Dietary restriction in ILSXISS mice is associated with widespread changes in splicing regulatory factor expression levels.

Running title: Splicing factor expression in DR mice

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Abstract

Dietary restriction (DR) represents one of the most reproducible interventions to extend lifespan and improve health outcomes in a wide range of species, but substantial variability in DR response has been observed, both between and within species. The mechanisms underlying this variation in effect are still not well characterised. Splicing regulatory factors have been implicated in the pathways linked with DR-induced longevity in *C. elegans* and are associated with lifespan itself in mice and humans.

We used qRT-PCR to measure the expression levels of a panel of 20 age- and lifespan-associated splicing regulatory factors in brain, heart and kidney derived from three recombinant inbred strains of mice with variable lifespan responses to short-term (2 months) or long-term (10 months) 40% DR to determine their relationship to DR-induced longevity.

We identified 3 patterns of association; i) splicing factors associated with DR alone, ii) splicing factors associated with strain alone or iii) splicing factors associated with both DR and strain. Tissue specific variation was noted in response to short term or long-term DR, with the majority of effects noted in brain following long term DR in the positive responder strain TejJ89. Association in heart and kidney were less evident, and occurred following short term DR.

Splicing factors associated with both DR and strain may be mechanistically involved in strain-specific differences in response to DR. We provide here evidence concordant with a role for some splicing factors in the lifespan modulatory effects of DR across different mouse strains and in different tissues.

1. Introduction

Since the lifespan extension effects of dietary restriction (DR) were first reported in the early 1900s (McCay and others 1928; Osborne and others 1917), intensive effort has focused on characterisation of the underlying mechanism(s) in model organisms (Gems and Partridge 2013; Mair and Dillin 2008; Speakman and Mitchell 2011). Several studies have shown the beneficial effects of DR in terms of extended lifespan to be conserved across many species ranging from single-celled organisms to non-human primates (Austad 1989; Kealy and others 2002; Masoro 2005; Mattison and others 2017). To date no lifespan data are available in humans, although there are many opinions as to the potential for DR to affect human lifespan (Cava and Fontana 2013; Ingram and others 2006; Phelan and Rose 2005; Speakman and Hambly 2007; Speakman and Mitchell 2011). Notwithstanding the reported effects on lifespan, there remains clear evidence that DR results in multiple health benefits in many organisms including humans (Cava and Fontana 2013; Heilbronn and others 2006; Larson-Meyer and others 2006; Smith and others 2010). These benefits could contribute to extended 'health span' (the period of life spent free from age-related chronic diseases) in ageing human populations, which is arguably far more relevant from a public health perspective than increasing lifespan alone. However, the exact nature of the mechanism(s) which lead to such benefits remains the subject of discussion. There is therefore a need to elucidate the pathways underlying the actions of DR in order to better understand how it could potentially be used to extend 'health span' in human populations.

When discussing DR as a potential intervention, it must be recognised that the universality of the beneficial effects is far from clear cut. In animal models, lifespan extension results vary with the experimental methodology used; animal husbandry conditions, level of DR imposed, age at initiation of DR and method of introduction of DR may all influence the amount of extension reported (Ingram and de Cabo 2017; Selman and Swindell 2018; Vaughan and others 2017). Genetics is clearly also an important factor to be considered, especially given that studies conducted across different species show highly variable effects, with several reports showing dietary restriction to have no effect, or even a negative effect on lifespan (Mockett and others 2006; Selman and Swindell 2018; Speakman and Mitchell 2011). However, such disparity is not limited to cross-species differences; two studies from 2010 (Liao and others 2010; Rikke and others 2010) tested a large number of ILSXISS recombinant

inbred mouse strains and reported wide variability in lifespan response to 40% DR, both lifespan extension and lifespan reduction were observed in similar numbers of strains in each of these experiments. It is currently unclear as to what caused the variation in response to DR, although a number of reasons have been suggested (Selman and Swindell 2018). However, the simple fact that such variation exists presents valuable opportunities to study the molecular mechanisms involved in differential lifespan response to dietary restriction.

One molecular mechanism with potential to play a role in the DR response is alternative mRNA splicing; components of the machinery that regulates this process have previously been implicated in DR in *C.elegans* (Heintz and others 2017) . Alternative splicing is known to be a contributor to cellular plasticity and is a key element of the homeostatic stress response, both of which are important factors in the ageing process (Kelemen and others 2013; Kourtis and Tavernarakis 2011). Dysregulated splicing is also a major feature of age-related diseases including Alzheimer's disease, Parkinson's disease and several tumour types (Danan-Gotthold and others 2015; Lisowiec and others 2015; Scuderi and others 2014). Regulation of alternative splicing events is complex and multifactorial, however trans-acting splicing factors are necessary to determine the outcome of any particular splicing event (Smith and Valcarcel 2000). The Serine Arginine-rich (SR) family of splicing factors and the heterogeneous nuclear ribonucleoprotein (HNRNP) family of splicing factors usually, but not exclusively, have stimulatory and inhibitory roles respectively in the determination of splice site usage (Cartegni and others 2002). We have previously shown that alternative splicing and splicing factor expression are deregulated during normal human ageing (Harries and others 2011) and that splicing factor expression levels are associated with lifespan in mice and humans (Lee and others 2016). We have also demonstrated changes in splicing factor expression in senescent cells from multiple human tissue types *in vitro* (Holly and others 2013; Latorre and others 2018b) and recently we reported the reversal of several senescent cell phenotypes through moderation of splicing factor expression levels using resveratrol analogues, hydrogen sulfide donors or inhibition of the ERK or AKT signalling pathways in cultured human cells (Latorre and others 2017; Latorre and others 2018a; Latorre and others 2018c).

Given the emerging importance of splicing factors in the ageing phenotype and links to longevity, we hypothesised that their expression may be altered under DR conditions, and may present some insight into the role of alternative splicing in the effects of DR. To explore this, we measured splicing factor transcript expression levels in three recombinant ILSXISS mouse strains with differential responses to short term or long term 40% DR. We identified striking tissue specificity in expression profiles. The expression of some splicing factors was associated with exposure to either short-term or long-term DR, or both, but demonstrated no associations with strain. Others demonstrated strain specific responses but were unrelated to DR status. Some splicing factors however demonstrated interactions between both strain and DR, and may underlie the observed strain specificity in DR response.

2. Methods

2.1. ILSXISS Mice

The mouse strains used in the present study have been extensively described elsewhere (Bennett and others 2002; Liao and others 2010; Mulvey and others 2017; Rikke and others 2010; Williams and others 2004). In brief, the ILSXISS recombinant inbred (RI) mouse strains were originally derived from a cross between inbred long sleep (ILS) and inbred short sleep (ISS) mice. These two strains were developed from an original eight-way cross using heterogeneous stock; A, AKR, BALB/c, C3H/2, C57BL, DBA/2, IsBi and RIII, the offspring of which were subsequently bred for differential ethanol sensitivity, giving the long and short sleep models. Over 20 successive generations of inbreeding of these progenitor strains (ILS X ISS) resulted in more than 75 ILSXISS RI lines, each genetically distinct from each other (Liao and others 2010). These lines have previously been shown to have variable lifespan responses to DR, making them ideal for exploration of the mechanisms underlying DR-induced lifespan extension (Liao and others 2010; Rikke and others 2010).

Mice from three of these strains were chosen for use in the present study, on the basis of replicable responses to 40% DR across two previous independent studies with no significant strain-specific differences in median lifespan under AL conditions (Liao and others 2010; Rikke and others 2010). Only female mice were used in the present study for consistency since one previous study (Rikke and

others 2010) did not include male mice. Lifespan measurements from the Liao study (Liao and others 2010) therefore could not be corroborated for both sexes. Mice were maintained in groups of 4 post-weaning in shoebox cages (48 cm × 15 cm × 13 cm), with AL access to water and standard chow (CRM(P), Research Diets Services, LBS Biotech, UK; Atwater Fuel Energy-protein 22%, carbohydrate 69%, fat 9%) and maintained on a 12L/12D cycle (lights on 0700–1900h) at 22 ± 2 °C.

One of the strains chosen showed an extension of lifespan under life-long 40% DR (TejJ89), one showed a lifespan reduction response to 40% DR (TejJ114) and one exhibited no response to 40% DR (TejJ48) relative to strain-specific *ad libitum* fed controls. There is some debate as to whether these strain responses truly reflect each strain's true potential for lifespan extension or simply that a 40% DR regime is sub-optimal in the cases of TejJ48 and TejJ114 (Selman and Swindell 2018). However for purposes of clarity, the strains will be referred to as positive-, negative- and non-responder strains since these are the responses that have previously been reported under 40% DR (Liao and others 2010; Mulvey and others 2017; Rikke and others 2010). Mice were introduced to DR in a graded fashion; at 10 weeks of age mice were exposed to 10% DR (90% of AL feeding), at 11 weeks this was increased to 20% DR, and from 12 weeks of age until the termination of the experiment mice were exposed to 40% DR, relative to their appropriate strain-specific AL controls. Mice were given either *ad libitum* (AL) feed or short- (2 months) or long-term (10 months) 40% DR, as previously published (Mulvey and others 2017). Brain, heart and kidney tissue samples were collected as part of a previous study, therefore full details of animal husbandry conditions, DR protocols and treatment of dissected tissues have all been previously described in Mulvey *et al* (Mulvey and others 2017). All experiments were carried out under a licence from the UK Home Office (Project Licence 60/4504) and followed the "principles of laboratory animal care" (NIH Publication No.86-23, revised 1985).

2.2. Splicing factor candidate genes for analysis

An *a priori* list of splicing factor candidate genes were chosen based on associations previously seen in multiple human aging cohorts and in senescent primary human cell lines (Harries and others 2011; Holly and others 2013; Latorre and others 2017; Latorre and others 2018b). Some of the splicing factors in this list have also been shown to associate with lifespan in both mice and humans (Lee and others

2016). The list of genes included the negative regulatory splicing factors *Hnrnpa0*, *Hnrnpa1*, *Hnrnpa2b1*, *Hnrnpd*, *Hnrnph3*, *Hnrnpk*, *Hnrnpm*, *Hnrnpul2*, the positive regulatory splicing enhancers *Pnisr*, *Srsf1*, *Srsf2*, *Srsf3*, *Srsf6*, *Tra2b* and the core components of the spliceosome *Sf1* and *Sf3b1*. Expression assays were obtained in single-tube TaqMan® Assays-on-Demand™ format (ThermoFisher, Waltham, MA, USA). Assay Identifiers are given in Supplementary Table S1.

2.3. RNA extraction

Snap-frozen tissues were first treated with RNA*later*™-ICE Frozen Tissue Transition Solution (ThermoFisher, Waltham, MA, USA) according to the manufacturer's instructions, in order to allow handling of the tissue without RNA degradation occurring due to thawing of sample. Tissue sections were then placed in 1 mL TRI Reagent® Solution (ThermoFisher, Waltham, MA, USA) supplemented with the addition of 10mM $MgCl_2$ to aid recovery of microRNAs (Kim and others 2012). Samples were then completely homogenized in a bead mill (Retsch Technology GmbH, Haan, Germany) at a frequency of 30 cycles per second for 15 mins. Phase separation was carried out using chloroform. Total RNA was precipitated from the aqueous phase by means of an overnight incubation at -20°C with isopropanol. 1.2µl Invitrogen™ GlycoBlue™ Coprecipitant (ThermoFisher, Waltham, MA, USA) was added prior to incubation to aid pellet recovery. RNA pellets were then ethanol-washed twice and re-suspended in 1X TE buffer, pH8.0. RNA quality and concentration were assessed by NanoDrop spectrophotometry (NanoDrop, Wilmington, DE, USA).

2.4. Reverse transcription

500ng of total RNA was reverse transcribed using EvoScript Universal cDNA Master kit (Roche LifeScience, Burgess Hill, West Sussex, UK) in 20µl reactions, according to the manufacturer's instructions except for a change to the extension phase of the reaction: a step of 30 minutes at 65°C was used instead of 15 minutes at 65°C. Resulting cDNA was then diluted to a final volume of 80µl with dH_2O to ensure sufficient volume for all subsequent qRT-PCR reactions.

2.5. Quantitative real-time PCR

1.0µl cDNA (reverse transcribed as indicated above) was added to a 5µl qRT-PCR reaction including 2.5µl TaqMan® Universal Master Mix II, no UNG (ThermoFisher, Waltham, MA, USA) and 0.125µl TaqMan® Assays-on-Demand™ probe and primer mix (corresponding to 450nM each primer and 125nM probe). Reactions were run in triplicate on 384-well plates using the QuantStudio 6 Flex Real-Time PCR System (ThermoFisher, Waltham, MA, USA). Amplification conditions were a single cycle of 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. As this study consisted of a collection of 288 samples, three separate plates were required to run all samples with each Taqman® assay. To mitigate the effects of plate-to-plate variation, two approaches were used. Firstly, samples were randomised before being assigned to a plate such that any given plate did not contain all the samples from one strain, tissue or DR condition. Secondly, internal calibrator samples were used: 6 samples were chosen at random from the collection and separate to the main workflow, each sample was reverse-transcribed 3 times and diluted as described above. The 3 resulting cDNA samples were then pooled for each sample, mixed thoroughly and then added as extra samples to each plate. These internal calibrator samples were then used in the downstream analysis to normalise across plates.

2.6. Data preparation

EDS files were uploaded to the ThermoFisher Cloud (ThermoFisher, Waltham, MA, USA) and analysed using the Relative Quantification qPCR App within the software (https://www.thermofisher.com/uk/en/home/cloud.html). This platform was used to manually set Baseline and Threshold for each assay (see Supplementary Table S1 for values) and to ensure there were no apparent outliers before further analysis. One sample was excluded from the TejJ89 dataset at this stage as expression data was missing for >50% of all genes measured. Output was imported into Excel (Microsoft, Redmond, WA, USA) and the C_T values used for analysis using the comparative C_T method. First, raw C_T values were corrected using the internal calibrator samples from each of the three plates. Corrected C_T data from all genes measured, endogenous controls, calculated averages and geometric means of these controls along with calculated ‘global’ averages and geometric means

across all genes measured were then uploaded to the RefFinder webtool (Xie and others 2012) to establish the most stable gene(s). This returned the 'global' geometric mean value across all genes measured as the most stable and thus the most appropriate for the ΔC_T normalisation step. At this point, $\Delta\Delta C_T$ expression calculations were performed for each strain separately; expression for each transcript was calculated relative to the average expression in the *ad-libitum* fed animals, for each tissue individually and separately for long-term and short-term treatments. Following the $\Delta\Delta C_T$ normalisation, the fold-changes were calculated using the $2^{-\Delta\Delta C_T}$ method, followed by an additional normalisation using the geometric mean expression of the non-responder strain (TejJ48) as a baseline.

This final normalisation step was intended to account for any minor changes in splicing factor expression caused by DR, but presumably unrelated to the lifespan-alteration response seen in the positive (TejJ89) and negative (TejJ114) responder strains. The expression profiles of splicing factors in the non-responder strain (TejJ48) under DR conditions are shown in Supplementary Figure S1 and Supplementary Table S2. As can be seen, there are very few significant alterations in expression levels (and none that meet multiple testing criteria), although a certain amount of deviation from zero can be seen. These deviations in expression are likely to be brought about through the imposition of a DR regime, however owing to the lack of response in this strain it is reasonable to assume that they are highly unlikely to be contributory to the responses seen in the other strains. As such, normalisation using these minor deviations should merely remove a certain amount of 'background' from the positive- and negative-responder strain data. As a consequence of this normalisation, the data from TejJ48 were effectively set as a zero point against which TejJ89 and TejJ114 were compared, so results for TejJ48 are presented only in supplementary data.

Data were log transformed to ensure normal distribution and outlier detection was then performed in SPSS (IBM, Armonk, NY, USA). Univariate outliers were identified using standardised z-scores, with any individual measures for each gene falling outside the cut-off (set at 3 standard deviations from the mean) being discarded. Multivariate outliers were identified using a regression model with Mahalanobis distance as an output, followed by comparison of the calculated Mahalanobis distances with the critical χ^2 value for the dataset (Rasmussen 1988). One sample from the TejJ89 dataset for which the Mahalanobis distance exceeded the critical χ^2 was discarded, leaving a total of n=286 samples to take

forward for statistical testing. The characteristics of this final set of samples are summarised in Table 1.

