

# Understanding Down syndrome as an Interferonopathy

Joaquín M. Espinosa, PhD  
Associate Director for Science  
Professor of Pharmacology  
University of Colorado School of Medicine



# Individuals with Trisomy 21 have a different 'disease spectrum'



**The >400,000 Americans with trisomy 21 may hold solutions  
to major medical conditions**

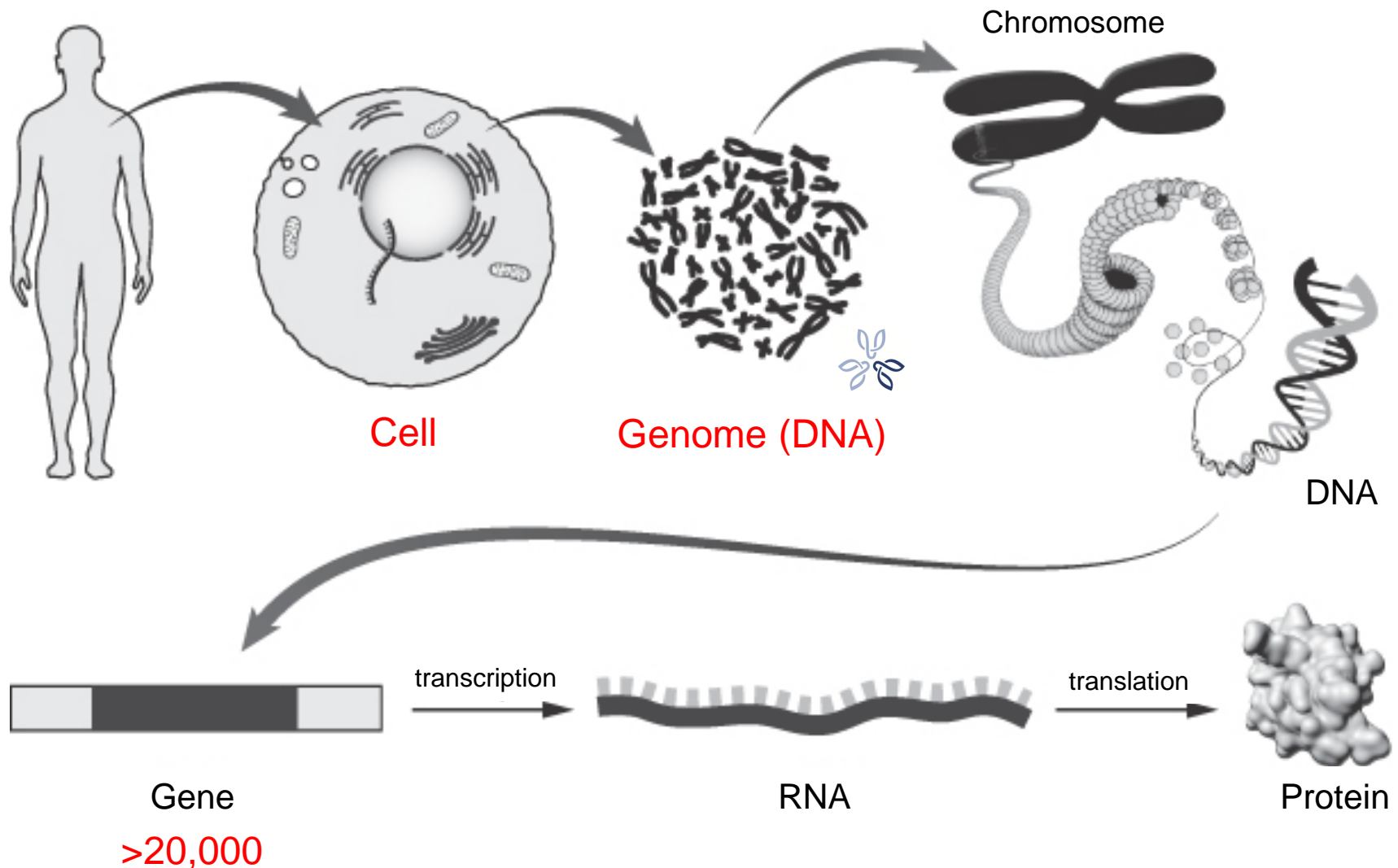
Each one of them is dealing with trisomy 21  
in their own unique, personal way



They are more awesome than different,  
yet they are **ALL** different

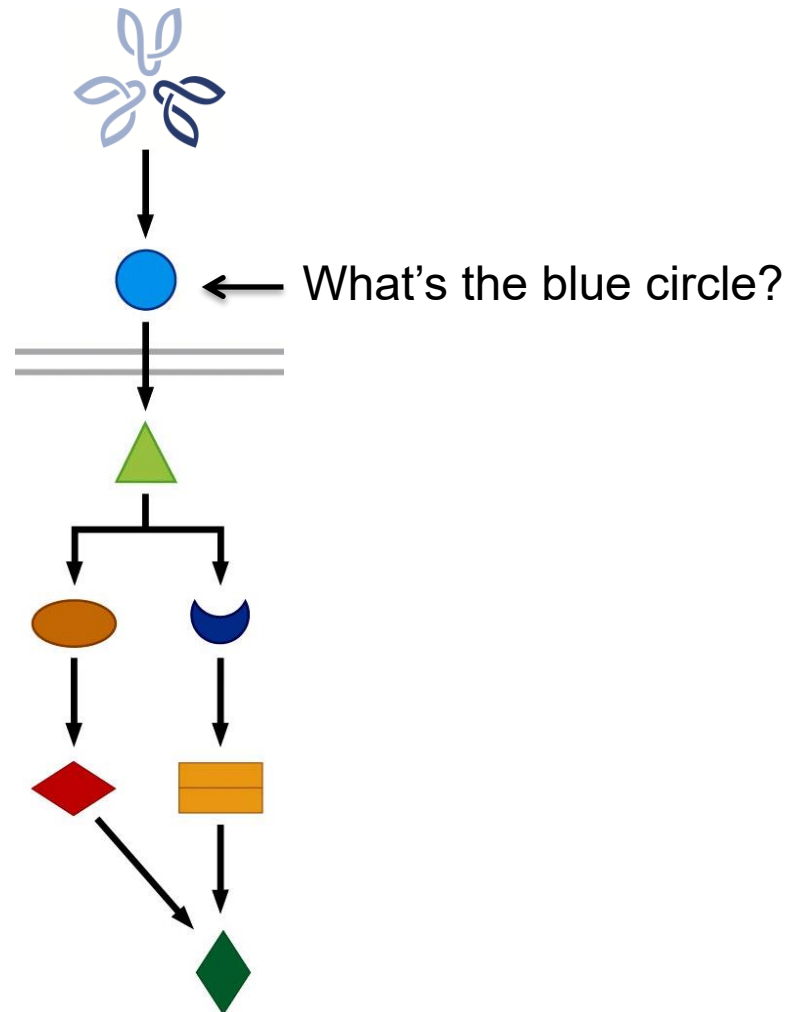
# Project Goal

To identify the consistent molecular events activated by trisomy 21

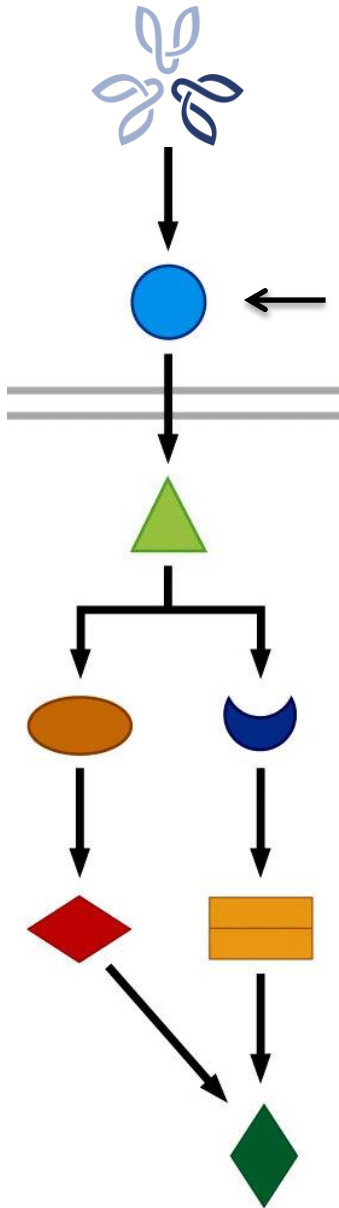


# Project Goal

To identify key signaling pathways **consistently** activated by trisomy 21



# The ideal scenario



Early molecular event that  
can be blocked with a drug

In the ideal scenario,  
the ill effects of trisomy 21 are  
caused by one, or a few, molecular  
pathways that can be blocked with  
FDA-approved drugs

# Approach

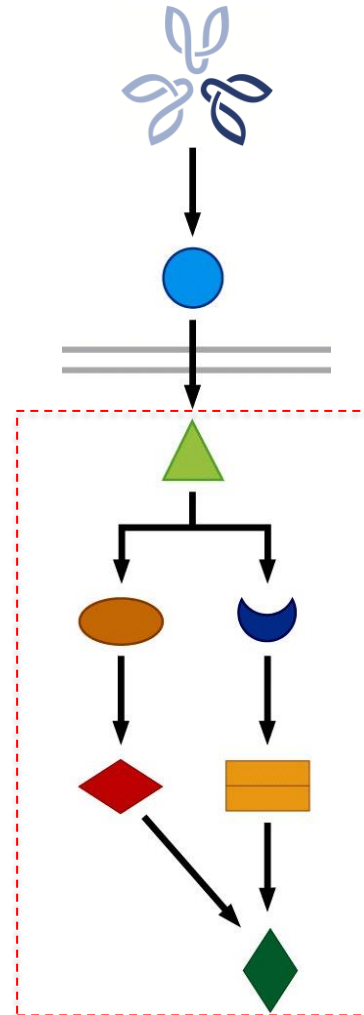
Functional genomics approaches to elucidate the molecular events activated by trisomy 21

