

# The Direct Addition of RetroNectin® Solutions in the Liquid Phase Improves the Efficiency of Lentiviral Gene Transduction.

Yu Okubo, Yoko Kudo, and <u>Sachiko Okamoto</u>, Technology Development Center, TAKARA BIO INC., Shiga, Japan

## **Abstract**

RetroNectin<sup>®</sup> (a recombinant human fibronectin fragment CH-296) significantly enhances the efficiency of viral-mediated gene transfer, and is widely used in various clinical applications involving gene-engineered cells, including T cells and hematopoietic cells.

However, there are several challenges in manufacturing clinical-grade gene-engineered T-cell products that must be addressed in order to standardize manufacturing methods and achieve consistent results. To simplify and streamline the gene transduction process, it is feasible to incorporate RetroNectin as a transduction enhancer directly into the liquid. This modification would reduce the number of steps involved and can be easily implemented in automated cell processing systems.

In this study, we evaluated the efficiency of enhancing gene transduction by directly adding RetroNectin into the liquid phase during the transduction process of T cells using lentiviral vectors. The results showed that the direct addition of RetroNectin and lentiviruses significantly improved transduction efficiency in T cells, with a 1.5 to more than 3-fold increase compared to viral infection without RetroNectin. Importantly, the addition of RetroNectin did not have any adverse effects on cell viability, cell growth, or cell phenotype.

# The Structure of RetroNectin® and the mechanism of RetroNectin-mediated transduction

## RetroNectin®

Recombinant human fibronectin fragment CH-296

### functional domains:

- Heparin-binding domain(H-domain) ⇒bind to viral particles
- Cell-binding domain (C-domain) ⇒bind to VLA-5\*
- CS-1 sequence ⇒bind to VLA-4\*

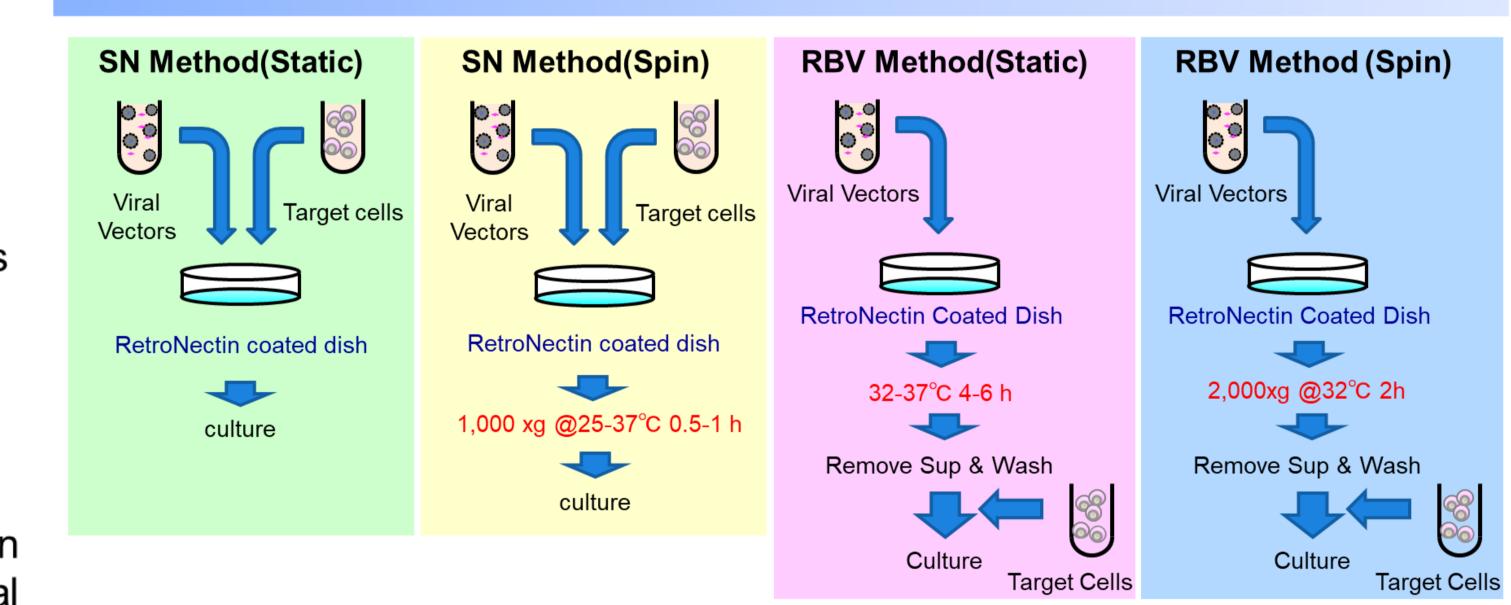
\*VLA-4&5= Integrin (Fibronectin Receptors)

Enhance lentiviral- and retroviral-mediated gene transduction by facilitating the colocalization of target cells and viral particles.

