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The effects of Cell Subset Isolation Method on Gene Expression in Leukocytes

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- Isolation of specific subsets of blood cells
 - Positive immunomagnetic selection
 - Negative immunomagnetic selection
 - FACS (fluorescence activated cell sorting)

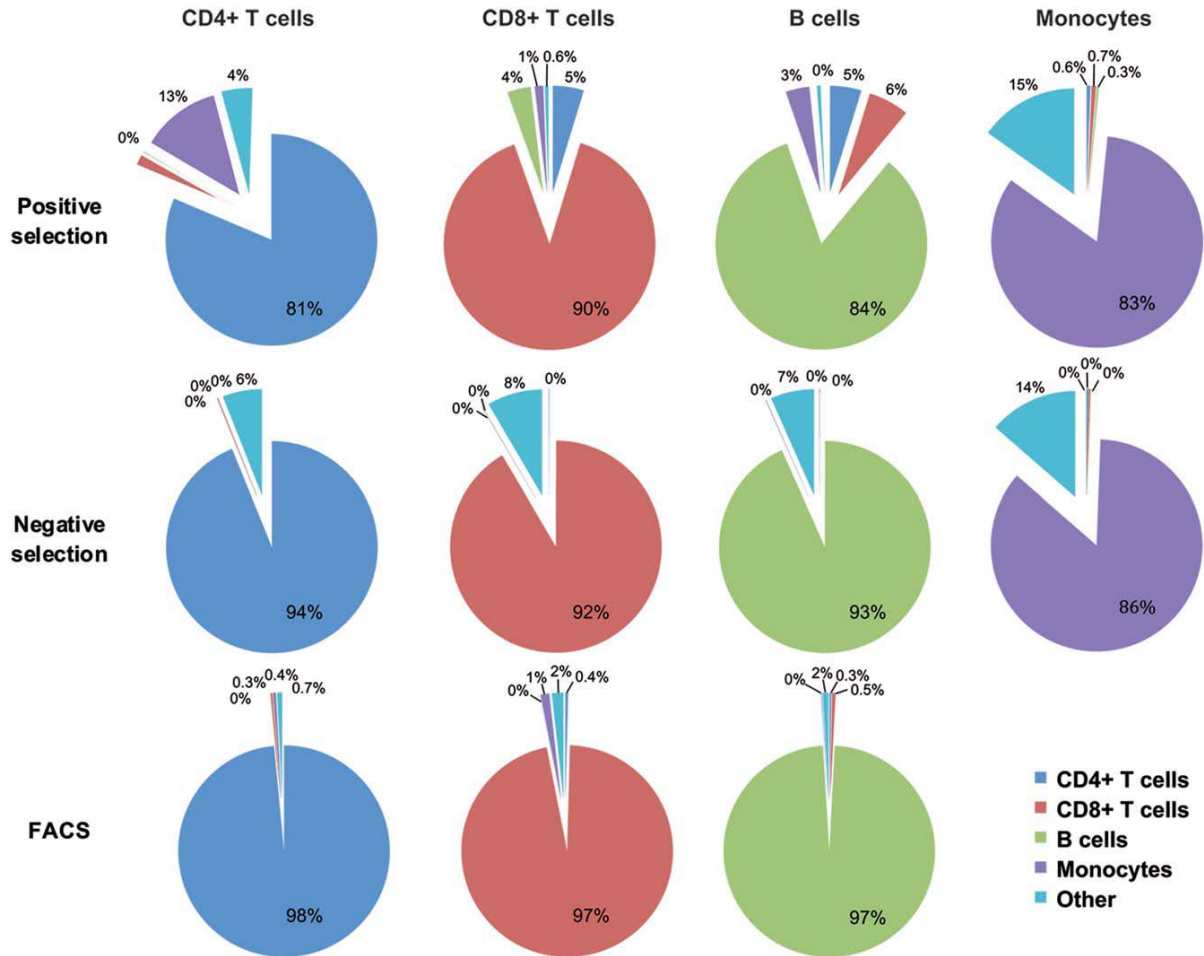
- Hypotheses

- Positive selection and FACS yield higher purity
- Positive selection and FACS have an impact on gene expression
- FACS may upregulate stress response genes

