IFITM3 Retrogenes with Intact ORF: Are They Functional?

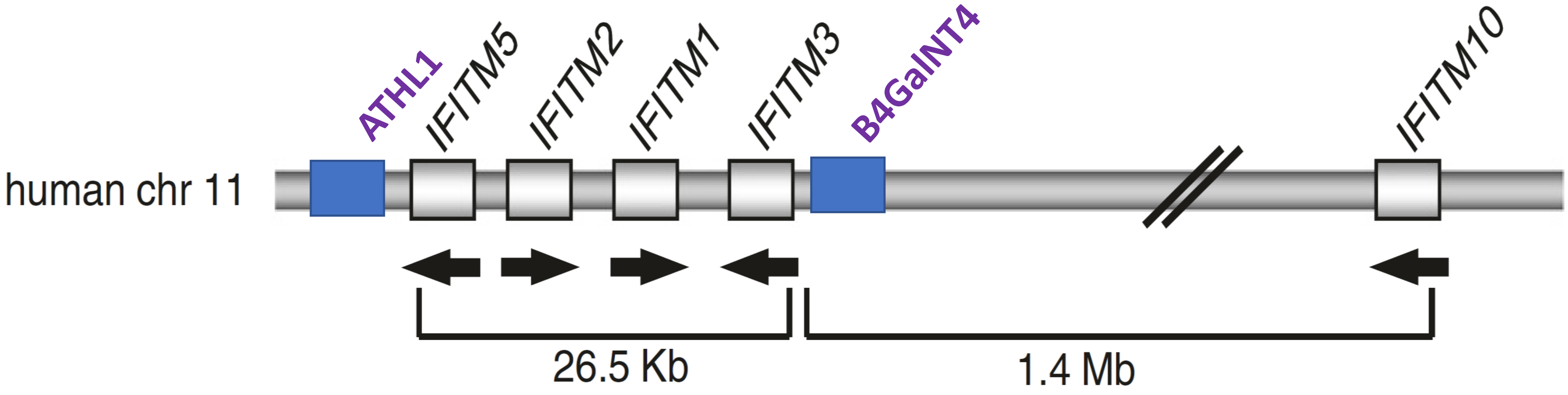
Kazi Rahman, PhD

National Cancer Institute

03/02/2018

IFITMs : Genomic and Syntenic Context

- Five different types: IFITM1, 2, 3, 5 and 10
- All are in Chromosome 11
- IFITM1, 2, and 3 are interferon-inducible and flanked by ATHL1 and BGalNT4 genes

