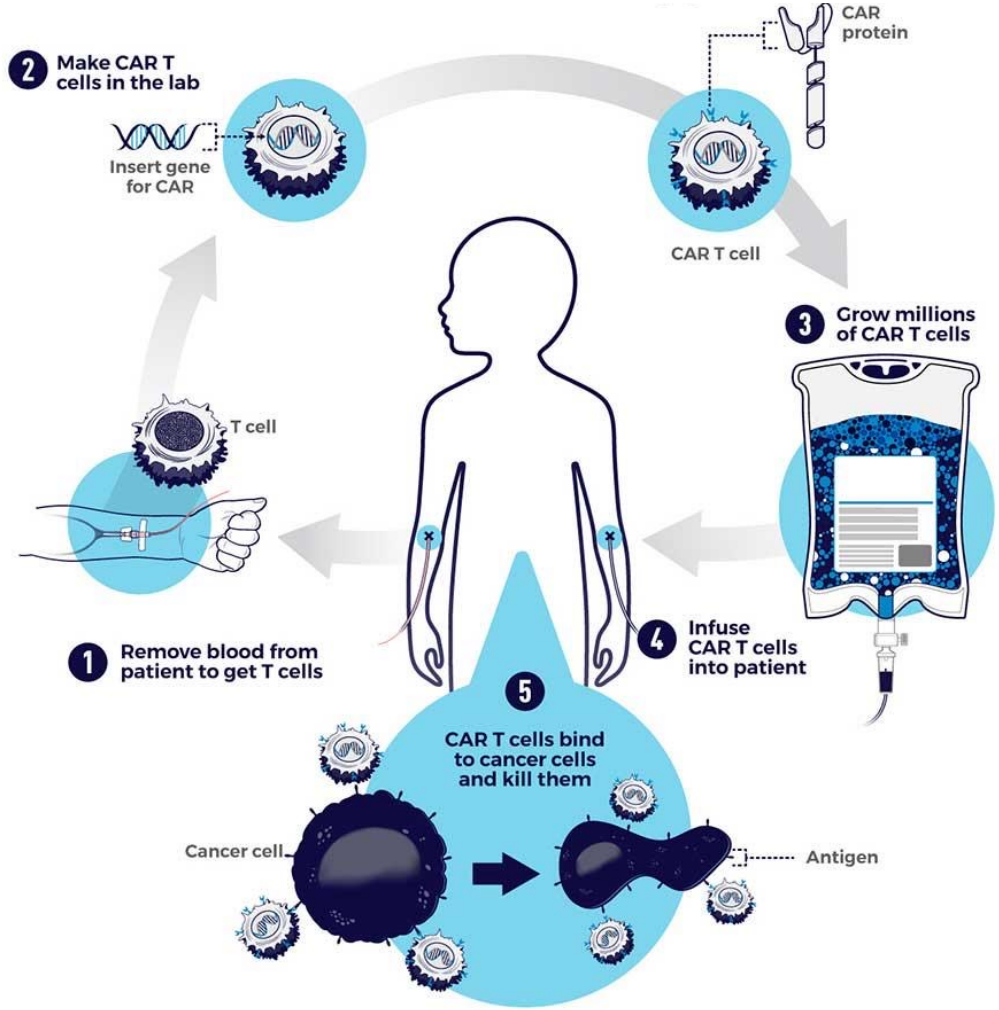
Single Cell Analysis of CAR-T Pre-Infusion Products Identifies Gene Markers Determining Complete Response

Kai Wang

What is CAR-T



(*National Cancer Institute*, 2022)

CAR-T therapy uses T cells engineered with CARs (chimeric antigen receptors) for cancer therapy.

It has been used to treat blood cancers (acute lymphocytic leukemia, multiple myeloma, and large B-cell lymphoma).

CAR-T therapy isolates the T-cells focused on fighting the virus and then genetically modifies those with CARs to target the surface markers of the cancer cell. (Bai et. al., 2022)

CAR-T therapy can not only kill tumor cells but also promote immune surveillance and assist tumor-infiltrating lymphocytes to attack the tumors. (June et. al., 2018)

Current Limitations

It has yet to be determined how the specific heterogeneities of the engineered T-cells mediate the effectiveness in therapy and whether it matches autologous therapies. (Bai et. al., 2021)

Currently, within 1 year of patients receiving CAR-T therapy, 30-60% of the treated patients relapse and nearly 20% of Acute Lymphoblastic Leukemia (ALL) patients and around 50% of patients with Large B Cell Lymphoma (LBCL) or Chronic Lymphocytic Leukemia (CLL) fail to enter initial remission (have no response). (Bai et. al., 2022)

In addition, some patients eventually fail CAR-T therapy due to a phenomenon called antigen escape. This causes tumors to reduce antigen expression which causes the immune system to not have the ability to respond to the cancer. (Shah et. al., 2021)

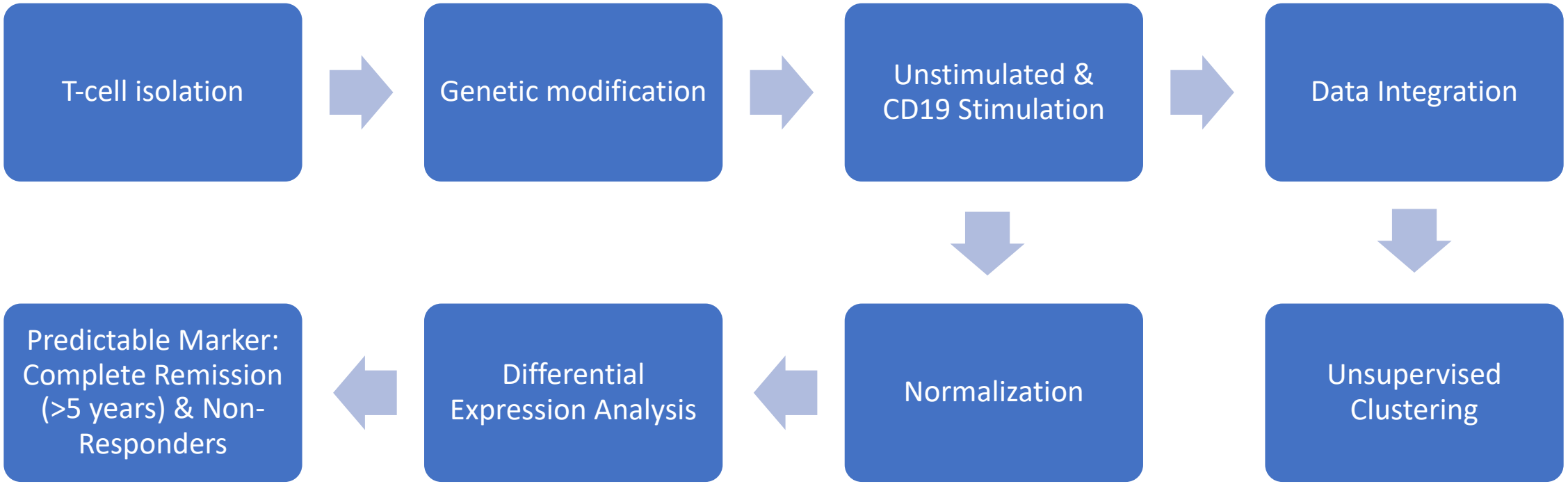
Background

- CAR-T products were collected from Children's Hospital of Philadelphia, and UPenn clinical trials.
- CAR-T cells were stimulated with CD-19 expressed 3T3 cells
- Patients are grouped into CR (complete remission after 5 years) and NR (no response)
  - CR sample IDs : 112, 118, 154, 165
  - NR sample IDs : 103, 108, 167
- Each patients has a BA (basal unstimulated) and a CD (CD-19 stimulated) dataset

