Developmental stage-specific gene expression distinguishes the murine $\gamma\delta$ T-cell lineage

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Abstract

It remains unclear whether $\gamma\delta$ T-cell fate is instructed or reinforced by the $\gamma\delta$ TCR. Prospective isolation of a multi-potent $\gamma\delta$ cell progenitor (“the $\gamma\delta$ stem cell”) and precisely assessing when the $\gamma\delta$ TCR is displayed in relation to developmental emergence of multi-lineage reconstitution could resolve this question but there is a lack of reliable cell surface markers for the $\gamma\delta$ stem cell. The double-negative 3a stage (“DN3a”) is a critical intermediate cell-state during the transition from non-committed T-lymphocyte progenitor to mature $\gamma\delta$ or $\alpha\beta$ T-cell, as $\gamma\delta$ Tcr rearrangements and β -selection can theoretically temporally co-exist during this time in lymphocyte development. In this study I compared the transcriptomes of murine stage DN3a thymocytes to that of $\gamma\delta$ T-cells ($T\gamma\delta$) to illustrate the key transcriptional differences during this developmental transition. The analyses here revealed a series of cell surface markers, transcription factors, cytokine receptors, cell adhesion genes and non-coding RNA that represent some of the major transcriptional differences between $\gamma\delta$ T-cells and the cells that they might arise from.

Keywords: $T\gamma\delta$, $\gamma\delta$ T-cell, $\gamma\delta$ T-cell development

Introduction

$\gamma\delta$ T-cells, or $T\gamma\delta$, are one of two types of T-lymphocytes. They are distinguished from T-cell subsets such as helper (CD4+) and cytotoxic (CD8+) by the type of T-cell receptor (TCR) displayed on the cell surface. $T\gamma\delta$ display a TCR generated using the Tcr gamma and delta gene products, while $T\alpha\beta$ display a TCR generated using the Tcr beta and alpha gene products.

$\gamma\delta$ T-cells reside in the blood and in our tissues, from fetal life to adulthood. The common ancestor of these cells, a T-cell that has lost the ability to choose the $T\alpha\beta$ lineage fate but retains the ability to differentiate into any cell of the $T\gamma\delta$ lineage (the $T\gamma\delta$ stem cell) remains unknown.

$T\gamma\delta$ and $T\alpha\beta$ both differentiate from a common T-lineage restricted lymphocyte. At stage DN3a (double negative 3a), T-cells undergo rearrangement Tcr genes at the (1,2). This is one of the final identifiable steps before $T\gamma\delta$ lineage is restricted, as once a lymphocyte progenitor rearranges a Tcr beta gene and displays an $\alpha\beta$ TCR, becoming a $T\alpha\beta$ (and vice-versa for rearrangements at the Tcr beta locus) (3,4,5). Comparing the global transcriptional profile of a $T\gamma\delta$ cell to that of a thymocyte at stage DN3a would enable the identification of transcripts that are uniquely up-regulated and down-regulated during this critical developmental period, when $T\gamma\delta$ and $T\alpha\beta$ fates are simultaneously emerging but in a mutually exclusive fashion.

The instructive model of $T\gamma\delta$ cell fate argues that the type of TCR displayed on the T-cell surface, a function of the Tcr rearrangement process occurring in DN3a thymocytes, dictates whether the cell chooses the $\alpha\beta$ or $\gamma\delta$ lineage (6,7). On the hand, the stochastic model of $T\gamma\delta$ cell fate argues that while expression of the $\gamma\delta$ TCR and $T\gamma\delta$ cell fate are linked, $T\gamma\delta$ cell fate is simply reinforced, and not dictated by the presence of the $\gamma\delta$ TCR (6,7).

