

A mitochondrial disease model is generated and corrected using engineered base editors in rat zygotes

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Supplementary Information

Supplementary Figure 1. Generation of pathogenic m.13875T>C mutations with eTdmABE variants in primary cells.

Supplementary Figure 2. Genotype analysis of Leigh syndrome rat models.

Supplementary Figure 3. Phenotype analysis of Leigh syndrome rat models.

Supplementary Figure 4. Correction of pathogenic m.13875T>C mutations with DdCBE variants in primary cells.

Supplementary Figure 5. Correction of pathogenic m.13875T>C mutations with engineered DdCBEs in rats.

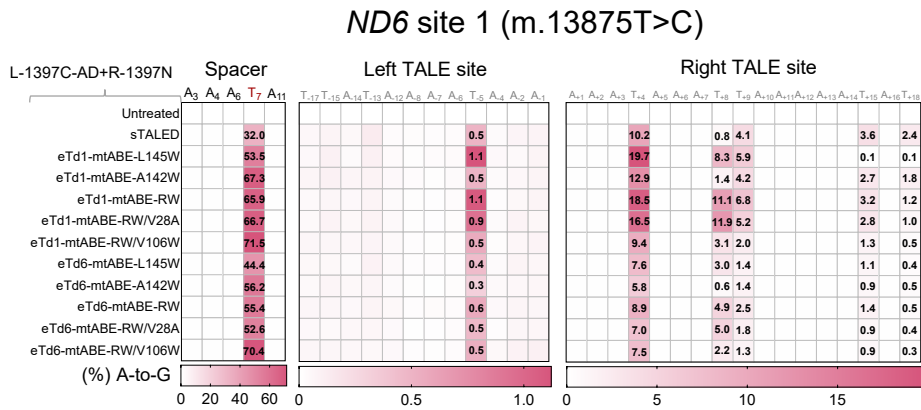
Supplementary Table 1. Primer sequences used in this study. Separate document.

Supplementary Table 2. Target sites used in this study. Separate document.

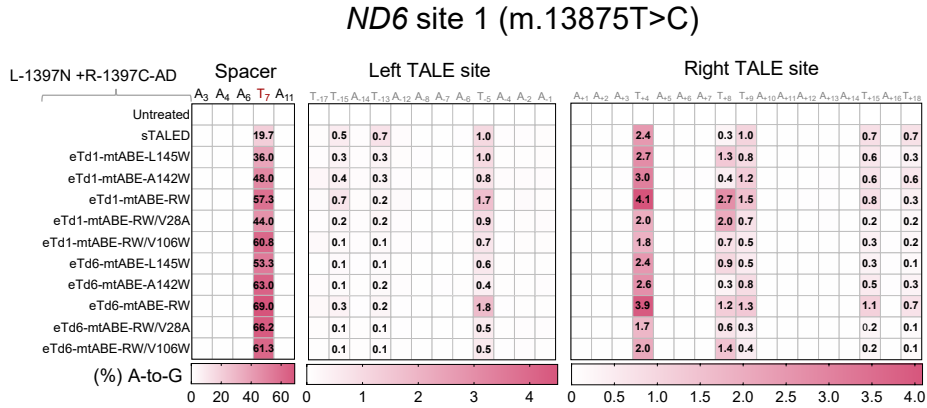
Supplementary Table 3. General architecture and DNA or amino acid sequences of plasmids used in this study. Separate document.

Supplementary Note. FACS gating examples for mCherry- and EGFP- double positive cell sorting conditions in the PC12 cells.

