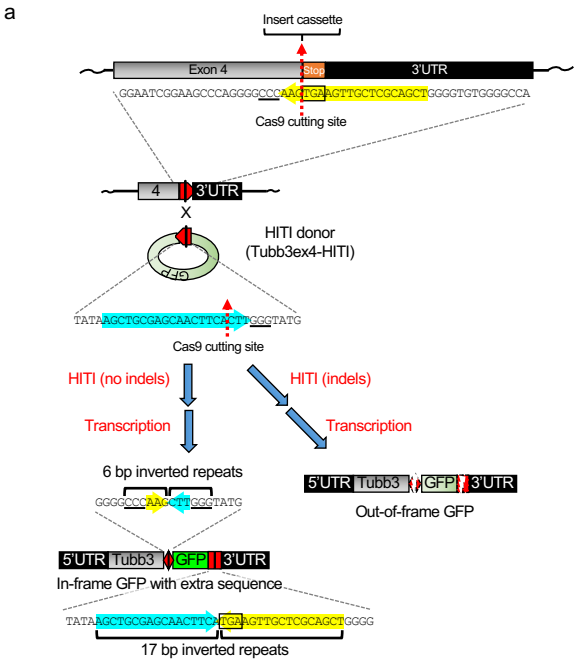
a



b

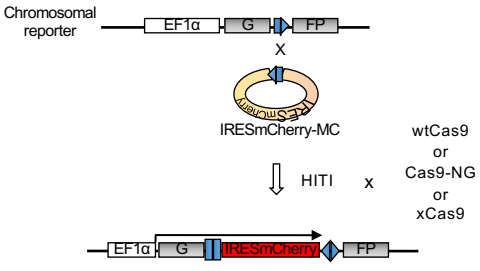
			# of gRNA design sites	
Target gene	Target site	Application	NGG PAM	NG PAM
Tubb3	Exon 4 (stop codon)*	GFP knock-in	1	1
	Intron 3**	GFP knock-in	185	648
Lmna	Exon 11 (c.1827C>T)***	Knockout .1827C>T mutation	0	2
	Intron 10**	Knock-in *Lmna* minigene	93	256

* Cut in front of stop codon

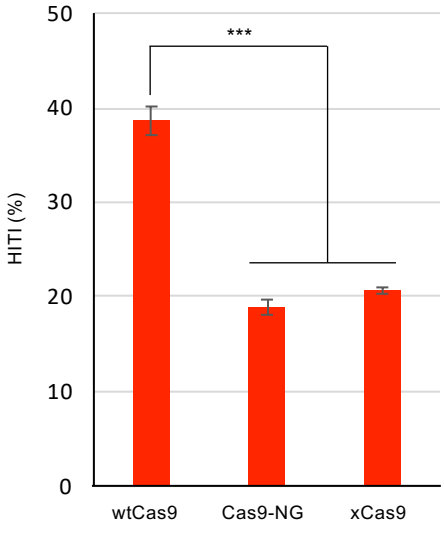
** Exclude each 100 bp from exon to avoid splicing site

*** To give the specificity for the mutation, the designed gRNA includes this mutation in PAM or within 6 bp from PAM

c



d



Supplementary Figure S3 | Schematic representation of HITI and intronic-targeting SATI strategies. a Scheme showing inserted DNA sequences with exon-targeting HITI donors via conventional HITI system. Red pentagon and yellow and light blue highlights, the 3' end of exon 4 gRNA target sequence. Black line within the red pentagon and red broken arrow, Cas9 cleavage site. When HITI can insert donor sequence without indel, the junction sequence of both ends are indicated as left below and GFP can express normally because of no frame-shift (left). The donor DNA is often integrated with small indels at junction sites when original HITI target at exon, resulting in out-of-frame mutation and cannot express GFP signal in the end (right). **b** Number of the design capacity of gRNA in this study. **c** Schematic representation of gene targeting by HITI with IRESmCherry-MC donor and different Cas9s in the GFP-correction HEK293 line. If IRESmCherry donor can be integrated into the targeted legion successfully by HITI, mCherry signal will be detected. **d** mCherry knock-in HITI efficiency (%) with Normal SpCas9 (wtCas9) and NG PAM Cas9 (Cas9-NG and xCas9) in HEK293. Data are represented as mean ± s.e.m. One-way ANOVA with Bonferroni's multiple comparison test for analysis, ***P<0.001.