Tissue engineering applied to lung tissue

INTO THE CLINIC : MIND THE GAP

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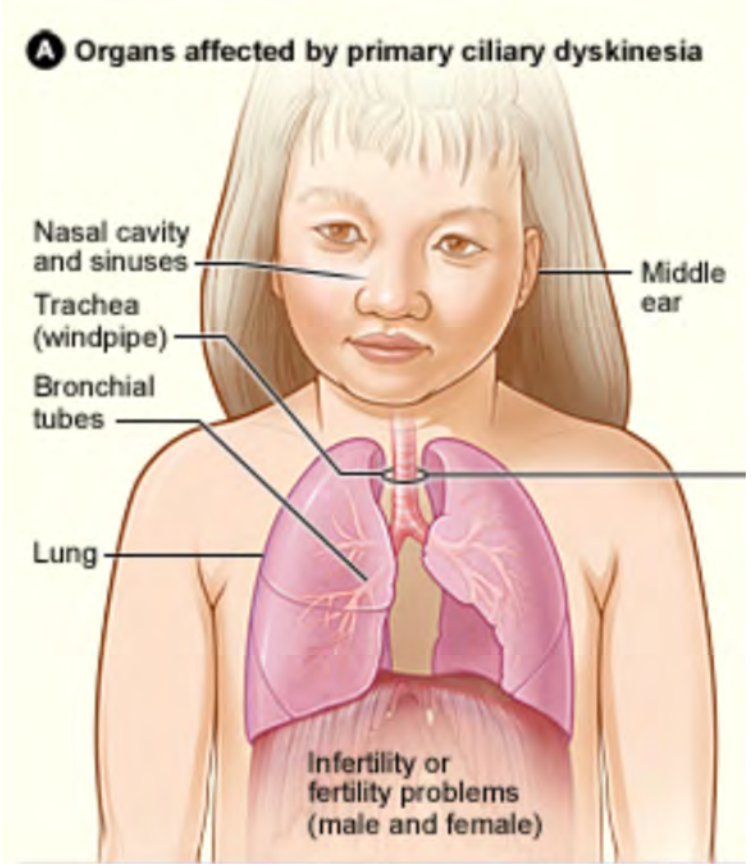
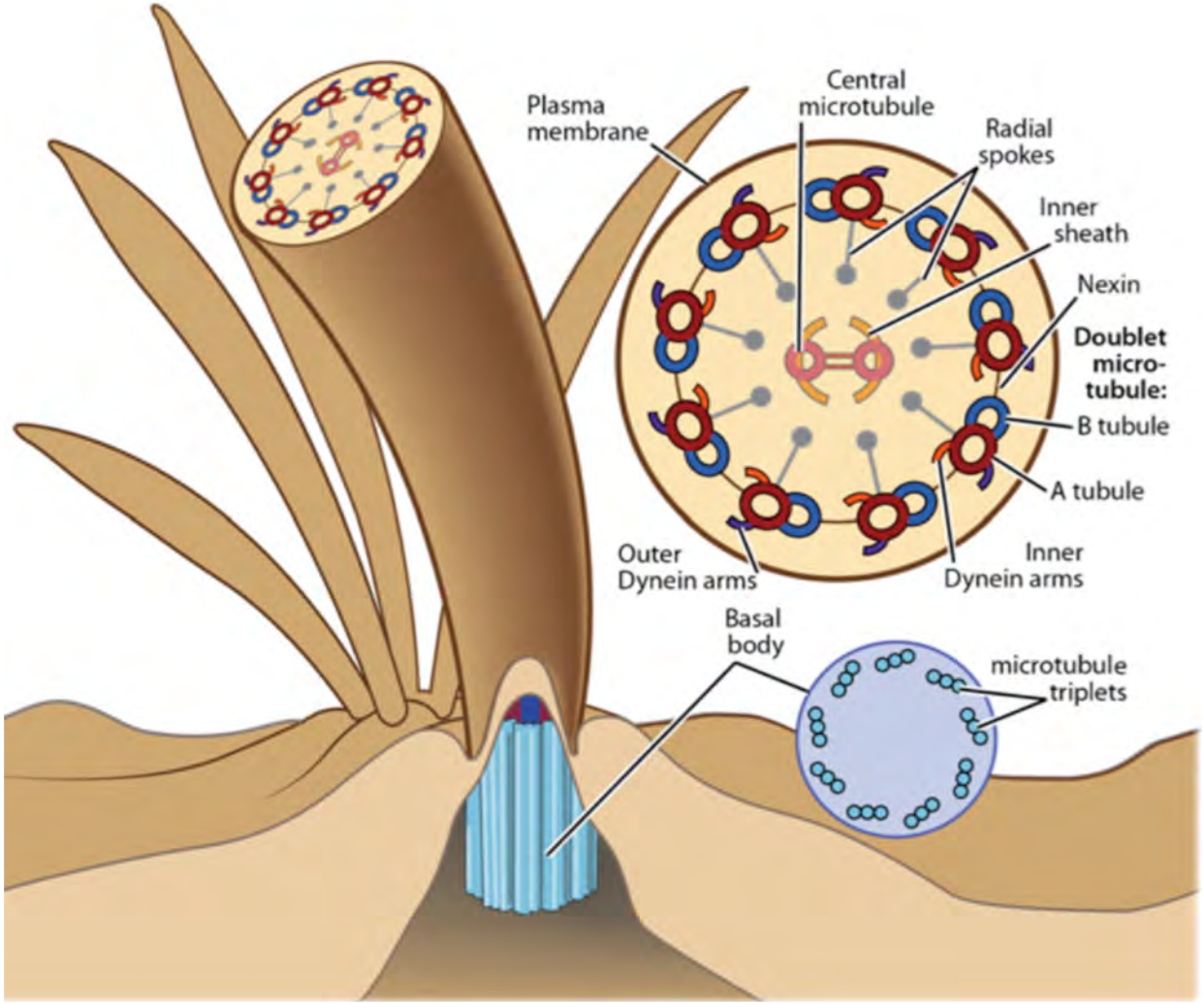
PRIMARY CILIARY DYSKINESIA

- Rare genetic condition (1/10,000)
- Ciliary dysfunction
- Heterogeneous: > 35 genes
- → mucus stasis, chronic respiratory infections, destruction of bronchi, death

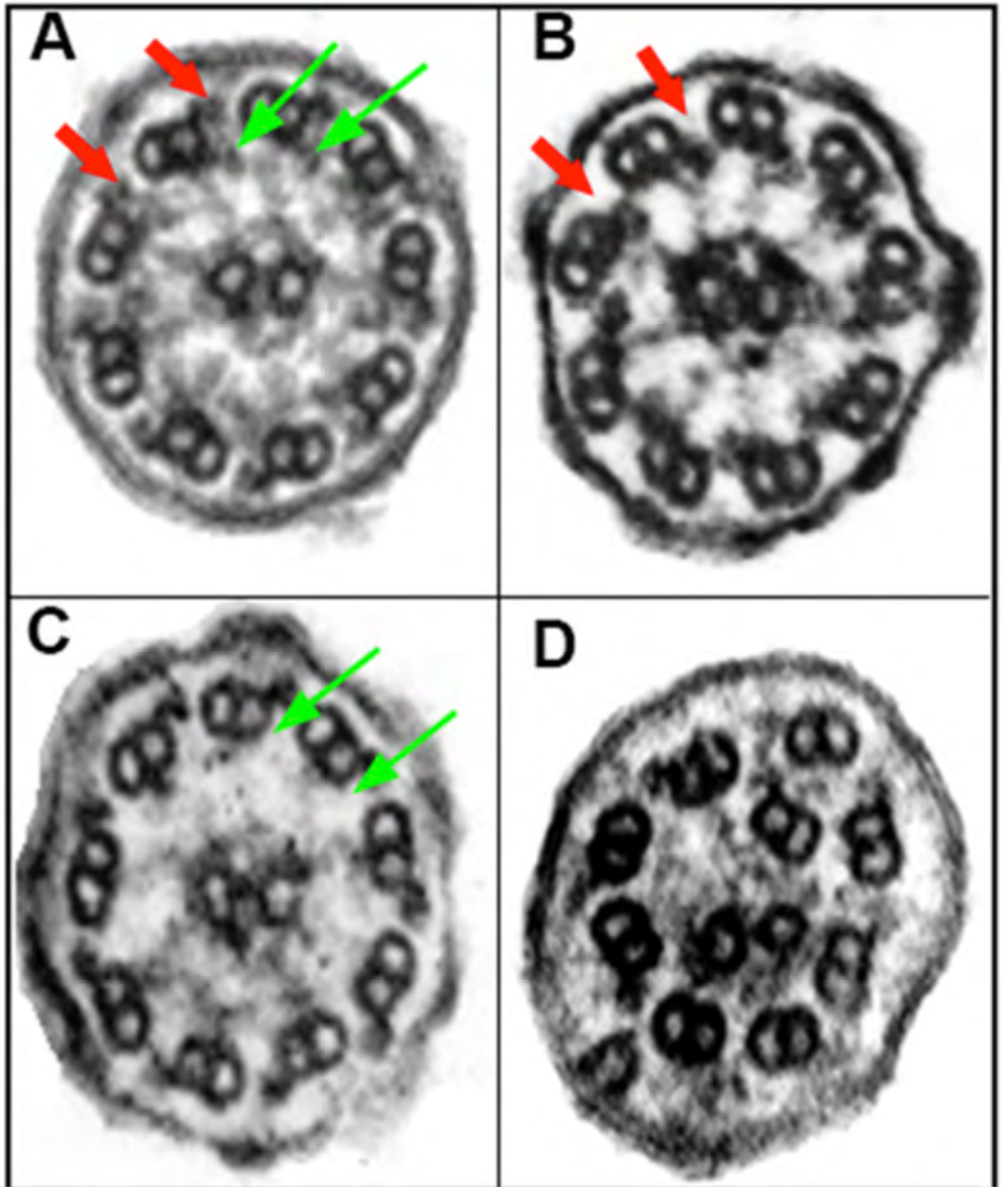


PRIMARY CILIARY DYSKINESIA



Knowles et al. (2013). *American Journal of Respiratory and Critical Care Medicine*

PRIMARY CILIARY DYSKINESIA



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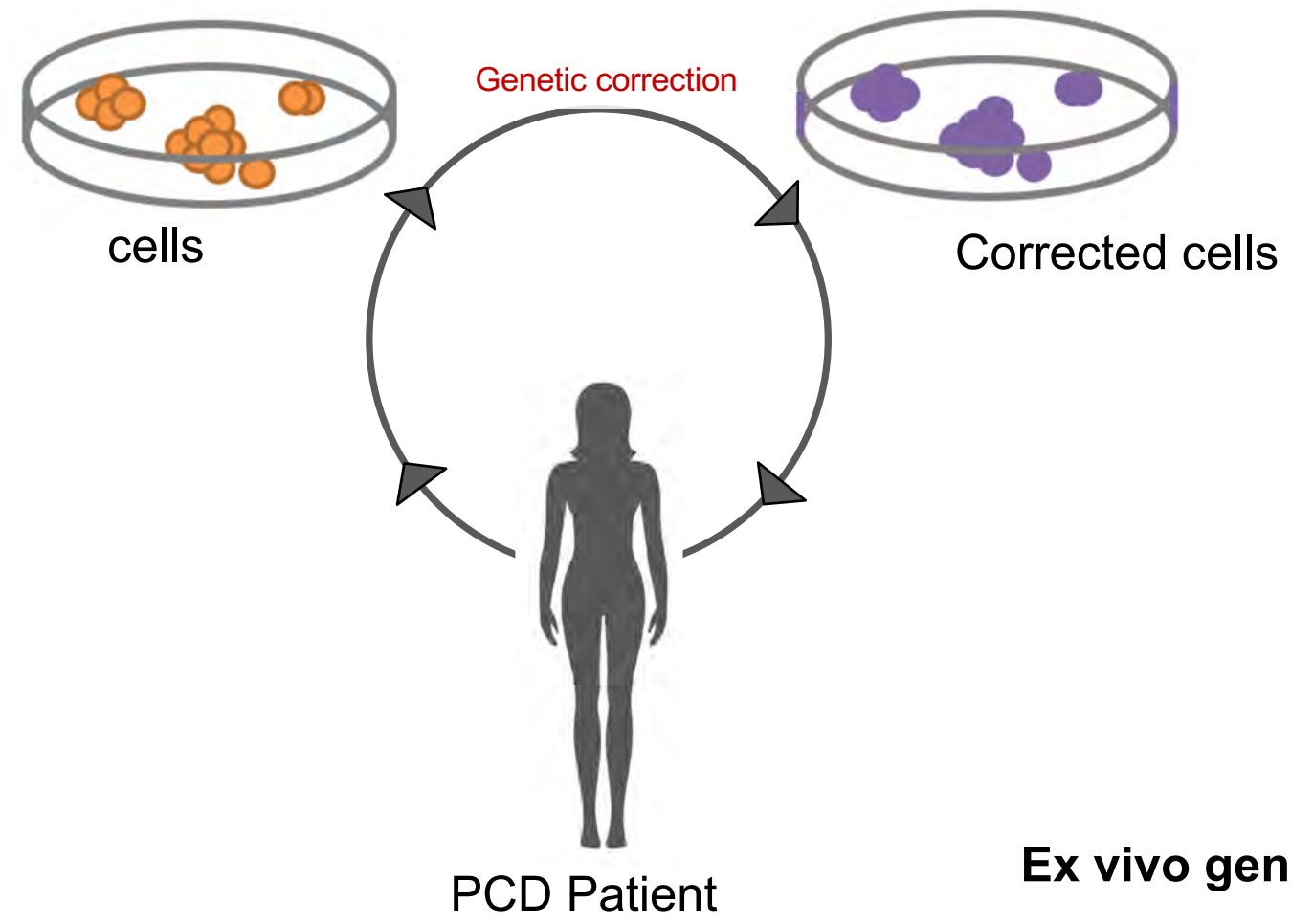
Gene and cell therapy for PCD



Ex vivo gene therapy

Regenerative medicine

- ?

Regenerative medicine

- Replace cell or tissues damaged by disease, with normal or corrected cells

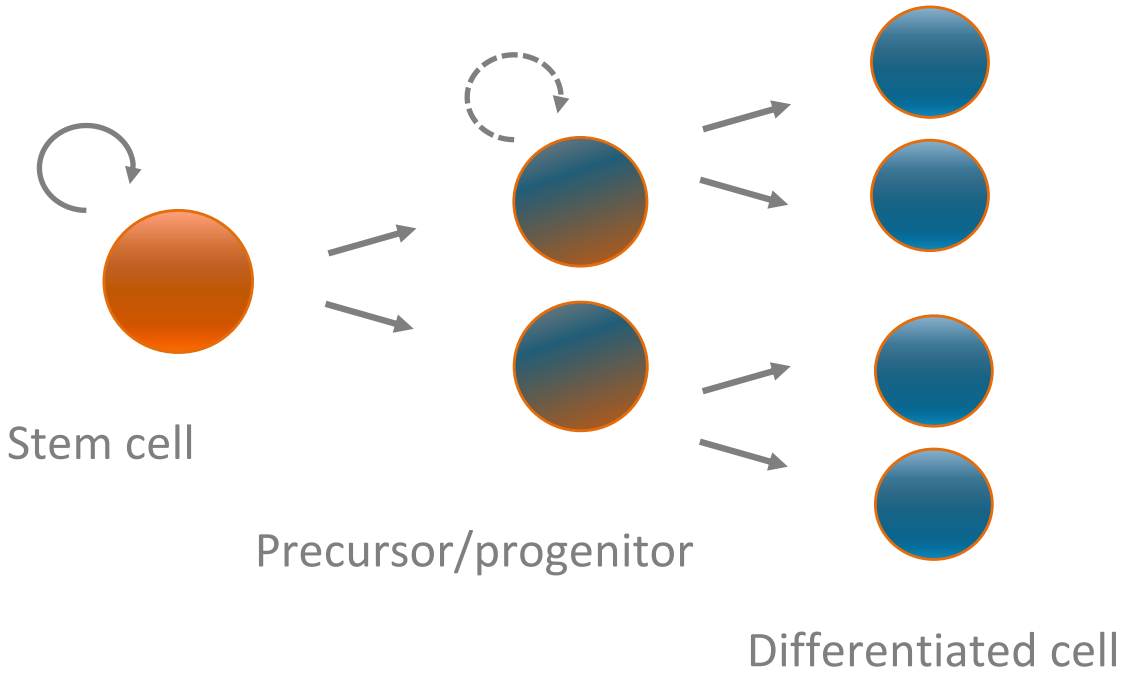
Regenerative medicine

- What you need:
  1. Define the cell of interest (stem cell or not?)
  2. How to obtain this cell
  3. Immunity issue (auto/allo/xeno)
  4. Safety
  5. How to provide the cells to the organ?

STEM CELLS

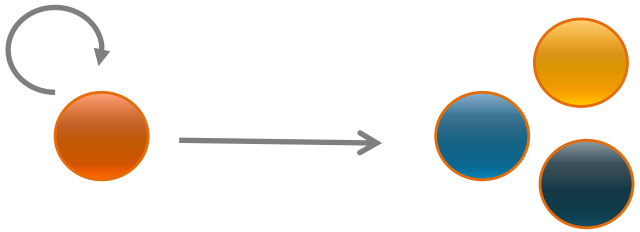
Stem Cells

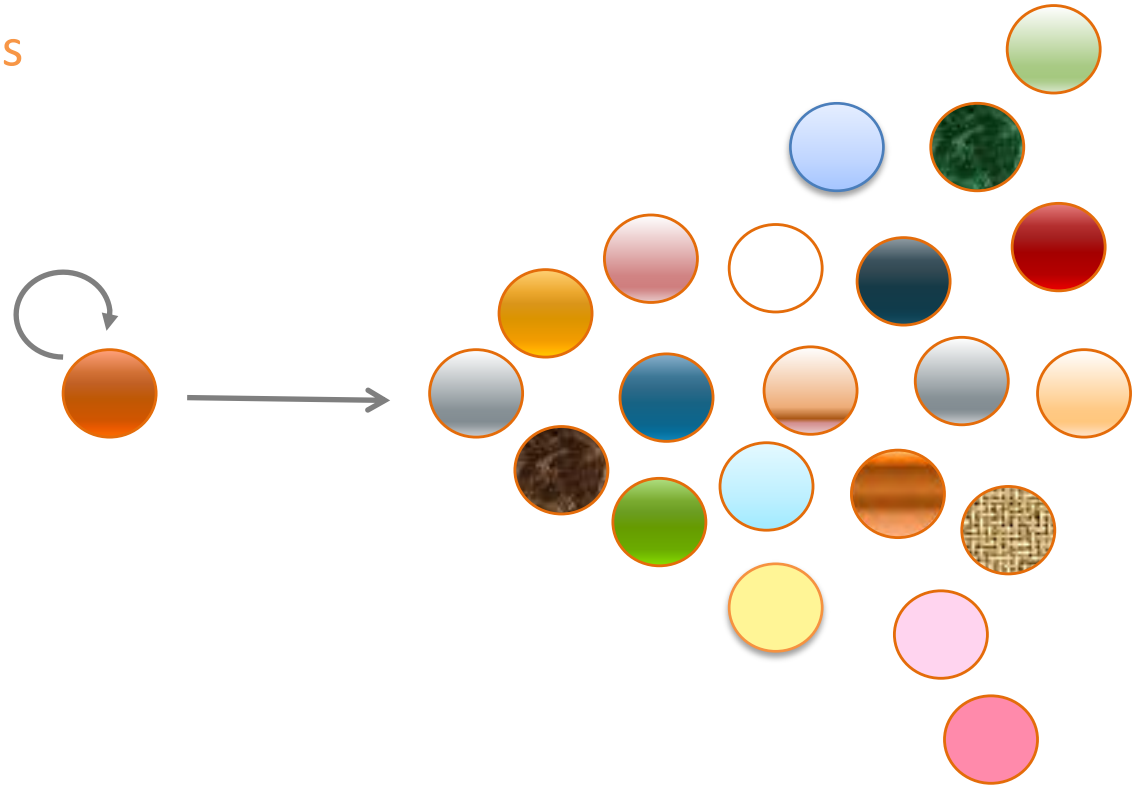
- Main properties :
  - Self renewal
  - Differentiation
  - Immaturity



Different stem cells

- Multipotent stem cells
  - Mesenchymal stem cells
  - Hematopoietic stem cells
  - Etc.



