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# sncRNA regulatory networks in $T_H$ cell activation, differentiation and HIV infection

BioSys PhD programme retreat

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Co-supervisor: Prof. Francisco Pinto

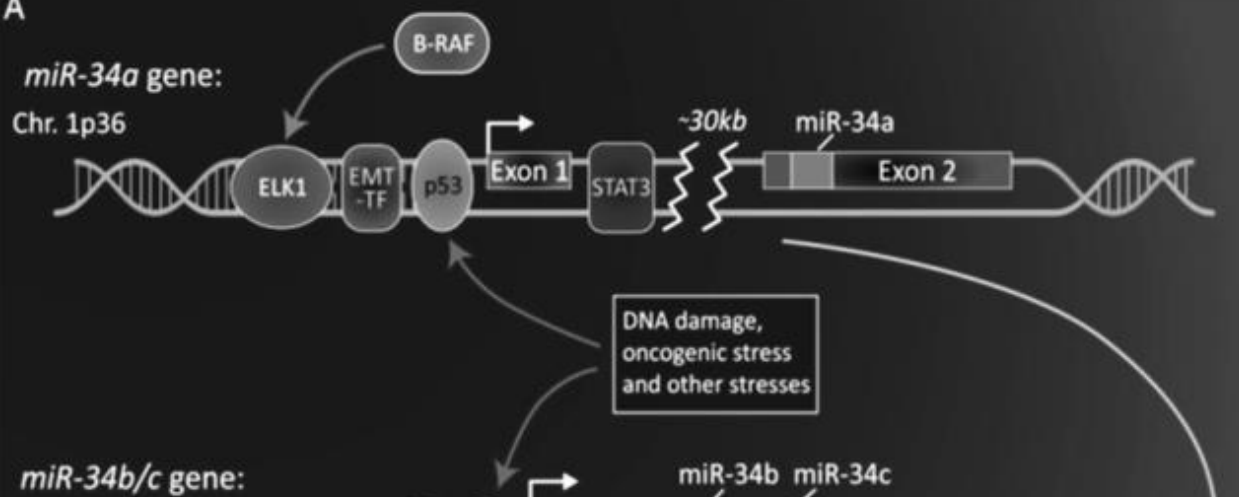


PD + F

FCT PhD PROGRAMMES

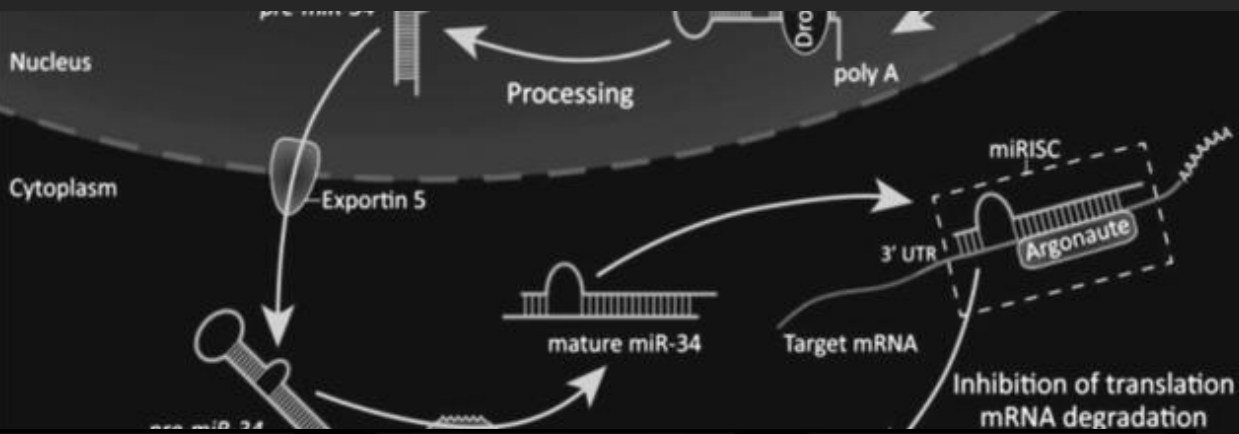
Beja, 7<sup>th</sup> October 2017

A

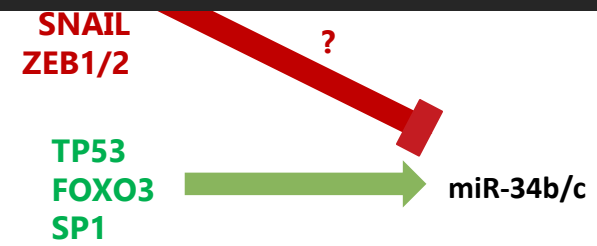


# miRNA biogenesis and miR-34 family

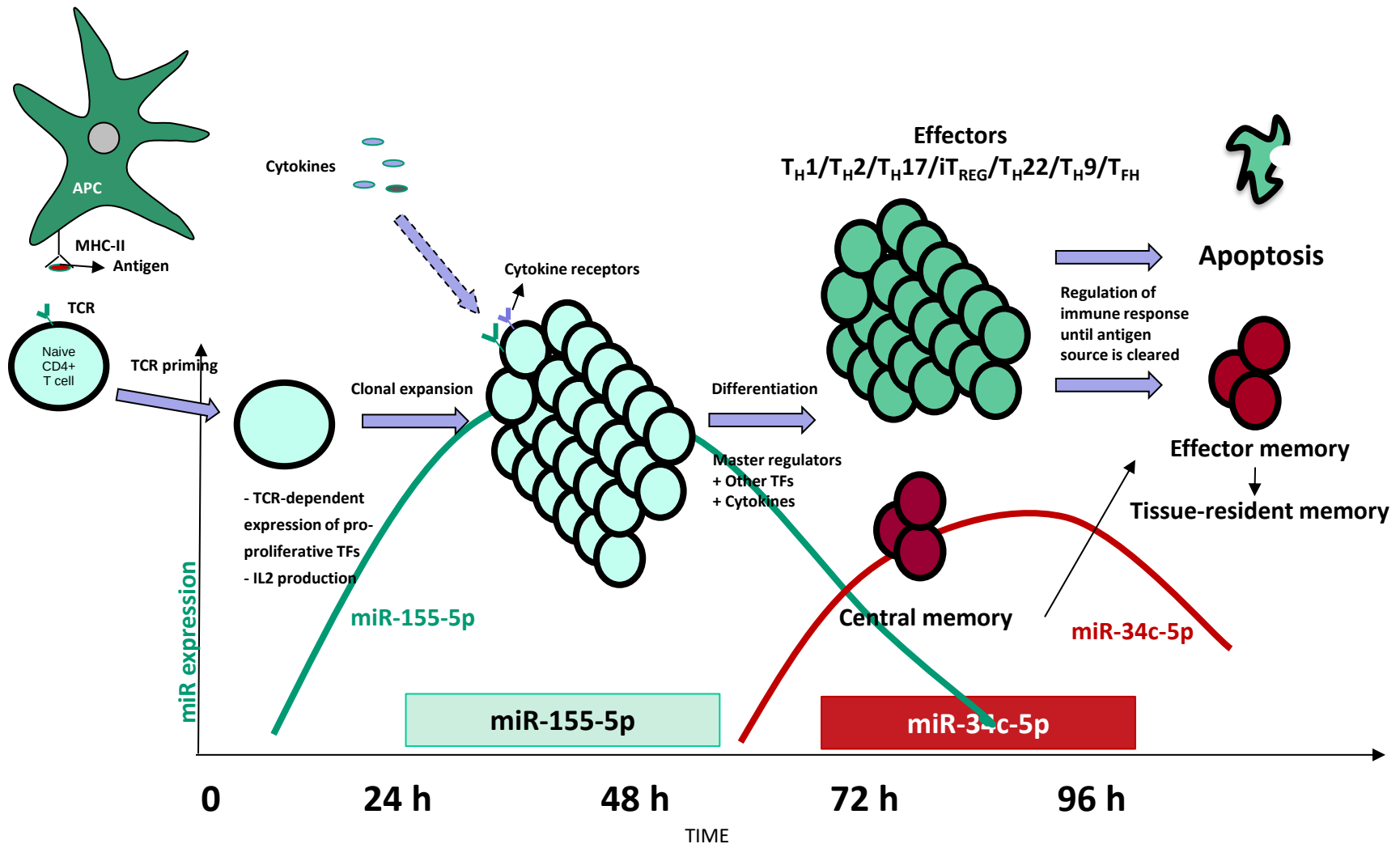