## Measurements performed:

- >16,000 RNAs
- 600 metabolites
- 654 kinases (important enzymes)
- >4,000 proteins

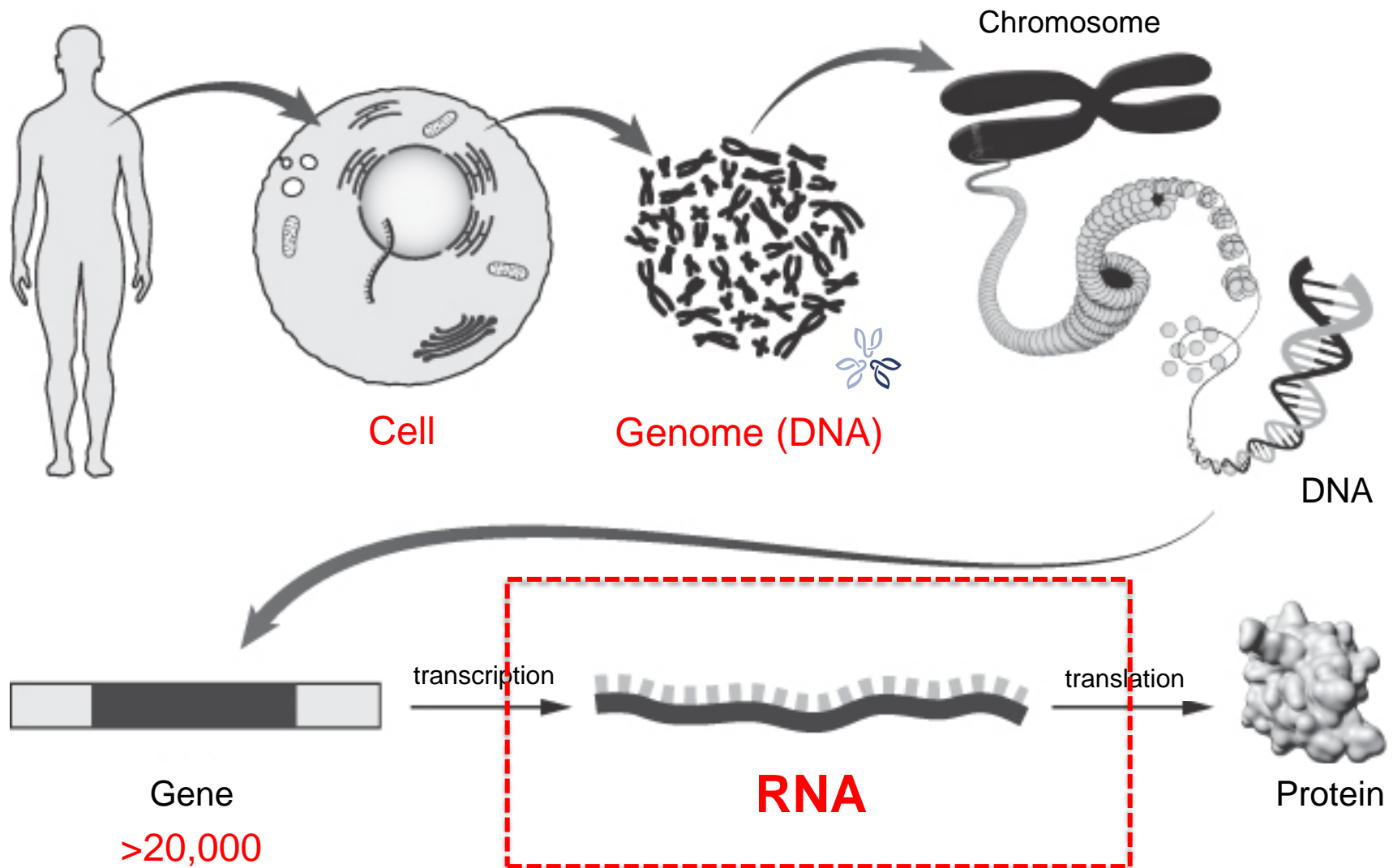
## Cell types employed:

- Skin Fibroblasts Cell Lines
- Lymphoblastoids Cell Lines (B cells)
- Circulating Monocytes
- Circulating T cells



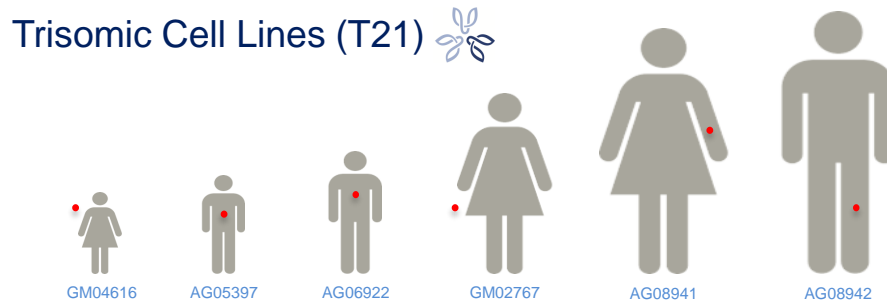
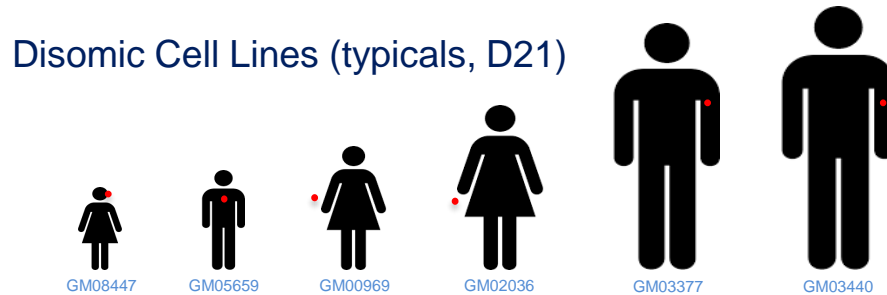
# First experiment

To measure thousands of RNAs from skin cells





# Employing highly diverse pools of skin fibroblasts to discover consistent effects caused by trisomy 21



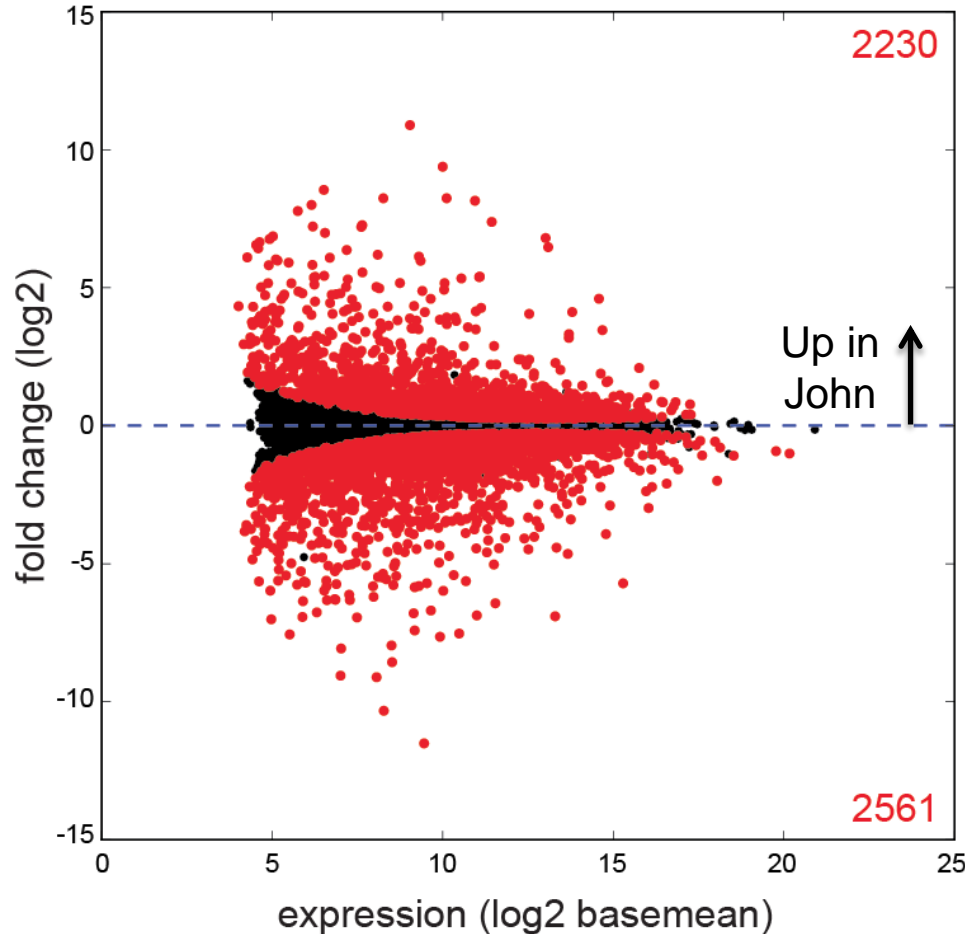
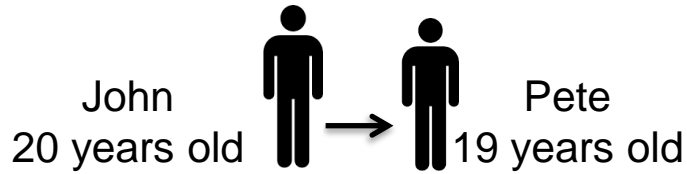
• biopsy site

