

HDR based gene-editing strategy for HSPCs gene therapy

- HSPCs remains as an ideal target for the gene therapy for a subset of hematological and immunological disorders
- Correction of defective gene by targeted gene manipulation is an attractive strategy in HSPC gene therapy
- HDR based strategy can be used for disease with cluster of mutations in a single gene, insertion of transgene at its endogenous locus/at any desired locus and for gene knock-out studies.
- β -hemoglobinopathies - β -Thalassemia and Sickle cell disease (SCD), Hemophilia, Wiskott-Aldrich syndrome, Pyruvate kinase deficiency, X-linked chronic granulomatous disease etc., includes a few identified disorders for which targeted therapeutic strategies are in high demand
- Establishing efficient HDR editing in HSPCs is an important criteria for attaining favorable therapeutic outcomes¹

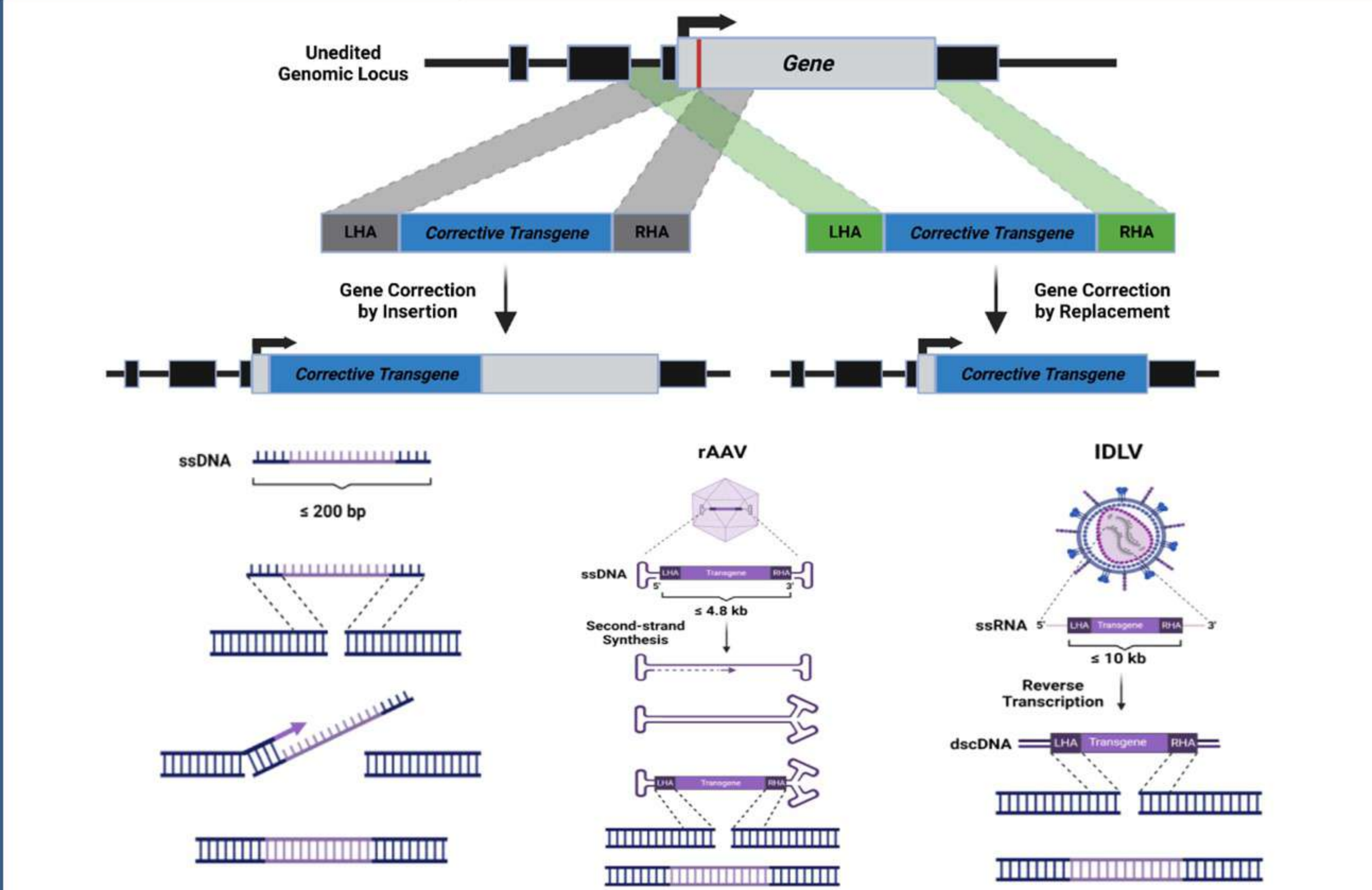


Fig 1. A Representation of HDR mechanism. Gene correction can be done using the repair template delivered as an AAV6 or IDLV for the large transgene knock-in or as ssDNA for creating point mutations

Non deletional HPFH beneficial mutations for gamma-globin upregulation

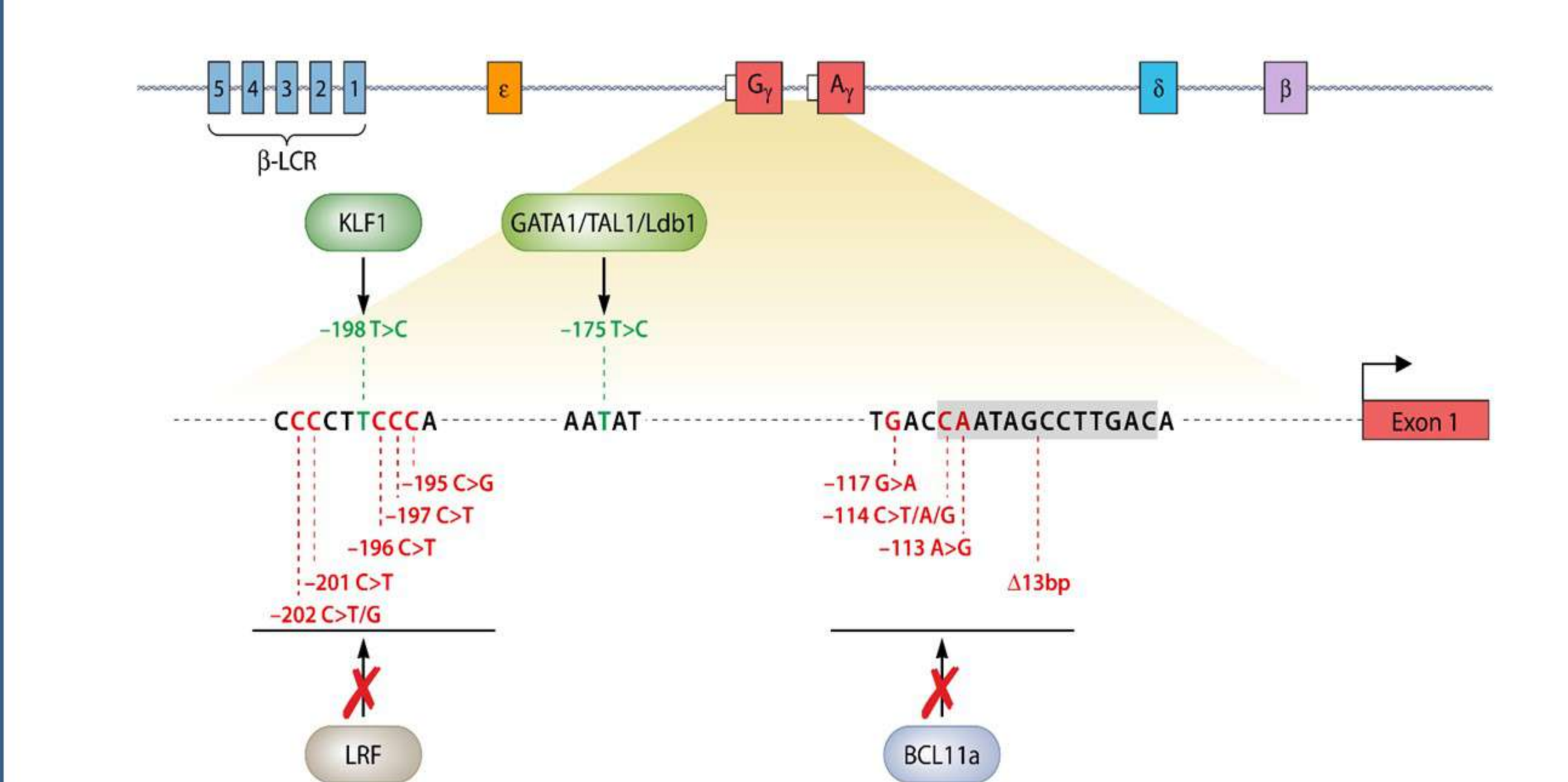


Fig 3. Schematic diagram of the γ -globin promoter harboring mutations/small deletions in individuals with persistent hemoglobin production

The -175 T>C and -158 C>T conversion for gamma globin reactivation

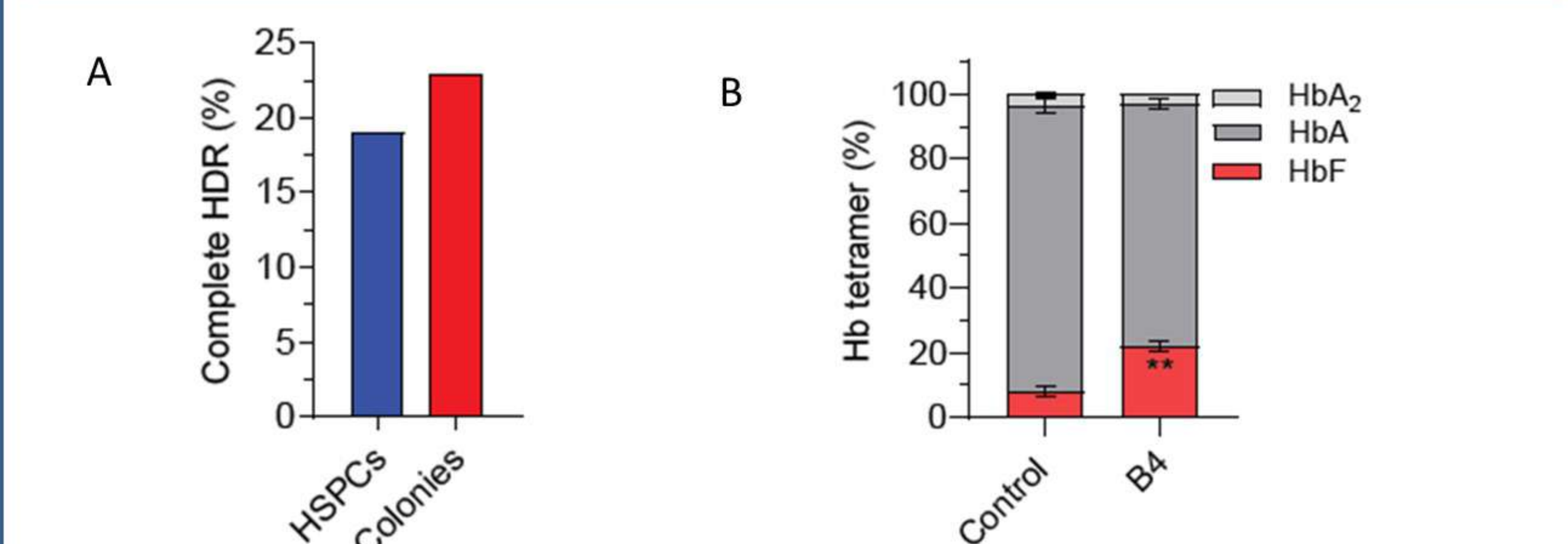


Fig 4. A. Complete HDR frequency (all 3 mutations) in the edited HSPC pool and corresponding pooled CFU colonies generated by 14-day MC culture. B. Proportion of hemoglobin (Hb) tetramers detected by HPLC analysis

Methodology

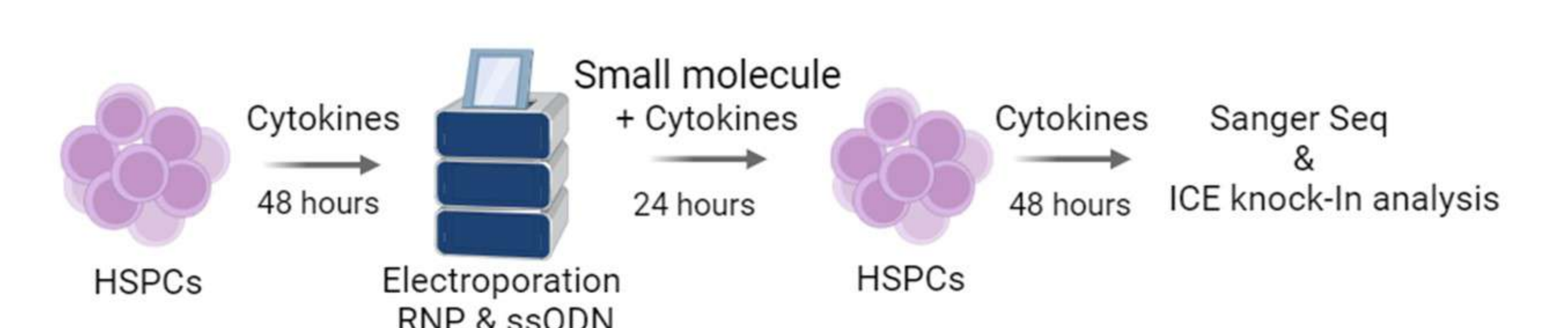


Fig 5. Schematic of the experimental workflow for small molecule screening to enhance HBG promoter targeted HDR conversion in HSPCs

Screening of small molecules in HSPCs

- To further increase the efficiency of HDR mediated gene editing in HSPCs, complete HDR conversion was monitored in the presence of 24 selected small molecules
- The molecules were known to regulate various cellular processes, such as the cell cycle, DNA repair, chromatin state, and immune responses
- The screen identified NU7441, entinostat, and ursolic acid as the top candidates that influenced the complete HDR conversion, with mean efficiencies of 44, 34, and 24%, respectively

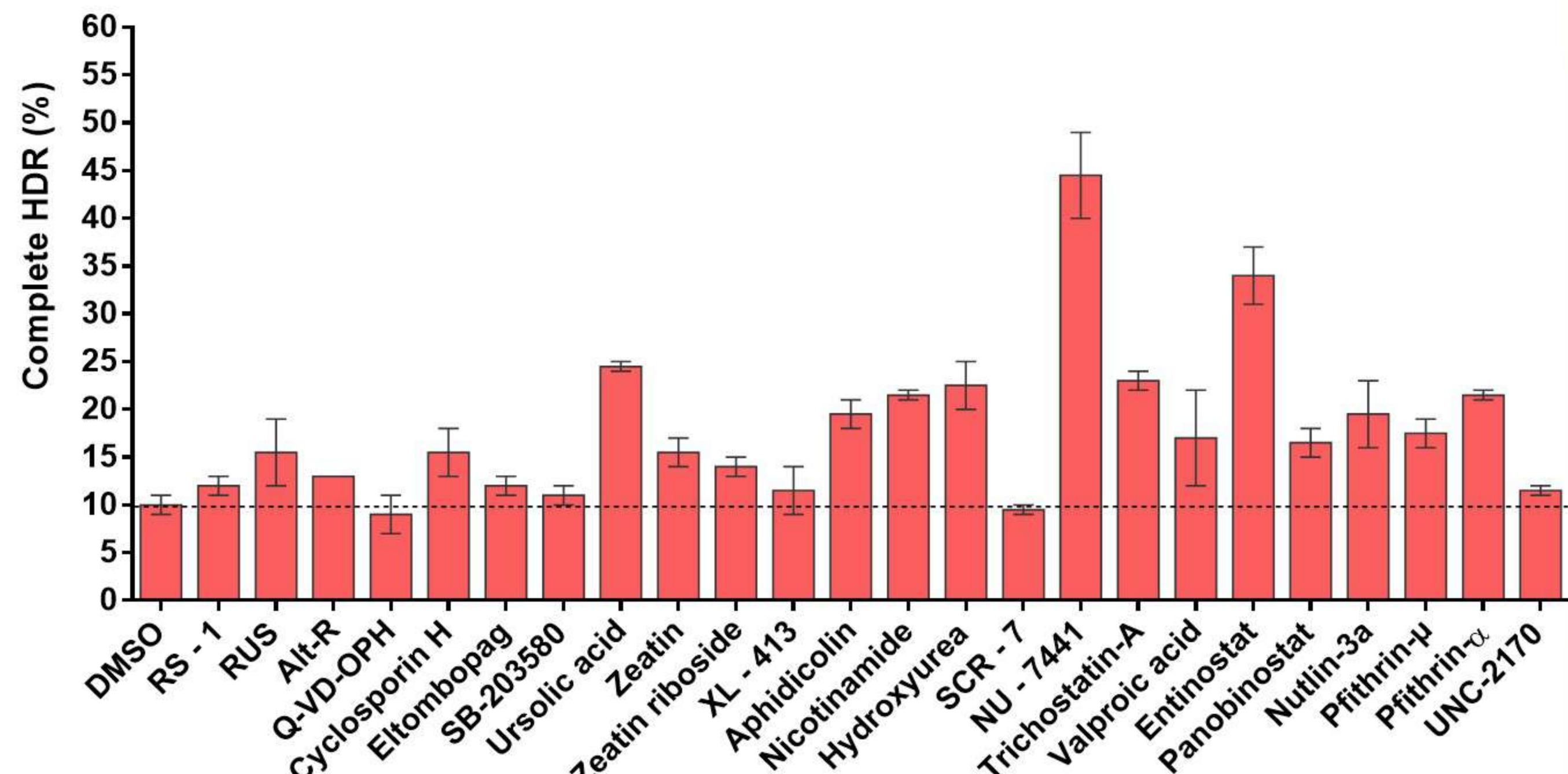


Fig 6. Schematic of the experimental workflow for small molecule screening to enhance HBG promoter targeted HDR conversion in HSPCs

Screening of different DNA-PKCs inhibitor

- NU-7441 promoted the highest HDR in the initial screening, we focused on DNA-PK inhibition, and whether any other DNA-PK inhibitor could outperform NU-7441 was questioned
- Consequently, the influence of various DNA-PK inhibitors- NU7441, AZD-7648, DNMB, LTURM 34, and NU-702 was analyzed
- Although AZD-7648 treatment induced very high HDR conversions and prevented InDels at a dose of 20 μ M, cellular toxicity was also exhibited
- To overcome the toxicity, the dose was reduced to 7 μ M and tested in the presence and absence of the RUS cocktail

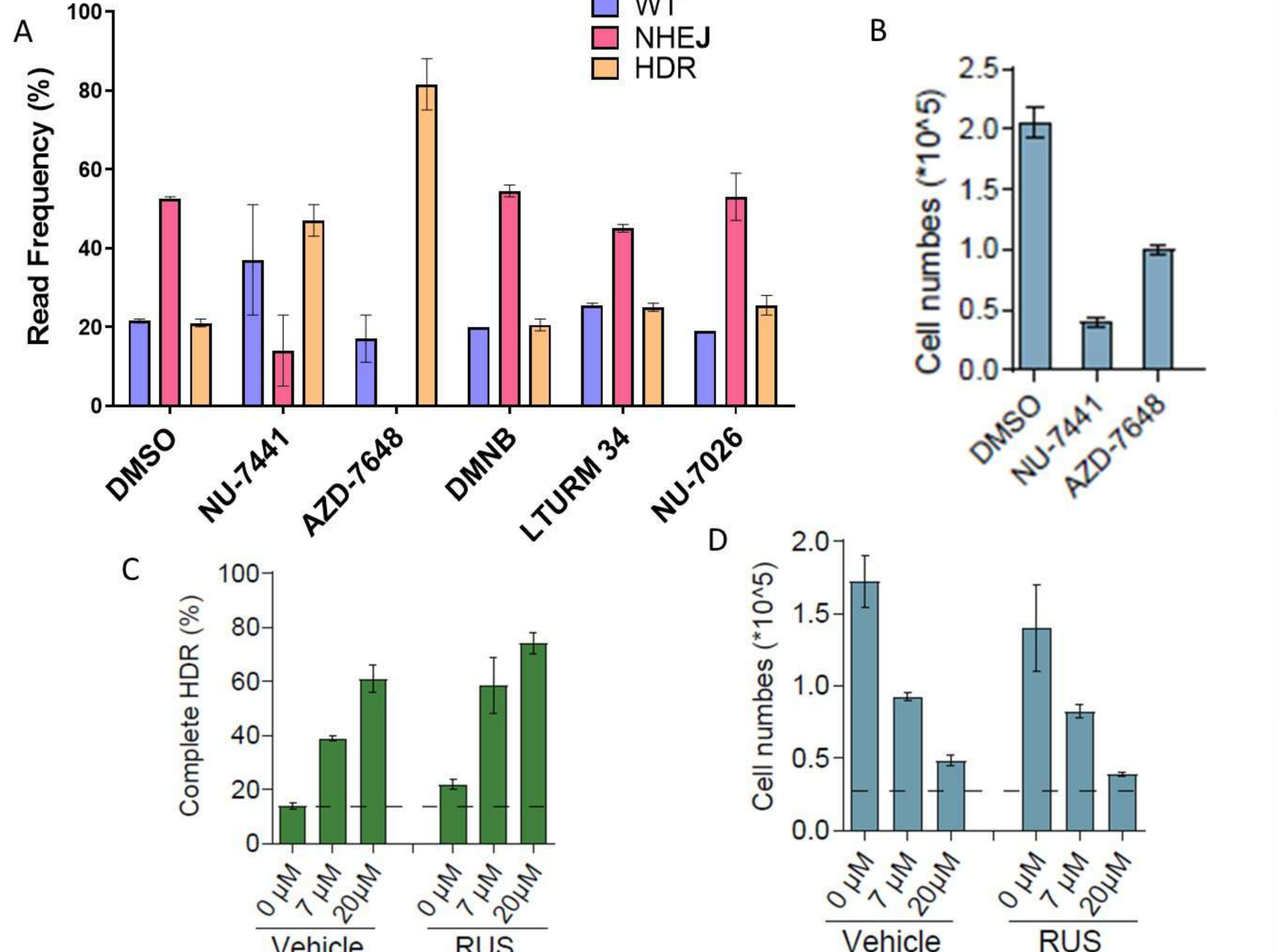


Fig 7. A. Complete HDR, NHEJ, and WT read frequencies observed post electroporation after transient exposure to various DNA-PK inhibitors. B. Absolute HSPC cell numbers with the DNA-PK inhibitors NU-7441 and AZD-7648. C. The frequency of complete HDR after treatment with different doses (0 μ M, 7 μ M, or 20 μ M) of AZD-7648 with and without RUS supplementation in HSPC culture media. D. Absolute cell numbers observed after treatment with different concentrations (0 μ M, 7 μ M, or 20 μ M) of AZD-7648

Effect of AZD-7648 on HSPCs sub-population

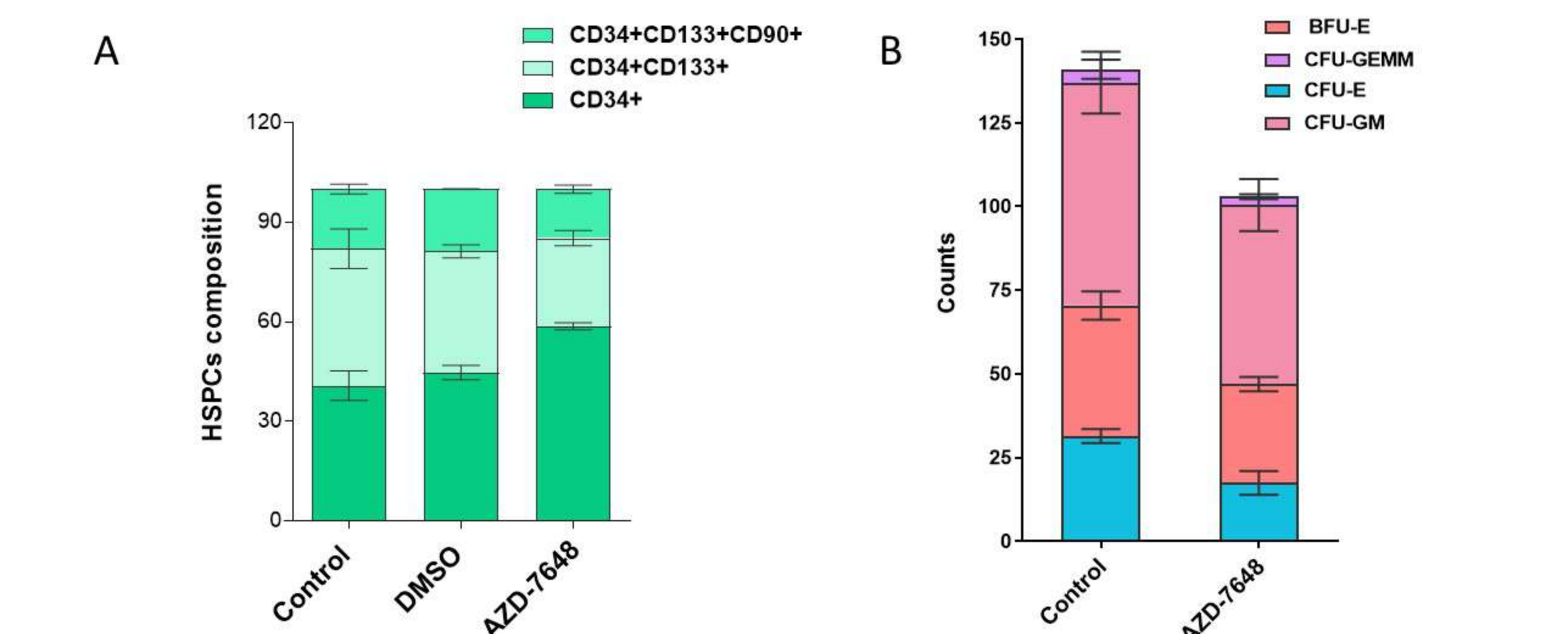


Fig 8. A. Edited HSPCs treated with AZD-7648 were collected analysed for subsets by flow cytometry analysis. B. Proportion of CFU-E, BFU-E, CFU-GM and CFU-GEMM colonies displayed by control and AZD-7648 treated HSPCs

Long-term engraftment analysis of AZD-7648 treated HSPCs

- HSPCs that had undergone electroporation for RNP-B4 ssODN (B4), along with AZD-7648 treatment were infused into NBSGW mice
- HbF+ve cells were analyzed among the FACS-purified CD235⁺ve erythroid cells, which exhibited a 2-fold increase after B4 editing and a 3-fold increase after B4-AZD-7648 editing

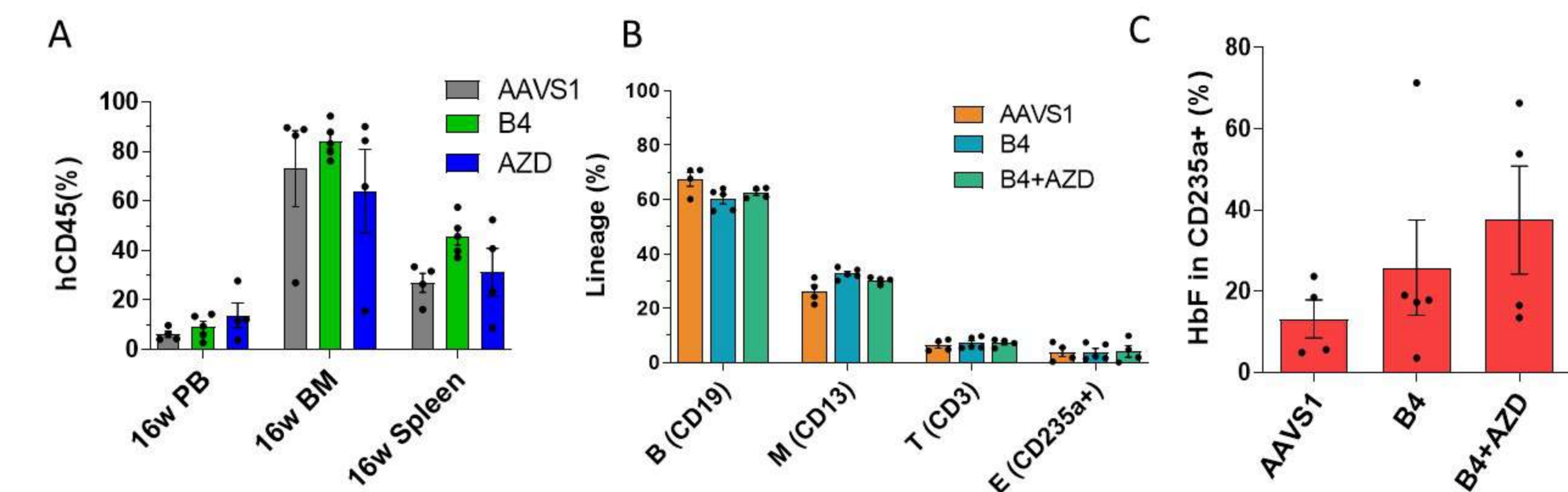


Fig 9. A. The frequency of human cell (hCD45+) engraftment in the peripheral blood, spleen and bone marrow. B. Sixteen-week multilineage repopulation output of edited cells detected by staining for B cells (CD19), myeloid cells (CD33), and T cells (CD3) C. Frequency of HbF⁺ cells among CD235a⁺ erythroblasts sorted from bone marrow.

MSCs co-culture mitigates the toxicity with HDR gene editing

- Efficiency of RNP-ssODN could be further improved if the toxicity associated with RNP-ssODN and AZD-7648 treatment was alleviated
- Anti-inflammatory factors produced by bone marrow MSCs overcome the toxicity associated with RNP-AAV6 HDR editing, resulting in better engraftment

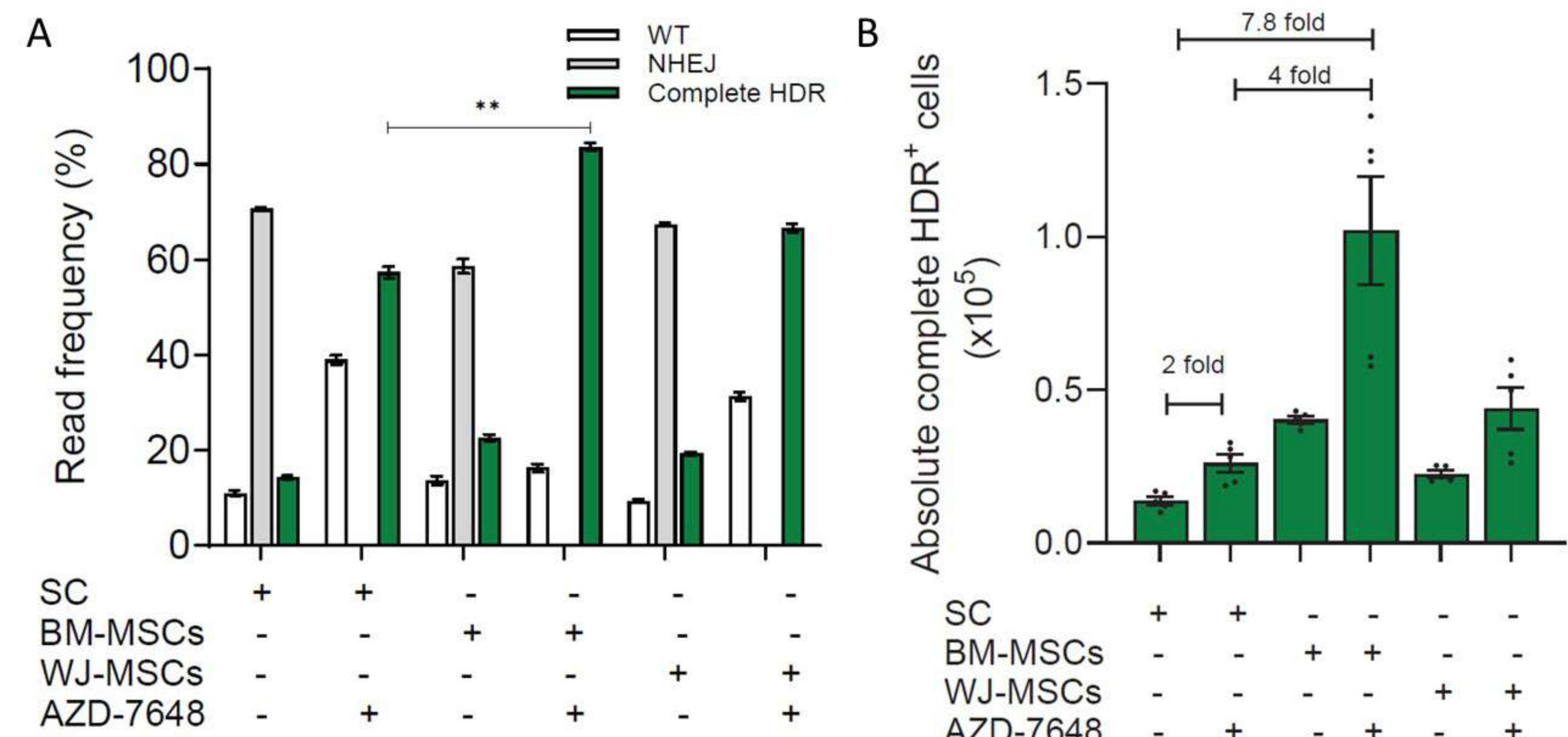


Fig 10. A. Frequency of reads corresponding to complete HDR (all 3 conversions), NHEJ indels, and wild-type sequences in edited samples. The standard condition (SC) refers to only cytokine culture. Mesenchymal stem cells (MSCs) are derived from either bone marrow (BM- MSCs) or Wharton's jelly (WJ-MSCs). B. Absolute number of complete HDR edited cells on day 3 post electroporation.

Conclusion

- Small molecule was identified to increase the HDR efficiency in HSPCs by decreasing the NHEJ events
- Efficient HDR rates over 80% was achieved *in vitro* in HSPCs
- Our study indicated that AZD-7648 treatment has a negligible impact on the long-term repopulation of HSPCs
- We mitigated the issue of reduction in cell numbers followed by ssODN editing by co-culturing HSPCs with bone marrow MSCs, which increased the absolute HDR cell frequency fourfold *in vitro*
- Thereby, we propose that our strategy using small molecule can be used to develop HDR based gene-edited HSPCs close to therapeutic level

References

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- Li G, Zhang X, Zhong C, Mo J, Quan R, Yang J, Liu D, Li Z, Yang H, Wu Z. Small molecules enhance CRISPR/Cas9-mediated homology-directed genome editing in primary cells. Scientific Reports. 2017 Aug 21;7(1):8943.
- Lee AB, Tan MH, Chai CL. Small-molecule enhancers of CRISPR-induced homology-directed repair in gene therapy: A medicinal chemist's perspective. Drug Discovery Today. 2022 Sep 1;27(9):2510-25.

Acknowledgements: Department of Biotechnology, India

Adaptive Quasi-Quiescence Induced Glioblastoma Survival on Emulsion Copolymer Matrix Surface

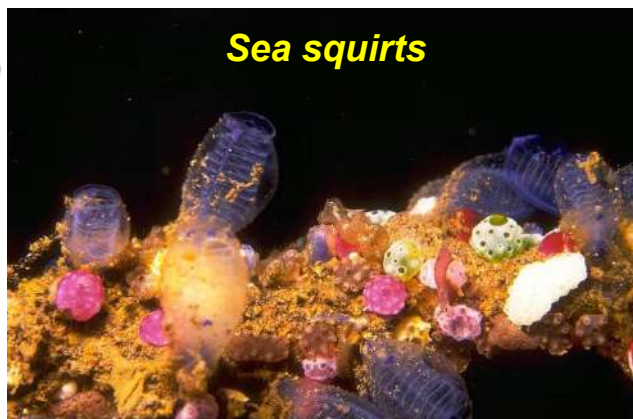
Presentation by
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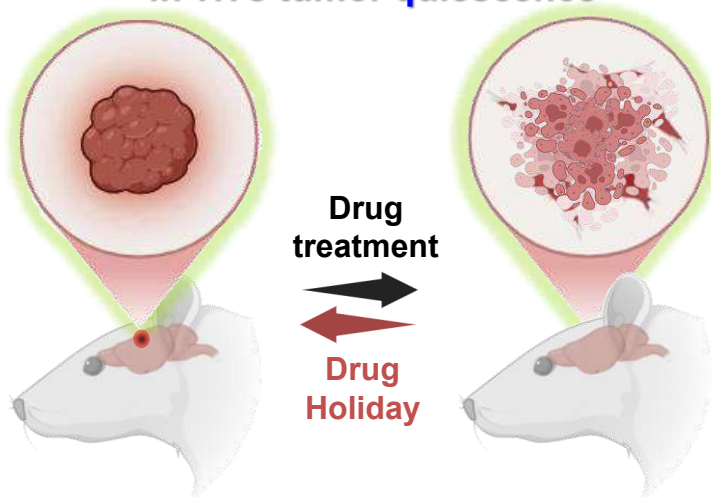
Introduction

In Nature



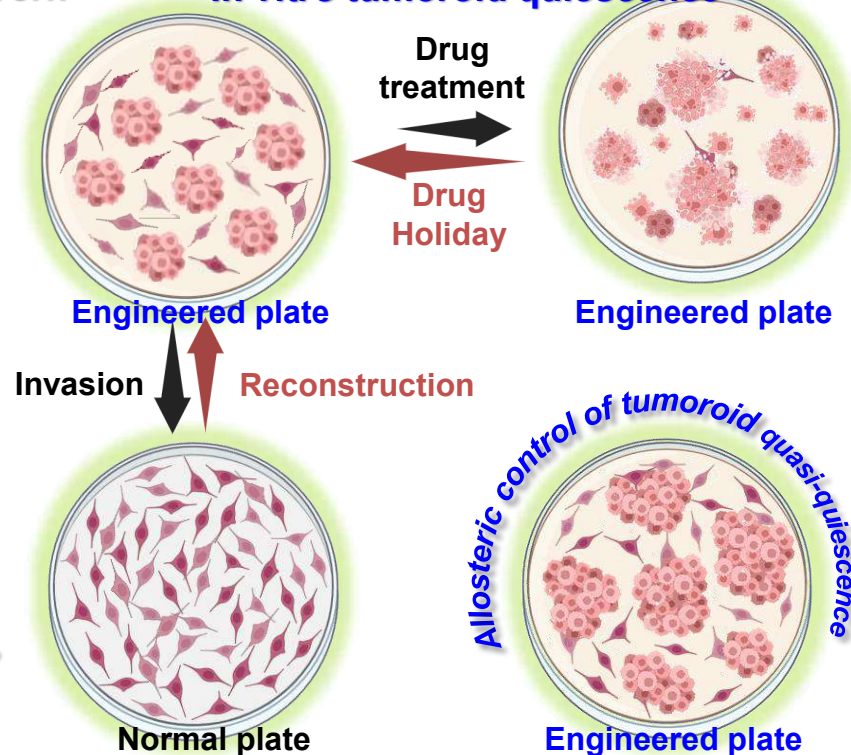
In Clinical Therapy

In vivo tumor quiescence



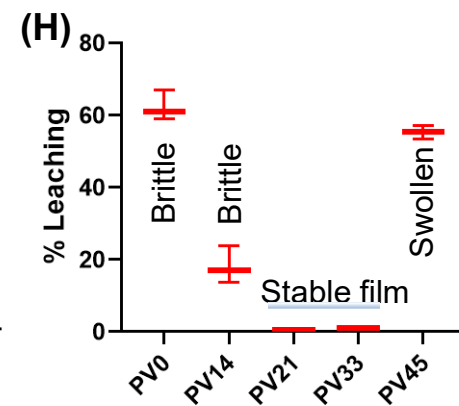
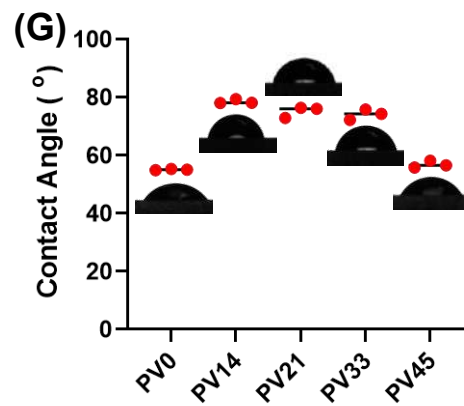
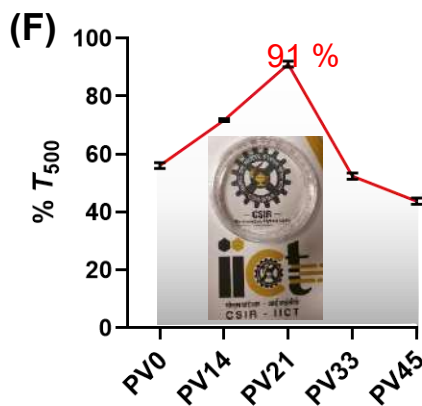
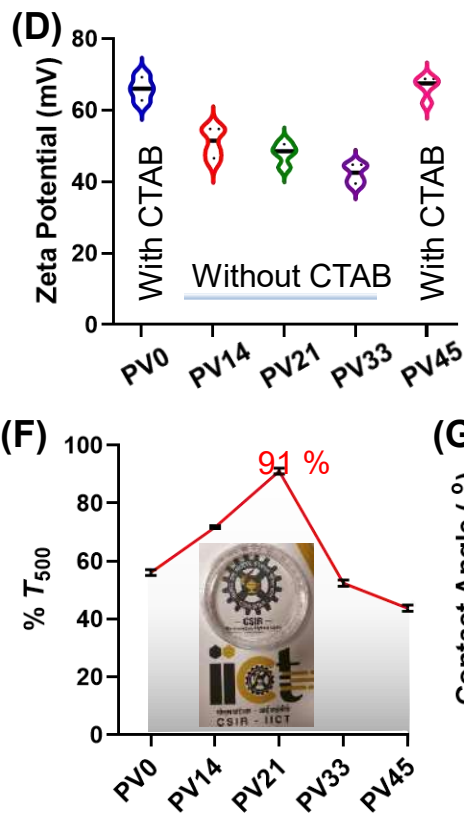
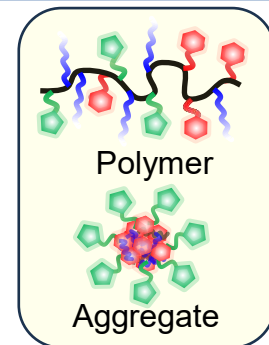
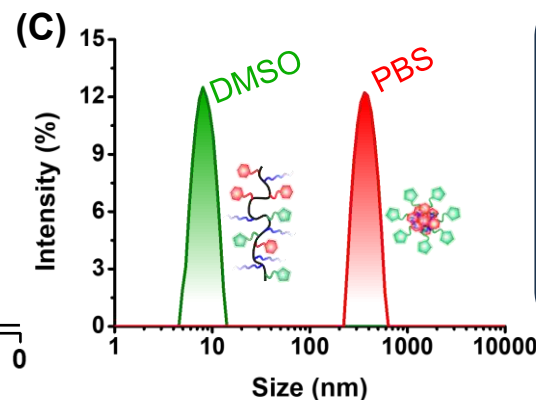
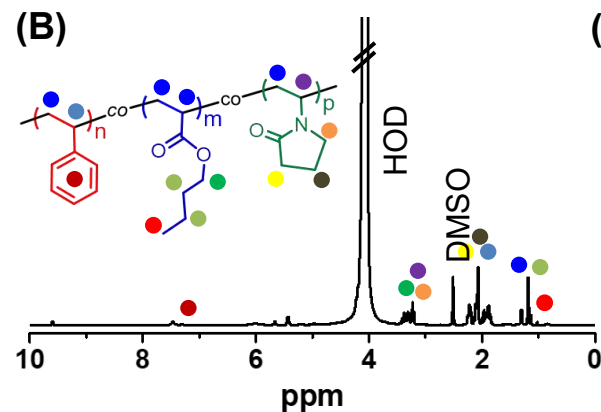
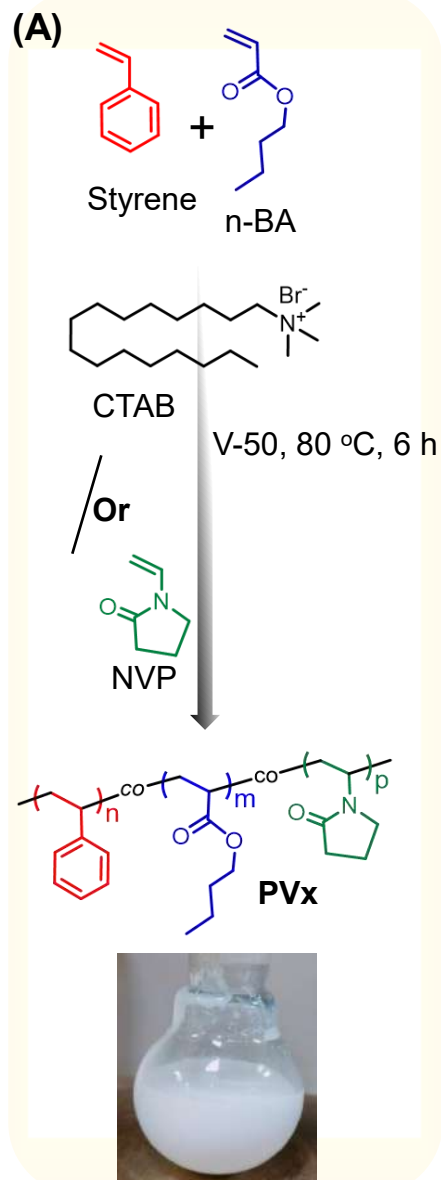
Our Work

In vitro tumoroid quiescence



In vitro mimic of in vivo glioblastoma quiescence

Synthesis & Characterizations



Development of Tumoroids

(A)



Emulsion polymer

Dropcast
Spin coating

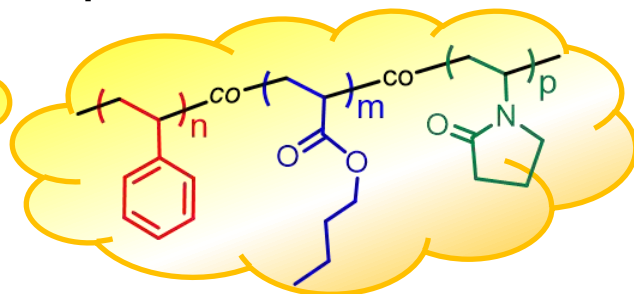
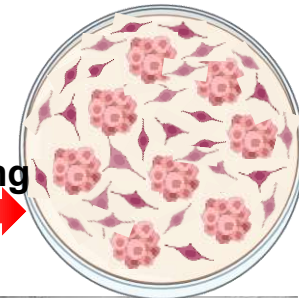


60 °C
2 h

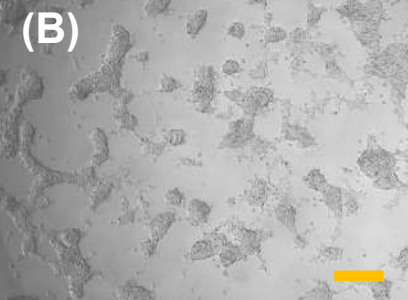


Coated plate

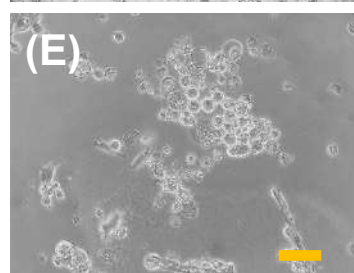
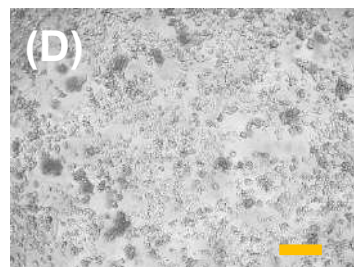
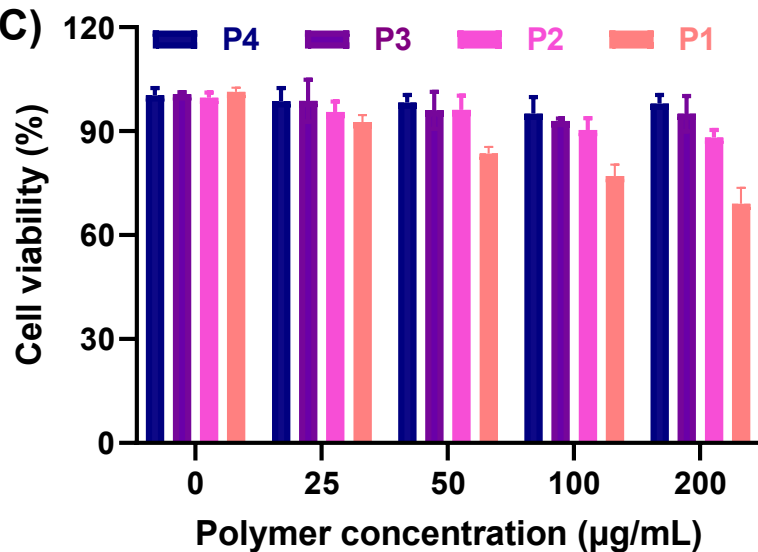
Cell seeding



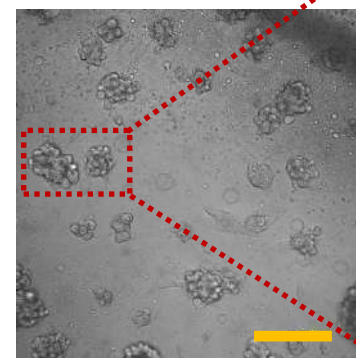
U87
tumoroids



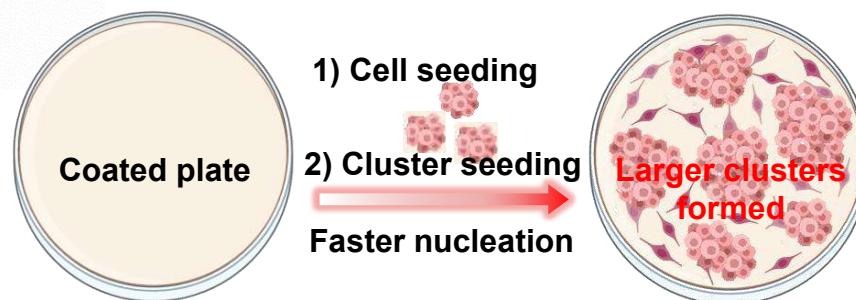
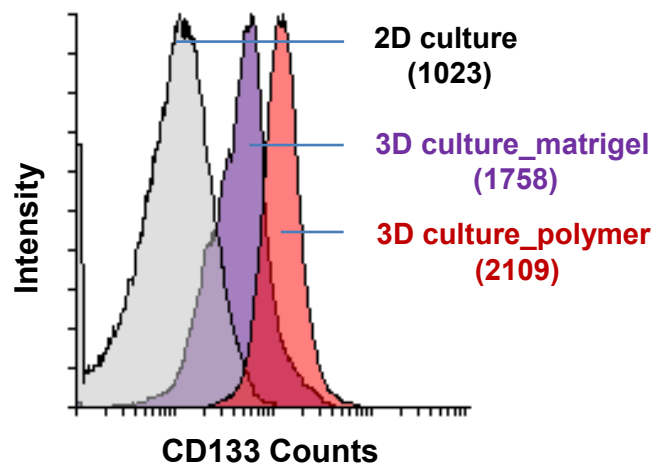
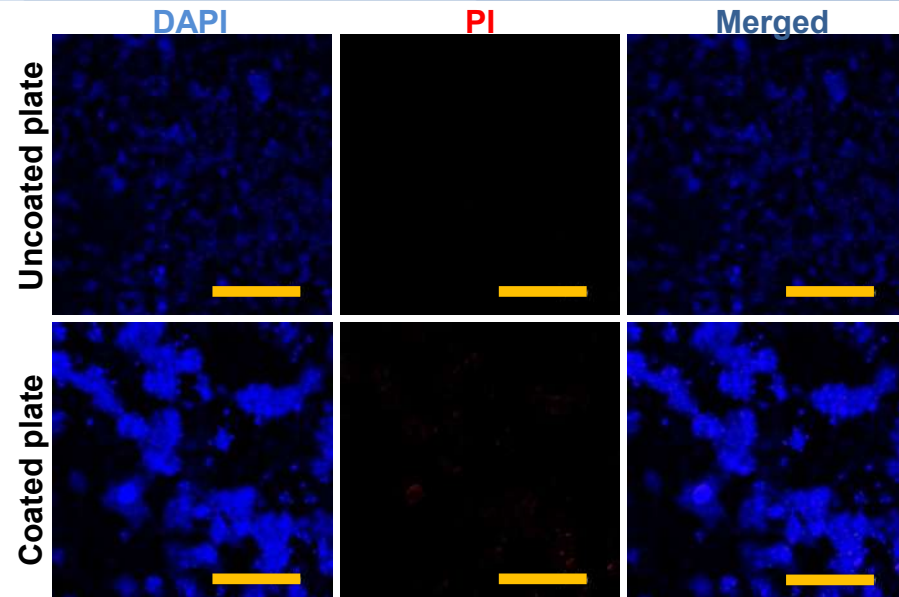
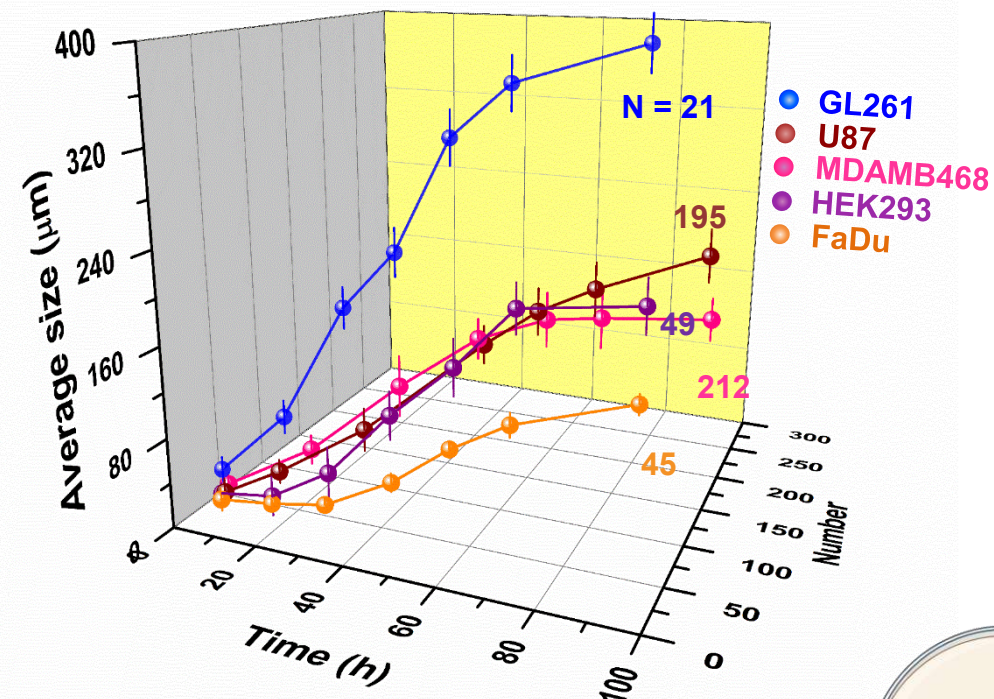
(C)



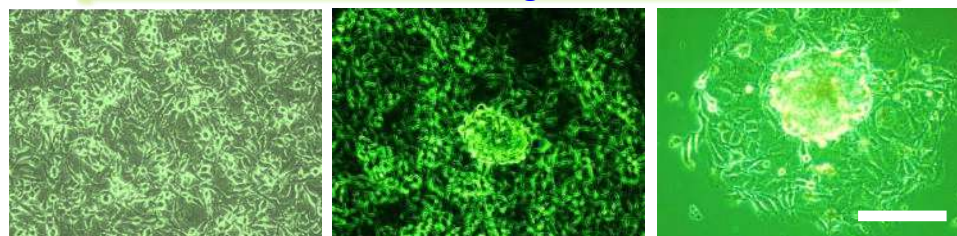
(F)



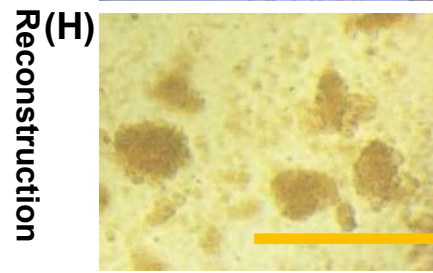
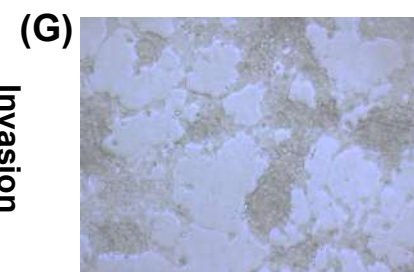
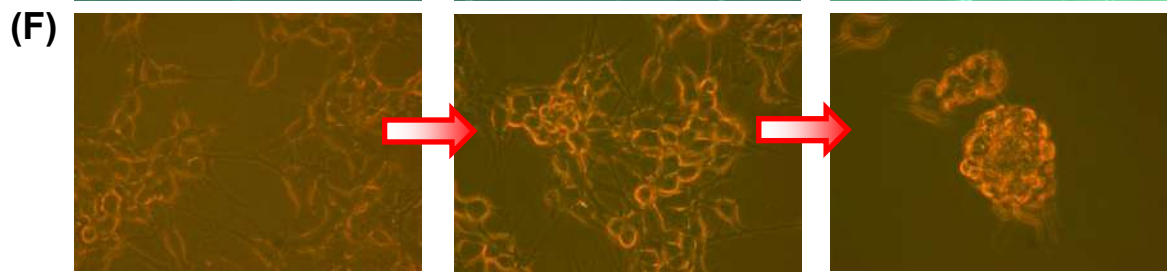
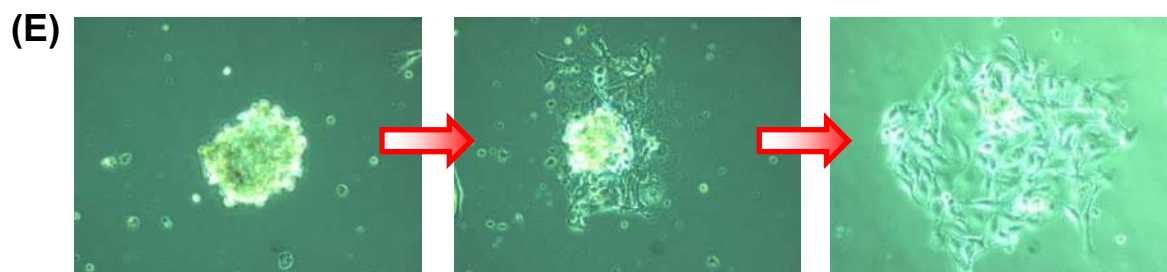
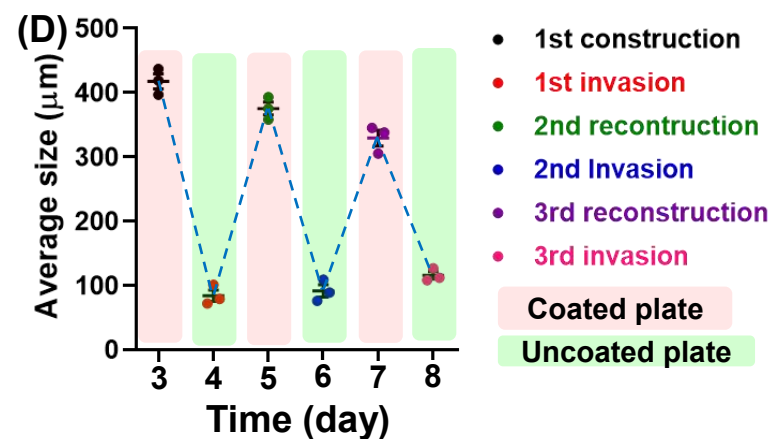
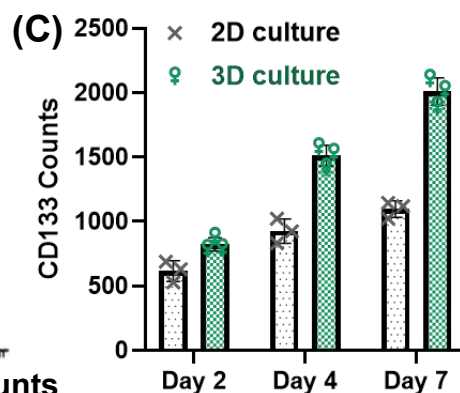
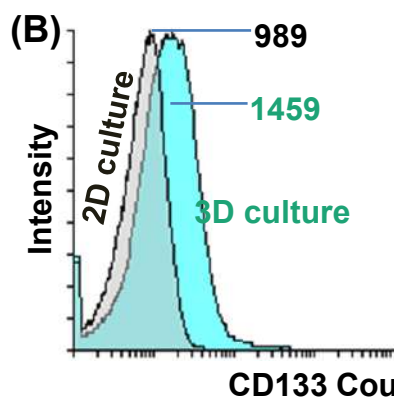
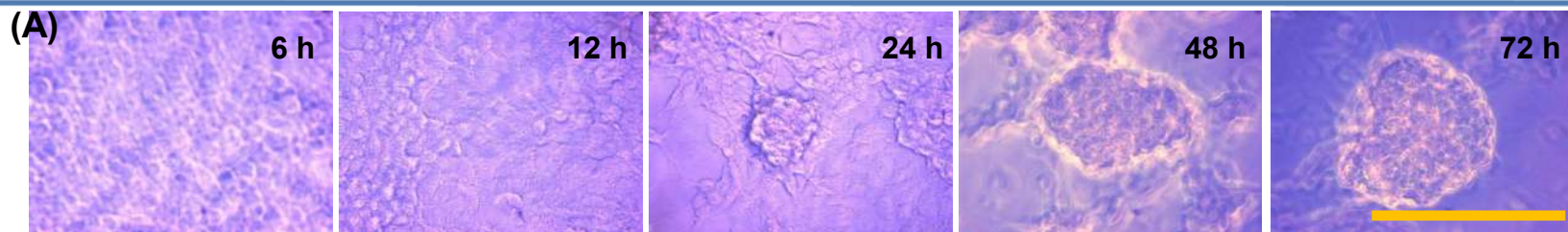
Tumoroid Production



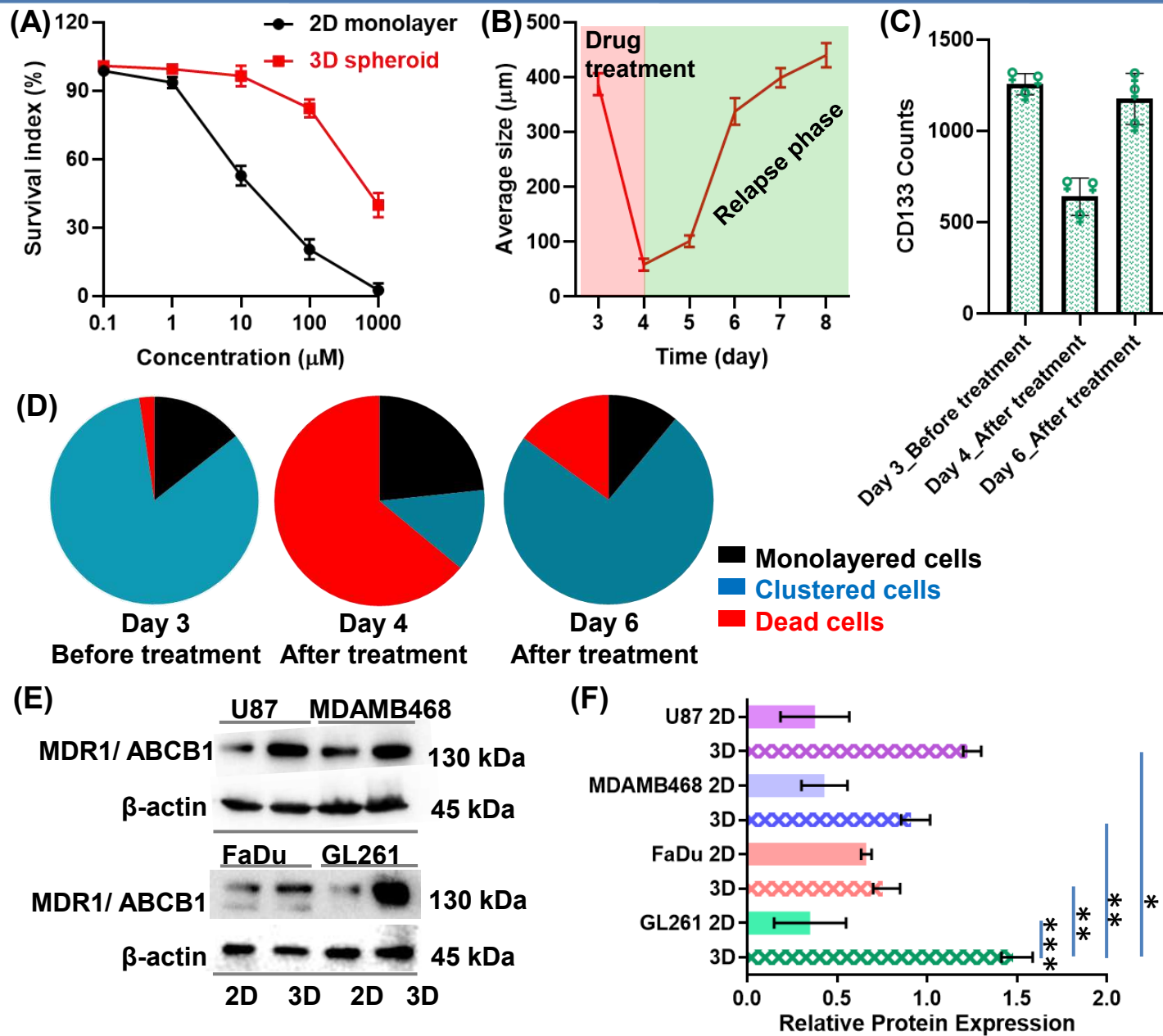
Larger clusters formed within 12 h post smaller clusters seeding with cells



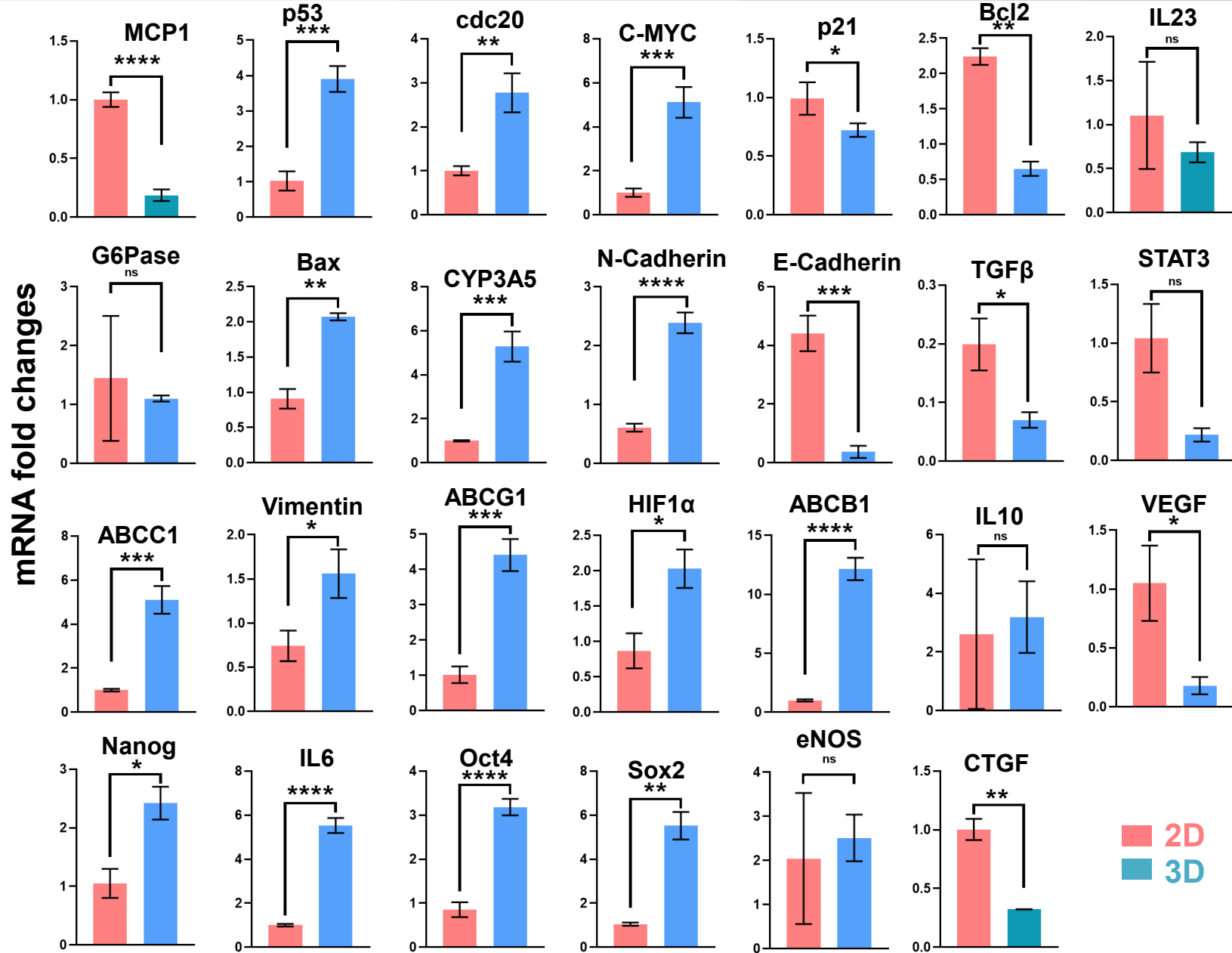
Key Features



Drug Treatment



Transcriptomic Analysis



Conclusions

Inventiveness

1. This is a cost-effective greener strategy for preparing stable polymeric coating that promotes the speedy and effective production of cancer spheroids on cell culture plates.
2. This coating material is reusable and recyclable. It can be used repeatedly to grow tumoroids without losing its effectiveness.
3. Ease of production makes it suitable for large-scale production.
4. The tumoroids can be reversibly engineered (in terms of stemness) using the coating platform.
5. The pre-formed small clusters of cells could accelerate/boost the formation of larger tumoroids on the coated surface.

Industrial Application Potential

- This invention holds high promise on commercial 3D tumoroid-based drug screening technology as well as for (to-be-tested) organoid tissue-model regeneration applications.

Outcomes

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2. Sahoo, S.; Ghosh, S.; Malik A. K. A.; Das, P.; Upadhyay, A.; Chakrabarty, A.; Banerjee, R. Emergence of In Vitro Glioblastoma Survival via Adaptive Quasi-Quiescence by Emulsion Copolymer Matrix. *Adv. Healthcare Mater. 2025, e01637*.

THANKS!



Inhibitors of the PQS Receptor (PqsR) in *Pseudomonas aeruginosa*: A Promising Anti-Virulence Therapeutic Approach

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INTRODUCTION

The *Pseudomonas* Quinolone Signal (PQS) system is a crucial component of the complex quorum sensing (QS) network in *Pseudomonas aeruginosa*, a multidrug-resistant opportunistic pathogen responsible for severe infections, particularly in immunocompromised individuals. The PQS system regulates the expression of various virulence factors, including pyochelin, pyoverdine and oxidative stress responses, through its interaction with the transcriptional regulator PqsR (also known as MvfR). PqsR is activated by alkyl quinolone signal molecules such as PQS and HHQ (2-heptyl-4-hydroxyquinoline), leading to the transcription of genes involved in virulence and further quinolone synthesis, forming a positive feedback loop. Given the essential role of PqsR in mediating virulence without affecting bacterial viability, it has emerged as a promising target for anti-virulence therapy.

The PQS System & PqsR (MvfR)

- The *Pseudomonas* Quinolone Signal (PQS) is a quorum sensing (QS) system that controls expression of virulence factors (pyocyanin, elastase), biofilm maturation, and inter-kingdom interactions.
- PqsR (also called MvfR) is a LysR-type transcriptional regulator that binds PQS family ligands (e.g., HHQ, PQS) and activates downstream virulence gene expression.
- Targeting PqsR blocks transcriptional activation of PQS-regulated genes → reduced virulence and biofilm formation without exerting bactericidal pressure.

Mechanism of Inhibition

Competitive antagonism

Conformational locking

Downstream readouts

Experimental Approaches to Validate Inhibitors

In vitro assay

Biophysical methods

In vivo models

Classes of PqsR Inhibitors

Sl no		
1	Small molecule antagonists	Structural analogs of PQS/HHQ that bind PqsR but fail to activate transcription. Example scaffolds: quinolone derivatives, benzamide and phenyl furanone analogs.
2	Allosteric inhibitors	Molecules that bind outside the ligand pocket, perturbing PqsR conformation.
3	Fragment-based leads	Low-MW fragments discovered by NMR/X-ray fragment screening, optimized by medicinal chemistry.
4	Natural product inhibitors	Plant flavonoids and microbial metabolites reported to interfere with PQS signaling.

Classes of PqsR Inhibitors

- Targets virulence, not viability — potentially reduces resistance development.
- Can be used as adjuvants to conventional antibiotics, improving efficacy against biofilm-embedded bacteria.
- May attenuate inflammation by lowering toxin production, improving clinical outcomes.

CONCLUSIONS

- Despite promising results, challenges remain in optimizing the pharmacokinetic and pharmacodynamic properties of PqsR inhibitors for clinical application. Issues such as bioavailability, metabolic stability, and off-target effects must be addressed through further medicinal chemistry optimization and preclinical evaluation.
- In conclusion, the PQS system and its receptor PqsR play a central role in regulating *P. aeruginosa* virulence. Targeting this system with specific inhibitors offers a novel, non-bactericidal approach to control infections by disarming the pathogen rather than killing it, thereby reducing selective pressure for resistance.
- Continued efforts in drug discovery, structural biology, and translational research are essential to advance PqsR inhibitors toward therapeutic use in managing *P. aeruginosa* infections, especially in the face of rising antibiotic resistance.

References

1. Vico-Oton, E. (2019). *Pseudomonas aeruginosa* PQS mediated virulence regulation and interference (Doctoral dissertation, University of Nottingham).
2. Aziz, M., Ahmed, K., & Ravichandran, V. (2025). PqsR-specific quorum sensing inhibitors targeting *Pseudomonas aeruginosa*: current advances and future directions. *Journal of Chemotherapy*, 1-18.
3. Soheili, V., Tajani, A. S., Ghodsi, R., & Bazzaz, B. S. F. (2019). Anti-PqsR compounds as next-generation antibacterial agents against *Pseudomonas aeruginosa*: A review. *European journal of medicinal chemistry*, 172, 26-35.

The logo of Pondicherry University is a circular emblem. It features a yellow sun with rays at the top, a pink lotus flower in the center, and blue horizontal stripes at the bottom. The text "PONDICHERRY UNIVERSITY" is written in a circle around the top, and "VERS LA LUMIERE" is written around the bottom. In the center, there is a Sanskrit motto: "वसुधैव कुटुम्बकम्" (Vasudhaiva Kutumbakam).

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- The alarming scenario of antimicrobial resistance made treatment of infections due to opportunistic pathogen *Pseudomonas aeruginosa* challenging.
- The cell-cell communication system- Quorum sensing (QS) and associated biofilm is an excellent target for the discovery of novel antimicrobial modalities.
- Plant flavonoids are known for its anti-QS and anti-biofilm efficacy. In this study, Derrisisoflavone-B (DIF-B), a diprenylated isoflavone, was analyzed for its anti-QS and antibiofilm potential through *in vitro*, *in vivo* and *in silico* approaches and compared with known QS inhibitory flavonoid Baicalein (BCL).

***In silico* analysis to predict drug likeliness, toxicity and pharmacokinetic properties**

Compound	Docking Score (kcal/mol)	No. of Hydrogen bonds	Hydrophobic interactions
LasR			
C₁₂-HSL	-9.442	4 (*TRP 60, *ASP 73, *ARG 61, *ARG 61)	*PHE 101, *TYR 56, *THR 75, *TRP 88, *LEU 36, *TYR 64, ALA 50, *LEU 125, *TYR 47, *ILE 52, *GLY 126, *CYS 79, *LEU 110, ALA 105
BCL	-9.864	4 (*THR 75, *ARG 61, *ARG 61, *TRP 60)	*TYR 64, *LEU 36, ALA 50, *TYR 47, *VAL 76, *ASP 73, *TRP 88, *TYR 56, *SER 129, ALA 127
DIF- B	-11.880	1 (*LEU 125)	*LEU 39, *LEU 40, *GLY 38, ALA 50, *TYR 64, *ARG 61, *LEU 36, *TRP 88, *LEU 110, *PHE 101, *TYR 56, ALA 105, *THR 75, *TYR 93, *TRP 60, *SER 129, *ASP 73, *TYR 47, *CYS 79, *VAL 76, *GLY 126, ALA 127
RhlR			
C₄-HSL	-6.066	1 (*TRP 68)	*LEU 69, *ASP 81, *TYR 72, ALA 44, *SER 135, *TYR 64, *ILE 84, *LEU 107, *TRP 108, *PHE 101, ALA 111
BCL	-9.277	-	*GLY 46, *VAL 60, *SER 135, *TYR 45, ALA 44, *ILE 84, *TYR 64, *ASP 81, *TRP 96, *PHE 101, *TYR 72, *LEU 69, *TRP 68
DIF- B	-3.282	-	*GLN 73, *VAL 60, *THR 58, ALA 79, *PRO 56, *PHE 53, *THR 54, GLY 78, *ASN 76, *TYR 72

A

OD (600 nm)

Time (Hrs)

Control BCL DIF-B

B

BCL

DIF-B

Pyocyanin
LasA Protease
LasB Elastase
Total protease
Biofilm formation
Cell Viability
EPS
Alginate
Rhamnolipid
MATH

80
70
60
50

C

Percentage inhibition

BCL DIF-B

D

Swimming diameter (cm)

Control BCL DIF-B

A

Control **BCL** **DIF-B**

B

Control **BCL** **DIF-B**

Figure 2 displays fluorescence microscopy images of *C. elegans* expressing GFP under different promoters. Panel A shows 3D surface plots of GFP expression in the head region for Control, BCL, and DIF-B conditions. The Control plot shows a high, uniform expression level across the head region. The BCL plot shows a significantly reduced expression level, and the DIF-B plot shows a further reduced expression level. Panel B shows 2D fluorescence images of the whole worm for the same conditions. The Control worm shows strong GFP expression throughout its body. The BCL worm shows a significantly reduced expression level, and the DIF-B worm shows a further reduced expression level. Scale bars are 20 μ m.

1. Salim, S.A., Mohan, M.S., Ranganathan, S., Parasuraman, P., Lee, J.K., Ramatchandirane, M., Suchiang, K. and Busi, S. (2025) Microb. Pathog. p.107738.
2. Mohan, M.S., Salim, S.A., Ranganathan, S., Parasuraman, P., Anju, V.T., Ampasala, D.R., Dyavaiah, M., Lee, J.K. and Busi, S (2024) Microb. Pathog. 189, 106609.



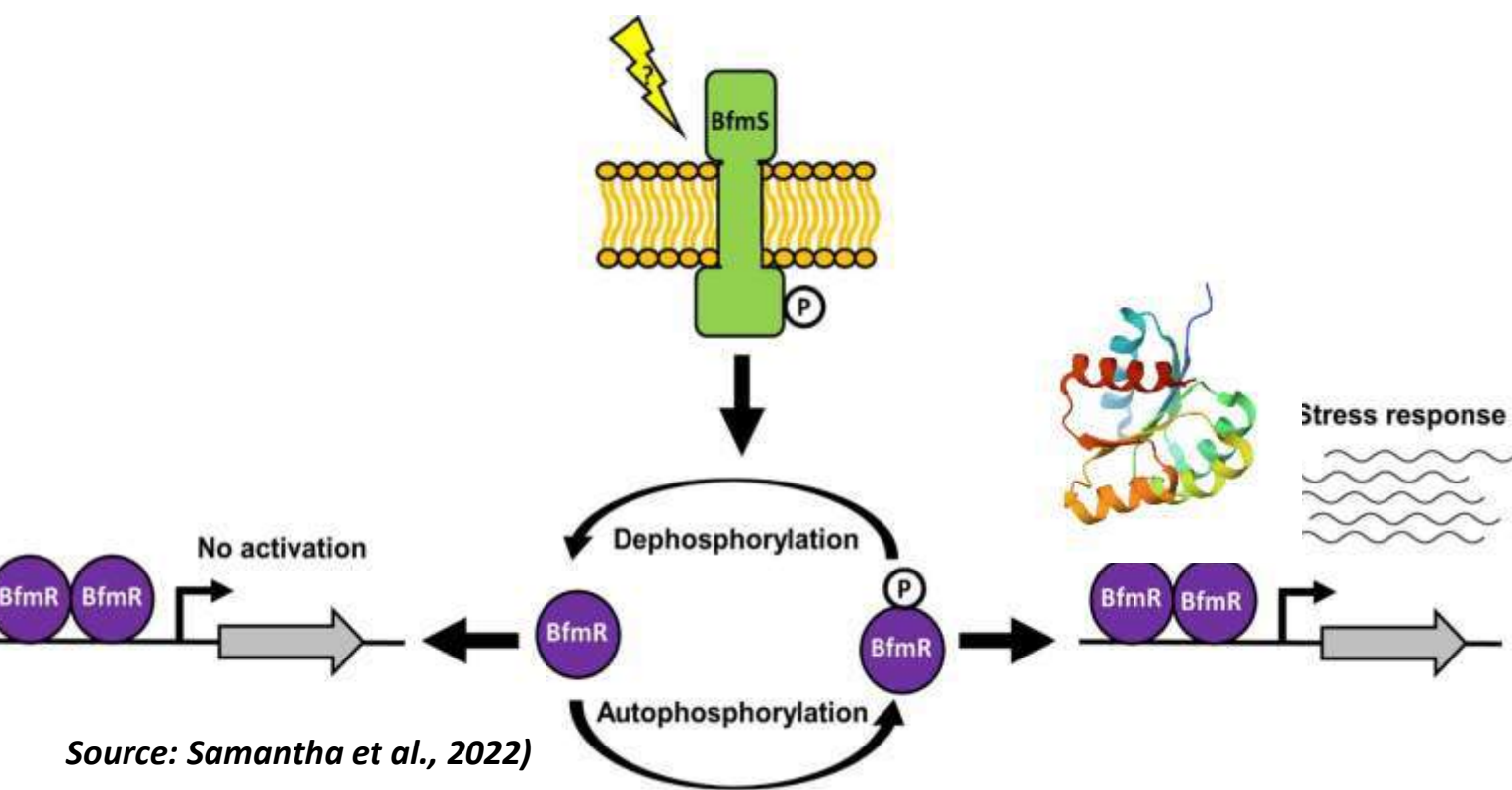
Introduction

Acinetobacter baumannii is an important Gram-negative nosocomial pathogen often associated with severe nosocomial infections, including ventilator-associated pneumonia, urinary tract infections, bacteremia and septicemia, cystic fibrosis etc.,

It is a member of the ESKAPE pathogens *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species

To address the advancement of *A. baumannii* resistance juncture, naturally occurring flavonoids which are the standalone compounds playing an essential role in downregulating biofilm formation by interfering with the response regulatory system (BfmR).

What is BfmR/S system?



Flavonoids

Sl.no	Flavonoids	Structure	Pub chem ID
01.	Curcumin		969516
02.	Chrysin		5281607
03.	Quercetin		5280343

References

Palethorpe, S., Farrow III, J. M., Wells, G., Milton, M. E., Actis, L. A., Cavanagh, J., & Pesci, E. C. (2022). *Acinetobacter baumannii* regulates its stress responses via the BfmRS two-component regulatory system. *Journal of bacteriology*, 204(2), e00494-21.

