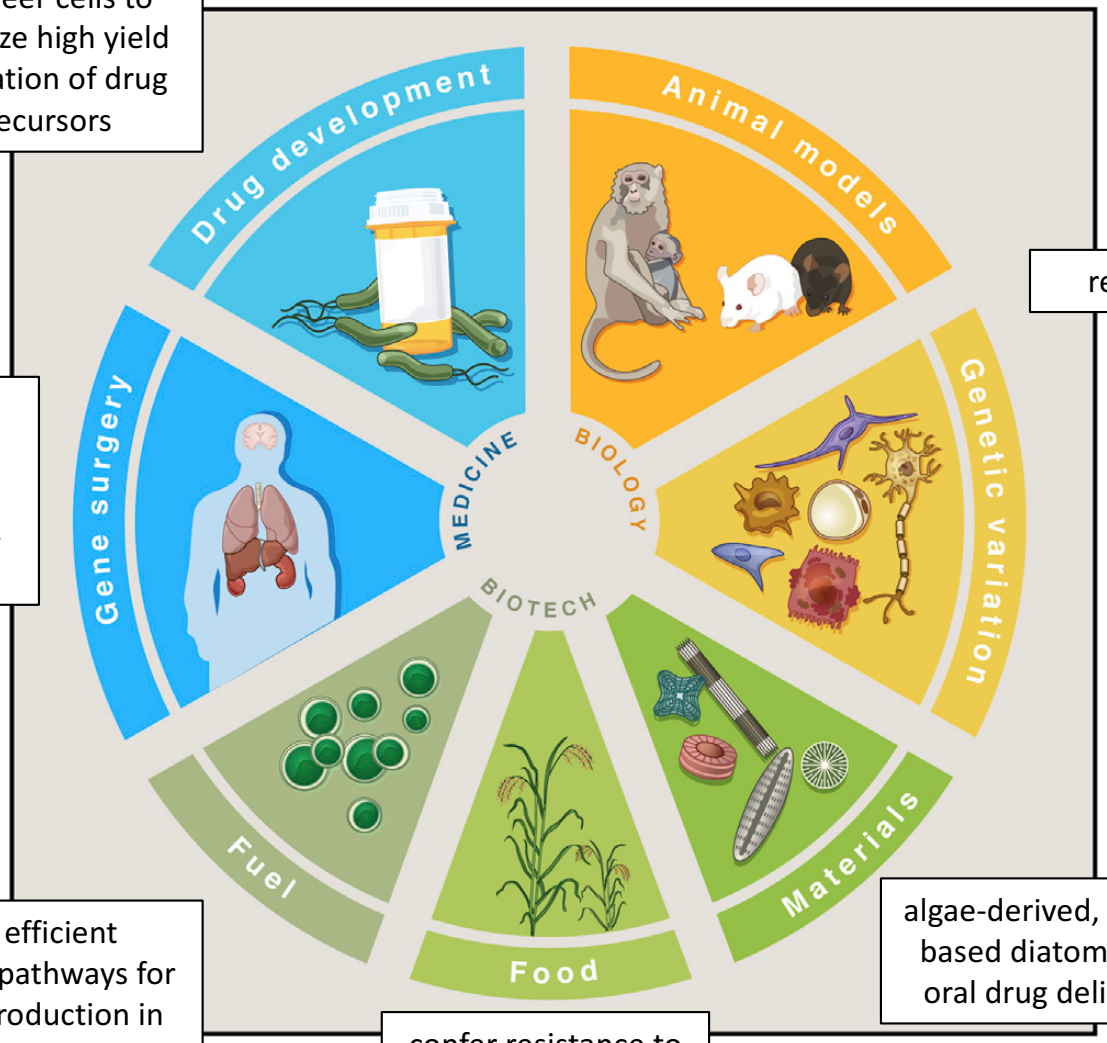
genome engineering

processes of making targeted modifications to the genome,
its contexts (e.g., epigenetic marks), or its outputs (e.g., transcripts).

Genome engineering technologies are enabling a broad range of applications

engineer cells to optimize high yield generation of drug precursors



reverse genetic

direct *in vivo* correction of genetic or epigenetic defects in somatic tissue

create efficient metabolic pathways for ethanol production in algae or corn

confer resistance to environmental deprivation or pathogenic infection

algae-derived, silica-based diatoms for oral drug delivery

(Hsu et al., Cell, 2014)

gene therapy

transfer of genetic material to a patient to treat a disease

AIM:

2.0 gene therapy

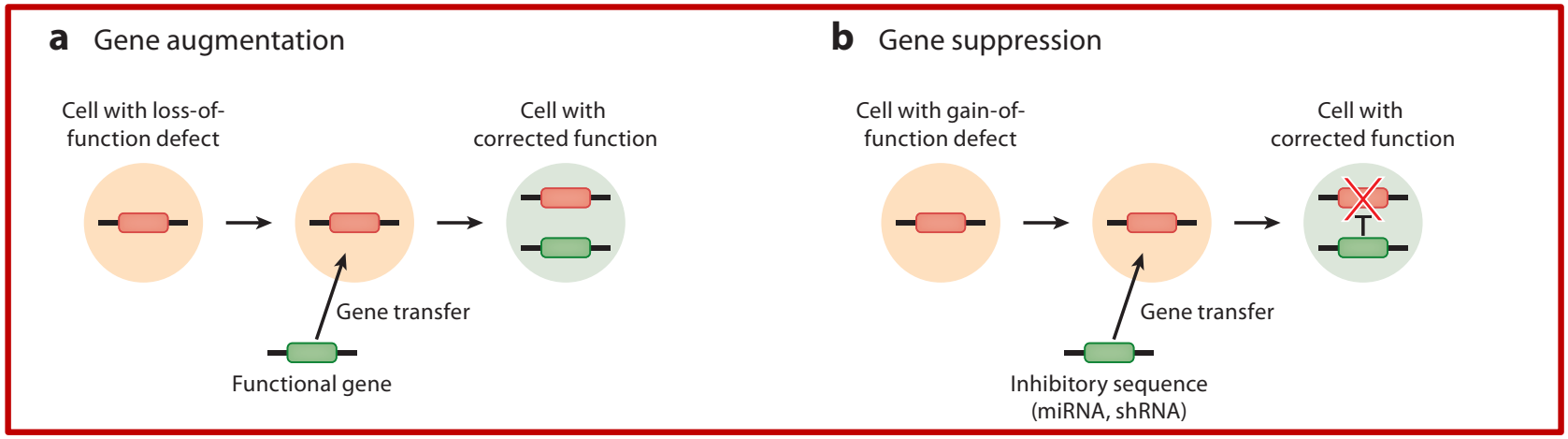
long- term expression of the transferred gene high enough to be therapeutic

3.0 gene therapy

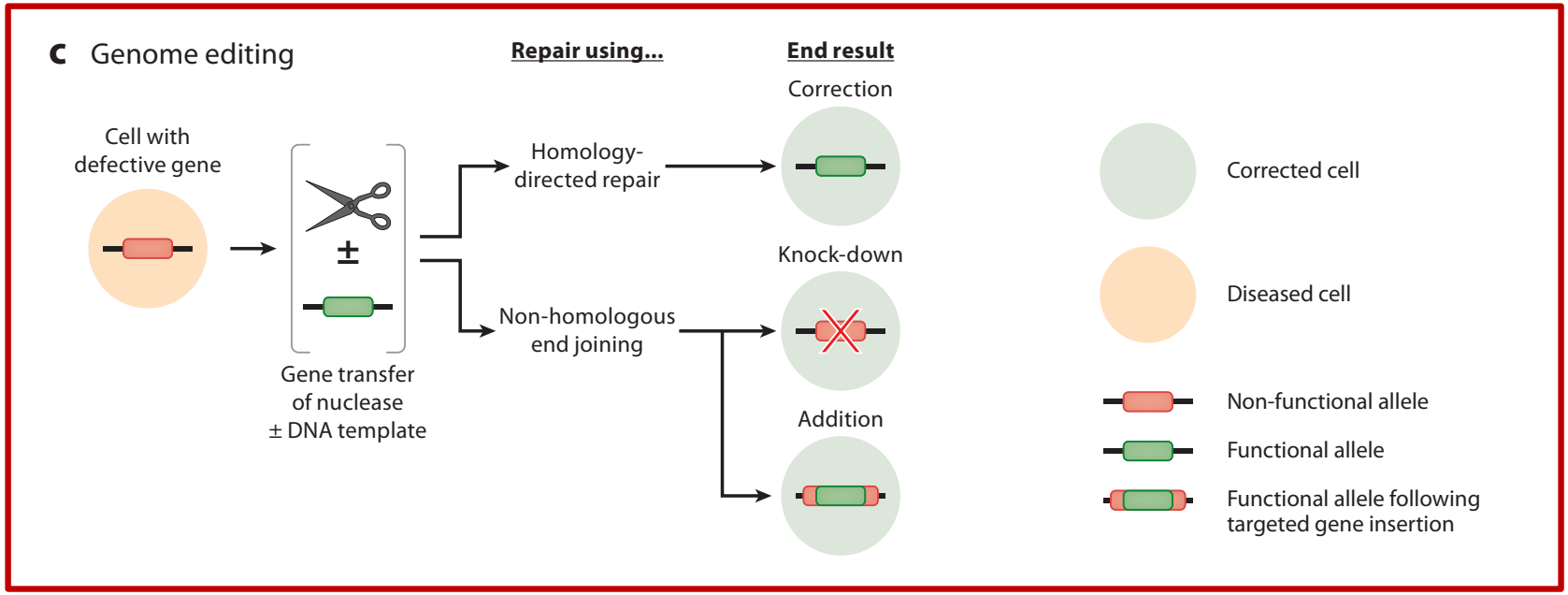
long- term correction of the 'edited' gene high enough to be therapeutic

2.0 gene therapy vs 3.0 gene therapy

2.0

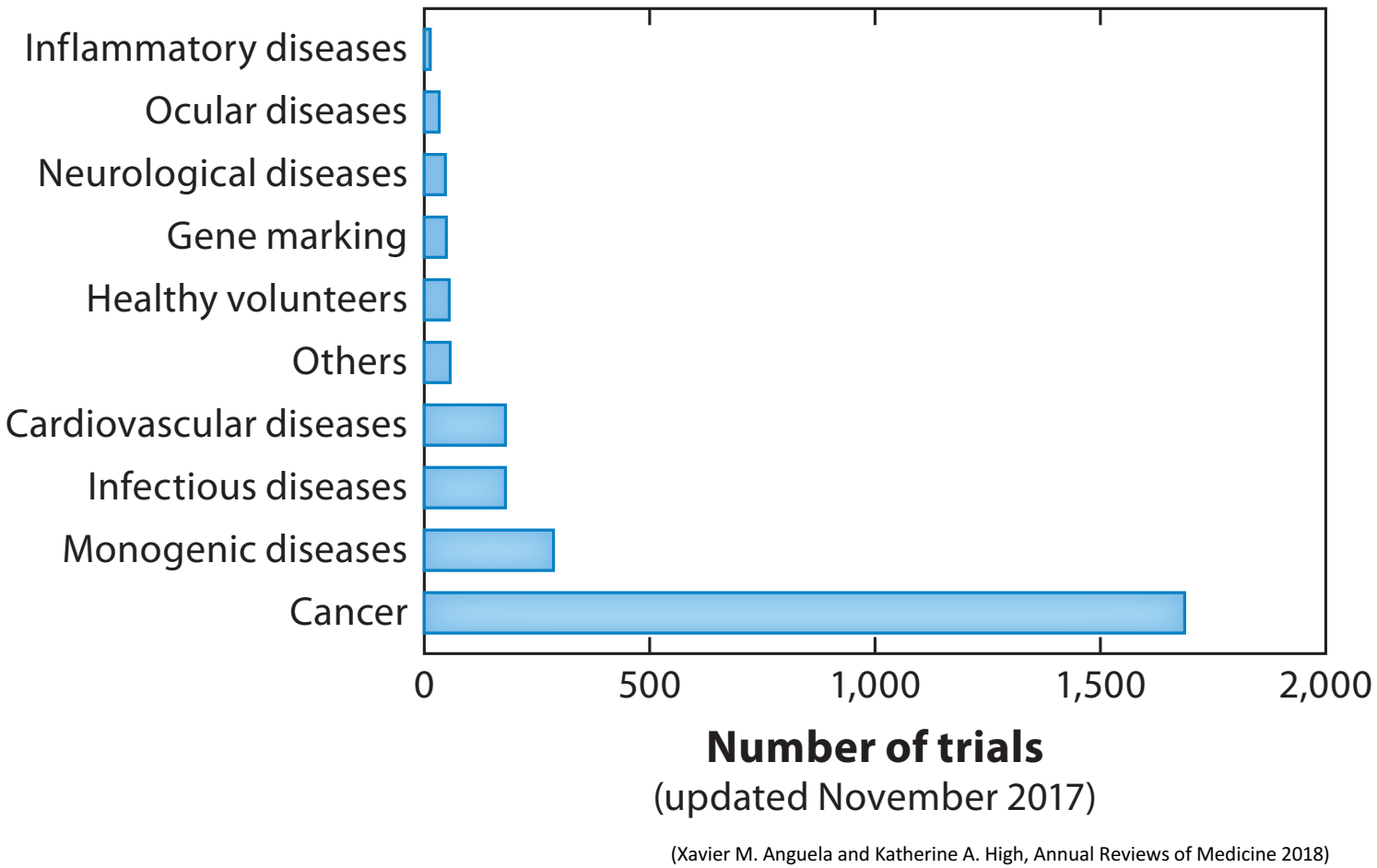


3.0

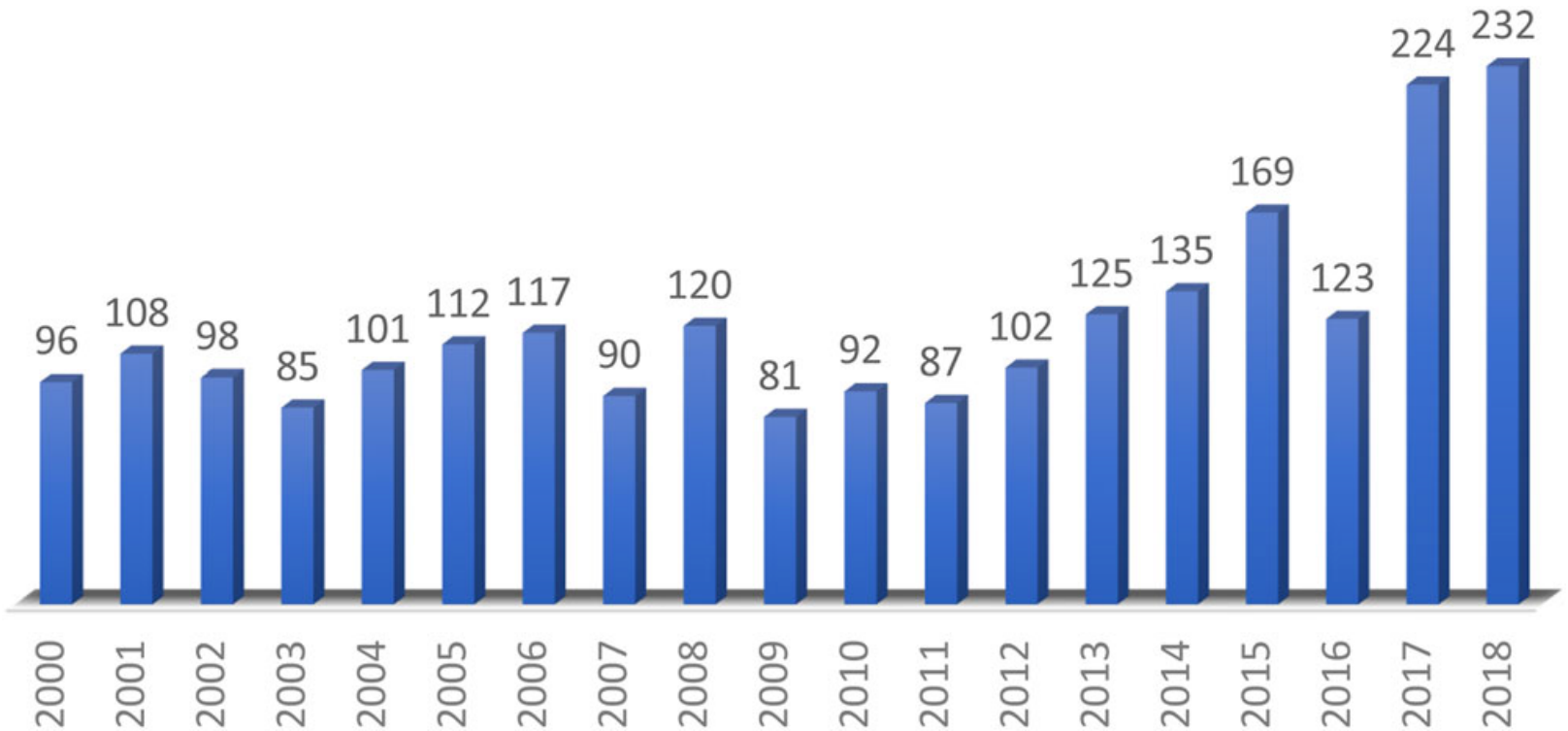


(Xavier M. Anguela and Katherine A. High, Annual Reviews of Medicine 2018)

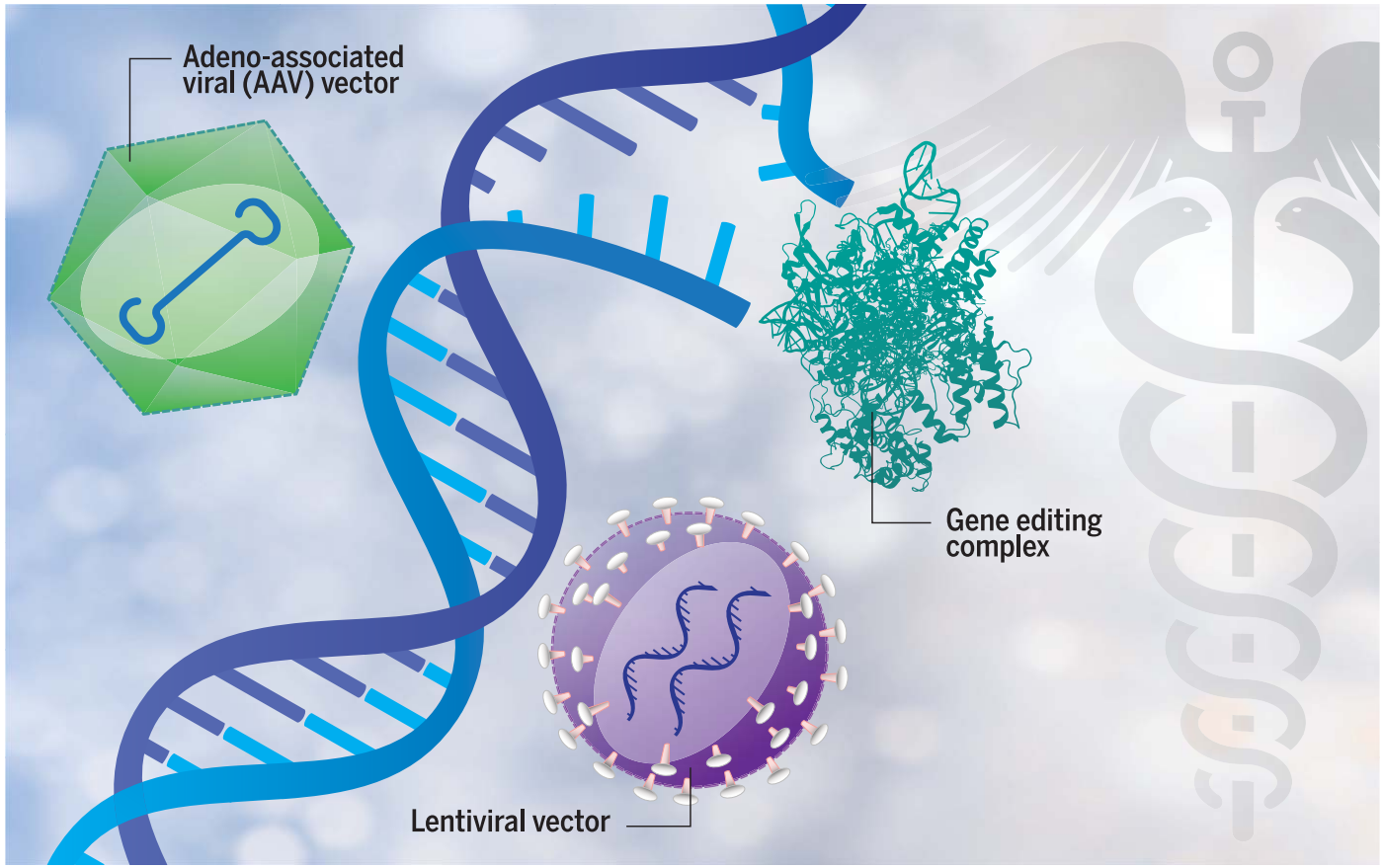
Monogenic disease and cancer gene therapy



(Xavier M. Anguela and Katherine A. High, Annual Reviews of Medicine 2018)



(Fazhan Wang et al., J Gene Med. 2019)

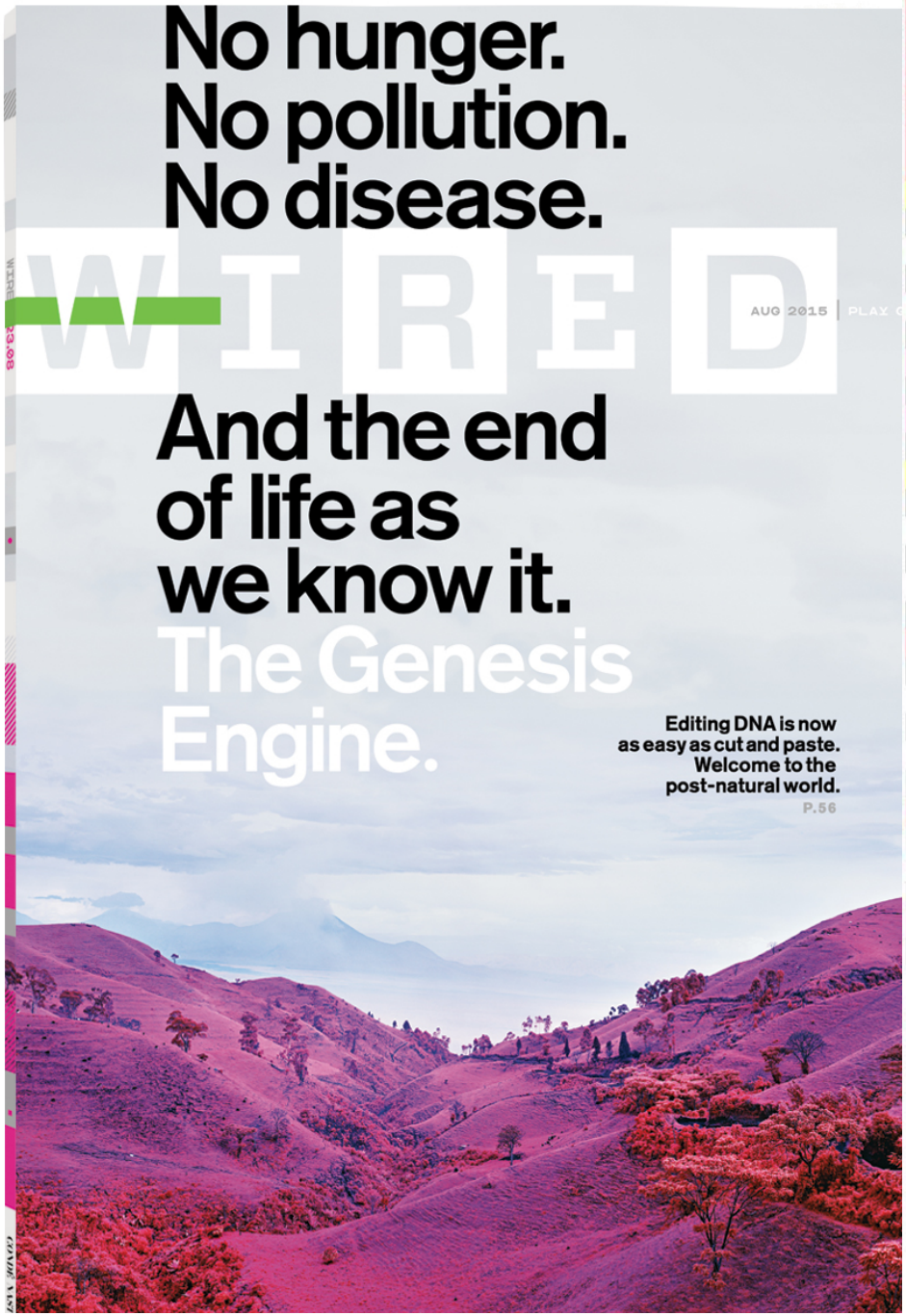


(Fazhan Wang et al., J Gene Med. 2019)

Bubble boy

CRISPR Revolution

2015


No hunger.
No pollution.
No disease.
WIRED
And the end of life as we know it.
The Genesis Engine.
Editing DNA is now as easy as cut and paste. Welcome to the post-natural world.
P.56

2019

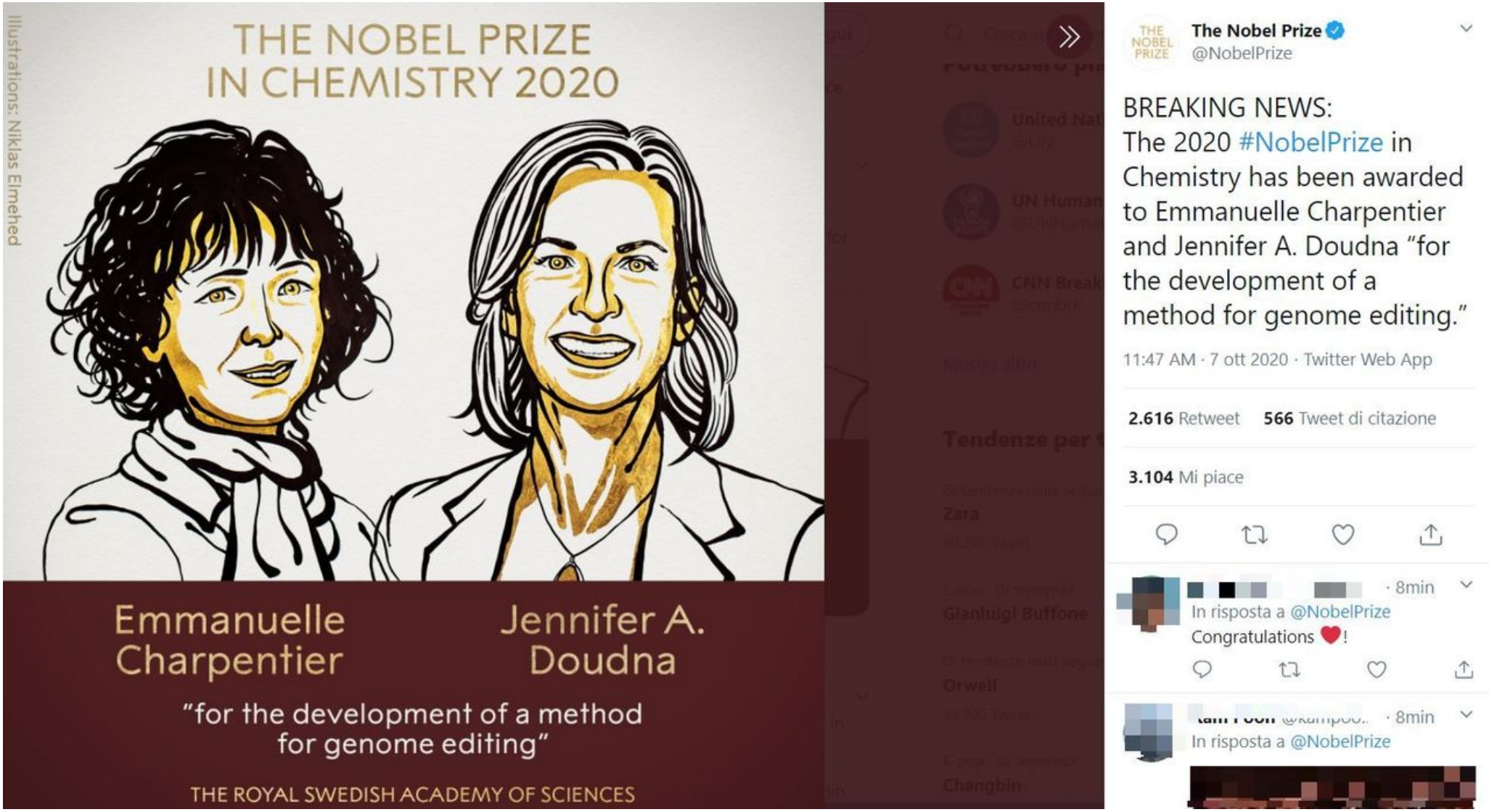

On-demand organs. Disease-proof babies. Horn-free cows.
WIRED
APR 2019 | CUT & PASTE
Crispr could give us a more humane world. Will humans let that happen?
Dairy cows often have their horns burned off with hot irons or caustic chemicals. Meet Princess, who was engineered never to grow them

