Supplemental Data

Supplemental Tables and Figures

Global Profiling of the Cellular Alternative RNA Splicing Landscape During Virus-host Interactions

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Table A. RNA-sequencing data

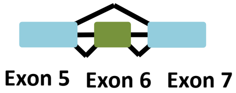
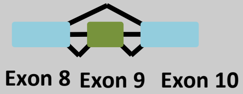
Sample	Number of bases	HiSeq Reads
Mock 1	9 270 066 200	46 350 331
Mock 2	9 400 946 800	47 004 734
Mock 3	7 880 753 600	39 403 768
Reovirus 1	10 499 367 400	52 496 837
Reovirus 2	10 352 922 800	51 764 614
Reovirus 3	8 850 997 600	44 254 988

RNA-seq was performed on uninfected (mock-infected) cells, and cells infected with reovirus (14 hours post-infection). Sequencing was done in triplicate.

Table B. Distribution of ΔPSI values in the 240 differentially spliced ASEs

ΔPSI	Number of ASEs modified
[-80,-70[	1
[-70,-60[	0
[-60,-50[	4
[-50,-40[	4
[-40,-30[	15
[-30,-20[	35
[-20,-10[	60
]10,20]	64
]20,30]	39
]30,40]	11
]40,50]	3
]50,60]	2
]60,70]	2

Table C: Sequencing results (Sanger) for splicing junctions confirmed by RT-PCR

Gene	ASE Form	Splicing junction	Predicted sequence	Sequencing results
Il34  	Short	5-7	CAGCGGAGCCT CATGGATGTGG AGATTGGC	CAGCGGAGCCT CATGGATGTGG AGATTGGC
	Long	5-6	NA	NA
	Long	6-7	NA	NA
hnRNPA2B1  	Short	8-10	GAGGAGGACCT GGAGGAAATTA TGGAAGTG	GAGGAGGACCT GGAGGAAATTA TGGAAGTG
	Long	8-9	GAGGAGGACCT GGAGGTGGCAA TTTTGGAG	GAGGAGGACCT GGAGGTGGCAA TTTTGGAG
	Long	9-10	ACAACTATGGA GGAGGAAATTA TGGAAGTG	ACAACTATGGA GGAGGAAATTA TGGAAGTG

NA: Splicing junction expressed at levels too low to allow sequencing

Table D. Bioinformatic prediction of functional changes caused by some identified ASEs

Gene	Delta-PSI	Event Type	Full name	Predicted residue variation caused by alternative splicing modification (ΔAA)	Predicted functional consequence
Abi1	-50.8	exon-cassette+alternate-3p+multiple-exon-cassette	abl-interactor 1	-29	Unknown
Adar	-25.1	alternate-5p	adenosine deaminase, RNA-specific	-26	Unknown
Agfg1	-27.6	alternate-3p	ArfGAP with FG repeats 1	-19	Unknown
Apol9a	22.9	alternate-5p	apolipoprotein L 9a	0	No predicted functional change
Arhgap11a	-42.9	exon-cassette+alternate-3p+intron-retention	Rho GTPase activating protein 11A	-515	NLS and RhoGAP domain loss in short isoform
Arhgap11a	16.7	exon-cassette+alternate-3p+intron-retention	Rho GTPase activating protein 11A	-489	Premature stop codon introduced by intron retention in long isoform
Asph	13.8	exon-cassette	aspartate-beta-hydroxylase	83	Unknown
Atat1	13.8	exon-cassette	alpha tubulin acetyltransferase 1	23	Unknown
Atxn7l1	-22.4	exon-cassette	ataxin 7-like 1	-51	Unknown
Bnip2	-18.3	exon-cassette	BCL2/adenovirus E1B interacting protein 2	-6	Unknown
Bora	37.5	exon-cassette	bora, aurora kinase A activator	-251	Premature stop codon in long isoform
Bptf	-22.9	alternate-5p	bromodomain PHD finger transcription factor	-281	Unknown
Calu	13.3	mutually-exclusive-exon	Calumenin	0	Unknown
Cbwd1	-23.1	alternate-5p	COBW domain containing 1	-121	CobW/HypB/UreG domain truncated in short isoform
Ccdc136	-10.2	exon-cassette	coiled-coil domain containing 136	-47	Unknown
Ccdc15	14.8	alternate-5p	coiled-coil domain containing 15	116	Unknown
Cdkn2aip	-18.2	alternate-5p	CDKN2A interacting protein	-444	Premature stop codon inducing a NLS loss in short isoform
Celf1	11.2	alternate-3p	CUGBP, Elav-like family member 1	1	Unknown

Table D

Gene	Delta-PSI	Event Type	Full name	Predicted residue variation caused by alternative splicing modification (ΔAA)	Predicted functional consequence
Cflar	-14.8	exon-cassette	CASP8 and FADD-like apoptosis regulator	-186	Loss of the two DED domains in short isoform
Cflar	27.6	exon-cassette	CASP8 and FADD-like apoptosis regulator	-186	Loss of the two DED domains in long isoform
Clip1	-18.9	alternate-5p	CAP-GLY domain containing linker protein 1	-76	Unknown
Clk4	11.9	exon-cassette+intron-retention	CDC like kinase 4	180	Gain of 10 NLS and full-length Pkinase domain in long isoform
Cwc22	21.5	alternate-5p	CWC22 spliceosome-associated protein homolog (S, cerevisiae)	-6	Unknown
Cwc22	19.9	exon-cassette	CWC22 spliceosome-associated protein homolog (S, cerevisiae)	6	Unknown
E2f3	-20.9	alternate-3p	E2F transcription factor 3	-6	Additionnal NLS in short isoform
Edc3	-26.5	exon-cassette	enhancer of mRNA decapping 3 homolog (S, cerevisiae)	-183	Loss of Edc3-linker domain, LSM14 domain and truncation of YjwF_N domain in short isoform
Eif4a2	-11.4	exon-cassette	eukaryotic translation initiation factor 4A2	-95	Truncation of DEAD box helicase domain in short isoform
Eif4h	-16.7	exon-cassette	eukaryotic translation initiation factor 4H	-20	Unknown
Erbb2ip	-30.0	exon-cassette+alternate-3p+multiple-exon-cassette+mutually-exclusive-exon	Erbb2 interacting protein	-39	Unknown
Erbb2ip	23.3	exon-cassette+alternate-3p+multiple-exon-cassette+mutually-exclusive-exon	Erbb2 interacting protein	-384	Premature stop codon, C-Term PDZ domain loss in short isoform
Fam179b	-30.2	exon-cassette	family with sequence similarity 179, member B	-50	Unknown

Table D (cont’d)

Gene	Delta-PSI	Event Type	Full name	Predicted residue variation caused by alternative splicing modification (ΔAA)	Predicted functional consequence
Flcn	35.8	alternate-3p	folliculin	-129	Premature stop codon, resulting in Folliculin domain truncation (174AA, C-term)
Foxk2	-29.7	exon-cassette	forkhead box K2	-401	Unknown
G2e3	-53.0	exon-cassette+alternate-3p+multiple-exon-cassette	G2/M-phase specific E3 ubiquitin ligase	116	Unknown
Gdap2	15.1	alternate-3p	ganglioside-induced differentiation-associated-protein 2	256	Complete CRAL and TRIO domains in long isoform
Gen1	-28.7	exon-cassette	Gen homolog 1, endonuclease (Drosophila)	-10	Earlier start codon in long isoform; Short isoform loses its XPG_N domain in N-term
Gls	11.3	alternate-3p	glutaminase	-376	Unknown
Gpbp1	25.6	exon-cassette	GC-rich promoter binding protein 1	20	Unknown
Hmga1	15.6	exon-cassette+alternate-5p+intron-retention	high mobility group AT-hook 1	0	5'UTR sequence change
Hnrnpa2b1	-13.6	exon-cassette	heterogeneous nuclear ribonucleoprotein A2/B1	-40	Additionnal HnRNPA1 domain in N-term of short isoform
Il34	58.9	exon-cassette	interleukin 34	-16	Frameshift introducing a premature stop codon in exon 8; truncation of Il34 domain
Ilf3	13.2	exon-cassette	interleukin enhancer binding factor 3	13	Unknown
Iqce	17.6	exon-cassette	IQ motif containing E	17	Unknown
Iqce	-19.2	exon-cassette	IQ motif containing E	-17	Unknown
Ivns1abp	30.8	exon-cassette+alternate-3p	influenza virus NS1A binding protein	-281	NLS and BTB domains loss in long isoform

Table D (cont'd)

Gene	Delta-PSI	Event Type	Full name	Predicted residue variation caused by alternative splicing modification (ΔAA)	Predicted functional consequence
Kat5	28.7	exon-cassette	K(lysine) acetyltransferase 5	52	Long isoform have two NLS instead of only one in the short isoform
Kat6a	-13.6	exon-cassette	K(lysine) acetyltransferase 6A	130	Unknown
Kmt2e	10.8	exon-cassette	lysine (K)-specific methyltransferase 2E	-1602	Loss of 6 NLS and SET and PHD domains in long isoform
Lrrfip1	13.1	exon-cassette	leucine rich repeat (in FLII) interacting protein 1	24	Truncation in double-stranded RNA binding protein DUF2051 in long isoform
Macf1	-25.2	multiple-exon-cassette	microtubule-actin crosslinking factor 1	-2027	Loss of all 9 Plectin repeat domains in short isoform
Matr3	12.3	alternate-5p	matrin 3	0	No predicted functional change
Max	11.3	exon-cassette	Max protein	-122	NLS and Helix-Loop-Helix domain loss in long isoform
Mbnl2	24.1	exon-cassette	muscleblind-like 2	18	Unknown
Mdm2	-58.8	exon-cassette+alternate-5p	transformed mouse 3T3 cell double minute 2	-197	SWIB domain in N-Term loss in short isoform
Mon2	38.3	exon-cassette	MON2 homolog (yeast)	7	6 residue gap in Mon2 domain in short isoform
Morn1	-16.3	alternate-5p	MORN repeat containing 1	-245	Loss of 4 MORN domain out of 8 in short isoform
Msh4	-38.7	alternate-5p	mutS homolog 4 (E, coli)	404	Premature stop codon in long isoform
Mtif3	25.0	exon-cassette+alternate-5p	mitochondrial translational initiation factor 3	0	Unknown
Nabp1	41.4	alternate-5p+intron-retention	nucleic acid binding protein 1	-91	Unknown
Naip2	21.8	exon-cassette	NLR family, apoptosis inhibitory protein 2	56	Unknown

Table D (cont'd)

Gene	Delta-PSI	Event Type	Full name	Predicted residue variation caused by alternative splicing modification (ΔAA)	Predicted functional consequence
Nasp	-16.5	exon-cassette	nuclear autoantigenic sperm protein (histone-binding)	-325	Unknown
Nde1	-16.6	alternate-3p	nuclear distribution gene E homolog 1 (A nidulans)	27	Unknown
Nek1	26.9	exon-cassette	NIMA (never in mitosis gene a)-related expressed kinase 1	29	Unknown
Neurl4	-19.2	exon-cassette	neuralized homolog 4 (Drosophila)	-22	Unknown
Nfx1	28.3	alternate-3p	nuclear transcription factor, X-box binding 1	-14	Unknown
Odf2l	-16.0	exon-cassette	outer dense fiber of sperm tails 2-like	-96	Unknown
Opa1	-14.5	exon-cassette	optic atrophy 1	-37	Unknown
Pan3	17.4	exon-cassette	PAN3 polyA specific ribonuclease subunit homolog (S, cerevisiae)	258	Unknown
Pcgf5	15.5	exon-cassette	polycomb group ring finger 5	20	C-term RAWUL domain truncated in long isoform
Phactr4	-16.8	exon-cassette	phosphatase and actin regulator 4	-27	RPEL repeat domain loss in short isoform
Piga	-33.8	alternate-5p	phosphatidylinositol glycan anchor biosynthesis, class A	-316	PIGA domain loss in short isoform
Pitpnb	-21.8	exon-cassette	phosphatidylinositol transfer protein, beta	-1	Unknown
Pnpla7	69.1	alternate-5p	patatin-like phospholipase domain containing 7	-886, -52, -454 or +380	Unknown
Polg2	-13.5	alternate-5p	polymerase (DNA directed), gamma 2, accessory subunit	-44	Unknown
Ppfibp1	-16.1	exon-cassette+intron-retention	PTPRF interacting protein, binding protein 1 (liprin beta 1)	-11	Unknown

Table D (cont’d)

Gene	Delta-PSI	Event Type	Full name	Predicted residue variation caused by alternative splicing modification (ΔAA)	Predicted functional consequence
Ppp1r12a	10.2	exon-cassette	protein phosphatase 1, regulatory (inhibitor) subunit 12A	422	Unknown
Pqlc1	50.9	exon-cassette	PQ loop repeat containing 1	18	Unknown
Prpf39	18.5	exon-cassette+multiple-exon-cassette	PRP39 pre-mRNA processing factor 39 homolog (yeast)	-201, -239	Loss of part of Tetratricopeptide-like helical domain and HAT reapeats in both C- and N-term
Pum2	-11.2	exon-cassette	pumilio RNA-binding family member 2	-79	Unknown
Ranbp3	-27.0	exon-cassette+multiple-exon-cassette	RAN binding protein 3	331	NLS loss and full RanBP1 domain in long isoform
Rfx5	34.7	exon-cassette+alternate-5p	regulatory factor X, 5 (influences HLA class II expression)	185	NLS gain and full-length RFX5 DNA-binding domain in long isoform
Rnf135	-31.1	exon-cassette	ring finger protein 135	-216	Zinc finger of C3HC4-type domain loss in short isoform
Rnps1	-25.7	exon-cassette+multiple-exon-cassette	ribonucleic acid binding protein S1	-23	Unknown
Rnps1	-14.6	exon-cassette+multiple-exon-cassette	ribonucleic acid binding protein S1	-23	Unknown
Scaf11	-11.6	alternate-5p	SR-related CTD-associated factor 11	-16	Unknown
Senp1	22.9	exon-cassette	SUMO1/sentrin specific peptidase 1	-142	Unknown
Sfswap	-24.8	alternate-5p	splicing factor, suppressor of white-apricot family	-724	47 AA truncation in Alternative splicing regulator domain and 2 Surp module domain loss in short isoform
Skil	-14.2	alternate-5p	SKI-like	-35	Unknown

Table D (cont’d)

Gene	Delta-PSI	Event Type	Full name	Predicted residue variation caused by alternative splicing modification (ΔAA)	Predicted functional consequence
Slc23a2	-12.2	alternate-3p	solute carrier family 23 (nucleobase transporters), member 2	-308	208 AA loss in the N-term of Xan_ur_permease domain in short isoform
Son	25.7	exon-cassette+alternate-5p	Son DNA binding protein	-2198	Premature stop codon in long isoform, introducing 4 NLS and G-patch, Arginine/Serine-Rich protein 1 and double-stranded RNA binding domain loss
Spef2	18.4	exon-cassette	sperm flagellar 2	923	Unknown
Spopl	27.8	exon-cassette	speckle-type POZ protein-like	56	Unknown
Stx3	39.5	exon-cassette+mutually-exclusive-exon	syntaxin 3	97	75 AA truncation of Syntaxin domain in long isoform
Tbp	-44.3	exon-cassette	TATA box binding protein	-56	TBP domain loss and truncation of first TBP domain's end in short isoform
Tcf12	30.7	exon-cassette+intron-retention	transcription factor 12	24	Unknown
Tmem132a	66.1	alternate-3p	transmembrane protein 132A	29	Unknown
Tmem168	-32.5	exon-cassette	transmembrane protein 168	-384	Unknown
Tmem19	-15.0	alternate-5p	transmembrane protein 19	12	Full lenght DUF92 integral membrane domain in long isoform
Tmpo	-38.3	exon-cassette+multiple-exon-cassette	thymopoietin	-40	Unknown
Tmpo	-11.5	exon-cassette+multiple-exon-cassette	thymopoietin	-72	Unknown
Tpp2	10.7	exon-cassette	tripeptidyl peptidase II	-130	Unknown
Tra2a	21.2	exon-cassette	transformer 2 alpha homolog (Drosophila)	97	Full-length RNA recognition motif in long isoform

