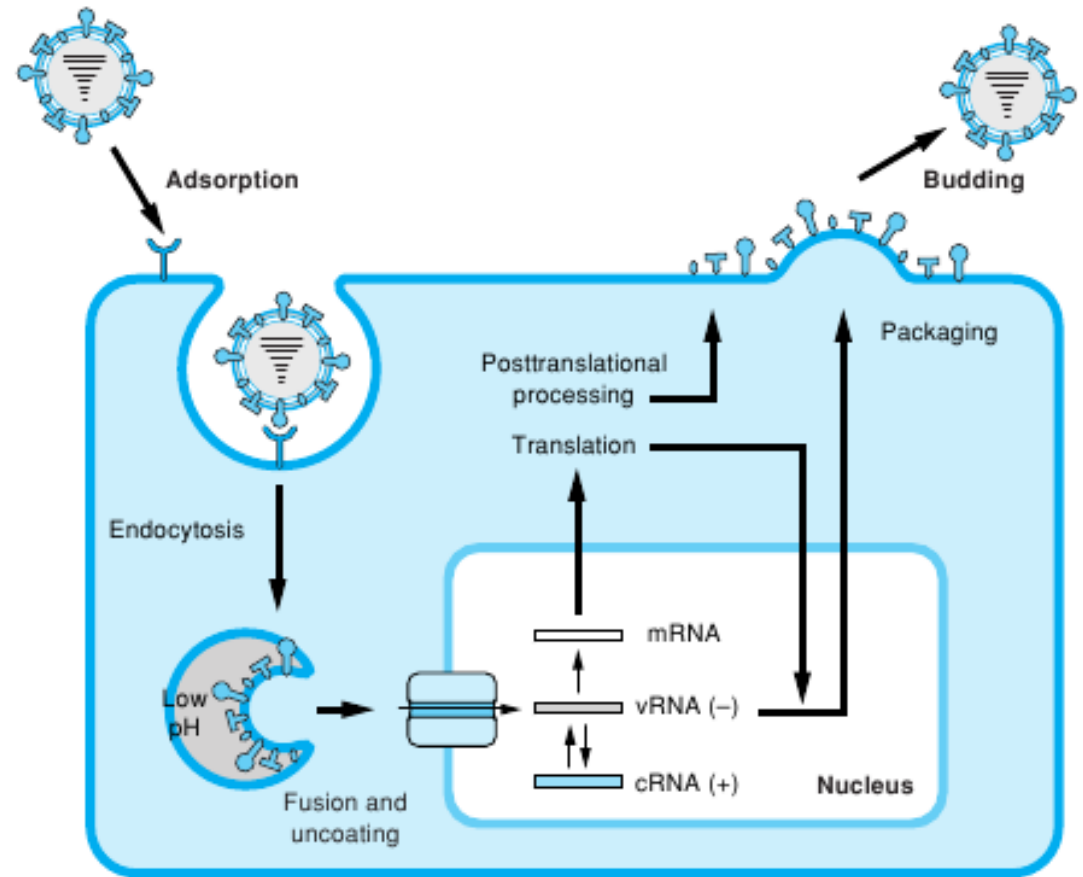
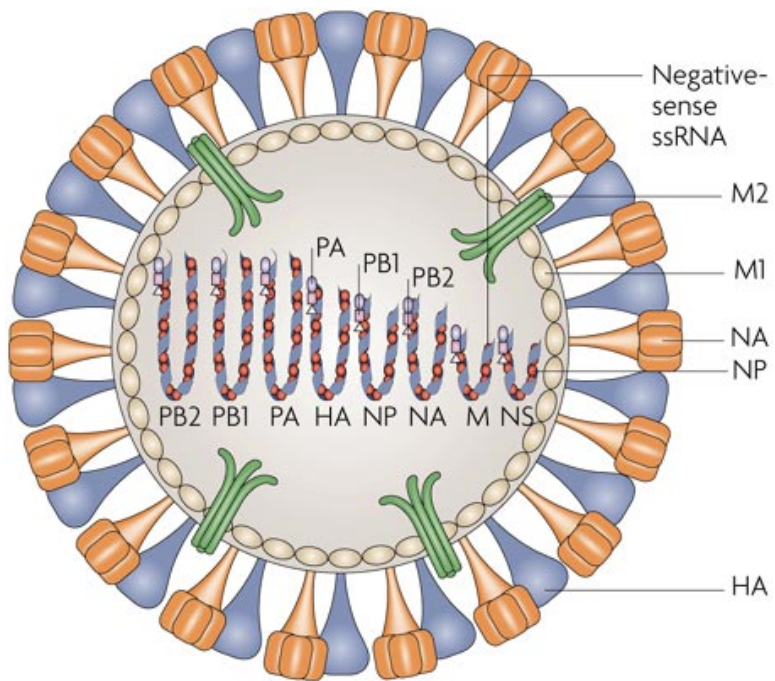


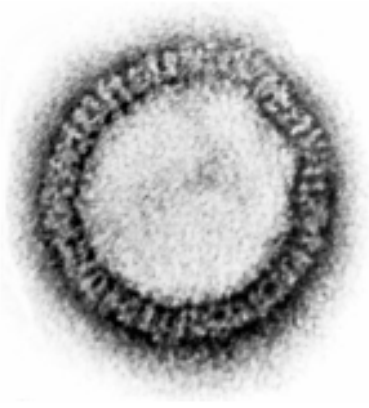
Interferon-inducing molecules for use as broad antivirals/**adjuvants**.

Luis Martinez-Gil, PhD
Dep. Biochemistry
Universidad d Valencia

Influenza A



From: Fields Virology

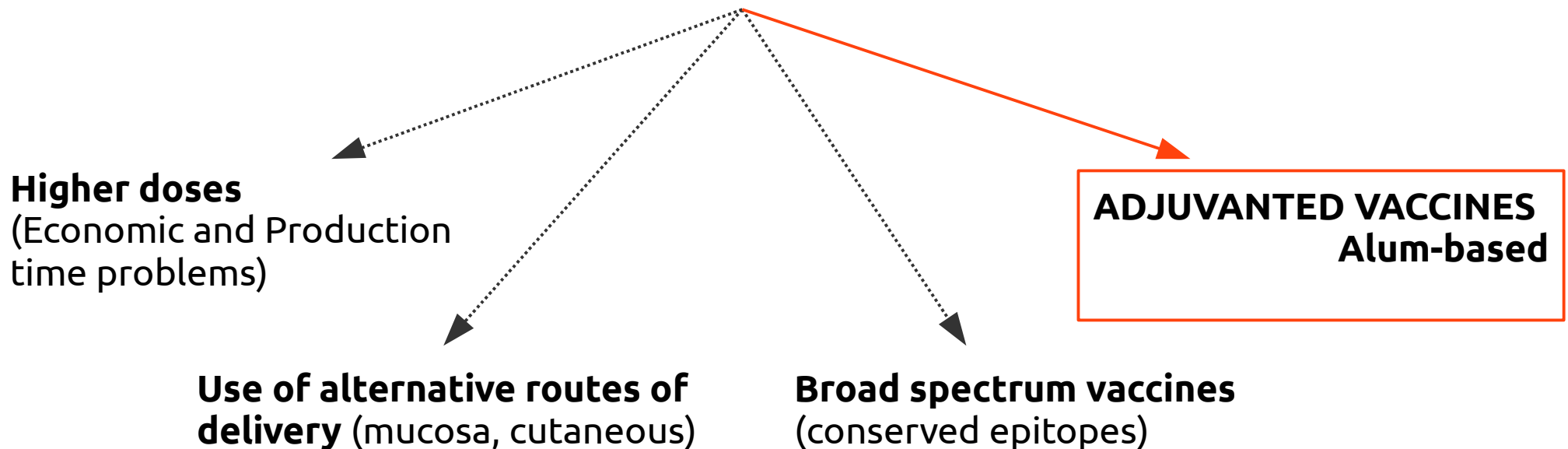


The need for better vaccines

- **Poor efficacy for some groups** (eg. Immunocompromised)
- **Little or no cross protection against significantly drifted strains or different subtypes** (eg. Influenza)
- **High doses required for protection**
90µg Avian H5N1 vs 15µg seasonal (Treanor et al. N. Eng. J. Med. 2006, 354:1343-1351)

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Interferon as vaccine adjuvant

An adjuvant is an ingredient of a vaccine that helps create a stronger immune response in the patient's body. In other words, adjuvants help vaccines work better.

Interferon activities :

- Broad spectrum antiviral
- Stimulator of a variety of cells of the immune system (T cells, NK cells, monocytes, macrophages and dendritic cells (Dcs)). Interferon as vaccine adjuvants.

Baccala et al. Nature Med. 2007; 13-5, 543

Tovey et al. Biol. Chem; 2008; 389, 541



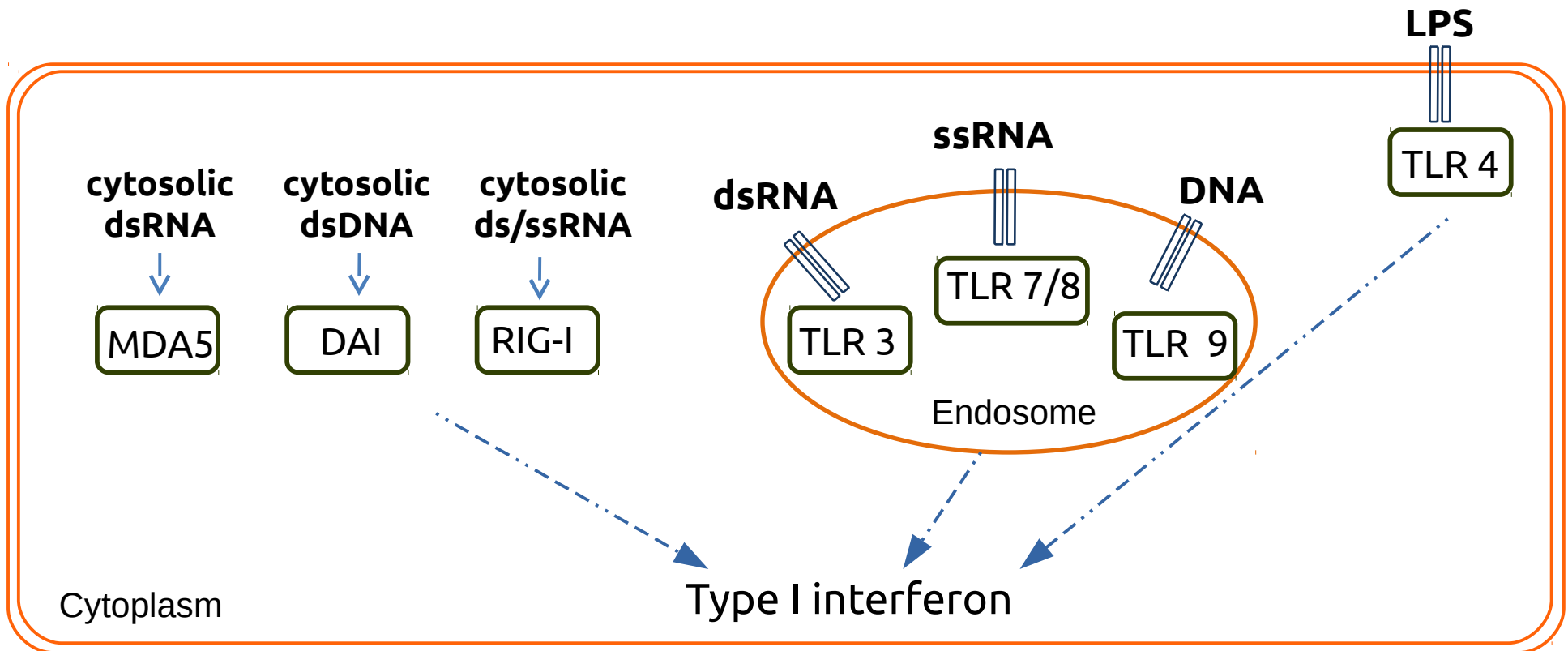
- Recombinant IFNs as vaccine adjuvants

Toporovski et al. Exprt. Opin. Biol. Ther; 2010, 1489

- IFN-inducing molecules have already been tested as adjuvants (poly I:C)

