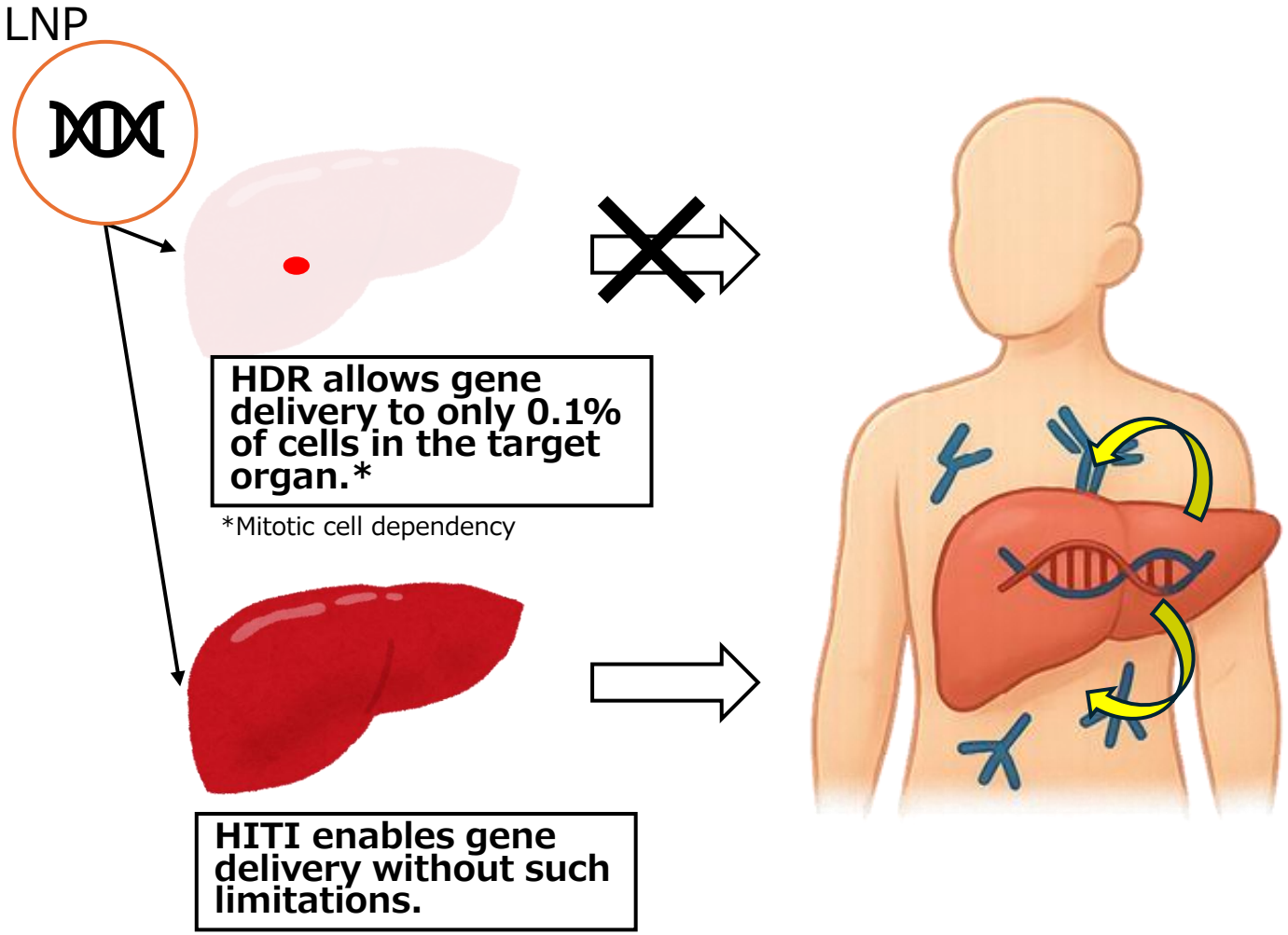
Engineering Long-Lasting In Vivo Bio-Pharmaceutical Expression Through HITI

The HITI method, which utilizes non-homologous end joining (NHEJ), enables gene delivery into non-dividing and terminally differentiated cells—an approach that has traditionally been challenging with conventional homology-directed repair (HDR). Here, we developed a HITI-specific cassette designed to optimize protein secretion and achieve sustained in vivo production of biopharmaceuticals. Specifically, by combining a specific signal peptide sequence and cleavage sites within the donor DNA, we have balanced efficient insertion at target loci with precise secretion control. This advancement holds promise not only for gene therapy and drug delivery applications but also as a research reagent enabling long-term expression of secreted proteins in target cells. Here, we demonstrate system validation using Exendin-4 (Exe4) as a model.

