IFITMs restrict the replication of multiple pathogenic viruses

Jill M. Perreira, **Christopher R. Chin**, **Eric M. Feeley**, and **Abraham L. Brass**[*]

Microbiology and Physiological Systems (MaPS) Department, University of Massachusetts Medical School, Albert Sherman Center 8 1001, 368 Plantation St., Worcester, MA 01655, USA

Abstract

The IFITM family of proteins inhibit a growing number of pathogenic viruses, among them influenza A virus, dengue virus, hepatitis C virus, and Ebola virus. This review covers recent developments in our understanding of the IFITM's molecular determinants, potential mechanisms of action, and impact on pathogenesis.

Introduction

To replicate viruses must gain access to the resource rich cytosol which lies beyond the cell's plasma membrane. Enveloped-viruses breach this barrier by using specialized fusion proteins. Three major classes of viral fusion proteins exist, all similarly containing a fusion peptide which inserts into the cytolemma, thereby anchoring the two membranes side by side [1]. Once transfixed, the juxtaposed membranes are forcefully distorted as the viral envelope protein undergoes a profound conformational change; the two outer leaflets are welded together to form a hemifusion intermediate which rapidly converts into a fusion pore through which the viral contents enter the cytosol. Any means by which the host can block fusion would confer an advantage by preventing both the emergence of an escalating number of progeny viruses and the deployment of viral countermeasures.

Restriction factors are a diverse group of host proteins which are united in the common goal of antagonizing viral replication. Multiple mechanisms of restriction have evolved, with some factors having activity against one virus and others acting broadly across several viral families. The expression of many restriction factors is transcriptionally controlled by the antiviral cytokine, interferon (IFN). Among such IFN stimulated genes (ISGs), the related restriction factors, IFITM1, 2 and 3, inhibit the replication of multiple pathogenic viruses, including Influenza A and B viruses (IAV, IBV), West Nile virus (WNV), dengue virus (DENV), SARS coronavirus (SARS CoV), hepatitis C virus (HCV) and the filoviruses, Ebola (EBOV) and Marburg (MARV, Table 1)[2-4]. The anti-viral properties of the IFITMs were discovered using orthologous functional genomic strategies [2,5-8]. Early worked showed that the IFITMs resided on the cytolemmal and endosomal membranes and specifically blocked viral pseudoparticles (pp) bearing the receptors of restricted viruses, demonstrating that they acted during an early envelope-dependent portion of the viral life cycle [2]. Further studies revealed that the IFITMs block viral replication by preventing

[*]To whom correspondence should be addressed abraham.brass@umassmed.edu.

viral-host membrane fusion subsequent to viral binding and endocytosis [3,9]. Imaging studies of these events revealed that the invading viruses were trapped by the IFITMs, leading to their ultimate destruction in the host cell's lysosomes and autolysosomes, both of which are expanded with IFITM expression (Fig. 1A, B)[9]. A range of viruses are restricted in this manner, including ones which exploit each of the host cell's endocytic pathways [10]. The kinetics of this entrapment are rapid, with viral entry usually occuring over a 5 to 30 minute time span, requiring the IFITMs to already be in place or to rapidly mobilize to meet such threats.

Consistent with an early role in intrinsic immunity, the IFITM1, 2 and 3 proteins are ubiquitously expressed, with IFITM2 and IFITM3 present at baseline in most primary and transformed cells (Table 2, 3). In contrast, IFITM1's basal expression is considerably less. The levels of these three IFITMs are increased by IFN-α or IFN-γ. Remarkably, the depletion of IFITM3 alone results in the loss of 50-80% of the *in vitro* anti-IAV actions of IFN [2,11]. Furthermore, mice null for only Ifitm3 are more susceptible to IAV infection, testifying to the importance of IFITM-mediated restriction *in vivo* [12,13]. In sum, these data suggest that the IFITMs may prevent or ameliorate multiple viral illnesses. Indeed such is the case for influenza infection, where a human allele of IFITM3, rs12252-C, is associated with worse influenza infections, suggesting that the IFITM3 status of a population may influence the course of seasonal influenza epidemics and pandemics [12,14] .

Recent studies regarding the IFITMs have focused on their structure-function as well as their potential mechanism of action. In addition, several efforts have reported new families of IFITM-sensitive viruses. Herein we discuss these results together with previous work, and compare and contrast the various models proposed to explain how the IFITMs protect our cells.

The IFITM and CD225 Families

In addition to IFITM3, four more members of the IFITM family are present in both man (IFITM1, 2, 5 and 10, Fig. 2A, Table 3) and mouse (Ifitm1, 2, 5 and 6). The IFITMs each contain two hydrophobic membrane-associated domains separated by a conserved intracellular loop (CIL, Fig. 2A). The IFITM family belongs to a larger family of membrane-associated proteins, the CD225/pfam04505 protein superfamily, with greater than 300 members sharing homology across their first membrane-associated domains and CILs (http://pfam.sanger.ac.uk/family/PF04505). Interestingly, while IFITM5 has been shown to prevent infection by multiple viruses, it also is restricted in its expression to osteoblasts, where it plays a role in bone formation *in vitro* and *in vivo* (15,16). Consistent with these data, a variant allele of IFITM5 is associated with a human brittle bone disease, osteogenesis imperfecta type V [11,17]. Remarkably, the protein encoded by this rare IFITM5 allele possesses an additional five amino acids at its N-terminus; this is in keeping with our unpublished data showing the IFITMs do not function in a wild-type manner when epitope tagged at their N-termini.

Additional human CD225 family members include PRRT1, PRRT2, TMEM91, TMEM233, TUSC5, SYNDIG1, and SYNDIG1L, with little if anything known about the functions of

these proteins (Fig. 3A, Table 3). However, PRRT2, which is expressed in the nervous system, has received considerable attention for its genetic association with multiple movement disorder syndromes, many of which arise in the setting of its haploinsufficiency [17-19]. Therefore, among the twelve CD225 family members present in humans, there are currently three proteins with human disease associations: IFITM3, PRRT2, and IFITM5 (Table 3). Many additional CD225 proteins are found among prokaryotes, including several plant pathogens, suggesting that the CD225 domain confers a selective advantage across kingdoms. Interestingly while both fish and amphibians express CD225 family members, none have been found in plants, fungi, insects or worms, leading to the fascinating hypothesis that a gene encoding a prokaryotic CD225 family member may have been transferred to an ancestral metazoan [20].

Membrane topology and cellular localization

Two hydrophobic domains are separated by a CIL in each of the IFITMs. Our appreciation of whether these hydrophobic domains are transmembrane or intramembrane has evolved over time [10,21]. Initial studies suggested that IFITM3 possessed two transmembrane domains predicated on the extracellular accessibility of epitopes located on either the N- or C-termini [2]. However, mass spectrometric (MS) analyses by several groups (Table 4, [21], PhosphoSite) have detected prevalent ubiquitinylation of IFITM3's Lysine-24, which could occur only if the N-terminal domain (NTD) was cytosolic (Table 4, [21]). Similarly, a tyrosine in the NTD of IFITM3, Y20, was also shown to undergo phosphorylation [22]. In support of these latter results, large scale MS studies have also reported detecting Y20-P, in addition to several other post-translational modifications occurring in the respective NTDs of the other human CD225 proteins. For example, PRRT2's S239 has been reported to undergo phosphorylation by nine groups at PhosphoSite Plus. Furthermore, an engineered IFITM3 possessing both an N-terminal myristoylation site and a C-terminal prenylation site was modified by these cytosolic-resident enzymes and restricted IAV [21]. Collectively, these data argue for both N- and C-termini residing intracellularly, similar to the reticulon and caveolin proteins [21,23], with both hydrophobic regions being intramembranous (IM1 and IM2, Fig 2B). We note that IFITM's residing in the cytosolic leaflet of the membrane would shield it both from neutralization by invading viruses and from degradation by lysosomal enzymes. An intramembranous topology also has potential implications for altering the membrane's biophysical properties, a point discussed below. Of importance, this topology remains controversial, and to reflect that we refer the reader to a recent review which also presents the alternatively proposed transmembrane topology in graphic form [10].

Endogenous IFITM1 is predominantly located in the plasma membrane and in early endosomes [3,9], where it resides in lipid-rafts and interacts with the cell-surface proteins CD19 and CD81, the latter being a co-receptor for HCV [24-26]. In contrast, the majority of endogenous IFITM2 and 3 are present in late endosomes, lysosomes and autolysosomes, colocalizing with RAB7, CD63 and LAMP1 [3,9,27]. Overexpression of IFITM1, 2 or 3, results in the exogenous proteins localizing to a highly acidified and expanded late endosomal and lysosomal compartment [3,9]. However, even when overexpressed, a substantial amount of IFITM1 can still be detected near the cell surface [3,4,11]. We have

observed that the overexpression of epitope-tagged IFITMs can produce partial mislocalization and diminution of their antiviral function. For example, epitope-tagged IFITM1 appears more in the late endosomes and lysosomes than the endogenous protein. In our experience N-terminal epitope tags alter the intracellular localization of the IFITMs more than C-terminal tags. Moreover, the addition of green fluorescence protein or *discosoma* species red protein to either terminus of IFITM3 results in chimeric proteins that do not restrict viral replication and are retained in the cellular interior (our unpublished data). The molecular determinants underlying IFITM cellular localization are discussed in the structure-function section.

Specificity of Action

IFITMs inhibit the entry of a number of viruses, with enveloped RNA viruses reported most frequently (Table 1). Remarkably, the IFITMs block the entry of viruses from each of the three classes of viral fusion proteins. Furthermore, susceptible viruses are prevented from entering via the cell surface, the early and late endosomes, as well from the lysosome. While overlap exists among which IFITMs inhibit which viruses, some specificity is apparent, with IFITM1 better preventing infection by early endosomal-entering viruses and IFITM3 exerting more resistance to viruses that enter in the late endosomes or lysosomes, including IAV, DENV, and the *bunyaviridae*, including Rift Valley fever virus. These late-entering viruses share common features including their dependence on greater endosomal acidification (pH<6) and the actions of RAB7, a host protein required for late endosomal trafficking and acidification [28]. IFITM2 behaves similarly to IFITM3 in terms of viral specificity, albeit with weaker effect, and so it will not be the focus of additional comment [2].

IFITM3 is less proficient than IFITM1 at inhibiting viruses that enter at the cell surface or in the early RAB5+ endosomes (pH >6). For example, IFITM1 prevents HIV-1 entry in both T cell lines and HeLa cells [6,8]. In contrast, we saw no effect on HIV-1 when IFITM3 was overexpressed in HeLa cells (TZM-bl cell line, NIH AIDS Reagent Resource), and only a two-fold effect with its overexpression in Jurkat T cells (our unpublished data, [2]), suggesting that it plays a minor role in regulating HIV-1 entry. An additional example is IFITM3's modest restriction of vesicular stomatitis virus-g (VSV-g) protein-mediated entry, which is RAB5-dependent and requires a pH of 6.5 or less to fuse. IFITM1 also blocks HCV, a hepacivirus which enters in RAB5+ endosomes with a pH requirement of 6.5 [4]. Our previous work overexpressing IFITM3 in the HCV JFH1-permissive cell line, Huh7.5.1, showed no effect on HCV infection [2]. Similarly, IFITM1, but not IFITM3, halted infection by pseudoparticles bearing the Jaagsiekte sheep retroviral (JSR) envelope, which also fuses in the RAB5+ early endosomes at pH 6.3 [29]. Furthermore, although bunyaviruses express a similar glycoprotein (GP) that mediates fusion, they differed in their respective susceptibilities to IFITM3 (Lacrosse virus>Rift Valley fever virus (RVFV)>Andes virus>Hantaan virus>Crimean Congo hemorrhagic fever virus (CCHF)) [30]. RVFV was only restricted by IFITM3, and CCHF showed no inhibition by IFITM1, 2 or 3. Interestingly, IFITM3 was equally as effective in blocking the same virus from infecting different cell types, while IFITM1's efficacy varied more across cell lines; this suggests that IFITM1's actions were cell type specific (Table 1). The difference in localization may

provide one explanation as to why the viral specificities of IFITM1 and 3 differ, as they may preferentially inhibit viruses that enter where they are located. However, an exception to this rule is IFITM1's outperforming IFITM3 in inhibiting the very late-entering viruses, SARS CoV, EBOV and MARV [3]. In addition, chimeric IFITM1 proteins containing the NTD of IFITM3 are located similarly to wild-type IFITM3 but do not curtail IAV as potently, suggesting that location alone may not fully explain these observations.

