502757 142505515
<i>Tnnt3</i>	SE	-0.118145	3.84E-08	7.79E-06	chr7:142505515-142505530 + 142504065 142508334
<i>Tnnt3</i>	SE	-0.12201	8.02E-06	0.0010375	chr7:142505287-142505299 + 142504065 142508334
<i>Tnnt3</i>	MXE	0.115	4.77E-05	0.0010069	chr7:142504047-142504065:142505515- 142505530 + 142502757 142508334
<i>Tnnt3</i>	MXE	0.103	0.0003129	0.0048725	chr7:142505287-142505299:142505515- 142505530 + 142502757 142508334
<i>Tnnt3</i>	MXE	-0.088	1.93E-11	1.15E-09	chr7:142511464-142511542:142512035- 142512149 + 142511373 142512255
<i>Ttn</i>	SE	0.2640386	2.13E-12	8.65E-10	chr2:76827482-76827560 - 76827078 76831787
<i>Ttn</i>	SE	0.1423995	1.94E-06	0.0002952	chr2:76857396-76857474 - 76852137 76857903
<i>Ttn</i>	SE	0.0785962	0.0009573	0.027761	chr2:76901840-76902122 - 76894423 76903062
<i>Ttn</i>	SE	0.0537143	0	0	chr2:76828318-76828402 - 76827078 76831787
<i>Ttn</i>	SE	-0.06525	0.0006838	0.0224722	chr2:76839404-76839482 - 76831874 76840232
<i>Ttn</i>	SE	-0.070643	1.29E-09	3.93E-07	chr2:76705669-76705972 - 76705329 76706439
<i>Ttn</i>	SE	-0.082573	8.05E-05	0.005303	chr2:76838568-76838643 - 76831874 76838811
<i>Ttn</i>	SE	-0.089494	0.0002532	0.0121937	chr2:76854386-76854467 - 76852137 76857396
<i>Ttn</i>	SE	-0.093934	2.50E-14	1.55E-11	chr2:76818735-76818816 - 76817863 76821115
<i>Ttn</i>	SE	-0.106261	2.31E-10	8.06E-08	chr2:76818735-76818816 - 76817075 76821115
<i>Ttn</i>	SE	-0.10804	0.0007561	0.0238606	chr2:76842770-76842848 - 76831874 76843375

<i>Ttn</i>	SE	-0.111524	0.000777	0.0239581	chr2:76844204-76844279 - 76831874 76852833
<i>Ttn</i>	MXE	0.308	0	0	chr2:76831787-76831874:76850305-76850380 - 76827078 76850569
<i>Ttn</i>	MXE	0.259	0	0	chr2:76831787-76831874:76854149-76854218 - 76827078 76854386
<i>Ttn</i>	MXE	0.225	0	0	chr2:76831787-76831874:76840232-76840313 - 76827078 76841089
<i>Ttn</i>	MXE	0.21	0	0	chr2:76831787-76831874:76847724-76847808 - 76827078 76850569
<i>Ttn</i>	MXE	0.207	6.73E-09	2.59E-07	chr2:76831787-76831874:76847387-76847471 - 76827078 76847724
<i>Ttn</i>	MXE	0.201	0	0	chr2:76831787-76831874:76853850-76853928 - 76827078 76854149
<i>Ttn</i>	MXE	0.148	1.01E-07	3.32E-06	chr2:76831787-76831874:76847112-76847196 - 76827078 76847387
<i>Ttn</i>	MXE	0.142	1.66E-12	1.21E-10	chr2:76856802-76856904:76857154-76857229 - 76856366 76857396
<i>Ttn</i>	MXE	0.139	3.43E-10	1.60E-08	chr2:76849256-76849334:76850305-76850380 - 76849071 76850569
<i>Ttn</i>	MXE	0.126	2.79E-09	1.14E-07	chr2:76848558-76848642:76850305-76850380 - 76848356 76850569
<i>Ttn</i>	MXE	0.106	2.04E-06	5.56E-05	chr2:76848558-76848642:76857154-76857229 - 76848356 76857396