## Growth arrest and differentiation

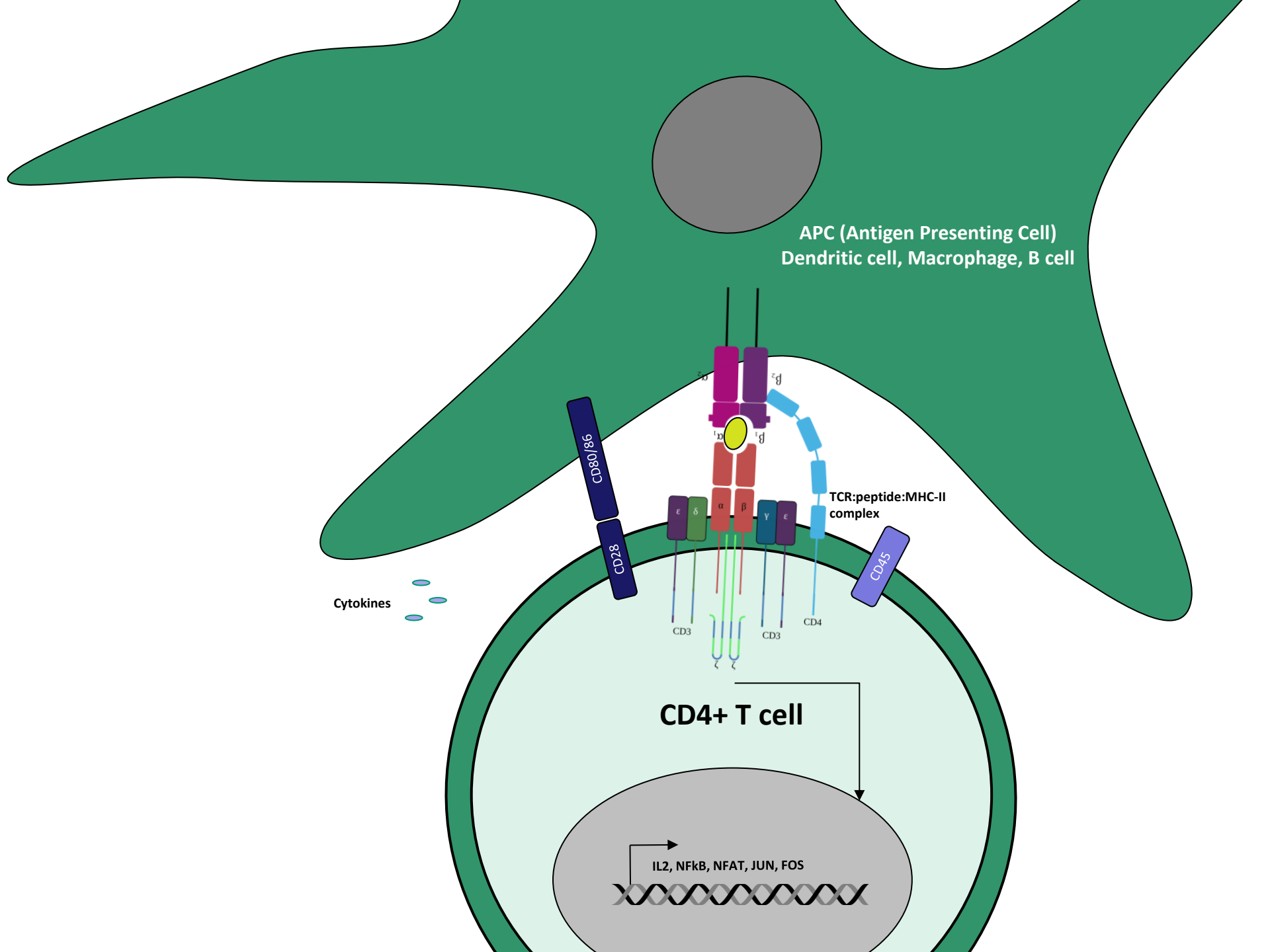


- CDK4/6, E2F3, CCND1, CCNE2,
- Arhgap2, AXL, MTA2, PDGFR $\alpha$ ,
- ALDH2, ATF1, BCL2,

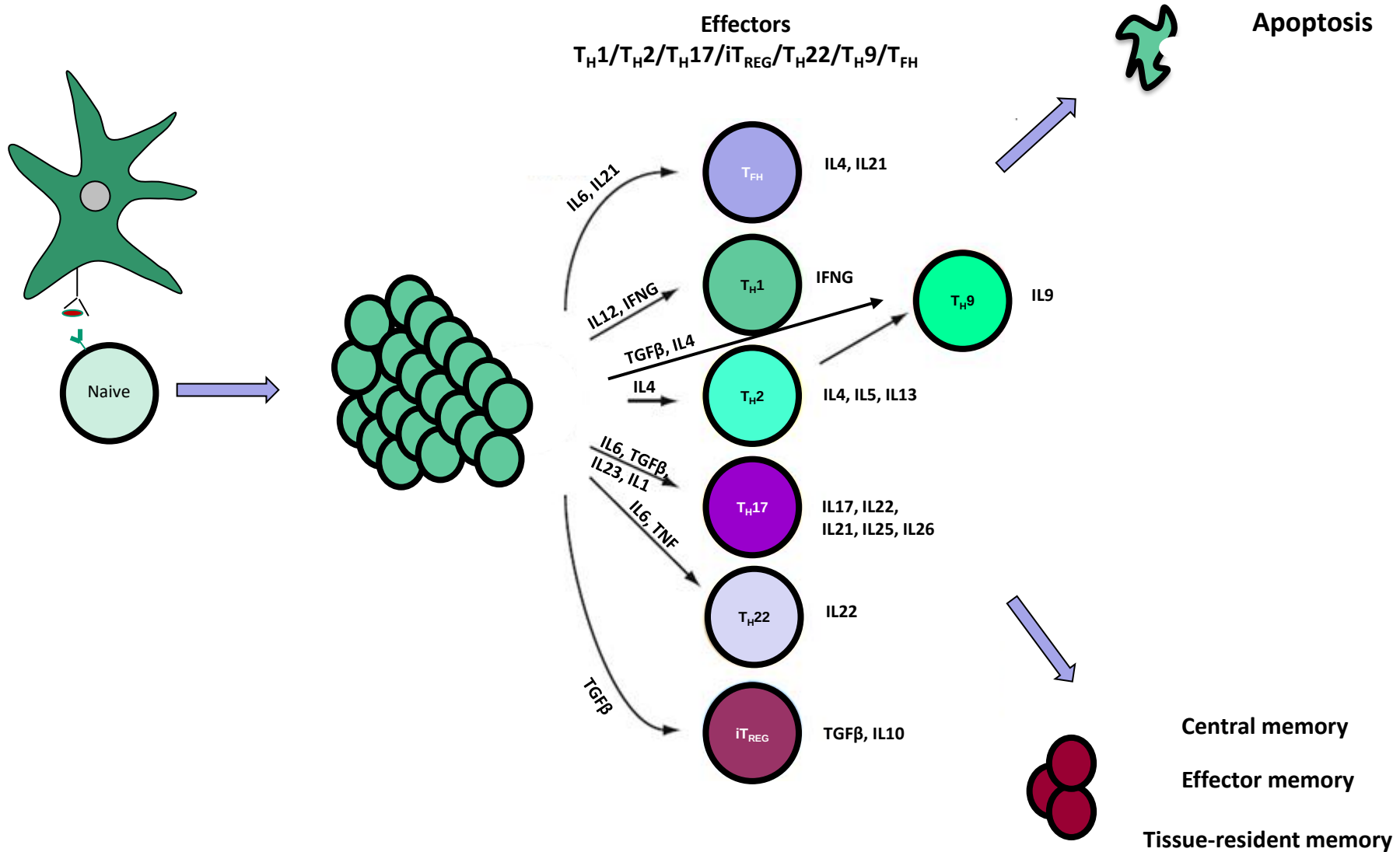


# CD4+ T cell ( $T_H$ ) response and immune miRs





# CD4+ T cell differentiation



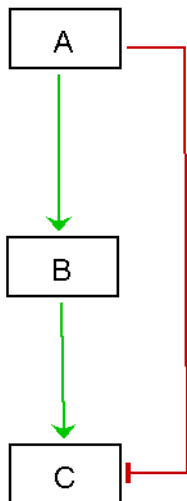
# Logical modelling framework

## Discrete logical models

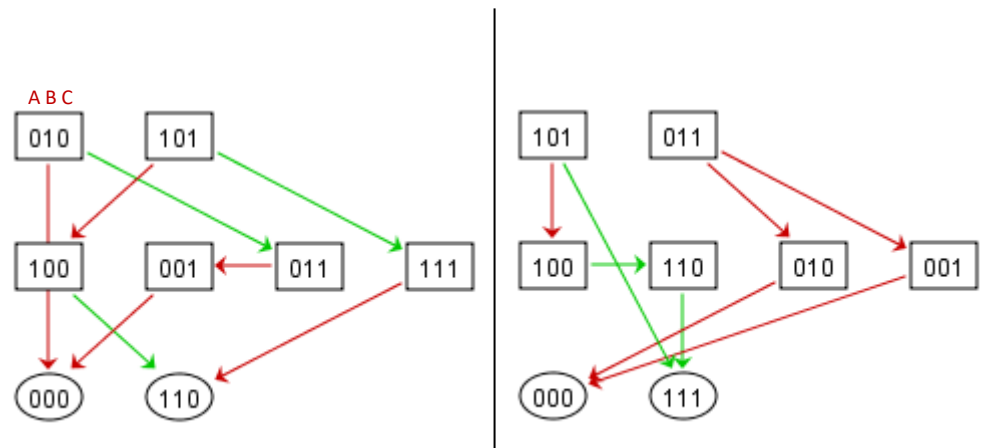
Simulate signalling and regulatory networks

### Static analysis

Node states 0 ; 1 ; (2) ...



