

Expanded View Figures

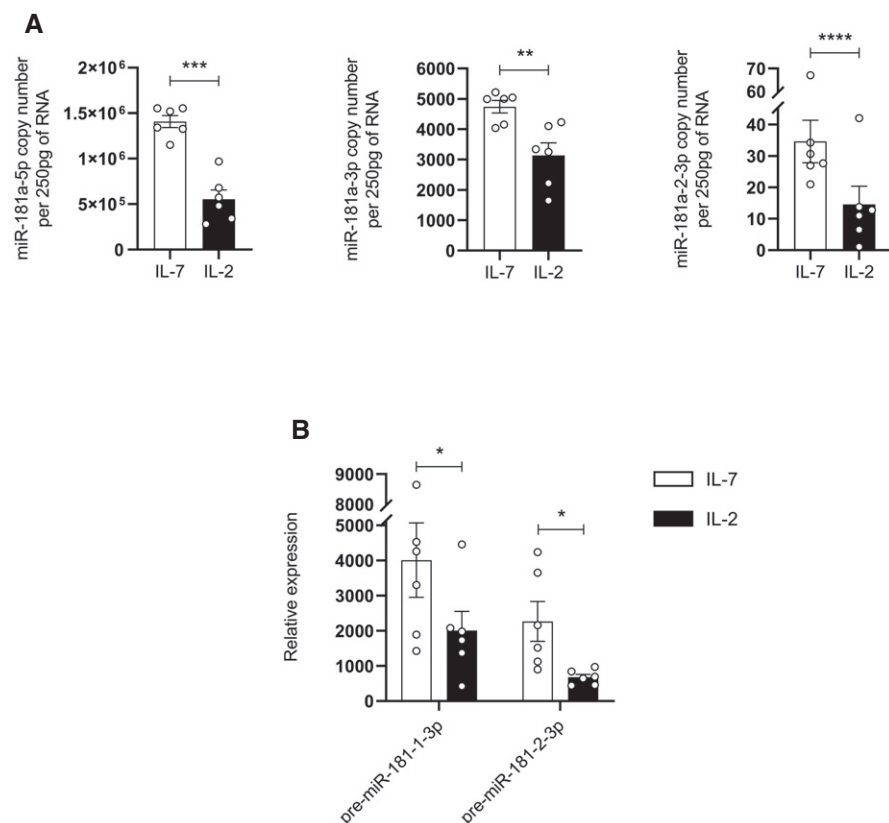


Figure EV1. miR-181a-5p and 3p strands are downregulated upon IL-2 stimulation.

A RT-PCR analysis of miR-181a-5p, miR-181a-1-3p and miR-181a-2-3p copy numbers in $\gamma\delta$ thymocytes cultured with IL-7 versus IL-2 ($n = 6$ independent biological samples).

B RT-PCR analysis of pre-miR-181a-1-3p and pre-miR-181a-2-3p expression in $\gamma\delta$ thymocytes cultured with IL-7 versus IL-2 ($n = 6$ independent biological samples).

Data information: Error bars represent the mean $\pm$ SEM. Statistical analysis was performed using the paired Student's *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. All experiments were performed with two technical replicates.

Source data are available online for this figure.

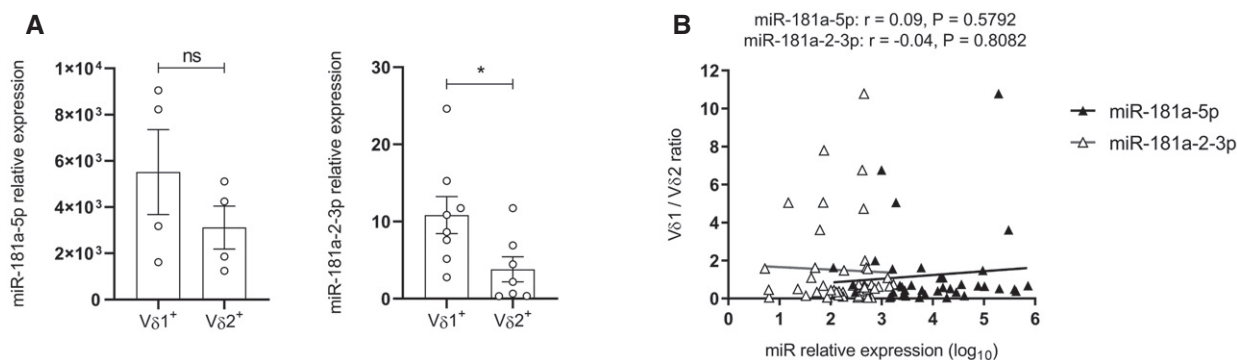


Figure EV2. miR-181a expression in V δ 1⁺ versus V δ 2⁺ $\gamma\delta$ T cells.