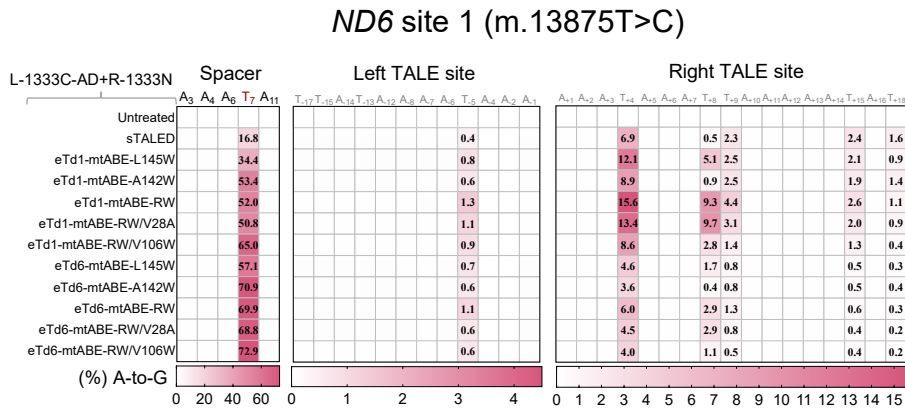
a



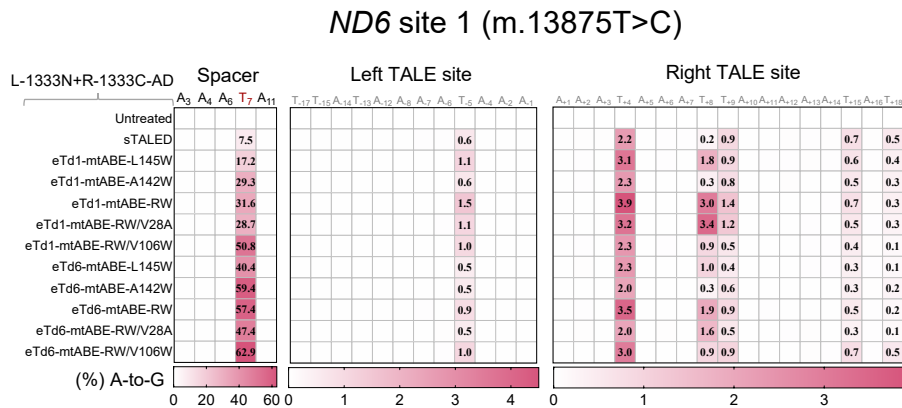
b



c



d

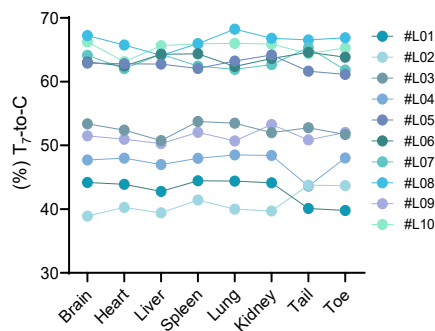


Supplementary Figure 1. Generation of pathogenic m.13875T>C mutations with eTd-mtABE variants in primary cells. a-d, Heatmap showing frequencies of mitochondrial A-to-G conversion treated with indicated editors targeting the ND6 site 1 (m.13875T>C) in PC12 cells. Data represent the mean of three biologically independent replicates.

a

Gene	Methods	No. of examined embryos	No. of transferred embryos (%)	No. of offspring (%)	Mutation rate (%)	
					No. of mutants/total pups (%)	No. of desired A-to-G mutants/total pups (%)
m.13875T>C	mRNA	262	160 (61)	49 (31)	49/49 (100)	49/49 (100)

