nature cell biology

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41556-018-0202-4

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CRISPR–Cas9-mediated base-editing screening in mice identifies DND1 amino acids that are critical for primordial germ cell development

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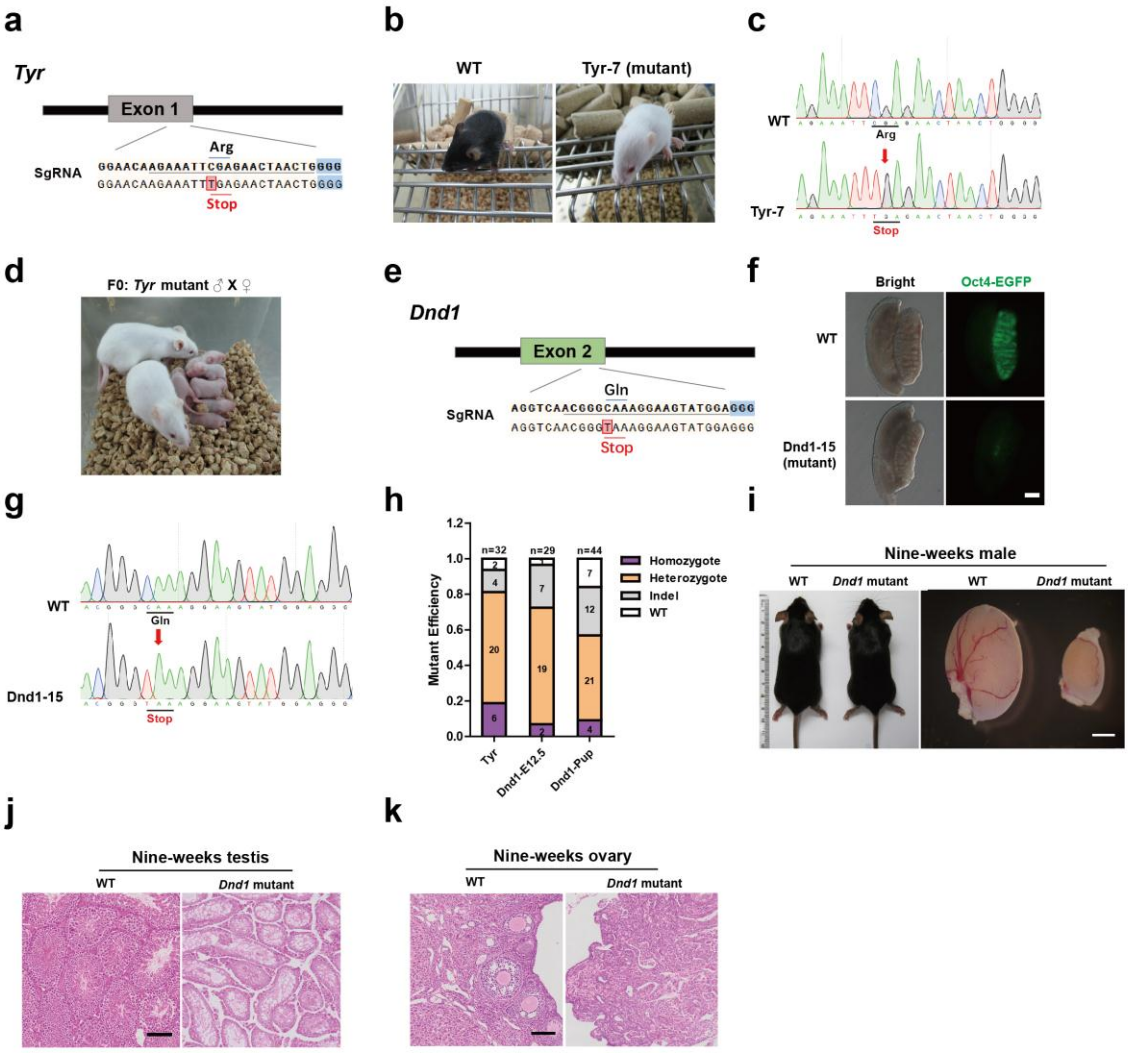
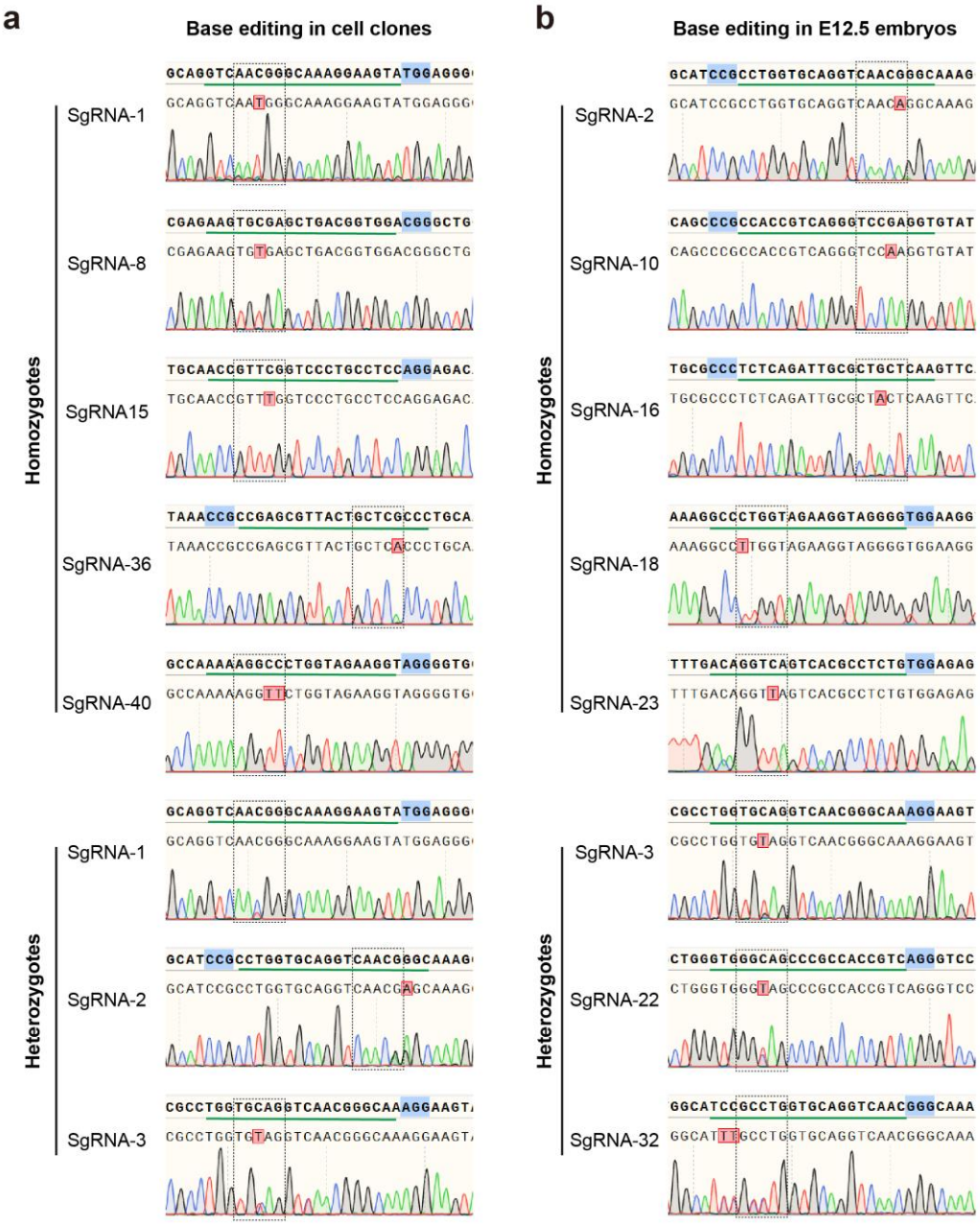