2.7. Statistical analysis

Differences in gene expression were tested using ANCOVA between 1) DR and AL feeding regimes and 2) TejJ89 and TejJ114 positive and negative responder strains under DR conditions. qRT-PCR plate was included as a co-variate in order to control for any batch effects across the 3 plates used for each gene expression assay. Linear regression models were then performed using DR status and responder strain as independent variables and including an interaction term to determine the presence of moderating effects between the two variables. ANCOVAs and regressions were carried out in STATA v15.1 (StataCorp, College Station, TX, USA). Benjamini, Krieger and Yekutieli false discovery rate (FDR) calculations (Benjamini and others 2006) were performed using GraphPad Prism 8.1.1 (GraphPad Software, San Diego, CA, USA), with the q-value set at 5%.

3. Results

3.1. Splicing factors demonstrate altered expression levels under DR conditions ('DR associated factors')

We identified that several splicing factors displayed differential expression levels with short-term or long-term DR, and that these differences displayed striking tissue specificity (Fig 1, Supplementary Tables S2, S3 & S4). In brain, most of the expression changes we observed were associated with long-term 40% DR, mainly in the positive responder strain TejJ89 and largely belonging to the Hnrnp class of splicing inhibitors. Expression levels of over half (9/16) of the splicing factors tested were significantly altered with DR at a nominal level, with 4 of these (*Hnrnpa0*, *Hnrnpa1*, *Hnrnph3* and *Hnrnpk*) remaining statistically significant after correction for multiple testing. Conversely, following short-term 40% DR in brain, differences were seen equally frequently in positively and negatively responding strains and mainly involved *Srsf* splicing activators or core spliceosome components, although only one (*Srsf6*) met multiple testing criteria (Fig 2a & 2b). In heart, we identified most alterations in conjunction with

short-term DR, with almost all differences being found in the negative responder strain TejJ114, involving both *Srsf* and *Hnrnp* splicing factors, the majority of which (*Hnrnpa1*, *Hnrnpa2b1*, *Hnrnpd*, *Srsf6* and *Sf1*) were significant after correcting for multiple testing (Fig 3a & 3b). Finally, in kidney, as we saw in the heart, most of the changes we identified were in conjunction with short-term DR but occurred in both positively and negatively responsive strains. Differences found involved mainly *Srsf* splicing activators or core components of the spliceosome, and 5 out of 14 of these (*Hnrnpa1*, *Srsf1*, *Srsf6*, *Tra2b* and *Sf1*) remained significant after correction for multiple testing. (Fig 4a & 4b).

3.2. Splicing factors demonstrate different patterns of expression with DR in positive and negative responder strains ('strain-associated factors')

We next identified splicing factors that demonstrated differences in expression patterns between the positive and negative responder strains under short-term or long-term 40% DR. With the exception of brain, most of the differential expression levels in the two strains were present under short-term DR conditions (Supplementary Table S5). In brain, only expression of *Hnrnpa0* and *Srsf2* differed between strains under short-term DR, and only *Srsf2* remained significant after correction for multiple testing (Fig 2a). Many more incidences where the positive and negative responder strains demonstrated differences in splicing factor expression were evident in brain in response to long-term DR; 11/16 genes exhibited differential expression between strains under these conditions, with 6 of these (*Hnrnpa2b1*, *Hnrnpd*, *Hnrnph3*, *Hnrnpk*, *Srsf6* and *Sf1*) meeting the multiple testing threshold (Fig 2b). Several differences between strains were apparent in heart under conditions of short-term DR, which involved both *Srsf* and *Hnrnp* transcripts (Fig 3a), although only one of these (*Hnrnpd*) was significant when corrected for multiple testing. Fewer expression differences were apparent overall under long-term DR in heart (Fig 3b), however 2 of these (*Hnrnpul2* and *Srsf3*) met multiple testing criteria. Kidney demonstrated fewer alterations than either brain or heart, with differences seen only in response to short-term DR, although 2 of these (*Hnrnpa1* and *Sf1*) met the multiple testing threshold (Fig 4a & 4b).

3.3. Expression levels of some splicing factors are associated with both lifespan effects and DR ('interacting factors')

Some of the most interesting associations are those in which splicing factor expression is associated with both DR and strain. In such cases it is reasonable to postulate that those transcripts may be involved in pathways which contribute to the observed responses to 40% DR within each strain, but are also playing some part in the differences seen in strain-specific lifespan response, and so these splicing factors may comprise part of the molecular mechanism behind the response to DR. We therefore sought to identify situations where a statistical interaction was apparent between DR, strain and splicing factor expression (Supplementary Table S6). In brain, only *Srsf2* displayed a nominal interaction under short-term DR conditions (Fig 2a), whereas under long-term DR, 9 of 16 splicing factors tested showed at least nominal interactions, with 4 of these (*Hnrnpa1*, *Hnrnpa2b1*, *Hnrnph3* and *Hnrnpk*) significant after correction for multiple testing (Fig 2b). In heart, far fewer interactions were apparent overall, with 3 of the 16 splicing factors having nominally significant interactions under short-term DR (Fig 3a) and only 1 nominal interaction was detected under long-term DR conditions (Fig 3b), however none of these were significant after correction for multiple testing. Finally, in kidney tissue only 2 transcripts were found to show interactions, and only under conditions of short-term DR, with one of these (*Sf1*) meeting the criteria for multiple testing (Fig 4a & 4b).

4. Discussion

Lifespan extension as a result of dietary restriction (DR) has been recognised for over a century (McCay and others 1928; Osborne and others 1917) and has since been the subject of intensive research. The relationship between DR and lifespan is however sometimes unclear, with variation in the lifespan effect reported both across and within species (Liao and others 2010; Mockett and others 2006; Rikke and others 2010; Selman and Swindell 2018; Speakman and Mitchell 2011). It is apparent therefore that our understanding of the mechanistic basis underpinning responses to DR is not complete, and that other influences exist which may explain some of the observed strain heterogeneity. One such influence may be the interface between the environmental stimulus (DR) and factors moderating the expression or activity of gene expression. While many such factors exist, one that is

highly likely to play a part is alternative splicing, as it is a fundamental component of the response of cells to external and internal stimuli (Mastrangelo and others 2012), and components of the splicing machinery have previously been implicated in response to DR (Heintz and others 2017; Swindell 2009). Here, we have measured transcript expression levels of an *a priori* panel of age- or senescence-related splicing regulatory factors in brain, heart and kidney tissue taken from three ILSXISS recombinant inbred mouse strains with previously reported different lifespan responses to 40% DR. Animals were exposed to both short-term and long-term 40% DR and subsequent analyses were performed to characterise expression differences related to DR alone, differences only related to strain, and effects attributable to both. Our results show that expression levels of several splicing factor transcripts are significantly affected by either short-term or long-term DR, that there are significant differences in expression levels of some transcripts between positive and negative responder strains, and that there are strong tissue specific influences on both effects. Furthermore, some splicing factors demonstrate statistical interactions between their expression, DR and strain lifespan response, which may indicate mechanistic involvement in the divergent lifespan response to DR observed in these mouse strains under DR conditions.

Dietary restriction has been shown to be linked to lifespan, with multiple pathways involved including those involved in genomic stability, proteostasis, inflammation, autophagy, mitochondrial function, oxidative damage and nutrient signalling pathways (IIS, IGF-1, SIRT, AMKP and mTOR) (Kenyon 2010; Picca and others 2017). It is known that the ability to respond to internal and external sources of cellular stress is an important factor in successful ageing (Kourtis and Tavernarakis 2011), and that transcriptomic responsiveness plays a large part in this, including the plasticity of response that is achieved through alternative splicing (Kelemen and others 2013). A recent study has shown that the splicing factor SF1 is necessary for lifespan extension by DR in *C. elegans*, specifically through the modulation of TORC1 pathway components (Heintz and others 2017). Our previous work has shown that both alternative splicing and more specifically the expression levels of splicing regulatory factors that control it, are associated with ageing in humans (Harries and others 2011), cellular senescence *in vitro* (Holly and others 2013; Latorre and others 2018b) and lifespan in animal models (Lee and others 2016). Recently we also showed that alteration of splicing factor levels using small molecules such as resveratrol analogues, hydrogen sulfide donors or inhibitors of ERK or AKT signalling can reverse

senescence phenotypes *in vitro* (Latorre and others 2017; Latorre and others 2018a; Latorre and others 2018c). Given this evidence, it is reasonable to hypothesise that regulation of alternative splicing may play a role in the lifespan modification response following DR.

The results presented here are consistent with a hypothesis that altered splicing regulation may form part of the mechanistic response to DR in mice. We propose that the splicing factors we tested can be classified into three broad classes: *1) DR-associated factors.* Expression of these splicing factors is significantly affected by DR, but no differences are apparent between strains, suggesting that although they may have some association to DR, they are unlikely to contribute to any strain-specific differences seen in the DR response. *2) Strain-associated factors.* Expression of these splicing factors is significantly different between strains but do not differ between AL and DR. *3) Interacting factors***.** Splicing factors showing statistically significant interactions between DR and strain lifespan response in terms of their expression. Where such interactions exist, the associations between splicing factor expression and either DR or responder strain (or both), coupled with a statistically significant mediation effect between the two variables (Fig 5), suggests that these splicing factors may be mechanistically involved in defining the divergent lifespan response observed in these mouse strains under 40% DR.

Splicing factors showing statistical interactions between strain and DR were very common in brain, particularly in response to long term DR. This may reflect a more pressing need for the brain to moderate gene output to maintain homeostatic control than is necessary in the other tissues. It is interesting to note that within the splicing factors affected in the brain, a preponderance of the differences noted between AL and DR (7 out of 8) are observed in the positive responder strain while only 3 of 8 are altered in the negative responder. Few associations were shared between tissues, with only *Srsf6* and *Hnrnpa1* showing patterns that were shared between brain and heart (*Srsf6*) or brain and kidney (*Hnrnpa1*).

Our study has several strengths, including a comprehensive assessment of strain-, tissue- and duration effects. There are of course also limitations to this work; it would have been advantageous to measure alternative isoform expression of target genes of these splicing factors to determine whether they could actively be affecting alternative splicing. Another caveat to the work is that optimally, protein levels of splicing factors would be informative. Unfortunately this was not possible due to limits on starting

material. We have used an FDR approach to account for multiple testing, following the two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli (Benjamini and others 2006). However, it must be recognised that although relatively modest, correlations do exist between expression levels of many splicing factors (Fig 6) and that further correlations are likely to exist between different DR treatments and indeed to an extent between the different mouse strains. All of this suggests that the tests performed here are not completely independent, which in turn greatly complicates any sensible application of multiple testing criteria. In addition, while groups of 8 animals per condition is reasonable for a study of this type, there may be an impact on statistical power which could result in Type II errors. Therefore, we recognise that the multiple testing threshold applied here may be overly severe, and as such have presented nominal findings alongside those which are FDR-corrected, although we recognise that careful interpretation must be applied to such results.

In summary, this study has shown that the expression of splicing factor transcripts shows widespread alterations in response to dietary restriction, and that these are highly tissue specific. It is also apparent that certain transcripts show interactions between the effects of DR, expression levels and strain lifespan response, which could therefore be involved in the mechanisms driving lifespan modulation via DR.

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6. Conflict of Interest

No conflicts of interest to declare.

7. Author contributions

LWH managed the project, designed the study and reviewed the manuscript. BPL coordinated experiments, performed the data analysis and wrote the manuscript. LM handled animal husbandry. GB, JG and EG all performed parts of the gene expression experiments. CS assisted with study design, provided the tissue samples and reviewed the manuscript.

8. References

Austad, S.N. Life extension by dietary restriction in the bowl and doily spider, Frontinella pyramitela. Experimental gerontology. 24:83-92; 1989

Benjamini, Y.; Krieger, A.M.; Yekutieli, D. Adaptive linear step-up procedures that control the false discovery rate. Biometrika. 93:491-507; 2006

Bennett, B.; Beeson, M.; Gordon, L.; Johnson, T.E. Reciprocal congenics defining individual quantitative trait Loci for sedative/hypnotic sensitivity to ethanol. Alcoholism, clinical and experimental research. 26:149-157; 2002

Cartegni, L.; Chew, S.L.; Krainer, A.R. Listening to silence and understanding nonsense: exonic mutations that affect splicing. Nature reviews Genetics. 3:285-298; 2002

Cava, E.; Fontana, L. Will calorie restriction work in humans? Aging. 5:507-514; 2013

Danan-Gotthold, M.; Golan-Gerstl, R.; Eisenberg, E.; Meir, K.; Karni, R.; Levanon, E.Y. Identification of recurrent regulated alternative splicing events across human solid tumors. Nucleic acids research. 43:5130-5144; 2015

Gems, D.; Partridge, L. Genetics of longevity in model organisms: debates and paradigm shifts. Annual review of physiology. 75:621-644; 2013

Harries, L.W.; Hernandez, D.; Henley, W.; Wood, A.R.; Holly, A.C.; Bradley-Smith, R.M.; Yaghootkar, H.; Dutta, A.; Murray, A.; Frayling, T.M.; Guralnik, J.M.; Bandinelli, S.; Singleton, A.; Ferrucci, L.; Melzer, D. Human aging is characterized by focused changes in gene expression and deregulation of alternative splicing. Aging cell. 10:868-878; 2011

Heilbronn, L.K.; de Jonge, L.; Frisard, M.I.; DeLany, J.P.; Larson-Meyer, D.E.; Rood, J.; Nguyen, T.; Martin, C.K.; Volaufova, J.; Most, M.M.; Greenway, F.L.; Smith, S.R.; Deutsch, W.A.; Williamson, D.A.; Ravussin, E. Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. Jama. 295:1539-1548; 2006

Heintz, C.; Doktor, T.K.; Lanjuin, A.; Escoubas, C.; Zhang, Y.; Weir, H.J.; Dutta, S.; Silva-Garcia, C.G.; Bruun, G.H.; Morantte, I.; Hoxhaj, G.; Manning, B.D.; Andresen, B.S.; Mair, W.B. Splicing factor

1 modulates dietary restriction and TORC1 pathway longevity in C. elegans. Nature. 541:102-106; 2017

Holly, A.C.; Melzer, D.; Pilling, L.C.; Fellows, A.C.; Tanaka, T.; Ferrucci, L.; Harries, L.W. Changes in splicing factor expression are associated with advancing age in man. Mechanisms of ageing and development. 134:356-366; 2013

Ingram, D.K.; de Cabo, R. Calorie restriction in rodents: Caveats to consider. Ageing research reviews. 39:15-28; 2017

Ingram, D.K.; Roth, G.S.; Lane, M.A.; Ottinger, M.A.; Zou, S.; de Cabo, R.; Mattison, J.A. The potential for dietary restriction to increase longevity in humans: extrapolation from monkey studies. Biogerontology. 7:143-148; 2006

Kealy, R.D.; Lawler, D.F.; Ballam, J.M.; Mantz, S.L.; Biery, D.N.; Greeley, E.H.; Lust, G.; Segre, M.; Smith, G.K.; Stowe, H.D. Effects of diet restriction on life span and age-related changes in dogs. Journal of the American Veterinary Medical Association. 220:1315-1320; 2002

Kelemen, O.; Convertini, P.; Zhang, Z.; Wen, Y.; Shen, M.; Falaleeva, M.; Stamm, S. Function of alternative splicing. Gene. 514:1-30; 2013

Kenyon, C.J. The genetics of ageing. Nature. 464:504-512; 2010

Kim, Y.K.; Yeo, J.; Kim, B.; Ha, M.; Kim, V.N. Short structured RNAs with low GC content are selectively lost during extraction from a small number of cells. Molecular cell. 46:893-895; 2012