The list of IFITM-resistant viruses is short by comparison, although a bias against reporting negative data may be contributory (Table 2). Both the Moloney leukemia virus (MLV) and Sendai virus (SeV) envelopes fuse at the cell surface in a pH-independent manner and both are immune to the IFITMs [2,31]. Among the reported pH-independent viruses that fuse at the plasmalemma only HIV-1 is blocked by IFITM1, making this perhaps the best site to avoid IFITM-mediated restriction. The largest enrichment of IFITM-resistant viral envelopes occurs among the arenaviruses, including both the old world (Lassa virus, lymphocytic choriomengitis virus (LCMV)) and new world (Machupo, Junin) classes. Due to the pathogenicity of these viruses, this work has been done almost exclusively using pseudoparticles bearing the viral glycoprotein (GP) receptors [2,3,30]. The GPs from both classes of arenaviruses fuse in the late endosomes at a pH of 5.5, placing them in the midst of IFITM3's sphere of restriction. To date, the arenaviruses and the bunyavirus CCHF, are the only late-endosomal entering viruses immune to IFITM3. Intriguingly, the only attribute shared between the two classes of arenavirus pseudoparticles is the GP spike because the old world arenaviruses enter in a clathrin-independent manner after binding to the α-dystroglycan receptor, while the new world viruses bind to the transferrin receptor and enter the host cell via clathrin-mediated endocytosis [32].

Structure and Function

Efforts to elucidate the structure and function of the IFITMs have focused on IFITM3. For brevity, we now highlight a few of these insights (Fig. 2).

IFITM3's NTD and Y20: The N-termini of IFITM2 and 3 share strong homology across their first 21 amino acids (aa), a region absent in IFITM1 (Fig. 2). Mutation of a single tyrosine, Y20, within this region produces a 50% or greater loss in restriction, coincident with the mutant isoform's mislocalization to the cell periphery [11,22]. This work suggests that Y20 is required for the proper trafficking of IFITM3 to the late endosomes and lysosomes, and thus is a major functional determinant within this IFITM2/3-specific portion of the NTD. Moreover, Y20 appears to be a key component of the aa cluster, PPNY, which is likely a clathrin-mediated endocytosis motif (CMEM), based on similarity to a bona fide CMEM needed for the localization of the low density lipoprotein receptor [33]. It follows that the lack of this CMEM within IFITM1 contributes to its differential localization, given that both IFITM1 and 3 are comparably palmitoylated on either C72 (IFITM3) or C50 (IFITM1, see section below) [27]. The localization of IFITM1/3 chimeras is also strongly influenced by their respective NTDs [11].

IFITM3's IM1 and IFITM complexes: While searching for proteins which interact with IFITM3 using affinity purification coupled with MS, it was noted that the IFITMs formed

homo- and heteromeric complexes [11]. These associations persisted after high speed ultracentrifugation, suggesting that they were direct in nature. Alanine-scanning (AS) mutagenesis identified two redundant phenylalanine residues in IFITM3's IM1, F75 and F78, which were needed for both complex formation and restriction. The existence of IFITM multimers whose disruption coincides with decreased restriction and whose formation is dependent on residues within IM1 suggests that such interactions may alter the properties of the host cell membrane.

Cysteine palmitoylation: To prevent viral fusion the IFITMs must traffic to vulnerable regions. As discussed, IFITM1 is primarily located in the plasma membrane and in early endosomes, and IFITM3 resides in the late endosomes and lysosomes. Palmitoylation of proteins on cysteines directs them to cellular membranes, and in some instances into the endosomal pathway [34]. Preventing the addition of a palmitate to C72 diminished IFITM3's anti-viral actions and resulted in the mutant's more central cellular location [11,27]. Similar results were also reported for murine Ifitm1 [31]. Thus palmitoylation of a single conserved residue in the CD225 domain plays an important part in preventing viral entry and correctly positioning IFITM1 and 3 [11,27].

The CD225 domain: While this domain defines one of the largest families of membrane-associated proteins, little data exists regarding its function. The structural and functional data for the CD225 domain of IFITM3, presented below, reveals a strong link between proper intracellular location and restriction; thus, lending support for a location-restriction rule. This simply predicts that any perturbation or mutation that alters the location of the IFITMs in respect to the cytosolic membrane or endosomal pathway will decrease restriction [2,9,11,21,27]. Alanine scan (AS) mutagenesis in six residue increments identified the CD225 domain as being the most important region of IFITM3 in terms of its cellular localization, and expression level [11]. The importance of F75 and F78 for IFITM complex formation, together with the role of C72's palmitoylation for localization, provides the earliest structural and functional associations for the CD225 domain and demonstrates this domain's importance for intrinsic immunity [2,9,11,27]. Additional CD225 domain molecular determinants required for IFITM3's restriction of IAV and DENV were found within IM1, with IFITM3 AS mutants 67AS (aa 67-72) and 73AS (aa 73-78) being defective in intracellular localization and antiviral activity; subsequent work has revealed that N69 is likely the most critical residue in this region for both functions (our unpublished data, [11] Fig 3). Again several mutations revealed a strong link between proper intracellular location and restriction. Within IFITM3's CIL, R85, R87, Y99, and K104 were all needed for viral restriction. Y99 has been reported to be phosphorylated on PhosphoSitePlus, however, similar to Y20 both the functional significance and the possible regulation of this PTM remains to be determined. Y99's mutation decreased restriction of IAV more so than DENV, demonstrating that differing structural requirements may be necessary for controlling various infections [11]. Interestingly, Y99A, along with R87A, were mutations which lowered restriction but had intracellular distributions indistinguishable from wild-type IFITM3, thus separating these phenotypes and demonstrating that the CD225 domain functions in both proper positioning and restriction.

To compare the prevalence of these functionally important residues the CLUSTAL and Weblogo programs were used to construct probability diagrams for aa in the IM1, CIL and IM2 regions of either the human IFITMs (IFITM1, 2, 3, 5 and 10, Fig 2C) with or without an additional seven human CD225 proteins (Fig 3B) [35]. These analyses revealed that across the IFITMs, N69, C72, F75, R85 and K104 were among the residues with the highest conservation, consistent with their functional importance to IFITM3-mediated restriction. D56, D86, and D92, are conserved across the CD225 proteins and were found to be required for IFITM3 expression suggesting important roles in stability [11]. D56 is situated immediately prior to IM1 and thus may maintain membrane topology. Though the IM1s of the IFITMs contain a higher percentage of conserved polar residues than found in the IM2s, only N69 was found to be required for restriction by IFITM3, suggesting an alternative functional role and/or redundancy (Fig 2C). IM2 is not a designated portion of the CD225 domain since its sequence conservation is low, however in all the human CD225 proteins the segment following the CIL is hydrophobic and in many instances a leucine/isoleucine zipper is demonstrable. Consideration should be given to extending the CD225 domain to include a distal hydrophobic segment predicted to be membrane-associated. AS mutagenesis of IFITM3's IM2 revealed that the amino acid side chains within this region were not required for either restriction or localization [11]. However because a hydrophobic-based mutagenesis strategy was employed, the resulting alterations may have been too conservative. Therefore, IM2 may indeed serve a role in decreasing membrane fluidity based on its hydrophobic properties and potential leucine zipper motif (discussed below).

IFITM3's role *in vivo*

IFITM3's potent restriction of IAV *in vitro* suggested that it might also play an important role in *vivo*; such is the case, as mice deficient in Ifitm3 succumb more readily to IAV infection when compared to wild-type littermates [12,13]. While these experiments involved the animals' first exposure to IAV, an additional *in vivo* role for Ifitm3 in protecting long term memory CD8+ T cells during re-infection has also been reported [36]. Notably, when Everitt et al. investigated how IFITM3 might influence the clinical course of humans infected with IAV, they found that a minor allele, SNP rs12252-C, was enriched in patients hospitalized due to pandemic H1N1/09 infection in England and Scotland [12]. While the mechanistic role of this allele remains under investigation, the rs12252 C/C SNP alters a predicted splice acceptor site suggesting several scenarios. While comparatively rare in Caucasian populations, the rs12252-C allele is considerably more prevalent in Han Chinese individuals. Therefore it was noteworthy when in an independent study rs12252-C was subsequently found to be enriched for in patients with severe influenza (69% severe influenza vs. 25% mild) [14]. Based on these results the rs12252 allele is estimated to confer a six-fold higher risk for severe influenza. Therefore, populations expressing higher percentages of the rs12252 allele such as in regions of China and Japan may be more at risk for seasonal influenza epidemics and pandemics [14]. It follows that IFITM3 genotyping may be beneficial to clinicians serving these appropriate populations because it could help with risk stratification including decisions for closer observation and administration of therapy. Moreover, these results suggest that IFITM levels and/or actions may influence the clinical course of additional illnesses caused by IFITM-sensitive viruses.

Mechanism of Action

IFITMs block viral fusion and prevent the cytosolic entry of viral genomes. Instead of successfully fusing with the host membrane, IFITM-sensitive viruses are arrested at the cell surface and trapped in the endosomal pathway, resulting in their traveling to the lysosomes or autolysosomes, where they are destroyed (Fig 1A, B). The end result, the sequential inhibition and destruction of the virion serves to lower the inoculum, and is an effective means of preventing invasion by an array of viruses which exploit the endocytic pathway.

How do the IFITMs prevent viral entry? In addressing this, several points must be considered; i) the IFITM's inhibition of an array of viruses that enter at various cell locations using divergent fusion machinery ii) the ability of some viruses to resist one IFITM but not another, iii) the IFITM-immunity of SeV, MLV and all of the arena virus envelopes thus tested iv) previous data showing that their overarching effect is the inhibition of viral fusion subsequent to viral binding and endocytosis and v) data concerning the molecular determinants required for restriction. Recently, we and others have postulated that the IFITMs alter the physical properties of the host cell's membrane thus interfering with viral fusion [11,29,31,37]. In this "tough-membrane" model, we postulate that intramembranous interactions between the IMI domains of adjacent IFITMs alter both the fluidity and the bending modulus of the host cell membrane making it resistant to the viral fusion machinery (Fig 4)[11]. Additionally, the intramembranous insertions of the IMs may asymmetrically compress the outer leaflet generating a curvature directed away from the viral fusion machinery's drawing force. Precedent for this last part comes from studies of the reticulons, which shape the endoplasmic reticulum (ER) into a tubular structure by inserting their two IM domains into the outer leaflet of the membrane [23]. The tough-membrane model predicts that IFITMs must be at the site of viral entry, which has been shown to be the case in mutagenesis studies. Moreover, it accounts for the specificity of IFITM actions, with IFITM1 primarily blocking early endosomal-entering viruses and IFITM3 acting on late endosomal-entering pathogens. In addition, this scenario would account for the rapid action of the IFITMs and their effective inhibition of diverse viruses.

Decreasing membrane fluidity may impede the lateral movements of membrane-associated proteins and thus inhibit both the sequential interaction of viral envelopes with multiple host receptors as is required for HIV-1 and HCV, and also the coalescence of multiple viral envelopes, as has been demonstrated for IAV entry (Fig 4A, B, C) [38]. It is also possible that viral membranes which originate from the host and contain IFITMs will have decreased fluidity. Although speculative, this scenario could also explain why some viruses are immune to the IFITMs; perhaps the IFITM-resistant viral fusion machinery possesses intrinsic properties (strength, cooperativity) that permit them to overcome the increased rigidity of the host membrane. Alternatively, viruses which either depend on a single host receptor (MLV and SeV), or more readily achieve the clustering of the required envelopes to fuse may also overcome restriction. For viruses which are susceptible to the IFITMs but only rely on one host receptor, i.e. JSRV, one possibility is that an additional co-receptor requirement may await detection. An obvious concern with this scenario is that while a more viral-resistant membrane would be advantageous, it could potentially interfere with normal

cellular physiology, e.g. endocytic trafficking, vesicular fusion, cytokinesis, all of which are impacted by membrane fluidity.