Identification of a multi-potent $T\gamma\delta$ stem cell requires the use of novel markers for their prospective isolation. I previously reported a set of genes that were most different between cells that express a $\gamma\delta$ TCR versus cells that express a $\alpha\beta$ TCR (8). In this study I performed a comparative transcriptome analysis of murine $T\gamma\delta$ to stage DN3a thymocytes, cells that are developmentally poised to choose the $T\gamma\delta$ lineage but have not yet. This mode of analysis has previously been used to identify the SLAM family of hematopoietic stem cell (HSC) markers that can prospectively isolate HSC with multi-potent lineage reconstitution capacity of the myeloid, lymphoid, erythroid and megakaryocytic fates (9, 10).

Here, I discovered a group of genes encoding cell surface receptors, transcription factors, cell adhesion genes and non-coding RNA that are specifically up-regulated as lymphocytes commit to the $T\gamma\delta$ cell fate. Some have previously been reported to as critical to the $T\gamma\delta$ cell lineage choice, but some have not been previously described in relation to the $T\gamma\delta$ cell fate.

Methods

Comparative Gene Expression Profiling

GEO (Gene Expression Omnibus) Dataset GSE 86495 was utilized for this analysis (11). The GEO2R web application identifies the most differentially expressed transcripts in a given comparison by ranking the log-fold change of RNA expression value between samples when

provided with a dataset. Log transformation of data was set to “Yes”, and the p-value, was set to “Benjamini-Hochberg”. The “Submitter supplied” category of platform annotation was used to identify gene/RNA names.

Statistics

Statistical analyses comparing the mean RNA expression value of each differentially expressed transcript from flow cytometry-sorted T γ δ (CD4- CD8- TCR γ δ + TCR β - NK1-1- and Gr1-) and stage DN3a thymocyte (CD4- CD8- TCR γ δ - TCR β - CD25hi CD44- CD28-) ($n=3$ mouse for each cell type) were performed using GraphPad Prism Version 8.0. A unpaired t-test with Welch's correction was performed for each differentially expressed transcript, comparing the mean expression RNA expression value of T γ δ to DN3a thymocytes. P-values for each comparison are denoted on each respective graph.

Results

Seven cluster of differentiation markers were specifically up-regulated between T γ δ and DN3a thymocytes.

CD2, CD6, CD7, CD5, CD52, CD226 and CD73 were all among the genes most differentially regulated when comparing the transcriptomes of T γ δ to DN3a thymocytes.

CD2 was expressed at significantly higher levels in T γ δ than in DN3a thymocytes (Figure 1, $p<0.0001$).

CD6 was expressed at significantly higher levels in T γ δ than in DN3a thymocytes (Figure 1, $p<0.0001$).

CD7 was expressed at significantly higher levels in T γ δ than in DN3a thymocytes (Figure 1, $p<0.0001$).

CD5 was expressed at significantly higher levels in T γ δ than in DN3a thymocytes (Figure 1, $p<0.0001$).

CD52 was expressed at significantly higher levels in T γ δ than in DN3a thymocytes (Figure 1, $p=0.0004$).

CD226 (Figure 1, $p<0.0001$) was expressed at significantly higher levels in T γ δ than in DN3a thymocytes.

CD73, or Nt5e was also expressed at significantly higher levels in T γ δ than in DN3a thymocytes (Figure 1, $p=0.0006$).

Together, these data reveal a set of markers that might be used in tandem, in any combination, in conjunction with other T γ δ -enriched markers to isolate homogenous fractions of T γ δ that might contain rare cells with long-term multi-lineage T γ δ -restricted reconstitution potential.

Proteins expressed at the plasma membrane both inform our understanding of the biology of the T γ δ as well as provide further potential targets for prospective isolation of multi-potent T γ δ stem cells. I found ten cell surface receptors from varying families whose expression was specifically enriched in T γ δ cells when compared to DN3a thymocytes.

Membrane-spanning 4-domain family member and CD20 homologue Ms4a4b was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 2, $p<0.0001$).

V-domain Ig suppressor of T-cell activation VISTA, also known as V-set immunoregulatory receptor Vsir, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 2, $p=0.0002$).

Scavenger receptor Scart2 was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 2, $p=0.0004$).

The tumor necrosis factor receptor superfamily member 25, Tnfrsf25, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 2, $p<0.0001$).

