Immune system adaptation during gender-affirming testosterone treatment

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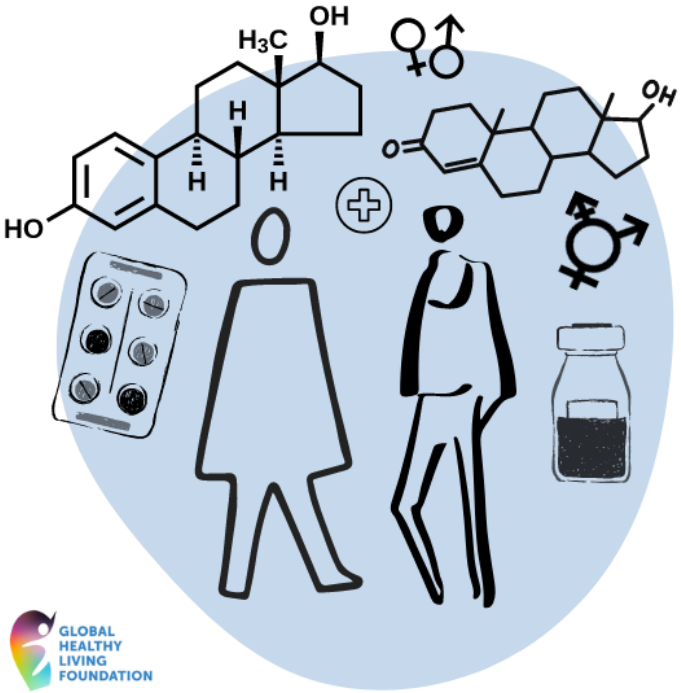
What is Gender Affirming Hormone Therapy (GAHT)?

- Administration of estrogen or testosterone based medicine
- Testosterone Therapy
  - Nebido
  - Administered once every 12 weeks
  - Patient doses = 1,000 mg or 750 mg based on body mass
- Study Setup
  - 23 adults assigned female at birth undergoing masculinizing gender affirming treatment
  - Sweden
  - No previous testosterone treatment, abnormal sex hormone concentrations, or confounding immune diseases/deficiencies

https://coimages.sciencemuseumgroup.org.uk/70/162/medium_2014_0566__0005_.jpg

GAHT & Immune Response



https://ghlf.org/wp-content/uploads/2024/06/1.png

What are the immunological impacts and risks of GAHT?

How do they manifest in individuals undergoing masculinizing testosterone treatment?

- Changes in sex hormones
- Active/inactive sex chromosomes

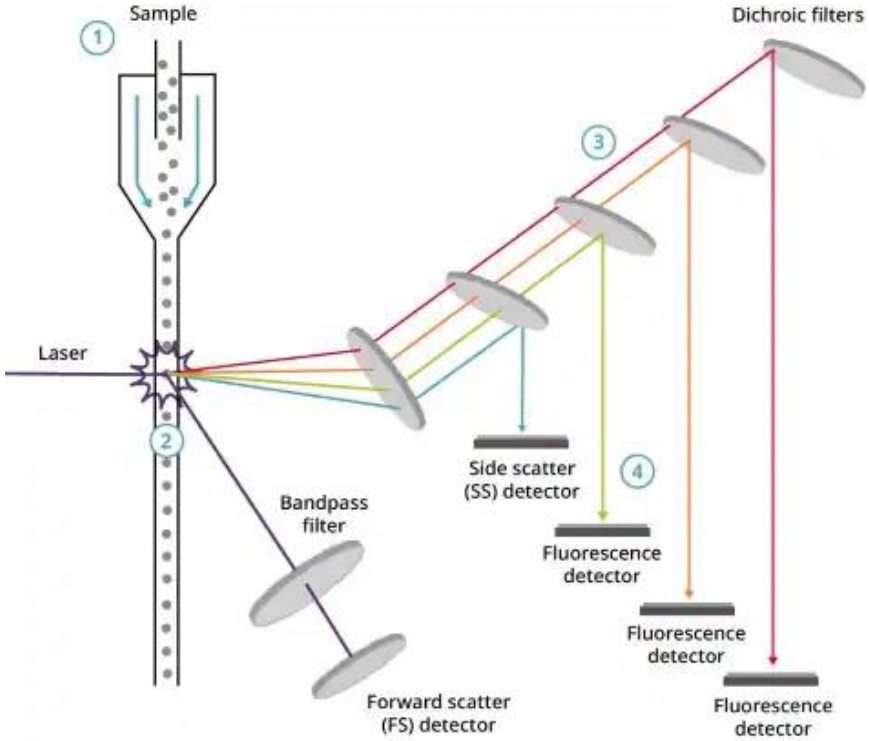
Systems Immunology Relevance

- Sex related immunological impacts
- Personalized medicine
- Human immunology
- Omics
  - Liquid chromatography
  - Mass spectrometry
  - Mass cytometry
  - **RNA sequencing**
  - **Flow cytometry**
  - Plasma protein profiling

https://media.istockphoto.com/id/1177896837/vector/restroom-gender-symbols.jpg?s=612x612&w=0&k=20&c=4vWuhz8yUfPQIqnHPbeS4UlTcti60cJKXZfBaNGTTCU=

https://biomedicalodyssey.blogs.hopkinsmedicine.org/files/2021/08/mrna-vaccine-development.jpg

