Cite as: E. Zuo *et al.*, *Science* 10.1126/science.aav9973 (2019).

Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos

Erwei Zuo[1,2]*, Yidi Sun[3]*, Wu Wei[3,4,5]*, Tanglong Yuan[2]*, Wenqin Ying[1], Hao Sun[6], Liyun Yuan[3], Lars M. Steinmetz[4,7,8]†, Yixue Li[3,9,10]†, Hui Yang[1]†

[1]Institute of Neuroscience, State Key Laboratory of Neuroscience, Key Laboratory of Primate Neurobiology, CAS Center for Excellence in Brain Science and Intelligence Technology, Shanghai Research Center for Brain Science and Brain-Inspired Intelligence, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China. [2]Center for Animal Genomics, Agricultural Genome Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518124, China. [3]Key Lab of Computational Biology, CAS-MPG Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai 200031, China. [4]Stanford Genome Technology Center, Stanford University, Palo Alto, CA 94304, USA. [5]Center for Biomedical Informatics, Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai 200040, China. [6]University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, 92093-0726, USA. [7]Department of Genetics, School of Medicine, Stanford University, Stanford, CA 94305, USA. [8]Genome Biology Unit, European Molecular Biology Laboratory (EMBL), 69117 Heidelberg, Germany. [9]Collaborative Innovation Center for Genetics and Development, Fudan University, Shanghai, China. [10]Shanghai Center for Bioinformation Technology, Shanghai Industrial Technology Institute, Shanghai, China.

*These authors contributed equally to this work. †Corresponding author. Email: lars.steinmetz@stanford.edu (L.M.S.); yxli@sibs.ac.cn (Y.L.); huiyang@ion.ac.cn (H.Y.)

Genome editing holds promise for correcting pathogenic mutations. However, it is difficult to determine off-target effects of editing due to single nucleotide polymorphism in individuals. Here, we developed a method named GOTI (Genome-wide Off-target analysis by Two-cell embryo Injection) to detect off-target mutations by editing one blastomere of two-cell mouse embryos using either CRISPR-Cas9 or base editors. Comparison of the whole genome sequences of progeny cells of edited vs. non-edited blastomeres at E14.5 showed that off-target single nucleotide variants (SNVs) were rare in embryos edited by CRISPR-Cas9 or adenine base editor, with a frequency close to the spontaneous mutation rate. In contrast, cytosine base editing induced SNVs with over 20-fold higher frequencies, requiring a solution to address its fidelity.

Genome editing holds great potential for treating genetic diseases induced by pathogenic mutations (*1*). A comprehensive analysis of off-target effects by genome editing is required for their utility (*2*). Multiple methods have been developed to detect genome-wide gene-editing off-target sites (*2*–*5*). However, these approaches are not applicable to detect SNVs in vivo. In this study, we developed a method named GOTI to evaluate the off-target effects induced by CRISPR-Cas9, cytosine base editor 3 (BE3, rAPOBEC1-nCas9-UGI) and adenine base editor 7.10 (ABE7.10, TadA-TadA*-nCas9), three commonly used gene-editing tools (*6*–*8*). Briefly, we injected CRISPR-Cas9, BE3, or ABE7.10, together with Cre mRNA into one blastomere of two-cell embryos derived from Ai9 (CAG-LoxP-Stop-LoxP-tdTomato) mice (*9*, *10*) (Fig. 1A). The progeny cells of the edited and non-edited blastomeres were then sorted by FACS based on tdTomato expression in gene-edited cells at embryonic day 14.5 (E14.5) (Fig. 1B), when the whole embryo could be readily digested to obtain enough single cells. Whole genome sequencing (WGS) was then performed separately on the tdTomato$^+$ and tdTomato$^-$ cells. SNVs and indels were called by three algorithms in the tdTomato$^+$ sample, with the tdTomato$^-$ sample from the same embryo as the reference (Fig. 1A).