<i>Ttn</i>	MXE	0.106	9.38E-10	4.09E-08	chr2:76857154-76857229:76857396-76857474 - 76856904 76857609
<i>Ttn</i>	MXE	0.098	3.87E-05	0.0008736	chr2:76847724-76847808:76850305-76850380 - 76847471 76850569
<i>Ttn</i>	MXE	0.092	0.0001903	0.0031917	chr2:76849994-76850087:76850305-76850380 - 76849334 76850569
<i>Ttn</i>	MXE	0.075	0.000577	0.0082033	chr2:76852833-76852917:76857154-76857229 - 76852137 76857396
<i>Ttn</i>	MXE	0.073	1.27E-05	0.0003067	chr2:76847724-76847808:76850569-76850644 - 76847471 76851554
<i>Ttn</i>	MXE	0.065	0.0001079	0.0019608	chr2:76848558-76848642:76853850-76853928 - 76848356 76854149
<i>Ttn</i>	MXE	0.063	1.65E-12	1.21E-10	chr2:76848816-76849071:76850305-76850380 - 76848642 76850569
<i>Ttn</i>	MXE	0.063	0.0001903	0.0031917	chr2:76849256-76849334:76849994-76850087 - 76849071 76850305
<i>Ttn</i>	MXE	-0.064	1.66E-05	0.0003884	chr2:76856264-76856366:76856802-76856904 - 76854467 76857154
<i>Ttn</i>	MXE	-0.082	7.71E-05	0.0014825	chr2:76817788-76817863:76821115-76821184 - 76817075 76822450
<i>Ttn</i>	MXE	-0.088	5.28E-05	0.001079	chr2:76841533-76841626:76847387-76847471 - 76841389 76847724
<i>Ttn</i>	MXE	-0.101	8.87E-14	8.29E-12	chr2:76817788-76817863:76818735-76818816 - 76817075 76821115

<i>Ttn</i>	MXE	-0.219	0	0	chr2:76826074-76826152:76826994-76827078 - 76822564 76831787
<i>Usp47</i>	MXE	0.102	0.0003653	0.0054299	chr7:112080171-112080231:112082429- 112082561 + 112077959 112083087
<i>Xirp2</i>	MXE	-0.121	8.75E-07	2.72E-05	chr2:67507908-67517173:67519219- 67519353 + 67507180 67524771

Supplemental Table 5. 99 statistically significant alternative splicing events were identified across spaceflight in the gastrocnemius, representing 32 alternatively spliced genes, respectively. Reported here are the gene symbol, event type, delta PSI, p value, and FDR.

Quadriceps					
Gene	Event Type	ΔPSI	PValue	FDR	Coorindates
43895	SE	0.1001325	0.0001339	0.0037934	chr19:37217182-37217313 + 37210873 37220302
Aamdc	SE	-0.20277	0.0001212	0.0034743	chr7:97558130-97558226 - 97550741 97565150
Add1	SE	-0.332069	1.15E-05	0.000611	chr5:34620831-34620918 + 34620134 34625278
Ank2	SE	0.0932034	0.0015663	0.0214353	chr3:126926802-126926894 - 126923971 126928664
Ankrd11	SE	-0.207885	0.0018744	0.0245097	chr8:122900601-122900698 - 122900149 122908667
Ap3b1	MXE	0.241	0.0003918	0.0065605	chr13:94501917-94502024:94528164-94528396 + 94493741 94531981
Aplp2	SE	-0.219005	3.36E-06	0.0002478	chr9:31167560-31167728 - 31164673 31168707
Art3	SE	0.0687899	9.12E-06	0.0005419	chr5:92405942-92405979 + 92404866 92409269
Art3	SE	0.0606602	0.0002472	0.0060829	chr5:92405943-92405979 + 92404866 92409269
Cacna2d1	SE	-0.072795	0.0024543	0.0286059	chr5:16341989-16342010 + 16340783 16342682
