

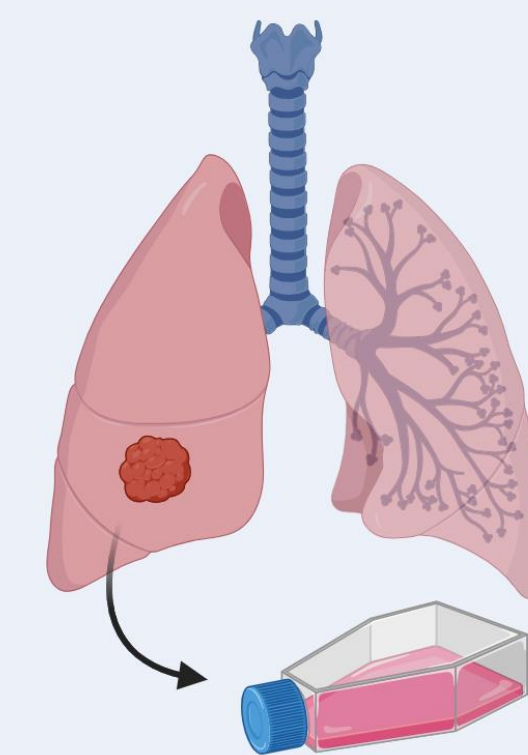
# Optimizing *in vitro* tumour cell growth for personalised patient care

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## Introduction

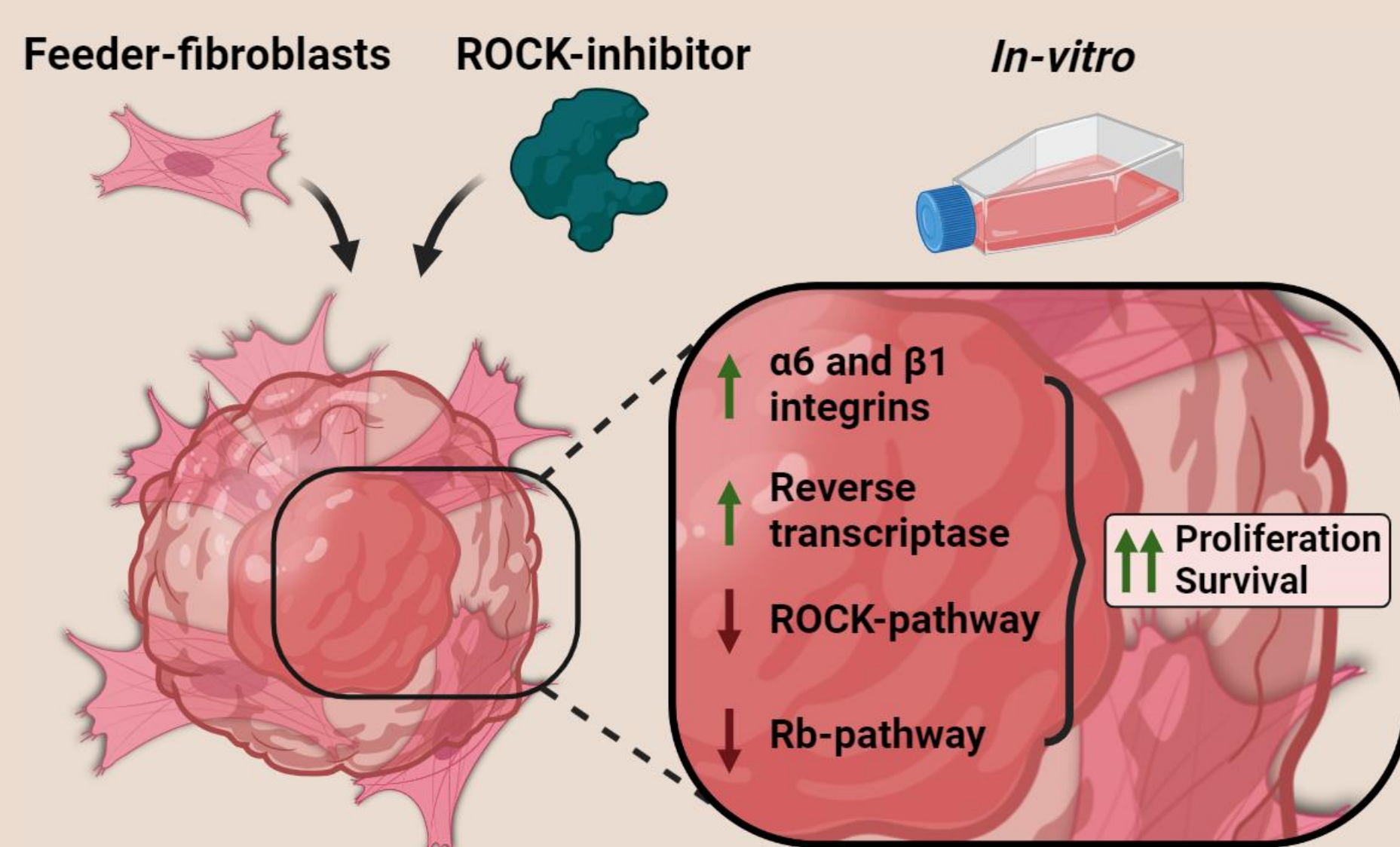
- Targeted treatment of non-small cell lung cancer (NSCLC) remains a challenge due to tyrosine kinase inhibitor (TKI) resistant mutants.
  - In vitro* TKI testing of patient-derived malignant cells could prove to be a relevant diagnostic tool. However, culturing of core biopsies has been historically difficult.
- **The aim of this study** is to optimize NSCLC tumour culture conditions by the use of conditional reprogramming.