We included twelve groups in our study: one Cre group (Cre only), six Cas9 groups with or without sgRNA (Cas9, Cas9-LacZ, Cas9-Pde6b, Cas9-Tyr-A, Cas9-Tyr-B and Cas9-Tyr-C), three BE3 groups with or without sgRNA (BE3, BE3-Tyr-C, BE3-Tyr-D) (*11*) and two ABE groups with or without sgRNA (ABE7.10, ABE7.10-Tyr-E). First, we validated the on-target efficiency of our approach in embryos at the 8-cell and E14.5 stages by Sanger sequencing (figs. S1 to S3). To further explore the on-target efficiency and potential genome-wide off-target effects, we performed WGS at an average depth of 47x on 46 samples from 23 E14.5 embryos (table S1). The activities of Cas9, BE3 and ABE7.10 in tdTomato$^+$ cells were confirmed by high on-target efficiencies to induce indels and nucleotide substitutions, respectively (Fig. 1C, fig. S4, and tables S2 and S3).

For the off-target editing effects, we found only 0 - 4 indels in embryos from all twelve groups (figs. S5 and S6 and tables S2 and S4), and none of them overlapped with the predicted off-target sites (fig. S5 and table S5). For all Cas9-treated embryos there was no significant difference among different Cas9 groups (12 SNVs/embryo on average) or in comparison with the "Cre-only" group (14 SNVs/embryo on average) (figs. S7 and S8 and tables S2 and S6). The SNVs detected in the Cre- or Cas9- treated samples were likely caused by spontaneous mutations during genome replication during

Downloaded from http://science.sciencemag.org/ on February 28, 2019

development, since the number of variants was within the range of simulated spontaneous mutations and no sequence similarity was observed between the adjacent sequences of the identified SNVs and the target sites (fig. S8 and methods) (*12*).

Surprisingly, we found on average 283 SNVs/embryo in BE3-treated embryos, a level at least 20 times higher than that observed in Cre- or Cas9-treated embryos (Fig. 2A, fig. S7, and tables S2 and S7). By contrast, ABE7.10 generated on average 10 SNVs/embryo, with a frequency close to the spontaneous mutation rate (Fig. 2A and table S2). We further compared the off-target sites identified in the "BE3 only" group with those of the BE3-Tyr-C or BE3-Tyr-D groups, and found that the presence of sgRNAs did not induce significantly higher SNVs (P = 0.21, Kruskal-Wallis test). Besides, these variants were specifically identified in tdTomato$^+$ cells rather than the tdTomato$^-$ cells (see methods, fig. S9, and table S8). Notably, more than 90% of the SNVs identified in the BE3-edited cells were mutated from G to A or C to T, a mutation bias not observed in Cre-, Cas9-, or ABE7.10-treated cells (Fig. 2, B and C, and fig. S10). This bias was the same as that of APOBEC1 itself (*13*), indicating that these mutations were not spontaneous but induced by BE3-editing. Previous studies have shown that the action of several members of the APOBEC family (including APOBEC1) require single-stranded DNA (*14*–*16*). Consistently, our analysis showed that SNVs induced by BE3 were significantly enriched in transcribed regions (Fig. 3A), especially in genes with high expression (Fig. 3B and fig. S11). Interestingly, none of the off-target sites were shared by any of the BE3-treated embryos or overlapped with predicted off-target mutations (Fig. 3, C and D). Besides, no similarity was observed between the off-target and on-target sequences, whereas the top predicted off-target sites showed high sequence similarity with BE3 on-target loci (fig. S12). Thus, the BE3 off-target SNVs were sgRNA-independent and likely caused by overexpression of APOBEC1.

Among 1698 SNVs in BE3-treated embryos, 26 were located on exons, 14 of which led to non-synonymous changes (fig. S13). We successfully amplified 20 of them by PCR and confirmed their presence by Sanger sequencing (fig. S14 and table S9). We also found that 1 SNV was located in a proto-oncogene and 13 SNVs in tumor suppressors (fig. S13), raising the concern about the oncogenic risk of BE3-editing. This risk might be reduced by expressing lower amounts of BE3. However, we found that the on-target efficiencies were progressively reduced with lower amounts of BE3 used (fig. S15 and table S10).

Intriguingly, we found that numerous de novo SNVs are induced by BE3, which was not reported in previous studies. A possible explanation is that our method, GOTI, examines the cell population derived from a single gene-edited blastomere, whereas previous studies used large pools of cells where editing is variable, resulting in loss of signal for random off-targets due to population averaging. Unlike BE3, ABE7.10 induced no increase in SNVs, probably due to lack of DNA binding ability of TadA (*17*). These results are consistent with a similar study in rice plants (*18*). The off-target effects of base editors may be reduced by decreasing the DNA binding ability of APOBEC1 or using different versions of cytidine deaminase (*19*–*21*). In summary, GOTI could be useful for examining off-target effects of various gene editing tools without the interference of SNPs present in different individuals.

