INTERNATIONAL COMMISSION ON THE
CLINICAL USE OF HUMAN GERMLINE GENOME EDITING

London
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Genome Editing in human embryos

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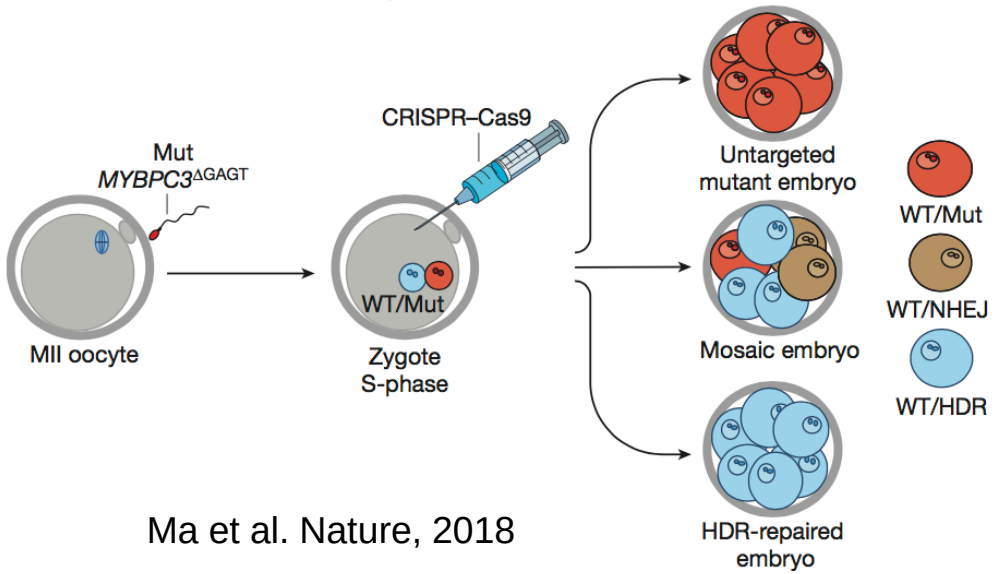
CAS Center for Excellence in Brain Science and Intelligence Technology (CEBSIT)

Gene Therapy Approaches

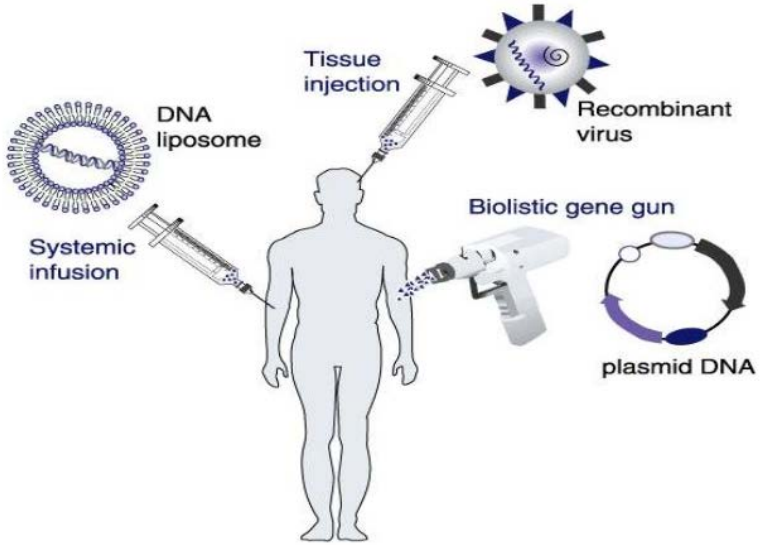
Germline Gene Therapy



Ma et al. Nature, 2018

- **Efficiency: >99%**
- **Off targets: None**

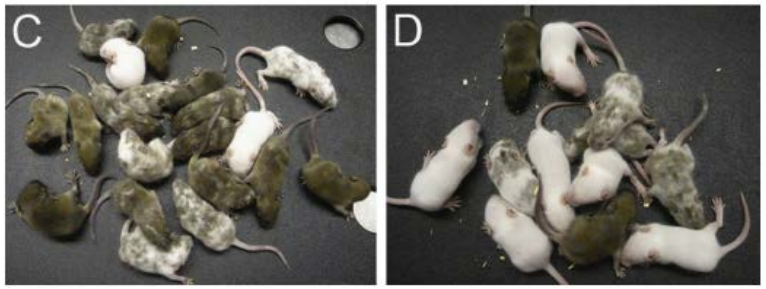
Somatic Gene Therapy



- **Efficiency: 1% - 100%**
- **Off targets: No oncogenic mutation**
- **Immunoresponse**
- **Deliver**

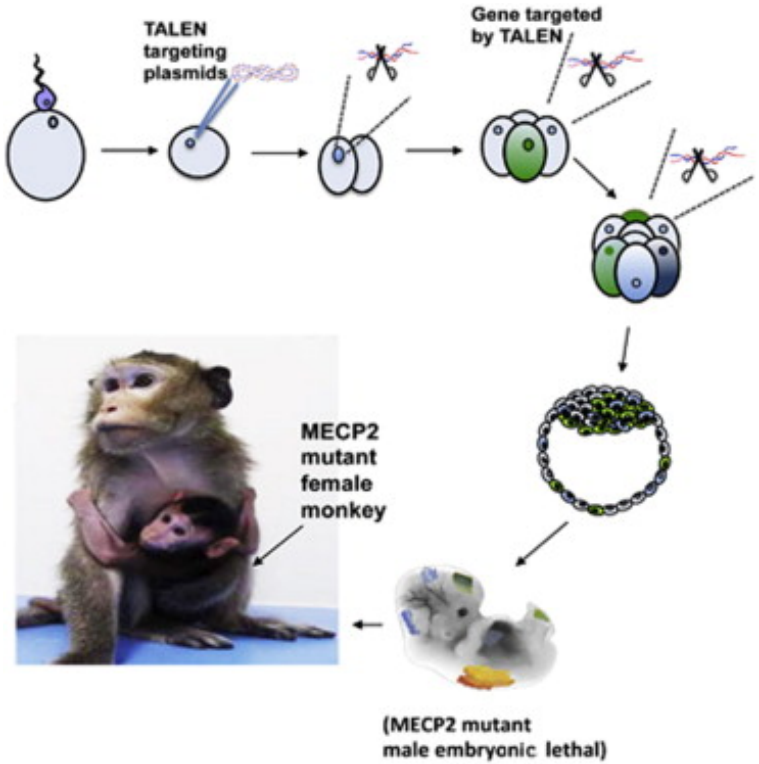
Mosaicism in CRISPR-generated mouse, monkey and human embryos