CRISPR/Cas9 - It all started with yogurt

2005-Rodolphe Barrangou discovered that S. thermophilus contained odd chunks of repeating DNA sequences—Crisprs

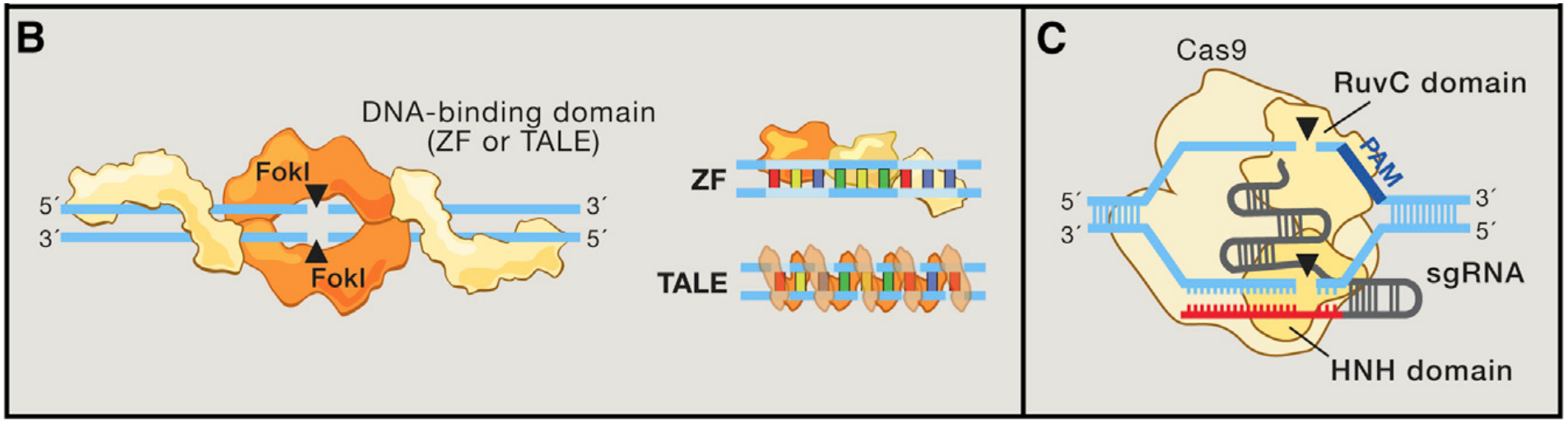
CRISPR/Cas9 - as a tool for genetic engineering

2012 : Jennifer Doudna and Emmanuelle Charpentier discovered S. pyogenes molecular mechanism



2020 - Nobel prize

Researchers can directly edit the function of DNA sequences in their endogenous context

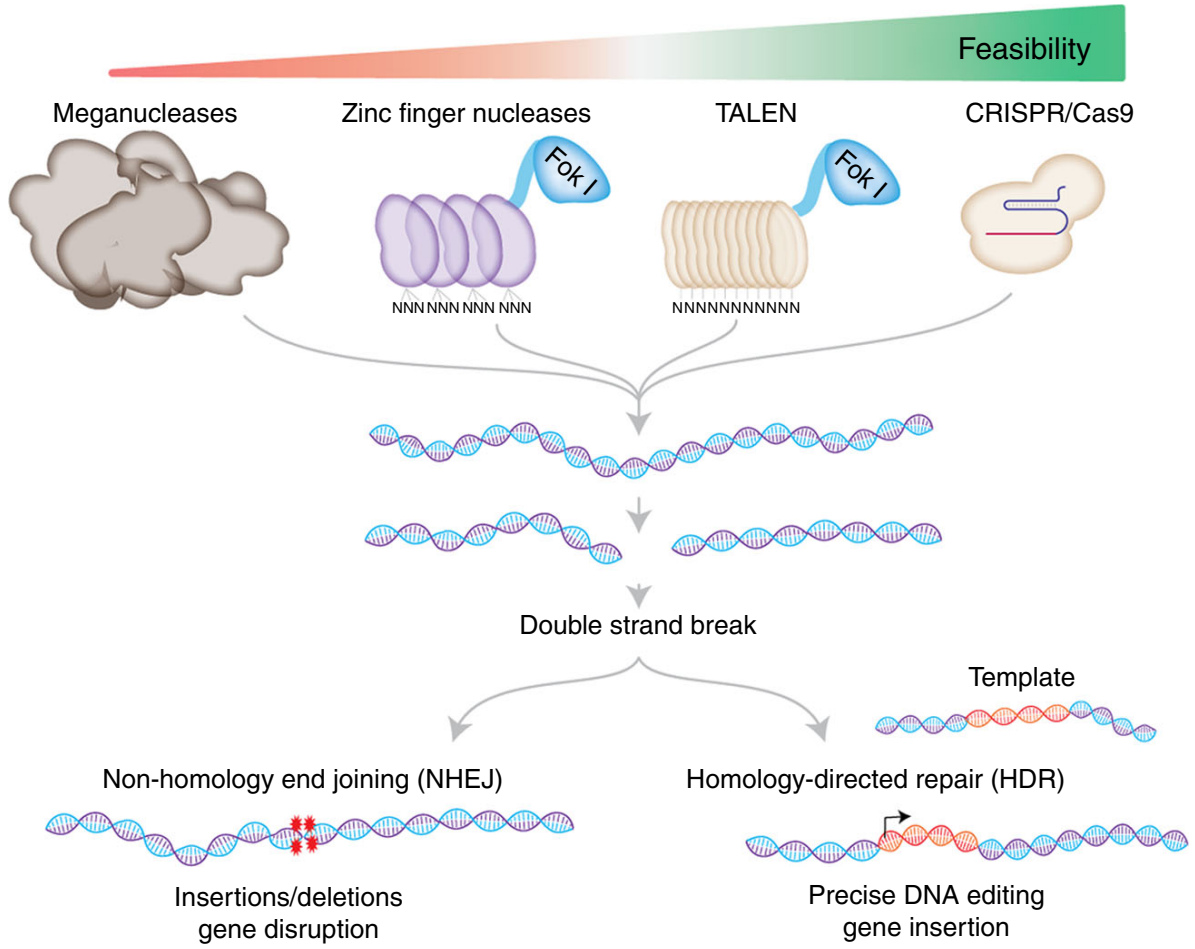


(Hsu et al., Cell, 2014)

	TALEN and ZFN	CRISPR/Cas9
Target binding principle	Protein-DNA specific recognition	Watson-Crick complementary rule
Working mode	Specifically recognizes the target DNA and dimeric Fok1 makes DSB	Guide RNA specifically recognizes the target DNA and Cas9 makes DSB
Essential components	Dimers of TALE/ZFN-Fok1 fusion protein	Guide RNA and Cas9
Target DNA lenght	14-18 bp	20 bp
Time consumption for construction	5-7 days	1-3 days
Multiple targeting	context-dependent binding (multiple proteins)	high specificity with multiple sgRNAs

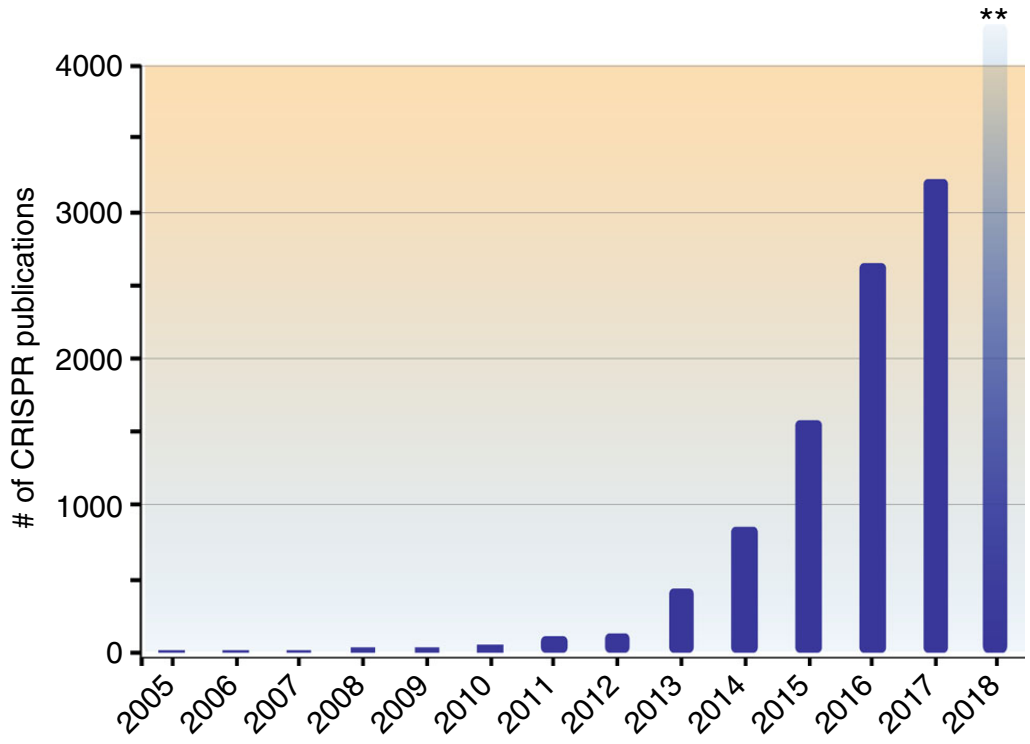
(Adapted from Wei C. et al., Journal of Genetics and Genomics, 2013)

CRISPR/Cas9 technology increased the feasibility of genome-editing technologies



(Adli M., Nature communications, 2018)

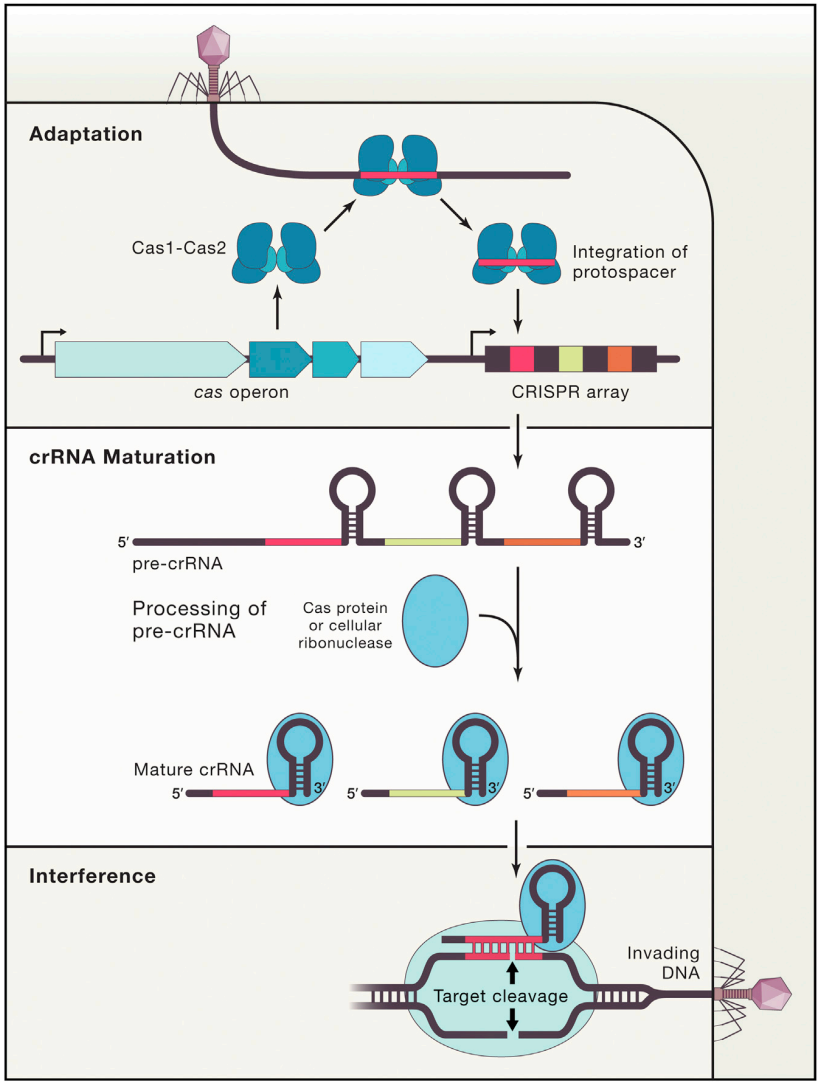
CRISPR/Cas9 technology increased the feasibility of genome-editing technologies



(Adli M., Nature communications, 2018)

CRISPR/Cas9

CRISPR system in prokariotes is an adaptive immunity system



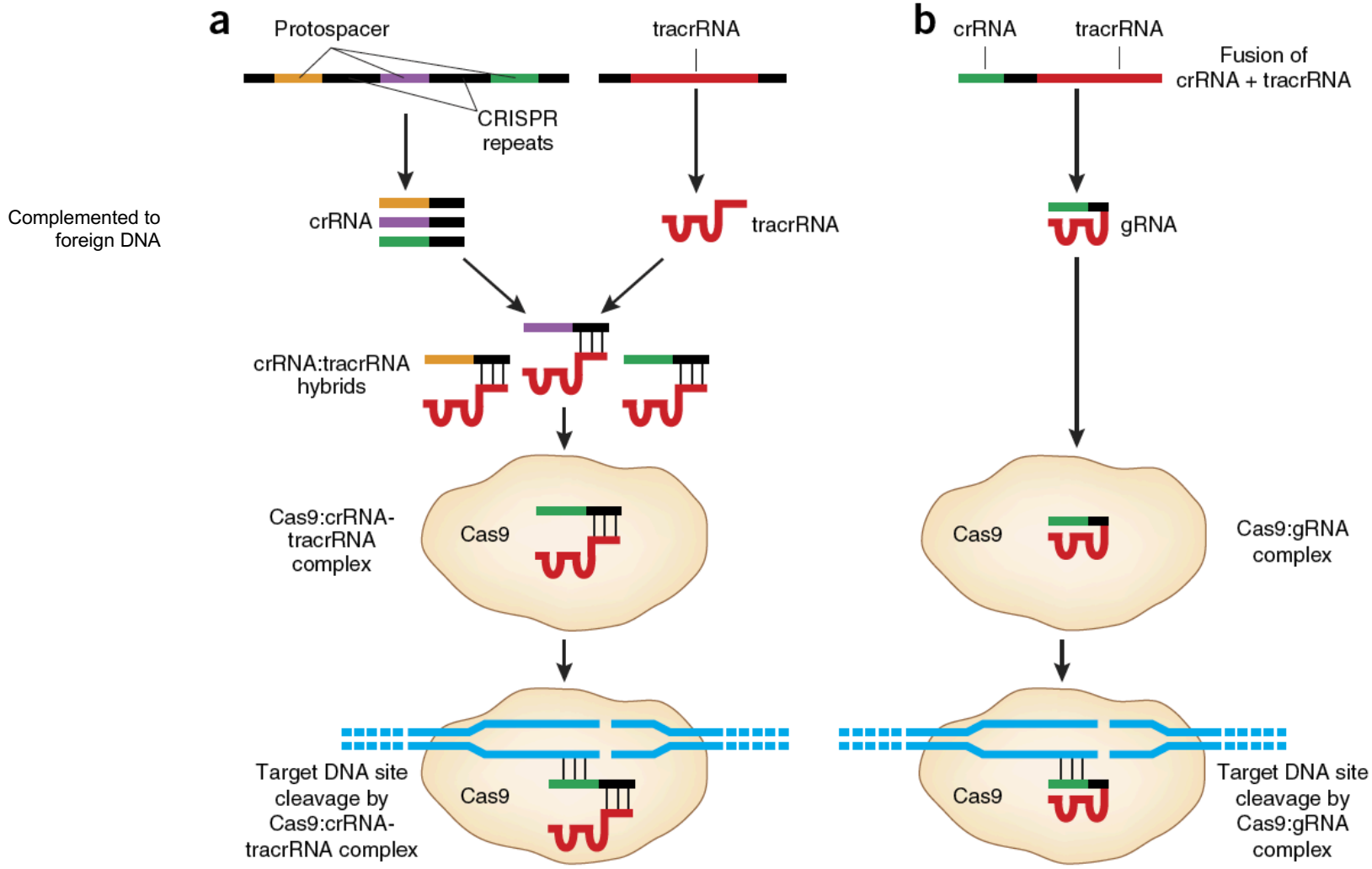
(Hille F. et al., Cell, 2018)

Engineered CRISPR-Cas9 system consists of a fusion between a crRNA and a part of the tracrRNA sequence: sgRNA

CRISPR system in prokariotes is an adaptive immunity system

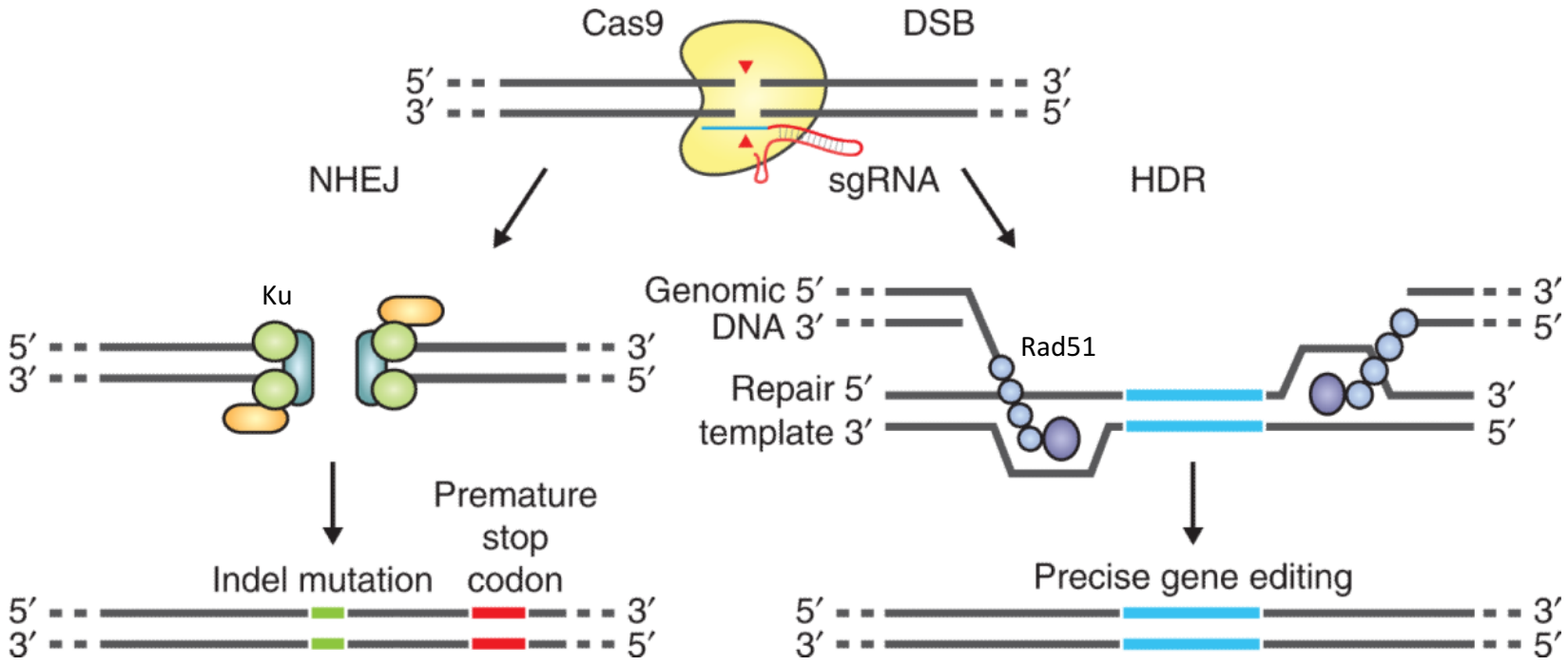
Naturally occurring CRISPR-Cas9 systems

Engineered CRISPR-Cas9 systems



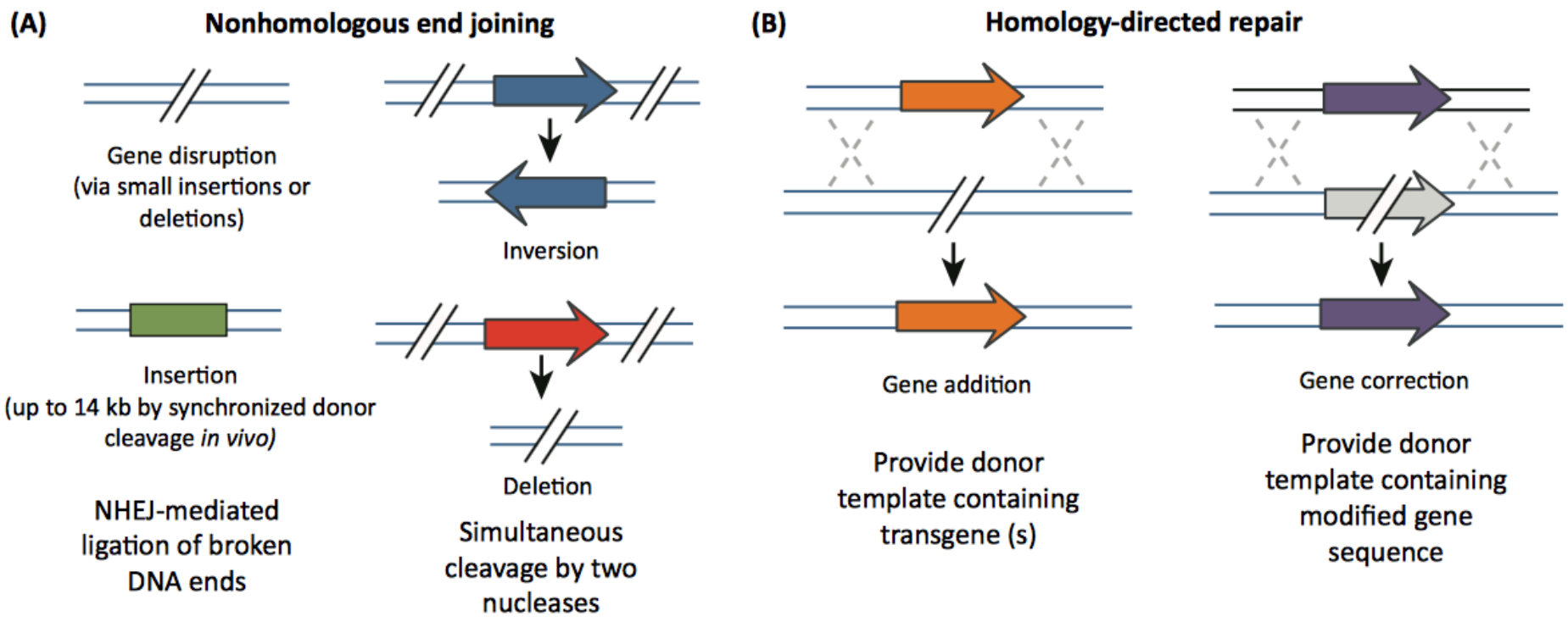
(Sander D. and Joung K., nature biotechnology, 2014)

CRISPR/Cas9 Genome editing tool exploit endogenous DNA repair machinery



(Ran et al, Nat Protoc. 2013)

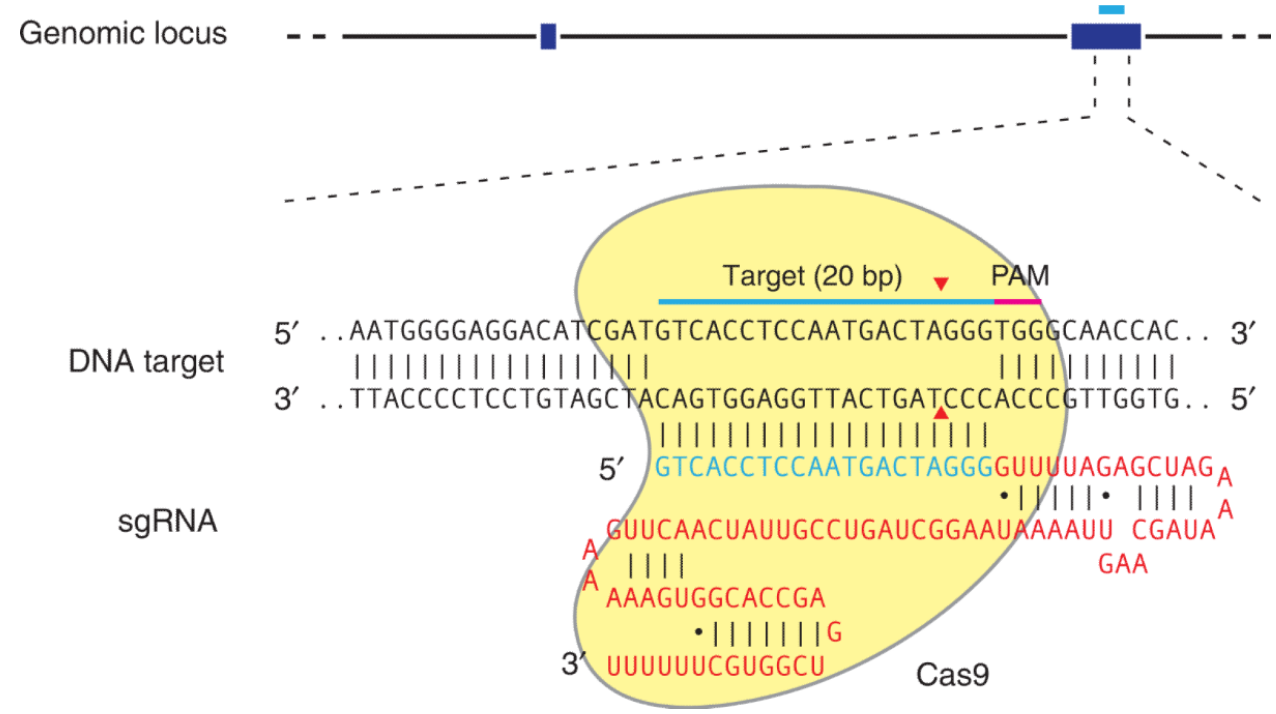
CRISPR/Cas9 Genome editing tool exploit endogenous DNA repair machinery



