

# microRNAs are key regulators of the development and functional differentiation of $\gamma\delta$ T cell subsets

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## Introduction

The ability of murine  $\gamma\delta$  T cells to rapidly produce the pro-inflammatory cytokines interleukin-17 (IL-17) or interferon- $\gamma$  (IFN- $\gamma$ ) underlies their crucial and non-redundant roles in several (patho)physiological contexts, such as tissue homeostasis, infection, autoimmunity and cancer. This capacity stems from a complex process of 'developmental pre-programming' in the thymus, after which a large fraction of  $\gamma\delta$  T cells migrate to peripheral sites already committed to producing IL-17 or IFN- $\gamma$ , unlike their  $\alpha\beta$  T cell counterparts. So far, several miRNAs have been implicated in the control of the differentiation and IFN- $\gamma$  and IL-17 levels by  $\alpha\beta$  Th1 and Th17 cells, respectively<sup>1</sup>. However, little is known about the action of these post-transcriptional regulators on  $\gamma\delta$  T cell differentiation. Schmolka *et al.* showed that miR-146a is selectively enriched in IL-17-biased CD27<sup>+</sup>  $\gamma\delta$  T cells and restricts their co-production of IFN- $\gamma$  by targeting *Nod1* mRNA, therefore regulating  $\gamma\delta$  T cell plasticity<sup>2</sup>. This isolated work illustrates the need of a more comprehensive study of the miRNA repertoires of  $\gamma\delta$  T cells and of the regulatory networks they take part in the control of IFN- $\gamma$  and IL-17 production by these cells.

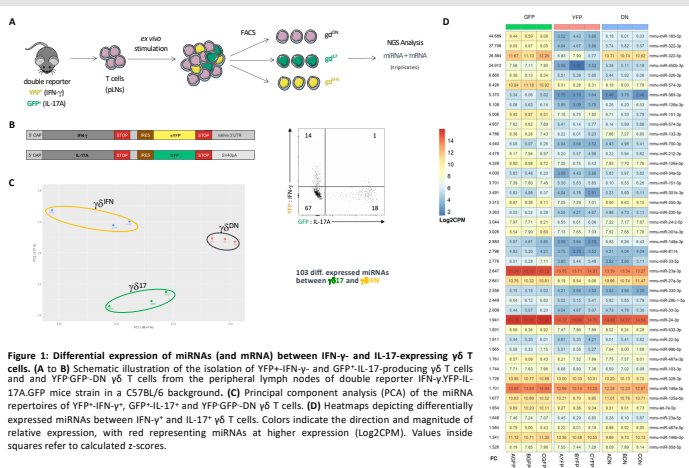
## Aims

To characterize the miRNA:mRNA regulatory networks that regulate IFN- $\gamma$  and IL-17 expression in  $\gamma\delta$  T cells subsets *in vivo*, we will:

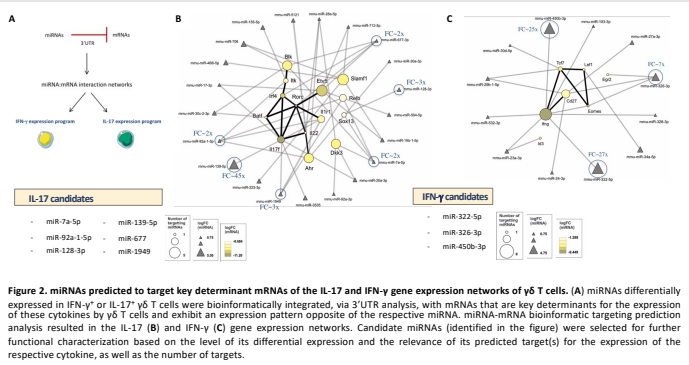
1. Identify the miRNA and mRNA repertoires of pure IL-17- and IFN- $\gamma$ -producing  $\gamma\delta$  T cells;
2. Determine the functional impact of specific miRNAs on  $\gamma\delta$  T cell differentiation;
3. Analyse the regulation of candidate miRNA expression in IFN- $\gamma$  and IL-17<sup>+</sup>  $\gamma\delta$  T cells;
4. Identify mRNA networks controlled by candidate miRNAs.

## Results

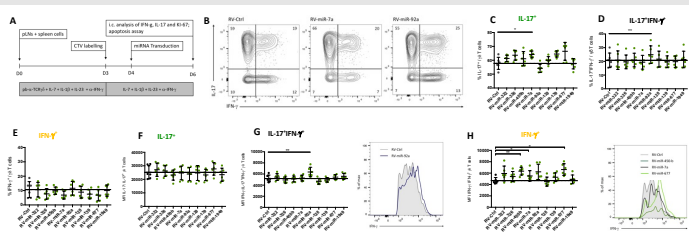
### 1. miRNA (and mRNA) profiling of IFN- $\gamma$ <sup>+</sup> and IL-17<sup>+</sup> $\gamma\delta$ T cells



