



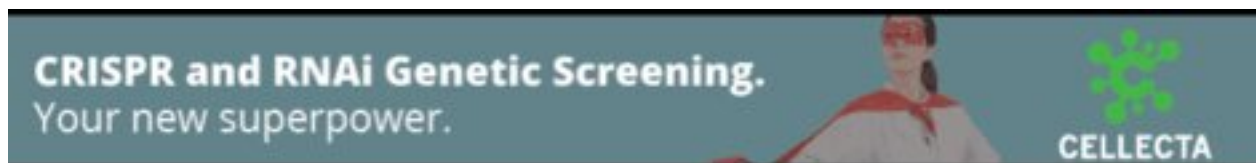
Genomic regulatory landscape underlying the antiviral response of Atlantic salmon

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1 **Genomic regulatory landscape underlying the antiviral response of Atlantic salmon**

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13 **Abstract**

14 All jawed vertebrates have a highly coordinated innate immune response to viral infections
15 driven by a core set of interferon responsive genes (ISGs), but interspecies variation
16 remains substantial. In this study, we examined the genome-wide regulatory basis for
17 antiviral gene expression responses in Atlantic salmon (*Salmo salar*), representing a teleost
18 family that underwent a whole genome duplication (WGD) event ~100 Mya. We stimulate
19 fish systemically with polyinosinic:polycytidylic acid (poly I:C), a synthetic viral mimic, and
20 profile transcriptomic and epigenomic responses in the primary haematopoietic and
21 lymphoid tissue. We used ATAC-seq and ChIP-seq (H3K27ac and H3K27me3), combined
22 with mRNA-seq, to comprehensively examine modifications in gene regulation following
23 stimulation. We identified a set of 197 ISGs with regulatory elements showing increased
24 chromatin accessibility and H3K27ac signal in concert with increased gene expression in
25 response to poly I:C. Fifty-four of these genes were conserved ISGs in rainbow trout,
26 zebrafish, and human. Our analysis provides evidence for conserved transcription factors
27 (TFs) driving the interferon response by binding ISG promoters, including IRF8, IRF9,
28 STAT1, and STAT2. Regulatory elements within differentially expressed genes were
29 enriched for predicted binding sites for STAT6, PRDM1, IRF6, JDP2, NR2E1, and BCL6,
30 suggesting a central role for these TFs in the antiviral response. Finally, we demonstrate
31 paralogue-specific enrichment of interferon-stimulated response element (ISRE) motifs in
32 poly I:C activated promoters of ISGs retained as duplicates from the salmonid WGD. Overall,
33 this study provides novel insights into the genomic regulatory landscape underlying antiviral
34 immunity in a farmed fish with a complex genome.

35 **Introduction**

36 The innate immune response to viral infection, which is mainly based on the type I interferon
37 (type I IFN) pathway is crucial to both disease progression and the outcome of infection.
38 Type I IFNs are present in all gnathostomes, and play a pivotal role in inducing an antiviral

39 state in host responding cells by the induction of IFN stimulated genes (ISGs) (Schoggins,
40 2019).

41 This antiviral response is triggered when viral pathogen-associated molecular patterns
42 (PAMPs) bind to pattern recognition receptors (PRRs) such as toll-like receptors (TLR3,
43 TLR7, TLR8) and RIG-I-like receptors (RLRs), activating downstream signalling cascades
44 that induce transcription of type I interferons (IFNs) and antiviral effectors (Thompson *et al.*,
45 2011). In mammals, teleost fish and other vertebrate species this response is regulated at
46 multiple levels, including complex transcriptional feedback loops and chromatin-mediated
47 control of key antiviral genes (Ivashkiv and Donlin, 2014; Castro *et al.*, 2014; Dehler *et al.*,
48 2019). Epigenetic modifications such as histone acetylation and methylation contribute to
49 this regulation by altering DNA accessibility at enhancers and promoters (Mostafavi *et al.*,
50 2016; Kan *et al.*, 2022). Transposase-accessible chromatin with sequencing (ATAC-seq)
51 and chromatin immunoprecipitation and sequencing (ChIP-seq) are widely used to map
52 open chromatin and histone modifications across the genome, enabling the identification of
53 putative regulatory elements. While these assays are powerful, they have limitations,
54 including the potential for false positives (regions that are accessible but not functionally
55 active) and false negatives (context-dependent enhancers that may be missed in bulk
56 analysis). Nonetheless, ATAC-seq and ChIP-seq have proven effective in both mammalian
57 and teleost systems for revealing the epigenomic architecture of immune tissues and
58 mapping regulatory elements associated with interferon responses (Cooper *et al.*, 2024;
59 Harvey *et al.*, 2024; Tan *et al.*, 2025).

60 The induction of type I IFN typically involves an initial detection and recognition of viral RNA
61 by cytoplasmic RNA helicases like RIG-I and Melanoma Differentiation-Associated protein 5
62 (MDA5) or TLRs (TLR3, TLR7/8) (Matsumoto *et al.*, 2003; Liu, 2005). In human or mouse,
63 this leads to the production and secretion of IFN α and β , which then bind to their cognate
64 receptors, interferon alpha/beta receptor 1 (IFNAR1) and 2 (IFNAR2) present on the surface
65 of target cells. Activation of these receptors induces a signalling cascade leading to the

66 phosphorylation of the Janus kinases Tyk2 and JAK1, as well as the “signal transducers and
67 activators of transcription” STAT1 and STAT2 (Platanias, 2005). These phosphorylated
68 STAT1 and STAT2 molecules then bind with a third subunit, interferon regulatory factor 9
69 (IRF9), to form the IFN stimulated gene factor 3 (ISGF3) complex (Platanitis *et al* 2019).
70 After translocation to the cell’s nucleus, ISGF3 binds to interferon-stimulated response
71 elements (ISRE) in the promoters of ISGs, rapidly activating diverse genes with effector and
72 regulatory immune function (Platanias, 2005; Ivashkiv and Donlin, 2014). The binding of
73 ISGF3 to ISREs is essential for the induction of ISGs and for establishing an antiviral cellular
74 state (Wang *et al.*, 2017). Multiple type I IFNs are present in and orchestrate antiviral innate
75 responses teleost fish (Langevin *et al.*, 2019). The ISG repertoire is more complex than in
76 mammals, with additional gene family member paralogues being retained following whole
77 genome duplication events (Collet *et al.*, 2009; Skjesol *et al.*, 2010; Briolat *et al.*, 2014;
78 Macqueen *et al.*, 2014; Levraud *et al.*, 2019; Gan *et al.*, 2020; Clark *et al.*, 2023). In addition
79 to the presence of mammalian orthologs and their paralogous genes resulting from whole
80 genome duplication, several gene families involved in antiviral immunity such as GCRV-
81 induced gene 1 (*gig1*), RNA-dependent protein kinase (*pkp/pkz*), and specific TRIM gene
82 subsets such as *finTRIMs* are unique to teleost fish. These teleost-specific ISGs likely
83 evolved during lineage-specific expansions and are thought to play important roles in
84 shaping fish-specific interferon responses or could have been lost in the lineages leading to
85 tetrapods (An *et al.*, 2025).

86 While the teleost IFN response has been extensively analysed at the transcriptomic level
87 (e.g. Martin *et al.*, 2007; Zou and Secombes, 2011; Briolat *et al.*, 2014; Levraud *et al.*, 2019;
88 Clark *et al.*, 2023) and functionality of ISREs has been proposed (Boudinot *et al.*, 2001;
89 Collet and Secombes, 2001; Castro *et al.*, 2008, 2010), the regulatory basis of the IFN
90 system, and more generally antiviral transcriptional responses, remains poorly understood.
91 To tackle this knowledge gap, we investigated the genomic regulatory mechanisms driving

92 core ISG expression responses in the head kidney of Atlantic salmon (*Salmo salar*), a
93 globally important aquaculture species.

94 We hypothesised that: (i) gene regulation in response to poly I:C stimulation involves
95 coordinated changes in chromatin accessibility and histone modifications, similar to
96 mechanisms described in higher vertebrates; (ii) regulatory divergence between paralogous
97 genes contributes to their differential expression during the antiviral response; and (iii)
98 expanded or salmonid-specific gene families exhibit distinct regulatory signatures that reflect
99 their functional roles in immunity.

100 To test these hypotheses, we integrated ATAC-seq, ChIP-seq and RNA-seq datasets
101 following *in vivo* stimulation with the viral dsRNA mimic poly I:C. ChIP-seq was performed
102 using the histone marks H3K27ac and H3K27me3. H3K27ac is an activating mark
103 associated with open chromatin and transcriptionally active genes, marking promoter and
104 enhancer regions (Wang *et al.*, 2008; Creyghton *et al.*, 2010). In contrast, H3K27me3 is a
105 repressive mark associated with polycomb-mediated transcriptional repression and gene
106 silencing (Bernstein *et al.*, 2006; Barski *et al.*, 2007), typically associated with regulatory
107 regions of inactive genes. As salmonid genomes are characterised by two rounds of whole
108 genome duplication (WGD) (Lien *et al.*, 2016; Robertson *et al.*, 2017), leading to the
109 expansion and functional diversification of many gene sets compared to mammals (including
110 ISGs: Clark *et al.*, 2023), we further compared the regulation of ISG paralogues to seek
111 evidence for changes in regulation or function. Our findings advance understanding of
112 immune function, providing insights into the evolution of teleost fish paralogous ISGs at the
113 regulatory level, while demonstrating functional conservation of key antiviral regulatory
114 elements across vertebrates.

115 **Results**

116 In this study we examine the modulation of gene expression, chromatin accessibility and
117 histone modifications in Atlantic salmon following stimulation using poly I:C, a mimic of

118 infection with an RNA virus. A major transcriptomic response was elicited driving the
119 upregulation of a core set of ISGs (Clark *et al.*, 2023), in this report we use the term ISGs to
120 include all genes inducible within the interferon response including those that are directly
121 upregulated by poly I:C stimulation. The aim of the current study is to elucidate epigenomic
122 changes in chromatin state following this stimulation and shed light on regulatory elements
123 and TFBSs influencing antiviral transcriptional responses in a complex teleost genome.

124 ***Genomic sequencing data and sample metadata***

125 Thirty-eight datasets were generated, comprising ATAC-seq (12 datasets: n=6 Poly I:C, n=6
126 PBS control) and ChIP-seq (12 datasets per histone mark: n=6 Poly I:C, n=6 PBS; along
127 with 2 input controls). For H3K27ac, one PBS sample was excluded from the analysis post-
128 sequencing, while for H3K27me3, one sample each for PBS and Poly I:C were excluded.
129 Full details regarding samples and sequence data are provided in Supplementary Table 1.
130 ATAC-seq provides genome-wide identification of open chromatin regions, and with six
131 control and six poly I:C stimulated fish, offers in-depth data to study regulatory elements,
132 TFBS, and changes in chromatin accessibility. ChIP-seq was performed using two histone
133 modifications: H3K27ac and H3K27me3. Promoters and enhancers were defined based on
134 chromatin accessibility, histone modifications, and ChromHMM annotations. Regions
135 classified as putative promoters were those overlapping transcription start sites (TSS) with
136 ATAC-seq consensus peaks and H3K27ac enrichment, indicative of active regulatory
137 activity. Specifically, candidate enhancers were identified as intergenic or intronic regions
138 that exhibited both ATAC-seq accessibility and H3K27ac modification, suggesting distal
139 regulatory elements. Peaks marked predominantly by H3K27me3 were classified as
140 polycomb-repressed regions, reflecting transcriptionally silent chromatin states. These
141 ChromHMM annotated enhancers and promoters, defined through the integration of ATAC-
142 seq and H3K27ac, were subsequently used for downstream TFBS analysis, allowing the
143 identification of key regulatory motifs involved in the antiviral response.

144 To further characterise the genomic distribution of ATAC-seq and H3K27ac peaks, we
145 annotated consensus peaks relative to gene features and transcription start sites (TSS). The
146 majority of peaks in both ATAC-seq and H3K27ac datasets were associated with genes
147 (Figure 1A and 1B) and enriched in promoter-proximal and enhancer regions, with a high
148 density observed within 50 kb of the TSS (Figure 1C and 1D). This confirms the utility of
149 these datasets for identifying active regulatory elements in the Atlantic salmon genome.

150

151 ***Immune stimulation remodels chromatin accessibility and histone modifications in***
152 ***Atlantic salmon head kidney***

153 Poly I:C stimulation led to widespread changes in chromatin accessibility, with an
154 approximately 25% increase in ATAC-seq peaks detected in treated fish ($53,102 \pm 6,142$)
155 compared to PBS controls ($39,869 \pm 11,858$). These accessible regions were distributed
156 across promoter-TSS (10%), exonic (13%), intronic (45%), and intergenic (26%) features of
157 the genome (Supplementary Figure 2A). To illustrate the data quality and consistency,
158 genome browser tracks at the housekeeping gene *elf1a* showed well-defined cis-regulatory
159 peaks in both conditions, matching the expected expression patterns (Figure 2A and B).

160 Differential analysis identified 613 regions (250 promoters, 363 enhancers) with increased
161 accessibility and 48 regions (6 promoters, 42 enhancers) with reduced accessibility after
162 poly I:C stimulation (fold change > 2, $p_{adj} < 0.05$) (Figure 2E; Supplementary Table 2).
163 Functional enrichment of the regions with increased accessibility, performed using rGREAT,
164 highlighted pathways central to antiviral immunity, including “regulation of cytokine-mediated
165 signalling,” “interferon-mediated signalling,” and “type I interferon response” (Figure 2C;
166 Supplementary Table 3A). Several terms related to JAK-STAT signalling and host-virus
167 interactions were also among the top enriched categories.

168 Chromatin immunoprecipitation profiling of H3K27ac and H3K27me3 marks further revealed
169 distinct patterns of histone modification following immune stimulation. Stimulated fish
170 showed an average of 72,490 ($\pm 21,211$) H3K27ac and 68,873 ($\pm 16,608$) H3K27me3 peaks,
171 compared to 78,816 ($\pm 31,106$) and 48,221 ($\pm 26,905$) in PBS controls. Analysis of differential
172 abundance identified 1,539 regions enriched for H3K27ac (528 promoters, 1,011 enhancers)
173 and 794 regions enriched for H3K27me3 (124 promoters, 670 enhancers), while a
174 comparable number of regions were lost for each respective mark in response to stimulation
175 (Figure 2F and G; Supplementary Table 4). A small subset of loci (45) exhibited reciprocal
176 regulation, gaining H3K27ac while losing H3K27me3, suggesting polycomb-mediated

177 repression was relieved to allow gene activation (Supplementary Figure 3C; Supplementary
178 Table 5). In contrast, only 30 regions showed concurrent increases in both marks, supporting
179 the notion that their regulation is largely independent in this context (Supplementary Figure
180 3A).

181 Gene set enrichment of regions gaining H3K27ac underscored their association with
182 immune processes, including “interferon-mediated signalling,” “cellular response to type I
183 interferon,” “response to virus,” and pathways involved in epithelial and endothelial cell
184 migration (Figure 2D; Supplementary 3B). Comparison of the enriched biological processes
185 revealed that 8 out of the top 15 GO terms identified in ATAC-seq peaks were also
186 significantly enriched among regions gaining H3K27ac. These shared terms included
187 hallmark antiviral processes such as “interferon-mediated signalling pathway” and “response
188 to type I interferon” and “regulation of receptor signalling pathway via JAK-STAT”, reflecting
189 coordinated chromatin accessibility and histone acetylation at critical immune loci. These
190 findings demonstrate a coordinated regulatory response to poly I:C, involving both chromatin
191 accessibility and activating histone modifications at key antiviral loci.

192

193 ***Multomics identifies epigenomic changes associated to the induction of core ISGs***

194 To uncover the regulatory mechanisms underlying the transcriptome response induced by
195 poly I:C, we integrated data from RNA-seq, ATAC-seq, and ChIP-seq for the active histone
196 mark H3K27ac and repressive histone mark H3K27me3. For this analysis, we defined 3,541
197 genes associated with changes across all four assays, split into five subsets with distinct
198 regulatory profiles (Figure 3A). Of interest we find different groups of genes that are
199 activated at different levels, these are: (1) Core genes (197 genes; set 1) were defined by
200 significant concurrent increases in ATAC-seq chromatin accessibility, H3K27ac signal, and
201 mRNA expression following poly I:C stimulation. The presence of both open chromatin
202 regions and H3K27 acetylation close to these genes suggests that these regions function as
203 candidate active enhancers and promoters, driving transcriptional activation in response to
204 stimulation (Figure 3B). The second gene set (1,348 genes; set 2) exhibited significant
205 increases in chromatin accessibility and H3K27ac signal without corresponding
206 transcriptional upregulation. These regions likely represent candidate active enhancers and

207 promoters that are primed for activation but may require additional regulatory inputs for
208 transcriptional induction (Figure 3C), (3) genes where mRNA expression is increased but
209 lacking changes in chromatin accessibility (1,008 genes; set 3) (Figure 3D), (4) genes
210 showing increased mRNA expression and chromatin openness, but lacking changes in
211 H3K27ac signal (52 genes; set 4) (Figure 3E) and (5) genes with significant increases in
212 H3K27ac signals alongside transcript-level upregulation (173 genes; set 5) (Figure 3F;
213 Supplementary Table 6). Canonical antiviral genes were mainly enriched in Set 1, which
214 exhibited coordinated increases in gene expression, chromatin accessibility, and H3K27ac.
215 This group included key effectors such as *TRIM25*, *RSAD2*, *IRF7*, *MX1*, and *IFI44*.
216 Additional antiviral genes, such as *STAT1* and *IRF8*, were found in Set 3 (RNA-seq only).
217 Few antiviral genes were detected in Sets 2, 4, or 5, underscoring Set 1 as the principal
218 group reflecting multi-layered antiviral gene regulation.

219 The core 197 genes (set 1 above) displayed increases in chromatin openness and H3K27ac
220 signal alongside significant transcript upregulation, underscoring their tight regulatory
221 coordination. Functional enrichment for GO terms revealed these genes are primarily
222 involved in early innate immune responses, with significant enrichment for terms such as
223 “*response to virus*”, “*innate immune response*”, and “*protein ubiquitination*” (Figure 3B).
224 Importantly, further analysis of this subset revealed that 54 (27%) of the 197 genes
225 overlapped with the core set of Atlantic salmon ISG induced in vivo that are conserved
226 between salmon and human (154 genes) (Clark *et al.*, 2023) (Supplementary Figure 4; Table
227 1). This is significantly higher (two-tailed Fisher’s exact test, $p < 0.0001$) than the conserved
228 ISG proportion among all poly I:C induced genes (154 out of 1,446 transcriptionally induced
229 genes i.e. 11%), this evolutionary conservation underscores the central importance of these
230 genes in antiviral immunity.

231 A subset of 1,348 genes (set 2 above) showed significant increases in chromatin
232 accessibility and H3K27ac signal without corresponding changes in transcript levels. To
233 further investigate this finding, we analysed the genomic context of the associated peaks.

234 Many peaks were located in intergenic or intronic regions, indicative of putative enhancers.
235 As enhancers often regulate distal genes rather than the closest annotated gene, this could
236 explain the absence of detectable changes in RNA levels for nearby loci. For peaks
237 associated with promoters, the increased chromatin accessibility and H3K27ac signal may
238 reflect transcriptional activation that is not captured in total RNA-seq data. This could occur if
239 small transcriptional changes are masked by the stability and abundance of pre-existing
240 transcripts in the cytoplasm. The corresponding gene set did not exhibit enrichment for
241 antiviral or interferon response pathways, suggesting potential involvement in alternative
242 biological processes (Figure 3C).

243 Third, we identified a subset of genes (set 3 above) that were differentially expressed in
244 response to poly I:C but did not exhibit changes in chromatin accessibility or histone mark
245 modifications. These genes were enriched for GO terms such as “*cellular response to virus*”,
246 “*positive regulation of NF-kappaB transcription factor activity*”, “*response to*
247 *lipopolysaccharide*”, “*cellular response to type II interferon*”, and “*inflammatory response*”
248 (Figure 3D), highlighting their pivotal role in the antiviral response.

249 Fourth, we identified a set of 52 genes (set 4 above) that exhibited significant changes in
250 chromatin accessibility and transcript levels, without corresponding alterations in H3K27ac
251 marks. GO term enrichment for these genes included “*T-helper 2 cell differentiation*” and
252 “*response to virus*” (Figure 3E). The lack of H3K27ac changes suggests that these genes
253 might be regulated through distal enhancers, changes in chromatin topology, or mechanisms
254 unrelated to histone acetylation.

