

Optimising Allogeneic Therapeutic Transduction at Scale

Marta Barisa
Senior Fellow UCL

8 Nov 2022, Cell UK



NHS

**Great Ormond Street
Hospital for Children**
NHS Foundation Trust

Cell Therapy at UCL GOS ICH

Immunotherapy Research at Great Ormond St



**Experimental
Paediatric
Oncology Group**

John Anderson

Honorary Consultant Paediatric
Oncologist

GOSH

Paediatric solid cancers

- Neuroblastoma
- Brain cancer(s)

Autologous manufacture

$\alpha\beta$ -CAR-T



**Allogeneic Innate
Immunotherapy
group**

Jonathan Fisher

Paediatric Oncologist

UCLH

Paediatric and adult solid cancers

- Sarcoma
- Neuroblastoma

Allogeneic manufacture

$\gamma\delta$ -CAR-T
Armoured- $\gamma\delta$ -T

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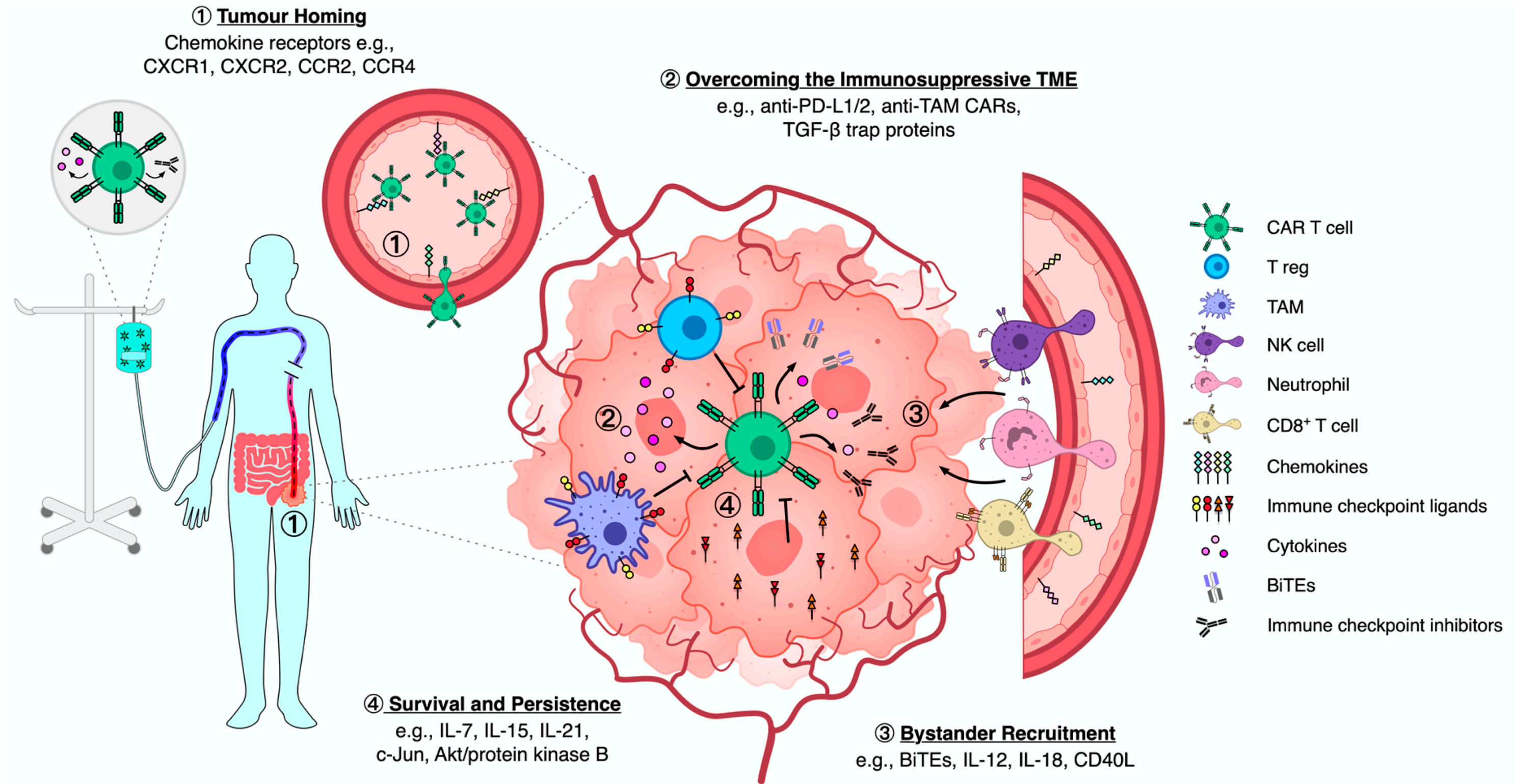
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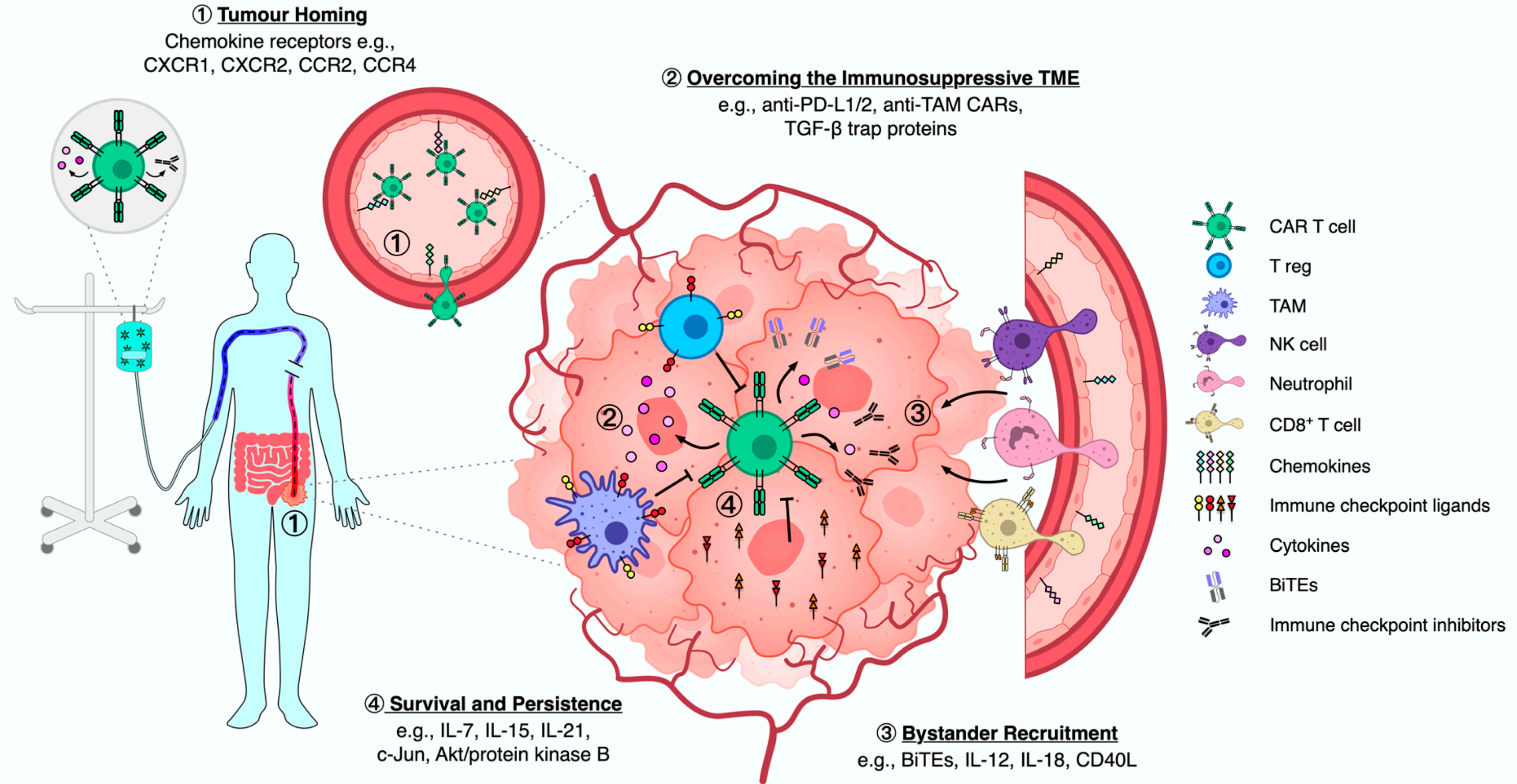
Gene engineering is a requirement

Unmodified lymphocytes can't compete with suppressive TME



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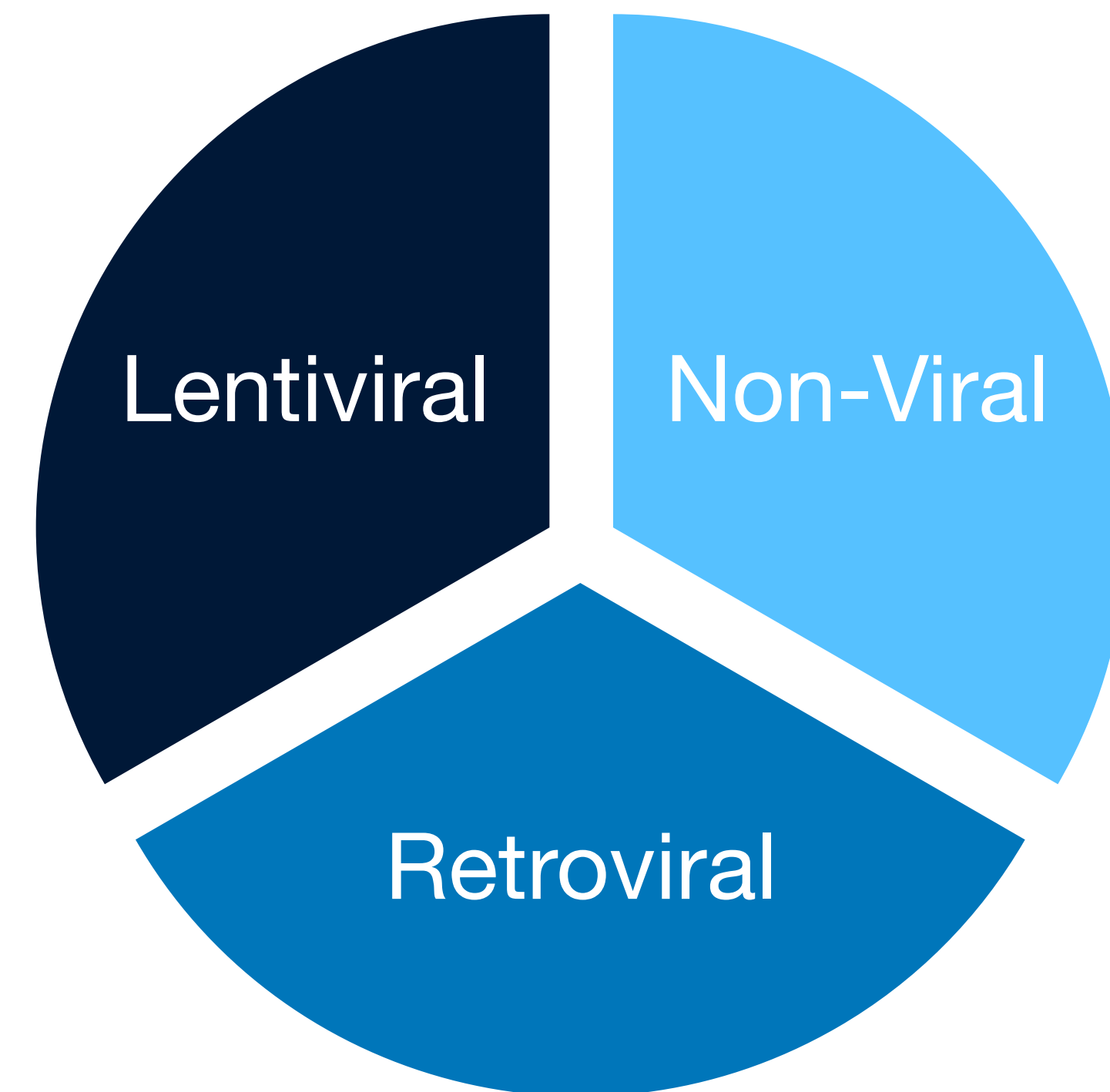
$\gamma\delta$ T

NK

What does “good” transduction look like?

Considerations for cell therapy manufacture

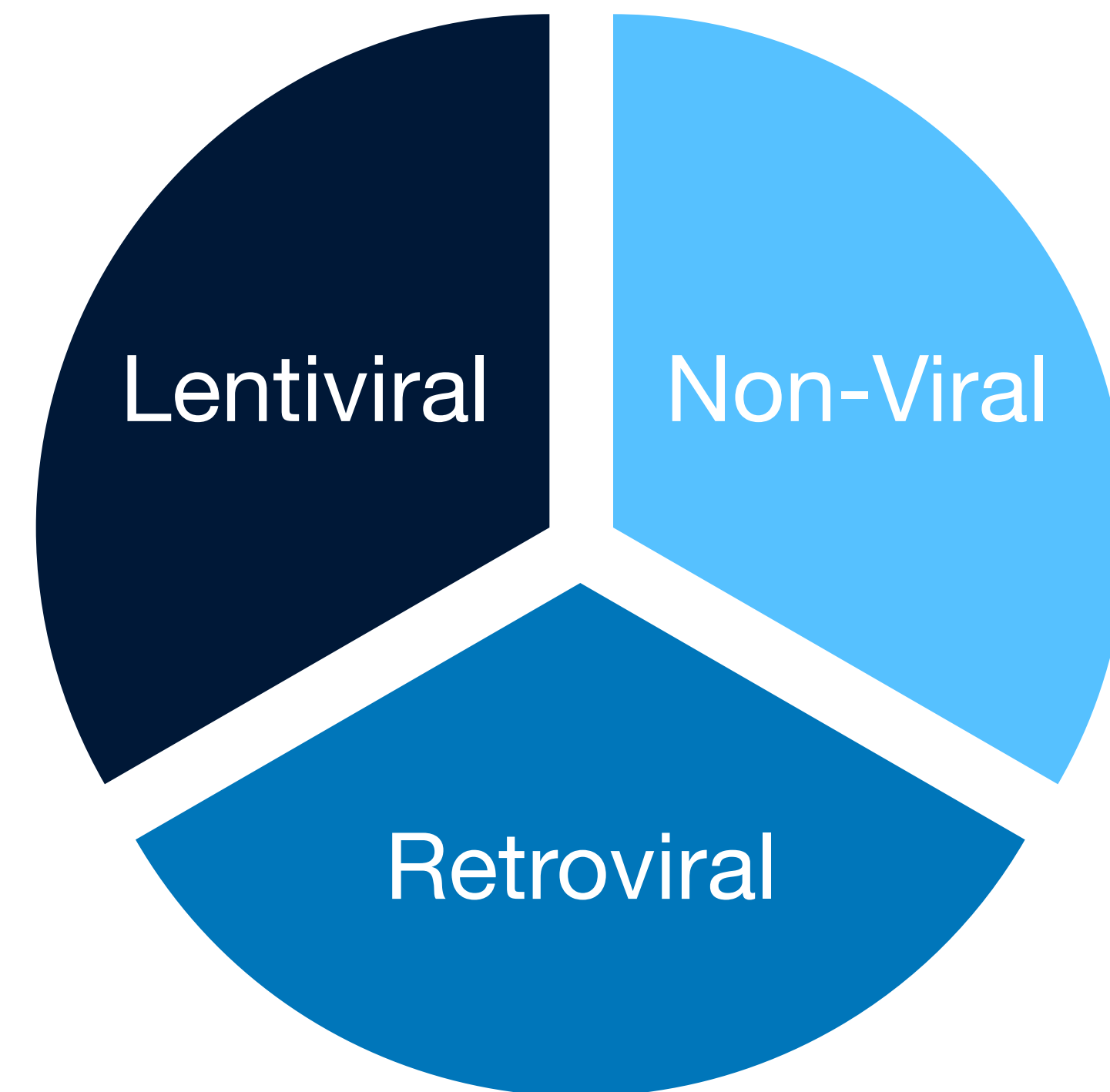
- Regulatory and clinical experience
- Scalability
- Product viability & health
- Large construct size
- Acceptable safety profile
- Reasonable cost
- Easy concentration & storage
- High transduction efficiency