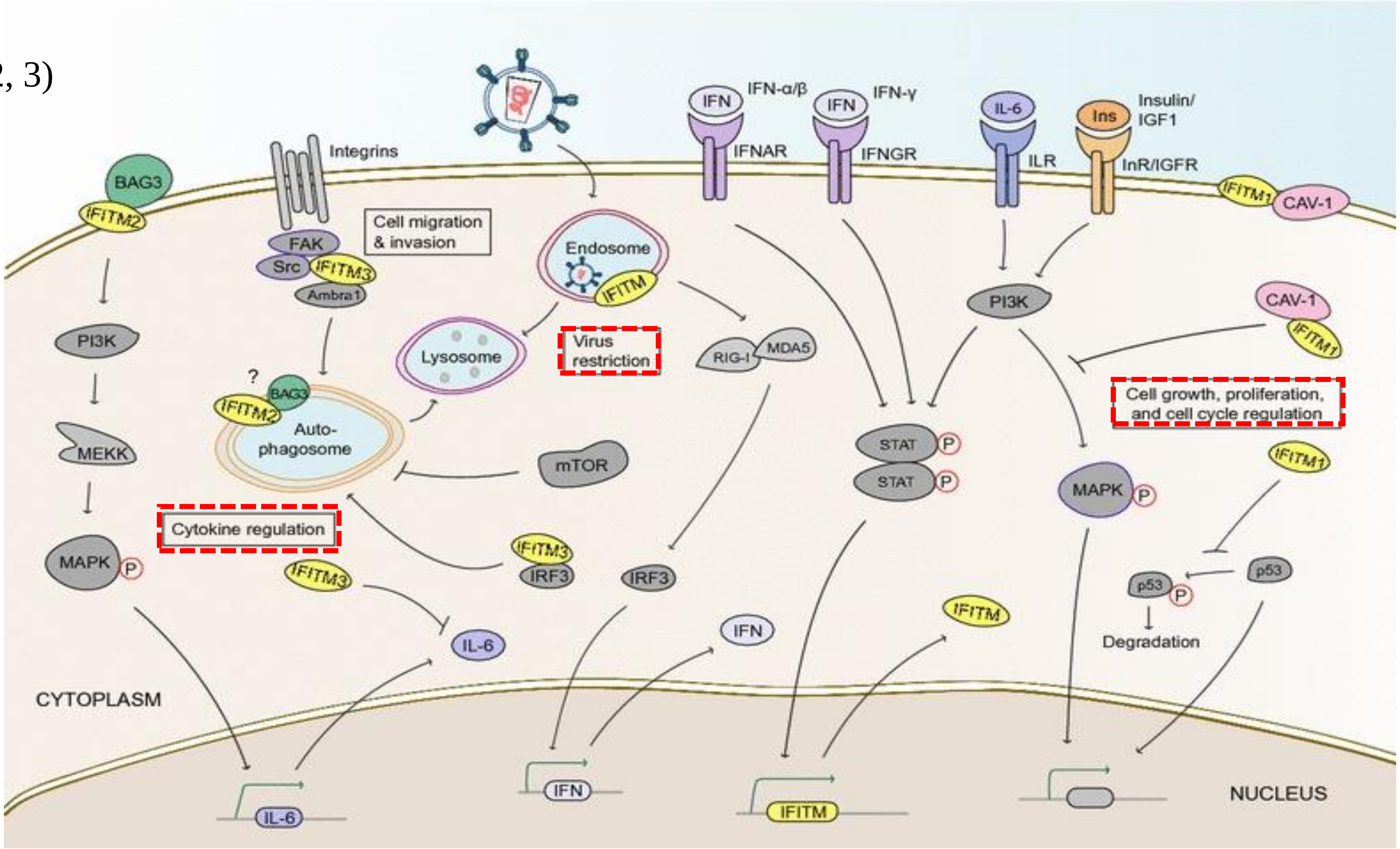


BMC Genomics 2012 **13**:155

Role of IFITMs in Various Cellular Processes

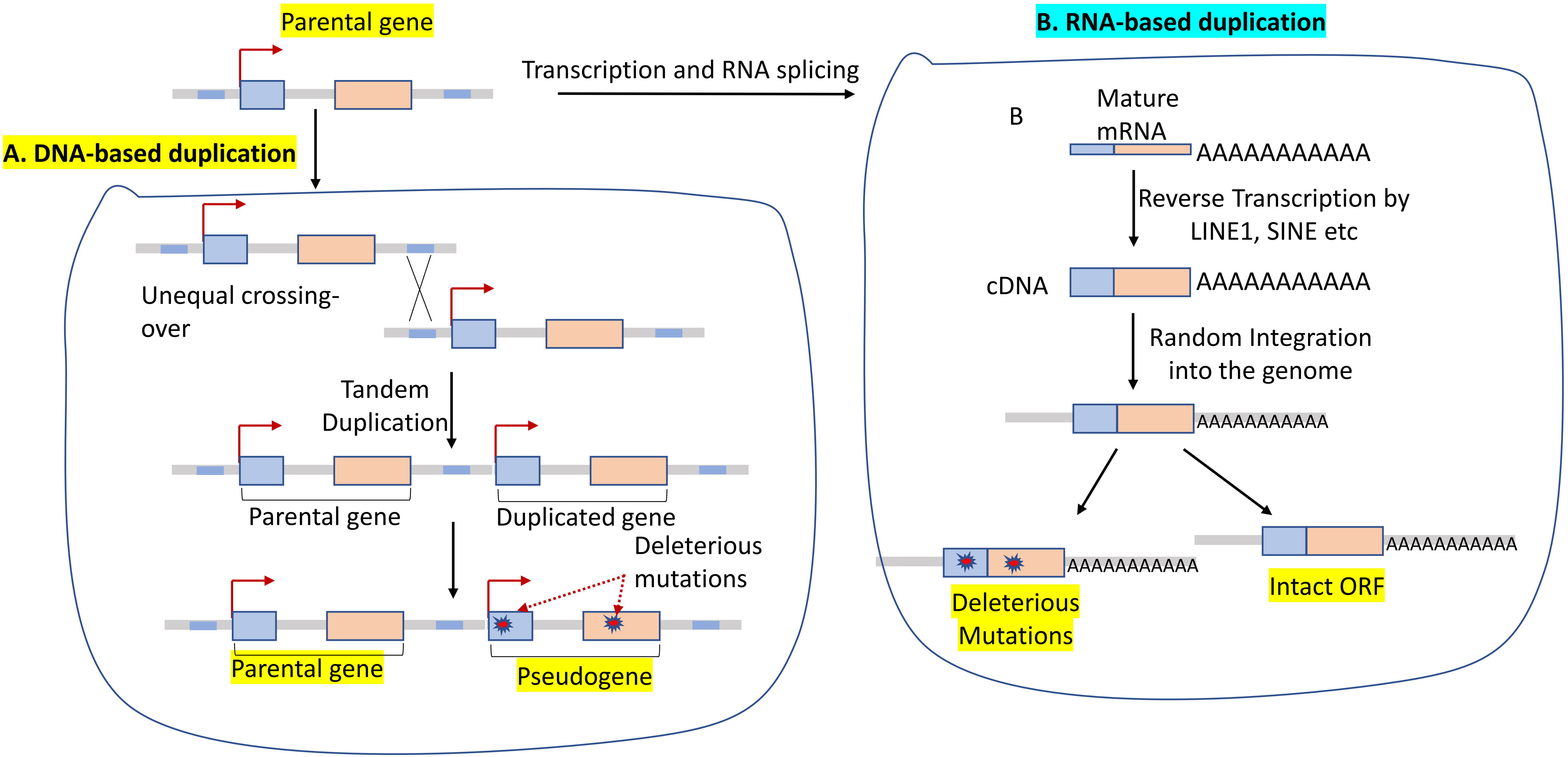
<u>Involved in</u>

A. Virus Restriction (IFITM1, 2, 3)
B. Cell Adhesion
C. Anti-Proliferation
D. Tumor Suppression
E. Germ cell and embryo
development