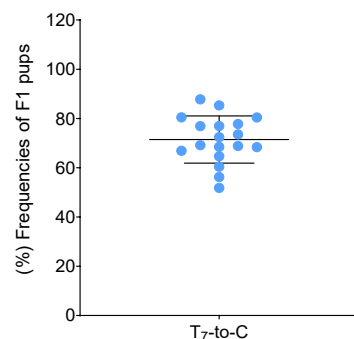
b



c

L-#47 ♀ × L-#15 ♂			
L#47 ♀ (F0)	CCAACA CCCCACCC	Frequency (%)	60.2
L#15 ♂ (F0)	CCAACA CCCCACCC		34.8
↓ F1			
#1 ♂	CCAACA CCCCACCC		71.1
#2 ♀	CCAACA CCCCACCC		65.1
#3 ♂	CCAACA CCCCACCC		71.4
#4 ♂	CCAACA CCCCACCC		61.9
#5 ♀	CCAACA CCCCACCC		65.0
#6 ♀	CCAACA CCCCACCC		67.2
#7 ♀	CCAACA CCCCACCC		66.5
#8 ♂	CCAACA CCCCACCC		66.5
#9 ♂	CCAACA CCCCACCC		49.1
#10 ♀	CCAACA CCCCACCC		62.2
#11 ♀	CCAACA CCCCACCC		50.6
#12 ♂	CCAACA CCCCACCC		76.7
#13 ♀	CCAACA CCCCACCC		66.8
#14 ♀	CCAACA CCCCACCC		85.3
#15 ♀	CCAACA CCCCACCC		73.3
#16 ♀	CCAACA CCCCACCC		82.9
#17 ♀	CCAACA CCCCACCC		49.7
#18 ♀	CCAACA CCCCACCC		74.8

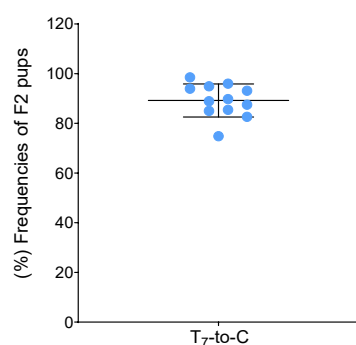
d



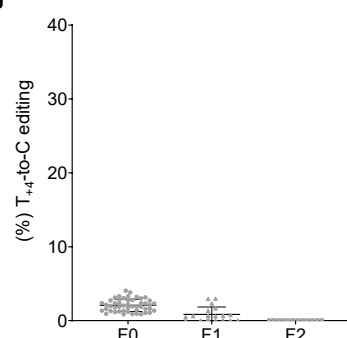
e

#14 ♀ × WT ♂			
#14 ♀ (F1)	CCAACA CCCCACCC	Frequency (%)	85.3
WT ♂	CCAACA TCCCACCC		0.0
↓ F2			
#1 ♀	CCAACA CCCCACCC		91.2
#2 ♂	CCAACA CCCCACCC		92.7
#3 ♀	CCAACA CCCCACCC		93.5
#4 ♀	CCAACA CCCCACCC		85.4
#5 ♂	CCAACA CCCCACCC		86.6
#6 ♀	CCAACA CCCCACCC		82.6
#7 ♂	CCAACA CCCCACCC		96.0
#8 ♀	CCAACA CCCCACCC		84.8
#9 ♀	CCAACA CCCCACCC		80.4
#10 ♂	CCAACA CCCCACCC		87.5
#11 ♀	CCAACA CCCCACCC		83.3
#12 ♀	CCAACA CCCCACCC		72.9