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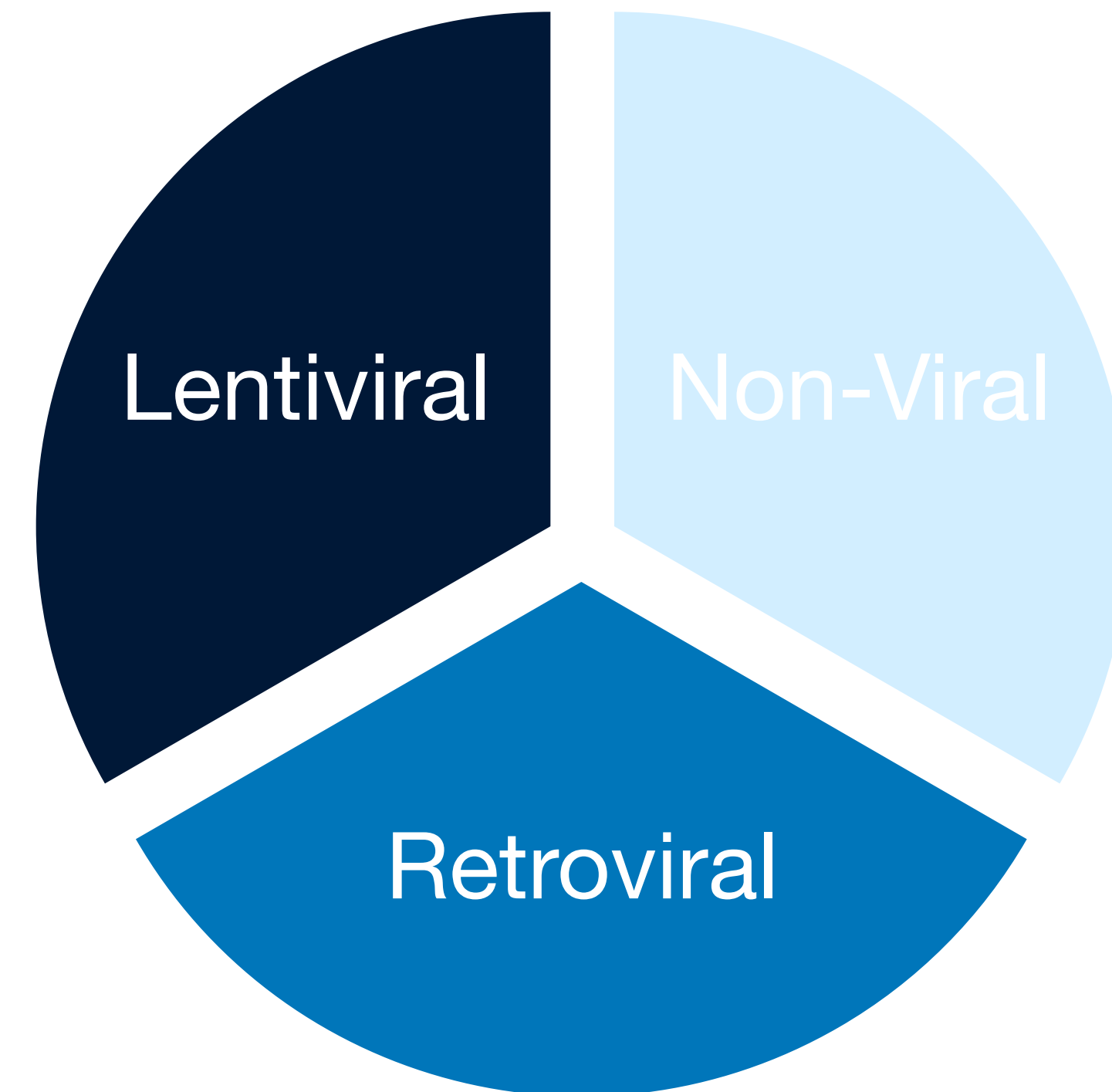
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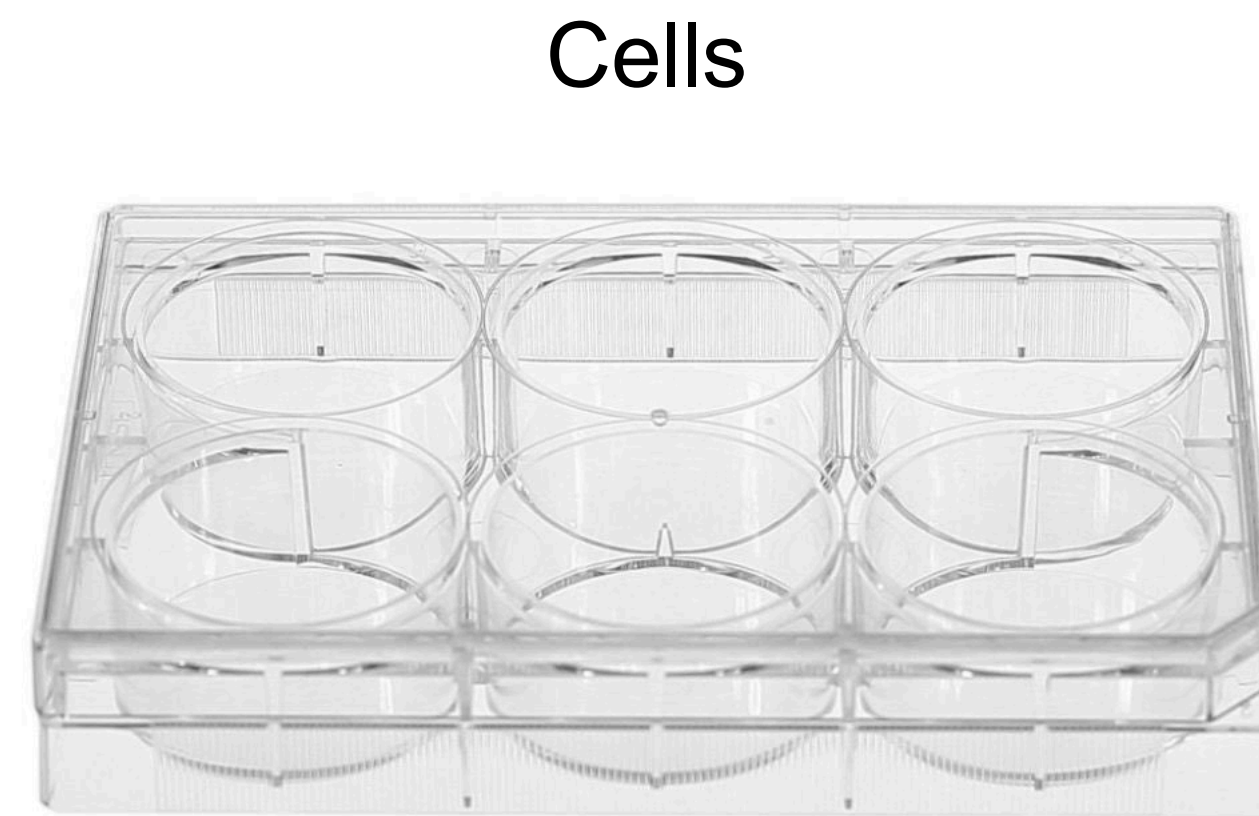
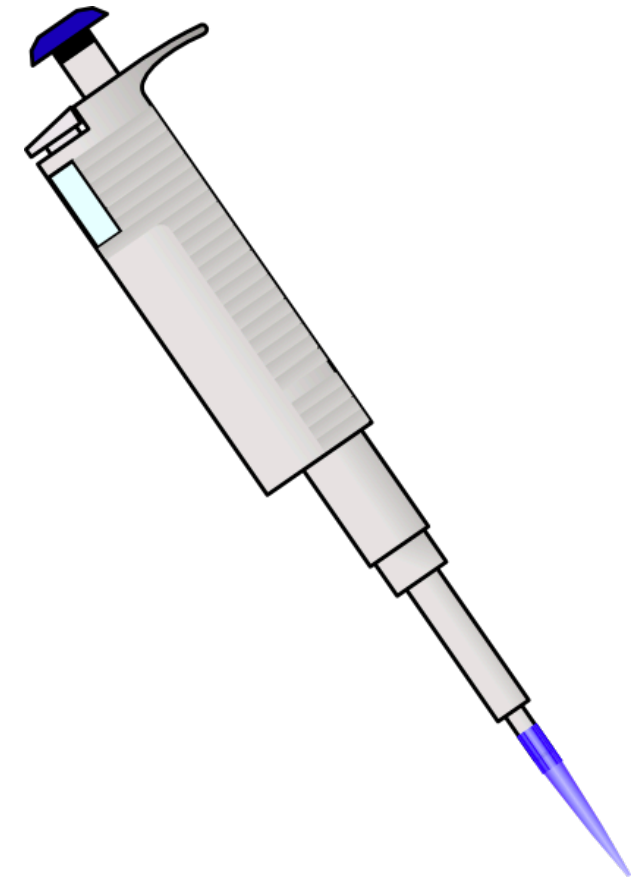
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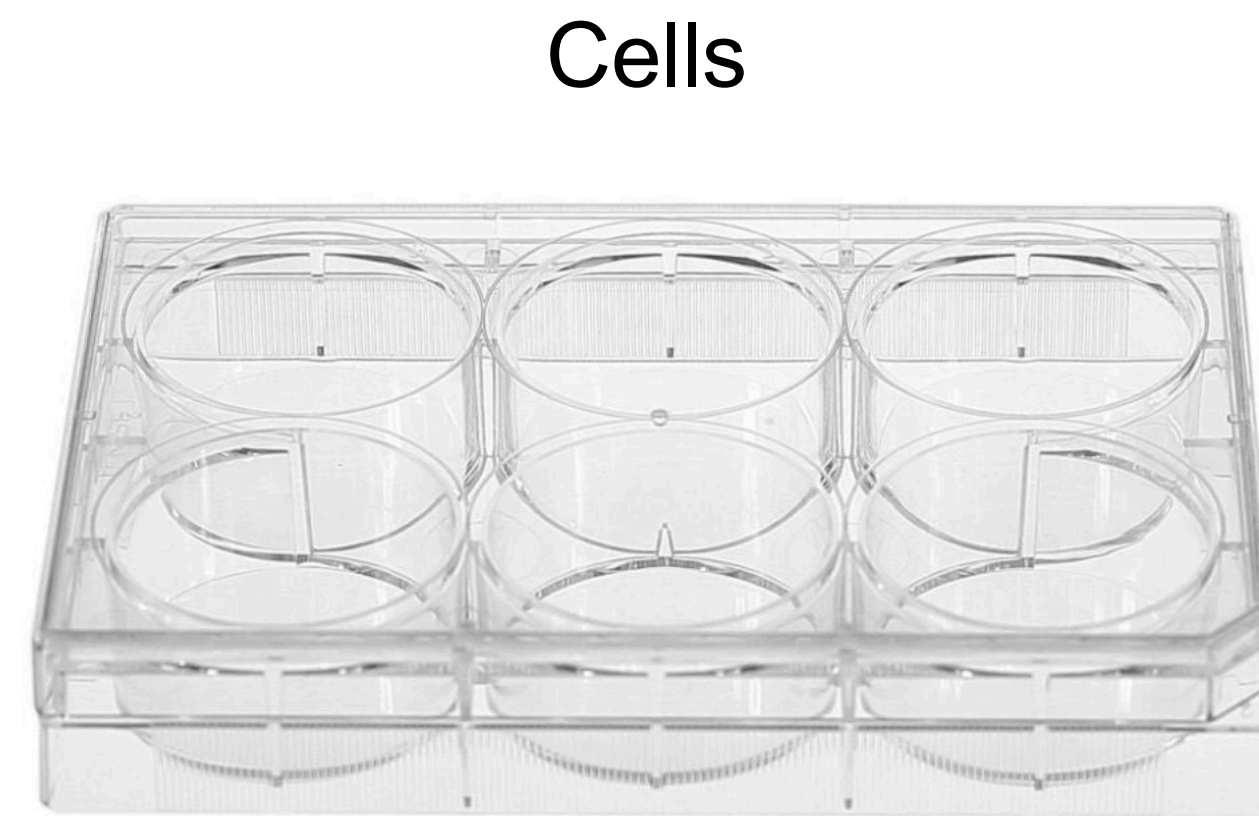
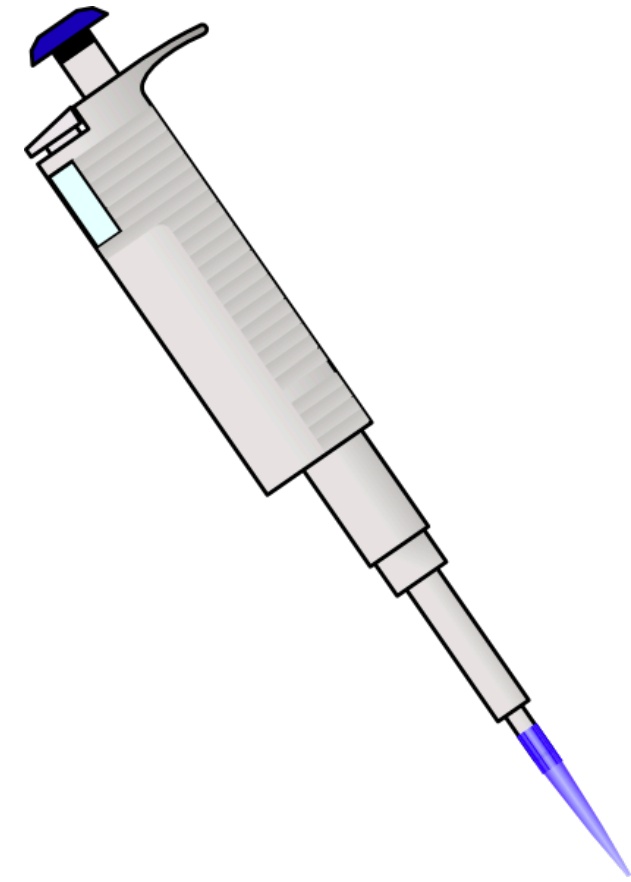
Lab reality



Our favourite solutions = moving liquids around

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Lab reality

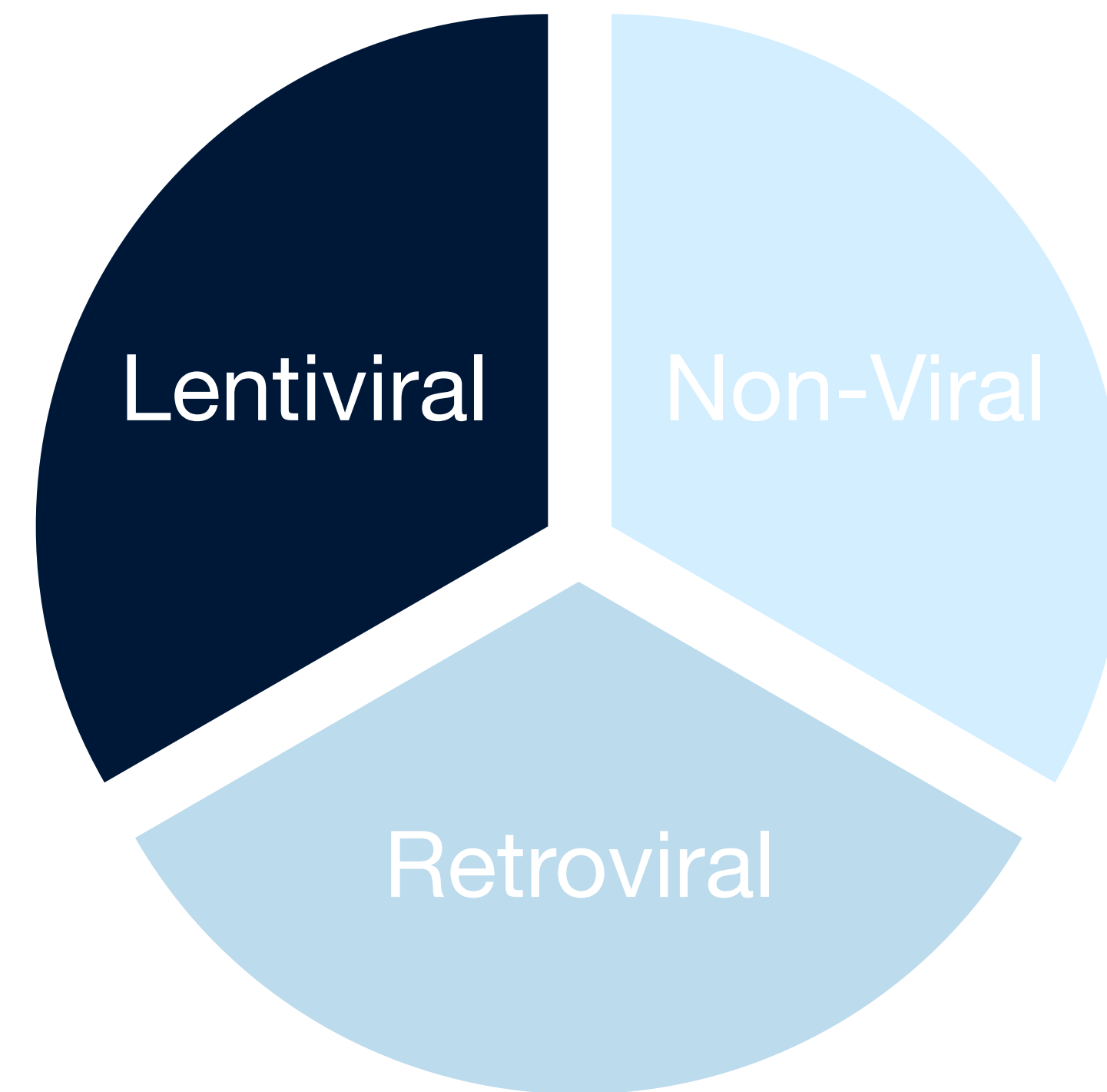


***9 of 10 times - the R&D
manufacturing transduction setup
will inform the clinical trial***

What does “good” transduction look like?