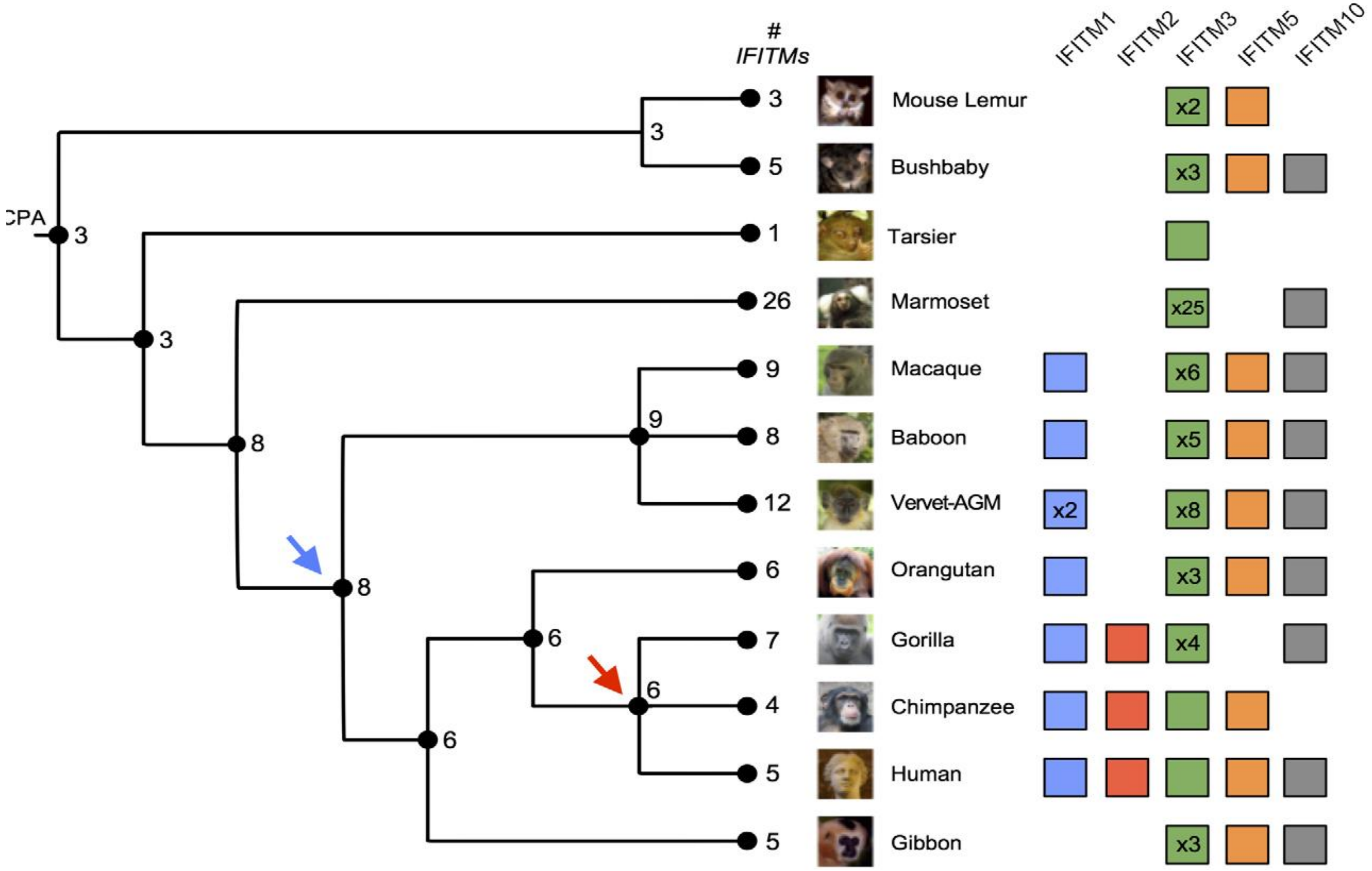


Scarlett et al, *Retrovirology 2017*

Origin of New Gene Copies through Gene Duplication



Recurrent Duplication of IFITM3 in Primates



Compton et al, EMBO Reports 2016

Questions

Are those IFITM3 retrogenes with intact ORF functional?

A. Do they undergo transcription and translation?

B. What is their spatio-temporal expression pattern (i.e. specific cells/tissues and timing of expression)?

C. Do they play redundant Roles of IFITM3 or gained neofunctionalism?

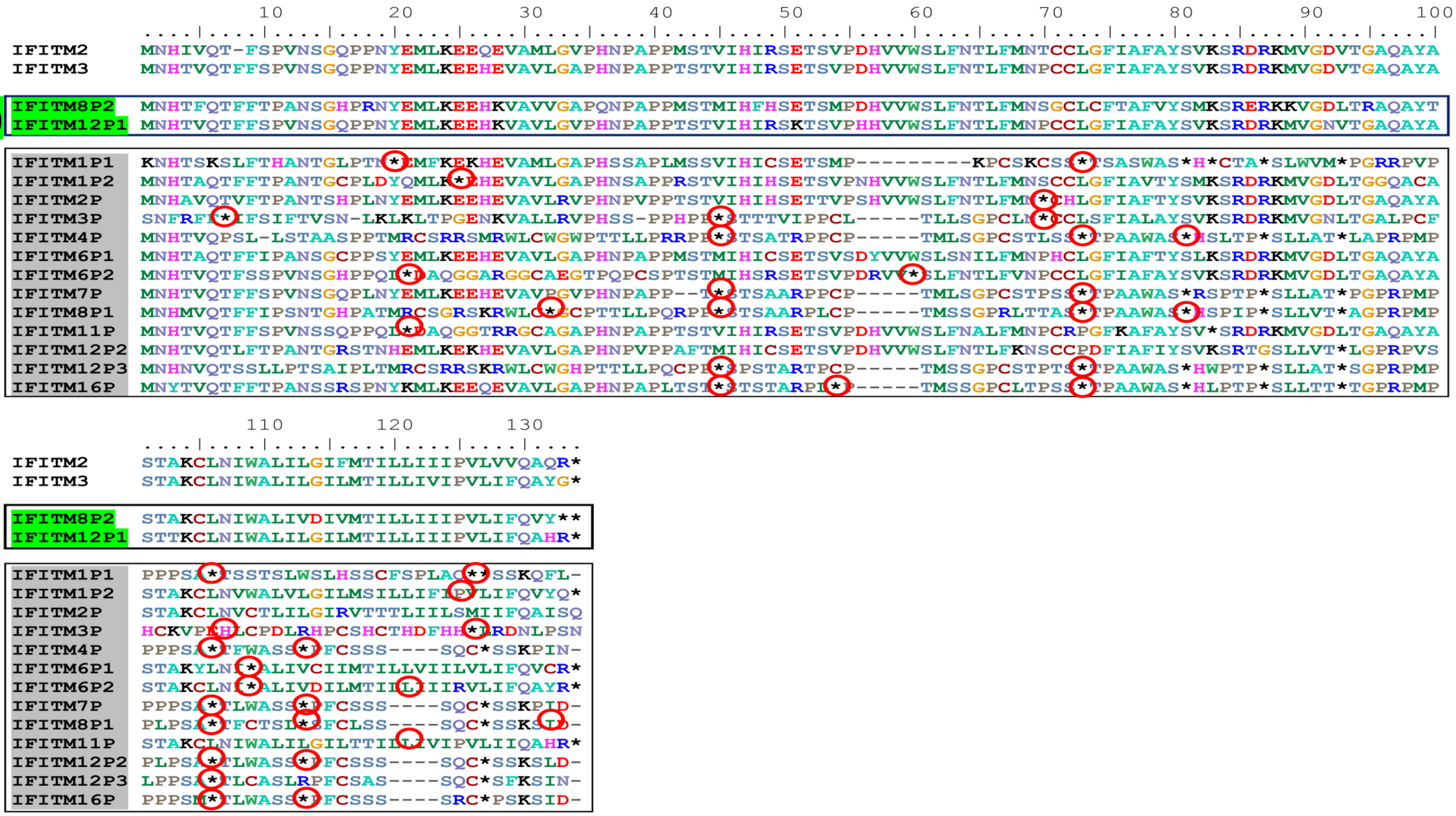
Investigating the function of human IFITM3 retrogenes.