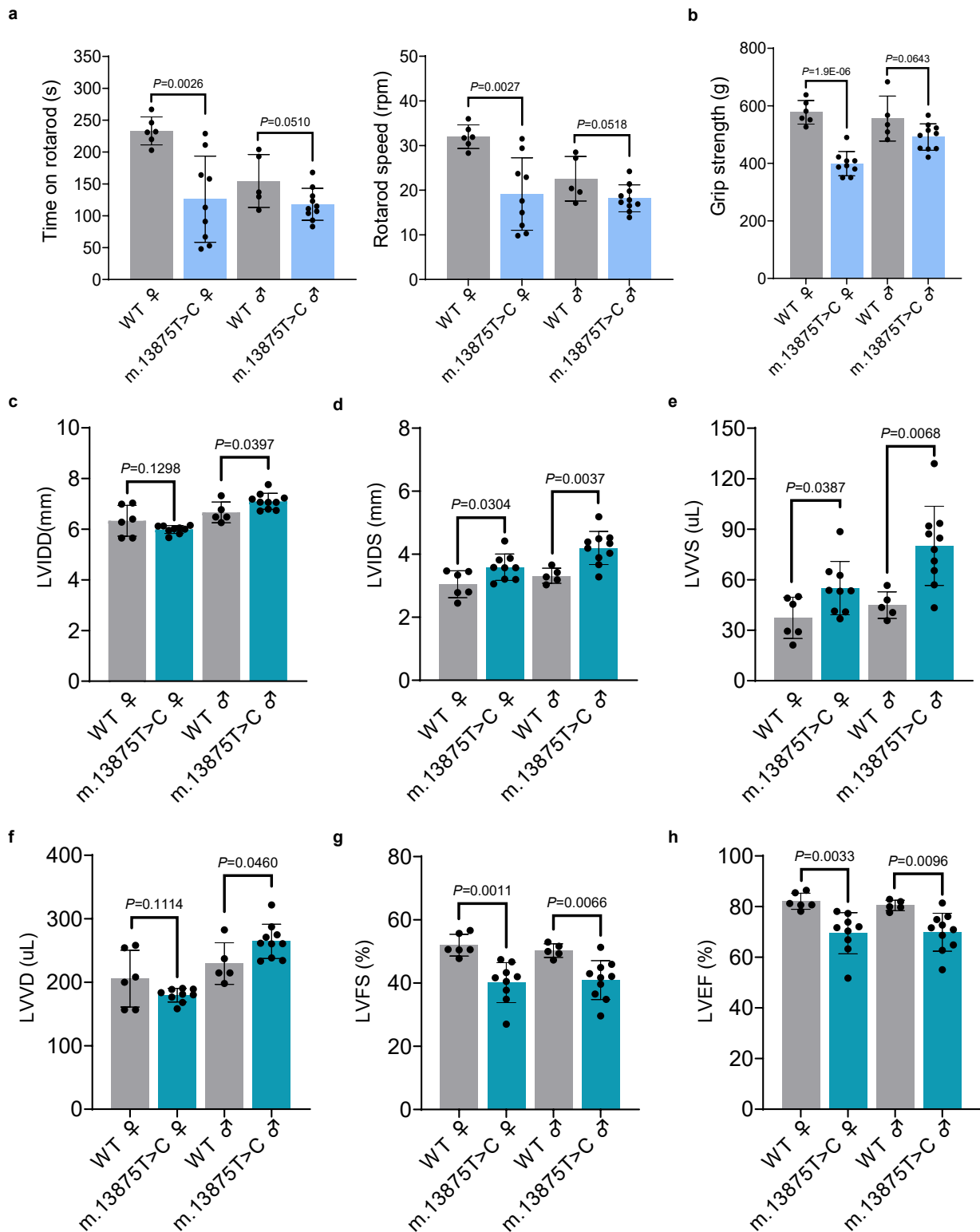
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