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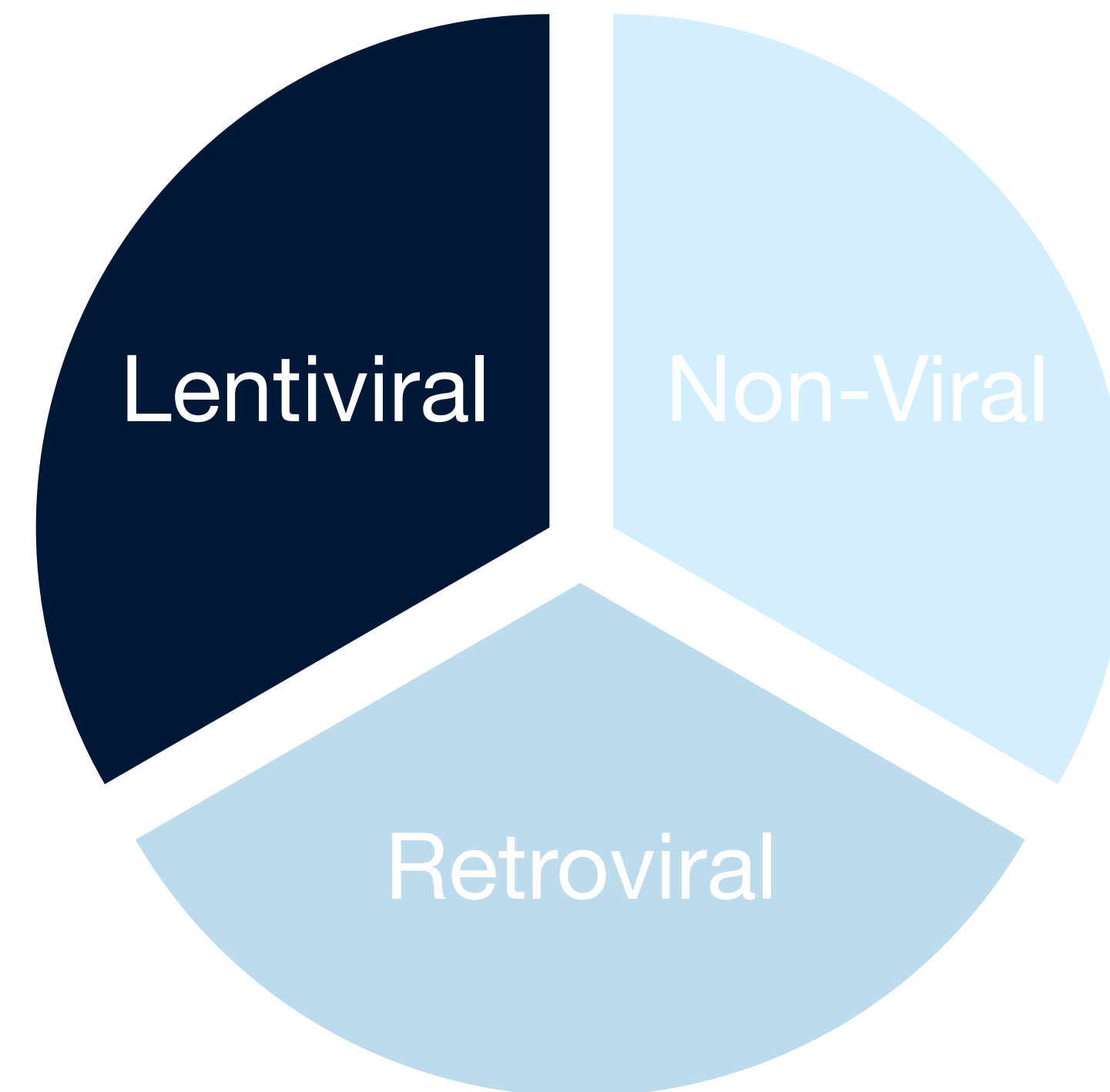
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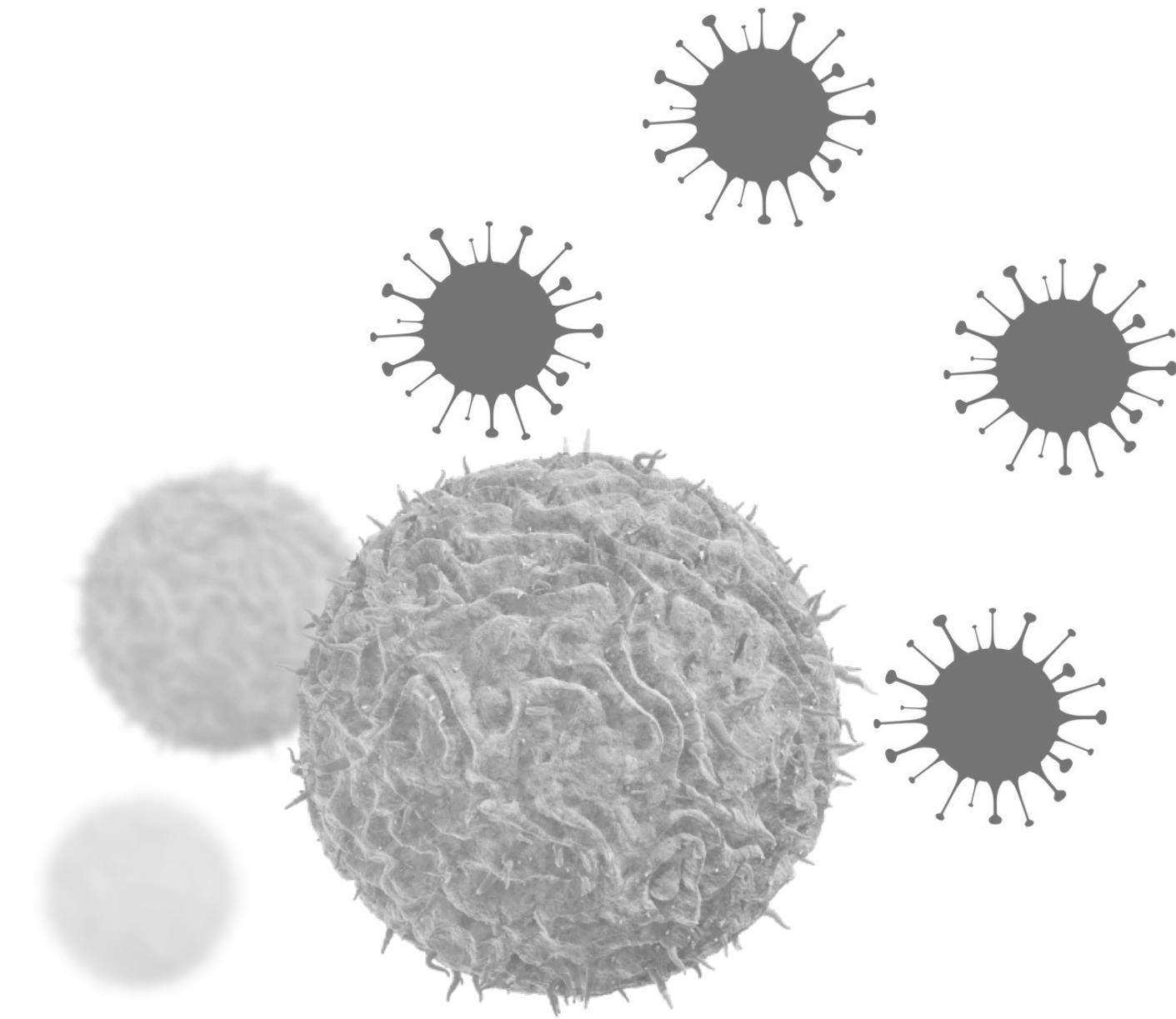
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Transducing Classic Peripheral $\alpha\beta$ T cells

Optimisation is always required

- Codon sequence
- Insert size?
- MOI
- Activation
- Transduction efficiency vs toxicity
- Automated or manual?
- Cost

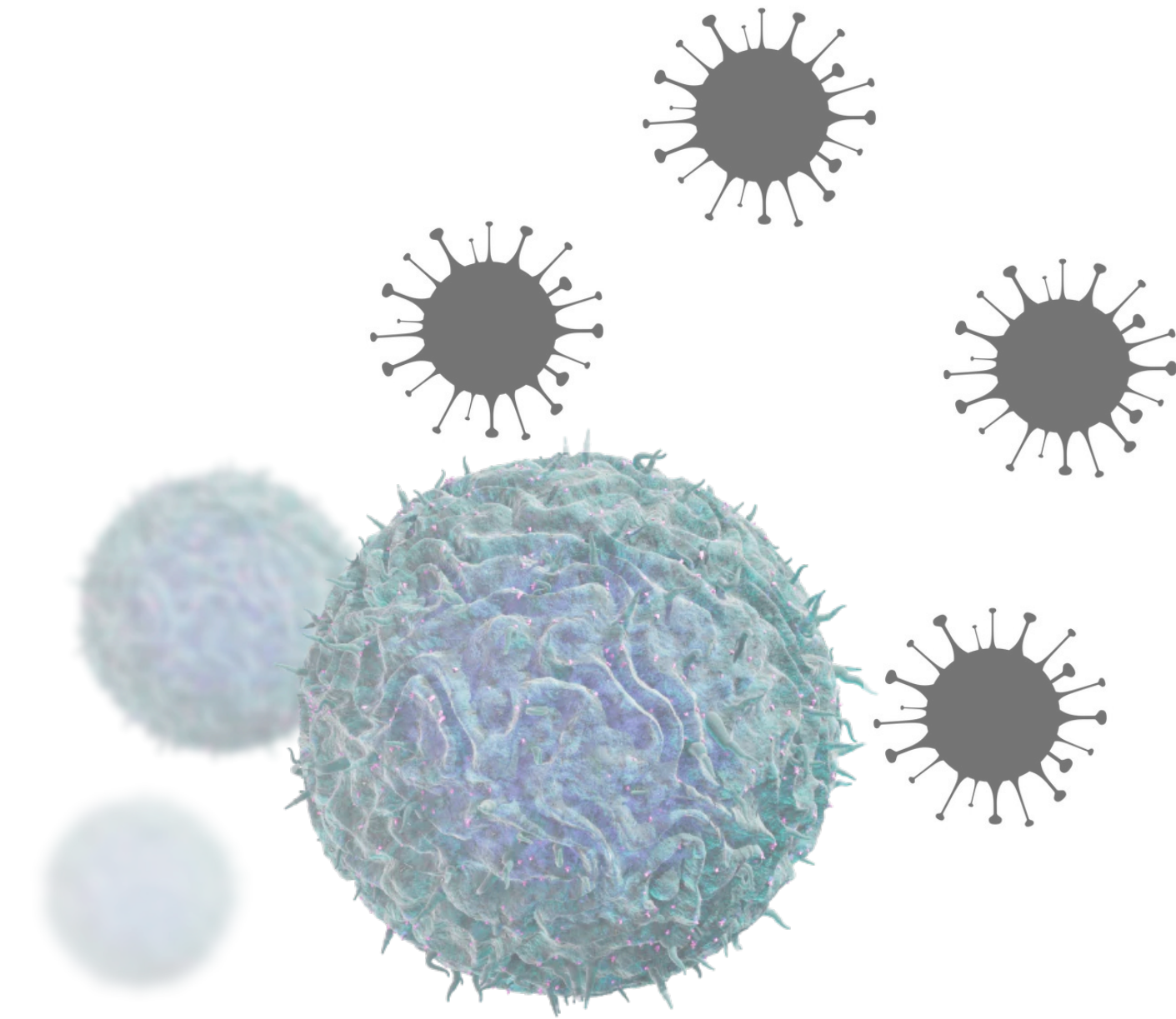


Leukapheresate
 $\alpha\beta$ T cell

Transducing innate lymphocytes is harder

Lentiviral options are few and far between

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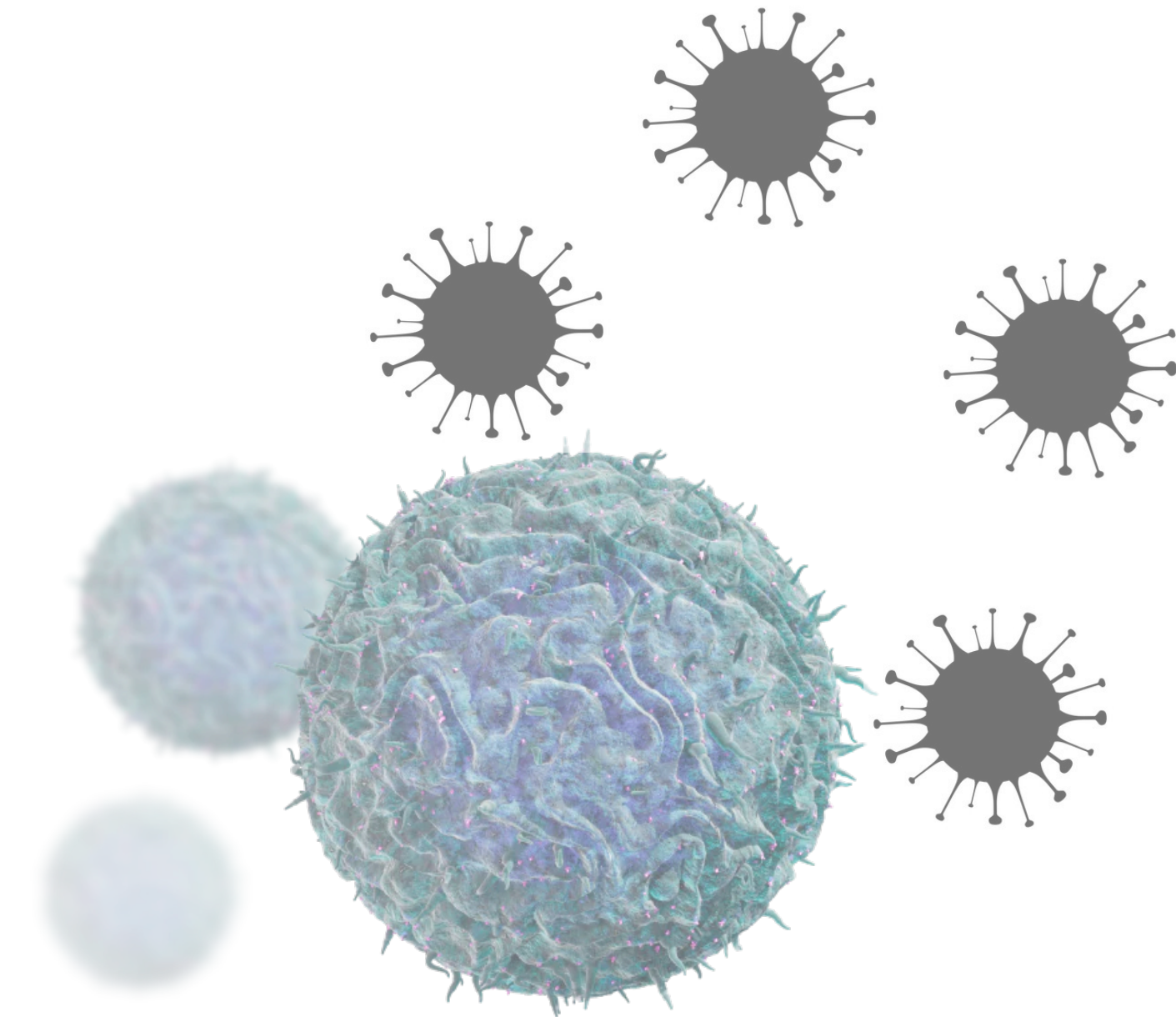
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NK cell or $\gamma\delta$ T cell

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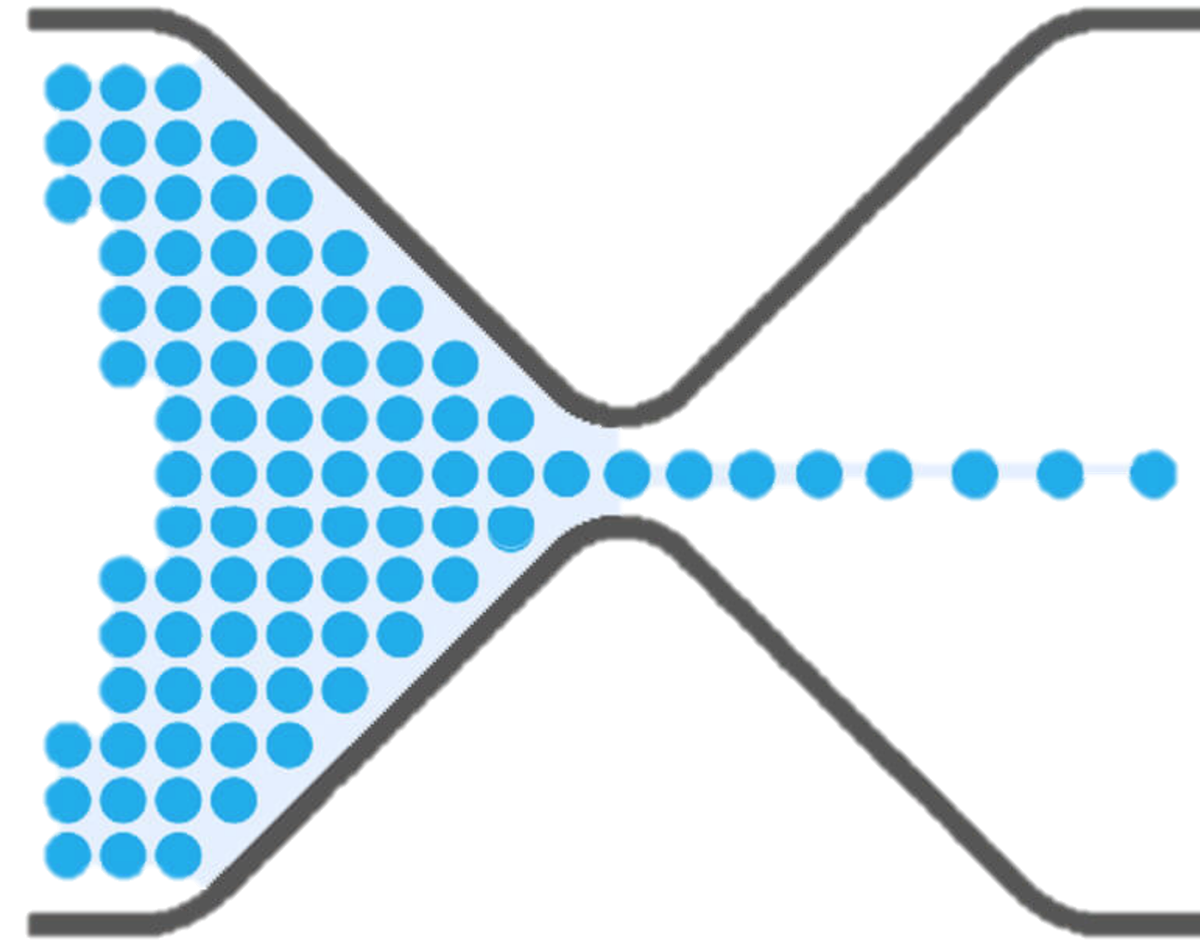
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***Requirement for
transduction boosters
and spinoculation***



Leukapheresate
NK cell or $\gamma\delta$ T cell

Transducing innate lymphocytes is harder



Optimal lentiviral innate lymphocyte transductions are a major bottleneck for allogeneic cell therapy development