### State transition graphs



Steady-states

PREDICT SYSTEM BEHAVIOURS

# OBJECTIVES

- I. Establish a modelling framework to study CD4+ T cell activation and differentiation with *in vitro* TCR and IL2 stimulation for prediction of miR-34c regulation and function
- II. Validate those mechanisms of miR-34c regulation by understanding which factors regulate its transcription and validate miRNA-mRNA interactions, and determine its role in CD4 T cells in Th1/Th2 polarizing conditions (TCR + IL2 stimulation)
- III. Determine whether the detected conserved miRNA-like sequences detected in HIV1 and HIV2 infected CD4 T cells are *de facto* HIV encoded sncRNAs

**model**

Cytokines

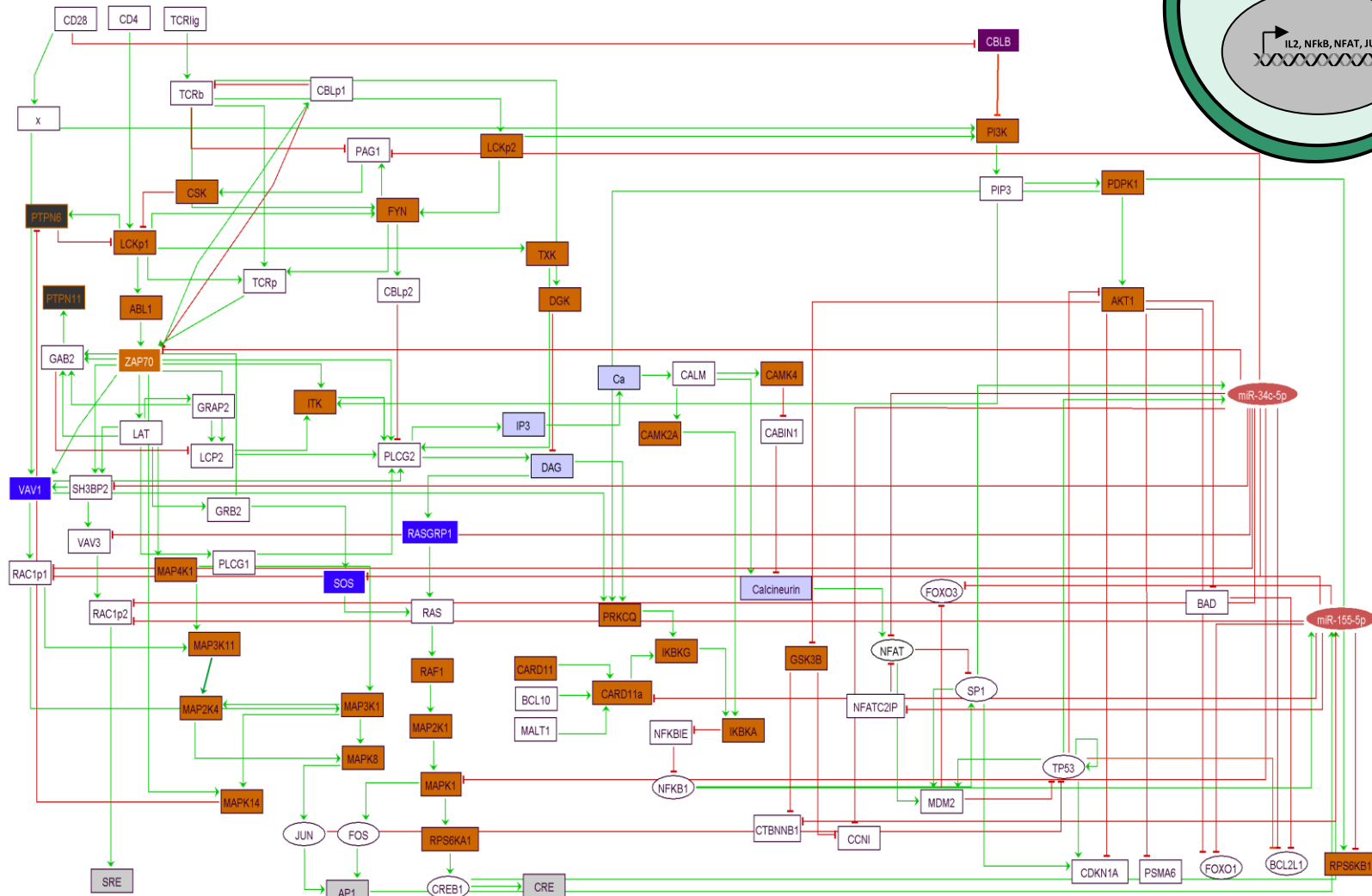
TCR: peptide:MHC-II complex

CD3

CD4

CD4+ T cell

IL2, NFkB, NFAT, JUN, FOS





# TOOLS

**R** – Steady-state analysis and simulations

**Linux Shell** – target prediction

**GINsim** – user interface for building logical networks

**multiMiR** – miRNA validated targets R package

**PITA, Miranda** – miRNA-mRNA target prediction

**mfold and RNAfold** – RNA structure prediction

**JASPAR** – TFBS predictions

# DATABASES

**Genome browsers from UCSC, Zenbu and ensembl**

**IHEC, Roadmap** – Epigenetic marks

**ENCODE** – ChIP-Seq data

**FANTOM5** – TSSs by CAGE-Seq

**Cytoscape (GeneMania) and STRING** – visualize networks

# T<sub>H</sub> cell activation model

| miRs                    | Activation model changes in state values                                                       |
|-------------------------|------------------------------------------------------------------------------------------------|
| miR-155-5p              | RAC1*, MAP3K11, MAPK14, CTNBB1, RPS6KB1, MAP3K1, MAP2K4, MAPK8, JUN**, NFKB                    |
| miR-34c-5p              | ZAP70**, RAC1 *, MAP3K11, MAPK14, CCNI, RPS6KB1, MAP3K1, MAP2K4, MAPK8, JUN, CREB, MAPK1**     |
| miR-155-5p + miR-34c-5p | ZAP70, RAC1 *, MAP3K11, MAPK14, CCNI, RPS6KB1, MAP3K1, MAP2K4, MAPK8, JUN, CREB, MAPK1, CTNBB1 |

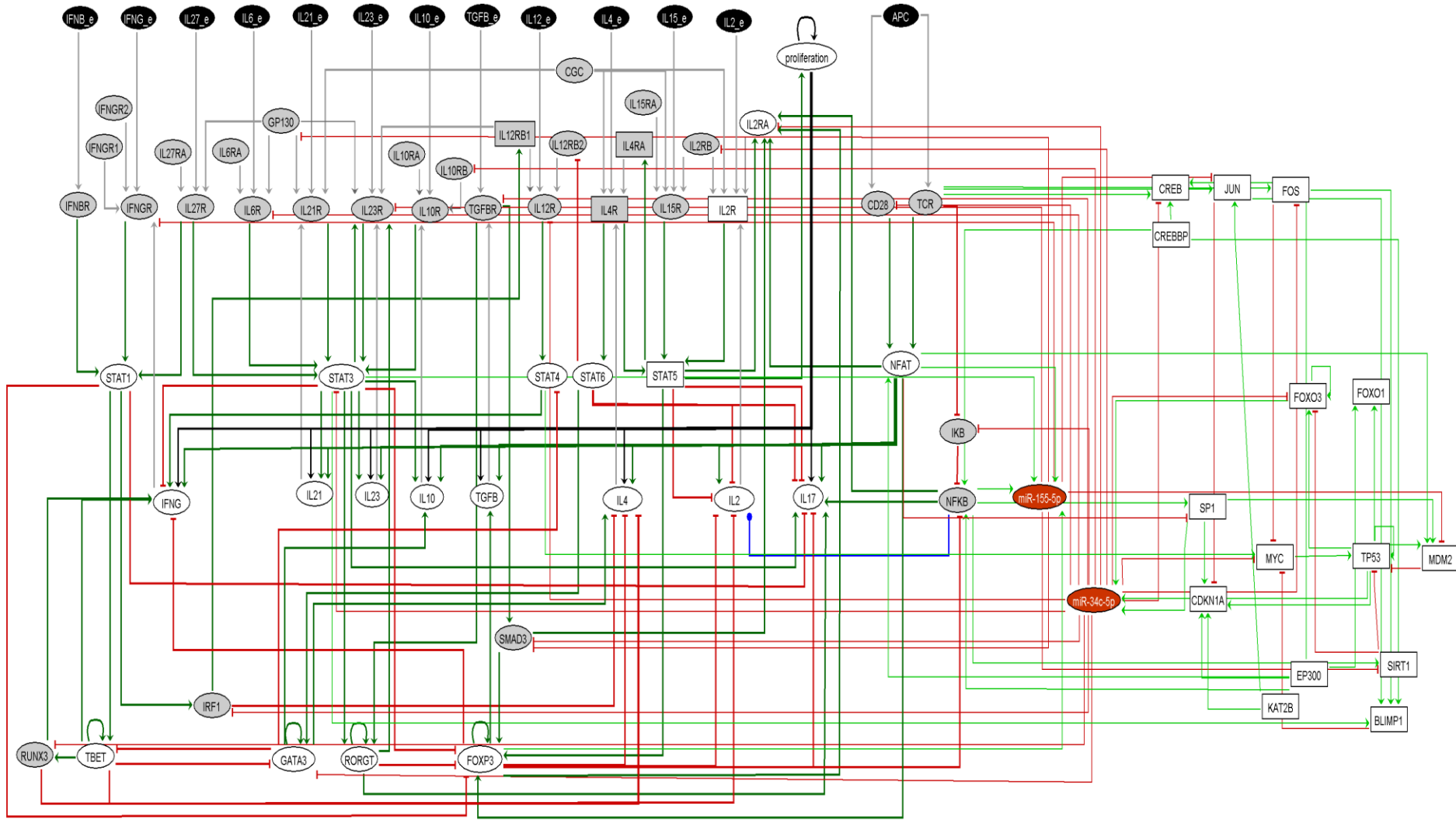
\*Necessary and sufficient target for the remaining changes of state value.

