

Supplementary Materials

CRISPR/Cas9-Mediated Knock-Out of dUTPase in Mice Leads to Early Embryonic Lethality

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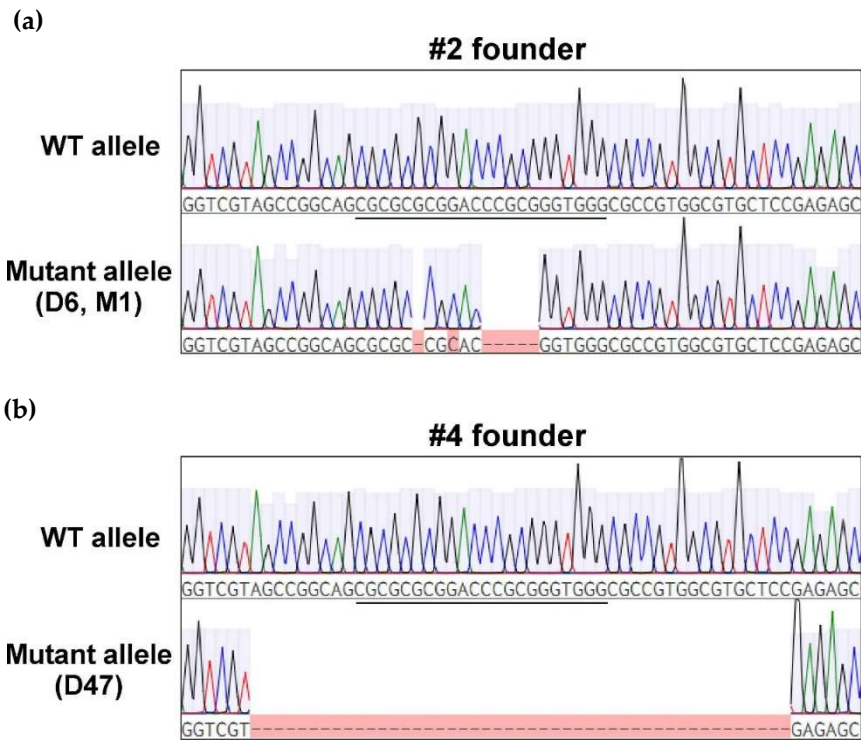


Figure S1. Sequencing analysis of *dut* gene from founder mouse #2 and #4. Sequencing results of *dut* gene from the mouse #2 (a) show 6 bp deletion and 1 bp substitution (D6, M1), while from the mouse #4 (b) show 47 bp deletion (D47). CRISPR target site including PAM sequence is underlined. CRISPR induced modifications are marked with red highlight.