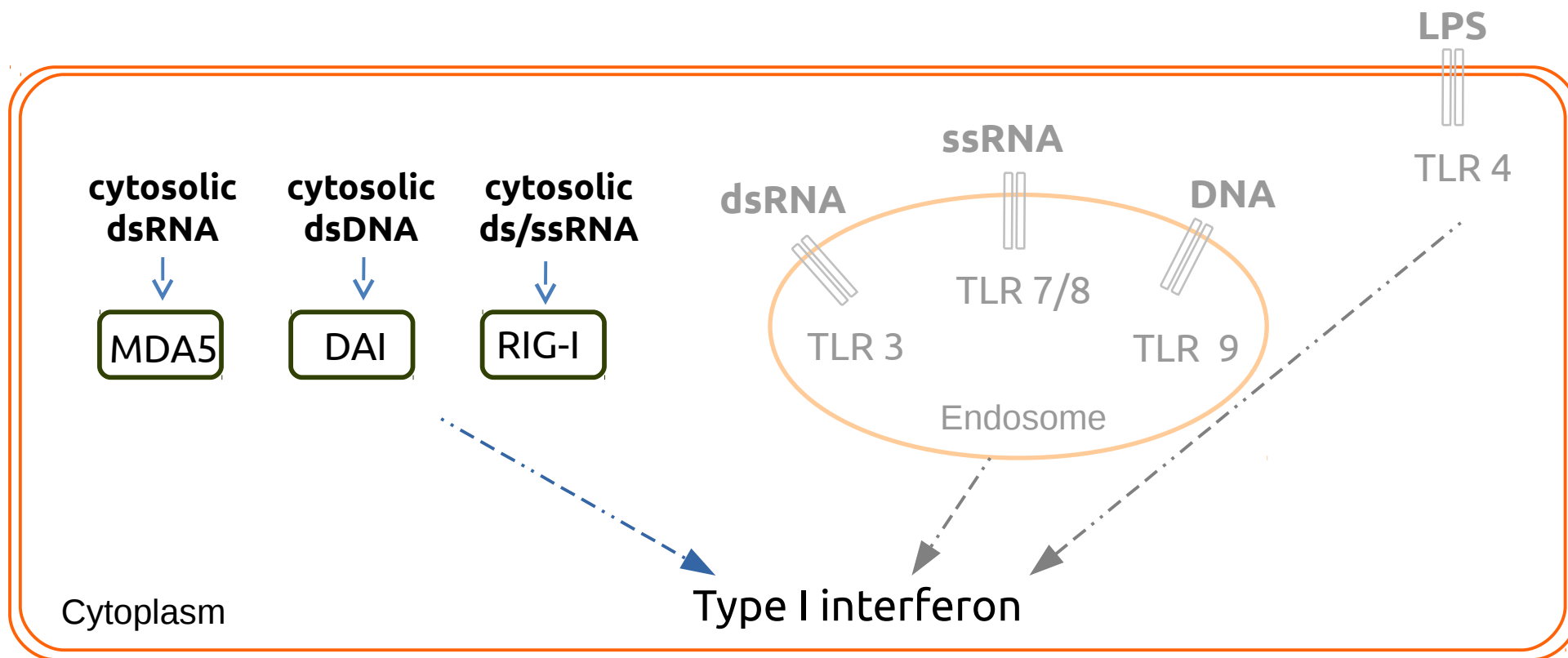
Field et al. Proc Natl Acad Sci USA. 1967; 58, 1004

Induction of interferon



Interferon-inducing molecules for use as broad antivirals/adjuvants

Induction of interferon



Interferon-inducing molecules for use as broad antivirals/adjuvants

**Interferon-inducing
small molecules**

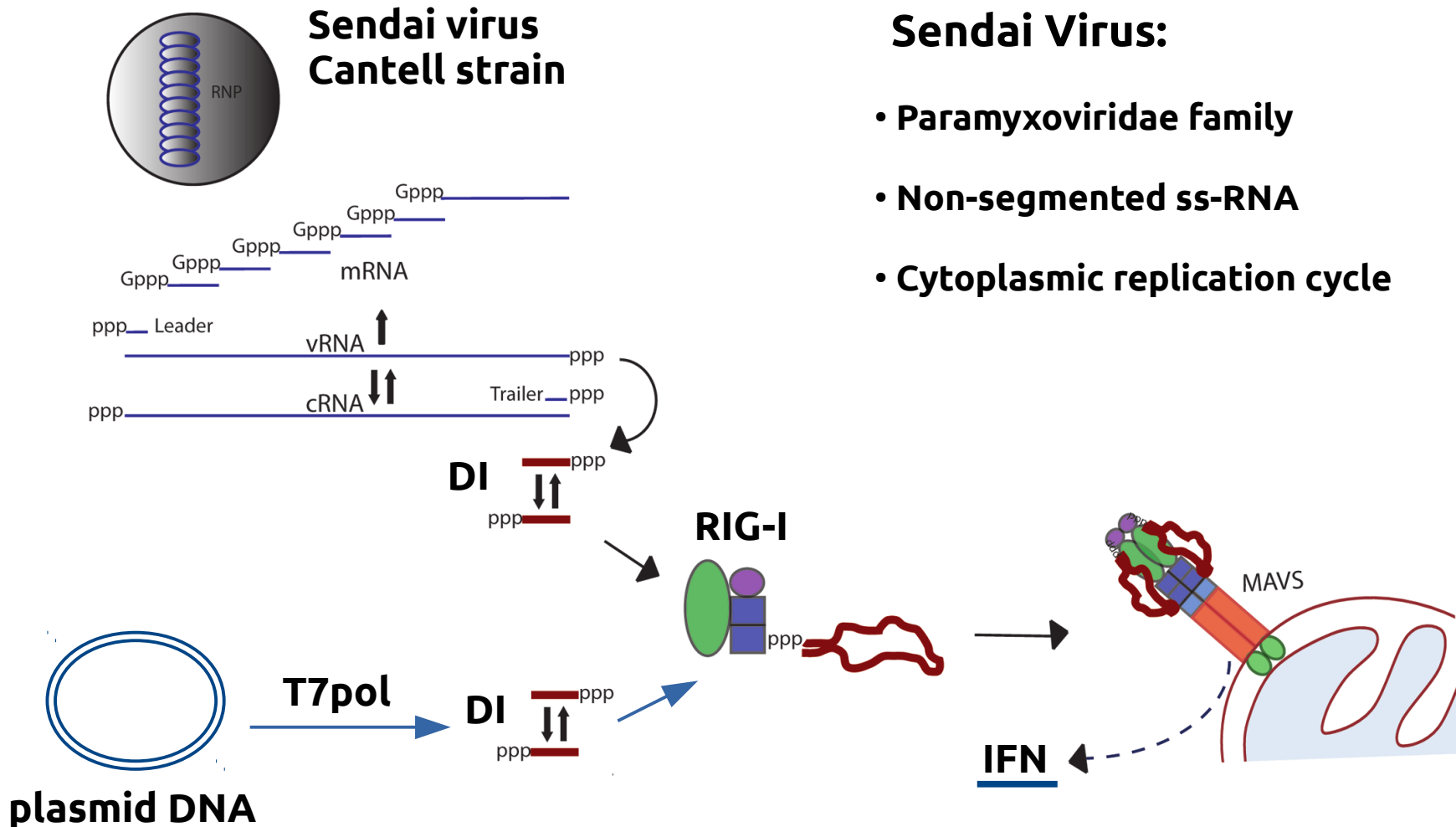
**Interferon-inducing RIG-I
binding dsRNA**

A Sendai Virus-Derived RNA Agonist of RIG-I as a Virus Vaccine Adjuvant

L. Martínez-Gil,^a P. H. Goff,^{a,b} R. Hai,^a A. García-Sastre,^{a,c} M. L. Shaw,^a P. Palese^{a,d}

Department of Microbiology,^a Graduate School of Biological Sciences,^b Institute of Global Health and Emerging Pathogens,^c Department of Medicine Division of Infectious Diseases,^d Mount Sinai School of Medicine, New York, New York, USA

Sendai virus DI particle is a strong RIG-I agonist



In vitro transcribed SeV DI is a potent inducer of interferon

1. Poly I:C

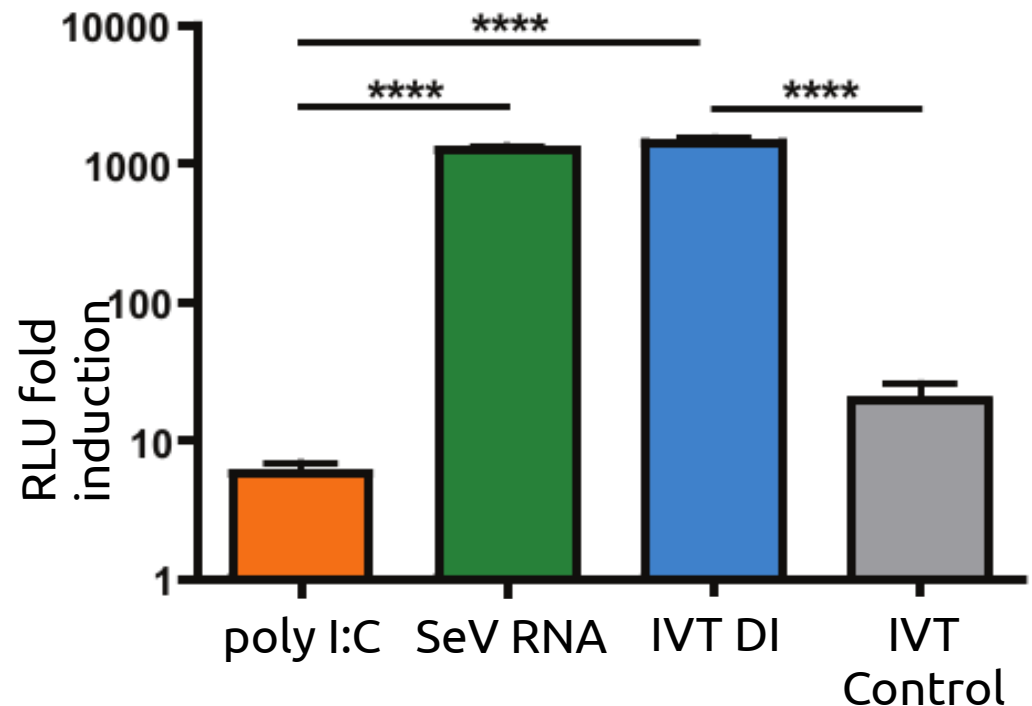
2. Sendai virus (SeV) RNA
RNA extracted from SeV egg grown virus. Mostly DI particles