\*\* Validated targets.

**miR-34c-5p affects AP1, NFKB and CREB activation**  
**RAC1 is the critical target of up-regulated miRs during activation**

## T<sub>H</sub> cell differentiation models

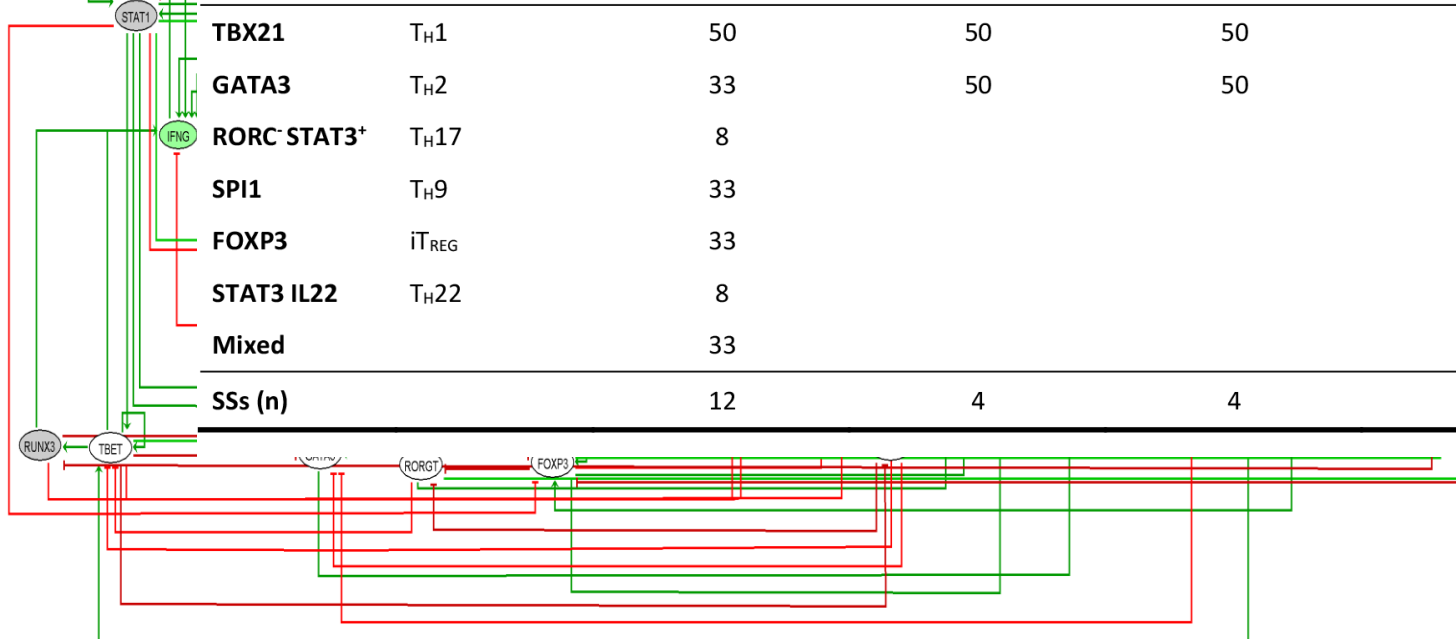
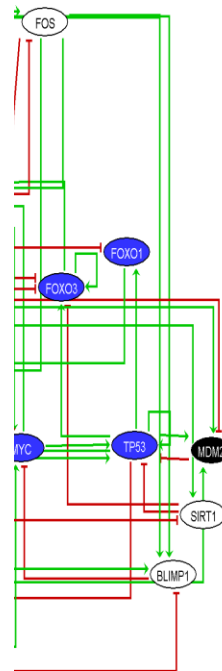
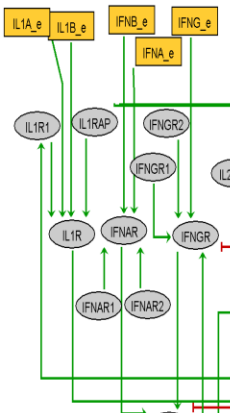
## Naldi et al. 2010 expanded – Static steady-state analysis



# T<sub>H</sub> cell differentiation models

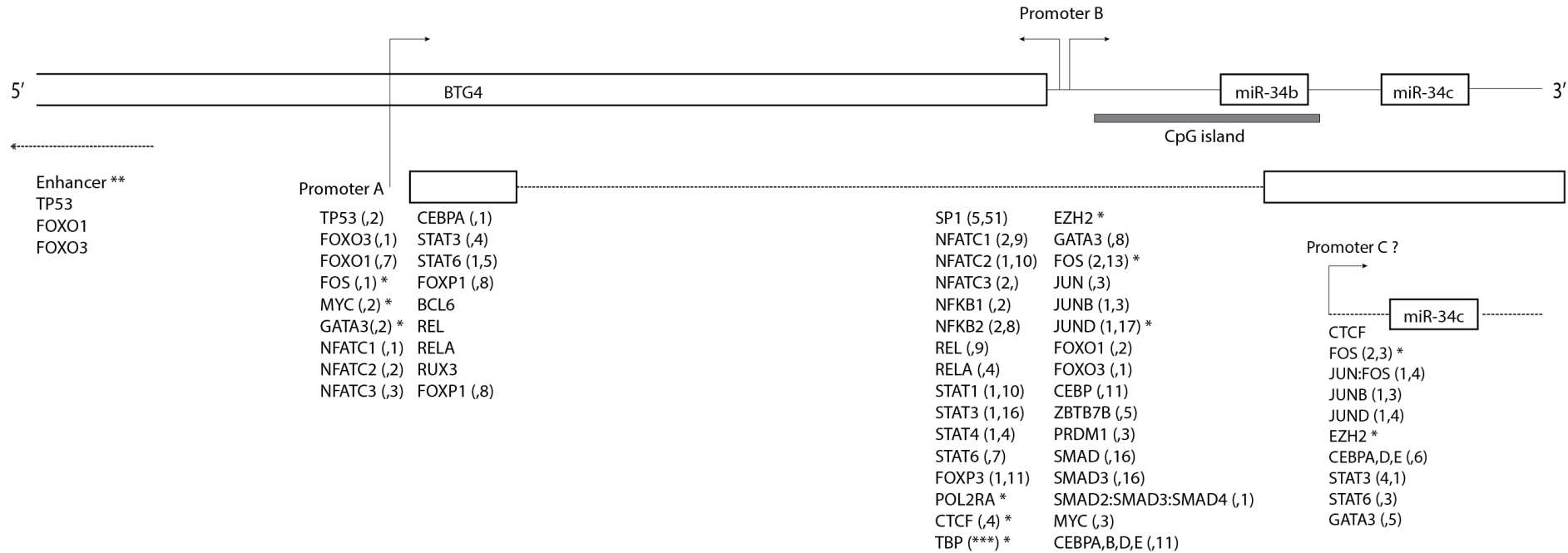
| Percentage of phenotypes acquired in Naldi model |                   |         |    |            |    |            |    |                   |    |
|--------------------------------------------------|-------------------|---------|----|------------|----|------------|----|-------------------|----|
| Master TFs/<br>Th subtypes                       |                   | No miRs |    | miR-155-5p |    | miR-34c-5p |    | miR-34c + miR-155 |    |
|                                                  |                   | A       | B  | A          | B  | A          | B  | A                 | B  |
| <b>TBX21</b>                                     | T <sub>H</sub> 1  | 42      | 42 | 44         | 44 | 50         | 50 | 50                | 50 |
| <b>GATA3</b>                                     | T <sub>H</sub> 2  | 17      | 17 | 22         | 11 | 50         | 50 | 50                | 50 |
| <b>RORC</b>                                      | T <sub>H</sub> 17 | 42      | 42 | 78         | 56 |            |    |                   |    |
| <b>FOXP3</b>                                     | iT <sub>REG</sub> | 33      | 33 | 44         | 44 |            |    |                   |    |
| <b>Mixed</b>                                     |                   | 33      | 42 | 89         | 56 |            |    |                   |    |
| <b>SSs (n)</b>                                   |                   | 12      |    | 9          |    | 2          |    | 2                 |    |

