UNIVERSITÀ DEGLI STUDI DI TRIESTE

An isoform of Dicer protects mammalian stem cells against multiple RNA viruses

Presented by Durante Tommaso, Klinger Maximilian

ANTIVIRAL DEFENSE

An isoform of Dicer protects mammalian stem cells against multiple RNA viruses

Enzo Z. Poirier[1]*, Michael D. Buck[1], Probir Chakravarty[2], Joana Carvalho[3]†, Bruno Frederico[1], Ana Cardoso[1], Lyn Healy[4], Rachel Ulferts[5], Rupert Beale[5,6], Caetano Reis e Sousa[1]*

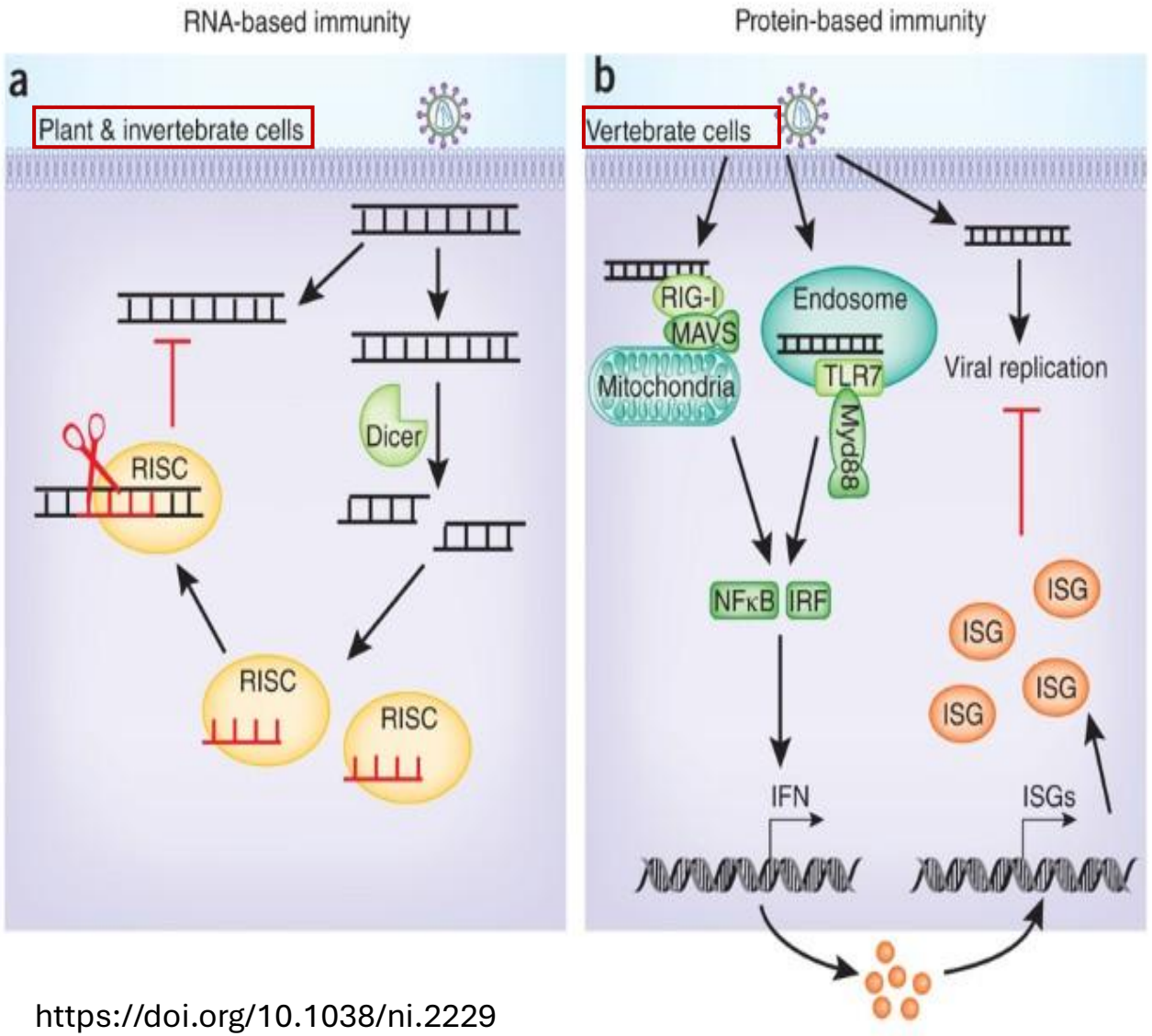
In mammals, early resistance to viruses relies on interferons, which protect differentiated cells but not stem cells from viral replication. Many other organisms rely instead on RNA interference (RNAi) mediated by a specialized Dicer protein that cleaves viral double-stranded RNA. Whether RNAi also contributes to mammalian antiviral immunity remains controversial. We identified an isoform of Dicer, named antiviral Dicer (aviD), that protects tissue stem cells from RNA viruses—including Zika virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—by dicing viral double-stranded RNA to orchestrate antiviral RNAi. Our work sheds light on the molecular regulation of antiviral RNAi in mammalian innate immunity, in which different cell-intrinsic antiviral pathways can be tailored to the differentiation status of cells.

PRESENTATION OUTLINE

1. THE «ANTIVIRAL PARADOX» IN MAMMALS
2. ANTIVIRAL RESPONSE IN MAMMALIAN STEM CELLS
3. ARTICLE PRESENTATION

THE «ANTIVIRAL PARADOX» IN MAMMALS

Cell intrinsic antiviral mechanisms in animals and plants



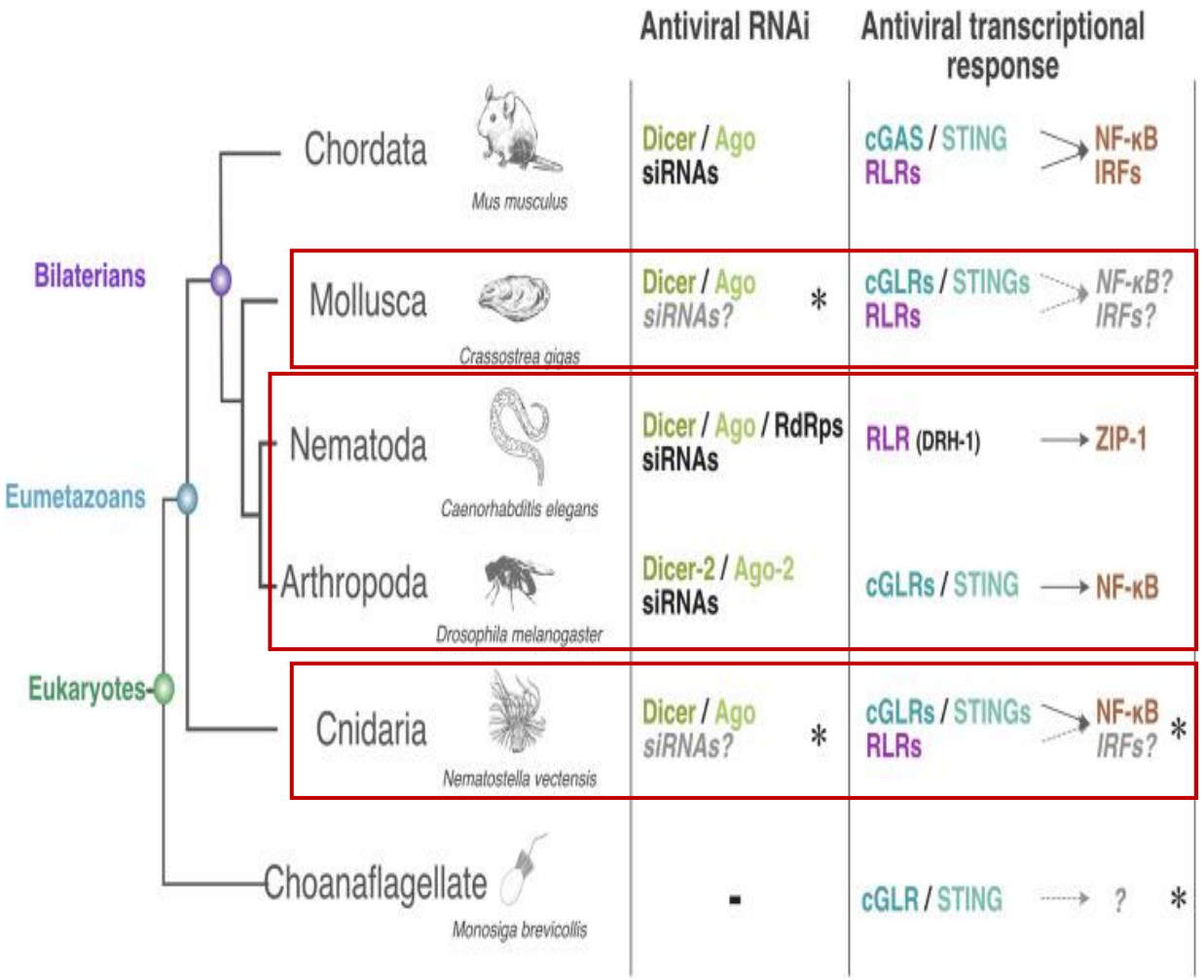
https://doi.org/10.1038/ni.2229

1. **Cell-intrinsic antiviral mechanisms** are part of the innate immune system and include:
   1. **RNA-based**
   2. **PROTEIN-based (transcriptional response)**

2. **Vertebrate= IFN response?**
3. **Invertebrate= RNAi?**

OLD BELIEF

In reality, almost all animals possess both RNA-based and a protein-based response

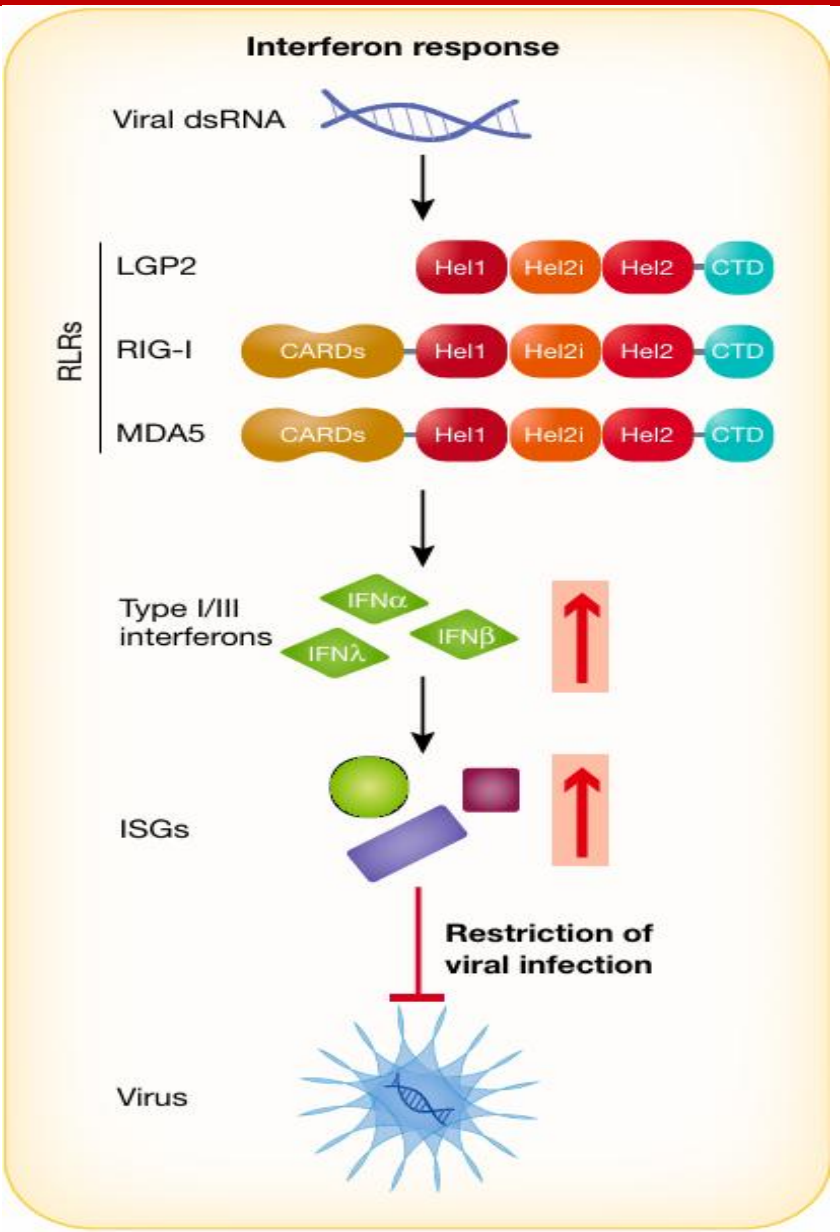


- **C. elegans** and **D. melanogaster** do **not have clear homologs to classical ISGs** and **IRFs.**

- **Cnidarians** and **mollusks** have an an **IFN-like response:**
  - Have genes with **homology** to classical **mammalian ISGs**.
  - Driven by **transcription factors** of the **IRF family**

https://doi.org/10.1016/j.celrep.2024.114678

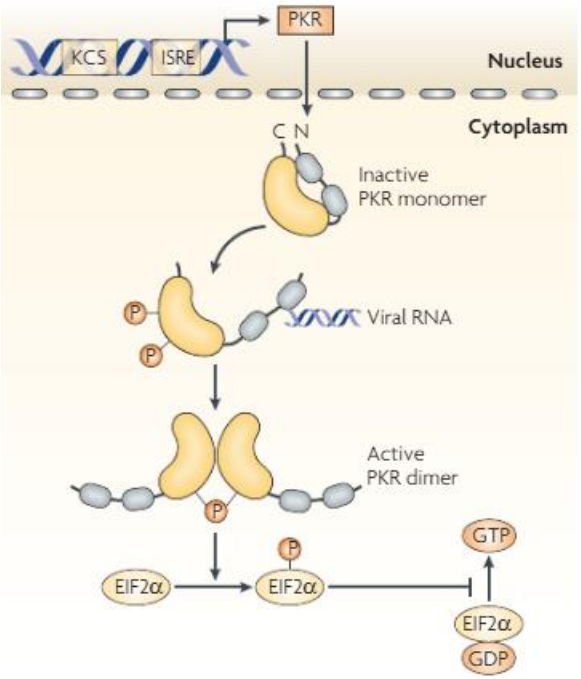
The interferon response triggered by dsRNA in vertebrates

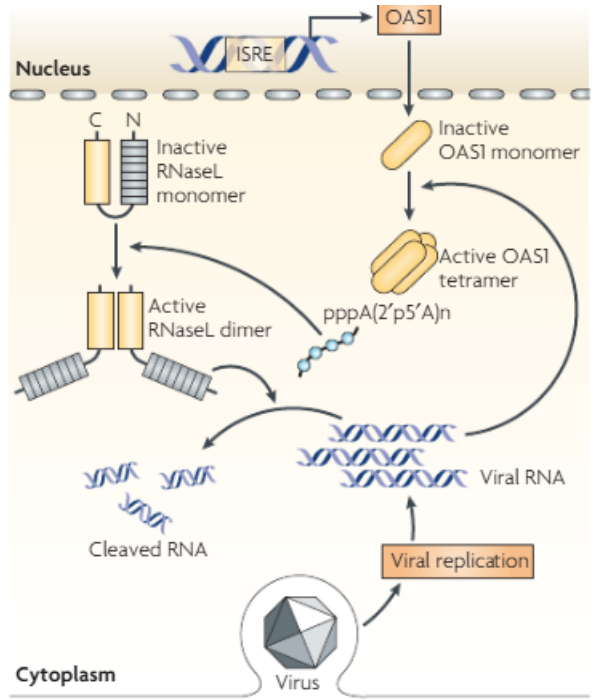


1. Characteristic of **vertebrate cells.**
2. Triggered by **dsRNA** and **other nucleic acids structures** associated with viral infection.
3. Their recognition leads to the **release of specific cytokines (interferons)**, which act in an **autocrine** and **paracrine** manner to establish an **antiviral state.**
4. This **antiviral state** is established by the signalling cascade induced by interferons which activate the **«Interferon stimulated genes (ISGs)».**

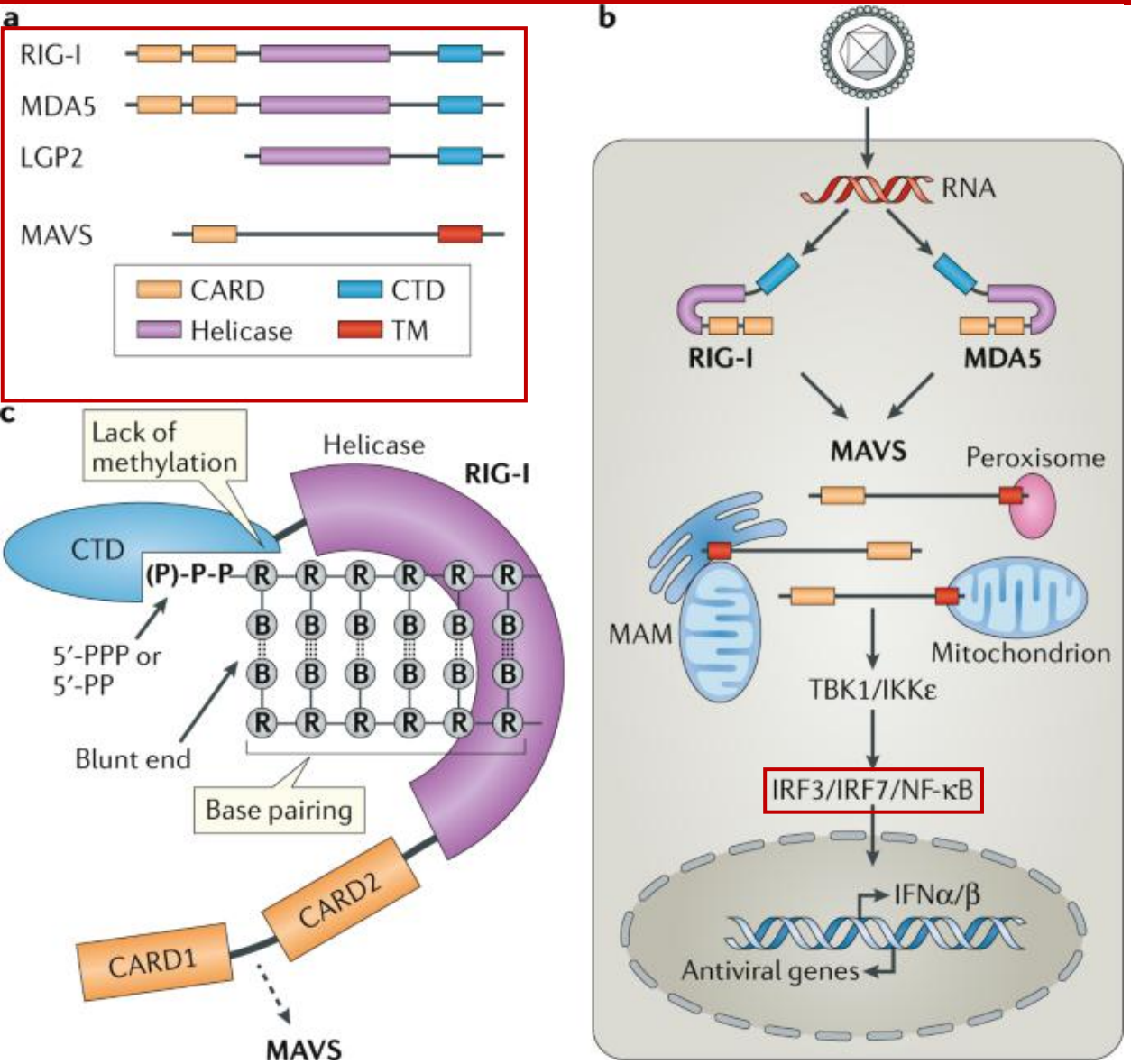
- **ISG examples:**

1) PKR
2) RNaseL





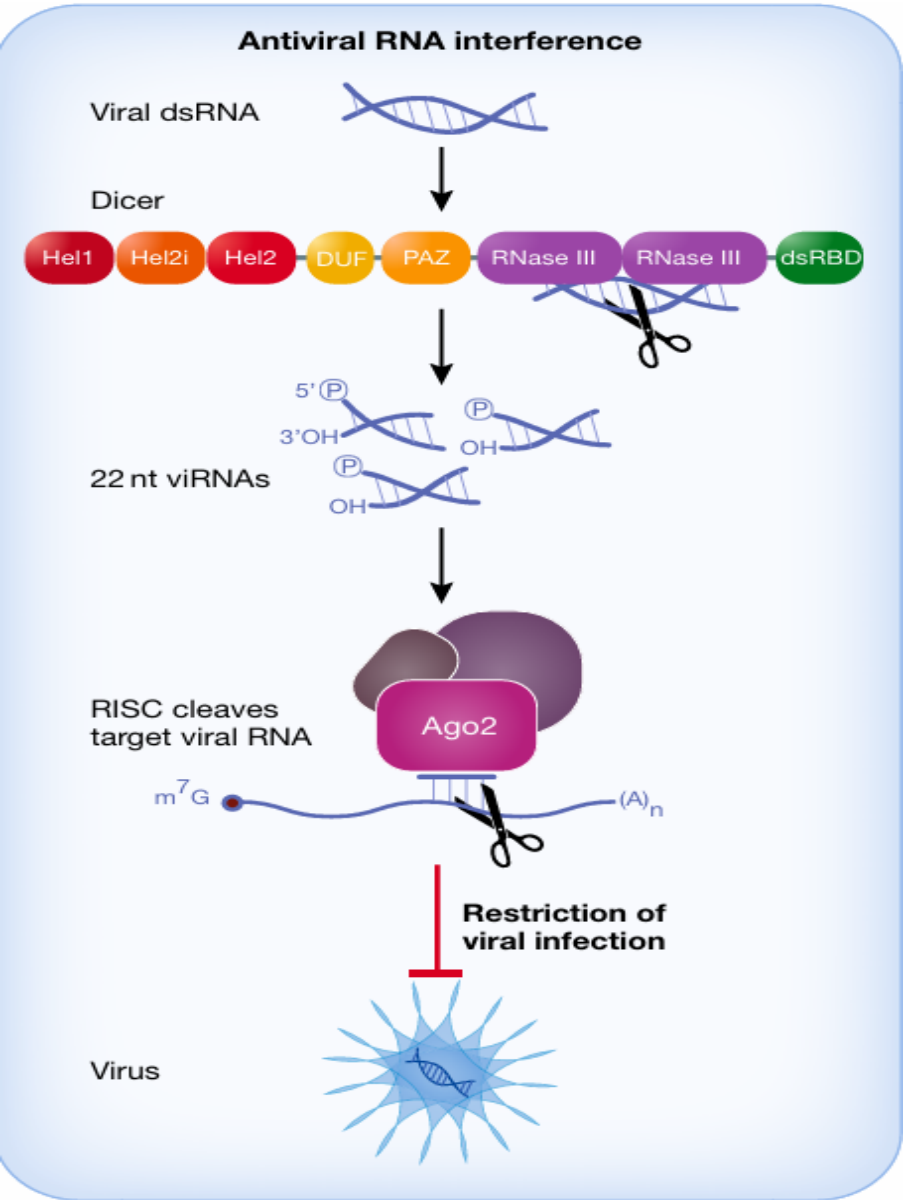
RIG-I like receptors are involved in the recognition of nucleic acids



https://doi.org/10.1038/s41577-020-0288-3

1. **RIG-I like receptors (RLRs)** (RIG-I, MDA5 and LGP2) **detect** virally derived nucleic acids.
2. **CARDs** domains of **RIG-I** and **MDA5** mediate **downstream signalling** via the adaptor protein **MAVS**
3. The signalling cascade activates the transcription factors **IRF3, IRF7** and **NFκ-B**.
4. These transcription factors drive the expression of **type I** and **type III IFNs** and can directly induce some **ISGs**
5. **LGP2** positively regulate the other RLRs receptors and **inhibits Dicer** activity