IFITM Duplicants in Human

- Total 15 IFITM duplicants in Human
- All are retrogenes –intronless/ PolyA-tailed

Intact ORF (2)

Deleterious Mutations (13)



Evolutionary Conservation of IFITM8P2/12P1

IFITM8P2
(Chr. 8)

```
                                   10        20        30        40        50        60        70        80        90
                           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
IFITMP8P2_Human            MNHTFQTFFTPANSGHPRNYEMLKEEHKVAVVGAPQNPAPPMSTMIHFHSETSMPDHVVWSLFNTLFMNSGCLCFTAFVYSMKSRERKKV
IFITMP8P2_Bonobo           MNHTFQTFFTPANSGHPRNYEMLKEEHEVAVVGAPQNPALPMSTMIHFHSETSMPDHVVWSLFNTLFMNSGCLGFIAFAYSLKSRERKKV
IFITMP8P2_Chimpanzee       MNHTFQTFFTPANSGHPRNYEMLKEEHEVAVVGAPQNPALPMSTMIHFHSETSMPDHVVWSLFNTLFMNSGCLGFIAFAYSLKSRERKKV
IFITMP8P2_Gorilla          MNHTFQTFFTPANSGHPRNYEMLKEEHEVAVVGVPQNPAPPMSTMIHFHSETSMPDHVVWSLFNTLFMNSGCLGFIAFVYSMKSRERKKV
IFITMP8P2_Orangutan        MNHIFQTFFTPANSGHPRNYEMLKEEHEVAVVGAPQNPAPLMSTMIHFHSETSMPDHVDWSLFNTLFMNSGCLGFIEFAYSKKSRGRKMV
IFITMP8P2_Green Monkey     MNHTFQTFFTPANSSRSRNYEMLKEEHEVAVLGAPHNSAPPMSTMIHFSSETSVPDHVVWSLFNTLFMNSSCLGFIAFAYSMTSRDRKIV
IFITMP8P2_Gibbon           MNHTFQTFFTPANSGHPCNYEMLKEEHEVAVVGARPTTLLPDVHHDPLPQRDLMPDHVIWSLFDALFMNSGCLG*SAFAYSMKCWTGRCE
IFITMP8P2_Crab-eating Macaque MNHTFQTFFTPANSSRPPQL*DAQGGA*GGCGGDTPQLYASNVHHDPLQQ*DLCARPCCLVAVQHHFHELQLPGLHSICLLHDV*GQEDG
IFITMP8P2_Rhesus Macaque   MNHTFQTFFTPANSSRPPQL*DAQGGA*GGCGGDTPQLYASNVHHDPLQQ*DLCARPCCLVAVQHHFHELQLPGLHSICLLHDV*GQEDG
IFITMP8P2_Olive baboon     MNHTFQTFFTPANSRRPPQL*DAQGGA*GGCGGDTPQLYSSNVHHDPLQQ*DLCARPCCLVAVQHPFHELQLPGLHSICLLHDV*GQEDG
```