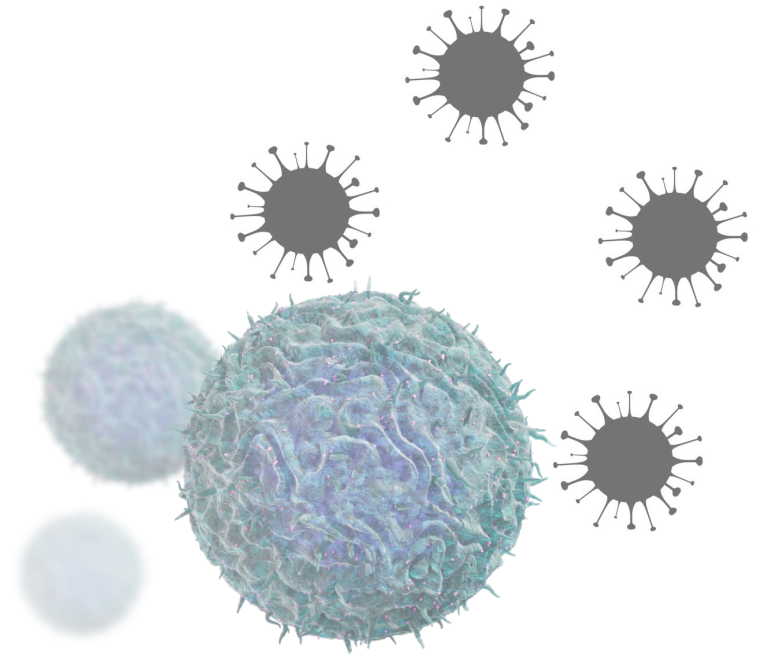
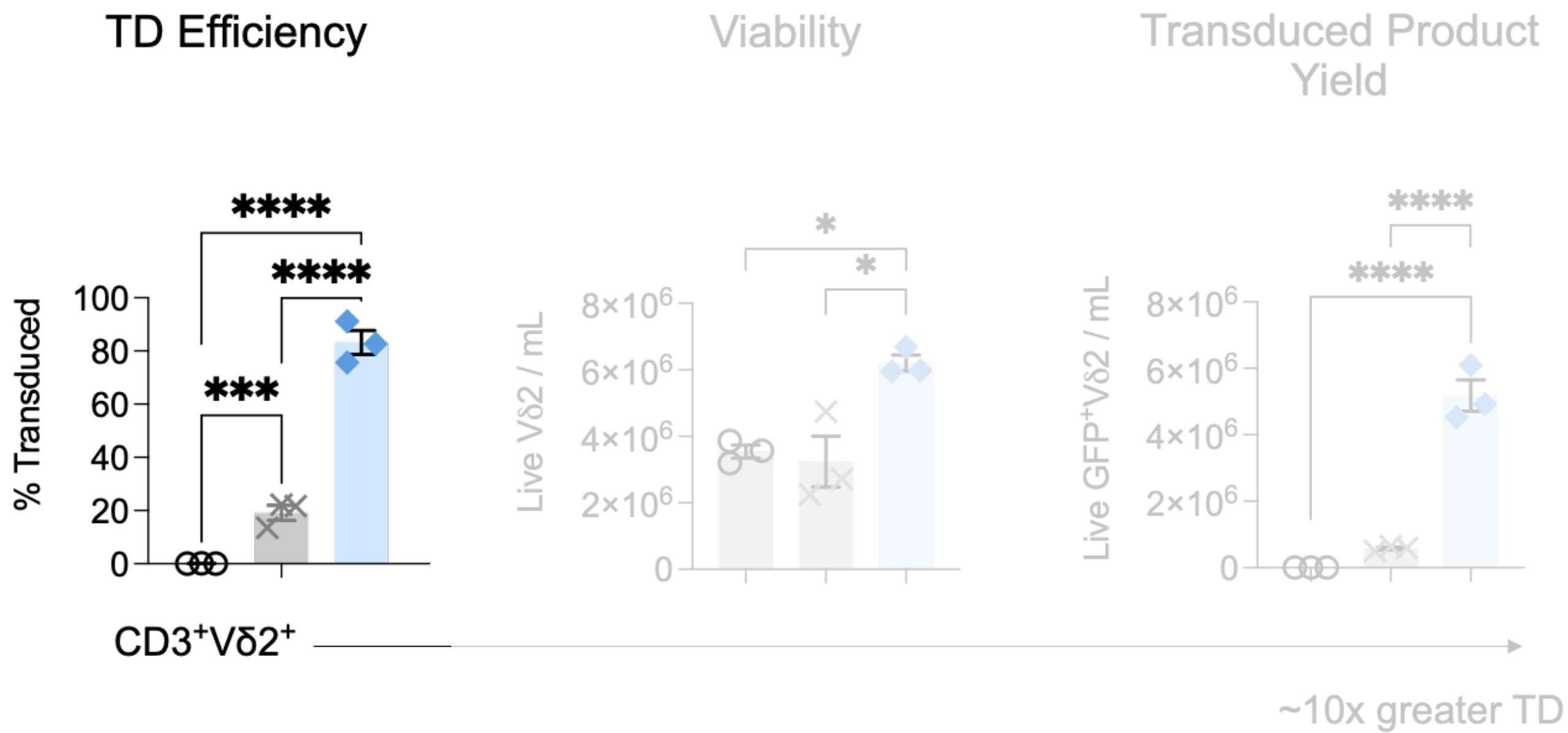
Our Journey to 'UCL Lenti'

An alternative to standard approaches

Test on Vγ9Vδ2 at standard MOI 15:

- Mock TD
- × VSVg
- ◆ UCL Lenti

Bicistronic
No *spinoculation*
No *enhancers*



Leukapheresate
γδT cell

Enhancers decreased product viability without significant impact on transduction efficiency

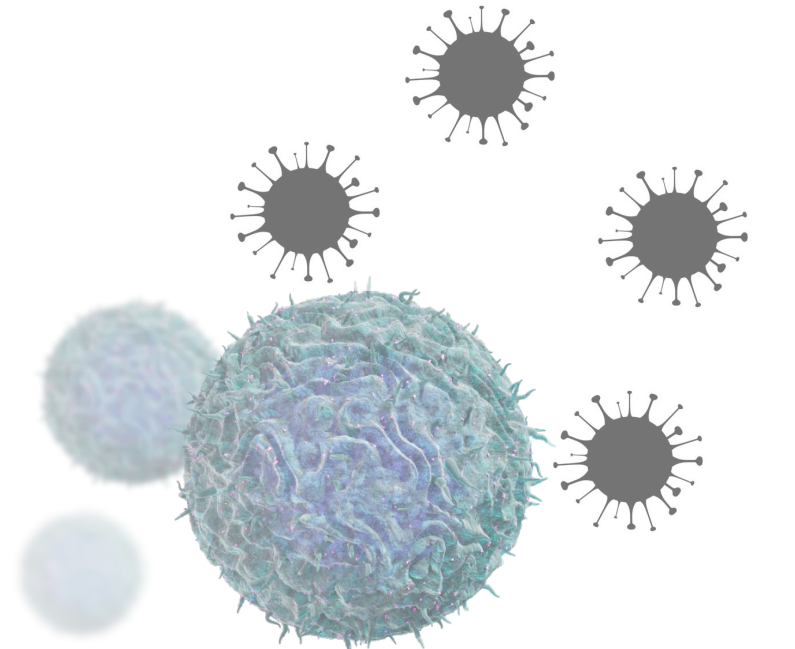
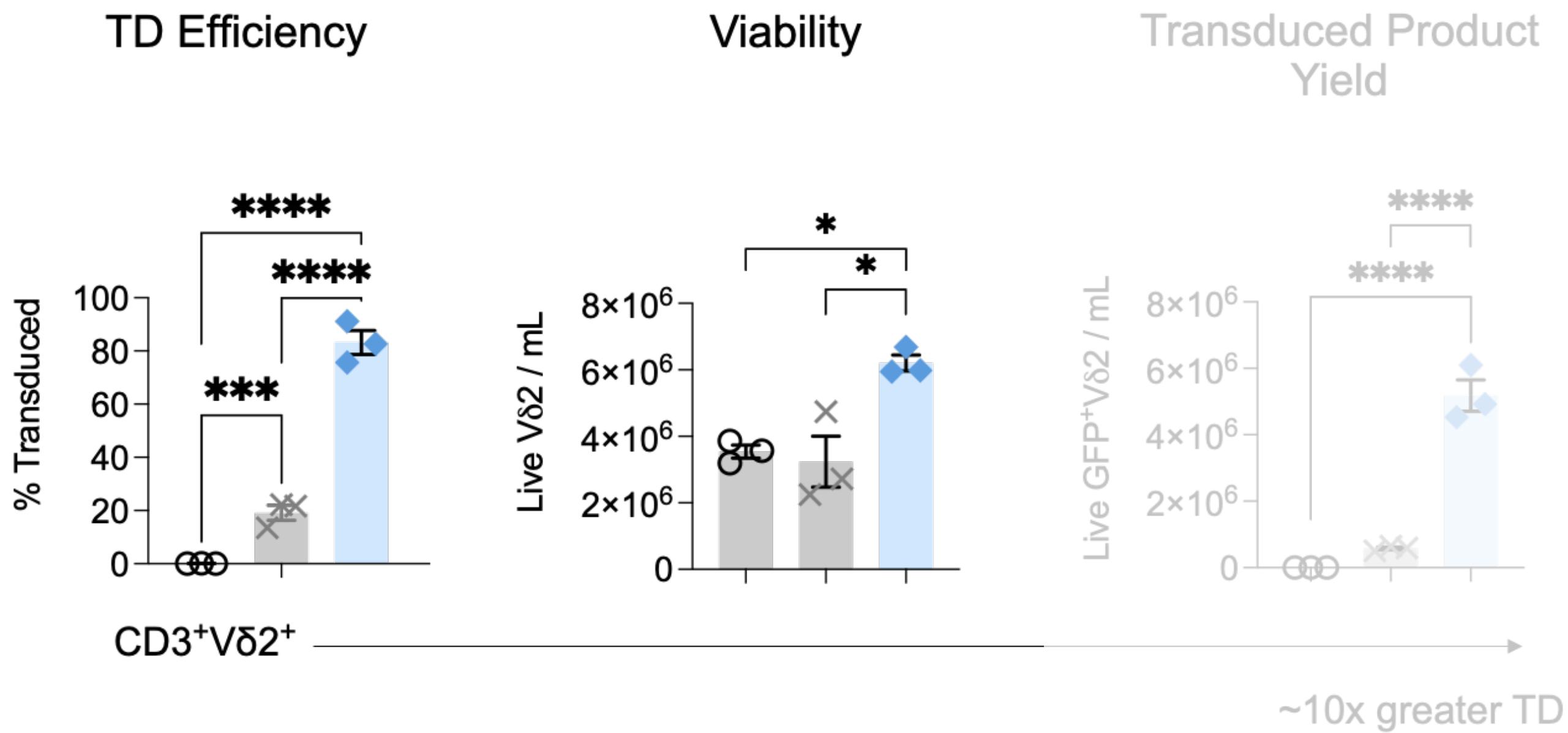
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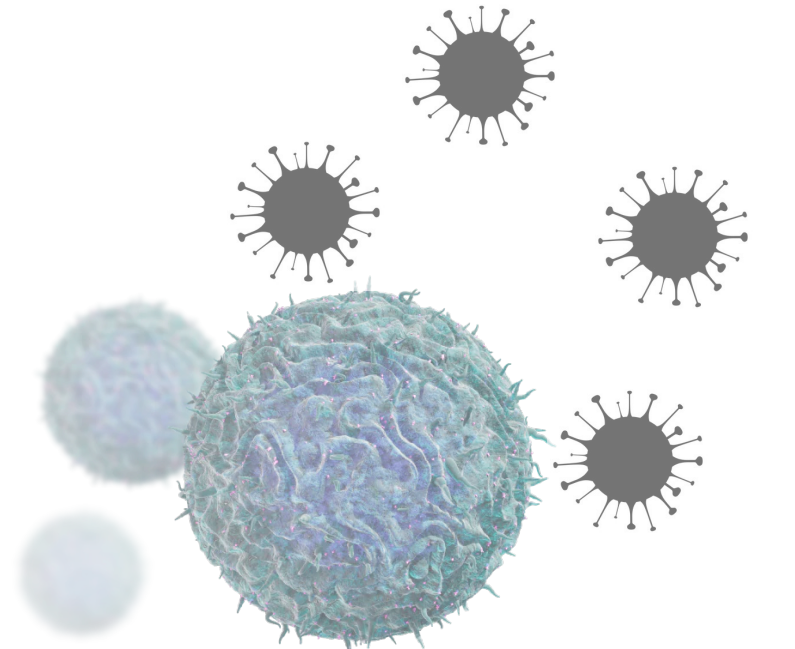
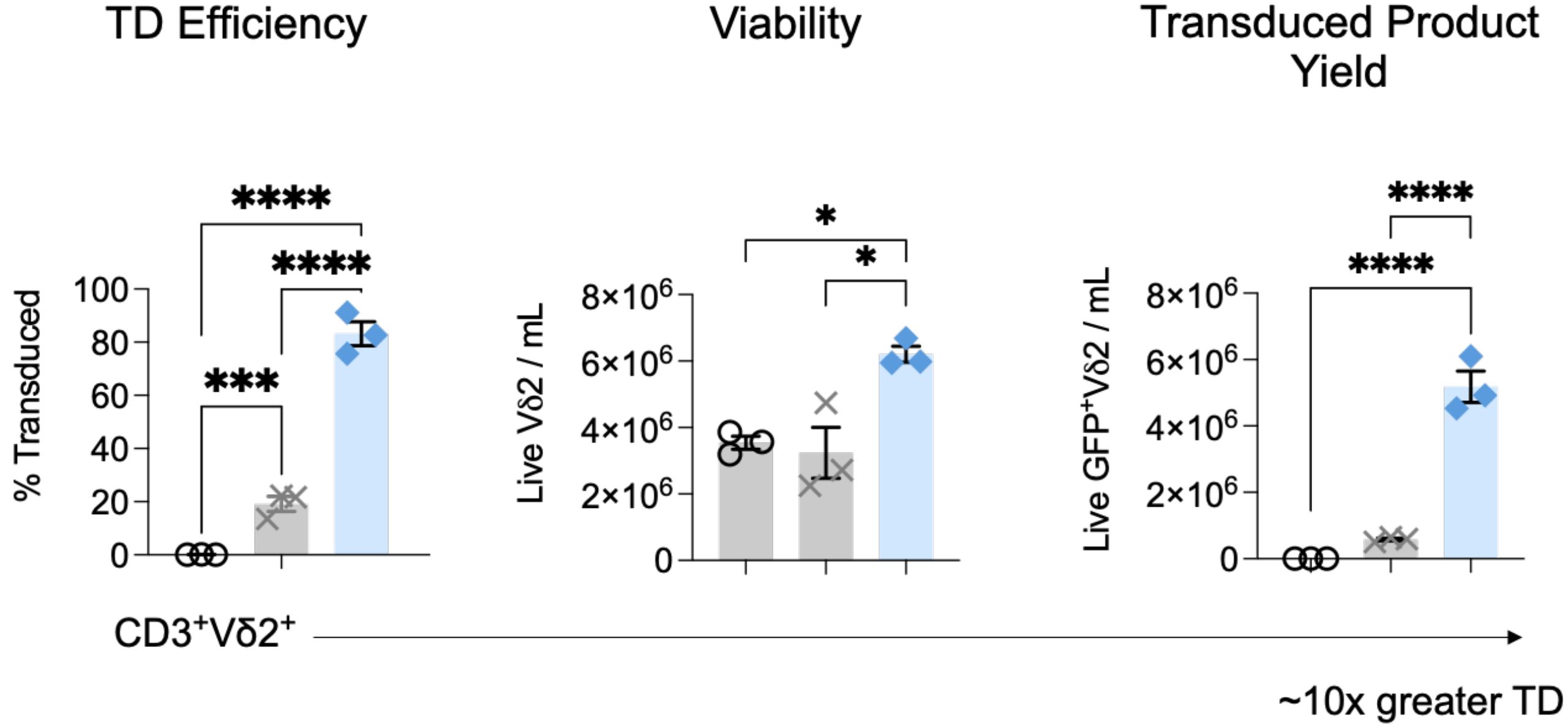
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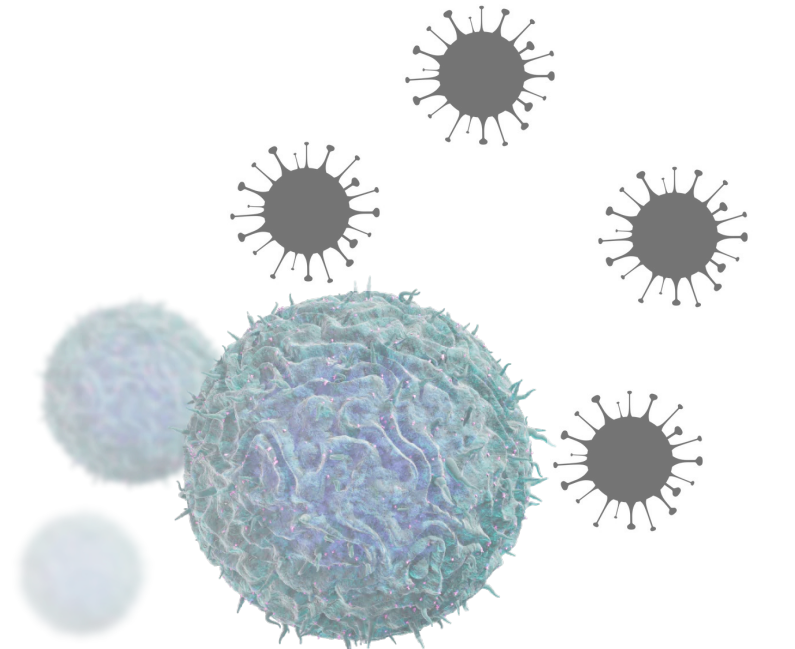
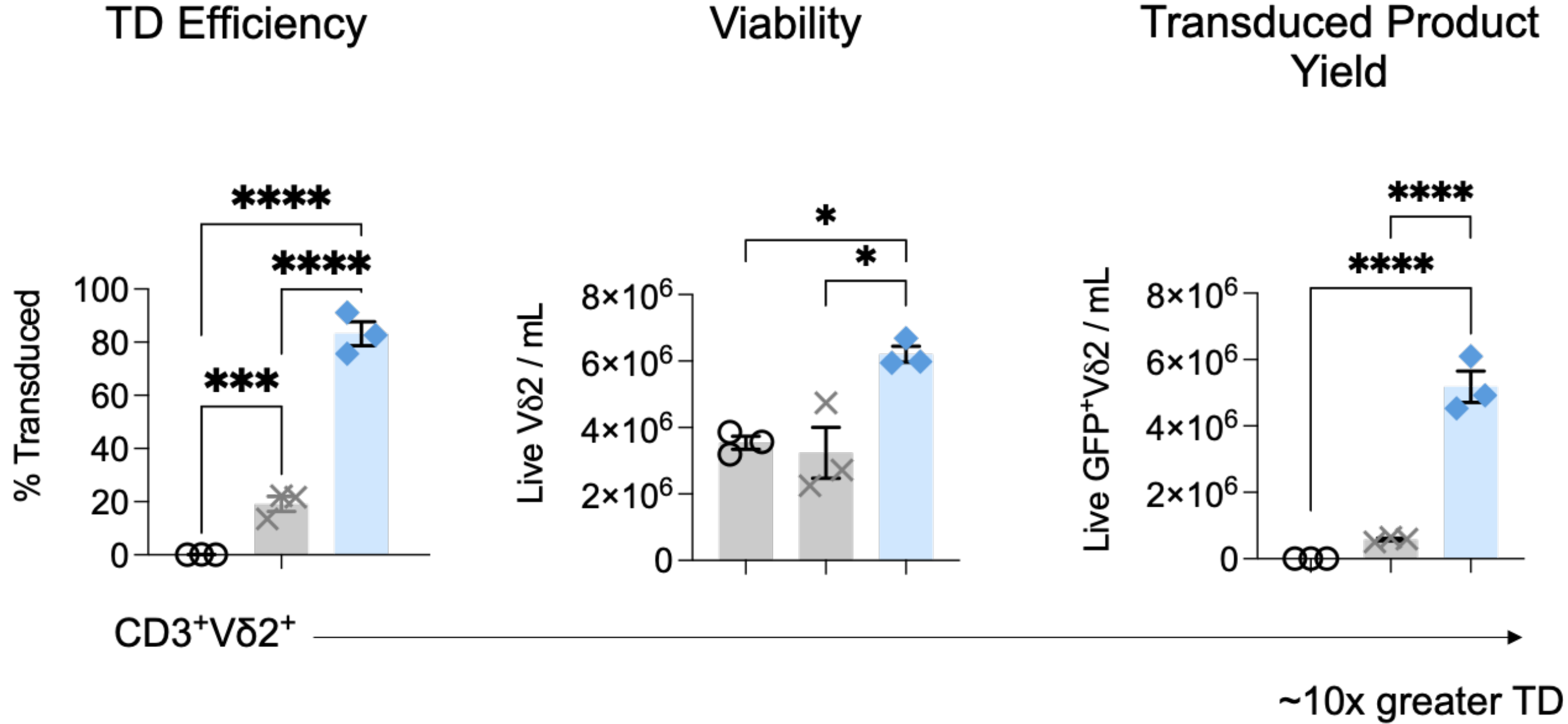
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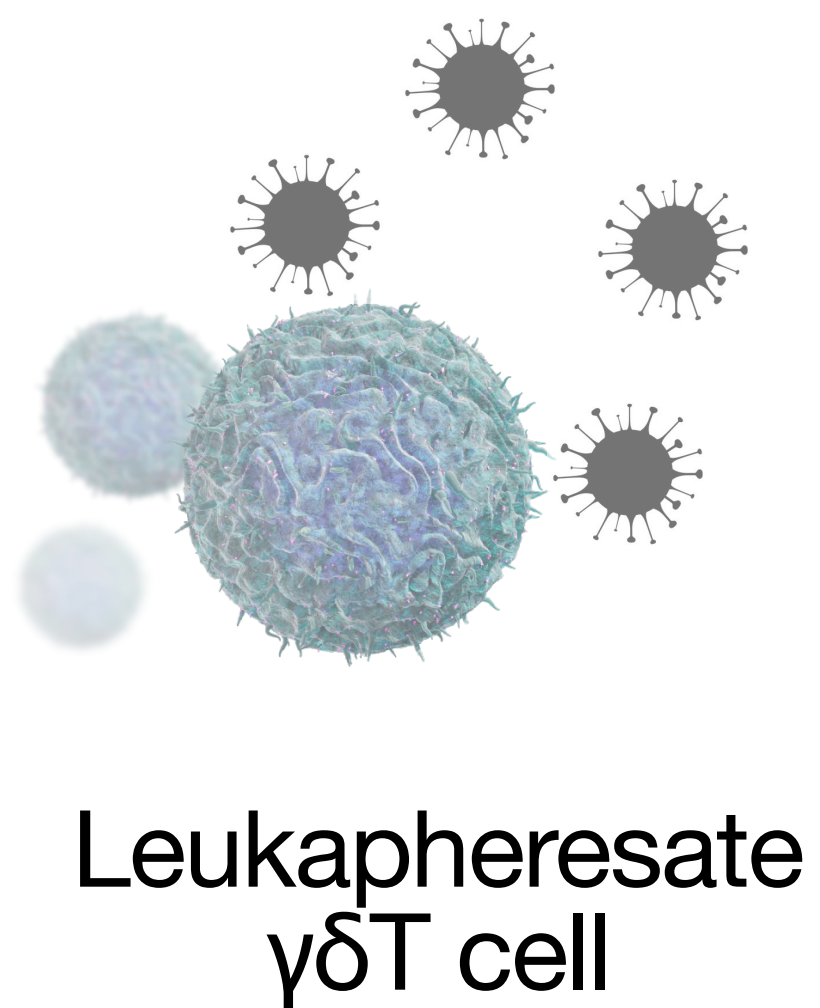
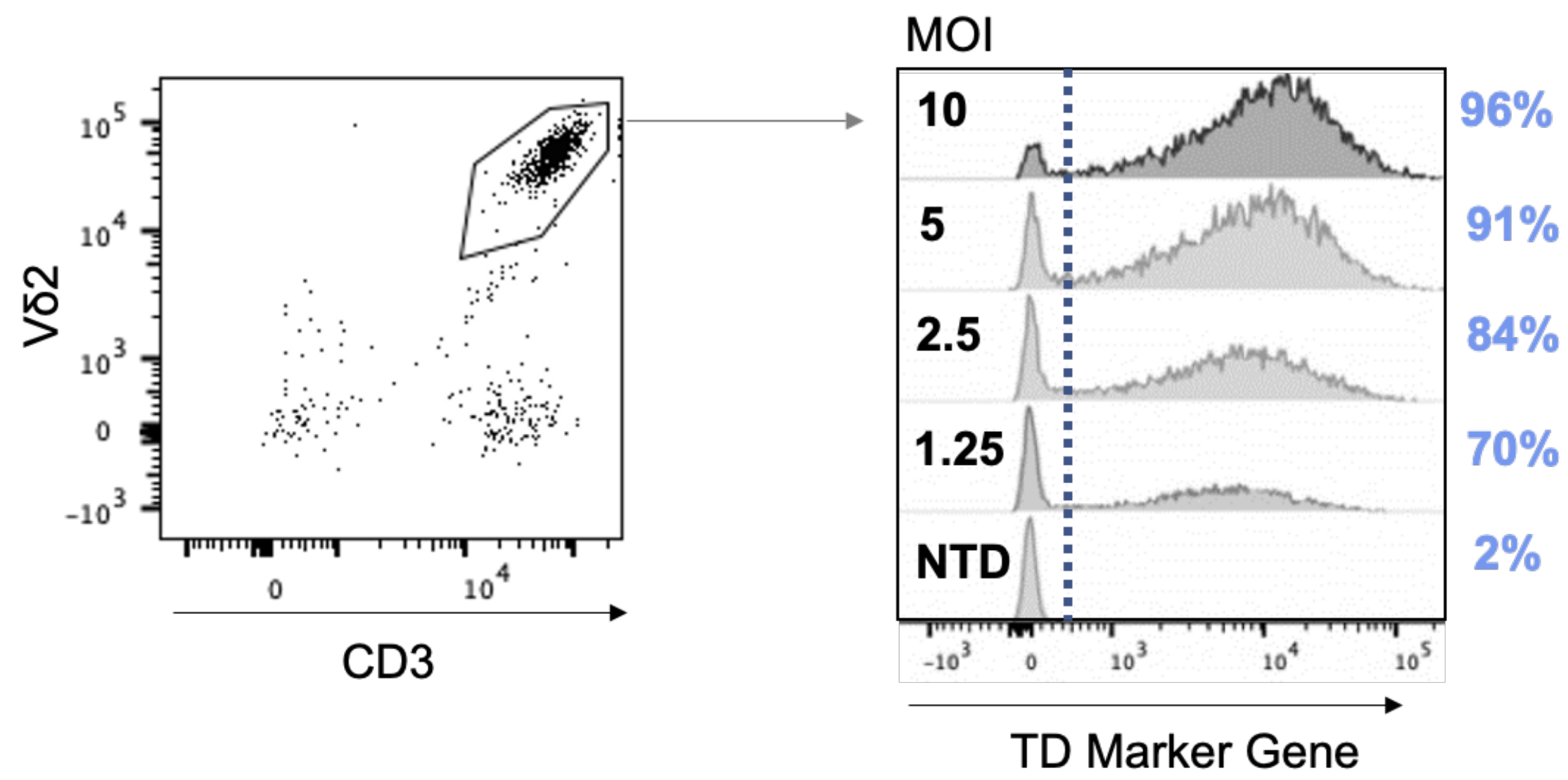
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How far could we push the system?