3. *In vitro* (IVT) DI RNA
T7 polymerase produced DI RNA

4. *In vitro* control RNA
T7 polymerase produced RNA

293T-FF

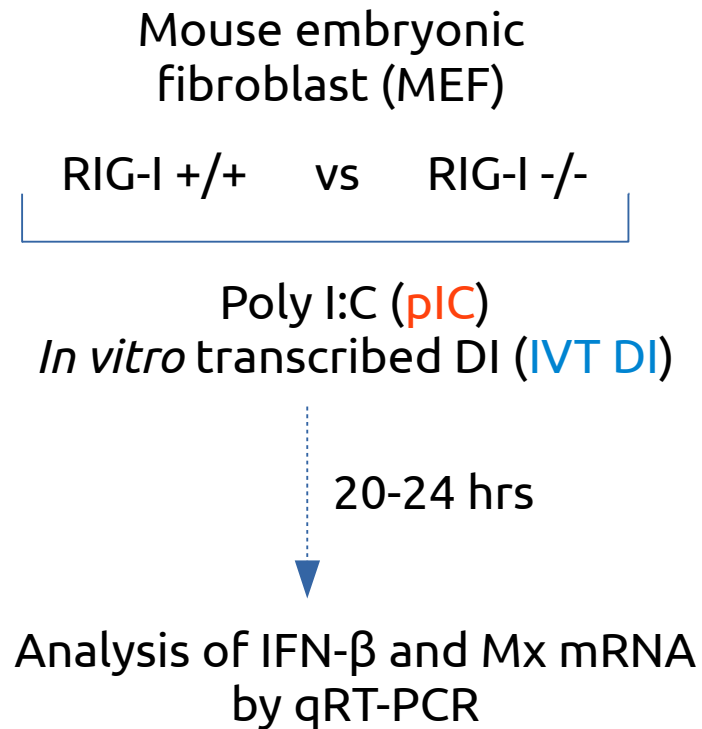
293T expressing firefly luciferase
under IFN- β promoter



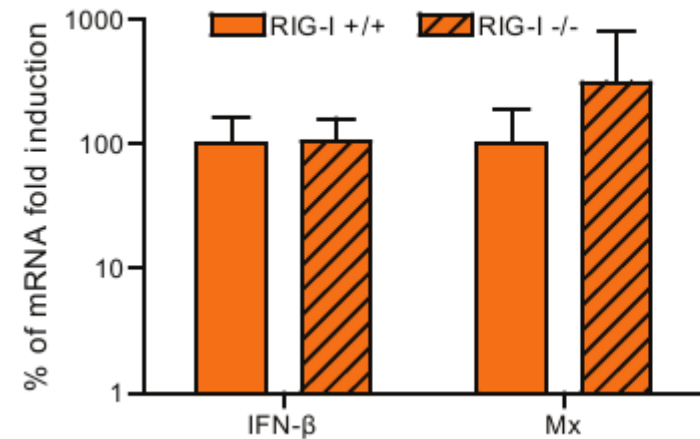
In vitro transcribed (IVT) DI is as potent as Sendai Virus RNA DI

NS not significant * pvalue<0.05 ** pvalue<0.01 *** pvalue<0.001 **** pvalue<0.0001

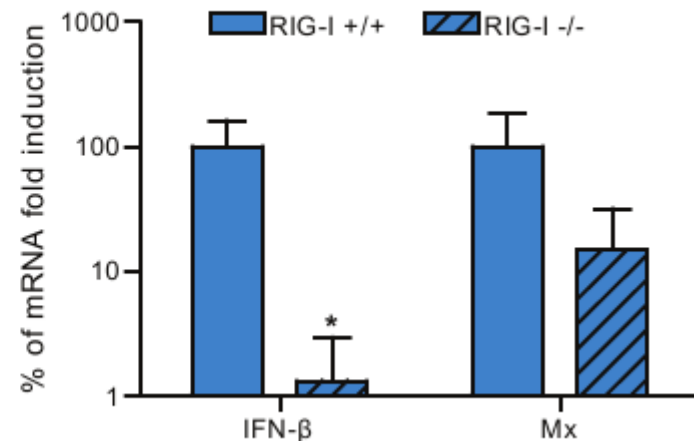
In vitro transcribed DI requires RIG-I for its IFN induction



pIC



IVT DI



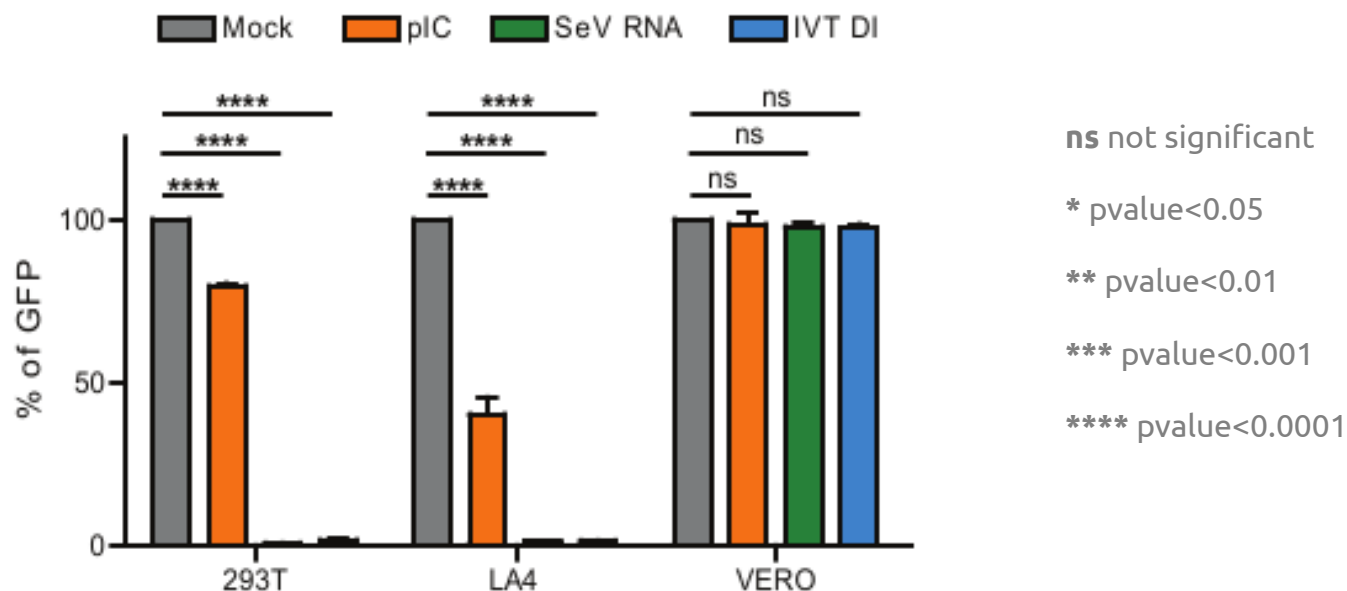
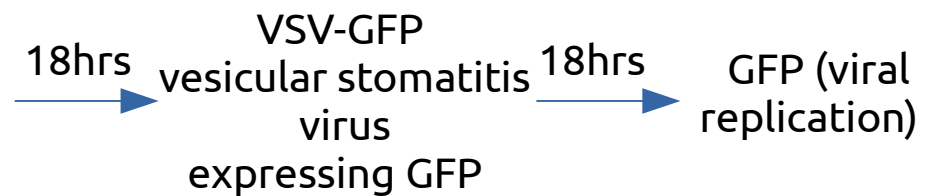
IVT DI can block viral infection *in vitro* via type I IFN

293T (human kidney derived)

LA4 (mouse lung adenoma)

VERO (Type 1 IFN deficient)

1. PIC
2. SeV RNA
3. IVT DI
4. PBS



IVT DI can induce IFN *in vivo*

Intra-muscular or intra-nasal (50μL) administration

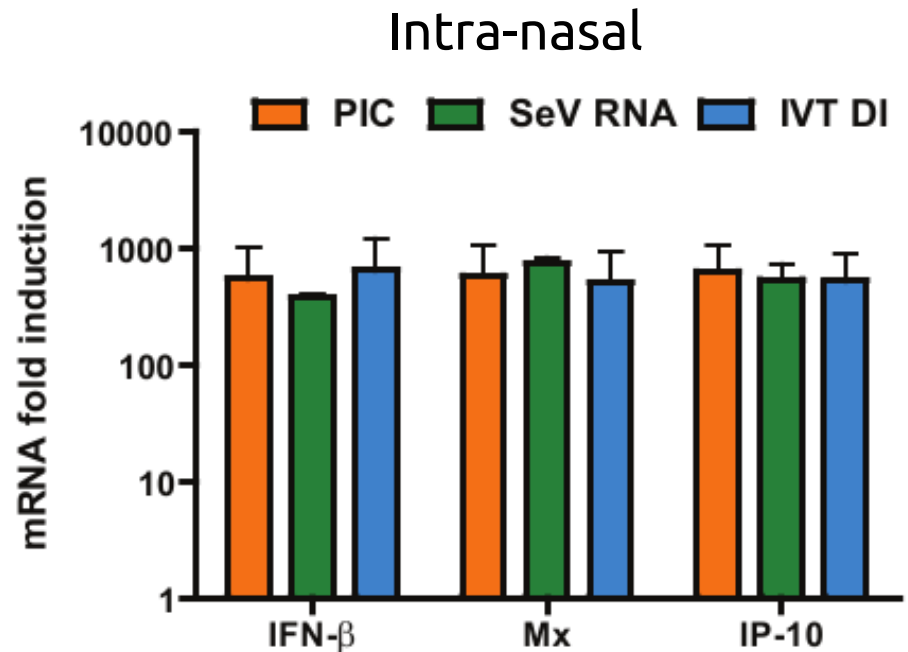
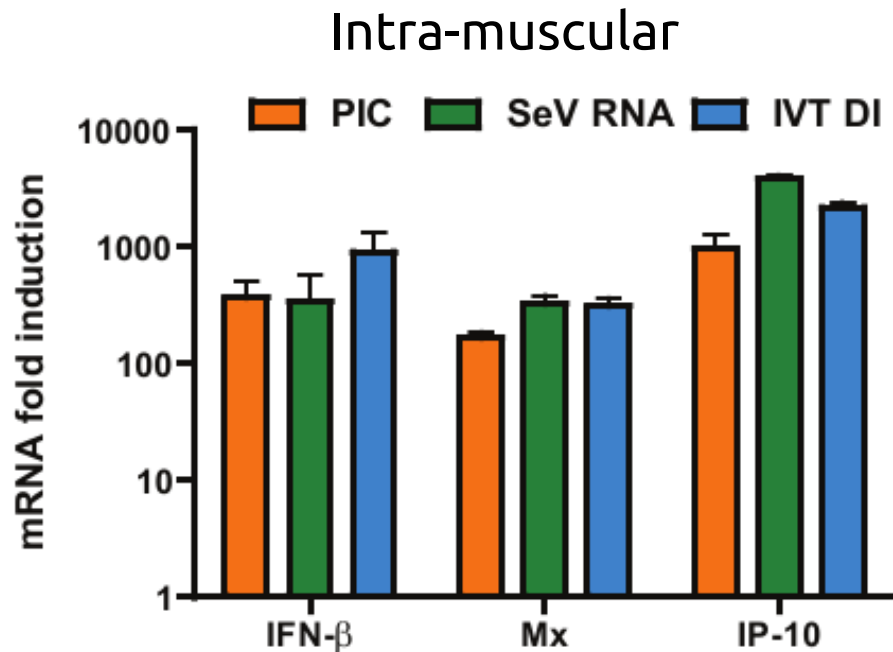
1. PIC
2. SeV RNA
3. IVT DI
4. PBS

6 hrs

Tissue collection (site of administration)

RNA extraction

mRNA quantification (qRT-PCR)