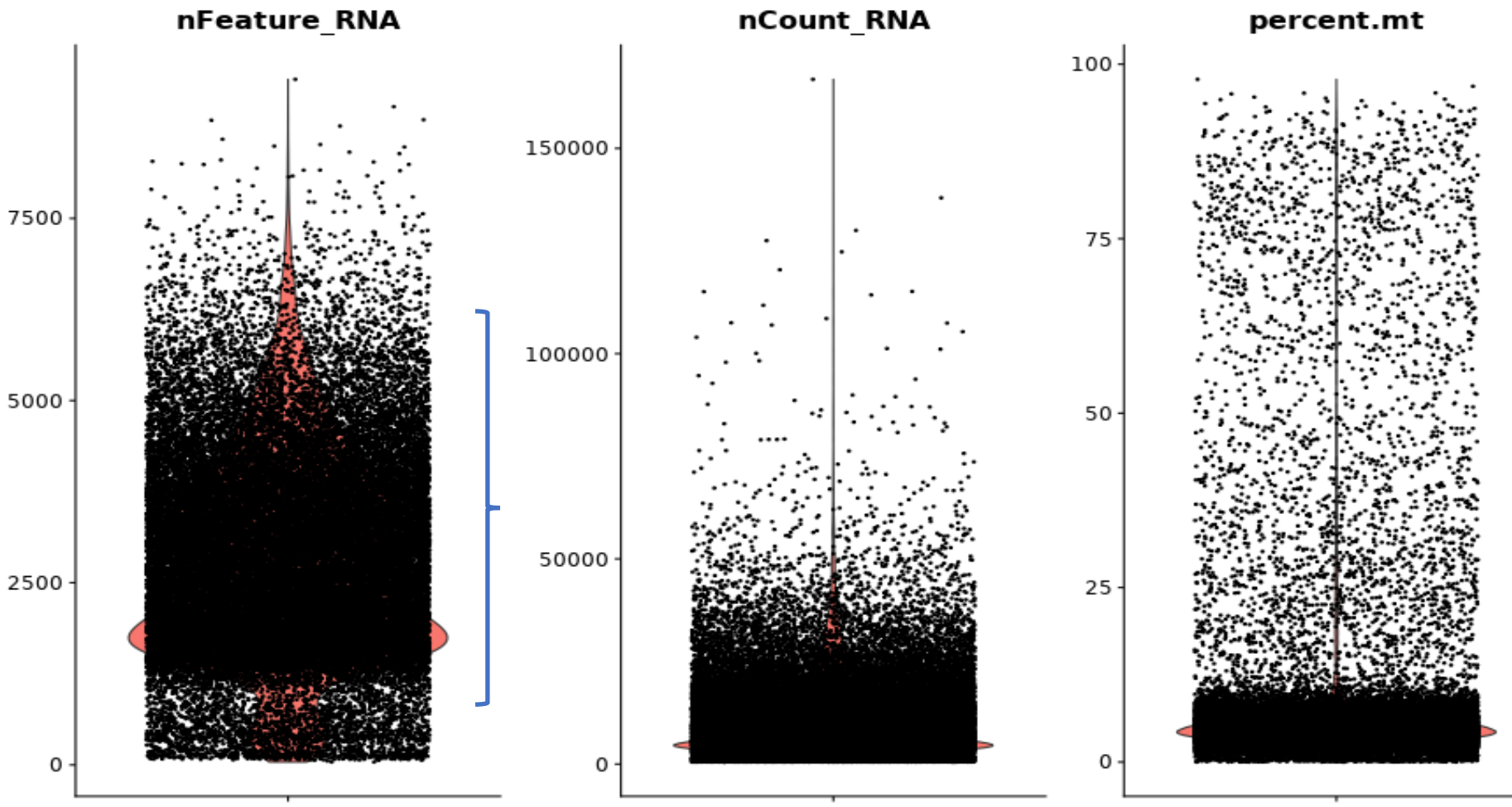
Objectives

- Learn R and Seurat for single cell RNA-seq analysis
- Perform integration analysis
  - Use reciprocal PCA (RPCA or reciprocal principal component analysis)
  - Use UMAP for visualization
- Perform differential expression analysis (DEA) to find significant genes
  - Build DEA pipeline
  - Validate with published data
  - Find significant genes between CR and NR

Methodology



Quality Control

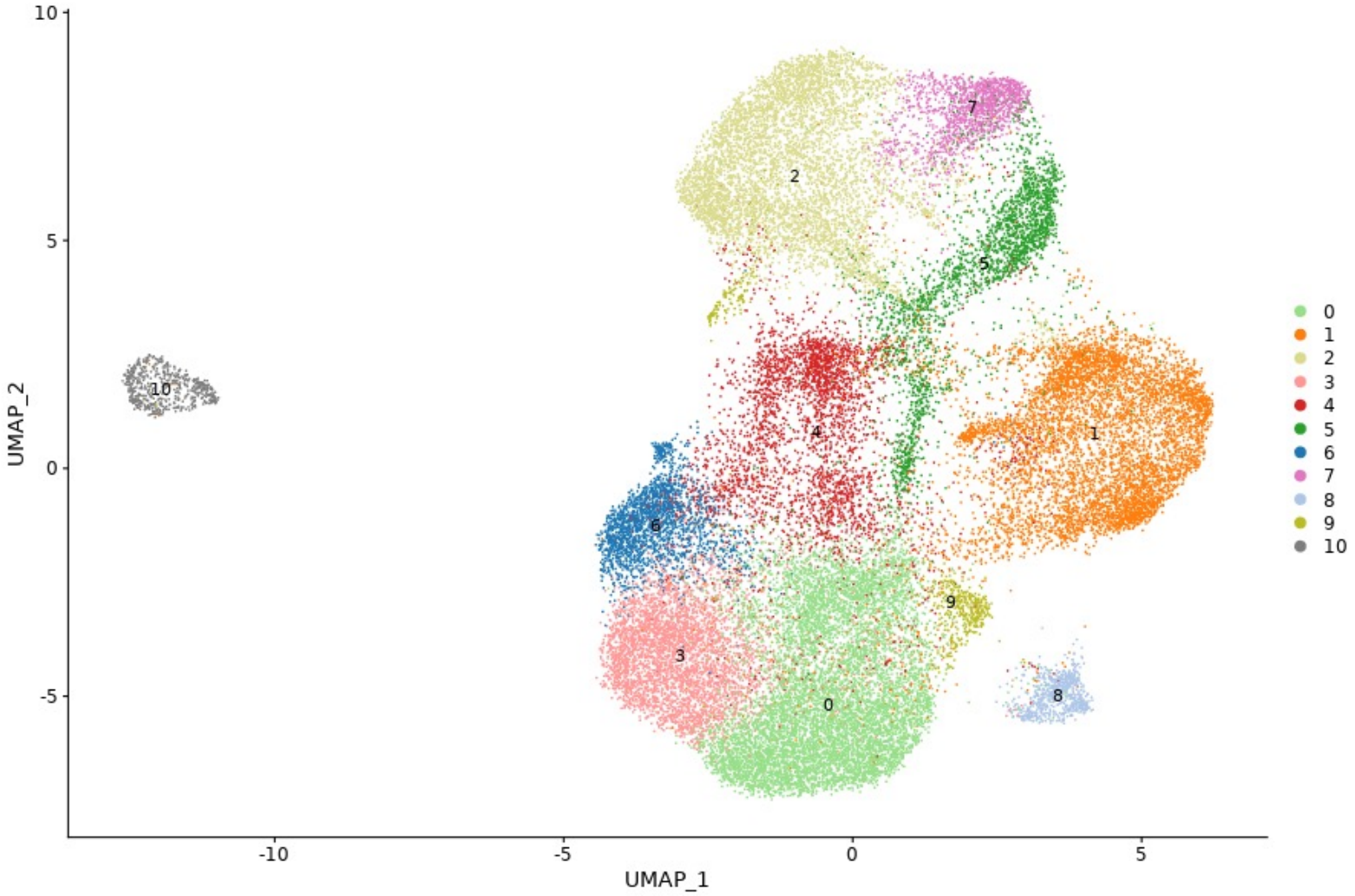


Filter out cells:

- Unique transcript counts below 600 or above 6,000
- Mitochondrial counts over 10%