- Gene expression signatures to investigate
 - disease pathogenesis
 - effects of therapeutic intervention

- PBMC gene expression profiling
 - Pro: Ease of sampling
 - Problem: mixture of different cells
 - HIV
 - Systemic lupus erythematosus

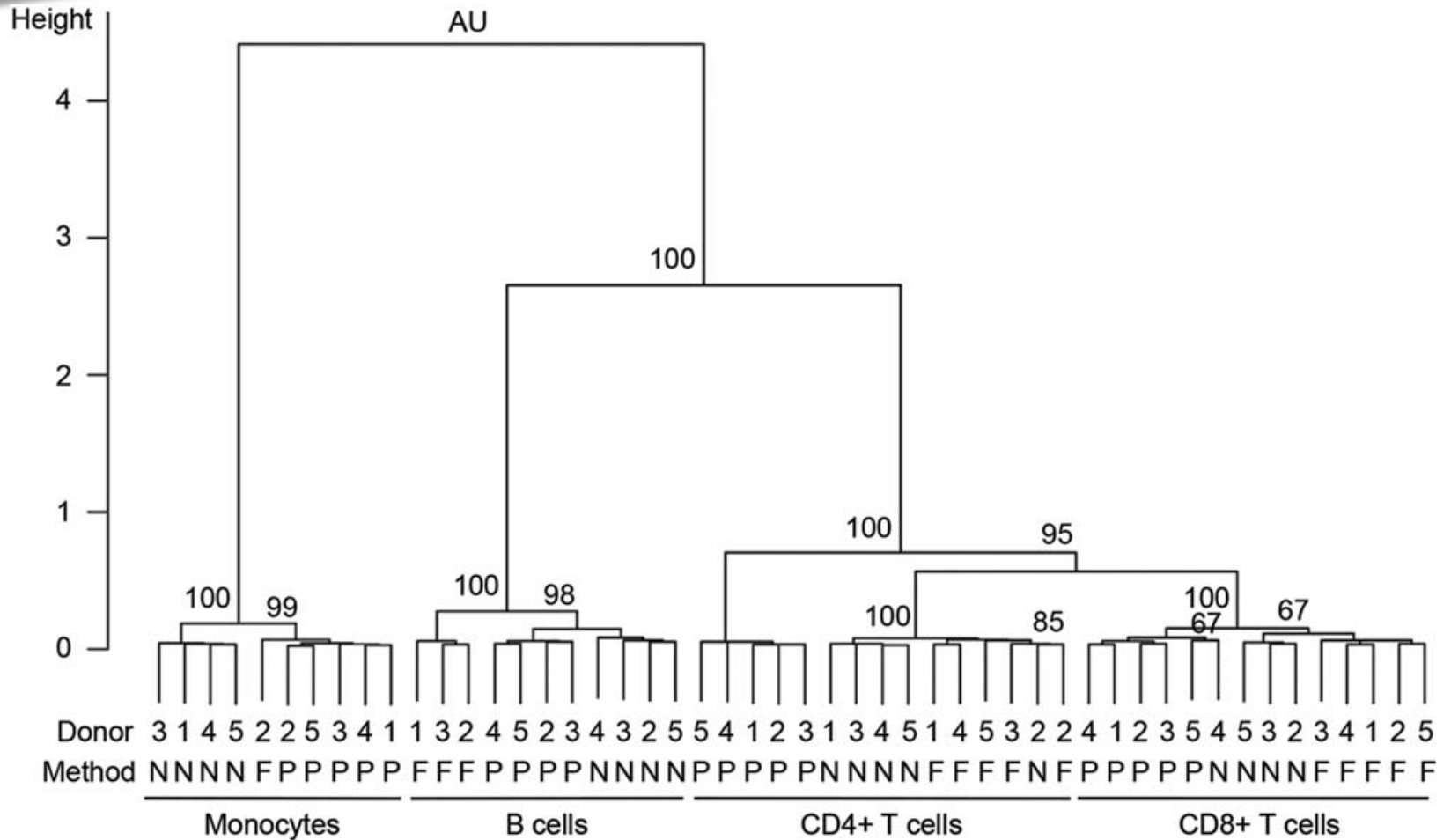
- Subsets of blood cells isolated from PBMCs
 - CD4+ -T-cells
 - CD8+-T-cells
 - B-cells
 - Monocytes