255 Fifth, a distinct set of 173 genes showed significant increases in H3K27ac signals alongside
256 transcript-level upregulation, but no ATAC-seq signal. These genes were enriched for GO
257 terms such as “*defence response to virus*”, “*innate immune response*”, “*protein*
258 *ubiquitination*”, and “*signal transduction*” (Figure 3F), reflecting their active involvement in the
259 antiviral response. The concurrent increase in H3K27ac and gene expression strongly

260 supports their role as actively transcribed loci, with acetylation marking a key feature of them
261 in response to poly I:C stimulation.

262 Finally, a subset of 747 genes was exclusively associated with increase in repressive
263 histone mark H3K27me3, which is typically linked to transcriptional silencing through
264 repressive polycomb complexes, with known roles in regulating immune responses
265 (Bosselut, 2016). However, GO analysis did not reveal any significantly enriched terms for
266 this subset, indicating a lack of strong functional enrichment for these genes.

267 ***A key ISRE motif, IRF8 drives the antiviral gene regulatory network***

268 To further investigate regulatory mechanisms driving the antiviral response, we focussed on
269 the core set of 197 genes identified above showing coordinated changes in expression,
270 chromatin accessibility and H3K27ac signal. TFBS enrichment analysis was performed on
271 the peaks or regions corresponding to those 197 genes containing both enhancers and
272 promoters, which revealed nine enriched TFBS (Figure 4A). The most enriched TFBS was
273 interferon regulatory factor 8 (IRF8), a highly characterised binding site utilised during
274 interferon responses (Mancino *et al.*, 2015). IRF8 binding site was identified in the regulatory
275 regions of 37 of the 197 genes (18.8%), and all these genes were also highly significantly
276 upregulated at the mRNA level, supporting the functional relevance of IRF8 motif enrichment
277 in the antiviral response (Table 2). The IRF8 binding site corresponds to the ISRE, a motif
278 that has core elements of nnGAAAnnGAAAnn (Figure 4B) shared with many other ISRE
279 elements found in or near interferon responsive genes (Tamura *et al.*, 2015). This ISG
280 subset includes key antiviral effectors such as *MX*, an IFN-inducible dynamin-like large
281 GTPases that is well characterised to mediate antiviral activities; a TRIM ubiquitin ligase
282 involved in viral neutralization; *MEFV*, a key regulator of innate immunity; and *PARP14*, a
283 poly(ADP-ribose) polymerase involved in transcriptional activation of ISGs (Haller *et al.*,
284 2007; Caprara *et al.*, 2018; Koepke *et al.*, 2021). The TFBS motif for IRF8-binding motifs
285 (Figure 4B) confirms the functional conservation of the ISRE sequence, consistent with its
286 binding preferences and regulatory function in ISGs. These findings are consistent with the

287 earlier observation of GO term enrichment for the 197 genes (Figure 3B), which were
288 associated with processes such as “*response to virus*” and “*innate immune response*”. The
289 prevalence of IRF8 motifs within these genes further supports this TF's role as a critical
290 transcription factor driving interferon-stimulated regulatory networks during poly I:C
291 stimulation.

292

293 ***Chromatin state dynamics and TFBS using whole ATAC-seq and ChIP-seq data sets***

294 To investigate the chromatin landscape underlying the antiviral immune response to poly I:C,
295 we applied ChromHMM modelling to the ATAC-seq, H3K27ac, and H3K27me3 datasets.
296 This integrative analysis identified five distinct chromatin states, annotated following the
297 framework established by Ernst and Kellis (2017) (Figure 5A, B and C). These chromatin
298 states were classified as follows: (1) ATAC Islands: regions characterized by high chromatin
299 accessibility, indicative of active regulatory activity lacking H3K27ac and H3K27me3 and the
300 presence of potential enhancer or promoter elements. (2) Quiescent/Low Regions: Areas
301 with minimal chromatin accessibility and low regulatory activity, suggesting these regions are
302 largely inactive under the conditions tested. (3) Repressed States: Regions dominated by
303 H3K27me3 with low ATAC-seq signal, indicative of transcriptional silencing mediated by
304 polycomb repression. (4) Poised Enhancer States: Regions marked by H3K27me3
305 (repressive mark) alongside H3K4me1, indicative of enhancers that are primed but not yet
306 active. These regions may transition to an active state upon specific stimuli through the loss
307 of H3K27me3 and the acquisition of H3K27ac, which marks active enhancers (Barral and
308 Déjardin, 2023). (5) Active Regulatory Elements (Enhancers/Promoters): Regions displaying
309 strong ATAC-seq and H3K27ac signals, reflecting robust regulatory activity and heightened
310 chromatin accessibility at enhancers and promoters. ChromHMM analysis revealed that
311 chromatin state 5, corresponding to active enhancers and promoters (enriched for ATAC-seq
312 and H3K27ac signals), was more prevalent in poly I:C stimulated samples compared to PBS
313 controls (Figure 5B and C), consistent with increased activation of regulatory regions during
314 the antiviral response. These findings provide a high-resolution view of chromatin state

315 dynamics, illustrating the diversity of regulatory landscapes and their functional relevance in
316 response to poly I:C stimulation.

317 To identify transcription factors potentially regulating these active regions, consensus open
318 chromatin regions representing ChromHMM state 5 (active regulatory elements) were
319 analysed for TFBS enrichment (Figure 5D). Sixteen TFBSs were either significantly enriched
320 or under-represented in poly I:C. Seven TFBSs were uniquely enriched in the poly I:C
321 stimulated group, including STAT6, IRF9, PRDM1, IRF6, JDP2, NR2E1, and BCL6. These
322 transcription factors play central roles in antiviral immunity and immune system regulation.
323 The presence of STAT6 (Li *et al.*, 2016), IRF9 and IRF6 demonstrates a set of key TFBS for
324 interferon responsive genes, with IRF9 sharing a binding site highly similar with IRF8
325 described earlier.

326 ***ISREs reside within regulatory elements driving the antiviral response***

327 The enrichment of TFBSs for conserved vertebrate interferon regulators including IRF3,
328 IRF7, IRF8, IRF9 and STAT2, implies the activation of ISGs through the ISRE pathway. In
329 this context, our focus was directed towards investigating the response induced by primary
330 viral recognition facilitated by the RIG-I pathway, which play a central role in initiating the
331 interferon response and establishment of an antiviral cellular state (Figure 6A). Specifically
332 examining the RIG-I pathway, we observed upregulation of *rig-i*, *mda5*, *irf3*, and *irf7* at the
333 mRNA level, with a concurrent significant increase in associated peaks in ATAC-seq and
334 H3K27ac datasets. However, while *mavs* was expressed, but as previously demonstrated
335 this gene was not induced by viral infection (Biacchesi *et al.*, 2009) (Figure 6B). Notably, *rig-i*
336 and *mda5* displayed reduced promoter H3K27me3 (repressive mark) signal in response to
337 poly I:C, suggesting their activation rather than suppression during this immune antiviral
338 response. Turning to the JAK-STAT pathway, we observed significant induction of *stat1*,
339 *stat2*, and *irf9* mRNAs, accompanied by a notable increase in promoter openness and
340 H3K27ac signal, and a visible decrease in promoter H3K27me3 signal for *stat2* and *irf9*.
341 These results clearly demonstrate combined chromatin activation and expression

342 upregulation of the major signalling transcription factor genes involved in interferon and
343 antiviral responses.

344 We further sought to identify ISREs within genes associated with the RIG-I and JAK-STAT
345 pathways. Our criteria for enrichment included the presence of ISREs within promoter
346 regions exhibiting increased accessibility and/or concurrent H3K27ac activity in response to
347 poly I:C. Notably, *rig1* and *mda5* share the ISRE consensus sequences *TTTCGGTTTC* and
348 *TTTCGTTTC* in their proximal promoters (Figure 6C), while *irf7* and *stat1* share
349 *TTTCACTTC* as their exclusive ISRE (Figure 6C and D). *irf3* featured a single ISRE,
350 *GAAAACGAAA*, whereas *stat2* and *irf9* harbour two ISREs within their proximal promoters
351 (Figure 6D).

352 We also searched for ISREs in additional key ISGs that were differentially expressed and
353 showed increased chromatin openness and H3K27ac activity upon poly I:C simulation. We
354 focused on five genes captured in multiple enriched GO terms for antiviral activity with strong
355 mRNA upregulation by poly I:C: *mx*, *rsad2*, *tapbp*, *isg15*, and *cd9*. ISREs were enriched in
356 the proximal promoter of these genes, including two for *mx* (*GAAATGAAA* and
357 *TTTCGATTTTC*), two for *cd9* (*TTTCATTTTC* and *TTTCTTTTC*), three for *rsad2*
358 (*GAAACGAAA*, *TTTCGTTTTTC* and *TTTCATTTTC*), three for *tapbp* (*GAAAGGAAA*,
359 *TTTCACTTTC* and *TTTCATTTTT*), and one for *isg15* (*GAAACCGAAA*) (Figure 7A and B).
360 The peaks associated with ISRE motifs in the H3K27me3 dataset were either significantly
361 lower or remained unchanged following poly I:C stimulation, suggesting that these regions
362 are repressed and do not undergo activation in response to the stimulus (Figure 7B).

363

364 ***ISRE enrichment is paralogue specific***

365 Salmonid fish have expanded gene families with paralogues retained from both ancestral
366 WGDs and tandem gene duplications. Our previous work has demonstrated differential
367 expression in response to poly I:C among paralogous ISGs (Clark *et al.*, 2023). To further

368 explore the regulation of these paralogues, we conducted paralogue-specific ISRE motif
369 analysis for key ISGs, including *mx*, *rsad2*, *irf9*, *irf7*, *dhx58*, *stat1*, and *cd9* (Supplementary
370 Figure 5).

371 Within the *mx* gene family, seven paralogues were identified, with four showing highly
372 induced gene expression (ENSSSAG00000077530, ENSSSAG00000117576,
373 ENSSSAG00000096170, and ENSSSAG00000051905) (Supplementary Figure 5A).
374 Notably, only the most upregulated *mx* paralogue (ENSSSAG00000077530) possessed
375 ISREs in its putative promoter (*GAAATGAAA* and *TTTCGATTTTC*). Four of these *mx*
376 paralogues are tandem repeats located on Chromosome 25.

377 The *rsad2* gene family comprised four paralogues, three of which were highly upregulated by
378 poly I:C (ENSSSAG00000048046, ENSSSAG00000108937, and ENSSSAG00000108840),
379 each harbouring two or three ISRE motifs with *GAAACGAAA* shared (Supplementary Figure
380 5B). Three *rsad2* genes were found as tandem repeats on Chromosome 9, two with
381 identified ISREs (ENSSSAG00000108840 and ENSSSAG00000108937) (Supplementary
382 Figure 5B).

383 *irf9* and *irf7* have two and three paralogues, respectively. Only one *irf9* paralogue
384 (ENSSSAG00000080439) exhibited ISRE motifs (*AAAACCGAAA* and *AAAAACGAAA*) in
385 regulatory elements activated by poly I:C (Supplementary Figure 5C). For *irf7* one paralogue
386 (ENSSSAG00000076373) contained the ISRE *TTTCACTTTC* in activated regulatory
387 elements (Supplementary Figure 5D). A second *irf7* paralogue (ENSSSAG00000066279)
388 displayed open chromatin at the promoter, though chromatin accessibility was not
389 significantly different between stimulated and unstimulated fish, suggesting alternative
390 regulatory mechanisms.

391 The core viral receptor gene family *rig-I* has three paralogues (ENSSSAG00000037858,
392 ENSSSAG00000003156, and ENSSSAG00000119673), all showing the presence of ISREs
393 within regulatory elements activated by poly I:C stimulation (Supplementary Figure 5E).

394 In the *stat1* family, three out of five paralogues harboured ISREs in upstream regulatory
395 regions activated by poly I:C stimulation (Supplementary Figure 5F). One copy
396 (ENSSSAG00000056374) had open chromatin without any ISRE, and another
397 (ENSSSAG00000102446) did not display open chromatin in the promoter.

398 The *cd9* family comprised eight paralogues, with only ENSSSAG00000059637 exhibiting
399 ISRE regions (*TTTCATTTTC* and *TTTCTTTTC*) in the proximal promoter, which was
400 associated with the strongest mRNA expression and upregulation by poly I:C
401 (Supplementary Figure 5G). Four of these paralogues were identified as contigs,
402 representing highly duplicated sequences not assignable to specific chromosomes due to
403 their repetitive nature.

404 Our paralogue specific ISRE motif analysis unveils distinct regulatory patterns and functional
405 implications within the innate immune response, shedding light on the intricate dynamics of
406 gene paralogues in antiviral defence mechanisms.

407

408 **Discussion**

409 The immune response to viral infections is a fundamental aspect of host defence
410 mechanisms conserved across vertebrates. In this study, we combined RNA-seq, ATAC-seq
411 and ChIP-seq to investigate the regulatory landscape underlying the antiviral response in
412 Atlantic salmon following poly I:C stimulation, a mimic of double-stranded RNA viruses. Our
413 aim was to elucidate the interplay between gene expression, chromatin accessibility, and
414 histone modifications during the early stages of the immune response.

415 Our analysis revealed significant alterations in both the transcriptome and 'regulome' in
416 response to poly I:C stimulation. Notably, these changes were observed in a subset of
417 conserved ISGs, which play an important role in antiviral defence in all vertebrates. ATAC-
418 seq provided valuable insights into chromatin accessibility alterations during the antiviral
419 response, revealing hundreds of regions exhibiting significant changes following poly I:C

420 stimulation. The functional significance of these open chromatin regions was supported by
421 enrichment of Gene Ontology terms related to viral response for the nearby genes they
422 presumably regulate. H3K27ac and H3K27me3 histone modifications are key players in
423 gene regulation (Creyghton *et al.*, 2010; Barski *et al.*, 2007). Our study revealed a significant
424 increase in H3K27ac activity in 1,539 genomic regions, indicative of gene activation.
425 Conversely, the upregulation of H3K27me3 in 795 regions suggests transcriptional
426 suppression and the formation of repressive chromatin structures. Only 30 genomic regions
427 exhibited concurrent changes in both H3K27ac and H3K27me3. While these marks are
428 typically considered mutually exclusive, their detection within the same genomic regions is
429 likely to reflect cell-type heterogeneity, where the same regulatory element is repressed
430 (H3K27me3) in some cell populations while being active (H3K27ac) in others. This may
431 indicate differential regulatory states across distinct cell subsets rather than a true bivalent
432 chromatin state (Barral and Déjardin, 2023). These regions may play critical roles in
433 maintaining essential cellular processes or fine-tuning immune response regulation,
434 potentially through mechanisms involving chromatin remodelling and transcription factor
435 recruitment. Collectively, the integration of ATAC-seq and ChIP-seq data provides
436 comprehensive insights into regulatory changes in Atlantic salmon in response to viral
437 stimulation.

438 We find 3,541 genes that show differential expression following poly I:C stimulation, which
439 together are consistent with different layers of regulatory changes representing different
440 functional groups of genes. The core set of 197 genes that are transcriptionally upregulated
441 and also have significant increased open chromatin and increased presence of activating
442 H3K27ac is enriched in ISGs conserved among vertebrates (Clark *et al.*, 2023). Of interest
443 there were also genes that showed chromatin remodelling, with no associated significant
444 change in gene expression. These genes may represent a group of loci with distinct
445 temporal regulation, potentially primed for expression either before or after the single time
446 point analysed in our study. For instance, genes that exhibited changes in chromatin

447 accessibility and gene expression without accompanying H3K27ac modifications, suggest
448 the involvement of distal enhancers or alternative chromatin dynamics such as topologically
449 associating domains (TADs) influencing their regulation (Tena and Pereira, 2021). The
450 genes that are defined as having chromatin openness and increased expression have very
451 clear interferon associated functionality, which could reflect different activation modes of
452 histone modification (H3K4me3 and H3K4me1), as in our study we examined a single
453 activating histone mark (Murphy *et al.*, 2024). The large number of genes that are
454 transcriptionally upregulated but lack significant changes in chromatin accessibility and
455 histone acetylation (H3K27ac), exhibit a very clear role in the IFN response. These genes
456 likely represent early response genes that are already in an accessible chromatin state,
457 allowing for rapid transcriptional activation upon stimulation (Bahrami and Diablos, 2016).
458 Alternatively, these genes may be regulated post-transcriptionally following poly I:C
459 stimulation, a mechanism often observed in innate immune responses, which could involve
460 mRNA stability, translation efficiency, or degradation pathways (Carpenter *et al.*, 2014).

461 Recent single-cell transcriptomic studies have highlighted the remarkable cellular
462 heterogeneity of Atlantic salmon immune cells, particularly in the head kidney (Andresen *et al.*,
463 2024) and in the liver (Taylor *et al.*, 2022). This diversity likely underlies the observed
464 largely disconnected regulation of H3K27ac and H3K27me3 marks in response to poly I:C,
465 as different immune cell subpopulations likely exhibit distinct patterns of chromatin
466 remodelling at the same loci. Although a detailed, locus-by-locus inspection was beyond the
467 scope of this study, our findings are consistent with the idea that cellular heterogeneity
468 shapes the chromatin and transcriptional responses to antiviral stimulation. Incorporating
469 such single-cell insights in future studies will be valuable for resolving how specific cell types
470 contribute to the epigenomic regulation of the interferon response in salmonid immunity.

471 ISREs are DNA sequence motifs responsible for the regulation of immune genes (Au *et al.*,
472 1995), which serve as recognition sites for transcription factors integral to the interferon
473 response pathway (Ivashkiv and Donlin, 2014). Our analysis identified a notable enrichment

474 of TFBSs specific to IRF8, IRF9, STAT1, and STAT2 in response to poly I:C stimulation.
475 These transcription factors are well-documented to bind to ISREs (Au-Yeung *et al.*, 2013;
476 Platanitis *et al.*, 2019), underlining the significance of transcription factor-mediated regulatory
477 mechanisms governing the antiviral immune response in Atlantic salmon. IRF8 exhibited the
478 highest level of enrichment in those genes which were identified across RNA-seq, ATAC-
479 seq, and H3K27ac datasets. These genes included key antiviral effectors, such as *TRIM21*,
480 *MEFV*, and *PARP14*, which are involved in protein ubiquitination, immune modulation, and
481 transcriptional activation of ISGs. IRF8, a transcription factor that binds to ISREs to regulate
482 antiviral gene expression, is critical for immune responses across vertebrates. In humans
483 and mice, IRF8 is crucial for the development and function of dendritic cells and
484 macrophages, which are key players in antiviral defence and type I interferon production
485 (Hambleton *et al.*, 2011; Laricchia-Robbio *et al.*, 2005). Studies on IRF8-deficient mice have
486 demonstrated increased susceptibility to viral infections, linked to compromised dendritic cell
487 populations and disrupted immune signalling pathways (Becker *et al.*, 2012; Moorman *et al.*,
488 2022). These findings, combined with the strong enrichment of IRF8 motifs in our dataset,
489 highlight its evolutionary conservation and central role in orchestrating the antiviral immune
490 response in Atlantic salmon.

491 Moreover, these transcription factors are known regulators of immune response genes
492 during viral infections (Ivashkiv and Donlin, 2014). Our analysis demonstrates that ISRE-
493 containing genomic regions undergo dynamic histone modifications during antiviral
494 signalling, indicating their functional relevance in gene regulation. This study also expands
495 upon previous research on ISREs in salmonids, focusing on gene families such as *mx*,
496 *rsad2*, *stat1*, *stat2*, and *cd9* which were previously examined primarily at the expression level
497 (Collet, 2004; Castro *et al.*, 2008, 2010; Holland *et al.*, 2008; Collins *et al.*, 2014; Dehler *et*
498 *al.*, 2023). Earlier studies included *in silico* promoter analyses and reporter gene assays, but
499 our integration of ATAC-seq and ChIP-seq datasets allows demarcation of genomic regions
500 with putative functional involvement in the early antiviral response. However, further

501 experimental validation, such as knockout or knock-in studies targeting the identified
502 regulatory elements, will be essential to confirm the molecular mechanisms underlying these
503 observations.