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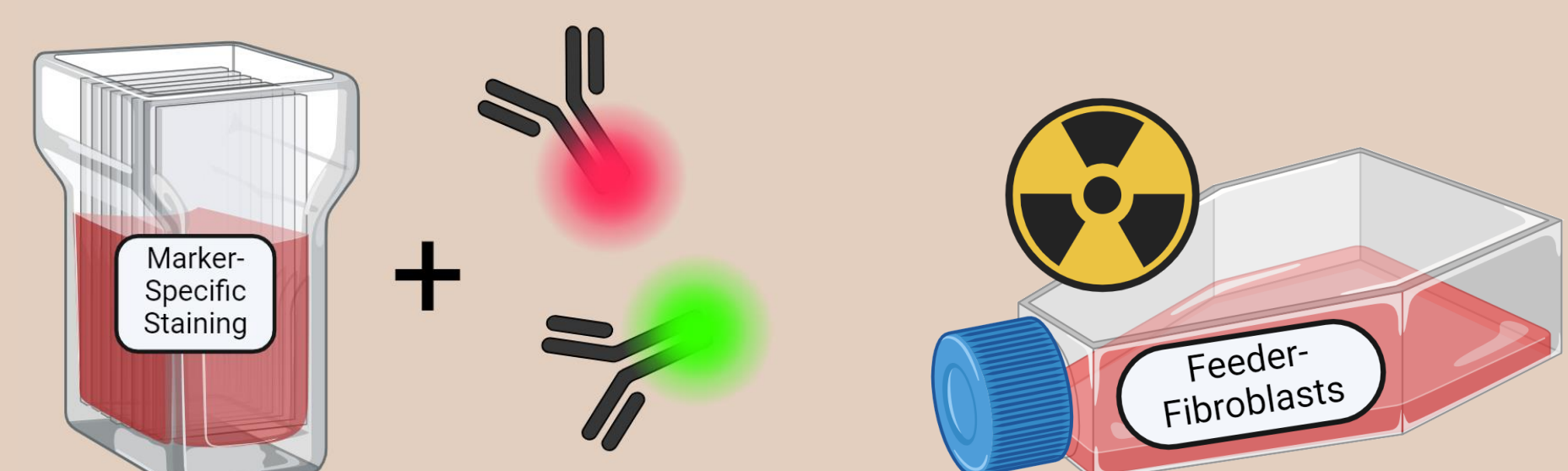


## Methods



### Conditional reprogramming

Conditional reprogramming is a culturing technique that can be used to rapidly establish epithelial cell cultures by increasing cell survival and growth.<sup>[1,2]</sup>



