

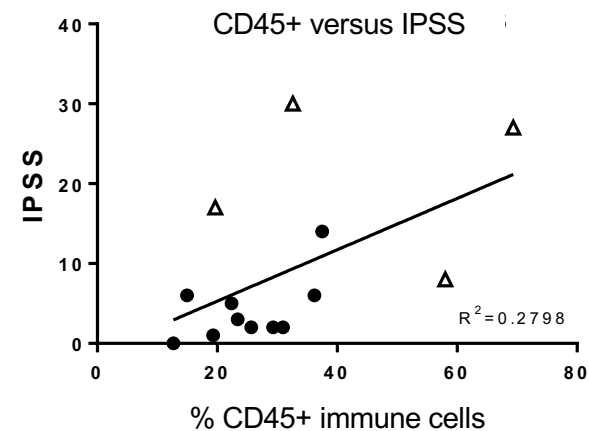
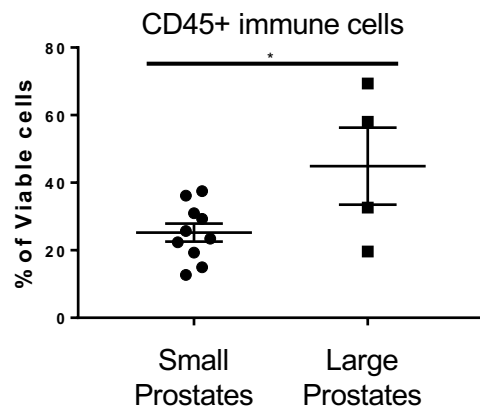
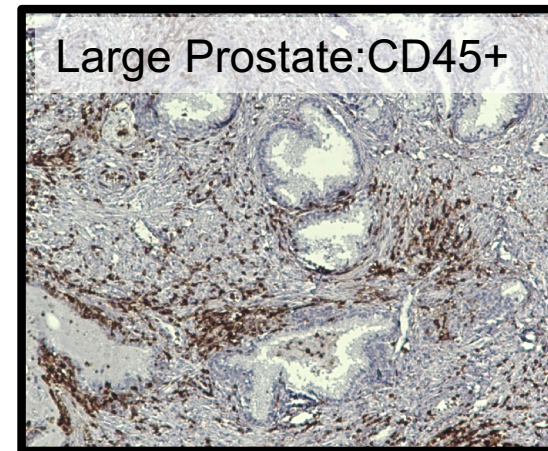
TNF-alpha antagonism as a potential approach to modify BPH pathogenesis

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Inflammation in BPH

- Inflammation is evident in histological sections of BPH
- Could contribute to fibrotic changes in the prostate (“stiff” prostates)
- Could contribute to prostate growth
- Goals
 - Define the profile of leukocytes and their changes during prostate enlargement based upon single-cell RNA-seq analysis
 - Identify cellular clusters that possess distinct pathway topologies in BPH progression
 - Identify potential interventional strategies

Inflammatory cells accumulate as prostates enlarge



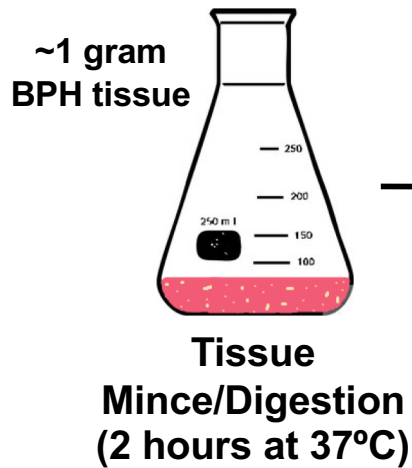
- Small Prostates
- △ Large Prostates

Renee Vickman

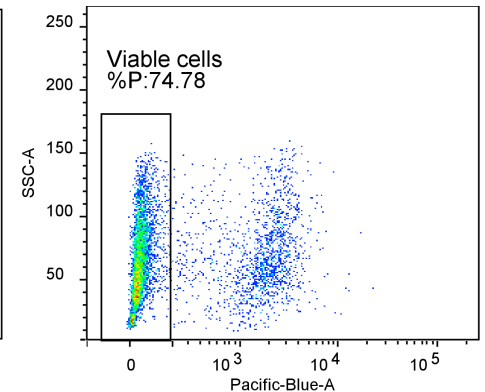
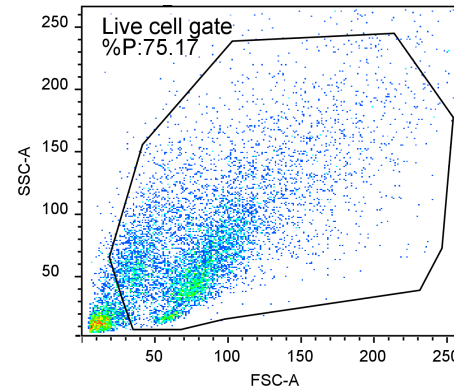
Rationale for scRNA-seq Studies

