

## **Gut mucosa dissociation protocols influence cell type proportions and single-cell gene expression levels**

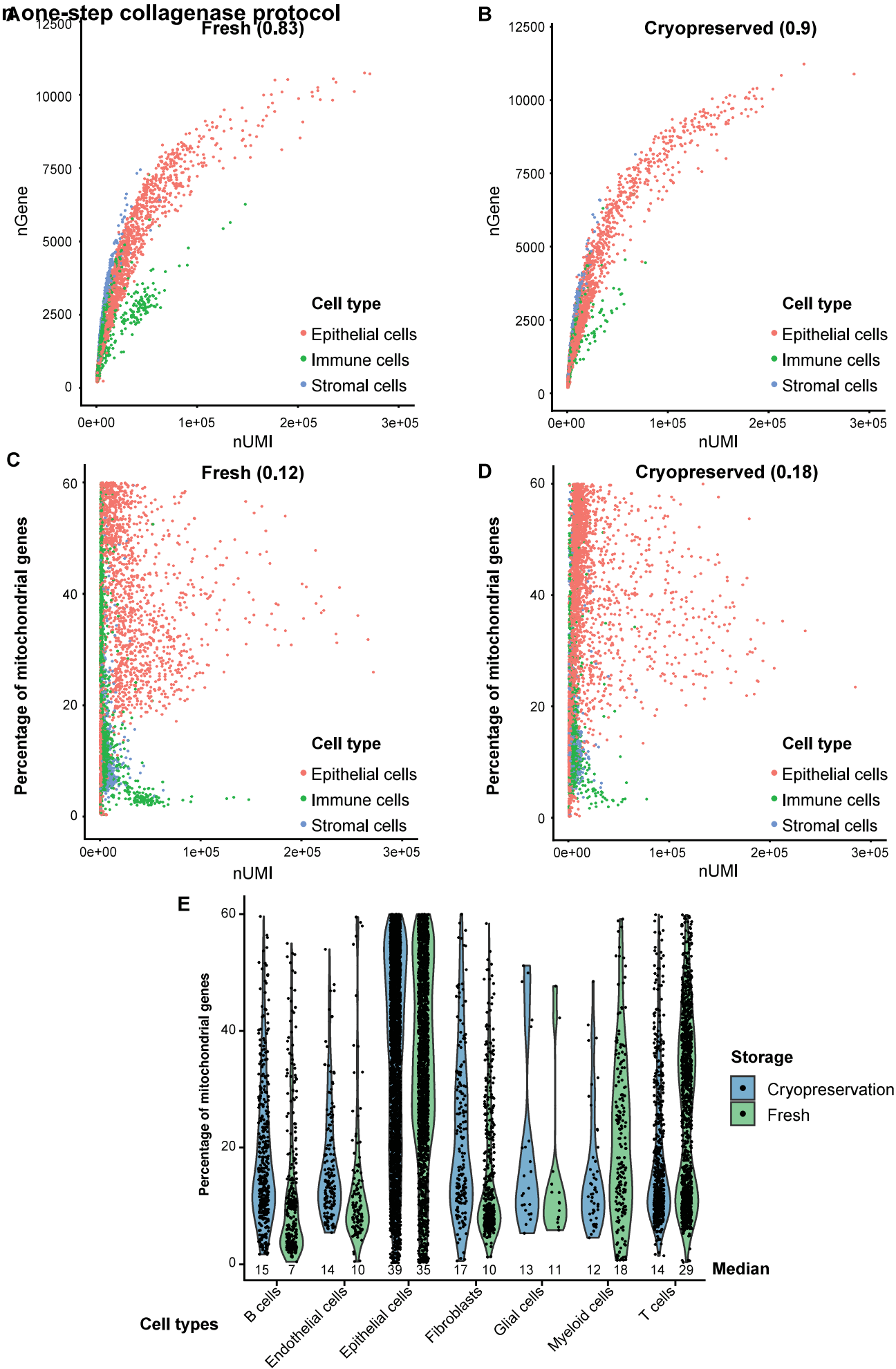
Werna T.C. Uniken Venema<sup>1,#</sup>, Aarón D. Ramírez-Sánchez<sup>2,#</sup>, Emilia V. Bigaeva<sup>1</sup>, Sebo Withoff<sup>2</sup>, Iris Jonkers<sup>2</sup>, Rebecca E. McIntyre<sup>X</sup>, M. Ghouraba<sup>X</sup>, Rinse K. Weersma<sup>2</sup>, Lude Franke<sup>1</sup>, Eleonora A.M. Festen<sup>1,\*</sup>, Monique G.P. van der Wijst<sup>2,\*</sup>