How do a relatively few IFITM molecules alter the cell's membrane surfaces? One explanation may come from IFITM1 locating to lipid rafts, an area of the membrane where many viruses bind to host receptors and are endocytosed [25]. This suggests that IFITMs may have been selected to concentrate at, or rapidly move to, viral attachment and entry zones that are enriched for host receptors; one means of achieving this may be seen with IFITM1's association with the tetraspanins, including CD19 and CD81, which reside in lipid rafts [4]. Binding to proteins that are enriched in lipid rafts could efficiently home the IFITMs to a location where they can block multiple viruses without needing to interact with each virus' host receptor; these associations could potentially involve a predicted leucine zipper in IM2. Together, the paired interactions of IM1 and 2 could forseeably tether each end of the IFITMs to create a protective mesh stretching across viral entry zones (Fig 4D). Indeed, a meshwork of tetraspanins, the tetraspanin web, has been previously proposed [39], however, in contrast to earlier pro-viral models of tetraspanin interactions, this scenario would instead be antiviral. For IFITM3, located in late endosomes, this model predicts its association with viral entry zones as the pathogen-bearing endosomes sequentially matures along the endocytic pathway.

While such a direct model of IFITM-induced restriction addresses some issues, we note that the IFITMs cause changes in the endosomal environment, including its expansion and acidification, suggesting that modulation of endosomal conditions may also contribute to viral inhibition [3,9,21,30,40]. In light of this it has recently been proposed that IFITMs antagonize the host cell's lipid homeostasis, resulting in the mislocalization of high levels of cholesterol to the late endosomes producing a block to viral fusion [37]. While cholesterol itself is required by many viruses for fusion, it also has can inhibit infection. IFITM1, 2 and 3 are reported to strongly bind to the endocytic trafficking protein, Vesicle-Associated Membrane Protein-A (VAPA). The IFITM-VAPA interaction interferes with the binding of VAPA to oxysterol binding protein (OSBP) [41]. OSBP is a known regulator of cholesterol trafficking, and has been shown to bind to numerous host proteins, including VAPA. Therefore a competition may exist between OSBP and the IFITMs for VAPA, with higher IFITM levels sending more cholesterol to the late endosomes and blocking viral fusion. An attractive aspect of this model is that the general alteration of membrane cholesterol levels readily explains how a relatively small number of IFITMs can prevent different viruses from entering from the late endosomes and lysosomes. However it is unclear how the mislocalization of cholesterol to the late endosomal compartment would inhibit early endosomal-entering viruses such as HIV-1, HCV, and JSRV. Similarly, this model cannot fully explain IFITM specificity because the interaction of either IFITM1 or 3 with VAPA does not appear to direct excess cholesterol to a particular compartment, i.e. IFITM1 is not shown to relocate cholesterol to the early endosomes to block HCV and JSRV. While these early attempts to explain the antiviral effects of the IFITMs may prove useful, much additional experimentation is required, perhaps leading to the integration of several components of these models. For example, increased cholesterol in combination with IFITM complexes could further toughen the host membrane against viral entry (Fig. 4D, right panel). These unanswered mechanistic issues notwithstanding, the IFITMs represent a

broadly acting and previously unappreciated class of restriction factor which traps and degrades invading pathogens, thereby protecting the host individually as well as at a population level.

Acknowledgments

References

1. Harrison SC. Mechanism of membrane fusion by viral envelope proteins. Adv Virus Res. 2005; 64:231–261. [PubMed: 16139596]

2. Brass AL, Huang IC, Benita Y, John SP, Krishnan MN, Feeley EM, Ryan BJ, Weyer JL, van der Weyden L, Fikrig E, et al. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. Cell. 2009; 139:1243–1254. [PubMed: 20064371]

3. Huang IC, Bailey CC, Weyer JL, Radoshitzky SR, Becker MM, Chiang JJ, Brass AL, Ahmed AA, Chi X, Dong L, et al. Distinct patterns of IFITM-mediated restriction of filoviruses, SARS coronavirus, and influenza A virus. PLoS Pathog. 2011; 7:e1001258. [PubMed: 21253575]

4. Wilkins C, Woodward J, Lau DT, Barnes A, Joyce M, McFarlane N, McKeating JA, Tyrrell DL, Gale M Jr. IFITM1 is a tight junction protein that inhibits hepatitis C virus entry. Hepatology. 2013; 57:461–469. [PubMed: 22996292]

5. Jiang D, Weidner JM, Qing M, Pan XB, Guo H, Xu C, Zhang X, Birk A, Chang J, Shi PY, et al. Identification of five interferon-induced cellular proteins that inhibit west nile virus and dengue virus infections. J Virol. 2010; 84:8332–8341. [PubMed: 20534863]

6. Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, Bieniasz P, Rice CM. A diverse range of gene products are effectors of the type I interferon antiviral response. Nature. 2011; 472:481–485. [PubMed: 21478870]

7. Shapira SD, Gat-Viks I, Shum BO, Dricot A, de Grace MM, Wu L, Gupta PB, Hao T, Silver SJ, Root DE, et al. A physical and regulatory map of host-influenza interactions reveals pathways in H1N1 infection. Cell. 2009; 139:1255–1267. [PubMed: 20064372]

8. Lu J, Pan Q, Rong L, He W, Liu SL, Liang C. The IFITM proteins inhibit HIV-1 infection. J Virol. 2011; 85:2126–2137. [PubMed: 21177806]

9. Feeley EM, Sims JS, John SP, Chin CR, Pertel T, Chen LM, Gaiha GD, Ryan BJ, Donis RO, Elledge SJ, et al. IFITM3 inhibits influenza A virus infection by preventing cytosolic entry. PLoS Pathog. 2011; 7:e1002337. [PubMed: 22046135]

10. Diamond MS, Farzan M. The broad-spectrum antiviral functions of IFIT and IFITM proteins. Nat Rev Immunol. 2013; 13:46–57. [PubMed: 23237964]

11. John SP, Chin CR, Perreira JM, Feeley EM, Aker AM, Savidis G, Smith SE, Elia AE, Everitt AR, Vora M, et al. The CD225 Domain of IFITM3 Is Required for both IFITM Protein Association and Inhibition of Influenza A Virus and Dengue Virus Replication. J Virol. 2013; 87:7837–7852. [PubMed: 23658454]

12. Everitt AR, Clare S, Pertel T, John SP, Wash RS, Smith SE, Chin CR, Feeley EM, Sims JS, Adams DJ, et al. IFITM3 restricts the morbidity and mortality associated with influenza. Nature. 2012; 484:519–523. [PubMed: 22446628]

13. Bailey CC, Huang IC, Kam C, Farzan M. Ifitm3 limits the severity of acute influenza in mice. PLoS Pathog. 2012; 8:e1002909. [PubMed: 22969429]

14. Zhang YH, Zhao Y, Li N, Peng YC, Giannoulatou E, Jin RH, Yan HP, Wu H, Liu JH, Liu N, et al. Interferon-induced transmembrane protein-3 genetic variant rs12252-C is associated with severe influenza in Chinese individuals. Nat Commun. 2013; 4:1418. [PubMed: 23361009]

15. Moffatt P, Gaumond MH, Salois P, Sellin K, Bessette MC, Godin E, de Oliveira PT, Atkins GJ, Nanci A, Thomas G. Bril: a novel bone-specific modulator of mineralization. J Bone Miner Res. 2008; 23:1497–1508. [PubMed: 18442316]

16. Hanagata N, Li X, Morita H, Takemura T, Li J, Minowa T. Characterization of the osteoblast-specific transmembrane protein IFITM5 and analysis of IFITM5-deficient mice. J Bone Miner Metab. 2010

17. Charlesworth G, Bhatia KP, Wood NW. The genetics of dystonia: new twists in an old tale. Brain. 2013; 136:2017–2037. [PubMed: 23775978]

18. Chen WJ, Lin Y, Xiong ZQ, Wei W, Ni W, Tan GH, Guo SL, He J, Chen YF, Zhang QJ, et al. Exome sequencing identifies truncating mutations in PRRT2 that cause paroxysmal kinesigenic dyskinesia. Nat Genet. 2011; 43:1252–1255. [PubMed: 22101681]

19. Meneret A, Gaudebout C, Riant F, Vidailhet M, Depienne C, Roze E. PRRT2 mutations and paroxysmal disorders. Eur J Neurol. 2013

20. Sallman Almen M, Bringeland N, Fredriksson R, Schioth HB. The dispanins: a novel gene family of ancient origin that contains 14 human members. PLoS One. 2012; 7:e31961. [PubMed: 22363774]

21. Yount JS, Karssemeijer RA, Hang HC. S-palmitoylation and ubiquitination differentially regulate interferon-induced transmembrane protein 3 (IFITM3)-mediated resistance to influenza virus. J Biol Chem. 2012; 287:19631–19641. [PubMed: 22511783]

22. Jia R, Pan Q, Ding S, Rong L, Liu SL, Geng Y, Qiao W, Liang C. The N-terminal region of IFITM3 modulates its antiviral activity by regulating IFITM3 cellular localization. J Virol. 2012; 86:13697–13707. [PubMed: 23055554]

23. Voeltz GK, Prinz WA, Shibata Y, Rist JM, Rapoport TA. A class of membrane proteins shaping the tubular endoplasmic reticulum. Cell. 2006; 124:573–586. [PubMed: 16469703]

24. Takahashi S, Doss C, Levy S, Levy R. TAPA-1, the target of an antiproliferative antibody, is associated on the cell surface with the Leu-13 antigen. J Immunol. 1990; 145:2207–2213. [PubMed: 2398277]

25. Bradbury LE, Kansas GS, Levy S, Evans RL, Tedder TF. The CD19/CD21 signal transducing complex of human B lymphocytes includes the target of antiproliferative antibody-1 and Leu-13 molecules. J Immunol. 1992; 149:2841–2850. [PubMed: 1383329]

26. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, Petracca R, Weiner AJ, Houghton M, Rosa D, Grandi G, et al. Binding of hepatitis C virus to CD81. Science. 1998; 282:938–941. [PubMed: 9794763]

27. Yount JS, Moltedo B, Yang YY, Charron G, Moran TM, Lopez CB, Hang HC. Palmitoylome profiling reveals S-palmitoylation-dependent antiviral activity of IFITM3. Nat Chem Biol. 2010; 6:610–614. [PubMed: 20601941]

28. Lozach PY, Huotari J, Helenius A. Late-penetrating viruses. Curr Opin Virol. 2011; 1:35–43. [PubMed: 22440565]

29. Li K, Markosyan RM, Zheng YM, Golfetto O, Bungart B, Li M, Ding S, He Y, Liang C, Lee JC, et al. IFITM proteins restrict viral membrane hemifusion. PLoS Pathog. 2013; 9:e1003124. [PubMed: 23358889]

30. Mudhasani R, Tran JP, Retterer C, Radoshitzky SR, Kota KP, Altamura LA, Smith JM, Packard BZ, Kuhn JH, Costantino J, et al. IFITM-2 and IFITM-3 but Not IFITM-1 Restrict Rift Valley Fever Virus. J Virol. 2013; 87:8451–8464. [PubMed: 23720721]

31. Hach JC, McMichael T, Chesarino NM, Yount JS. Palmitoylation on conserved and non-conserved cysteines of murine IFITM1 regulates its stability and anti-influenza A virus activity. J Virol. 2013

32. Nunberg JH, York J. The curious case of arenavirus entry, and its inhibition. Viruses. 2012; 4:83–101. [PubMed: 22355453]

33. Chen WJ, Goldstein JL, Brown MS. NPXY, a sequence often found in cytoplasmic tails, is required for coated pit-mediated internalization of the low density lipoprotein receptor. J Biol Chem. 1990; 265:3116–3123. [PubMed: 1968060]