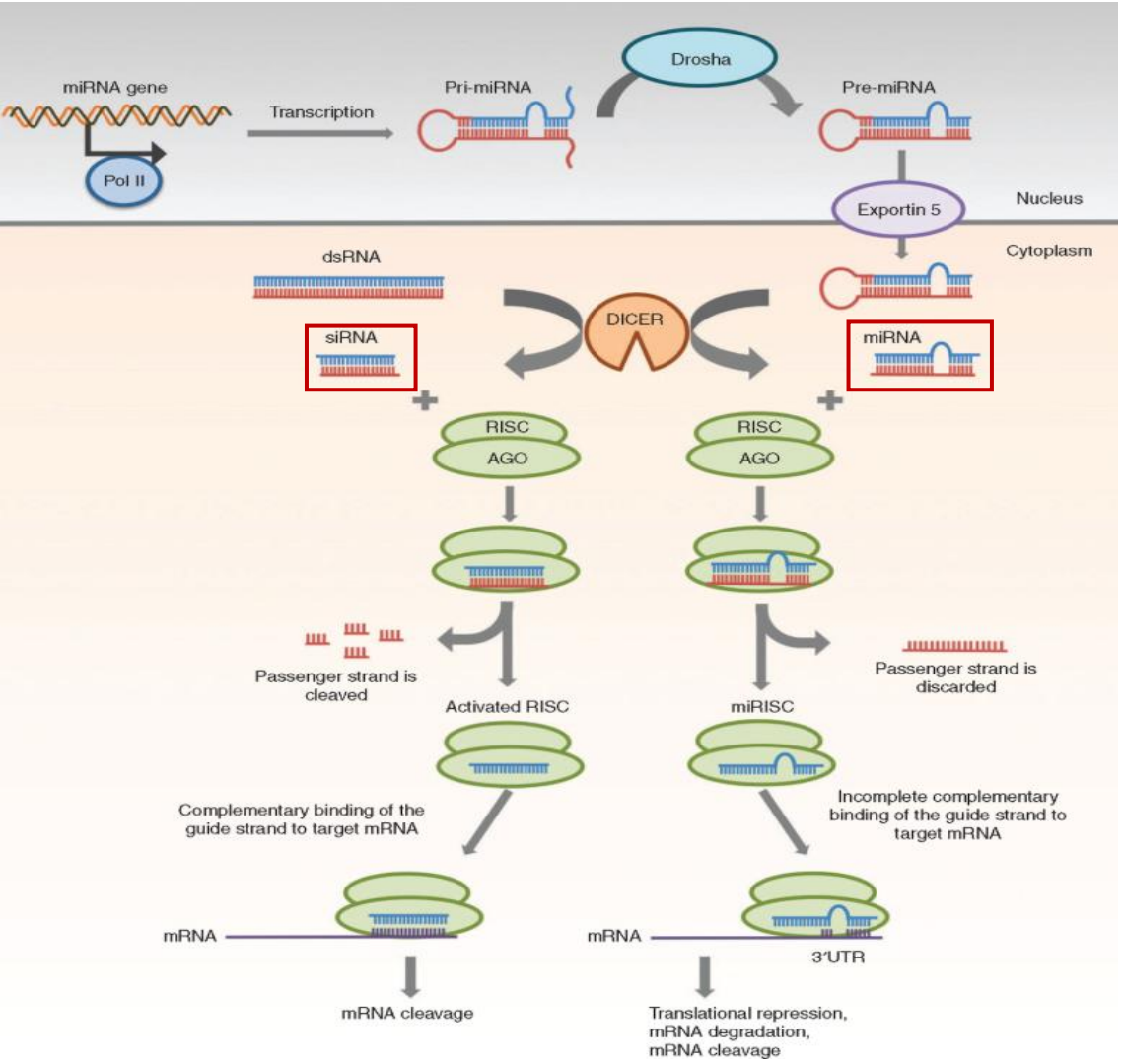
Antiviral RNA interference (RNAi)



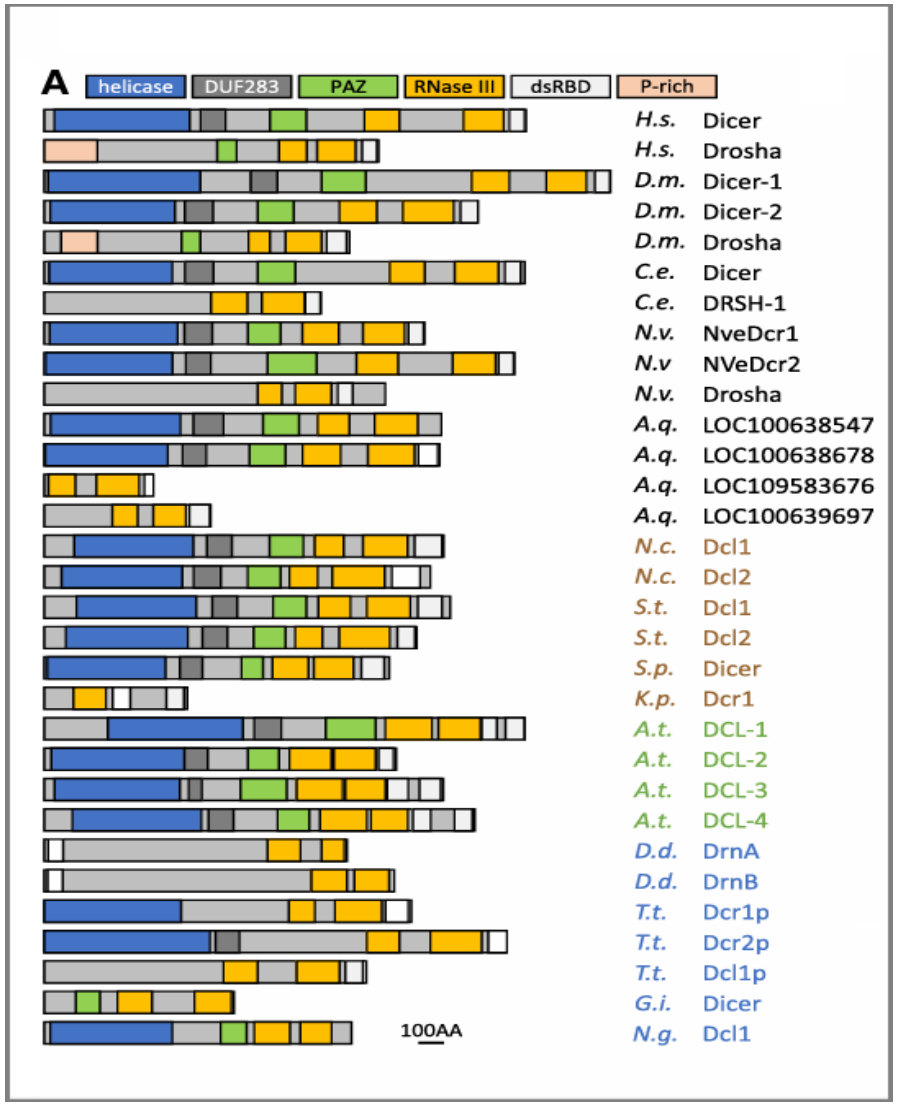
1. In RNAi, **long dsRNA** is cleaved by the type III endoribonuclease **Dicer** into small interfering RNA (**siRNAs**), RNA duplexes of
   - **21–24 nucleotides** in length,
   - with **3' 2-nt overhangs** and
   - a **5' mono-phosphate** and a **3' hydroxyl group on both strands**
2. One strand of each siRNA duplex is bound by an **Argonaute (Ago) protein**, which, together with accessory proteins, forms the **RNA-induced silencing complex (RISC)**
3. The RISC complex then mediates the **endonucleolytic cleavage** ("slicing") of complementary **target RNAs**

Dicer: function and distribution

❑ DICER FUNCTIONS:



❑ DICER across ORGANISMS:



DOI: 10.1038/mtna.2015.23

Dicer conservation in common organisms

Arabidopsis has four **«Dicer-like proteins»**

- **DCL1** involved in **miRNA** generation
- **DCL4**, **DCL2** and **DCL3** **endogenous siRNAs**, and **virally-derived siRNAs**

Drosophila has two specialized Dicer enzymes:

- **Dicer-1** involved in **miRNA** generation.
- **Dicer-2** involved in **siRNA** generation.

Mouse has two Dicer:

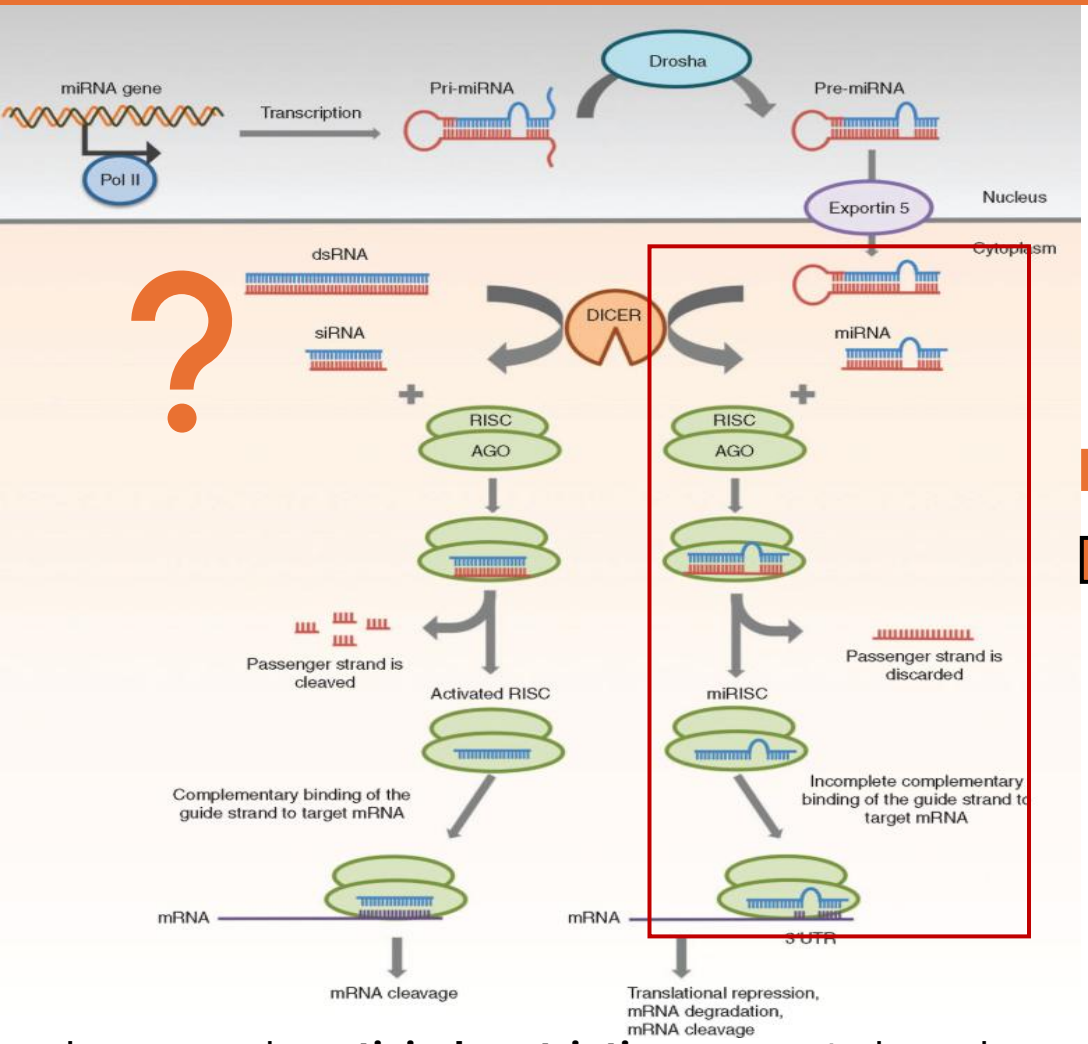
- **Dicer1** involved **miRNA** generation
- **DicerO**, a **truncated** isoform

Human:

- **Dicer1** involved in **miRNA** generation.
- A **truncated** isoform **(aviD)**, the topic of the article

siRNAs?

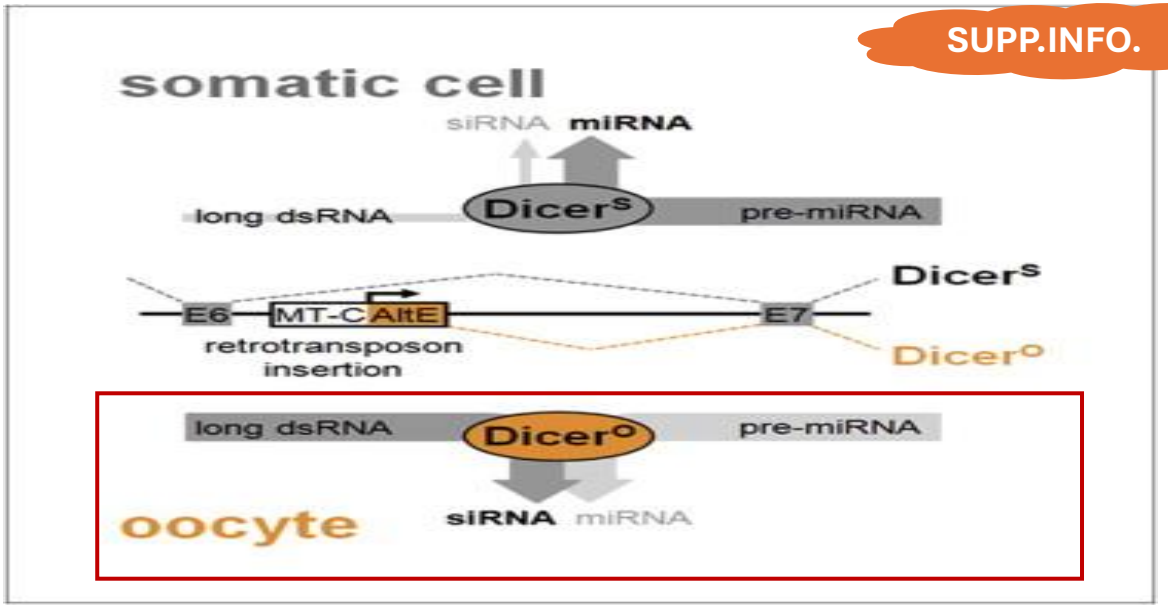
In mammals Dicer *seems* to be involved only in miRNA generation



- In mammals **antiviral restriction** seems to be only based on the **IFN response**
- **Dicer** seems to be involved **only in pre-miRNA processing**

BUT...

❑A DICER ISOFORM IN MICE

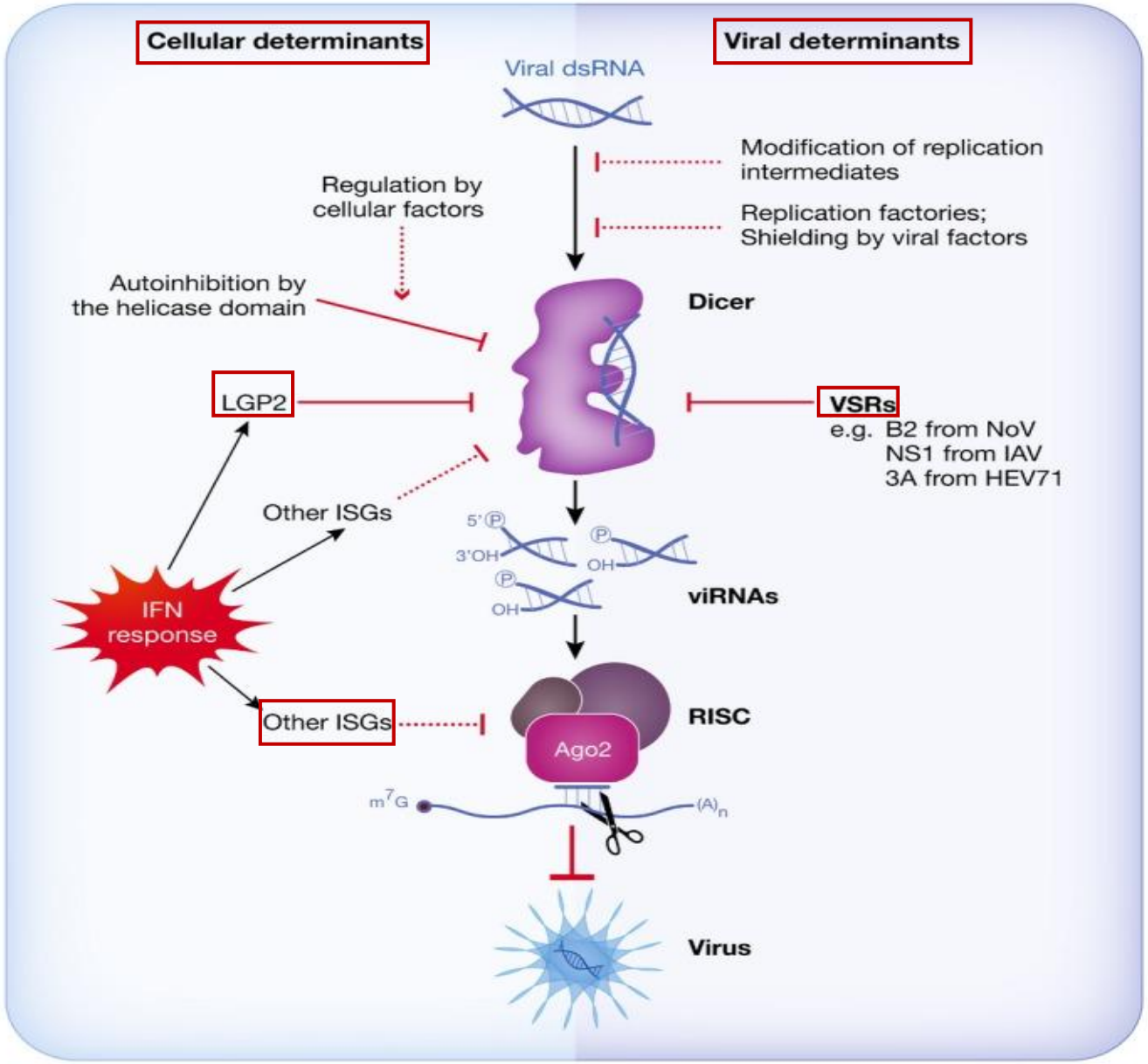


https://doi.org/10.1016/j.cell.2013.10.001

- **Active** in **mice oocytes**, but **not in somatic cells**
- **Lacks the N-terminal DExD helicase** domain
- Efficiently **produces siRNA from long dsRNA**.

- **Endo-siRNAs important for oocytes development**

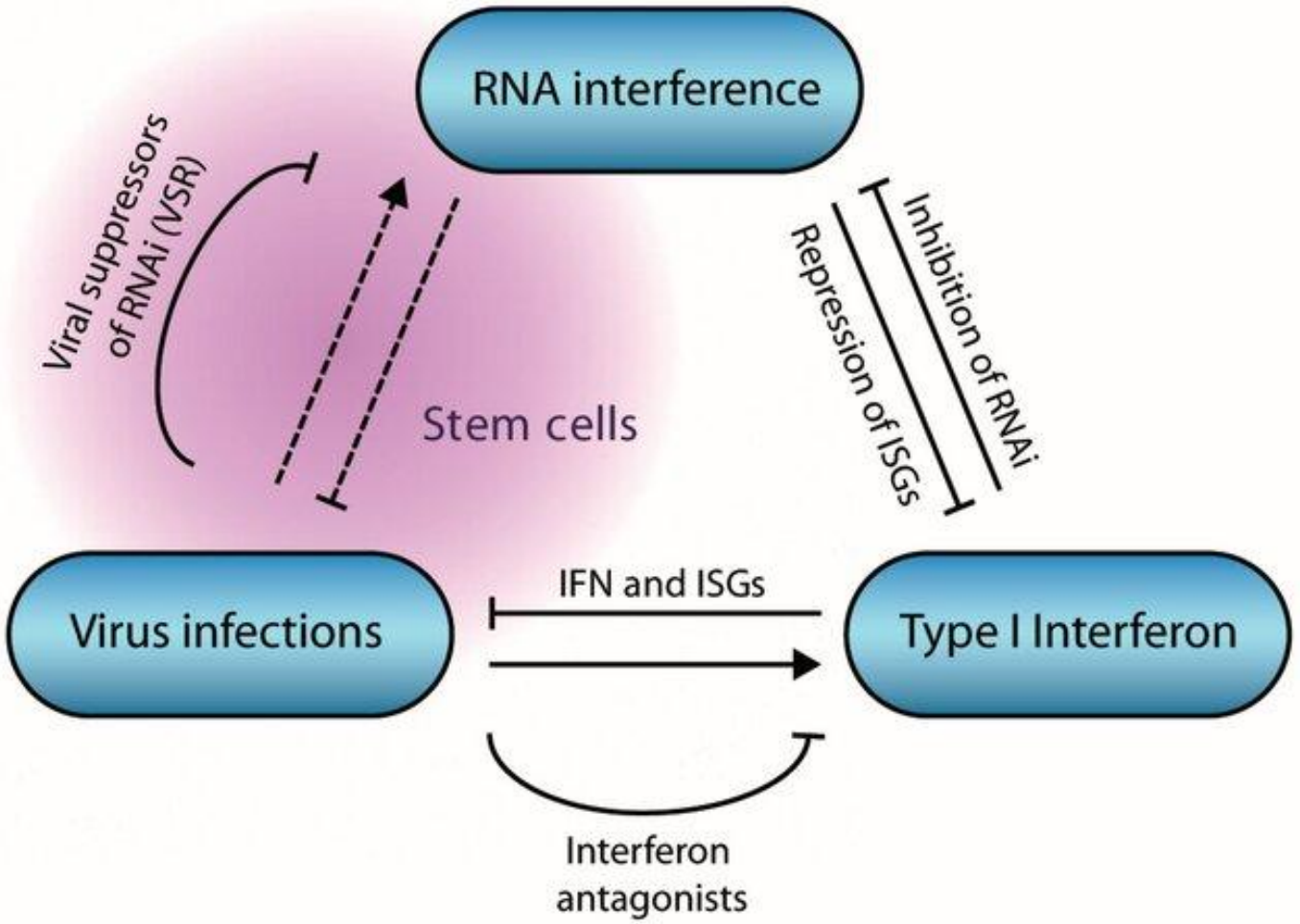
Have vertebrates really abandoned antiviral RNAi?



