Arg-tRNA synthetase links inflammatory metabolism to RNA splicing via nuclear condensates

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Summary Cells respond to perturbations such as inflammation by sensing changes in metabolite levels. Especially prominent is arginine, which has long known connections to the inflammatory response. Here we show that depletion of arginine during inflammation decreased levels of arginyl-tRNA synthetase (ArgRS) in the nucleus. We found that nuclear ArgRS interacted with serine/arginine repetitive matrix protein 2 (SRRM2) in membrane-less, condensate-like, SRRM2-dependent nuclear speckles. This interaction impeded SRRM2 speckle trafficking and resulted in changes in alternative mRNA splicing. Splice site usage was regulated in opposite directions by ArgRS and SRRM2. These ArgRS- and SRRM2-dependent splicing changes cumulated in synthesis of different protein isoforms that altered cellular metabolism and peptide presentation to immune cells. Our findings delineate a novel mechanism whereby a tRNA synthetase responds to a metabolic change and modulates the splicing machinery via condensate trafficking for cellular responses to inflammatory injury.

Keywords: aminoacyl-tRNA synthetase, tRNA, nuclear speckle, condensate, multi-synthetase complex, arginine, mRNA processing, inflammation, immune regulation

Introduction

Inflammation is commonly associated with diseases, including cancer, neurodegeneration and auto-immune disorders (Netea et al., 2017). One of the metabolic hallmarks of inflammation is a decrease in systemic arginine levels due to its increased consumption by pro- and anti-inflammatory enzymes, with plasma arginine recovering when the inflammatory insult is resolved (Bronte and Zanovello, 2005; Murray, 2016). Consequently, arginine is a key regulator of cellular signaling pathways, including the central metabolic regulator mTOR, which is necessary for immune cell activation during inflammation (Bar-Peled and Sabatini, 2014; Weichhart et al., 2015; Jones and Pearce, 2017). In addition to its physiological decrease during inflammation, therapeutic arginine depletion by recombinant enzymes is in clinical trials for cancer treatment (Patil et al., 2016).

More generally, sensing and responding to external metabolic stimuli are essential for cells. The function of aminoacyl-tRNA synthetases (aaRSs) in cell signal transduction has come into focus in recent years. tRNA synthetases are highly conserved proteins that are essential for mRNA translation, where they catalyze the covalent attachment of amino acids to the 3' end of their cognate tRNAs (Schimmel and Söll, 1979). Eight aaRSs with nine distinct catalytic activities form the multisynthetase complex (MSC) together with 3 designated adapter proteins. While the MSC does not contribute to protein synthesis, complex formation is crucial for the intracellular distribution of aaRSs, specifically for their nuclear localization (Cui et al., 2021). The MSC is dynamic and can act as a reservoir to release aaRSs for extra-translational functions (Lee et al., 2004; Ray et al., 2007; Duchon et al., 2017).

aaRSs play a prominent role in mediating extracellular and nuclear cell signaling pathways. Although nuclear-localized aaRSs were historically seen as quality control agents for the

correct processing of nascent tRNAs before their export to the cytoplasm (Lund and Dahlberg, 1998; Sarkar et al., 1999), they have also been linked to other functions (Guo and Schimmel, 2013; Shi et al., 2017; Kwon et al., 2019) including mediation of small molecule sensing (Sajish and Schimmel, 2015) and regulation of gene expression (Yannay-Cohen et al., 2009; Shi et al., 2014). In addition to freely diffusing nuclear aaRSs, the MSC is also found in the nucleus (Kaminska et al., 2009; Nathanson and Deutscher, 2000).

The cell nucleus is organized by biomolecular condensates, membraneless organelles with the characteristics of phase-separated liquids (Shin and Brangwynne, 2017; Hnisz et al., 2017). Despite their lack of a membrane and constant exchange with the surrounding nucleoplasm, these subnuclear domains display structural integrity (Zhu and Brangwynne, 2015). In the nucleus, DNA-sparse interchromatin clusters termed nuclear speckles are condensates that sequester proteins with repetitive sequences of low complexity and high disorder, among them factors necessary for mRNA maturation and mRNA splicing (Galganski et al., 2017; Saitoh et al., 2004; Spector and Lamond, 2011). Polymerases and splicing factors are in steady exchange between condensates and the surrounding nucleoplasm (Guo et al., 2019), raising the possibility that nuclear condensate trafficking regulates their functions.

Different protein isoforms can be derived from the same coding sequence by alternative splicing, potentially resulting in different functions (Galarza-Muñoz et al., 2017). Intron retention alternative splicing events can post-transcriptionally regulate mRNA levels by impeding transport to the cytoplasm and increasing RNA degradation (Braunschweig et al., 2014). 20% of cassette exons alternative splicing events (where splice sites are variably recognized leading to an exon being either retained or spliced out) are evolutionarily

conserved between human and mouse, suggesting functional consequences (Pan et al., 2004).

Nuclear speckles have been suggested to store mRNA maturation factors for mRNA processing at their periphery and/or be hubs for mRNA processing (Chen and Belmont, 2019). A series of studies has identified SRRM2 (serine/arginine repetitive matrix protein 2, also known as SRm300, gene name *SRRM2*) as a component of spliceosomal complexes (Blencowe et al., 1998, 2000; Zhang et al., 2017) as well as an integral and essential protein for nuclear speckle formation (Miyagawa et al., 2012; Hu et al., 2019; Ilik et al., 2020). During mRNA splicing, the conserved N-terminal domain of SRRM2 engages with exon sequences close to the exon-junction complex and regulates splice site selection (Gautam et al., 2015; Zhang et al., 2017). In line with its importance, SRRM2 mutations, truncations and misregulation have been identified in developmental disorders (Kaplanis et al., 2020), cancer (Tomsic et al., 2015) and Parkinson's disease (Shehadeh et al., 2010).

Given the role of arginine metabolism in inflammation (Bronte and Zanovello, 2005), we initiated our studies of the physiological role of nuclear aaRSs with arginyl-tRNA synthetase (ArgRS, gene name: *RARS*). We discovered a pathway through which cells respond to arginine starvation during inflammation through reduced interaction between ArgRS and SRRM2 in nuclear condensates which results in changes in alternative mRNA splicing. These alternative splicing changes culminated in altered cellular metabolism and peptide presentation to the immune system. This work supports a role distinct from previously known functions of aaRSs and extends the reach of aaRSs to regulation of speckle protein trafficking and alternative mRNA splicing in response to a metabolic signal.

Results

Arginine promotes nuclear localization of arginyl-tRNA synthetase

Previous work showed that ArgRS localizes to the nucleus as part of the MSC (Nathanson and Deutscher, 2000). To begin, we re-investigated the nuclear localization of ArgRS using four model systems: (i) human hepatic carcinoma cell line HepG2, (ii) human embryonic kidney endothelial cell line 293T, (iii) murine embryonic fibroblasts, and (iv) mouse liver. We confirmed nuclear ArgRS in all four samples (Figure 1A, Supplementary Figure S1A). As arginine levels regulate central cellular signaling pathways (Bronte and Zanovello, 2005; Saxton et al., 2016), we tested whether nuclear ArgRS levels were affected by a reduction of extracellular arginine. We starved HEK 293T cells of arginine for 6 h by incubation in arginine-free medium and found a reversible decrease of nuclear ArgRS, while cytoplasmic levels were unchanged (Figure 1B).

Arginine depletion and inflammation reduce nuclear ArgRS *in vivo*

Next, we verified the effect of arginine starvation on nuclear ArgRS levels in a more physiological model by reducing systemic arginine in mice by intraperitoneal injection of recombinant murine Arginase-1. Plasma arginine was reduced to 30 - 50% (Supplementary Figure S1B), while nuclear ArgRS in the spleen (mostly from immune cells) of the arginine-depleted mice was reduced to < 40% of PBS controls (Figure 1C, $p = 0.03$).

As plasma arginine concentration was known to decrease during inflammation (Murray, 2016), we tested whether systemic inflammation in mice would similarly affect nuclear ArgRS levels by using the well-established liver toxin carbon tetrachloride (CCl_4) (Horiguchi et al., 2010). Plasma arginine levels dropped to 50% of vehicle controls (Supplementary Figure S1C) and nuclear ArgRS levels in the spleen were reduced by ~60% (Figure 1D). These findings indicated that a decrease in systemic arginine by itself and as a result of

inflammation induced by liver injury decreased nuclear ArgRS levels and focused our attention on the function of nuclear ArgRS (Figure 1E).

The ArgRS interactome contains nuclear proteins

To identify interaction partners of nuclear ArgRS, which could offer clues to its function, we enriched ArgRS from the nuclear fraction of HepG2 cells using ArgRS antibodies and characterized its protein interactome using mass spectrometry (Keilhauer et al., 2015). We used label-free quantification (LFQ) to compare intensities against an antibody isotype control and calculated p-values from three replicates (Supplementary Table S1-4). Proteins with a p-value < 0.05 were listed as significantly enriched (Figure 2A). All MSC proteins, except leucyl-tRNA synthetase (LeuRS, gene name *LARS*), were retrieved from the HepG2 nuclear fraction (Figure 2A, orange, Supplementary Table S1), suggesting that LeuRS may be absent or sub-stoichiometric in the nuclear MSC. Aminoacyl tRNA Synthetase Complex Interacting Multifunctional Protein 3 (AIMP3) was enriched but with a p-value > 0.05. Thus, by enriching only for ArgRS, we captured nearly all MSC interactions. This result suggests that the MSC is mostly intact in the nucleus of HepG2 cells but may be in a more dynamic form than in the cytoplasm.

Besides MSC components, we found several proteins with predominantly nuclear localization in the interactome of nuclear ArgRS (Figure 2A, yellow). To extend these results and avoid artifacts due to the isolation of nuclei, we repeated the experiment using whole cell lysates. Specific and reproducible binding (p-value < 0.05) to nuclear proteins was confirmed (Figure 2A, Supplementary Table S2), despite ArgRS being a predominantly cytoplasmic protein. Consistent with the known cytoplasmic composition of the MSC

(Kaminska et al., 2009), LeuRS was retrieved by ArgRS immunoprecipitation from the whole cell lysates of HepG2 cells (Figure 2A).

To test if these interactions are limited to the liver carcinoma cell line HepG2, we used the same approach on human embryonic kidney 293T cells (Figure 2A, Supplementary Table S3). Affinity enrichment of ArgRS retrieved the MSC and several proteins with predominately nuclear localization (Figure 2A, B). Comparison between the ArgRS interactomes in the two cell types showed significant overlap but also distinct binding partners (Supplementary Figure S1D). In contrast, the same experimental procedure in 293T cell lysates with an antibody specific to methionyl-tRNA synthetase (MetRS, gene name *MARS*), which is localized to another subcomplex within the MSC (Kim and Kim, 2020), yielded exclusively MSC proteins as significant interactors (p-value < 0.05, Figure 2A, Supplementary Table S4, Figure 2B, lower right).

ArgRS and SRRM2 interact and colocalize in nuclear speckles

Among the ArgRS binding partners, SRRM2 was highly and reproducibly enriched in both 293T and HepG2 cells (Figure 2A, B, upper right and lower left, Supplementary Table S5). We subsequently focused on SRRM2 because of its significance as a part of the spliceosome (Blencowe et al., 2000; Zhang et al., 2017; Bertram et al., 2020) and as an essential component for nuclear speckle formation (Hu et al., 2019; Ilik et al., 2020; Miyagawa et al., 2012). We confirmed the interaction between ArgRS and SRRM2 in both HepG2 and 293T cell lysates by reciprocal co-immunoprecipitation and western blotting (Supplementary Figure S1E, F), while MetRS, another component of the nuclear MSC, was not enriched by SRRM2 pulldown (Supplementary Figure S1G). These findings, together

with the lack of SRRM2 enrichment in the MetRS interactome (Figure 2B, lower right), indicated that ArgRS interacts with SRRM2 independently of the MSC (Figure 2C).

We next assessed where ArgRS and SRRM2 colocalized in intact cells. To verify that SRRM2 localizes to nuclear speckles, we visualized the speckle marker SC35 (also named SRSF2) using confocal microscopy with an antibody raised against an SC35 peptide. SRRM2 staining showed close to 75% overlap with SC35 in distinct structures in the nucleus, as expected for nuclear speckle proteins (Supplementary Figure S2A, Supplementary Video S1).

Immunofluorescence staining of ArgRS localized ArgRS in SRRM2-rich areas (Figure 3A, Supplementary Video S2, Supplementary Figure S2B, C for staining specificity and additional images). ArgRS colocalized with SRRM2 preferentially at the periphery of SRRM2 condensates (Figure 3A, Supplementary Video S2) as opposed to SC35, which co-localized with SRRM2 throughout its condensate-like structure (Supplementary Video S1). The Manders overlap coefficient, which denotes the correlation of absolute intensities, was calculated for individual cell nuclei and was on average over 0.6 for SRRM2 in ArgRS/SRRM2 co-staining (Figure 3D), suggesting that SRRM2 consistently colocalized with ArgRS. If the Costes method (Costes et al., 2004) was used for thresholding, colocalization increased to > 0.8 for both ArgRS and SRRM2 in the nucleus (Supplementary Figure S2D). In contrast, colocalization of another nuclear protein, the paraspeckle protein SFPQ, with ArgRS, led to a Manders coefficient of < 0.3 (Supplementary Figure S2E).