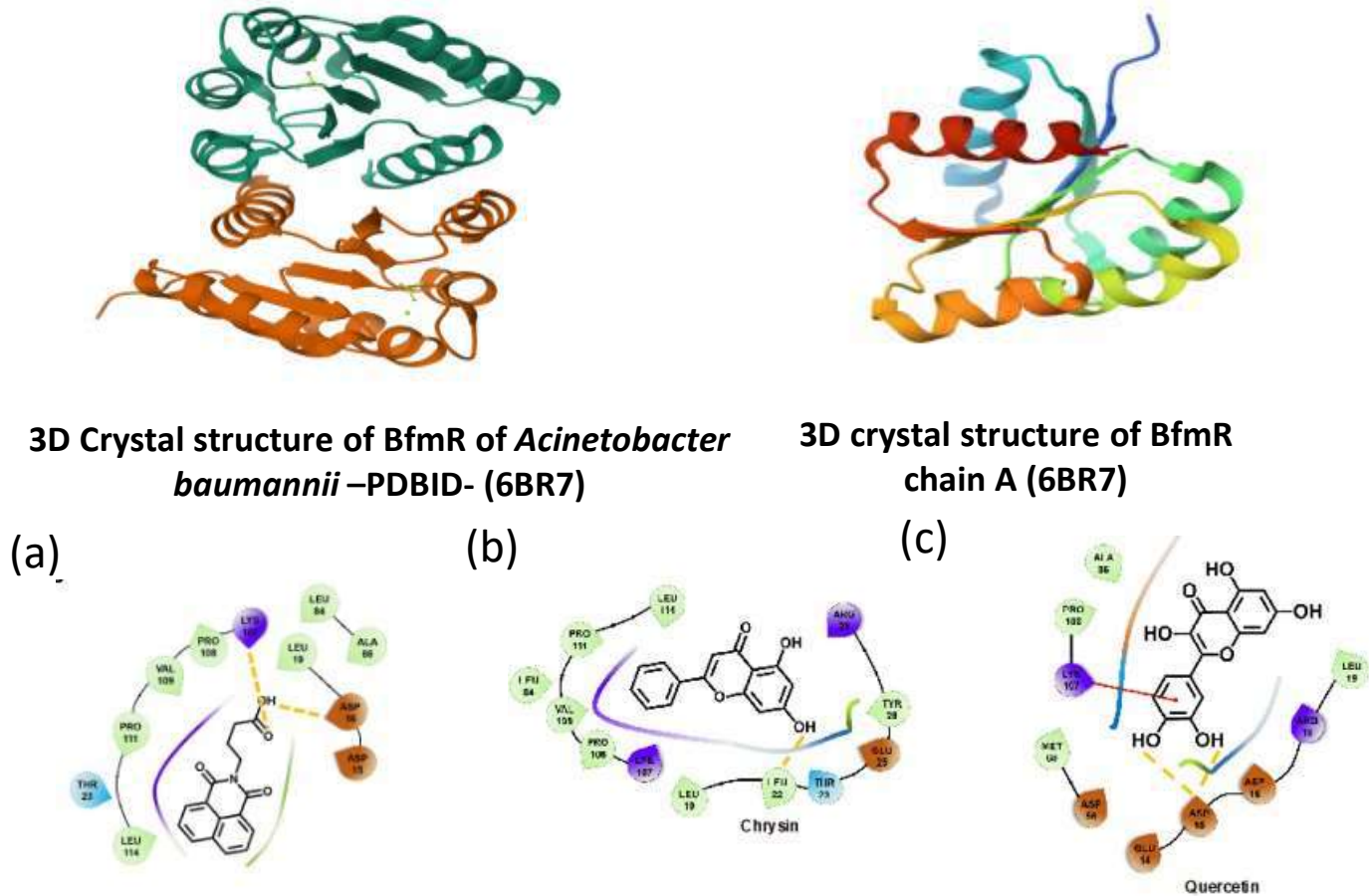
Raorane, C. J., Lee, J. H., Kim, Y. G., Rajasekharan, S. K., García-Contreras, R., & Lee, J. (2019). Antibiofilm and antivirulence efficacies of flavonoids and curcumin against *Acinetobacter baumannii*. *Frontiers in microbiology*, 10, 990.

In-vivo studies

- Agar well diffusion method (*Abdhulla et al., 2024*)
- Minimum inhibitory Concentration (*Shu-Yun Wei et al., 2025*)
- MATH assay (*Anthonymuthu Selvaraj et al., 2020*)
- EPS extraction and Quantification (*Jothipandian et al., 2022*)
- Biofilm and Pellicle Formation Assays (*Oh,M.H., et al., 2020*)
- PNAG production (*Choi et al., 2009*).
- Surface motility (*Hyo kim et al., 2022*).
- Sub-surface Twitching assay (*Luo et al., 2015*)
- Bioassay for the detection of Autoinducers (*Hyo kim et al., 2022*)

In- silico studies

Molecular Docking Simulations of Flavonoids With BfmR



2D Interaction patterns of flavonoids with the N-terminal domain of BfmR (a) Curcumin (b) Chrysin (c) Quercetin
Source: (*Raorane et al., 2019*)

Conclusion

Flavonoids plays a major role in inhibiting the biofilm formation by directly interacting with the BfmR response regulator of *Acinetobacter baumannii*.

This approach helps in addressing anti-virulence strategies against *A.baumannii* infections.

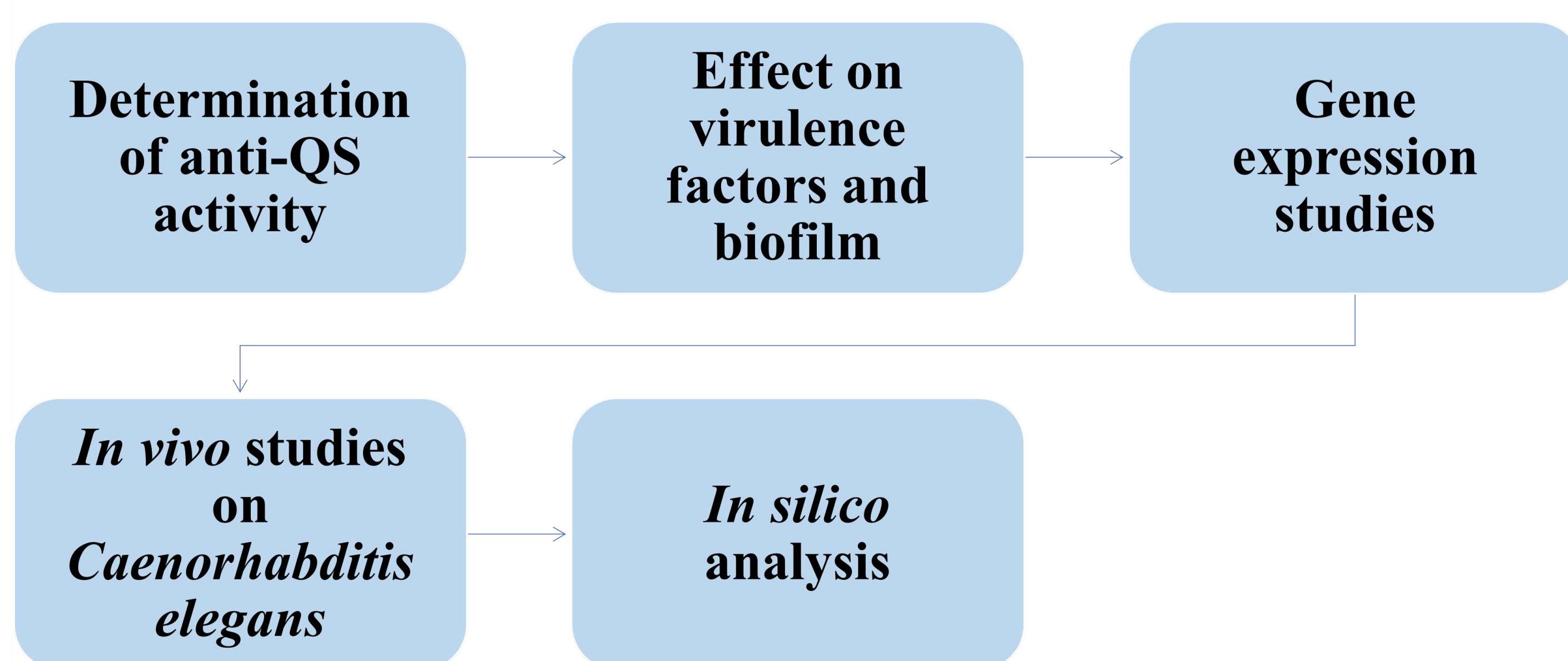
Acknowledgement

Milleny N Y is grateful to Pondicherry university for the research fellowship

INTRODUCTION

- Quorum Sensing plays an important role in *Pseudomonas aeruginosa* virulence and pathogenesis
- Targeting QS signaling mechanism can overcome the virulence and antibiotic resistance mechanisms of *P. aeruginosa*
- Phytochemicals are known for their ability to target QS signaling pathways and reduces the pathogenesis
- Artocarpesin (ACN), a 6-prenylated flavone inhibited virulence factors by targeting QS mechanisms

METHODS



RESULTS

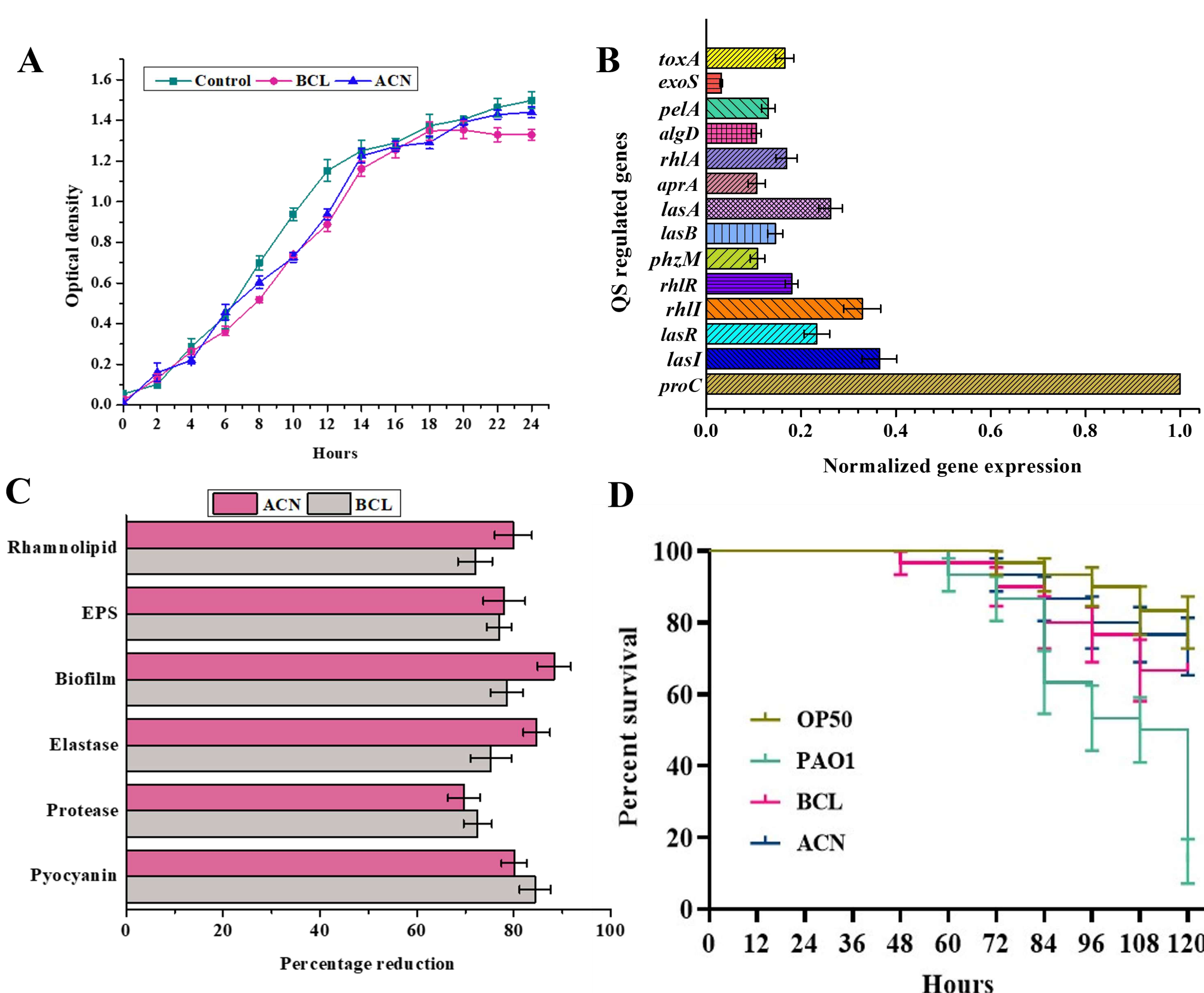


Fig 1. (A) Growth curve of *P. aeruginosa* PAO1 treated with ACN and positive control, BCL at $\frac{3}{4}$ MIC concentration; (B) Normalized gene expression of QS genes (*lasI/lasR*, *rhII/rhIR*) and related virulence genes are downregulated after treatment with ACN, which was compared with housekeeping gene, *proC*. (C) Percentage reduction of virulence factor and biofilm determinants production in *P. aeruginosa* when treated with ACN, which was compared with positive control BCL; (D) Kaplan-Meier graph represents the survival of *Caenorhabditis elegans* worms infected with PAO1, and treatment with ACN and BCL.

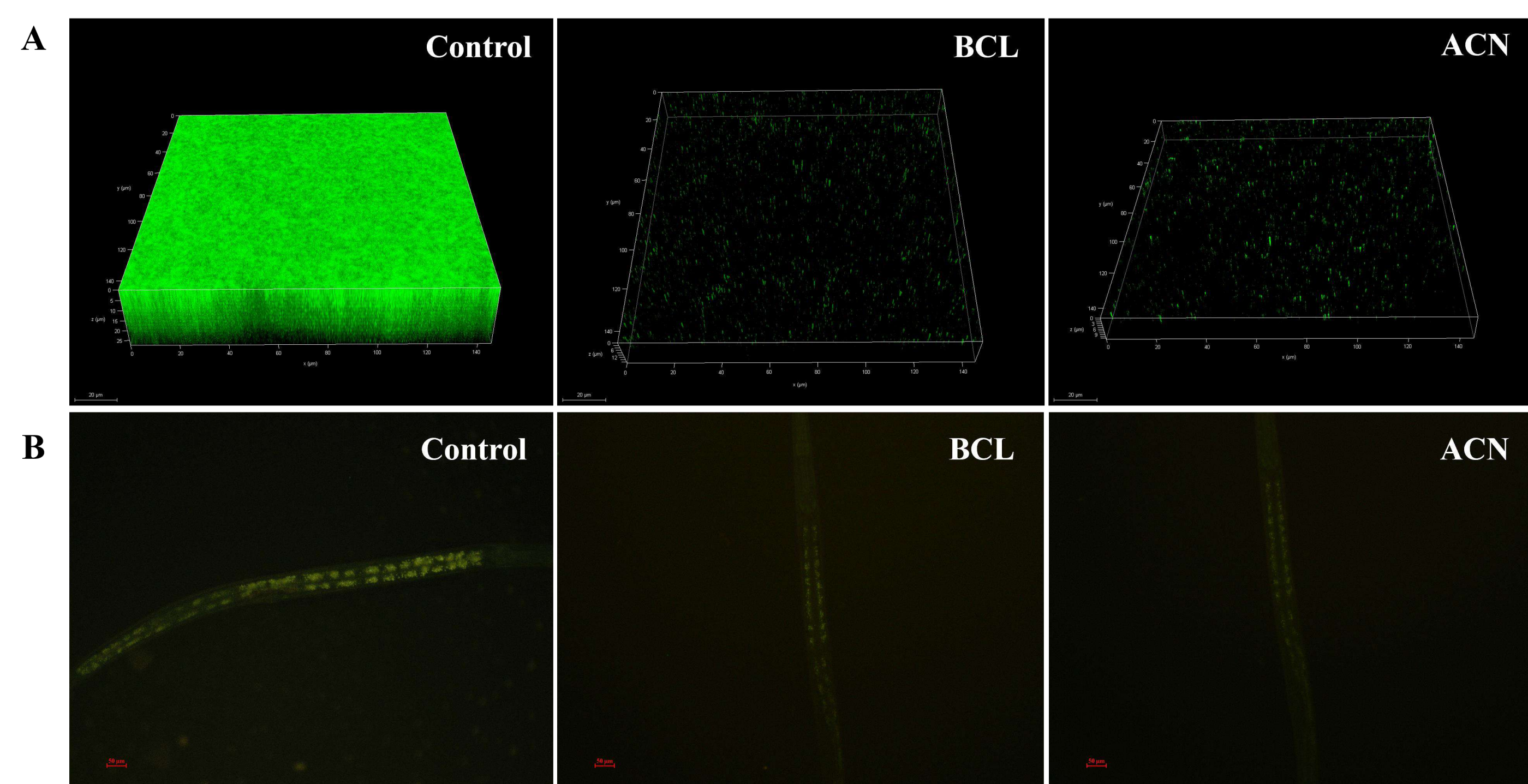


Fig 2. (A) CLSM images of biofilm formation after treatment with ACN which was compared with control and BCL treated; (B) Reduction in the colonization of *P. aeruginosa* in the gut of *C. elegans* treated with ACN and BCL at sub-MIC concentration.

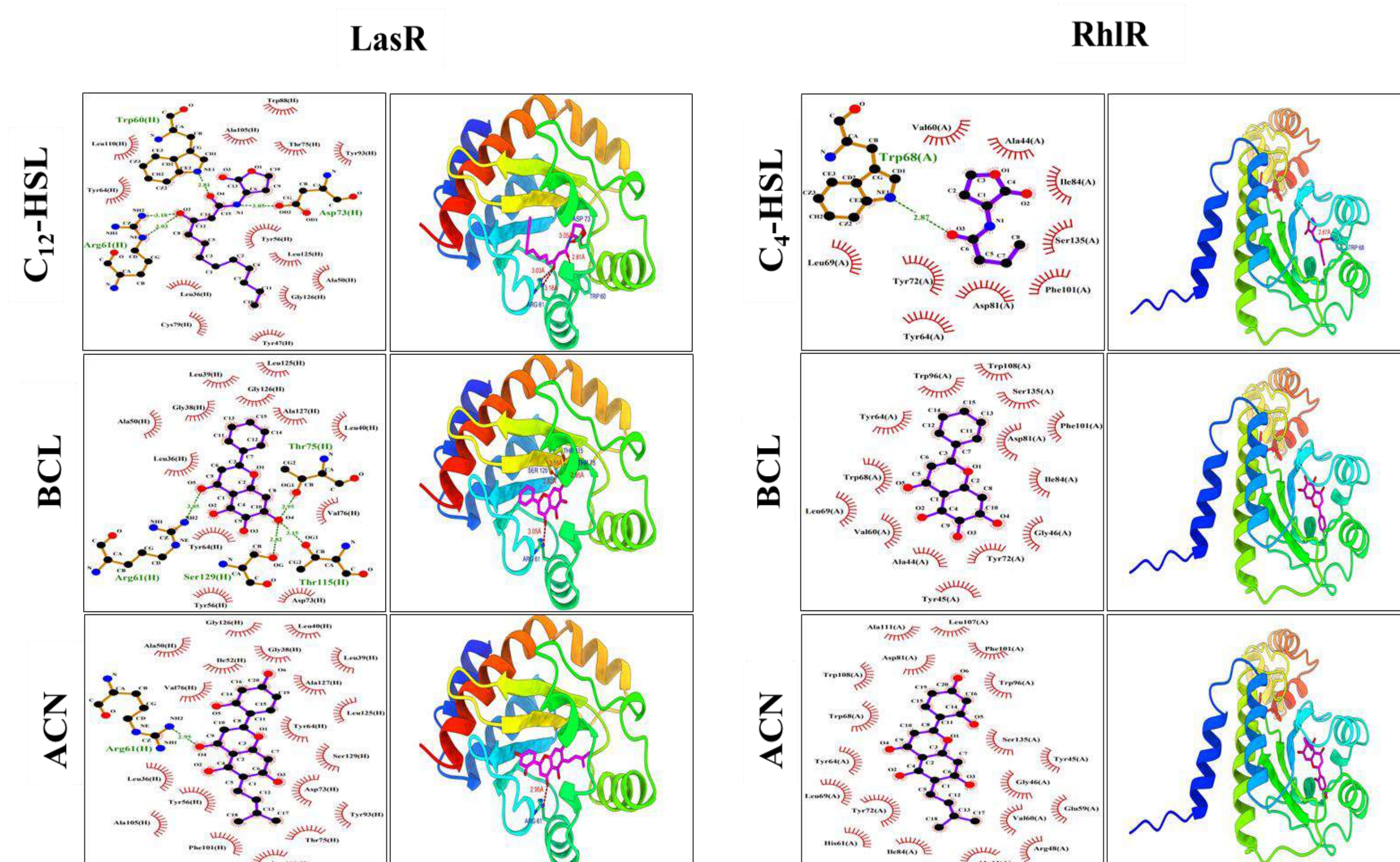


Fig 3. The interaction between ACN with QS receptors, LasR and RhIR was studied using molecular docking.

CONCLUSION

- ✓ Sub-MIC concentration of ACN is non bactericidal in nature
- ✓ Virulence factor production and biofilm formation was reduced in *P. aeruginosa* after treatment with ACN
- ✓ Downregulation of QS genes was observed after treatment with ACN
- ✓ ACN improves the survival of *C. elegans* infected with PAO1
- ✓ ACN competitively binds with the QS receptors than the natural ligands, evident from the molecular docking studies

References:

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Acknowledgements: Mahima S Mohan was grateful to Department of Science and Technology, Govt of India INSPIRE program for providing fellowship (DST/Inspire Fellowship/2021/IF210457).

Glucocorticoid receptor-targeted liposomal delivery of wnt/ β -catenin inhibitor selectively modulates tumor-microenvironment for efficient colorectal tumor regression

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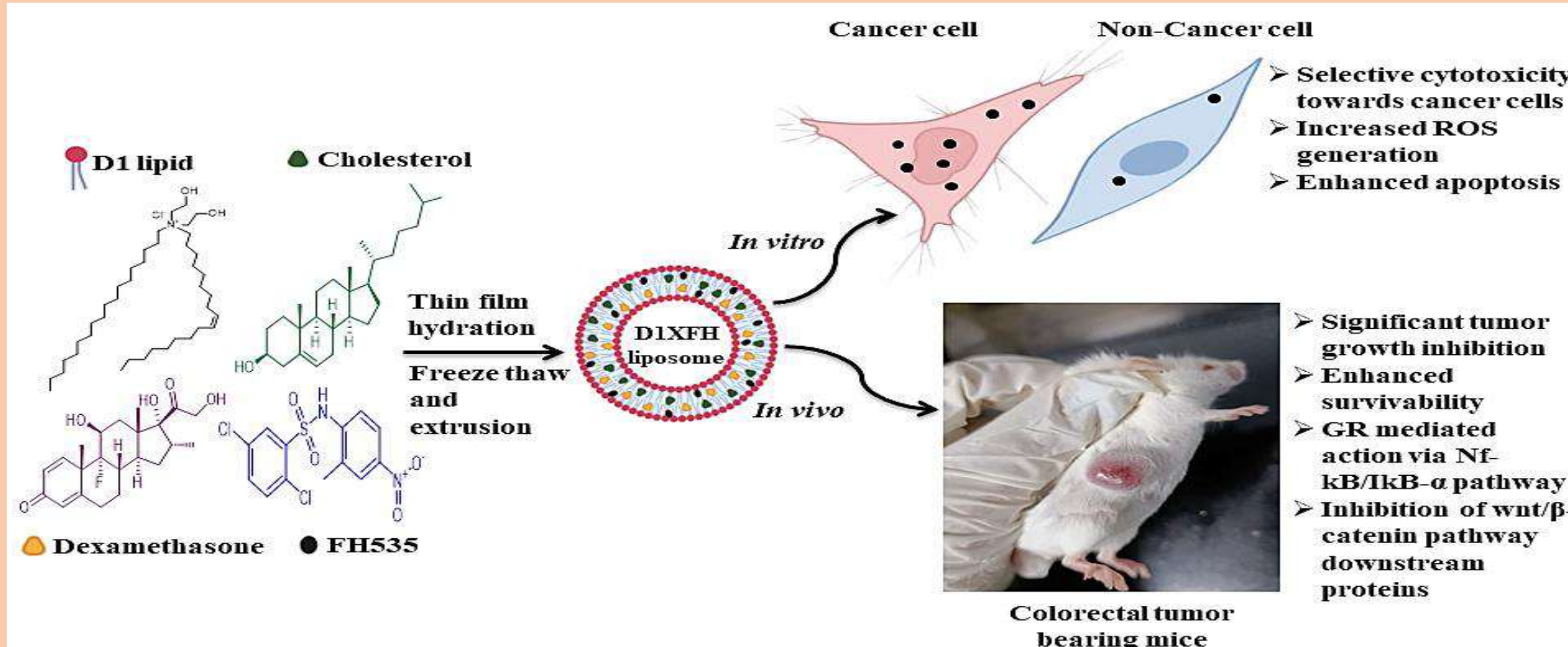
^cApplied Biology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad, 500007, India

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GR mediated selective targeting of wnt/ β -catenin pathway in colorectal cancer

- Wnt/ β -catenin signaling pathway is a highly conserved developmental pathway which is extremely challenging to selectively target.
- GR is a nuclear hormone receptor; present ubiquitously in all the cells and thus its cancer cell-selective targeting is also difficult.
- However, we showed that cancer cell-associated GR can be selectively targeted owing to their aberrant transactivation behavior in cancer cells.
- Based on this unique feature, here we intend to co-formulate a GR-targeted cationic liposomal formulation for simultaneous targeting of both the GR and wnt/ β -catenin pathway.

The rationale behind D1XFH formulation preparation



Materials and Methods

Liposomal formulations of FH535 were prepared by thin film hydration method

The final liposome was produced after 5 cycles of freeze thaw followed by extrusion (0.2 μ m)

Liposomes were characterized by DLS analysis and TEM imaging

Several *in vitro* and *in vivo* studies were performed

In vitro studies

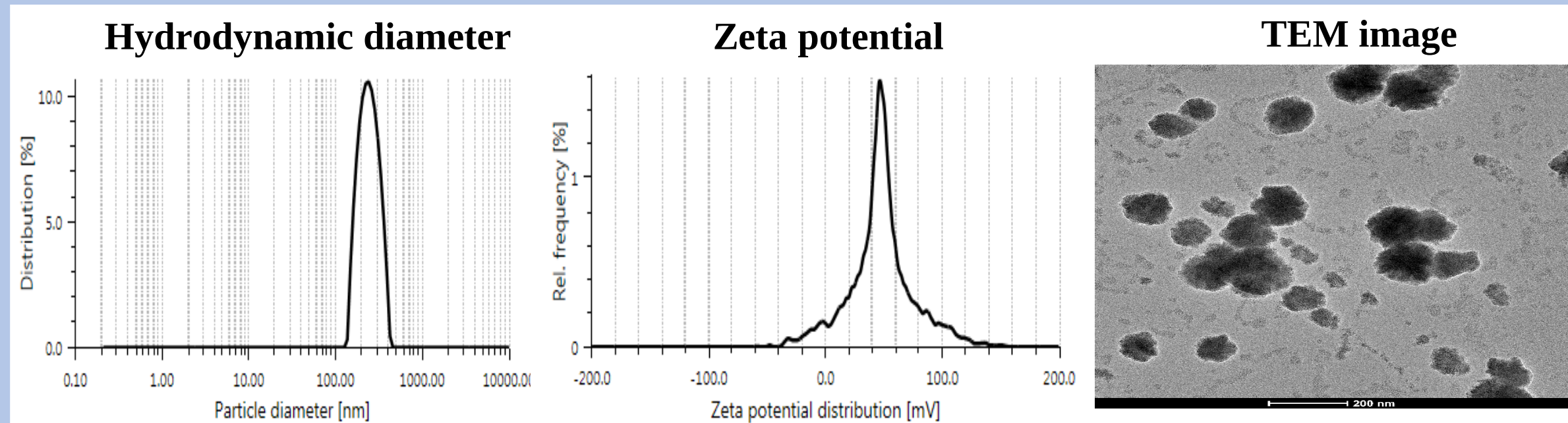
- Cellular cytotoxicity
- Cellular and nuclear uptake
- GR-transactivation
- ROS generation
- Apoptosis or cell death

In vivo studies

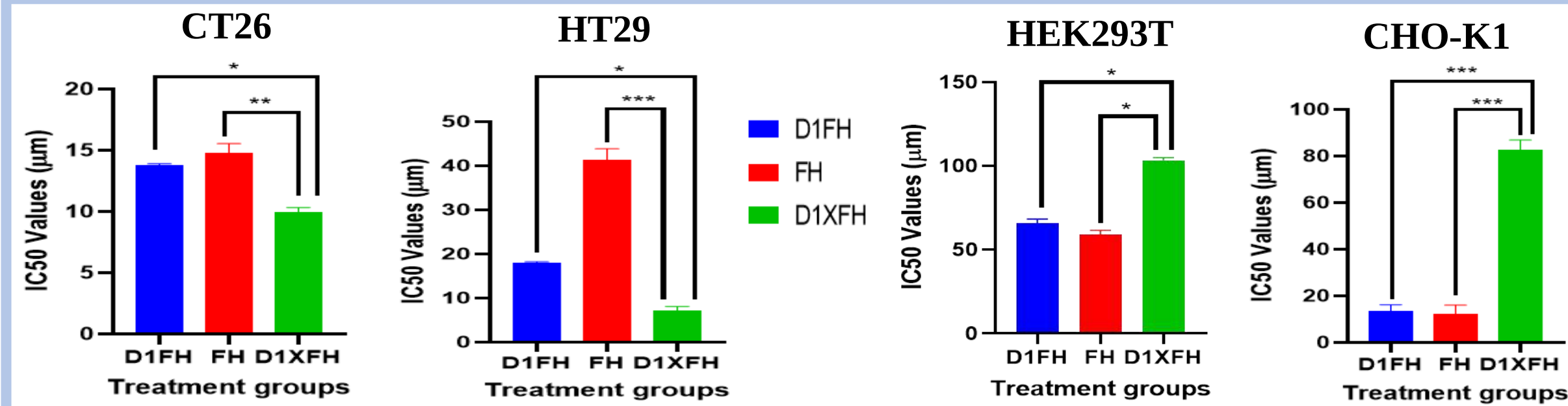
- Survivability
- Biodistribution
- Tumor regression
- Sub-chronic toxicity
- Protein expression

Results

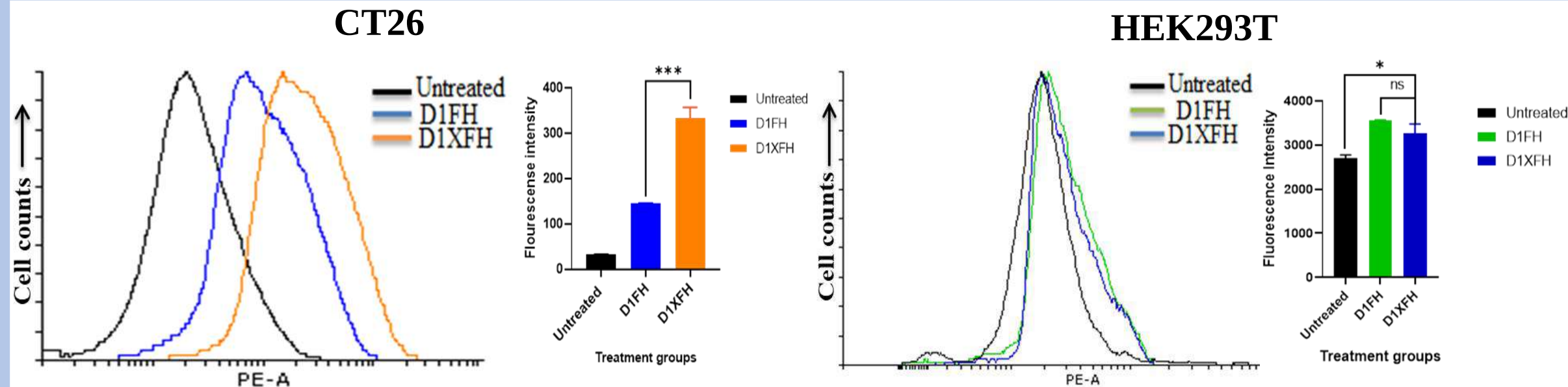
Characterization studies of D1XFH liposomal formulation



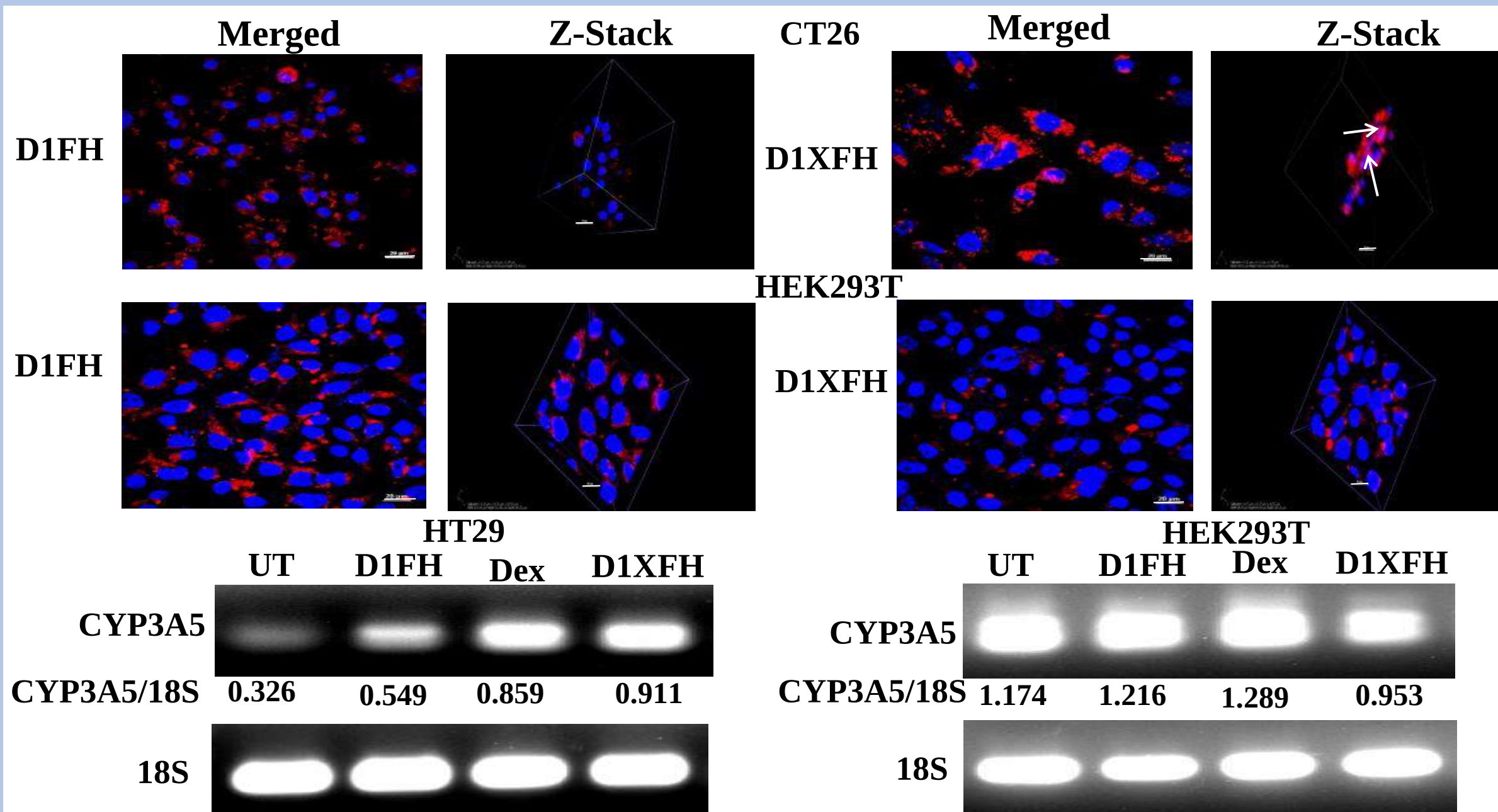
Selective cytotoxicity towards colorectal cancer cells



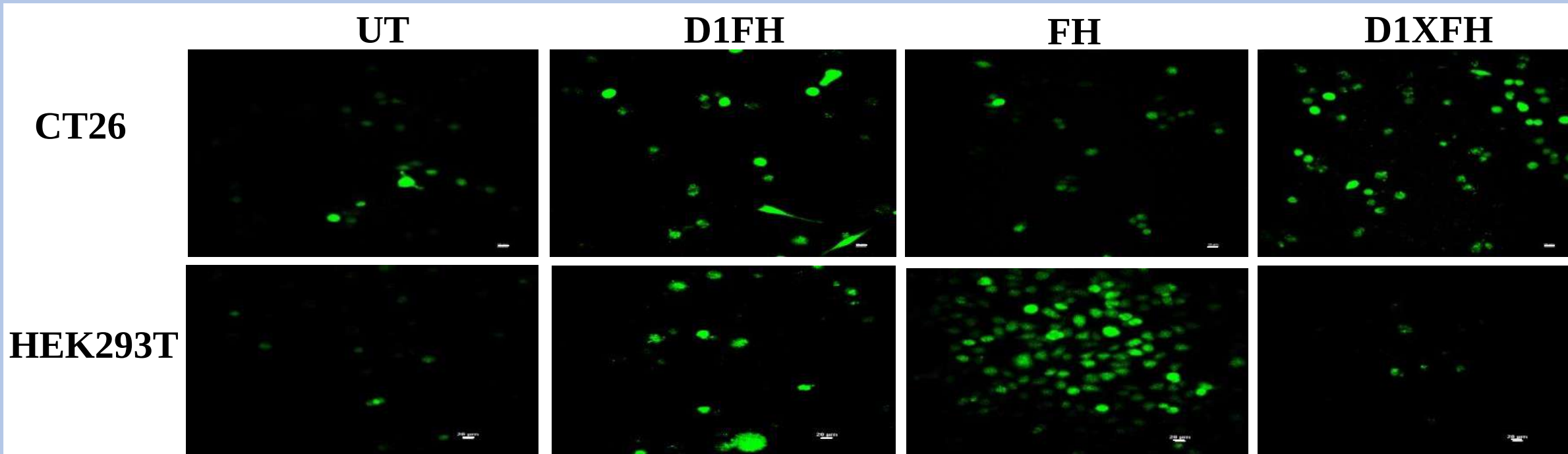
Enhanced cellular uptake in colorectal cancer cells



Selective nuclear uptake and GR transactivation in cancer cells



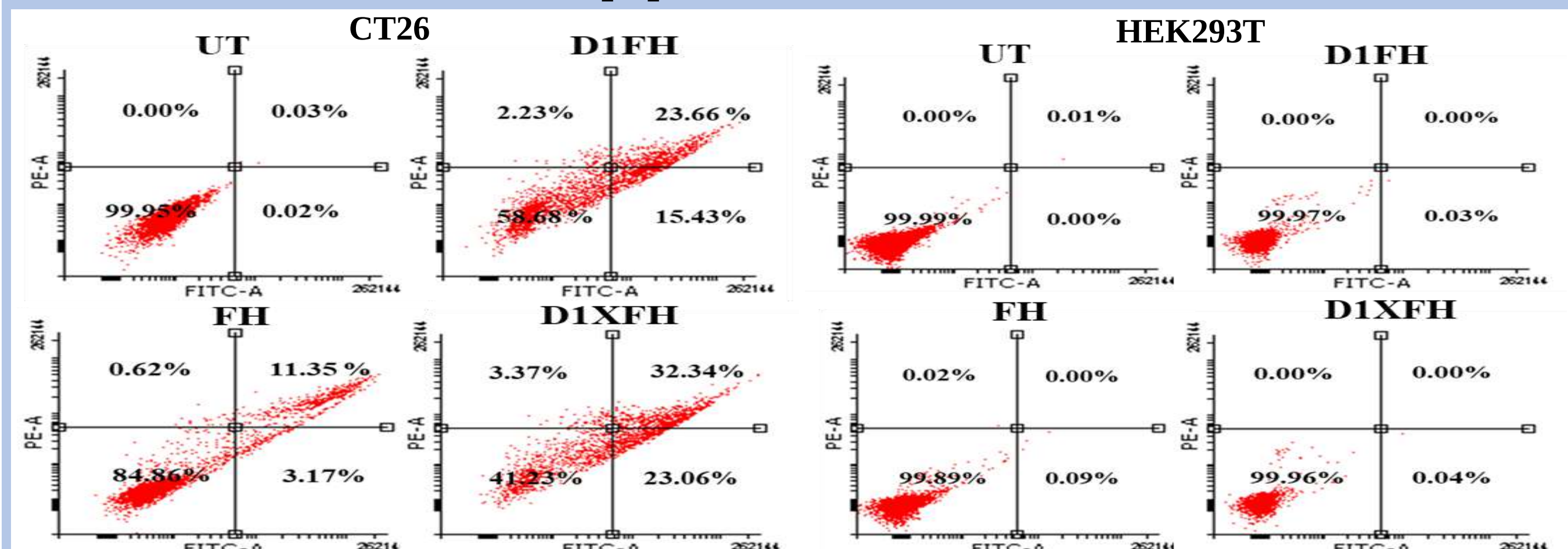
Increased ROS generation in cancer cells



Conclusion

- The highly sensitive wnt/ β -catenin developmental pathway for the first time is selectively targeted for TME immuno-modulation.
- The simultaneous targeting of GR and wnt/ β -catenin led to significant tumor growth inhibition with reversal of EMT and pro-tumorigenic immune response.
- The toxicological analysis of this formulation indicates its biocompatible nature towards animal model.
- The data clearly shows that GR is safe for repurposing various other drugs/ inhibitor molecules targeted to different pathways.

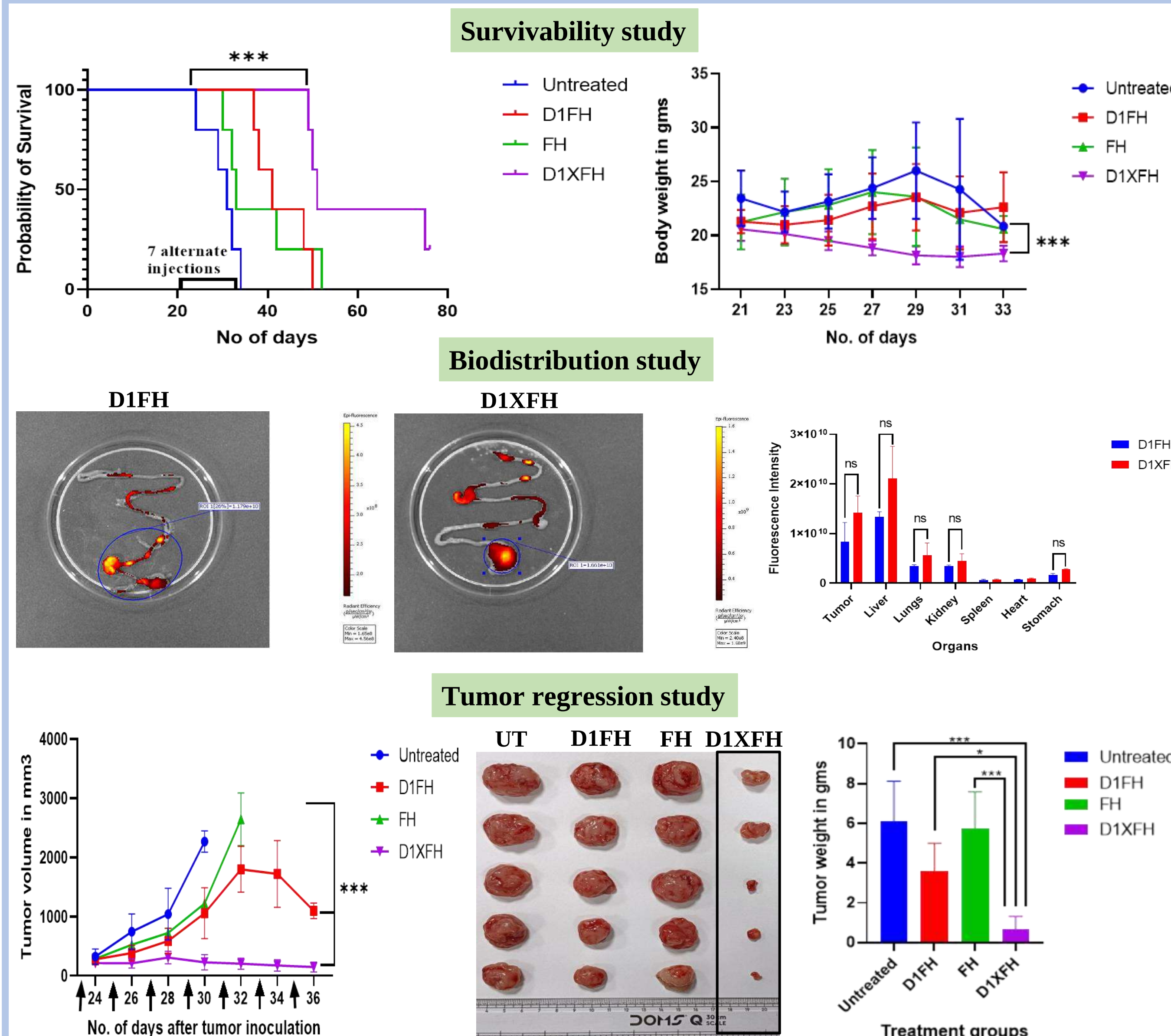
Enhanced apoptosis in colorectal cancer cells



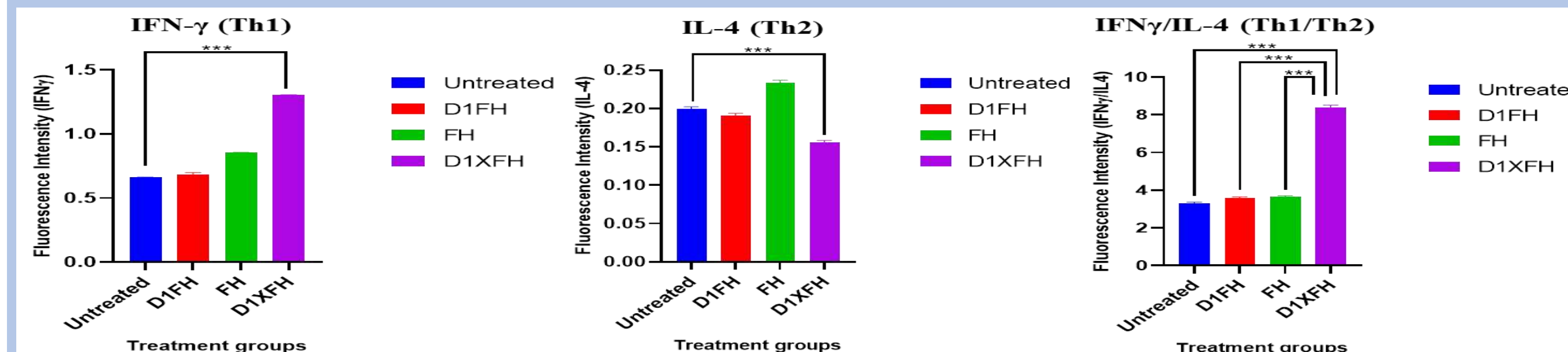
Key findings

- Here, we find that the D1XFH liposomal formulation is able to selectively kill colorectal cancer cells by generating excess ROS, which in turn induces apoptosis.
- Further, our *in-vivo* data shows that D1XFH treatment leads to significant tumor growth inhibition and increased survivability signifying its efficacy and biocompatibility in mice model. This is also corroborated with the protein expression analysis denoting reduction in cellular proliferation, enhancement of EMT-reversal and an inhibition of various downstream proteins of wnt/ β -catenin pathway.
- Thus, our GR-targeted D1XFH liposomal formulation can be used for selective targeting of this particular developmental pathway in cancer cells, indicating a potential new drug-sensitization strategy against colorectal cancer.

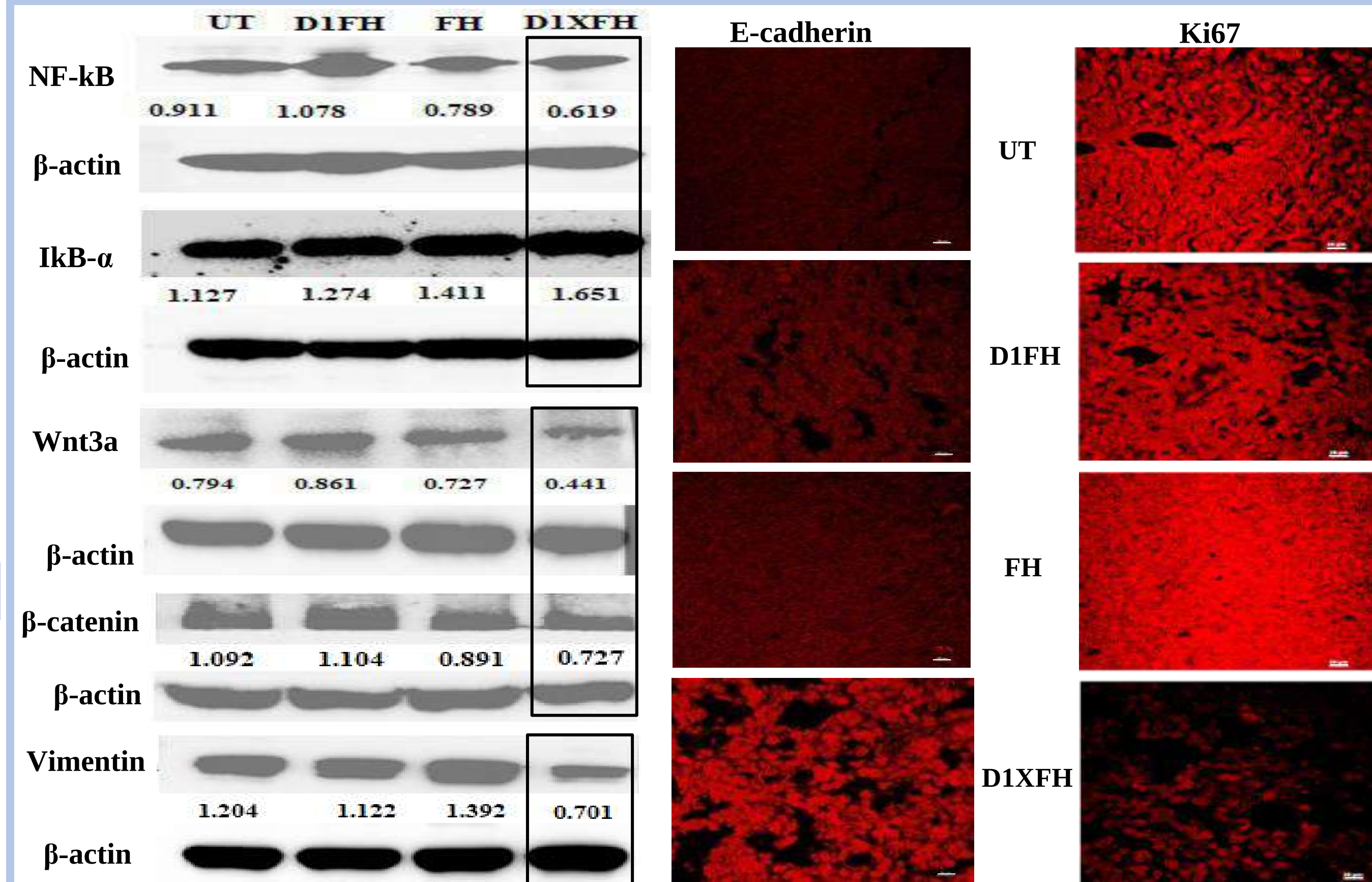
In vivo efficacy evaluation of D1XFH formulation



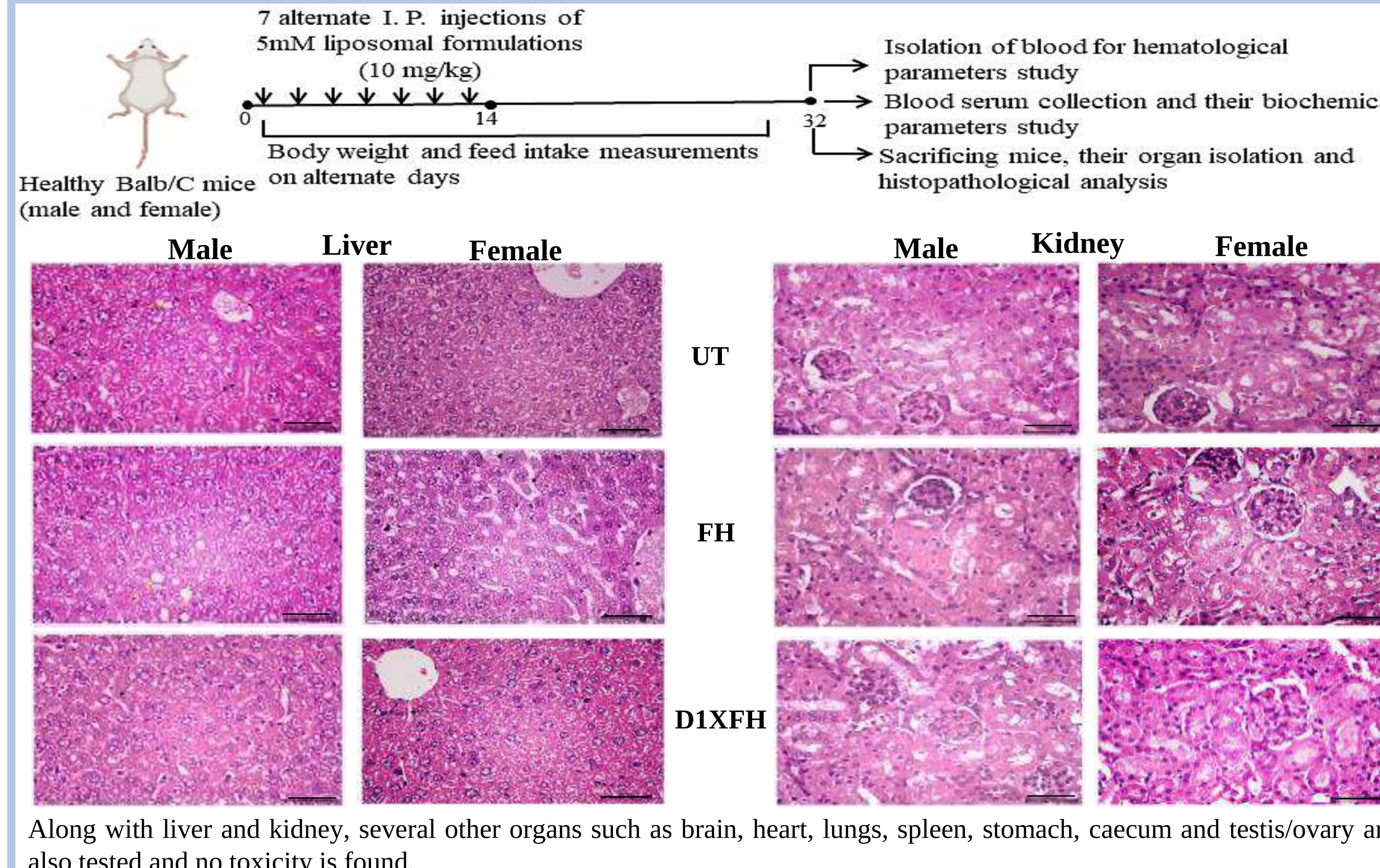
Increased Th1/Th2 ratio indicating effective anti-tumor immune response



D1XFH treatment inhibits wnt/ β -catenin pathway, induces EMT-reversal and reduces tumor cell proliferation



Histopathological analysis of vital organs reveals no toxicity



Along with liver and kidney, several other organs such as brain, heart, lungs, spleen, stomach, caecum and testis/ovary are also tested and no toxicity is found.

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Acknowledgements

- Dr. Rajkumar Banerjee**, Ph.D. Supervisor, Chief Scientist, Dept. of Oils, Lipids Science and Technology (DOLST), CSIR-Indian Institute of Chemical Technology (IICT), Hyderabad.
- Director**, CSIR-IICT, Hyderabad.
- HoDs** of DOLST and Applied Biology Div.
- CSIR (Council of Scientific and Industrial Research), India** for my doctoral fellowship.
- CSIR-IICT, Hyderabad**.



Combination of transfection incompetent lipids having strikingly different aliphatic chain lengths in a liposome demonstrates superior transfection and produces high titre lentivirus.

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¹ CCRAS, Arumbakkam, Chennai, Centre for Stem Cell Research (CSCR), Bagayam, Vellore.

Introduction: Amino acid-based liposome and its Gene delivery application

- This study introduces a liposomal gene delivery system using ionizable lipids from glycine, phenylalanine, and tryptophan.
- Combined with cationic lipid and cholesterol, the formulations showed strong transfection efficiency across cell lines.
- The optimized system also enhanced lentiviral vector production, supporting affordable gene therapy.

Importance of liposomes and their application

- Liposomal platforms have also demonstrated improved delivery and functional expression of mRNA and siRNA in vivo.
- mRNA-2416, encoding OX40L, is a liposomal mRNA drug currently in clinical trials for cancer immunotherapy due to its strong antitumor immune activation.
- mRNA-2752, encoding OX40L, IL-23, and IL-36y, is also under evaluation for its ability to elicit robust immune responses against tumors.

Physicochemical Characterization

- DLS were used to analysis the zeta size and zeta potential of at lipid-to-DNA charge ratios of 1:1, 2:1, 4:1, and 8:1, using plasmid DNA as the payload.
- Lipoplexes containing heterocyclic aromatic amino acids (e.g., Lipo3 and Lipo6) exhibited comparatively lower surface charges than those with simple phenyl moieties (e.g., Lipo2 and Lipo5), and markedly lower than those with non-aromatic headgroups (e.g., Lipo1 and Lipo4).

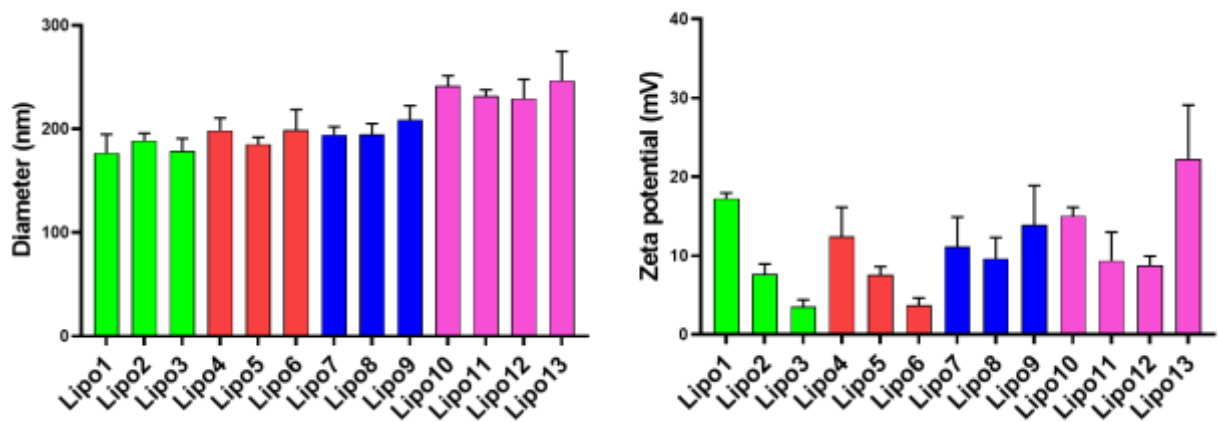


Fig. 1. Characterization of lipoplexes by (A) Particle size (Diameter) and (B) Zeta potential

Aim

- 1) Future studies will assess liposomal lentiviral vectors delivering therapeutic transgenes, aiming to enhance clinical efficacy.
- 2) Key focus areas include reducing cytotoxicity and improving formulation stability for safer, more robust gene therapy.

Characterization of Lipids

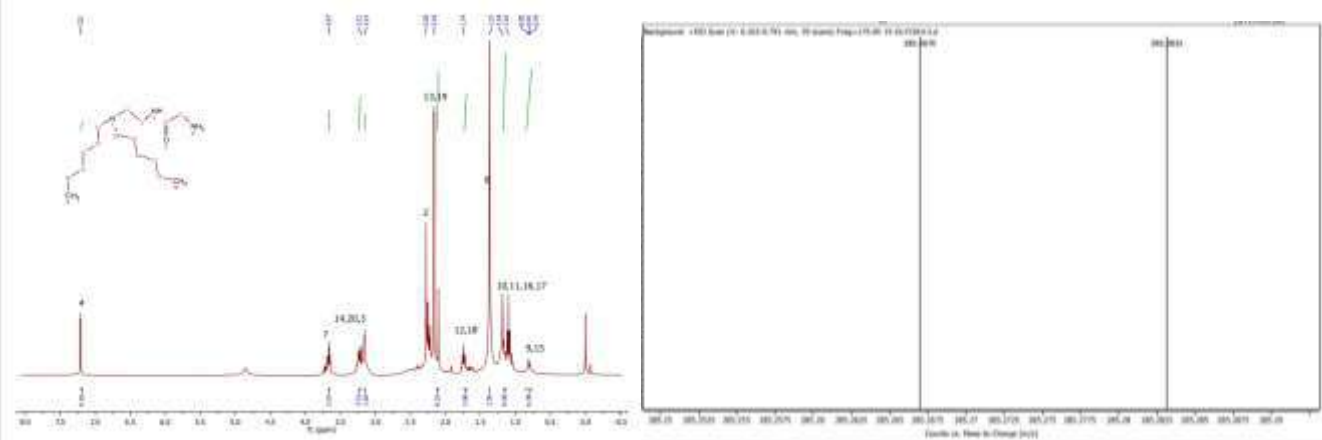


Fig .2. H¹-NMR, HR-MS spectrum of C6G

Transfection analysis of liposomes

- Fluorescence microscopy image and flow cytometry analysis of liposome transfection in different cell lines.
- Incorporating hydrophobic aromatic moieties into lipid head groups enhances membrane interaction and boosts transgene expression.
- Multiple factors, including particle size, surface charge, head group design, and intracellular trafficking, influence transfection efficiency.
- Further cellular uptake studies are needed to clarify the underlying mechanisms.

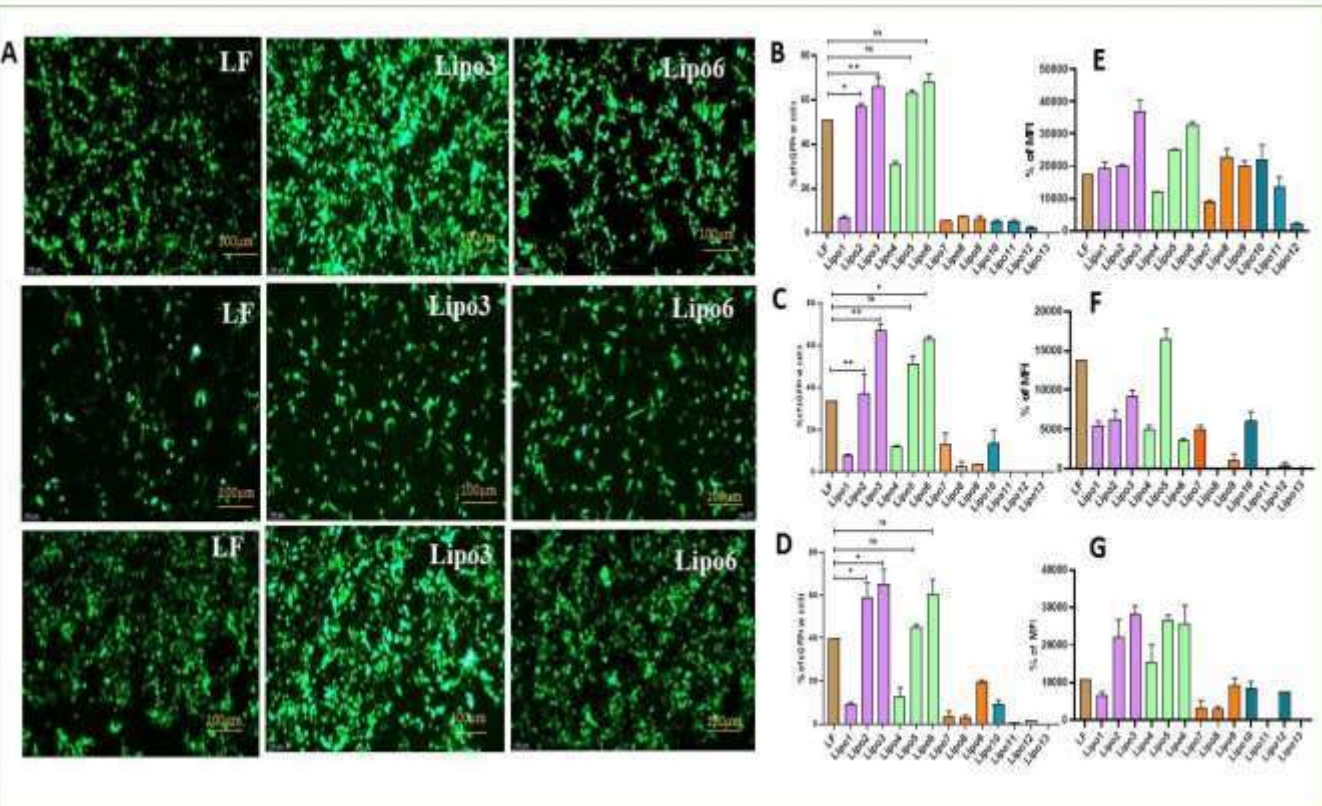


Fig. 3. illustrates the transfection efficiency of liposome-pDNA complexes (2:1 charge ratio) in HEK293T, SKHEP, and HEPA cells via fluorescence microscopy and flow cytometry. Lipofectamine 3000 served as a positive control, with statistical analysis confirming significant transfection across all cell lines.

Methodology

Transfection

Cellular uptake study

Lentiviral vector

Lentiviral vector Characterization

Functional Characterization Transduction

Physical Characterization P24 Elisa

Amino acid based liposome, Gene delivery, Lentiviral vector.

Cellular uptake and expression studies & FRET Assay

- Despite similar internalisation profiles, Lipo2 and Lipo3 achieved higher transfection rates, emphasising the importance of post-uptake processes in gene delivery.
- As shown in Fig.5(C), the cationic liposomes of Lipo3 exhibited approximately 3.5-fold higher biomembrane fusogenicity compared with those of Lipo10 and Lipo13.

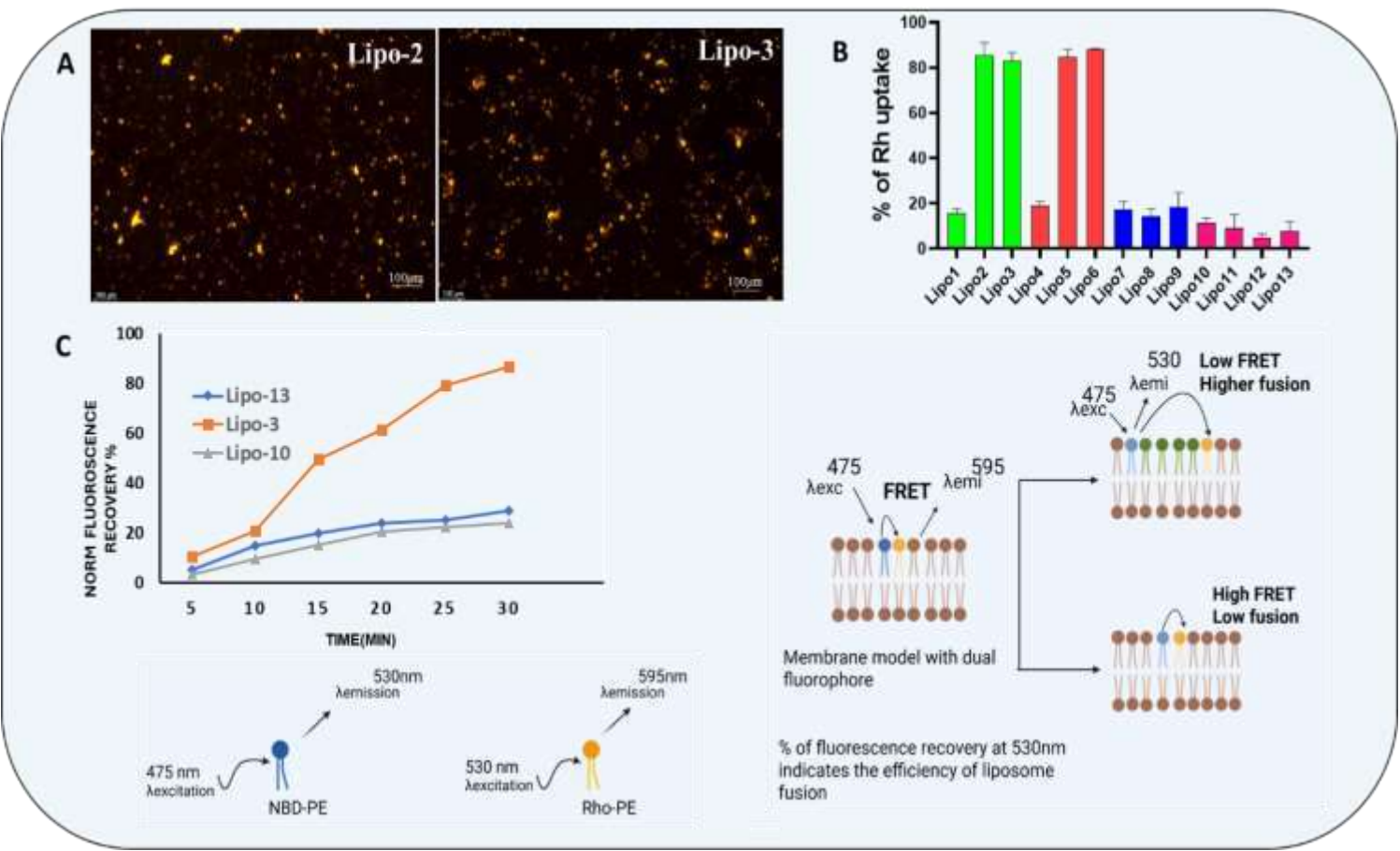


Fig.4. shows Rhodamine-PE-labelled liposome uptake and RFP-positive HEK293T cell quantification via flow cytometry at optimal lipid/DNA ratios. Fusogenicity of Chol/lipid 3, lipid 4, and their combination was evaluated using FRET-based bio-membrane fusion assays

Lentiviral vector Post-Transduction Evaluation and Optimization

- HEK293T cells were transduced with lentiviral vectors and harvested post-trypsinization for flow cytometry analysis of ZsGreen expression.
- Transduction efficiency was measured via FITC channel fluorescence using a BD Celesta cytometer. A titration study compared Lipofectamine™ (LFV) and Lipo-3 at a fixed N/P ratio of 2:1.
- Lipo-3 outperformed LFV at 10 μ L input, yielding higher ZsGreen-positive cell percentages.
- demonstrate the enhanced transduction efficiency and stronger fluorescent signal intensity achieved with Lipo-3.

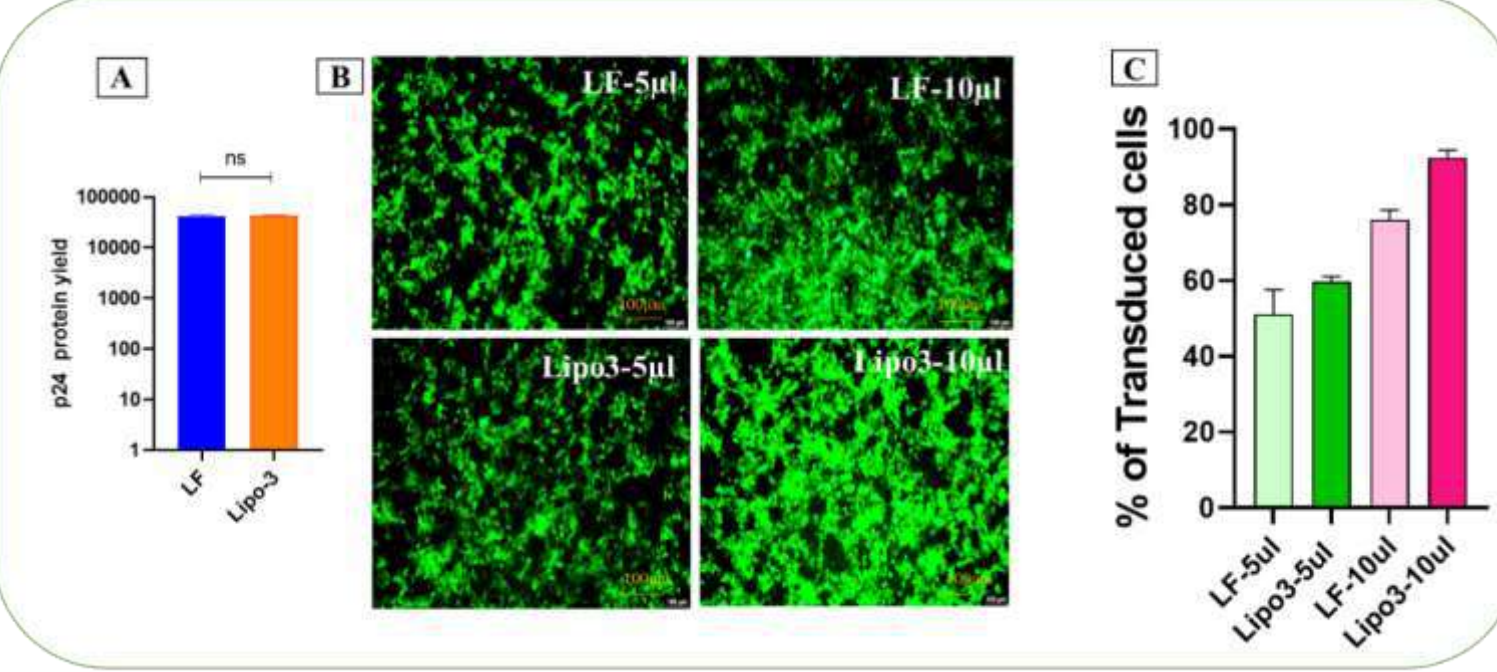


Fig. 5. (A) Quantification of pseudoviral particles by p24 ELISA and the vector map of the lentiviral transfer plasmid used in this study. (B) Pseudoviral infectivity was assessed 72 hours post-transduction by fluorescence microscopy. (C) Transduction efficiency is quantified as transduction units, calculated from the percentage of ZsGreen-positive cells measured by flow cytometry. Data are presented as mean $\pm$ SEM (n = 3)

Conclusion

- Thirteen liposomal formulations were developed for gene delivery, with Lipo2, Lipo3, Lipo5, and Lipo6 showing superior transfection efficiency over Lipofectamine™ 3000.
- Lipo-3 lentiviral vectors achieved higher transduction and ZsGreen expression in target cells.
- Enhanced delivery was linked to circular nucleic acids and hydrophobic R-group structures. These formulations offer promising, safer, and cost-effective options for gene therapy and vaccines.

References:

- [1] K. Zhang, H. Fang, G. Shen, J.S. Taylor, K.L. Wooley, Well-defined cationic shell crosslinked nanoparticles for efficient delivery of DNA or peptide nucleic acids, Proceedings of the American Thoracic Society, 6 (2009) 450-457, <https://doi.org/10.1513/pats.200902-010AW>.
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- [3] D. Pozzi, G. Caracciolo, Looking Back, Moving Forward: Lipid Nanoparticles as a Promising Frontier in Gene Delivery, ACS Pharmacology & Translational Science, 6 (2023) 1561-1573, <https://doi.org/10.1021/acpsptsci.3c00185>.

Acknowledgements:

Dr. Srujan Marepally, Dr. R.Ilavarasan



Microfluidic triple-co-culture model of age-related macular degeneration for advancing retinal drug development

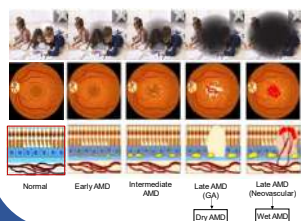
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Introduction



World Health Organization (WHO) estimates that 314 million people have visual impairment worldwide, of whom 269 million have low vision and 45 million are blind. Most of the retinal diseases involve an irreversible damage to outer retina.

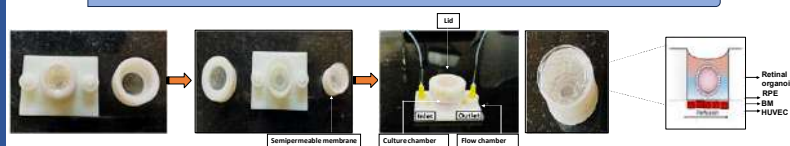


Limitations of Traditional Models: Static cell cultures are nonphysiological, lack ECM, and use single cell types. Animal models such as rat and mice have non-human physiology (e.g., lack of macula/trichromacy) and present ethical concerns. Ex-vivo explants suffer from a short lifespan and inter-donor variability.

Organ-on-a-Chip: The Most Advanced Model: This microfluidic system overcomes limitations of traditional model by incorporating vasculature-like perfusion for constant nourishment, a physiological RPE barrier, an ECM, and all retinal cell types, providing the most physiological structure and cell-cell interactions.

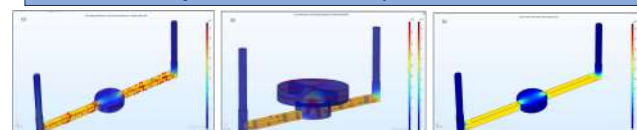
Materials & Methods

01. Microfluidic device fabrication



- Device design:** Device were designed using Fusion 360 software and printed using SLA 3D printer; standard resin
- Sterilization Process:** Devices were given Ethylene Oxide (EtO) treatment followed by 70% IPA treatment for 1/2 an hour. Further sterilize by UV 20 mins

02. Computational validation: Velocity & Pressure distribution

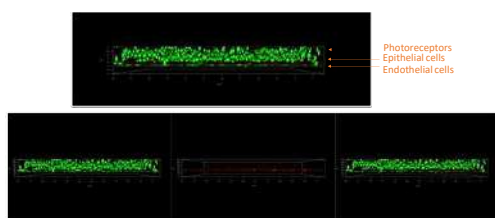


- Fluid flow did not result in any dead zones within the proposed device
- Hence maximum reach of the nutrient medium to the cells adhered over the membrane of the basolateral flow chamber In fig (b), we observed **secondary flow behavior** within the cell culture chamber, secondary flow aids in smooth nutritional flow within the semi-permeable membrane
- The pressure generated by the nutrient medium fluid flow exerts no significant effect on cellular proliferation, making it suitable for cellular studies

1. Development of triple co-culture

1.1. Development of triple co-culture using flow device

Optimized culture conditions:
Seeding Density and incubation time:
(A) HUVEC cells seeded on the basolateral side: 1e5, 72 Hr of incubation
(B) Retinal Epithelial layer on apical side: 1e5, 48 Hr of incubation
(C) Photoreceptor seeded with LC Matrigel (1:1): 3e4, 24 Hr of incubation



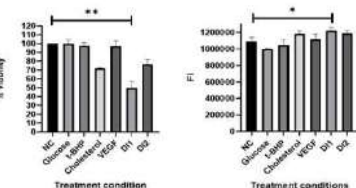
3. Induction of AMD disease condition

3.1. Optimization of Disease induction



Reagent	Concentration
Glucose	21mM
t-BHP	100nM
Cholesterol	12.5 ug
VEGF	100ng
DI1 (Glu, t-BHP, Cholesterol)	21mM, 100nM, 12.5 ug
DI2 (Glu, t-BHP, Cholesterol, VEGF)	21mM, 100nM, 12.5 ug, 100ng

3.2. Reactive Oxygen Species Quantification



Results

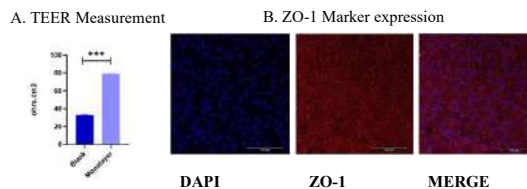
2. Characterization of developed triple-co-culture

2.1. Live/ Dead analysis of all three layers:

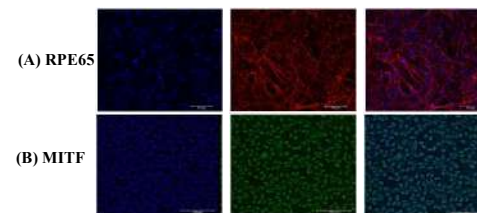


Figure: Live/dead analysis; (A) HUVEC layer seeded on the basolateral side (B) ARPE19 cells seeded at the apical side, (C) 661W cells embedded in the Matrigel

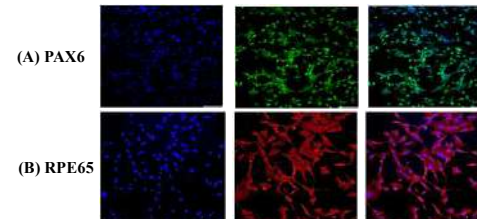
2.2. Tight junction analysis of Epithelial cells:



2.3. Surface marker analysis using ICC; ARPE19 cells



2.3. Surface marker analysis using ICC; Photoreceptor



Conclusion

- This investigation focuses on the development of triple-culture of retinal epithelial, endothelial and photoreceptor cells to recapitulate age related macular degeneration (AMD) model using microfluidic platform.
- Retina on a chip prototype was designed and computationally validated for pressure and velocity of fluid flow.
- The device was then operated at the computationally derived flow rate of 1 μ L/min and the triple-co-culture was established.
- Developed triple-culture was analysed for cellular viability and proliferation, marker expression, barrier integrity and permeability in order to characterize the developed model.
- A disease induction protocol was established to mimic the pathophysiological conditions of AMD, further ROS level were quantified with respect to disease induced model as compared to healthy cells.

References

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Future Outlook

- The retina- on-a chip model will serve as an alternative to animal model with a high throughput drug screening platform.
- In vitro disease model of AMD can further be used for testing several drugs for reversing the disease.
- These model can therefore be more relevant for drug screening than traditional animal model used.

Acknowledgements



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Molecular Links Between Circadian Genes and Breast Cancer Prognosis

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Introduction

Dysregulation of sleep-related and circadian genes—including PER2, BMAL1, CLOCK, CRY, SIRT1, NF-κB, and ROR—contributes to the development, progression, prognosis, and cancer stem cell maintenance in breast cancer. Modulation or correction of these genes is hypothesized to offer novel therapeutic strategies to impede tumor growth and recurrence

Materials and Methods

This review integrates published data (2015–2025) from patient genomic studies, preclinical models, and clinical outcome analyses. Sources include transcriptomic and methylation datasets, circadian disruption paradigms, gene knockout/overexpression in cell and animal models, and gene-targeted therapies (e.g., gene editing, chronotherapy, melatonin supplementation). Single-cell and lineage-tracing techniques were used to evaluate gene-specific impacts on cancer stem cells.