REFERENCES AND NOTES

1. G. J. Knott, J. A. Doudna, CRISPR-Cas guides the future of genetic engineering. *Science* **361**, 866–869 (2018). doi:10.1126/science.aat5011 Medline
2. S. Q. Tsai, J. K. Joung, Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. *Nat. Rev. Genet.* **17**, 300–312 (2016). doi:10.1038/nrg.2016.28 Medline
3. C. R. Lazzarotto, N. T. Nguyen, X. Tang, J. Malagon-Lopez, J. A. Guo, M. J. Aryee, J. K. Joung, S. Q. Tsai, Defining CRISPR-Cas9 genome-wide nuclease activities with CIRCLE-seq. *Nat. Protoc.* **13**, 2615–2642 (2018). doi:10.1038/s41596-018-0055-0 Medline
4. K. R. Anderson, M. Haeussler, C. Watanabe, V. Janakiraman, J. Lund, Z. Modrusan, J. Stinson, Q. Bei, A. Buechler, C. Yu, S. R. Thamminana, L. Tam, M.-A. Sowick, T. Alcantar, N. O'Neil, J. Li, L. Ta, L. Lima, M. Roose-Girma, X. Rairdan, S. Durinck, S. Warming, CRISPR off-target analysis in genetically engineered rats and mice. *Nat. Methods* **15**, 512–514 (2018). doi:10.1038/s41592-018-0011-5 Medline
5. D. Kim, K. Lim, S.-T. Kim, S. H. Yoon, K. Kim, S.-M. Ryu, J.-S. Kim, Genome-wide target specificities of CRISPR RNA-guided programmable deaminases. *Nat. Biotechnol.* **35**, 475–480 (2017). doi:10.1038/nbt.3852 Medline
6. T. I. Cornu, C. Mussolino, T. Cathomen, Refining strategies to translate genome editing to the clinic. *Nat. Med.* **23**, 415–423 (2017). doi:10.1038/nm.4313 Medline
7. H. A. Rees, D. R. Liu, Base editing: Precision chemistry on the genome and transcriptome of living cells. *Nat. Rev. Genet.* **19**, 770–788 (2018).
8. N. M. Gaudelli, A. C. Komor, H. A. Rees, M. S. Packer, A. H. Badran, D. I. Bryson, D. R. Liu, Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature* **551**, 464–471 (2017). doi:10.1038/nature24644 Medline
9. L. Madisen, T. A. Zwingman, S. M. Sunkin, S. W. Oh, H. A. Zariwala, H. Gu, L. L. Ng, R. D. Palmiter, M. J. Hawrylycz, A. R. Jones, E. S. Lein, H. Zeng, A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat. Neurosci.* **13**, 133–140 (2010). doi:10.1038/nn.2467 Medline
10. L. Wang, M.-Y. Li, C. Qu, W.-Y. Miao, Q. Yin, J. Liao, H.-T. Cao, M. Huang, K. Wang, E. Zuo, G. Peng, S.-X. Zhang, G. Chen, Q. Li, K. Tang, Q. Yu, Z. Li, C. C. L. Wong, G. Xu, N. Jing, X. Yu, J. Li, CRISPR-Cas9-mediated genome editing in one blastomere of two-cell embryos reveals a novel Tet3 function in regulating neocortical development. *Cell Res.* **27**, 815–829 (2017). doi:10.1038/cr.2017.58 Medline
11. K. Kim, S.-M. Ryu, S.-T. Kim, G. Baek, D. Kim, K. Lim, E. Chung, S. Kim, J.-S. Kim, Highly efficient RNA-guided base editing in mouse embryos. *Nat. Biotechnol.* **35**, 435–437 (2017). doi:10.1038/nbt.3816 Medline
12. J. W. Drake, B. Charlesworth, D. Charlesworth, J. F. Crow, Rates of spontaneous mutation. *Genetics* **148**, 1667–1686 (1998). Medline
13. A. C. Komor, Y. B. Kim, M. S. Packer, J. A. Zuris, D. R. Liu, Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* **533**, 420–424 (2016). doi:10.1038/nature17946 Medline
14. R. S. Harris, S. K. Petersen-Mahrt, M. S. Neuberger, RNA editing enzyme APOBEC1 and some of its homologs can act as DNA mutators. *Mol. Cell* **10**, 1247–1253 (2002). doi:10.1016/S1097-2765(02)00742-6 Medline
15. S. Rebhandl, M. Huemer, R. Greil, R. Geisberger, AID/APOBEC deaminases and cancer. *Oncoscience* **2**, 320–333 (2015). doi:10.18632/oncoscience.155 Medline
16. L. B. Alexandrov, S. Nik-Zainal, D. C. Wedge, S. A. J. R. Aparicio, S. Behjati, A. V. Biankin, G. R. Bignell, N. Bolli, A. Borg, A.-L. Børresen-Dale, S. Boyault, B. Burkhardt, A. P. Butler, C. Caldas, H. R. Davies, C. Desmedt, R. Eils, J. E. Eyfjörd, J. A. Foekens, M. Greaves, F. Hosoda, B. Hutter, T. Ilicic, S. Imbeaud, M. Imielinski, N. Jäger, D. T. W. Jones, D. Jones, S. Knappskog, M. Kool, S. R. Lakhani, C. López-