Different ages, gender and biopsy sites

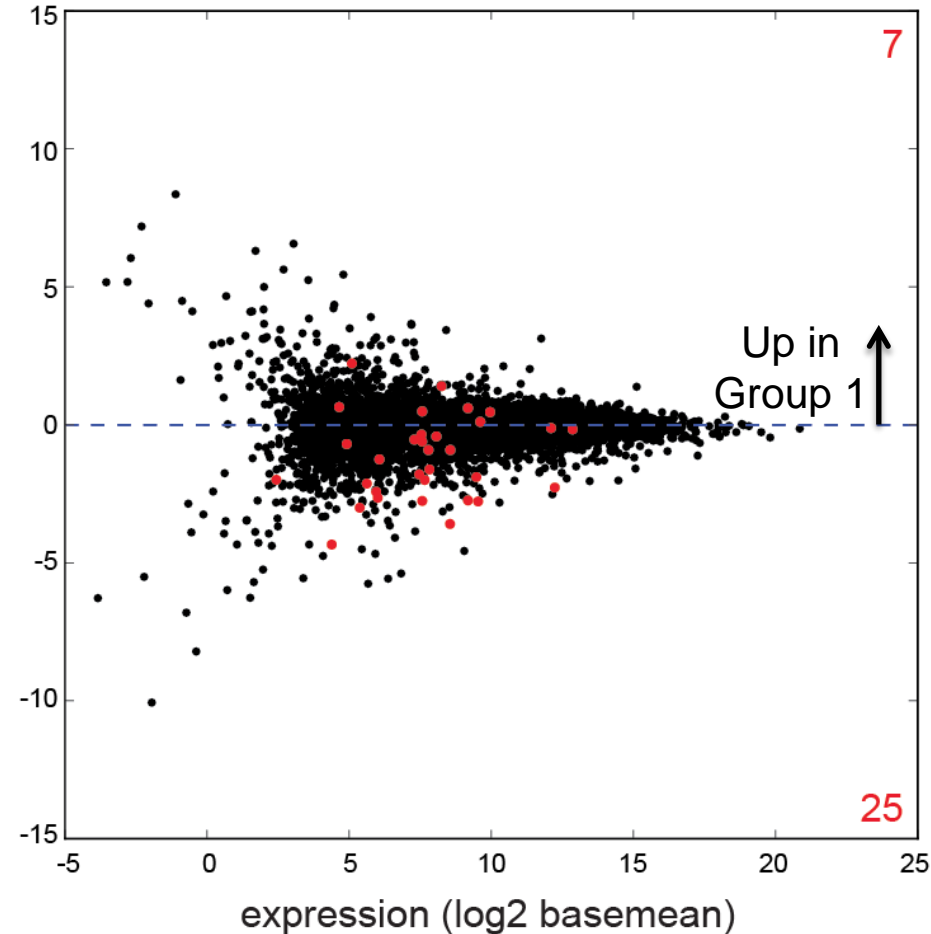
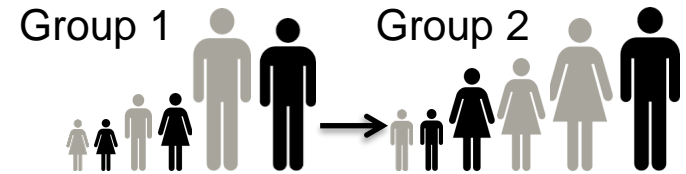
**Protocol:** grow cells in the lab, extract their RNA,  
then measure >16,000 RNAs with *RNA-seq* technology

# The power of inter-individual variation

Two Disomic Males

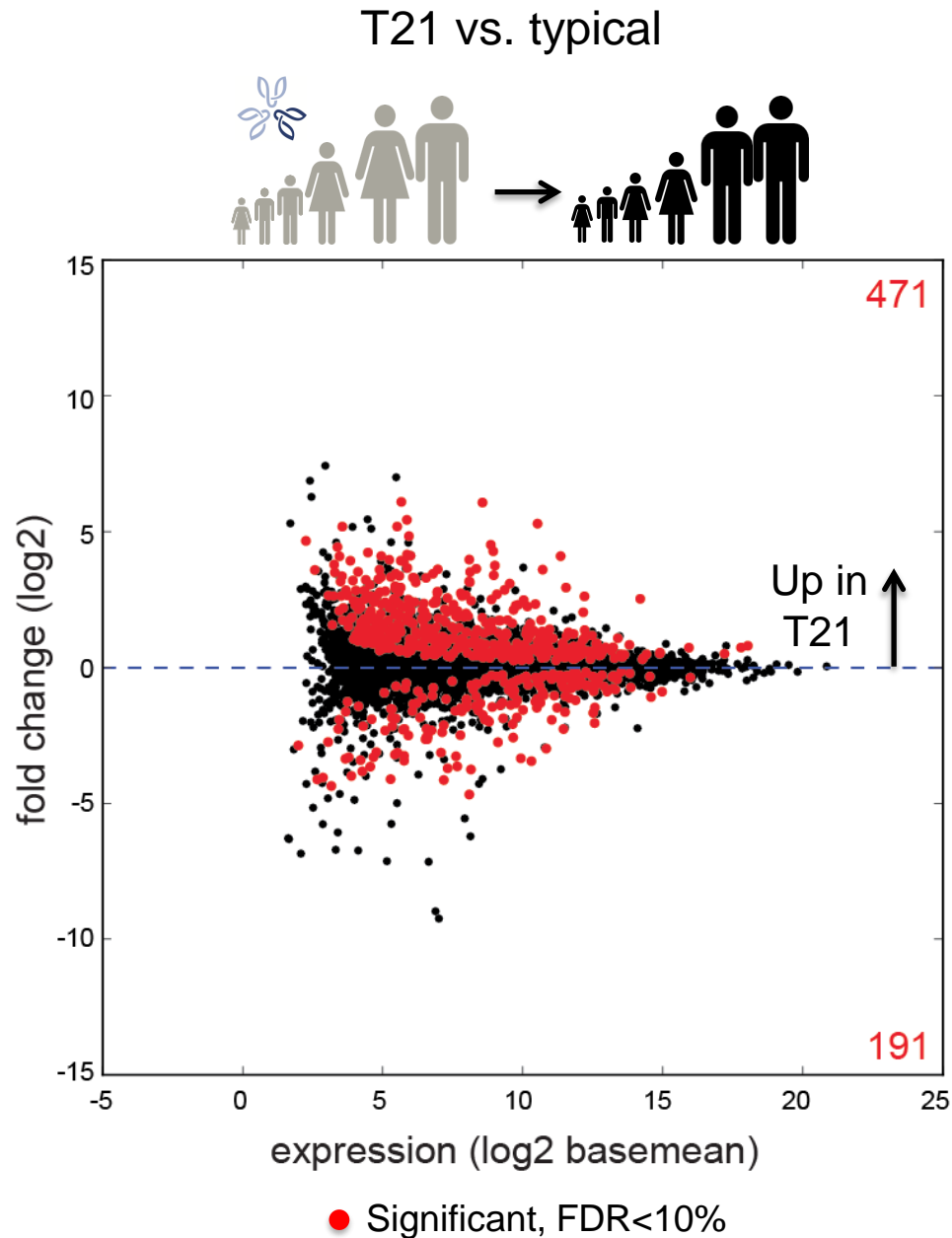


Control Grouping

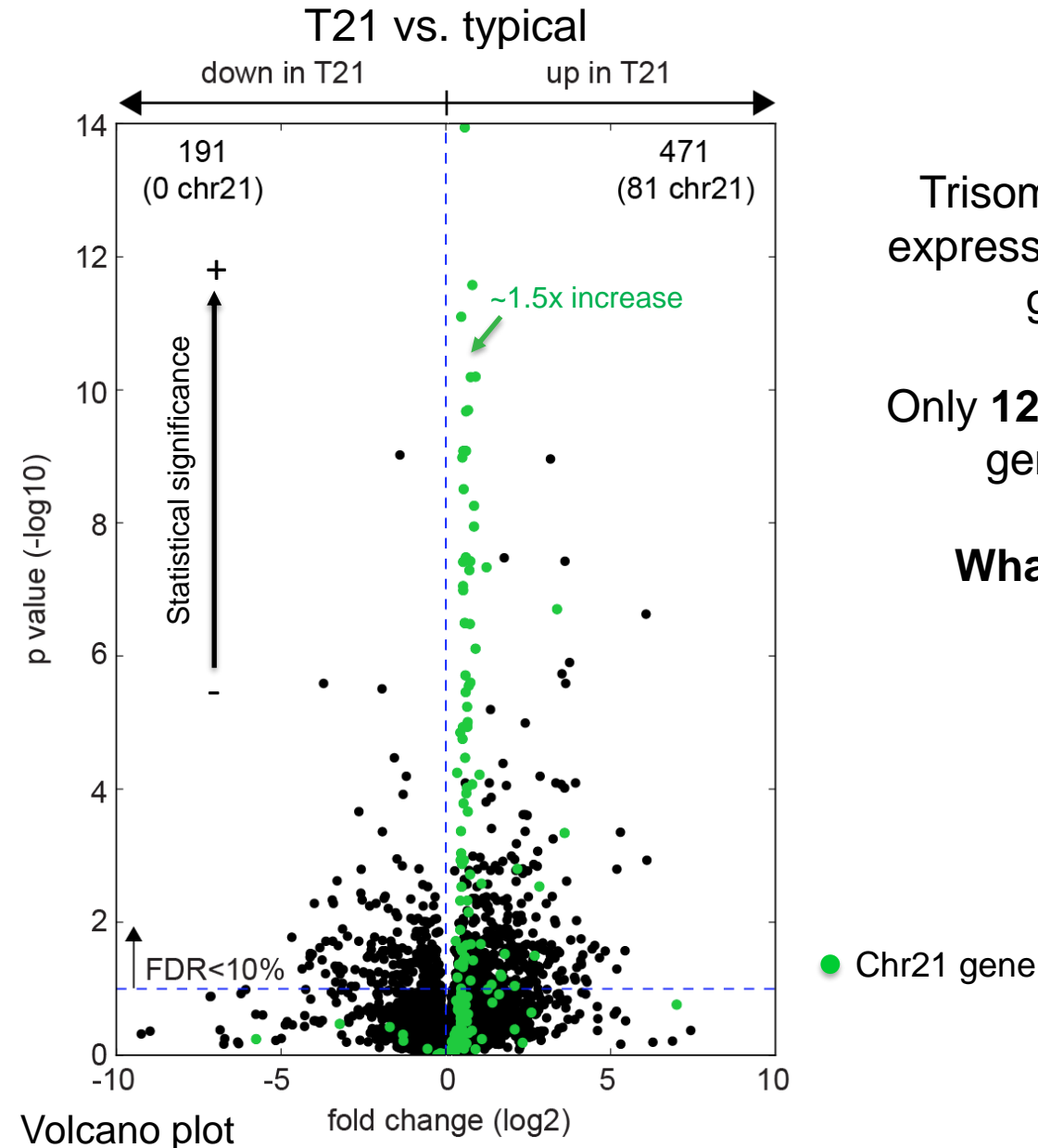


● Significant, FDR<10%

# Trisomy 21 causes consistent changes in RNA abundance



# Trisomy 21 causes consistent changes in RNA expression that withstand variations in age, gender and site of biopsy



Trisomy 21 causes a consistent gene expression signature that withstands age, gender and site of biopsy...

