

**B** sgRNA-Tyr-B+C+D+E Tail #5

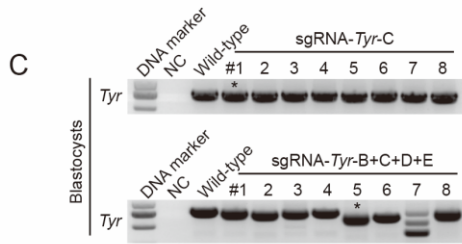
CGAAGGCCACCGCCCTCTTTGG//CCAGAAGCCAATGCACCTATCGG//CTTCATAACATCCAAGGATCTGG//TACAGTACCTCCAAGAGTCAGG WT

CGAAGCCAt-----//-----ATCTGG//TACAGTACCTCCAAGAGTCAGG -118 X6

GCC-----//-----ATCTGG//TACAGTACCTCCAAGAGTCAGG -127 X3

CCAT-----//-----ATCTGG//TACAGTACCTCCAAGAGTCAGG -118 X1

GCGAAGGCA-----//-----TCTGG//TACAGTACCTCCAAGTCAGG -122 X1



**D** sgRNA-Tyr-C Blastocyst #1

AGTTTACCCAGAAGCCAATGCACCTATCGGCCATAAC

AGTTTACCCAGAAGCCAATGCAC-TATCGGCCATAAC -1 X3

AGTTTACCCAGAAGCCAATGCACCTATCGGCCATAAC WT X2

AGTTTACCCAGAAGCCAATGC-----CATAAC -10 X3

GTTTACCCAGAAGCCAATGCACCcTATCGGCCATAAC +1 X3

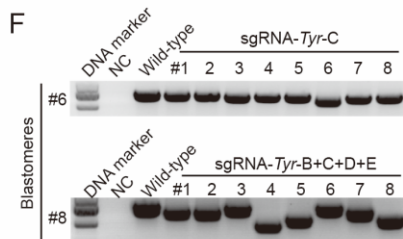
**E** sgRNA-Tyr-B+C+D+E Blastocyst #5

GCGAAGGCCACCGCCCTCTTTGG//CCAGAAGCCAATGCACCTATCGG//CTTCATAACATCCAAGGATCTGG//TACAGTACCTCCAAGAGTCAGG

CGAAGGCCACCGCCCA-----G//CCAGAAGCCAATGCAC-----//----- -220 X6

CGAAGA-----//-----AGAAGCCAATGCACC-----//-----GTCAGG -137 X1

GCGAAGG-----//-----//----- -158 X2



**G** 16-cell embryo #4 (sgRNA-Tyr-C)

GCCCTCTTTTGGAAAGTTTACCCAGAAGCCAATGCACCTATCGGCCATAACAGAGACTCTTACA

#1 GCCCTCTTTTGGAAAGTTTACCCAGAAGCCAATGCAC--ATCGGCCATAACAGAGACTCTTACA -2

2 GCCCTCTTTTGGAAAGTTTACCCAGAAGCCAATGCAC--ATCGGCCATAACAGAGACTCTTACA -2

3 GCCCTCTTTTGGAAAGTTTACCCAGAAGCCAATGCAC-TATCGGCCATAACAGAGACTCTTACA -1

4 GCCCTCTTTTGGAAAGTTTACCCAGAAGCCAATGCACCTATCGGCCATAACAGAGACTCTTACA WT -149

5 GCCCTCTTTTGGAAAGTTTACCCAGAAGCCAATGCAC-----TCTTACA -20

6 GCCCTCTTTTGGAAAGTTTACCCAGAAGCCAATGCACCTATCGGCCATAACAGAGACTCTTACA WT

7 GCCCTCTTTTGGAAAGTTTACCCAGAAGCCAATGCAC--ATCGGCCATAACAGAGACTCTTACA -2

8 GCCCTCTTTTGGAAAGTTTACCCAGAAGCCAATGCACCTATCGGCCATAACAGAGACTCTTACA WT

**H** 16-cell embryo #6 (sgRNA-Tyr-B+C+D+E)

GCGAAGGCCACCGCCCTCTTTGG//CCAGAAGCCAATGCACCTATCGG//CTTCATAACATCCAAGGATCTGG//TACAGTACCTCCAAGAGTCAGG

#1 G-----G//CCAGAAGCCAATGCACCTATCGG//CTTCATAACATCCAAG-----G//TACAGTACCTCCAAG-GTCAGG -28

CGAAGGCCACCGCCCTCT-----//-----ATCGG//CTTCATAACATCCAATccGGATCTGG//TACAGTAcCCTCCAAGAGT -27

2 CGAAGGCCACCGCCCC--TTTGG//CCAGAAGCCAAT-----//-----//TACAGTAcCCTCCAAGAGT -376

3 CGAAG--//-----ATCGG//CTTCATAACATCCAATccGGATCTGG//TACAGTAcCCTCCAAGAGT -56