Downloaded from http://science.sciencemag.org/ on February 28, 2019

Otín, S. Martin, N. C. Munshi, H. Nakamura, P. A. Northcott, M. Pajic, E. Papaemmanuil, A. Paradiso, J. V. Pearson, X. S. Puente, K. Raine, M. Ramakrishna, A. L. Richardson, J. Richter, P. Rosenstiel, M. Schlesner, T. N. Schumacher, P. N. Span, J. W. Teague, Y. Totoki, A. N. J. Tutt, R. Valdés-Mas, M. M. van Buuren, L. van 't Veer, A. Vincent-Salomon, N. Waddell, L. R. Yates, J. Zucman-Rossi, P. A. Futreal, U. McDermott, P. Lichter, M. Meyerson, S. M. Grimmond, R. Siebert, E. Campo, T. Shibata, S. M. Pfister, P. J. Campbell, M. R. Stratton; Australian Pancreatic Cancer Genome Initiative; ICGC Breast Cancer Consortium; ICGC MMML-Seq Consortium; ICGC PedBrain, Signatures of mutational processes in human cancer. *Nature* **500**, 415–421 (2013). doi:10.1038/nature12477 Medline

17. H. C. Losey, A. J. Ruthenburg, G. L. Verdine, Crystal structure of *Staphylococcus aureus* tRNA adenosine deaminase TadA in complex with RNA. *Nat. Struct. Mol. Biol.* **13**, 153–159 (2006). doi:10.1038/nsmb1047 Medline
18. S. Jin, Y. Zong, Q. Gao, Z. Zhu, Y. Wang, P. Qin, C. Liang, D. Wang, J.-L. Qiu, F. Zhang, C. Gao, Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. *Science*, 10.1126/science.aaw7166 (2019).
19. Y. B. Kim, A. C. Komor, J. M. Levy, M. S. Packer, K. T. Zhao, D. R. Liu, Increasing the genome-targeting scope and precision of base editing with engineered Cas9-cytidine deaminase fusions. *Nat. Biotechnol.* **35**, 371–376 (2017). doi:10.1038/nbt.3803 Medline
20. X. Wang, J. Li, Y. Wang, B. Yang, J. Wei, J. Wu, R. Wang, X. Huang, J. Chen, L. Yang, Efficient base editing in methylated regions with a human APOBEC3A-Cas9 fusion. *Nat. Biotechnol.* **36**, 946–949 (2018). Medline
21. J. M. Gehrke, O. Cervantes, M. K. Clement, Y. Wu, J. Zeng, D. E. Bauer, L. Pinello, J. K. Joung, An APOBEC3A-Cas9 base editor with minimized bystander and off-target activities. *Nat. Biotechnol.* **36**, 977–982 (2018). Medline
22. E. Zuo, Y.-J. Cai, K. Li, Y. Wei, B.-A. Wang, Y. Sun, Z. Liu, J. Liu, X. Hu, W. Wei, X. Huo, L. Shi, C. Tang, D. Liang, Y. Wang, Y.-H. Nie, C.-C. Zhang, X. Yao, X. Wang, C. Zhou, W. Ying, Q. Wang, R.-C. Chen, Q. Shen, G.-L. Xu, J. Li, Q. Sun, Z.-Q. Xiong, H. Yang, One-step generation of complete gene knockout mice and monkeys by CRISPR/Cas9-mediated gene editing with multiple sgRNAs. *Cell Res.* **27**, 933–945 (2017). doi:10.1038/cr.2017.81 Medline
23. L. Swiech, M. Heidenreich, A. Banerjee, N. Habib, Y. Li, J. Trombetta, M. Sur, F. Zhang, In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. *Nat. Biotechnol.* **33**, 102–106 (2015). doi:10.1038/nbt.3055 Medline