Kourtis, N.; Tavernarakis, N. Cellular stress response pathways and ageing: intricate molecular relationships. The EMBO journal. 30:2520-2531; 2011

Larson-Meyer, D.E.; Heilbronn, L.K.; Redman, L.M.; Newcomer, B.R.; Frisard, M.I.; Anton, S.; Smith, S.R.; Alfonso, A.; Ravussin, E. Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. Diabetes care. 29:1337-1344; 2006

Latorre, E.; Birar, V.C.; Sheerin, A.N.; Jeynes, J.C.C.; Hooper, A.; Dawe, H.R.; Melzer, D.; Cox, L.S.; Faragher, R.G.A.; Ostler, E.L.; Harries, L.W. Small molecule modulation of splicing factor expression is associated with rescue from cellular senescence. BMC Cell Biol. 18:31; 2017

Latorre, E.; Ostler, E.O.; Faragher, R.G.A.; Harries, L.W. FOXO1 and ETV6 genes may represent novel regulators of splicing factor expression in cellular senescence FASEB Journal. 33:1086-1097; 2018a

Latorre, E.; Pilling, L.C.; Lee, B.P.; Bandinelli, S.; Melzer, D.; Ferrucci, L.; Harries, L.W. The VEGFA156b isoform is dysregulated in senescent endothelial cells and may be associated with prevalent and incident coronary heart disease. Clinical science (London, England : 1979). 132:313-325; 2018b

Latorre, E.; Torregrossa, R.; Wood, M.E.; Whiteman, M.; Harries, L.W. Mitochondria-targeted hydrogen sulfide attenuates endothelial senescence by selective induction of splicing factors HNRNPD and SRSF2. Aging. 10:1666-1681; 2018c

Lee, B.P.; Pilling, L.C.; Emond, F.; Flurkey, K.; Harrison, D.E.; Yuan, R.; Peters, L.L.; Kuchel, G.A.; Ferrucci, L.; Melzer, D.; Harries, L.W. Changes in the expression of splicing factor transcripts and variations in alternative splicing are associated with lifespan in mice and humans. Aging cell. 15:903-913; 2016

Liao, C.Y.; Rikke, B.A.; Johnson, T.E.; Diaz, V.; Nelson, J.F. Genetic variation in the murine lifespan response to dietary restriction: from life extension to life shortening. Aging cell. 9:92-95; 2010

Lisowiec, J.; Magner, D.; Kierzek, E.; Lenartowicz, E.; Kierzek, R. Structural determinants for alternative splicing regulation of the MAPT pre-mRNA. RNA biology. 12:330-342; 2015

Mair, W.; Dillin, A. Aging and survival: the genetics of life span extension by dietary restriction. Annual review of biochemistry. 77:727-754; 2008

Masoro, E.J. Overview of caloric restriction and ageing. Mechanisms of ageing and development. 126:913-922; 2005

Mastrangelo, A.M.; Marone, D.; Laido, G.; De Leonardis, A.M.; De Vita, P. Alternative splicing: enhancing ability to cope with stress via transcriptome plasticity. Plant science : an international journal of experimental plant biology. 185-186:40-49; 2012

Mattison, J.A.; Colman, R.J.; Beasley, T.M.; Allison, D.B.; Kemnitz, J.W.; Roth, G.S.; Ingram, D.K.; Weindruch, R.; de Cabo, R.; Anderson, R.M. Caloric restriction improves health and survival of rhesus monkeys. Nat Commun. 8:14063; 2017

McCay, C.M.; Bing, F.C.; Dilley, W.E. FACTOR H IN THE NUTRITION OF TROUT. Science. 67:249-250; 1928

Mockett, R.J.; Cooper, T.M.; Orr, W.C.; Sohal, R.S. Effects of caloric restriction are species-specific. Biogerontology. 7:157-160; 2006

Mulvey, L.; Sands, W.A.; Salin, K.; Carr, A.E.; Selman, C. Disentangling the effect of dietary restriction on mitochondrial function using recombinant inbred mice. Molecular and cellular endocrinology. 455:41-53; 2017

Osborne, T.B.; Mendel, L.B.; Ferry, E.L. THE EFFECT OF RETARDATION OF GROWTH UPON THE BREEDING PERIOD AND DURATION OF LIFE OF RATS. Science. 45:294-295; 1917

Phelan, J.P.; Rose, M.R. Why dietary restriction substantially increases longevity in animal models but won't in humans. Ageing research reviews. 4:339-350; 2005

Picca, A.; Pesce, V.; Lezza, A.M.S. Does eating less make you live longer and better? An update on calorie restriction. Clinical interventions in aging. 12:1887-1902; 2017

Rasmussen, J.L. Evaluating Outlier Identification Tests: Mahalanobis D Squared and Comrey Dk. Multivariate behavioral research. 23:189-202; 1988

Rikke, B.A.; Liao, C.Y.; McQueen, M.B.; Nelson, J.F.; Johnson, T.E. Genetic dissection of dietary restriction in mice supports the metabolic efficiency model of life extension. Experimental gerontology. 45:691-701; 2010

Scuderi, S.; La Cognata, V.; Drago, F.; Cavallaro, S.; D'Agata, V. Alternative splicing generates different parkin protein isoforms: evidences in human, rat, and mouse brain. BioMed research international. 2014:690796; 2014

Selman, C.; Swindell, W.R. Putting a strain on diversity. The EMBO journal. 37; 2018

Smith, C.W.; Valcarcel, J. Alternative pre-mRNA splicing: the logic of combinatorial control. Trends in biochemical sciences. 25:381-388; 2000

Smith, D.L., Jr.; Nagy, T.R.; Allison, D.B. Calorie restriction: what recent results suggest for the future of ageing research. European journal of clinical investigation. 40:440-450; 2010

Speakman, J.R.; Hambly, C. Starving for life: what animal studies can and cannot tell us about the use of caloric restriction to prolong human lifespan. The Journal of nutrition. 137:1078-1086; 2007

Speakman, J.R.; Mitchell, S.E. Caloric restriction. Molecular aspects of medicine. 32:159-221; 2011

Swindell, W.R. Genes and gene expression modules associated with caloric restriction and aging in the laboratory mouse. BMC genomics. 10:585; 2009

Vaughan, K.L.; Kaiser, T.; Peaden, R.; Anson, R.M.; de Cabo, R.; Mattison, J.A. Caloric Restriction Study Design Limitations in Rodent and Nonhuman Primate Studies. The journals of gerontology Series A, Biological sciences and medical sciences. 73:48-53; 2017

Williams, R.W.; Bennett, B.; Lu, L.; Gu, J.; DeFries, J.C.; Carosone-Link, P.J.; Rikke, B.A.; Belknap, J.K.; Johnson, T.E. Genetic structure of the LXS panel of recombinant inbred mouse strains: a powerful resource for complex trait analysis. Mammalian genome : official journal of the International Mammalian Genome Society. 15:637-647; 2004

Xie, F.; Xiao, P.; Chen, D.; Xu, L.; Zhang, B. miRDeepFinder: a miRNA analysis tool for deep sequencing of plant small RNAs. Plant molecular biology; 2012

Table 1. Details of mice used in the study.

Shown here are the numbers of animals included in each feeding regime and diet for each tissue in each strain of mouse used in the current study.

Strain	Tissue	Diet	Regime	n
TejJ48	Brain	AL	2 month	8
			10 month	8
		DR	2 month	8
			10 month	8
	Heart	AL	2 month	8
			10 month	7
		DR	2 month	8
			10 month	8
	Kidney	AL	2 month	7
			10 month	8
		DR	2 month	7
			10 month	8
TejJ89	Brain	AL	2 month	8
			10 month	8
		DR	2 month	8
			10 month	8
	Heart	AL	2 month	8
			10 month	8
		DR	2 month	9
			10 month	8
	Kidney	AL	2 month	8
			10 month	10
		DR	2 month	8
			10 month	6
TejJ114	Brain	AL	2 month	8
			10 month	8
		DR	2 month	8
			10 month	8
	Heart	AL	2 month	8
			10 month	8
		DR	2 month	8
			10 month	8
	Kidney	AL	2 month	8
			10 month	7
		DR	2 month	9
			10 month	8

Figure Legends

Figure 1: Tissue-specificity of splicing factor expression under 40% DR conditions

Heatmaps depicting post-ANCOVA marginal effects for log fold-change in 40% DR expression levels of each transcript (when compared to AL). Data from short-term and long-term 40% DR regimes are shown for each tissue separately. Panel **a** shows data for the positive responder (TejJ89) and panel **b** for the negative responder (TejJ114). Transcripts up-regulated in 40% DR are shown in green while those that are down-regulated are shown in red.

Figure 2: Effects of 40% DR on splicing factor expression in brain tissue

Shown here are transcript expression levels in ILSXISS mouse brain tissue under short-term and long-term DR conditions. Panel **a** shows expression under short-term 40% DR, panel **b** shows expression under long-term 40% DR. Plots show post-estimation marginal effects from the linear regressions used for interaction analysis. Data points represent log fold-change in DR expression levels of each transcript (when compared to AL), separately for the two mouse strains. Significant differences are denoted with stars: * = p<0.05, ** = p<0.01, *** = p<0.001. Stars indicated in black denote associations which meet the multiple testing threshold, while those in grey represent nominal associations. Data for the positive responder strain (TejJ89) is shown as solid points and line in black, while the negative responder strain (TejJ114) is shown as open points and dashed line in grey. The null point is indicated by a dotted line. Error bars represent 95% confidence intervals.

Figure 3: Effects of 40% DR on splicing factor expression in heart tissue

Shown here are transcript expression levels in ILSXISS mouse heart tissue under short-term and long-term DR conditions. Panel **a** shows expression under short-term 40% DR, panel **b** shows expression under long-term 40% DR. Plots show post-estimation marginal effects from the linear regressions used for interaction analysis. Data points represent log fold-change in DR expression levels of each transcript (when compared to AL), separately for the two mouse strains. Significant differences are denoted with stars: * = p<0.05, ** = p<0.01, *** = p<0.001. Stars indicated in black denote associations which meet the multiple testing threshold, while those in grey represent nominal associations Data for the positive responder strain (TejJ89) is shown as solid points and line in black,

while the negative responder strain (TejJ114) is shown as open points and dashed line in grey. The null point is indicated by a dotted line. Error bars represent 95% confidence intervals.

Figure 4: Effects of 40% DR on splicing factor expression in kidney tissue

Shown here are transcript expression levels in ILSXISS mouse kidney tissue under short-term and long-term DR conditions. Panel **a** shows expression under short-term 40% DR, panel **b** shows expression under long-term 40% DR. Plots show post-estimation marginal effects from the linear regressions used for interaction analysis. Data points represent log fold-change in DR expression levels of each transcript (when compared to AL), separately for the two mouse strains. Significant differences are denoted with stars: $*$ = p<0.05, $**$ = p<0.01, $***$ = p<0.001. Stars indicated in black denote associations which meet the multiple testing threshold, while those in grey represent nominal associations. Data for the positive responder strain (TejJ89) is shown as solid points and line in black, while the negative responder strain (TejJ114) is shown as open points and dashed line in grey. The null point is indicated by a dotted line. Error bars represent 95% confidence intervals.

Figure 5: Directionality of effects and potential moderating interactions

This figure shows the likely interplay between the variables measured in the present study. Direct effects are shown as solid black arrows, while interactions where one variable could be moderating the effect exerted between other variables are shown as dashed grey arrows.

Figure 6: Correlations between splicing factor expression levels

Pearson correlations of relationships between expression levels of all splicing factors measured.

Figure 1

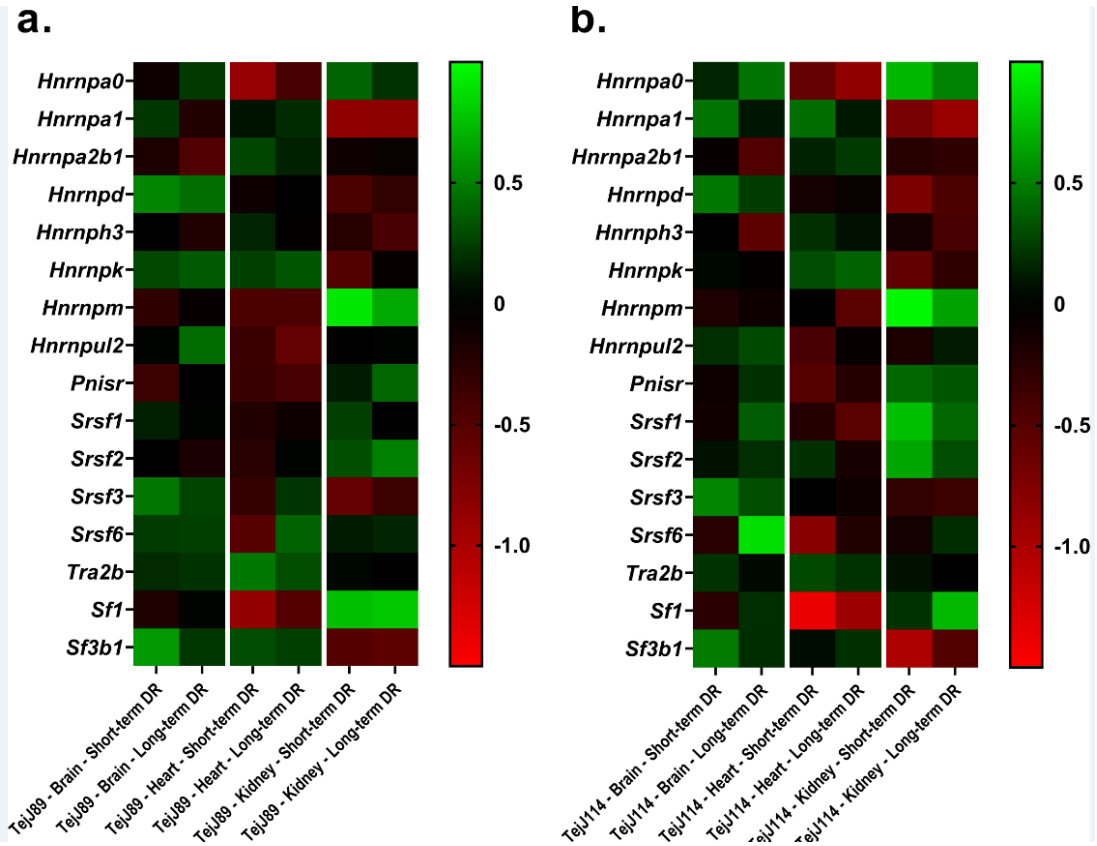

a.
Hnrnpa0
Hnrnpa1
Hnrnpa2b1
Hnrnpd
Hnrnph3
Hnrnpk
Hnrnpm
Hnrnpul2
Pnisr
Srsf1
Srsf2
Srsf3
Srsf6
Tra2b
Sf1
Sf3b1
0.5
0
-0.5
-1.0
TejJ89 - Brain - Short-term DR
TejJ89 - Brain - Long-term DR
TejJ89 - Heart - Short-term DR
TejJ89 - Heart - Long-term DR
TejJ89 - Kidney - Short-term DR
TejJ89 - Kidney - Long-term DR
b.
Hnrnpa0
Hnrnpa1
Hnrnpa2b1
Hnrnpd
Hnrnph3
Hnrnpk
Hnrnpm
Hnrnpul2
Pnisr
Srsf1
Srsf2
Srsf3
Srsf6
Tra2b
Sf1
Sf3b1
0.5
0
-0.5
-1.0
TejJ114 - Brain - Short-term DR
TejJ114 - Brain - Long-term DR
TejJ114 - Heart - Short-term DR
TejJ114 - Heart - Long-term DR
TejJ114 - Kidney - Short-term DR
TejJ114 - Kidney - Long-term DR