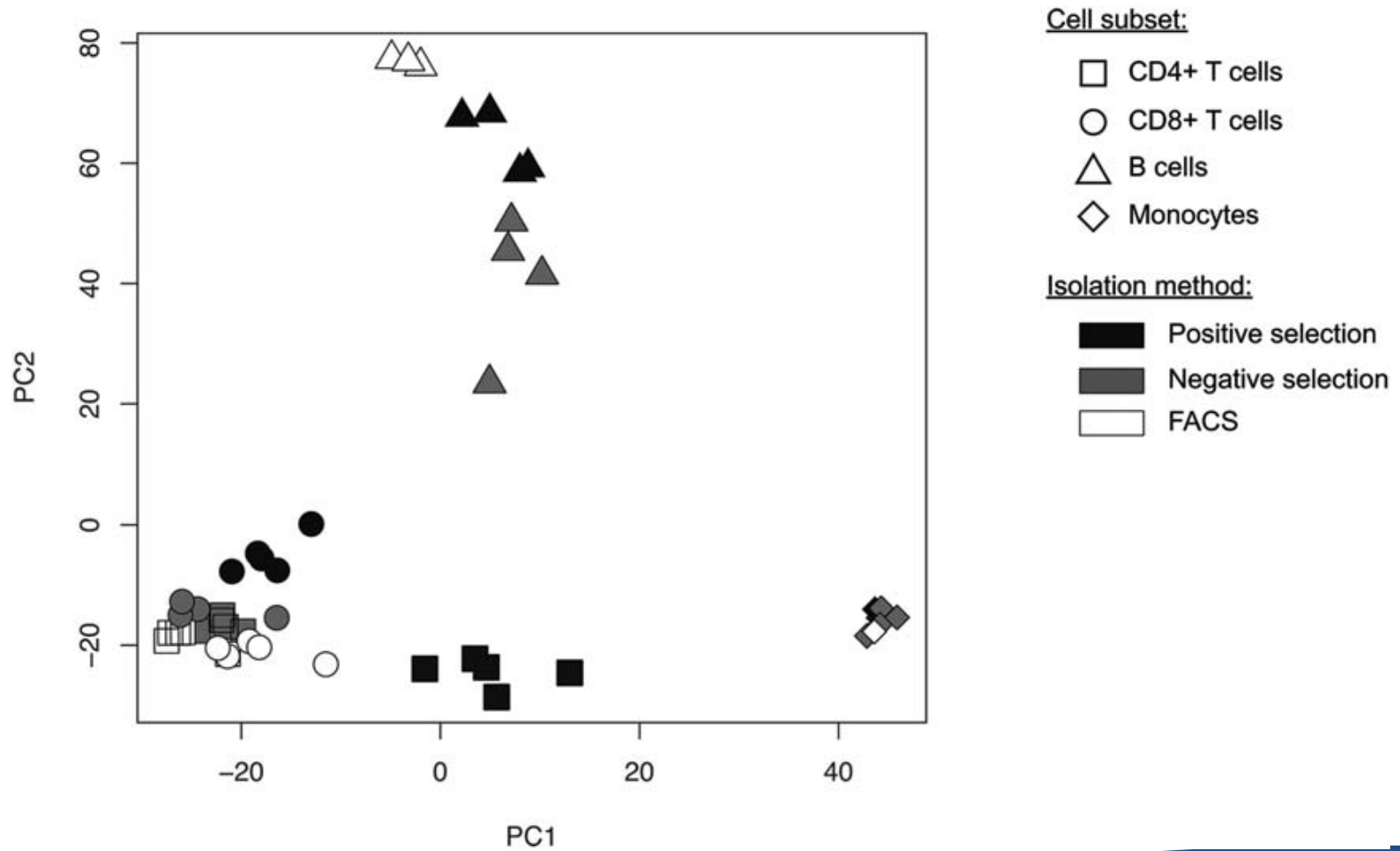


	POSITIVE SELECTION	NEGATIVE SELECTION	FACS
CD4+ T cells	Donors 1,2,3,4,5	Donors 1,2,3,4,5	Donors 1,2,3,4,5
CD8+ T cells	Donors 1,2,3,4,5	Donors _,2,3,4,5	Donors 1,2,3,4,5
B cells	Donors _,2,3,4,5	Donors _,2,3,4,5	Donors 1,2,3,_,_
Monocytes	Donors 1,2,3,4,5	Donors 1,_,3,4,5	Donor _,2,_,_,_

- RNA extraction
