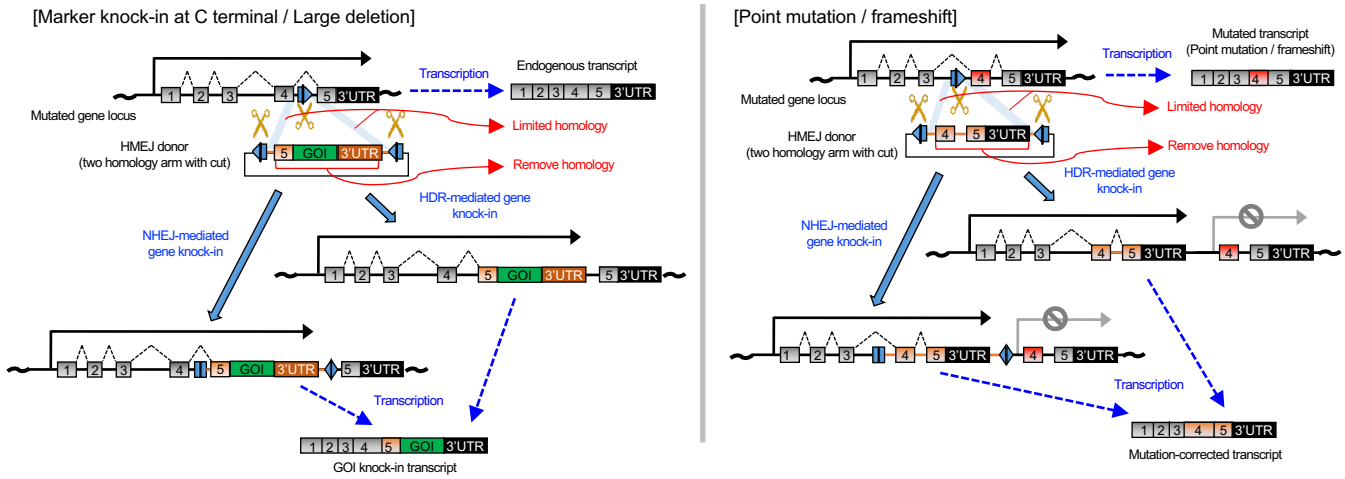
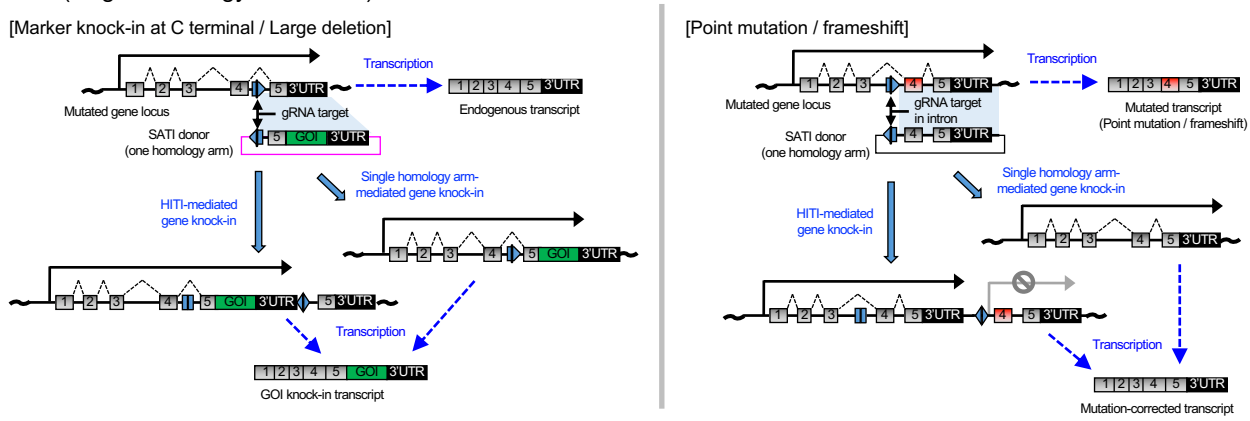
a

HMEJ (Homology-mediated end joining)

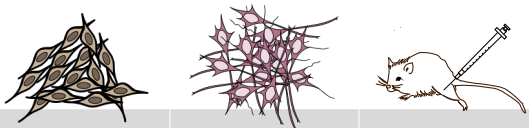


b

SATI (single homology arm donor)



c



Applicability	Dividing cells	Non-dividing cells	*In vivo*	Note
HDR	○	×	△	Less efficiency in non-dividing cells
HITI	○	○	○	Cannot apply for point mutation
HMEJ	○	○	△	Less flexibility of donor design, targeting locus and cell types
SATI	○	○	○	

Supplementary Figure S2 | Schematic representation of HMEJ and intron-targeting SATI methods. a Schematic representation of the HMEJ-mediated intronic gene-knock-in method. The donor DNA includes an inserting cassette, two DSB induction sites and two-homology arms where is identical to target genome. In order to avoid undesired recombination, it is important to lack any homology sequences from the inserting cassette (i.e. splicing acceptor, exon (s), GOI and 3'UTR). Furthermore, in order to avoid undesired splicing when the insert is integrated by NHEJ, the left homology arm should not include splicing acceptor. HMEJ allows DNA knock-in via conventional HDR or NHEJ. Under the above limitations for donor design, HMEJ-mediated gene knock-in is also able to target a broad range of mutations and cell types although less efficient in diving cells due to competition of conventional HDR. Furthermore, it is necessary to carry two homology arms, which may beyond the capacity of AAV and limit the application for *in vivo*. **b** Schematic representation of the new intronic gene-knock-in method, SATI. The donor DNA includes DSB induction site and one-homology arm where is identical to target genome. SATI allows DNA knock-in via single homology arm mediated HDR (oaHDR) or homology independent NHEJ-based HITI, enabling to target a broad range of mutations and cell types. **c** Summary for difference of applicability between gene editing methods used in this study. Red circle means "fully applicable," red triangle means "partially applicable," and red cross means "difficult to apply." Weak points of each gene editing method are indicated in the note (right).