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GENERATION OF CELLULAR MODELS TO IDENTIFY SMALL MOLECULES INHIBITORS OF THE ANTI-INTERFERON PROTEINS OF HUMAN PATHOGENIC RNA VIRUSES

International Conference on Innate Immunity July 20-21, 2015 Barcelona, Spain
Innate Immunity: An Emerging Science

- **Innate Immunity** represents the first line of defense of multicellular organisms against infections and evolved as a survival response against pathogens.

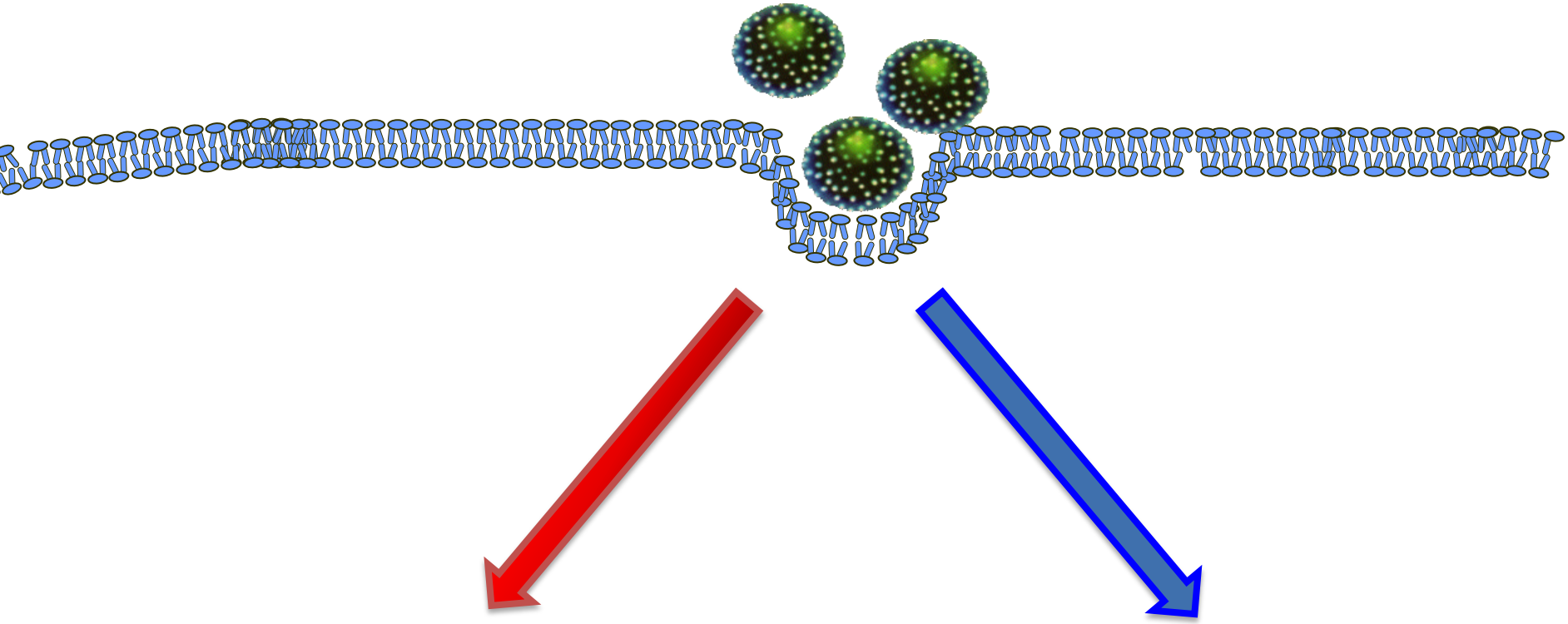
- **Innate Immunity** senses and responds to pathogens (PAMP) via pattern recognition receptors (PRR) & also recognizes and eliminates damaged and dead cells.

- **Innate Immunity** also primes and stimulates adaptive immune responses.

- The similarity between components of **Innate Immunity** belonging to plants, insects and mammalians indicates that they appeared in a common ancestor, long time ago in evolution.

- **Innate Immunity** probably evolved just after the development of complex multicellular organisms (750 million years ago), long before the appearance of Adaptive Immune Responses in vertebrates.

Innate immunity stimulates two main types of responses to protect the host



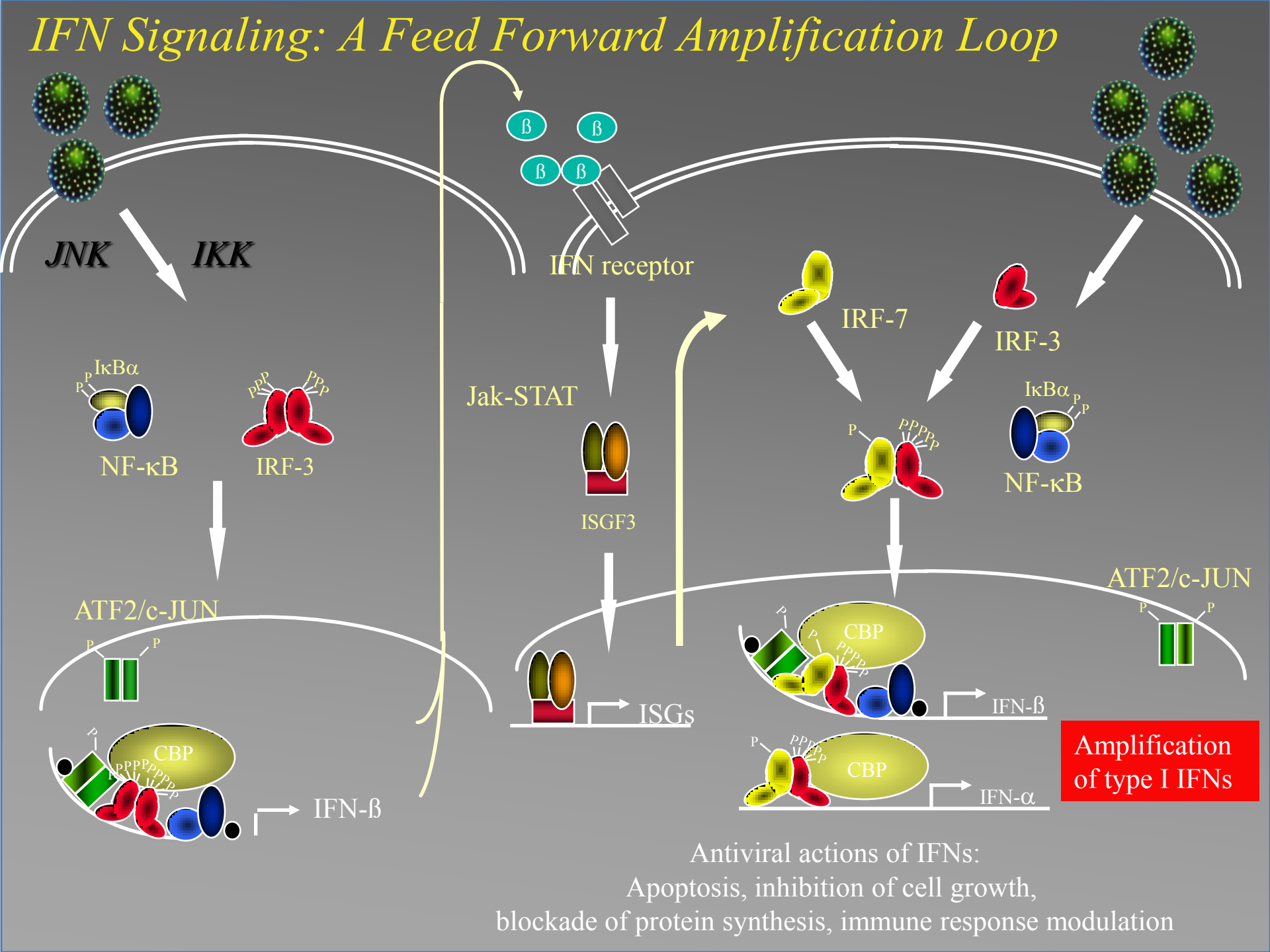
Inflammation

It is required to bring leukocytes and plasmatic proteins to the site of infection and to activate them