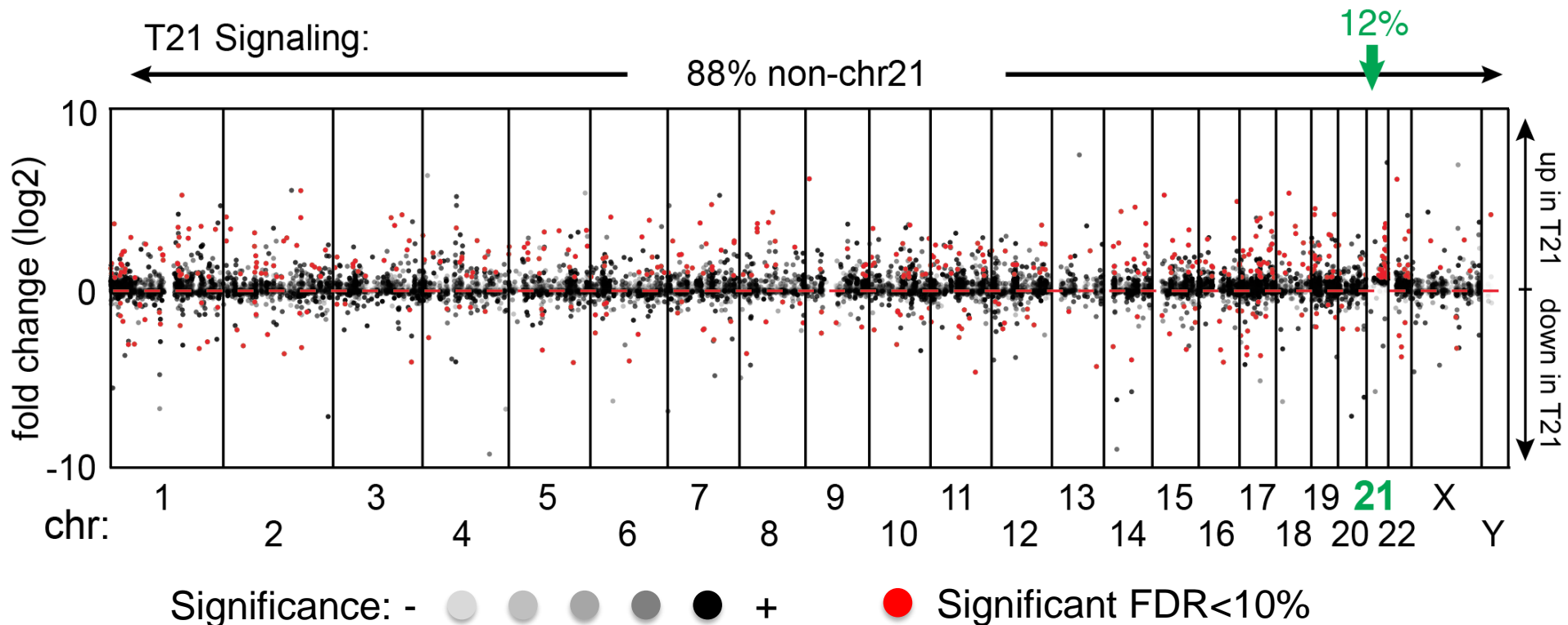
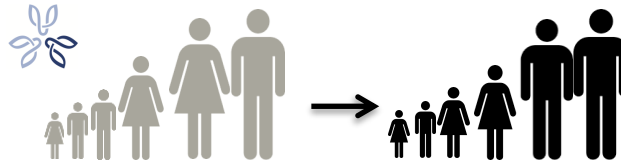
Only **12%** can be accounted by increased gene dosage due to the trisomy

**What drives the remaining 88%?**

# Signal amplification across the genome

Trisomy 21 causes a **consistent** gene expression signature (even **outside of chr21**) that withstands age, gender and site of biopsy...

T21 vs. typical

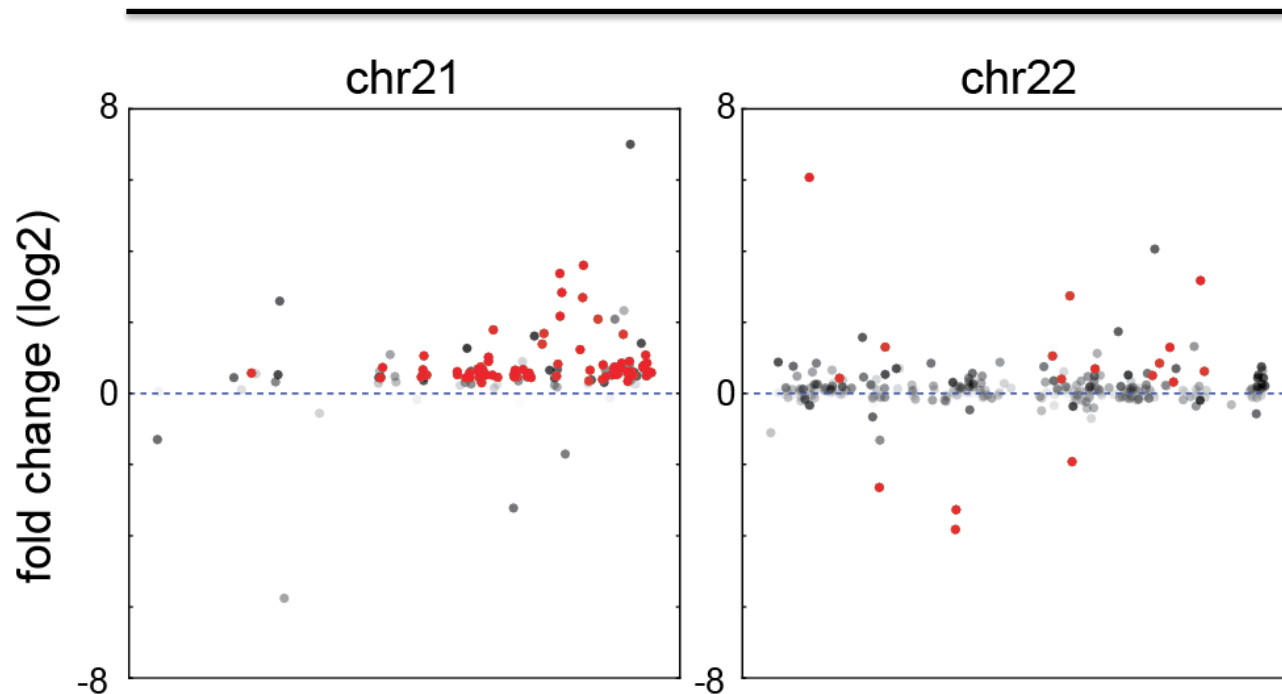


Manhattan plot

# Signal amplification across the genome

Trisomy 21 causes a **consistent** gene expression signature (even **outside of chr21**) that withstands age, gender and site of biopsy.