- This has become an **area of controversy**, with some investigators suggesting that

1. **RNAi** can be a relevant means of **cell intrinsic restriction** to **virus** infection in mammals
2. While others argue that it is an **epiphenomenon with no role in antiviral resistance**

Antagonism between the IFN response and dsRNAi



doi:10.3390/v11050448

MAVS/IFNAR deficient cells resulted in:

- **Dicer-dependent** accumulation of **siRNAs.**
- **Ago2-dependent** sequence-specific **gene silencing.**

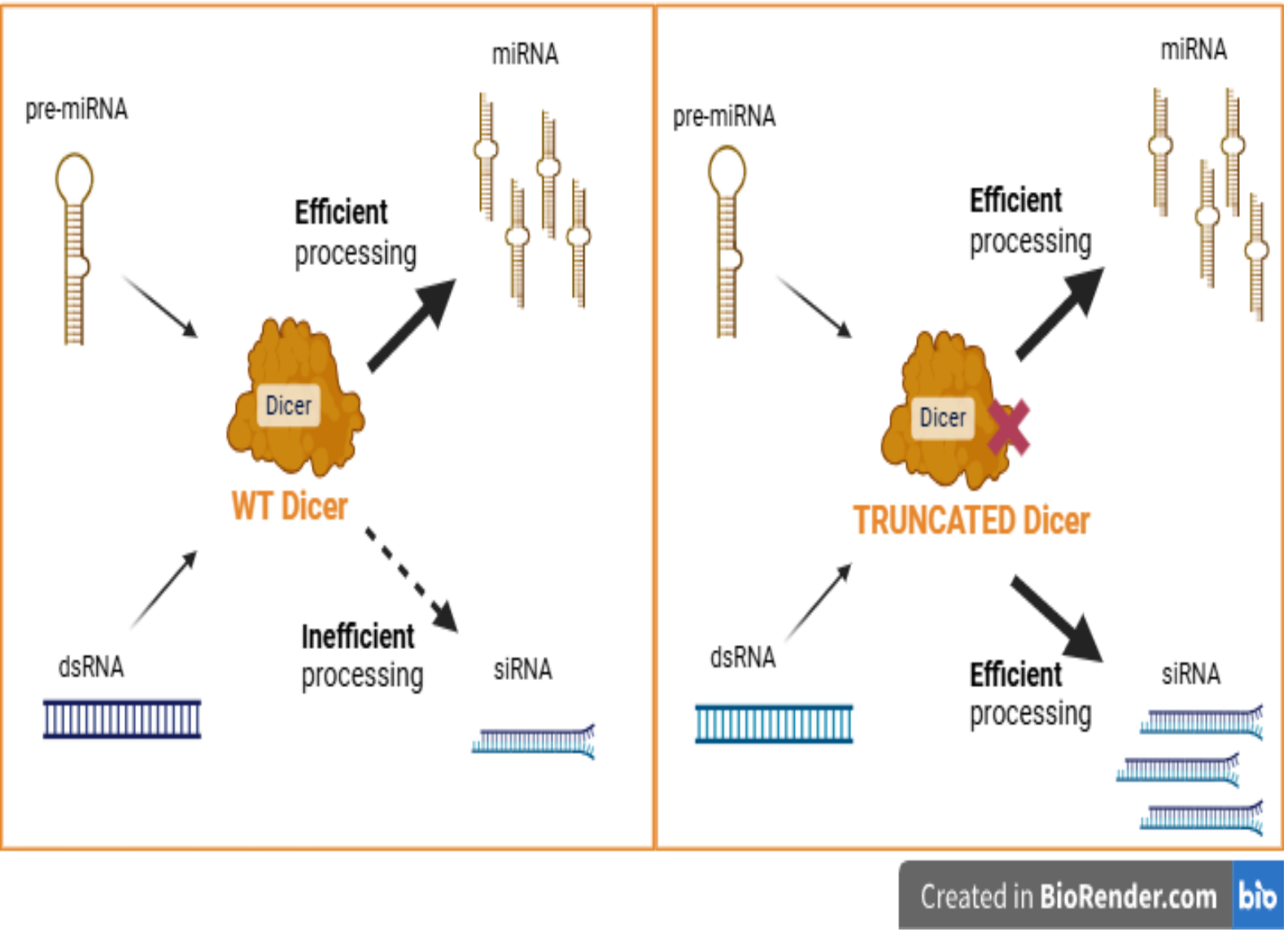
Experimental evidence also shows:

- **Active inhibition of of dsRNAi** by IFN through induction of **LGP2**.
- **Viral infection/poly(I:C)** induce **poly-ADP-ribosylation** of **Ago2** and other **RISC components.**

EXPLANATION:

- Inhibition of Dicer/RISC **prevents loss of dsRNA** substrates for **RLR activation**.
- **Preservation of dsRNA** ensures **activity of ISG-encoded antiviral proteins** is not compromised.

Intrinsic inefficiency of mammalian Dicer in processing long dsRNA



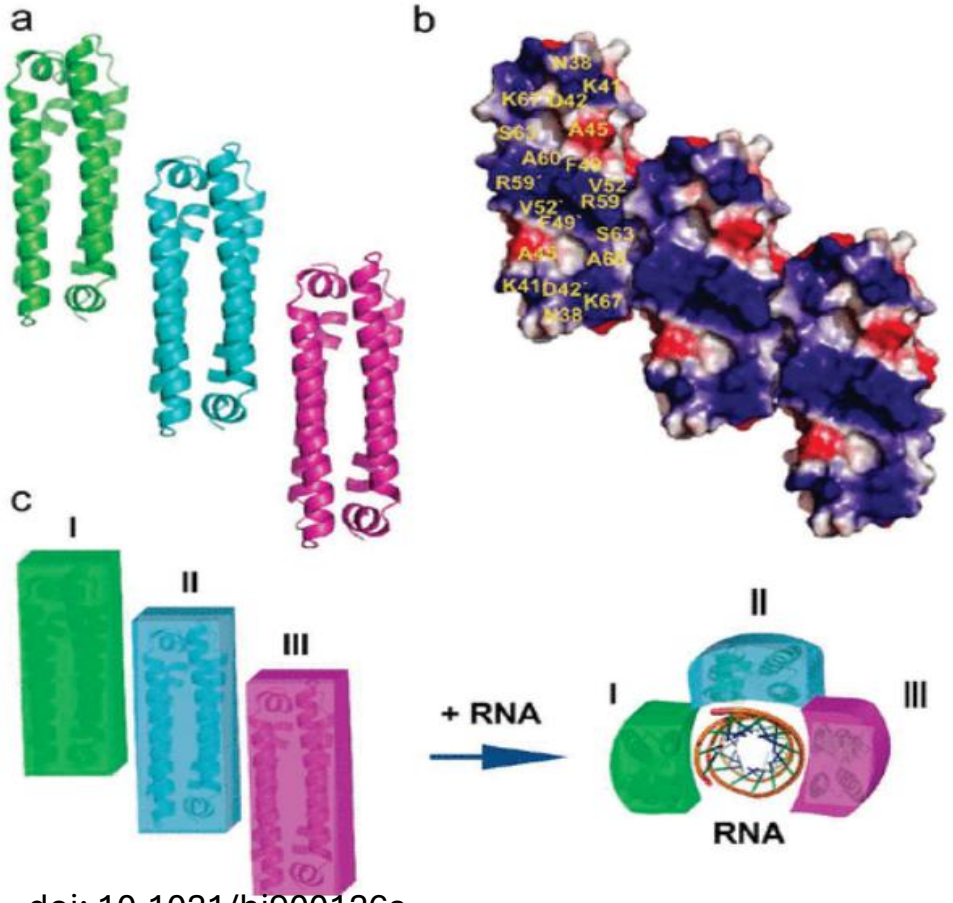
❑ EXPERIMENTAL OBSERVATIONS:

1. **Deletion** or **partial proteolysis** of the **helicase domain** increases rate of **dsRNA cleavage**, while only modestly affecting the cleavage of pre-miRNAs. It **conferred dsRNAi activity to engineered cells**
2. Finally, **mouse oocytes**, in which **dsRNAi is active**, express a **shortened isoform of Dicer (Dicer^{O})** that **lacks the N-terminal helicase domain** and processes endogenous or ectopically expressed long hairpin RNAs more efficiently

Viral suppressors of RNAi (VSRs) are viral proteins that can inhibit RNAi

❑ B2 PROTEIN OF NODAMA VIRUS

- NMV B2 acts as a **suppressor of RNA interference** (RNAi) by **shielding dsRNA** and **siRNA**



doi: 10.1021/bi900126s

- This proteins are expressed in **plants** and **invertebrates RNA viruses**, but also in **some mammalian viruses.**

❑HYPOTHESIS:

- **Viruses** might have **evolved** under the **selective pressure of RNAi**
- **BUT** since **dsRNA is a potent inducer of the IFN pathway**, most of these VSRs also act as **IFN antagonists**

❑ "CONCLUSION"

- **It is therefore unclear whether these viral proteins specifically evolved to block RNAi or whether their VSR activity is a byproduct of their role as IFN antagonists**

Niches for antiviral RNAi?

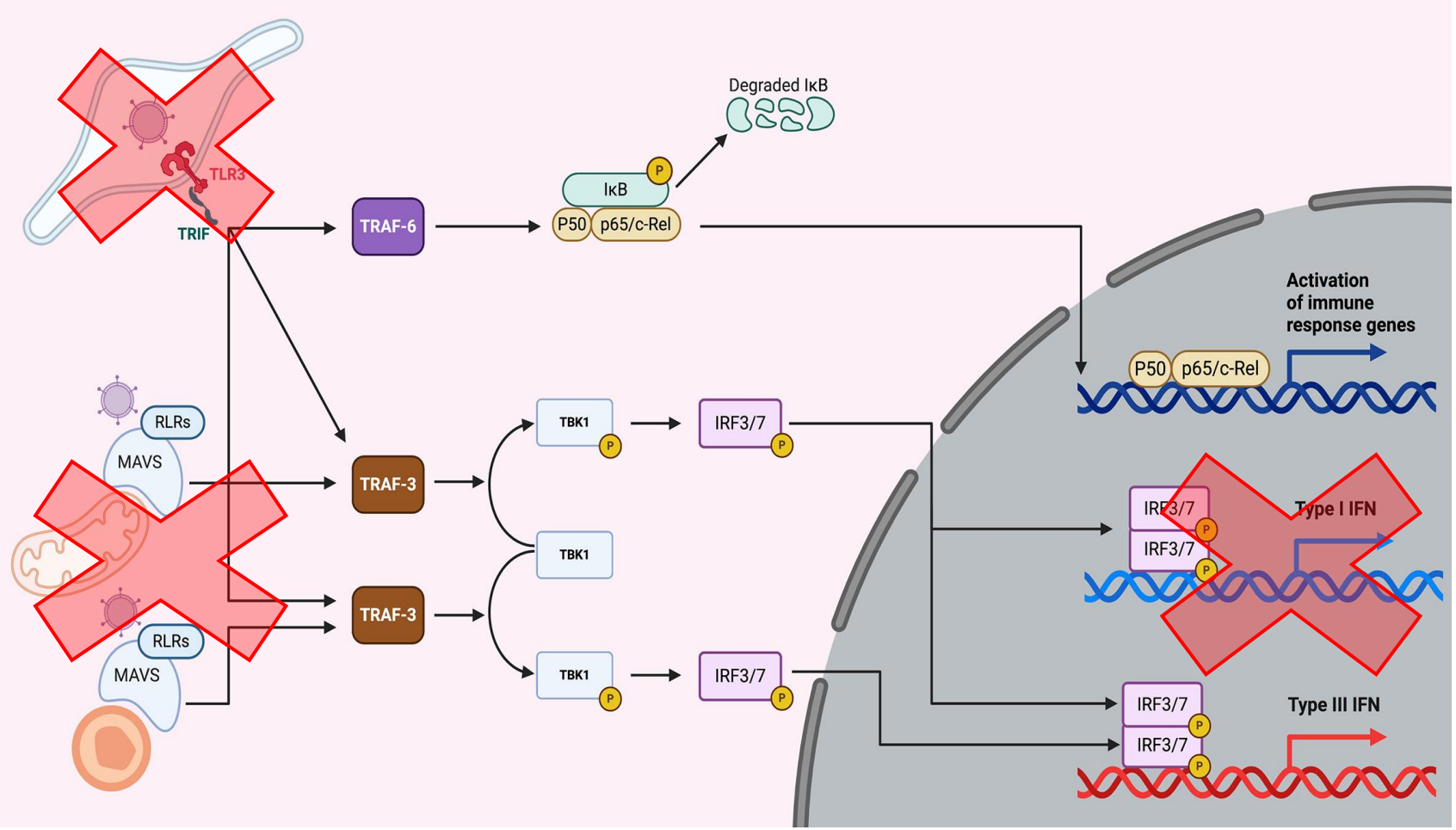
- These general observations suggests that:

antiviral RNAi may be especially important in cellular niches in which the induction of or the response to IFN is limited. One of those niches might be stem cells.

2. ANTIVIRAL RESPONSE IN MAMMALIAN STEM CELLS

Pluripotent stem cells possess an «attenuated innate immune response

❑ ANTIVIRAL DEFENSE IN ESC AND iPSC

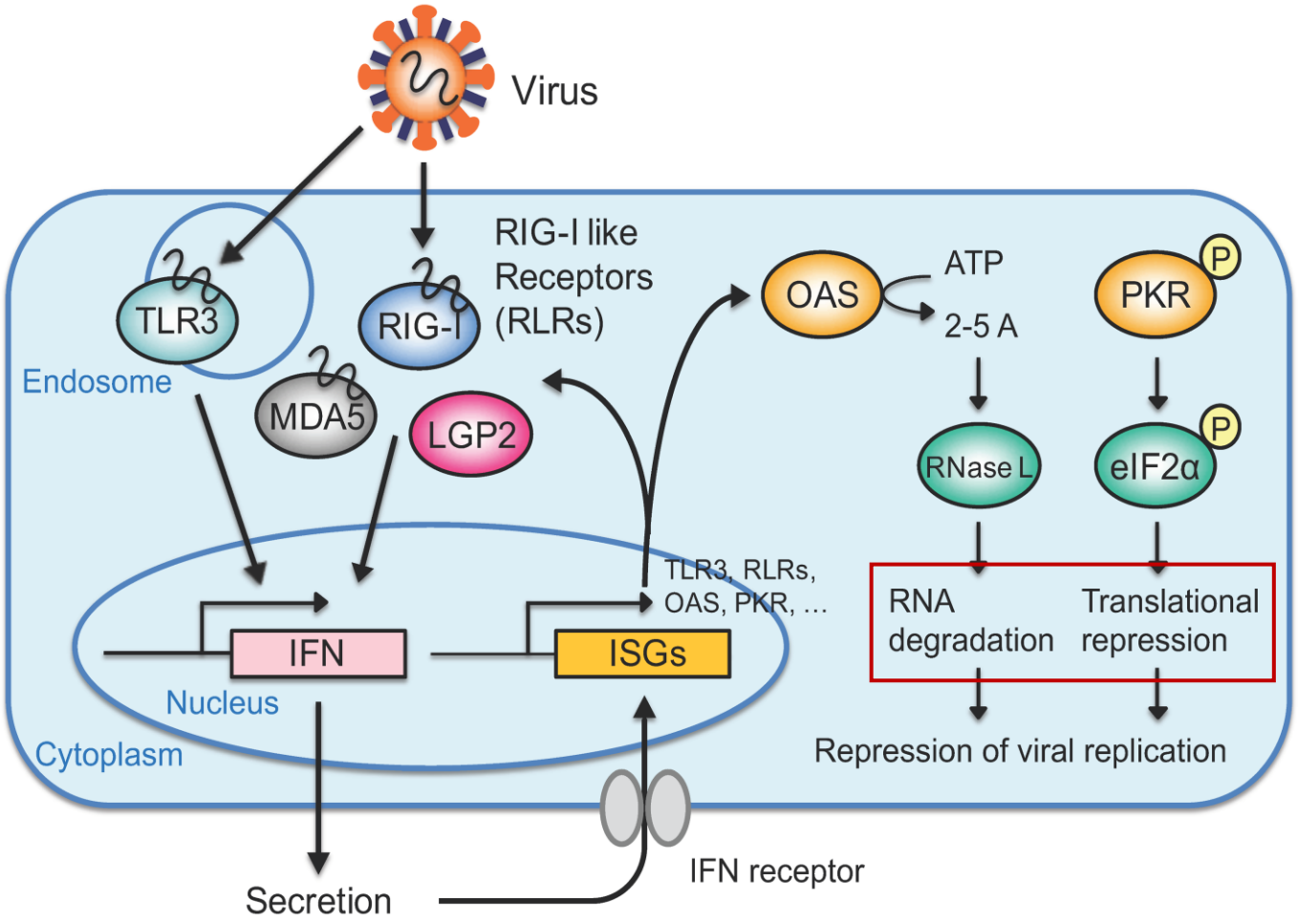


https://doi.org/10.3389/fimmu.2020.608645https://doi.org/10.3390/ijms21041348

- **No IFN expression** to viral stimuli.
- No respsonse/attenutated response to **PAMPs and inflammatory cytokines**.
- Responsiveness to cytokines and IFN expression is **acquired with differentiation.**

- ESC are not defenseless, might use **alternative mechanism** for innate defense.
- More studies on mESC, **limited knowledge on hESC**.

ESCs might prevent «Immunological citotoxicity» by suppressing IFN and cytokine responses



https://doi.org/10.3390/ijms21041348

❑ EFFECTS OF IMMUNE RESPONSE

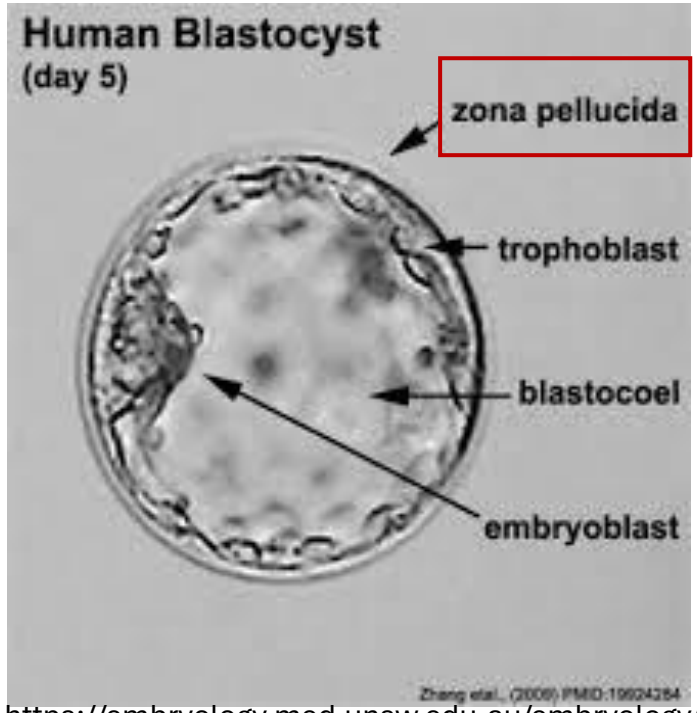
- **double-edged sword**: defence against pathogen, but it induces damage to the host cell.
- Absence of IFN in ESC might be due to **incompatibility** between **self-renewal and** anti-proliferative/**pro-apoptotic effects of the cytokines**.

❑ MOLECULAR BASIS

- **receptors for viral RNA** (TLR3, RIG-I, and MDA5), **LPS** (TLR4), and **TNFα** (TNFR1) **expressed at low level or non-functional.**
- **NF-κB** master mediator of immune response is **inactive in ESC**.

ESCs may adopt «intrinsic» antiviral mechanisms

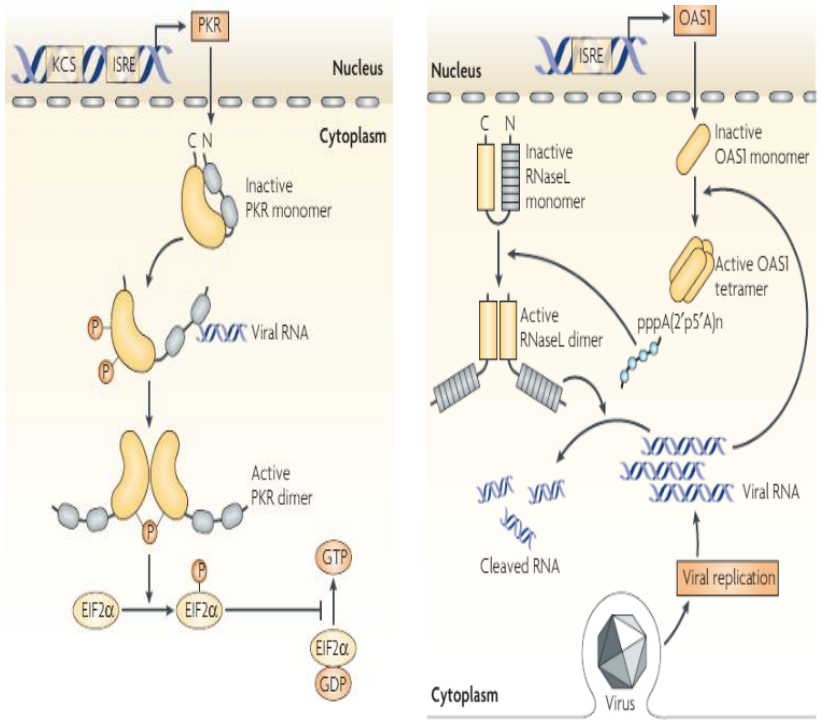
❑ ZONA PELLUCIDA



https://embryology.med.unsw.edu.au/embryology/index.php?title=Blastocyst_Development

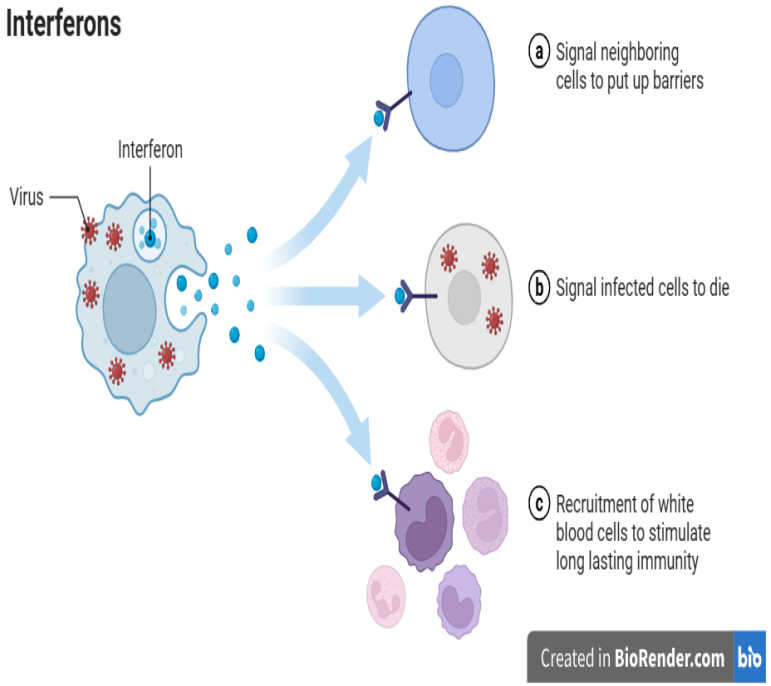
- Thick **extracellular coat** layer that can provide a physical **barrier** to microbial pathogens to the **preimplantation blastocyst**

❑ INTRINSIC ISGs



- Intrinsic ISGs in ESCs are **constitutively** expressed in the **absence of IFN**

❑ LOW IFN RESPONSE



- Despite their **inability to produce IFNs,** they present **low level responses to IFNα and IFNβ** and **express ISGs.**

3. ARTICLE PRESENTATION

ANTIVIRAL DEFENSE

An isoform of Dicer protects mammalian stem cells against multiple RNA viruses

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OUTLINE of the EXPERIMENTAL STEPS

1. **IDENTIFICATION and EXPRESSION PROFILING of an ISOFORM of DICER**
2. **BIOCHEMICAL CHARACTERIZATION IN VITRO**
3. **BIOCHEMICAL CHARACTERIZATION in ENGINEERED CELL LINES**
4. **CHARACTERIZATION in EX VIVO STEM CELLS and ORGANOIDS**

1) IS THERE AN ALTERNATIVE VERSION OF THE DICER ENZYME?