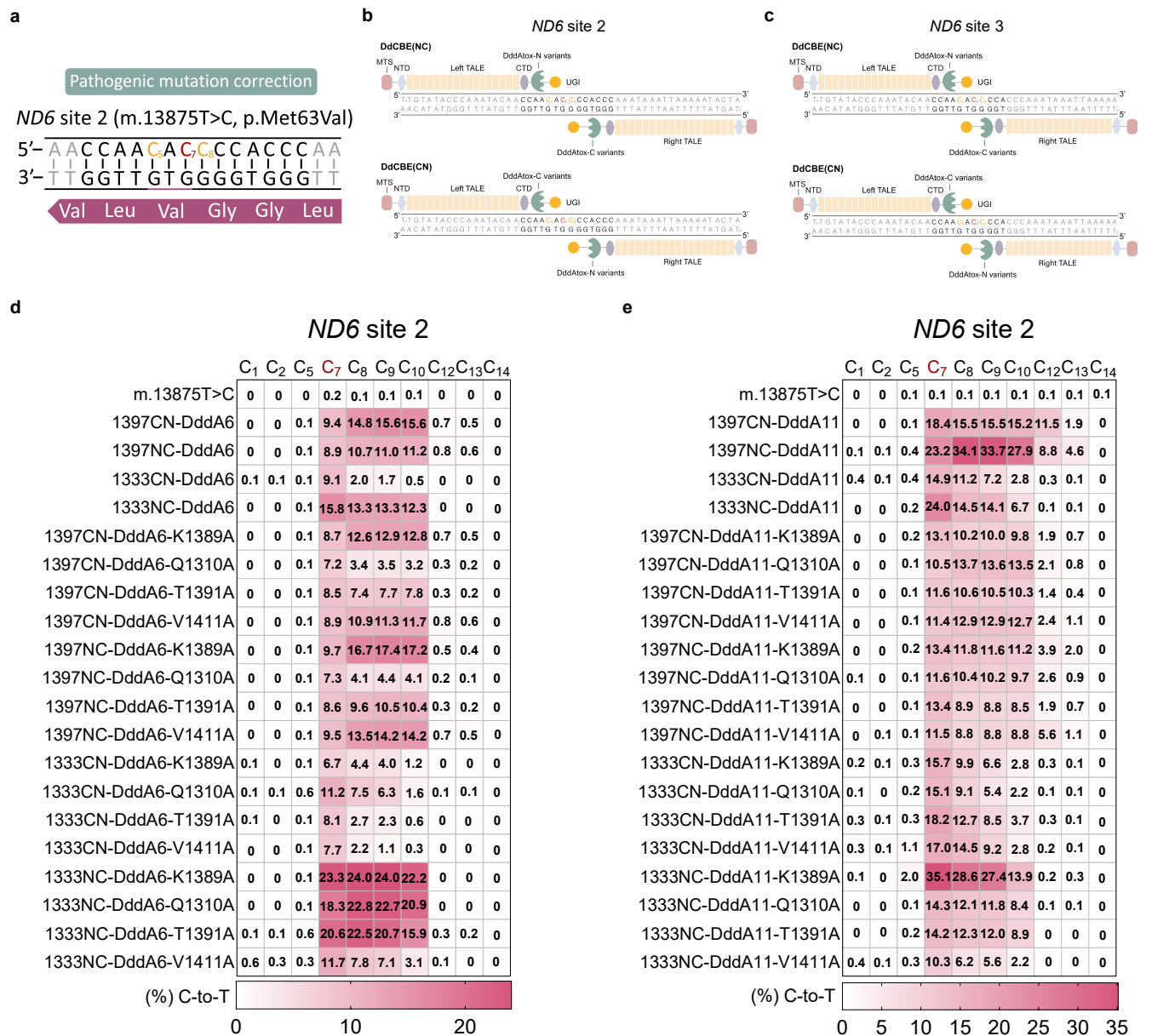
g