In vivo PIC and IVT DI show equal potency

IVT DI requires RIG-I for its IFN induction *in vivo*

RIG-I +/+ RIG-I -/-

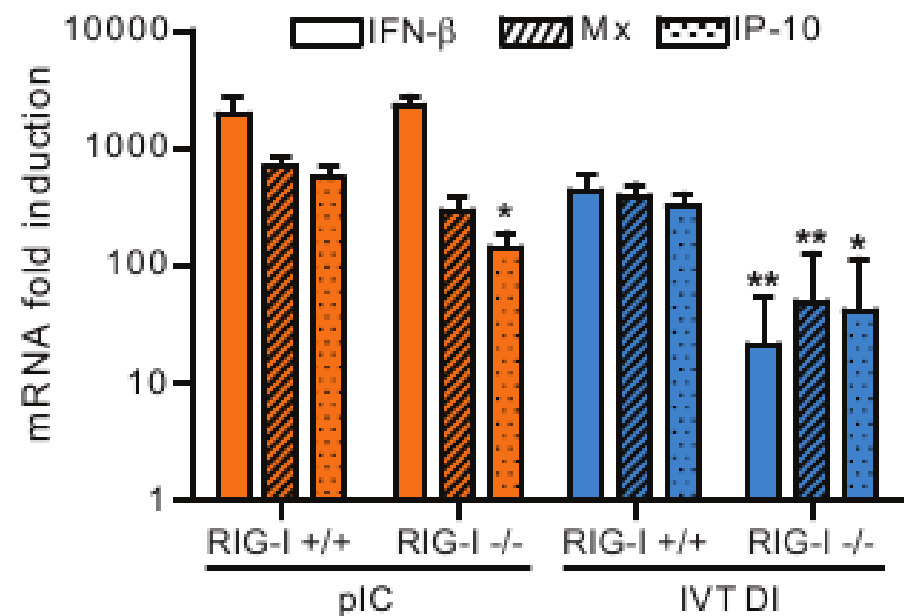
Intra-muscular (IM) administration

4 µg poly I:C (PIC)
4µg *in vitro* transcribed DI (IVT DI)
Mock treatment (PBS)

6 hrs

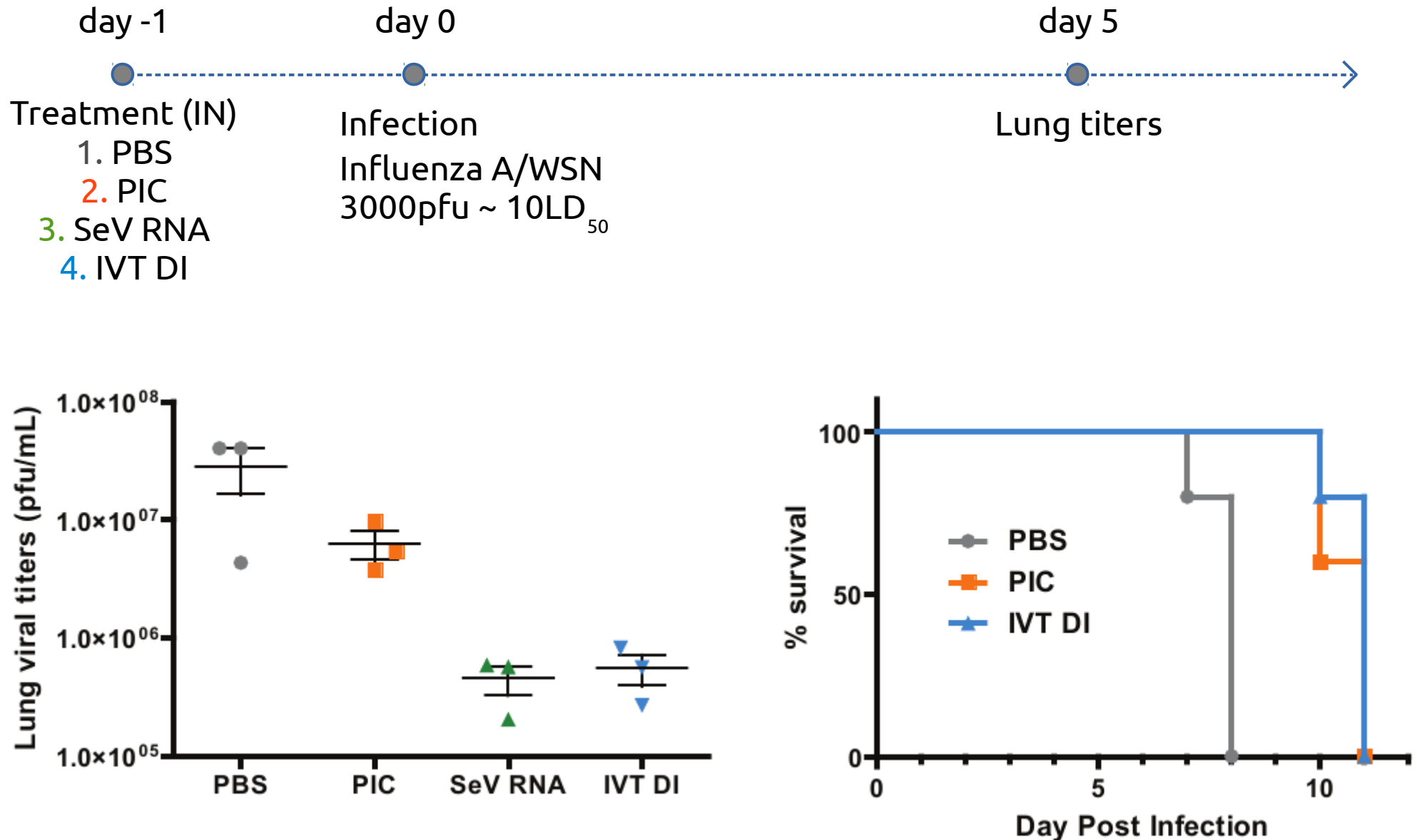


Tissue collection (site of administration)
RNA extraction
mRNA quantification (qRT-PCR)

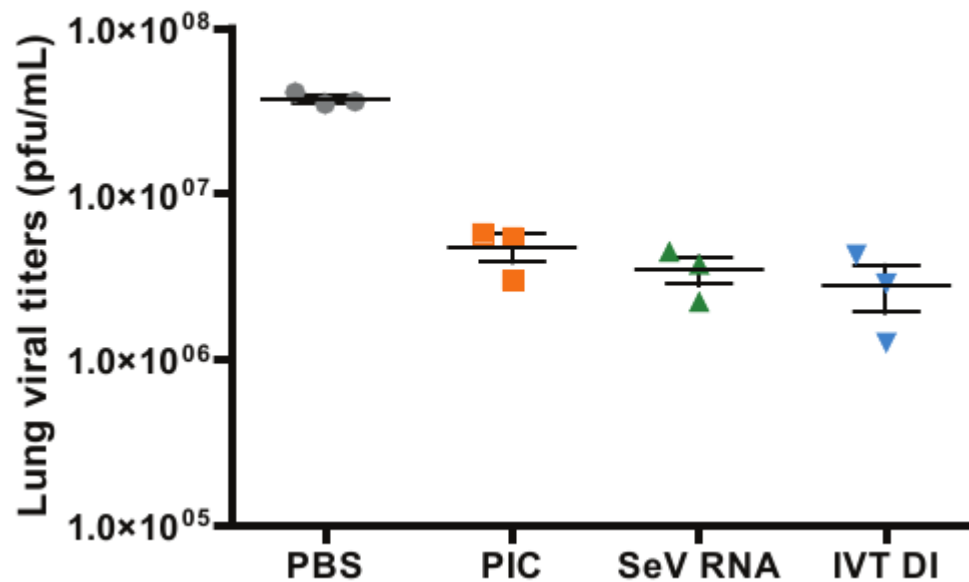
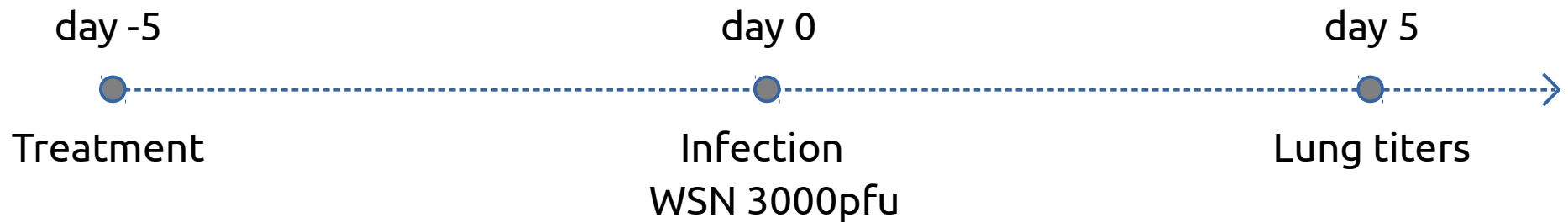


ns not significant * pvalue<0.05 ** pvalue<0.01 *** pvalue<0.001 **** pvalue<0.0001

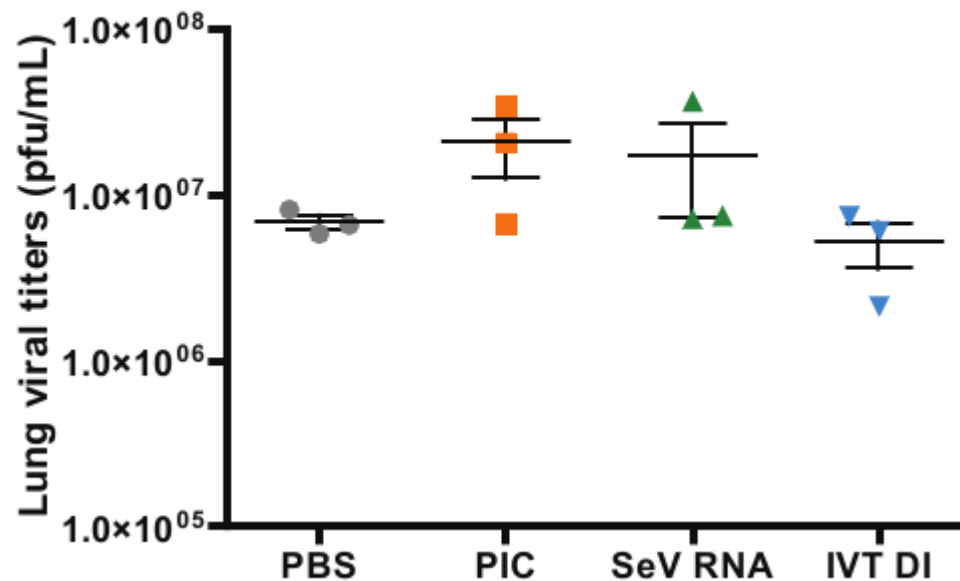
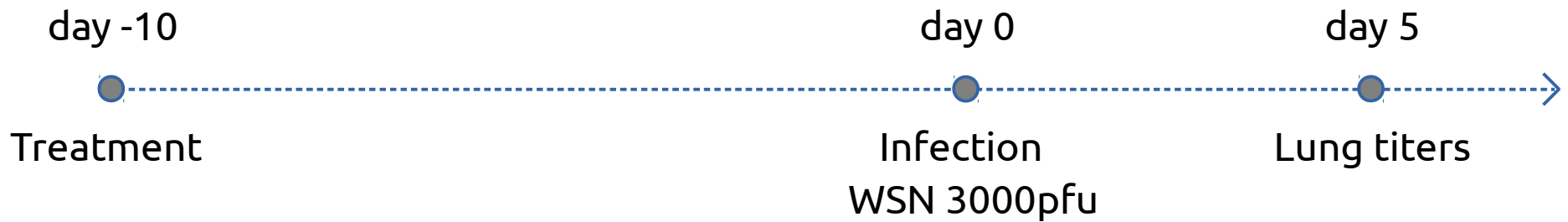
IVT DI can reduce viral growth *in vivo*