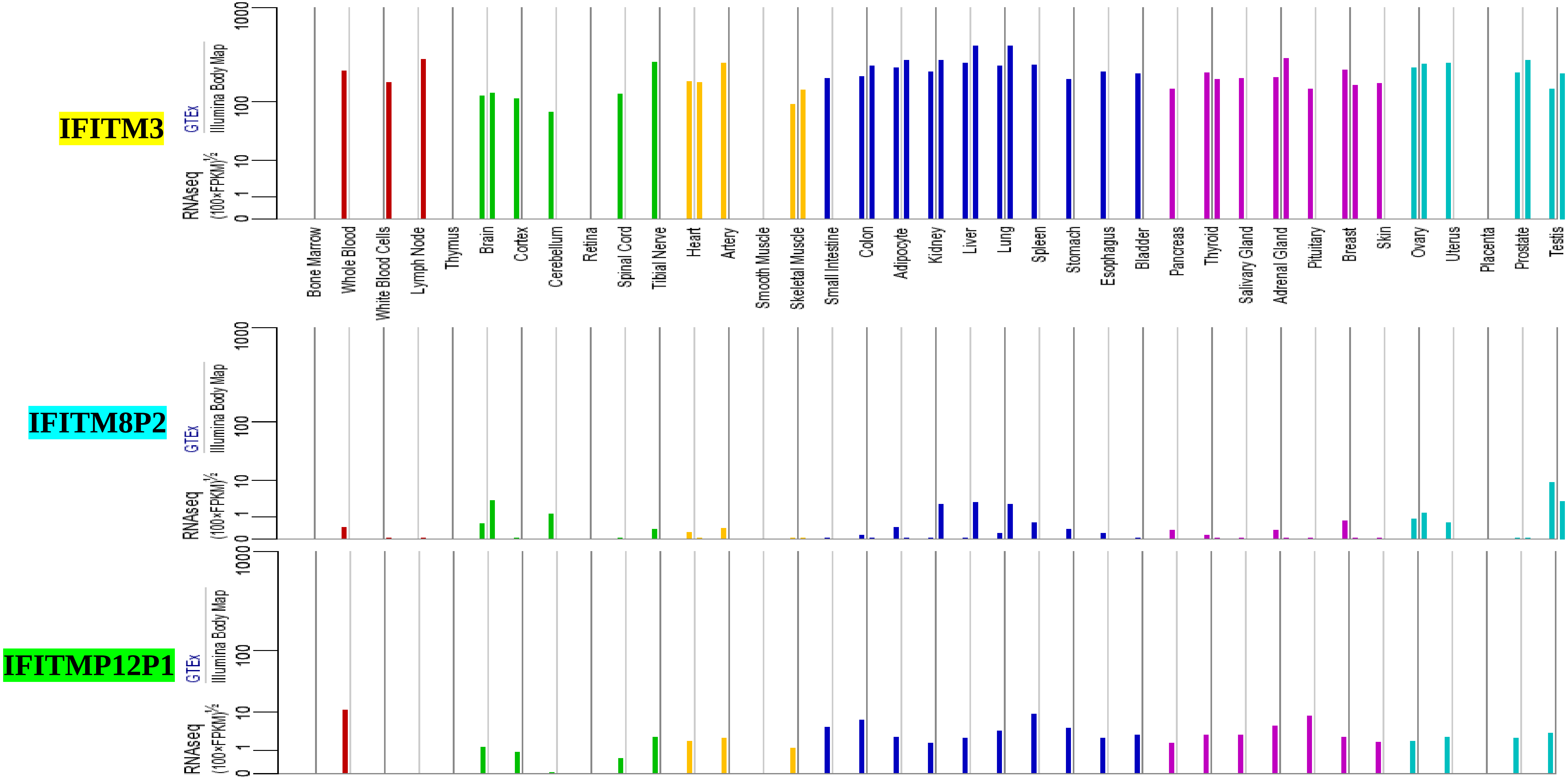
IFITMP12P1
(Chr. 12)

```
IFITMP12P1_Human           MNHTVQTFFSPVNSGQPPNYEMLKEEHKVAVLGVPHNPAPPTSTVIHIRSKTSVPHHVVWSLFNTLFMNPCCLGFIAFAYSVKSRDRKMV
IFITMP12P1_Bonobo          MNHTVQTFFSPVNSGQPPNYEMLKEEHKVAVLGVPHNPAPPTSTVIHIHSKTSVPHCVVWSLFNTLFMNPCCLGFIAFAYSVKSRDRKMV
IFITMP12P1_Chimpanzee      MNHTVQTFFSPVNSGQPPNYEMLKEEHKVAVLGVPHNPAPPTSTVIHIHSKTSVPHCVVWSLFNTLFMNPCCLGFIAFAYSVKSRDRKMV
IFITMP12P1_Gorilla         MNHTVQT-FSPVNSGQPPNYEMLKEEHKVAVLGVPHNPAPPTSTVIHIRSKTSVPHHVVWSLFNTLFMNSCCLGFIAFAYSVKSRDRKMV
IFITMP12P1_Orangutan       MNHTVQTFFSPVNSGQHPQ---V*DTQGGAQGGCAEGTPQPCSPDVH-RSETSVPHHVVWSLFNTLFRNPCCLGFIAFAYSVKSRDRKMV
```

```
                                  100       110       120       130
                           ....|....|....|....|....|....|....|....|....
IFITMP8P2_Human            GDLTRAQAYTSTAKCLNIWALIVDIVMTILLIIIPVLIFQVY**
IFITMP8P2_Bonobo           GDLTRAQAYTSTAKCLNIWALIVDIVMTILLIIIPVLIFQVY**
IFITMP8P2_Chimpanzee       GDLTRAQAYTSTAKCLNIWALIVDIVMTILLIIIPVLIFQVY**
IFITMP8P2_Gorilla          GDLTRAQAYASTAKCLNIWALIVDIVMTILLSIIPVLIFQVY**
IFITMP8P2_Orangutan        GDLTRAQAYASTAKCLNIWALIVDIVMTILLIIIPVLIFQVY**
IFITMP8P2_Green Monkey     GDLTRAQAYASTAKCLNIWALIVGIVMTVLLIIIPVLIFQVY**
IFITMP8P2_Gibbon           PTCPPLKAISP*T*DRYTGP*YSIPS*KFE*HNMC*SSESID--
IFITMP8P2_Crab-eating Macaque W*PDQGPGLCLHHQVPEHLGPDCGHCHDRSAHHHPGVDLPSLLI
IFITMP8P2_Rhesus Macaque   W*PDQGPGLCLHHQVPEHLGPDCGHCHDRSAHHHPGVDLPSLLI
IFITMP8P2_Olive baboon     W*PDQAPMPPPPSA*TSGP*LWALS*PFCSSSSRC*SSKSID
```

```
IFITMP12P1_Human           GNVTGAQAYASTTKCLNIWALILGILMTILLIIIPVLIFQAHR*
IFITMP12P1_Bonobo          GNVTGAQAYASTTKCLNIWALILGILMTILLIIIPVLIFQAHR*
IFITMP12P1_Chimpanzee      GNVTGAQAYASTAKCLNIWALILGILMTILLIIIPVLIFQAHR*
IFITMP12P1_Gorilla         GNVTGAQAYSSTAKCLNIWALILGILMTILLIVIPVLIFQAHR*
IFITMP12P1_Orangutan       GDLTGAQAYASTAKCLNIWALILGILMTILLIVIPILIFQAHR*
```

mRNA Expression In Normal Human Tissues From Genotype-Tissue Expression (GTEx) Consortium