Mouse



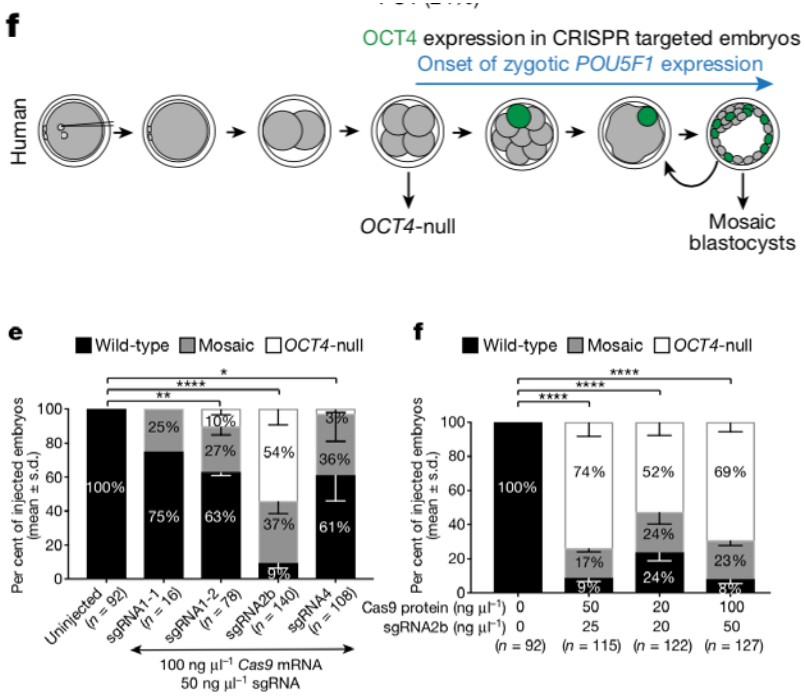
Yen, ST. et al. 2014

Monkey



Liu, HL. et al. 2014

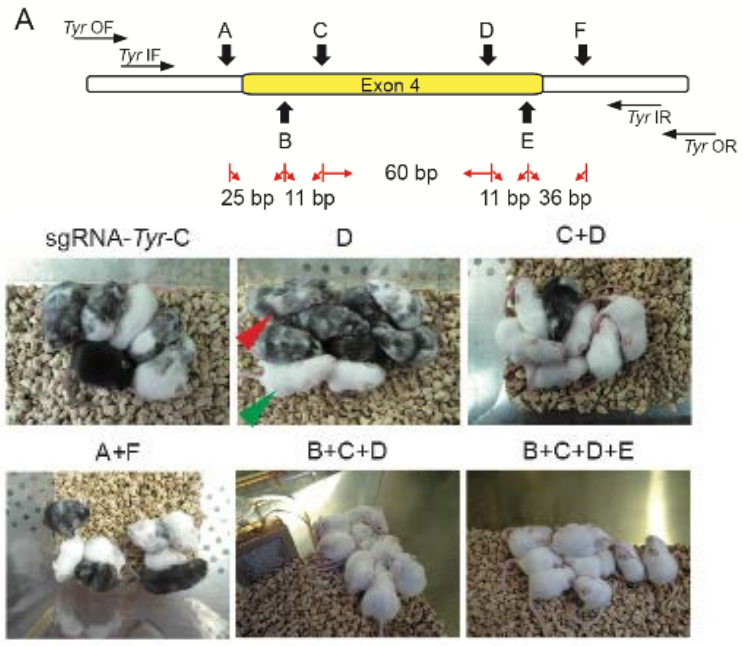
Human

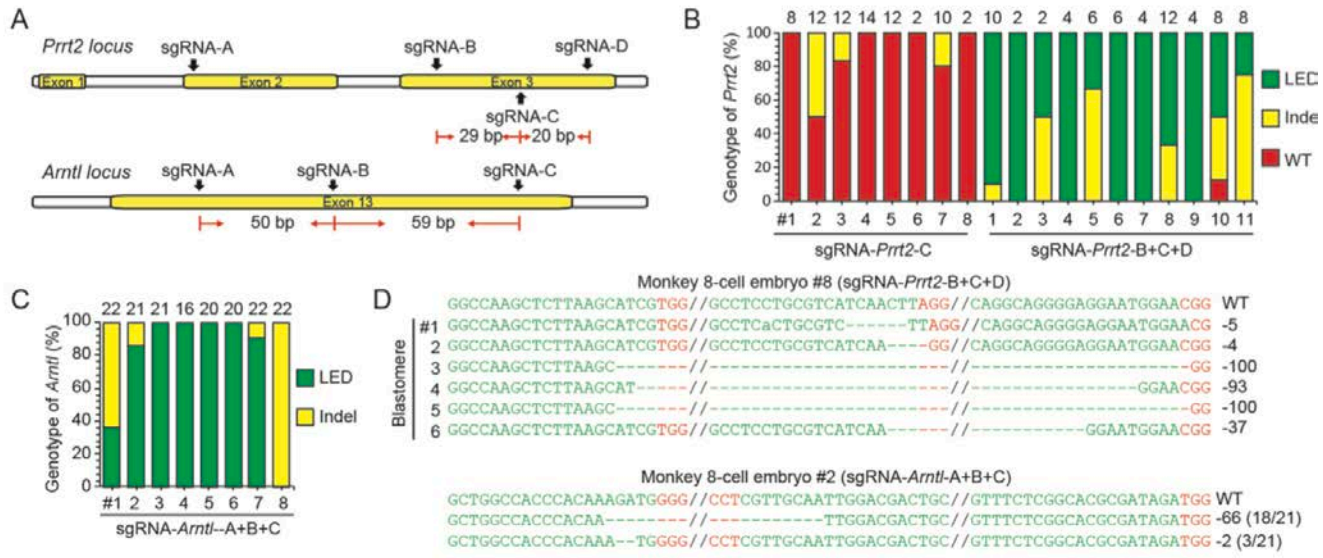


Fogarty, NM. Et al. 2017

Generation of complete knockout mice by C-CRISPR

Erwei Zuo





Zuo et al, ***Cell research***, 2017

Mouse embryos

Monkey embryos

- **Multiple DSBs** ⟹
  - **Chromosome rearrangement** ↑
  - **Chromosome deletion** ↑
  - **Off-target indels** ↑

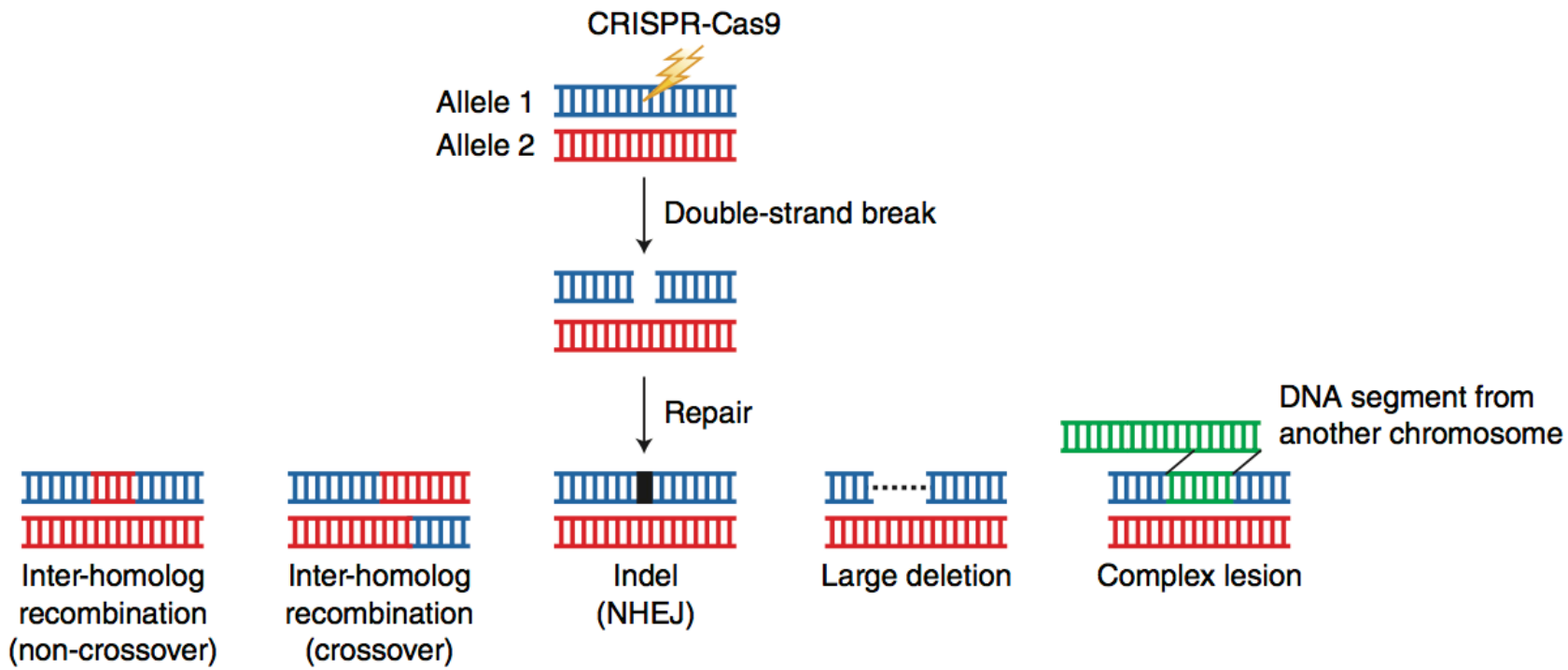
- **Mosaic genotype**
- **Embryonic development**

Unexpected CRISPR on-target effects

Hyunji Lee & Jin-Soo Kim

Cas9 can induce extensive on-target damage, including large deletions, inversions, and insertions.

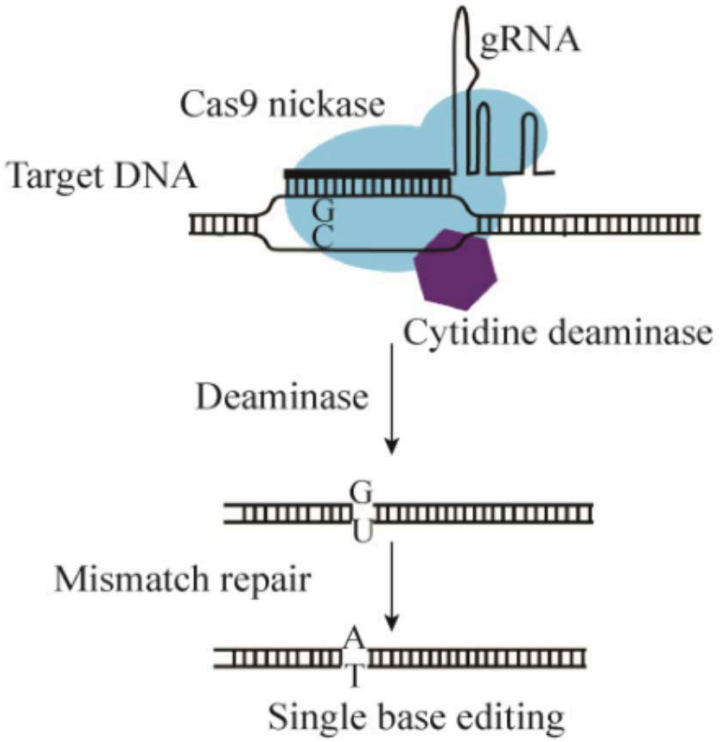
CRISPR–Cas9 genome editing induces megabase-scale chromosomal truncations