24. W. H. Wu, Y.-T. Tsai, S. Justus, T.-T. Lee, L. Zhang, C.-S. Lin, A. G. Bassuk, V. B. Mahajan, S. H. Tsang, CRISPR repair reveals causative mutation in a preclinical model of retinitis pigmentosa. *Mol. Ther.* **24**, 1388–1394 (2016). doi:10.1038/mt.2016.107 Medline
25. K. Cibulskis, M. S. Lawrence, S. L. Carter, A. Sivachenko, D. Jaffe, C. Sougnez, S. Gabriel, M. Meyerson, E. S. Lander, G. Getz, Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat. Biotechnol.* **31**, 213–219 (2013). doi:10.1038/nbt.2514 Medline
26. A. Wilm, P. P. K. Aw, D. Bertrand, G. H. T. Yeo, S. H. Ong, C. H. Wong, C. C. Khor, R. Petric, M. L. Hibberd, N. Nagarajan, LoFreq: A sequence-quality aware, ultra-sensitive variant caller for uncovering cell-population heterogeneity from high-throughput sequencing datasets. *Nucleic Acids Res.* **40**, 11189–11201 (2012). doi:10.1093/nar/gks918 Medline
27. C. T. Saunders, W. S. W. Wong, S. Swamy, J. Becq, L. J. Murray, R. K. Cheetham, Strelka: Accurate somatic small-variant calling from sequenced tumor-normal sample pairs. *Bioinformatics* **28**, 1811–1817 (2012). doi:10.1093/bioinformatics/bts271 Medline
28. H. Fang, E. A. Bergmann, K. Arora, V. Vacic, M. C. Zody, I. Iossifov, J. A. O'Rawe, Y. Wu, L. T. Jimenez Barron, J. Rosenbaum, M. Ronemus, Y. H. Lee, Z. Wang, E. Dikoglu, V. Jobanputra, G. J. Lyon, M. Wigler, M. C. Schatz, G. Narzisi, Indel variant analysis of short-read sequencing data with Scalpel. *Nat. Protoc.* **11**, 2529–2548 (2016). doi:10.1038/nprot.2016.150 Medline
29. S. Bae, J. Park, J. S. Kim, Cas-OFFinder: A fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. *Bioinformatics* **30**, 1473–1475 (2014). doi:10.1093/bioinformatics/btu048 Medline
30. M. Haeussler, K. Schönig, H. Eckert, A. Eschstruth, J. Mianné, J.-B. Renaud, S. Schneider-Maunoury, A. Shkumatava, L. Teboul, J. Kent, J.-S. Joly, J.-P. Concordet, Evaluation of off-target and on-target scoring algorithms and integration into the guide RNA selection tool CRISPOR. *Genome Biol.* **17**, 148 (2016). doi:10.1186/s13059-016-1012-2 Medline
31. K. Wang, M. Li, H. Hakonarson, ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* **38**, e164 (2010). doi:10.1093/nar/gkq603 Medline
32. E. Zuo, X. Huo, X. Yao, X. Hu, Y. Sun, J. Yin, B. He, X. Wang, L. Shi, J. Ping, Y. Wei, W. Ying, W. Wei, W. Liu, C. Tang, Y. Li, J. Hu, H. Yang, CRISPR/Cas9-mediated targeted chromosome elimination. *Genome Biol.* **18**, 224 (2017). doi:10.1186/s13059-017-1354-4 Medline
33. D. Kim, S. Kim, S. Kim, J. Park, J. S. Kim, Genome-wide target specificities of CRISPR-Cas9 nucleases revealed by multiplex Digenome-seq. *Genome Res.* **26**, 406–415 (2016). doi:10.1101/gr.199588.115 Medline