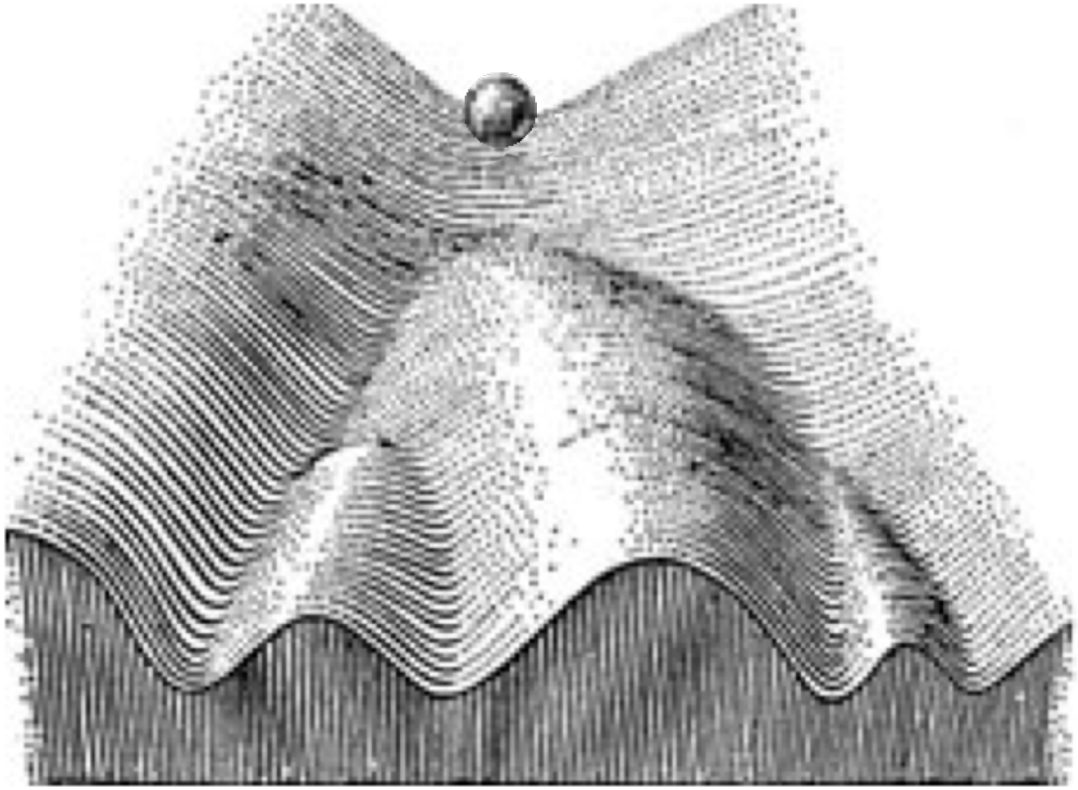


- Pluripotent stem cells
  - Embryonic stem cells
  - iPS

iPS cells

Irreversible differentiation

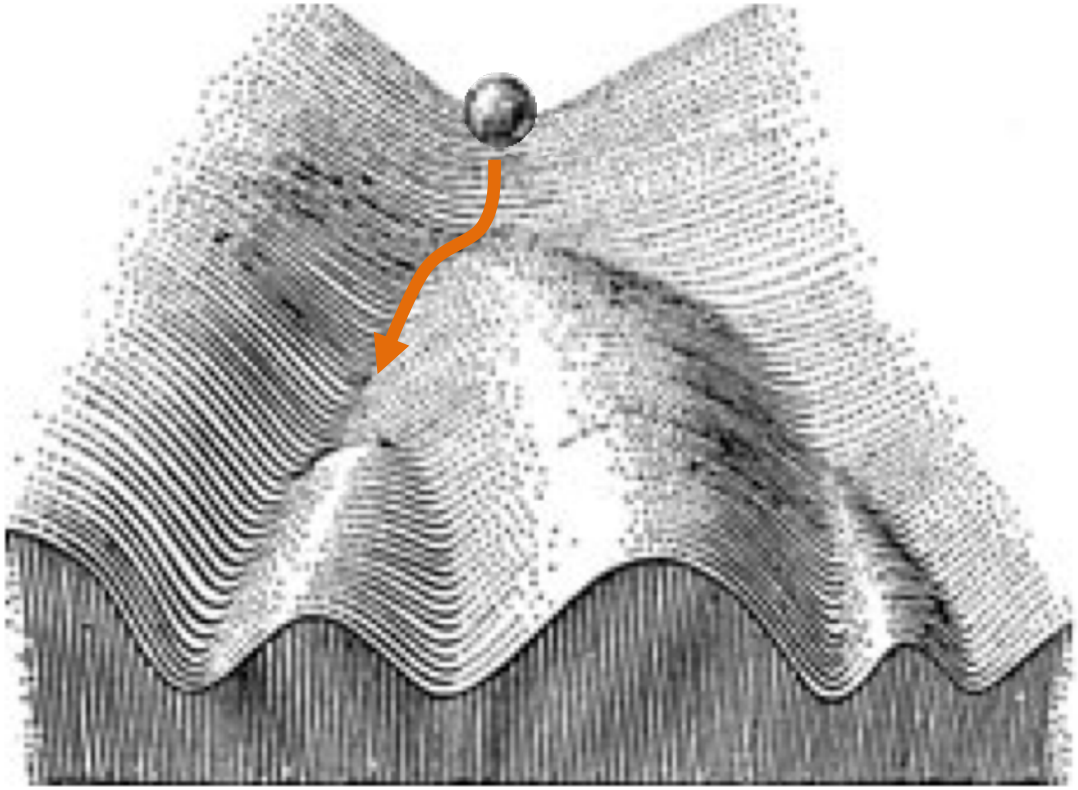
- Waddington Diagram



Waddington, C. H., 1956, Principles of Embryology, op. cit., p. 412

Irreversible differentiation

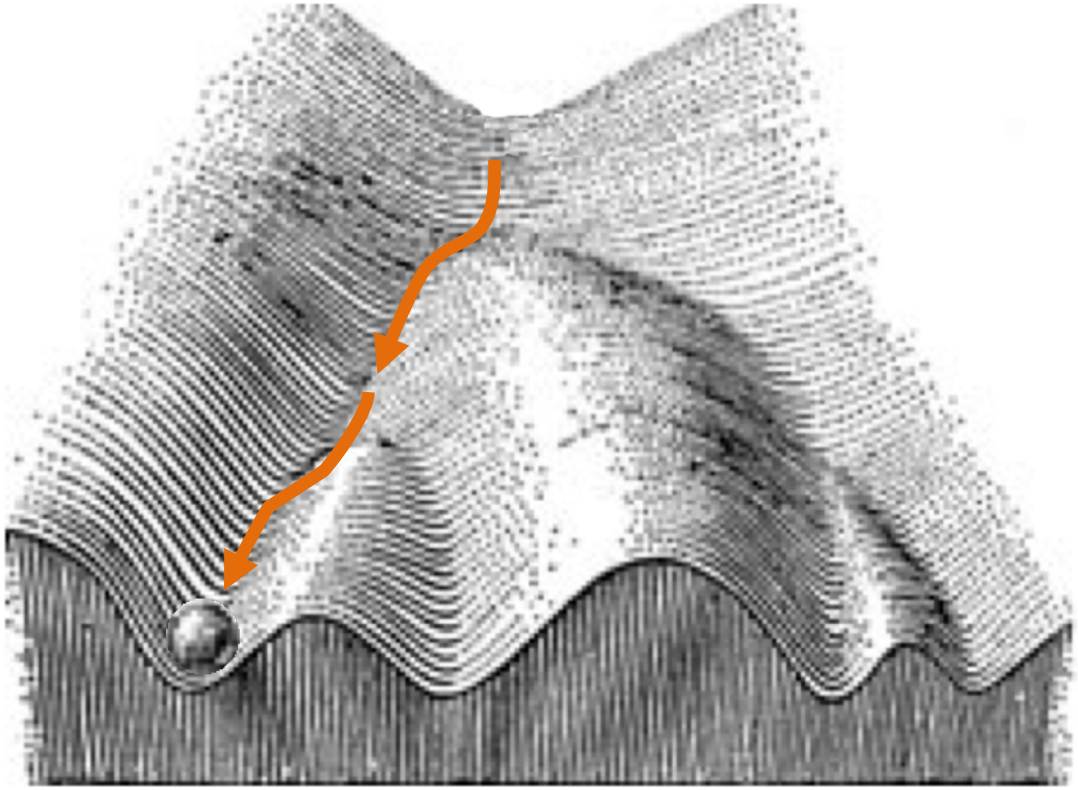
- Transcription factor network



Waddington, C. H., 1956, Principles of Embryology, op. cit., p. 412

Irreversible differentiation

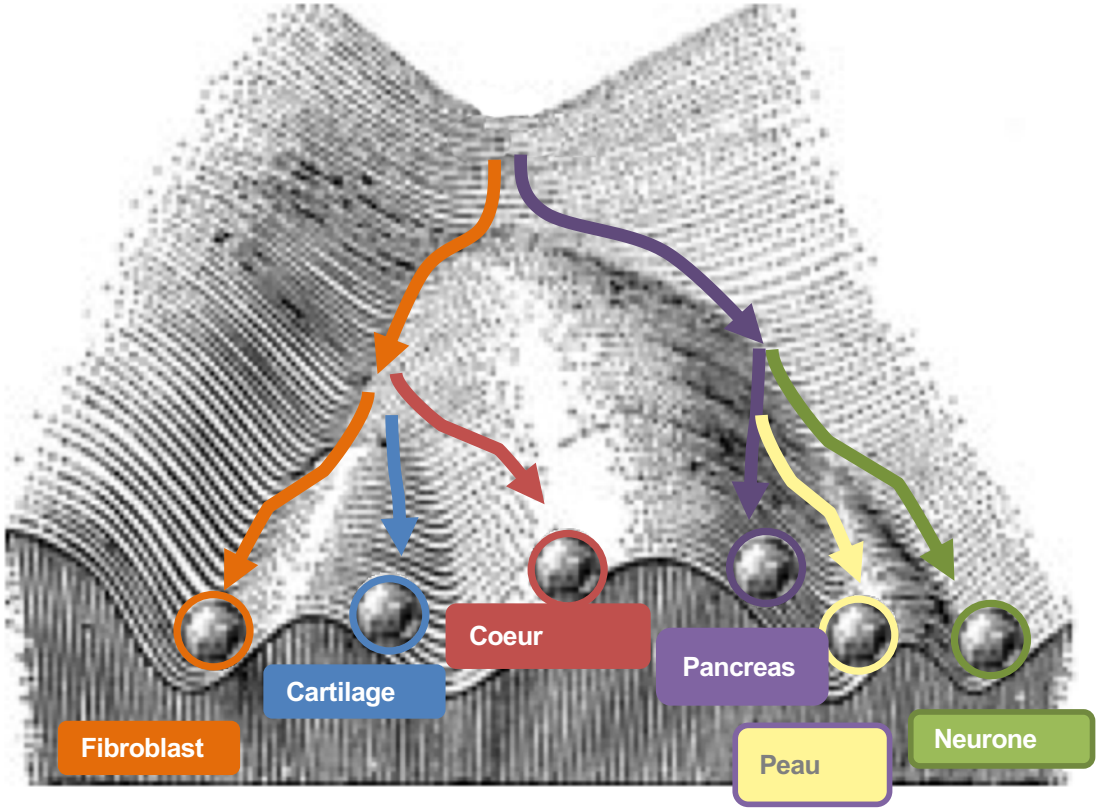
- Transcription factor network
- DNA methylation, histones modifications
- miRNA, lncRNA



Waddington, C. H., 1956, Principles of Embryology, op. cit., p. 412

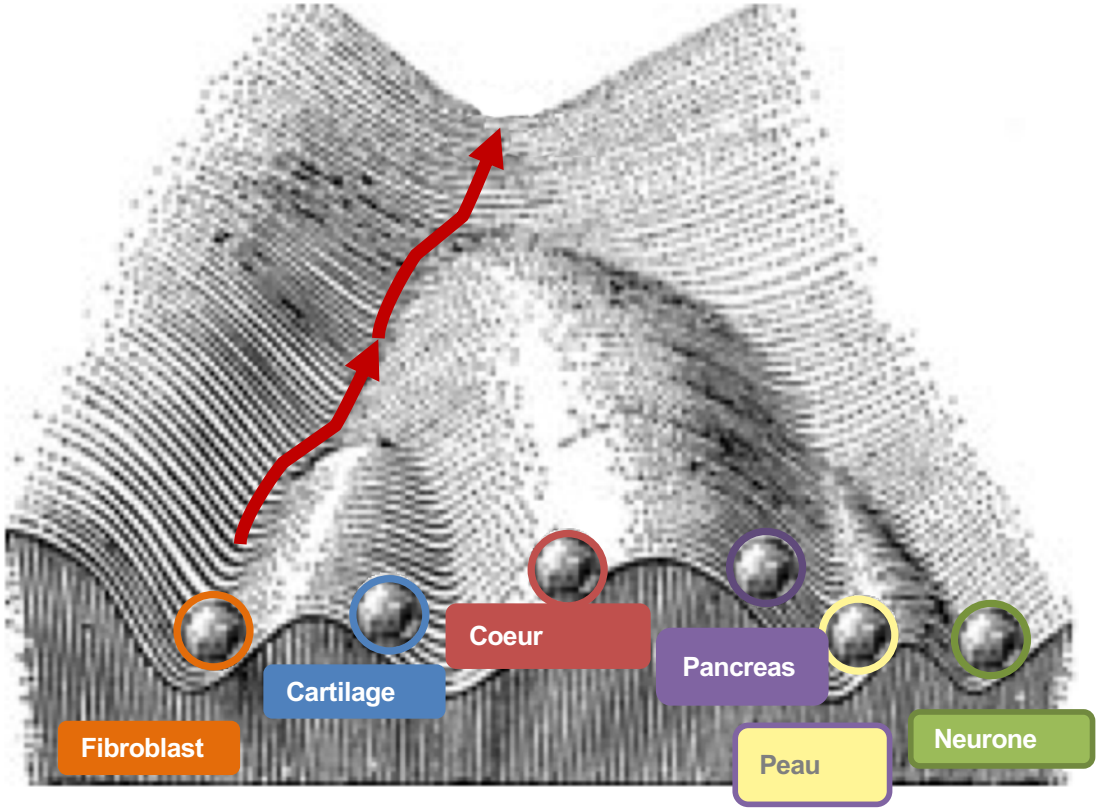
Irreversible differentiation

- Differentiation is irreversible
- Necessary to :
  - Prevent uncontrolled proliferation
  - Prevent accidental cell fate change
- Cells do not have the liberty to decide their fate!



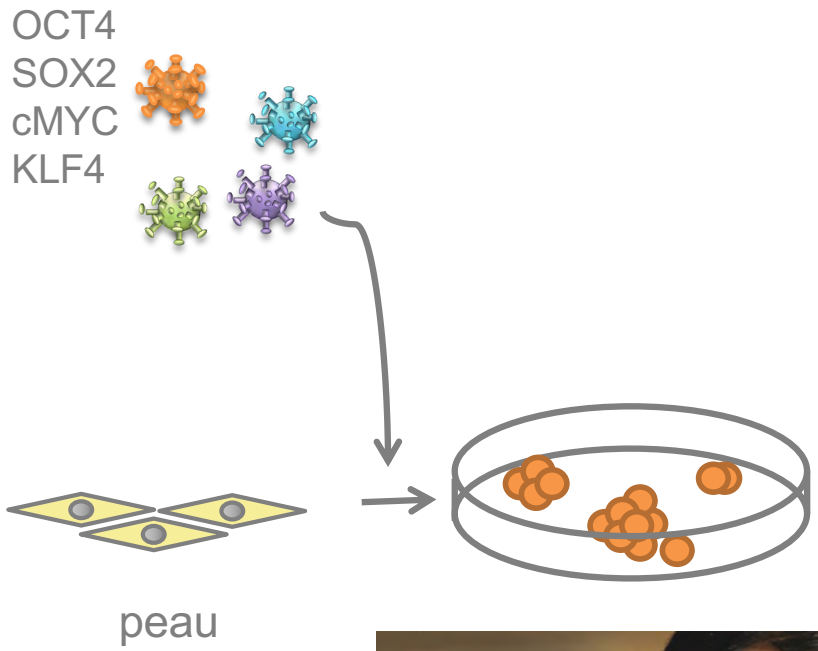
Waddington, C. H., 1956, Principles of Embryology, op. cit., p. 412

Cell reprogramming



Waddington, C. H., 1956, Principles of Embryology, op. cit., p. 412

INDUCED PLURIPOTENT STEM CELLS (IPS)



2006 S Yamanaka – Nobel Price 2012

K. Takahashi and S. Yamanaka. *Cell*, 126:663, 2006.

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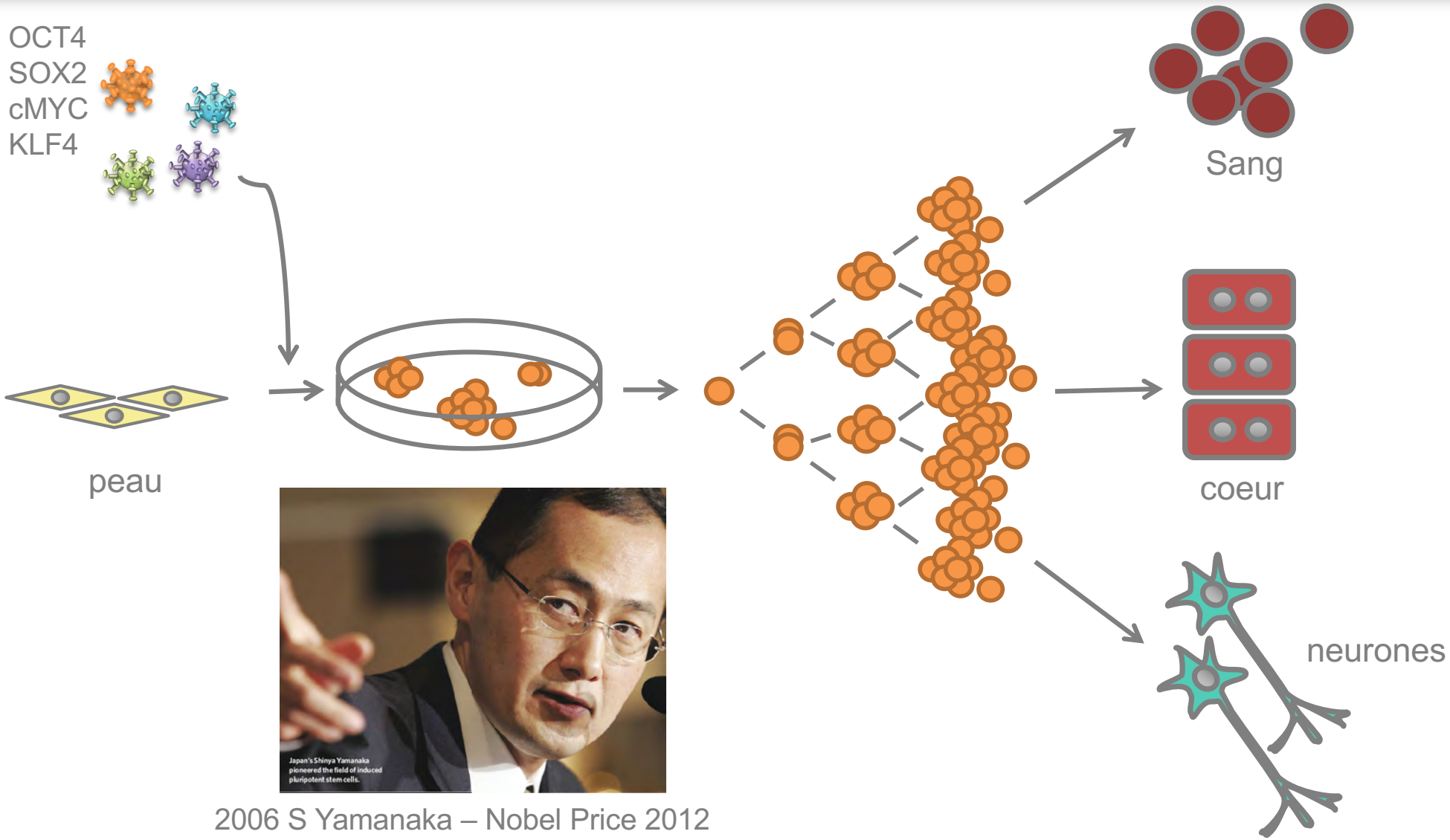