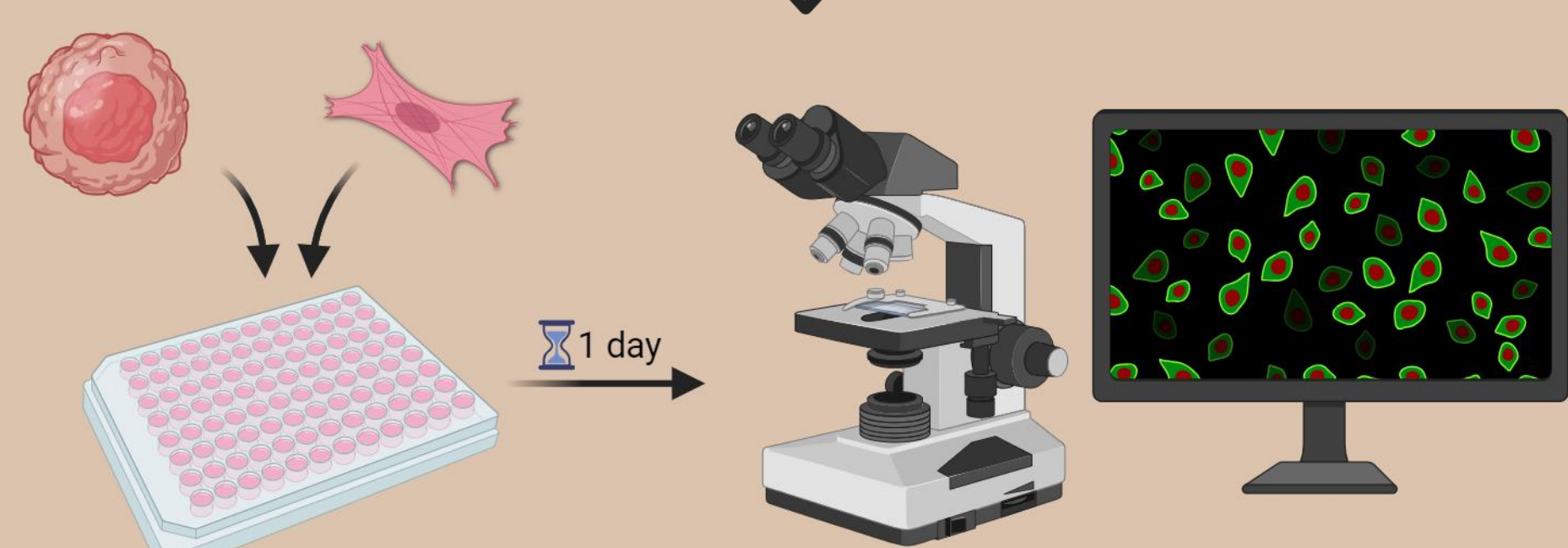
### Preparation & Optimization

#### Staining of tumour-specific markers.

- ROS1+ NSCLC cell-line (HCC78).
- Immunohistochemical & fluorescent.
- TTF-1 & EpCAM.

#### Optimization of feeder-fibroblast irradiation.

- Feeder-fibroblasts (VH25).
- 50, 75 & 100 Gy.