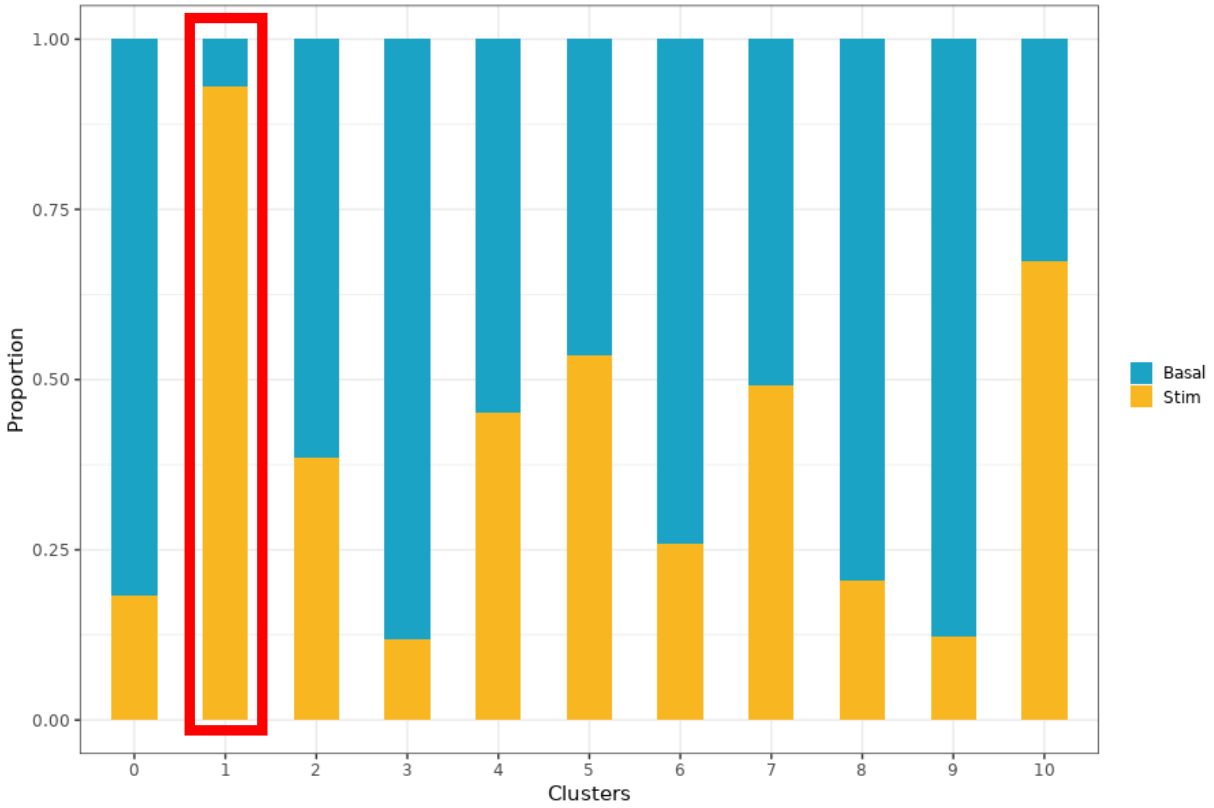
Integration

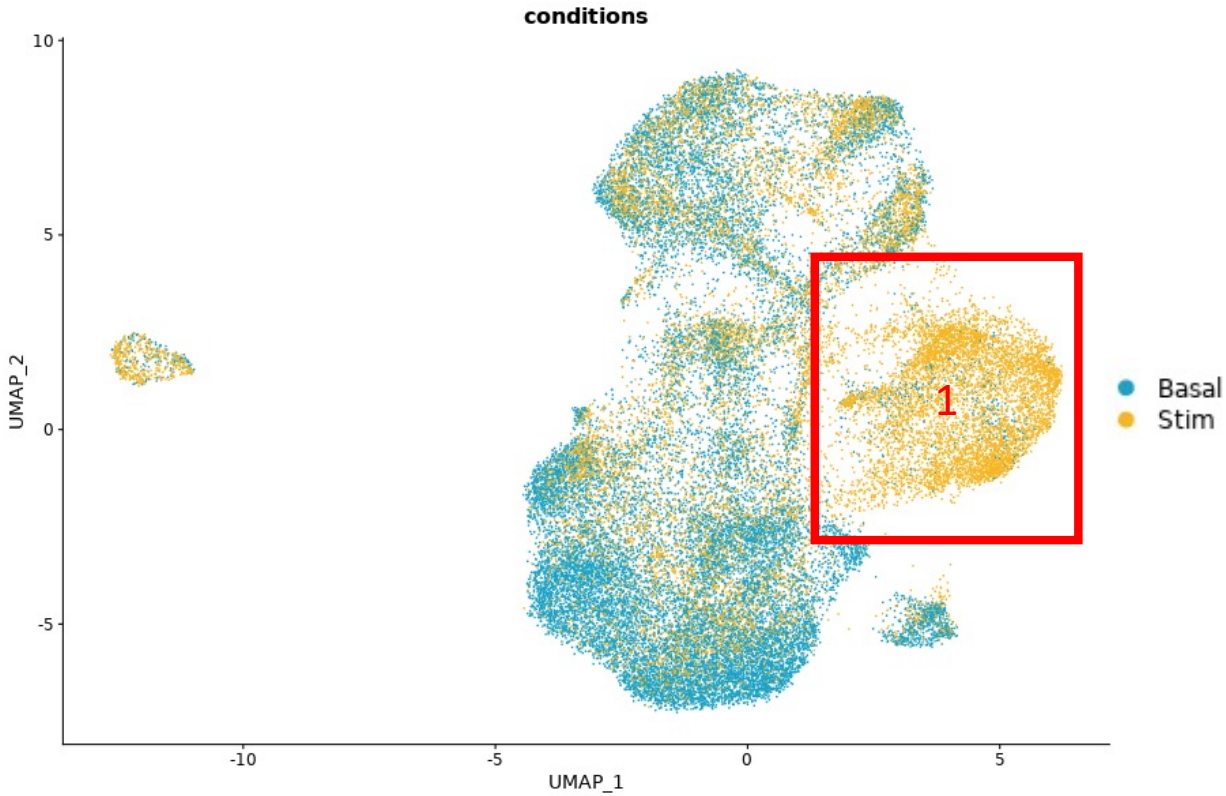
Integrating 14 scRNA-seq data of 7 patients



* Used a resolution of 0.35 and get 11 clusters

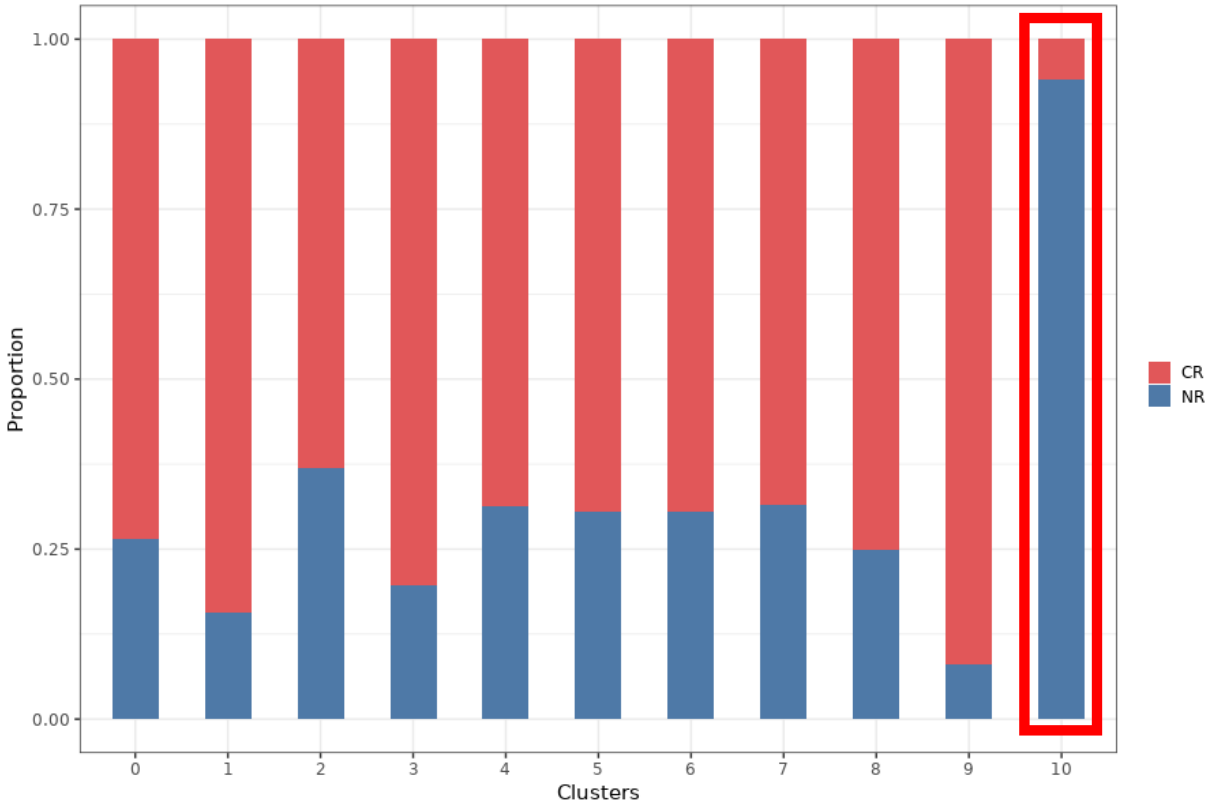
Percentage of conditions in each cluster