- The currently defined subpopulations of immune cells in BPH are limited to known markers which may not include all intercellular signaling profiles
- Based on traditional M1/M2 polarization markers we know that as BPH progresses to surgery the ratio between these phenotypes become more pro-inflammatory. However, the M1/M2 continuum is not well-defined
- We want to understanding extracellular signaling pathways altered throughout BPH progression to identify potential therapeutic targets

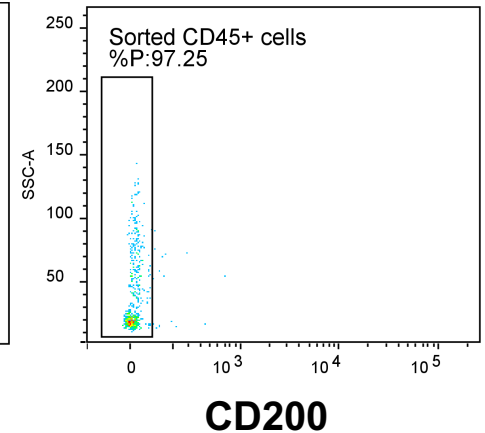
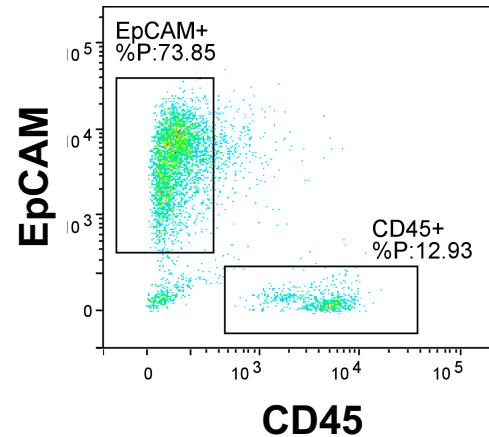
Tissue processing and CD45+ cell sorting