a
Tyr
Exon 1
Arg
SgRNA
GGAACAAGAAATTCGAGAACTAACTGGGG
GGAACAAGAAATTTGAGAACTAACTGGGG
Stop
b
WT
Tyr-7 (mutant)
c
WT
Arg
Tyr-7
Stop
d
F0: Tyr mutant ♂ X ♀
e
Dnd1
Exon 2
Gln
SgRNA
AGGTCAACGGGCAAAGGAAGTATGGAGGG
AGGTCAACGGGTAAAGGAAGTATGGAGGG
Stop
f
Bright
Oct4-EGFP
WT
Dnd1-15
(mutant)
g
WT
Gln
Dnd1-15
Stop
h
Mutant Efficiency
n=32
n=29
n=44
Homozygote
Heterozygote
Indel
WT
Tyr
Dnd1-E12.5
Dnd1-Pup
i
Nine-weeks male
WT
Dnd1 mutant
WT
Dnd1 mutant
j
Nine-weeks testis
WT
Dnd1 mutant
k
Nine-weeks ovary
WT
Dnd1 mutant

Supplementary Figure 1

One-step generation of mouse models carrying point mutations via direct injection of BE3 into zygotes.

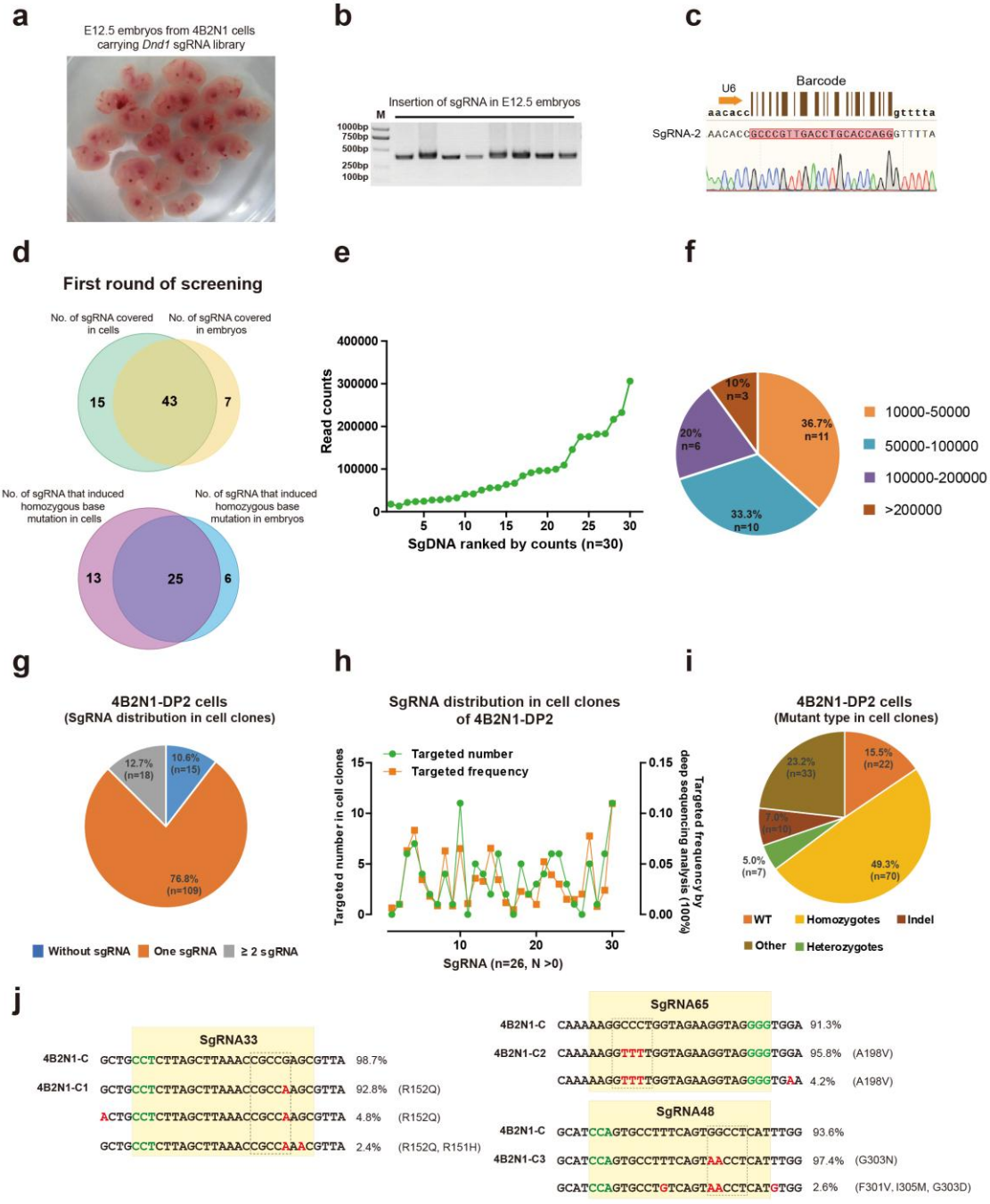
a, The target sequence in *Tyr* exon 1. The target sequence is underlined. The PAM sequence and the intended base mutation are shown in blue and red, respectively.
b, A mutant mouse (Tyr-7, 2-weeks old, right) produced by intracytoplasmic injection of BE3 mRNA and *Tyr* sgRNA.
c, Genotyping analysis of the mutant mouse in (**b)** showing a base mutation in *Tyr*, leading to the replacement of Arginine by a termination codon.
d, Progeny with the mutant parents carrying $TYR^{R223stop/R223stop}$. All 24 pups exhibit an albino phenotype.
e, The target sequence in *Dnd1* exon 2. The target sequence is underlined. The PAM sequence and the expected base mutation are shown in blue and red, respectively.
f, The representative images of WT and mutant (Dnd1-15) gonads carrying an *Oct4-EGFP* transgene (E12.5). Left, bright-field image of the gonads. Right, the same gonads under fluorescent illumination. Two independent mutants were analyzed. Scale bar, 200 μm.
g, Genotyping analysis of the mutant gonad in (**f)** showing a base mutation in *Dnd1*, leading to the replacement of Glutamine by a termination codon.
h, Summary of base editing efficiency in embryos or mice generated through direct injection of BE3 mRNA and *Tyr* sgRNA or *Dnd1* sgRNA into zygotes.
i, An adult mouse (9-weeks old) carrying a point mutation in *Dnd1* and its testis. Scale bar, 1.0 mm.
j, Histological analysis of the *Dnd1* mutant testis shown in **i**. No germ cells in seminiferous tubules. Scale bar, 100 μm.
k, Histological analysis of the ovary from one adult *Dnd1* mutant mouse, indicating no germ cells. Scale bar, 100 μm. Two independent mutants were analyzed for each group in **i-k**.