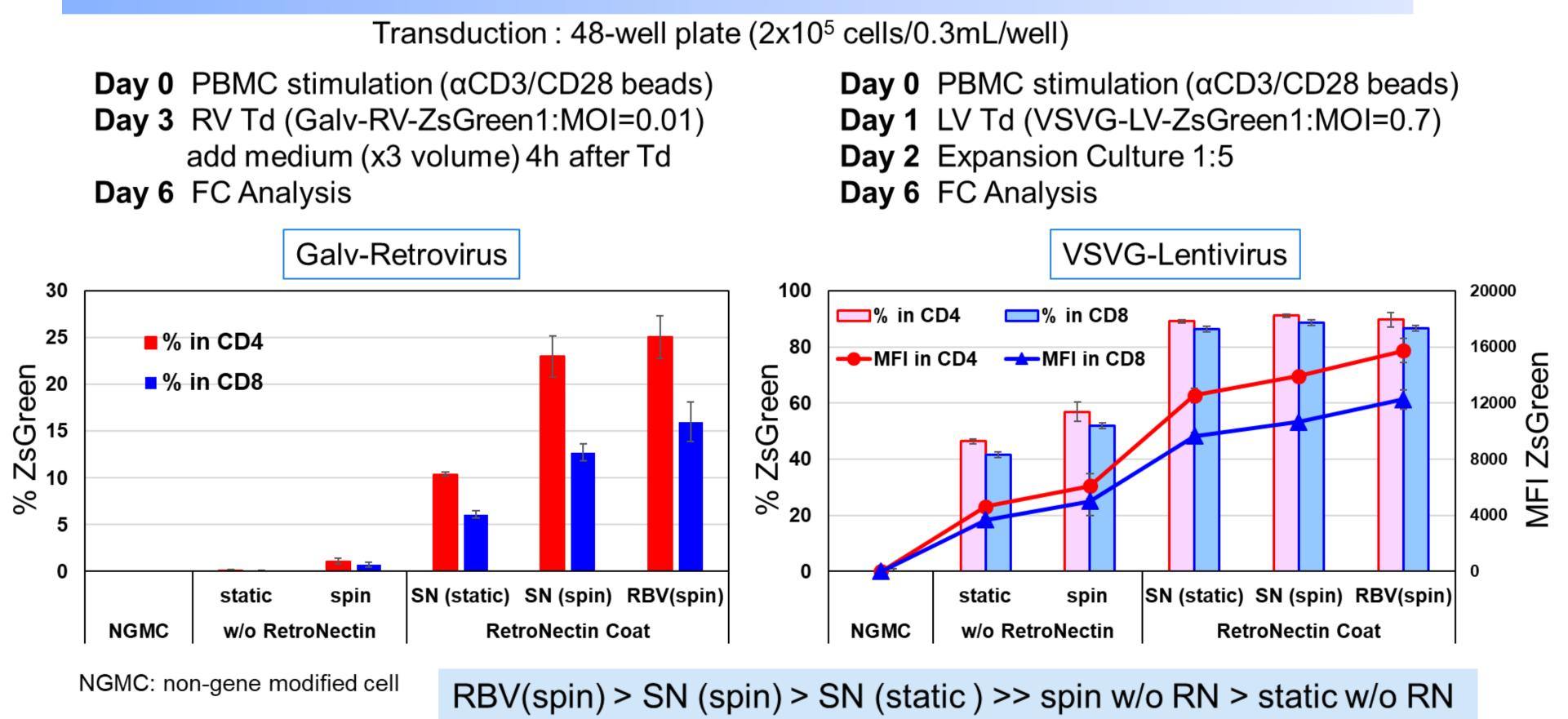
# Target Cell VLA-5 (α 5 β 1) receptor VLA-4 (α 4 β 1) Cell-binding domain (C-Domain; RGDS) RetroNectin (CH-296) RetroNectin (CH-296)