Table D (cont’d)

Gene	Delta-PSI	Event Type	Full name	Predicted residue variation caused by alternative splicing modification (ΔAA)	Predicted functional consequence
Trappc13	-29.9	exon-cassette	trafficking protein particle complex 13	-6	DUF974 domain truncation of 6 AA in short isoform
Ubap1	-21.4	exon-cassette	ubiquitin-associated protein 1	-61	Unknown
Ubr3	11.3	alternate-3p	ubiquitin protein ligase E3 component n-recognin 3	4	Unknown
Upf1	-23.4	alternate-5p	UPF1 regulator of nonsense transcripts homolog (yeast)	-11	Unknown
Wsb1	19.9	alternate-3p	WD repeat and SOCS box-containing 1	-177	Premature stop codon in long isoform, resulting in a truncated protein containing only a WD, G-beta domain instead of 4 WD, G-beta domain and a SOCS box domain
Xdh	-25.6	alternate-5p	xanthine dehydrogenase	-599	NLS appearance in short isoform
Zfp207	-12.5	exon-cassette	zinc finger protein 207	-6	Unknown
Zfp788	-32.4	exon-cassette+alternate-3p+multiple-exon-cassette	zinc finger protein 788	-532	Unknown
Zmynd11	-17.1	exon-cassette	zinc finger, MYND domain containing 11	-54	Unknown
Zyx	13.8	exon-cassette	zyxin	31	Unknown
1700057G04Rik	-31.1	exon-cassette	RIKEN cDNA 1700057G04 gene	-76	Truncation in Scramblase domain in short isoform
2310057M21Rik	-15.9	alternate-5p	RIKEN cDNA 2310057M21 gene	77	Unknown
4631405J19Rik	-45.7	alternate-3p	RIKEN cDNA 4631405J19 gene	-100	Unknown
4930511M06Rik	-23.6	exon-cassette	RIKEN cDNA 4930511M06 gene	NA	Non-coding RNA

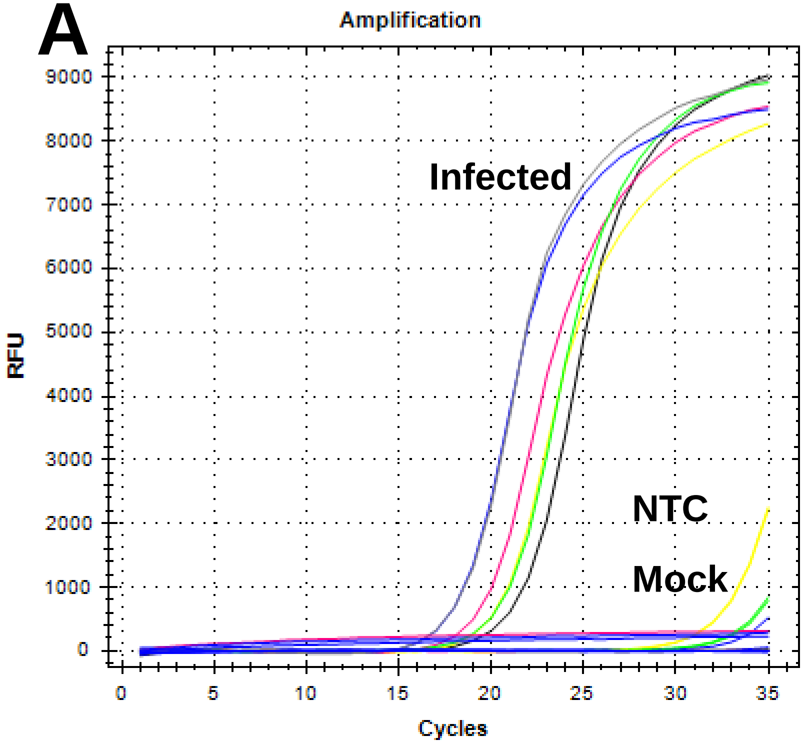
Table D (cont’d)

Figure A. Detection of reovirus genes in infected cells by qRT-PCR.

(A) Total RNA was extracted from both uninfected and infected cells (14 h post-infection). Viral infection was confirmed by qRT-PCR using specific primers for three viral genes (M1, S1, L1). A control reaction without template (NTC: no template control) was also performed. All three viral genes were detected in the infected cells samples but were undetectable in the uninfected (mock) samples (absence of amplification or nonspecific amplification). Cq: quantification cycle.

(B) Primers used for qPCR detection.

A



qRT-PCR data on amplification of viral genes in uninfected (mock) and infected cells

Orthoreovirus Primer	Cq Mock	Cq infected	Cq NTC	Color
L1_1	Undetected	20.52	Undetected	Black
L1_2	Undetected	19.22	Undetected	Yellow
M1_1	Undetected	18.57	Undetected	Pink
M1_2	Undetected	19.68	Undetected	Green
S1_1	Undetected	16.93	Undetected	Blue
S1_2	Undetected	17.00	Undetected	Gray

B

Primers design for qRT-PCR amplification of reovirus genes

Name	Primer forward	Sequence	Primer reverse	Sequence
L1_1	orthoreovirus_t3d_seg_L1.F1	GGCAAAGACGGTGTCGGGTC	orthoreovirus_t3d_seg_L1.R1	TGCGTCCGCTTCTGACTCCT
L1_2	orthoreovirus_t3d_seg_L1.F2	CTAGGGGTGGATCTGGCGCA	orthoreovirus_t3d_seg_L1.R2	TCGCTAATTGTGCCGCCTGG
M1_1	orthoreovirus_t3d_seg_M1.F1	AGCGCGCAGCCTAAATGGTT	orthoreovirus_t3d_seg_M1.R1	ACGTAGATGCCGGGTCTGCT
M1_2	orthoreovirus_t3d_seg_M1.F2	AGATGAGGCTTTCGTGCGGC	orthoreovirus_t3d_seg_M1.R2	GCATGTCACGGATCACGCCA
S1_1	orthoreovirus_t3d_seg_S1.F1	GGTCGGATGGATCCTCGCCT	orthoreovirus_t3d_seg_S1.R1	TCGAGTCCCTGGGTGATCCG
S1_2	orthoreovirus_t3d_seg_S1.F2	CCTCCGGATCACCCAGGGAC	orthoreovirus_t3d_seg_S1.R2	TGTCCCACTCGAGCACCCAA

Figure A

Figure B. Heatmap representation of gene expression of total cellular genes in both infected and uninfected (mock) cells.

RNA sequencing was done in triplicate for each condition. The map represents the number of transcripts sequences (TPM: transcripts per million) in a color-coded logarithmic scale. Blue and green indicates high levels of gene expression while yellow indicates low levels of gene expression.

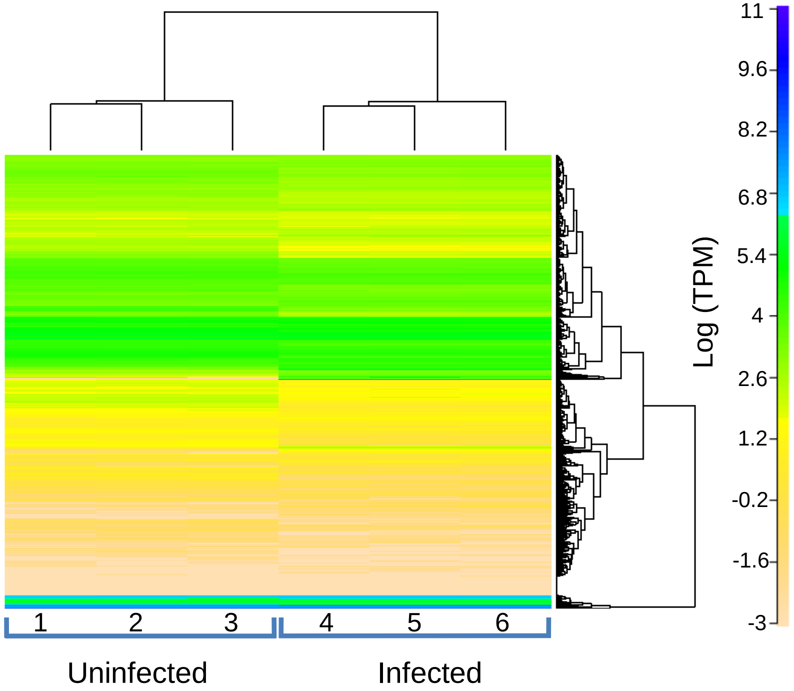
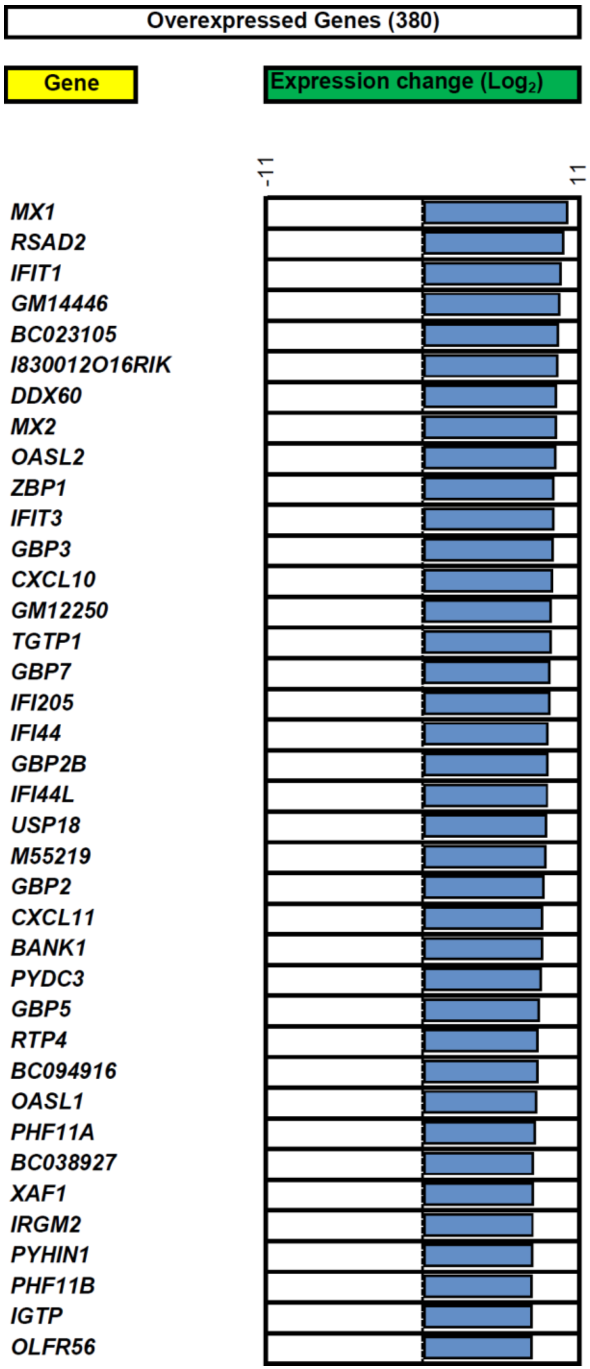

11
9.6
8.2
6.8
5.4
4
2.6
1.2
-0.2
-1.6
-3
Log (TPM)
1
2
3
4
5
6
Uninfected
Infected

Figure B

Figure C. List of the 570 genes for which the expression was the most significantly modified following viral infection.

The list of genes is shown with the corresponding expression profile of each gene. The level of gene expression is presented on a logarithmic scale (Log_2)


Overexpressed Genes (380)
Gene
Expression change (Log$_2$)
-11
11
MX1
RSAD2
IFIT1
GM14446
BC023105
I830012O16RIK
DDX60
MX2
OASL2
ZBP1
IFIT3
GBP3
CXCL10
GM12250
TGTP1
GBP7
IFI205
IFI44
GBP2B
IFI44L
USP18
M55219
GBP2
CXCL11
BANK1
PYDC3
GBP5
RTP4
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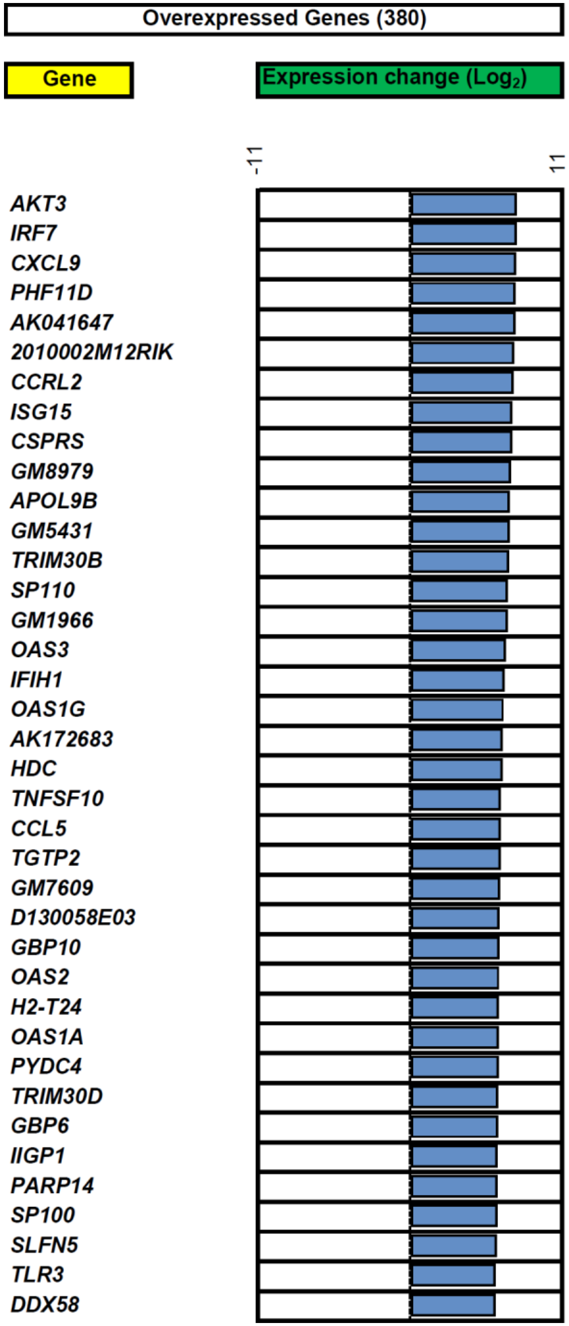
IRGM2
PYHIN1
PHF11B
IGTP
OLFR56