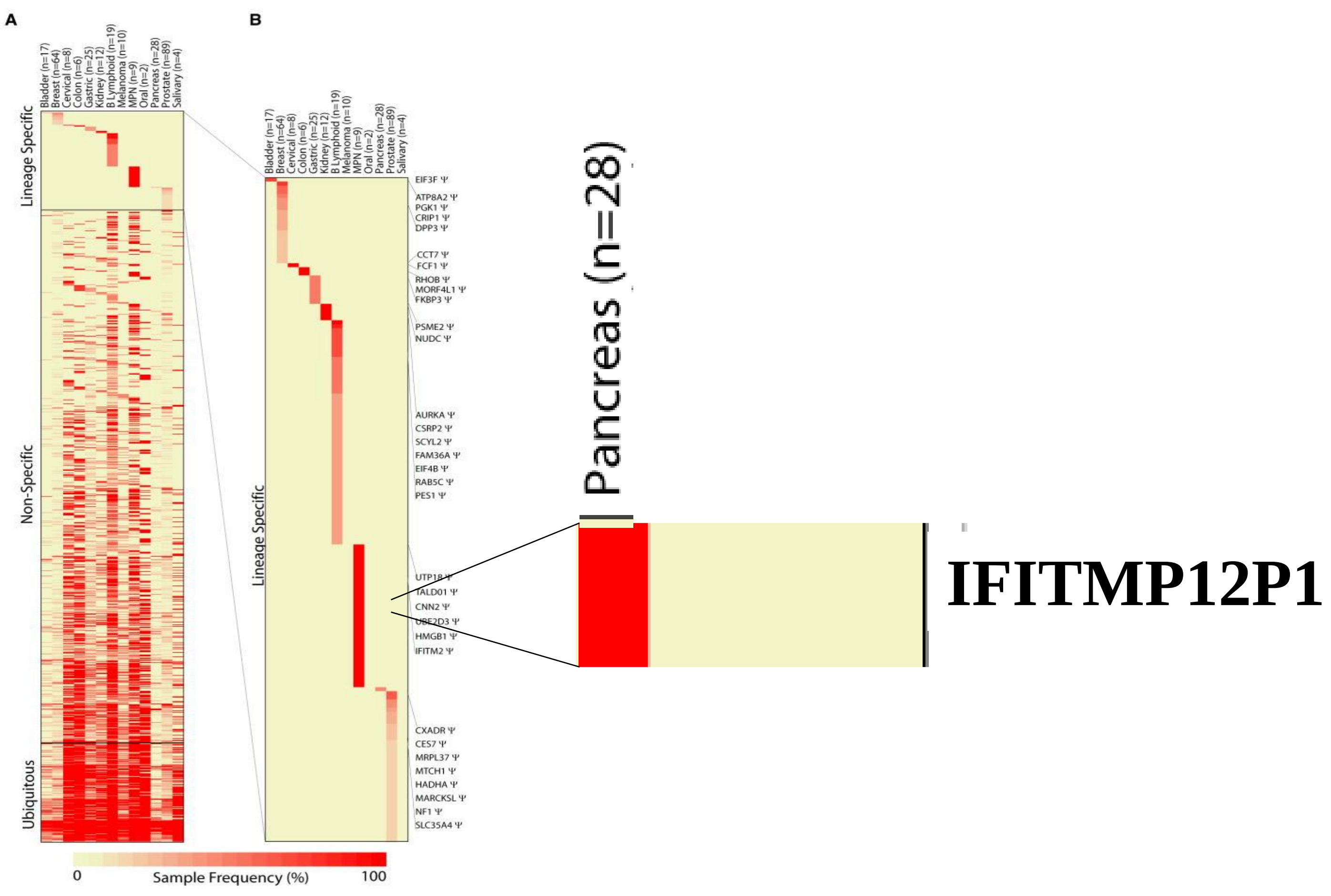
Transcriptomic Evidence of IFITM12P1 Expression

Tissue/Lineage-Specific Pseudogene Expression Profiles

Cell

Expressed Pseudogenes in the Transcriptional Landscape of Human Cancers

Cell. 2012 Jun 22; 149(7): 1622–1634.



Proteomics Evidence of IFITM12P1 Expression

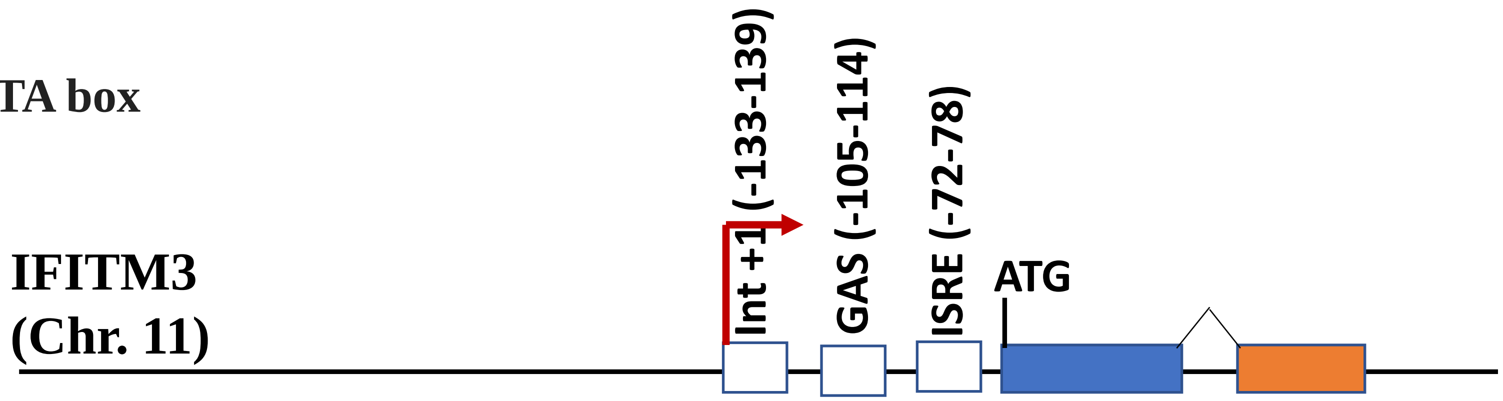
```
IFITM3       1  MNHTVQTFFSPVNSGQPPNYEMLKEEHEVAVLGAPHNPAPPTSTVIHIRSETSVPDHVV
IFITM12P1    1  MNHTVQTFFSPVNSGQPPNYEMLKEEHKVAVLGVPHNPAPPTSTVIHIRSKTSVPHHVV

IFITM3      60  WSLFNTLFMNPCCLGFIAFAYSVKSRDRKMVGDVTGAQAYASTAKCLNIWALILGILMTI
IFITM12P2   60  WSLFNTLFMNPCCLGFIAFAYSVKSRDRKMVGNVTGAQAYASTTKCLNIWALILGILMTI

IFITM3     120  LLIVIPVLIFQAYG
IFITM12P3  120  LLIIIPVLIFQAHR
```

Unique peptide

J. Proteomic Research: *2016 Mar 4;15(3):945-55*: Myoblastic cell lines
Mol. Cell Proteomics: 2016 Mar;15(3):1072-82: Pancreatic carcinoma
***Scientific Data* volume2:** Article number: 150022 : Colon cancer
Mol. Cell Proteomics: 2012 Mar;11(3) :deep proteomics –in Jurkat cells

Potential Adoption of Regulatory Sequences in IFITM12P1

- **IFITM3: NO TATA box**



ISRE: Interferon-stimulated response element (for IFN-alfa/beta)
GAS: Interferon-Gamma Activated Sequence
Inr+1 : Transcription Start Site (TSS)

Aim-1: Is endogenous IFITM12P1 undergoes transcription and if these exapted regulatory sequences are active?

