The use of single-cell RNA-Seq to understand virus-host interactions

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University of Lausanne, Switzerland

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Palau Macaya, Barcelona

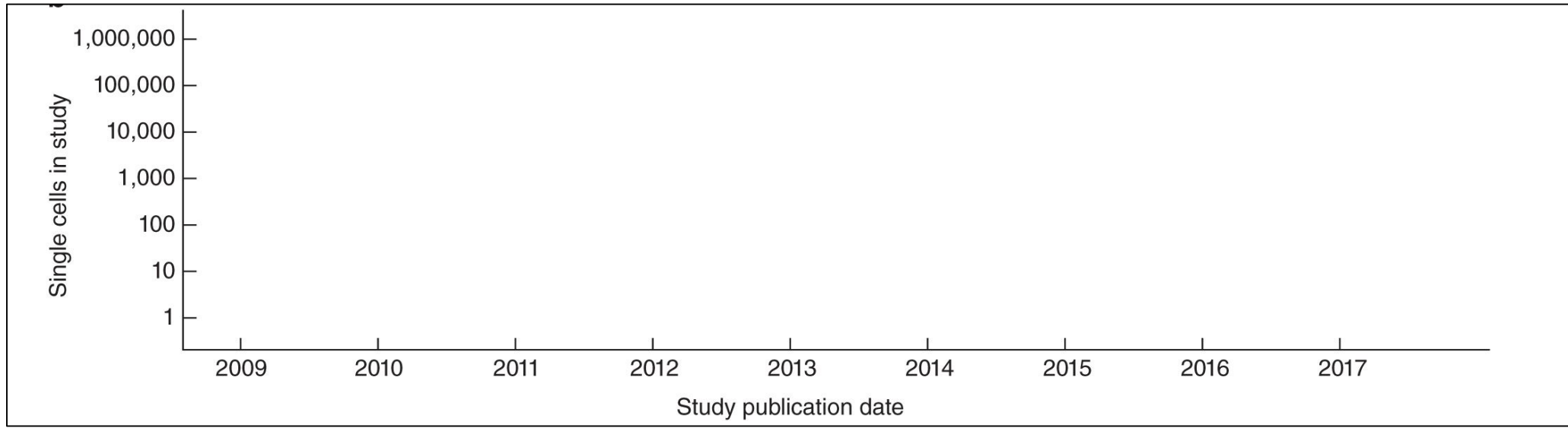**HETEROGENEITY** : complex cell populations, phenotype diversity (in response to virus), rare events

HETEROGENEITY : complex cell populations, phenotype diversity (in response to virus), rare events

-> single cell isolation
-> lysis
-> RT
-> amplification
-> Sequencing (NGS)

Svensson et al, Nature Protocols 2018

HETEROGENEITY : complex cell populations, phenotype diversity (in response to virus), rare events
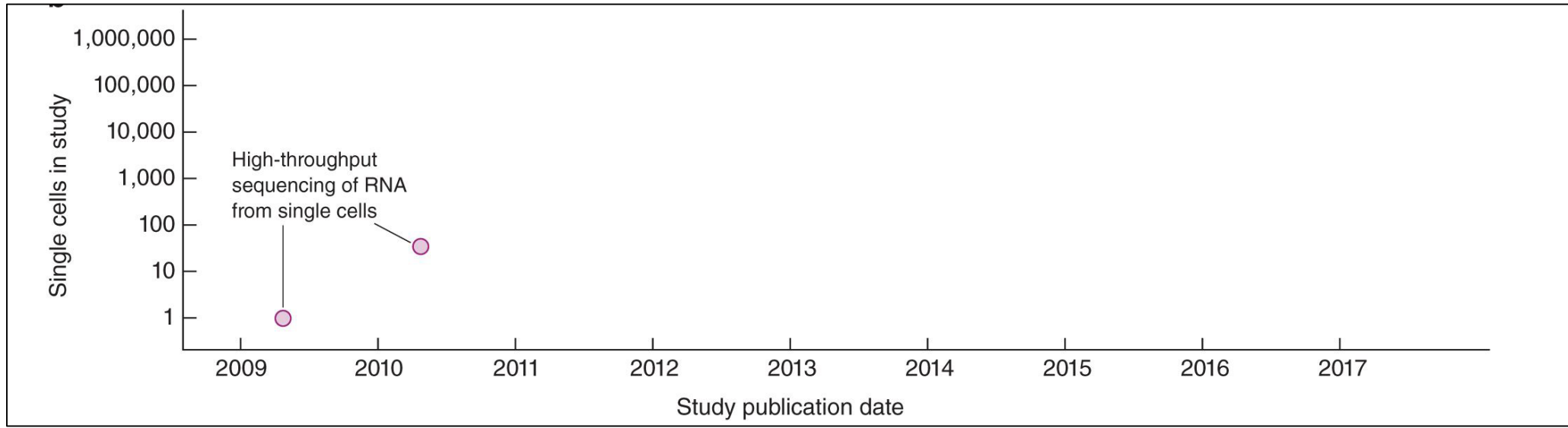
-> single cell isolation (manual, physical)
-> lysis
-> RT
-> amplification
-> Sequencing (NGS)

Manual

Tang *et al.* 2009[18]

1 cell

Svensson et al, Nature Protocols 2018

HETEROGENEITY : complex cell populations, phenotype diversity (in response to virus), rare events

-> single cell isolation (multiplexing, automated, physical)
-> lysis
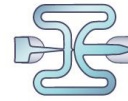-> RT
-> amplification (IVT, PCR)
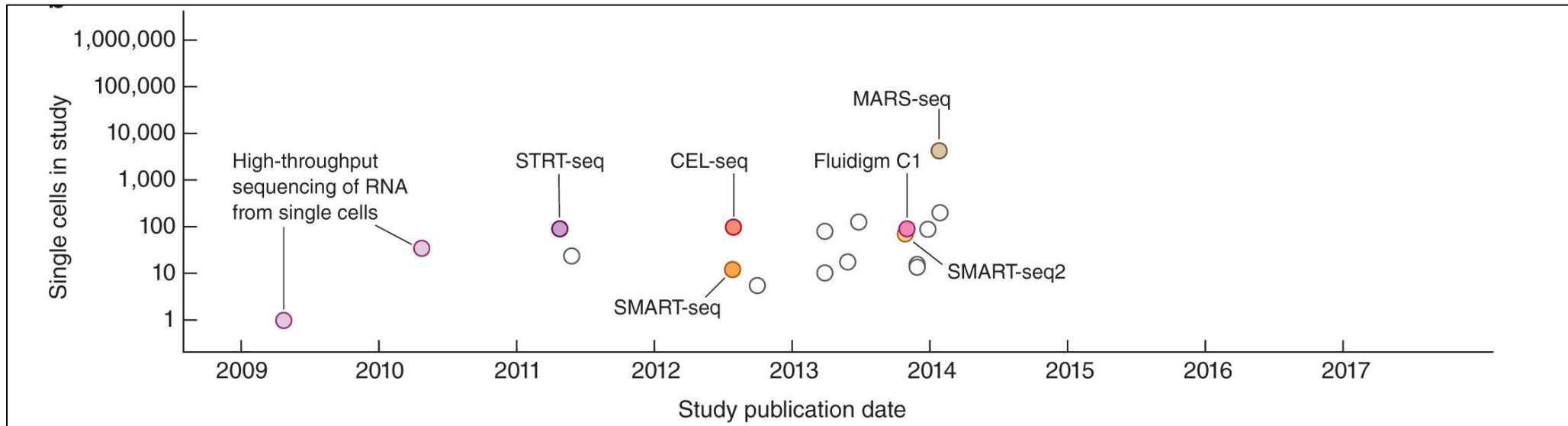-> Sequencing (NGS)

Manual

Tang *et al.* 2009[18]
1 cell

Integrated fluidic circuits
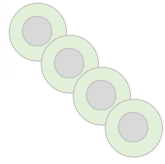
Brennecke *et al.* 2013[64]
~100 cells

Cell number

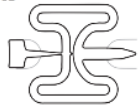*Svensson et al, Nature Protocols 2018*

Fluidigm technology

C1 Autoprep system
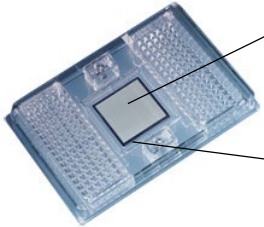
10-17 µm small plate

1 plate = 96 capture sites

-> Isolation of up to 96 single cells

Cells flow one by one in a serpentine pattern through each capture site to be isolated.

If the capture site is occupied then the cells travel through the bypass wings to the next chamber.

Microfluidics

4.5 nL   ~300 nL

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Fluidigm technology

C1 Autoprep system

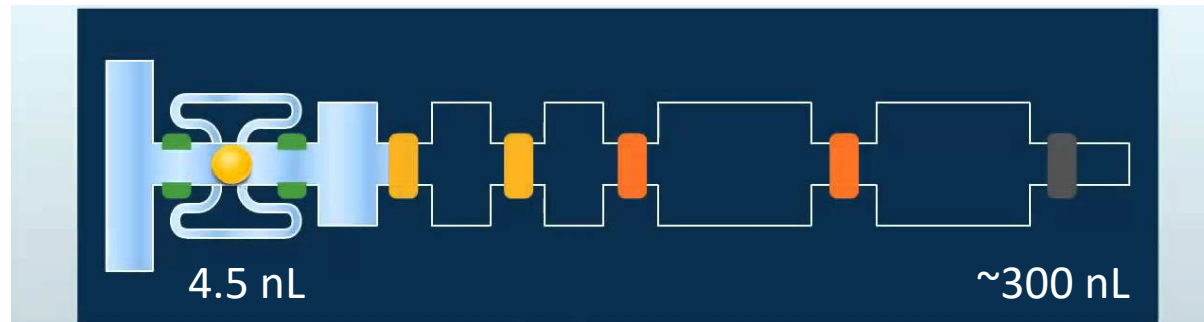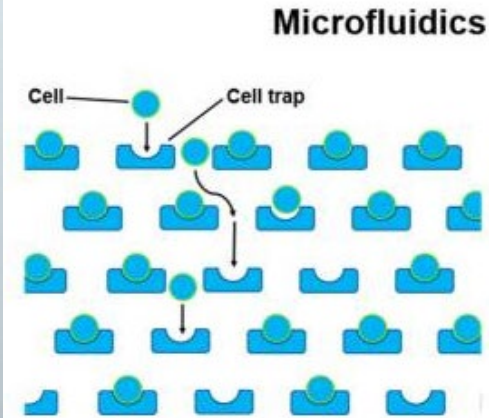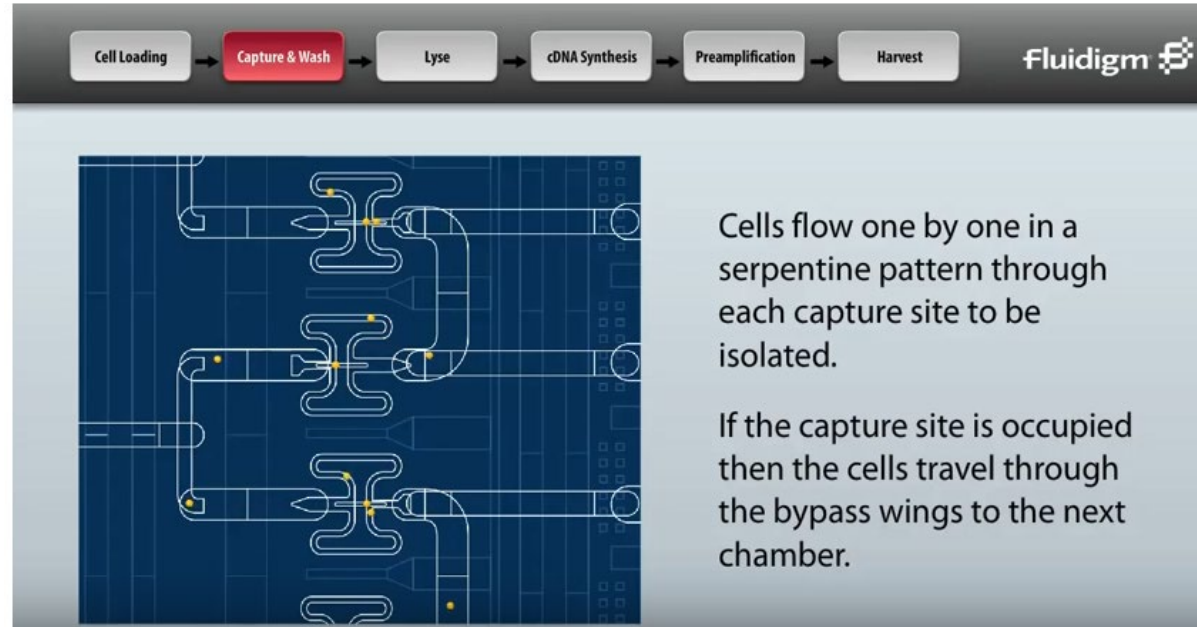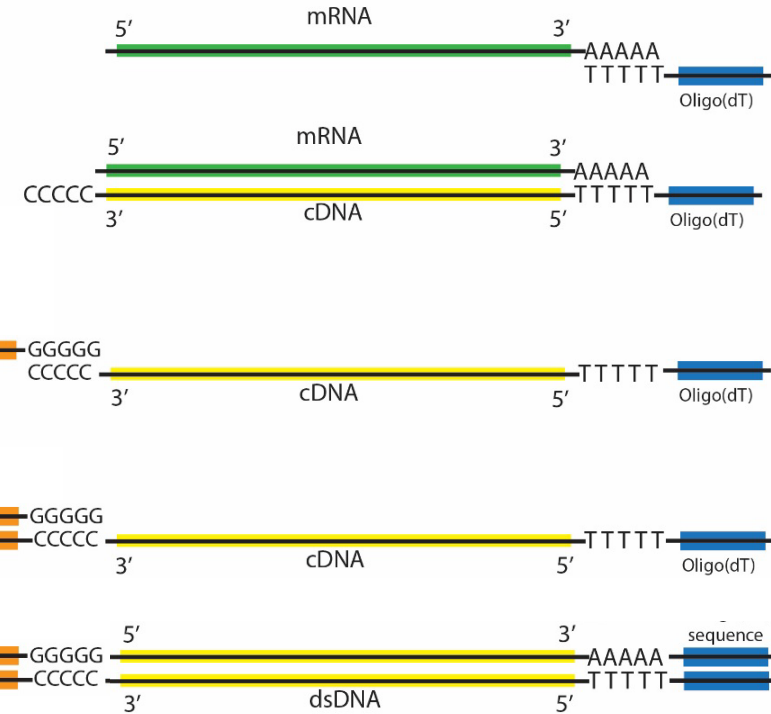**Lysis**