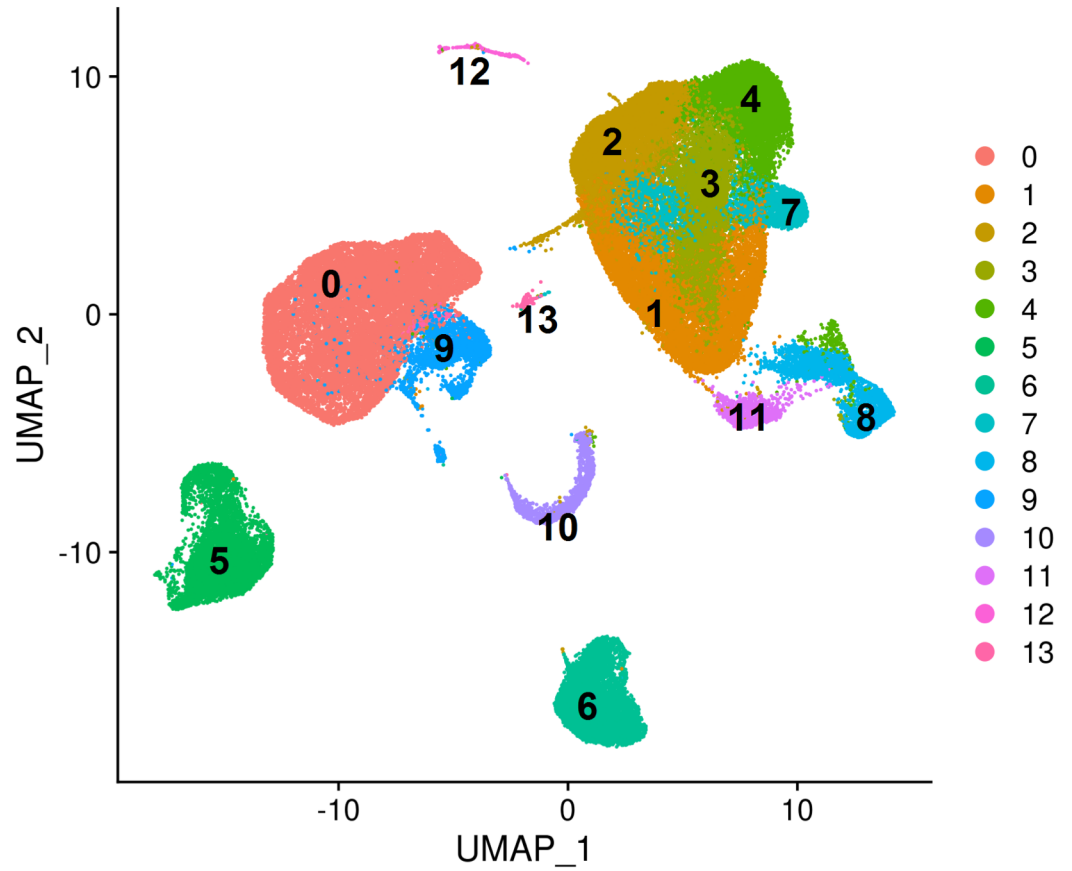
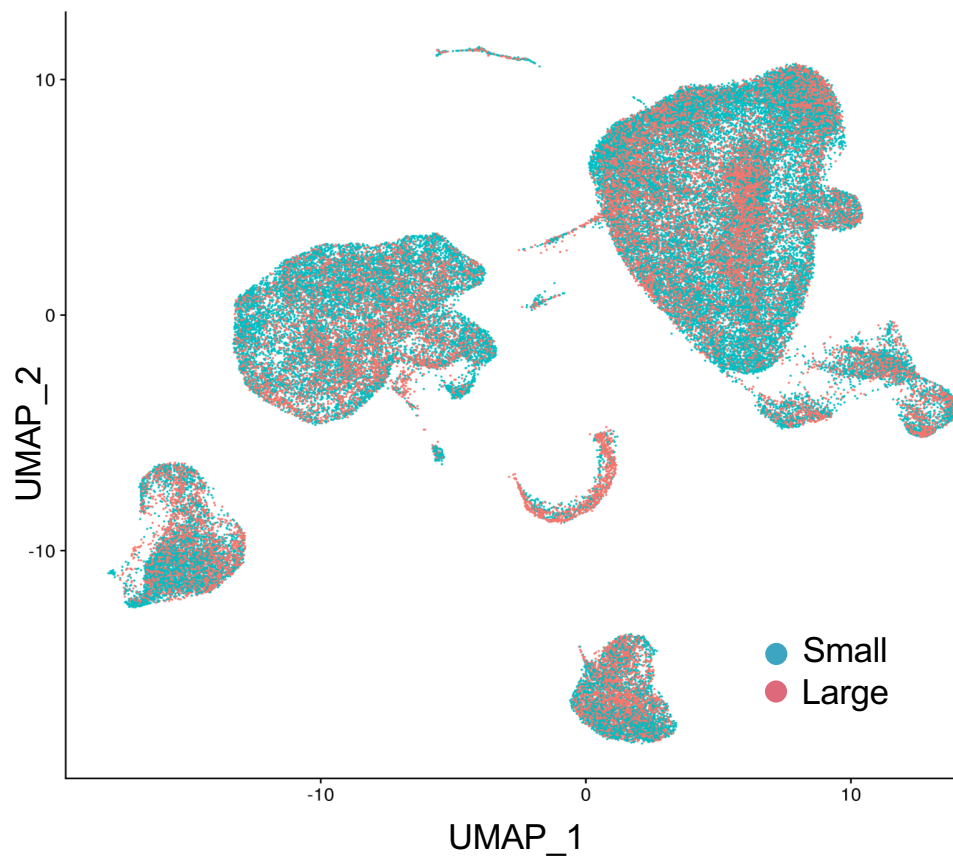
**Prep and
Staining for
Cell Sorting**



Selection for non-epithelial (EpCam-)
non-endothelial (CD200-) CD45+
immune cells