Experiments:

A. Determine the level of IFITM12P1 RNA expression in different cells/tissues by RT-PCR

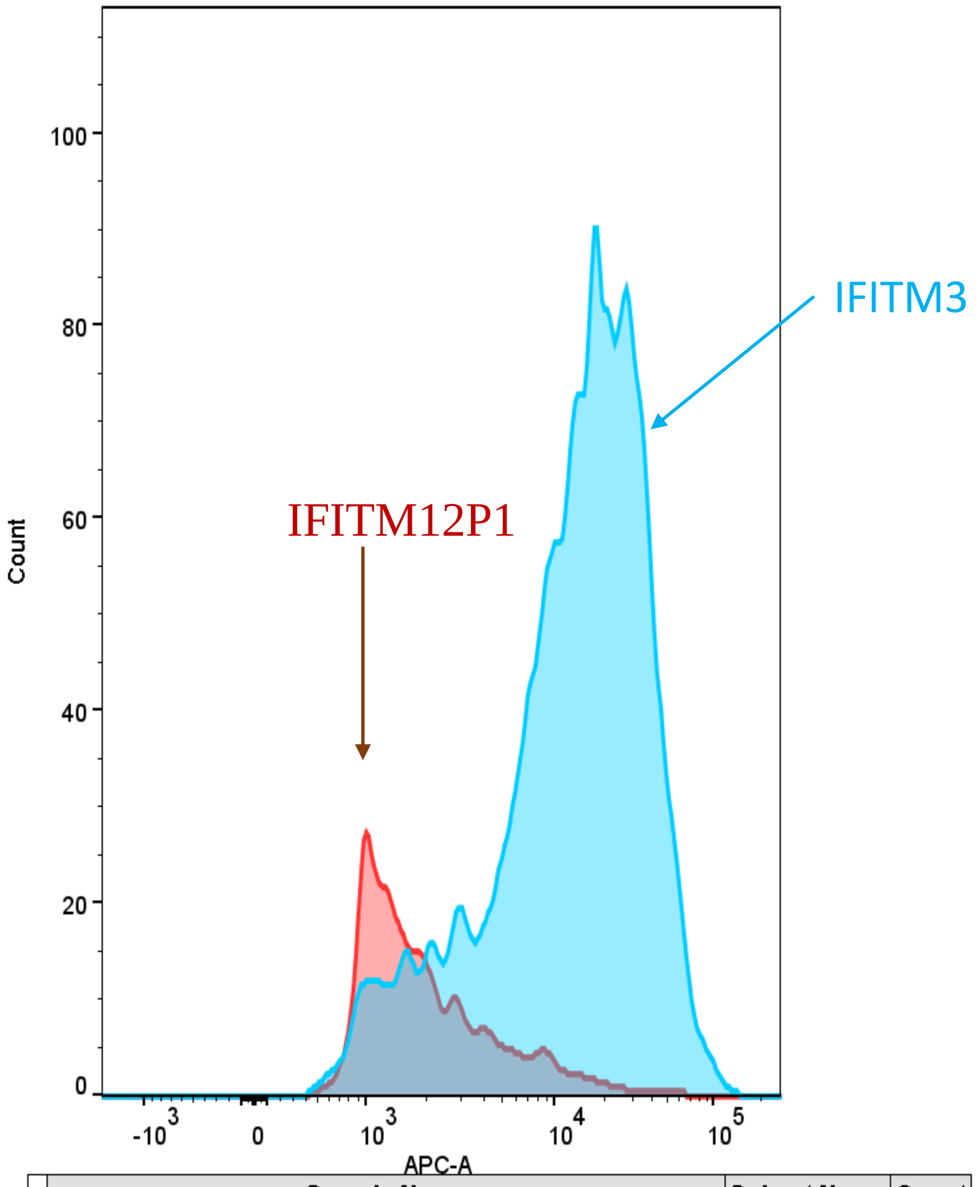
B. Endogenous Tagging of IFITM12P1 by CRISPR/Cas9