| Percentage of phenotypes acquired in Abou-Jaoudé model |                   |         |   |            |   |            |   |                   |   |
|--------------------------------------------------------|-------------------|---------|---|------------|---|------------|---|-------------------|---|
| Master TFs/<br>Th subtypes                             |                   | No miRs |   | miR-155-5p |   | miR-34c-5p |   | miR-34c + miR-155 |   |
|                                                        |                   | A       | B | A          | B | A          | B | A                 | B |
| <b>TBX21</b>                                           | T <sub>H</sub> 1  | 50      |   | 50         |   | 50         |   | 50                |   |
| <b>GATA3</b>                                           | T <sub>H</sub> 2  | 33      |   | 50         |   | 50         |   | 50                |   |
| <b>RORC STAT3<sup>+</sup></b>                          | T <sub>H</sub> 17 | 8       |   |            |   |            |   |                   |   |
| <b>SPI1</b>                                            | T <sub>H</sub> 9  | 33      |   |            |   |            |   |                   |   |
| <b>FOXP3</b>                                           | iT <sub>REG</sub> | 33      |   |            |   |            |   |                   |   |
| <b>STAT3 IL22</b>                                      | T <sub>H</sub> 22 | 8       |   |            |   |            |   |                   |   |
| <b>Mixed</b>                                           |                   | 33      |   |            |   |            |   |                   |   |
| <b>SSs (n)</b>                                         |                   | 12      |   | 4          |   | 4          |   | 4                 |   |

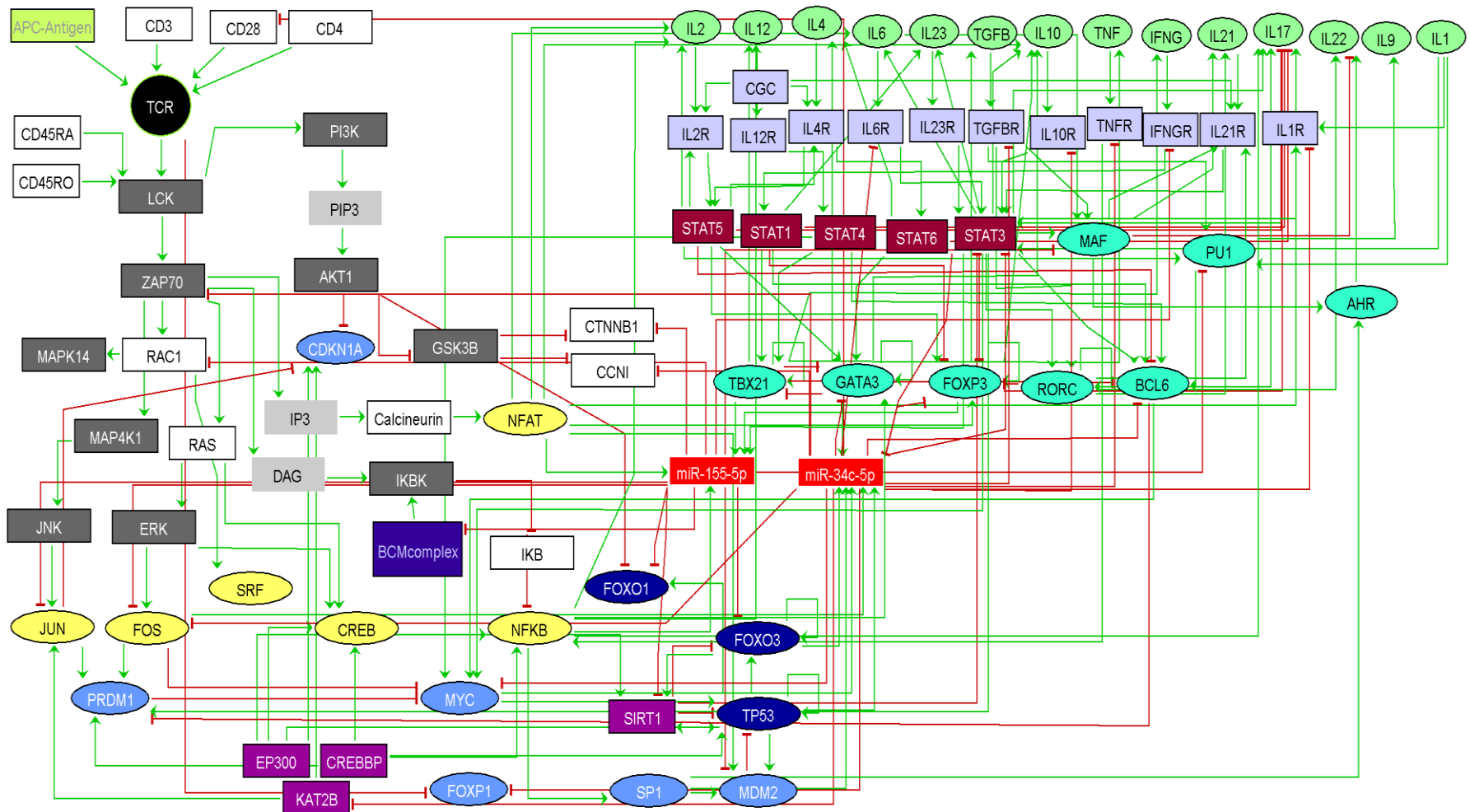


| Relevant predicted miR-34c-5p targets |   |                                                                                                                                |       |       |        |           |        |            |       |           |          |       |        |      |
|---------------------------------------|---|--------------------------------------------------------------------------------------------------------------------------------|-------|-------|--------|-----------|--------|------------|-------|-----------|----------|-------|--------|------|
| Activation                            | A | CD28                                                                                                                           | CALM3 | PRKCQ | NFATC4 | DGKH      | DGKB   | IKBKE      | PTEN  |           |          |       |        |      |
|                                       | B | CD28                                                                                                                           | CALM3 | CBL   | PRKCQ  | MAPK1     | FOSB   | REL        | RELN  | NFATC4    | DGKH     | IKBKE | SH3BP2 | PAG1 |
|                                       | C | REL; PRKCA,B,E,H,Q; AKT2; PTEN; NFKBIA,E; IKBKAB,E,G,AP; CALM3; CREB5; FOS; MAPK1; MAP2K1; KRAS; PTPN11; CDKN1B,C; ABL1; GRAP2 |       |       |        |           |        |            |       |           |          |       |        |      |
| Receptors                             | A | IL10RB                                                                                                                         | IL6ST | IL9R  | IL6R   | TGFB1,2,3 |        |            |       |           |          |       |        |      |
|                                       | B | IL10RB                                                                                                                         | IL7R  | IL21R | IL6R   | IL17RD    | IL31RA | IL1RN      | IL21R | TGFB1,2,3 |          |       |        |      |
|                                       | C | IL9R; IL21R; IL2RB; TGFB1,2,3; IL1R1; IL22RA2; IL31RA                                                                          |       |       |        |           |        |            |       |           |          |       |        |      |
| Cytokines                             | A | -                                                                                                                              |       |       |        |           |        |            |       |           |          |       |        |      |
|                                       | B | -                                                                                                                              |       |       |        |           |        |            |       |           |          |       |        |      |
|                                       | C | TGFB3                                                                                                                          |       |       |        |           |        |            |       |           |          |       |        |      |
| STATs                                 | A | STAT3                                                                                                                          |       |       |        |           |        |            |       |           |          |       |        |      |
|                                       | B | STAT1                                                                                                                          | STAT3 |       |        |           |        |            |       |           |          |       |        |      |
|                                       | C | STAT3                                                                                                                          |       |       |        |           |        |            |       |           |          |       |        |      |
| SOCS                                  | A | SOCS7                                                                                                                          | SOCS5 | SOCS2 |        |           |        |            |       |           |          |       |        |      |
|                                       | B | SOCS4                                                                                                                          | SOCS7 |       |        |           |        |            |       |           |          |       |        |      |
|                                       | C | SOCS2,3,5,6,7                                                                                                                  |       |       |        |           |        |            |       |           |          |       |        |      |
| Master TFs                            | A | GATA3                                                                                                                          | FOXP3 |       |        |           |        |            |       |           |          |       |        |      |
|                                       | B | FOXP3                                                                                                                          |       |       |        |           |        |            |       |           |          |       |        |      |
|                                       | C | GATA3, FOXP3, BCL6                                                                                                             |       |       |        |           |        |            |       |           |          |       |        |      |
| Others                                | A | MYCN                                                                                                                           | RUNX3 | FOXP1 | IRF1   | IRF4      | PRDM1  | NOTCH1,2,4 |       |           |          |       |        |      |
|                                       | B | MYCN                                                                                                                           | MAFF  | FOXP1 | SMAD2  | SMAD7     | SMAD4  | RUNX2      | IRF4  | PRDM1     | NOTCH1,2 |       |        |      |
|                                       | C | SMAD2,3,4,7; MYB; RUNX2,3; IRF1,4; PRDM1; NOTCH1,2,3,4                                                                         |       |       |        |           |        |            |       |           |          |       |        |      |
| VALIDATED                             |   | ZAP70; NOTCH1,4; MYC; RUNX2; BCL2                                                                                              |       |       |        |           |        |            |       |           |          |       |        |      |