Antiviral responses

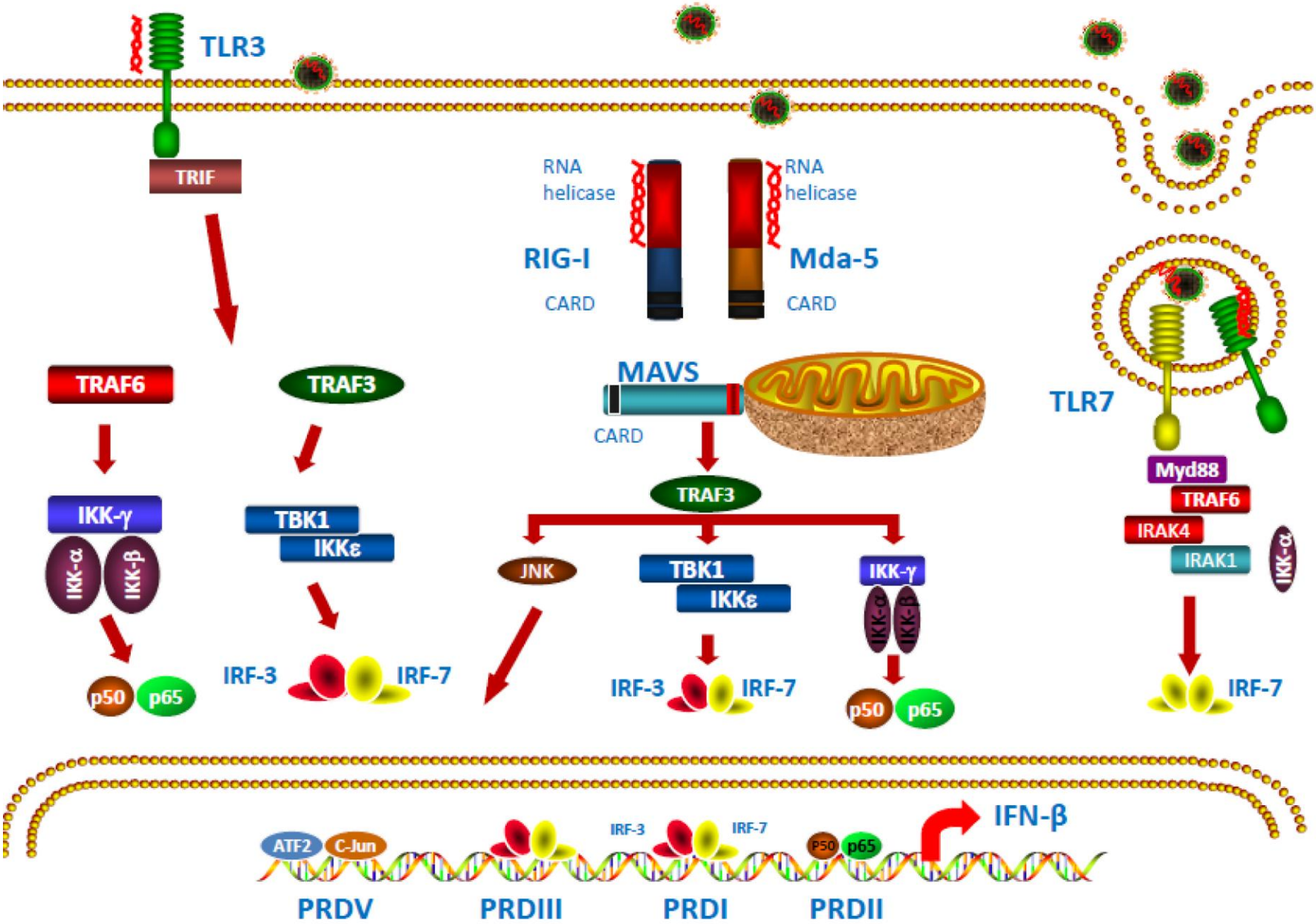
Inhibition of viral replication through the secretion of type I Interferons (IFN) by infected cells and the expression of Interferon Stimulated Genes (ISGs) in bystander cells

IFN Signaling: A Feed Forward Amplification Loop

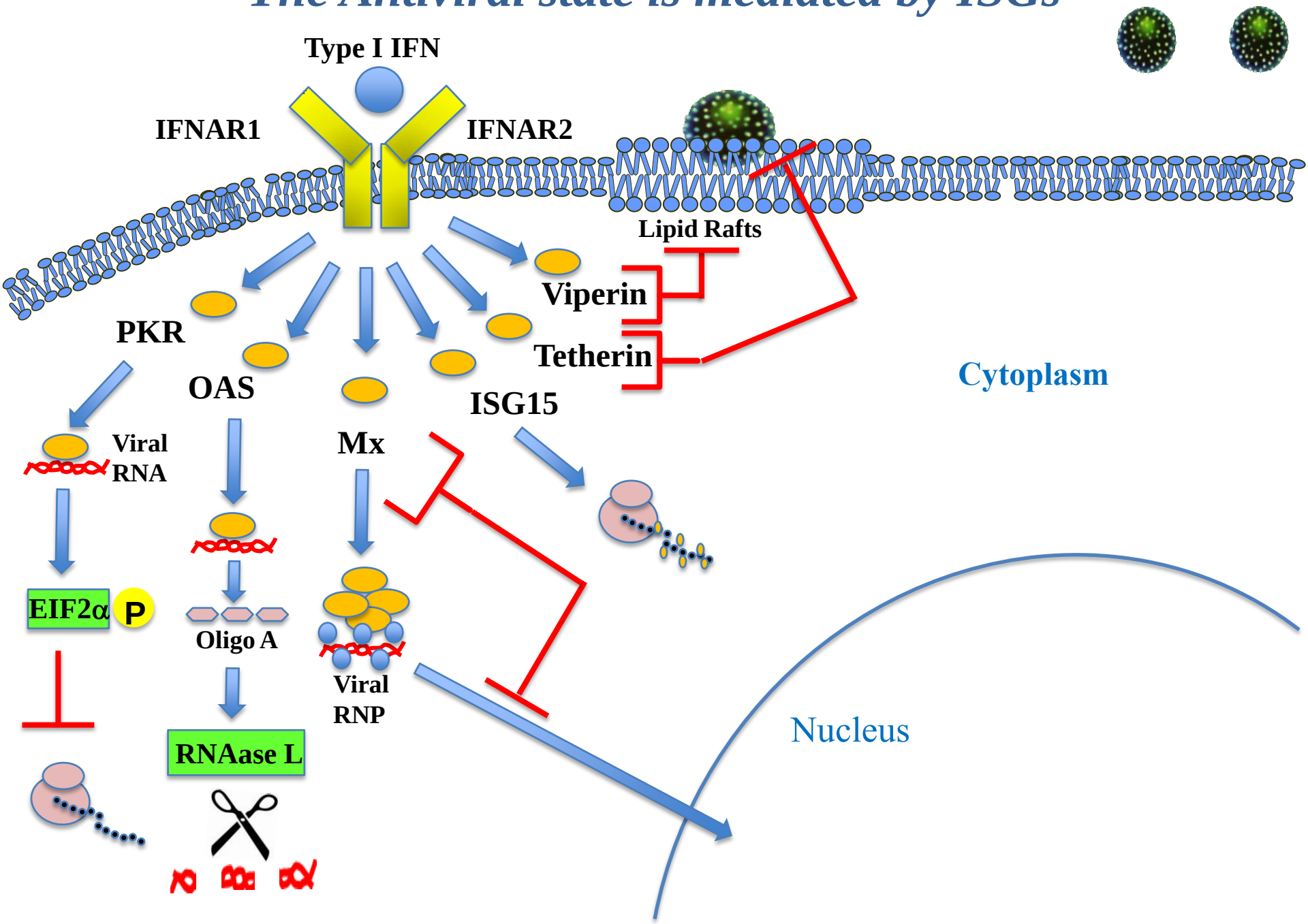


Antiviral actions of IFNs:
Apoptosis, inhibition of cell growth,
blockade of protein synthesis, immune response modulation