34. Chamberlain LH, Lemonidis K, Sanchez-Perez M, Werno MW, Gorleku OA, Greaves J. Palmitoylation and the trafficking of peripheral membrane proteins. Biochem Soc Trans. 2013; 41:62–66. [PubMed: 23356259]

35. Crooks GE, Hon G, Chandonia JM, Brenner SE. WebLogo: a sequence logo generator. Genome Res. 2004; 14:1188–1190. [PubMed: 15173120]

36. Wakim LM, Gupta N, Mintern JD, Villadangos JA. Enhanced survival of lung tissue-resident memory CD8(+) T cells during infection with influenza virus due to selective expression of IFITM3. Nat Immunol. 2013; 14:238–245. [PubMed: 23354485]

37. Amini-Bavil-Olyaee S, Choi YJ, Lee JH, Shi M, Huang IC, Farzan M, Jung JU. The antiviral effector IFITM3 disrupts intracellular cholesterol homeostasis to block viral entry. Cell Host Microbe. 2013; 13:452–464. [PubMed: 23601107]

38. Ivanovic T, Choi JL, Whelan SP, van Oijen AM, Harrison SC. Influenza-virus membrane fusion by cooperative fold-back of stochastically induced hemagglutinin intermediates. Elife. 2013; 2:e00333. [PubMed: 23550179]

39. Boucheix C, Rubinstein E. Tetraspanins. Cell Mol Life Sci. 2001; 58:1189–1205. [PubMed: 11577978]

40. Wee YS, Roundy KM, Weis JJ, Weis JH. Interferon-inducible transmembrane proteins of the innate immune response act as membrane organizers by influencing clathrin and v-ATPase localization and function. Innate Immun. 2012; 18:834–845. [PubMed: 22467717]

41. Wyles JP, McMaster CR, Ridgway ND. Vesicle-associated membrane protein-associated protein-A (VAP-A) interacts with the oxysterol-binding protein to modify export from the endoplasmic reticulum. J Biol Chem. 2002; 277:29908–29918. [PubMed: 12023275]

42. Leung HS, Li OT, Chan RW, Chan MC, Nicholls JM, Poon LL. Entry of influenza A Virus with a alpha2,6-linked sialic acid binding preference requires host fibronectin. J Virol. 2012; 86:10704–10713. [PubMed: 22837202]

43. Chen YC, Wang SY, King CC. Bacterial lipopolysaccharide inhibits dengue virus infection of primary human monocytes/macrophages by blockade of virus entry via a CD14-dependent mechanism. J Virol. 1999; 73:2650–2657. [PubMed: 10074110]

44. Zaitseva E, Yang ST, Melikov K, Pourmal S, Chernomordik LV. Dengue virus ensures its fusion in late endosomes using compartment-specific lipids. PLoS Pathog. 2010; 6:e1001131. [PubMed: 20949067]

45. Smit JM, Moesker B, Rodenhuis-Zybert I, Wilschut J. Flavivirus cell entry and membrane fusion. Viruses. 2011; 3:160–171. [PubMed: 22049308]

46. Chan YK, Huang IC, Farzan M. IFITM proteins restrict antibody-dependent enhancement of dengue virus infection. PLoS One. 2012; 7:e34508. [PubMed: 22479637]

47. Sanchez-San Martin C, Liu CY, Kielian M. Dealing with low pH: entry and exit of alphaviruses and flaviviruses. Trends Microbiol. 2009; 17:514–521. [PubMed: 19796949]

48. Harris HJ, Clerte C, Farquhar MJ, Goodall M, Hu K, Rassam P, Dosset P, Wilson GK, Balfe P, Ijzendoorn SC, et al. Hepatoma polarization limits CD81 and hepatitis C virus dynamics. Cell Microbiol. 2013; 15:430–445. [PubMed: 23126643]

49. Inoue Y, Tanaka N, Tanaka Y, Inoue S, Morita K, Zhuang M, Hattori T, Sugamura K. Clathrin-dependent entry of severe acute respiratory syndrome coronavirus into target cells expressing ACE2 with the cytoplasmic tail deleted. J Virol. 2007; 81:8722–8729. [PubMed: 17522231]

50. Hunt CL, Lennemann NJ, Maury W. Filovirus entry: a novelty in the viral fusion world. Viruses. 2012; 4:258–275. [PubMed: 22470835]

51. Carette JE, Raaben M, Wong AC, Herbert AS, Obernosterer G, Mulherkar N, Kuehne AI, Kranzusch PJ, Griffin AM, Ruthel G, et al. Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. Nature. 2011; 477:340–343. [PubMed: 21866103]

52. Cote M, Misasi J, Ren T, Bruchez A, Lee K, Filone CM, Hensley L, Li Q, Ory D, Chandran K, et al. Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. Nature. 2011; 477:344–348. [PubMed: 21866101]

53. Harmon B, Schudel BR, Maar D, Kozina C, Ikegami T, Tseng CT, Negrete OA. Rift Valley fever virus strain MP-12 enters mammalian host cells via caveola-mediated endocytosis. J Virol. 2012; 86:12954–12970. [PubMed: 22993156]

54. Hollidge BS, Nedelsky NB, Salzano MV, Fraser JW, Gonzalez-Scarano F, Soldan SS. Orthobunyavirus entry into neurons and other mammalian cells occurs via clathrin-mediated

endocytosis and requires trafficking into early endosomes. J Virol. 2012; 86:7988–8001. [PubMed: 22623766]

55. Arikawa J, Takashima I, Hashimoto N. Cell fusion by haemorrhagic fever with renal syndrome (HFRS) viruses and its application for titration of virus infectivity and neutralizing antibody. Arch Virol. 1985; 86:303–313. [PubMed: 3933458]

56. McNulty S, Flint M, Nichol ST, Spiropoulou CF. Host mTORC1 signaling regulates andes virus replication. J Virol. 2013; 87:912–922. [PubMed: 23135723]

57. Jin M, Park J, Lee S, Park B, Shin J, Song KJ, Ahn TI, Hwang SY, Ahn BY, Ahn K. Hantaan virus enters cells by clathrin-dependent receptor-mediated endocytosis. Virology. 2002; 294:60–69. [PubMed: 11886265]

58. McCaughey C, Shi X, Elliot RM, Wyatt DE, O'Neill HJ, Coyle PV. Low pH-induced cytopathic effect--a survey of seven hantavirus strains. J Virol Methods. 1999; 81:193–197. [PubMed: 10488778]

59. Weidner JM, Jiang D, Pan XB, Chang J, Block TM, Guo JT. Interferon-induced cell membrane proteins, IFITM3 and tetherin, inhibit vesicular stomatitis virus infection via distinct mechanisms. J Virol. 2010; 84:12646–12657. [PubMed: 20943977]

60. Finkelshtein D, Werman A, Novick D, Barak S, Rubinstein M. LDL receptor and its family members serve as the cellular receptors for vesicular stomatitis virus. Proc Natl Acad Sci U S A. 2013; 110:7306–7311. [PubMed: 23589850]

61. Zhu R, Wang J, Lei XY, Gui JF, Zhang QY. Evidence for Paralichthys olivaceus IFITM1 antiviral effect by impeding viral entry into target cells. Fish Shellfish Immunol. 2013; 35:918–926. [PubMed: 23850425]

62. Zhang QY, Tao JJ, Gui L, Zhou GZ, Ruan HM, Li ZQ, Gui JF. Isolation and characterization of Scophthalmus maximus rhabdovirus. Dis Aquat Organ. 2007; 74:95–105. [PubMed: 17432038]

63. Yoshii H, Kamiyama H, Goto K, Oishi K, Katunuma N, Tanaka Y, Hayashi H, Matsuyama T, Sato H, Yamamoto N, et al. CD4-independent human immunodeficiency virus infection involves participation of endocytosis and cathepsin B. PLoS One. 2011; 6:e19352. [PubMed: 21541353]

64. Sloan RD, Kuhl BD, Mesplede T, Munch J, Donahue DA, Wainberg MA. Productive entry of HIV-1 during cell-to-cell transmission via dynamin-dependent endocytosis. J Virol. 2013; 87:8110–8123. [PubMed: 23678185]

65. Bertrand P, Cote M, Zheng YM, Albritton LM, Liu SL. Jaagsiekte sheep retrovirus utilizes a pH-dependent endocytosis pathway for entry. J Virol. 2008; 82:2555–2559. [PubMed: 18094164]

66. Cote M, Zheng YM, Liu SL. Receptor binding and low pH coactivate oncogenic retrovirus envelope-mediated fusion. J Virol. 2009; 83:11447–11455. [PubMed: 19726505]

67. Anafu AA, Bowen CH, Chin CR, Brass AL, Holm GH. Interferon Inducible Transmembrane Protein 3 (IFITM3) Restricts Reovirus Cell Entry. J Biol Chem. 2013

68. Danthi P, Holm GH, Stehle T, Dermody TS. Reovirus receptors, cell entry, and proapoptotic signaling. Adv Exp Med Biol. 2013; 790:42–71. [PubMed: 23884585]

69. Zhan QY, Xiao F, Li ZQ, Gui JF, Mao J, Chinchar VG. Characterization of an iridovirus from the cultured pig frog Rana grylio with lethal syndrome. Dis Aquat Organ. 2001; 48:27–36. [PubMed: 11843137]

70. Rojek JM, Kunz S. Cell entry by human pathogenic arenaviruses. Cell Microbiol. 2008; 10:828–835. [PubMed: 18182084]

71. Kunz S. Receptor binding and cell entry of Old World arenaviruses reveal novel aspects of virus-host interaction. Virology. 2009; 387:245–249. [PubMed: 19324387]

72. Burri DJ, Pasqual G, Rochat C, Seidah NG, Pasquato A, Kunz S. Molecular characterization of the processing of arenavirus envelope glycoprotein precursors by subtilisin kexin isozyme-1/site-1 protease. J Virol. 2012; 86:4935–4946. [PubMed: 22357276]

73. Moraz ML, Pythoud C, Turk R, Rothenberger S, Pasquato A, Campbell KP, Kunz S. Cell entry of Lassa virus induces tyrosine phosphorylation of dystroglycan. Cell Microbiol. 2013; 15:689–700. [PubMed: 23279385]

74. Coffin JM. Virions at the gates: receptors and the host-virus arms race. PLoS Biol. 2013; 11:e1001574. [PubMed: 23723739]

75. Zhang Y, Li L, Liu X, Dong S, Wang W, Huo T, Guo Y, Rao Z, Yang C. Crystal structure of Junin virus nucleoprotein. J Gen Virol. 2013

76. Helguera G, Jemielity S, Abraham J, Cordo SM, Martinez MG, Rodriguez JA, Bregni C, Wang JJ, Farzan M, Penichet ML, et al. An antibody recognizing the apical domain of human transferrin receptor 1 efficiently inhibits the entry of all new world hemorrhagic Fever arenaviruses. J Virol. 2012; 86:4024–4028. [PubMed: 22278244]

77. Martinez MG, Forlenza MB, Candurra NA. Involvement of cellular proteins in Junin arenavirus entry. Biotechnol J. 2009; 4:866–870. [PubMed: 19548229]

78. Kumar P, Nachagari D, Fields C, Franks J, Albritton LM. Host cell cathepsins potentiate Moloney murine leukemia virus infection. J Virol. 2007; 81:10506–10514. [PubMed: 17634228]

79. Voelkel C, Galla M, Dannhauser PN, Maetzig T, Sodeik B, Schambach A, Baum C. Pseudotype-independent nonspecific uptake of gammaretroviral and lentiviral particles in human cells. Hum Gene Ther. 2012; 23:274–286. [PubMed: 22010882]

80. Lee S, Zhao Y, Anderson WF. Receptor-mediated Moloney murine leukemia virus entry can occur independently of the clathrin-coated-pit-mediated endocytic pathway. J Virol. 1999; 73:5994–6005. [PubMed: 10364351]

