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Advances in analyzing viral-induced alterations of host-cell splicing

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Keywords

Alternative splicing

Virus-host interaction

Genome-wide transcriptomics

Systems biology

35 **Abstract**

36 Alteration of host cell splicing is a common feature of many viral infections which is
37 underappreciated because of the complexity and technical difficulty of studying alternative
38 splicing regulation. Recent advances in RNA sequencing technologies revealed that up to
39 several hundreds of host genes can show altered mRNA splicing upon viral infection. The
40 observed changes in alternative splicing events can either be a direct consequence of viral
41 manipulation of the host splicing machinery or result indirectly from the viral-induced innate
42 immune response or cellular damages. Analysis at a higher resolution with single-cell
43 RNAseq and a higher scale with the integration of multiple omics datasets in a systems
44 biology perspective will be needed to further comprehend this complex facet of virus-host
45 interactions.

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48 **Alteration of cellular splicing: a complex facet of virus-host interactions**

49 In higher eukaryotic cells most genes are transcribed as precursor messenger RNAs (pre-
50 mRNAs) that undergo splicing, a maturation process through which RNA sequences (introns)
51 are removed and the remaining sequences (exons) are ligated together. Splicing occurs in
52 the nucleus and is catalyzed by the spliceosome, a large and highly dynamic
53 ribonucleoprotein complex [1, 2]. Most mammalian pre-mRNAs are subject to alternative
54 splicing (AS), and human genes contain on average 8.8 exons and 7.8 introns per gene, giving
55 rise to an average of 3.4 alternatively spliced isoforms [3, 4]. The most common types of AS
56 events are the use of alternative donor and acceptor splice sites, exon skipping, alternative
57 use of mutually exclusive exons, and intron retention. Alternative splicing expands the
58 diversity of proteins that can be expressed from a given gene, and can also modify cis-
59 regulatory elements that govern the stability and translation of mRNAs. In recent years,
60 head-to-tail back-splicing events that result in the formation of non-coding circular RNAs
61 (circRNAs) have also been observed to play key regulatory roles in a variety of biological
62 processes, including antiviral immunity [5, 6]. Splicing is tightly coupled to transcription, and
63 is controlled by cis- and trans-acting elements as well as through chromatin structure and
64 signalling pathways [3, 5, 7]. The advent of high-throughput RNA sequencing technologies
65 (RNAseq) has opened up a new era in studying how AS is regulated and shapes the cellular
66 proteome in response to changes in environmental conditions (e.g. [8]).

67 Viruses modulate host gene expression in order to favor viral replication and evade antiviral
68 responses. They have evolved mechanisms to affect cellular transcription, mRNA processing
69 and nuclear export, mRNA decay, and translation [9-11]. RNAseq, proteomic and
70 interactomic studies are now beginning to provide a global view of viral-induced alterations
71 of cellular splicing and insights into how they may impact viral pathogenesis. Herein, we
72 review the recent developments in the field, we discuss how current limitations could be
73 overcome in the future, and what advances may be expected from the integration of splicing
74 isoforms datasets into a systems biology perspective.

75

76 **Viral manipulation of the host splicing machinery**

77 Viruses that replicate in the nucleus of infected cells and gain access to the splicing
78 machinery (e.g. adenoviruses, herpesviruses, and influenza viruses) have evolved an

79 expansion of their coding capacity by producing spliced viral mRNAs. However, manipulation
80 of the host splicing machinery is not exclusive to nuclear viruses and has also been observed
81 with viruses that replicate in the cytoplasm such as picornaviruses or flaviviruses (Table 1).
82 This can be accounted for by the nucleo-cytoplasmic shuttling of some viral proteins (e.g. the
83 dengue virus NS5 protein [12]) and splicing factors (e.g. SR proteins [13]), increased nuclear
84 permeability upon viral infection [14] or signaling pathways triggered by viral infection [15].
85 Components of the splicing machinery commonly targeted across different virus families are
86 the small nuclear ribonucleoproteins (snRNPs), serine/arginine-rich (SR) proteins, and
87 heterogeneous nuclear ribonucleoproteins (hnRNPs). The U1 to U6 snRNPs are core
88 components of the spliceosome, whereas SR and hnRNP proteins are involved in the
89 regulation of constitutive and alternative splicing. Different viruses appear to induce similar
90 types of alterations, *i.e.* changes in the level of expression, protein-protein or protein-RNA
91 interaction pattern, localization, phosphorylation and/or intrinsic activity of splicing factors
92 (Table 1).

93 Among the genes that appear differentially expressed upon viral infections in transcriptomic
94 and proteomic datasets, the overall enrichment of splicing-related genes is generally not
95 reported. However, particular splicing factors can undergo significant changes in expression
96 upon viral infection. As an example, expression of EFTUD2, a U5-snRNP associated factor,
97 was found to be decreased upon HCV infection of cultured cells and in liver samples from
98 HCV-infected patients; downregulation of EFTUD2 impairs the splicing of RIG-I and MDA5
99 pre-mRNAs and therefore enables the virus to circumvent the innate antiviral response [16].
100 Phosphoproteomic profiling provides an additional layer of information by revealing viral-
101 induced changes in the phosphorylation status of host proteins. Dynamic
102 phosphorylation/dephosphorylation is known to regulate the function of splicing factors,
103 most notably SR proteins [17]. HIV-1 entry triggers early changes in the phosphorylation of
104 five SR proteins, including SRRM2 which regulates the splicing of HIV-1 transcripts [18]. Later
105 in infection there is evidence that the HIV-1 protein Vpr modulates the activity of SR protein-
106 specific kinases and the phosphorylation of SRRM2 [19]. In the pre-omics era, the
107 herpesvirus ICP27, adenovirus E4-ORF4 and papillomavirus E1[^]E4 proteins have also been
108 reported to regulate SR proteins phosphorylation in order to facilitate their replication [20-

109 22] (Table 1). Altogether these findings point to changes in SR phosphorylation as a
110 mechanism commonly triggered by multiple viruses to co-opt the splicing machinery.
111 Interrogation of the VirHostNet 2.0 public database dedicated to virus-host protein-protein
112 interactions (PPIs) [23] reveals that a large proportion of known splicing factors have at least
113 one reported interaction with a viral protein, pointing to the spliceosome as a frequent viral
114 target (Figure 1A and 1B). Most of the data derive from a yeast two-hybrid or affinity-
115 purification mass spectrometry screen with no systematic experimental validation of the
116 interactions and therefore cannot be assumed *a priori* as high confidence data [14]. However
117 data integration helps reveal splicing factors which are found to be associated with multiple
118 viral species and represent potentially relevant targets for onward studies, e.g. DDX5 and
119 FUS (Figure 1C). Some PPIs have been identified in low throughput studies and are well
120 documented such as the recently identified interaction between the reovirus protein $\mu 2$ and
121 the SR protein SRSF2, which alters SRSF2 function and determines the virus ability to
122 counteract the interferon response [24]. Additional PPIs and associated effects on the host
123 splicing machinery are listed in Table 1. Furthermore, viral RNAs can act on the splicing
124 machinery, as exemplified by the changes in AS events observed upon sequestration of the
125 HuR protein by the 3'UTR of Sindbis virus [25], (Table 1).

126

127 **Peering into viral-induced alterations of host alternative splicing events**

128 To date there are only a few studies that performed transcriptome-wide micro-array or
129 RNAseq analysis of cellular AS events in virus-infected cells (Table 2). They concern
130 herpesviruses [26-28], reoviruses [24, 29], dengue viruses (DENV) [12, 30], Zika virus [31]
131 and influenza viruses [32], and reveal several hundreds of host genes that show altered
132 mRNA splicing upon infection. When examined, no correlation was found between changes
133 in AS events and changes in mRNA expression levels. Validation by RT-PCR or RT-qPCR of a
134 selected subset of the predicted differential AS events is compulsory and usually performed
135 to sort out and confirm RNAseq findings. Validation rates can only be determined if a large
136 enough number of AS events is assayed, and are likely to depend on the metrics and
137 statistical analysis that are used. In the few studies which provide GO terms enrichment
138 analysis on the list of differentially spliced genes in virus-infected cells, an enrichment in
139 genes related to cell cycle, gene expression and/or RNA metabolism was reported [24, 27,

140 30-32]. Consistently with previous reports that the AS landscape varies between human
141 tissues [33], neural progenitor cells infected with the human cytomegalovirus (HCMV)
142 exhibited fewer AS changes compared to fibroblasts and only half of altered exon-skipping
143 overlapped in both cell types [26]. Exon-skipping, which is most frequently detected in the
144 human transcriptome [33] accounts for the largest share of infection-altered AS events in all
145 except the DENV5 study [12]. Interestingly intron retention (IR) which, although identified
146 early on in spliced viral mRNAs, was until recently considered a rare event in mammalian
147 cells, was found to represent a substantial proportion (> 20%) of infection-altered AS events
148 in herpes simplex virus-1 (HSV-1) and DENV5-infected cells [12, 27]. These observations align
149 with recent findings that IR is actually a common AS event in mammalian cells, although the
150 fate of intron-retaining mRNAs regarding nucleo-cytoplasmic export or nonsense mediated
151 decay is not fully understood [34]. Taken together with a recent report that influenza virus
152 NS1 protein primarily binds intronic sequences [35], they suggest that some viruses may
153 have evolved specific mechanisms to alter host cell expression through increased IR. Finally,
154 within the last couple of years the first reports of viral-induced alterations of cirRNAs were
155 published. Notably, cirRNAs were found to be expressed and associated with the
156 NF90/NF110 factor at lower levels upon Vesicular Stomatitis Virus (VSV) infection, which
157 results in increased NF90/NF110 binding to viral mRNAs in the cytoplasm and thereby likely
158 contributes to the antiviral immune response [36]. A global dysregulation of circRNAs was
159 observed upon infection with HSV-1, which could potentially modulate the cellular
160 transcriptional responses through the miRNA sponge function of circRNAs [37].

161 Splicing alterations observed upon infection potentially result from the combination of two
162 types of mechanisms: i) they can be caused by a viral manipulation of the splicing machinery,
163 as described above; or ii) they can be related to viral-induced cellular damages or innate
164 immune responses (Figure 2). Indeed, there is increasing evidence that AS is a mechanism to
165 regulate the immune responses to pathogens [38-40] (Box 1), as well as apoptosis [41], DNA
166 damage response [42] and endoplasmic reticulum stress response [43]. Integration of multi-
167 omics data will probably be key to distinguishing between direct and indirect effects of viral
168 infection on the cellular splicing machinery and uncovering of the complex mechanisms by
169 which the host-cell splicing landscape is modified (see below).