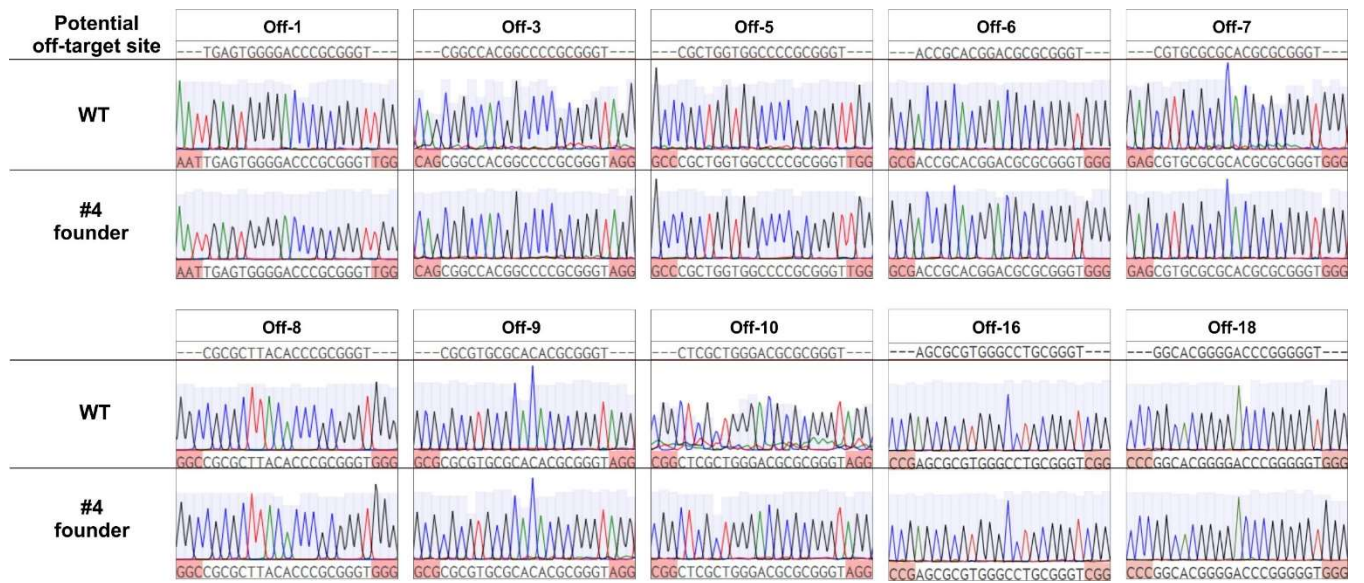
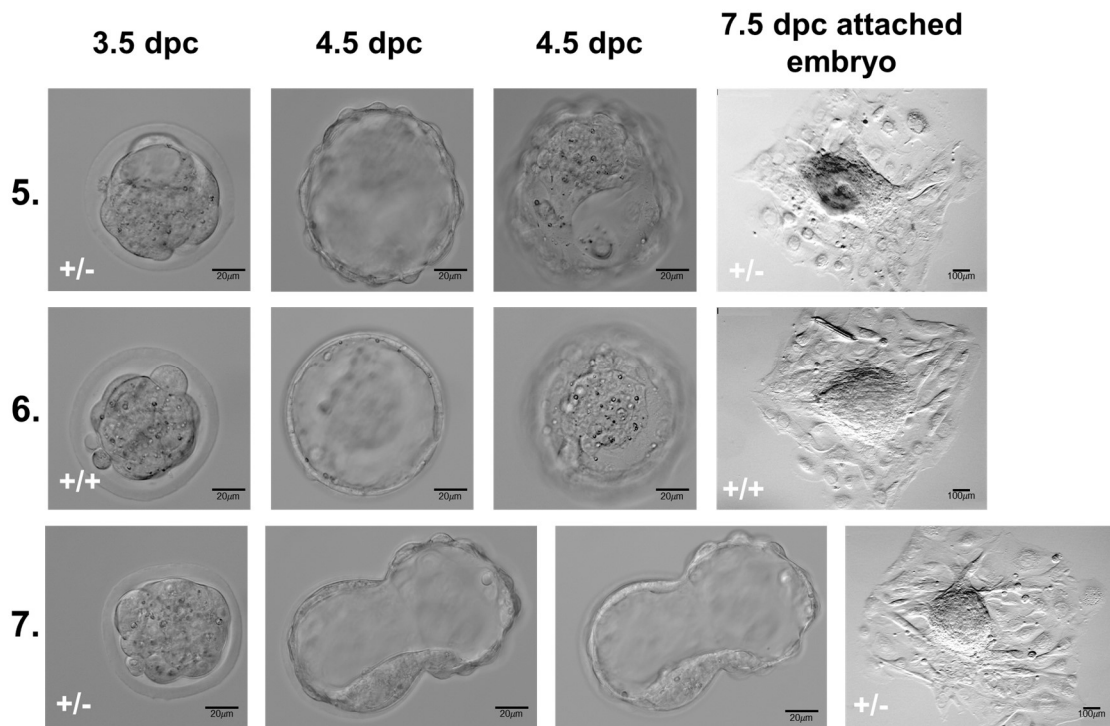
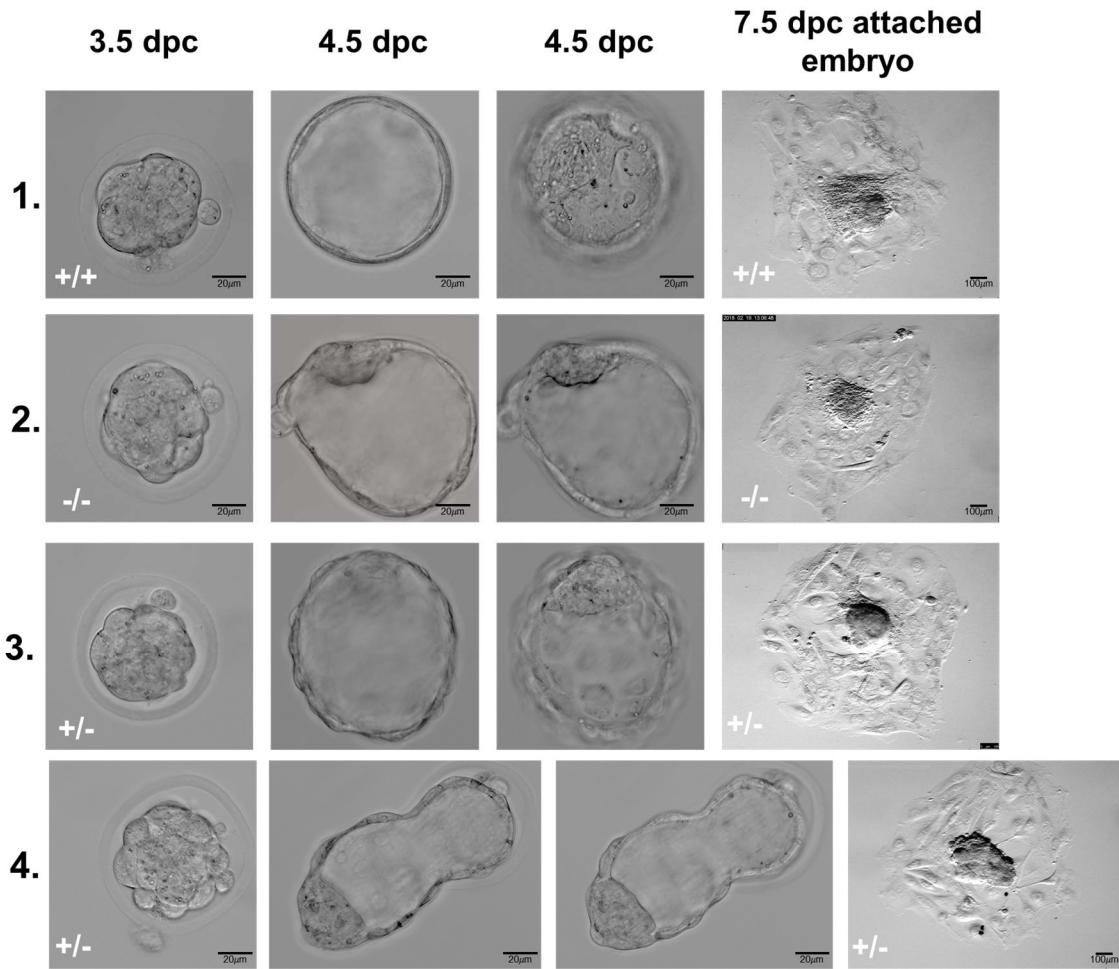


Figure S2. Alignment of the sequencing results from wild type and mouse #4 animals. Sequences are fully identical at these potential off-target sites identified by the CRISPR/Cas9 target online predictor software CCTop.



