Single cell characterisation of cattle NK cell subpopulations

Immunogenetics

Biotechnology and Biological Sciences Research Council

Pirbright receives strategic funding from BBSRC UKRI

Isolation of cattle NK cells for 10X genomics

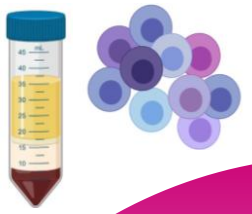
Challenge: How do we isolate without anti-NKp46?

x 6

2 x A14
2 x A18
2 x A31

May 2021
Jan 2024

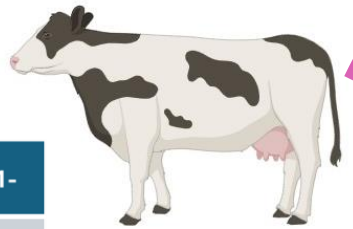
16-26 months



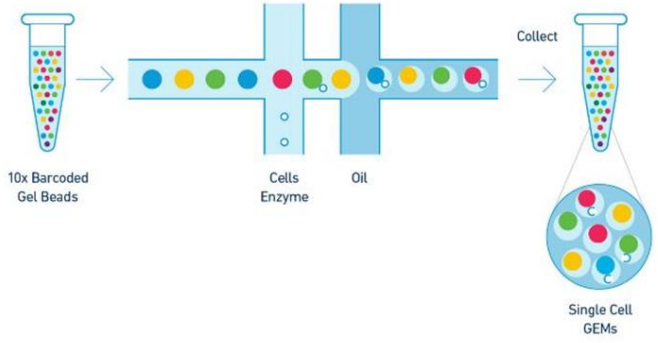
anti-NKp46 FITC

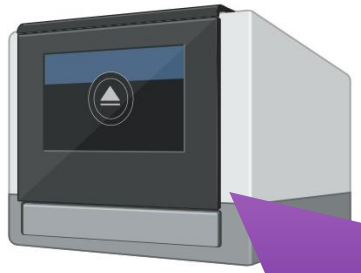
anti-non-NK APC

α-MHC-II
α-CD14
α-IgL
α-CD3(αβ)
α-γδTCR

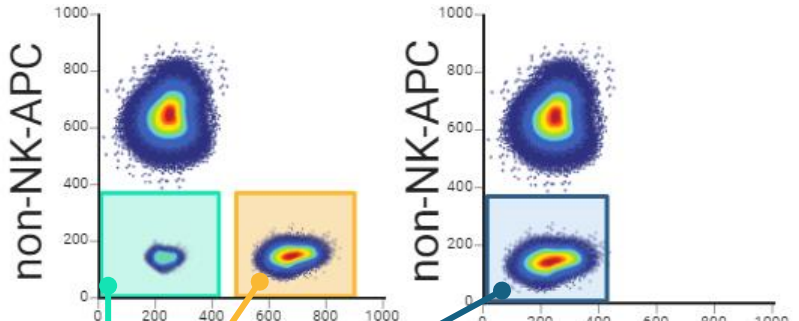


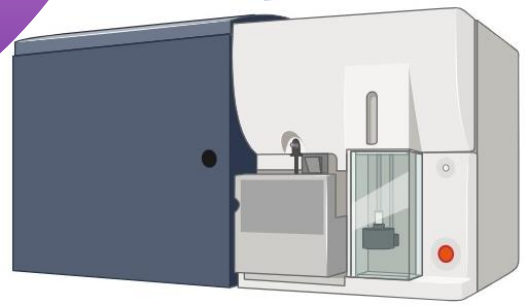
Animal Number	Total NK	NCR1 +	NCR1-
51	✓	×	×
51	✓	✓	✓
25	✓	✓	~
26	✓	✓	✓
27	✓	✓	✓
28	✓	✓	✓