170 In some instances, differences in viral-induced splicing changes were reported when viral
171 variants were compared. Reovirus strain T1L, compared to strain T3D which differs in that its
172 $\mu 2$ protein does not localize to nuclear speckles, triggered more splicing changes *i.e.* 369
173 compared to 142, with an overlap of only 35 changes. The cellular processes which were
174 most affected by T1L infection, *i.e.* gene expression and RNA post-transcriptional
175 modification, were not strongly affected by T3D infection [24]. Comparison of a subset of AS
176 events upon infection with DENV2 and DENV4 revealed differences, suggesting a possible
177 serotype specificity in AS alteration [12]. However the molecular mechanisms that lead to
178 specific alterations of the splicing landscape remain largely unknown. The only evidence for
179 a sequence recognition mechanism comes from Tang et al. who showed that the ICP27
180 protein of HSV-1 mediates splicing alterations in genes that are GC-rich, with suboptimal
181 splicing sites and cytosine-rich sequences close to the 5' splice site [44]. The presence of a
182 conserved 41 nucleotide motif was observed in 93 out of 240 AS events that were
183 dysregulated upon infection with the reovirus strain T3D [29], however this motif does not
184 correspond to a predicted RNA regulatory motif and the significance of this observation
185 remains to be demonstrated.

186 Importantly, transcriptomics studies revealed that not only splicing but also other features of
187 cellular mRNAs can be altered upon viral infection. For instance, DENV1 was found to induce
188 alterations in the relative usage of transcriptional start sites, in addition to splicing changes
189 [30]. Infection with herpesviruses was shown to trigger widespread disruption of
190 transcription termination of cellular mRNAs [26-28], which in the case of HCMV infection
191 was attributed to a strong induction of the host RNA-binding protein CPEB1 [26].
192 Transcription extends over thousands of nucleotides beyond poly(A) sites and into
193 downstream genes, it interferes with the analysis of transcriptional and splicing regulation of
194 the downstream gene and can generate novel intergenic splicing between exons of two
195 neighbouring genes [28]. Defective transcription termination of cellular mRNAs was also very
196 recently reported in influenza virus-infected cells [45-47]. While most studies provide
197 information about the steady-state levels of mRNA isoforms, a few of them rely on the NET-
198 Seq method [45] or on 4sU tagging of mRNAs [28] to provide a real-time view of viral-
199 induced changes in co-transcriptional RNA processing. As these methods allow to detect
200 actively or newly transcribed mRNAs, they can also help avoid biases due to isoform-specific

201 differences in the stability of mRNA. Transcriptomic analysis and mechanistic understanding
202 of how viral infection impacts cellular mRNA co- and post-transcriptional processing is an
203 expanding field, constantly evolving in response to progress in cellular biology (e.g. the
204 recent findings on the biogenesis and function of circRNAs) and in technologies (as discussed
205 below).

206

207 **Towards a more accurate view: methodological challenges**

208 RNAseq transcriptomic analysis of AS is a challenging issue, and even more so when
209 performed on a virus-host system. Accurate quantification of isoform abundance requires a
210 high read number (about 50 millions paired-end reads of at least 75 bp are recommended
211 for the human transcriptome), which to date can only be provided by the Illumina
212 technology. However a serious limitation of Illumina RNAseq is that it relies on short reads,
213 so that the resolution of exon connectivity and full-length isoform structure cannot be
214 achieved (Box 2). Upon viral infection the reads mapping to viral mRNAs may represent 5-
215 75% of the total number of reads (Table 2), therefore the sequencing depth of virus- and
216 mock-infected samples must be adjusted accordingly. The degree of variability of AS viral-
217 induced perturbations also need to be taken into account when setting the sequencing
218 depth and the number of biological replicates, in order to differentiate biologically relevant
219 changes from transcriptional noise.

220 Many viruses induce host-cell transcription shut-off [10] although specific genes (e.g. IFN-
221 stimulated genes, cytokines) can escape this shut-off and be up-regulated. Marked
222 differences between the gene expression profiles of mock- and virus-infected cells poses a
223 challenge for accurate comparison of isoform abundance. Commonly used RNAseq
224 normalization methods [48] make the assumption that a core set of genes is not
225 differentially expressed and mainly correct for sequencing depth. Normalization methods
226 taking into account other variability factors (e.g. the average amount of cellular RNA per cell)
227 besides sequencing depth for host-pathogen dual RNAseq experiments need to be
228 developed [49].

229 Another methodological challenge of viral-host RNAseq lies in the ability to deal with cell-to-
230 cell heterogeneity during infection. Usually high multiplicities of infection are used and it is
231 verified or assumed that almost all cells are infected. However, single-cell RNAseq

232 (scRNAseq) experiments revealed extensive variability from one individual cell to another in
233 terms of production of intracellular viral RNAs and cellular responses [50-52]. RNAseq
234 experiments on polyadenylated mRNAs or circRNAs extracted from bulk cell cultures or
235 tissues are providing an average measure of isoform abundance; they are potentially
236 masking heterogeneity that occurs in the dynamics of infection and/or specific
237 transcriptomic profiles in a subset of cells which could be relevant to the viral phenotype.
238 Single cell RNAseq can be used for transcriptome-wide differential splicing isoform
239 quantification, however this requires specific bioinformatics tools and normalization
240 procedures to be developed, to cope with the low reads counts, the heterogeneity and noise
241 in the datasets [53-55].

242 So far, most RNAseq analyses of splicing events in virus-infected cells have been conducted
243 with cancer cell lines and/or viral laboratory strains. Such experimental conditions have
244 practical advantages (ready availability, ability to infect almost 100% of the cells, and higher
245 reproducibility), however they may not accurately reflect physiological infection. The
246 development of scRNAseq, which allows discrimination between infected and uninfected
247 cells, will probably encourage the use of field viral isolates and more relevant cellular
248 systems such as primary cells or tissue explants. Novel perspectives will be opened by the
249 use of induced human pluripotent cells (iPS) which upon differentiation can serve as
250 convenient surrogates for primary cells that are difficult to isolate and culture [56], and 3D
251 organoid cultures which can provide an accurate model for the micro-anatomy of an organ
252 [57].

253

254 **Integration of isoforms datasets in a systems biology perspective**

255 The rapid advancement of high-throughput technologies has led to the development of the
256 systems biology field, which aims at modeling the properties of complex biological systems,
257 and predicting their response to biological or chemical perturbations. The commonly used
258 “top down” systems biology approach turns RNAseq measurement of expression levels into
259 a variable which can be included in a mathematical model, such as a generalized linear
260 model or a multivariate analysis (Principal Component Analysis, Singular Value
261 Decomposition, Partial Least Square). The variance across genes or isoforms, samples and
262 conditions is computed to identify statistically significant transcriptomics signatures, *i.e.*

263 particular combinations of thousands of genes or isoform expression levels. The mechanistic
264 interpretation of these signatures and the prioritization of candidate genes for downstream
265 experimental validation remain challenging tasks. However, the use of transcriptomic
266 signatures has already allowed genetically close viral strains to be robustly distinguished
267 [58], species-specific responses to infection outlined [59] and the outcome of viral infections
268 predicted [60]. It can also pave the way towards the discovery of relevant biomarkers [61].
269 Transcriptomic signature analysis has become a routine at the gene level, but little has been
270 done so far at the isoform-level. Recent methodological developments - such as splicing
271 signature comparison workflows for the discovery of candidate splicing regulatory elements
272 - have been applied to psoriasis studies [62, 63] and could be transferred to the analysis of
273 infected-cells in the future. Further progress in the field will also be enhanced by the
274 accessibility and the integration of published RNAseq datasets within open access
275 knowledge bases (e.g. ArrayExpress, Gene Expression Omnibus), which allows meta-analyses
276 to be conducted.

277 The integration of RNAseq datasets with other omics datasets, so far mostly proteomics,
278 interactomics and metabolomics datasets, has become a new way to rationalize the
279 deconvolution of the transcriptomics signal, and has been applied to virus-infected cells [64-
280 66] or patients [67]. To facilitate this integration step, network-based methods such as
281 correlation network (e.g. WGCNA) or probabilistic models (e.g. MERLIN) are currently being
282 investigated (reviewed in [68]). The main objective is to infer the transcriptional gene
283 regulatory network and to prioritize a set of candidate genes, transcriptional factors or
284 functional modules that are involved in viral infection. A growing number of studies combine
285 newly generated experimental data with published datasets available in open access
286 databases to identify the most relevant molecular pathways. For example, by combining
287 proteomics and RNA-seq experimental data along with available gene-regulatory and
288 protein-protein interaction networks Sychev *et al.* successfully implemented a
289 computational model based on the Prize-Collecting Steiner Forest algorithm, which
290 highlighted peroxisome lipid metabolism as an important function involved in KSHV (Kaposi's
291 sarcoma associated herpesvirus) latent infection [65].

292 One should stress that integration is for now only performed at the gene-level. Gene
293 annotation knowledge bases (GO, Interpro, and KEGG) are exploited to investigate

294 enrichment of specific biological or molecular functions (GSEA). Although they are
295 continuously being improved, gene annotations are still far from being complete [69], and
296 even more so at the isoform level and for non-model organisms. Gene annotations often
297 relate to a “reference isoform” which is assumed to be the predominant one, whereas the
298 relative proportion of splicing isoforms may differ from one tissue to another. Alternative
299 splicing may lead to the gain or loss of functional domains, catalytic sites and/or protein-
300 protein interfaces. However, the full set of alternative isoforms that effectively contribute to
301 proteomic diversity and represent “functional alloforms” remains to be characterized [70,
302 71]. Available Exon Ontology resources can be used to readily identify enriched functions of
303 gene isoform subsets but they restrict the analysis at the exon-level [72]. The systematic
304 characterization of AS isoforms function is a challenging task and might be accelerated
305 through bioinformatics prediction [72-74] or experimentally by using interactomics
306 approaches [75, 76].

307 As molecular approaches to study virus-host interactions at a high level of mechanistic detail
308 are also making steady progress, the “bottom up” systems biology approach also seem
309 promising. In this approach the knowledge generated by the molecular and biochemical
310 characterization of a subsystem and its response to perturbations is used to generate
311 specific sub-networks, which can subsequently be integrated together with high-throughput
312 data into larger molecular interaction networks [77-79]. In the case of the spliceosome
313 machinery, subnetwork modeling is making progress through Bayesian probability modeling
314 [80], food-web modeling [81], or deep-learning approaches [82], which offers advanced
315 tools for studying its involvement and vulnerability upon viral infection. In return, research
316 on the virus-spliceosome interplay will likely contribute to a better definition of the complex
317 set of rules that can predict the splicing pattern of each isoform based on a comprehensive
318 catalogue of cis-regulatory elements and their functional molecular interactions in various
319 physiological and pathological conditions.