RT (Smart-Seq)

Preamplification

Collect 96 individual samples

-> 96 library prep -> Illumina sequencing

5′   mRNA   3′—AAAAA
                           TTTTT
                             Oligo(dT)

5′   mRNA   3′—AAAAA
CCCCC   TTTTT
3′   cDNA   5′   Oligo(dT)

—GGGGG
—CCCCC   TTTTT
    3′   cDNA   5′   Oligo(dT)

—GGGGG
—CCCCC   TTTTT
    3′   cDNA   5′   Oligo(dT)

5′   3′   sequence
—GGGGG   AAAAA
—CCCCC   TTTTT
    3′   dsDNA   5′

Modified from *Serra et al, Bio-Protocols 2018*

https://www.youtube.com/watch?v=TF4NJRE4Xg4

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Cell heterogeneity for permissiveness to HIV
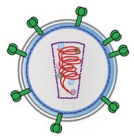
CD4+ T cells
(isolated from individuals)

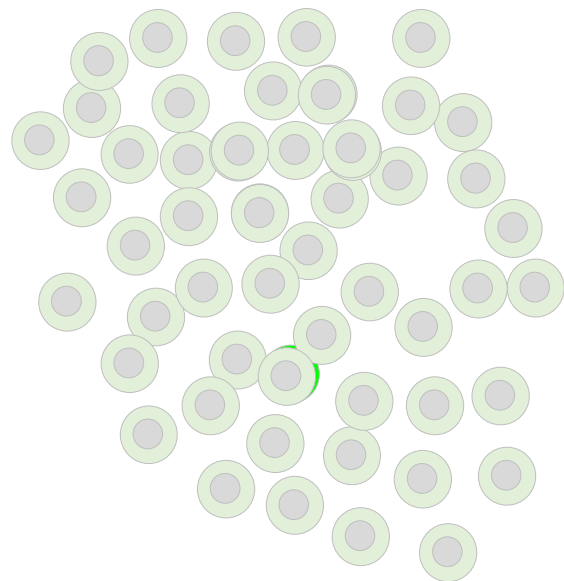Replication kinetics for HIV-1 in native CD4 T cells isolated from 128 healthy blood donors

Native CD4 T cells

Cellular permissiveness to HIV infection differs between cells from the SAME donor

Highly permissive

Poorly permissive
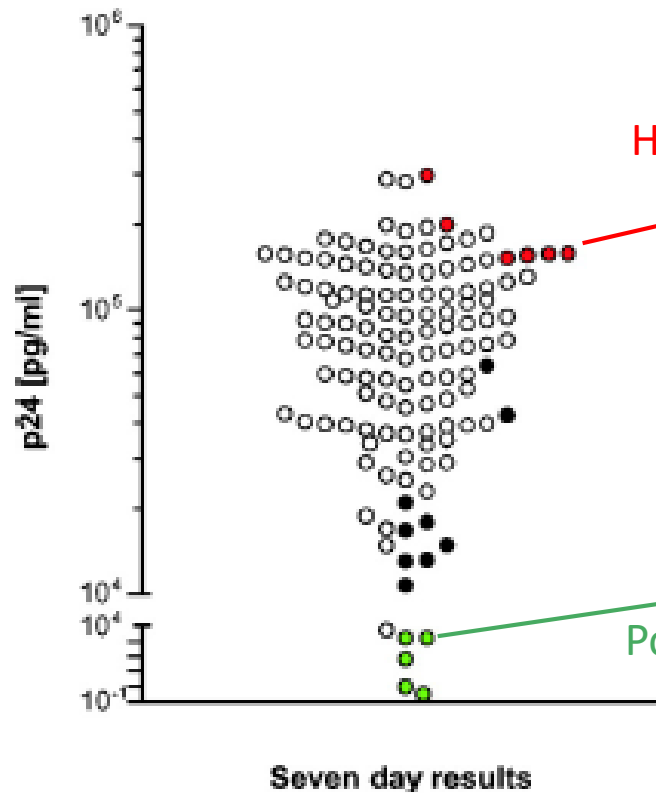
Ciuffi et al, Journal of Virology 2004
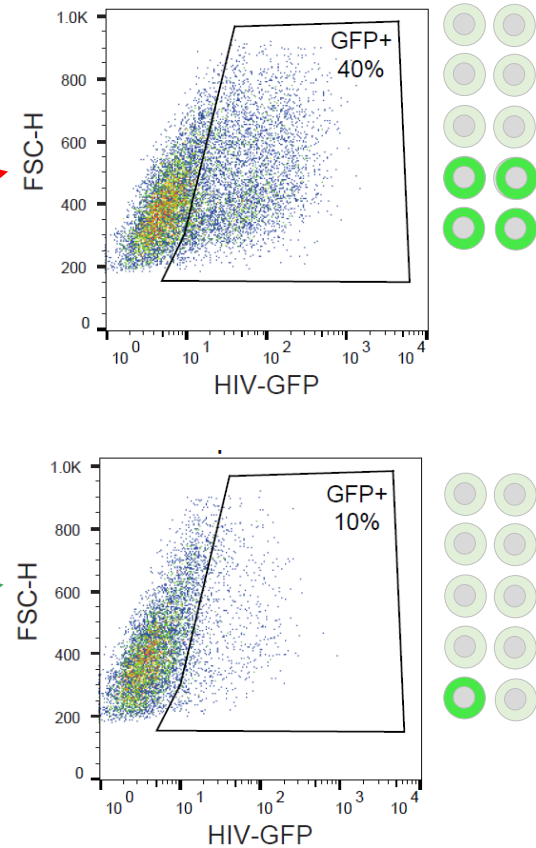
Rato et al, PLoS Pathogens 2017

How to identify determinants of permissiveness ?

Diversity of outcomes

Non infected

Latently infected

Productively infected

T-cell activation
Biomarkers ?

Cell heterogeneity : determinants of HIV permissiveness

Assessing differential HIV permissiveness between two donors:

Resting CD4+T Cells

TCR stimulation

Activated CD4+ T Cells

Fluidigm

highly permissive
85 single cells

poorly permissive
81 single cells

Single Cell RNA-Seq:
50 bp paired-end, Illumina,
~25 $\pm$ 7 mio reads/cell,
6.6 $\pm$ 2.7 mio uniquely mapped reads
~640-10,000 genes/cell

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CHUV

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Resting cells

Resting CD4+ T cells

Activated CD4+ T cells   8h   24h   72h

PC2 (5.4%)

PC1 (18.5%)

Activated cells

density

PC2 coordinate

"Poorly Permissive"

"Highly Permissive"

Population   Single cells

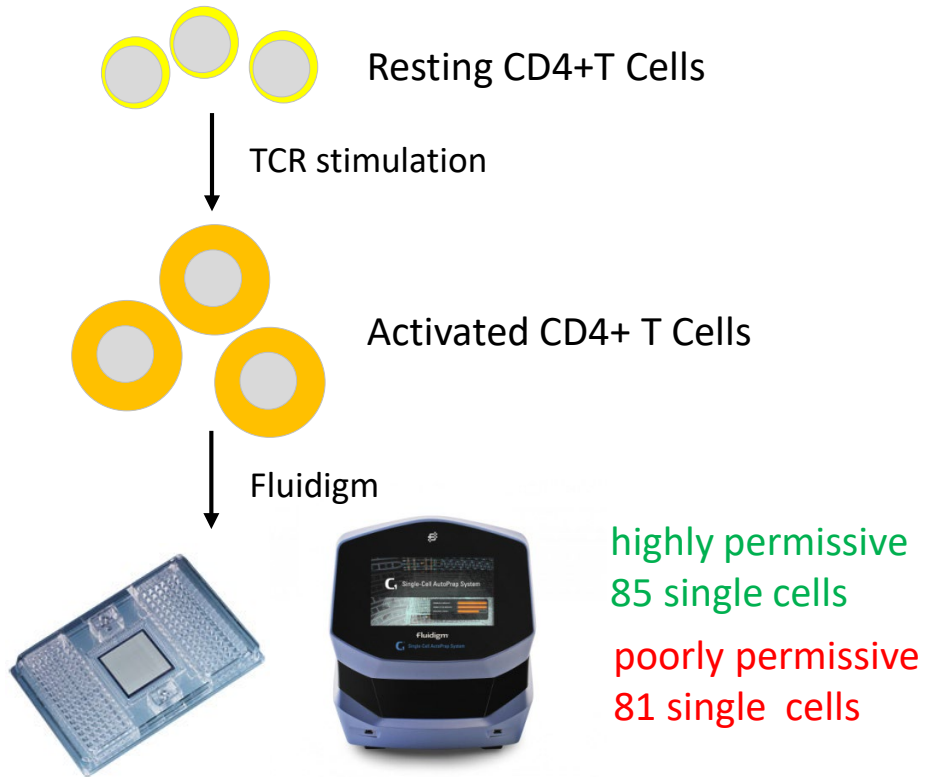=> T cell activation is a major driver of cell heterogeneity

Rato et al, PLoS Pathogens 2017

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Cell heterogeneity : determinants of HIV permissiveness