~20,000 cells



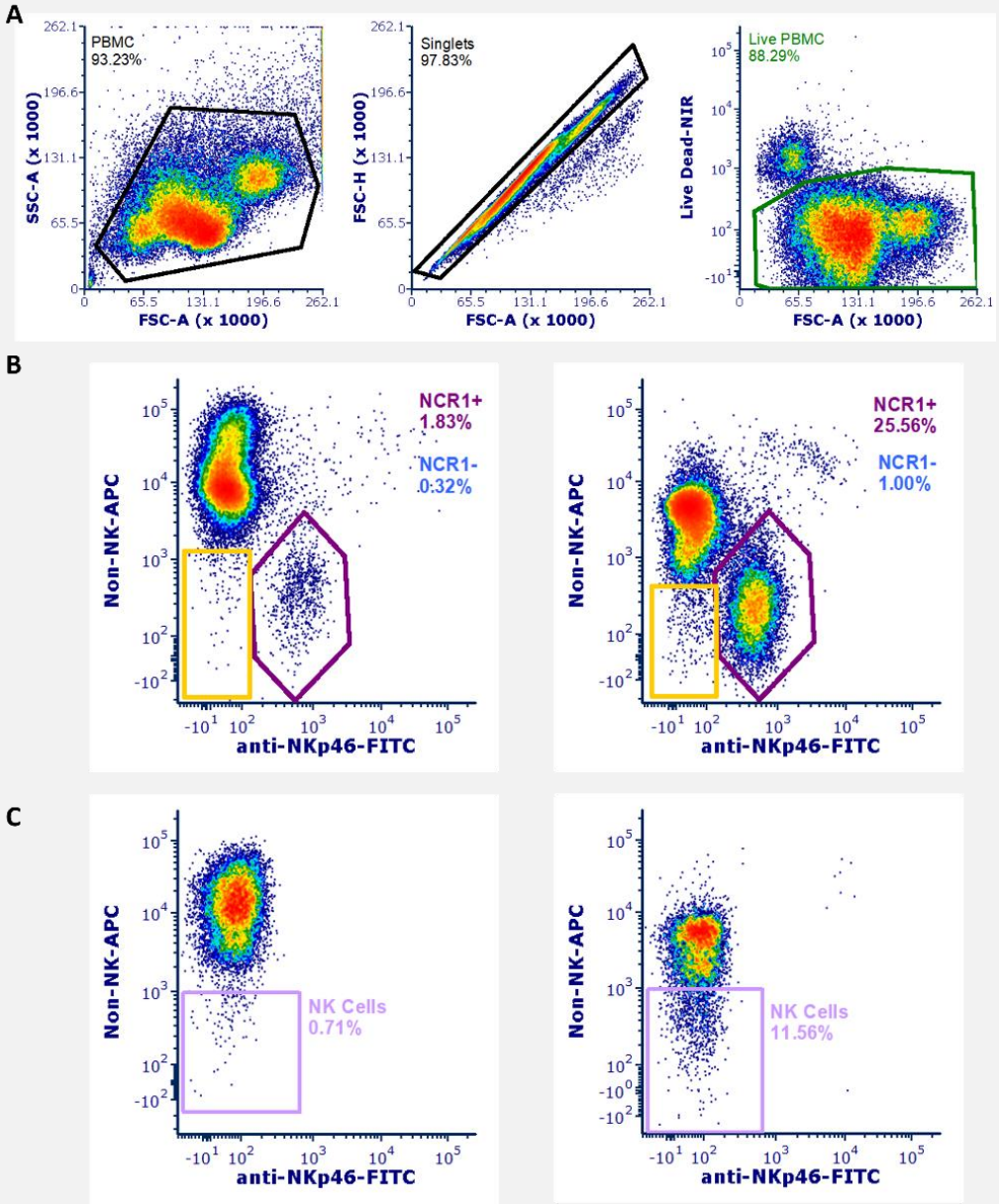


Total NK
NKp46+
NKp46-

Results section of the paper

1. Optimised flow cytometry panel
2. Unsupervised clustering of NKp46+ and NKp46- cell sorts indicate NKp46- cells are a transcriptomically distinct NK cell subset.
3. NKp46- cells exhibit characteristics consistent with NK cell identity.
4. NK cells or ILCs?
5. NK cell subtypes
6. NKC and LRC gene expression