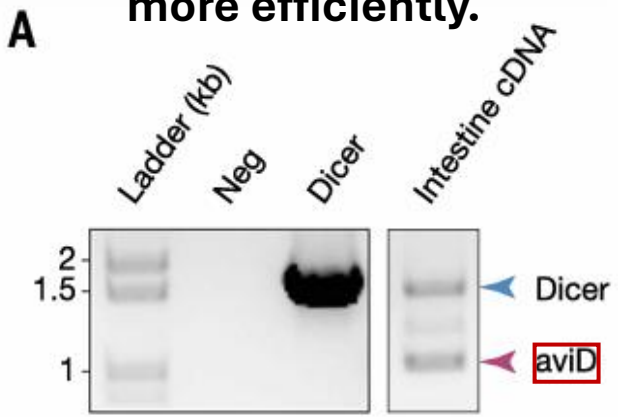
aviD: a Dicer isoform that lacks an helicase domain

❑ OBSERVATIONS:

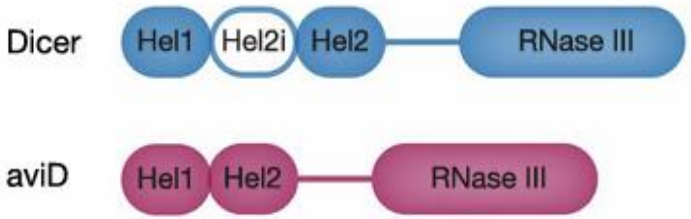
1) dsRNA mediated RNAi may be relevant in cells that are **hyporesponsive to IFNs**, such as **stem cells.**
2) An **isoform of Dicer** has been observed in **mouse oocytes**.

❑ HYPOTHESIS:

- Antiviral RNAi in mammals may involve expression of an **isoform of Dicer** that **processes dsRNA more efficiently.**

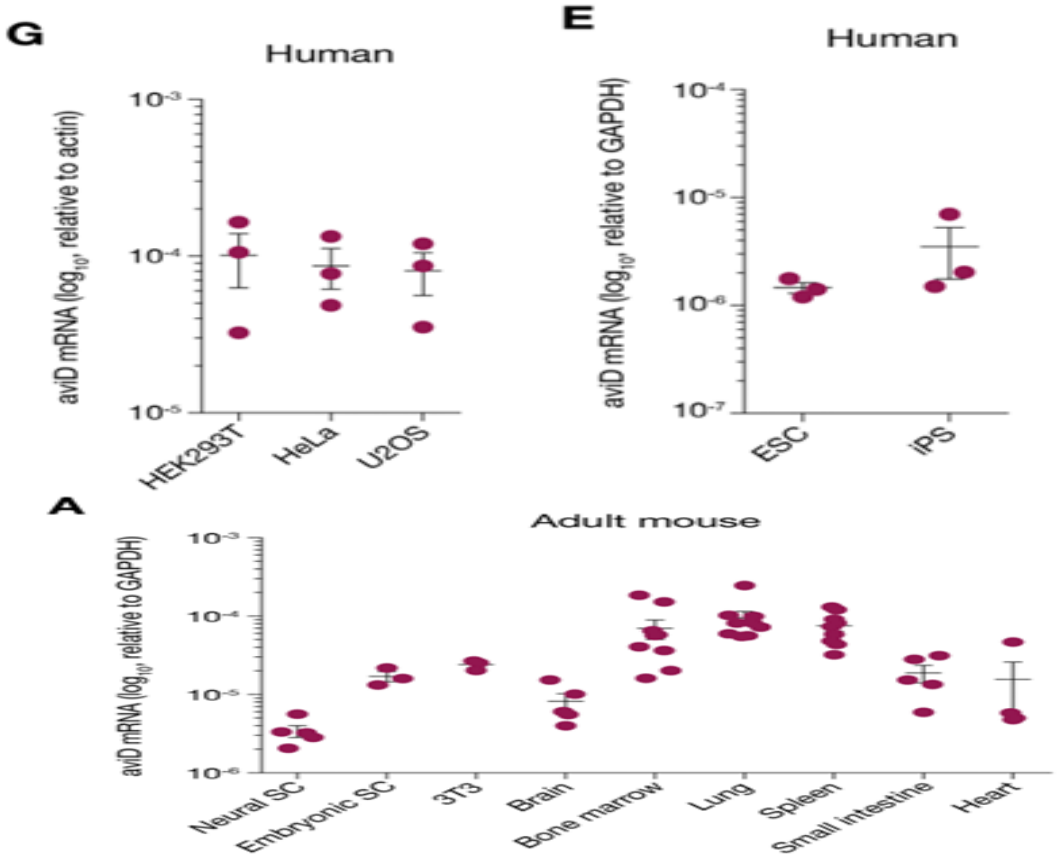


- **RNA** extraction + **cDNA** synthesis
- **PCR on total cDNA** from mouse small intestine and they identified an **alternatively spliced** in-frame transcript of Dicer **missing exons 7** and **8**



- **In silico translation** of this transcript resulted in a **truncated Dicer protein** in which the central **Hel2i domain** of the **N-terminal helicase** segment is **absent**

aviD RNA and protein levels are detected at low levels

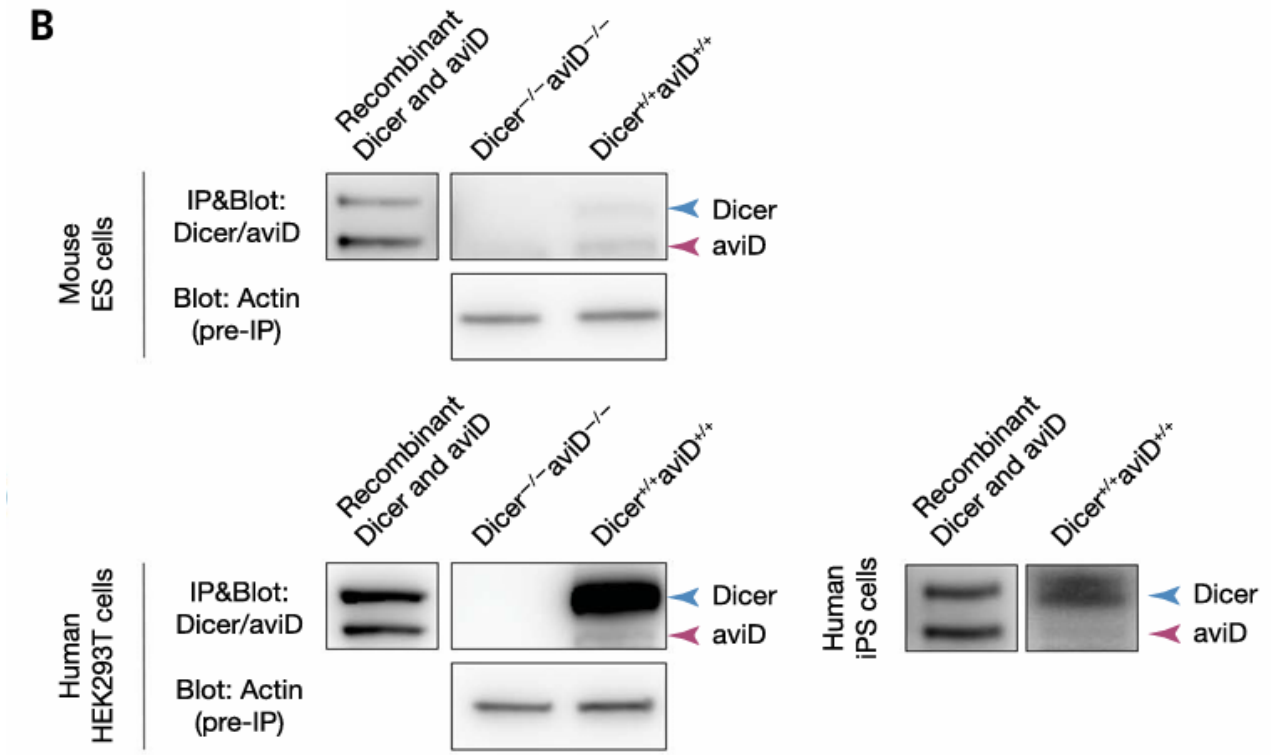


❑ PROTEIN

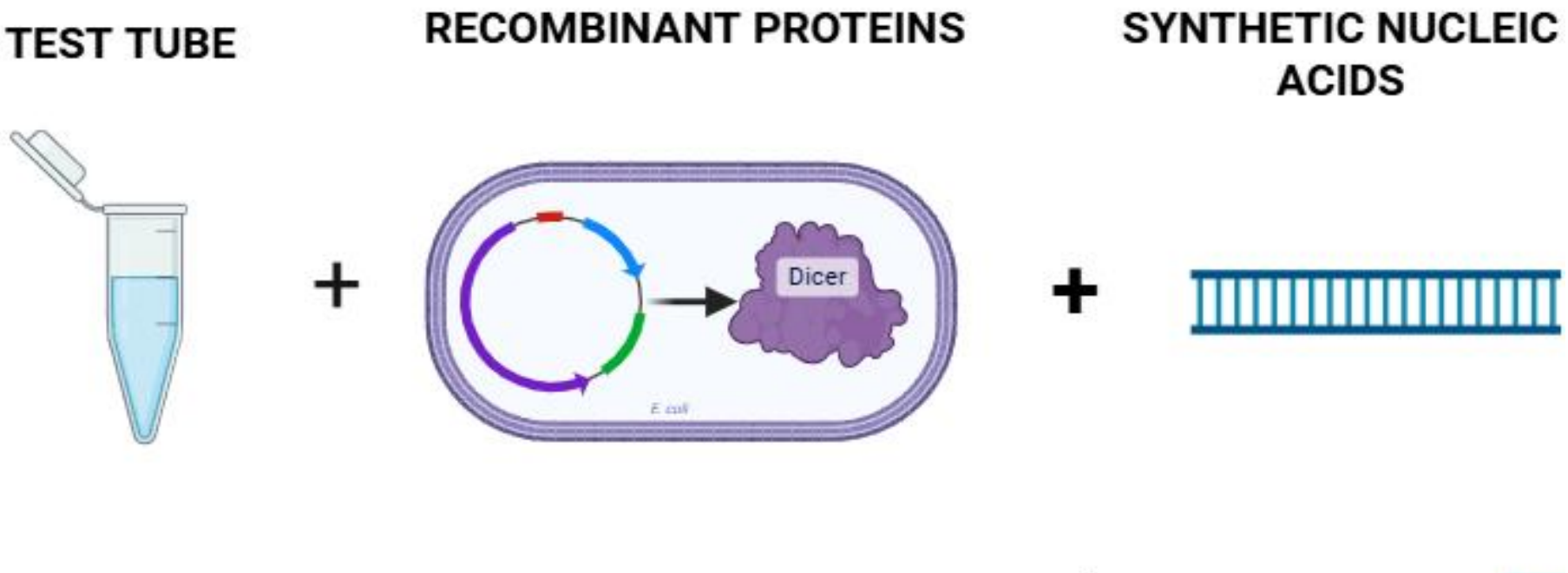
- **Immunoprecipitation** using an antibody that **dually recognizes** Dicer and aviD
- As a **negative control** used cells Dicer deficient (Dicer—/—aviD—/—) cells

❑ RNA

- **RT-qPCR** assay that **distinguishes aviD** and Dicer mRNA, both isoforms could be detected and in general, transcripts encoding **aviD appeared to be less abundant** than transcripts encoding full-length Dicer by at least a factor of 10

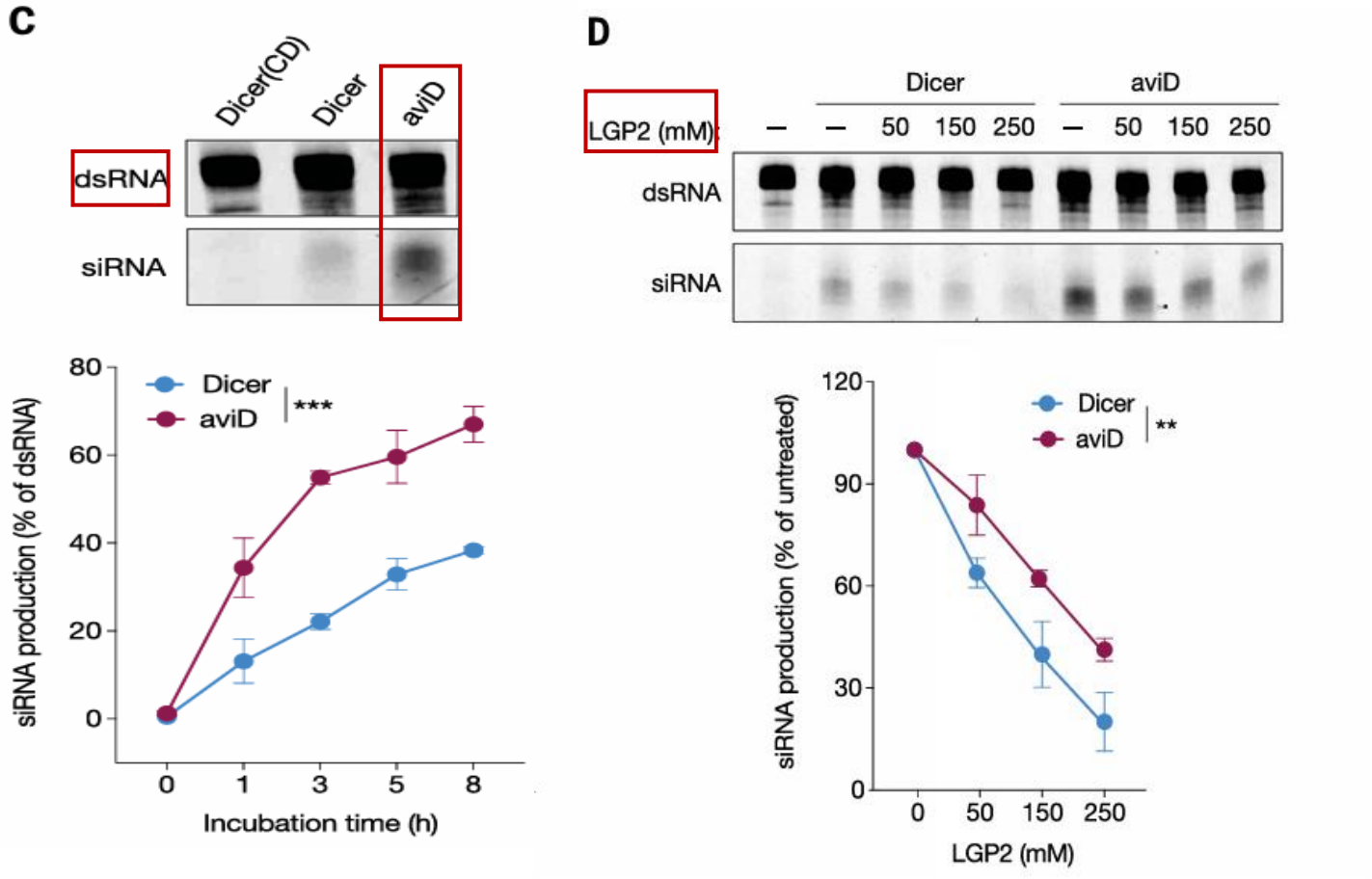


2) WHAT IS THE BIOCHEMICAL ACTIVITY OF aviD IN VITRO?



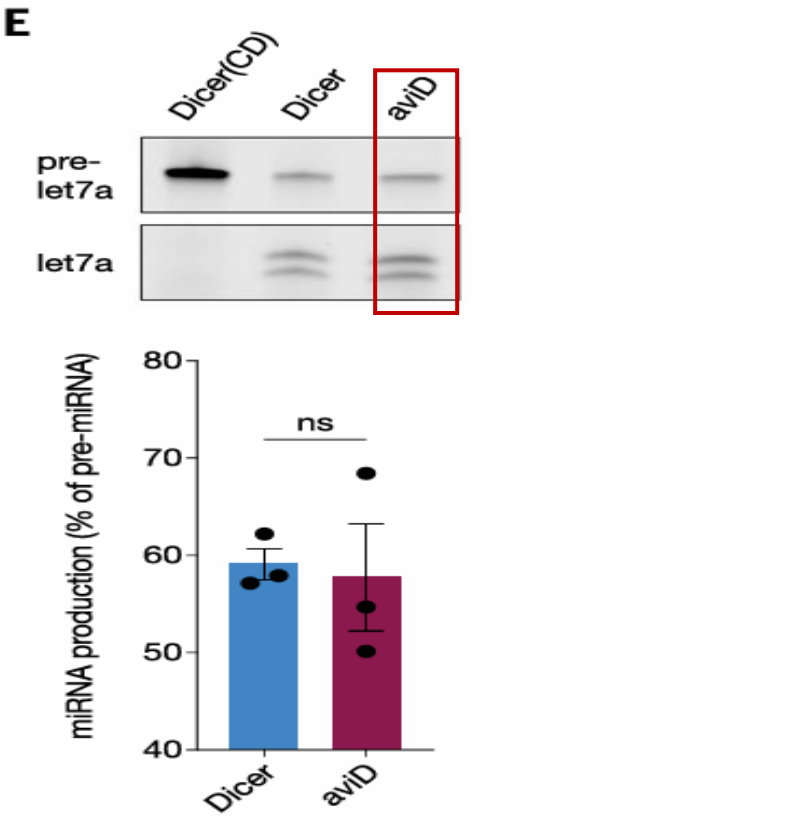
aviD processes dsRNA more efficiently and can still process pre-miRNA

❑ siRNA PROCESSING



- Recombinant aviD produced about twice as much siRNA from **synthetic dsRNA** and is more **resistant to LGP2**

❑ miRNA PROCESSING



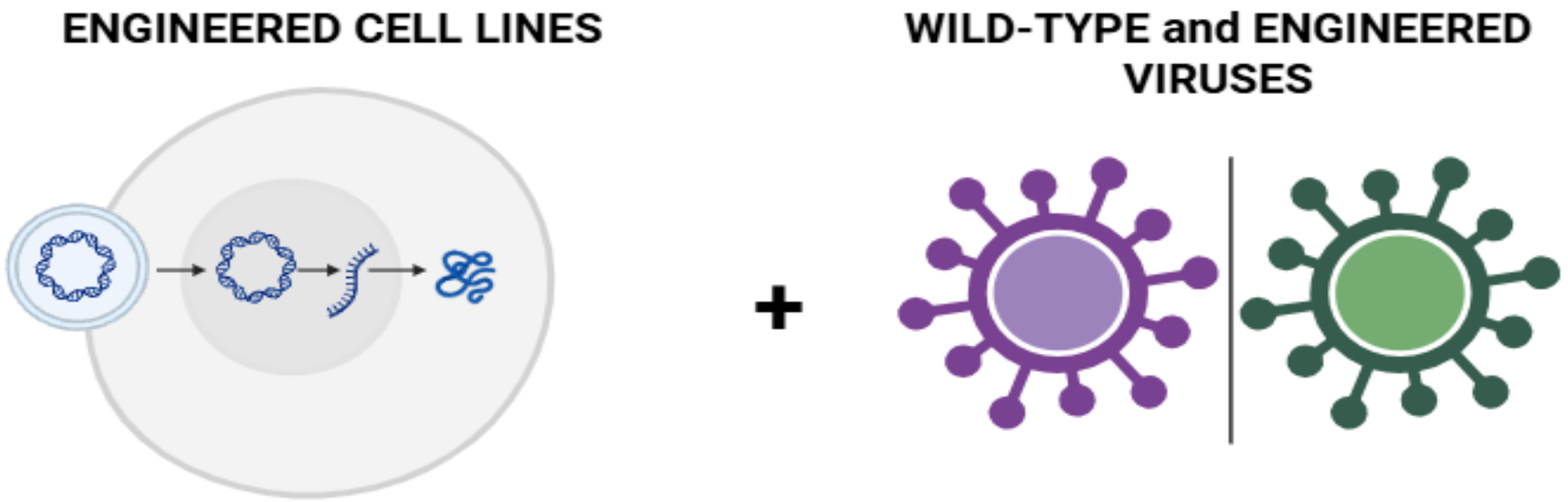
- Both Dicer and aviD generated **equivalent amounts** of let-7a miRNA from **pre-miRNA**

KEY POINTS

- These results suggest that **loss of the Hel2i domain:**

1) Does **not impair** the ability of aviD to **process miRNA precursors.**
2) Confers **enhanced capacity to dice dsRNA** into siRNAs

3) IS aviD ABLE TO MEDIATE ANTIVIRAL RNAi IN ENGINEERED CELL LINES?