# shared first author, \* corresponding authors

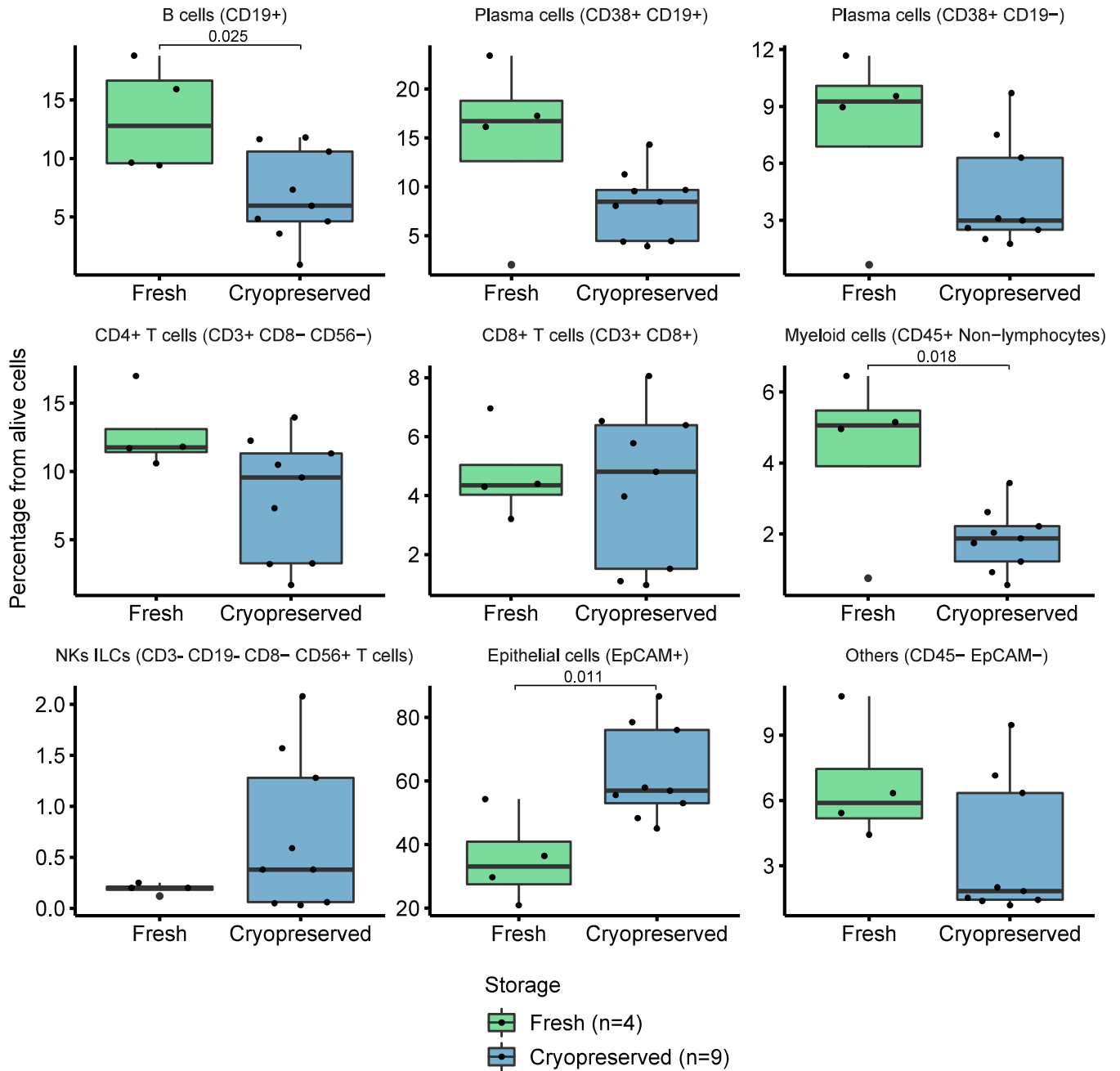
<sup>1</sup> University of Groningen, University Medical Center Groningen, Department of Gastroenterology and Hepatology, Groningen, The Netherlands

<sup>2</sup> University of Groningen, University Medical Center Groningen, Department of Genetics, Groningen, the Netherlands.

**Supplementary Figure 1. Parameters of quality control in scRNAseq after cryopreservation in a one-step collagenase protocol**

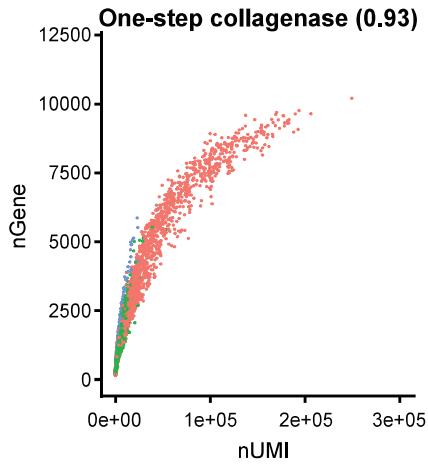


**Supplementary Figure 2. Comparison of main cell types recovered after cryopreservation by one-step collagenase protocol assessed by FACS**