a
Base editing in cell clones
Homozygotes
SgRNA-1
SgRNA-8
SgRNA15
SgRNA-36
SgRNA-40
Heterozygotes
SgRNA-1
SgRNA-2
SgRNA-3
b
Base editing in E12.5 embryos
Homozygotes
SgRNA-2
SgRNA-10
SgRNA-16
SgRNA-18
SgRNA-23
Heterozygotes
SgRNA-3
SgRNA-22
SgRNA-32

Supplementary Figure 2

The chromatograms of Sanger sequencing show the homozygous and heterozygous mutations in cell clones and SC embryos from 4B2N1-DP1 cells.

a, b, Sanger sequencing chromatograms of DNA from cell clones (**a**) and SC embryos (**b**) obtained from 4B2N1-DP1 cells showing the homozygous (one peak at the target site) and heterozygous (two peaks at the target site) mutations. The nucleotides in red shadow indicate the substituted nucleotide. The PAM sequences are shown in blue shadow and the sgRNA-targeted sequences are underlined. The editing window is shown with dotted orthogon.


a
E12.5 embryos from 4B2N1 cells carrying Dnd1 sgRNA library
b
Insertion of sgRNA in E12.5 embryos
M
1000bp
750bp
500bp
250bp
100bp
c
U6
Barcode
aacacc
gtttta
SgRNA-2 AACACCGCCCGTTGACCTGCACCAGGGTTTTA
d
First round of screening
No. of sgRNA covered in cells
No. of sgRNA covered in embryos
15
43
7
No. of sgRNA that induced homozygous base mutation in cells
No. of sgRNA that induced homozygous base mutation in embryos
13
25
6
e
Read counts
SgDNA ranked by counts (n=30)
f
10%
n=3
36.7%
n=11
20%
n=6
33.3%
n=10
10000-50000
50000-100000
100000-200000
>200000
g
4B2N1-DP2 cells
(SgRNA distribution in cell clones)
10.6%
(n=15)
12.7%
(n=18)
76.8%
(n=109)
Without sgRNA
One sgRNA
≥ 2 sgRNA
h
SgRNA distribution in cell clones of 4B2N1-DP2
Targeted number
Targeted frequency
Targeted number in cell clones
Targeted frequency by deep sequencing analysis (100%)
SgRNA (n=26, N >0)
i
4B2N1-DP2 cells
(Mutant type in cell clones)
15.5%
(n=22)
23.2%
(n=33)
7.0%
(n=10)
5.0%
(n=7)
49.3%
(n=70)
WT
Homozygotes
Indel
Other
Heterozygotes
j
SgRNA33
4B2N1-C GCTGCCTCTTAGCTTAAACCGCCGAGCGTTA 98.7%
4B2N1-C1 GCTGCCTCTTAGCTTAAACCGCCAAGCGTTA 92.8% (R152Q)
ACTGCCTCTTAGCTTAAACCGCCAAGCGTTA 4.8% (R152Q)
GCTGCCTCTTAGCTTAAACCGCCAAACGTTA 2.4% (R152Q, R151H)
SgRNA65
4B2N1-C CAAAAAGGCCCTGGTAGAAGGTAGGGGTGGA 91.3%
4B2N1-C2 CAAAAAGGTTTTGGTAGAAGGTAGGGGTGGA 95.8% (A198V)
CAAAAAGGTTTTGGTAGAAGGTAGGGGTGAA 4.2% (A198V)
SgRNA48
4B2N1-C GCATCCAGTGCCTTTCAGTGGCCTCATTTGG 93.6%
4B2N1-C3 GCATCCAGTGCCTTTCAGTAACCTCATTTGG 97.4% (G303N)
GCATCCAGTGCCTGTCAGTAACCTCATGTGG 2.6% (F301V, I305M, G303D)