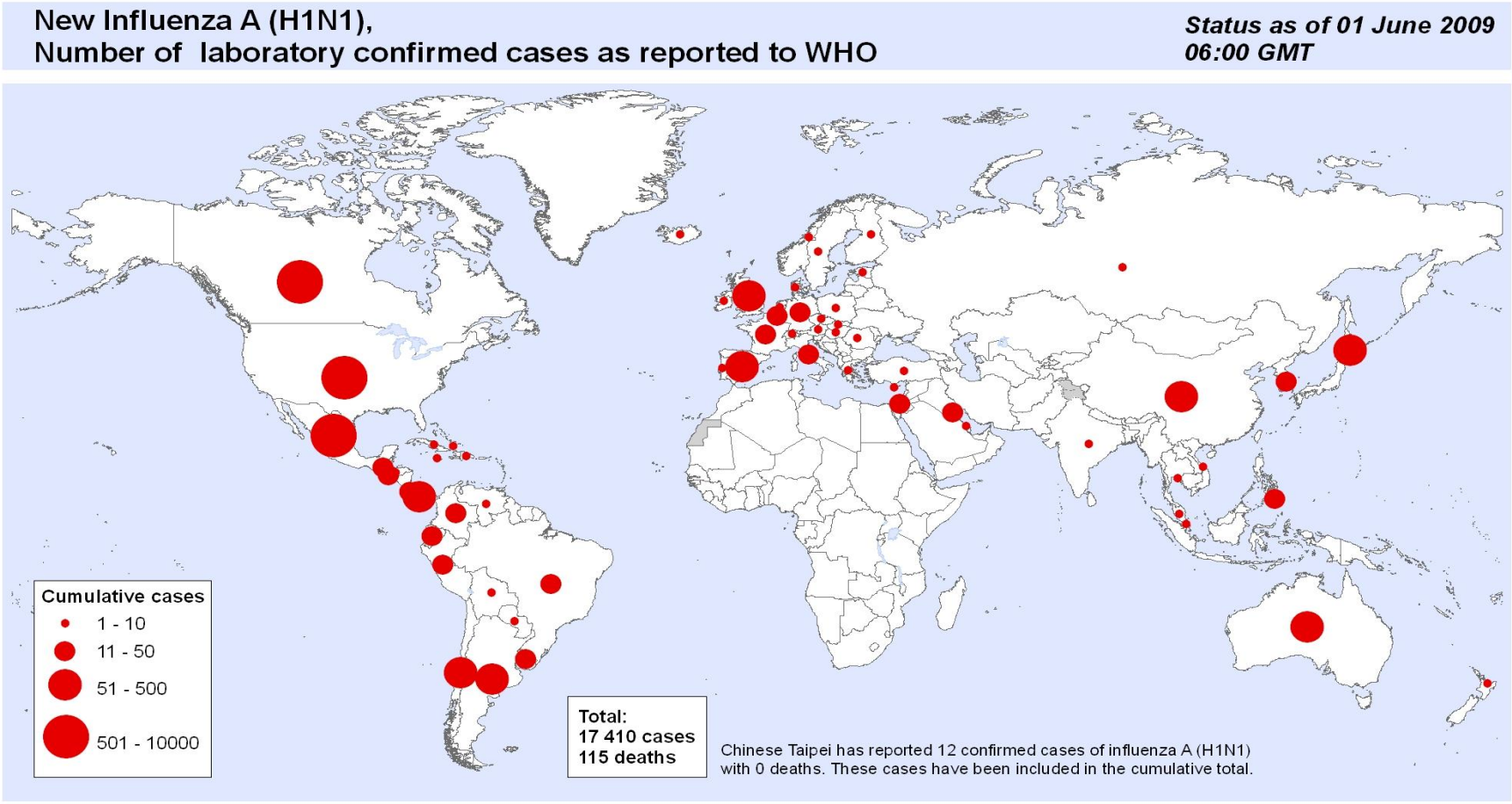
Innate Immune Response to RNA Viruses



The Antiviral state is mediated by ISGs



2009 ‘Swine Influenza’ A (H1N1) pandemic



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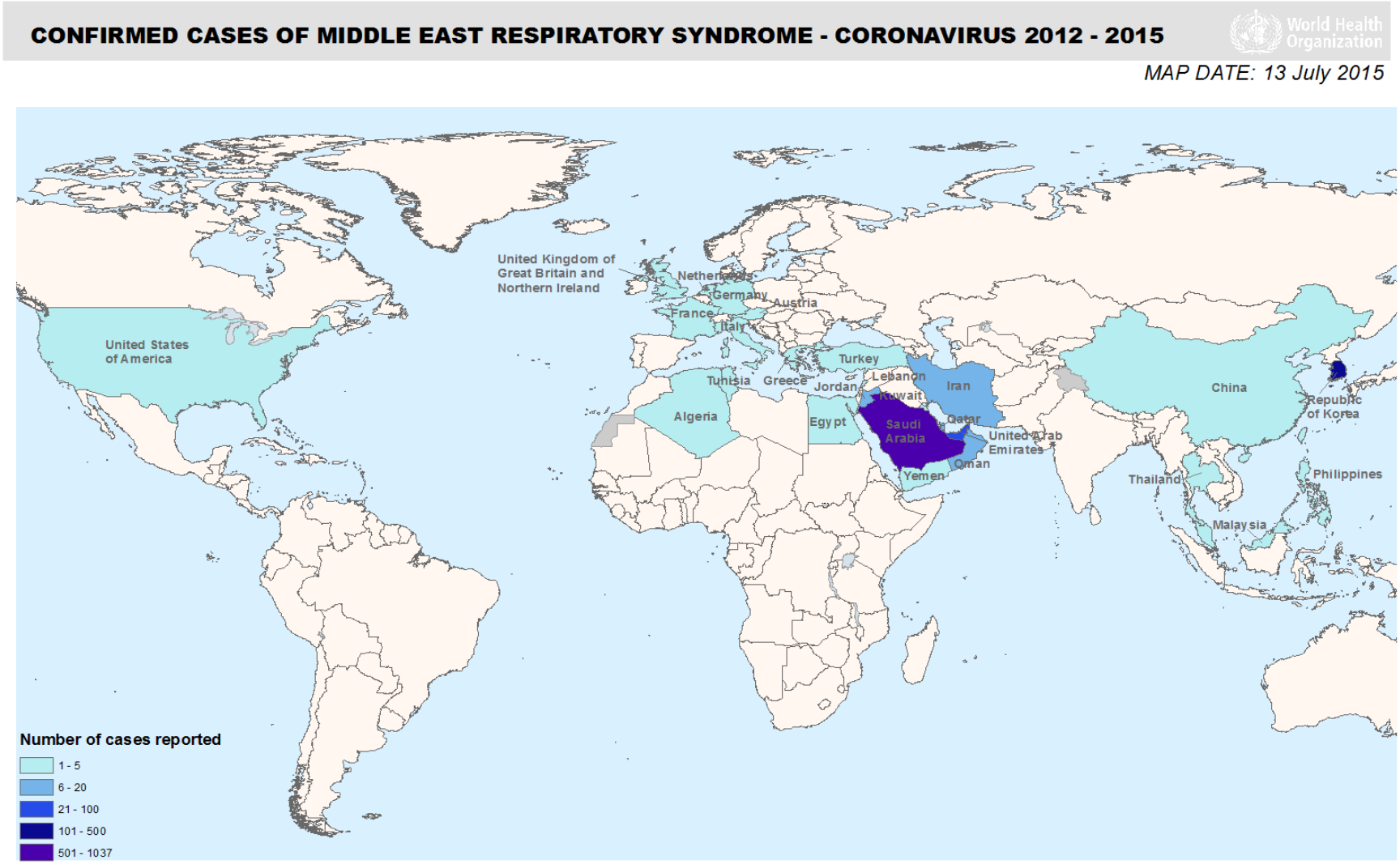
Map produced: 01 June 2009 06:46 GMT

Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

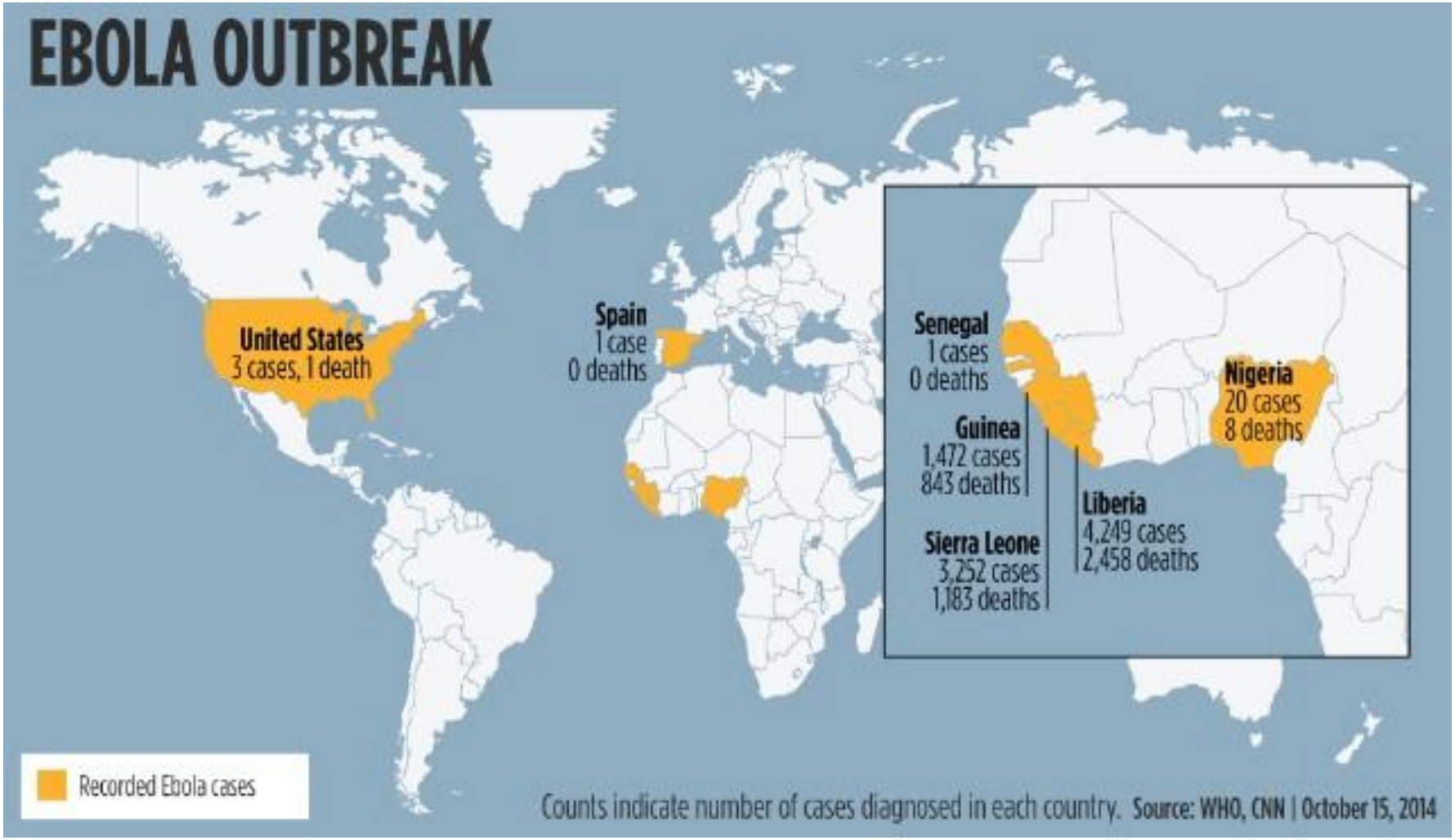
World Health Organization

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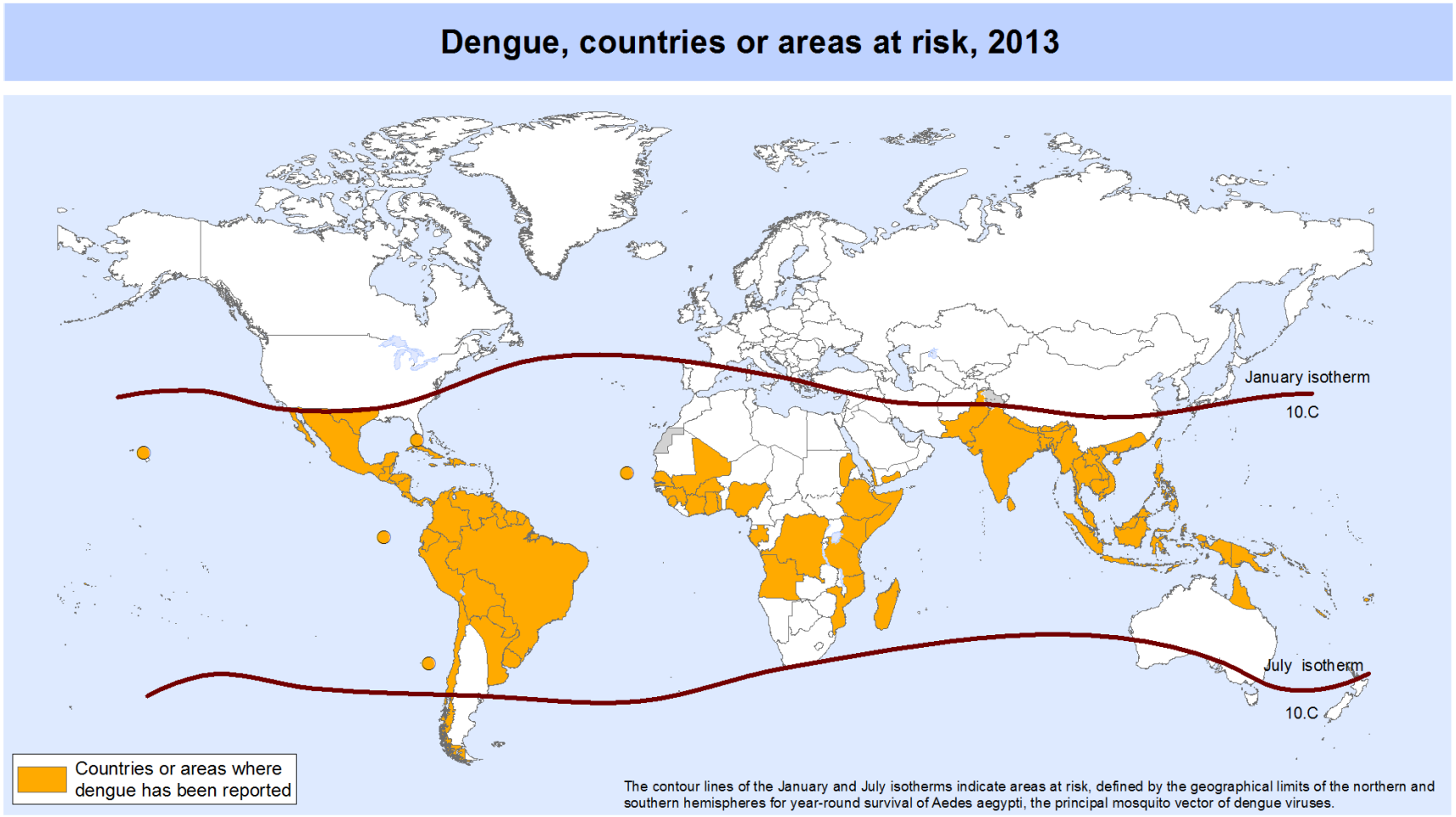
2012-15 MERS-Coronavirus outbreaks



Ebola Virus 2014 outbreak



Dengue Virus distribution



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Data Source: World Health Organization
Map Production: Health Statistics and Information Systems (HSI)
World Health Organization

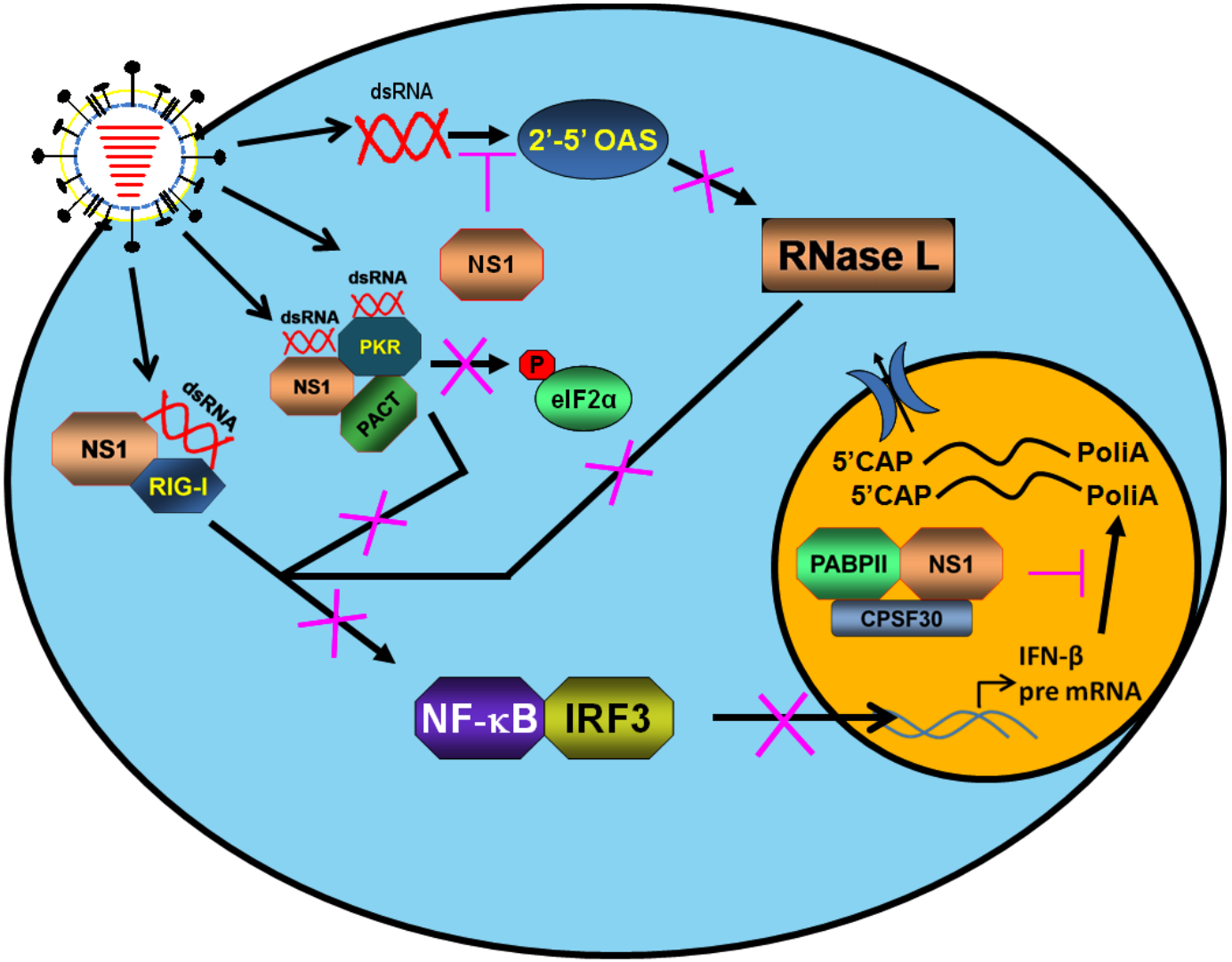
World Health Organization

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Anti-IFN proteins produced by pathogenic RNA viruses

Influenza A Viruses (Othomyxoviridae)	**NS1**
MERS CoV (Coronaviridae)	**Papain-like protease, M protein, accessory proteins 4a, 4b, and 5**
Ebola Virus (Filoviridae)	**Vp35, Vp24**
Dengue virus (Flaviviridae)	**NS2B3 (protease), NS5 NS4a NS4b, NS2a**

Influenza A virus multifunctional NS1 protein



Influenza anti-IFN protein NS1 is essential for viral fitness

Functional genomic and serological analysis of the protective immune response resulting from vaccination of macaques with an NS1-truncated influenza virus.
Baskin CR et al J Virol. 81:11817-27 (2007)

Live attenuated influenza viruses containing NS1 truncations as vaccine candidates against H5N1 highly pathogenic avian influenza.
Steel J et al J Virol. 83:1742-53 (2009)

NS-based live attenuated H1N1 pandemic vaccines protect mice and ferrets.
Zhou B et al Vaccine. 28:8015-25 (2010)

Preclinical evaluation of a replication-deficient intranasal DeltaNS1 H5N1 influenza vaccine.
Romanova J et al PLoS One. 4:e5984. doi: 10.1371/journal.pone.0005984. (2009)

A novel type of influenza vaccine: safety and immunogenicity of replication-deficient influenza virus created by deletion of the interferon antagonist NS1.
Wacheck V et al J Infect Dis. 201:354-62 (2010)

Workflow for a rapid 'Scientific Response' to a new Emerging virus

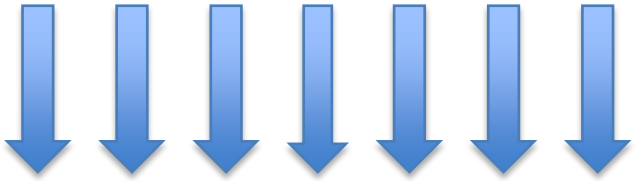
RNA Virus X outbreak anywhere in the world