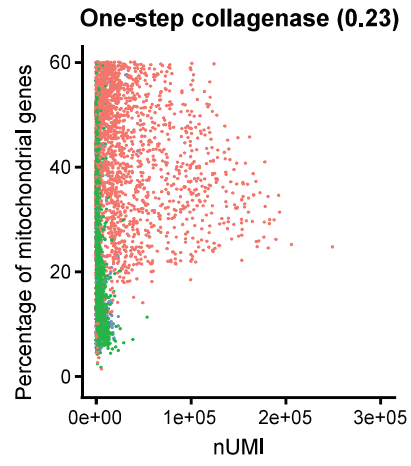


# Supplementary Figure 3. Parameters of quality control in scRNAseq per cell-dissociation protocol

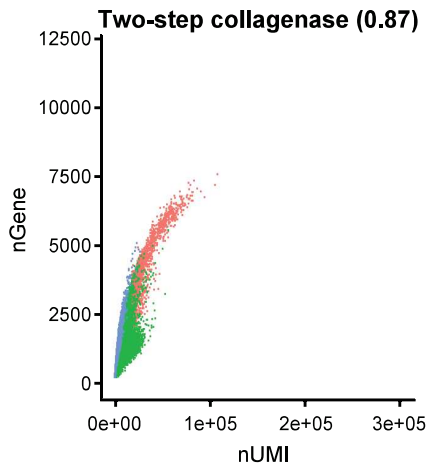
**A**



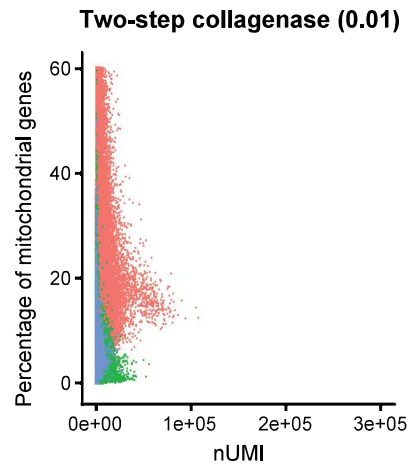
