2nd International Summit on Human Genome Editing

Towards precise genome engineering *in vivo*

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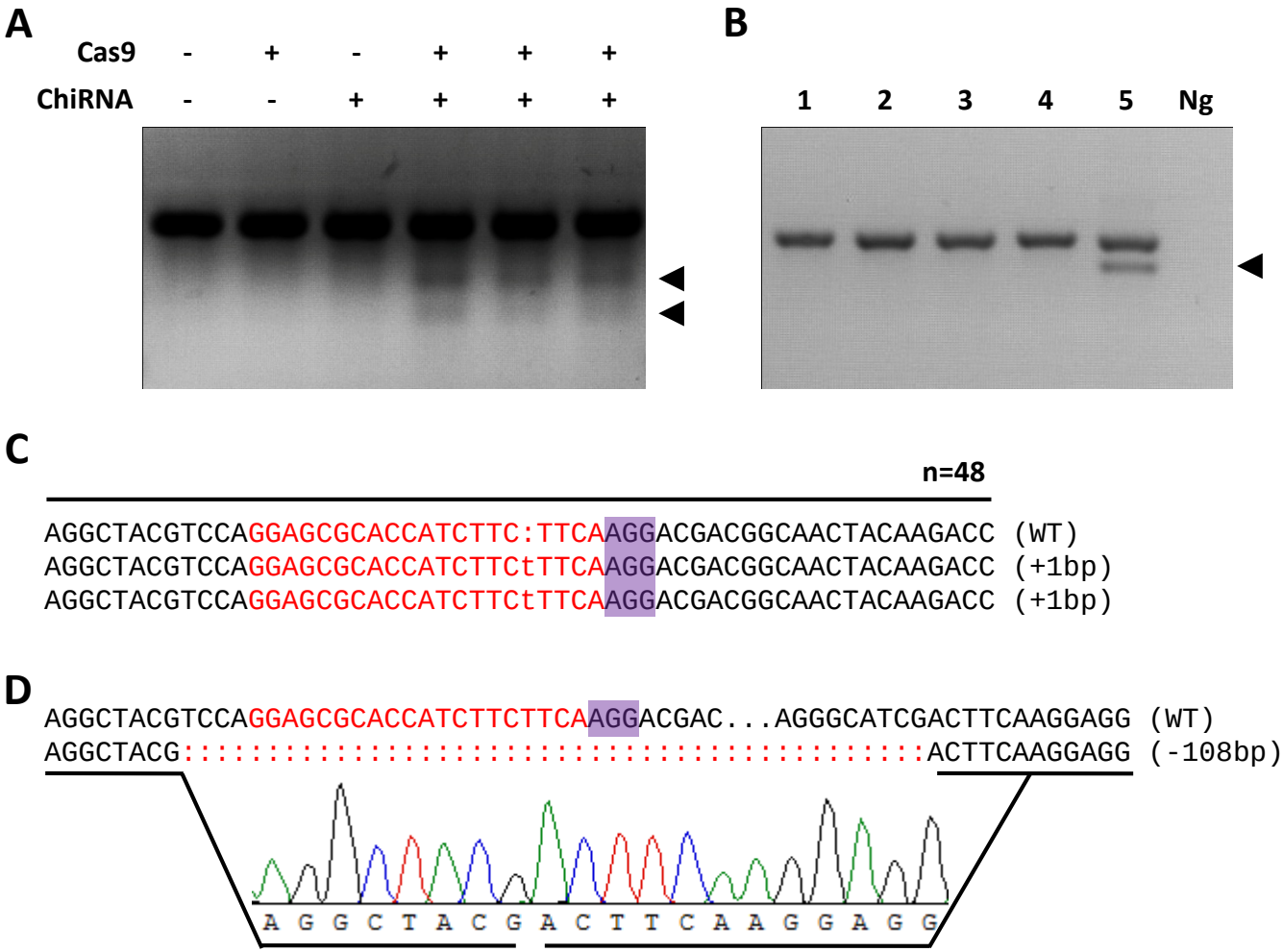
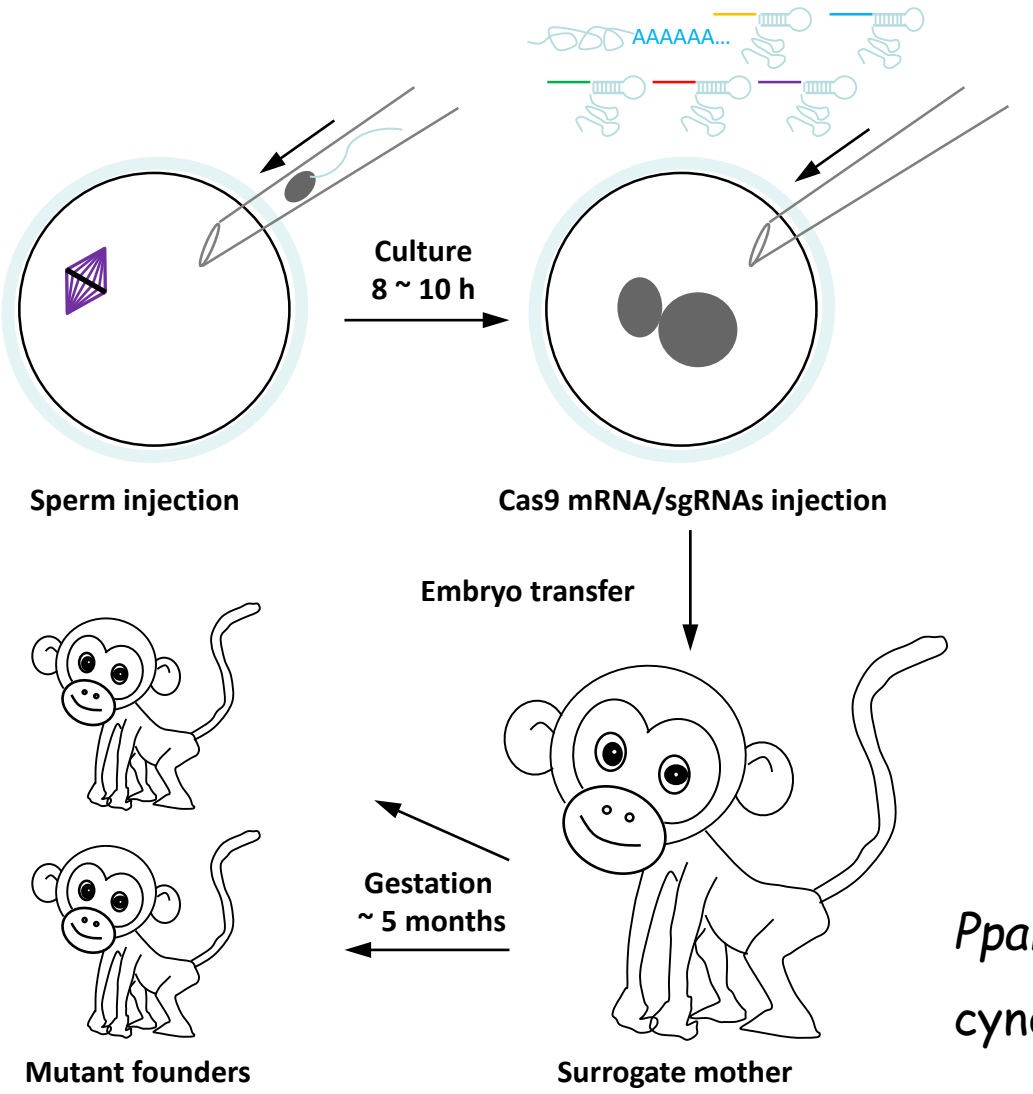
First *in vivo* CRISPR genome editing in mice



Shen B, et al. *Cell Res.* 2013, 23(5): 720-723.

Successful *in vivo* genome editing in monkeys

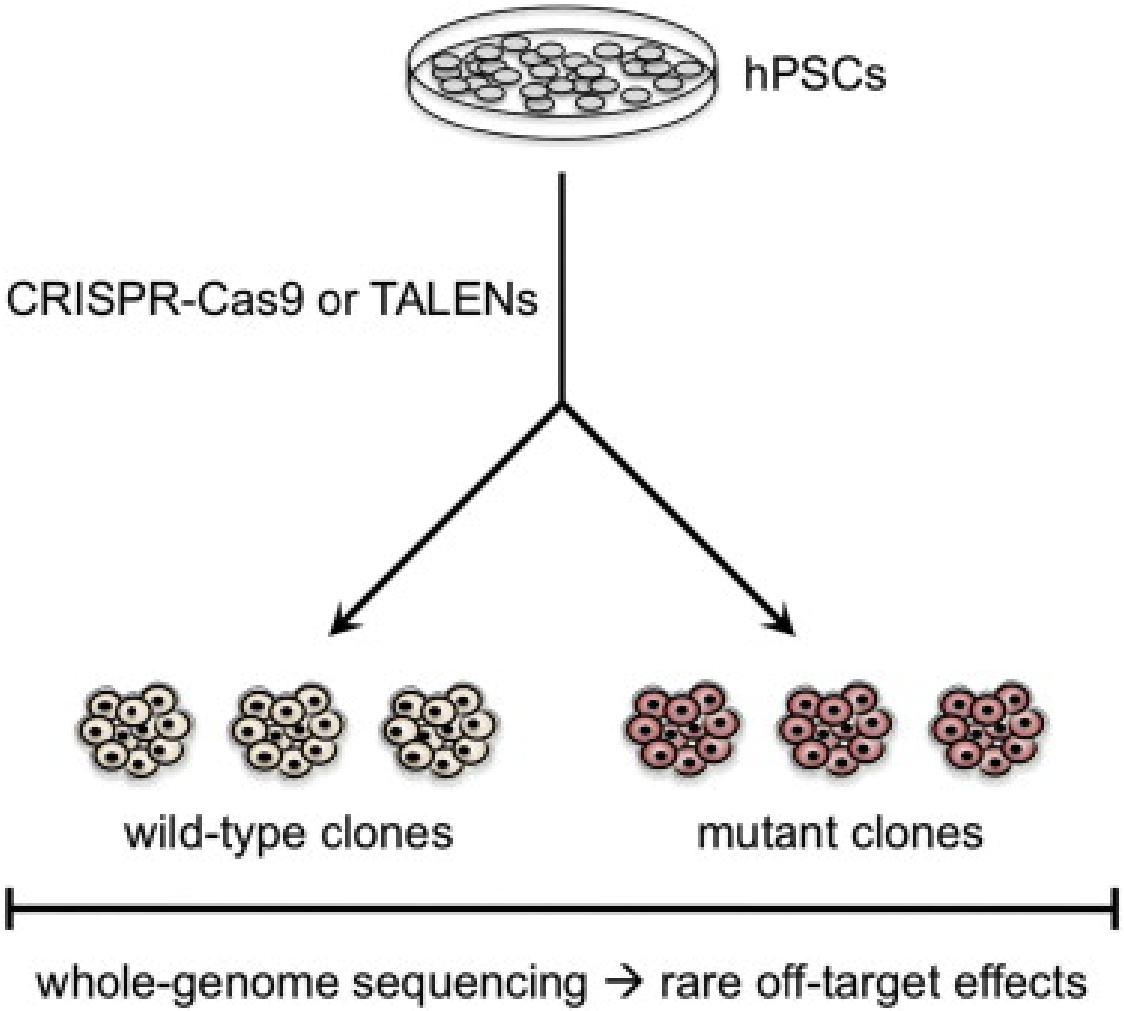


Ppar-y & Rag1

cynomolgus monkey

Niu Y, et al. *Cell* 2014. 156(4): 836-843.

The off-target cleavage in individual clone



Kiskinis E, et al. *Cell Stem Cell* 2014.
Smith C, et al. *Cell Stem Cell* 2014.
Suzuki K, et al. *Cell Stem Cell* 2014.
Veres A, et al. *Cell Stem Cell* 2014.