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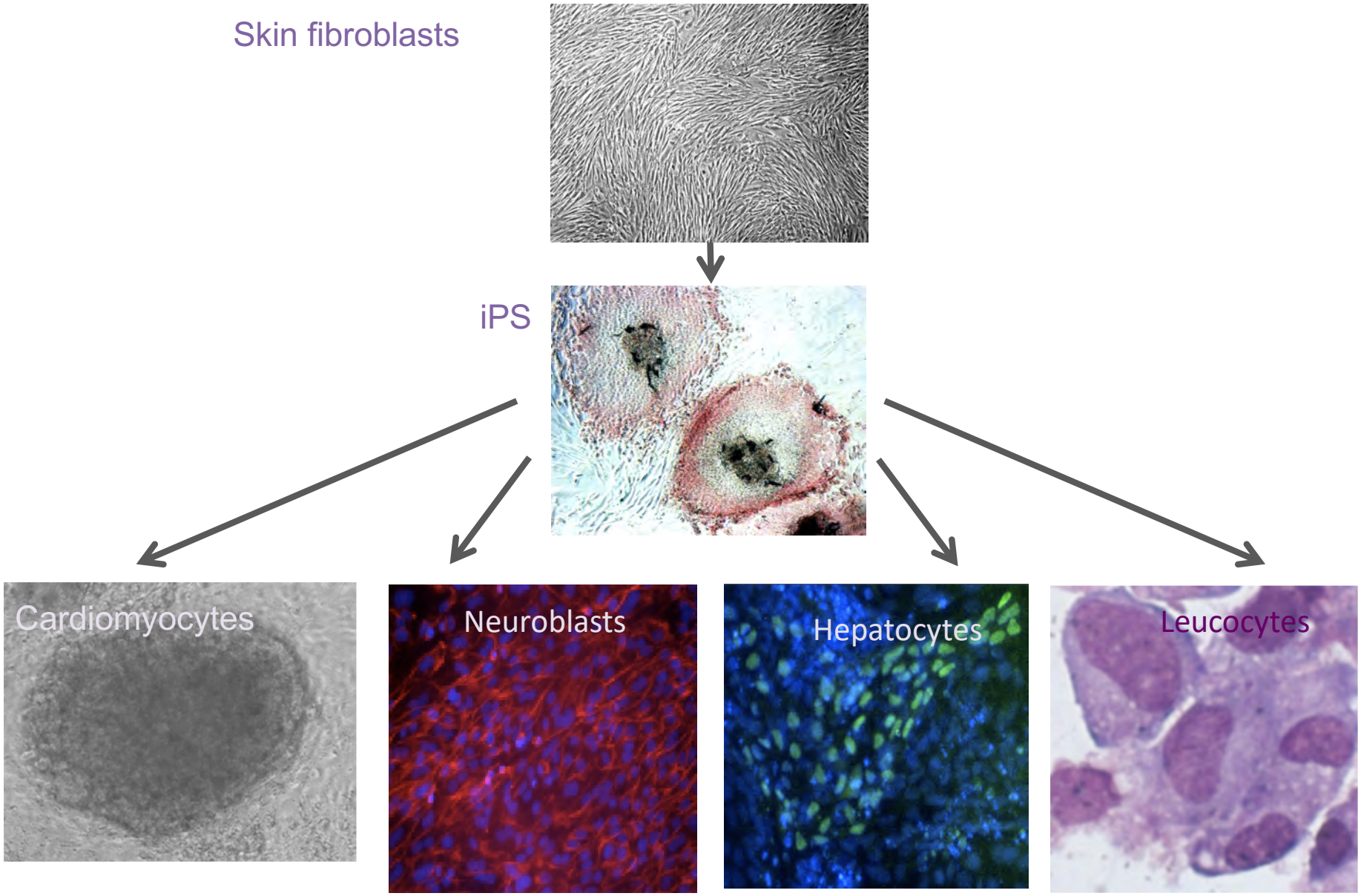
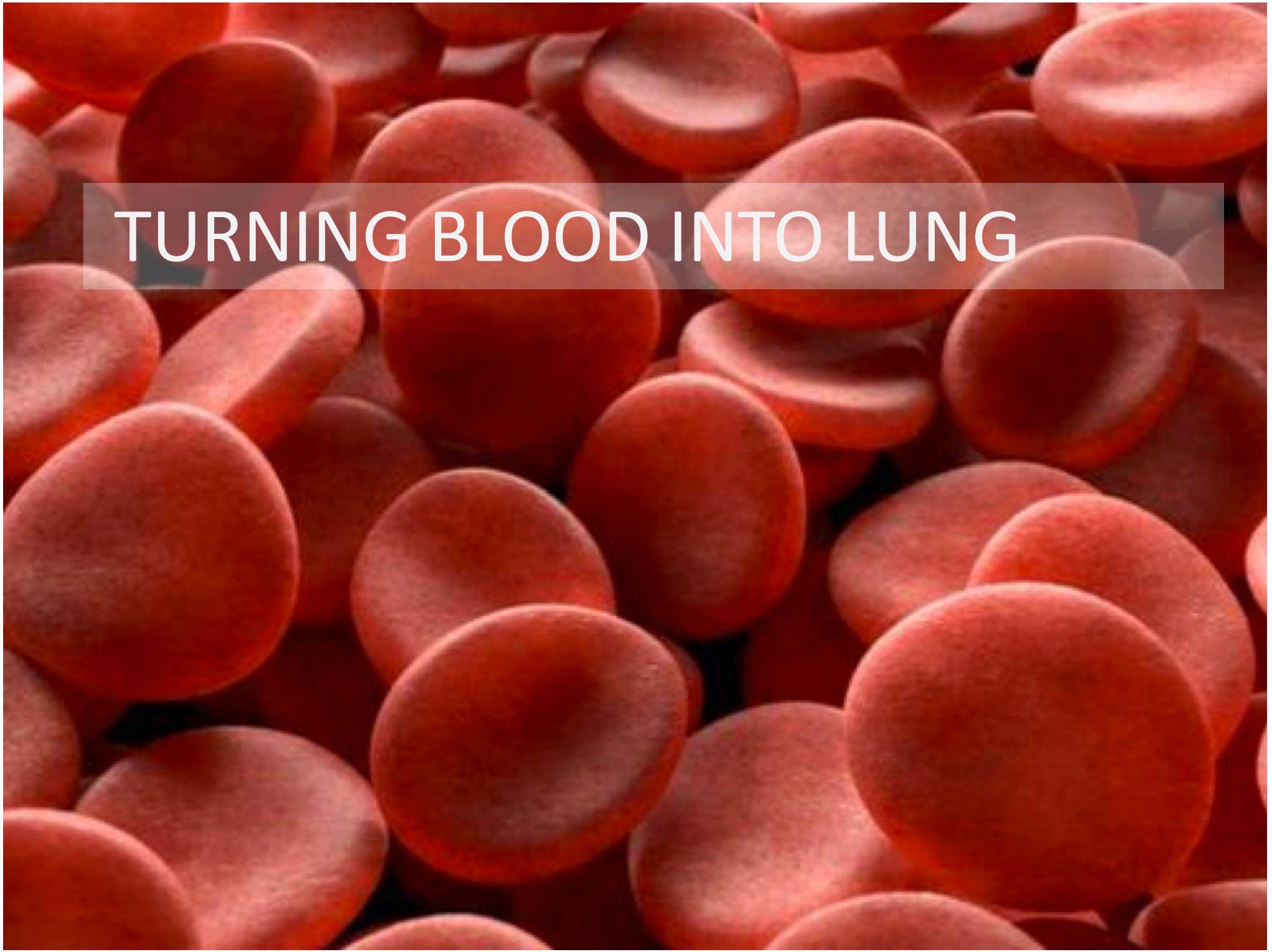
K. Takahashi and S. Yamanaka. *Cell*, 126:663, 2006.

Reproducible results



Applications des iPS

1. In vitro modelling of human normal development and genetic diseases
2. An unlimited source of cells for autologous regenerative medicine
3. A tool to rejuvenate aged or senescent cells

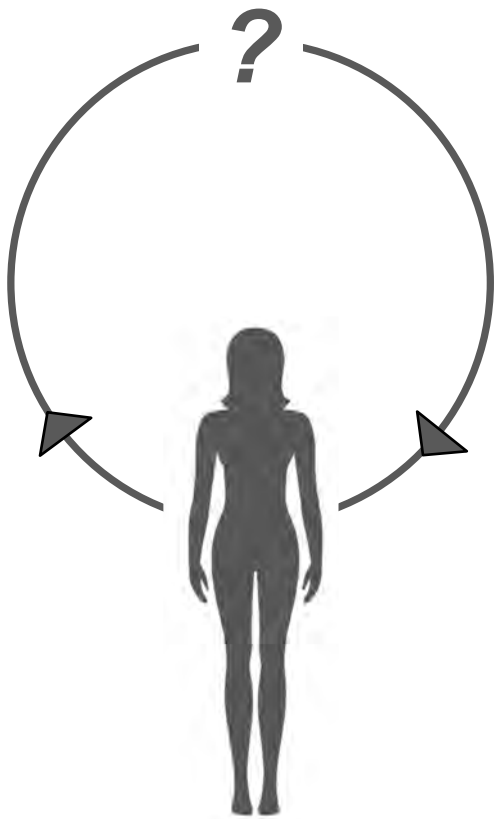

TURNING BLOOD INTO LUNG

LUNG ORGANOIDS

- 3D or … 2D
- Applications
  - Models
    - Infectious diseases
    - Pollution, tobacco
    - Genetic diseases
    - Screening for new treatments
    - Etc
  - Cell source for cell therapy

PRIMARY CILIARY DYSKINESIA IN THE LUNG: AN IPSC MODEL (iALI)

