Supplemental Material

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Supplementary Table 1: Hits from CRISPRa screen for negative regulators of the type I IFN response.

Gene	**Induced by IFN >2x** (Interferome)	**HGNC Official Full Name**
C1GALT1	Yes	core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase 1
CASC3	No	CASC3 exon junction complex subunit
ETV7	Yes	ETS variant transcription factor 7
GGT1	No	gamma-glutamyltransferase 1
GOLGA6D	No	golgin A6 family member D
IMPAD1	No	inositol monophosphatase domain containing 1
LAPTM5	No	lysosomal protein transmembrane 5
NUP153	Yes	nucleoporin 153
PCP2	No	Purkinje cell protein 2
RRM2B	No	ribonucleotide reductase regulatory TP53 inducible subunit M2B

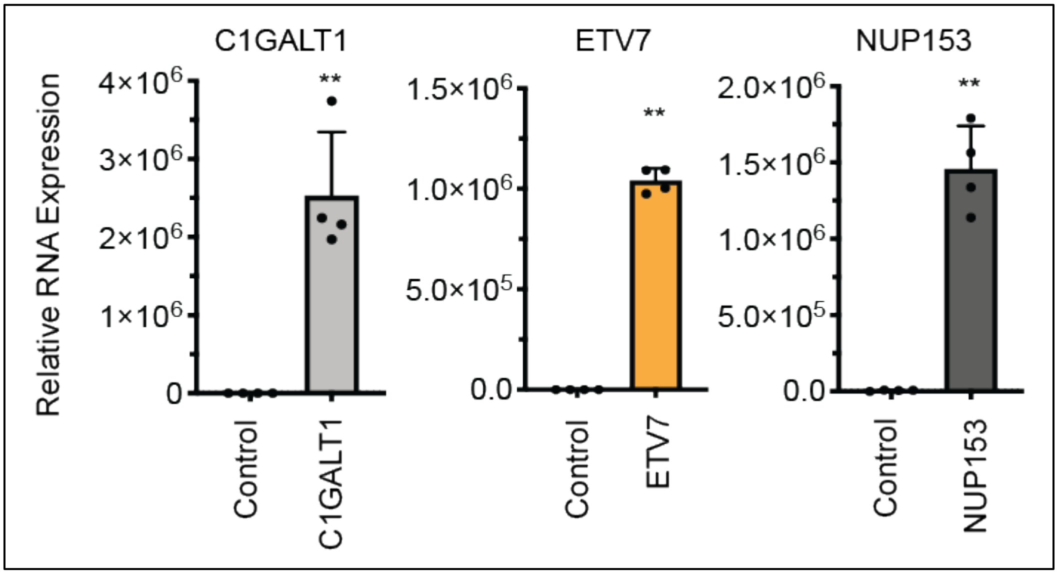
Ten hits identified using overlap between two replicate screens. Hits selected for validation were determined to be induced at least two-fold after IFN treatment in the Interferome database (1).

Supplementary Table 2: ISRE sequences identified in the literature.

Gene	ISRE sequence	Reference	Ref #
Consensus ISRE	`N GAAA NN GAAA CT`	Bluyssen et al. (1994)	(2)
BST2	`G GAAA CT GAAA CT`	Ohtomo et al. (1999)	(3)
IFI35	`G GAAA  T GAAA GT`	Yang et al. (2012)	(4)
IFIT1	`G GAAA GT GAAA CT` `G GAAA CC GAAA GG`	Bluyssen et al. (1994) Wathelet et al. (1998)	(2) (5)
IFIT2	`G GAAA GT GAAA CT`	Levy et al. (1988)	(6)
IRF9	`A GAA  CT GAAA CT`	Testoni et al. (2011)	(7)
ISG15	`G GAAA CC GAAA CT` `G GAAA GG GAAA CC`	Testoni et al. (2011) Testoni et al. (2011)	(7) (7)
OAS1	`G GAAA  C GAAA CC`	Rutherford et al. (1988)	(8)
OAS2	`G GAAA CT GAAA CT`	Wang and Floyd-Smith (1997)	(9)
OAS3	`G GAAA AC GAAA CC` `C GAAA CT GAAA GC`	Rebouillat et al. (2000) Rebouillat et al. (2000)	(10) (10)
OASL	`A GAA  TC GAAA CT`	Wang et al. (2010)	(11)

Consensus ISRE with potential ETS sites highlighted in blue. ISGs and their identified ISRE sequences with ETS sites highlighted in yellow and non-ETS sites highlighted in gray.