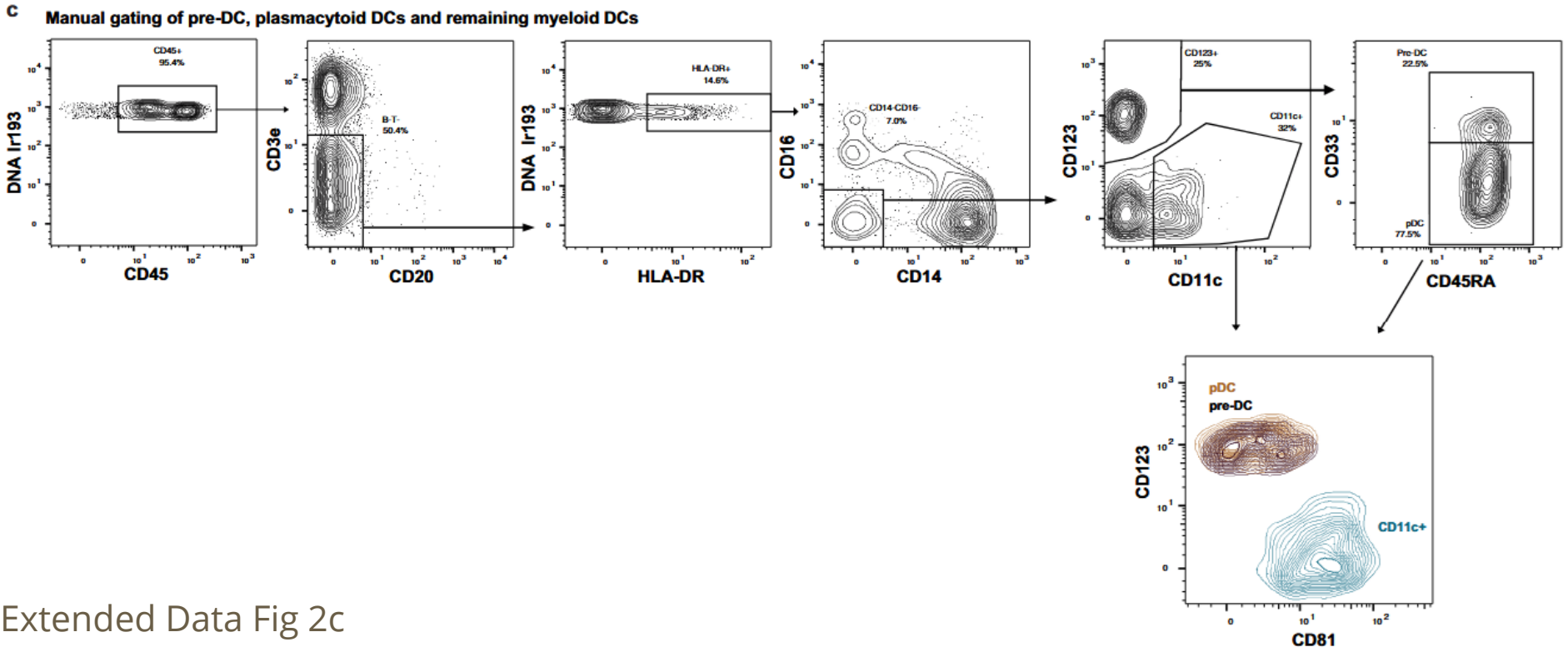
Flow Cytometry → What? Why? How?



https://a.static-abcam.com/CmsMedia/Media/multicolor-flow-cytometer-472-px.jpg

- Fluorescence detection of labelled antibodies or gene sequences
- Events = cells
- Forward vs side scatter
- Laser excitations

Flow Cytometry → What? Why? How?



Extended Data Fig 2c

Flow Cytometry → What? Why? How?