1. A panel for enrichment and negative sorting of bovine NK cells



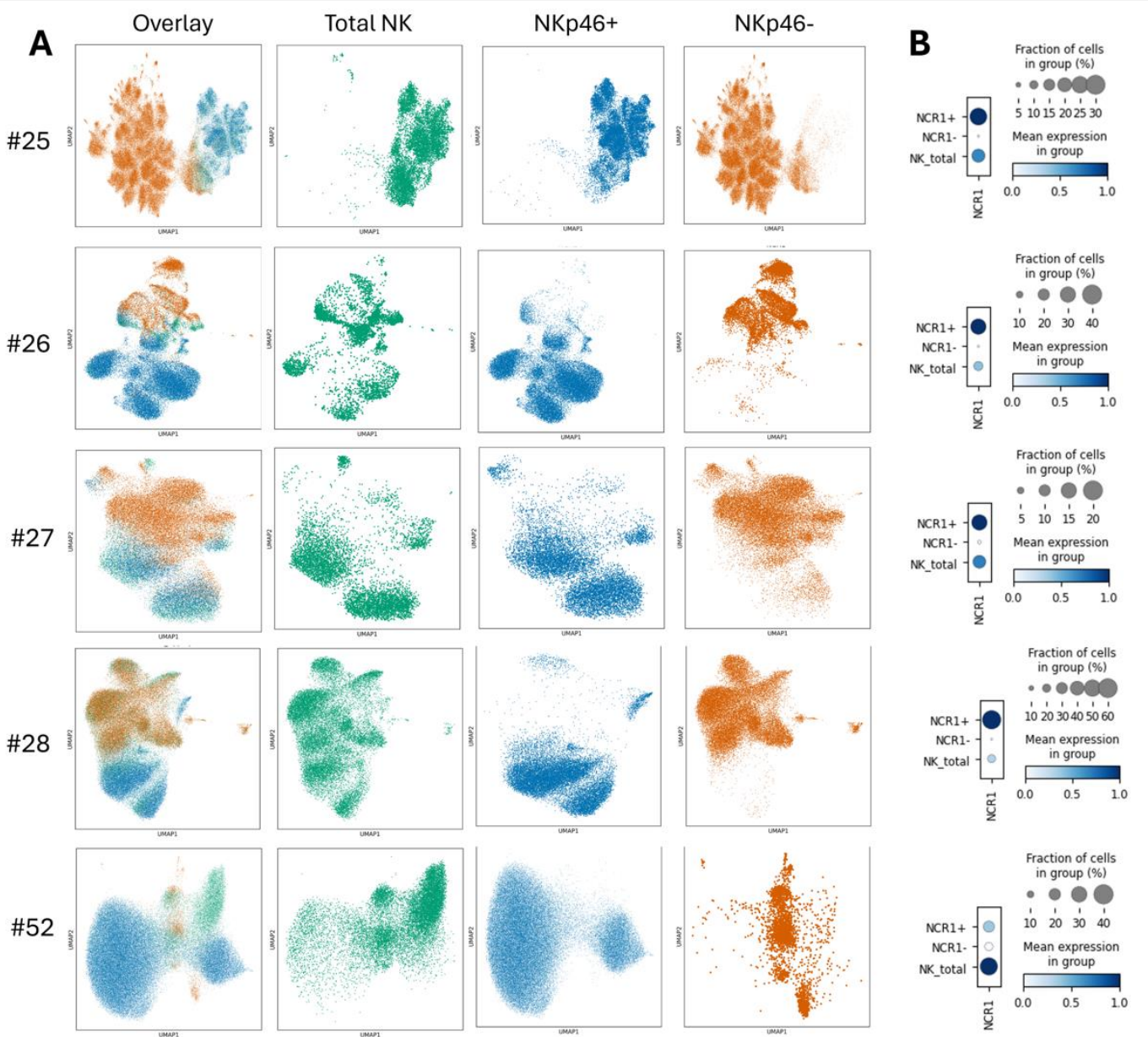
A panel for enrichment and negative sorting of bovine NK cells

- Shows gating strategy for Total NK cells, NCR1+ and NCR1- cells.

Shows validation of successfully enriching NK population

- Gating strategy used for sorting

2. Unsupervised clustering of NKp46+ and NKp46- cell sorts indicate NKp46- cells are a transcriptomically distinct NK cell subset.