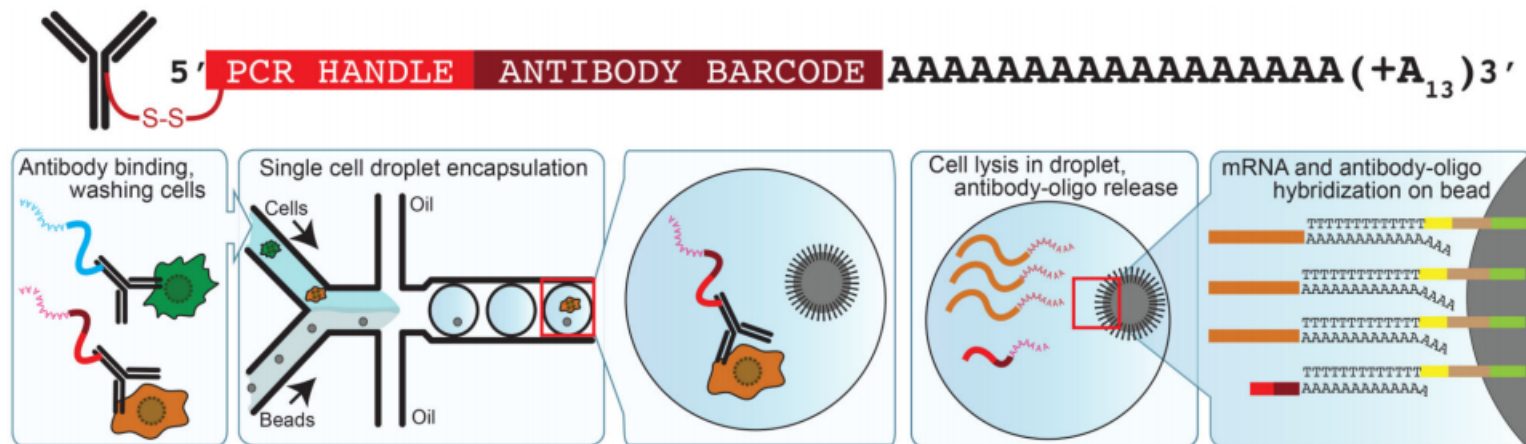


Clustering based on scRNA-seq



Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITE-seq)

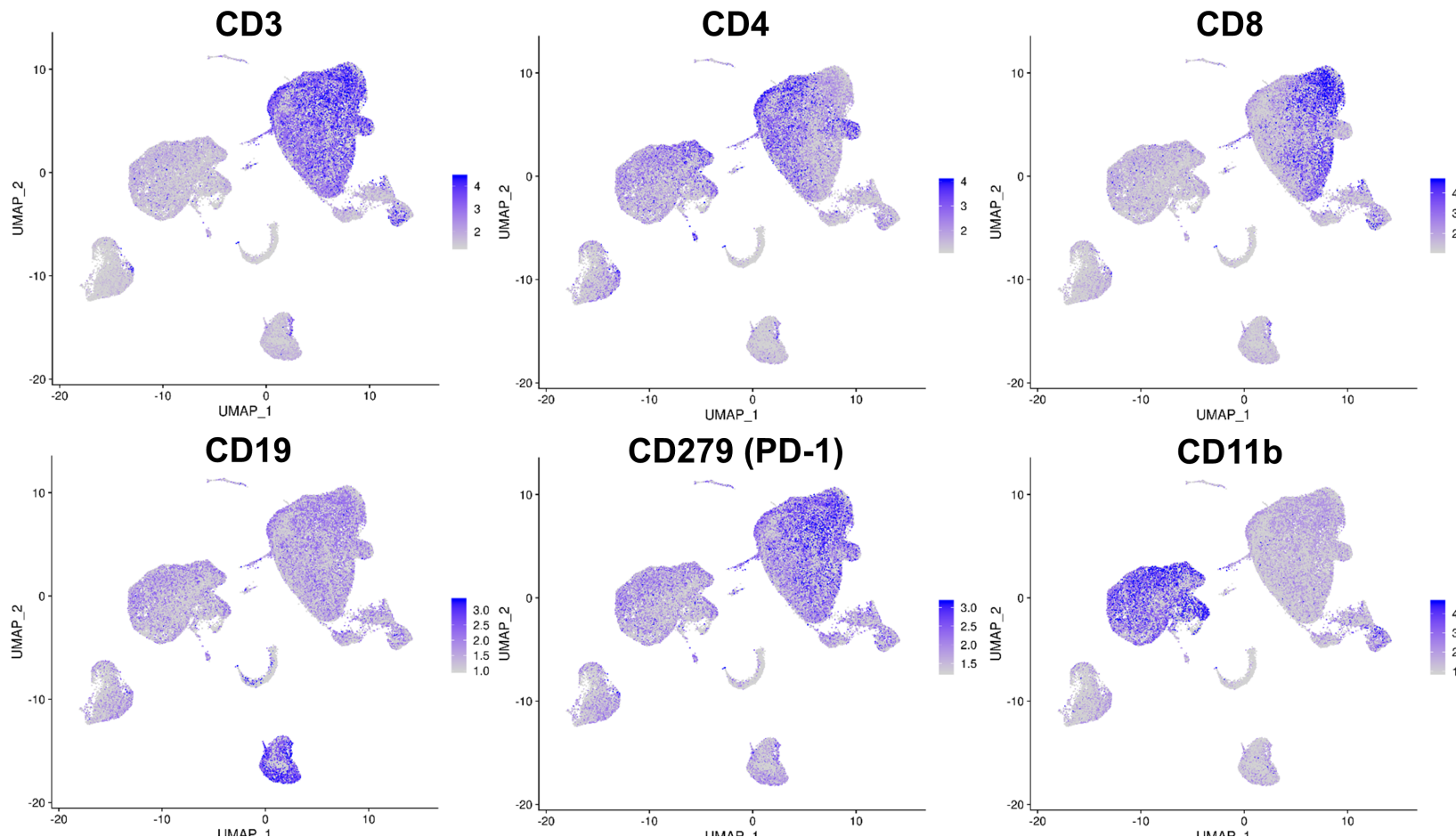
- Simultaneous gene expression and surface protein expression at the single cell level
- Validation of immune cell categorization after scRNA-seq