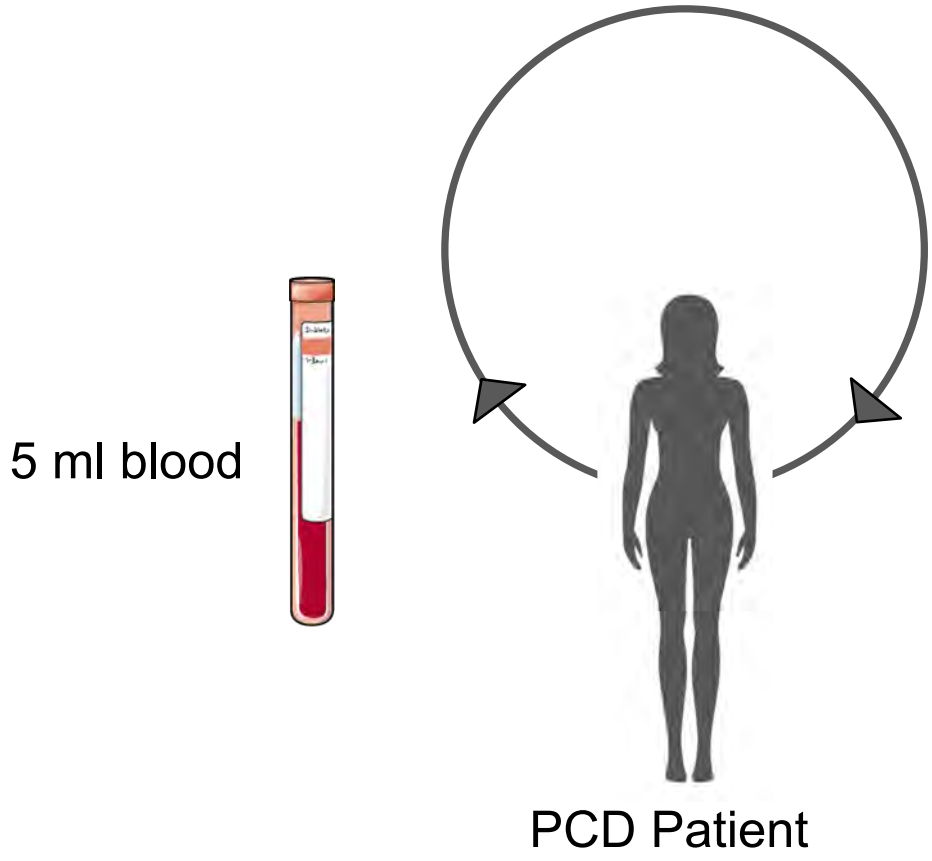
Gene and cell therapy for PCD



PCD Patient

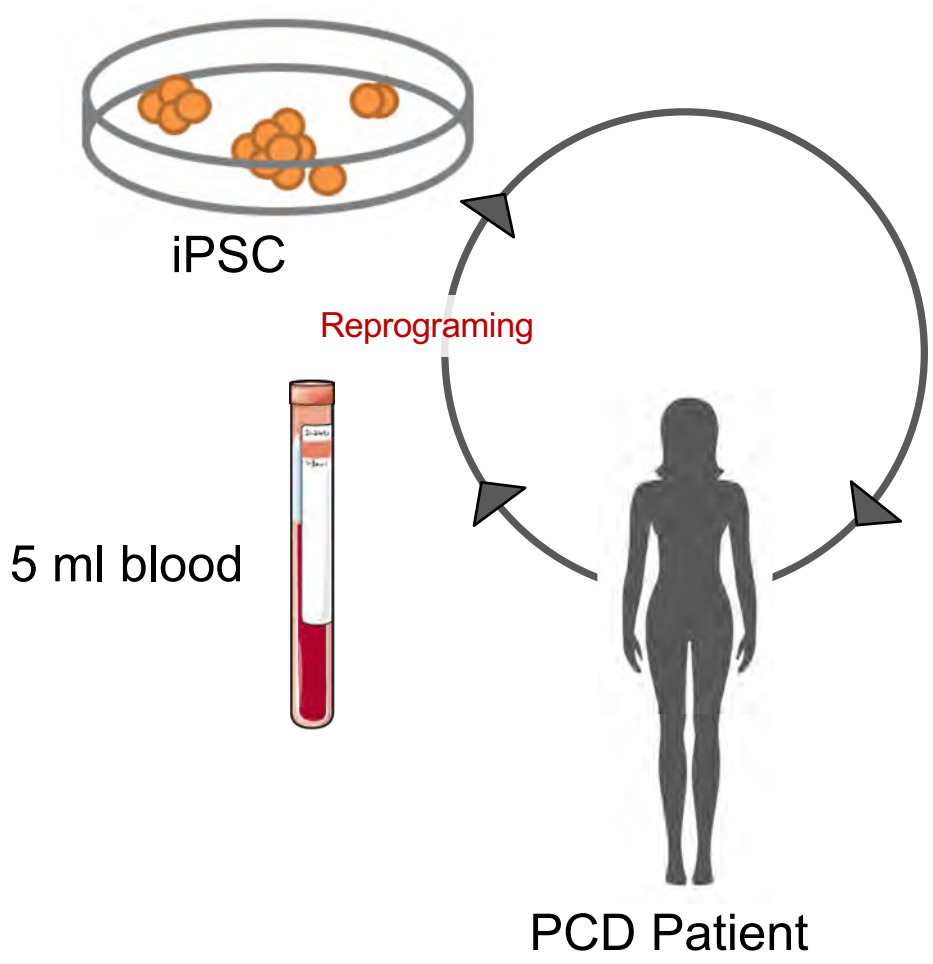
Ex vivo gene therapy

Gene and cell therapy for PCD



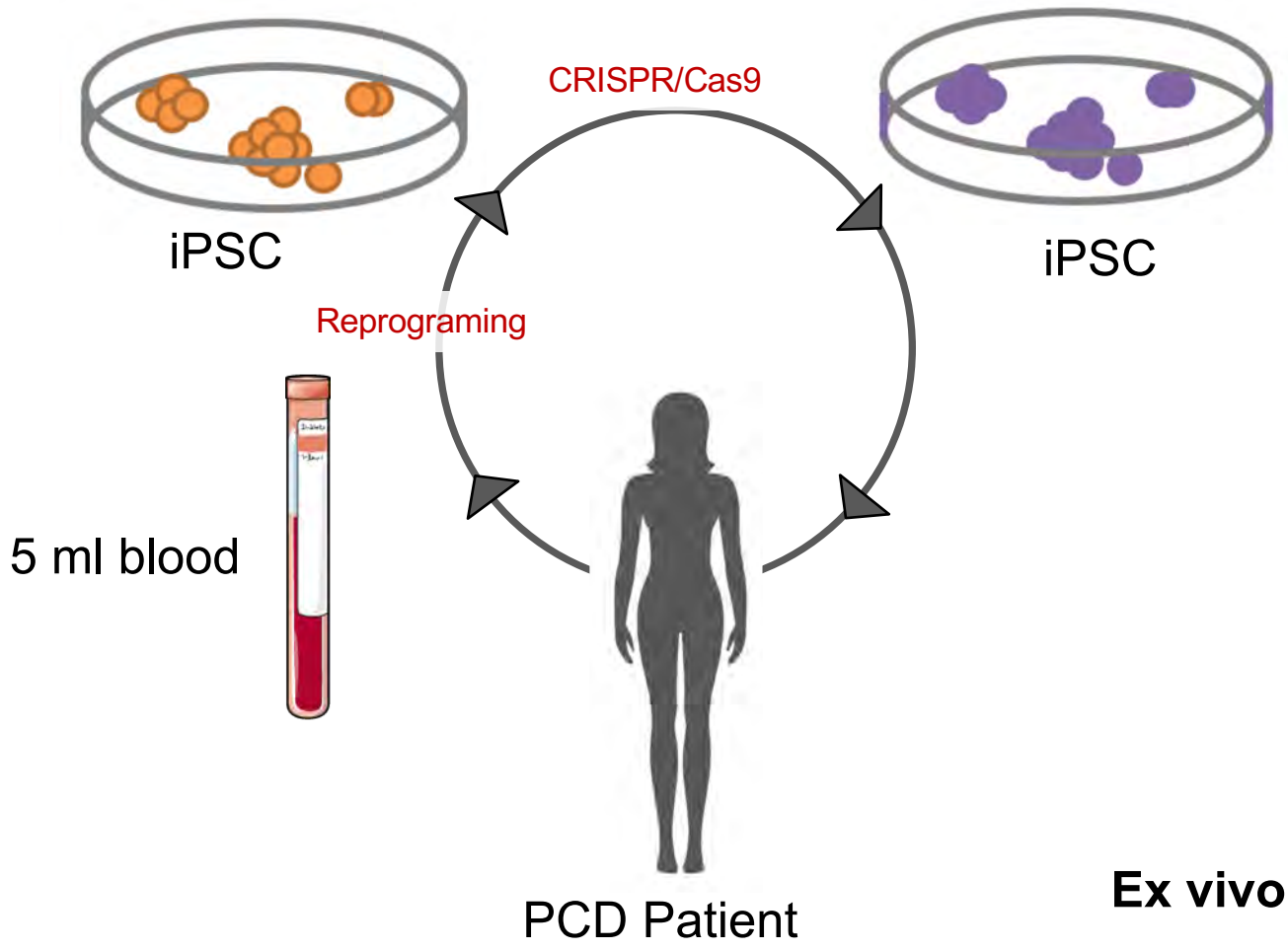
Ex vivo gene therapy

Gene and cell therapy for PCD



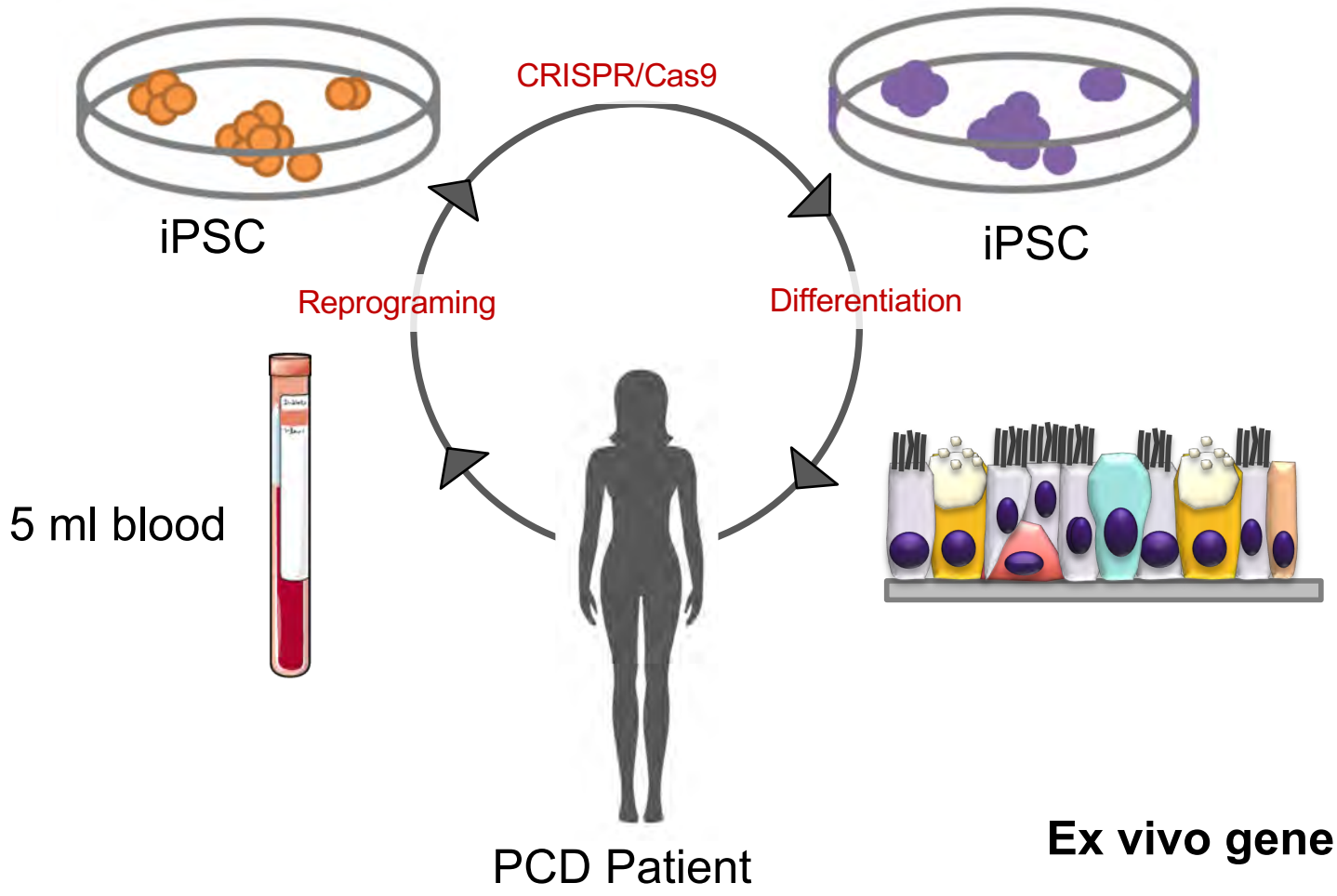
Ex vivo gene therapy

Gene and cell therapy for PCD

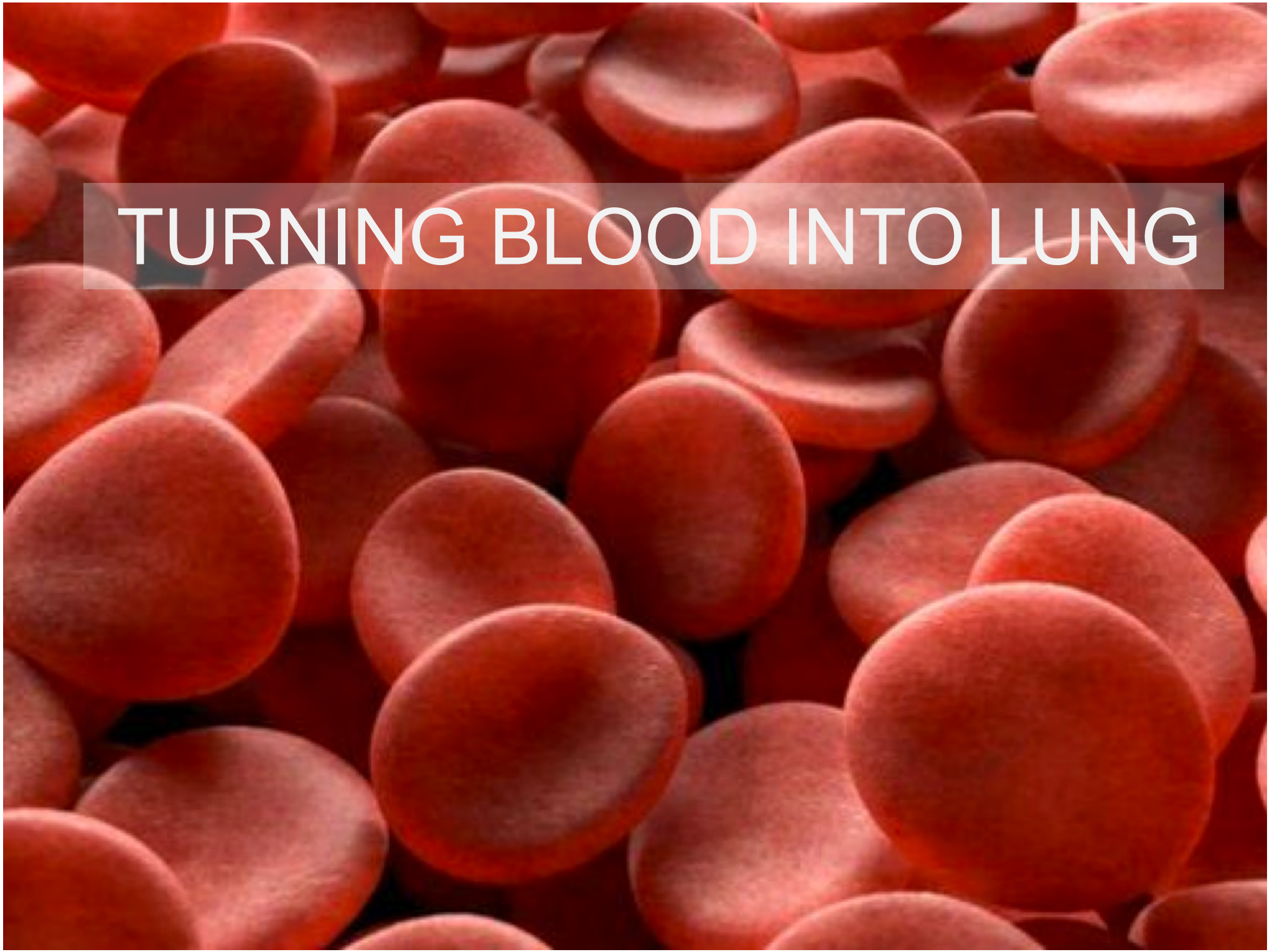


Ex vivo gene therapy

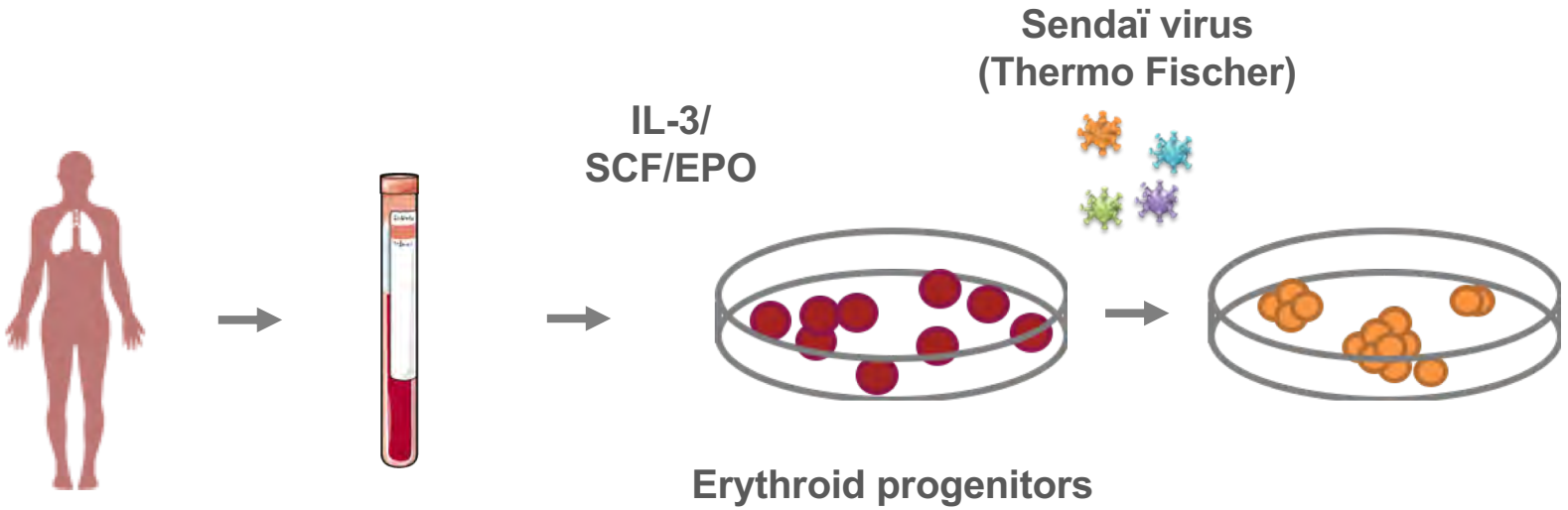
Gene and cell therapy for PCD



Ex vivo gene therapy


TURNING BLOOD INTO LUNG

TURNING BLOOD INTO LUNG

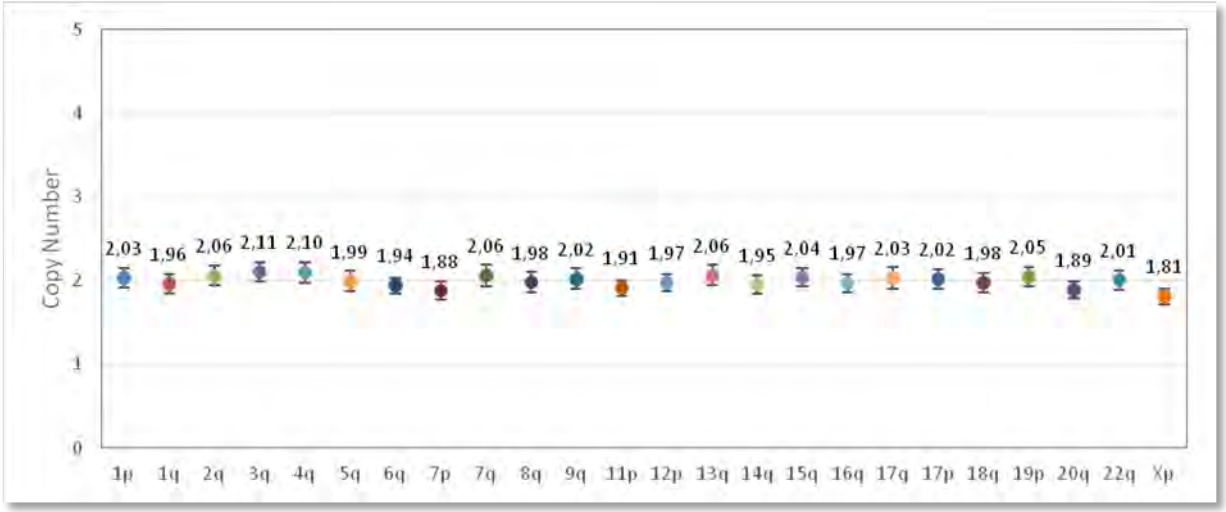


Ahmed E, bioRxiv
https://www.biorxiv.org/content/10.1101/2020.11.29.400358v1

Engi Ahmed, CCA
en pneumologie

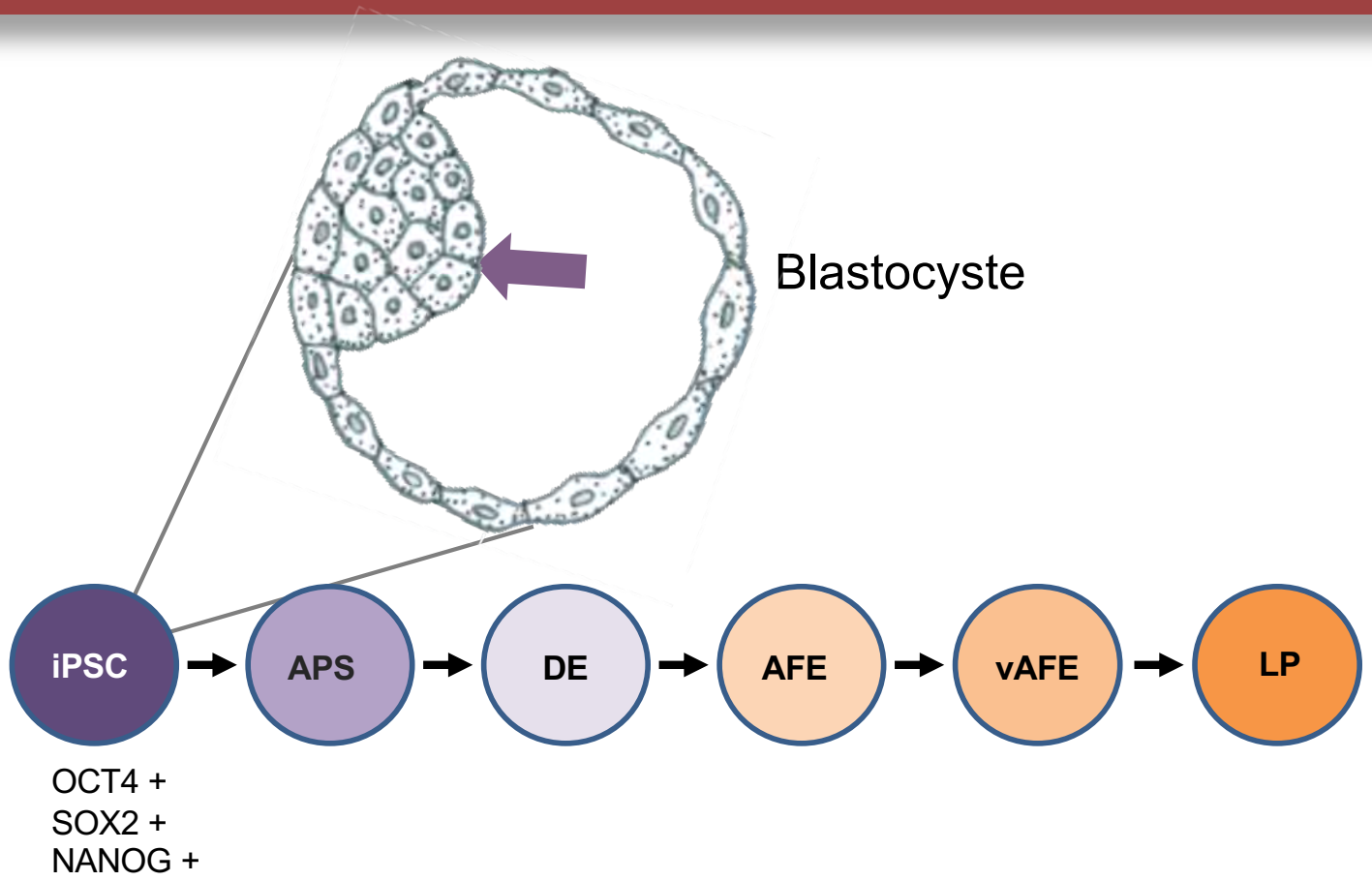
iPSC DERIVATION

- 7 lines of which:
  - 1 healthy donor
  - 4 COPD, 1 heavy smoker without COPD
  - 1 primary ciliary dyskinesia (CCDC40)
- Quality controls including genetic integrity



stemgenomics cell integrity

DIFFERENTIATION: MIMIC HUMAN DEVELOPPEMENT



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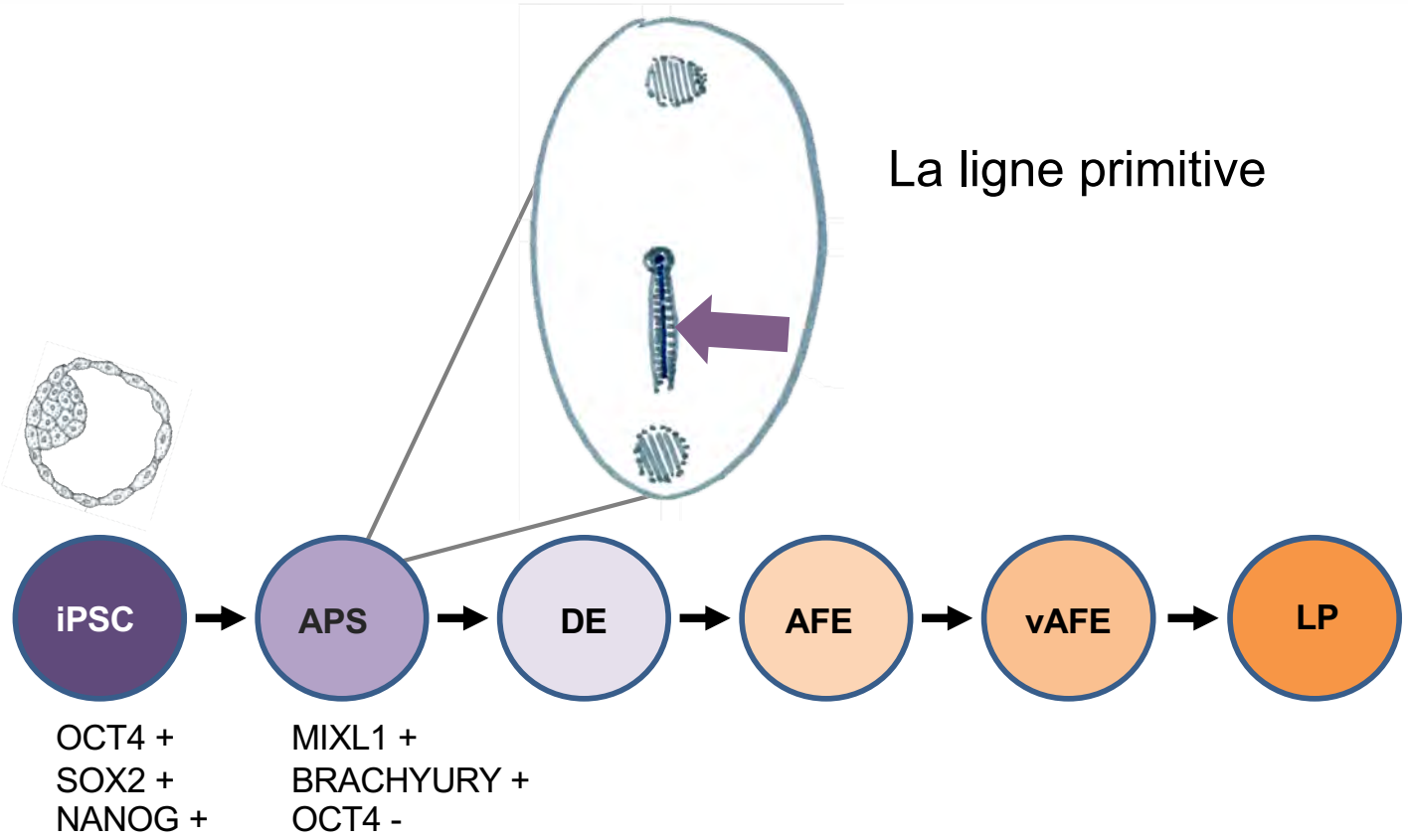

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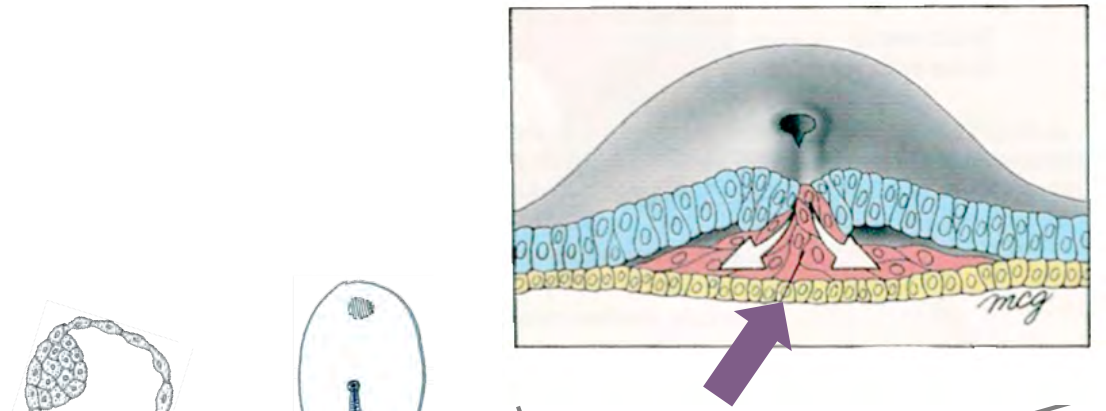
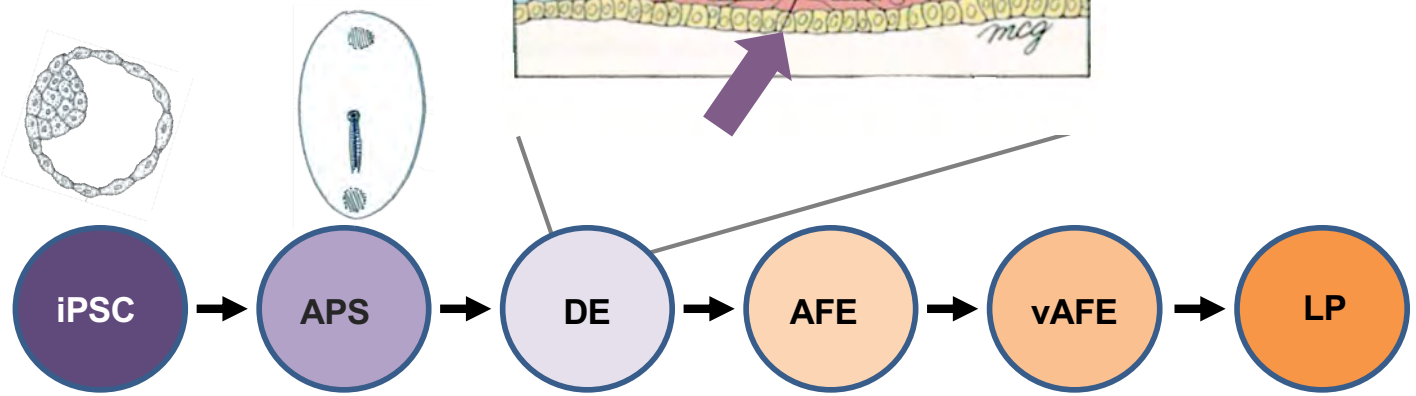


Endoderme définitif



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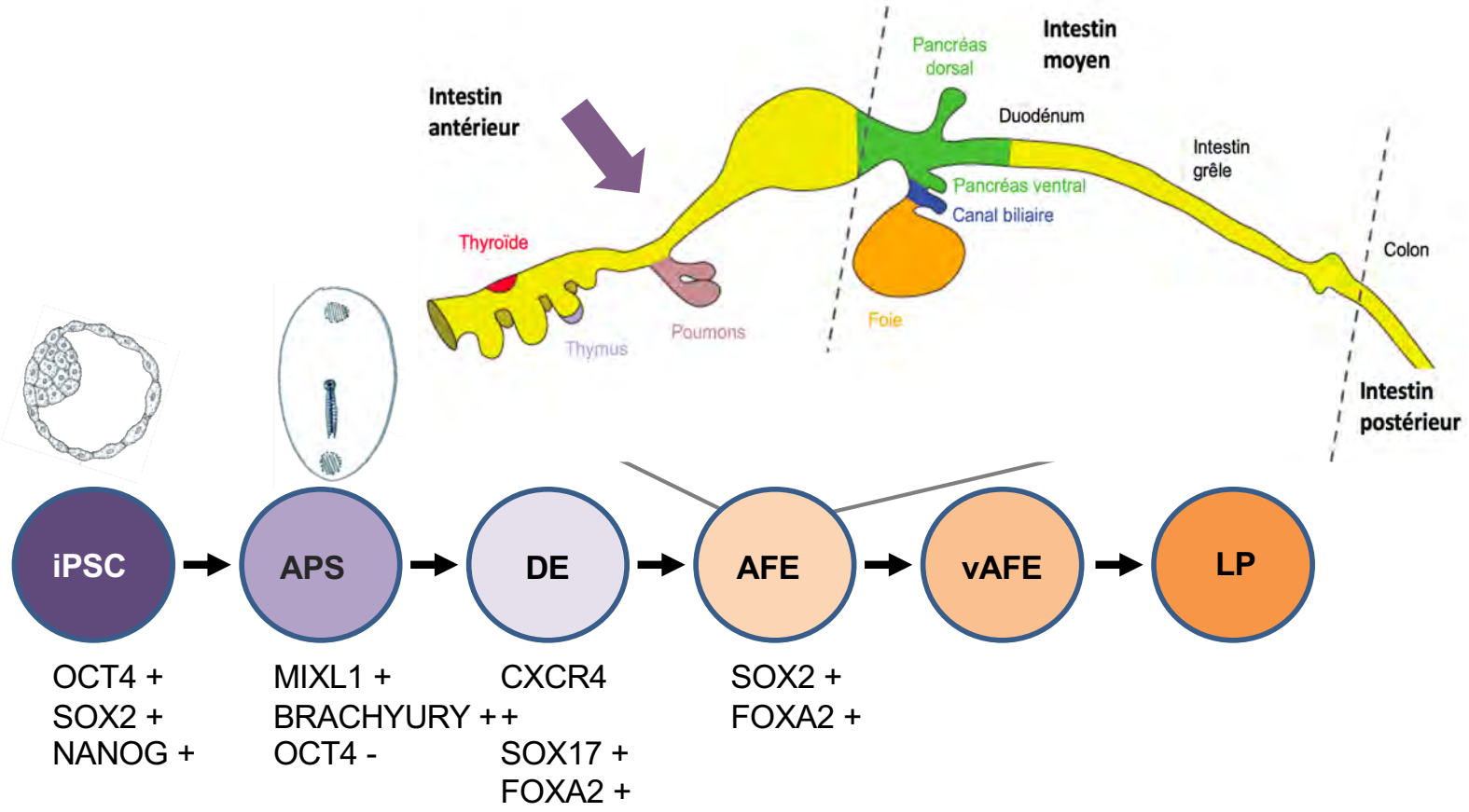
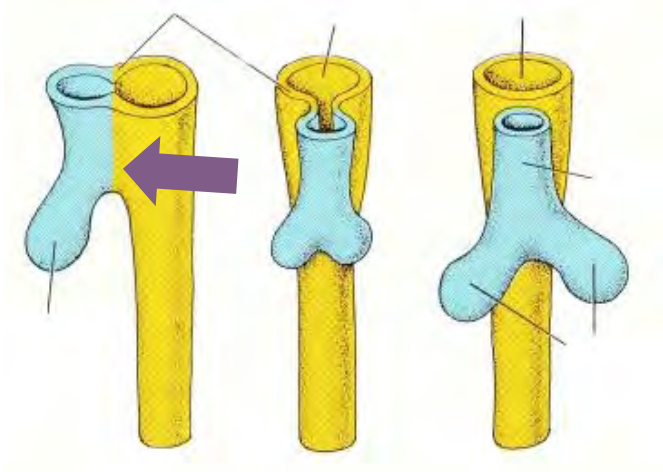
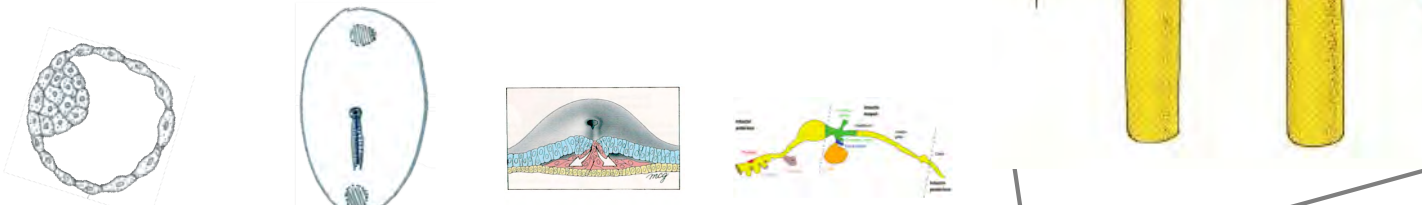