Figure C



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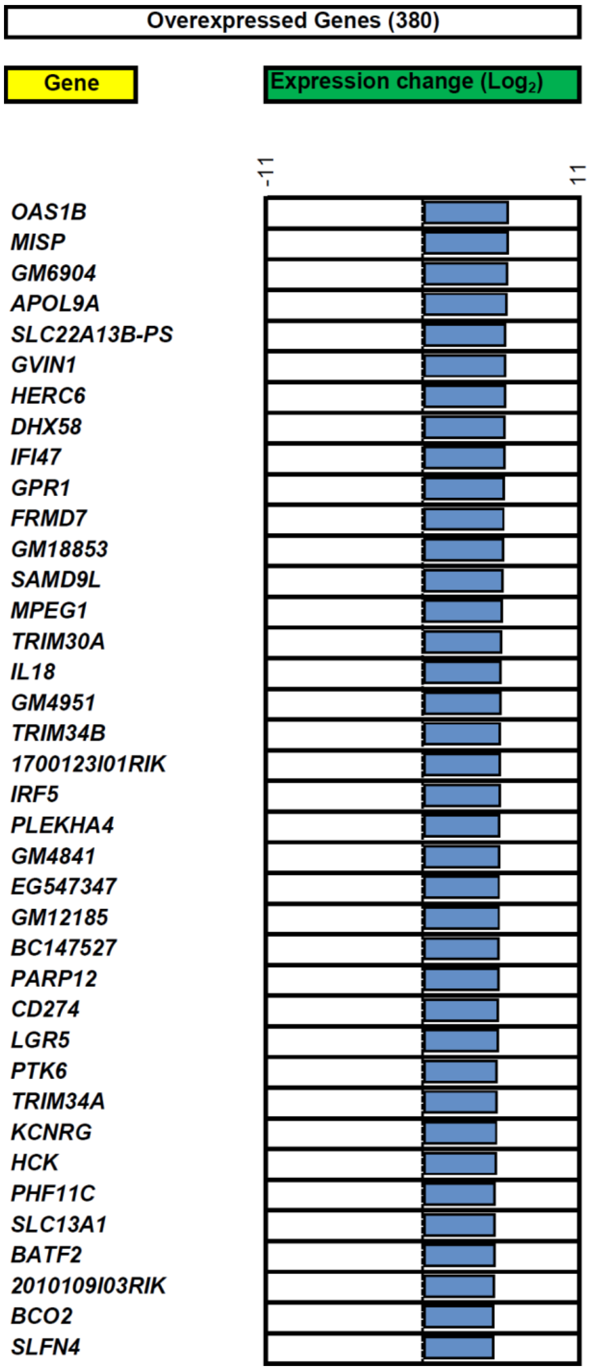

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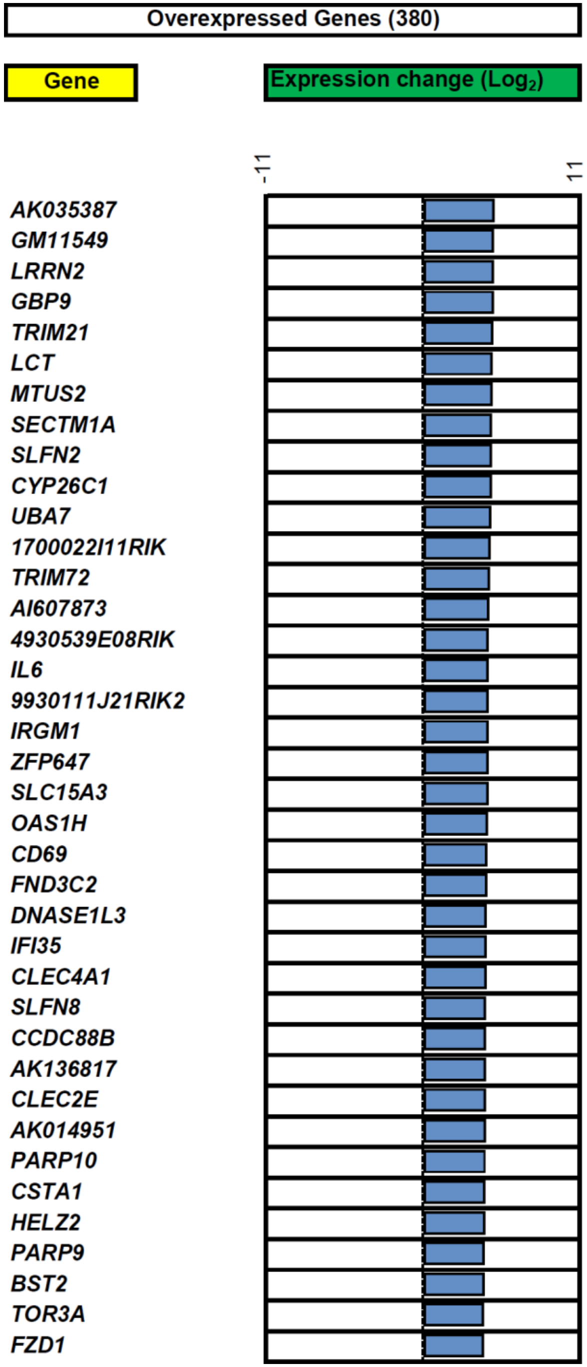

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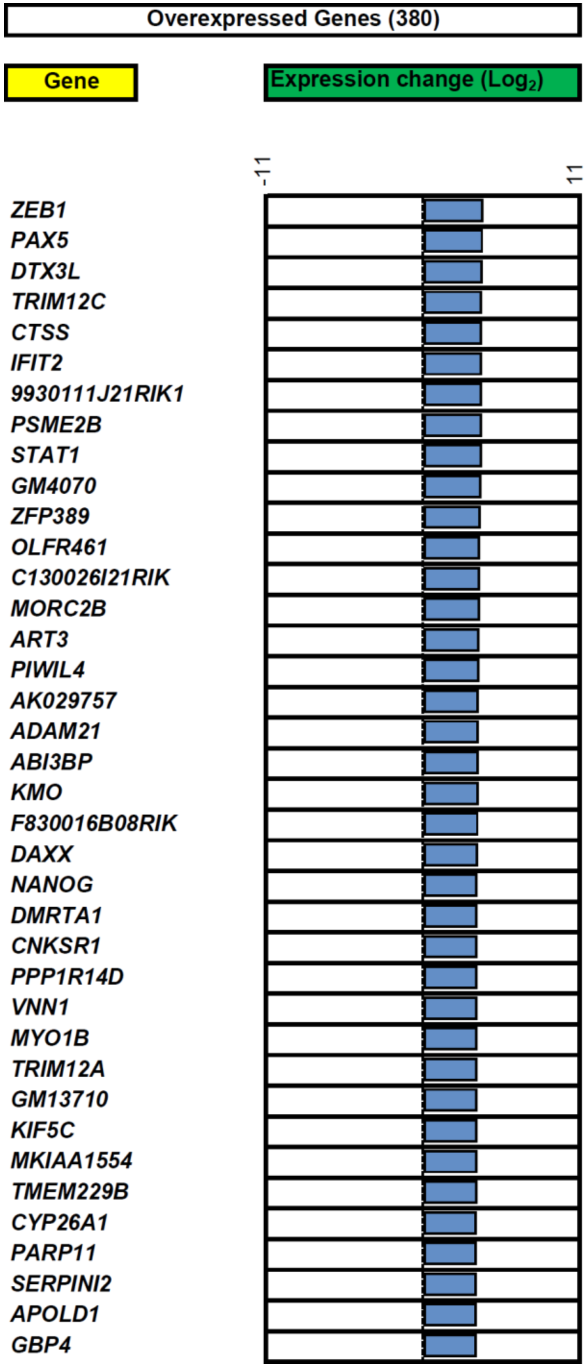

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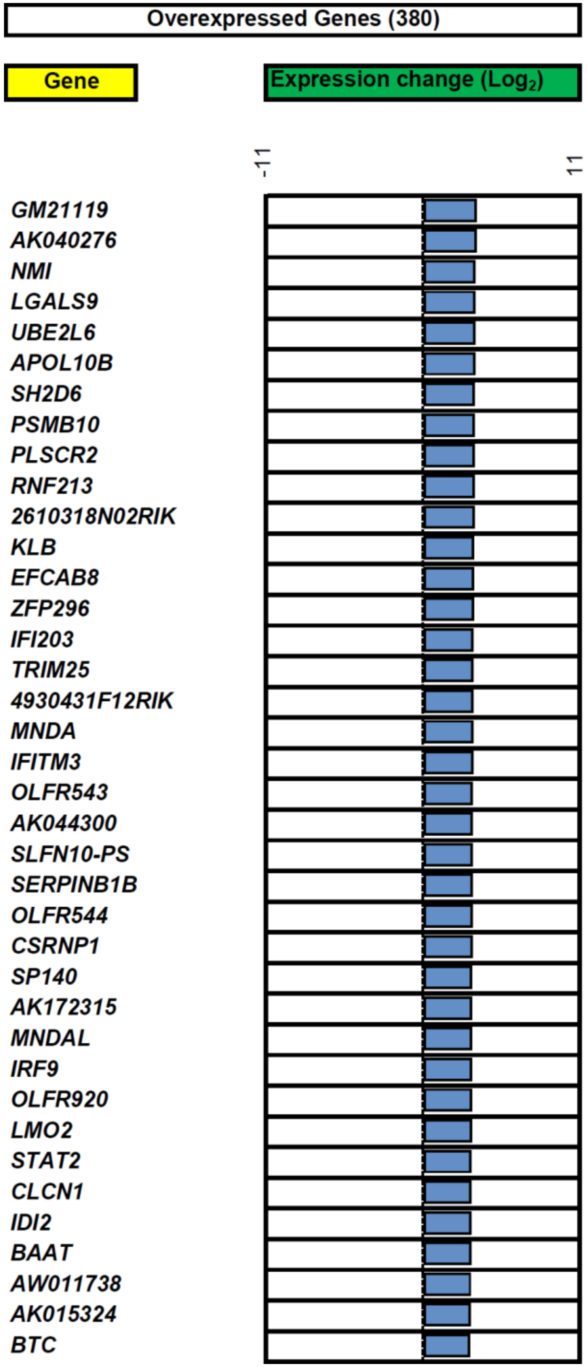


Figure C (cont'd)

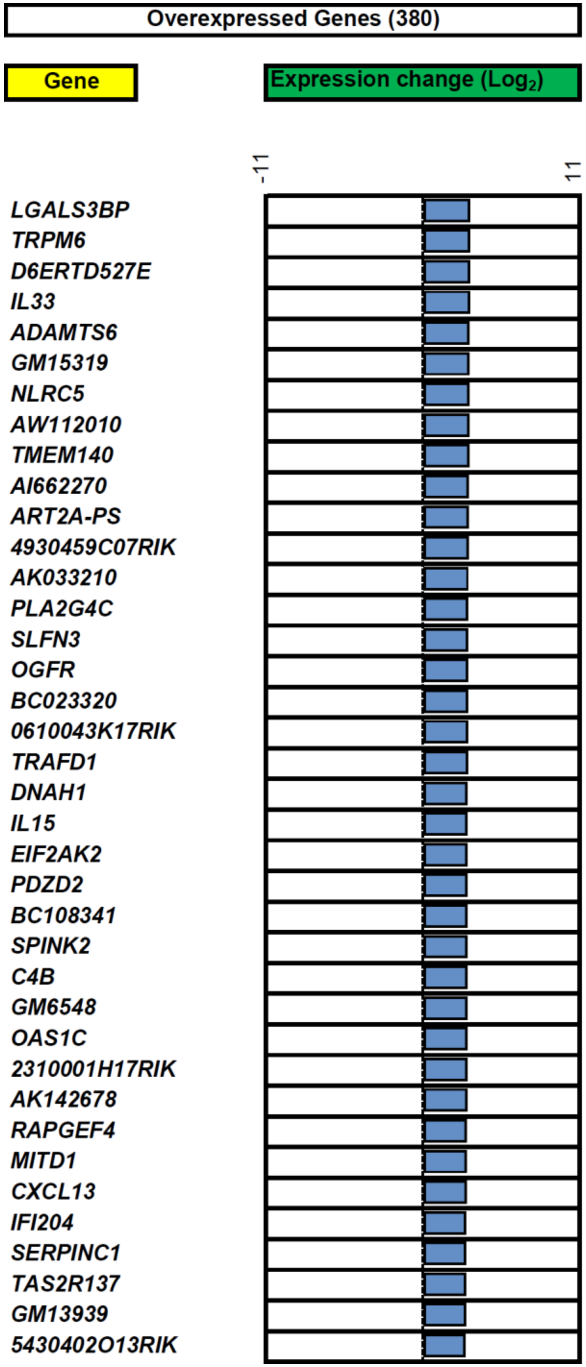


Figure C (cont’d)

Overexpressed Genes (380)

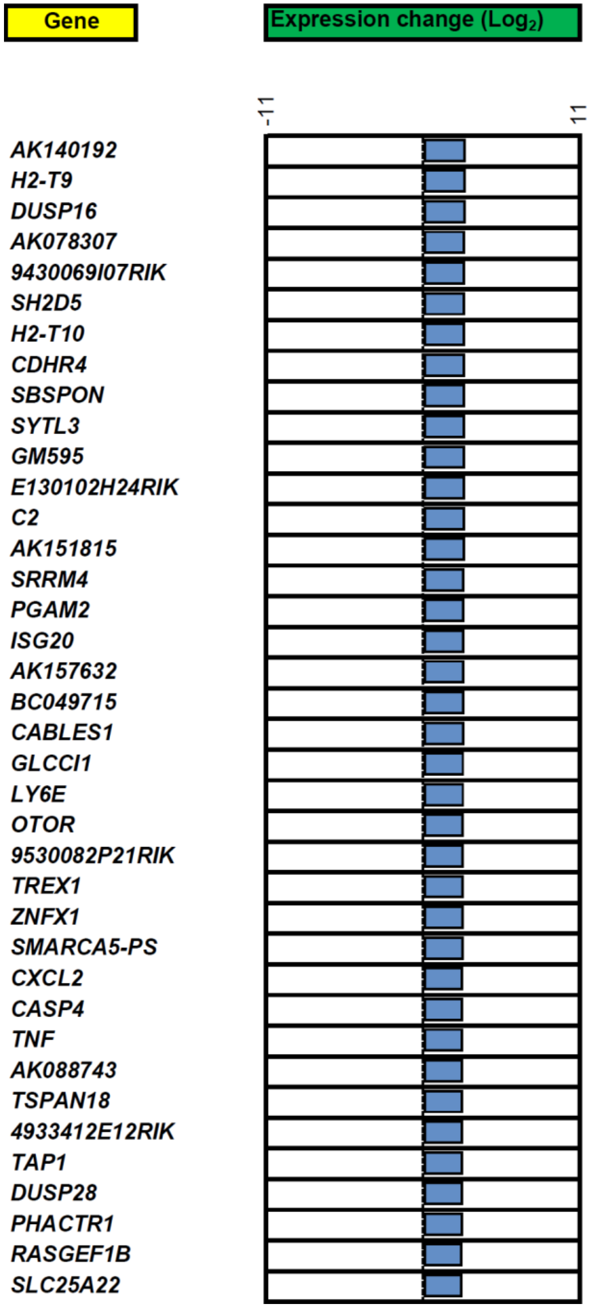
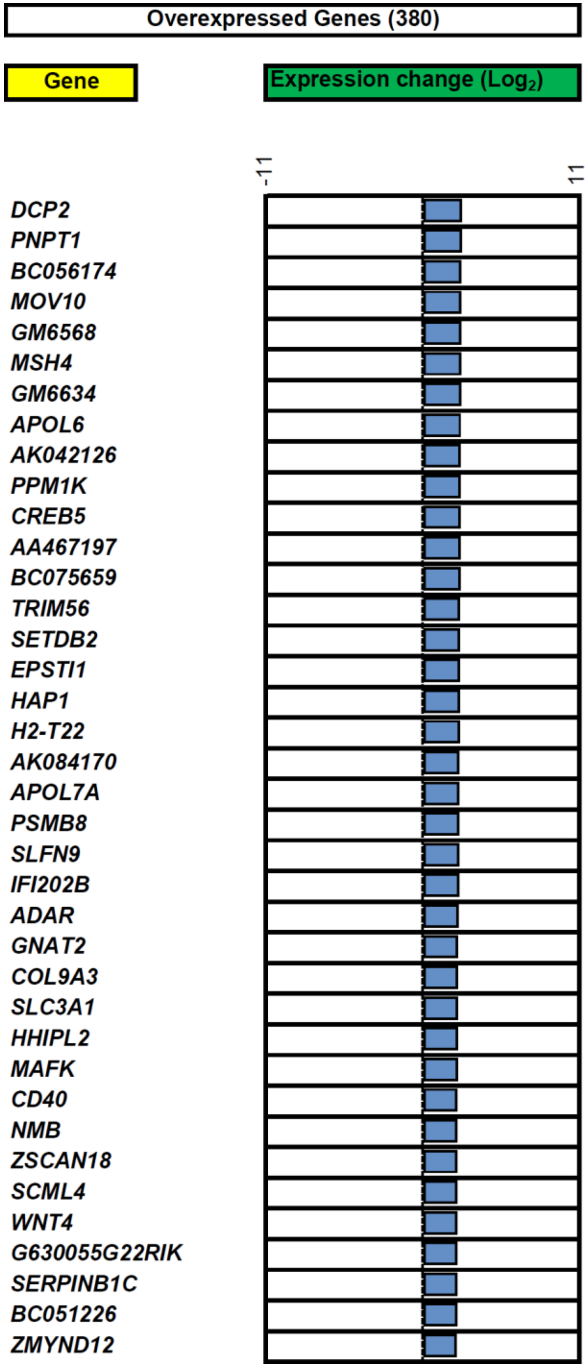