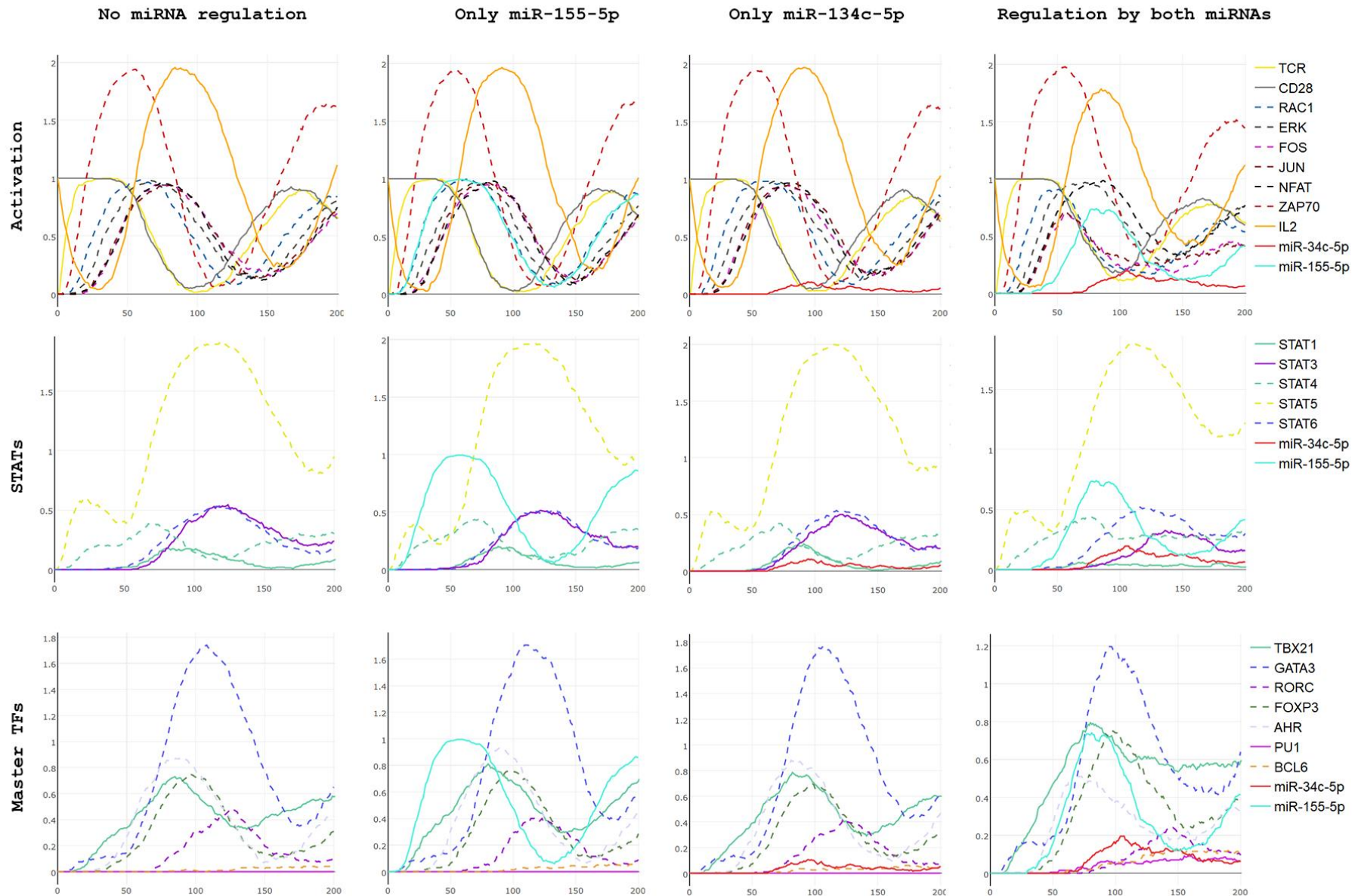
# Promoter analysis



# Activation and differentiation of T<sub>H</sub> cells with TCR and IL2 stimulation model



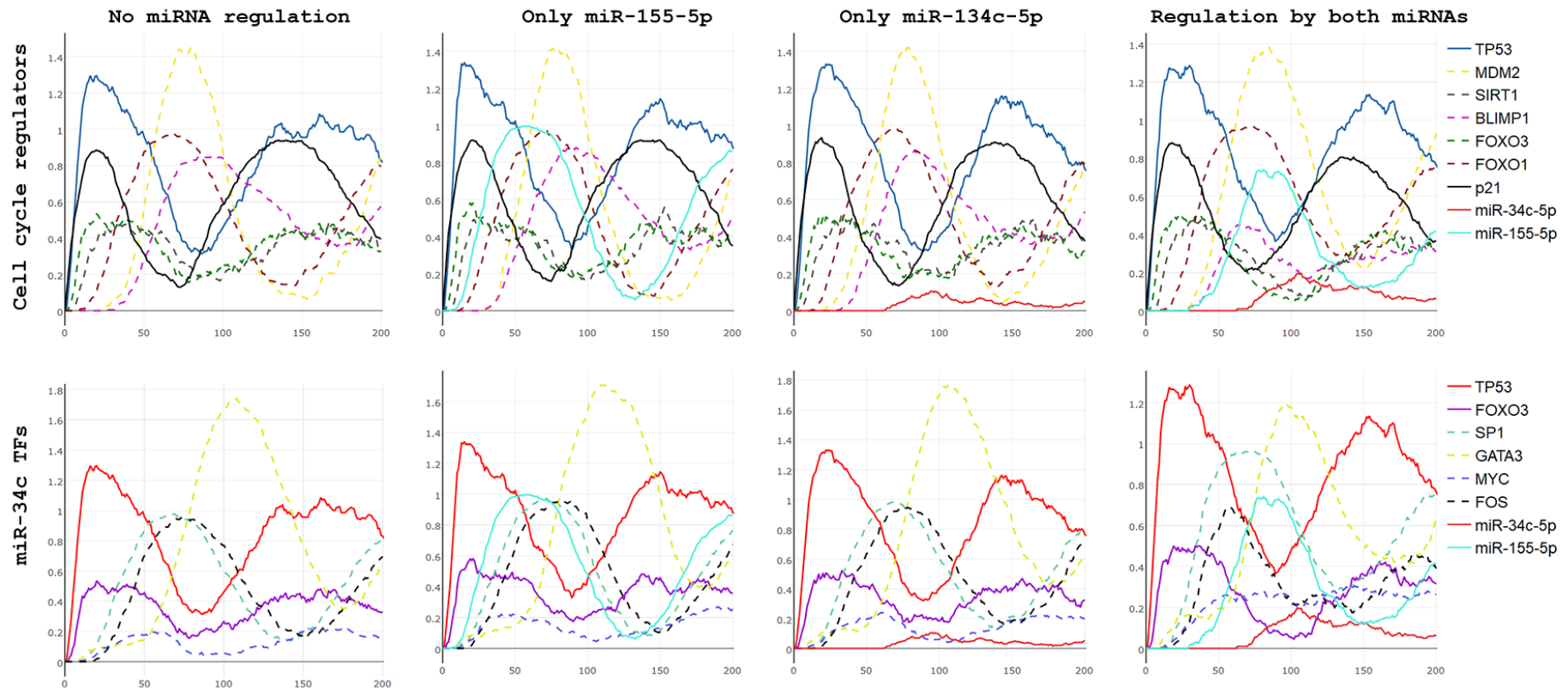
# Activation and differentiation of T<sub>H</sub> cells with TCR and IL2 stimulation model