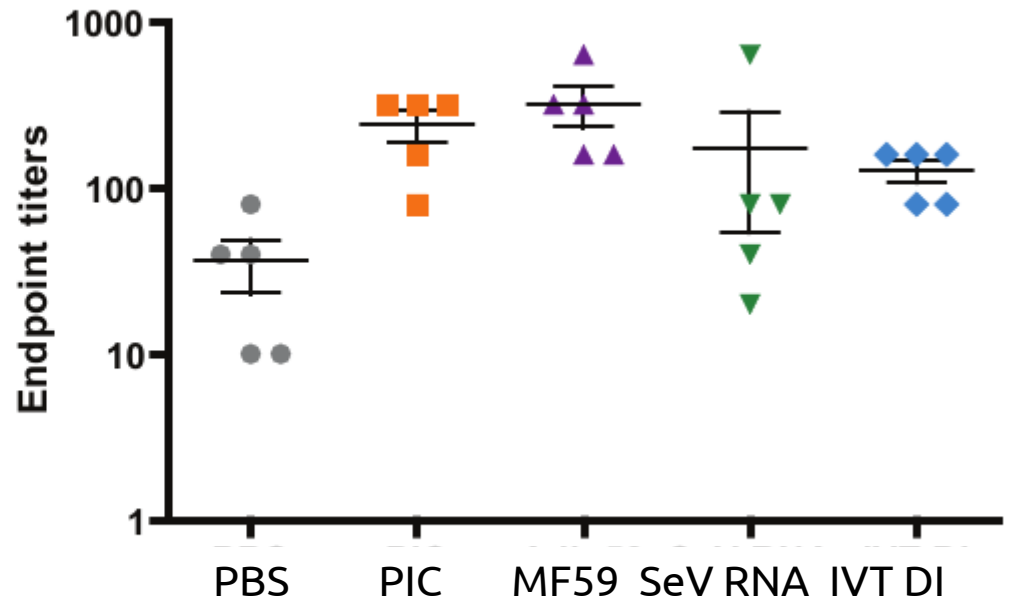
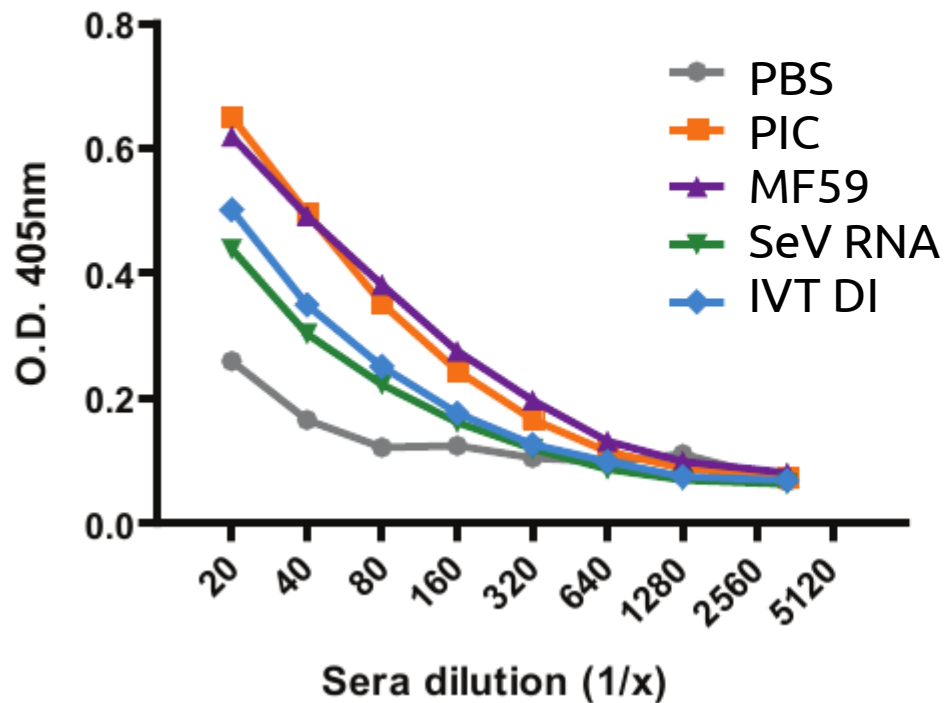
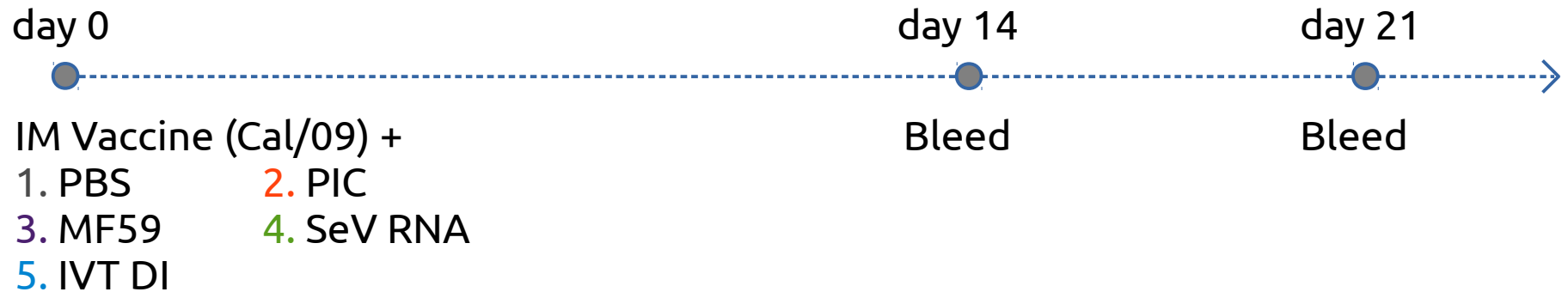
The antiviral effect of IVT DI disappears over the time



The antiviral effect of IVT DI disappears over the time



Intra-muscular administration of IVT DI increases Ab titers



Intra-nasal administration of IVT DI increases Ab titers

day 0

IN Vaccine (Cal/09) +

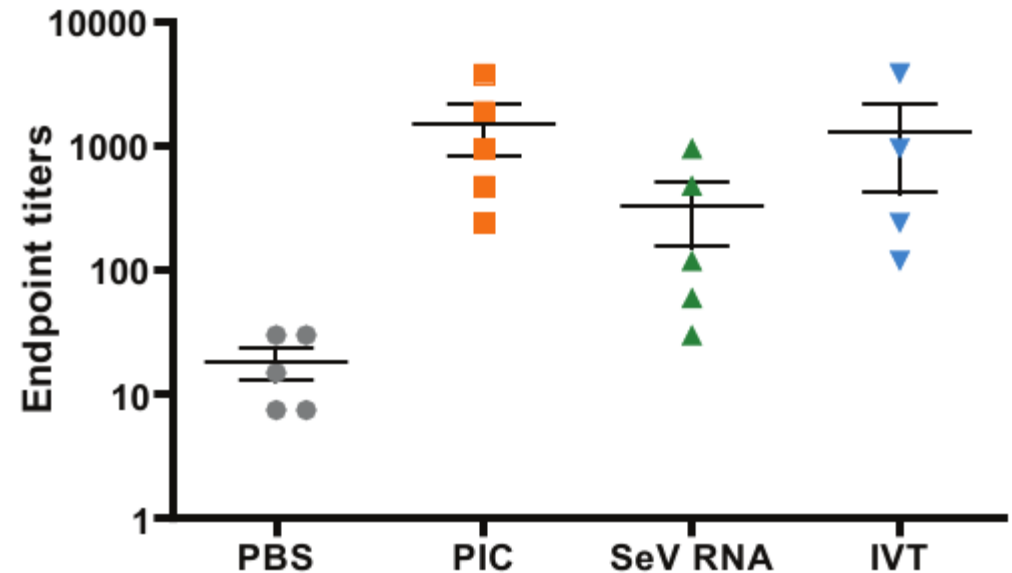
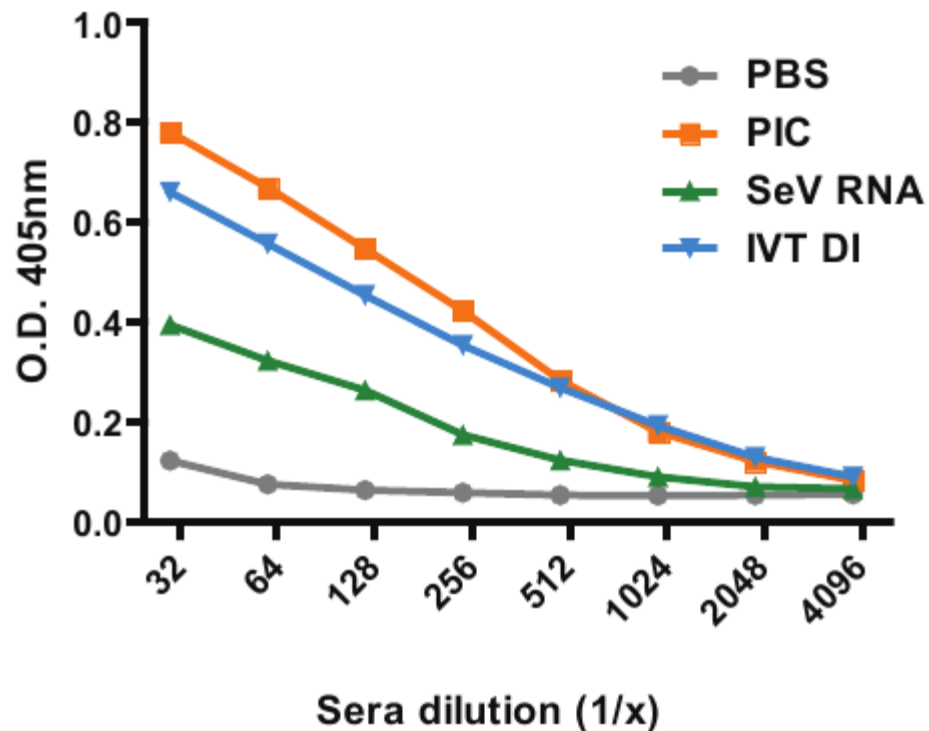
1. PBS 2. PIC
3. SeV RNA 4. IVT DI