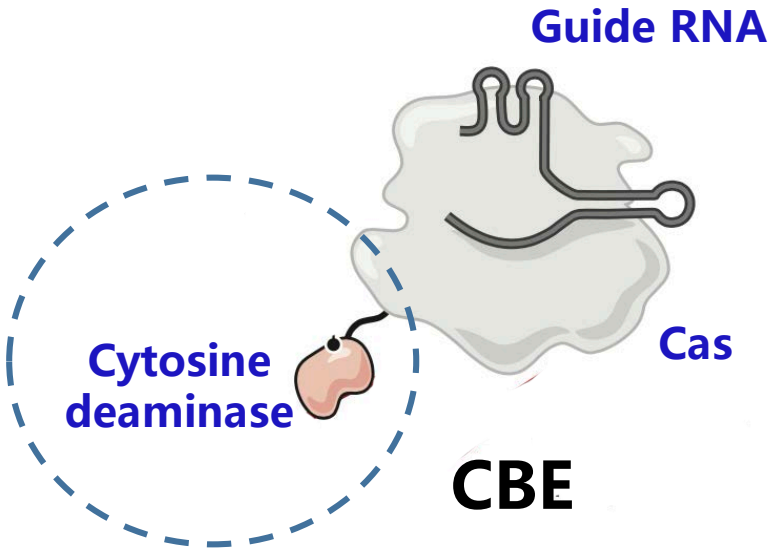


DSB -

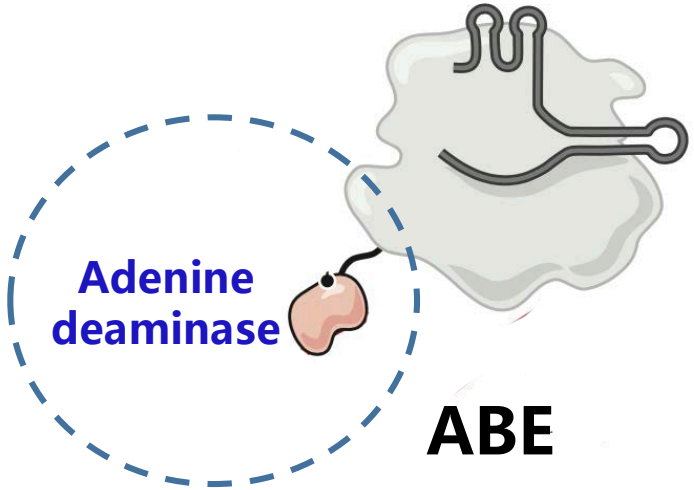
Template -

Base editing



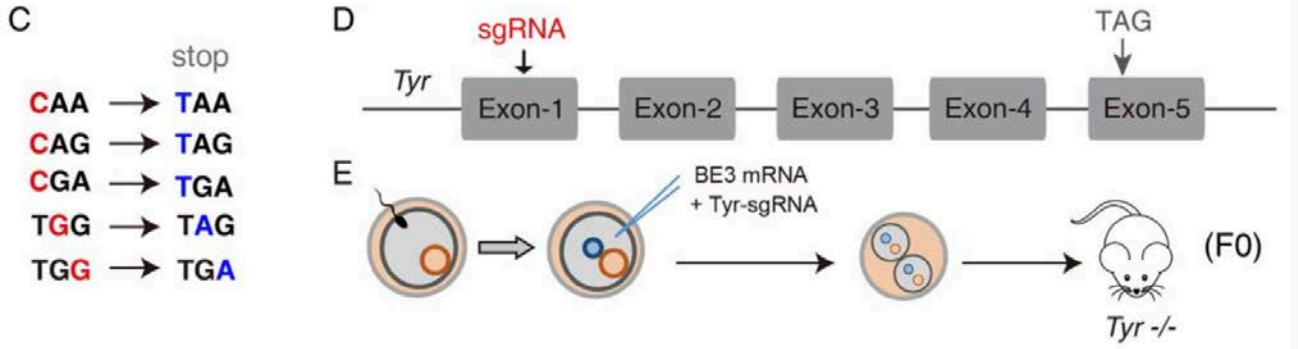


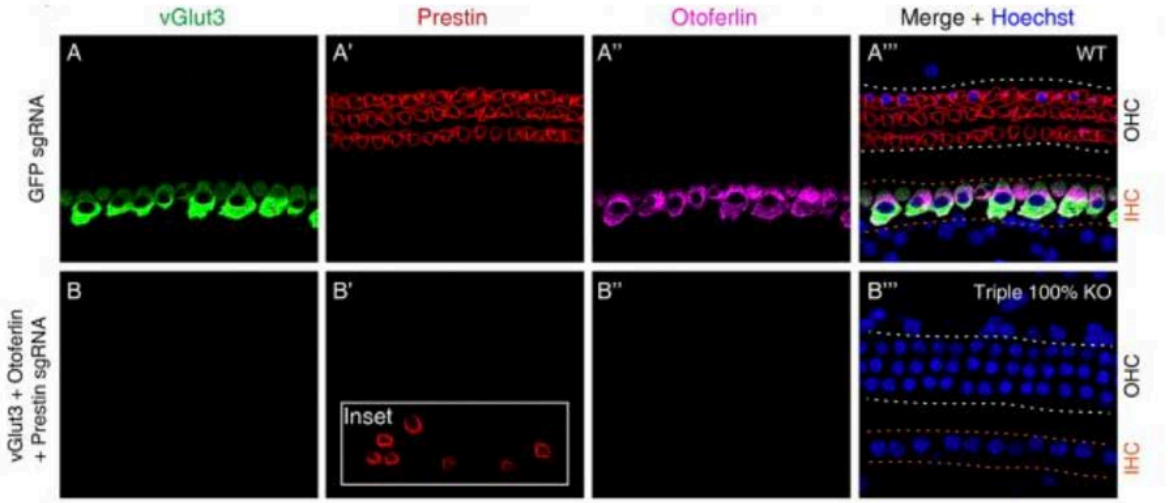
C to T or G to A conversions

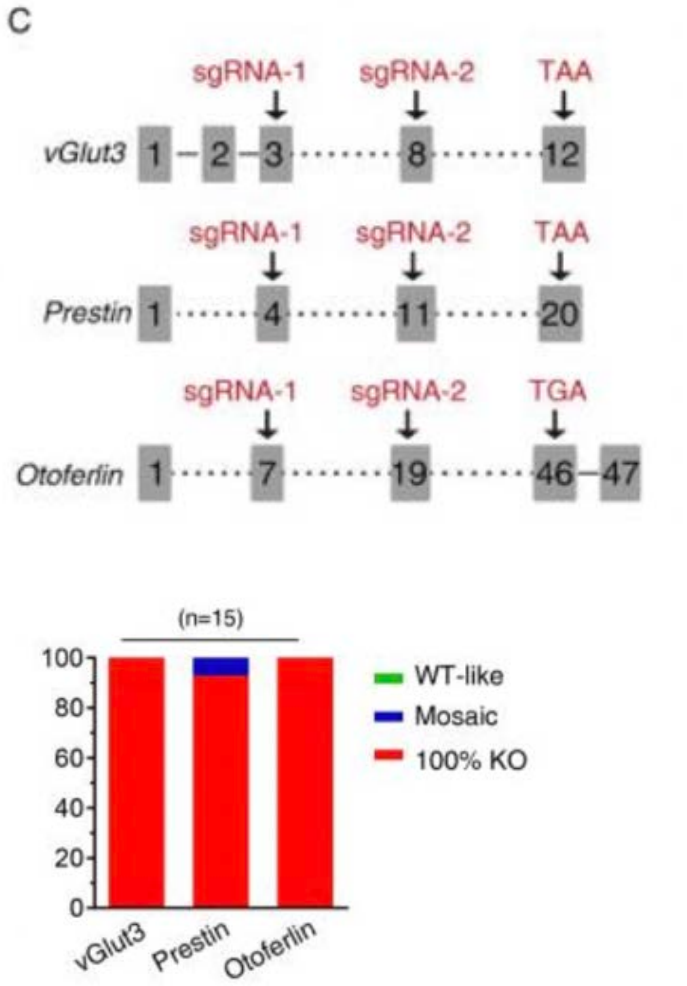


T to C or A to G conversions

Simultaneous inactivation of multiple genes by base editing



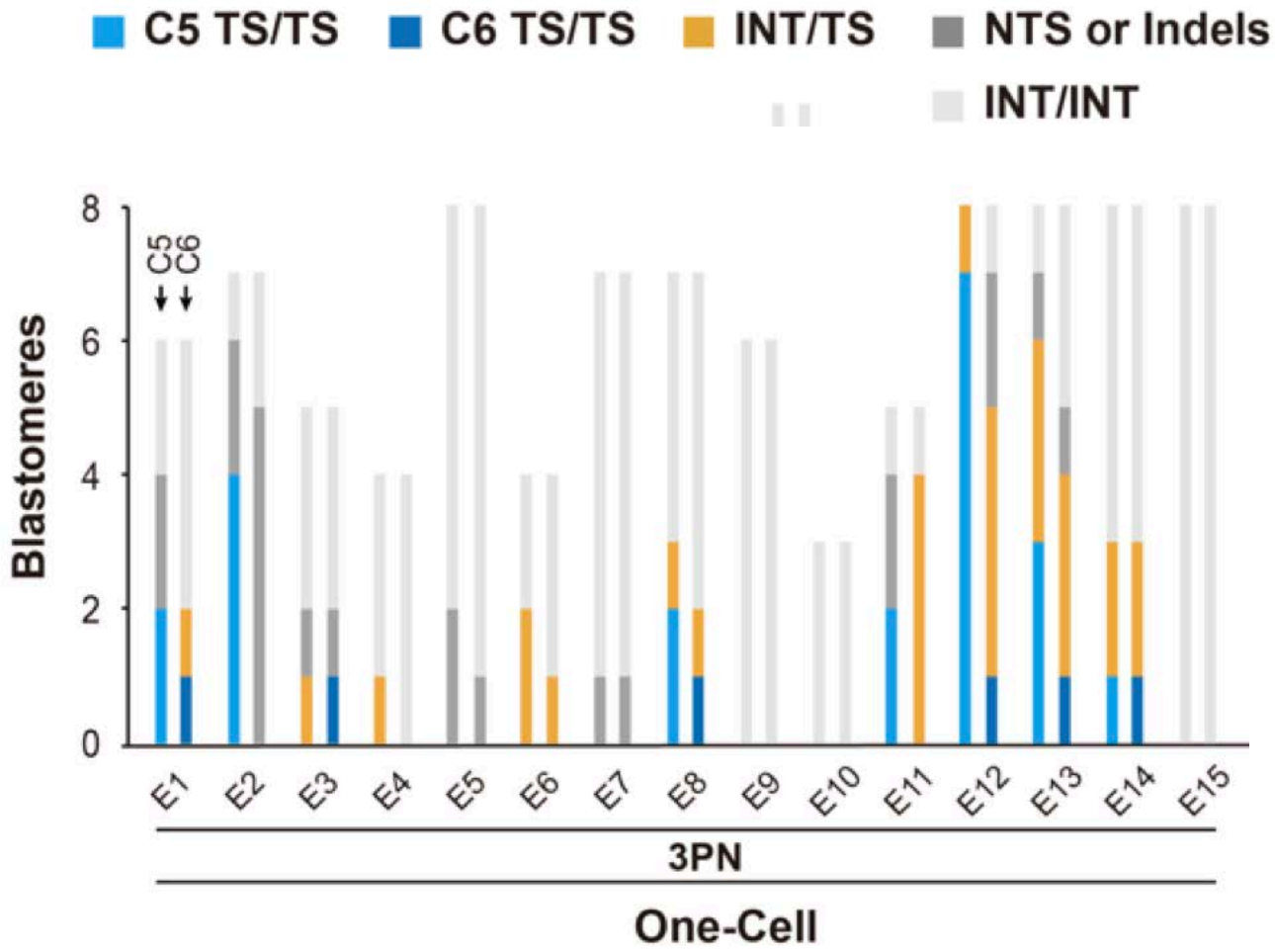




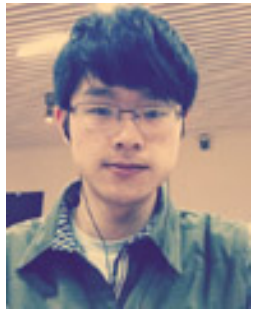
Zhang, H. et al., **Yang, H**[#] & Zhiyong Liu[#], ***Development***, 2018