Gene	Primary Role	Breast Cancer Impact	Stem Cell Influence
PER2	Clock, tumor suppressor	Low expression increases risk, worsens prognosis	High levels suppress CSC pool
BMAL1	Circadian factor	Disruption = high risk, poor survival	Knockout increases CSC features
CLOCK	Master regulator	Mutation/disruption increases risk, metastasis	Directs stemness via cell cycle
CRY1/2	Clock, DNA repair	Loss: increased risk, aggressive type	Regulates via p21/cell cycle
SIRT1	Epigenetic, repair	Overactivity: proliferation/chemo resistance	Maintains CSCs in stress
NF-κB	Inflammatory, survival	Overactivation: relapse, poor prognosis	Increases stem marker expression
ROR	Nuclear receptor	Mutation: tumorigenesis, advanced stage	Promotes CSC renewal

Results/Observations

- Knockdown/mutation of PER2, BMAL1, CLOCK, and CRY accelerates tumorigenesis, metastasis, and worsens prognosis.
- SIRT1 and ROR dysregulation alters cell cycle, apoptosis, DNA repair, and increases treatment resistance.
- Disruption, especially NF-κB pathway changes, enhances inflammatory signaling and cancer stem cell renewal.
- Circadian disruption increases breast cancer stem-like cell markers (CD44⁺/CD24[−]).
- Clinical data link poor sleep/circadian disruption to reduced survival, higher recurrence, and chemoresistance.
- Restoring PER2/BMAL1 expression or using chronotherapy/melatonin can suppress tumor growth and enhance therapeutic response.

Conclusion

Circadian and sleep genes significantly regulate breast cancer etiology and the dynamics of cancer stem cells through direct transcriptional, epigenetic, and immunological pathways. Their dysregulation drives cancer initiation, progression, and therapy resistance by expanding CSC pools. Targeting these genes through gene therapy, chronomodulated drugs, or lifestyle adjustments shows promising translational opportunities for breast cancer management.

References

Based on data and reviews published between 2015 and 2025 in the field of circadian biology, cancer genomics, and clinical interventions .

Glucocorticoid Receptor-Mediated Drug Delivery System Against Aggressive Oral Cancer

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10th Annual
CELL AND GENE THERAPY SYMPOSIUM

Introduction

Given the challenges associated with conventional oral squamous cell carcinoma (OSCC) treatments, including non-targeted cytotoxicity, systemic toxicity, and high relapse rates, receptor-mediated targeted delivery systems offer a promising alternative. This study explores the glucocorticoid receptor (GR) as a therapeutic target for oral cancer treatment.

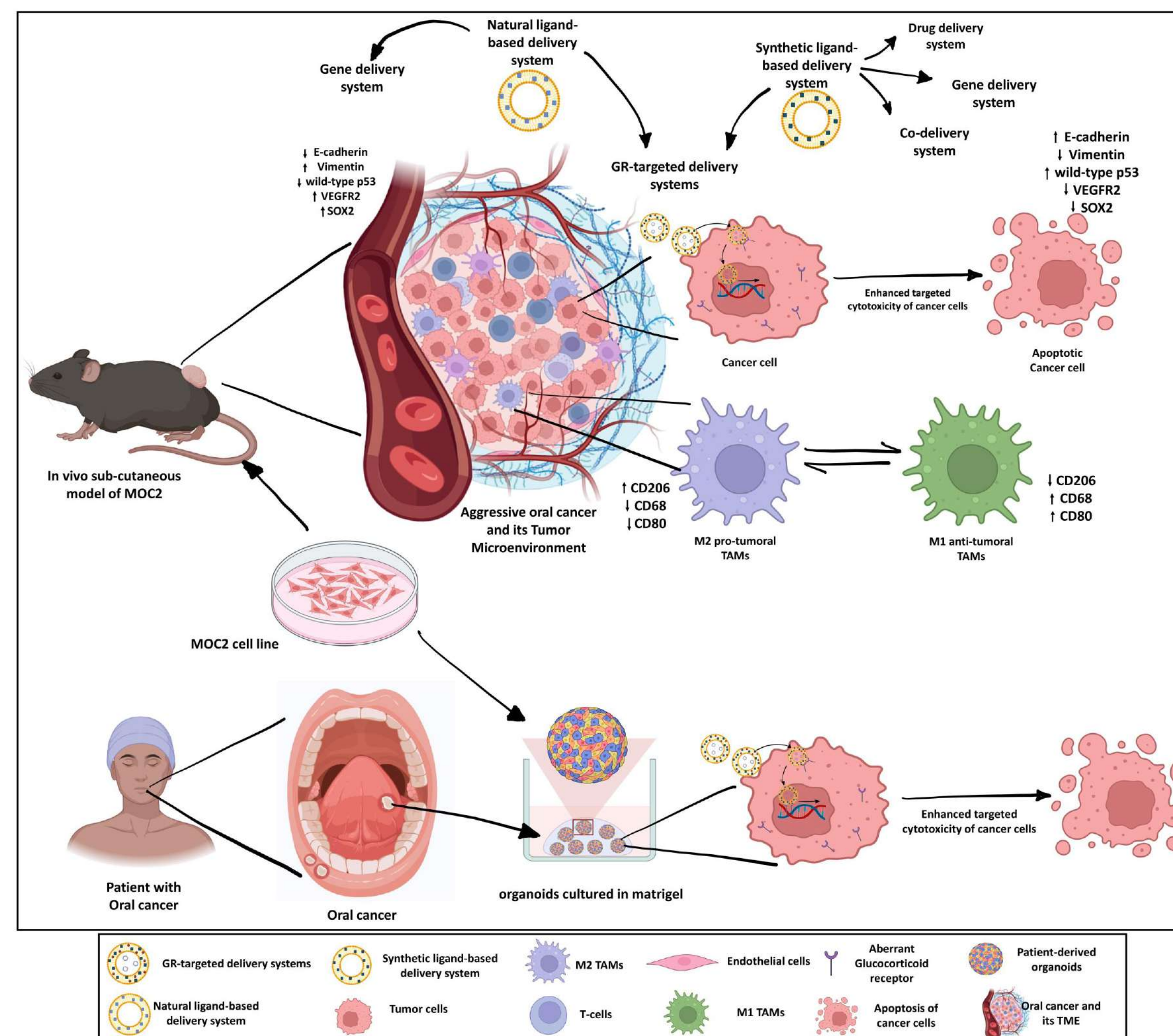
This study thus aims to target Glucocorticoid receptors of oral cancer cells mediated through liposomal delivery systems using both natural and synthetic ligands, considering the following objectives.

➤ To selectively target oral cancer through a GR-mediated liposomal delivery system employing plant-derived saponins as a GR-ligand and to test their efficacy as a gene delivery system on *in vitro* and *in vivo* models.

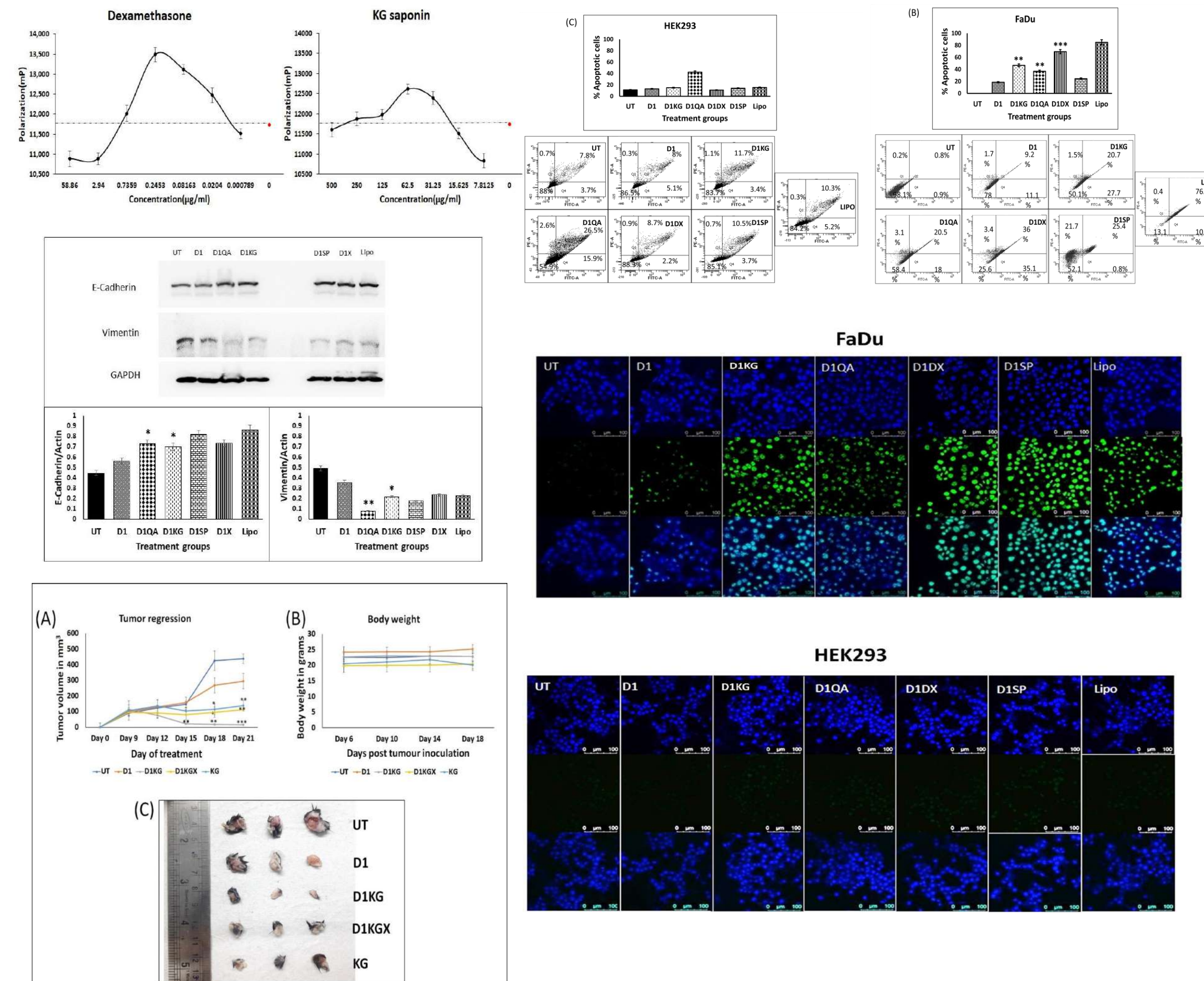
➤ To employ dexamethasone-based liposome as a gene, drug, and co-delivery system carrying paclitaxel and wild-type p53 and to test the efficacy of this co-delivery system on *in vitro*, *ex vivo*, and *in vivo* model systems.

➤ To isolate and culture primary cells and tumoroids from patient samples, and to test the efficacy of the drug delivery system on these patient-derived primary cells and tumoroids.

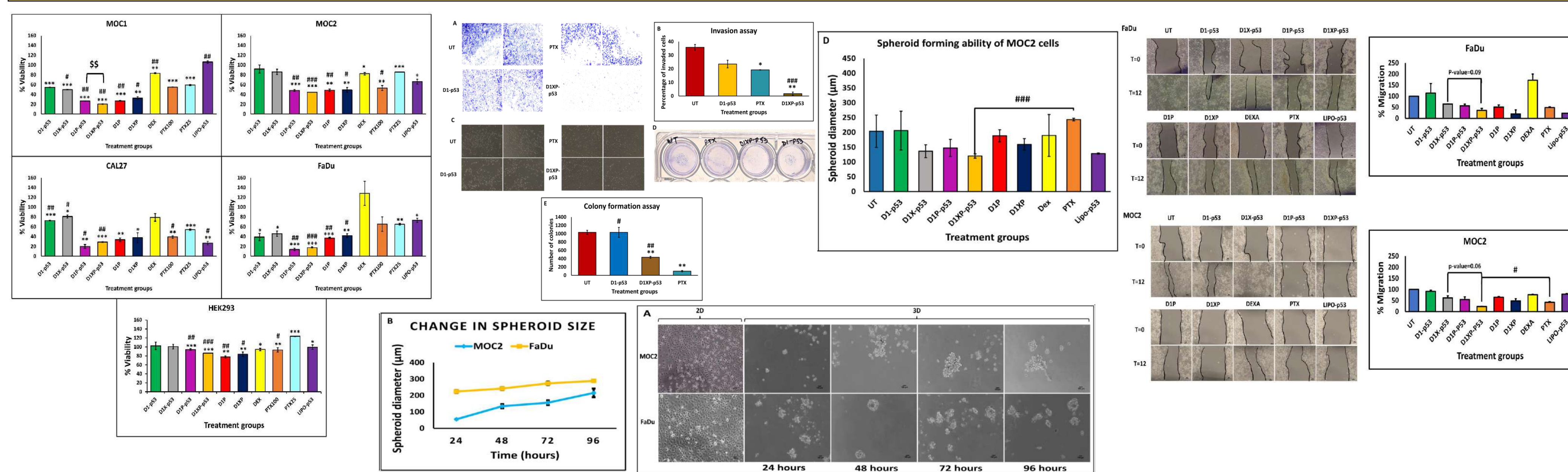
Graphical abstract & methodology



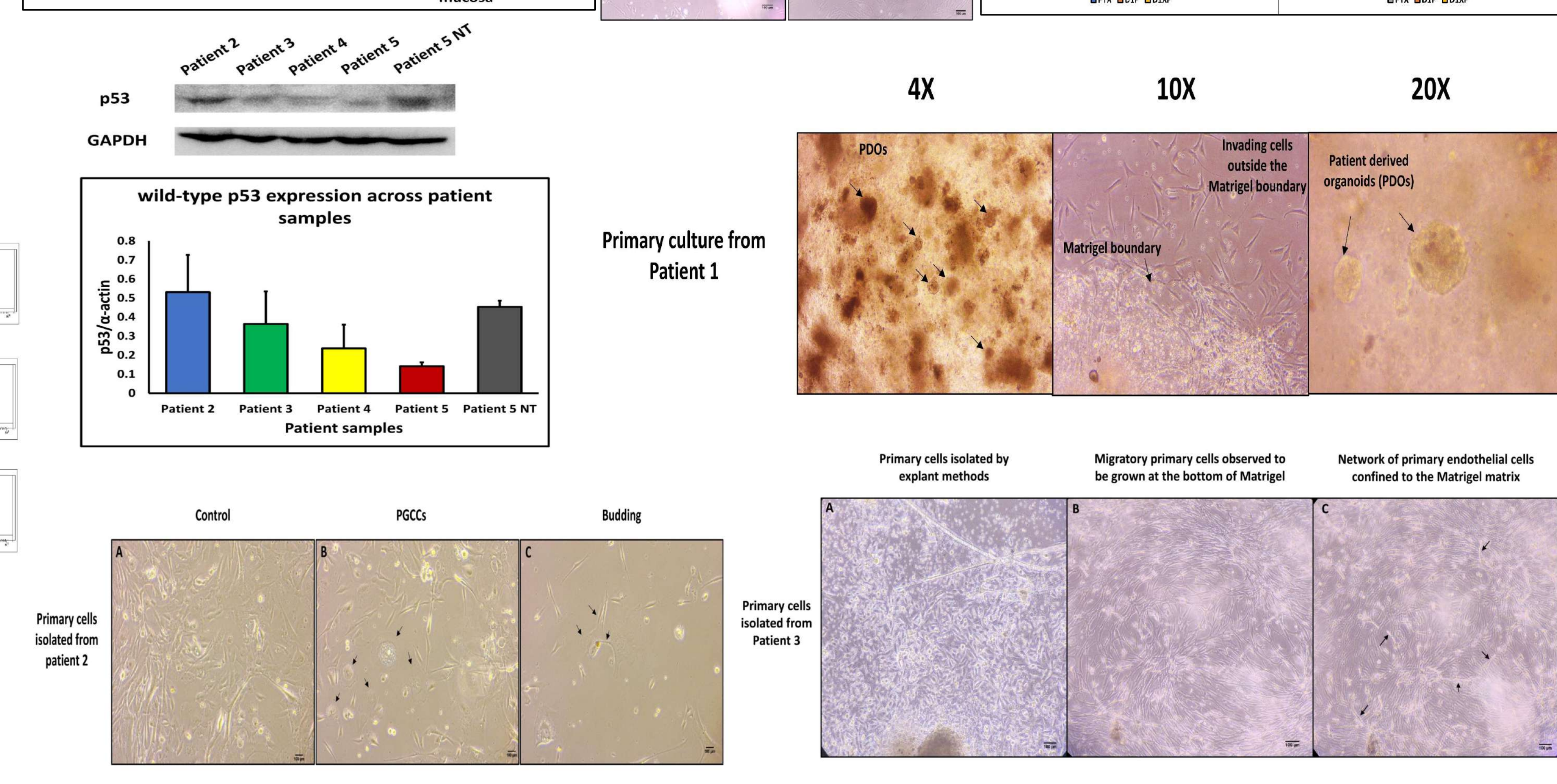
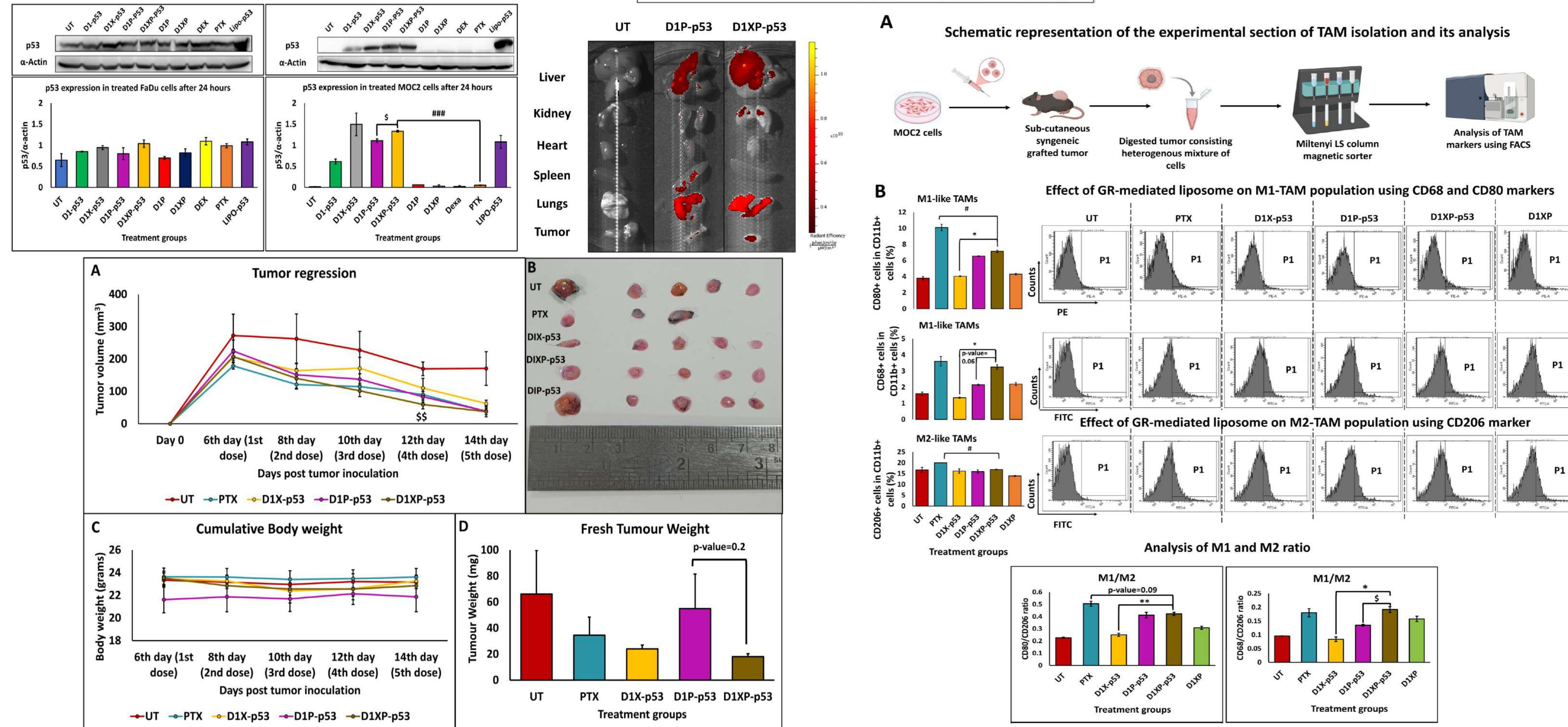
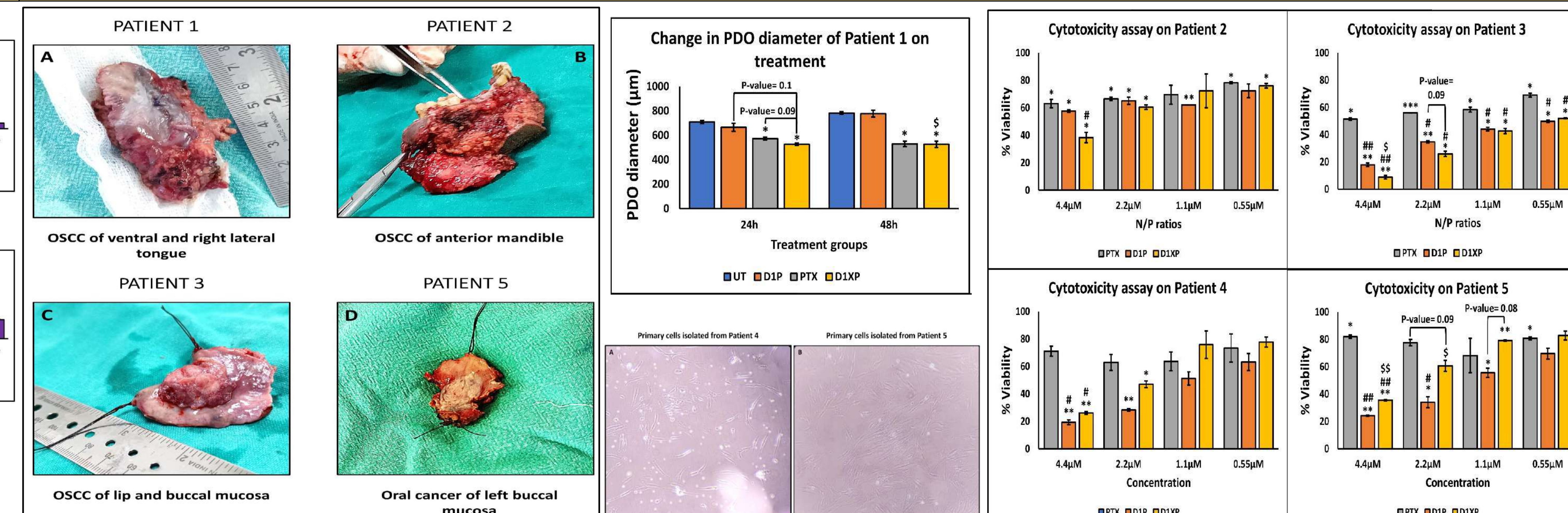
Saponin-based liposomal gene delivery system for OSCC



Synthetic ligand-based co-delivery system targets tumor and TME



Need for personalized anti-cancer treatment of OSCC



Summary & Conclusion

- The crude saponins from plant extract were observed to target the GR of cancer cells, providing an economical and natural source of GR ligands. The natural ligand-based liposomal gene delivery system played a role in targeted cytotoxicity along with the indications of EMT reversal.
- The synthetic ligand-based delivery system also influenced the immune microenvironment of *in vivo* tumors by enhancing the ratio of anti-tumoral to pro-tumoral tumor-associated macrophages (TAMs), highlighting its potential as a combinatorial therapy targeting both tumor and the tumor microenvironment.
- The patient-derived models effectively recapitulated the heterogeneity of oral cancer in vitro conditions. They suggested a potential link between p53 mutations and their aggressive nature, indicative of the development of personalized anti-cancer therapy for oral cancer.

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Acknowledgement

- Director, BITS Pilani, Hyderabad campus
- Department of Oils, Lipids Science and Technology, CSIR-IICT, Hyderabad
- DBT, India, for funding
- Lady Tata Memorial Trust for fellowship



Drug Resistance in iPSCs: Targeting PI3K-AKT-NFκB Signaling to Enhance Reprogramming Efficiency

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INTRODUCTION

Induced pluripotent stem cells (iPSCs) illustrate unharnessed potential in precision therapeutics and personalized medicine. However, low reprogramming efficiency and cell turnover remain to be critical challenges, limiting their clinical translation, with traditional methods achieving success rates of only 0.0006-3%. This inefficiency increases production costs and compromises the scalability of iPSC-based therapies.

While small molecules such as valproic acid, CHIR99021, and ascorbic acid have been introduced to reprogramming protocols as they are known to increase efficiency by 100 fold, responsiveness of iPSCs to these chemical reprogramming agents remains to be low and is observed to diminish over time. This phenomenon of drug resistance is particularly concerning due to the critical role they play in modern reprogramming protocols and their service as safer alternatives to viral delivery systems.

While recent evidence point to drug resistance in cancer cells via expression of survival pathways, such mechanisms are not well defined in iPSCs. We suggest that Integrins play a central role in this resistance mechanism. Other gene families, especially BIRC and CXCR have been identified as critical determinants of drug resistance across multiple cancer types, with its expression correlating inversely with treatment sensitivity. Integrin dysregulation represents a critical and relatively underexplored mechanism contributing to reprogramming inefficiency. Integrins are known to mediate mechanomodulation, whose disruption may impair the cellular machinery responsible for small molecule uptake, creating a physiological barrier to effective reprogramming.

The interaction between Integrins, CXCR family members and BIRC survival signaling cascades thus needs to be studied as it can be hypothesized that they are responsible for creating robust resistance networks that protect cells from external perturbations. Here we can refer to the PI3K-AKT-NFκB signaling pathway which serves as a central hub integrating these resistance mechanisms.

Hyperactivation of PI3K-AKT signaling pathway may be creating a "resistance state" characterized by enhanced cell survival but reduced responsiveness to external reprogramming factors. Understanding these interconnected resistance mechanisms is crucial for developing strategies to enhance reprogramming efficiency and advance iPSC-based therapeutics toward clinical reality.

We conducted a study comparing iPSCs with embryonic stem cells (ESCs) using publicly available data from Gene Expression Omnibus (GEO), as indicated in table 1.

Dataset	Organism	Comparisons	Reprogramming state of generated iPSCs
GSE38265	Homo sapiens	ESC Vs iPSCs	Early
GSE69626	Homo sapiens	ESCs vs generated iPSCs	Middle
GSE239446	Homo sapiens	Derived NSCs vs hiPSCs	Late

Table 1: Datasets chosen with their details given.

MATERIALS & METHODOLOGY

Data acquisition

All of the RNA-seq files were obtained online at the Gene Expression Omnibus (GEO) to conduct this integrative analysis.

Data Processing

Raw read counts were obtained from GEO. These underwent cross-platform normalisation to minimise batch effects. This normalised data was used for global PCA clustering and t-SNE plot. Simultaneously, raw read counts were used as input for global data visualization and differential expression analysis by the DESeq2 Bioconductor package (v1.8.1) in the R environment (v4.1.1). For differential gene expression of, |log2FC|> 1 and adjusted p-value (padj, Benjamini–Hochberg correction) <0.05 were used as the cut-off to define statistically significant differentially expressed genes (DEGs).

Data Analysis

We did two types of analyses, we did within-dataset analysis and then compared them post hoc and we also performed combined differential expression and network/pathway analysis across datasets. Over-Representation Analysis was done using clusterProfiler package in the same R environment. The functions of up- or down-regulated genes in iPSCs vs. comparative cell type were investigated by using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v 6.7 based on gene ontology (GO) annotations.

Data visualisation

Visualisation was done in the form of heatmaps, volcano plots, cluster graphs and differential statistical representations. Global expression profiles were assessed using t-SNE plots, hierarchical clustering, distribution of gene expression clustering and visualisation of gene expression profiles.

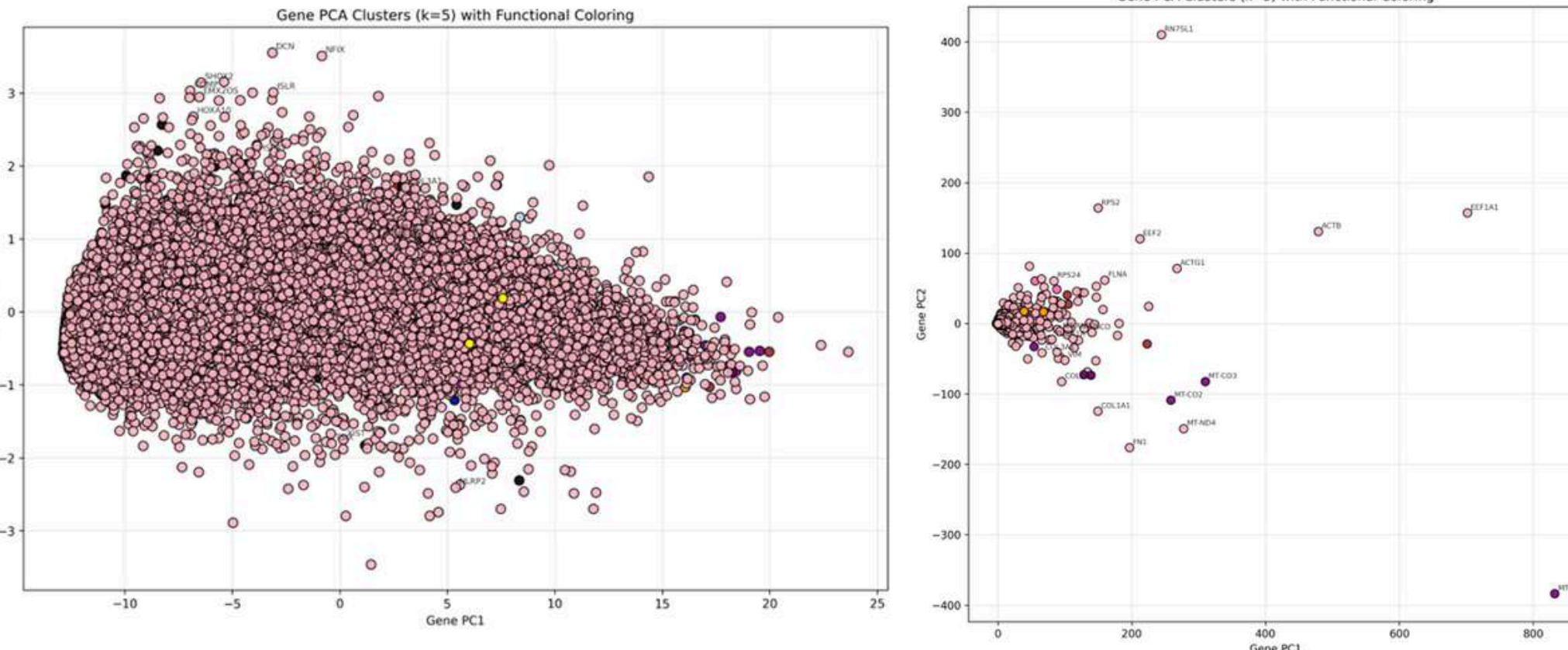


Fig 1: The statistical significance of cross platform normalisation. *Left* represents global PCA clustering of genes with cross platform normalisation and *right* represents global PCA clustering of genes without cross platform normalisation.

DISCUSSION

Mechanistic Insights into iPSC Drug Resistance

Our transcriptomic analysis provides compelling evidence for a coordinated resistance network in iPSCs that significantly impacts reprogramming efficiency. Data suggests a bi-modal resistance pattern with GSE69626 and GSE239446 indicating a high-survival mode and GSE38265 depicting a compensated survival mode. In GSE69626 and GSE239446 BIRC3 upregulation and CXCR6 upregulation imply strong anti-apoptotic signaling and inflammatory survival cues which would have a net effect of enhanced cell survival but a reduced plasticity for reprogramming.

Implications of Integrin Dysregulation for Therapeutic Applications

The 3-6 fold dysregulation in integrin expression represents a lesser appreciated mechanism in reprogramming. Since integrins serve as critical mediators of mechanotransduction, the observed dysregulation implies compromised cellular mechanosensitivity that may reduce responsiveness to environmental cues essential for efficient reprogramming.

Upregulation of key integrins seems to be a part of a compensatory response to PI3K pathway disturbances, reinforcing downstream Akt activation to support cell survival under stress from the induced reprogramming. Upregulation of certain integrin subunits increases FAK-mediated PI3K/Akt signaling, sustaining pro-survival cascades that can delay apoptotic priming in iPSCs.

Integrin-mediated mechanotransduction governs endocytic trafficking and membrane tension where the dysregulated expression profiles signal impair the mechanosensitivity that diminishes cellular uptake of reprogramming small molecules. Elevated expression also stiffens focal adhesion complexes, reducing membrane deformability and endocytic vesicle formation, thereby imposing a physical barrier to chemical reprogramming agents. Therapeutic modulation of integrin–cytoskeleton interactions which can be through FAK/Src inhibitors or mechanical conditioning regimen could restore membrane dynamics and enhance small molecule delivery in fibroblast-derived iPSC cultures.

Implications for Regenerative Medicine

The clinical implications of drug resistance in iPSCs extend beyond reprogramming efficiency to encompass safety and therapeutic efficacy. Cells with enhanced survival characteristics may exhibit increased tumorigenic potential, necessitating careful evaluation of resistance pathway activation in therapeutic applications. However, the same mechanisms that create resistance barriers may also provide enhanced engraftment potential following transplantation, suggesting complex trade-offs in therapeutic optimization

Our findings suggest the need for therapeutic intervention strategies that can help elevate reprogramming efficiency. Targeted inhibition of BIRC proteins using SMAC mimetics could potentially overcome resistance while maintaining cell viability. Similarly, intracellular CXCR4 modulation rather than surface expression may enhance cellular responsiveness without compromising survival. While our recommendation implies that iPSCs need to be treated similar to drug resistant cancer cells, the prevalence of similar expression patterns prerequisite such drastic protocol changes. The coordinated nature of resistance pathways suggests that combination approaches targeting multiple components simultaneously may achieve superior outcomes.

RESULTS

Transcriptomic Analysis Reveals Coordinated Expression of Resistance Pathways

Our comprehensive transcriptomic analysis identified a consistent dysregulation of BIRC family genes in iPSCs compared to embryonic stem cells. BIRC2 and BIRC5 are consistently downregulated with an average fold change of -1.5. GSE239446 shows hyper activation of BIRC3, with an upregulation of 4. CXCR chemokine receptor expression analysis revealed striking dysregulation patterns across multiple family members. CXCR4, CXCR5 and CXCR6 expression is highly varied ranging between a 5 fold upregulation to a 3 fold downregulation (p<0.05) while others show minimal biological variation and statistical insignificance.

PI3K-AKT-NFκB Pathway Activation in Drug-Resistant iPSCs

Integrative pathway enrichment analysis using REACTOME revealed significant enrichment of PI3K-AKT signaling components in iPSCs displaying MET activation of PI3K-AKT and its corresponding events in ERBB2 signaling. Key pathway components, as indicated in table 2, show elevated expression, as high as up to logFC ~ 9.

Gene	PI3K Role	Pathway position	Functional Impact
GAB1	Direct Activator	Upstream scaffolding	High
SMAD2	Cross talk partner	TGF-β/PI3K integration	High
CCND2	Downstream partner	G1-S cell cycle control	High
PPP1R12B	Negative regulator	Upstream inhibition	Moderate
KCNJ13	Indirect supporter	Ion homeostasis	Low

Table 2: Common significant genes in all datasets with their functional summary.

Integrin Expression Dysregulation

Integrin family analysis revealed substantial dysregulation affecting multiple family members critical for cellular adhesion and mechanotransduction. GSE38265 demonstrated selective upregulation, which was reproduced in GSE69626, implying experimental reproducibility of results, even from different labs. GSE239446 exhibited broader integrin dysregulation with 13 significantly altered genes (padj < 0.05), including 12 upregulated and 1 downregulated integrin subunit. This dysregulation pattern was consistently observed across multiple iPSC lines derived from fibroblast source cells.

The most dramatically affected integrins show a 3-6 fold dysregulation in expression representing a lesser appreciated mechanism in reprogramming. Such dysregulation is contributing to the stemness, ECM remodeling, cell adhesion, migration and basement membrane organization.

Functional Annotation

Functional annotation shows that dysregulated integrins are primarily involved in extracellular matrix interactions and mechanotransduction pathways (GO enrichment p < 0.05). The altered integrin profile is predicted to diminish cellular mechanosensitivity and small molecule uptake efficiency.

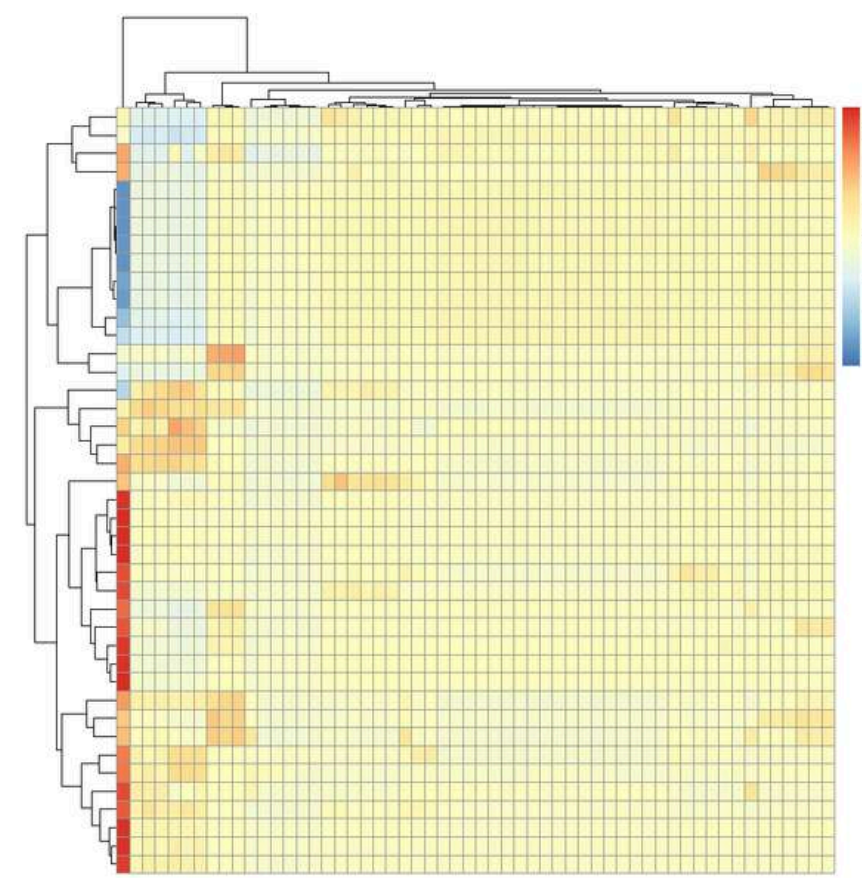


Figure 2: Heatmap of expression of top significant genes in all datasets

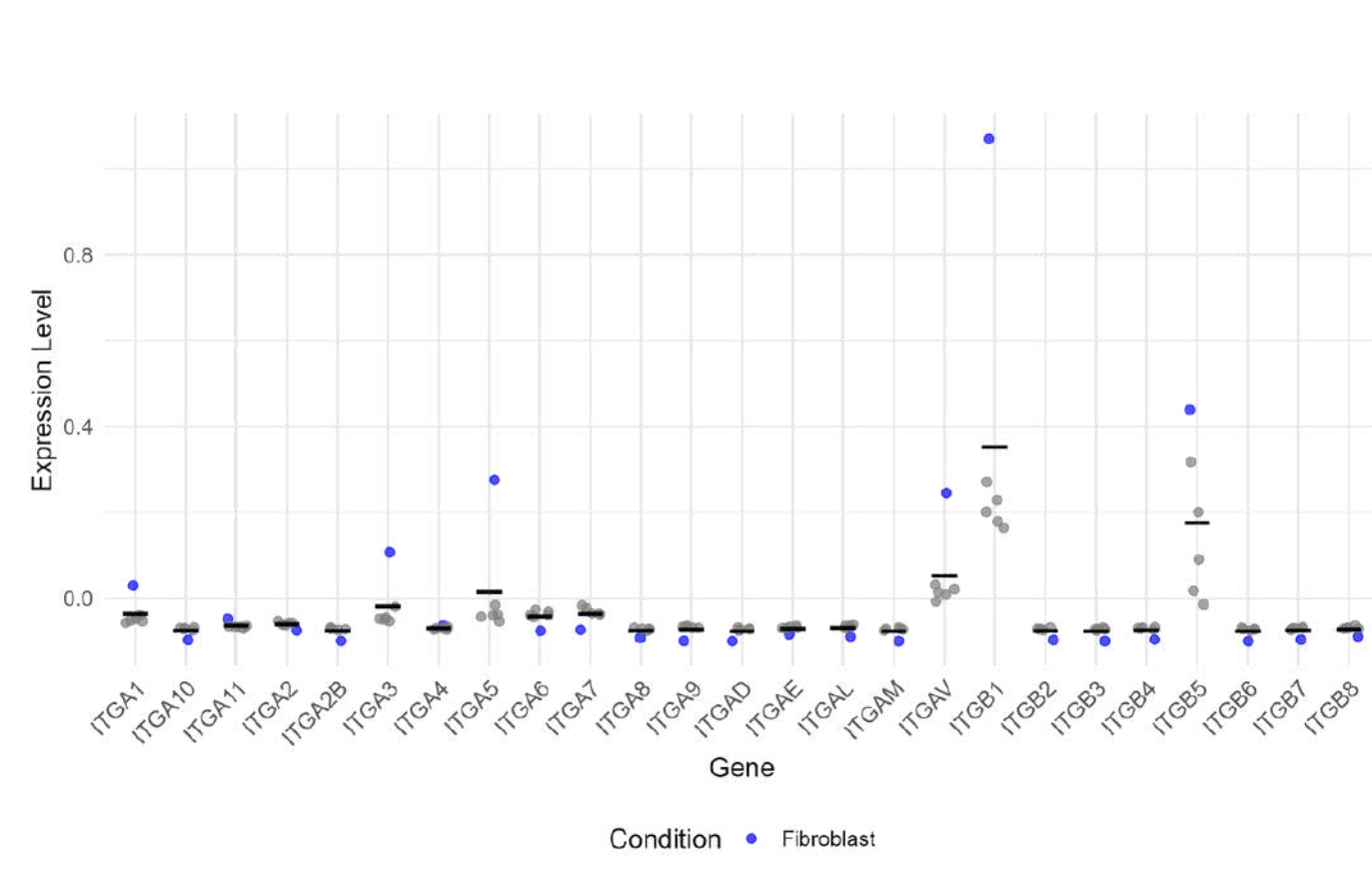


Figure 3: Gene Expression Profiles of Integrins and other important genes in all datasets

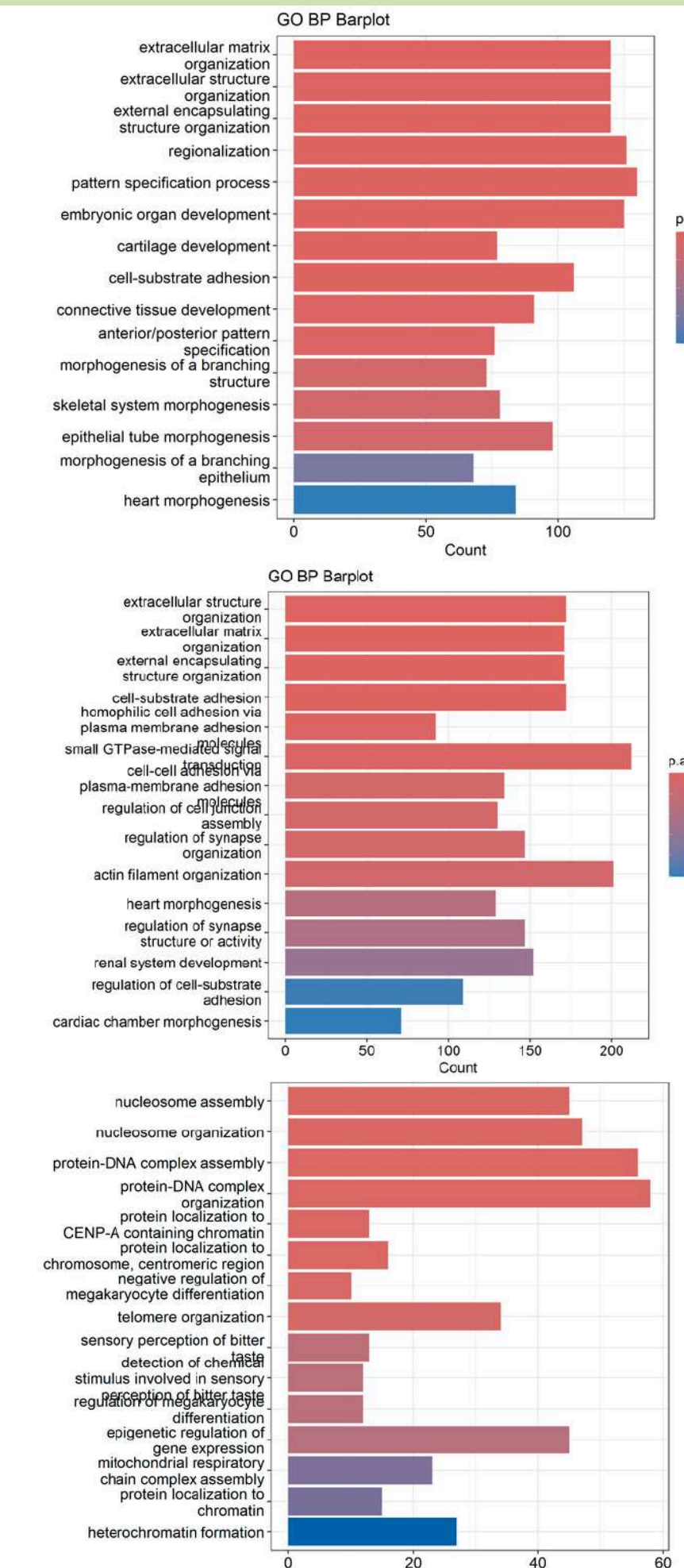


Fig 4 : ORA Bar Plots for all datasets; Data suggest global changes in ECM, cell fate and protein transport

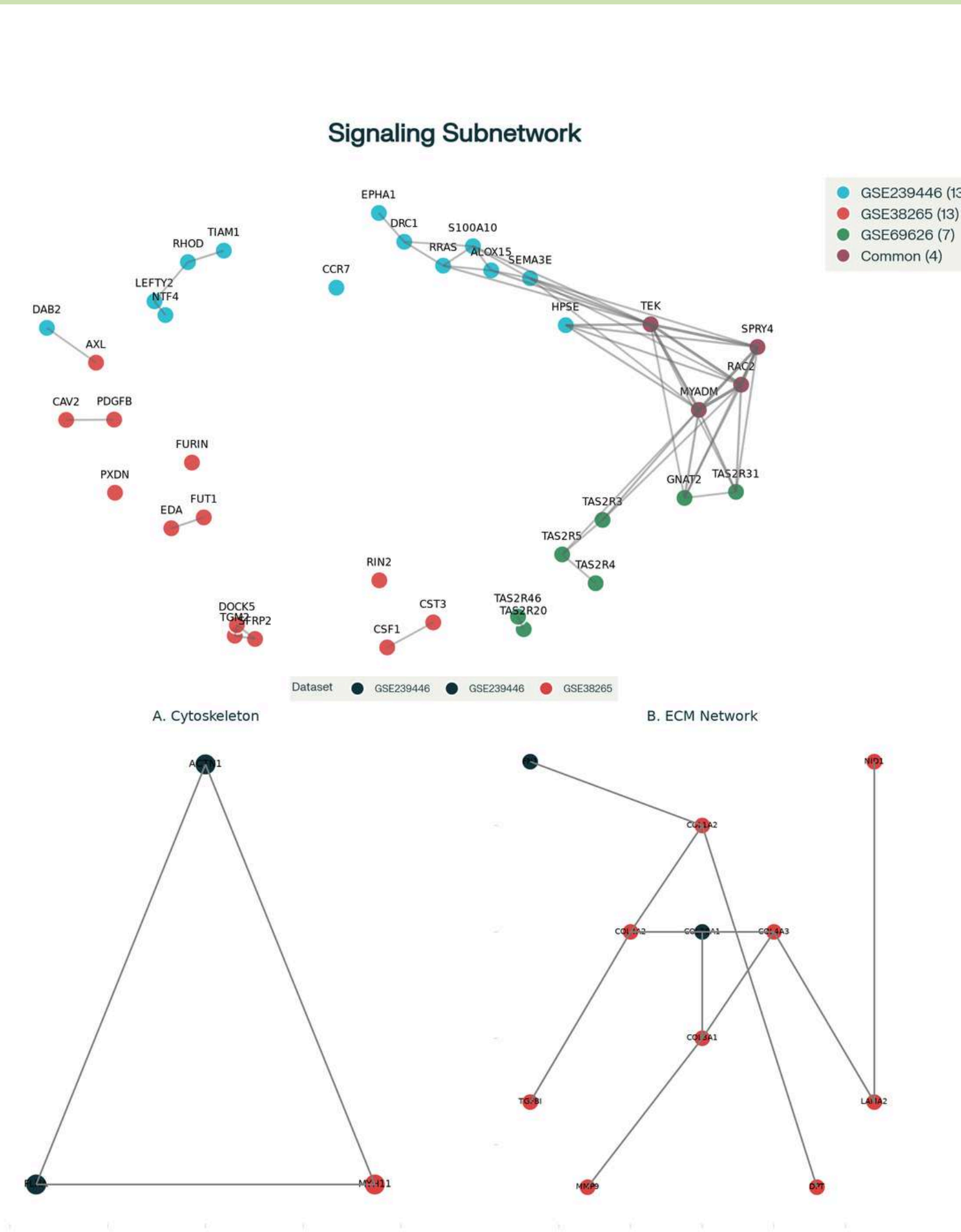


Fig 5 :Signaling and Cell Adhesion subnetworks from all datasets

ABOUT ME



ACKNOWLEDGEMENT

I would like to appreciate the encouragement and support of my university, Manipal Institute of Technology, MAHE, for assisting me in pursuing academic excellence. Additionally I would like to thank my parents for their relentless support.

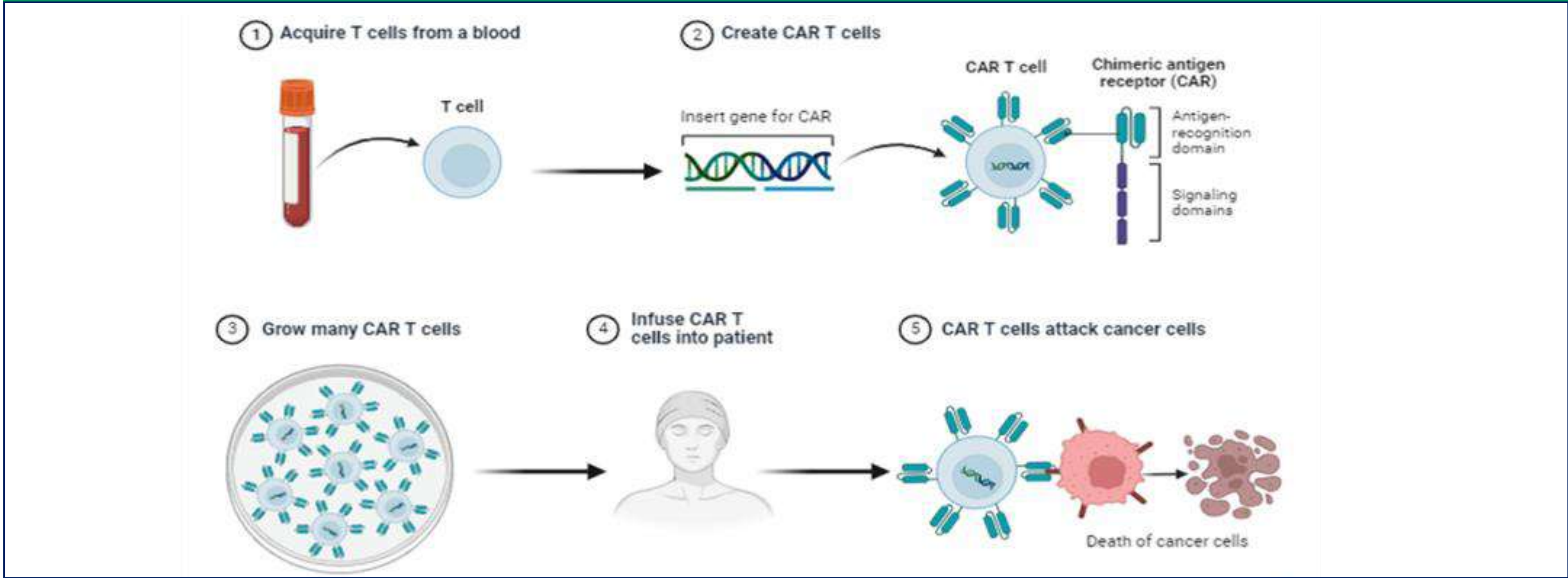
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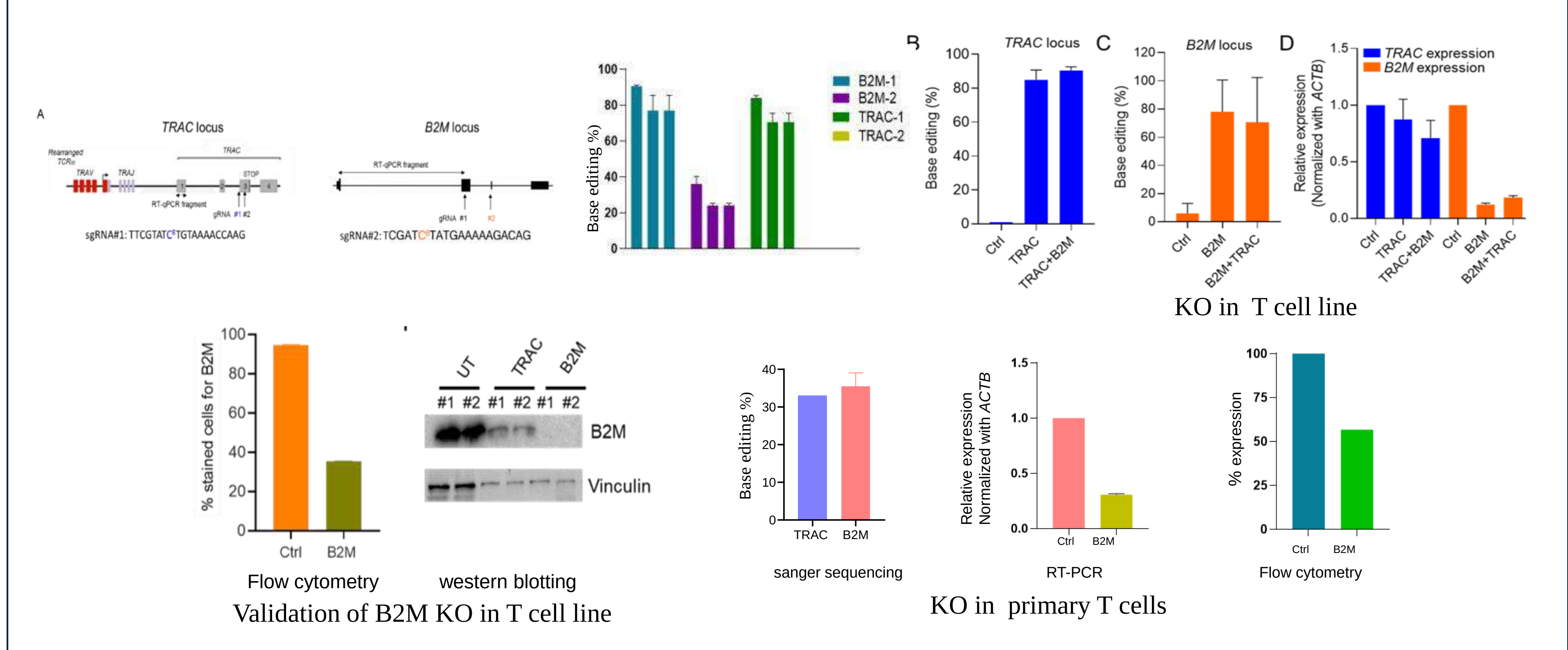
INTRODUCTION

- CAR-T cell therapy revolutionized cancer treatment by achieving remarkable success in B-cell malignancies. Yet, challenges persist such as manufacturing delay and antigen escape.
- To address these challenges multitargeted “off-the-shelf” products are being investigated.
- Genome editing with CRISPR/Cas9 supports allogeneic CAR-T cell development but poses several risks including double strand DNA breaks, genomic rearrangements and chromothripsis.
- Base editing, by contrast, enables precise nucleotide changes without double strand DNA breaks, reducing genotoxicity and expanding therapeutic potential.

CAR-T CELL THERAPY OVERVIEW



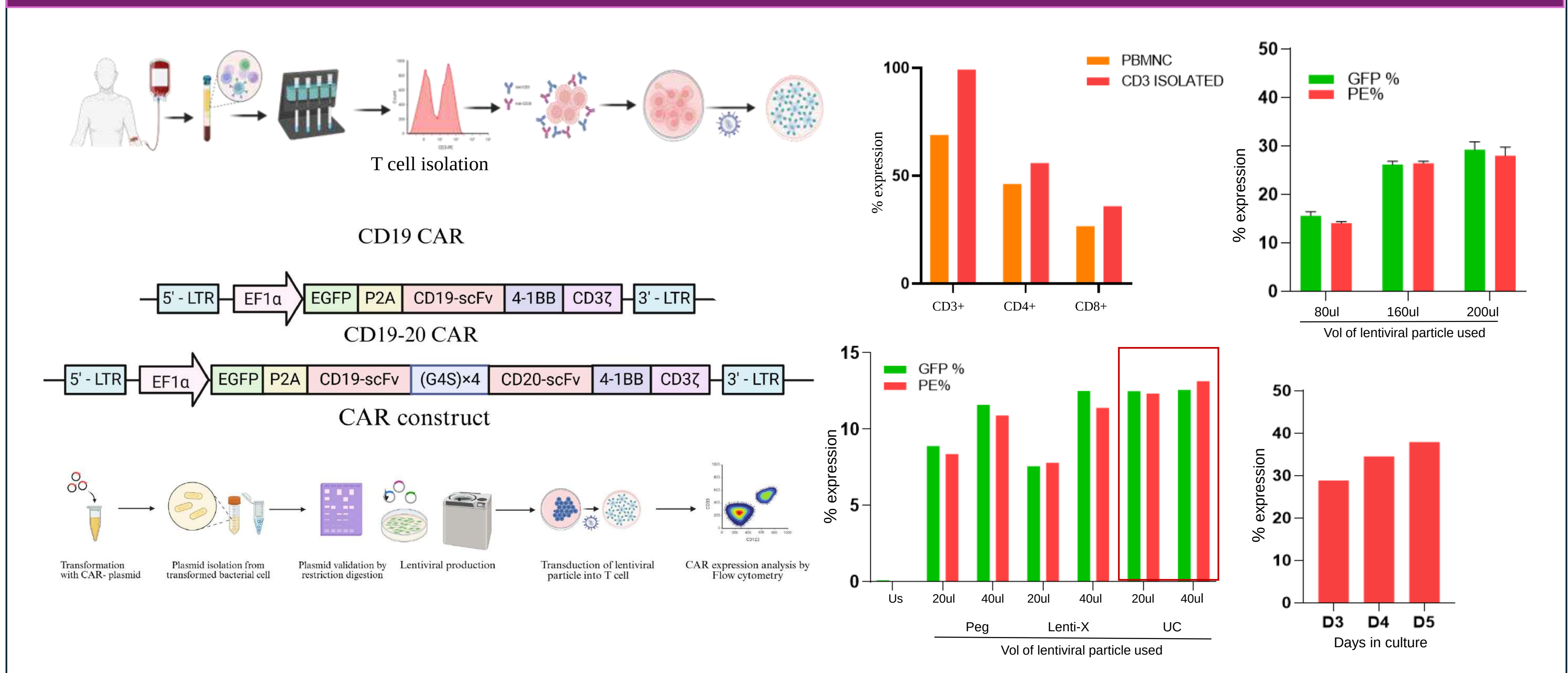
BASE EDITING TO KNOCK OUT TRAC AND B2M



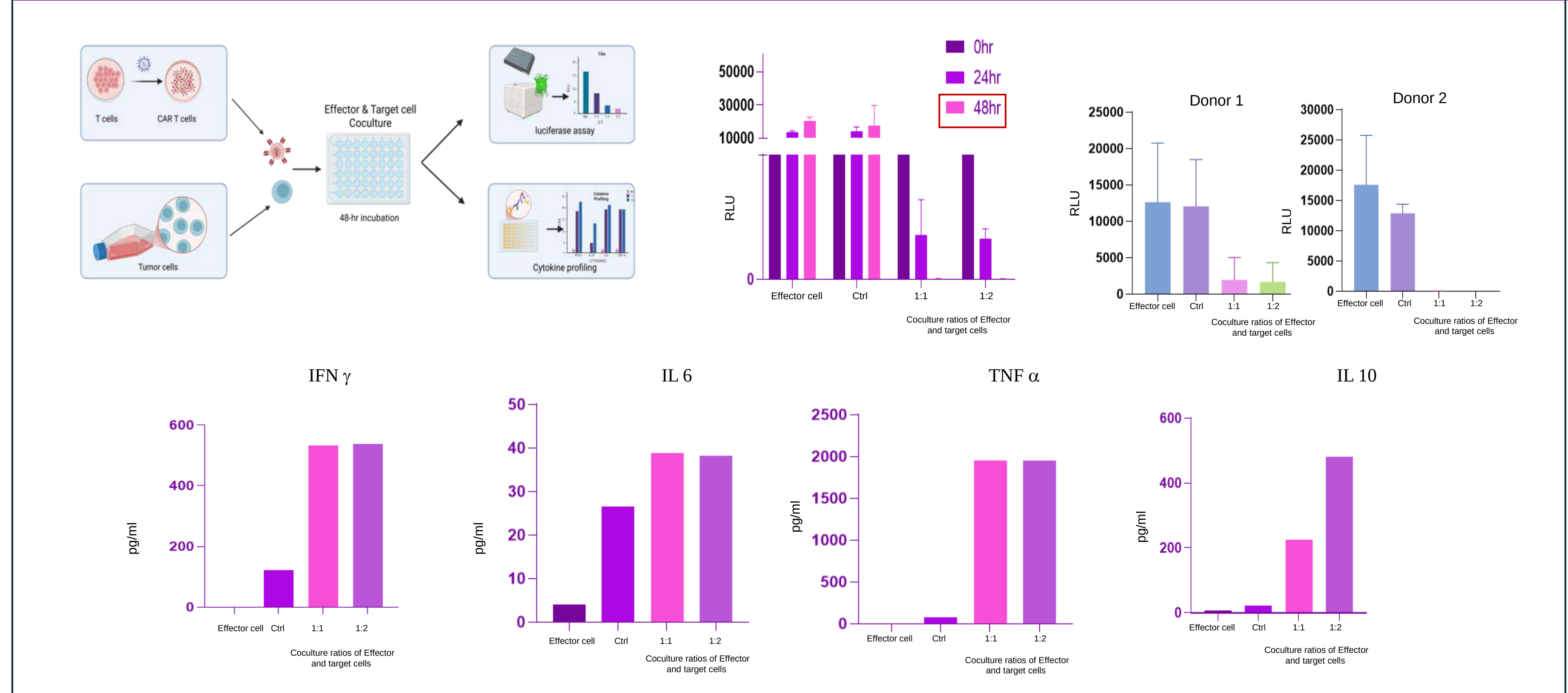
CONCLUSION

- The CD19 CAR construct achieved ~40% expression upon transduction into primary T cells derived from healthy donors .
- Engineered CAR-T cells from healthy donors demonstrated potent killing of CD19-expressing malignant cells (NALM6).
- CD19 CAR-T cells exhibited cytokine production upon co-culture with tumor cells.
- We designed the sgRNA and optimized a protocol to efficiently knock out the TRAC and B2M genes using cytosine base editors in a T-cell line.
- We confirmed the functional knock-out of the B2M gene in base-edited cells by assessing transcript levels using qRT-PCR and protein expression through western blot analysis.
- We aim to refine the transduction protocol to improve efficacy, perform base editing in primary T cells and investigate functional knock-out of the TRAC gene in cell lines

DEVELOPING CAR CONSTRUCTS



VALIDATING THE POTENCY OF ALLOGENIC CAR-T CELLS



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ACKNOWLEDGEMENTS

- Department Of Biotechnology, Government of India
- Centre for Stem Cell Research (A unit of BRIC-inStem, Bengaluru), Christian Medical College Campus, Vellore

Computationally Designed Bi-specific and Tri-specific CD19, CD20, and CD22 CARs with AI-Guided Models for Longer Persistence and Resistance to Antigen Escape

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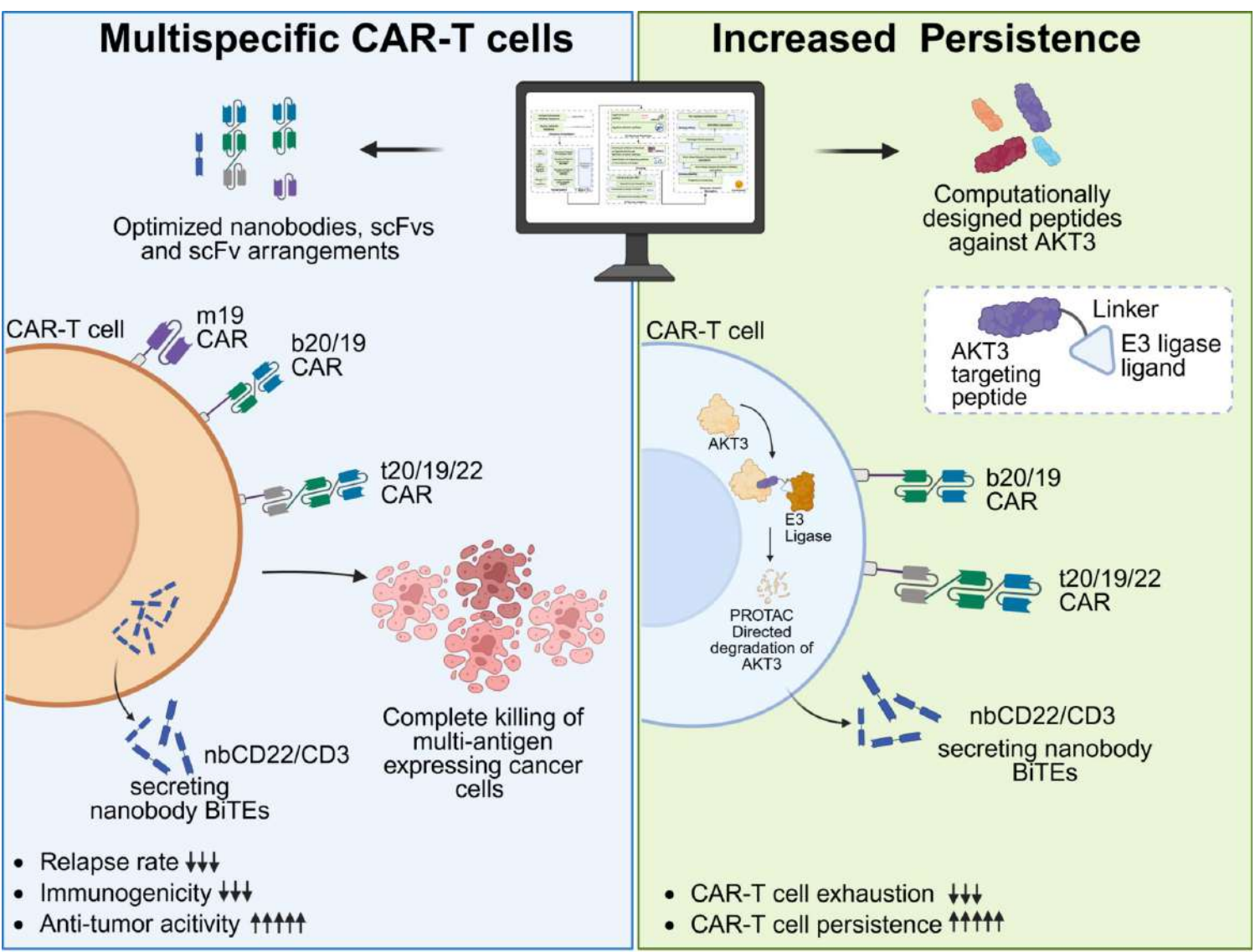
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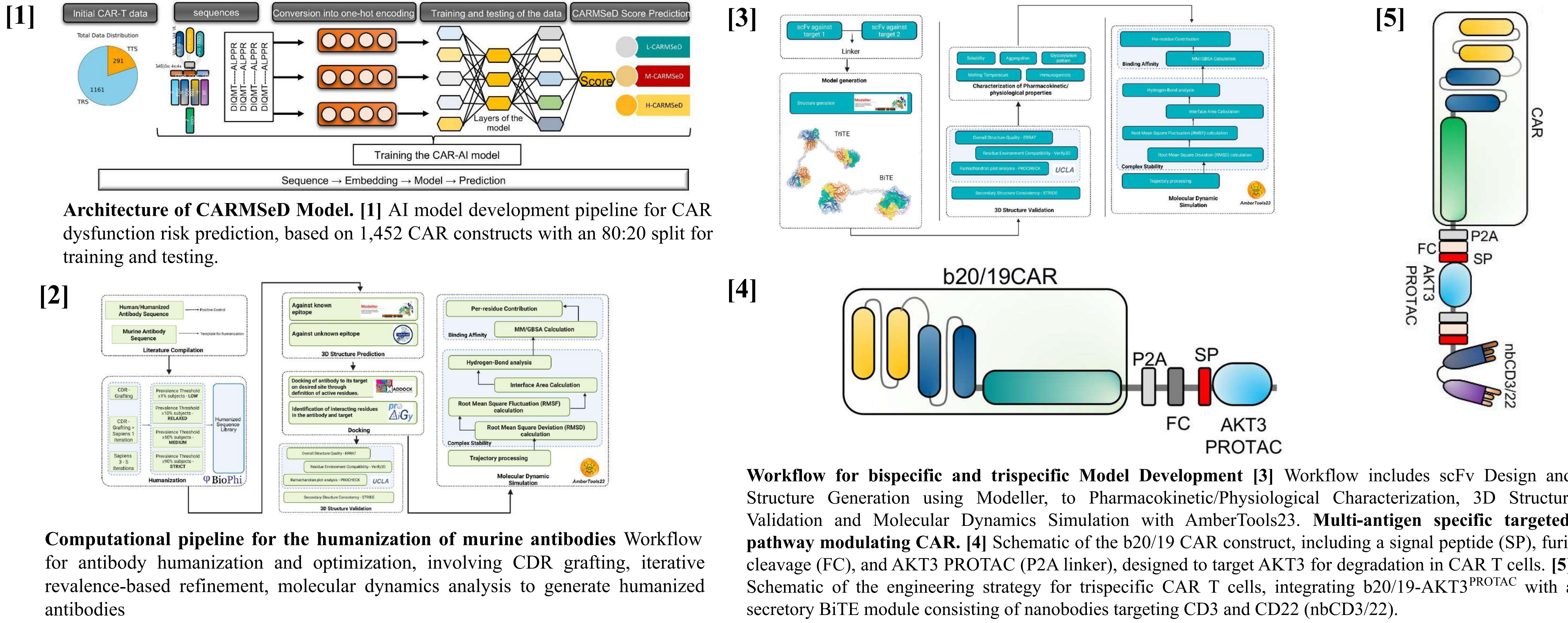
INTRODUCTION

CAR T cell therapy transforms hematologic malignancy treatment but faces T cell exhaustion, metabolic issues, and antigen escape, causing relapse in ~50% of cases. We hypothesized AI-guided multi-specific CAR design would optimize signaling, curb self-destruction, and boost persistence via exhaustion countermeasures. We created an in silico library of 10,824 CARs targeting CD19/20/22 in mono-/bi-/tri-specific formats, varying linkers, co-stim domains (e.g., 4-1BB/ICOS), and transmembrane setups. In vitro screening of 1,452 variants in donor T cells assessed activation, exhaustion, and CARMSed via fluorescence.

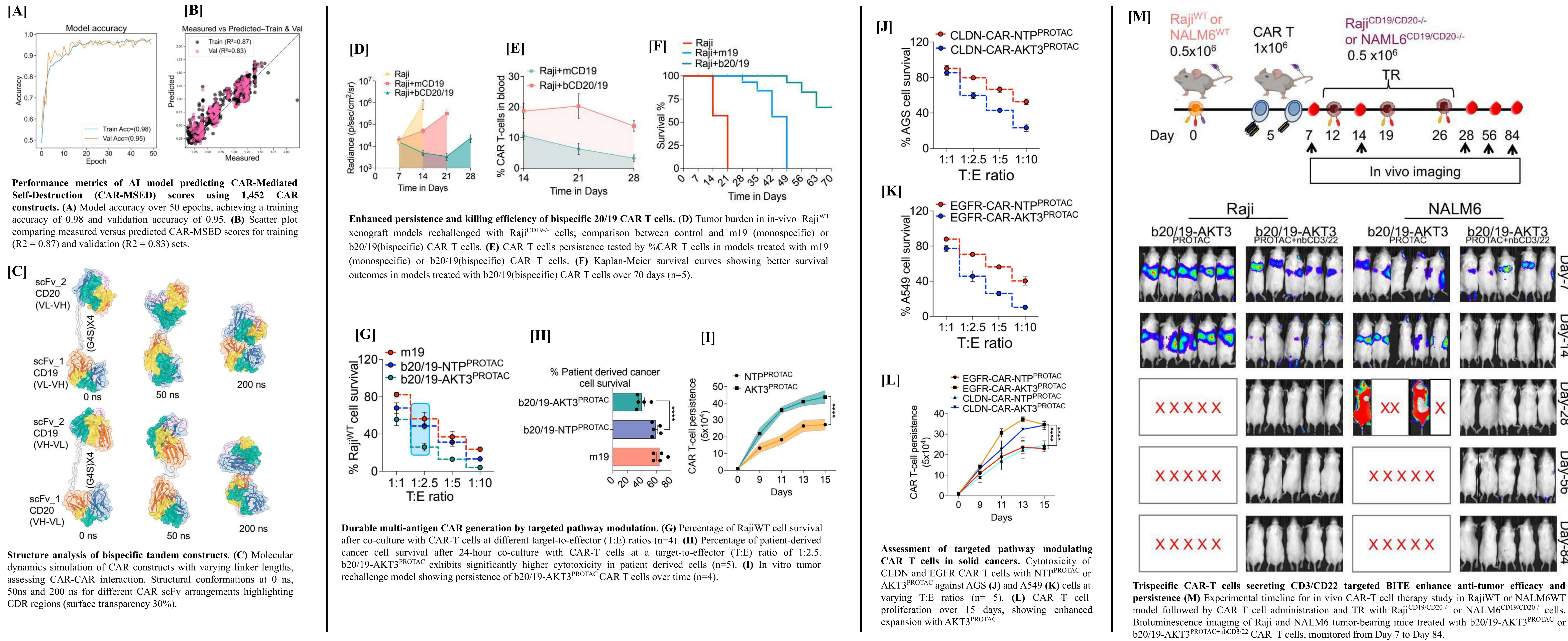
An AI model (95% accuracy) prioritized bispecific CD19/20 CARs with ICOS TM, VH-VL orientation, and 3-4 G4S linkers; AI-humanized scFvs reduced immunogenicity. Transcriptomics identified AKT3 upregulation in exhausted cells; PROTAC degraders targeted AKT3 to enhance mitochondrial fitness and memory (T_{em}) formation. In co-cultures, re-challenge models (CRISPR Raji), and NSG xenografts, optimized bispecific CARs outperformed CD19-mono specifics in cytotoxicity, proliferation, and persistence, eliminating antigen-loss escapes and curbing relapse. AKT3 degradation upregulated FOXO4, inhibited mTOR, and sustained IFN- γ /Granzyme B, prolonging survival. A trispecific CD3/CD22 engager extended efficacy to heterogeneous patient leukemia/solid tumors (gastric/lung). This AI strategy delivers durable, multi-antigen CAR T cells for advanced immunotherapy.



METHODS



RESULTS



CONCLUSION AND FUTURE DIRECTIONS

This study presents an integrated AI-driven and mechanistically guided approach to overcome two major barriers in CAR T cell therapy: antigen escape, and limited cellular persistence. By combining large-scale in-silico CAR design screening through the CARMSeD model with PROTAC-mediated AKT3 degradation, we demonstrated substantial improvements in CAR T cell metabolic fitness, memory differentiation, and antitumor durability. The activation of the AKT3-FOXO4 regulatory axis emerged as a key determinant of long-term CAR T cell function. Furthermore, extending this design to a trispecific CD19/CD20/CD22 platform incorporating a secretory BiTE system provided a powerful solution to antigen heterogeneity without compromising safety. Building on these findings, future studies will focus on:

- Clinical translation of AKT3-PROTAC-engineered CAR T cells through GMP-grade manufacturing and first-in-human trials for refractory B-cell malignancies.
- Expanding the PROTAC platform to target other exhaustion regulators such as PD-1, LAG-3, and TIGIT to fine-tune CAR T functionality.
- Integrating synthetic gene circuits and temporal degradation switches for controlled modulation of signaling and metabolic pathways.
- Evaluating efficacy in solid tumor models using tumor-specific antigens like Claudin18.2 and EGFR under hypoxic and immunosuppressive microenvironments.
- AI model enhancement with multi-omics data to improve predictive accuracy of CARMSeD for personalized CAR design and patient stratification.

ACKNOWLEDGMENTS

We thank the Jamia Millia Islamia (JMI), Indian Institute of Technology Kanpur (IITK), CSIR-Indian Institute of Chemical Biology (CSIR-IICB), and Christian Medical College (CMC) Vellore for providing essential facilities, animal model support, and ethical compliance. We also acknowledge the collaborative contributions of Apollo Hospitals, New Delhi, for providing clinical samples and technical expertise.

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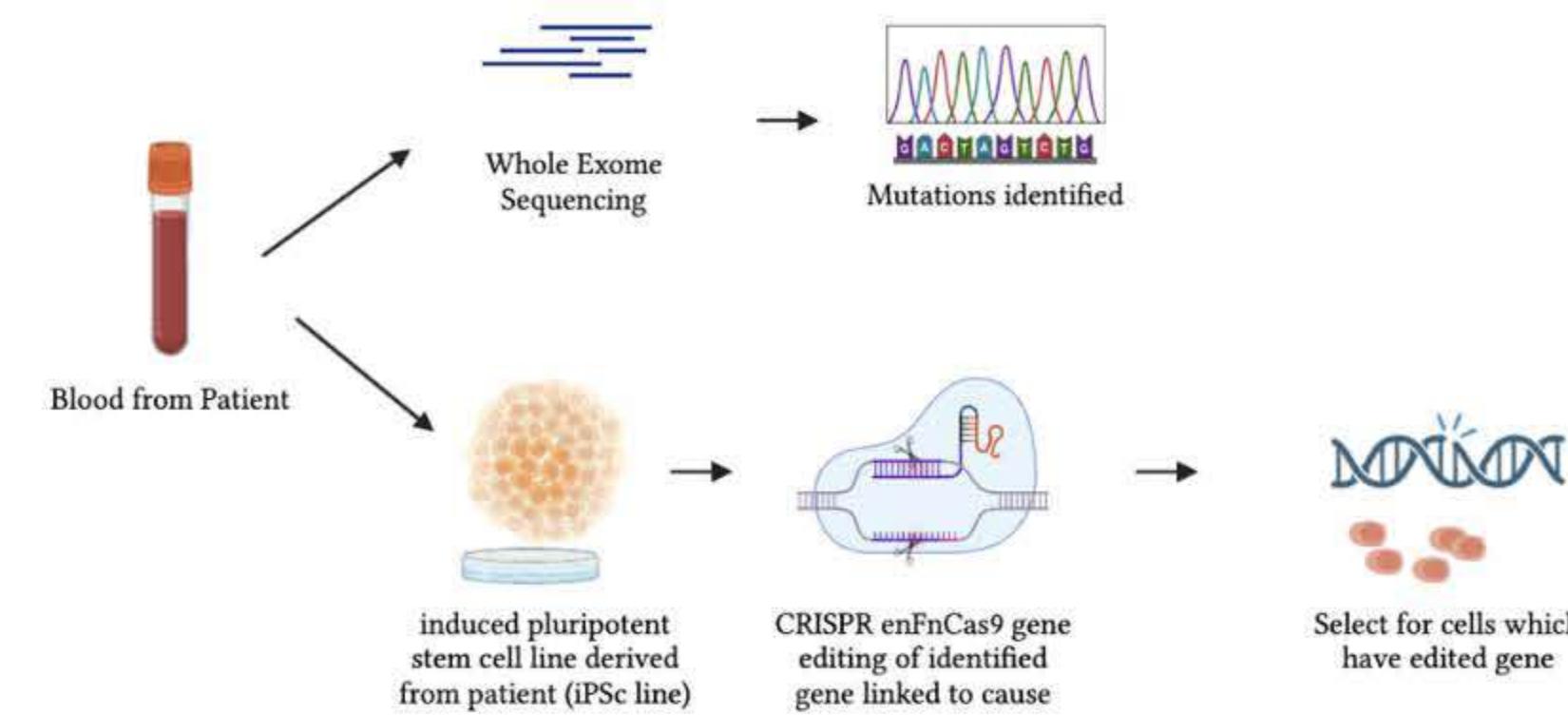
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INTRODUCTION

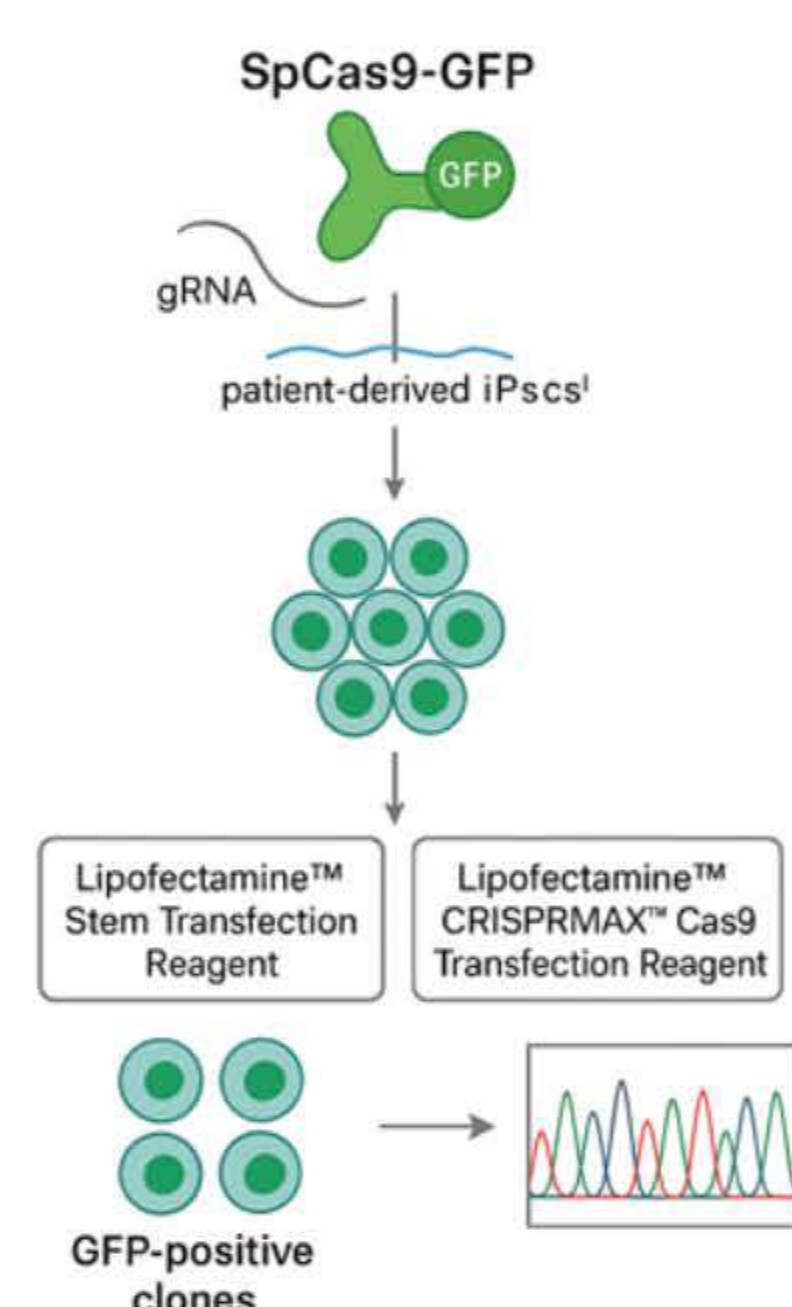
Rare diseases are a major healthcare challenge in India, defined by the NPRD, 2021, as conditions affecting fewer than 1 in 2,500 individuals. Despite the policy, over 300 patients still lack access to essential care due to limited awareness, high costs, and weak research infrastructure. CRISPRBITS aims to tackle these barriers using CRISPR-based genome editing to develop affordable therapeutic solutions. Its core technology, CurieCrisp, is a proprietary platform built on enhanced FnCas9 (enFnCas9). CurieCrisp enables precise modelling and correction of rare genetic diseases using patient-derived iPSCs.