Camk2g	SE	0.0967742	0.0014488	0.0207278	chr14:20764860-20764955 - 20764268 20765395
Capn3	SE	0.0775825	0.0005289	0.0102248	chr2:120501210-120501324 + 120498985 120502102
Cct4	MXE	-0.264	0.0002768	0.005364	chr11:22994297-22994387:22995929-22996038 + 22993319 22996314
Cdk11b	SE	0.1818182	0.0003939	0.0084176	chr4:155634088-155634225 + 155630332 155638519
Clasp1	SE	0.1933501	3.58E-05	0.0014065	chr1:118512174-118512222 + 118510376 118521826
Clip1	SE	-0.099705	0.0003826	0.0083219	chr5:123581591-123581597 - 123580124 123582719
Cluh	SE	-0.119396	0.0023525	0.0278791	chr11:74665692-74665777 + 74665426 74665922
Crim1	SE	-0.143041	0.0012891	0.01975	chr17:78302986-78303108 + 78281366 78313123
Cyld	SE	-0.082737	0.0011642	0.01818	chr8:88718289-88718298 + 88709996 88719297
D5Ertd579e	SE	0.0516864	0.0049821	0.0468582	chr5:36604510-36604608 - 36602803 36607937
Dcaf6	SE	0.1139712	0.0002503	0.006097	chr1:165374061-165374235 - 165367505 165388657
Dctn2	SE	-0.098361	0.0006157	0.0113624	chr10:127277254-127277320 + 127276849 127277486
Dkk3	SE	0.0558092	0.0034104	0.036688	chr7:112148960-112149044 - 112121682 112150610
Dnm1l	SE	0.0666186	0.0039128	0.0407337	chr16:16332000-16332132 - 16330009 16333828
Dnm2	MXE	0.163	0.0005295	0.0080412	chr9:21478898-21479037:21480406-21480545 + 21478348 21481332
Dpf2	SE	0.1120724	0.0027459	0.0310362	chr19:5903730-5903772 - 5902768 5904239
Dysf	SE	-0.080056	0.0031253	0.0346737	chr6:84064470-84064563 + 84063435 84064875
Eif4g3	SE	-0.062229	0.0036373	0.0386918	chr4:138103314-138103412 + 138097359 138105313
Eif4g3	SE	-0.170042	0.0030375	0.0339416	chr4:138120345-138120454 + 138105436 138125809
Eif4g3	SE	-0.190956	0.0025403	0.0293272	chr4:138120348-138120454 + 138105436 138125809
Eif4g3	SE	-0.357571	0.0004492	0.0091946	chr4:138087532-138087565 + 138082988 138096751
Eif4h	SE	-0.194364	2.38E-09	1.94E-06	chr5:134624440-134624500 - 134622086 134625365
Epn1	SE	0.1034345	2.18E-05	0.000967	chr7:5093311-5093386 + 5092965 5093898
Erc1	SE	0.1420103	3.91E-06	0.0002574	chr6:119734080-119734092 - 119722308 119743287
Fez2	SE	-0.259848	0.0049161	0.04682	chr17:78386990-78387071 - 78384819 78400629
Fnip1	SE	-0.194084	5.74E-05	0.0019416	chr11:54487711-54487795 + 54482586 54489268
Gapvd1	SE	0.2097948	0.005158	0.04753	chr2:34722937-34723018 - 34720728 34725042
Gfm2	SE	0.1714286	0.00036	0.0079005	chr13:97160086-97160167 + 97155600 97161018
Hnrnpd	SE	-0.196543	0.0001472	0.0040697	chr5:99963730-99963877 - 99962612 99963983
Huwe1	SE	0.1323714	0.0013209	0.0199852	chrX:151859454-151859688 + 151857913 151860166

<i>Irak1</i>	SE	-0.116324	0.0011586	0.01818	chrX:74021848-74021963 - 74021061 74022184