Arginine starvation significantly decreased the Pearson coefficient, which denotes the correlation of relative intensities, between ArgRS and SRRM2 ($p = 0.01$; Supplementary Figure S2F), in agreement with our finding that nuclear ArgRS levels are decreased by

arginine starvation. In accordance with our mass spectrometry results (Figure 2), the Manders coefficient for SRRM2/MetRS colocalization was only 0.13, as opposed to 0.65 for SRRM2/ArgRS (Figure 3B, D, Supplementary Video S3, $p < 0.0001$). If ArgRS interacts with SRRM2 while in the MSC, MetRS would be expected to colocalize with SRRM2 as well (Figure 3B, 3D). The majority of nuclear ArgRS did not colocalize with nuclear MetRS while cytoplasmic ArgRS and MetRS showed high colocalization (Manders coefficients 0.08 vs 0.72, Figure 3C, 3D, Supplementary Video S4), suggesting that the majority of nuclear ArgRS is not bound to the MSC. These results point to an ArgRS-specific, MSC-independent association with SRRM2 within or on the periphery of nuclear speckles (Figure 3E).

ArgRS depletion enhances SRRM2 mobility in nuclear speckles

Nuclear speckles contain splicing factors that are in steady exchange with the surrounding nucleoplasm to facilitate splicing (Galganski et al., 2017; Chen and Belmont, 2019). ArgRS co-localized with the core nuclear speckle protein SRRM2 in the periphery of SRRM2-dense signals. We wondered whether ArgRS might thereby influence SRRM2 mobility and trafficking based on the interaction we observed via mass spectrometry, co-immunoprecipitation, and co-localization via microscopy. To address this question, we used fluorescence recovery after photobleaching (FRAP) to monitor SRRM2 flux (Figure 4A). For FRAP measurements, we fluorescently labeled endogenous SRRM2 by introducing an mVenus-tag four amino acids from the C-terminus of SRRM2 (Supplementary Figure S2G). As expected, SRRM2-mVenus in 293T cells localized to nuclear regions in the same distinct pattern as antibody-stained endogenous SRRM2 (Supplementary Figure S2H). For FRAP experiments, regions of interest (ROIs) were chosen that contain one nuclear speckle including its surrounding nucleoplasm (Figure 4A). Bleaching of SRRM2-mVenus in

ROIs was followed by a time-dependent recovery of fluorescence that plateaued within 60 s (Supplementary Figure S2I) through influx of SRRM2 from the surrounding SRRM2-rich condensates and nucleoplasm (Figure 4A, Supplementary Video S5-7). About 50% of SRRM2 in the ROIs was immobile (Supplementary Figure S2J). A one-exponential curve fit of background-subtracted, normalized fluorescent recordings estimated a recovery half-time of 17 s in arginine-rich medium (Figure 4B, D). This half-time of recovery is 5x longer than that for the nuclear speckle marker protein ASF/SRSF1 (3 s, Phair and Misteli, 2000). If SRRM2 and SRSF1 have dynamic movements that reflect simple diffusion kinetics, where the rate of diffusion varies inversely with the square root of the molecular weight, then the 10-fold higher molecular weight of SRRM2 would predict a 3x difference in their dynamics for spherical objects. From this perspective, given the disordered structure of both proteins, but especially of SRRM2, a 5-fold higher half-time of recovery seems reasonable. Arginine starvation, which decreased the amount of nuclear ArgRS (Figure 1B), increased SRRM2 mobility as indicated by the shorter half-time of recovery of SRRM2 (11 s vs 18 s) (Figure 4B, Supplementary Figure S2K).

The above findings suggested that interaction of ArgRS with SRRM2 in the nucleus decreases SRRM2 mobility. To further investigate this possibility, we stably introduced shRNAs directed against the 3' UTR (shRARS_1) or 5' end (shRARS_2) of *RARS* mRNA. With both shRNAs, we reduced the ArgRS protein level in SRRM2-mVenus-expressing 293T cells to ~40% of that in cells expressing a non-targeting shRNA control (Figure 4C). This level of ArgRS knock down did not decrease cell viability or proliferation (Supplementary Figure S2L), in agreement with findings for knock downs of other aminoacyl-tRNA synthetases (Hayano et al., 2016; Ofir-Birin et al., 2013) and in a haplo-insufficient

mouse (Seburn et al., 2006). SRRM2 protein expression levels were not reduced by the ArgRS knock downs (Supplementary Figure S2M). In both ArgRS knock down cell lines the half-time of recovery in SRRM2-mVenus regions decreased significantly to ~70% of the control ($p = 0.048, 0.007$) (Figure 4D, Supplementary Video S5-7), consistent with the increased mobility of SRRM2 seen for arginine starvation. Collectively, these findings indicate that the SRRM2-ArgRS interaction slowed SRRM2 speckle-trafficking, and that this interaction is modulated by the external arginine concentration, which in turn controls the amount of ArgRS in the nucleus (Figure 4E).

ArgRS affects processing of mRNAs but not small ncRNAs

As SRRM2 is both a speckle organizer and spliceosome component, we hypothesized that arginine-mediated changes in the level of nuclear ArgRS, which impact SRRM2 exchange between speckles and nucleoplasm, could affect RNA processing. Because manipulation of arginine triggers several cross-talking cellular signaling pathways (Bar-Peled and Sabatini, 2014; Dong et al., 2000), we used ArgRS and SRRM2 knock downs as surrogates to assess how changes in SRRM2 mobility due to its interaction with ArgRS might affect gene expression. For this purpose, we stably knocked down ArgRS in HepG2 cells (to ~ 50% on the protein level and ~ 40% on the RNA level, Supplementary Figure S3A, B) and reset the cellular response to arginine by 6 h of arginine starvation followed by 2 h of arginine supplementation. In previous experiments, we lowered total cellular ArgRS by 90% without affecting tRNA aminoacylation or mRNA translation (Cui et al., 2021). Consequently, the milder knock down of ArgRS to 50% was not expected to affect ArgRS' housekeeping function in protein synthesis. We isolated RNA from a control shRNA-expressing cell line

(shSCR ctrl) and one expressing a shRNA against ArgRS (shRARS) and confirmed that the knock down did not impair cell proliferation (Supplementary Figure S3C).

For a comprehensive view of the effect of ArgRS depletion on cellular RNA synthesis, we used TGIRT-seq of chemically fragmented, rRNA-depleted cellular RNAs. This method employs the novel template-switching activity of the TGIRT enzyme for 3' RNA-seq adapter addition and enables profiling of coding and non-coding RNAs simultaneously (Figure 5A) (Qin et al., 2016; Nottingham et al., 2016; Boivin et al., 2018).

Comparing 21,540 different coding and non-coding RNAs showed relatively little difference in the proportion of different RNA biotypes between the ArgRS shRNA knock down and control shRNA (Figure 5B). Most differentially expressed genes were protein-coding genes (Figure 5C, D). The ArgRS knock down also resulted in numerous differences in exon usage for these genes (Supplementary Figure S3D). In contrast, we found only minor differences in the levels of small ncRNAs (Figure 5E-H) with only a small change in the relative abundance of only one tRNA (tRNA^{Asn}-GTT-10-1, p-value = 0.01) and no significant changes in the relative abundance of other tRNAs, including tRNA^{Arg} isodecoders (Figure 5H, Supplementary Table S6).

ArgRS knock down alters mRNA processing and protein isoforms

Due to the higher representation of small ncRNAs, the sequencing depth we achieved with TGIRT-seq for mRNA exon-exon junctions was insufficient to draw conclusions about the significance of changes in splice junction usage. Thus, we prepared and sequenced libraries from the same RNA preparations after pre-selection for poly(A)-containing mRNAs. We used vast-tools (Tapial et al., 2017) to evaluate previously annotated splice junctions based on reads spanning exons (in the following referred to as “alternative splicing events”). We first

identified alternative splicing events that were affected by ArgRS knock down compared to the shSCR control (Han et al., 2017; Irimia et al., 2014). For this purpose, we used the minimum value of the difference (denoted MV) as a measure of significance, where $MV > 0$ means a ≥ 0.95 probability that splice junction usage changed between the ArgRS knock down and the control (Han et al., 2017) (see Methods). These events were then further classified by $|\Delta PSI|$, which is defined as the % difference spliced-in. We thus identified 393 events in 345 genes with alternative splicing changes between the ArgRS knock down and control with $MV > 0$ (Figure 6A shows events with $|\Delta PSI| > 0.1$, Supplementary Table S7). These alternative splicing events corresponded predominantly to the inclusion or skipping of cassette exons and increased retained introns, while inclusion or skipping of microexons (3-27 nt) or use of alternative 5' or 3' splice sites were relatively unaffected by the ArgRS knock down (Fig. 6A).

We also analyzed differential transcript usage with DEXseq (Anders et al., 2012), which compares expression levels of individual exons to the rest of the gene as opposed to quantifying splice junction spanning reads. By using this complementary method, we identified 1,004 exons in 533 genes that were differentially expressed upon ArgRS knock down compared to the control ($p_{adj} < 0.05$, Figure 6B, Supplementary Table S8).

By using RT-PCR, we directly confirmed alternative splicing events upon ArgRS knock down for *FGFR3* and *PLEKHG5* in HepG2 cells (Supplementary Figure S4A, B). These genes displayed the same regulation in HEK 293T cells upon ArgRS knock down (Supplementary Figure S4A, B). Rescue of ArgRS knock down by overexpression of a *RARS* transgene restored the ratio of *FGFR3* splice variants to that of control cells (Supplementary Figure S4C, D).

To investigate if these splicing changes upon ArgRS knockdown led to an altered proteome, we assessed splice variants using mass spectrometry. As proteins derived from alternative splicing can only be identified from individual peptides (Blencowe, 2017), we sought to increase the sensitivity of detection by enriching these peptides. Therefore, we focused on events where alternative splicing would give rise to distinct N-termini and enriched N-terminal peptides by removing all other (tryptic) peptides (Kleifeld et al., 2011). We identified 167 peptides from 135 proteins (Supplementary Table S9) that were detected at higher or lower intensity upon ArgRS knock down ($|\text{pdiff}| > 1$). Ten proteins were encoded by genes with differential exon usage and seven by genes with alternative splicing events upon ArgRS knockdown (Supplementary Figure S4E), including splicing changes that affected peptides in the proximity of potential alternative translation start sites (Supplementary Figure S4F). For the splicing factor PTBP1, we saw an increase of the N-terminal peptide for one isoform while the N-terminal peptide of another isoform was simultaneously reduced (Supplementary Figure S4F). Differences in PTBP1 isoform usage were confirmed by western blots (Supplementary Figure S4G). Collectively, these findings indicate that ArgRS knock down at levels that do not affect cell proliferation results in alternative splicing changes that lead to different protein isoforms.

Identification of SRRM2-dependent splicing changes

Nuclear ArgRS interacts with and impedes SRRM2 mobility in the nucleus (Figure 4) and could thereby reduce the levels of SRRM2 available for RNA splicing. To identify alternative splicing events that are sensitive to decreasing levels of functional SRRM2 and might therefore be regulated by its interaction with ArgRS, we knocked down SRRM2 by 50% (RNA and protein level, Supplementary Figure S5A). This knockdown led to 1,033

alternative splicing events in 807 genes with $MV > 0$ (Figure 6C shows events with $|\Delta PSI| > 0.1$, Supplementary Table S10) and 42,140 differential exon usage events in 8,563 genes that were changed relative to the control shRNA (Figure 6D, Supplementary Table S11, $p_{adj} < 0.05$). The numbers of changed alternative splicing and differential exon usage events were much higher than those for the ArgRS knock down (393 and 1,004, respectively) (Figure 6A), as expected for a spliceosome component, whose activity might be subject to multiple modes of regulation. Cassette exons were changed in both directions upon SRRM2 knock down while introns were increasingly spliced out (Figure 6C). In order to rule out that the alternative splicing changes were due to off-target effects of the SRRM2 knock down with a single shRNA, we transfected 293T cells with a pool of siRNAs against SRRM2. Alternative splicing of *FGFR3* and *CDK5RAP3* genes could also be observed upon the reduction of SRRM2 with an heterogenous pool of siRNAs, suggesting that this was a direct consequence of lowered SRRM2 availability (Supplementary Figure S5B).

ArgRS and SRRM2 modulate alternative splicing of a set of genes in opposite directions

After obtaining lists of genes that were sensitive to reduction of either SRRM2 or ArgRS, we next set out to identify which genes were shared between both and might be regulated by the interaction between ArgRS and SRRM2 described above. To control for indirect effects due to knockdown of an aaRSs, we also knocked down MetRS to 10% on RNA and protein level (which is lower than the ArgRS knock down, Figure S3A) as a control (Supplementary Figure S6A, Supplementary Table S12). We identified 602 alternative splicing events in 493 genes ($|MV| > 0$, events with $|\Delta PSI| > 0.1$ shown in Supplementary Figure S6B) and 50,896

differentially expressed exons in 7,668 genes that changed upon MetRS knock down (Supplementary Figure S6B).