day 21

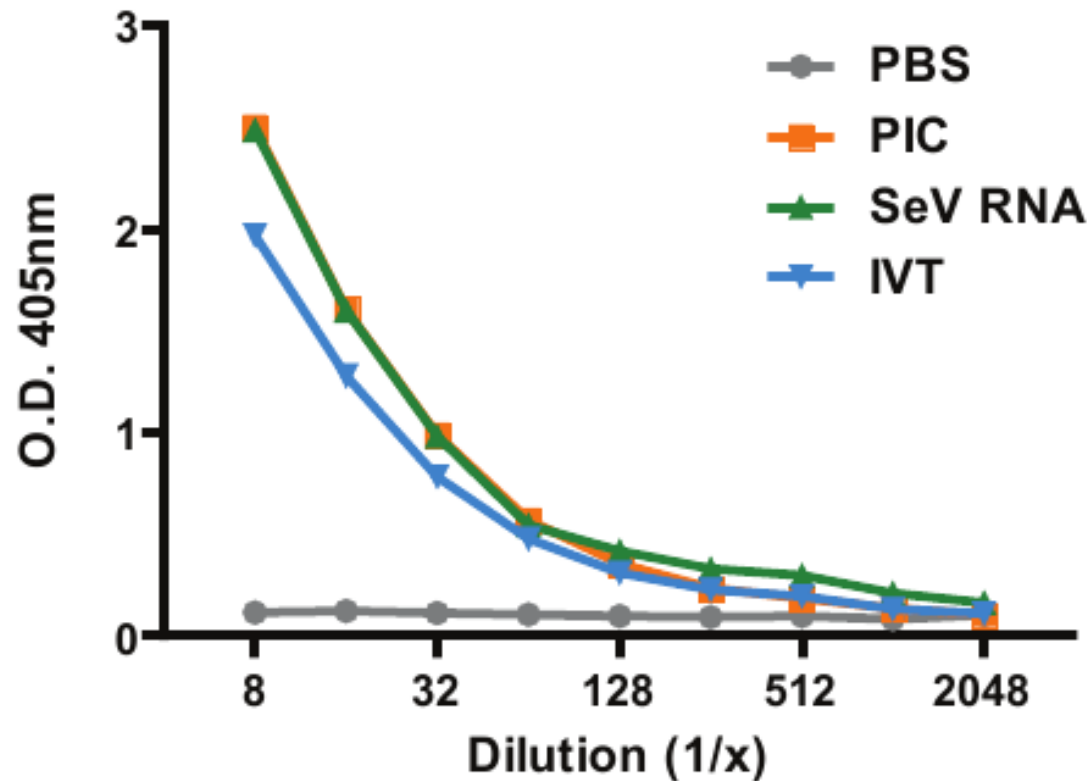
Boost

day 28

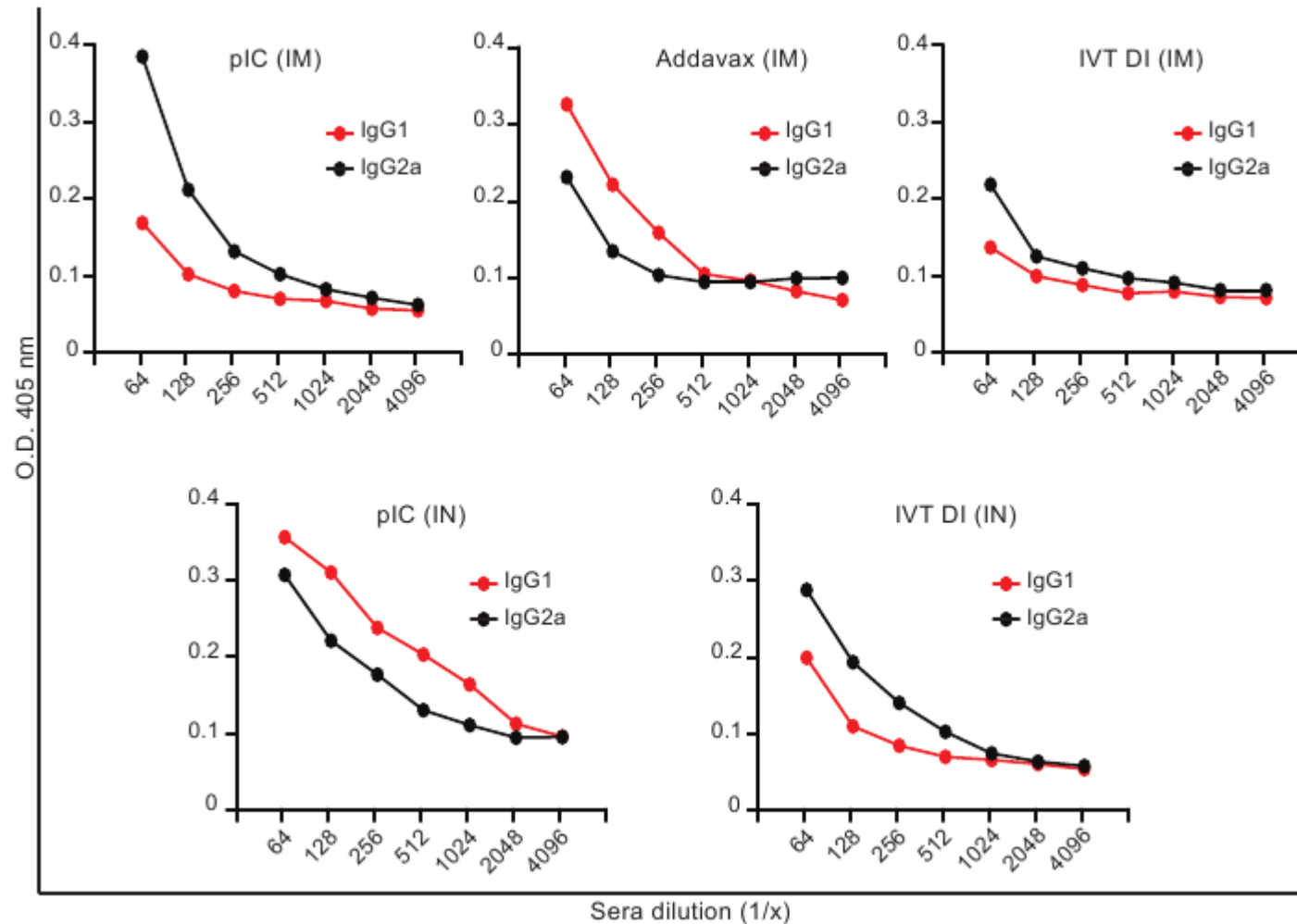
Bleed
Lung lavage



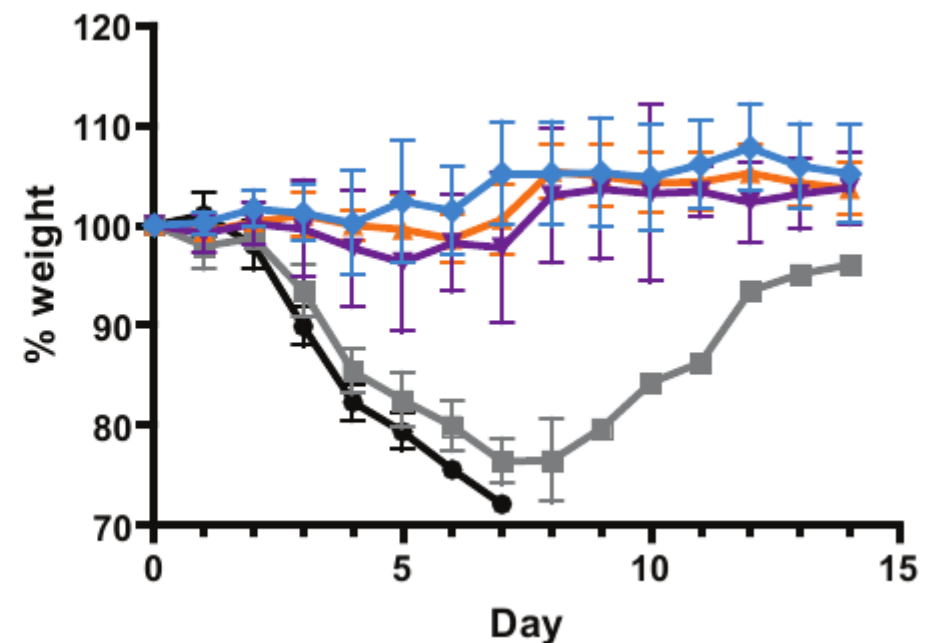
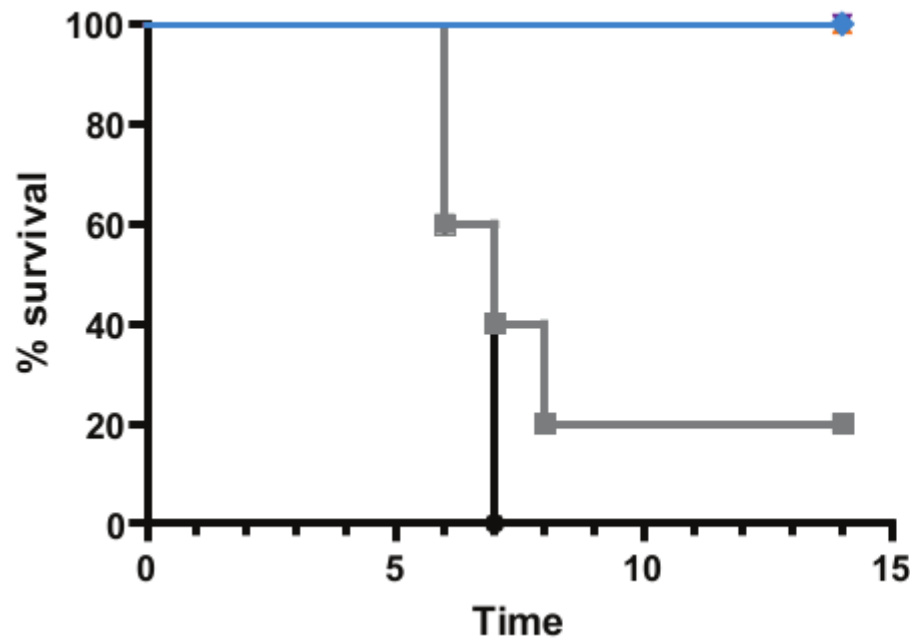
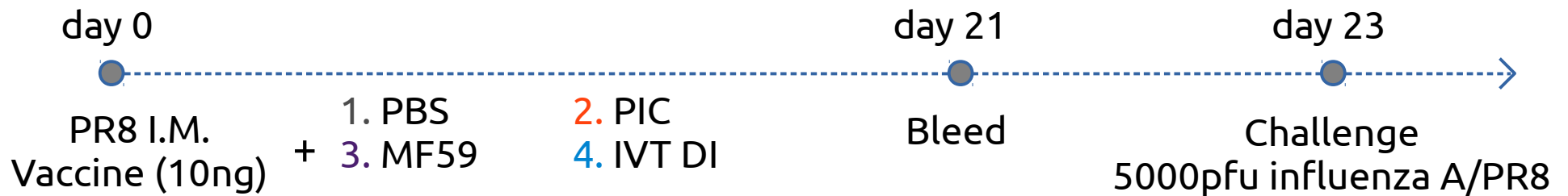
Intra-nasal administration of IVT DI induces a potent IgA response