Figure 2

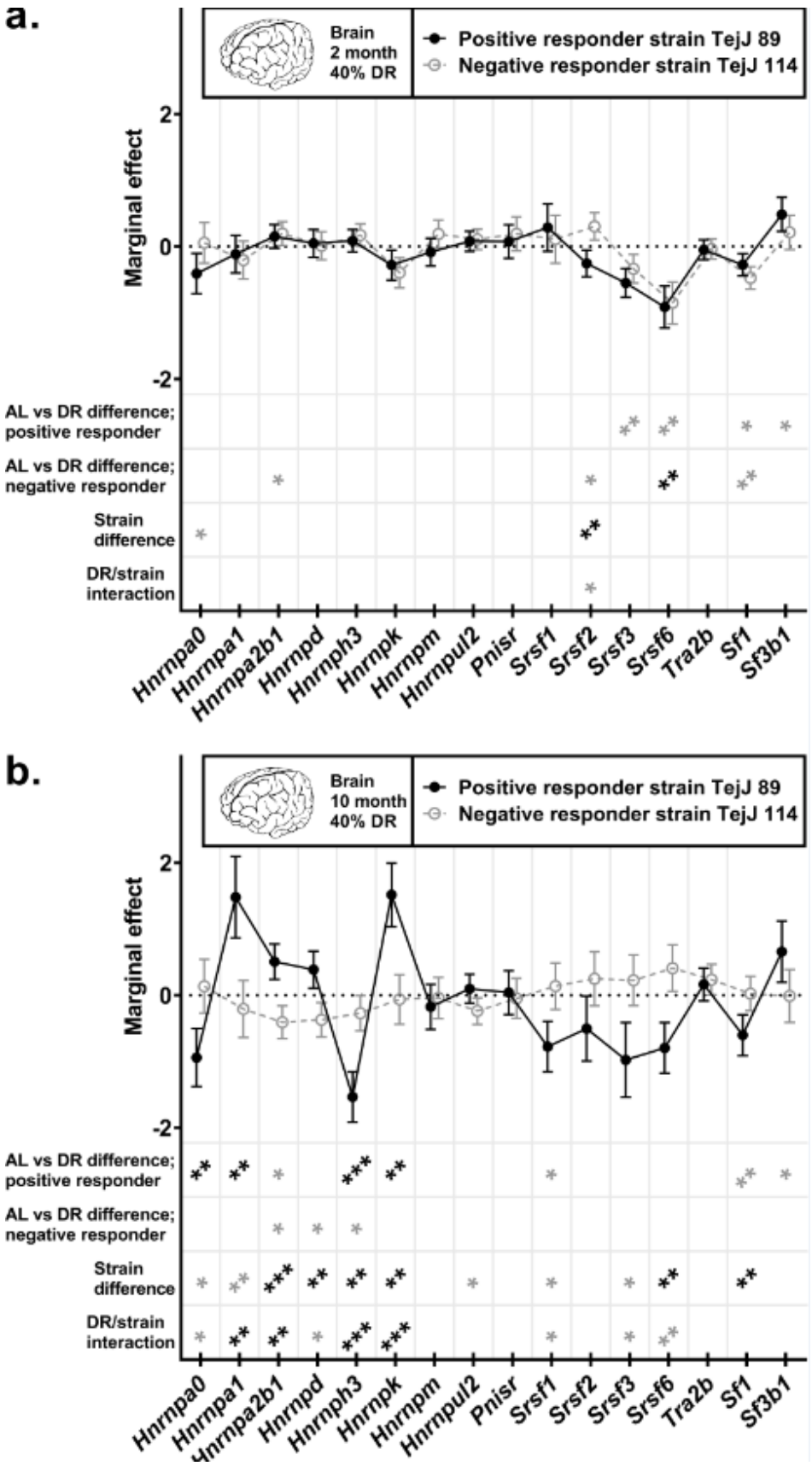

a.
Brain
2 month
40% DR
Positive responder strain TejJ 89
Negative responder strain TejJ 114
Marginal effect
2
0
-2
AL vs DR difference; positive responder
AL vs DR difference; negative responder
Strain difference
DR/strain interaction
Hnrnpa0
Hnrnpa1
Hnrnpa2b1
Hnrnpd
Hnrnph3
Hnrnpk
Hnrnpm
Hnrnpul2
Pnisr
Srsf1
Srsf2
Srsf3
Srsf6
Tra2b
Sf1
Sf3b1
b.
Brain
10 month
40% DR
Positive responder strain TejJ 89
Negative responder strain TejJ 114
Marginal effect
2
0
-2
AL vs DR difference; positive responder
AL vs DR difference; negative responder
Strain difference
DR/strain interaction
Hnrnpa0
Hnrnpa1
Hnrnpa2b1
Hnrnpd
Hnrnph3
Hnrnpk
Hnrnpm
Hnrnpul2
Pnisr
Srsf1
Srsf2
Srsf3
Srsf6
Tra2b
Sf1
Sf3b1

Figure 3

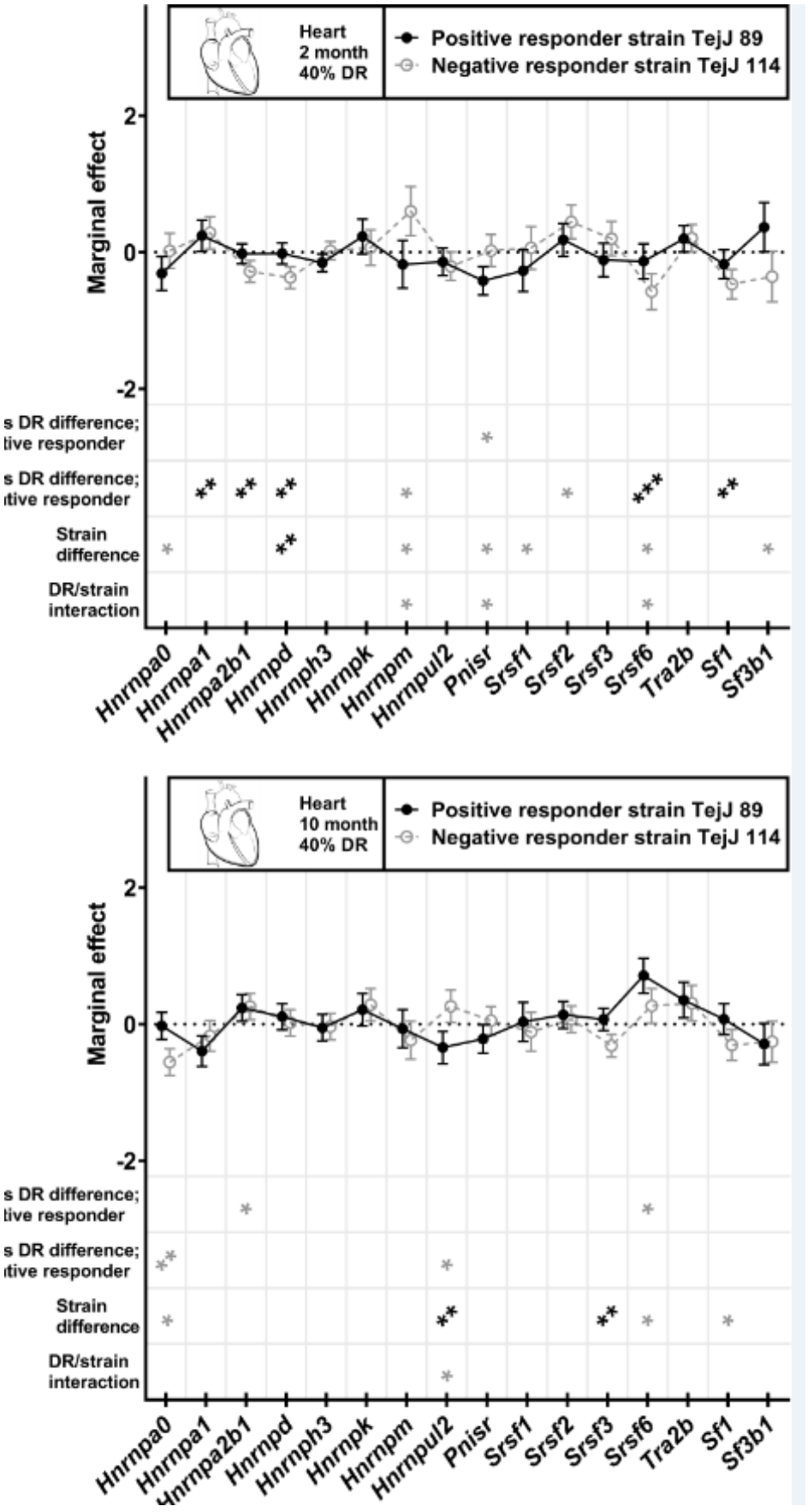


Figure 4

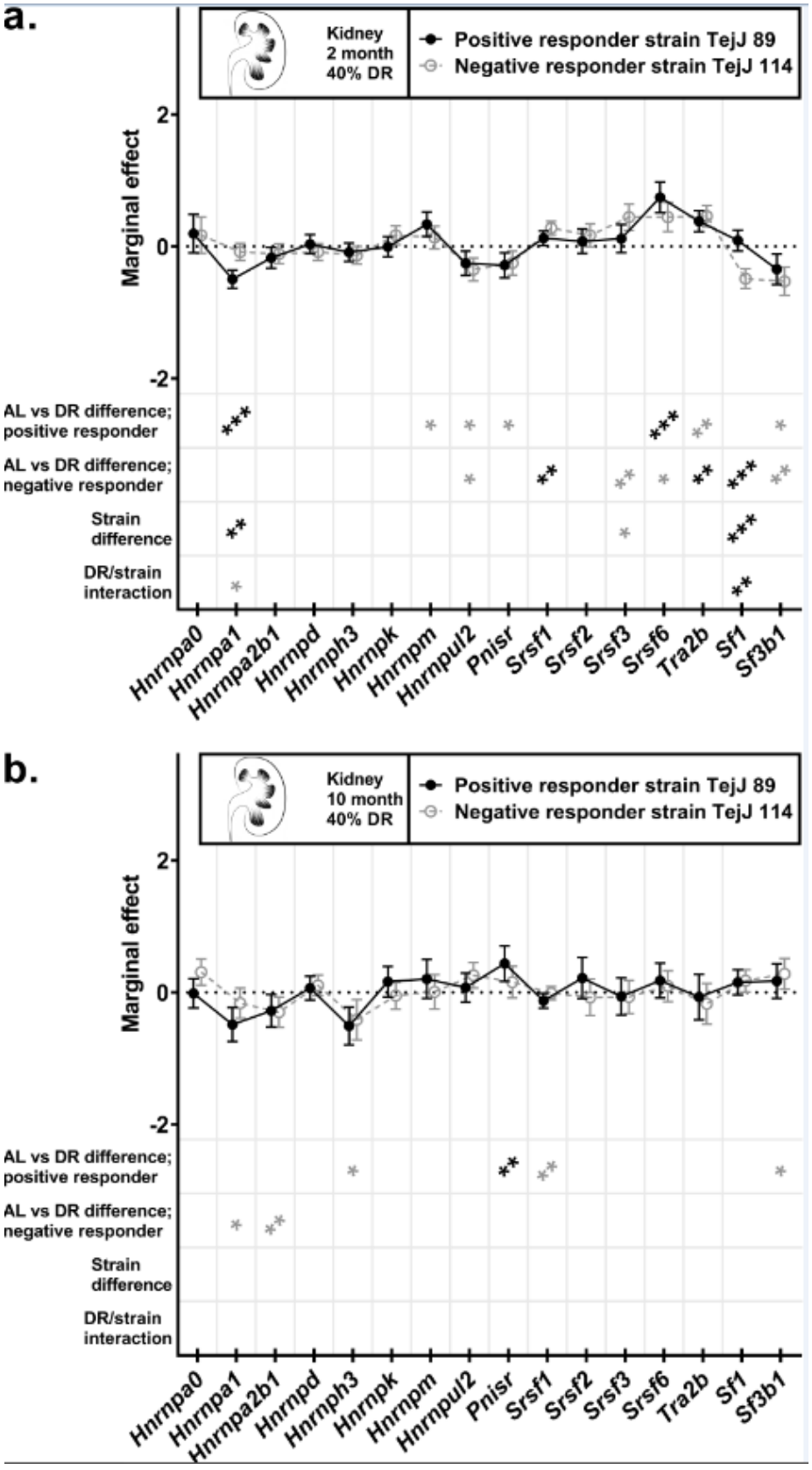

a.
Kidney
2 month
40% DR
Positive responder strain TejJ 89
Negative responder strain TejJ 114
Marginal effect
2
0
-2
AL vs DR difference; positive responder
AL vs DR difference; negative responder
Strain difference
DR/strain interaction
Hnrnpa0
Hnrnpa1
Hnrnpa2b1
Hnrnpd
Hnrnph3
Hnrnpk
Hnrnpm
Hnrnpul2
Pnisr
Srsf1
Srsf2
Srsf3
Srsf6
Tra2b
Sf1
Sf3b1
b.
Kidney
10 month
40% DR
Positive responder strain TejJ 89
Negative responder strain TejJ 114
Marginal effect
2
0
-2
AL vs DR difference; positive responder
AL vs DR difference; negative responder
Strain difference
DR/strain interaction
Hnrnpa0
Hnrnpa1
Hnrnpa2b1
Hnrnpd
Hnrnph3
Hnrnpk
Hnrnpm
Hnrnpul2
Pnisr
Srsf1
Srsf2
Srsf3
Srsf6
Tra2b
Sf1
Sf3b1