- 5 donors, 3 isolation methods, 4 cell types
- RNA quality measurement
 - RNA integrity number (RIN)
- Unsupervised clustering



- Samples clustered primarily by cell type
- Secondarily by isolation method
 - Not by donor
- Significant difference between Isolation method

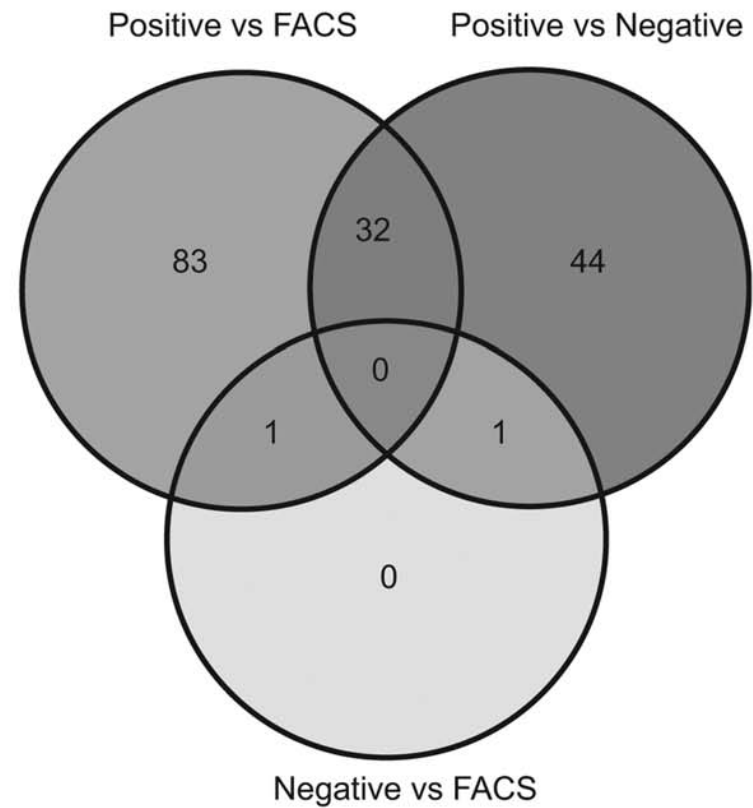
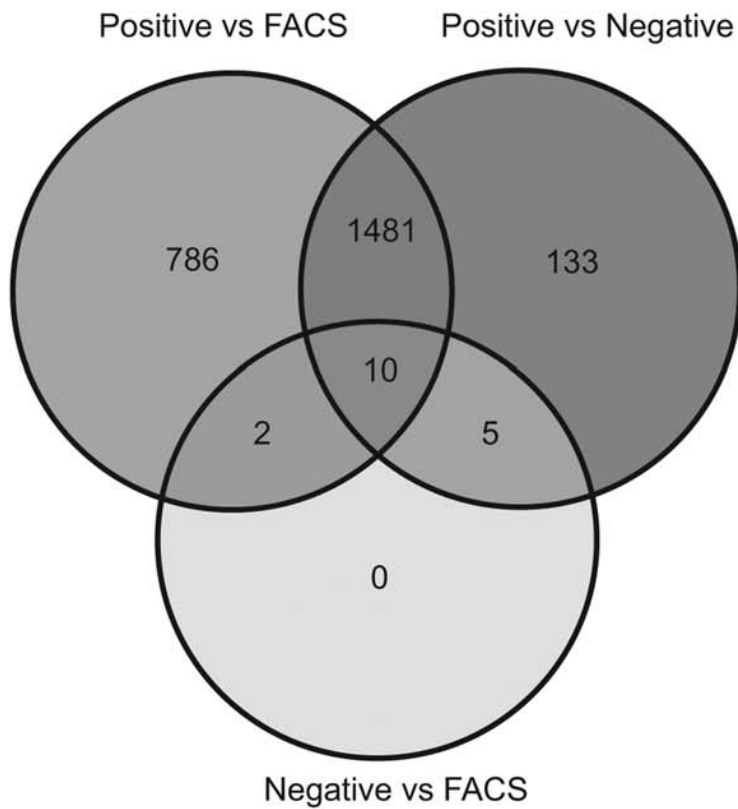