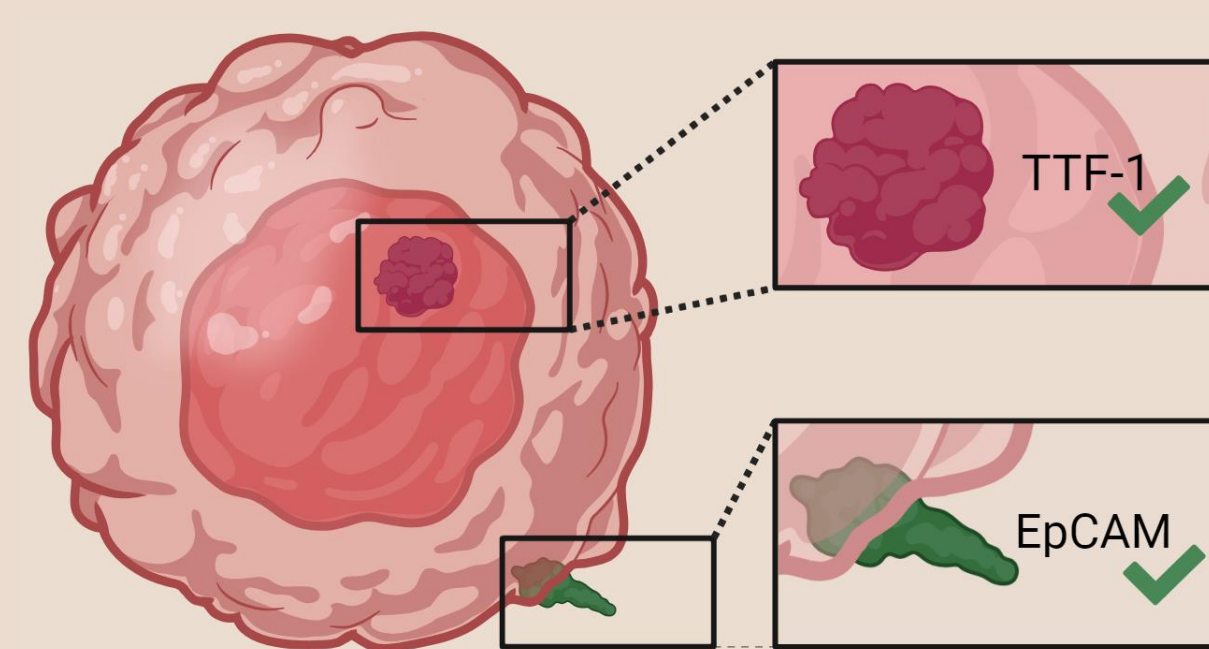


### Co-Culture Identification

- Fluorescent staining of tumour-specific markers; identification of HCC78 cells.
- Development of QuPath script.

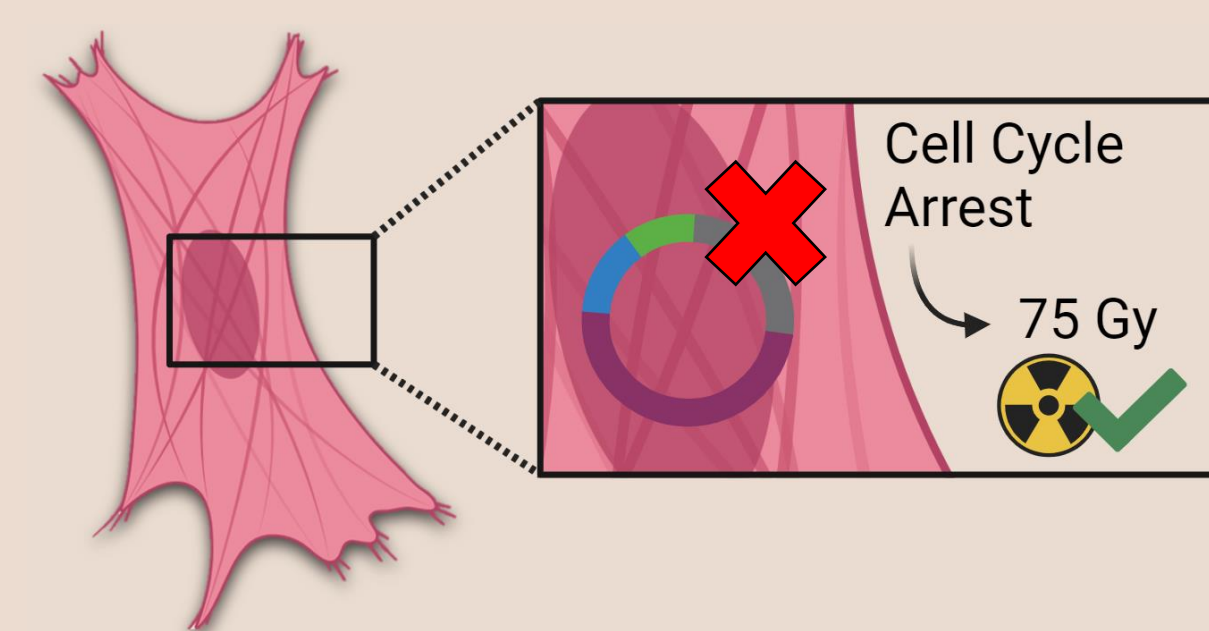
### Stage 1: Marker Stainings

Fluorescent staining of TTF-1 & EpCAM showed positive staining in tumour cells and negative staining in fibroblast cells on cytopspots, allowing for microscopical identification.