504 The enrichment of TFBSs associated with IRF3 and IRF7 underscores their important role in
505 the antiviral response of Atlantic salmon. The enrichment of TFBSs for PRDM1, IRF6, JDP2,
506 NR2E1, and BCL6 highlights the diverse regulatory landscape governing the immune
507 response in Atlantic salmon following poly I:C stimulation. PRDM1 (also known as BLIMP-1)
508 is known for regulating type I IFN and differentiation of B cells into plasma cells (Lin *et al.*,
509 1997), but also orchestrates plasma cell differentiation by extinguishing the mature B cell
510 gene expression program (Shaffer *et al.*, 2002), while BCL6 is involved in B cell
511 development in mammalian germinal centres (Tsai *et al.*, 2019). IRF6 further contributes to
512 antiviral responses (Liang *et al.*, 2022). JDP2 has a core role in modulating inflammatory
513 signalling pathways (Maruyama *et al.*, 2012) and NR2E1 is involved in neural development
514 and has a crucial role in regulating the proliferation, differentiation, and maintenance of
515 neural progenitor cells in various regions of the brain (Islam and Zhang, 2015). Despite our
516 TFBS analysis relying on the JASPER database, primarily employing experimental data on
517 mammalian transcription factors as the background, the enrichment of these TFBSs in
518 response to poly I:C suggests their evolutionary conservation across vertebrate species,
519 underscoring their relevance in understanding immune responses beyond Atlantic salmon.

520 Our analysis of enrichment in paralogue-specific ISRE motifs unveiled distinctive ISRE
521 sequences exclusive to specific paralogues. Consequences of four rounds of whole-genome
522 duplication (two in early vertebrates, one in the teleost ancestor, and one salmonid-specific)
523 can be detected in the Atlantic salmon genome, a phenomenon well-documented in the
524 literature (Lien *et al.*, 2016). This process has given rise to multiple paralogous genes that
525 have evolved distinct regulation (e.g. Gillard *et al.*, 2021), as observed for many ISG families
526 (Clark *et al.*, 2023). We identified paralogue-specific alterations in the chromatin accessibility
527 of key ISGs, including genes such as *mx*, *rsad2*, *cd9*, *rig1*, *stat1*, *irf7*, and *irf9*. One outcome

528 following gene duplication and frequent subsequent silencing of the particular paralogue
529 could relate to insertion of transposable elements into the regulatory regions of ISGs as was
530 suggested for a decrease transcriptional activity across salmonid genomes where
531 ohnologues showing decreased expression had great abundance of transposable elements
532 (Gillard *et al.*, 2021). In line with this genomic complexity, immune gene families exhibit
533 heterogeneous distribution patterns, with some, such as finTRIMs, in gene groups dispersed
534 across the genome while others, including MHC class I genes, form a few or a unique cluster
535 on a specific location (Van der Aa *et al.*, 2009; Lukacs *et al.*, 2010). These findings
536 underscore the importance of considering paralogue-specific responses in investigations of
537 the interferon response in salmonids and other species with a history of WGDs.

538 This study has demonstrated the activation of putative regulatory elements initiating the
539 antiviral response in a teleost fish by integrating transcriptomics and regulomics. We
540 identified a conserved set of ISGs showing increased mRNA expression following antiviral
541 stimulation, that is likely a product of transcriptional changes induced by remodelling of
542 chromatin state and histone modifications. We further discovered the importance of
543 paralogue-specific changes in key transcription factor binding sites, indicating that the
544 regulatory control of key immune genes has been altered in the recent evolutionary history of
545 salmonids. This work provides a new reference for functional analysis of immune response
546 of Atlantic salmon / fish using transcriptomics, genomics and frames it in an evolutionary
547 perspective.

548

549 **Materials and Methods**

550 ***Animal studies***

551 Juvenile Atlantic salmon (~70g) of commercial origin were maintained in 250L freshwater
552 tanks in the Zoology building aquarium at the University of Aberdeen (School of Biological
553 Sciences). Water temperature was maintained at 14°C, and fish were fed a commercial

554 pellet diet. The water flow rate was approximately 1000L/h and photoperiod was 12:12
555 light:dark. All procedures described complied with the Animal (Scientific Procedures) Act of
556 1986 under the UK Home Office license PFF8CC5BE and were approved by the ethics
557 committee at the University of Aberdeen, Scotland, United Kingdom.

558 ***Stimulation of fish with viral mimic***

559 Poly I:C (Sigma, P1530) was diluted to 5 mg/mL in PBS, aliquoted and stored at -20 °C.
560 Prior to being used for immune stimulation, poly I:C was heated to 55 °C for 15 minutes and
561 then allowed to cool at room temperature for 20 minutes. Fish were anaesthetised using 2-
562 phenoxyethanol (2.5 mL in 10 L water/ 0.0025 % v/v) and given an intraperitoneal injection
563 of either PBS (1x, 0.1 mL) (n=6, controls) or poly I:C (500 µg in 0.1 mL, per fish) (n=6). 24
564 hours after stimulation, fish were euthanised by overexposure to 2-phenoxyethanol followed
565 by destruction of the brain using a scalpel. Head kidney tissue was sampled – chosen for
566 this study as the primary haematopoietic tissue in teleosts, rich in different leucocyte
567 populations. For RNA-seq, samples were stored in RNAlater (Sigma) for 24 hrs at 4°C
568 followed by long-term storage at -80 °C. For ATAC-seq and CHIP-seq, fresh head kidney
569 tissue was placed in L15 media (Gibco) containing 2 % FBS (Labtech) and 0.02 % EDTA
570 (Sigma) and placed on ice until processing. For each fish (n=6 per PBS and poly I:C injected
571 groups) one RNA-seq, one ATAC-seq and two CHIP-seq libraries (H3K27ac and H3K27me3
572 histone marks; see later section) were constructed from the same head kidney sample to
573 facilitate data integration (Supplementary Figure 1).

574 ***ATAC-seq library preparation***

575 ATAC-seq library preparation was adapted from Buenrostro *et al.* (2015). Head kidney tissue
576 (~20 mg) was placed into a 50 mL tube (tube 1) containing 20 mL extraction media (L15, 2
577 % FBS and 0.02 % EDTA, pH 8). The tissue was gently passed through a 100 µm nylon
578 mesh (Starlab) and placed in a new 50 mL tube using a sterile pipette and spatula.
579 Extraction media was continually applied to prevent cells from drying out and assist with their

580 transit through the mesh. This step disrupted the tissue, and cells were dissociated in the
581 media. The final suspension of cells was made up to 20 mL in extraction media. An aliquot of
582 10 μ L of these cells was mixed with trypan blue (Sigma) at a 1:5 ratio and counted using a
583 haemocytometer. An aliquot of 75,000 cells for each sample was transferred in a 1.5 mL
584 loBind Eppendorf tube for ATAC-seq library preparation, and the remaining cells were
585 retained for ChIP-seq library generation as described in a later section.

586 Head kidney nuclei were isolated by pelleting the cells by centrifugation (500 g, 5 min, 4 °C).
587 The supernatant was discarded carefully by aspiration to avoid cell loss. The cells were
588 washed by resuspending then gentle pipetting in 1 mL PBS containing protease inhibitor
589 cocktail (PIC) (Sigma). The cells were pelleted again (5 mins at 500 g at 4 °C), and the
590 supernatant removed, to which 50 μ L of cold ATAC-resuspension buffer L (ATAC-RSB-L)
591 containing 0.1 % Tween-20, 0.1 % NP-40 and 0.01 % Digitonin (Promega) was added to the
592 cell pellet, suspended by gently pipetting up and down three times. The ATAC-RSB cold
593 buffer was made up of 1 M Tris pH 7.4 (Sigma), 5M NaCl (Sigma), 1M MgCl₂ (Sigma) and
594 dH₂O. Cells were incubated on ice for 3 mins in cold ATAC-RSB-L to lyse the cells and
595 release intact nuclei. Immediately after 3 mins, 1 mL of ATAC-RSB-W (ATAC-RSB-W
596 contains ATAC-RSB and 10% Tween-20) with 0.1 % Tween-20 was added to the lysed cells.
597 The tube was inverted three times and then centrifuged for 10 mins at 800 g at 4 °C to pellet
598 the nuclei, before the supernatant was aspirated carefully to avoid losing the pellet.

599 The remaining ATAC-seq library preparation steps, such as transposase reaction, clean-up
600 using MinElute PCR purification, library amplification and library clean-up, were performed
601 according to Buenrostro *et al.* (2015).

602 ***ChIP-seq library preparation***

603 ChIP-seq libraries were generated using the ChIPmentation Kit for Histones (Diagenode:
604 Cat. No. C010110009). After tissue disruption and dissociation of cells in the extraction
605 media, as described above for ATAC-seq, 2 $\times 10^6$ cells were transferred into a 1.5 mL tube

606 and pelleted for 10 mins at 800 g at 4 °C before the supernatant was carefully removed.
607 Next, 1 mL of formaldehyde solution 1 % (Sigma) was added, and the pellet was then
608 resuspended by pipetting up and down and incubated under constant rotation at room
609 temperature for 5 mins to crosslink the DNA and proteins. The reaction was quenched by the
610 addition of 125 µL of 1 M glycine (0.125 M final) and incubated for 10 mins at room
611 temperature under constant rotation. The crosslinked cells were centrifuged for 5 mins at
612 1,000 g at 4 °C, and the supernatant removed. Next, cells were resuspended in 1 mL of PBS
613 with PIC by pipetting and centrifuged for 5 mins at 1,000 g at 4 °C, before the supernatant
614 was removed and the cell pellet was stored at -80 °C.

615 Sonication using a Bioruptor Pico device (Diagenode) was optimised at 20 cycles and 30
616 sec/30 sec on/off to disrupt the cells and shear the DNA. The sonicated chromatin was
617 processed using the MinElute PCR purification kit (Qiagen). 20 µL sonicated chromatin was
618 added to 180 µL elution buffer along with 2 µL of RNase A (100 mg/mL) and incubated for
619 10 mins at 37 °C. 5 µL Proteinase K (20mg/mL) was added before further incubation for 1
620 hour at 68 °C under constant rotation. DNA purification was performed using the MinElute
621 PCR purification kit (Qiagen). DNA was then quantified using NanoDrop (One/One^o
622 Microvolume UV-Vis Spectrophotometer, ThermoFisher Scientific) and assessed by
623 Tapestation (4200) (Agilent Technologies) with a high-sensitivity DNA kit to determine DNA
624 fragment size. A fragment size range of 100-700 bp was achieved for all downstream ChIP-
625 seq library preparations. The remaining steps followed Diagenode's instructions for the
626 ChIPmentation Kit. The two antibodies used were H3K27ac (Diagenode: C15410196) and
627 H3K27me3 (Diagenode: C15410195). For the control input, an IgG antibody (Diagenode
628 code: C15400001-15) was used in each series of ChIP reactions. The input samples
629 consisted of a pooled mixture of all six samples within the respective group.

630 ***Sequencing and data analysis***

631 For ATAC-seq and ChIP-seq, paired-end 150bp libraries were sequenced using an Illumina
632 NovaSeq 6000 platform by Novogene Ltd, aiming to generate 40 million reads per sample.

633 The raw reads were deposited in the European Nucleotide Archive (EBI) and are accessible
634 in the FAANG data coordination centre under accession numbers PRJEB50077 and
635 PRJEB56698 for ATAC-seq and ChIP-seq, respectively. For RNA-seq, we used the
636 differentially expressed data published in Clark *et al.* (2023), which was from matched
637 samples (EBI accession number: PRJEB50076).

638 For ATAC-seq and ChIP-seq, the processing of raw reads, mapping of reads, peak calling
639 and quantification was performed using the nextflow pipelines, *nf-core/atacseq* (v2.0) and *nf-*
640 *core/chipseq* (v2.0) (Patel *et al.*, 2020). Raw reads were trimmed using Trim Galore
641 (v0.6.10) and adapters removed. All reads were mapped to the current Atlantic salmon
642 reference genome (GCA_905237065.2) downloaded from the Ensembl genome browser
643 release Ssal_v3.1 (https://www.ensembl.org/Salmo_salar/Info/Index). Mapping was carried
644 out using BWA (v0.7.17) (Li and Durbin, 2009), consensus peak calling for ATAC-seq and
645 ChIP-seq were performed using MACS2 (Zhang *et al.*, 2008). Peaks were annotated using
646 HOMER (v4.9.1) (Heinz *et al.*, 2010) and consensus peaks were quantified using
647 featureCounts (Liao *et al.*, 2014). Input controls were included in the peak-calling process to
648 normalize background signal and enhance the specificity of peak identification. The
649 sequence-specific mapping and individually selected genes were visualised using IGV tools
650 (Thorvaldsdottir *et al.*, 2013).

651 The quantified ATAC-Seq and ChIP-Seq peaks were imported into R v4.2.2 (R Core Team,
652 2022) and differential peak analysis was performed using DESeq2 (Love *et al.*, 2014).
653 Significantly different peaks among groups were identified using the Wald test in DESeq2
654 following False Discovery Rate (FDR) correction using the Benjamini–Hochberg approach,
655 with adjusted *P*-value < 0.05 and up- or down- fold change > 2.

656 ***Gene set enrichment analysis***

657 To identify biological processes associated with differentially accessible or modified
658 chromatin regions, we performed region-centric enrichment analysis using the R package

659 rGREAT (Gu, 2022), which interfaces with the Genomic Regions Enrichment of Annotations
660 Tool. This approach allows functional enrichment analysis directly from genomic coordinates
661 without requiring prior gene assignment, preserving the spatial resolution of the ATAC-seq
662 and ChIP-seq datasets.

663 Differentially accessible ATAC-seq regions and differentially abundant H3K27ac regions
664 were supplied to rGREAT as query sets in BED format and imported into R as GRanges
665 objects. For each analysis, the background set was defined as the full set of consensus
666 ATAC-seq or H3K27ac peaks, respectively, to control for assay-specific genomic coverage
667 and avoid biases from using the whole genome as background. Enrichment was tested
668 against Gene Ontology Biological Process (GO:BP) terms using the Atlantic salmon genome
669 annotation (GTF file) and the Ensembl *Salmo salar* dataset (ssalar_gene_ensembl) provided
670 by rGREAT. GO terms with an adjusted P -value < 0.05 were considered significantly
671 enriched. In addition, gene expression–centric enrichment analysis was performed using the
672 Database of Annotation, Visualization, and Integrated Discovery (DAVID) (v2023q4) (Huang
673 *et al.*, 2009) on the list of differentially expressed genes identified by RNA-seq. Human
674 orthologs of the Atlantic salmon genes were identified following the approach of Clark *et al.*
675 (2023). In cases where multiple paralogs were expressed for a single gene, the paralog with
676 the highest expression was selected. The background gene set for DAVID was the human
677 gene repertoire. GO terms with fold enrichment > 2 and FDR-adjusted $p < 0.05$ were
678 considered enriched.

679 ***Chromatin state discovery***

680 ChromHMM (v1.24) was used to identify genome-wide chromatin states using the integrated
681 ATAC-seq, H3K27ac and H3K27me3 ChIP-seq datasets. ChromHMM uses a multivariate
682 Hidden Markov Model that considers the spatial and combinatorial arrangements of various
683 chromatin marks to learn and describe these states (Ernst and Kellis, 2012). Firstly, the
684 aligned reads for each sample were converted into a binarized format using the BinarizeBam
685 function using the following command: `java -mx4000M -jar ChromHMM.jar BinarizeBam`

686 CHROMSIZES/Ssal_v3.1.txt inputbam samplefile.txt binarize outputdir. We then used the
687 LearnModel function to develop models of chromatin states based on these binary files,
688 automatically generating chromatin state segmentation across the genome. To start model
689 learning and have automatic enrichments computed after the model is learned, the
690 LearnModel command was computed: java -mx4000M -jar ChromHMM.jar LearnModel -p 64
691 inputdir outputdir 5 Ssal_v3.1. Chromatin states were annotated by ChromHMM using the
692 five-state model based on enrichment of ATAC-seq and H3K27ac signals (active) or
693 H3K27me3 signal (repressive). Promoter regions were defined as the chromatin segments
694 annotated by ChromHMM as promoter-like states and overlapping the annotated
695 transcription start sites (TSSs) from the *Salmo salar* genome annotation. TSS coordinates
696 were derived directly from the reference GTF and correspond to the 5' end of each
697 transcript.

698 ***Transcriptional factor binding site analysis***

699 For the transcription factor binding site (TFBS) prediction and analysis we used
700 GimmeMotifs (Bruse and van Heeringen, 2018) with the JASPAR2022 vertebrate core
701 database (Castro-Mondragon *et al.*, 2022), the database comprises curated, non-redundant
702 binding motifs derived from experimental evidence. Motif enrichment was assessed using
703 active regulatory regions from ChromHMM state 5 (active enhancers and promoters) in poly
704 I:C-stimulated and control PBS samples. To reduce false positives, background-matched
705 genomic regions were used, and a Z-score threshold of >2 was applied to retain only
706 significantly enriched motifs associated with antiviral responses.

707

708 **Data access**

709 The raw sequence data has been submitted to the European Nucleotide Archive (ENA)
710 (<https://www.ebi.ac.uk/ena/browser/home>) under accession numbers PRJEB50076 (RNA-
711 seq), PRJEB50077 (ATAC-seq), and PRJEB56698 (ChIP-seq). Metadata from this study

712 can be found at the AQUA-FAANG Data Portal (<https://data.faang.org/projects/AQUA->
713 FAANG).

714 **Competing interest statement**

715 The authors declare no competing interests.

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732

733

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1071

1072 *Table 1. 54 conserved ISGs common between ATAC-seq, ChIP-seq (H3K27ac) and RNA-seq. Considered ISG*
 1073 *data was obtained from Clark et al, (2023).*