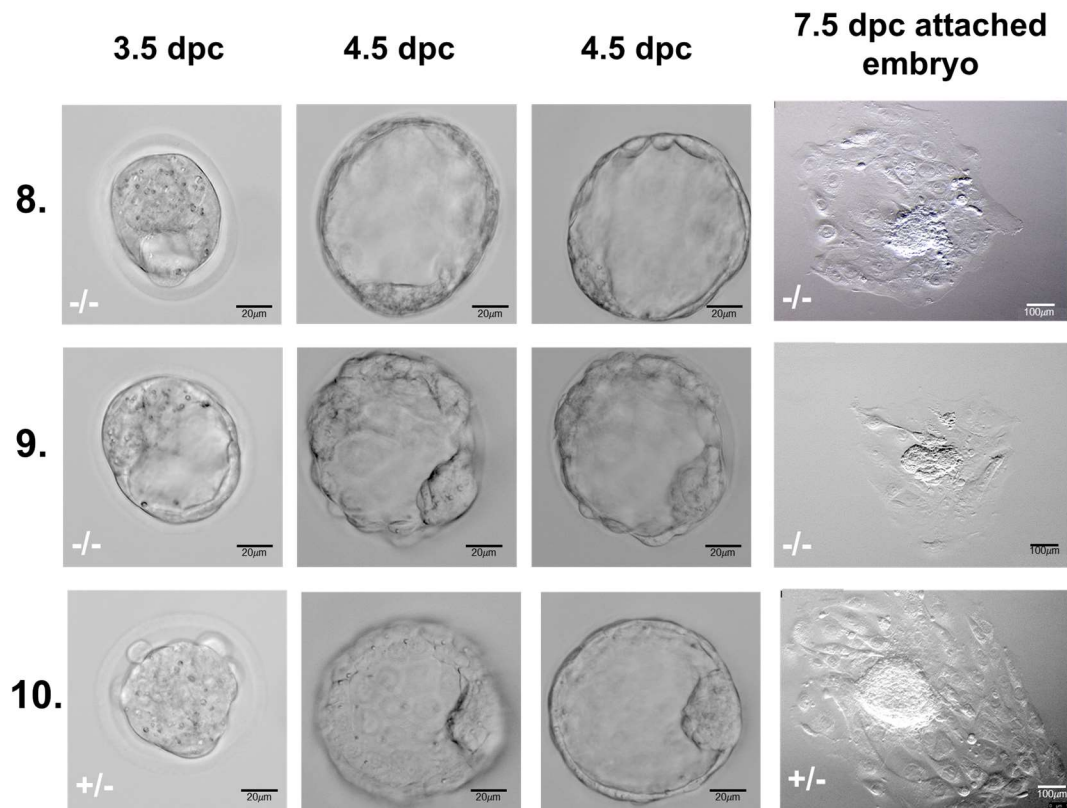
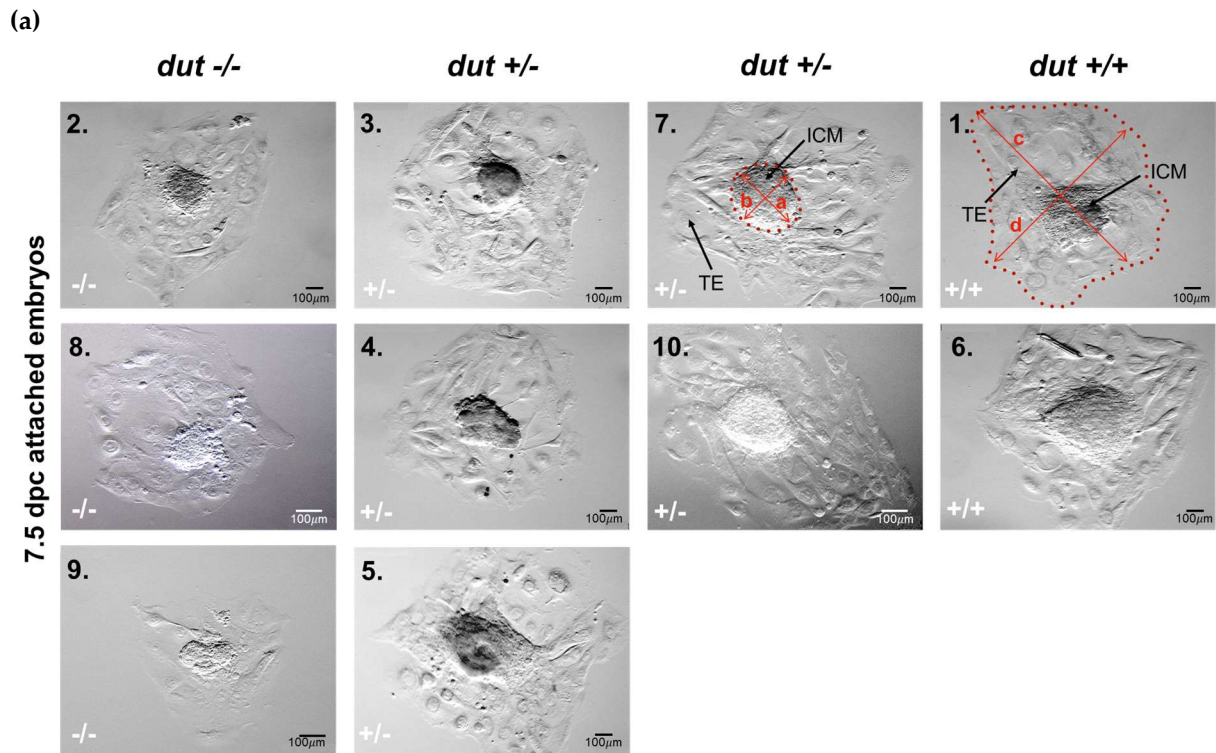


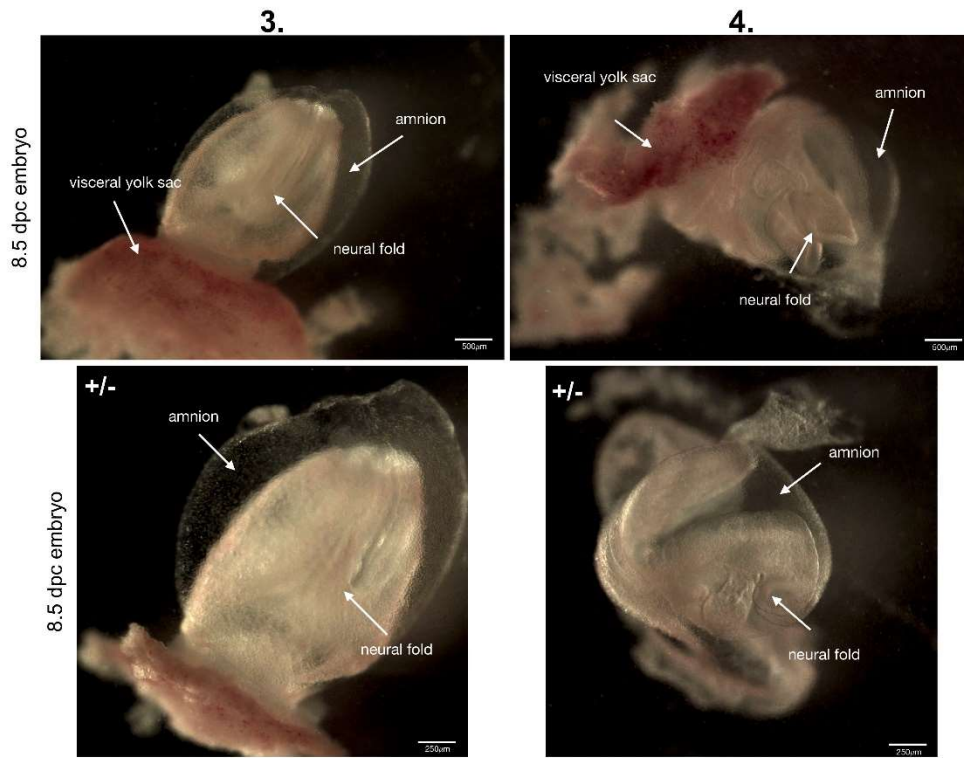
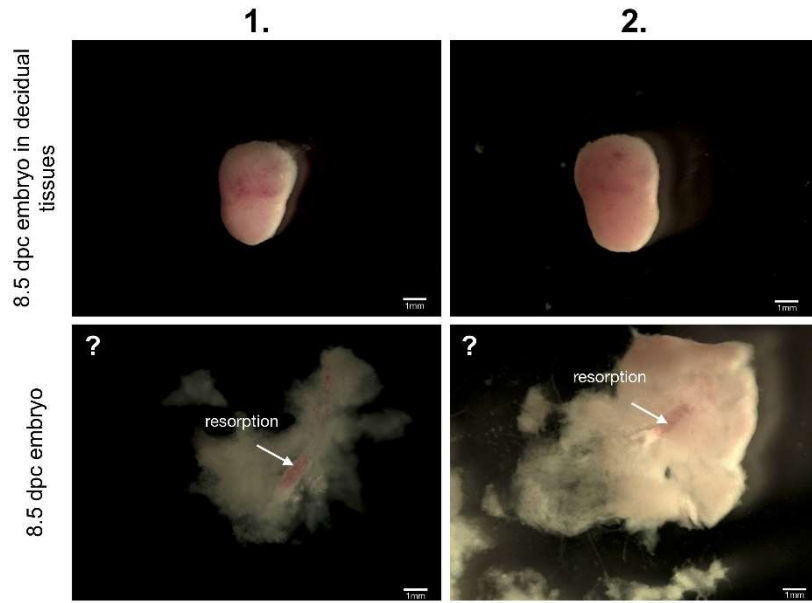
Figure S3. Phase contrast images of embryos obtained by crossing D47 heterozygous mice. Homozygous (*dut*^{-/-}: 2, 8, 9), heterozygous embryos (*dut*^{+/-}: 3, 4, 5, 7, 10), and wild type blastocysts (*dut*^{+/+}: 1, 6) in *in vitro* culture. The first column shows embryos at 3.5 dpc after flushing from oviducts. The second and third columns show the embryos one day later focusing on the trophoblast cells or the inner cell mass (ICM). Scale bar, 20 µm. The last columns present outgrowths after 4 days in culture (7.5 dpc). Scale bar, 100 µm.