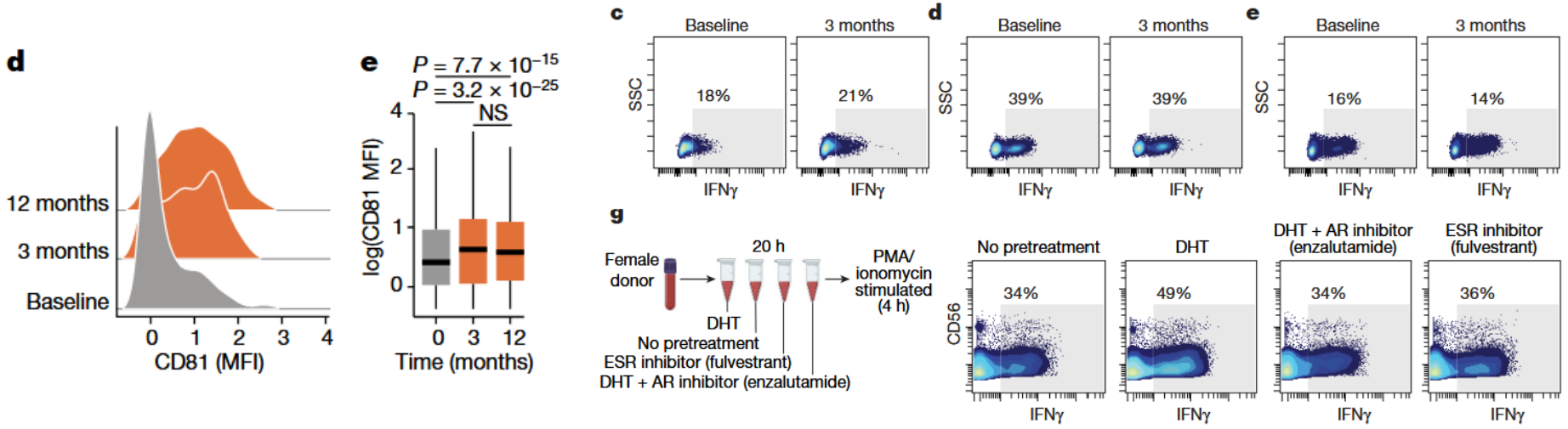
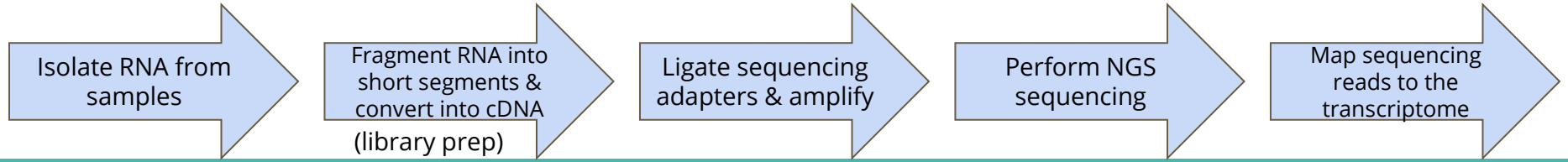


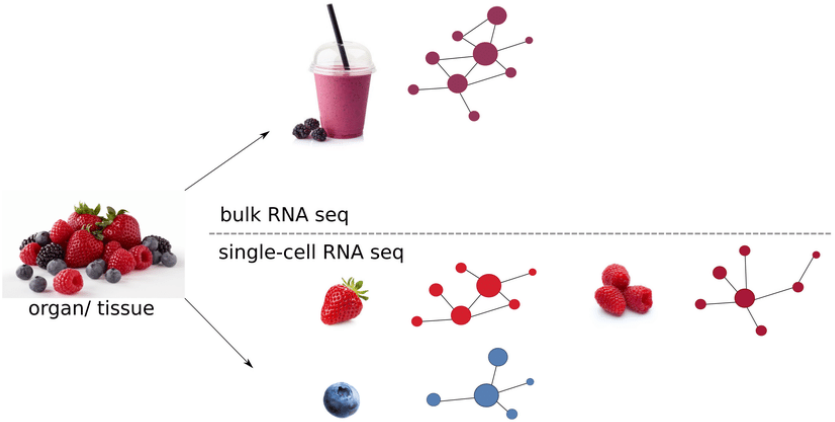
Fig 2d, 2e & Fig 4c-e, 4g

What is mRNA sequencing?

- Technique that uses next-generation sequencing to reveal the presence and quantity of RNA molecules in a biological sample, providing a snapshot of gene expression in the sample, also known as transcriptome
- Facilitates the ability to look at changes in gene expression over time



Bulk vs single-cell RNA sequencing



https://www.researchgate.net/figure/Difference-in-resolution-from-bulk-and-single-cell-RNA-seq-data-on-the-level-of-gene_fig1_350593672

Bulk RNA seq:
Inferred networks would reflect an average of all the signals detected in the mixture of, potentially different, cells

Single-cell RNA seq:
Preserves information of different cell types and would allow for cell type specific gene correlation networks

Bulk RNA seq

PAXgene blood collection tube

https://www.bdbiosciences.com/en-us/products/blood-collection/blood-collection-tubes/paxgene-blood-rna-tube.762165

Sample preparation:

- Using PAXgene blood RNA kit
- Quality assessed before cDNA library prep
  - Measure RNA concentration
  - Determine RNA integrity number