A RT-PCR analysis of the expression of miR-181a(-5p and -2-3p) in freshly isolated V δ 1⁺ versus V δ 2⁺ sorted $\gamma\delta$ PBLs ($n = 4-8$ independent biological samples).

B Correlation between V δ 1/V δ 2 ratio versus miR-181a(-5p and -2-3p) expression in freshly isolated $\gamma\delta$ PBLs ($n = 39-46$ independent biological samples).

Data information: (A) Error bars represent the mean $\pm$ SEM. Statistical analysis was performed using the unpaired Student's *t*-test. (B) The Pearson's correlation coefficient (*r*) was used to measure the strength of association between two variables. ns, not significant. * $P < 0.05$. All experiments were performed with two technical replicates.

Source data are available online for this figure.

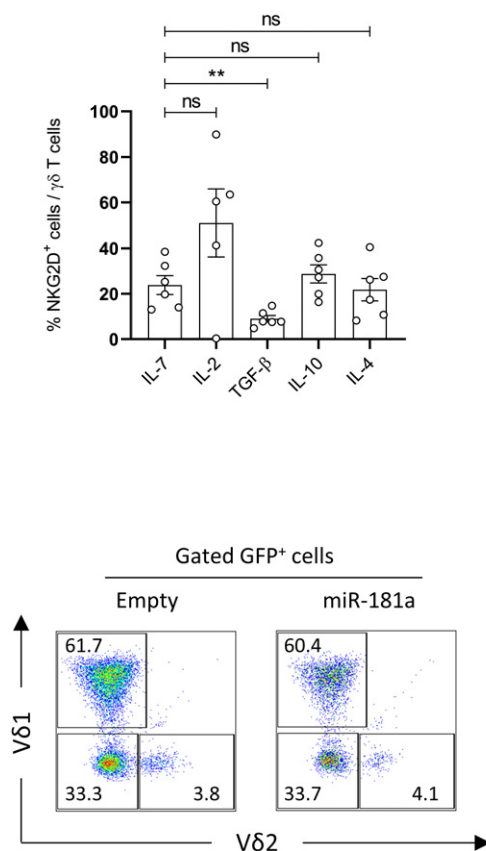


Figure EV3. TGF-β signals reduce NKG2D expression.

FACS analysis of the expression of NKG2D in γδ T cells isolated from PBLs cultured with the indicated cytokines for 4–6 days ($n = 5–6$ independent biological samples, paired Student's *t*-test). Data represent the mean $\pm$ SEM. ns, not significant. $**P < 0.01$. All experiments were performed with two technical replicates.

Source data are available online for this figure.

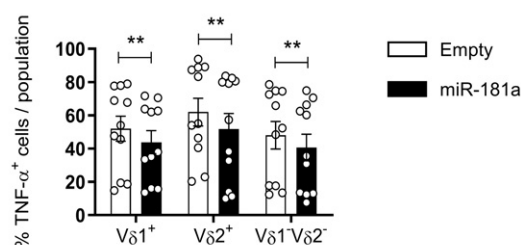
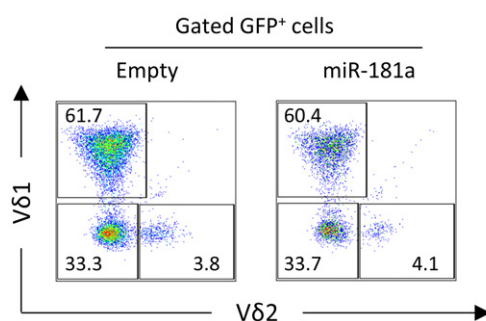


Figure EV4. miR-181a overexpression impact on TNF-α cytokine production in different γδ T cell subpopulations.

Gating strategy for the identification of the Vδ1 versus Vδ2 subpopulations in miR-181a versus empty transduced (GFP⁺) γδ thymocytes (left panel) and TNF-α expression gated on either Vδ1⁺, Vδ2⁺, or Vδ1⁺Vδ2⁻ populations, in (GFP⁺) miR-181a versus empty transduced γδ thymocytes, cultured with IL-7 plus IL-2 for 11 days (right panel, $n = 11$ independent biological samples).

Data Information: Error bars represent the mean $\pm$ SEM. Statistical analysis was performed using the paired Student's *t*-test. $**P < 0.01$. All experiments were performed with two technical replicates.

Source data are available online for this figure.