320

321 **Concluding remarks**

322 The importance of characterizing the transcriptome landscape of virus-infected cells at the
323 splicing level is highlighted by recent studies, which reveal significant AS alterations in
324 response to viral infections. The observed changes in AS events, which are regulated through

325 a very complex protein-RNA interaction network, can either be a direct consequence of viral
326 manipulation of the host splicing machinery or result indirectly from viral-induced immune
327 responses or cellular damages. Integration of multiple omics datasets in a systems biology
328 perspective will be needed to comprehend this complex facet of virus-host interactions.
329 Beyond proteomics and interactomics, which have been most commonly combined with
330 RNAseq analysis so far, epigenomics and epitranscriptomics would also be relevant. Indeed,
331 viral infections may induce epigenetic [83, 84] or epitranscriptomic [85] modifications, and
332 both types of modifications may in turn affect splicing [86, 87]. Genetic mutations that affect
333 AS, and consequently may determine phenotypic variability and individual susceptibility to
334 viral diseases, is also an interesting direction for future research [88]. Species-dependent AS
335 patterns of cellular genes could possibly be determinant for viral host-range, as suggested
336 recently for influenza viruses [89]. As technological advances in RNA sequencing and RNA
337 genomics will allow to study the interplay between cellular AS and viral infections at an
338 increasing scale and resolution, major challenges in terms of computational analysis and
339 storage of the corresponding datasets will need to be addressed, and the building of
340 pluridisciplinary research teams along the lines of the European Virus Bioinformatics Center
341 (<http://evbc.uni-jena.de>) [90] will be paramount. Integration of splicing isoform datasets
342 with other omics data may well contribute to the development of personalized prognosis
343 and management of infectious diseases and lead to therapeutic innovations.
344

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352

353 **Box 1 : Role of alternative splicing in shaping immune responses.**

354 In the recent years there has been growing evidence for a role of AS in shaping both innate
355 and adaptative immune responses [38-40]. Different isoforms have been found for key
356 players of the antiviral innate immunity, including pattern-recognition receptors (e.g. TLRs,
357 RIG-I, and MDA5), downstream signaling proteins (e.g. MyD88, MAVS, STING, TBK1, and
358 IRF3), and effectors (IFN Type I, IFNAR, cytokines and chemokines). The splicing factors
359 involved are known in a few cases : EFTUD2 regulates the splicing of RIG-I, MDA5 and
360 MyD88 [91], while SF3A1 affects the splicing of several genes of the TLR signaling pathway
361 including MyD88 [92]. In several instances, splice variants exert a negative feedback loop on
362 the signaling pathway, thereby probably controlling the intensity and duration of the
363 antiviral and inflammatory responses. Notably, RIG-I and STING splice variants whose
364 expression are up-regulated upon viral infection strongly inhibit RIG-I and STING signaling
365 pathways, respectively [93, 94]. A viral-induced, alternatively spliced isoform of TPK-1
366 disrupts the interaction between RIG-I and MAVS and inhibits IFN-beta signaling [95]. Short
367 isoforms of MAVS negatively regulate TLR3-mediated nucleic acid sensing [96], and limit self-
368 aggregation of the full-length MAVS protein thereby preventing accidental antiviral innate
369 immune signaling [97]. The contribution of AS to the regulation of humoral and cellular
370 adaptative immune responses is also clearly recognized, however the mechanisms involved
371 remain largely unknown. In B cells two mechanisms were recently uncovered : the HuR
372 protein, by regulating mRNA splicing upon B cell activation, is essential for antibody
373 response to a variety of antigens [98], while the ZFP318 factor regulates the AS-dependent
374 balance between IgM and IgD immunoglobulins [99]. In T cells, one of the best documented
375 examples is the AS of CD45 in response to antigen receptor-mediated signaling, which is
376 differentially regulated depending on the T cell lineage and the stage of activation (reviewed
377 in [39, 40]).

378

379

380

381 **Box 2 : Resolving alternative splicing by short- or long-reads sequencing technologies.**

382 Currently Illumina is the most commonly used RNAseq technology for transcriptome-wide
383 analysis of AS. However Third Generation Sequencing (TGS) technologies, such as the Pacific
384 Biosciences (PacBio) and Oxford Nanopore (ON) technologies, are emerging as alternative
385 platforms for AS analysis [100].

386 The major advantage of Illumina over the TGS technologies is its higher sequencing depth
387 (up to 400 million reads instead of 1 million for TGS), even more so in the case of dual
388 RNAseq when both the viral and host transcriptome need to be sequenced. High depth is
389 needed for the detection of minor isoforms and the robust quantification and statistical
390 analysis of AS events [101]. Another advantage of Illumina resides in its higher sequencing
391 accuracy (about 0.1% error rates instead of 10-15% for TGS), which is particularly
392 advantageous when working on poorly annotated genomes of non-model organisms.
393 However, a major limitation of Illumina is that it generates short reads (75-300 bp) which
394 provides only local information about AS events and entails a challenging computational
395 reconstruction of full-length isoforms. The strength of TGS is their read length (1-100 kb),
396 which allows the direct characterisation of full-length transcripts with information not only
397 on alternative splicing and the coordination of distant exons [102] but also on alternative
398 transcription start and termination sites [103]. Moreover long reads allow to resolve
399 repetitive sequences, that are posing a major challenge for sequence assembly or alignment
400 from short-read datasets.

401 Future advances may lie in the Hybrid-Seq approach which combines short and long-read
402 technologies [101], the Synthetic Long-Read technology which exploits the assets of Illumina
403 with a short-read barcoding system to reconstruct full-length transcripts [104], or the very
404 swift evolution of long-read technologies. Particularly promising is the perspective of direct
405 RNA sequencing proposed by ON, which would avoid reverse-transcription- and
406 amplification-related biases in isoforms quantification [105].

407

408

409

410 **Table 1. Viral targeting of the splicing machinery.**

411
412

Virus	Viral factor	Cellular Target	Associated cellular changes^a	References
HSV-1	ICP27	Binding to, relocalisation and inhibition of SRPK1	Altered phosphorylation of SR proteins	[22]
HSV-1	ICP27	Binding to SF3B2*	ND	[106]
HIV-1	Vpr	Binding to SF3B2*	Altered splicing of pre-mRNAs	[107]
EBV	SM	Binding to SRSF3	Altered splicing of STAT1 pre-mRNA	[108]
HPV1	E1 [^] E4	Binding to and inhibition of SRPK1	Altered phosphorylation of SR proteins	[21]
HPV16	E2	Transactivation of SRSF1-3 promoters	ND	[109]
Adenovirus	E4-ORF4	Binding to SRSF1, SRSF9	Modulation of pre-mRNA splicing	[20]
Influenza V	NS1	Binding to U6snRNA	Inhibition of pre-mRNA splicing	[110]
Influenza V	NS1	Relocalisation of SRSF2	ND	[111]
Influenza V	NS1	Binding to and relocalisation of NS1-BP	Altered splicing of some pre-mRNAs regulated by NS1-BP	[112, 113]
Poliovirus	2A	Relocalisation of HuR, TIA-1, TIAR	Modulation of Fas6 pre-mRNA splicing	[114]
Poliovirus	2A	Relocalisation of SRSF3	ND	[115]
EV-71	3D (Pol)	Binding to and inhibition of PRPF8**	Blockage of pre-mRNA splicing and mRNA synthesis	[116]
FMDV	3C (Pro)	Cleavage and relocalisation of Sam68	ND	[117]
Reovirus	μ2	Binding to and relocalisation of SRSF2	Altered splicing of pre-mRNAs	[24]
Rotavirus	NSP2, NSP5	Association with and relocalisation of hnRNPs and HuR	ND	[118]
Sindbis V	nsP2	Binding to hnRNPK	ND	[119]
Alphaviruses	3' UTR	Binding to and relocalisation of HuR	Altered stability, splicing and polyadenylation of mRNAs	[25, 120]
HCV	3'UTR	Binding to and relocalisation of HuR	ND	[121]
DENV-1	NS5 protein	Binding to CD2BP2 DDX23**	Altered pre-mRNA splicing in vitro	[12]
VSV	M	Relocalisation of hnRNPH	ND	[122]

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^aND: not determined, * component of the U2 snRNP, ** component of the U5 snRNP,

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Table 2. Transcriptome-wide analyses of AS in virus-infected cells.

	Rutkowski et al. 2015 [28]	Hu et al. 2016 [27]	Batra et al. 2016 [26]	Boudreault et al. 2016 [29]	Rivera-S et al. 2017 [24]	Sessions et al. 2013 [30]	De Maio et al. 2016 [12]	Hu et al. 2017 [31]	Fabozzi et al. 2018 [32]
Virus	HSV-1	HSV-1	HCMV	Reovirus	Reovirus	Dengue V	Dengue V	Zika V	Influenza V
Host-cell model^a	Human Fibroblasts	Human Fibroblasts	Human Fibroblasts & NPC	Murine L929 cells	Murine L929 cells	Human HuH7 cells	Human A549 cells	Human NPC	Human BEAS-2B cells
Number of replicates	n=2	n=3 pooled	n=1	n=3	n>1 pooled	n=3	n=3	n=2	n=3
Sequencing library	ribo-depleted	polyA+	polyA+	polyA+	polyA+	polyA+	polyA+	polyA+	ribo-depleted
Sequencing platform	HiSeq 2500	HiSeq 2000	HiSeq	HiSeq 2000	NextSeq 500	HiSeq 2000/GA II x	HiSeq 4000	NextSeq 500	HiSeq 2000
Reads features^a	2 x 101 nt	2 x 90 nt	1 x 101 nt	2 x 100 nt	2 x 50 nt	2 x 75 nt	2 x 90 nt	2 x 75 nt	2 x 50 nt
Sequencing depth	> 25 x 10 ⁶ reads	~ 26 x 10 ⁶ reads*	~ 130-230 x 10 ⁶ reads	> 40 x 10 ⁶ reads	NA	~ 20-100 x 10 ⁶ reads	> 20 x 10 ⁶ reads	~ 7 x 10 ⁶ reads	~ 30 x 10 ⁶ reads
Fraction of viral reads^b	27%	30%	22-68%	NA	NA	NA	30-40%	NA	~5-75%**
Mapping	Context Map	TopHat2	GSNAP	Bowtie2	TopHat	TopHat	TopHat2	TopHat2	TopHat2
Gene expression analysis	RPKM	Cufflinks	RPKM	RSEM	NA	Cufflinks	NA	Cufflinks	DESeq2
Alternative splicing analysis	Home-made scripts	Cufflinks ASD DaPars	Olego Quantas MISO	RSEM	MISO	Cufflinks MISO	ASpli edgeR	Cufflinks ASD	MISO
Data availability^b	GSE59717	NA	GSE74250	GSE81017	NA	NA	GSE84285	GSE78711	GSE61517

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^a NPC: Neural Progenitor Cells

^b nt: nucleotide

^c NA: not available

* number of reads for the pooled samples, ** depending on the viral strain and time-point

426 **Figure legends.**

427

428 **Figure 1. Virus-spliceosome protein-protein interaction network.**

429 **A.** Summary of virus-spliceosome protein-protein interactions, as recorded in the VirHostNet
430 database, January release 2018 [23]. For each viral family, the number of viral species (as
431 defined by the International Committee on Taxonomy of Viruses and the National Center for
432 Biological Information or NCBI) and viral proteins (as defined by the NCBI Reference
433 Sequence and the UniProt databases) reported to interact with at least one cellular
434 spliceosomal factor (among the list of 244 factors defined in [123]) is indicated. The number
435 of viral proteins obtained upon merging of homologous proteins derived from different
436 strains or isolates of a single viral species, grouped thereafter under the name “viral proteins
437 types” and represented as a single node in B, are indicated into brackets.