Acvr2a, the gene encoding the activin receptor 2a was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 2, $p=0.0033$).

The folate receptor Folr was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 2, $p=0.0006$).

Transmembrane protein 20, or Tmem20 was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 2, $p<0.0001$).

S1pr1, the gene encoding the sphingosine-1 phosphate receptor, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 2, $p<0.0001$).

Fcrl1 (Fc receptor-like 1) was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 2, $p<0.0001$).

Icos was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 2, $p=0.0003$).

Gain of Ms4a4b, Vsir/VISTA, Scart2, Tnfrsf25, Acvr2a, Folr, Tspan32, S1pr1, Fcrl1, and Icos are distinguishing features of the developmental transition reflected in the genes whose expression is most significantly different between $\text{Ty}\delta$ and DN3a thymocytes.

Transcription factors can bind to coordinate sets of elements to orchestrate cell-fate decisions, in T-cells and in cells throughout the hematopoietic spectrum. These genes are most often specifically up-regulated in a developmental stage-specific fashion as the cell expressing this gene chooses the fate in question. As such, comparative gene expression analyses between committed and uncommitted cells along the same continuum can be useful in identifying transcription factors that guide lineage choice.

Nine transcription factors were among the genes most differentially regulated when comparing the transcriptomes of $\text{Ty}\delta$ and DN3a thymocytes.

Zbtb16, or PLZF (promyelocytic leukemia zinc finger) was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 3, $p<0.0001$).

Etv5 was expressed at significantly higher levels in $\text{Ty}\delta$ DN3a thymocytes (Figure 3, $p=0.0003$).

Rorc was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 3, $p=0.0004$).

Runx3 was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 3, $p < 0.0001$).

Sox13, previously reported to be critical for the development of IL17-producing $\text{Ty}\delta$, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 3, $p < 0.0001$).

Maf was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 3, $p = 0.0005$).

Nr4a1 was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 3, $p < 0.0001$).

Ikzf was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 3, $p < 0.0001$).

Zfp821, a relatively uncharacterized molecule with predicted transcription factor functions was also expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 3, $p = 0.0004$).

The signals that lymphocytes transduce are dependent upon the soluble factors that are present in the lymph and in the bloodstream and their cognate receptors expressed on the lymphocyte. Seven interleukin receptors or receptor subunits, three chemokine receptors and the chemokine Xcl1 were among the genes whose expression most widely varied between $\text{Ty}\delta$ and DN3a thymocytes.

Il18r1, one of the genes that encode for the interleukin 18 receptor, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 4, $p < 0.0001$).

Il21r, the gene encoding the interleukin 21 receptor, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 4, $p < 0.0001$).

Il23r, the gene encoding the interleukin 23 receptor, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 4, $p < 0.0001$).

Il4ra, the gene encoding the interleukin 4 receptor alpha subunit, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 4, $p < 0.0001$).

Il1r1, one of the genes that encode for the interleukin 1 receptor, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 4, $p = 0.0032$).

Il6ra, the gene encoding the alpha subunit of the interleukin 6 receptor, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 4, $p = 0.0009$).

Il2rb, the gene encoding the beta subunit of the interleukin 2 receptor, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 4, $p < 0.0001$).

The chemokine ligand Xcl1, or lymphotactin, was expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 4, $p = 0.0003$).

Ccr9, the gene encoding the C-C chemokine receptor type 9, and Ccr8, the gene encoding the C-C chemokine receptor type 8 were both expressed at significantly higher levels in $\text{Ty}\delta$ than in DN3a thymocytes (Figure 4, $p < 0.0001$ and $p = 0.0001$, respectively).

The C-X-C chemokine receptor type 6, *Cxcr6*, was expressed at significantly higher levels in $\gamma\delta$ than in DN3a thymocytes (Figure 4, $p=0.0013$).

Similarly, cell adhesion genes were also among the most differentially expressed genes in $\gamma\delta$ cells relative to DN3a thymocytes.