Blue: binding domain

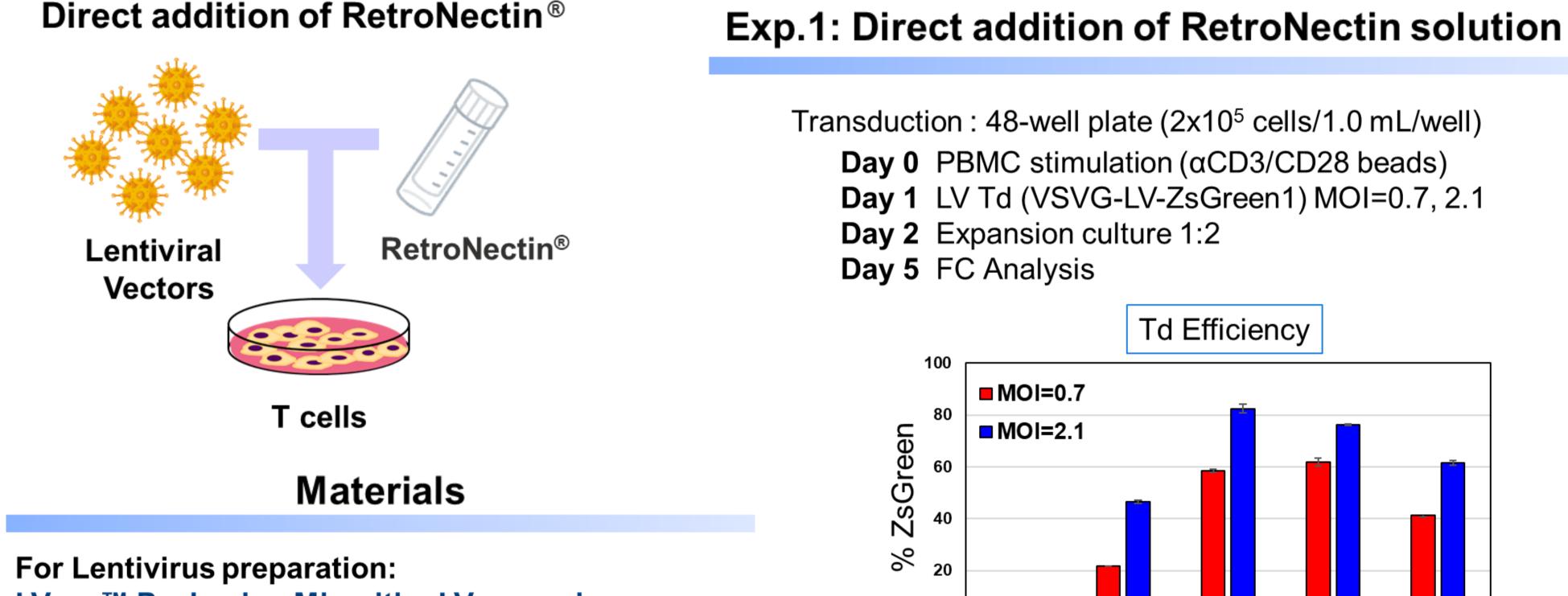
# The standard protocols for RetroNectin mediated transduction