(Gaj T. at al., Trends Biotechnol, 2013)

Cas9 nuclease from *S. pyogenes* is targeted to genome by an sgRNA consisting of a 20-nt guide sequence and a scaffold

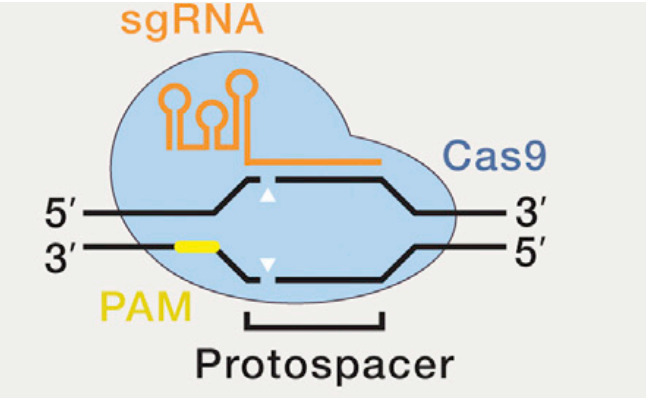
Genetic GPS



(Ran et al, Nat Protoc. 2013)

The only restriction for targeting is that the sequence must be followed by **PAM motif**

RNA-programmed endonucleases offer a variety of genome editing-options



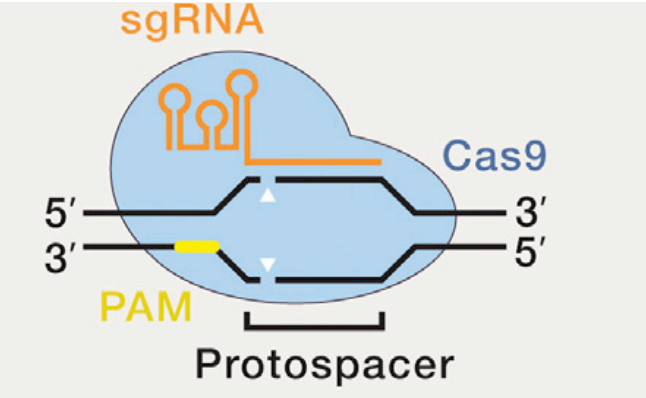
SpCas9:

- More characterized;
- Balance between PAM complexity and construct size;
- Tested in a variety of contexts

(Komor A.C. et al., Cell, 2017)

Enzyme name	Size (residues)	PAM requirement and cleavage pattern
SpCas9 / FnCas9	1368 / 1629	5′-NGG-3′ / 3′-NCC-5′
St1Cas9	1121	5′-NNAGAAW-3′ / 3′-NNTCTTW-5′
St3Cas9	1409	5′-NGGNG-3′ / 3′-NCCNC-5′
NmCas9	1082	5′-NNNNGATT-3′ / 3′-NNNNCTAA-5′
SaCas9	1053	5′-NNGRRT-3′ / 3′-NNCYYA-5′
AsCpf1 / LbCpf1	1307 / 1228	5′-TTTN-3′ / 3′-AAAN-5′
VQR SpCas9	1368	5′-NGA-3′ / 3′-NCT-5′
EQR SpCas9	1368	5′-NGAG-3′ / 3′-NCTC-5′
VRER SpCas9	1368	5′-NGCG-3′ / 3′-NCGC-5′
RHA FnCas9	1629	5′-YG-3′ / 3′-RC-5′
KKH SaCas9	1053	5′-NNNRRT-3′ / 3′-NNNYYA-5′

RNA-programmed endonucleases offer a variety of genome editing-options



Cpf1s:

- Use naturally crRNA;
- TTTN PAM at 5’ end of the protospacer;
- Cleave the two DNA in a stagger configuration

Enzyme name	Size (residues)	PAM requirement and cleavage pattern
SpCas9 / FnCas9	1368 / 1629	5′-…NGG-3′ / 3′-…NCC-5′ (18 / 17 / 20)
St1Cas9	1121	5′-…NNAGAAW-3′ / 3′-…NNTCTTW-5′ (18 / 17 / 20)
St3Cas9	1409	5′-…NGGNG-3′ / 3′-…NCCNC-5′ (18 / 17 / 20)
NmCas9	1082	5′-…NNNNGATT-3′ / 3′-…NNNNCTAA-5′ (22 / 21 / 24)
SaCas9	1053	5′-…NNGRRT-3′ / 3′-…NNCYYA-5′ (19 / 18 / 21)
AsCpf1 / LbCpf1	1307 / 1228	5′-TTTN…-3′ / 3′-AAAN…-5′ (24 / 19)
VQR SpCas9	1368	5′-…NGA-3′ / 3′-…NCT-5′ (18 / 17 / 20)
EQR SpCas9	1368	5′-…NGAG-3′ / 3′-…NCTC-5′ (18 / 17 / 20)
VRER SpCas9	1368	5′-…NGCG-3′ / 3′-…NCGC-5′ (18 / 17 / 20)
RHA FnCas9	1629	5′-…YG-3′ / 3′-…RC-5′ (18 / 17 / 20)
KKH SaCas9	1053	5′-…NNNRRT-3′ / 3′-…NNNYYA-5′ (19 / 18 / 21)

(Komor A.C. et al., Cell, 2017)

The amazing CRISPR enzyme clan

Cas9 | The OG
Good at cutting DNA, great for knockouts. Already being replaced by newer base pair editors with more fine-tuned control.

Cpf1 | The Stickler
Like Cas9 but not as sloppy. It leaves "sticky" DNA ends, which are easier to work with when making edits.

Cas13 | The Cowboy
Cuts RNA not DNA. Could knock down protein levels without permanently changing your genome. Pair it with a reporter signal and you've got a diagnostic.

Cas3 | The Gobbler
Cas3 gives zero f***. It offers no repair mechanism—once it finds that target DNA sequence it just starts cutting till there ain't no DNA left.

CasX/CasY | The X/Y Factor
Just discovered in an abandoned silver mine, we don't know yet what these tiny enzymes' superpowers will be.

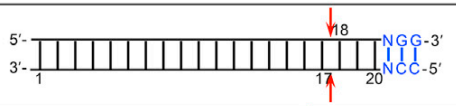
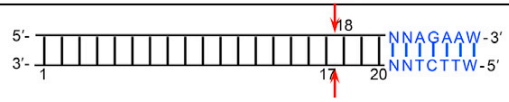
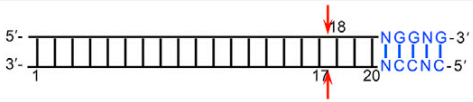
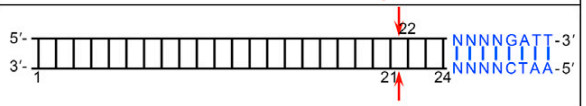
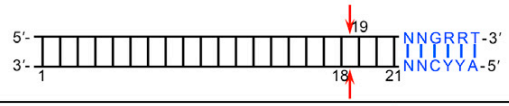
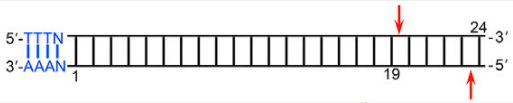
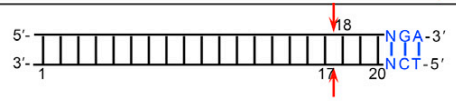
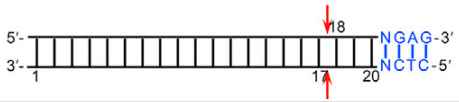
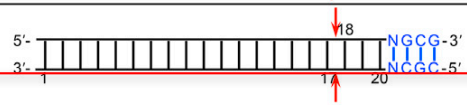
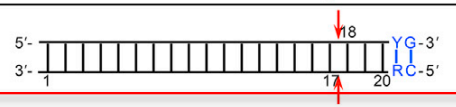
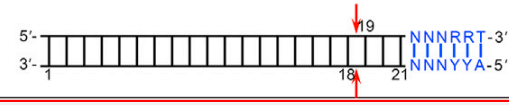
(Wired, march 2019)

RNA-programmed endonucleases offer a variety of genome editing-options

PRO	CONS
- Target design simplicity; - Higly efficiency - Fast (4 weeks for mice);	- **fidelity** - **delivery** - **targeting scope**

- OPEN QUESTIONS:
- **Immunogenicity of nucleases *in vivo* (?)**
- **Ethics (?)**

I - targeting scope

Enzyme name	Size (residues)	PAM requirement and cleavage pattern
SpCas9 / FnCas9	1368 / 1629	
St1Cas9	1121	
St3Cas9	1409	
NmCas9	1082	
SaCas9	1053	
AsCpf1 / LbCpf1	1307 / 1228	
VQR SpCas9	1368	
EQR SpCas9	1368	
VRER SpCas9	1368	
RHA FnCas9	1629	
KKH SaCas9	1053	

RHA FnCas9 requires only a YG PAM

(Komor A.C. et al., Cell, 2017)

KKH SaCas9 shows Relaxed PAM specifities

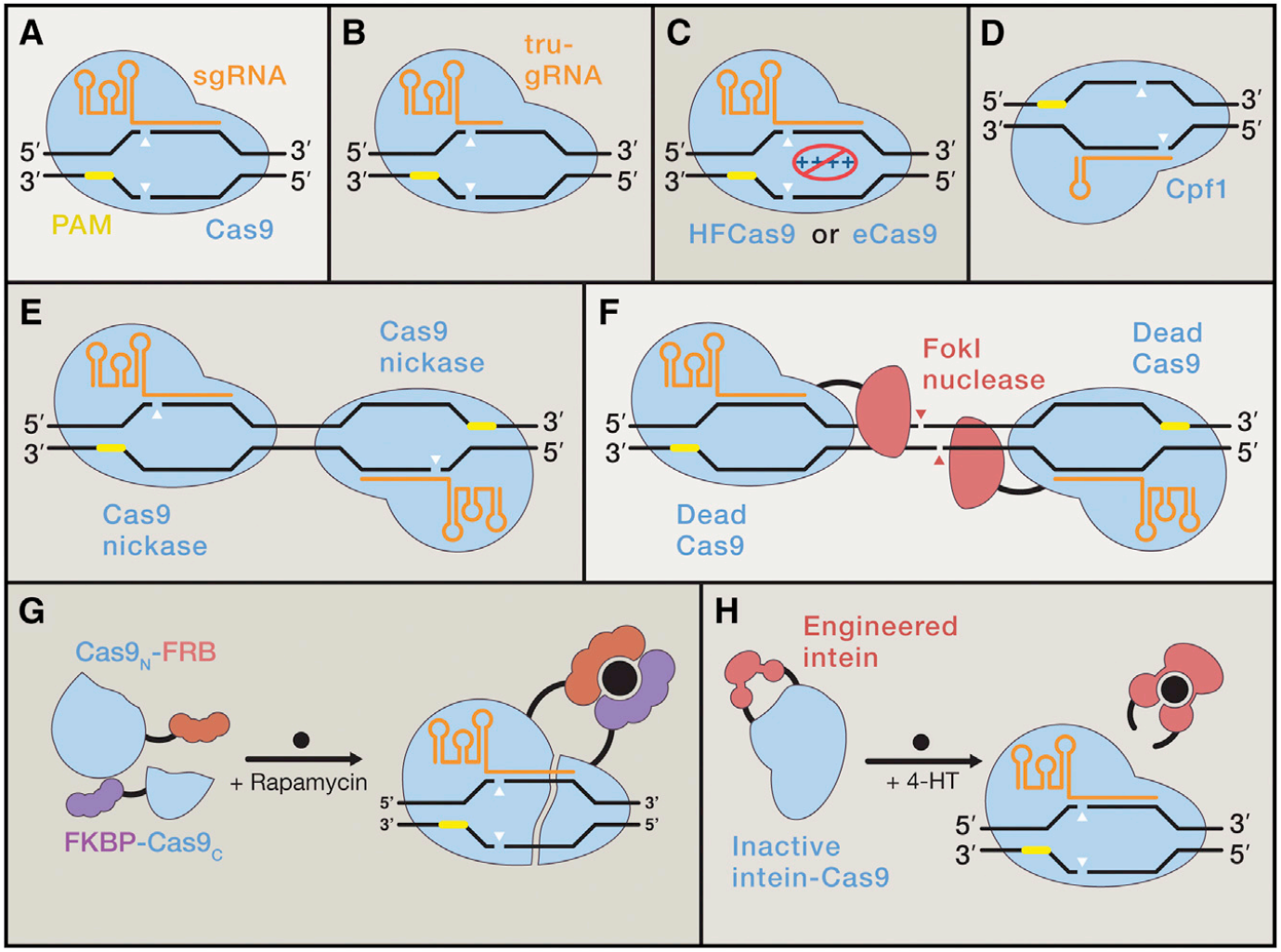
II - fidelity

How to check?

- Whole genome deep sequencing;
- BLESS
- GUIDE-Seq
- Digenome-Seq

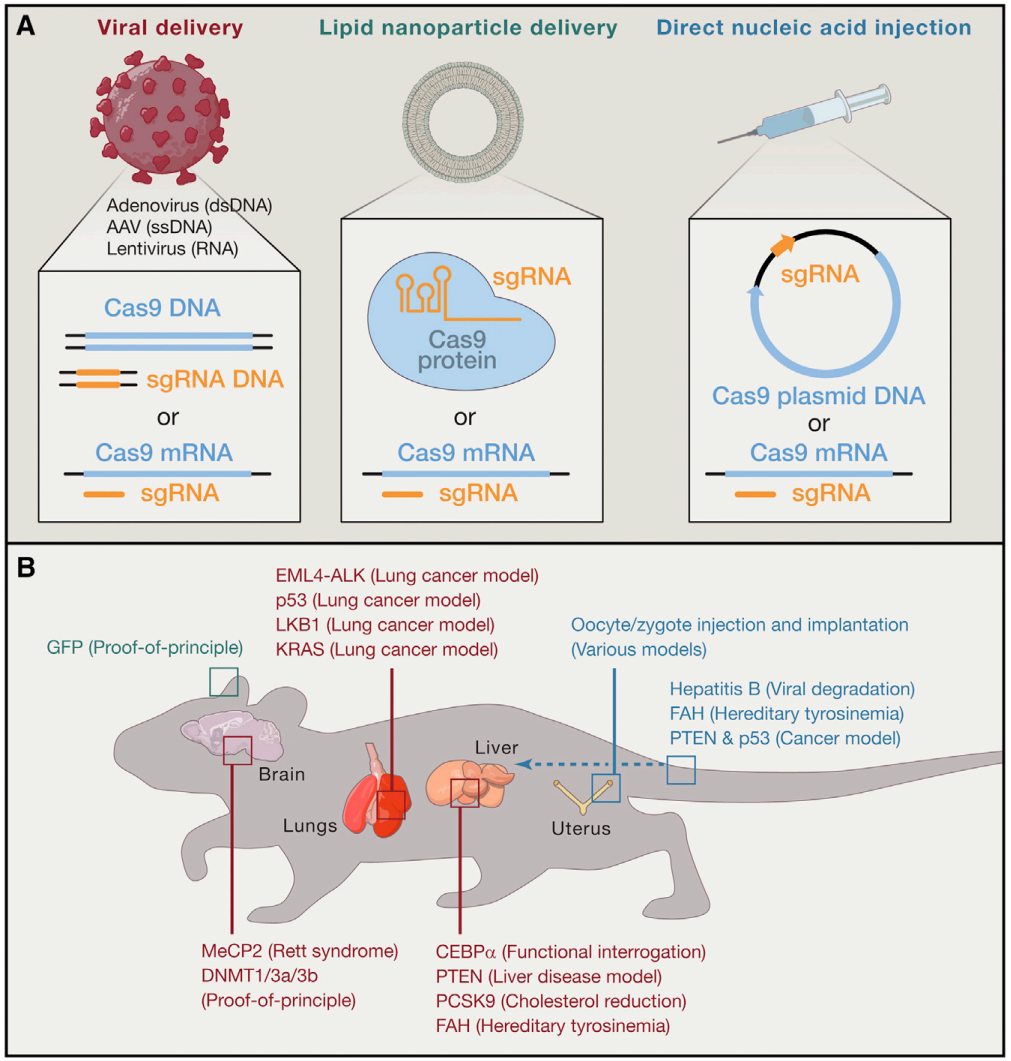
II - Fidelity

How to improve?



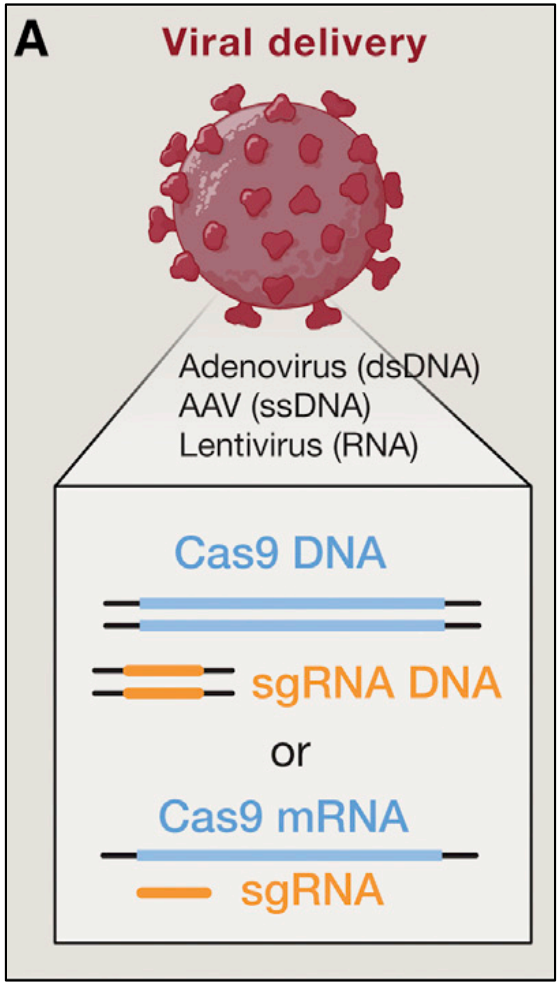
(Komor A.C. et al., Cell, 2017)

III – delivery



(Komor A.C. et al., Cell, 2017)

III – delivery



(Komor A.C. et al., Cell, 2017)

Lentivirus:

- infects non dividing cells;
- Packaging limit **~8.5 kb** (package Cas9 genes, gRNA, promoter and regulatory sequences)

Adenovirus:

- infects dividing and non dividing cells;
- Do not integrate DNA;
- Elicits strong immune response in animals;

AAV variants:

- infect both dividing and non-dividing cells;
- do not integrate;
- do not elicit immune response in the host;
- A variety of serotypes of AAV are known,

- AAV has a packaging limit of **~4.5** kb of foreign DNA

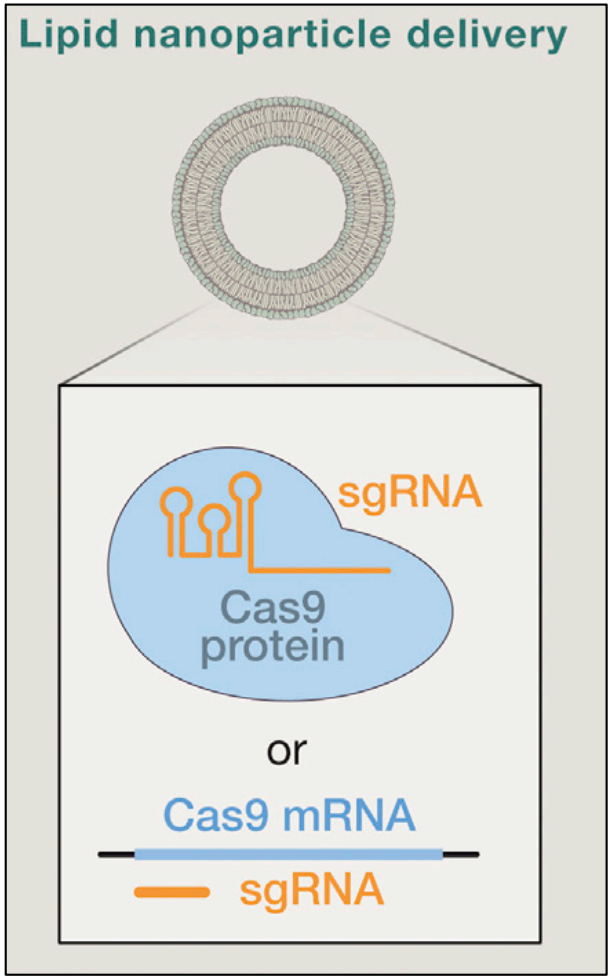
III – delivery

Table 1 Naturally occurring major CRISPR-Cas enzymes

	Size	PAM sequence	Size of sgRNA guiding sequence	Cutting site	Reference
spCas9	1368	NGG	20 bp	~ 3 bp 5′ of PAM	Jinek et al.[42] Gasiunas et al.[43]
FnCas9	1629	NGG	20 bp	~ 3 pb 5′ of PAM	Hirano et al.[60]
SaCas9	1053	NNGR RT	21 bp	~ 3 pb 5′ of PAM	Mojica et al.[57]
NmCas9	1082	NNNNG ATT	24 bp	~ 3 bp 5′ of PAM	Hou et al.[53]
St1Cas9	1121	NNAGA AW	20 bp	~ 3 bp 5′ of PAM	Gasiunas et al.[43] Cong et al.[45]
St3Cas9	1409	NGGNG	20 bp	~ 3 bp 5′ of PAM	Gasiunas et al.[43] Cong et al.[45]
CjCas9	984	NNNNACAC	22 bp	~ 3 bp 5′ of PAM	Kim et al.[56]
AsCPf1	1307	TTTV	24 bp	19/24 bp 3′ of PAM	Yamano et al.[50] Kim et al. 2016
LbCpf1	1228	TTTV	24 bp	19/24 bp 3′ of PAM	Yamano et al.[50] Kim et al. 2016
Cas13	Multiple orthologs	RNA targeting	28 bp		Abudayyeh et al. 2017

(Adli M., Nature communications, 2018)

III – delivery

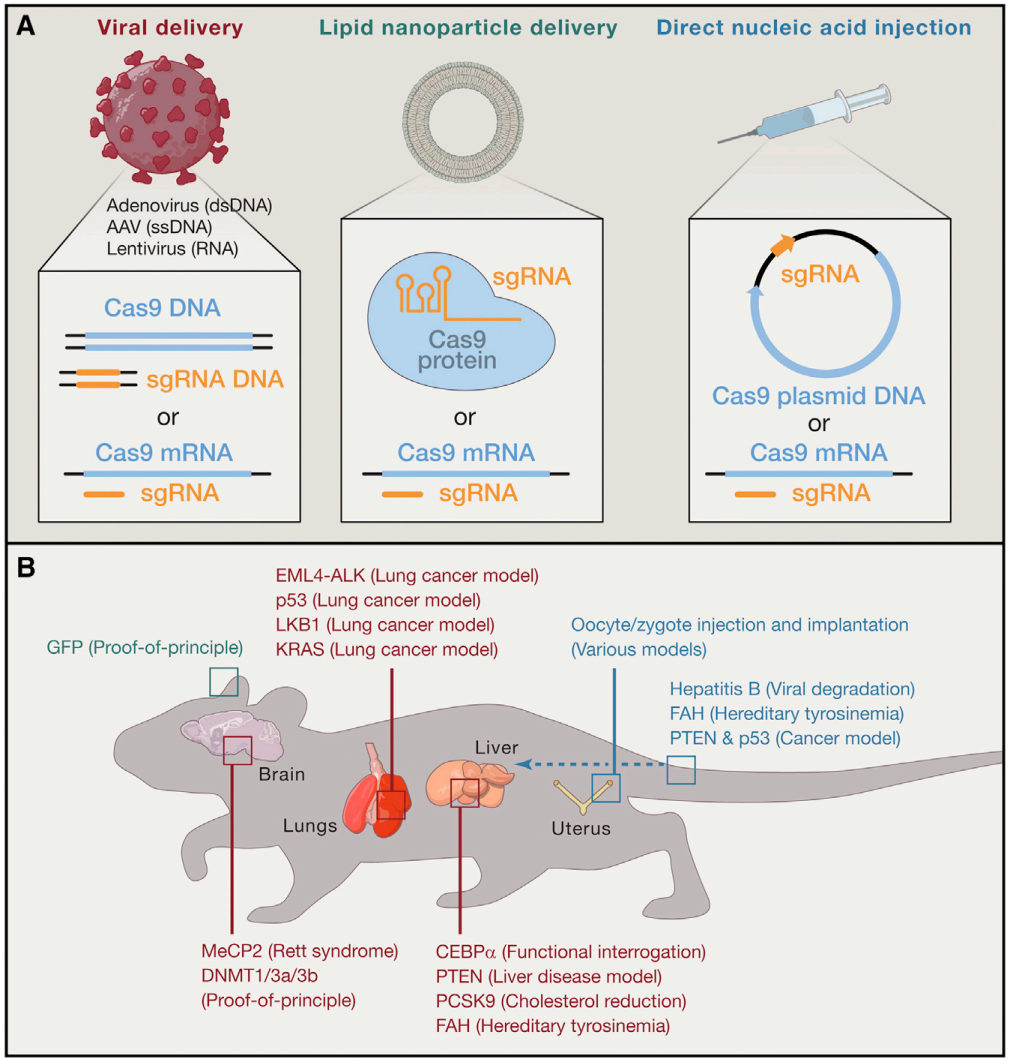


Lipid nanoparticle delivery:

- more transient
- higher DNA specificity
- less off-target editing

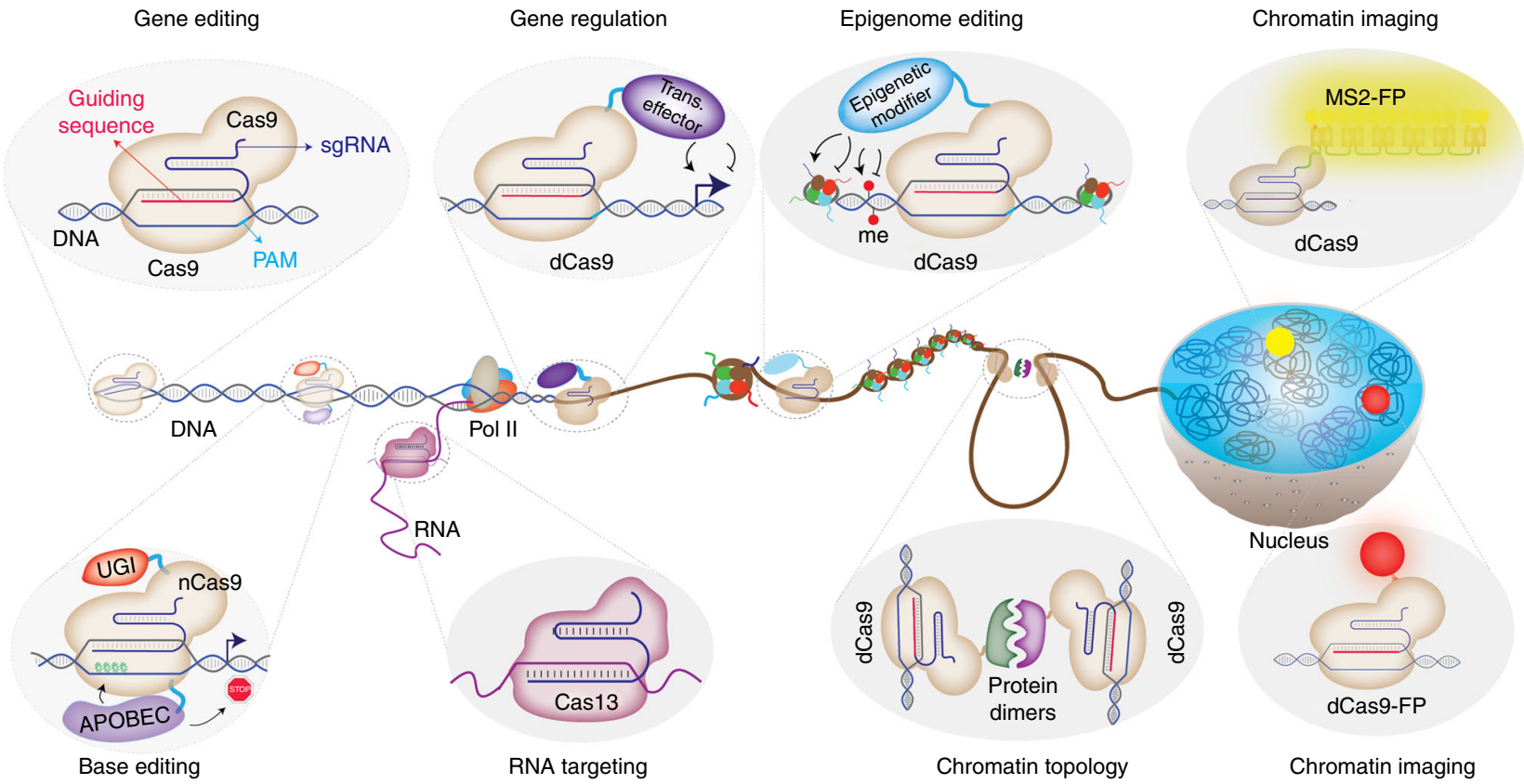
(Komor A.C. et al., Cell, 2017)