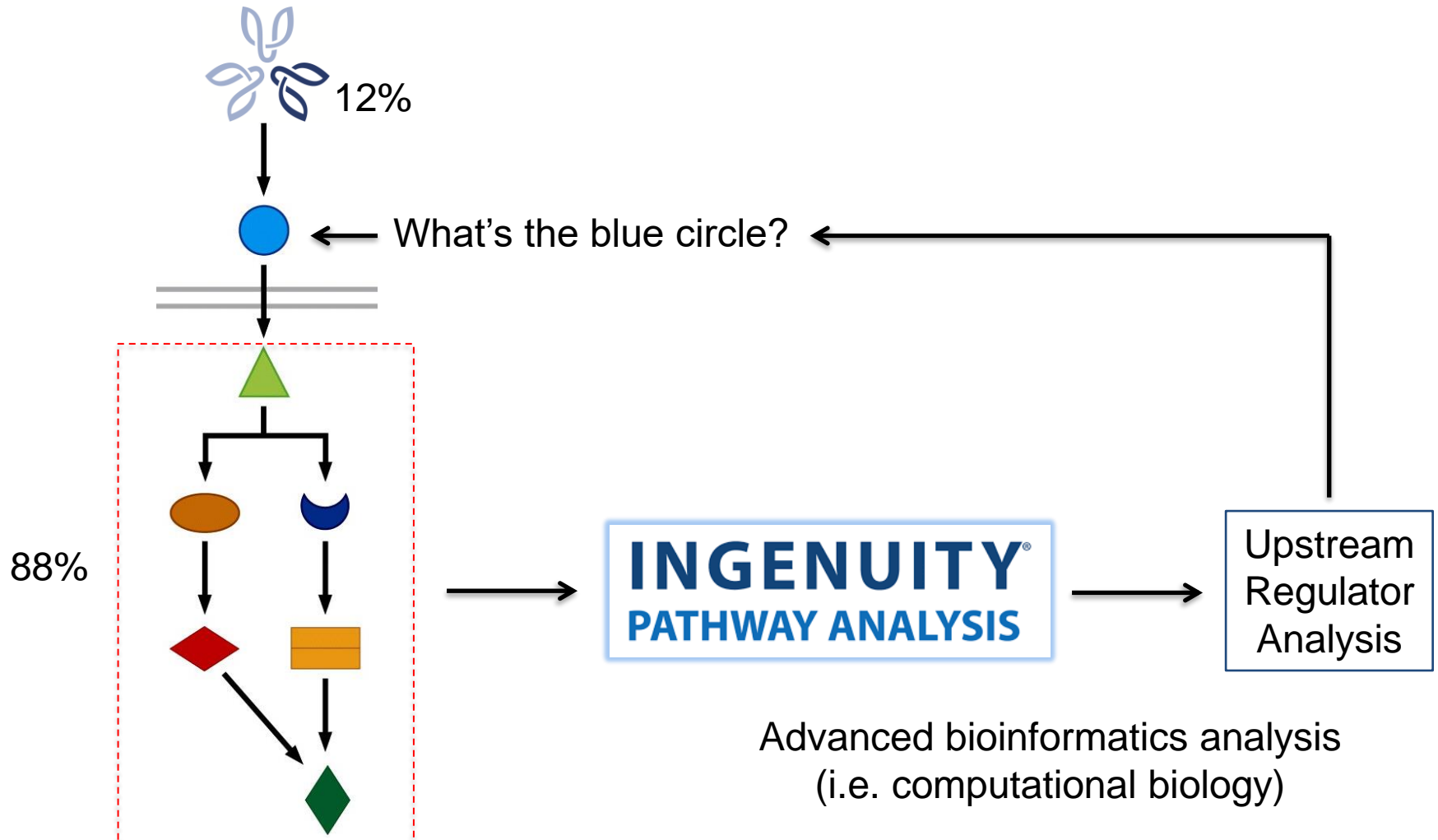
T21 vs. typical



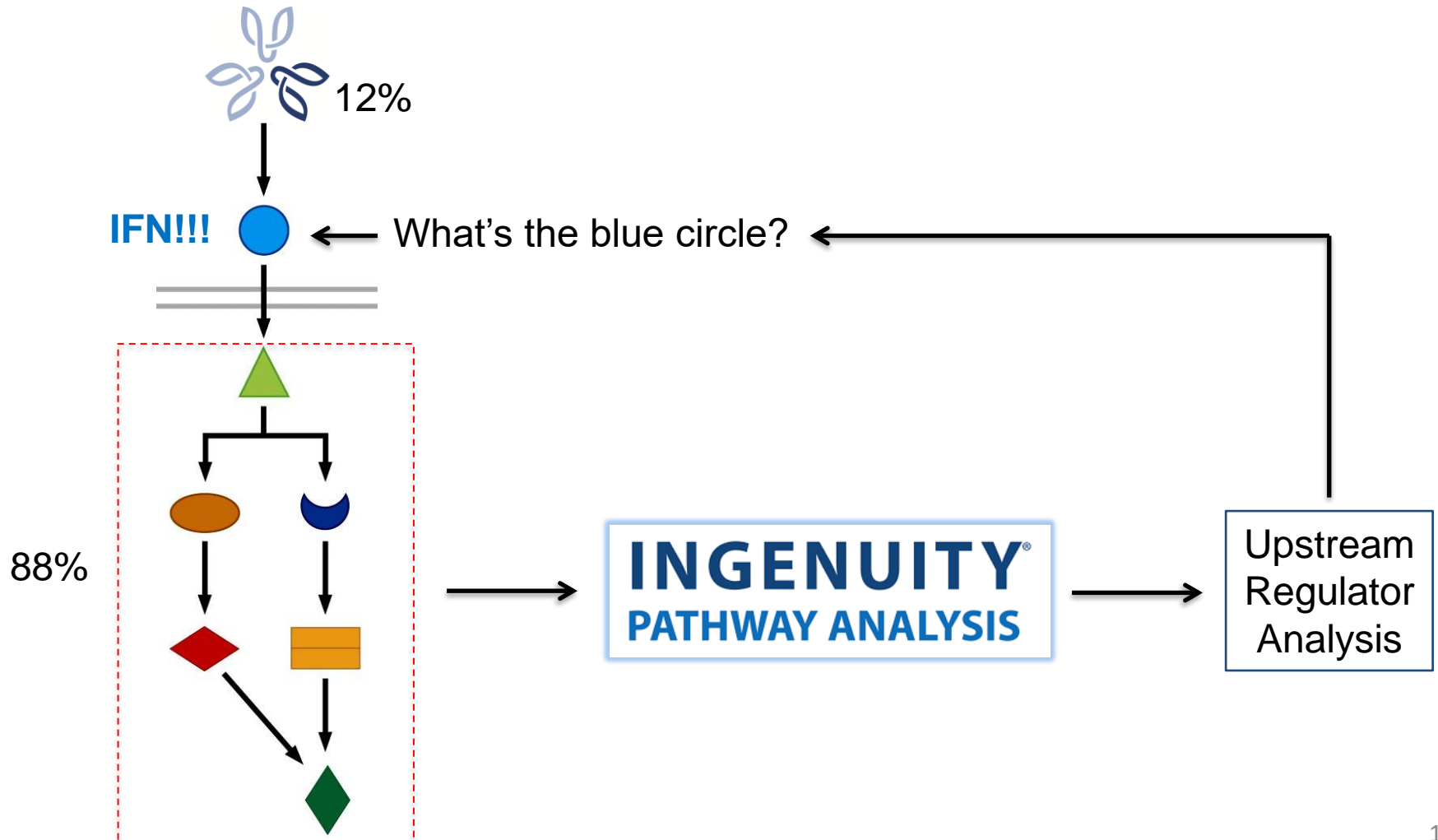
Significance: -     +  Significant FDR<10%

# What is the signal amplifier?

Upstream Regulator Analysis of the **consistent** gene expression signature activated by trisomy 21



# Upstream regulator analysis identifies the Interferon pathway (IFN) as the key mediator of the gene expression changes caused by trisomy 21





# Interferon

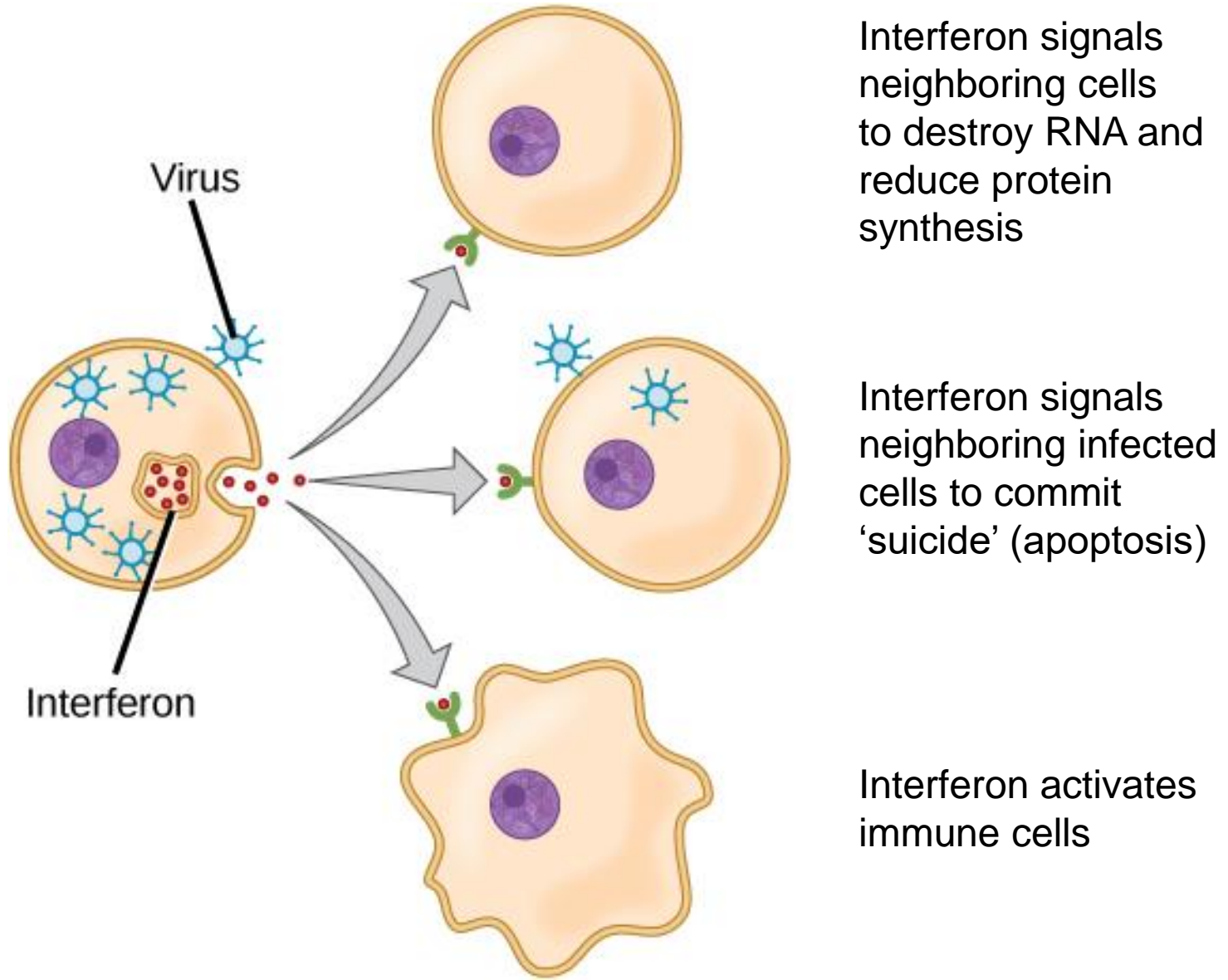
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From Wikipedia, the free encyclopedia