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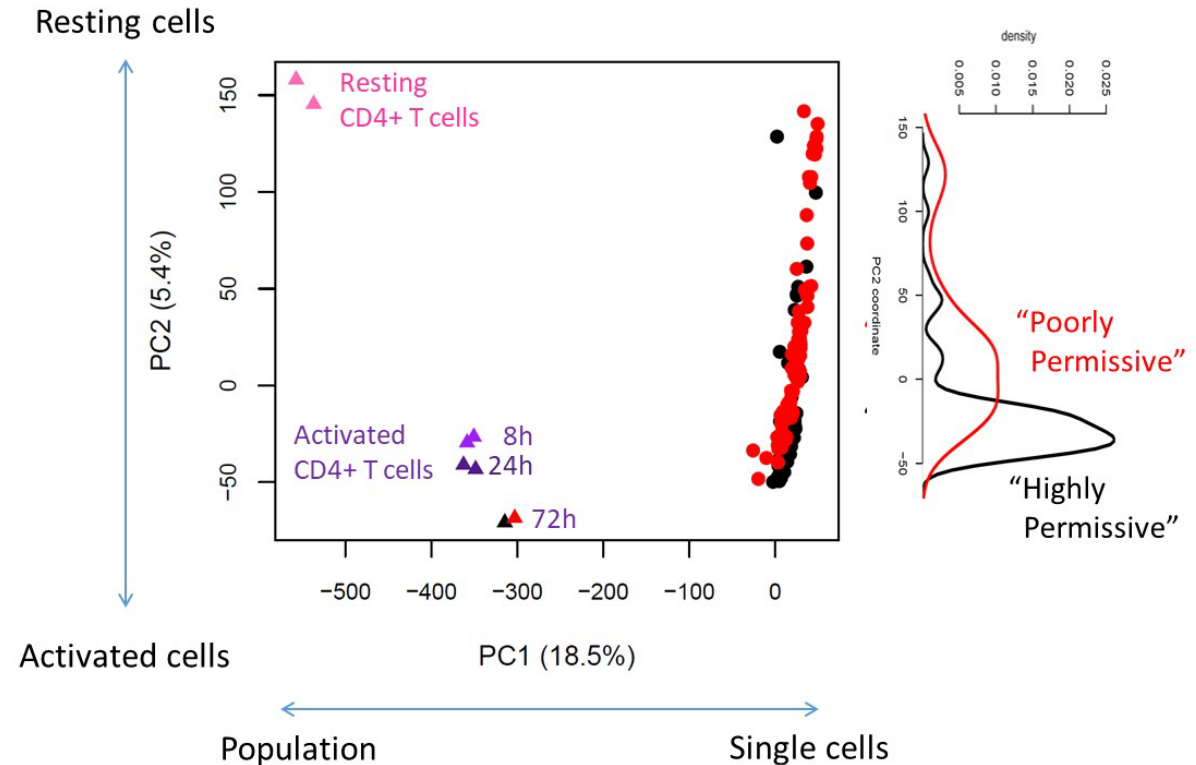6.6 ± 2.7 mio uniquely mapped reads
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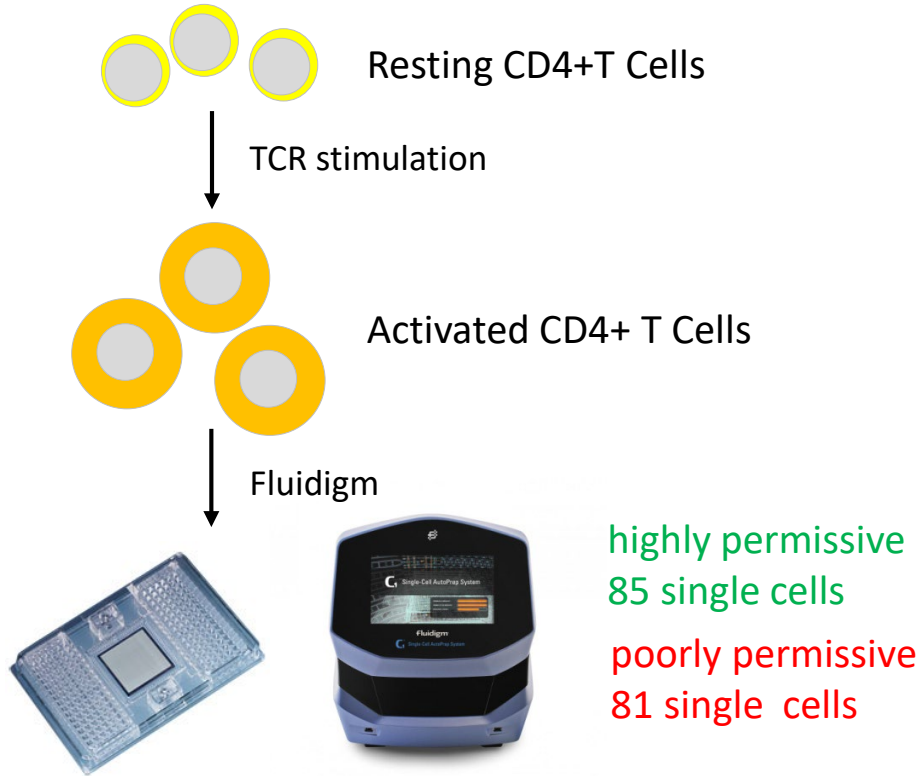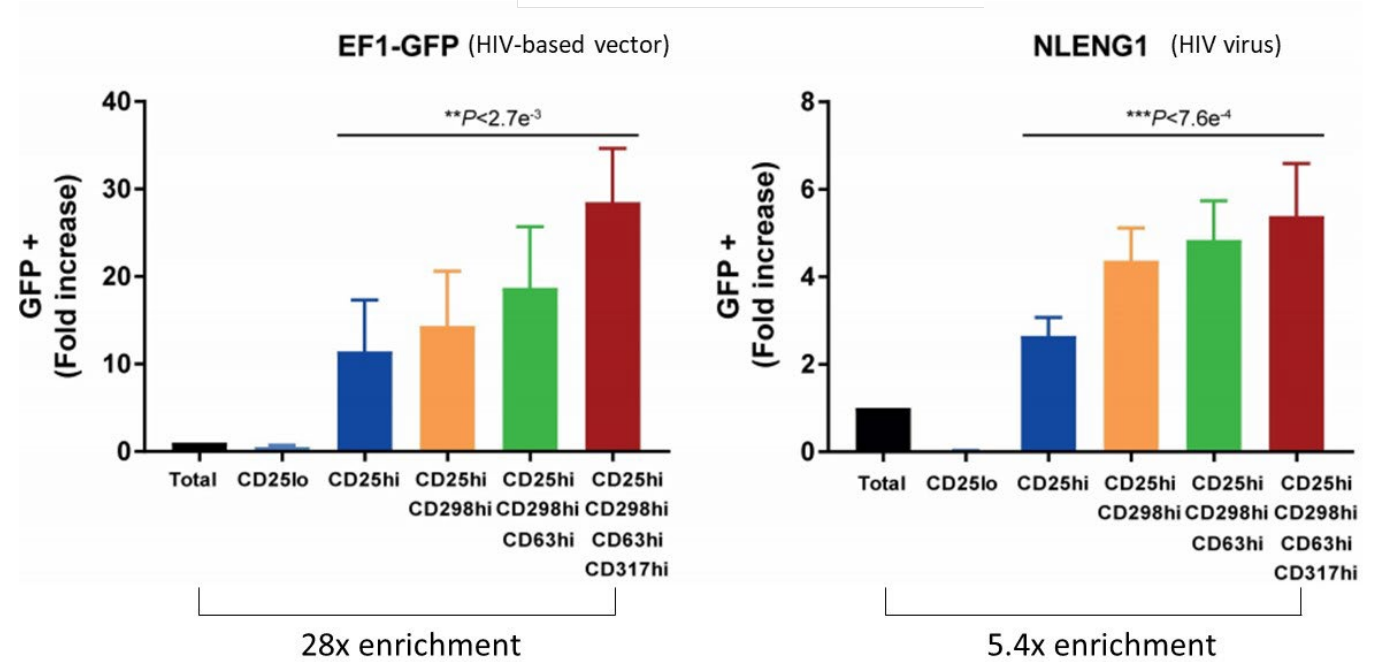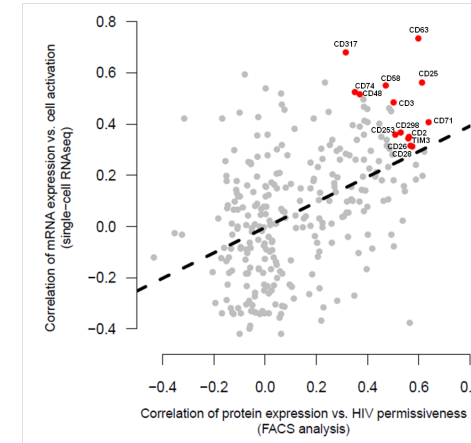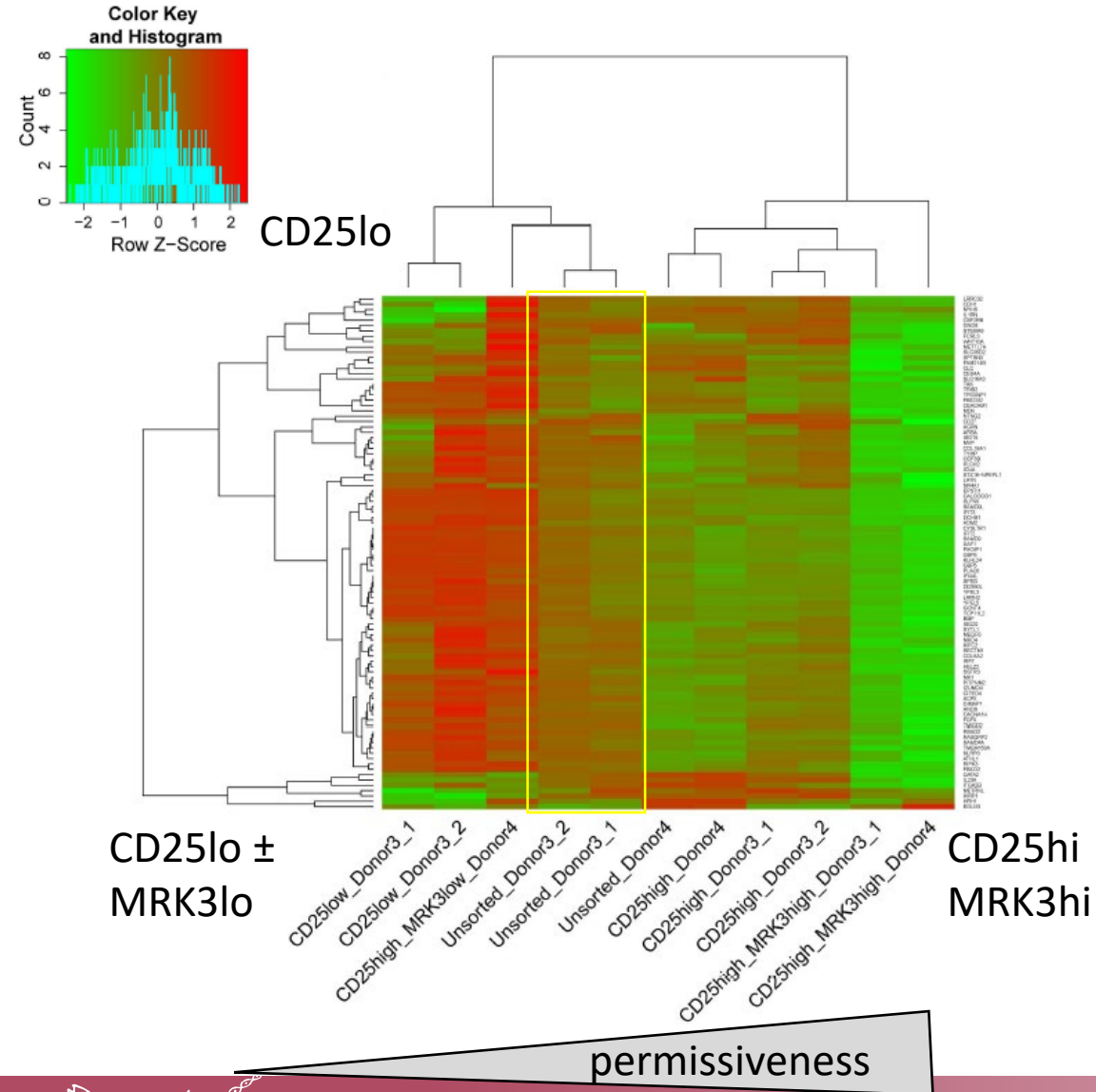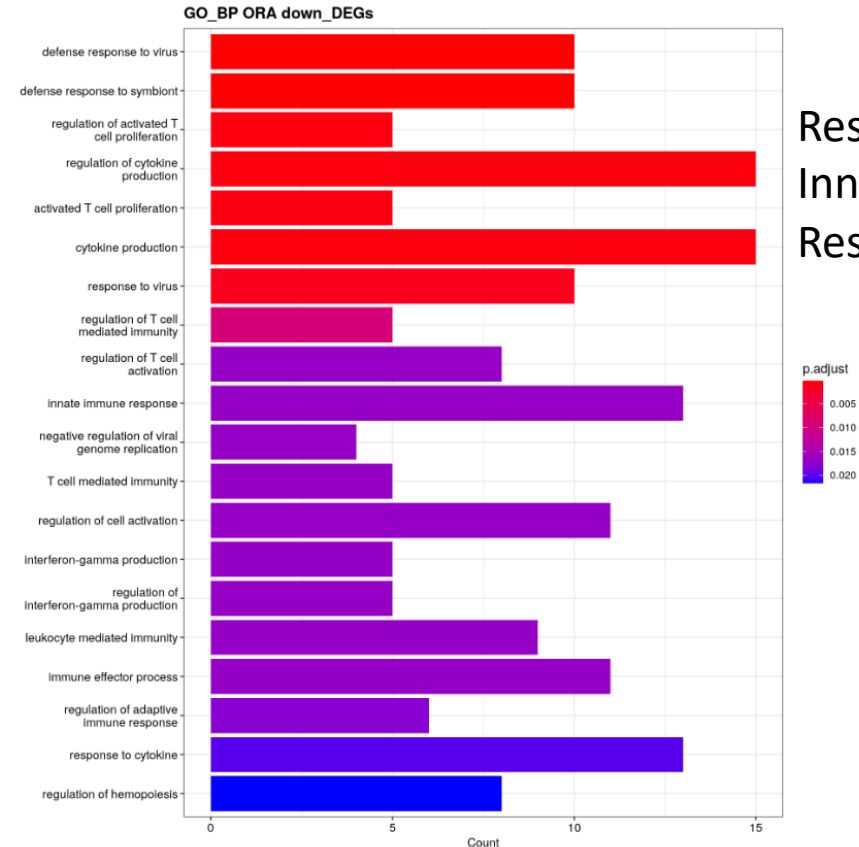*Rato et al, PLoS Pathogens 2017*