III – delivery



(Komor A.C. et al., Cell, 2017)

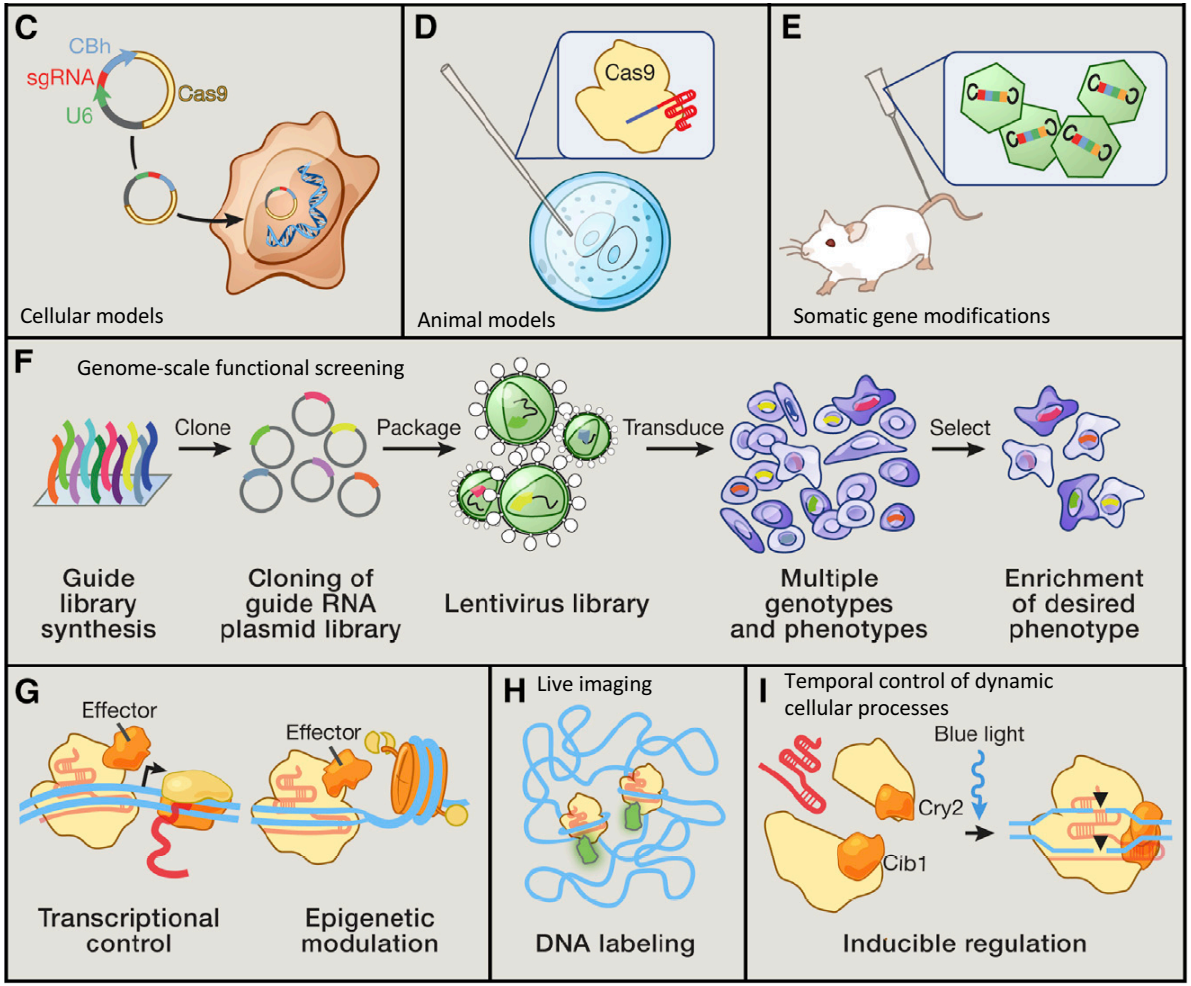
CRISPR/Cas9 technologies beyond genome editing are based mainly on dead-Cas9



(Adli M., Nature communications, 2018)

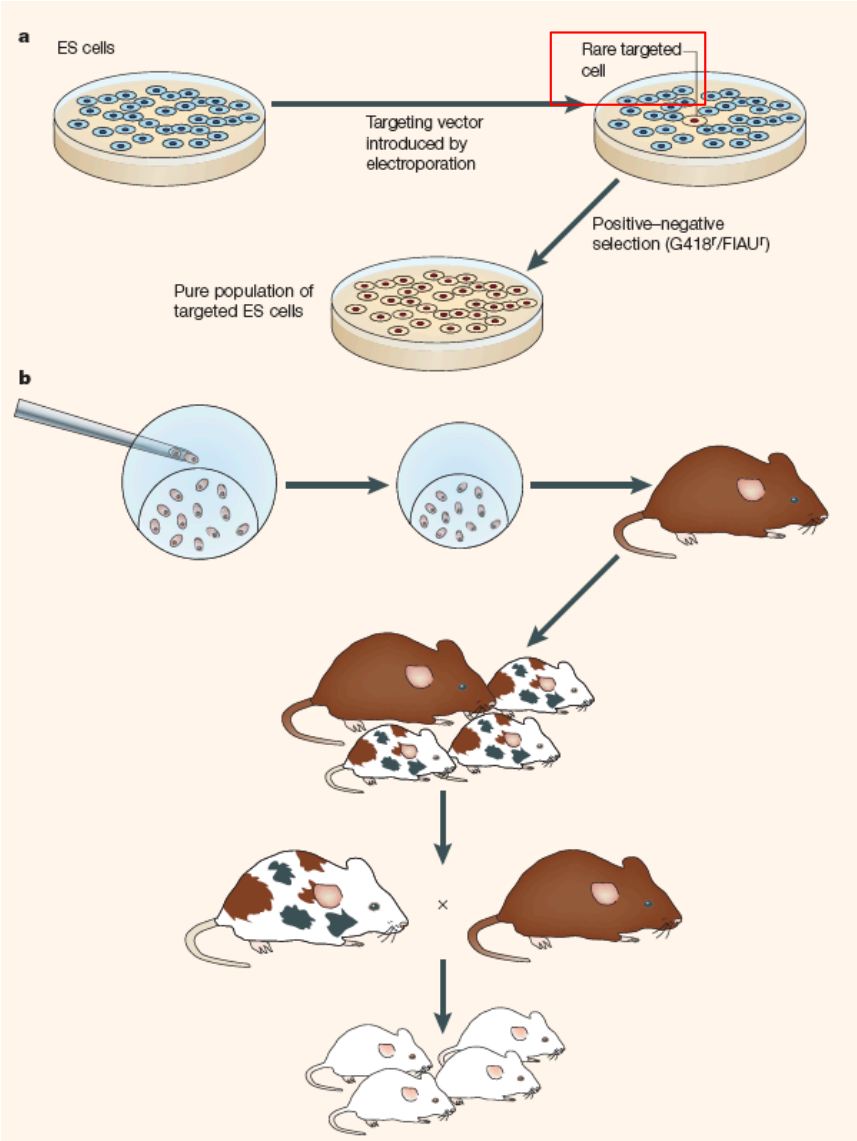
CRISPR/Cas9 APPLICATIONS

CRISPR/Cas engineering is enabling a broad range of applications



(Hsu et al., Cell, 2014)

Why develop new tools for genome editing?

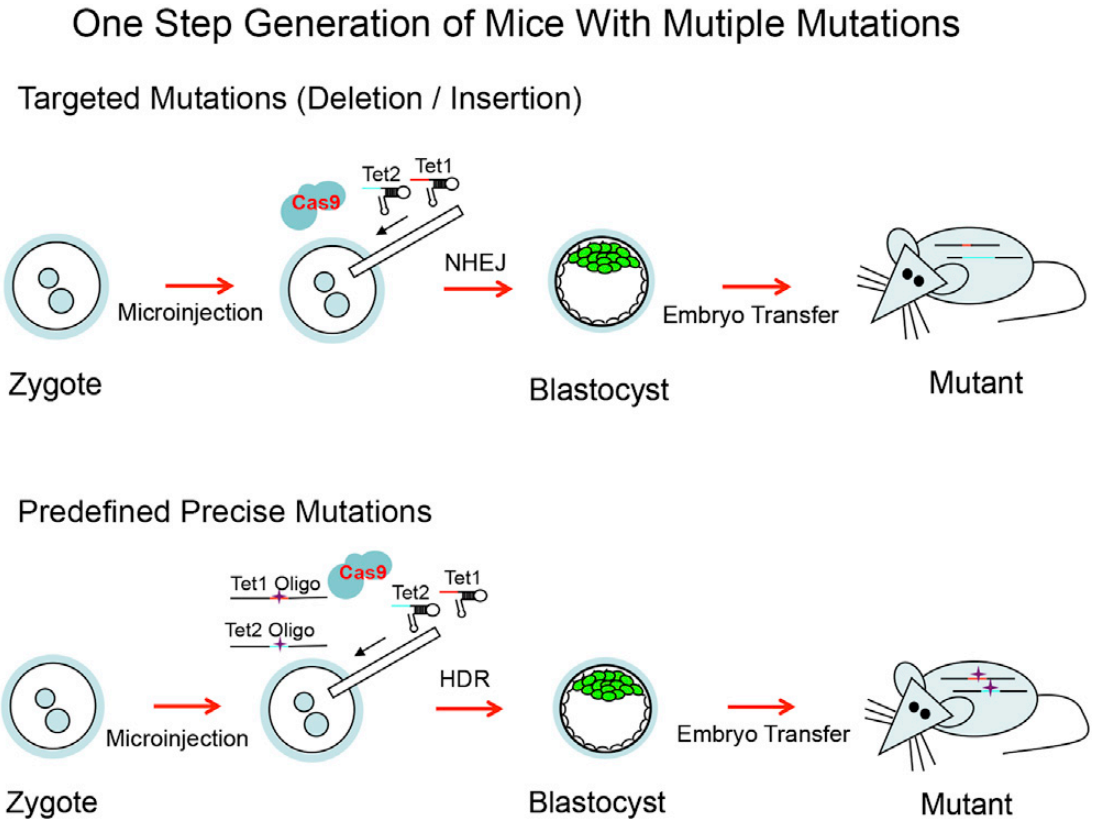


(Capecchi, Nature 2005)

HR - Classical transgenesis:

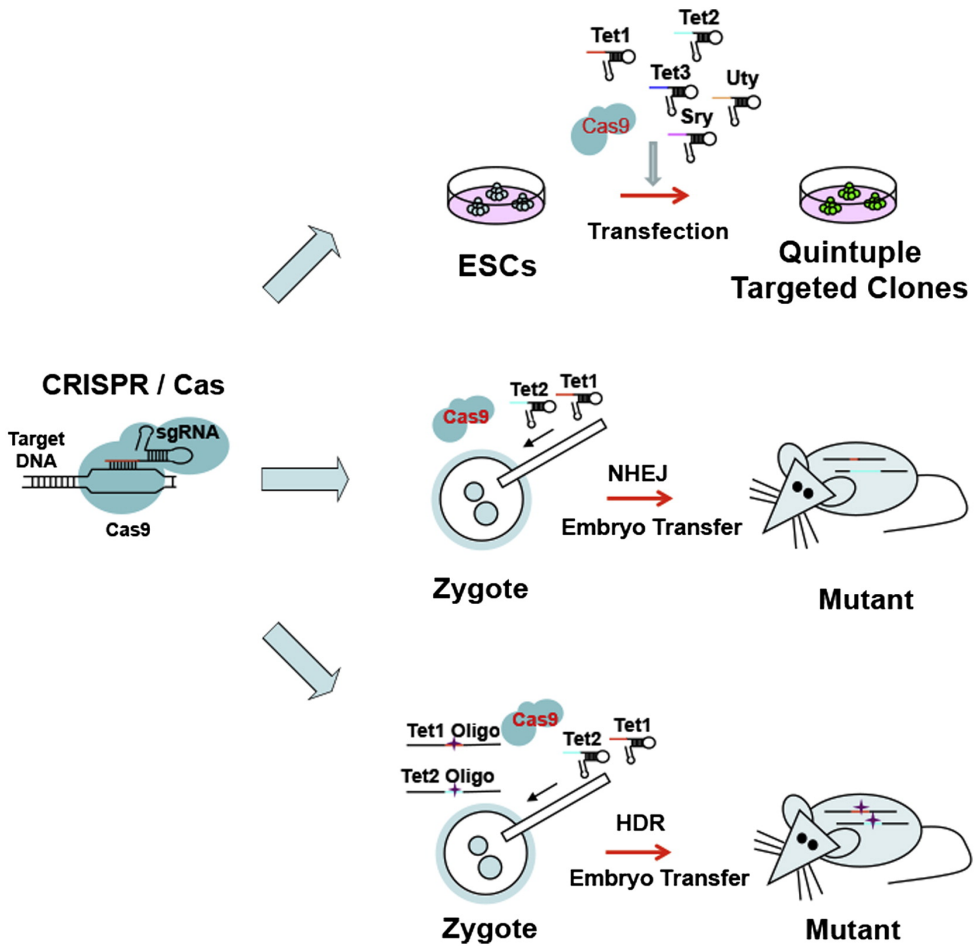
- HR is a rare event (1 in 10^6–10^9 cells – Capecchi, Nature, 1989);
- Time consuming (up to 6-12 months);
- Expensive;
- In most mammalian species no established ES cell lines;
- Difficult to target multiple genes.

Why develop new tools for genome editing?



(Wang et al., Cell 2013)

Why develop new tools for genome editing?



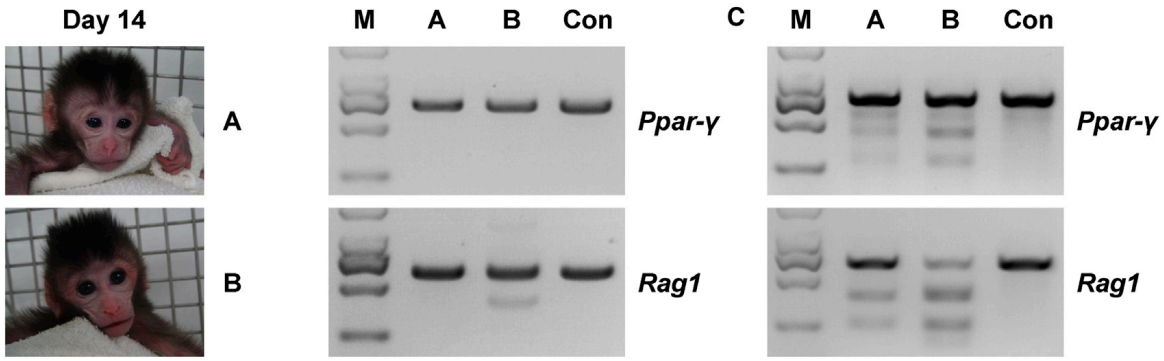
(Wang et al., Cell 2013)

CRISPR/Cas9-mediated transgenesis

- Target design simplicity;
- Higly efficiency: directly injecting RNAs encoding the Cas9 protein and gRNA into zygote (no need for ES);
- Fast (4 weeks for mice);
- Multiplexed mutations

CRISPR/Cas9 system can be used in other mammals?

In vivo




```
Ppar-γ ACTCCTTTGACATCAAGCCCTTCACTACTGTTGACTTCTCCAGCATTTCTGCTCCAC (WT)
  A    ACTCCTTTGACATCAAGCCCTTC^aCTACTGTTGACTTCTCCAGCATTTCTGCTCCAC (+1,2/23)
  B    ACTCCTTTGACATCAAGCCCTTC^aCTACTGTTGACTTCTCCAGCATTTCTGCTCCAC (+1,6/23)

Rag1   CGCTATGATTCAGCTTTGGTGTCTGCTTTGATGGACATGGAAGAAGACATCTTGGAA (WT)
  A    CGCTATGATTCAGCTTTGGTGTCTGCTTTGATGG::::::AAGAAGACATCTTGGAA (-6,3/20)
  B    CGCTATGATTCAGCTTTGGTGTCTGCTTT::::GACATGGAAGAAGACATCTTGGAA (-4,2/18)
       CGCTATGATTCAGCTTTGGTGTCTGCTTT:::::::::::::::::::::::::::: (-203,2/18)
       CGCTATGATTCAGCTTTGGTGTCTGCTTTGATGG^gaagaaGACATGGAAGAAGACATCTTGGAA (+6,7/18)
```

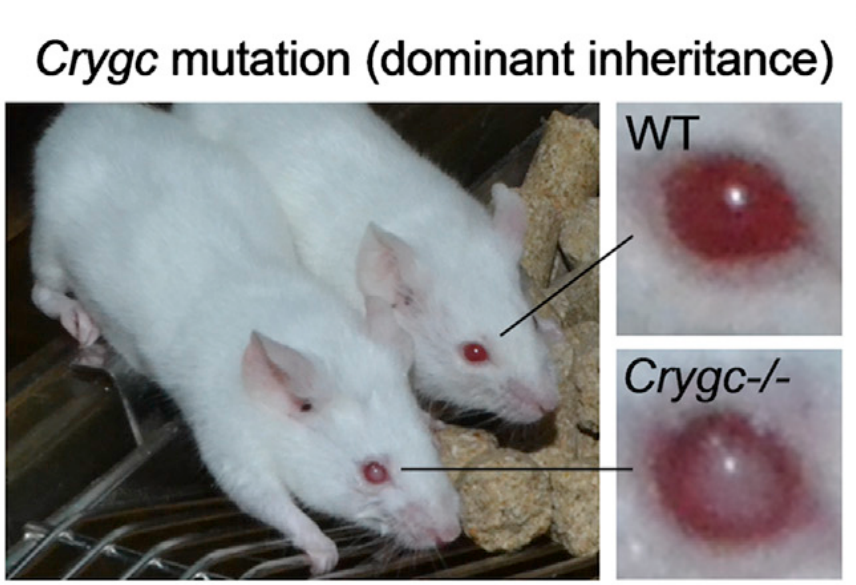
(Niu et al., Cell 2013)

CRISPR/Cas can be used to insert *multiple* genes mutations in monkeys zygotes

Genome editing based on CRISPR/Cas9 nucleases is in its translational and clinical infancy

(up to 2018)

Can CRISPR/Cas9 be used for correct genetic disorders?

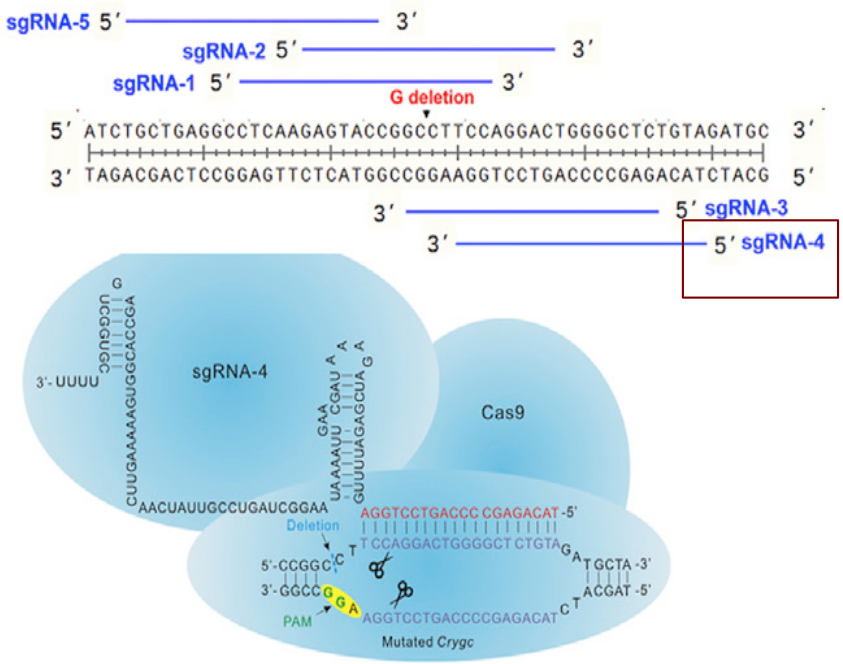


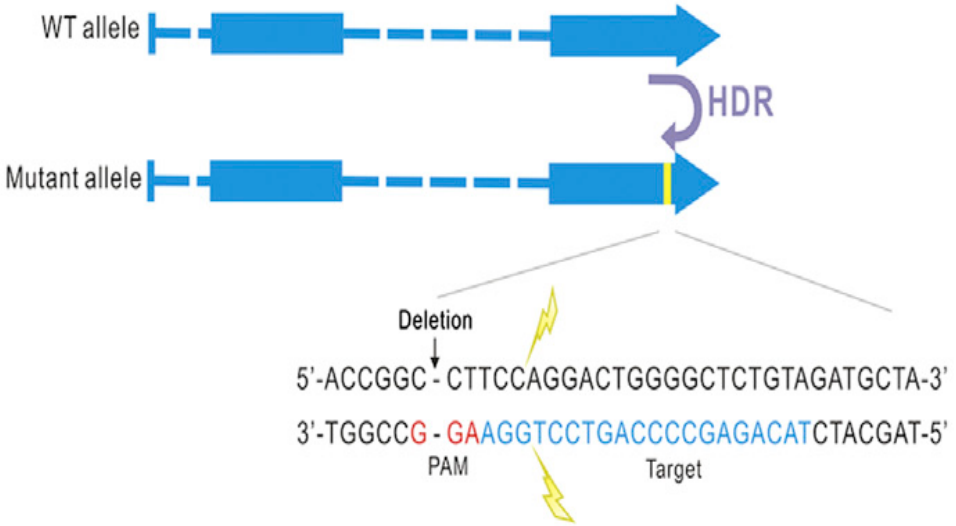
(Wu et al., Cell 2013)

1 bp deletion in exon 3 of Crygc gene leads to cataract

Can CRISPR/Cas9 be used for correct genetic disorders?

In vitro





(Wu et al., Cell 2013)

Can CRISPR/Cas9 be used for correct genetic disorders?

In vitro

sgRNA leads to HDR mediated repair

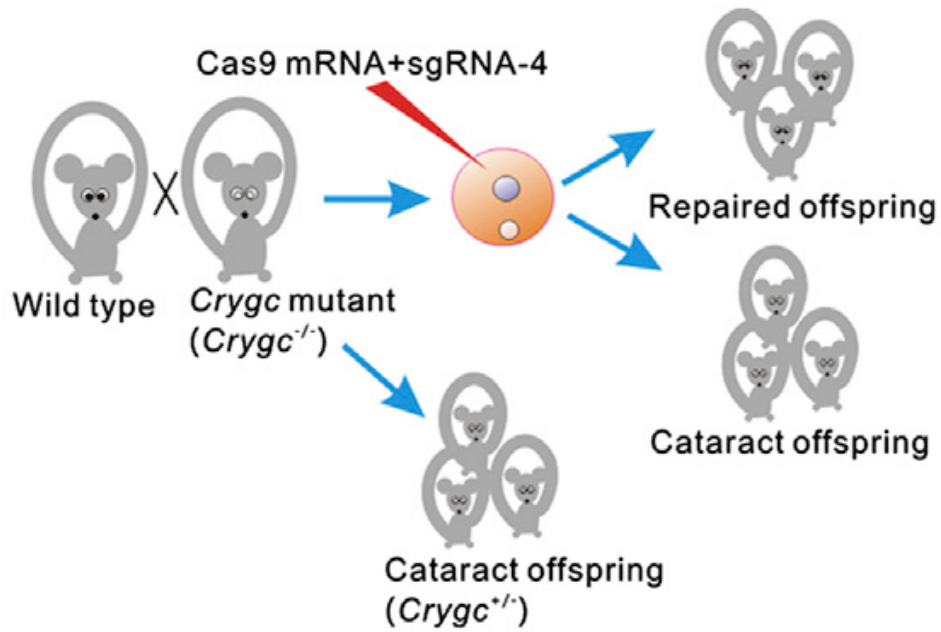
sgRNA	E14 ESC clones		m*Crygc* (*Crygc*$^{+/-}$) ESC clones		
	Cleavage at 1 Allele/Total	Cleavage at 2 Alleles/Total	Cleavage at WT Allele/Total	Cleavage at Mutant Allele/Total	HDR-mediated Repair/Total
sgRNA-1	4/36	0/36	0/36	10/36	7/36
sgRNA-2	23/36	7/36	17/36	25/36	2/36
sgRNA-3	3/36	0/36	0/36	7/36	5/36
sgRNA-4	0/36	0/36	0/36	11/36	16/36
sgRNA-5	4/36	26/36	27/36	26/36	0/36

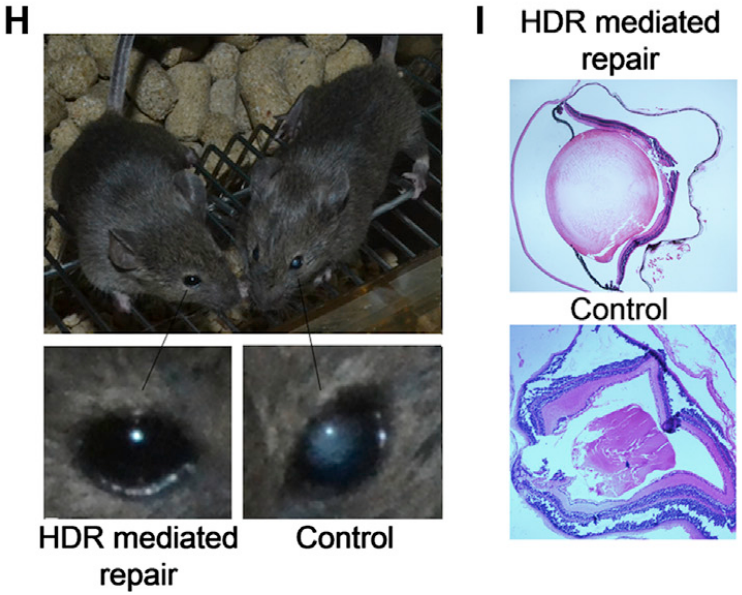
(Wu et al., Cell 2013)

sgRNA4 show high specificity for mCrygc allele and mediates HDR

Can CRISPR/Cas9 be used for correct genetic disorders?