**B**



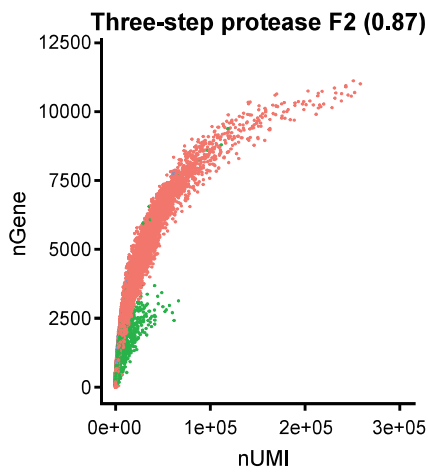
**C**



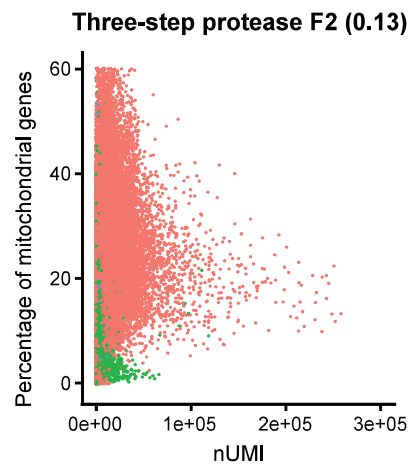
**D**



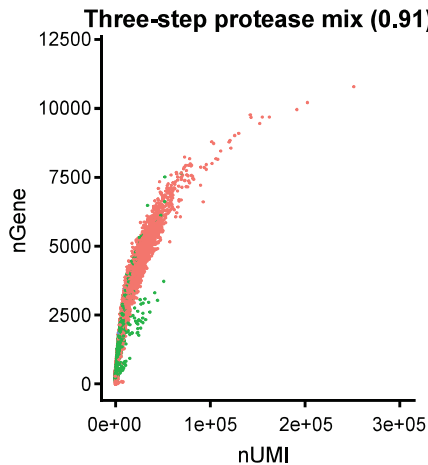
**E**



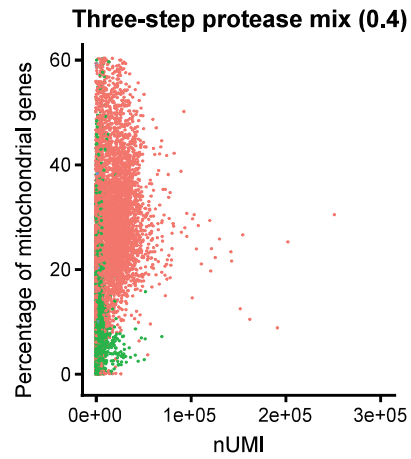
**F**



**G**



**H**

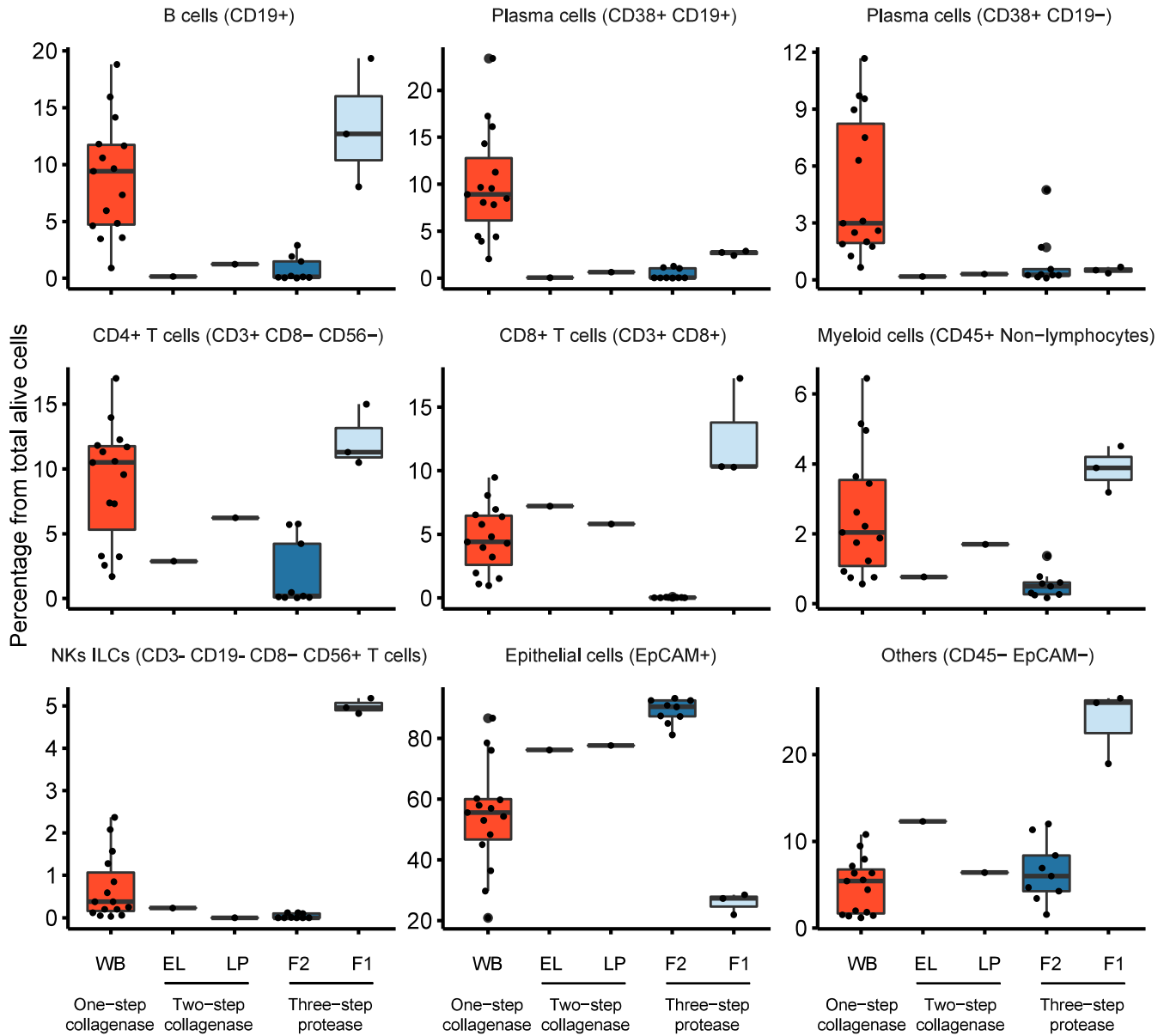