<i>Kdm3b</i>	SE	0.2127018	3.82E-06	0.0002574	chr18:34803449-34804049 + 34799359 34808240
<i>Klf1b</i>	SE	0.0906904	0.0022399	0.0270502	chr4:149220541-149220790 - 149215025 149222226
<i>Limch1</i>	SE	-0.113189	0.0032303	0.0352866	chr5:66975558-66975819 + 66974662 66993142
<i>Limd1</i>	SE	0.1626016	0.001309	0.0199289	chr9:123500130-123500232 + 123480621 123510507
<i>Lrrfip1</i>	SE	-0.079205	0.0001197	0.0034712	chr1:91110354-91110447 + 91108672 91112155
<i>Lsm14a</i>	SE	-0.096018	0.0022992	0.0274551	chr7:34357765-34357942 - 34357496 34365614
<i>Mapk12</i>	SE	0.1193465	3.44E-05	0.0013748	chr15:89135122-89135170 - 89134726 89135426
<i>Mbnl1</i>	SE	-0.132852	9.05E-06	0.0005419	chr3:60528755-60529804 + 60501423 60595593
<i>Mbnl1</i>	SE	-0.467391	0.0007891	0.0137367	chr3:60528755-60528978 + 60501423 60595593
<i>Micu1</i>	SE	-0.113555	0.003182	0.0350735	chr10:59756705-59756717 + 59750547 59768137
<i>Mlip</i>	SE	0.1263441	4.70E-05	0.0017101	chr9:77190323-77190383 - 77181396 77190671
<i>Mlip</i>	SE	-0.108333	0.001514	0.0209795	chr9:77229484-77230954 - 77217048 77239391
<i>Mlip</i>	SE	-0.125874	0.00092	0.0154557	chr9:77229484-77231029 - 77217048 77239391
<i>Mllt10</i>	SE	-0.166667	0.0041487	0.041761	chr2:18109781-18109891 + 18071204 18122244
<i>Mllt3</i>	SE	0.0520503	0.0049755	0.0468582	chr4:87782286-87782358 - 87774146 87783648
<i>Mmadhc</i>	SE	-0.094397	6.92E-05	0.0022188	chr2:50280572-50280658 - 50280402 50281102
<i>Mob4</i>	SE	0.1494253	0.0004832	0.0096491	chr1:55148318-55148361 + 55145224 55148547
<i>Msi2</i>	SE	0.0648935	0.0051438	0.04753	chr11:88347052-88347125 - 88344660 88366784
<i>Mybpc1</i>	SE	-0.098199	9.01E-05	0.0027088	chr10:88582006-88582048 - 88573529 88585659
<i>Myo18b</i>	SE	-0.068727	1.56E-05	0.0007624	chr5:112716986-112717031 - 112715518 112723806
<i>Nde1</i>	SE	0.185641	0.0009938	0.0163571	chr16:14175290-14175439 + 14170491 14183475
<i>Ndufaf1</i>	SE	-0.101571	0.0025595	0.0294104	chr2:119658273-119658459 - 119656442 119660001
<i>Neb</i>	SE	0.2404687	0.001051	0.0170684	chr2:52156354-52156447 - 52153148 52157290
<i>Neb</i>	SE	-0.058353	0.0034022	0.036688	chr2:52157831-52157924 - 52149697 52159476
<i>Neb</i>	SE	-0.075052	0.000434	0.0089834	chr2:52163936-52164050 - 52163009 52164591
<i>Neb</i>	SE	-0.078293	0.0037627	0.039812	chr2:52161370-52161475 - 52160748 52162067
<i>Neb</i>	SE	-0.082203	2.01E-05	0.0009048	chr2:52165166-52165277 - 52164702 52167559
<i>Neb</i>	SE	-0.129247	6.01E-11	1.46E-07	chr2:52153055-52153148 - 52151456 52159476
<i>Neb</i>	SE	-0.241795	2.46E-07	4.48E-05	chr2:52185270-52185375 - 52183836 52186011