Figure C (cont'd)



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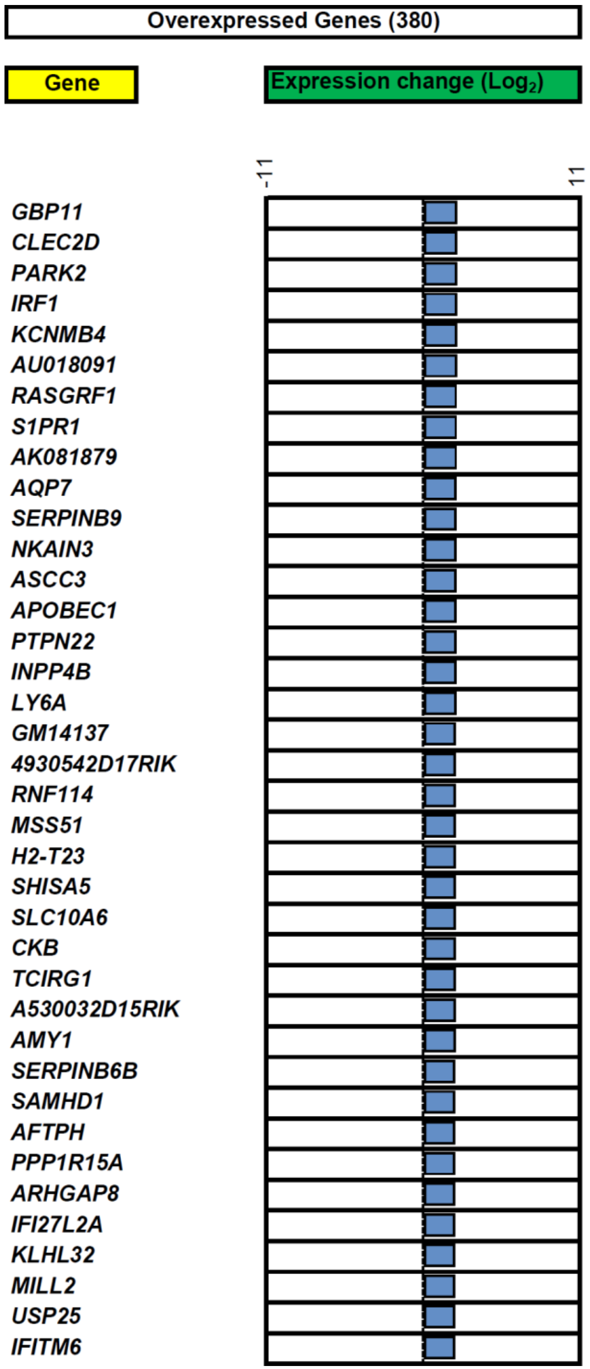


Figure C (cont’d)

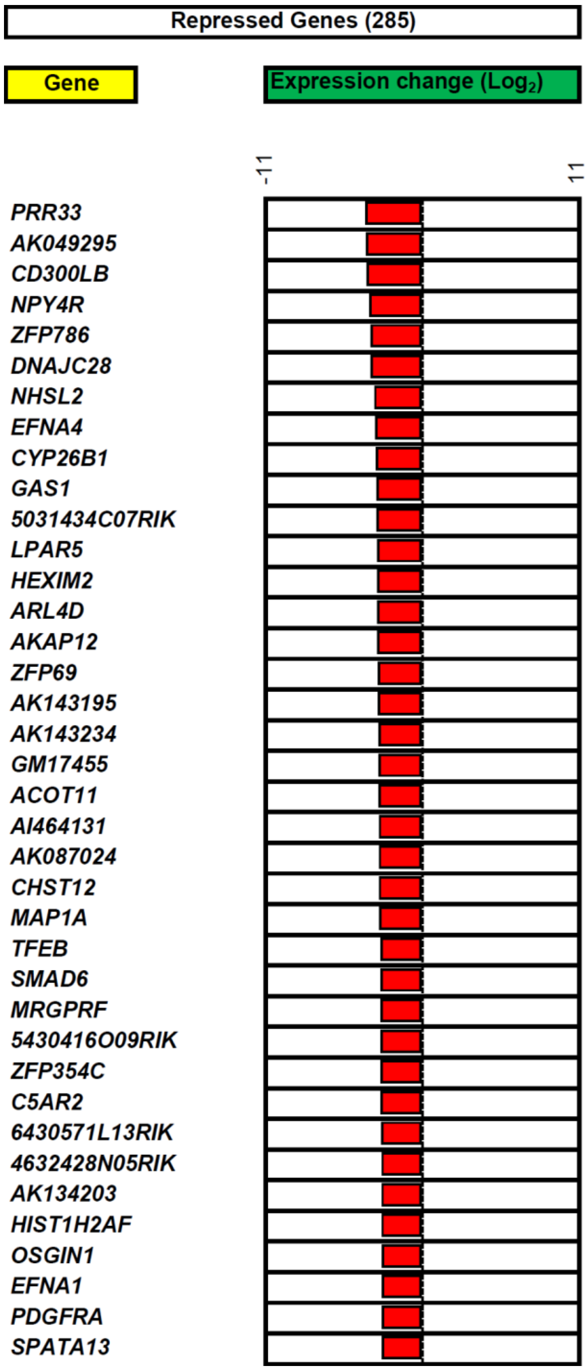


Figure C (cont’d)

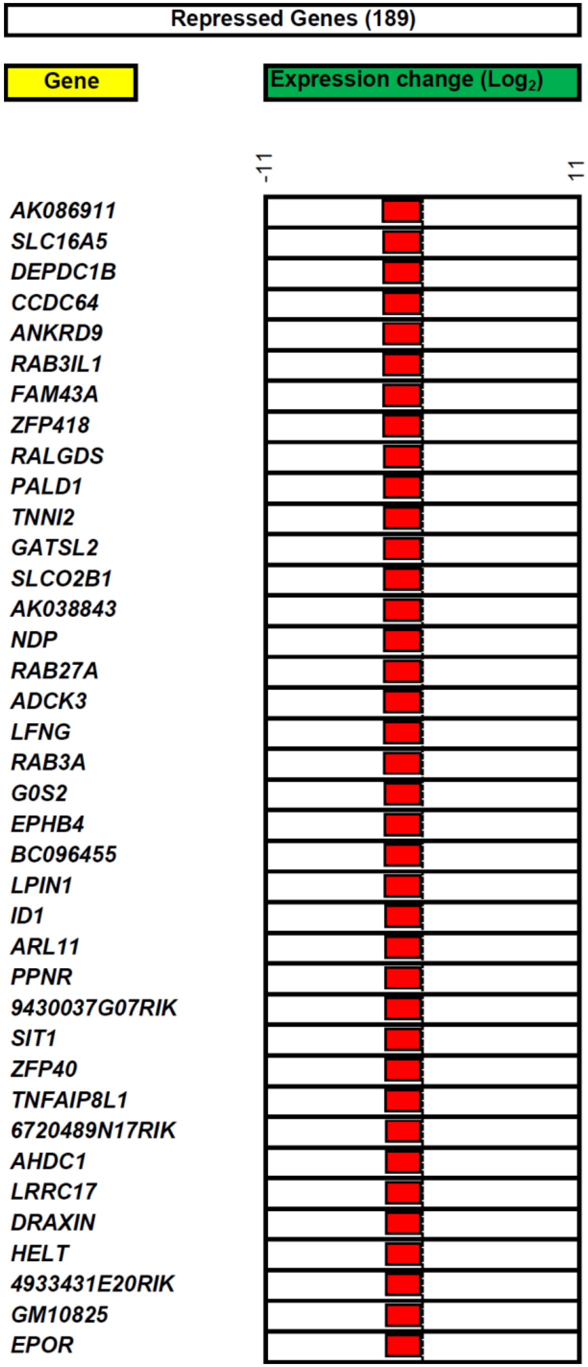


Figure C (cont'd)

Repressed Genes (189)

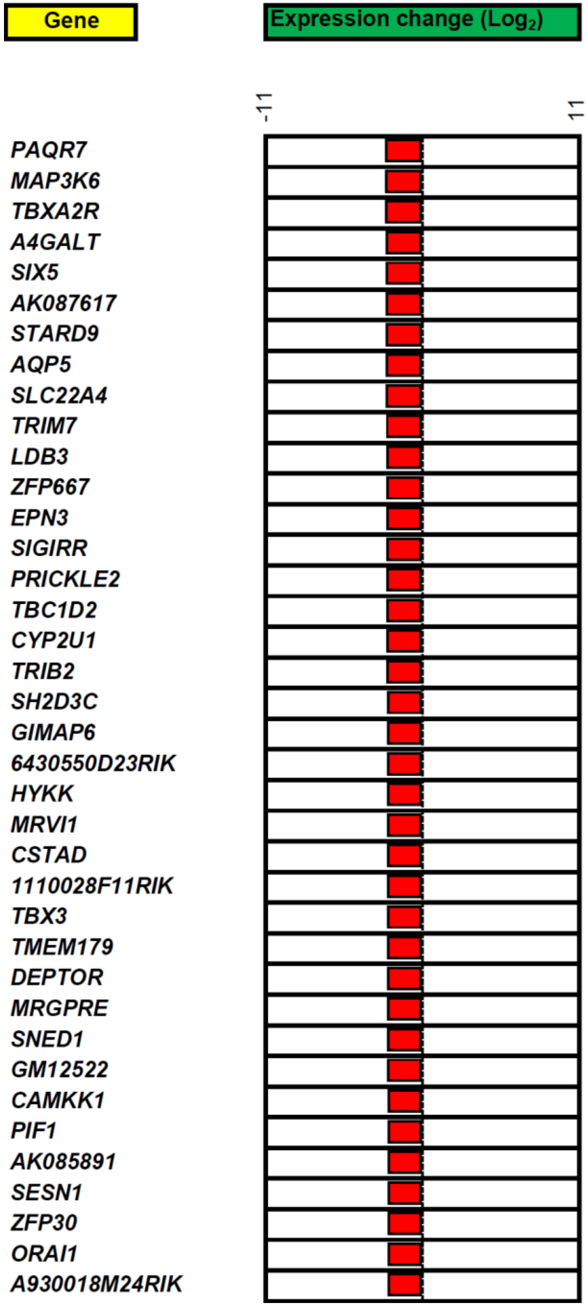


Figure C (cont'd)

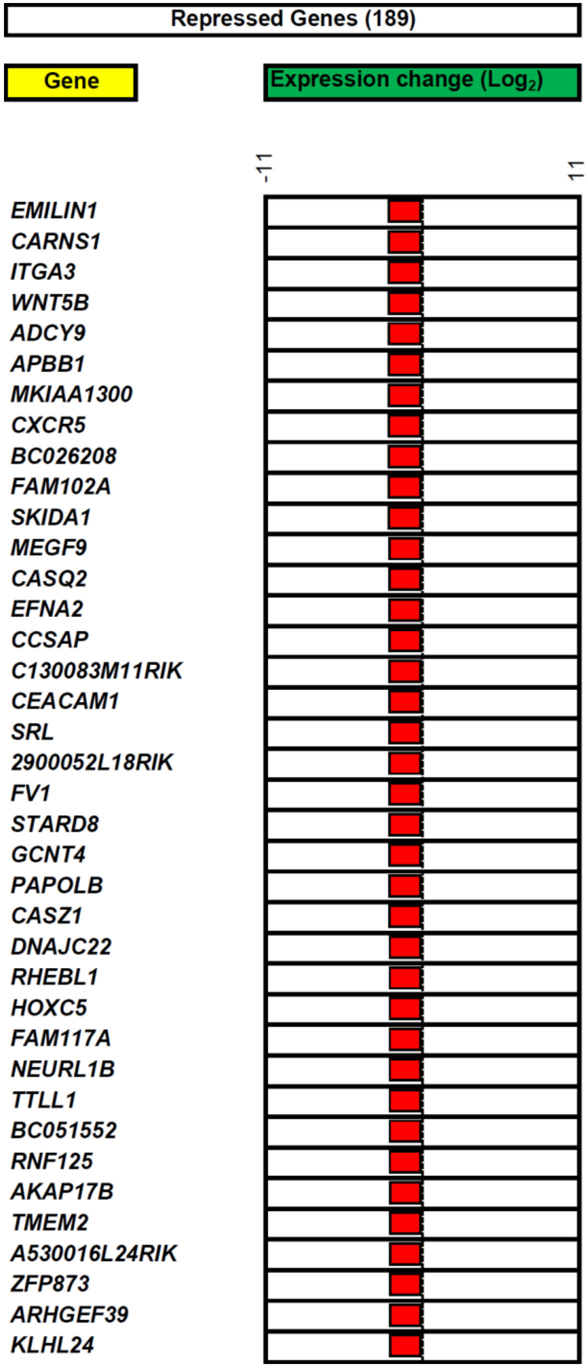


Figure C (cont'd)