The off-target cleavage *in vivo*

Species	Number of sgRNA	Tentative off-target sites	Authentic off-target sites
Mouse	7	97	9
Rat	4	119	1
Pig	1	8	0
Goat	4	13	2
Monkey	5	84	0

The human embryo editing

Protein Cell 2015, 6(5):363–372
DOI 10.1007/s13238-015-0153-5

CrossMark Protein & Cell

RESEARCH ARTICLE

CRISPR/Cas9-mediated gene editing in human tripronuclear zygotes

Puping Liang, Yanwen Xu, Xiya Zhang, Chenhui Ding, Rui Huang, Zhen Zhang, Jie Lv, Xiaowei Xie, Yuxi Chen, Yujing Li, Ying Sun, Yaofu Bai, Zhou Songyang, Wenbin Ma, Canquan Zhou✉, Junjiu Huang✉

Guangdong Province Key Laboratory of Reproductive Medicine, the First Affiliated Hospital, and Key Laboratory of Gene Engineering of the Ministry of Education, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China
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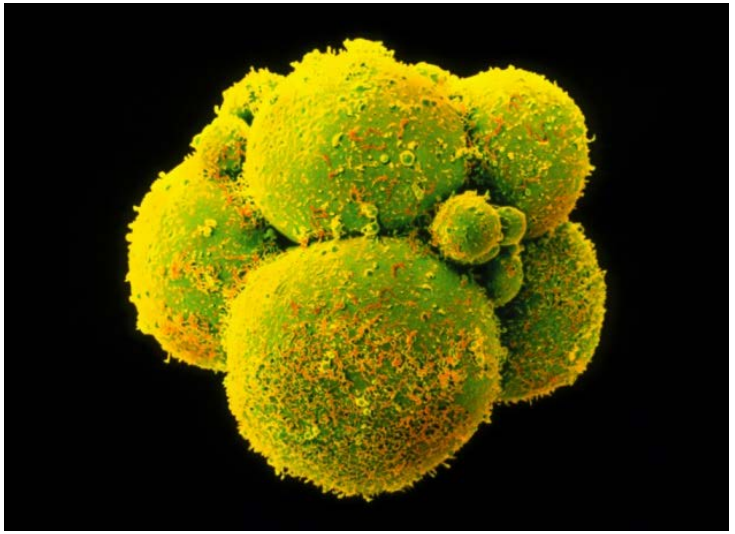
Received March 30, 2015 Accepted April 1, 2015

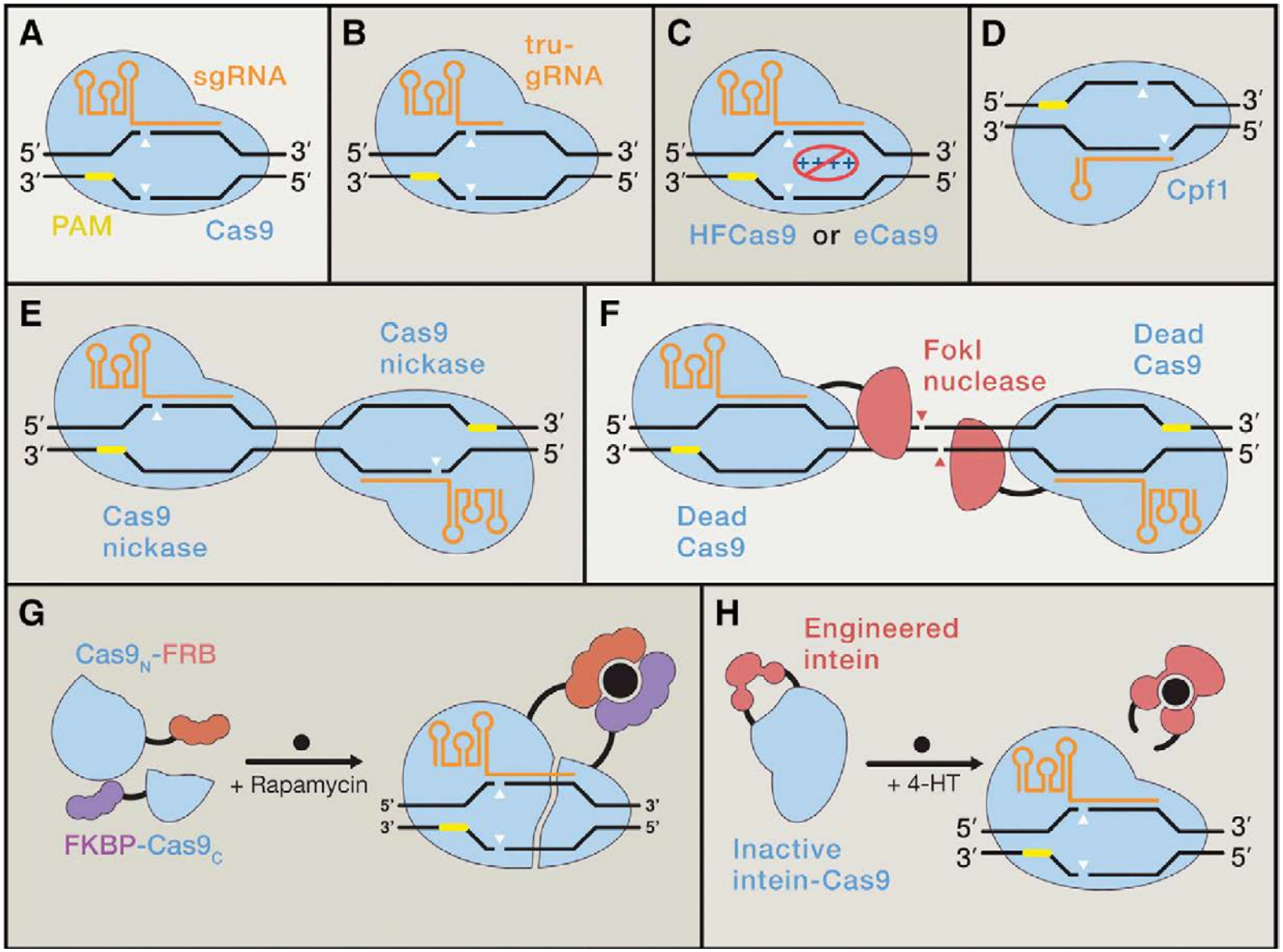
Concerns:

- Efficacy
- Safety
- Mosaicism



http://www.nature.com/news/embryo-editing-sparks-epic-debate-1.17421

Strategies for improving the specificities



Komor A, et al. *Cell* 2017, 168: 20-36.

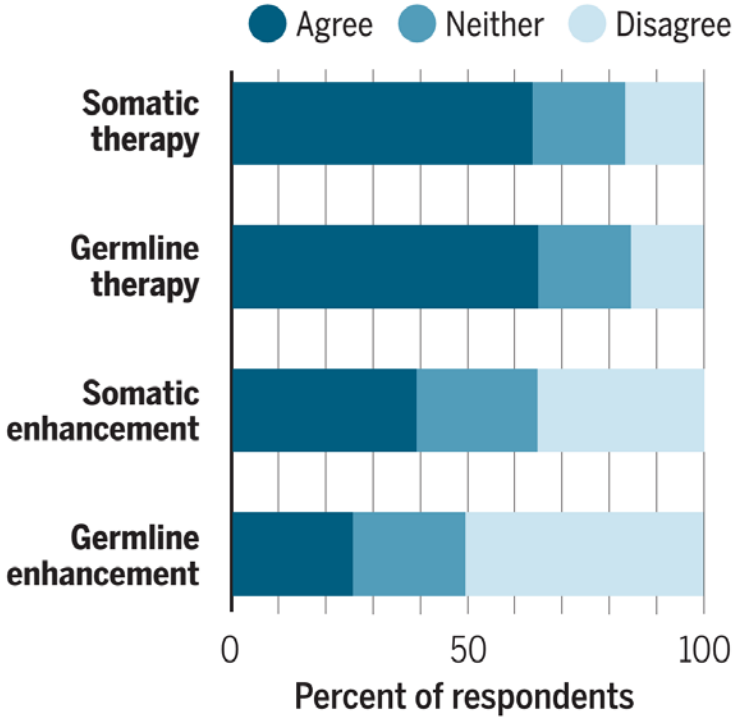
More CRISPR genome editing in human embryos

- Kang et al. Introducing precise genetic modifications into human 3PN embryos by CRISPR/Cas-mediated genome editing. ***Journal Assisted Reproduction Genetics*** 2016, *33*: 581-588.
- Tang et al. CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein. ***Molecular Genetics Genomics*** 2016, *292*: 525-533.
- Ma et al. Correction of a pathogenic gene mutation in human embryos. ***Nature*** 2017, *548*: 413-419.
- Fogarty et al. Genome editing reveals a role for OCT4 in human embryogenesis. ***Nature*** 2017, *550*: 67-73.

Attitudes on human genome editing

- **U.S. panel gives yellow light to embryo editing.** Feb. 14, 2017

http://www.sciencemag.org/news/2017/02/us-panel-gives-yellow-light-human-embryo-editing

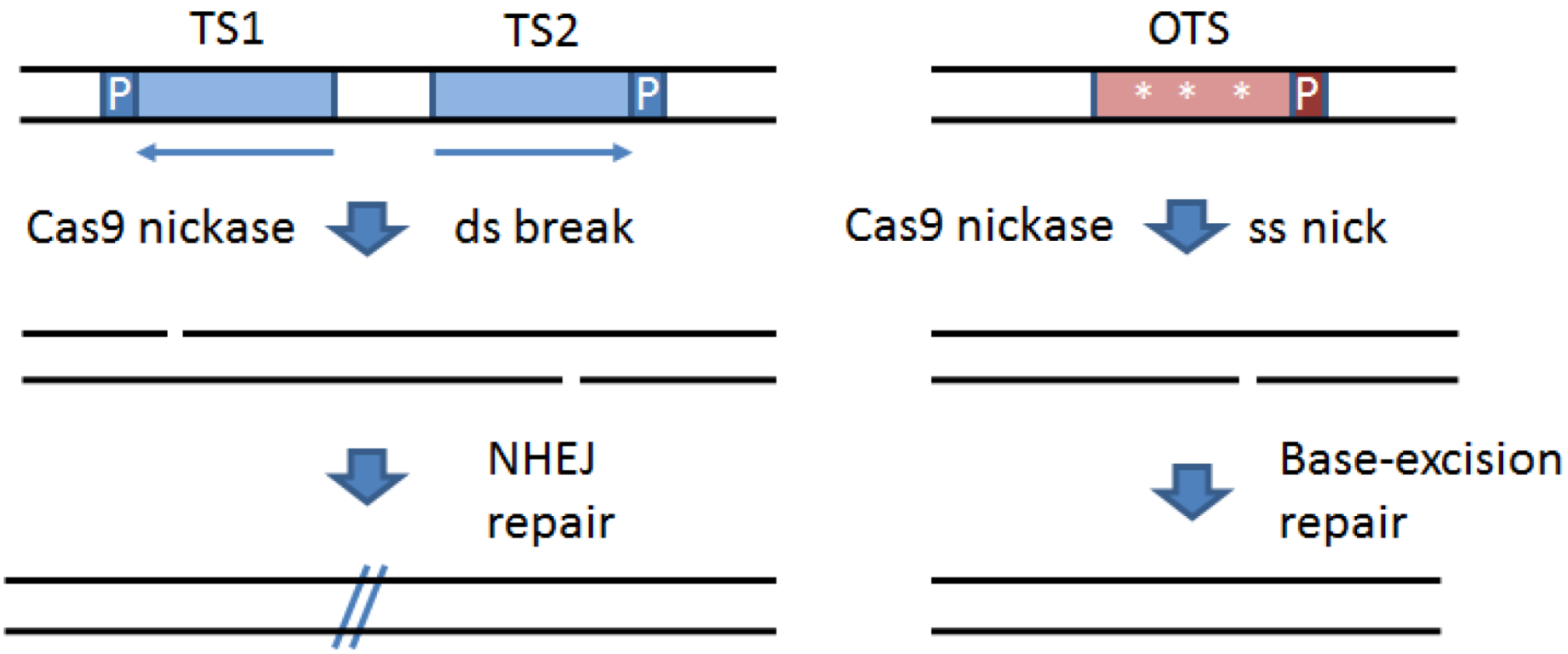


Aug. 11, 2017

- A majority finds use of human genome editing for therapeutic purposes acceptable, including somatic and germline edits. Public opposition increases for applications aimed at enhancement.

http://science.sciencemag.org/content/357/6351/553.full

Minimizing off-target mutagenesis using Cas9-nickase/dual sgRNAs



http://www.sanger.ac.uk/htgt/wge/find_crisprs

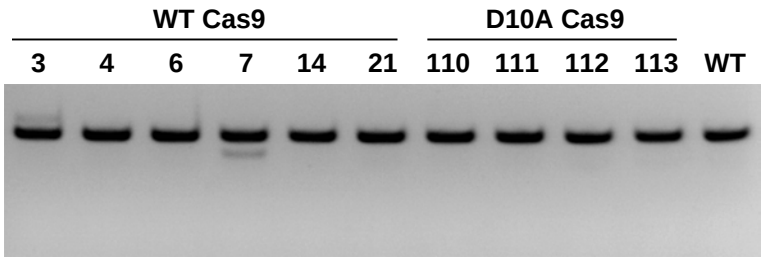
Shen B, et al. *Nature Methods* 2014, 11(4): 399-402.