A total of 282 alternative splicing events overlapped between the ArgRS, SRRM2, and MetRS knock downs (Supplementary Figure S6C, Venn diagram, $MV > 0$, no criterion for $|\Delta PSI|$ to depict all events regardless of magnitude). Of these, 70 splicing events were shared between the SRRM2 and ArgRS knock downs (Supplementary Figure S6C) and their $\Delta PSIs$ were negatively correlated ($R = -0.29$, $p = 0.037$). We then compared the directionality of the overlapping splicing events between ArgRS and SRRM2 knock downs and identified 27 of the 70 shared alternative splicing events (38.6%) that were inversely regulated by ArgRS and SRRM2, meaning that the knock down of ArgRS led to a $\Delta PSI > 0$, while knock down of SRRM2 led to a $\Delta PSI < 0$ or vice versa (Supplementary Figure S6D, inner brown circle, $MV > 0$, hypergeometric test for overrepresentation $p = 0.01$). In contrast, only 12/185 splicing events (6.2%) were inversely regulated by MetRS and SRRM2 (Supplementary Figure S6D, $MV > 0$, hypergeometric test $p = 1$). We confirmed splicing changes by RT-PCR for prominently changed events (*CDK5RAP3*, *IL32*, *RAD52*, and *STX16*, all Supplementary Figure S6E).

If focusing on genes that were alternatively spliced and shared between the ArgRS and SRRM2 knock downs (instead of individual splicing events within a gene that were shared), close to 500 genes had splicing events or differentially expressed exons that were inversely regulated by ArgRS and SRRM2 (72 and 475, respectively, Figure 6E, F). These findings indicate that specific genes are inversely regulated by ArgRS and SRRM2 in their alternative splicing and could lead to different protein isoforms (Figure 6G).

ArgRS and SRRM2-regulated splicing alters cellular metabolism and communication

To explore the possible biological consequences of the ArgRS/SRRM2 interaction, we examined the enrichment of pathways and GO terms in the genes changed in opposite directions in the ArgRS and SRRM2 knock downs (Figure 7A, B, Supplementary Figure S7A-E). Among these were genes that might benefit the cellular response to inflammation such as the pro-inflammatory cytokine IL32 (Supplementary Figure S6E) (Aass et al., 2021). Overrepresented pathways included the mTORC1-pathway, which can be activated by arginine (Figure 7B).

To investigate the functional consequences of differential exon usage that might be regulated by the ArgRS/SRRM2 interaction, we chose several mTORC1-associated genes (Supplementary Table S13, Supplementary Figure S7F) and compared the enzymatic activity of their encoded proteins after ArgRS or SRRM2 knock down. The enzyme Isocitrate dehydrogenase 1 (IDH1) responds to cell stress by regulating intracellular NADP/NADPH levels (Itsumi et al., 2015). Differential exon usage in *IDH1* correlated with an increase in IDH enzymatic activity in cell lysates upon SRRM2 knock down while ArgRS knock down led to decreased activity (Figure 7C). Differential exon usage was also found for genes encoding components of the ubiquitin/proteasome system (UPS), including the 19S proteasomal subunit de-ubiquitinating enzyme UCHL5 and the catalytically active proteasomal subunit PSMB5 ($\beta 5$). These correlated with increased $\beta 5$ activity upon SRRM2 knock down and decreased $\beta 5$ activity upon ArgRS knock down (Figure 7D).

The UPS provides peptides that are presented on the cell surface by the major histocompatibility complex I (MHCI) and alternative splicing of UPS components, such as the $\beta 5$ proteasomal subunit, could result in differences in peptide processing and consequentially peptides presented to immune cells by MHCI. Additionally, other splicing

changes due to ArgRS and SRRM2 knock down that result in different protein isoforms could yield alternative peptides directly through differences in amino acid sequence and alternative protease cleavage sites. These MHCI-bound peptides communicate the status of the presenting cell to immune cells (Rock et al., 2016).

To investigate whether communication to immune cells through MHCI might be changed by ArgRS and SRRM2 knock down (Figure 7E), we compared their MHCI-bound peptidome using mass spectrometry. The detected MHCI-bound peptides matched previously described MHCI-bound peptides in length and a preference for the amino acids Tyr and Leu in position 2 (Bassani-Sternberg et al., 2015) (Supplementary Figure S7G). Overall, MHCI protein levels and the spectral counts for the measured peptides were comparable between all treatments (Supplementary Figure S7G, Figure 7F). However, upon sequencing of the peptides presented by MHCI by mass spectrometry, we found that ArgRS knock down substantially reduced the peptide diversity displayed on MHCI (20 vs 104). In contrast, SRRM2 knock down led to the presentation of a comparable number of different peptides by MHCI as the shSCR control (81 vs 104, Figure 7F, see also Supplementary Figure S7H). Nineteen of these peptides were unique for the SRRM2 knock down cell line and 41 were unique for shSCR control, suggesting that SRRM2 knock down led to the presentation of an altered set of MHCI-bound peptides (Figure 7F, Supplementary Figure S7H).

In order to predict how these MHCI peptide pools would influence communication with immune cells, we calculated MHCI immunogenicity scores (Figure 7F). A higher score predicts that these peptides would be more likely recognized by T cells and therefore more likely to elicit an immune response (Calis et al., 2013). Consistent with our model, in which ArgRS knock down mimics inflammation, MHCI peptides presented in ArgRS knock down

cells had a higher median score than peptides that were exclusively presented by SRRM2 knock down cells (Figure 7F). Collectively, these findings support the hypothesis that ArgRS and SRRM2 regulate cellular metabolism and communication to contribute to the cellular response to inflammation (Figure 7G).

Discussion

Here we found that a portion of ArgRS localizes to the cell nucleus and that nuclear ArgRS levels fluctuate in an arginine-dependent manner (Figure 1). To characterize the function of ArgRS in the nucleus, we assessed the ArgRS interactome and found that ArgRS interacted with the serine/arginine repetitive matrix protein SRRM2 (Figure 2) and that SRRM2 and ArgRS colocalized to nuclear condensates (Figure 3). We showed that both arginine starvation and depletion of ArgRS increased SRRM2 mobility (Figure 4), suggesting that the nuclear presence of ArgRS impedes SRRM2 trafficking. Upon ArgRS knock down, depletion of nuclear ArgRS and the resulting altered SRRM2 mobility correlated with numerous changes in SRRM2-dependent alternative splicing, while in contrast, small noncoding RNAs were largely unaffected by the ArgRS knock down (Figure 5, 6). We found further that SRRM2 and ArgRS knock downs modulated alternative splicing of a subset of genes in opposite directions, supporting the hypothesis that ArgRS-SRRM2 interaction affects SRRM2's function (Figure 6). Two inversely regulated genes, *IDH1*, which encodes isocitrate dehydrogenase 1, and *PSMB5*, which encodes the $\beta 5$ subunit of the 20S proteasome, showed that the inversely regulated alternative splicing changes had opposite effects on the biochemical activities of the encoded proteins. These changes in enzymatic activity impacted cellular metabolism and, together with alternative splicing changes in other

proteins, led to altered MHCI peptide presentation in a manner consistent with a cellular response to inflammation (Figure 7).

Arginine levels decrease during inflammation as pro- and anti-inflammatory enzymes compete for arginine as a substrate (Bronte and Zanovello, 2005). Eventually, the depletion of arginine leads to the resolution of inflammation due to a restraining effect on immune cells, especially T cells (Geiger et al., 2016; Murray, 2016). Therefore, the ArgRS/SRRM2 interaction and its consequences could have alternative outcomes at different stages of inflammation. Additionally, arginine is a key regulator of the mTOR pathway (Saxton et al., 2016; Wyant et al., 2017), and we found that expression of functionally diverse splice variants of mTORC1 pathway-associated genes (Figure 7B) - driven by the ArgRS/SRRM2 interaction - had consequences for multiple functions of that pathway. Because manipulation of arginine triggers several cross-talking cellular signaling pathways (Bar-Peled and Sabatini, 2014; Dong et al., 2000), we focused on using ArgRS knock downs to specifically study ArgRS' relationship with SRRM2. The observed downregulation of IDH1 activity and thereby NADPH production by ArgRS knock down for example would support the metabolic shutdown initiated by mTOR inhibition upon arginine depletion.

We showed previously that ArgRS' presence in the nucleus is dependent on its presence in the MSC as nuclear ArgRS was strongly reduced upon exclusion of ArgRS from the MSC (Cui et al., 2021). Here, we found that ArgRS – once in the nucleus – interacted with SRRM2 independently of the MSC, as affinity enrichment of ArgRS, but not MetRS, another component of the MSC, retrieved SRRM2 (Figure 2B, lower right). These finding suggest

that in addition to tRNA quality control (Lund and Dahlberg, 1998), the nuclear MSC might act as a reservoir of nuclear aaRSs with regulatory functions that are sequestered until a cue induces their release. This concept is similar to that suggested for the cytoplasmic MSC (Ray et al., 2007). Operationally, this function of the MSC resembles that of nuclear speckles, where highly disordered, low complexity proteins are sequestered (Galganski et al., 2017). We hypothesize that both the MSC and nuclear speckles are dynamic reservoirs in steady exchange with their surroundings that enable the correct localization and the controlled release of their components. ArgRS, with changing arginine levels as a cue, thus connects two distinct protein organizational structures with central roles in transcription and translation. Due to their ability to sense and respond to changes in amino acids levels and interact with a variety of other proteins, aaRSs are uniquely poised to function in metabolite sensing and signal transduction (Netzer et al., 2009; Sajish and Schimmel, 2015; Son et al., 2019).

ArgRS and SRRM2 colocalized more towards the interface between nuclear speckles and nucleoplasm as compared to SRRM2 and SC35, another speckle marker (Supplementary Video S1, S2). mRNA processing and transcription have been shown to take place at or in close proximity to nuclear speckles (Chen and Belmont, 2019). A recent model suggested that splice site selection is regulated by partial immersion of unspliced pre-mRNAs in condensates at the interface between nuclear speckles and nucleoplasm (Liao and Regev, 2021). The interaction between ArgRS and SRRM2 could therefore impact alternative mRNA splicing directly by disrupting SRRM2's function within the spliceosome and/or by regulating trafficking of SRRM2 to the nuclear speckle-nucleoplasm interface.

489

490 SRRM2 is essential for nuclear speckle formation (Ilik et al., 2020) and simultaneously a
 491 component of active spliceosomes (Bertram et al., 2020). The N-terminal 1% of SRRM2 is
 492 immobilized sufficiently in the spliceosome to be resolved by cryo-EM while the remaining
 493 99% is disordered (Bertram et al., 2020). SRRM2 would therefore be an ideal candidate to
 494 anchor spliceosomes to the nuclear speckle interface. Due to its large size and many
 495 phosphorylation sites, SRRM2 provides ample interaction surface for other splicing
 496 regulatory proteins to gather in the proximity of the spliceosome and in nuclear speckles.
 497 Our results suggest that these functions of SRRM2, which are dependent on its constant
 498 trafficking, could be impeded by SRRM2 sequestration by ArgRS (Figure 3).

499

500 A previous study in a murine liver cell line, in which SRRM2 was more severely depleted
 501 than in our study, found that immune response genes were upregulated in their expression
 502 and mRNA splicing (Hu et al., 2019). Here, we show that modulation of SRRM2 levels
 503 impacted cellular metabolism and MHC I peptide processing (Figure 7). MHC I presentation
 504 allows cells to communicate with the immune system (Rock et al., 2016). In pathological
 505 scenarios, such as viral infection and cancer, an altered MHC I peptidome could induce a
 506 different immune response. We found that ArgRS knock down, which mimics arginine
 507 starvation by leading to decreased concentrations of ArgRS in the nucleus, led to a decrease
 508 in peptide diversity without reducing the total amount of detectable MHC I-presented
 509 peptides (Figure 7F). These peptides were also predicted to be more immunogenic (Figure
 510 7F) and might therefore be preferentially recognized for example by cytotoxic T cells during
 511 cancer and viral infections, in which inflammation is induced. During inflammatory

conditions, the reduction of peptide diversity and increase in immunogenicity presented by MHCI upon ArgRS knock down could enable more efficient identification by the immune system. On the other hand, SRRM2 knock down led to the presentation of a different set of MHCI peptides as compared to control (Figure 7F), suggesting that sequestration of SRRM2 by ArgRS could potentially increase the diversity of MHCI peptide presentation. Those MHCI peptides exclusively found on SRRM2 knock down cells were predicted to be less immunogenic (Figure 7F). Recently, splicing inhibitors have been used to modulate the MHCI peptidome to increase sensitivity to immune therapy in cancer (Lu et al., 2021) and our findings suggest that a comparable mechanism to modulate and diversify MHCI peptide variety is employed during inflammation. Therefore, the interplay of arginine, ArgRS, and SRRM2 would enable cells to not only detect ongoing extracellular inflammation by picking up metabolic cues but also by responding adequately by adapting their proteome and tuning the communication with immune cells during inflammation.