**Cell type**

- Epithelial cells
- Immune cells
- Stromal cells



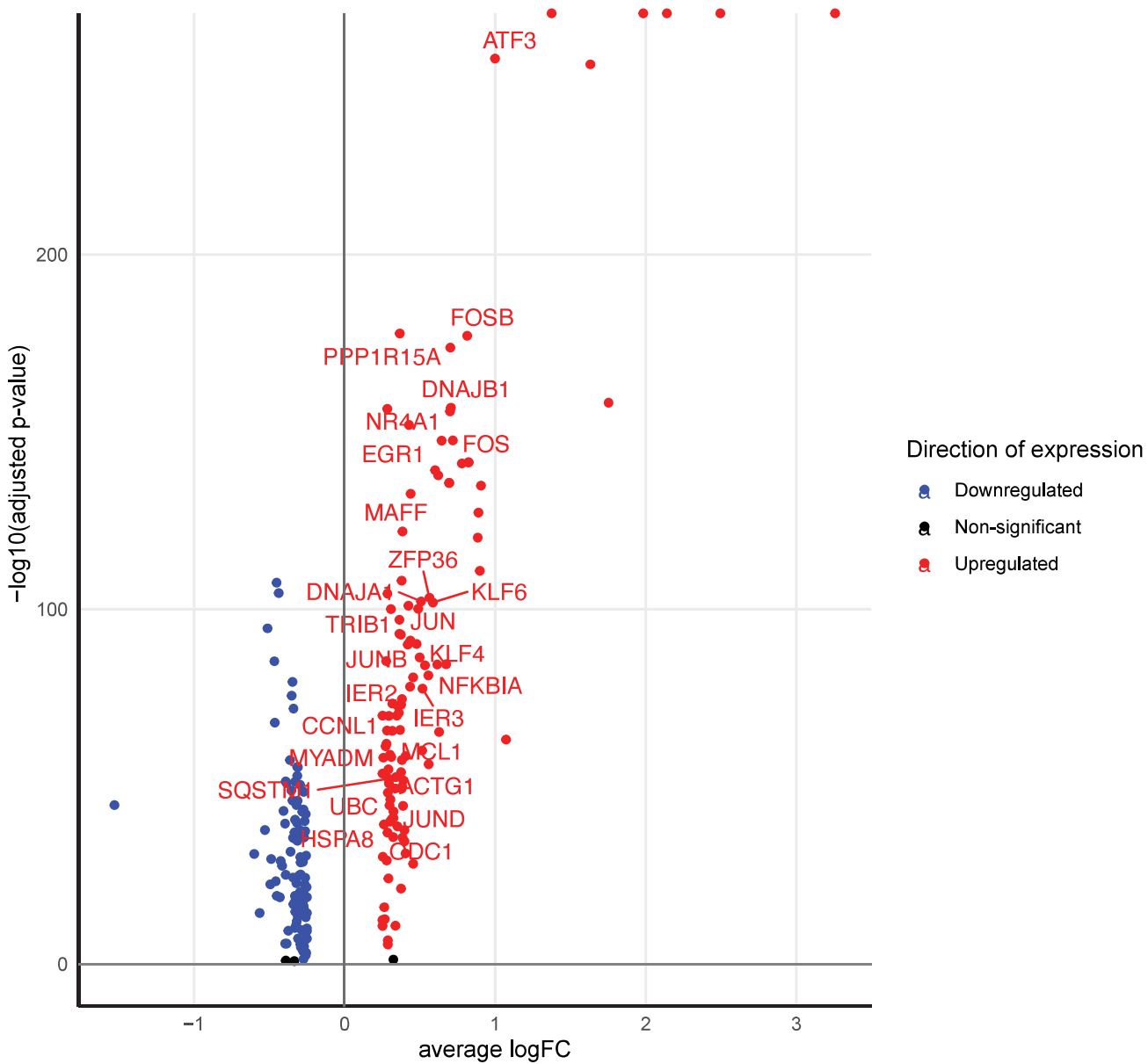
# Supplementary Figure 4. Differences in cell type proportions recovered in each protocol by FACS