438 **B and C.** Interaction network between viral proteins (B) or viral species (C) and cellular
439 spliceosomal proteins. The network was built with Cytoscape (version 3.2.1). Nodes and
440 edges between nodes represent protein and protein-protein interactions, respectively. Core
441 and regulatory spliceosomal factors are represented by square and triangular black nodes,
442 respectively. **B.** Viral proteins types, as defined in A, are represented by colored nodes (the
443 color code is according to the Table in A). The size of the viral protein nodes is proportional
444 to their degree of connectivity (i.e. the number of interacting partners of a protein) and the
445 layout is done according to their centrality in the network. **C.** Viral species are represented
446 by colored nodes (the color code is according to the Table in A). The size of the spliceosomal
447 factor nodes is proportional to their degree of connectivity and the layout is done according
448 to their centrality. The five spliceosomal factors showing the highest degree of connectivity
449 (interaction with 11 to 15 distinct viral species) are indicated with a white star and edges
450 representing their protein-protein interactions with viral species are highlighted in red.

451

452 **Figure 2. Direct and indirect mechanisms for viral-induced splicing alterations.**

453 Red arrows represent viral manipulation of the splicing machinery and the resulting AS
454 changes, which have in turn the potential to modulate innate immunity [16, 95] or apoptosis
455 [124]. Blue arrows represent viral-induced cell damages and innate immune responses and
456 the subsequent AS changes in the infected cell [40-43]. Grey arrows represent viral-induced

457 B- and T-cell immune responses, which are subject to AS-mediated regulation [38-40]. UPR:
458 Unfolded Protein Response. DDR : DNA Damage Response.
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462 **References**

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- 464 1. Fica SM, Nagai K. Cryo-electron microscopy snapshots of the spliceosome: structural insights
465 into a dynamic ribonucleoprotein machine. *Nat Struct Mol Biol.* 2017;24(10):791-9. Epub
466 2017/10/06. doi: 10.1038/nsmb.3463. PubMed PMID: 28981077.
- 467 2. Papasaikas P, Valcarcel J. The Spliceosome: The Ultimate RNA Chaperone and Sculptor.
468 *Trends Biochem Sci.* 2016;41(1):33-45. Epub 2015/12/20. doi: 10.1016/j.tibs.2015.11.003. PubMed
469 PMID: 26682498.
- 470 3. Lee Y, Rio DC. Mechanisms and Regulation of Alternative Pre-mRNA Splicing. *Annu Rev*
471 *Biochem.* 2015;84:291-323. Epub 2015/03/19. doi: 10.1146/annurev-biochem-060614-034316.
472 PubMed PMID: 25784052; PubMed Central PMCID: PMC4526142.
- 473 4. Sakharkar MK, Chow VT, Kanguene P. Distributions of exons and introns in the human
474 genome. *In Silico Biol.* 2004;4(4):387-93. Epub 2004/06/26. PubMed PMID: 15217358.
- 475 5. Li X, Yang L, Chen LL. The Biogenesis, Functions, and Challenges of Circular RNAs. *Mol Cell.*
476 2018;71(3):428-42. Epub 2018/07/31. doi: 10.1016/j.molcel.2018.06.034. PubMed PMID: 30057200.
- 477 6. Wang M, Yu F, Wu W, Zhang Y, Chang W, Ponnusamy M, et al. Circular RNAs: A novel type of
478 non-coding RNA and their potential implications in antiviral immunity. *Int J Biol Sci.*
479 2017;13(12):1497-506. Epub 2017/12/13. doi: 10.7150/ijbs.22531. PubMed PMID: 29230098;
480 PubMed Central PMCID: PMC5723916.
- 481 7. Kornblihtt AR, Schor IE, Allo M, Dujardin G, Petrillo E, Munoz MJ. Alternative splicing: a
482 pivotal step between eukaryotic transcription and translation. *Nat Rev Mol Cell Biol.* 2013;14(3):153-
483 65. Epub 2013/02/07. doi: 10.1038/nrm3525. PubMed PMID: 23385723.
- 484 8. Richards AL, Watza D, Findley A, Alazizi A, Wen X, Pai AA, et al. Environmental perturbations
485 lead to extensive directional shifts in RNA processing. *PLoS Genet.* 2017;13(10):e1006995. Epub
486 2017/10/13. doi: 10.1371/journal.pgen.1006995. PubMed PMID: 29023442; PubMed Central PMCID:
487 PMC5667937.
- 488 9. Harwig A, Landick R, Berkhout B. The Battle of RNA Synthesis: Virus versus Host. *Viruses.*
489 2017;9(10). Epub 2017/10/27. doi: 10.3390/v9100309. PubMed PMID: 29065472; PubMed Central
490 PMCID: PMC5691660.
- 491 10. Herbert KM, Nag A. A Tale of Two RNAs during Viral Infection: How Viruses Antagonize
492 mRNAs and Small Non-Coding RNAs in The Host Cell. *Viruses.* 2016;8(6). Epub 2016/06/09. doi:
493 10.3390/v8060154. PubMed PMID: 27271653; PubMed Central PMCID: PMC4926174.
- 494 11. Rivas HG, Schmaling SK, Gaglia MM. Shutoff of Host Gene Expression in Influenza A Virus and
495 Herpesviruses: Similar Mechanisms and Common Themes. *Viruses.* 2016;8(4):102. Epub 2016/04/20.
496 doi: 10.3390/v8040102. PubMed PMID: 27092522; PubMed Central PMCID: PMC4848596.
- 497 12. De Maio FA, Risso G, Iglesias NG, Shah P, Pozzi B, Gebhard LG, et al. The Dengue Virus NS5
498 Protein Intrudes in the Cellular Spliceosome and Modulates Splicing. *PLoS Pathog.*
499 2016;12(8):e1005841. Epub 2016/08/31. doi: 10.1371/journal.ppat.1005841. PubMed PMID:
500 27575636; PubMed Central PMCID: PMC5004807.
- 501 13. Twyffels L, Gueydan C, Krays V. Shuttling SR proteins: more than splicing factors. *FEBS J.*
502 2011;278(18):3246-55. Epub 2011/07/29. doi: 10.1111/j.1742-4658.2011.08274.x. PubMed PMID:
503 21794093.
- 504 14. Cohen S, Etingov I, Pante N. Effect of viral infection on the nuclear envelope and nuclear pore
505 complex. *Int Rev Cell Mol Biol.* 2012;299:117-59. Epub 2012/09/11. doi: 10.1016/B978-0-12-394310-
506 1.00003-5. PubMed PMID: 22959302.
- 507 15. Avota E, Harms H, Schneider-Schaulies S. Measles virus induces expression of SIP110, a
508 constitutively membrane clustered lipid phosphatase, which inhibits T cell proliferation. *Cell*
509 *Microbiol.* 2006;8(11):1826-39. Epub 2006/07/11. doi: 10.1111/j.1462-5822.2006.00752.x. PubMed
510 PMID: 16824039.

511 16. Zhu C, Xiao F, Hong J, Wang K, Liu X, Cai D, et al. EFTUD2 Is a Novel Innate Immune Regulator
512 Restricting Hepatitis C Virus Infection through the RIG-I/MDA5 Pathway. *J Virol.* 2015;89(13):6608-
513 18. Epub 2015/04/17. doi: 10.1128/JVI.00364-15. PubMed PMID: 25878102; PubMed Central PMCID:
514 PMCPMC4468487.

515 17. Howard JM, Sanford JR. The RNAissance family: SR proteins as multifaceted regulators of
516 gene expression. *Wiley Interdiscip Rev RNA.* 2015;6(1):93-110. Epub 2014/08/27. doi:
517 10.1002/wrna.1260. PubMed PMID: 25155147; PubMed Central PMCID: PMCPMC4268343.

518 18. Wojcechowskyj JA, Didigu CA, Lee JY, Parrish NF, Sinha R, Hahn BH, et al. Quantitative
519 phosphoproteomics reveals extensive cellular reprogramming during HIV-1 entry. *Cell Host Microbe.*
520 2013;13(5):613-23. Epub 2013/05/21. doi: 10.1016/j.chom.2013.04.011. PubMed PMID: 23684312;
521 PubMed Central PMCID: PMCPMC4104530.

522 19. Lapek JD, Jr., Lewinski MK, Wozniak JM, Guatelli J, Gonzalez DJ. Quantitative Temporal
523 Viromics of an Inducible HIV-1 Model Yields Insight to Global Host Targets and Phospho-Dynamics
524 Associated with Protein Vpr. *Mol Cell Proteomics.* 2017;16(8):1447-61. Epub 2017/06/14. doi:
525 10.1074/mcp.M116.066019. PubMed PMID: 28606917; PubMed Central PMCID: PMCPMC5546197.

526 20. Estmer Nilsson C, Petersen-Mahrt S, Durot C, Shtrichman R, Krainer AR, Kleinberger T, et al.
527 The adenovirus E4-ORF4 splicing enhancer protein interacts with a subset of phosphorylated SR
528 proteins. *EMBO J.* 2001;20(4):864-71. Epub 2001/02/17. doi: 10.1093/emboj/20.4.864. PubMed
529 PMID: 11179230; PubMed Central PMCID: PMCPMC145406.

530 21. Prescott EL, Brimacombe CL, Hartley M, Bell I, Graham S, Roberts S. Human papillomavirus
531 type 1 E1^{E4} protein is a potent inhibitor of the serine-arginine (SR) protein kinase SRPK1 and
532 inhibits phosphorylation of host SR proteins and of the viral transcription and replication regulator
533 E2. *J Virol.* 2014;88(21):12599-611. Epub 2014/08/22. doi: 10.1128/JVI.02029-14. PubMed PMID:
534 25142587; PubMed Central PMCID: PMCPMC4248925.

535 22. Sciabica KS, Dai QJ, Sandri-Goldin RM. ICP27 interacts with SRPK1 to mediate HSV splicing
536 inhibition by altering SR protein phosphorylation. *EMBO J.* 2003;22(7):1608-19. Epub 2003/03/28.
537 doi: 10.1093/emboj/cdg166. PubMed PMID: 12660167; PubMed Central PMCID: PMCPMC152910.