Data availability

TGIRT-seq data are deposited in the NCBI sequence read archive and accessible through the BioProject number PRJNA561913. Poly(A) RNA-seq data are deposited GEO GSE165513. Mass spectrometry data is deposited on ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifiers PXD015692 (interactomes), PXD024091 (N-terminomics), and PXD027531 (MHCI peptidome).

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Author contributions

HC planned and conducted experiments and analyzed data. JKD, JJM, and JRY performed, analyzed, or supervised mass spectrometry experiments. DCW, RMN, and AML performed, analyzed, or supervised TGIRT-seq and RNA-seq library preparation and sequencing. JJL and BB analyzed RNA-seq results. PS, AML, and HC designed research and wrote the manuscript with input and contributions from all authors.

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Declaration of Interests

Thermostable Group II Intron Reverse Transcriptase (TGIRT) enzymes and methods for their use are the subject of patents and patent applications that have been licensed by the University of Texas and East Tennessee State University to InGex, LLC. AML and the University of Texas are minority equity holders in InGex, and AML, some members of AML's laboratory, and the University of Texas receive royalty payments from the sale of TGIRT enzymes and the licensing of intellectual property by InGex to other companies.

Figure legends

Figure 1: Arginyl-tRNA synthetase (ArgRS) localized to the nucleus in response to external arginine levels. (A) ArgRS in the cytoplasmic and nuclear fractions of human hepatocellular carcinoma HepG2 or human embryonic kidney (HEK) 293T cells. (B) ArgRS in the nucleus (left) and cytoplasm (right) following cell fractionation. Nuclear ArgRS could

be restored by addition of external arginine (0x/10x). Arginine concentrations are relative to DMEM. $p = *0.02$. (C) Injection of recombinant Arginase-1 reduced nuclear ArgRS in the spleen of mice. $p = *0.03$. (D) Systemic inflammation by CCl₄ reduced nuclear ArgRS in the spleen of mice. (A-D) Western blots and densitometric quantification. GAPDH: cytoplasmic marker, Tubulin: cytoplasmic marker, Histone H3: nuclear marker. (E) Scheme: Reduction of arginine during inflammation leads to reduced nuclear ArgRS.

Figure 2: Characterization of the ArgRS interactome revealed nuclear proteins as interaction partners. (A) Comparison between the ArgRS interactome in nucleus, HepG2 whole cell lysate, 293T whole cell lysate, and with the interactome of MetRS, another tRNA synthetase. Multisynthetase complex (MSC) components: orange. Nuclear proteins: yellow. Proteins are identified by gene names and listed in order of enrichment over a non-targeted antibody of the same isotype with fold-change noted in parentheses. (B) Volcano plots of ArgRS or MetRS interactomes. MSC proteins are highlighted by larger symbol size. Axes denote enrichment (Welch's t-test enrichment, x-axis) and reproducibility between 3 repeats (Welch's t-test significance, y-axis). Color: number of unique peptides detected. Line: p -value = 0.05 (C) Scheme: The MSC is intact in the nucleus and ArgRS interacts with SRRM2 independent of the MSC.

Figure 3: SRRM2 colocalized with ArgRS but not with MetRS. (A-C) Confocal microscopy following immunofluorescent staining of ArgRS/SRRM2 (A), MetRS/SRRM2 (B), or ArgRS/MetRS (C), respectively. A single slice of a series of z-stacks is shown. Colocalized areas in yellow. Bar: A, B: 2 μ m. C: 5 μ m. (D) Manders coefficients corresponding to panels A-C. Hoechst staining was used to identify the cell nucleus. Manders coefficient was calculated for individual cell nuclei (A, B, $n = 11, 12$) or for an

average for 2-3 cells (C, n = 8). (E) Scheme: ArgRS leaves the multisynthetase complex in the nucleus to interact with SRRM2 in the proximity of nuclear speckles.

Figure 4: ArgRS knock down increased SRRM2 mobility. (A) Fluorescence recovery after photobleaching (FRAP). SRRM2-mVenus-dense areas were bleached, and recovery was monitored over 90 s. SRRM2-mVenus is displayed as a 16-color lookup table with white and red indicating the highest intensity and blue and green the lowest. (B) Arginine starvation reduced SRRM2-mVenus FRAP half-time $t_{1/2}$ to 60% as compared to high arginine. Arginine concentrations relative to DMEM, n = 8, p = **0.001. (C) Stable knock down of ArgRS in SRRM2-mVenus cell lines with two different shRNAs (shRARS_1, shRARS_2) reduced ArgRS protein levels to 40% of a non-targeting shRNA control, shown by western blot. Relative *RARS* mRNA levels quantified by qRT-PCR: shSCR: 1.00 ± 0.17 ; shRARS_1: 0.20 ± 0.17 ; shRARS_2: 0.07 ± 0.06 . (D) Left panel: Recovery of fluorescent signal after photobleaching. Errors bars depict standard error of the mean. Right panel: $t_{1/2}$ of SRRM2-mVenus recovery time after bleaching was reduced to 70% by stable ArgRS knock down. shSCR: n = 10, shRARS_1: n = 11, shRARS_2: n = 12, p = *0.048, **0.007. (E) Scheme: ArgRS impedes SRRM2 trafficking by slowing the exchange between condensates and nucleoplasm.

Figure 5: ArgRS knock down primarily affected mRNAs but not non-coding RNAs. (A) Stacked bar graphs showing RNA biotype distributions. (B) Scatter plot comparing RNAs. ρ is the Spearman's correlation coefficient. (C-H) Volcano plots comparing all and different RNA biotypes in ArgRS knock down versus control HepG2 cells. Vertical dashed lines depict a 1.5-fold change and horizontal dashed line a $p_{adj} < 0.05$. Differential expression of (C) all RNAs, (D) mRNAs, (E) small non-coding RNAs, (F) microRNAs, (G) small nucleolar RNAs,

and (H) transfer RNAs. (A-H): ctrl: HepG2 shSCR control. shRARS: HepG2 ArgRS knock down. Three replicate TGIRT-seq datasets.

Figure 6: Differential mRNA processing upon ArgRS knock down. (A) Splicing event changes upon ArgRS knock down (shRARS). (B) Volcano plot of differentially expressed exons upon ArgRS knock down (shRARS). (C) Splicing event changes upon SRRM2 knock down. (D) Volcano plot of differential exon usage upon SRRM2 knock down. (A, C): Splicing events divided by event type, relative to shSCR control, as detected by RNA-seq. Events with a $|\Delta\text{PSI}| > 0.1$, $\text{MV} > 0$ are shown. Increased event frequency shown in cyan, events with decreased frequency in pink. PSI: percent spliced in. $\text{MV} > 0$: significant splicing changes. Cassette: cassette exons/exon skipping, microexons: 3-15 nucleotide exons, alt 3': alternative 3' end splice site, alt 5': alternative 5' end splice site, intron: intron retention. 115814 (shRARS) and 111346 (shSRRM2) events were detected in total. (B, D) Dashed vertical lines: 2-fold change, dashed horizontal line: p-value $1\text{e-}6$. (E, F) Venn diagram of genes with shared alternative splicing events or differentially expressed exons upon ArgRS and SRRM2 knock down. Genes with inversely regulated (E) splicing events ($\Delta\text{PSI} > 0$ for shRARS and $\Delta\text{PSI} < 0$ for shSRRM2 or vice versa) or (F) differentially expressed exons ($\log_2\text{fold change} > 0$ for shRARS or < 0 for shSRRM2 or vice versa) in brown circle. Hypergeometric test for enrichment of inversely correlated, ArgRS regulated splicing events. (A-F) Data was derived from sequencing of 3 replicates. All changes relative to shSCR control. (G) ArgRS slows down SRRM2 trafficking and thereby disrupts SRRM2-dependent mRNA splicing changes, leading to the regulation of alternative mRNA splicing in opposite directions by ArgRS and SRRM2.

Figure 7: SRRM2- and ArgRS-regulated mRNA processing led to metabolic changes and an altered MHCI peptidome. (A) MSigDB and Gene Ontology (GO) analysis of genes with inversely regulated splicing events upon ArgRS and SRRM2 knock down. (B) MSigDB and GO analysis of genes with inversely regulated differentially expressed exons upon ArgRS and SRRM2 knock down. (A, B) GO terms associated with RNA-interacting proteins in fuchsia, GO terms associated with cellular organization and organelles in blue. (C) IDH1 enzyme activity. IDH activity assay: representative of 3 repeats, 12 technical replicates, $p < ****0.0001$. (D) PSMB5 differential exon usage, $\beta 5$ proteasomal subunit (PSMB5 protein) activity. Quantification of differentially used exon: $\beta 5$ activity: representative of 3 repeats, 4 replicates, $p = **0.006$, $p < ****0.0001$. (E) Model of ArgRS and SRRM2 knock down, their connection to inflammation, and consequences on their splice targets. (F) Total spectral counts (upper left) correspond to peptide quantity. Venn diagrams of identified MHCI peptides by mass spectrometry following isolation of MHCI complex (upper right) found in all replicates upon ArgRS or SRRM2 knock down. Bottom: Violin plot of MHCI immunogenicity score for peptides found in all samples, shSCR ctrl, ArgRS knock down, and SRRM2 knock down only. Median MHCI immunogenicity score is indicated by a black line. (A-F) shSCR ctrl: HepG2 shSCR control. shRARS: HepG2 ArgRS knock down. shSRRM2: HepG2 shSRRM2 knock down. (G) Model of cellular response to inflammation by arginine/ArgRS/SRRM2.

Material and Methods

Further information and requests for resources and reagents should be directed to and will be fulfilled by Prof. Paul Schimmel (schimmel@scripps.edu).

Materials Availability

All unique reagents generated in this study are available from Prof. Paul Schimmel, with a completed Materials Transfer Agreement.

Data and Code Availability

TGIRT-seq data are deposited in the NCBI sequence read archive and accessible through the BioProject number PRJNA561913. Poly(A) RNA-seq data are deposited at GEO (GSE165513). Mass spectrometry search results and raw data are deposited in the PRIDE archive as PXD015692 (interactomes), PXD024091 (N-terminomics), and PXD027531 (MHCI peptidome).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mouse model

6-8-week-old, female C57BL6/J mice from the Scripps Research breeding facility were used. Animals with the same birthdate were randomly assigned to experimental groups. All animals were sacrificed in accordance with animal protocol 19-0023 which was approved by the Scripps Research IACUC Department. Staff and researchers were wearing scrubs, facemasks, and gloves while handling animals. The rooms were kept between 69-78 degrees Fahrenheit. Individually ventilated rack caging system consisted of a cage bottom, wire bar lid, and filter top, which were sanitized in a cage wash. Feed was commercially

available laboratory grade rodent diet. Purified water through reverse osmosis was provided through an automatic watering system. Cages were changed approximately every 10-14 days. Groups consisted of mice injected with (I) 100 μ l 35 mg/ml murine Arginase, (II) 100 μ l PBS control, (III) 50 μ l 20% CCl₄ in olive oil, or (IV) 50 μ l olive oil.

Cell lines

HepG2 human hepatocellular carcinoma cells and human embryonic kidney (HEK) 293T cells were cultured in high glucose DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS, Omega Scientific) and penicillin/streptomycin (Gibco). Both cell lines were regularly tested for mycoplasma contamination with a PCR-based, in-house assay and were consistently negative. HepG2 and 293T cells were verified by the ATCC Cell Line Authentication service using STR Profiling Results. Both cell lines were found to be a 93% match with the reference cell line profile. HepG2 cells were originally derived from a male donor and HEK 293T cells from a female embryo. Murine embryonic fibroblasts (MEF) cells were a kind gift from Yao Tong (Scripps Research). MEFs were generated from C57Bl/6J embryos around E13.5 and used at cell passage 3. The sex of the embryos that were used for MEF generation was not determined.

Statistics

Student's t-test was used to determine significance between groups, except for mass spectrometry data for which Welch's t-test was used. Repeats are different cell passages if not noted otherwise. For densitometric analysis, paired t-test were used as repeats were run on individual gels. GraphPad Prism 8 was used for testing except for large datasets. Significance in large datasets was determined with Perseus for mass spectrometry results, DESeq2 in TGIRT-seq and RNA-seq, DEXSeq in exon analysis, and Vast-Tools in splice

junction usage. Correlation coefficients were calculated with Kendall Rank correlation or Pearson correlation and enrichment of gene sets by hypergeometric testing, both in R. If not stated otherwise, significance was defined as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Arginine depletion in mice

To enzymatically deplete systemic arginine, mice were i.p. injected with 35 mg/kg recombinant, LPS-free (6.15 EU/mg) murine Arginase-1 purified from Clear *E. coli* (Lucigen) and sacrificed 5-7 hours later. Arginase-1 activity was tested by measuring conversion of arginine to urea. Purified Arginase-1 was mixed with a reaction buffer containing 320 mM arginine, 100 mM Hepes pH 8.5, and 10 mM CoCl_2 . The reaction was incubated for 45 min at 37°C in a thermocycler. Urea was detected by a colorimetric assay monitoring the conversion of α -Isonitrosopropiophenone. α -Isonitrosopropiophenone was dissolved in ethanol (2 g/50 ml) and freshly diluted 1:10 in 1:1:3 water:sulphuric acid:phosphoric acid to make urea detection reagent. Urea detection reagent was mixed with the Arginase-1 reaction product or a urea standard 5:1 and incubated in a thermocycler for 60 min at 100°C followed by 15 min at 25°C.