The current research focuses on Leber Congenital Amaurosis (LCA), a group of inherited retinal dystrophies causing progressive photoreceptor degeneration. One form of LCA is linked to mutations in RDH12, an enzyme vital for retinoid metabolism in the visual cycle. A specific nonsense mutation in exon 3 of RDH12 (C>A, p.S13Ter) leads to early-onset retinal degeneration and severe vision loss. CRISPRBITS hypothesizes that correcting this mutation in patient-derived iPSCs can restore RDH12 expression and activity. This correction could normalize retinoid metabolism and enhance photoreceptor survival and function in retinal cells or organoids.

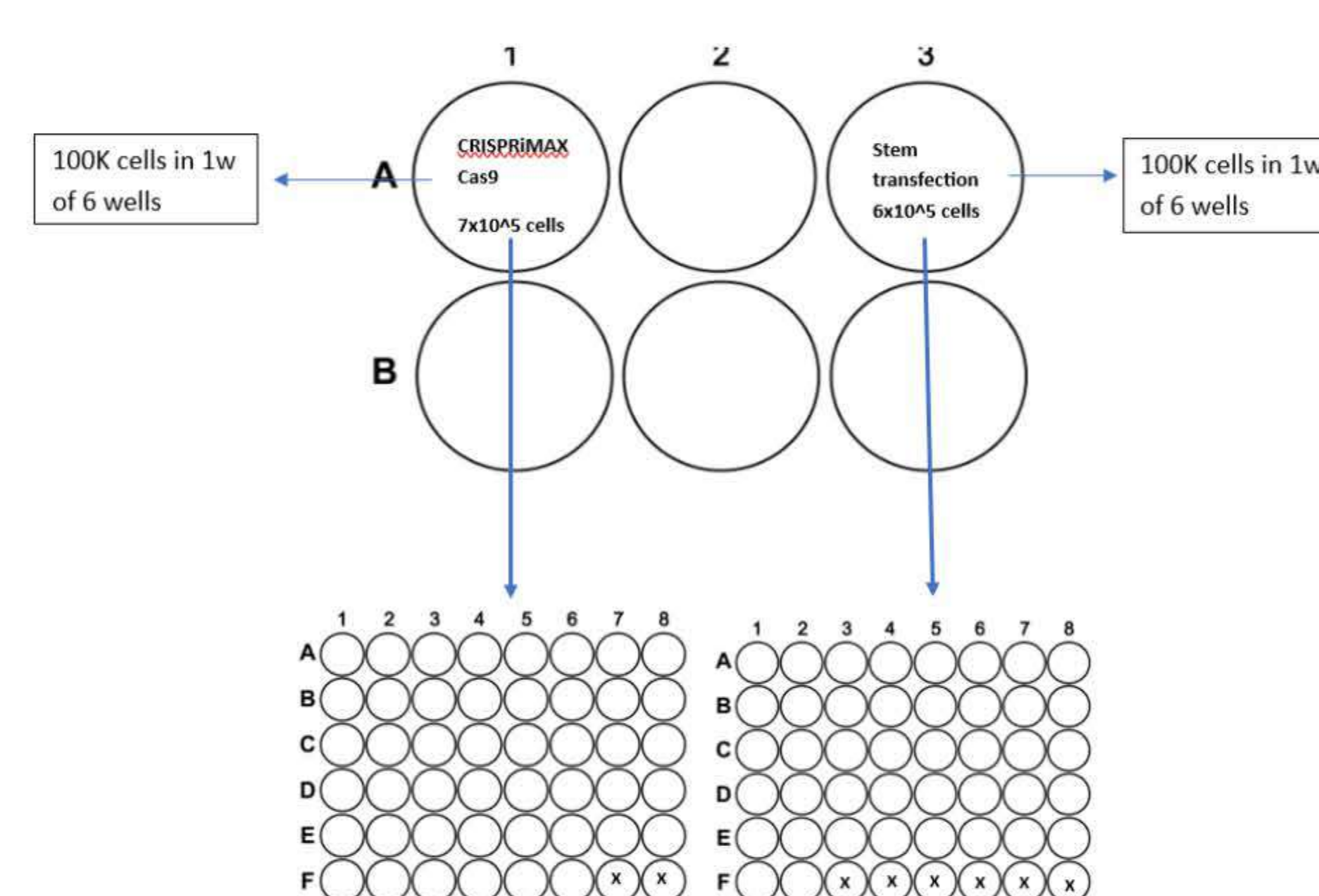


METHODOLOGY

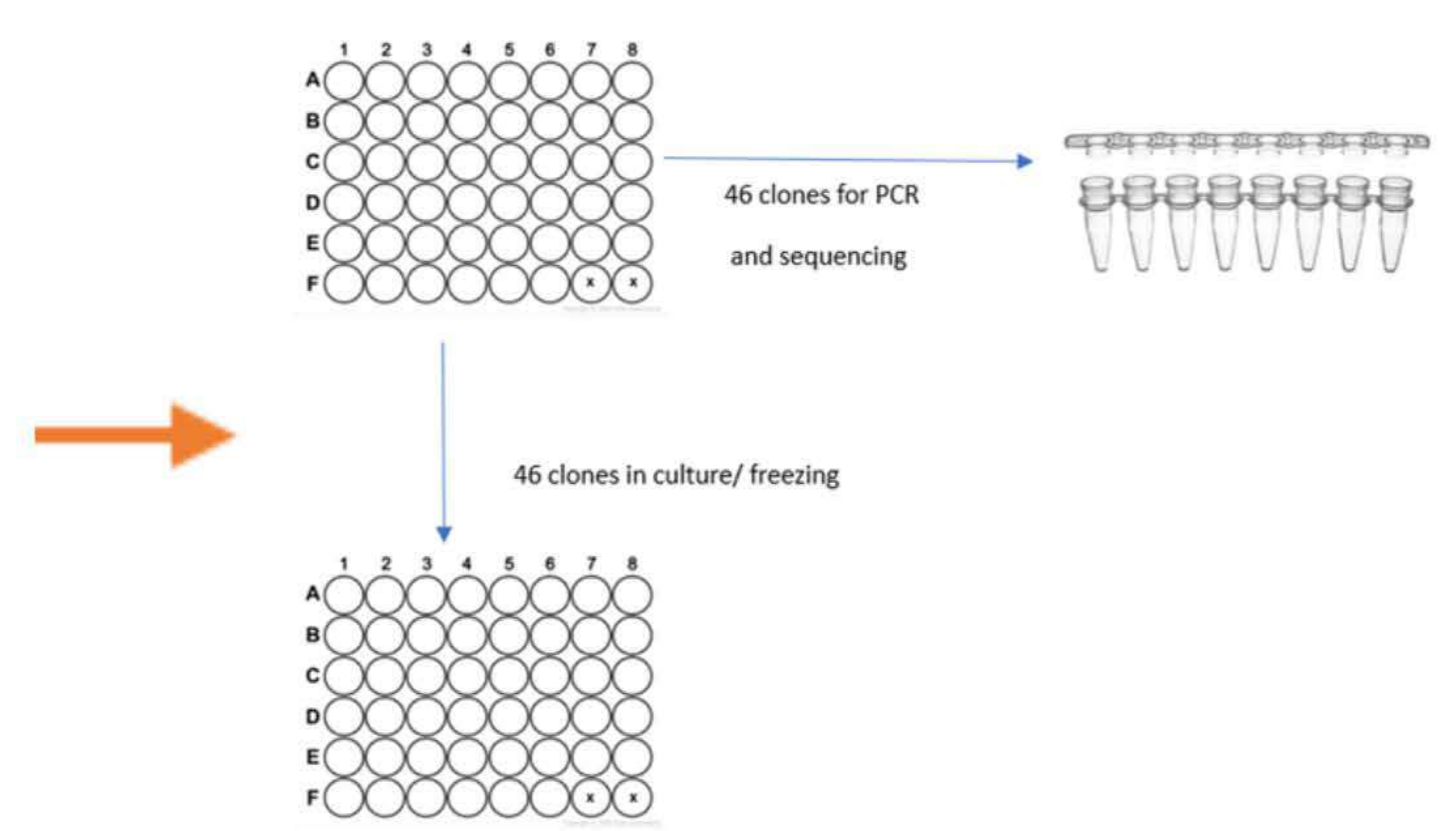
Overview of Lipofection of RNP complex



Isolation of bulk clones in a 48 well plate



Freezing and PCR of bulk clones



Patient derived iPSc cells were transfected using Lipofectamine reagents and CRISPR RNP Complex and ssODN for correcting the mutation. After selecting for Cas9-GFP expressing cells and serial dilutions we obtained a heterozygous corrected clone.

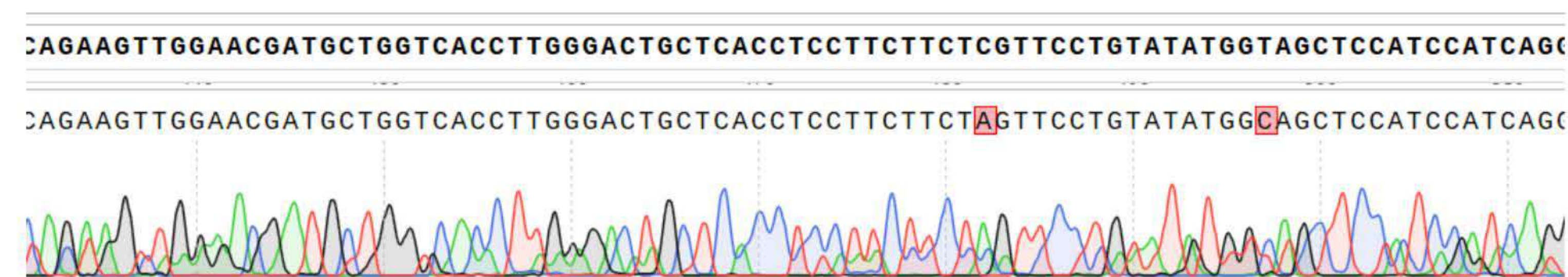
RESULTS



Representative Image of fluorescence microscopy showing GFP expression 24 hours post-lipofection at 10x magnification.

CAGAAAGTTGGAACGATGCTGGTACACCTTGGGACTGCTCACCTCCTTCTTCTCGTTCTCTGTATATGGTAGCTCCATCCATCAGG
GTCTTCAACCTTGCTACGACCAAGTGGAAACCTGACGAGTGGAGGAAGAAGAGCAAGGACATATACCATCGAGGTAGGTAGTCT

C>A mut



Isolation and Screening of Clonal Populations

Twenty-four hours post-lipofection, four initial clones were isolated and subsequently bulk-sorted into 42 individual clones. After 10 days of culture, each clone was split — half of the well was cryopreserved, and the remaining cells were analyzed by PCR and sequencing. Of the 42 clones analyzed, 26 exhibited heterozygous correction.

CONCLUSIONS

Although the observed correction was limited and mosaic, the results underscore the potential of CRISPR-based strategies for precise genome editing in rare retinal disorders. Further optimization of transfection conditions and HDR parameters will be required to enhance efficiency.

Corrected iPSC lines generated in this study may serve as a foundation for retinal organoid modelling and the development of personalized gene therapies for RDH12-associated Leber Congenital Amaurosis.

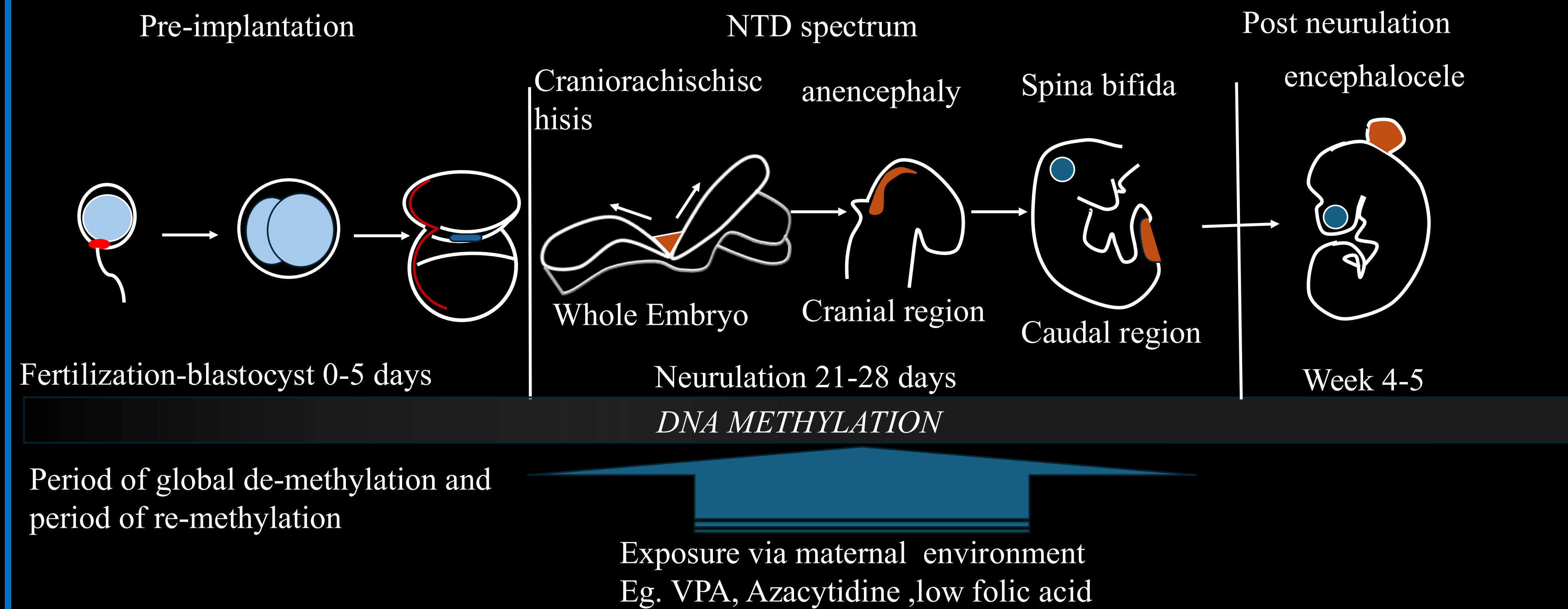
This is our first proof of principle study for evaluation of our CurieCrisp platform and we are excited to see how the heterozygous corrected line will behave upon differentiation.

ACKNOWLEDGEMENTS

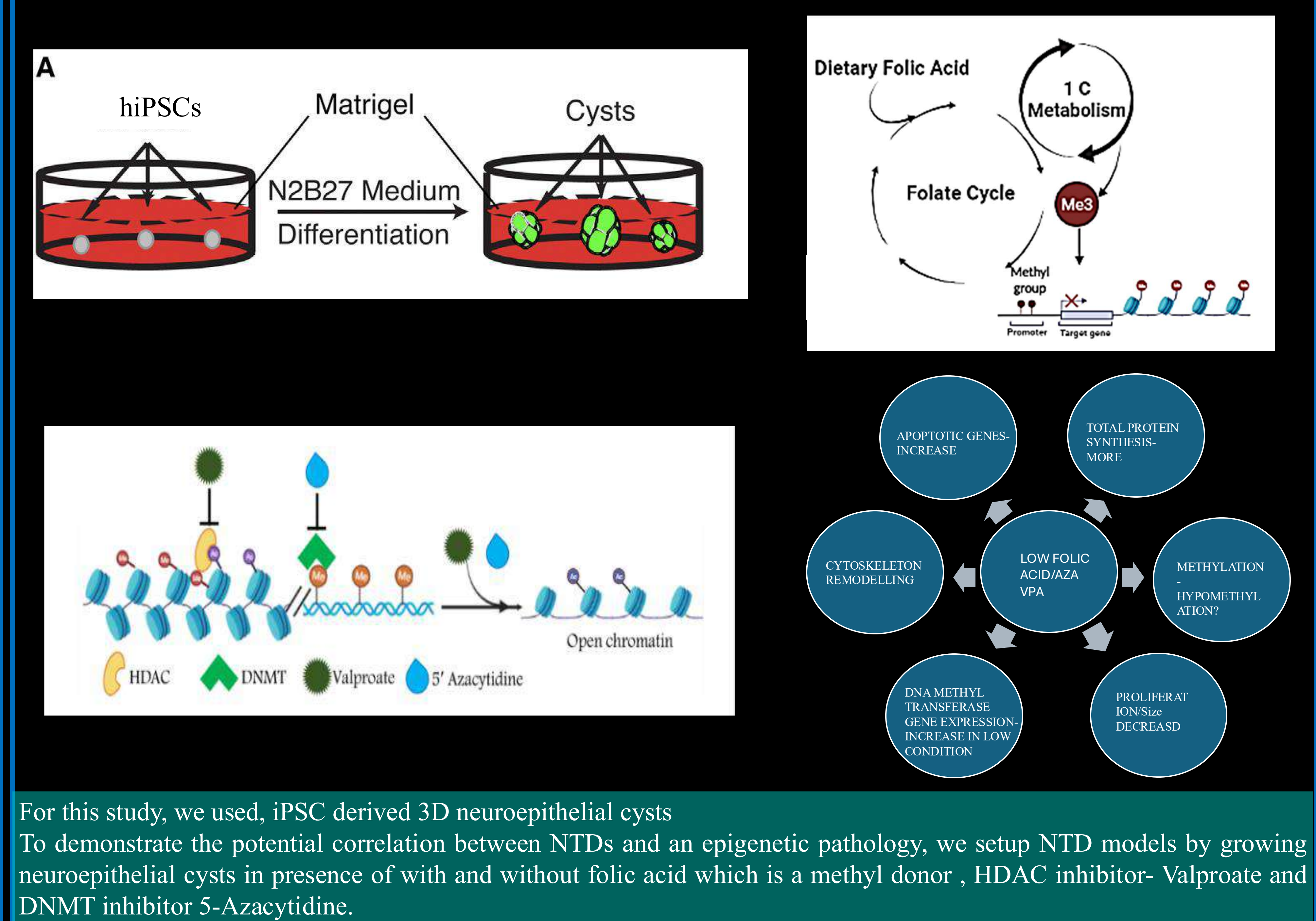
I would like to thank Janavi Subramani, Niharika Patolla, Rajashri Pal, Jogin Desai of Eyestem for providing with patient derived iPSCs. I also acknowledge CRISPRBITS team for their guidance and valuable discussions.

Background

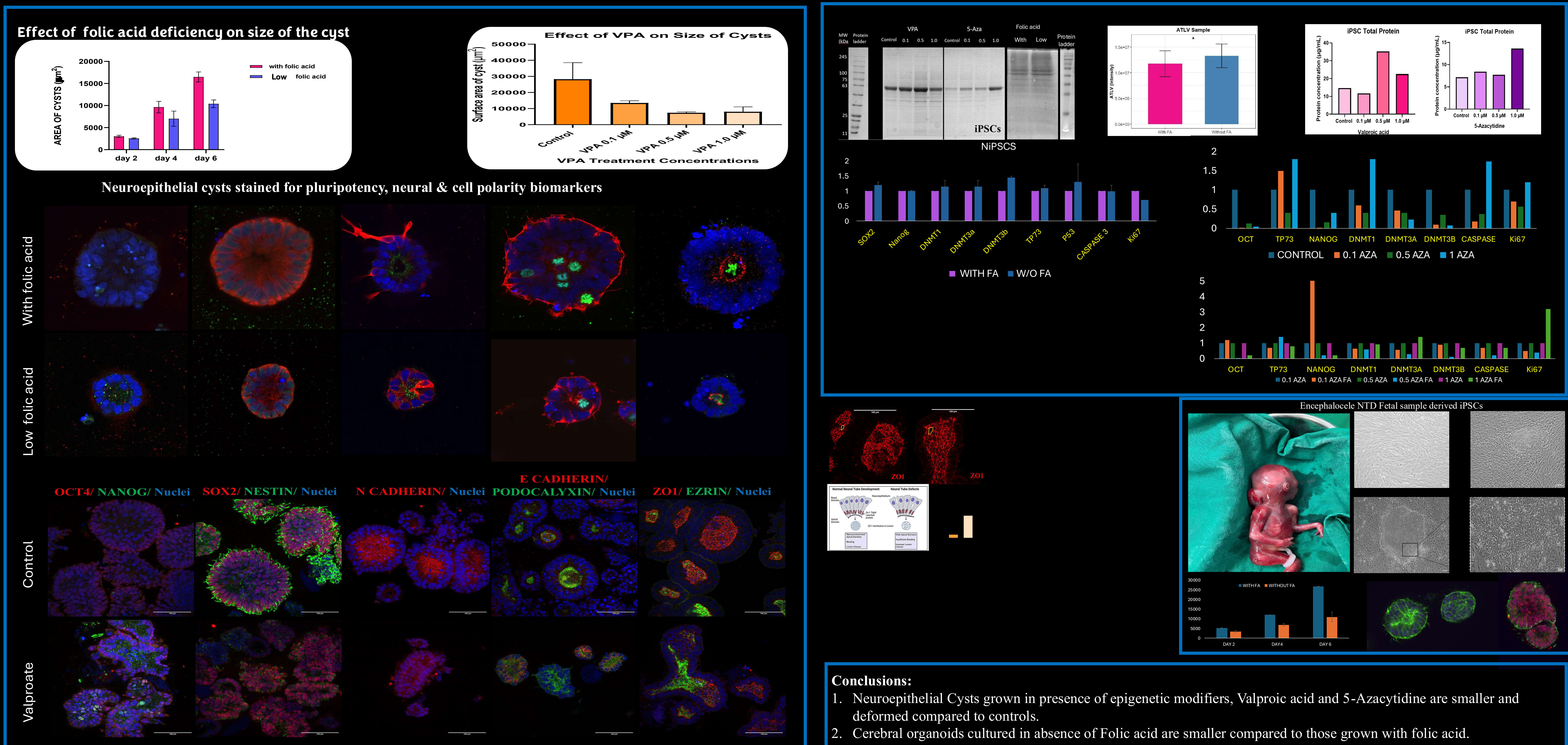
- Neural tube defects [NTDs] are severe congenital malformations in humans.
- The etiology of NTDs is poorly understood.
- NTDs are considered to have multifactorial etiology which include genetic mutations and environmental factors .
- The present animal model studies do not exemplify human NTDs completely as there are differences in genomes, molecular mechanisms, timing and cellular process of Neural tube[NT] formation.
- Dysregulated epigenetics particularly during embryogenesis results in abnormal genome wide methylation which has been linked to NTDs.
- Maternal exposure to teratogens like valproic acid(VPA), azacytidine(AZA) and dietary folic acid(FA) deficiencies lead to neural tube defects.



Materials and methods



Results



Conclusions:

- Neuroepithelial Cysts grown in presence of epigenetic modifiers, Valproic acid and 5-Azacytidine are smaller and deformed compared to controls.
- Cerebral organoids cultured in absence of Folic acid are smaller compared to those grown with folic acid.
- SH-SY5Y cells and iPSCs treated with Valproic acid and 5-Azacytidine have increased total cellular proteins.
- Neural cysts treated with Valproic acid have a more expanded apical surface [ZO1 Staining] indicating insufficient apical constriction required for neural plate bending.

AI Assisted Residual *E. coli* DNA Quantification in Plasmids for GMP Grade Lentiviral Manufacturing

Cellogen Therapeutics Pvt. Ltd.

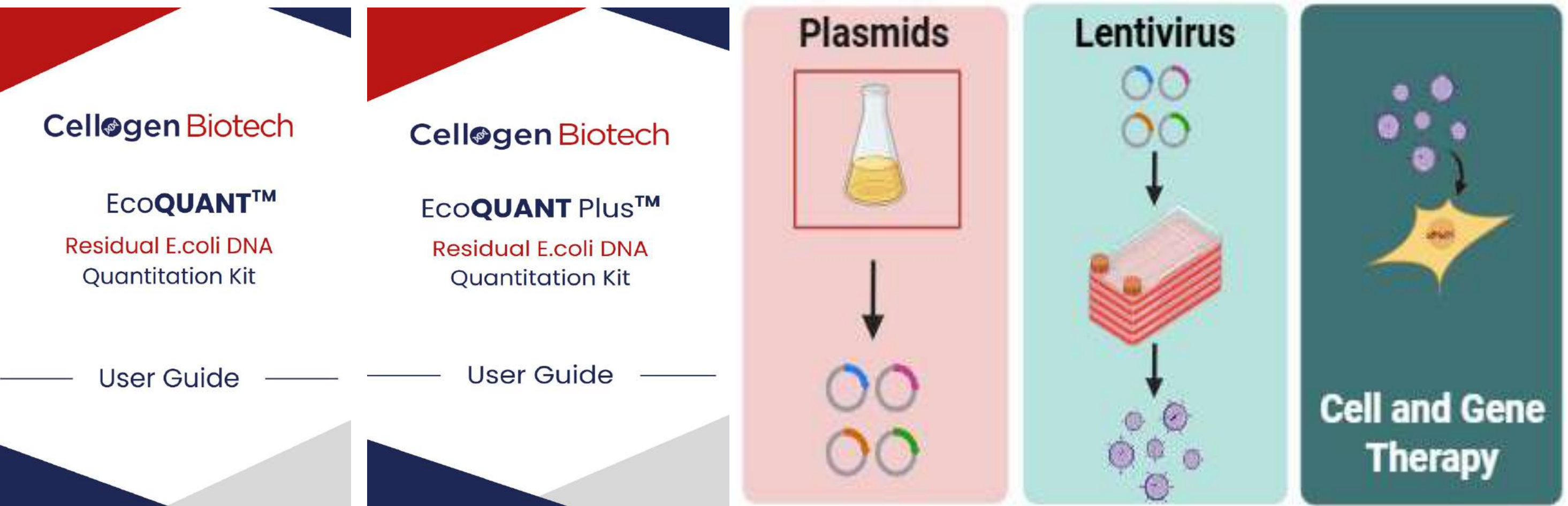
Aswathi CB, Syed Ahmad Ali, Tanveer Ahmad, Suresh Madheswaran*

Cellogen Biotech

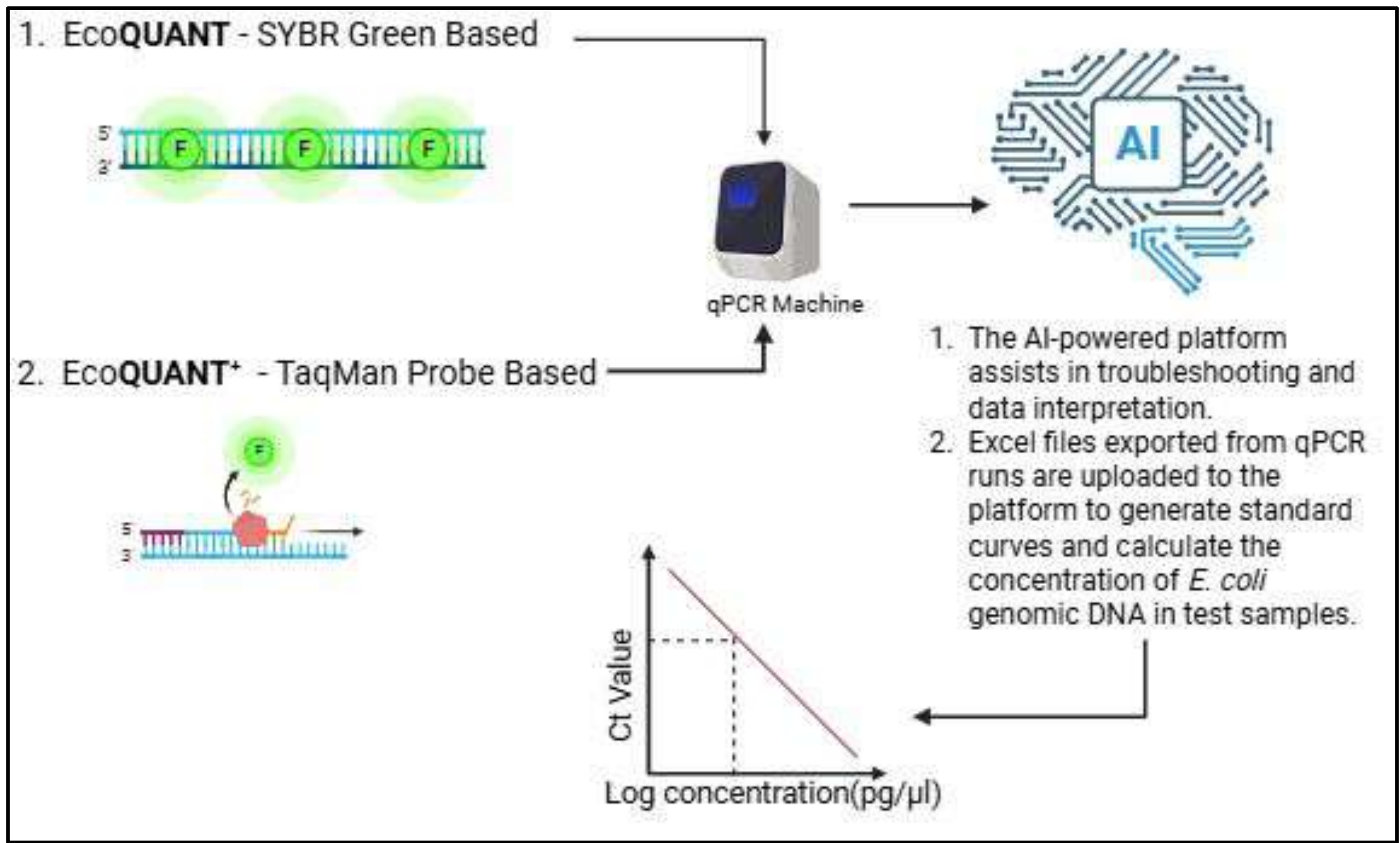
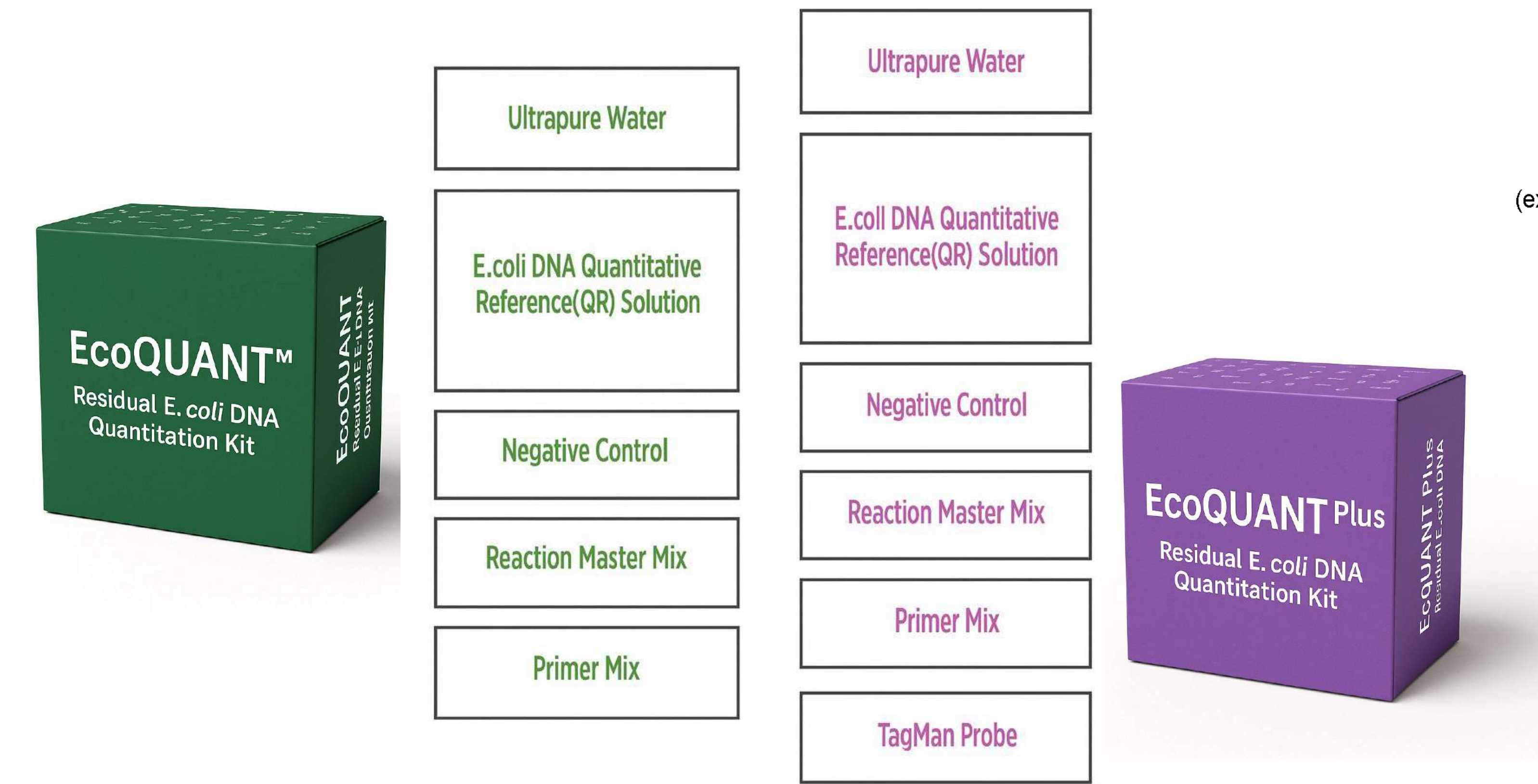
Cellogen
Therapeutics

INTRODUCTION

Cell and gene therapies have transformed the management of previously incurable diseases, such as B-cell malignancies, by offering durable and potentially curative outcomes. A critical backbone of these therapies is plasmid DNA, used for lentiviral vector (LVV) manufacturing to generate high-titer, functional viral particles in HEK293T cells. Since plasmids are propagated in *E. coli*, quantifying residual host genomic DNA (gDNA) is critical to verify that contamination remains within WHO-approved regulatory limits, ensuring GMP-grade plasmid quality and safe LVV production.

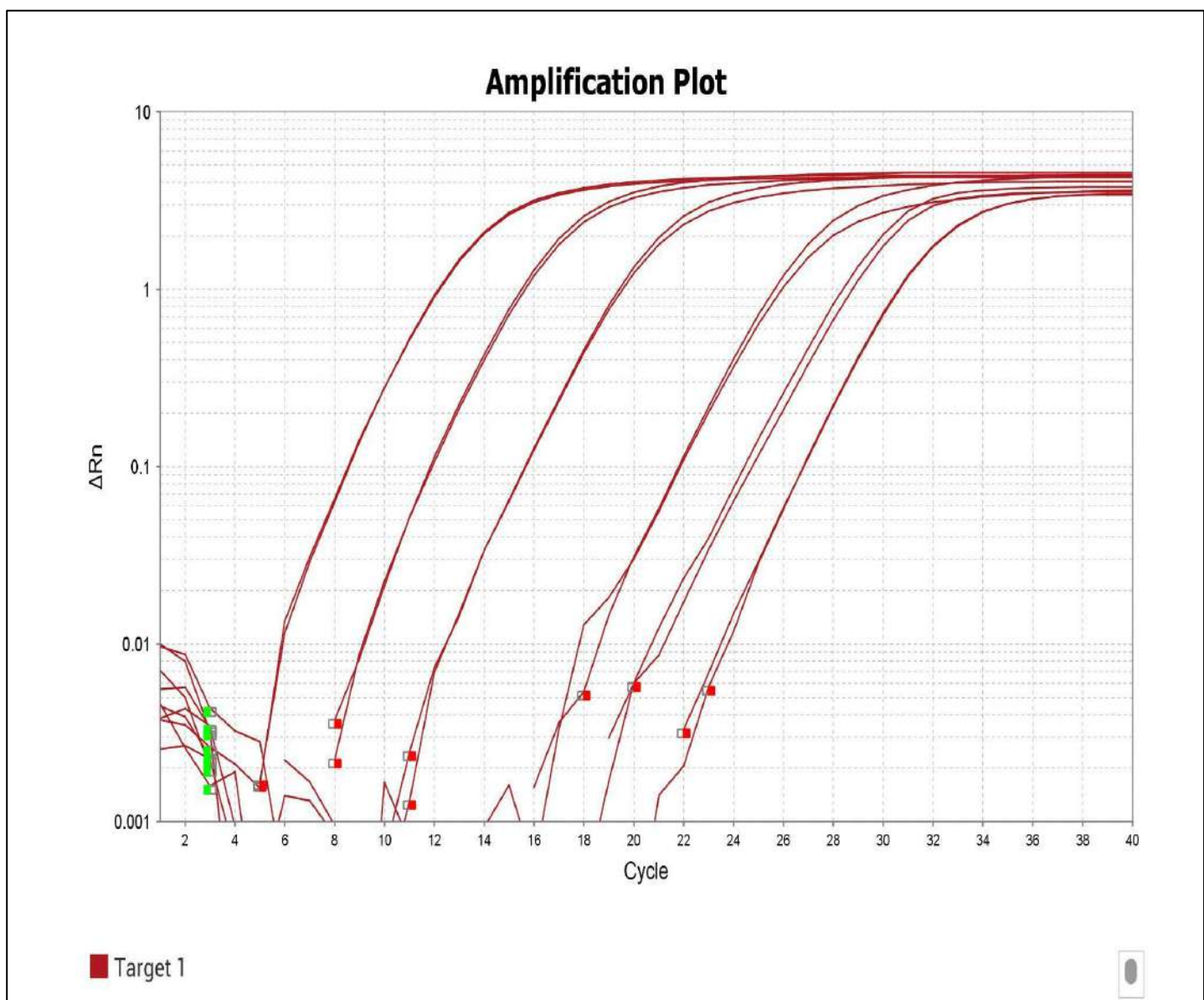


METHODS



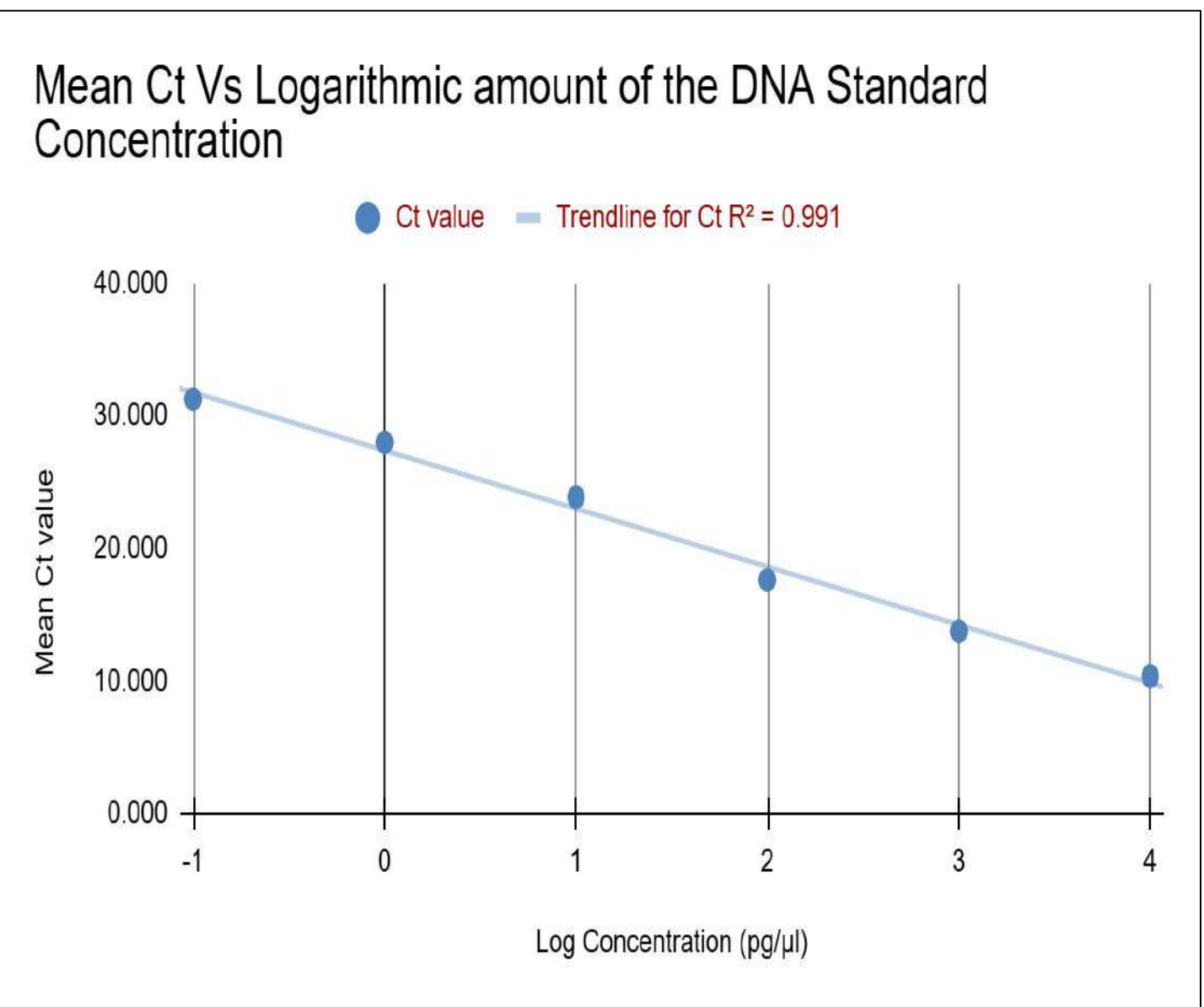
RESULTS

Representative qPCR amplification plot of the samples and standard



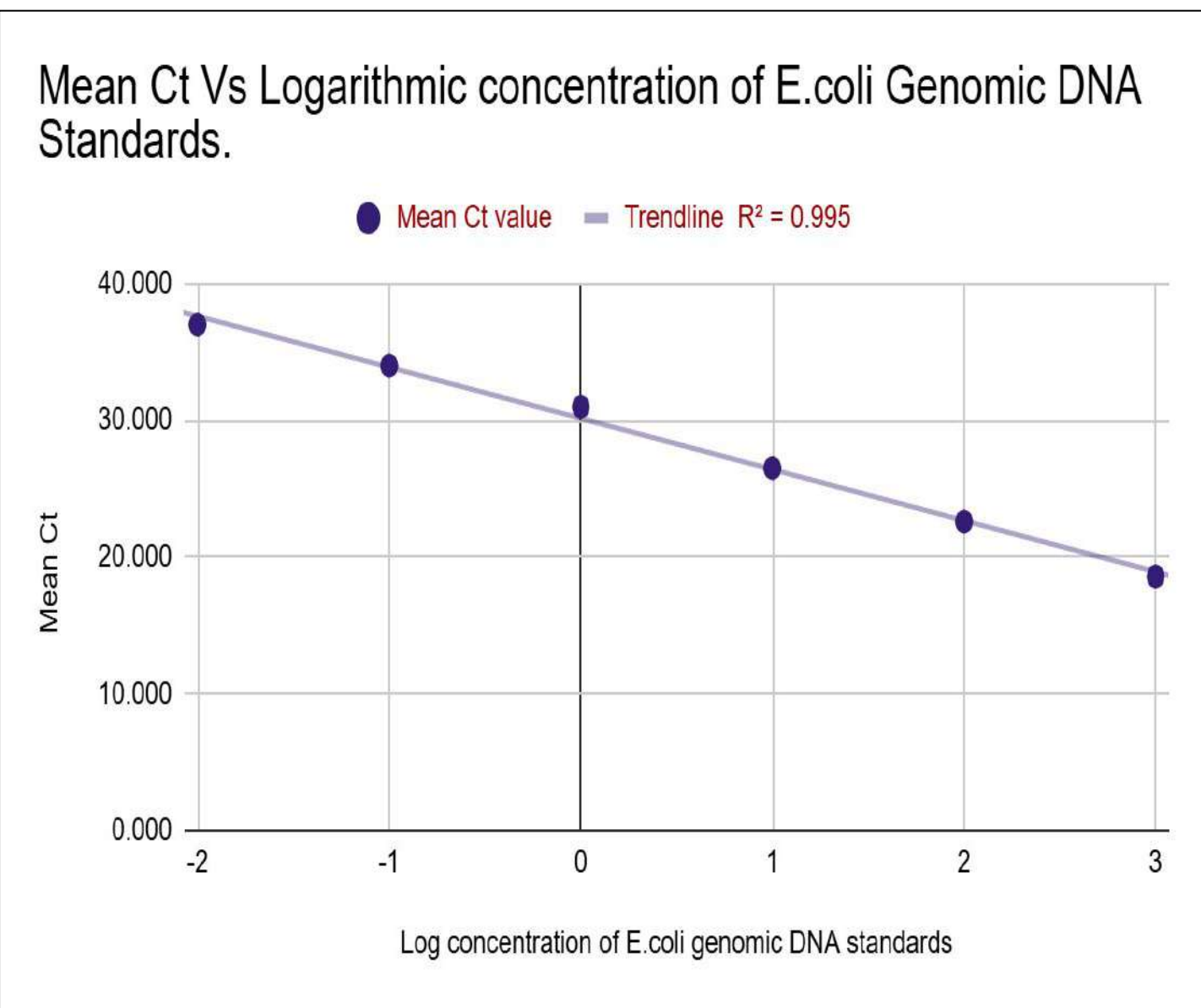
Standards from 0.1 pg/μl to 10,000 pg/μl had an amplification with good repeatability.

Standard Curve analysis of samples using EcoQUANT™



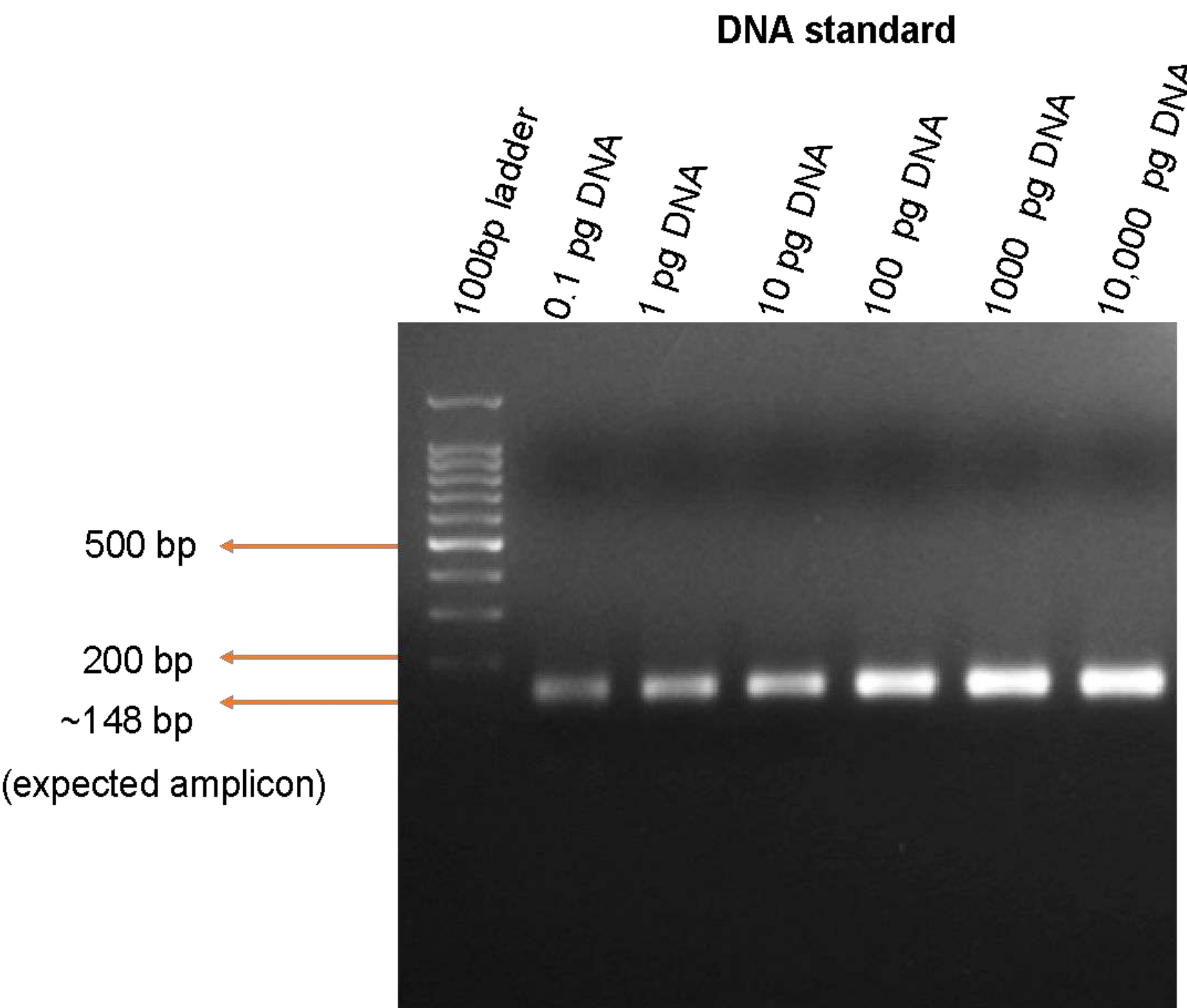
The R² of the standard curve is 0.991 with acceptable amplification efficiency (95.2 %).

Standard Curve analysis of samples using EcoQUANT™ Plus



The R² of the standard curve is 0.991 with acceptable amplification efficiency (95.2 %).

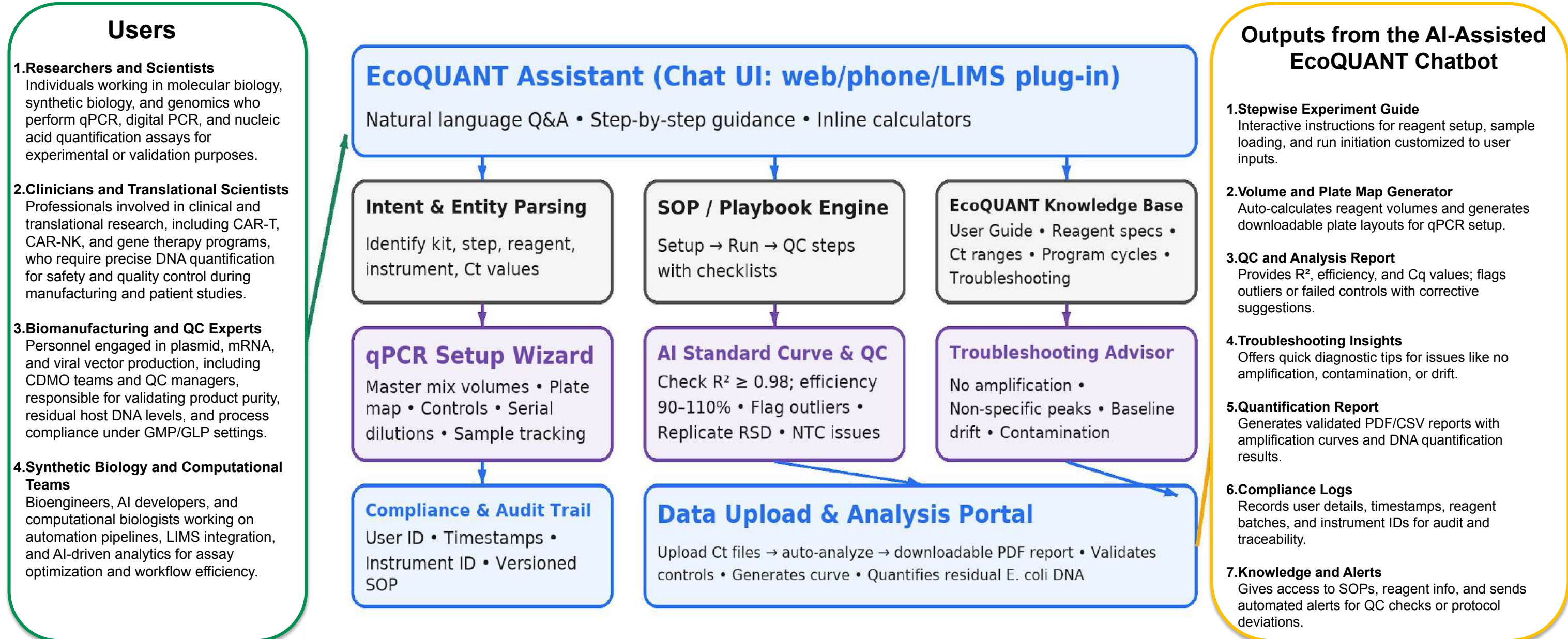
Gel picture of the standard *E. coli* DNA



Sensitivity and Specificity of the samples

Validation Method	EcoQUANT™	EcoQUANT™™
Limit of detection (LOD)	0.01pg/ μl	0.001 pg/ μl
Amplification Efficiency	99.2%	99.92%
Mean Recovery	~99.84	~99.34
Accuracy	~99.1	~99.8

AI-Assisted Chatbot for EcoQUANT Kits — System Schema (Aligned)



CONCLUSIONS

The EcoQUANT™ and EcoQUANT+™ kits demonstrated higher specificity, accuracy, and reliability compared to chromogenic-based methods. Furthermore, the TaqMan probe-based EcoQUANT+™ showed superior amplification efficiency over the SYBR Green-based approach.

ACKNOWLEDGEMENT

Cellogen Therapeutics
Cellogen Biotech

Dr Gaurav Kharya
Mohan Ranganathan
Deeksha Joshi
Aparna Ramachandran
Jahnvi Hora
Entire Cellogen team



<https://www.cellogentherapeutics.com/>

<https://www.cellogenbiotech.com/>

Cellogen Biotech

Cellogen
Therapeutics

Integrative *In Silico* and Toxicogenomic Study Reveals Cyhalothrin-Mediated Perturbation of Estrogen Signaling in Breast Cancer

S. Monica Jannett and S. Sumathi

Department of Biochemistry, Biotechnology and Bioinformatics
Avinashilingam Institute for Home Science and Higher Education for Women

INTRODUCTION

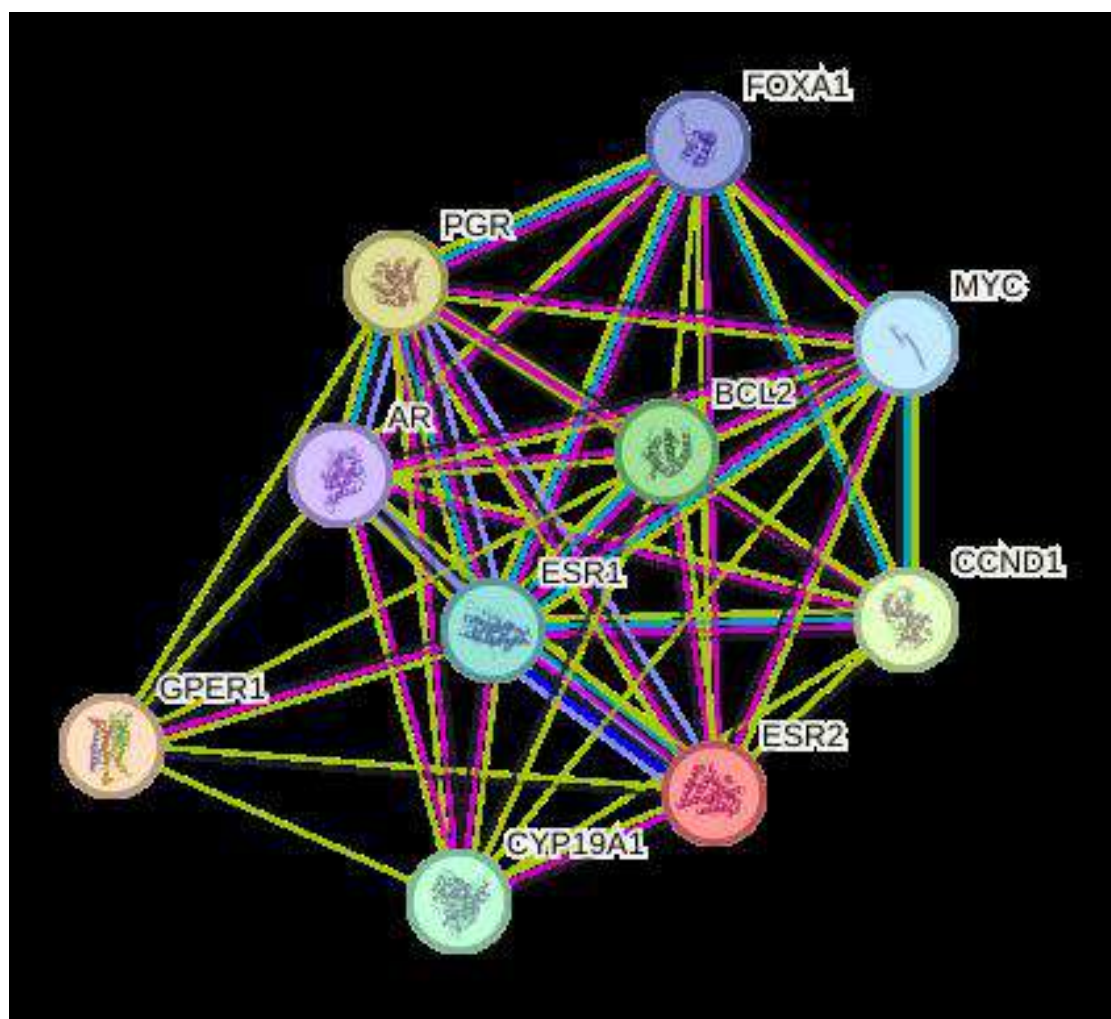
- Breast cancer -**heterogeneous disease** influenced by genetic, hormonal, and environmental factors.
- Endocrine-disrupting pesticides (EDPs)** mimic or interfere with natural hormone signaling.
- Estrogen receptor alpha (ER- α)** -regulator of breast cancer tumor behavior.
- Environmental exposures, such as pesticides, may **initiate and promote breast cancer progression**.
- Understanding **pesticide–ER- α interactions** can provide insights into disease risk and therapeutic targets.

RESULTS

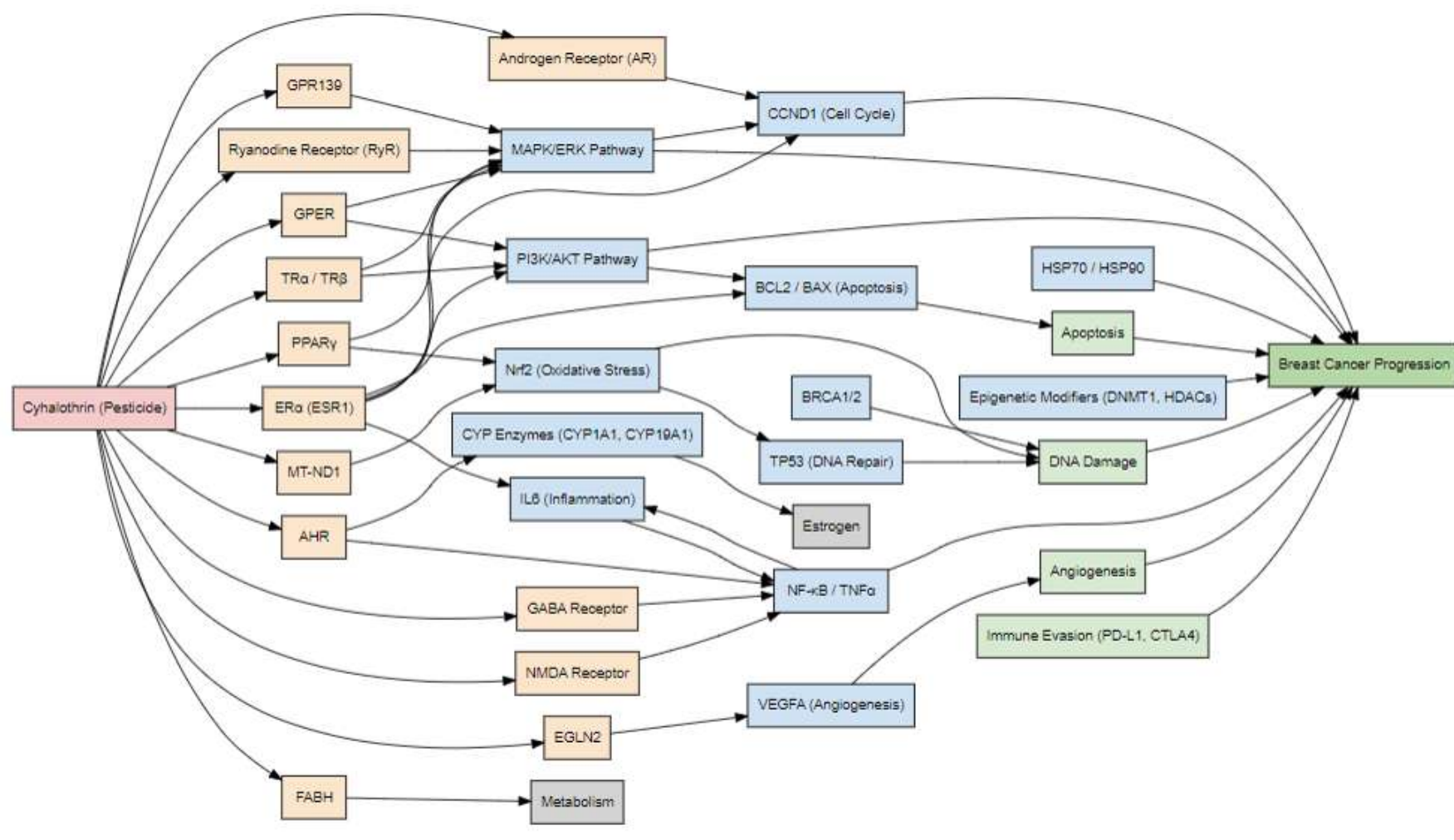
Docking of Cyhalothrin Pesticides with ER- α ”

LIGAND	CAVITY	VINA SCORE	CONTACT RESIDUES	INTERACTION TYPE
Cyhalothrin	C4	-8.6	GLU353	HYDROPHOBIC CONTACT
Fenvalerate	C4	-8.5	ARG394	HYDROPHOBIC CONTACT & HYDROGEN BOND
Dtransallethrin	C4	-8.2	ARG394	HYDROPHOBIC CONTACT
Cyfluthrin	C4	-8	ARG394	WEAK HYDROGEN BOND
Deltamethrin	C4	-8	ARG394	CATION-PI BOND & HYDROPHOBIC CONTACT

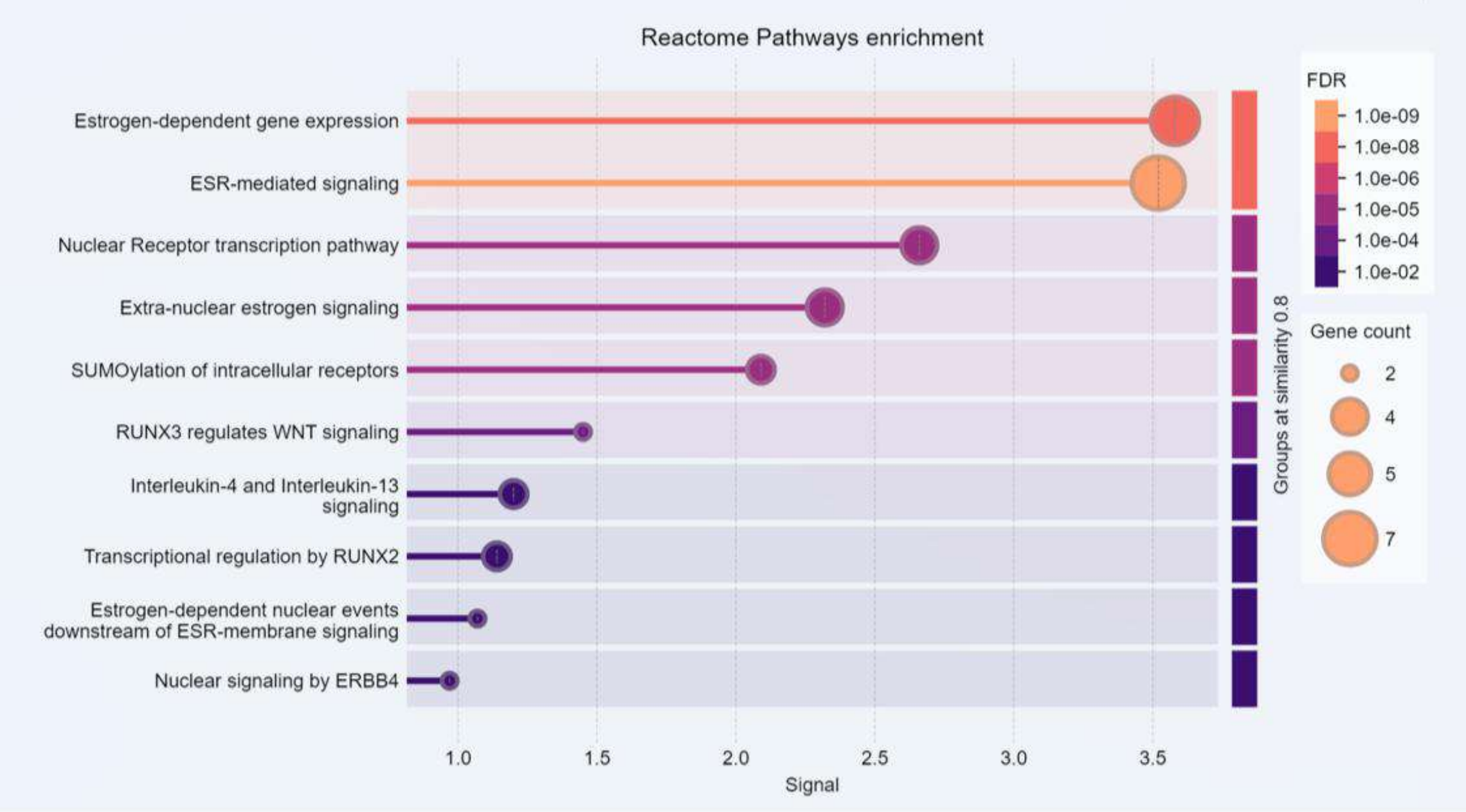
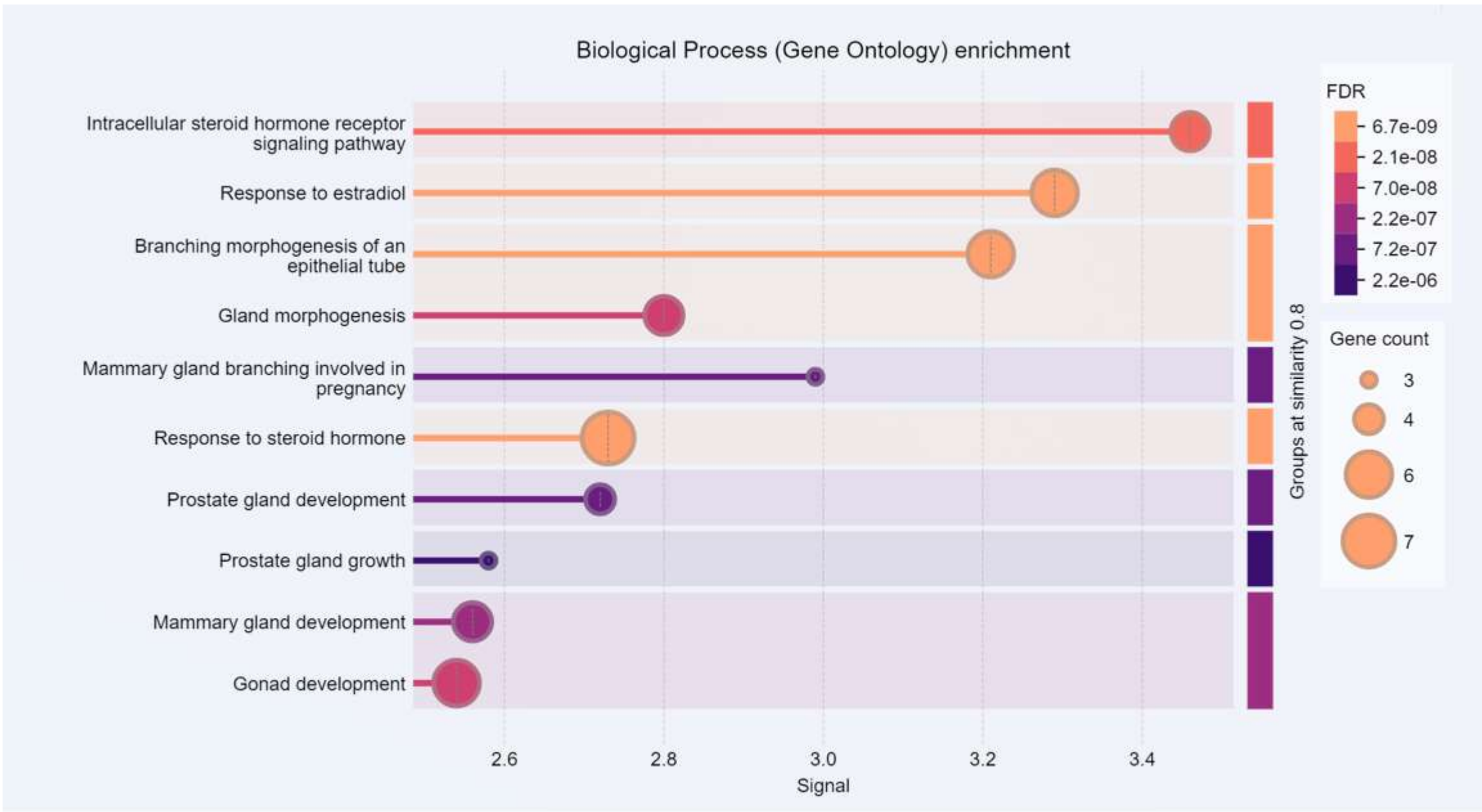
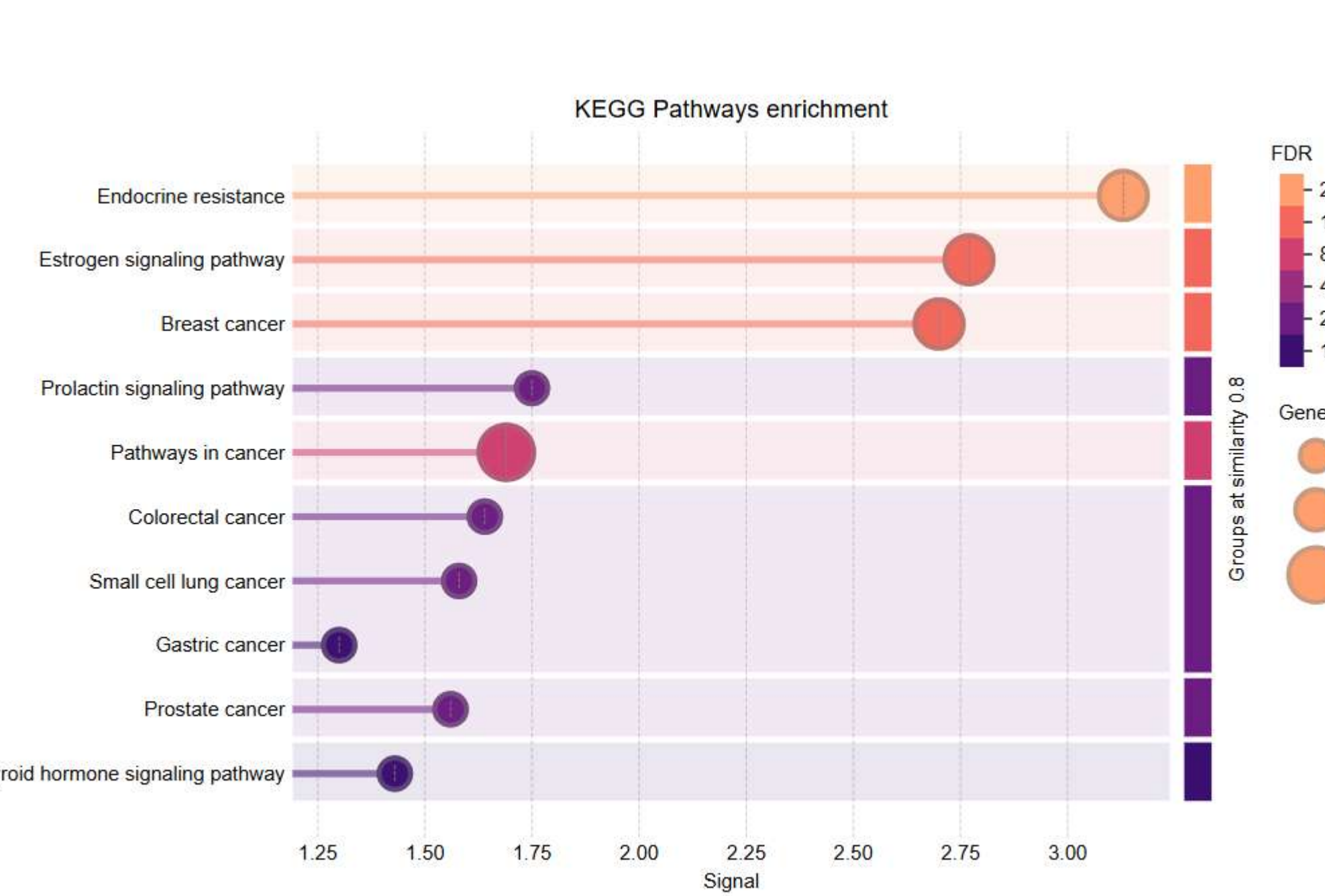
PPI Network Of breast Cancer Associated Genes



SWISS Target Prediction



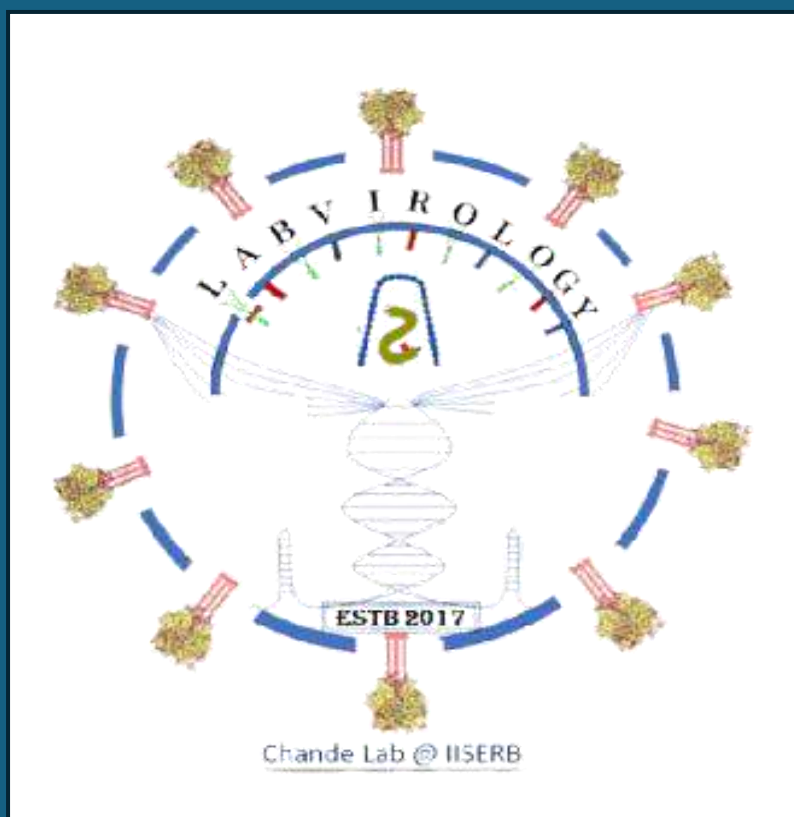
Therapeutic Translation: Vaccine Design Approach



Conclusion :Cyhalothrin showed strong binding affinity with ER- α (–8.6), indicating potent endocrine-disrupting potential.

Toxicogenomic analysis revealed dysregulation of key breast cancer–related genes involved in cell cycle, oxidative stress, and inflammation.

Identified molecular targets provide a foundation for **vaccine-based and cell-based therapeutic strategies** against pesticide-induced breast carcinogenesis.



Lentiviral Vectors derived from T/F HIV-1 for gene delivery



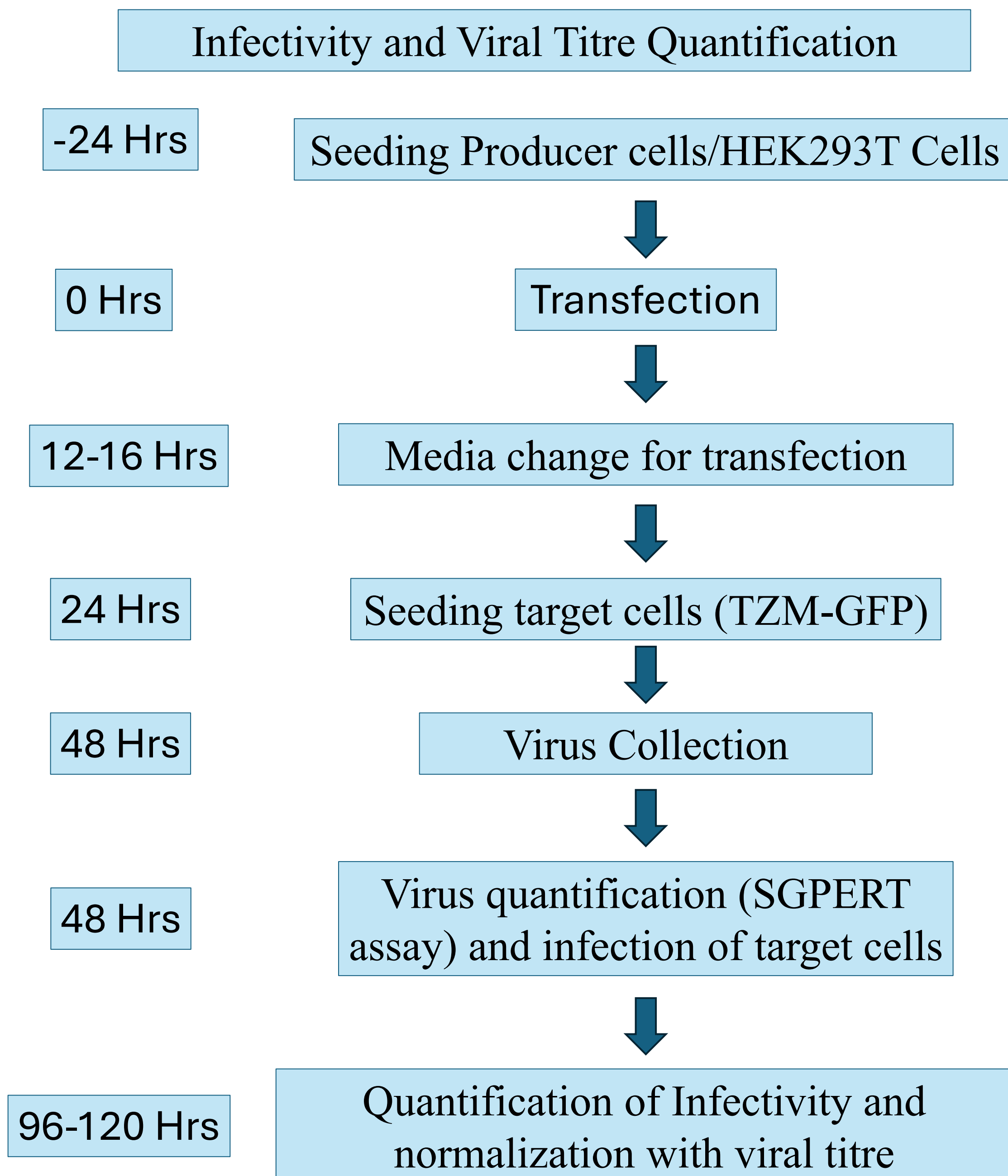
Kaushik Muralidharan, Rishikesh Dalavi, Ajit Chande

Laboratory of Molecular Virology and Genome Editing, IISER Bhopal

INTRODUCTION

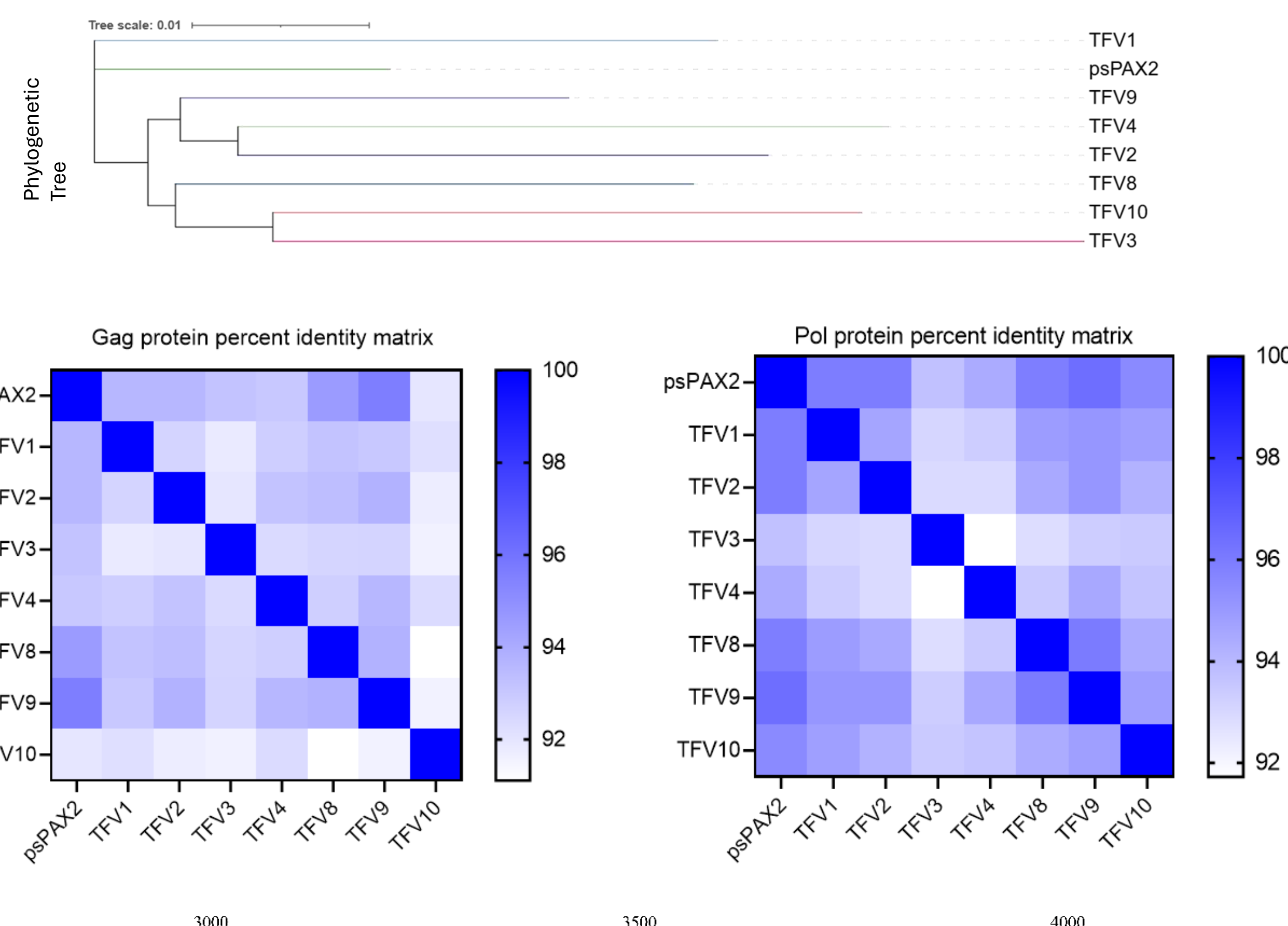
Cell and gene therapies as corrective clinical tools have gained a lot of traction in recent years. Since 2017, many such therapies have been FDA approved, largely due to the development of viral vector-based gene delivery, which exhibit tissue specific tropism and versatility across diseases. Gene therapy via lentiviral vectors is attractive because of their ability to stably transduce non-dividing cells. This enables targeting of a broad range of cell types and long-term stable expression of therapeutics. However, the treatment may induce an immune reaction within the host. To make these therapies less immunogenic, generation and optimization of novel lentiviral vectors is required. Our work focuses on generating third-generation lentiviral vectors. By utilizing the molecular clones of Transmitted/Founder (T/F) HIV-1, we generated a panel of packaging vectors to identify candidates for further rational engineering. By obtaining insights into the protein sequence and function of these vector candidates, we strive to devise a vector suitable for efficient gene delivery applications.