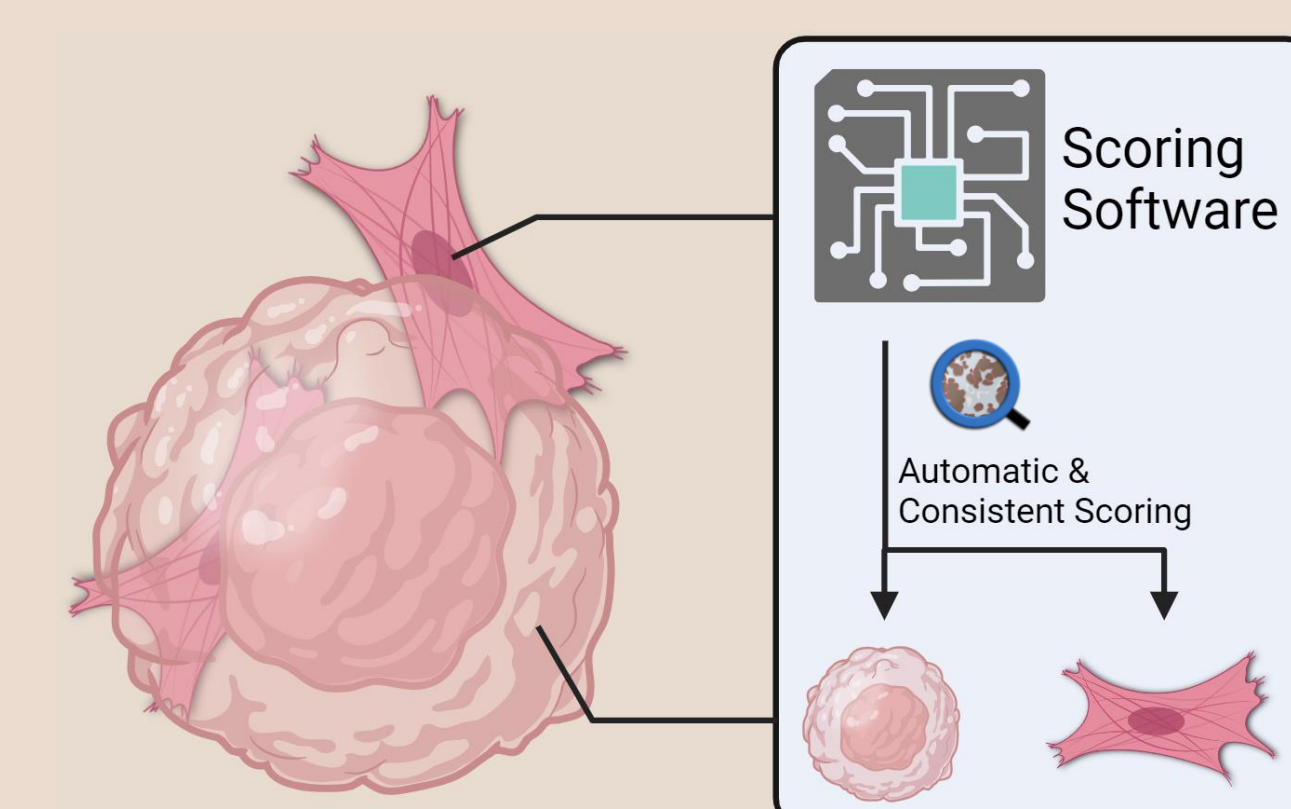
### Stage 2: Irradiation

Conditional reprogramming requires fibroblasts which do not proliferate, achievable through irradiation. VH25 cells achieved this state at a doses of 75 Gy.