Next-Generation Therapeutics via In Vivo Biopharmaceutical Production and Secretion



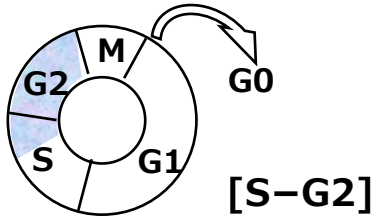
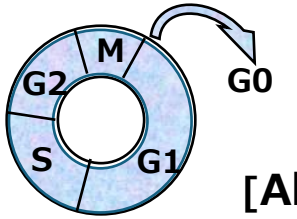
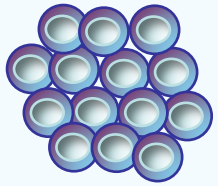
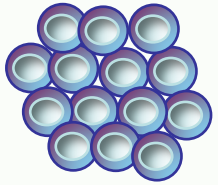
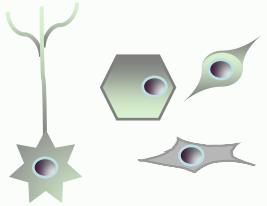
Efficient gene integration
↓
Persistent bioproduction in the liver
↓
Optimized secretion
↓
Bloodstream-mediated drug delivery
↓
Self-Healing from Within
Long-Term Prevention and Therapy

A HITI-based cassette with secretion signals and cleavage sites is essential.

【Patent pending】

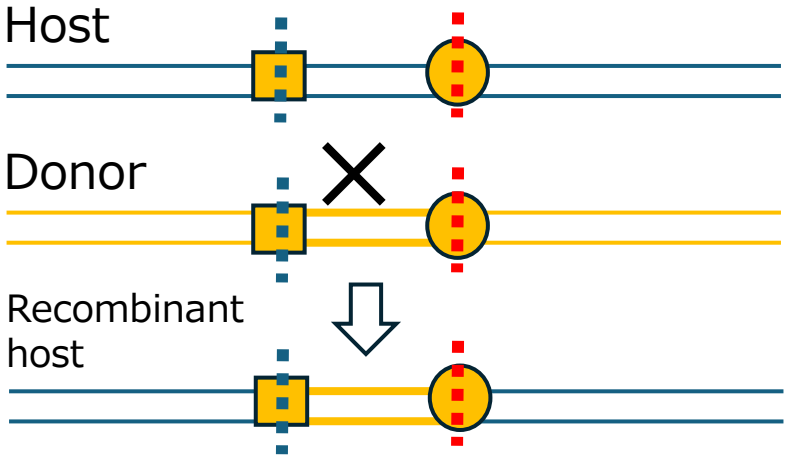
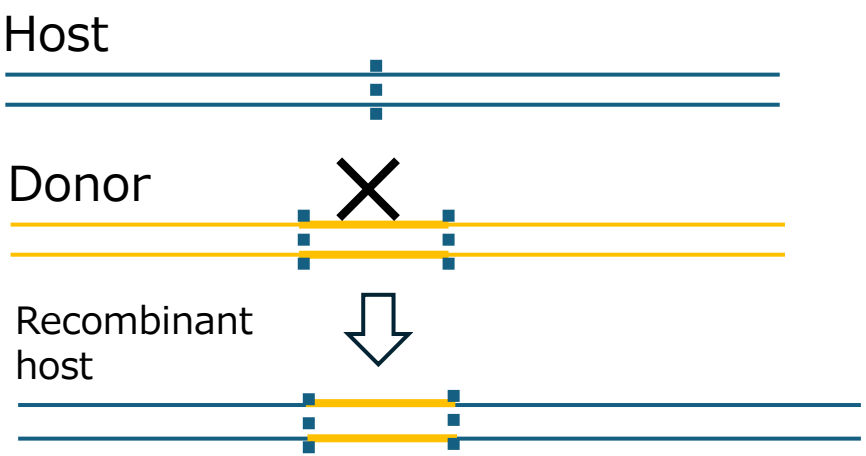
Unrestricted by the cell cycle —
the power of HITI

What is HITI?