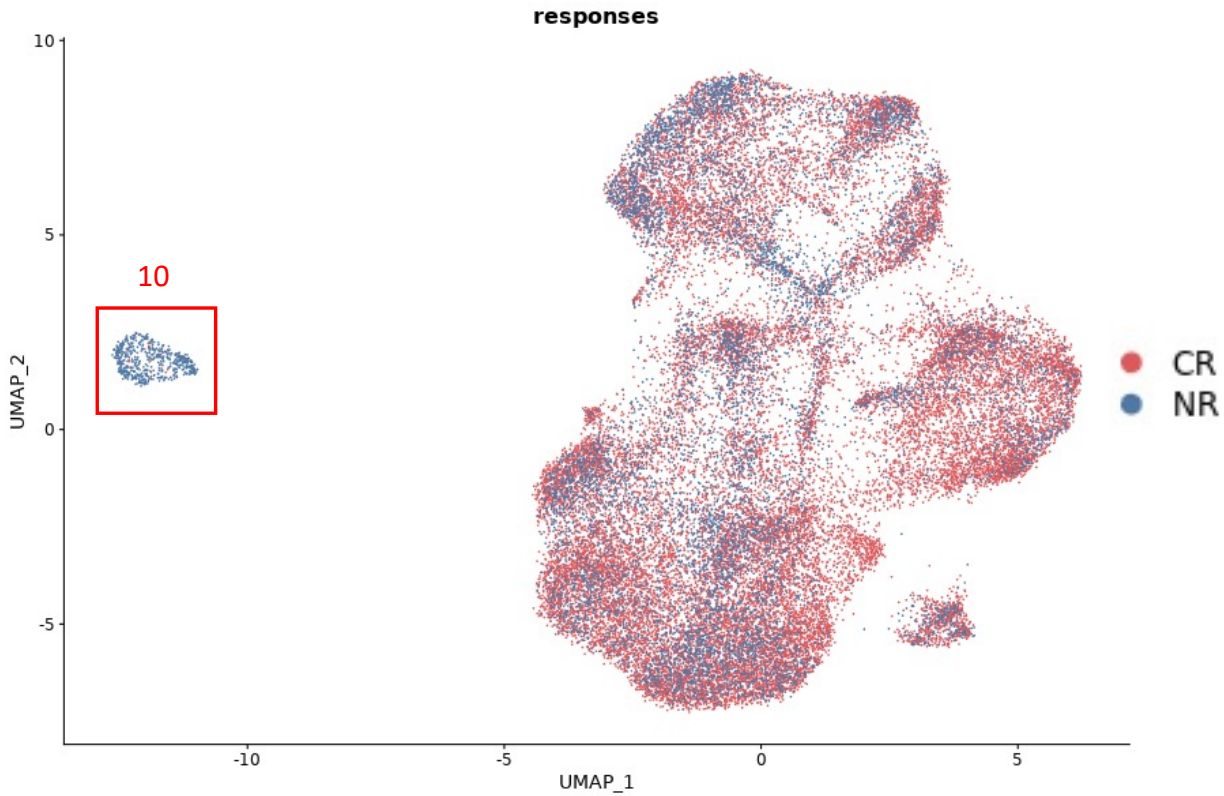




Integration removed batch effects

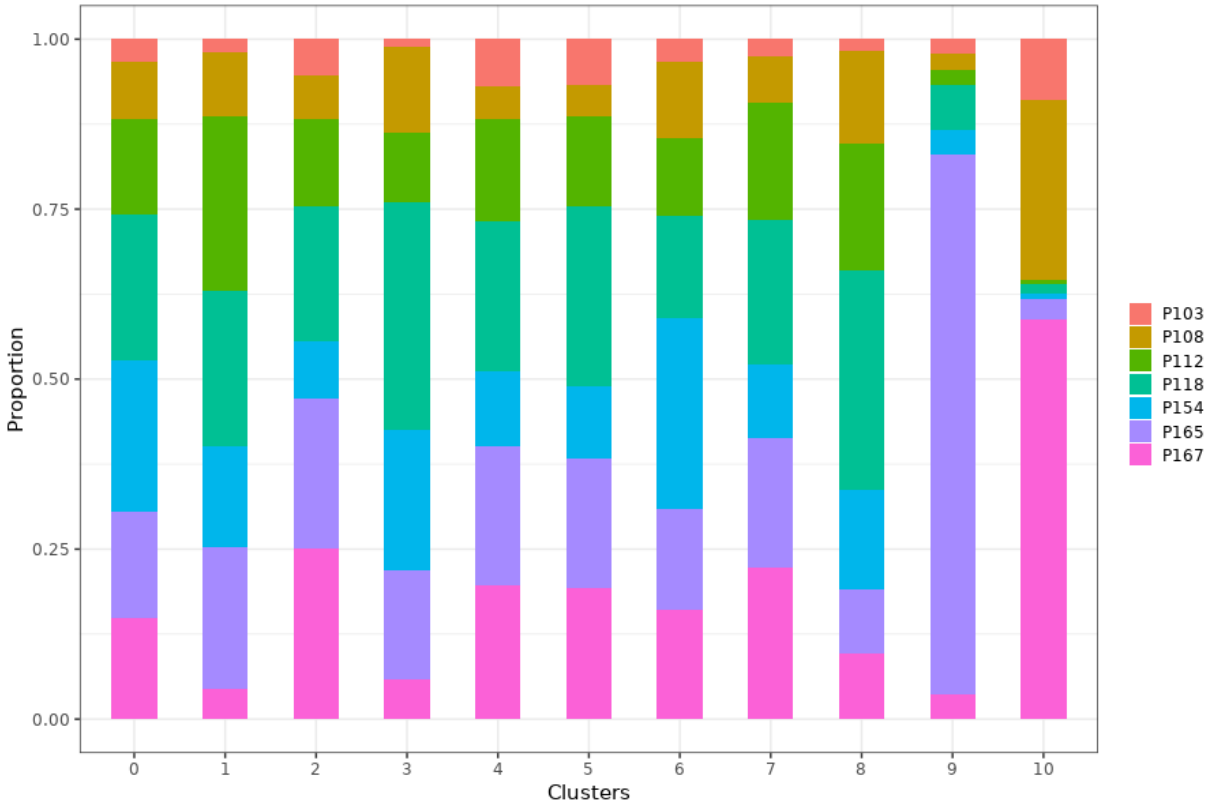
Percentage of responses in each cluster

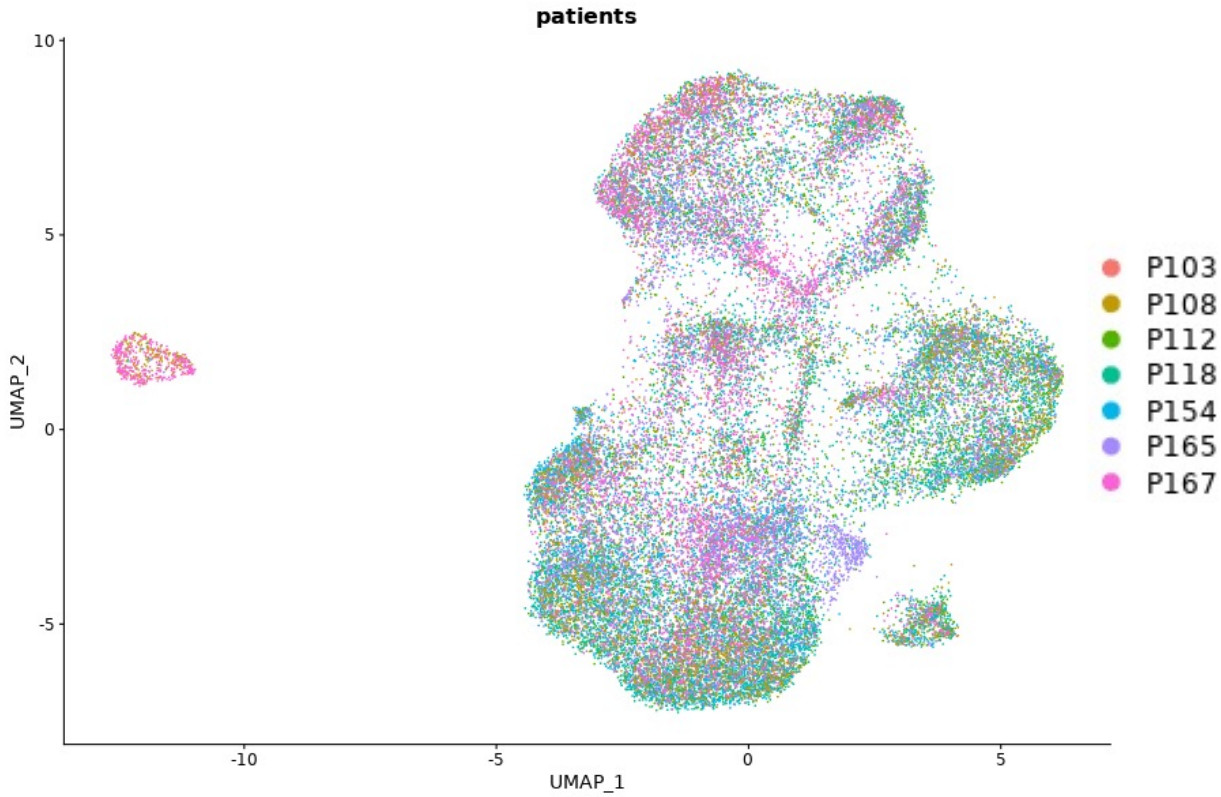




Integration removed batch effects

Percentage of patients in each cluster

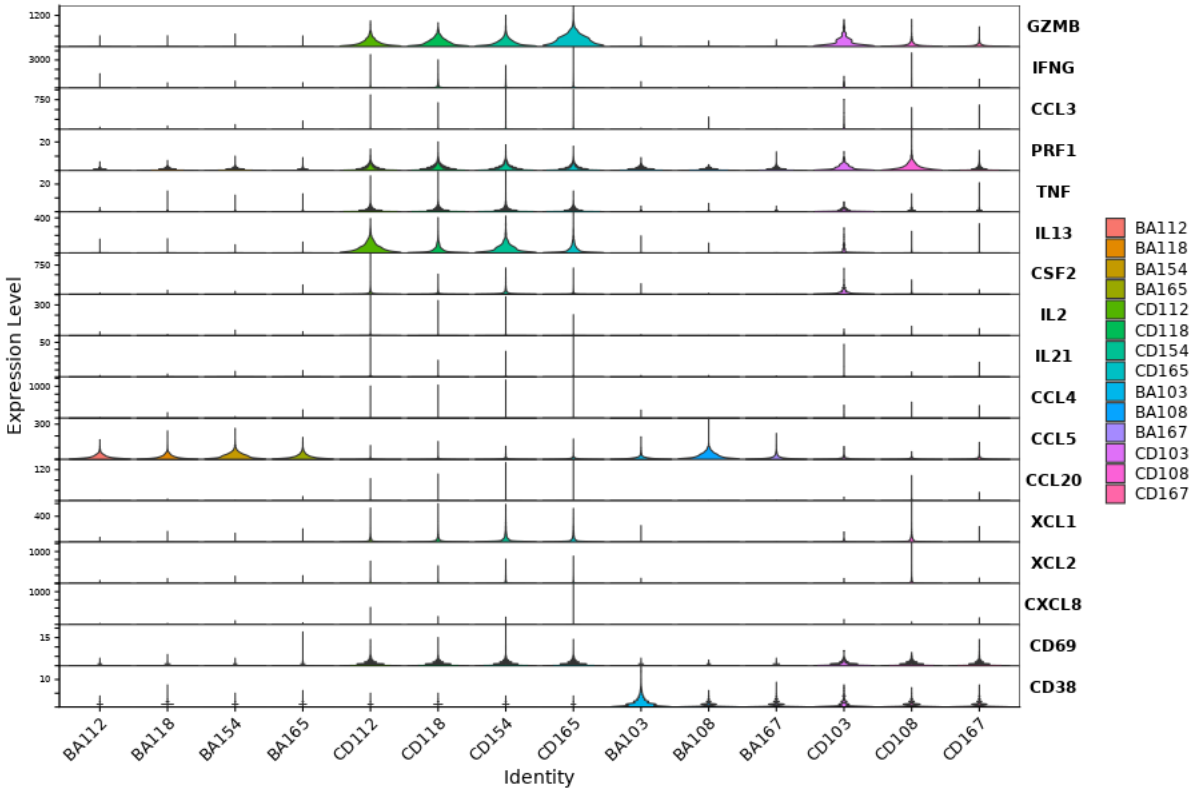




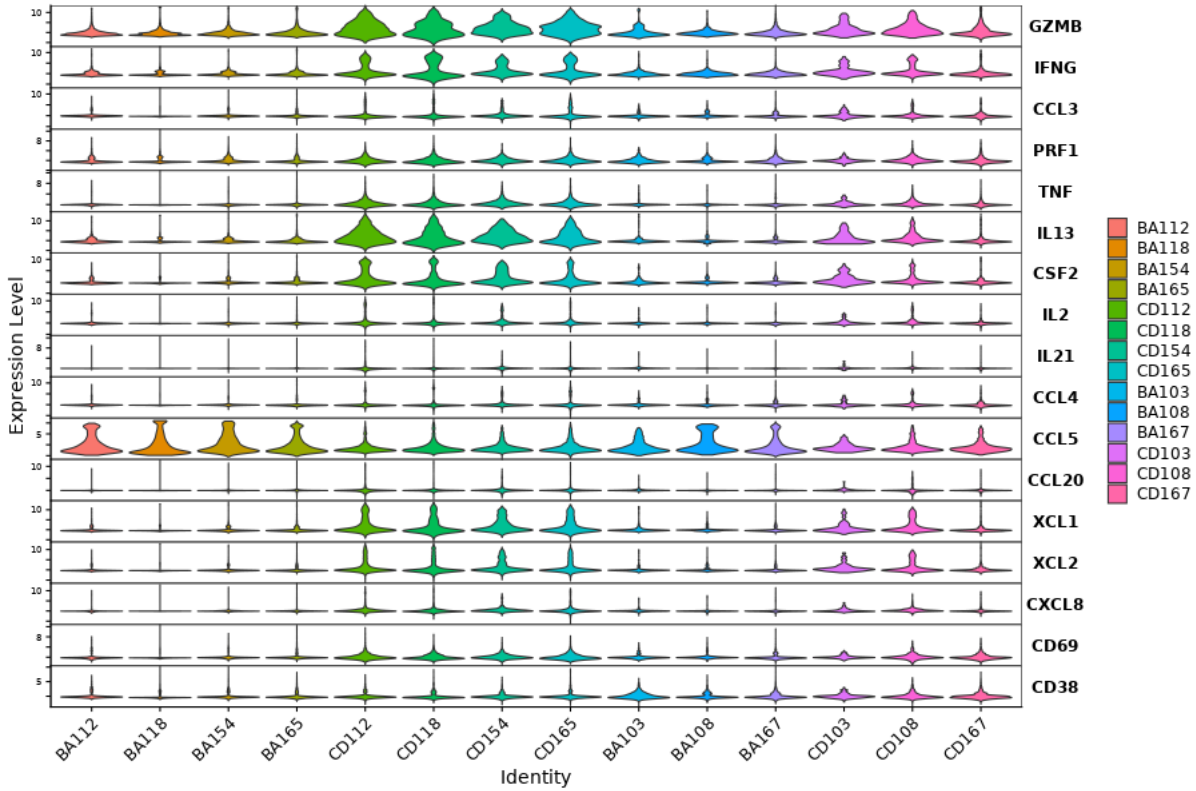
Integration removed batch effects

Single-cell expression level violin plot of immunologically relevant genes