C. 5'-RACE (Rapid amplification of cDNA ends) analysis to check the TSS.

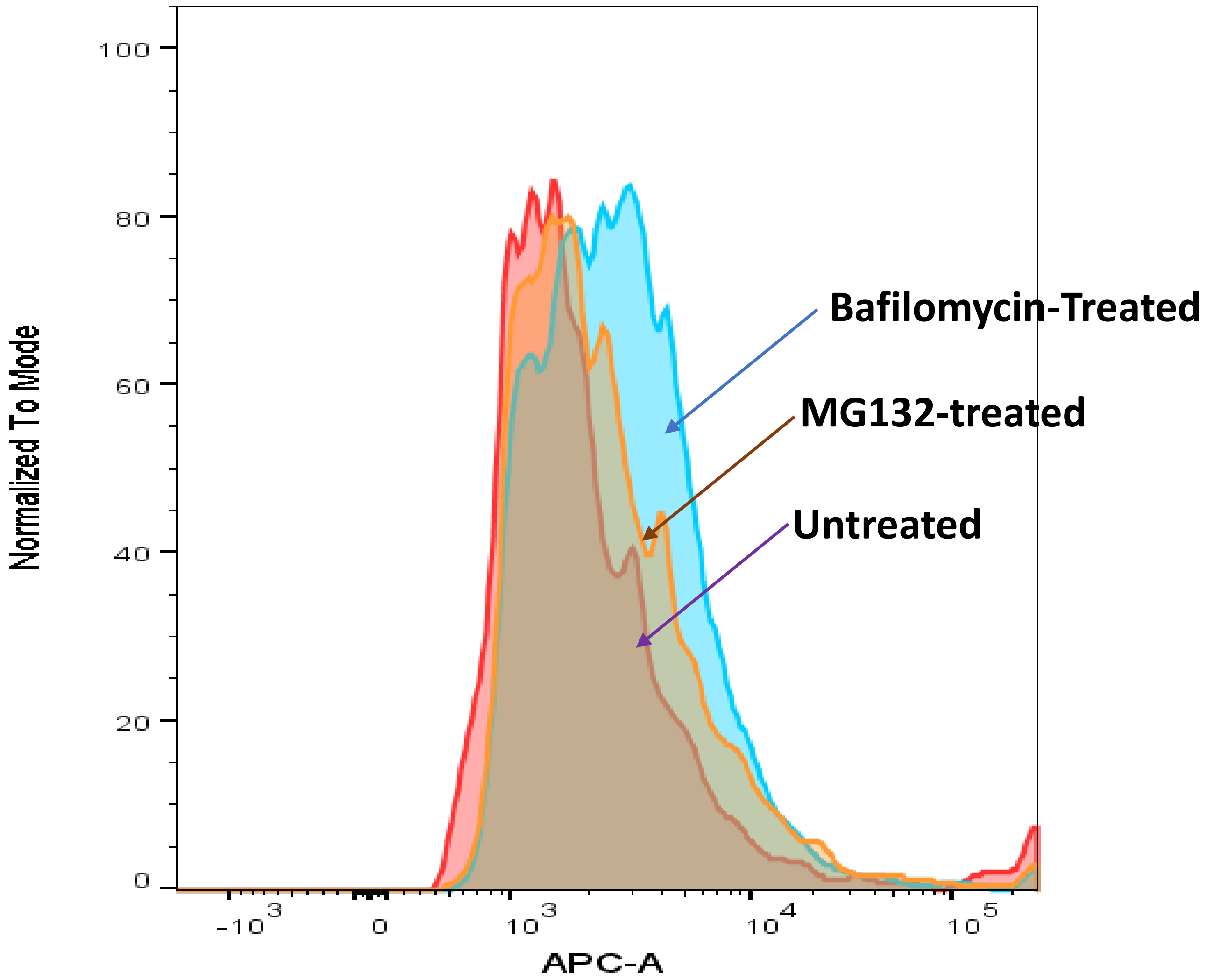
Aim-2: Investigating the role of IFITM12P1

Ectopically Expressed IFITM12P1 Shows Instability

Stable 293-T cells expressing FLAG-IFITM12P1/IFITM3



IFITM12P1 Might Degrade by Lysosome



Why IFIM12P1 shows Instability in comparison to IFITM3?

Sequence Variations among IFITM12P1 and IFITM2/IFITM3

E27K E50K D54H

IFITM2	1	MNHIVQT-FSPVNSGQPPNYEMLKEEQEVAMLGVPHNPAPPMSTVIHIRSETSPDHVVWS
IFITM3	1	MNHTVQTFFSPVNSGQPPNYEMLKEEHEVAVLGAPHNPAPPTSTVIHIRSETSPDHVVWS
Human IFITM12P1	1	MNHTVQTFFSPVNSGQPPNYEMLKEEHKVAVLGVPHNPAPPTSTVIHIRSKTSPHHVVWS
Gorilla- IFITM12P1	1	MNHTVQT-FSPVNSGQPPNYEMLKEEHKVAVLGVPHNPAPPTSTVIHIRSKTSPHHVVWS
Chimpange- IFITM12P1	1	MNHTVQTFFSPVNSGQPPNYEMLKEEHKVAVLGVPHNPAPPTSTVIHIHSKTSPHCVVWS

D91N

IFITM2	60	LFNTLFMNTCCLGFIAFAYSVKSRDRKMVGDVTGAQAYASTAKCLNIWALILGIFMTILL
IFITM3	61	LFNTLFMNPCCLGFIAFAYSVKSRDRKMVGDVTGAQAYASTAKCLNIWALILGILMTILL
Human- IFITM12P1	61	LFNTLFMNPCCLGFIAFAYSVKSRDRKMVGNVTGAQAYASTTKCLNIWALILGILMTILL
Gorilla- IFITM12P1	60	LFNTLFMNSCCLGFIAFAYSVKSRDRKMVGNVTGAQAYSSTAKCLNIWALILGILMTILL
Chimpange- IFITM12P1	61	LFNTLFMNPCCLGFIAFAYSVKSRDRKMVGNVTGAQAYASTAKCLNIWALILGILMTILL

IFITM2	120	IIIPVLVVQAQR
IFITM3	121	IVIPVLIFQAYG
Human- IFITM12P1	121	IIIPVLIFQAHR
Gorilla- IFITM12P1	120	IVIPVLIFQAHR
Chimpange- IFITM12P1	121	IIIPVLIFQAHR

Which amino acids are responsible for IFITM12P1 instability?

Aim-2a: Which amino acid(s) render IFITM12P1 unstable?

Experiments:

A. SDM of each modified a.a. and testing their impact on expression pattern.

B. Determine if that specific mutation is a characteristic pattern of other retrogenes to find for a universal code of retrogene instability

Aim-2b: What is the endogenous role of IFITM12P1 in cellular immunity against virus and/or in other cellular processes?

<u>Experiments:</u>

A. **Redundant function of IFITMs?**

Knocking out the IFITM12P1 by CRISPR/Cas9 in different cell types and test for mutants against virus challenge.

B. **Inhibiting the expression of IFITMs by anti-sense RNA?**

Co-expression of IFITM3 and IFITM12P1 and check the impact on IFITM3 expression

C. **Enhancing the expression of IFITMs by binding with the IFIMT3 miRNAs?**

Expression of IFITM12P1 3’-UTR in Hela cells and explore the expression level of IFITM3

Knocking-out of IFITMP121 by CRISPR attempt

Experimental progress:

-Made stable 293T cells and Hela cells expressing Cas9 protein.

Thanks