High mosaicism in base-editing human embryos

BE3 *OCT4* GGCTAAGCTTC$_5$C$_6$AAGGCCCTCCTGGAGGGCCAGG



Changyang Zhou



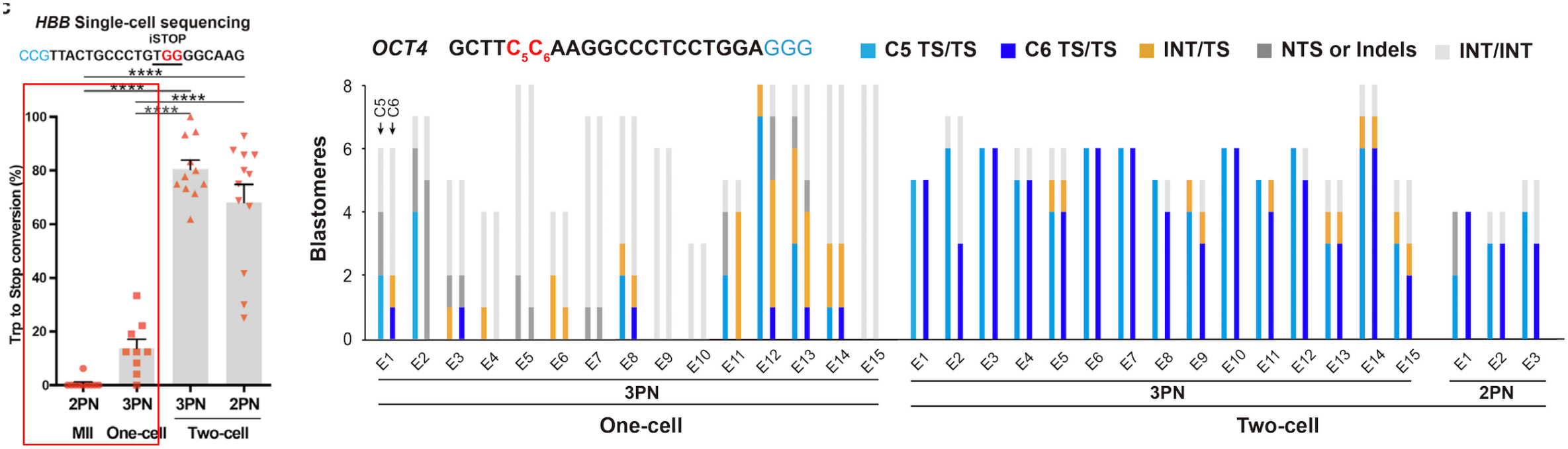
Cooperated with Zi-Jiang Chen

Injection of base editors at different embryonic stages

Changyang Zhou

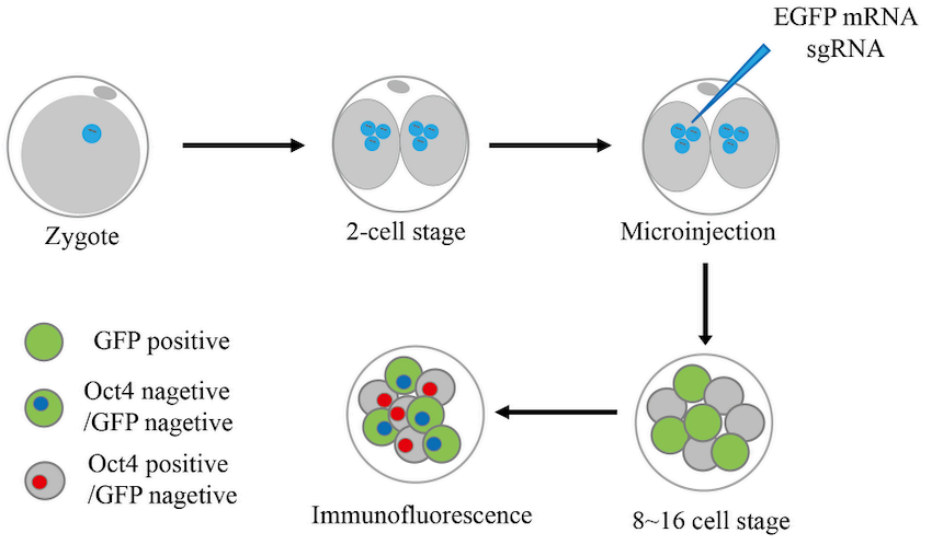
Zhang et al, ***Genome Biology***, 2019

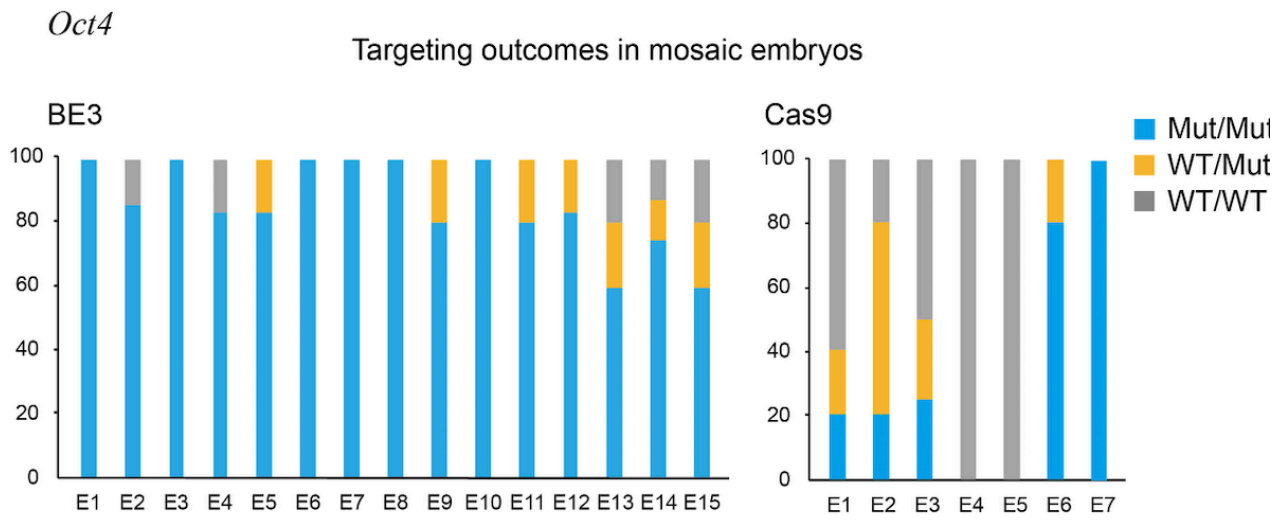
Improved base-editing efficiency in human cleaving embryos compared with MII oocytes and zygotes