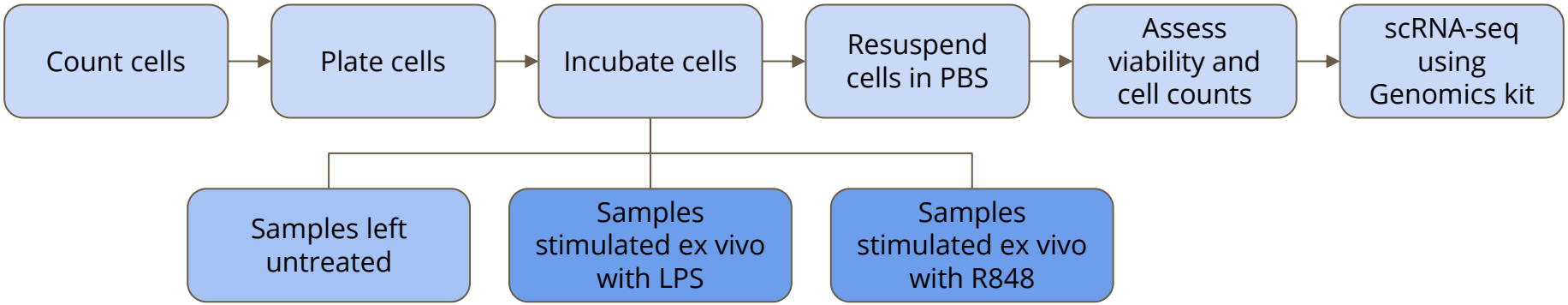
Genes filtered out before assessing differential gene expression:

- Genes with <100 reads across samples
- Genes without a normalized count of 10 in at least ¼ samples

Single-cell RNA seq

- Uses cryopreserved Peripheral blood mononuclear cells (PBMCs)
  - Obtained at baseline and 3 months after treatment

Sample preparation:



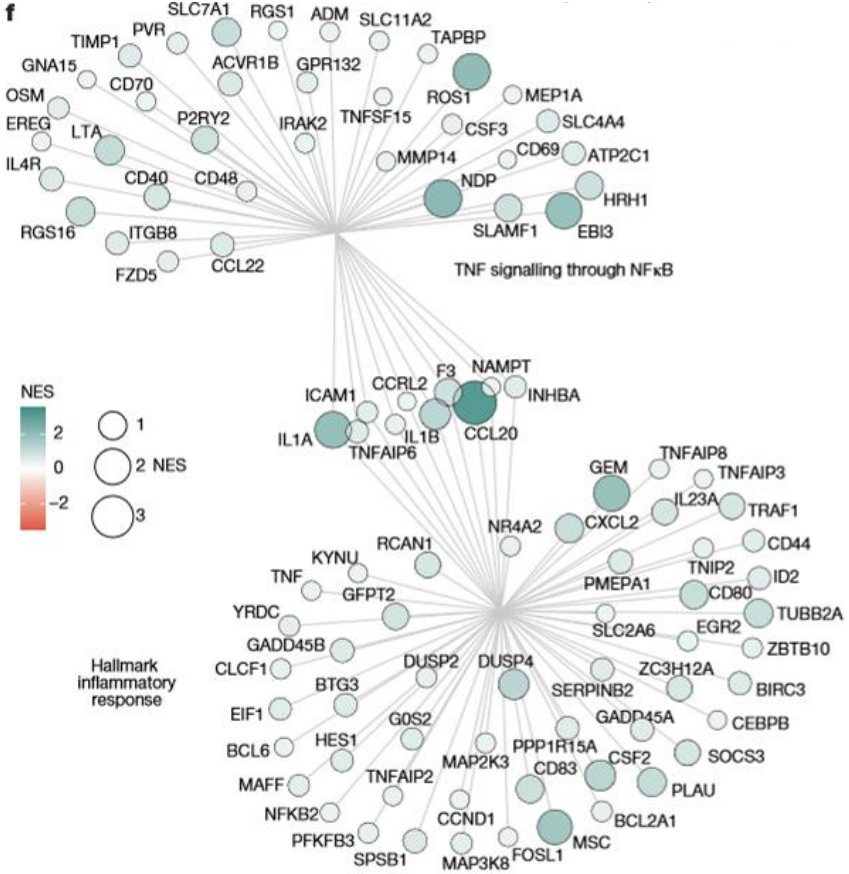
Hallmark Pathways

A collection of gene sets that summarize information about biological processes by highlighting genes that are expressed together

Uses:

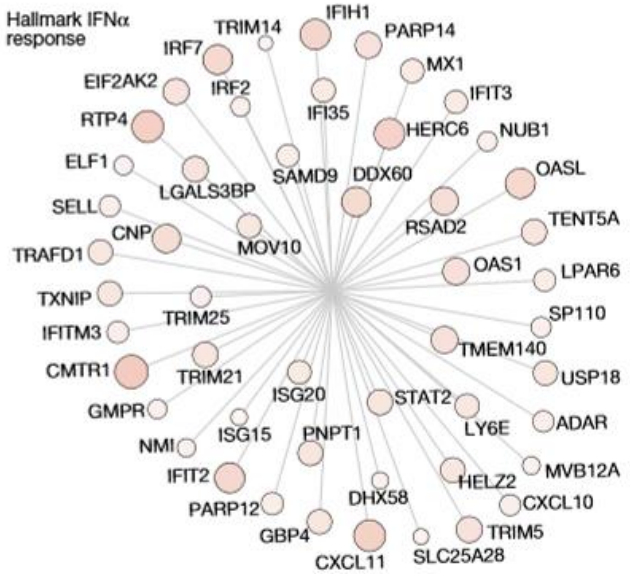
1. Analyzing gene sets
2. Validating methods
3. Identifying key signaling pathways
4. Understanding disease