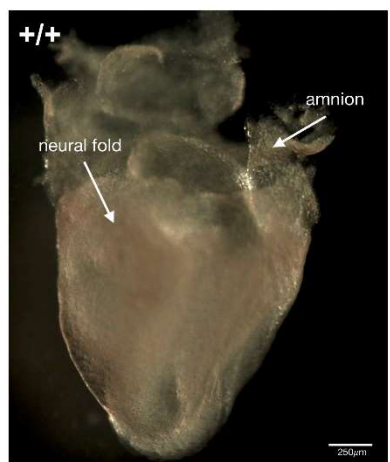
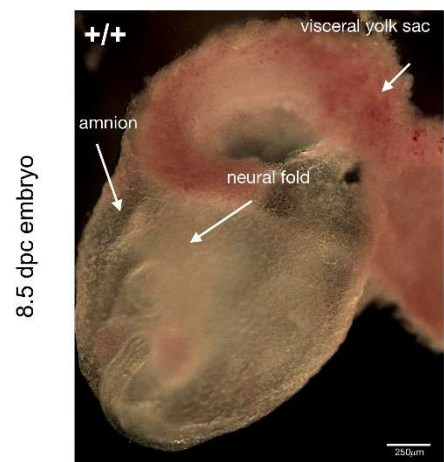
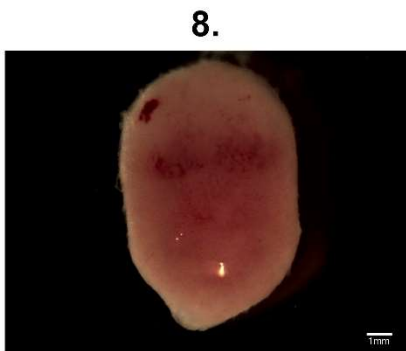
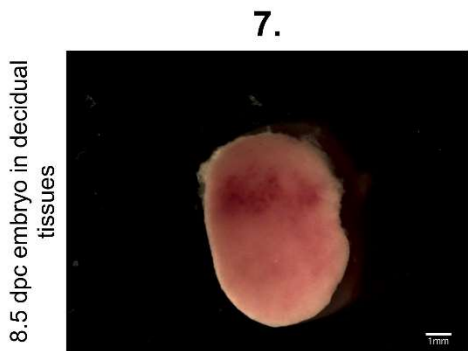
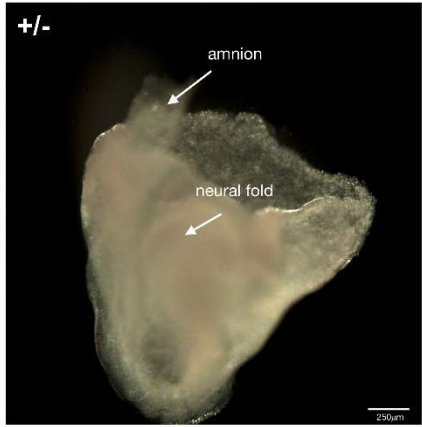
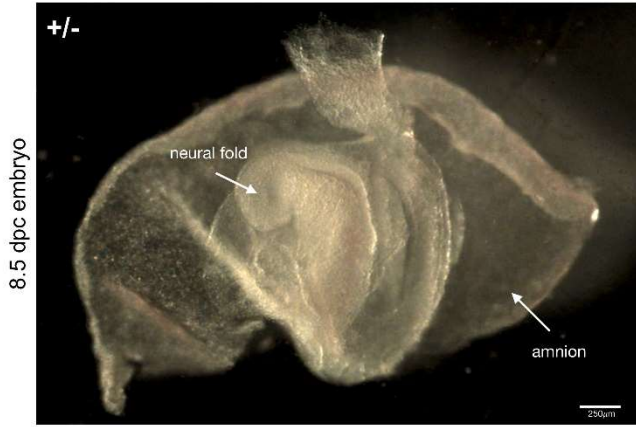
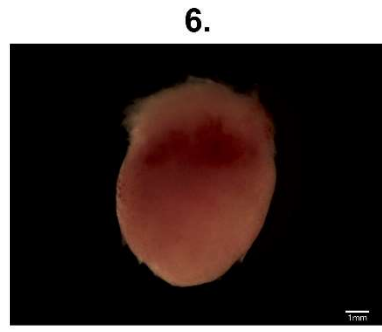
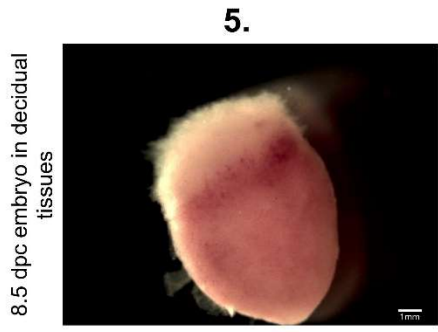