<i>Neb</i>	MXE	0.375	3.82E-08	4.16E-06	chr2:52154569-52154662:52156354-52156447 - 52153775 52157290
<i>Neb</i>	MXE	0.355	3.14E-13	1.03E-10	chr2:52153055-52153148:52156354-52156447 - 52151456 52157290
<i>Neb</i>	MXE	0.3	4.80E-11	1.04E-08	chr2:52153682-52153775:52156354-52156447 - 52153148 52157290
<i>Neb</i>	MXE	0.18	0.0002793	0.005364	chr2:52153055-52153148:52156354-52156447 - 52149697 52157290
<i>Neb</i>	MXE	0.166	0.0005229	0.0080412	chr2:52150536-52150629:52156354-52156447 - 52149697 52157290
<i>Neb</i>	MXE	0.105	3.08E-09	5.03E-07	chr2:52245280-52245388:52251706-52251814 - 52244153 52255531
<i>Neb</i>	MXE	0.081	6.55E-15	4.28E-12	chr2:52185270-52185375:52186011-52186116 - 52183836 52187410
<i>Neb</i>	MXE	-0.105	0.000188	0.0042603	chr2:52151363-52151456:52154569-52154662 - 52150629 52156354

<i>Nrd1</i>	SE	-0.052759	0.0042161	0.0419198	chr4:109047596-109047716 + 109046681 109049066
<i>Nrip1</i>	SE	-0.206439	0.0040669	0.041226	chr16:76330745-76330864 - 76295015 76352471
<i>Obscn</i>	SE	0.0838678	0.0015158	0.0209795	chr11:59062646-59062910 - 59062294 59064186
<i>Obscn</i>	SE	0.0687418	0.0051842	0.04753	chr11:59054161-59054425 - 59053837 59067065
<i>Obscn</i>	SE	0.0599842	0.0005515	0.0104145	chr11:59064186-59064450 - 59053837 59067065
<i>Pbrm1</i>	SE	-0.217925	0.0045251	0.0440927	chr14:31045329-31045425 + 31044709 31046331
<i>Pcgf3</i>	SE	-0.068312	0.0054099	0.0488093	chr5:108486139-108486247 + 108478358 108487835
<i>Pcmt1</i>	SE	-0.073179	0.0002718	0.0064282	chr10:7648466-7648660 - 7647621 7649050
<i>Pecam1</i>	SE	-0.10069	0.0018916	0.0245097	chr11:106681031-106681085 - 106679611 106682879
<i>Phactr4</i>	SE	-0.110002	0.005046	0.0472775	chr4:132383281-132383362 - 132378563 132386891
<i>Pibf1</i>	SE	0.2651852	1.95E-07	4.16E-05	chr14:99105168-99105269 + 99101301 99107298
<i>Preb</i>	SE	-0.191393	0.0019278	0.0247167	chr5:30955929-30956089 - 30955737 30956261
<i>Preb</i>	SE	-0.209668	0.0019673	0.0247943	chr5:30955931-30956089 - 30955737 30956261
<i>Rab28</i>	SE	-0.069276	0.0042842	0.0424238	chr5:41626986-41627081 - 41625862 41633172
<i>Rars2</i>	SE	-0.152453	0.0002408	0.0060474	chr4:34625400-34625484 + 34623513 34630472
<i>Rbfox1</i>	SE	0.0901038	0.0003458	0.0077287	chr16:7363864-7363904 + 7353064 7369800
<i>Rexo4</i>	SE	-0.147373	0.0005094	0.0100077	chr2:26960223-26960417 - 26958567 26960602
<i>Rorc</i>	SE	0.0925926	0.0043039	0.0424469	chr3:94391644-94391755 + 94391487 94393030
<i>Rps24</i>	SE	-0.066426	0.0016098	0.0219074	chr14:24494051-24494069 + 24493493 24495429
<i>Rsu1</i>	SE	-0.112713	0.0040664	0.041226	chr2:13219008-13219127 - 13217271 13224304
<i>Ryr1</i>	SE	0.0920597	6.97E-06	0.000447	chr7:29056233-29056248 - 29055059 29056923