Titering down

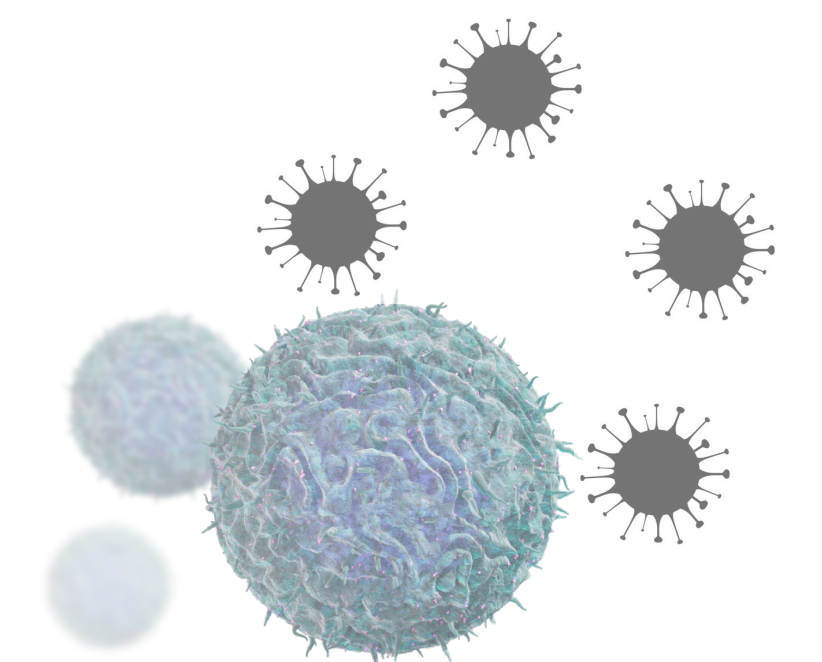
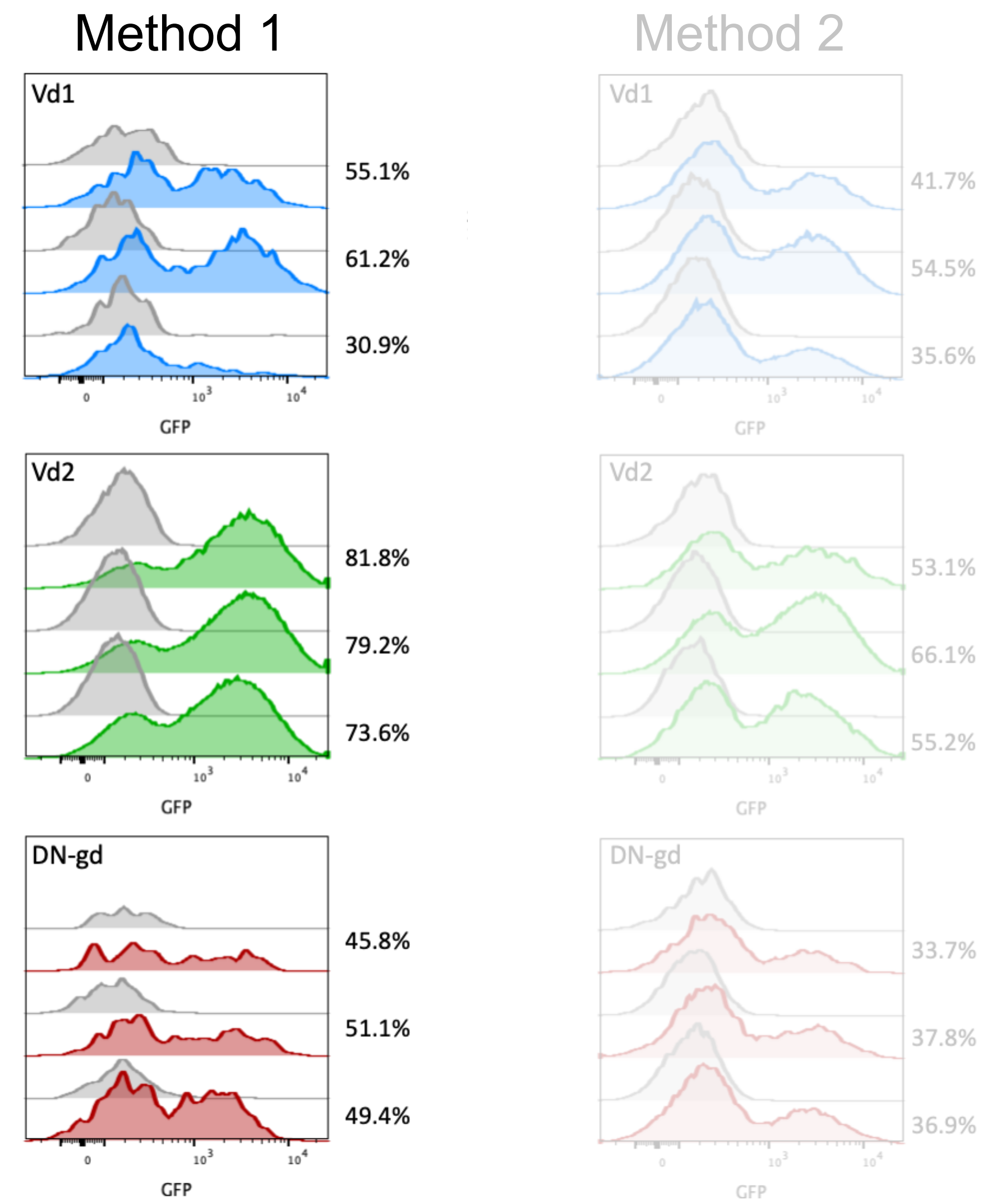


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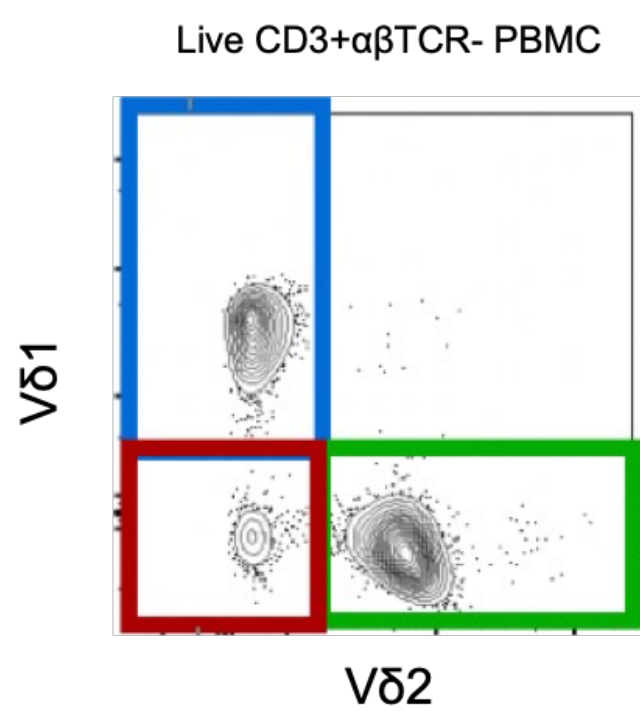
How far could we push the UCL Lenti system?