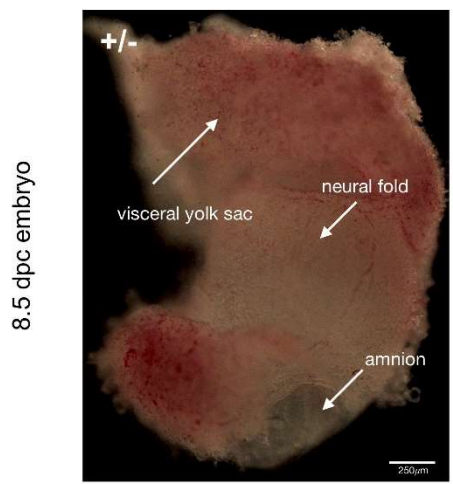
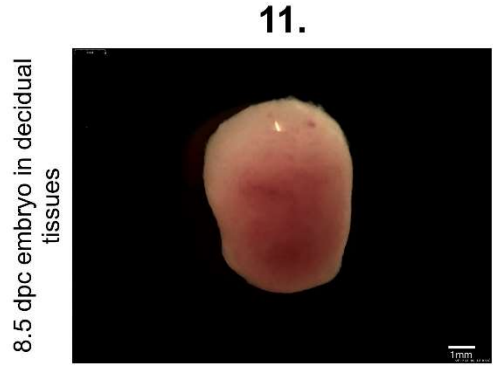
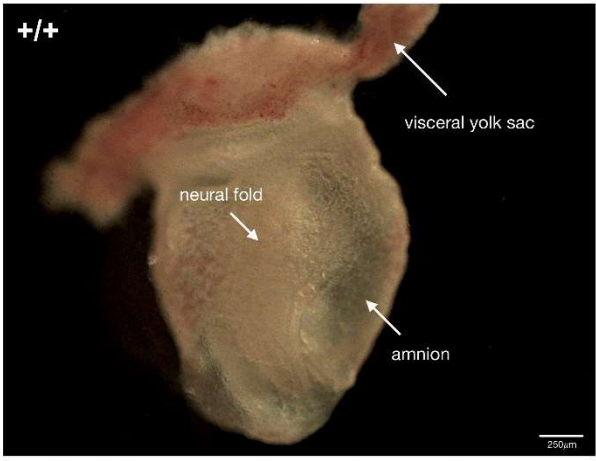
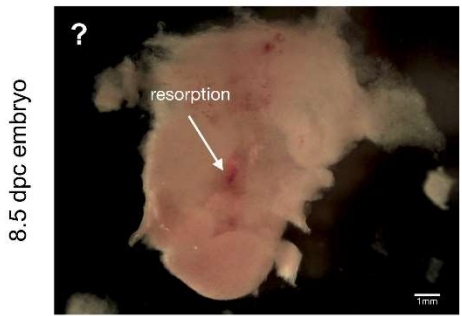
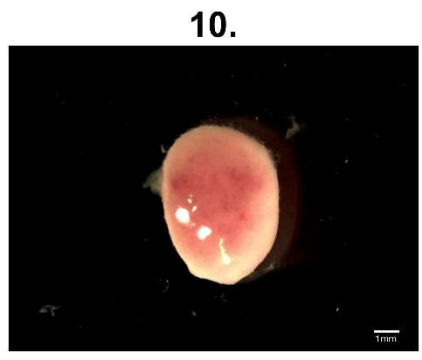
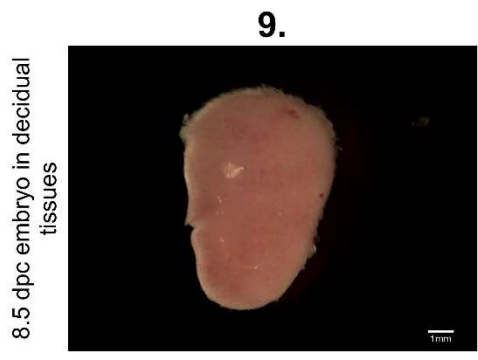
(b)

genotype	number of blastocysts	ICM size (a*b) mm ²		TE size (c*d) mm ²	
		average	SD(±)	average	SD(±)
<i>dut -/-</i>	3	0.078	0.006	0.964	0.156
<i>dut +/-</i>	5	0.138	0.036	1.425	0.177
<i>dut +/+</i>	2	0.189	0.044	1.314	0.055

Figure S4. Outgrowth assay of preimplantation embryos obtained by intercrossing D47 heterozygous mice. (a) The images represent homozygous (*dut -/-*: 2, 8, 9); heterozygous (*dut +/-*: 3, 4, 5, 7, 10); wild type (*dut +/+*: 1, 6) blastocysts attached to the surface of tissue culture dishes and their outgrowth in *in vitro* cultures for 4 days (7.5 dpc). Scale bar, 100 μm. Red arrows (a, b, c, d, respectively) show the parameters used for outgrowth size measurements. (b) Table contains the result of the blastocysts outgrowth size measurements: average size of inner cell mass (ICM) and trophoblastic ectoderm (TE). SD indicates the standard deviation. n = 3 for (*dut -/-*), n = 5 for (*dut +/-*), and n = 2 for (*dut +/+*).

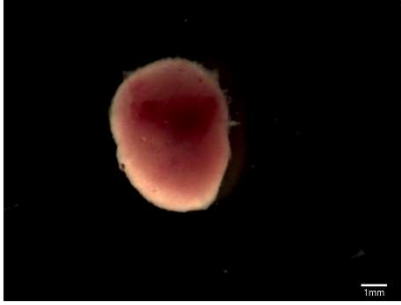






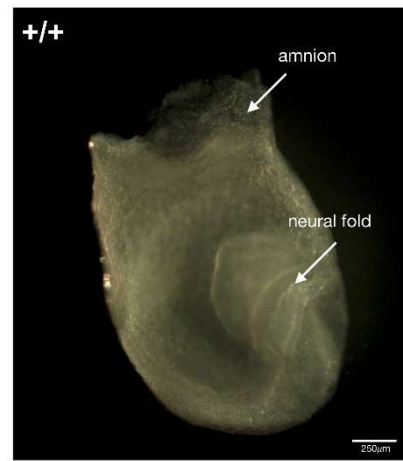
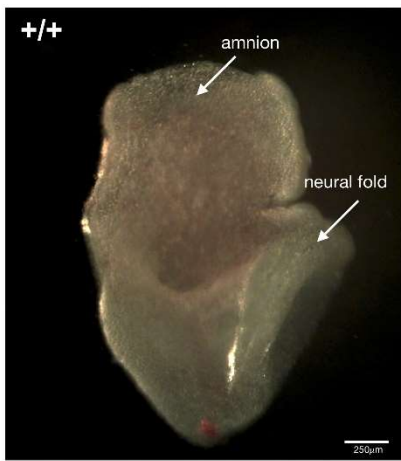
12.