mRNA Trends

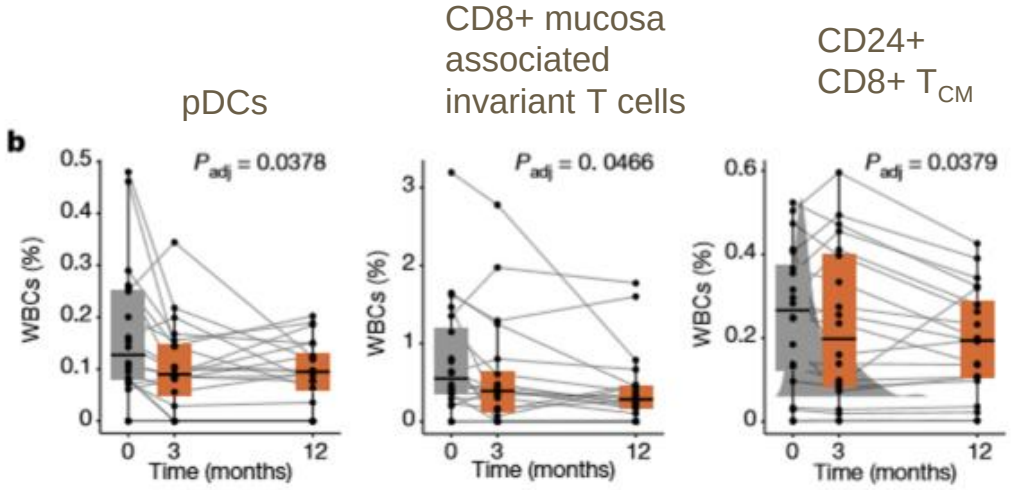


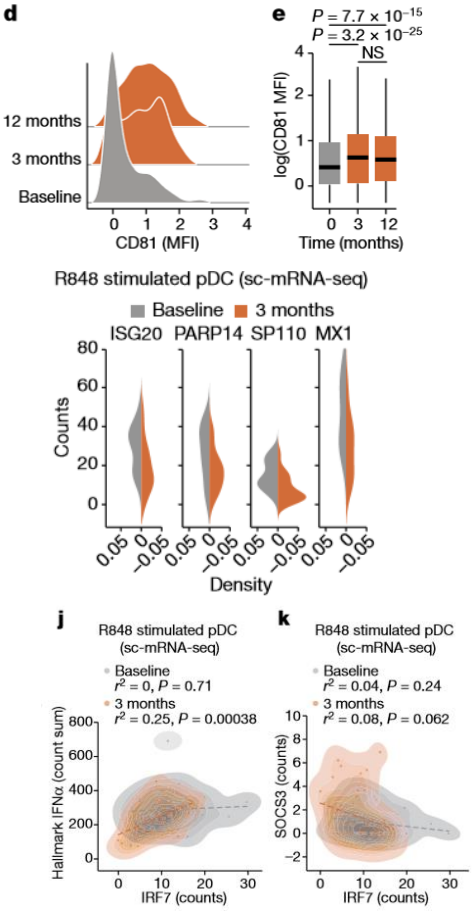
60 whole blood samples

- Inflammation up
- IFNα down



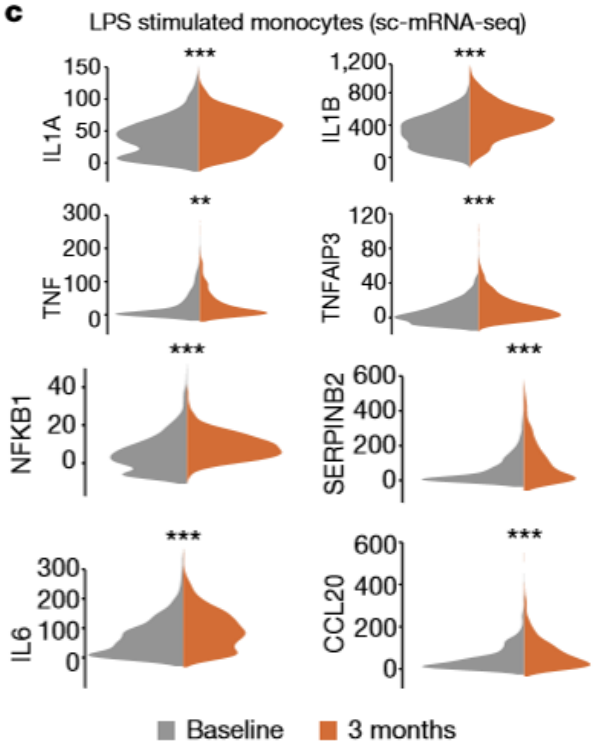
Explaining IFN attenuation





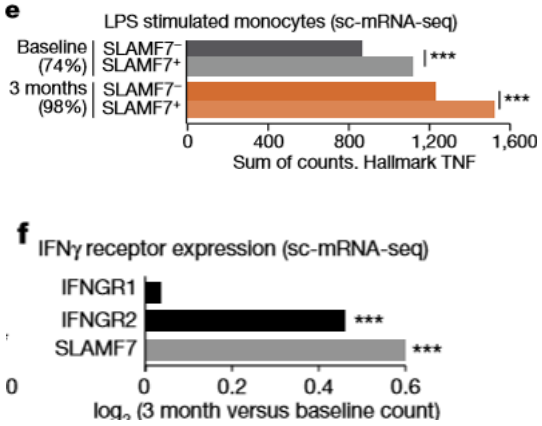
- CD81 expression increased
