

SUMO inhibition reveals temporally distinct intron retention programs shaping the immunopeptidome in acute myeloid leukemia

Lea Christophe^{1,2}, Javier Anleu Alegria^{1,3}, Mirela Pascariu¹, Chantal Durette¹, Eric Bonneil¹, Joel Lanoix¹, Mathieu Courcelles¹, Marie-Pierre Hardy¹, Krystel Vincent¹, Trang Hoang^{1,4}, Claude Perreault^{1,5} and Pierre Thibault^{1,2}

¹Institute of Research in Immunology and Cancer, Université de Montréal, Montréal, Canada.

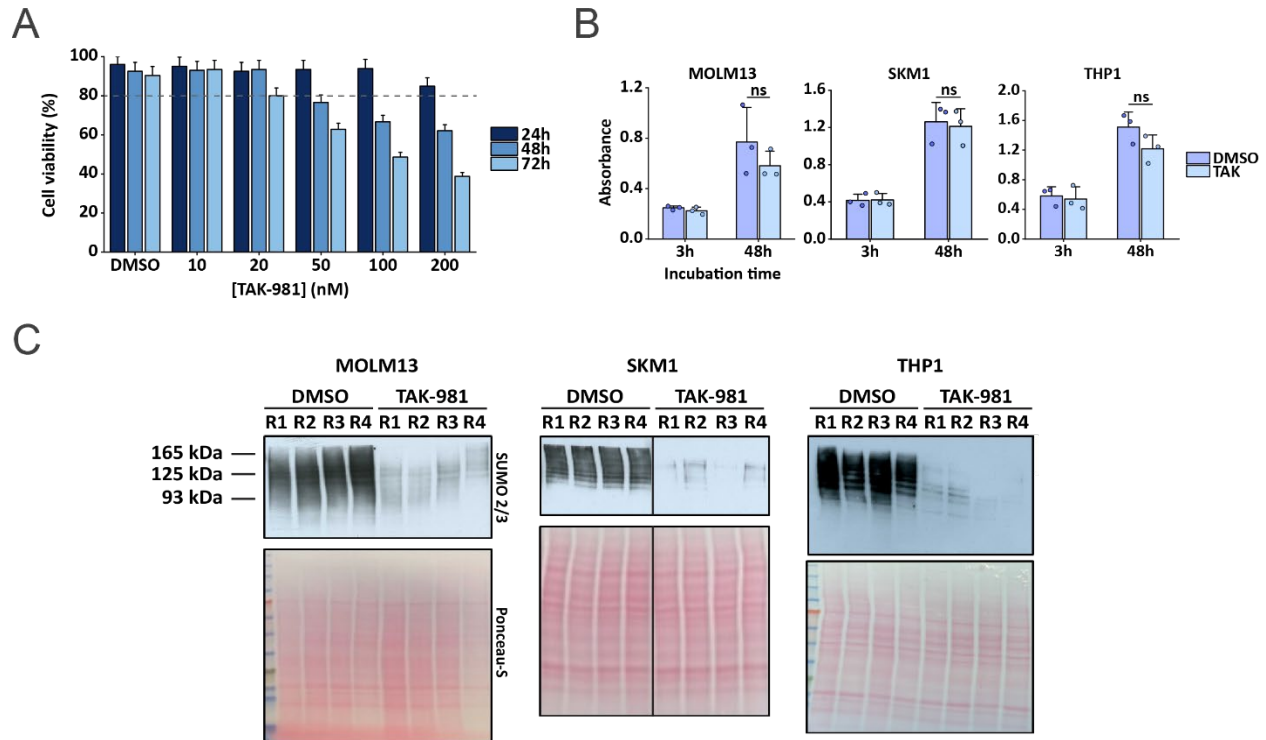
²Department of Chemistry, Université de Montréal, Montréal, Canada.

³Molecular Biology program, Université de Montréal, Montréal, Canada.