Integrin $\beta 2$, Integrin $\beta 3$, and Integrin $\beta 7$ were all expressed at significantly higher levels in $\gamma\delta$ than in DN3a thymocytes (Figure 5, $p=0.0001$, $p<0.0001$, and $p<0.0001$, respectively).

Interestingly, unlike Integrins $\beta 2$, $\beta 4$ and $\beta 7$, Integrin $\beta 4$ was expressed at significantly lower levels in $\gamma\delta$ than in DN3a thymocytes (Figure 5, $p<0.0001$). Similarly, Cadherin 1 and Cadherin 23 were also expressed at significantly lower levels in $\gamma\delta$ than in DN3a thymocytes (Figure 5, $p=0.0002$ and $p<0.0001$, respectively).

RNA that do not encode for protein products were also among the genes most differentially expressed in $\gamma\delta$ when compared to DN3a thymocytes.

Two long non-coding RNA (lncRNA) were expressed in a stage-selective manner in $\gamma\delta$. *Gm14178*, or lncRNA $\gamma\delta 1$, and *AK014756* or lncRNA $\gamma\delta 2$, was expressed in significantly higher quantities in $\gamma\delta$ than in DN3a thymocytes (Figure 6, $p=0.0001$).

A microRNA, *mir342*, was also expressed at significantly higher levels in $\gamma\delta$ than in DN3a thymocytes (Figure 6, $p=0.0021$).

Discussion

I report here a group of genes whose expression marks the $\gamma\delta$ lineage. These expression of these genes is specifically increased during the developmental transition from $\gamma\delta$ TCR- DN3a thymocyte to $\gamma\delta$ TCR+ T-cell ($\gamma\delta$), and these genes are among the most significantly differentially expressed genes when comparing the transcriptomes of $\gamma\delta$ to DN3a thymocytes.

It has been suggested by at least one group that the $\alpha\beta$ and $\gamma\delta$ T-cell lineages diverge at DN2 (12). If this were the case, that would mean that DN3a cells (cells that are capable of $\gamma\delta$ lineage choice) would have lost the ability to choose $\alpha\beta$ cell fate. DN3a cells have not even completed the process of β -selection at this point, so TCR β expression and $\alpha\beta$ lineage potential have not been lost. It follows that $\alpha\beta$ and $\gamma\delta$ lineages have not yet diverged at DN2.

CD7 expression was previously reported to precede β -selection (13, 14). This suggests that CD7, a gene that is differentially expressed in $\gamma\delta$ cells (quite significantly compared to DN3a thymocytes), if used to prospectively enrich $\gamma\delta$ cells, would positively enrich for TCR β -cells.

The identification of *Sox13* provides valuable validation to application of this technique to this dataset, as well the potentially of the other genes identified to serve in similarly critical roles. Deletion of *Sox13* in mice specifically impairs the development of $\gamma\delta$ versus T $\alpha\beta$ (15).

While β -integrins *Itgb2*, *Itgb3*, and *Itgb7* were expressed at significantly higher levels in $\gamma\delta$ cells, β -integrin *Itgb4* and cadherins *Cdh1* and *Cdh23* were expressed at significantly lower levels in $\gamma\delta$ cells. Thus, cell adhesion genes are both “turned off” and “turned on” during the $\gamma\delta$ lineage choice.

The advent of fluorescent proteins across the visible spectrum as well as CRISPR knock-in technology enables the generation of transgenic mice whose endogenous loci are tagged in a manner that allows for *in vivo* lineage tracing in conjunction with flow cytometry. Using fluorescent proteins like GFP, RFP, BFP, and YFP, individual transcription factors identified here can be endogenously tagged *in vivo* and when crossed, could generate mice that provide cells whose endogenous loci for these transcription factors is tagged in tandem array.