Ensembl id	FC ATAC	FCH 3K27ac	FC RNA	HGNC	Description
ENSSSAG00000068365	4.16	1.62	5.64	IFIT5	interferon induced protein with tetratricopeptide repeats 5
ENSSSAG00000064740	3.35	2.16	4.49	UBB	ubiquitin B
ENSSSAG00000077530	2.79	2.35	4.58	MX1	MX dynamin like GTPase 1
ENSSSAG00000041408	2.75	3.29	4.12	RNF213	ring finger protein 213
ENSSSAG00000043101	2.72	1.62	5.39	BATF	Basic Leucine Zipper ATF-Like Transcription Factor
ENSSSAG000000108395	2.58	3.09	4.54	SAMD9	sterile alpha motif domain containing 9
ENSSSAG000000118579	2.55	3.88	4.88	SOCS1	suppressor of cytokine signaling 1
ENSSSAG000000104513	2.44	3.46	4.79	SOCS1	suppressor of cytokine signaling 1
ENSSSAG00000043265	2.40	1.84	4.91	HERC3	HECT and RLD domain containing E3 ubiquitin protein ligase 3
ENSSSAG00000007886	2.39	2.24	5.22	CMPK2	cytidine/uridine monophosphate kinase 2
ENSSSAG00000073332	2.21	1.55	3.01	HELZ2	helicase with zinc finger 2
ENSSSAG000000121778	2.04	1.12	5.49	UBB	ubiquitin B
ENSSSAG000000058932	2.02	1.95	3.22	PARP14	poly(ADP-ribose) polymerase family member 14
ENSSSAG00000078539	2.02	1.55	1.66	RNF213	ring finger protein 213
ENSSSAG000000056446	1.98	1.86	3.79	NLRCS	NLR family CARD domain containing 5
ENSSSAG00000077480	1.96	1.71	4.64	NAMPT	nicotinamide phosphoribosyltransferase
ENSSSAG00000043036	1.94	1.54	4.24	ZNF1	zinc finger NFX1-type containing 1
ENSSSAG00000075304	1.92	1.69	2.98	EIF2AK2	eukaryotic translation initiation factor 2 alpha kinase 2
ENSSSAG000000005181	1.85	1.48	3.31	SAMD9L	sterile alpha motif domain containing 9 like
ENSSSAG000000054938	1.82	1.85	2.51	PARP12	poly [ADP-ribose] polymerase 12-like
ENSSSAG00000071823	1.77	2.65	2.48	XCL2	X-C motif chemokine ligand 2
ENSSSAG000000110347	1.77	1.32	5.65	TREX	three prime repair exonuclease
ENSSSAG000000079036	1.74	2.05	4.92	IFIT5	interferon induced protein with tetratricopeptide repeats 5
ENSSSAG00000072910	1.70	2.31	3.17	IFI44L	interferon induced protein 44 like
ENSSSAG000000068298	1.66	1.59	1.48	NLRCS	NLR family CARD domain containing 5
ENSSSAG000000059637	1.65	1.19	1.75	TSPAN33	tetraspanin 33
ENSSSAG00000004551	1.63	1.74	3.08	XAF1	XIAP associated factor 1
ENSSSAG000000003156	1.62	1.66	5.13	DHX58	DEXH-box helicase 58
ENSSSAG00000075036	1.62	2.35	3.40	LGALS3BP	galectin 3 binding protein
ENSSSAG000000081672	1.61	1.21	3.48	PARP9	poly(ADP-ribose) polymerase family member 9
ENSSSAG000000049790	1.55	1.82	3.25	LGALS9C	galectin 9C
ENSSSAG000000046065	1.53	1.59	3.50	USP20	ubiquitin specific peptidase 20
ENSSSAG000000051388	1.52	1.69	4.17	NCOA7	nuclear receptor coactivator 7
ENSSSAG000000005381	1.51	2.31	3.94	PARP12	poly(ADP-ribose) polymerase family member 12
ENSSSAG00000078885	1.48	1.48	2.73	IFIH1	interferon induced with helicase C domain 1
ENSSSAG000000119673	1.47	1.64	2.54	DDX58	DEXD/H-box helicase 58
ENSSSAG00000072880	1.46	1.82	2.93	TRAFD1	TRAF-type zinc finger domain containing 1
ENSSSAG000000054674	1.46	1.43	1.88	RNF213	ring finger protein 213
ENSSSAG000000045256	1.45	1.18	2.69	IFI44	interferon-induced protein 44-like
ENSSSAG000000045959	1.45	1.69	1.94	PARP12	poly(ADP-ribose) polymerase family member 12
ENSSSAG00000048046	1.36	1.39	6.43	RSAD2	radical S-adenosyl methionine domain containing 2
ENSSSAG000000020083	1.34	1.84	2.96	PARP12	poly [ADP-ribose] polymerase 12-like
ENSSSAG000000002526	1.28	1.36	2.92	USP20	ubiquitin specific peptidase 20
ENSSSAG00000077407	1.26	1.25	1.53	TAPBP	TAP binding protein
ENSSSAG000000042163	1.22	1.77	2.42	PARP9	poly(ADP-ribose) polymerase family member 9
ENSSSAG000000037936	1.15	2.03	1.49	LGALS3BP	galectin 3 binding protein
ENSSSAG000000119808	1.14	1.68	2.13	OGFR	opioid growth factor receptor
ENSSSAG000000006364	1.12	1.65	1.76	ADAR	adenosine deaminase RNA specific
ENSSSAG000000001032	1.11	1.50	1.94	RNF114	ring finger protein 114
ENSSSAG00000076373	1.08	1.75	3.37	IRF7	interferon regulatory factor 7
ENSSSAG00000074138	1.06	1.17	2.29	CGAS	cyclic GMP-AMP synthase
ENSSSAG000000058809	1.04	1.59	2.38	PARP15	poly(ADP-ribose) polymerase family member 15
ENSSSAG00000070425	1.03	1.31	1.31	MOV10	Mov10 RISC complex RNA helicase
ENSSSAG000000047562	1.01	1.78	1.58	RNF213	ring finger protein 213

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1075

1076 *Table 2. Genes with enriched IRF8 (ISRE) motifs identified in a subset of 197 genes that are common among*
 1077 *ATAC-seq, H3K27ac ChIP-seq, and RNA-seq datasets. FC RNA indicate the fold change in response to Ploy I:C*
 1078 *at mRNA level.*

Ensembl_id	HGNC	Description	FC RNA	FDR
ENSSSAG00000099839	ENSSSAG00000099839		3.26	0.000
ENSSSAG00000010621	EDIL3	EGF like repeats and discoidin domains 3	3.33	0.000
ENSSSAG00000044071	CARS1	cysteinyl-tRNA synthetase 1	2.71	0.000
ENSSSAG00000078539	RNF213	ring finger protein 213	3.16	0.000
ENSSSAG00000058809	PARP15	poly(ADP-ribose) polymerase family member 15	5.20	0.000
ENSSSAG00000058932	PARP14	poly(ADP-ribose) polymerase family member 14	9.32	0.000
ENSSSAG00000004437	TDRD3	Tudor Domain Containing 3	12.70	0.000
ENSSSAG000000115088	ENSSSAG000000115088		4.54	0.000
ENSSSAG00000098558	MX1	MX dynamin like GTPase 1	10.61	0.000
ENSSSAG00000074112	ENSSSAG00000074112		4.95	0.000
ENSSSAG00000072910	IFI44L	interferon induced protein 44 like	9.01	0.000
ENSSSAG000000112886	RTP4	receptor transporter protein 4	35.48	0.000
ENSSSAG00000085797	RTP3	receptor transporter protein 3	52.11	0.000
ENSSSAG00000045959	PARP12	poly(ADP-ribose) polymerase family member 12	3.82	0.000
ENSSSAG00000089774	ARI6	Putative E3 ubiquitin-protein ligase SINA-like 6	13.42	0.000
ENSSSAG00000061910	TRIM21	tripartite motif containing 21	12.17	0.000
ENSSSAG00000098931	CPRAS1	circularly permuted Ras protein 1-like	5.49	0.000
ENSSSAG00000032869	CALCOCO2	calcium binding and coiled-coil domain 2	2.23	0.000
ENSSSAG00000064740	UBB	ubiquitin B	22.54	0.000
ENSSSAG000000121778	UBB	ubiquitin B	44.79	0.000
ENSSSAG00000078885	IFIH1	interferon induced with helicase C domain 1	6.64	0.000
ENSSSAG00000074688	GIG2P	grass carp reovirus (GCRV)-induced gene 2p	7.09	0.000
ENSSSAG00000042324	TLR7	toll like receptor 7	4.90	0.000
ENSSSAG000000112790	ENSSSAG000000112790		2.39	0.000
ENSSSAG00000001032	RNF114	ring finger protein 114	3.82	0.000
ENSSSAG000000119632	ENSSSAG000000119632		5.56	0.000
ENSSSAG00000008629	HERC3	HECT and RLD domain containing E3 ubiquitin protein ligase 3	3.89	0.000
ENSSSAG00000040462	MEFV	MEFV innate immunity regulator, pyrin	5.21	0.000
ENSSSAG000000120148	URGCP	upregulator of cell proliferation	2.88	0.027
ENSSSAG00000053722	FMR1	FMRP translational regulator 1	2.73	0.000
ENSSSAG00000067713	ZC3H7B	zinc finger CCCH-type containing 7B	2.29	0.000
ENSSSAG00000003892	ENSSSAG00000003892		11.81	0.001
ENSSSAG00000005950	FRK	fyn related Src family tyrosine kinase	5.99	0.000
ENSSSAG00000009694	TRIM35	tripartite motif containing 35	18.23	0.000
ENSSSAG00000043265	HERC3	HECT and RLD domain containing E3 ubiquitin protein ligase 3	30.05	0.000
ENSSSAG00000043072	HERC3	HECT and RLD domain containing E3 ubiquitin protein ligase 3	22.19	0.000
ENSSSAG00000063287	MAVS	mitochondrial antiviral-signaling protein-like	8.54	0.000

1079

1080 **Figure Legends:**

1081 Figure 1. Genomic distribution of ATAC-seq and H3K27ac ChIP-seq peaks relative to gene
 1082 features and transcription start sites (TSS). Barplots showing the number of consensus
 1083 peaks associated with genes or unassociated to genes in ATAC-seq (A) and H3K27ac (B)
 1084 datasets. Distribution of peak–gene associations binned by signed distance to the nearest
 1085 TSS in ATAC-seq (C) and H3K27ac (D) datasets. Most peaks are located within 50 kb of a
 1086 TSS, supporting their annotation as promoters or enhancers.

1087 Figure 2. Integrative analysis of chromatin accessibility, histone modifications, and gene
 1088 expression following poly I:C stimulation in Atlantic salmon. (A) Genome browser tracks
 1089 showing ATAC-seq, RNA-seq, and gene annotation at the *elf1a* locus in PBS (control) and
 1090 poly I:C–stimulated samples. The housekeeping gene *elf1a* is shown as a representative
 1091 locus to illustrate the data quality and consistency across assays. (B) Genome browser
 1092 tracks at the same locus showing H3K27ac ChIP-seq signal, indicating histone modification
 1093 in both control and poly I:C stimulated samples. (C) Gene Ontology (GO) biological process
 1094 enrichment of genes associated with upregulated ATAC-seq peaks. Dot size reflects the
 1095 number of observed regions; colour scale indicates adjusted *P*-values. (D) GO biological
 1096 process enrichment of genes associated with upregulated H3K27ac peaks, displayed as in
 1097 (C). For GO analysis following filtrations were carried out: fold enrichment > 5, observed hits
 1098 ≥ 10 and *p* adjusted < 0.05. (E) Volcano plot of differentially accessible ATAC-seq regions
 1099 between PBS and poly I:C. Red points indicate significantly upregulated regions, green
 1100 points indicate downregulated regions, black points are not significant. (F) Volcano plot
 1101 of differentially enriched H3K27ac-marked regions. (G) Volcano plot of differentially enriched
 1102 H3K27me3-marked regions.

1103 Figure 3. Comparative analysis of the ATAC, ChIP, and RNA-seq data following poly I:C
 1104 stimulated Atlantic salmon. (A) Venn diagram illustrating the overlap of upregulated genes
 1105 among the different assays. (B) Gene Ontology analysis of the upregulated genes shared
 1106 between ATAC, RNA-seq, and ChIP H3K27ac, providing insights into the biological
 1107 processes associated with these genes and their involvement in the antiviral response. (D)
 1108 Genes with significant ATAC and H3K27ac presence at regulatory regions but were not
 1109 significantly expressed at transcript level. (E) Genes which were upregulated at transcript
 1110 level however their regulatory regions were not significantly activated. (F) Genes which were
 1111 significantly expressed and had significant H3K27ac peaks. GO analysis was performed
 1112 using DAVID, with stringent filtering criteria of FDR < 0.05 and fold enrichment > 2.

1113 Figure 4. TFBS analysis of key set of antiviral genes. (A) TFBS identified in a subset of 197
 1114 genes that are common among ATAC-seq, H3K27ac ChIP-seq, and RNA-seq datasets.
 1115 Distribution of TFBS showing that over 20% of the genes contain IRF8 binding sites; the
 1116 accompanying table 2 lists the gene IDs associated with these IRF8 binding sites. (B)
 1117 Sequence logo representing the binding motif of IRF8, highlighting the conserved nucleotide
 1118 positions crucial for IRF8 binding specificity. Transcription factor binding sites were identified
 1119 using GimmeMotifs from the enriched regions in ATAC-seq and H3K27ac, corresponding to
 1120 active enhancers and promoters; the JASPER database was used as background.

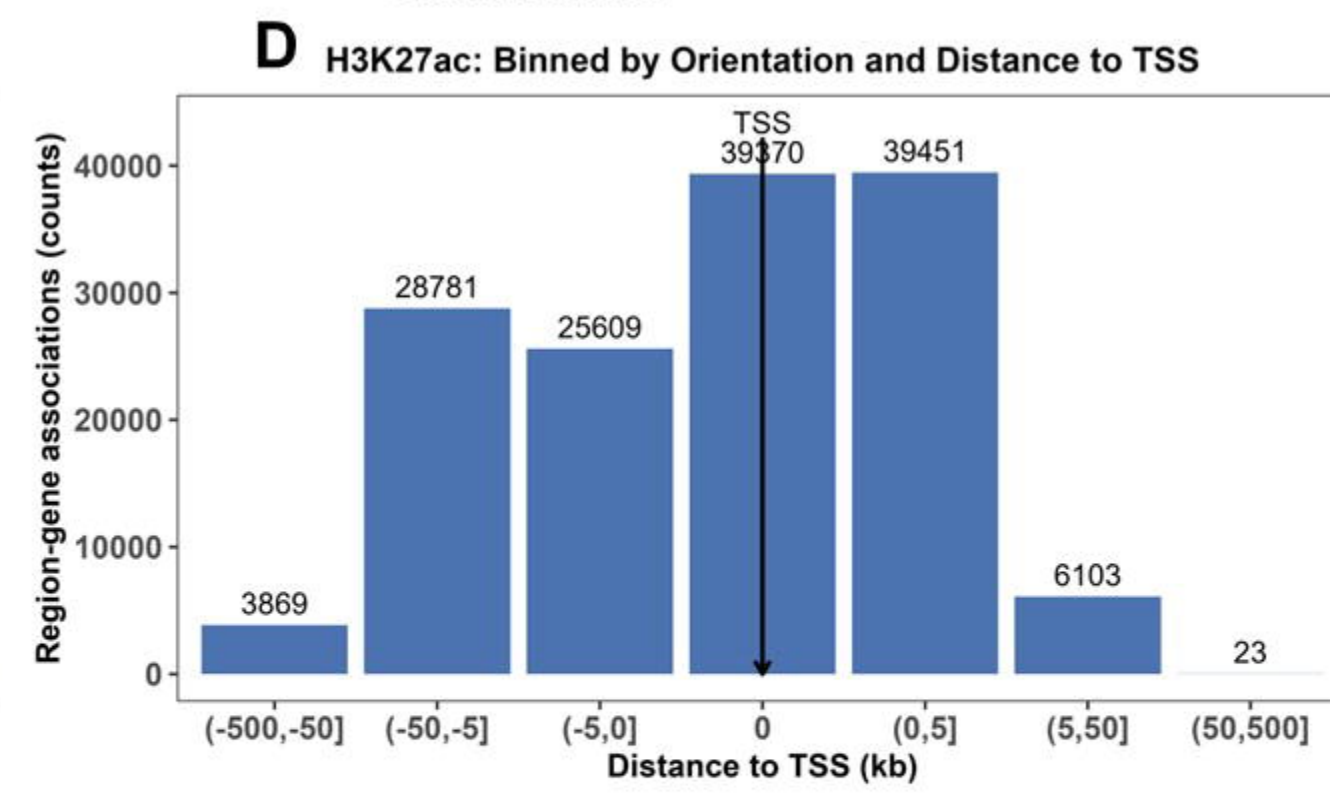
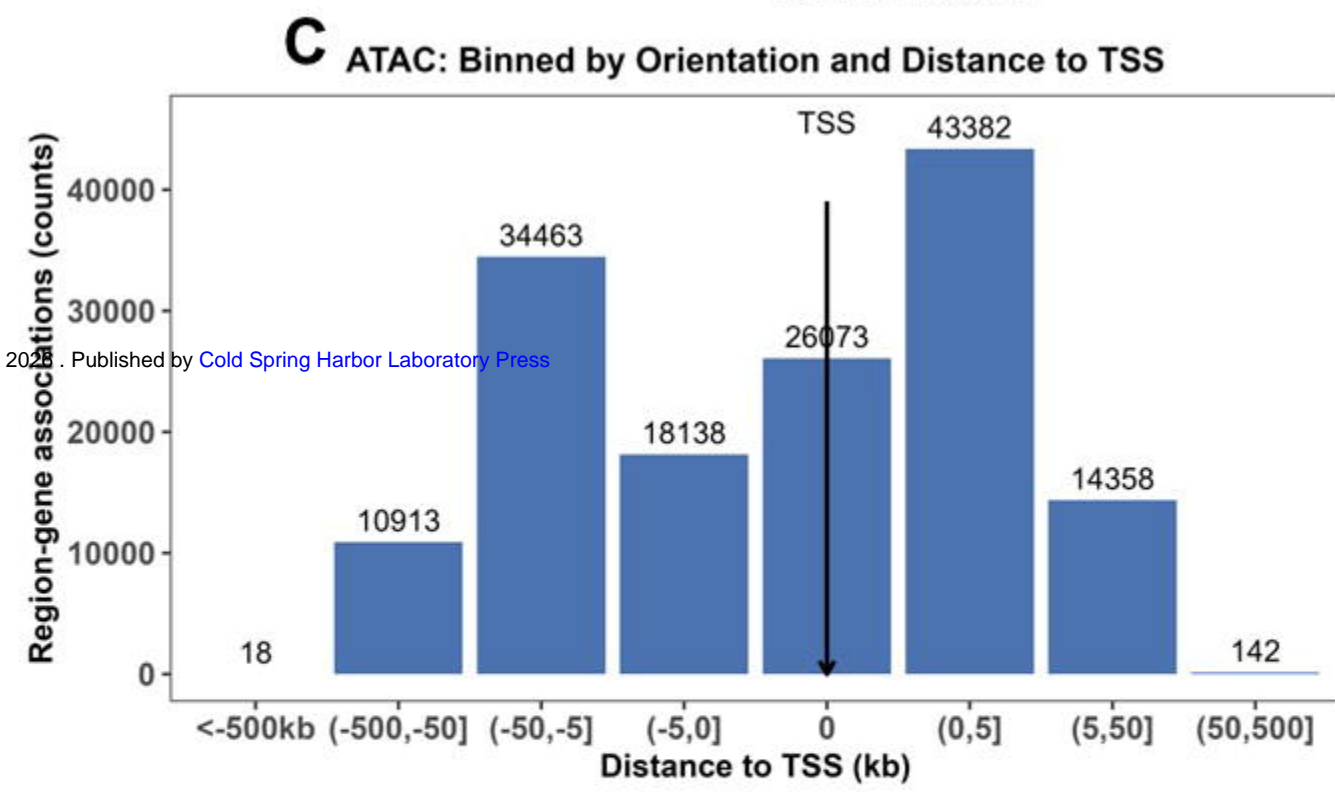
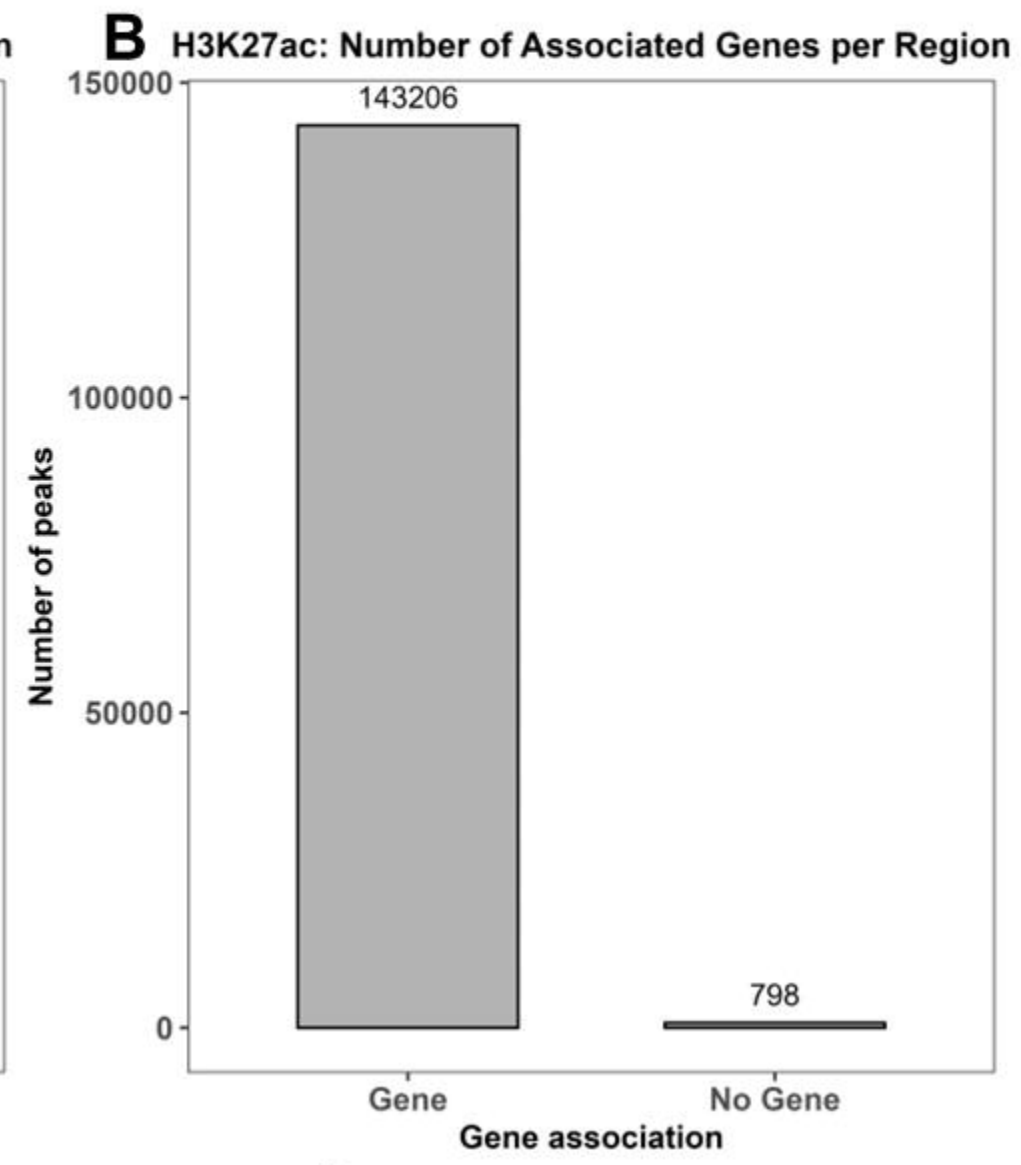
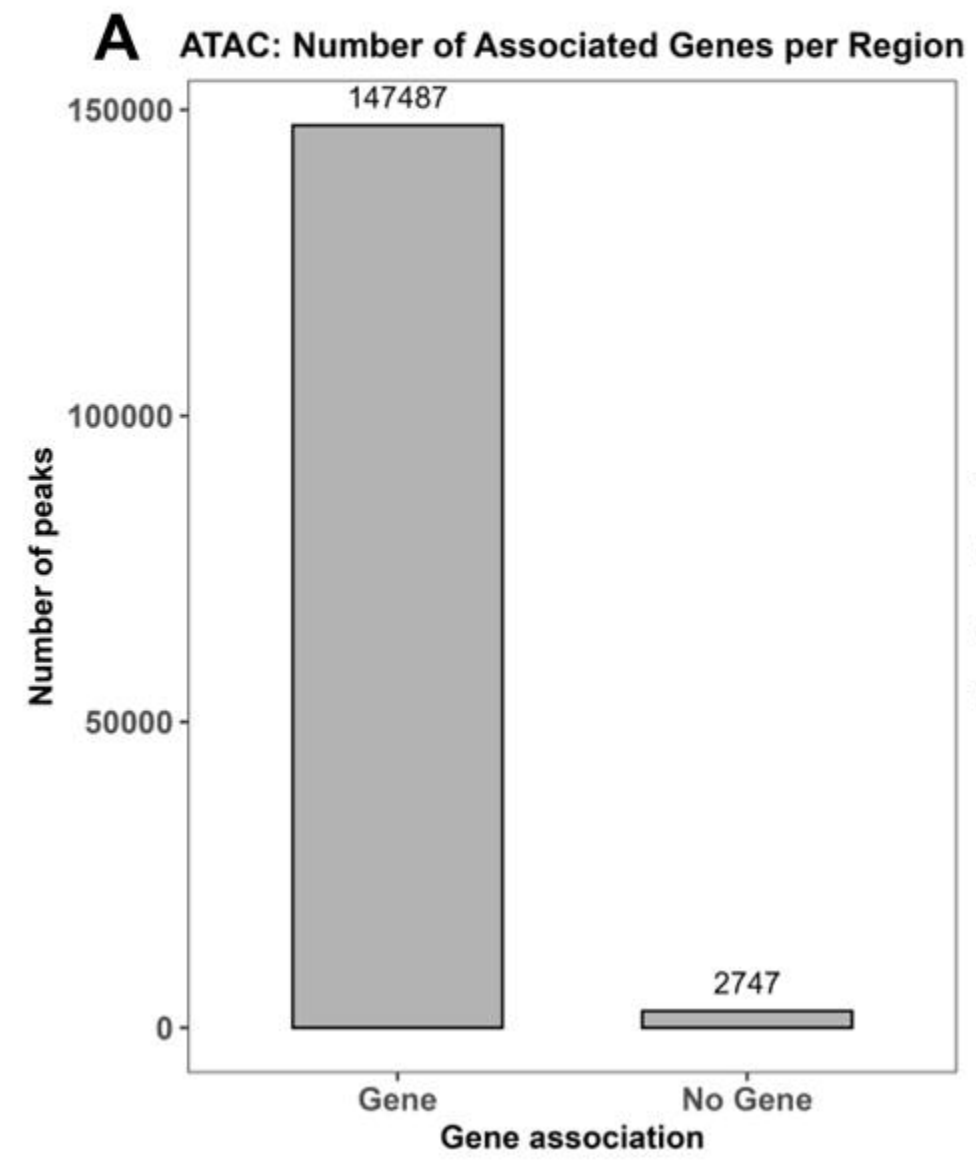
1121 Figure 5. Transcription factor binding analysis. A, B, and C present the results of
 1122 ChromHMM chromatin state discovery using ATAC-seq and two ChIP marks (H3K27ac and
 1123 H3K27me3). (A) The five states predicted by the model are shown and annotated based on
 1124 existing literature. (B) The genomic location of the segments in each state is depicted for the
 1125 PBS control samples. (C) The genomic location of the segments in each state is depicted for
 1126 the poly I:C stimulated samples. (D) Transcription factor binding sites were identified using
 1127 GimmeMotifs from the enriched regions in state 5, corresponding to active enhancers and