538 23. Guirimand T, Delmotte S, Navratil V. VirHostNet 2.0: surfing on the web of virus/host
539 molecular interactions data. *Nucleic Acids Res.* 2015;43(Database issue):D583-7. Epub 2014/11/14.
540 doi: 10.1093/nar/gku1121. PubMed PMID: 25392406; PubMed Central PMCID: PMCPMC4383936.

541 24. Rivera-Serrano EE, Fritch EJ, Scholl EH, Sherry B. A Cytoplasmic RNA Virus Alters the Function
542 of the Cell Splicing Protein SRSF2. *J Virol.* 2017;91(7). Epub 2017/01/13. doi: 10.1128/JVI.02488-16.
543 PubMed PMID: 28077658; PubMed Central PMCID: PMCPMC5355601.

544 25. Barnhart MD, Moon SL, Emch AW, Wilusz CJ, Wilusz J. Changes in cellular mRNA stability,
545 splicing, and polyadenylation through HuR protein sequestration by a cytoplasmic RNA virus. *Cell*
546 *Rep.* 2013;5(4):909-17. Epub 2013/11/12. doi: 10.1016/j.celrep.2013.10.012. PubMed PMID:
547 24210824; PubMed Central PMCID: PMCPMC3849337.

548 26. Batra R, Stark TJ, Clark E, Belzile JP, Wheeler EC, Yee BA, et al. RNA-binding protein CPEB1
549 remodels host and viral RNA landscapes. *Nat Struct Mol Biol.* 2016;23(12):1101-10. Epub
550 2016/11/01. doi: 10.1038/nsmb.3310. PubMed PMID: 27775709; PubMed Central PMCID:
551 PMCPMC5140759.

552 27. Hu B, Li X, Huo Y, Yu Y, Zhang Q, Chen G, et al. Cellular responses to HSV-1 infection are
553 linked to specific types of alterations in the host transcriptome. *Sci Rep.* 2016;6:28075. Epub
554 2016/06/30. doi: 10.1038/srep28075. PubMed PMID: 27354008; PubMed Central PMCID:
555 PMCPMC4926211.

556 28. Rutkowski AJ, Erhard F, L'Hernault A, Bonfert T, Schilhabel M, Crump C, et al. Widespread
557 disruption of host transcription termination in HSV-1 infection. *Nat Commun.* 2015;6:7126. Epub
558 2015/05/21. doi: 10.1038/ncomms8126. PubMed PMID: 25989971; PubMed Central PMCID:
559 PMCPMC4441252.

- 560 29. Boudreault S, Martenon-Brodeur C, Caron M, Garant JM, Tremblay MP, Armero VE, et al.
561 Global Profiling of the Cellular Alternative RNA Splicing Landscape during Virus-Host Interactions.
562 PLoS One. 2016;11(9):e0161914. Epub 2016/09/07. doi: 10.1371/journal.pone.0161914. PubMed
563 PMID: 27598998; PubMed Central PMCID: PMC5012649.
- 564 30. Sessions OM, Tan Y, Goh KC, Liu Y, Tan P, Rozen S, et al. Host cell transcriptome profile during
565 wild-type and attenuated dengue virus infection. PLoS Negl Trop Dis. 2013;7(3):e2107. Epub
566 2013/03/22. doi: 10.1371/journal.pntd.0002107. PubMed PMID: 23516652; PubMed Central PMCID:
567 PMC597485.
- 568 31. Hu B, Huo Y, Yang L, Chen G, Luo M, Yang J, et al. ZIKV infection effects changes in gene
569 splicing, isoform composition and lncRNA expression in human neural progenitor cells. Virol J.
570 2017;14(1):217. Epub 2017/11/09. doi: 10.1186/s12985-017-0882-6. PubMed PMID: 29116029;
571 PubMed Central PMCID: PMC568814.
- 572 32. Fabozzi G, Oler AJ, Liu P, Chen Y, Mindaye S, Dolan MA, et al. Strand-Specific Dual RNA
573 Sequencing of Bronchial Epithelial Cells Infected with Influenza A/H3N2 Viruses Reveals Splicing of
574 Gene Segment 6 and Novel Host-Virus Interactions. J Virol. 2018;92(17). Epub 2018/07/07. doi:
575 10.1128/JVI.00518-18. PubMed PMID: 29976658; PubMed Central PMCID: PMC6096831.
- 576 33. Wang ET, Sandberg R, Luo S, Khrebtkova I, Zhang L, Mayr C, et al. Alternative isoform
577 regulation in human tissue transcriptomes. Nature. 2008;456(7221):470-6. Epub 2008/11/04. doi:
578 10.1038/nature07509. PubMed PMID: 18978772; PubMed Central PMCID: PMC2593745.
- 579 34. Rekosh D, Hammarskjold ML. Intron retention in viruses and cellular genes: Detention,
580 border controls and passports. Wiley Interdiscip Rev RNA. 2018;9(3):e1470. Epub 2018/03/07. doi:
581 10.1002/wrna.1470. PubMed PMID: 29508942; PubMed Central PMCID: PMC5910242.
- 582 35. Zhang L, Wang J, Munoz-Moreno R, Kim M, Sakthivel R, Mo W, et al. Influenza Virus NS1
583 Protein RNA-Interactome Reveals Intron Targeting. J Virol. 2018. Epub 2018/09/28. doi:
584 10.1128/JVI.01634-18. PubMed PMID: 30258002.
- 585 36. Li X, Liu CX, Xue W, Zhang Y, Jiang S, Yin QF, et al. Coordinated circRNA Biogenesis and
586 Function with NF90/NF110 in Viral Infection. Mol Cell. 2017;67(2):214-27 e7. Epub 2017/06/20. doi:
587 10.1016/j.molcel.2017.05.023. PubMed PMID: 28625552.
- 588 37. Shi J, Hu N, Mo L, Zeng Z, Sun J, Hu Y. Deep RNA Sequencing Reveals a Repertoire of Human
589 Fibroblast Circular RNAs Associated with Cellular Responses to Herpes Simplex Virus 1 Infection. Cell
590 Physiol Biochem. 2018;47(5):2031-45. Epub 2018/07/05. doi: 10.1159/000491471. PubMed PMID:
591 29972822.
- 592 38. Chang MX, Zhang J. Alternative Pre-mRNA Splicing in Mammals and Teleost Fish: A Effective
593 Strategy for the Regulation of Immune Responses Against Pathogen Infection. Int J Mol Sci.
594 2017;18(7). Epub 2017/07/18. doi: 10.3390/ijms18071530. PubMed PMID: 28714877; PubMed
595 Central PMCID: PMC5536018.
- 596 39. Martinez NM, Lynch KW. Control of alternative splicing in immune responses: many
597 regulators, many predictions, much still to learn. Immunol Rev. 2013;253(1):216-36. Epub
598 2013/04/05. doi: 10.1111/imr.12047. PubMed PMID: 23550649; PubMed Central PMCID:
599 PMC3621013.
- 600 40. Schaub A, Glasmacher E. Splicing in immune cells-mechanistic insights and emerging topics.
601 Int Immunol. 2017;29(4):173-81. Epub 2017/05/13. doi: 10.1093/intimm/dxx026. PubMed PMID:
602 28498981; PubMed Central PMCID: PMC5890895.
- 603 41. Paronetto MP, Passacantilli I, Sette C. Alternative splicing and cell survival: from tissue
604 homeostasis to disease. Cell Death Differ. 2016;23(12):1919-29. Epub 2016/10/01. doi:
605 10.1038/cdd.2016.91. PubMed PMID: 27689872; PubMed Central PMCID: PMC5136496.
- 606 42. Shkreta L, Chabot B. The RNA Splicing Response to DNA Damage. Biomolecules.
607 2015;5(4):2935-77. Epub 2015/11/04. doi: 10.3390/biom5042935. PubMed PMID: 26529031;
608 PubMed Central PMCID: PMC4693264.

609 43. Tsalikis J, Pan Q, Tattoli I, Maisonneuve C, Blencowe BJ, Philpott DJ, et al. The transcriptional
610 and splicing landscape of intestinal organoids undergoing nutrient starvation or endoplasmic
611 reticulum stress. *BMC Genomics*. 2016;17:680. Epub 2016/08/27. doi: 10.1186/s12864-016-2999-1.
612 PubMed PMID: 27561422; PubMed Central PMCID: PMC5000506.

613 44. Tang S, Patel A, Krause PR. Herpes simplex virus ICP27 regulates alternative pre-mRNA
614 polyadenylation and splicing in a sequence-dependent manner. *Proc Natl Acad Sci U S A*.
615 2016;113(43):12256-61. Epub 2016/10/30. doi: 10.1073/pnas.1609695113. PubMed PMID:
616 27791013; PubMed Central PMCID: PMC5087043.

617 45. Bauer DLV, Tellier M, Martinez-Alonso M, Nojima T, Proudfoot NJ, Murphy S, et al. Influenza
618 Virus Mounts a Two-Pronged Attack on Host RNA Polymerase II Transcription. *Cell Rep*.
619 2018;23(7):2119-29 e3. Epub 2018/05/17. doi: 10.1016/j.celrep.2018.04.047. PubMed PMID:
620 29768209; PubMed Central PMCID: PMC5972227.

621 46. Heinz S, Texari L, Hayes MGB, Urbanowski M, Chang MW, Givarkes N, et al. Transcription
622 Elongation Can Affect Genome 3D Structure. *Cell*. 2018;174(6):1522-36 e22. Epub 2018/08/28. doi:
623 10.1016/j.cell.2018.07.047. PubMed PMID: 30146161; PubMed Central PMCID: PMC6130916.

624 47. Zhao N, Sebastiano V, Moshkina N, Mena N, Hultquist J, Jimenez-Morales D, et al. Influenza
625 virus infection causes global RNAPII termination defects. *Nat Struct Mol Biol*. 2018;25(9):885-93.
626 Epub 2018/09/05. doi: 10.1038/s41594-018-0124-7. PubMed PMID: 30177761.

627 48. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-
628 seq data with DESeq2. *Genome Biol*. 2014;15(12):550. Epub 2014/12/18. doi: 10.1186/s13059-014-
629 0550-8. PubMed PMID: 25516281; PubMed Central PMCID: PMC4302049.

630 49. Westermann AJ, Barquist L, Vogel J. Resolving host-pathogen interactions by dual RNA-seq.
631 *PLoS Pathog*. 2017;13(2):e1006033. Epub 2017/02/17. doi: 10.1371/journal.ppat.1006033. PubMed
632 PMID: 28207848; PubMed Central PMCID: PMC5313147.

633 50. Cristinelli S, Ciuffi A. The use of single-cell RNA-Seq to understand virus-host interactions.
634 *Curr Opin Virol*. 2018;29:39-50. Epub 2018/03/21. doi: 10.1016/j.coviro.2018.03.001. PubMed PMID:
635 29558678.