Off-target mutations are rare *in vivo*

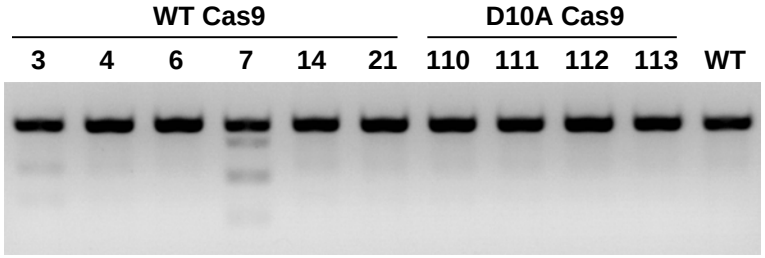
Iver V, et al. *Nature Methods* 2015, 12(6): 479.

nCas9/dual sgRNAs strategy reduces off-target

a



b



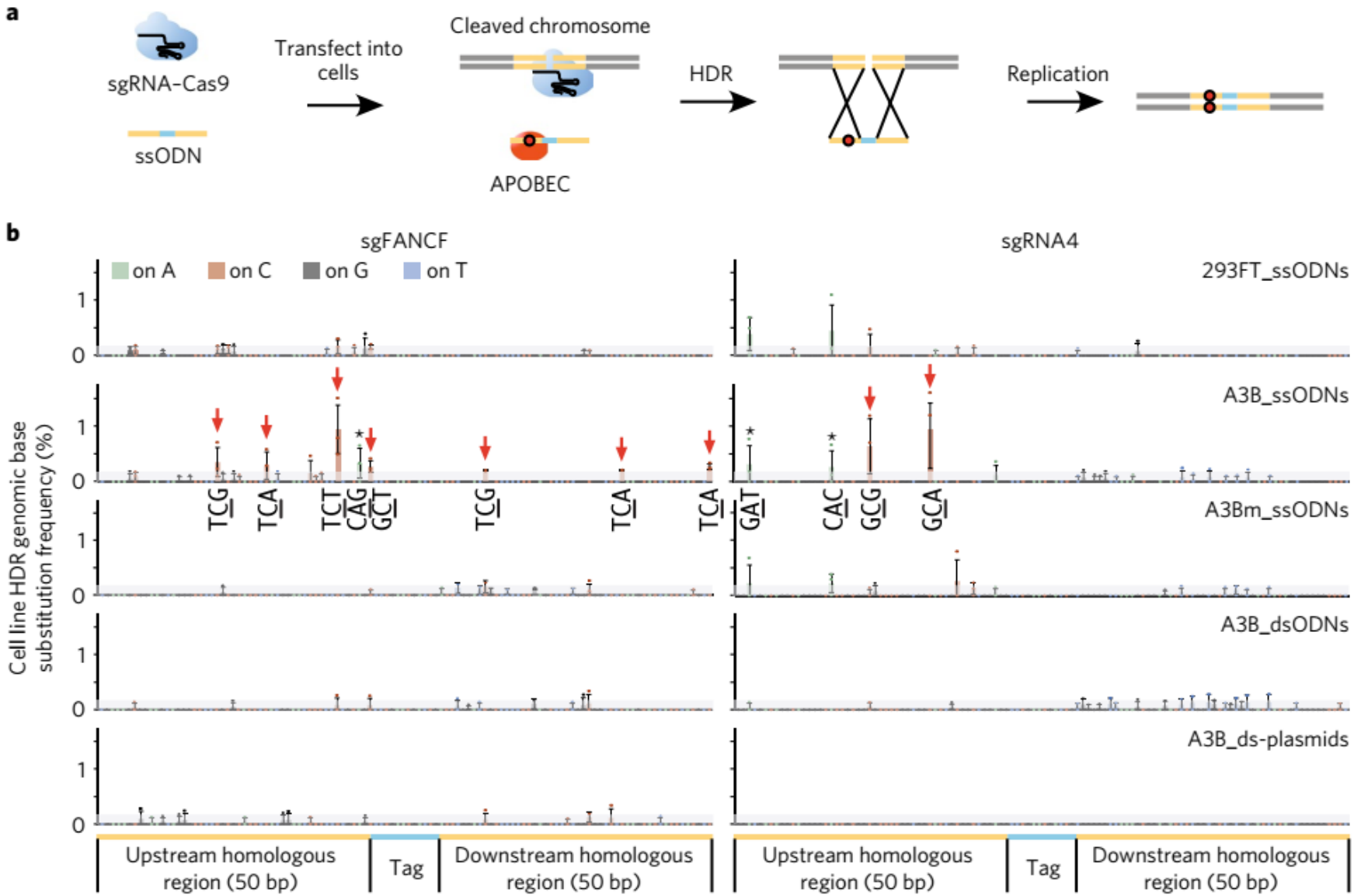
c

	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	N	G	G
RAG1 sgRNAE	G	G	T	G	T	C	T	G	C	T	T	T	G	A	T	G	G	A	C	A	T	G	G
PP13	T	A	T	G	T	C	T	G	C	A	T	T	G	A	T	G	G	A	C	A	A	G	G

```
PP13 CTTGGAGAAGGGGTCTTCTTTCCCCCCTTGTCCATCAATGCAGACATAGCTAGGGCTTCTCCTAAAGAAGCTC (WT)
#7   CTTGGAGAA----------------------------------------------------------GCTC (-60, 3/21)
```

Unpublished data

APOBEC3 mediates the formation of unexpected indel mutations near SSBs



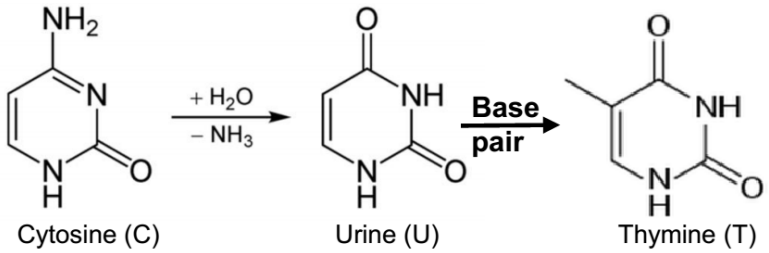
Lei et al. *NSMB* 2018, 25: 45-52.

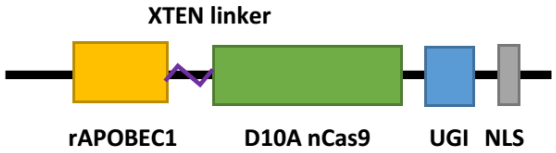
Base editing provides a more precise strategy

CBE: cytosine base editor

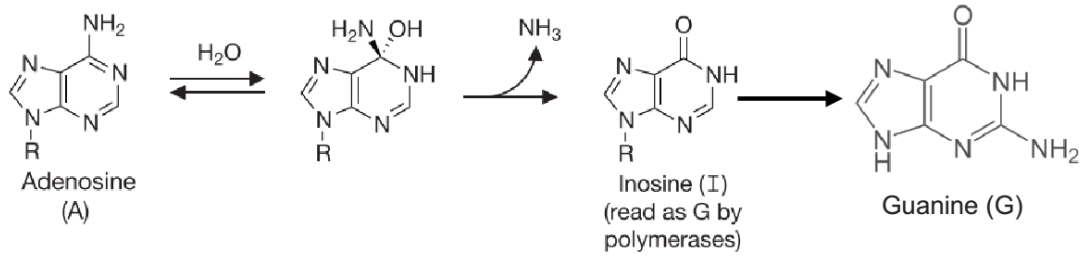
ABE: adenine base editor

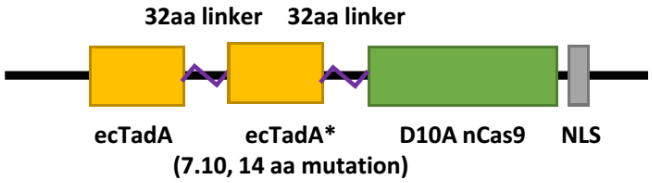
BE3





ABE

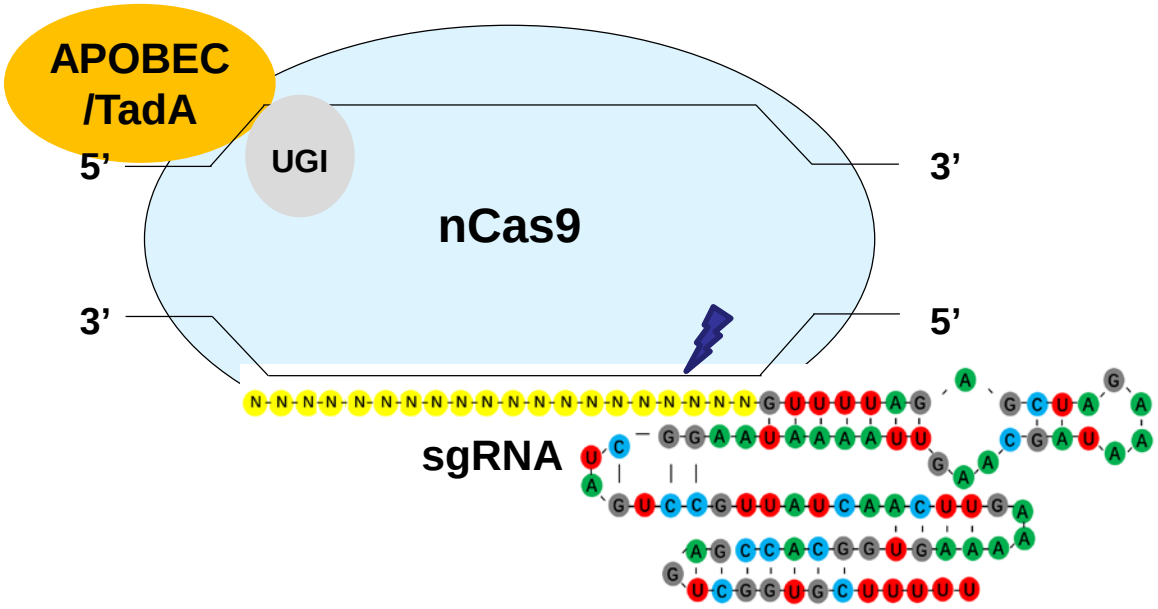




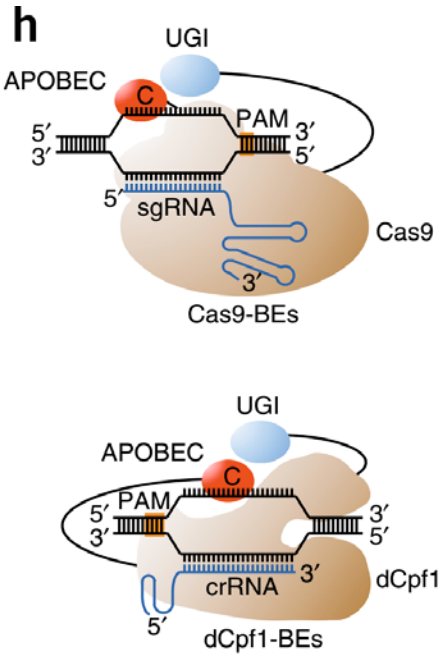
Perform programmable editing of a target base in genome by deamination without double-stranded DNA cleavage.