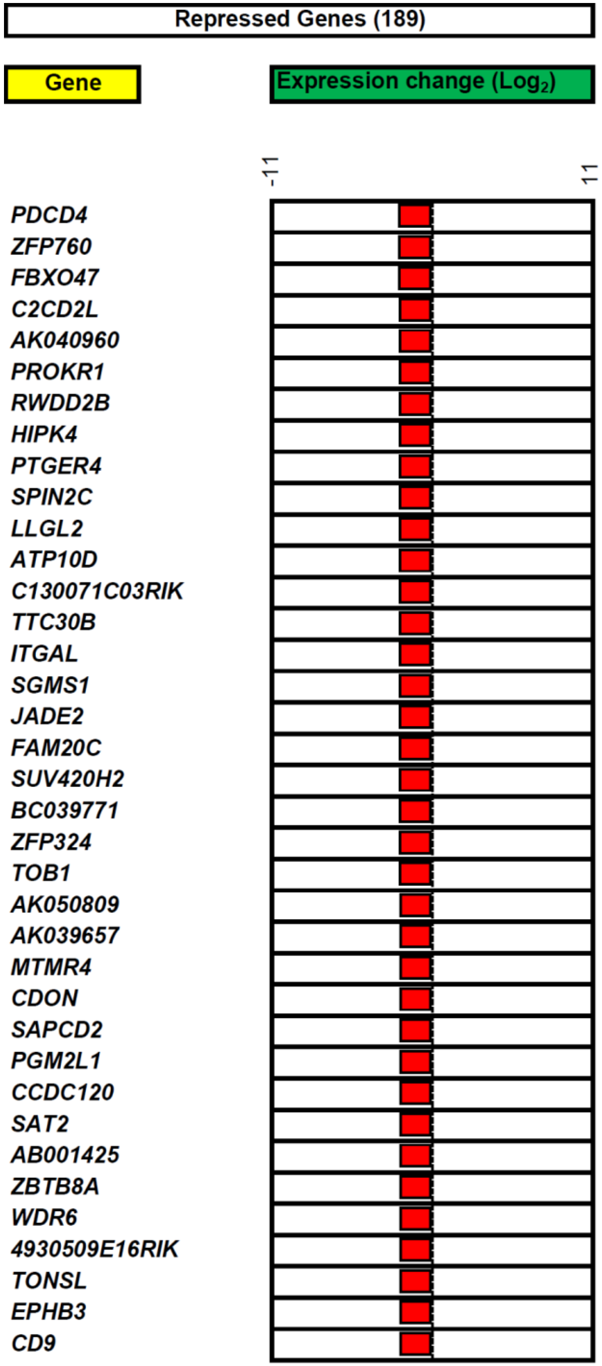


Figure C (cont’d)

Figure D. Overexpressed genes interaction network during reovirus infection.

Interaction network for the 380 cellular genes for which the expression was upregulated upon viral infection. The network was determined by uploading the genes into STRING. Solid lines: direct known interactions; dashed lines: suspected or indirect interactions.

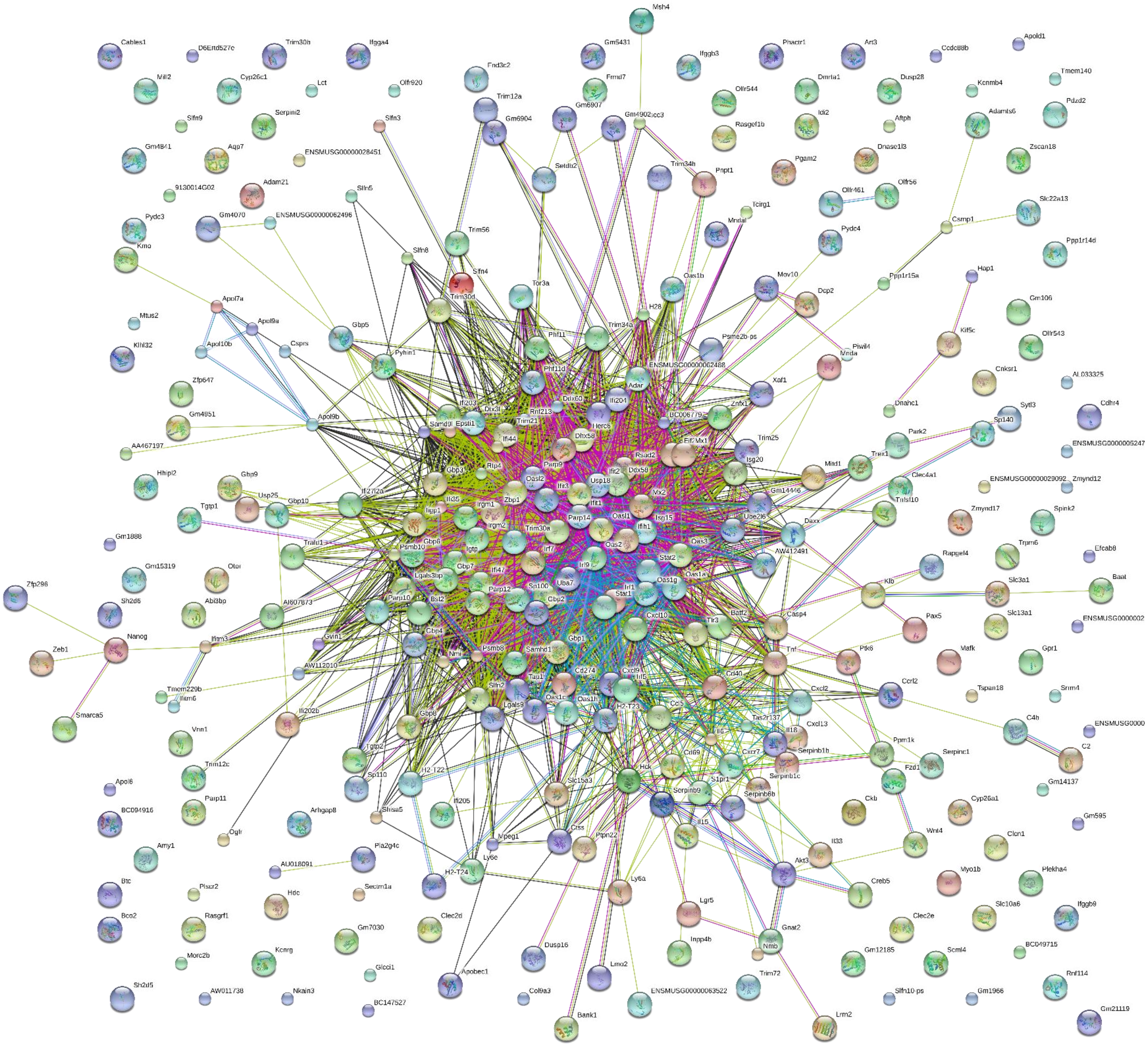


Figure D

Figure E. 240 ASEs are modified during viral infection with reovirus.

Heatmap representation with a sequential color scale of the 240 ASEs that are differentially spliced upon viral infection. RNA sequencing was done in triplicate for both the uninfected (mock) and infected cells. Darker shades indicates high ΔPSI values and lighter shades indicates low ΔPSI values between infected and mock cells. More than one ASEs can impact the same gene.

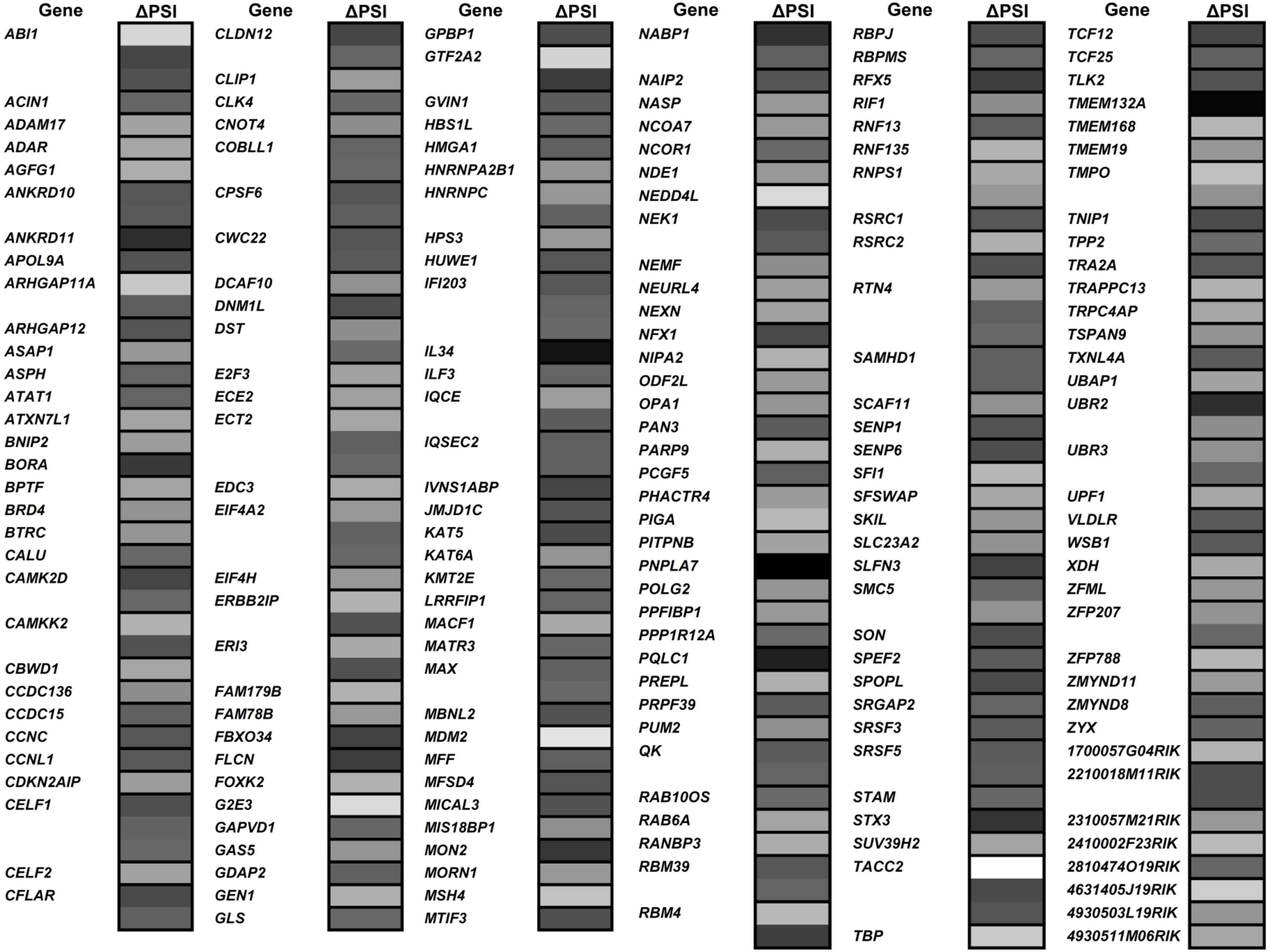

Gene
ΔPSI
ABI1
ACIN1
ADAM17
ADAR
AGFG1
ANKRD10
ANKRD11
APOL9A
ARHGAP11A
ARHGAP12
ASAP1
ASPH
ATAT1
ATXN7L1
BNIP2
BORA
BPTF
BRD4
BTRC
CALU
CAMK2D
CAMKK2
CBWD1
CCDC136
CCDC15
CCNC
CCNL1
CDKN2AIP
CELF1
CELF2
CFLAR
Gene
ΔPSI
CLDN12
CLIP1
CLK4
CNOT4
COBLL1
CPSF6
CWC22
DCAF10
DNM1L
DST
E2F3
ECE2
ECT2
EDC3
EIF4A2
EIF4H
ERBB2IP
ERI3
FAM179B
FAM78B
FBXO34
FLCN
FOXK2
G2E3
GAPVD1
GAS5
GDAP2
GEN1
GLS
Gene
ΔPSI
GPBP1
GTF2A2
GVIN1
HBS1L
HMGA1
HNRNPA2B1
HNRNPC
HPS3
HUWE1
IFI203
IL34
ILF3
IQCE
IQSEC2
IVNS1ABP
JMJD1C
KAT5
KAT6A
KMT2E
LRRFIP1
MACF1
MATR3
MAX
MBNL2
MDM2
MFF
MFSD4
MICAL3
MIS18BP1
MON2
MORN1
MSH4
MTIF3
Gene
ΔPSI
NABP1
NAIP2
NASP
NCOA7
NCOR1
NDE1
NEDD4L
NEK1
NEMF
NEURL4
NEXN
NFX1
NIPA2
ODF2L
OPA1
PAN3
PARP9
PCGF5
PHACTR4
PIGA
PITPNB
PNPLA7
POLG2
PPFIBP1
PPP1R12A
PQLC1
PREPL
PRPF39
PUM2
QK
RAB10OS
RAB6A
RANBP3
RBM39
RBM4
Gene
ΔPSI
RBPJ
RBPMS
RFX5
RIF1
RNF13
RNF135
RNPS1
RSRC1
RSRC2
RTN4
SAMHD1
SCAF11
SENP1
SENP6
SFI1
SFSWAP
SKIL
SLC23A2
SLFN3
SMC5
SON
SPEF2
SPOPL
SRGAP2
SRSF3
SRSF5
STAM
STX3
SUV39H2
TACC2
TBP
Gene
ΔPSI
TCF12
TCF25
TLK2
TMEM132A
TMEM168
TMEM19
TMPO
TNIP1
TPP2
TRA2A
TRAPPC13
TRPC4AP
TSPAN9
TXNL4A
UBAP1
UBR2
UBR3
UPF1
VLDLR
WSB1
XDH
ZFML
ZFP207
ZFP788
ZMYND11
ZMYND8
ZYX
1700057G04RIK
2210018M11RIK
2310057M21RIK
2410002F23RIK
2810474O19RIK
4631405J19RIK
4930503L19RIK
4930511M06RIK

Figure E

Figure F. Profiling of the cellular alternative splicing landscape during reovirus infection.

(A) Interaction network for genes encoding the 240 differentially spliced transcripts upon viral infection. The network was determined by uploading the corresponding genes into STRING. Solid lines: direct known interactions; dashed lines: suspected or indirect interactions.

(B) Gene ontology analysis of the 240 differentially spliced transcripts upon viral infection. The corresponding genes were imported into the DAVID gene ontology suite of programs at the NIAID. Ontological functions were determined determined for biological processes using all ASEs caracterized as background.