8.5 dpc embryo in decidual tissues



13.

8.5 dpc embryo



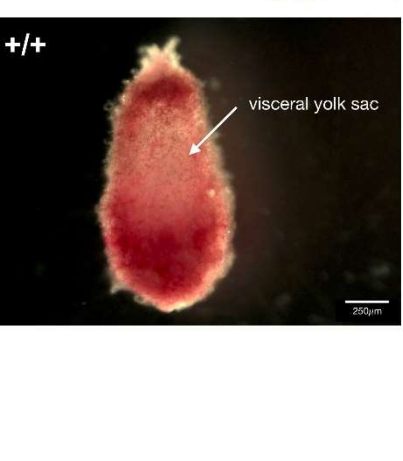
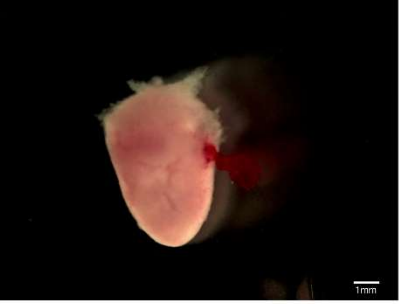
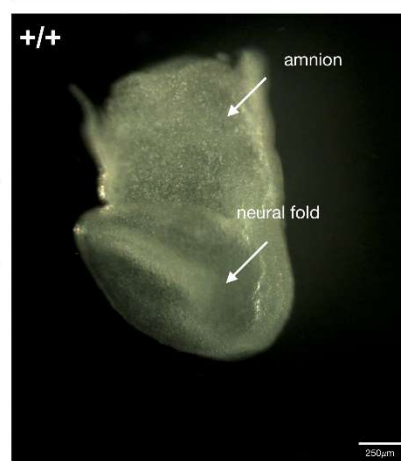
14.

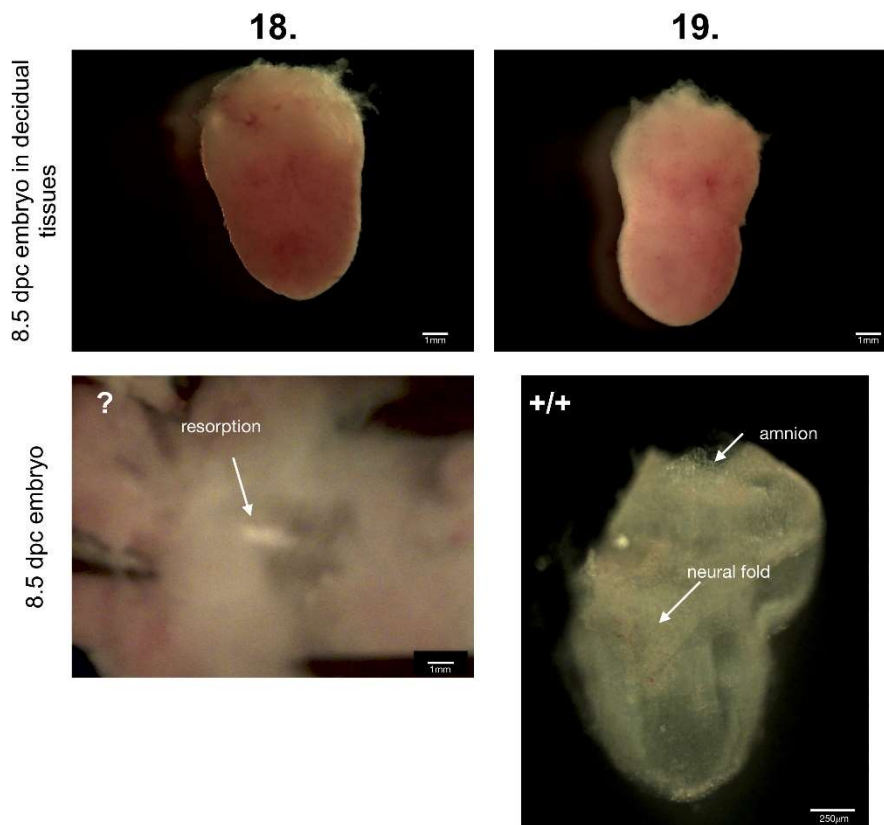
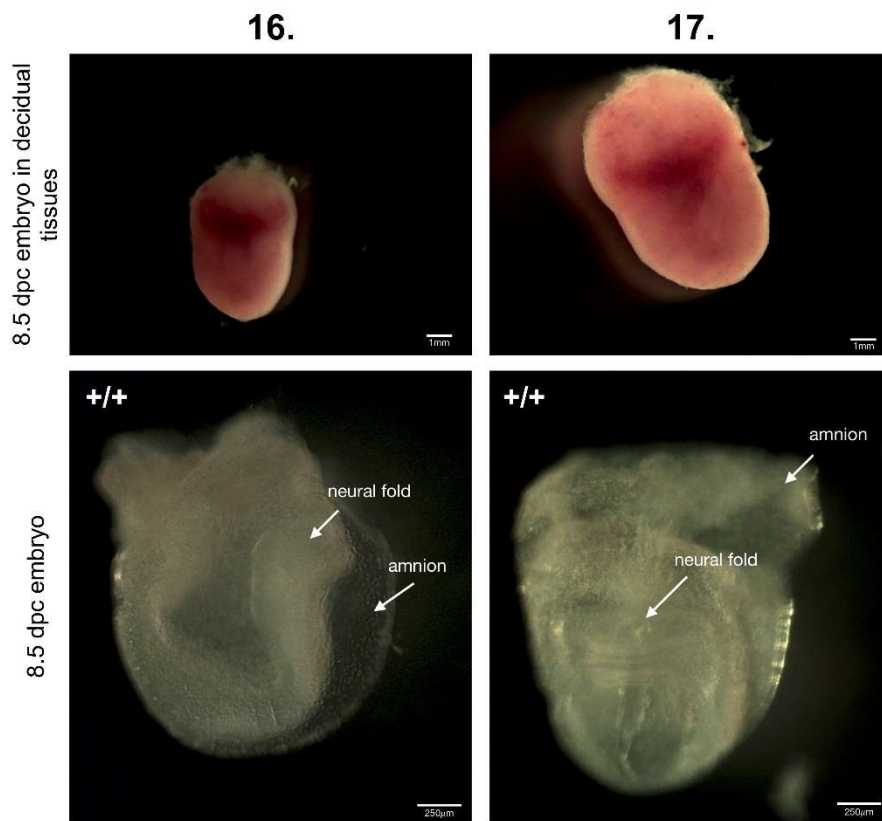
8.5 dpc embryo in decidual tissues



15.