I propose a novel gene set, comprised of transcripts that are specifically up-regulated in $\text{T}\gamma\delta$ when compared to (a) cells that are TCR negative (this manuscript), and (b) cells that are TCR+ but of the $\alpha\beta$ lineage (8). These genes, eighty in total, can be harnessed for their potential to aid in prospective isolation of the $\text{T}\gamma\delta$ stem cell. Importantly, isolation of a $\text{T}\gamma\delta$ stem cell would enable direct experimental assessment of the long-standing question between the instructive and stochastic models of $\text{T}\gamma\delta$ cell fate choice, which is whether the $\gamma\delta\text{TCR}$ directs or supports $\text{T}\gamma\delta$ cell fate. Moreover, this could be accomplished without supra physiological over-expression of TCR components. Specifically, enabling the precise identification of the time point in which $\gamma\delta\text{TCR}$ expression emerges is critical, in order to determine whether this precedes or follows the time point in which $\text{T}\gamma\delta$ cell multipotency dissipates. The existence of cells that gain $\gamma\delta\text{TCR}$ expression after the emergence of $\text{T}\gamma\delta$ cell multi-lineage reconstitution potential would be a clear demonstration that the $\gamma\delta\text{TCR}$ reinforces, but does not instruct $\text{T}\gamma\delta$ cell fate.

References

1. Kreslavsky, T., Gleimer, M., Miyazaki, M., Choi, Y., Gagnon, E., Murre, C., Sicinski, P. and von Boehmer, H., 2012. β -Selection-induced proliferation is required for $\alpha\beta$ T cell differentiation. *Immunity*, 37(5), pp.840-853.
2. Taghon, T., Yui, M.A., Pant, R., Diamond, R.A. and Rothenberg, E.V., 2006. Developmental and molecular characterization of emerging β - and $\gamma\delta$ -selected pre-T cells in the adult mouse thymus. *Immunity*, 24(1), pp.53-64.
3. Ishida, I., Verbeek, S., Bonneville, M., Itohara, S., Berns, A. and Tonegawa, S., 1990. T-cell receptor gamma delta and gamma transgenic mice suggest a role of a gamma gene silencer in the generation of alpha beta T cells. *Proceedings of the National Academy of Sciences*, 87(8), pp.3067-3071.
4. Saint-Ruf, C., Panigada, M., Azogui, O., Debey, P., Von Boehmer, H. and Grassi, F., 2000. Different initiation of pre-TCR and $\gamma\delta$ TCR signalling. *Nature*, 406(6795), p.524.
5. Hayes, S.M. and Love, P.E., 2002. Distinct structure and signaling potential of the $\gamma\delta$ TCR complex. *Immunity*, 16(6), pp.827-838.
6. Haks, M.C., Lefebvre, J.M., Lauritsen, J.P.H., Carleton, M., Rhodes, M., Miyazaki, T., Kappes, D.J. and Wiest, D.L., 2005. Attenuation of $\gamma\delta$ TCR signaling efficiently diverts thymocytes to the $\alpha\beta$ lineage. *Immunity*, 22(5), pp.595-606.
7. Hayes, S.M., Li, L. and Love, P.E., 2005. TCR signal strength influences $\alpha\beta/\gamma\delta$ lineage fate. *Immunity*, 22(5), pp.583-593.
8. Mamoor, S., 2019. A new family of markers to characterize the human $\gamma\delta$ T-cell lineage.
9. Kiel, M.J., Yilmaz, Ö.H., Iwashita, T., Yilmaz, O.H., Terhorst, C. and Morrison, S.J., 2005. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*, 121(7), pp.1109-1121.
10. Oguro, H., Ding, L. and Morrison, S.J., 2013. SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. *Cell stem cell*, 13(1), pp.102-116.
11. Yang, K., Blanco, D.B., Chen, X., Dash, P., Neale, G., Rosencrance, C., Easton, J., Chen, W., Cheng, C., Dhungana, Y. and Anil, K.C., 2018. Metabolic signaling directs the reciprocal lineage decisions of $\alpha\beta$ and $\gamma\delta$ T cells. *Science immunology*, 3(25), p.eaas9818.