CGAAGGCCACCGCCCTCT-----//-----ATCGG//CTTCATAACATCCAATccGGATCTGG//TACAGTAcCCTCCAAGAGT -27

4 CGAAGGCCACCGCC--TTTGG//CCAGAAGCCAAT-----//-----//TACAGTAcCCTCCAAGAGT -376

5 G-----G//CCAGAAGCCAATGCACCTATCGG//CTTCATAACATCCAAG-----G//TACAGTACCTCCAAG-GTCAGG -28

6 CGAAGGCCACCGCCCTCT-----//-----ATCGG//CTTCATAACATCCAATccGGATCTGG//TACAGTAcCCTCCAAGAGT -27

7 CGAAGGCCACCGCCCTCT-----//-----ATCGG//CTTCATAACATCCAATccGGATCTGG//TACAGTAcCCTCCAAGAGT -27

8 CGAAGGCCACCGCC--TTTGG//CCAGAAGCCAAT-----//-----//TACAGTAcCCTCCAAGAGT -376

G-----G//CCAGAAGC-----TATCGG//CTTCATAACATCCAAG-----G//TACAGTAcCCTCCAAG-GTCAGG -38

**Supplementary information, Figure S1.** Genotyping analysis of gene-edited mice by C-CRISPR.

(A) PCR products from nine mice with sgRNA-*Tyr-B+C+D+E* targeting. PCR products of mice #1 to #6 were TA cloned and sequenced. PCR products of tail marked with \* were sequenced shown in Figure S1B. NC, negative control.

(B) Representative sequences from mouse tail #5 with sgRNA-*Tyr-B+C+D+E* targeting. The sgRNA targeting sequences are labeled in green and PAMs are labeled in red; deleted nucleotides are indicated by hyphens. Dashed lines mark the region omitted for clarity.

(C) Representative PCR products from whole embryos at blastocyst stage with sgRNA-*Tyr-C* or sgRNA-*Tyr-B+C+D+E* targeting. PCR products of blastocysts marked with \* were further sequenced and shown in Figure S1D and S1E.

(D & E) Representative sequences from Blastocyst #1 (D) with sgRNA-*Tyr-C* targeting and Blastocyst #5 (E) with sgRNA-*Tyr-B+C+D+E* targeting.

(F) Representative PCR products from single blastomeres of 8- to 16-cell embryos with sgRNA-*Tyr-C* or sgRNA-*Tyr-B+C+D+E* targeting.

(G & H) Representative sequences for individual blastomeres from a single 16-cell embryo with sgRNA-*Tyr-C* targeting (G) and sgRNA-*Tyr-B+C+D+E* targeting (H). About 50% blastomeres of each embryo were successfully amplified and sequenced. The sgRNA targeting sequences are labeled in green and the PAM sequences are labeled in red; deleted nucleotides are indicated by hyphens. Dashed lines mark the region omitted for clarity.