Applicable across different subsets & activation methods

- MOI 2-4
- Translationally-relevant **tricistronic** construct
- No spinoculation
- No enhancers



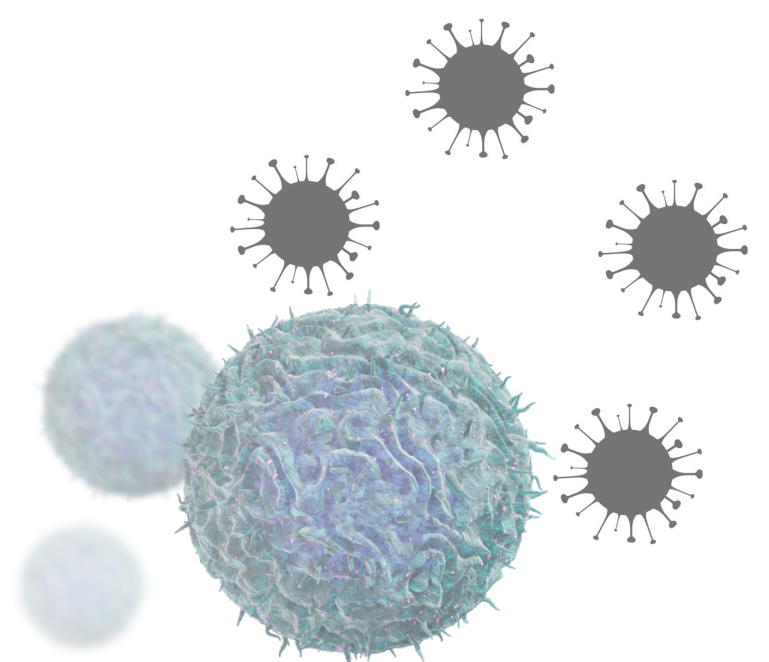
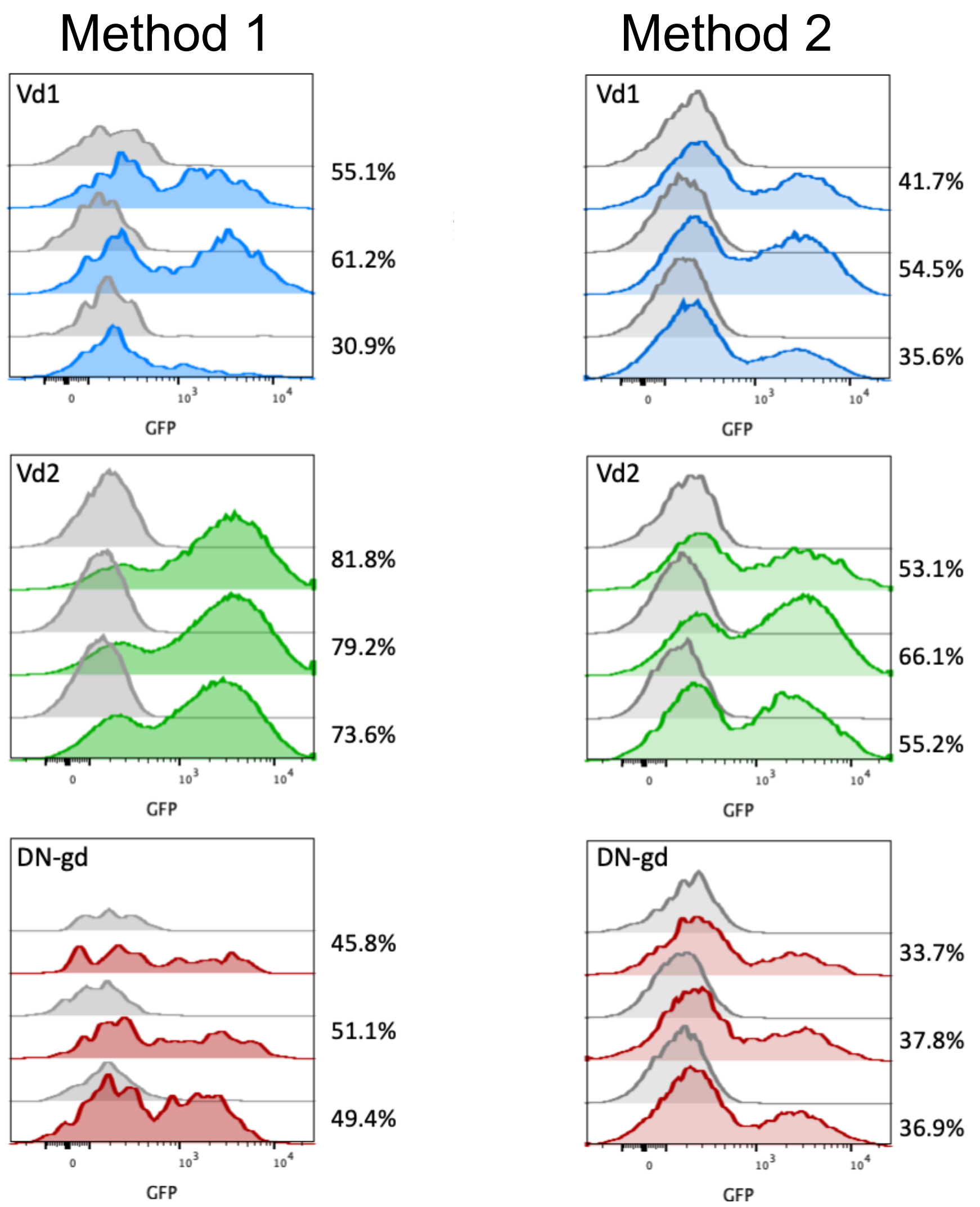
Leukapheresate $\gamma\delta$ T cell



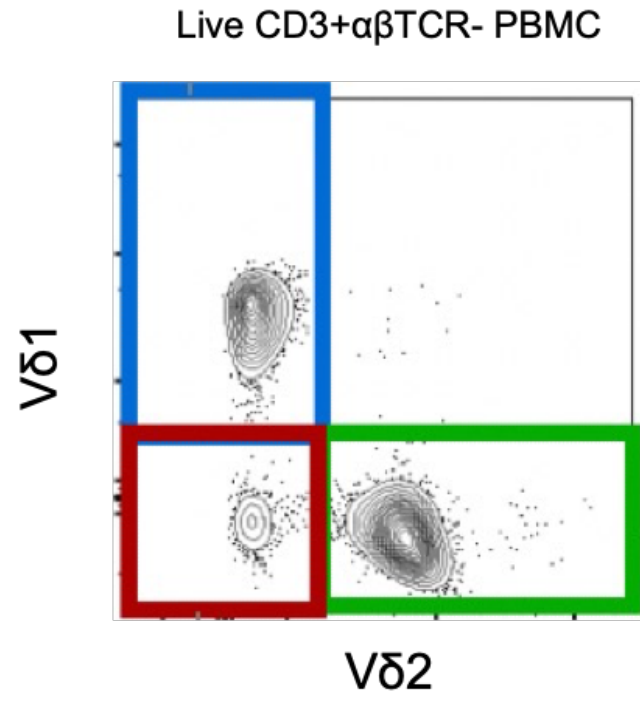
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And NK cells?

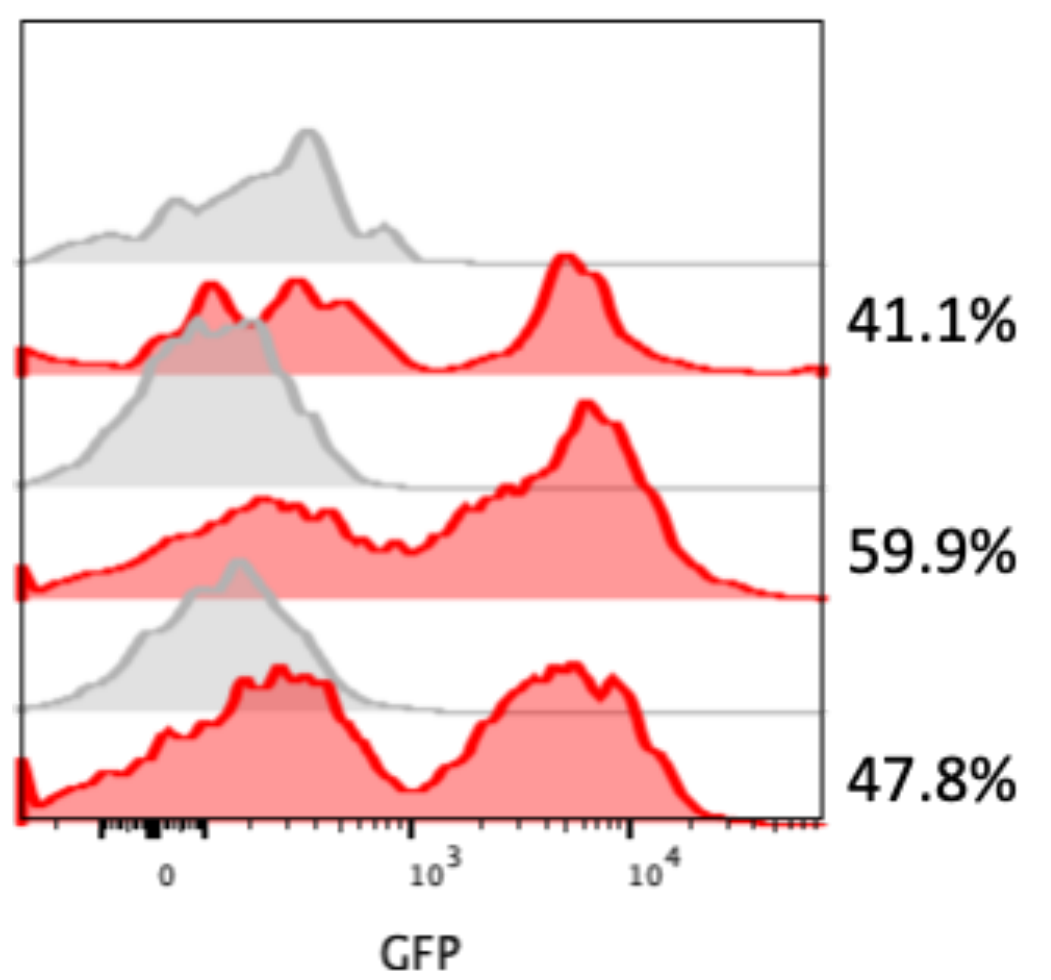
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Translationally-relevant **tricistronic** construct

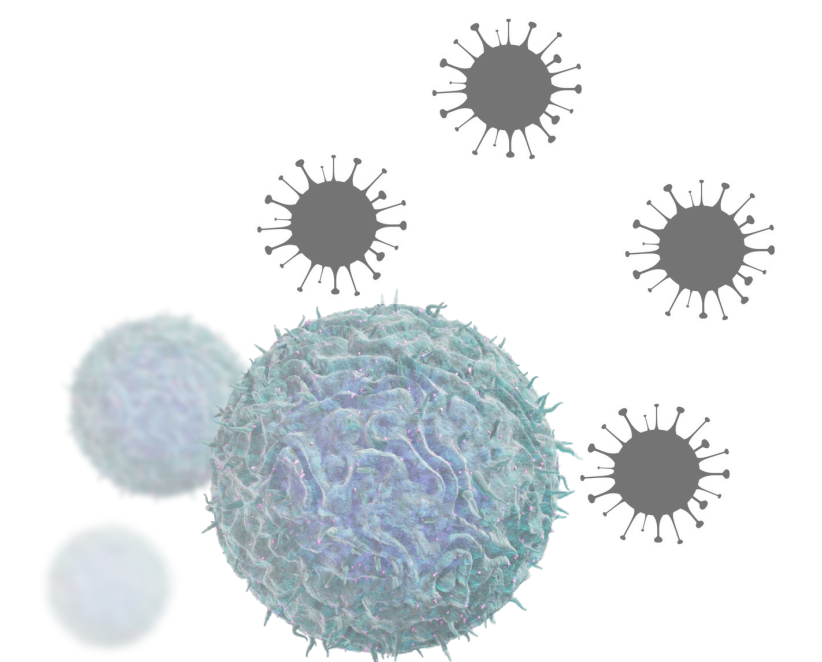
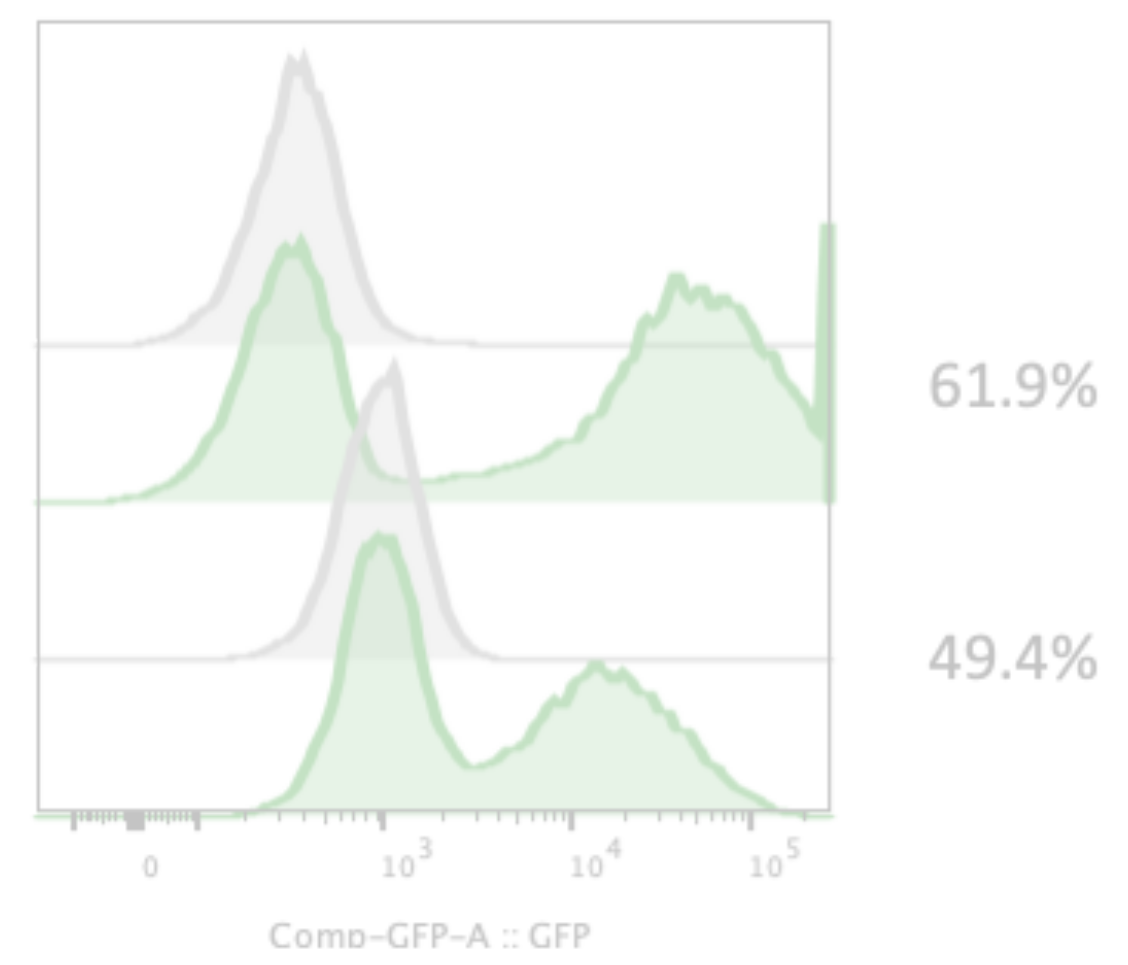
No spinoculation