On site sequencing of Virus X genome



***De novo* gene synthesis anywhere in the world**

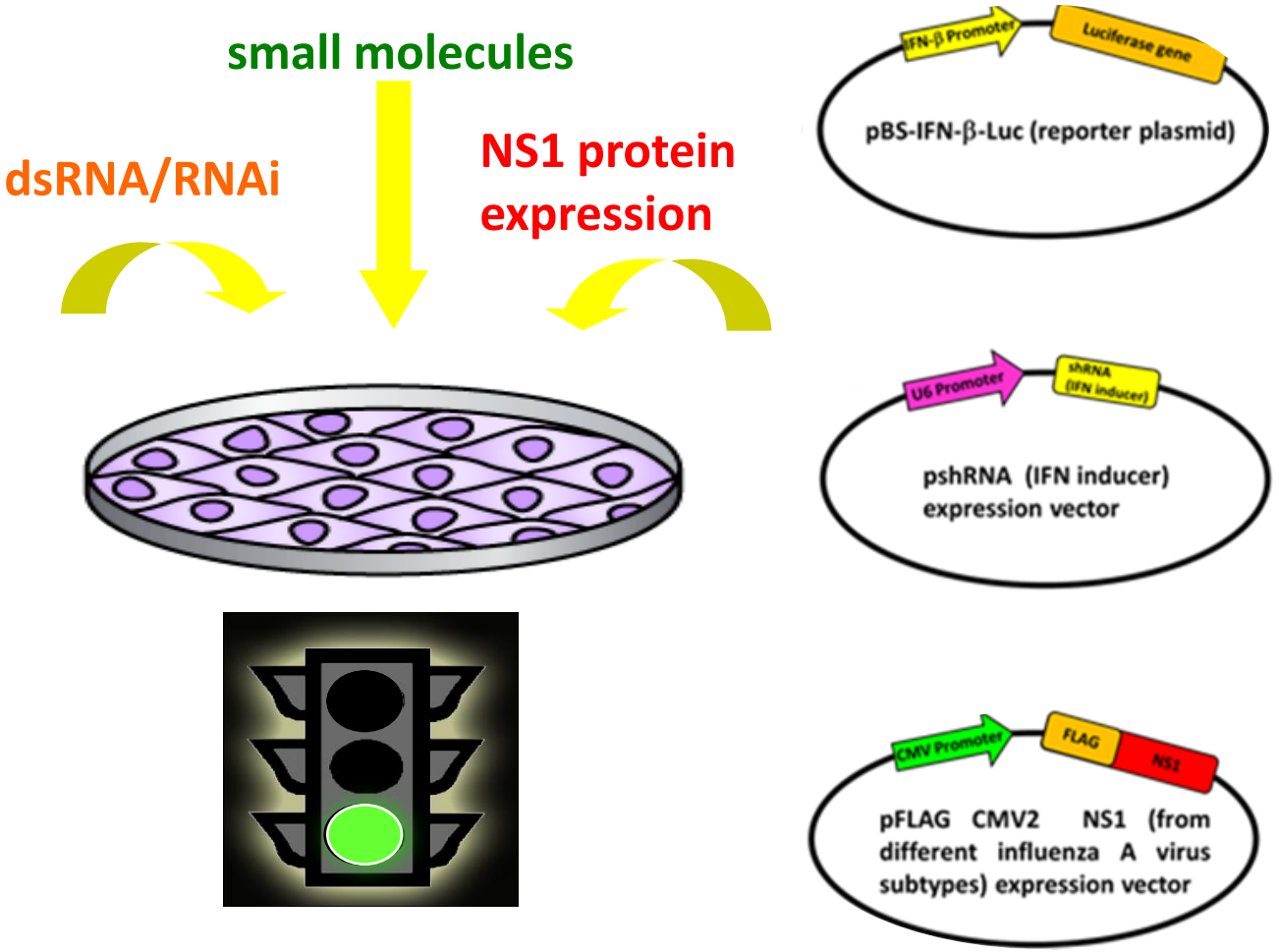


Several labs throughout the world are able to work on viral proteins without the need for any biological material from the outbreak site

Development of cell-based assays to evaluate the ability of compounds to inactivate biological effects of NS1

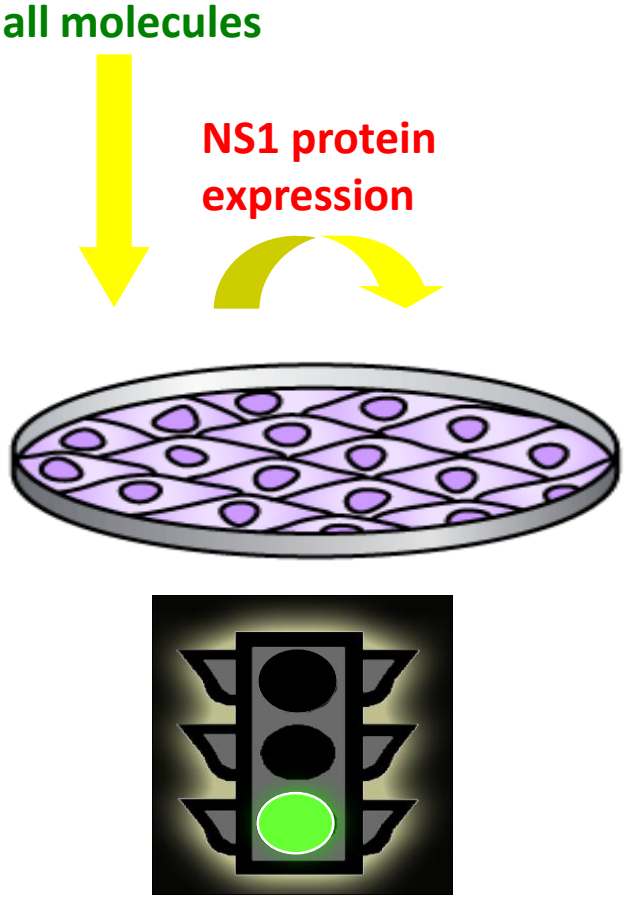
Assay no.1:

Inhibition of IFN-beta gene promoter-driven transcriptional activation



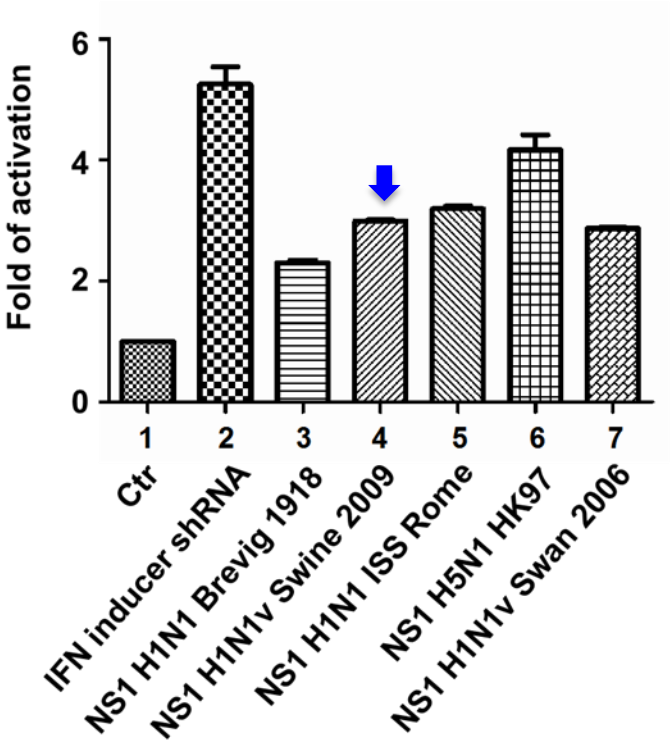
Development of cell-based assays to evaluate the ability of compounds to inactivate biological effects of NS1

Assay no.2:
Inhibition of cellular mRNA export

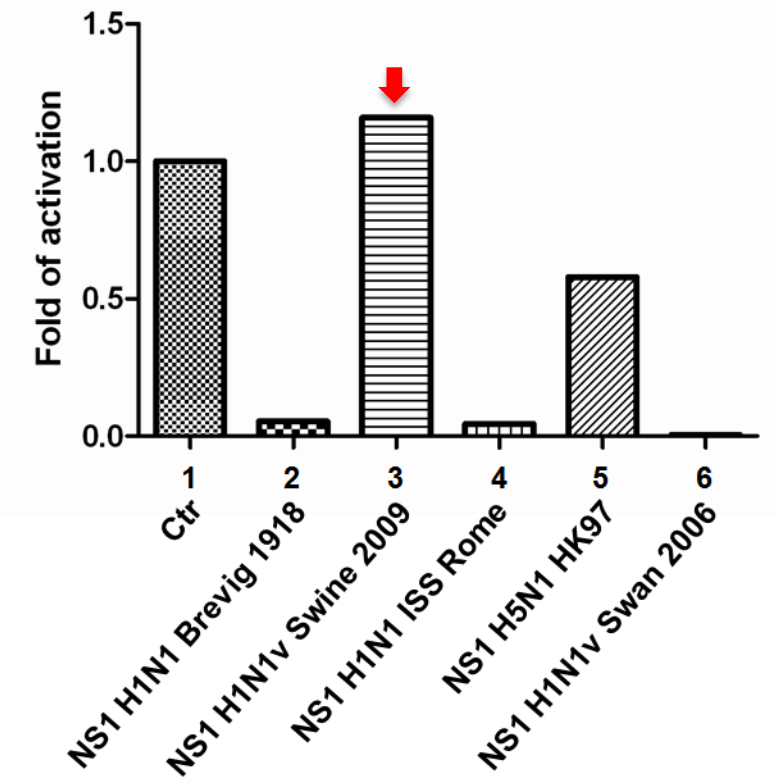


NS1 from different subtypes display a differential capacity to inhibit Type 1 IFN expression and mRNA nuclear export

