

**IFITM Proteins Mediate the Innate Immune Response to Influenza A H1N1 Virus,
West Nile Virus and Dengue Virus**

Sinu P. John¹, I-Chueh Huang⁴, Yair Benita⁵, Manoj N. Krishnan⁶, Eric M. Feeley¹, Bethany Ryan¹, Jessica L. Weyer⁴, Louise van der Weyden⁷, Erol Fikrig^{6,8}, David J. Adams⁷, Ramnik J. Xavier^{2,5}, Michael Farzan⁴, Stephen J. Elledge³ Abraham L. Brass^{1,2,3#*}

¹ Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology, and Harvard Medical School, Charlestown, MA 02129, USA

² Gastrointestinal Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA.

³ Department of Genetics, Harvard Medical School, Division of Genetics, Brigham and Women's Hospital, Howard Hughes Medical Institute, Boston, MA 02115, USA

⁴ Department of Microbiology and Molecular Genetics, Harvard Medical School, New England Primate Research Center, Southborough, MA 01772, USA.

⁵ Center for Computational and Integrative Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

⁶ Section of Infectious Diseases, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut, 06520 USA.

⁷ Experimental Cancer Genetics, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton Cambridge, CB10 1SA U.K.

⁸ Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut, New Haven, CT 06520.

Corresponding author: abrass@partners.org

ABSTRACT

Influenza viruses exploit host cell machinery to replicate, resulting in epidemics of respiratory illness. In turn, the host expresses anti-viral restriction factors to defend against infection. To identify host cell modifiers of viral infection, we undertook a functional genomic screen and found that the interferon inducible transmembrane protein (IFITM) family can strongly inhibit influenza A virus infection. The IFITM proteins confer basal resistance to influenza A virus, but are also inducible by interferons type I and II, and are critical for 40-70% of interferon's virustatic actions. Overexpression of IFITM3 rendered multiple cell lines, including primary cells, resistant to influenza A virus infection. Mouse embryonic fibroblast cells lacking all IFITM proteins were more susceptible to influenza A virus infection, and the reinstatement of IFITM3 expression in the null cells rescued their resistance to viral infection. IFITM proteins inhibited all strains of influenza virus tested, including the current seasonal vaccine strains, A/Brisbane/59/07 H1N1 and A/Uruguay/716/07 H3N2, as well as a strain isolated from the Hong Kong flu pandemic of 1968, A/Aichi/2/68 H3N2. Further analysis using pseudotyped viruses revealed that cells over-expressing IFITM3 blocked the influenza A virus at the entry stage of infection. The IFITM proteins were also found to inhibit the infection of flaviviruses, including dengue virus and West Nile virus. Collectively this work identifies a new family of anti-viral restriction factors that mediate cellular resistance to at least three major human pathogens.