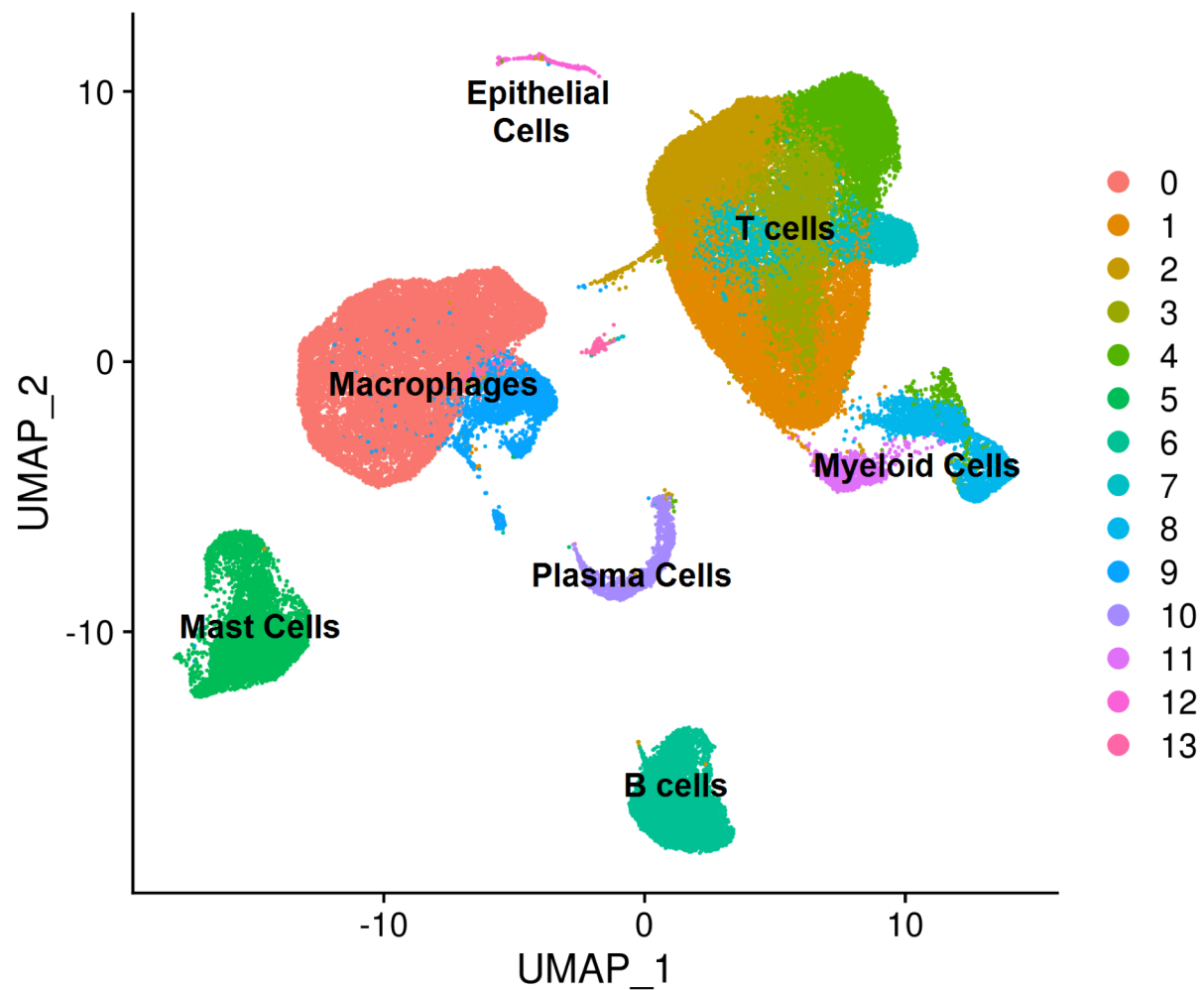
Marketed as TotalSeq by Biolegend

New York Genome Center
Stoeckius, et. al, 2017 *Nature Methods*

Correlation of transcriptomes and cell surface proteins (scRNA-seq plus CITE-seq)