Komor et al. *Nature* 2016; Gaudelli et al. *Nature* 2017

To develop *in vivo* base editing toolbox



High-fidelity C/T base editing mediated by Cpf1-cytidine deaminase fusion

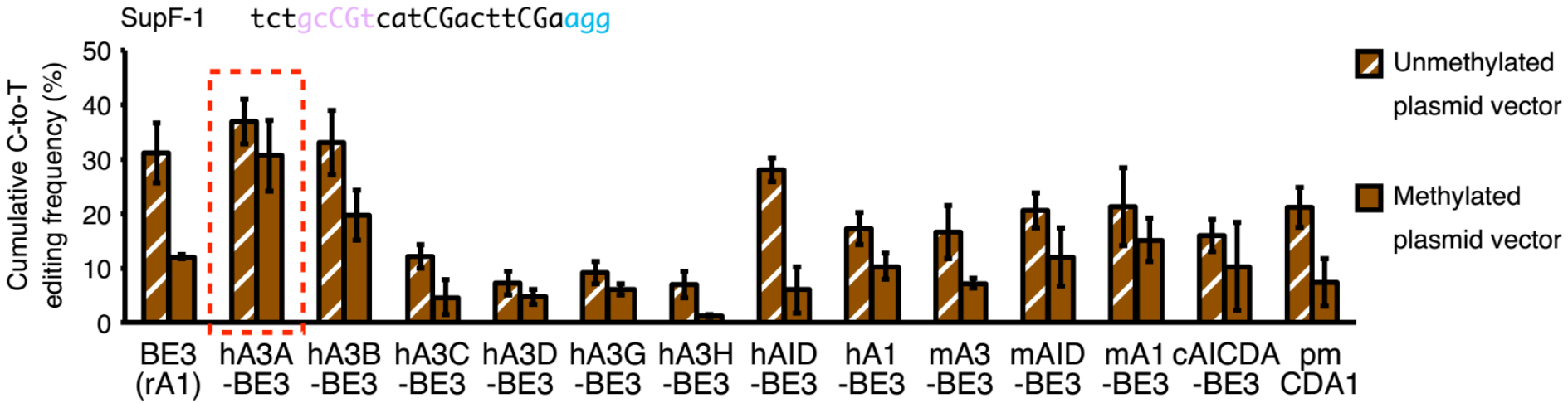


Name	Cas	PAM	APOBEC	N-terminal NLS	Fused UGI	Free UGI	Editing window	Editing efficiency	C-to-T fraction
dCas9-BE2	dCas9	NGG	rA1	–	+	–	4–8	~9–16%	~91–98%
nCas9-BE3	nCas9	NGG	rA1	–	+	–	4–8	~21–46%	~82–99%
dCpf1-BE0	dCpf1	TTTV	rA1	–	+	–	8–13	~10–31%	~89–99%
dCpf1-BE	dCpf1	TTTV	rA1	+	+	–	8–13	~20–44%	~88–99%
dCpf1-BE-YE	dCpf1	TTTV	rA1-YE	+	+	–	10–12	~2–29%	~92–98%
dCpf1-eBE	dCpf1	TTTV	rA1	+	+	+++	8–13	~15–30%	~97–99%
dCpf1-eBE-YE	dCpf1	TTTV	rA1-YE	+	+	+++	10–12	~2–28%	~95–99%

Based on the DYRK1A-, FANCF- and RUNX1-target sites

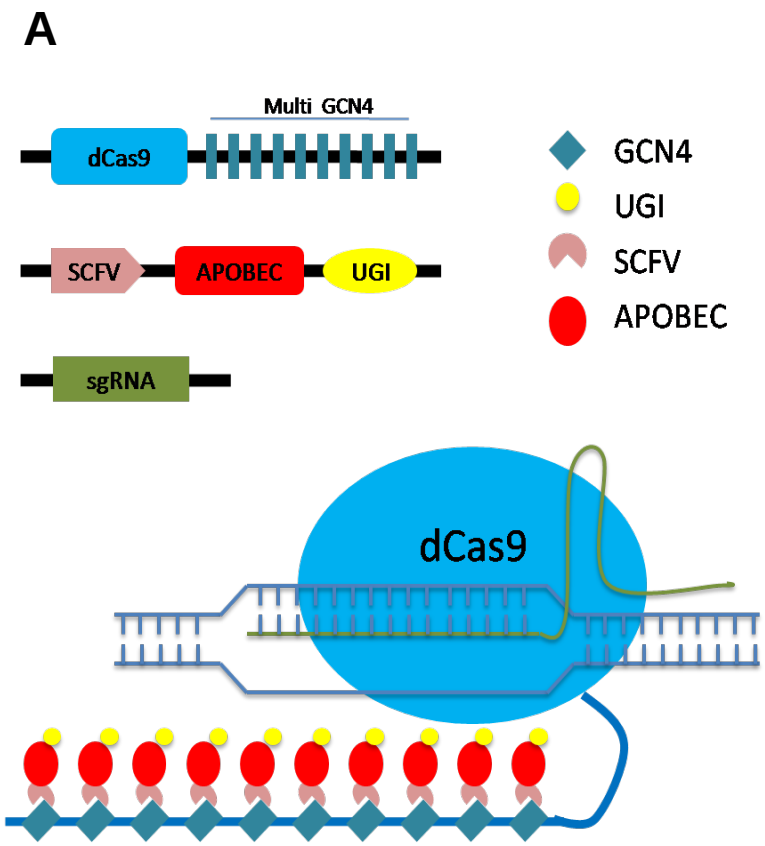
Li et al. *Nature Biotechnol.* 2018, 36(4): 324-327.

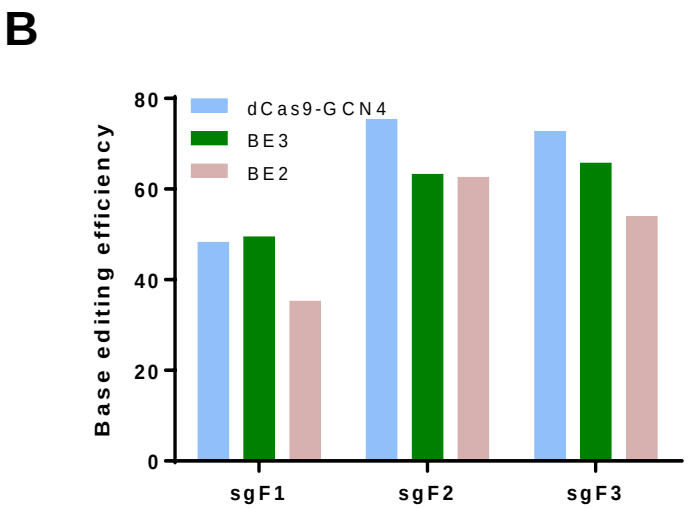
Comprehensive C/T base editing mediated by a new deaminase, A3A

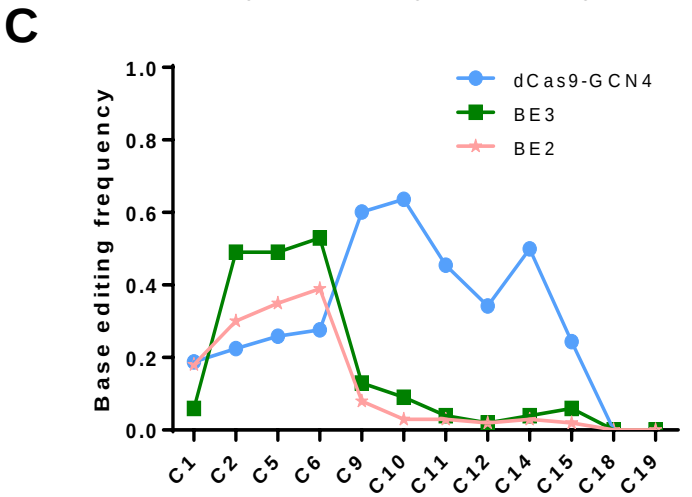


Wang et al. *Nature Biotechnol.* 2018, 36(10): 946-949.

BE-PLUS, to increase the editing scope & precision

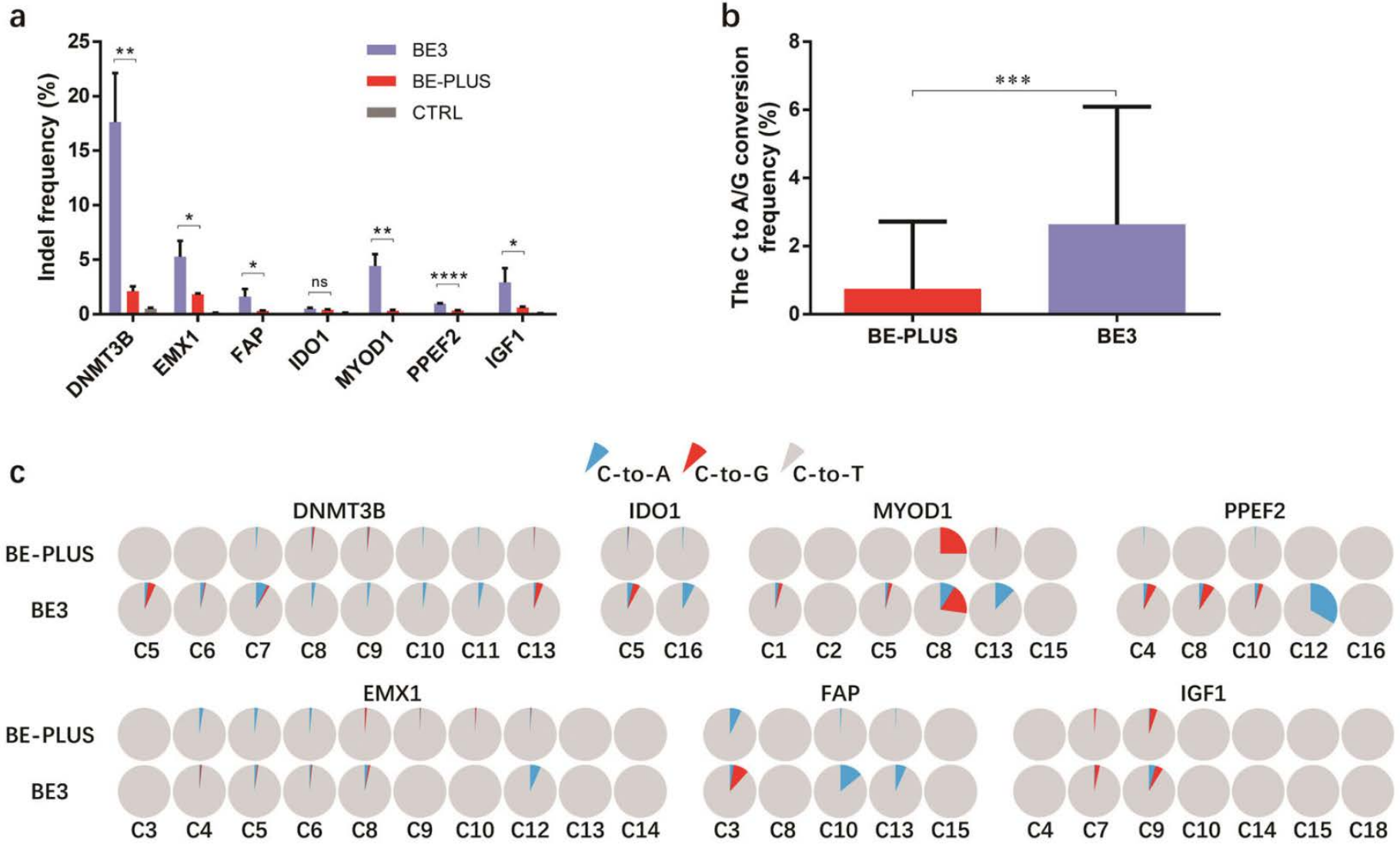






Jiang et al. *Cell Res.* 2018, 28(8): 855-861.