<i>Scarb1</i>	SE	-0.206349	3.84E-06	0.0002574	chr5:125281104-125281233 - 125277865 125283898
<i>Sei1l</i>	SE	0.1136842	0.0032095	0.0352175	chr12:91833054-91833222 - 91831674 91841637
<i>Sh2b1</i>	SE	0.3195352	5.76E-07	6.53E-05	chr7:126467949-126468049 - 126467773 126468419
<i>Sh3pxd2a</i>	SE	0.2054079	1.01E-05	0.0005737	chr19:47320275-47320320 - 47314166 47343439
<i>Slc12a2</i>	SE	-0.126639	0.0011391	0.01818	chr18:57934950-57934998 + 57932605 57936307
<i>Slc4a1ap</i>	MXE	-0.259	4.85E-05	0.0015843	chr5:31540385-31540497:31543669-31543913 + 31536324 31546179
<i>Slc9a8</i>	MXE	0.423	0.0006248	0.0092724	chr2:167445505-167445607:167451206-167451350 + 167443532 167457326
<i>Smarca4</i>	SE	0.0796417	0.0003333	0.0075881	chr9:21677952-21678051 + 21677677 21678315
<i>Speg</i>	SE	0.1416807	0.0005273	0.0102248	chr1:75384038-75384068 + 75375833 75384655
<i>Speg</i>	SE	0.1329991	0.0002989	0.0069342	chr1:75384033-75384068 + 75375833 75384655
<i>Stxbp3</i>	SE	-0.135169	0.00041	0.0086846	chr3:108809876-108810001 - 108805333 108812877
<i>Tars2</i>	MXE	0.185	3.94E-05	0.0013959	chr3:95750866-95750984:95751505-95751630 - 95750765 95753006
<i>Thoc7</i>	SE	0.0824593	0.0009429	0.0156259	chr14:13950298-13950365 - 13950201 13951205
<i>Tmem56</i>	SE	0.0915068	0.0007771	0.0137181	chr3:121211297-121211371 - 121207470 121228309
<i>Tnnt1</i>	SE	0.0965447	0.0021284	0.0261854	chr7:4513580-4513613 - 4512283 4513810
<i>Tnnt3</i>	SE	0.1473814	4.51E-07	6.46E-05	chr7:142504249-142504264 + 142504065 142505515
<i>Tnnt3</i>	SE	0.0855241	3.79E-05	0.0014444	chr7:142513664-142513705 + 142512622 142514325
<i>Tnnt3</i>	SE	-0.07201	2.19E-06	0.0001979	chr7:142505515-142505530 + 142504065 142508334
<i>Tnnt3</i>	SE	-0.079683	1.71E-06	0.0001664	chr7:142514325-142514366 + 142513705 142515775
<i>Tnnt3</i>	SE	-0.083149	3.30E-07	5.02E-05	chr7:142502739-142502757 + 142501842 142504047
<i>Tnnt3</i>	MXE	0.145	2.57E-06	0.0001862	chr7:142504249-142504264:142505515-142505530 + 142504065 142508334

<i>Tnnt3</i>	MXE	0.091	8.73E-06	0.0004752	chr7:142513664-142513705:142514325-142514366 + 142512622 142515775
<i>Tnnt3</i>	MXE	0.05	1.38E-05	0.0006623	chr7:142504249-142504264:142505515-142505530 + 142504065 142507749
<i>Tnrc6b</i>	SE	-0.069251	0.0014655	0.0207549	chr15:80890105-80890276 + 80889155 80894328
<i>Trappc10</i>	SE	-0.230469	2.76E-07	4.48E-05	chr10:78222663-78222860 - 78220384 78228778
<i>Ttn</i>	SE	0.3177899	0.0005343	0.0102483	chr2:76840232-76840313 - 76832184 76850569
<i>Ttn</i>	SE	0.2253098	7.55E-06	0.0004718	chr2:76832100-76832184 - 76831874 76841089
<i>Ttn</i>	SE	0.2137322	0.0026069	0.0296748	chr2:76838568-76838643 - 76831874 76847112