81. Bitzer M, Lauer U, Baumann C, Spiegel M, Gregor M, Neubert WJ. Sendai virus efficiently infects cells via the asialoglycoprotein receptor and requires the presence of cleaved F0 precursor proteins for this alternative route of cell entry. J Virol. 1997; 71:5481–5486. [PubMed: 9188621]

82. Chambers R, Takimoto T. Trafficking of Sendai virus nucleocapsids is mediated by intracellular vesicles. PLoS One. 2010; 5:e10994. [PubMed: 20543880]

83. Plattet P, Plemper RK. Envelope protein dynamics in paramyxovirus entry. MBio. 2013:4.

84. Xiao X, Feng Y, Zhu Z, Dimitrov DS. Identification of a putative Crimean-Congo hemorrhagic fever virus entry factor. Biochem Biophys Res Commun. 2011; 411:253–258. [PubMed: 21723257]

85. Simon M, Johansson C, Mirazimi A. Crimean-Congo hemorrhagic fever virus entry and replication is clathrin-, pH- and cholesterol-dependent. J Gen Virol. 2009; 90:210–215. [PubMed: 19088291]

86. Garrison AR, Radoshitzky SR, Kota KP, Pegoraro G, Ruthel G, Kuhn JH, Altamura LA, Kwilas SA, Bavari S, Haucke V, et al. Crimean-Congo hemorrhagic fever virus utilizes a clathrin- and early endosome-dependent entry pathway. Virology. 2013; 444:45–54. [PubMed: 23791227]

87. Yang G, Xu Y, Chen X, Hu G. IFITM1 plays an essential role in the antiproliferative action of interferon-gamma. Oncogene. 2007; 26:594–603. [PubMed: 16847454]

88. Zhang Z, Liu J, Li M, Yang H, Zhang C. Evolutionary dynamics of the interferon-induced transmembrane gene family in vertebrates. PLoS One. 2012; 7:e49265. [PubMed: 23166625]

89. Daniel-Carmi V, Makovitzki-Avraham E, Reuven EM, Goldstein I, Zilkha N, Rotter V, Tzehoval E, Eisenbach L. The human 1-8D gene (IFITM2) is a novel p53 independent pro-apoptotic gene. Int J Cancer. 2009; 125:2810–2819. [PubMed: 19544527]

90. Seo GS, Lee JK, Yu JI, Yun KJ, Chae SC, Choi SC. Identification of the polymorphisms in IFITM3 gene and their association in a Korean population with ulcerative colitis. Exp Mol Med. 2010; 42:99–104. [PubMed: 19946179]

91. Shen C, Wu XR, Jiao WW, Sun L, Feng WX, Xiao J, Miao Q, Liu F, Yin QQ, Zhang CG, et al. A Functional Promoter Polymorphism of IFITM3 Is Associated with Susceptibility to Pediatric Tuberculosis in Han Chinese Population. PLoS One. 2013; 8:e67816. [PubMed: 23874452]

92. Hanagata N, Li X. Osteoblast-enriched membrane protein IFITM5 regulates the association of CD9 with an FKBP11-CD81-FPRP complex and stimulates expression of interferon-induced genes. Biochem Biophys Res Commun. 2011; 409:378–384. [PubMed: 21600883]

93. Semler O, Garbes L, Keupp K, Swan D, Zimmermann K, Becker J, Iden S, Wirth B, Eysel P, Koerber F, et al. A mutation in the 5′-UTR of IFITM5 creates an in-frame start codon and causes autosomal-dominant osteogenesis imperfecta type V with hyperplastic callus. Am J Hum Genet. 2012; 91:349–357. [PubMed: 22863195]

94. Rauch F, Moffatt P, Cheung M, Roughley P, Lalic L, Lund AM, Ramirez N, Fahiminiya S, Majewski J, Glorieux FH. Osteogenesis imperfecta type V: marked phenotypic variability despite the presence of the IFITM5 c.-14C>T mutation in all patients. J Med Genet. 2013; 50:21–24. [PubMed: 23240094]

95. Cho TJ, Lee KE, Lee SK, Song SJ, Kim KJ, Jeon D, Lee G, Kim HN, Lee HR, Eom HH, et al. A single recurrent mutation in the 5′-UTR of IFITM5 causes osteogenesis imperfecta type V. Am J Hum Genet. 2012; 91:343–348. [PubMed: 22863190]

96. Takagi M, Sato S, Hara K, Tani C, Miyazaki O, Nishimura G, Hasegawa T. A recurrent mutation in the 5′-UTR of IFITM5 causes osteogenesis imperfecta type V. Am J Med Genet A. 2013; 161:1980–1982. [PubMed: 23813632]

97. Kasaai B, Gaumond MH, Moffatt P. Regulation of the bone-restricted IFITM-like (Bril) gene transcription by Sp and Gli family members and CpG methylation. J Biol Chem. 2013; 288:13278–13294. [PubMed: 23530031]

98. Grover M, Campeau PM, Lietman CD, Lu JT, Gibbs RA, Schlesinger AE, Lee BH. Osteogenesis imperfecta without features of type V caused by a mutation in the IFITM5 gene. J Bone Miner Res. 2013

99. Zhang Z, Li M, He JW, Fu WZ, Zhang CQ, Zhang ZL. Phenotype and genotype analysis of chinese patients with osteogenesis imperfecta type v. PLoS One. 2013; 8:e72337. [PubMed: 23977282]

100. Shapiro JR, Lietman C, Grover M, Lu JT, Nagamani SC, Dawson BC, Baldridge DM, Bainbridge MN, Cohn DH, Blazo M, et al. Phenotypic variability of osteogenesis imperfecta type V caused by an IFITM5 mutation. J Bone Miner Res. 2013; 28:1523–1530. [PubMed: 23408678]

101. Siegrist F, Ebeling M, Certa U. The small interferon-induced transmembrane genes and proteins. J Interferon Cytokine Res. 2011; 31:183–197. [PubMed: 21166591]

102. Hanagata N, Li X, Morita H, Takemura T, Li J, Minowa T. Characterization of the osteoblast-specific transmembrane protein IFITM5 and analysis of IFITM5-deficient mice. J Bone Miner Metab. 2011; 29:279–290. [PubMed: 20838829]

103. Liu Y, Liu H, Titus L, Boden SD. Natural antisense transcripts enhance bone formation by increasing sense IFITM5 transcription. Bone. 2012; 51:933–938. [PubMed: 22884724]

104. Balasubramanian M, Parker MJ, Dalton A, Giunta C, Lindert U, Peres LC, Wagner BE, Arundel P, Offiah A, Bishop NJ. Genotype-phenotype study in type V osteogenesis imperfecta. Clin Dysmorphol. 2013; 22:93–101. [PubMed: 23612438]

105. Marini JC, Blissett AR. New Genes in Bone Development: What's New in Osteogenesis Imperfecta. J Clin Endocrinol Metab. 2013; 98:3095–3103. [PubMed: 23771926]

106. Lazarus S, Moffatt P, Duncan EL, Thomas GP. A brilliant breakthrough in OI type V. Osteoporos Int. 2013

107. Merritt MA, Parsons PG, Newton TR, Martyn AC, Webb PM, Green AC, Papadimos DJ, Boyle GM. Expression profiling identifies genes involved in neoplastic transformation of serous ovarian cancer. BMC Cancer. 2009; 9:378. [PubMed: 19849863]

108. Shanks NF, Savas JN, Maruo T, Cais O, Hirao A, Oe S, Ghosh A, Noda Y, Greger IH, Yates JR 3rd, et al. Differences in AMPA and kainate receptor interactomes facilitate identification of AMPA receptor auxiliary subunit GSG1L. Cell Rep. 2012; 1:590–598. [PubMed: 22813734]

109. Hedera P, Xiao J, Puschmann A, Momcilovic D, Wu SW, LeDoux MS. Novel PRRT2 mutation in an African-American family with paroxysmal kinesigenic dyskinesia. BMC Neurol. 2012; 12:93. [PubMed: 22985072]

110. Heron SE, Ong YS, Yendle SC, McMahon JM, Berkovic SF, Scheffer IE, Dibbens LM. Mutations in PRRT2 are not a common cause of infantile epileptic encephalopathies. Epilepsia. 2013; 54:e86–89. [PubMed: 23566103]

111. Silveira-Moriyama L, Gardiner AR, Meyer E, King MD, Smith M, Rakshi K, Parker A, Mallick AA, Brown R, Vassallo G, et al. Clinical features of childhood-onset paroxysmal kinesigenic dyskinesia with PRRT2 gene mutations. Dev Med Child Neurol. 2013; 55:327–334. [PubMed: 23363396]

112. Shi CH, Sun SL, Wang JL, Liu AQ, Miao W, Avinash C, Mao X, Tang BS, Xu YM. PRRT2 gene mutations in familial and sporadic paroxysmal kinesigenic dyskinesia cases. Mov Disord. 2013; 28:1313–1314. [PubMed: 23456995]

113. Jing XY, Li XH, Yuan P, Deng J, Hu B, Wang Y. A novel mutation and functional implications of 5 variants in the PRRT2 gene in 20 paroxysmal kinesigenic dyskinesia pedigrees. Parkinsonism Relat Disord. 2013; 19:639–642. [PubMed: 23529024]

114. Fabbri M, Marini C, Bisulli F, Di Vito L, Elia A, Guerrini R, Mei D, Tinuper P. Clinical and polygraphic study of familial paroxysmal kinesigenic dyskinesia with PRRT2 mutation. Epileptic Disord. 2013; 15:123–127. [PubMed: 23771590]

115. Wang JL, Mao X, Hu ZM, Li JD, Li N, Guo JF, Jiang H, Shen L, Li J, Shi YT, et al. Mutation analysis of PRRT2 in two Chinese BFIS families and nomenclature of PRRT2 related paroxysmal diseases. Neurosci Lett. 2013

116. Oort PJ, Warden CH, Baumann TK, Knotts TA, Adams SH. Characterization of Tusc5, an adipocyte gene co-expressed in peripheral neurons. Mol Cell Endocrinol. 2007; 276:24–35. [PubMed: 17689857]

117. Bubnov V, Moskalev E, Petrovskiy Y, Bauer A, Hoheisel J, Zaporozhan V. Hypermethylation of TUSC5 genes in breast cancer tissue. Exp Oncol. 2012; 34:370–372. [PubMed: 23302999]

118. Lovero KL, Blankenship SM, Shi Y, Nicoll RA. SynDIG1 promotes excitatory synaptogenesis independent of AMPA receptor trafficking and biophysical regulation. PLoS One. 2013; 8:e66171. [PubMed: 23785483]

119. de Chaldee M, Brochier C, Van de Vel A, Caudy N, Luthi-Carter R, Gaillard MC, Elalouf JM. Capucin: a novel striatal marker down-regulated in rodent models of Huntington disease. Genomics. 2006; 87:200–207. [PubMed: 16359841]

120. Kim W, Bennett EJ, Huttlin EL, Guo A, Li J, Possemato A, Sowa ME, Rad R, Rush J, Comb MJ, et al. Systematic and quantitative assessment of the ubiquitin-modified proteome. Mol Cell. 2011; 44:325–340. [PubMed: 21906983]

121. Wagner SA, Beli P, Weinert BT, Nielsen ML, Cox J, Mann M, Choudhary C. A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles. Mol Cell Proteomics. 2011; 10:M111 013284.

122. Shi Y, Chan DW, Jung SY, Malovannaya A, Wang Y, Qin J. A data set of human endogenous protein ubiquitination sites. Mol Cell Proteomics. 2011; 10:M110 002089.

123. Olsen JV, Vermeulen M, Santamaria A, Kumar C, Miller ML, Jensen LJ, Gnad F, Cox J, Jensen TS, Nigg EA, et al. Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. Sci Signal. 2010; 3:ra3. [PubMed: 20068231]

Highlights

- IFITMs are host intramembrane proteins that block viral fusion resulting in virus degradation.

- IFITMs inhibit a growing number of pathogenic viruses, including Influenza A virus (IAV), dengue virus, and Ebola virus.

- Mice null for Ifitm3 exhibit increased disease severity when infected with IAV.