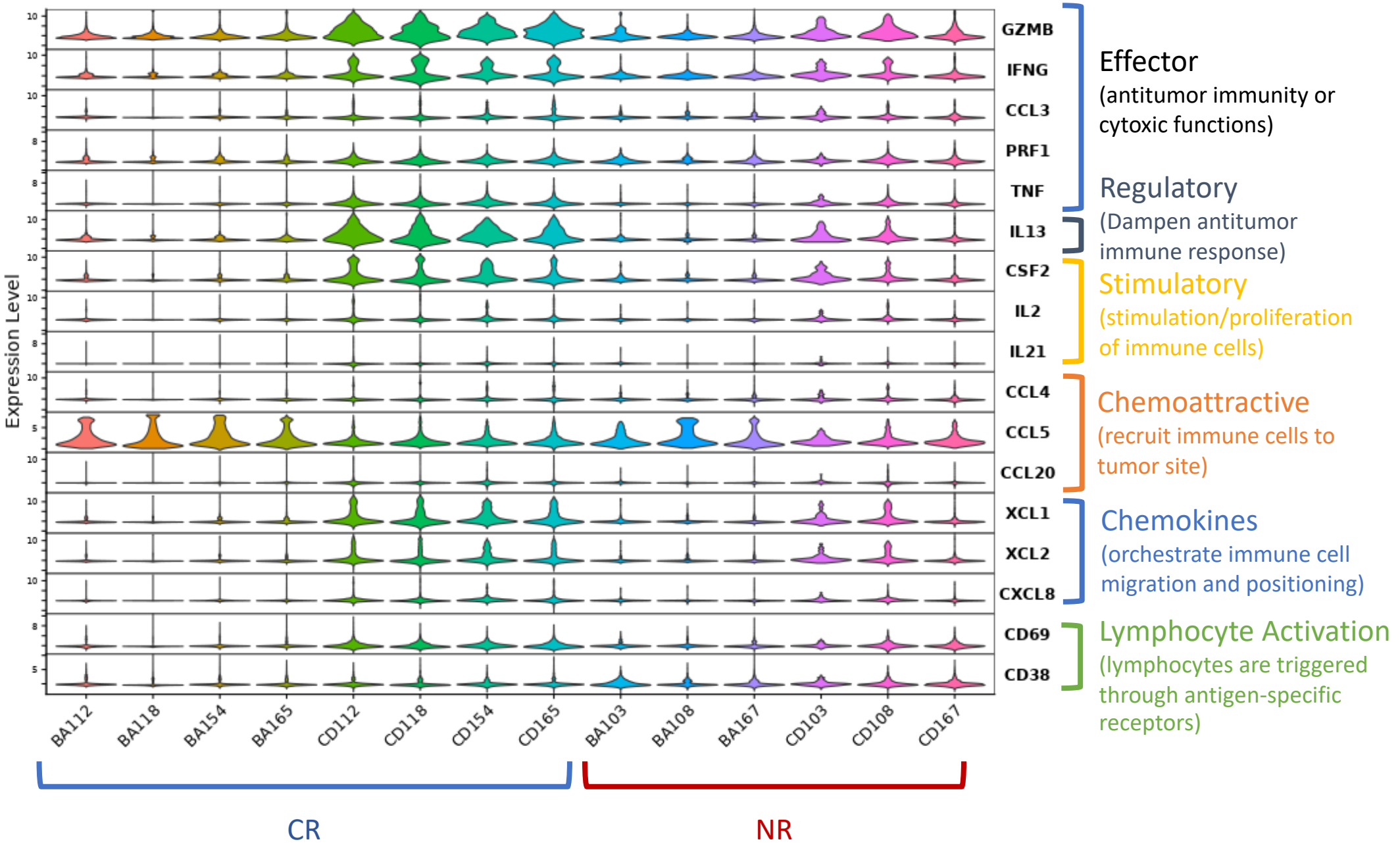
Using RNA data



Using Integrated data

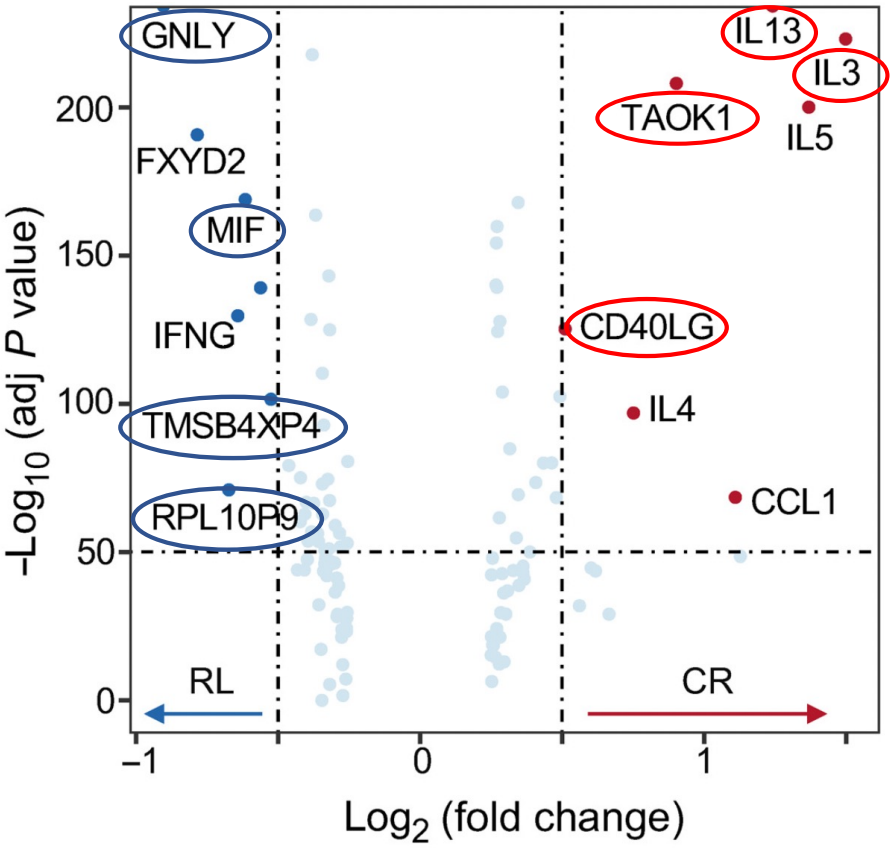


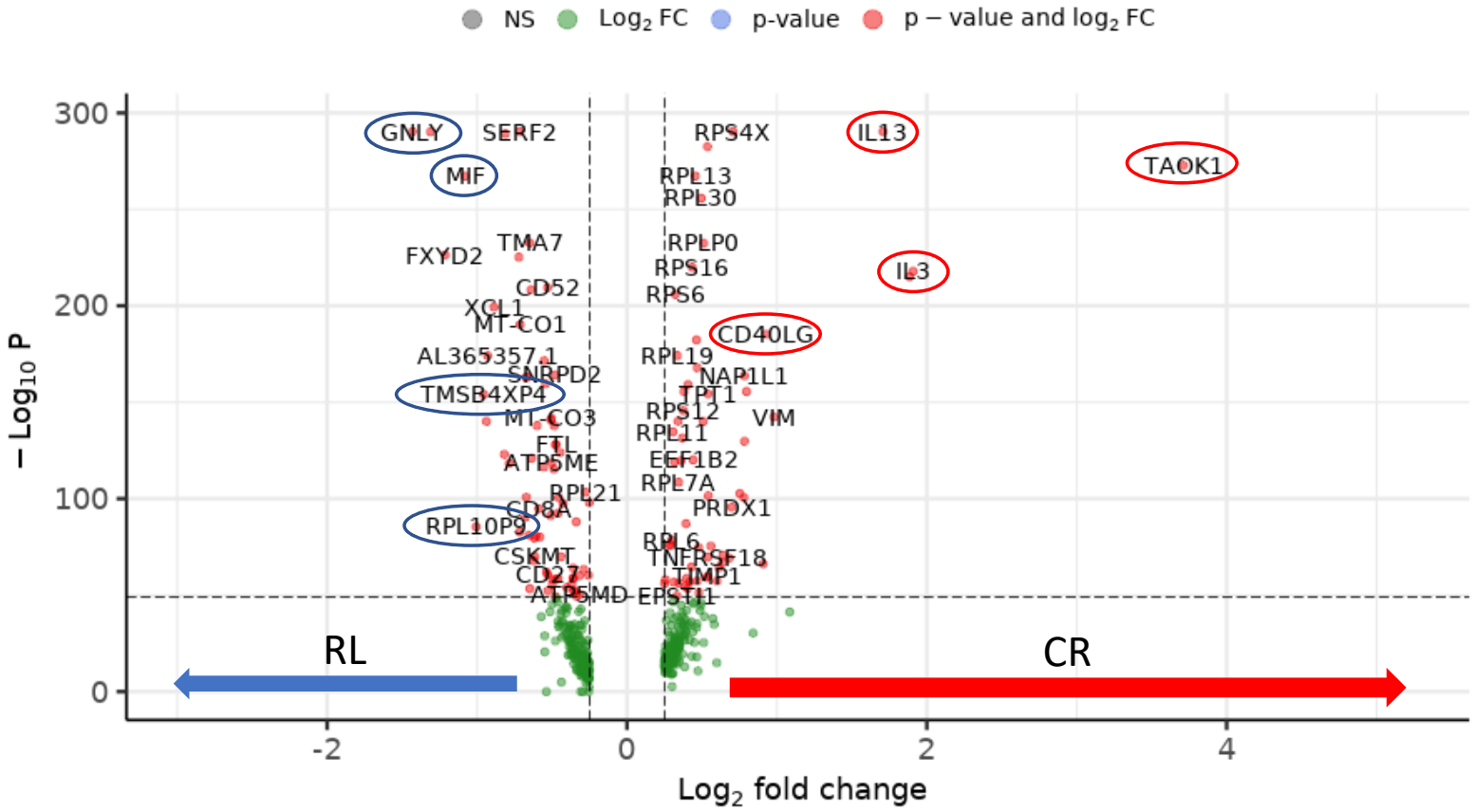
Integration removed batch effects


Expression Level
GZMB
IFNG
CCL3
PRF1
TNF
IL13
CSF2
IL2
IL21
CCL4
CCL5
CCL20
XCL1
XCL2
CXCL8
CD69
CD38
BA112
BA118
BA154
BA165
CD112
CD118
CD154
CD165
BA103
BA108
BA167
CD103
CD108
CD167
CR
NR
Effector