CCl_4 (Sigma Aldrich) was used to elicit systemic inflammation. Mice were i.p. injected with 50 μl 20% CCl_4 in olive oil, or olive oil only, and sacrificed 30 h later. In all experiments, blood was collected from the inferior vena cava with EDTA-coated syringes and plasma was separated by centrifugation at 500 x g for 3 minutes at 4°C.

Arginine determination in plasma

5 μl 250 μM $^{13}\text{C}_6$ -arginine (Pierce) was spiked in 20 μl plasma as an internal control. Plasma was precipitated with 200 μl 80% ice-cold methanol and dried in a speedvac. Metabolites were resuspended in 40 μl water and incubated with 20 μl freshly-made 1% Marfey's reagent

in acetone (w/v) and 4 μ l 1 M sodium bicarb for 1 h at 40°C in a thermocycler. Derivatized amino acids were dried in a speedvac and resuspended in 40 μ l 40% methanol. Samples were measured in the Scripps Research open access mass spectrometry core using an Agilent 6125 single quadrupole mass spectrometer coupled to an Agilent 1260 LC stack. The column used was an Agilent SB-C8 5 μ m 300 A 4.6x50 mm running at a flowrate of 0.5 ml/min. Mobile phases consisted of A (H₂O/0.1% formic acid) and B (ACN/0.1% formic acid). Spray chamber conditions were dry gas flowrate = 12 L/min at 350 C, nebulizer pressure = 35 psi and capillary voltage = 4000 V for pos mode, and 3500 V for neg mode. 20 μ l sample were injected and eluted with a 15 ml 10-35% A to B gradient. Derivatized arginine eluted at 9.3 ml. Spectra were collected in a mass range of 150-600 for both positive and negative mode and additionally in the 420-430 Da and 430-440 Da range.

Modulation of arginine levels

For the modulation of arginine in tissue culture, SILAC DMEM with dialyzed FBS and substituted with lysine was used as a base medium. Arginine concentrations are given as relative to 1x DMEM (0.084 g/l L-arginine monohydrochloride, dissolved in PBS). For 0x, arginine concentrations were under the detection limit of the assay described above. Cells were deprived of arginine for 4-6 hours and arginine was added back at the indicated concentrations for 2 h.

Cell fractionation

Cultured cells were seeded at a density of 5 x 10⁶ cells per 10 cm tissue culture dish the day before (293T, MEF) or at 2.5 x 10⁶ per 10 cm tissue culture dish two days prior (HepG2) to harvest in 500 μ L cell fractionation buffer (20 mM HEPES, pH 7.5, 10 mM KCl, 2 mM MgCl₂, 2 mM EDTA, 1 mM dithiothreitol (DTT), protease inhibitor). Spleens of arginine-depleted or

control mice were homogenized by passing through a 40 μ m cell sieve. Mouse liver was harvested from 4-week old C57Bl/6J mice and homogenized using a 40 μ m cell sieve. The cell suspension was left on ice for 20 minutes for osmotic lysis and 100 μ L were taken as whole cell lysate. The remaining cells were passed 10 times through a 27-gauge needle. Nuclei and cytoplasm were separated by centrifugation at 750x g for 5 minutes. The supernatant was taken as cytoplasmic fraction and the nuclear pellet washed three times with cell fractionation buffer. 10x RIPA buffer (0.5 M Tris/HCl, pH 7.4, 1.5 M NaCl, 2.5% Deoxycholate, 10% IGE-PAL CA-630, 10 mM EDTA) was added to 2x final concentration to all fractions. Complete lysis was achieved by freezing and thawing. Lysates were spun down at 14,000x g for 20 min to remove insoluble components and SDS loading buffer was added before western blot analysis.

Affinity enrichment/co-immunoprecipitation

Two days prior to the experiment, 1×10^7 293T or 5×10^6 HepG2 cells were seeded on a 15 cm tissue culture dish. Cells were lysed for 20 minutes under mild conditions in 1% IGE-PAL CA-630 (Sigma Aldrich) in Tris-buffered saline (TBS) with protease (Pierce Protein Biology) and phosphatase inhibitors (Pierce Protein Biology). Insoluble components were pelleted by centrifugation at 14,000x g for 20 minutes. Protein A/G agarose beads (30 μ L, Santa Cruz Biotechnology) were equilibrated in TBS. Cell lysate was added to the beads together with 2 μ L antibody against ArgRS (Biorbyt). For immunoprecipitation of SRRM2, antibody against SRRM2 (Santa Cruz Biotechnology) was conjugated to beads using the Co-Immunoprecipitation kit (Pierce Protein Biology) according to the manufacturer's instructions. Immunoprecipitation was performed for 3 h (ArgRS) or overnight (SRRM2). After incubation, the beads were washed twice with TBS containing 0.1% IGE-PAL CA-630

and twice with TBS only. Precipitated proteins were either eluted using 0.1 M glycine, pH 3, for western blot or by tryptic digestion for interactome analysis.

Western blot

Cell lysate from different cellular fractions or immunoprecipitated proteins were run on a 4-15% gradient gel (Bolt, ThermoFisher Scientific) and subsequently blotted on a PVDF membrane using an iBlot or iBlot2 device (ThermoFisher Scientific). Membranes were blocked for at least one hour with 5% bovine serum albumin (BSA) or 1% dry milk in TBS-T. Primary antibody was incubated overnight at 4°C at a 1:5000 (ArgRS: Biorbyt, GAPDH, Tubulin, and Histone H3: Cell Signaling Technology, MetRS: Abcam, GFP, PTBP1: Proteintech Group) or a 1:100 (SRRM2, Santa Cruz Biotechnology) dilution in 5% BSA/TBS-T. The next day, blots were washed three times for at least 10 minutes per wash in TBS-T and HRP-labeled secondary antibody (Invitrogen) was added at a 1:10,000 (anti-rabbit) or 1:5000 (anti-mouse) dilution in BSA/TBS-T or milk/TBS-T for at least 90 minutes. After three additional washes, membranes were developed with ProSignal Dura ECI (Genesee Scientific) and imaged with a FluorChem M (Proteinsimple).

ArgRS interactome

Proteomes were eluted by incubation with 25 µL 2 M urea, 5 ng/µL trypsin (Pierce Protein Biology), 1 mM DTT in 50 mM Tris/HCl, pH 7.5. After 30 minutes at room temperature, 5 mM chloroacetamide (Sigma Aldrich) was added for alkylation of cysteine residues to a total volume of 125 µL. Samples were digested overnight at 37°C and quenched with 0.5% trifluoroacetic acid (Sigma Aldrich) the next morning. Stage tip desalting was done using C18 spin tips with a 100 µL bed (Pierce Protein Biology) according to the manufacturer's instructions. The digested samples were analyzed on a Q Exactive mass spectrometer

(Thermo). Approximately 250 ng of digest was injected directly onto a 20 cm, 100 μ m ID column packed with Aqua 3 μ m C18 resin (Phenomenex). Samples were separated at a flow rate of 400 nl/min on an Easy nLCII (Thermo). Buffer A and B were 0.1% formic acid in 5% acetonitrile and 0.1% formic acid in 80% acetonitrile, respectively. A gradient of 1-35% B over 80 minutes, an increase to 80% B over 25 minutes and held at 80% B for 5 minutes prior to returning to 1% B was used for 120 minutes total run time. Column was re-equilibrated with buffer A prior to the injection of sample. Peptides were eluted directly from the tip of the column and nanosprayed directly into the mass spectrometer by application of 2.5 kV voltage at the back of the column. The Q Exactive was operated in a data dependent mode. Full MS¹ scans were collected in the Orbitrap at 70 K resolution with a mass range of 400 to 1800 m/z. The 10 most abundant ions per cycle were selected for MS/MS and dynamic exclusion was used with exclusion duration of 15 seconds.

Three biological replicates were measured sequentially. Mass spectrometry data was processed with MaxQuant 1.5.7.0 (Cox and Mann, 2008) and Perseus 1.6.2.3 (Tyanova et al., 2016) as described previously (Keilhauer et al., 2015) to identify ArgRS interaction partners. In brief, raw files were searched against *Homo sapiens* reference proteome UP000005640_9606 (UniProt Consortium, 2019) (Uniprot) with the Andromeda search engine integrated in MaxQuant. Default settings were used for label-free quantification (LFQ). Phosphorylated peptides (STY) were included when searching for nuclear interaction partners of ArgRS. The resulting protein groups file was loaded into Perseus and filtered for “reverse”, “potential contaminants”, and “only identified by site”. The log₂ values of LFQ intensities were calculated and all proteins with less than two valid values/group discarded.

Missing values were replaced from a normal distribution (width 0.3, downshift 1.8) and Welch's t-test was used to calculate t-test significance and difference.

Generation of SRRM2-mVenus cell line

A gRNA (CCAUGAGACACCGCUCCUCC) targeted to the second last exon of SRRM2 (exon 14) was inserted into pSpCas9(BB)-2A-GFP. pSpCas9(BB)-2A-GFP (PX458) was a gift from Feng Zhang, Addgene plasmid #48138; <http://n2t.net/addgene:48138>; RRID:Addgene_48138 (Ran et al., 2013)). mVenus (mVenus C1) was a gift from Steven Vogel, Addgene plasmid #27794; <http://n2t.net/addgene:27794>; RRID:Addgene_27794 (Koushik et al., 2006). The donor vector for insertion of mVenus was based on pcDNA6. The CMV promoter and multiple cloning site of pcDNA6 were substituted for mVenus flanked by 800 bp upstream and downstream of the Cas9 cleavage site. Cells were transfected with both plasmids at a 1:2 ratio gRNA/Cas9:donor vector and sorted for GFP expression after two days (successful expression of pSpCas9-GFP) by the Flow Cytometry core at Scripps Research. Single cells were expanded into monoclonal cell lines and evaluated for mVenus expression after expansion by flow cytometry. About 1/3 of all tested clones showed mVenus fluorescence. Correct genomic integration was assessed by PCR on isolated genomic DNA (DNeasy Blood and Tissue Kit, Qiagen, Supplementary Figure S2G). PCR primers: GTGGTGCCTGAGGTGGTGCC/CCACTCCCAAATGGGGCCG

Successful labeling of SRRM2 only was confirmed by the reduction of mVenus by two individual shRNAs directed against SRRM2 (Supplementary Figure S5A).

ArgRS, MetRS, and SRRM2 knock down

shRNAs against ArgRS (shRARS_1: gtggacacaagcataagtaaa, shRARS_2: ggagcagttacaagaagaaaa) were cloned into a pLKO.1 vector (pLKO.1 - TRC cloning vector

was a gift from David Root, Addgene plasmid #10878; <http://n2t.net/addgene:10878>; RRID:Addgene_10878 (Moffat et al., 2006)). A non-targeting control shRNA pLKO.1 shSCR was a gift from Sheila Stewart (Addgene plasmid #17920; <http://n2t.net/addgene:17920>; RRID:Addgene_17920 (Saharia et al., 2008)). shRNAs against *SRRM2* and *MARS* were acquired from The RNAi Consortium via Sigma Aldrich (shSRRM2_1: cgccacctaacagaaatc, shSRRM2_2: gttgggactggaggttgta, shMARS_1: caaggaaacattgtccgagaac, shMARS_2: tcgacatggcaaccaatatatc). Plasmids were co-transfected into 293T cells together with lentiviral packaging plasmids pRSV-rev, pMDLg/pRRE, and pMD2.G (plasmids were a gift from Didier Trono (Addgene plasmid #12253 and #12251; <http://n2t.net/addgene:12253> and [:12251](http://n2t.net/addgene:12251); RRID:Addgene_12253 and _12251 (Dull et al., 1998)), and a gift from the Torbett lab at Scripps Research) using Lipofectamine 2000 (Invitrogen). Medium was changed after overnight transfection and collected two days later. Supernatant containing viral particles was filtered through a 0.45 µm syringe filter, and either used directly or stored at -80°C. For viral transduction, SRRM2-mVenus-tagged 293T or HepG2 cells were seeded a day prior on 6 cm tissue culture dishes and incubated with 1 ml undiluted viral particles in the presence of 8 µg polybrene (EMD Millipore) for two hours. Afterwards, fresh medium was added, and the infection left to proceed for two days. Successfully transduced cells were selected with 10 µg/ml (293T) or 50 µg/ml (HepG2) puromycin (AdipoGen Life Science). ArgRS knock down was confirmed via western blot and quantitative real-time PCR.