DIFFERENTIATION: MIMIC HUMAN DEVELOPPEMENT

Intestin antérieur ventralisé





iPSC → APS → DE → AFE → vAFE → LP

OCT4 +
SOX2 +
NANOG +

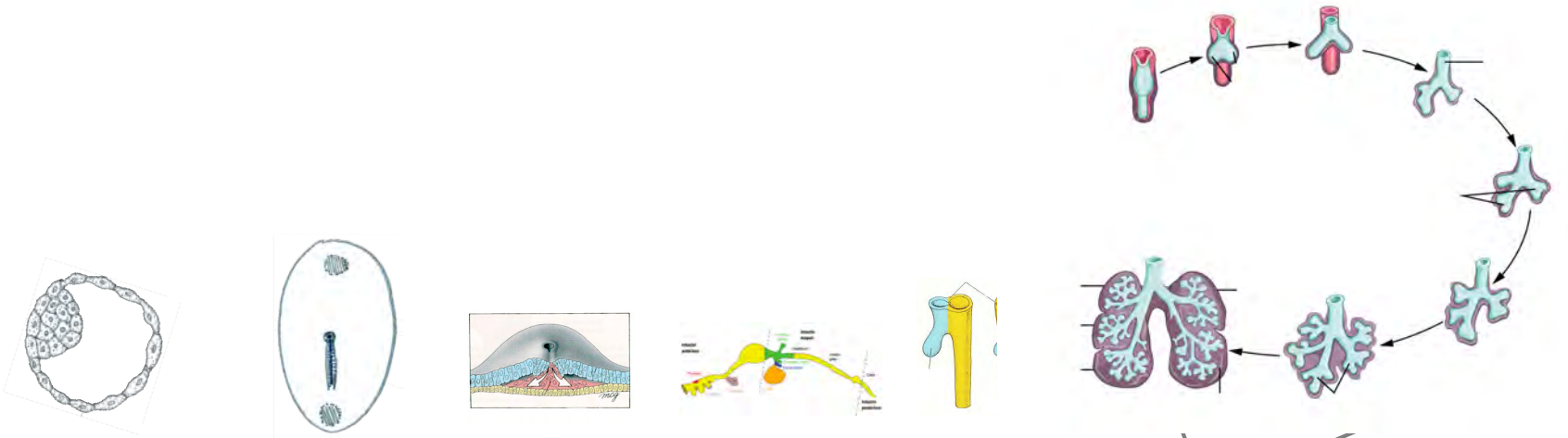
MIXL1 +
BRACHYURY + +
OCT4 -

CXCR4
SOX17 +
FOXA2 +

SOX2 +
FOXA2 +

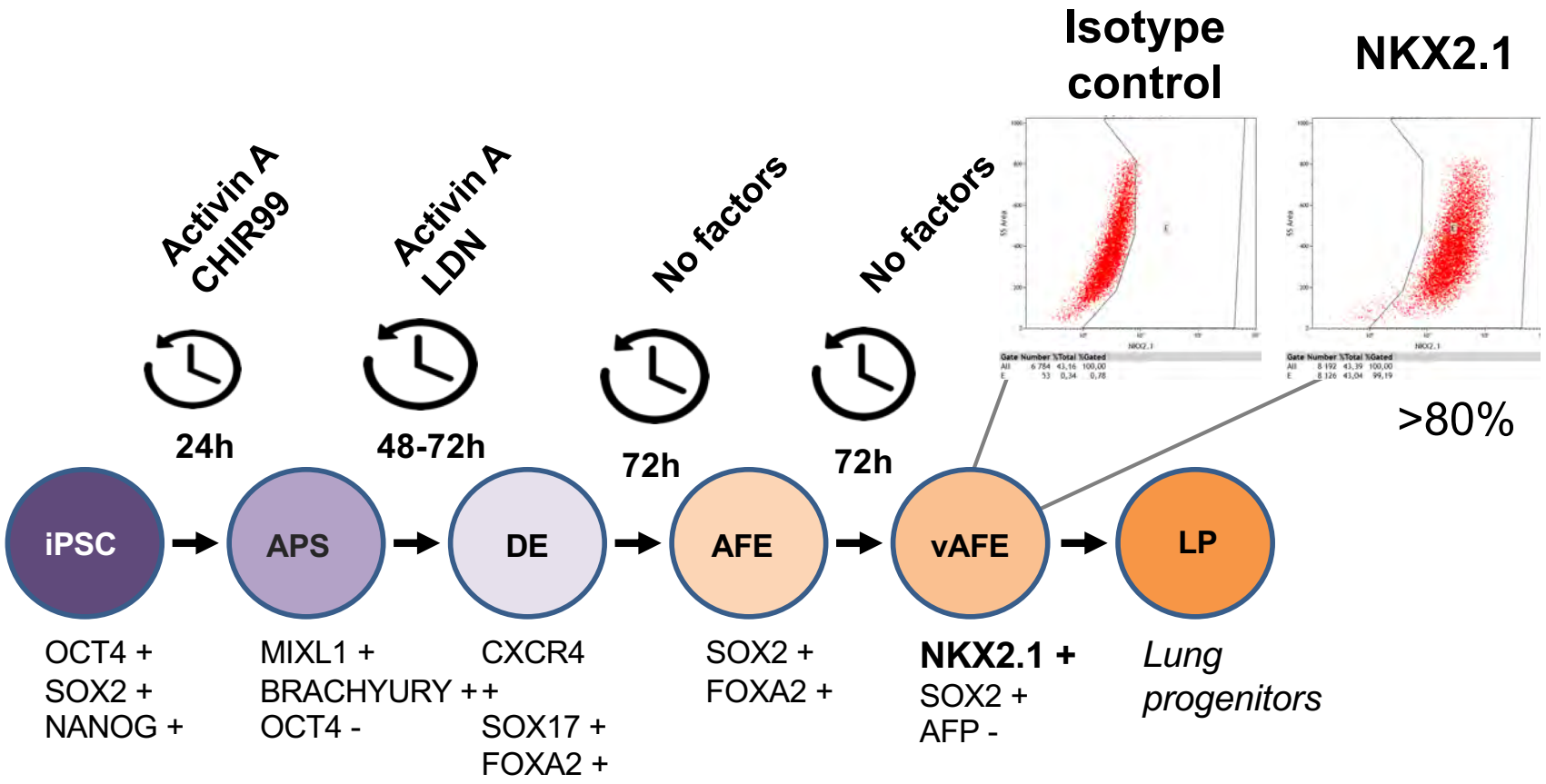
NKX2.1 +
SOX2 +
AFP -

DIFFERENTIATION: MIMIC HUMAN DEVELOPPEMENT



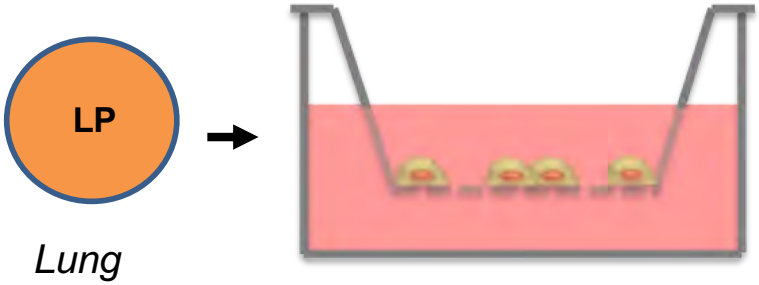
DIFFERENTIATION: MIMIC HUMAN DEVELOPPEMENT

Engi Ahmed



DIFFERENTIATION: MIMIC HUMAN DEVELOPPEMENT

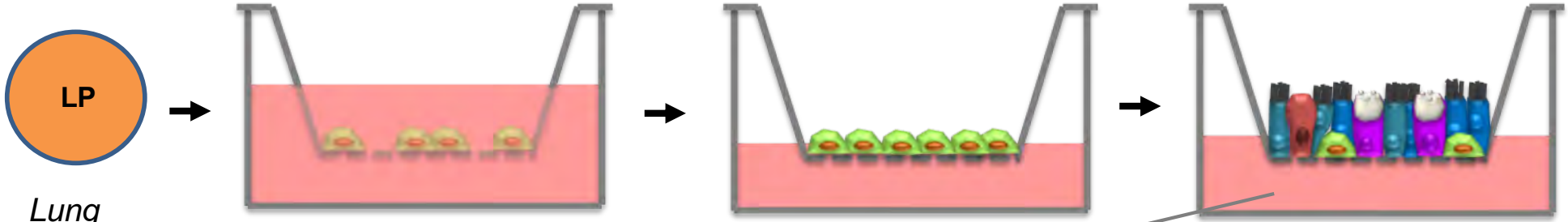
Mathieu Fieldès
Interne en pharmacie



Lung progenitors

iALI

Mathieu Fieldès
Interne en pharmacie

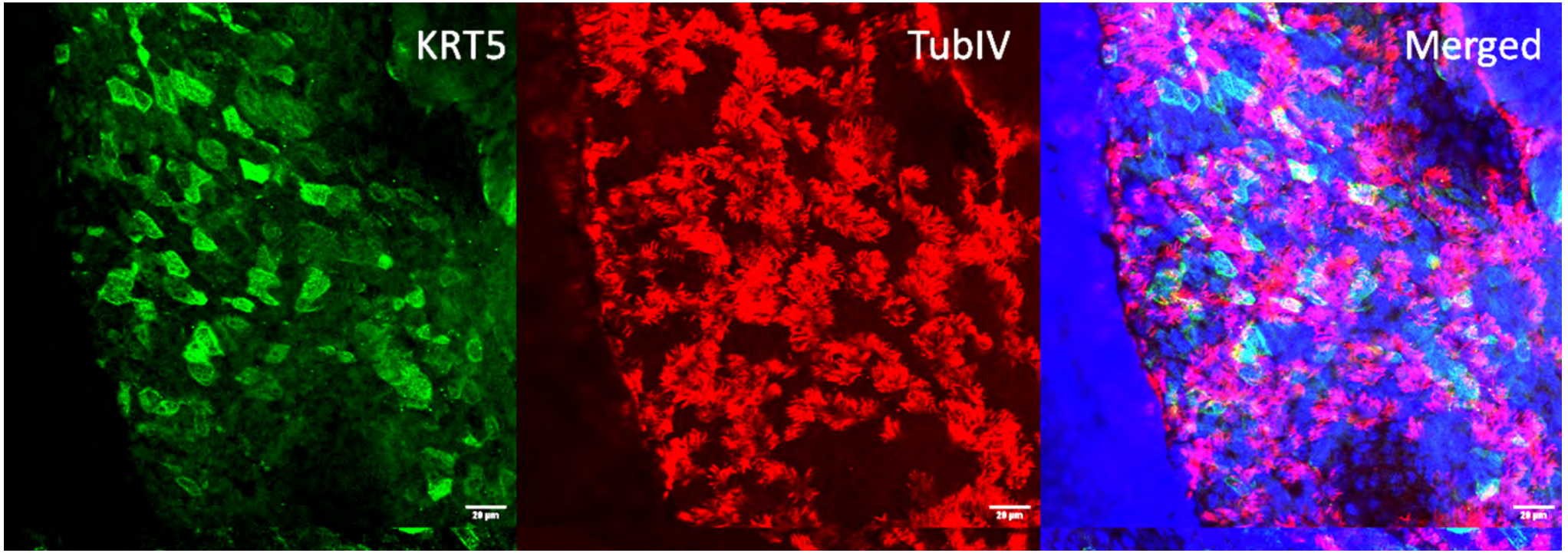


Lung progenitors

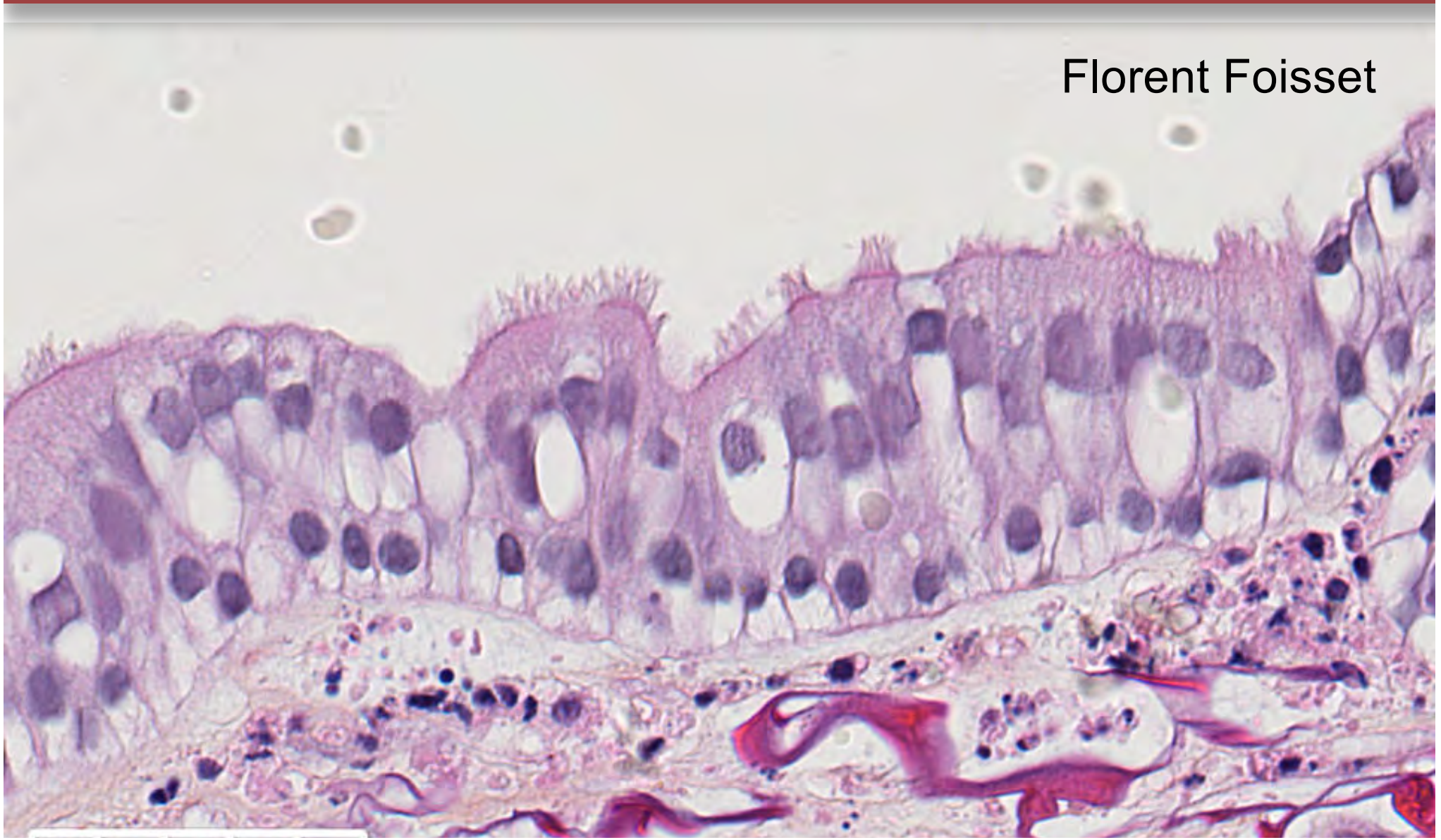
PneumaCult (Stem Cells Technologies)
+ DAPT