636 51. Russell AB, Trapnell C, Bloom JD. Extreme heterogeneity of influenza virus infection in single
637 cells. *Elife*. 2018;7. Epub 2018/02/17. doi: 10.7554/eLife.32303. PubMed PMID: 29451492; PubMed
638 Central PMCID: PMC5826275.

639 52. Steuerman Y, Cohen M, Peshes-Yaloz N, Valadarsky L, Cohn O, David E, et al. Dissection of
640 Influenza Infection In Vivo by Single-Cell RNA Sequencing. *Cell Syst*. 2018;6(6):679-91 e4. Epub
641 2018/06/11. doi: 10.1016/j.cels.2018.05.008. PubMed PMID: 29886109.

642 53. Huang Y, Sanguinetti G. BRIE: transcriptome-wide splicing quantification in single cells.
643 *Genome Biol*. 2017;18(1):123. Epub 2017/06/29. doi: 10.1186/s13059-017-1248-5. PubMed PMID:
644 28655331; PubMed Central PMCID: PMC5488362.

645 54. Song Y, Botvinnik OB, Lovci MT, Kakaradov B, Liu P, Xu JL, et al. Single-Cell Alternative Splicing
646 Analysis with Expedition Reveals Splicing Dynamics during Neuron Differentiation. *Mol Cell*.
647 2017;67(1):148-61 e5. Epub 2017/07/05. doi: 10.1016/j.molcel.2017.06.003. PubMed PMID:
648 28673540; PubMed Central PMCID: PMC5540791.

649 55. Vallejos CA, Risso D, Scialdone A, Dudoit S, Marioni JC. Normalizing single-cell RNA
650 sequencing data: challenges and opportunities. *Nat Methods*. 2017;14(6):565-71. Epub 2017/05/16.
651 doi: 10.1038/nmeth.4292. PubMed PMID: 28504683; PubMed Central PMCID: PMC5549838.

652 56. Schobel A, Rosch K, Herker E. Functional innate immunity restricts Hepatitis C Virus infection
653 in induced pluripotent stem cell-derived hepatocytes. *Sci Rep*. 2018;8(1):3893. Epub 2018/03/03. doi:
654 10.1038/s41598-018-22243-7. PubMed PMID: 29497123; PubMed Central PMCID:
655 PMC5832748.

656 57. Ramani S, Crawford SE, Blutt SE, Estes MK. Human organoid cultures: transformative new
657 tools for human virus studies. *Curr Opin Virol*. 2018;29:79-86. Epub 2018/04/16. doi:
658 10.1016/j.coviro.2018.04.001. PubMed PMID: 29656244; PubMed Central PMCID: PMC5944856.

659 58. Ljungberg K, McBrayer A, Camp JV, Chu YK, Tapp R, Noah DL, et al. Host gene expression
660 signatures discriminate between ferrets infected with genetically similar H1N1 strains. *PLoS One*.
661 2012;7(7):e40743. Epub 2012/07/19. doi: 10.1371/journal.pone.0040743. PubMed PMID: 22808249;
662 PubMed Central PMCID: PMC3396591.

663 59. Holzer M, Krahlting V, Amman F, Barth E, Bernhart SH, Carmelo VA, et al. Differential
664 transcriptional responses to Ebola and Marburg virus infection in bat and human cells. *Sci Rep*.
665 2016;6:34589. Epub 2016/10/08. doi: 10.1038/srep34589. PubMed PMID: 27713552; PubMed
666 Central PMCID: PMC5054393.

667 60. Liu X, Speranza E, Munoz-Fontela C, Haldenby S, Rickett NY, Garcia-Dorival I, et al.
668 Transcriptomic signatures differentiate survival from fatal outcomes in humans infected with Ebola
669 virus. *Genome Biol*. 2017;18(1):4. Epub 2017/01/20. doi: 10.1186/s13059-016-1137-3. PubMed
670 PMID: 28100256; PubMed Central PMCID: PMC5244546.

671 61. Gliddon HD, Herberg JA, Levin M, Kafrou M. Genome-wide host RNA signatures of infectious
672 diseases: discovery and clinical translation. *Immunology*. 2018;153(2):171-8. Epub 2017/09/19. doi:
673 10.1111/imm.12841. PubMed PMID: 28921535; PubMed Central PMCID: PMC5765383.

674 62. Badr E, ElHefnawi M, Heath LS. Computational Identification of Tissue-Specific Splicing
675 Regulatory Elements in Human Genes from RNA-Seq Data. *PLoS One*. 2016;11(11):e0166978. Epub
676 2016/11/20. doi: 10.1371/journal.pone.0166978. PubMed PMID: 27861625; PubMed Central PMCID:
677 PMC5115852.

678 63. Li J, Yu P. Genome-wide transcriptome analysis identifies alternative splicing regulatory
679 network and key splicing factors in mouse and human psoriasis. *Sci Rep*. 2018;8(1):4124. Epub
680 2018/03/09. doi: 10.1038/s41598-018-22284-y. PubMed PMID: 29515135; PubMed Central PMCID:
681 PMC5841439.

682 64. Chasman D, Walters KB, Lopes TJ, Einfeld AJ, Kawaoka Y, Roy S. Integrating Transcriptomic
683 and Proteomic Data Using Predictive Regulatory Network Models of Host Response to Pathogens.
684 *PLoS Comput Biol*. 2016;12(7):e1005013. Epub 2016/07/13. doi: 10.1371/journal.pcbi.1005013.
685 PubMed PMID: 27403523; PubMed Central PMCID: PMC4942116.

686 65. Sychev ZE, Hu A, DiMaio TA, Gitter A, Camp ND, Noble WS, et al. Integrated systems biology
687 analysis of KSHV latent infection reveals viral induction and reliance on peroxisome mediated lipid
688 metabolism. *PLoS Pathog*. 2017;13(3):e1006256. Epub 2017/03/04. doi:
689 10.1371/journal.ppat.1006256. PubMed PMID: 28257516; PubMed Central PMCID:
690 PMC5352148.

691 66. Tisoncik-Go J, Gasper DJ, Kyle JE, Einfeld AJ, Selinger C, Hatta M, et al. Integrated Omics
692 Analysis of Pathogenic Host Responses during Pandemic H1N1 Influenza Virus Infection: The Crucial
693 Role of Lipid Metabolism. *Cell Host Microbe*. 2016;19(2):254-66. Epub 2016/02/13. doi:
694 10.1016/j.chom.2016.01.002. PubMed PMID: 26867183; PubMed Central PMCID: PMC5271177.

695 67. Einfeld AJ, Halfmann PJ, Wendler JP, Kyle JE, Burnum-Johnson KE, Peralta Z, et al. Multi-
696 platform 'Omics Analysis of Human Ebola Virus Disease Pathogenesis. *Cell Host Microbe*.
697 2017;22(6):817-29 e8. Epub 2017/11/21. doi: 10.1016/j.chom.2017.10.011. PubMed PMID:
698 29154144; PubMed Central PMCID: PMC5730472.

699 68. van Kampen AH, Moerland PD. Taking Bioinformatics to Systems Medicine. *Methods Mol*
700 *Biol*. 2016;1386:17-41. Epub 2015/12/18. doi: 10.1007/978-1-4939-3283-2_2. PubMed PMID:
701 26677177.

702 69. Haynes WA, Tomczak A, Khatri P. Gene annotation bias impedes biomedical research. *Sci*
703 *Rep*. 2018;8(1):1362. Epub 2018/01/24. doi: 10.1038/s41598-018-19333-x. PubMed PMID:
704 29358745; PubMed Central PMCID: PMC5778030.

705 70. Liu Y, Gonzalez-Porta M, Santos S, Brazma A, Marioni JC, Aebersold R, et al. Impact of
706 Alternative Splicing on the Human Proteome. *Cell Rep*. 2017;20(5):1229-41. Epub 2017/08/03. doi:
707 10.1016/j.celrep.2017.07.025. PubMed PMID: 28768205; PubMed Central PMCID: PMC5554779.

708 71. Tress ML, Abascal F, Valencia A. Alternative Splicing May Not Be the Key to Proteome
709 Complexity. *Trends Biochem Sci.* 2017;42(2):98-110. Epub 2016/10/08. doi:
710 10.1016/j.tibs.2016.08.008. PubMed PMID: 27712956.

711 72. Tranchevent LC, Aube F, Dulaurier L, Benoit-Pilven C, Rey A, Poret A, et al. Identification of
712 protein features encoded by alternative exons using Exon Ontology. *Genome Res.* 2017;27(6):1087-
713 97. Epub 2017/04/20. doi: 10.1101/gr.212696.116. PubMed PMID: 28420690; PubMed Central
714 PMCID: PMC5453322.

715 73. Li HD, Menon R, Omenn GS, Guan Y. The emerging era of genomic data integration for
716 analyzing splice isoform function. *Trends Genet.* 2014;30(8):340-7. Epub 2014/06/22. doi:
717 10.1016/j.tig.2014.05.005. PubMed PMID: 24951248; PubMed Central PMCID: PMC4112133.

718 74. Li W, Liu CC, Kang S, Li JR, Tseng YT, Zhou XJ. Pushing the annotation of cellular activities to a
719 higher resolution: Predicting functions at the isoform level. *Methods.* 2016;93:110-8. Epub
720 2015/08/05. doi: 10.1016/j.jymeth.2015.07.016. PubMed PMID: 26238263.

721 75. Corominas R, Yang X, Lin GN, Kang S, Shen Y, Ghamsari L, et al. Protein interaction network of
722 alternatively spliced isoforms from brain links genetic risk factors for autism. *Nat Commun.*
723 2014;5:3650. Epub 2014/04/12. doi: 10.1038/ncomms4650. PubMed PMID: 24722188; PubMed
724 Central PMCID: PMC3996537.

725 76. Yang X, Coulombe-Huntington J, Kang S, Sheynkman GM, Hao T, Richardson A, et al.
726 Widespread Expansion of Protein Interaction Capabilities by Alternative Splicing. *Cell.*
727 2016;164(4):805-17. Epub 2016/02/13. doi: 10.1016/j.cell.2016.01.029. PubMed PMID: 26871637;
728 PubMed Central PMCID: PMC4882190.

729 77. Diner BA, Li T, Greco TM, Crow MS, Fuesler JA, Wang J, et al. The functional interactome of
730 PYHIN immune regulators reveals IFIX is a sensor of viral DNA. *Mol Syst Biol.* 2015;11(1):787. Epub
731 2015/02/11. doi: 10.15252/msb.20145808. PubMed PMID: 25665578; PubMed Central PMCID:
732 PMC4358659.