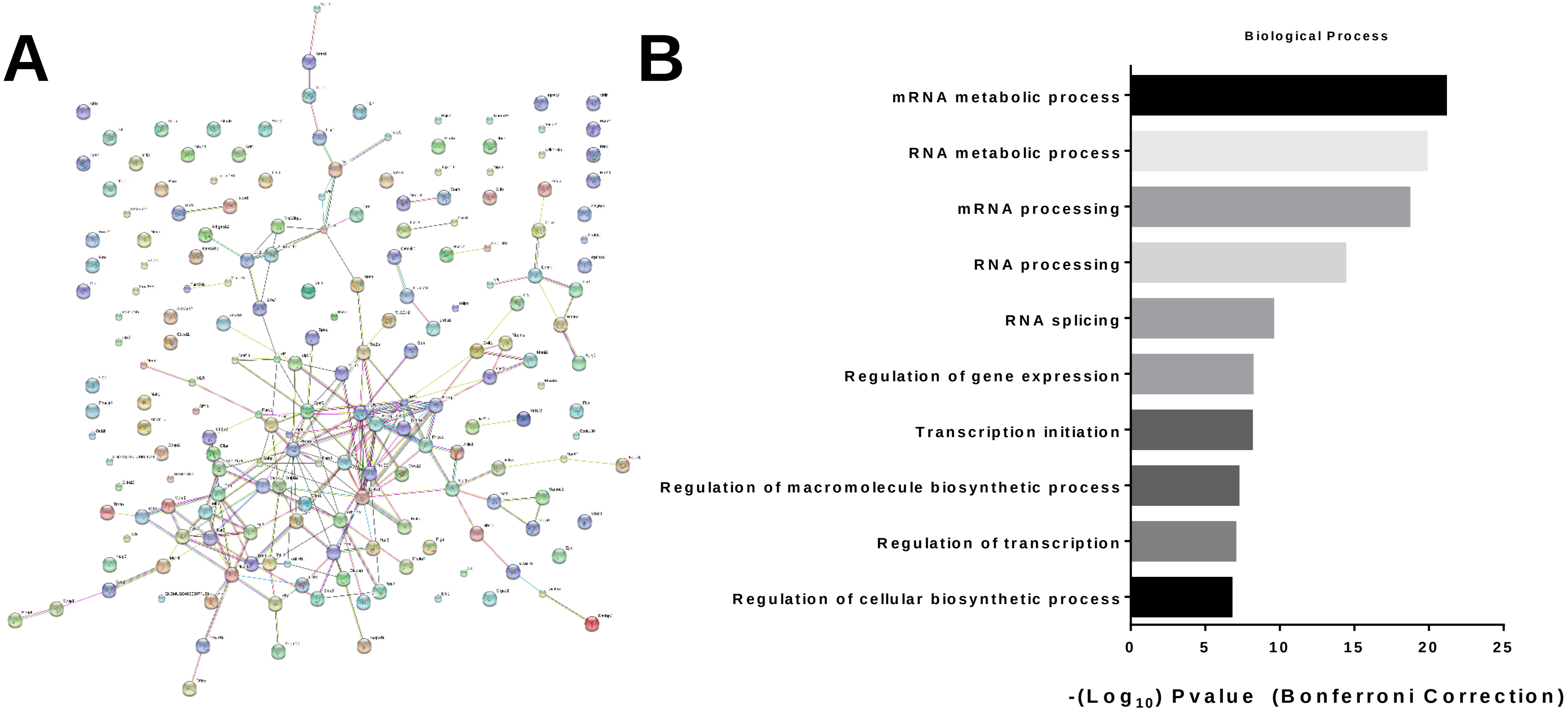

A
B
Biological Process
mRNA metabolic process
RNA metabolic process
mRNA processing
RNA processing
RNA splicing
Regulation of gene expression
Transcription initiation
Regulation of macromolecule biosynthetic process
Regulation of transcription
Regulation of cellular biosynthetic process
0
5
10
15
20
25
-(Log$_{10}$) Pvalue (Bonferroni Correction)

Figure F

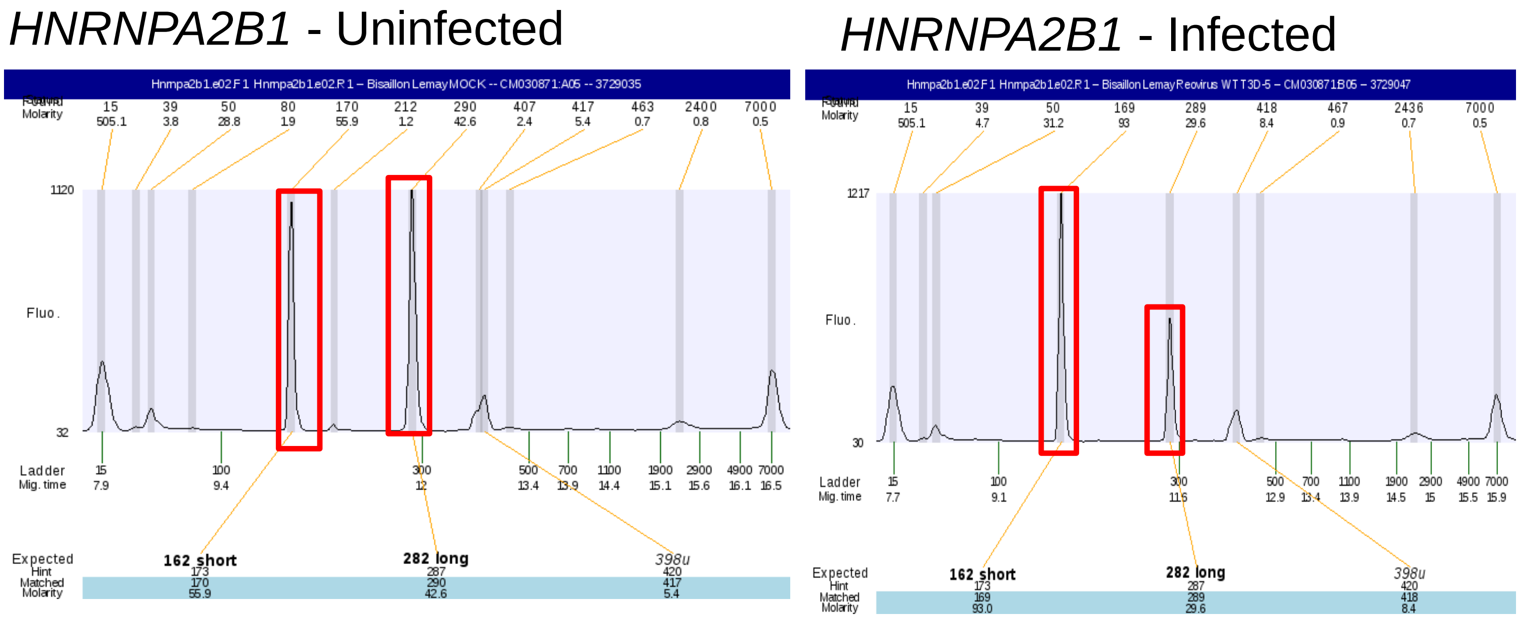
Figure G. Additional validation of ASEs dysregulated in infected cells.

(A, C, E) Overview of two isoforms encoded by the *HNRNPA2B1, EIF4A2,* and *IL34* genes. Exons are depicted in red and and the intervening introns are shown as thin black lines (not to scale). The primers used to detect the ASEs by RT-PCR assays are shown in gray and the sizes of the expected amplicons (162 nt and 282 nt, 112 nt and 219 nt, 256 nt and 374 nt, respectively) are also indicated. The genomic coordinates of the two isoforms are also indicated.

(B, D, F) Cellular mRNAs isolated from both uninfected and infected cells were analyzed by RT-PCR using specific primers to detect both forms of the ASE encoded on various genes. The amplified products were analyzed by automated chip-based microcapillary electrophoresis. Capillary electrophoregrams of the PCR reactions are shown. The positions and the amplitude of the detected amplicons are highlighted by red boxes. The positions of the internal markers are also indicated.

A

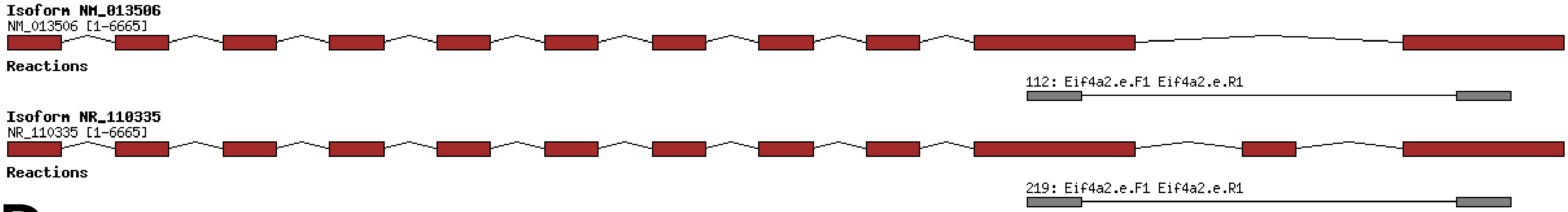
B


HNRNPA2B1 - Uninfected

HNRNPA2B1 - Infected

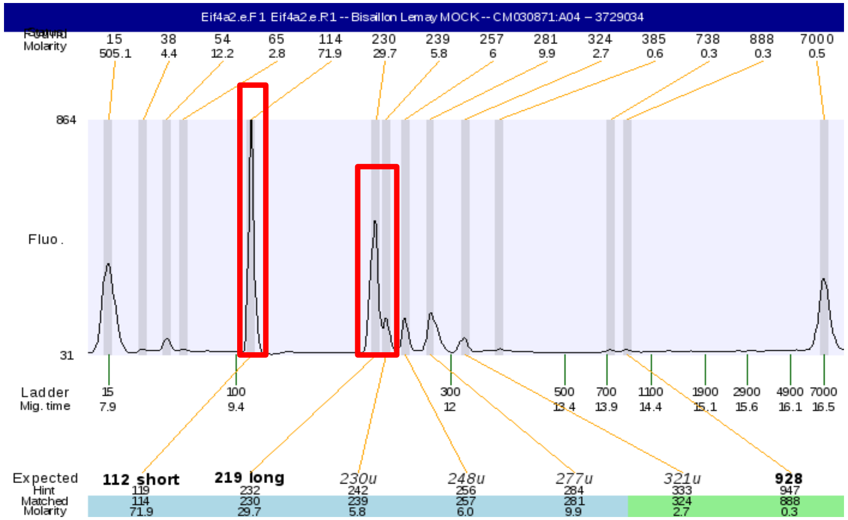
Figure G

C



D

EIF4A2 - Uninfected



EIF4A2 – Infected

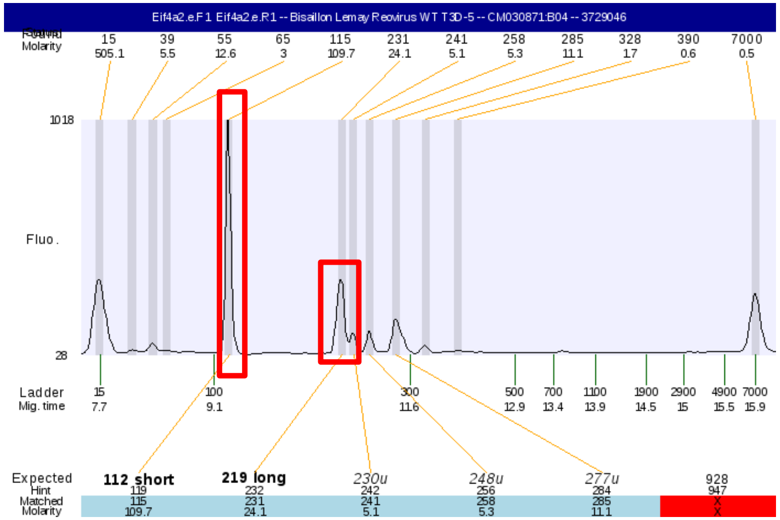
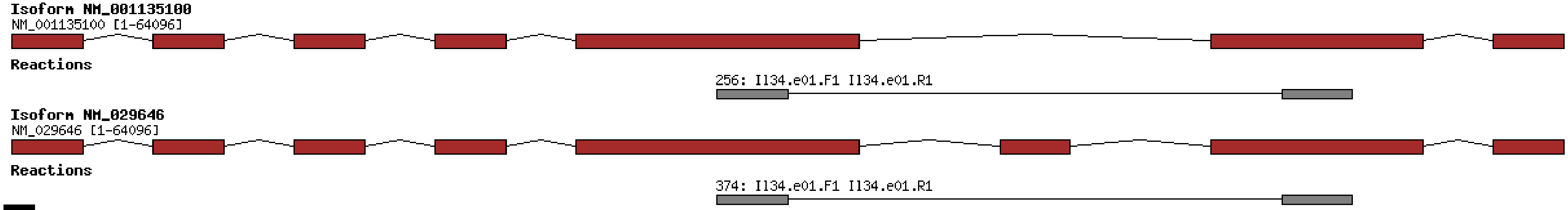


Figure G (cont’d)

E



F

IL34 – Uninfected

IL34 – Infected

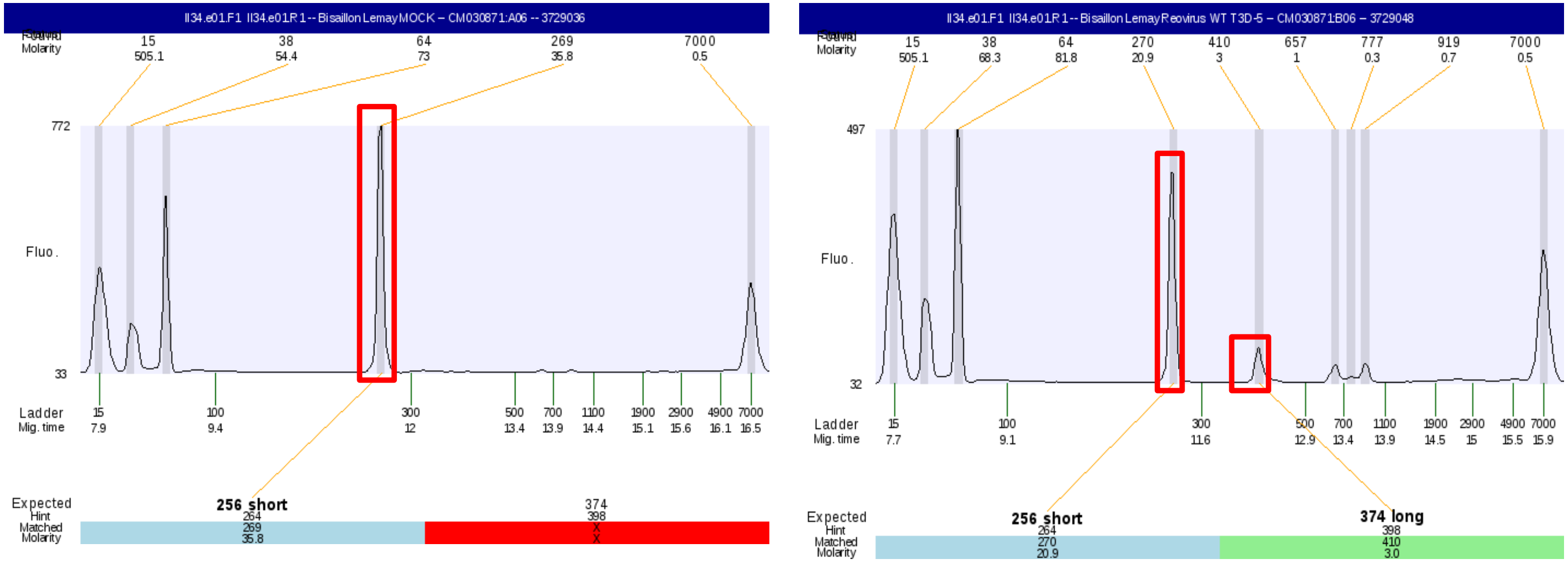


Figure G (cont'd)

Figure H. List of the differentially ASEs associated with viral infection.

The list displays the 240 differentially spliced transcripts with the corresponding Delta PSI values, the associated gene expression (in Log_2), and the associated biological processes.