- Patients expressing a variant allele of IFITM3 are more likely to develop severe influenza infection.

- Recent work suggests that IFITMs may toughen the host membrane thus preventing viral membrane fusion.
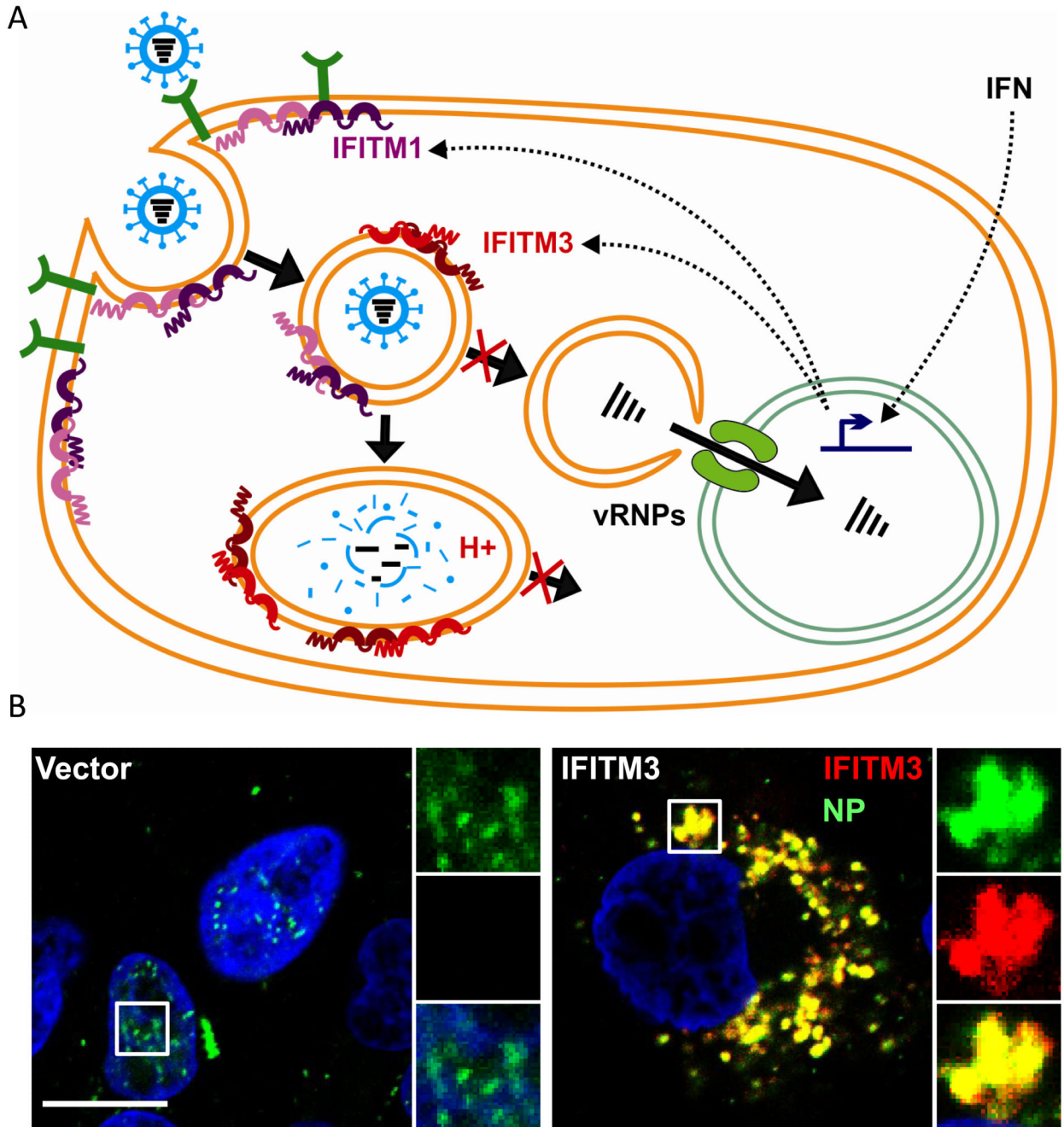
Figure 1.

(A) Model of IFITM3-mediated restriction of viral replication. Influenza A viruses (blue with genomes in black) first interact with a cell surface receptor (green) and then enter the cell through endocytosis. IFITM1 (purple, with light and dark hues representing two distinct IFITM1 molecules), located in the cytosolic leaflet of the plasma membrane and endosomal membrane, prevents the fusion of viruses that enter in the early endosomes (i.e. HCV) as well as viruses fusing later in the endosomal pathway (i.e. IAV). IFITM3 (red, with dark and light hues representing two distinct IFITM3 molecules) resides in the late endosomal

membranes and lysosomal membranes and prevents viral fusion of viruses that enter from those compartments. Because of the block to fusion there is no release of viral RNPs (vRNPs), and therefore no viral replication. As a result of IFITM-mediated restriction the trapped viral particles are destroyed in the lysosomes and/or autolysosomes. IFITM3 is present at baseline, however, interferon (IFN) upregulates its levels, as well as inducing the expression of IFITM1 (dashed lines).

(B) IFITM3 prevents IAV cytosolic entry and nuclear entry. Confocal images of Mardin Darby canine kidney cells stably transduced with the empty retroviral vector, pQCXIP (Vector, Clontech), or one expressing IFITM3, that were infected with IAV (A/Puerto Rico/ 8/1934 (H1N1) (PR8, Charles River Labs)) for 90 min. The cells were then washed, fixed, permeablized and immunostained for IAV nucleoprotein (NP, green), IFITM3 (red) or nuclear DNA (blue) [9]. In the Vector cells the IAV NP can be seen in the host cell nucleus (left panel). In contrast, in the IFITM3 cells the virus is prevented from entering the cytosol and instead the NP is seen sequestered in the IFITM3-positive endosomal compartments. Scale bar=10 μm.
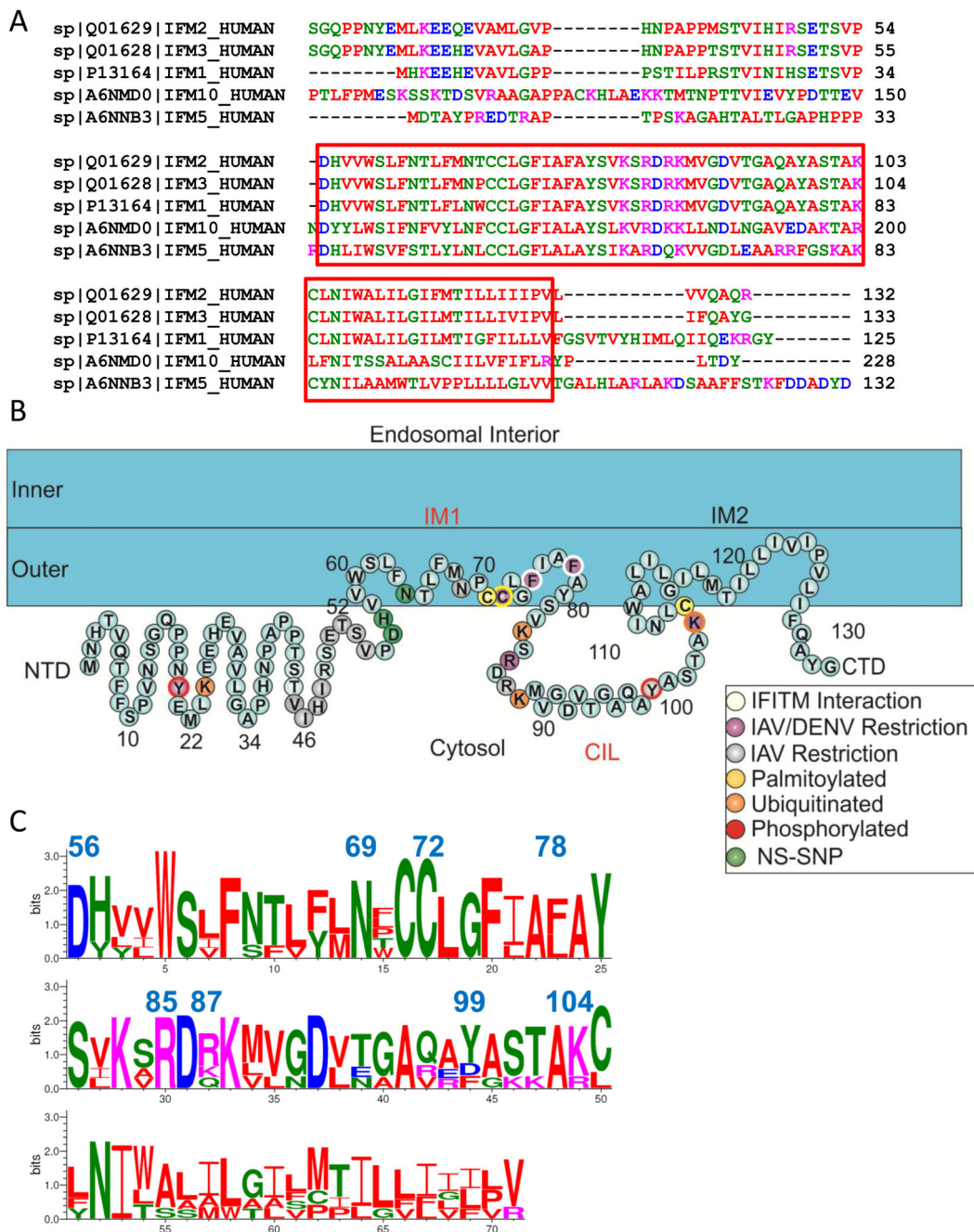
Figure 2.

(A) Alignment of the human IFITM1, IFITM2, IFITM3, IFITM5 and IFITM10 protein sequences (ClustalW2). The amino acids are color coded as follows: red, hydrophobic amino acids; green, polar amino acids and basic amino acids; blue, acidic amino acids. The CD225 domain and the adjacent IM2s of the aligned proteins are outlined in red. Gaps introduced to maximize alignment are indicated by dashes.

(B) Cartoon of IFITM3 in the endosomal membrane. The molecular determinants required for the IFITM3-mediated restriction, sites of post-translational modifications and

nonsynonymous single-nucleotide polymorphisms (NS-SNP) are indicated in the key (bottom right). The N-terminal domain (NTD), intramembrane domain 1 (IM1), conserved intracellular loop (CIL), and intramembrane domain 2 (IM2), are indicated. IM1 and the CIL are in red font to convey that they comprise the canonical CD225 domain. The outer and inner leaflets of the endosomal membrane are noted.

(C) Sequence logo for the CD225 domains and IM2s of the human IFITMs in (A). The respective amino acid properties color coded as above. Blue numbers indicate residues in IFITM3 found to be required for restriction, localization, or expression. These and similar figures were generated using Weblogo and the CLUSTALW freeware programs.