2 - 3 weeks

BASAL & CILIATED CELLS

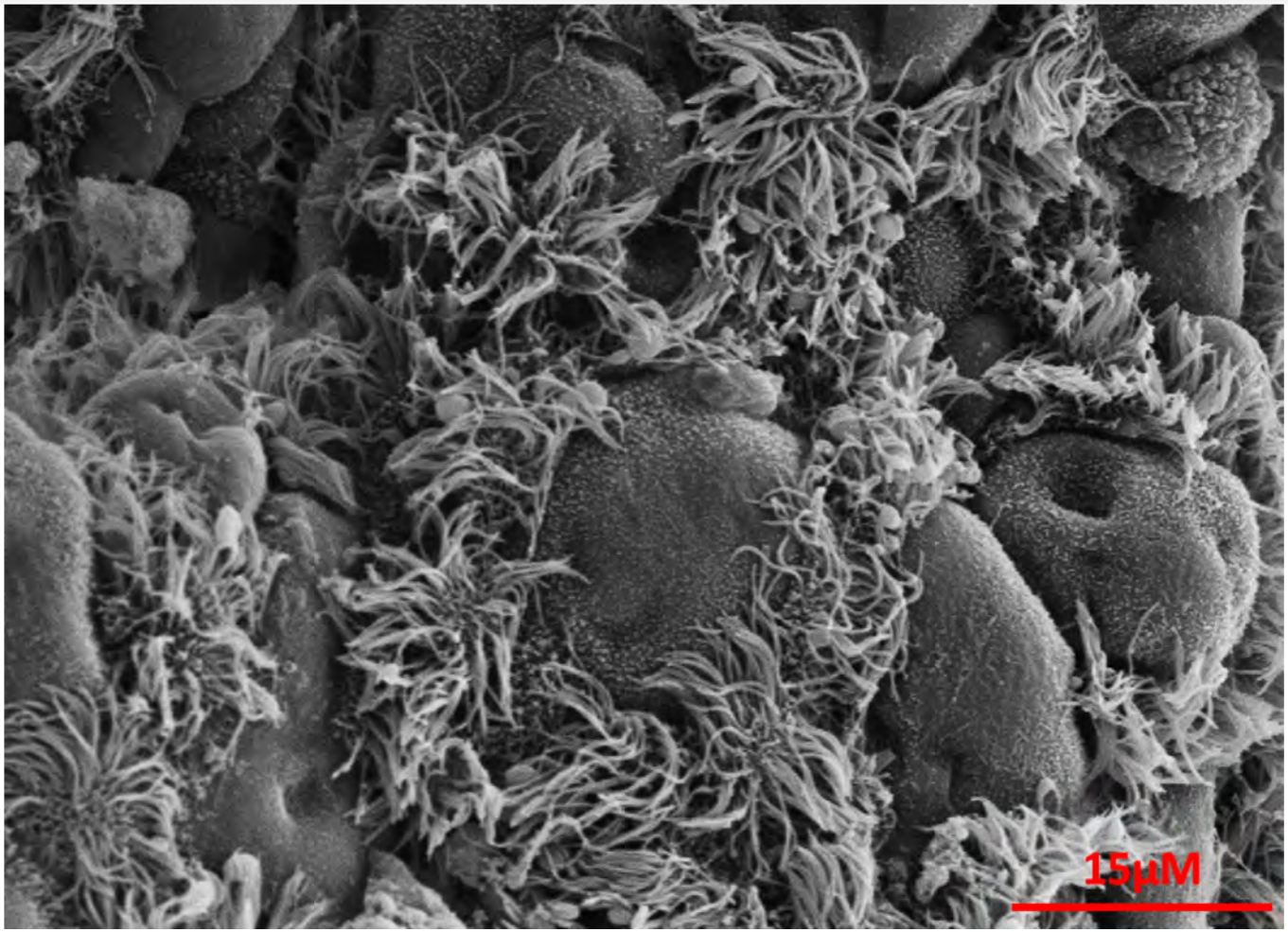


CILIATED CELLS

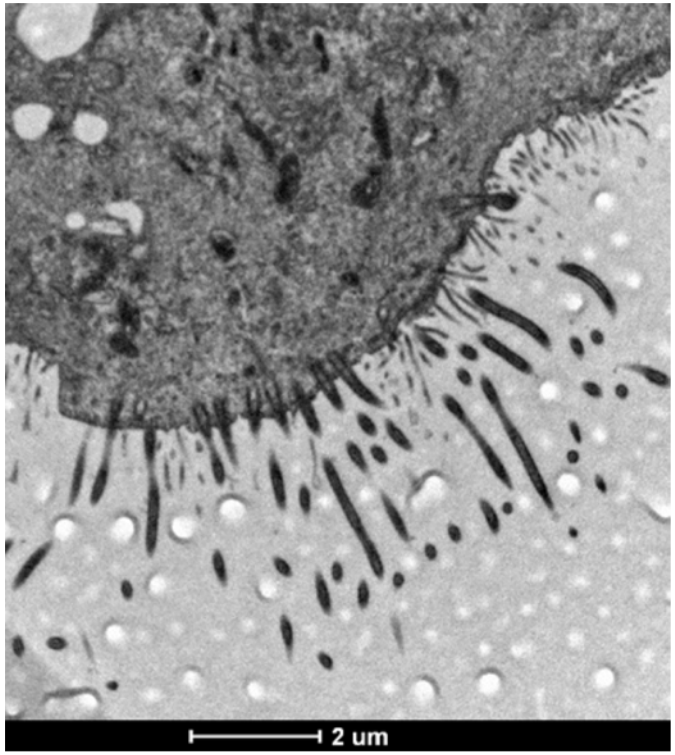


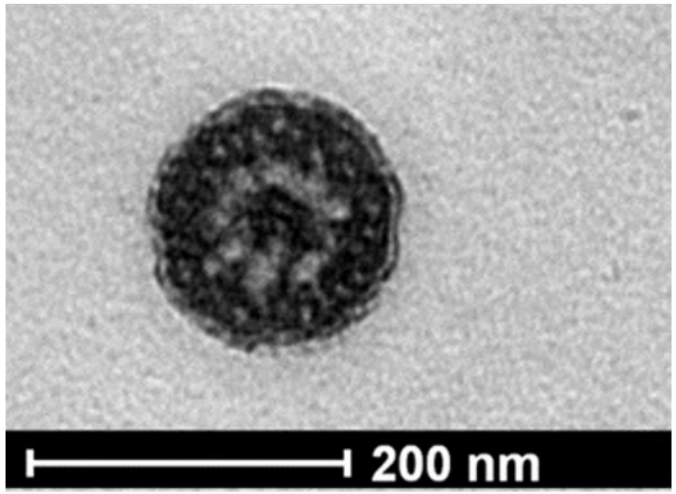
CILIATED CELLS

Scanning electron microscopy



CILIATED CELLS

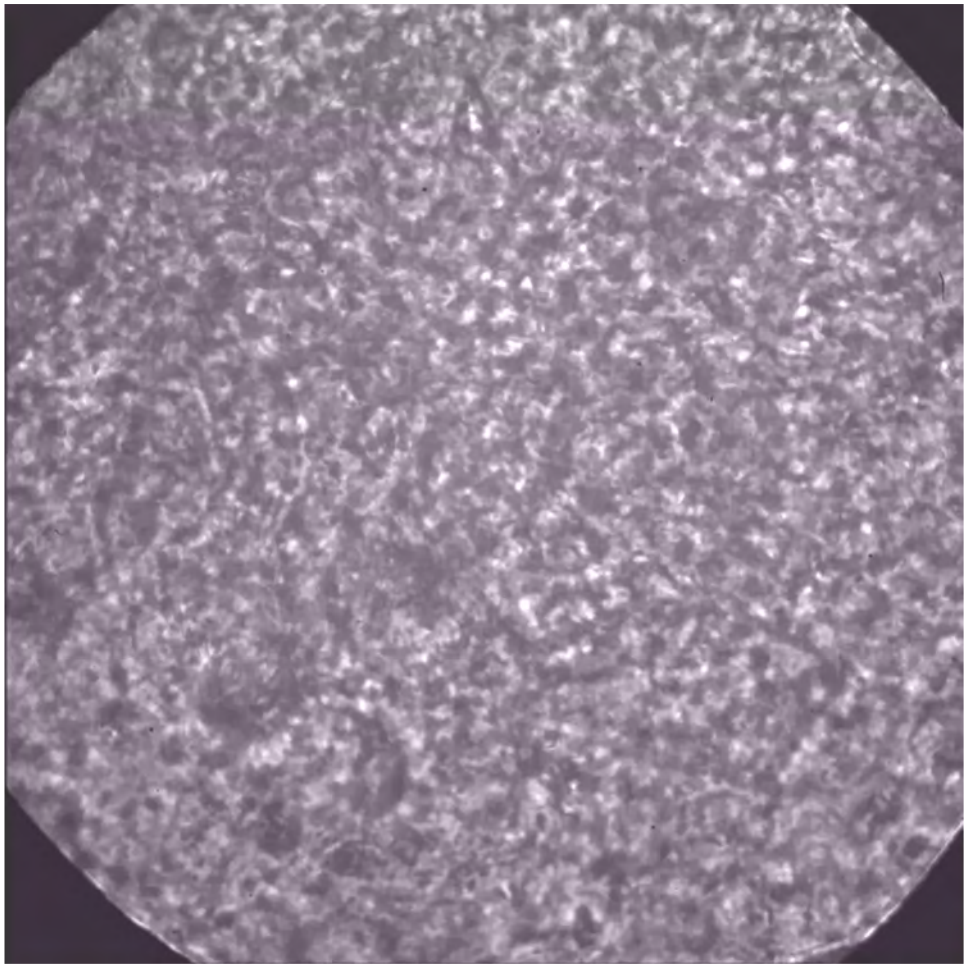




TEM

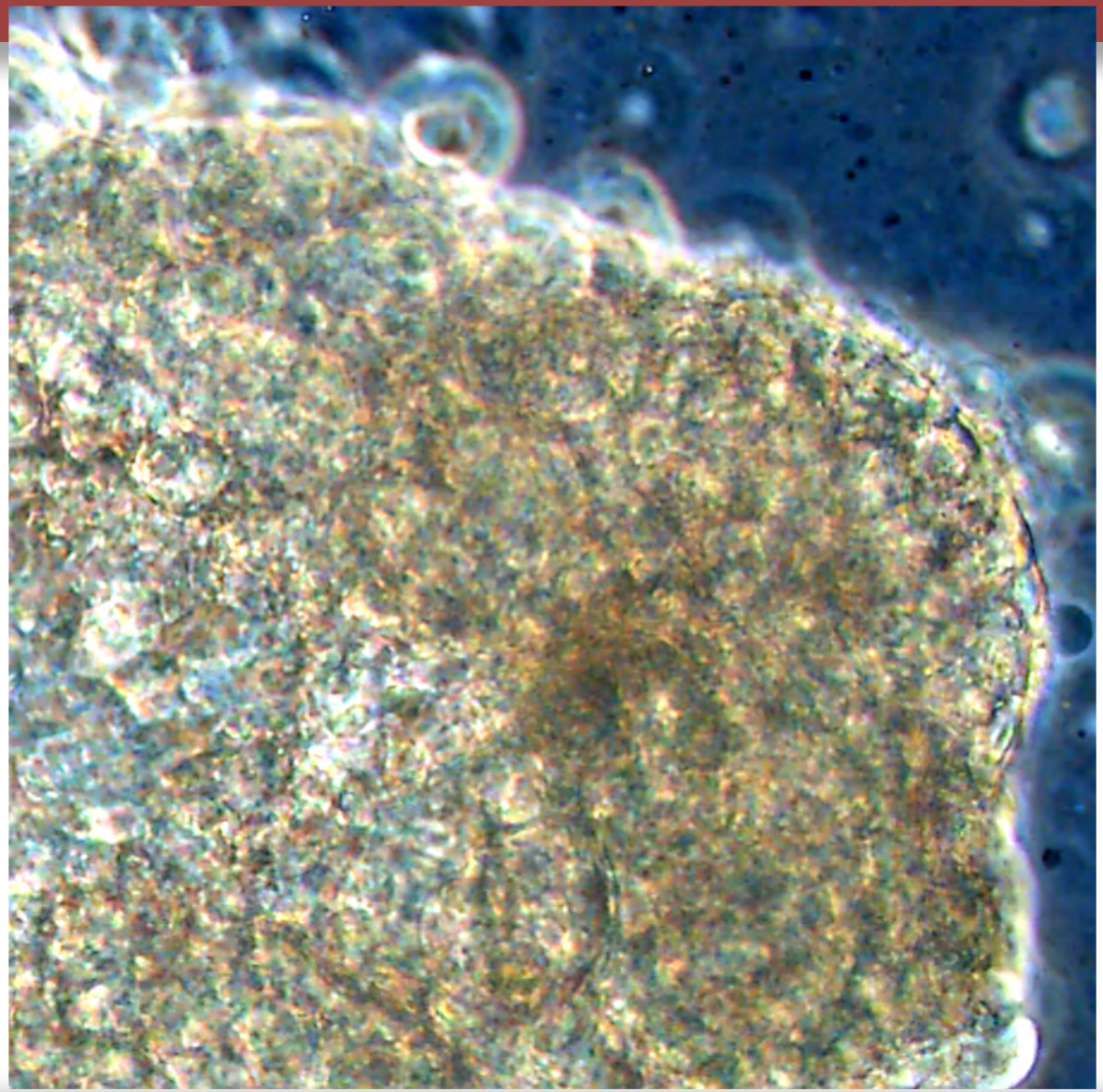
CILIATED CELLS

Digital high-speed camera footage



CILIATED CELLS

Digital high-speed camera footage



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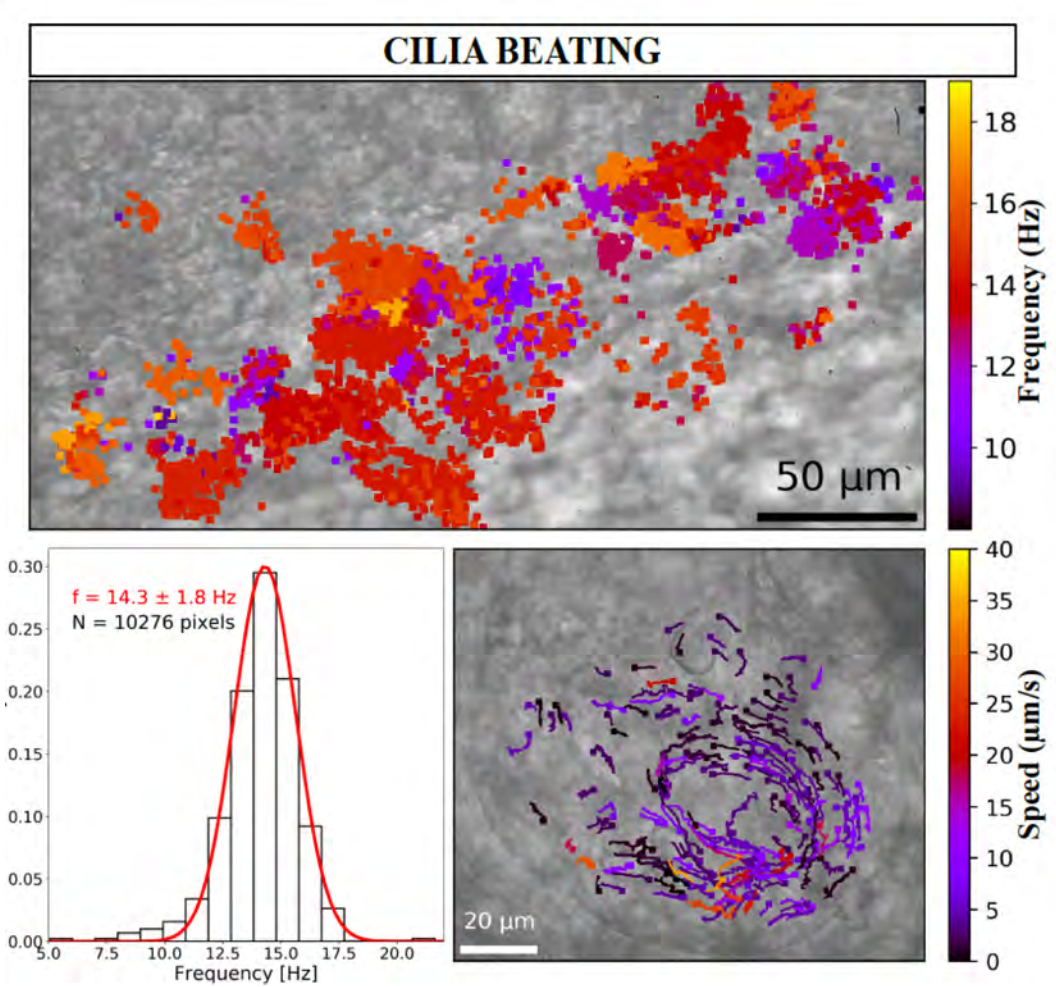
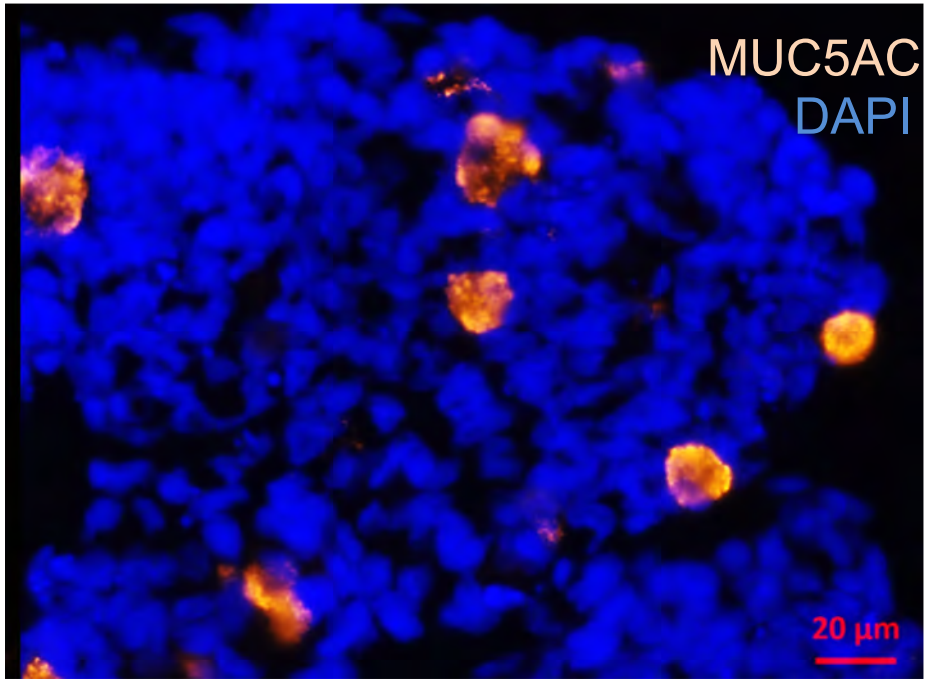
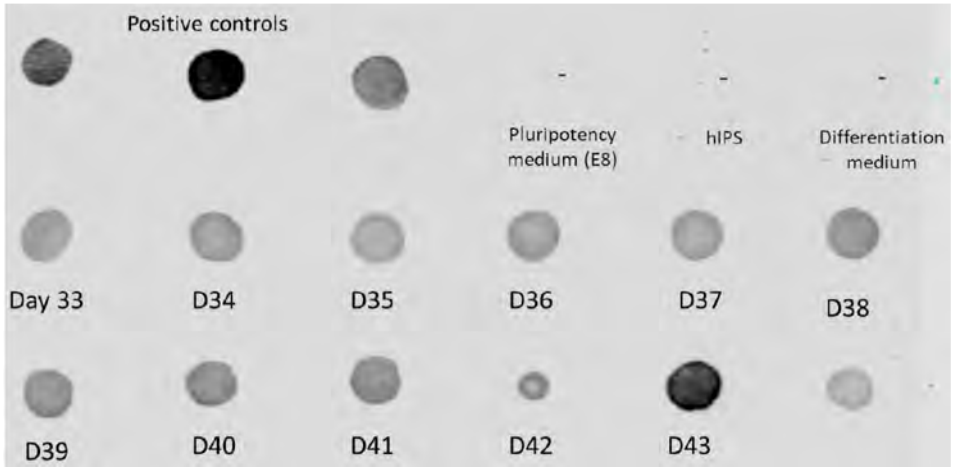


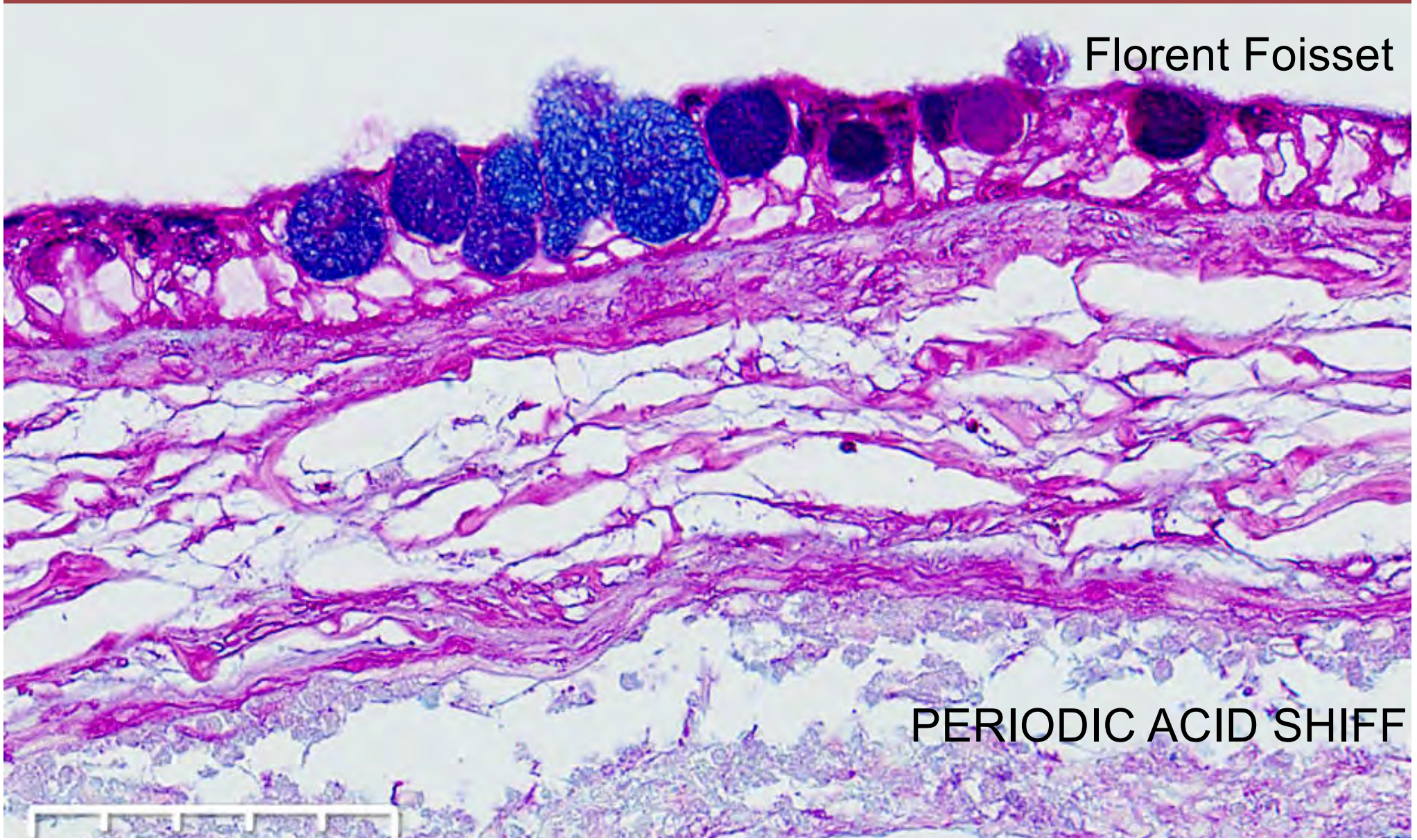
GOBLET CELLS



Dot Blot MUC5AC

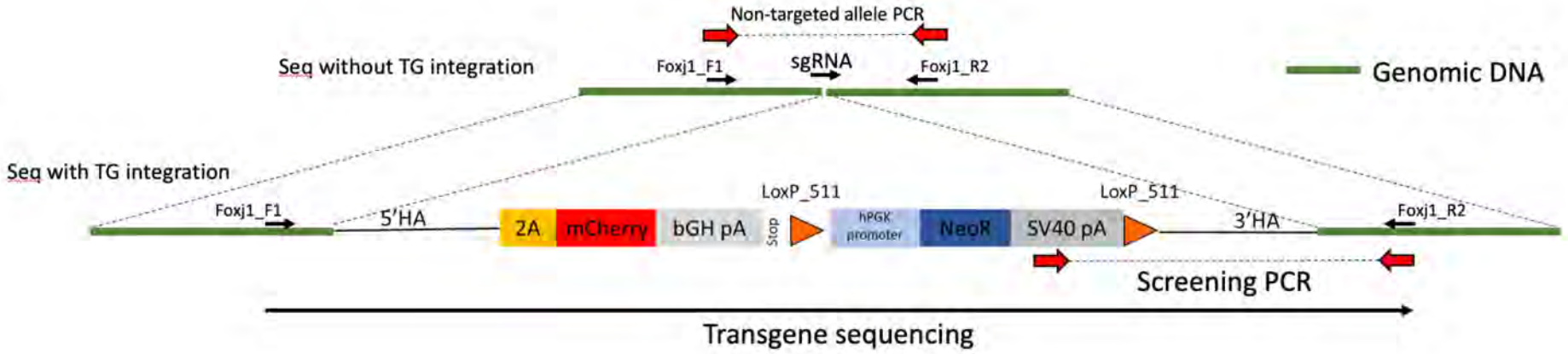


GOBLET CELLS

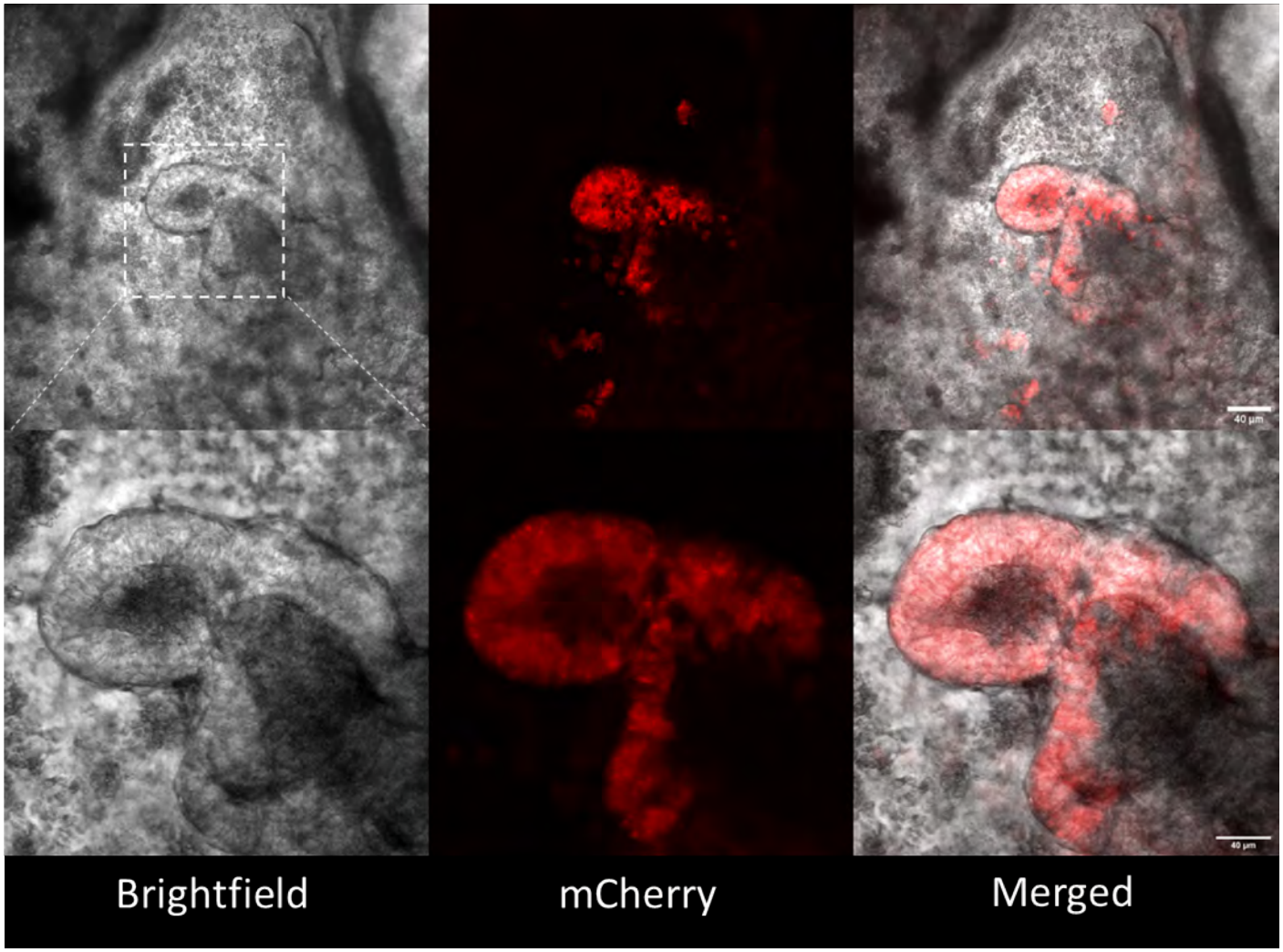


Lignée Foxj1-mCherry

- FOXJ1: transcription factor important for ciliogenesis
- ➔ FOXJ1-mCherry using CRISPR/Cas9



iALI : organoids « 2D$^{1/2}$ »