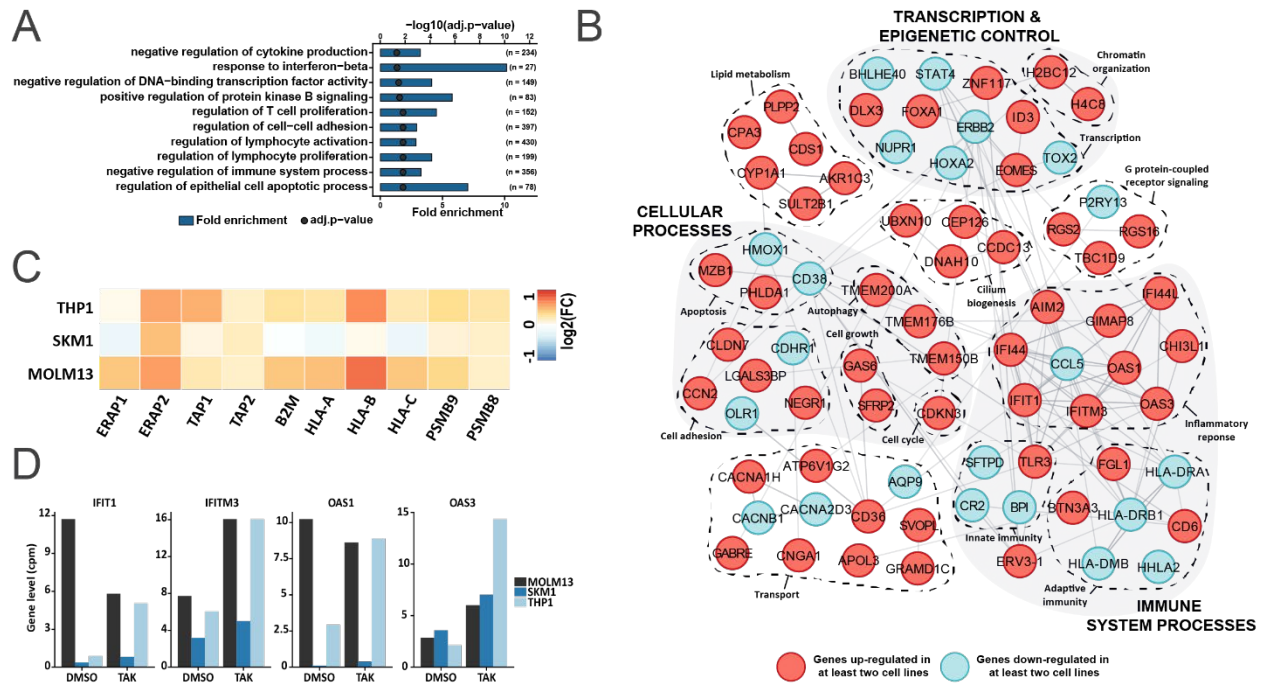
⁴Department of Pharmacology and Physiology, Université de Montréal, Montréal, Canada.

⁵Department of Medicine, Université de Montréal, Montréal, Canada.