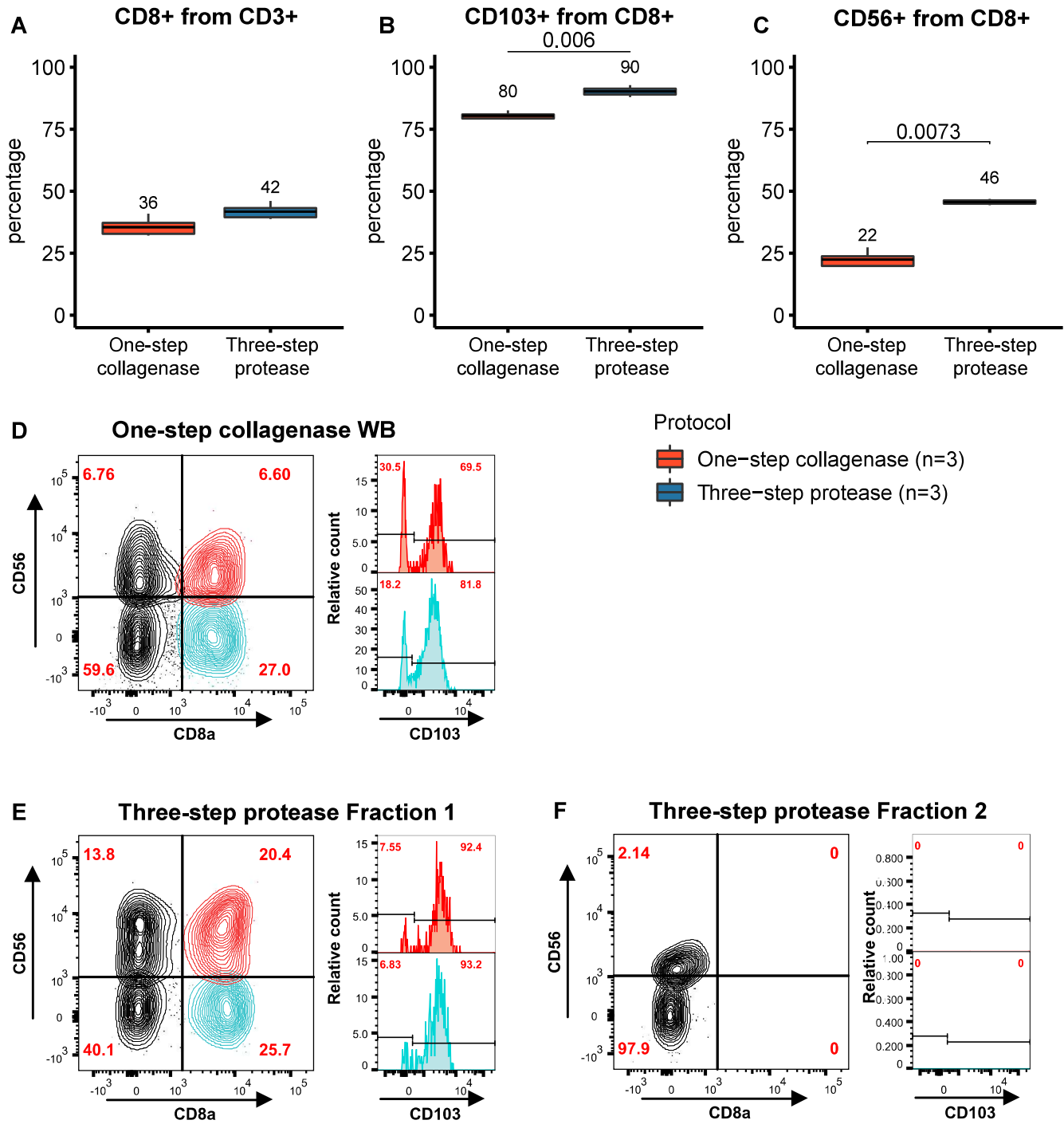
## Protocol and compartment

- One-step collagenase, Whole-Biopsy (n=16)
- Two-step collagenase, Epithelial Layer (n=1)
- Two-step collagenase, Lamina Propria (n=1)
- Three-step protease, Fraction 2 (n=9)
- Three-step protease, Fraction 1 (n=3)