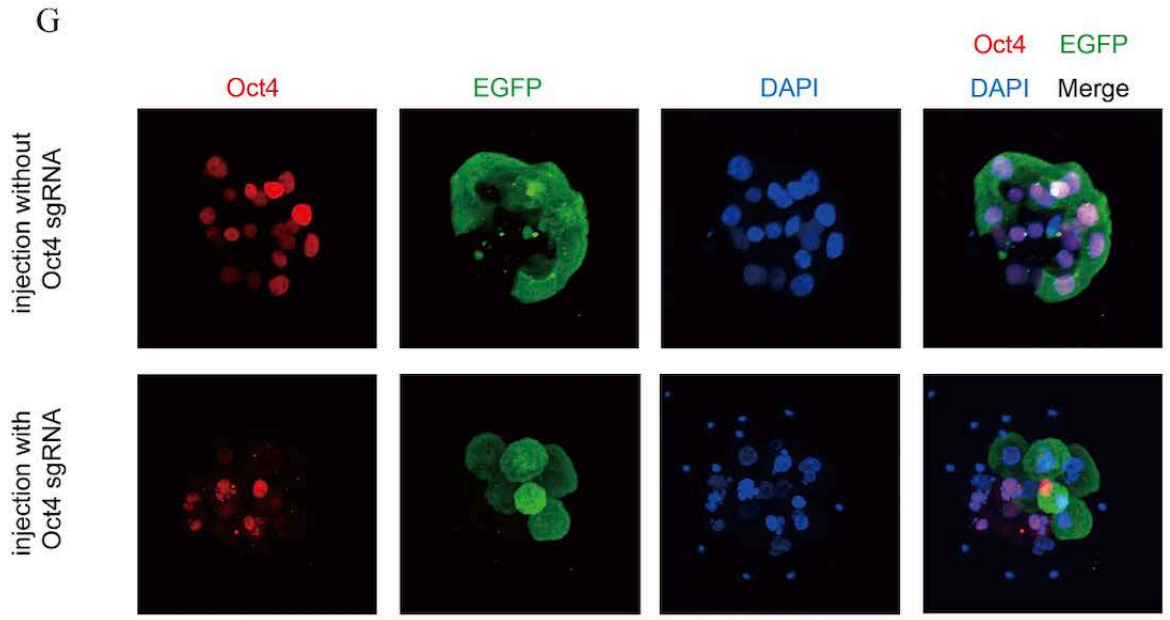


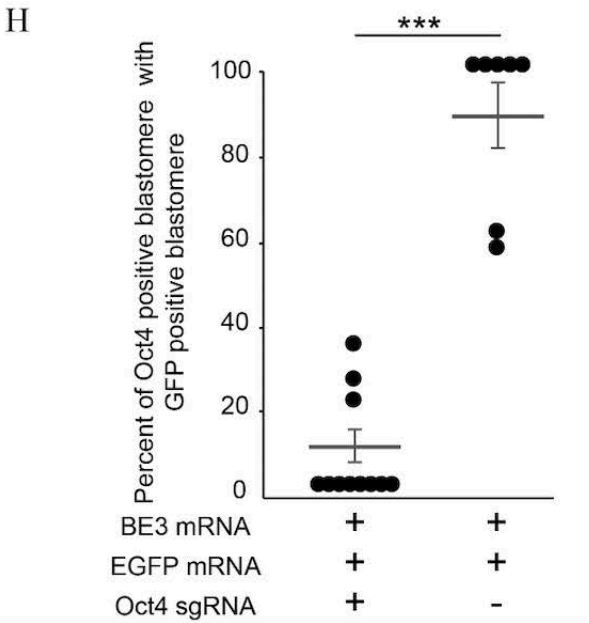
Zhang et al, ***Genome Biology***, 2019

Reveal OCT4 function by base editors



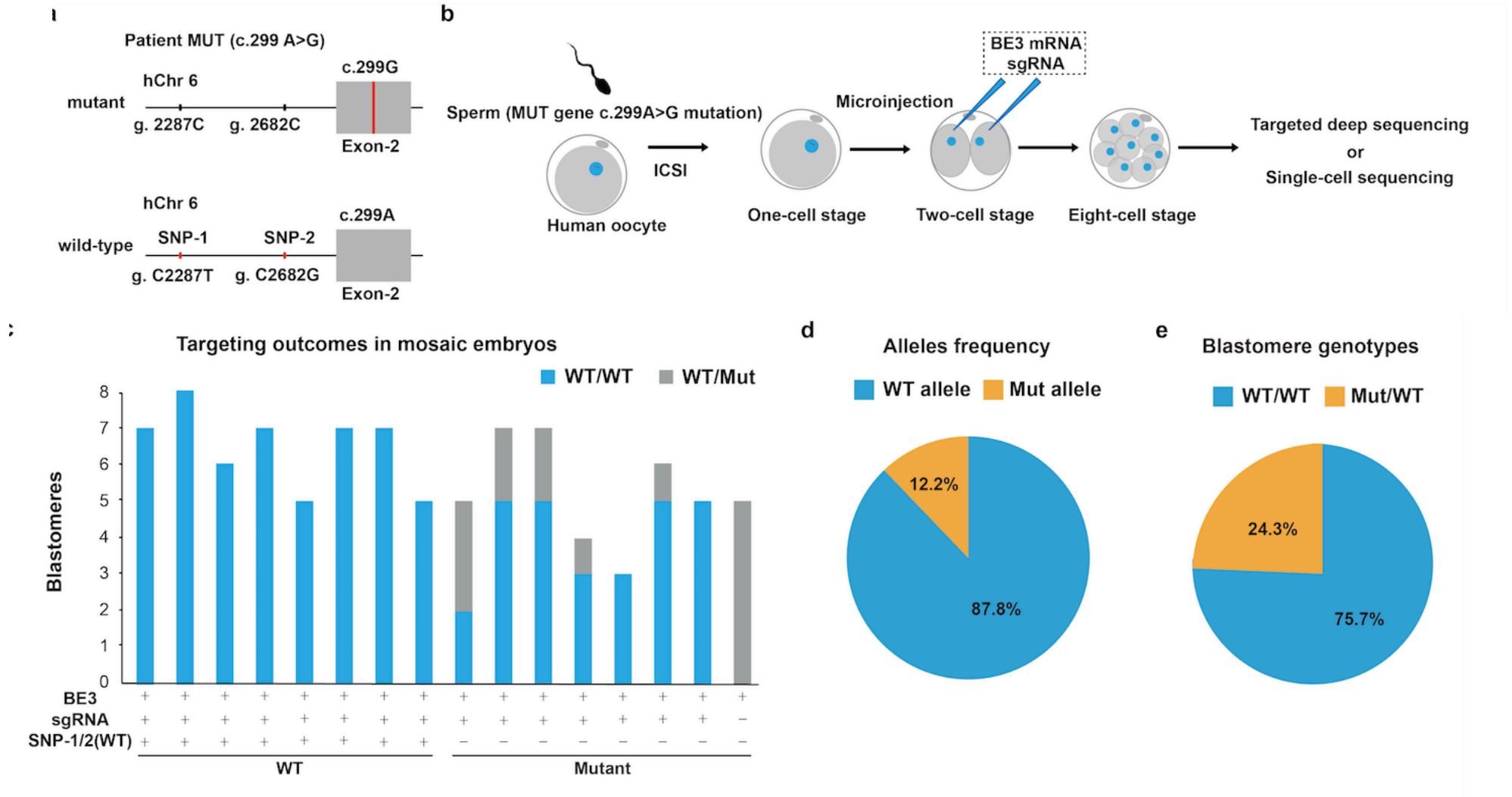






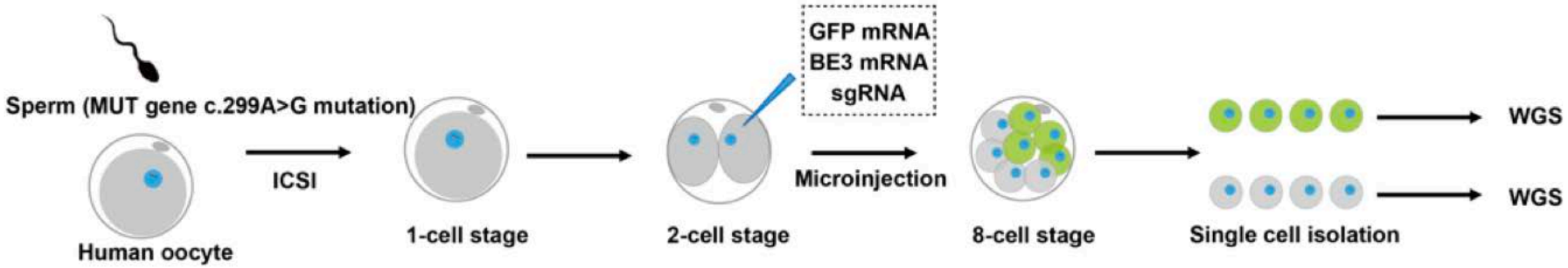
Unpublished data

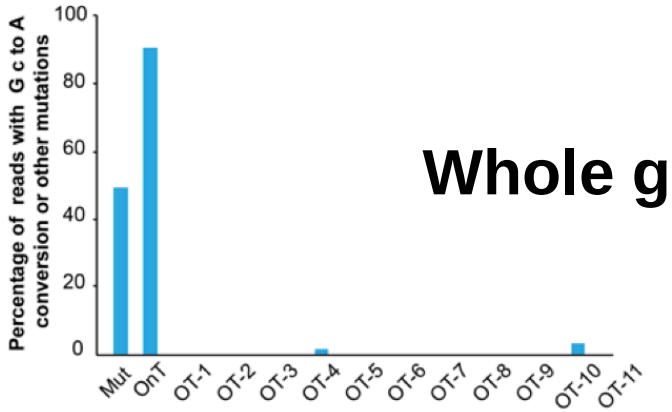
Correction of a pathogenic heterozygous mutation in human embryos with base editors



Zhang et al, ***Genome Biology***, 2019

Low off-target effects by base editing in human embryos





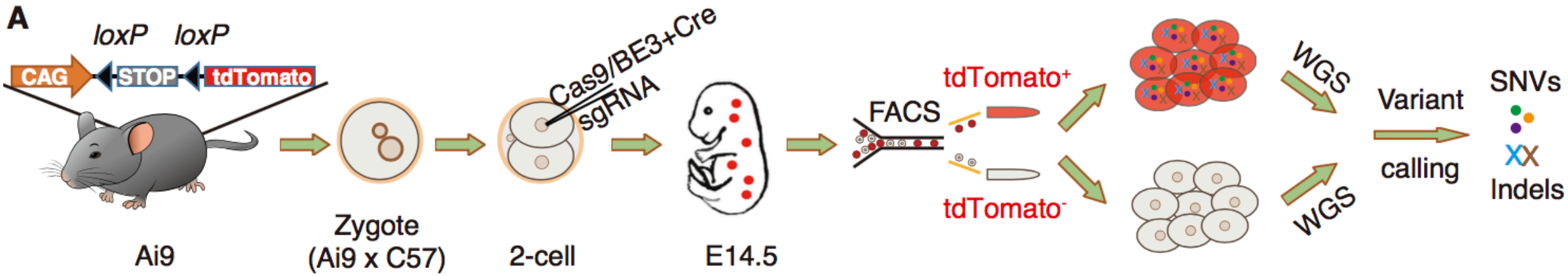
Whole genome amplification

Random off-targets

Sample name	E1		E2	
	SNPs	Indels	SNPs	Indels
Variants supported by three algorithms	15345	442	1616	10
Filter repeats and microsatellites	6966	192	729	3
exons	242	3	35	0
Protein coding	168	2	21	0
Variants in both samples	0	0	0	0
Predicted offtarget sites (10611)	0	0	0	0

Zhang et al, ***Genome Biology***, 2019

GOTI (Genome-wide Off-target analysis by Two-cell embryo Injection)



- The same genetic background
- Without genome amplification
- Genome wide and no bias
- **Single-cell resolution**
- Detection of various mutations