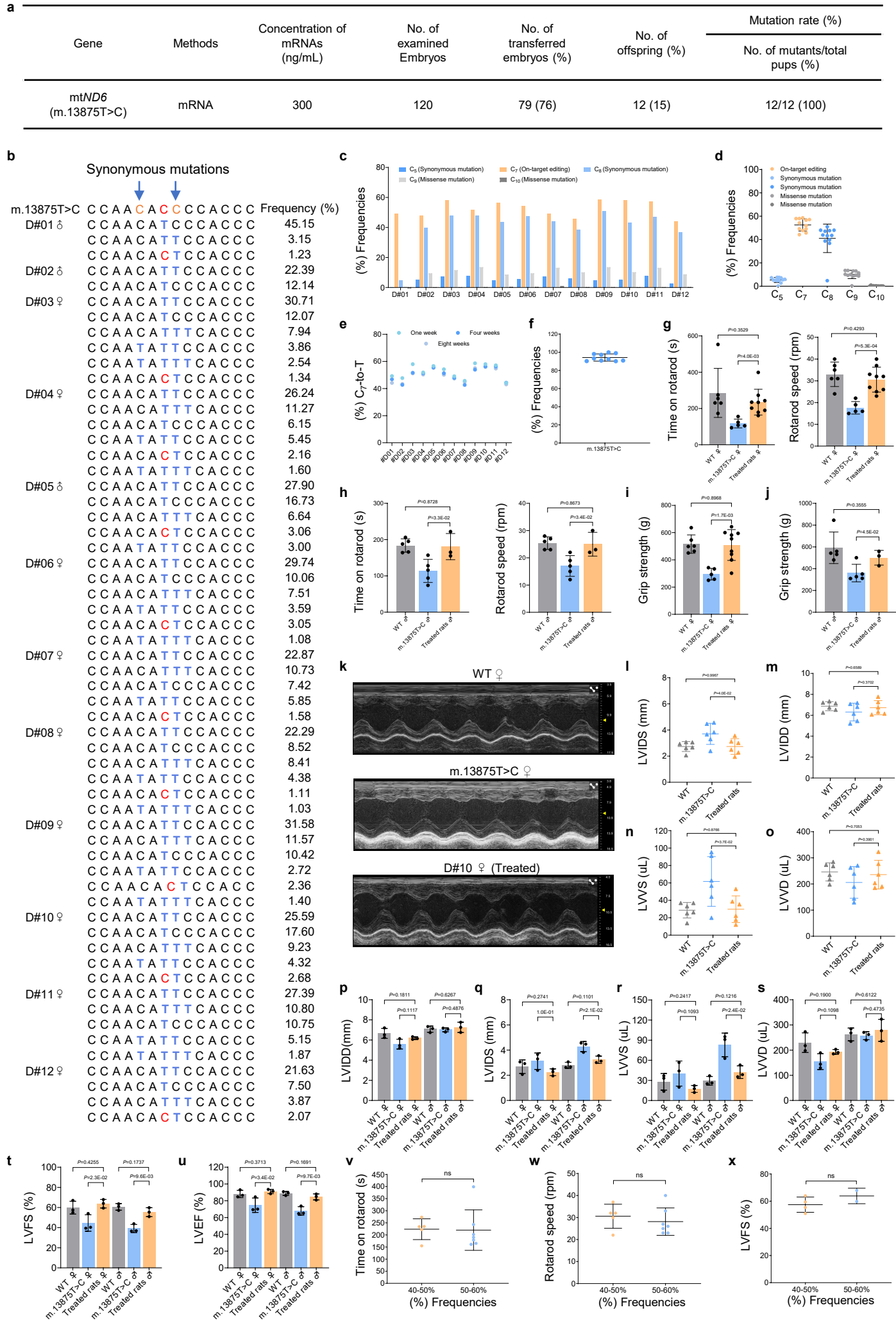
Supplementary Figure 2. Genotype analysis of Leigh syndrome rat models. **a**, The numbers of embryos used and pups generated after microinjection of eTd-mtABEs mRNA. **b**, Desired T₇-to-C editing frequencies in indicated tissues from representative F0 rats. **c**, HTS alignments of mutant sequences from F1 pups. **d**, Desired T₇-to-C editing frequencies of F1 pups (n = 18). **e**, HTS alignments of mutant sequences from F2 pups generated by mating F1 L#14 (♀) with WT (♂). **f**, Desired T₇-to-C editing frequencies of F2 pups (n = 12). **g**, Comparison of the bystander T₊₄-to-C editing frequencies induced by F0, F1 and F2 pups. In **d**, **f** and **g**, data represent the mean ± s.d., and each data point represents an individual rat.