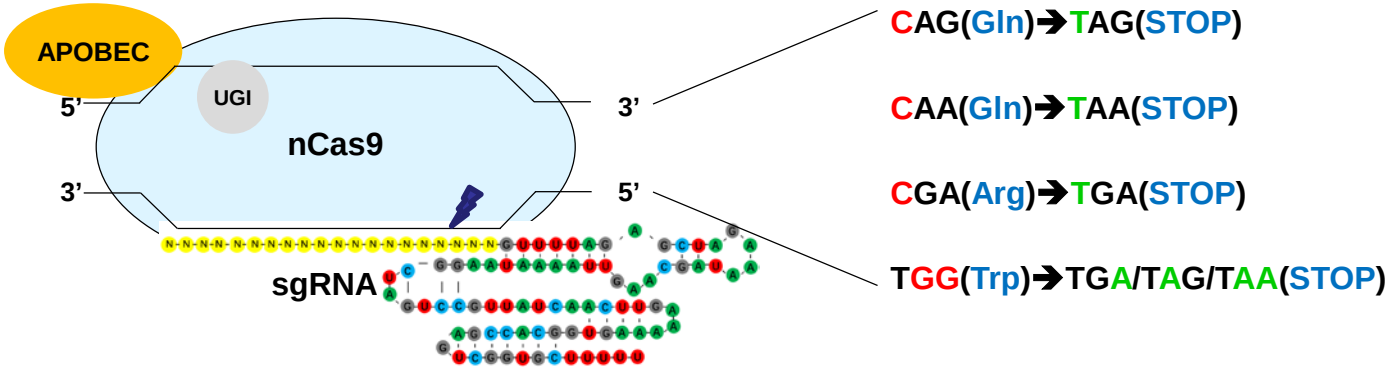
BE-PLUS enhances the fidelity of C/T base editing



Jiang et al. *Cell Res.* 2018, 28(8): 855-861.

BE3 mediates isogenic C/T gene editing *in vivo*

a



b

***VISTA* sp-1**

```
CAGCACCACGGAAGCCACCTGAAAGCCAACGCCAGCCATGACC6AGCCCCAGAAGCATGGGCTAGAGCTAGCTTCTGAC (WT)
CAGCACCACGGAAGCCACCTGAAAGCCAACGCCAGCCATGATT6AGCCCCAGAAGCATGGGCTAGAGCTAGCTTCTGAC (14/16,6/14 homozygote)
CAGCACCACGGAAGCCACCTGAAAGCCAACGCCAGCCATGACT6AGCCCCAGAAGCATGGGCTAGAGCTAGCTTCTGAC (1/16,0/1 homozygote)
CAGCACCACGGAAGCCACCTGAAAGCCAACGCCAGCCATGATC6AGCCCCAGAAGCATGGGCTAGAGCTAGCTTCTGAC (1/16,0/1 homozygote)
```

***CD160* sp-2**

```
AGCATCCCAGAAAGGAGGGCGACTGGACCTCACCTGTACTTTGTG6G5CACAAGAAAGACGAAGCTGAGGGGCTAATACT (WT)
AGCATCCCAGAAAGGAGGGCGACTGGACCTCACCTGTACTTTGTA6G5CACAAGAAAGACGAAGCTGAGGGGCTAATACT (3/12,2/3 homozygote)
AGCATCCCAGAAAGGAGGGCGACTGGACCTCACCTGTACTTTGTA6A5CACAAGAAAGACGAAGCTGAGGGGCTAATACT (2/12,0/2 homozygote)
```

Table 1 Summary of the microinjections

Target genes	Methods		No. of examined embryos	No. of two-cell stage embryos (%)	No. of blastocysts (%)	Mutant ratio (%)	
						No. of mutants/total blastocysts	No. of homozygotes/total mutants
VISTA	*VSITA* mRNA+BE3 mRNA	Microinjection	20	18 (90)[a]	16 (89)[b]	16 (100)	6/16 (38)
CD160	*CD160* mRNA+BE3 mRNA	Microinjection	20	17 (85)[a]	12 (71)[b]	9 (75)	2/9 (22)

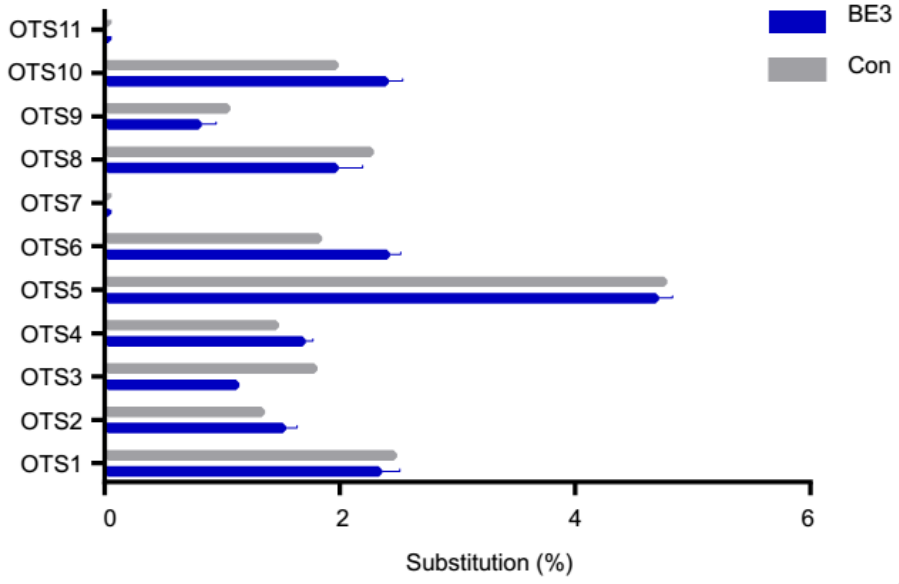
Manuscript accepted

Off-target analysis by deep sequencing

A

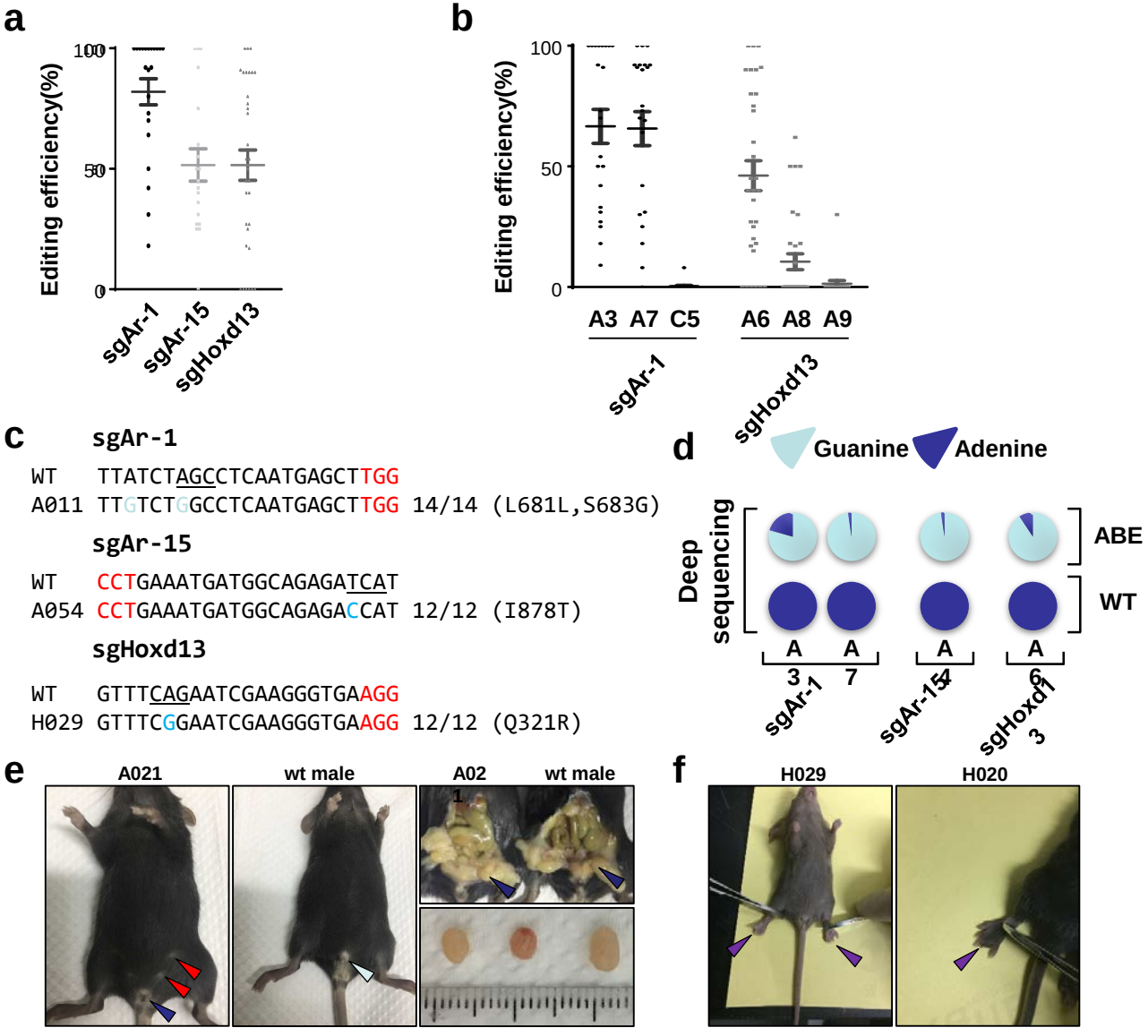
Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	PAM			Chr.
On-Target	A	T	G	A	C	C	A	G	C	C	C	C	A	G	A	A	G	C	A	T	G	G	G	10
OTS1	A	T	G	A	a	C	A	t	C	C	C	C	A	G	A	A	G	C	A	a	A	G	G	3
OTS2	A	T	t	A	C	C	A	G	C	C	C	a	A	G	A	A	G	C	c	T	A	G	G	7
OTS3	A	T	G	A	C	C	A	a	C	C	C	C	A	G	A	t	G	C	t	T	T	G	G	4
OTS4	A	g	G	A	C	C	A	G	a	C	C	C	A	G	A	A	c	C	A	T	A	G	G	4
OTS5	c	T	G	A	C	C	A	G	a	C	C	C	A	G	A	A	G	C	A	g	G	G	G	5
OTS6	t	a	G	A	C	C	A	G	C	C	C	C	A	G	A	A	G	C	A	a	A	G	G	5
OTS7	A	T	G	g	C	t	g	G	C	C	C	C	A	G	A	A	G	C	A	T	T	G	G	2
OTS8	A	T	a	c	C	C	A	G	C	C	C	C	A	G	A	A	G	C	c	T	G	G	G	6
OTS9	A	a	a	A	C	C	A	G	C	C	t	C	A	G	A	A	G	C	A	T	G	G	G	14
OTS10	A	T	G	A	t	C	A	G	t	C	C	C	A	G	g	A	G	C	A	T	G	G	G	14
OTS11	A	T	G	A	a	C	A	G	C	t	C	C	A	G	A	A	t	C	A	T	T	G	G	9