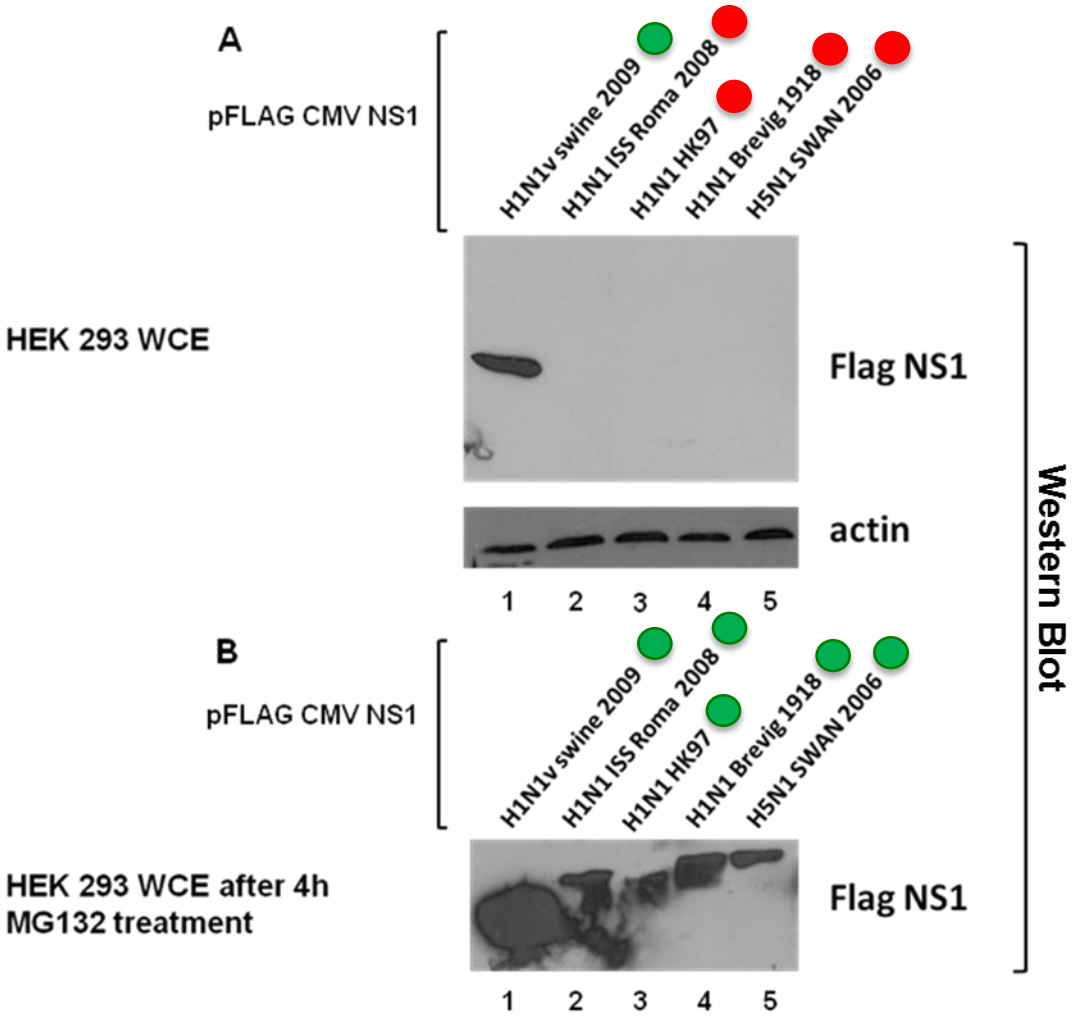
IFN-β Luc



RSV Luc



Evaluation of the expression of NS1 proteins derived from different influenza A subtypes upon transient transfection of pFLAG NS1 expression vectors in HEK293 cells



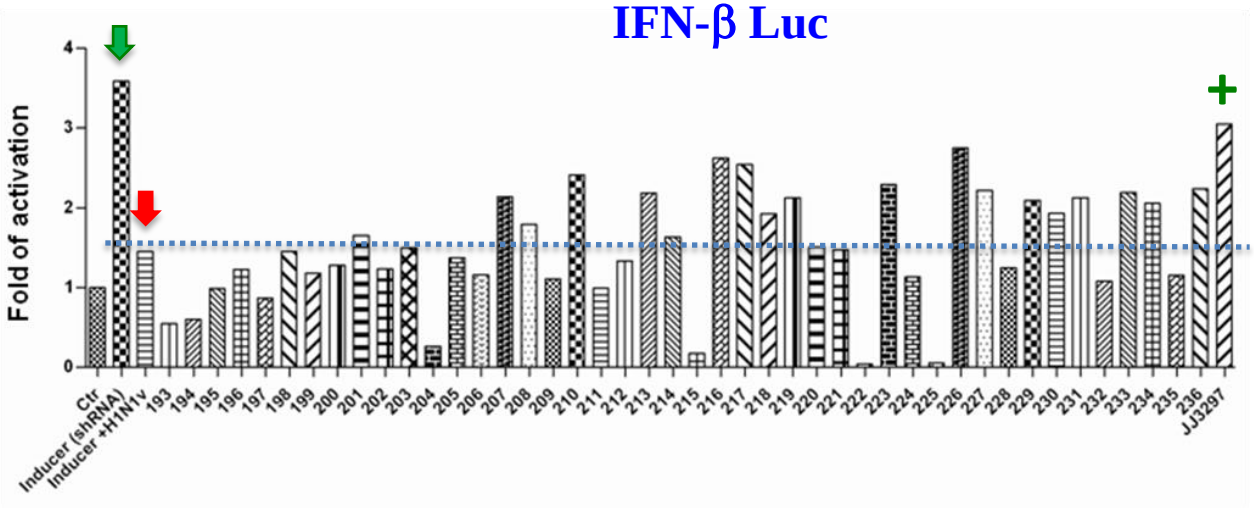
- *The selection of a **diverse library** of small molecules allows to test a collection of molecules having different properties, ideally able to cover the entire **'drug-like chemical space'** on different targets.*

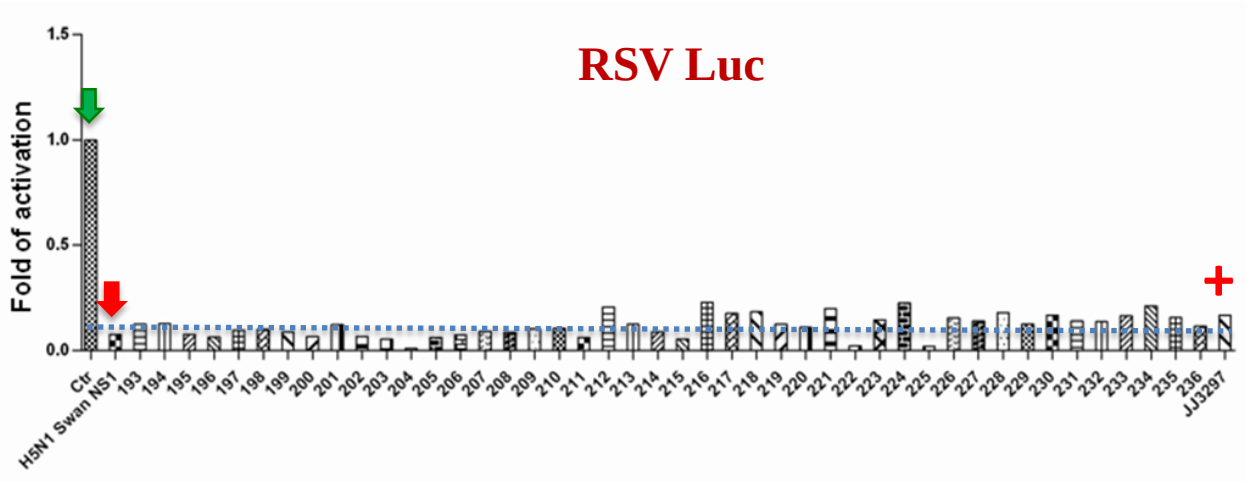
- *The assumption is that **molecules with similar properties** share **common biological activities**.*

- ***Filtering** protocols can be applied to diverse libraries to remove compounds containing specific chemical sub-structures associated with poor stability or toxicity*

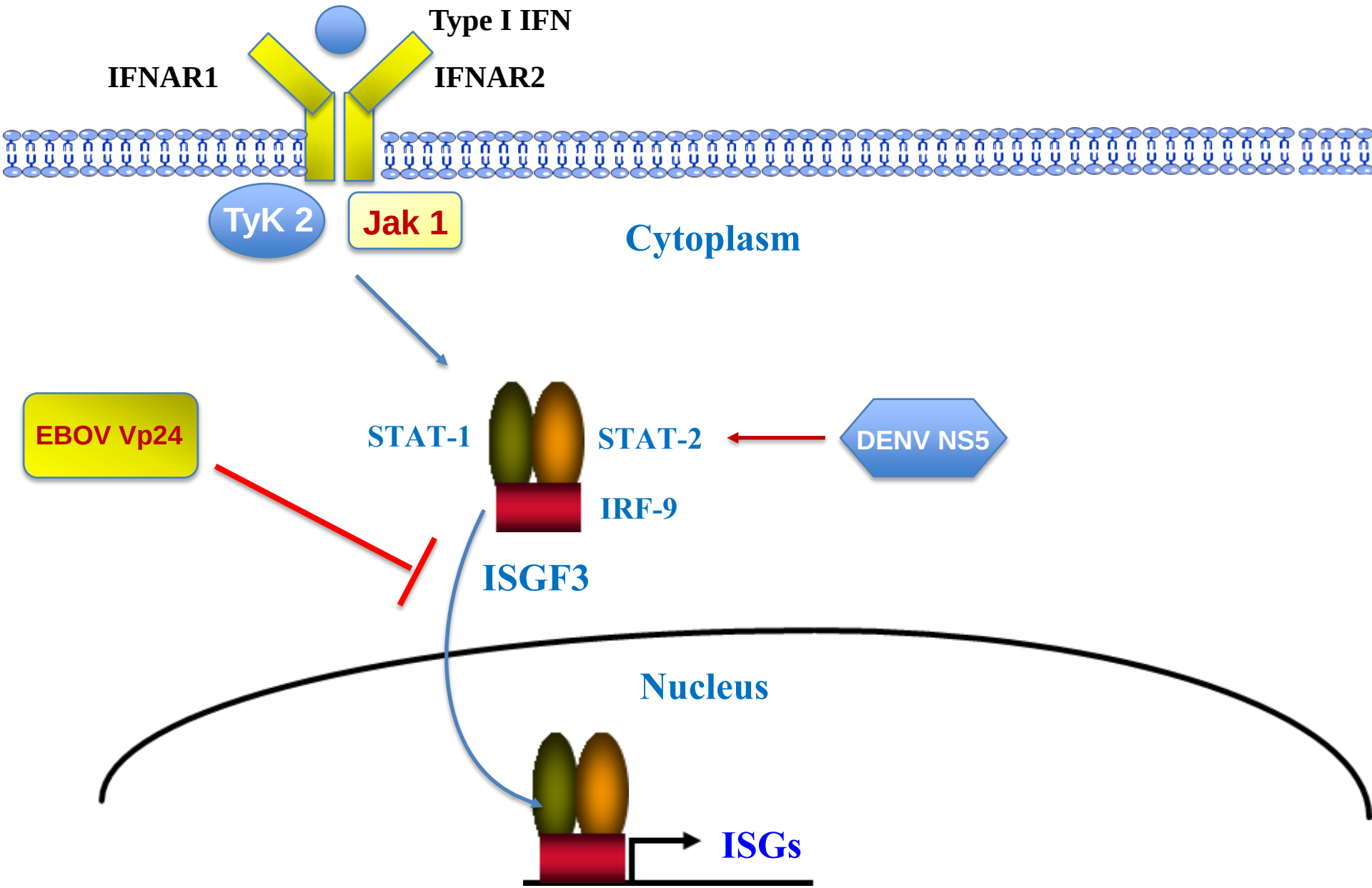
- *This approach, in the first stage of drug discovery, could avoid costly late-stage preclinical and clinical failures .*