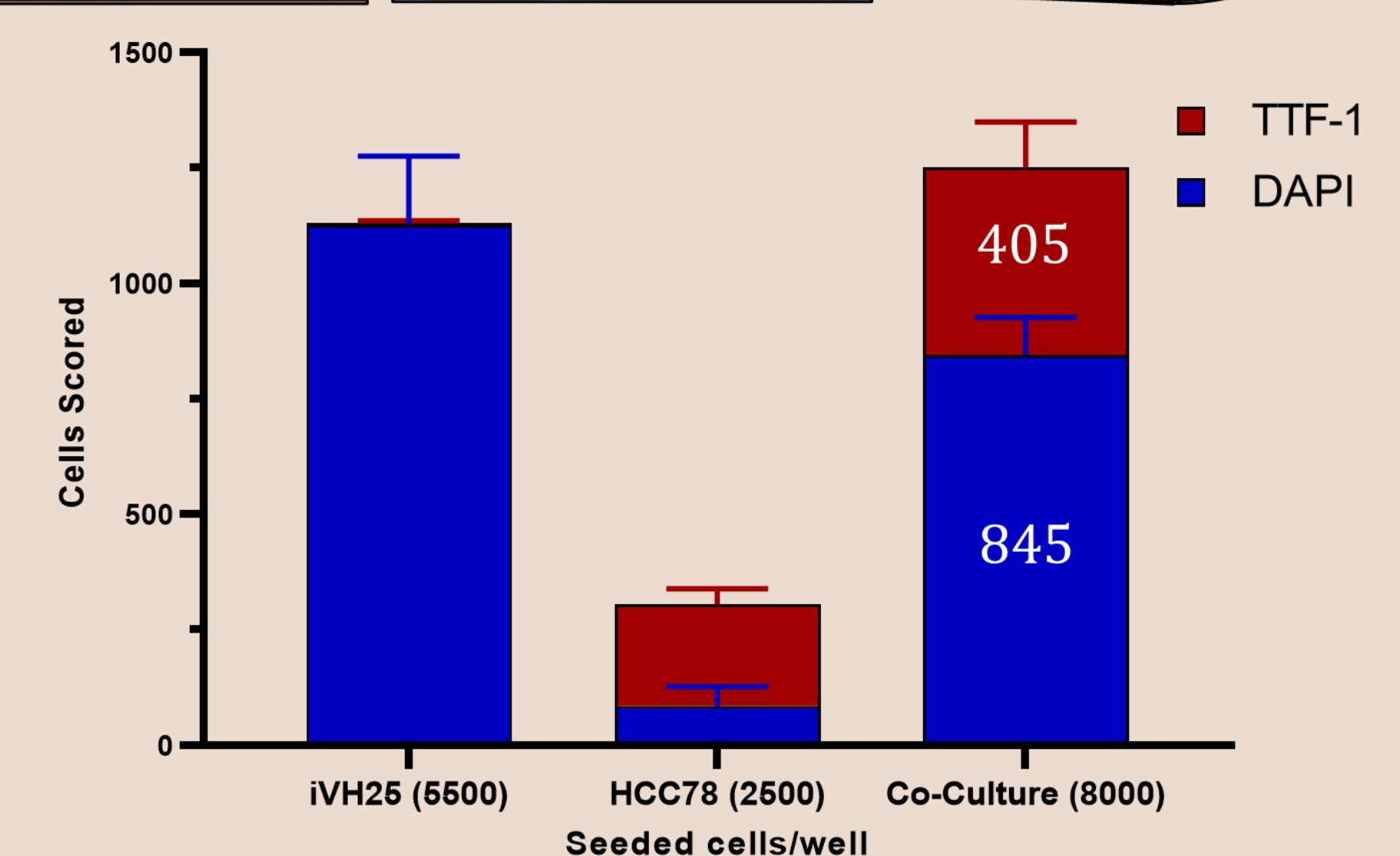
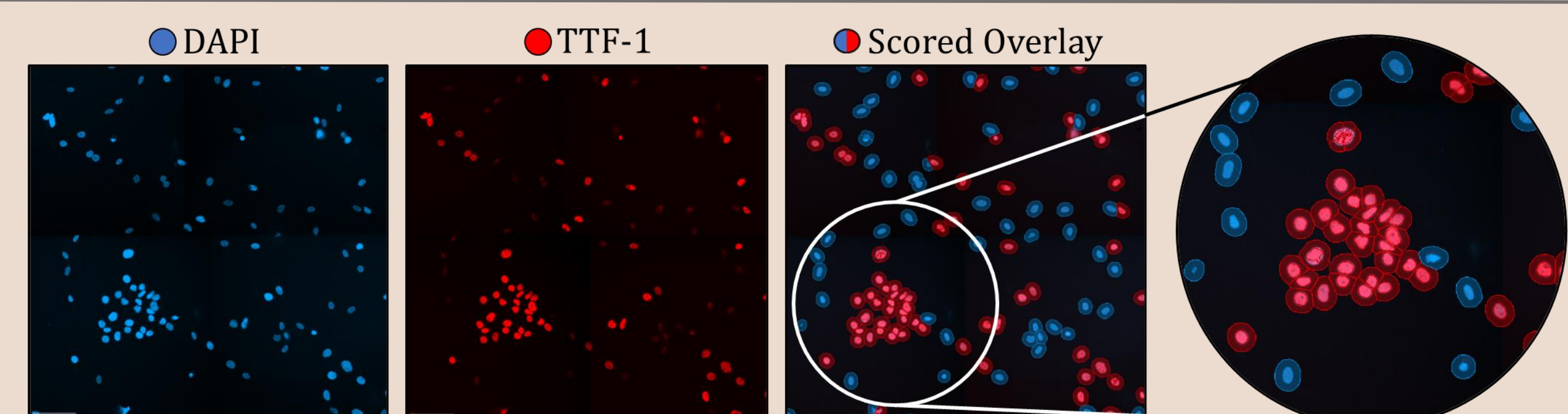