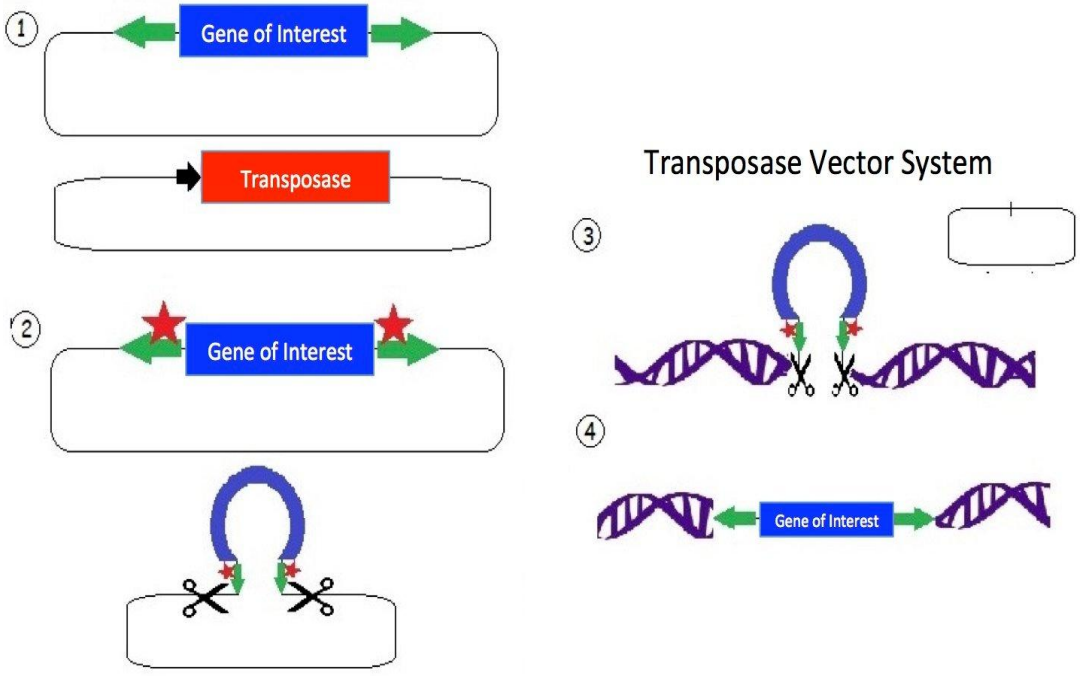
Generation of engineered cells lines

- In the next slides the following **HEK293T** or **ES cells lines** will be cited:

1. **Dicer–/–aviD–/–** → **Dicer deficient cells**
2. **Dicer+/+aviD–/–**
3. **Dicer–/–aviD+/+**

«Rescued» cells: knock out cells transfected with either **Flag-Dicer** or **aviD**

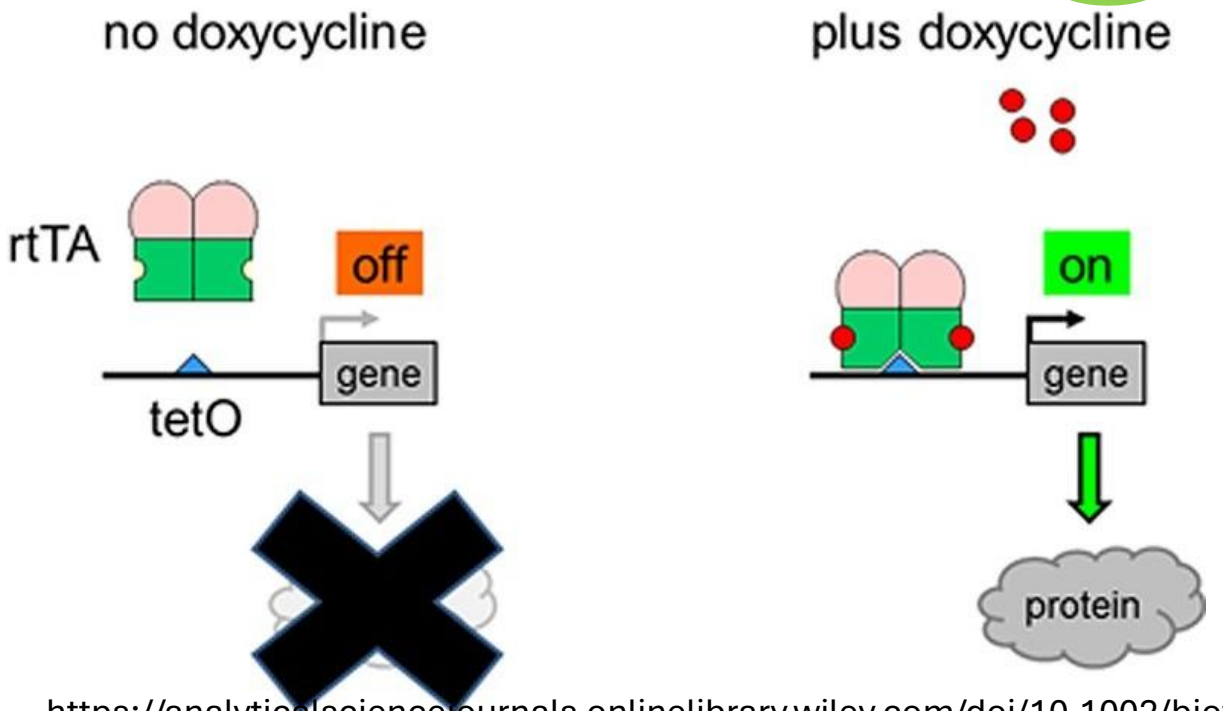
❑ Dicer–/–aviD–/– COMPLEMENATION



https://blog.addgene.org/sleeping-beauty-awakens-for-genome-engineering

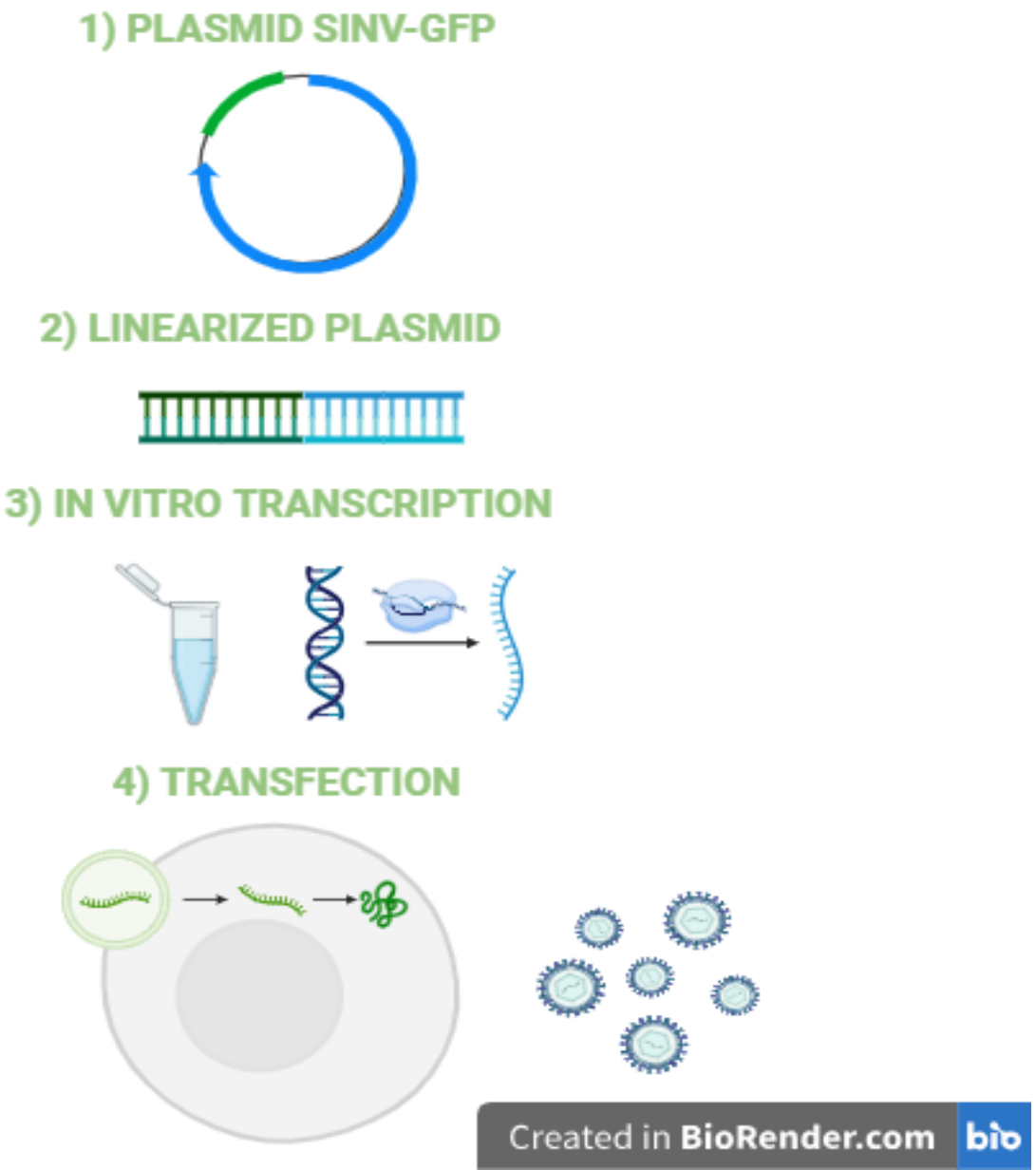
❑ Tet-On SYSTEM

SUPP.INFO.



https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/biot.201500236

Generation of engineered viruses



❑ INFECTIOUS DNA CLONE:

1. **dsDNA copy of the viral genome** carried in a bacterial **plasmid**.
2. DNA is **linearized**
3. **In vitro transcribed** into RNA
4. RNA is **transfected** in permissive cells where the **viral replication cycle begins**

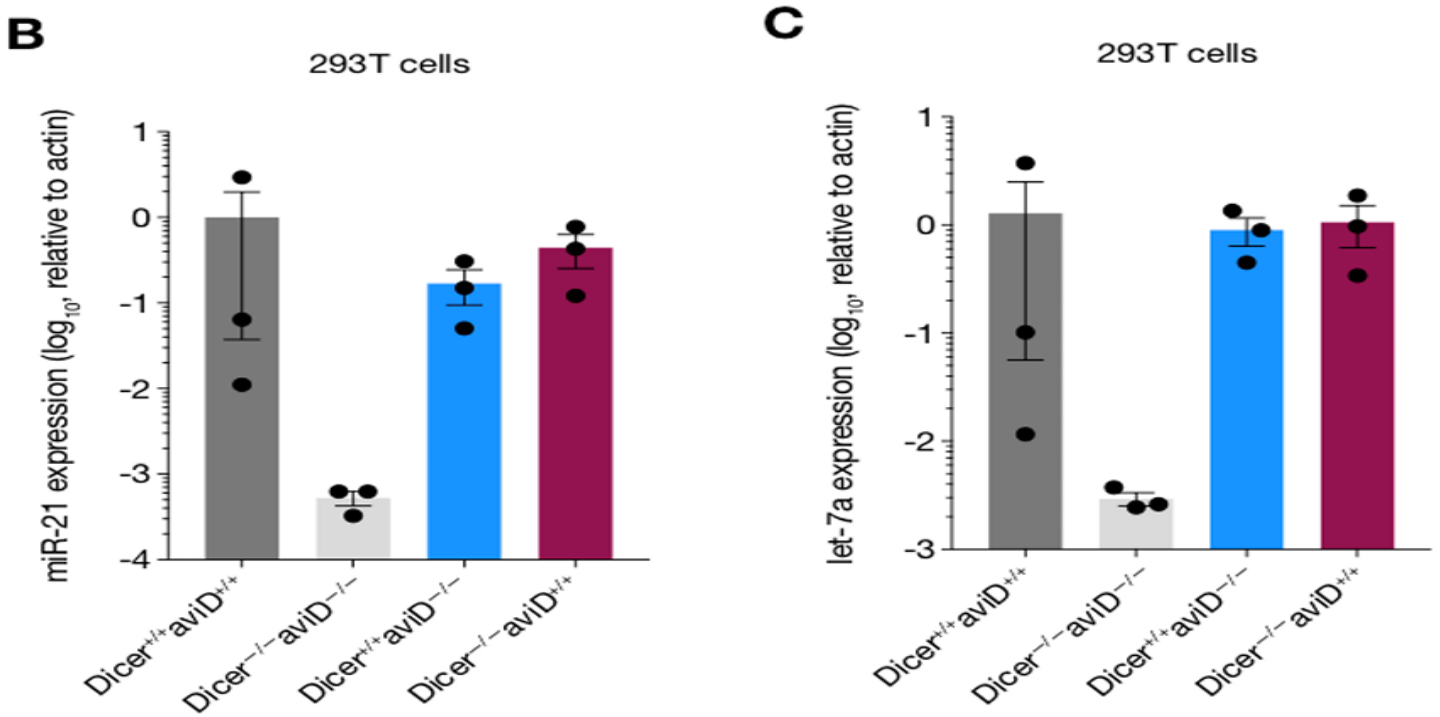
- The presence of specific **cloning sites** flanking GFP, could allow to **clone a different protein.**
- In our case, the **NMV B2** was introduced.

«Rescue» of aviD restores miRNA production

❑ RESCUE EXPERIMENTS

- To assess the **ability of aviD to mediate antiviral RNAi**, they **complemented** Dicer gene deficient **(Dicer–/–aviD–/–)** HEK293T "NoDice" cells by stable transfection with constructs encoding **FLAG-tagged Dicer** or **aviD.**

❑ miRNA PROCESSING



- Expression of **either Dicer** or **aviD** was **sufficient to restore miRNA production** to Dicer–/–aviD–/ "NoDice" cells

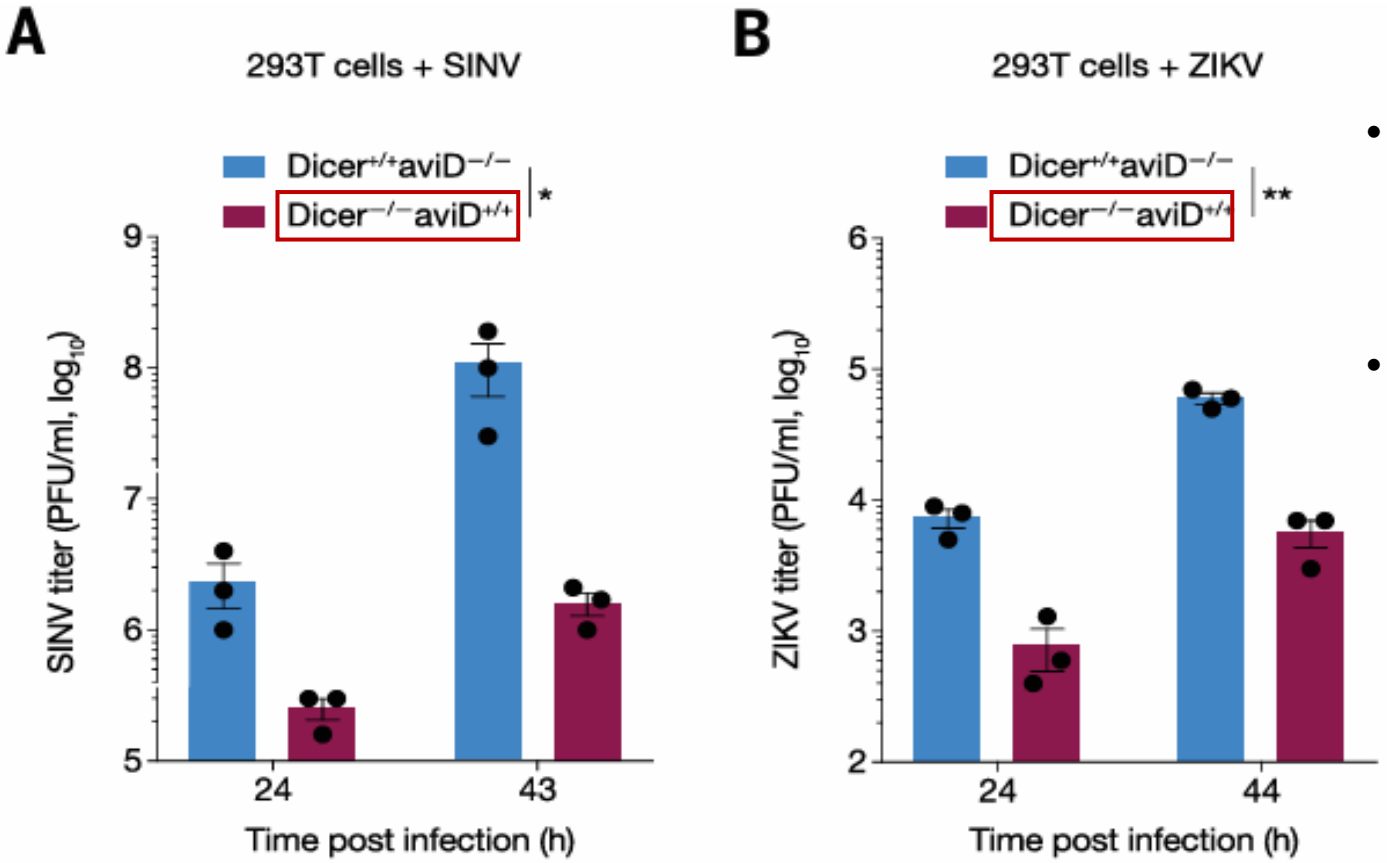
❑ CONCLUSION

- **aviD can also process pre-miRNAs and compensates for Dicer loss in miRNA generation when it is the only isoform expressed in the cell.**

«Rescue» of aviD reduces viral titer in infected cells

- **SINV** and **ZIKV** viruses were chosen because they have a **genome** consisting of **positive single-stranded RNA (+ ssRNA).** It can immediately act as a **messanger RNA** and be **translated**.
- One of the protein encoded is a **RNA-dependent RNA polymerase** generate **–ssRNA**. This last passage is relevant because it leads to **transient formations of dsRNAs**

❑ VIRAL TITER OF INFECTED CELLS



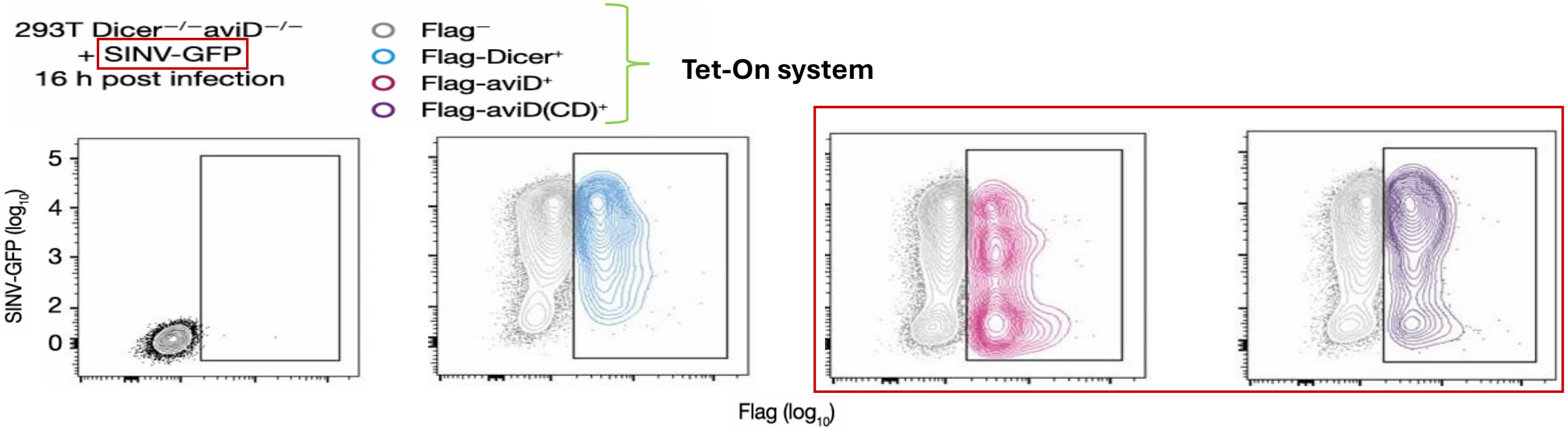
- Both **“rescued” cells** lines were transfected with the **SINV virus** or **ZIKV**

- Cells expressing only **aviD displayed lower production** of SINV and ZIKV **virus progeny** than did cells that only expressed Dicer.

❑ CONCLUSION

- **<u>aviD can restrain viral replication more efficiently than Dicer</u>**

Catalytic deficient (CD) aviD is not able to restrain viral infection



- **NO FLAG TRANSFECTION**
- **Flag-Dicer+**
- **Flag-aviD+**
- **Flag-aviD(CD)+**

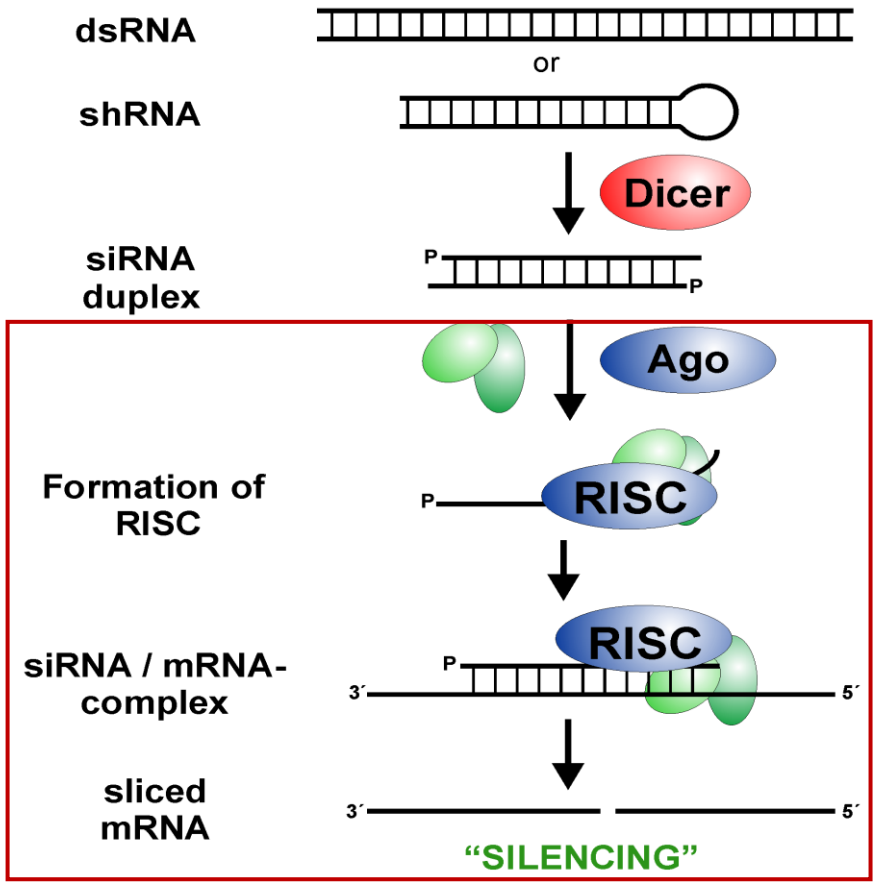
❑CONCLUSION

- **<u>These data demonstrate that aviD but not Dicer possesses antiviral function that is dependent on its catalytic domain, consistent with a role in RNAi</u>**

Ago2 is important for aviD-mediated viral restriction

❑ HYPOTHESIS:

- They wanted to verify if this **siRNAs**, produced by the activity of aviD, **carry out their antiviral role** through the **RNAi machinery**
- Mammals encode **four Ago** proteins, **all** of which can mediate **miRNA**-driven gene silencing. However, **only <u>Ago2 possesses endonuclease activity</u>** to mediate target **"slicing" in antiviral RNAi.**



https://www.gene-quantification.de/si-rna.html

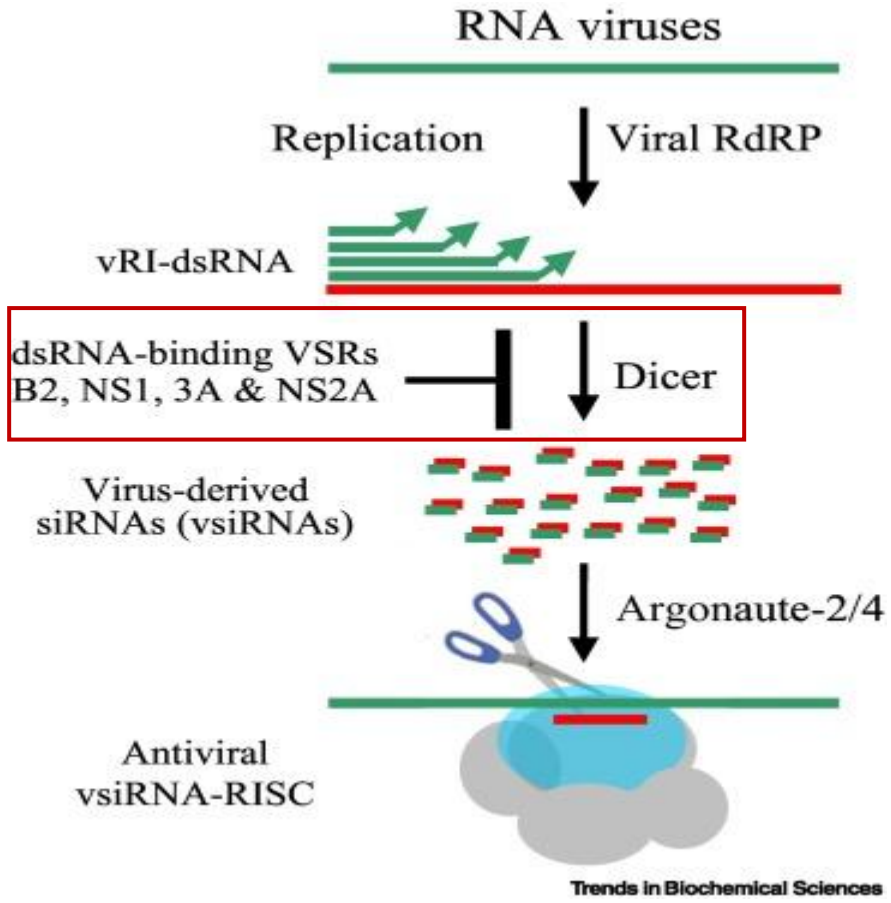
❑ INFECTED CELLS TRANSFECTED WITH siRNAS