Figure 5

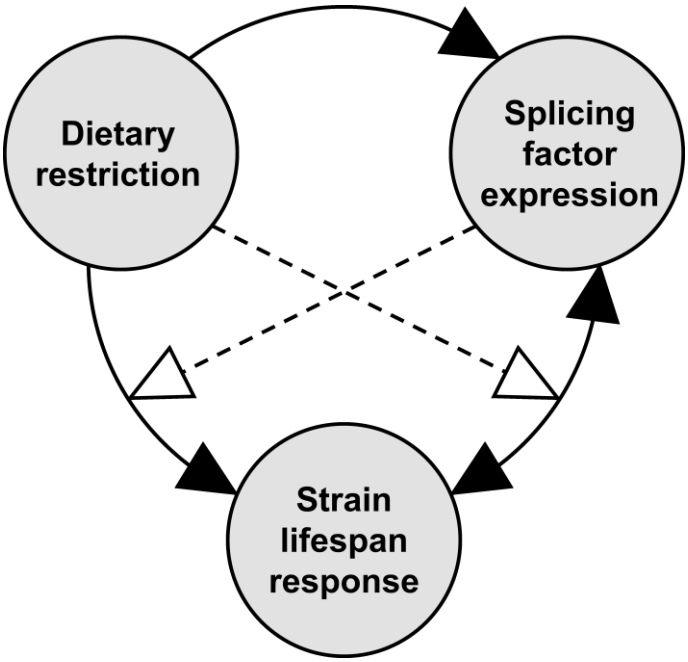

Dietary restriction
Splicing factor expression
Strain lifespan response

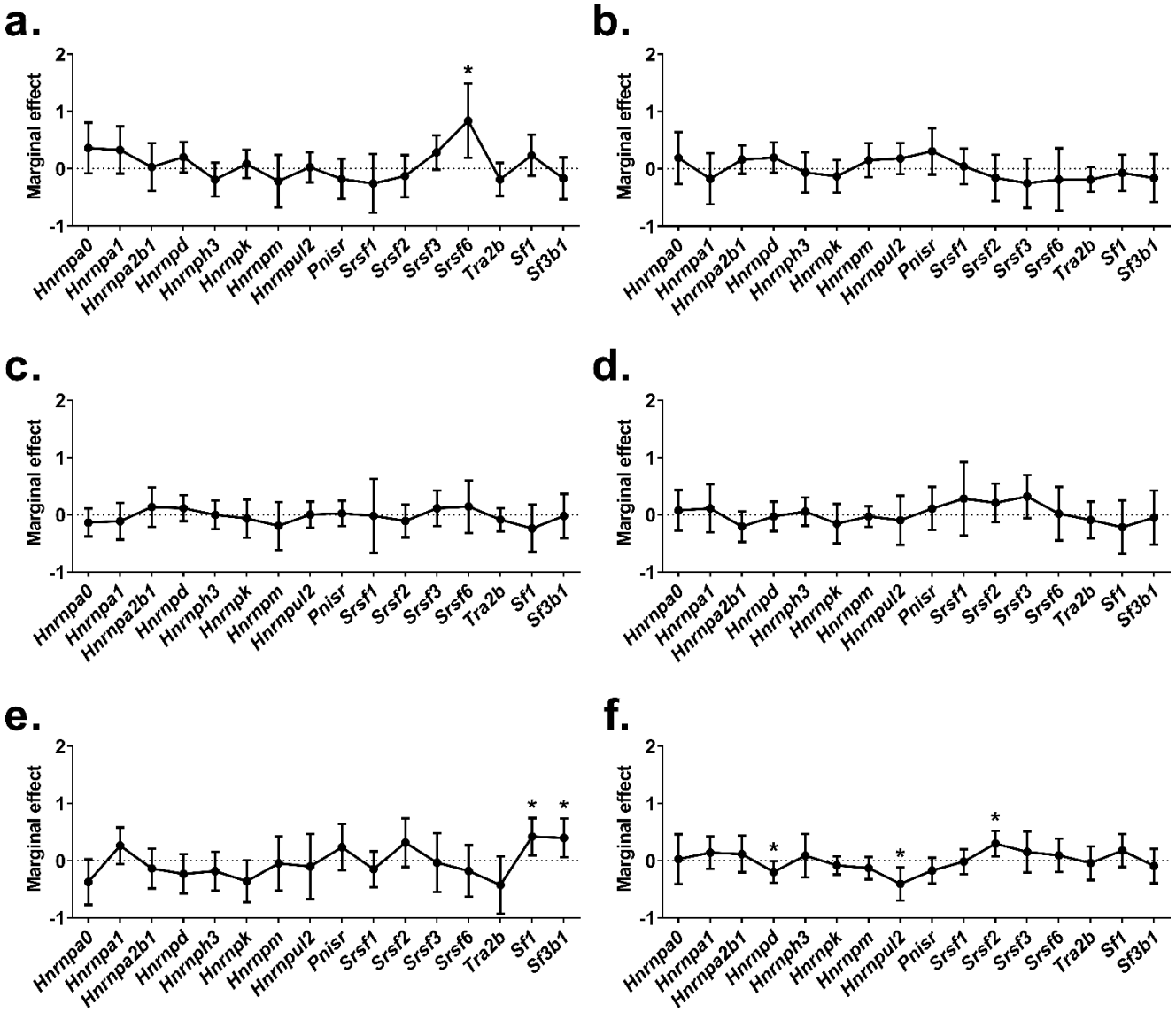
Figure 6

	Hnrnpa0	*Hnrnpa1*	*Hnrnpa2b1*	*Hnrnpd*	*Hnrnph3*	*Hnrnpk*	*Hnrnpm*	*Hnrnpul2*	*Pnisr*	*Srsf1*	*Srsf2*	*Srsf3*	*Srsf6*	*Tra2b*	*Sf1*	*Sf3b1*
Hnrnpa0	1.000															
Hnrnpa1	-0.109	1.000														
Hnrnpa2b1	-0.361	0.384	1.000													
Hnrnpd	-0.310	0.460	0.567	1.000												
Hnrnph3	-0.062	-0.306	-0.006	-0.017	1.000											
Hnrnpk	-0.490	0.007	0.150	0.151	-0.037	1.000										
Hnrnpm	0.202	-0.378	-0.375	-0.388	0.043	-0.101	1.000									
Hnrnpul2	-0.140	-0.032	0.071	0.123	0.053	-0.158	0.131	1.000								
Pnisr	0.080	-0.105	-0.032	-0.086	-0.064	-0.332	0.258	0.529	1.000							
Srsf1	0.308	-0.434	-0.379	-0.438	0.249	-0.360	0.262	0.032	0.255	1.000						
Srsf2	0.239	-0.157	-0.312	-0.347	0.046	-0.155	0.120	-0.354	-0.130	0.206	1.000					
Srsf3	-0.002	-0.164	-0.328	-0.210	0.014	0.203	-0.038	-0.494	-0.384	0.124	0.352	1.000				
Srsf6	0.109	-0.396	-0.319	-0.188	0.037	0.189	0.078	-0.298	-0.307	0.153	0.211	0.490	1.000			
Tra2b	-0.292	-0.330	-0.142	-0.208	0.107	0.337	-0.067	-0.354	-0.383	0.023	0.109	0.482	0.412	1.000		
Sf1	0.180	-0.145	-0.150	0.011	-0.007	-0.065	0.081	0.076	0.030	0.003	-0.077	0.045	0.382	-0.206	1.000	
Sf3b1	-0.152	0.518	0.358	0.507	-0.236	0.044	-0.424	0.036	0.019	-0.407	-0.197	-0.235	-0.429	-0.427	0.128	1.000

Supplementary information

Supplementary Figure S1: Changes in splicing factor expression in non-responder strain (TejJ48) under DR conditions

Plots illustrating changes in splicing factor expression with DR in the non-responder strain of ILSXISS mice (TejJ48). Plot **a** shows mean differences between AL and DR in brain tissue under short-term DR, **b** shows mean differences between AL and DR in brain tissue under long-term DR, **c** shows mean differences between AL and DR in heart tissue under short-term DR, **d** shows mean differences between AL and DR in heart tissue under long-term DR, **e** shows mean differences between AL and DR in kidney tissue under short-term DR and **f** shows mean differences between AL and DR in kidney tissue under long-term DR. Error bars represent 95% confidence intervals and significant differences in splicing factor expression are denoted by stars: ∗ = p<0.05.



Supplementary Table S1: Taqman® Assays.

Splicing factor target genes, assay IDs and qPCR software settings for each transcript included in the current study. Endogenous control genes used are shown in bold italics.

Target	Assay ID	Threshold	Baseline Start	Baseline End
Hnrnpa0	Mm03809085_s1	0.075	3	22
Hnrnpa1	Mm02528230_g1	0.098	3	18
Hnrnpa2b1	Mm01325931_g1	0.145	3	18
Hnrnpd	Mm01201314_m1	0.112	3	21
Hnrnph3	Mm01032120_g1	0.095	3	24
Hnrnpk	Mm01349462_m1	0.129	3	18
Hnrnpm	Mm00513070_m1	0.068	3	21
Hnrnpul2	Mm01230949_m1	0.114	3	21
Pnisr	Mm01219239_m1	0.052	3	20
Srsf1	Mm00557620_m1	0.123	3	21
Srsf2	Mm00448705_m1	0.040	3	20
Srsf3	Mm00786953_s1	0.044	3	23
Srsf6	Mm00471475_m1	0.074	3	21
Tra2b	Mm00833637_mH	0.031	3	21
Sf1	Mm00496060_m1	0.104	3	19
Sf3b1	Mm00473100_m1	0.044	3	19
Gusb	***Mm01197698_m1***	***0.092***	***3***	***21***
Idh3b	***Mm00504589_m1***	***0.112***	***3***	***18***
Ppia	***Mm03024003_g1***	***0.068***	***3***	***17***

Supplementary Table S2: Changes in splicing factor expression with long-term and short-term 40% DR in non-responder mice.

Changes in splicing factor expression levels with long-term and short-term DR in brain, heart and kidney tissues from mice which display no change in lifespan under 40% DR conditions (TejJ89), by ANCOVA. A positive mean difference denotes an increase in expression levels under 40% DR conditions when compared to AL feeding. Transcripts showing nominal associations (p<0.05) are shown in italic and underlined. SE: standard error, 95% CI: 95% confidence intervals.

		Brain – Short-term DR					Brain – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	0.360	0.203	0.102	0.803	-0.083	0.188	0.208	0.383	0.641	-0.264
	Hnrnpa1	0.326	0.187	0.109	0.739	-0.086	-0.175	0.204	0.407	0.269	-0.619
	Hnrnpa2b1	0.029	0.192	0.882	0.447	-0.389	0.161	0.113	0.182	0.408	-0.086
	Hnrnpd	0.200	0.121	0.123	0.463	-0.063	0.194	0.121	0.136	0.459	-0.070
	Hnrnph3	-0.192	0.135	0.181	0.103	-0.487	-0.063	0.161	0.701	0.287	-0.413
	Hnrnpk	0.082	0.112	0.482	0.329	-0.165	-0.131	0.131	0.335	0.153	-0.416
	Hnrnpm	-0.220	0.211	0.318	0.240	-0.679	0.150	0.136	0.293	0.447	-0.147
	Hnrnpul2	0.027	0.121	0.830	0.291	-0.238	0.179	0.125	0.178	0.451	-0.094
	Pnisr	-0.181	0.161	0.284	0.171	-0.532	0.305	0.186	0.126	0.709	-0.099
	Srsf1	-0.257	0.235	0.294	0.254	-0.769	0.044	0.144	0.766	0.357	-0.270
	Srsf2	-0.130	0.168	0.456	0.237	-0.496	-0.157	0.186	0.414	0.248	-0.562
	Srsf3	0.283	0.137	0.063	0.584	-0.018	-0.252	0.197	0.225	0.177	-0.681
	Srsf6	*0.837*	*0.297*	*0.015*	*1.483*	*0.190*	-0.187	0.251	0.470	0.359	-0.734
	Tra2b	-0.190	0.133	0.179	0.100	-0.480	-0.188	0.100	0.085	0.030	-0.406
Core	*Sf1*	0.232	0.163	0.183	0.590	-0.127	-0.070	0.146	0.638	0.247	-0.388
	Sf3b1	-0.170	0.168	0.330	0.195	-0.536	-0.162	0.191	0.414	0.255	-0.578

		Heart – Short-term DR					Heart – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	-0.135	0.111	0.249	0.108	-0.378	0.077	0.161	0.642	0.430	-0.277
	Hnrnpa1	-0.115	0.146	0.448	0.204	-0.433	0.112	0.190	0.566	0.531	-0.306
	Hnrnpa2b1	0.135	0.158	0.411	0.480	-0.210	-0.205	0.121	0.119	0.062	-0.473
	Hnrnpd	0.115	0.104	0.292	0.341	-0.112	-0.029	0.117	0.810	0.228	-0.286
	Hnrnph3	0.000	0.114	0.997	0.247	-0.248	0.056	0.113	0.630	0.303	-0.192
	Hnrnpk	-0.065	0.153	0.681	0.269	-0.398	-0.156	0.157	0.340	0.189	-0.501
	Hnrnpm	-0.195	0.193	0.332	0.225	-0.615	-0.029	0.080	0.721	0.149	-0.208
	Hnrnpul2	0.003	0.104	0.980	0.230	-0.224	-0.096	0.196	0.632	0.334	-0.527
	Pnisr	0.023	0.102	0.826	0.244	-0.199	0.109	0.171	0.535	0.485	-0.266
	Srsf1	-0.020	0.297	0.948	0.627	-0.666	0.280	0.290	0.355	0.918	-0.358
	Srsf2	-0.109	0.131	0.423	0.176	-0.394	0.208	0.154	0.205	0.547	-0.132
	Srsf3	0.113	0.142	0.439	0.422	-0.195	0.318	0.171	0.091	0.695	-0.059
	Srsf6	0.143	0.210	0.511	0.601	-0.316	0.020	0.212	0.928	0.487	-0.448
	Tra2b	-0.086	0.093	0.375	0.117	-0.288	-0.091	0.143	0.541	0.229	-0.410
Core	*Sf1*	-0.239	0.190	0.232	0.174	-0.652	-0.219	0.213	0.327	0.250	-0.687
	Sf3b1	-0.021	0.175	0.905	0.363	-0.406	-0.048	0.214	0.826	0.423	-0.519

		Kidney – Short-term DR					Kidney – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	-0.371	0.176	0.064	0.027	-0.769	0.028	0.199	0.891	0.462	-0.406
	Hnrnpa1	0.263	0.143	0.096	0.581	-0.056	0.141	0.130	0.300	0.426	-0.143
	Hnrnpa2b1	-0.137	0.153	0.395	0.210	-0.483	0.117	0.145	0.436	0.435	-0.201
	Hnrnpd	-0.230	0.155	0.168	0.115	-0.575	*-0.196*	*0.086*	*0.041*	*-0.010*	*-0.383*
	Hnrnph3	-0.182	0.151	0.254	0.154	-0.519	0.088	0.174	0.624	0.467	-0.292
	Hnrnpk	-0.360	0.165	0.054	0.007	-0.727	-0.082	0.074	0.290	0.079	-0.242
	Hnrnpm	-0.047	0.213	0.829	0.428	-0.522	-0.129	0.090	0.178	0.067	-0.325
	Hnrnpul2	-0.101	0.256	0.703	0.469	-0.671	*-0.403*	*0.134*	*0.011*	*-0.112*	*-0.695*
	Pnisr	0.237	0.182	0.222	0.643	-0.169	-0.170	0.103	0.124	0.054	-0.394
	Srsf1	-0.148	0.141	0.316	0.165	-0.461	-0.016	0.100	0.874	0.202	-0.235
	Srsf2	0.316	0.191	0.128	0.740	-0.109	*0.299*	*0.102*	*0.012*	*0.521*	*0.077*
	Srsf3	-0.035	0.231	0.882	0.479	-0.549	0.154	0.165	0.367	0.513	-0.205
	Srsf6	-0.179	0.202	0.398	0.272	-0.630	0.093	0.133	0.500	0.383	-0.198
	Tra2b	-0.424	0.225	0.089	0.077	-0.925	-0.041	0.136	0.769	0.255	-0.336
Core	*Sf1*	*0.420*	*0.145*	*0.016*	*0.744*	*0.097*	0.178	0.132	0.202	0.465	-0.109
	Sf3b1	*0.400*	*0.151*	*0.025*	*0.737*	*0.063*	-0.092	0.137	0.514	0.206	-0.390

Supplementary Table S3: Changes in splicing factor expression with long-term and short-term 40% DR in positive responder mice.

Changes in splicing factor expression levels with long-term and short-term DR in brain, heart and kidney tissues from mice which display lifespan extension under 40% DR conditions (TejJ89), by ANCOVA. A positive mean difference denotes an increase in expression levels under 40% DR conditions when compared to AL feeding. Transcripts showing nominal associations (p<0.05) are shown in italic and underlined, those which meet correction for multiple testing (p<0.0045) are shown in bold italic and underlined. SE: standard error, 95% CI: 95% confidence intervals.