12. Mengrelis, K., Lau, C.I., Rowell, J., Solanki, A., Norris, S., Ross, S., Ono, M., Outram, S. and Crompton, T., 2019. Sonic Hedgehog Is a determinant of $\gamma\delta$ T-Cell differentiation in the Thymus. *Frontiers in immunology*, 10.
13. Pittaluga, S., Raffeld, M., Lipford, E.H. and Cossman, J., 1986. 3A1 (CD7) expression precedes T beta gene rearrangements in precursor T (lymphoblastic) neoplasms. *Blood*, 68(1), pp.134-139.
14. Pittaluga, S., Raffeld, M., Lipford, E.H. and Cossman, J., 1986. 3A1 (CD7) expression precedes T beta gene rearrangements in precursor T (lymphoblastic) neoplasms. *Blood*, 68(1), pp.134-139.
15. Melichar, H.J., Narayan, K., Der, S.D., Hiraoka, Y., Gardiol, N., Jeannet, G., Held, W., Chambers, C.A. and Kang, J., 2007. Regulation of $\gamma\delta$ Versus $\alpha\beta$ T Lymphocyte Differentiation by the Transcription Factor SOX13. *Science*, 315(5809), pp.230-233.

A.

B.

Figure 1: CD2, CD6, CD7, CD5, CD52, CD226, and CD73 are expressed in a developmental stage-selective manner in $\gamma\delta$ T-cells.

A. The mean RNA expression value for each gene is graphically represented by floating bars. $n=3$, from minimum to maximum value.

B. The mean RNA expression value for each gene is individually graphically represented in bar graphs, with error bars representing the standard deviation ($n=3$). The p -value for an unpaired t-test with Welch's correction comparing the mean RNA expression value is indicated on each respective graph.

A.

B.

Figure 2: A new family of cell surface receptors expressed by $\gamma\delta$ T-cells.

A. The mean RNA expression value for each gene is graphically represented by floating bars. $n=3$, from minimum to maximum value.

B. The mean RNA expression value for each gene is individually graphically represented in bar graphs, with error bars representing the standard deviation ($n=3$). The p -value for an unpaired t-test with Welch's correction comparing the mean RNA expression value is indicated on each respective graph.

A

B

Figure 3: Nine transcription factors distinguish the murine γδ T-cell lineage from DN3a thymocytes

A. The mean RNA expression value for each gene is graphically represented by floating bars. $n=3$, from minimum to maximum value.

B. The mean RNA expression value for each gene is individually graphically represented in bar graphs, with error bars representing the standard deviation ($n=3$). The p -value for an unpaired t-test with Welch's correction comparing the mean RNA expression value is indicated on each respective graph.

A.

B.

Figure 4: Ten receptors for cytokines and chemokines and the chemokine Xcl1 distinguish the Tyδ lineage from DN3a thymocytes.

A. The mean RNA expression value for each gene is graphically represented by floating bars. $n=3$, from minimum to maximum value.

B. The mean RNA expression value for each gene is individually graphically represented in bar graphs, with error bars representing the standard deviation ($n=3$). The p -value for an unpaired t-test with Welch's correction comparing the mean RNA expression value is indicated on each respective graph.

A.

Cell Adhesion Molecules

B.

Figure 5: Cell adhesion molecules are regulated in an opposing fashion during Tγδ development

A. The mean RNA expression value for each gene is graphically represented by floating bars. $n=3$, from minimum to maximum value.

B. The mean RNA expression value for each gene is individually graphically represented in bar graphs, with error bars representing the standard deviation ($n=3$). The p -value for an unpaired t-test with Welch's correction comparing the mean RNA expression value is indicated on each respective graph.

A.

B.

Figure 6: Three non-coding RNAs distinguish are expressed at higher levels and among the most differentially expressed genes in T $\gamma\delta$ cells.

- A. The mean RNA expression value for each gene is graphically represented by floating bars. $n=3$, from minimum to maximum value.
- B. The mean RNA expression value for each gene is individually graphically represented in bar graphs, with error bars representing the standard deviation ($n=3$). The p -value for an unpaired t-test with Welch's correction comparing the mean RNA expression value is indicated on each respective graph.