<i>Ttn</i>	SE	0.2021182	0.0049395	0.04682	chr2:76846625-76846916 - 76831874 76847112
<i>Ttn</i>	SE	0.1919323	6.61E-05	0.0021482	chr2:76832100-76832184 - 76831874 76841767
<i>Ttn</i>	SE	0.1808616	0.0004352	0.0089834	chr2:76828485-76828569 - 76828402 76831787
<i>Ttn</i>	SE	0.1706664	0.0019746	0.0247943	chr2:76827691-76827766 - 76827078 76841089
<i>Ttn</i>	SE	0.1612842	0.0002371	0.0060165	chr2:76838811-76838895 - 76831874 76841767
<i>Ttn</i>	SE	0.1612345	0.0046637	0.0450822	chr2:76832100-76832184 - 76831874 76844204
<i>Ttn</i>	SE	0.1481673	0.001396	0.0206152	chr2:76844204-76844279 - 76831874 76849994
<i>Ttn</i>	SE	0.1412963	0.0014048	0.0206152	chr2:76847387-76847471 - 76827078 76850569
<i>Ttn</i>	SE	0.1278022	2.33E-06	0.0002028	chr2:76838568-76838643 - 76831874 76841767
<i>Ttn</i>	SE	0.1234253	2.30E-05	0.0010025	chr2:76847112-76847196 - 76840313 76847387
<i>Ttn</i>	SE	0.1037963	1.83E-05	0.0008402	chr2:76838811-76838895 - 76831874 76850569
<i>Ttn</i>	SE	0.1018808	0.0038578	0.0403327	chr2:76848558-76848642 - 76847808 76854149
<i>Ttn</i>	SE	0.0916991	5.21E-05	0.0018146	chr2:76852833-76852917 - 76852137 76857903
<i>Ttn</i>	SE	0.0864042	0.0010262	0.0167774	chr2:76852833-76852917 - 76827078 76853604
<i>Ttn</i>	SE	0.0834746	1.06E-08	6.48E-06	chr2:76841089-76841389 - 76840313 76847387
<i>Ttn</i>	SE	0.0823917	0.0012019	0.0186479	chr2:76850305-76850380 - 76831874 76850569
<i>Ttn</i>	SE	0.0705831	4.60E-05	0.0017101	chr2:76846625-76846916 - 76843492 76847112
<i>Ttn</i>	SE	0.0652384	0.0027647	0.0310362	chr2:76850305-76850380 - 76847808 76850569
<i>Ttn</i>	SE	0.0643234	0.0002295	0.0058858	chr2:76832100-76832184 - 76831874 76850569
<i>Ttn</i>	SE	0.0639113	0.0038506	0.0403327	chr2:76849256-76849334 - 76849071 76849994
<i>Ttn</i>	SE	0.0628139	0.0014359	0.0206972	chr2:76843375-76843492 - 76831874 76850569
<i>Ttn</i>	SE	0.0623565	3.03E-05	0.0012509	chr2:76846625-76846916 - 76844279 76847112
<i>Ttn</i>	SE	0.0519173	0.0021067	0.0261035	chr2:76838568-76838643 - 76831874 76838811
<i>Ttn</i>	SE	0.0507641	6.87E-08	2.27E-05	chr2:76850305-76850380 - 76848642 76850569
<i>Ttn</i>	SE	-0.0591	0.0022514	0.0270502	chr2:76831787-76831874 - 76828956 76832100
<i>Ttn</i>	SE	-0.088624	5.31E-07	6.46E-05	chr2:76818735-76818816 - 76817863 76821115
<i>Ttn</i>	SE	-0.107875	0.0002094	0.0055455	chr2:76841767-76841836 - 76831874 76843375
<i>Ttn</i>	SE	-0.123104	1.26E-05	0.0006506	chr2:76836732-76836813 - 76832184 76838568
<i>Ttn</i>	SE	-0.144154	0.0051752	0.04753	chr2:76844204-76844279 - 76831874 76853604
<i>Ttn</i>	SE	-0.195175	1.86E-06	0.0001741	chr2:76847387-76847471 - 76843492 76850569