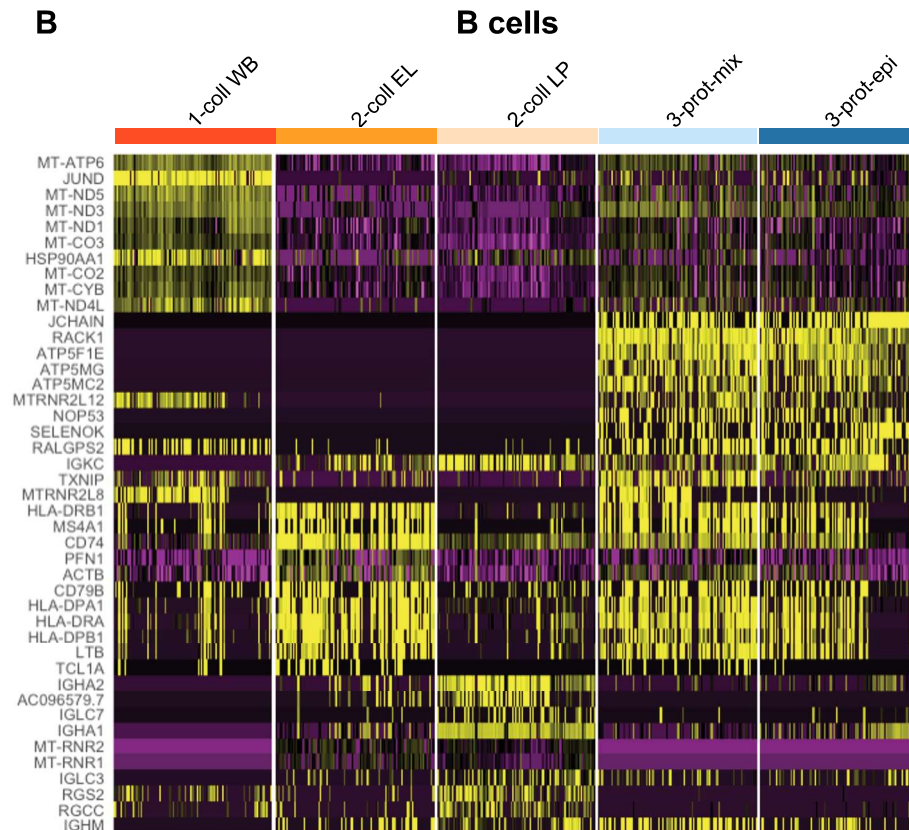
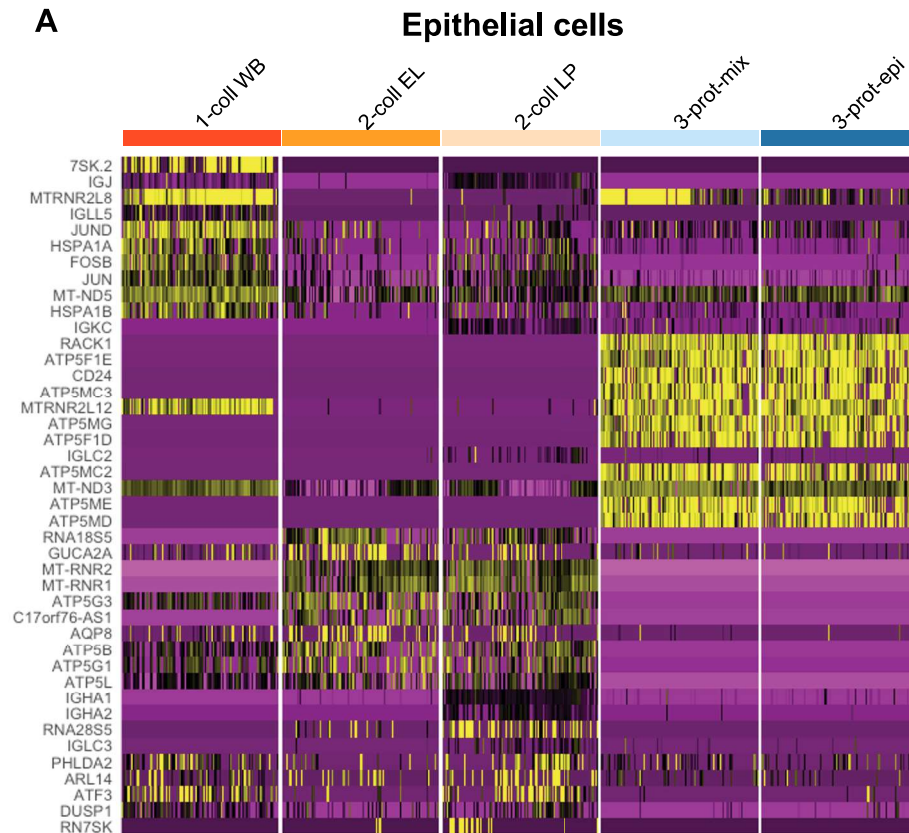
**Supplementary Figure 5. Differential expressed genes in epithelial cells from epithelial layer versus lamina propria in the two-step collagenase protocol**



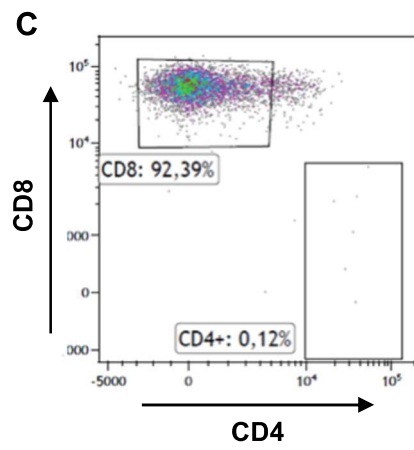
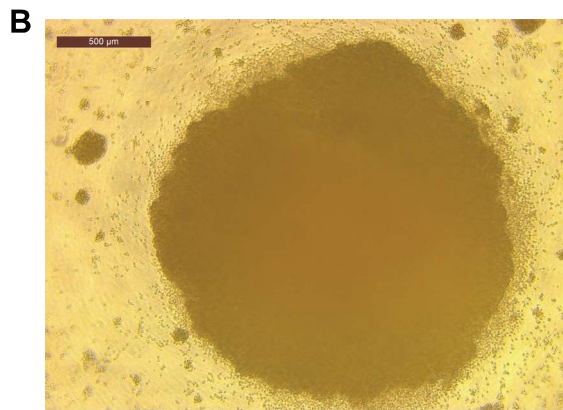
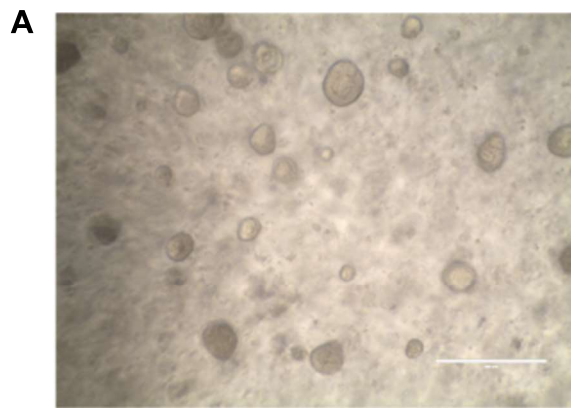
**Supplementary Figure 6. Recovery of CD8 T cells and IELs is different between protocols**