733 78. Ghosh S, Kumar GV, Basu A, Banerjee A. Graph theoretic network analysis reveals protein
734 pathways underlying cell death following neurotropic viral infection. *Sci Rep.* 2015;5:14438. Epub
735 2015/09/26. doi: 10.1038/srep14438. PubMed PMID: 26404759; PubMed Central PMCID:
736 PMC4585883.

737 79. Gregoire IP, Richetta C, Meyniel-Schicklin L, Borel S, Pradezynski F, Diaz O, et al. IRGM is a
738 common target of RNA viruses that subvert the autophagy network. *PLoS Pathog.*
739 2011;7(12):e1002422. Epub 2011/12/17. doi: 10.1371/journal.ppat.1002422. PubMed PMID:
740 22174682; PubMed Central PMCID: PMC3234227.

741 80. Akerman M, Fregoso OI, Das S, Ruse C, Jensen MA, Pappin DJ, et al. Differential connectivity
742 of splicing activators and repressors to the human spliceosome. *Genome Biol.* 2015;16:119. Epub
743 2015/06/07. doi: 10.1186/s13059-015-0682-5. PubMed PMID: 26047612; PubMed Central PMCID:
744 PMC4502471.

745 81. Pires MM, Cantor M, Guimaraes PR, de Aguiar MA, Dos Reis SF, Coltri PP. The network
746 organization of protein interactions in the spliceosome is reproduced by the simple rules of food-web
747 models. *Sci Rep.* 2015;5:14865. Epub 2015/10/08. doi: 10.1038/srep14865. PubMed PMID:
748 26443080; PubMed Central PMCID: PMC4595644.

749 82. Jha A, Gazzara MR, Barash Y. Integrative deep models for alternative splicing. *Bioinformatics.*
750 2017;33(14):i274-i82. Epub 2017/09/09. doi: 10.1093/bioinformatics/btx268. PubMed PMID:
751 28882000; PubMed Central PMCID: PMC5870723.

752 83. Marazzi I, Garcia-Sastre A. Interference of viral effector proteins with chromatin,
753 transcription, and the epigenome. *Curr Opin Microbiol.* 2015;26:123-9. Epub 2015/08/02. doi:
754 10.1016/j.mib.2015.06.009. PubMed PMID: 26232586.

755 84. Menachery VD, Schafer A, Burnum-Johnson KE, Mitchell HD, Eisfeld AJ, Walters KB, et al.
756 MERS-CoV and H5N1 influenza virus antagonize antigen presentation by altering the epigenetic

757 landscape. *Proc Natl Acad Sci U S A*. 2018;115(5):E1012-E21. Epub 2018/01/18. doi:
758 10.1073/pnas.1706928115. PubMed PMID: 29339515; PubMed Central PMCID: PMC5798318.

759 85. Gonzales-van Horn SR, Sarnow P. Making the Mark: The Role of Adenosine Modifications in
760 the Life Cycle of RNA Viruses. *Cell Host Microbe*. 2017;21(6):661-9. Epub 2017/06/16. doi:
761 10.1016/j.chom.2017.05.008. PubMed PMID: 28618265; PubMed Central PMCID: PMC5555051.

762 86. Knuckles P, Buhler M. Adenosine methylation as a molecular imprint defining the fate of
763 RNA. *FEBS Lett*. 2018. Epub 2018/05/22. doi: 10.1002/1873-3468.13107. PubMed PMID: 29782652.

764 87. Naftelberg S, Schor IE, Ast G, Kornblihtt AR. Regulation of alternative splicing through
765 coupling with transcription and chromatin structure. *Annu Rev Biochem*. 2015;84:165-98. Epub
766 2015/06/04. doi: 10.1146/annurev-biochem-060614-034242. PubMed PMID: 26034889.

767 88. Park E, Pan Z, Zhang Z, Lin L, Xing Y. The Expanding Landscape of Alternative Splicing
768 Variation in Human Populations. *Am J Hum Genet*. 2018;102(1):11-26. Epub 2018/01/06. doi:
769 10.1016/j.ajhg.2017.11.002. PubMed PMID: 29304370; PubMed Central PMCID: PMC5777382.

770 89. Baker SF, Ledwith MP, Mehle A. Differential Splicing of ANP32A in Birds Alters Its Ability to
771 Stimulate RNA Synthesis by Restricted Influenza Polymerase. *Cell Rep*. 2018;24(10):2581-8 e4. Epub
772 2018/09/06. doi: 10.1016/j.celrep.2018.08.012. PubMed PMID: 30184493; PubMed Central PMCID:
773 PMC56157632.

774 90. Ibrahim B, McMahon DP, Hufsky F, Beer M, Deng L, Mercier PL, et al. A new era of virus
775 bioinformatics. *Virus Res*. 2018;251:86-90. Epub 2018/05/12. doi: 10.1016/j.virusres.2018.05.009.
776 PubMed PMID: 29751021.

777 91. De Arras L, Laws R, Leach SM, Pontis K, Freedman JH, Schwartz DA, et al. Comparative
778 genomics RNAi screen identifies Eftud2 as a novel regulator of innate immunity. *Genetics*.
779 2014;197(2):485-96. Epub 2013/12/24. doi: 10.1534/genetics.113.160499. PubMed PMID: 24361939;
780 PubMed Central PMCID: PMC4063909.

781 92. O'Connor BP, Danhorn T, De Arras L, Flatley BR, Marcus RA, Farias-Hesson E, et al. Regulation
782 of toll-like receptor signaling by the SF3a mRNA splicing complex. *PLoS Genet*. 2015;11(2):e1004932.
783 Epub 2015/02/07. doi: 10.1371/journal.pgen.1004932. PubMed PMID: 25658809; PubMed Central
784 PMCID: PMC4450051.

785 93. Gack MU, Kirchhofer A, Shin YC, Inn KS, Liang C, Cui S, et al. Roles of RIG-I N-terminal tandem
786 CARD and splice variant in TRIM25-mediated antiviral signal transduction. *Proc Natl Acad Sci U S A*.
787 2008;105(43):16743-8. Epub 2008/10/25. doi: 10.1073/pnas.0804947105. PubMed PMID: 18948594;
788 PubMed Central PMCID: PMC2575490.

789 94. Wang PH, Fung SY, Gao WW, Deng JJ, Cheng Y, Chaudhary V, et al. A novel transcript isoform
790 of STING that sequesters cGAMP and dominantly inhibits innate nucleic acid sensing. *Nucleic Acids*
791 *Res*. 2018;46(8):4054-71. Epub 2018/03/17. doi: 10.1093/nar/gky186. PubMed PMID: 29547894;
792 PubMed Central PMCID: PMC5934658.

793 95. Deng W, Shi M, Han M, Zhong J, Li Z, Li W, et al. Negative regulation of virus-triggered IFN-
794 beta signaling pathway by alternative splicing of TBK1. *J Biol Chem*. 2008;283(51):35590-7. Epub
795 2008/11/04. doi: 10.1074/jbc.M805775200. PubMed PMID: 18977754.

796 96. Lakhdari O, McAllister CS, Wang M, Mineev I, Prince LS, Eckmann L, et al. TLR3 signaling is
797 downregulated by a MAVS isoform in epithelial cells. *Cell Immunol*. 2016;310:205-10. Epub
798 2016/09/07. doi: 10.1016/j.cellimm.2016.08.010. PubMed PMID: 27593154; PubMed Central PMCID:
799 PMC5125873.

800 97. Qi N, Shi Y, Zhang R, Zhu W, Yuan B, Li X, et al. Multiple truncated isoforms of MAVS prevent
801 its spontaneous aggregation in antiviral innate immune signalling. *Nat Commun*. 2017;8:15676. Epub
802 2017/06/14. doi: 10.1038/ncomms15676. PubMed PMID: 28607490; PubMed Central PMCID:
803 PMC5474743.

804 98. Diaz-Munoz MD, Bell SE, Fairfax K, Monzon-Casanova E, Cunningham AF, Gonzalez-Porta M,
805 et al. The RNA-binding protein HuR is essential for the B cell antibody response. *Nat Immunol*.

806 2015;16(4):415-25. Epub 2015/02/24. doi: 10.1038/ni.3115. PubMed PMID: 25706746; PubMed
807 Central PMCID: PMCPMC4479220.

808 99. Enders A, Short A, Miosge LA, Bergmann H, Sontani Y, Bertram EM, et al. Zinc-finger protein
809 ZFP318 is essential for expression of IgD, the alternatively spliced Igh product made by mature B
810 lymphocytes. *Proc Natl Acad Sci U S A*. 2014;111(12):4513-8. Epub 2014/03/13. doi:
811 10.1073/pnas.1402739111. PubMed PMID: 24616512; PubMed Central PMCID: PMCPMC3970522.

812 100. van Dijk EL, Jaszczyszyn Y, Naquin D, Thermes C. The Third Revolution in Sequencing
813 Technology. *Trends Genet*. 2018. Epub 2018/06/27. doi: 10.1016/j.tig.2018.05.008. PubMed PMID:
814 29941292.

815 101. Weirather JL, de Cesare M, Wang Y, Piazza P, Sebastiano V, Wang XJ, et al. Comprehensive
816 comparison of Pacific Biosciences and Oxford Nanopore Technologies and their applications to
817 transcriptome analysis. *F1000Res*. 2017;6:100. Epub 2017/09/07. doi:
818 10.12688/f1000research.10571.2. PubMed PMID: 28868132; PubMed Central PMCID:
819 PMCPMC5553090.

820 102. Anvar SY, Allard G, Tseng E, Sheynkman GM, de Klerk E, Vermaat M, et al. Full-length mRNA
821 sequencing uncovers a widespread coupling between transcription initiation and mRNA processing.
822 *Genome Biol*. 2018;19(1):46. Epub 2018/03/31. doi: 10.1186/s13059-018-1418-0. PubMed PMID:
823 29598823; PubMed Central PMCID: PMCPMC5877393.

824 103. Oikonomopoulos S, Wang YC, Djambazian H, Badescu D, Ragoussis J. Benchmarking of the
825 Oxford Nanopore MinION sequencing for quantitative and qualitative assessment of cDNA
826 populations. *Sci Rep*. 2016;6:31602. Epub 2016/08/25. doi: 10.1038/srep31602. PubMed PMID:
827 27554526; PubMed Central PMCID: PMCPMC4995519 and the MinION instrument and R7, R7.3
828 flowcells were received free of charge.

829 104. Levy SE, Myers RM. Advancements in Next-Generation Sequencing. *Annu Rev Genomics Hum*
830 *Genet*. 2016;17:95-115. Epub 2016/07/01. doi: 10.1146/annurev-genom-083115-022413. PubMed
831 PMID: 27362342.

832 105. Garalde DR, Snell EA, Jachimowicz D, Sipos B, Lloyd JH, Bruce M, et al. Highly parallel direct
833 RNA sequencing on an array of nanopores. *Nat Methods*. 2018;15(3):201-6. Epub 2018/01/16. doi:
834 10.1038/nmeth.4577. PubMed PMID: 29334379.