ACKNOWLEDGMENTS

We thank Drs. M. Poo, D. Li, G. Xu and K. Roy for helpful discussions and insightful comments on this manuscript, and FACS facility in ION. **Funding:** R&D Program of China (2018YFC2000100 and 2017YFC1001302 to HY, 2017YFC0908405 to WW), CAS Strategic Priority Research Program (XDB32060000), National Natural Science Foundation of China (31871502, 31522037), Shanghai Municipal Science and Technology Major Project (2018SHZDZX05), Shanghai City Committee of science and technology project (18411953700, 18JC1410100) and NIH P01 Center grant (P01HG00020527 to L.M.S.). **Author contributions:** EZ designed and performed experiments. YS, WW, HS and LY performed data analysis. TY performed PCR analysis. WY performed mouse embryo transfer. HY, YL and L.M.S. supervised the project and designed experiments. All the authors wrote the paper. **Competing interests:** We declare no competing financial interests. **Data and materials availability:** All the sequencing data were deposited in NCBI Sequence Read Archive (SRA) under project accession SRP119022 and https://www.biosino.org/node/project/detail/OEP000195.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/cgi/content/full/science.aav9973/DC1
Materials and Methods
Figs. S1 to S15
Tables S1 to S12
References (*22*–*33*)

8 November 2018; accepted 17 February 2019
Published online 28 February 2019
10.1126/science.aav9973

Downloaded from http://science.sciencemag.org/ on February 28, 2019

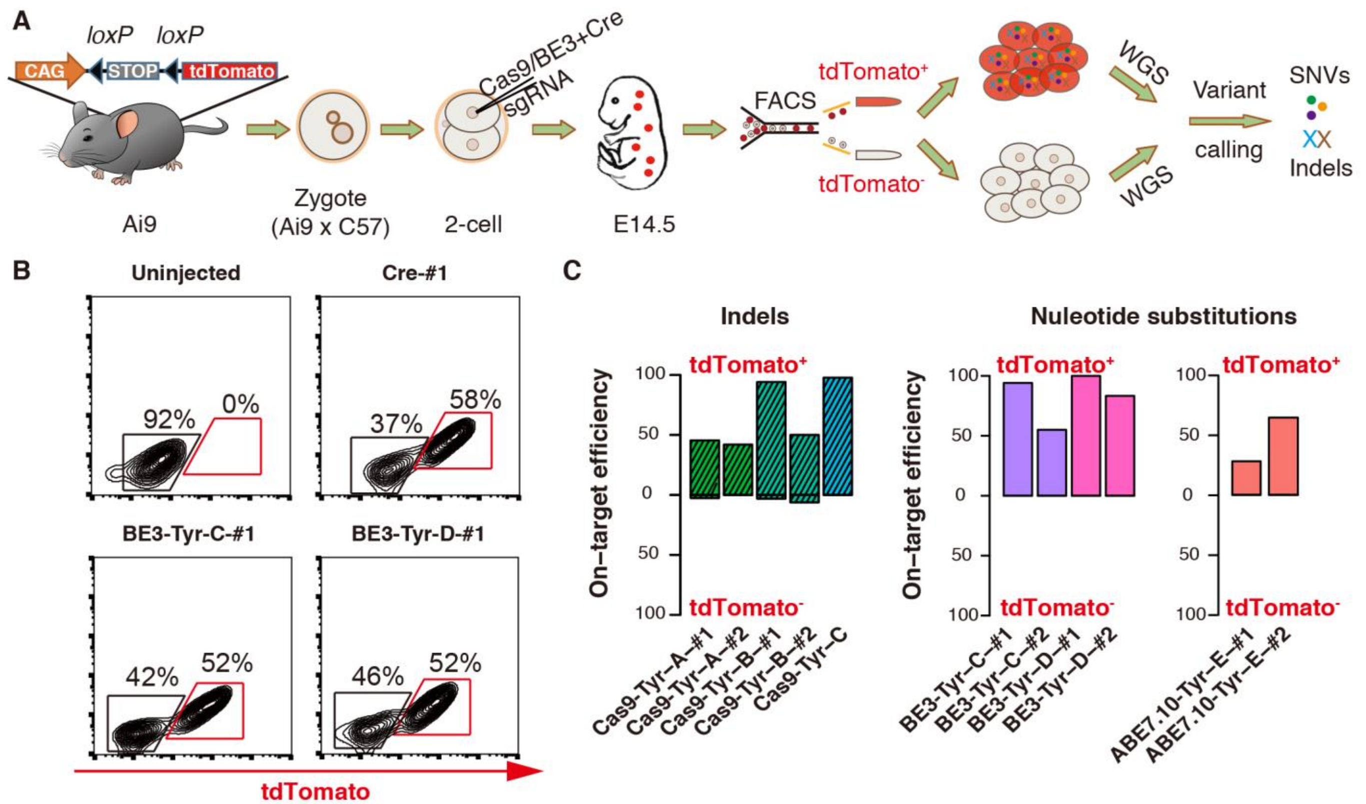

Fig. 1. CRISPR-Cas9-, BE3- or ABE7.10-mediated gene editing in one blastomere of 2-cell embryos. (**A**) Experimental design. (**B**) FACS analysis in indicated embryos. (**C**) On-target efficiency for tdTomato^{+} and tdTomato^{-} cells based on WGS. On-target efficiencies of Cas9, BE3 and ABE7.10 in tdTomato+ cells were 66% +/− 12% indels (SEM, n = 5), 83% +/− 10% (SEM, n = 4) and 47% +/− 18% (SEM, n = 2) nucleotide substitutions, respectively.