METHODOLOGY

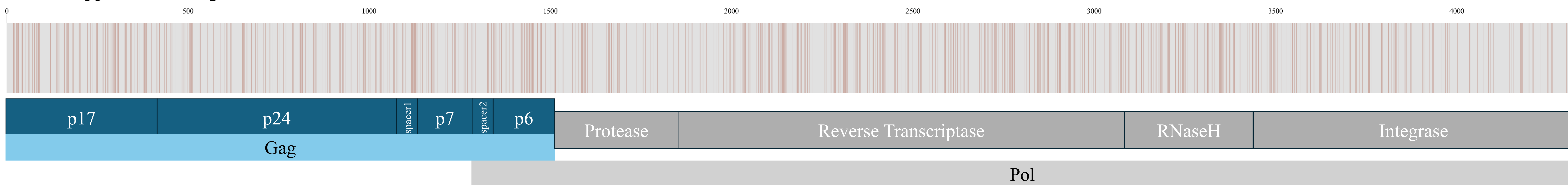


SEQUENCE ANALYSIS

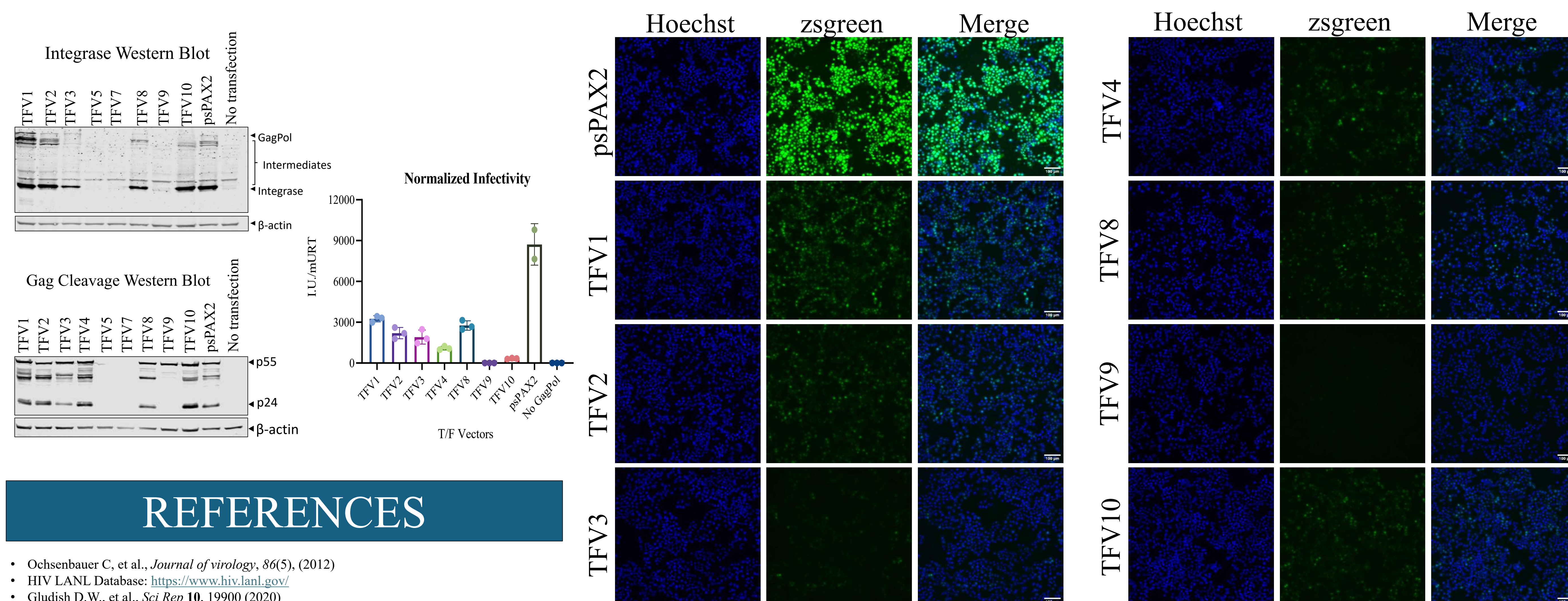
- Sequences were analysed by Multiple Sequence Alignment at the DNA and protein level using Clustal Omega to check SNP's and conserved sequences.
- To visualize the closeness of viral sequences, a phylogenetic tree was built using HIV-LANL Database tools (FindModel and IQ-TREE).



SNP's mapped across GagPol



RESULTS



REFERENCES

- Ochsenbauer C, et al., *Journal of virology*, 86(5), (2012)
- HIV LANL Database: <https://www.hiv.lanl.gov/>
- Gludish D.W., et al., *Sci Rep* 10, 19900 (2020)
- Pizzato, Massimo, et al., *Journal of virological methods*, 156(1-2) (2009)

CONCLUSION

- Based on this data, we observe that T/F derived vectors show a fraction of the infectivity of psPAX2 lentiviral vector.
- This study served as an initial comparison between the infectivity of T/F virus derived vectors and psPAX2 based on infectivity data from T2M-gfp reporter cells.
- Further experiments are underway to enhance the infectivity by rational mutagenesis backed by insights from sequence and structure-based data.

ACKNOWLEDGEMENT



This research was supported by Indian Institution of Science Education and Research (IISER) Bhopal and Indian Council of Medical Research (ICMR).

Optimization of a High Titer 3rd Generation Lentivirus Production Platform for Cell and Gene Therapy Applications

Umasish Mohanty^{1,2}, Pruthviraj Mahadeo Chavan¹, Sri Prajna Mula¹, Debasis Nayak ¹
1 Indian Institute of Science Education and Research Bhopal (IISERB), Madhya Pradesh, India
2 Cytoforge Therapeutics Pvt. Ltd., Bhopal, Madhya Pradesh, India

A. Background & Aim

Advances in the field of cell and gene therapy has caused diversification of different types of vectors with distinct capabilities for generation of novel biotherapeutics. But viral vectors still remains the major tool for genetic modification because of their efficient gene delivering capabilities. The broad aspect of these engineering marvels is to directly or indirectly support the development of therapeutic modalities for not only oncological conditions but also for orphan diseases and rare genetic disorders. Towards this, development of a cost-effective approach for production of these vectors is a crucial element which needs immediate addressing. Lentiviruses (LV), the vector standard workhorses of CAR based cell therapy development, are among a key cost driver—typically comprising 20–30% of total drug product costs. This substantial cost underscores the importance of LV in facilitating these therapies and the pressing demand for creative, lower-cost approaches. We have developed a novel cost-effective approach for generation of high titer LV production at par with the industrial standards. The goal is to develop a potent, scalable and cost-effective LV vector particles (LVVP) manufacturing process for easy translation into cGMP settings for clinical grade production.

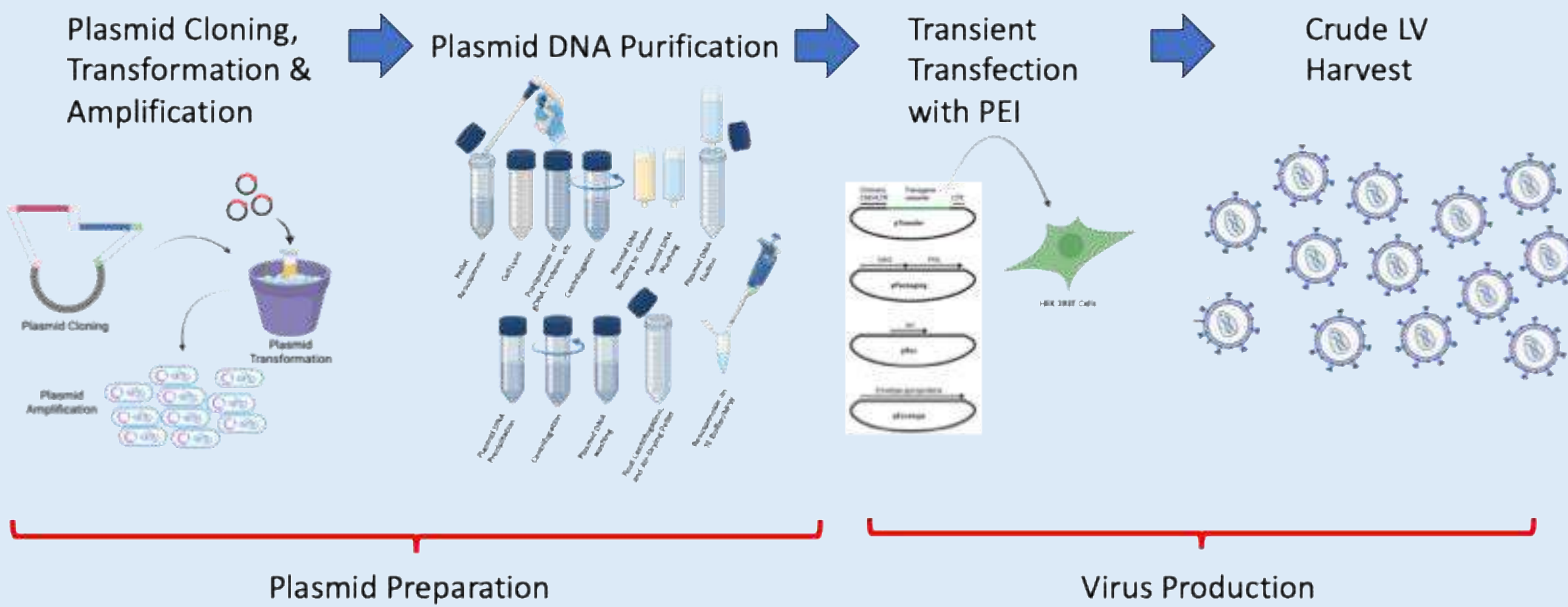
B. Materials & Methods

Materials used:

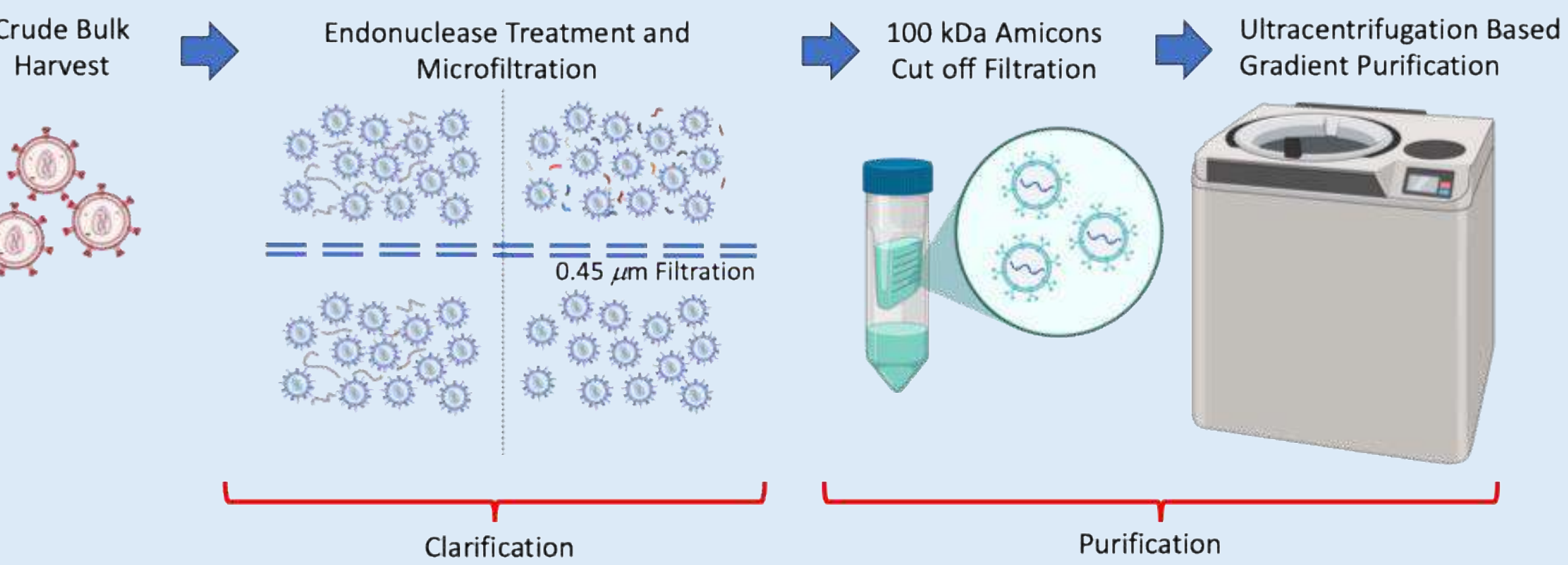
Instruments	Consumables (Plasticwares)	Consumables (Media & Chemicals)	Cell Lines/ Cells
Biosafety Cabinet	Cell culture plates and flasks	DMEM	HEK 293T
Cell Culture Shaker incubator	Serological Pipette	OptiMEM	HEK293T (Serum free suspension adapted)
AKTA FPLC	100 KDa cutoff filters	FBS	BHK21
Ultra Centrifuge	Ultra centrifugation tubes	PBS	CHO
Hemocytometer	MCTs	Pluronic F68	SKN-SH-SY-SY
qRT PCR	Conical bottom tubes	PEI MAX	VERO
Flow Cytometer		Sheath Fluid	mHSCs (Primary cells)

The 3rd generation LVVP was produced using a proprietary vector platform by transient transfection of the 4 different plasmids (Transfer plasmid, 2 helper plasmids and 1 envelop plasmid) using polyethylenimine (PEI) at an optimized ratio. The downstream purification process involved benzonase treatment of the harvested LVVP, followed by 0.45µm filtration, concentration using 100 KDa cutoff filter followed by ultracentrifugation by sucrose gradient cushioning. The purified LVVP was transduced in different cell lines and transduction efficiency was noted for each.

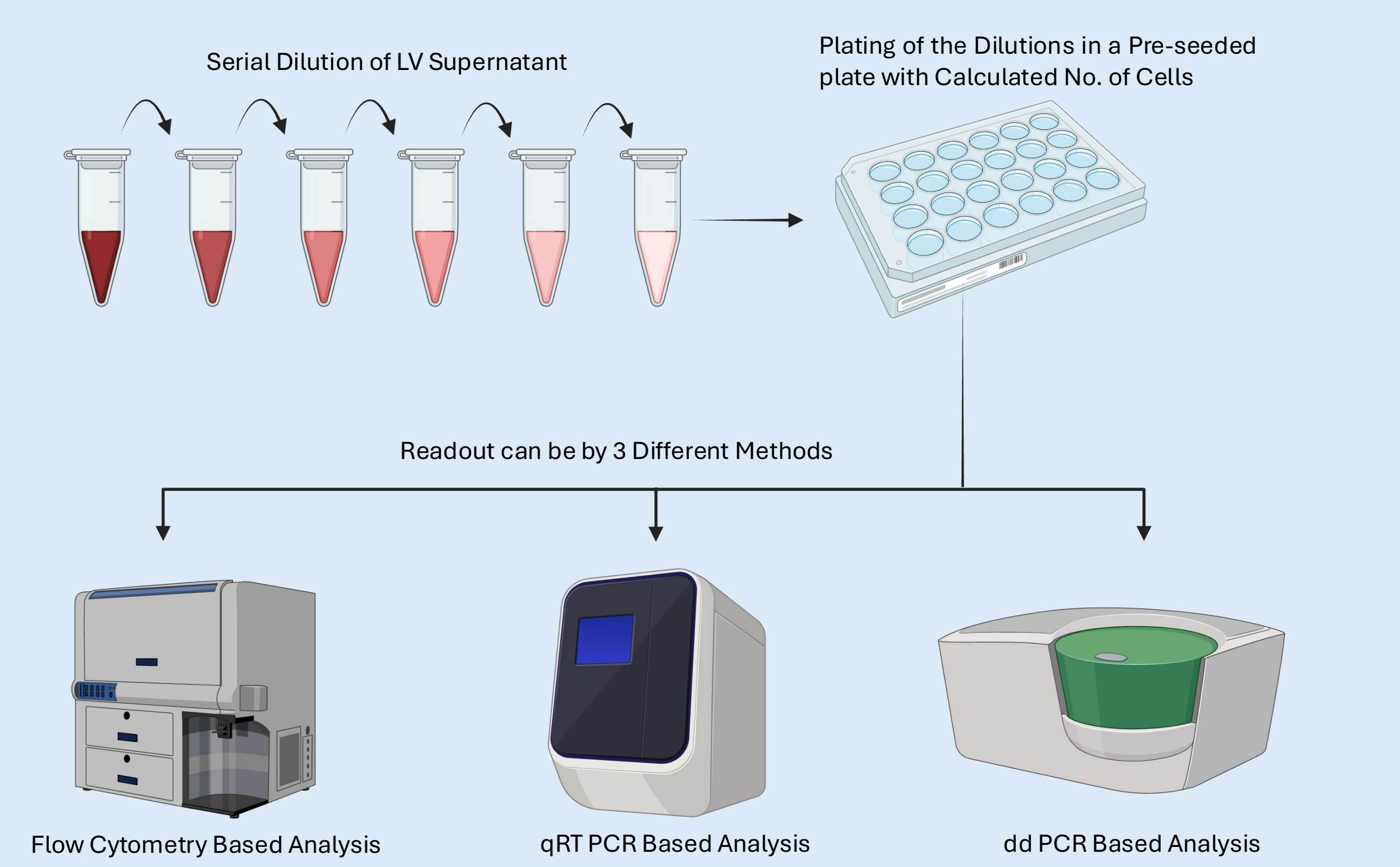
Optimizing Upstream Workflows for High-Yield Lentiviral Vector



Downstream Processing of LV Vectors for Ensuring Minimal Viral Titer Loss in Each Step



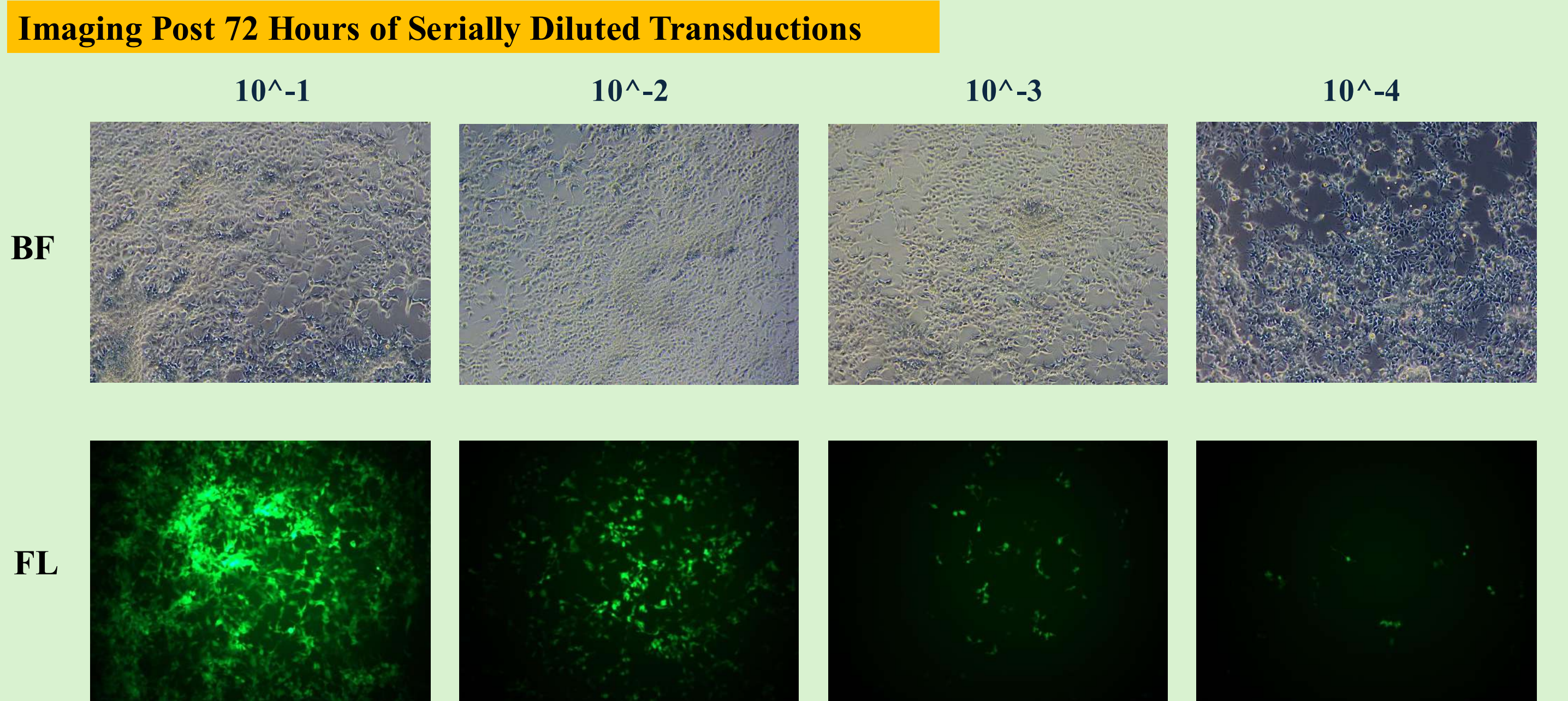
Functional Titer Assay Workflow



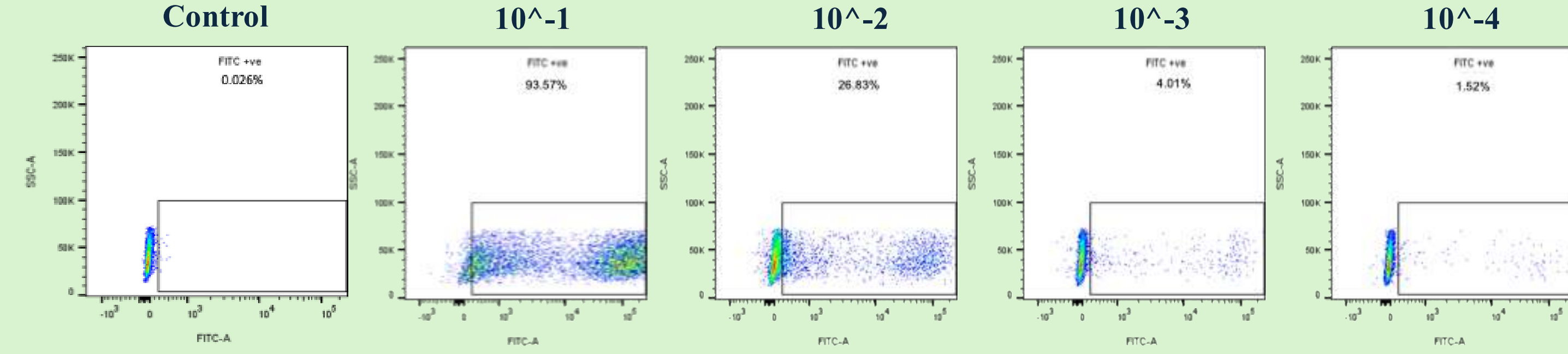
C. Results:

The lentiviral plasmids plenti_EGFP, pGAGPOL, pREV and pVSVG were transfected in the ratios given below and the titer was calculated using flowcytometric analysis.

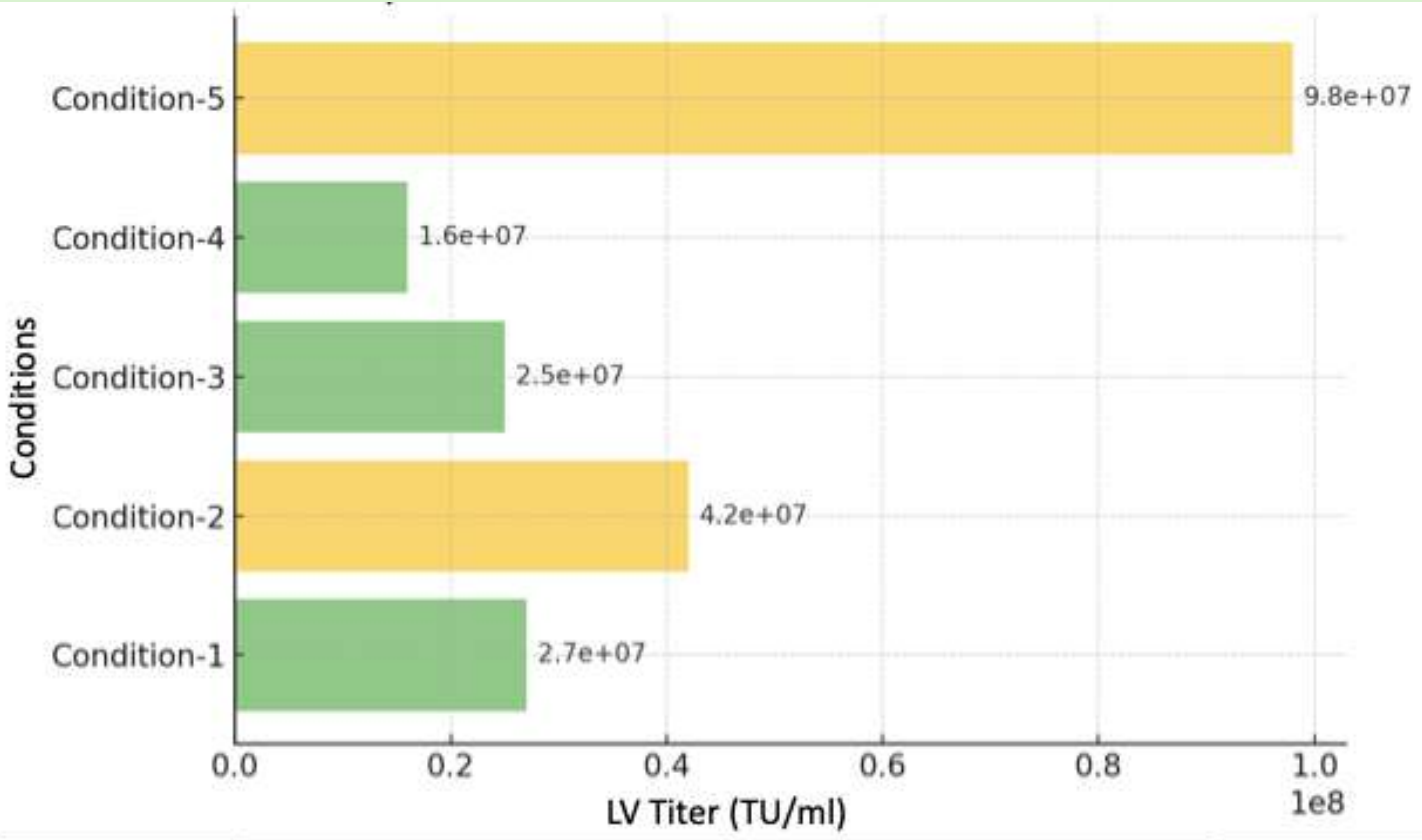
Plasmid Ratios	5:3:1:1	7:3:2:1	4:2:1:0.5	
DNA:PEI	1:3	1:5	1:7	
DNA (For 100mm Plate)	20 µg	25 µg	30 µg	35 µg



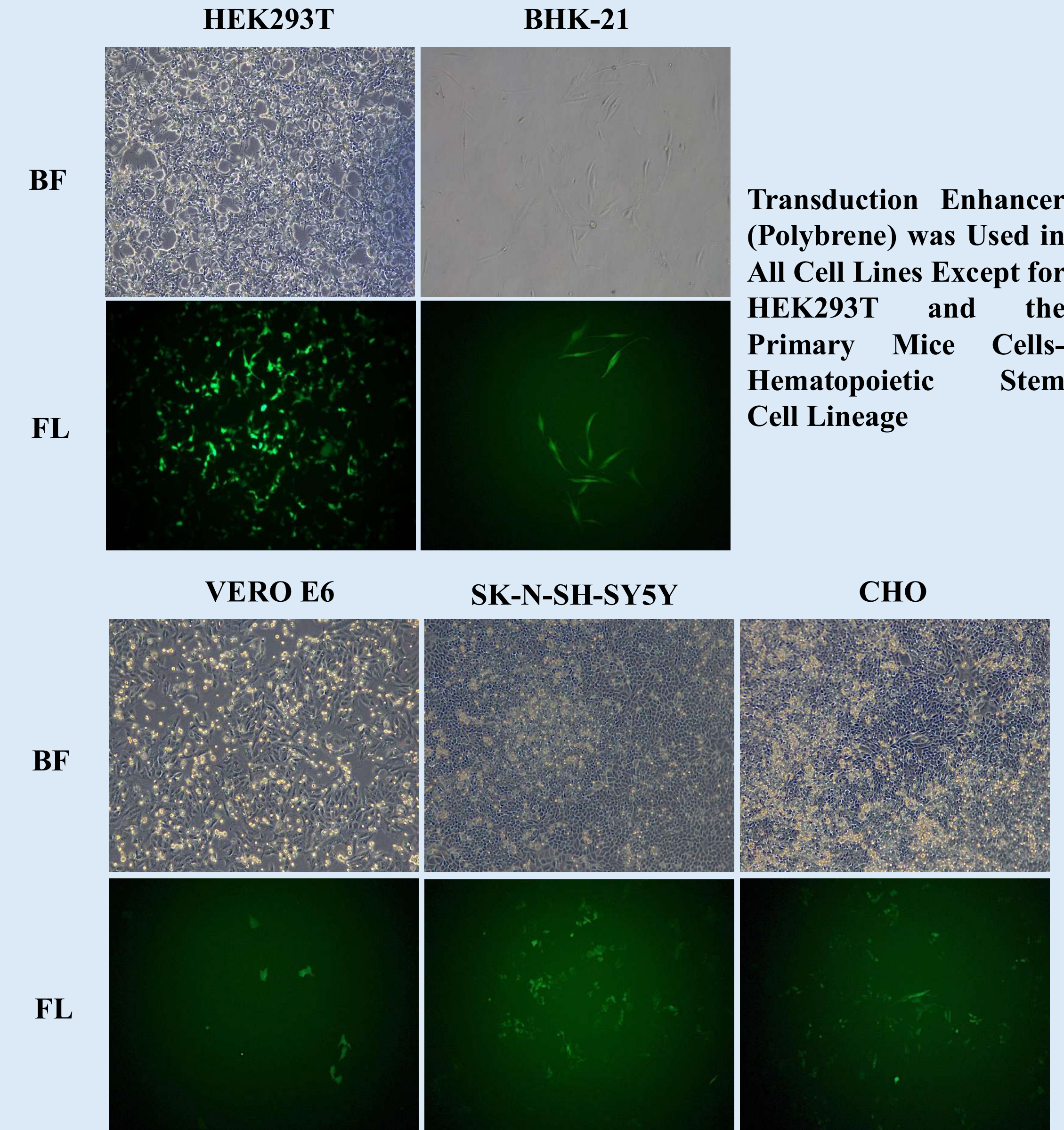
Flow Cytometric Analyses of the Serially Diluted Transductions



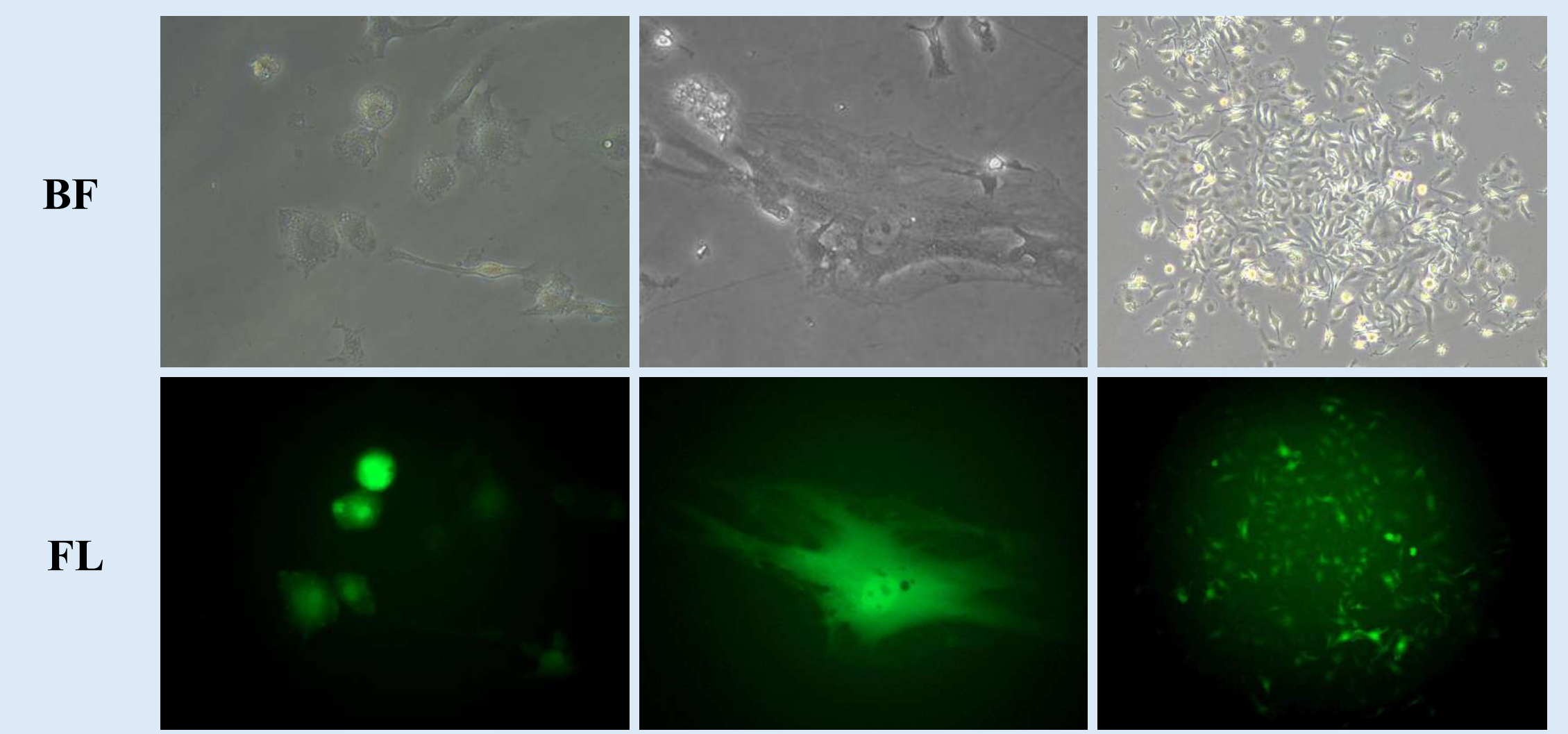
Comparison of LV Titers Across Different Plasmid Ratios, DNA to PEI Ratios & Amount of DNA per 1 Million Cells



Different Cell Lines Transduced at 2.5 MOI for Checking the Transduction Efficiency

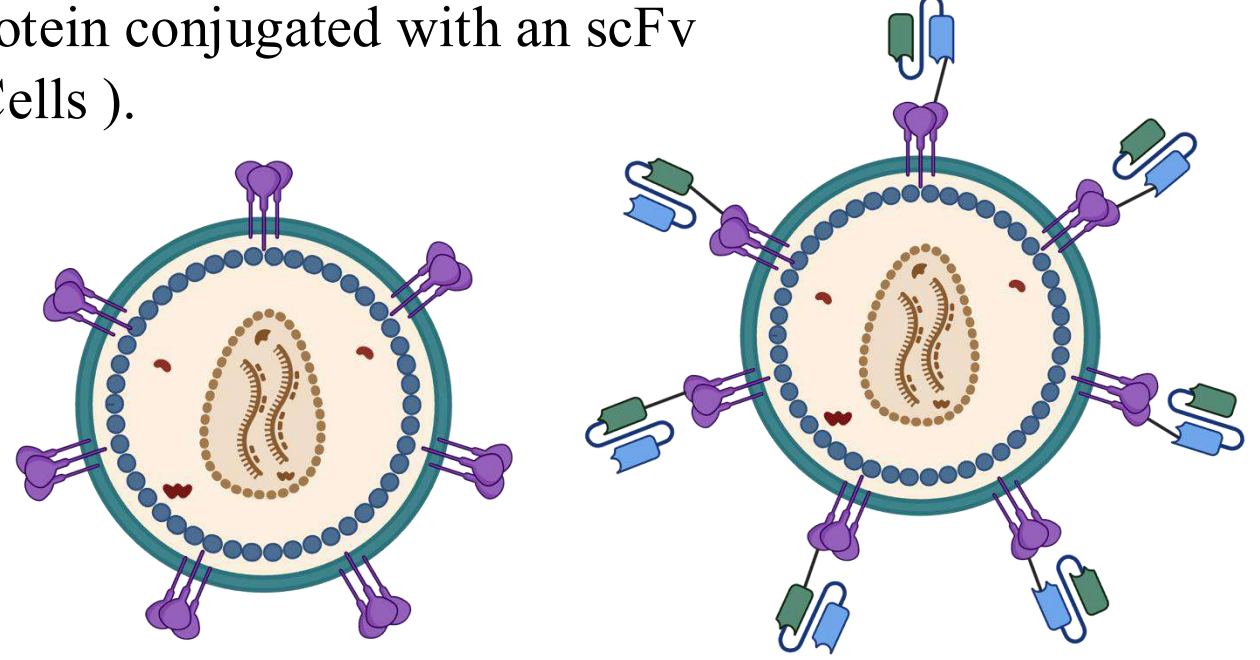


Mice Hematopoietic Stem Cells Transduced at MOI=5 after 12 Days of Culture



D. Discussion:

A high titer LV platform was established using adherent culture system but experiments are ongoing to establish a serum free suspension adapted producer cell line for a more higher titer LV production as well as establishment of an up scalable platform which can be used in bioreactor scales. Moreover, for making cell and gene therapies, cost effective, there is an urgent need for development of *in-vivo* CAR generation for which we are trying to pseudotype LV with a safe non targeting viral glycoprotein conjugated with an scFv for cell specific targeting (T-cells or NK Cells). Further research also includes generating LVs with reduced phagocytosis by macrophages for efficient gene transfer.



E. Conclusion:

The optimized protocol yielded a high titer LVVP in the crude harvest itself. Moreover, along with the downstream purification strategies used to obtain a highly pure LVVP with minimal loss in all the processes exemplifies a cost-effective approach. The particles being highly potent which is demonstrated by its transducing capabilities of different cell lines including primary cells, without the use of any transduction enhancer makes it a robust system for cell and gene therapy applications.

F. References:

- Klimpel, Maximilian, et al. "Generation of stable suspension producer cell lines for serum-free lentivirus production." *Biotechnology Journal* 19.5 (2024): 2400090.
- Valkama, Annina J., et al. "Development of large-scale downstream processing for lentiviral vectors." *Molecular Therapy Methods & Clinical Development* 17 (2020): 717-730.

G. Acknowledgement:

ABCC6-Deficient iPSC-Derived Liver Organoids as a Platform for Metabolic and Therapeutic Investigation in Pseudoxanthoma Elasticum



Devika N S, Aiswarya S , Bismi Basheer, Gayathri Vijayakumar, Vidhya J , Indulekha Pillai
School of Biotechnology, Amrita Viswa Vidyapeetham, Kollam, Kerala

INTRODUCTION

Pseudoxanthoma Elasticum (PXE) is a rare genetic disorder caused by loss of functional ABCC6, leading to symptoms such as angioid streaks, vascular problems, and yellowish skin papules. Although ABCC6 is mainly expressed in the liver and kidney, the disease affects tissues that lack its expression. Currently, there is no specific treatment, and suitable human models are limited; therefore, disease modeling is crucial for uncovering mechanisms and developing better therapies.

METHODOLOGY

iPSCs are knocked out using CRISPR-Cas9 technology

Gene knock-out confirmed using qRT-PCR

iPSCs differentiated to specific hepatic lineages and confirmed by hepatic-specific markers by qRT-PCR.

Metabolic changes in the liver cells are analysed using untargeted metabolomics

RESULTS

qRT-PCR using ABCC6-specific primers confirmed the successful gene knock-out in iPSCs. The differentiated hepatic lineages express different markers at various stages. Since ABCC6 is said to be a metabolic disease, the untargeted metabolomics of KO revealed a significant upregulation of Kynurenic acid and Hippuric acid. This data suggests that the metabolic changes seen in PXE may be caused by an integrated role of Hippuric and kynurenic acid-mediated metabolic shifts.

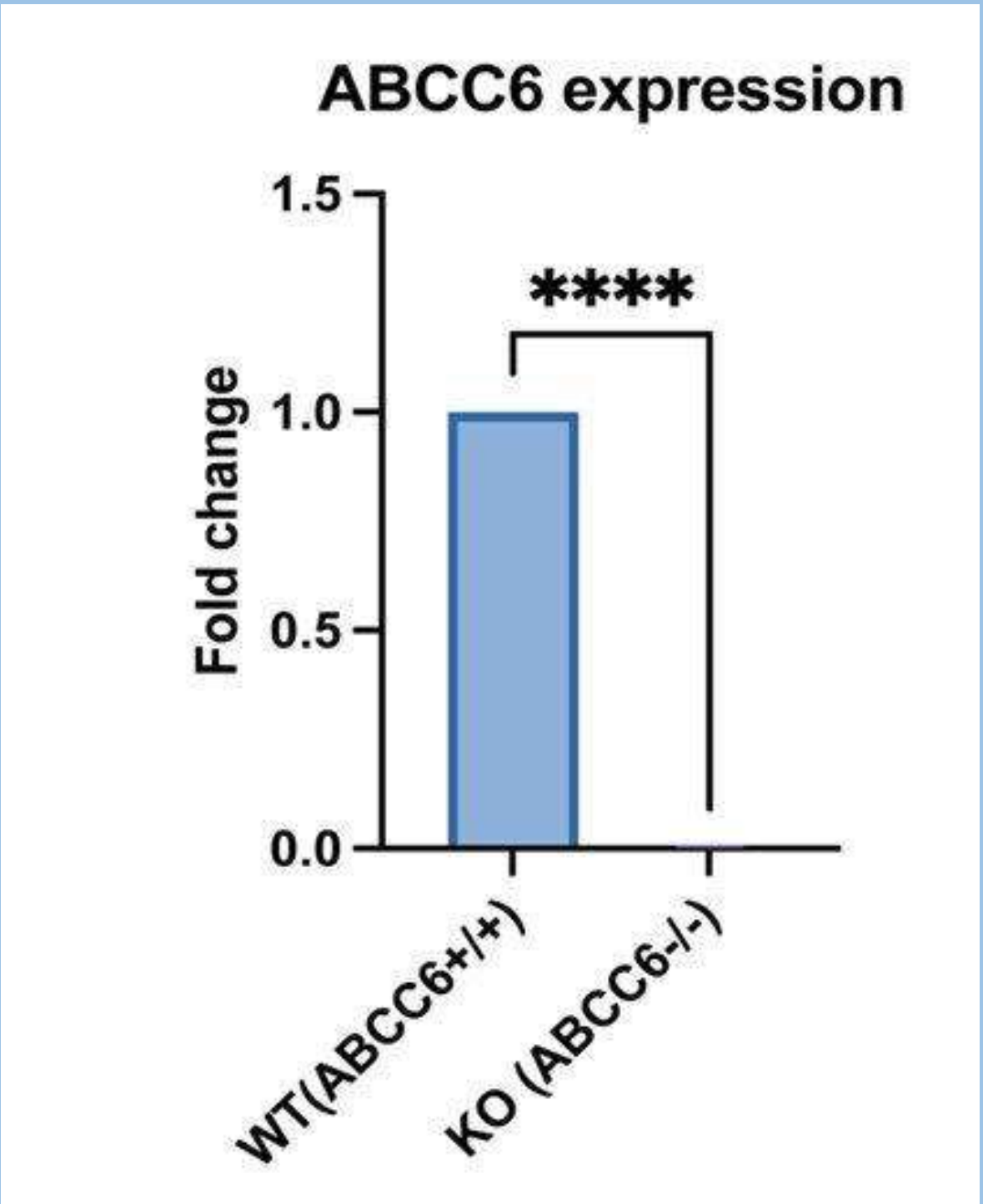


Figure 1: Knockout confirmed by qRT-PCR

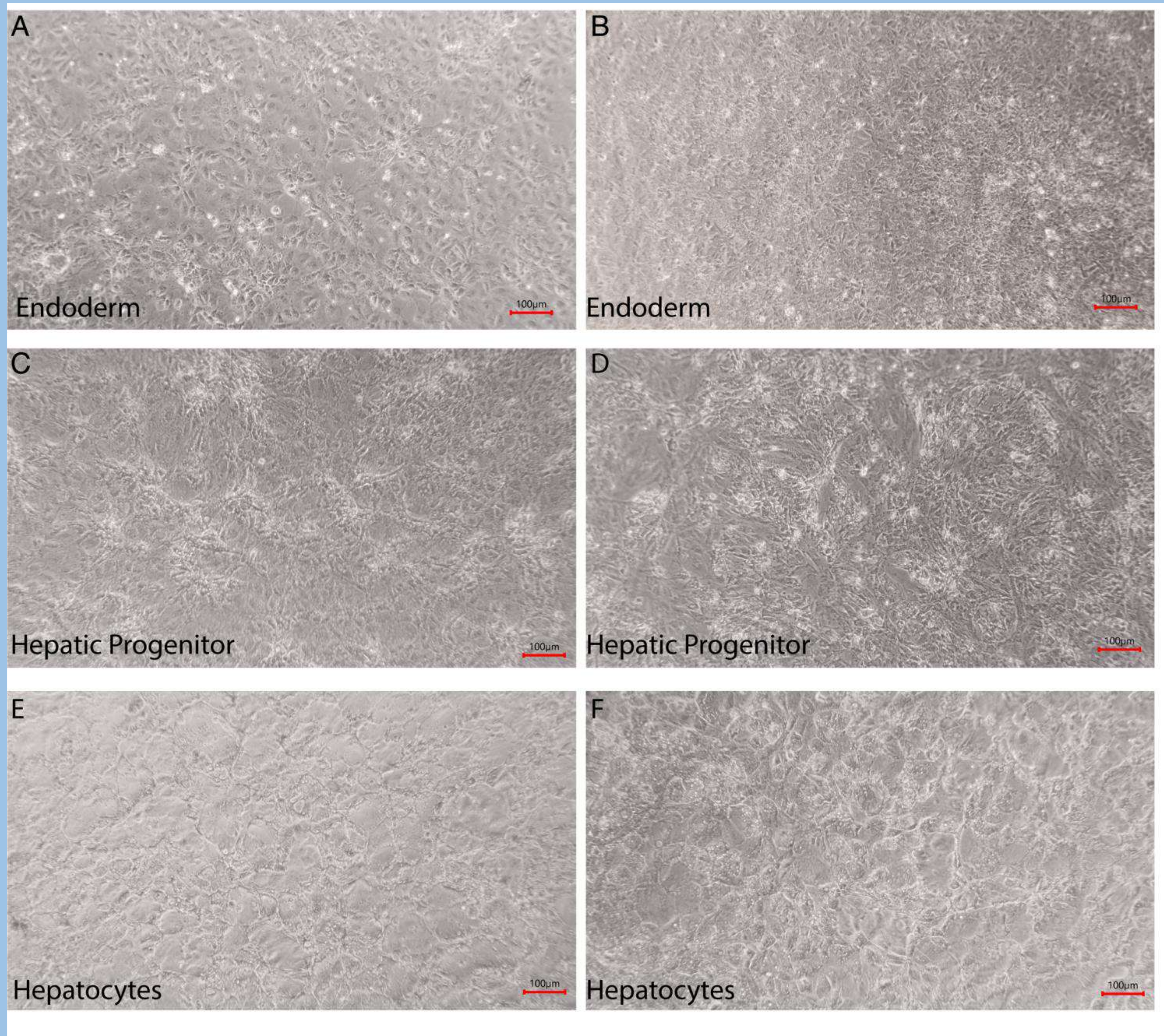


Figure 2: Both wild-type and knockout hiPSCs differentiated into different hepatic lineages

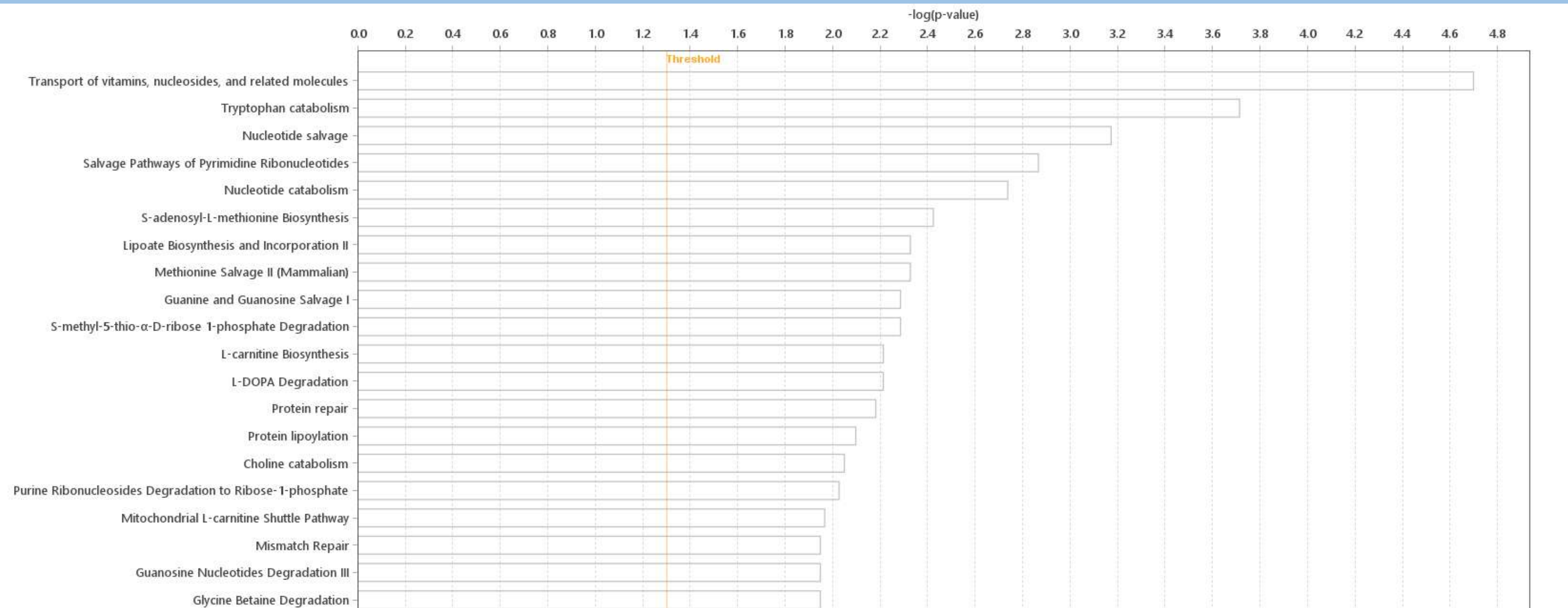


Figure 3: IPA Analysis data

CONCLUSION AND FUTURE PERSPECTIVE

Understanding the molecular mechanisms in Pseudoxanthoma elasticum provides new therapeutic targets and insights relevant to PXE, GACI, and chronic kidney disease. Building on this, ongoing targeted metabolomics and gain-of-function studies in ABCC6 KO liver organoids aim to confirm the role of kynurenic acid and Wnt/ β -catenin signaling in disease progression.

REFERENCES

- Jansen, Robert S., et al. “ABCC6 prevents ectopic mineralization seen in pseudoxanthoma elasticum by inducing cellular nucleotide release.” *Proceedings of the National Academy of Sciences* 110.50 (2013): 20206-20211.
- Plümers, Ricarda, et al. “Investigation on ABCC6-Deficient Human Hepatocytes Generated by CRISPR–Cas9 Genome Editing.” *Cells* 14.8 (2025): 576.

ACKNOWLEDGEMENT

We are immensely grateful for the funding provided by Agilent Technologies. We also thank the Amrita School of Biotechnology for the constant support and infrastructure provided.



In-silico screening of Target Genes in Gastric cancer and identification of Probiotic Therapeutics

Archana.R and S.Sumathi

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Coimbatore-641043, TN, India
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ABSTRACT

Gastric cancer is the fourth most prevalent and fifth most common cause of mortality globally, with over half of the population infected with *Helicobacter pylori* infection. In India, high rates of gastric infection increase the risk of enteric gastroenterology disease. Probiotic have emerged as potential agent in adjuvant therapy in managing GC associated with *H.pylori* infection. An *in-silico* approach was used to identify key genes - ATP4A, CUX2, CXCL8, CHIA, ESRRG, GPER1, and PTGER3 and *H.pylori* growth limiting probiotic candidates *Streptococcus thermophilus*, *Streptococcus bulgaris*, *Lactobacillus plantarum*, and *Lactobacillus rhamonosasus* for managing *H.pylori*-associated gastric cancer, which will be confirmed by *in-vitro* studies.

OBJECTIVE

- To identify prognostic genes for *H.pylori* associated gastric cancer.
- To demonstrate interaction between selected probiotic and pathogen *H.pylori* by analyzing *H.pylori* growth limiting property of probiotic species.
- To study the docking interaction between CXCL8, CUX2 genes and *Streptococcus thermophilus*, *Streptococcus bulgaris*, *Lactobacillus plantarum* and *Lactobacillus rhamonosasus* that might contribute as adjuvant therapeutic to progression of *H.pylori* associated gastric cancer.

INTRODUCTION

- Probiotic are live, beneficial microorganism that are administered to improve overall health.
- In particular it is found useful to help improve the *H.pylori* gastric cancer adjuvant therapy.
- Considering *H.pylori*, a class I carcinogen classified by WHO, is associated with severe gastrointestinal diseases and chronic inflammation, probiotics are being researched as an adjuvant therapy to increase the effectiveness of *H. pylori* eradication.
- To identify specific probiotic strains that are most effective against various *H. pylori* strains.
- H. pylori* cannot adhere to the stomach lining when probiotics occupy binding sites on the gastric epithelium. By lowering the bacterial load, this competitive inhibition supports eradication attempts.

METHODS

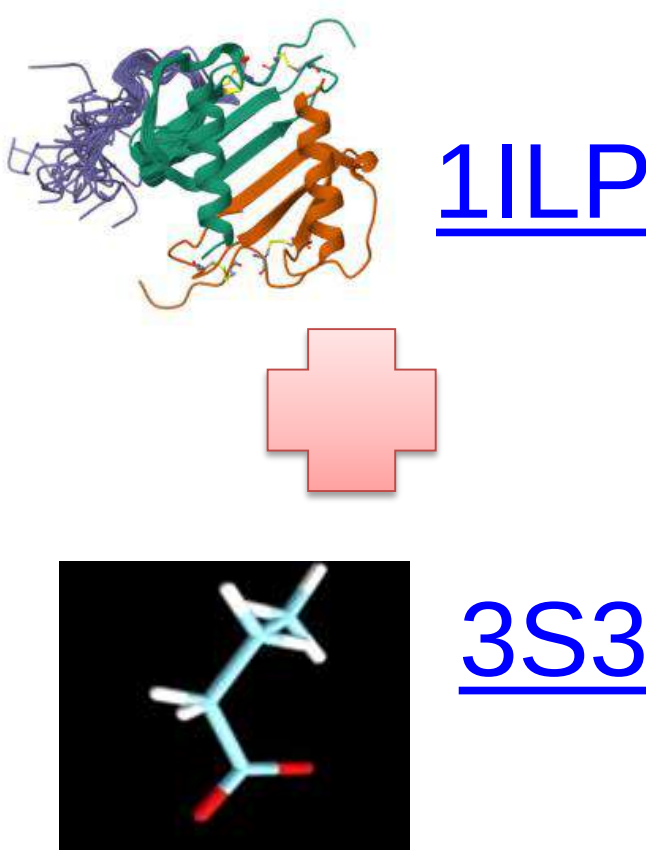
COLLECT AND IDENTIFY THE TARGETS OF H.PYLORI ASSOCIATED GASTRIC CANCER

GENOME SCALE METABOLIC MODEL – USING MODEL SEED AND VISUALISED THE MODEL USING VANTED TOOL

MOLECULAR DOCKING OF KEY GENE RECEPTOR AND PROBIOTIC LIGAND

KEY GENES

GENOME SCALE METABOLIC MODEL – USING MODEL SEED AND VISUALISED THE MODEL USING VANTED TOOL



RESULTS

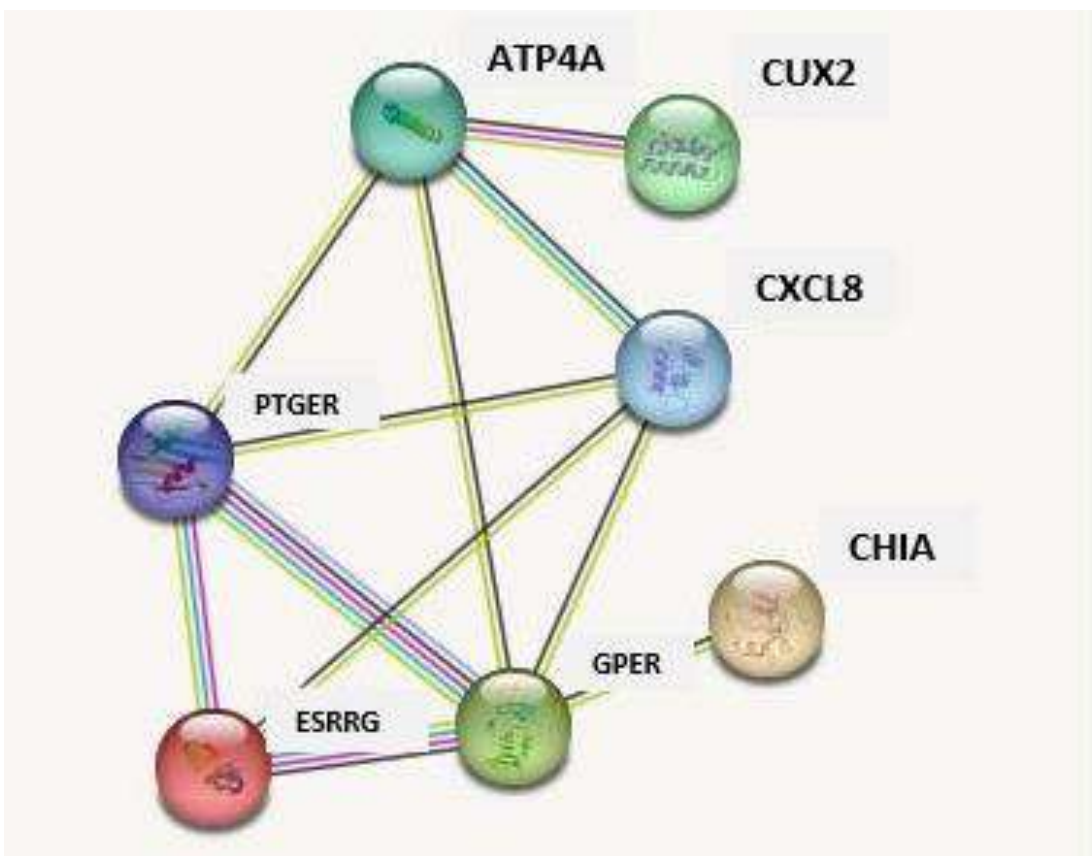


Fig: 1 INTERACTION ANALYSIS OF UPREGULATED GENE

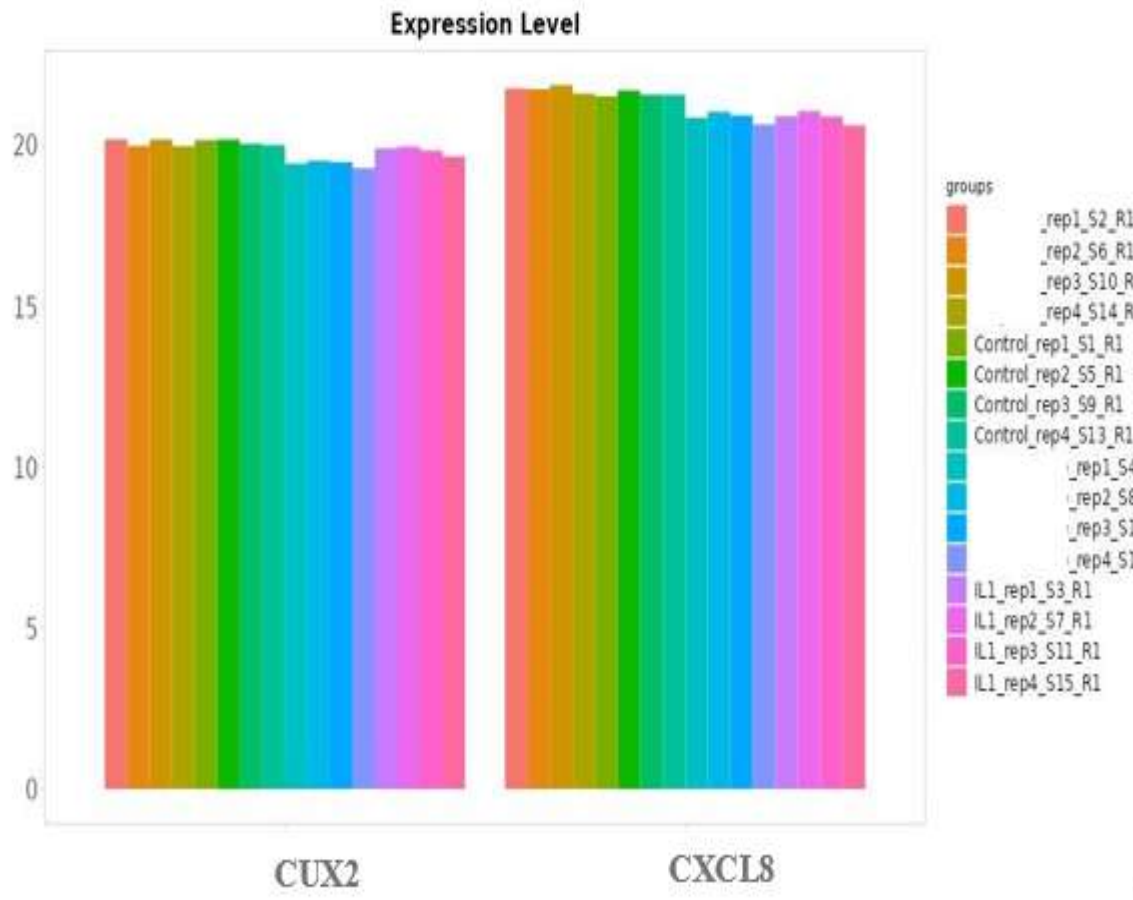


Fig: 2 BAR PLOT SHOWING DIFFERENTIAL GENE EXPRESSION

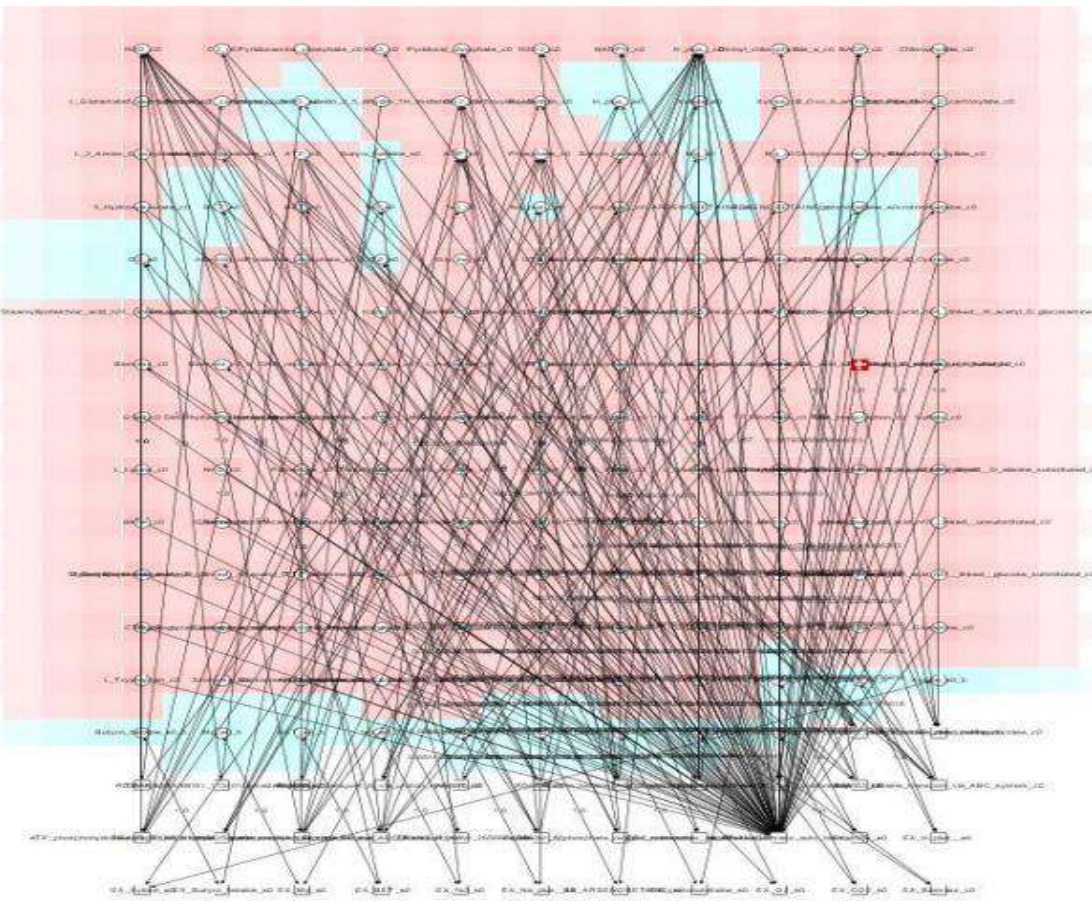


Fig: 3a GENOMIC MODEL OF *HELICOBACTER PYLORI*

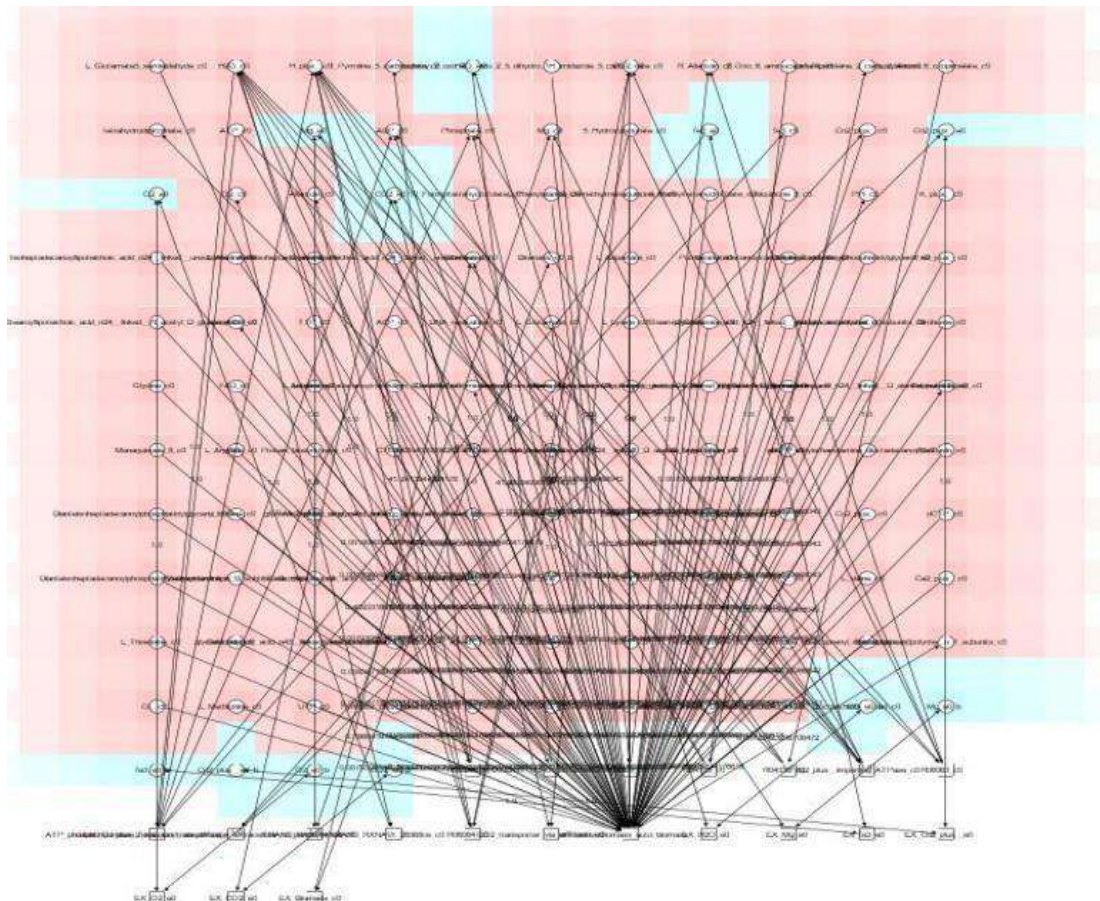


Fig: 3b GENOMIC MODEL OF *LACTOBACILLUS PLANTARUM*

smetana medium smetana score...species1.xml	
Arb	0.000
Man	0.002
Lb	0.000

smetana medium smetana score...species2.xml	
Arb	0.88
Man	0.92
Lb	1.00

Fig: 4 SMETANA TOOL OUTPUT SHOWING THE SCORES

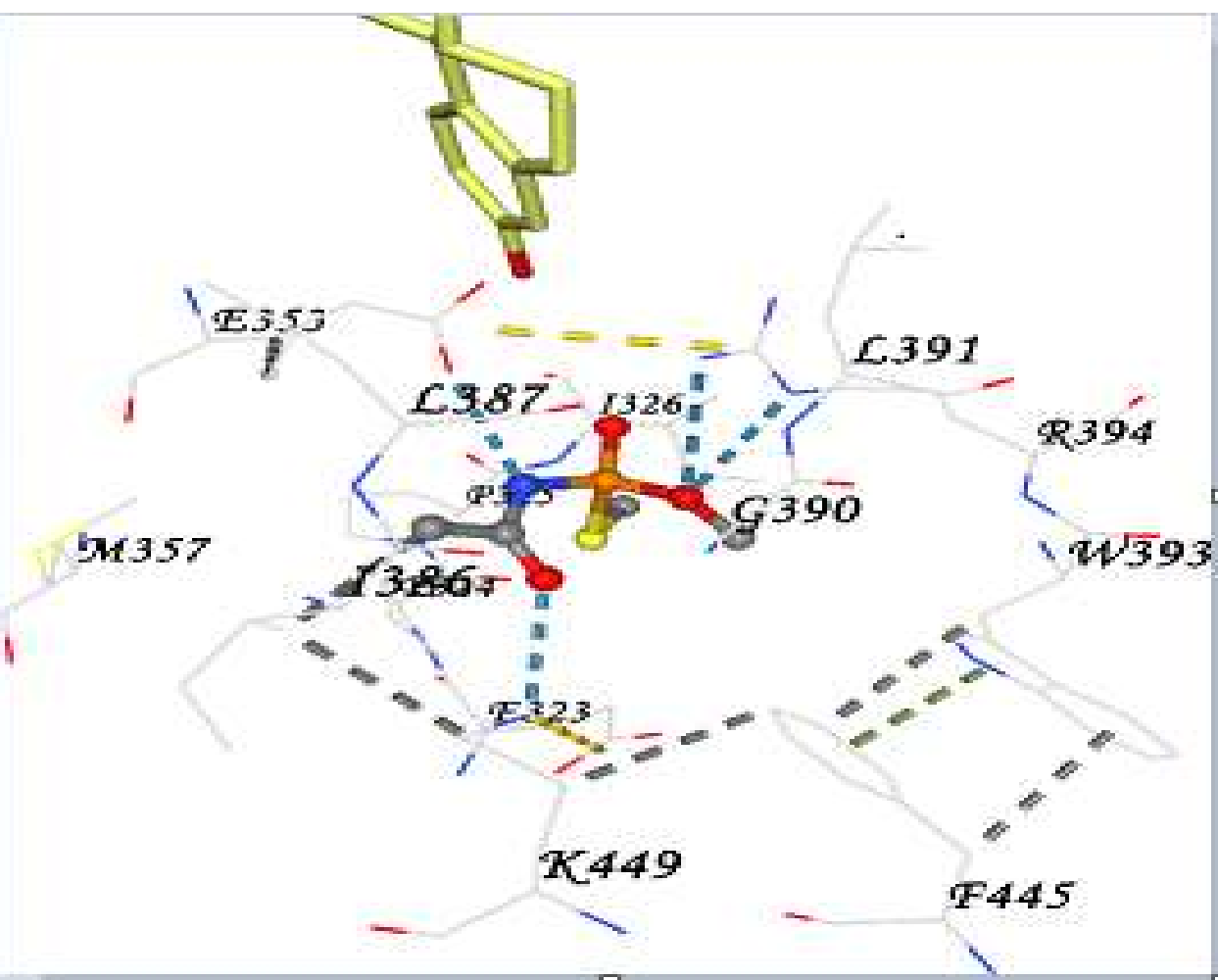


Fig: 5 CXCL8 GENE RECEPTOR DOCKED PROTEIN FROM *LACTOBACILLUS PLANTARUM*

Differential Expression Analysis: Using DESeq2, CUX2 and CXCL8 were identified as key genes overexpressed in *H. pylori*-associated gastric cancer. CXCL8, known for promoting inflammation and angiogenesis, was significantly up regulated. **STRING Network Analysis:** Seven genes closely linked to CXCL8 and CUX2 pathways were identified, with strong associations with inflammatory signalling and gastric cancer pathways. **Metabolic Modelling:** Comparative metabolic models of *H. pylori* and *Lactobacillus plantarum* revealed metabolic competition, particularly in pathways influencing lactic acid and ammonia production. This suggests the potential for probiotics to suppress *H. pylori* growth through metabolic interference. **Docking Analysis:** Molecular docking of CXCL8's overexpressed receptor with metabolites produced by *Lactobacillus plantarum* demonstrated high binding affinities that infers specific probiotic metabolite modulate inflammatory pathways and reduce gastric carcinogenic signalling.

CONCLUSION

Our study could help to bridge a gap between *H. pylori*-associated gastric cancer progression and the therapeutic potential of probiotic *Lactobacillus plantarum*. By integrating gene expression analysis, metabolic modeling, and docking studies combined approach targeting CXCL8-mediated pathways and metabolic interactions with *Lactobacillus plantarum* serve as a complementary strategy to inhibit tumor-promoting mechanisms of *H. pylori*-associated gastric cancer progression.

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Council of Scientific & Industrial Research (CSIR), GOI.

Title: iPSC Based Disease Modelling and Therapeutic Development for Diamond-Blackfan Anemia

Authors: Riswan N, Dr. Dinesh Babu, Dr. RV Shaji

Diamond-Blackfan Anemia (DBA) is a rare congenital bone marrow failure syndrome caused primarily by heterozygous mutations in ribosomal protein (RP) genes, with RPS19 being the most frequently affected. These mutations impair ribosome biogenesis, activate stress pathways such as p53, and result in erythroid failure. While patient-derived iPSCs have advanced our understanding of DBA, their use is limited by the scarcity of samples, variability in genetic backgrounds, and heterogeneity in disease severity. To overcome these challenges, we established isogenic iPSC-based models using a dual strategy of inducible complementation and precise genome editing. Codon-optimized RP transgenes were integrated into the AAVS1 safe-harbor locus under doxycycline (Dox)-inducible control, allowing exogenous RP expression during editing. Adenine base editors (ABE8e) were employed to introduce splice-site mutations into endogenous RP genes, which otherwise caused strong negative selection and loss of edited alleles in the absence of complementation. A dual-fluorescent allele-tagging system (eGFP/dTomato) enabled bulk isolation of monoallelic and biallelic haploinsufficient populations without the need for single-cell cloning. Functional assays confirmed that RP haploinsufficiency led to apoptosis and defective erythroid differentiation, while Dox complementation restored viability and maturation. This standardized, scalable platform faithfully models DBA pathophysiology and provides a robust resource for mechanistic studies and high-throughput therapeutic discovery.

Engineering Hydrogels for Hematopoietic Stem Cells Expansion: Current Progress and Future Directions

Pavitrareeti V, Harsh Agrawal, Satish Khurana*

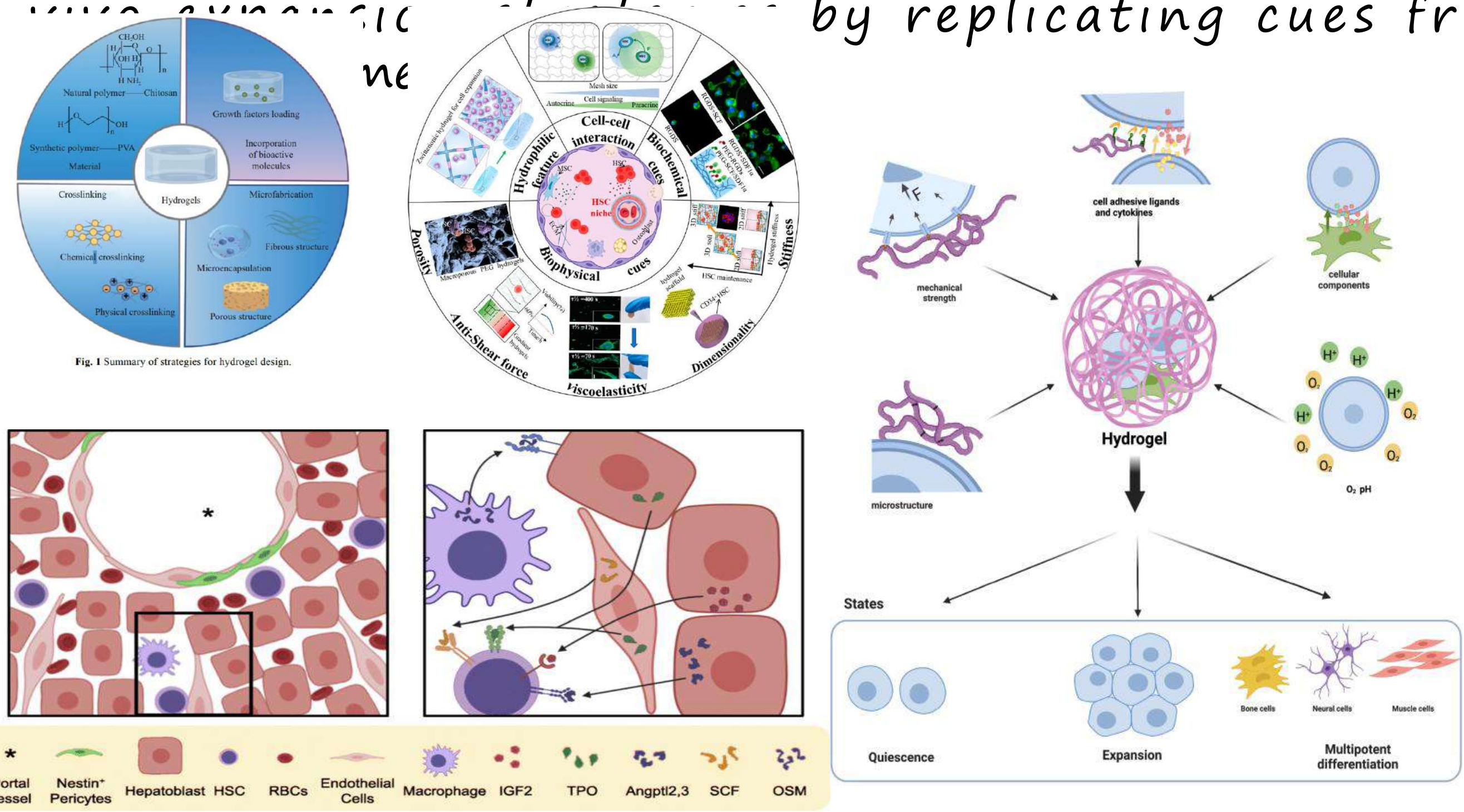
School of Biology, Indian Institute of Science Education and Research, Thiruvananthapuram, 695551, Kerala, India

*corresponding author



Introduction

Hematopoietic stem cells (HSCs) are foundational for lifelong blood and immune system renewal, with transplantation serving as a treatment for various hematological conditions. However, the limited availability of transplantable HSCs is a critical challenge. This poster offers an overview of recent advances in engineering hydrogels to mimic the natural HSC niche, aiming to improve ex vivo expansion by replicating cues from the fetal liver



Objective

Development of biomimetic hydrogels with fetal liver-like components to assess the growth and expansion of hematopoietic stem cells

Materials and Methods

Cell Sources

Stem/progenitor and other Niche cells were used as model systems.

Culture Systems

Cells were grown in different hydrogel-based environments to mimic the extracellular matrix. Variations in composition and stiffness were explored for their effect on cell behavior.

Imaging & Morphology

Microscopy techniques were used to monitor cell shape, growth, and 3D organization. Morphometric analysis quantified cell spreading and structural features.