Transcriptional heterogeneity : Signature of HIV permissive cell

Differential expression analysis : 96-gene signature

Response to virus,
Innate immune response,
Response to IFN-γ

permissiveness

Rato et al, PLoS Pathogens 2017

scRNA-Seq : single cell isolation – RNA-Seq

HETEROGENEITY : complex cell populations, phenotype diversity (in response to virus), rare events

-> single cell isolation (multiplexing, automated, oil droplets)
-> lysis
-> RT
-> amplification (IVT, PCR)
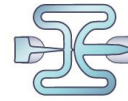-> Sequencing (NGS)

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~100 cells
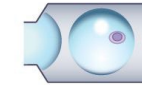
Nanodroplets
Klein *et al.* 2015[34]
Macosko *et al.* 2015[40]
~1000 cells
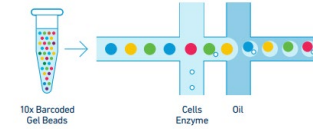
Cell Indexing / Barcoding
>10,000 cells   3000 TXP/cell

7500 FL TXP/cell

Svensson et al, Nature Protocols 2018

Cell number
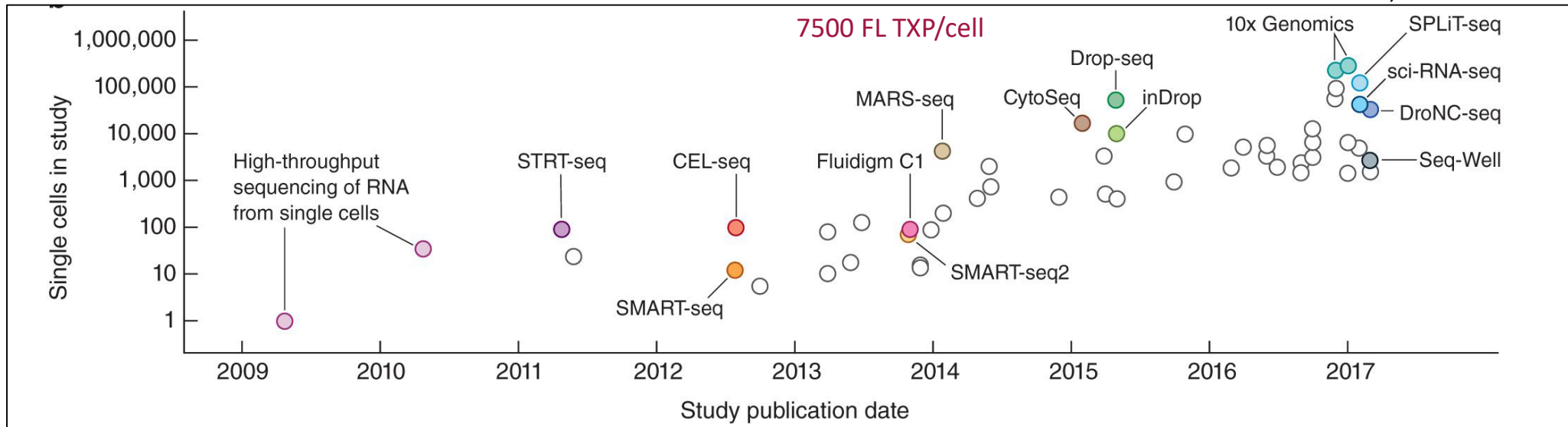
10x genomics technology

10x Barcoded Gel Beads

Cells Enzyme

Oil

10,000 cells
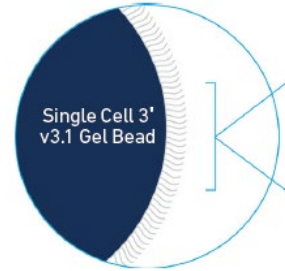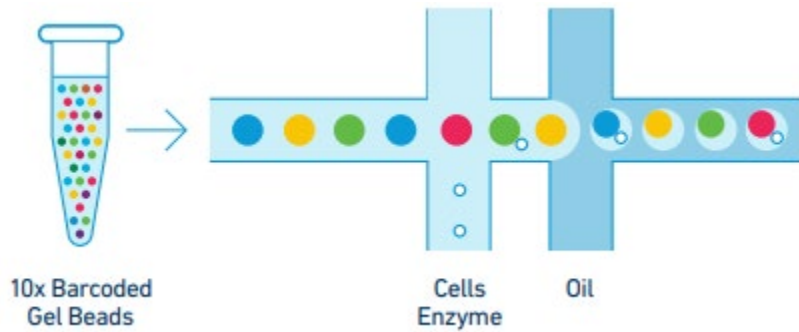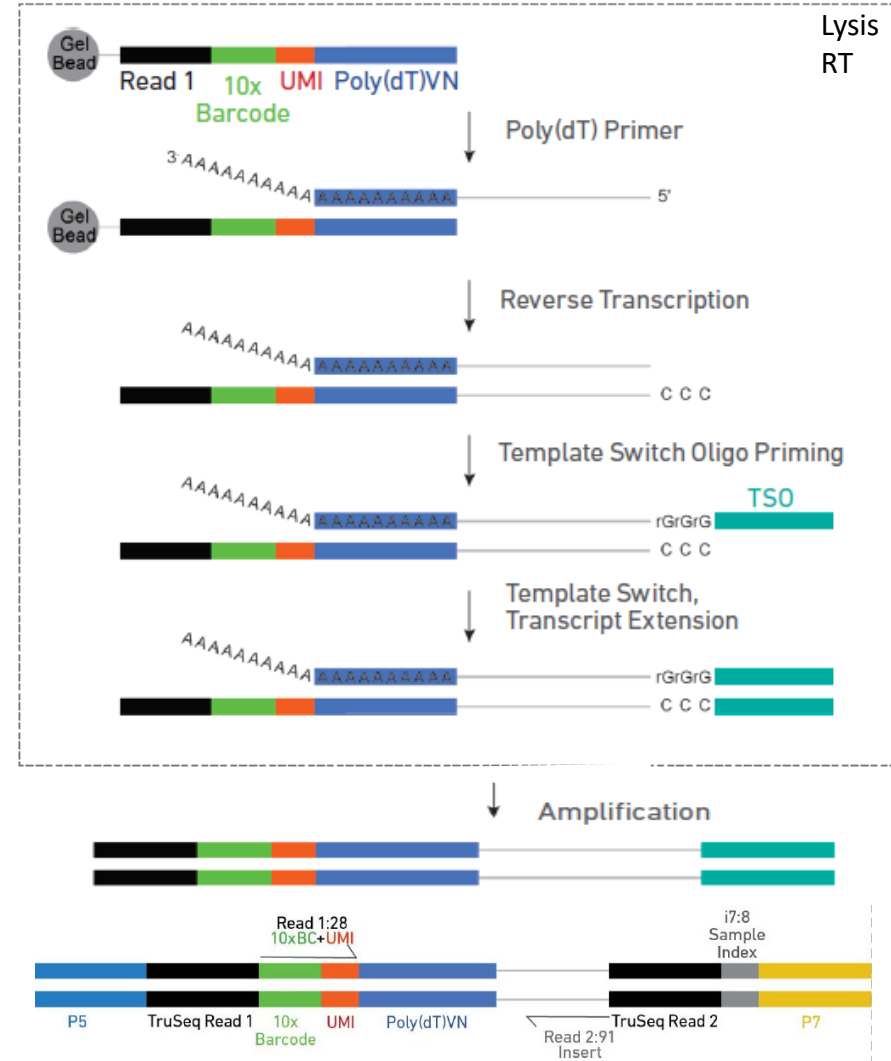
Lysis
RT

Collect & Pool
Library Preparation and Sequencing
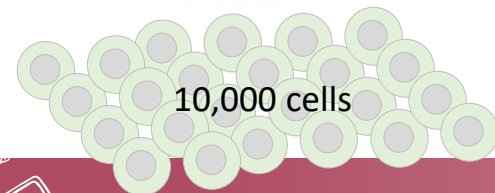
Lysis
RT

10xGenomics.com

https://wp.10xgenomics.com/instruments/chromium-controller/

Cell (subset) heterogeneity : determinants of HIV permissiveness

Assessing differential HIV permissiveness of cells from a single donor:

Resting CD4+T Cells

TCR stimulation

Activated CD4+ T Cells

Non infected (1919 cells)

Infected (3342 cells)

10x genomics

Single Cell RNA-Seq
180 total mio reads
~25,000 – 59,000 reads/cell
200 – 10,000 genes/cell

10x Barcoded
Gel Beads

Cells
Enzyme

Oil

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10x genomics

10x Barcoded Gel Beads   Cells Enzyme   Oil

Panel of 228 antibodies (CITE-Seq)

5' PCR Handle Antibody Barcode AAAAAAAAAAAAAAAAA (+A$_{11}$)3'

5' Flanking Sequence   15 bp Barcode   3' Flanking Sequence

TotalSeq™-A (4-digit code)