Supplementary Figure 3

Analyses of the sgRNA coverage and base-mutation efficiency in cell clones and SC embryos obtained from 4B2N1 cells with an sgRNA library.

a, E12.5 SC embryos from 4B2N1 cells carrying *Dnd1* sgRNA library.

b, Identification of sgRNA in E12.5 SC embryos by PCR. All tested SC embryos carried sgRNA transgenes. Data represent 3 independent experiments.

c, Determination of the inserted sgRNA by Sanger sequencing of RCR products in **b**. Each sgRNA carried a specific sequence that can be recognized as a "barcode" of the SC embryo. The sgRNA transcription is driven by U6 promoter.

d, Top, the number of identified sgRNAs in cell clones and SC embryos from 4B2N1-DP1 cells. Bottom, the number of the sgRNAs that induced homozygous base mutations in cell clones and SC embryos determined by Sanger sequencing.

e, Deep-sequencing of sgRNAs in 4B2N1-DP2 cells carrying an sgRNA library consisting of 30 sgRNAs . The horizontal axis represents the sgRNA number. n = 30 sgRNAs.

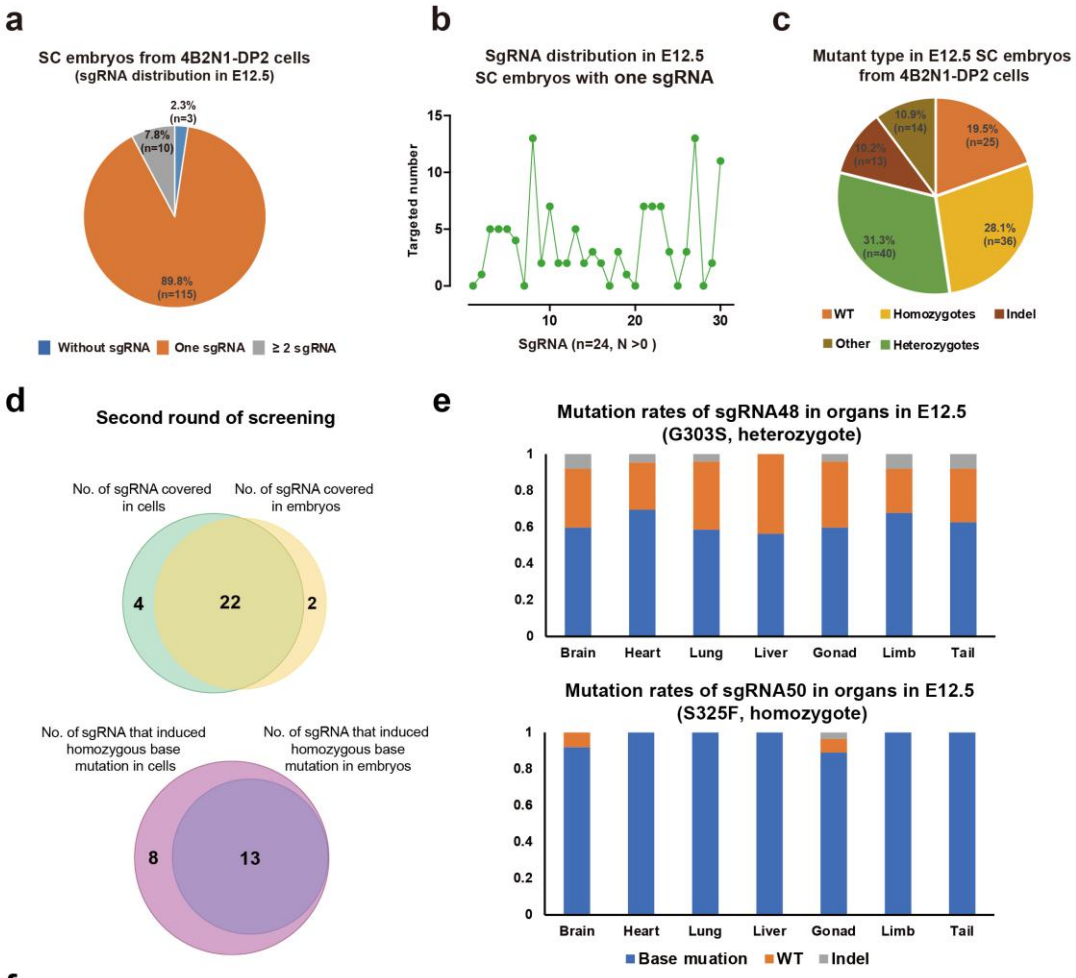
f, Distribution of sgRNAs in 4B2N1-DP2 cells according to their read counts.

g, SgRNA distribution in 4B2N1-DP2 cells. Out of 142 cell clones, 76.8%carried only one sgRNA.

h, Similar sgRNA distribution patterns revealed by cell-clone and deep-sequencing of 4B2N1-DP2 cells. 109 cell clones with only one sgRNA covering 26 sgRNAs were analyzed. The horizontal axis represents the sgRNA number. The left vertical axis represents the number of cell clones with a specific sgRNA. The right vertical axis represents the frequency of the corresponding sgRNA through deep-sequencing.

i, Summary of the mutations in tested cell clones through Sanger sequencing of sgRNA-targeted sites in cell clones with only one sgRNA from 4B2N1-DP2 cells. Other represents the cell clones without sgRNA or with 2 or more sgRNAs.

j, The whole-exome sequencing data of three 4B2N1-DP2 cell clones (4B2N1-C1, C2 and C3) from showed a low rate of mosaicism at the target sites of *Dnd1*. The target sequences are shown with yellow shadow. The PAM sites and substituted nucleotides are shown in green and red, respectively. The expected editing windows are shown with dotted orthogon. The columns on the right indicate frequencies of mutant alleles.