No enhancers

Method 1

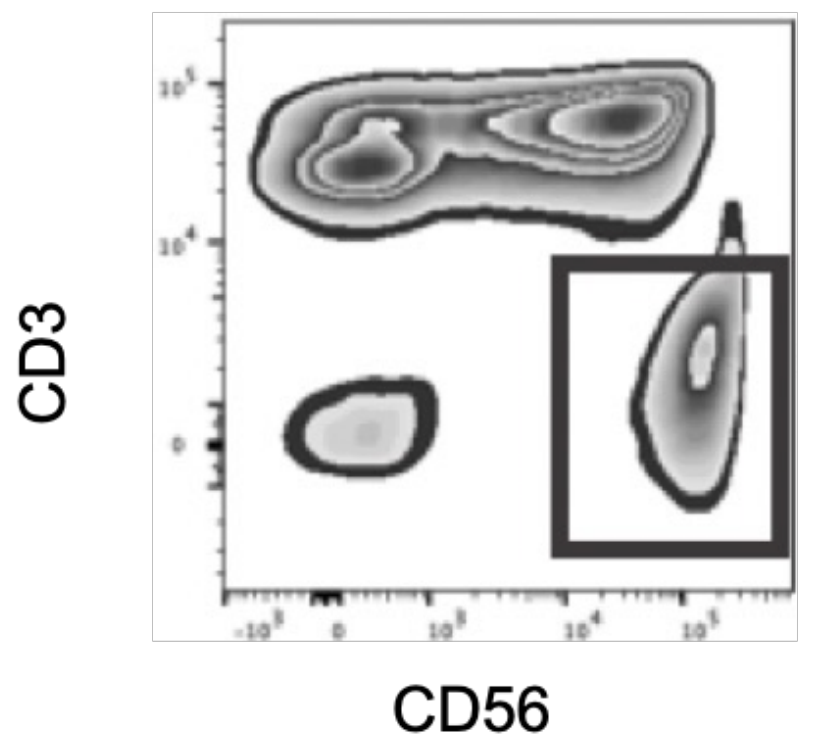


Method 2



Leukapheresate
NK cell

Live PBMC



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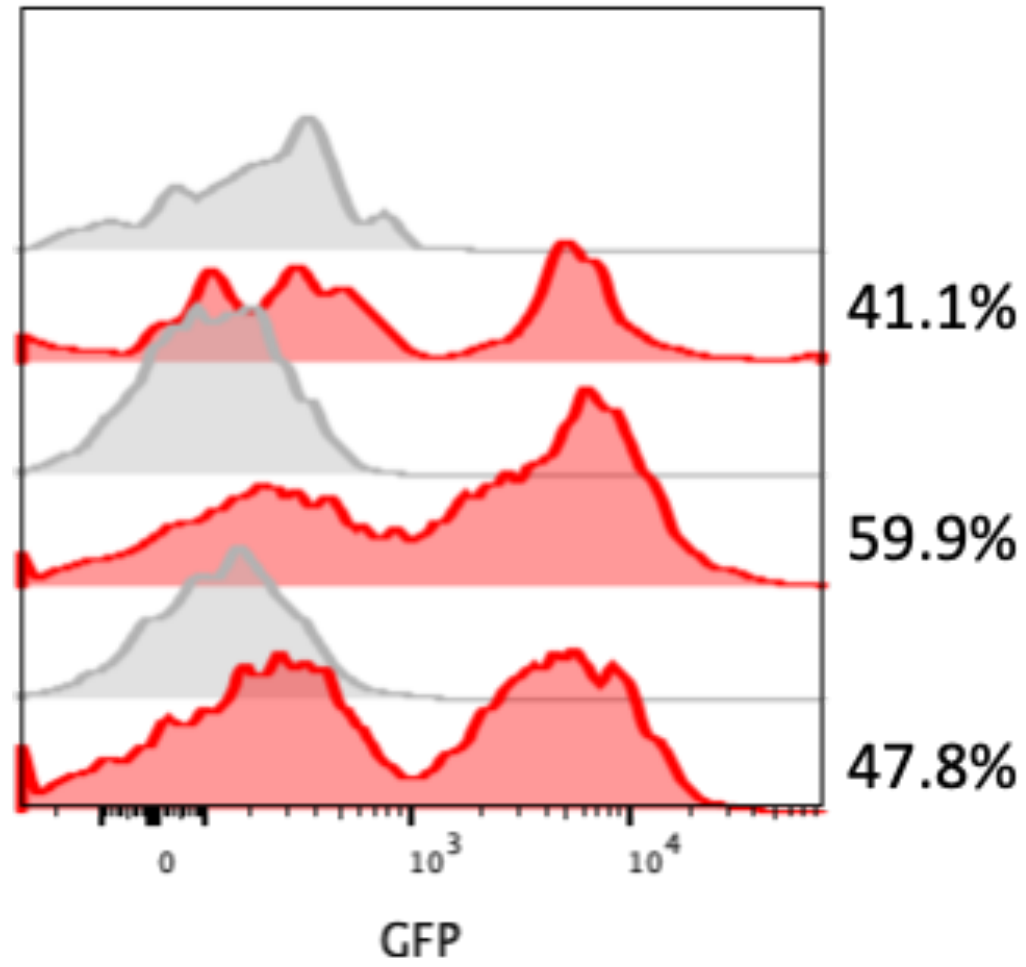
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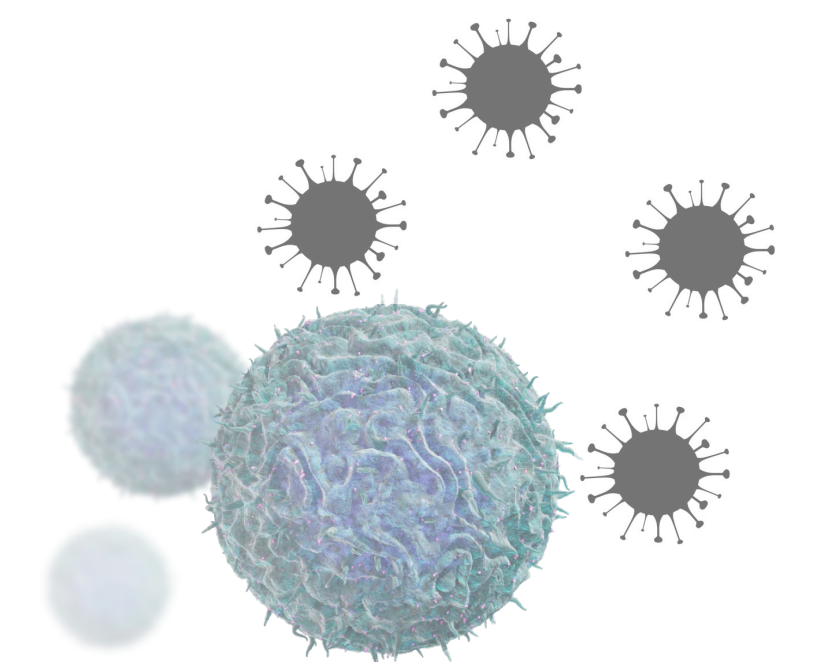
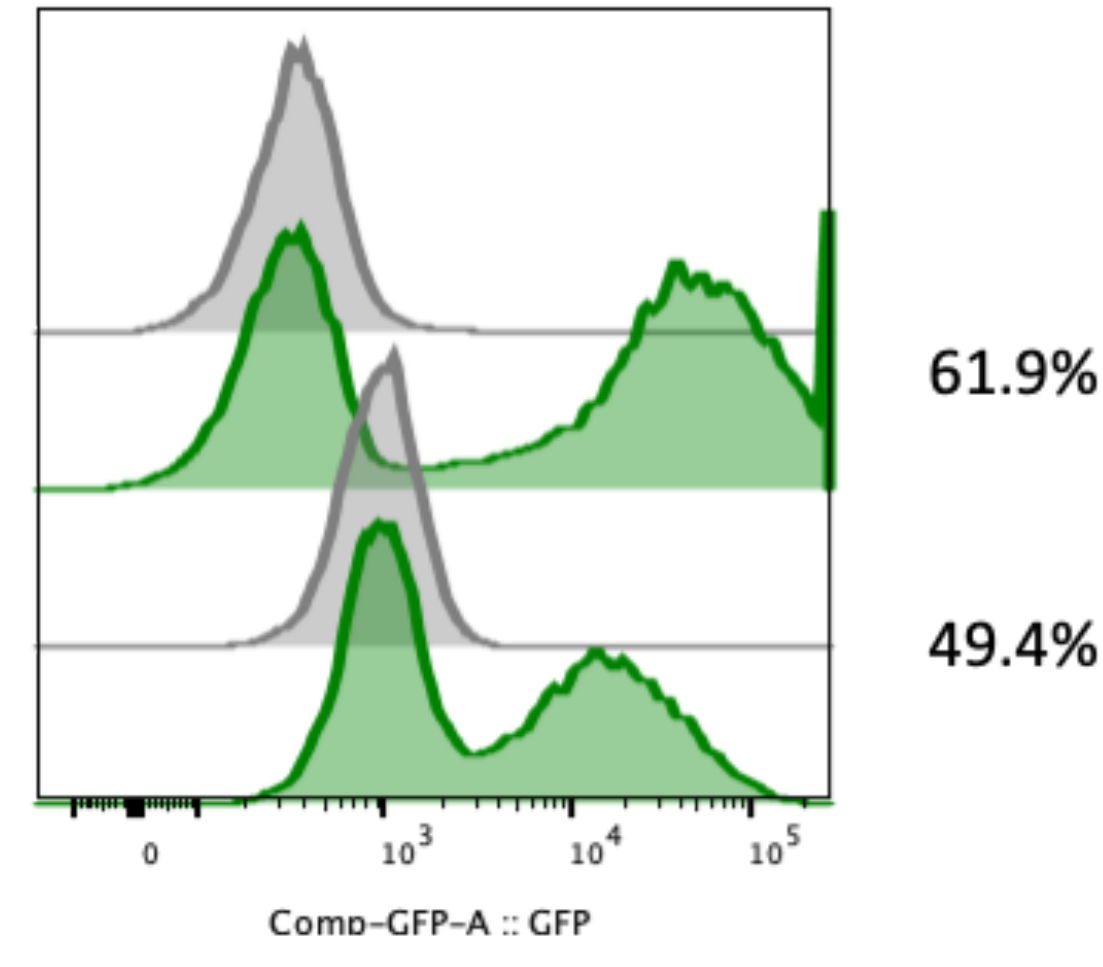
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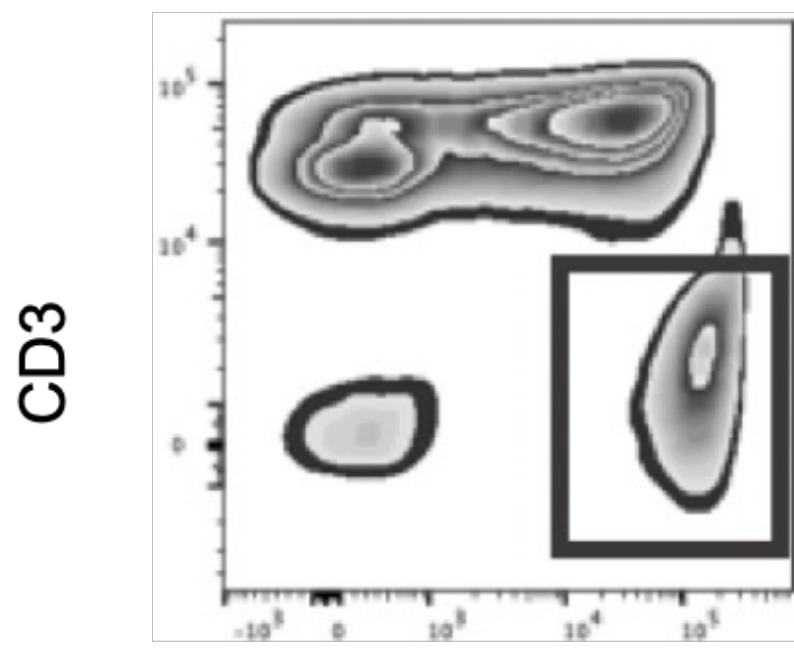
Method 2



Leukapheresate
NK cell

Feeder free

Live PBMC



CD56

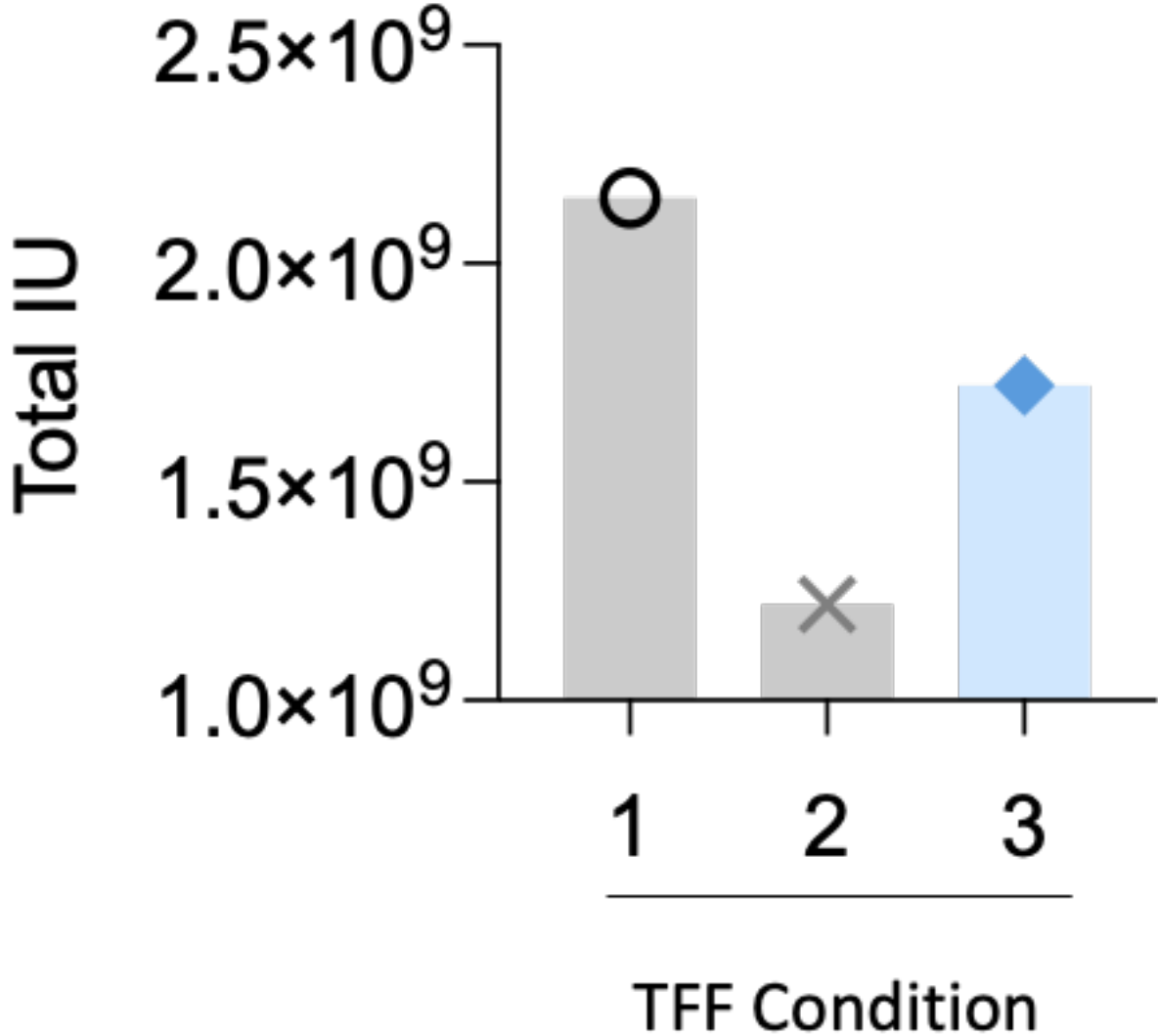
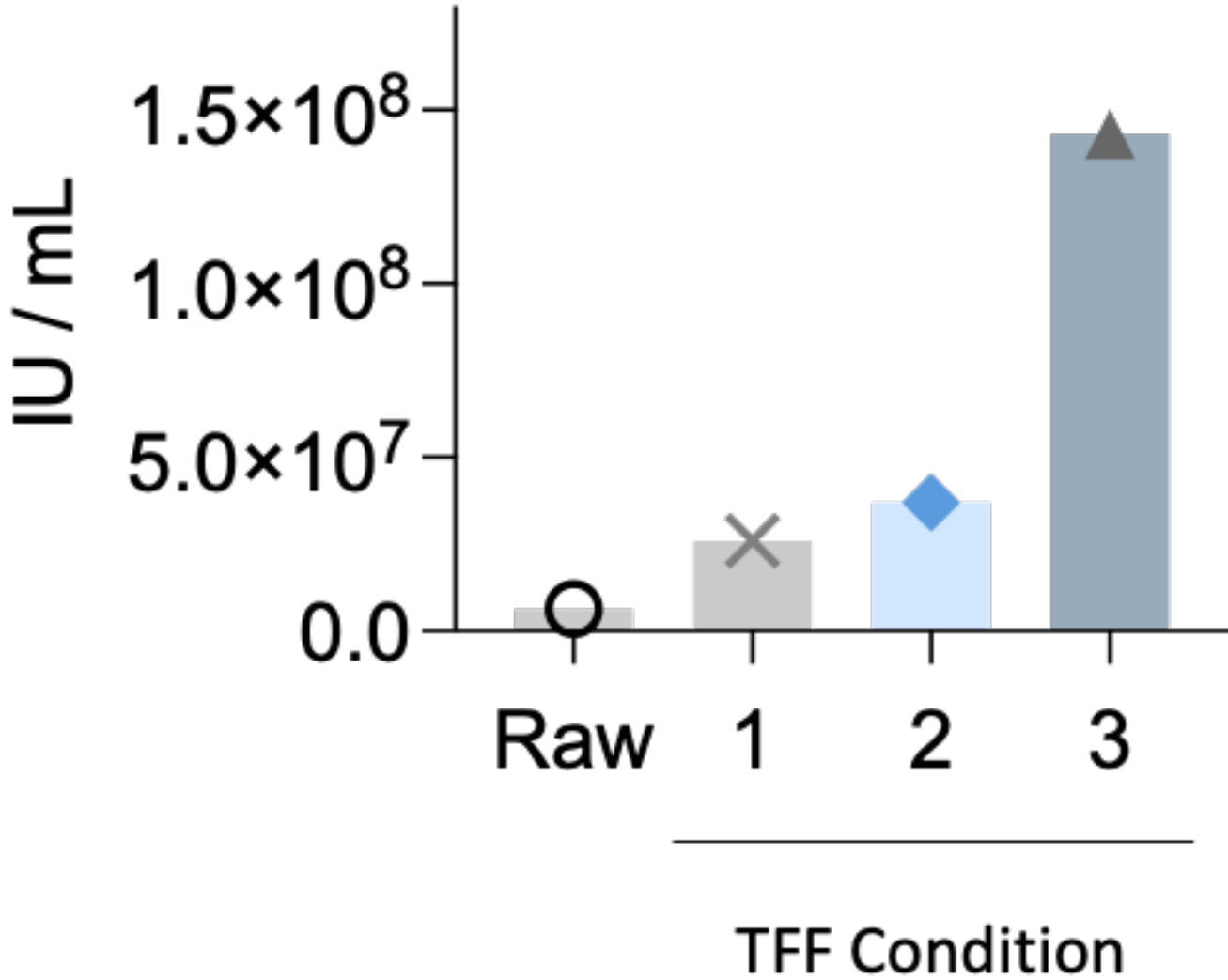
UCL Lenti Manufacture

Where are we now?

Medium scale optimisations

Superc concentration & storage

Infectious unit yield optimisation: similar yield to VSVg



- Optimised transfection
- Optimised collection media
- Optimised concentration protocol

What next?