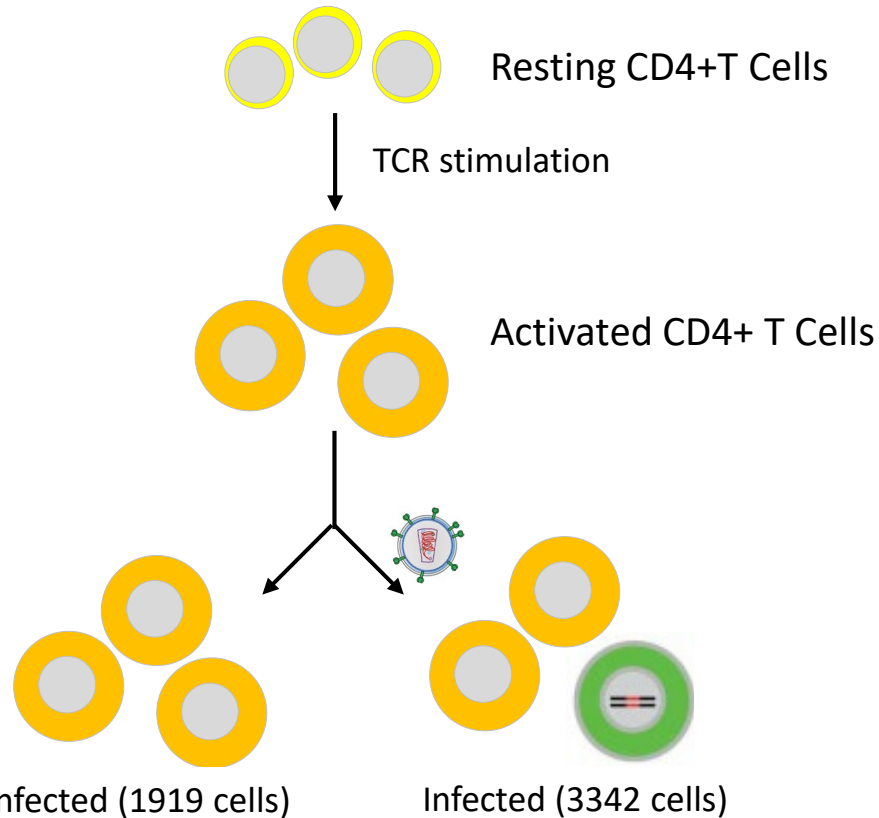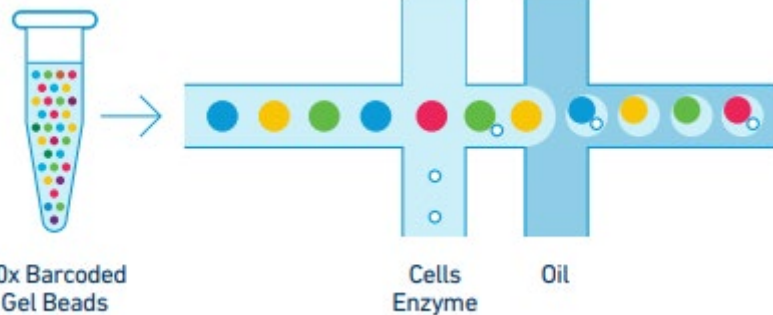161,784 PBMC

41,001 CD4+ T cells

pDC
CD14 Mono
cDC2
ASDC
cDC1
Doublet
CD16 Mono
Platelet
Eryth
HSPC
CD8 TEM
dnT
CD8 TCM
CD8 Naive
gdT
MAIT
NK
CD8 Proliferating
NK Proliferating
CD4 Proliferating
Plasmablast
B memory
B intermediate
NK_CD56bright
ILC
B naive

CD4 Naive
CD4 TCM
CD4 TEM
Treg
CD4 CTL

WUMAP_2
WUMAP_1

Hao et al., 2021, Cell 184:3573, https://doi.org/10.1016/j.cell.2021.04.048

Combining our data with the reference dataset

Combined datasets (CD4+ T cells)

In vitro stimulated cells

Reference single cell atlas
(CD4+ T cells only)
(Hao et al, Cell 2021)

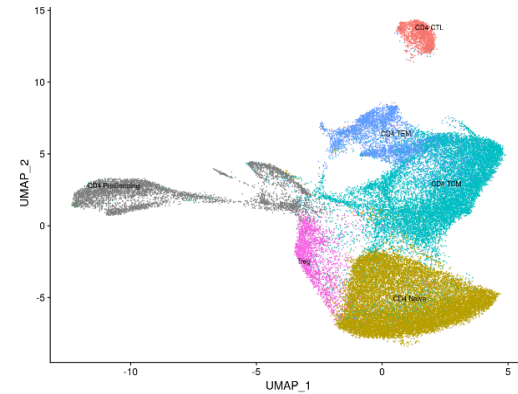

CTL:0.5%

TEM:0.2%
TCM:28.9%

TN:9.2%

Prolif.:54.8%   Treg:6.5%

- CD4 CTL
- CD4 Naive
- CD4 Proliferating
- CD4 TCM
- CD4 TEM
- Treg
- NA

CTL:4.2%

TEM:10.4%

TCM:36.3%

Proliferating:0.3%

Treg:6.1%

TN:42.6%
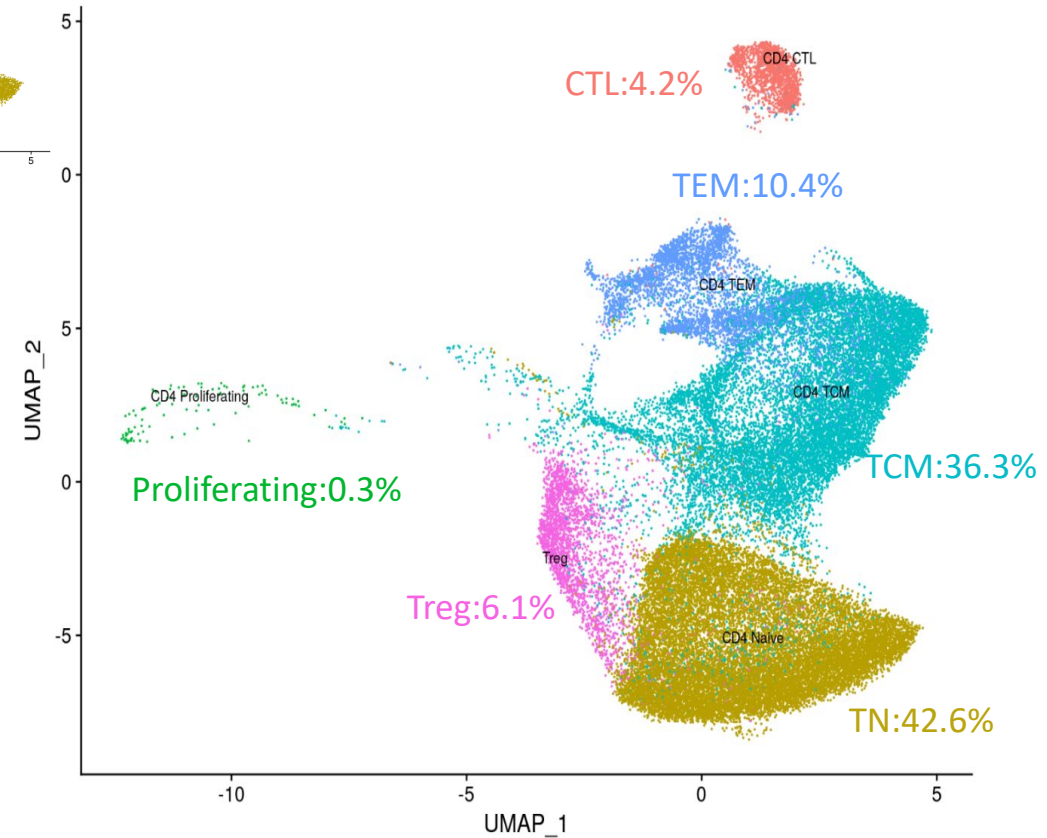
Unpublished data

Stimulation kinetcs / CD4+ dynamics
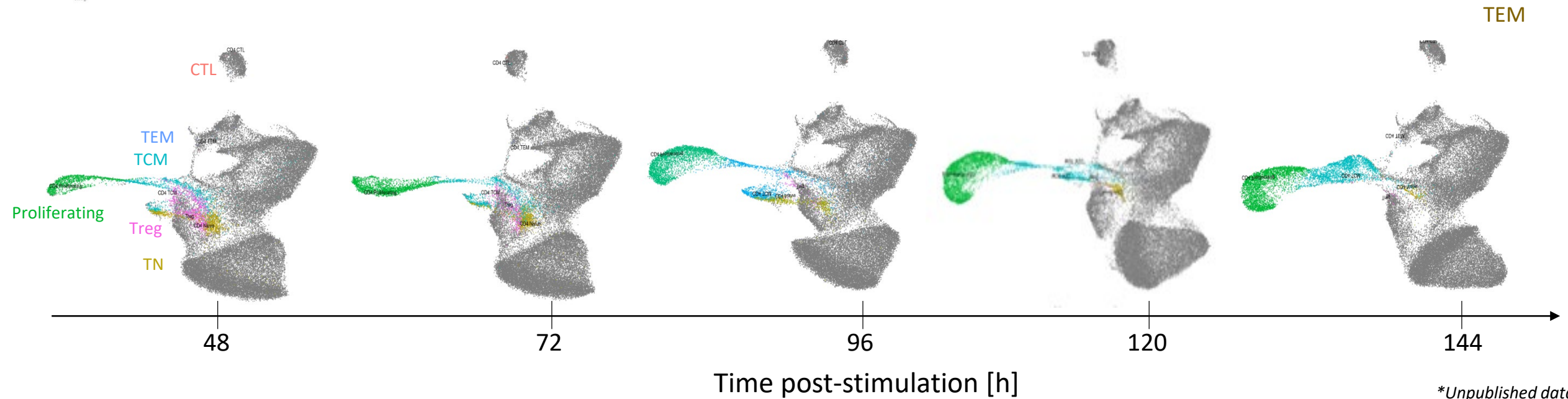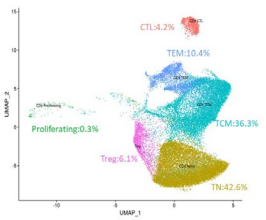
TEM

CTL

TEM
TCM

Proliferating

Treg

TN

48   72   96   120   144

Time post-stimulation [h]

*Unpublished data

Cell (subset) heterogeneity : determinants of HIV permissiveness

Globally :
HIV infection success in
exposed cells : 86%

HIV sample (3342 cells)	HIV- [counts]	HIV+ [counts]	HIV- [%]	HIV+ [%]	
Proliferating	56	2128	2.6	97.4	Highly permissive
CTL	2	8	20.0	80.0	NA
TEM	1	1	50.0	50.0	
Treg	51	111	31.5	68.5	Cell heterogeneity
TNaive	34	47	42.0	58.0	
TCM	314	589	34.8	65.2	

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Cell (subset) heterogeneity : determinants of HIV permissiveness

Globally :
HIV infection success in
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HIV-exposed cells

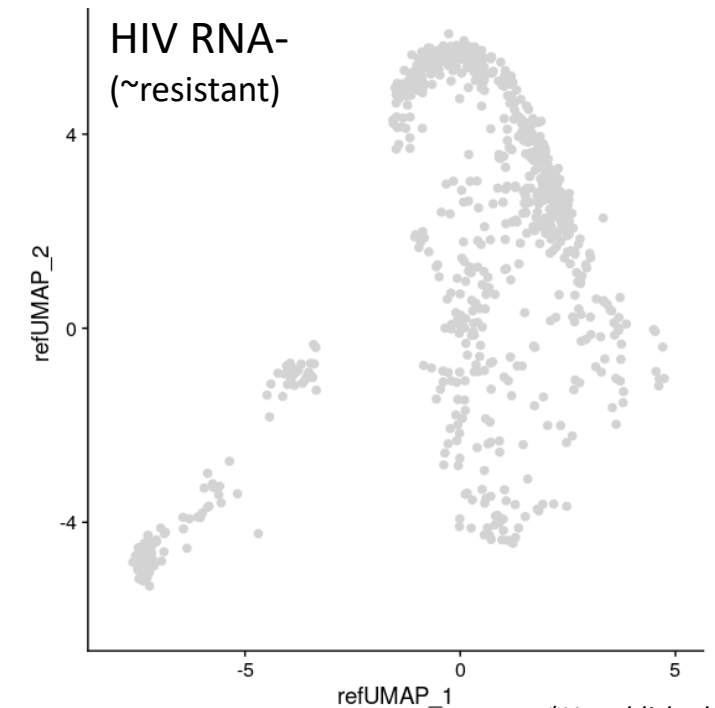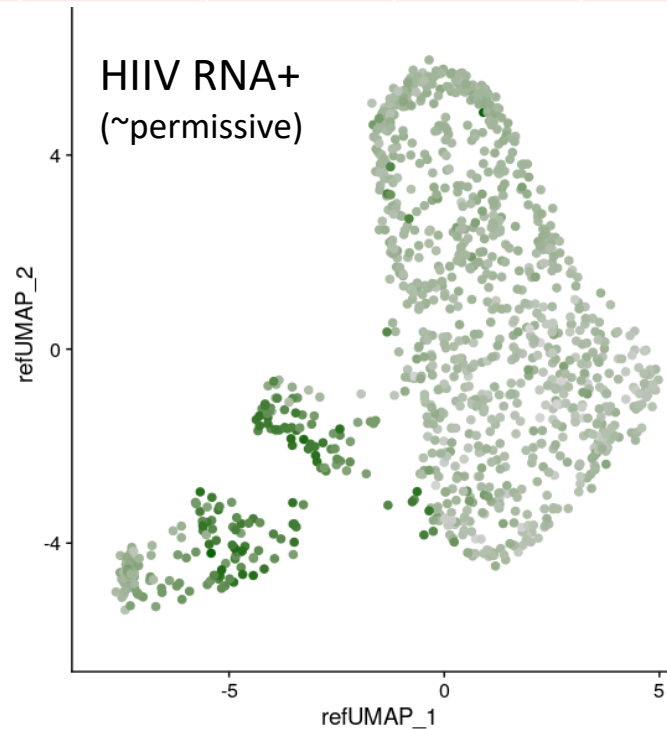| HIV sample (3342 cells)	HIV- [counts]	HIV+ [counts]	HIV- [%]	HIV+ [%]	
~~Proliferating~~	~~56~~	~~2128~~	~~2.6~~	~~97.4~~	Highly permissive
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TCM	314	589	34.8	65.2	