8.5 dpc embryo





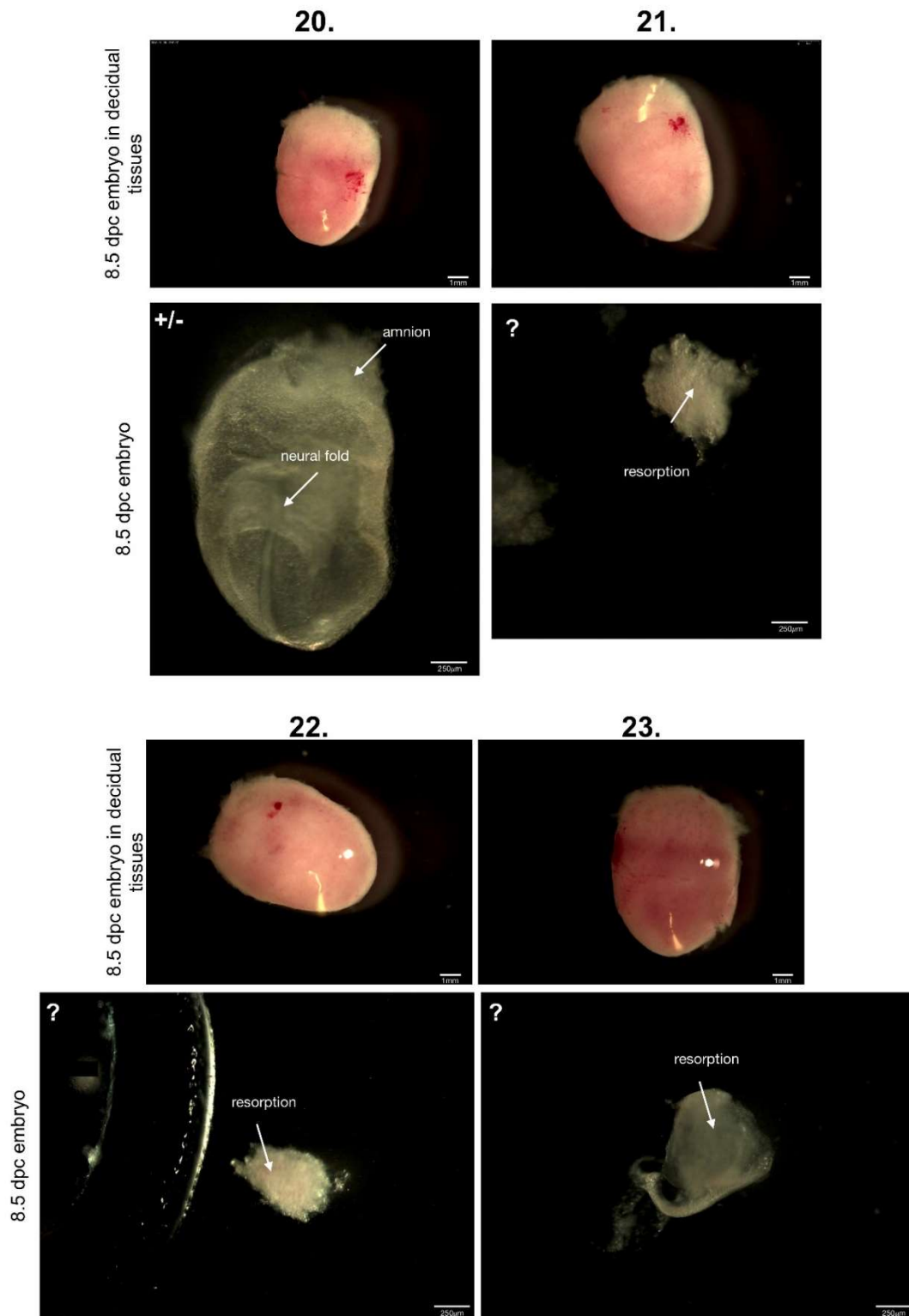


Figure S5. Images of embryos at 8.5 dpc obtained by crossing D47 heterozygous mice. Heterozygous embryos (*dut +/-*: 3, 4, 5, 6, 11, 20) and wild type embryos (*dut +/+*: 7, 8, 10, 12, 13, 14, 15, 16, 17, 19). The resorbed embryos (1, 2, 9, 18, 21, 22, 23) could not be genotyped (indicated with „?“). Upper panels show embryos in the intact decidual tissues. Scale bar, 1 mm. Lower panels present the embryos dissected from decidual tissues. Arrows represent the embryonic regions (neural fold) and extra-embryonic tissues (amnion, yolk sac) of embryos. Scale bar, 250 µm.