Supplementary Figure 1.



Supplementary Figure 1: Overexpression of screen hits for validation. RT-qPCR of three screen hits, C1GALT1, NUP153, and ETV7, in 293T cells confirms their overexpression after transfection of a pLex vector containing the respective gene. Data shown are representative of two independent experiments. P-values calculated using unpaired, two-tailed Student's t-tests (*p<0.05, **p<0.001) compared to mCherry-expressing control samples.

Supplementary Figure 2.

ETV7 KO1 genomic locus **Peptide Length**

```
WT       5′ G TAC TCT CTG CCA TGC ACC GCG GAG CAC GGG TTC GAG ATG AAC GGA CGC GCC CTC TGC AT      3′      WT
   N-term      Y   S   L   P   C   T   A   E   H   G   F   E   M   N   G   R   A   L   C      C-term  342aa

KO1 c1   5′ G TAC TCT CTG CCA TGC ACC GCG GAA GCA CGG GTT CGA GAT GAA CGG ACG CGC CCT CTG CAT     3′   trunc
   N-term      Y   S   L   P   C   T   A   E   A   R   V   R   D   E   R   T   R   P   L   H C-term  104aa

KO1 c2   5′ G TAC TCT CTG CCA TGC ACC GCG GAA GCA CGG GTT CGA GAT GAA CGG ACG CGC CCT CTG CAT     3′   trunc
   N-term      Y   S   L   P   C   T   A   E   A   R   V   R   D   E   R   T   R   P   L   H C-term  104aa
```

ETV7 KO2 genomic locus **Peptide Length**

```
WT       5′ TC CCC GCG ATG CCG CAG GCC CCC ATT GAC GGC AGG ATC GCT Ggtgagtgggaggctgttc          3′      WT
   N-term       P   A   M   P   Q   A   P   I   D   G   R   I   A                         C-term  342aa

KO2 c1   5′ TC CCC GCG ATG CCG CAG GCC CCC ATT GAC G-- --- --- -CT Ggtgagtgggaggctgttc          3′     del
   N-term       P   A   M   P   Q   A   P   I   D               A                         C-term  333aa

KO2 c2   5′ TC CCC GCG ATG CCG CAG GCC CCC ATT GAC GGC AGG --- --- --tgagtgggaggctgttc          3′     lss
   N-term       P   A   M   P   Q   A   P   I   D   G   R                                 C-term unknown
```