Administration of IVT DI induces a Th1 response

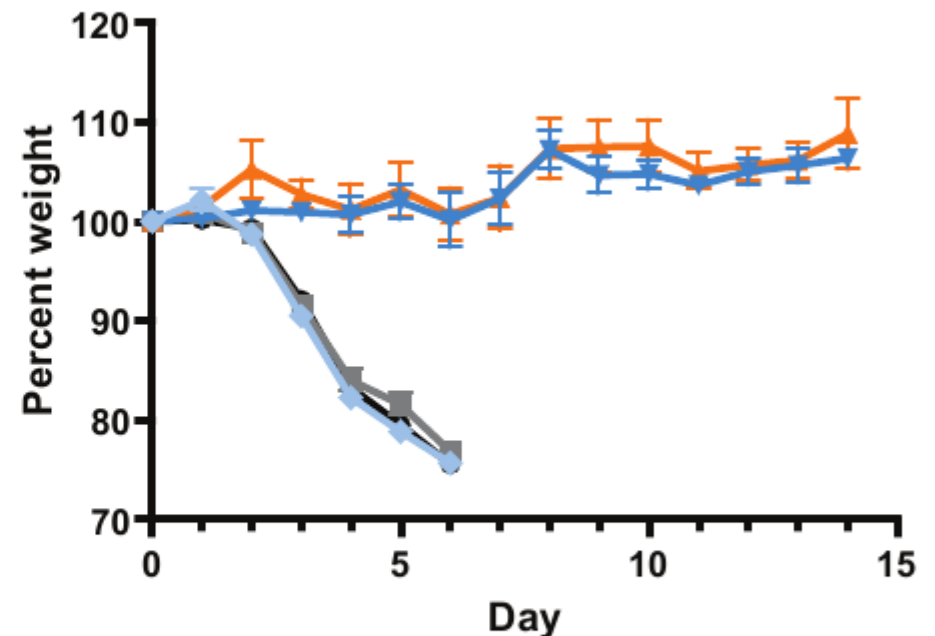
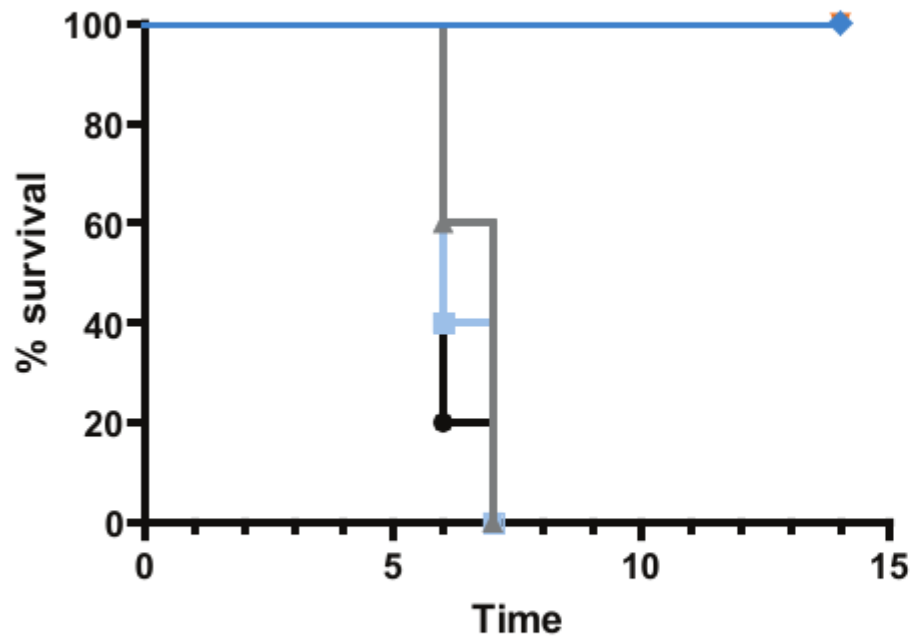


Intra-muscular administration of IVT DI increases vaccine efficiency



- No Vaccine
- Vaccine + PBS
- ▲ Vaccine + PIC
- ▼ Vaccine + Adju59
- ◆ Vaccine + IVT DI

Intra-nasal administration of IVT DI increases vaccine efficiency



- No Vaccine
- IVT DI (No Vacc)
- PBS + Vaccine
- PIC + Vaccine
- IVT + Vaccine

RIG-I participates in IVT DI adjuvancy

day 0

IM Vaccine (Cal/09) +

1. PBS 2. MF59

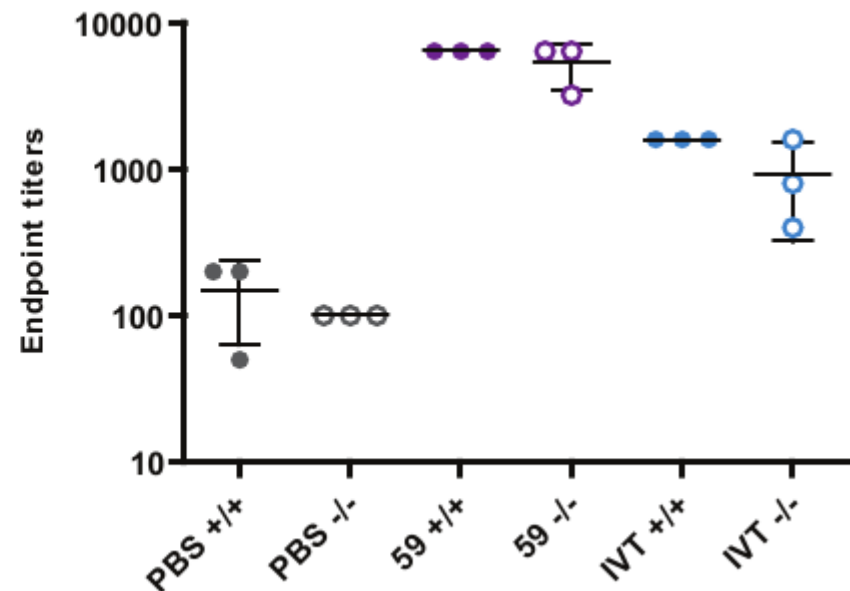
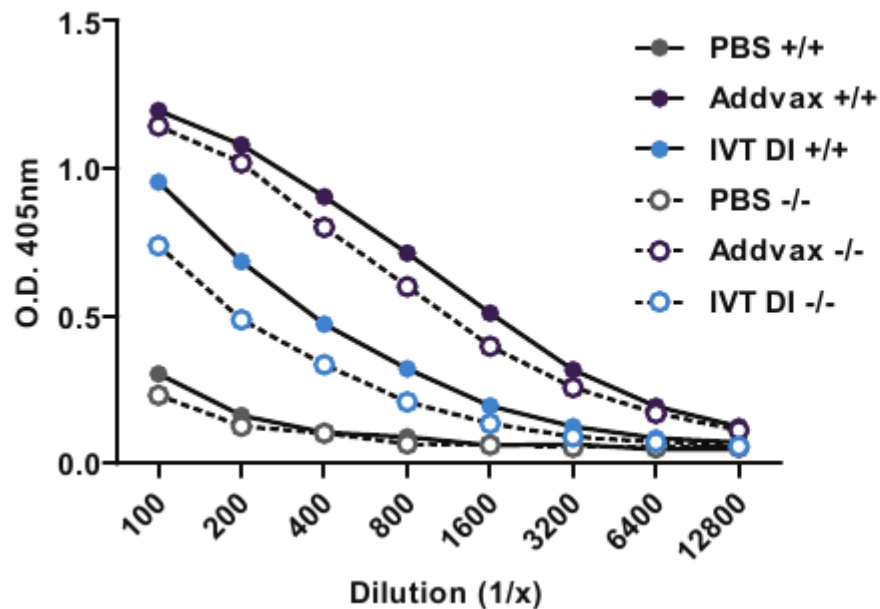
3. IVT DI (4 μ g)

day 14

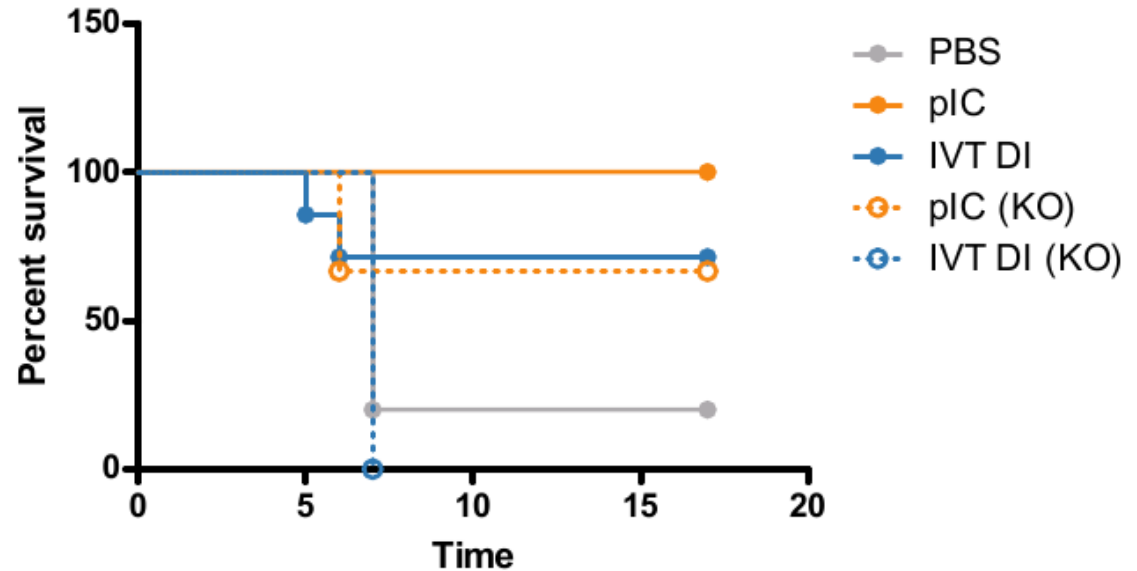
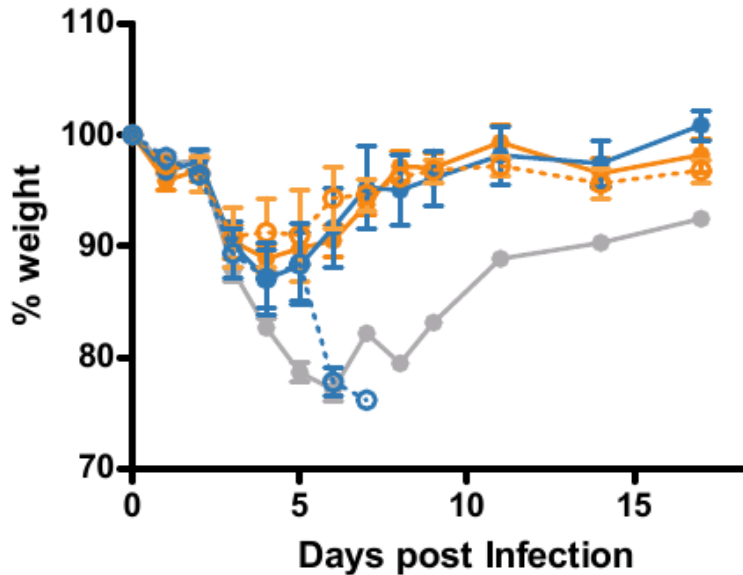
Bleed

day 21

Bleed



RIG-I participates in IVT DI adjuvancy

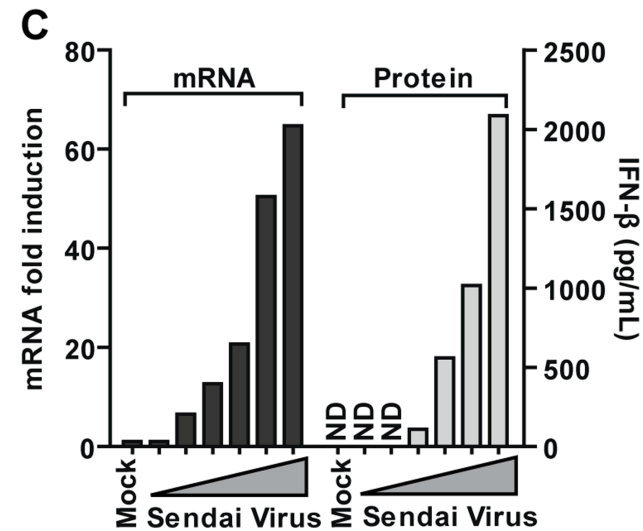
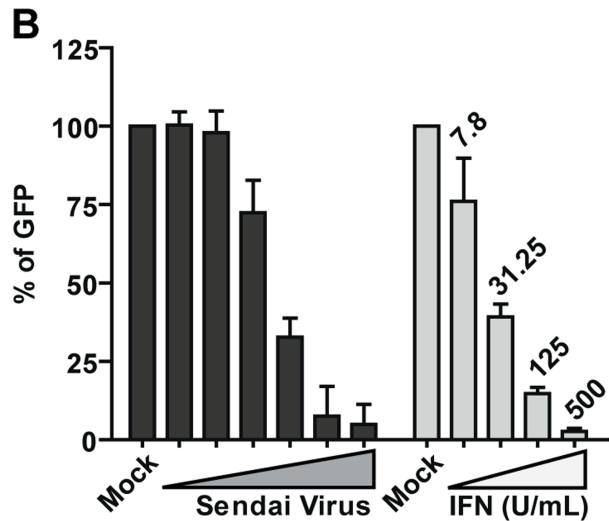
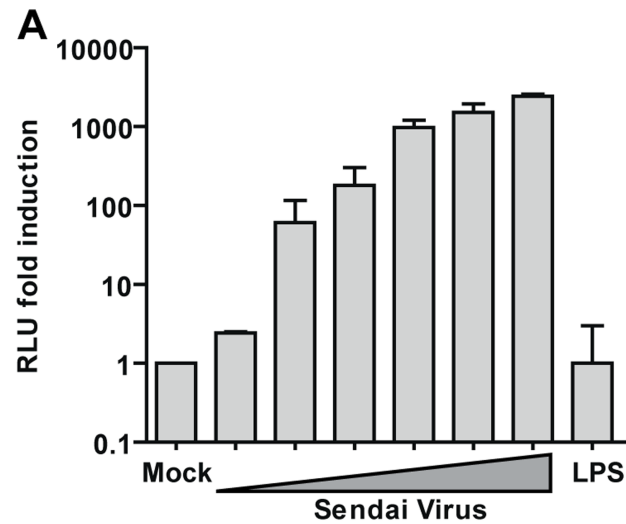