f

Summary of SC embryos derived from 4B2N1 cells carrying an sgRNA library

Donor cells	No. of SC embryos (E12.5)	No. of SC embryos without sgRNA	No. of SC embryos carrying sgRNAs (n ≥ 2)	No. of SC embryos carrying one sgRNA	No. of SC embryos with base mutations (% of all embryos)	No. of SC embryos with homozygous base mutations (% of all embryos)	No. of SC embryos with indel mutations
4B2N1-DP1 cells	198	12	43	143	108 (54.5)	75 (37.9)	22
4B2N1-DP2 cells	128	3	10	115	76 (59.4)	36 (28.1)	13

Supplementary Figure 4

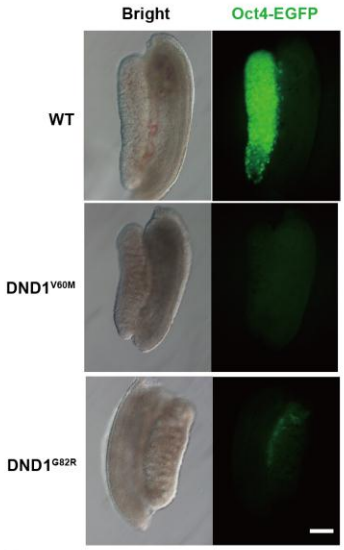
Analyses of intended and non-intended base mutations in SC embryos from 4B2N1-DP2 cells and embryos obtained by injection of BE3 into zygotes.

a, SgRNA distribution in a total of 128 E12.5 SC embryos obtained by ICAHCI of 4B2N1-DP2 cells, showing that a majority of the SC embryos (89.8%) carried one sgRNA.
b, SgRNA distribution in 115 embryos with one sgRNA, covering a total of 24 different sgRNAs. The horizontal axis represents the sgRNA number. The vertical axis represents the number of SC embryos with a specific sgRNA.
c, Summary of the mutations in tested SC embryos with one sgRNA obtained through ICAHCI of 4B2N1-DP2 cells. Other represents the SC embryos without sgRNA or with 2 or more sgRNAs.
d, Top, the number of identified sgRNAs in cell clones and SC embryos obtained from 4B2N1-DP2 cells. Bottom, the number of the sgRNAs that induced homozygous base mutations in cell clones and SC embryos determined by Sanger sequencing analyses.
e, Blunt Cloning and sequencing analysis of different organs in one SC embryo carrying a heterozygous base mutation (top) and one with a homozygous base mutation (bottom). More than 20 clones were tested for each organ.
f, Summary of the generation of E12.5 SC embryos from 4B2N1 cells carrying *Dnd1* sgRNA libraries.

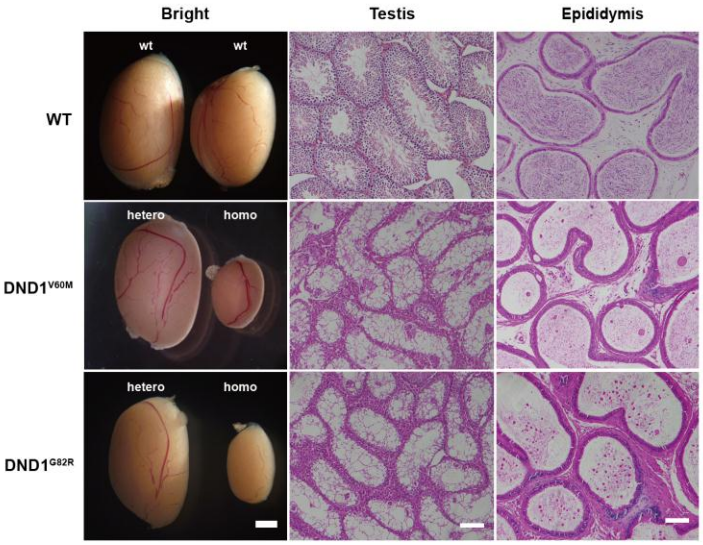
a

Microinjection	Intended amino acid mutation	No. of E12.5 embryos	No. of embryos lost PGCs	No. of embryos with a base mutation	No. of embryos with a indel mutation
Dnd1 sgRNA10+ BE3 mRNA	E59K	52	21	21	0
Dnd1 sgRNA57+ BE3 mRNA	V60M	85	80	56	24
Dnd1 sgRNA58+ BE3 mRNA	P76L	41	38	30	8
Dnd1 sgRNA75+ BE3 mRNA	G82R	96	31	20	11