A

```
sp|Q01628|IFM3_HUMAN    DHVVWSLFNTLFMNPCCLGFIAFAYSVKSRDRKMVGDVTGAQAYASTAKCLNIWALILGILMTILLIVIPV
sp|Q01629|IFM2_HUMAN    DHVVWSLFNTLFMNTCCLGFIAFAYSVKSRDRKMVGDVTGAQAYASTAKCLNIWALILGIFMTILLIIIPV
sp|P13164|IFM1_HUMAN    DHVVWSLFNTLFLNWCCLGFIAFAYSVKSRDRKMVGDVTGAQAYASTAKCLNIWALILGILMTIGFILLLV
sp|A6NNB3|IFM5_HUMAN    DHLIWSVFSTLYLNLCCLGFLALAYSIKARDQKVVGDLEAARRFGSKAKCYNILAAMWTLVPPLLLLGLVV
sp|A6NMD0|IF10_HUMAN    DYYLWSIFNFVYLNFCCLGFIALAYSLKVRDKKLLNDLNGAVEDAKTARLFNITSSALAASCIILVFIFLR
sp|B4DJY2|TM233_HUMAN   NYLWLTIVSCFCPAYPINIVALVFSIMSLNSYNDGDYEGARRLGRNAKWVAIASIIIGLLIIGISCAVHFT
sp|Q8IXB3|TUSC5_HUMAN   DYLILAVVACFCPVWPLNLIPLIISIMSRSSMQQGNVDGARRLGRLARLLSITLIIMGIVIIMVAVTVNFT
sp|Q7Z6L0|PRRT2_HUMAN   DYIILAILSCFCPMWPVNIVAFAYAVMSRNSLQQGDVDGAQRLGRVAKLLSIVALVGGVLIIIASCVINLG
sp|A6NDD5|SYN1L_HUMAN   DHLGLTLFSMLCCFWPLGIAAFYFSQGTSKAISKGDFRLASTTSRRALFLATLAIAVGAGLYVAVVVALAA
sp|Q9H7V2|SYNG1_HUMAN   DHLGLSVFSMLCCFWPLGIAAFYLSHETNKAVAKGDLHQASTSSRRALFLAVLSITIGTGVYVGVAVALIA
sp|Q6ZNR0|TMM91_HUMAN   DHLGLAVFSMLCCFWPVGIAAFCLAQKTNKAWAKGDIQGAGAASRRAFLLGVLAVGLGVCTYAAALVTLAA
sp|Q99946|PRRT1_HUMAN   DYMPIAVLTTICCFWPTGIIAIFKAVQVRTALARGDMVSAEIASREARNFSFISLAVGIAAMVLCTILTVV
```

B
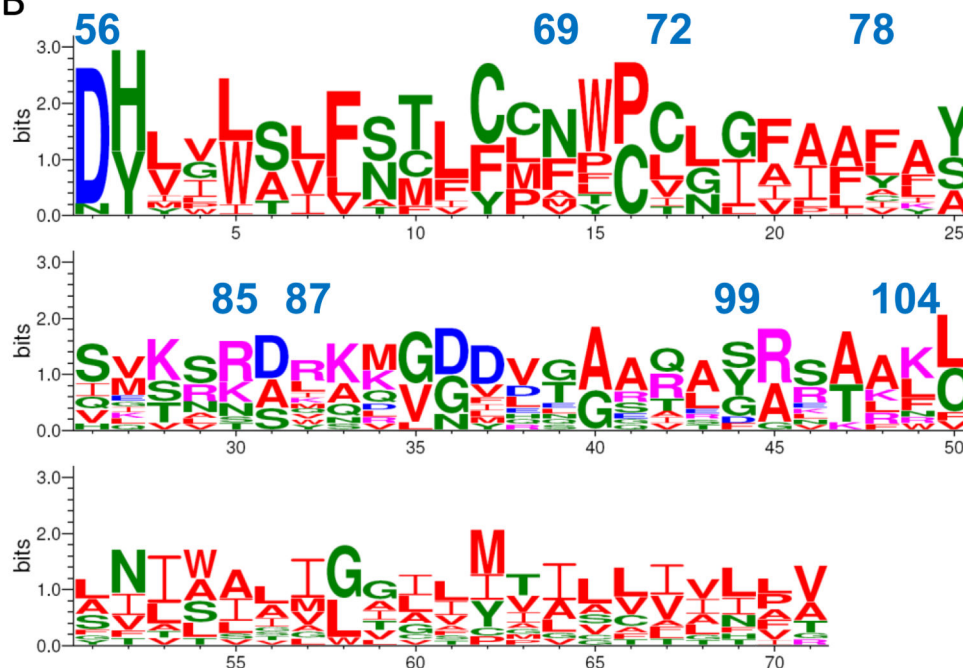
Figure 3.

(A) Alignment of the CD225 domains and IM2s of the human CD225 proteins (ClustalW2). The amino acids are color coded as follows: red, hydrophobic amino acids; green, polar amino acids and basic amino acids; blue, acidic amino acids.

(B) Sequence logo for the human CD225 proteins. The respective amino acid properties color coded as in (A). Blue numbers indicate residues in IFITM3 found to be required for restriction, localization, or expression. This figure was generated using Weblogo.
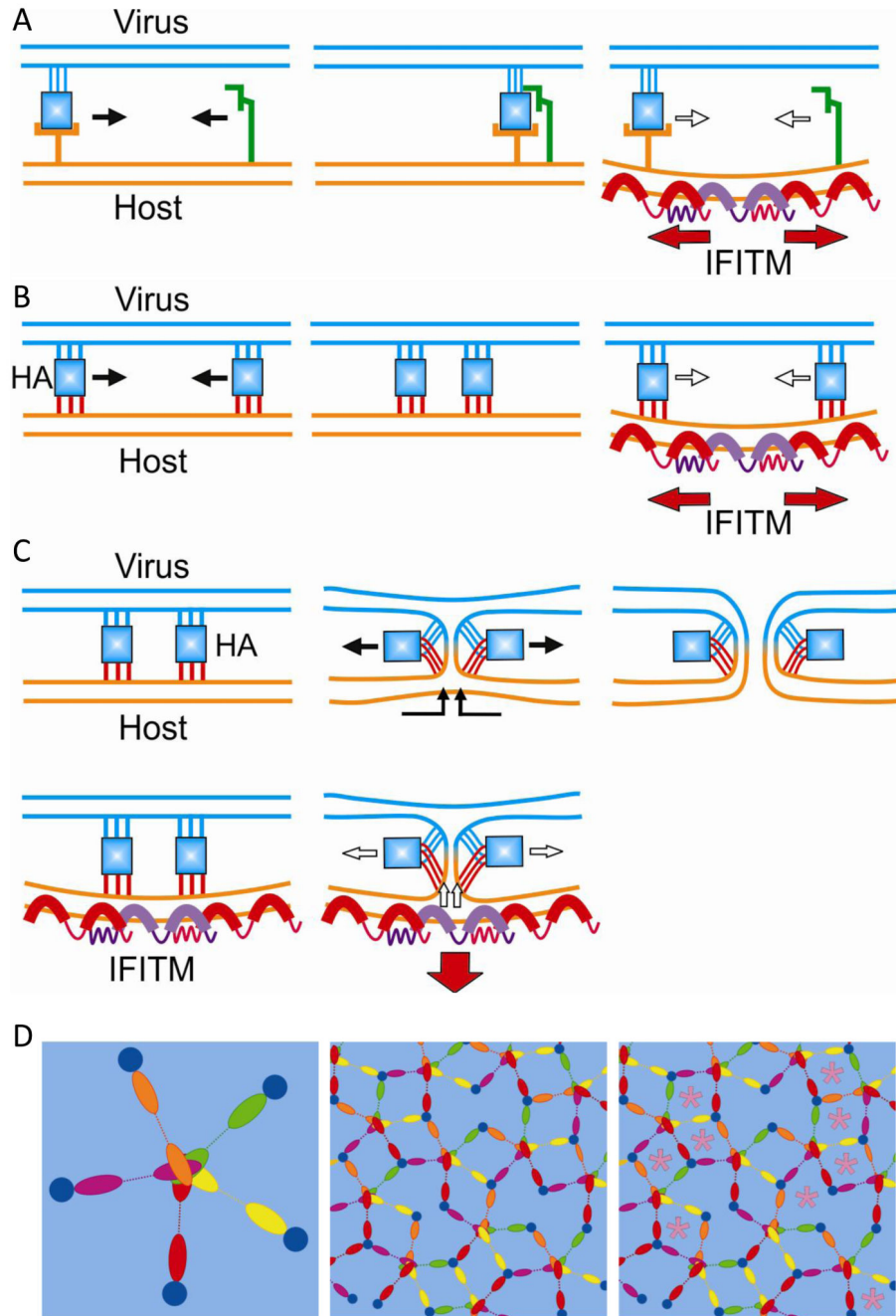
**Figure 4. Model of IFITM antiviral action**
We postulate that one or more of the following IFITM-mediated events "toughens" the host cell membrane and prevents viral fusion. **(A)** Inhibition of sequential host co-receptor interactions. After binding of the viral receptor-binding protein (blue, downward facing) to a host receptor (orange, upward facing, left panel) the pair then moves through the membrane surface until one or more required co-receptors (green) are encountered (middle panel), ultimately triggering fusion peptide insertion (not shown). In contrast, we postulate that adjacent IFITMs interact via their IM1s to decrease membrane fluidity and restrict the lateral

movement of the partially assembled receptor complex. Black arrows: movement through the membrane; white arrows: diminished movement; red arrows: resistance within the membrane generated by IFITMs.

(B) Inhibition of viral envelope protein clustering. Two HA receptors (blue) are shown with their fusion peptides (red) inserted into the host membrane (left panel). The engaged receptors then move through the membrane and coalesce (middle panel). This juxtapositioning permits the HA receptors to coordinately generate a fusion pore (C)[38]. Similar to (A), the presence of intramembranous IFITMs decreases HA receptor movement and prevents their effective association. Arrows are as above.

(C) HA-mediated fusion: Top row: As in (B) but in the absence of IFITMs, the HA receptors have now been able to coordinately generate a fusion pore (right panel). However, IFITMs residing in the membrane alter the properties of the host membrane due to intramolecular interactions and assymetric membranous insertions. The association of IFITMs via their IM1s decreases membrane fluidity. Furthermore the insertions of the IM domains of each IFITM into the outer leaflet of the membrane produces a curvature directed away from the HA receptors drawing force. We envision that this induced curvature would require that greater force be exerted by the viral fusion machinery, thus preventing the formation of a pore. Arrows are as above.

(D) Superior view of IFITM-IFITM interactions occurring in the membrane. We speculate that IFITMs multiplex via their IM1s (assorted-color ovals binding to one another at a central IM1-generated hub, left panel). A pentamer is shown as one possible IFITM complex. Radiating from this common interaction point, the more distal IM2s form interactions with the transmembrane or intramembrane domains of additional proteins (blue), i.e. the tetraspanins. When such IFITM units are symmetrically multiplied, they could possibly form an integrated matrix that alters the membrane's fluidity and bending modulus (right panel). This meshwork may also enhance the membrane-rigidifying properties of cholesterol (pink stars, [37]) The CILs that connect IM1 and IM2 and lie in the cytosol are represented by dotted lines.

Table 1

Viruses Inhibited by IFITM proteins

Virus	Family	Host Receptor	Endocytic Pathway	Where the virus enters the host cell	pH Requirement*	Cathepsin Processing	IFITM specificity	References
Influenza A virus	Orthomyxovirus	α2,6-linked sialic acid (human)	clathrin-mediated endocytosis, macropinocytosis	RAB7+ Late endosomes	pH 5.5	No	IFITM3>IFITM2>IFIT M1	[2,3,9,29,33,42]
Dengue Virus	Flavivirus	CD14	clathrin-mediated endocytosis	RAB7+ Late endosomes	pH 5.5	No	K562: IFITM3/IFITM1>IFIT M2	[2,5,43-46]
West Nile Virus	Flavivirus	Unknown	clathrin-mediated endocytosis	RAB7+ Late Endosomes	pH 5.5	No	IFITM3>IFITM1>IFIT M2	[2,5,6,45,47]
Yellow Fever Virus	Flavivirus	Unknown	clathrin-mediated endocytosis	RAB7+ Late Endosomes	pH 5.5	No	IFITM3>IFITM1>IFIT M2	[2,5,6,45,47]
Omsk Hemorrhagic Fever Virus	Flavivirus	Unknown	clathrin-mediated endocytosis	RAB7+ Late Endosomes	pH 5.5	No	IFITM3>IFITM1>IFIT M2	[2,5,6,45,47]
Hepatitis C Virus	Flavivirus	CD81, Occludin, SB-RI, Claudin-1	clathrin-mediated endocytosis	RAB5+ Early Endosomes	pH 6.5	No	IFITM1,notIFITM3	[2,4,6,47,48]
Severe Acute Respiratory Syndrome Coronavirus	Coronavirus	Angiotensin-converting enzyme 2 (ACE2)	clathrin-mediated endocytosis	Lysosomes (pH-dependent cleavage: Cathepsin B and L)	pH 4.5	yes	A549: IFITM3/IFITM2 IFIT M1 Vero E6: IFITM1>IFITM2>IFIT M3	[3,49]
Marburg Virus	Filovirus	Neimann-Pick C1 (NPC1), T-cell immunoglobulin mucin domain-1 (TIM-1), C-type lectins	Macropinocytosis	NPC1+ Lysosomes (pH-dependent cleavage: Cathepsins B and L)	pH 4.5	yes	A549: IFITM3>IFITM1>IFIT M2 Vero E6: IFITM1>IFITM2/IFIT M3 HUVEC: IFITM3>IFITM1>IFIT M2 293T: IFITM3>IFITM2>IFIT M1	[3,50]
Ebola Virus	Filovirus	Neimann-Pick C1 (NPC-1), T-cell immunoglobulin mucin domain-1 (TIM-1), C-type	Macropinocytosis	NPC1+ Lysosomes (pH-dependent cleavage: Cathepsins B and L)	pH 4.5	yes	A549: IFITM1/IFITM3>IFIT M2 Vero E6: IFITM1>IFITM2/IFIT M3 HUVEC: IFITM1/IFITM3>IFIT M2 293T: IFITM3>IFITM1/IFIT	[3,50-52]