Supplementary Figure 3. Phenotype analysis of Leigh syndrome rat models. **a**, Motor coordination of male and female WT or F0 founders at 11-week-old was evaluated by rotating rod test, with time on rotarods (left panel) and rotarod speed (right panel). **b**, Grip strength of male and female WT or F0 founders was measured by grip strength test at 11-week-old. **c-h**, Echocardiographic measurements of male and female WT or F0 founders at 12-week-old. Tests include LVDD (c), LVIDS (d), LVVS (e), LVDD (f), LVFS (g) and LVEF (h). In c-h, data represent the mean $\pm$ s.d., and each data point represents an individual rat. Male WT rats (n=5) and female WT rats (n=6) are as control, male F0 founders (n=10) and female F0 founders (n=9) are as experimental group. P values were evaluated with two-tailed Student's t-tests.



Supplementary Figure 4. Correction of pathogenic m.13875T>C mutations with DdCBE variants in primary cells. **a**, Diagram of the target site of the pathogenic mutation (*ND6* site 2, m.13875T>C) in Leigh syndrome rat cells to be corrected by DdCBE. The red letter indicates the target mutation, and yellow letters indicate synonymous mutations. **b,c** DdCBE variants designed to target the *ND6* site 2 (**b**) and *ND6* site 3 (**c**) in the m.13875T>C disease cells. Fusion of split-DddAtox-N to the left TALE array is on the top panel (DdCBE-NC), and fusion of split-DddAtox-N to the right TALE array is on the bottom panel (DdCBE-CN). TALE binding sites and spacers are shown. **d,e**, Heatmap showing the editing efficiency of indicated editors with DddA6_{tox} split (**d**) or DddA11_{tox} split (**e**) targeted to *ND6* site 2 in the m.13875T>C disease cells. Data represent the mean of three biologically independent replicates.