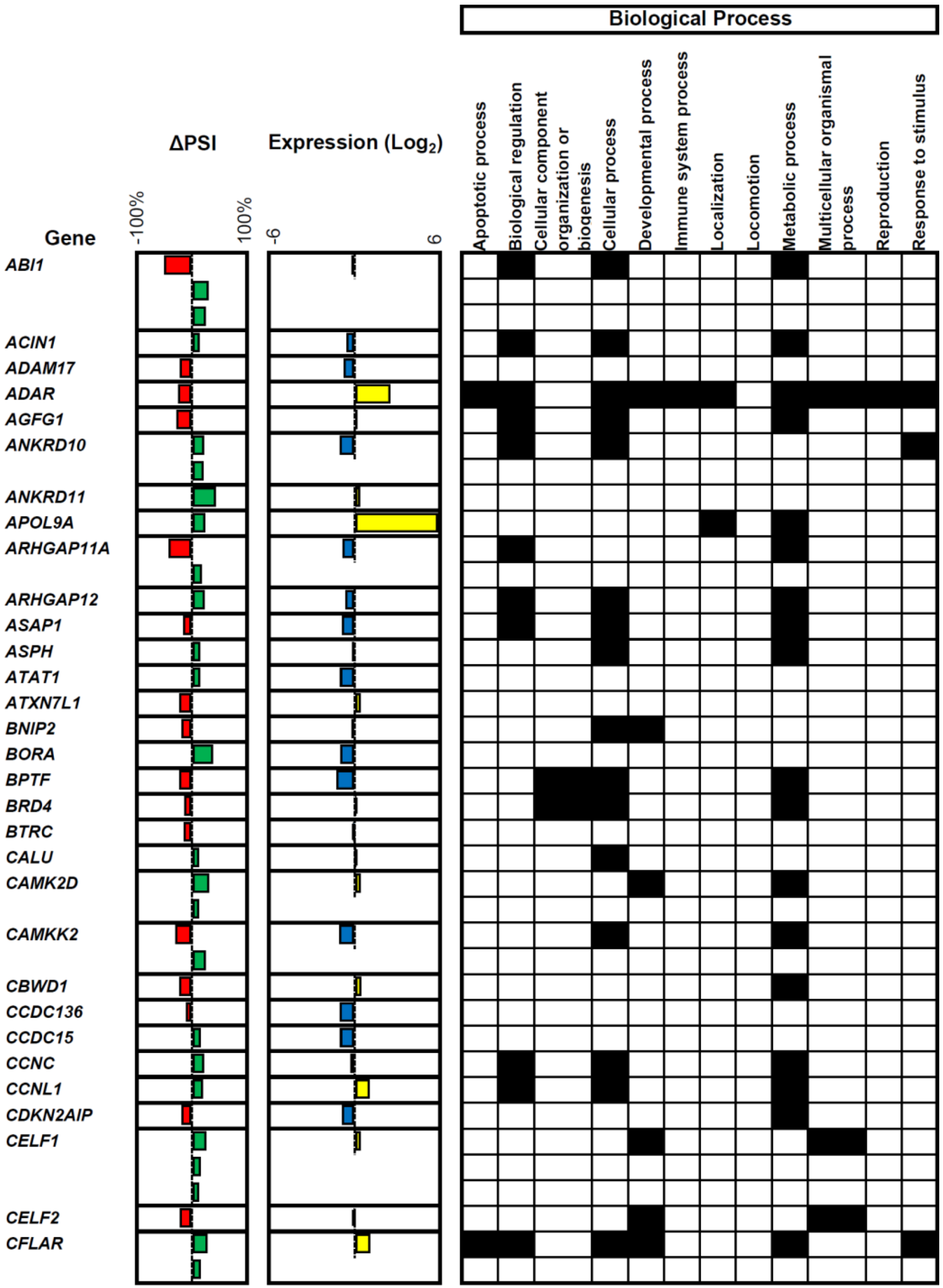
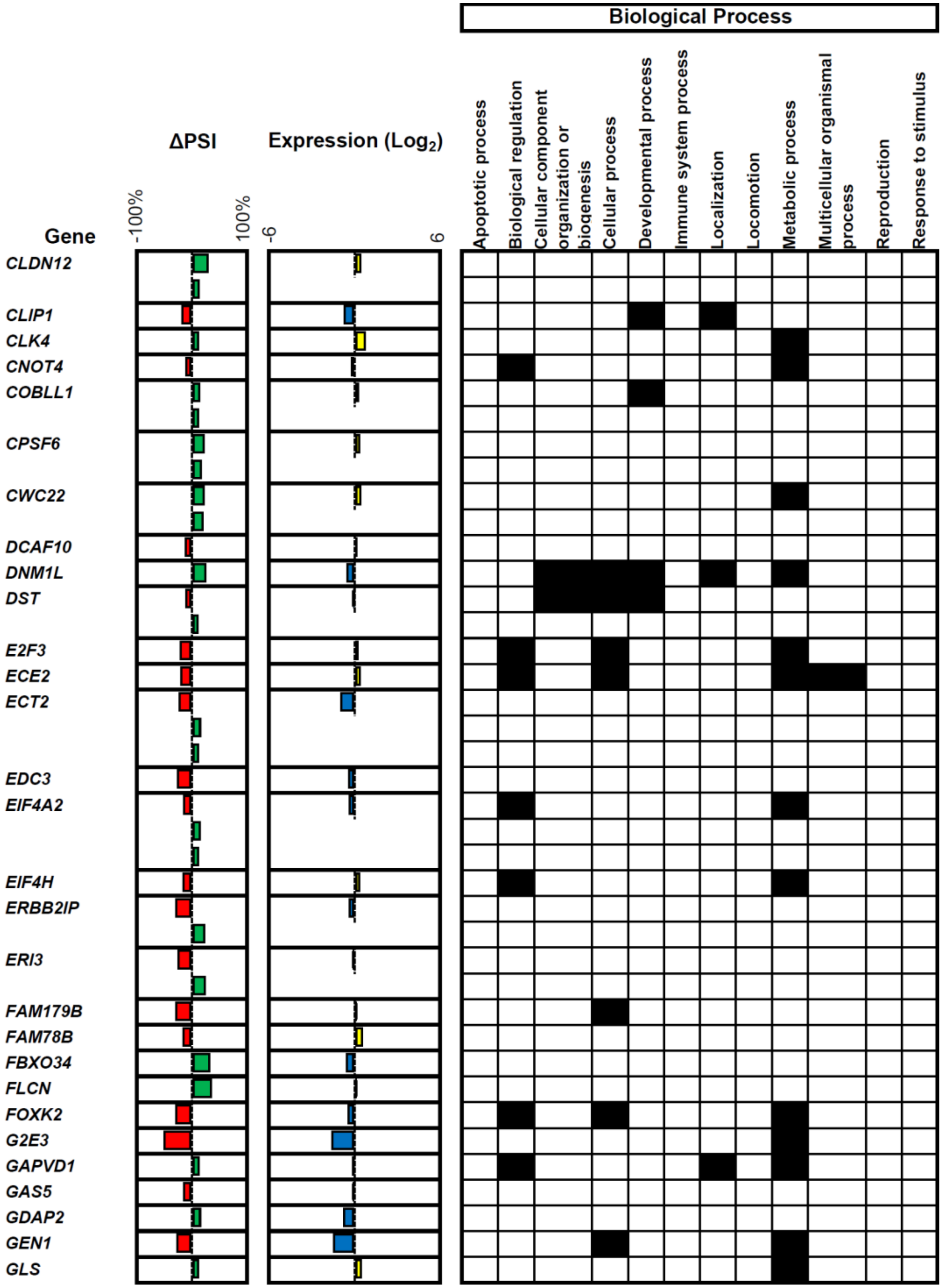

Gene
ΔPSI
-100%
100%
Expression (Log2)
-6
6
Biological Process
Apoptotic process
Biological regulation
Cellular component organization or biogenesis
Cellular process
Developmental process
Immune system process
Localization
Locomotion
Metabolic process
Multicellular organismal process
Reproduction
Response to stimulus
ABI1
ACIN1
ADAM17
ADAR
AGFG1
ANKRD10
ANKRD11
APOL9A
ARHGAP11A
ARHGAP12
ASAP1
ASPH
ATAT1
ATXN7L1
BNIP2
BORA
BPTF
BRD4
BTRC
CALU
CAMK2D
CAMKK2
CBWD1
CCDC136
CCDC15
CCNC
CCNL1
CDKN2AIP
CELF1
CELF2
CFLAR

Figure H



Gene	Apoptotic process	Biological regulation	Cellular component organization or biogenesis	Cellular process	Developmental process	Immune system process	Localization	Locomotion	Metabolic process	Multicellular organismal process	Reproduction	Response to stimulus
CLDN12												
CLIP1					■		■					
CLK4									■			
CNOT4		■							■			
COBLL1					■							
CPSF6												
CWC22									■			
DCAF10												
DNM1L			■	■	■		■		■			
DST			■	■	■							
E2F3		■		■					■			
ECE2		■		■					■	■		
ECT2												
EDC3												
EIF4A2		■							■			
EIF4H		■							■			
ERBB2IP												
ERI3												
FAM179B				■								
FAM78B												
FBXO34												
FLCN												
FOXK2		■		■					■			
G2E3									■			
GAPVD1		■					■		■			
GAS5												
GDAP2												
GEN1				■					■			
GLS									■			

Figure H (cont’d)

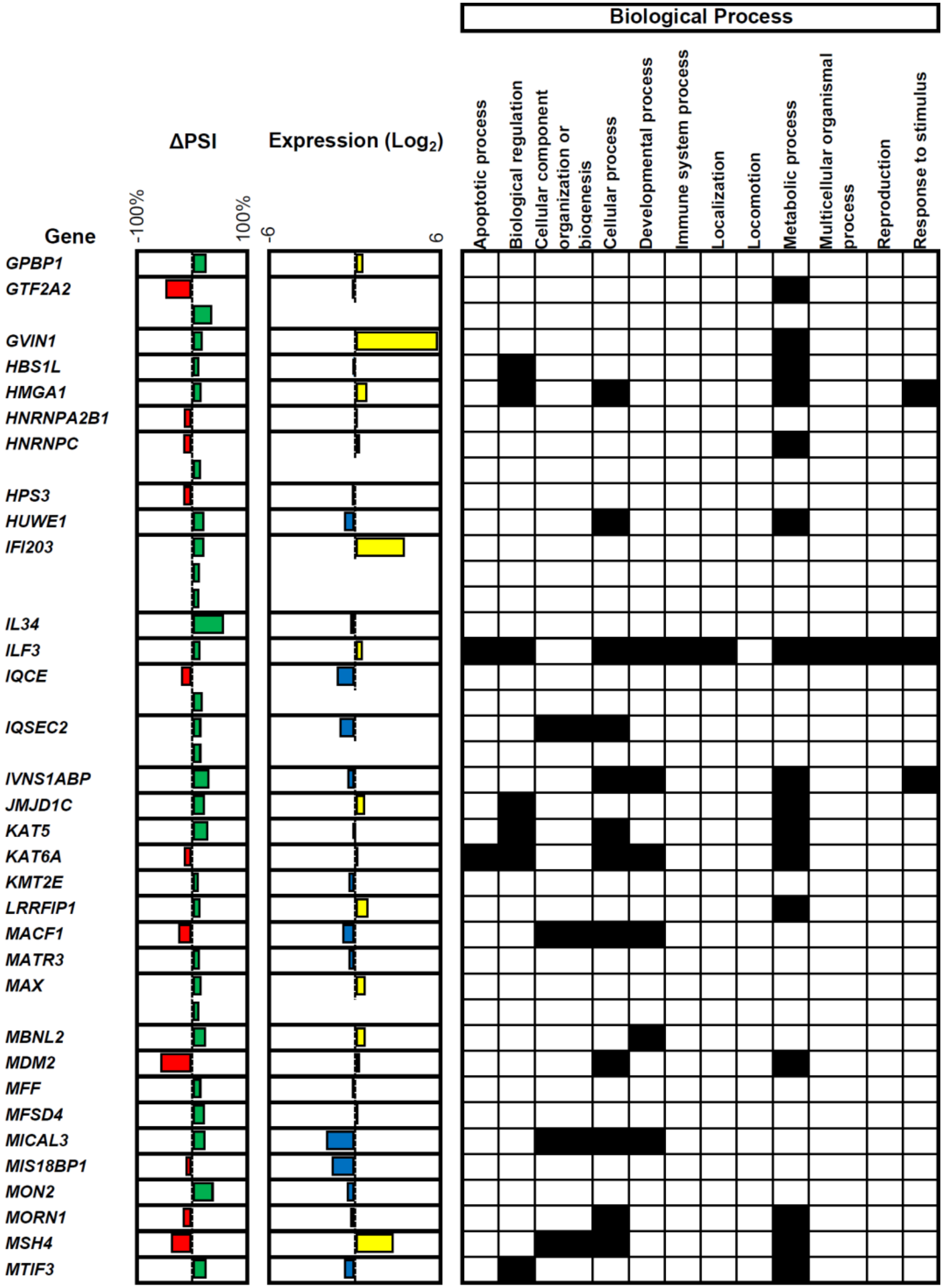
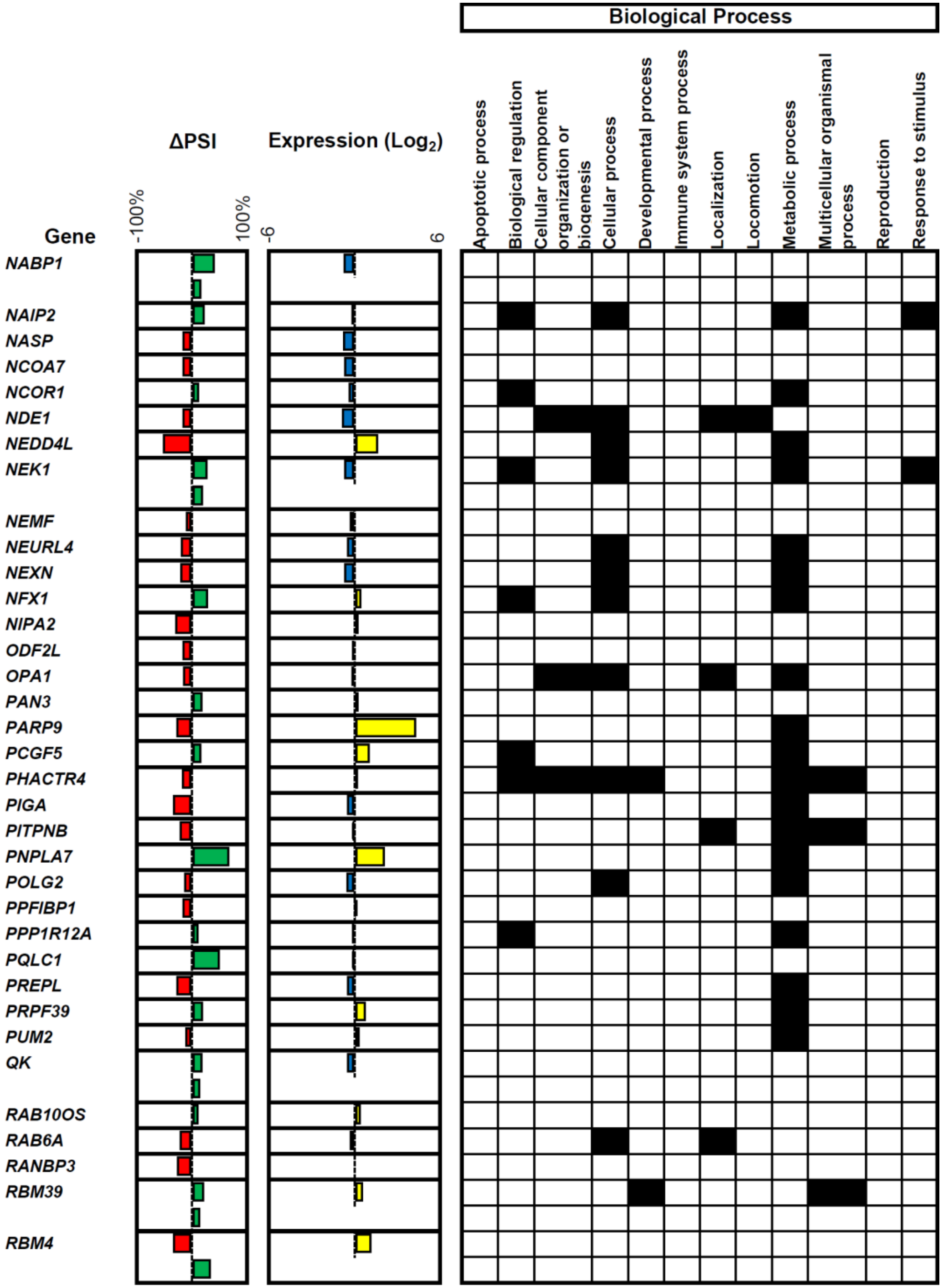