		Brain – Short-term DR					Brain – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	-0.424	0.252	0.119	-0.974	0.126	***-0.954***	***0.235***	***0.002***	***-1.478***	***-0.429***
	Hnrnpa1	-0.240	0.187	0.223	-0.647	0.167	***1.554***	***0.389***	***0.004***	***0.658***	***2.450***
	Hnrnpa2b1	0.128	0.125	0.324	-0.143	0.400	*0.508*	*0.228*	*0.047*	*0.007*	*1.009*
	Hnrnpd	0.021	0.179	0.909	-0.370	0.412	0.360	0.204	0.106	-0.090	0.810
	Hnrnph3	0.085	0.135	0.542	-0.210	0.380	***-1.595***	***0.293***	***<0.001***	***-2.272***	***-0.918***
	Hnrnpk	-0.312	0.179	0.107	-0.703	0.079	***1.512***	***0.343***	***0.002***	***0.736***	***2.287***
	Hnrnpm	0.061	0.164	0.717	-0.296	0.418	-0.167	0.195	0.409	-0.597	0.262
	Hnrnpul2	0.039	0.134	0.777	-0.254	0.331	0.122	0.142	0.407	-0.190	0.435
	Pnisr	0.078	0.168	0.651	-0.287	0.443	0.024	0.268	0.929	-0.566	0.615
	Srsf1	0.316	0.214	0.166	-0.150	0.783	*-0.828*	*0.272*	*0.011*	*-1.427*	*-0.229*
	Srsf2	-0.187	0.139	0.202	-0.489	0.115	-0.510	0.390	0.220	-1.379	0.359
	Srsf3	*-0.433*	*0.128*	*0.005*	*-0.712*	*-0.154*	-1.020	0.507	0.079	-2.189	0.148
	Srsf6	*-0.674*	*0.220*	*0.010*	*-1.153*	*-0.195*	-0.742	0.363	0.065	-1.540	0.056
	Tra2b	0.100	0.091	0.290	-0.097	0.298	0.160	0.159	0.335	-0.189	0.510
Core	*Sf1*	*-0.290*	*0.126*	*0.040*	*-0.564*	*-0.016*	*-0.637*	*0.188*	*0.007*	*-1.055*	*-0.219*
	Sf3b1	*0.373*	*0.155*	*0.033*	*0.035*	*0.710*	*0.737*	*0.254*	*0.016*	*0.172*	*1.302*

		Heart – Short-term DR					Heart – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	-0.331	0.180	0.089	-0.720	0.058	0.091	0.158	0.574	-0.254	0.436
	Hnrnpa1	0.210	0.199	0.310	-0.219	0.639	-0.208	0.156	0.206	-0.548	0.132
	Hnrnpa2b1	-0.022	0.109	0.841	-0.257	0.212	*0.287*	*0.129*	*0.046*	*0.006*	*0.569*
	Hnrnpd	-0.017	0.115	0.884	-0.265	0.231	0.169	0.120	0.184	-0.092	0.431
	Hnrnph3	-0.123	0.086	0.173	-0.309	0.062	-0.007	0.157	0.964	-0.349	0.334
	Hnrnpk	0.234	0.191	0.244	-0.180	0.647	0.142	0.151	0.367	-0.188	0.471
	Hnrnpm	-0.149	0.293	0.621	-0.783	0.485	-0.127	0.137	0.372	-0.425	0.171
	Hnrnpul2	-0.126	0.151	0.420	-0.453	0.201	-0.266	0.195	0.197	-0.692	0.159
	Pnisr	*-0.402*	*0.135*	*0.011*	*-0.694*	*-0.110*	-0.156	0.177	0.396	-0.541	0.229
	Srsf1	-0.241	0.235	0.323	-0.748	0.266	0.004	0.269	0.988	-0.581	0.589
	Srsf2	0.171	0.178	0.353	-0.212	0.555	0.115	0.175	0.523	-0.266	0.496
	Srsf3	-0.103	0.135	0.458	-0.396	0.189	-0.051	0.131	0.704	-0.336	0.234
	Srsf6	-0.064	0.201	0.753	-0.498	0.369	*0.607*	*0.220*	*0.017*	*0.128*	*1.086*
	Tra2b	0.230	0.130	0.100	-0.051	0.511	0.220	0.181	0.247	-0.174	0.614
Core	*Sf1*	-0.147	0.155	0.361	-0.482	0.188	0.044	0.111	0.702	-0.198	0.285
	Sf3b1	0.323	0.300	0.301	-0.326	0.972	-0.160	0.166	0.355	-0.522	0.202

		Kidney – Short-term DR					Kidney – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	0.203	0.214	0.362	-0.264	0.669	0.152	0.088	0.108	-0.039	0.343
	Hnrnpa1	***-0.438***	***0.093***	***<0.001***	***-0.640***	***-0.237***	-0.332	0.216	0.150	-0.803	0.138
	Hnrnpa2b1	-0.158	0.085	0.086	-0.343	0.026	-0.205	0.196	0.315	-0.632	0.221
	Hnrnpd	0.063	0.087	0.483	-0.129	0.256	0.012	0.132	0.928	-0.276	0.301
	Hnrnph3	-0.085	0.075	0.277	-0.248	0.078	*-0.627*	*0.212*	*0.012*	*-1.088*	*-0.166*
	Hnrnpk	-0.025	0.066	0.715	-0.169	0.119	-0.020	0.150	0.898	-0.346	0.307
	Hnrnpm	*0.330*	*0.140*	*0.037*	*0.024*	*0.635*	0.098	0.195	0.625	-0.327	0.522
	Hnrnpul2	*-0.237*	*0.105*	*0.043*	*-0.465*	*-0.008*	-0.192	0.161	0.258	-0.545	0.162
	Pnisr	*-0.289*	*0.121*	*0.034*	*-0.552*	*-0.026*	***0.522***	***0.149***	***0.004***	***0.198***	***0.846***
	Srsf1	0.133	0.066	0.068	-0.012	0.278	*-0.189*	*0.057*	*0.006*	*-0.314*	*-0.064*
	Srsf2	0.069	0.101	0.509	-0.151	0.288	0.353	0.232	0.155	-0.153	0.859
	Srsf3	0.094	0.160	0.569	-0.256	0.444	-0.186	0.180	0.322	-0.578	0.206
	Srsf6	***0.692***	***0.144***	***<0.001***	***0.378***	***1.005***	0.054	0.175	0.763	-0.327	0.435
	Tra2b	*0.343*	*0.109*	*0.008*	*0.106*	*0.580*	-0.265	0.173	0.152	-0.641	0.112
Core	*Sf1*	0.064	0.115	0.592	-0.188	0.315	0.147	0.122	0.251	-0.118	0.413
	Sf3b1	*-0.327*	*0.150*	*0.049*	*-0.653*	*-0.001*	*0.328*	*0.151*	*0.050*	*0.000*	*0.656*

Supplementary Table S4: Changes in splicing factor expression with long-term and short-term 40% DR in negative responder mice.

Changes in splicing factor expression levels with long-term and short-term DR in brain, heart and kidney tissue from mice which display lifespan reduction under 40% DR conditions (TejJ114), by ANCOVA. A positive mean difference denotes an increase in expression levels under 40% DR conditions when compared to AL feeding. Transcripts showing nominal associations (p<0.05) are shown in italic and underlined, those which meet correction for multiple testing (p<0.0045) are shown in bold italic and underlined. SE: standard error, 95% CI: 95% confidence intervals.

		Brain – Short-term DR					Brain – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	0.062	0.200	0.761	-0.373	0.497	0.258	0.332	0.453	-0.472	0.988
	Hnrnpa1	0.032	0.215	0.886	-0.436	0.499	-0.254	0.314	0.435	-0.937	0.430
	Hnrnpa2b1	*0.268*	*0.114*	*0.037*	*0.019*	*0.517*	*-0.437*	*0.153*	*0.014*	*-0.769*	*-0.104*
	Hnrnpd	0.093	0.116	0.436	-0.158	0.345	*-0.462*	*0.186*	*0.028*	*-0.867*	*-0.058*
	Hnrnph3	0.089	0.100	0.392	-0.129	0.307	*-0.342*	*0.151*	*0.043*	*-0.670*	*-0.013*
	Hnrnpk	-0.281	0.159	0.102	-0.627	0.065	-0.196	0.269	0.481	-0.781	0.390
	Hnrnpm	0.010	0.147	0.949	-0.310	0.329	0.163	0.255	0.535	-0.393	0.719
	Hnrnpul2	0.112	0.086	0.214	-0.074	0.298	-0.193	0.166	0.266	-0.554	0.168
	Pnisr	-0.008	0.174	0.965	-0.388	0.372	0.123	0.184	0.517	-0.278	0.524
	Srsf1	-0.247	0.317	0.453	-0.945	0.451	0.222	0.235	0.365	-0.291	0.734
	Srsf2	*0.348*	*0.157*	*0.046*	*0.007*	*0.689*	0.237	0.277	0.408	-0.366	0.840
	Srsf3	-0.334	0.184	0.094	-0.735	0.067	0.172	0.204	0.417	-0.273	0.616
	Srsf6	***-0.808***	***0.208***	***0.002***	***-1.262***	***-0.354***	0.261	0.149	0.105	-0.064	0.586
	Tra2b	-0.037	0.102	0.727	-0.259	0.186	0.156	0.189	0.427	-0.257	0.568
Core	*Sf1*	*-0.407*	*0.125*	*0.007*	*-0.680*	*-0.134*	-0.023	0.213	0.916	-0.487	0.441
	Sf3b1	0.417	0.213	0.074	-0.047	0.882	-0.009	0.340	0.979	-0.750	0.732

		Heart – Short-term DR					Heart – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	0.067	0.174	0.705	-0.312	0.447	*-0.344*	*0.099*	*0.005*	*-0.561*	*-0.127*
	Hnrnpa1	***0.407***	***0.113***	***0.004***	***0.161***	***0.653***	-0.236	0.170	0.189	-0.606	0.134
	Hnrnpa2b1	***-0.287***	***0.073***	***0.002***	***-0.448***	***-0.127***	0.258	0.151	0.114	-0.071	0.587
	Hnrnpd	***-0.316***	***0.087***	***0.003***	***-0.505***	***-0.127***	0.005	0.156	0.975	-0.334	0.344
	Hnrnph3	0.010	0.077	0.895	-0.158	0.178	-0.015	0.116	0.899	-0.268	0.238
	Hnrnpk	-0.006	0.165	0.971	-0.365	0.353	0.368	0.181	0.066	-0.028	0.763
	Hnrnpm	*0.567*	*0.187*	*0.011*	*0.158*	*0.975*	-0.221	0.249	0.393	-0.763	0.321
	Hnrnpul2	-0.202	0.135	0.159	-0.495	0.091	*0.275*	*0.126*	*0.049*	*0.001*	*0.550*
	Pnisr	0.060	0.166	0.724	-0.305	0.425	0.043	0.120	0.725	-0.218	0.304
	Srsf1	0.054	0.189	0.779	-0.357	0.465	-0.079	0.126	0.543	-0.353	0.196
	Srsf2	*0.460*	*0.166*	*0.017*	*0.099*	*0.821*	0.071	0.111	0.531	-0.170	0.313
	Srsf3	0.147	0.205	0.489	-0.301	0.594	-0.005	0.107	0.967	-0.240	0.231
	Srsf6	***-0.685***	***0.151***	***<0.001***	***-1.014***	***-0.355***	0.318	0.151	0.057	-0.012	0.648
	Tra2b	0.092	0.144	0.534	-0.221	0.405	0.338	0.195	0.109	-0.088	0.763
Core	*Sf1*	***-0.511***	***0.138***	***0.003***	***-0.812***	***-0.211***	-0.342	0.187	0.092	-0.750	0.065
	Sf3b1	-0.259	0.205	0.230	-0.705	0.188	-0.305	0.253	0.252	-0.857	0.247

		Kidney – Short-term DR					Kidney – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	0.147	0.195	0.466	-0.276	0.569	0.202	0.194	0.320	-0.225	0.630
	Hnrnpa1	-0.129	0.100	0.216	-0.345	0.086	*-0.325*	*0.125*	*0.025*	*-0.600*	*-0.050*
	Hnrnpa2b1	-0.105	0.129	0.433	-0.384	0.175	*-0.419*	*0.125*	*0.007*	*-0.698*	*-0.140*
	Hnrnpd	-0.095	0.098	0.350	-0.307	0.117	0.013	0.103	0.905	-0.214	0.239
	Hnrnph3	-0.122	0.111	0.293	-0.362	0.118	-0.345	0.201	0.120	-0.800	0.110
	Hnrnpk	0.198	0.128	0.146	-0.079	0.474	0.134	0.170	0.448	-0.240	0.507
	Hnrnpm	0.148	0.109	0.196	-0.087	0.384	-0.054	0.215	0.805	-0.527	0.419
	Hnrnpul2	*-0.314*	*0.135*	*0.037*	*-0.605*	*-0.022*	0.171	0.129	0.212	-0.113	0.454
	Pnisr	-0.261	0.144	0.094	-0.573	0.051	0.182	0.224	0.432	-0.310	0.674
	Srsf1	***0.285***	***0.073***	***0.002***	***0.128***	***0.442***	-0.015	0.081	0.857	-0.193	0.164
	Srsf2	0.151	0.139	0.297	-0.149	0.451	0.048	0.134	0.728	-0.247	0.343
	Srsf3	*0.456*	*0.140*	*0.006*	*0.155*	*0.758*	0.126	0.218	0.574	-0.354	0.606
	Srsf6	*0.449*	*0.177*	*0.025*	*0.066*	*0.832*	0.283	0.191	0.168	-0.139	0.704
	Tra2b	***0.487***	***0.119***	***0.001***	***0.228***	***0.746***	0.015	0.307	0.961	-0.660	0.691
Core	*Sf1*	***-0.495***	***0.104***	***<0.001***	***-0.720***	***-0.270***	0.173	0.145	0.257	-0.145	0.491
	Sf3b1	*-0.557*	*0.168*	*0.005*	*-0.919*	*-0.195*	0.223	0.204	0.297	-0.225	0.672

Supplementary Table S5: Splicing factor expression according to mouse strain.

Differences in splicing factor expression between the lifespan extension (TejJ89) and lifespan reduction (TejJ114) responder strains under 40% DR by ANCOVA. A positive mean difference denotes higher expression levels in TejJ89 relative to TejJ114 under 40% DR conditions. Transcripts showing nominal associations (p<0.05) are shown in italic and underlined, those which meet correction for multiple testing (p<0.0045) are shown in bold italic and underlined. SE: standard error, 95% CI: 95% confidence intervals.

		Brain – Short-term DR					Brain – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	*-0.446*	*0.170*	*0.022*	*-0.816*	*-0.077*	*-1.150*	*0.358*	*0.011*	*-1.960*	*-0.340*
	Hnrnpa1	0.089	0.208	0.675	-0.364	0.542	*1.788*	*0.510*	*0.008*	*0.613*	*2.963*
	Hnrnpa2b1	-0.044	0.156	0.780	-0.384	0.295	***0.963***	***0.199***	***<0.001***	***0.526***	***1.400***
	Hnrnpd	0.032	0.127	0.808	-0.245	0.309	***0.825***	***0.215***	***0.003***	***0.351***	***1.300***
	Hnrnph3	-0.105	0.125	0.418	-0.377	0.168	***-1.289***	***0.314***	***0.003***	***-2.014***	***-0.565***
	Hnrnpk	0.102	0.160	0.534	-0.246	0.450	***1.691***	***0.411***	***0.003***	***0.761***	***2.621***
	Hnrnpm	-0.281	0.148	0.082	-0.604	0.041	-0.200	0.264	0.465	-0.780	0.381
	Hnrnpul2	-0.017	0.109	0.876	-0.254	0.220	*0.309*	*0.109*	*0.016*	*0.070*	*0.549*
	Pnisr	-0.119	0.160	0.471	-0.468	0.230	0.144	0.190	0.465	-0.274	0.562
	Srsf1	0.229	0.278	0.426	-0.377	0.836	*-0.881*	*0.294*	*0.012*	*-1.528*	*-0.234*
	Srsf2	***-0.533***	***0.133***	***0.002***	***-0.823***	***-0.243***	-0.801	0.371	0.056	-1.628	0.026
	Srsf3	-0.219	0.135	0.131	-0.513	0.076	*-1.281*	*0.431*	*0.018*	*-2.274*	*-0.288*
	Srsf6	-0.084	0.227	0.718	-0.578	0.410	***-1.180***	***0.286***	***0.002***	***-1.810***	***-0.550***
	Tra2b	0.008	0.103	0.940	-0.216	0.231	-0.119	0.143	0.424	-0.434	0.196
Core	*Sf1*	0.186	0.138	0.204	-0.115	0.487	***-0.687***	***0.184***	***0.004***	***-1.098***	***-0.276***
	Sf3b1	0.245	0.155	0.141	-0.094	0.584	0.715	0.398	0.103	-0.172	1.602