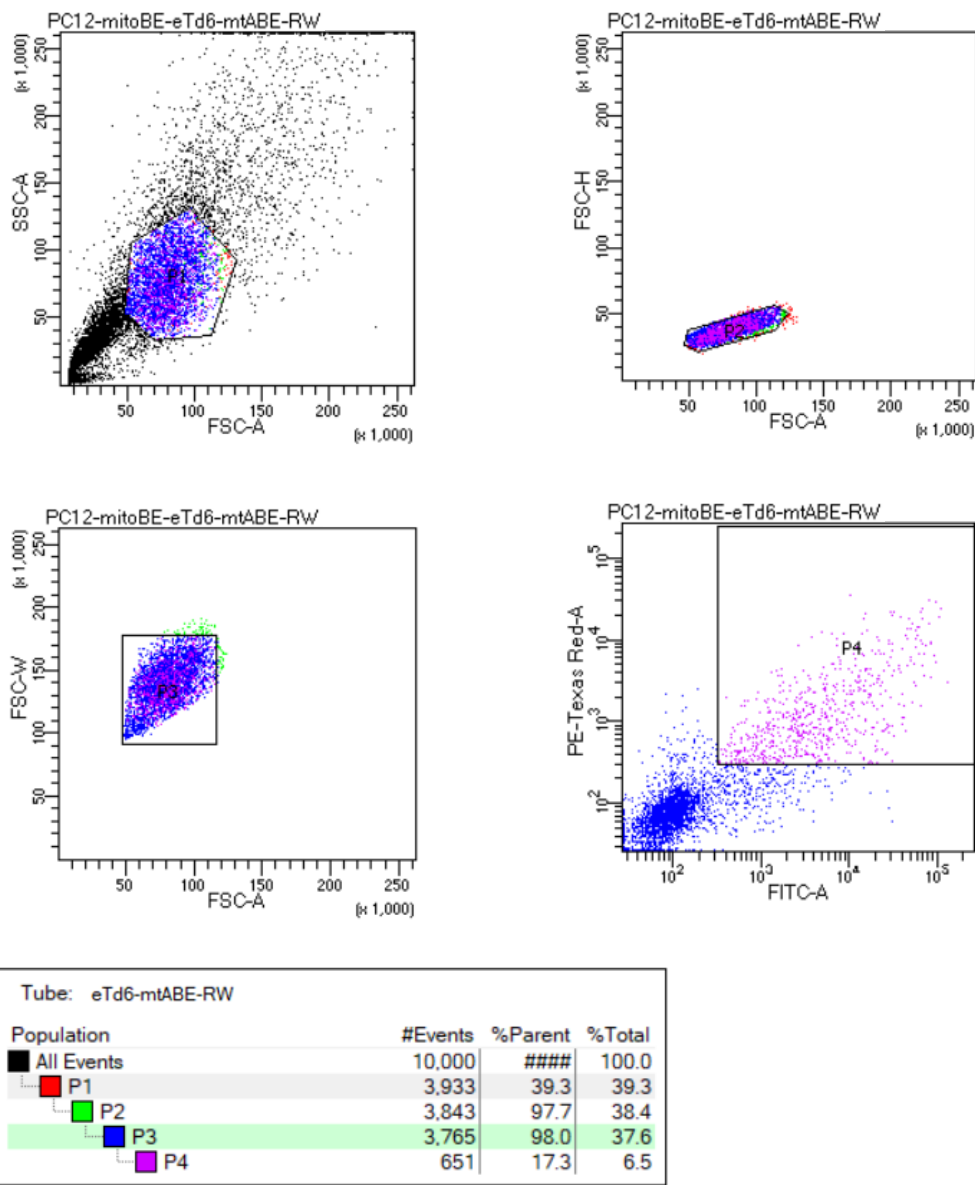


Supplementary Figure 5. Correction of pathogenic m.13875T>C mutations with engineered DdCBEs in rats. **a**, The numbers of embryos used and pups generated after microinjection of engineered DdCBE mRNA. **b**, Genotyping of representative F0 founders treated with engineered DdCBE mRNA targeting the *ND6* site 3. C-to-T frequencies of mutant alleles were determined by HTS. **c**, The frequencies of C₇ (on-target editing), C₅ or C₈ (synonymous mutation), and C₉ (missense mutation) at individual F0 founders treated with engineered DdCBE mRNA. **d**, The frequencies of C₇ (on-target editing), C₅ or C₈ (synonymous mutation), and C₉ (missense mutation) at all F0 founders treated with engineered DdCBE mRNA. **e**, C₇-to-T editing frequencies of treated rats injected with engineered DdCBE at one-week-old, four-week-old and eight-week-old. **f**, T₇-to-C editing frequencies of Leigh syndrome rats with m.13875T>C mutation as control. **g,h**, Motor coordination of wild-type, m.13875T>C and treated female rats in (**g**) and male rats in (**h**) at 8-week-old was evaluated by rotating rod test, with time on rotarods (left) and rotarod speed (right). **i,j**, Grip strength of wild-type, m.13875T>C and treated female rats in (**i**) and male rats in (**j**) was measured by Grip Strength Test at 8-week-old. **k**, Snapshots of M-mode of echocardiographic measurements including a wild-type, a representative m.13875T>C and a treated rat with engineered DdCBE mRNA at 9-week-old. **l-o**, Echocardiographic measurements including LVIDS (**l**), LVIDD (**m**), LVVS (**n**) and LVVD (**o**) of wild-type (n=6), m.13875T>C (n=6) and treated (n=6) rats at 9-week-old. **p-u**, Echocardiographic measurements including LVVD (**p**), LVIDS (**q**), LVVS (**r**), LVDD (**s**), LVFS (**t**) and LVEF (**u**) with indicated female and male rats at 9-week-old, respectively. **v-x**, The time on rotarods (**v**), rotarod speed (**w**) and LVFS (**x**) of treated rats with 40-50% or 50-60% editing frequencies. In **d**, **f-j** and **l-x**, data represent the mean $\pm$ s.d., and each data point represents an individual rat. In **g-j** and **l-x**, *P* values were evaluated with two-tailed Student's t-tests.

Supplementary Note

PC12: eTd6-mtABE-RW

BD FACSDiva 9.4



Supplementary Note. FACS gating examples for mCherry- and EGFP- double positive cell sorting conditions in the PC12 cells.