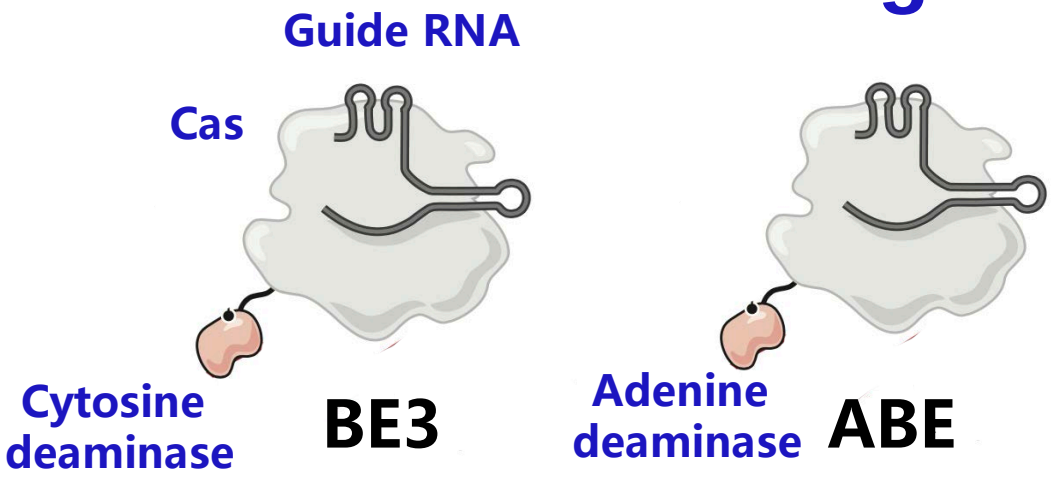
Erwei Zuo

Yidi Sun

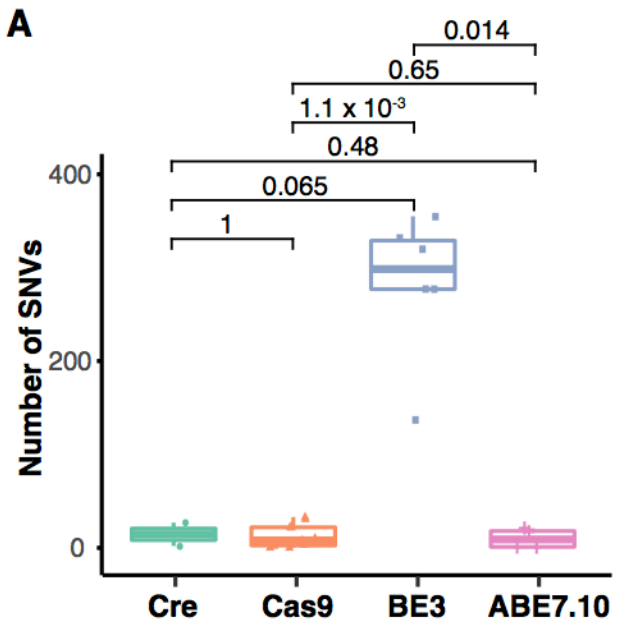
GOTI : 14 SNVs

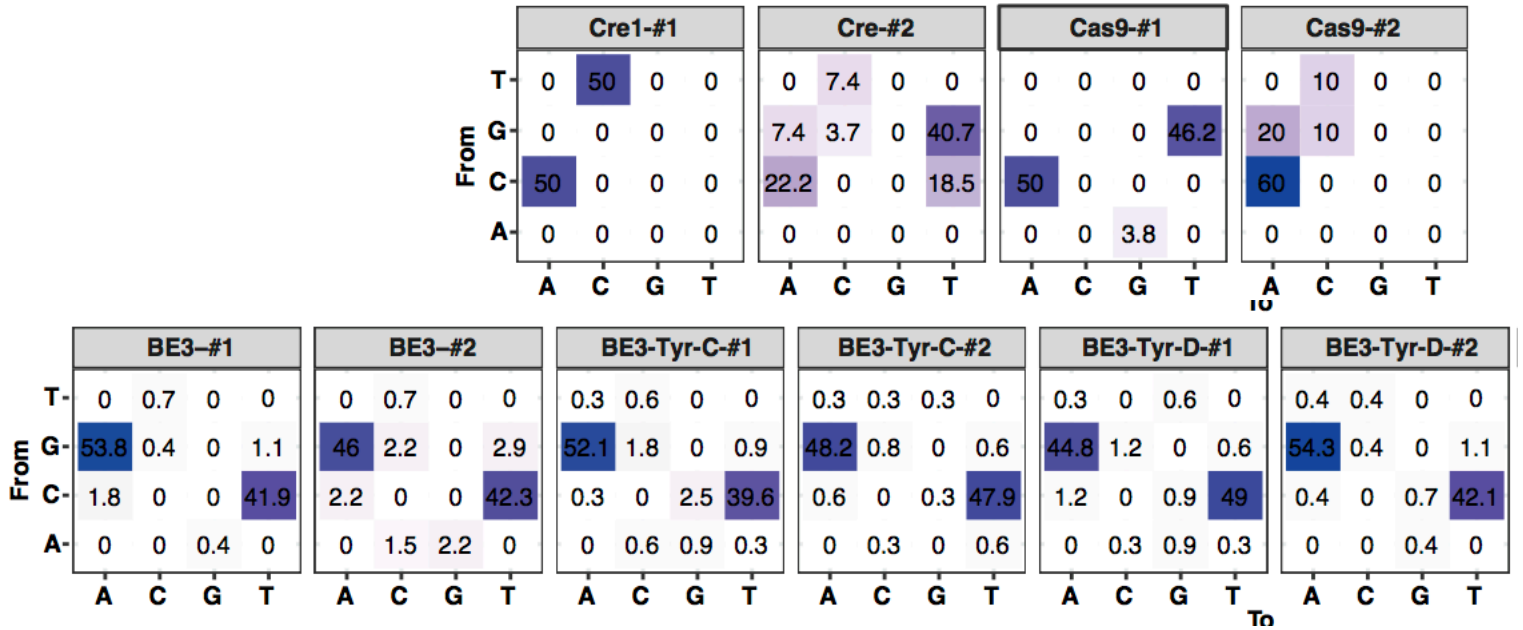
Traditional methods : 3706 SNVs

Cytosine base editor generates substantial off-target single nucleotide variants



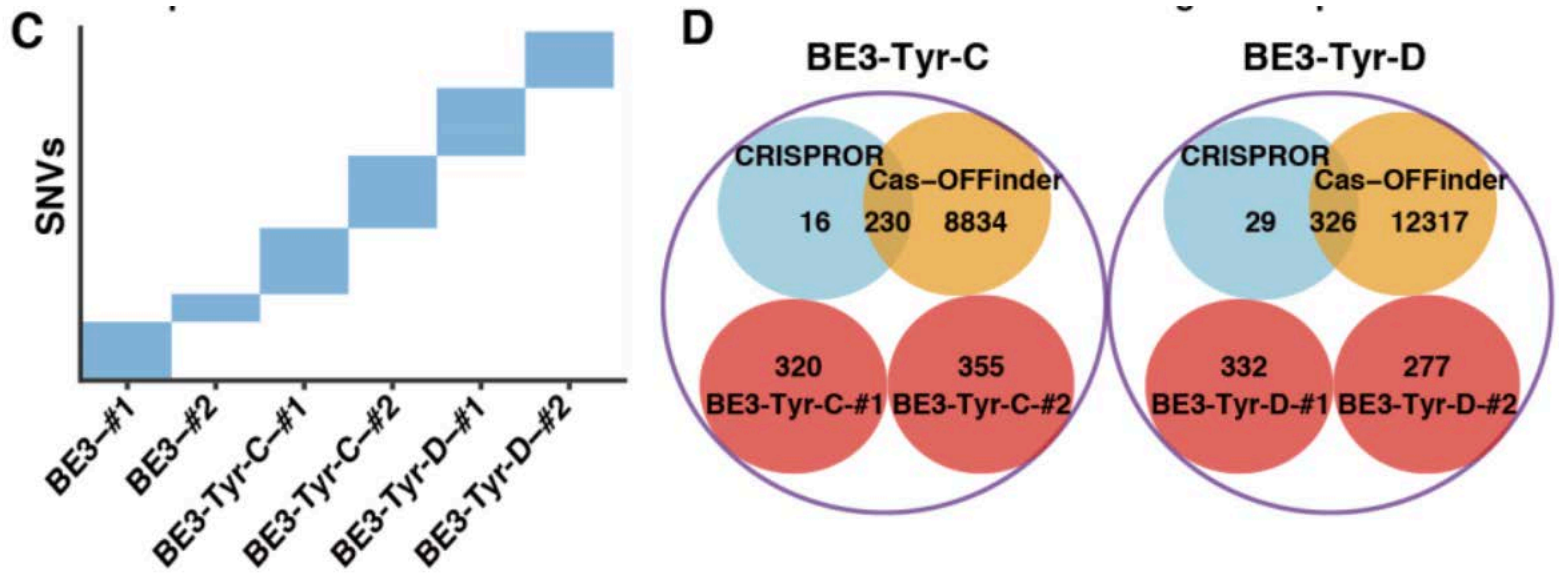
CBEs induced SNVs at more than 20-fold higher frequencies



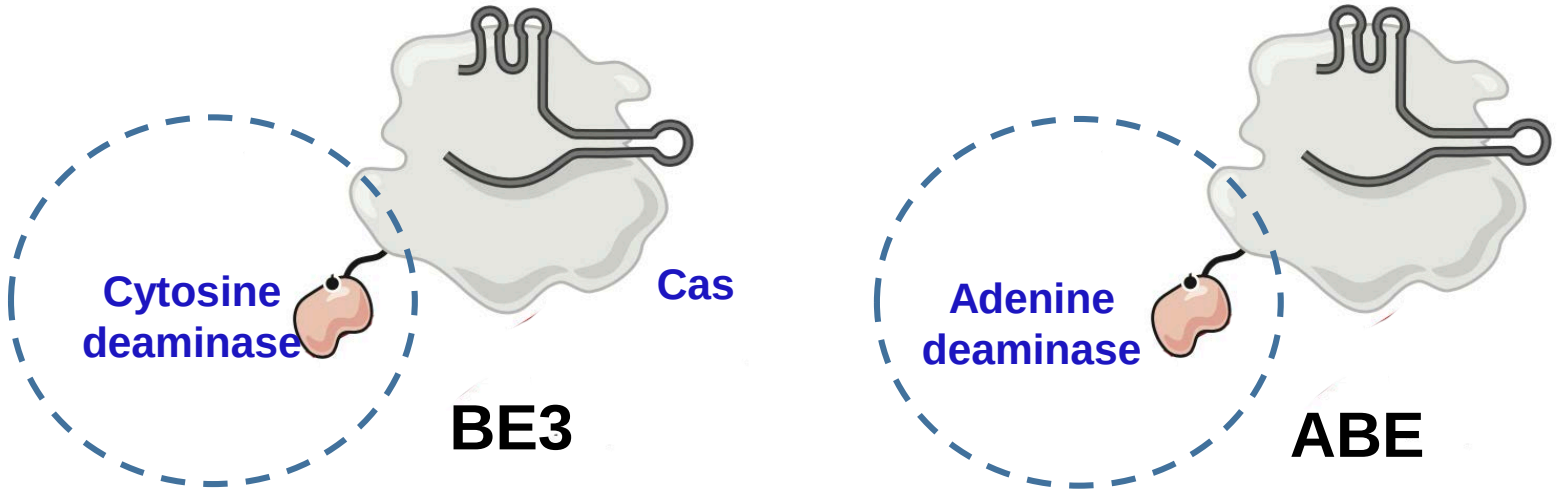


Zuo E et al., *Science*, 2019

BE3 off-target SNVs were sgRNA-independent



Zuo E et al., *Science*, 2019

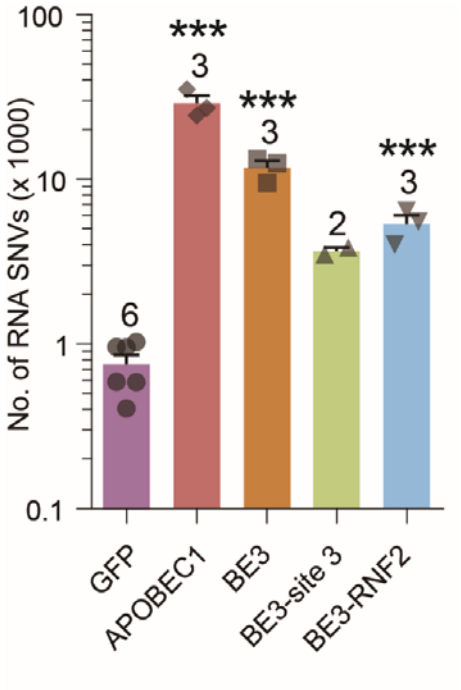


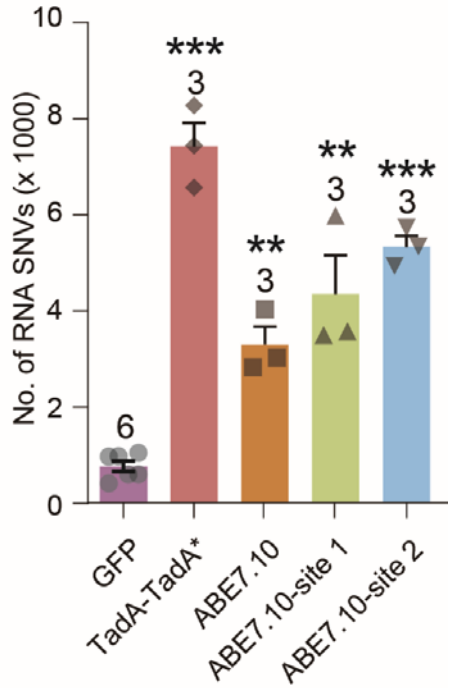
- **RNA off-targets?**
- **High fidelity base editors?**