Reverse transcription and quantitative real-time PCR (RT-qPCR)

RNA was isolated using Trizol (Invitrogen) according to the manufacturer's instructions. 500 ng RNA was reverse-transcribed using MultiScribe Reverse Transcriptase (Applied

Biosystems) according to instructions in the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) with random hexamers (Qiagen) as primers. qRT-PCR was performed with Power SYBR Green Master Mix (Thermo Fisher Scientific) on a StepOnePlus (Applied Biosystems). The following primers were used:

RARS: GAGAGTATAAGCCGCCTCTTTG/TCCTCCTCAGTATCAAACCTCT;

SRRM2: TGACAGCAAATCTCGACTATCC/GGTTCAGGAGAGGAATCAGAAC

18S: CTGAGAAACGGCTACCACATC/GCCTCGAAAGAGTCCTGTATTG

Immunofluorescence

Glass coverslips (12 mm, 1.5 thickness, Neuvitro Corporation) were sterilized with 70% ethanol, coated with 0.01% Collagen I (MP Biomedicals Inc) overnight, and stored in PBS until use. Two days prior to staining, 10,000 HepG2 or 293T cells were seeded on coverslips. Cells were washed, fixed with 4% PFA in PBS for 20 minutes, permeabilized in 0.25% Triton X-100 for 5 minutes, blocked with 1% FCS, 2% BSA in PBS (IF buffer) for a minimum of 15 minutes, and stained overnight with anti-SRRM2 antibody or anti-ArgRS antibody (both SCBT, 1:100, all dilutions in IF buffer) at 4°C. Cells were washed twice with PBS and once with IF buffer between all steps. Anti-mouse AlexaFluor 488 secondary antibody (Abcam) was diluted 1:250 and added to the cells for 45 minutes at room temperature. Anti-SC35/SFRS2 (Proteintech), Methionine-tRNA Synthetase (MetRS, Abcam), or Arginyl-tRNA Synthetase antibody (Biorbyt) were added at a 1:100 dilution (ArgRS) or 1:200 dilution (MetRS, SC35) and incubated at room temperature for 3 hours. Cross-adsorbed anti-rabbit Alexa Fluor 568 secondary antibody (Abcam) was added for 45 minutes at room temperature at a dilution of 1:250. Hoechst 33342 (AdipoGen Life Science) was added at 1:500 in PBS for 15 minutes. Cover slips were washed in PBS, dipped in water, mounted on soft-set

Vectashield (Vectorlabs), and sealed with gel glue or nailpolish. Slides were stored at 4°C until imaging.

Confocal microscopy and colocalization

Microscopes were maintained by the Core Microscopy Facility at Scripps Research. For co-localization of SRRM2, ArgRS, SFPQ, and MetRS, a Zeiss LSM 880 Airyscan confocal laser scanning microscope with a 100x oil objective was used for imaging in airyscan mode. Confocal images were recorded with a pixel dwell time of 1.5 μ s in 16-bit depth with a scaling of 0.034 μ m (x, y) and 0.145 μ m (z). Z-stacks covering the nucleus were taken with a motorized stage. Images were processed with “Airyscan Processing” in Zen (Zeiss). Estimation of colocalization was done with Imaris (Oxford Instruments). First, the nuclear volume of each cell was determined by using Hoechst DNA staining as a mask to delineate the cell nucleus, with an adequate threshold to filter spillover of Hoechst signal. Second, the nuclear signal of two antibody stains were colocalized by comparing nuclear volumes in individual cells and the percentage of colocalized volume was noted as well as Manders overlap coefficients and Pearson correlation coefficient. Manders overlap coefficient calculates the overlap between two colors from their absolute intensities and results in individual coefficients for each color (co-occurrence of color1 with color2 and vice versa). In contrast, Pearson correlation coefficient uses the deviation from the mean and results therefore in one coefficient for the correlation between both colors (relationship between the signal intensities). Background for the antibody staining was determined by comparison of signal intensity to a control in which cells were stained with secondary antibody only or by Costes thresholding in Imaris, if stated so in the figure legend. Videos demonstrating colocalization (Supplementary Videos 1-4) were recorded with Imaris (Oxford Instruments).

Supplementary Figure S2H was taken on a Zeiss LSM 710 confocal laser scanning microscope with a 63x oil objective.

Fluorescence recovery after photobleaching (FRAP)

SRRM2-mVenus 293T cells expressing either a non-targeting control shRNA (shSCR ctrl) or shRNA against ArgRS (shRARS) were seeded on collagen-I coated (as described above for cover slips) glass bottom 35 mm dishes with 1.5 thickness (World Precision Instrument) two days prior to imaging. Live cell imaging was performed on a Zeiss LSM 780 or a LSM 880 confocal laser scanning microscope at 37°C, 5% CO₂ with a 63x water objective. Fluorescent bleaching was achieved by 20 cycles of 100% intensity at 514 nm. Pictures were taken every 2 s for 75 cycles with a pixel dwell time of 1.58 µs and fluorescence in regions of interest (ROIs) were recorded with ZEN (Zeiss). The resulting data was subtracted from background, divided by fluorescence intensity of the same area before bleaching (normalization), and the geometric mean of ROIs was fitted with a one-exponential model to calculate the half-time of fluorescence recovery (GraphPad Prism 5). The mean of individual measurements was calculated and plotted together with standard error means. Pictures were exported with Fiji/ImageJ. Immobile fractions were calculated from dedicated measurements with images taken every 10 s (as opposed to every 2 s for the calculation of half-time of recovery) to minimize photobleaching during imaging (at the cost of temporal resolution). FRAP recordings were excluded if sample drift was noted during the recording. Significance was tested with a two-tailed Student's t-test (GraphPad Prism 5). We compared SRRM2-mVenus fluorescence recovery half-times to known literature values of ASF/SRSF1 (Phair and Misteli, 2000) recovery. Secondary structure prediction of both proteins was carried out with Sable (Adamczak et al., 2004).

Flow cytometry and fluorescence activated cell sorting

Flow cytometry was performed on instruments maintained by the Flow Cytometry core at Scripps Research. 293T cells were detached using Trypsin/EDTA and resuspended in DMEM with 10% FBS. The cell suspension was mixed 1:1 with sorting buffer (2.5 mM EDTA, 25 mM HEPES, pH 7.0, 1% FBS, 1% penicillin/streptomycin in PBS) and analyzed on an LSR II analytical flow cytometer (BD Bioscience). mVenus fluorescence was detected in the FITC channel (filter 525/50) after excitation by a 488 nm laser. Flow cytometry results were analyzed with FlowJo version 10.06 or higher. Fluorescence activated cell sorting was performed by members of the Scripps Research Flow Cytometry core on a MoFlo Astrios EQ jet-in-air sorting flow cytometer (Beckman Coulter). Two days after transfection, cells were detached using Accutase (Stemcell Technologies), resuspended in DMEM with FBS, and spun down to remove serum to prevent cells from adhering. Following a wash with PBS, cells were diluted in sorting buffer to 1×10^6 cells/ml. Single cells were sorted into a 96 well plate prefilled with 100 μ L medium and expanded to monoclonal cell lines. For quantification of MHCII expression, cells were detached with EDTA and stained with 1 μ g/ml HB-95 W6/32 for 20 minutes on ice followed by Alexa 647-cojugated anti-mouse secondary antibody for 20 minutes on ice, both in sorting buffer.

Cell viability

Cell viability and proliferation was assessed by measuring AlamarBlue (AbD Serotec) conversion. HepG2 or 293T cells were seeded in 96-well plates (10,000 cells/well) and 1:100 AlamarBlue was added two hours prior to measurement on a Synergy H1 (Biotek, λ_{exc} 530 nm, λ_{em} 580 nm). All measurements were done in technical quadruplicates.

RNA isolation and TGIRT-seq library preparation

2.5x10⁶ HepG2 cells expressing either a non-targeting shRNA control (shSCR) or a shRNA directed against ArgRS (gene name: *RARS*, shRARS) were seeded on 10 cm tissue culture dishes two days prior to RNA isolation. Cells were starved off arginine for 6 hours and supplemented with 10x arginine two hours prior to harvest. RNA was isolated by using a mirVana miRNA isolation kit (Invitrogen) with phenol extraction following the manufacturer's instruction for total RNA isolation. RNA integrity was evaluated with a Bioanalyzer (Agilent). Extracted total RNA was treated with TURBO DNase (Thermo Fisher) according to the manufacturer's instruction, and the DNase-treated RNA was then ribo-depleted by using a Ribo-Zero Gold (Human/Mouse/Rat) kit (Illumina). For each library, 50 ng of ribo-depleted RNA was fragmented by using an RNA Fragmentation Module kit (New England Biolabs) to a median size of 55-75 nt, and 3' phosphates were removed by treatment with T4 polynucleotide kinase (Epicentre).

TGIRT-seq libraries were prepared essentially as described (Qin et al., 2016) using a modified R2R adapter that decreases adapter dimer formation (Xu et al., 2019). Reverse transcription reactions contained fragmented, dephosphorylated cellular RNA, 100 nM R2 RNA/R2R DNA starter duplex, and 1 µM TGIRT-III (InGex) in reaction buffer (20 mM Tris-HCl, pH 7.5, 450 mM NaCl, 5 mM MgCl₂, 5 mM DTT). Reactions were pre-incubated at room temperature for 30 min and then initiated by addition of 1 mM dNTPs (an equimolar mix of 1 mM dATP, dCTP, dGTP, and dTTP) and raising the temperature to 60°C. Reverse transcription by TGIRT-III is initiated by template switching from a starting duplex consisting of a 35 nt DNA primer encoding the reverse complement of the Illumina Read 2 sequencing primer binding site (R2R) annealed to a 34-nt complementary RNA oligonucleotide (R2), leaving a single nucleotide 3' DNA overhang composed of an equimolar mixture of A, G, C

997 TGIRT-seq bioinformatic analysis

sequentially aligned to the human genome (hg19) using a splice-aware aligner (HISAT2) (Kim et al., 2015) and a sensitive local aligner (BOWTIE2) (Langmead and Salzberg, 2012). Finally, gene quantification was done on genomic loci, except for tRNA and rRNA, which required an additional step of re-aligning and counting (Supplementary Table S14). Differential gene expression analysis was done using DESeq2 (Love et al., 2014). Exon quantification was done by FeatureCount (Liao et al., 2014) and differential exon usage was quantified using DEXSeq (Anders et al., 2012). DEXSeq uses a negative binomial model to test for differential exon usage between two samples while normalizing for the differences in gene expression (Anders et al., 2012). Gene names were assigned using biomaRt (Durinck et al., 2009).

Poly(A) RNA-seq library preparation and bioinformatic analysis

Poly(A) RNA-seq libraries were prepared and sequenced by the Genomic Sequencing and Analysis Facility at the University of Texas at Austin. RNAs were selected by using a Poly(A) Purist MAG Kit (Thermo Fisher, AM1922) using the manufacturer's protocol followed by library preparation using a NEBNext Ultra II Directional RNA Library Kit for Illumina (New England Biolabs, E7760), also following the manufacturer's protocol. Samples were sequenced on a NovaSeq 6000 instrument using SP flowcells (2x150 nt, paired end reads). Reads were trimmed with trimgalore and aligned to grch37 with STAR (Dobin et al., 2013) (Supplementary Table S15). Counts were generated with Subread (Liao et al., 2014). Differential exon usage analysis was performed with DEXSeq (Anders et al., 2012). If exons were assigned to overlapping genes, gene symbols were assigned to the first gene and counted as one gene except in the direct comparison between DEXSeq results and N-terminal peptides, where all genes were used. Differentially expressed genes were identified

with DESeq2 (Love et al., 2014). Enrichment analysis was performed using Panther (Mi et al., 2019) and ShinyGO (Ge et al., 2020). For Panther, all genes mapped to grch37 were used as a reference list. Vast-tools was used to identify differentially spliced events, including cassette exons, microexons, 3' and 5' alternative splice sites, and retained splice sites (Irimia et al., 2014). Untrimmed reads were first aligned to the hg19 assembly and PSI values for all annotated splicing events were measured for each sample. Events were considered if, in at least half of the profiled samples, they had ≥ 15 reads overlapping the included/excluded splice junctions and a ratio of reads mapping to the upstream and downstream junctions (a balance score) of < 2 or 2 to 5 . These events were then processed through the vast-tools diff module (Han et al., 2017), which calculates the predicted Δ PSI between samples and assigns a minimum Δ PSI value (MV), with a ≥ 0.95 probability, for each event. For example, $MV > 0$ $|\Delta$ PSI ≥ 0.1 indicates ≥ 0.95 probability of at least a 10% difference in splice junction usage in either direction.

RT-PCR for verification of splicing events

RNA was reverse transcribed and amplified using OneStep RT-PCR (Qiagen) according to the manufacturer's instructions but in a reduced volume (10 μ l final). 40 ng of HepG2 RNA or 100 ng of 293T RNA were transcribed per reaction. Primers were designed using VastDB (Tapial et al., 2017) for splice junction changes and manually for differential exon usage. Amplified regions of different splice isoforms were separated on a 2.5% agarose/TAE gel and visualized after staining with SybrSafe on a Biorad ChemiDoc Imager.