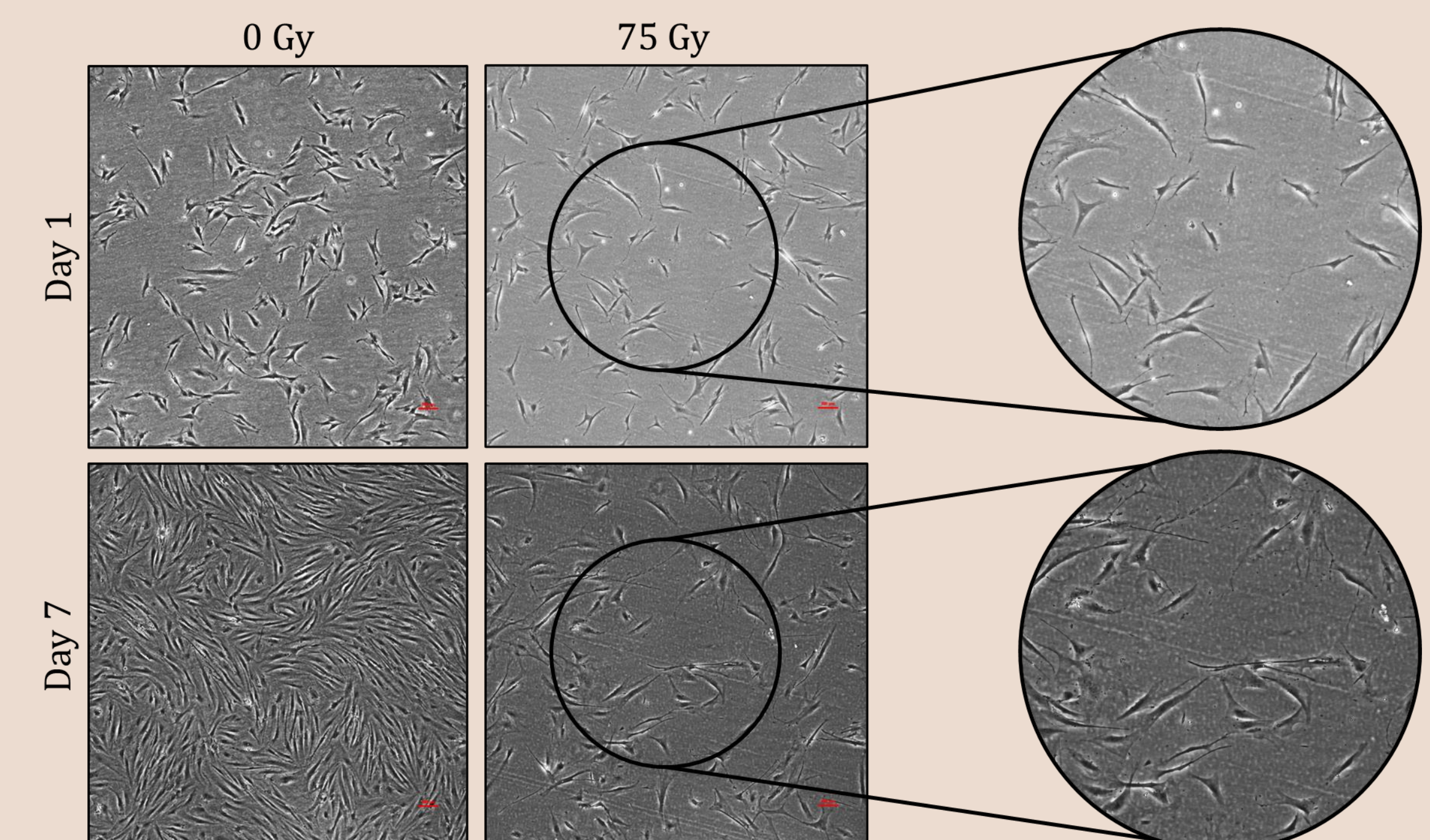
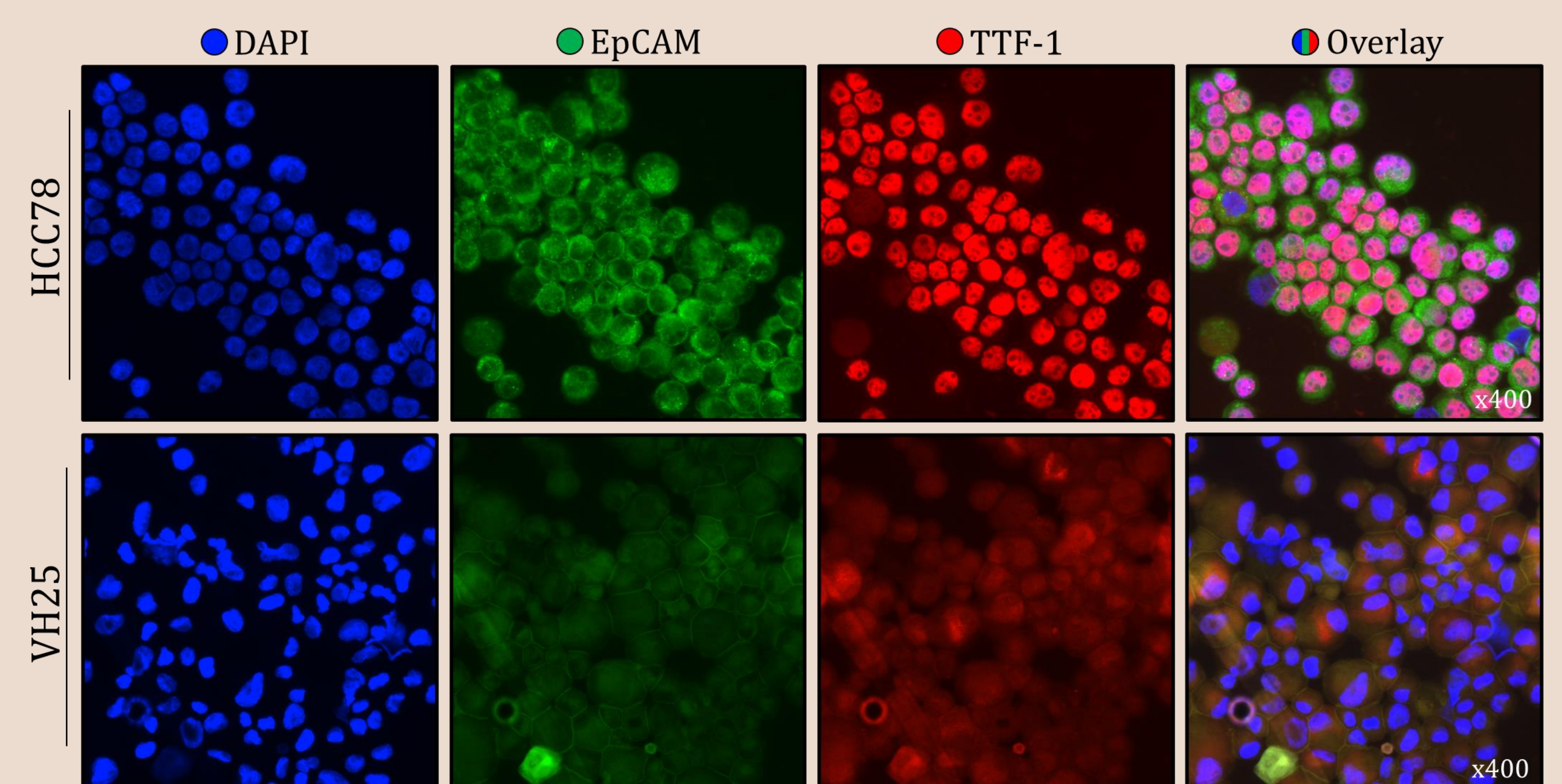