DNA base editors generate substantial RNA off-targets

Changyang Zhou

Yidi Sun

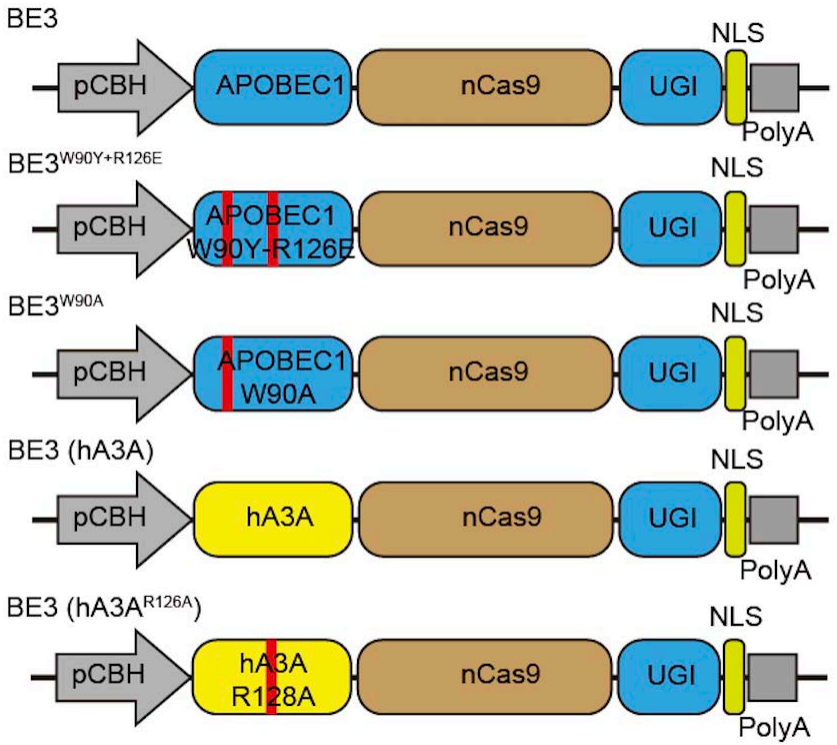


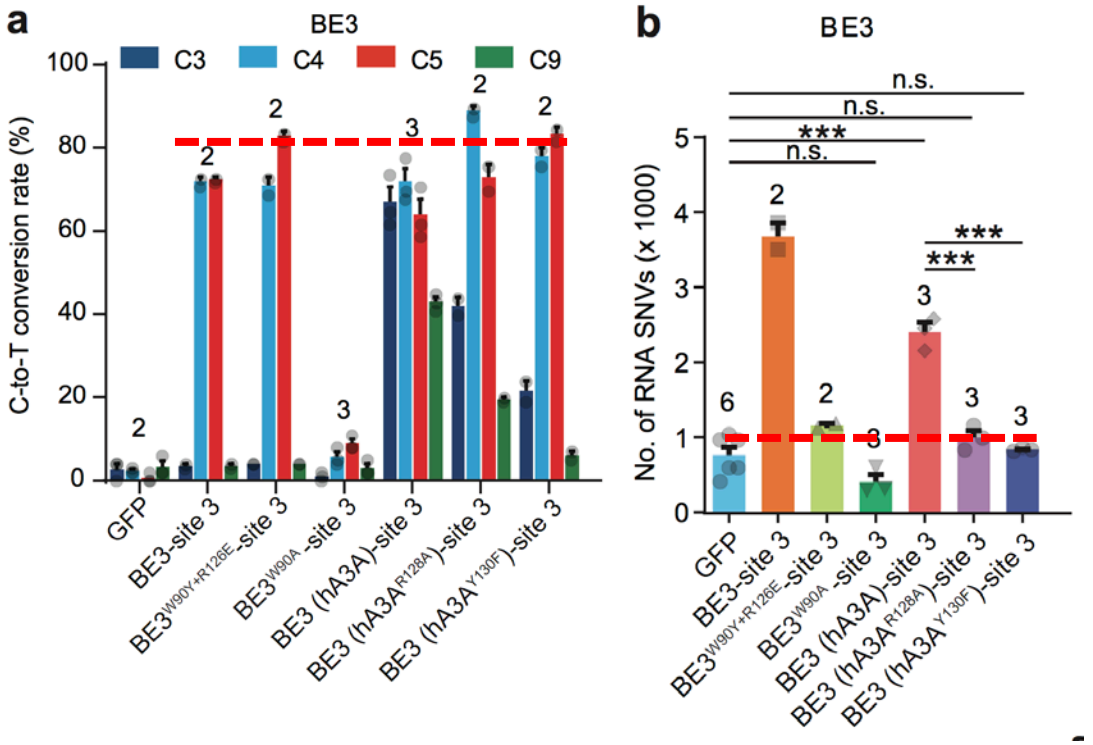


- **BE3 and ABE induce RNA SNVs**
- **RNA SNVs are caused by APOBEC1 and TadA**

Zhou et al, ***Nature***, 2019

Eliminate RNA off-targets by mutagenesis





On-target

Off-target

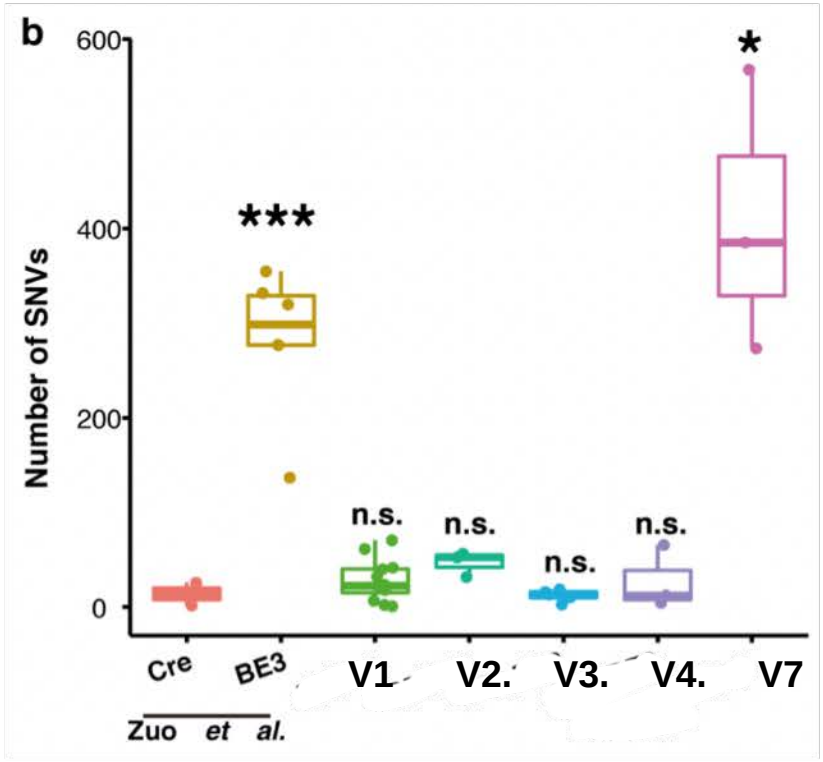
Destabilizing the RNA binding capacity of APOBEC1 and TadA