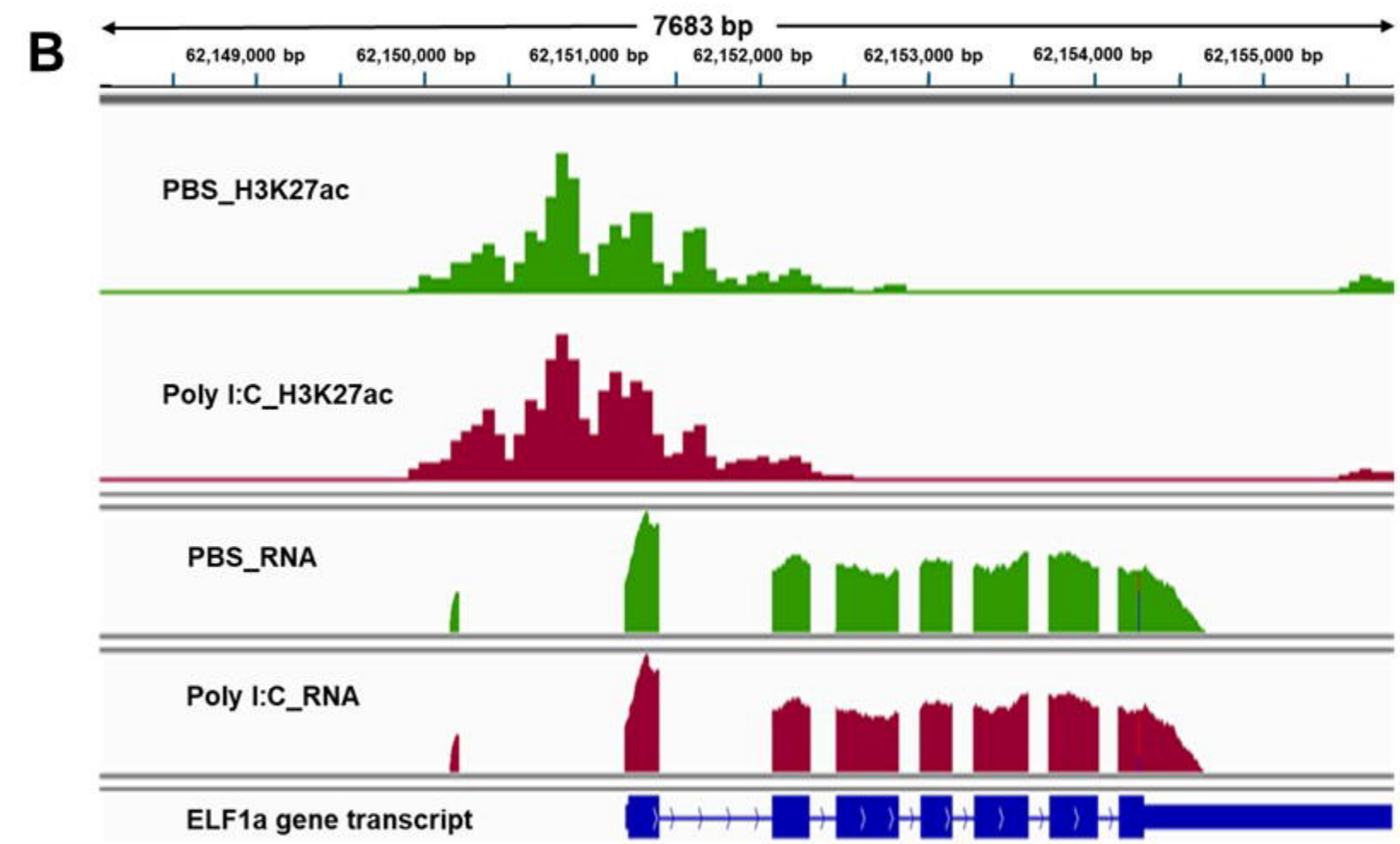
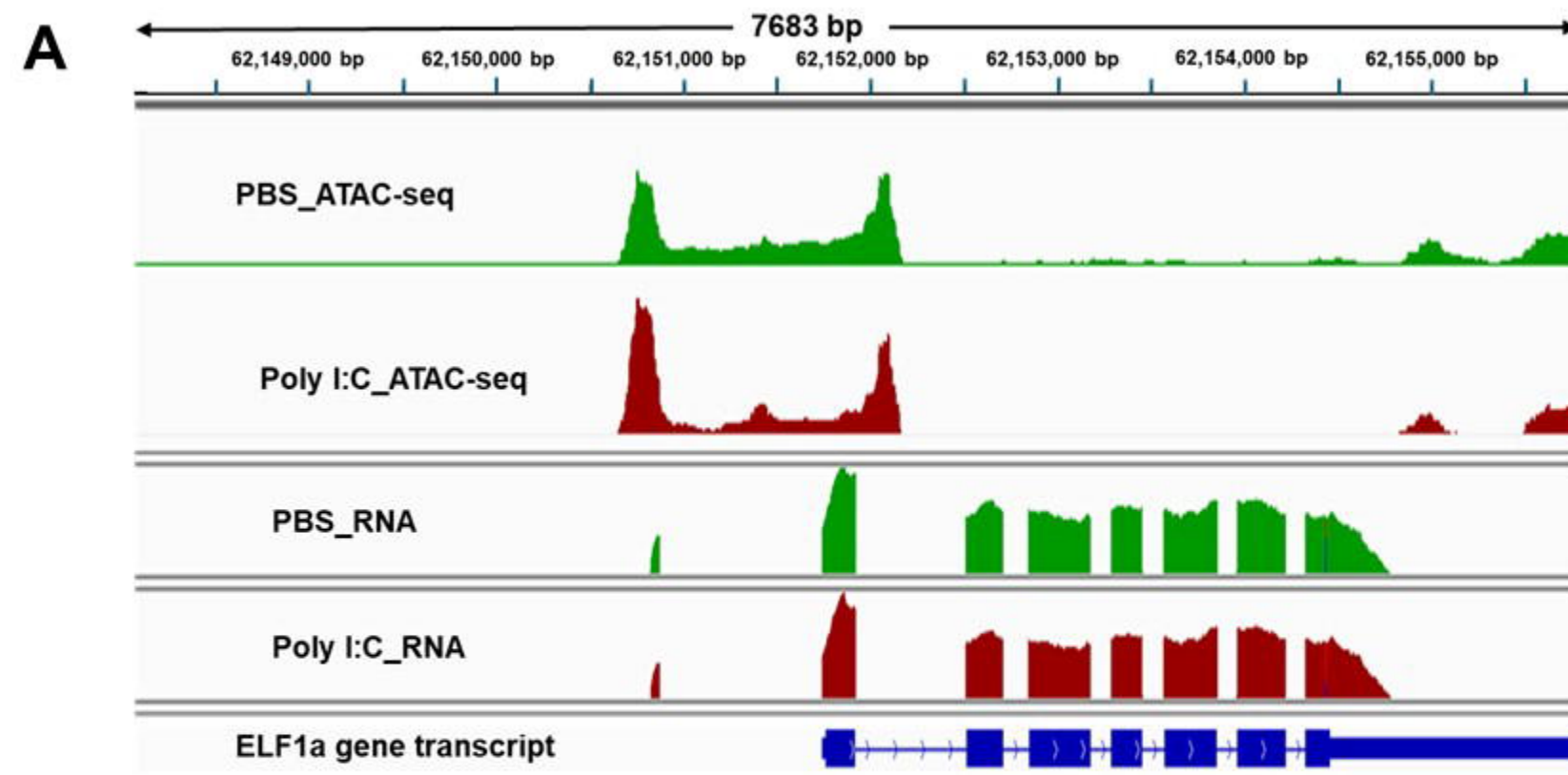
1128 promoters; the JASPER database was used as background. These transcription factors
1129 have binding sites enriched in ATAC and H3K27ac, however, GimmeMotif also predicted
1130 binding sites of transcription factors enriched from our dataset. For the list of predicted
1131 transcription factors, refer to supplementary table 7.

1132 Figure 6. Anti-viral IFN response processes. (A) A schematic view illustrating the type I IFN
1133 signalling pathway, encompassing both primary and secondary responses. (B) A table
1134 displaying the genes involved in the RIG-I and JAK-STAT pathways, along with their fold
1135 change values from the differential gene expression analysis. The colour coding indicates
1136 upregulation (red), downregulation (green), and no response (grey). (C) and (D) IGV
1137 visualisations of the RIG-I and JAK-STAT pathway genes derived from the ATAC-seq, ChIP-
1138 seq, and RNA-seq datasets. The visualisations also include the enriched ISRE motifs
1139 associated with each gene. The direction of the gene transcription is indicated by the black
1140 arrow below the gene structure.

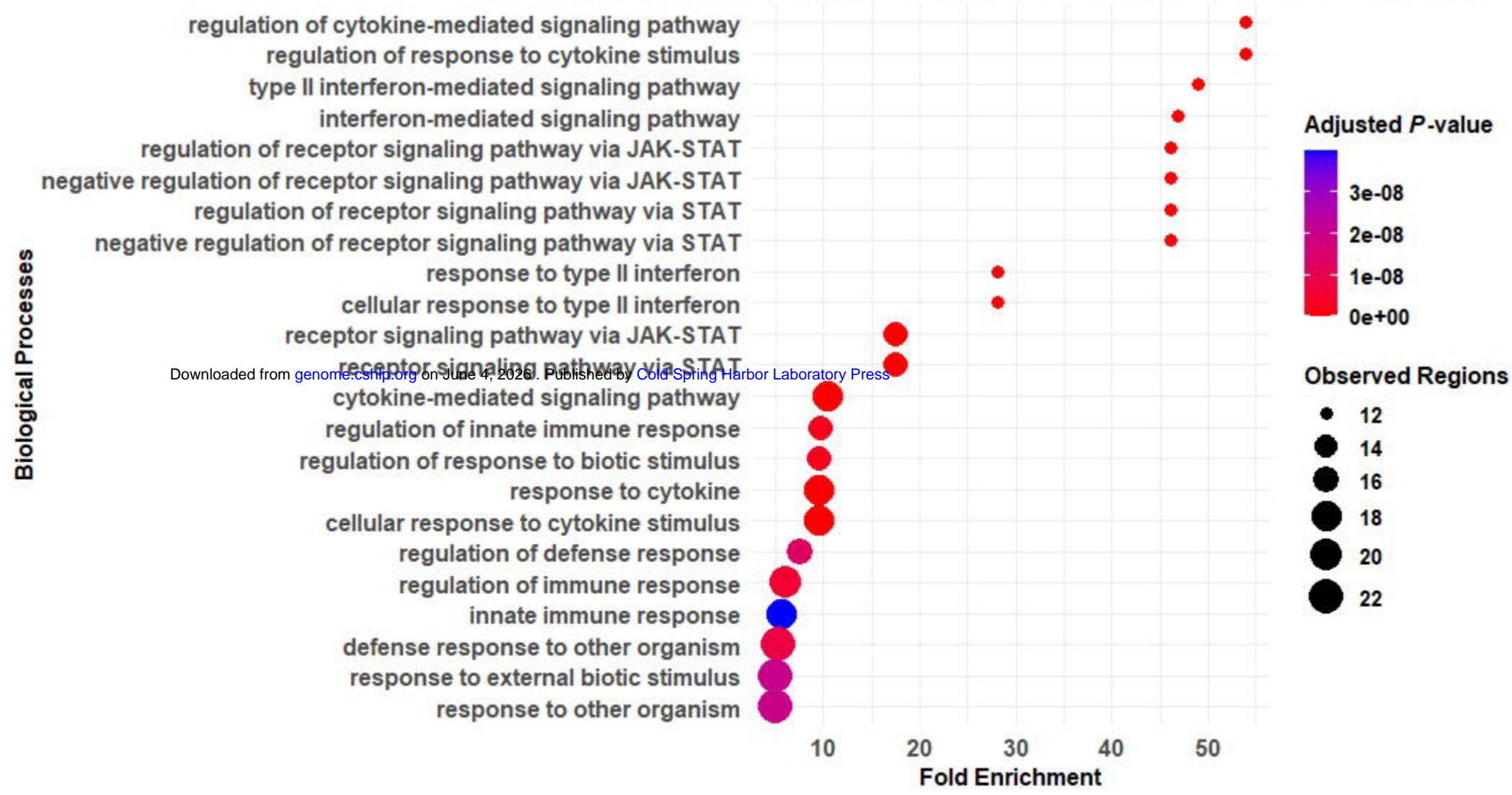
1141 Figure 7. Visualisation of key anti-viral response genes. (A) Shows the IGV visualisations of
1142 ATAC-seq, ChIP-seq, and RNA-seq data for the genes MX2, RSAD2, TAPBP, ISG15, and
1143 CD9. The visualisations illustrate the chromatin accessibility, histone modification profiles,
1144 and gene expression patterns associated with each gene. Enriched ISRE motifs specific to
1145 each gene are identified, indicating their regulatory importance in driving the anti-viral
1146 response. Arrows next to the transcripts indicate the direction of the sequence. (B) Presents
1147 a table summarising the gene expression data for MX2, RSAD2, TAPBP, ISG15, and CD9.
1148 Red indicates upregulation, green indicates downregulation, and grey indicates no response.
1149 The table provides a concise overview of the transcriptional changes exhibited by these key
1150 anti-viral response genes following stimulation with poly I:C.