		Heart – Short-term DR					Heart – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	*-0.374*	*0.165*	*0.041*	*-0.731*	*-0.017*	*0.516*	*0.175*	*0.012*	*0.134*	*0.899*
	Hnrnpa1	-0.039	0.164	0.817	-0.393	0.316	-0.230	0.167	0.194	-0.593	0.134
	Hnrnpa2b1	0.194	0.130	0.162	-0.089	0.478	-0.015	0.108	0.893	-0.249	0.220
	Hnrnpd	***0.344***	***0.088***	***0.002***	***0.154***	***0.534***	0.107	0.121	0.393	-0.156	0.371
	Hnrnph3	-0.168	0.093	0.096	-0.369	0.034	-0.001	0.130	0.996	-0.284	0.283
	Hnrnpk	0.214	0.163	0.211	-0.138	0.566	-0.093	0.146	0.535	-0.412	0.225
	Hnrnpm	*-0.733*	*0.255*	*0.013*	*-1.283*	*-0.183*	0.159	0.243	0.526	-0.371	0.690
	Hnrnpul2	0.056	0.158	0.731	-0.286	0.397	***-0.572***	***0.134***	***0.001***	***-0.863***	***-0.282***
	Pnisr	*-0.457*	*0.183*	*0.028*	*-0.856*	*-0.058*	-0.248	0.139	0.099	-0.550	0.054
	Srsf1	*-0.338*	*0.144*	*0.035*	*-0.648*	*-0.028*	0.165	0.217	0.462	-0.307	0.636
	Srsf2	-0.224	0.173	0.219	-0.599	0.151	0.073	0.144	0.624	-0.241	0.386
	Srsf3	-0.248	0.152	0.126	-0.576	0.080	***0.359***	***0.093***	***0.002***	***0.156***	***0.561***
	Srsf6	*0.425*	*0.172*	*0.028*	*0.053*	*0.797*	*0.429*	*0.177*	*0.032*	*0.043*	*0.815*
	Tra2b	0.022	0.177	0.904	-0.361	0.404	0.034	0.156	0.834	-0.307	0.374
Core	*Sf1*	0.191	0.169	0.279	-0.175	0.557	*0.402*	*0.183*	*0.048*	*0.005*	*0.800*
	Sf3b1	*0.657*	*0.234*	*0.015*	*0.151*	*1.162*	0.008	0.231	0.974	-0.495	0.510

		Kidney – Short-term DR					Kidney – Long-term DR				
		Mean Difference	SE	*p*-value	95% CI lower	95% CI upper	Mean Difference	SE	*p*-value	95% CI lower	95% CI upper
Splicing Factors	*Hnrnpa0*	-0.013	0.208	0.950	-0.463	0.436	-0.264	0.153	0.115	-0.604	0.076
	Hnrnpa1	***-0.371***	***0.092***	***0.001***	***-0.569***	***-0.173***	-0.206	0.156	0.217	-0.554	0.142
	Hnrnpa2b1	-0.065	0.150	0.672	-0.389	0.259	-0.001	0.156	0.996	-0.354	0.352
	Hnrnpd	0.123	0.089	0.191	-0.070	0.316	-0.022	0.120	0.856	-0.289	0.245
	Hnrnph3	0.055	0.103	0.605	-0.169	0.278	-0.148	0.247	0.566	-0.718	0.422
	Hnrnpk	-0.151	0.132	0.274	-0.436	0.135	0.229	0.148	0.154	-0.101	0.559
	Hnrnpm	0.188	0.111	0.114	-0.051	0.427	0.193	0.237	0.435	-0.335	0.721
	Hnrnpul2	0.093	0.147	0.538	-0.224	0.410	-0.177	0.153	0.274	-0.518	0.164
	Pnisr	-0.059	0.149	0.698	-0.381	0.263	0.259	0.160	0.136	-0.097	0.614
	Srsf1	-0.174	0.086	0.065	-0.361	0.012	-0.089	0.100	0.394	-0.311	0.133
	Srsf2	-0.112	0.116	0.352	-0.362	0.138	0.282	0.224	0.236	-0.217	0.781
	Srsf3	*-0.280*	*0.115*	*0.030*	*-0.528*	*-0.033*	-0.006	0.234	0.979	-0.527	0.514
	Srsf6	0.279	0.186	0.157	-0.122	0.681	0.072	0.143	0.627	-0.247	0.390
	Tra2b	-0.059	0.090	0.525	-0.254	0.137	0.096	0.263	0.724	-0.491	0.682
Core	*Sf1*	***0.552***	***0.116***	***<0.001***	***0.301***	***0.802***	-0.031	0.112	0.785	-0.281	0.218
	Sf3b1	0.178	0.160	0.285	-0.167	0.524	-0.102	0.187	0.597	-0.520	0.315

Supplementary Table S6: Interactions between strain effects and 40% DR effects on splicing factor expression.

Shown here are the interaction coefficients between strain effects and DR effects on splicing factor transcript expression. A positive coefficient denotes combinatorial effects contributing to higher expression levels in TejJ89 relative to TejJ114 under 40% DR conditions. Also shown are the postestimation marginal effects for each strain. Positive margins denote an increase in expression levels in the respective strain under 40% DR conditions when compared to AL feeding. Transcripts showing nominal associations (p<0.05) are shown in italic and underlined, those which meet correction for multiple testing (p<0.0045) are shown in bold italic and underlined. SE: standard error, 95% CI: 95% confidence intervals.

Brain – Short-term DR							
			Coefficient	**SE**	**95% CI lower**	**95% CI upper**	***p*-value**
Splicing Factors	*Hnrnpa0*	**Interaction coefficient**	-0.495	0.308	-1.128	0.138	0.120
		TejJ89 – Marginal effect	-0.407	0.148	-0.711	-0.103	
		TejJ114 – Marginal effect	0.056	0.149	-0.251	0.362	
	Hnrnpa1	**Interaction coefficient**	-0.175	0.289	-0.769	0.418	0.549
		TejJ89 – Marginal effect	-0.112	0.139	-0.397	0.172	
		TejJ114 – Marginal effect	-0.204	0.140	-0.491	0.083	
	Hnrnpa2b1	**Interaction coefficient**	-0.083	0.179	-0.452	0.285	0.646
		TejJ89 – Marginal effect	0.153	0.086	-0.024	0.330	
		TejJ114 – Marginal effect	0.198	0.087	0.020	0.377	
	Hnrnpd	**Interaction coefficient**	-0.026	0.211	-0.460	0.409	0.904
		TejJ89 – Marginal effect	0.048	0.101	-0.160	0.257	
		TejJ114 – Marginal effect	0.012	0.102	-0.199	0.222	
	Hnrnph3	**Interaction coefficient**	-0.055	0.170	-0.406	0.295	0.748
		TejJ89 – Marginal effect	0.087	0.082	-0.081	0.255	
		TejJ114 – Marginal effect	0.175	0.082	0.005	0.344	
	Hnrnpk	**Interaction coefficient**	-0.012	0.230	-0.485	0.462	0.960
		TejJ89 – Marginal effect	-0.278	0.111	-0.505	-0.051	
		TejJ114 – Marginal effect	-0.394	0.112	-0.624	-0.165	
	Hnrnpm	**Interaction coefficient**	0.019	0.212	-0.418	0.455	0.930
		TejJ89 – Marginal effect	-0.085	0.102	-0.294	0.125	
		TejJ114 – Marginal effect	0.189	0.103	-0.022	0.400	
	Hnrnpul2	**Interaction coefficient**	-0.081	0.155	-0.400	0.238	0.607
		TejJ89 – Marginal effect	0.080	0.074	-0.073	0.233	
		TejJ114 – Marginal effect	0.107	0.075	-0.048	0.261	
	Pnisr	**Interaction coefficient**	-0.002	0.257	-0.530	0.526	0.994
		TejJ89 – Marginal effect	0.075	0.123	-0.178	0.329	
		TejJ114 – Marginal effect	0.192	0.124	-0.064	0.448	
	Srsf1	**Interaction coefficient**	0.493	0.368	-0.265	1.251	0.193
		TejJ89 – Marginal effect	0.287	0.174	-0.072	0.646	
		TejJ114 – Marginal effect	0.110	0.175	-0.252	0.471	
	Srsf2	**Interaction coefficient**	*-0.491*	*0.206*	*-0.914*	*-0.067*	*0.025*
		TejJ89 – Marginal effect	*-0.254*	*0.099*	*-0.457*	*-0.051*	

		TejJ114 – Marginal effect	*0.305*	*0.100*	*0.100*	*0.510*	
	Srsf3	**Interaction coefficient**	-0.061	0.218	-0.509	0.387	0.782
		TejJ89 – Marginal effect	-0.548	0.105	-0.763	-0.333	
		TejJ114 – Marginal effect	-0.335	0.106	-0.552	-0.118	
	Srsf6	**Interaction coefficient**	0.249	0.320	-0.409	0.907	0.444
		TejJ89 – Marginal effect	-0.913	0.154	-1.228	-0.597	
		TejJ114 – Marginal effect	-0.850	0.155	-1.169	-0.532	
	Tra2b	**Interaction coefficient**	0.189	0.152	-0.123	0.502	0.224
		TejJ89 – Marginal effect	-0.049	0.073	-0.199	0.102	
		TejJ114 – Marginal effect	-0.038	0.074	-0.189	0.114	
Core Spliceosome	*Sf1*	**Interaction coefficient**	0.134	0.170	-0.215	0.482	0.439
		TejJ89 – Marginal effect	-0.276	0.081	-0.444	-0.109	
		TejJ114 – Marginal effect	-0.474	0.082	-0.643	-0.305	
	Sf3b1	**Interaction coefficient**	-0.012	0.260	-0.546	0.522	0.964
		TejJ89 – Marginal effect	0.485	0.125	0.229	0.741	
		TejJ114 – Marginal effect	0.212	0.126	-0.046	0.471	

11 **Supplementary Table S6: Continued.**

			Brain – Long-term DR				
			Coefficient	**SE**	**95% CI lower**	**95% CI upper**	***p*-value**
Splicing Factors	*Hnrnpa0*	**Interaction coefficient**	*-1.057*	*0.408*	*-1.902*	*-0.213*	*0.016*
		TejJ89 – Marginal effect	*-0.941*	*0.212*	*-1.379*	*-0.503*	
		TejJ114 – Marginal effect	*0.134*	*0.197*	*-0.273*	*0.541*	
	Hnrnpa1	**Interaction coefficient**	***1.652***	***0.479***	***0.659***	***2.644***	***0.002***
		TejJ89 – Marginal effect	***1.478***	***0.296***	***0.865***	***2.091***	
		TejJ114 – Marginal effect	***-0.208***	***0.208***	***-0.639***	***0.223***	
	Hnrnpa2b1	**Interaction coefficient**	***0.896***	***0.258***	***0.365***	***1.426***	***0.002***
		TejJ89 – Marginal effect	***0.507***	***0.130***	***0.239***	***0.775***	
		TejJ114 – Marginal effect	***-0.408***	***0.120***	***-0.655***	***-0.161***	
	Hnrnpd	**Interaction coefficient**	*0.717*	*0.270*	*0.161*	*1.273*	*0.014*
		TejJ89 – Marginal effect	*0.385*	*0.136*	*0.104*	*0.666*	
		TejJ114 – Marginal effect	*-0.370*	*0.126*	*-0.629*	*-0.112*	
	Hnrnph3	**Interaction coefficient**	***-1.241***	***0.296***	***-1.855***	***-0.627***	***<0.001***
		TejJ89 – Marginal effect	***-1.536***	***0.183***	***-1.915***	***-1.156***	
		TejJ114 – Marginal effect	***-0.272***	***0.129***	***-0.539***	***-0.005***	
	Hnrnpk	**Interaction coefficient**	***1.632***	***0.421***	***0.760***	***2.504***	***0.001***
		TejJ89 – Marginal effect	***1.515***	***0.232***	***1.035***	***1.995***	
		TejJ114 – Marginal effect	***-0.063***	***0.180***	***-0.436***	***0.309***	
	Hnrnpm	**Interaction coefficient**	-0.164	0.326	-0.834	0.507	0.620
		TejJ89 – Marginal effect	-0.175	0.165	-0.514	0.164	
		TejJ114 – Marginal effect	-0.042	0.152	-0.354	0.271	
	Hnrnpul2	**Interaction coefficient**	0.376	0.210	-0.055	0.808	0.084
		TejJ89 – Marginal effect	0.096	0.106	-0.122	0.314	

		TejJ114 – Marginal effect	-0.239	0.098	-0.440	-0.038	
	Pnisr	**Interaction coefficient**	0.026	0.318	-0.629	0.681	0.936
		TejJ89 – Marginal effect	0.040	0.161	-0.291	0.371	
		TejJ114 – Marginal effect	-0.048	0.148	-0.353	0.257	
	Srsf1	**Interaction coefficient**	*-0.876*	*0.366*	*-1.629*	*-0.123*	*0.024*
		TejJ89 – Marginal effect	*-0.776*	*0.185*	*-1.157*	*-0.396*	
		TejJ114 – Marginal effect	*0.136*	*0.170*	*-0.214*	*0.487*	
	Srsf2	**Interaction coefficient**	-0.845	0.433	-1.738	0.049	0.063
		TejJ89 – Marginal effect	-0.504	0.237	-0.993	-0.014	
		TejJ114 – Marginal effect	0.247	0.198	-0.161	0.655	
	Srsf3	**Interaction coefficient**	*-1.204*	*0.449*	*-2.135*	*-0.274*	*0.014*
		TejJ89 – Marginal effect	*-0.976*	*0.272*	*-1.539*	*-0.412*	
		TejJ114 – Marginal effect	*0.224*	*0.184*	*-0.158*	*0.606*	
	Srsf6	**Interaction coefficient**	*-1.052*	*0.367*	*-1.807*	*-0.296*	*0.008*
		TejJ89 – Marginal effect	*-0.795*	*0.185*	*-1.176*	*-0.413*	
		TejJ114 – Marginal effect	*0.408*	*0.171*	*0.056*	*0.760*	
	Tra2b	**Interaction coefficient**	-0.064	0.239	-0.555	0.428	0.792
		TejJ89 – Marginal effect	0.163	0.121	-0.086	0.411	
		TejJ114 – Marginal effect	0.236	0.111	0.007	0.465	
Core Spliceosome	*Sf1*	**Interaction coefficient**	-0.481	0.274	-1.047	0.085	0.092
		TejJ89 – Marginal effect	-0.603	0.150	-0.914	-0.293	
		TejJ114 – Marginal effect	0.024	0.125	-0.235	0.282	
	Sf3b1	**Interaction coefficient**	0.665	0.422	-0.206	1.536	0.128
		TejJ89 – Marginal effect	0.658	0.224	1.120	0.000	
		TejJ114 – Marginal effect	-0.013	0.193	-0.412	0.386	

14 **Supplementary Table S6: Continued.**

			Heart – Short-term DR				
			Coefficient	**SE**	**95% CI lower**	**95% CI upper**	***p*-value**
Splicing Factors	*Hnrnpa0*	**Interaction coefficient**	-0.407	0.242	-0.904	0.090	0.104
		TejJ89 – Marginal effect	-0.310	0.121	-0.560	-0.061	
		TejJ114 – Marginal effect	0.023	0.125	-0.233	0.279	
	Hnrnpa1	**Interaction coefficient**	-0.197	0.221	-0.651	0.258	0.383
		TejJ89 – Marginal effect	0.242	0.111	0.015	0.470	
		TejJ114 – Marginal effect	0.284	0.114	0.050	0.518	
	Hnrnpa2b1	**Interaction coefficient**	0.280	0.144	-0.016	0.575	0.063
		TejJ89 – Marginal effect	-0.019	0.071	-0.165	0.126	
		TejJ114 – Marginal effect	-0.281	0.078	-0.440	-0.121	
	Hnrnpd	**Interaction coefficient**	0.291	0.151	-0.018	0.600	0.064
		TejJ89 – Marginal effect	-0.017	0.075	-0.172	0.138	
		TejJ114 – Marginal effect	-0.371	0.077	-0.530	-0.212	
	Hnrnph3	**Interaction coefficient**	-0.148	0.127	-0.410	0.113	0.254
		TejJ89 – Marginal effect	-0.153	0.064	-0.284	-0.022	
		TejJ114 – Marginal effect	0.023	0.066	-0.111	0.158	
	Hnrnpk	**Interaction coefficient**	0.277	0.247	-0.230	0.785	0.272
		TejJ89 – Marginal effect	0.231	0.124	-0.023	0.486	
		TejJ114 – Marginal effect	0.068	0.127	-0.194	0.329	
	Hnrnpm	**Interaction coefficient**	*-0.750*	*0.339*	*-1.446*	*-0.054*	*0.036*
		TejJ89 – Marginal effect	*-0.176*	*0.170*	*-0.525*	*0.173*	
		TejJ114 – Marginal effect	*0.603*	*0.175*	*0.244*	*0.961*	
	Hnrnpul2	**Interaction coefficient**	0.059	0.196	-0.344	0.462	0.767
		TejJ89 – Marginal effect	-0.140	0.098	-0.342	0.063	
		TejJ114 – Marginal effect	-0.205	0.101	-0.412	0.003	
	Pnisr	**Interaction coefficient**	*-0.441*	*0.205*	*-0.864*	*-0.019*	*0.041*
		TejJ89 – Marginal effect	*-0.417*	*0.102*	*-0.626*	*-0.208*	
		TejJ114 – Marginal effect	*0.026*	*0.113*	*-0.206*	*0.259*	
	Srsf1	**Interaction coefficient**	-0.338	0.294	-0.942	0.267	0.262
		TejJ89 – Marginal effect	-0.270	0.148	-0.573	0.033	
		TejJ114 – Marginal effect	0.064	0.152	-0.247	0.375	
	Srsf2	**Interaction coefficient**	-0.275	0.233	-0.753	0.203	0.249
		TejJ89 – Marginal effect	0.183	0.117	-0.056	0.423	
		TejJ114 – Marginal effect	0.446	0.120	0.199	0.692	
	Srsf3	**Interaction coefficient**	-0.219	0.238	-0.708	0.270	0.366
		TejJ89 – Marginal effect	-0.113	0.120	-0.358	0.133	
		TejJ114 – Marginal effect	0.202	0.123	-0.050	0.454	
	Srsf6	**Interaction coefficient**	*0.584*	*0.248*	*0.074*	*1.093*	*0.026*
		TejJ89 – Marginal effect	*-0.131*	*0.125*	*-0.386*	*0.125*	
		TejJ114 – Marginal effect	*-0.576*	*0.128*	*-0.838*	*-0.314*	