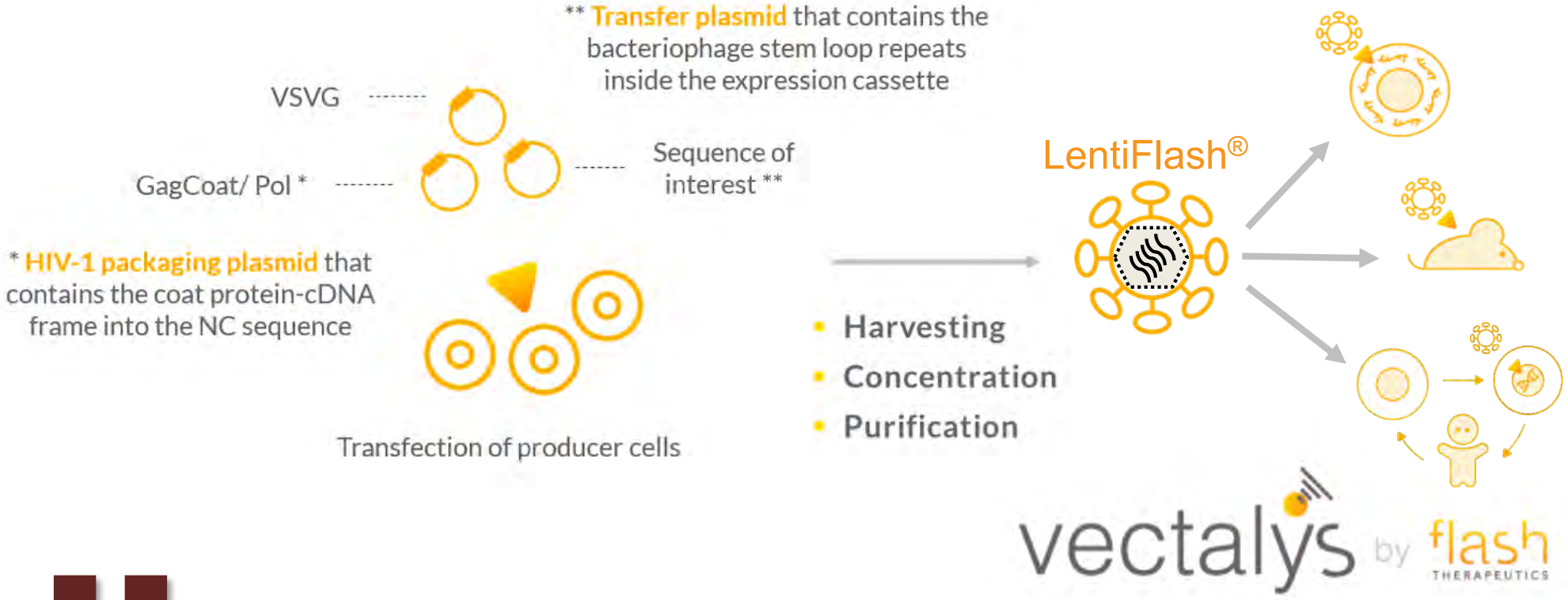
GENE THERAPY OF PRIMARY CILIARY DYSKINESIA IN THE LUNG

Genetic engineering of iPSC is still a challenge

- Induced pluripotent stem cells (iPSC) are a promising tool for cell (and gene) therapy
- But iPSC genetic engineering at high rate is challenging (apoptosis, low rate of gene modification)

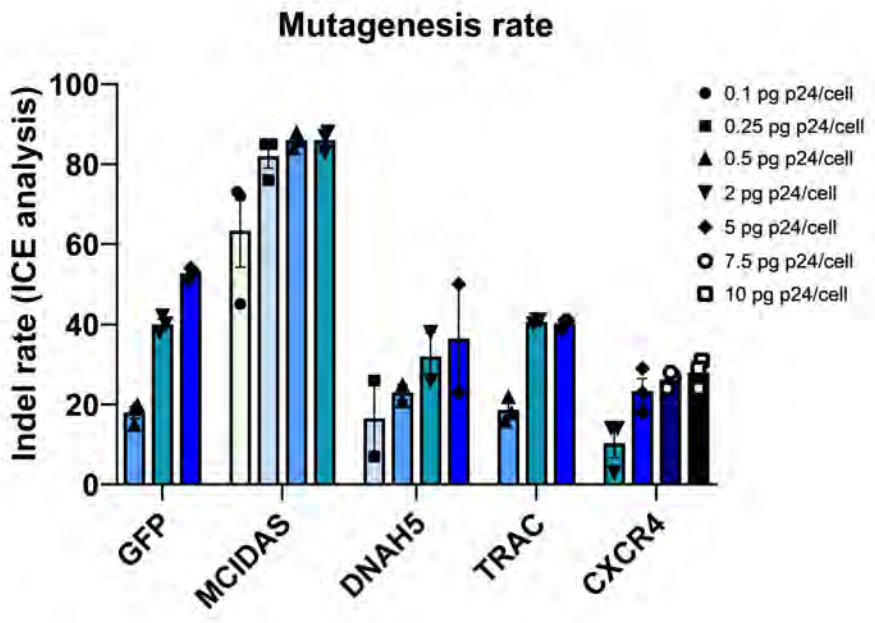
LentiFlash® vectors

- Use of nonintegrative bacteriophage-chimeric retrovirus-like particles (LentiFlash®) for RNA delivery



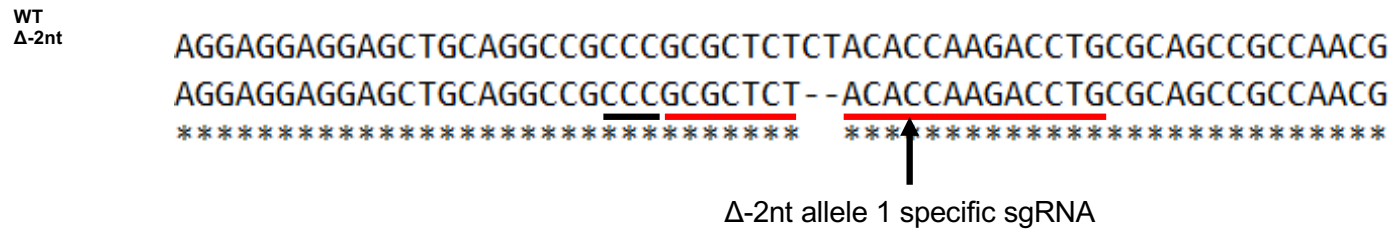
Gene KO

- A healthy iPS cell line (HY03)
- LentiFlash® particles that carry a sgRNA targeting two genes involved in motile ciliary biology (MCIDAS and DNAH5) and two endogenous genes implicated in T-cell biology (TRAC and CXCR4).



Similar data for two other iPSC lines (PCD_02:30 and iCODP9_B27)

Allele-specific gene editing induces interallelic gene conversion in hiPSC



CCDC40 gene

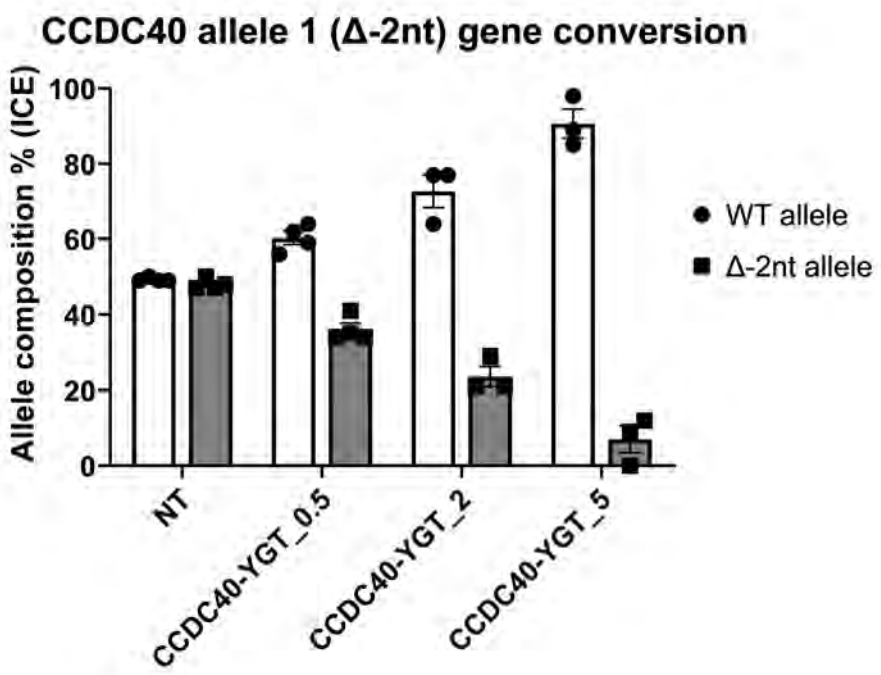
Allele-specific gene editing induces interallelic gene conversion in hiPSC

WT
Δ-2nt

```
AGGAGGAGGAGCTGCAGGCCGCCCGCGCTCTCTACACCAAGACCTGCGCAGCCGCCAACG
AGGAGGAGGAGCTGCAGGCCGCCCGCGCTCT--ACACCAAGACCTGCGCAGCCGCCAACG
**********************************  ****************************
```

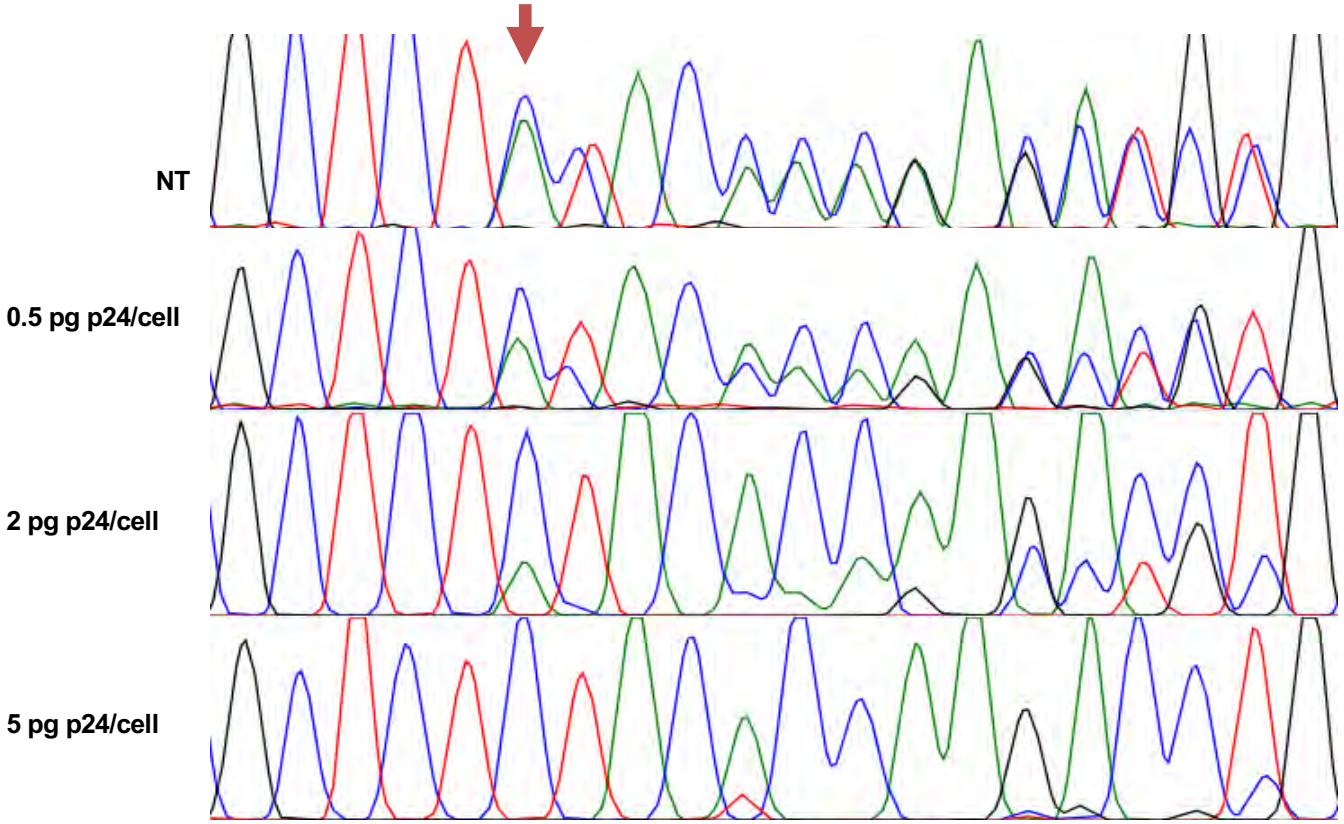
CCDC40 gene

Δ-2nt allele 1 specific sgRNA

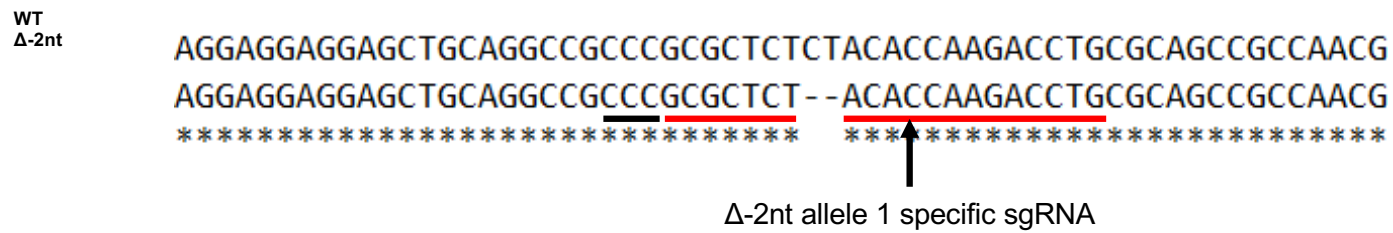


Allele-specific gene editing induces interallelic gene conversion in hiPSC

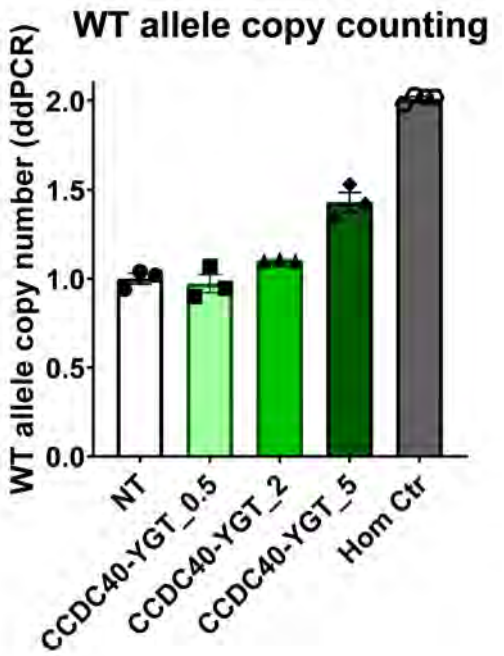
Gene Conversion



Allele-specific gene editing induces interallelic gene conversion in hiPSC

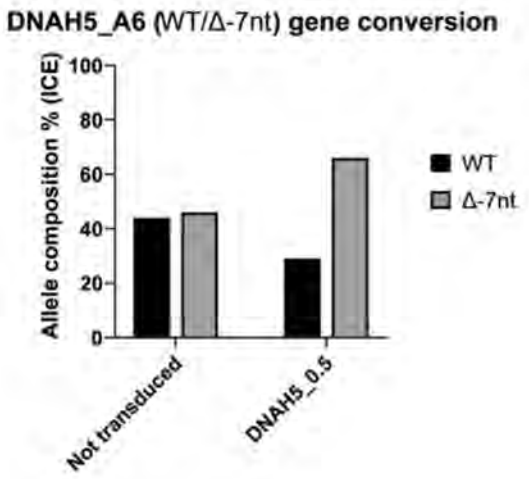


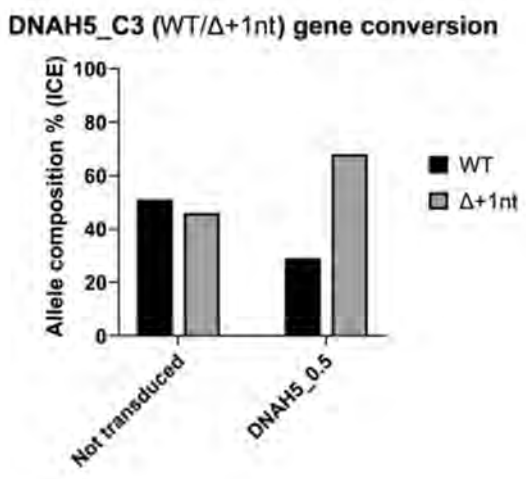
CCDC40 gene

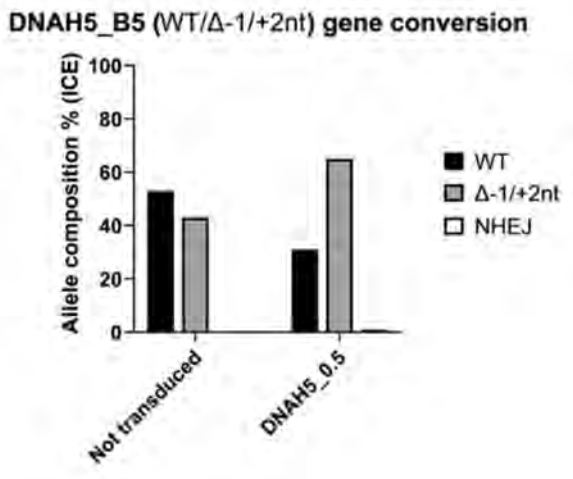


Allele-specific gene editing induces interallelic gene conversion in hiPSC

- Confirmation for three other mutation in another locus (DNAH5)