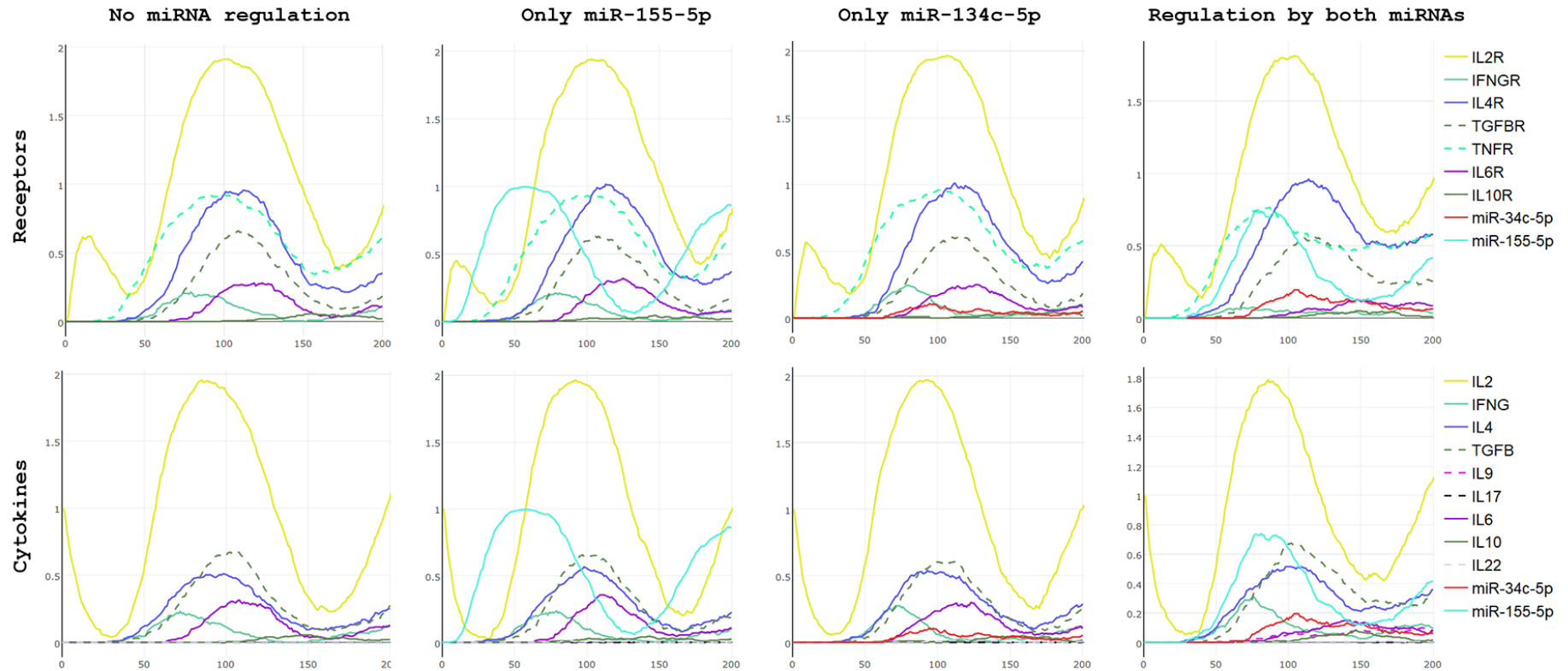
# Activation and differentiation of T<sub>H</sub> cells with TCR and IL2 stimulation model