B



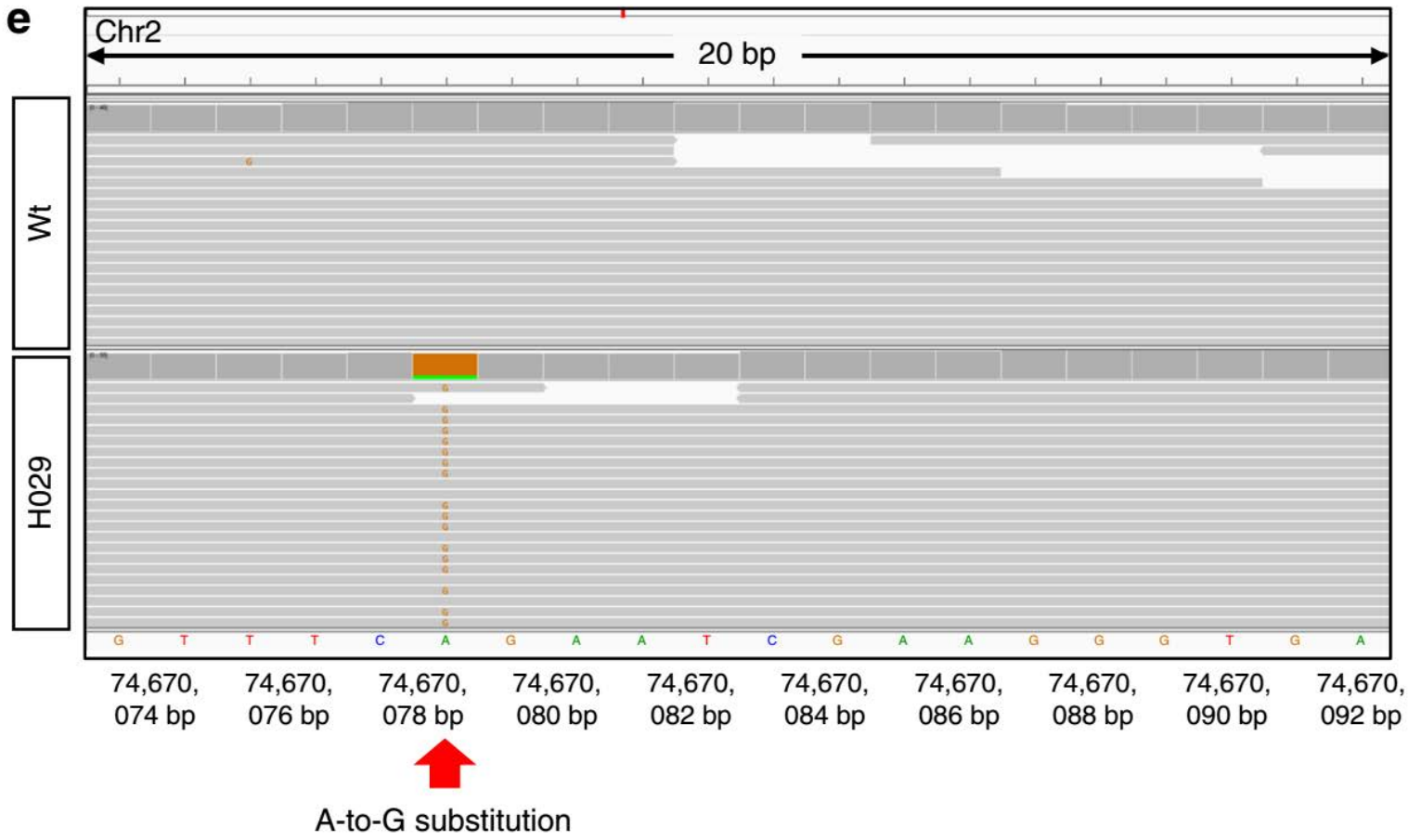
Manuscript accepted

ABE mediates efficient A/G base editing *in vivo*



Liu et al. *Nature Commun.* 2018, 9(1): 2338.

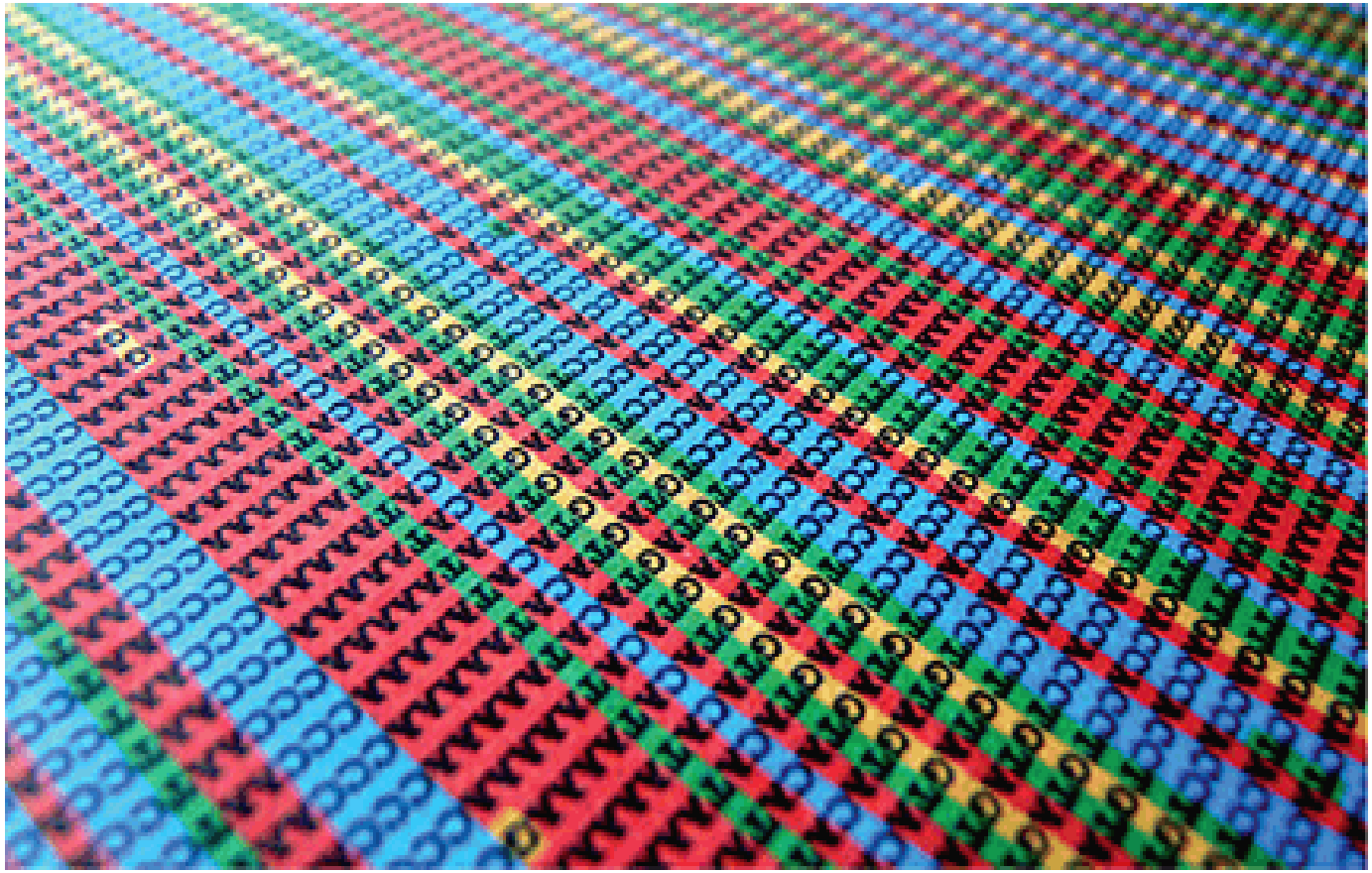
On-target and off-target analysis



Liu et al. *Nature Commun.* 2018, 9(1): 2338.

A safe & efficient *in vivo* genome editing approach

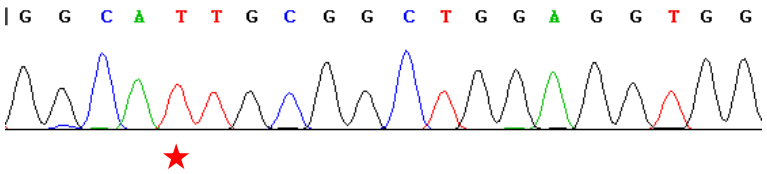
BE: pinpoint gene editing



http://vis.sciencemag.org/breakthrough2017/

Efficient & pure *in vivo* C/T conversion by BE

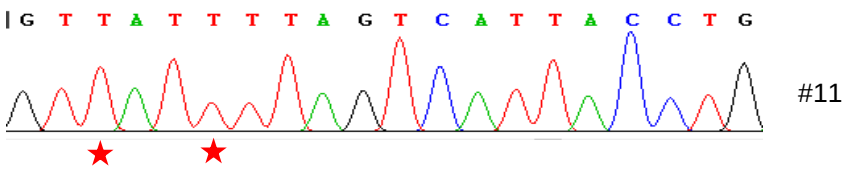
a



b

```
GCGCCCCGGTGGCACTGCGGCTGGAGGTGGGGGTTAAAGC   (wt)
GCGCCCCGGTGGCACTGCGGCTGGAGGTGGGGGTTAAAGC   (wt, 1/8)
GCGCCCCGGTGGCATTGCGGCTGGAGGTGGGGGTTAAAGC   (mt, 7/8)
```

c



d

```
CCCCTTGGCAGTCATCTTAGTCATTACCTGAGGTGTTCGT   (wt)
CCCCTTGGCAGTCATCTTAGTCATTACCTGAGGTGTTCGT   (wt, 1/8)
CCCCTTGGCAGTTATTTTAGTCATTACCTGAGGTGTTCGT   (mt, 7/8)
```

Table 1 Summary of the microinjections

	Injected mixture	Target sites	No. of examined tripronuclear zygotes	No. of mutants/total zygotes	Editing efficiency
Experiment 1	mRNA+sgRNA	SITE 4	8	7/8	87.5%
Experiment 2	mRNA+sgRNA	RNF2	8	7/8	87.5%
Experiment 3	mRNA+sgRNA	SITE4	9	9/9	100%
		RNF2	9	9/9	100%

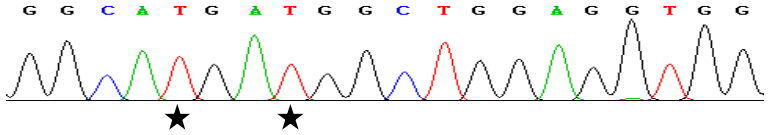
Li G, et al. *Protein & Cell* 2017, 8(10): 776-779.