- Provoked pDCs induce IFN-associated genes less
- IFNα response diminished in correlation with decrease in IRF7

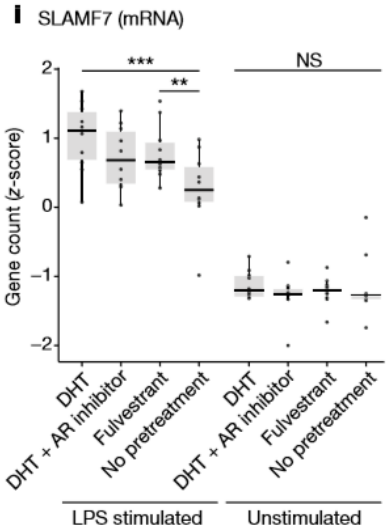
Explaining TNF stimulation



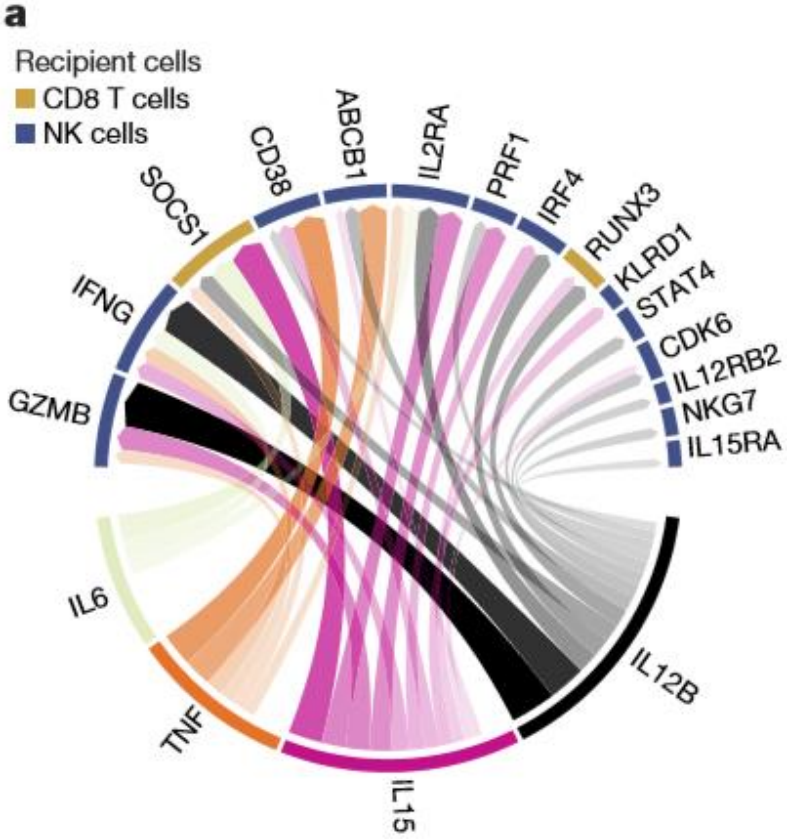
- Provoked monocytes show upregulation of Hallmark TNF response