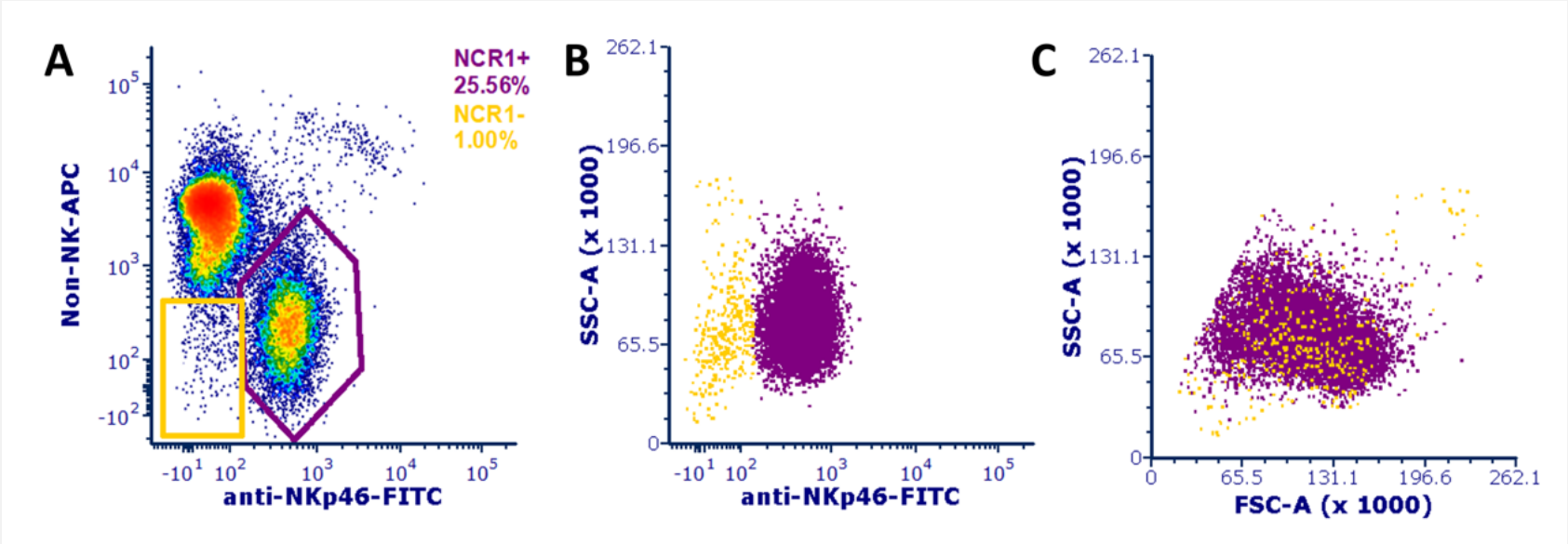


NKp46- cells form transcriptomically distinct clusters

3. NKp46- cells exhibit characteristics consistent with NK cell identity

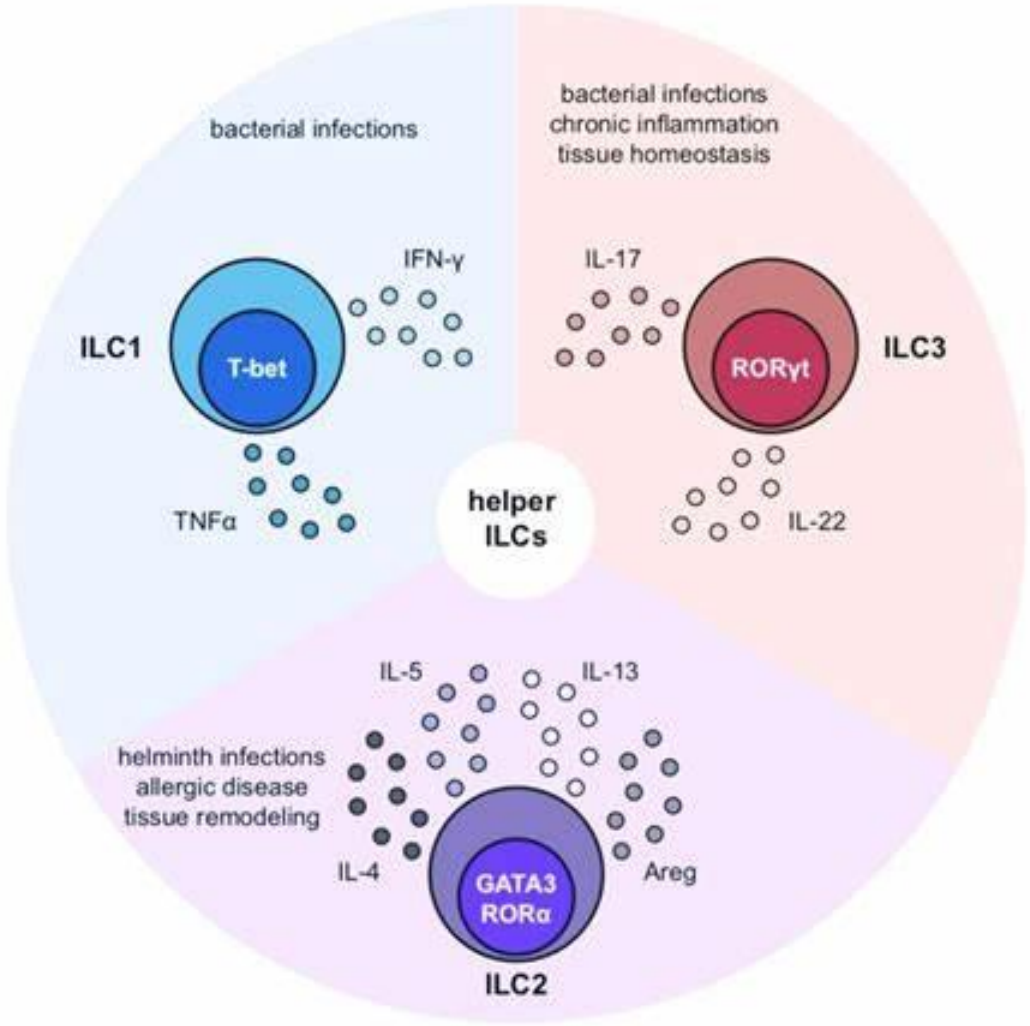
- **NKp46- cells exhibit the same distribution along the FSC (size) and SSC (granularity) axes indicating a basic cell phenotype consistent with a bovine NK cell**