Figure H (cont'd)



Gene	Apoptotic process	Biological regulation	Cellular component organization or biogenesis	Cellular process	Developmental process	Immune system process	Localization	Locomotion	Metabolic process	Multicellular organismal process	Reproduction	Response to stimulus
NABP1												
NAIP2		■		■					■			■
NASP												
NCOA7												
NCOR1		■							■			
NDE1			■	■			■	■				
NEDD4L				■					■			
NEK1		■		■					■			■
NEMF												
NEURL4				■					■			
NEXN				■					■			
NFX1		■		■					■			
NIPA2												
ODF2L												
OPA1			■	■			■		■			
PAN3												
PARP9									■			
PCGF5		■							■			
PHACTR4		■	■	■	■				■	■		
PIGA									■			
PITPNB							■		■	■		
PNPLA7									■			
POLG2				■					■			
PPFIBP1												
PPP1R12A		■							■			
PQLC1												
PREPL									■			
PRPF39									■			
PUM2									■			
QK												
RAB10OS												
RAB6A				■			■					
RANBP3												
RBM39					■					■		
RBM4												

Figure H (cont'd)

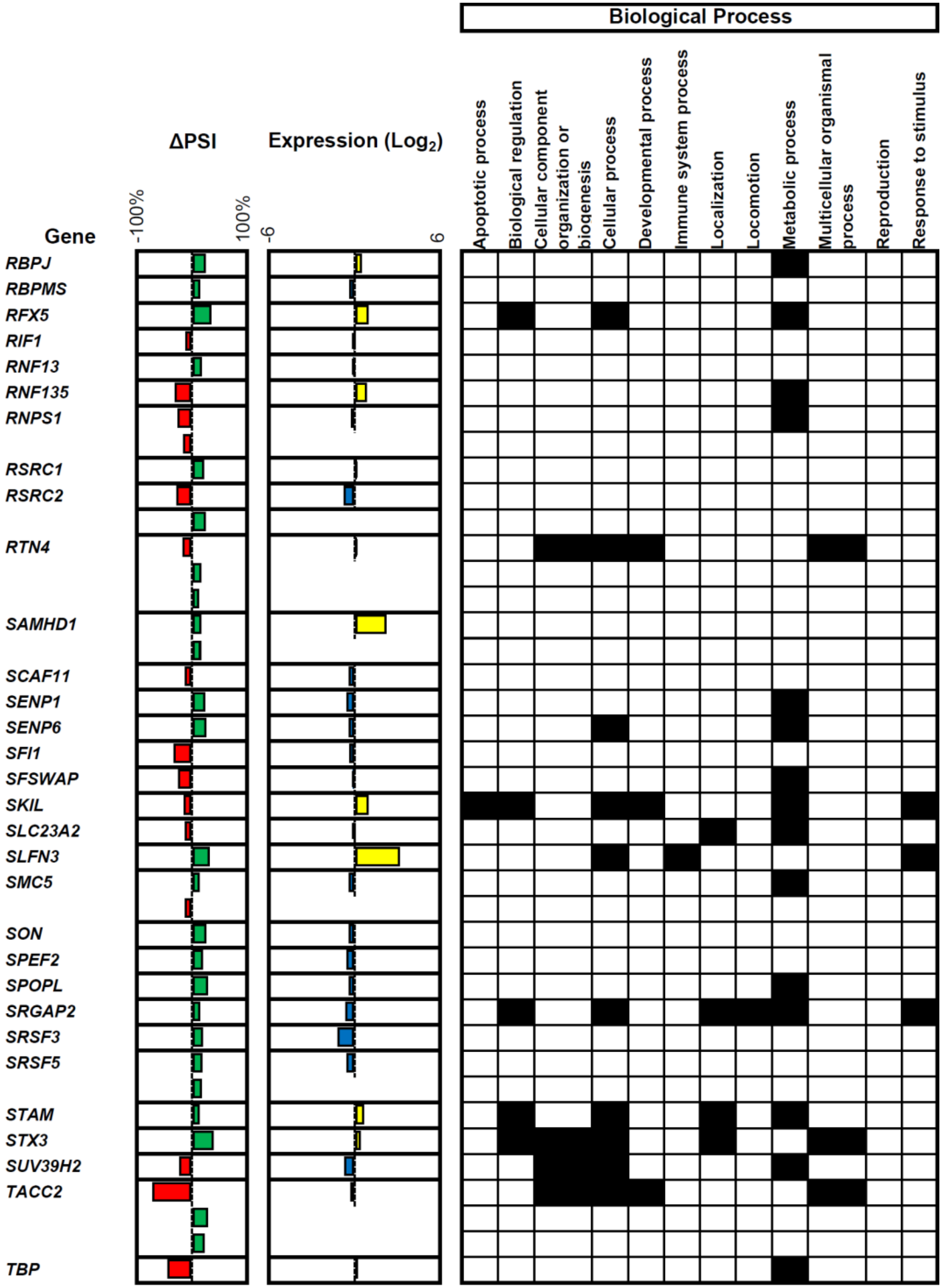
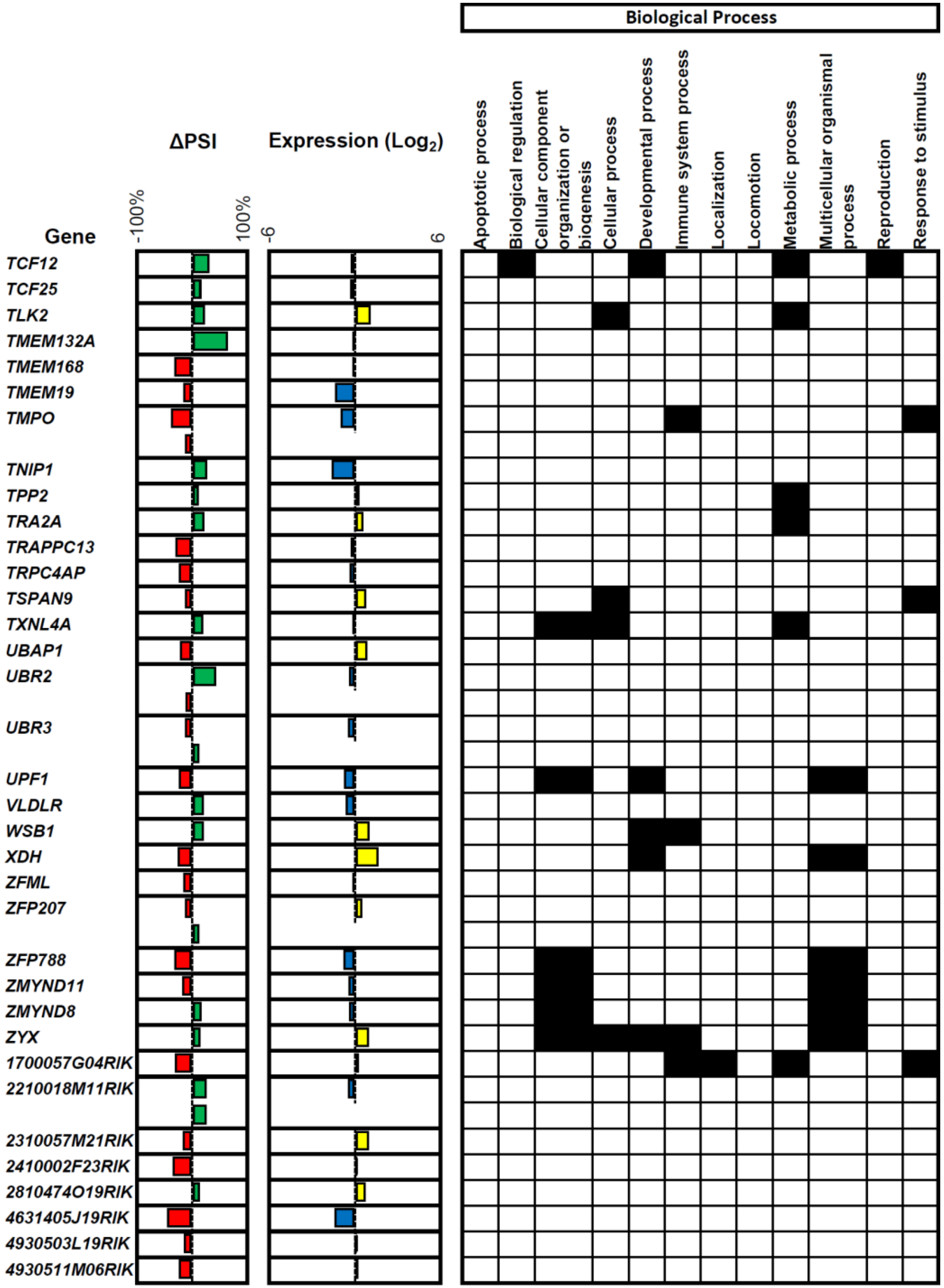


Figure H (cont’d)



Gene	Apoptotic process	Biological regulation	Cellular component organization or biogenesis	Cellular process	Developmental process	Immune system process	Localization	Locomotion	Metabolic process	Multicellular organismal process	Reproduction	Response to stimulus
TCF12		■			■				■		■	
TCF25												
TLK2				■					■			
TMEM132A												
TMEM168												
TMEM19												
TMPO						■						■
TNIP1												
TPP2									■			
TRA2A									■			
TRAPPC13												
TRPC4AP												
TSPAN9				■								■
TXNL4A			■	■					■			
UBAP1												
UBR2												
UBR3												
UPF1			■		■					■		
VLDLR												
WSB1					■	■						
XDH					■					■		
ZFML												
ZFP207												
ZFP788			■							■		
ZMYND11			■							■		
ZMYND8			■							■		
ZYX			■	■	■	■				■		
1700057G04RIK						■	■		■			■
2210018M11RIK												
2310057M21RIK												
2410002F23RIK												
2810474O19RIK												
4631405J19RIK												
4930503L19RIK												
4930511M06RIK												

Figure H (cont’d)

Figure I. Common ASEs modified between WT reovirus and P4L-12 mutant show a strong correlation.

Correlation between PSI values for WT and P4L-12 mutant reovirus.

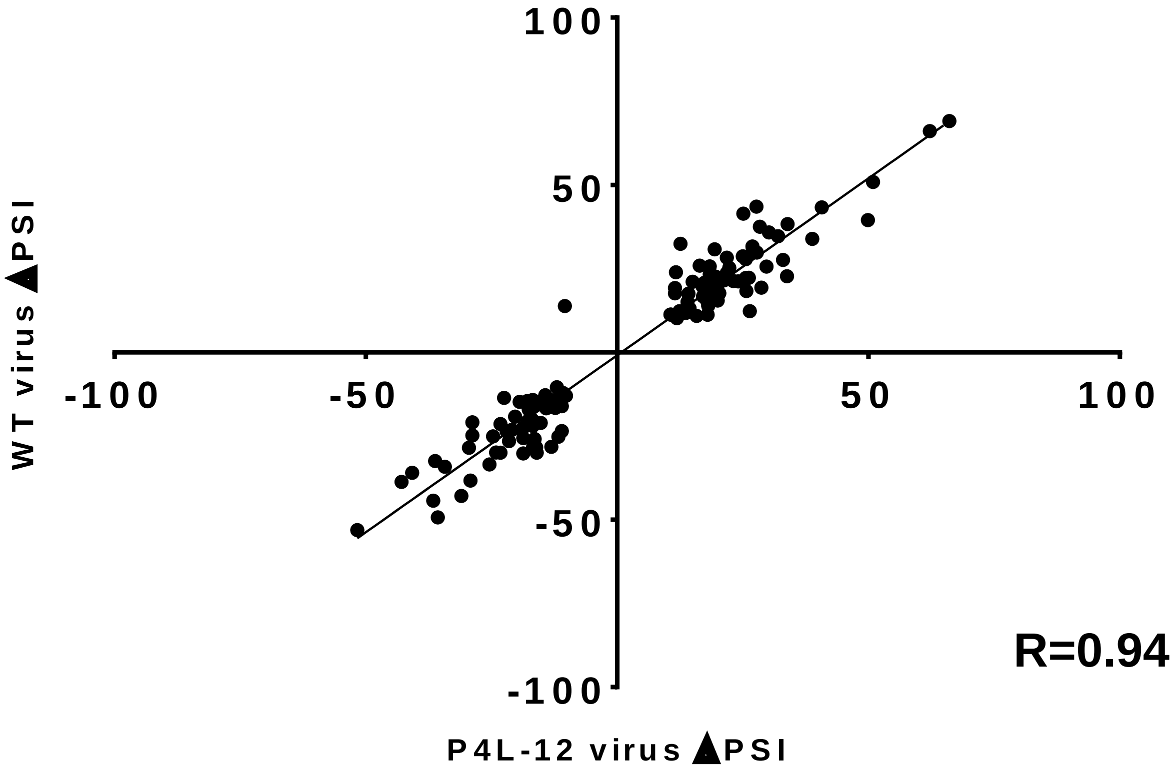


Figure I

Figure J. Analysis of the 240 differnentially spliced transcripts highlights a consensus sequence.

A significant enrichment of a 41 nt motif in the vicinity of many splice regions was found in transcripts for which AS was altered upon viral infection. The motif was found in 93 of the 240 transcripts that were differentially spliced upon viral infection (e value = $1,3e\text{-}10^{49}$). The diffentially spliced exon and its flanking introns were used to search for this motif.

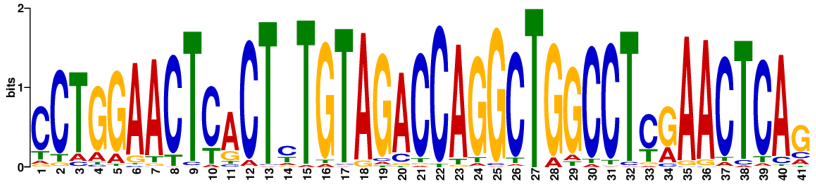

2
1
0
bits
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41

Figure J