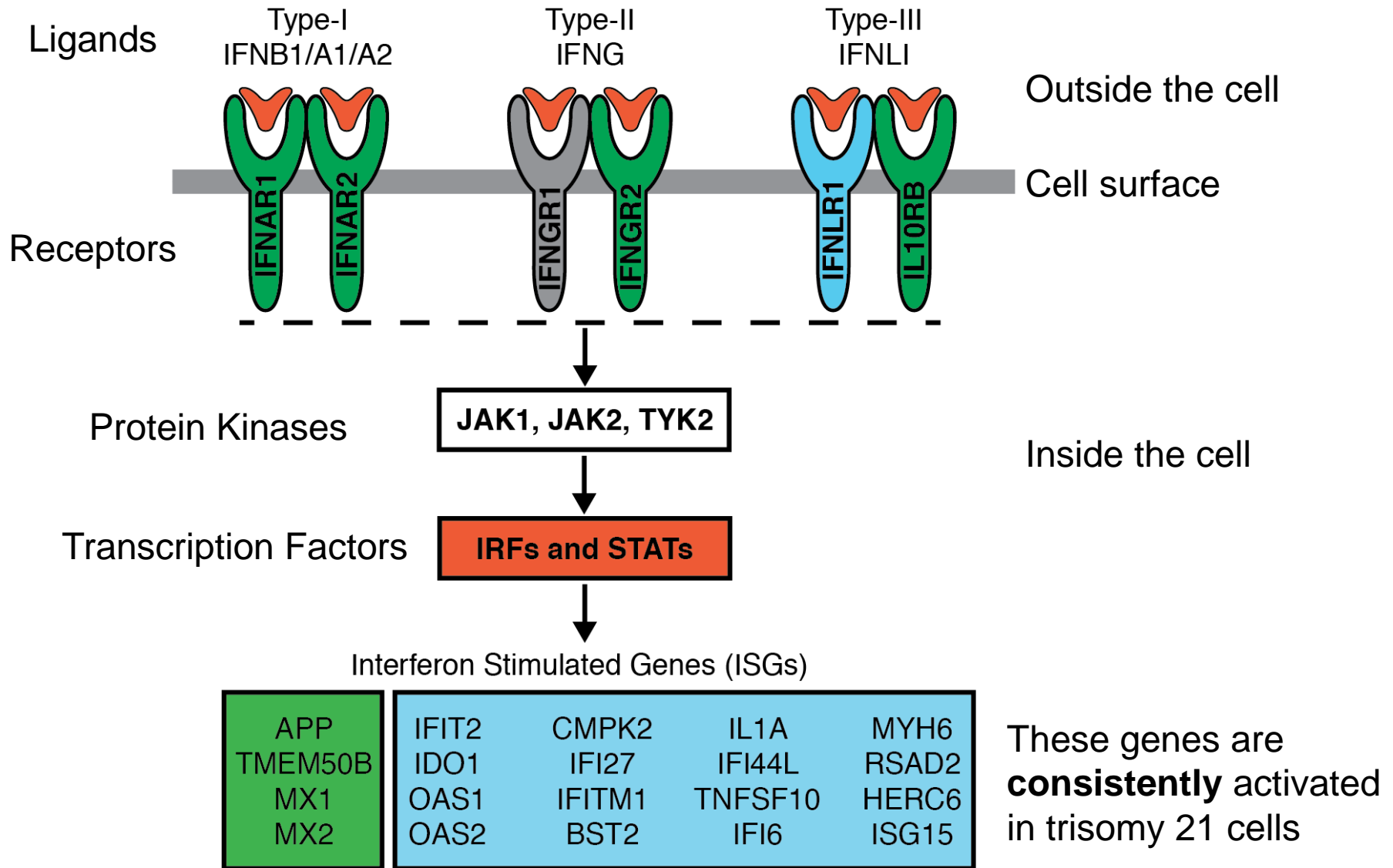
**Interferons (IFNs)** are a group of [signaling proteins](#)<sup>[1]</sup> made and released by [host cells](#) in response to the presence of several [pathogens](#), such as [viruses](#), [bacteria](#), [parasites](#), and also [tumor](#) cells. In a typical scenario, a virus-infected cell will release interferons causing nearby cells to heighten their anti-viral defenses.

IFNs belong to the large class of proteins known as [cytokines](#), molecules used for communication between cells to trigger the protective defenses of the [immune system](#) that help eradicate pathogens.<sup>[2]</sup> Interferons are named for their ability to "interfere" with [viral replication](#)<sup>[2]</sup> by protecting cells from [virus infections](#). IFNs also have various other functions: they activate [immune cells](#), such as [natural killer cells](#) and [macrophages](#); they increase host defenses by up-regulating [antigen presentation](#) by virtue of increasing the expression of [major histocompatibility complex](#) (MHC) antigens. Certain symptoms of infections, such as [fever](#), [muscle pain](#) and "flu-like symptoms", are also caused by the production of IFNs and other cytokines.