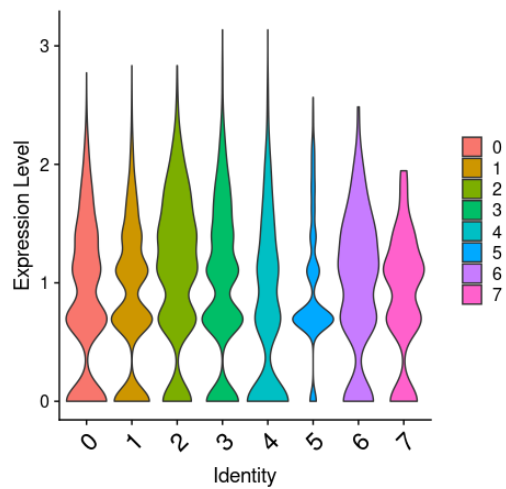
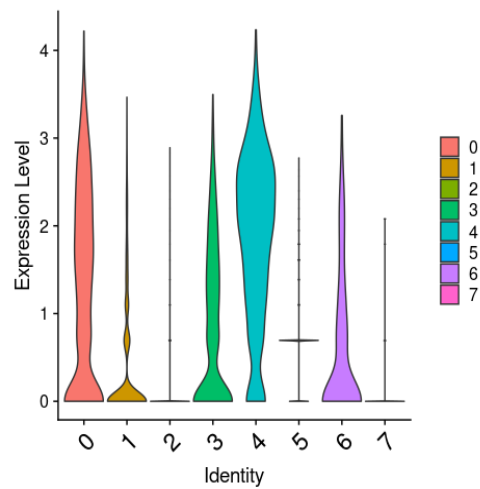
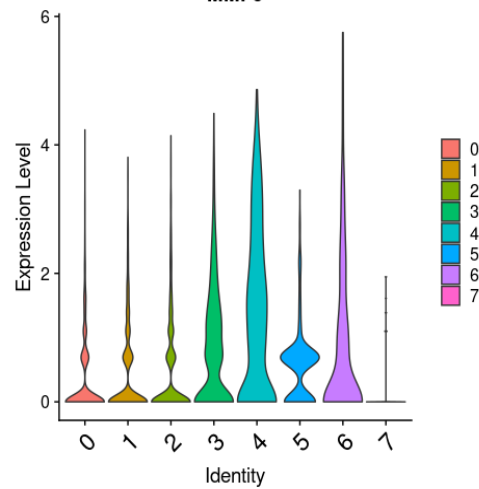
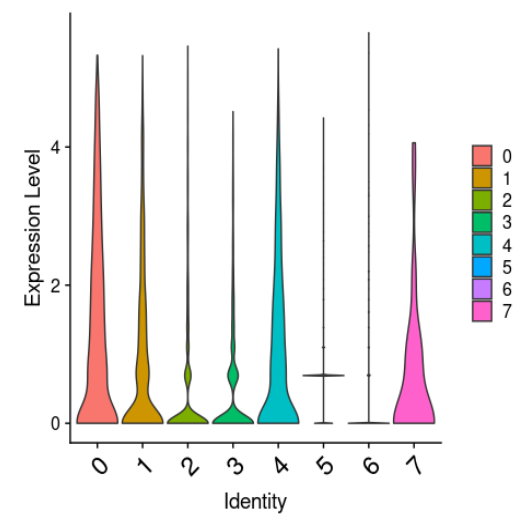
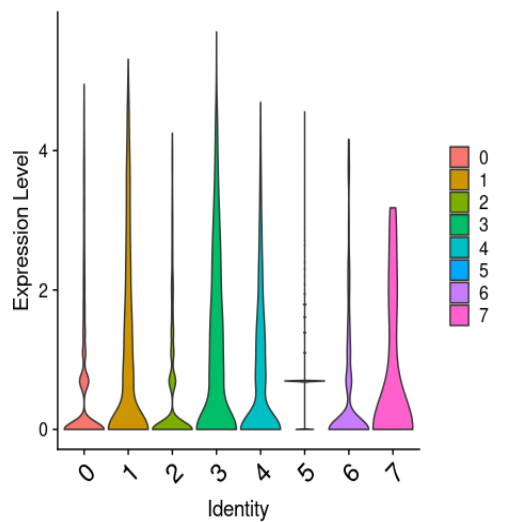
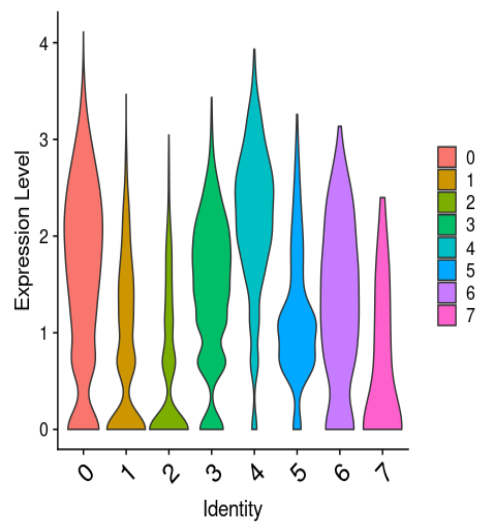
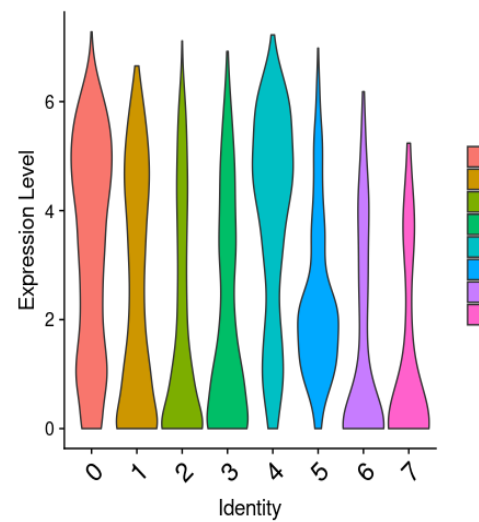
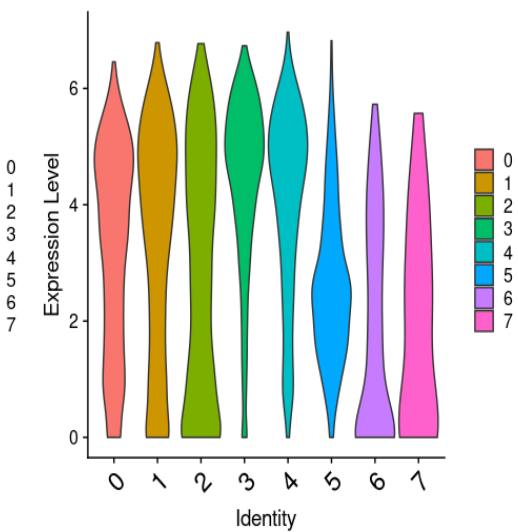
Provisional assignment of mRNA clusters