HIIV RNA+
(~permissive)

HIV RNA-
(~resistant)

Unpublished data

Cell (subset) heterogeneity : determinants of HIV permissiveness

HIV-non exposed cells
(1919 cells)

Resistant

Permissive

refUMAP_2

refUMAP_1

Permissive and resistant cells

predicted.celltype.l2
predicted.ref.clusters

DE: 91 genes

DE genes signature (91 genes)

Signature score

Permissive
Resistant

Permissive   Resistant

Unpublished data

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Cell (subset) heterogeneity : determinants of HIV permissiveness

HIV-non exposed cells (1919 cells)

Permissive and resistant cells

DE: 91 genes

DE genes signature (91 genes)

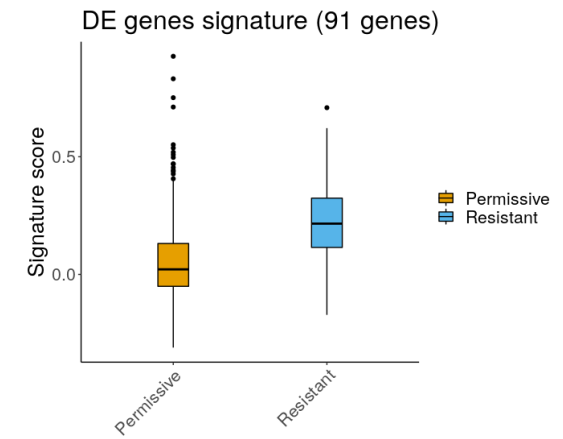negative DE genes signature (57 genes)
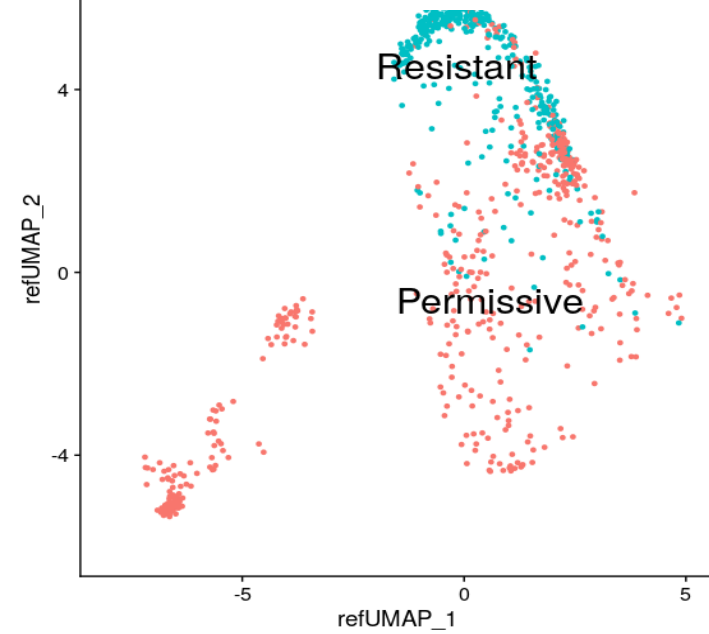
positive DE genes signature (34 genes)
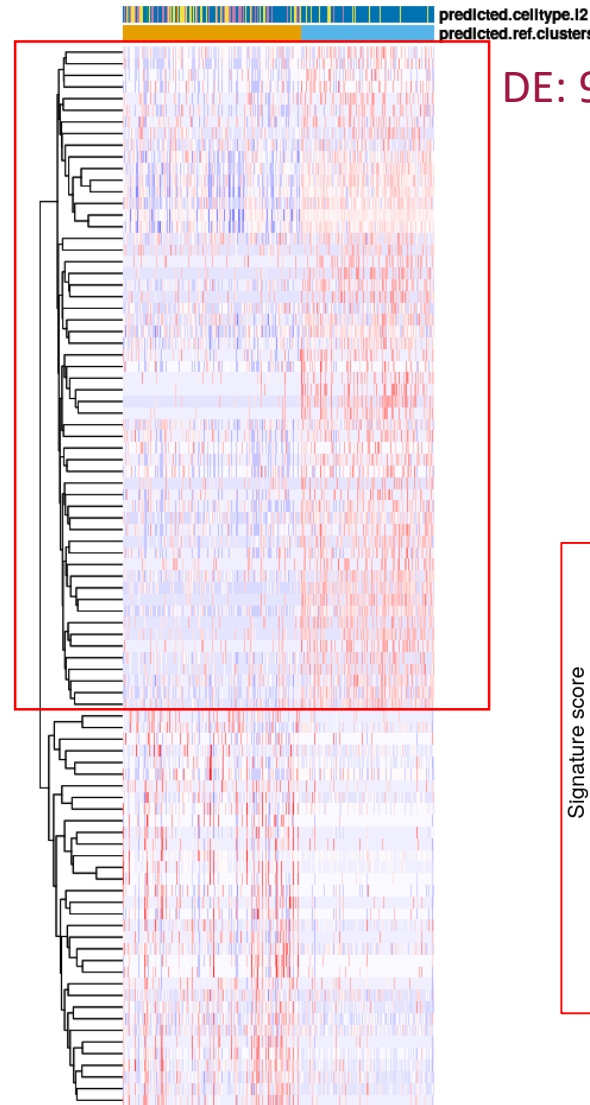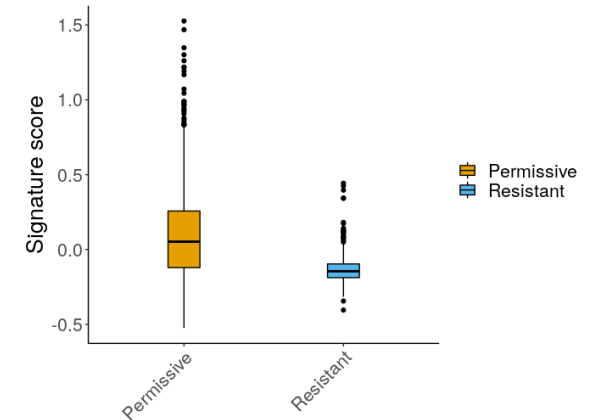
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Cell (subset) heterogeneity : determinants of HIV permissiveness

HIV-non exposed cells
(1919 cells)
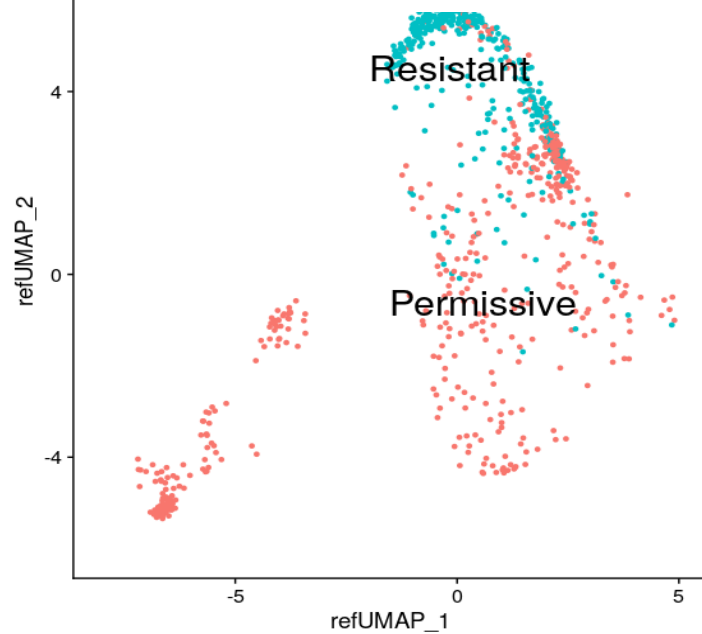
Permissive and resistant cells
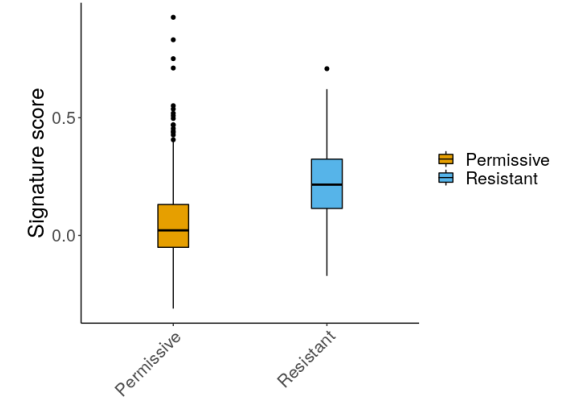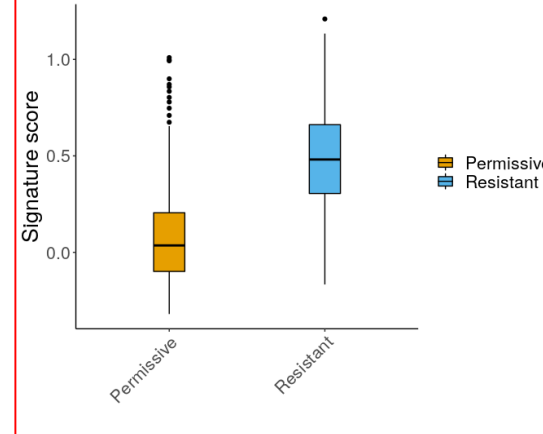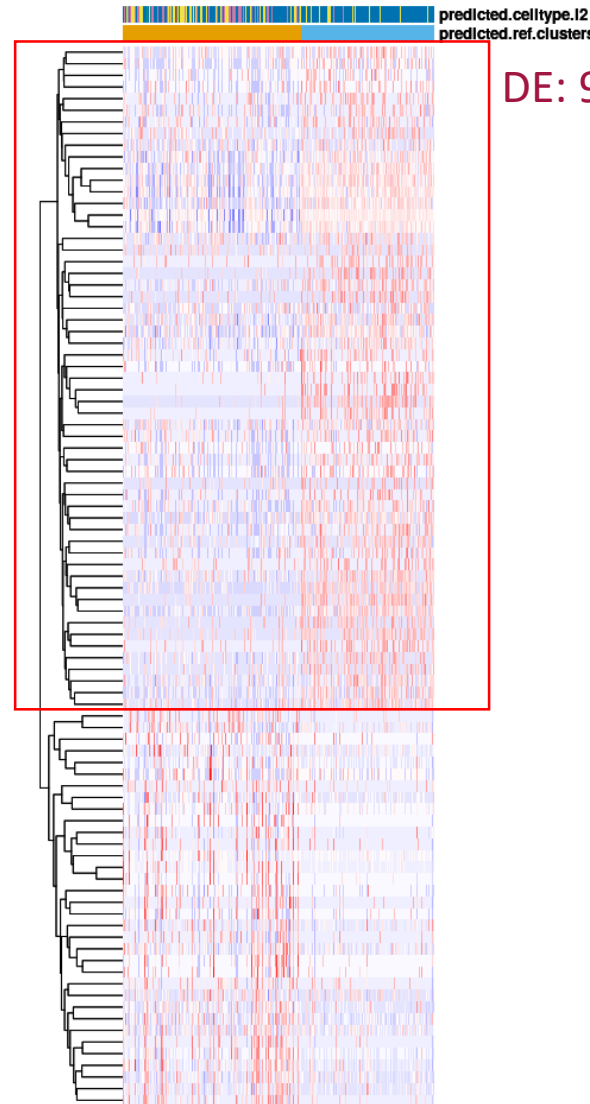
DE: 91 genes