- Preliminary analysis of transcriptomic signature shows NKp46- cells exhibit characteristics consistent with NK cell identity



4. NK cells or ILCs?

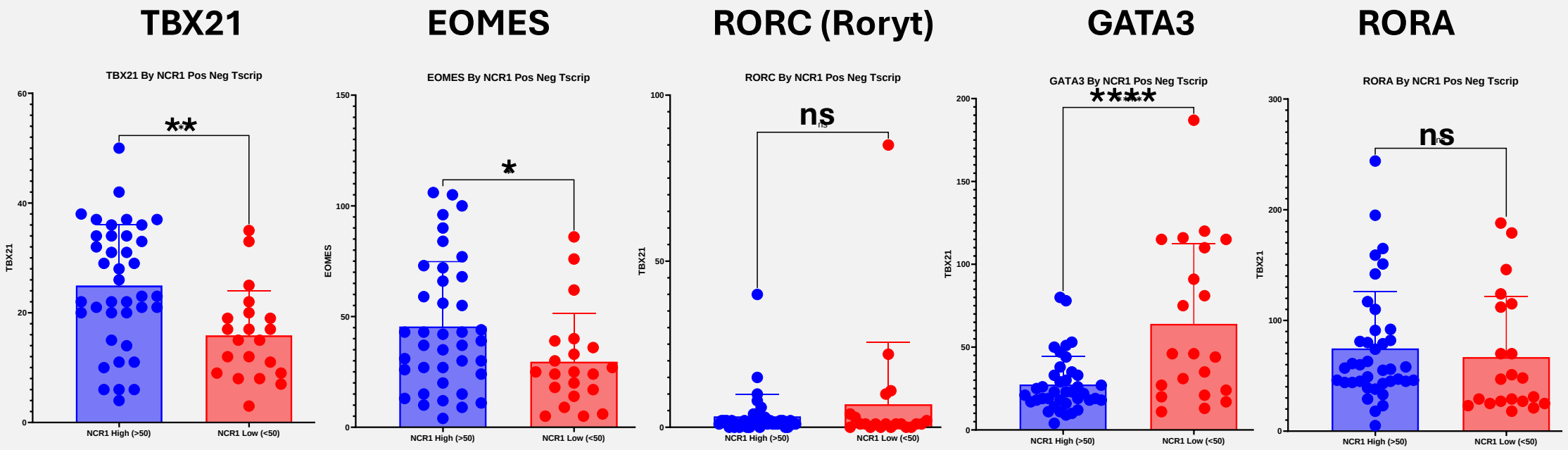
ILC Markers



10.3389/fimmu.2019.00930