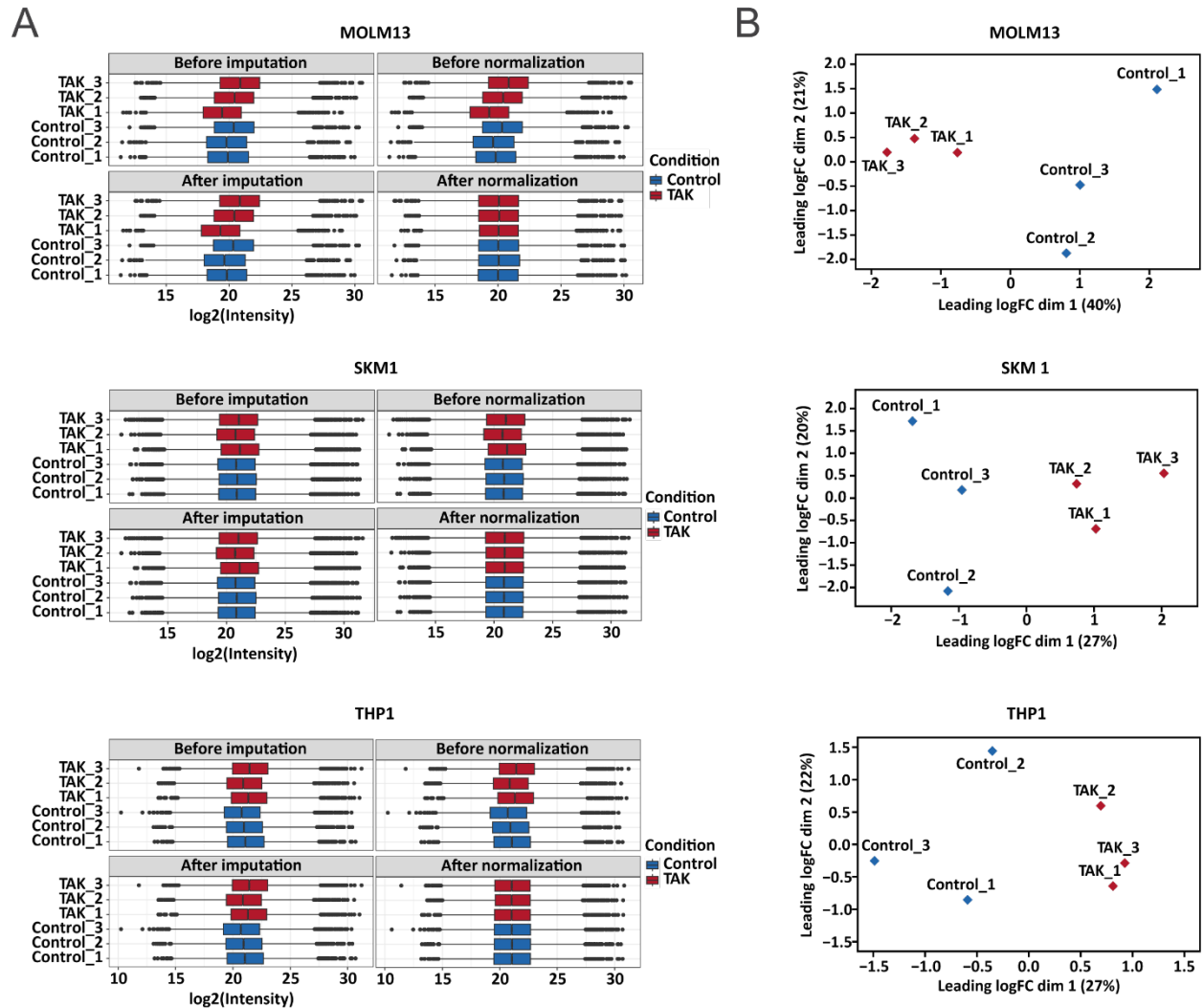
Corresponding authors: Dr. Pierre Thibault, Institute for Research in Immunology and Cancer, PO Box 6128, Downtown Station, Montreal, QC, H3C 3J7, Canada. Phone: 514-343-6910; Fax: 514-343-6843; E-mail: pierre.thibault@umontreal.ca and Claude Perreault, Institute for Research in Immunology and Cancer, P.O. Box 6128, Downtown Station Montreal, Québec H3C 3J7 CANADA. Phone: 514-343-6126; Fax: 514-343-5839; E-mail: claudio.perreault@umontreal.ca