With very rare off-target

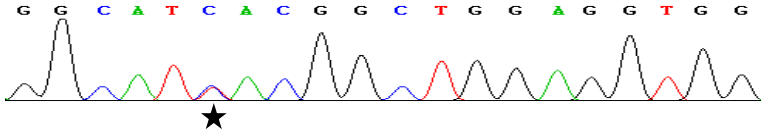
a

	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	N	G	G
SITE4	G	G	C	A	C	T	G	C	G	G	C	T	G	G	A	G	G	T	G	G	G	G	G
OTS3	G	G	C	A	C	G	A	C	G	G	C	T	G	G	A	G	G	T	G	G	A	G	G
OTS4	G	G	C	A	T	C	A	C	G	G	C	T	G	G	A	G	G	T	G	G	A	G	G

b Off-target-3



c Off-target-4



d

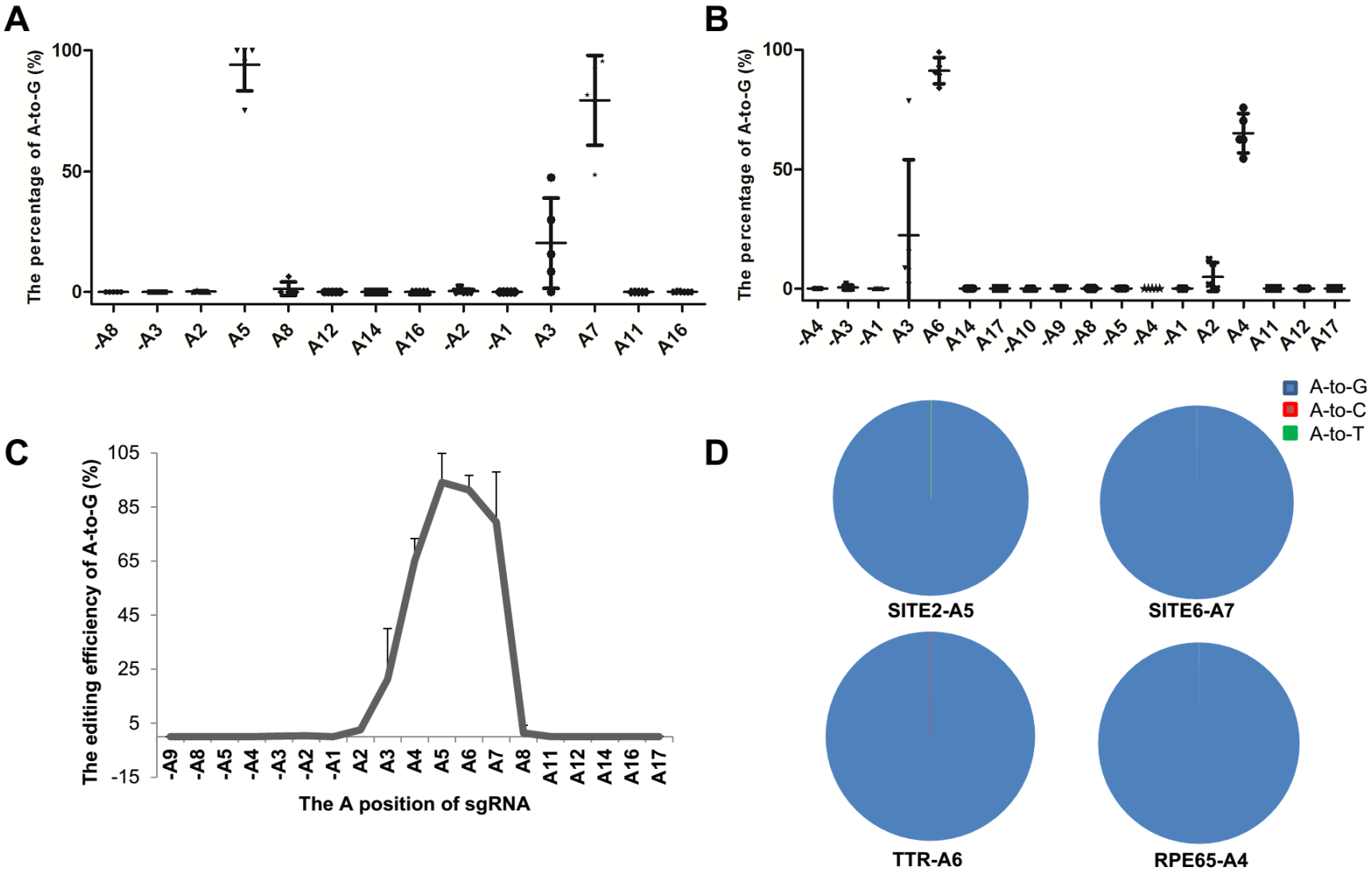
Off-target-3	G	G	C	A	C	G	A	C	G	G	C	T	G	G	A	G	G	T	G	G	G	G	G
#2			0.00		0.01			0.00			0.00												
#3			0.00		0.76			0.67			0.00												
#18			0.00		0.18			0.01			0.00												

e

Off-target-4	G	G	C	A	T	C	A	C	G	G	C	T	G	G	A	G	G	T	G	G	A	G	G
#2			0.00			0.00		0.02			0.00												
#3			0.00			0.36		0.00			0.00												
#18			0.00			0.01		0.00			0.00												

Li G, et al. *Protein & Cell* 2017, 8(10): 776-779.

Efficient & pure *in vivo* A/G conversion by ABE



Manuscript under review

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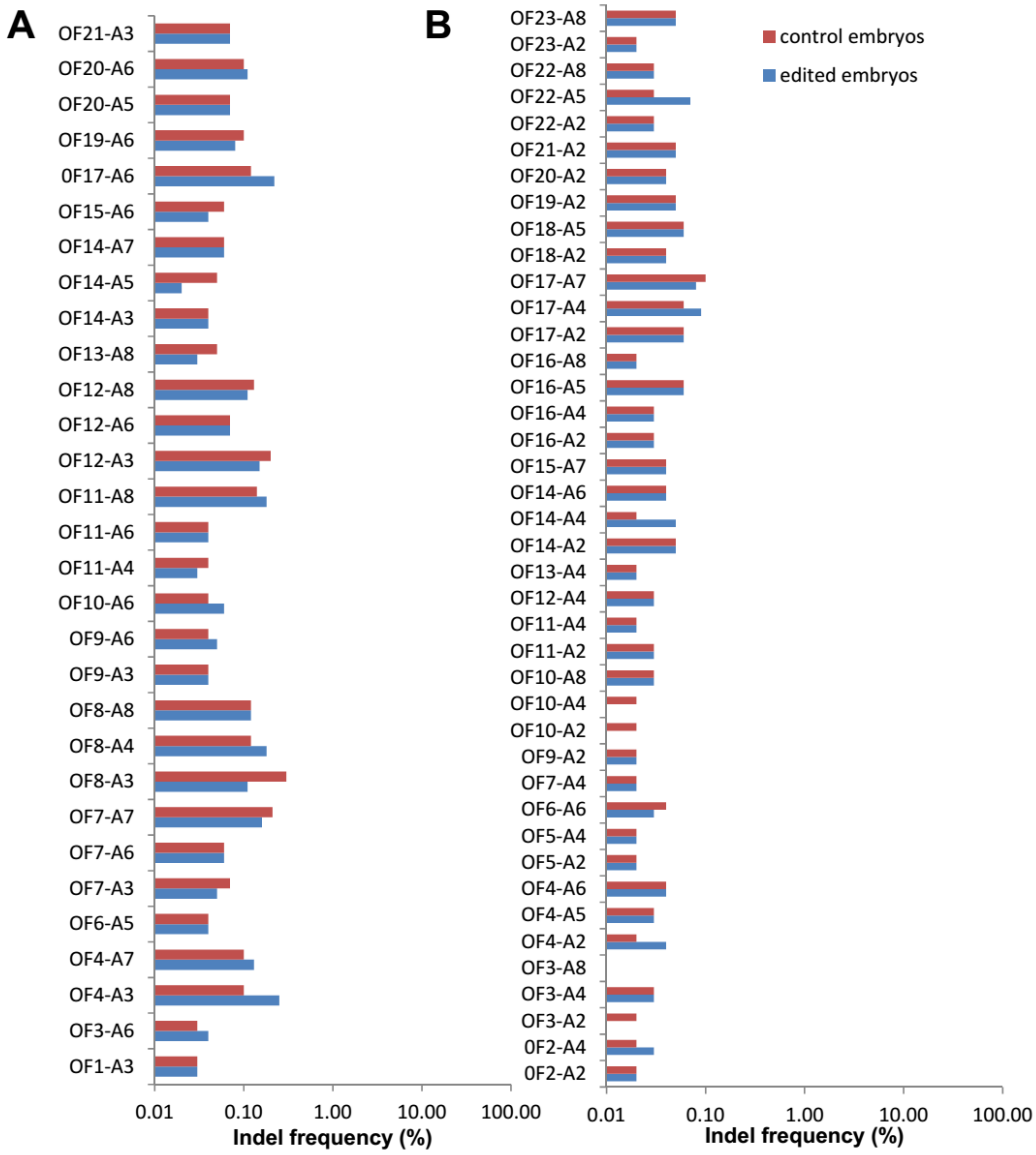
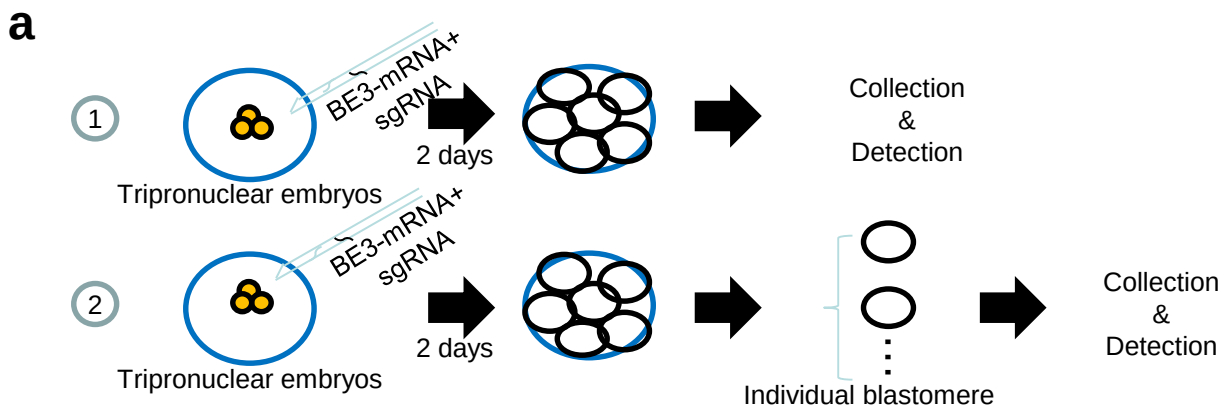
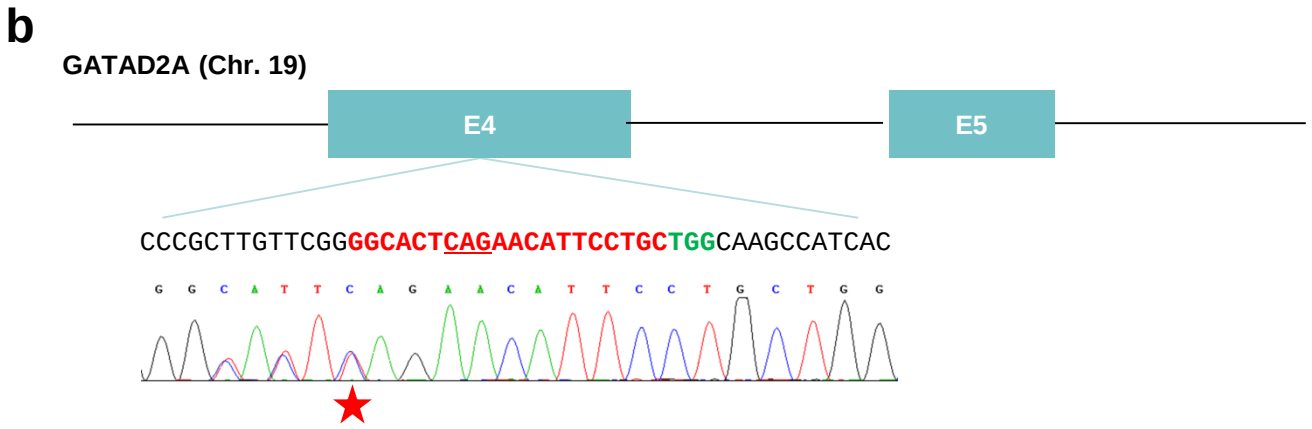