KEY

c1, c2 = copy 1, copy 2

UPPERCASE = exonic

lowercase = intronic

underlined = sgRNA sequence

highlighted = insertion

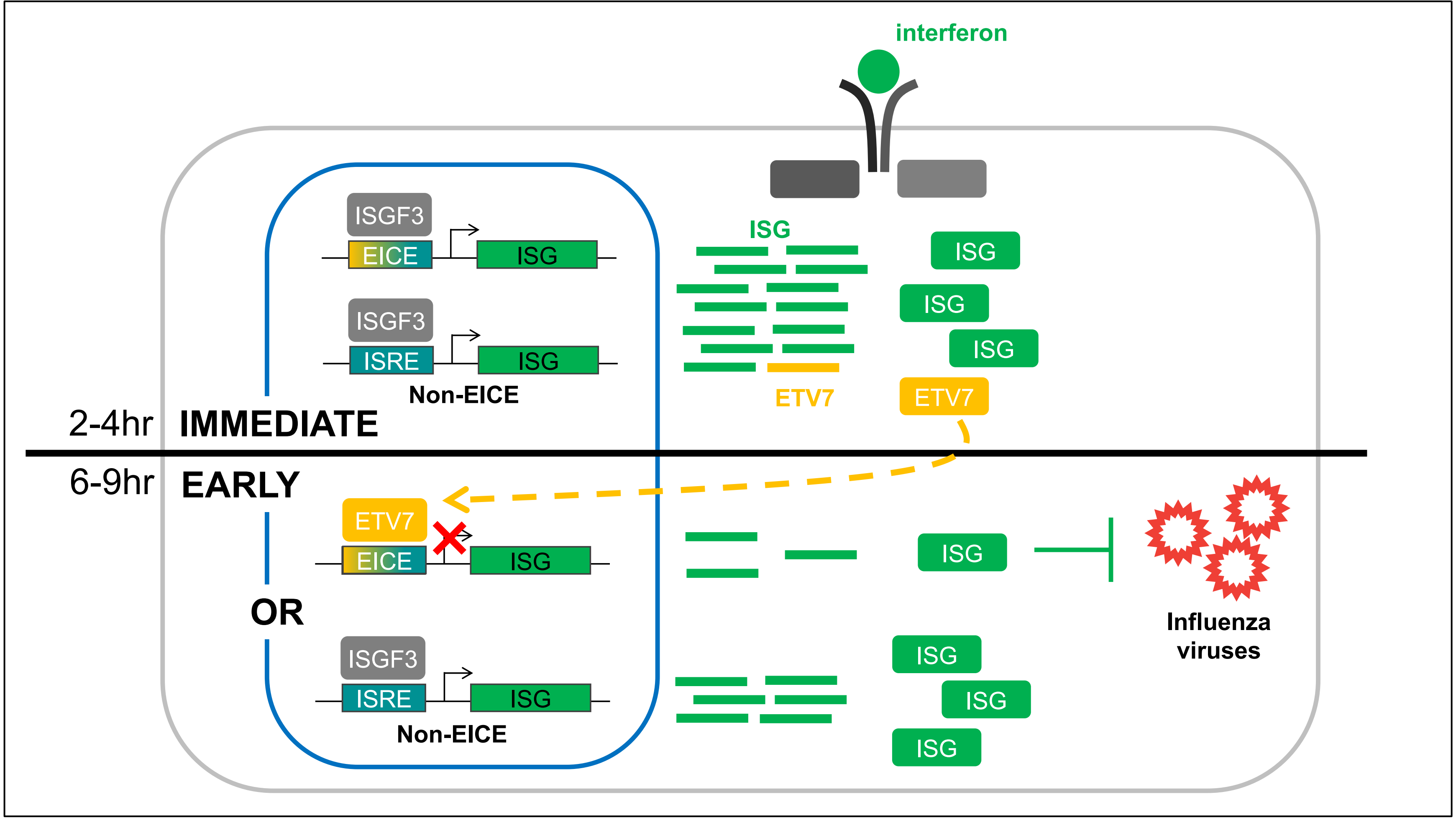
--dashes-- = deletion

italicized = description of resulting peptide (see below)

- *trunc* = truncation
- *del* = deletion
- *lss* = loss of splice site

Supplementary Figure 2: DNA lesions in ETV7 KO cell lines. Sequences of the ETV7 KO target regions and the predicted impact on the peptide sequence.

Supplementary Figure 3.



Supplementary Figure 3: Model of ETV7 activity during the type I IFN response. IFNα/β binds its receptor, resulting in the translocation of STAT1/2 and IRF9 (ISGF3) into the nucleus where they bind ISREs, including EICEs, inducing ISG expression. ETV7 is expressed during this initial round of gene induction to then bind EICEs, suppressing further induction of specific ISGs, early during the IFN response. Many of the ISGs most affected by ETV7 negatively regulate influenza virus replication (12).

Supplemental References

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