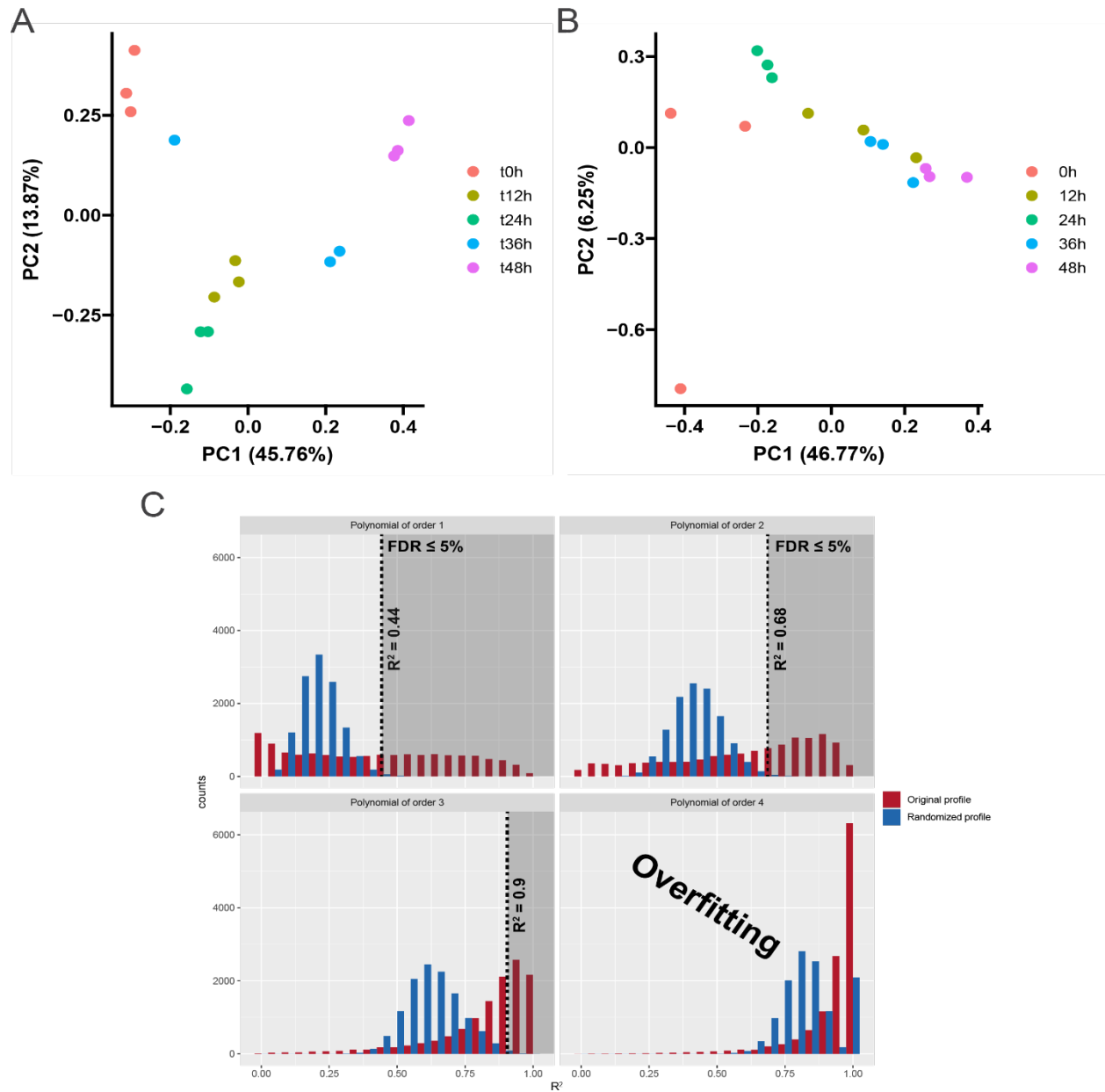
Supplementary Figure 1: TAK-981 inhibits SUMO2/3 conjugation in leukemic cells in a dose- and time-dependent manner. A) Preliminary cell viability analysis of THP-1 cells by flow cytometry. Cells were treated with increasing concentrations of TAK-981 (10, 20, 50, 100, and 200 nM) and assessed at 24 h, 48 h, and 72 h post-treatment. DMSO-treated cells served as controls. A horizontal line denotes the 80% viability threshold. Error bars represent the 95% confidence interval. B) WST-1 assay measuring proliferation and viability in three AML cell lines (MOLM-13, SKM-1, and THP-1) following treatment with 20 nM TAK-981 or DMSO for 48 h. Data are presented as mean $\pm$ standard deviation (SD) from three biological replicates. Statistical significance was determined using Student's *t*-test. C) Western blot analysis of SUMO2/3-conjugated proteins in AML cell lysates after 48 h treatment with 20 nM TAK-981 or DMSO. Ponceau S staining was used to confirm equal protein loading and transfer efficiency. Blots represent four biological replicates per condition. For the SKM-1 blot, non-adjacent lanes are indicated. *ns*, not significant.