### Stage 3: Co-Culture

HCC78 cells were identified based on fluorescent staining in co-culture. A software script proved capable of identification and showed expected results according to seeding amount.



## Results



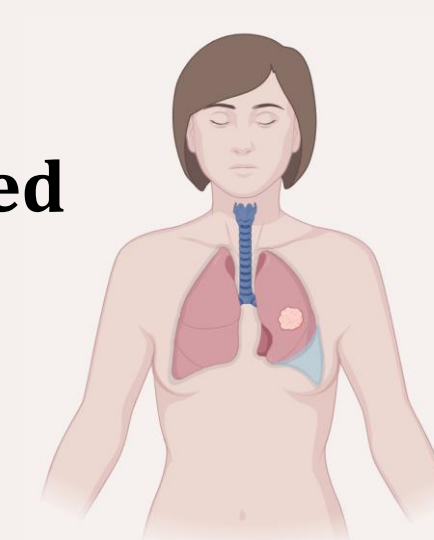
## Conclusion & Prospects

### A successful set-up for conditional reprogramming.

- Fluorescent staining allows for insight on tumour growth.
- Conditions are optimized for HCC78 (NSCLC) cell-line.

### Next step: personalized patient care.

- Testing of patient material.



## Sources

- Liu X, Ory V, Chapman S, et al. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. *Am J Pathol*. PMID: 22189618.
- Supryniewicz FA, Upadhyay G, Krawczyk E, et al. Conditionally reprogrammed cells represent a stem-like state of adult epithelial cells. *Proc Natl Acad Sci USA*. PMID: 23169653.

A Very Special Thanks To:

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