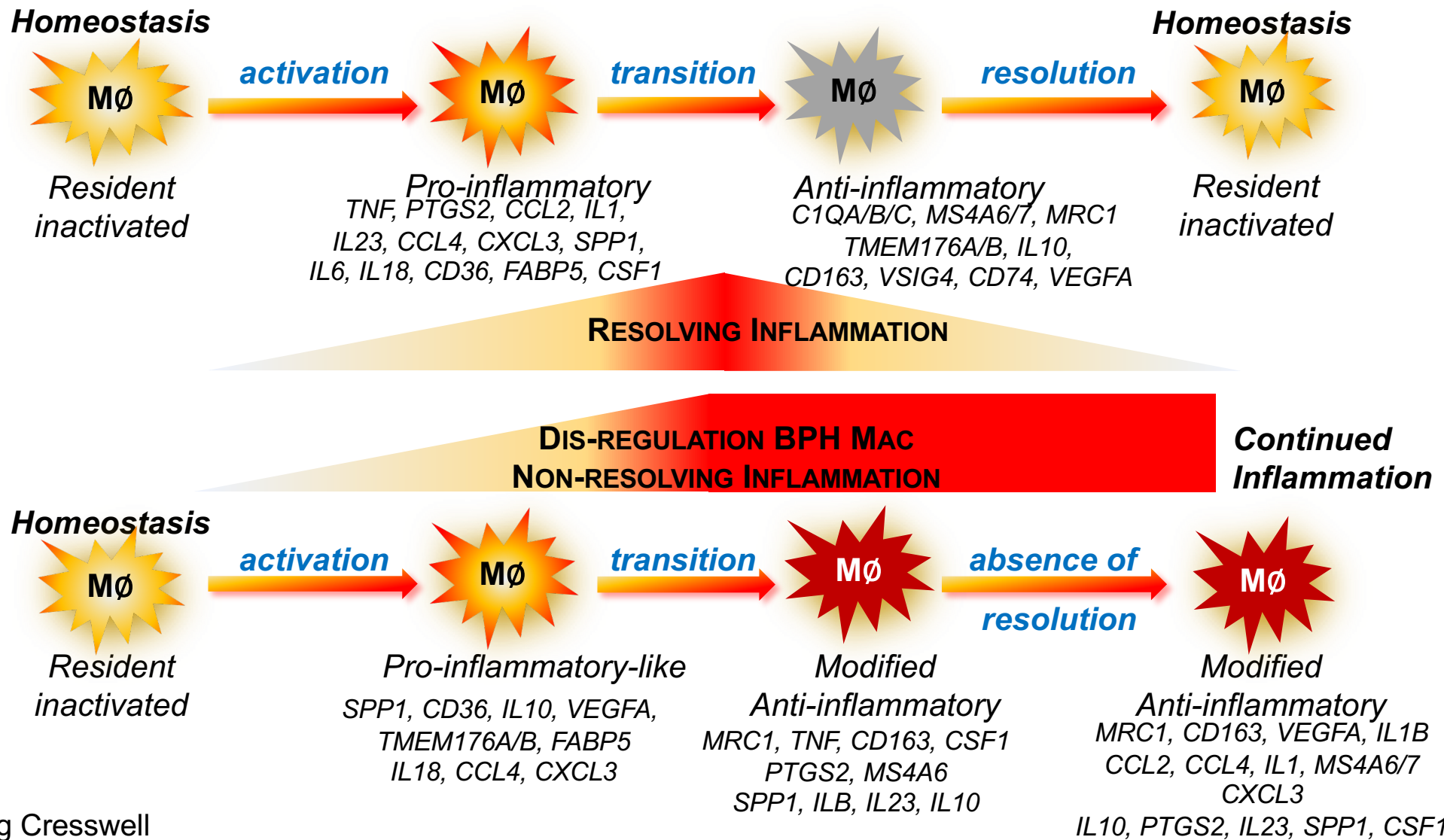


Early hints about macrophage populations

General dogma regarding macrophage action in injury:

- 1) Inflammation (*IL1B, TNF, SPP1, CD36, FABP4/5, CCL2, CXCL3, and PTGS2*)
- 2) Anti-inflammation (wound repair)(*IL10, IL1RN, C1QA/B/C, MRC1, CD163*)
- 3) Resolution (*C1QA/B/C, MRC1, CD74, CD163, TGFB, IL10, VEGFA, and IL4*)

TGFB1**MRC1****MMP9****TNF****IL10****CD163****CCL4****IL1B**



**Men with autoimmune inflammatory (AI) diseases
have more BPH diagnoses. Men treated for AI
diseases have less BPH diagnoses**

All Men, N (%)

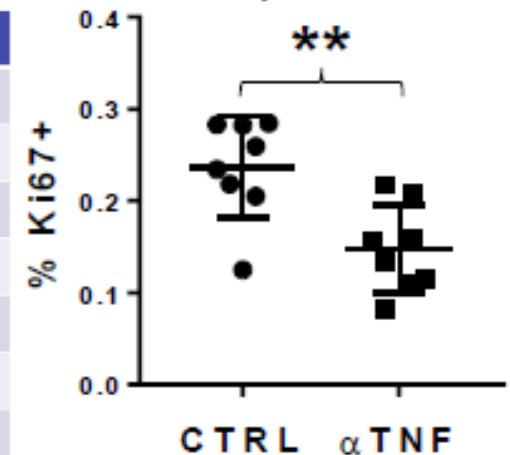
Autoimmune 10,796 (9.6)		No Autoimmune 101,383 (90.4)		P-Value
BPH 3,294 (30.6)	No BPH 7,475 (69.4)	BPH 20,586 (20.3)	No BPH 80,797 (79.7)	
				<0.001

Men where Autoimmune occurred PRIOR TO BPH Diagnosis, N (%)

Autoimmune 9,274 (8.4)		No Autoimmune 101,383 (91.6)		P-Value
BPH 1,799 (19.4)	No BPH 7,475 (80.6)	BPH 20,586 (20.3)	No BPH 80,797 (79.7)	
				0.037

Effects of TNF-antagonists on human BPH

Predictors	Odds Ratio	95% CI	P-Value
Methotrexate	1.05	0.79-1.40	0.745
TNF-α antagonists	0.67	0.50-0.91	0.010
Age 60+	4.92	1.30-5.63	<0.001
Race			
Caucasian	Reference		
African-American	1.16	0.81-1.65	0.427
Hispanic	0.72	0.45-1.17	0.186



Multivariable Regression Model Predicting BPH in Men with an Autoimmune Condition Prior to BPH Diagnosis

Effects of TNF antagonist therapy on epithelial Ki67 labeling index in human BPH.
p<0.01

Ongoing and Planned Studies

- BPH is a T-cell dominant environment. We want to produce a clear picture of the B and T cell populations present in BPH.
- Develop the pathway analysis to determine how intercellular communication changes with progression, including communication with stromal and epithelial cells.
- Androgens are generally somewhat immunosuppressive. Therapies such as 5ARIs reduce androgen availability. We wish to quantify these effects on leukocyte populations in patient tissue.
- Explore the potential of immune signaling modifiers (such as TNF antagonists) in clinical BPH.

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