- Silencing Ago2 in Dicer–/–**aviD+/+** cells **rescued ZIKV particle production** to levels similar to those in Dicer+/+aviD–/ cells treated with control or Ago2 siRNA

The viral suppressor of RNAi B2 impairs the action of aviD

❑ HYPOTHESIS

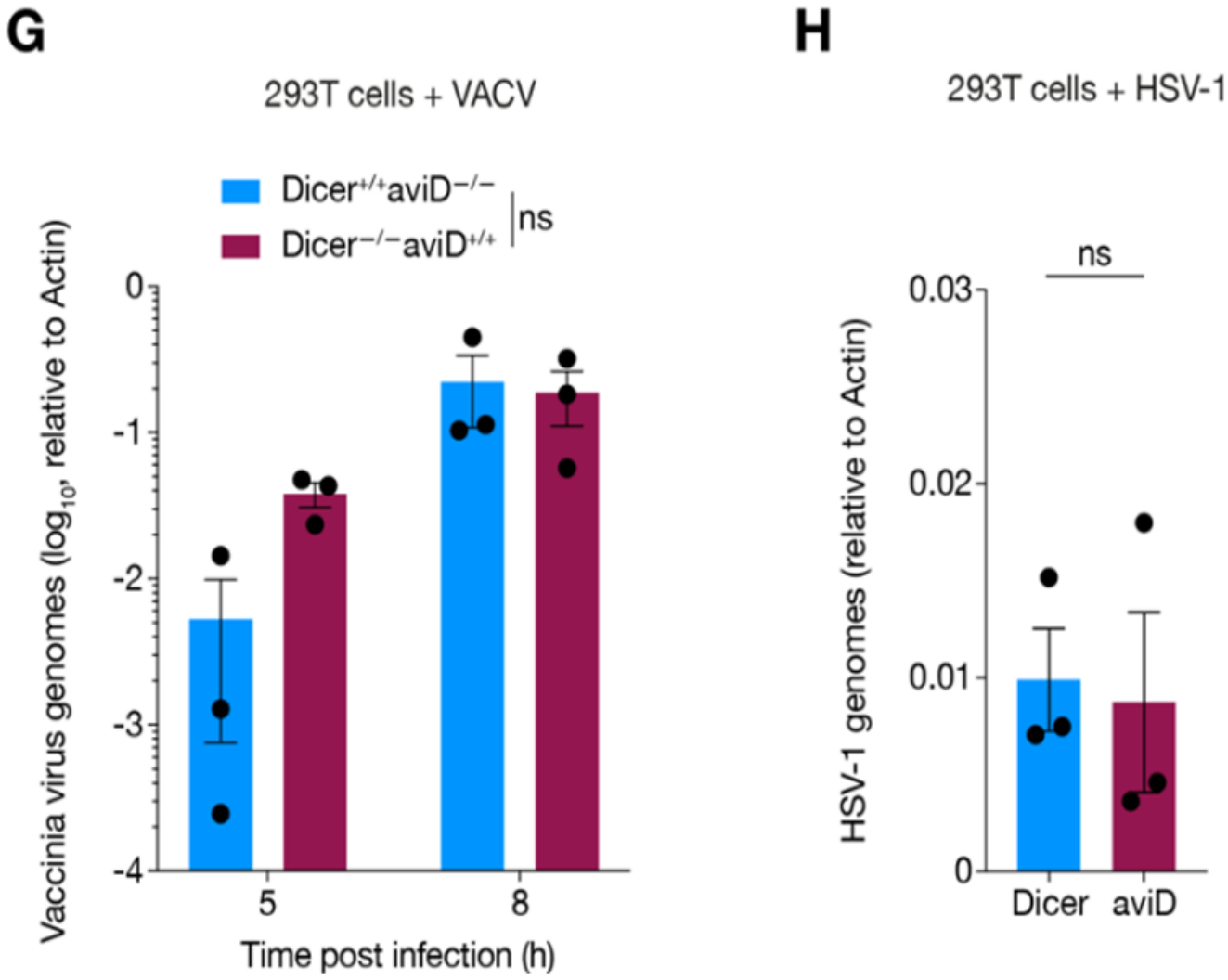
- If the antiviral activity of aviD is based on RNAi, **VSR** (Viral suppressors of RNA interference), which bind dsRNA and siRNA, should **inhibit** its action.
- To study these aspects, they **enginered the SINV** virus to express **GFP** or **B2** (a VSR) and infected the cells.



- **B2 protein inhibits aviD,** and so antiviral RNAi, enabling an **higher viral replication**

https://doi.org/10.1016/j.tibs.2022.05.001

aviD does not restrict the replication of two DNA viruses



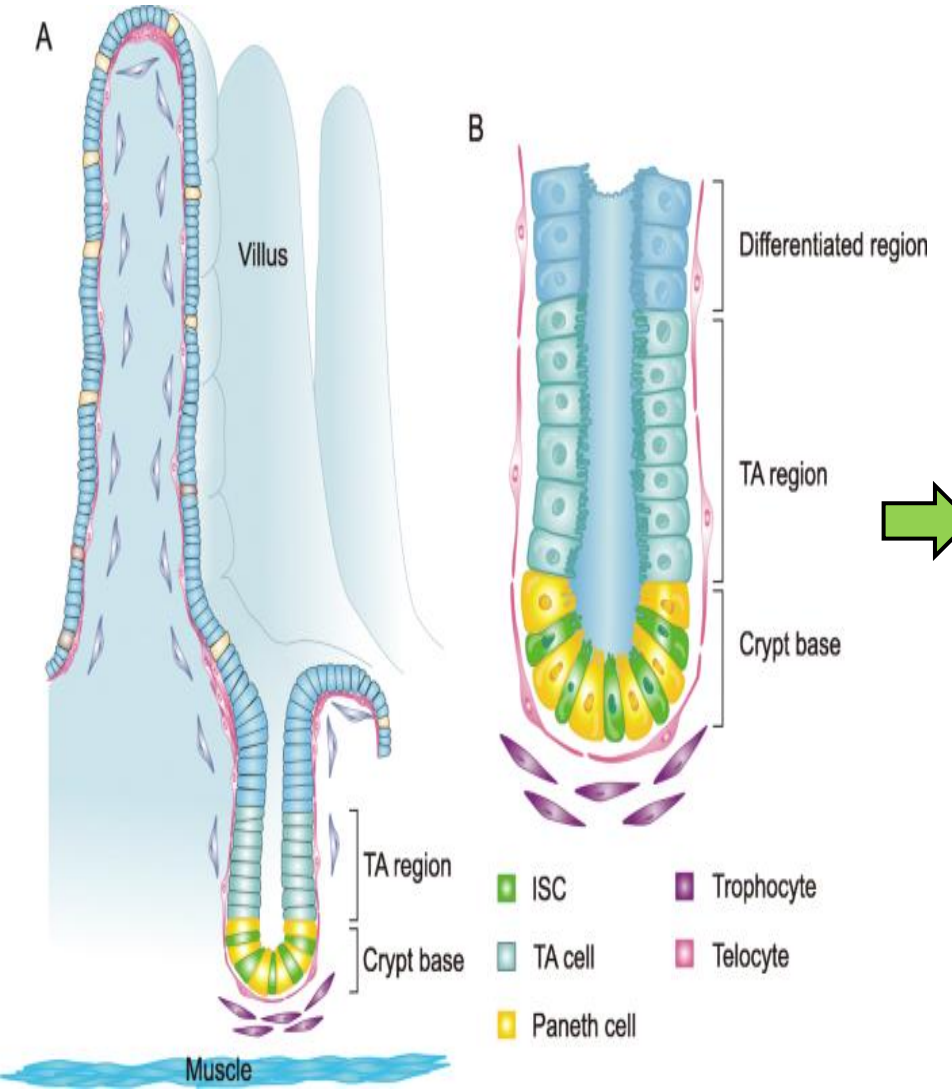
- In contrast, **replication of two DNA viruses**, **vaccinia** virus and **herpes** simplex virus 1, was **similar** in Dicer+/+aviD–/– and Dicer–/–aviD+/+ cells.

❑ CONCLUSION

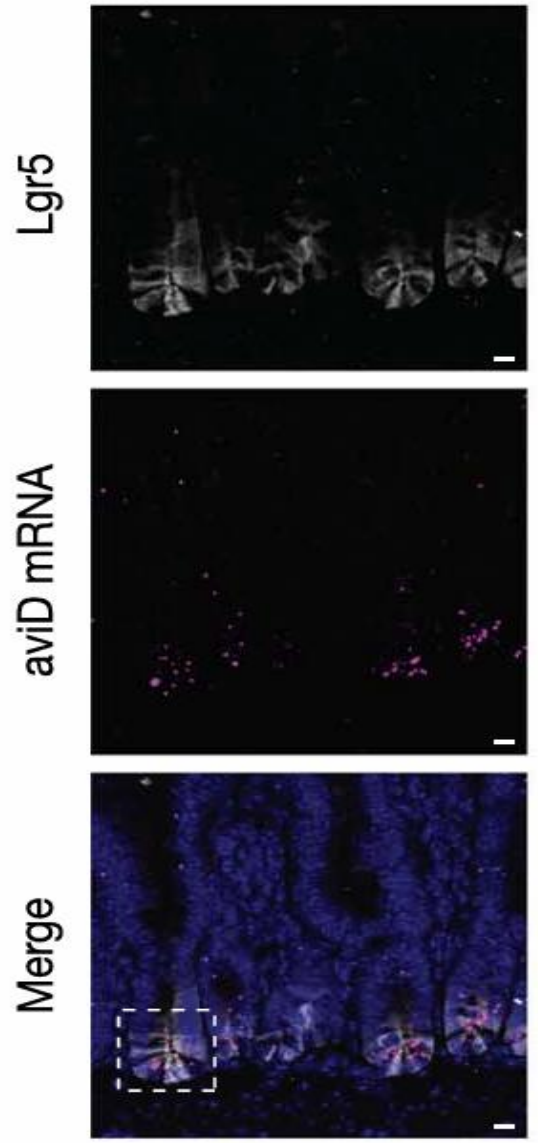
- **Together, these data reveal that expression of aviD allows for an antiviral RNAi response that restricts replication of several RNA viruses.**

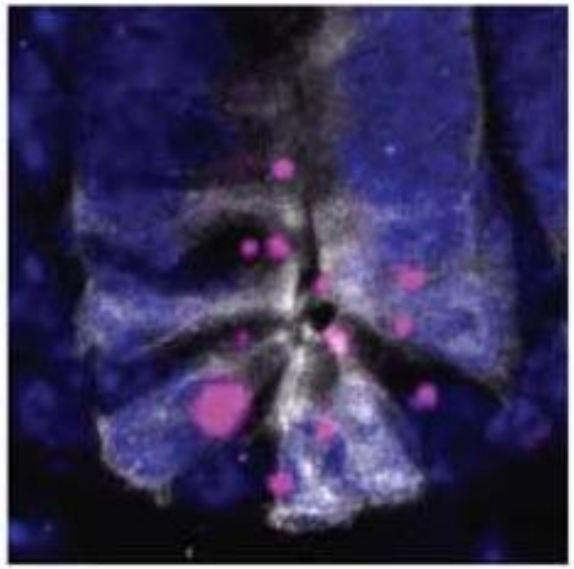
4) IS aviD RELEVANT ALSO IN EX-VIVO STEM CELLS?

aviD preferentially co-localizes with intestinal stem cells



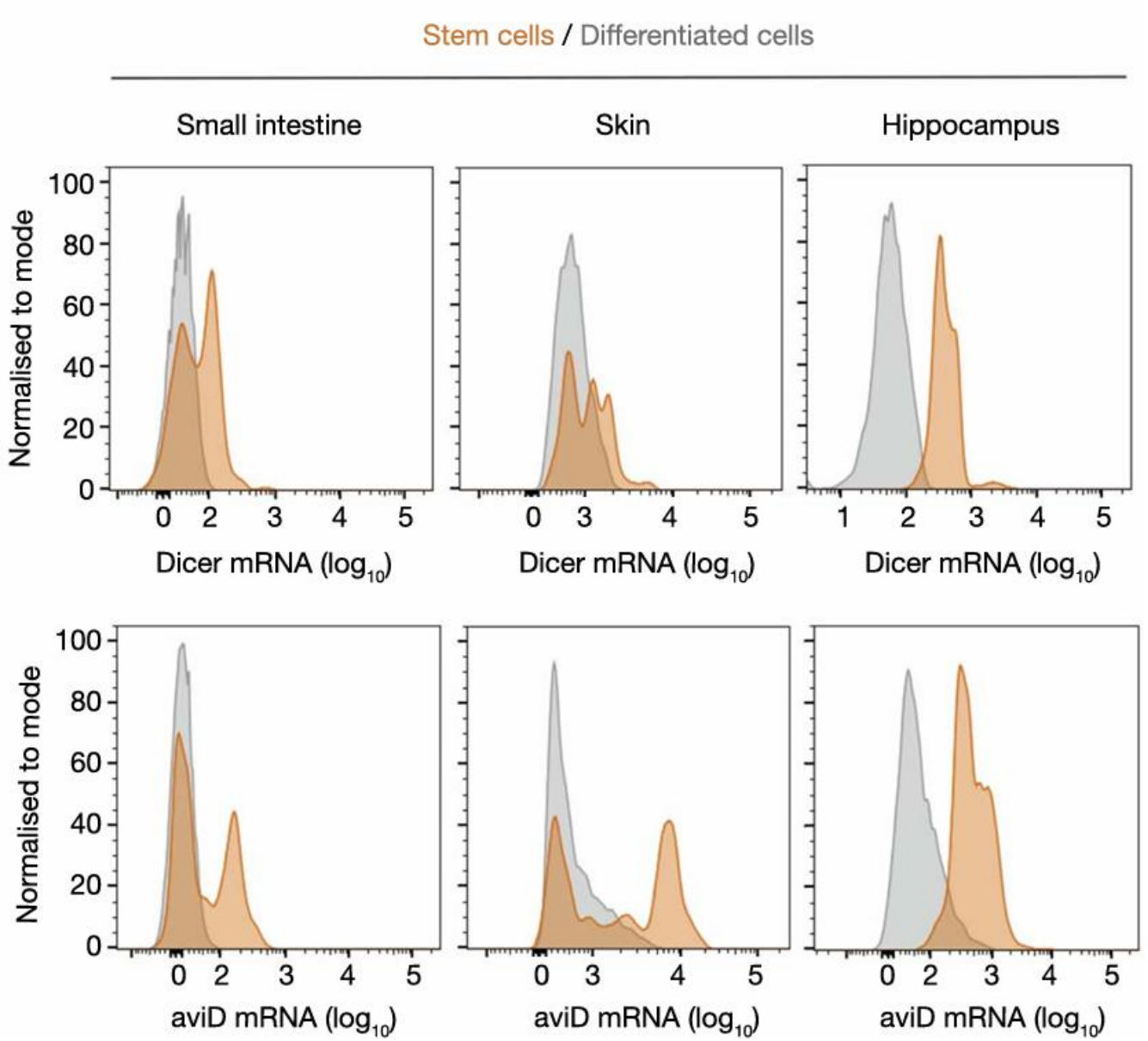
https://doi.org/10.1186/s13619-020-00061-5





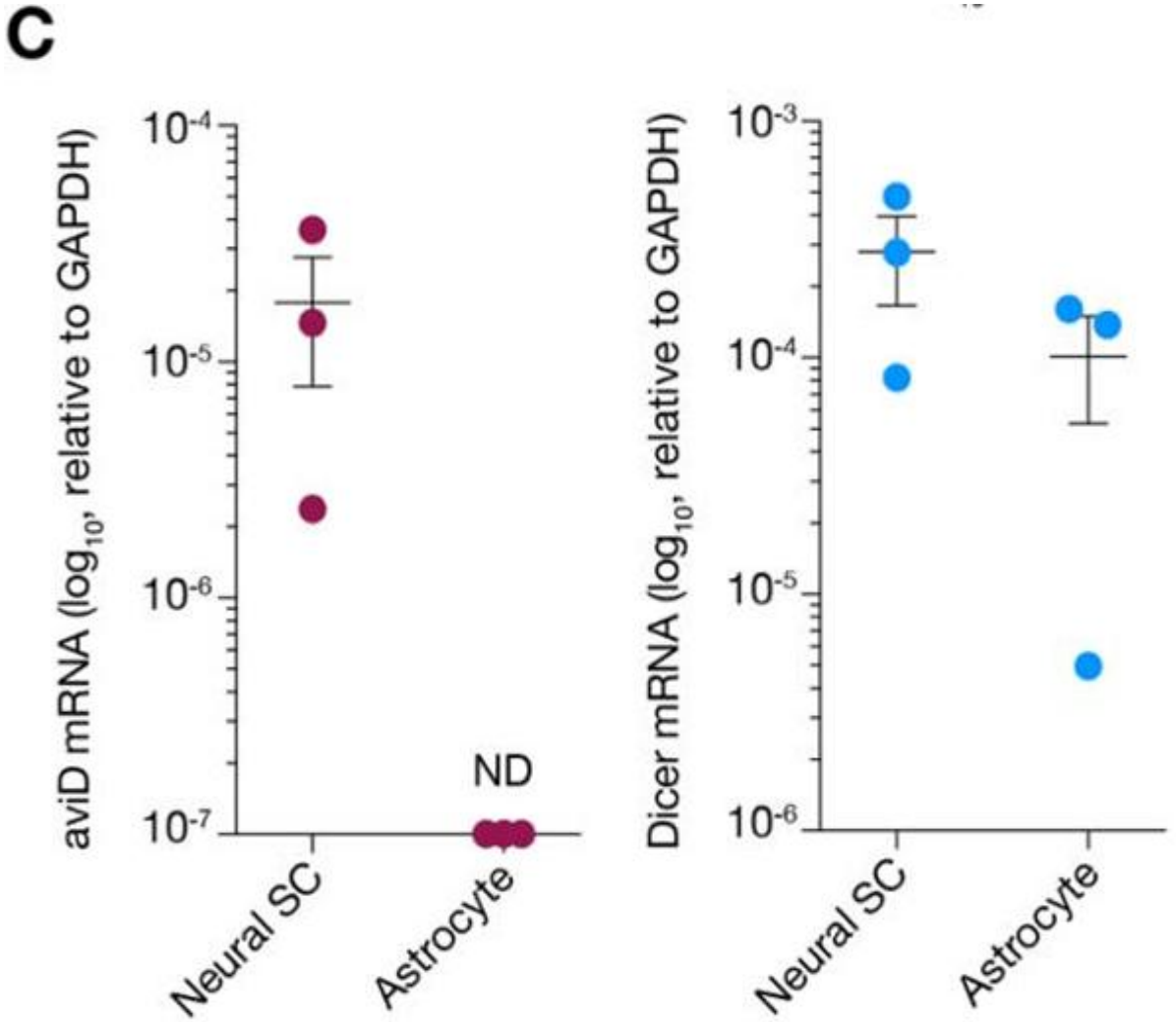
- The **aviD probe** was designed to detect the **exon-exon junction specific to aviD**
- **aviD transcripts colocalized with Lgr5**, a marker of intestinal stem cells, but were **not found in differentiated** cells along the **villi**.

aviD is preferentially expressed in stem cells of different body structures



- **aviD mRNA** was found to be predominantly expressed in a fraction of **Lgr5+ stem cells** in the intestine, as well as in **Lgr5+ hair follicle stem** cells of the skin and in **Sox2+ neural stem cells** of the hippocampus.

aviD expression changes through differentiation

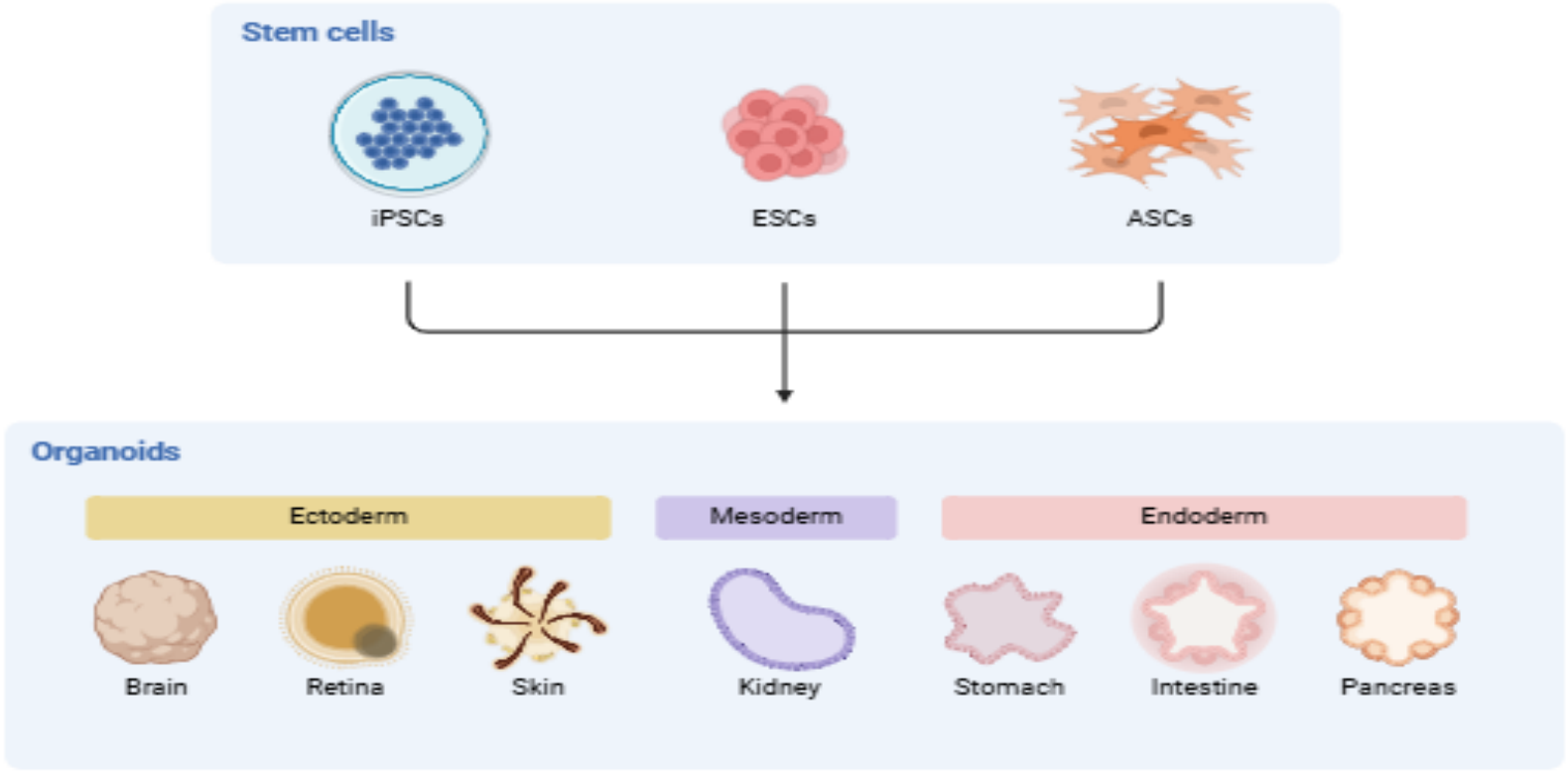


- Astrocytes differentiated from neural stem cells in **culture.**
- aviD expression by **RT-qPCR** was found in **cultured neural stem cells** but, unlike Dicer mRNA, was **lost when the cells were made to differentiate into astrocytes.**

❑ CONCLUSION:

These data suggest that aviD is expressed preferentially by stem cells rather than differentiated cells within adult mouse tissues.

