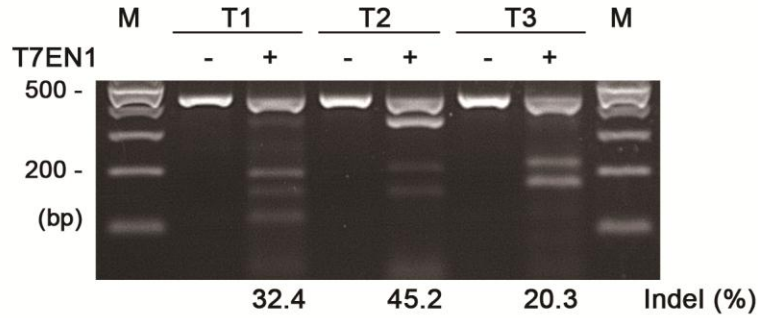


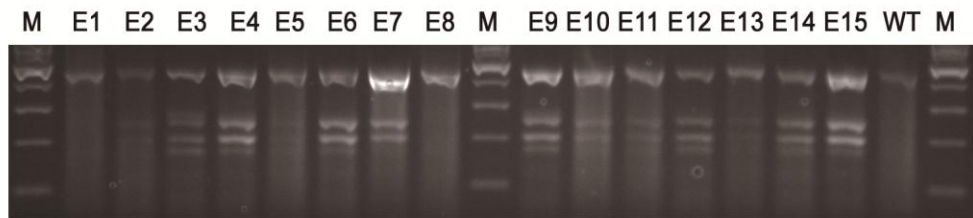
**A**



**B**

mRNA (ng/μl)	MII oocytes	2-cell embryos	Morula/blastocyst (% of 2-cell)	Embryos sequenced	Monoallelic mutation	Biallelic mutation	Wild type
20:20	10	8	5 (62.5%)	8	3 (37.5%)	1 (12.5%)	4 (50.0%)
50:50	13	8	4 (50.0%)	7	6 (85.7%)	0 (0%)	1 (14.3%)

**C**



**D**

WT: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA

E1: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (6/6)

E2: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (8/8)

E3: =====TACCAGAGCACTTGG=====GCTCGAAA Δ51 (4/8)  
TTATCTTCAAAGATGGCTACCA===== Δ164 (4/8)

E4: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (4/10)  
=====TACCAGAGCACTTGG=CAAAGCTCGAAA Δ49 (6/10)

E5: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (8/8)

E6: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (5/8)  
TTATCTTCAAAGATGGCTA=gccatct=TTGGGACAAAGCTCGAAA Δ9bp +7bp (3/8)

E7: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (6/8)  
TTATCTTCAAAGATGGCTACCAGAGCA==TGGGACAAAGCTCGAAA Δ2 (1/8)  
TTATCTTCAAAGATGGCT=====TGGGACAAAGCTCGAAA Δ11 (1/8)

E8: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (6/6)

E9: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (4/8)  
TTATCTTCAAAGATGGCTACCAGA==ACTTGGGACAAAGCTCGAAA Δ2 (4/8)

E10: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (6/8)  
TTATCTTCAAAGATGGCTACCA=====ACTTGGGACAAAGCTCGAAA Δ4 (2/8)

E11: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (8/10)  
TTATCTTCAAAGATGGCTACCAaAGCACTTGGGACAAAGCTCGAAA Δ1bp +1bp (2/10)

E12: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (5/9)  
TTATCTTCAAAGATGGCTACCAG===== large fragment (4/9)

E13: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (8/8)

E14: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (11/15)  
TTATCTTCAAAGATGGCTACCAGAGCACcTGGGACAAAGCTCGAAA Δ1bp +1bp (4/15)

E15: TTATCTTCAAAGATGGCTACCAGAGCACTTGGGACAAAGCTCGAAA WT (7/12)  
TTATCTTCAAAGATGGCTACCA=====CT=GGGACAAAGCTCGAAA Δ6bp (5/12)

**Figure S1. Evaluation of TALEN-mediated mutagenesis in COS-7 cells and cynomolgus**

## monkey embryos

(A) T7EN1 assay of the indel rate (shown below the relevant lanes) of three TALENs targeting the cynomolgus monkey *MCPHI* gene. The TALEN-targeting sequences are labeled as T1, T2 and T3; M, Marker.

(B) Effects of TALEN mRNA injection at different concentrations on the mutagenesis efficiency in cynomolgus monkey embryos.

(C) Genomic DNA extracted from 15 cynomolgus monkey embryos injected with *MCPHI-T2* mRNAs were subjected to PCR and T7EN1 assay. M, Marker; embryos E1-E8 received 20:20 ng  $\mu\text{l}^{-1}$ , while embryos E9-E15 received 50:50 ng  $\mu\text{l}^{-1}$ .

(D) Sanger sequencing of PCR products from 15 monkey embryos injected with *MCPHI-T2* mRNAs. Embryos E1-E8 received 20:20 ng  $\mu\text{l}^{-1}$  and embryos E9-E15 received 50:50 ng  $\mu\text{l}^{-1}$ . The targeting sequence is shown in red uppercase. The targeted integration and the sizes of each insertion (+, in lowercase) and deletion ( $\Delta$ ) are presented to the right of the corresponding allele. The fractions indicate the number of mutant sequencing reads (numerator) divided by number of total sequencing reads (denominator) of each allele.