Manuscript under review

BE mediates isogenic genome editing *in vivo*





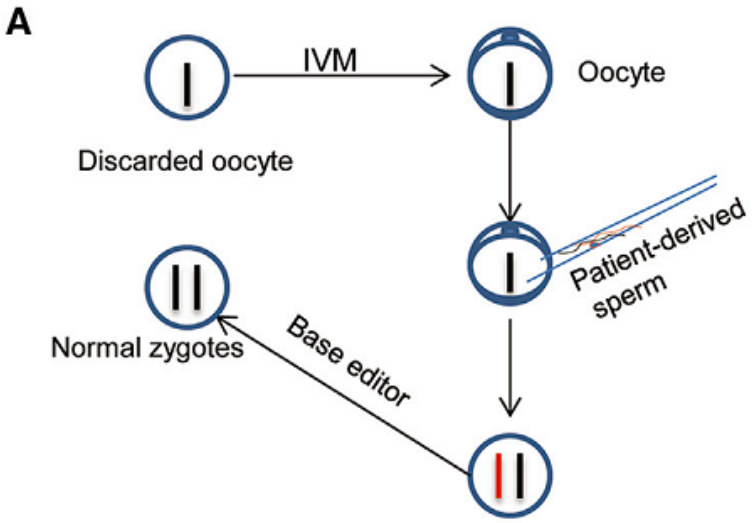
Unpublished data

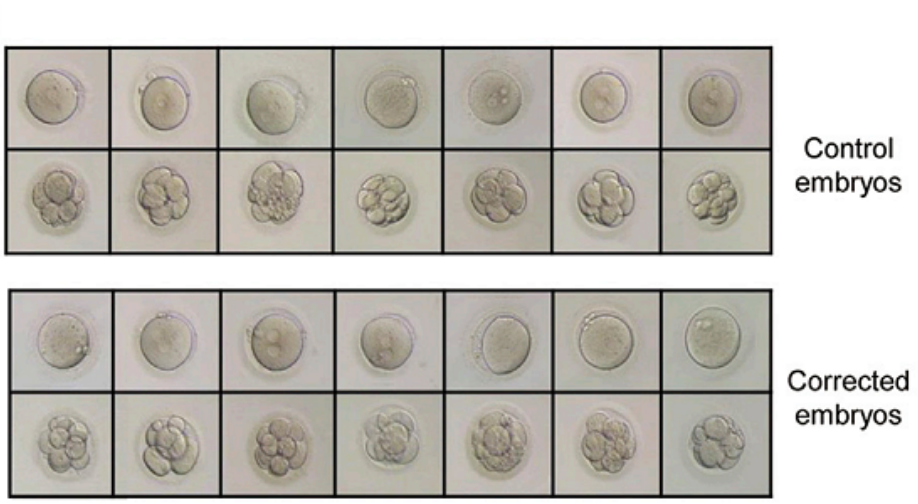
To correct pathogenic mutation of genetic disease

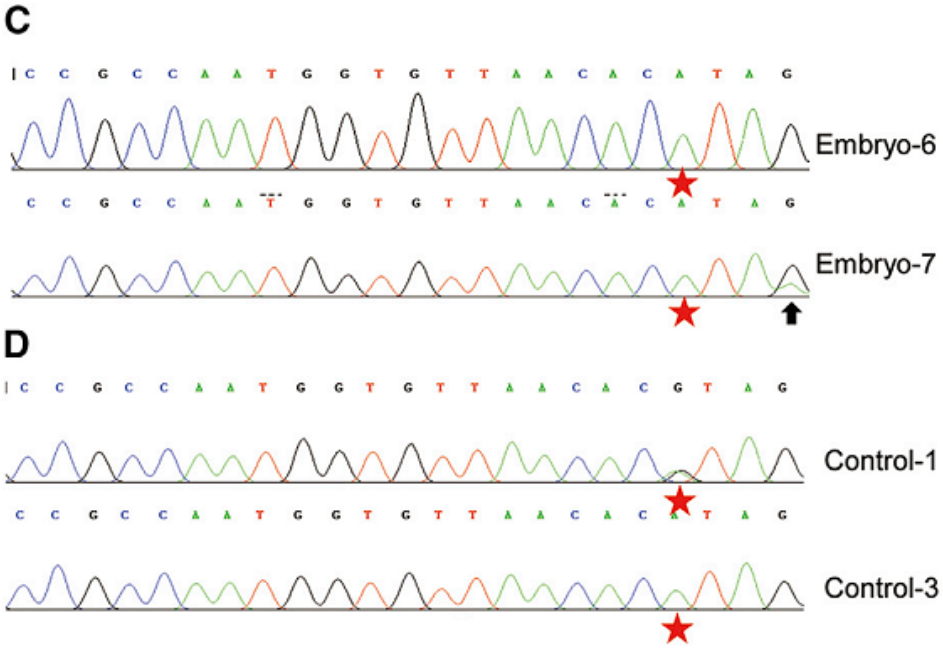
- There are an estimated 6,000~8,000 rare diseases. The rare diseases are estimated to collectively affect more than 200 million people worldwide;
- Three-quarters affect children, over half are life limiting;
- Over 80% of rare diseases are genetic in origin. Most are caused by defects in a single gene;
- Both the central nervous system (CNS), and peripheral organs and tissues are affected in most rare diseases;
- There are treatments for only 6% of rare diseases, of which fewer than 1% are curative;
- It was too expensive, at an average of $1 million per treatment.

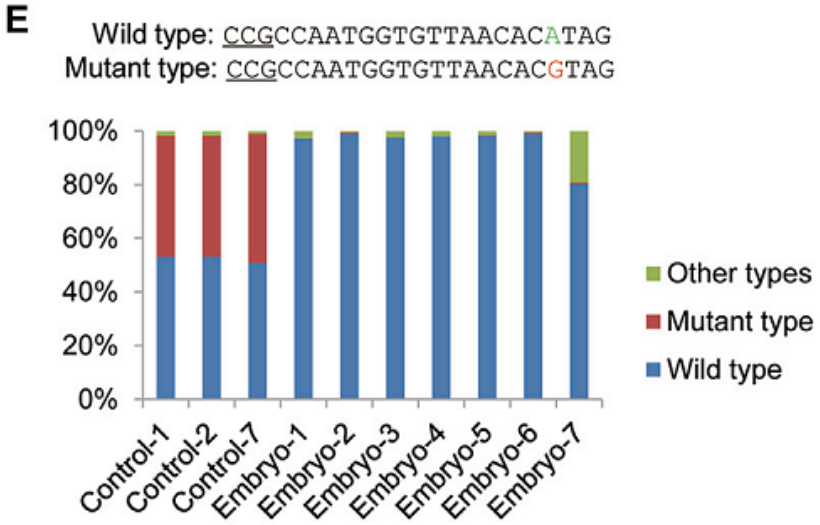
Orkin & Reilly. *Science* 2016, 352: 1059-1061.
Melnikova. *Nat Rev Drug Dis.* 2012, 11: 267-268.
Boycott & Ardgo. *Nat Rev Drug Dis.* 2018, 17: 151-153.

Correction of the MFS pathogenic FBN1^{T7498C} mutation by BE in tripronuclear human embryos



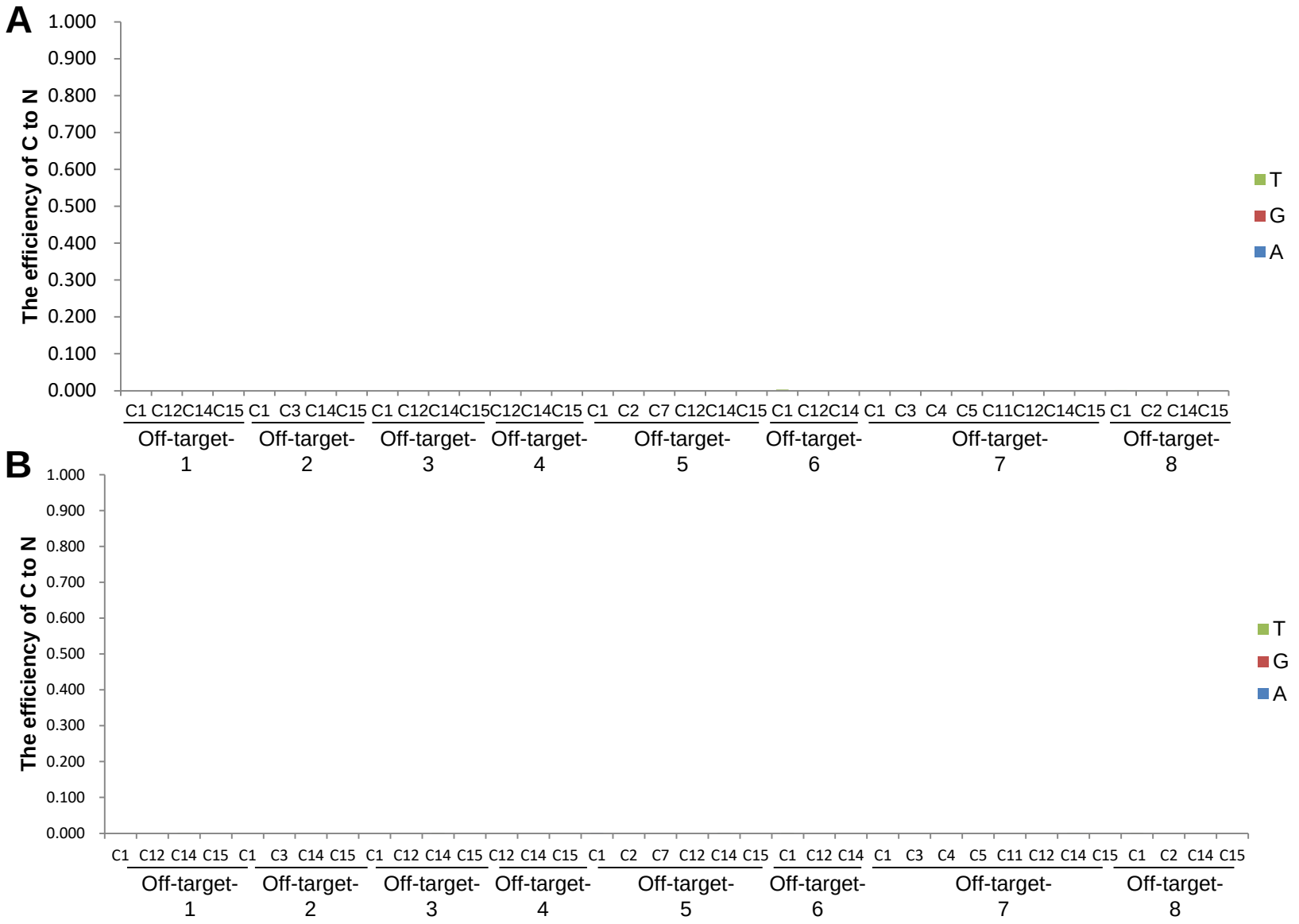






Zeng et al. *Mol Ther.* 2018, 26(11): 2631-2637.

No off-target



Zeng et al. *Mol Ther.* 2018, 26(11): 2631-2637.

BE: a precise strategy for correction of pathogenic mutations in a appropriate way

- Ethically: strict regulation ("really the last reasonable option")
- Technically: efficacy, safety, mosaicism
- Translational cooperation
- … …

Pei et al. *Cell Stem Cell* 2017, 21: 423-426.

http://www.sciencemag.org/news/2017/02/us-panel-gives-yellow-light-human-embryo-editing

- Easy to develop correction for rare genetic disease pathogenic mutations;
- Once intervention with good effect;
- Low cost.

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THANK YOU

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