<i>Ttn</i>	MXE	0.111	0.0004688	0.0074662	chr2:76847387-76847471:76850305-76850380 - 76831874 76850569
<i>Ttn</i>	MXE	0.107	4.71E-09	6.15E-07	chr2:76831787-76831874:76840232-76840313 - 76827078 76841089
<i>Ttn</i>	MXE	0.086	0.0001261	0.0032941	chr2:76848816-76849071:76849256-76849334 - 76848642 76849994

<i>Ttn</i>	MXE	0.076	0.0002555	0.0052144	chr2:76856264-76856366:76857396-76857474 - 76854467 76857609
<i>Ttn</i>	MXE	0.065	0.0001324	0.0033255	chr2:76847724-76847808:76850305-76850380 - 76847471 76850569
<i>Ttn</i>	MXE	0.064	0.0001892	0.0042603	chr2:76856802-76856904:76857154-76857229 - 76856366 76857396
<i>Ttn</i>	MXE	0.062	0.0001261	0.0032941	chr2:76857154-76857229:76857396-76857474 - 76856904 76857609
<i>Ttn</i>	MXE	0.061	0.0001069	0.0030351	chr2:76847724-76847808:76853154-76853238 - 76847471 76853604
<i>Ttn</i>	MXE	-0.06	4.06E-05	0.0013959	chr2:76841533-76841626:76847387-76847471 - 76841389 76847724
<i>Ttn</i>	MXE	-0.071	5.10E-05	0.0015852	chr2:76852056-76852137:76854149-76854218 - 76851764 76854386
<i>Ttn</i>	MXE	-0.074	0.0003148	0.0057108	chr2:76852833-76852917:76857154-76857229 - 76852137 76857396
<i>Ttn</i>	MXE	-0.085	1.87E-05	0.0008141	chr2:76848278-76848356:76854149-76854218 - 76847808 76854386
<i>Ttn</i>	MXE	-0.104	0.0002989	0.0055773	chr2:76846625-76846916:76847112-76847196 - 76846313 76847387
<i>Ttn</i>	MXE	-0.115	8.12E-05	0.002411	chr2:76832100-76832184:76847724-76847808 - 76831874 76854149
<i>Uap1</i>	SE	-0.136399	0.0020577	0.0257051	chr1:170147995-170148046 - 170143463 170149270
<i>Ubc1</i>	SE	-0.074131	0.0001487	0.0040697	chr2:26015954-26016035 - 26014965 26016295
<i>Ube2d-ps</i>	SE	0.0976761	0.0018565	0.0245097	chr11:5782184-5782357 + 5780635 5783891
<i>Ube2g1</i>	SE	0.0654511	0.0004562	0.0092234	chr11:72645536-72645652 + 72607635 72663070
<i>Wnk1</i>	SE	-0.282682	0.0043373	0.0426033	chr6:119953770-119953902 - 119952969 119956070
<i>Wnk1</i>	SE	-0.286403	0.0021669	0.0263928	chr6:119953770-119954232 - 119952969 119956070
<i>Zbtb20</i>	SE	0.2906839	0.0018859	0.0245097	chr16:43193464-43193538 + 43146943 43232758
<i>Zdhc3</i>	SE	-0.079122	0.0006379	0.0116838	chr9:123098922-123098974 - 123089156 123100262
<i>Zfr</i>	MXE	-0.178	0.0002322	0.0049391	chr15:12146148-12146396:12149527- 12149719 + 12140652 12150172
<i>Zfyve9</i>	SE	-0.175388	0.0025192	0.0292228	chr4:108693319-108693496 - 108691791 108695768

Supplemental Table 6. 181 statistically significant alternative splicing events were identified across spaceflight in the quadriceps, representing 103 alternatively spliced genes, respectively. Reported here are the gene symbol, event type, delta PSI, p value, and FDR.