In vivo

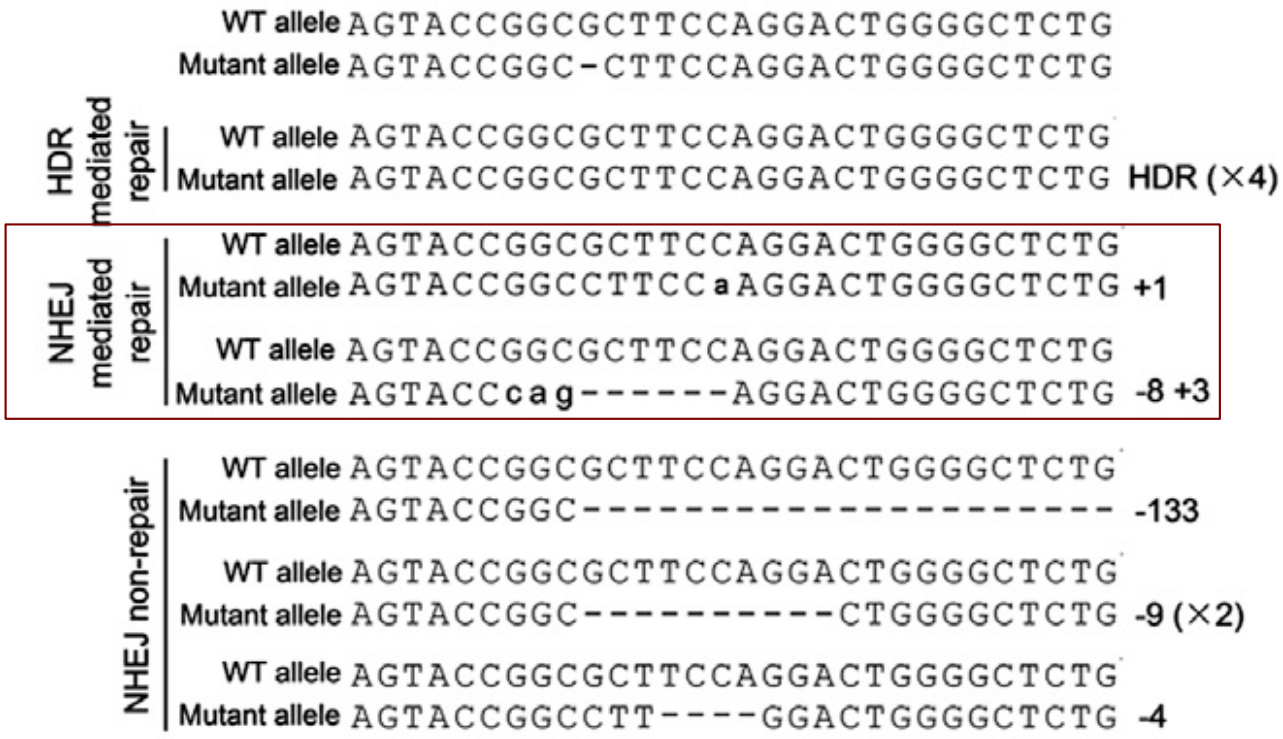




(Wu et al., Cell 2013)

CRISPR/Cas9 system leads to gene correction via HDR using wt allele on the homologous chromosome

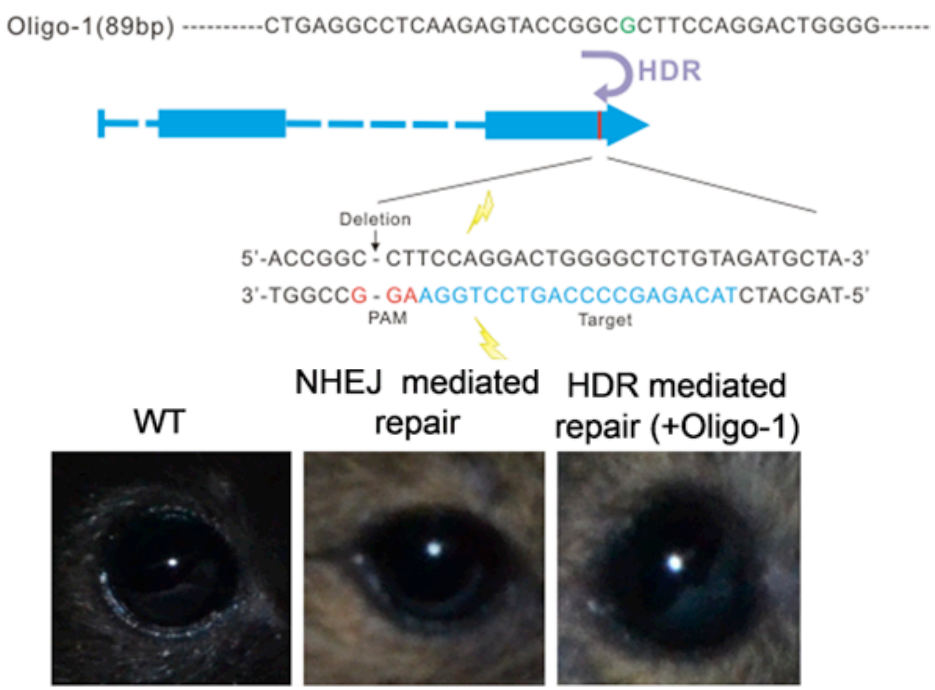
Can CRISPR/Cas9 be used for correct genetic disorders?

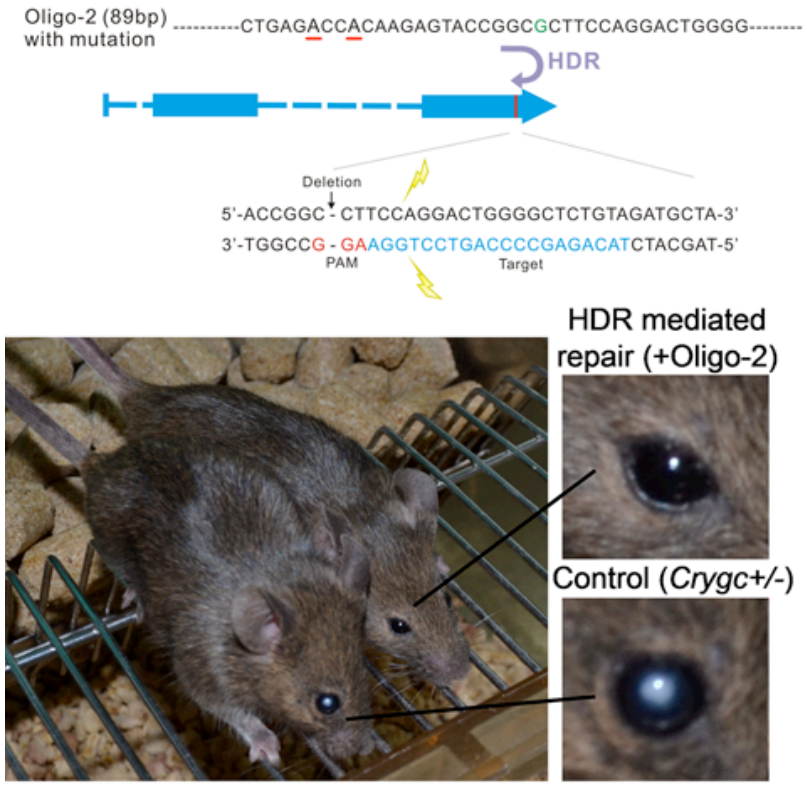


(Wu et al., Cell 2013)

NHEJ events can lead to correct reading frame

Is it possible to improve CRISPR/Cas9 sgRNA4 gene correction?





(Wu et al., Cell 2013)

Insertion of Oligo-1 that mimic wt allele and Oligo-2 that contains specific in frame mutation

Is it possible to improve CRISPR/Cas9 sgRNA4 gene correction?

Table 1. CRISPR-Cas9-Mediated Gene Correction in Cataract Mice

Oligo	Injected Embryos	Blastocysts (Percentage of Injected Embryos)	Transferred Blastocysts	Live-Born Pups	Genetic Modification		NHEJ-Mediated Repair/Nonrepair	HDR-Mediated Repair
					WT allele	mutant allele		
-	172	157 (91%)	135	22	0	10	2/4	4
Oligo-1	245	213 (87%)	178	29	0	14	4/5	5
Oligo-2	221	190 (86%)	159	27	0	12	5/3	4

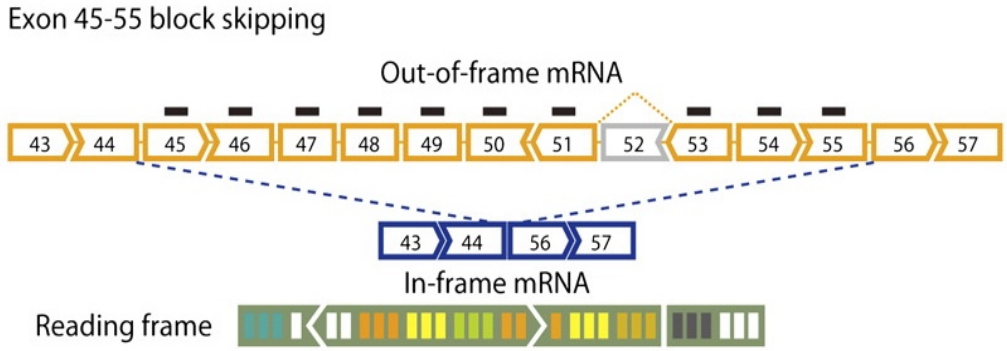
3/4 HDR used oligo-2 donor template

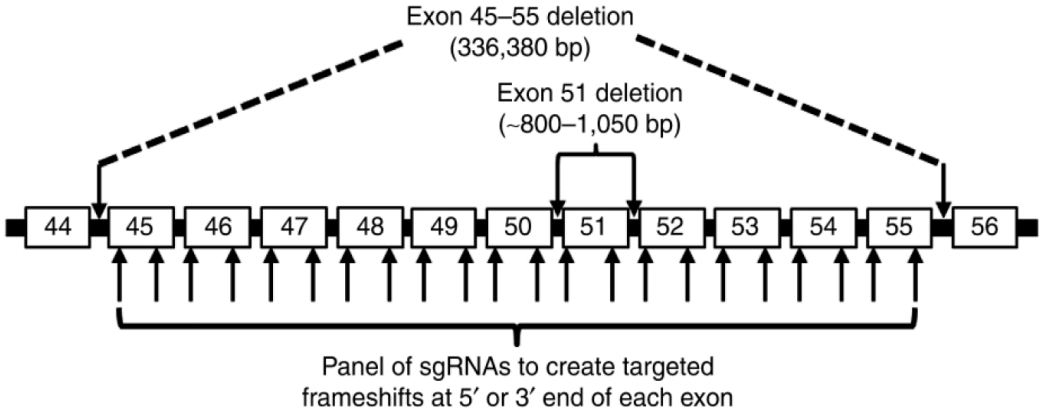
(Wu et al., Cell 2013)

Supplying of exogenous template seems to be not necessary, but it could be useful in homozygous genetic disease

Can CRISPR/Cas9 be used for gene therapy?

Duchenne Muscolar Dystrophy (DMD):
– most common hereditary disease;
– progressive muscle wasting;
– no effective treatment
DMD molecular mechanism:
– out of frame mutations in dystrophin gene (loss of function);
– common deletions in the **exons 45-55** maintain correct reading frame (still functional dystrophin)

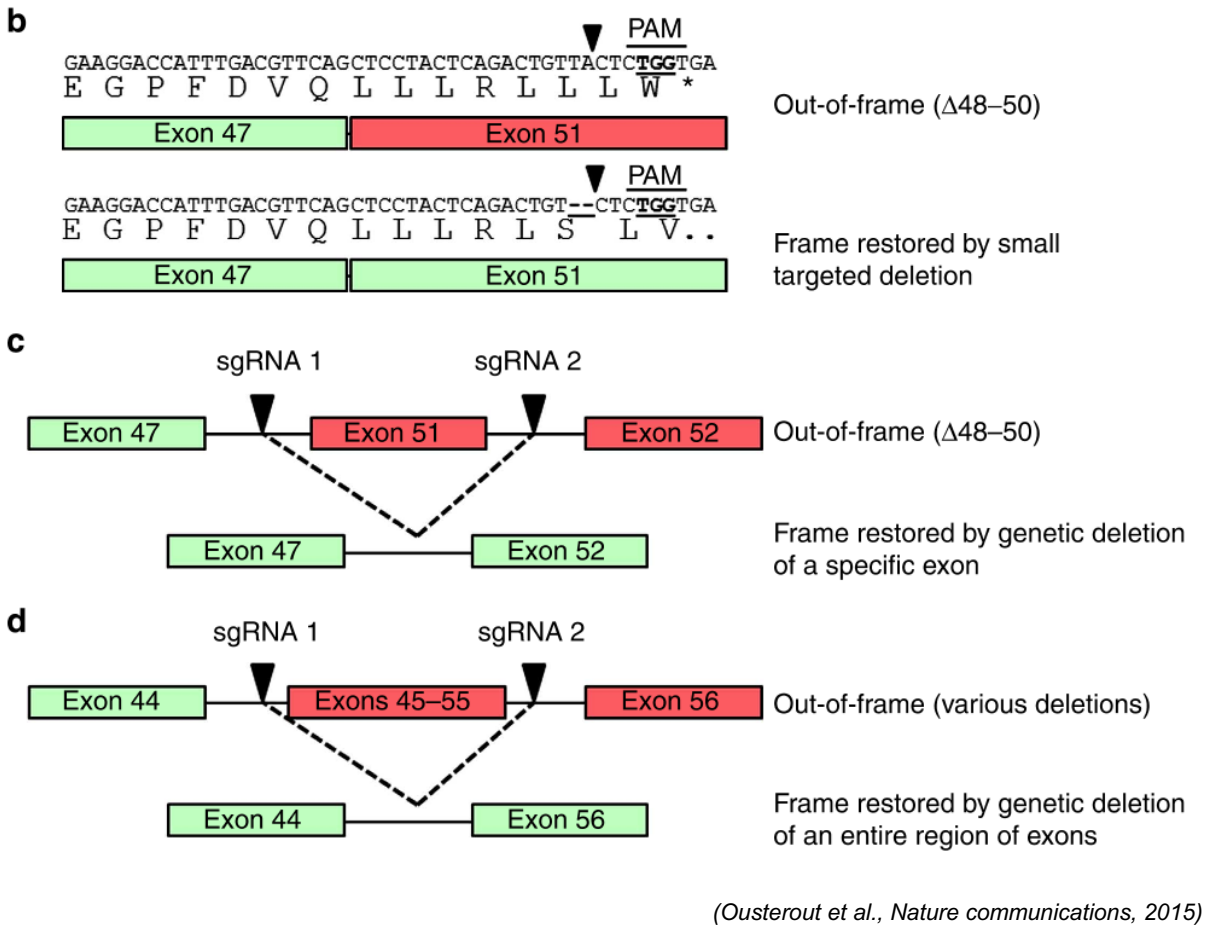




(Ousterout et al. Nature communications 2015)

Targeting hotspot region (45-55 Ex) of dystrophin gene with sgRNA to restore correct reading frame

Can CRISPR/Cas9 be used for gene therapy?



(Ousterout et al., Nature communications, 2015)

sgRNA are designed to restore dystrophin reading frame

Are the sgRNA able to edit the genome?

Table 1 | Measured activity of sgRNAs in human cells.

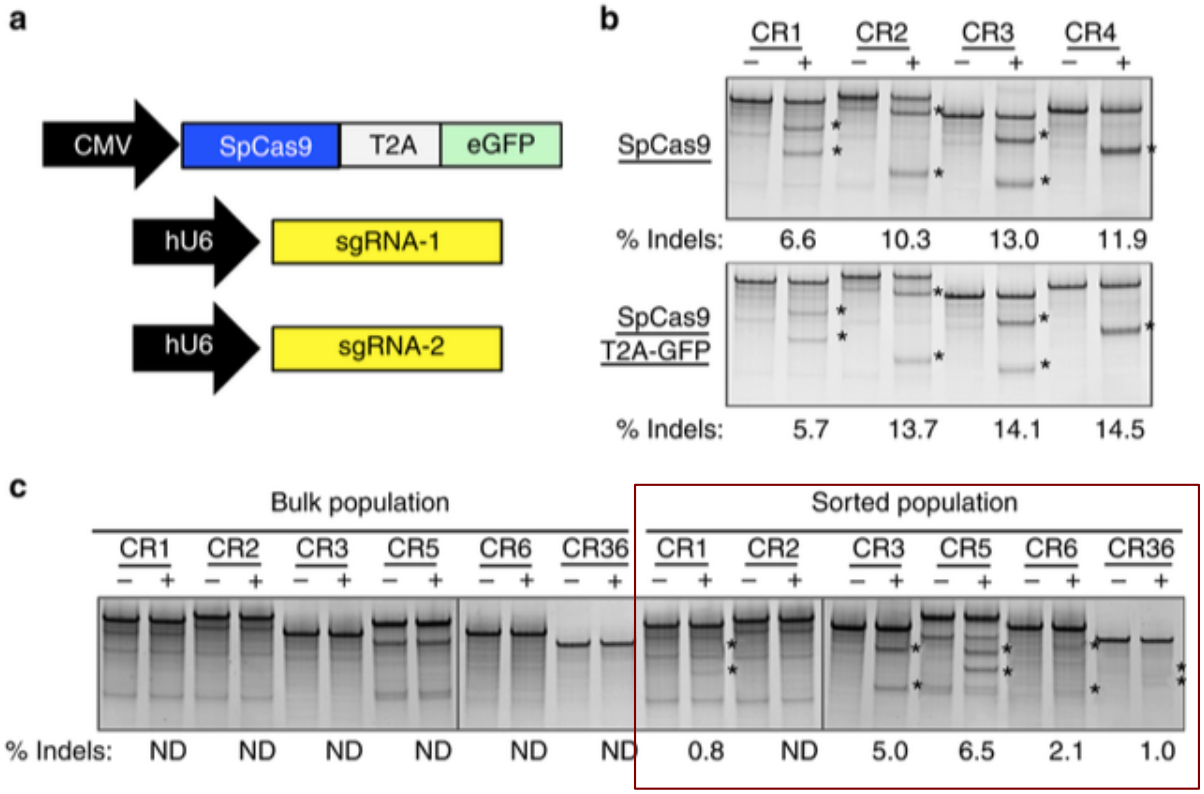
Target	sgRNA #	% Modified alleles at day 3	% Modified alleles at day 10	% Change day 10/day 3
Multiplex deletion of exon 51				
Int 50	CR1	6.6	9.3	41.8
Int 50	CR2	10.3	14.0	36.2
Ex 51	CR4	11.9	14.4	21.3
Int 51	CR5	12.4	13.3	7.8
Multiplex deletion of exons 45–55				
Int 44	CR6	16.1	16.9	4.3
Int 44	CR33	1.3	<1	n.d.
Int 44	CR34	13.2	11.0	−16.6
Int 55	CR7	6.8	7.1	5.3
Int 55	CR35	22.5	20.9	−7.1
Int 55	CR36	26.4	24.7	−6.4
Targeted frameshifts				
Ex 45	CR10	14.9	16.3	9.3
Ex 45	CR11	<1	<1	n.d.
Ex 46	CR12	<1	<1	n.d.
Ex 46	CR13	16.9	18.4	9.2
Ex 47	CR14	17.2	17.6	2.9
Ex 47	CR15	15.4	15.3	−0.9
Ex 48	CR16	11.5	10.9	−5.0
Ex 48	CR17	<1	<1	n.d.
Ex 49	CR18	1.8	2.2	20.1
Ex 49	CR19	33.7	38.4	13.9
Ex 50	CR20	14.9	13.7	−7.6
Ex 50	CR21	24.1	20.8	−13.5
Ex 51	CR3	13.0	16.7	28.0
Ex 51	CR31	18.9	16.9	−10.2
Ex 52	CR22	25.9	20.3	−21.6
Ex 52	CR23	25.2	24.0	−4.8
Ex 53	CR24	24.8	23.6	−4.6
Ex 53	CR25	2.6	2.9	9.5
Ex 54	CR26	24.5	22.0	−10.1
Ex 54	CR27	13.4	12.6	−5.9
Ex 55	CR28	21.6	19.8	−8.4
Ex 55	CR29	19.2	19.6	2.2

sgRNA, single guide RNA.
HEK293Ts were transfected with constructs encoding human codon-optimized SpCas9 and the indicated sgRNA. Each sgRNA was designed to modify the dystrophin gene as indicated. The frequency of gene modification at day 3 or day 10 post transfection was determined by the Surveyor assay. The ratio of measured Surveyor signal at day 3 and day 10 was calculated to quantify the stability of gene editing frequencies for each sgRNA in human cells.

(Ousterout et al., Nature communications, 2015)

29 out of 32 of sgRNA were able to mediate efficient gene modification

Is possible to correct specific mutations in DMD patient myoblasts cell lines?



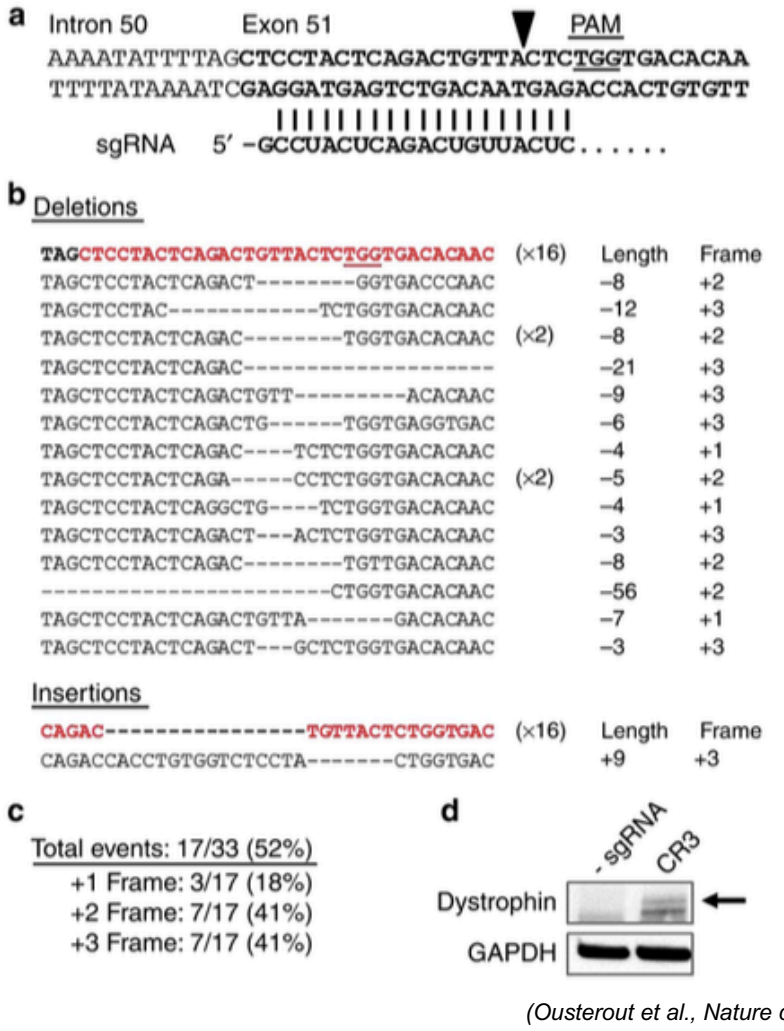
HEK293T cells

DMD myoblasts

(Ousterout et al., Nature communications, 2015)

In DMD sorted cells there is detectable level of sgRNA activity

Are the indels created by NHEJ able to restore dystrophin expression?



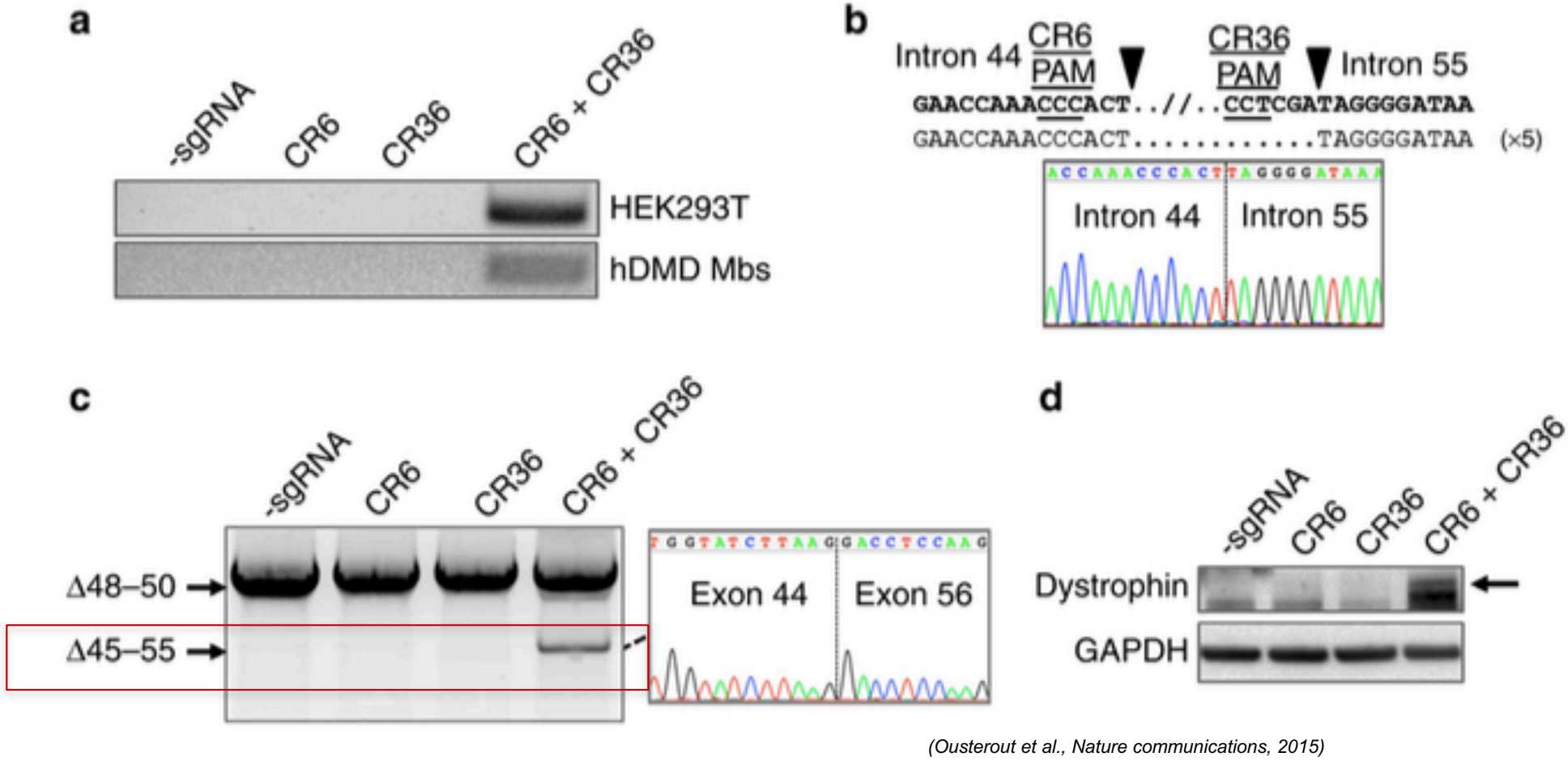
Sanger sequencing

differentiated DMD myoblasts

(Ousterout et al., Nature communications, 2015)

sgRNA CR3 is able to restore dystrophin reading frame by the introduction of indels within exon 51

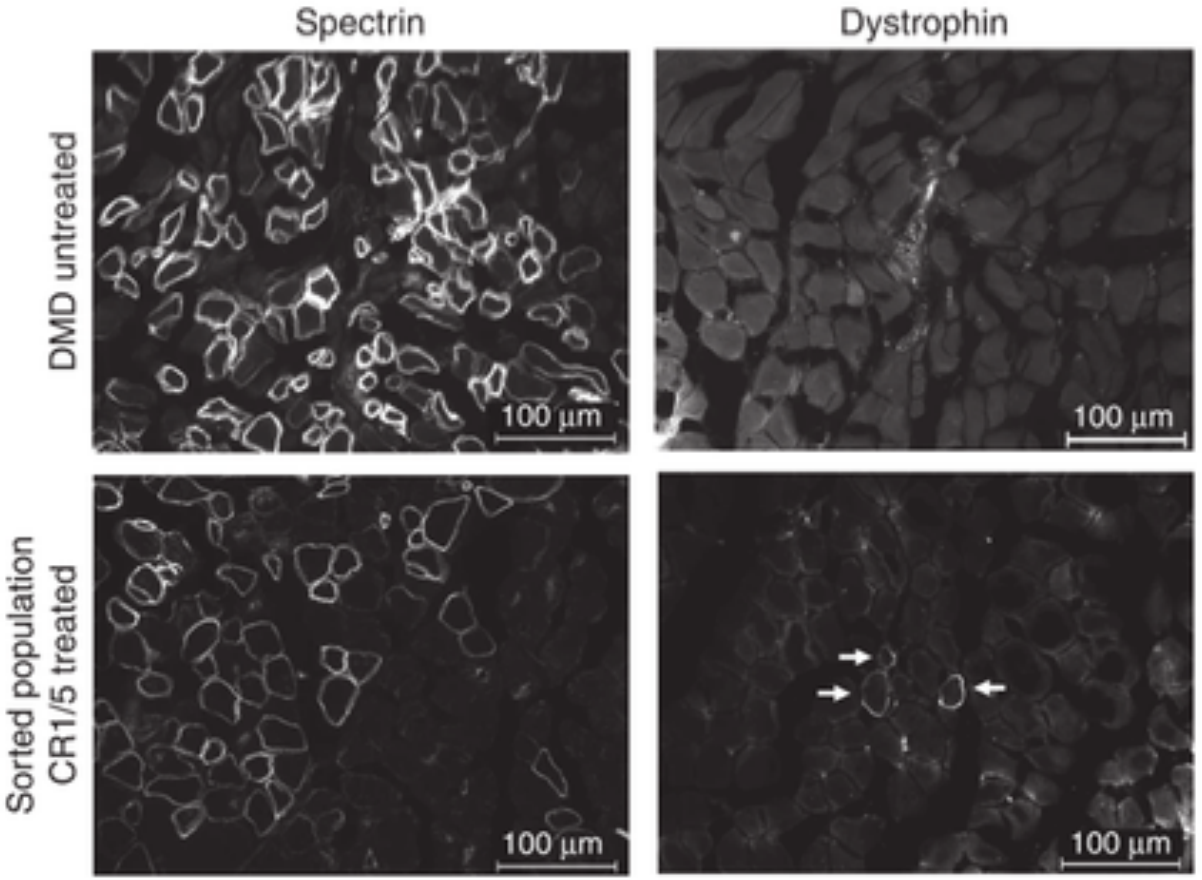
Is it possible to develop a single method that can address different common patients deletions?