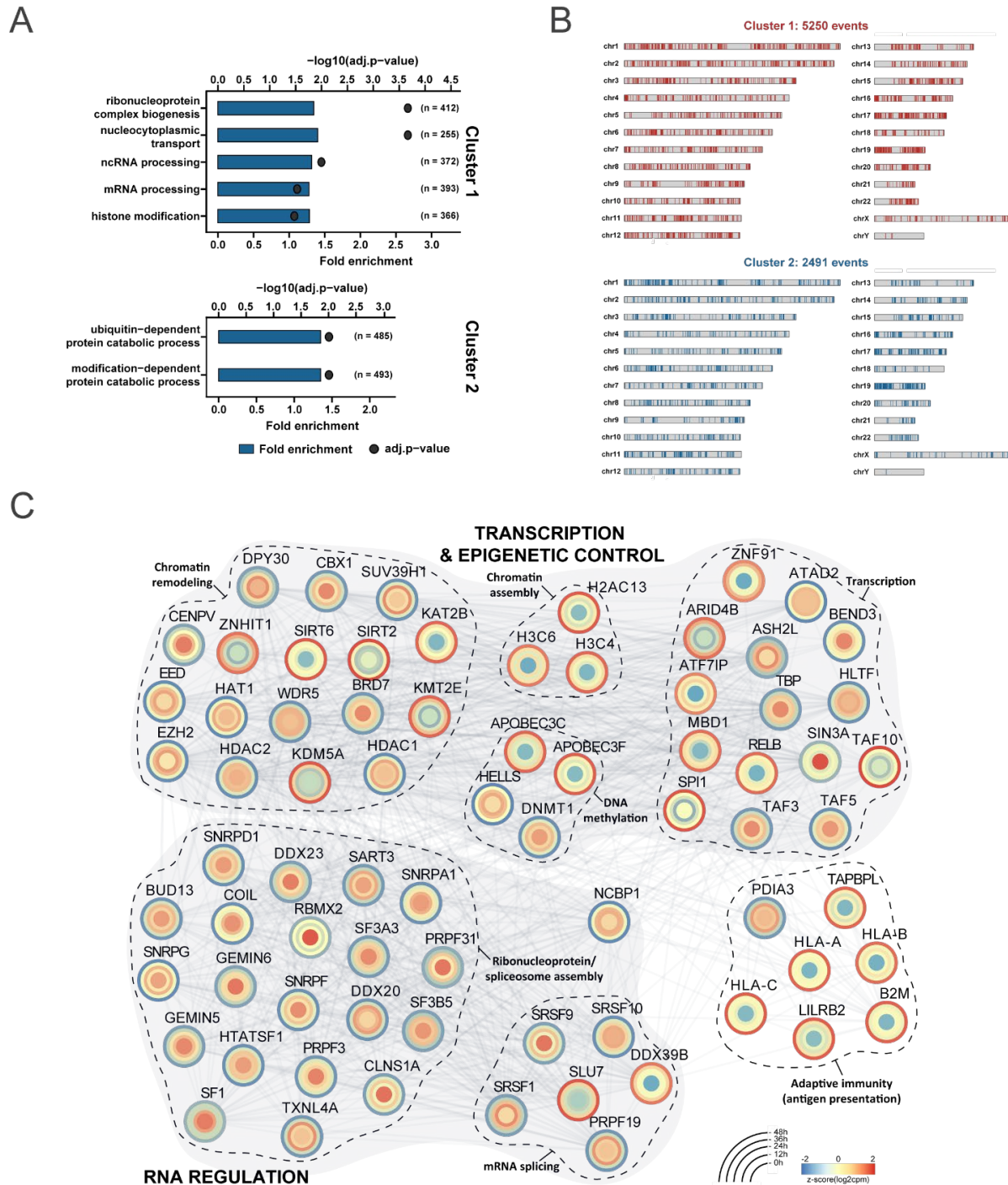
Supplementary Figure 2: AML cells treated with a SUMOylation inhibitor exhibit a transcriptomic signature indicative of an inflammatory response. A) Gene Ontology (GO) overrepresentation analysis of biological processes enriched among commonly differentially expressed genes. Adjusted p-values were calculated using the Benjamini-Hochberg (B-H) correction. B) Protein-protein interaction network of genes commonly upregulated or downregulated (shared by at least two AML cell lines) following TAK-981 treatment. Subnetworks are clustered based on Panther Protein Class annotations. C) Heatmap showing the normalized $\log_2$ fold change of genes involved in the MHC class I antigen processing and presentation pathway following TAK-981 treatment. D) Expression levels of selected interferon-stimulated genes in DMSO- and TAK-981-treated cells. Gene expression values are presented as counts per million (cpm).