- CD4+-cells contaminated with monocytes
- B-cell samples largest spread between isolation methods
- CD8+-cells and monocytes
 - tight clusters indicate less effect of contamination and isolation method

- Effect of Isolation method on gene Expression
 - Positive Selection utilizes cell receptor antibodies
 - Potential activation cascade
 - Receptor blockade
 - Negative Selection using antibody cocktails
 - FACS antibodies stain detected cells

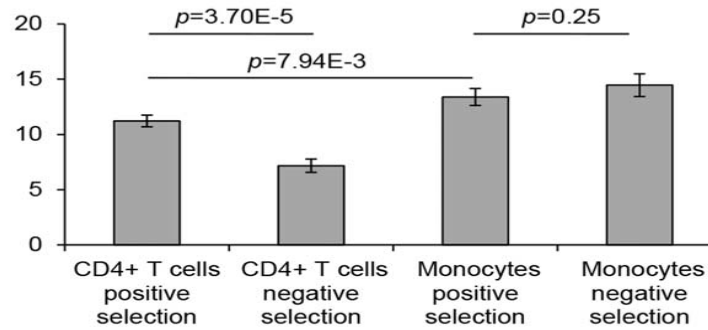
CD4+ T Cells DEGs

CD8+ T Cells DEGs



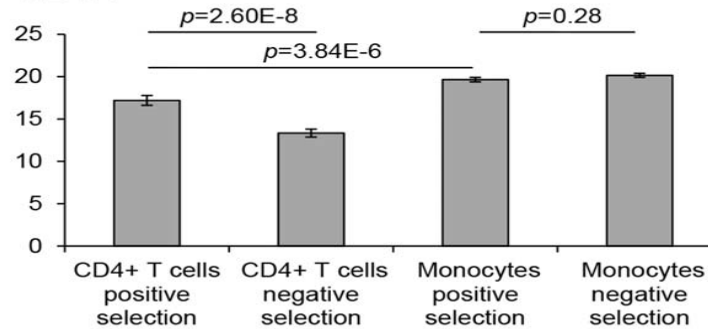
- Stress genes have the lowest expression in FACS-isolated cells
- In monocytes isolated by positive and negative selection, platelet contamination affected expression of stress genes

SPI1

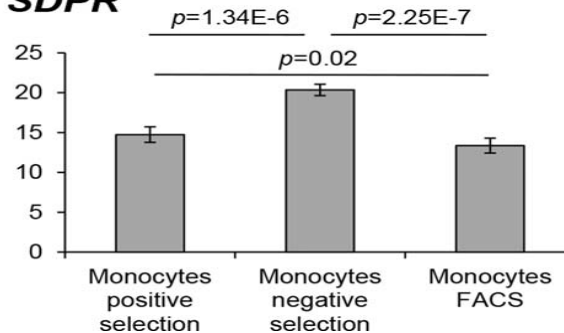


Log₂ copies per 10⁶ RPL27

CD14



SDPR



Discussion

- FACS is the optimal method for PBMC subset separation for gene expression profiling studies
 - High purity
 - Little impact on cellular transcription

- Exception: Monocytes
 - Low yields were obtained by FACS
 - Concentration of 10millions/ml used
- Long term effects not examined



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Thank you for your attention!