Screening of a diverse library of compounds in NS1 cell-based assays





Type I IFN pathway is targeted by both Ebola (Vp24) and Dengue Virus (NS5) proteins



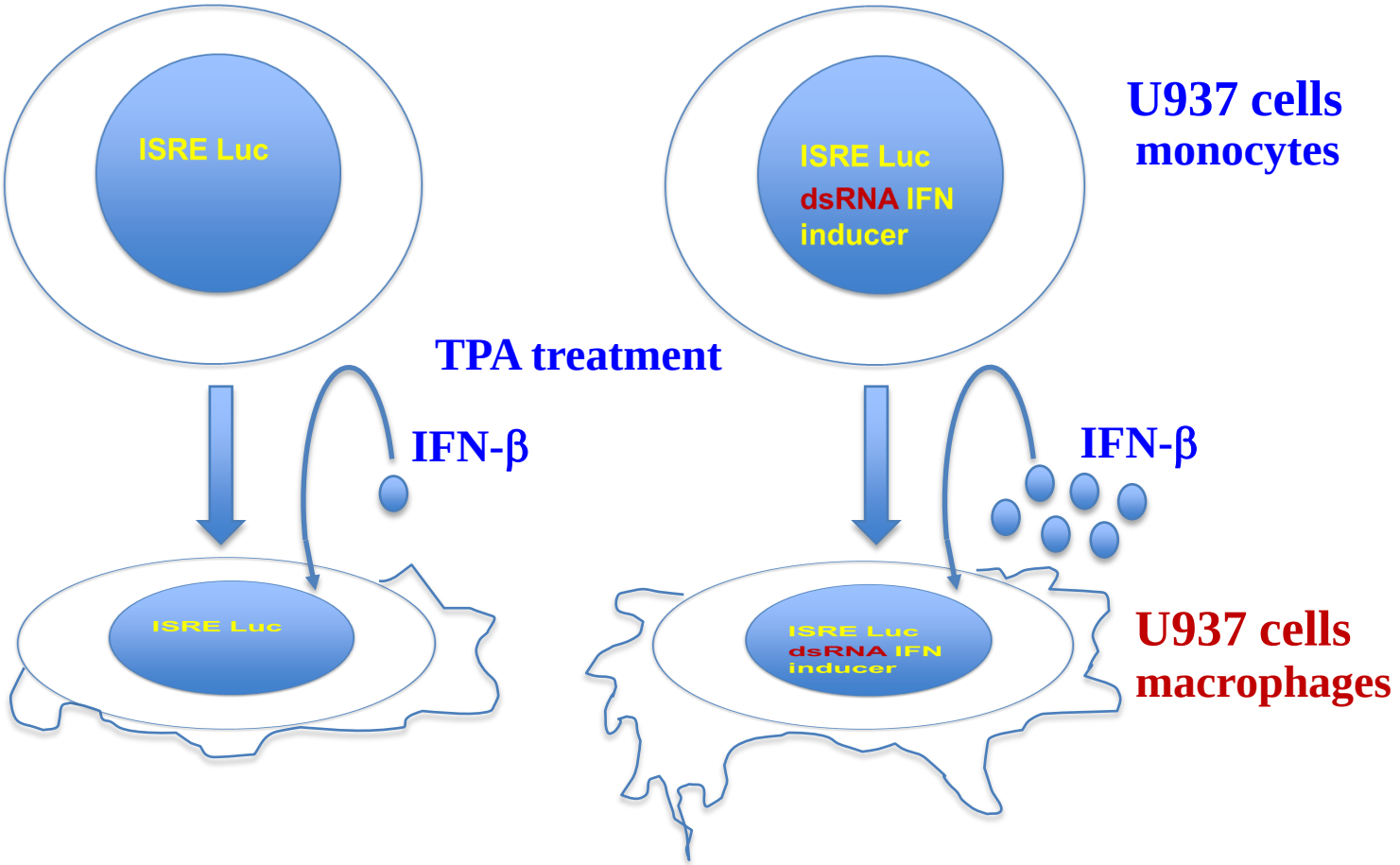
- **Both Ebola and Dengue Viruses are capable of causing hemorrhagic infections in humans.**

- **Monocytes, tissue macrophages and dendritic cells are the early targets and preferred sites of Ebola virus replication.**

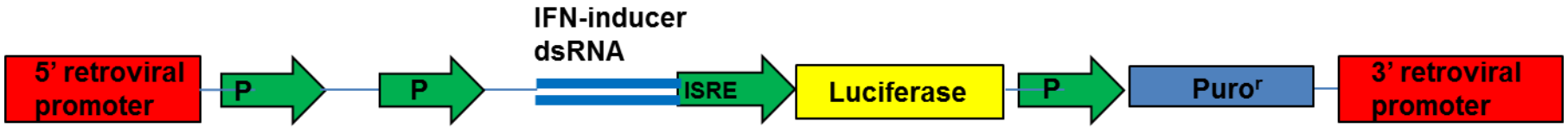
- **Dengue shock syndrome (DSS) occurs when monocytes and macrophages are infected with higher efficiency through the antibody dependent enhancement (ADE).**

- **To create a physiological cellular model to test compounds potentially able to counteract Ebola and Dengue anti-type I IFN proteins, cells of the myeloid lineage should be preferred.**

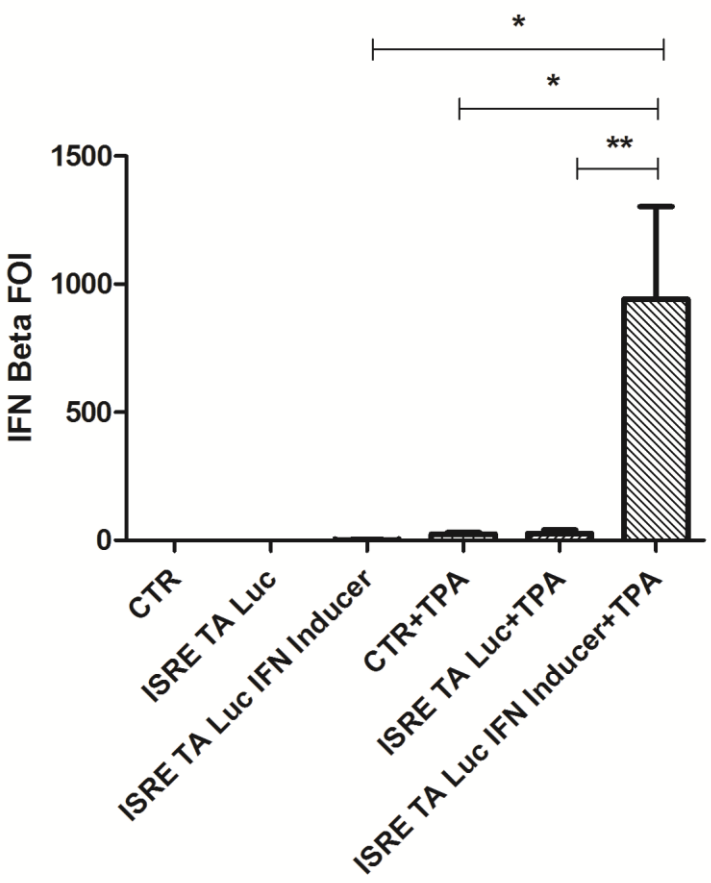
Monocytic U937 reporter cell model for inducible Type I IFN production