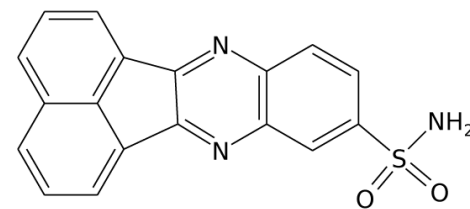
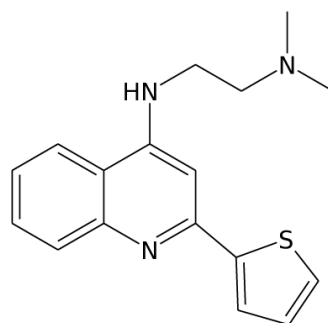
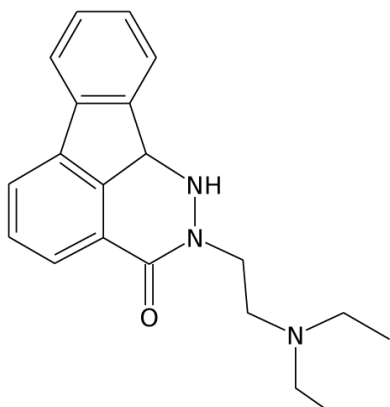
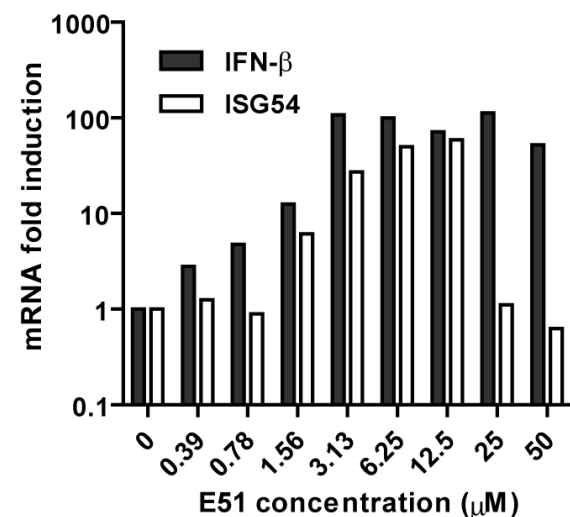
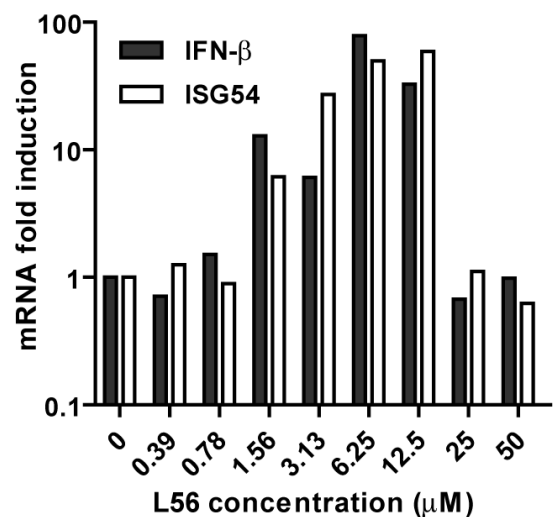
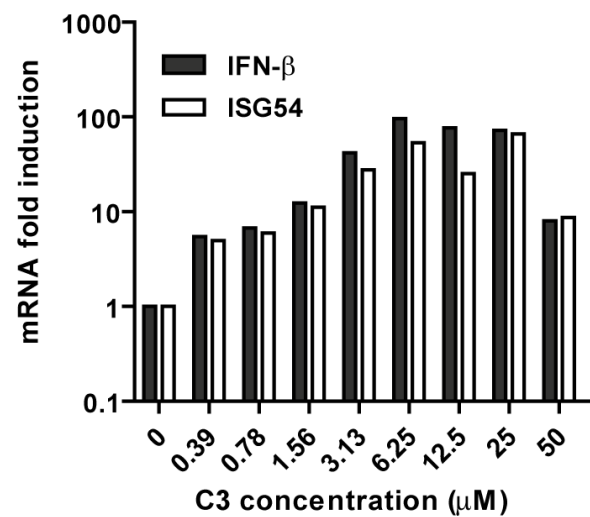
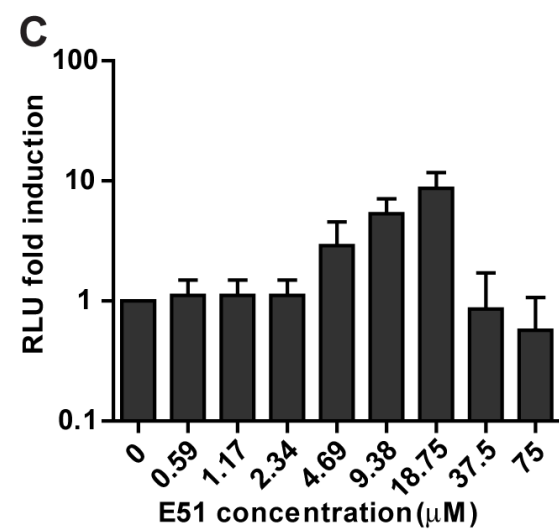
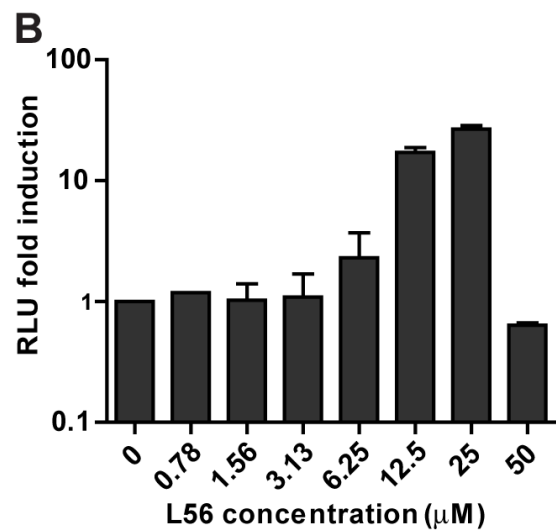
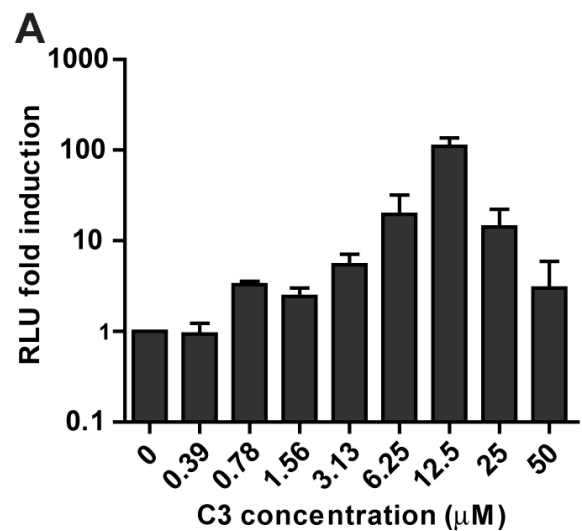
Identification of Small Molecules with Type I Interferon Inducing Properties by High-Throughput Screening

Luis Martínez-Gil¹, Juan Ayllon¹, Mila Brum Ortigoza¹, Adolfo García-Sastre^{1,2,3}, Megan L. Shaw¹, Peter Palese^{1,3*}

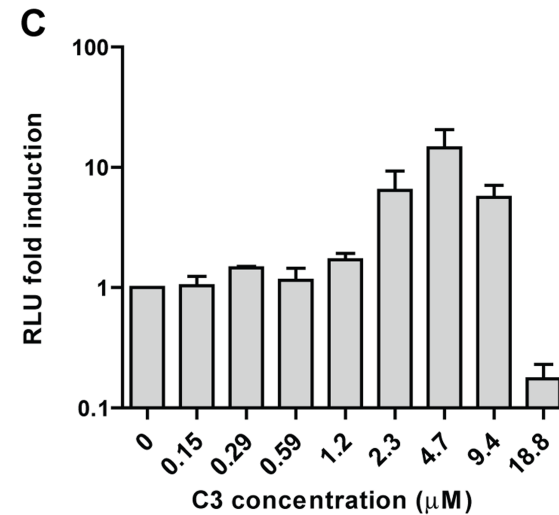
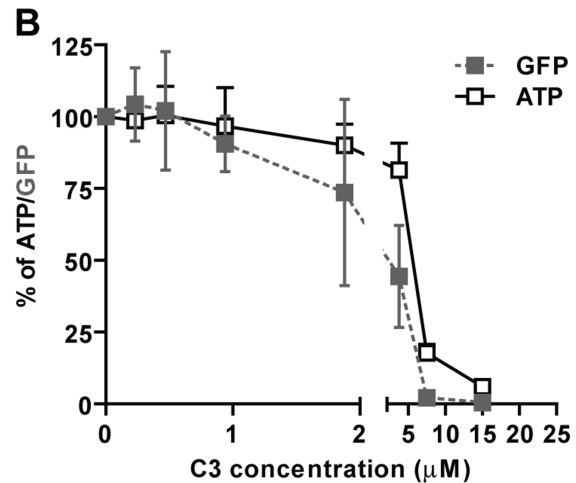
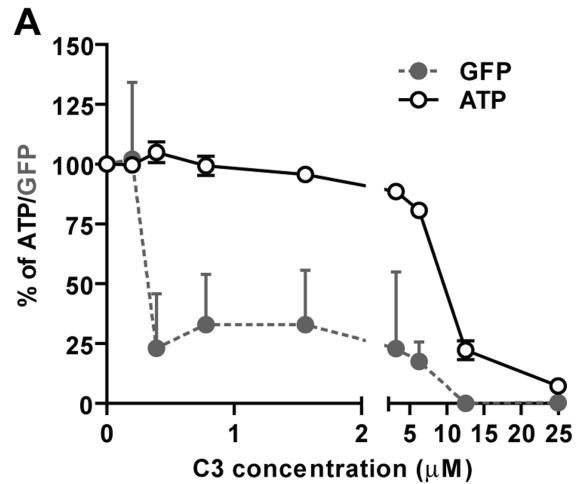
1 Department of Microbiology, Mount Sinai School of Medicine, New York, New York, United States of America, **2** Institute of Global Health and Emerging Pathogens, Mount Sinai School of Medicine, New York, New York, United States of America, **3** Department of Medicine, Division of Infectious Diseases, Mount Sinai School of Medicine, New York, New York, United States of America

Creation of a IFN-reporter cell line





C3 IFN induction confirmation



C3 IFN induction is not inhibited by viral antagonist