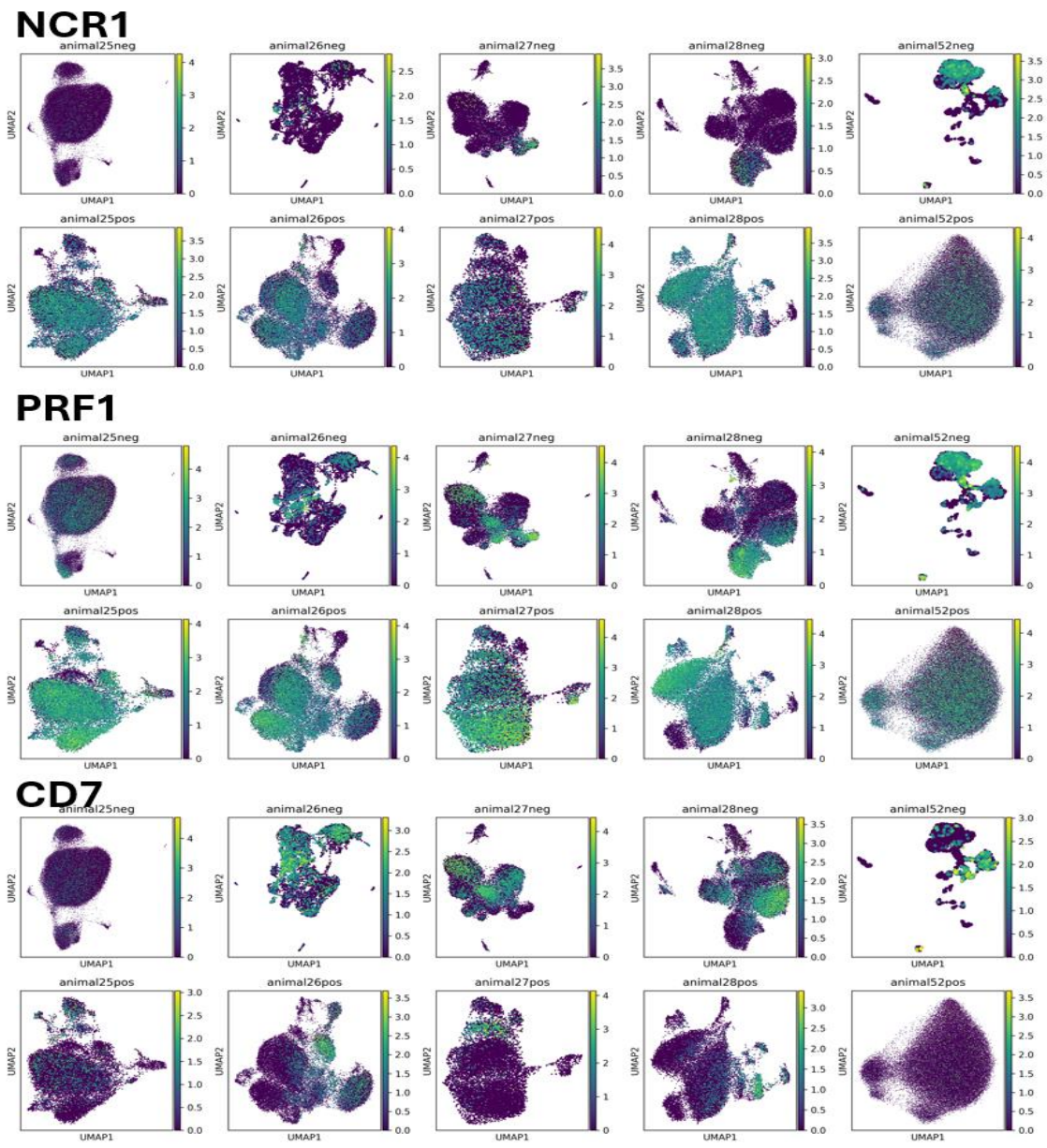
- NCR1+ and NCR1- aggregate dataset to answer question
- Preliminary finding show hybrid population based on markers observed in mice and humans

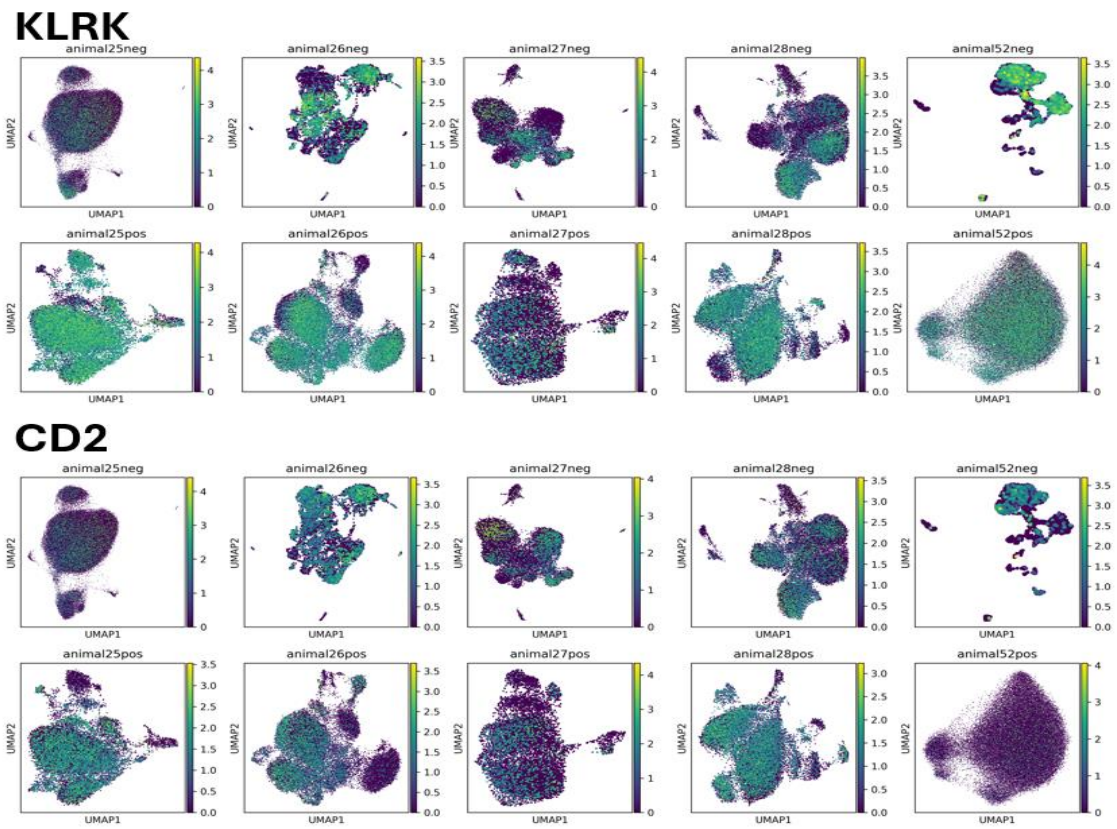
Transcription Factors. NK or ILC?