Virus	Family	Host Receptor	Endocytic Pathway	Where the virus enters the host cell	pH Requirement*	Cathepsin Processing	IFITM specificity	References
		Lectins					M2	
Rift Valley Fever Virus	Bunyavirus	Unknown	dynamin II-dependent caveolin-1-mediated endocytosis	RAB7+ Late Endosomes	pH 5.5	No	IFITM2/IFITM3	[30,53]
La Crosse Virus	Bunyavirus	Unknown	clathrin-mediated endocytosis	RAB7+ Late Endosomes	pH 5.5	No	IFITM1/IFITM2/IFITM3	[30,54,55]
Andes Virus	Bunyavirus	Unknown	Unknown	RAB7+ Late Endosomes	pH 5.5	No	IFITM1/IFITM2/IFITM3	[30,56]
Hantaan Virus	Bunyavirus	Unknown	clathrin-dependent endocytosis	RAB7+ Late Endosomes	pH 5.5	No	IFITM1/IFITM2/IFITM3	[30,57,58]
Vesicular stomatitis Indiana virus	Rhabdovirus	LDL receptor	clathrin-mediated endocytosis	RAB5+ Early Endosomes	pH 6.5	No	IFITM3>IFITM1>IFITM2	2,29,30,53,59,60]
Scophthalmus maximus rhabdovirus	Rhabdovirus	Unknown	Unknown	Unknown	Stable infectivity between pH4 and pH9.	Unknown	IFITM1 (*Paralichthys olivaceus*)	[61,62]
Human Immunodeficiency Virus 1	Lentivirus	CD4, CXCR4 or CCR5	Unknown	Cell surface, Early Endosomes	none	No	SUPT1: IFITM1>IFITM3/IFITM2, IFITM3 doesnot block HIV-1 from infecting TZM-bl HeLa cells	[6,8,22,63,64]
Jaagsiekte Sheep Retrovirus	Betaretrovirus	Hyaluronidase 2 (Hyal2)	dynamin associated endocytosis	RAB5+ Early Endosomes	pH 6.5	No	HTX: IFITM1>IFITM2>IFITM3 293: IFITM1>IFITM3>IFITM M2 Cos7: IFITM1>IFITM3	[29,65,66]
Reovirus	Reovirus	Proteinaceous receptor junction adhesion molecule A (JAM-A)	clathrin-mediated endocytosis	RAB7+/RAB9+ Late Endosomes	pH 5.5	No	IFITM3	[67,68]
Rana grylio Virus	Iridoviridae	Unknown	Caveolin-mediated endocytosis	Unknown	pH-dependent	Unknown	IFITM1 (*Paralichthys olivaceus*)	[61,69]

*
pH values provided have been standardized based on reports of the values found for the relevant endosomal compartments in the primary literature: early endosome: pH 6.5, late endosomes: pH 5.5, Lysosome: pH 4.5.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Table 2

Viruses Not Inhibited by IFITM proteins

Virus	Family	Host Receptor	Endocytic Pathway	Where the virus enters the host cell	pH Requirement*	Cathepsin Processing	IFITM specificity	References
Lymphocytic Choriomeningitis Virus	Arenavirus	Dystroglycan (DG)	Clathrin-independent, caveolin-independent, dynamin-independent	RAB7+ Late Endosomes after acidic pH induced dissociation of GP1 and GP2	pH 5.5	No	Immune	[2,70-73]
Lassa Virus	Arenavirus	Preferred: Dystroglycan(DG); Potential: DC-SIGN, LSECtin, Tyro3/Axl/Mer (TAM)	Clathrin-independent, caveolin-independent, dynamin-independent	RAB7+ Late Endosomes after acidic pH induced dissociation of GP1 and GP2	pH 5.5	No	Immune	[2,71-73]
Machupo Virus	Arenavirus	Transferrin receptor 1 (TfR1)	clathrin-mediated endocytosis	RAB7+ Late Endosomes after acidic pH induced dissociation of GP1 and GP2	pH 5.5	No	Immune	[2,70,72,74]
Junin Virus	Arenavirus	Unknown	clathrin-mediated endocytosis	RAB7+ Late Endosomes after acidic pH induced dissociation of GP1 and GP2	pH 5.5	No	Immune	[75-77]
Moloney Leukemia Virus	Gammaretrovirus	Murine cationic amino acid transporter-1 (mCAT-1)	Clathrin-independent	Cell Surface	none	No	Immune	[2,78-80]
Sendai Virus	Paramyxovirus	Sialic acid-containing ganglioside receptors (SA-R); alternate: asialoglycoprotein receptor (ASGP-R)	Clathrin-independent	Cell Surface	none	No	Immune	[31,81-83]

Virus	Family	Host Receptor	Endocytic Pathway	Where the virus enters the host cell	pH Requirement*	Cathepsin Processing	IFITM specificity	References
Crimean Congo Hemorrhagic Fever Virus	Bunyavirus	Nucleolin	clathrin-mediated endocytosis	RAB5+ Early Endosomes	pH 6.5	No	Immune	[30,84-86]

Table 3

Human CD225 Protein Superfamily Members

Gene Name	Entrez Gene ID	Function	Cellular Expression Pattern	Tissue Expression Pattern	Human Genetic Determinants	References
IFITM1	8519	Viral restriction; Cell adhesion; Tumor suppression	Cell surface, early endosomes> late endosomes	Ubiquitous	Unknown	[2,11,87,88]
IFITM2	10581	Viral restriction; Cell adhesion; Tumor suppression	Late Endosomes and Lysosomes	Ubiquitous	Unknown	[11,88,89]
IFITM3	10410	Viral restriction; Cell adhesion; Tumor suppression	Late Endosomes and Lysosomes	Ubiquitous	rs12252-C; rs3888188 Increase in IAV disease severity	[2,11,12,14,22,88,90,91]
IFITM5	387733	Bone mineralization; Regulates association of CD9 with FK506 binding protein (FKBP11)-CD81-prostaglandin F2 receptor negative regulator(FPRP) complex; Facilitates increased expression of interferon- induced genes	Cell Surface, most intense membrane localization at cell-to-cell junctions (HEK293 Cells overexpressing the mouse homolog)	Bone; Localized to mineralizing nodules (WT protein expressed in Rat osteoblast cultures)	*c.-14C>T, Five amino acid N-terminal addition.* Associated witgha brittle bone disease Osteogenesis Imperfecta Type 5	[3,15,92-106]
IFITM10	402778	Unknown	Unknown	Adrenal gland, Blood, Bone, Brain, Connective tissue, Eye, Intestine, Kidney, Larynx, Liver, Lung, Mammary gland, Mouth, Pancreas, Placenta, Prostate, Salivary gland, Skin, Testis, Trachea	Unknown	
PRRT1	80863	Interacts with AMPA receptors; oncogenesis	Unknown	Brain, Cervix, Esophagus, Intestine, Kidney	Unknown	[107,108]

Gene Name	Entrez Gene ID	Function	Cellular Expression Pattern	Tissue Expression Pattern	Human Genetic Determinants	References
PRRT2	112476	Unknown; Truncating mutations cause of paroxysmal kinesigenic dystonia (PKD) and infantile convulsions with choreoathetosis (ICCA) syndrome	Unknown	Adrenal gland, Brain, Embryonic tissue, Eye, Heart, Intestine, Kidney, Lung, Muscle, Pancreas, Parathyroid, Pharynx, Placenta, Prostate, Stomach, Testis, Umbilical cord, Uterus, Vascular	rs199662641; rs200926711; rs76335820; c.649–650insC; c.776dupG; c.649dupC; Associated with paroxysmal kinesigenic dyskinesia (PKD), benign familial infantile seizure/epilepsy (BFIS)/(BFIE), infantile convulsions with choreoathetosis syndrome (ICCA),	[17-19,109-115]
TUSC5	286753	Responsive to insulin, glucose, glucocorticoids and/r PPARγ agonists; Potential role in fat cell physiology; Down-regulated in breast adenocarcinoma,	Unknown	Adipose tissue, Bone, Mammary gland, Muscle, Testis,	Unknown	[116,117]
TMEM91	641649	Unknown	Unknown	Blood, Bone, Brain, Cervix, Connective tissue, Eye, Heart, Intestine, Kidney, Lung, Placenta, Prostate, Skin, Spleen, Testis, Thyroid, Uterus	Unknown	
TMEM233	387890	Unknown	Unknown	Bone, Brain, Eye, Lung, Muscle, Pancreas, Thyroid	Unknown	
SYNDIG1	79953	Regulates synaptic AMPAR content; Regulates AMPAR- and NMDAR- mediated transmission; May play a role in regulating synaptogenesis	Unknown	Brain, Embryonic tissue, Esophagus, Eye, Heart, Intestine, Kidney, Lung, Ovary, Pancreas, Prostate, Stomach, Testis, Thymus, Umbilical cord	Unknown	[118]
SYNDIG1L	646658	Unknown; Possible role in the pathogenesis of Huntington disease; suggested role in striatal function	cis-Golgi (Mouse protein fused to yellow fluorescence protein in Chinese ovary cells and HeLa cells)	Brain, Connective tissue, Eye, Kidney, Lung, Nerve, Pancreas,	Unknown	[119]

Table 4

IFITM Protein Family Posttranslational modifications

Protein	Posttranslational Modification (PTM)	Functional Role	Reference*
IFITM1	Mouse: S-palmitoylation Cysteines 49, 50, 83, 103	Localization to membrane	[31]
	Phosphorylation Lysine 67	Unknown	[120-122]
IFITM2	Phosphorylation Serine 9	Unknown	[123]
	Ubiquitination Lysine 87	Unknown	[120-122]
IFITM3	Phosphorylation Tyrosine 20	Required for proper localization to late endosomes and lysosomes; Required for restriction of IAV and DENV	[11,22], PhosphoSitePlus
	Ubiquitination Lysine 24	Most robustly ubiquitinated of the four lysines found in IFITM3. The combined mutation of K24, K83, K88 and K104 to alanine increased protein stability, augmented the formation of autolysosomes, and promoted restriction of IAV	[21,31], PhosphoSitePlus
	S-palmitoylation Cysteines 71,72,105	Mutation of C72 to alanine disrupts proper cellular localization and decreases restriction for IAV and DENV. Mutation of either C71 or C105 to alanine has no effect on these attributes.	[11,27]
	Ubiquitination Lysine 83	Mutation to alanine had no effect on restriction	[11,21]
	Ubiquitination Lysine 88	Mutation to alanine had no effect on restriction	[11,21], PhosphoSitePlus
	Phosphorylation Tyrosine 99	Mutation to alanine decreased restriction of DENV more than IAV but did not alter localization	[11], PhosphoSitePlus
	Ubiquitination Lysine 104	Mutation to alanine decreased restriction (IAV>DENV) without altering localization	[11,21], PhosphoSitePlus

*
For PTMs with greater than 3 citations on PhosphoSitePlus the reader is referred to the website using the following link where those citations are visible upon clicking the specific PTM (http:// www.phosphosite.org/homeAction.do).