(antitumor immunity or cytoxic functions)
Regulatory
(Dampen antitumor immune response)
Stimulatory
(stimulation/proliferation of immune cells)
Chemoattractive
(recruit immune cells to tumor site)
Chemokines
(orchestrate immune cell migration and positioning)
Lymphocyte Activation
(lymphocytes are triggered through antigen-specific receptors)

Differential Expression Analysis

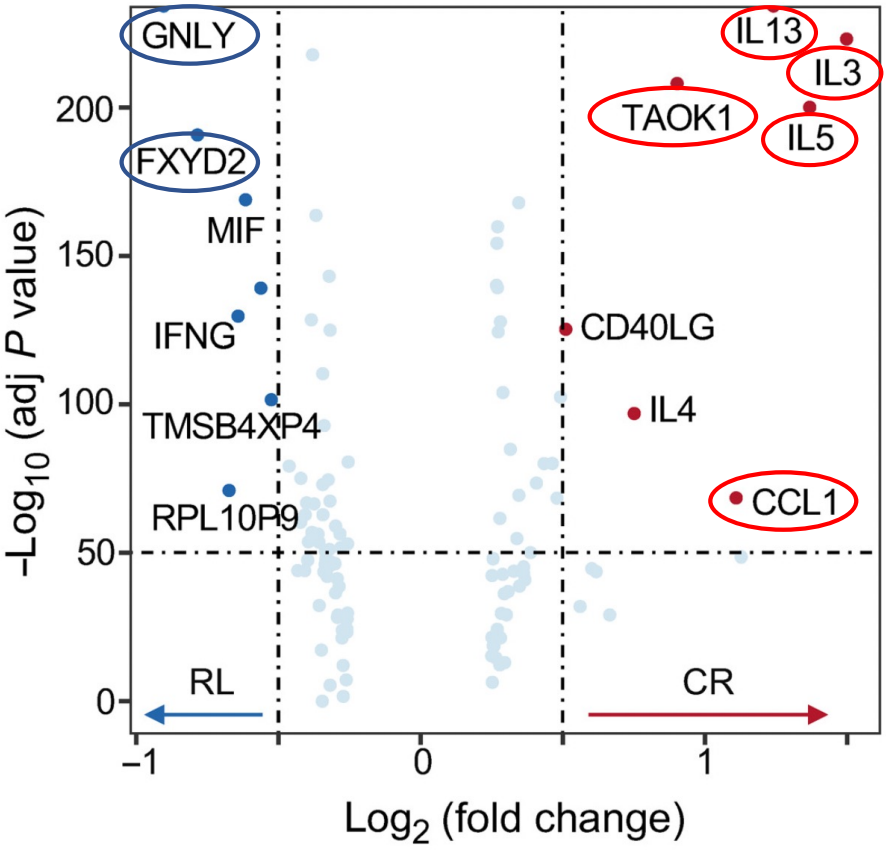
Validation of published data (Trial A)