## T cell transduction using various protocols



# Results: Direct addition of RetroNectin® solution in T cell transduction using lentiviral vector

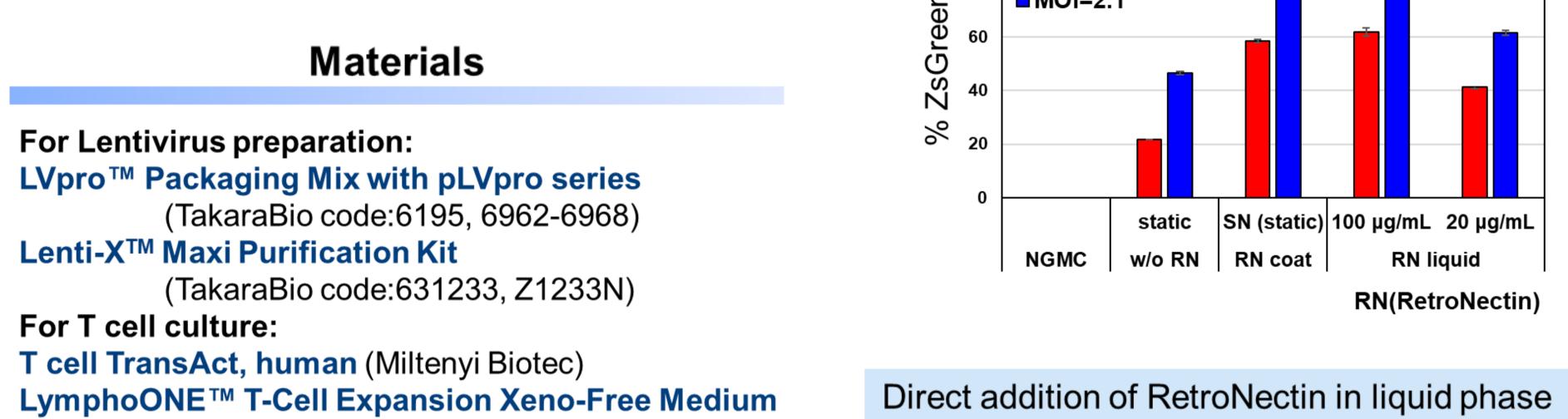


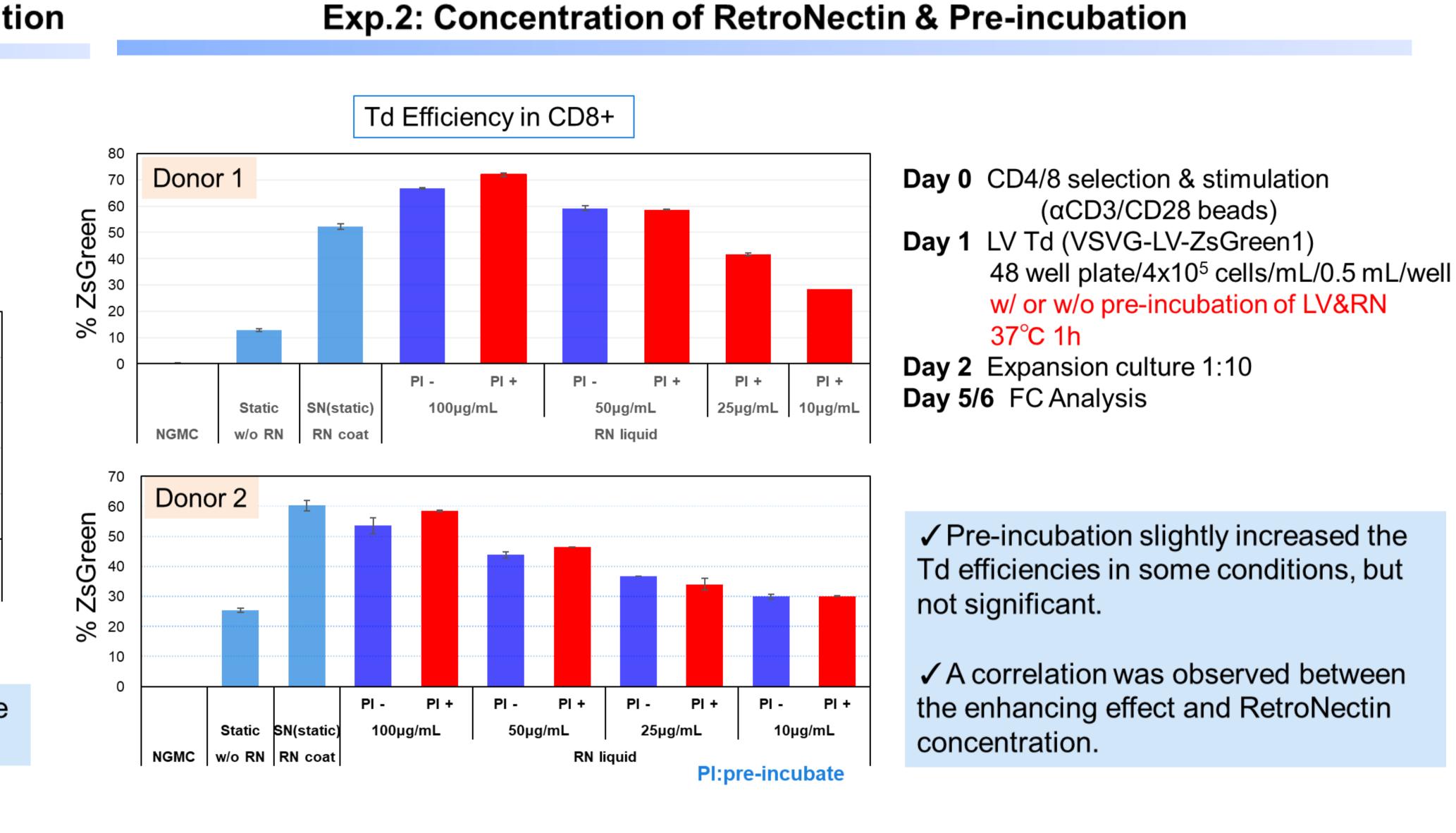
(TakaraBio, code WK552S)

Transduction Enhancer A&B (commercially available)