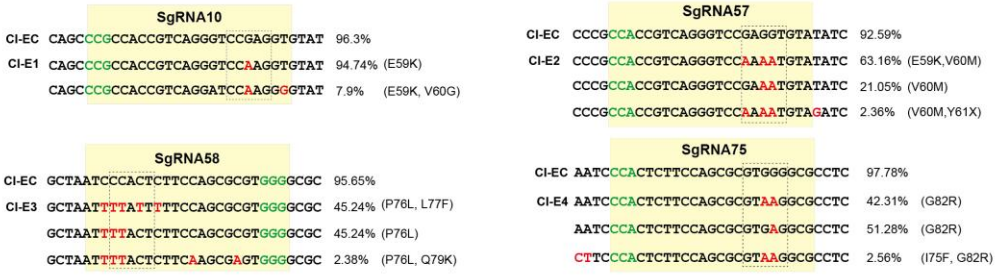
b



c



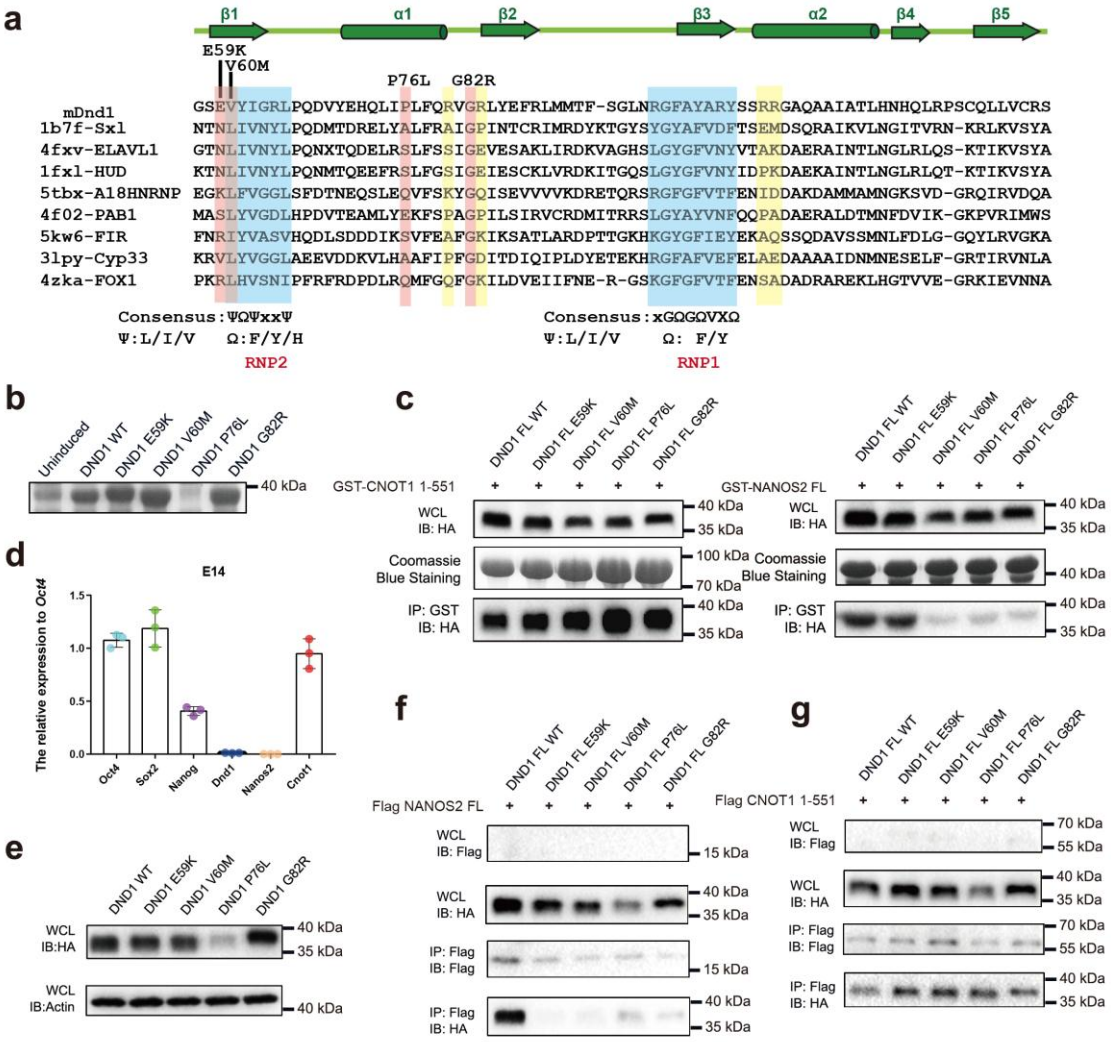
d



Supplementary Figure 5

Functional validation of identified amino acids of DND1 *in vivo*.

a, Summary of validation experiments. All E12.5 embryos carrying an expected point mutation lost PGCs in gonads.
b, Rare PGCs in gonads of E12.5 embryos carrying the V60M (*n* = 21) or G82R (*n* = 3) in DND1 obtained by injection of BE3 mRNA and corresponding sgRNA into zygotes (*n* = the number of mouse for analysis). Scale bar, 200 μm.
c, Morphological and histological analyses of the testis and epididymis of the mutant mice. Note no germ cells in the mutant testis and epididymis with V60M or G82R in DND1. Two adult mice (8-weeks old) were used for each group. Scale bar in left column, 1.0 mm; scale bars in middle and right columns, 100 μm.
d, The whole-exome sequencing data of four embryos (CI-E1, E2, E3 and E4) generated by injection of BE3 mRNA and corresponding sgRNA into zygotes shows a low rate of mosaicism at the target sites of *Dnd1*. CI-EC is a wild-type control embryo. The target sequences are shown with yellow shadow. The PAM sites and substituted nucleotides are shown in green and red, respectively. The expected editing windows are shown with dotted orthogon. The columns on the right indicate frequencies of mutant alleles.