1151

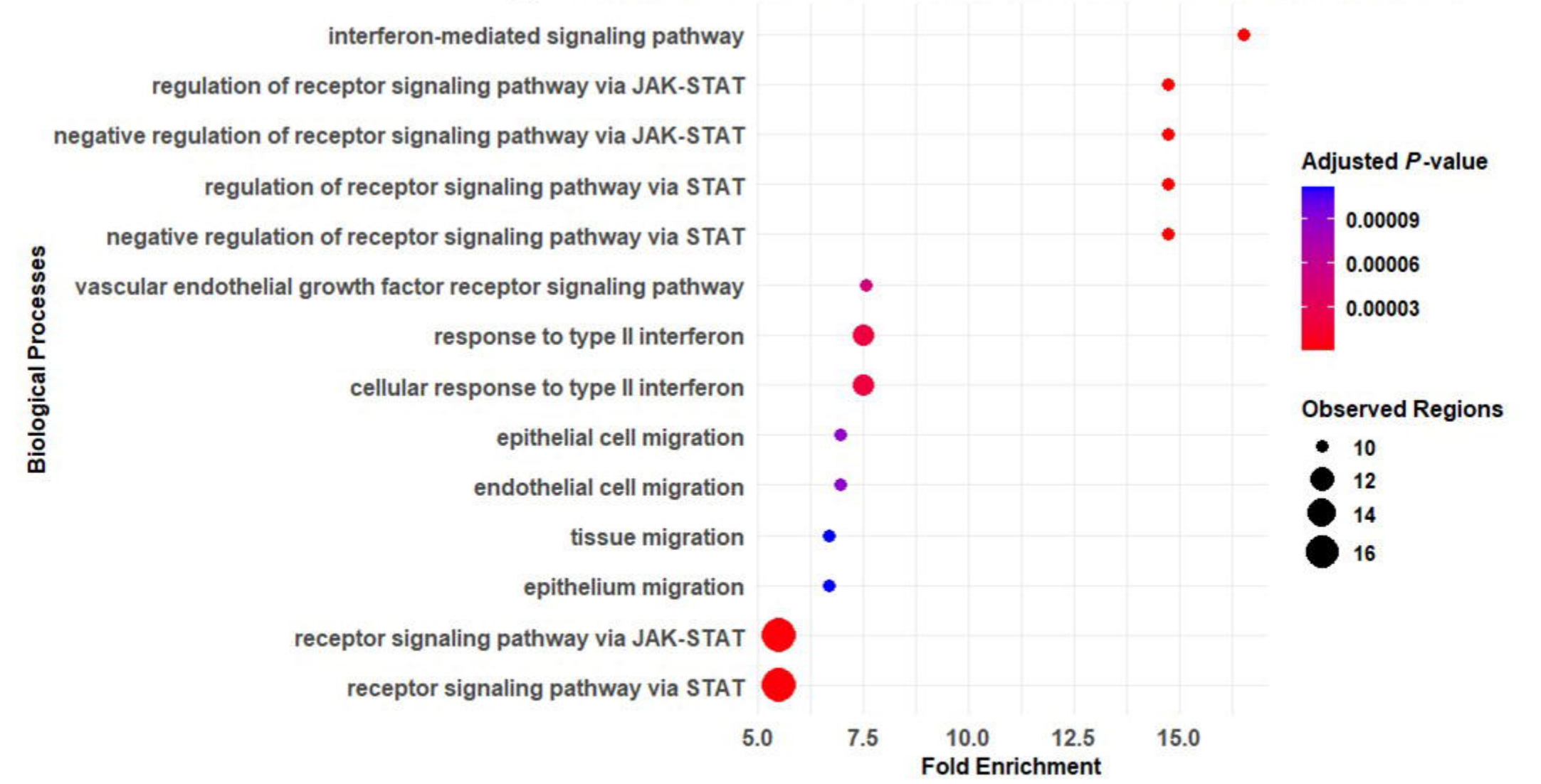




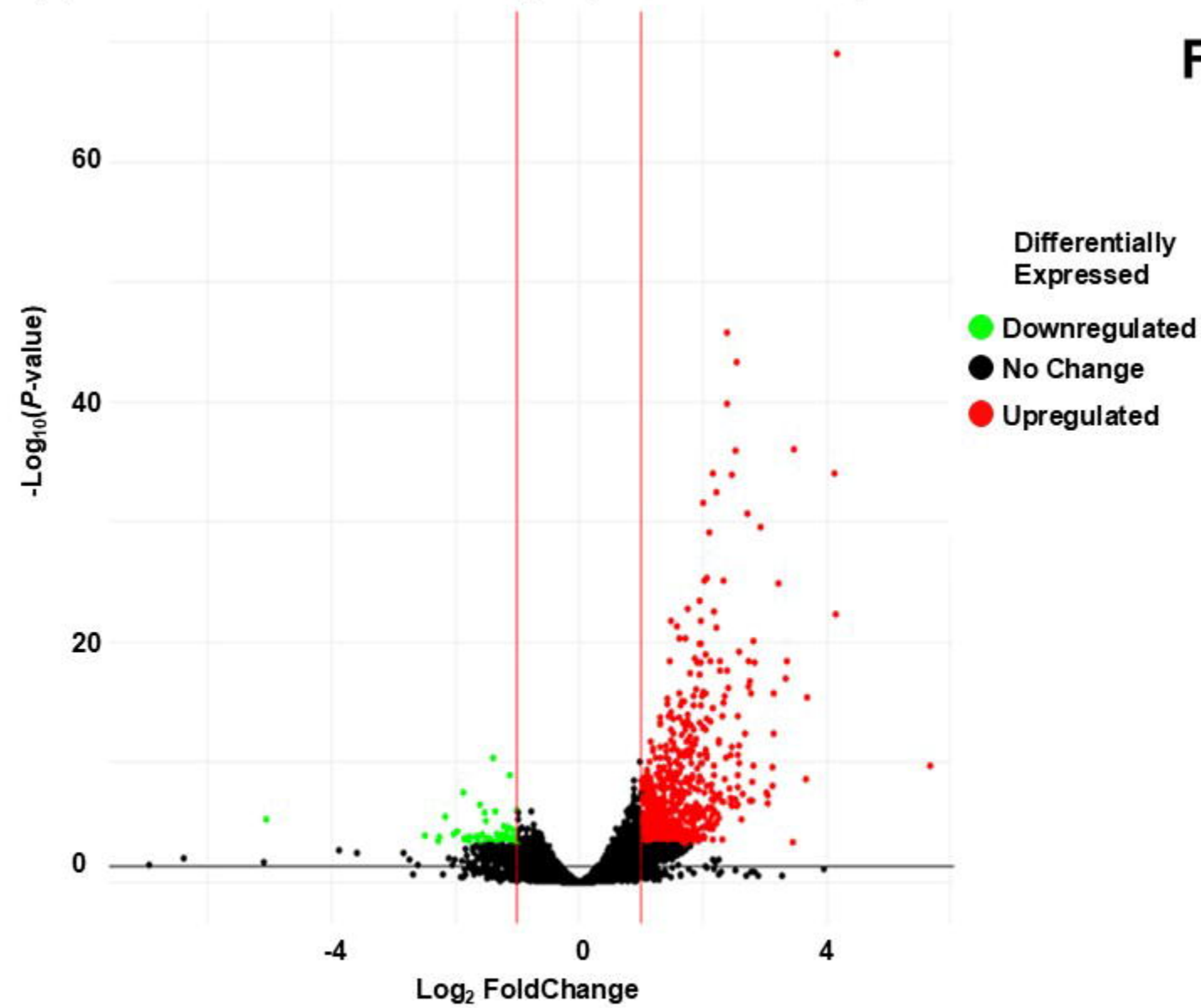
C GO:Biological Processes Enrichment of ATAC-seq Upregulated Peaks



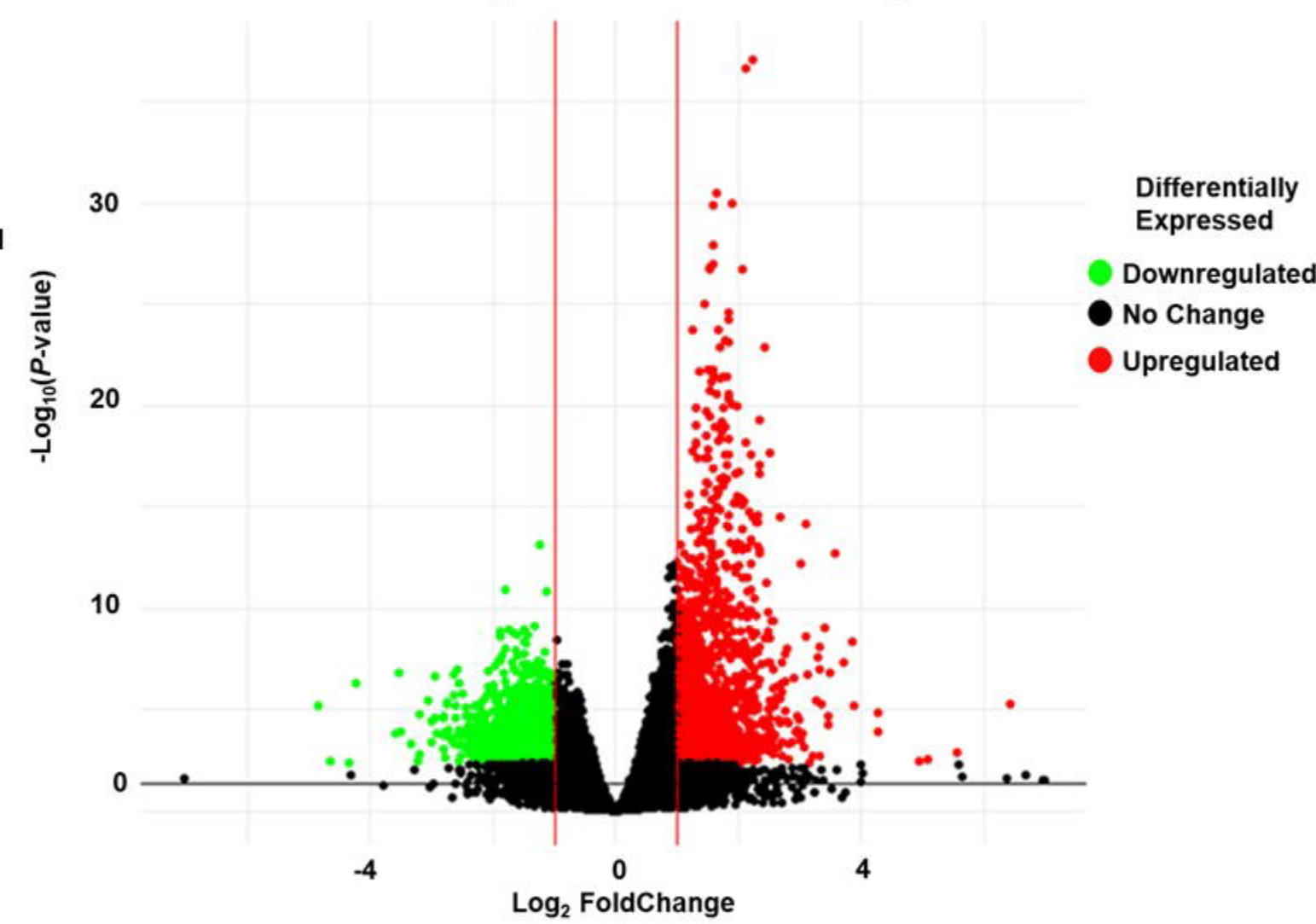
D GO:Biological Processes Enrichment of H3K27ac Upregulated Peaks



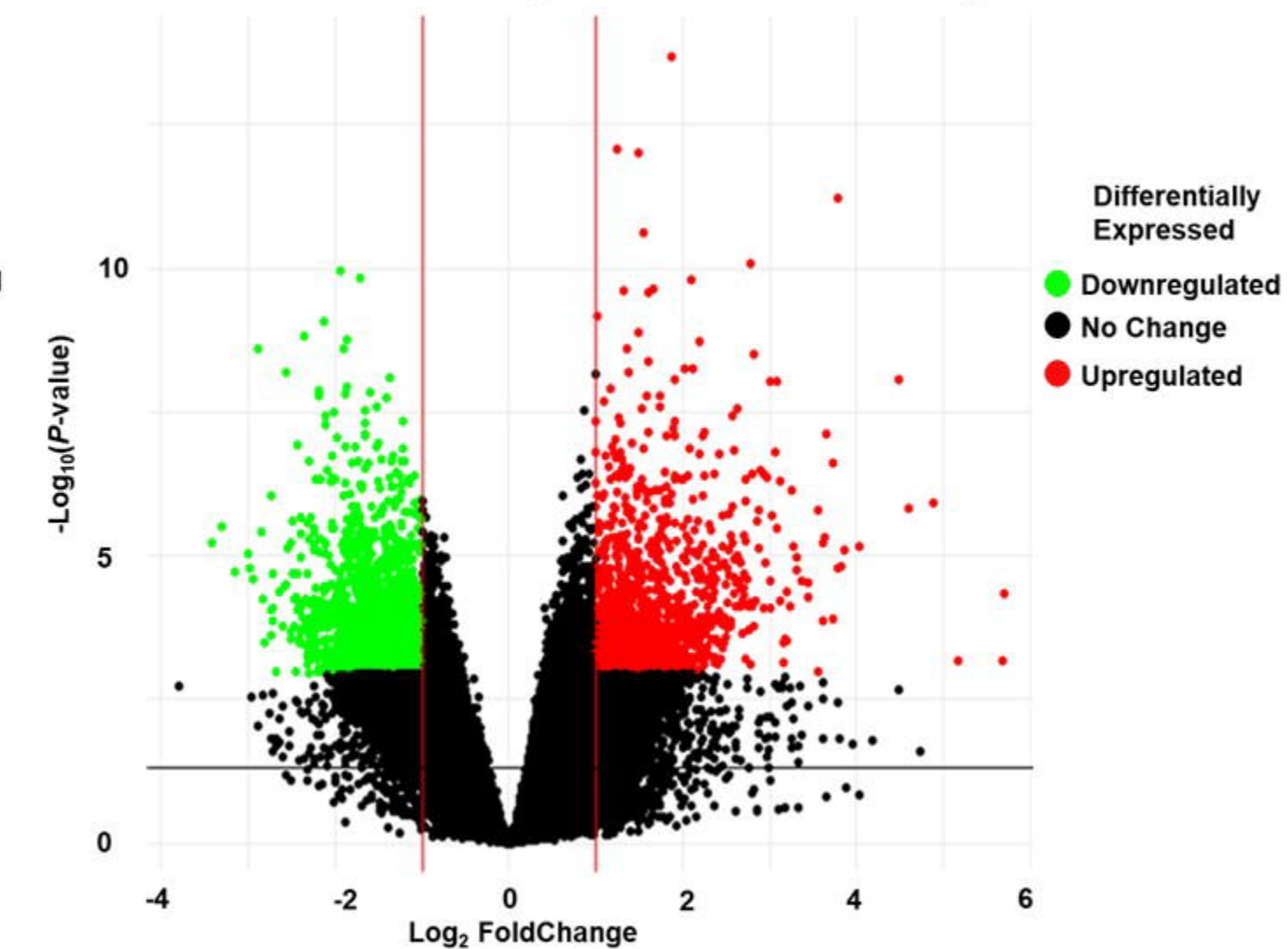
E Volcano Plot of Differentially Expressed ATAC Regions