Downloaded from http://science.sciencemag.org/ on February 28, 2019

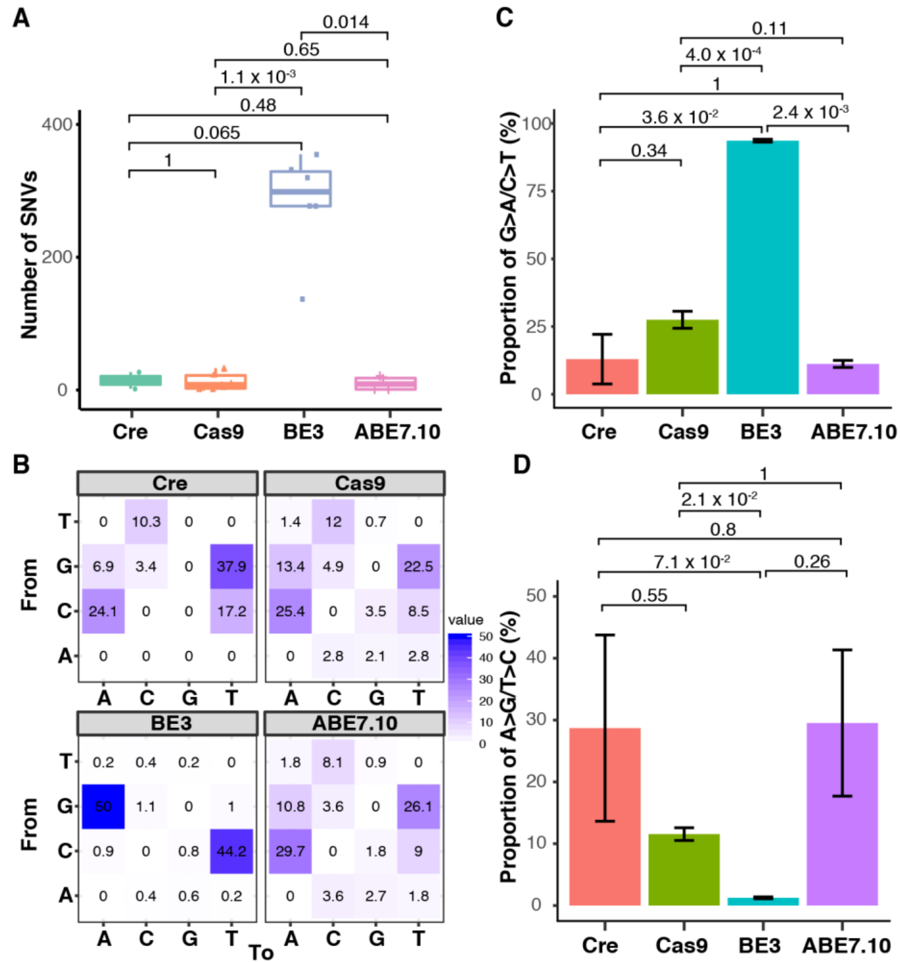

Fig. 2. Substantial off-target SNVs generated in BE3-treated mouse embryos. (A) Comparison of the total number of detected off-target SNVs. The number of SNVs for Cre-, Cas9-, BE3- and ABE7.10-treated embryos were 14 +/− 12 (SEM, n = 2), 12 +/− 4 (SEM, n = 11), 283 +/− 32 (SEM, n = 6) and 10 +/− 5 SNVs (SEM, n = 4), respectively. (B) Distribution of mutation types. The number in each cell indicates the proportion of a certain type of mutation among all mutations. (C) Proportion of C>T and G>A mutations for Cre, Cas9, BE3 and ABE7.10 groups. (D) Proportion of A>G and T>C mutations for Cre, Cas9, BE3 and ABE7.10 groups. Two Cre, 11 Cas9, 6 BE3 and 4 ABE7.10 samples were analyzed. *P* values were calculated by two-sided Wilcoxon rank sum test in (A), (C), and (D).

Downloaded from http://science.sciencemag.org/ on February 28, 2019

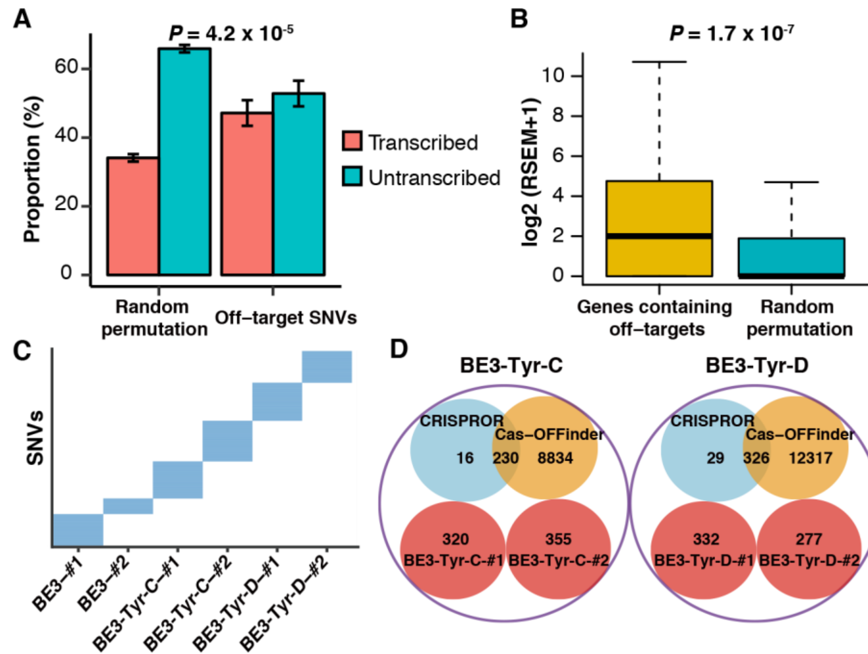

Fig. 3. Characteristics of BE3 induced off-target SNVs. (**A**) Off-target SNVs are enriched in the transcribed regions of the genome compared to random permutation. (**B**) Genes containing off-target SNVs were significantly higher expressed than random simulated genes in 4-cell embryos. (**C**) SNVs identified from