(Ousterout et al., Nature communications, 2015)

Multiplexed CRISPR/Cas9 is able to generate efficient deletion of the exon 45-55 locus

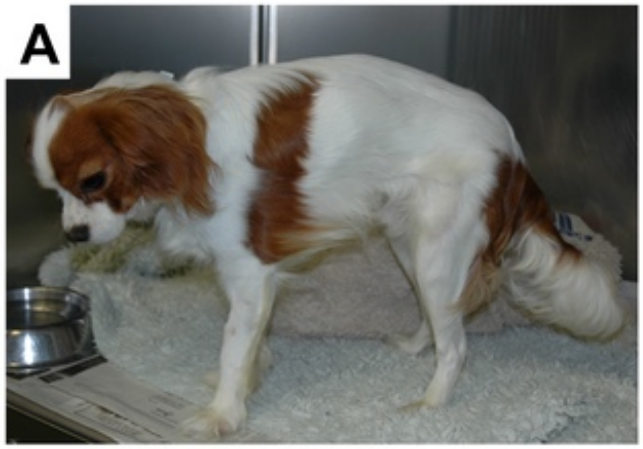
Can CRISPR/Cas9 be used for correct DMD *in vivo*?



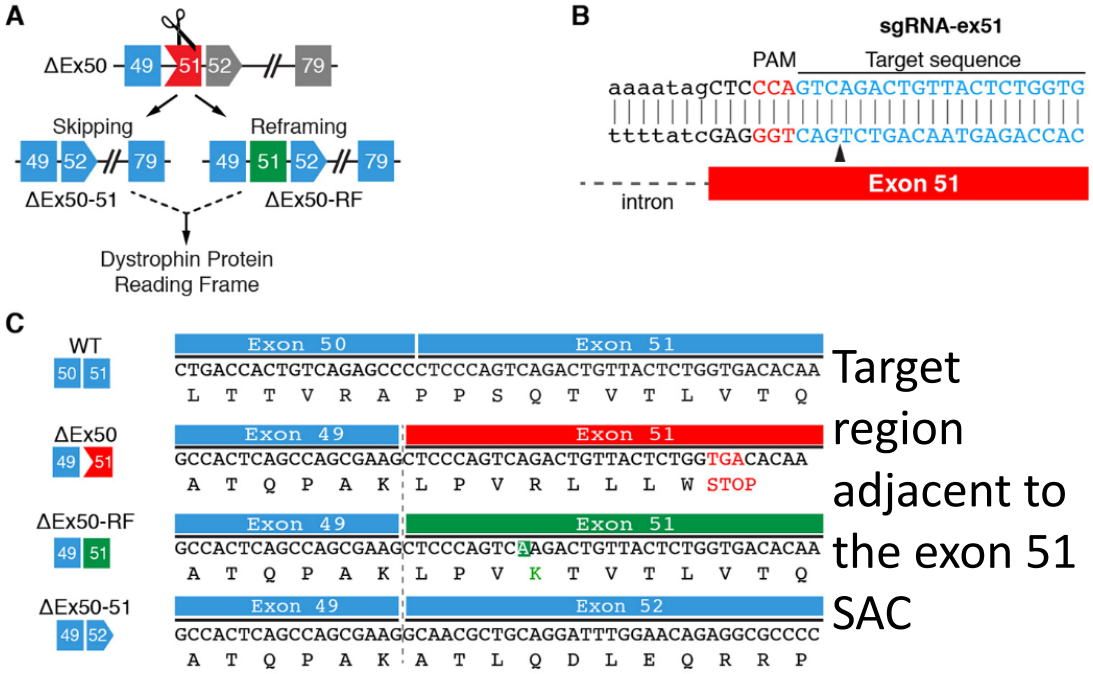
(Ousterout et al., Nature communications, 2015)

DMD sgRNAs treated myoblasts implanted in nude mice express human spectrin and dystrophin

Can CRISPR/Cas9 be used for correct DMD *in vivo*?



(Walmsley G.L., et al., PlosOne, 2010)



Target region adjacent to the exon 51 SAC

(Amoasii L. et al., Science, 2018)

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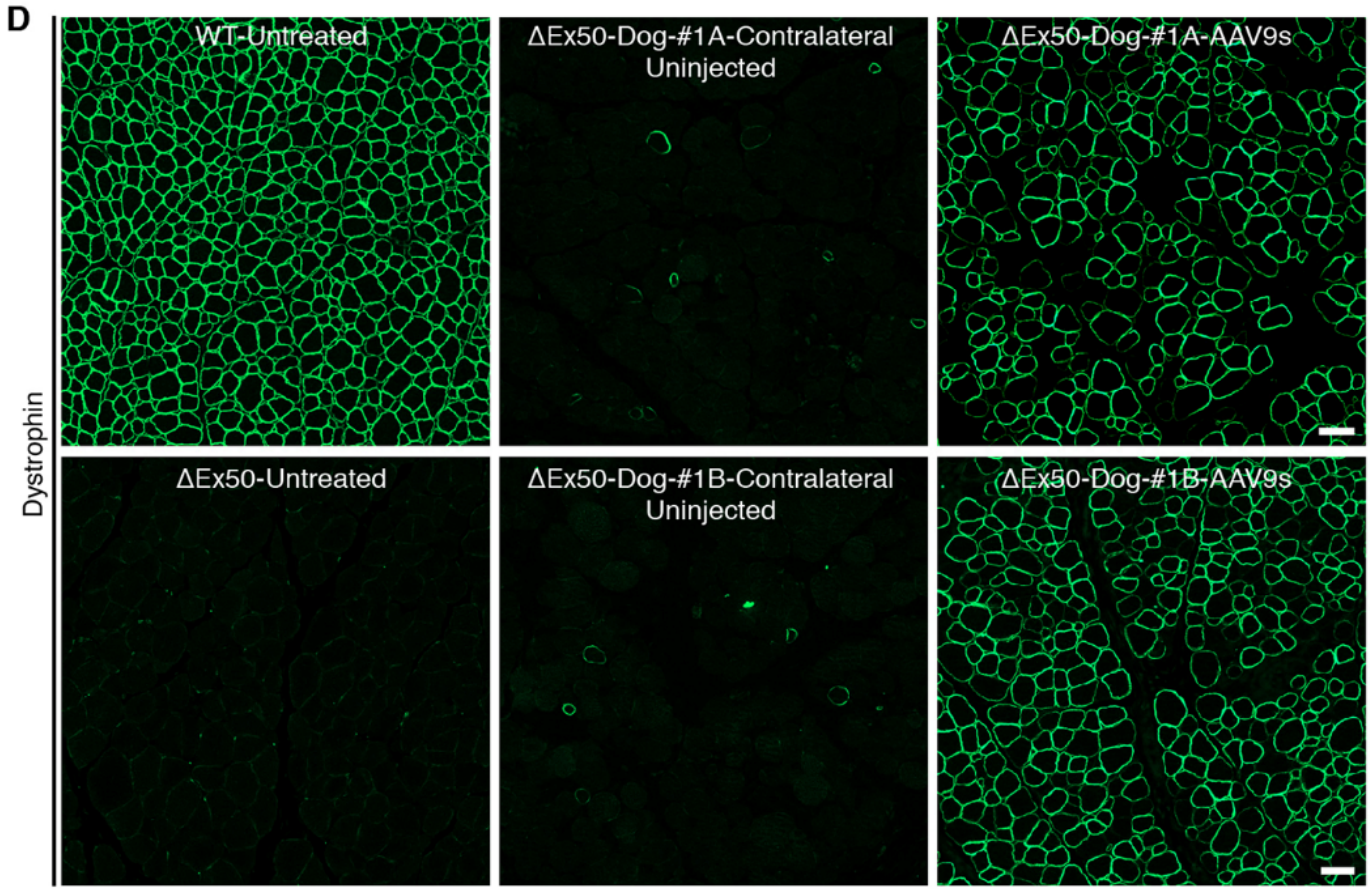

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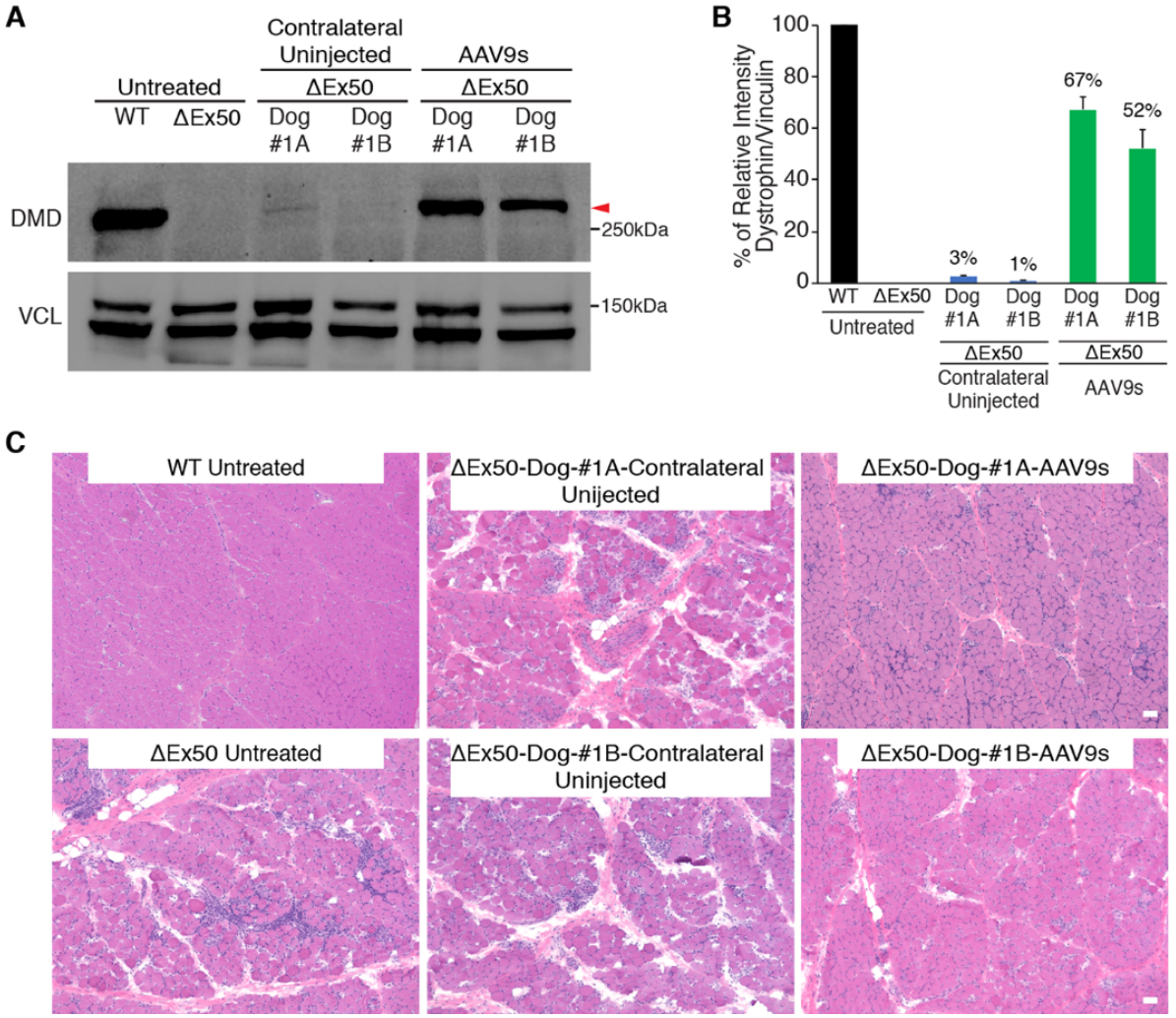

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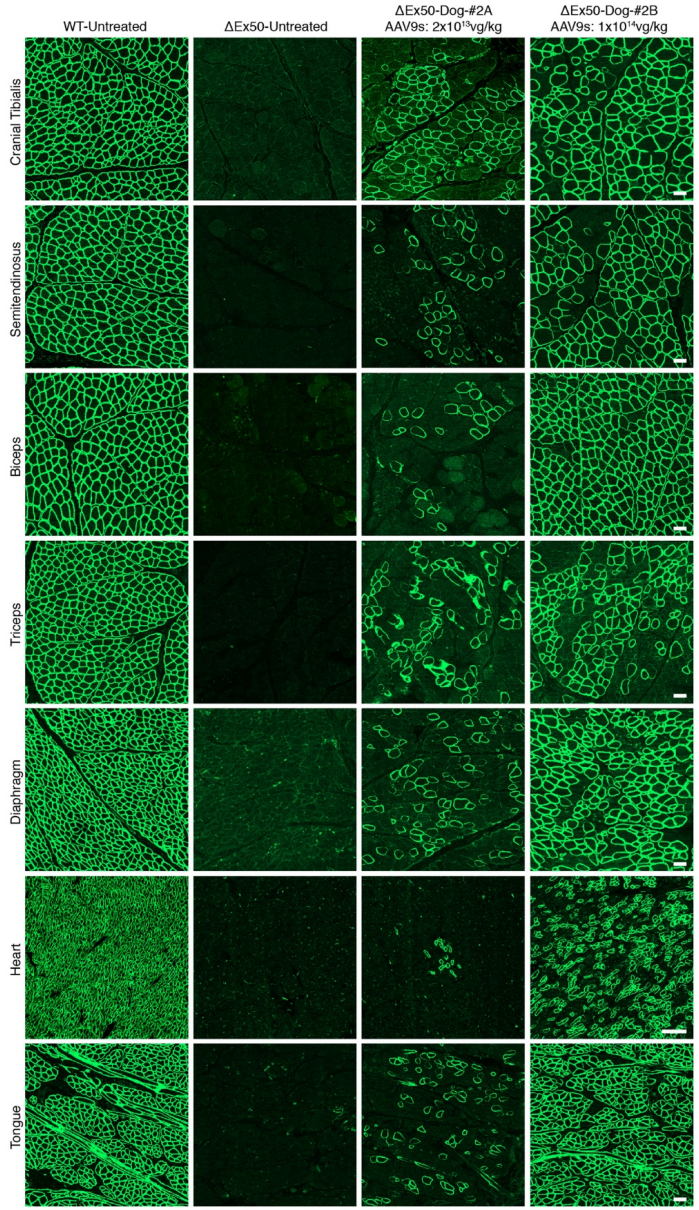
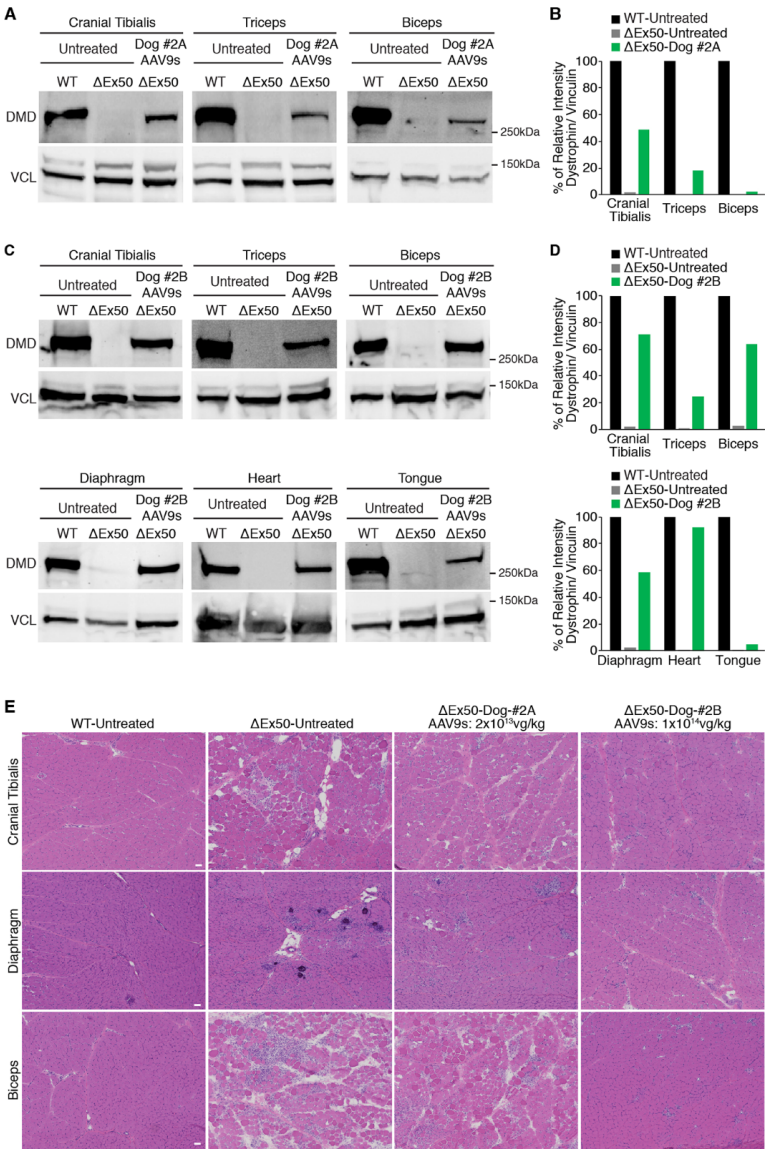
Can CRISPR/Cas9 be used for correct DMD *in vivo*?



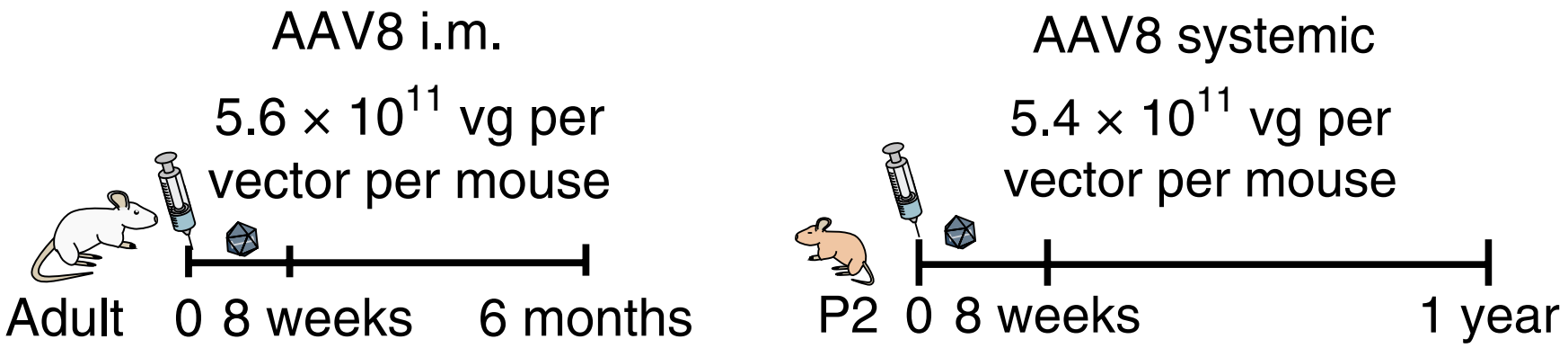
(Amoasii L. et al., Science, 2018)

CRISPR/Cas9 can be used for correct DMD *in vivo*?



(Amoasii L. et al., Science, 2018)

Can CRISPR/Cas9 sustain long term dystrophin expression?



(Nelson C.E. et al., Nature medicine letters, 2019)

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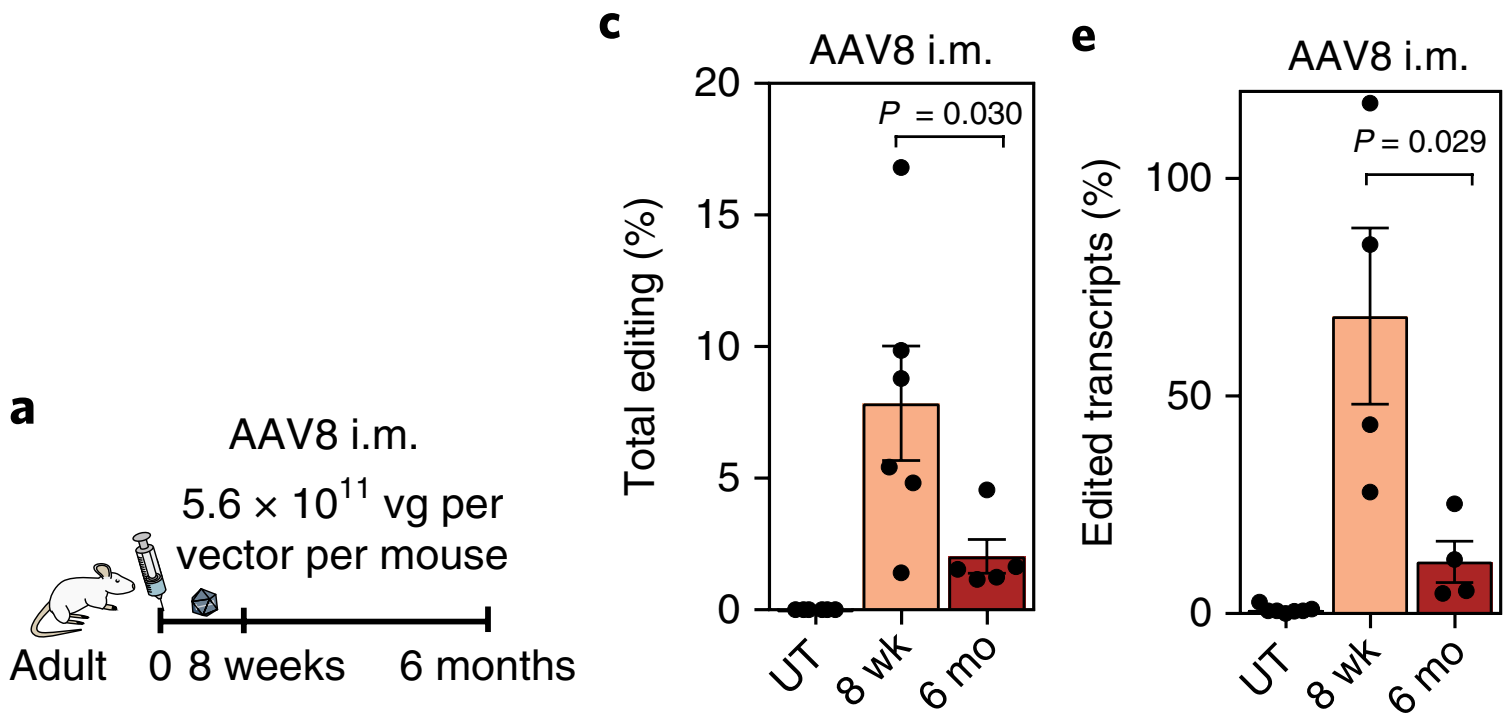

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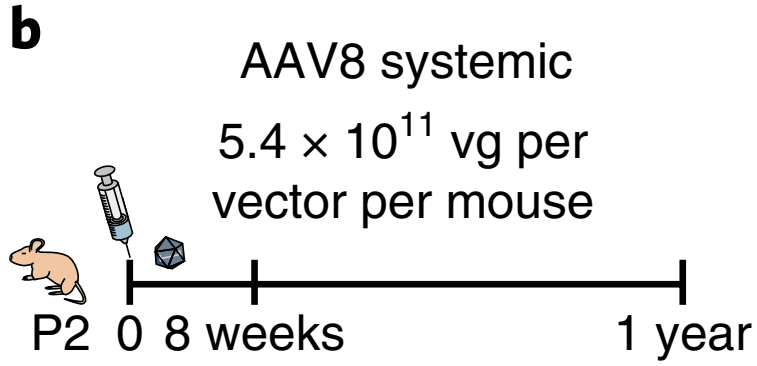