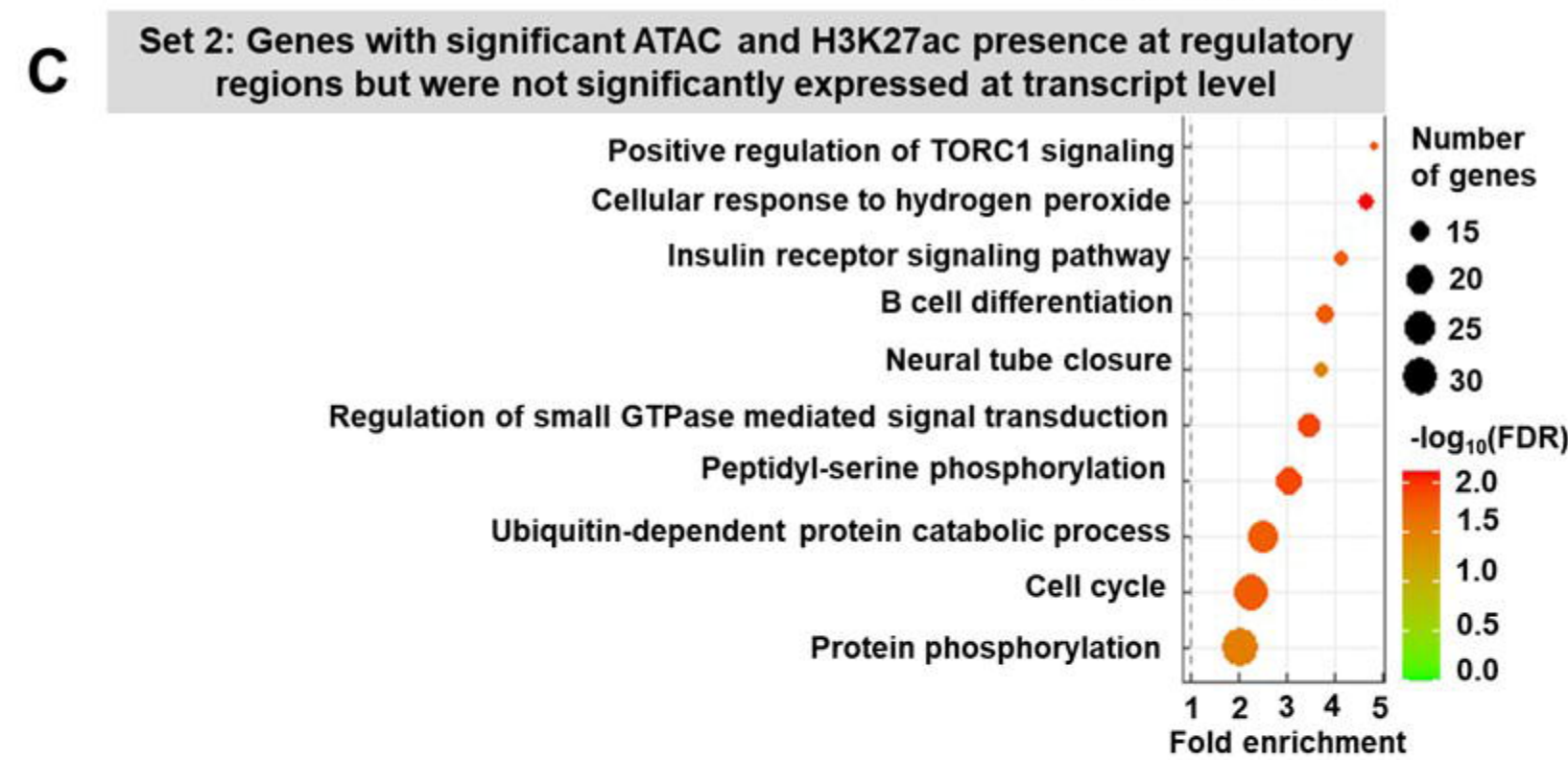
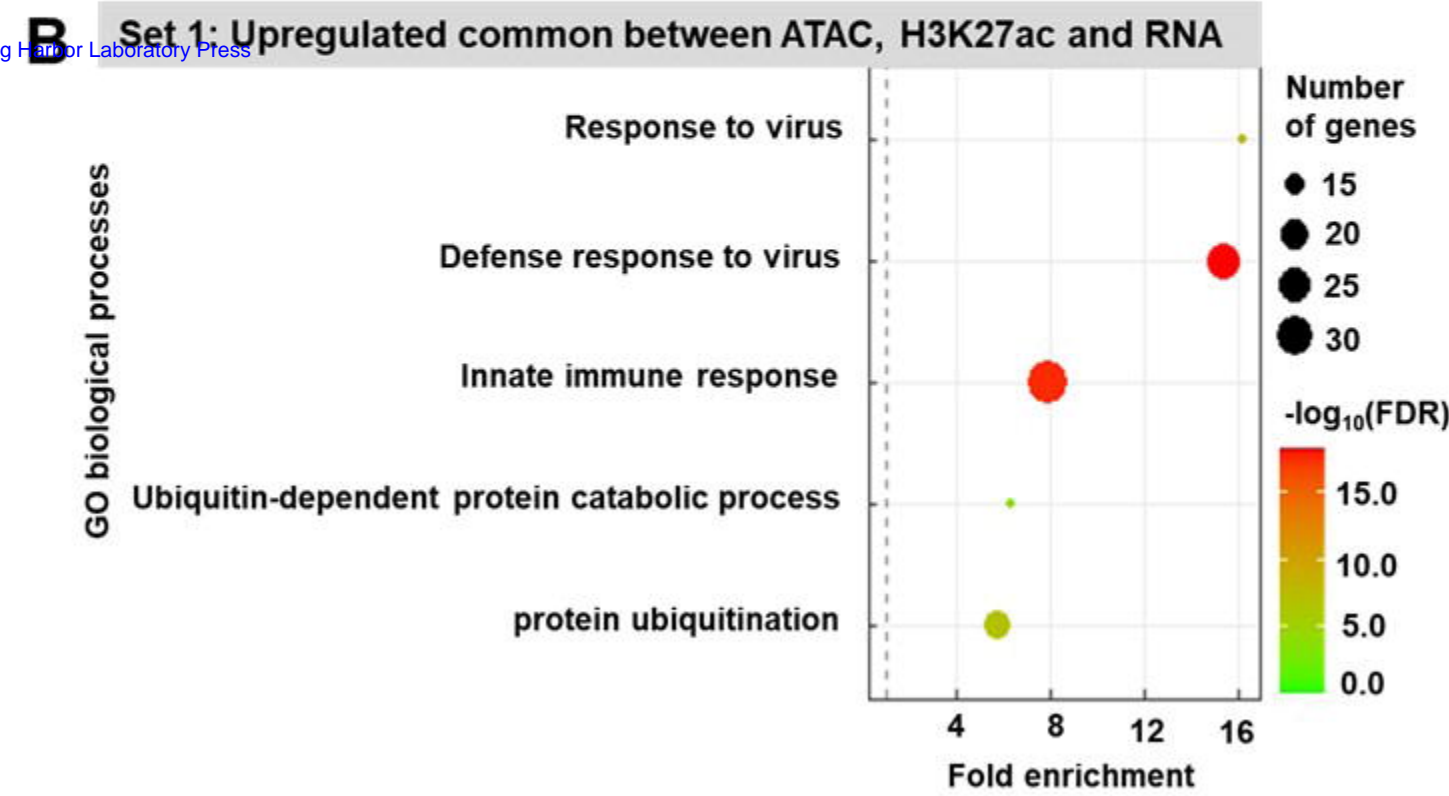
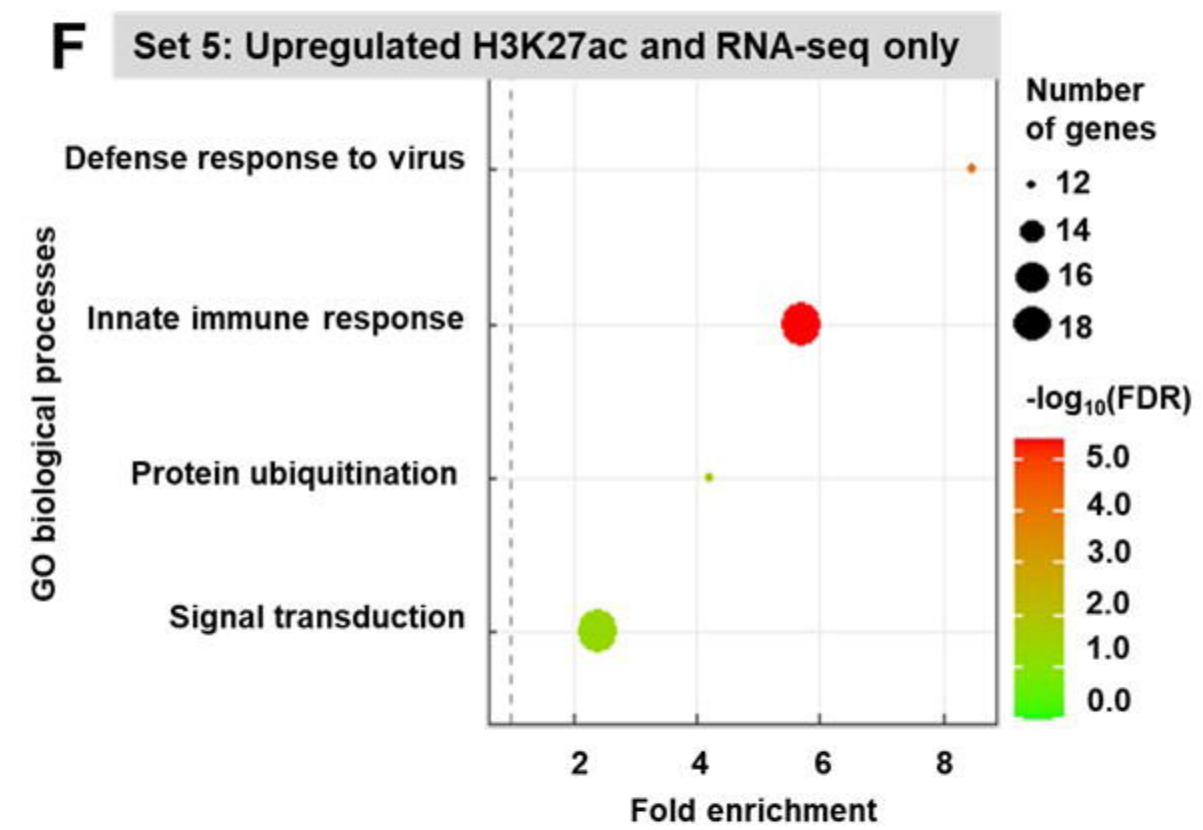
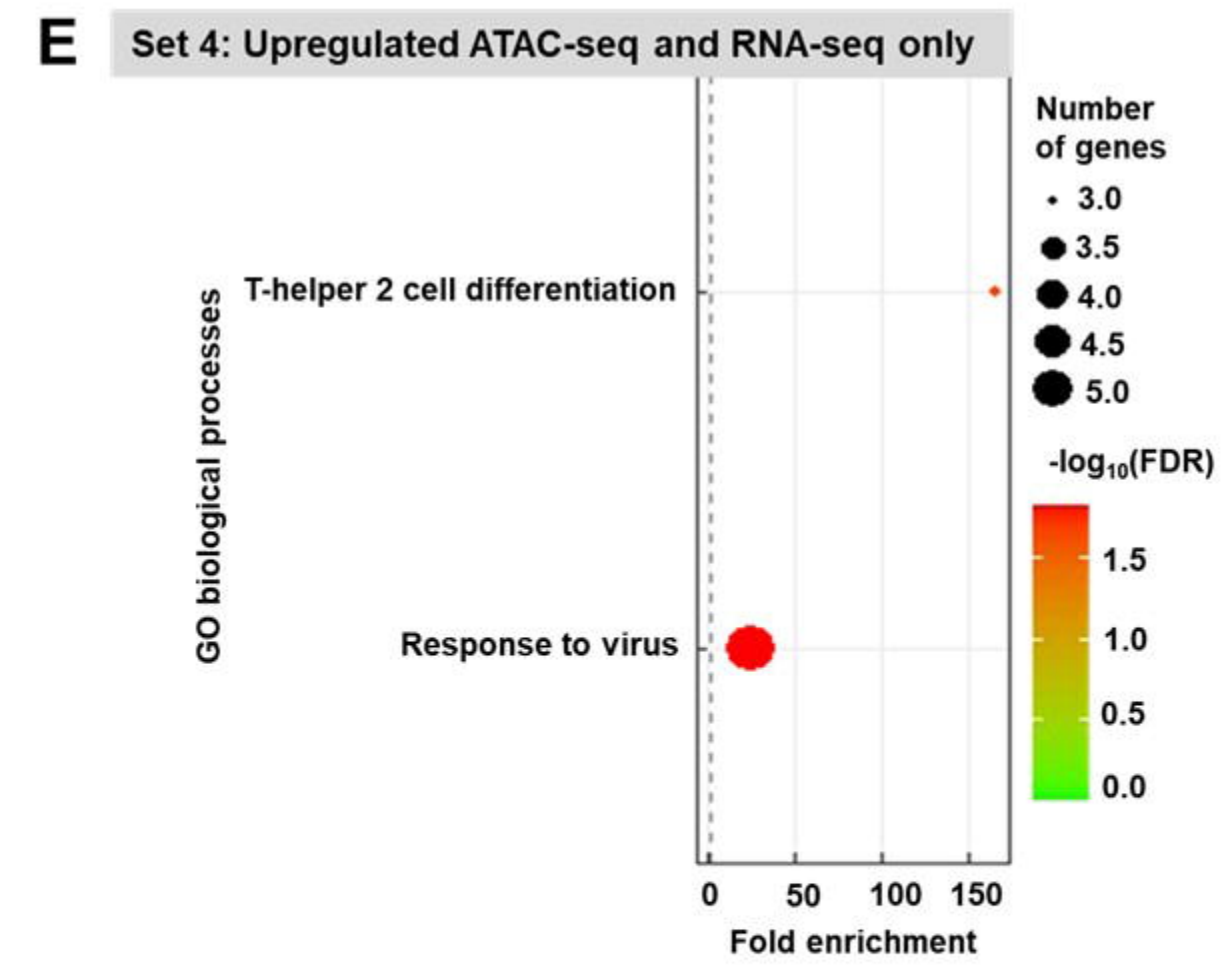
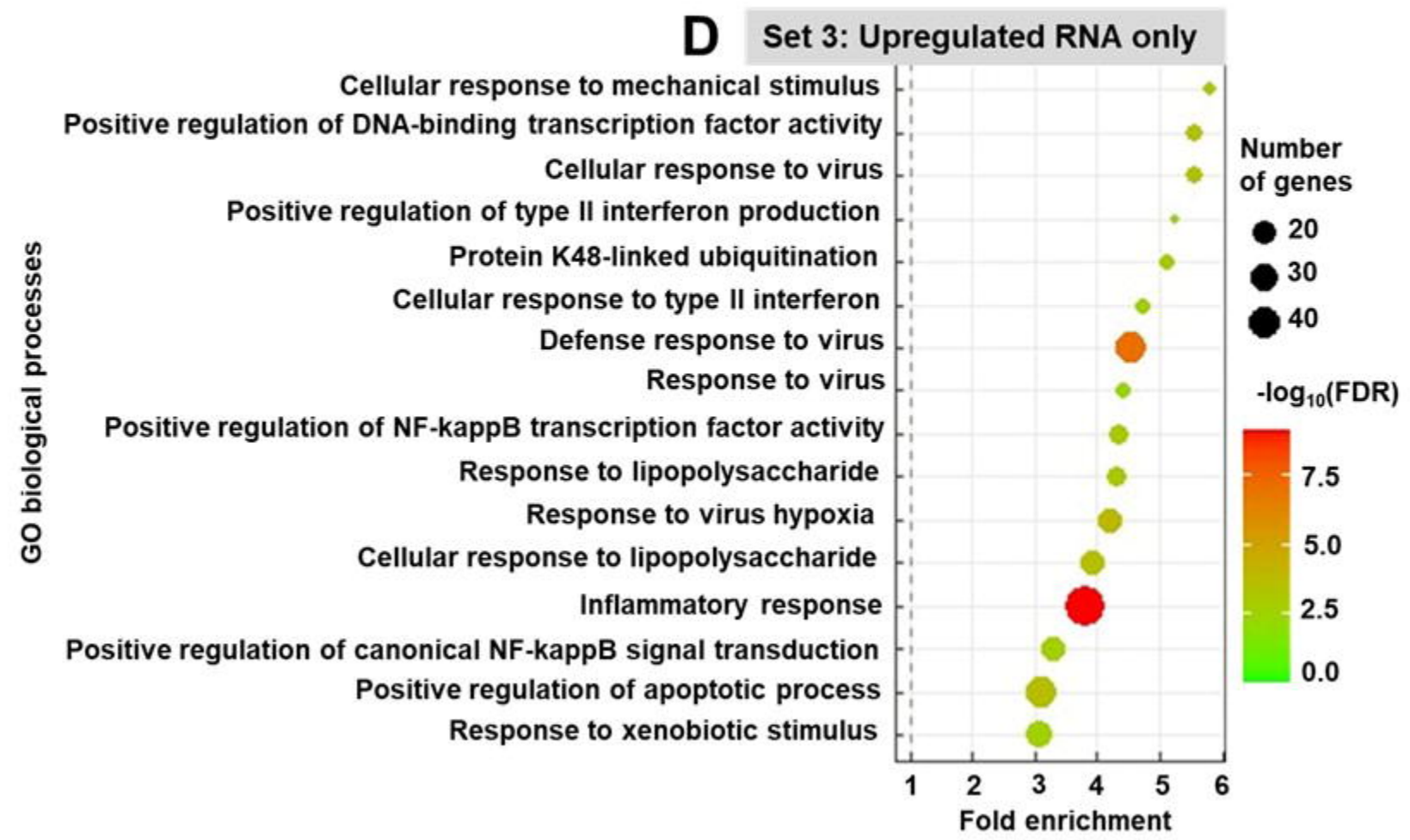
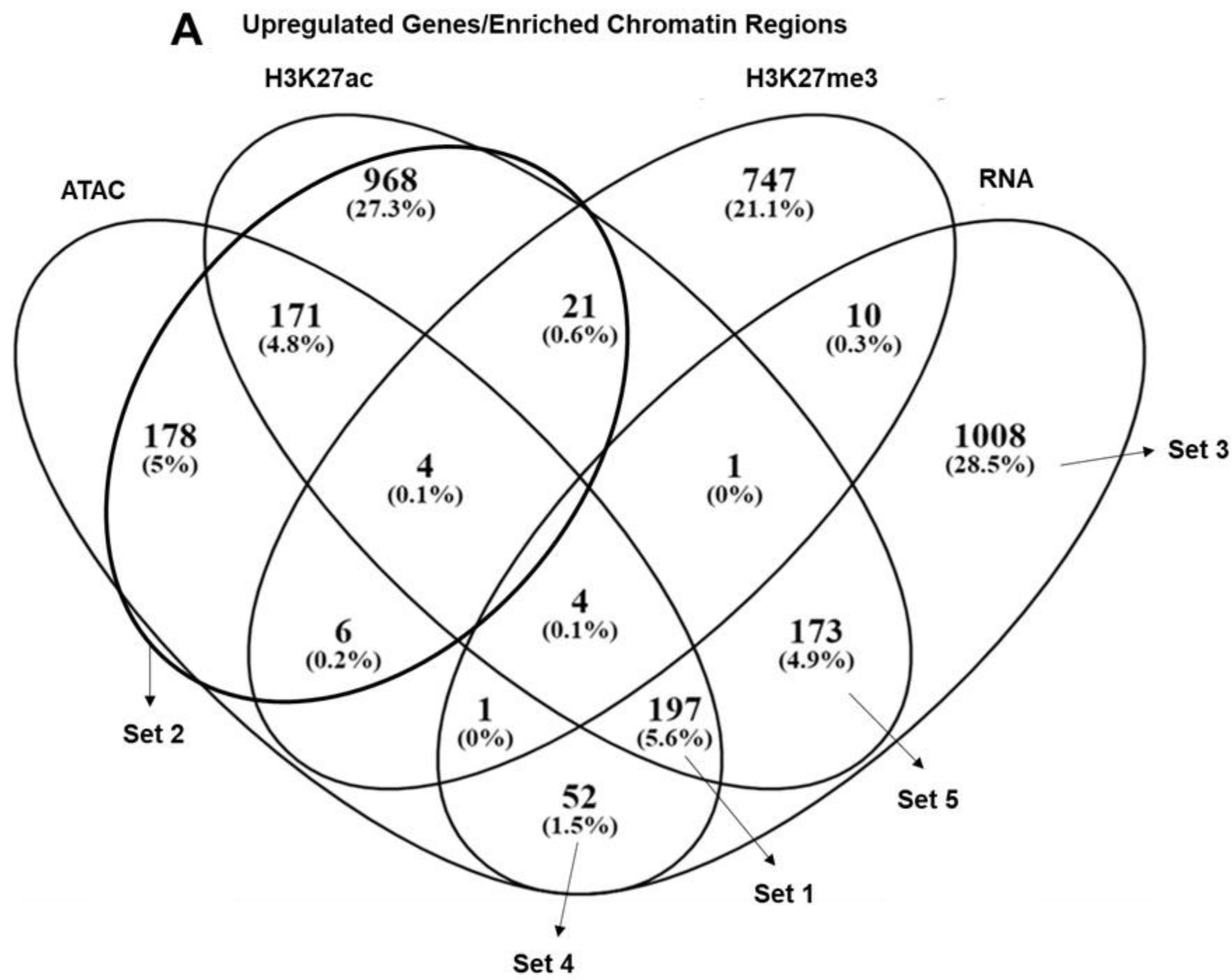


F Volcano Plot of Differentially abundance H3K27ac Regions

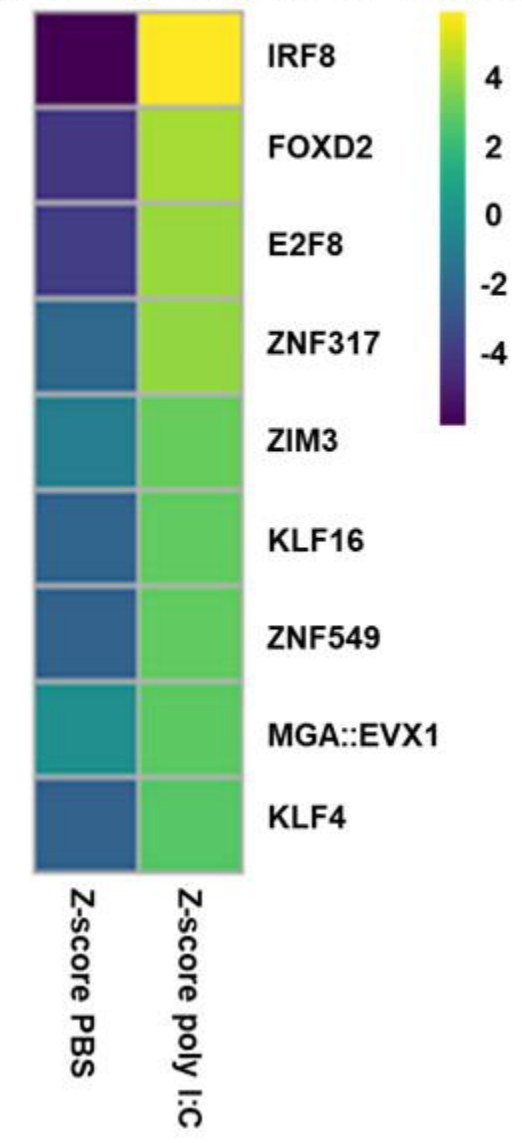


G Volcano Plot of Differentially abundance H3K27me3 Regions

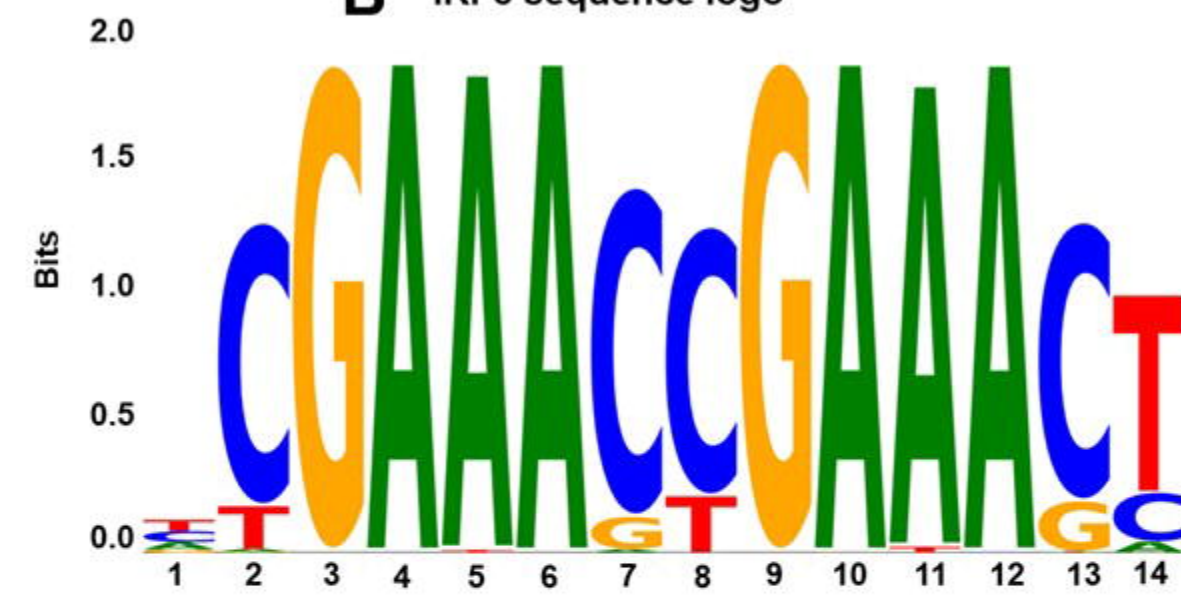


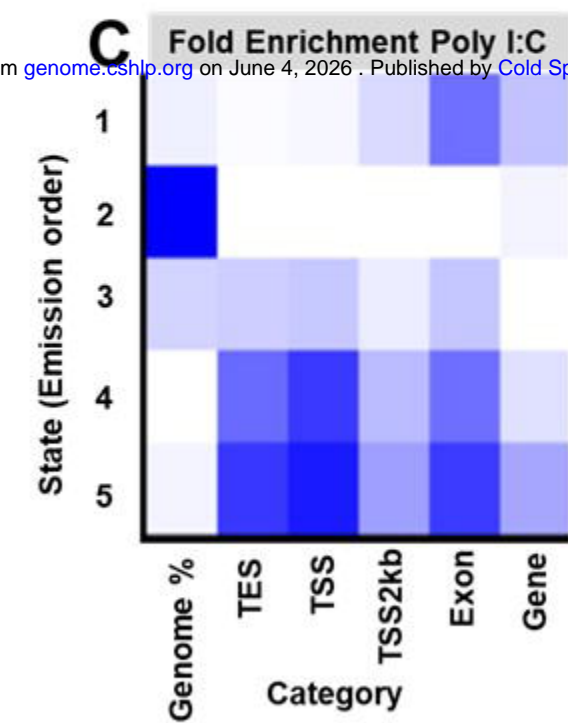
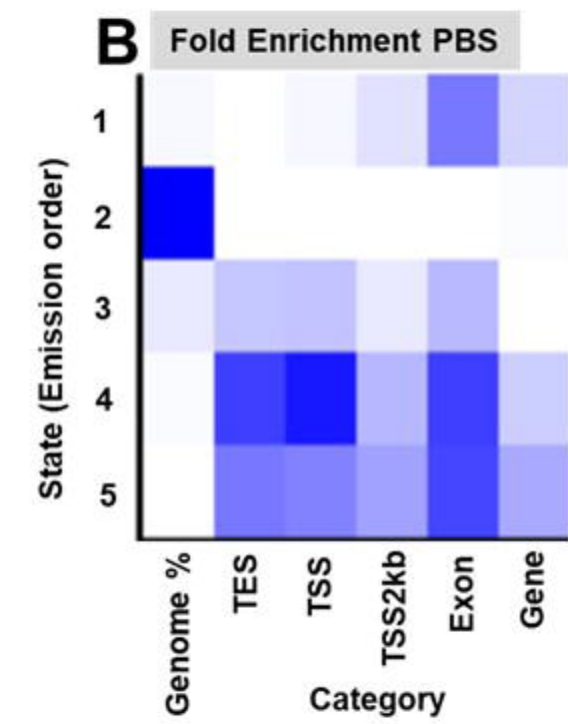
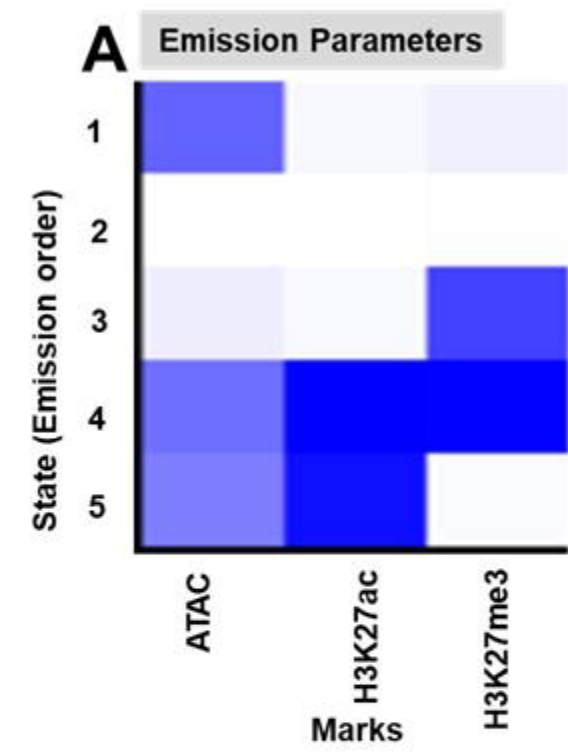