Viability & Proliferation

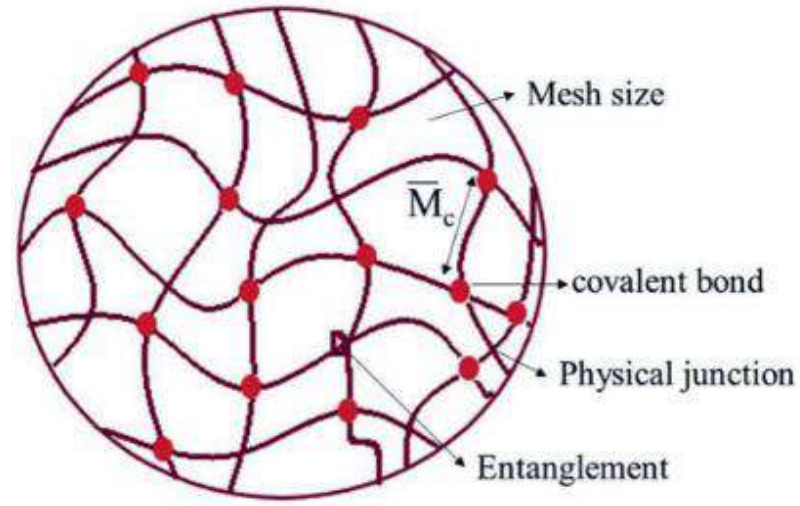
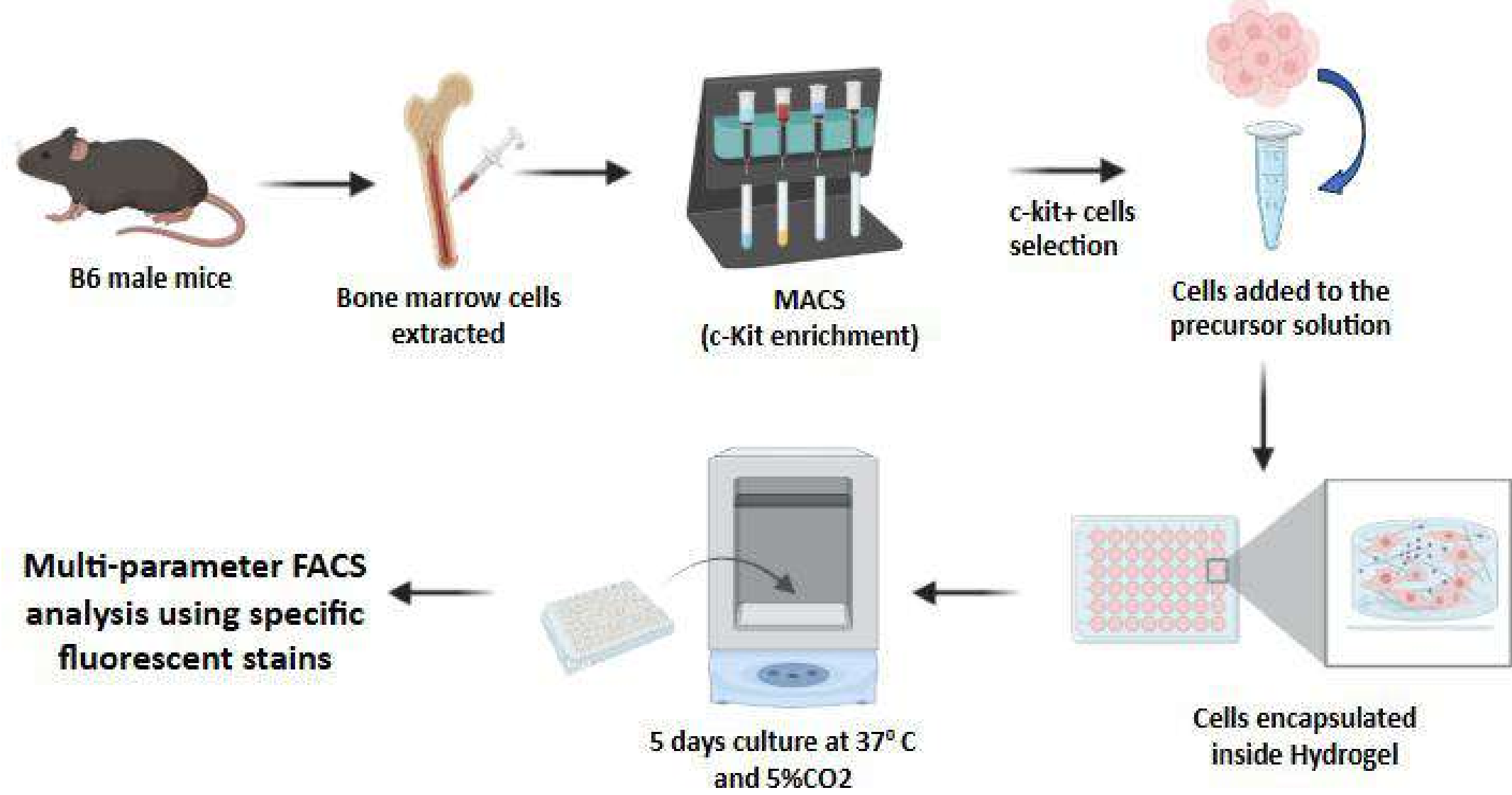
Standard staining and biochemical assays were applied to assess survival and growth. Matrix properties were correlated with cell retention and proliferation.

Cell-Cell Interactions

Spheroid formation and junction integrity were evaluated under different conditions. Both suspension and adherent cultures were compared.

Overall Approach

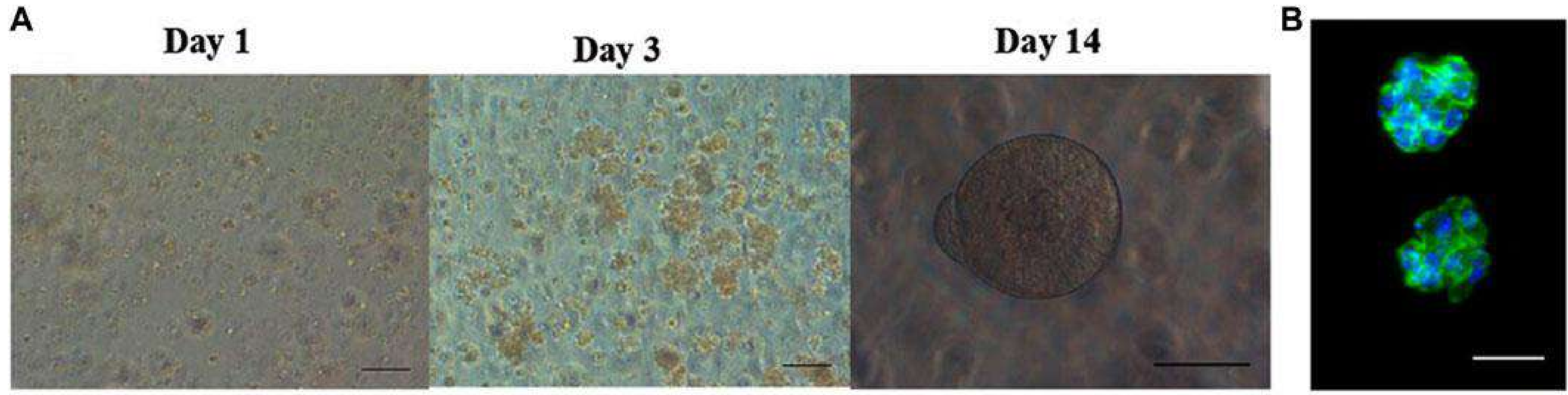
A combination of imaging, biochemical, and analytical tools provided insights. Findings emphasize how matrix design influences cell fate in engineered systems.



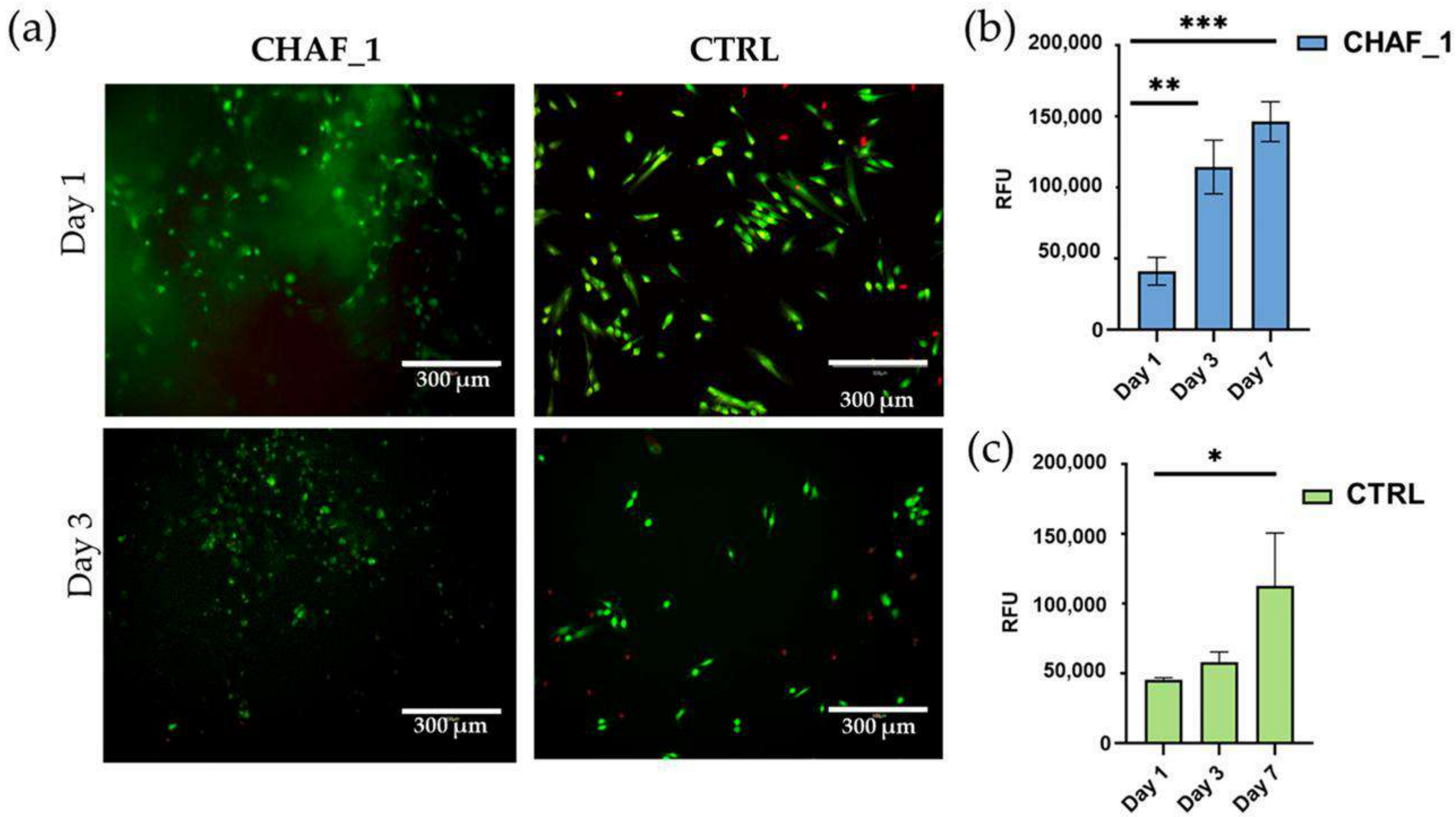
Hydrogel Scaffold

Results from Literature

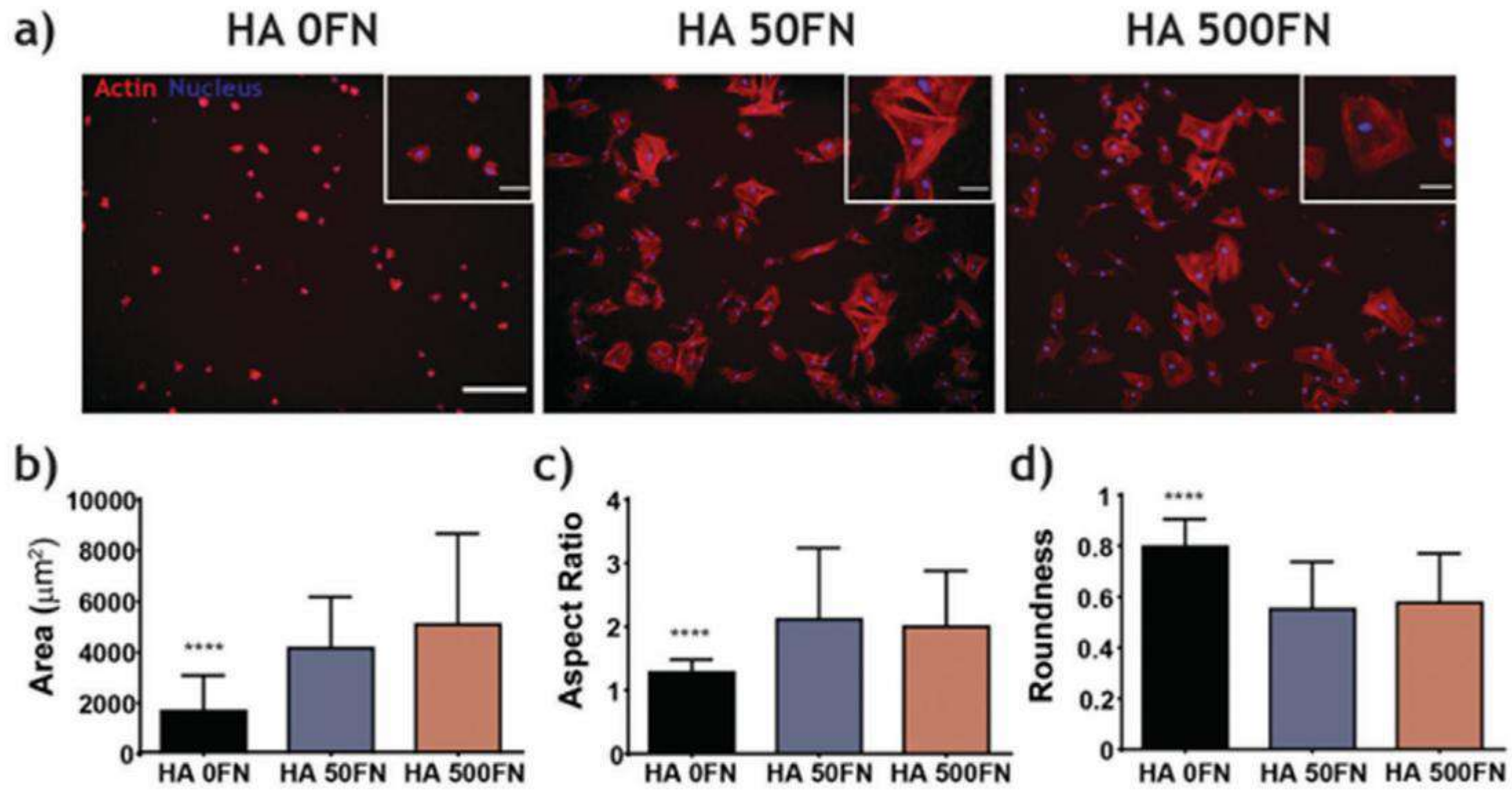
Relevant results from various literature:



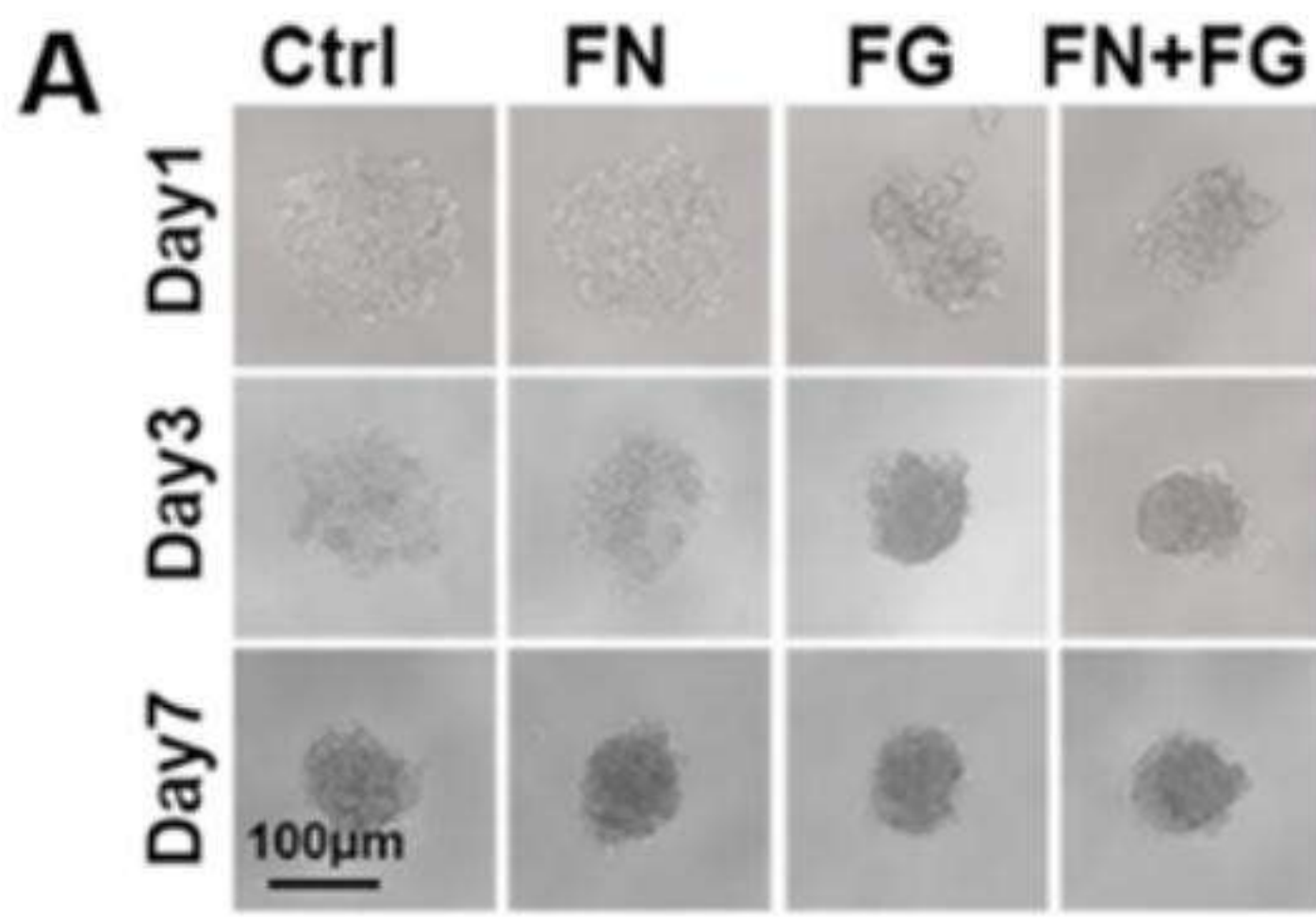
Phase contrast images of HSCs encapsulated within bioactive PEG hydrogels at days 1, 3, and 14, showing the proliferation and round shape retention of HSCs. Scale bar: 100 mm (day 1, day 3), 50 mm (day 14). (B) DAPI (blue) and phalloidin (green) staining of HSCs after 14 days of expansion showing the cell morphology retention and gel structure integrity. Scale bar: 25 mm. (Cuchiara et al., 2016)



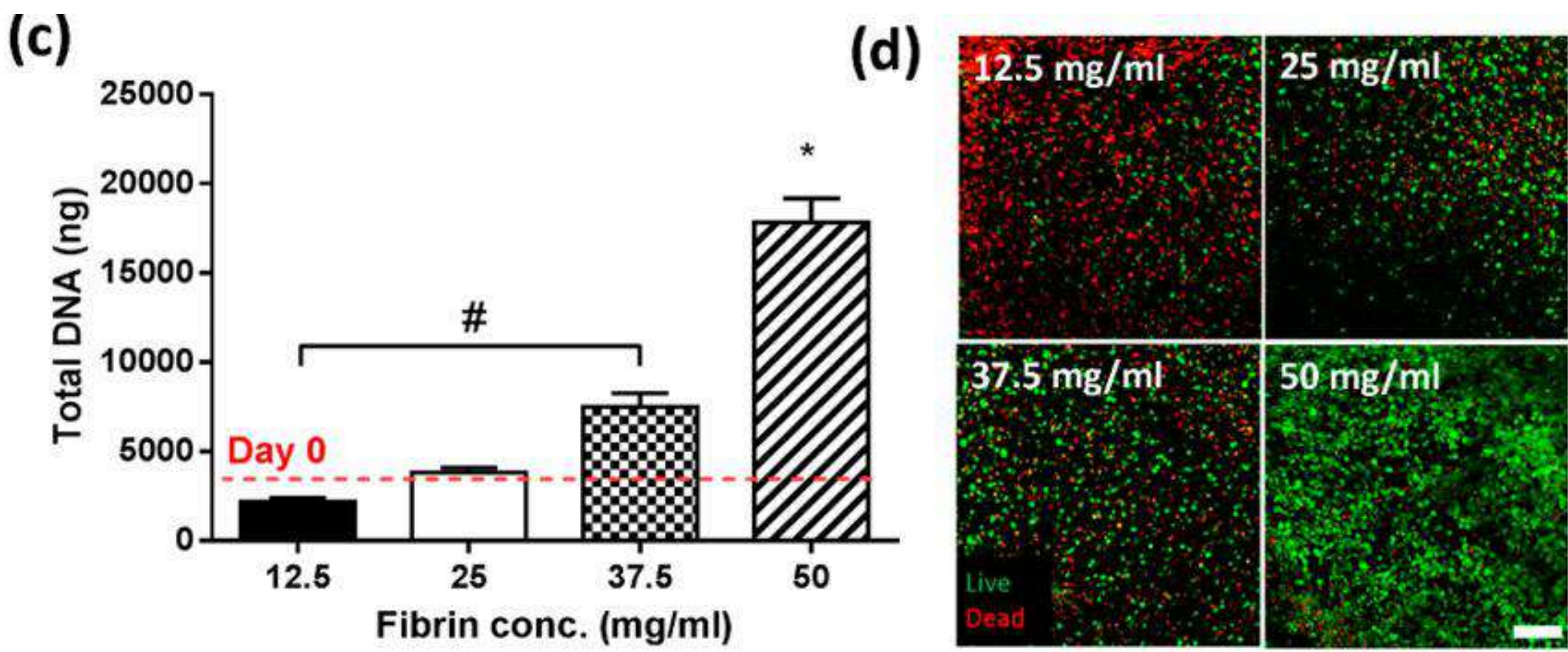
(a) Live/dead stained images of FBs encapsulated within CHAF_1 and cells on tissue culture plastic as a control (CTRL). Imaging was performed on days 1 and 3. Live cells are stained in green, and the dead cells in red. (b) PrestoBlue assay results for FBs encapsulated within CHAF_1 at Figure 8. (a) Live/dead stained images of FBs encapsulated within CHAF_1 and cells on tissue culture plastic as a control (CTRL). Imaging was performed on days 1 and 3. Live cells are stained in green, and the dead cells in red. (b) PrestoBlue assay results for FBs encapsulated within CHAF_1 at 1, 3, and 7 days. (c) FBs seeded on tissue culture plastic are used as a control (CTRL). (Bindi B et al., 2023)



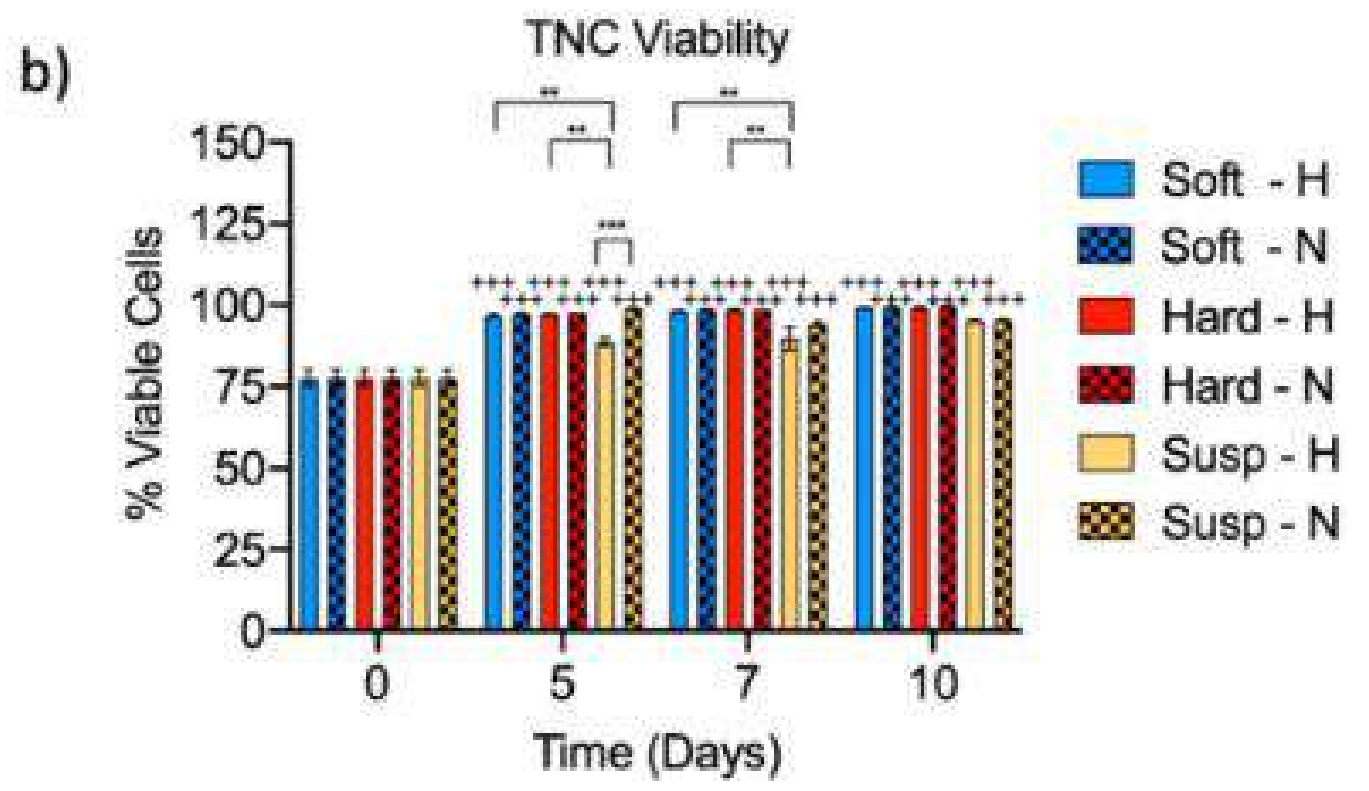
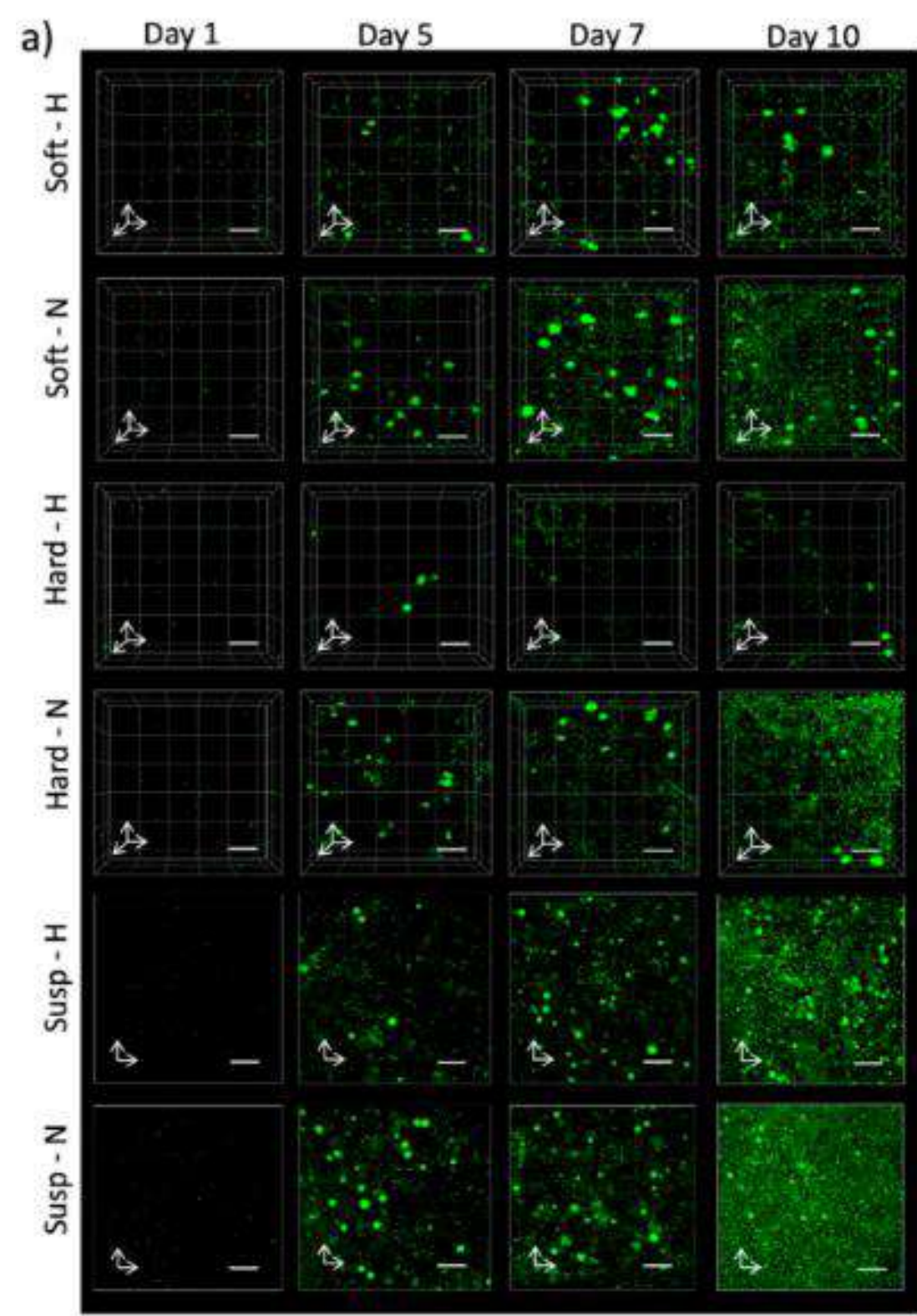
(a) Human MSCs were seeded on FN-HA hydrogels containing different amounts of FN. Quantification of cell spreading was performed by measuring cell b) spread area (μm^2), c) aspect ratio, and d) roundness (mean $\pm$ SD, $n > 100$ cells, conditions in triplicate). (Trujillo S et al., 2020)



Fibrinogen promotes cell aggregation in the establishment of cell-cell junctions. A) Macroscopic images and analyses of roundness and area of spheroids treated with fibrinectin and fibrinogen at 72 h. Scale bar = 100 μm . (Li R et al., 2022)



(c) Biochemical analysis of DNA content in constructs. # indicates significant difference between 12.5 and 37.5 mg/mL ($p < 0.01$) and * indicates a significant difference compared to all other groups ($p < 0.001$); (d) Live/dead analysis showing a high degree of cell death for the lowest concentration (12.5 mg/mL) and increased cell viability in the highest concentrations (50 mg/mL). Scale bar = 200 μm . (Gansau J et al., 2018)



Fibrin hydrogels support the viability of Lin-/cKit+ cells: (a) confocal images of live (green) and dead (red) cells in soft and hard fibrin hydrogels as well as in suspension culture (Susp) grown in hypoxia (H) or normoxia (N) and (b) quantification of viability of Lin-/cKit+ progeny using the NucleoCounter. Day 0 values are from the starting cell suspension. (Garcia-Abrego C et al., 2020)

Conclusion

- Biomimetic hydrogels provide supportive microenvironments for hematopoietic stem cell growth and expansion.
- Replicating niche-like cues helps address current limitations in ex vivo HSC culture.
- These approaches offer promising directions for transplantation and regenerative medicine.

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- Yin, S., et al. "Hydrogels for Large-Scale Expansion of Stem Cells," Acta Biomaterialia, 2021. This review contains figures summarizing the properties, composition, and applications of hydrogels for stem cell culture.
- Hong, J., et al. "Bone marrow-inspired hydrogel/graphene composite scaffolds to support in vitro expansion of hematopoietic stem cells," Journal of Materials Chemistry B, 2024. This work provides illustrative schematics of composite hydrogels for HSC expansion.
- Zhu, M., et al. "Hydrogel-based microenvironment engineering for hematopoietic stem/progenitor cell expansion," Frontiers in Bioengineering and Biotechnology, 2023. This review discusses, with schematics, hydrogel features crucial for HSC behavior

Future Directions

- Develop hydrogels enriched with ECM components that closely mimic the structural and biochemical features of the fetal liver microenvironment to provide a supportive niche for HSC growth.
- Establish co-culture systems with niche cells to enhance hematopoietic stem cell maintenance, self-renewal, and expansion in a physiologically relevant setting.



Precise Adenine and Cytosine base editing facilitates generation of isogenic iPSC lines for modeling rare and polygenic disorder

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¹Center for Stem Cell Research (A Unit of inStem, Bengaluru), Christian Medical College, Vellore, Tamil Nadu, 632002, India

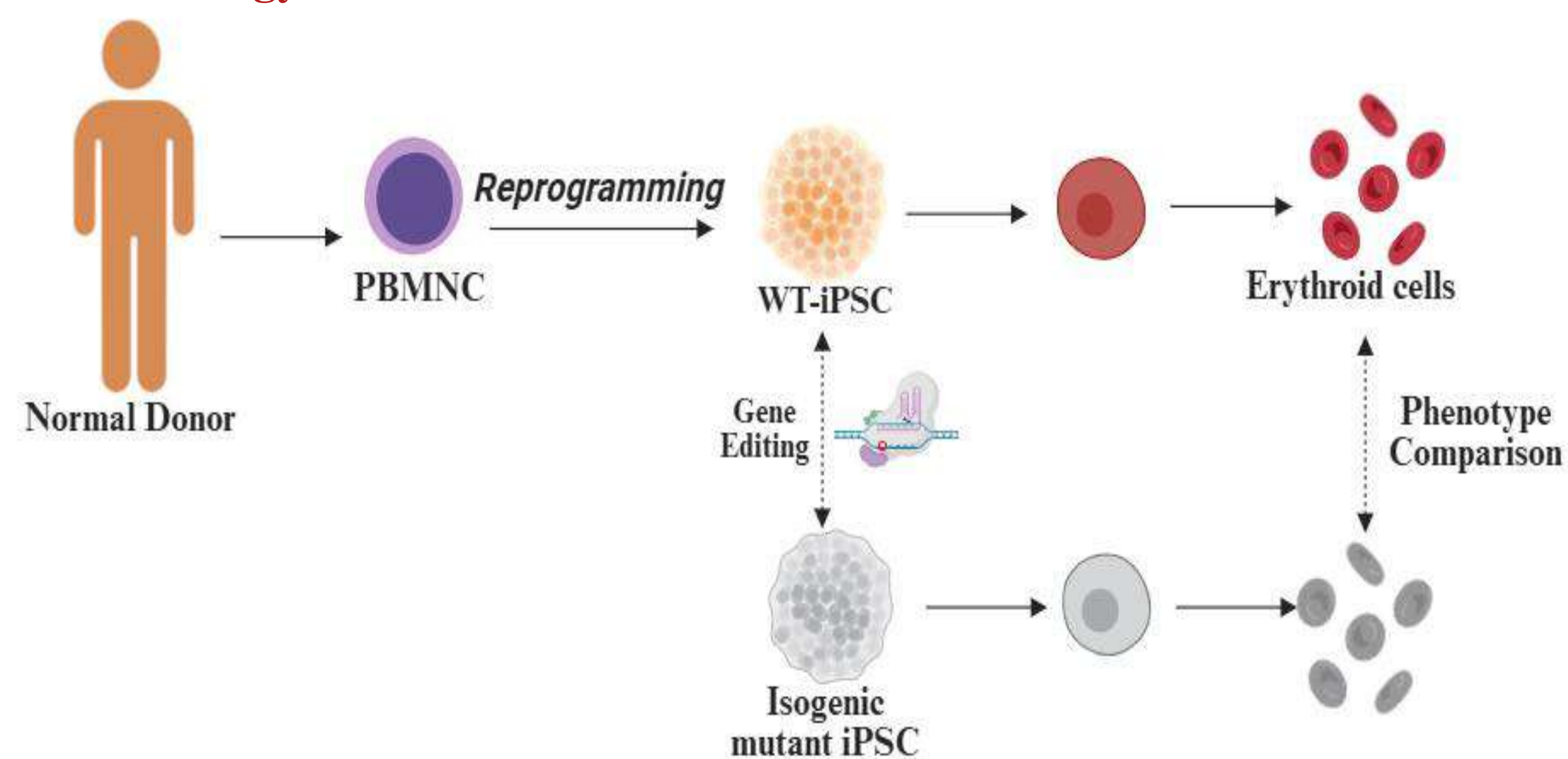
²Department of Haematology, Christian Medical College, Vellore, Tamil Nadu, 632004, India



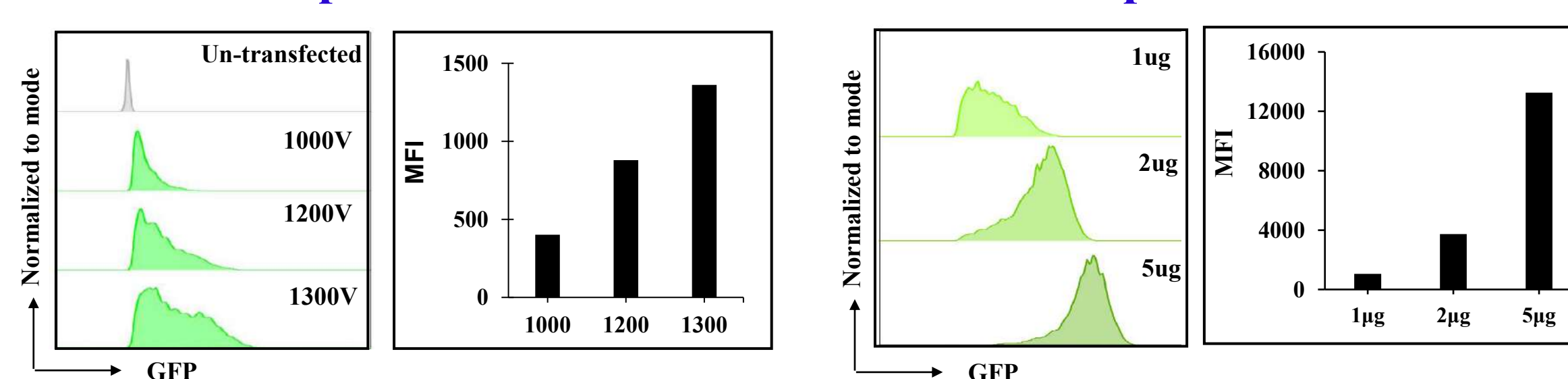
Introduction:

Induced pluripotent stem cells (iPSCs) offer powerful opportunities for disease modeling, particularly for studying genetic disorders. However, generating iPSC models for rare diseases remains challenging due to limited patient availability and complex genetic characterization. Recent advancements have focused on developing isogenic iPSC lines to ensure genetic consistency and eliminate background variability. Conventional CRISPR/Cas9 genome editing relies on double-strand breaks (DSBs) and homology-directed repair (HDR), which often result in low editing efficiency, unintended insertions or deletions, and labor-intensive clonal screening. Base editors (BEs) overcome these limitations by enabling precise single-nucleotide conversions without generating DSBs. Adenine base editors (ABEs) mediate A•T to G•C substitutions, while cytosine base editors (CBEs) catalyze C•G to T•A transitions. However, their delivery efficiency, editing window characteristics, and comparative performance in iPSCs have not been systematically evaluated. In this study, we established a high-efficiency, mRNA-based base editing platform for iPSCs using optimized electroporation conditions. By comparing multiple ABE and CBE variants, we achieved highly efficient, scarless editing across single and multiplexed disease-relevant loci, enabling the rapid generation of isogenic iPSC models for rare hematological disorders.

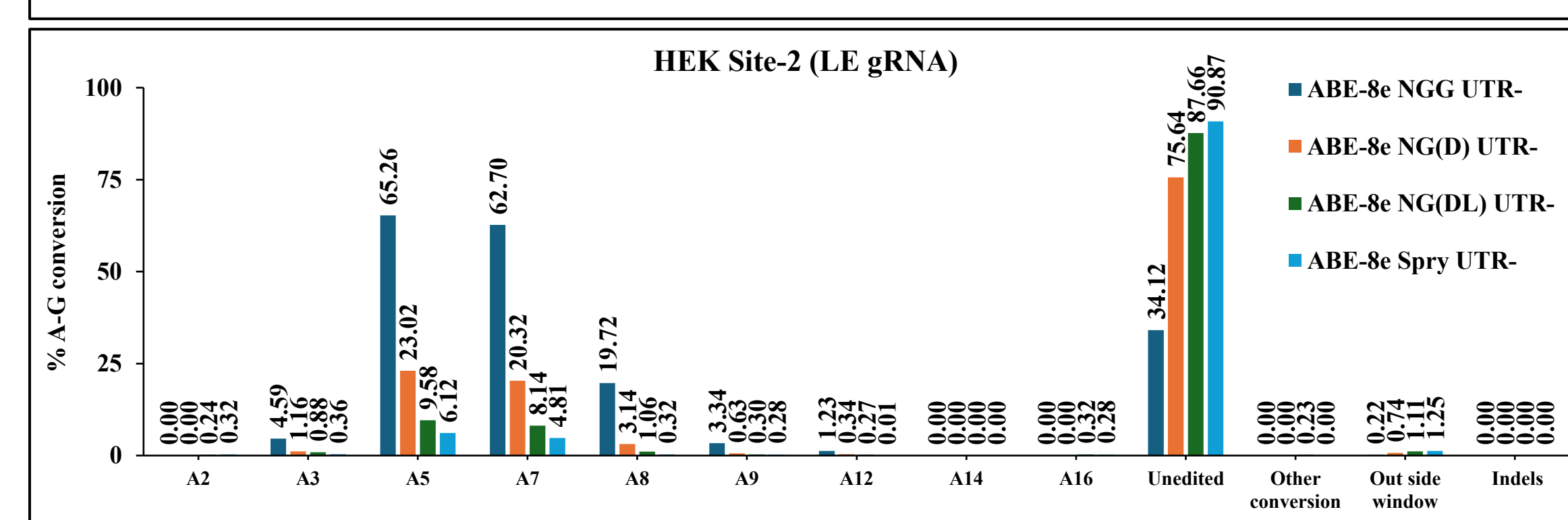
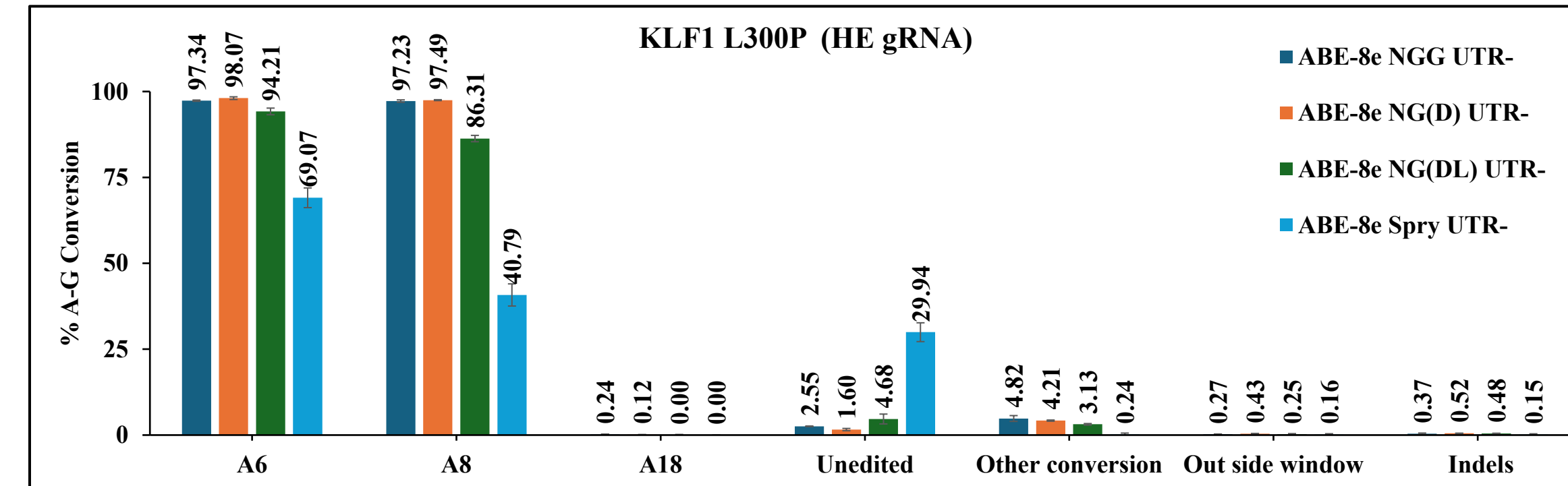
Methodology:



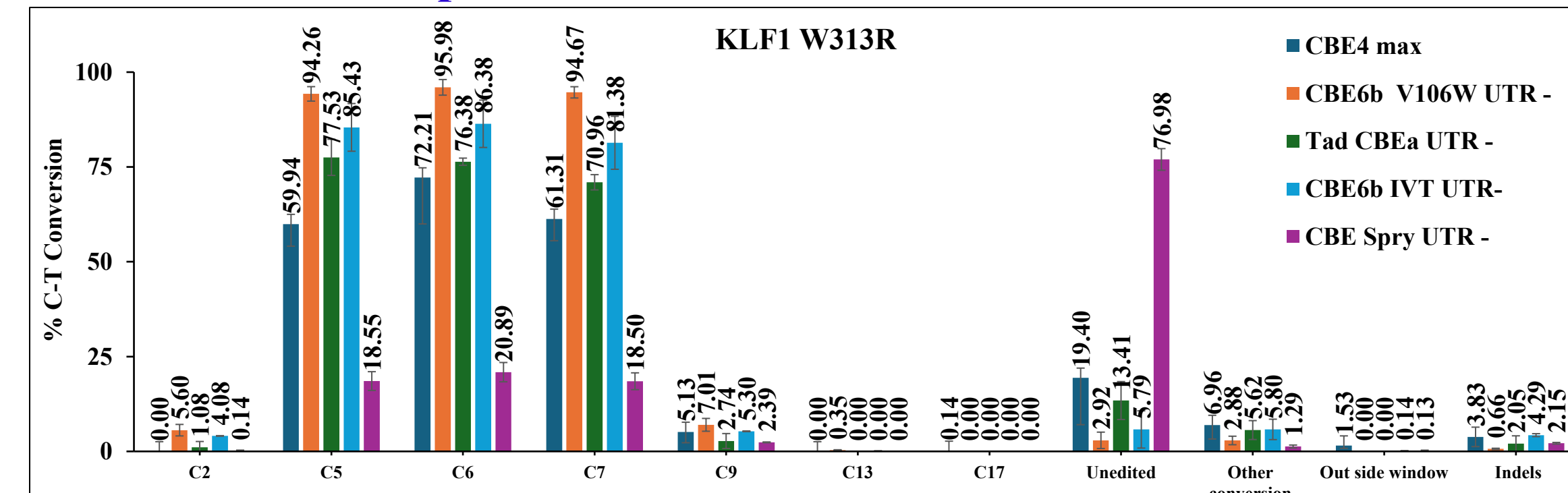
Results: Optimization of efficient mRNA electroporation



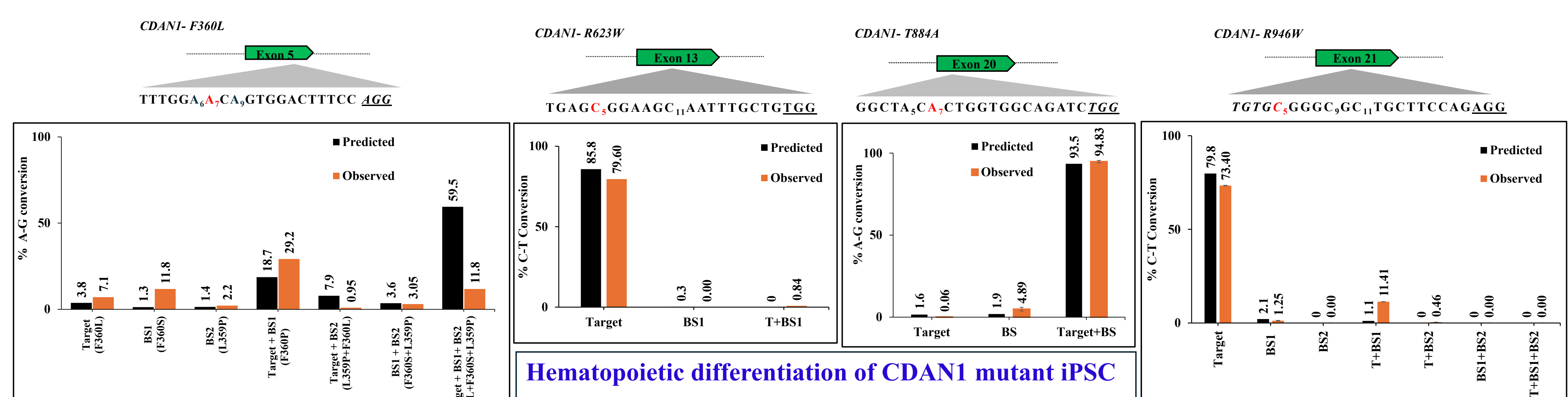
Comparison of ABE variant in K562 cells



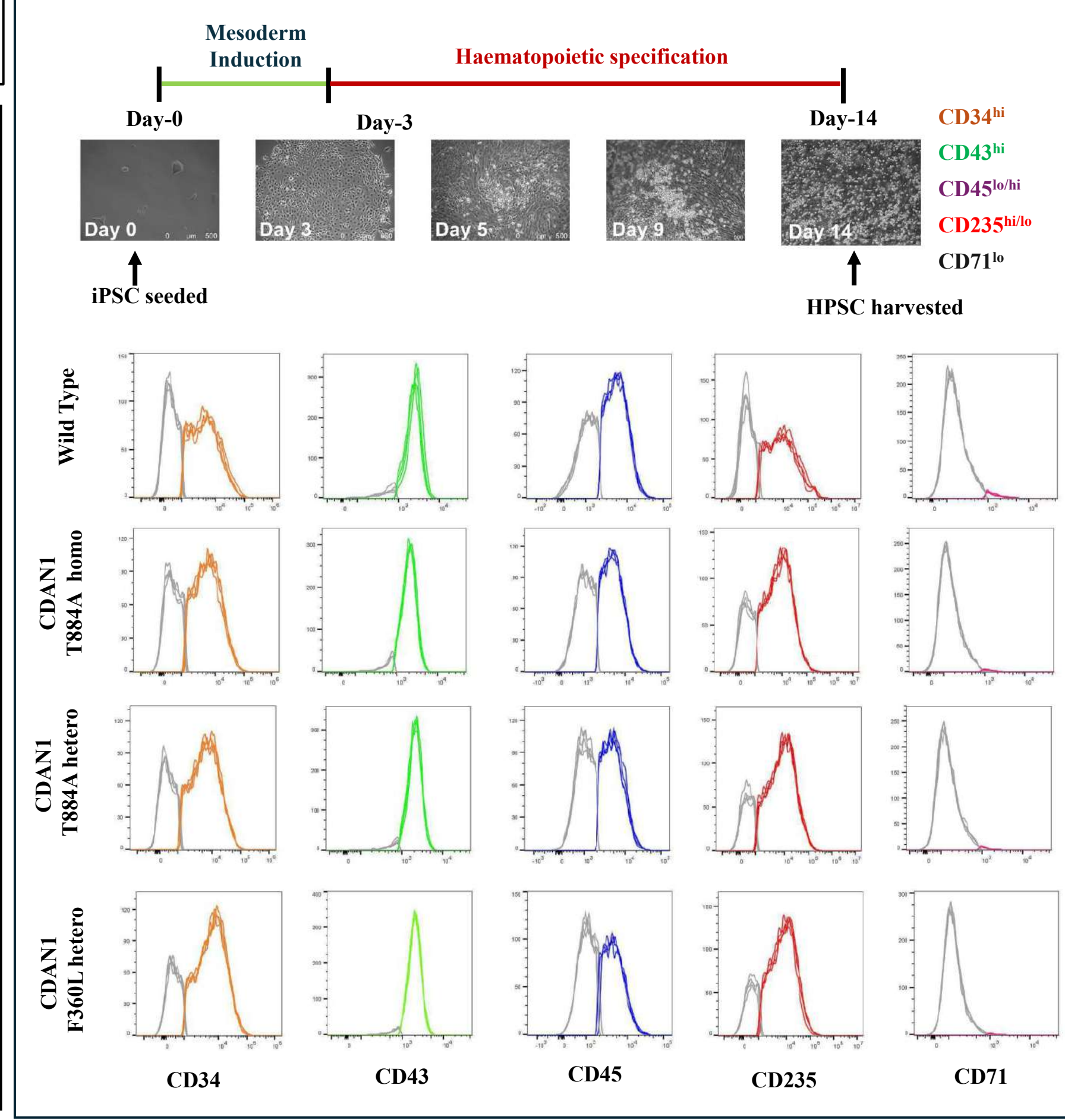
Comparison of CBE variant in K562 cells



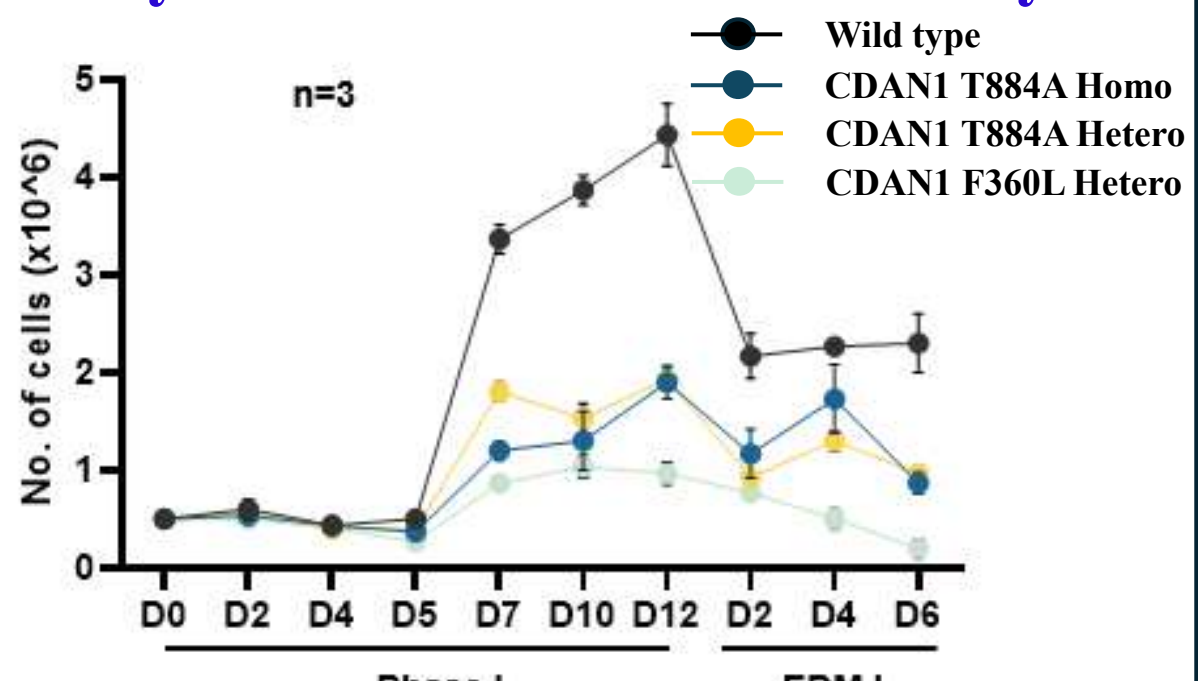
Generation of isogenic iPSC for modelling Congenital Dyserythropoietic Anemia type-1 (CDA-1)



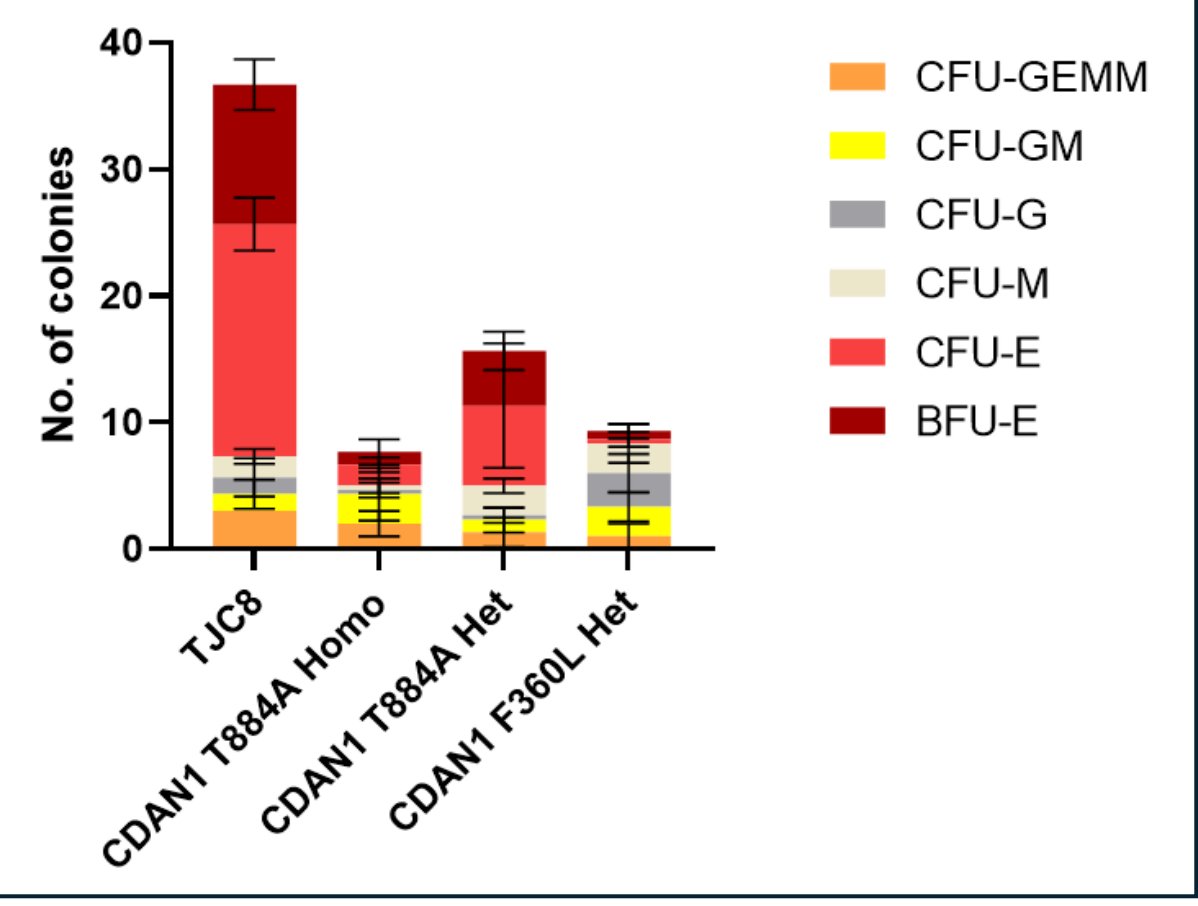
Hematopoietic differentiation of CDAN1 mutant iPSC



Erythroid differentiation analysis



Colony formation analysis



Conclusion:

- Established a robust, integration-free mRNA-based base editing platform in iPSCs.
- Achieved near-complete ABE and CBE editing efficiencies with high precision.
- Demonstrated the ability to modulate editing efficiency to create heterozygous or homozygous mutations using gRNAs with predicted editing efficiency.
- Enabled efficient single and multiplexed editing of disease-relevant mutations, including challenging loci.
- Facilitated rapid generation of isogenic iPSC models for disease modeling and drug discovery applications.

References:

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Acknowledgement:

- Wellcome-DBT Alliance Senior Research Fellowship.
- Department of Biotechnology, Ministry of Science & Technology, Government of India.

1. Department of Biotechnology, Vellore Institute of Technology (VIT), Vellore. 2. Center for Stem Cell Research (CSCR), Vellore.

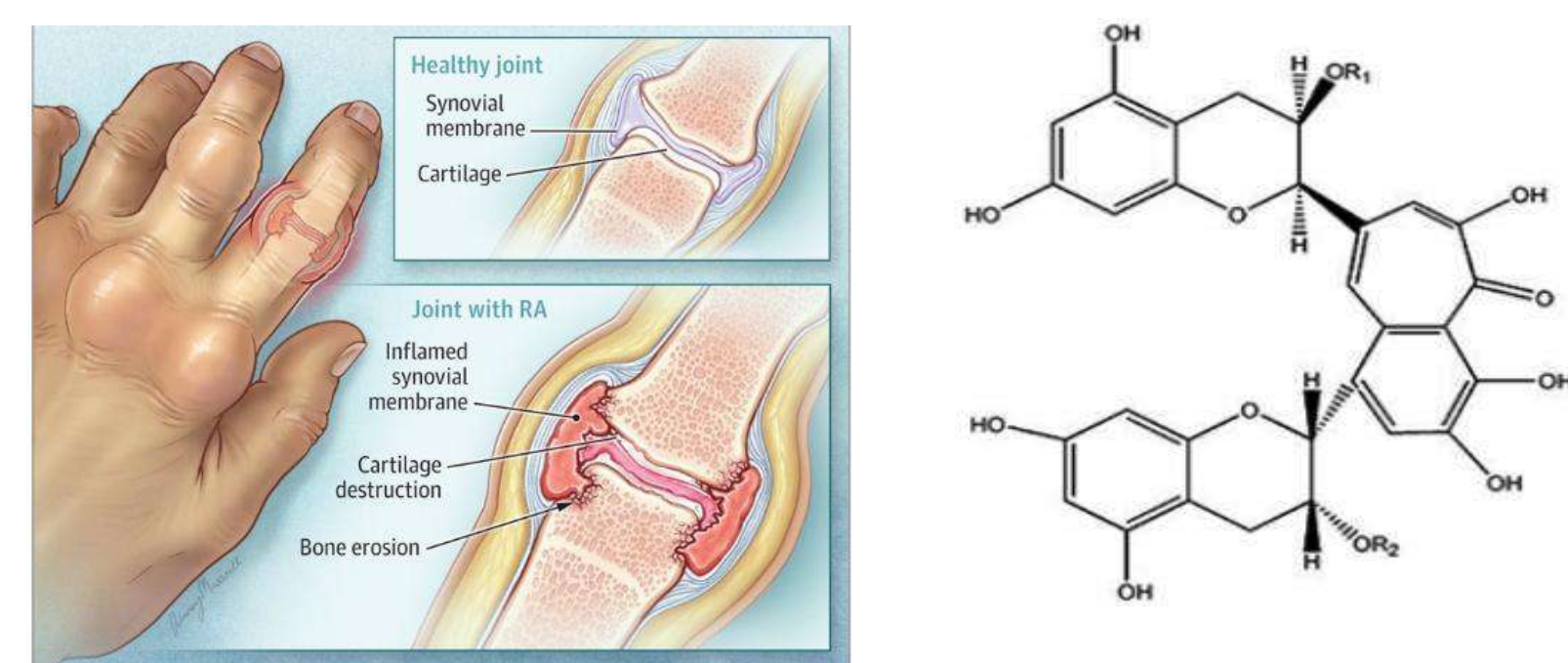
Introduction

Global Impact: Rheumatoid arthritis (RA) affects 1–2% of the global population, predominantly women.

Disease Burden: causes joint pain, deformity, and disability, severely affecting quality of life.

RA Current Treatments: DMARDs and NSAIDs offer low efficacy with notable side effects.

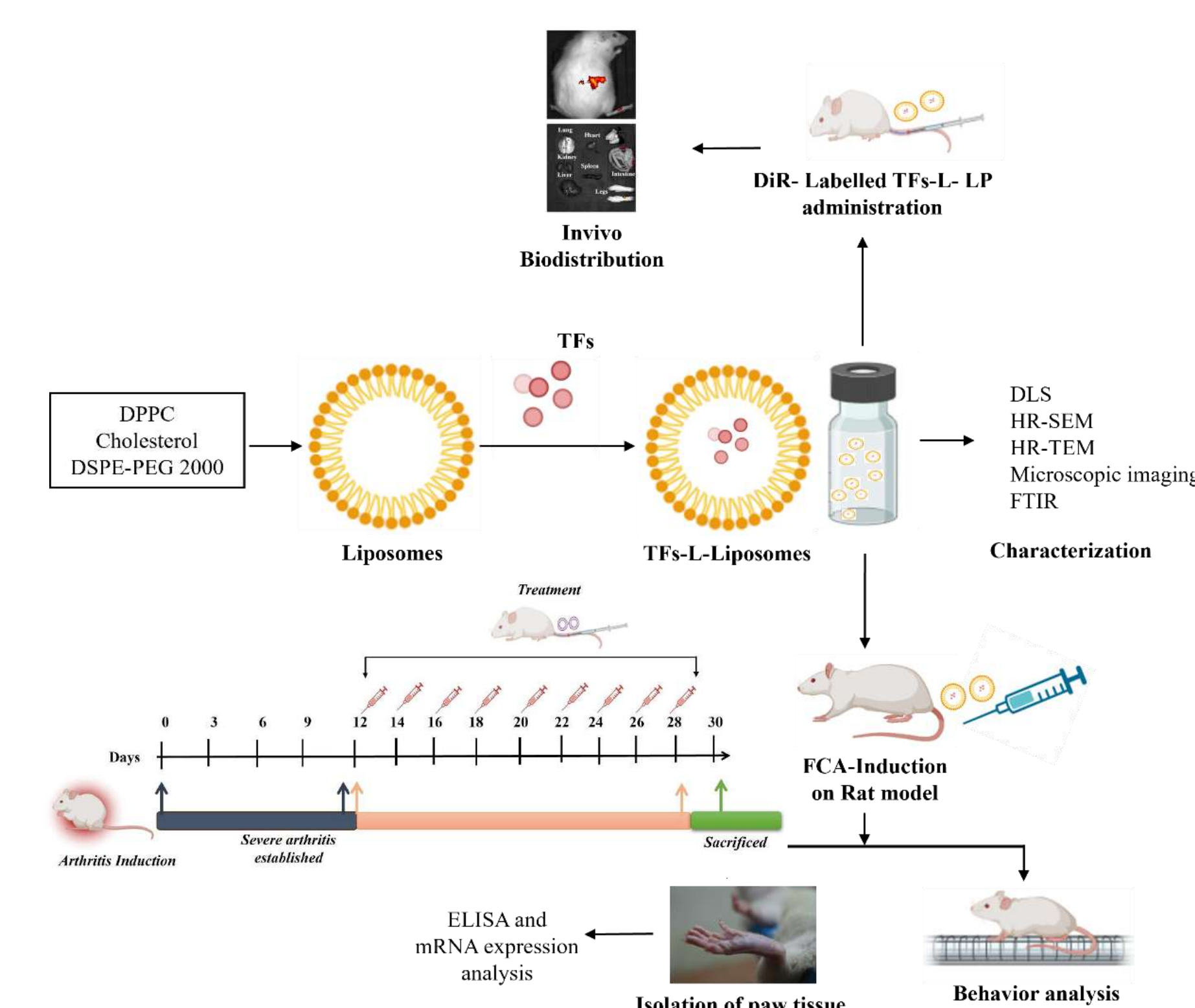
Need for Innovation: Novel therapies are needed to target immune dysregulation and improve outcomes.



(Smolen et al., 2016)

theaflavins

Methodology



Results

Physio chemical Parameters

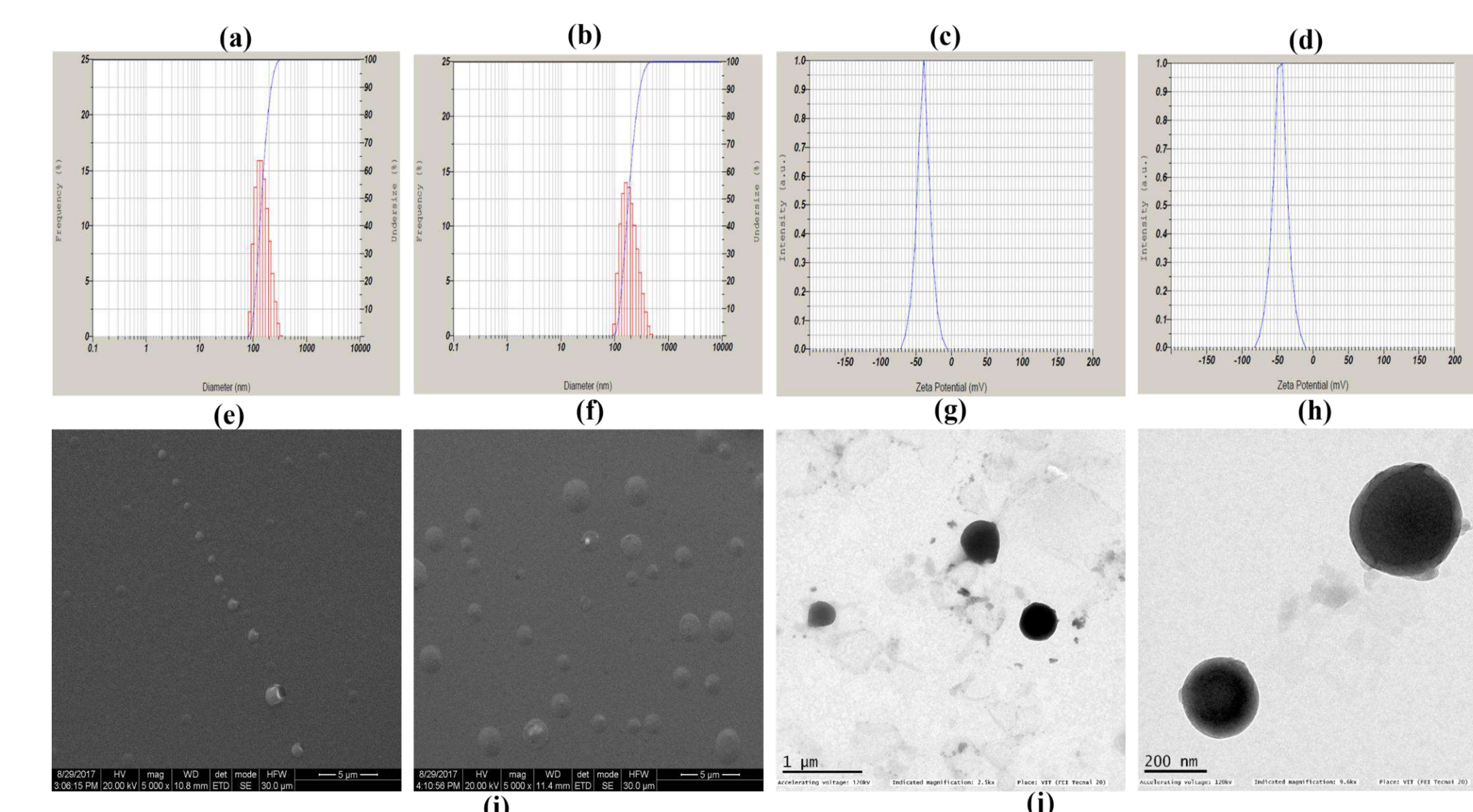


Figure 1 (above)¹ : The particle size of (A) U-LP, (B) TFS-L-LP, 111, 115nm. Zeta potential (C) U-LP and (D) TF-L-LP. -39 and -46mV. SEM-115 and 105nm; TEM-222 and 213nm.

Results

Physio chemical Parameters

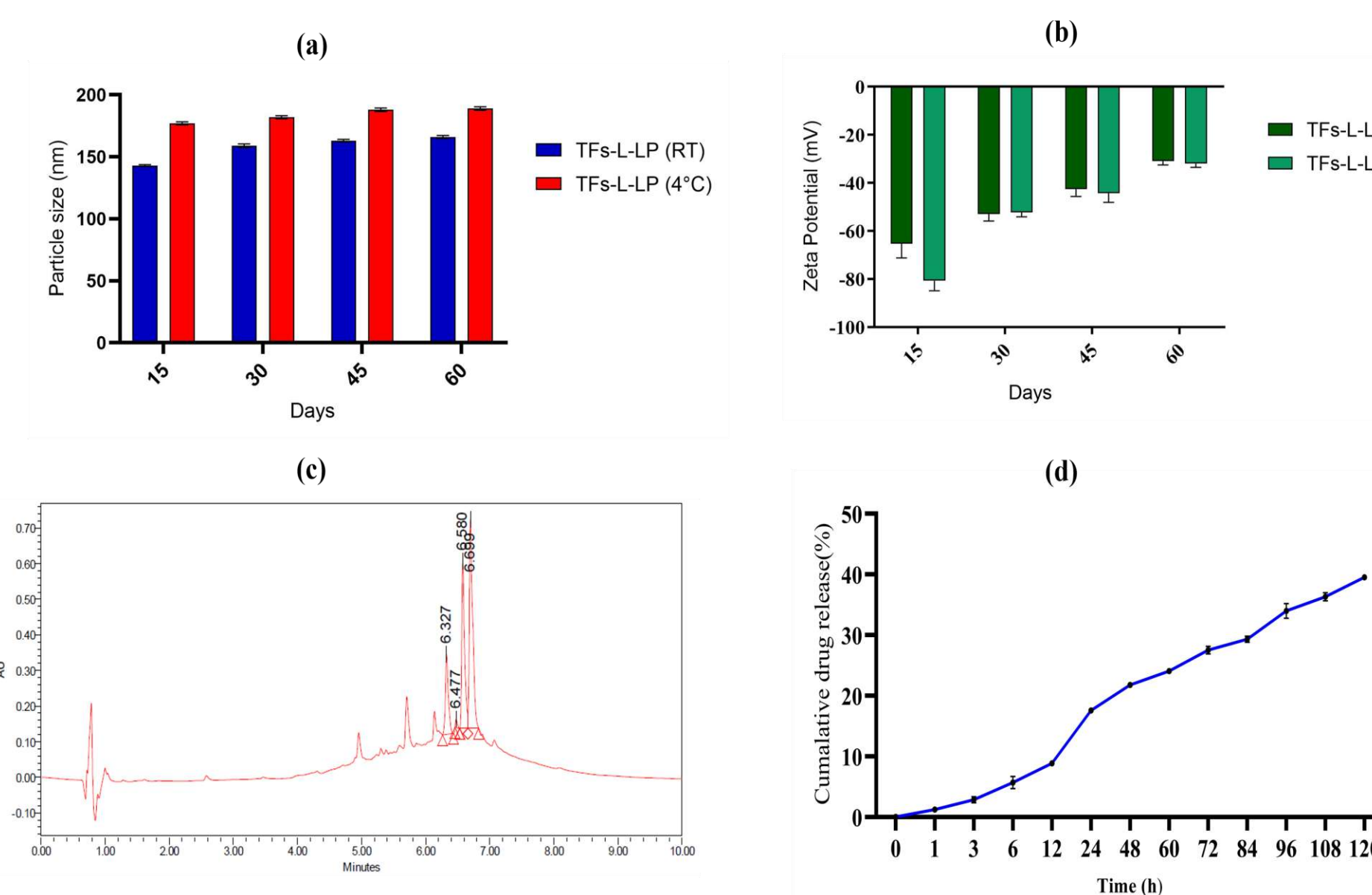


Figure 2 (above)¹ : Storage stability (A) Particle Size (B) Zeta Potential (C) Chromatograms of In vitro release from TFs-L-LP (D) In vitro release

In vivo biodistribution

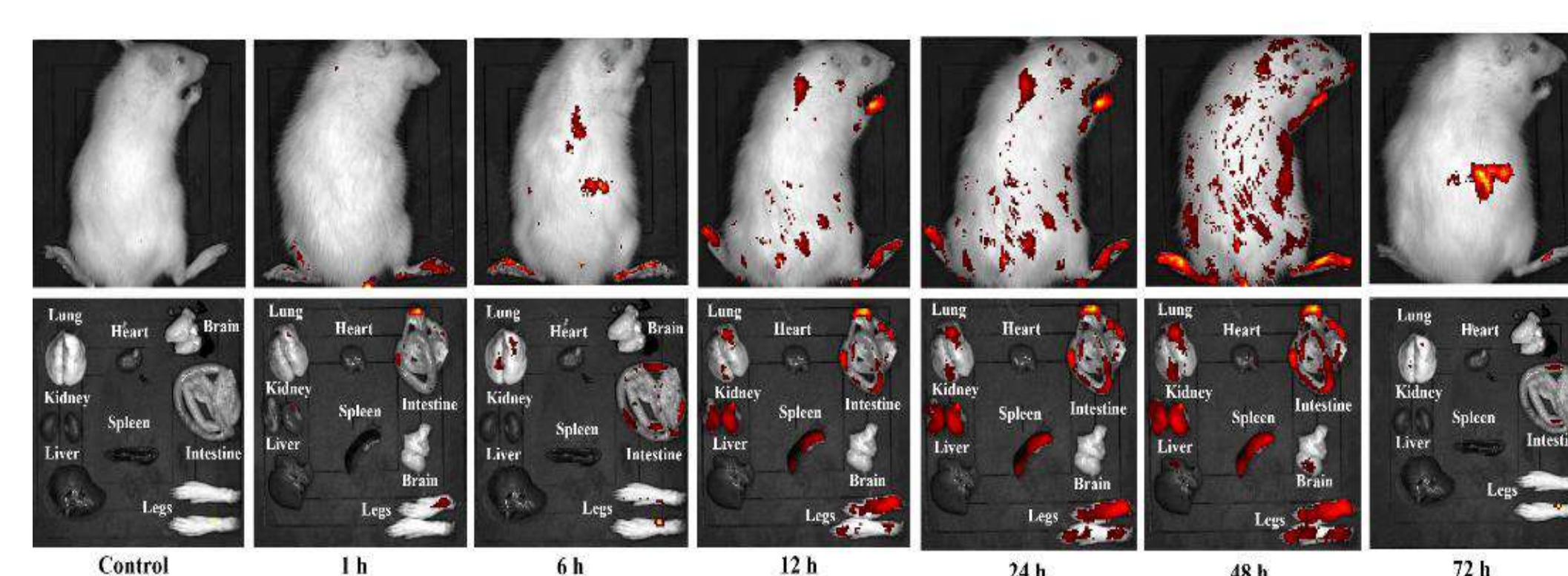


Figure 3 : *In vivo* and *ex vivo* DiR labelled liposomes

Toxicity Analysis Histopathology

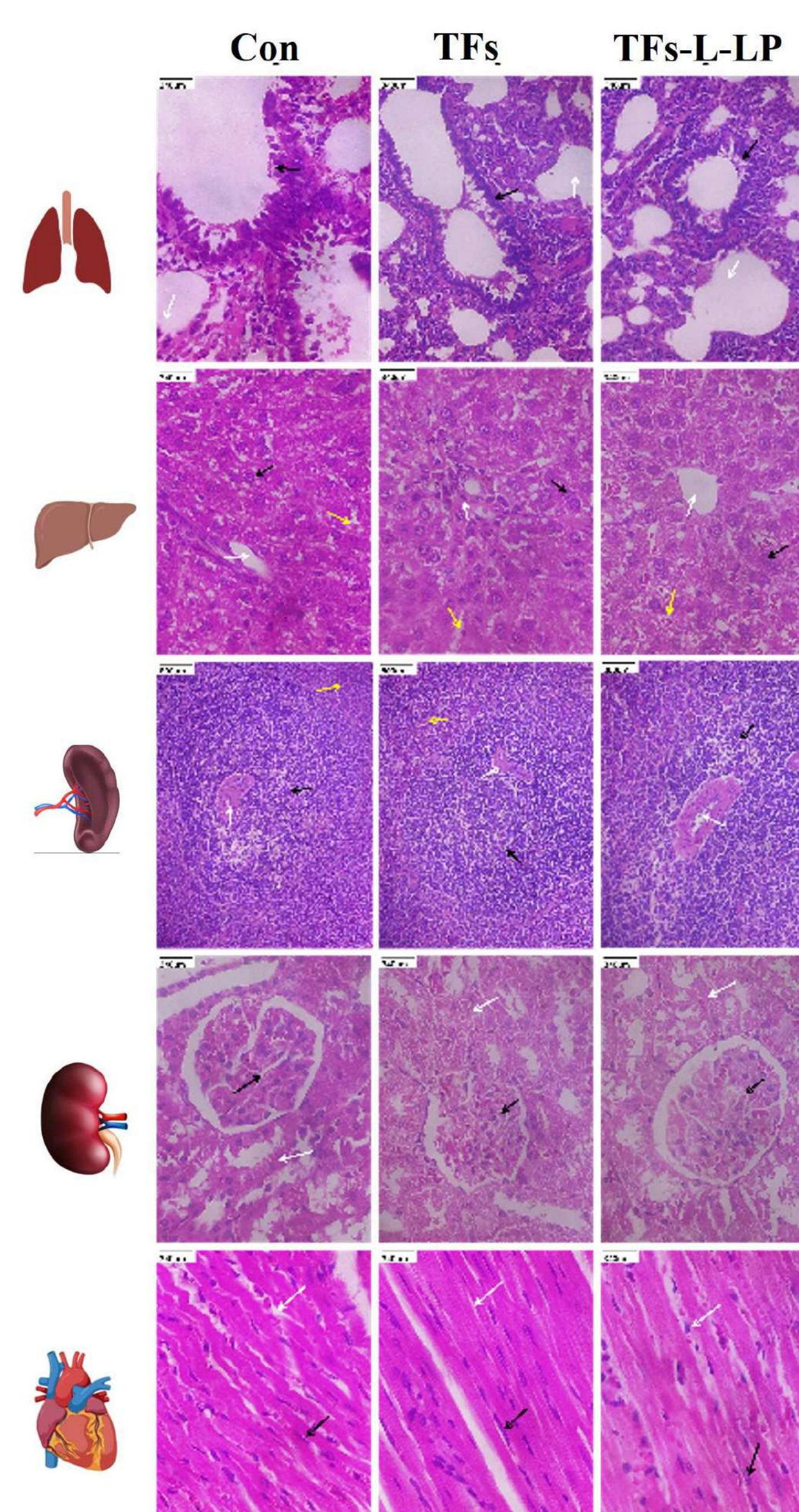


Figure 4 (above) ¹: Toxicity of TFs-L-LP in rats

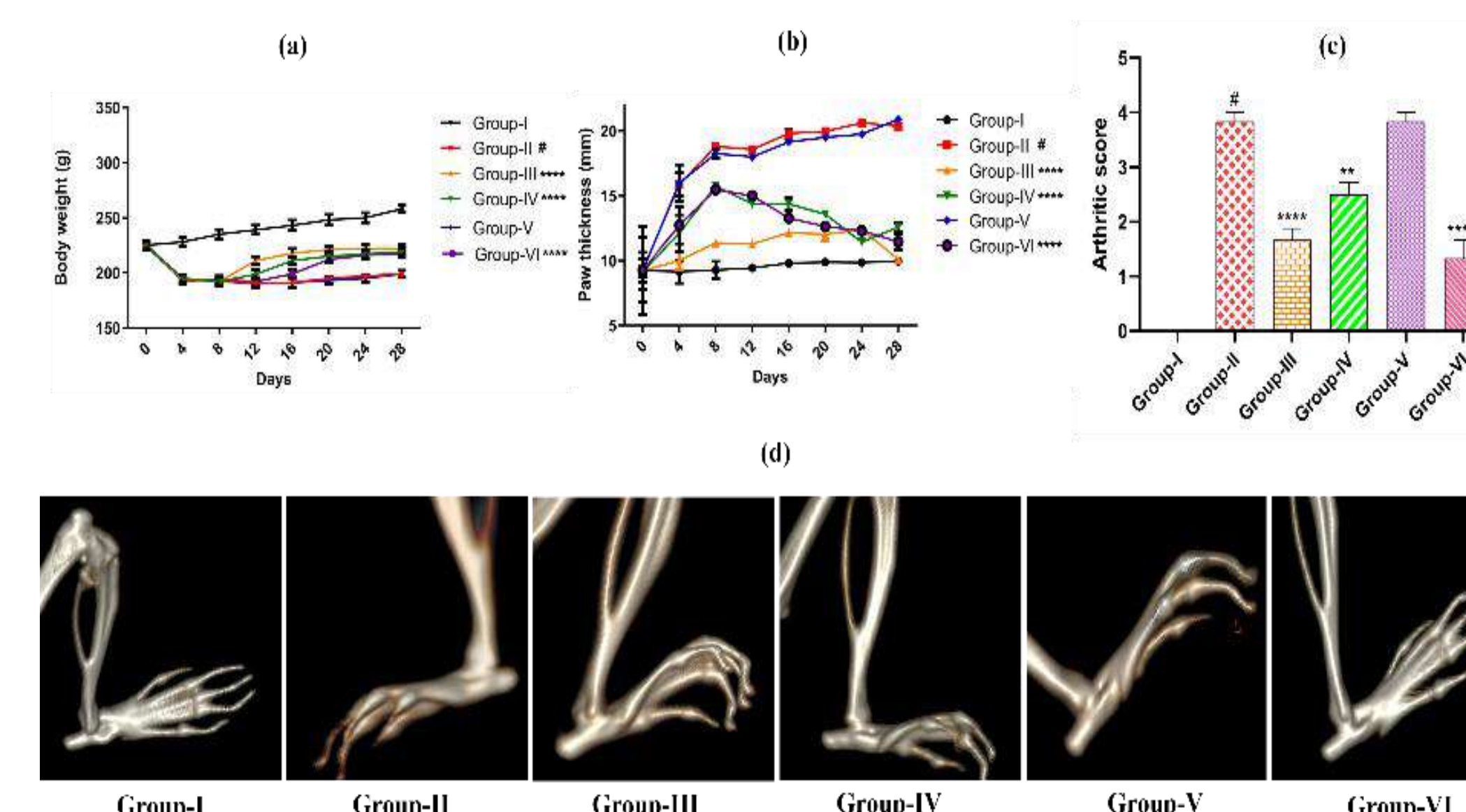
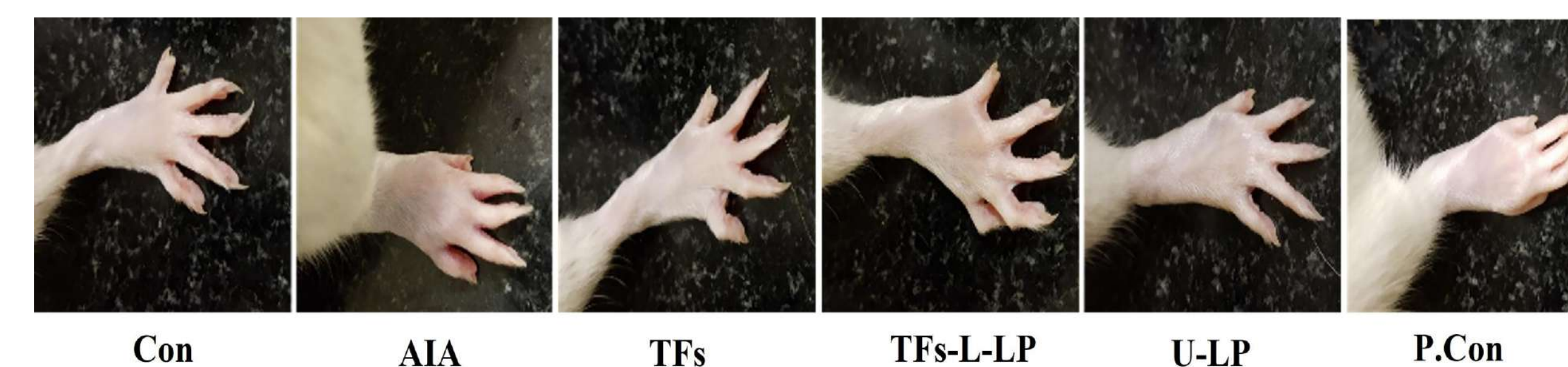