# Interferons are key components of the innate immune system

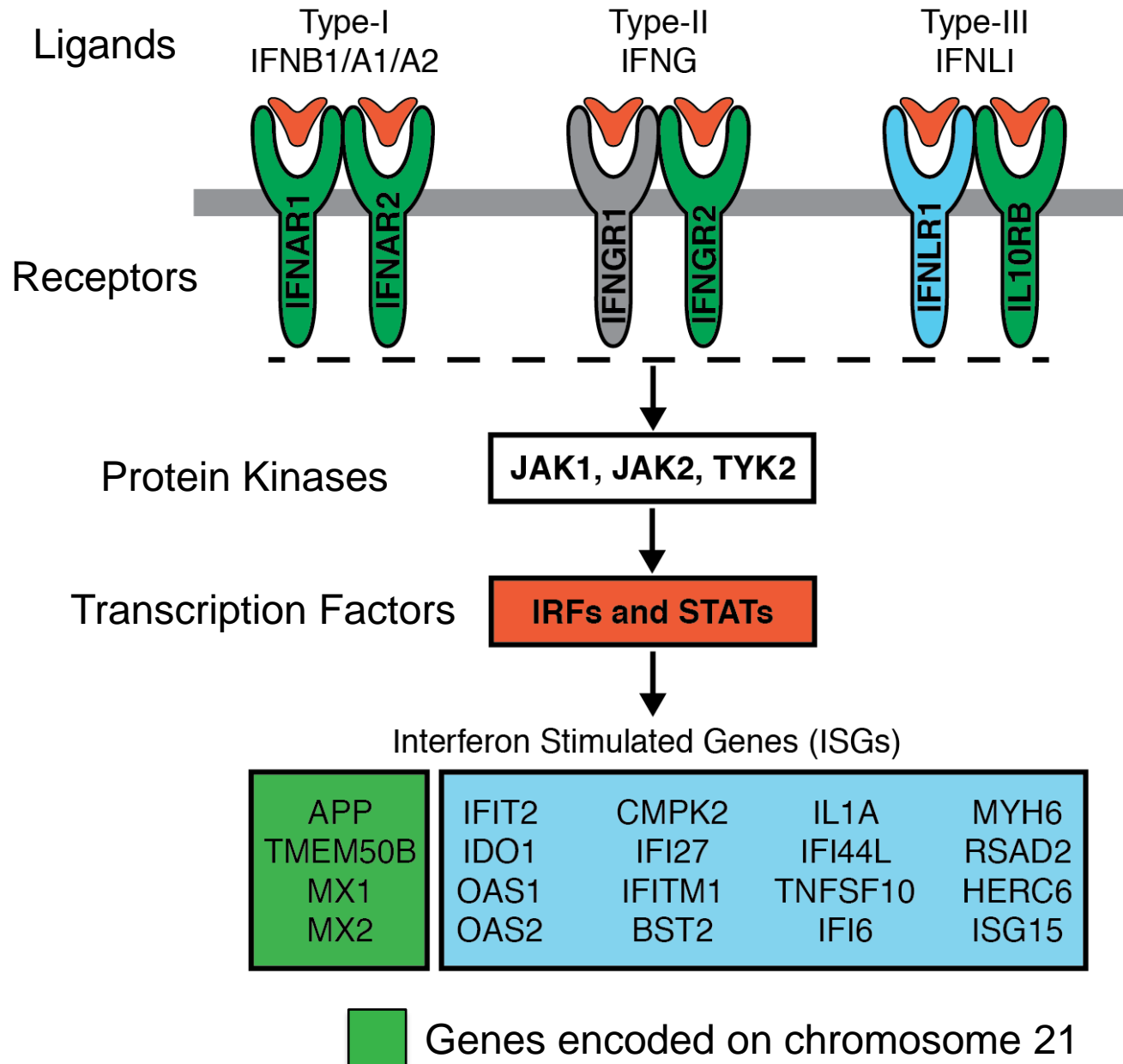


# Three types of Interferon

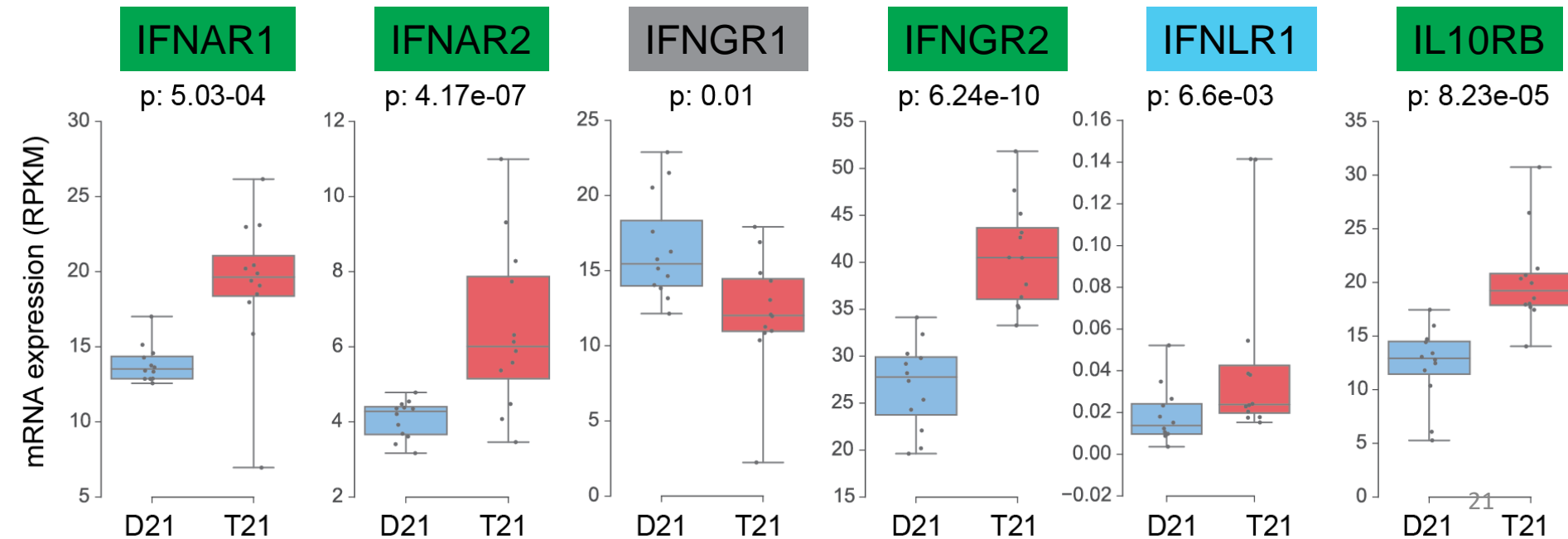
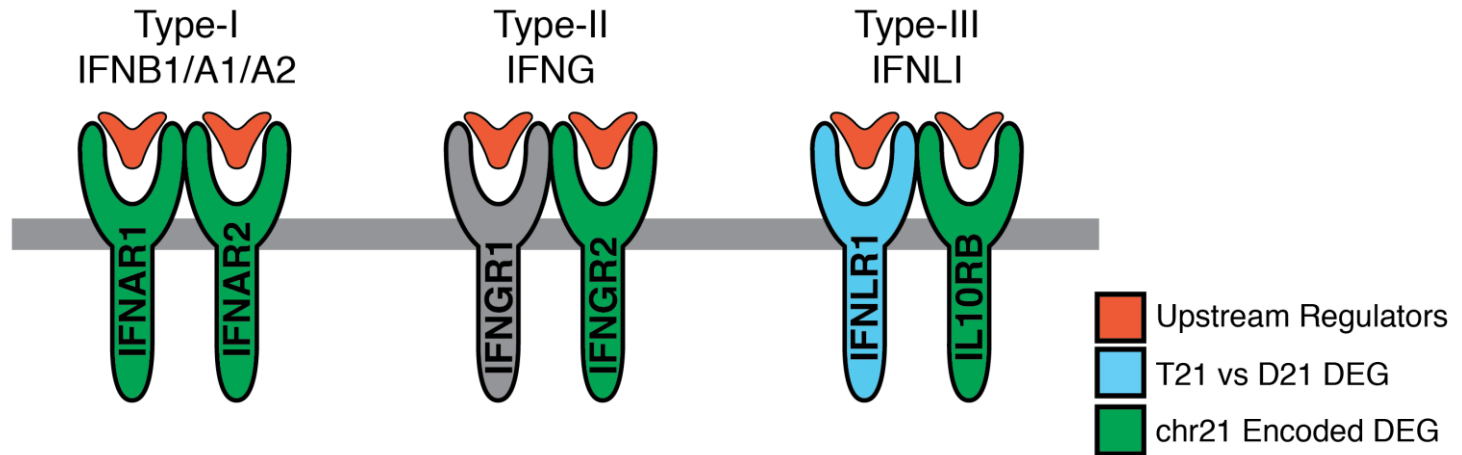


Why are **Interferon Stimulated Genes** activated in trisomy 21 cells?

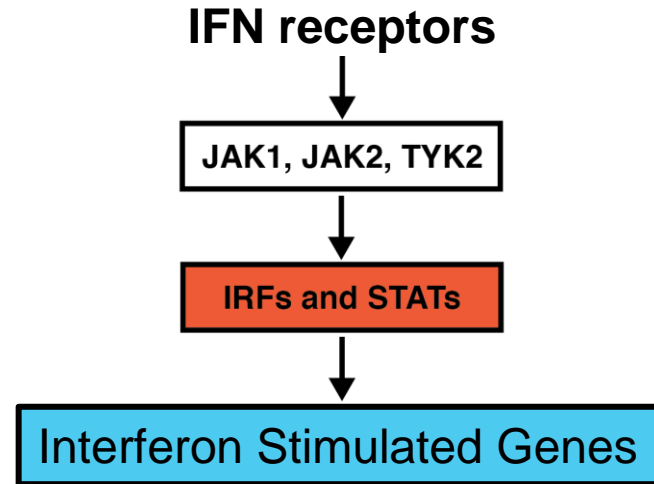
# 4 out of 6 IFN receptors are encoded on chr21!



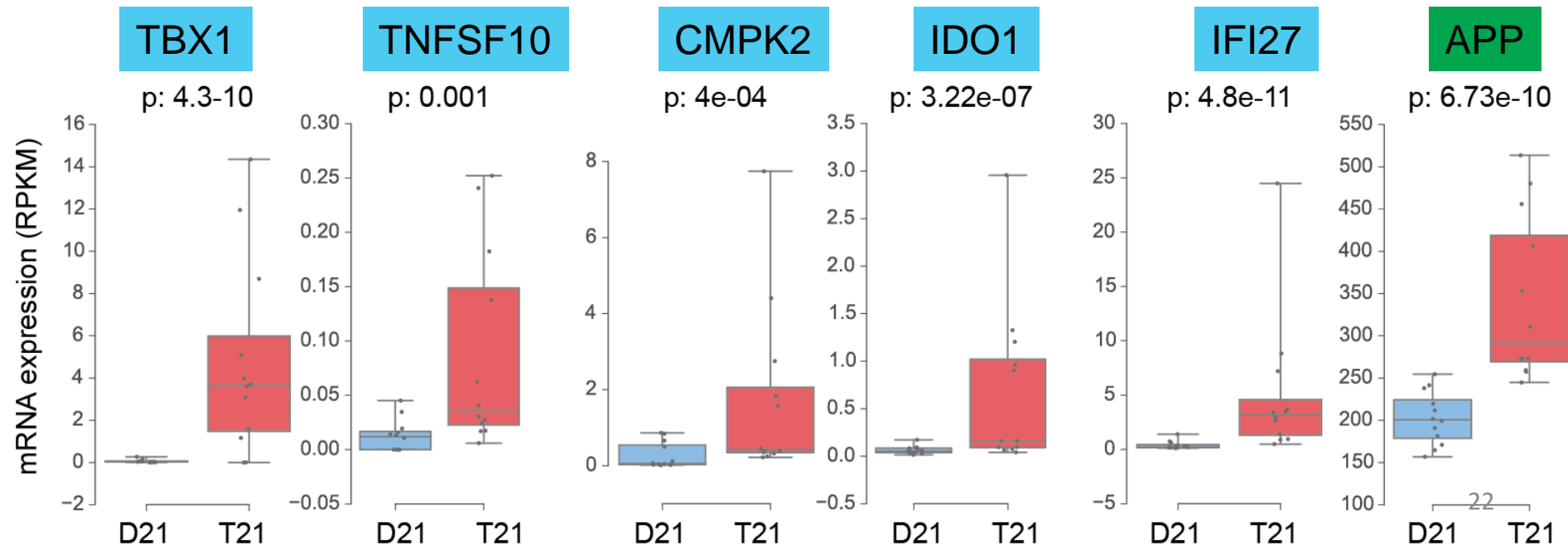
# 4 out of 6 Interferon receptors are encoded on chr21 and 'induced'



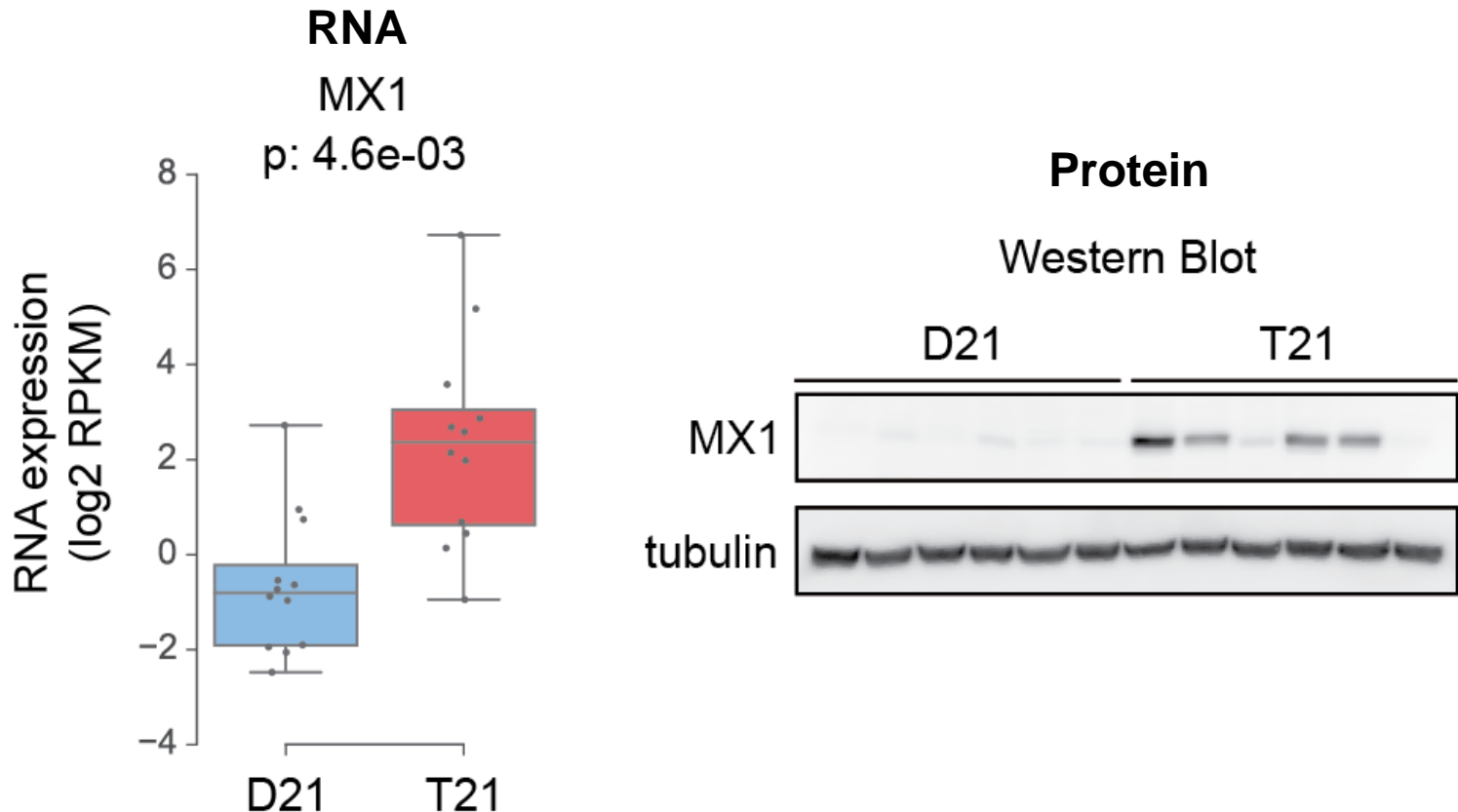
# Trisomy 21 cells show massive induction of Interferon Inducible Genes (ISGs)