each embryo were non-overlapping. (**D**) Overlap among SNVs detected by GOTI with predicted off-targets by Cas-OFFinder and CRISPOR. *P* values were calculated by two-sided Wilcoxon rank sum test in (A) and (B).

Downloaded from http://science.sciencemag.org/ on February 28, 2019

Science

Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos

Erwei Zuo, Yidi Sun, Wu Wei, Tanglong Yuan, Wenqin Ying, Hao Sun, Liyun Yuan, Lars M. Steinmetz, Yixue Li and Hui Yang

published online February 28, 2019

ARTICLE TOOLS	http://science.sciencemag.org/content/early/2019/02/27/science.aav9973
SUPPLEMENTARY MATERIALS	http://science.sciencemag.org/content/suppl/2019/02/27/science.aav9973.DC1
RELATED CONTENT	http://science.sciencemag.org/content/sci/early/2019/02/27/science.aaw7166.full
REFERENCES	This article cites 32 articles, 3 of which you can access for free http://science.sciencemag.org/content/early/2019/02/27/science.aav9973#BIBL
PERMISSIONS	http://www.sciencemag.org/help/reprints-and-permissions

Use of this article is subject to the Terms of Service

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. 2017 © The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works. The title *Science* is a registered trademark of AAAS.

Downloaded from http://science.sciencemag.org/ on February 28, 2019