BEs with reduced off-target RNA SNVs while maintaining DNA on-target

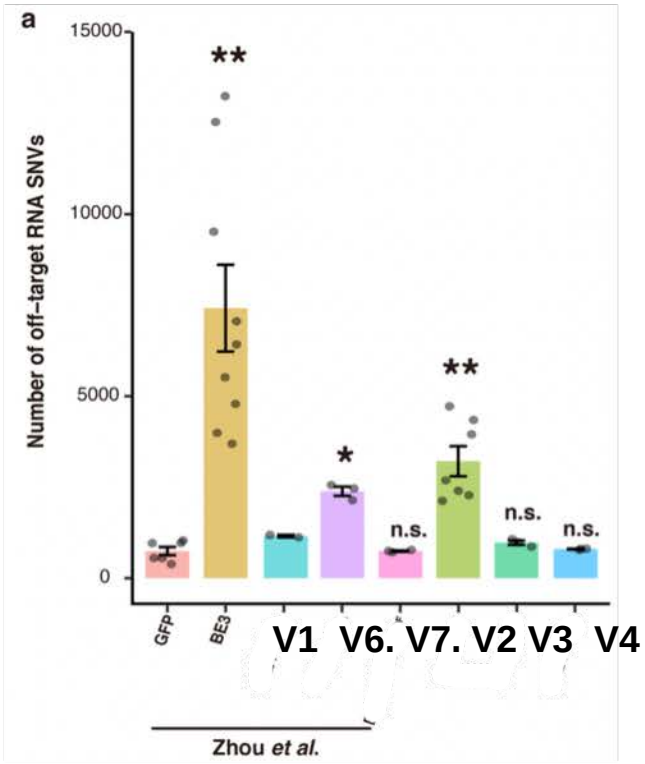
Zhou et al, ***Nature***, 2019

CBEs with no DNA and RNA off targets

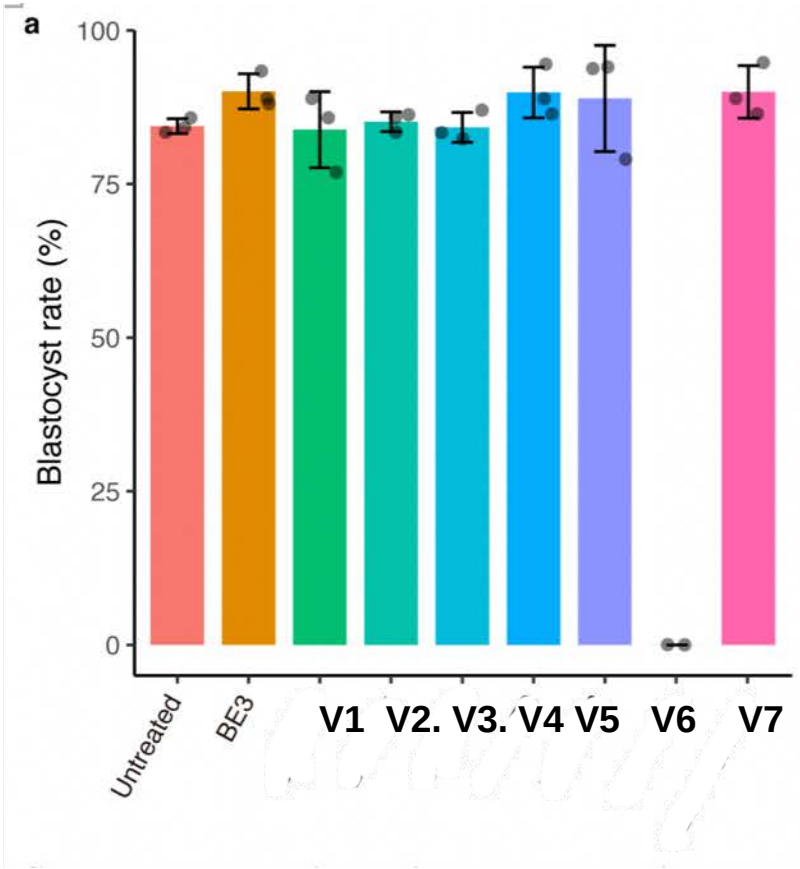
DNA off targets



RNA off targets



Embryonic development



Unpublished data

Off-target analysis in human embryos

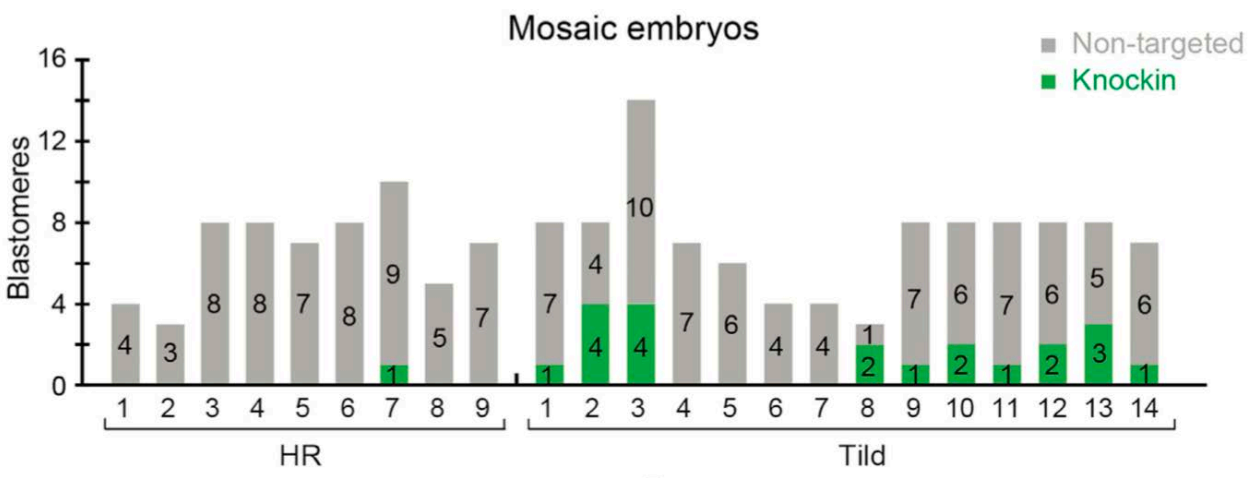
- **Combined methods**
  - **sgRNA-dependent** Cycle-Seq, Di-Genome, Chip-Seq …

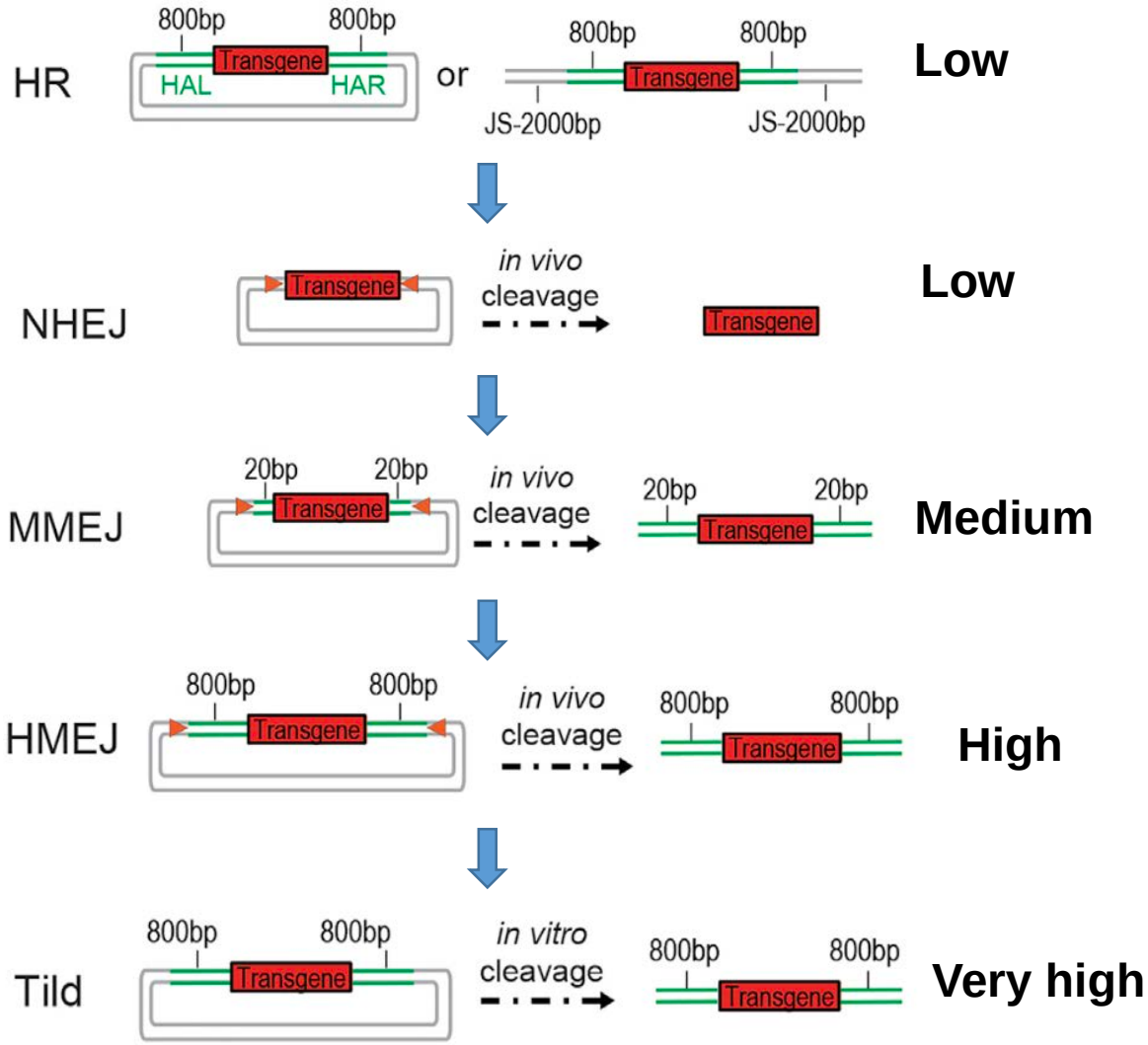
    High fidelity Cas9
  - **sgRNA-independent** GOTI, RNA-seq …

    High fidelity fused protein
- **New methods**
  - **Single-cell whole genome sequence**

Improve editing efficiency in human embryos

- **CRISPR-mediated HDR**
  - **Recombination factor RAD51 …**
  - **Cell cycle**
  - **Donor type ssDNA, dsDNA …**
  - **Unwanted alleles**





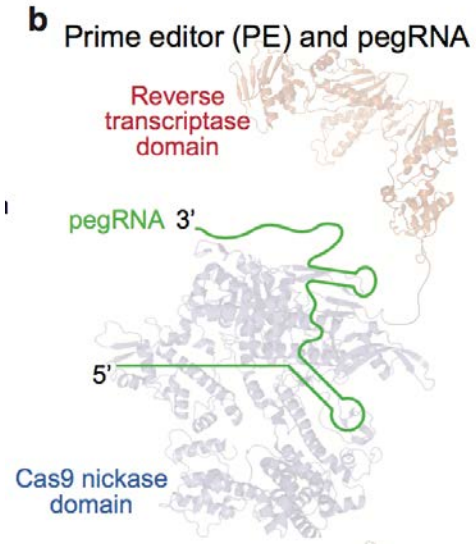
Yao, X. et al. **Yang H.**#, ***Dev Cell***, 2018

Improve editing efficiency in human embryos

➢ Base editing

- Different type of conversions A to C or A to T
- +1 or -1 editing
- Efficacy Different version of deaminases

➢ Prime Editing



- Different type of conversions
- No DSB and no template
- Off targets: reverse transcriptase
- Efficacy and indels

Non-human primates for human germline gene editing

➢ Embryonic development

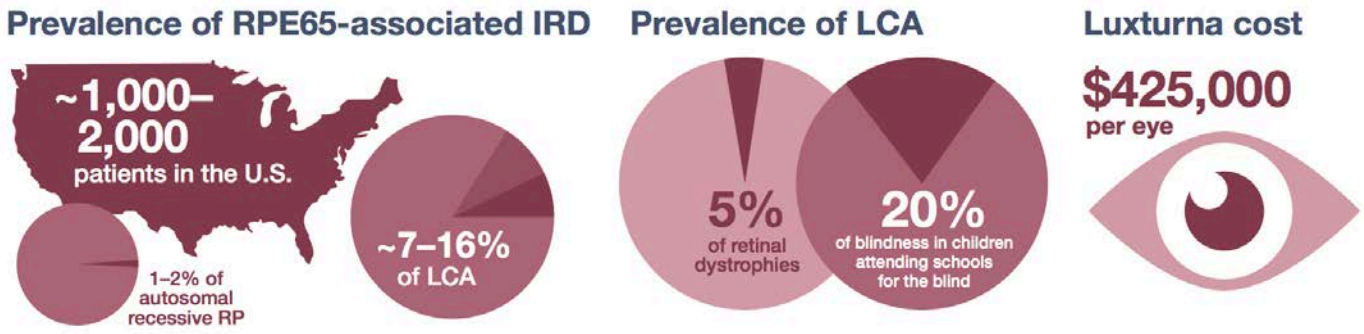
- **Tolerance for gene editing**

Gene	Injected embryos	Transferred embryos	Recipients	Pregnancy	Fetus	Live-birth
Leptin	163	88	47	6	6	2

➢ Off-target evaluation

- **Sequence similarity**

Somatic Gene Therapy Now, Germline Gene Therapy in Future?



30,000-50,000 patients in China

New born:	1/5000 – 1/10000
New add per year:	1500 – 2500, (SMA1, 555- 925)
Total:	30,000-50,000, (SMA1, 1110-1850)

Treatment for SMA

Drug	Type	Targeting	Administration	Phase	Price	Sponser
Spinraza	ASO	SMN2 mRNA splicing	intrathecal	FDA approved	$125,000-per-vial	Biogen, Ionis
Zolgensma (AVXS-101)	scAAV	SMN1 replace	intravenous	FDA approved	$2,100,000	AveXis, Inc (Novartis)
Branaplam	small molecular	SMN2 mRNA splicing	oral	Phase 1/2		Novartis Pharmaceuticals
RG7916	small molecular	SMN2 mRNA splicing	oral	Phase 2/3		Hoffmann-La Roche

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ShanghaiTech

Pengyu Huang

Shangdong Hospital

Zi-jiang Chen

Meiling Zhang

CAS-MPG

Yixue Li

Yidi Sun

All lab members

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