a
β1
α1
β2
β3
α2
β4
β5
E59K
V60M
P76L
G82R
mDnd1 GSEVYIGRLPQDVYEHQLIPLFQRVGRLYEFRLMMTF-SGLNRGFAYARYSSRRGAQAAIATLHNHQLRPSCQLLVCRS
1b7f-Sxl NTNLIVNYLPQDMTDRELYALFRAIGPINTCRIMRDYKTGYSYGYAFVDFTSEMDSQRAIKVLNGITVRN-KRLKVSYA
4fxv-ELAVL1 GTNLIVNYLPQNXTQDELRSLFSSIGEVESAKLIRDKVAGHSLGYGFVNYVTAKDAERAINTLNGLRLQS-KTIKVSYA
1fxl-HUD KTNLIVNYLPQNMTQEEFRSLFGSIGEIESCKLVRDKITGQSLGYGFVNYIDPKDAEKAINTLNGLRLQT-KTIKVSYA
5tbx-A18HNRNP EGKLFVGGLSFDTNEQSLEQVFSKYGQISEVVVVKDRETQRSRGFGFVTFENIDDAKDAMMAMNGKSVD-GRQIRVDQA
4f02-PAB1 MASLYVGDLHPDVTEAMLYEKFSPAGPILSIRVCRDMITRRSLGYAYVNFQQPADAERALDTMNFDVIK-GKPVRIMWS
5kw6-FIR FNRIYVASVHQDLSDDDIKSVFEAFGKIKSATLARDPTTGKHKGYGFIEYEKAQSSQDAVSSMNLFDLG-GQYLRVGKA
3lpy-Cyp33 KRVLYVGGLAEEVDDKVLHAAFIPFGDITDIQIPLDYETEKHRGFAFVEFELAEDAAAAIDNMNESELF-GRTIRVNLA
4zka-FOX1 PKRLHVSNIPFRFRDPDLRQMFGQFGKILDVEIIFNE-R-GSKGFGFVTFENSADADRAREKLHGTVVE-GRKIEVNNA
Consensus:ΨΩΨxxΨ
Ψ:L/I/V Ω:F/Y/H
RNP2
Consensus:xGΩGΩVXΩ
Ψ:L/I/V Ω: F/Y
RNP1
b
Uninduced
DND1 WT
DND1 E59K
DND1 V60M
DND1 P76L
DND1 G82R
40 kDa
c
DND1 FL WT
DND1 FL E59K
DND1 FL V60M
DND1 FL P76L
DND1 FL G82R
GST-CNOT1 1-551
WCL IB: HA
Coomassie Blue Staining
IP: GST IB: HA
40 kDa
35 kDa
100 kDa
70 kDa
GST-NANOS2 FL
d
E14
The relative expression to Oct4
1.5
1.0
0.5
0.0
Oct4
Sox2
Nanog
Dnd1
Nanos2
Cnot1
e
WCL IB:HA
WCL IB:Actin
f
Flag NANOS2 FL
WCL IB: Flag
IP: Flag IB: Flag
IP: Flag IB: HA
15 kDa
g
Flag CNOT1 1-551
70 kDa
55 kDa

Supplementary Figure 6

The E59K, V60M, P76L and G82R of DND1 disrupt the protein stability and protein-protein interaction.

a, Sequence alignment of RRM domains from various proteins. G82 is well conserved in RRM domains.
b, *In vitro* purification of wild-type and mutant RRM domains (1-217) of DND1 in *E. coli*.
c, GST pull-down assay for the interactions of GST-NANOS2 or GST-CNOT1 (1-551) with DND1 and DND1 mutant proteins.
d, Transcription analysis of *Oct4*, *Sox2*, *Nanog*, *Dnd1*, *Nanos2* and *Cnot1* genes in mouse ESCs (E14). The expression values were normalized to that of *Gapdh*. Data are shown as the average mean ± s.d. (n = 3 three independent experiments). The source data can be found in Supplementary Table 13.
e, Analysis of expression levels of DND1 and DND1 mutant proteins in ESCs by transfection of HA-tagged *Dnd1* plasmids.
f, g, Co-immunoprecipitation (Co-IP) analysis in mouse ESCs shows that the mutation of DND1 disrupt the interaction with NANOS2 but did not affect the binding with CNOT1. Data in **b, c** and **e-g** represent 3 independent experiments and their unprocessed images of gels are shown in Supplementary Fig. 7.

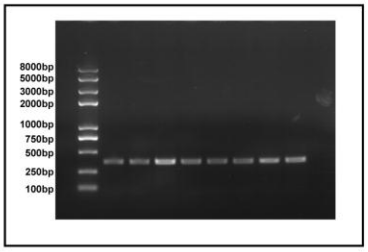


Figure 3f

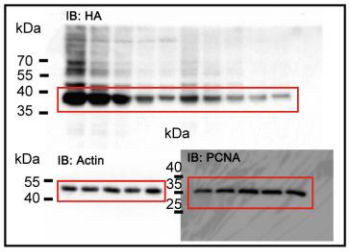


Figure 7a

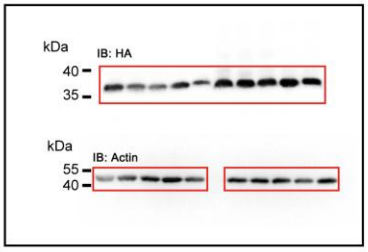


Figure 7b

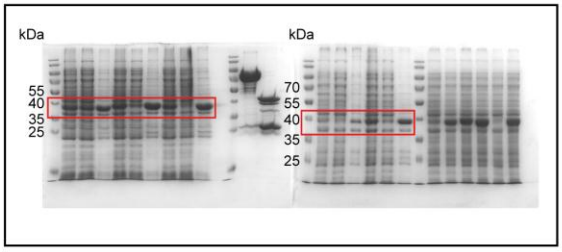


Figure 7c

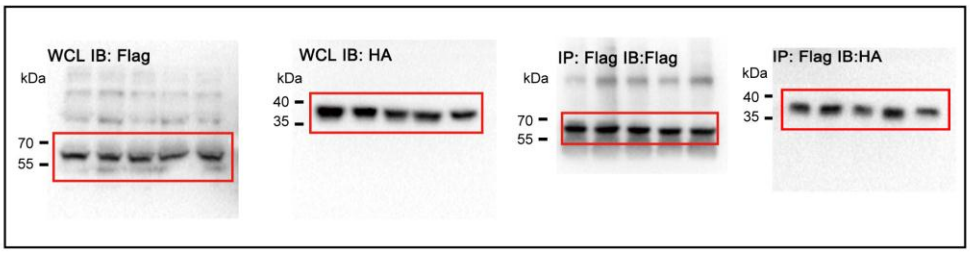


Figure 7e left panel

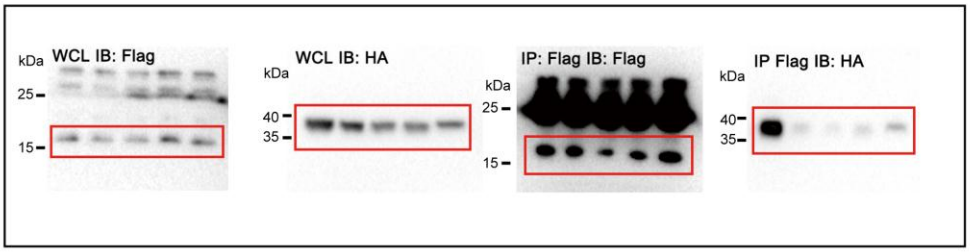
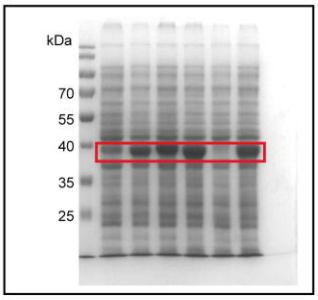