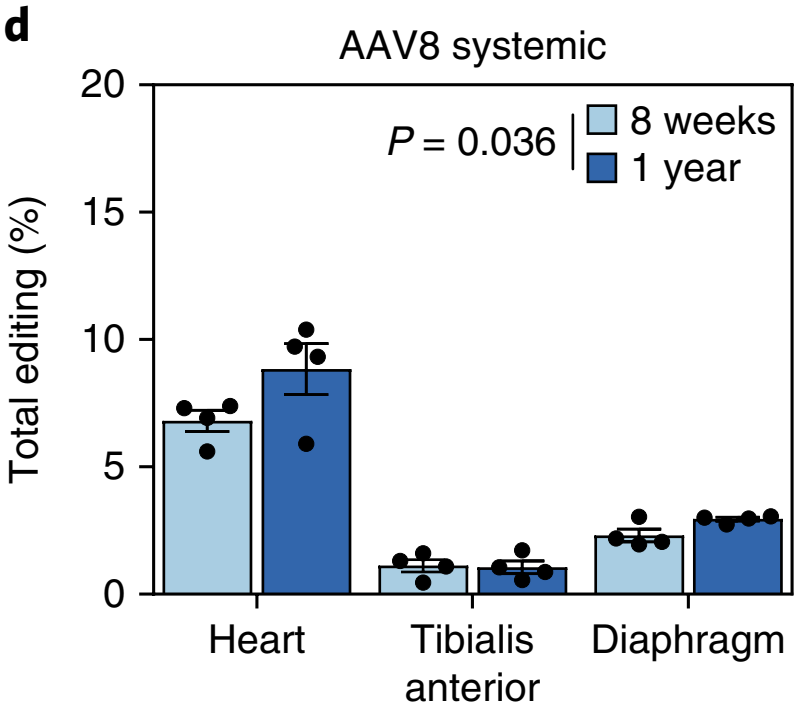

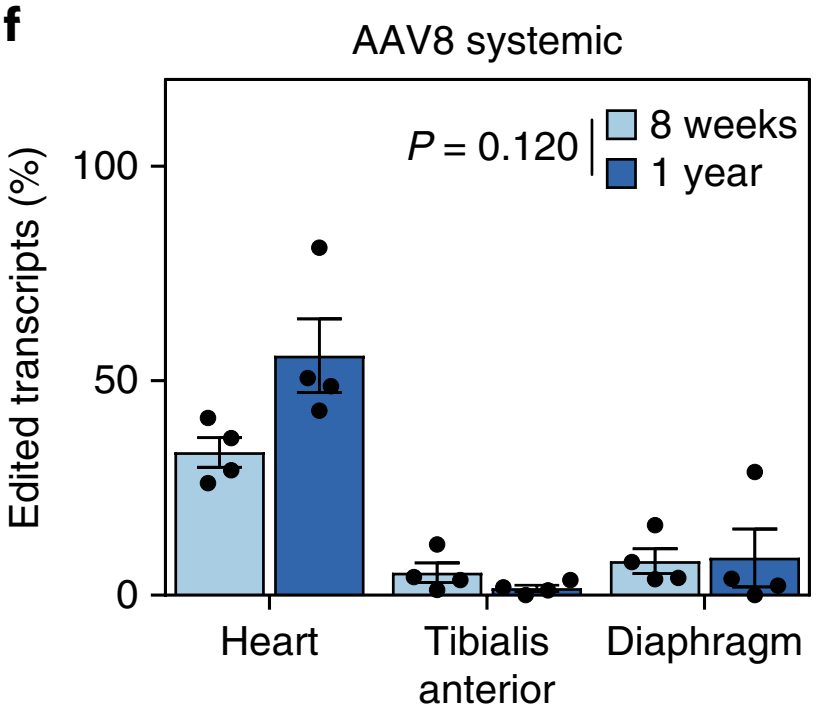

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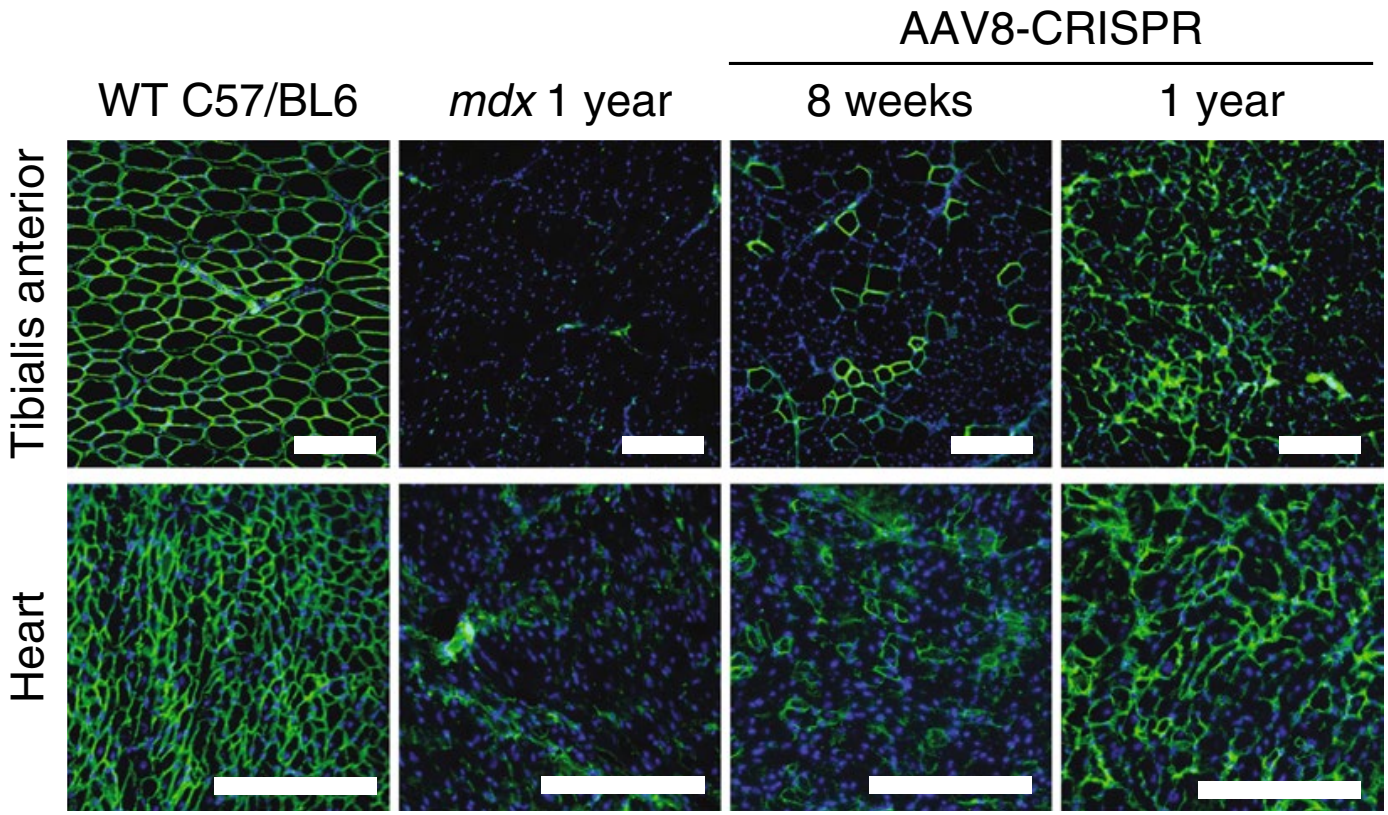

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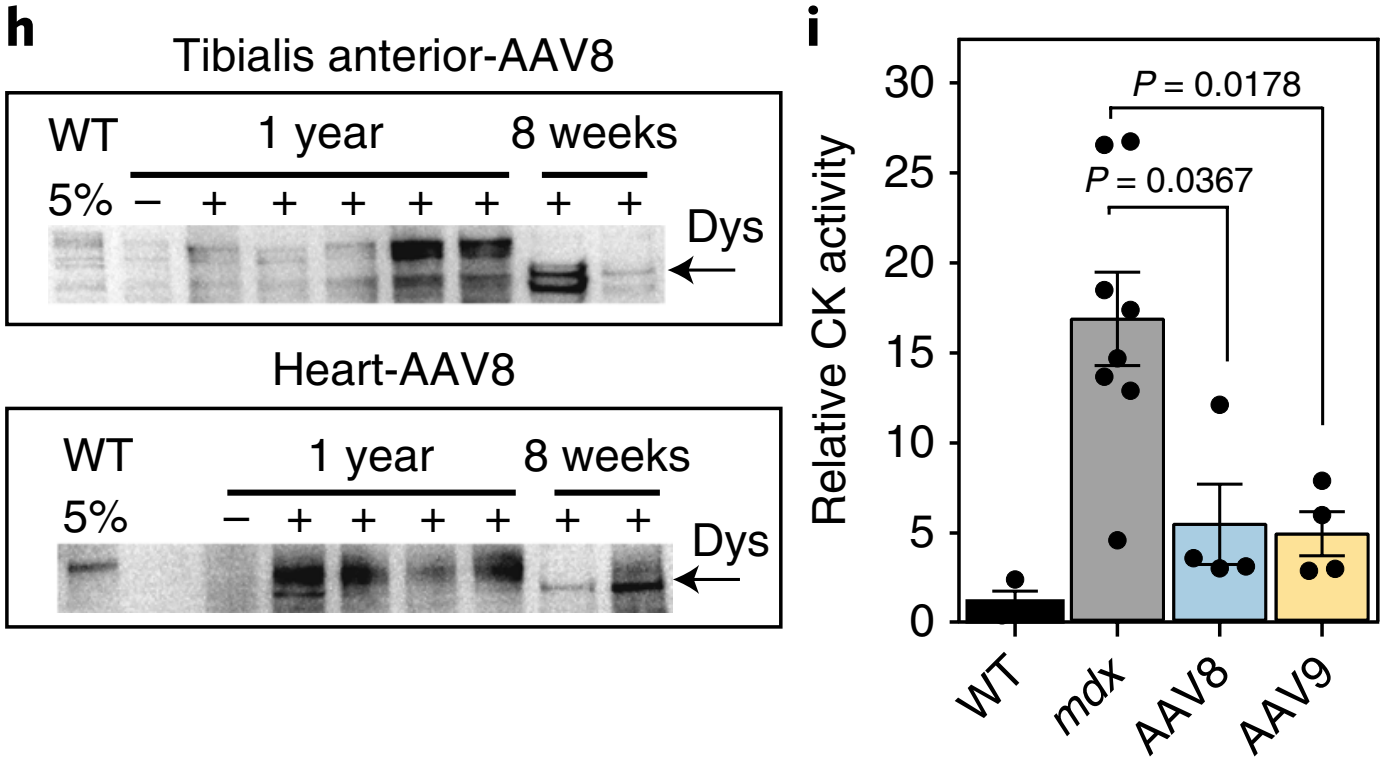

(Nelson C.E. et al., Nature medicine letters, 2019)

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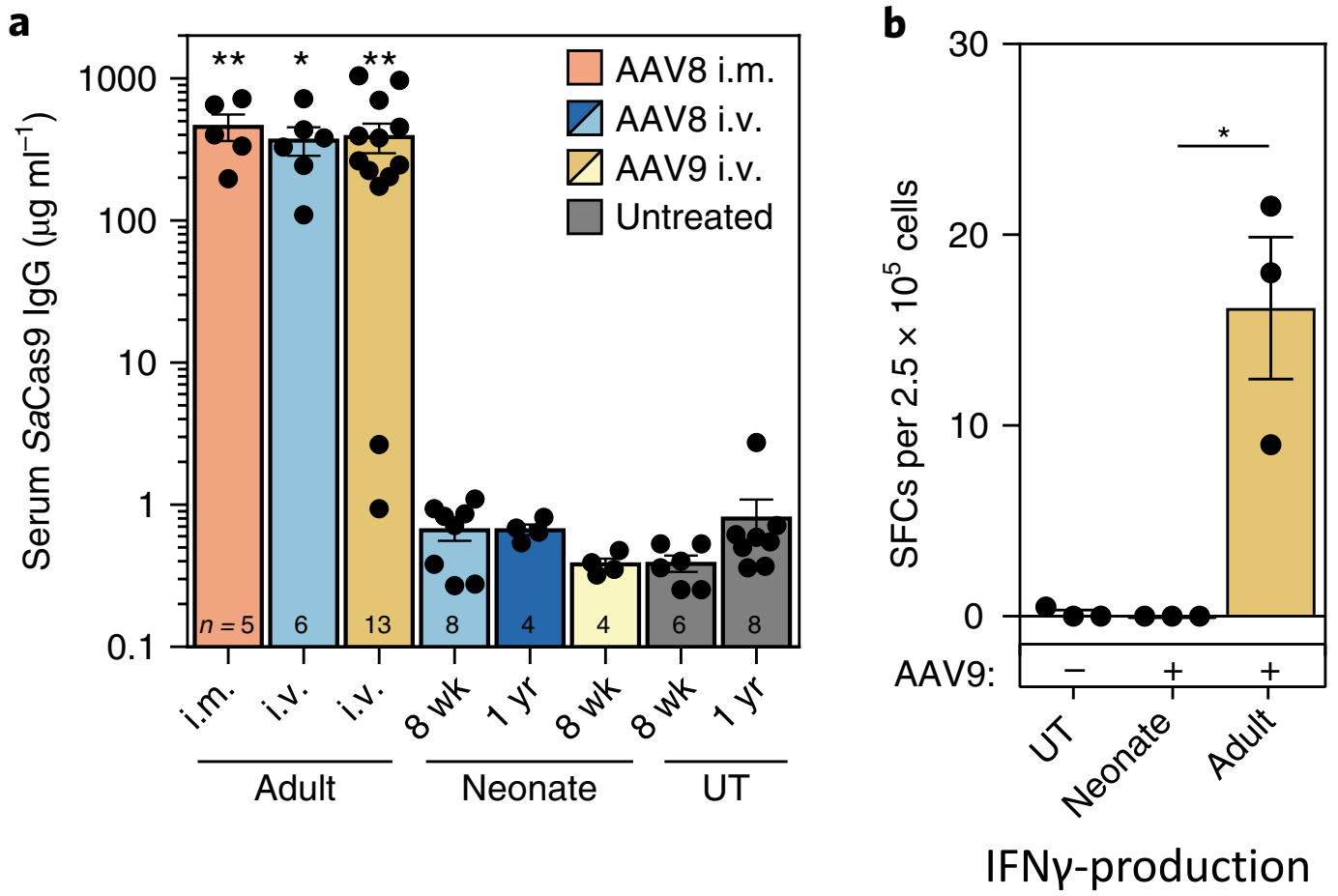

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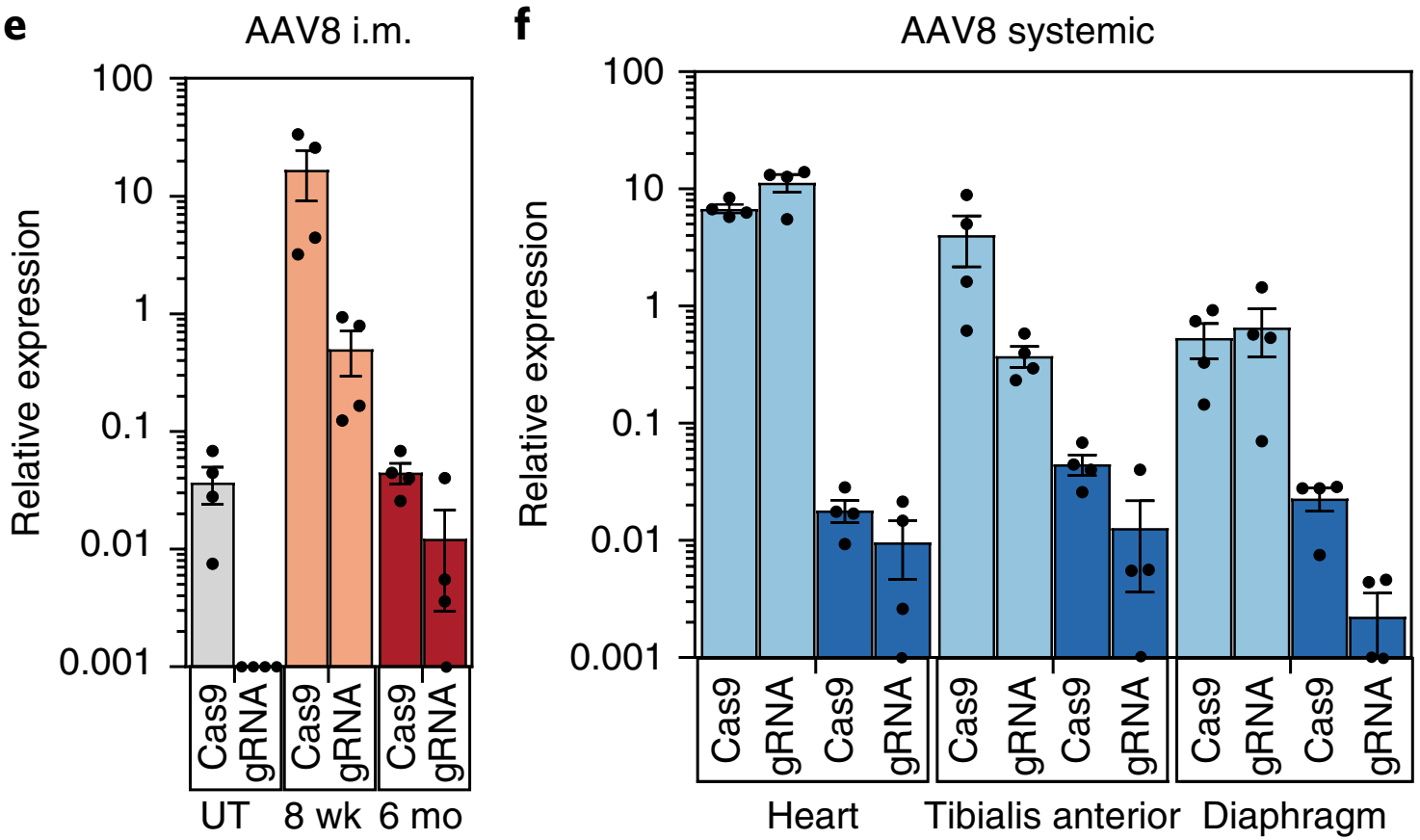

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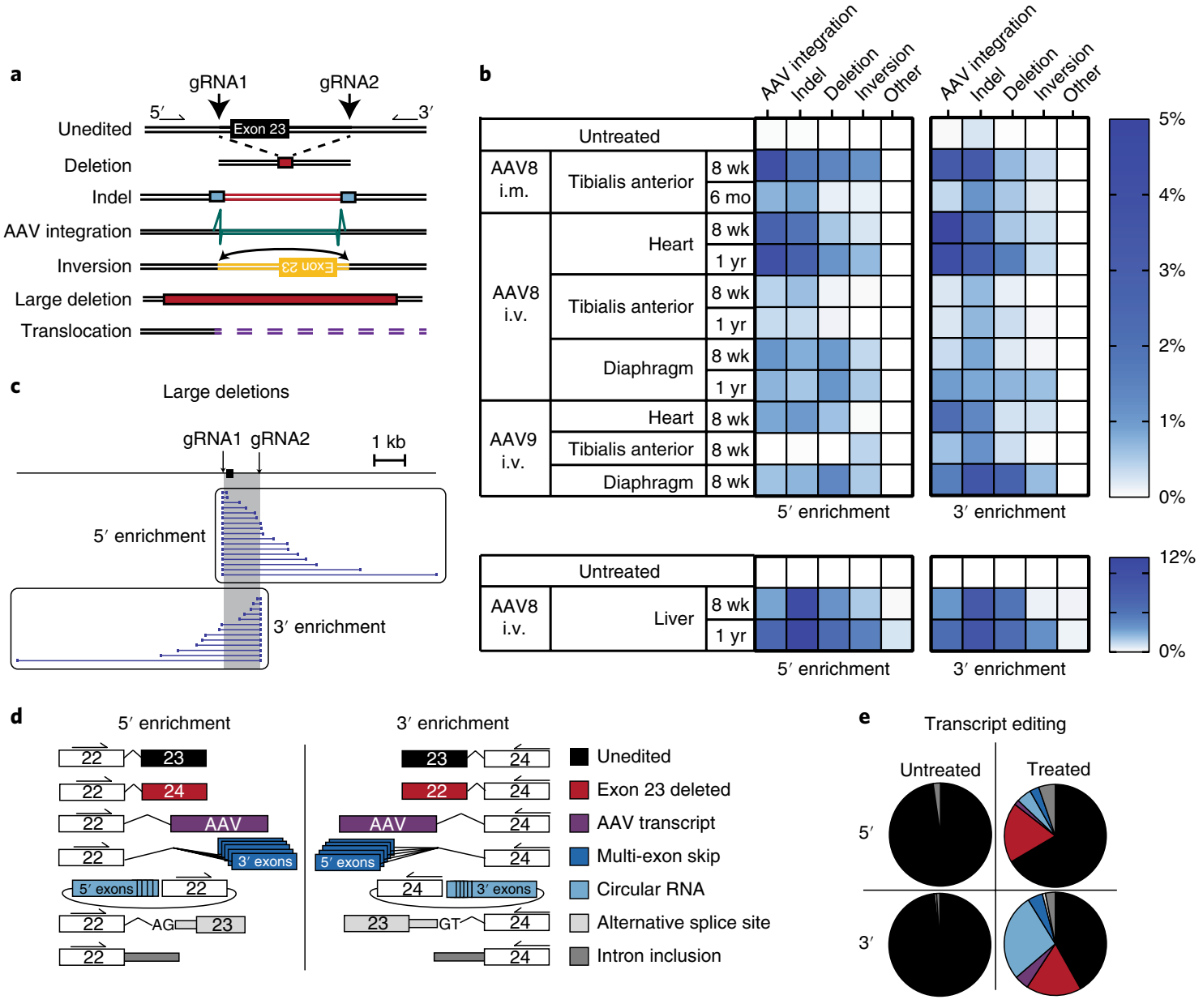
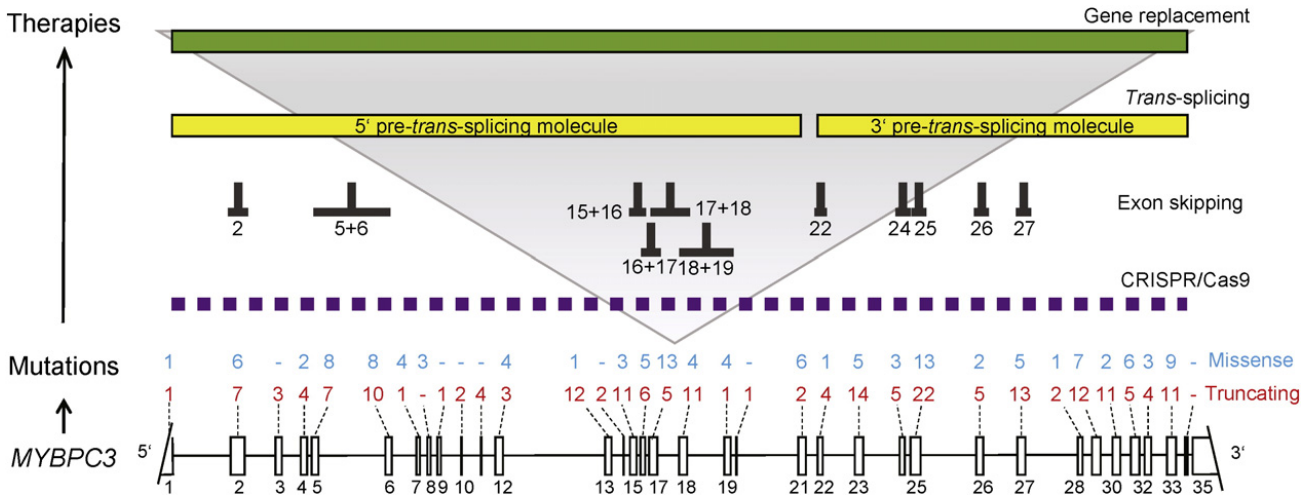


(Nelson C.E. et al., Nature medicine letters, 2019)

CRISPR/Cas9 correction in human embryos?

CRISPR/Cas9 correction in human embryos?