- SLAMF7 and IFNγ related to TNF upregulation



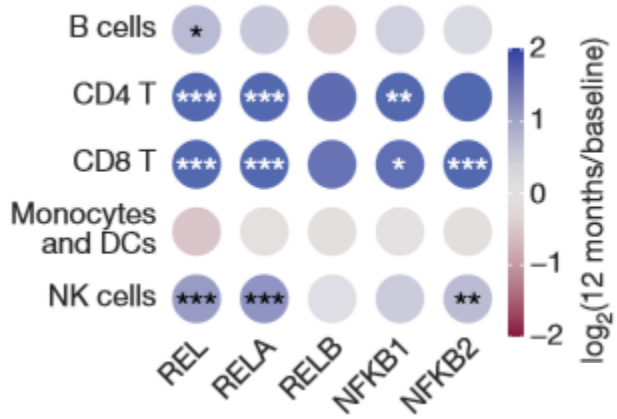


Considering downstream effects



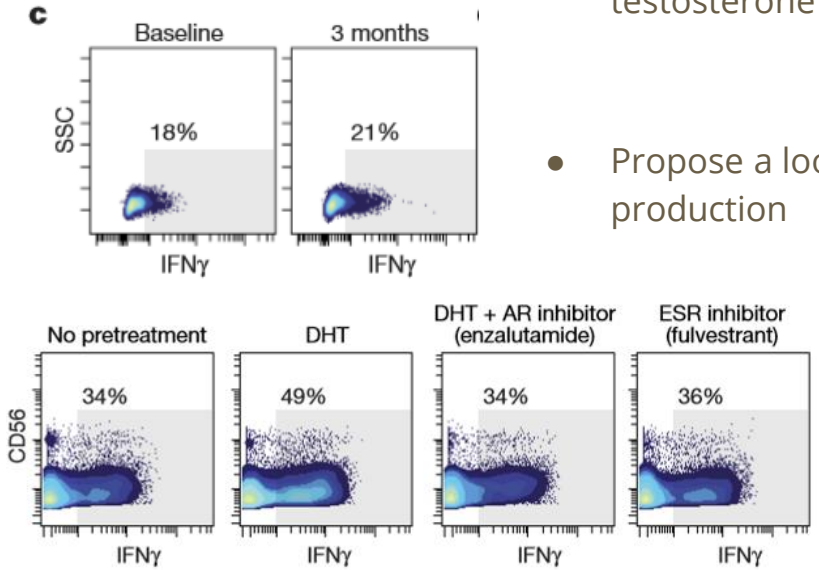
- NicheNet analysis infers downstream effects

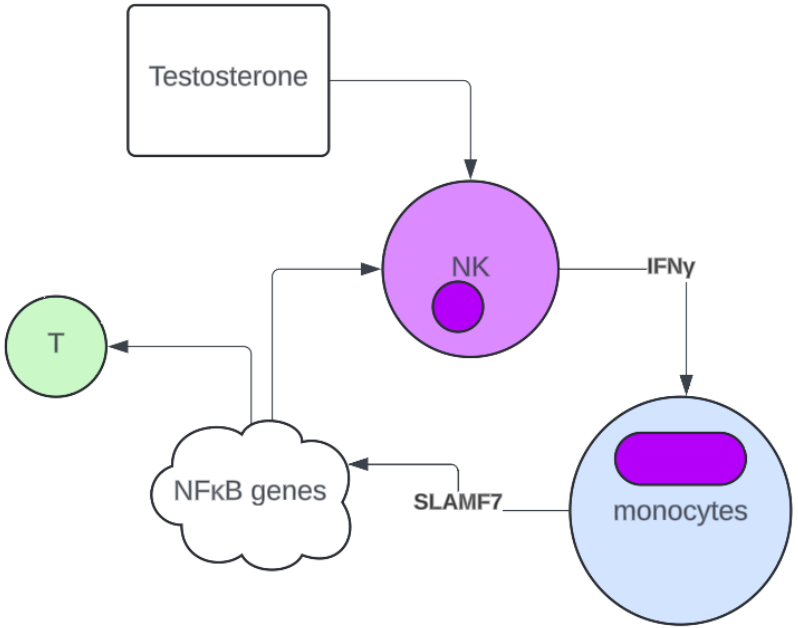
- sc-ATAC-seq reveals increased TF activity in NFκB binding sites