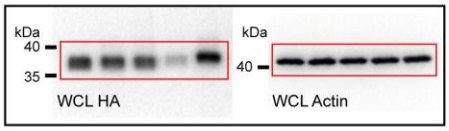
Figure 7e right panel

Supplementary Figure 7

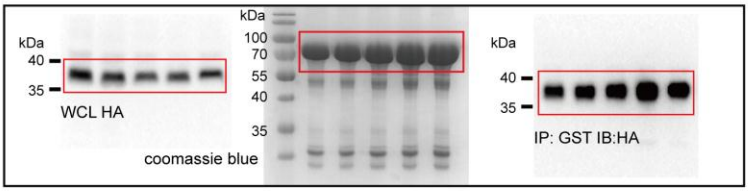
Unprocessed images of key blots/gels in whole study.



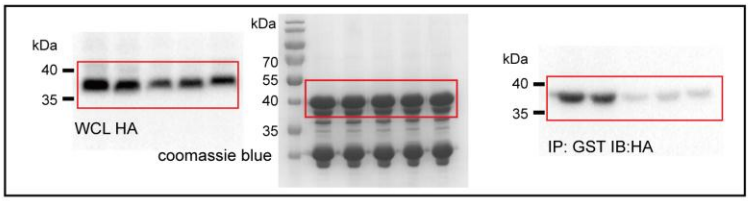
Supplementary Figure 6b



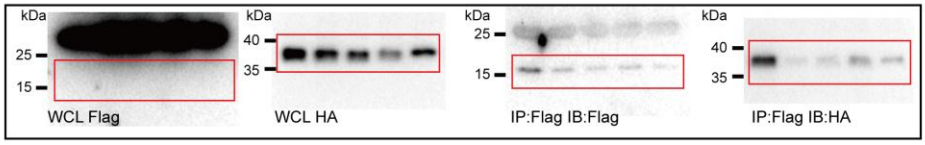
Supplementary Figure 6e



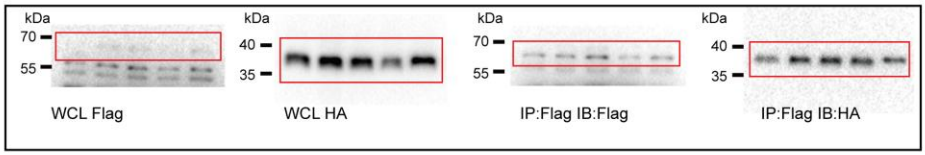
Supplementary Figure 6c left



Supplementary Figure 6c right



Supplementary Figure 6f



Supplementary Figure 6g

Supplementary Figure 7 continued

Unprocessed images of key blots/gels in whole study.

Supplementary Table 1. Detailed information of mutant mice generated by direct injection of BE3 into zygotes.

Detailed information of the mice generated through cytoplasm injection of BE3 mRNA and sgRNA targeting *Tyr* or *Dnd1* into mouse zygotes. Related to **Supplementary Fig. 1**.

Supplementary Table 2. Results of Blunt cloning and sequencing analysis of base editing in diploid and haploid ESCs.

The base-editing results in mouse ESCs using BE3 with different number of NLS. Related to **Fig. 1e,f** and **Fig. 2d**.

Supplementary Table 3. Detailed information of mutant SC blastocysts or mice produced by ICAHCI of 4B2N1 cells with an sgRNA.

The base editing results in SC blastocysts or mice produced by ICAHCI of 4B2N1 cells with an sgRNA. Related to **Fig. 2c, f**.

Supplementary Table 4. List of sgRNAs designed for targeting four exons of *Dnd1*.

The detailed information of a total of 77 sgRNAs targeting *Dnd1* coding sequence (CDS) that were designed using GuideScan software.

Supplementary Table 5. Detailed information of SC mice generated by ICAHCI of 4B2N1 cells carrying an sgRNA library.

The genotyping results of SC mice generated in two rounds of ICAHCI experiments using 4B2N1-DP1 and 4B2N1-DP2 cells respectively. Related to **Fig. 4d** and **Supplementary Fig. 4c**.

Supplementary Table 6. List of the samples for off-target analysis.

Samples collected for off-target analysis.

Supplementary Table 7. Results of off-target analysis through Sanger sequencing of the potential sites.

Analysis of the off-target sites of different sgRNAs in collected samples through Sanger sequencing, followed by confirmation according to whole-exome sequencing data.

Supplementary Table 8. Detailed information of off-target analysis through whole-exome sequencing.
Lists of the off-target sites identified from whole-exome sequencing results after filtering out naturally-occurring variants in the SNP database (dbSNPs), excluding SNPs/indels also found in the control sequence, and removing the SNPs/indels sites that are not predicted by Cas-OFFinder considering mismatch up to 7-bp. The remaining SNPs/indels sites were further verified by Sanger sequencing analysis.

Supplementary Table 9. Summary of off-target results from whole-exome sequencing.
Summary of whole-exome sequencing analysis in 14 different samples.

Supplementary Table 10. Results of Blunt cloning and sequencing analysis of base editing in different organs.
The base editing efficiency in different organs of three SC embryos generated from 4B2N1-DP2 cells. Related to **Supplementary Fig. 4e.**

Supplementary Table 11. Detailed information of mutant embryos generated by direct injection of BE3 and sgRNA into zygotes for validation.
The results of base editing in mice generated through zygote injection of BE3 mRNA and sgRNA targeting four sites of *Dnd1*. Related to **Supplementary Fig. 5a.**

Supplementary Table 12. Primer information.
List of primers used in this study.

Supplementary Table 13. Statistics source data.
Source data for Fig. 1c, 2b, 5e and Supplementary Fig. 6d.