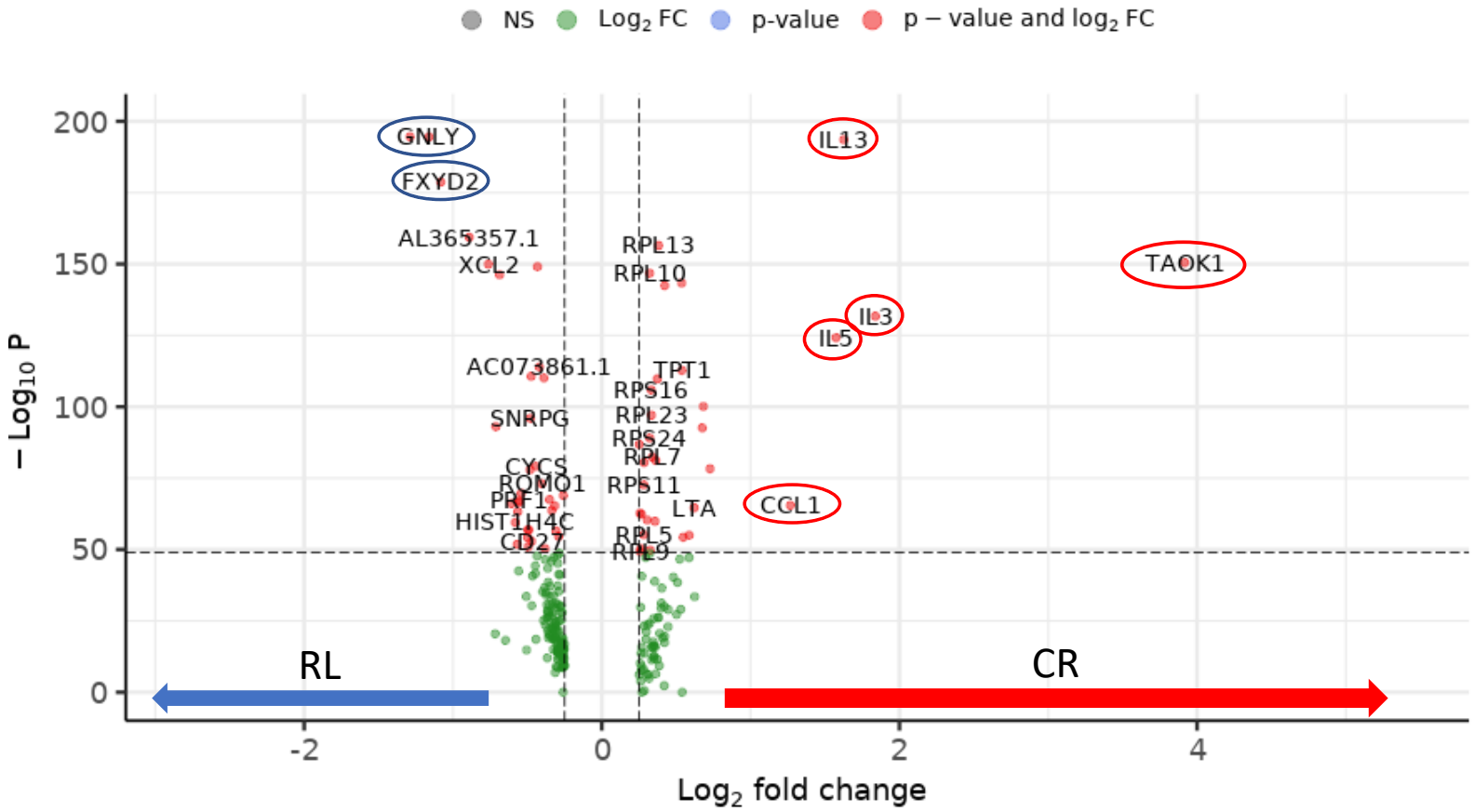




(Bai et. al., 2022)

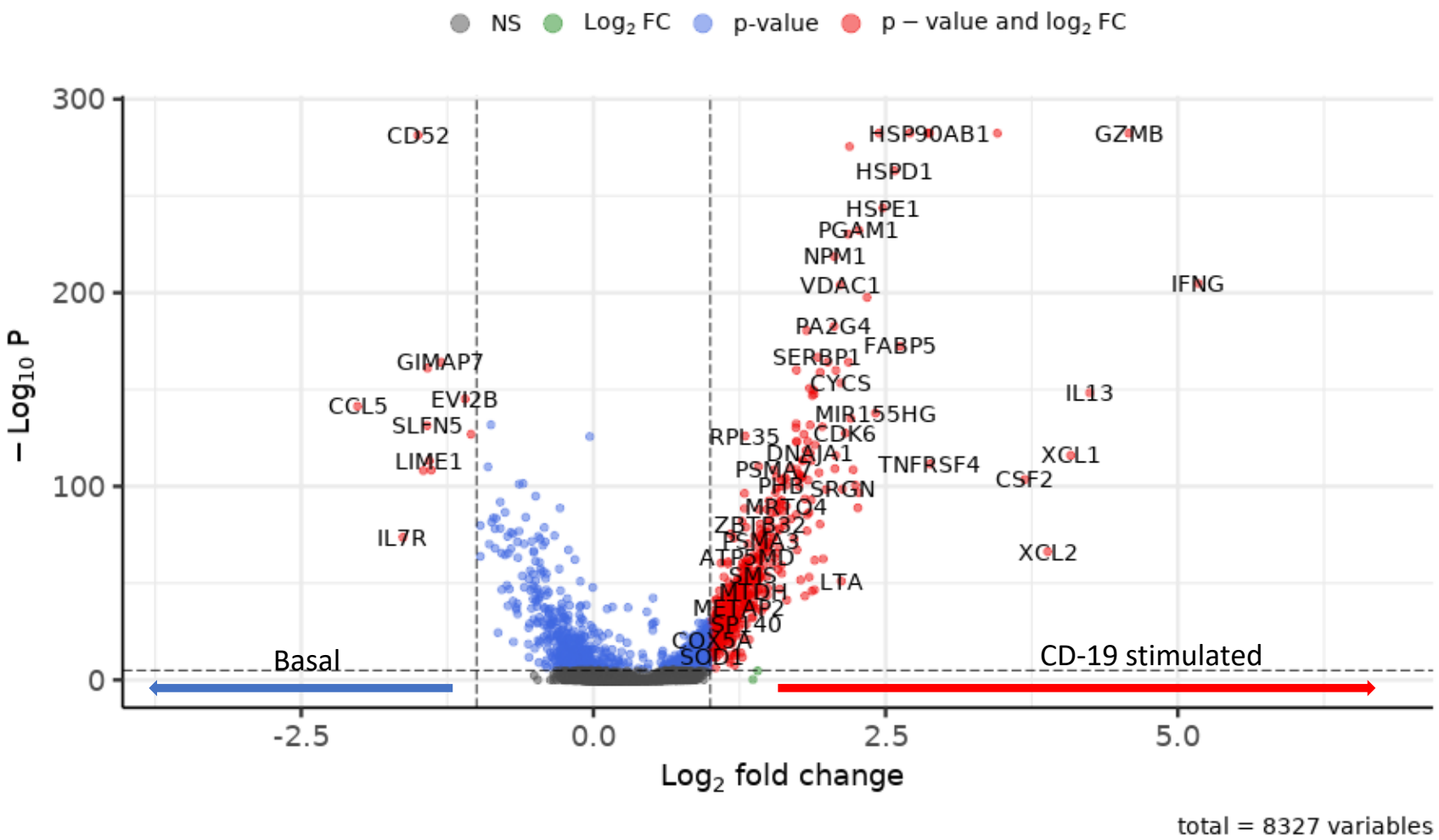
Validation of published data (Trial B)