Detection of N-terminal peptides

A modified protocol for the Terminal amine isotopic labeling of substrate (TAILS) was used to enrich for N-terminal peptides (Kleifeld et al., 2011). Cells were lysed, proteins were

extracted by a methanol/chloroform precipitation, and N-termini were blocked overnight with formaldehyde. Proteins were extracted again with methanol/chloroform to avoid carry-over of reactive formaldehyde and digested with trypsin overnight. HPG-ALD Polymers were acquired through Flintbox and added to the peptides at a 1:50 ratio to deplete peptides with unblocked reactive N-termini derived from the trypsin digest. The next day, the reaction was quenched, and free peptides separated from the polymer with a 10 kDa spin filter. Peptides were fractionated with Pierce High pH Reversed-Phase Peptide Fractionation kit (Thermo) according to the manufacturer's instructions, all 9 fractions were dried down, resuspended in 5% acetonitrile and 0.1% formic acid and were analyzed on the Q Exactive mass spectrometer as described above. Raw data was processed with MaxQuant (Cox and Mann, 2008) with dimethyllysine and dimethylated N-termini as a fixed modification. Peptides were searched against a reference proteome (Uniprot) with free N-termini and ArgC as protease and label free quantification was used with default settings. The resulting intensities and LFQ values were filtered in Perseus (Tyanova et al., 2016) against a contaminant database. Reverse and peptides preceded by arginine, which are likely derived from trypsin cleavage, were excluded. MaxQuant (version 1.6.7.0) was run on the Scripps High Performance Computing core. Genes with overlapping differential exon usage (DEXSeq padj < 0.05) and N-terminal peptides (Student's t-test difference > $|\Delta 1|$) were further manually analyzed to identify the position of the differential exon in the gene and the resulting peptide sequence.

IDH and proteasome activity

10,000 HepG2 cells were seeded in white 96 well plates two days prior to both assays. For IDH activity, cells were lysed in co-immunoprecipitation lysis buffer and mixed with an end concentration of 100 μ M NADP, 2 mM $MnCl_2$, and 5 mM isocitrate. IDH activity was

measured by monitoring fluorescence at 460 nm after excitation at 340 nm, once per minute for 10 minutes at 37°C. Proteasomal subunit $\beta 5$ activity was measured using the ProteasomeGlo assay (Promega) according to the manufacturer's instructions in the same format. Total protein concentration (IDH, with BCA assay) or cell count ($\beta 5$ activity, with alamar blue assay) were used to normalize between treatment conditions.

MHCI peptidome

5×10^8 cells were seeded per replicate 2 days prior to harvest and frozen at -80°C until MHCII peptide enrichment. HB-95 W6/32 hybridoma were acquired by ATCC, cultured in DMEM with 10% FBS, and diluted in HyClone CDM4MAb serum free media stepwise till 10 % DMEM was reached. Cells were left to condition the serum-reduced media for 5 days. 300 ml of medium were loaded on 3 ml of Protein A, washed with PBS, and eluted with 100 mM glycine, pH 3.0, into a final concentration of 130 mM Hepes, pH 7.5 in 5 column volumes. Coupling of antibody to protein A was performed as described previously (Purcell et al., 2019). Cell lysis followed instructions by Purcell et al., but centrifugation speed was changed to $40,000 \times g$ for 30 minutes. Separation of MHCII from MHCII-bound peptides was performed as described previously (Bassani-Sternberg et al., 2015). Peptides were measured on a QE-HFX and searched against the human proteome with ProLuCID (Xu et al., 2015) without protease specificity and no requirements for tryptic peptides for protein identification. The percentage of arginine in the proteins from which the MHCII peptides originated was not significantly changed between groups. MHCII immunogenicity score was calculated as described previously (Calis et al., 2013).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
ArgRS	Biorbyt	orb247357
SRRM2	SCBT	sc-390315
MetRS	Abcam	ab31541
GFP	Proteintech	10087-514
SFSR2	Proteintech	20371-1-AP
GAPDH	CST	2118L
Histone H3	CST	9715S
Tubulin	CST	2144S
Anti-Rabbit HRP	Invitrogen	31464
Anti-Mouse HRP	Invitrogen	PI31432
Anti-Rabbit Alexa 568	Abcam	ab175696-500ug
Anti-Mouse Alexa 488	Abcam	ab150113-500ug
Bacterial and Virus Strains		
DH5alpha	NEB	C2987H
Top10	Invitrogen	C404010
ClearColi	Lucigen	89428-536
Biological Samples		
Chemicals, Peptides, and Recombinant Proteins		
Marfey's Reagent	Pierce	PI48895
Murine Arginase-1	This paper	N/A
Hoechst 33342	Adipogen	50596053
Carbon tetrachloride (CCl ₄)	Sigma-Aldrich	270652-100ML
¹³ C6-arginine	Pierce	PI88210
Alamar Blue	Biorad	BUF012A
HPG-ALD Polymers	Flintbox UBC	N/A
T4 Polynucleotide Kinase, Cloned	Lucigen	P0503K
TGIRT-III	Ingex	TGIRT™-III Enzyme
Thermostable 5' App DNA/RNA Ligase	New England Biolabs	M0319
Phusion High Fidelity PCR Master Mix with HF Buffer	Thermo Scientific	F531
TGIRT III	Ingex	TGIRT™-III Enzyme
Critical Commercial Assays		
Co-IP kit	Pierce	PI26149
mirVana RNA isolation	Life Technologies	AM1560
OneStep RT PCR	Qiagen	210210
NEBNext Magnesium RNA Fragmentation Module	New England Biolabs	E6150S
5' DNA Adenylation Kit	New England Biolabs	E2610
NEBNext Ultra II Directional RNA Kit for Illumina	New England Biolabs	E7760
Poly(A)Purist MAG Kit	Invitrogen	AM1922
RNA Clean & Concentrator-5 Kit	Zymo Research	R1013
Oligo Clean & Concentrator Kit	Zymo Research	D4060
MinElute Reaction Cleanup Kit	Qiagen	28206
Pierce High pH Reversed-Phase Peptide Fractionation kit	Thermo Scientific	84868
Proteasome-Glo Assays	Promega	G8621
Deposited Data		
TGIRT-seq data	This paper	SRA PRJNA561913

Poly(A) RNA-seq	This paper	GEO GSE165513
ArgRS interactome	This paper	Pride PXD015692
MetRS interactome	Cui et al., 2020	Pride PXD021527
N-terminomics	This paper	Pride PXD024091
MHCI peptidome	This paper	Pride PXD027531
Experimental Models: Cell Lines		
HepG2	ATCC	HB-8065, verified by ATCC
HEK 293T	ATCC	CRL-3216, verified by ATCC
W6/32	ATCC	HB-95
Murine embryonic fibroblasts	Dr. Yao Tong Scripps Research	N/A
Experimental Models: Organisms/Strains		
C57Bl/6J	Scripps Breeding Colony	N/A
Oligonucleotides		
gRNA SRRM2 CCAUGAGACACCGCUCCUCC	Eton Bioscience	N/A
shRNA RARS (3'UTR) gtggacacaagcataagtaaa	Eton Bioscience	N/A
shRNA RARS (CDS) ggagcagttacaagaagaaaa	Eton Bioscience	N/A
All other oligos	Eton Bioscience	N/A
TGIRT-seq: starting molecule Read2 oligonucleotides	IDT Bioscience, Xu et al. 2019	NTT R2R DNA/R2 RNA
TGIRT-seq: 5' phosphorylated Read1 oligonucleotide	IDT Bioscience, Xu et al. 2019	R1R DNA
Recombinant DNA		
pSpCas9(BB)-2A-GFP_SRRM2	This paper	N/A
mVenus donor for SRRM2	This paper	N/A
pLKO.1 shRARS_3UTR	This paper	N/A
pLKO.1 shRARS_2	This paper	N/A
pSpCas9(BB)-2A-GFP (PX458)	Ran et al., 2013	Addgene 48138
pLKO.1 shSCR	Saharia et al., 2008	Addgene 17920
pLKO.1 shSRRM2_1, _2	Sigma Aldrich	TRCN0000314562, TRCN0000314504
pLKO.1 shMARS_1, _2	Sigma Aldrich	TRCN0000298212, TRCN0000293830
Software and Algorithms		
Imaris	Bitplane	https://imaris.oxinst.com/
Fiji	Schindelin et al., 2012	https://imagej.net/Fiji
Hisat2	Kim et al., 2015	http://daehwankimlab.github.io/hisat2/
Bowtie2	Langmead and Salzberg, 2012	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
Trimalore	Felix Krueger	https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/

Subread	Liao et al., 2014	https://bioconductor.org/packages/release/bioc/html/Rsubread.html
DESeq2	Love et al., 2014	http://bioconductor.org/packages/release/bioc/html/DESeq2.html
DEXseq	Anders et al., 2012	https://bioconductor.org/packages/release/bioc/html/DEXSeq.html
Vast-Tools	Irimia et al., 2014	https://github.com/vastgroup/vast-tools
Vast Tools Diff module	Han et al., 2017	https://github.com/vastgroup/vast-tools
VastDB	Tapial et al., 2017	http://vastdb.crg.eu/wiki/Main_Page
Panther	Mi et al., 2019	http://pantherdb.org/
ShinyGO	Ge et al., 2020	http://bioinformatics.sdstate.edu/go/
Maxquant	Cox,J. and Mann,M., 2008	https://www.maxquant.org/
Perseus	Tyanova et al., 2016	https://maxquant.net/perseus/
GraphPad Prism 7-9	GraphPad Software LLC	https://www.graphpad.com/
R Studio	R Studio	https://rstudio.com/
Tidyverse	Tidyverse/RStudio	https://www.tidyverse.org/
biomaRt	Durinck S et al., 2009	https://bioconductor.org/packages/release/bioc/html/biomaRt.html
EnhancedVolcano	Blighe K, Rana S, Lewis M, 2020	https://bioconductor.org/packages/release/bioc/html/EnhancedVolcano.html
TGIRTseq pipeline	Douglas C. Wu	https://github.com/wckdouglas/tgirt_map
ggpubr	Alboukadel Kassambara	https://CRAN.R-project.org/package=ggpubr
Subread to DEXSeq	Vivek Bhardwaj	https://github.com/vivekbhr/Subread_to_DEXSeq
ProLuCID	Xu et al., 2015	http://ip2.scripps.edu
Class I Immunogenicity	Calis et al., 2013	http://tools.iedb.org/immunogenicity/
Other		

1099

1100 **Supplementary Information**

1101 **Supplementary Figures 1-7**

1102 **Supplementary tables**

1103 Table S1: ArgRS nuclear interactome in HepG2.

1104 Table S2: ArgRS interactome in HepG2 whole cell lysate.

1105 Table S3: ArgRS interactome in 293T whole cell lysate.

1106 Table S4: MetRS interactome in 293T whole cell lysate.

1107 Table S5: Proteins shared between interactomes.

1108 Table S6: tRNA expression upon ArgRS knock down as calculated from TGIRTseq.

1109 Table S7: Splice junctions with $MV > 0$ and $|\Delta PSI| > 0.1$ upon ArgRS knock down.

1110 Table S8: Differentially expressed exons upon ArgRS knock down ($p_{adj} > 0.05$).

1111 Table S9: Peptides identified after enrichment of N-terminal peptides with $|pDiff| > 1$ upon

1112 ArgRS knock down.

1113 Table S10: Splice junctions with $MV > 0$ and $|\Delta PSI| > 0.1$ upon SRRM2 knock down.

1114 Table S11: Differentially expressed exons upon SRRM2 knock down ($p_{adj} > 0.05$).

1115 Table S12: Splice junctions with $MV > 0$ and $|\Delta PSI| > 0.1$ upon MetRS knock down.

1116 Table S13: Genes associated with Hallmark and GO terms that are inversely modulated by

1117 ArgRS and SRRM2.

1118 Table S14: Mapping statistics for TGIRT-seq. Numbers on the top of each cell represent

1119 numbers of read pairs. Numbers within parentheses represent percentages. Raw pairs

1120 indicate the number of read pairs for the sample after Illumina sample demultiplexing.

1121 Trimmed pairs indicate the number of read pairs that passed adapter- and quality- trimmings,

1122 and length-cutoffs, number in parentheses indicates percentage of trimmed pairs respect to

1123 raw pairs. tRNA/rRNA pairs indicates number of read pairs that can be concordantly aligned

to ribosomal RNA repeat units (GenBank accession numbers: X12811.1 and U13369.1)) or tRNA (GtRNAdb; (Chan and Lowe, 2016), percentage is computed respect to trimmed pairs. Non tRNA/rRNA pairs indicate the number of read pairs that are not mappable to rRNA or tRNA, percentage is computed respect to trimmed pairs. HISAT2 mapped pairs indicates number of read pairs that can be aligned concordantly to human genome (hg19) by HISAT2 (Kim et al., 2015), percentage is computed respect to non tRNA/rRNA pairs. BOWTIE2 mapped pairs indicates number of read pairs that aligned to human genome by BOWTIE2 (Langmead and Salzberg, 2012), percentage is computed respect to non tRNA/rRNA pairs. Mapping rate is computed by the sum of percentage of HISAT mapper pairs and BOWTIE2 mapped pairs. Uniquely mapped indicates number of read pairs that are aligned to the human genome at a single location without ambiguity by HISAT2 (with alignment tag NH:1) or BOWTIE2 (MAPQ = 255). Unique map rate is computed respective to the sum of HISAT mapped pairs and BOWTIE2 mapped pairs. Splice read rate is computed by dividing the number of read pairs with either read being split by HISAT2 aligner (with a N cigar string) to the sum of HISAT mapped pairs and BOWTIE2 mapped pairs.