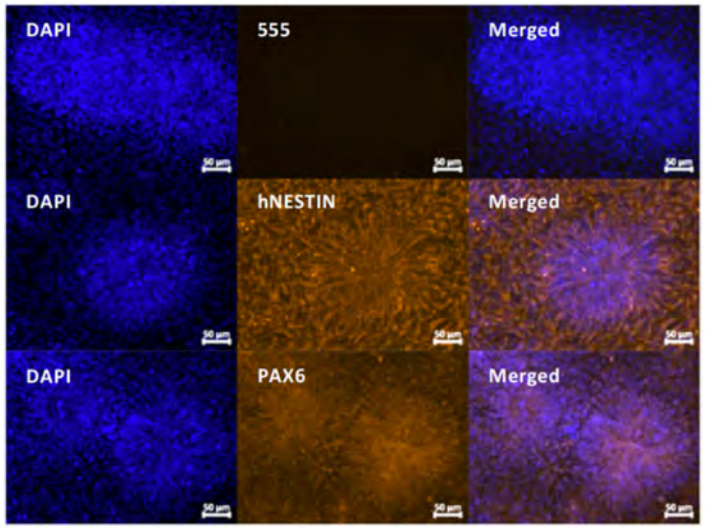


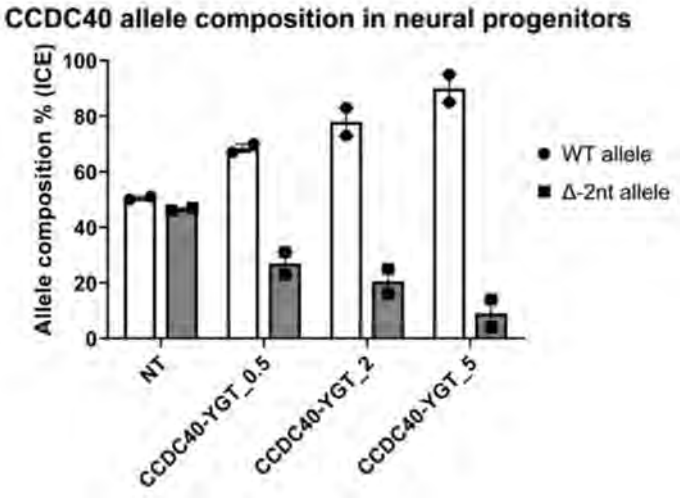


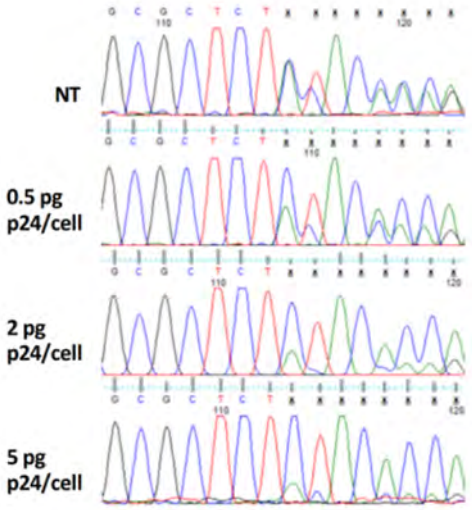


Allele-specific gene editing induces interallelic gene conversion in neural progenitors

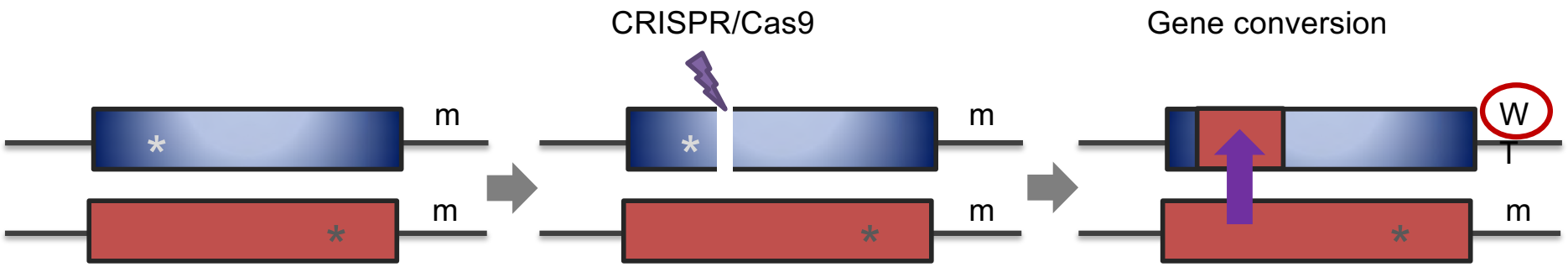
- Not restricted to pluripotent stem cells: neural progenitors







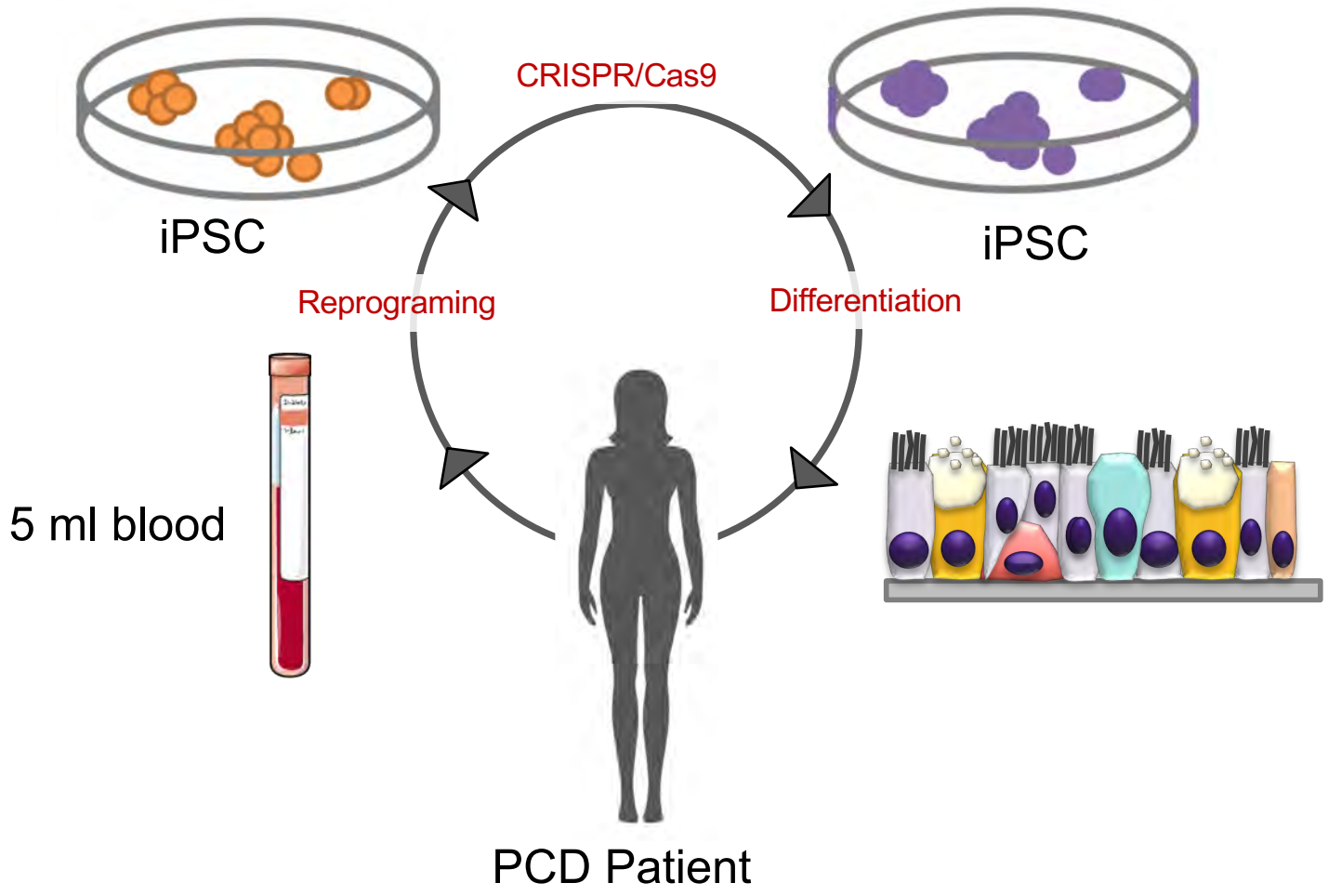
Interallelic gene conversion in hiPSC



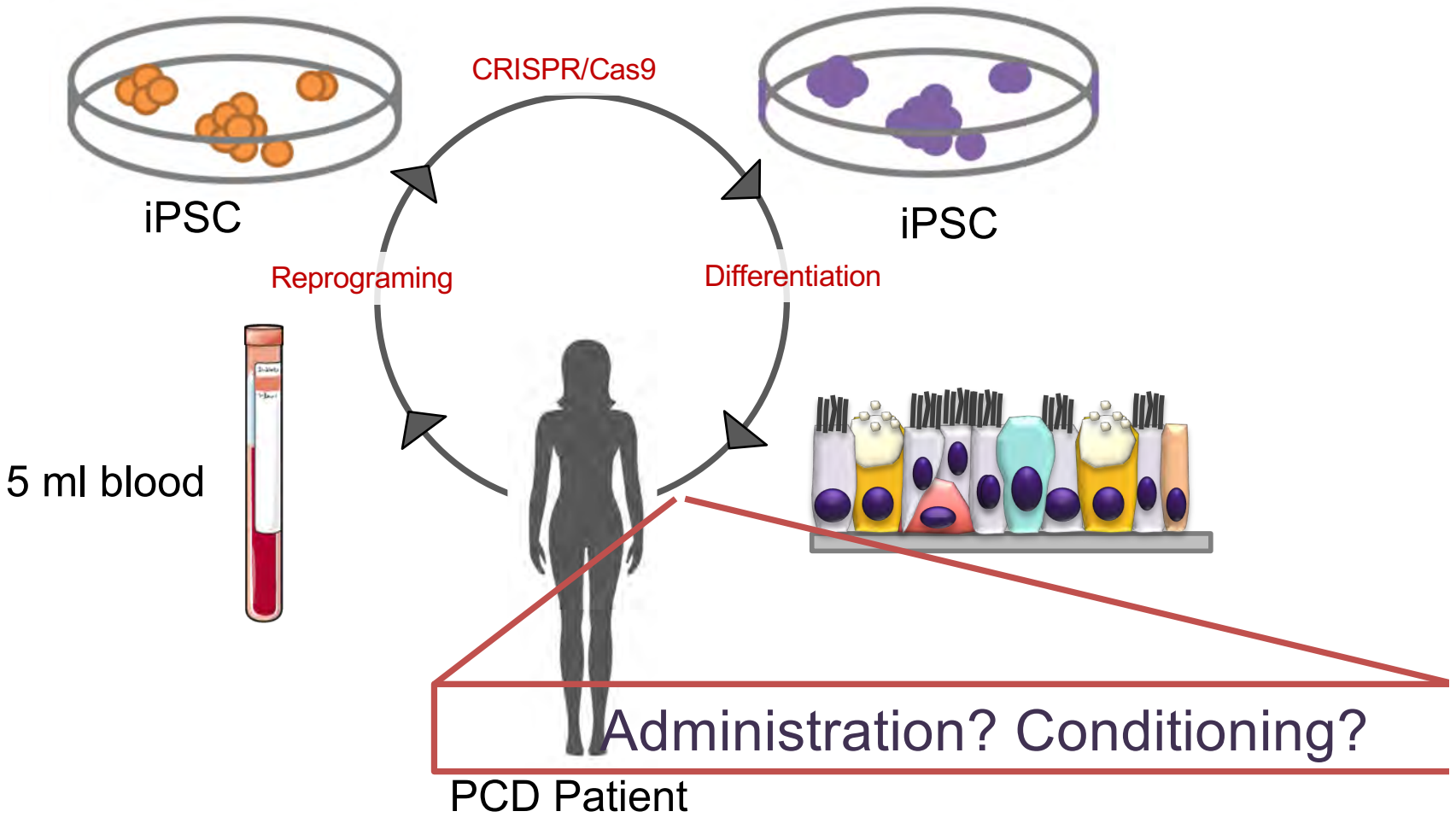
CONCLUSION

TOWARD GENE AND CELL THERAPY OF PRIMARY CILIARY DYSKINESIA

Gene and cell therapy for PCD

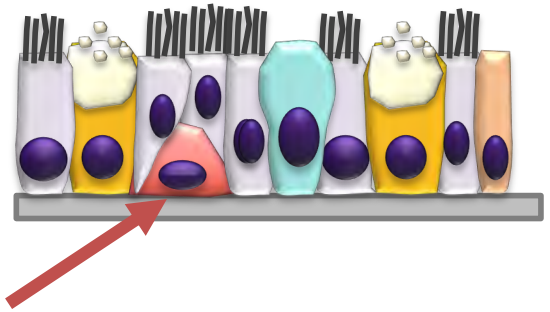


Gene and cell therapy for PCD



Towards a gene and cell therapy of DCP

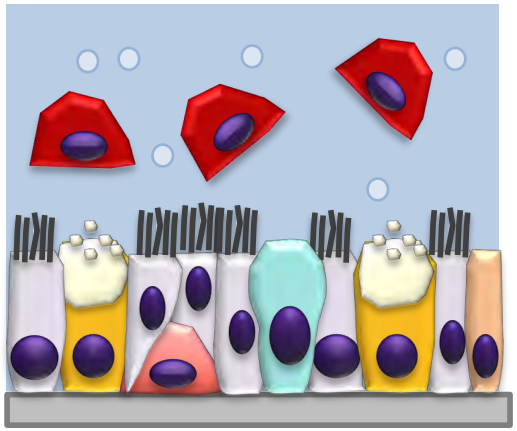
- Which cell type?



Basal cell (~lung stem cell)

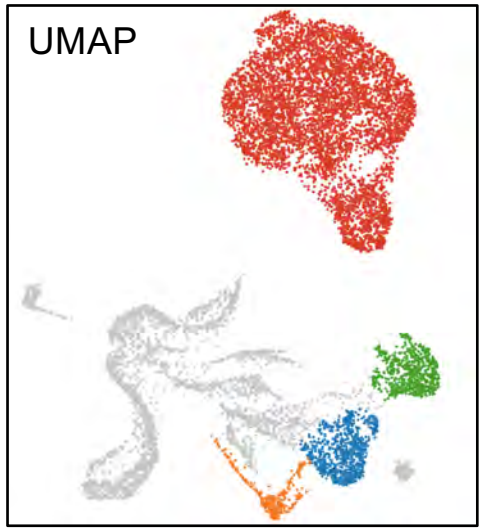
- Administration?

→ Flooding one bronchi at a time



Identification of extracellular markers

- Single cell RNAseq analysis



Cells population are defined by the expression of known markers

- Mesenchymal cells
- Goblet cells
- Basal cells → Up-regulated gene expression.
- Ciliated cells

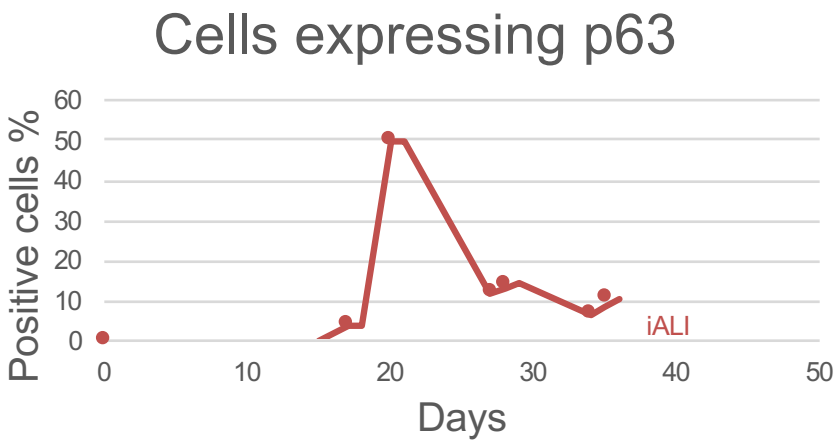
Amel Nasri

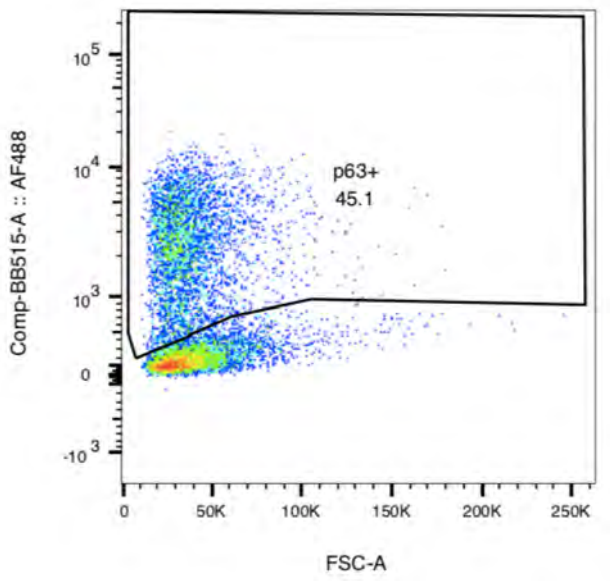
Identification of extracellular markers

Carine Bourdais

- Follow-up of p63 expression during differentiation

Flow cytometry with intracellular staining of p63 at different days of the differentiation process.



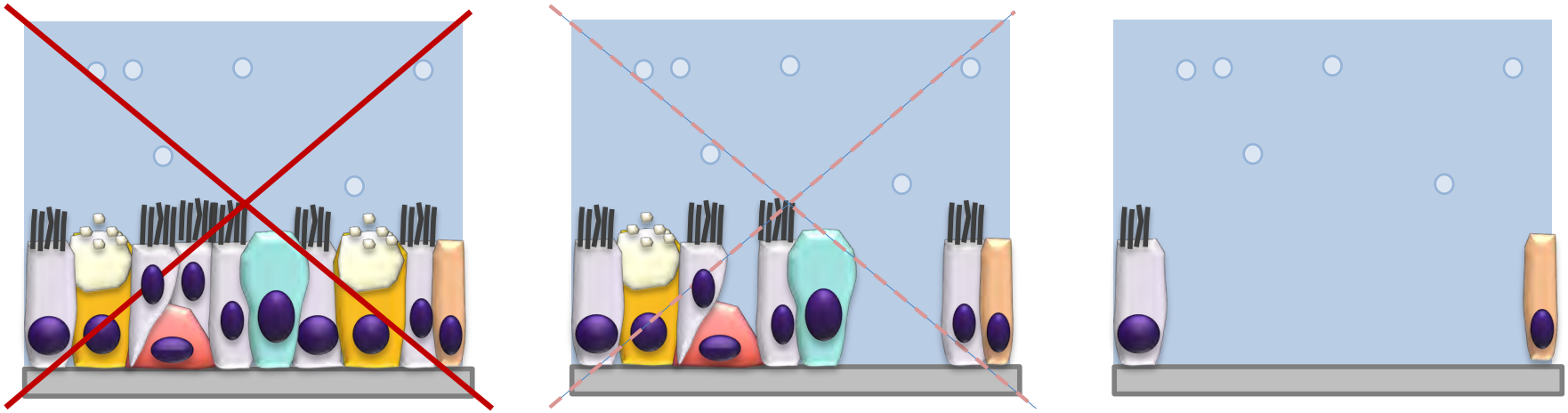


Higher expression level at Day 20 of differentiation !

Candidates co-expression with p63 in iALI should be analysed at D20.

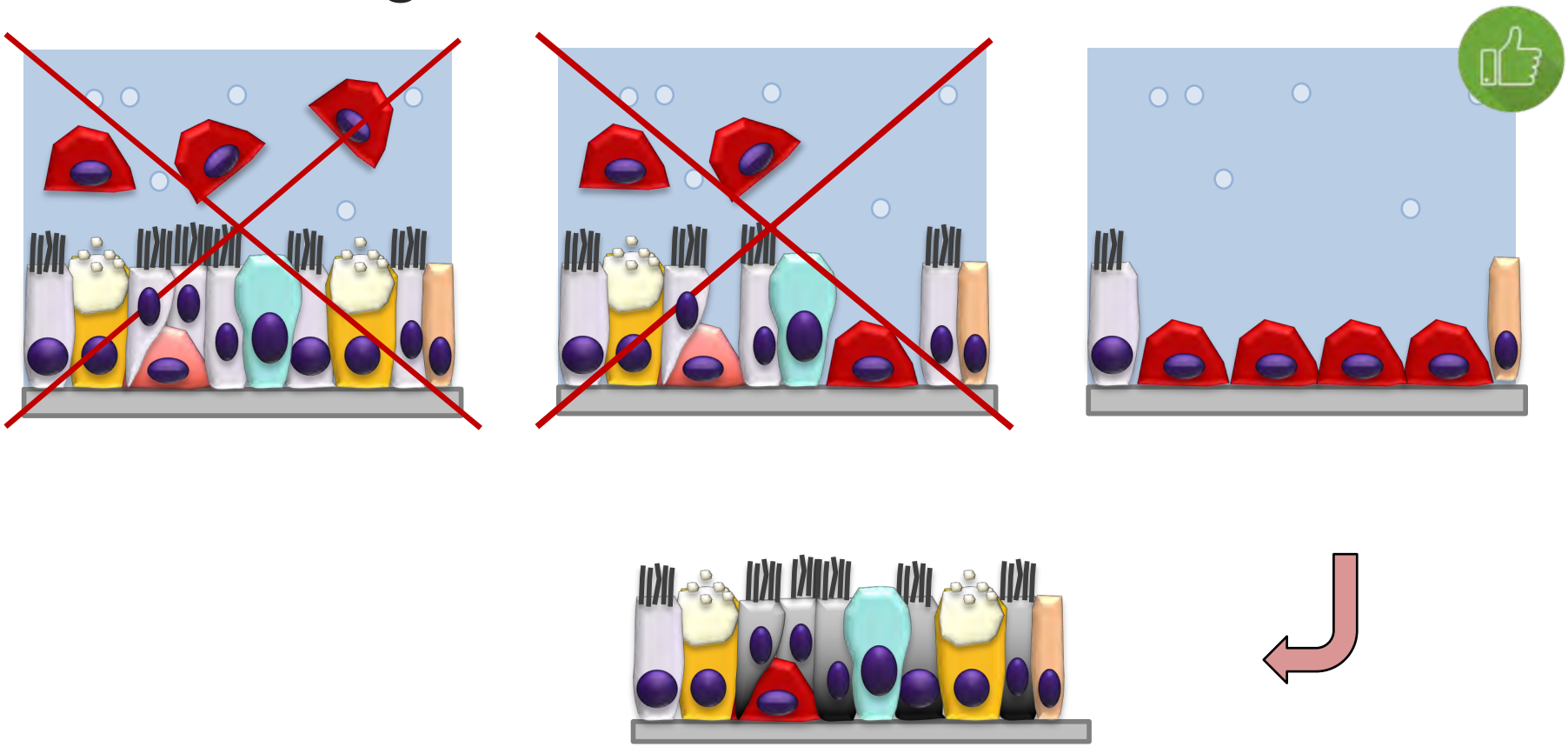
Towards a gene and cell therapy of DCP

- Conditioning?



Towards a gene and cell therapy of DCP

- Conditioning?



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- Mathilde VOLPATO, Master degree

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Others co-workers:

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- Chantal CAZEVIEILLE, ***INM*** (PhD)