	NK/ILC1/Th1	NK	ILC3/Th17	ILC2/Th2	ILC2
	T-bet	EOMES	RORyt	GATA3	RORA
NCR1 High	Higher	Higher	Same	Lower	Same
NCR1 Low	Lower	Lower	Same	Higher	Same

4. NK cells or ILCs?



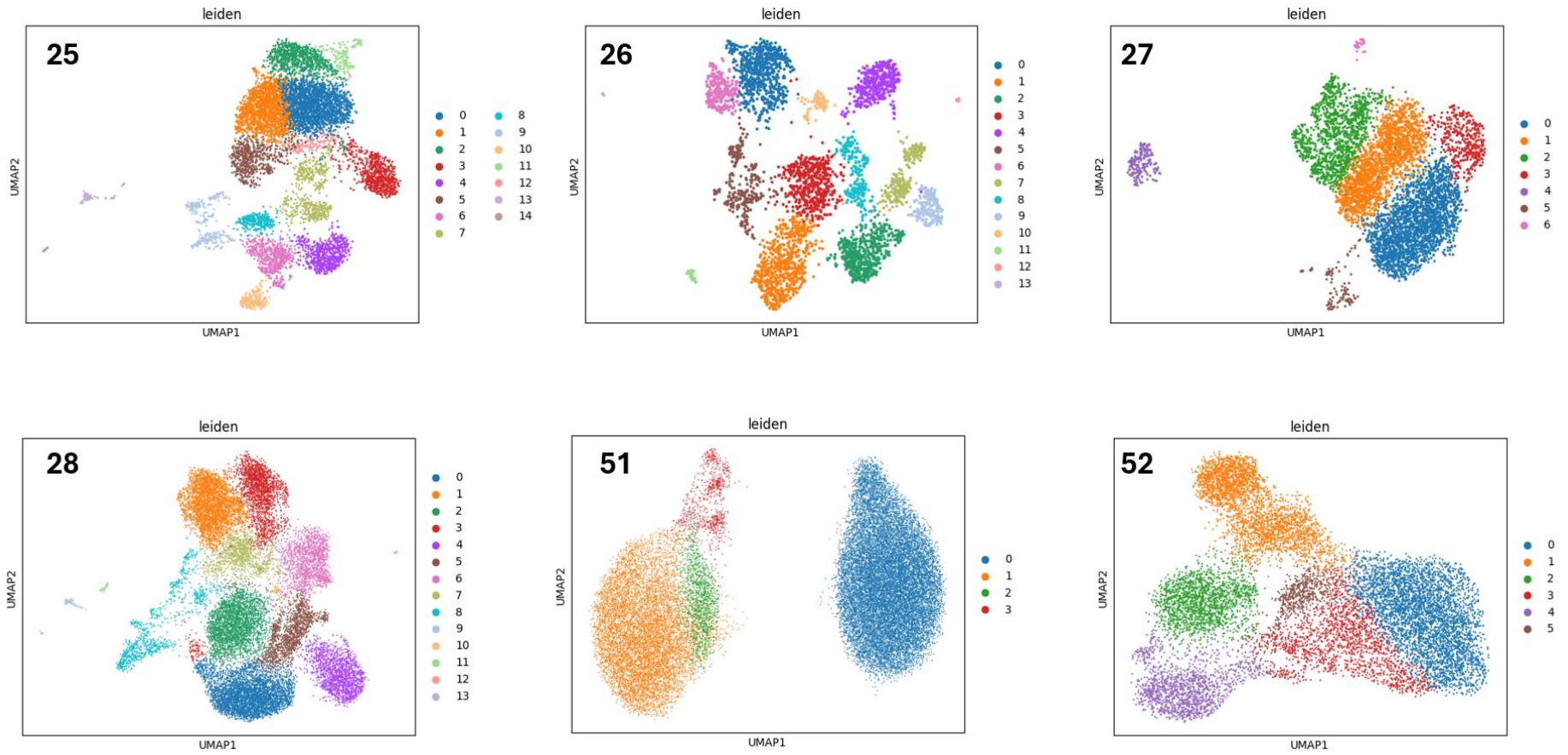


NK Cell Markers

Preliminary Conclusion: NKp46- cells are NK cells not ILCs

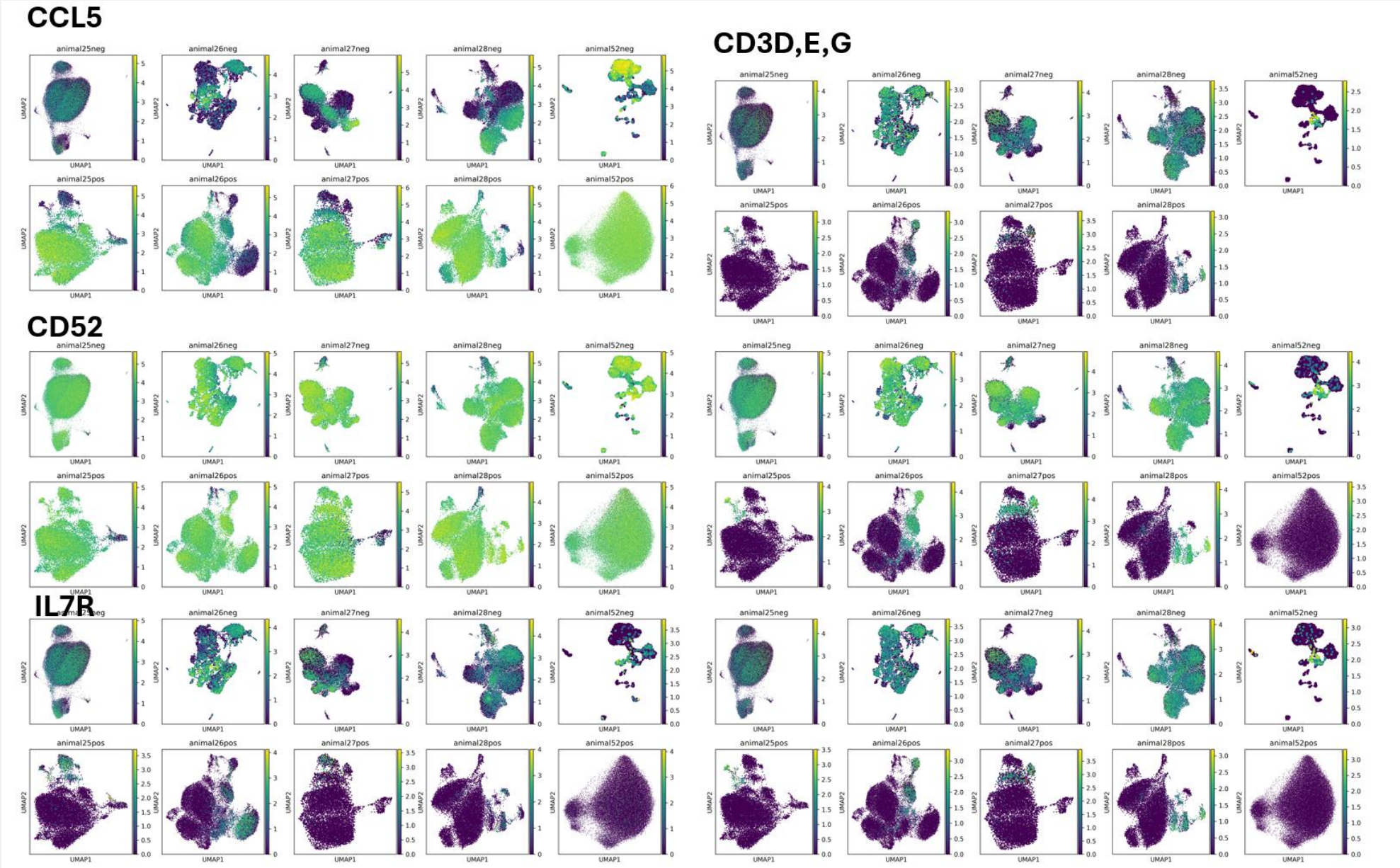
5. Defining NK cell subtypes

Unsupervised Clustering & DEG (Work in progress)



- **Defining NK cell subtypes based on?**
  - Cytotoxic/cytokine?
  - NK1/NK2/NK3?
  - Mitochondrial markers?
  - Memory/adaptive/ Mature/ Terminal/ Inflamed/ Active/ Transitional
- **Aggregating all 6 animals data set together**
  - **Try and define NK cell subtype that is consistent across all animals (regardless of animal-animal variation**

NCR1+ and NCR1- aggregated dataset interesting observations



6. NKC and LRC gene expression