Table S15: Mapping statistics for RNA-seq.

Supplementary videos

Video S1: SC35/SRRM2 distribution in the nucleus. Visualization of SC35 and SRRM2 signal in a HepG2 hepatocellular carcinoma cell by immunofluorescent staining. Blue: Hoechst staining of DNA; green: SRRM2; red: SC35, yellow: colocalization. Sequence in video: SC35 only – SRRM2 only – SC35 and SRRM2 – SC35 and SRRM2, colocalized areas highlighted in yellow.

1147 Video S2: ArgRS/SRRM2 colocalization in the nucleus. Visualization of ArgRS and SRRM2
 1148 nuclear signal in a HepG2 hepatocellular carcinoma cell by immunofluorescent staining. The
 1149 cell nucleus was identified by Hoechst staining. Blue: Hoechst staining of DNA; red: ArgRS;
 1150 green: SRRM2; yellow: colocalization between ArgRS and SRRM2. Sequence in video:
 1151 ArgRS only - SRRM2 only - ArgRS and SRRM2 – ArgRS and SRRM2, colocalized areas
 1152 highlighted in yellow.

1153 Video S3: MetRS/SRRM2 colocalization in the nucleus. Visualization of MetRS and SRRM2
 1154 nuclear signal in a HepG2 hepatocellular carcinoma cell by immunofluorescent staining. The
 1155 cell nucleus was identified by Hoechst staining. Blue: Hoechst staining of DNA; red: MetRS;
 1156 green: SRRM2; yellow: colocalization between MetRS and SRRM2. Sequence in video:
 1157 MetRS only - SRRM2 only - MetRS and SRRM2 – MetRS and SRRM2, colocalized areas
 1158 highlighted in yellow.

1159 Video S4: MetRS/ArgRS colocalization in the cell. Visualization of ArgRS and MetRS in a
 1160 HepG2 hepatocellular carcinoma cell by immunofluorescent staining. The cell nucleus was
 1161 identified by Hoechst staining. Blue: Hoechst staining of DNA; red: MetRS; green: ArgRS;
 1162 yellow: colocalization between ArgRS and SRRM2. Sequence in video: MetRS only - ArgRS
 1163 only - ArgRS and MetRS – ArgRS and MetRS, colocalized areas highlighted in yellow.

1164 Video S5: Fluorescence recovery after photobleaching (FRAP) of SRRM2-mVenus
 1165 fluorescence in 293T cells expressing a non-targeting shRNA (ctrl). Bleaching occurs
 1166 between frame 4 and 5 (1 s into the video).

1167 Video S6: Fluorescence recovery after photobleaching (FRAP) of SRRM2-mVenus
 1168 fluorescence in 293T cells expressing a shRNA against the 3'UTR of ArgRS (shRARS_1).
 1169 Bleaching occurs between frame 4 and 5 (1 s into the video).

1170 Video S7: Fluorescence recovery after photobleaching (FRAP) of SRRM2-mVenus
1171 fluorescence in 293T cells expressing a shRNA against the coding region of ArgRS
1172 (shRARS_2). Bleaching occurs between frame 4 and 5 (1 s into the video).

1173

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Supplementary Figure 1: ArgRS localizes to the nucleus in murine cells and tissues, plasma arginine levels, and characterization of the ArgRS interactome. (A) ArgRS in different cellular compartments of murine embryonic fibroblasts (MEF, left) and mouse liver (right) probed by western blot following cell fractionation. GAPDH: cytoplasmic marker, Histone H3: nuclear marker. lys: lysate, cyt: cytoplasm, nuc: nucleus. (B, C) LC/MS measurement of plasma arginine after (B) treatment with recombinant Arginase-1, an arginine-degrading enzyme, or (C) by induction of systemic inflammation with the liver toxin CCl₄. n=3 or 4 mice in each group. p= **0.002, p= *** p= 0.0009. (D) Venn diagrams comparing 4 different ArgRS interactomes. Statistical significance of overrepresentation by hypergeometric test: [nuc] vs [HepG2]: p=7.6e-10, [HepG2] vs [293T]: p=1.9e-10, [ArgRS 293T] vs [MetRS 293T]: p=2.0e-7. (E, F) SRRM2 interaction with ArgRS was verified by immunoprecipitation (IP) of ArgRS and vice versa. Detection of ArgRS and SRRM2 by western blot. Ctrl: Non-targeting antibody of the same isotype. (E) Input: HepG2 cell lysate. (F) Input: HEK 293T cell lysate. (G) SRRM2 immunoprecipitation did not retrieve MetRS but ArgRS. Detection of ArgRS, MetRS, and SRRM2 by western blot.

Supplementary Figure 2: Fluorescent microscopy to assess SRRM2 colocalization and dynamics. (A) Colocalization (yellow) of SRRM2 (green) and speckle marker SC35 (red) by immunofluorescent staining and imaging by confocal microscopy. Close to 75% of all SRRM2 colocalized with SC35. (B) Maximum intensity projections of ArgRS immunofluorescent staining in HepG2 shSCR control and shRARS_1 cells to show antibody specificity. (C) Immunofluorescent staining of ArgRS and SRRM2 in representative nuclei. Bar: 5 μ m. (D) Manders coefficient of ArgRS and SRRM2 colocalization when Costes thresholding was used. (E) Colocalization of ArgRS (green) and paraspeckle protein SFPQ (red). Manders coefficient of colocalization after Costes thresholding. (F) Pearson correlation coefficient of SRRM2 and ArgRS colocalization by immunofluorescence staining upon arginine starvation (0x arginine) and high arginine (5x arginine). Representative immunofluorescent staining of ArgRS and SRRM2. $p=0.01$, $n=14$, 9. Arginine concentration relative to DMEM. (G) Labeling of endogenous SRRM2 with the fluorescent protein mVenus. Verification of genomic mVenus insertion by PCR. Predicted amplification length of wildtype amplicon: 238 bp; predicted length of SRRM2-mVenus amplicon: 2071 bp. (H) Labeling with mVenus did not alter SRRM2 localization in 293T cells. Endogenous, unlabeled SRRM2 was visualized by immunofluorescence (upper panel). SRRM2-mVenus fluorescence displayed a similar pattern with fluorescence restricted to the cell nucleus and defined foci (middle panel). In comparison, stable expression of mVenus alone did not display a distinct localization (lower panel). (I) Fluorescence Recovery After Photobleaching (FRAP) measured over 90 s. Recovery reached its plateau after 60 s. (J) Absolute immobile SRRM2-mVenus fraction calculated from one-exponential curve fits of FRAP measurements and relative immobile fraction calculated by subtraction of residual fluorescence directly after bleaching. (K) Recovery of fluorescent signal is faster in the absence of arginine. Fluorescent signal was corrected for background and normalized. Each measurement included 14 regions of interest and the geometric mean of 8 measurements is shown. Errors bars depict standard error of the mean. (L) Cell viability upon ArgRS knock down in SRRM2-mVenus cells, measured with Alamar Blue. Technical quadruplets are shown. (M) Knock down of ArgRS did not affect SRRM2-mVenus protein levels measured by flow cytometry.

Supplementary Figure 3: Characterization of ArgRS knock down. (A) ArgRS knock down was confirmed on protein level by western blot and (B) on mRNA level by RT-qPCR and RNA-seq. A representative out of 3 repeats is shown. (C) Cell viability and proliferation of HepG2 cells was not affected by ArgRS knock down. Alamar blue based cell viability assay, quadruplets of technical replicates are shown. (D) Volcano plot of differentially expressed exons upon ArgRS knock down as found by TGIRT-seq. Dashed vertical lines: 2-fold change, dashed horizontal line: p-value $1e-6$. (A-D) ctrl: shSCR control, shRARS: ArgRS knock down.

Supplementary Figure 4: ArgRS knock down affected mRNA processing and protein isoforms. (A, B) RT-PCR of splice isoforms for (A) *FGFR3* and (B) *PLEKHG5*. Genes were detected as alternatively spliced in HepG2 cells by RNA-seq and confirmed for HEK 293T cells. A representative image from 3 or more replicates is shown as well as the densitometric quantification for 293T. PSI: percent spliced in. Ctrl: shSCR control. $p=0.001$, $n=5$, 5. $p=0.03$, $n=4$, 4. (C) Rescue of ArgRS knock down by overexpression of ArgRS-GFP by transient transfection in 293T cells, shown by western blot. (D) Rescue of *FGFR3* splicing by overexpression of ArgRS-GFP. Densitometric analysis of 3 repeats is shown. $p=0.003$, $n=3$, 3, 3, 3. (E) Genes and their corresponding proteins shared between differentially expressed exons or splice junction changes (RNA-seq) and N-terminal peptides (proteomics) in ArgRS knock down vs control cells. (F) Peptides found for PTBP1 in mass spectrometry following the enrichment of N-terminal peptides. (G) Western blot verification of preference for a higher molecular weight form of PTBP1 after ArgRS knock down. $p=0.003$, $n=4$, 4.

Supplementary Figure 5: Verification of stable and transient SRRM2 knock down and its effects on mRNA splicing. (A) Left to right: Knock down of SRRM2 in 293T_SRRM2_mVenus cells to verify shRNAs later used in HepG2 cells, assessed by flow cytometry. < 50% medium fluorescence intensity reduction upon SRRM2 knock down. Knock down of SRRM2 in HepG2 verified by qRT-PCR, three repeats, each measured in triplicates. Normalized SRRM2 counts in RNAseq. (B) Left: Verification of transient SRRM2 knock down by a heterogenous pool of siRNAs (esiRNA) in 293T_mVenus cells using flow cytometry. esi_SRRM2 1 and 2 are different batches of the same pool. ctrl: non-targeting pool of siRNAs. Right: RT-PCR splicing assays of *CDK5RAP3* and *FGFR3* upon transient knock down of SRRM2 with a pool of esiRNA in 293T cells. *CDK5RAP3*: p = *0.03, n=4, 4. *FGFR3*: p = **0.01, *0.04, n=3, 3, 3.

Supplementary Figure 6: Characterization of MetRS knock down and verification of splicing events regulated by ArgRS and SRRM2 in opposite directions. (A) Verification of MetRS knock down (shMARS) by western blot and RNAseq. (B) Splicing events with $|\Delta\text{PSI}| > 0.1$, $\text{MV} > 0$ and differential exon usage ($\text{padj} < 0.05$). Dashed vertical lines: 2-fold change, dashed horizontal line: $1e-6$. (C) Venn diagram of shared splicing event changes upon knock down of ArgRS, SRRM2, or MetRS. All splicing event changes relative to shSCR control. $\text{MV} > 0$, no criterion for $|\Delta\text{PSI}|$. (D) Inversely correlated alternative splicing events shared between ArgRS and SRRM2 or MetRS and SRRM2. Hypergeometric test for overrepresentation. (E) Knock down of ArgRS and SRRM2 had opposite effects on *CDK5RAP3*, *RAD52*, and *STX16* processing as shown by RT-PCR. Representatives of 4 or 5 replicates shown with quantification by densitometry. Scheme of SRRM2 sequestration by ArgRS and resulting altered mRNA splicing. *CDK5RAP3*: $p = *0.02$, $n = 5, 4, 5$. *IL32*: $p = *0.03$, $n = 4, 4, 3$. *RAD52*: $p = *0.02, *0.04$, $n = 5, 5, 5$. *STX16*: $p = *0.04, *0.04$, $n = 4, 4, 4$.

Supplementary Figure 7: SRRM2 and ArgRS induced changes and their effects on cellular signaling. (A) Comparison of genes with differential gene expression, differential exons, and splice junctions for shSRRM2 and shRARS. (B) Genes with differentially expressed exons enriched in MSigDB hallmark categories upon SRRM2 and ArgRS knock down ($p_{adj} < 0.05$) in HepG2. (C) Gene Ontology (GO) enrichment of genes with differentially expressed exons upon SRRM2 and ArgRS knock down ($p_{adj} < 0.05$) in HepG2. (D) GO enrichment of genes with changed splice junctions upon SRRM2 and ArgRS knock down ($MV > 0$) in HepG2. (C, D) All expressed genes were used as reference. (E) MSigDB hallmark categories and GO enrichment of genes with differential gene expression shared between SRRM2 and ArgRS knock down. (B-E) Circle size and Venn diagram visualize the proportion of shared exons, junctions, or genes. (F) Quantification of differentially expressed exons by qRT-PCR. PSMB5: $p = *0.03$, $*0.04$. UBE2D3: $p = **0.006$, $**0.008$. (G) (top left) Length distribution histogram of MHC I peptides confirmed that isolated peptides consisted predominantly of 9 amino acid peptides. (top right) Flow cytometry of MHC I on HepG2 shSCR ctrl, ArgRS knock down, and SRRM2 knock down cells by antibody staining. (bottom). Comparable overrepresentation of amino acids at specific positions (2, 4) were found with WebLogo in 9 amino acid-long peptides. (H) Venn diagrams of identified MHC I peptides by mass spectrometry following isolation of MHC I complex, displayed as proteins corresponding to the detected peptides found in all replicates (upper panel) and peptides found in at least one replicate (lower panel).