### 2. miRNA:mRNA interaction networks



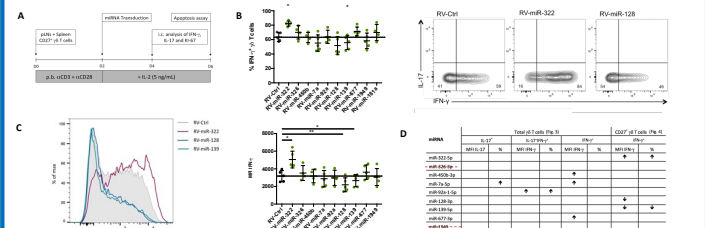
### 3. Candidate miRNA overexpression during in vitro gamma delta T cell expansion



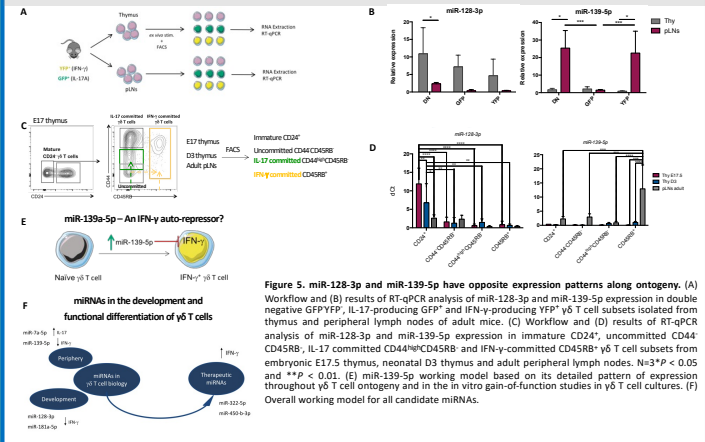
## References

1. Tan L, Inácio D, Prins J and Silva Santos B. New insights on murine  $\gamma\delta$  T cells from single-cell multi-omics. *Sci Bulletin*. 2022. doi:10.1016/j.scib.2022.03.008
2. Inácio D, Amado T, Silva Santos B, Gomes AQ. Control of T cell effector functions by miRNAs. *Cancer Lett*. 2018;427:83-93. doi:10.1016/j.canlet.2018.04.024
3. Schmolka M, Papotto PH, Romero PV *et al.* MicroRNA-146a controls functional plasticity in  $\gamma\delta$  T cells by targeting *NOD1*. *Sci Immunol*. 2018;3(2):eaao1392. doi:10.1126/sciimmunol.aao1392

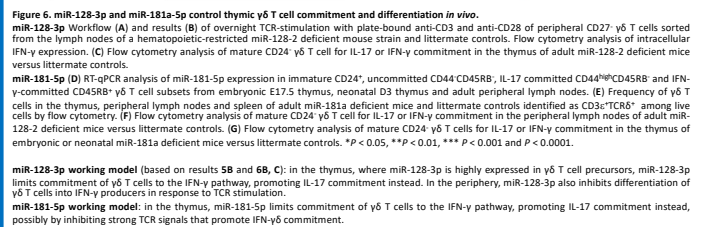
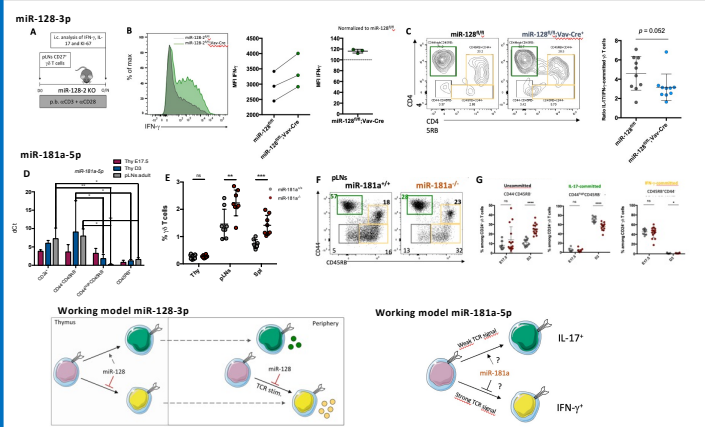
### 4. miR-128-3p and miR-139-5p restrict IFN-gamma production in peripheral gamma delta T cells



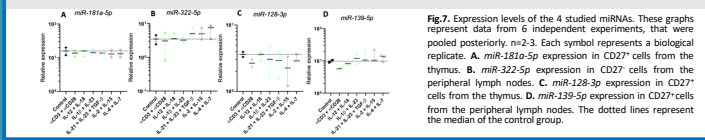
### 5. miR-128-3p and miR-139-5p have opposite expression patterns along ontogeny



### 6. miR-128-3p and miR-181a-5p control thymic gamma delta T cell commitment and differentiation in vivo



### 7. Modulation of candidate miRNA levels by extracellular cues



## Open questions/future work

1. Does miR-139 function as an IFN- $\gamma$  auto-repressor *in vivo*?
2. What is the  $\gamma\delta$  T cell phenotype of embryonic/neonatal miR-128-2 KO mice?
3. What are the relevant mRNA targets of the candidate miRNAs?