GO_BP ORA all_down_genes
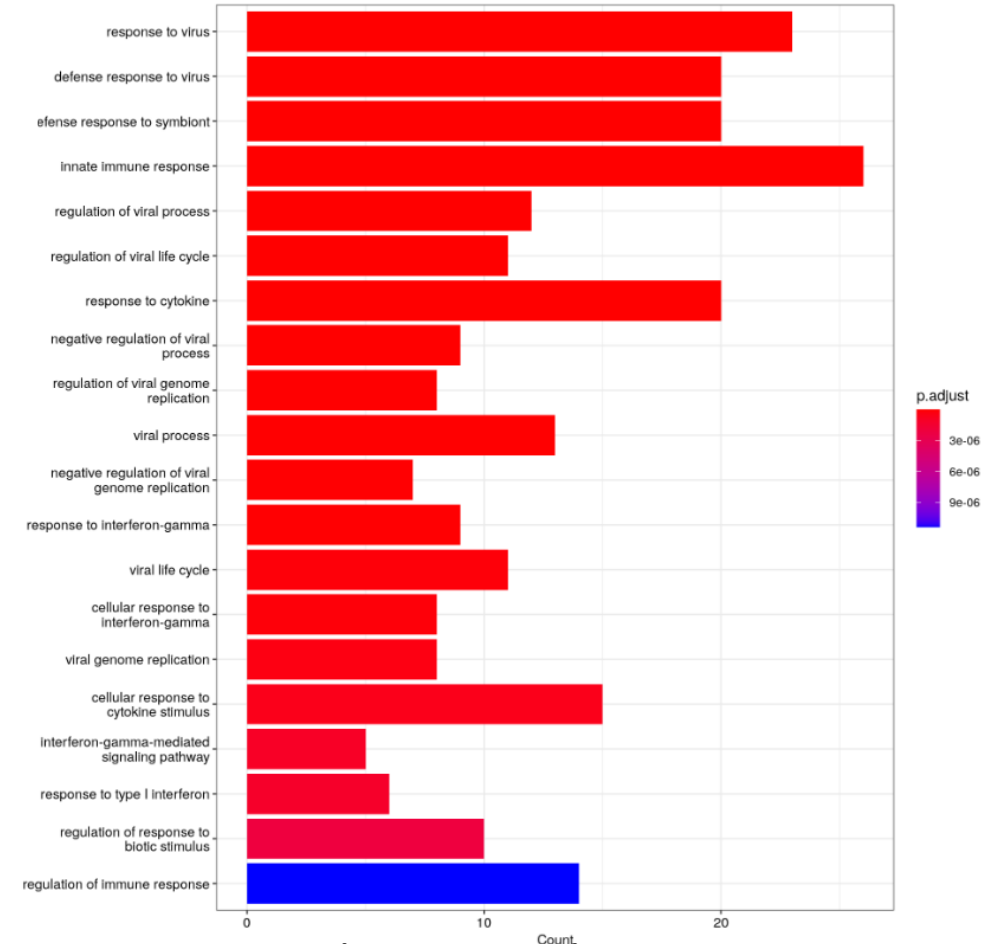
Response to virus, Innate immune response,
Response to IFN-γ
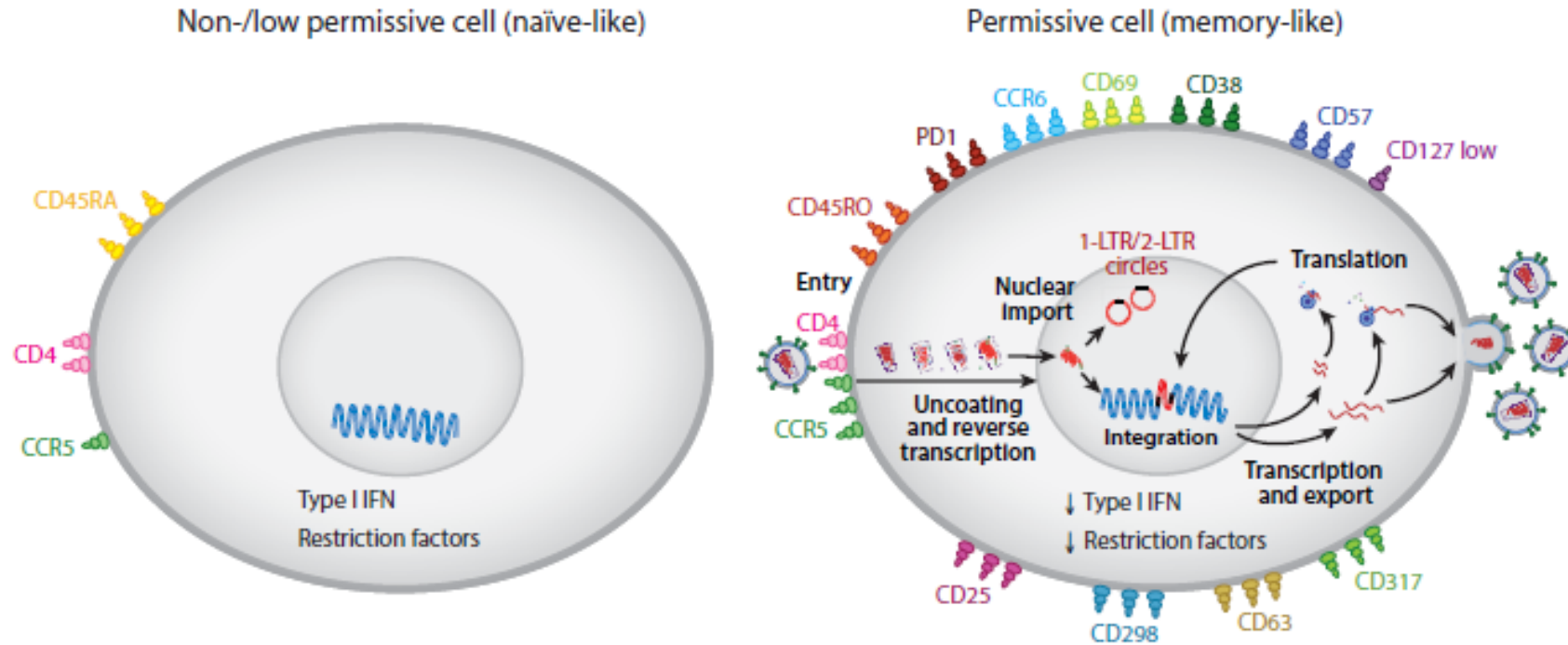
Unpublished data

Brandt et al, Annual Reviews 2020

Signature for HIV permissiveness prediction
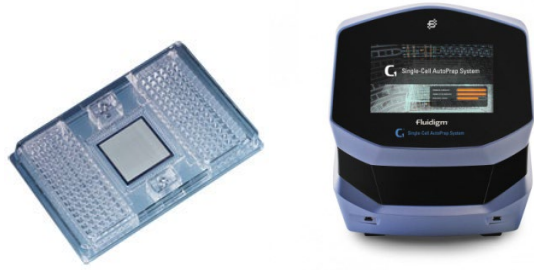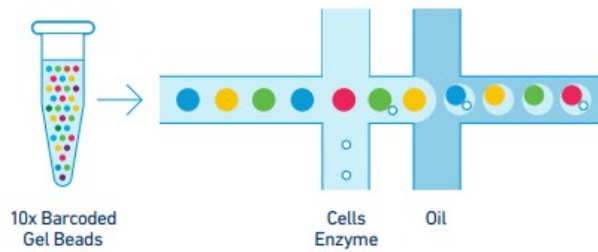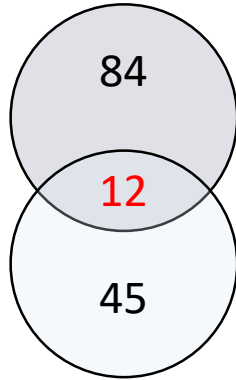
96-gene signature

57-gene signature

96 genes signature

negative DE genes signature (57 genes)

Reference annotations

Reference annotations
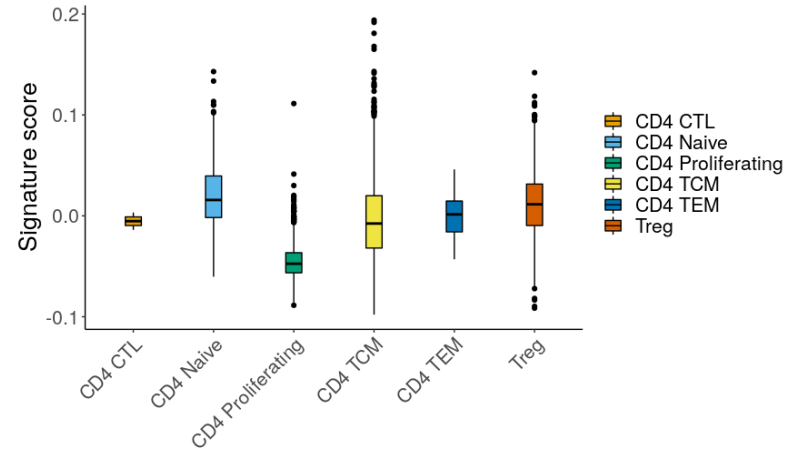
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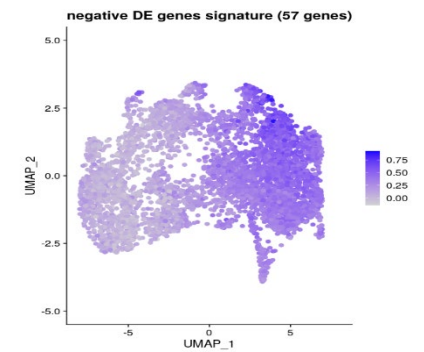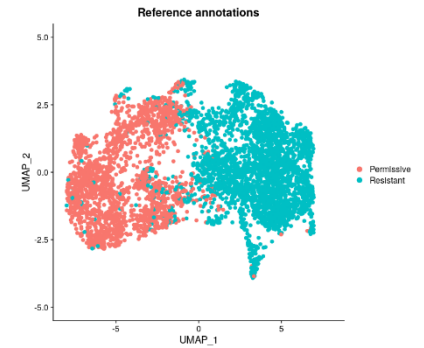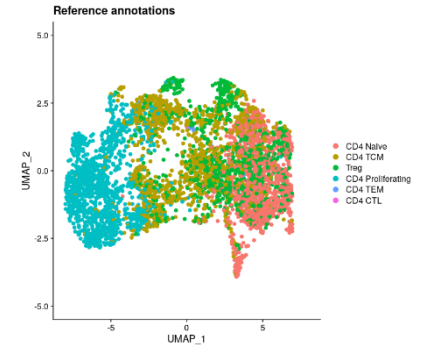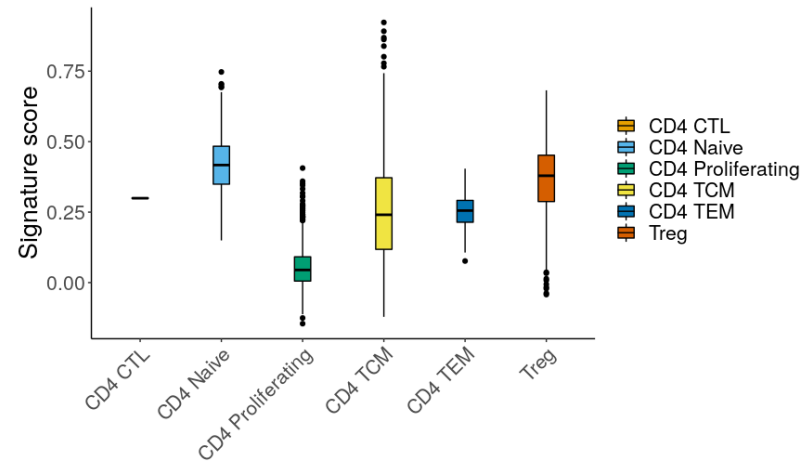
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Signature for HIV permissiveness prediction

96-gene signature

84

12

45

Machine learning

?