A Enriched TFBSs by common 197 active regions



B IRF8 sequence logo



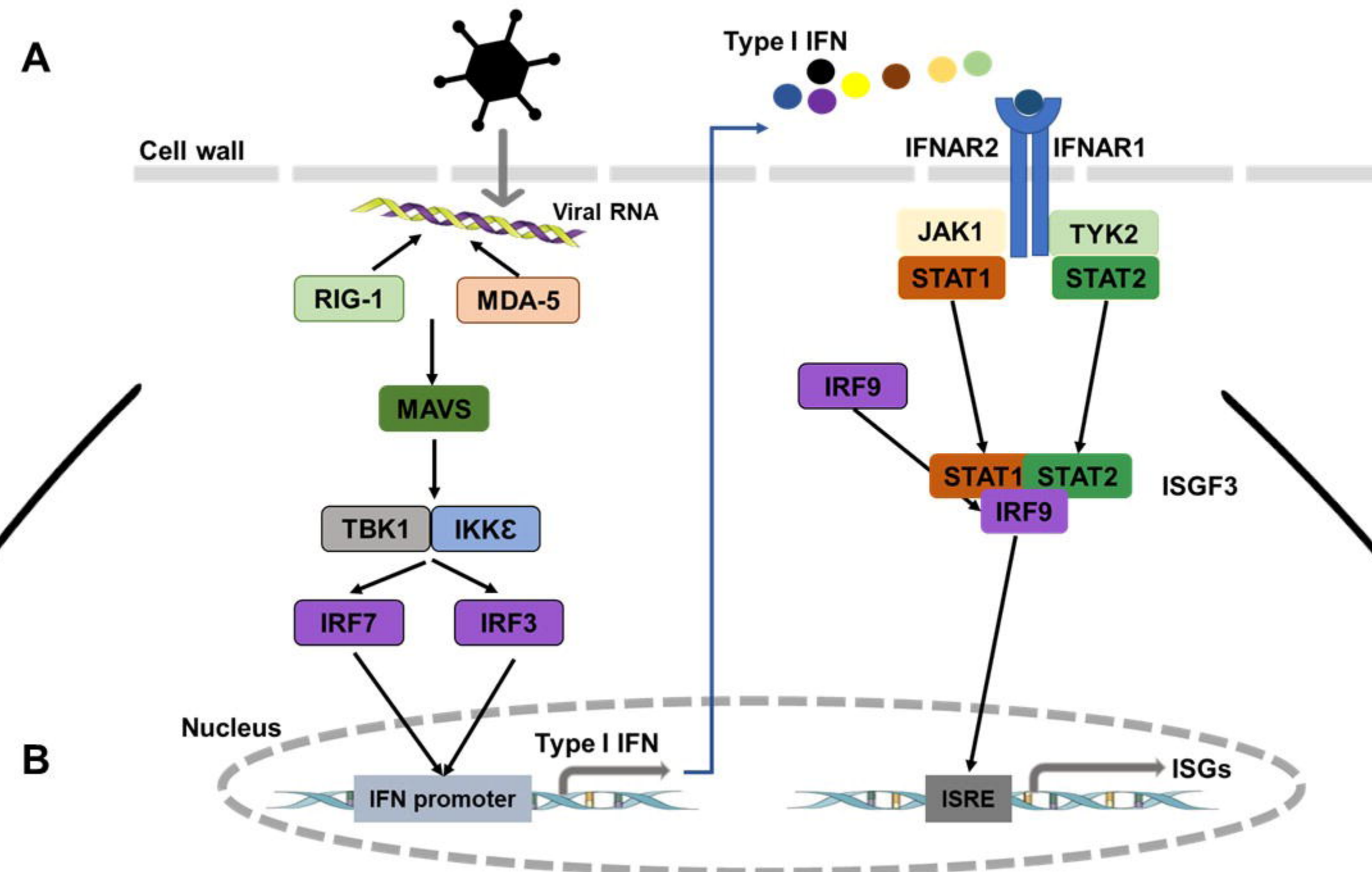


State Annotation

1. ATAC islands
2. Quiescent/Low
3. Repressed state
4. Poised promoter
5. Active enhancers and promoters

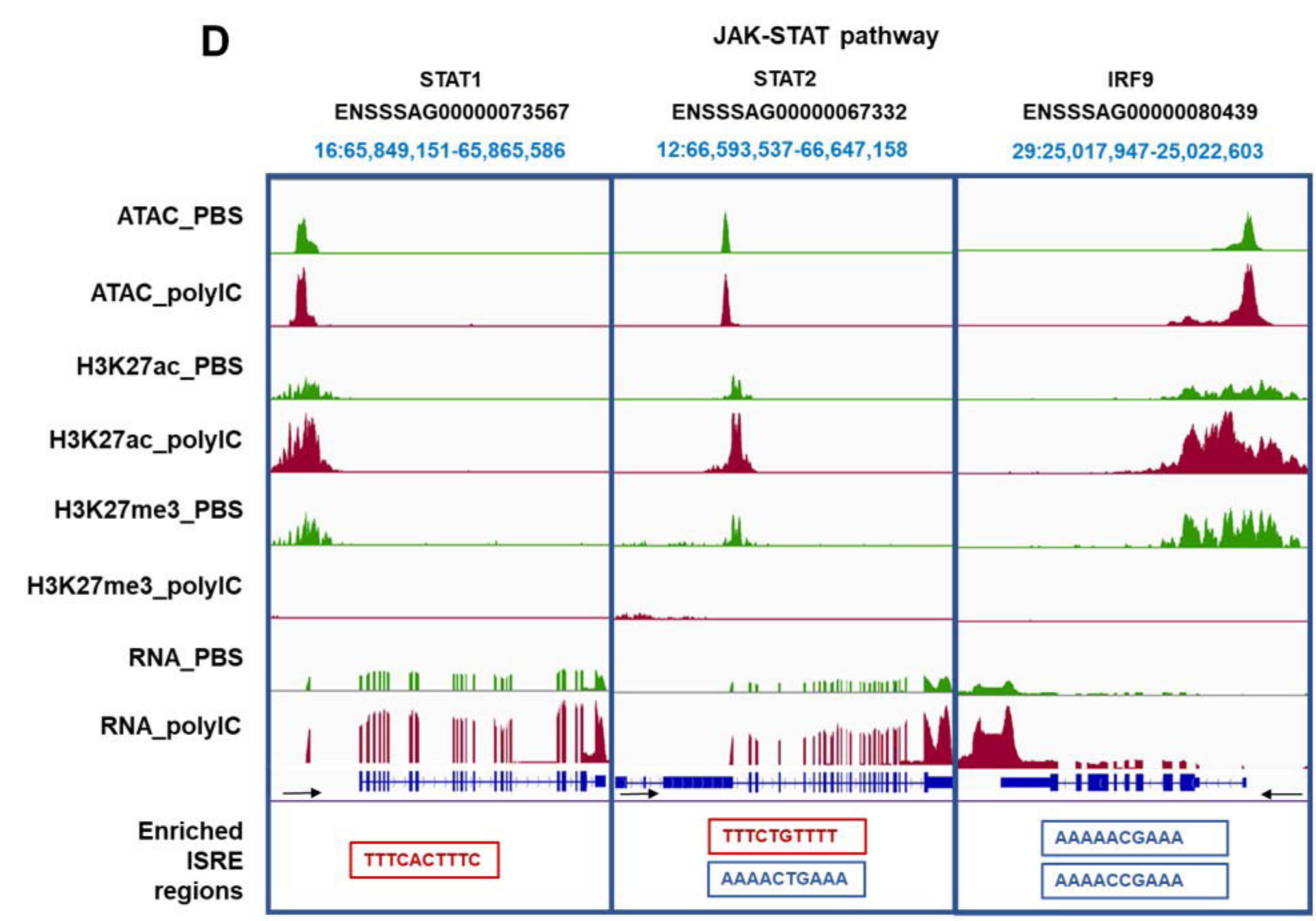
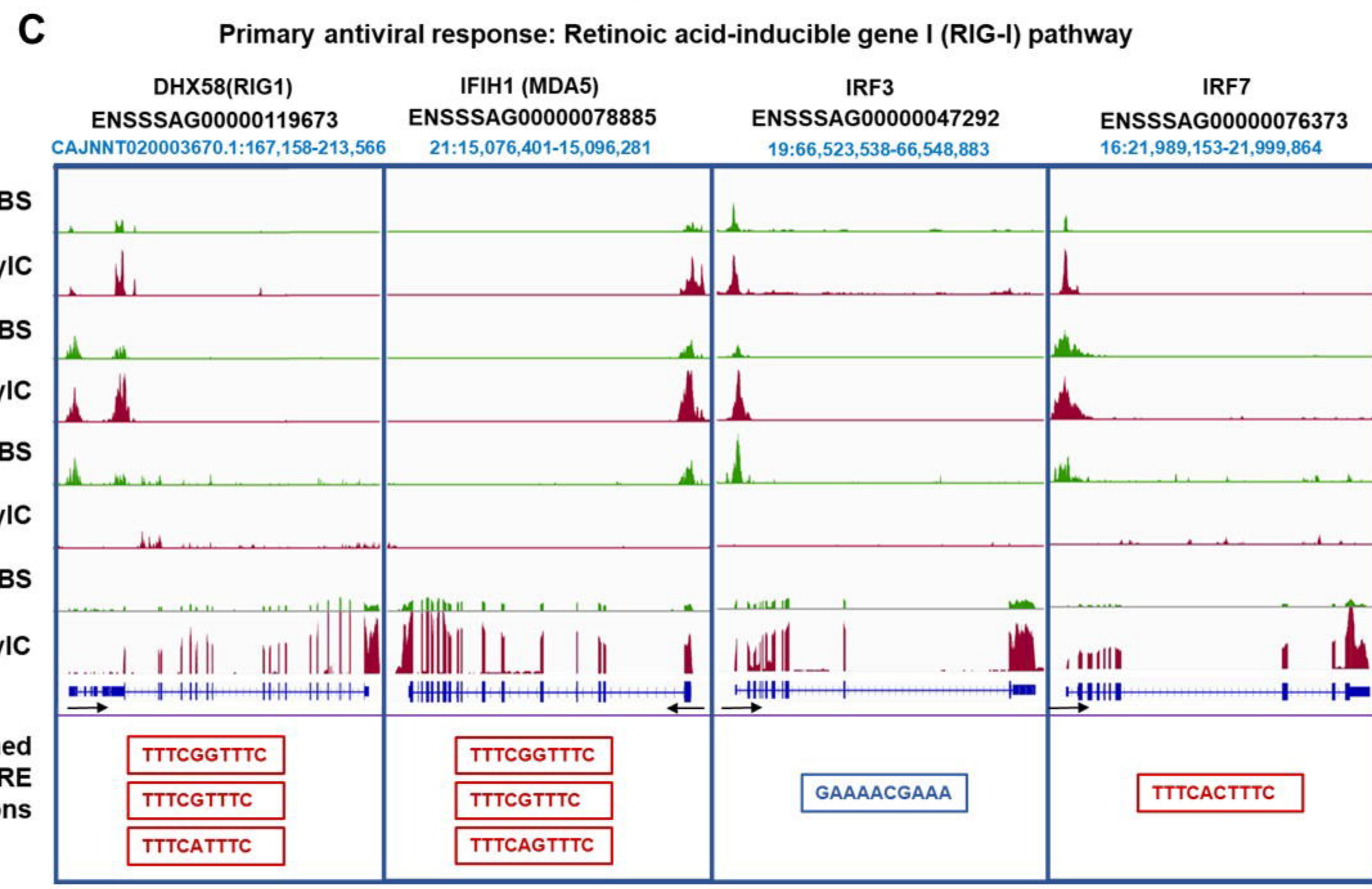
D TFBS identified using GimmeMotifs from the enriched regions in state 5, corresponding to active enhancers and promoters

Transcription factors	Z-score PBS	Z-score polyI:C	% with motif	Logo
STAT6	-2.43	4.56	2	
IRF9	-5.39	4.45	2	
PRDM1	-2.72	3.89	2	
IRF6	-3.18	3.6	2	
JDP2	-4.04	3.39	2	
Nr2e1	-1.99	3.09	<1	
BCL6	-3.25	2.66	<1	
GATA5	3.34	-1.9	2	
ONECUT2	3.56	-2.31	1	
POU1F1	3.32	-2.39	1	
KLF9	3.7	-2.68	2	
RFX5	3.34	-3.07	3	
TBX19	1.66	-3.08	2	
ZNF93	2.59	-3.17	2	
ZSCAN4	4.09	-3.38	2	
FOXF2	4.15	-4.27	1	



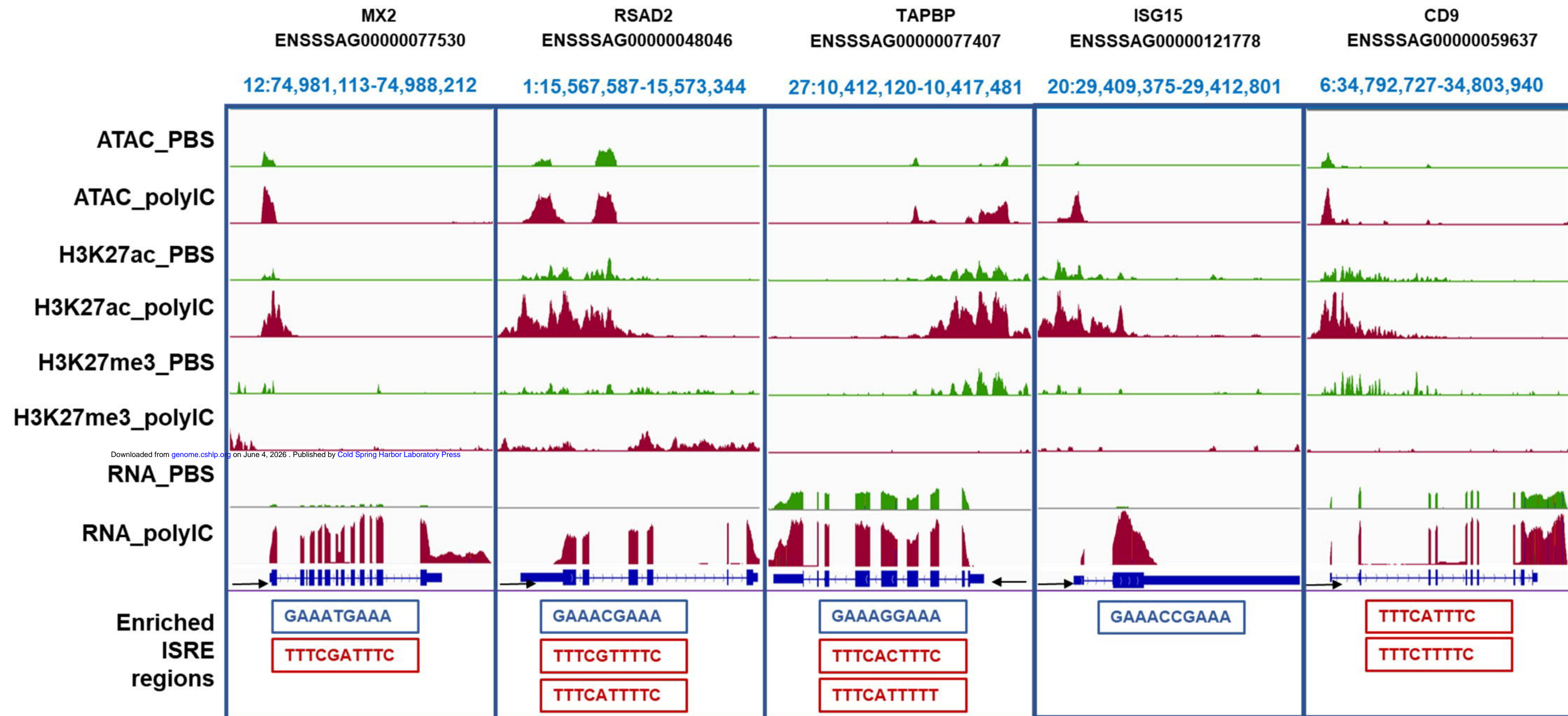
Gene_id	Ensembl_id	ATAC_FC	H3K27ac_FC	H3K27me3_FC	RNA_FC
DHX58(RIG1)	ENSSSAG00000119673	2.78	3.11	-2.43	5.83
IFIH1 (MDA5)	ENSSSAG00000078885	2.80	2.79	-1.62	6.64
IRF3	ENSSSAG00000047292	1.53	3.26	0.00	6.02
IRF7	ENSSSAG00000076373	2.12	3.36	0.00	10.33
STAT1	ENSSSAG00000073567	1.30	2.28	1.16	3.33
STAT2	ENSSSAG00000067332	1.40	2.51	-1.26	3.77
IRF9	ENSSSAG00000080439	1.78	2.53	-1.24	3.70

Downloaded from genome.cshlp.org on June 4, 2025. Published by Cold Spring Harbor Laboratory Press



A

Key anti-viral response genes



B

Gene_id	Ensembl_id	ATAC_FC	H3K27ac_FC	H3K27me3_FC	RNA_FC
MX2	ENSSSAG00000077530	6.91	4.56	1.77	23.84
RSAD2	ENSSSAG00000048046	2.56	2.62	-2.05	86.03
TAAPBP	ENSSSAG00000077407	2.40	2.38	0.00	2.89
ISG15	ENSSSAG00000121778	4.12	2.17	0.00	44.79
CD9	ENSSSAG00000059637	2.21	2.17	-1.46	3.36