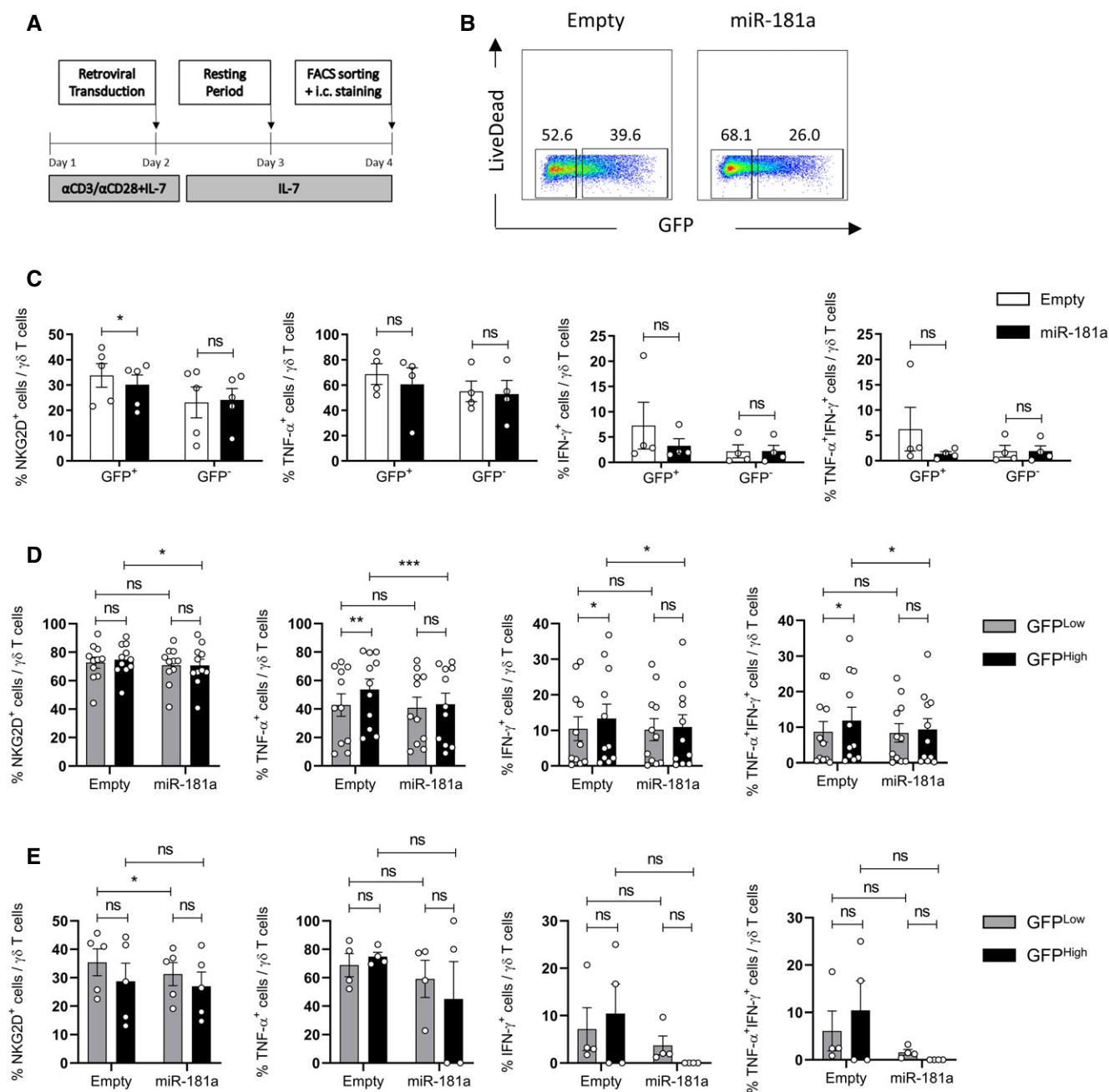


Figure EV5. Absence of dose effect of miR-181a-bearing vector transduction in $\gamma\delta$ T PBLs and thymocytes.

A Retroviral transduction workflow for $\gamma\delta$ PBLs.

B Gating strategy for the identification of the GFP⁺ versus GFP⁻ cells.

C FACS analysis of the expression of indicated surface and intracellular markers in miR-181a versus empty transduced (GFP⁺) and untransduced (GFP⁻) $\gamma\delta$ PBLs, cultured with IL-7 for 4 days ($n = 4-5$ independent biological samples).

D, E Impact of miR-181a levels on $\gamma\delta$ T cells isolated from (D) thymus and (E) PBLs. GFP^{Low} and GFP^{High} subsets were analyzed in miR-181a versus empty transduced $\gamma\delta$ T cells for their expression of NKG2D, TNF- α and IFN- γ . $\gamma\delta$ T thymocytes were cultured with IL-7 plus IL-2 for 11 days and $\gamma\delta$ PBLs were cultured with IL-7 for 4 days ($n = 11$ and $n = 4-5$ independent biological samples, respectively).

Data Information: (C–E) Error bars represent the mean $\pm$ SEM. Statistical analysis was performed using the paired Student's t-test. ns, not significant. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. All experiments were performed with two technical replicates.

Source data are available online for this figure.