RetroNectin® (TakakaBio, code T100A)

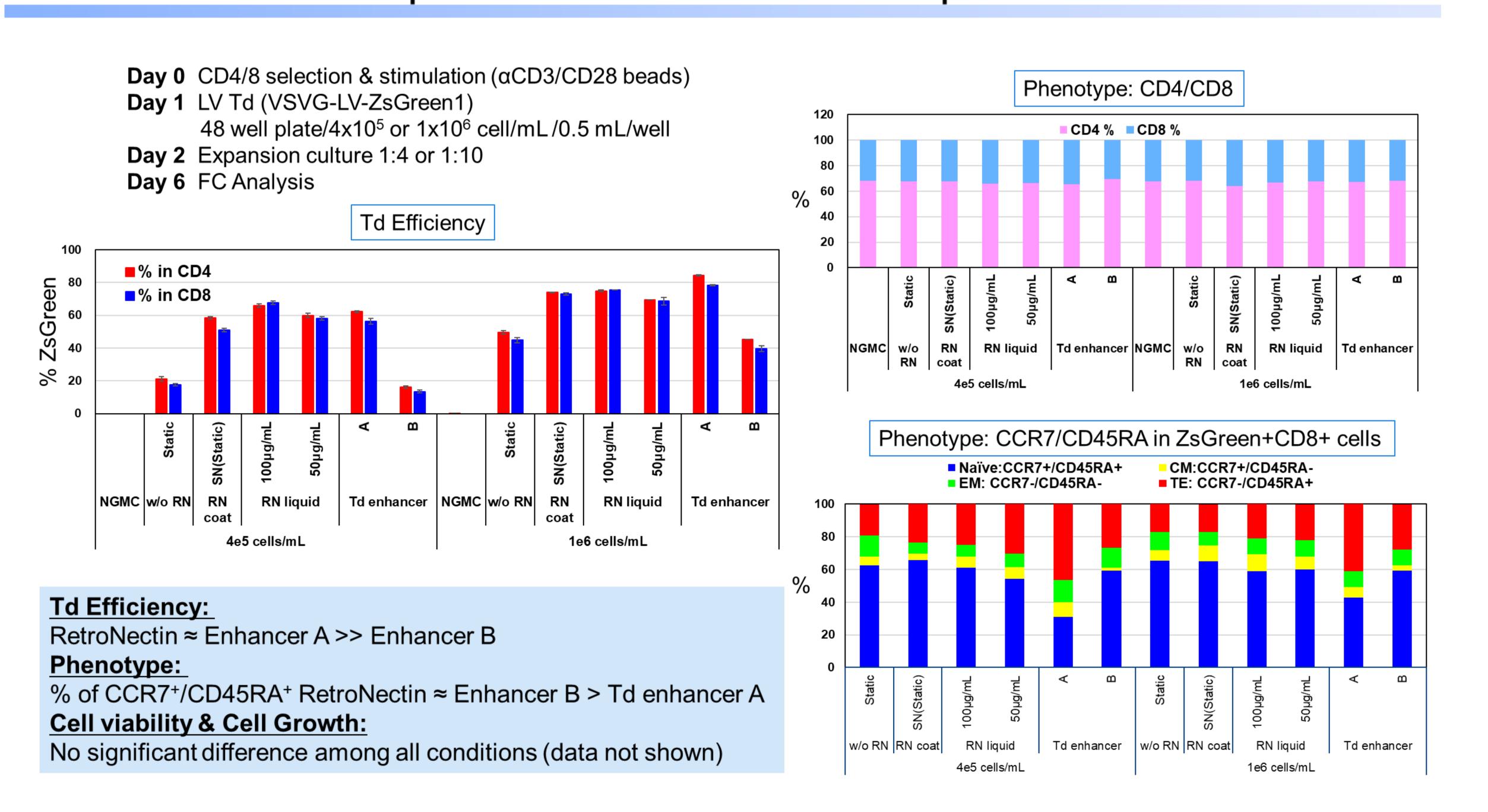
For Transduction:





Exp.3: RetroNectin vs. other Td enhancer products

improved the Td efficiencies



## Conclusion

Direct addition of RetroNectin solution can improve the Lentiviral Td efficiencies in T cells

- 1.5 to more than 3-fold increase compared to viral infection without RetroNectin
- Increasing the RN concentration leads to a greater enhancement effect
- No adverse effects on cell viability, cell growth, or cell phenotype

New RetroNectin protocol is simple & can be easily integrated into automated cell processing systems.

COI: Y.O., Y.K., and S.O are employees of TakaraBio Inc.