Mutational repair approach	Establishe method	HITI method (Homology-Independent Targeted Integration)
Repair pathway	Homologous recombination (HDR)	Non-homologous end joining(NHEJ) Suzuki et al, ***Nature*** 2016
Cell Cycle	 [S–G2]	 [All period]
Putative Target cells	 Mitotic cells (0.1%) Certain in vivo cells	 分裂細胞 (0.1%) +  非分裂細胞 [神経細胞・心筋細胞など] (99.9%) The majority of in vivo cells

➢ **HITI: Gene Delivery Unrestricted by Cell Type or Cycle**

HITI法の特徴

Mutational repair approach	Establishe method	HITI method
		(Homology-Independent Targeted Integration)
Repair pathway	Homologous recombination (HDR)	Non-homologous end joining(NHEJ)
		Suzuki et al, ***Nature*** 2016
Scheme		
	Adding homologous arms to both ends of the donor allows precise insertion of the target sequence at a specific site.	Donor sequences are inserted via Cas9-dependent blunt ends, with the insertion site directed by guide RNA. Furthermore, special sequence designs within the donor prevent re-cleavage after successful recombination.

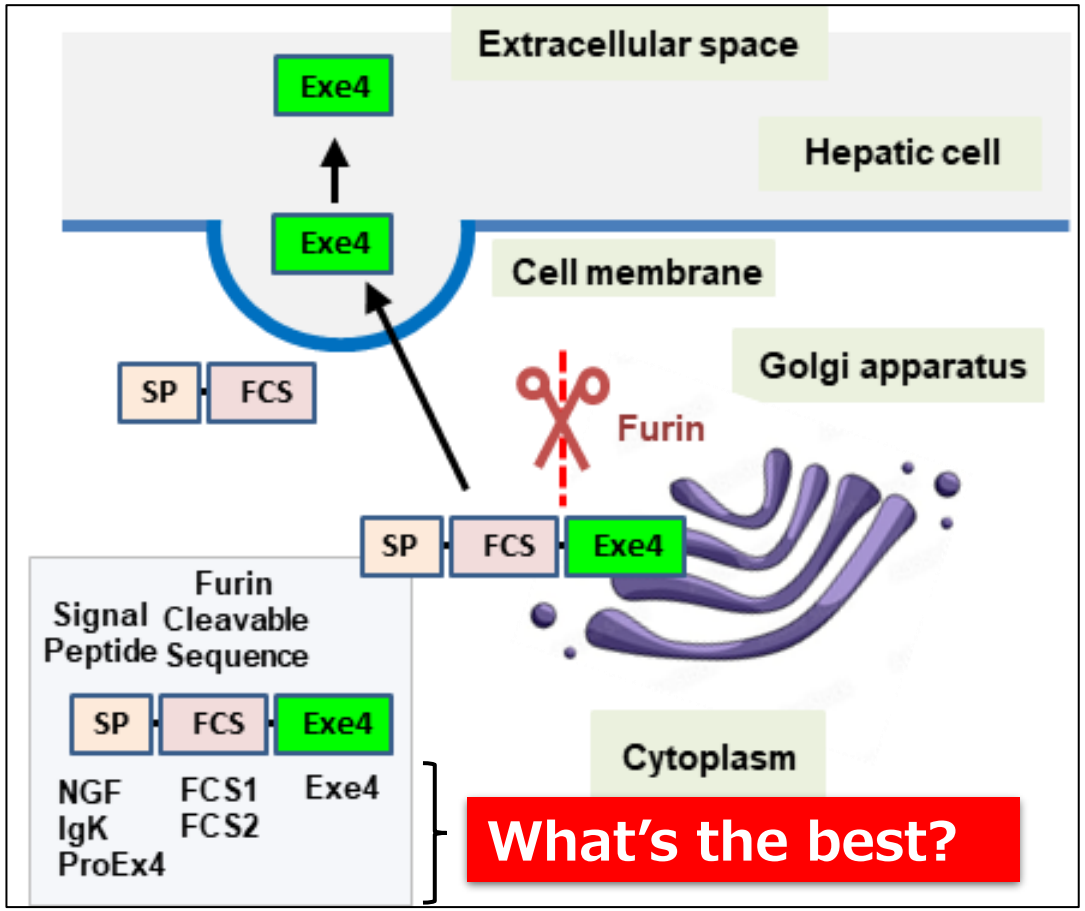
: Homologous sequence

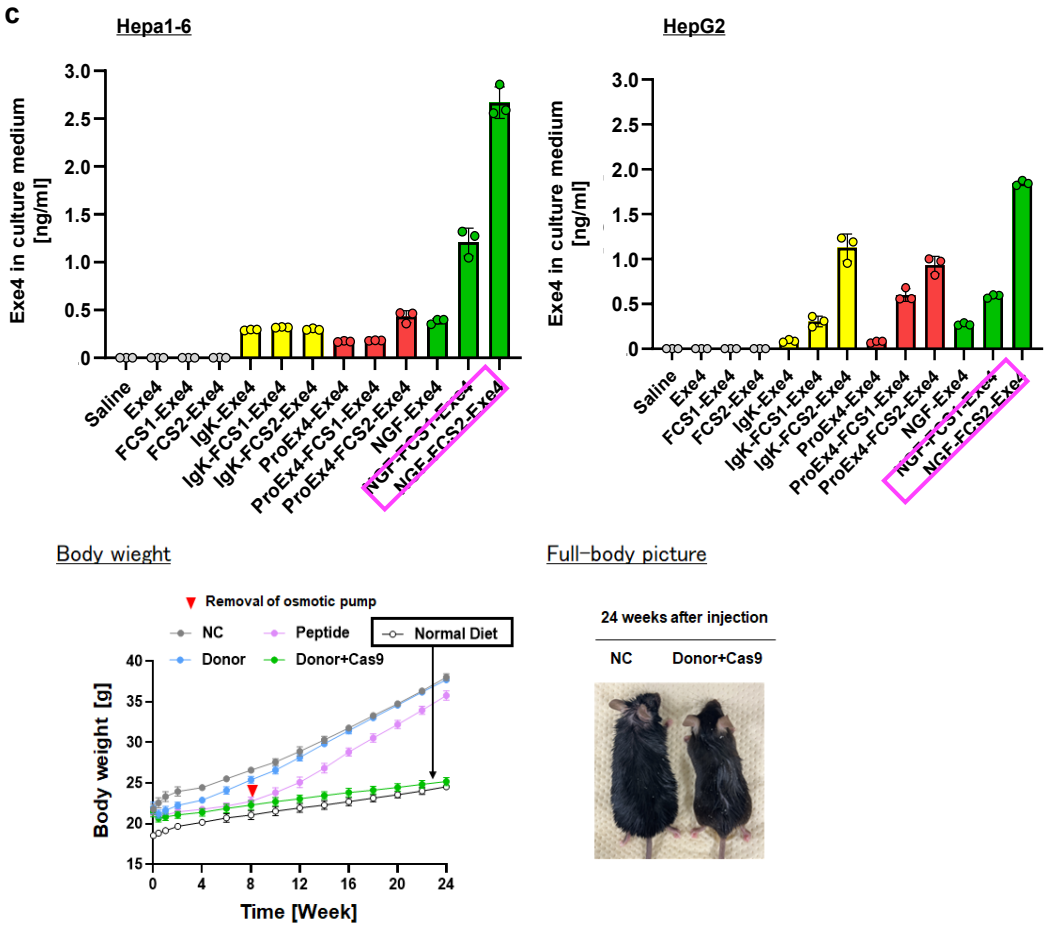
: Cut site

: Cut site

➢ **Optimized donor design enables efficient secretion of target proteins**