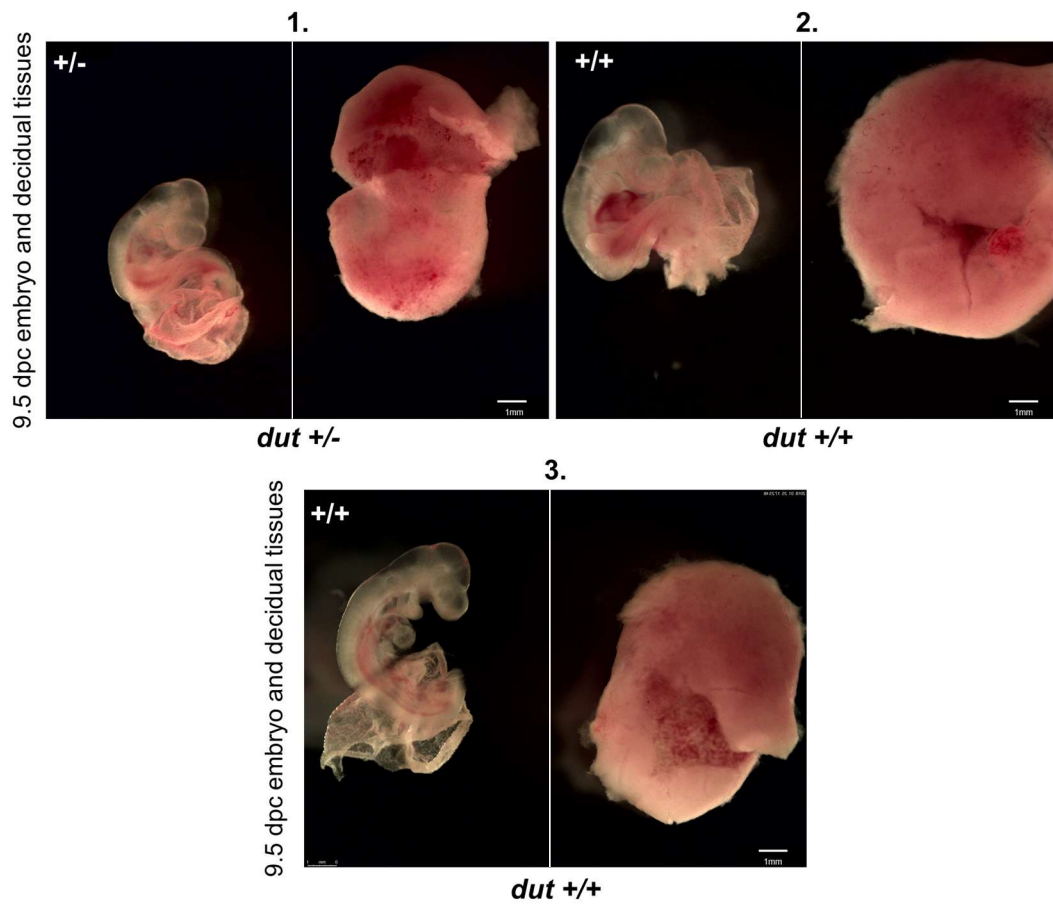


Figure S6. Images of embryos at 9.5 dpc obtained by crossing D47 heterozygous mice. Heterozygous embryos ($dut +/-$: 1) and wild type embryos ($dut +/+$: 2, 3). Right panels show embryos in decidual tissues. Left panels present the embryos dissected from decidual tissues. Scale bar, 1 mm.

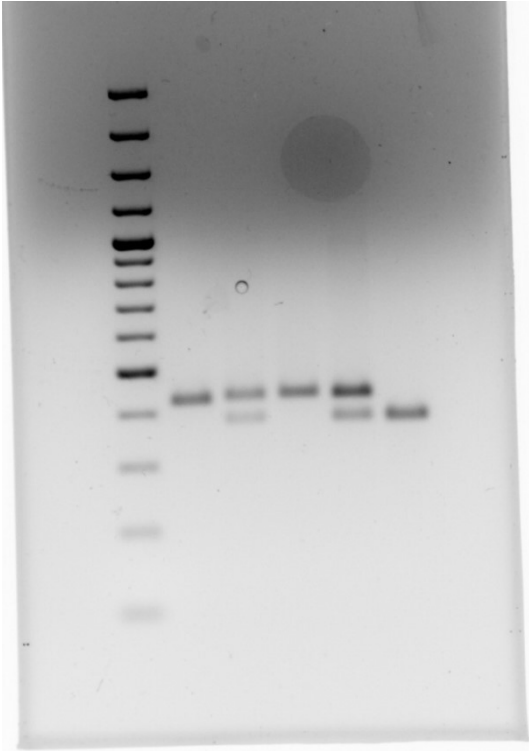


Figure S7. Full-length agarose gel of Figure 2b.

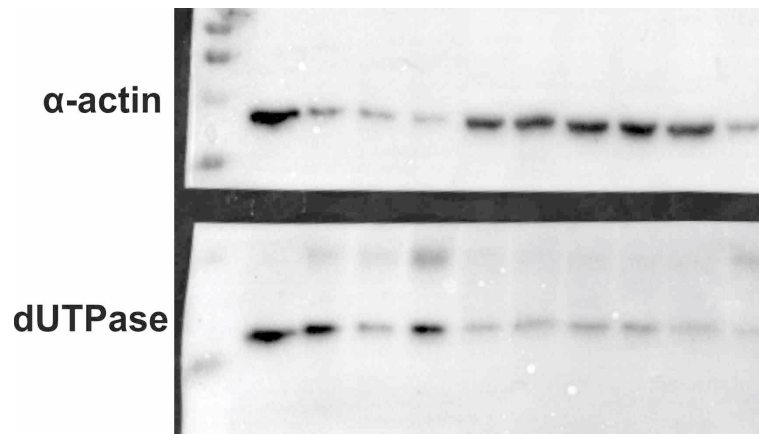


Figure S8. Full-length membrane of Figure 5a.

Table S1. Oligonucleotides used in this study. Restriction sites are underlined.

Sequence (5' - 3')	Name	Experiment
CTGGTCTGAGCAGGTAACGG	Cel-1-F	CEL 1 assay
TAACGTGCACACTGCAGACA	Cel-1-R	
GGTCGGTGCCTCCTCTAG	Dut-gen-F	Genotyping
AATAAGCCTTGACATCCGG	Dut-gen-R	
GGAGATTTTCGGCGGGTAGG	Dut-nest-F	
TCCGGT <u>CGAC</u> CTGGTCTGAGCAGGTAACGG	pBS-F	Cloning
CTAGGA <u>AATTC</u> TAACGTGCACACTGCAGACA	pBS-R	
AGCTTTTACTCATTGCTACTCTCT	Off-1-F	Off-target analysis
ATTGTGAAGTGTGGCTTATGGG	Off-1-R	
CCCCGTTTCACAGAACCGTA	Off-3-F	
CCAAGGACCTAGCCGACATC	Off-3-R	
AACAAGAGCTAGAGGCAGCG	Off-5-F	
CAACAGCAGCTGGGTGTCA	Off-5-R	
GGTGCGTGTGGCCAGTG	Off-6-F	
GAGAGAAGCAAGCAGATGCG	Off-6-R	
GTCACTATGCAAGAAGTTTCCTTAT	Off-7-F	
CCTACCACGTGCCCTGTAAG	Off-7-R	
CACCTCAGCAGTACCAAACAAAG	Off-8-F	
TTGATGGTAAATTCTCCTCTCTTCA	Off-8-R	
GCAGAATCGAAGGTGGTGGT	Off-9-F	
CAGGGCATAGGATACCTGCT	Off-9-R	
CAAAGGGAATGCCACTTCTCG	Off-10-F	
CGCGCGCACTACTGGAG	Off-10-R	
CGAATGGGATGGTAGCAAGT	Off-16-F	
TTTTGCTGTCAGTCCGTGTT	Off-16-R	
CTTCCTCGCCCTCATCGC	Off-18-F	
GAATGAGCGTTCAGCAGTGG	Off-18-R	