HIV Dependency Factors (HDF)

STOP

Restriction Factors (innate immunity)

57-gene signature

10x Barcoded Gel Beads

Cells Enzyme

Oil
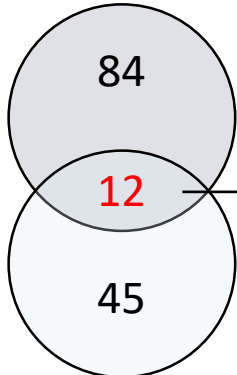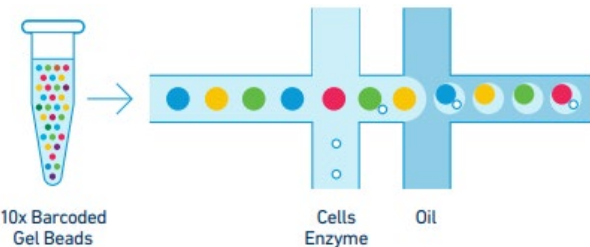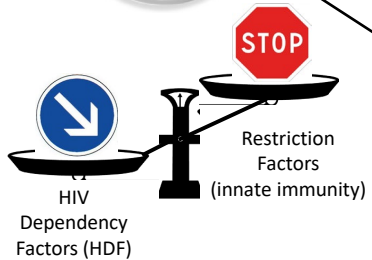
Diversity of outcomes
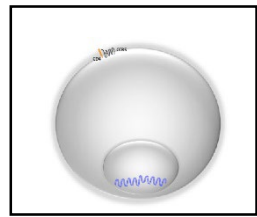
Non infected (resistant)
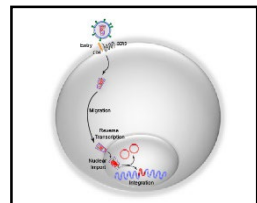
Latently infected
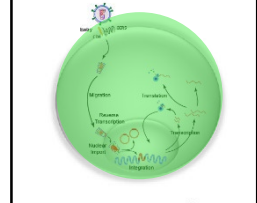
Productively infected (permissive)

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ScRNA-Seq in the field of HIV

Examples of investigation:
Features of the HIV permissive cells
Latent reservoir cells
Latency & reactivation features
Inducible vs non induced phenotype
Host adaptive immune response

Bengsch et al. (104)
Bohn-Wippert et al. (71)
Bradley et al. (72)
Cohn et al. (73)
Coindre et al. (79)
Farhadian et al. (109)
Francis & Melikyan (51)
Golumbeanu et al. (74)
Hansen et al. (98)
Martin-Gayo et al. (111)
Ukah et al. (75)
Zhang et al. (84)

Baxter et al. (78)
Boullé et al. (50)
Bui et al. (77)
Martrus et al. (80)
Pocock et al. (56)

Razooky et al. (46)
Suspène & Meyerhans (54)

Porichis et al. (102)
Petravic et al. (47)

2012 2013 2014 2015 2016 2017 2018 2019 2020

Josefsson et al. (90)
Lusic et al. (94)

Holmes et al. (48)
Marini et al. (49)
Razooky et al. (96)
Rouzine et al. (99)

Aull et al. (97)
Bolton et al. (SIV) (115)
Cavrois et al. (57)
Chen et al. (93)
Corneau et al. (103)
Grau-Expósito et al. (86)
Passaes et al. (82)
Puray-Chavez et al. (52)
Rato et al. (58)
Shan et al. (83)
Stultz et al. (55)
Wiegand et al. (92)
Yucha et al. (89)

Angin et al. (106)
Bruner et al. (85)
Coindre et al. (110)
de Armas et al. (107)
Einkauf et al. (95)
Grau-Expósito et al. (81)
Lee et al. (91)
Lucic et al. (53)
Nguyen et al. (105)
Pardons et al. (88)
Telwatte et al. (76)

Topics
Permissiveness/HIV life cycle
Latency/reservoir
Host response

Svensson et al, Nature Protocols 2018

Brandt et al, Annual Reviews 2020

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Nucleus

Genome → scDNA-Seq, SGS, SNS, SCI-seq

Epigenome → scATAC-Seq, scBS-seq, snmC-seq, sci-MET, scRRBS, scChIP-seq, scHI-C-seq, scNOMe-seq

Transcription

Transcriptome → scRNA-Seq, FISH-Flow, sc RT-qPCR

RNA modifications

Epitranscriptome → NA

Nuclear Export

Translatome → NA

Translation

Proteome → CyTOF, CITE-seq, SCoPE-MS

Protein modifications

Cytoplasm

Modified proteome → SCoPE-MS

Brandt et al, Annual Reviews 2020

Bengsch et al. (104)
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et al. (86)
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et al. (52)

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Yucha et al. (89)

Aamer et al, PLoS Pathog 2020,
Gantner et al, Nat Comm 2020
Léon-Riverta et al, mBio 2020
Liu et al, Sci Transl Met 2020 (Sort-Seq)
Wang et al, Emerg Microbes Infect 2020

Rao et al, Nat Comm 2021
Cole et al, Nat comm 2021
Puertas et al, mBio 2021
Ratnapriya et al, Cell Rep 2021
Sannier et al, Cell Rep 2021
De Armas, Frontiers Immunol 2021
Mboyne et al, PLoS Pathog 2021
Saluzzo et al, Immunity 2021
Simonetti et al, J Clin Invest 2021
Lian et al, Sci Transl Med 20021)
Matszuda et al, Cell Rep Methods, 2021
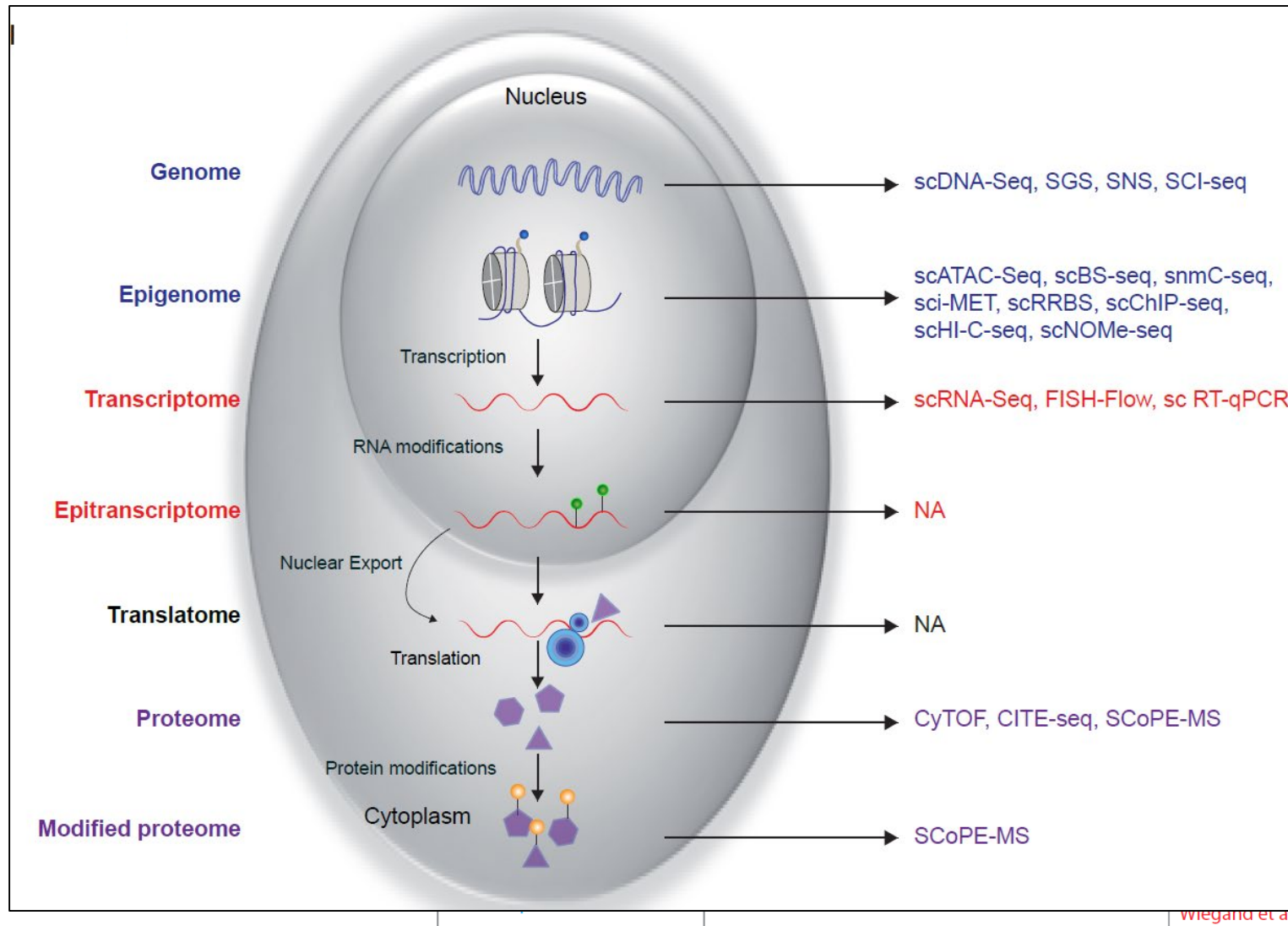Mellors et al, Sci Adv 2021

Claireaux et al, Nat Comm 2022
Dalecki et al, J virol 2022
Cao et al, Frontier Immunol 2022
Lin et al, Virulence 2022
Ramirez et al, Nat Comm 2022
Falcinelli, J Clin Invest 2022
Lindkvist et al, PLoS Pathog 2022
Janssens et al, mBio, 2022
Lee et al, Nature 20221
Duette et al, J Clin Invest 2022
Collora et al, Immunity 2022 (ECCITE-Seq)
Einkauf et al, Cell 2022 (PRIP-Seq)
...

Nucleus

Genome → scDNA-Seq, SGS, SNS, SCI-seq

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Cytoplasm

Modified proteome → SCoPE-MS

Brandt et al, Annual Reviews 2020

Examples of investigation:
Features of the HIV permissive cells
Latent reservoir cells
Latency & reactivation features
Inducible vs non induced phenotype
Host adaptive immune response
Genotyping
Integration landscape
LRA susceptibility
Clonality / Clonal expansion
Cell persistence and survival
Longitudinal analysis /dynamics
Spatial analysis
...

Bengsch et al. (104)
Bohn-Wippert et al. (71)
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Cohn et al. (73)
Coindre et al. (79)
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Martin-Gayo et al. (111)
Ukah et al. (75)
Zhang et al. (84)

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Angin et a
V) (115)   Bruner et al. (85)   Lin et al, Virulence 2022
7)   Coindre et al. (110)   Ramirez et al, Nat Comm 2022
              de Armas et al. (107)   Falcinelli, J Clin Invest 2022
103)   Einkauf et al. (95)   Lindkvist et al, PLoS Pathog 2022
t al. (86)   Grau-Expósito et al.   Janssens et al, mBio, 2022
2)   Lee et al. (91)   Lee et al, Nature 20221
t al. (52)   Lucic et al. (53)   Simonetti et al, J Clin Invest 2021
              Nguyen et al. (105)   Lian et al, Sci Transl Med 20021)
              Pardons et al. (88)
              Telwatte et al. (76)

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Duette et al, J Clin Invest 2022
Collora et al, Immunity 2022 (ECCITE-Seq)
Einkauf et al, Cell 2022 (PRIP-Seq

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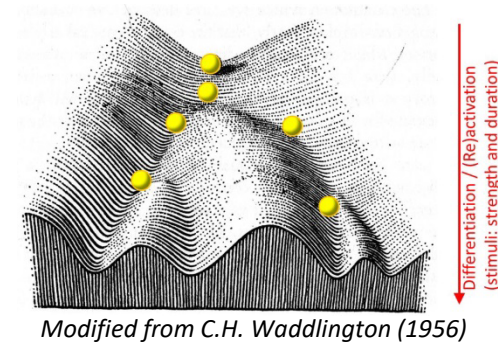Faculté de biologie et de médecine

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- Flow Cytometry Facility (FCF), University of Lausanne / CHUV

Institute of Microbiology

Modified from C.H. Waddlington (1956)