Supplementary Figure 3: Impact of preprocessing steps on the immunopeptidomics dataset and multidimensional scaling (MDS) analysis. A) Missing values were imputed using the Random Forest algorithm. Peptide intensity values were $\log_2$ -transformed and subsequently normalized using variance stabilizing normalization (VSN). Boxplots illustrate the distribution of peptide intensities before and after imputation and normalization. B) MDS plots of AML cell lines treated with TAK-981 or DMSO for 48 hours. Samples segregate into two distinct clusters along dimension 1 (Dim 1), reflecting the separation between control and TAK-981-treated conditions. Some intra-group variability is observed among biological replicates. Distances in the MDS plots represent leading $\log_2$ -fold changes between samples.



Supplementary Figure 4: Quality assessment of transcriptomic and alternative splicing profiles and determination of R^2 thresholds for profile fitting. A) Principal component analysis (PCA) of transcriptomic gene expression profiles. B) PCA of intron-retention (IR) alternative splicing events based on percent-spliced-in (PSI) values. C) Distribution of profiles (randomized vs original) across polynomial orders, used to determine the R^2 thresholds corresponding to a false discovery rate (FDR) of 5%.



Supplementary Figure 5: Characterization of clustered intron retention events and associated temporal transcriptional programs in TAK-treated cells. A) Gene Ontology (GO) enrichment analysis of source genes unique to each cluster. Only biological process terms are shown. Adjusted p-values were calculated using the Benjamini-Hochberg (B-H) method. B) Chromosomal distribution of clustered IR events. C) Protein-protein interaction network of selected genes involved in RNA regulation, transcription and epigenetic control, and antigen presentation. Each node represents a gene and is represented with

concentric rings corresponding to temporal transcriptomic expression. The innermost ring denotes the baseline (0h), while the outermost ring corresponds to 48h, with intermediate rings marking the in-between time points. Ring colors are scaled according to normalized $\log_2(\text{cpm})$ expression values. Subnetworks are clustered based on UniProt functional annotations and GO terms annotations.