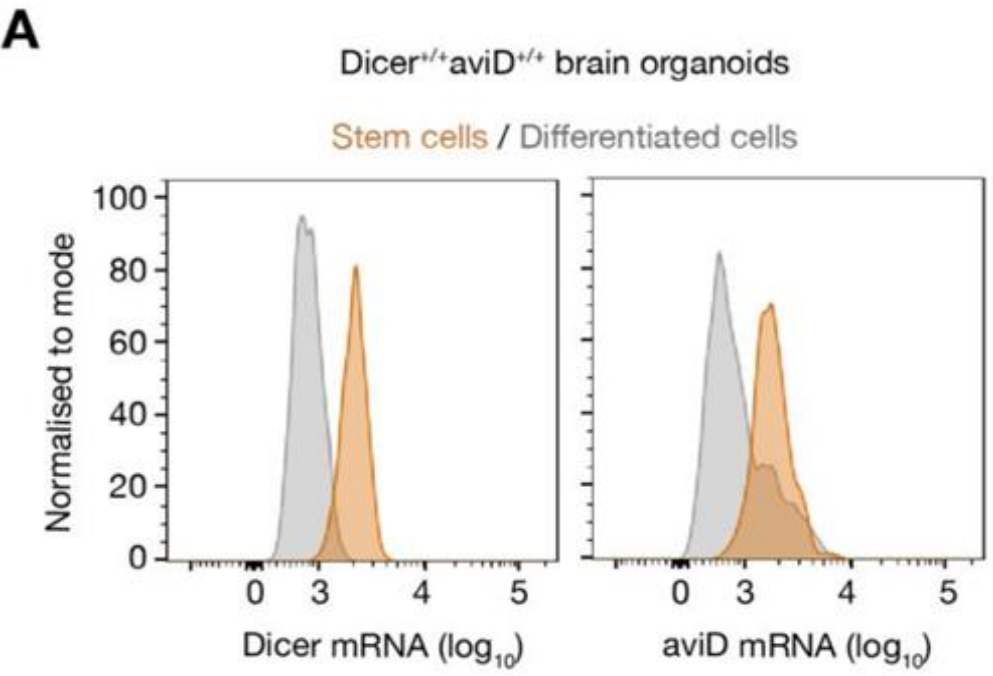
5) A FURTHER LEVEL OF COMPLEXITY: BRAIN ORGANOIDS



aviD is expressed at high levels in Sox2+ neural stem cells

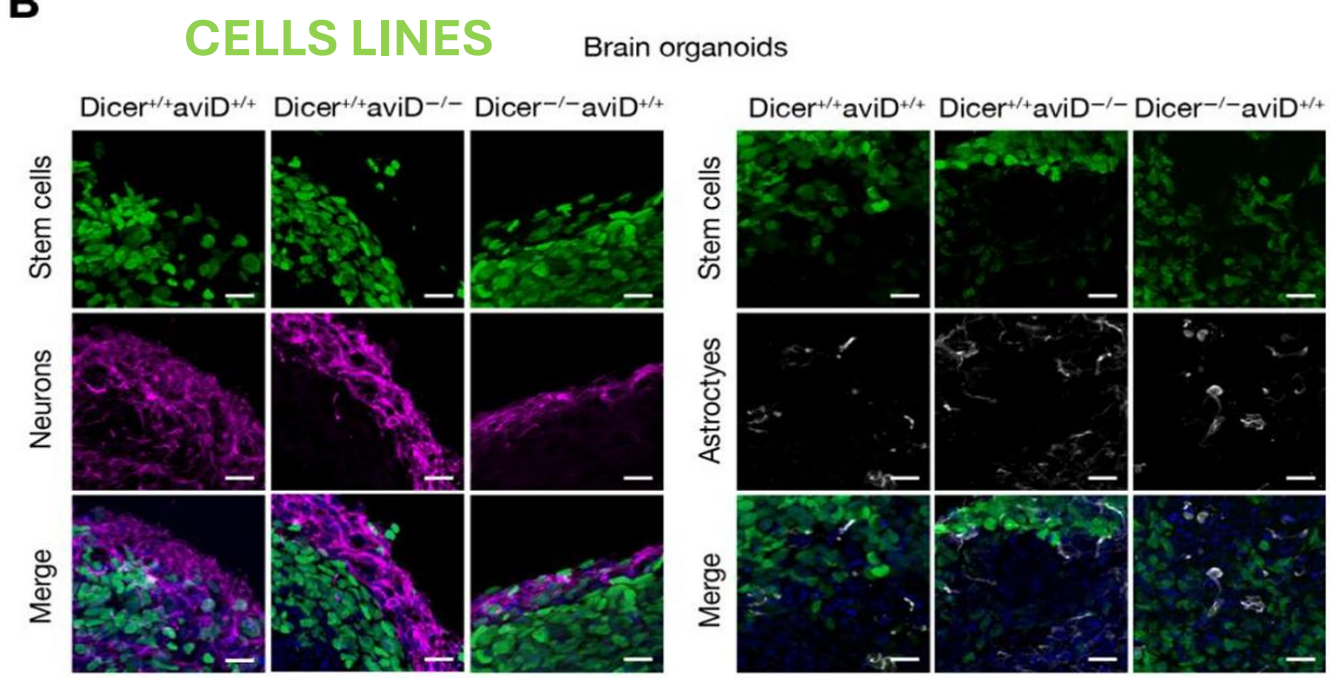
- To study the activity of aviD **brain organoids** were derived from **ES cells** and **recapitulate** the overall organization of the adult brain.

❑ aviD EXPRESSION



- **Sox2+ neural stem cells** present in organoids derived from **wild-type ES cells** expressed **more aviD and Dicer transcripts** than differentiated cells in the same tissue.

❑ ORGANOIDS DERIVING FROM DIFFERENT ES CELLS LINES

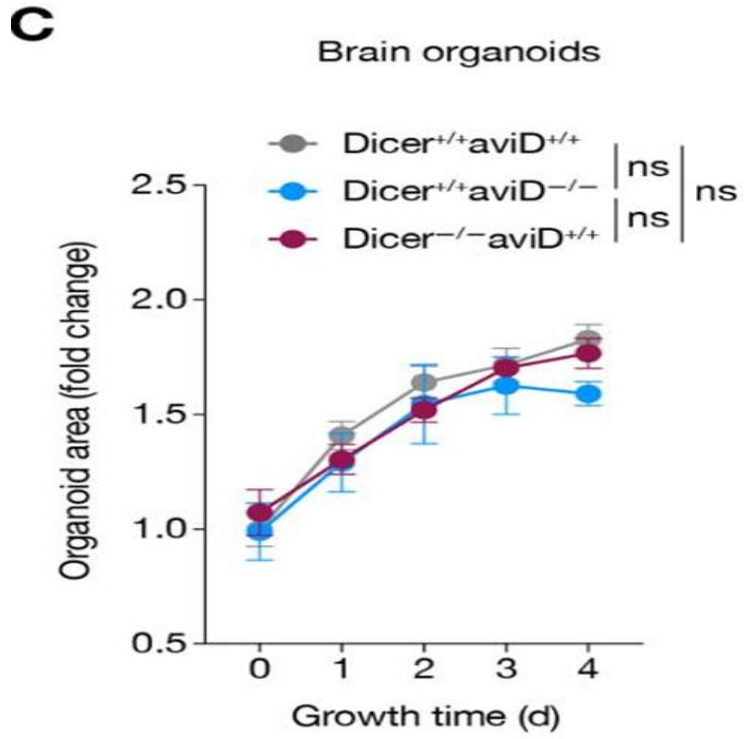


- **Both** Dicer–/– aviD+/+ and Dicer+/+aviD–/– ES cells generated **organoids similar to those made by wild-type** Dicer+/+aviD+/+ ES cells, including differentiated neuronal layers and astrocytes.

Brain organoid's growth is affected by ZIKV infection

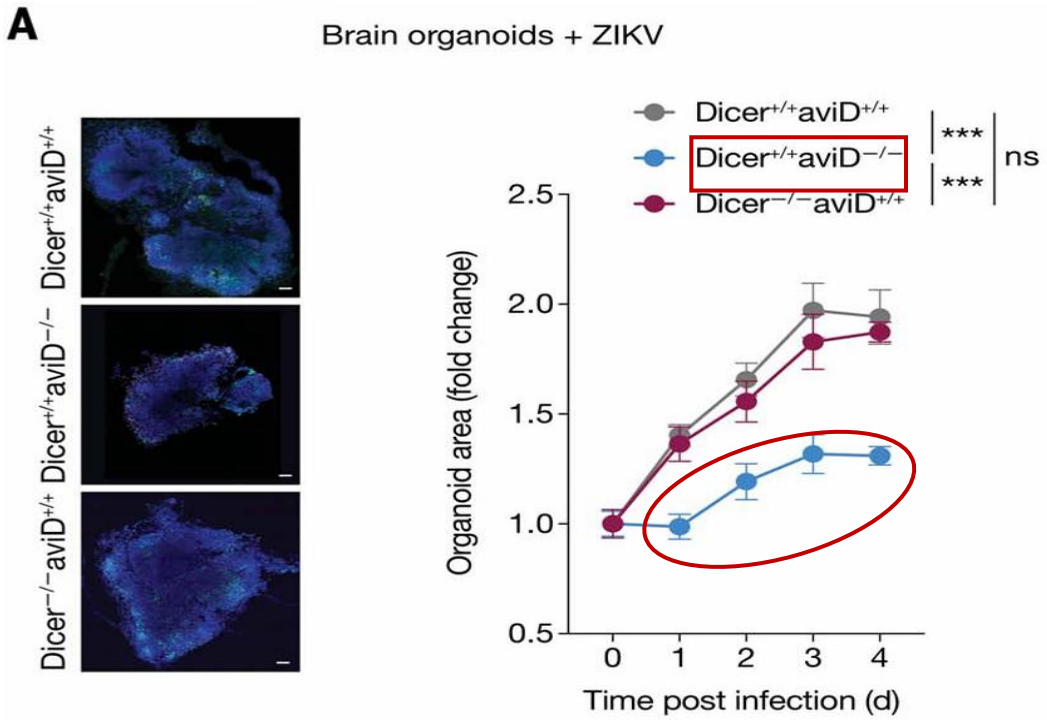
- **ZIKV infection** of brain organoids **preferentially targets Sox2+ stem cells**, resulting in **slower organoid growth** and increased stem cell demise by apoptosis.

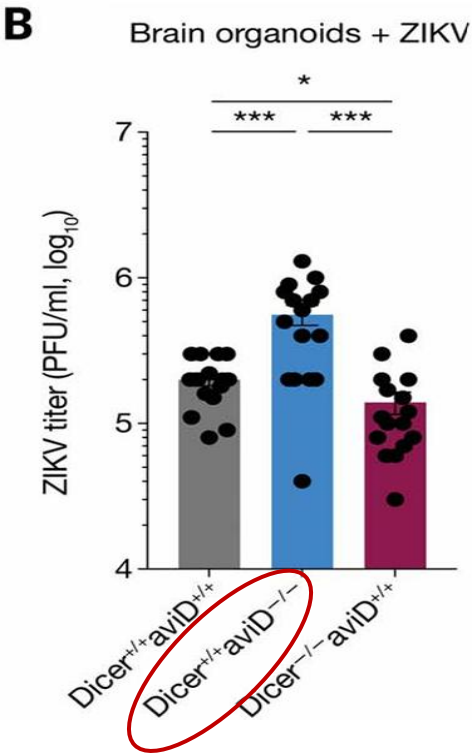
❑ UNINFECTED ORGANOIDS



- Uninfected organoids **grew similarly irrespective** of genotype.

❑ INFECTED ORGANOIDS



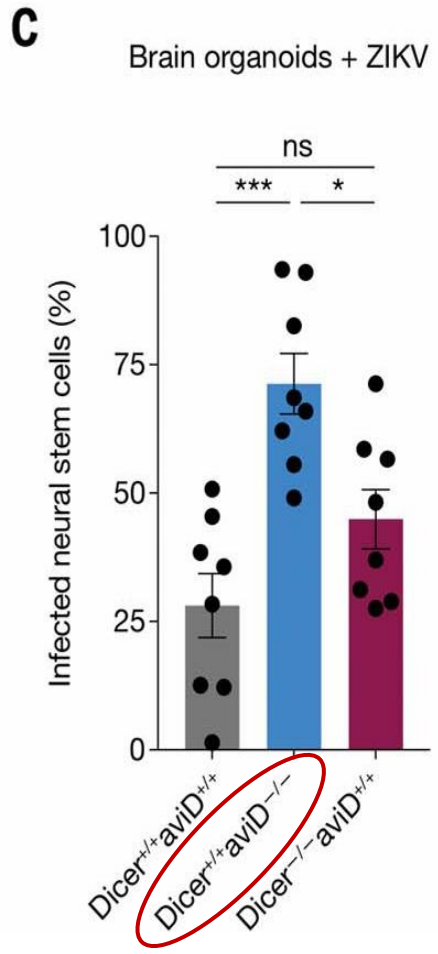


- Dicer+/+aviD–/– organoids
  - **grew more slowly** than Dicer+/+aviD+/+ and Dicer–/– aviD+/+ organoids.
  - **produced more infectious viral** particles.

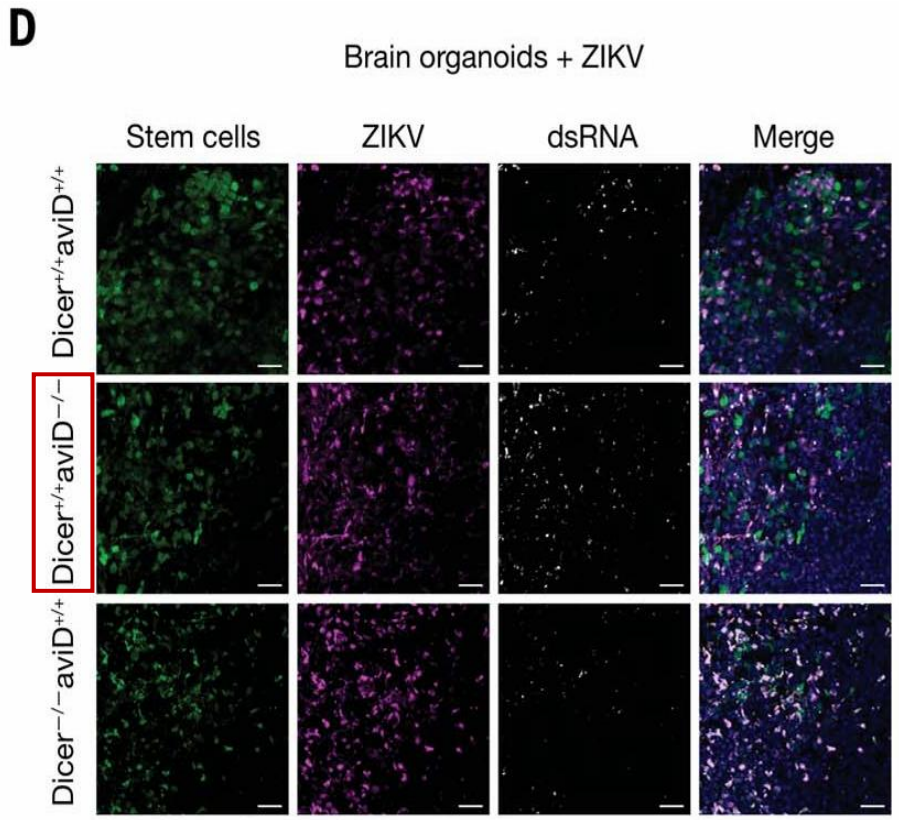
Absence of aviD compromises viral resistance of Sox2+ stem cells

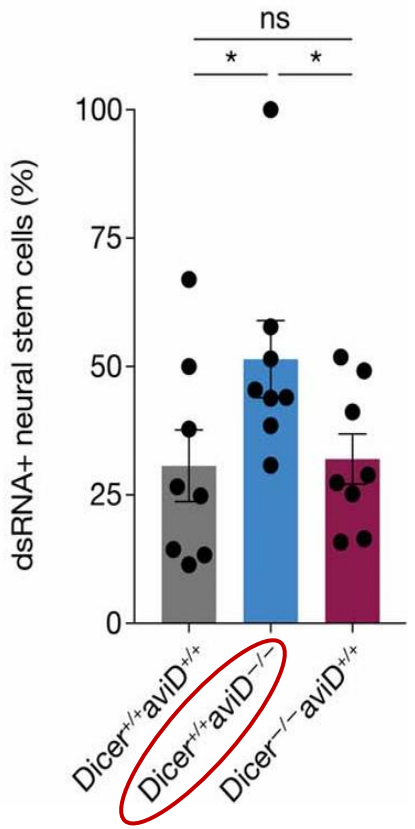
- Stem cells with an **impaired viral resistance (aviD-/-)** show: **higher levels of infections**, **accumulation of dsRNA** and **decreased proliferation.**

❑ INFECTED CELLS

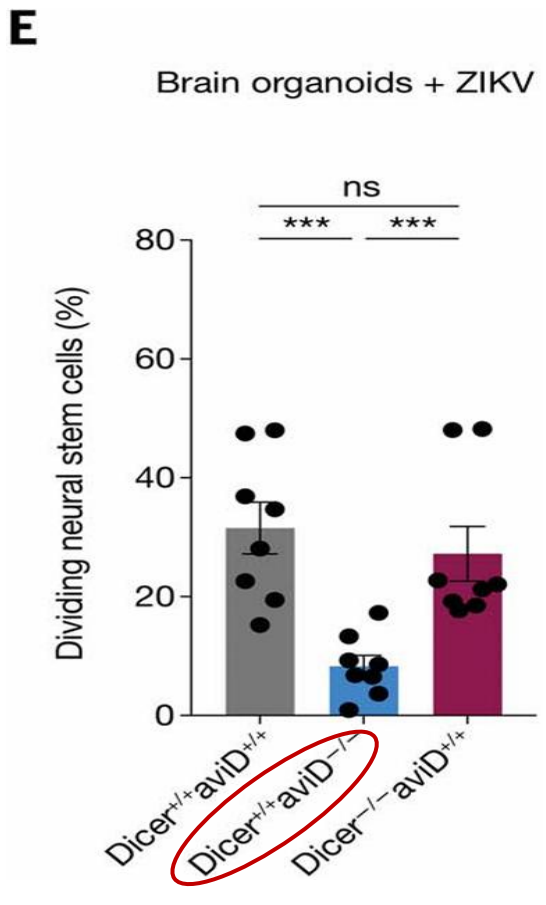


❑ dsRNA ACCUMULATION



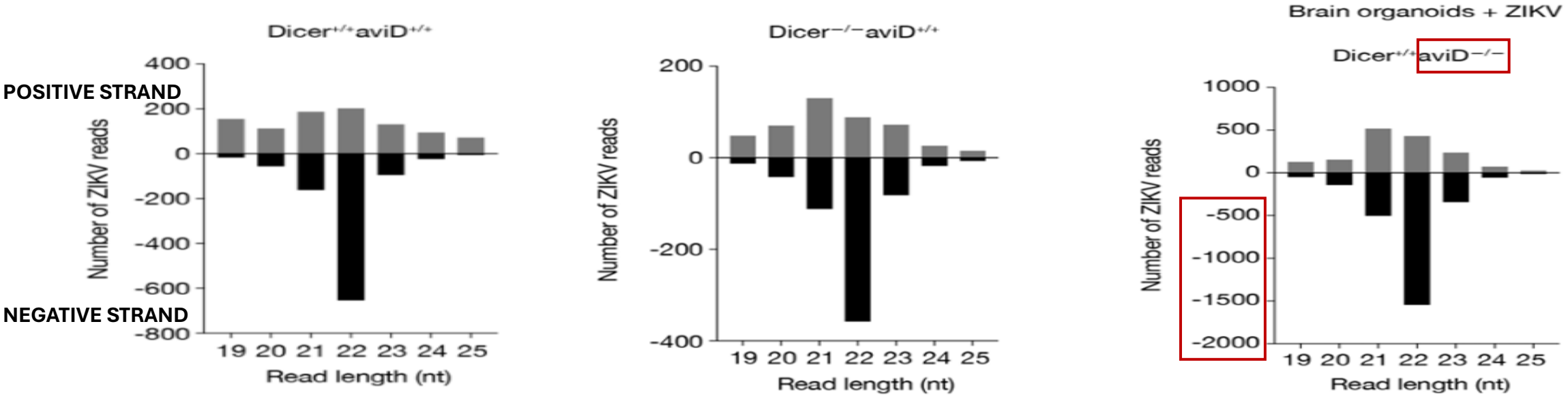


❑ EdU INCORPORATION



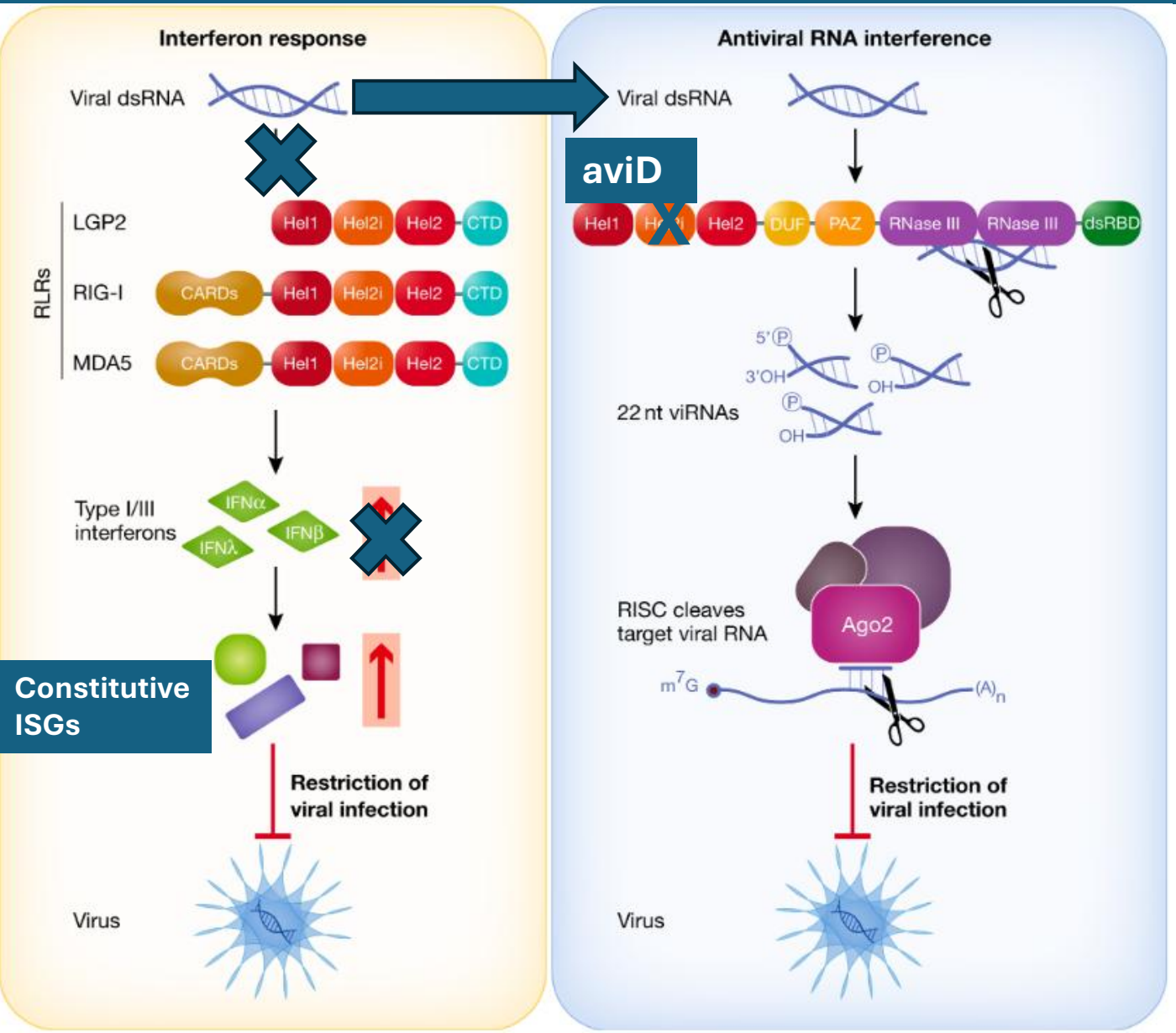
Small RNA sequencing confirms the ability of aviD to restrict viral infection

- **ZIKV-derived small RNAs** from **infected organoids** displayed canonical features of **viral siRNAs**, such as a predominant **length of 22 nucleotides** (nt).
- The **number of viral reads** define the **efficinency** of the **antiviral mechanism:**
  - **High:** failed restriction.
  - **Low:** successful restriction.