Figure 5 (above)¹: physical assessment parameters

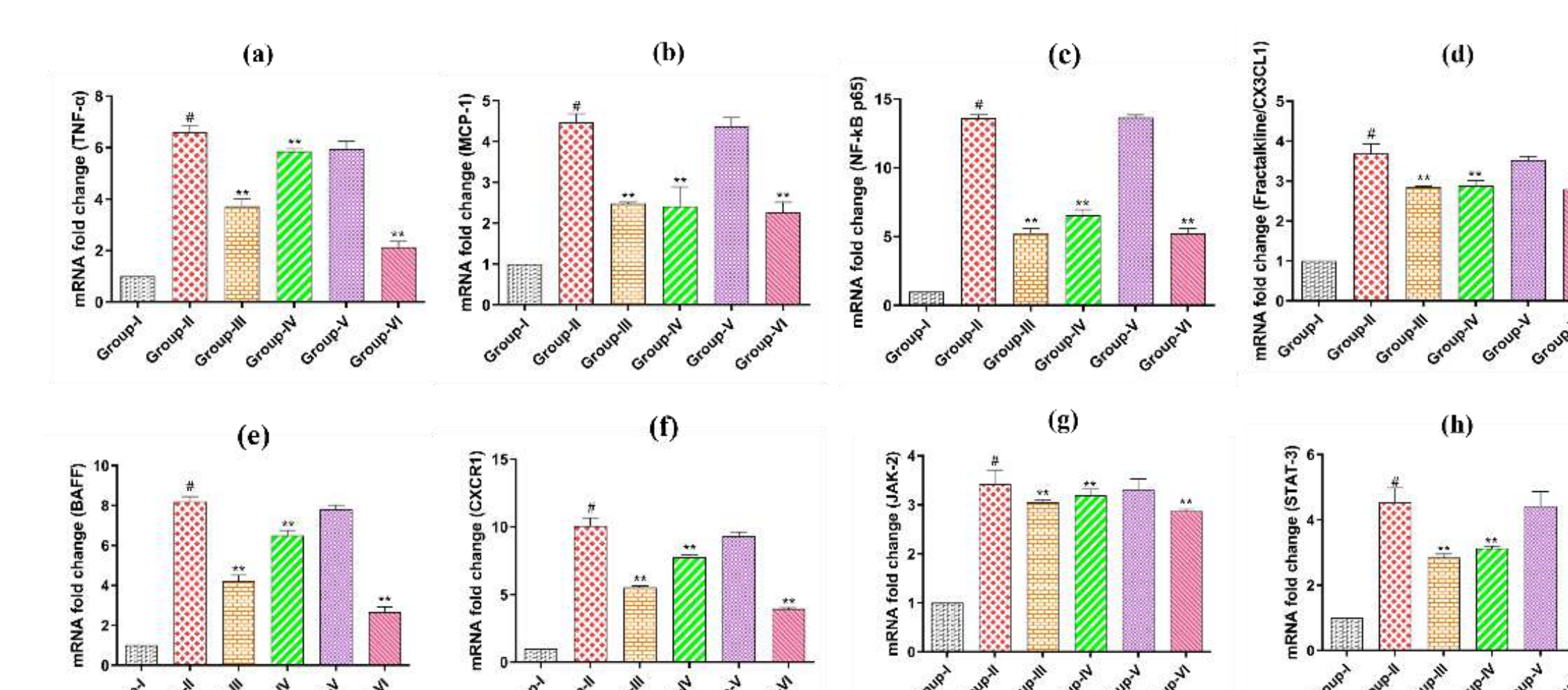
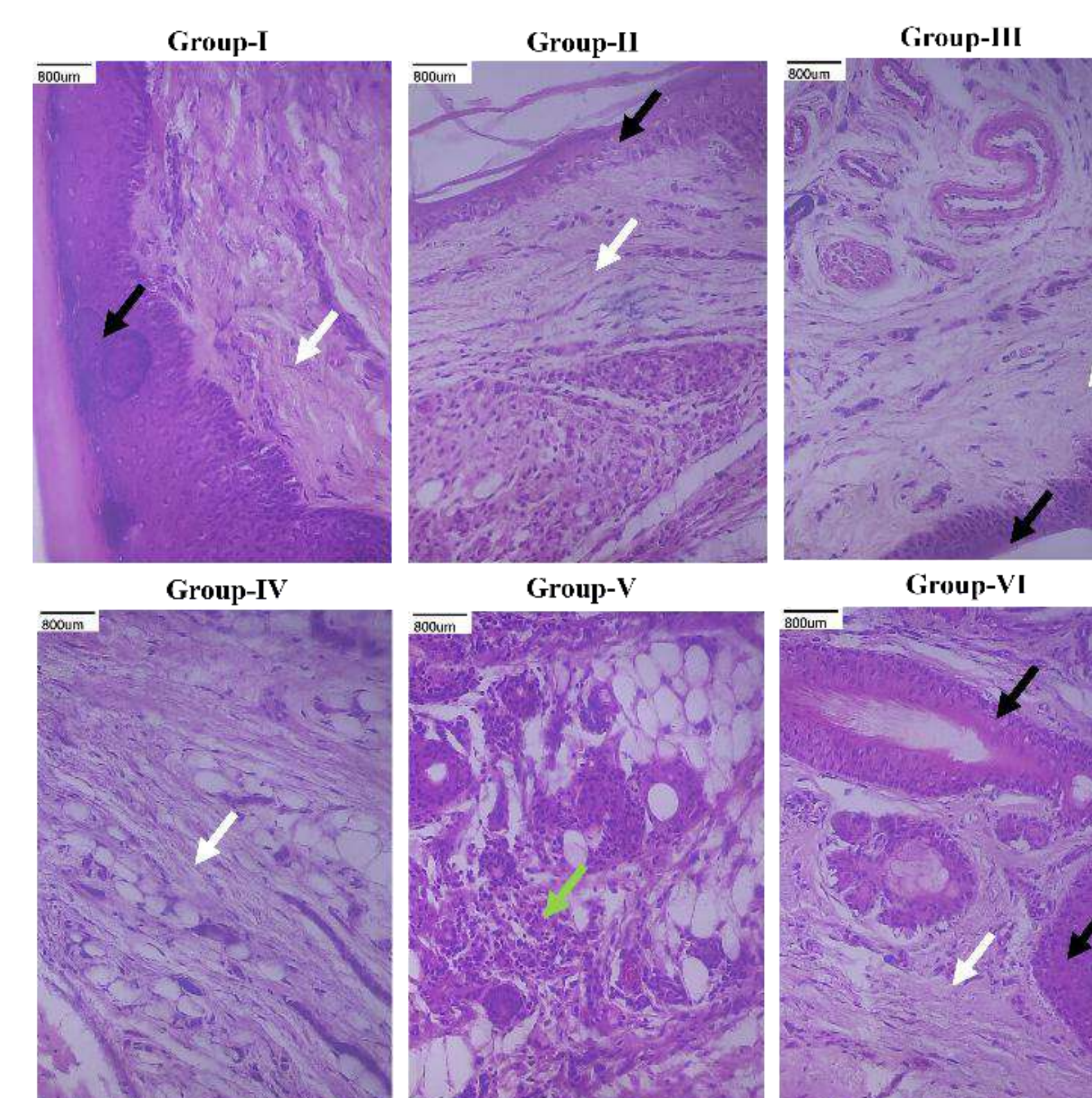
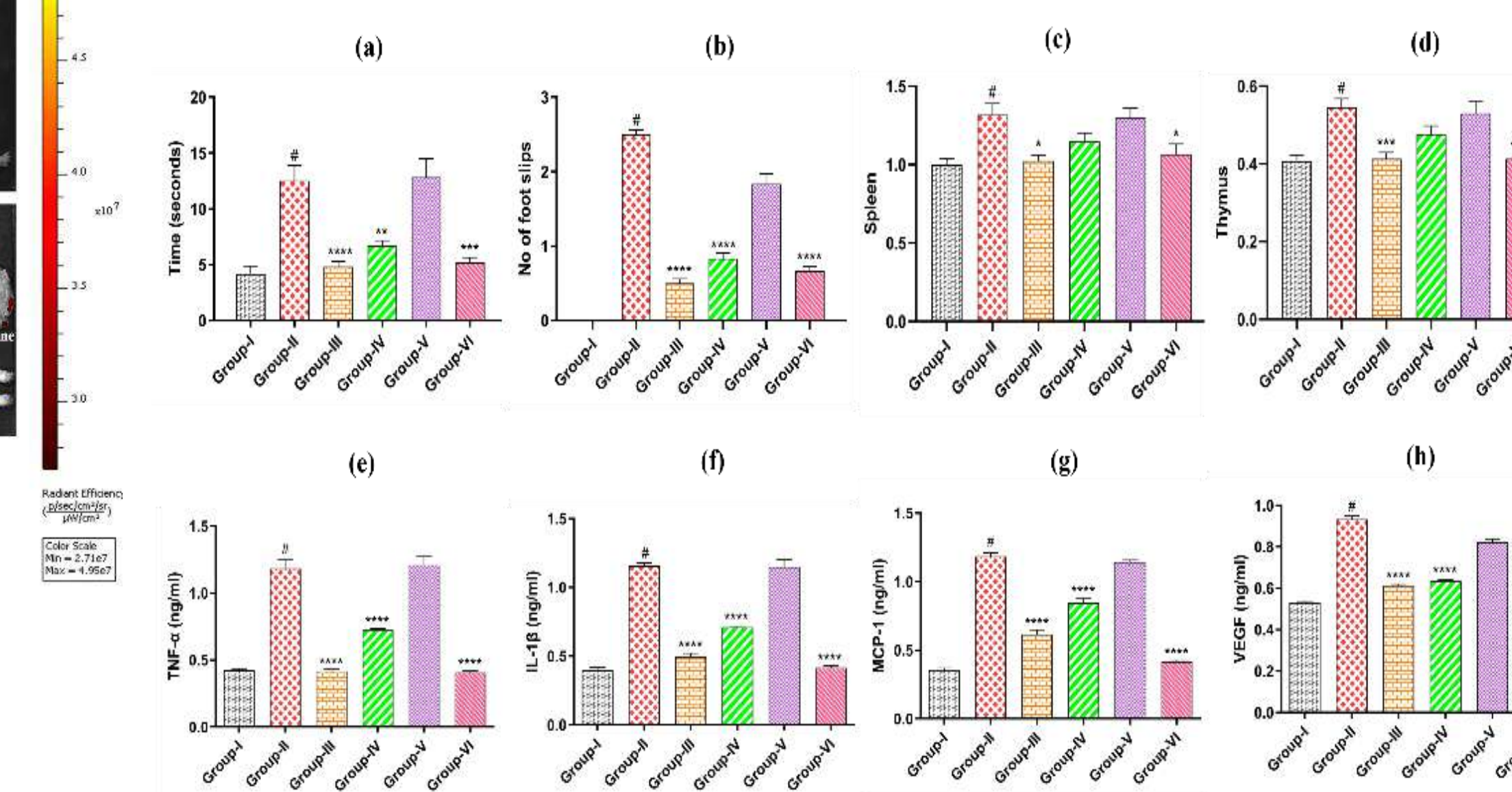


Figure 6: Molecular mechanisms of TFs-L-LP

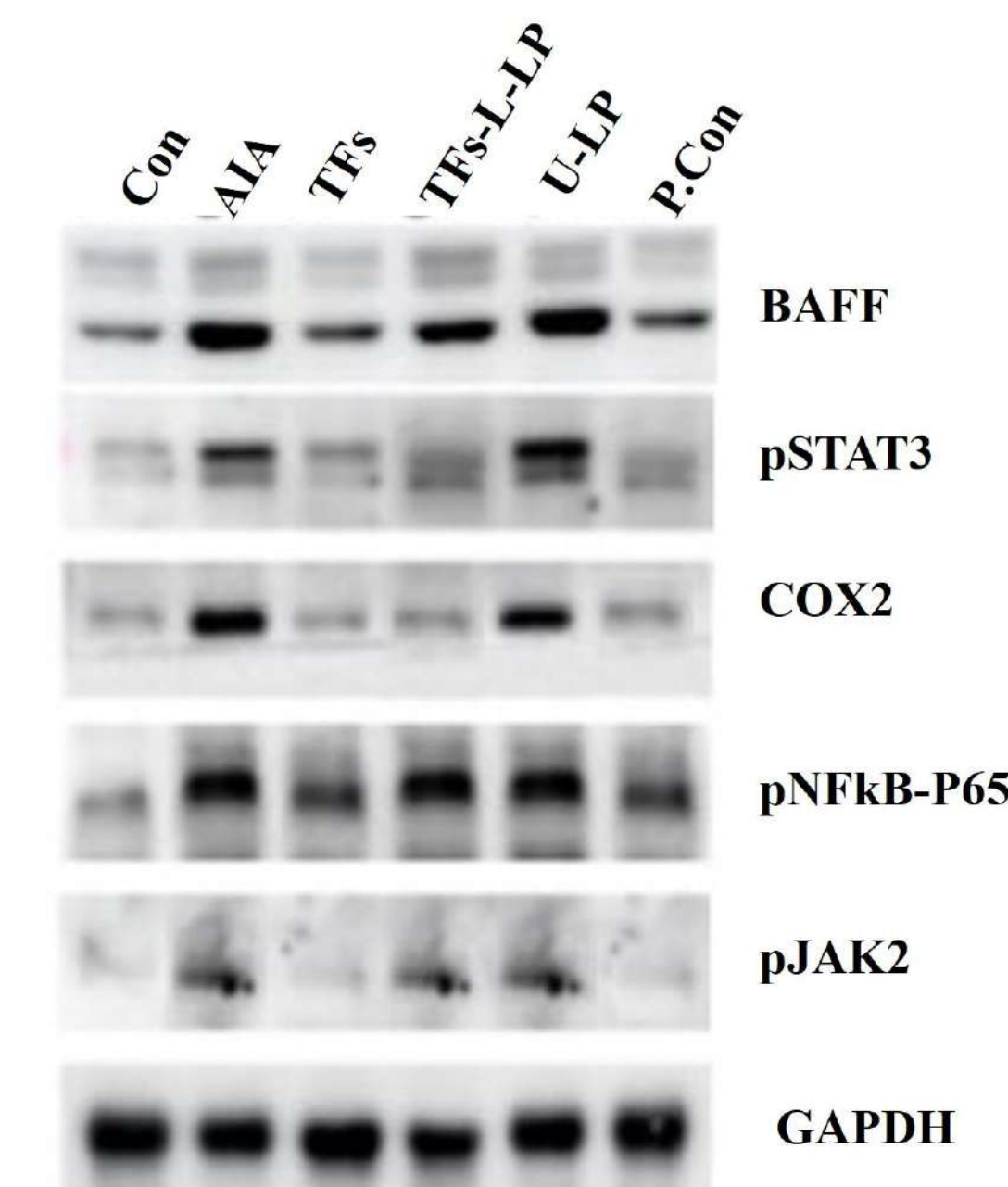
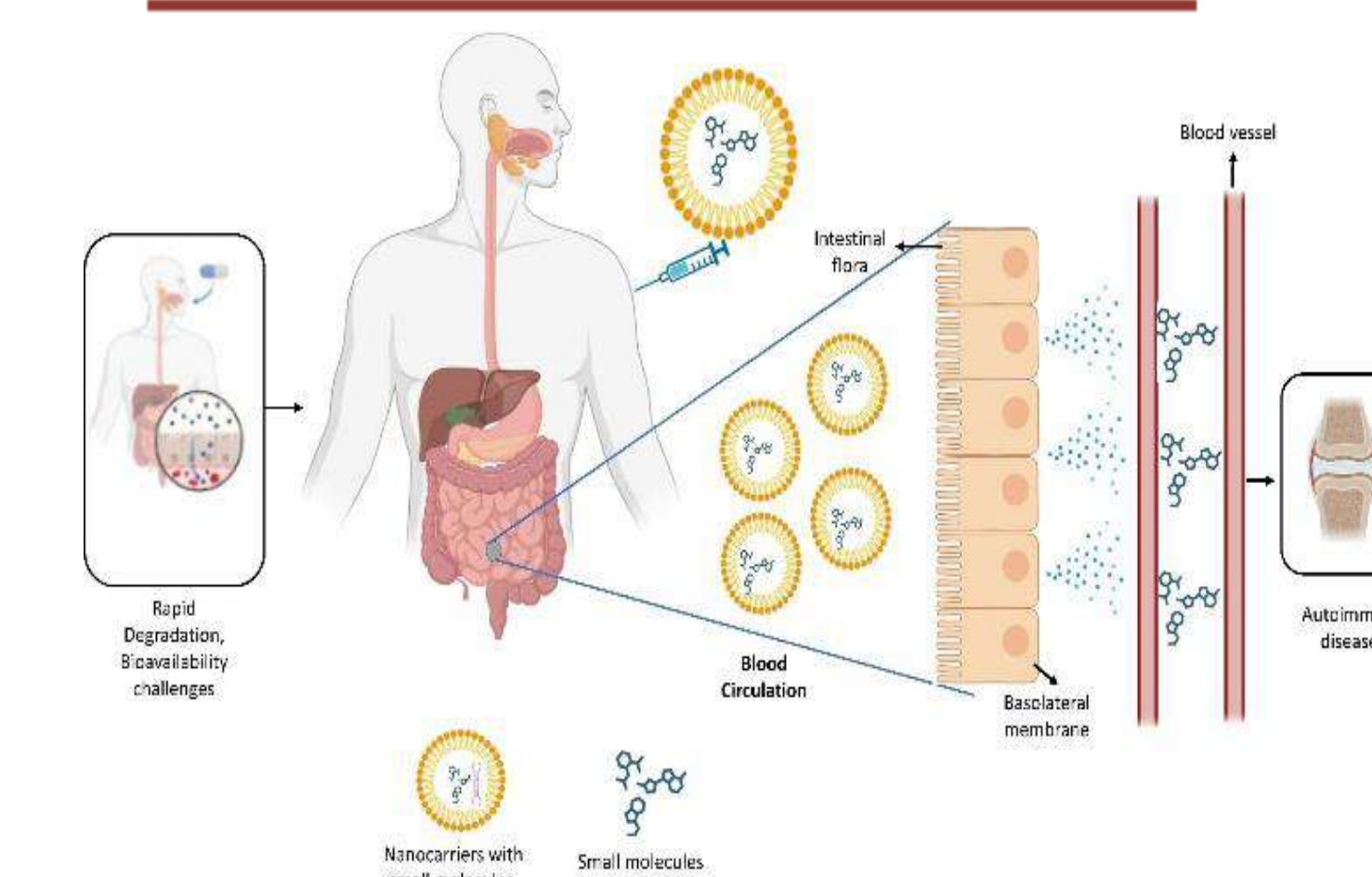
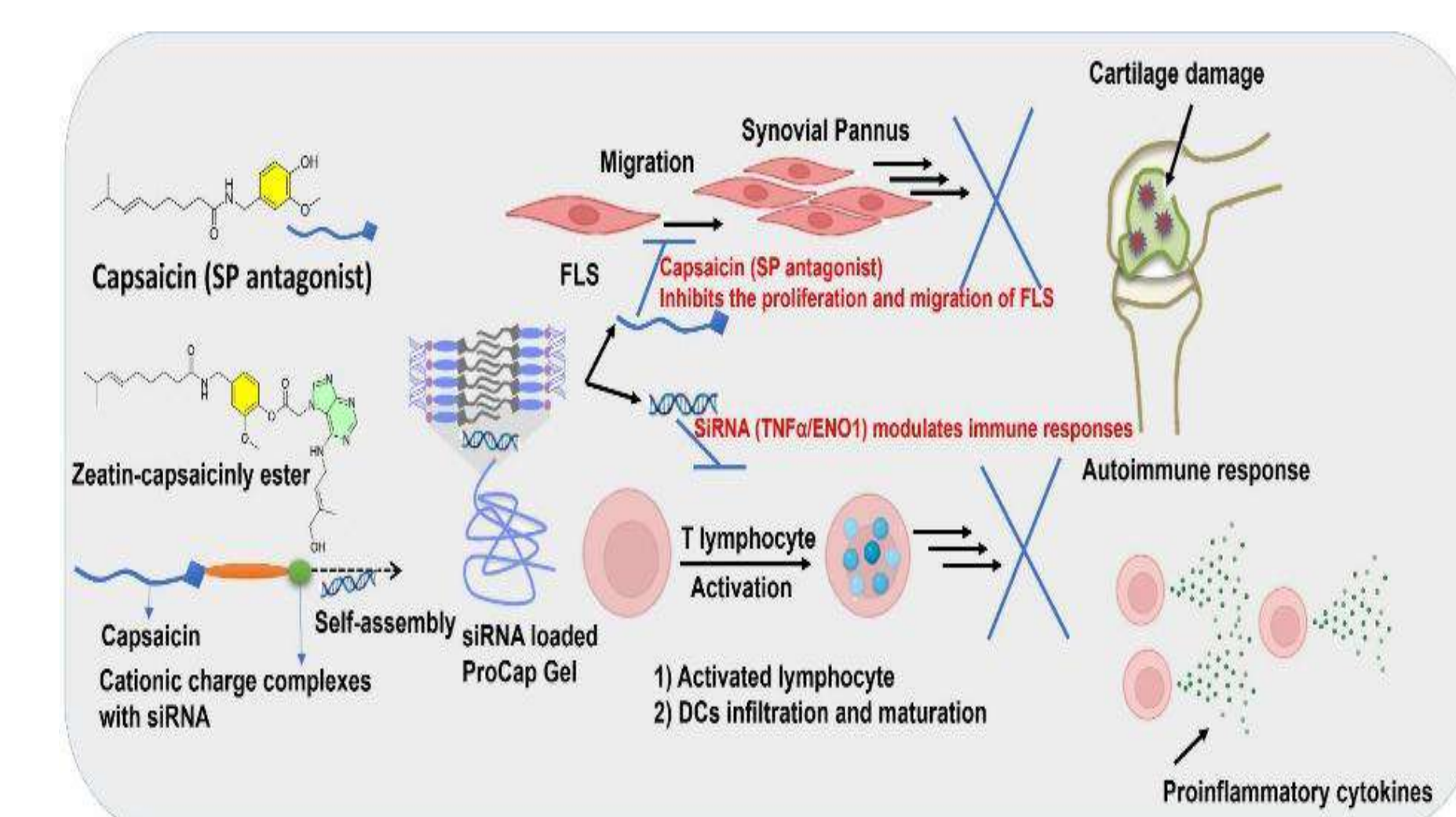
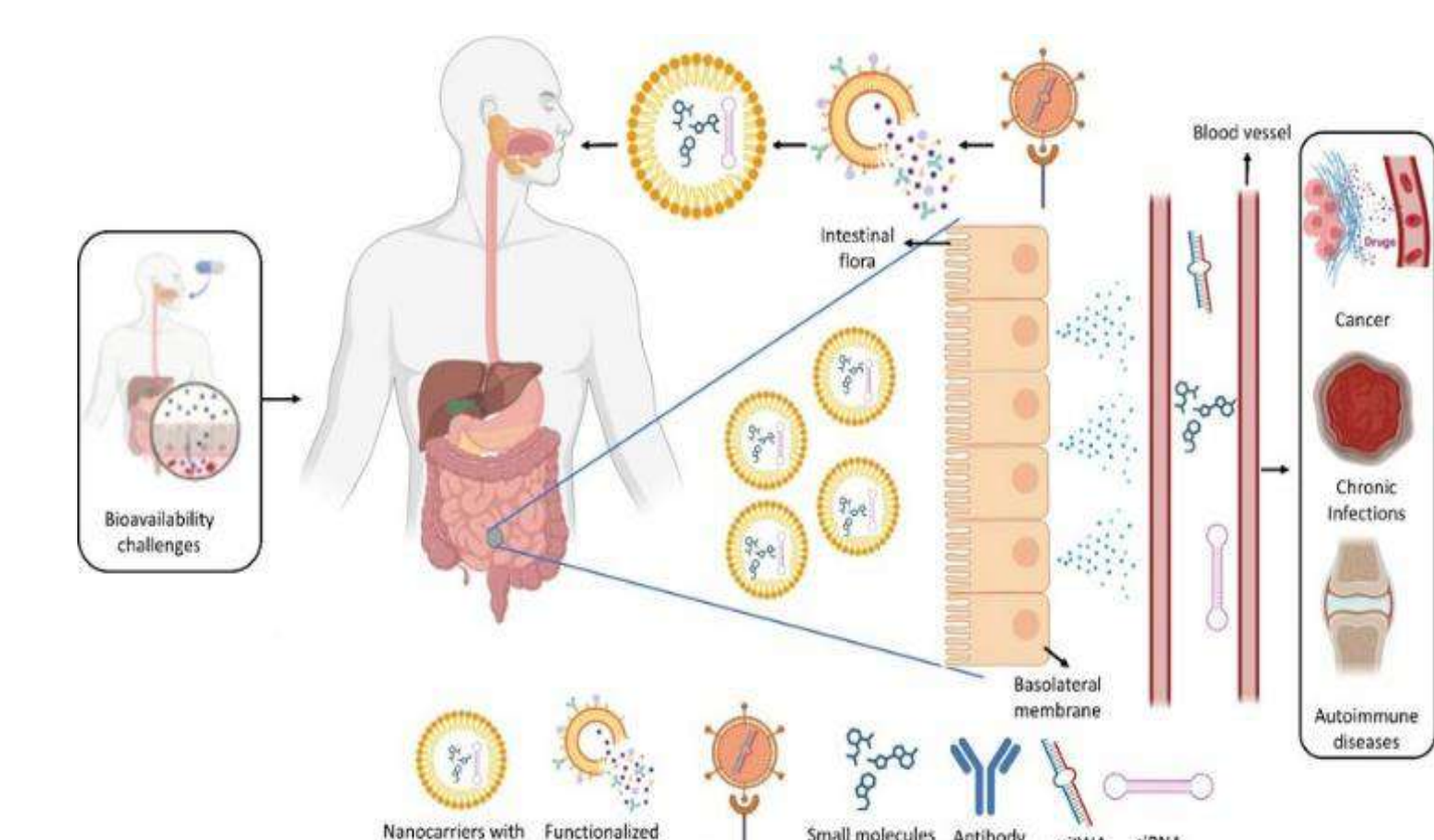


Figure 7: Protein expression of TFs-L-Lp

Conclusion



Future Directions



Focus on siRNA in Prodrug-based hydrogels will be utilized to prevent the formation of synovial pannus and reduce immune response

Acknowledgements

Thank you to:

- Dr. Abul Kalam Azad Mandal
- Dr. Srujan Marepally
- And the entire team

References

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Zhou B, et al., (2019) Therapeutic effects of a novel BAFF blocker on arthritis. *Signal Transduct Target Ther* 4:19

PRECLINICAL SAFETY STUDIES WITH GENE-EDITED HEMATOPOIETIC STEM AND PROGENITOR CELLS FOR THE TREATMENT OF BETA-HEMOGLOBINOPATHIES

Abhijith K^{1,2} & Saravanabhavan Thangavel¹

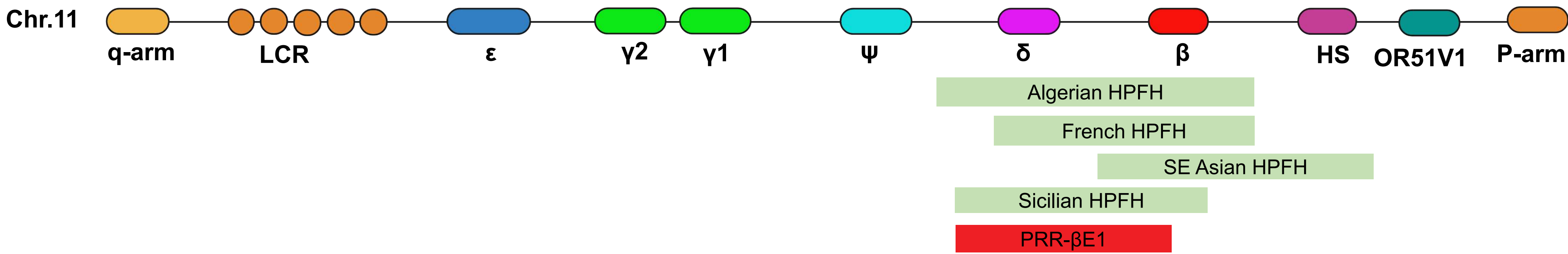
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²Manipal Academy of Higher Education, Manipal

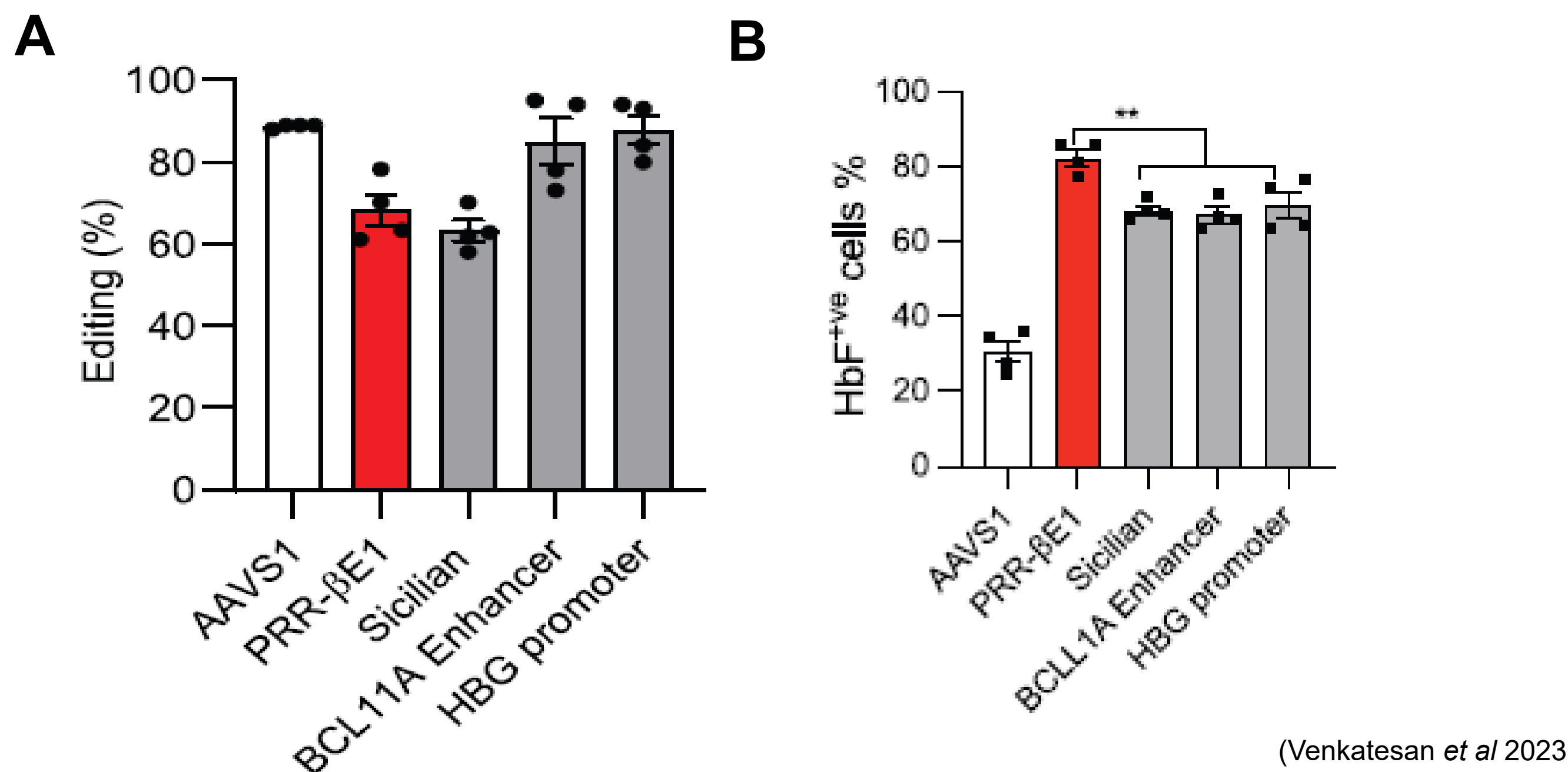


β-hemoglobinopathies

- Sickle cell disease (SCD) affecting approximately 0.2 million individuals in India and an estimated 7.7 million individuals globally.¹
- β-thalassemia accounts for around 0.3 million cases globally, with India contributing substantially to the disease burden, as nearly 40,000 affected infants are born annually.²
- Reactivation of fetal hemoglobin (HbF) is a well-recognized approach to ameliorate β-hemoglobinopathies. Elevated HbF can compensate for defective or insufficient β-globin production
- PRR-βE1 strategy is a bi-functional gene editing of the beta-globin locus for activation of fetal hemoglobin and downregulation and knock-out of defective adult globin³
- CRISPR/Cas9-induced double-strand breaks (DSBs) have the potential to cause genomic alterations, including chromosomal rearrangements, large unintended deletions, and translocations.⁴
- Optimizing the scale-up process to maintain efficiency, functionality, and safety through the use of clinical-grade reagents is essential for clinical transplantation applications

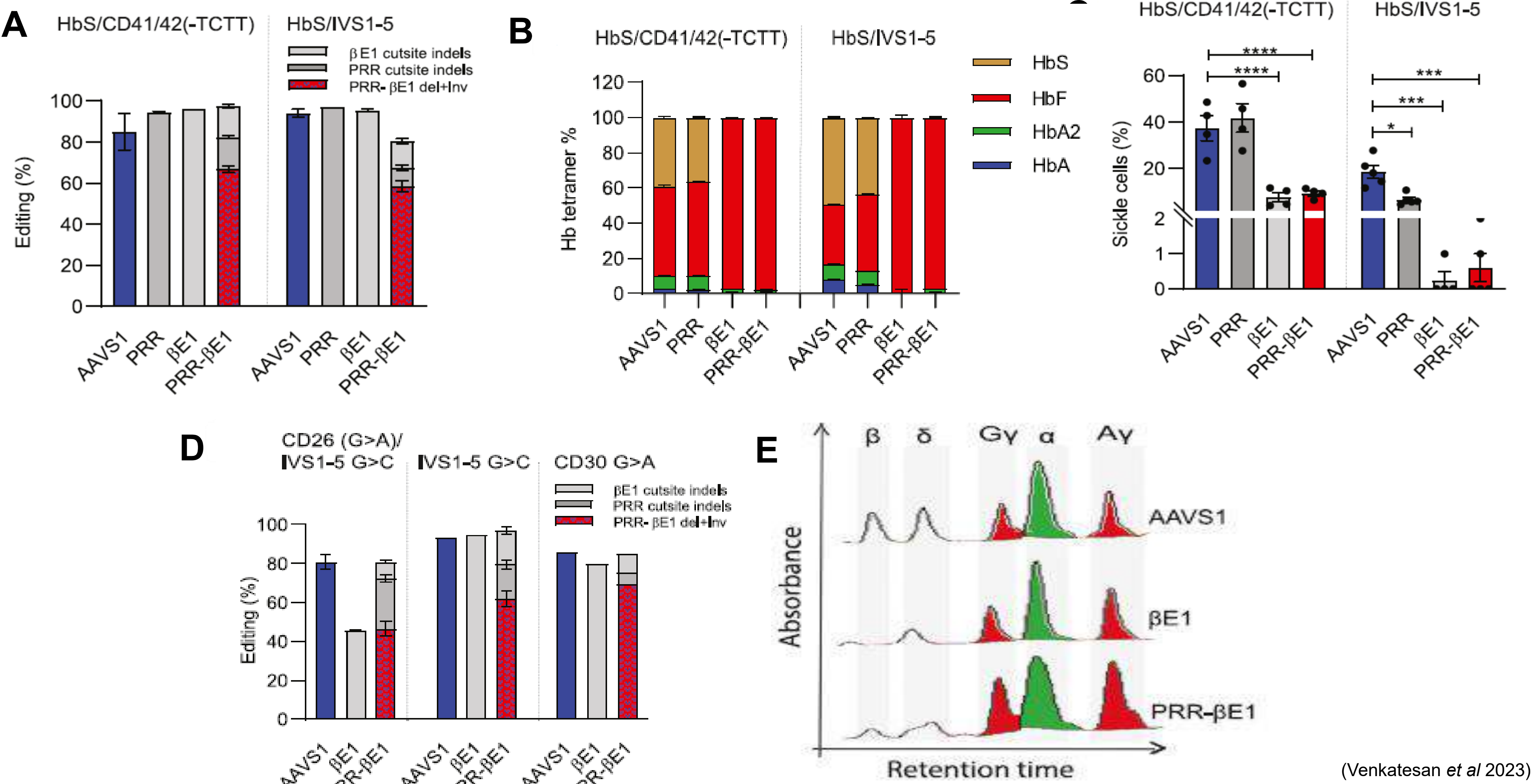


PRR-βE1 gene editing promotes HbF induction



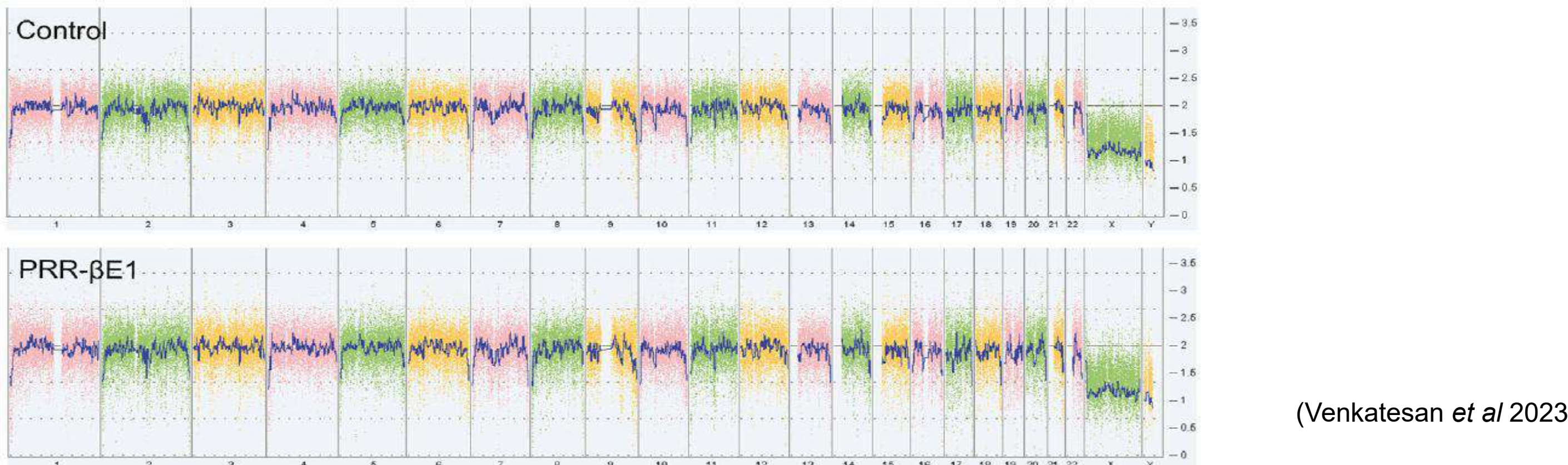
(A) Percentage of gene manipulation as measured by ddPCR for quantifying deletions in PRR-bE1 and Sicilian HPFH. Indel analysis of AAVS1, BCL11A enhancer, and HbG promoter by ICE. (B) percentage of HbF⁺ cells in erythroblasts generated from edited cells

PRR-βE1 gene-editing reverse the Sickle cell disease and β-Thalassemia phenotype



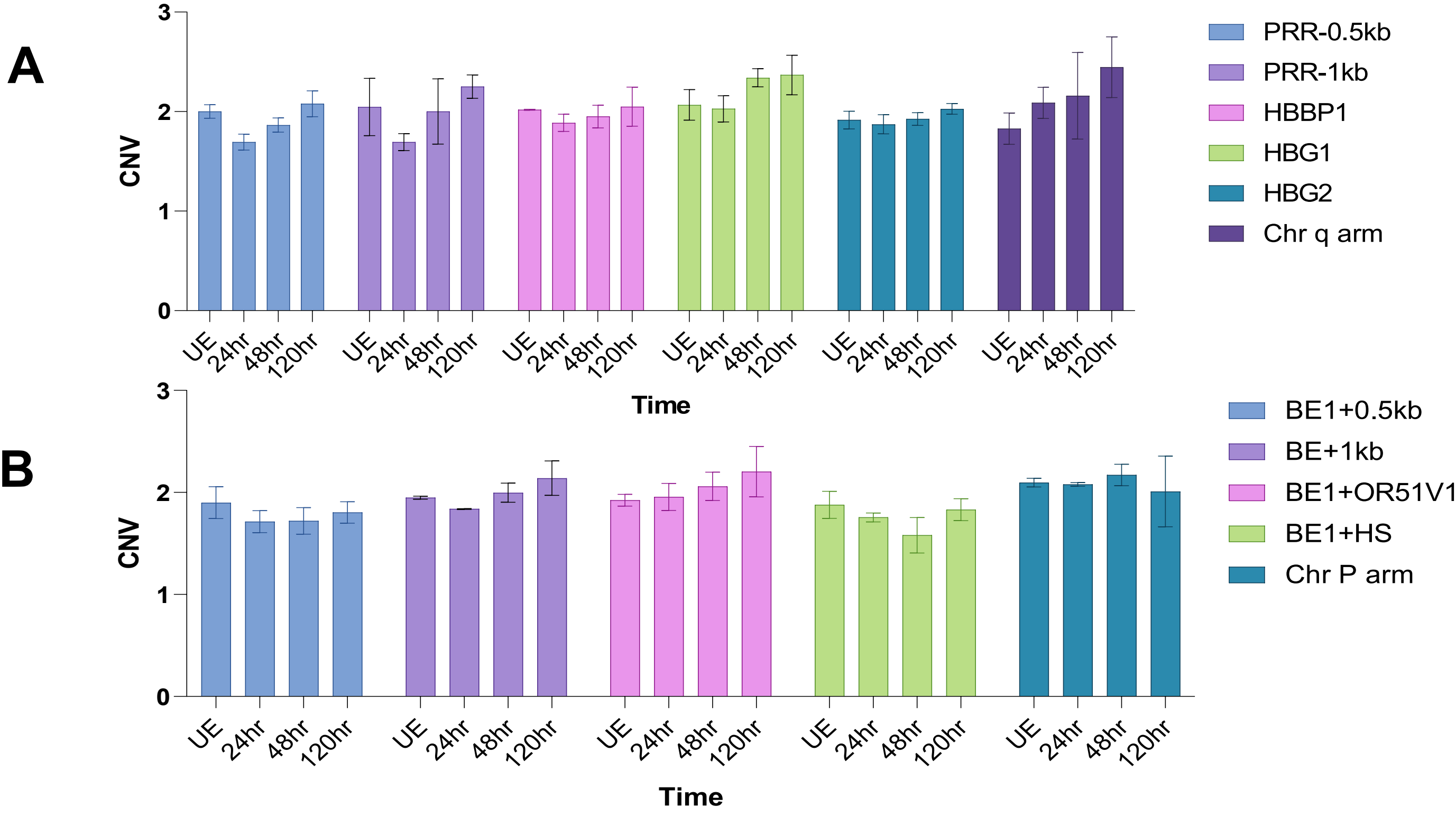
(A) Percentage of gene editing. Indels measured by Sanger sequencing and ICE analysis (B) Proportion of hemoglobin tetramer by variant HPLC. (C) Percentage of sickle cells. Gene-edited patient HSPCs were differentiated into erythroblasts in hypoxia (5% O₂) and the FACS sorted reticulocytes were treated with 1.5% sodium metabisulfite. (D) Percentage of gene editing in HSPCs. Deletion/Inversion (Del+Inv) in PRR-βE1 as quantified by ddPCR. Indels of the cut sites PRR and βE1 were measured by ICE analysis. (E) Representative globin chain HPLC chromatograms

KaryoStat™ analysis reveals no detectable structural abnormalities



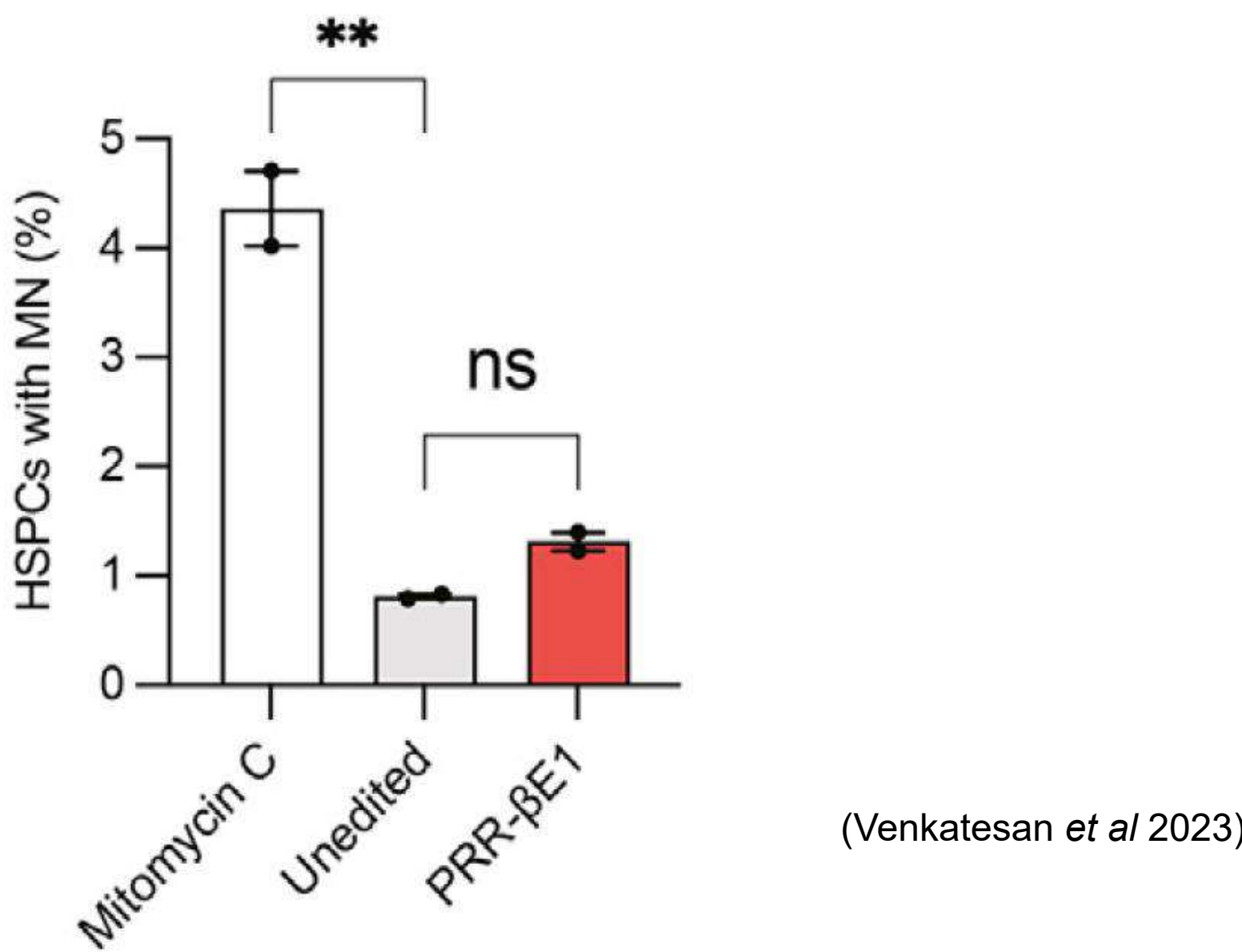
KaryoStat™ analysis of healthy donor derived HSPCs following CRISPR/Cas9-mediated PRR-βE1 deletion. The whole-genome coverage analysis with a resolution of >1 Mb revealed that the PRR-bE1 gene-edited HSPCs exhibited neither loss nor gain of chromosomal copy number

ddPCR analysis reveals no significant loss within the editing window



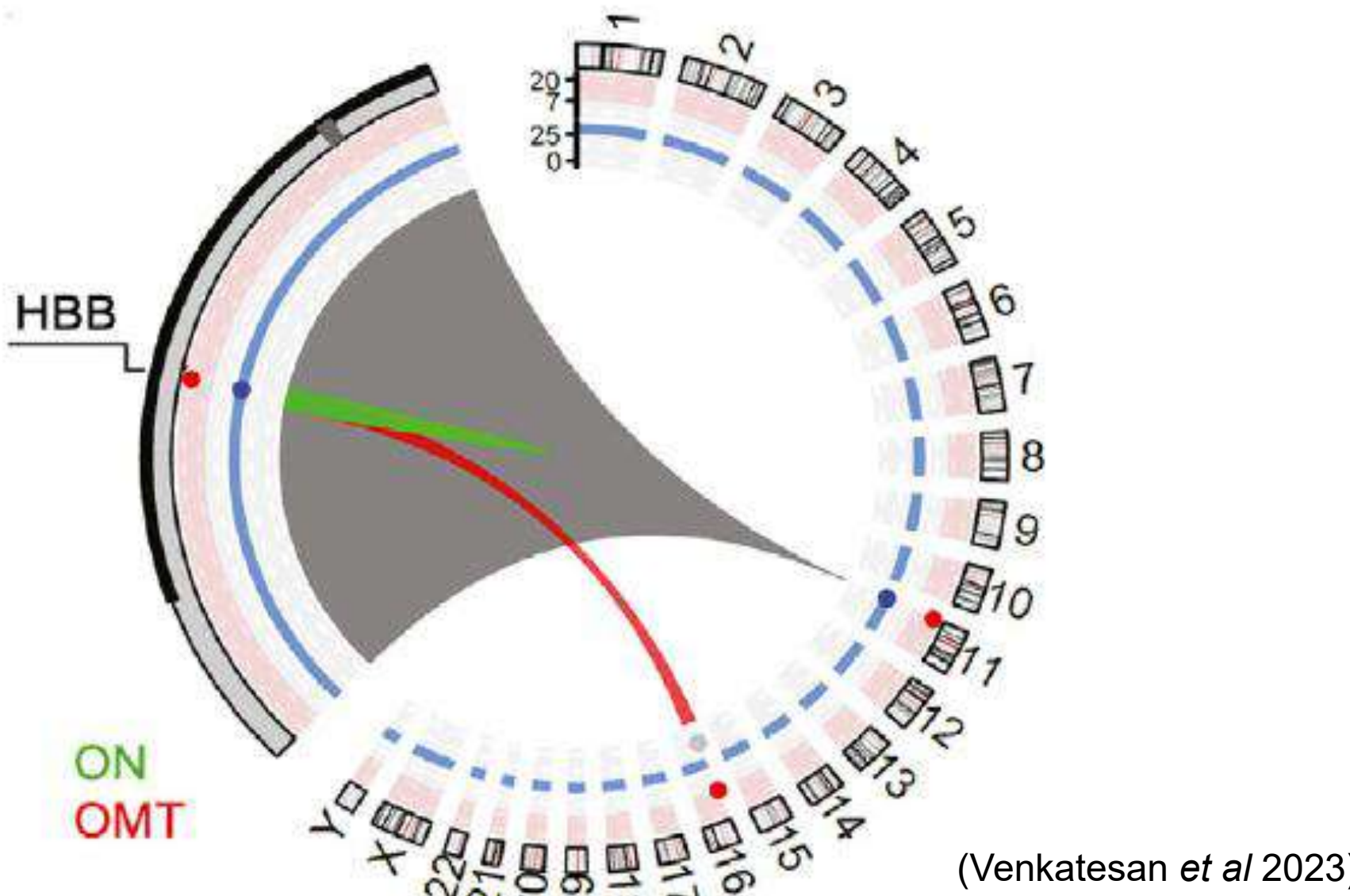
ddPCR copy number variation analysis showing that there is no significant loss in the editing window (A) Showing the extended deletion upstream to the PRR cut sites, (B) Showing the extended deletion upstream to the BE1 cut site

PRR-βE1 gene editing does not induce micronucleus formation in HSPCs



Percentage of micronucleus (MN) in Mitomycin C treated, unedited, and PRR-βE1 gene-edited HSPCs scored 48 h post nucleofection after staining with Giemsa was not significantly greater than in unedited control HSPCs

CAST-Seq analysis reveals no off-target mediated translocations



Circos plot showing off-target mediated translocation between the PRR-βE1 on-target site and βE1 off-target site in PRR-βE1 edited samples present in Chr16 identified by CAST-Seq

Summary

- Our study highlights the critical role of the PRR-βE1 region in the regulation of fetal hemoglobin
- (HbF) Establishes this locus as a prime target for gene editing strategies aimed at robust HbF induction.
- To facilitate the clinical translation of this approach, the development of optimized, Good Manufacturing Practice (GMP)-compliant protocols for large scale editing and production of PRR-βE1-edited HSPCs is essential. P
- Preliminary data indicate that our gene-editing strategy is safe, further comprehensive analyses are warranted to fully characterize its safety profile.

Reference

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Precision Biomarker Detection in Cell and Gene Therapy: Matrix-Validated ELISA Methods for Cytokine Quantification

Authors: Dr. Kalpesh Jain, Atul Gadhawe, Krishna Jain

ABSTRACT

Background: Reliable immune biomarker measurement is essential for the safe and effective development of cell and gene therapies (CGT). Cytokines are critical indicators of therapeutic response, immune activation, and treatment-related toxicity, including cytokine release syndrome (CRS) and neuroinflammation. However, cytokine quantification across serum, plasma, and cerebrospinal fluid (CSF) remains challenging due to matrix-dependent interference, low analyte abundance, and assay variability, limiting the correlation of preclinical data with clinical outcomes and reducing regulatory confidence.

Method: A suite of matrix-validated ELISA assays was developed for reproducible cytokine detection across serum, plasma, and CSF. Each assay was validated for accuracy, sensitivity, precision, linearity, and recovery using matrix-matched calibrators and optimized diluents to reduce interference. Calibration was aligned with NIBSC international standards to ensure traceability and harmonization across studies and laboratories.

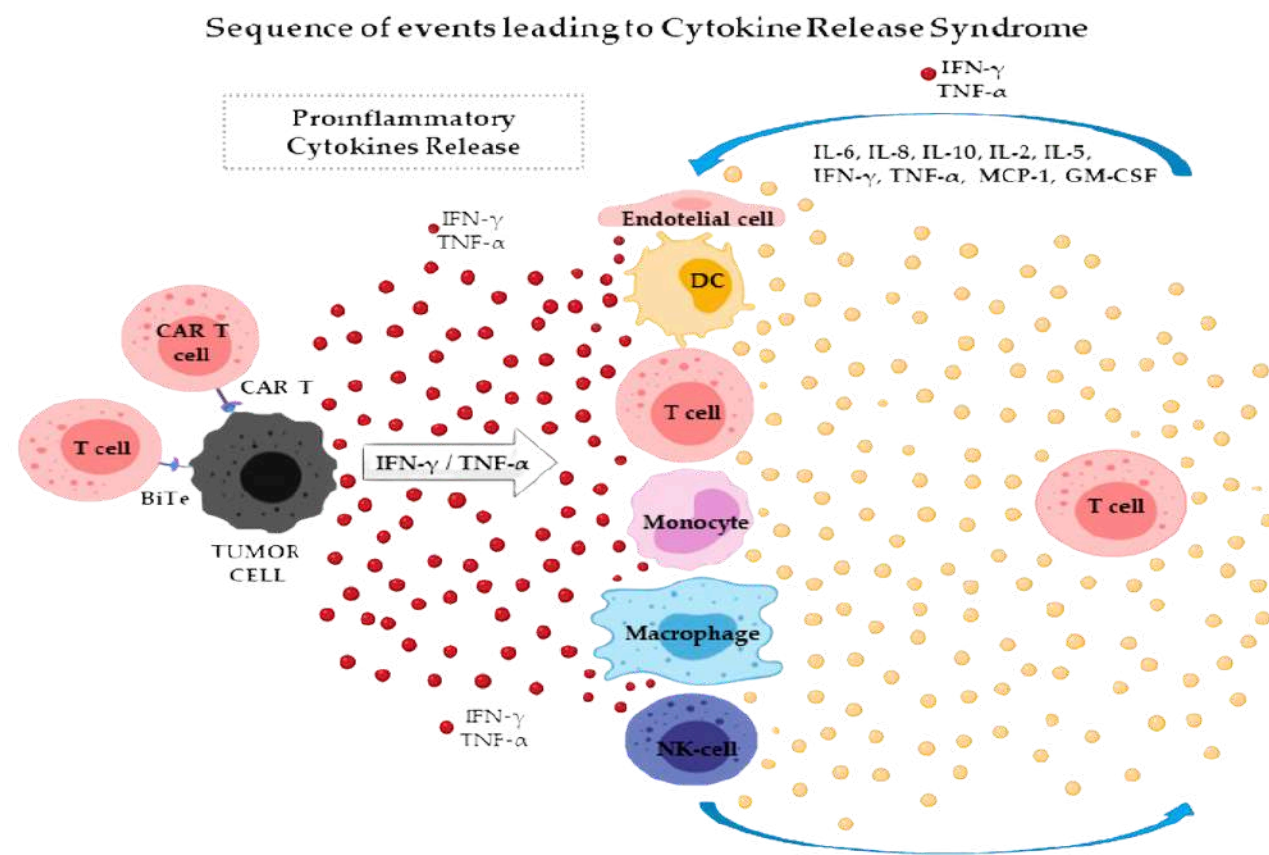
Results: The validated assays demonstrated high reproducibility and sensitivity for key cytokines relevant to CGT - including but not limited to IL-6, IL-10, IFN- γ , TNF- α , and GM-CSF. Enhanced detection enabled reliable quantification of low-abundance cytokines in CSF, supporting applications in neurotoxicity and CNS-targeted therapies. Cross-matrix validation confirmed strong correlation between sample types, enabling consistent biomarker interpretation across preclinical and clinical studies. Alignment with international standards improved inter-laboratory reproducibility and data comparability for multi-centre trials.

Clinical Impact and Conclusion: By integrating matrix validation and standardized calibration, these assays close a critical translational gap—the lack of harmonized, comparable cytokine data across studies and institutions. Standardized quantification enhances dose-response modeling, improves real-time safety monitoring, and enables data-driven clinical decisions. The use of international reference materials aligns with regulatory expectations and advances global standardization of immune monitoring in CGT. By ensuring analytical comparability across sample types, laboratories, and studies, these assays strengthen regulatory confidence and accelerate the safe, standardized translation of cell and gene therapies from research to clinical practice.

INTRODUCTION

Cell and gene therapies (CGT) have transformed therapeutic development by enabling precise, durable immune modulation in cancer, rare disease, and regenerative medicine. However, these innovative modalities also induce complex immune responses that must be tightly monitored to ensure patient safety and treatment efficacy. Cytokines such as IL-6, IL-10, IFN- γ , TNF- α , and GM-CSF serve as critical biomarkers for assessing therapeutic activity and detecting immune-related toxicities, including cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS).

Yet, quantifying cytokines accurately across biological matrices - serum, plasma, and cerebrospinal fluid (CSF) - remains a major analytical bottleneck. Protein complexity, matrix interference, and low analyte abundance often cause poor recovery and reproducibility. Most commercial cytokine kits are validated only in serum or plasma, offering limited sensitivity or validation in CSF or other translational matrices. This lack of matrix validation leads to inconsistent data between preclinical and clinical studies, reducing confidence in immune monitoring and delaying regulatory acceptance.



To address this translational gap, we developed and validated matrix-optimized ELISA assays, aligned with NIBSC International Cytokine Standards, for accurate and reproducible cytokine detection across multiple matrices. This approach establishes analytical traceability, supports inter-laboratory harmonization, and enables meaningful immune biomarker interpretation in CGT research and clinical trials.

This poster presents the development of matrix-validated, NIBSC-aligned ELISA assays that ensure accurate and reproducible cytokine quantification across serum, plasma, and CSF. By addressing the limitations of current commercial kits, these assays enable reproducible, regulatory-ready cytokine data across studies and laboratories. Ultimately, this work supports faster and safer translation of next-generation CGT products from bench to bedside.

RESULTS AND VALIDATION

SENSITIVITY AND CLINICAL APPLICABILITY

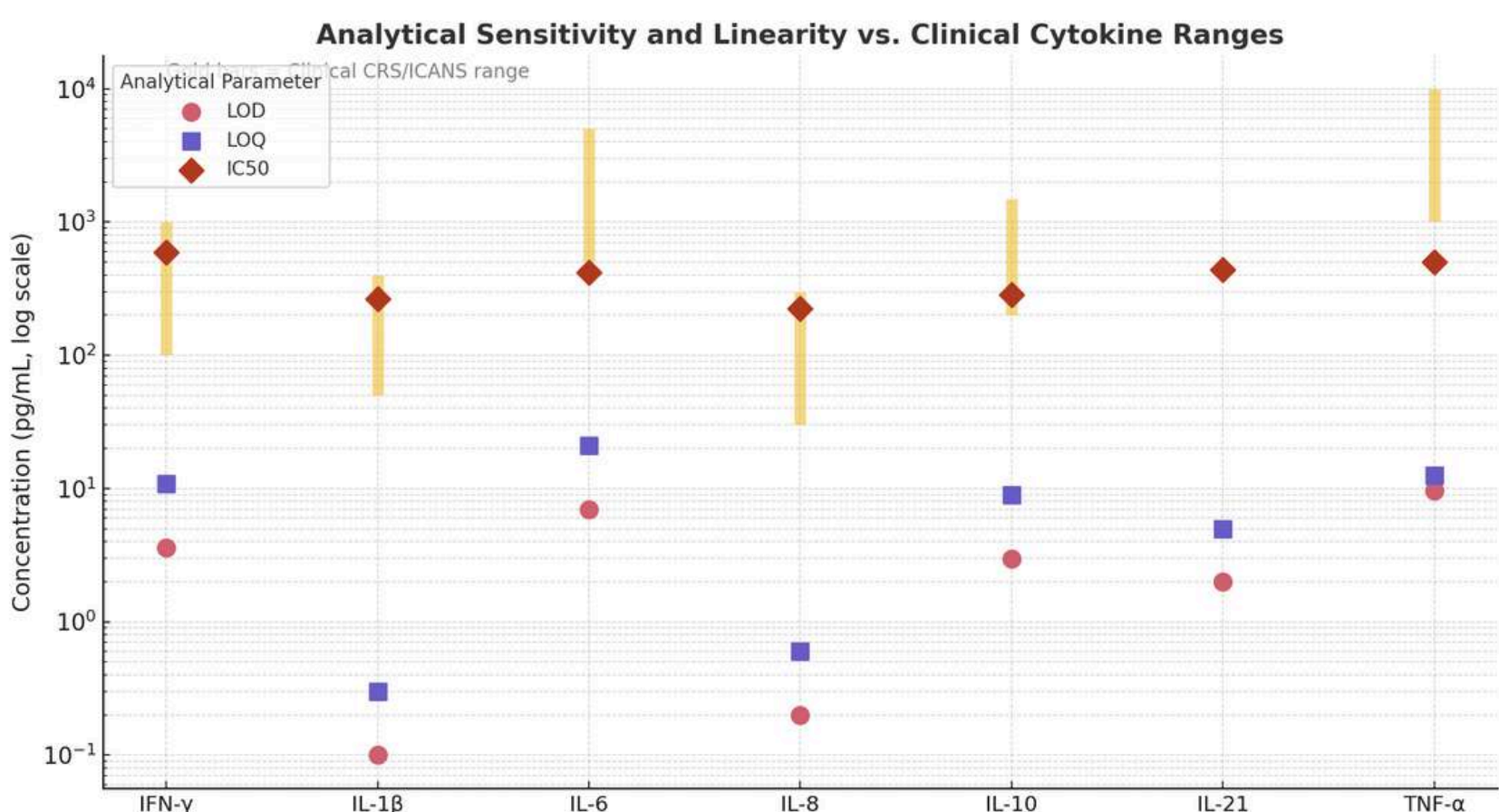


Figure 1: Assay dynamic ranges were compared against reported clinical Cmax values for key cytokines, including IL-1 β , IL-6, IL-8, IL-10, IL-12, IL-15, IFN- γ , and GM-CSF. Figure 2: The working range of each PrecisionBind™ Human Cytokine ELISA overlapped with or exceeded the expected cytokine concentrations observed across healthy, inflammatory, and cytokine storm conditions.

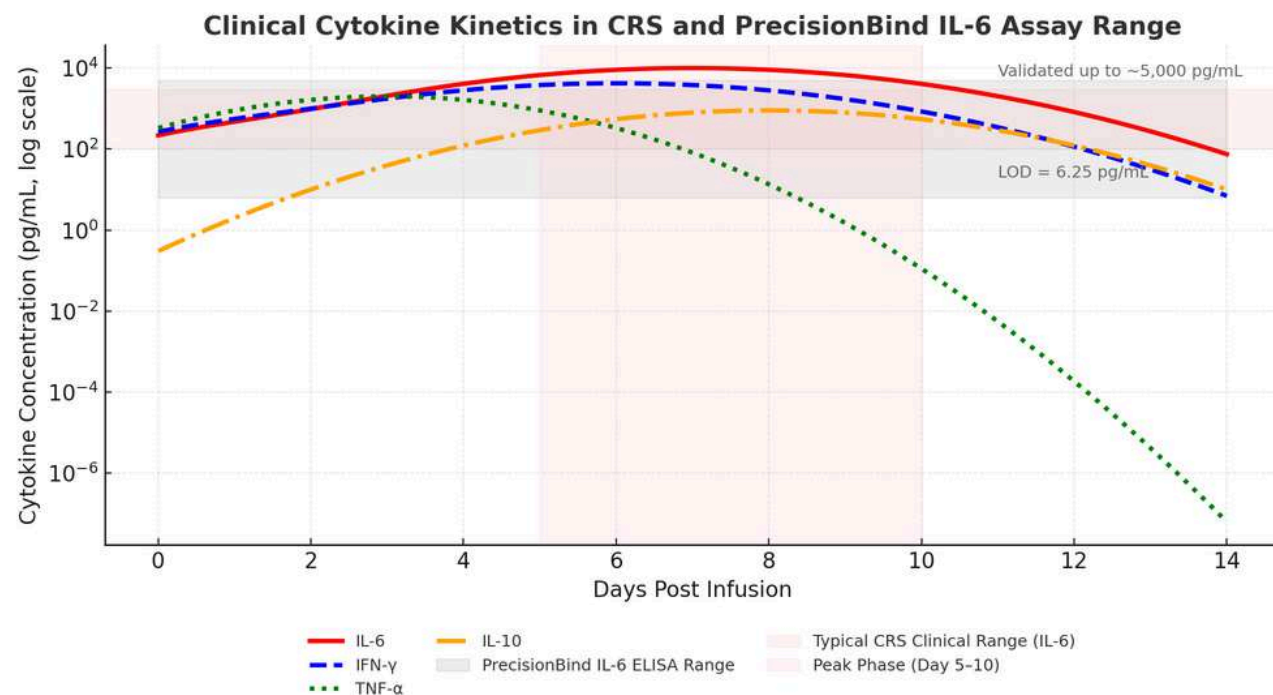
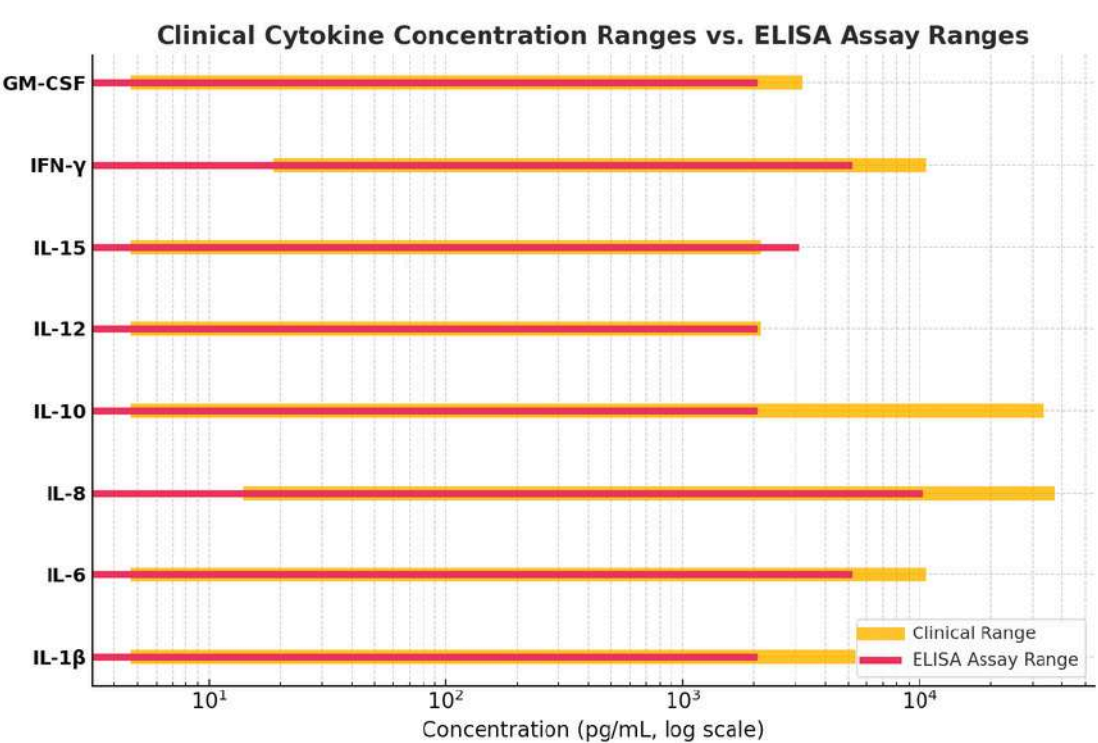


Figure 3: Standard Curve with Clinical Relevance

Assay dynamic ranges were compared against reported clinical Cmax values for key cytokines, including IL-1 β , IL-6, IL-8, IL-10, IL-12, IL-15, IFN- γ , and GM-CSF. As shown in Figure X, the working range of each PrecisionBind™ Human Cytokine ELISA overlapped with or exceeded the expected cytokine concentrations observed across healthy, inflammatory, and cytokine storm conditions.

RECOVERY AND MATRIX EFFECT

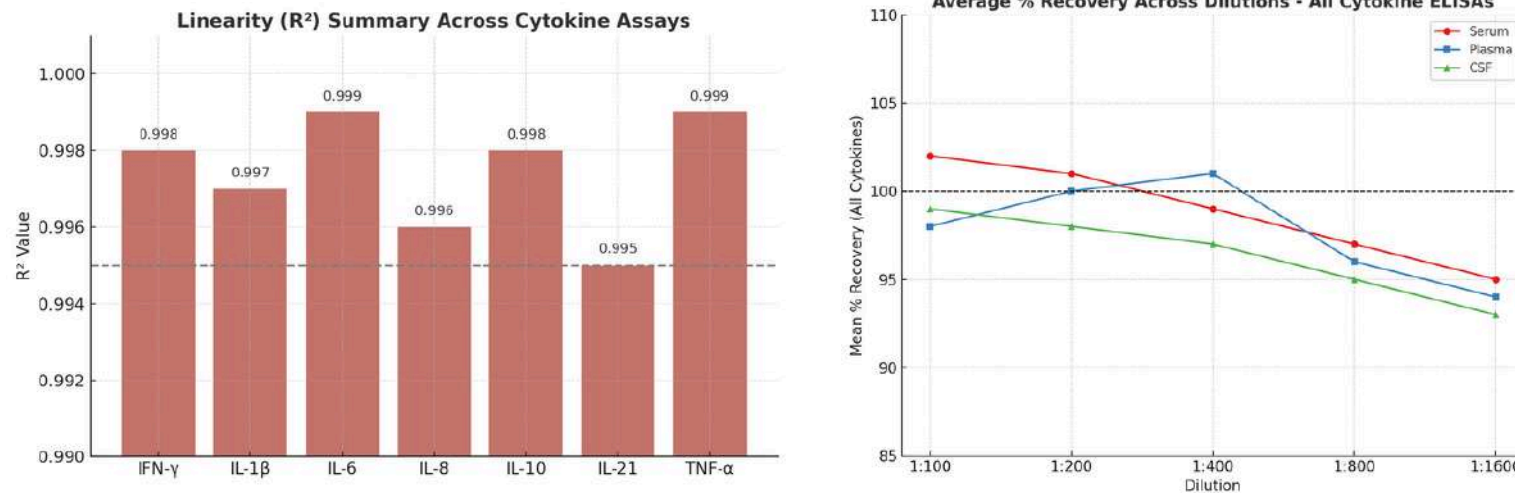


Figure 4: Linearity (R^2) summary across cytokine assays.

Figure 5: Mean recovery across dilutions in serum, plasma, and CSF.

Spiking and dilutional recovery studies across serum, plasma, and CSF confirmed excellent linearity ($R^2 \geq 0.995$) and consistent recovery within $\pm 10\%$ of the expected concentration across multiple cytokine assays. The data demonstrate traceable, reproducible quantification of cytokines across complex biological fluids.

LOT VARIABILITY

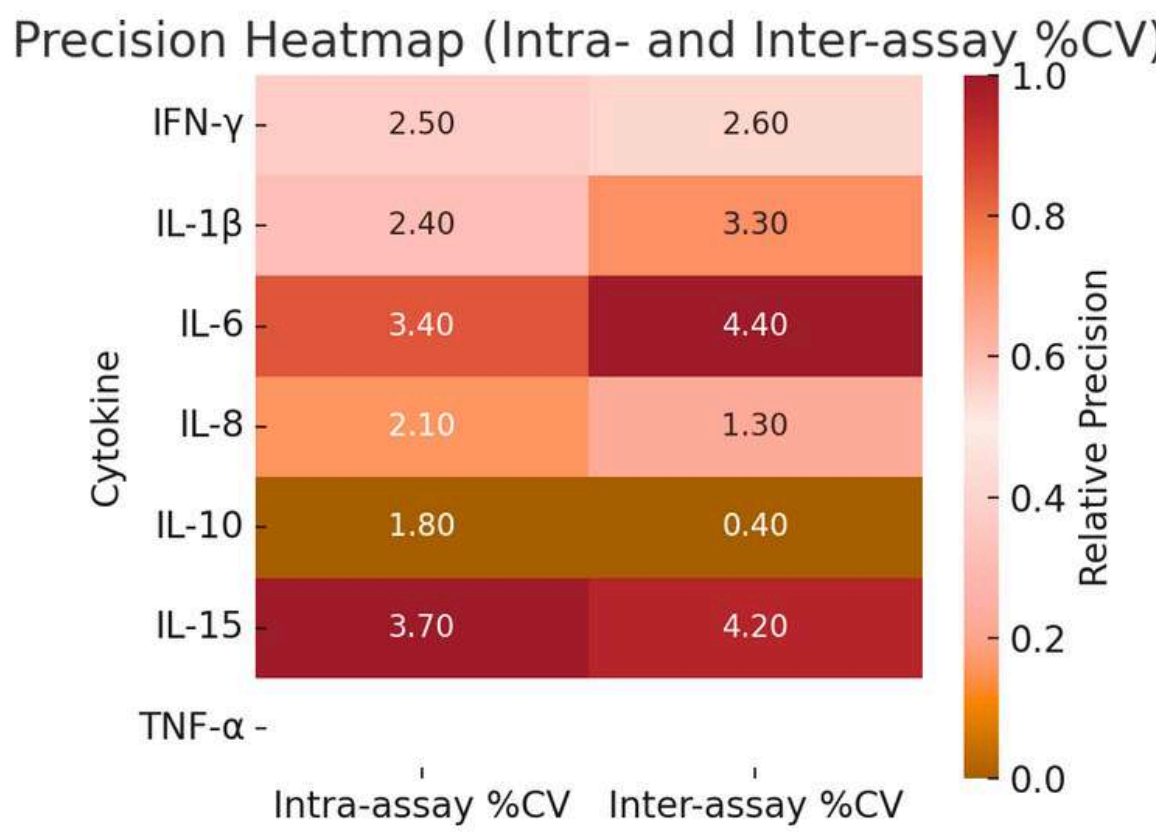


Figure 6: This heatmap summarizes the reproducibility performance for each cytokine, where greener values indicate higher precision (lower %CV). Minimal variability was observed between assay runs and manufacturing batches, confirming the robustness and stability of the assay formulations.

PARALLELISM AND ANALYTICAL LINEARITY

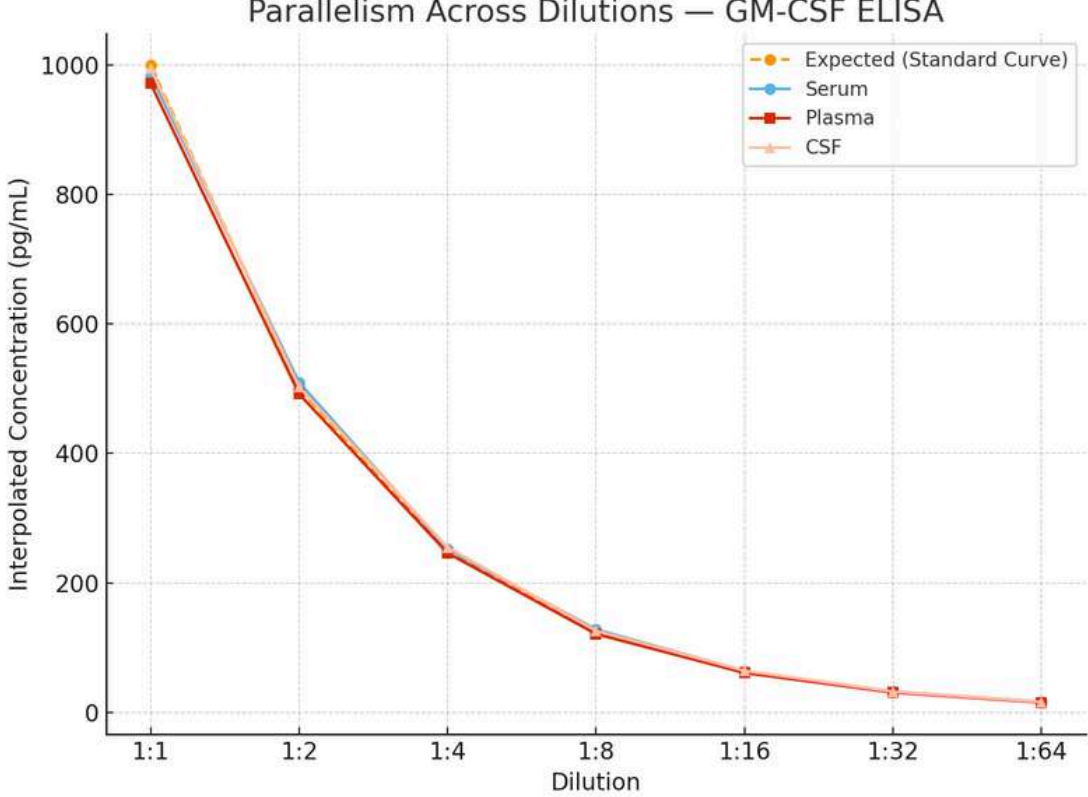
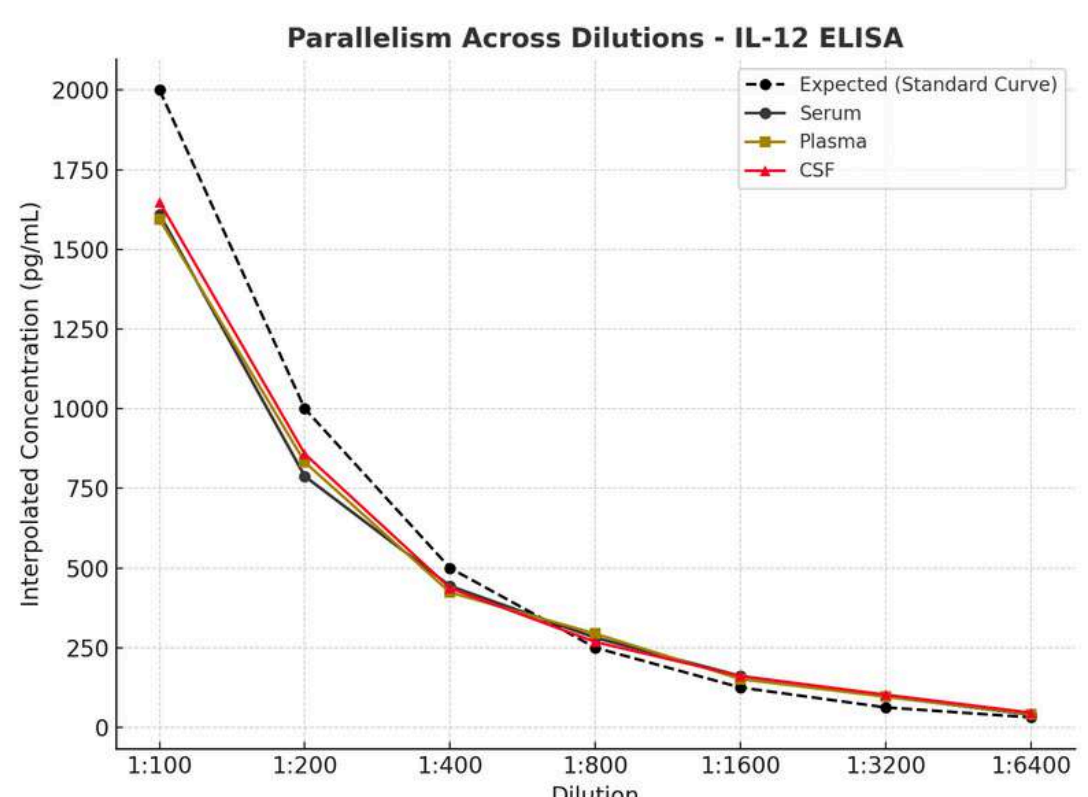


Figure 7 and 8. Parallelism across serial dilutions for IL-12 and GM-CSF ELISA respectively.