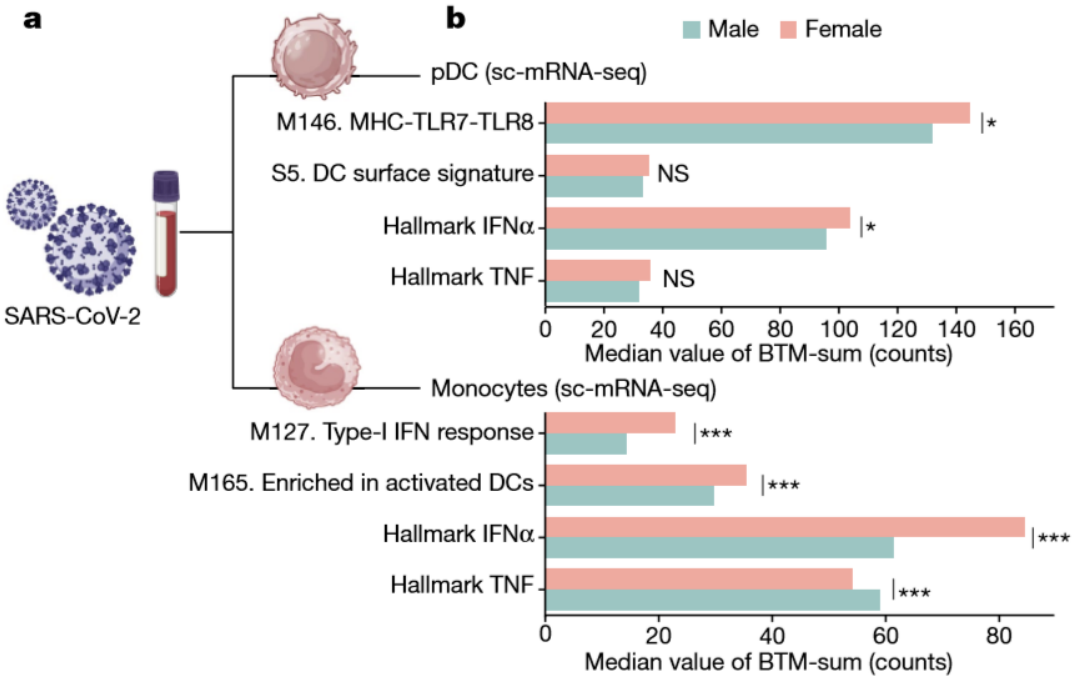
IFNγ Potentiation

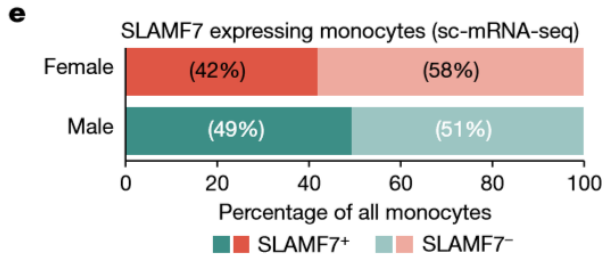
- Stronger IFNγ responses after testosterone treatment
- Propose a loop of IFNγ production

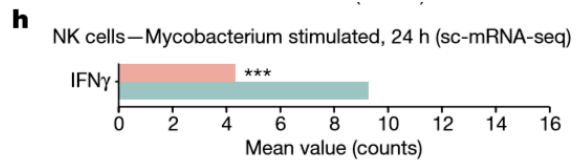




Database comparison during infection







Advantages of Flow cytometry

- High throughput
  - ESR Expression: One million live cells per test
  - Useful for identifying changes at scale in immune cells during testosterone therapy.
- Provides quantitative data - can do statistical analysis on changes

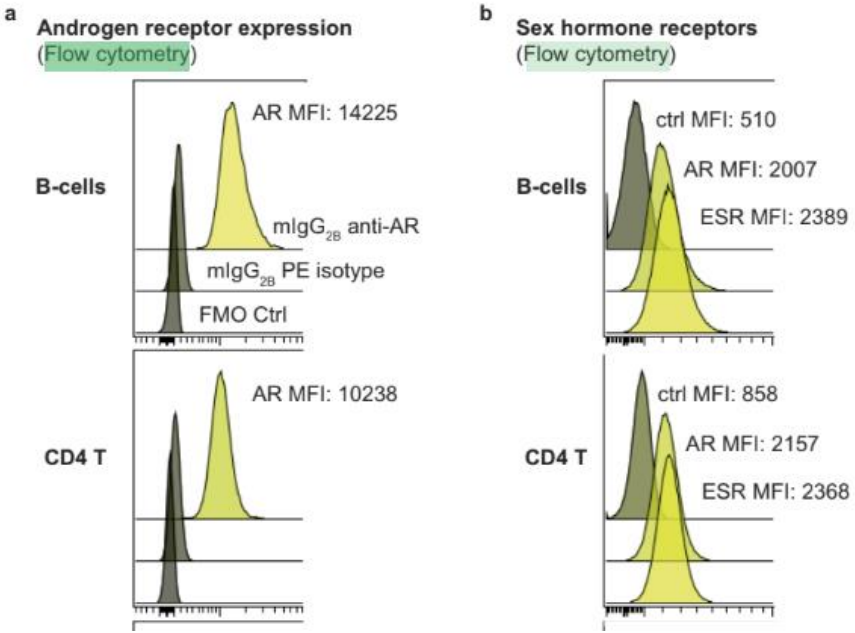


Fig. 6a,b

Limitations of Flow cytometry

- Need to pre-select parameters to measure
  - Cannot measure many at once
  - Have to avoid overlaps in fluorescent labelling and/or compensate data
- Many cells disappear during flow - can lose valuable data
  - Will lose all cells after a complete run
- Can be complex and costly (time and money)

Advantages of mRNA sequencing

- Highly sensitive and accurate means of quantifying gene expression
- Can identify both known and novel transcript isoforms, gene fusions, and other features as well as allele-specific expression
- Delivers a complete view of the coding transcriptome
- Can be applied across a wide range of species

Limitations of mRNA sequencing

- Expensive
- Time consuming
- Often requires complex bioinformatics analysis
  - Lots of data generated
- Additional steps to reduce background RNA and/or enrich for mRNAs can deplete the amount of original sample

Overall Strengths

- Valuable for understanding the effects of testosterone on the immune response.
- Provides insights on changes over time in response to hormone therapy.
- Can generalize results to explain differences in immune cell phenotypes across gender.
- Using human participants is important as model organisms have different sex hormone regulation systems.

Overall Limitations

- Blood samples only collected at baseline, 3 months, and 12 months following testosterone injection.
- Relatively small cohort (23 participants)
- Difficult to isolate the effects of gonal steroids from secondary effects.
- Different immune cell types have varying expression of AR and ESR proteins.

Thank you!

Questions/ Comments?