- In the **absence of aviD viral replications is not efficiently restricted** and the **number of reads increase drammatically**.
- The abundance of **negative strand-reads** represents the presence of a **higher number of replication intermediates** that represent an **higher rate of genome synthesis.**

CONCLUSIONS



1) **DICER gene** can generate an **alternative transcript that encodes aviD**, a truncated Dicer that helps protect mouse and human stem cells against **RNA virus infection** and **compensates in part for stem cell hyporesponsiveness to innate IFNs**

2) **Mammals,** like plants or insects**, can produce at least two Dicer proteins**, one of which is superior at initiating antiviral RNAi, but **aviD can also process pre-miRNAs** and **compensates for Dicer loss** in miRNA generation when it is the only isoform expressed in the cell.

3) The action of **aviD could deplete infected cells of viral dsRNA**, thereby **eliminating a key trigger of dsRNA-activated proteins of the IFN response pathway** such as RIG-I, MDA5, PKR, or ribonuclease (RNase) L. This is **less important for stem cells** that are **not reliant on the IFN pathway** for **antiviral resistance.**

FUTURE PERSPECTIVES

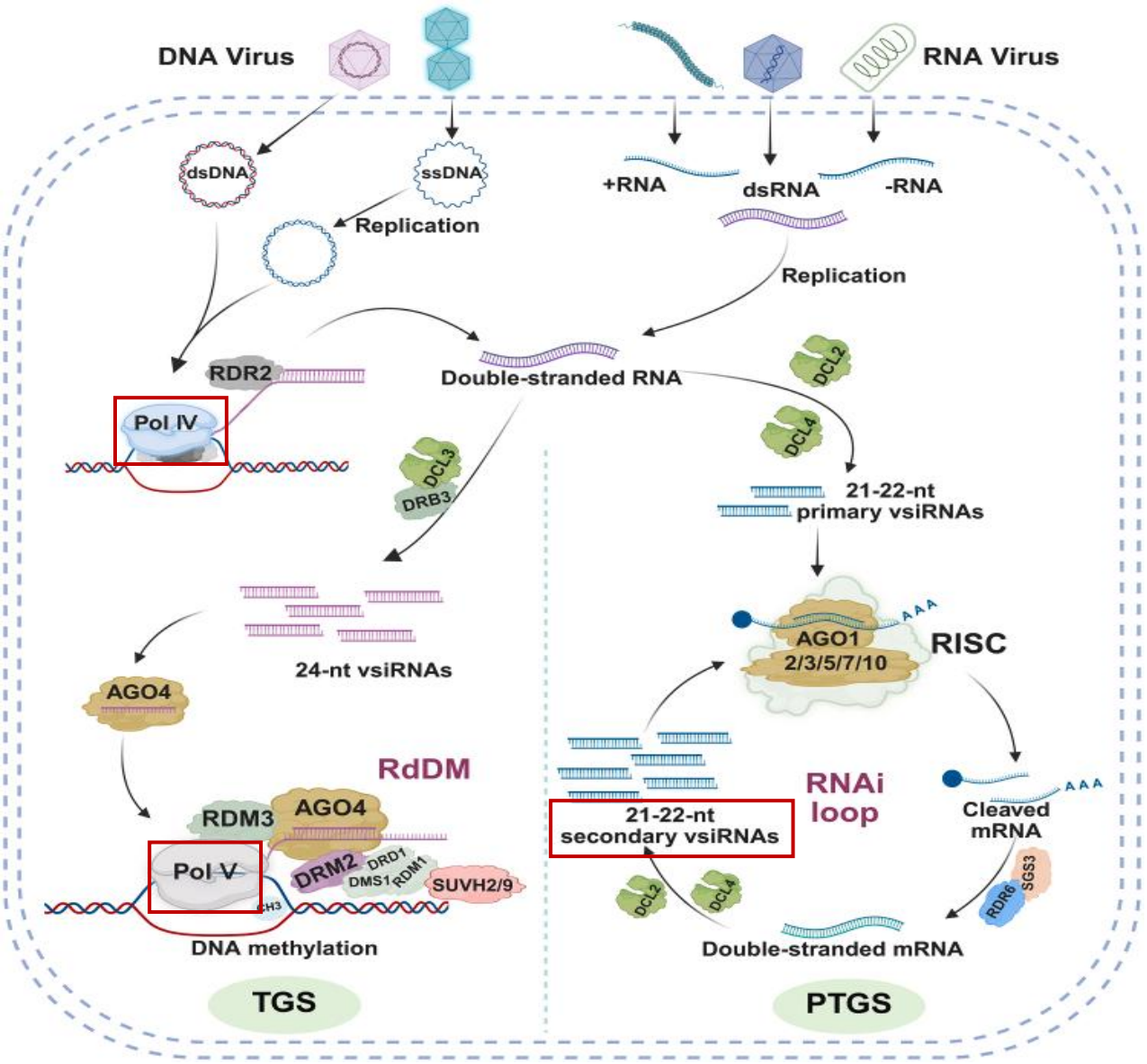
- Exploring and characterizing the **crosstalk** between **RNAi** and other **innate immune pathways** against viral infections through both catalytic and non-catalytic methods.
- Gene silencing induced by **RNAi** represents a **promising antiviral strategy** against acute and chronic infections. However, such technology is **still in its infancy**.
- An **aviD-specific KO mouse** will help to delineate the nonredundant contributions of these distinct strategies and help to design future therapeutic options.

THANK YOU FOR YOUR ATTENTION

QUESTIONS?

SUPPLEMENTARY INFORMATIONS

FOCUS: Antiviral RNAi in plants, not only slicing



doi: 10.1016/j.xplc.2025.101490

1. **Two additional RNA Polymerases** (IV and V), involved in **non coding RNA transcription**

2. **RNA-dependent RNA Polymerases**

3. **RNAi** can be subdivide in **two branches:**
   1. **PTGS** (Post Transcriptional Gene Silencing)
   2. **TGS** (Transcriptional Gene Silencing)

❑ PTGS

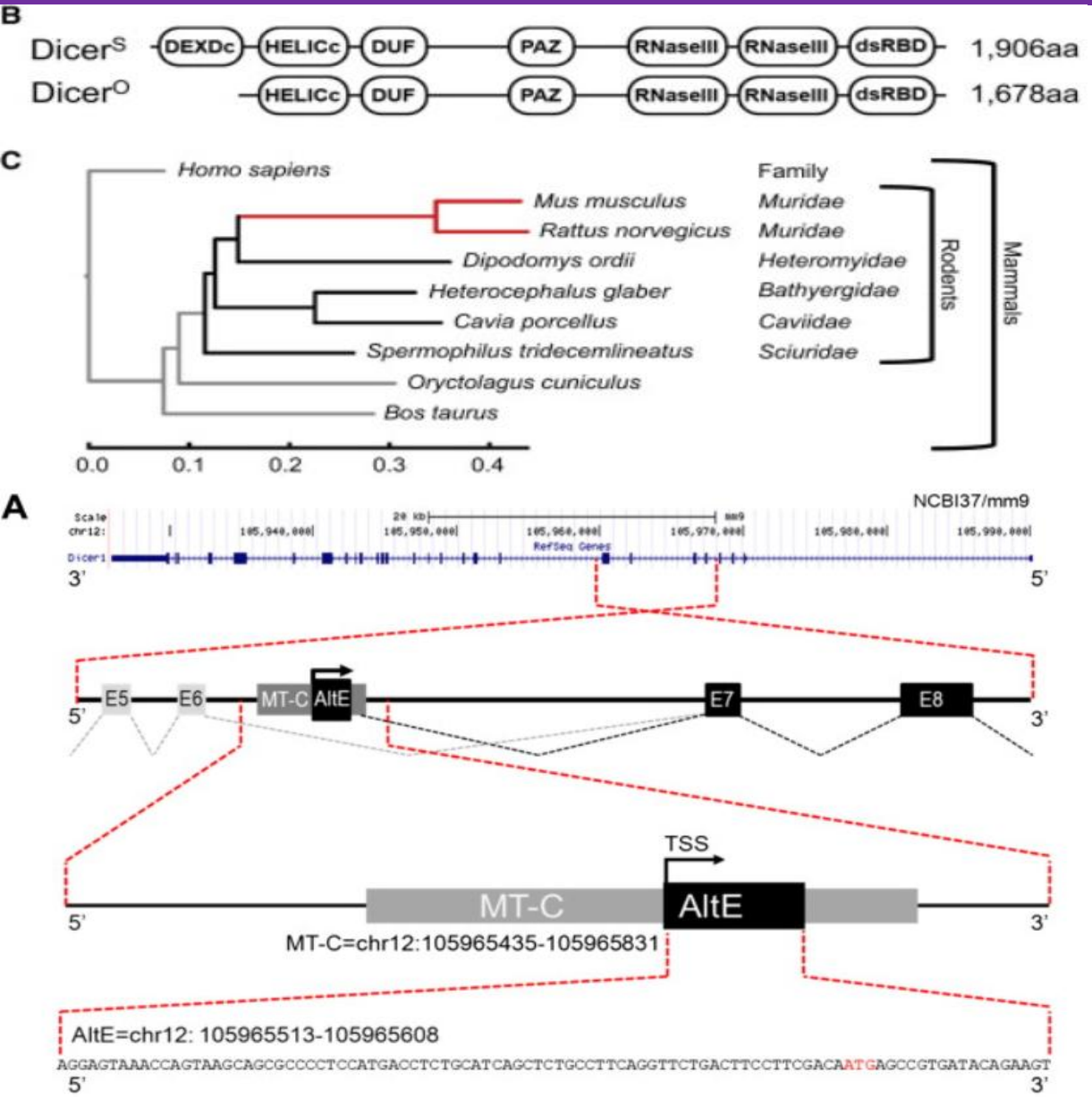
1. **«Canonical»** pathway
2. **«Additional»** pathway: **products of initial RISC cleavage** as templates to **synthesize additional dsRNAs (secondary)**

❑ TGS

- Pathway against **viral genomes integrated** in plants genome.
- Guides **DNA methylation of viral genomes** through the **RdDM (RNA-dependent DNA methylation) pathway**

«A Retrotransposon-Driven Dicer Isoform Directs Endogenous Small Interfering RNA Production in Mouse Oocytes»

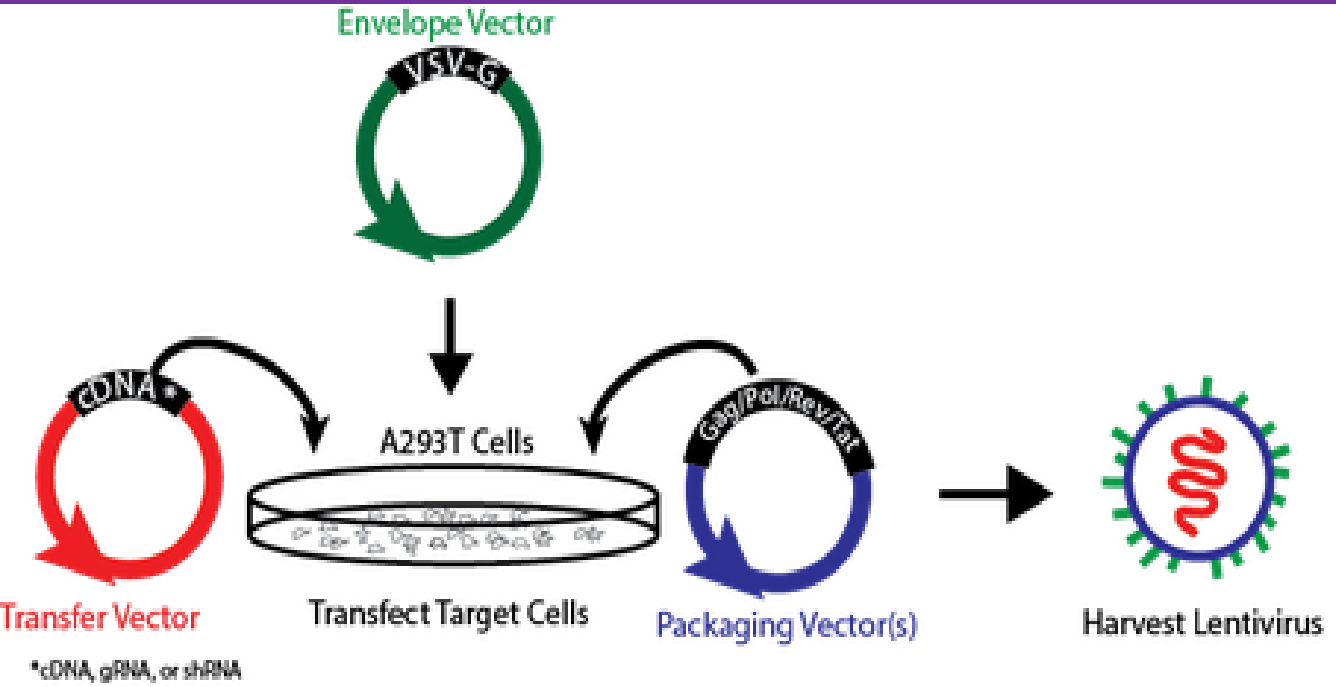
https://doi.org/10.1016/j.cell.2013.10.001



- **Comparative genomic analysis** suggests that the **MT insertion** giving rise to Dicer^{O} occurred relatively recently in the **Muridae family**.
- The MT insertion is **found only in mouse and rat** and is absent from genomes of other sequenced members of other rodent families

- An **alternative exon (AltE)** located **within intron 6** of the Dicer gene.
- AltE is **derived from an MT-C retroelement** of the mammalian apparent long terminal repeat retrotransposon family.
- **MT elements** are **expressed in oocytes** and can serve as **alternative promoters** for adjacent genes.
- AltE serves as the **first exon spliced** at a conserved donor site in frame with exon 7, leading to production of Dicer^{O}

VSV-G pseudotyped lentiviruses



❑ **PURPOSE:**

1. **CUSTOMIZED**: Create **customized viral particles** that can **infect efficiently a specific cell line**
2. **NON-REPLICATIVE:** This viral particle contains **only the gene of interest as genetic material**, so it can not replicate inside the cells, but can only deliver the GOI.

- Use of **3 type of plasmids:**

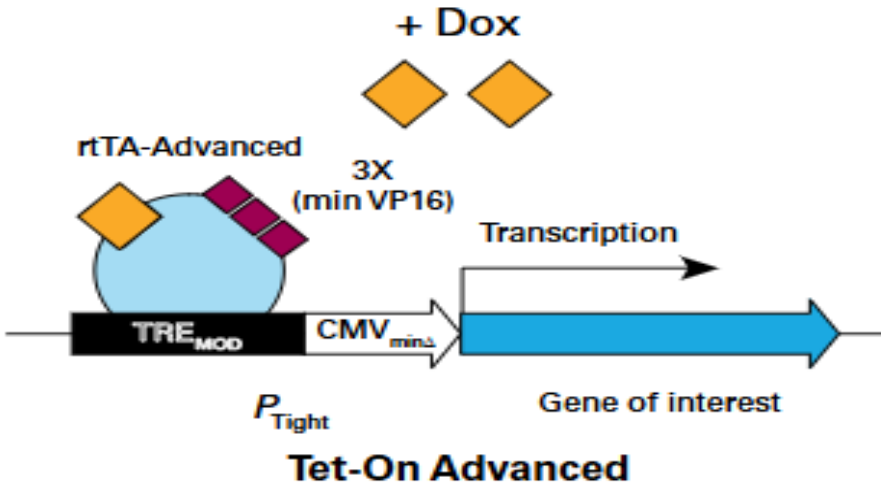
1. **Transfer**: cDNA of interest
2. **Envelope**: envelope of the viral particle
3. **Packaging**: structural proteins and enzymes

→ **NOTE:** There is **NO VIRAL GENOME** = viral particles **CAN'T REPLICATE**, they are a **DEAD END**

→ **STILL DANGEROUS:** they can **enter human cells** and **deliver the GOI**

Lenti-X Tet-On Advanced Inducible Expression System

❑ PLASMIDS for the Tet-On SYSTEM



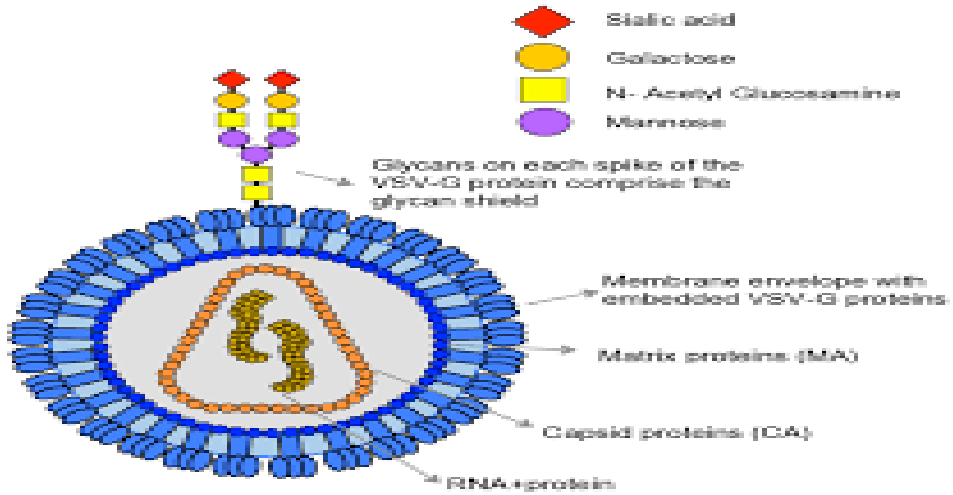
1) pLVX-Tet-On Advanced:

- Constitutively expresses the **tetracycline-controlled transactivator**, rtTA-Advanced

2) pLVX-Tight-Puro:

- Contains **PTight**, an **inducible promoter**
- It consists of:
  - a **modified Tet-Responsive Element** (TREMod) joined to a **modified minimal CMV promoter** (PminCMVΔ)
  - **lacks binding sites for endogenous mammalian TFs**

❑ PLASMIDS for VIRAL PACKAGING



1) pMD2.G

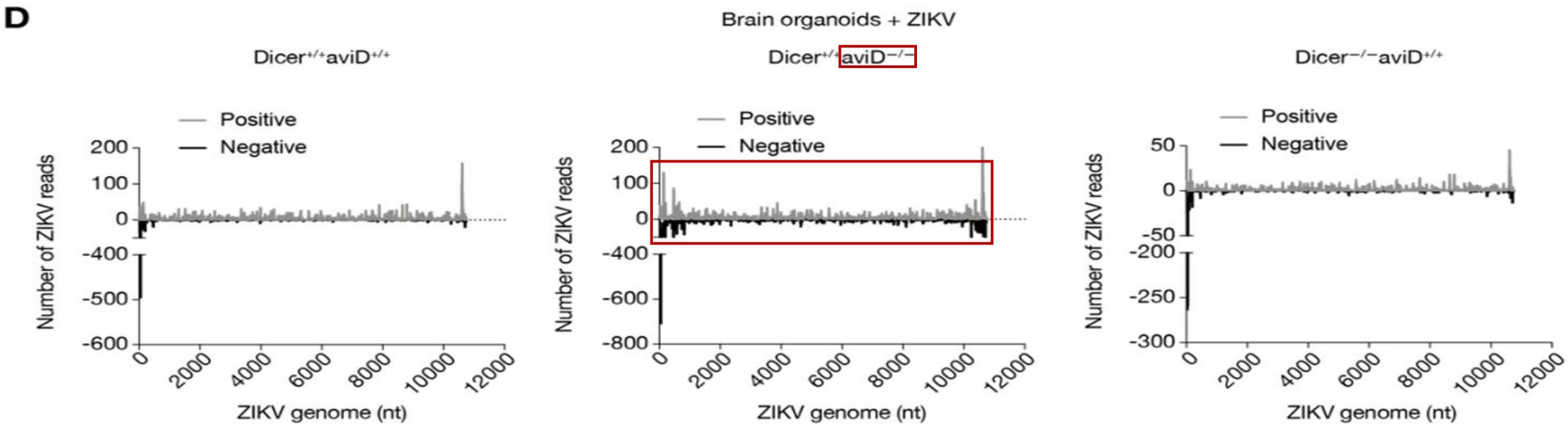
- Contains the **VSV-G protein** (Vesicular Stomatitis Virus Glycoprotein).

2) psPAX2

- internal **structural proteins** and **enzymes** derived from HIV

ZIKV genome sequencing conferms the aviD mediated restriction of viral progeny

- Another aspects that confirms the importance of aviD for viral restriction is represented by the **distributions of 22nt siRNAs reads** deriving from the **positive** and **negative strands** of **ZIKV genome.**



- The **presence of aviD** is correlated to the **low abundance of negative strand-reads**. aviD produces **more siRNAs** from viral dsRNA, which **load into Ago2**. **Ago2 slicing reduces viral replication**, limiting production of negative-strand intermediates.
- In the **absence of aviD**, virus can **replicate more efficiently** and so **many negative strands** can be observed.

Also SARS-CoV-2 can be restricted by aviD