## Cell cycle factors and miR-34 TFs

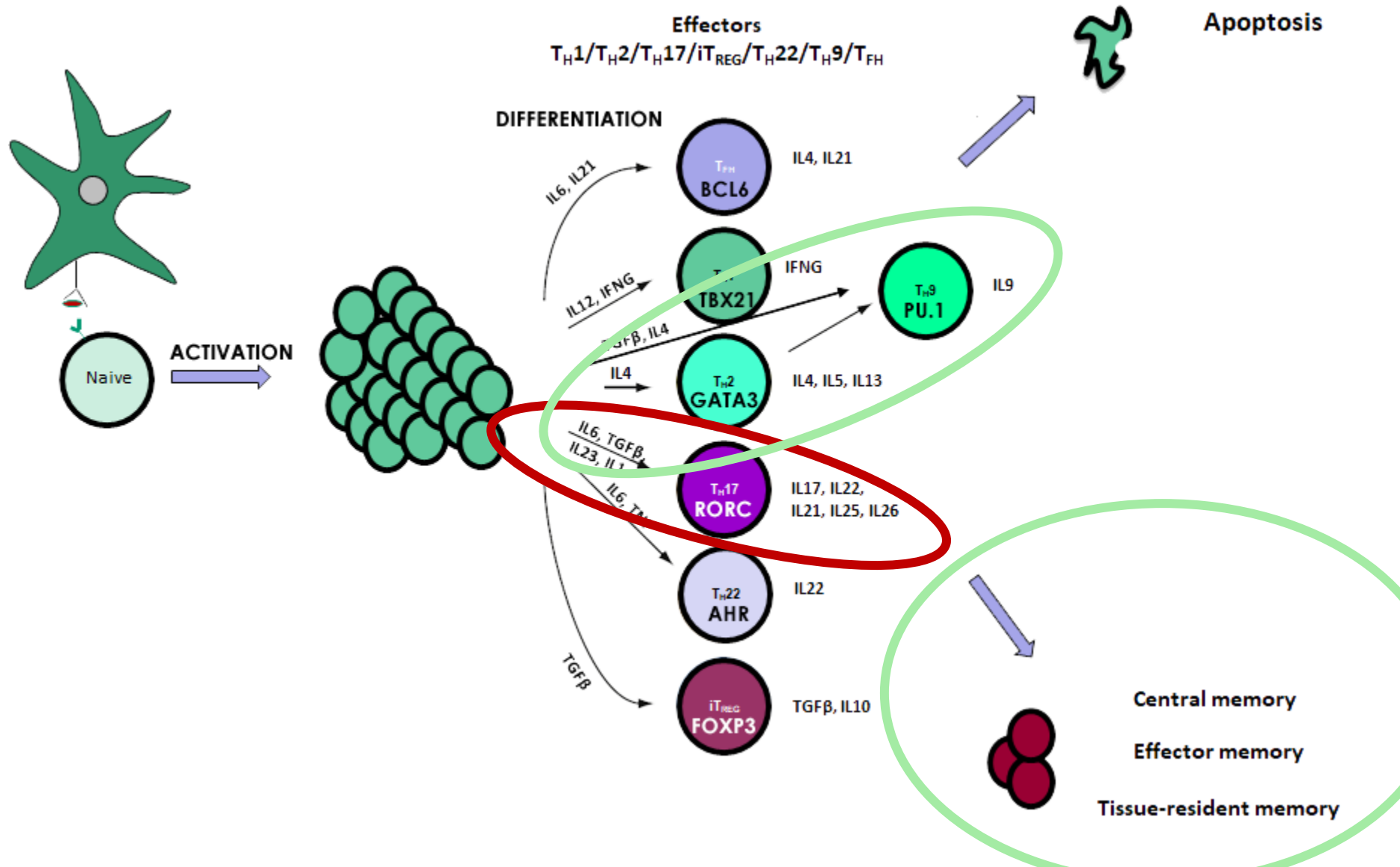


# Activation and differentiation of T<sub>H</sub> cells with TCR and IL2 stimulation model

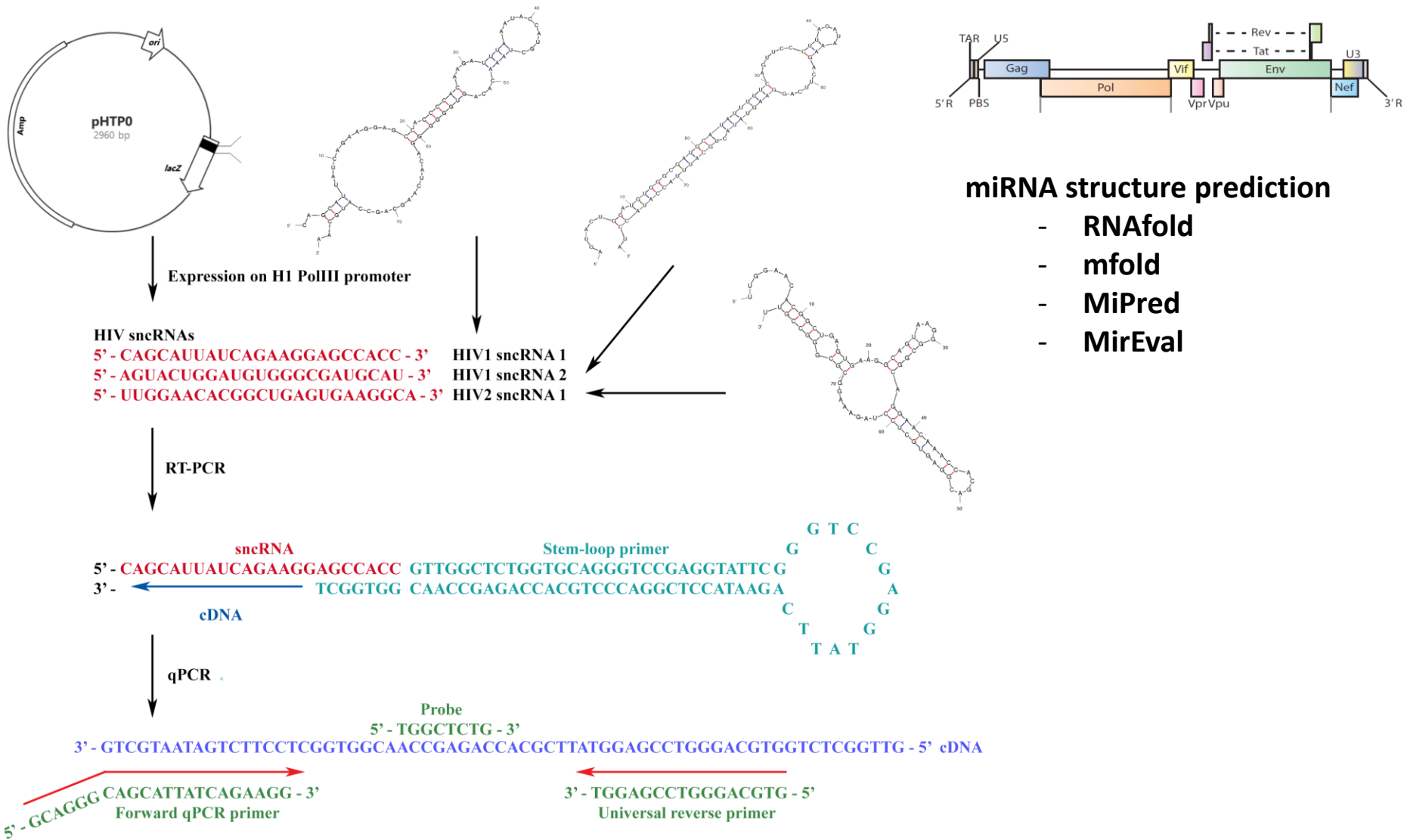
## Cytokines and their receptors



# miR-34c-5p predicted function

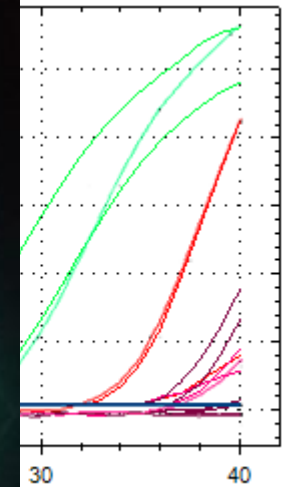
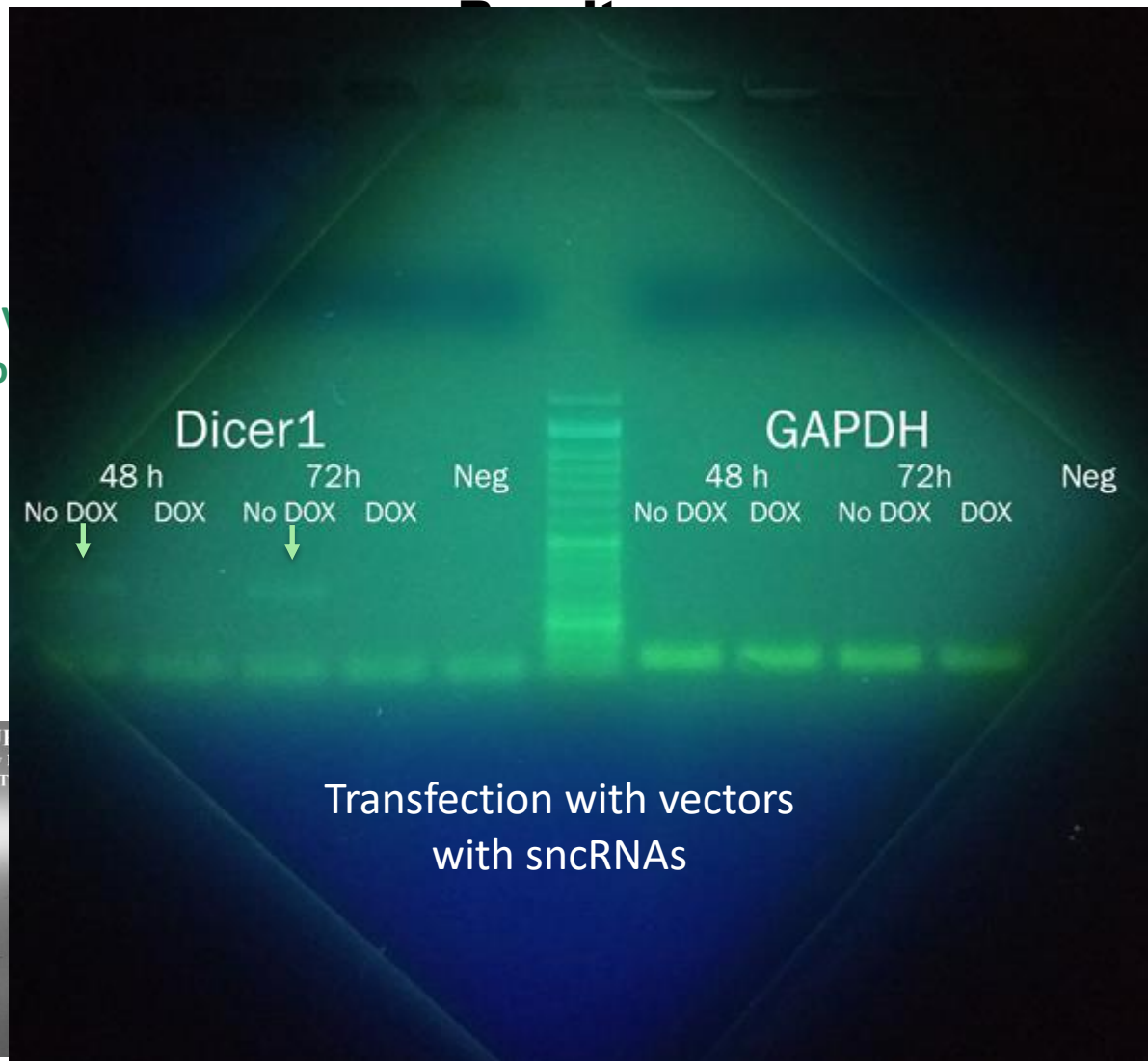


# HIV putative sncRNAs - METHODS



# HIV putative sncRNAs - RESULTS

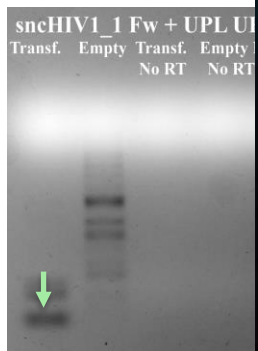
All 3 HIV  
stem-loops



A 1\_1, 1\_2 and 2\_1

ected cells

plate controls



ST  
DICER KD assays

# Conclusions



-> Effects on activation processes seem to be less pronounced than in differentiation, but RAC1 is suggested to be a relevant target of up-regulation of miR-155-5p and miR-34c-5p. In models of differentiation of more Th subtypes, steady-states are obtained only for Th1 and Th2 phenotypes. Therefore, it is likely that it is in one of these that miR-34c expression gets activated, particularly in Th2 cells.

-> Only when both miR-155-5p and miR-34c-5p are modulating the system, their pattern of expression is similar to what was observed experimentally in primary CD4 T cells, and with TP53, FOXO3, SP1, GATA3 and MYC controlling its expression.

-> Only with both miRs PU.1 (SPI1) activity is up-regulated, and a Th9 reprogramming from Th2 is suggested.

->The IL6/STAT3/RORC axis seems to be the most down-regulated.

->Quiescence seems to be promoted by miR-34c-5p (CDKN1A, FOXO3 and TP53 up-regulation), probably in a GATA3<sup>hi</sup> TBX21<sup>me</sup> PU1<sup>lo</sup> cell state of mostly IL4 and TGFβ secreting CD4 T cells. These GATA3 Th2 cells are down-regulating GATA3, directing the cells to a quiescent state. Whether it may be an effector memory phenotype remains to be assessed.

-> HIV seems to encode for its own miRNAs.

# Ongoing...

**DICER KD assays**

**Dual reporter assays to assess GATA3 effect on miR-34c-5p promoter(s)**

**Methylation specific PCR – CpG island state**

**NEXT:**

Target validation

ChIP-seq for histone marks and TFBSs

# Acknowledgements



Margarida Gama-Carvalho



Francisco Pinto