- Upstream Regulators
- T21 vs D21 DEG
- chr21 Encoded DEG



# Significant inter-individual variation, even for Interferon Stimulated Genes encoded on chr21



**MX1:** Myxovirus Resistance Protein 1

An Interferon Stimulated Gene involved in the antiviral response encoded on chr21

This protein is key to fight off the flu virus

# Additional results:

- Trisomy 21 causes **consistent** activation of the Interferon pathway in lymphoblastoid B cells, monocytes and T cells (all immune cells).
- Activation of Interferon Inducible Genes is also observed at the **protein** level
- Activation of Interferon Inducible Genes is also observed in 'Down syndrome' mice
- Activation of Interferon Inducible Genes is also observed in **brain samples** from both individuals with Down syndrome and Down syndrome mice



# Implications

Down Syndrome could be classified as an Interferonopathy, along with other genetic conditions caused by hyper-activation the Interferon pathway.

The ill effects of Down Syndrome could be ameliorated, even perhaps eliminated, with available inhibitors of the IFN pathway.

# Jobyna

4 years old



- Severe neurological dysfunction
- Severe developmental delay
- Less white matter in the brain
- Seizures
- Cerebellar atrophy
- Spastic diplegia, a form of cerebral palsy (CP), a chronic neuromuscular condition of hypertonia and spasticity
- Dystonic posturing
- Hyper- or hypotonia
- Profound psychomotor difficulties
- Thrombocytopenia (deficiency of platelets)
- CSF lymphocytosis (too many white blood cells in the spinal fluid)
- Systemic immune abnormalities, strong predisposition to autoimmunity
- Hypocomplementia
- Common skin lesions (e.g. acrosyanosis)

Jobyna  
4 years old



Aicardi-Goutieres Syndrome (AGS)

AGS is a 'Type I Interferonopathy'

What is an Interferonopathy?

Interferonopathies are a group of genetic disorders characterized by upregulation of the Interferon response

What if Down syndrome is also an  
Interferonopathy?

To what degree the increased  
dosage of 4 Interferon receptors  
contributes to the various aspects of  
Down syndrome?

# Five lines of evidence from the literature

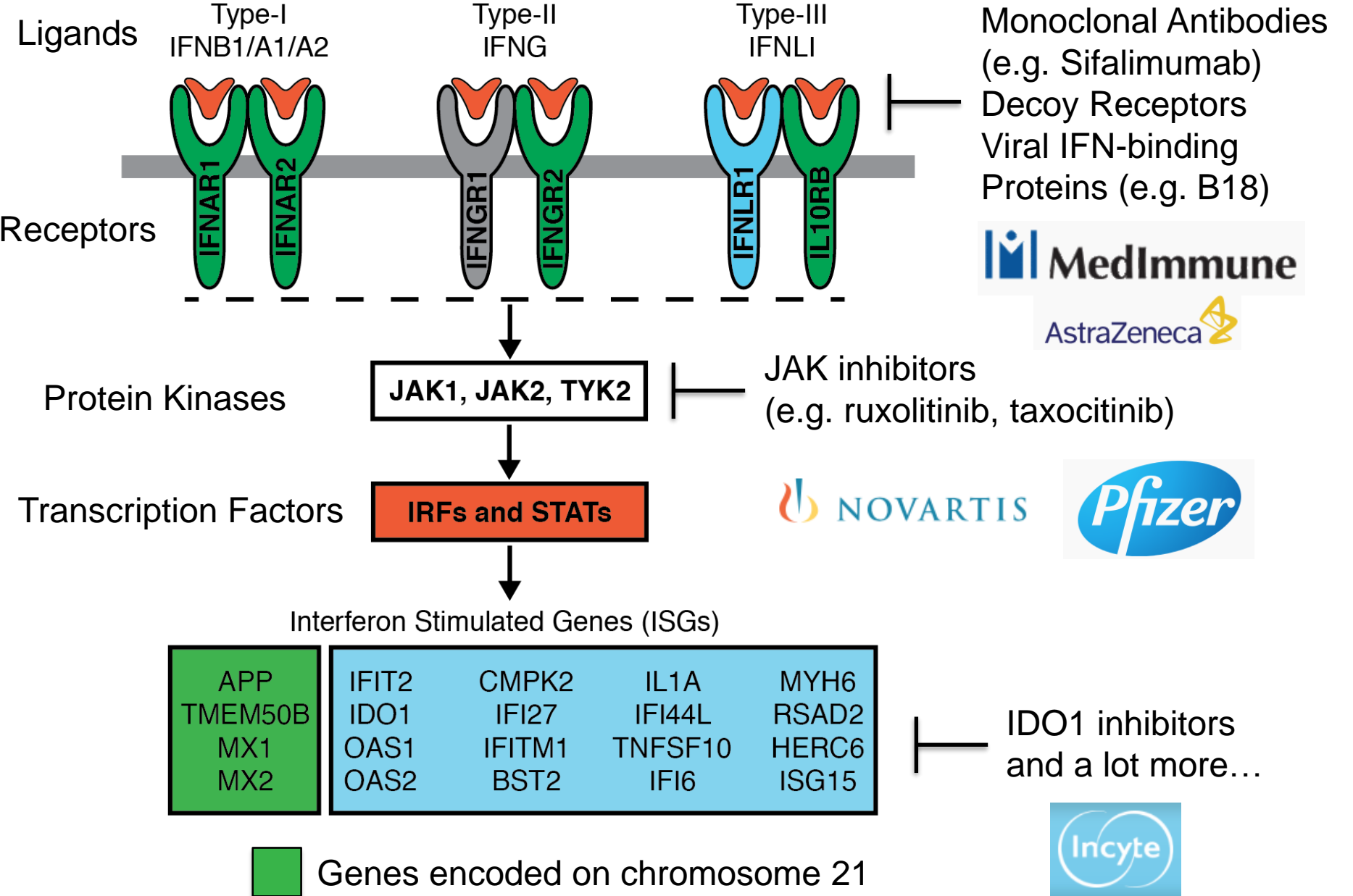
1. Cells from people with trisomy 21 are hyper-sensitive to Interferons (Tan, Epstein and Ruddell, 1970's)
2. In mouse models of Down syndrome, treatment with anti-Interferons, or reduction of the copy number for IFN receptors, decreases the ill effects of the trisomy (Dr. Maroun's work in the 1980's and 1990's)
3. If you give typical people Interferons, they start showing symptoms associated with Down syndrome (20 decades of research on the side effects of Interferon treatment).

# **Five lines of evidence from the literature**

4. If a person is born with an extra copy of interferon receptors, but without trisomy 21, they develop Down syndrome (segmental duplication of a short fragment of chr21 reported by Wensfeld-Adams et al, Genomics Medicine 2016)

**5. Drugs that block Interferon cure some of the conditions associated with Down syndrome!**

# Blocking the Interferon pathway with available drugs



# Alopecia areata, treated with Interferon antagonists

Alopecia Areata (autoimmune hair loss) is one of the many autoimmune conditions more prevalent in people with trisomy 21



baseline



3 months



4 months

Ruxolitinib: An FDA-approved JAK inhibitor

*Clynes et al, Nature Medicine 2014*



# Future Directions

To fully dissect the role of hyperactive Interferon signaling in the development of Down syndrome.

To define the therapeutic potential of Interferon antagonists to ameliorate, or even perhaps eliminate, the ill effects of trisomy 21.

# Credits

**Kelly  
Sullivan**



**Ahwan  
Pandey**



**Amanda  
Hill**



**Hannah  
Lewis**



Keith Smith, Matthew Galbraith, Zdenek Andrysik, James DeGregori, Alexander Ligget,  
Eliana Gomez, Leisa Jackson, Joseph Cabral

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# Upcoming publication



eLIFE

elifesciences.org

## Trisomy 21 consistently activates the interferon response

**Kelly D Sullivan<sup>1,2,3,4\*</sup>, Hannah C Lewis<sup>1,2</sup>, Amanda A Hill<sup>1,2</sup>, Ahwan Pandey<sup>1,2,3,4</sup>,  
Leisa P Jackson<sup>1,3,4</sup>, Joseph M Cabral<sup>1,3,4</sup>, Keith P Smith<sup>1</sup>, L Alexander Liggett<sup>1,5</sup>,  
Eliana B Gomez<sup>1,3,4</sup>, Matthew D Galbraith<sup>1,2,3,4</sup>, James DeGregori<sup>1,5,6,7,8,9</sup>,  
Joaquín M Espinosa<sup>1,2,3,4\*</sup>**

<https://elifesciences.org/content/5/e16220>