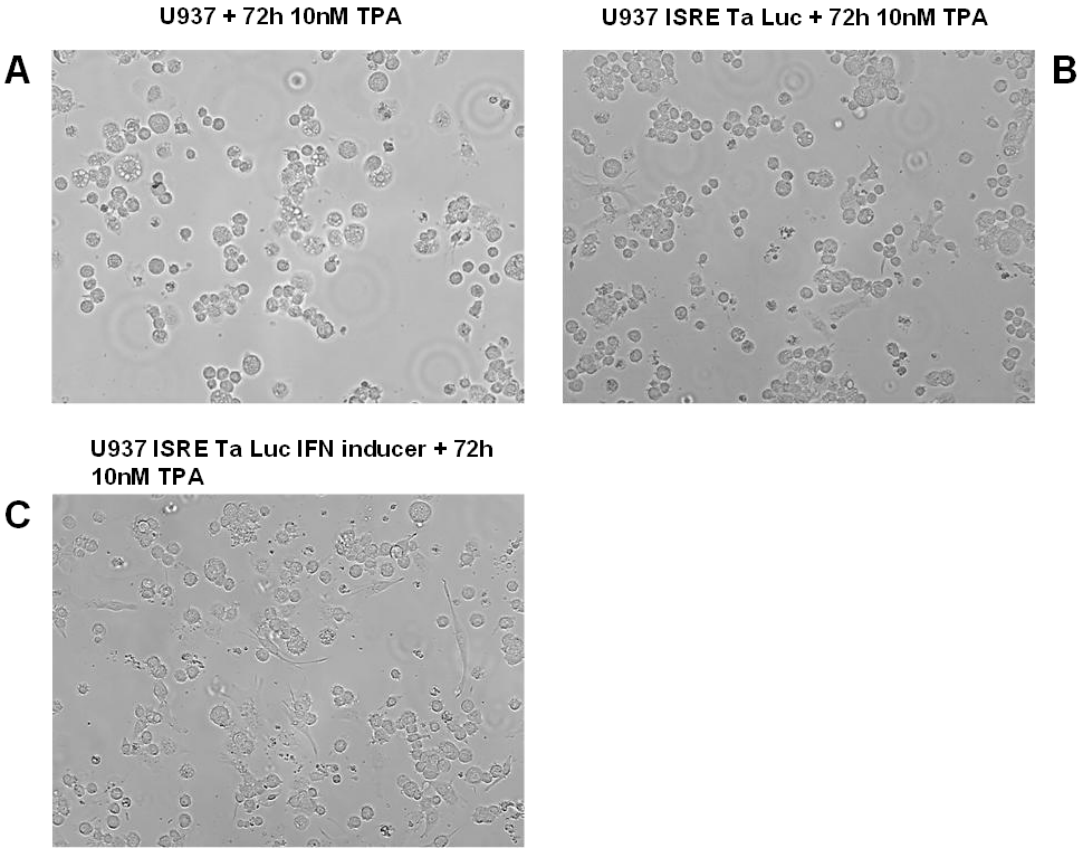




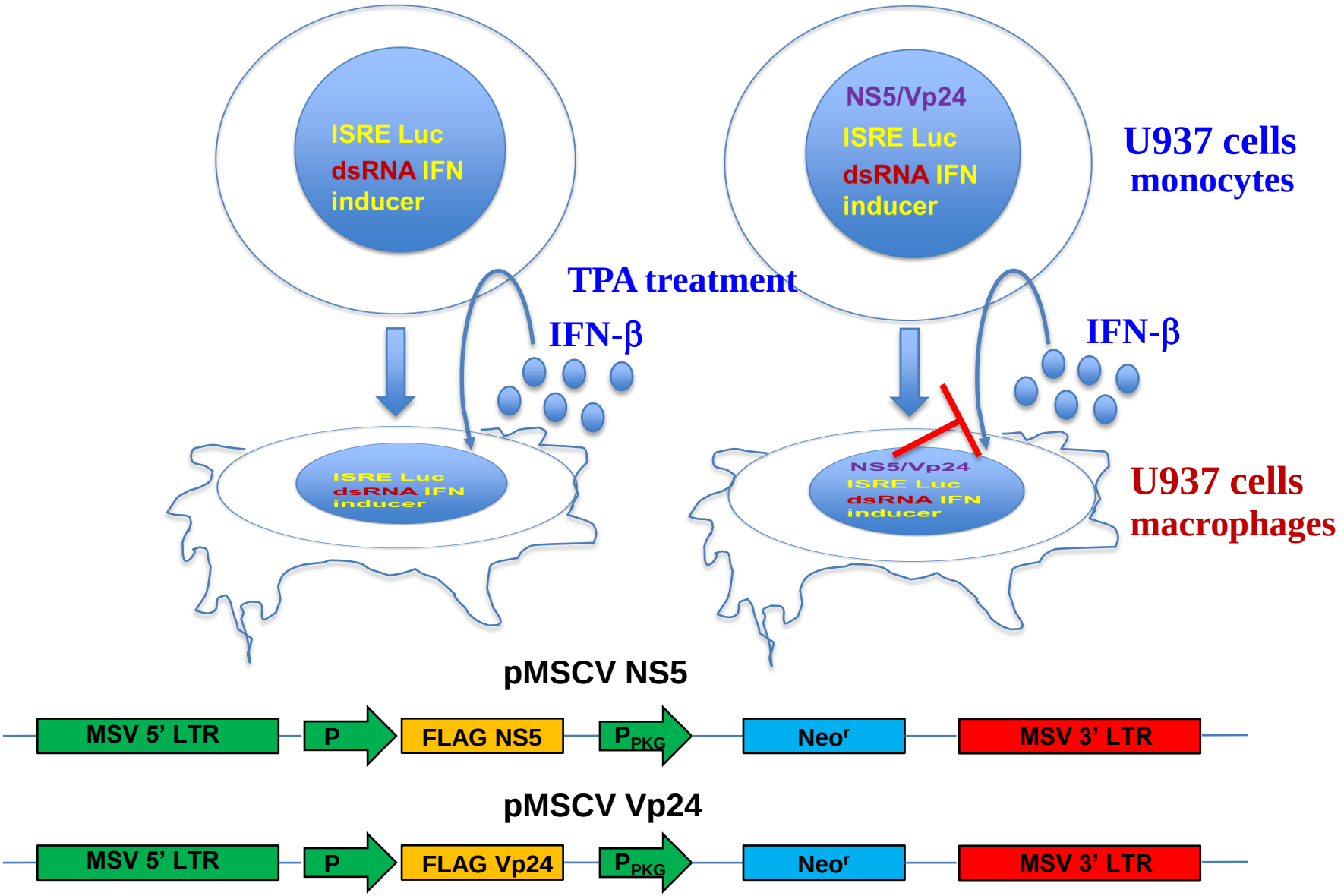


Monocytic U937 reporter cell model for inducible Type I IFN production

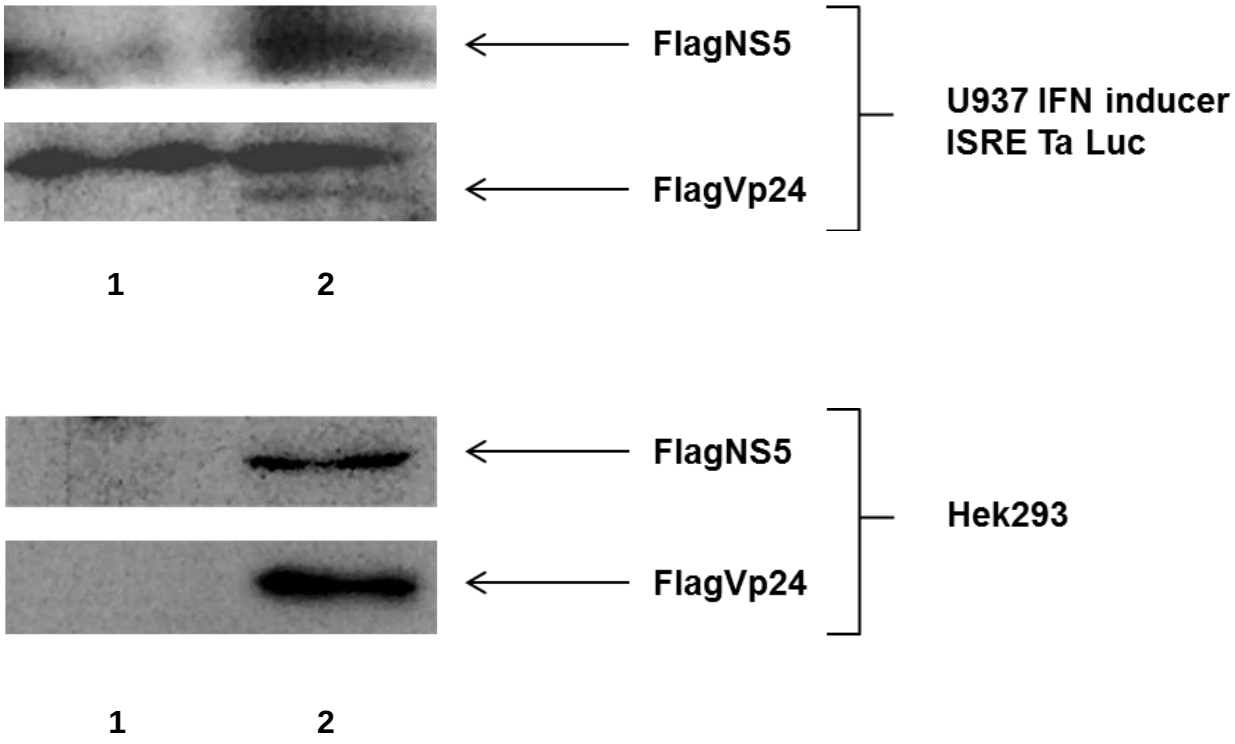




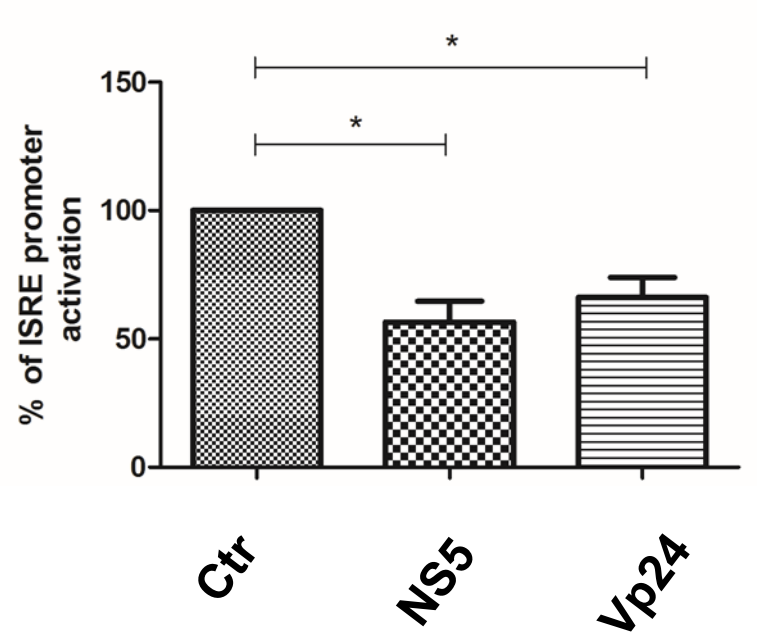
Expression of Dengue NS5 and Ebola Vp 24 in the monocytic U937 reporter model for inducible Type I IFN production



Expression of Dengue NS5 and Ebola Vp 24 in the monocytic U937 reporter model for inducible Type I IFN production



Inhibition of ISRE promoter activity in the monocytic U937 reporter cell model for inducible Type I IFN production due to the expression of Dengue NS5 and Ebola Vp24



Conclusions

Life threatening RNA viruses evolved to express anti-Type I IFN proteins, reaching the goal of evading innate immune responses of the host.

RNA virus anti-IFN proteins represent an ideal target for potential therapeutic intervention, as demonstrated by the Influenza A virus delNS1 mutant viruses.

The development of cell-based assays, able to recapitulate the ability of RNA viruses to inhibit type I IFN production and signaling, can help the identification of new potential drug candidates.

Such approach could also allow the identification of new mechanisms through which a certain viral protein, with anti-IFN properties, exerts its action.

For this purpose a tailored choice of the good cellular candidate can be decisive.

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