Next-gen therapies through in vivo biopharmaceutical production and secretion





Hirose J et al, *Commun Med* **5**, 269 (2025)

- ✓ NGF-FCS2 demonstrated the highest secretion efficiency.
- ✓ Exe4 in the culture medium showed biological activity comparable to synthetic peptide products.
- ✓ Therapeutic efficacy was also confirmed in vivo.

【関連文献】

1. Samson, S. L., Gonzalez, E. V, Yechoor, V., Bajaj, M., Oka, K., Chan, L. Gene therapy for diabetes: metabolic effects of helper-dependent adenoviral exendin 4 expression in diet-induced obesity mouse model. Molecular Therapy 16, 1805-1812 (2008).

2. Parsons, G. B., Souza, D. W., Wu, H., Yu, D., Wadsworth, S. G., Gregory, R. J., Armentano, D. Ectopic expression of glucagon-like peptide 1 for gene therapy of type II diabetes. Gene Therapy 14, 36-48 (2007).

3. DiPasquale, G., Dicembrini, I., Raimondi, L., Pagano, C., Egan, J. M., Cozzi, A., Cinci, L., Loreto, A., Manni, M. E., Berretti, S., Morelli, A., Zheng, C., Michael, D. G., Maggi, M., Vettor, R., Chiorini, J. A., Mannucci, E., Rotella, C. M. Sustained Exendin-4 secretion through gene therapy targeting salivary glands in two different rodent models of obesity/type 2 diabetes. PLoS One 7, e40074 (2012).