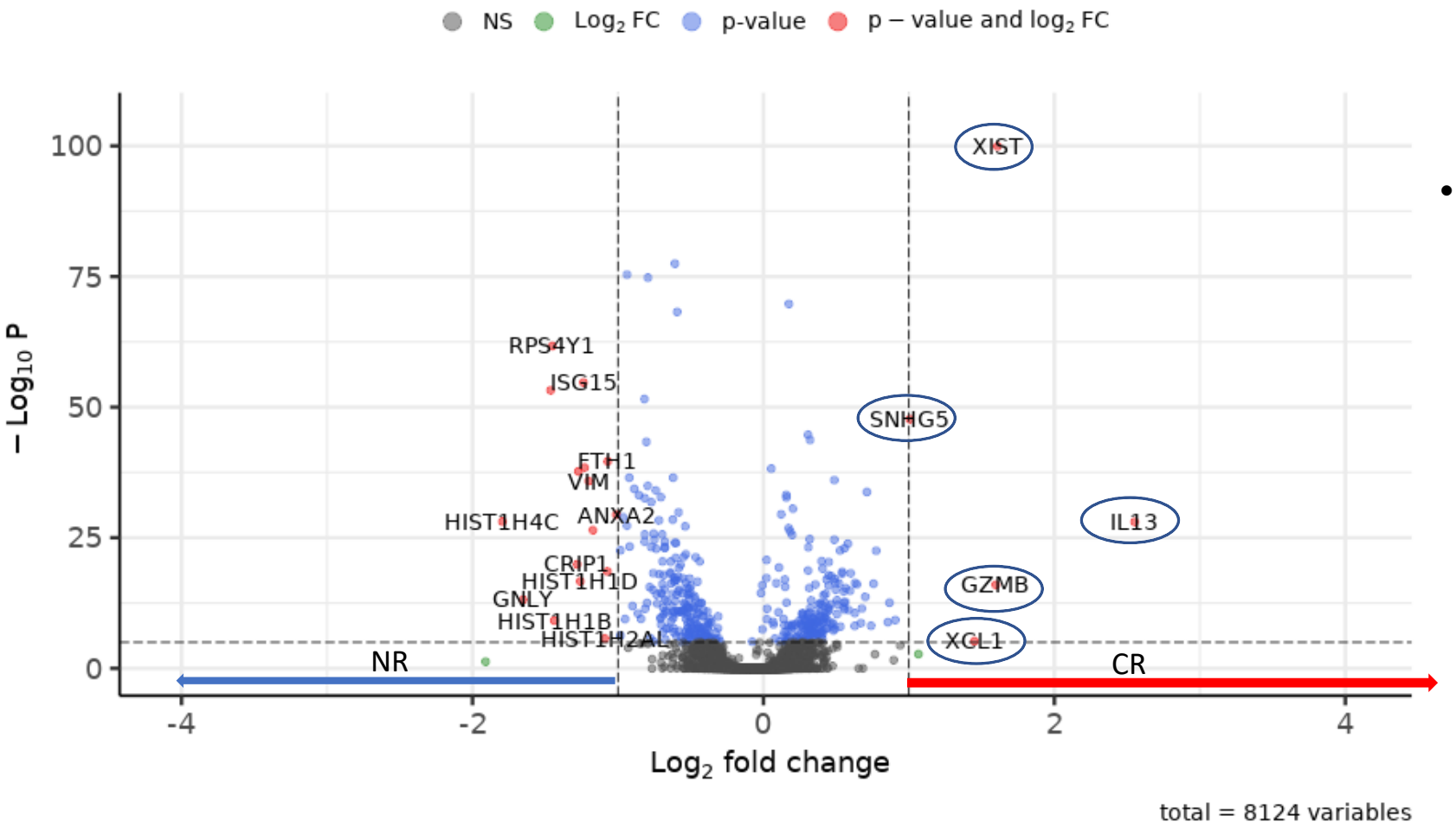


(Bai et. al., 2022)

Stimulated vs. Basal



Complete Remission vs. No Response

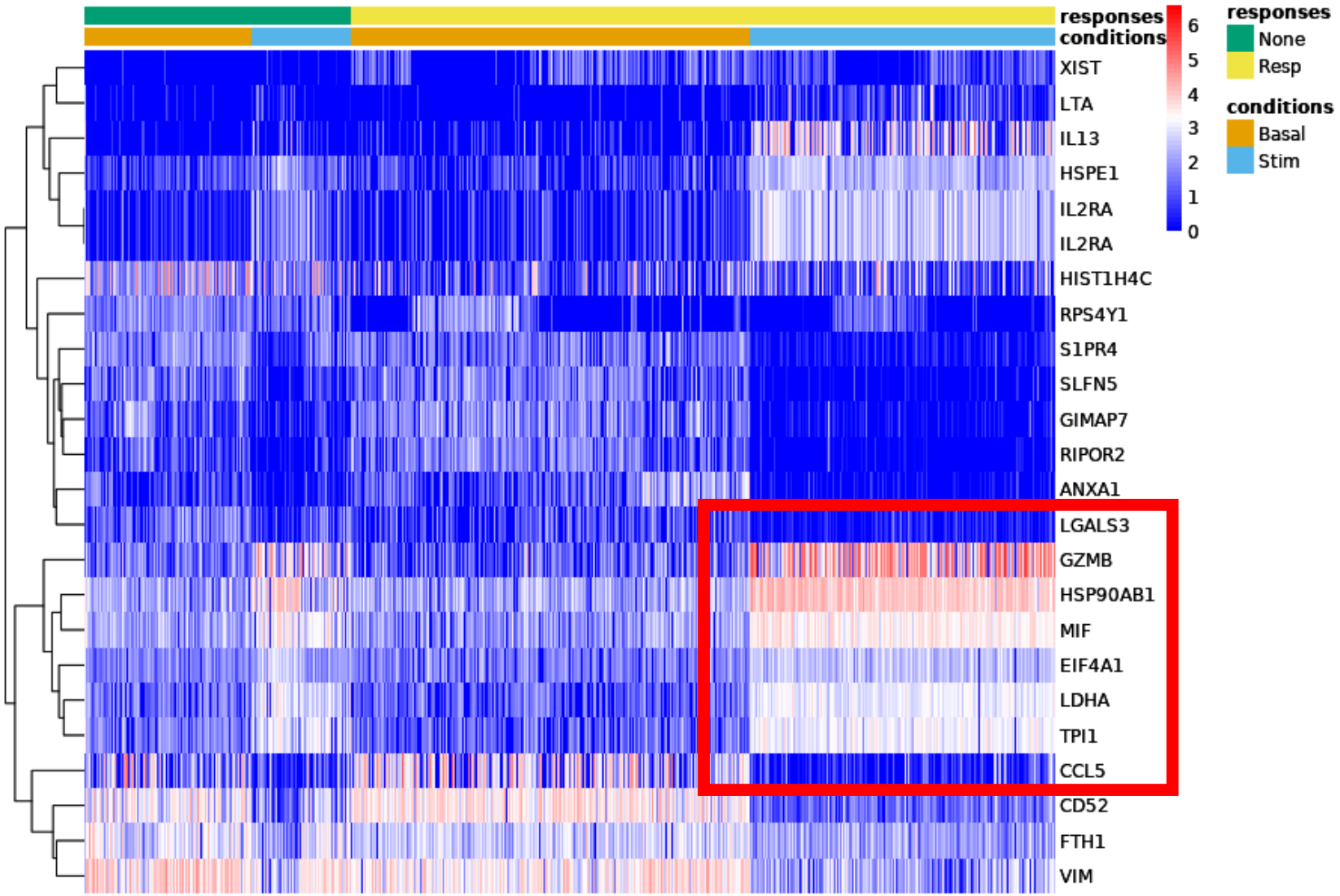


- From comparing CAR-T cells between CR patients and NR patients XIST, SNHG5, IL13, GZMB, and XCL1 have been shown to be up-regulated in complete remission

Positive DEGs in CR vs NR

	P_val	Avg_log2FC	Pct.1	Pct.2	P_val_adj
XIST	3.25e-105	1.609	0.586	0.048	1.19e-100
SNHG5	5.57e-53	1.008	0.880	0.62	2.04e-48
IL13	2.57e-33	2.552	0.514	0.192	9.40e-29
GZMB	3.01e-21	1.596	0.836	0.925	1.10e-16
XCL1	1.75e-10	1.451	0.380	0.184	6.40e-6

Heatmap of DEGs



- This heatmap uses the most biologically significant genes determined from the volcanoplot of the CR and NR patients
- This highlighted section shows the genes active in CD-19 stimulated patient cells which have reached complete remission (>5 years)

Conclusion

- XIST, SNHG5, IL13, GZMB, and XCL1 may be significant genes in distinguishing a patient entering complete remission rather than no response

Citations

- Bai, Z., Lundh, S., Kim, D., Woodhouse, S., Barrett, D. M., Myers, R. M., Grupp, S. A., Maus, M. V., June, C. H., Camara, P. G., Melenhorst, J. J., & Fan, R. (2021). Single-cell multiomics dissection of basal and antigen-specific activation states of CD19-targeted CAR T cells. *Journal for ImmunoTherapy of Cancer*, *9*(5), e002328. https://doi.org/10.1136/jitc-2020-002328
- Bai, Z., Woodhouse, S., Zhao, Z., Arya, R., Govek, K., Kim, D., Lundh, S., Baysoy, A., Sun, H., Deng, Y., Xiao, Y., Barrett, D. M., Myers, R. M., Grupp, S. A., June, C. H., Fan, R., Camara, P. G., & Melenhorst, J. J. (2022). Single-cell antigen-specific landscape of CAR T infusion product identifies determinants of CD19-positive relapse in patients with ALL. *Science Advances*, *8*(23), eabj2820. https://doi.org/10.1126/sciadv.abj2820
- June, C. H., & Sadelain, M. (2018). Chimeric Antigen Receptor Therapy. *New England Journal of Medicine*, *379*(1), 64–73. https://doi.org/10.1056/NEJMra1706169
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- Shah, N. N., & Fry, T. J. (2019). Mechanisms of resistance to CAR T cell therapy. *Nature Reviews. Clinical Oncology*, *16*(6), 372–385. https://doi.org/10.1038/s41571-019-0184-6

Acknowledgments

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