MYBPC3 mutations account for ~40% of all genetic defects causing hypertrophic cardiomyopathy

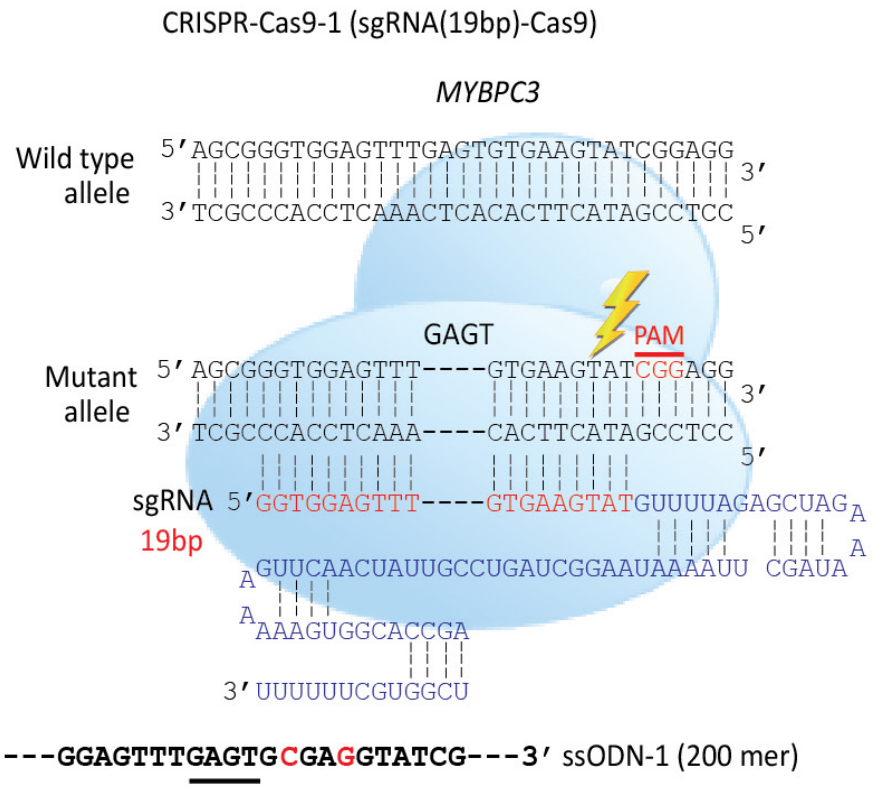


(Carrier L. et al., Gene review, 2015)

Heart failure in healthy individuals
Mostly autosomal dominant

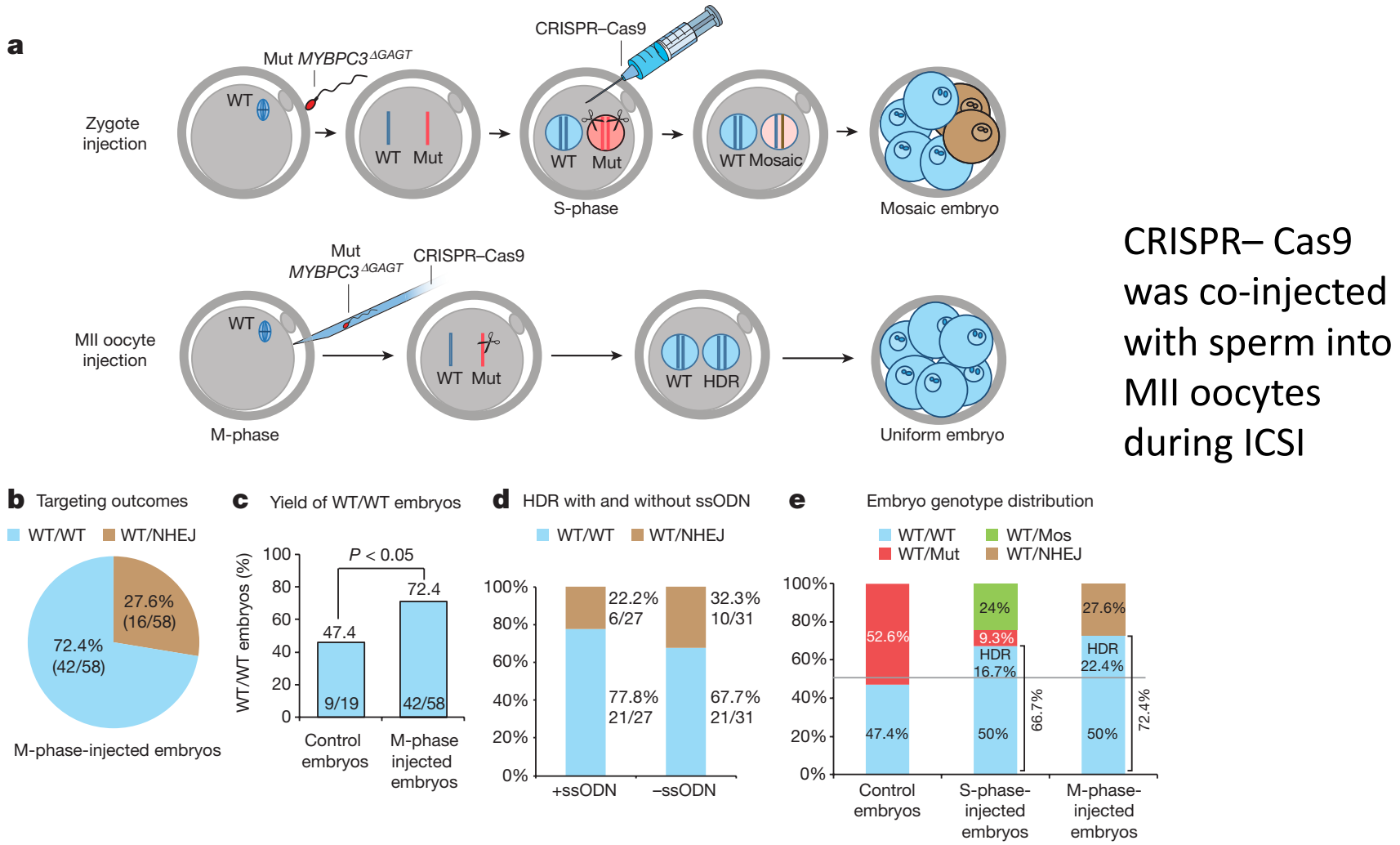
CRISPR/Cas9 correction in human embryos?

correct a heterozygous dominant 4 bp deletion in MYBPC3 ($MYBPC3^{\Delta GAGT}$)



(Ma H., et al., Nature 2017)

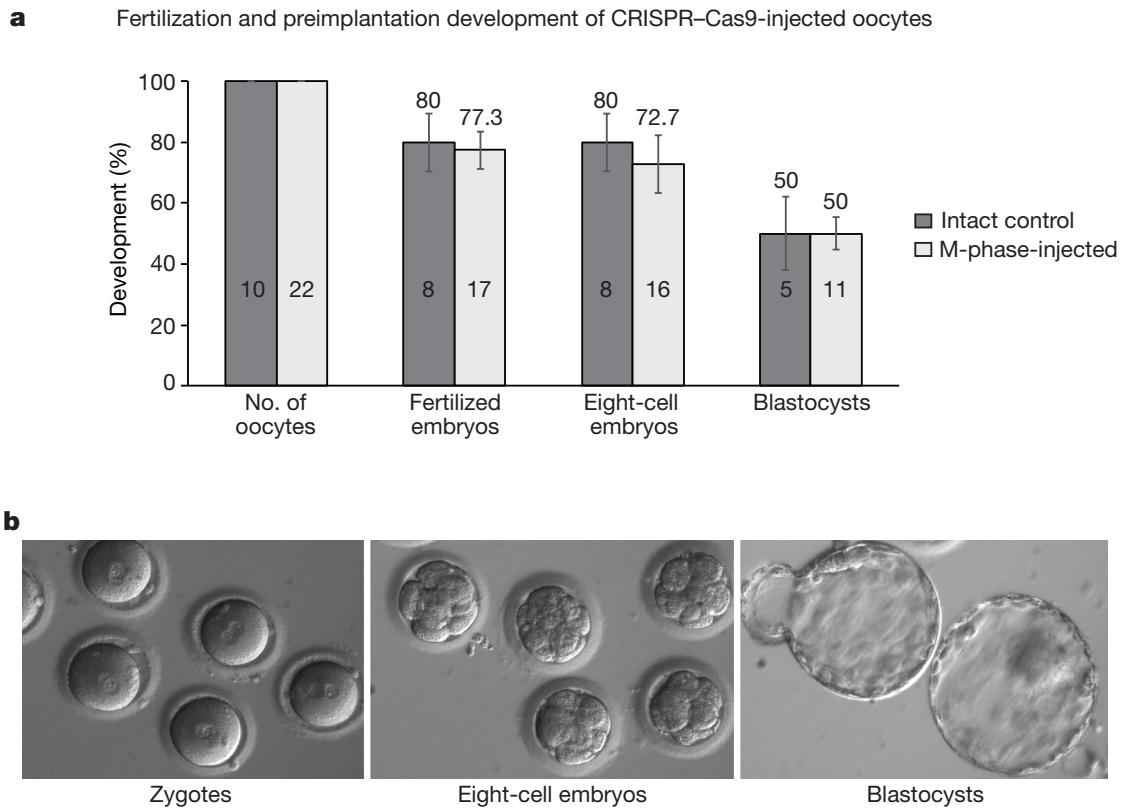
CRISPR/Cas9 correction in human embryos?



CRISPR– Cas9 was co-injected with sperm into MII oocytes during ICSI

(Ma H., et al., Nature 2017)

CRISPR/Cas9 correction in human embryos?



Origin and genotypes of ES cells derived from CRISPR-Cas9 injected embryos

ES cell line designation	Treatment	Karyotype	On target genotype	Egg donor
ES-WT1	M-phase injection	46,XX	WT/WT	Egg donor 1
ES-WT2	M-phase injection	46,XX, inv(10)(p11.2q21.2)	WT/WT	Egg donor 2
ES-WT3	M-phase injection	46,XY, inv(10)(p11.2q21.2)	WT/WT	Egg donor 2
ES-WT4	M-phase injection	46,XX	WT/WT	Egg donor 2
ES-Mut1	M-phase injection	46,XX	WT/NHEJ	Egg donor 1
ES-Mut2	M-phase injection	46,XX	WT/NHEJ	Egg donor 2
ES-C1	Intact control	46,XY, inv(10)(p11.2q21.2)	WT/WT	Egg donor1

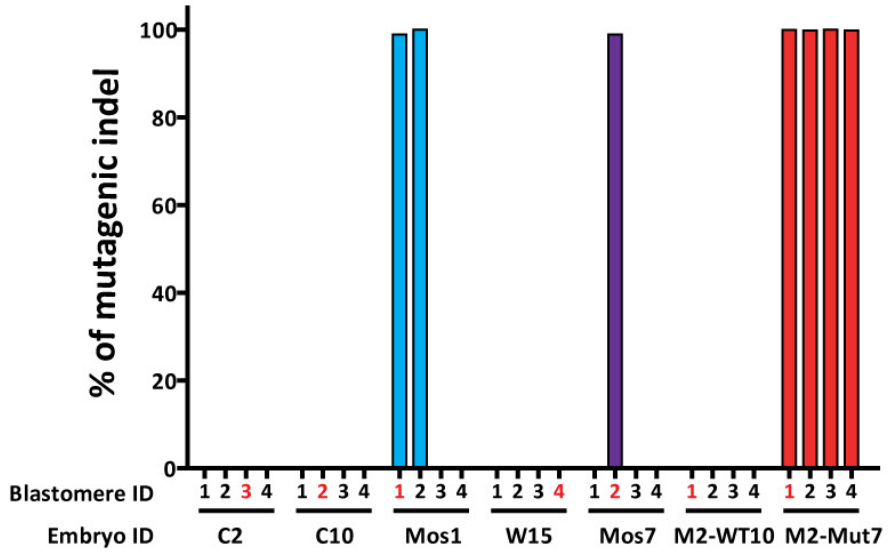
Corrected ES from blastocysts

(Ma H., et al., Nature 2017)

CRISPR/Cas9 correction in human embryos?

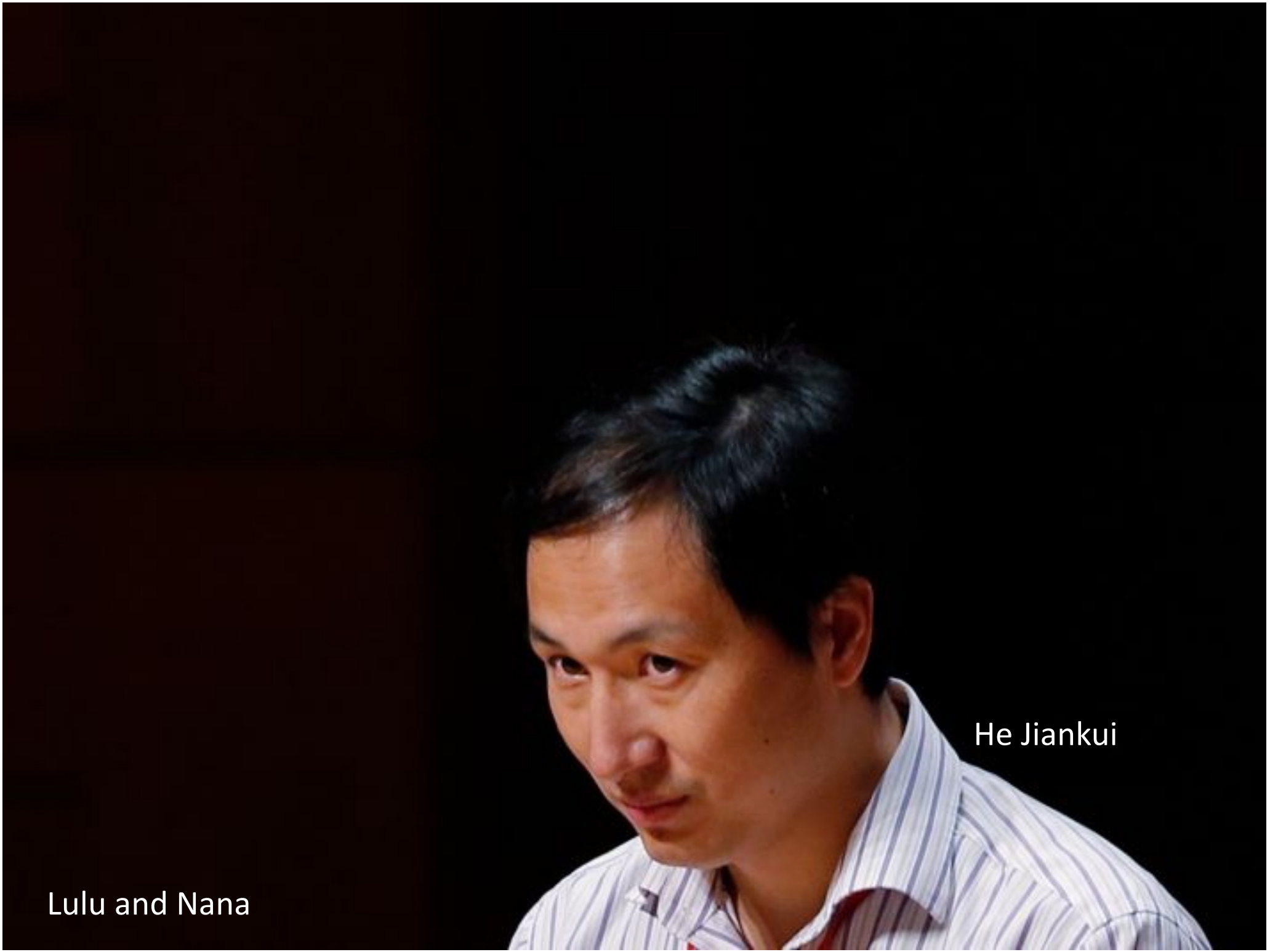
No off targets events analyzed by:

- Whole genome deep sequencing;
- BLESS
- GUIDE-Seq
- Digenome-Seq



(Ma H., et al., Nature 2017)

Έτσι, δεν γνωρίζω’
Socrate


He Jiankui
Lulu and Nana

ETHICS

RECOMMENDATION 5-1. Clinical trials using heritable genome editing should be permitted only within a robust and effective regulatory framework that encompasses

- **the absence of reasonable alternatives;**
- **restriction to preventing a serious disease or condition;**
- **restriction to editing genes that have been convincingly demonstrated to cause or to strongly predispose to that disease or condition;**
- **restriction to converting such genes to versions that are prevalent in the population and are known to be associated with ordinary health with little or no evidence of adverse effects;**
- **the availability of credible preclinical and/or clinical data on risks and potential health benefits of the procedures;**
- **ongoing, rigorous oversight during clinical trials of the effects of the procedure on the health and safety of the research participants;**
- **comprehensive plans for long-term, multigenerational followup that still respect personal autonomy;**
- **maximum transparency consistent with patient privacy;**
- **continued reassessment of both health and societal benefits and risks, with broad ongoing participation and input by the public; and**
- **reliable oversight mechanisms to prevent extension to uses other than preventing a serious disease or condition.**

National Academies of Sciences, Engineering, and Medicine, Human Genome Editing: Science, Ethics and Governance (National Academies Press, Washington, DC, 2017).

HUMAN GENE EDITING

March 2015: Chinese researchers become the first to edit genes in a human embryo.

June 2016: He Jiankui launches a project to edit genes in human embryos, with the goal of a live birth.

March 2017: He starts recruiting couples (each with an HIV-positive father) for the experiments.

Early November 2018: Gene-edited twin girls are reportedly born, and a second pregnancy with a third gene-edited embryo is established.

25–26 November 2018: The *MIT Technology Review* reveals the existence of the research programme; the Associated Press quickly goes public with the story of the girls' birth.

28 November 2018: He offers details about his work at a gene-editing summit in Hong Kong and is roundly criticized.

November–December 2018: China's National Health Commission orders an investigation into He's work.

January 2019: He is censured by the Guangdong health ministry and fired from his university.

18 March 2019: A World Health Organization committee will meet to set guidelines for human gene editing.

August 2019: Third gene-edited baby expected.

(Cyranoski D., March 2019, Nature)

NEWS · 12 DECEMBER 2018 nature

Baby gene edits could affect a range of traits

Gene targeted for its role in HIV is linked to increased severity of other infectious diseases — and has implications for learning in mice.

The CCR5 protein is expressed on the surface of some immune cells, and HIV takes advantage of it to sneak into the cells. In 1996, scientists identified a mutation, known as *CCR5-Δ32*, that makes carriers highly resistant to HIV

found naturally in about 10% of Europeans

Scientists analysing his presentation slides say that, instead, He seems to have produced three different mutations in the girls. It is expected that these mutations will have disabled the gene.

Slides from He's presentation suggest that both copies of the gene were disabled in one of the twins. The other twin seems to have at least one working copy

NEWS · 12 DECEMBER 2018 nature

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CCR5 also helps to protect the lungs, liver and brain during some other serious infections and chronic diseases.

Philip Murphy, an immunologist at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, has done experiments that show that people without a functional CCR5 gene are four times more likely than those with the gene to develop these serious conditions. “CCR5 deficiency is not benign,” he says.

Influenza could also pose a greater risk to the twins . Work in mice has shown that the CCR5 protein helps to recruit key immune cells to fight the virus in the lungs

Scientists have also found that, among people with multiple sclerosis, those with the CCR5-Δ32 deletion are twice as likely to die early than are people without the mutation

NEWS · 12 DECEMBER 2018 nature

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Gene targeted for its role in HIV is linked to increased severity of other infectious diseases — and has implications for learning in mice.

But, on the basis of the information in the consent form, none of these effects seems to have been communicated to the parents of the girls, or to other couples that participated in He's experiments.

He's informed-consent procedure "was a disaster", says Megan Allyse, a bioethicist at the Mayo Clinic in Rochester, Minnesota.

He has not responded to Nature's multiple requests for comment

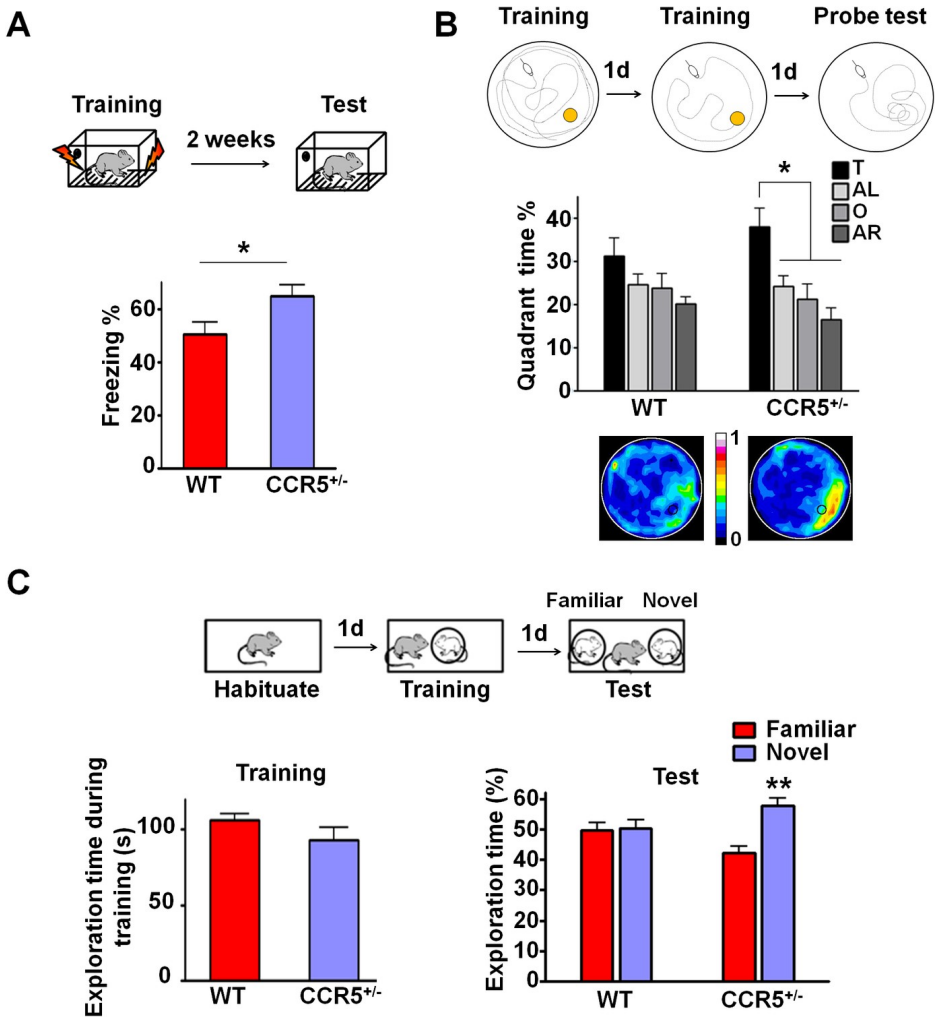
NEWS · 12 DECEMBER 2018 nature

Baby gene edits could affect a range of traits

Gene targeted for its role in HIV is linked to increased severity of other infectious diseases — and has implications for learning in mice.

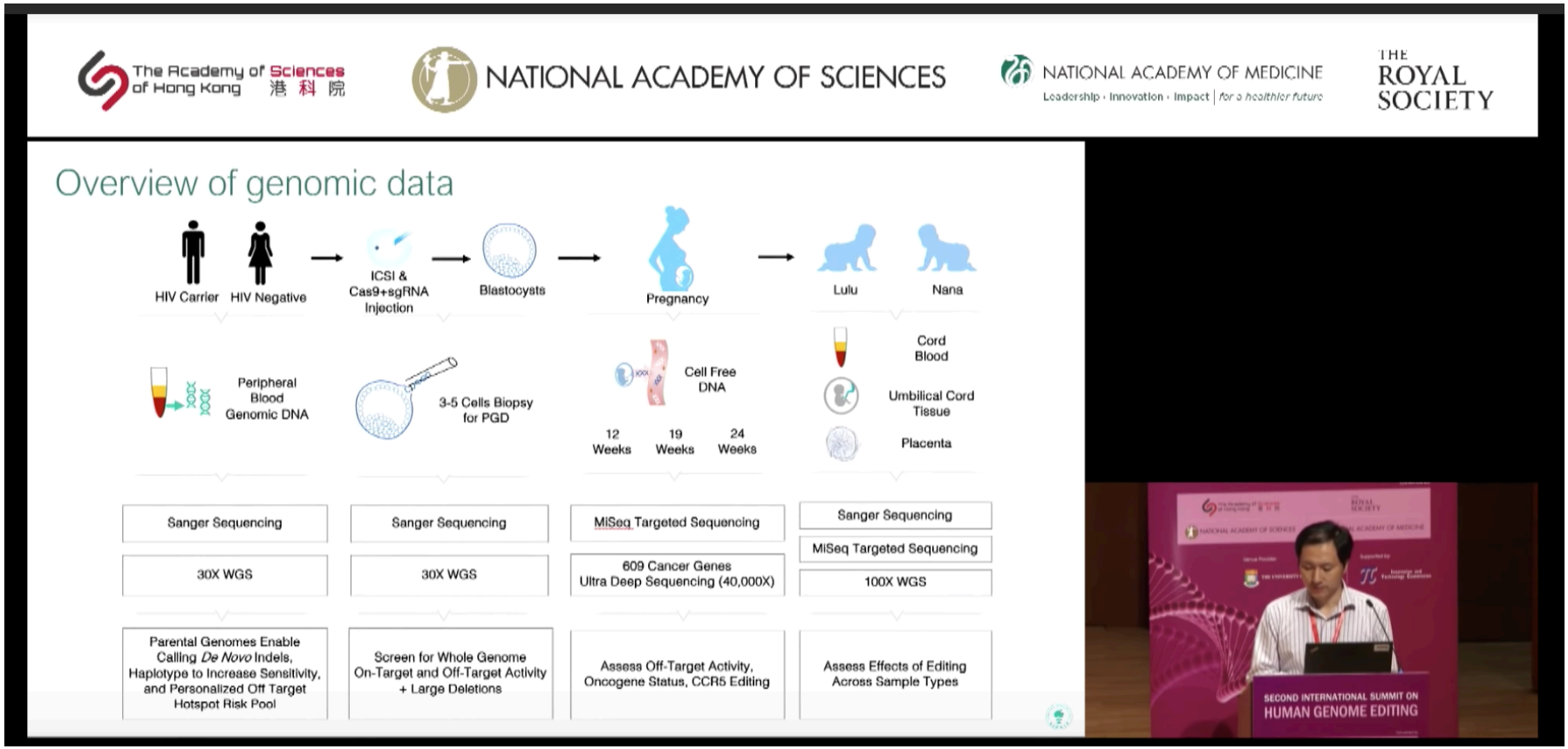
Brain enhancement?

Ccr5$^{+/-}$ mice show enhanced memory in multiple memory tasks.



(Zhou M., Elife, 2016)

https://www.youtube.com/watch?v=tLZufCrjrN0&feature=youtu.be&t=1644



https://www.youtube.com/watch?v=th0vnOmFltc

BIOTECHNOLOGY

The creator of the CRISPR babies has been released from a Chinese prison

He Jiankui created the first gene-edited children. The price was his career. And his freedom.

By Antonio Regalado

April 4, 2022

MIT Technology Review
https://www.technologyreview.com/2022/04/04/1048829/he-jiankui-prison-free-crispr-babies/?utm_source=Nature+Briefing&utm_campaign=94700c0bdc-briefing-dy-20220405&utm_medium=email&utm_term=0_c9dfd39373-94700c0bdc-45882746


2019 - beta-thalassemia
first authorized clinical trial - turn the fetal hemoglobin gene back on
https://clinicaltrials.gov/ct2/show/NCT03655678

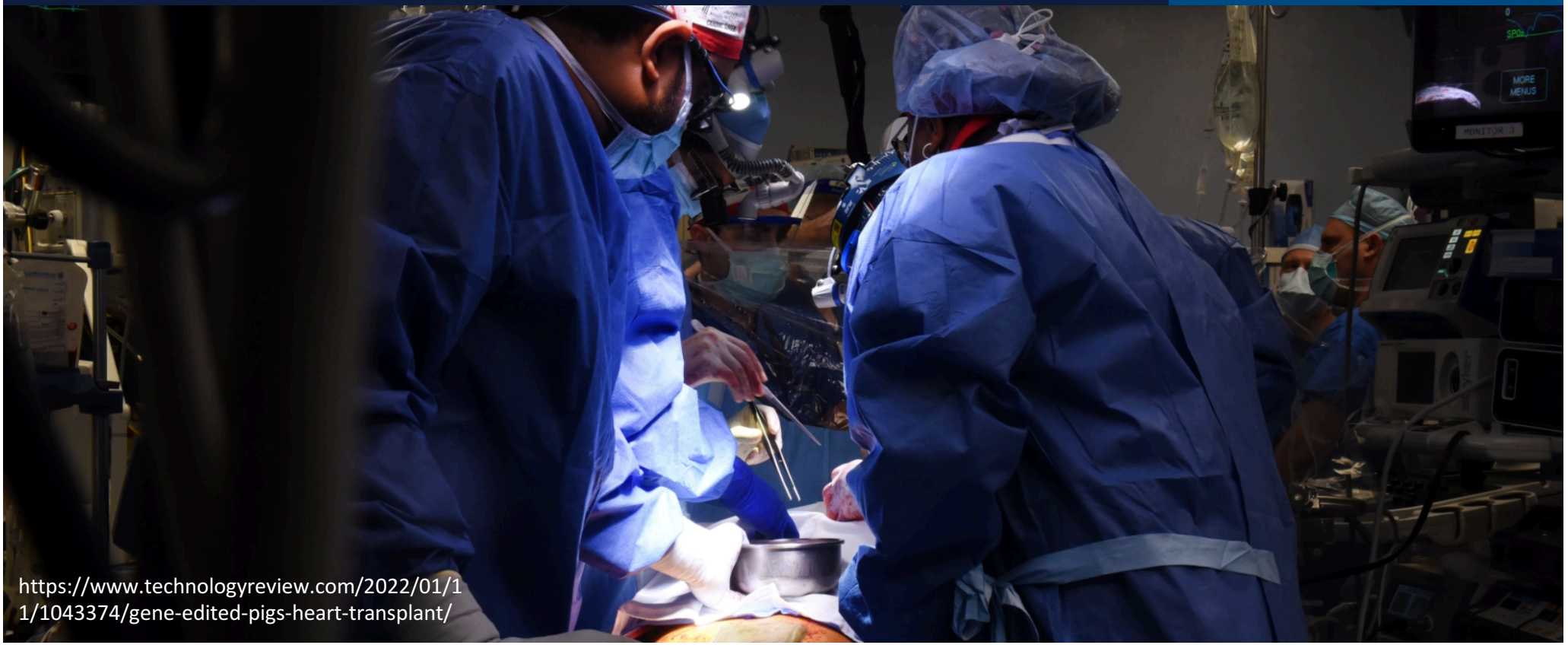
BIOTECHNOLOGY

A gene-edited pig's heart has been transplanted into a human for the first time

The procedure is a one-off, and highly experimental, but the technique could help reduce transplant waiting lists in the future.

By Charlotte Jee

January 11, 2022



https://www.technologyreview.com/2022/01/11/1043374/gene-edited-pigs-heart-transplant/