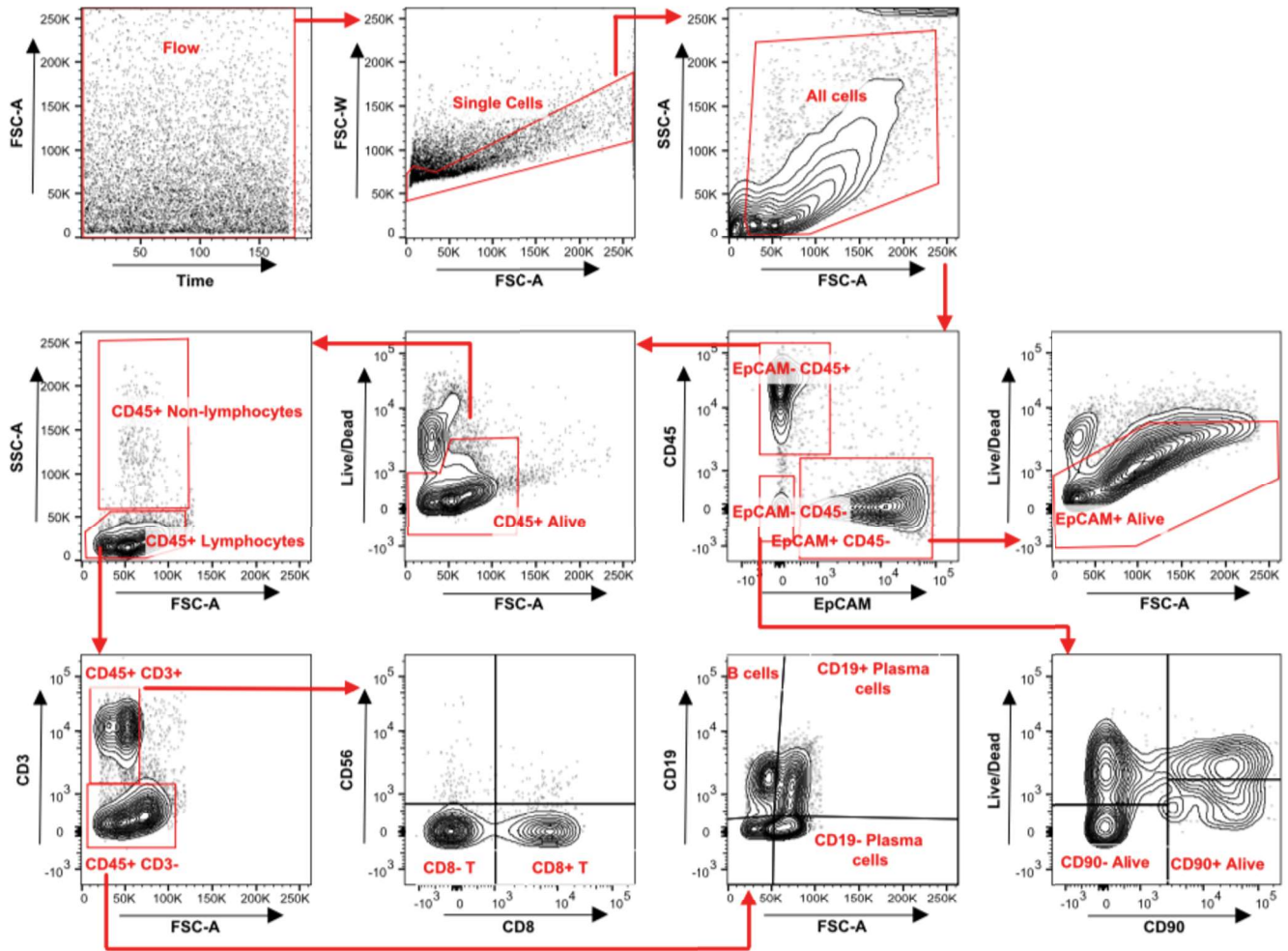
**Supplementary Figure 7. Differentially expressed genes between epithelial cells and B cells isolated with different protocols**



Supplementary Figure 8. Functional experiments to grow organoids and expand IELs.



# Supplementary Figure 9. Gating strategy used to identify cells by FACS.





# Supplementary Figure 10. Gene markers specific for cell types