Serum, plasma, and CSF samples show dilutional linearity parallel to the standard curve, confirming consistent response and absence of matrix effects.

Serial dilutions of serum, plasma, and CSF samples demonstrated parallelism with the expected standard curve for both IL-12 and GM-CSF ELISAs. The comparable slopes across matrices and observed linearity confirms robust assay performance, traceable quantification, and elimination of matrix interference - a critical requirement for reliable cytokine measurement in Cell and Gene Therapy (CGT)-related samples.

These results reinforce the traceability and analytical reliability of the PrecisionBind™ cytokine ELISAs, ensuring accurate quantification of clinical and translational samples within physiologically relevant ranges.

NIBSC CALIBRATION

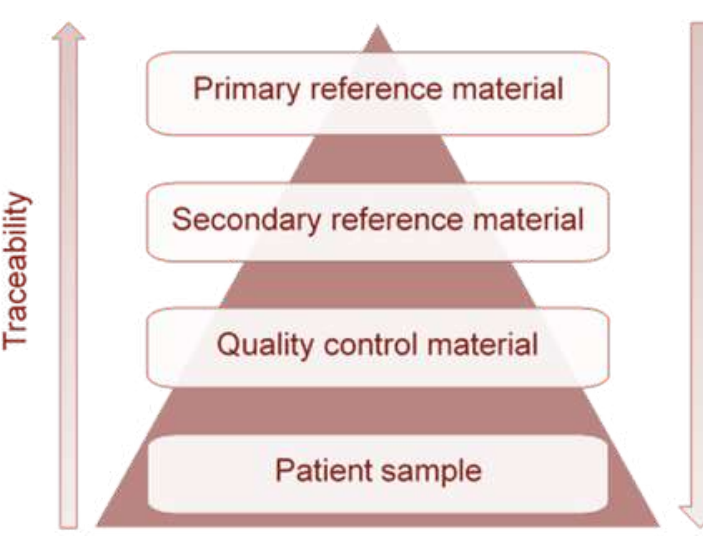


Figure 9. Traceability hierarchy showing how measurement uncertainty increases from primary reference materials to patient samples (eg. NIBSC WHO International Standards).

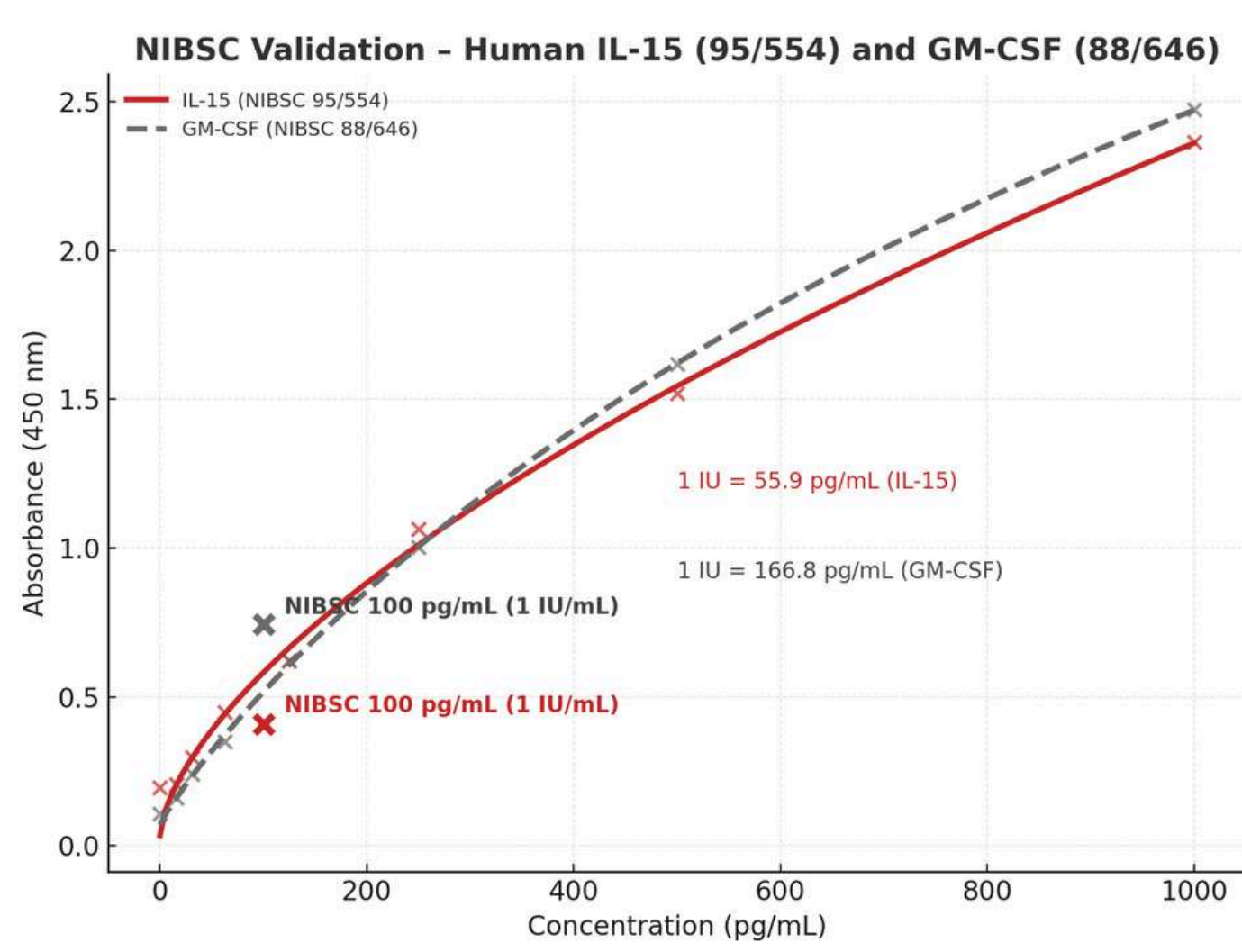


Figure 10. NIBSC Validation - Human IL-15 (95/554) and GM-CSF (88/646): Calibration curves demonstrate assay traceability to NIBSC reference standards (X), confirming equivalence of 1 IU = 55.9 pg/mL for IL-15 and 1 IU = 166.8 pg/mL for GM-CSF. Similar NIBSC Validation was conducted for all PRECISIONBIND cytokine ELISA.

PRECISION

Cytokine	LOD (pg/mL)	LOQ (pg/mL)	Intra-assay %CV	Inter-assay %CV
IFN- γ	3.6	10.9	2.5	2.6
IL-1 β	0.1	0.3	2.4	3.3
IL-6	3	5	3.4	4.4
IL-8	0.2	0.6	2.1	1.3
IL-10	2.97	9.01	1.8	0.4
IL-15	3.62	10.97	3.7	4.2

Figure 6: Distribution of OD450 values per standard across all lots, highlighting close signal spread with minimal outliers.

Intra- and inter-assay precision was evaluated across more than 50 independent production lots for each cytokine. The results demonstrate exceptional lot-to-lot consistency, with mean intra-assay CVs below 4% and inter-assay CVs below 5% across all analytes tested. Across seven cytokine ELISAs, intra- and inter-assay precision remained below 10% at all tested levels (12.5–800 pg/mL), confirming reproducibility across dynamic range and matrix types. Variation decreased with increasing concentration, aligning with theoretical expectations for immunoassays. This consistency enables reliable biomarker quantification in both preclinical and clinical CGT studies.

CROSS MATRIX CORRELATION AND COMPARABILITY (SERA, PLASMA, CSF)

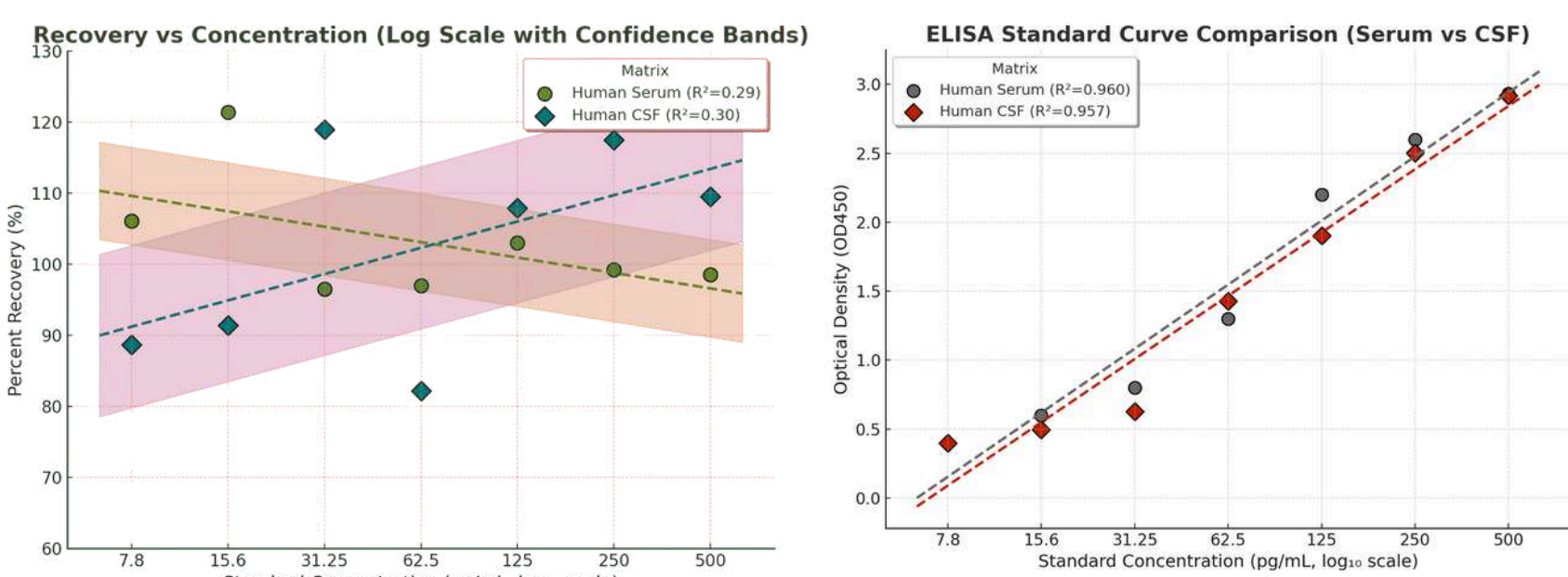


Figure 11, 12: Comparative graphs for Human TNF Alpha for Human Serum and Human CSF runs - showing recovery against standard concentration - with both matrices maintain stable recovery independent of concentration

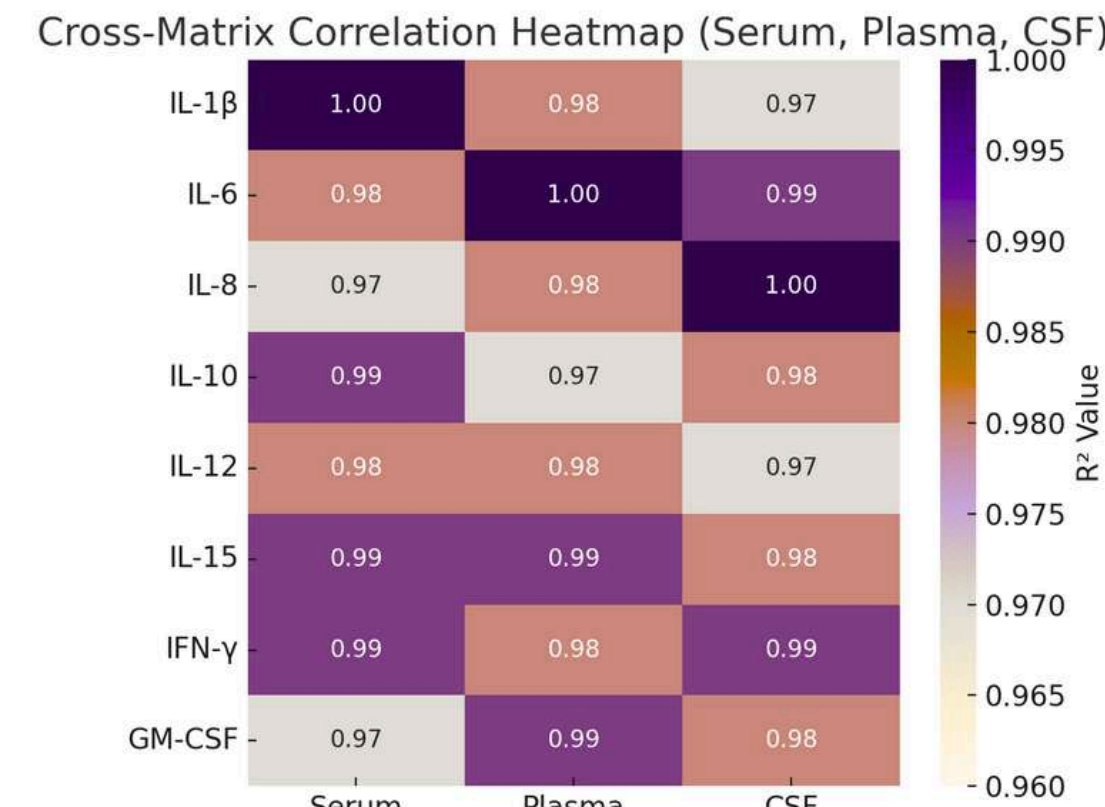
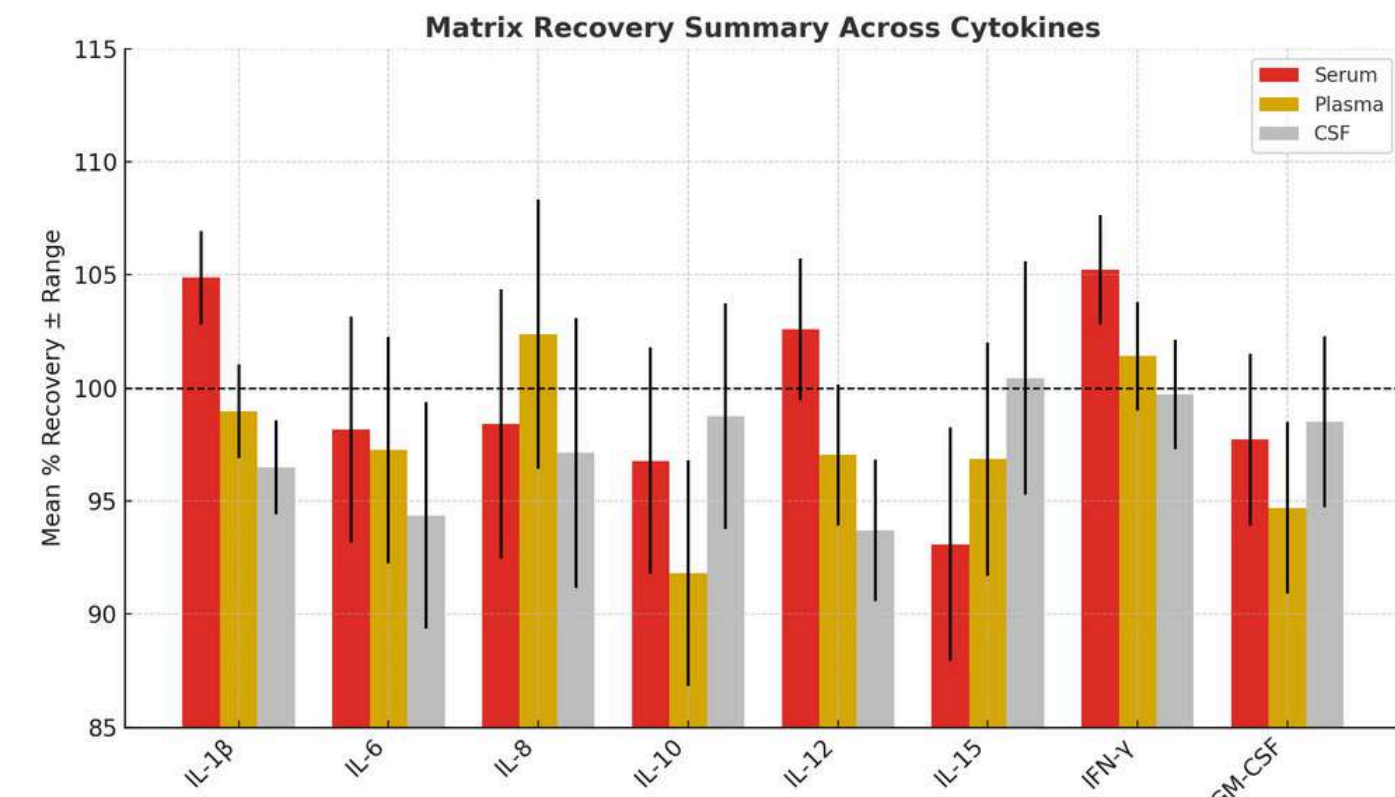


Figure 13: Interpolated concentration (% recovery). The grey box indicates ideal 100 $\pm$ 20% accepted recovery as per FDA Bioassay Guidelines.

Figure 14: Mean recovery $\pm$ range across serum, plasma, and CSF matrices for eight cytokine ELISAs. Bars represent average recovery, and error lines denote assay variation.

Figure 15: Regression comparison of serum versus plasma and serum versus CSF recovery profiles. Near-unity slopes and high R^2 values demonstrate strong matrix equivalence across sample types.

CONCLUSION

Matrix-validated and internationally standardized ELISA methods address a fundamental unmet need in CGT analytics - the absence of reliable, matrix-compatible commercial assays capable of detecting cytokines reproducibly across diverse biological samples.

By integrating matrix optimization with NIBSC-aligned calibration, these assays deliver:

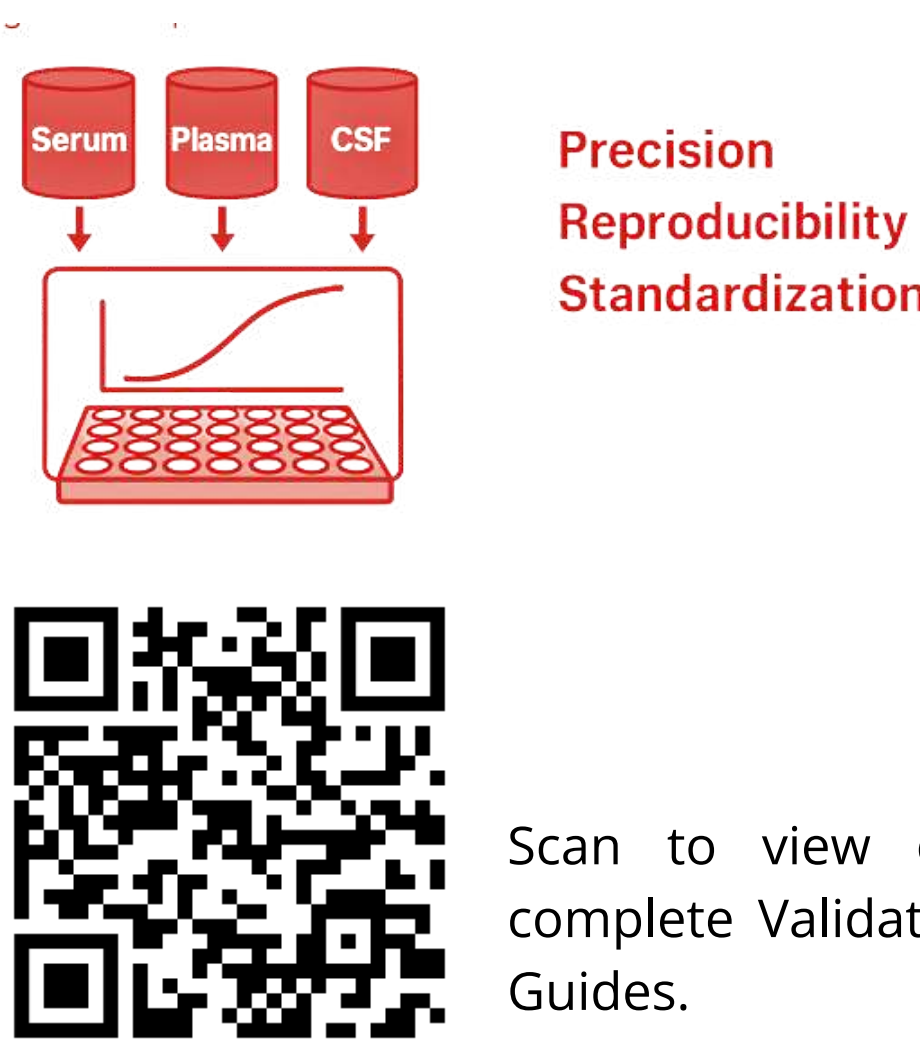
- Consistent, sensitive cytokine quantification across serum, plasma, and CSF
- Accurate differentiation between systemic (CRS) and CNS-related (ICANS) inflammation
- Reduced assay variability and enhanced inter-lab comparability
- Regulatory-ready, traceable data supporting harmonization across studies and sites

Clinically, this approach empowers investigators to detect toxicity earlier, tailor therapeutic dosing, and generate reproducible immune monitoring data across the CGT development continuum - transforming cytokine measurement from a variable research assay into a globally standardized translational tool, advancing the precision and safety of next-generation cell and gene therapies.

About Krishgen Biosystems:

Established 2003, Krishgen is an immunoassay manufacturer based out of Mumbai, India. Our key products include assays for mAbs, bispecific antibodies, antibody-drug conjugates, peptides as well as a wide range of cytokine and biomarker ELISA across various species.

Our products are well validated, sensitive, robust and competitively priced. As of April 2025, Krishgen ELISA have been cited in 5000+ publications worldwide. **Learn more about Krishgen and ELISA we offer at www.krishgen.com or reach out to us at kbiinfo@krishgen.com**



Scan to view our complete Validation Guides.



Adenoviral CRISPR-Based Targeted ATOH1 Integration for Hair Cell Regeneration: A Novel Strategy to Rescue Auditory Function

Pankaj Keshari¹, Ayesha Rahman¹, Arvind Kumar Kairo², Riyaz Ahmad Mir^{1*}

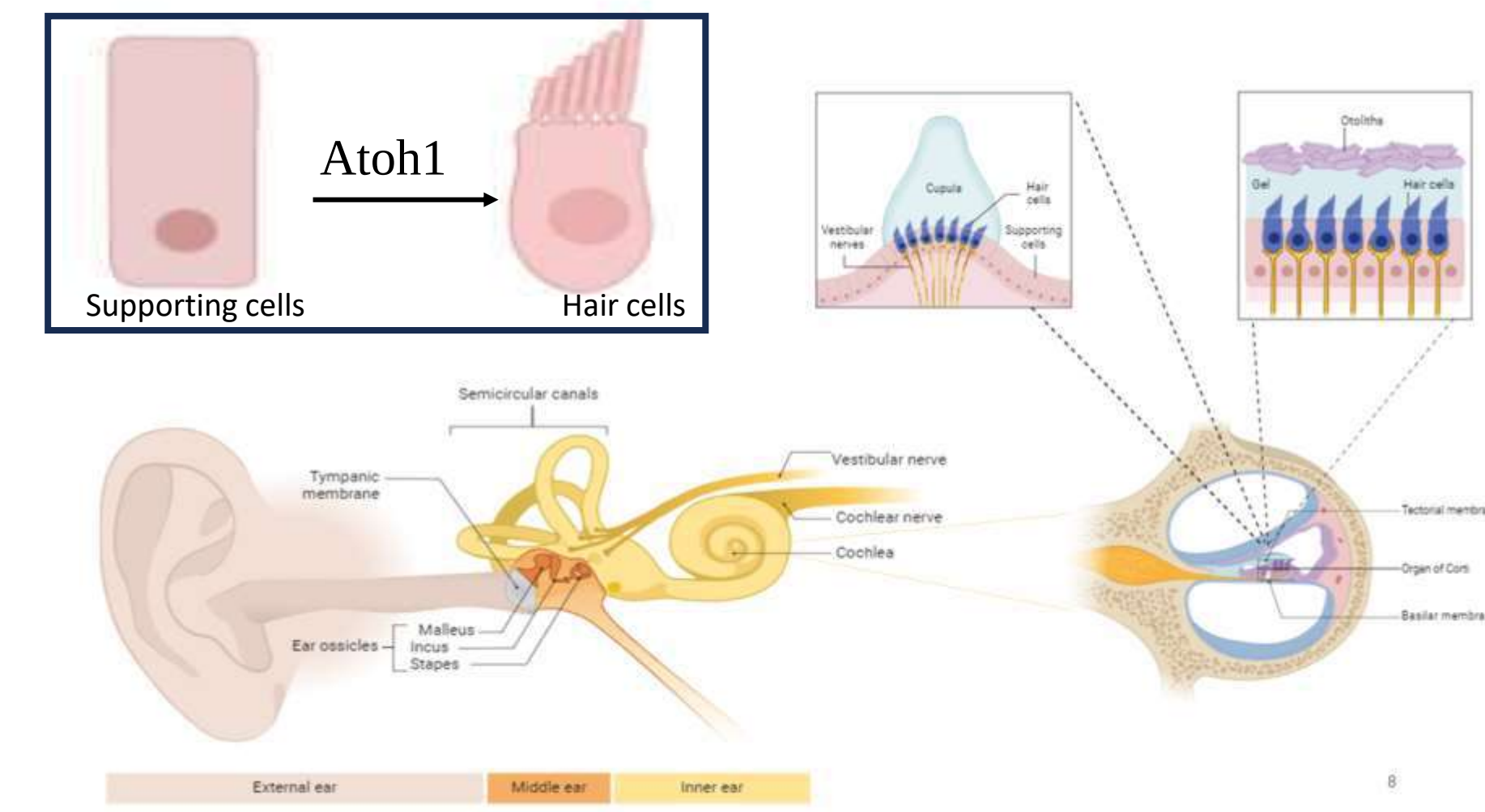
Department of Biochemistry¹, Department of Otorhinolaryngology², All India Institute of Medical Sciences, New Delhi 110029, India

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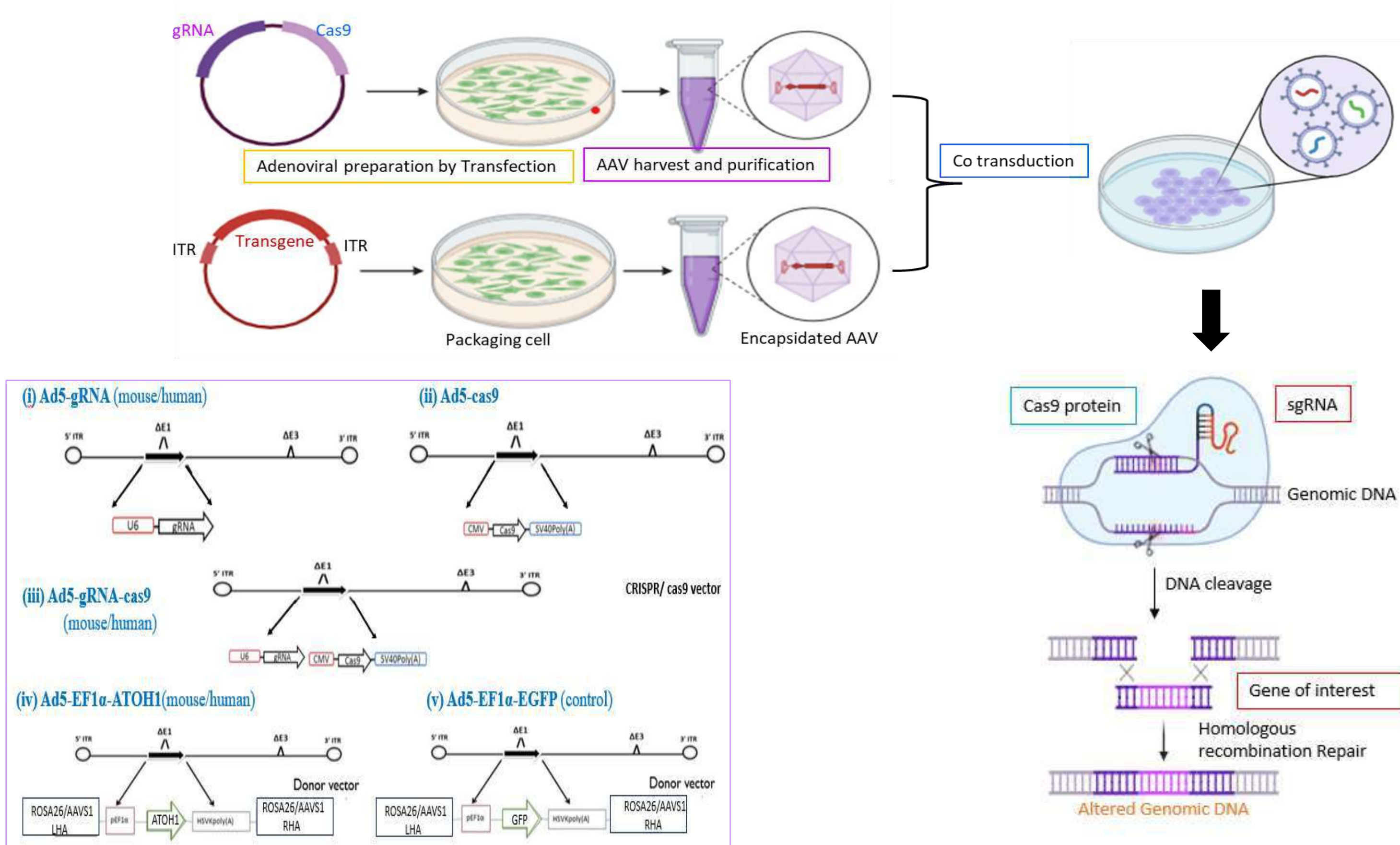
Introduction

Sensorineural hearing loss (SNHL) is the most common form of permanent hearing impairment in mammals, caused by the irreversible loss of sensory hair cells in the cochlea and vestibular system. This degeneration may occur due to ototoxic drugs, aging, or acoustic trauma. Unlike non-mammalian vertebrates, mammalian hair cells lack regenerative potential, making hearing loss permanent. Current interventions such as cochlear implants provide partial restoration but do not replace lost sensory cells. Studies in non-mammalian models have shown that ATOH1, a basic helix-loop-helix transcription factor, can induce supporting cells to transdifferentiate into functional hair cells. Building on these findings, this study explores a regenerative approach using CRISPR/Cas9-mediated site-specific integration of ATOH1 into supporting cells of the mammalian inner ear. Adenoviral knock-in vectors carrying ATOH1 were successfully constructed and validated in vitro, demonstrating efficient vector generation. This system will be evaluated in deaf mouse models to determine long-term expression and supporting cell transdifferentiation into hair cells. The approach aims to establish a foundation for gene-based hair cell regeneration, offering a promising and potentially durable strategy for restoring hearing function in mammals.

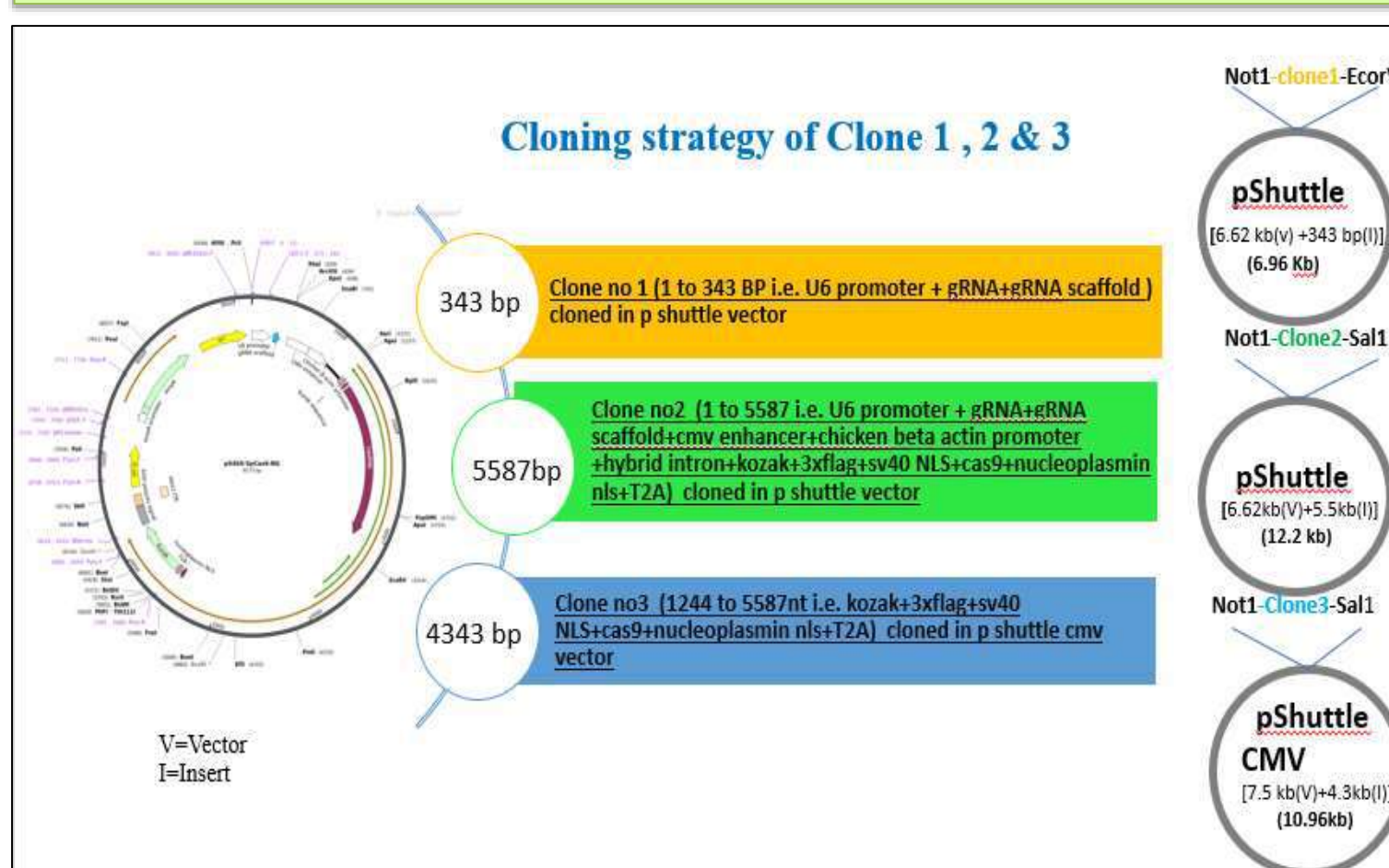


Methodology

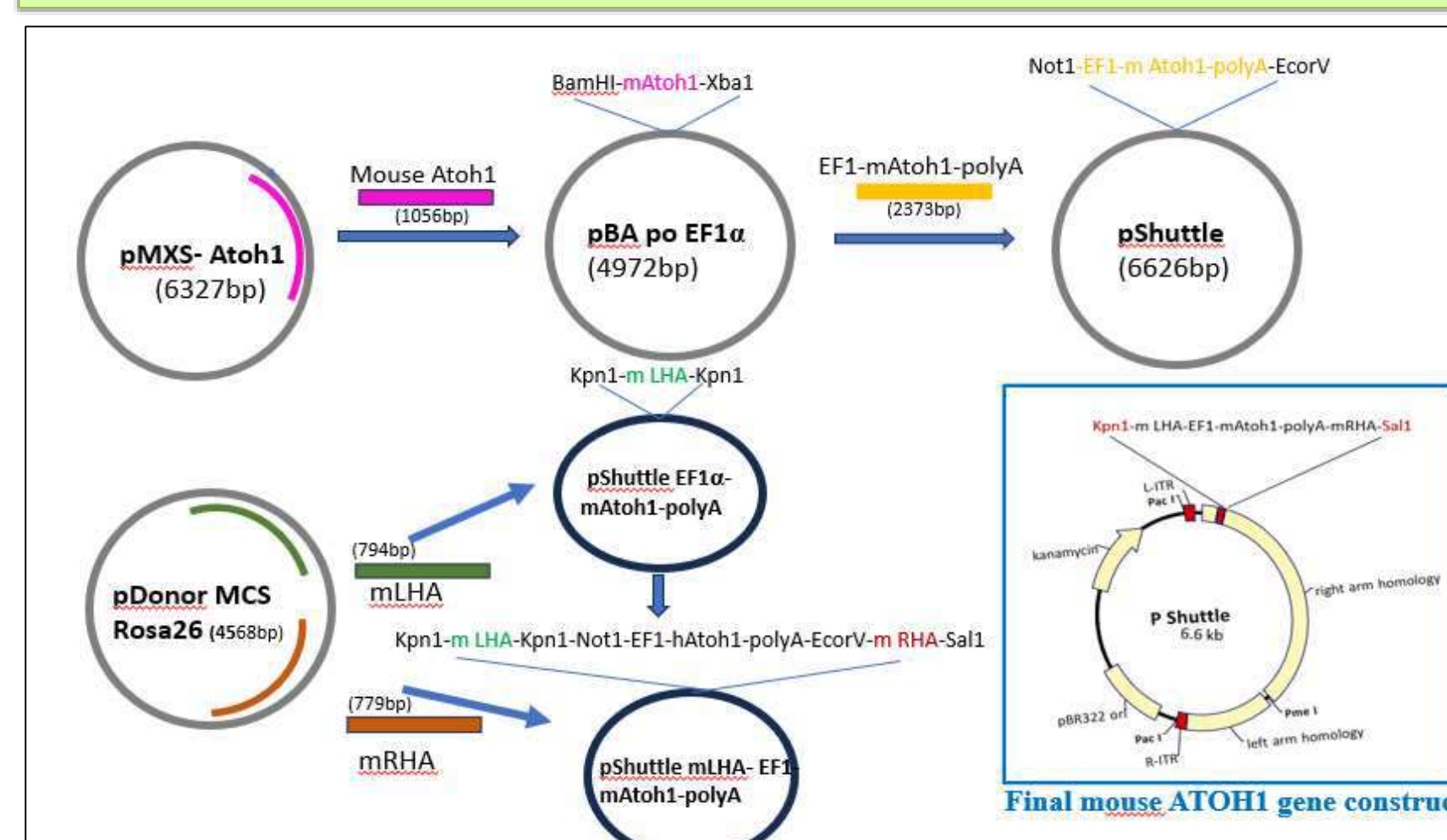
CRISPR/Cas9 mediated knock-in of Atoh1 cDNA for gene editing



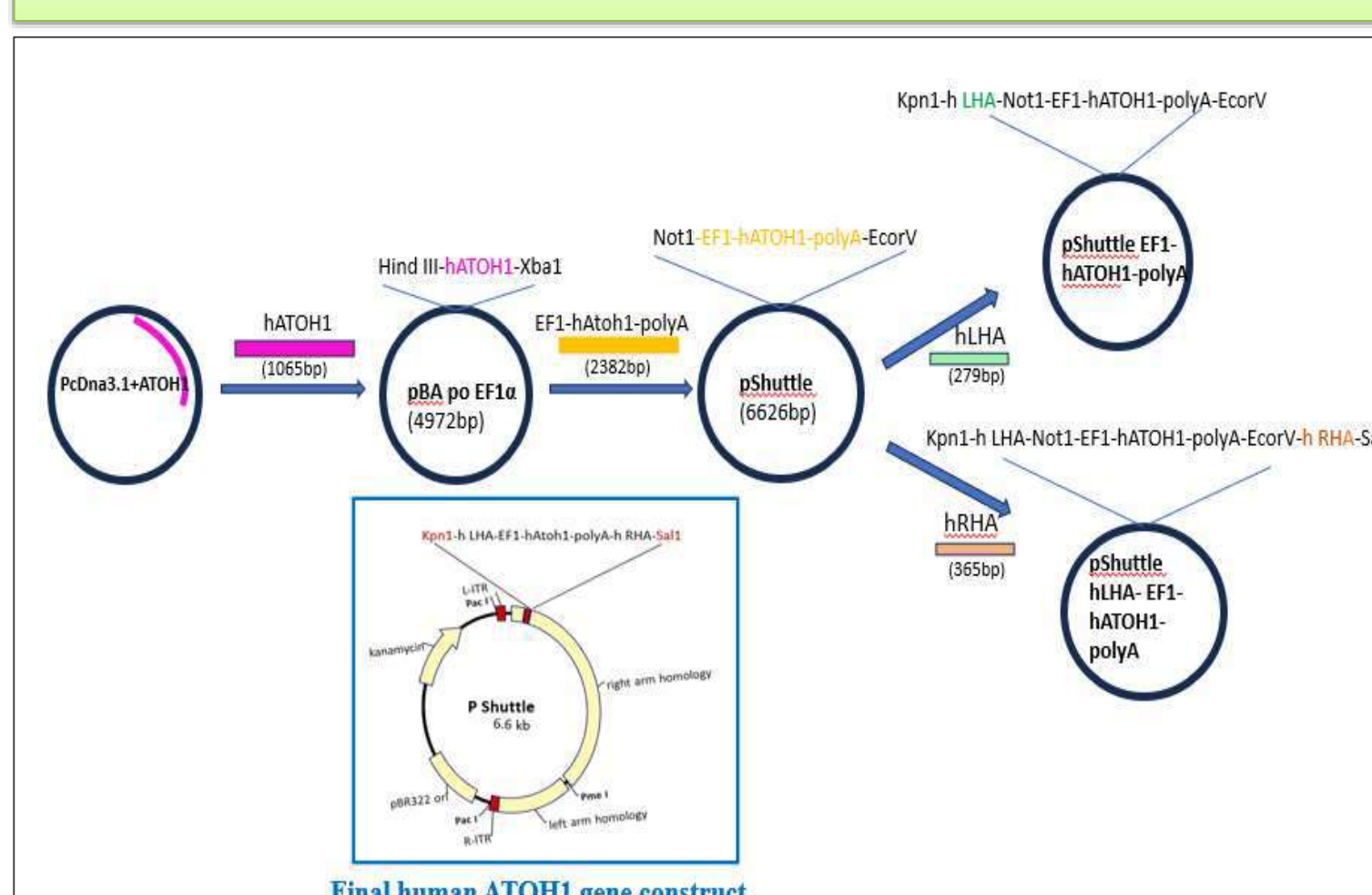
Cloning of gRNA and Cas9 protein in pShuttle Vector



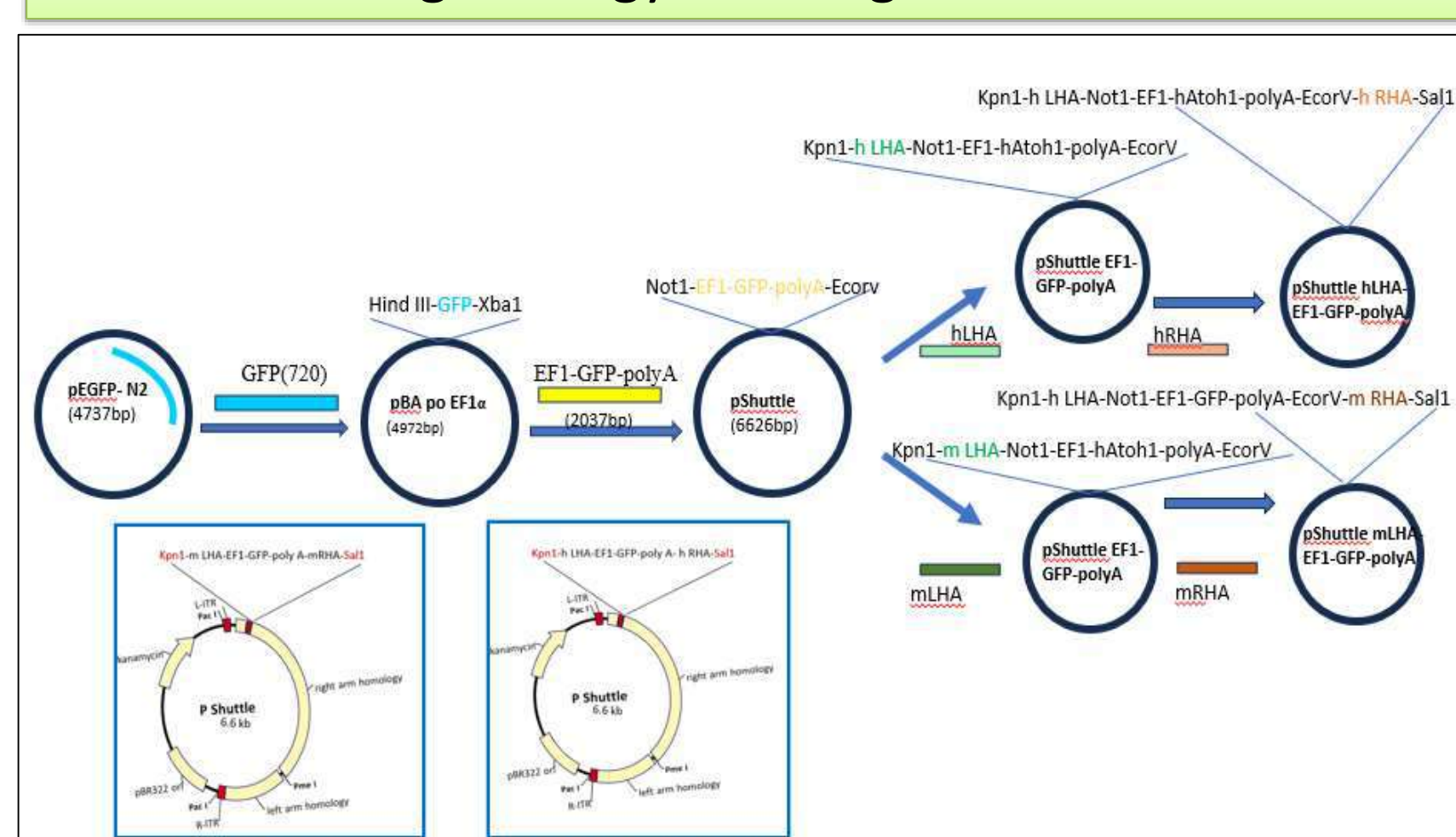
Cloning strategy of Mouse Atoh1 gene construct



Cloning strategy of Human Atoh1 gene construct



Cloning strategy of GFP gene construct



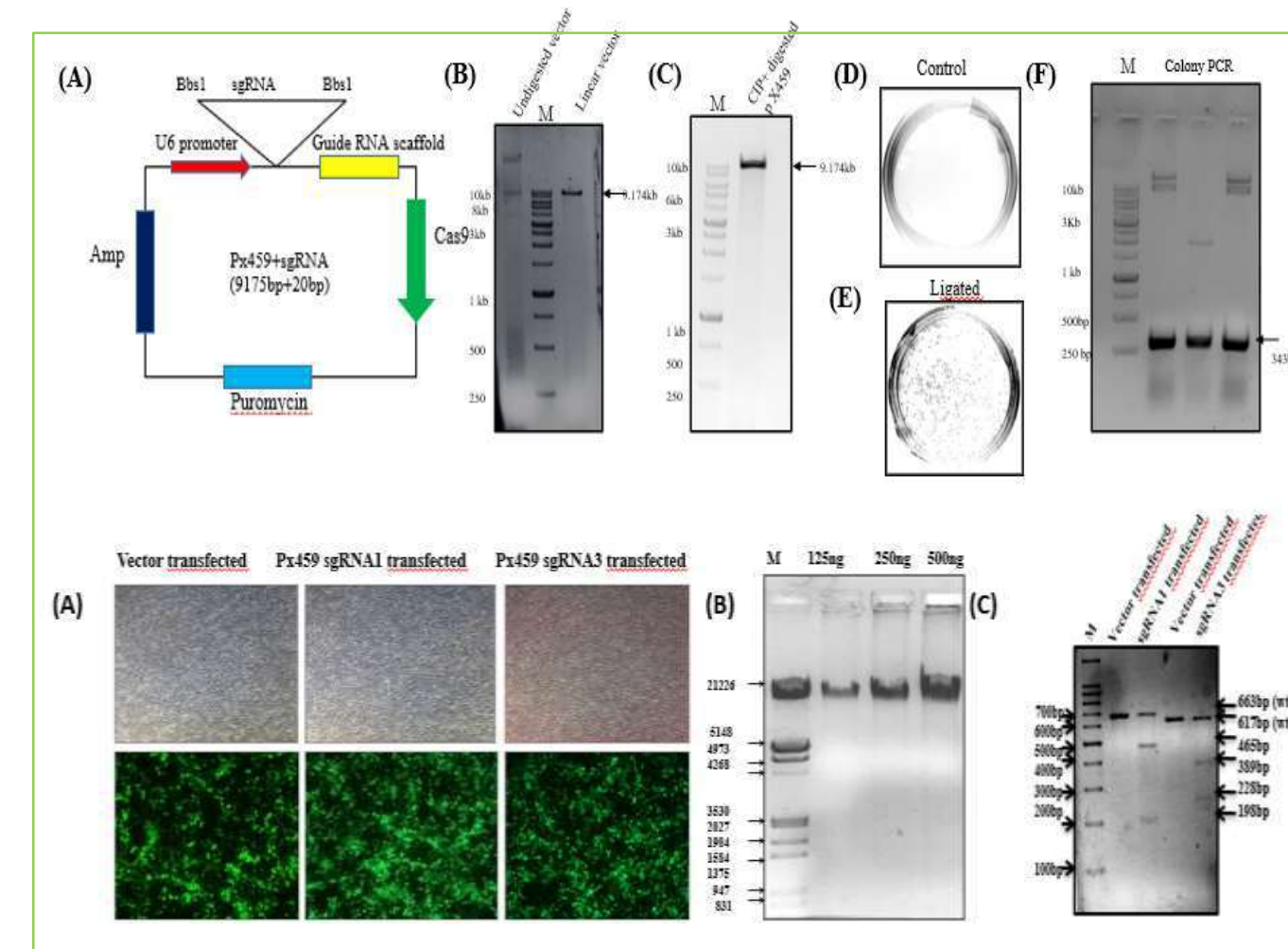
Discussion & Conclusion

- To knock in Atoh1 into the safe harbor locus of mouse (ROSA26) and human (AAVS1), we used standard cloning methods and generated vectors with Atoh1 (mouse and human). These vectors also included left and right homology arms for the respective safe harbor locus to facilitate homology-based recombination for gene integration.
- Five clones were generated, namely: clone 1-pShuttle-U6-msgRNA, clone 1-pShuttle-U6-hsgRNA, clone 2-pShuttle-U6-msgRNA-Cas9, clone 2-pShuttle-U6-hsgRNA-Cas9, and clone-pShuttle-CMV-Cas9 for gRNA control and CRISPR.
- We recombined these constructs with Ad-Easy vector to generate the respective viral vectors.

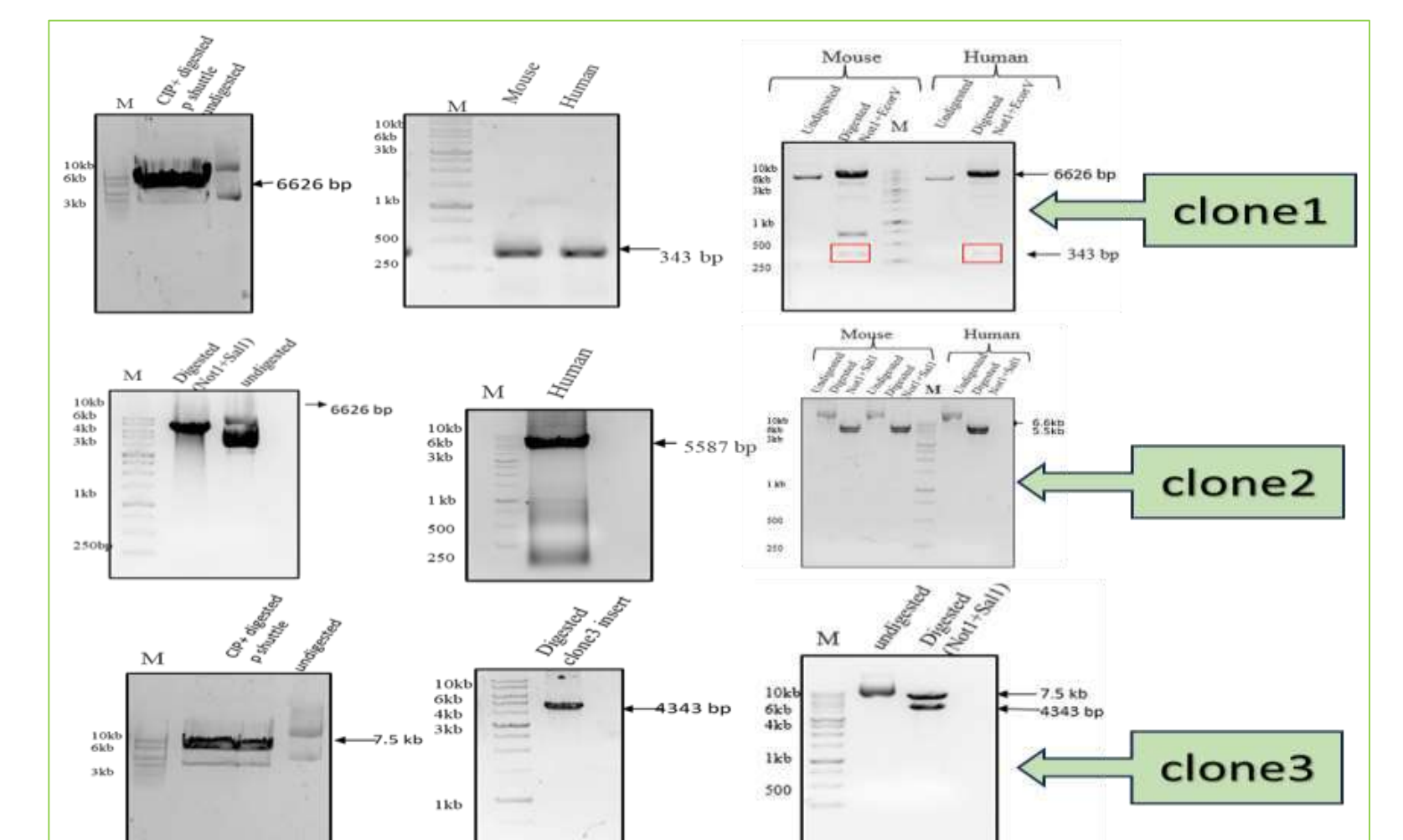
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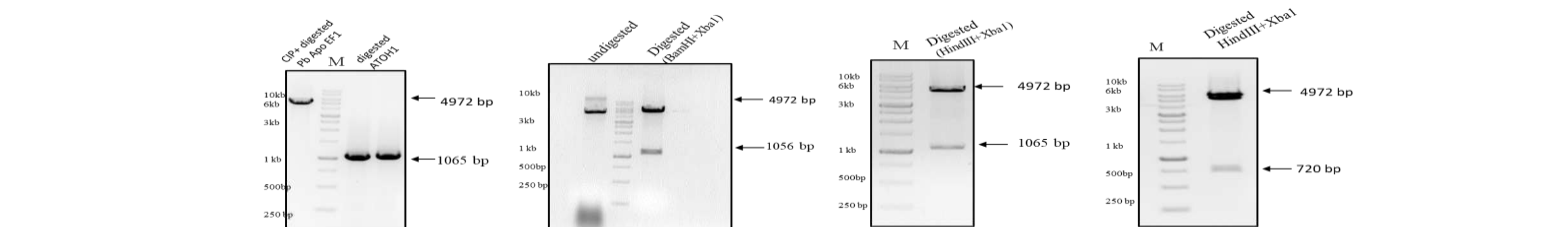
Result



Cloning of sgRNA in PX459 vector and cutting site verification by T7 Endonuclease Assay



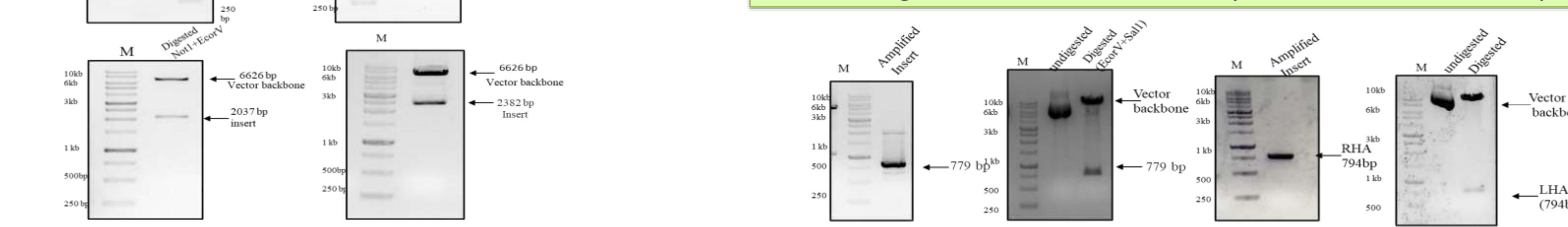
Cloning of Cas9 & Mouse gRNA and Human gRNA



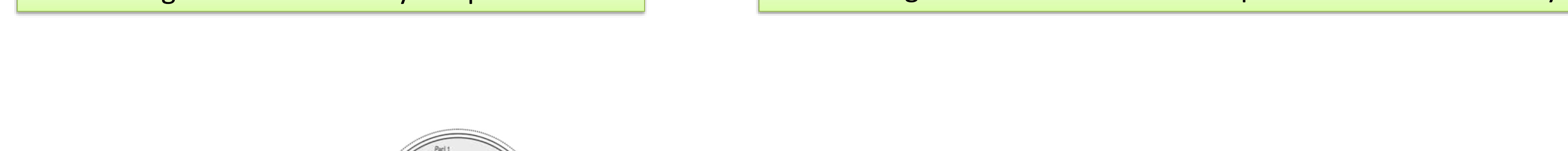
Cloning of human Atoh1, mouse Atoh1, and GFP in pBApo-EF1α vector



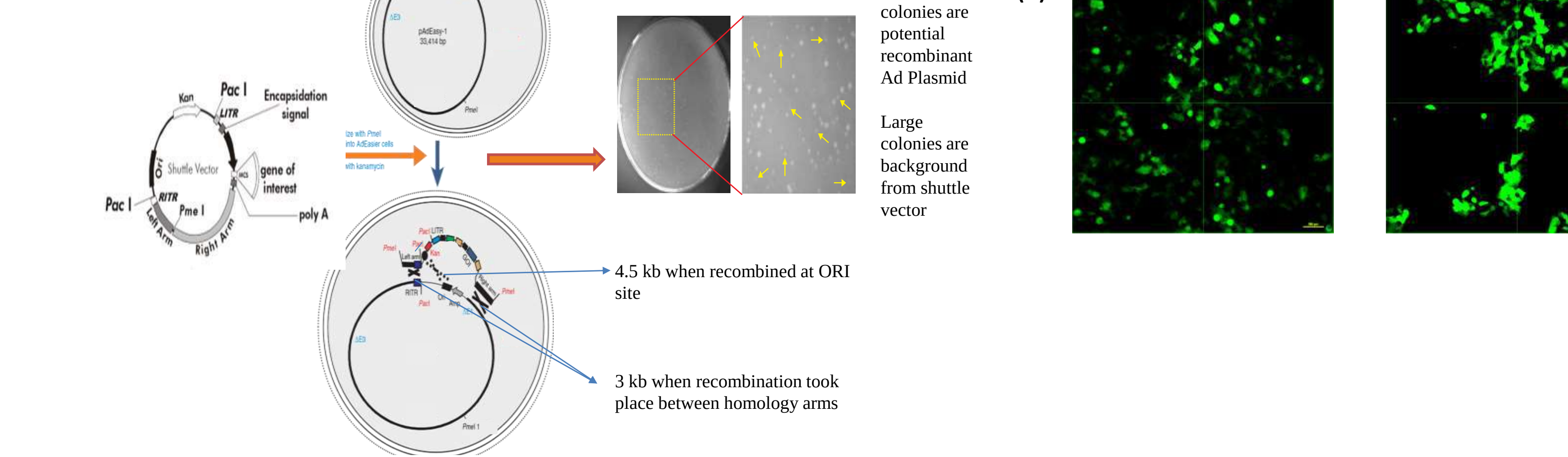
Cloning of Human LHA & RHA in pShuttle-EF1-ATOH-PolyA



Cloning of Mouse LHA & RHA in pShuttle-EF1-ATOH-PolyA



Cloning of EF1-Atoh1-PolyA in pShuttle



Cloning of GFP gene construct

Summary of Clones

No.	Clones (Mouse construct)	Insert	Vector	Update
1	spCas9-msgRNA	msgRNA(20 bp)	sp Cas9(px459)9.17kb	Cloned ✓
2	Clone 1 pShuttle-U6-msgRNA	U6-msg RNA - Scaffold(343bp)	pShuttle(6626 bp)	Cloned ✓
3	Clone2 -pshuttle -U6 msgRNA-cas9	U6 -msgRNA-cas9(5587 bp)	pShuttle(6626 bp)	Cloned ✓
4	Clone3 -pshuttle CMV -cas9	cas9 (4343bp)	pShuttle CMV (7.5 kb)	Cloned ✓
5	pBApro-mAtoh1	mAtoh1(1056bp)	pBA po EF1α (4972 bp)	Cloned ✓
6	pshuttle -EF1α-mAtoh1-polyA	EF1α-mAtoh1-polyA (2373bp)	pShuttle(6626 bp)	Cloned ✓
7	pshuttle m LHA-EF1α-mAtoh1-polyA	m LHA(794 bp)	pshuttle -EF1α-mAtoh1-polyA(8999 bp)	Cloned ✓
8	pshuttle -m LHA-EF1α-mAtoh1-polyA-m RHA	m RHA(779 bp)	pshuttle m LHA-EF1α-mAtoh1-polyA(9287)	Cloned ✓
9	pBApro-GFP	GFP(720 bp)	pBA po EF1α (4972 bp)	Cloned ✓
10	pshuttle -EF1α-GFP-polyA	EF1α-GFP-polyA(2037 bp)	pShuttle(6626 bp)	Cloned ✓
11	pshuttle h LHA-EF1α-GFP-polyA	h LHA(794 bp)	pshuttle -EF1α-GFP-polyA(8663)	Cloned ✓
12	pshuttle -m LHA-EF1α-GFP-polyA-m RHA	m RHA(779 bp)	pshuttle m LHA-EF1α-GFP-polyA(9457)	Cloned ✓

No.	Clones (Human construct)	Insert	Vector	Update
1	spCas9-hsgRNA	hsgRNA(20 bp)	sp Cas9(px459)9.17kb	Cloned ✓
2	Clone 1 pShuttle-U6-hsgRNA	U6-hsg RNA - Scaffold(343bp)	pShuttle(6626 bp)	Cloned ✓
3	Clone2 -pshuttle -U6 hsgRNA-cas9	U6 hsgRNA-cas9(5587 bp)	pShuttle(6626 bp)	Cloned ✓
4	Clone3 -pshuttle CMV -cas9	cas9 (4343bp)	pShuttle CMV (7.5 kb)	Cloned ✓
5	pBApro-hAtoh1	hAtoh1(1056bp)	pBA po EF1α (4972 bp)	Cloned ✓
6	pshuttle -EF1α-hAtoh1-polyA	EF1α-hAtoh1-polyA (2373bp)	pShuttle(6626 bp)	Cloned ✓
7	pshuttle hLHA-EF1α-hAtoh1-polyA	h LHA(794 bp)	pshuttle -EF1α-hAtoh1-polyA(9008 bp)	Cloned ✓
8	pshuttle -hLHA-EF1α-hAtoh1-polyA-hRHA	h RHA(779 bp)	pshuttle h LHA-EF1α-hAtoh1-polyA(9287)	Cloned ✓
9	pBApro-GFP	GFP(720 bp)	pBA po EF1α (4972 bp)	Cloned ✓
10	pshuttle -EF1α-GFP-polyA	EF1α-GFP-polyA(2037 bp)	pShuttle(6626 bp)	Cloned ✓
11	pshuttle h LHA-EF1α-GFP-polyA	h LHA(794 bp)	pshuttle -EF1α-GFP-polyA(8663)	Cloned ✓
12	pshuttle -h LHA-EF1α-GFP-polyA-h RHA	h RHA(779 bp)	pshuttle h LHA-EF1α-GFP-polyA(8942)	Cloned ✓

Acknowledgements

We gratefully acknowledge ICMR and AIIMS for funding and SRF to Pankaj Keshari

Identification of Noncanonical TGACCA Motifs Reveals Possible Additional Roles of BCL11A in Fetal Hemoglobin Regulation

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BACKGROUND

The zinc-finger transcription factor BCL11A is one of the major repressors of γ -globin expression and disrupting it as a therapeutic strategy is limited by its essential roles in survival, erythroid differentiation, broader hematopoietic and neural functions, making global inhibition clinically unsafe. BCL11A recognizes a preferred DNA sequence motif (TGACCA) that is duplicated within the HBG (γ -globin) promoters and it binds preferentially to the distal TGACCA motif (-115 cluster). Targeting this distal motif is an established therapeutic strategy for β -hemoglobinopathies with multiple ongoing trials. In addition, studies have shown that BCL11A also binds to other TGACCA motifs and therefore, we screened the entire β -globin locus for altering TGACCA motifs using adenosine and cytosine base editors in the human erythroid progenitor cell line.

METHODOLOGY

Guide RNAs with NGG PAM targeting the TGACCA motifs in the entire β -globin locus were designed, cloned in lentiviral plasmids and transduced in erythroid cell line expressing ABE/CBE base editors (Fig.1). The expanded cells were checked for editing percentage and further terminally differentiated into erythroid lineage for characteristics assays.

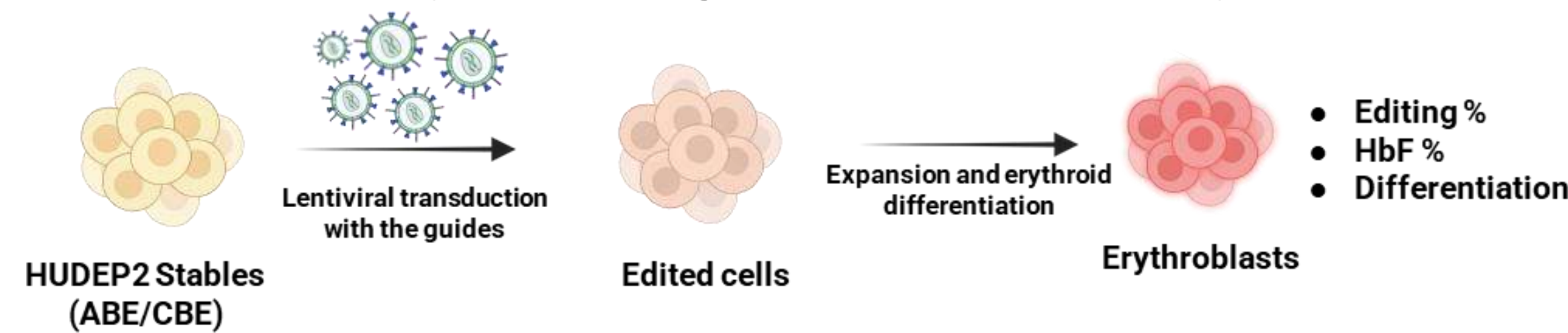


Fig.1 Schematic representation of the screening experiment workflow using lentivirus transduction in HUDEP2 stable cells

Further, the guide RNA that induced higher fetal globin was validated with transient editing using base editor mRNA and synthego gRNA (Fig.2).

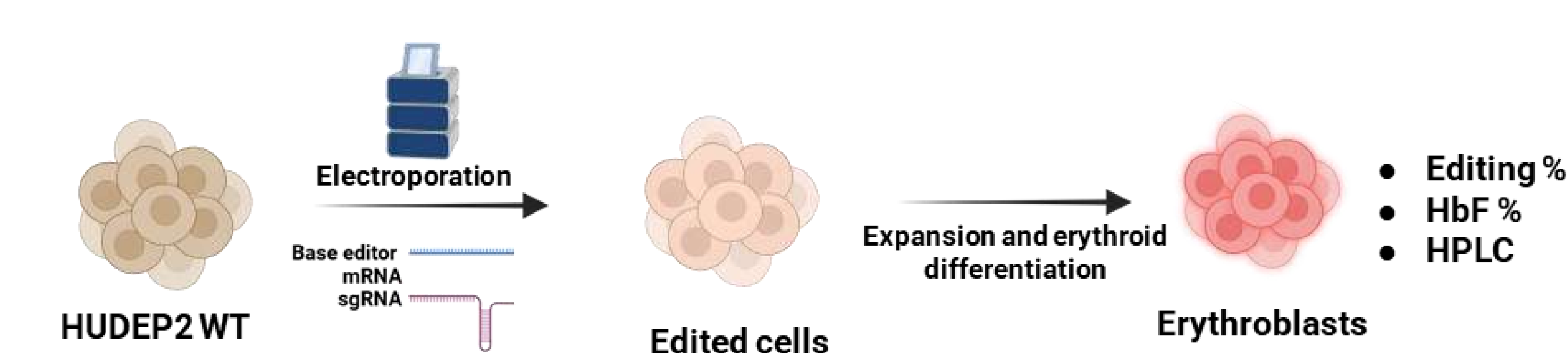


Fig.2 Schematic representation of the experiment workflow using electroporation of mRNA and sgRNA

DISCUSSION

To identify functional TGACCA targets within the β -globin cluster, we screened multiple gRNAs in HUDEP2 cells stably expressing ABE7.10. Among these, one candidate site showed a notable increase in fetal globin levels relative to the control (Fig. 3). Substitution of ABE7.10 with the more active ABE8e variant markedly enhanced editing efficiency to $\sim 88\%$, resulting in a moderate but reproducible elevation in HbF expression compared with the AAVS1 (Fig. 4). A similar HbF induction was observed with CBE (Fig. 5). Transient editing with ABE8e mRNA and synthetic sgRNA achieved $>80\%$ editing efficiency, accompanied by a nearly twofold increase in HbF levels relative to the AAVS1 control, as determined by flow cytometry and HPLC analyses (Fig. 6). These findings suggest that BCL11A may exert additional regulatory effects through its interaction with distinct TGACCA motifs across the β -globin locus, beyond its well-characterized binding at the distal TGACCA element within the HBG promoter region. The precise mechanistic basis of BCL11A recognition and repression via these alternative TGACCA motifs remains to be elucidated and warrants further investigation.

ACKNOWLEDGEMENT

We sincerely thank the Department of Biotechnology and Teams Science Grant for their funding and the CSCR core for the instrumentation facilities.

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RESULTS

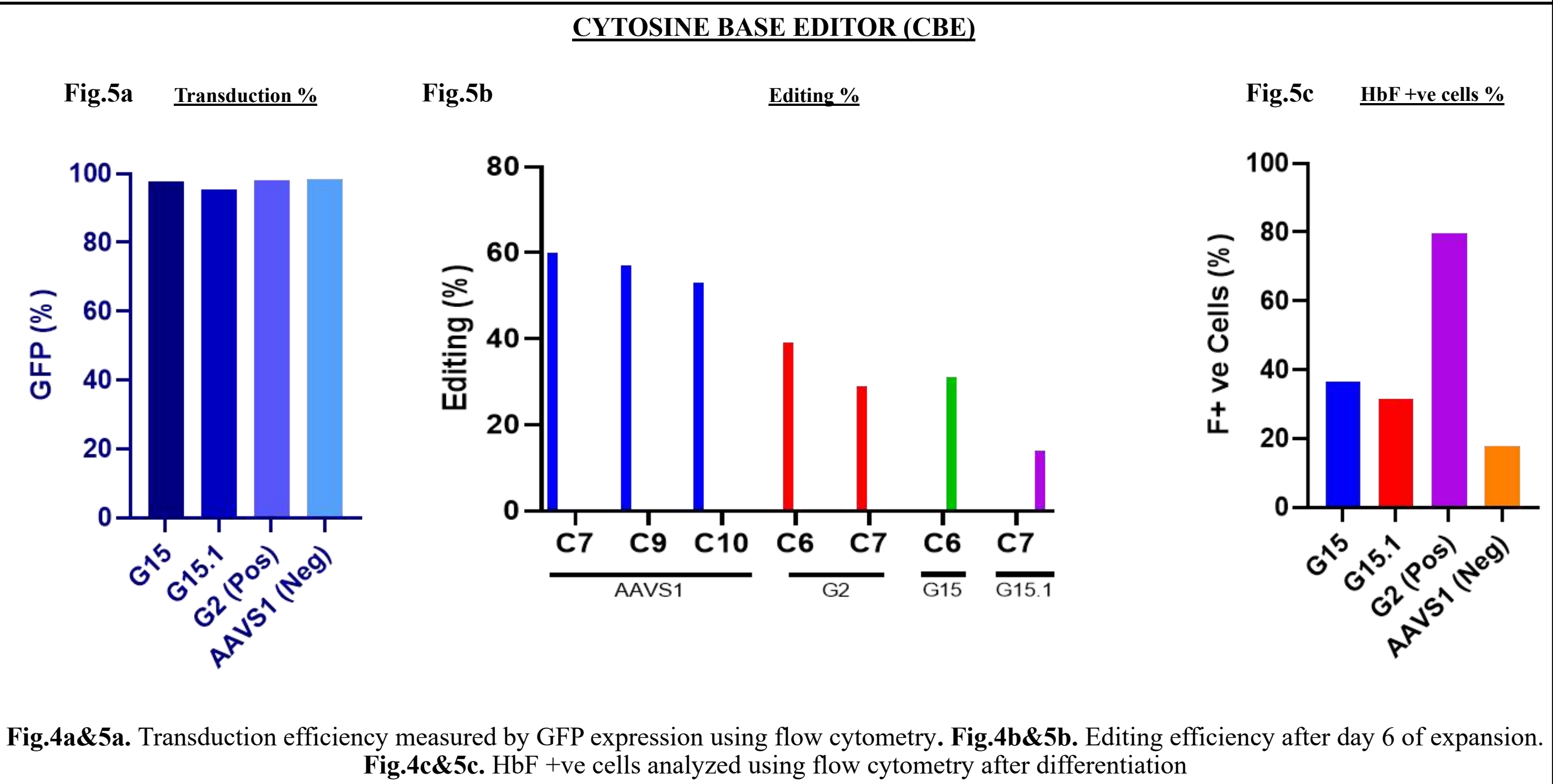
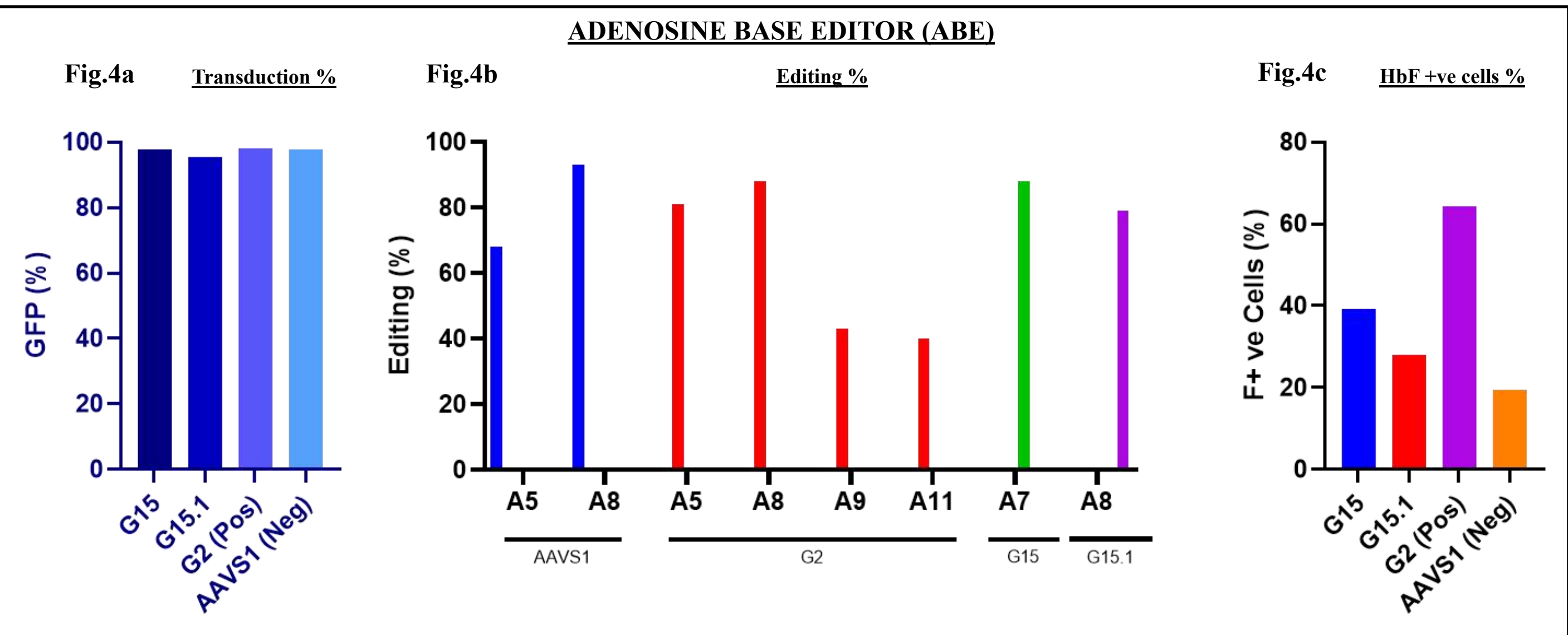
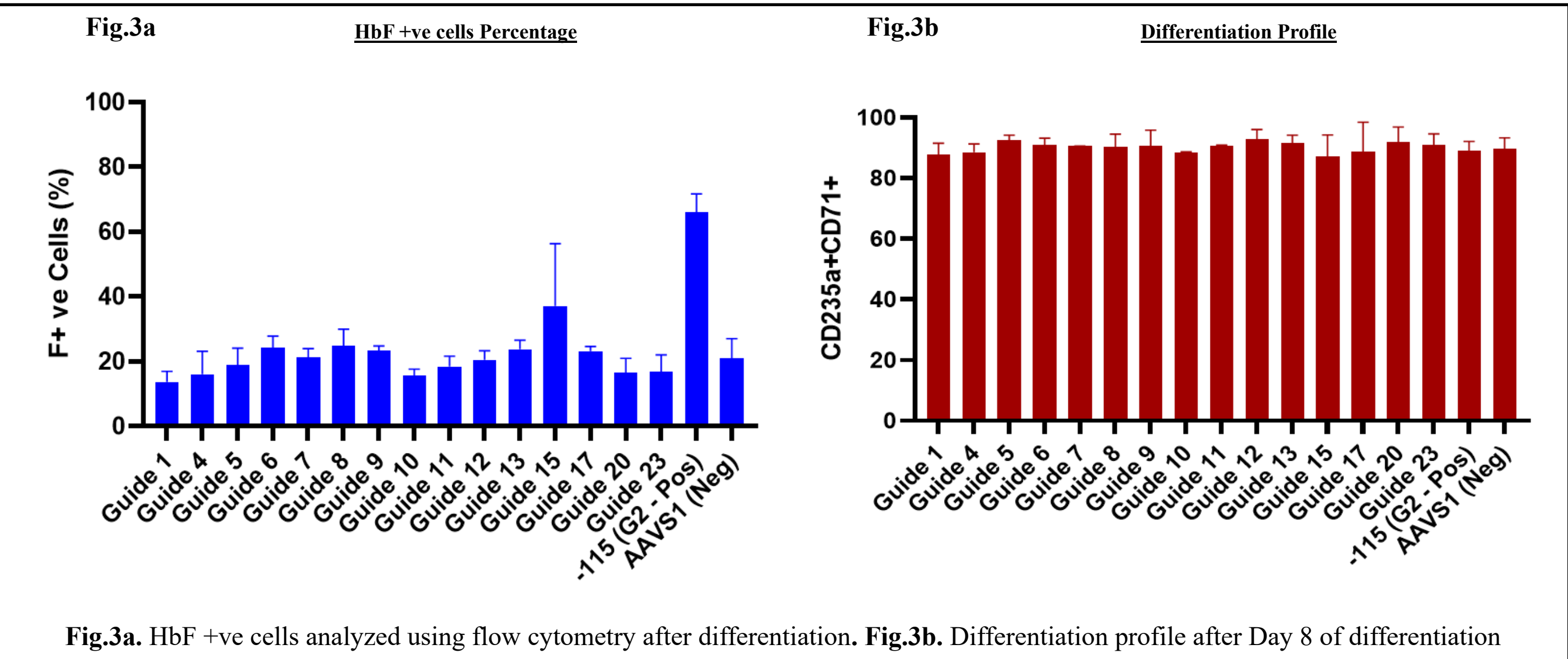


Fig.4a&5a. Transduction efficiency measured by GFP expression using flow cytometry. Fig.4b&5b. Editing efficiency after day 6 of expansion. Fig.4c&5c. HbF +ve cells analyzed using flow cytometry after differentiation

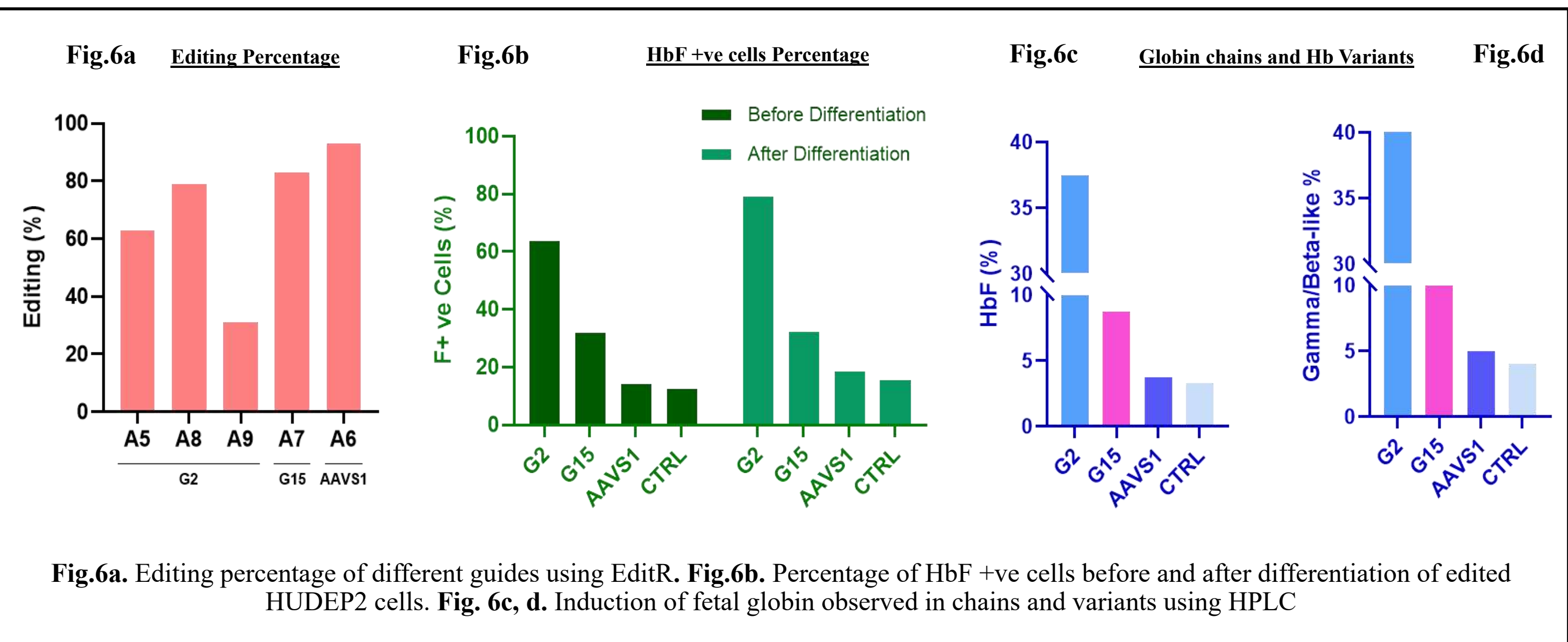


Fig.6a. Editing percentage of different guides using EditR. Fig.6b. Percentage of HbF +ve cells before and after differentiation of edited HUDEP2 cells. Fig. 6c, d. Induction of fetal globin observed in chains and variants using HPLC