835 106. Bryant HE, Wadd SE, Lamond AI, Silverstein SJ, Clements JB. Herpes simplex virus IE63 (ICP27)
836 protein interacts with spliceosome-associated protein 145 and inhibits splicing prior to the first
837 catalytic step. *J Virol*. 2001;75(9):4376-85. Epub 2001/04/05. doi: 10.1128/JVI.75.9.4376-4385.2001.
838 PubMed PMID: 11287586; PubMed Central PMCID: PMCPMC114182.

839 107. Hashizume C, Kuramitsu M, Zhang X, Kurosawa T, Kamata M, Aida Y. Human
840 immunodeficiency virus type 1 Vpr interacts with spliceosomal protein SAP145 to mediate cellular
841 pre-mRNA splicing inhibition. *Microbes Infect*. 2007;9(4):490-7. Epub 2007/03/10. doi:
842 10.1016/j.micinf.2007.01.013. PubMed PMID: 17347016.

843 108. Verma D, Bais S, Gaillard M, Swaminathan S. Epstein-Barr Virus SM protein utilizes cellular
844 splicing factor SRp20 to mediate alternative splicing. *J Virol*. 2010;84(22):11781-9. Epub 2010/09/03.
845 doi: 10.1128/JVI.01359-10. PubMed PMID: 20810723; PubMed Central PMCID: PMCPMC2977868.

846 109. Klymenko T, Hernandez-Lopez H, MacDonald AI, Bodily JM, Graham SV. Human
847 Papillomavirus E2 Regulates SRSF3 (SRp20) To Promote Capsid Protein Expression in Infected
848 Differentiated Keratinocytes. *J Virol*. 2016;90(10):5047-58. Epub 2016/03/11. doi: 10.1128/JVI.03073-
849 15. PubMed PMID: 26962216; PubMed Central PMCID: PMCPMC4859725.

850 110. Qiu Y, Nemeroff M, Krug RM. The influenza virus NS1 protein binds to a specific region in
851 human U6 snRNA and inhibits U6-U2 and U6-U4 snRNA interactions during splicing. *RNA*.
852 1995;1(3):304-16. Epub 1995/05/01. PubMed PMID: 7489502; PubMed Central PMCID:
853 PMCPMC1369083.

854 111. Fortes P, Lamond AI, Ortin J. Influenza virus NS1 protein alters the subnuclear localization of
855 cellular splicing components. *J Gen Virol.* 1995;76 (Pt 4):1001-7. Epub 1995/04/01. doi:
856 10.1099/0022-1317-76-4-1001. PubMed PMID: 9049349.

857 112. Mor A, White A, Zhang K, Thompson M, Esparza M, Munoz-Moreno R, et al. Influenza virus
858 mRNA trafficking through host nuclear speckles. *Nat Microbiol.* 2016;1(7):16069. Epub 2016/08/31.
859 doi: 10.1038/nmicrobiol.2016.69. PubMed PMID: 27572970; PubMed Central PMCID:
860 PMCPMC4917225.

861 113. Thompson MG, Munoz-Moreno R, Bhat P, Roytenberg R, Lindberg J, Gazzara MR, et al. Co-
862 regulatory activity of hnRNP K and NS1-BP in influenza and human mRNA splicing. *Nat Commun.*
863 2018;9(1):2407. Epub 2018/06/21. doi: 10.1038/s41467-018-04779-4. PubMed PMID: 29921878;
864 PubMed Central PMCID: PMCPMC6008300.

865 114. Fitzgerald KD, Chase AJ, Cathcart AL, Tran GP, Semler BL. Viral proteinase requirements for
866 the nucleocytoplasmic relocation of cellular splicing factor SRp20 during picornavirus infections. *J*
867 *Virol.* 2013;87(5):2390-400. Epub 2012/12/21. doi: 10.1128/JVI.02396-12. PubMed PMID: 23255796;
868 PubMed Central PMCID: PMCPMC3571363.

869 115. Alvarez E, Castello A, Carrasco L, Izquierdo JM. Poliovirus 2A protease triggers a selective
870 nucleo-cytoplasmic redistribution of splicing factors to regulate alternative pre-mRNA splicing. *PLoS*
871 *One.* 2013;8(9):e73723. Epub 2013/09/26. doi: 10.1371/journal.pone.0073723. PubMed PMID:
872 24066065; PubMed Central PMCID: PMCPMC3774746.

873 116. Liu YC, Kuo RL, Lin JY, Huang PN, Huang Y, Liu H, et al. Cytoplasmic viral RNA-dependent RNA
874 polymerase disrupts the intracellular splicing machinery by entering the nucleus and interfering with
875 Prp8. *PLoS Pathog.* 2014;10(6):e1004199. Epub 2014/06/27. doi: 10.1371/journal.ppat.1004199.
876 PubMed PMID: 24968230; PubMed Central PMCID: PMCPMC4072778.

877 117. Lawrence P, Schafer EA, Rieder E. The nuclear protein Sam68 is cleaved by the FMDV 3C
878 protease redistributing Sam68 to the cytoplasm during FMDV infection of host cells. *Virology.*
879 2012;425(1):40-52. Epub 2012/01/28. doi: 10.1016/j.virol.2011.12.019. PubMed PMID: 22280896.

880 118. Dhillon P, Tandra VN, Chorghade SG, Namsa ND, Sahoo L, Rao CD. Cytoplasmic Relocalization
881 and Colocalization with Viropasms of Host Cell Proteins, and Their Role in Rotavirus Infection. *J Virol.*
882 2018;92(15). Epub 2018/05/18. doi: 10.1128/JVI.00612-18. PubMed PMID: 29769336; PubMed
883 Central PMCID: PMCPMC6052293.

884 119. Burnham AJ, Gong L, Hardy RW. Heterogeneous nuclear ribonuclear protein K interacts with
885 Sindbis virus nonstructural proteins and viral subgenomic mRNA. *Virology.* 2007;367(1):212-21. Epub
886 2007/06/15. doi: 10.1016/j.virol.2007.05.008. PubMed PMID: 17561226.

887 120. Dickson AM, Anderson JR, Barnhart MD, Sokoloski KJ, Oko L, Opyrchal M, et al.
888 Dephosphorylation of HuR protein during alphavirus infection is associated with HuR relocalization to
889 the cytoplasm. *J Biol Chem.* 2012;287(43):36229-38. Epub 2012/08/24. doi:
890 10.1074/jbc.M112.371203. PubMed PMID: 22915590; PubMed Central PMCID: PMCPMC3476290.

891 121. Shwetha S, Kumar A, Mullick R, Vasudevan D, Mukherjee N, Das S. HuR Displaces
892 Polypyrimidine Tract Binding Protein To Facilitate La Binding to the 3' Untranslated Region and
893 Enhances Hepatitis C Virus Replication. *J Virol.* 2015;89(22):11356-71. Epub 2015/09/05. doi:
894 10.1128/JVI.01714-15. PubMed PMID: 26339049; PubMed Central PMCID: PMCPMC4645635.

895 122. Redondo N, Madan V, Alvarez E, Carrasco L. Impact of Vesicular Stomatitis Virus M Proteins
896 on Different Cellular Functions. *PLoS One.* 2015;10(6):e0131137. Epub 2015/06/20. doi:
897 10.1371/journal.pone.0131137. PubMed PMID: 26091335; PubMed Central PMCID:
898 PMCPMC4474437.

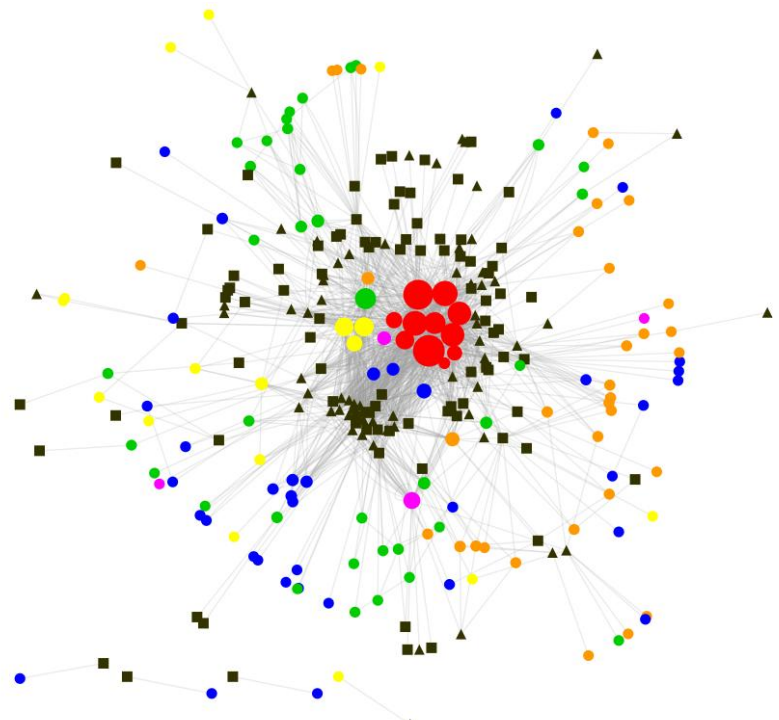
899 123. Hegele A, Kamburov A, Grossmann A, Sourlis C, Wowro S, Weimann M, et al. Dynamic
900 protein-protein interaction wiring of the human spliceosome. *Mol Cell.* 2012;45(4):567-80. Epub
901 2012/03/01. doi: 10.1016/j.molcel.2011.12.034. PubMed PMID: 22365833.

902 124. Liu W, Lin YT, Yan XL, Ding YL, Wu YL, Chen WN, et al. Hepatitis B virus core protein inhibits
903 Fas-mediated apoptosis of hepatoma cells via regulation of mFas/FasL and sFas expression. *FASEB J.*
904 2015;29(3):1113-23. Epub 2014/12/04. doi: 10.1096/fj.14-263822. PubMed PMID: 25466893.
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Viral family	Viral species	Viral proteins (non-redundant)
Papillomaviridae	9	34(34)
Retroviridae	8	17(17)
Herpesviridae	5	49(36)
Flaviviridae	4	9(4)
Togaviridae	3	3(3)
Paramyxoviridae	2	4(3)
Polyomaviridae	2	3(3)
Coronaviridae	2	2(2)
Parvoviridae	2	2(2)
Rhabdoviridae	2	2(2)
Orthomyxoviridae	1	27(11)
Poxviridae	1	13(12)
Filoviridae	1	2(2)
Pneumoviridae	1	2(2)
Adenoviridae	1	1(1)
Circoviridae	1	1(1)
Hepadnaviridae	1	1(1)
Peribunyaviridae	1	1(1)
Phenuiviridae	1	1(1)
Reoviridae	1	1(1)
Total	49	175(139)

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