	Tra2b	**Interaction coefficient**	0.138	0.189	-0.250	0.525	0.472
		TejJ89 – Marginal effect	0.198	0.095	0.004	0.392	
		TejJ114 – Marginal effect	0.205	0.097	0.006	0.404	
Core Spliceosome	*Sf1*	**Interaction coefficient**	0.335	0.203	-0.082	0.751	0.111
		TejJ89 – Marginal effect	-0.174	0.102	-0.383	0.035	
		TejJ114 – Marginal effect	-0.466	0.105	-0.681	-0.252	
	Sf3b1	**Interaction coefficient**	0.604	0.351	-0.117	1.324	0.097
		TejJ89 – Marginal effect	0.367	0.176	0.006	0.729	
		TejJ114 – Marginal effect	-0.354	0.181	-0.725	0.017	

17 **Supplementary Table S6: Continued.**

			Heart – Long-term DR				
			Coefficient	**SE**	**95% CI lower**	**95% CI upper**	***p*-value**
Splicing Factors	*Hnrnpa0*	**Interaction coefficient**	0.368	0.199	-0.043	0.778	0.077
		TejJ89 – Marginal effect	-0.025	0.096	-0.222	0.172	
		TejJ114 – Marginal effect	-0.557	0.096	-0.754	-0.360	
	Hnrnpa1	**Interaction coefficient**	0.016	0.221	-0.437	0.470	0.941
		TejJ89 – Marginal effect	-0.395	0.108	-0.617	-0.172	
		TejJ114 – Marginal effect	-0.172	0.108	-0.394	0.051	
	Hnrnpa2b1	**Interaction coefficient**	0.019	0.191	-0.374	0.412	0.922
		TejJ89 – Marginal effect	0.238	0.094	0.045	0.431	
		TejJ114 – Marginal effect	0.258	0.094	0.065	0.451	
	Hnrnpd	**Interaction coefficient**	0.180	0.192	-0.215	0.574	0.358
		TejJ89 – Marginal effect	0.109	0.094	-0.084	0.303	
		TejJ114 – Marginal effect	0.019	0.094	-0.175	0.212	
	Hnrnph3	**Interaction coefficient**	-0.008	0.193	-0.406	0.390	0.968
		TejJ89 – Marginal effect	-0.052	0.095	-0.247	0.143	
		TejJ114 – Marginal effect	-0.036	0.095	-0.231	0.159	
	Hnrnpk	**Interaction coefficient**	-0.247	0.235	-0.730	0.236	0.303
		TejJ89 – Marginal effect	0.212	0.115	-0.025	0.450	
		TejJ114 – Marginal effect	0.284	0.115	0.047	0.521	
	Hnrnpm	**Interaction coefficient**	0.109	0.276	-0.458	0.675	0.696
		TejJ89 – Marginal effect	-0.068	0.135	-0.346	0.210	
		TejJ114 – Marginal effect	-0.235	0.135	-0.513	0.043	
	Hnrnpul2	**Interaction coefficient**	*-0.547*	*0.236*	*-1.033*	*-0.062*	*0.029*
		TejJ89 – Marginal effect	*-0.342*	*0.116*	*-0.581*	*-0.104*	
		TejJ114 – Marginal effect	*0.261*	*0.116*	*0.023*	*0.499*	
	Pnisr	**Interaction coefficient**	-0.228	0.207	-0.652	0.197	0.280
		TejJ89 – Marginal effect	-0.217	0.101	-0.426	-0.009	
		TejJ114 – Marginal effect	0.051	0.101	-0.157	0.259	
	Srsf1	**Interaction coefficient**	0.079	0.281	-0.499	0.657	0.780
		TejJ89 – Marginal effect	0.034	0.138	-0.250	0.318	
		TejJ114 – Marginal effect	-0.112	0.138	-0.396	0.171	
	Srsf2	**Interaction coefficient**	0.051	0.196	-0.351	0.453	0.796
		TejJ89 – Marginal effect	0.136	0.096	-0.062	0.333	
		TejJ114 – Marginal effect	0.070	0.096	-0.127	0.267	
	Srsf3	**Interaction coefficient**	-0.014	0.166	-0.357	0.329	0.934
		TejJ89 – Marginal effect	0.069	0.080	-0.096	0.233	
		TejJ114 – Marginal effect	-0.314	0.080	-0.478	-0.149	
	Srsf6	**Interaction coefficient**	0.303	0.253	-0.217	0.824	0.242
		TejJ89 – Marginal effect	0.710	0.124	0.454	0.965	
		TejJ114 – Marginal effect	0.266	0.124	0.011	0.521	
	Tra2b	**Interaction coefficient**	-0.089	0.257	-0.618	0.441	0.733

		TejJ89 – Marginal effect	0.352	0.126	0.092	0.612	
		TejJ114 – Marginal effect	0.307	0.126	0.047	0.566	
Core Spliceosome	*Sf1*	**Interaction coefficient**	0.411	0.225	-0.052	0.874	0.079
		TejJ89 – Marginal effect	0.073	0.111	-0.154	0.301	
		TejJ114 – Marginal effect	-0.307	0.110	-0.534	-0.080	
	Sf3b1	**Interaction coefficient**	0.109	0.298	-0.504	0.723	0.717
		TejJ89 – Marginal effect	-0.290	0.146	-0.591	0.011	
		TejJ114 – Marginal effect	-0.257	0.146	-0.558	0.044	

Supplementary Table S6: Continued.

			Kidney – Short-term DR				
			Coefficient	**SE**	**95% CI lower**	**95% CI upper**	***p*-value**
Splicing Factors	*Hnrnpa0*	**Interaction coefficient**	0.051	0.280	-0.523	0.625	0.856
		TejJ89 – Marginal effect	0.196	0.142	-0.094	0.486	
		TejJ114 – Marginal effect	0.171	0.134	-0.104	0.445	
	Hnrnpa1	**Interaction coefficient**	*-0.310*	*0.132*	*-0.580*	*-0.039*	*0.027*
		TejJ89 – Marginal effect	*-0.496*	*0.067*	*-0.633*	*-0.359*	
		TejJ114 – Marginal effect	*-0.081*	*0.063*	*-0.210*	*0.049*	
	Hnrnpa2b1	**Interaction coefficient**	-0.054	0.153	-0.368	0.260	0.725
		TejJ89 – Marginal effect	-0.172	0.077	-0.331	-0.013	
		TejJ114 – Marginal effect	-0.112	0.073	-0.262	0.038	
	Hnrnpd	**Interaction coefficient**	0.152	0.131	-0.117	0.422	0.255
		TejJ89 – Marginal effect	0.036	0.069	-0.107	0.179	
		TejJ114 – Marginal effect	-0.082	0.062	-0.209	0.045	
	Hnrnph3	**Interaction coefficient**	0.033	0.136	-0.246	0.312	0.812
		TejJ89 – Marginal effect	-0.086	0.069	-0.227	0.055	
		TejJ114 – Marginal effect	-0.133	0.065	-0.266	0.000	
	Hnrnpk	**Interaction coefficient**	-0.226	0.146	-0.526	0.074	0.133
		TejJ89 – Marginal effect	-0.004	0.074	-0.155	0.148	
		TejJ114 – Marginal effect	0.171	0.070	0.028	0.314	
	Hnrnpm	**Interaction coefficient**	0.192	0.178	-0.173	0.558	0.290
		TejJ89 – Marginal effect	0.336	0.090	0.151	0.521	
		TejJ114 – Marginal effect	0.136	0.085	-0.039	0.311	
	Hnrnpul2	**Interaction coefficient**	0.088	0.177	-0.275	0.452	0.622
		TejJ89 – Marginal effect	-0.254	0.090	-0.438	-0.070	
		TejJ114 – Marginal effect	-0.349	0.085	-0.523	-0.175	
	Pnisr	**Interaction coefficient**	-0.029	0.183	-0.405	0.346	0.873
		TejJ89 – Marginal effect	-0.284	0.093	-0.474	-0.094	
		TejJ114 – Marginal effect	-0.251	0.088	-0.431	-0.072	
	Srsf1	**Interaction coefficient**	-0.159	0.105	-0.375	0.057	0.143
		TejJ89 – Marginal effect	0.127	0.053	0.018	0.237	
		TejJ114 – Marginal effect	0.280	0.050	0.177	0.383	
	Srsf2	**Interaction coefficient**	-0.077	0.178	-0.443	0.289	0.668
		TejJ89 – Marginal effect	0.079	0.090	-0.106	0.264	
		TejJ114 – Marginal effect	0.169	0.085	-0.006	0.344	
	Srsf3	**Interaction coefficient**	-0.363	0.204	-0.781	0.055	0.086
		TejJ89 – Marginal effect	0.119	0.103	-0.092	0.331	
		TejJ114 – Marginal effect	0.445	0.097	0.245	0.645	
	Srsf6	**Interaction coefficient**	0.238	0.225	-0.223	0.699	0.298
		TejJ89 – Marginal effect	0.744	0.114	0.511	0.978	
		TejJ114 – Marginal effect	0.444	0.107	0.224	0.664	

	Tra2b	**Interaction coefficient**	-0.145	0.156	-0.466	0.177	0.364
		TejJ89 – Marginal effect	0.382	0.078	0.222	0.542	
		TejJ114 – Marginal effect	0.461	0.078	0.301	0.621	
Core Spliceosome	*Sf1*	**Interaction coefficient**	***0.554***	***0.153***	***0.241***	***0.867***	***0.001***
		TejJ89 – Marginal effect	***0.091***	***0.077***	***-0.068***	***0.249***	
		TejJ114 – Marginal effect	***-0.488***	***0.073***	***-0.637***	***-0.338***	
	Sf3b1	**Interaction coefficient**	0.219	0.222	-0.236	0.674	0.332
		TejJ89 – Marginal effect	-0.346	0.112	-0.576	-0.115	
		TejJ114 – Marginal effect	-0.526	0.106	-0.744	-0.309	

23 **Supplementary Table S6: Continued.**

			Kidney – Long-term DR				
			Coefficient	**SE**	**95% CI lower**	**95% CI upper**	***p*-value**
Splicing Factors	*Hnrnpa0*	**Interaction coefficient**	-0.067	0.211	-0.501	0.368	0.755
		TejJ89 – Marginal effect	-0.016	0.108	-0.237	0.206	
		TejJ114 – Marginal effect	0.306	0.095	0.110	0.503	
	Hnrnpa1	**Interaction coefficient**	0.014	0.245	-0.490	0.519	0.954
		TejJ89 – Marginal effect	-0.486	0.125	-0.744	-0.229	
		TejJ114 – Marginal effect	-0.164	0.111	-0.392	0.064	
	Hnrnpa2b1	**Interaction coefficient**	0.184	0.239	-0.309	0.677	0.449
		TejJ89 – Marginal effect	-0.277	0.120	-0.524	-0.030	
		TejJ114 – Marginal effect	-0.300	0.112	-0.531	-0.069	
	Hnrnpd	**Interaction coefficient**	-0.045	0.170	-0.396	0.305	0.792
		TejJ89 – Marginal effect	0.064	0.087	-0.114	0.243	
		TejJ114 – Marginal effect	0.105	0.077	-0.053	0.264	
	Hnrnph3	**Interaction coefficient**	-0.336	0.284	-0.924	0.252	0.250
		TejJ89 – Marginal effect	-0.509	0.139	-0.796	-0.222	
		TejJ114 – Marginal effect	-0.414	0.149	-0.722	-0.107	
	Hnrnpk	**Interaction coefficient**	-0.146	0.218	-0.595	0.303	0.508
		TejJ89 – Marginal effect	0.162	0.111	-0.067	0.391	
		TejJ114 – Marginal effect	-0.050	0.099	-0.253	0.153	
	Hnrnpm	**Interaction coefficient**	0.106	0.280	-0.472	0.683	0.709
		TejJ89 – Marginal effect	0.202	0.143	-0.092	0.497	
		TejJ114 – Marginal effect	0.009	0.127	-0.251	0.270	
	Hnrnpul2	**Interaction coefficient**	-0.385	0.216	-0.831	0.061	0.087
		TejJ89 – Marginal effect	0.074	0.106	-0.145	0.294	
		TejJ114 – Marginal effect	0.259	0.093	0.067	0.452	
	Pnisr	**Interaction coefficient**	0.384	0.257	-0.145	0.913	0.147
		TejJ89 – Marginal effect	0.436	0.131	0.167	0.706	
		TejJ114 – Marginal effect	0.155	0.116	-0.084	0.394	
	Srsf1	**Interaction coefficient**	-0.212	0.107	-0.432	0.008	0.058
		TejJ89 – Marginal effect	-0.127	0.054	-0.239	-0.015	
		TejJ114 – Marginal effect	-0.012	0.048	-0.111	0.088	
	Srsf2	**Interaction coefficient**	0.365	0.296	-0.245	0.975	0.229
		TejJ89 – Marginal effect	0.218	0.151	-0.093	0.529	
		TejJ114 – Marginal effect	-0.076	0.134	-0.351	0.200	
	Srsf3	**Interaction coefficient**	-0.307	0.269	-0.862	0.248	0.265
		TejJ89 – Marginal effect	-0.062	0.137	-0.345	0.221	
		TejJ114 – Marginal effect	-0.068	0.122	-0.319	0.182	
	Srsf6	**Interaction coefficient**	-0.237	0.251	-0.754	0.279	0.353
		TejJ89 – Marginal effect	0.180	0.128	-0.083	0.444	
		TejJ114 – Marginal effect	0.093	0.113	-0.140	0.327	
	Tra2b	**Interaction coefficient**	-0.269	0.329	-0.946	0.408	0.421

		TejJ89 – Marginal effect	-0.072	0.168	-0.417	0.273	
		TejJ114 – Marginal effect	-0.173	0.149	-0.479	0.133	
Core Spliceosome	*Sf1*	**Interaction coefficient**	-0.049	0.180	-0.421	0.322	0.787
		TejJ89 – Marginal effect	0.154	0.092	-0.036	0.343	
		TejJ114 – Marginal effect	0.176	0.082	0.008	0.344	
	Sf3b1	**Interaction coefficient**	0.114	0.250	-0.400	0.629	0.651
		TejJ89 – Marginal effect	0.170	0.127	-0.092	0.432	
		TejJ114 – Marginal effect	0.281	0.113	0.048	0.513	