Transfer to suspension system

- Bioreactors
- Harvest and concentration optimisation
- Producer & packaging cell lines

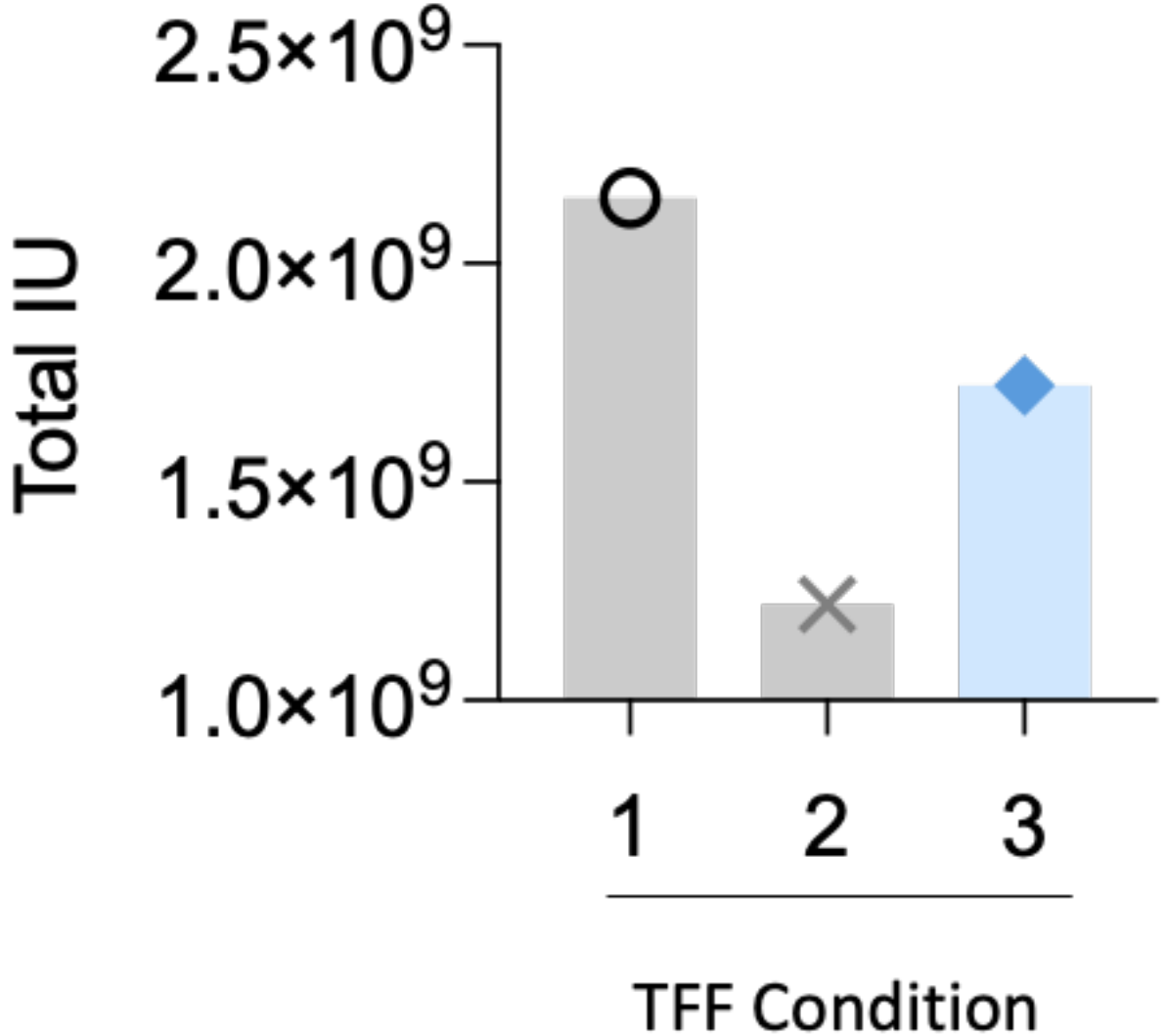
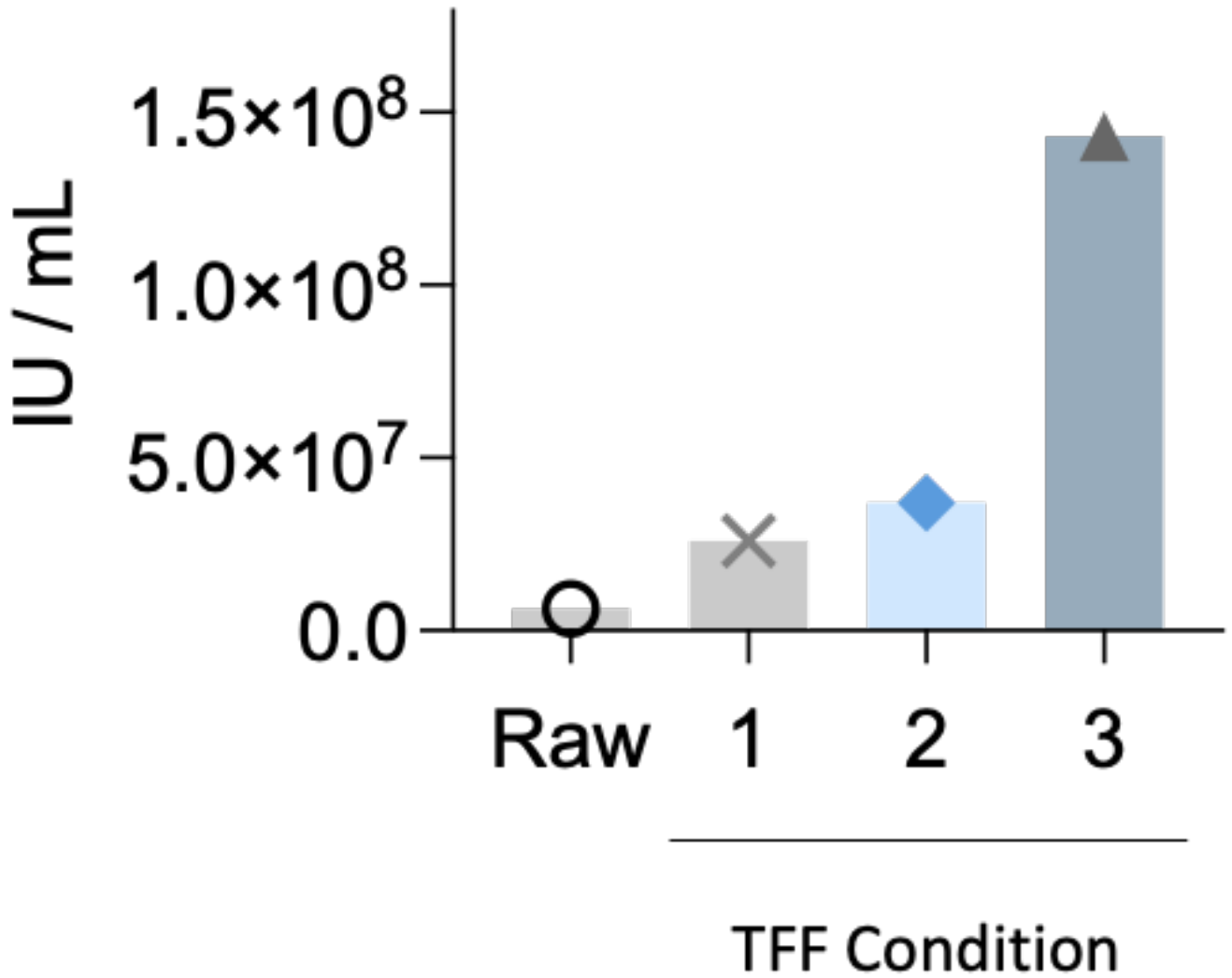
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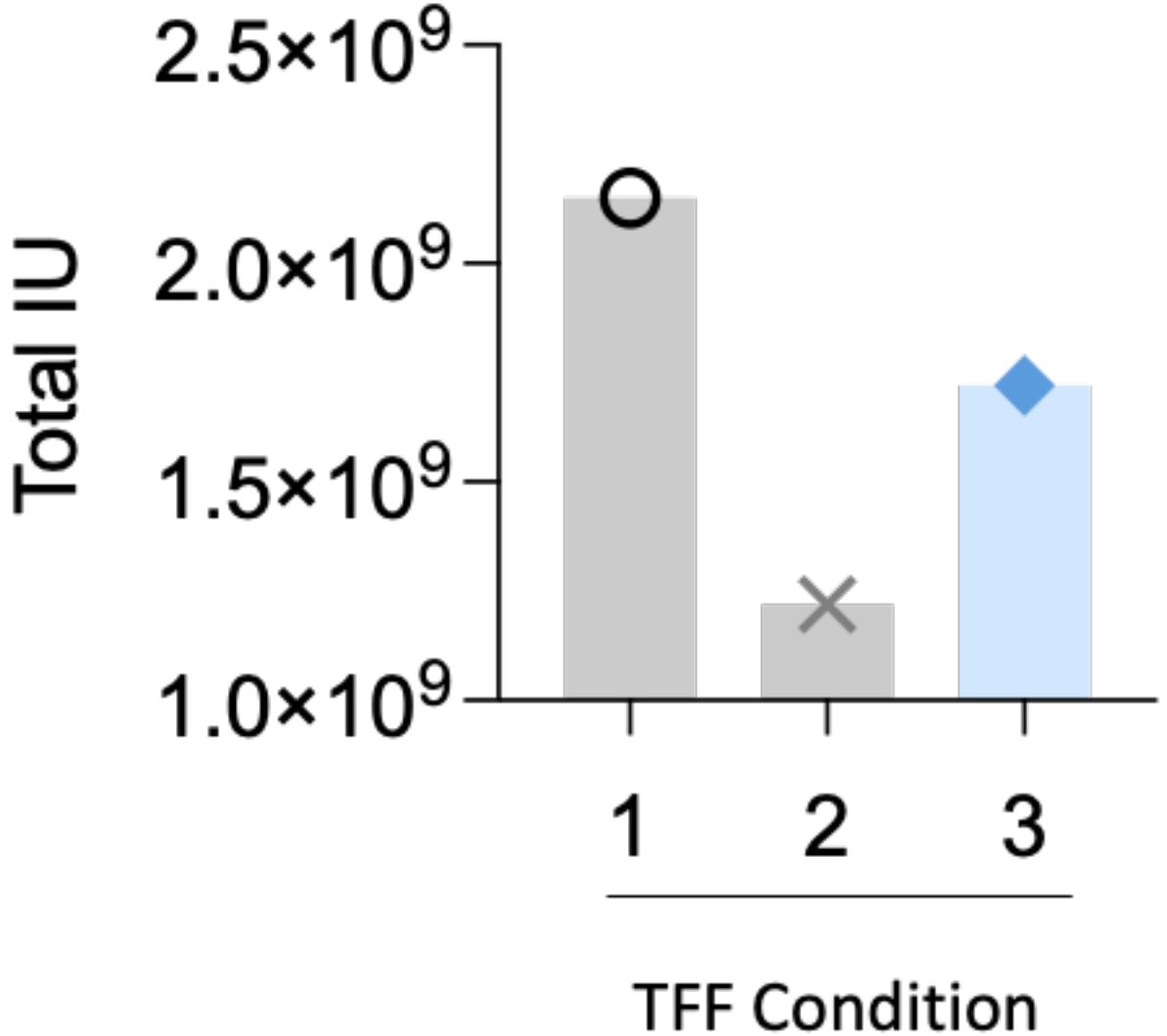
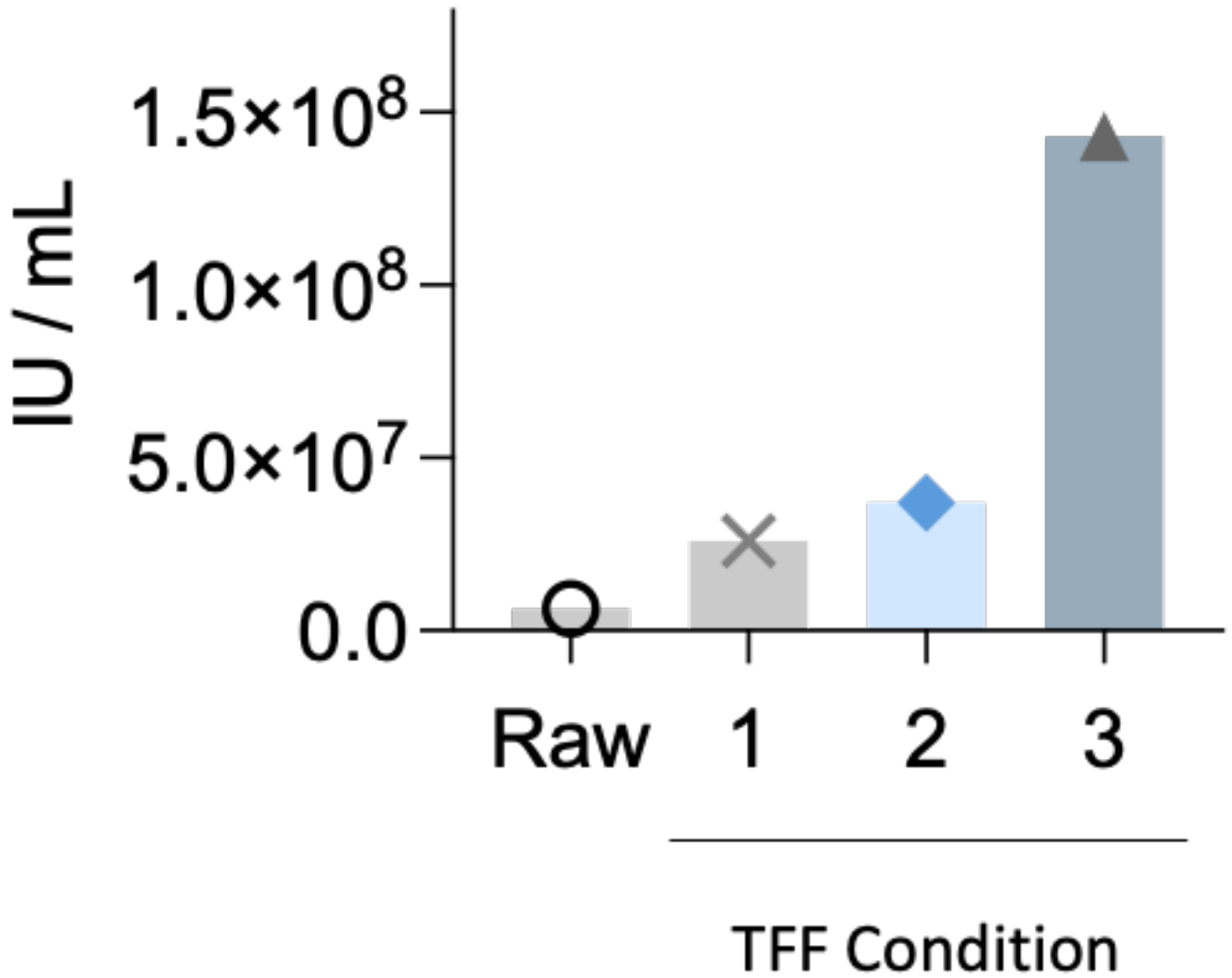
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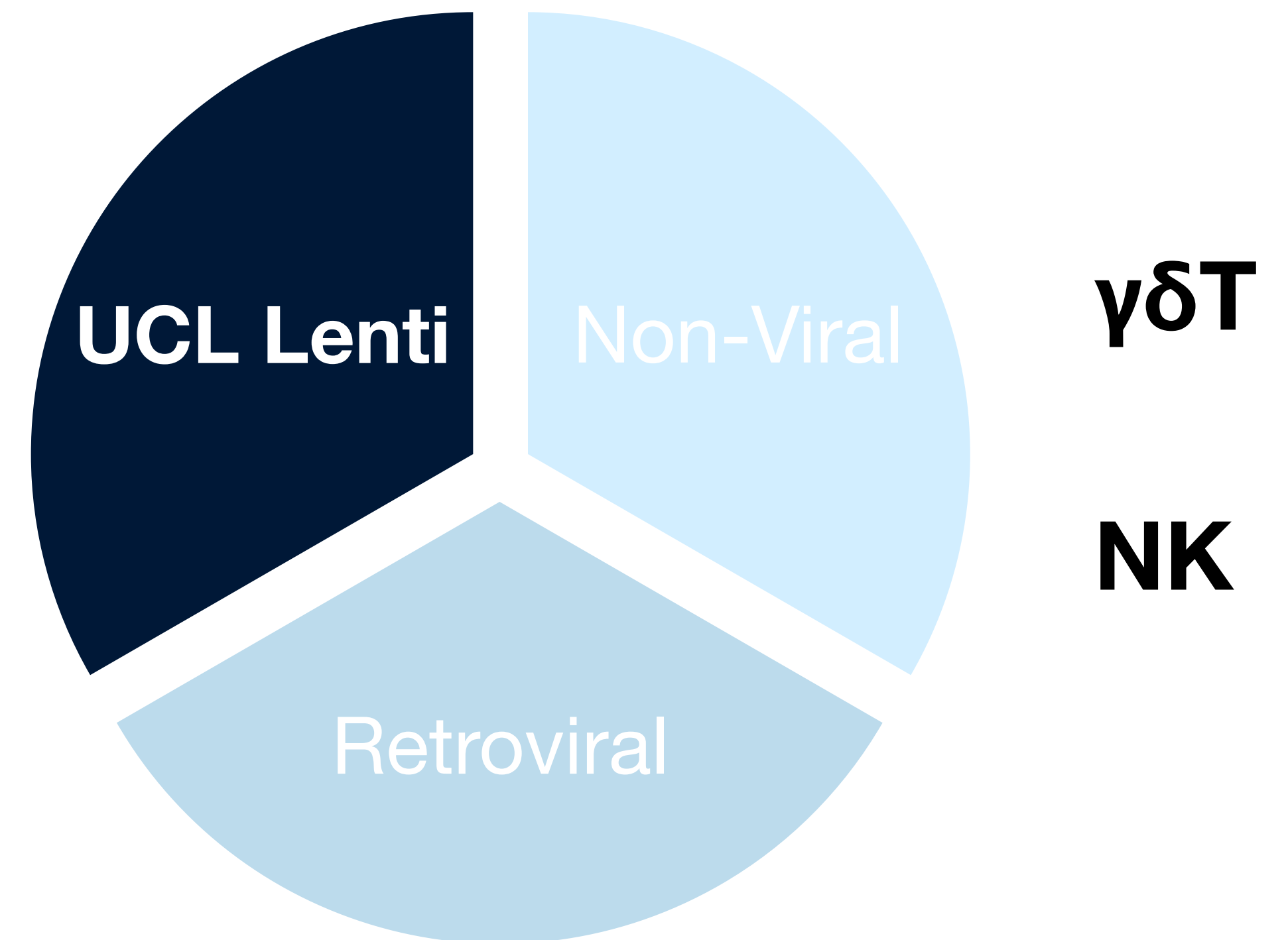
Transfer to suspension system

- Bioreactors
- Harvest and concentration optimisation
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Summary

We think we have our solution

- Regulatory and clinical experience
- Scalability
- Product viability & health
- Large construct size
- Acceptable safety profile
- Reasonable cost
- Easy concentration & storage
- High transduction efficiency



Thank you!

Always happy to chat: m.barisa@ucl.ac.uk



**Jon
Fisher**



**Marta
Barisa**



**Dan
Fowler**



**Callum
Nattress**



**Alba
Southern**



**Elina
Vassalou**



**Rumeysa
Tuna Deveci**



**Angeliki
Kanouta**



**Andrea
Farkas**



**Tessa
de Mooij**



**GREAT ORMOND STREET
INSTITUTE OF CHILD HEALTH**