# *Focus on CRISPR*

17/11/20

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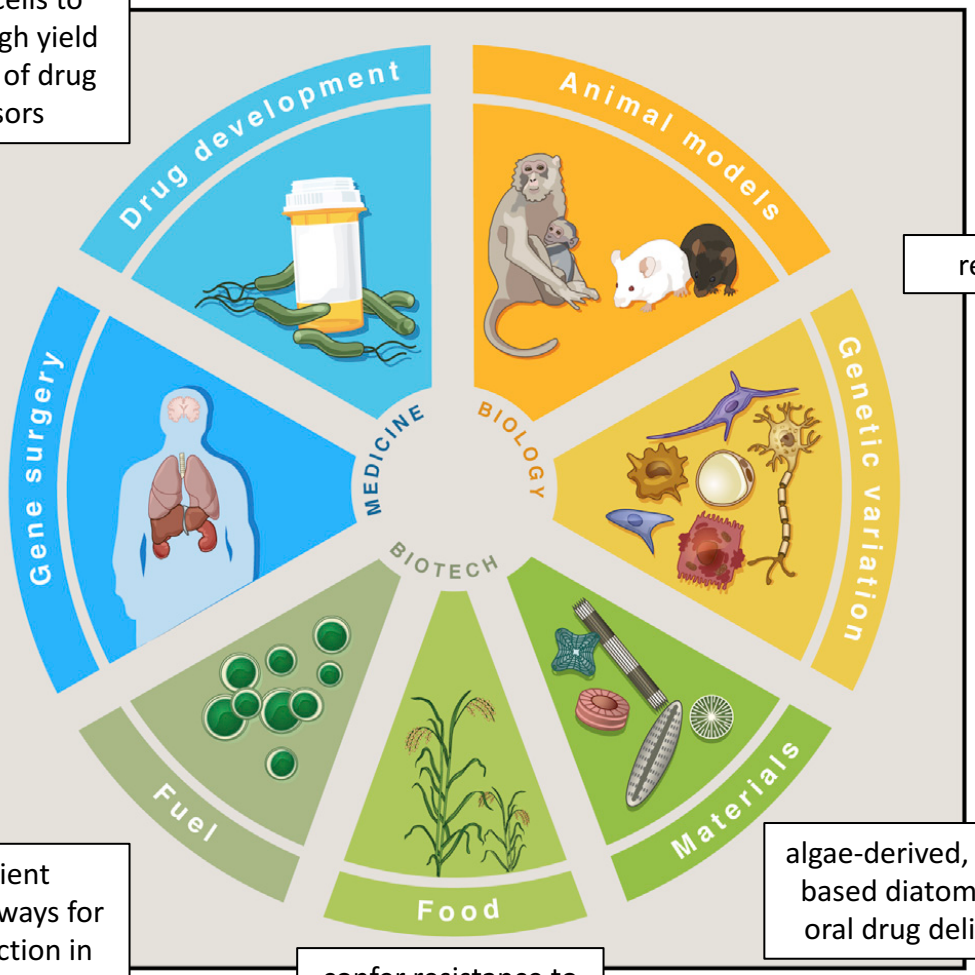
PostDoc
*Saggiolab*

*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***

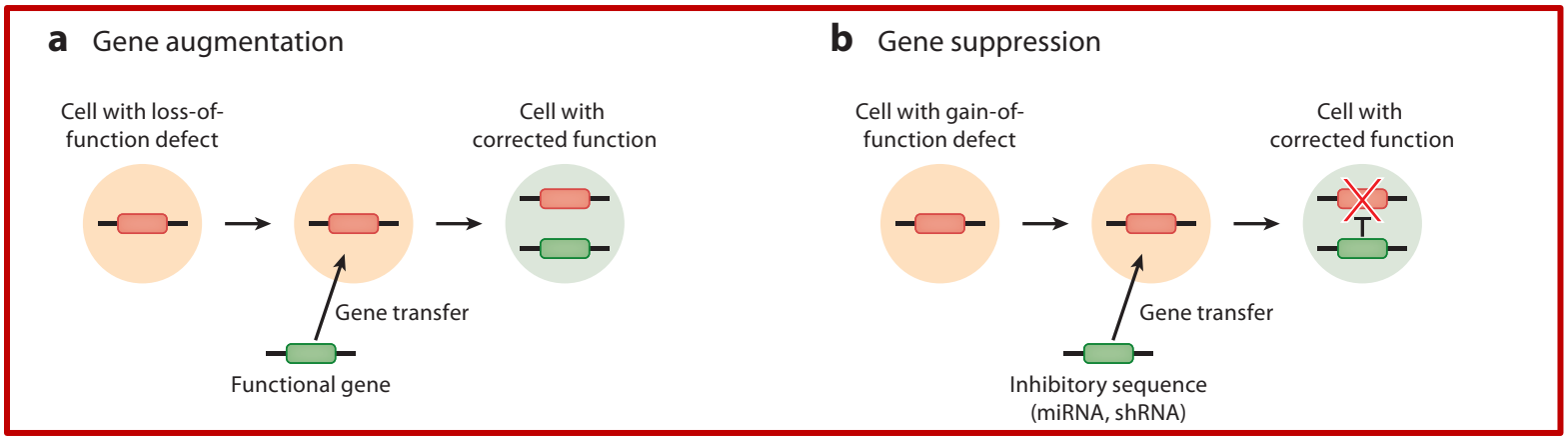
*long- term expression of the transferred gene high enough to be therapeutic*

***3.0 gene therapy***

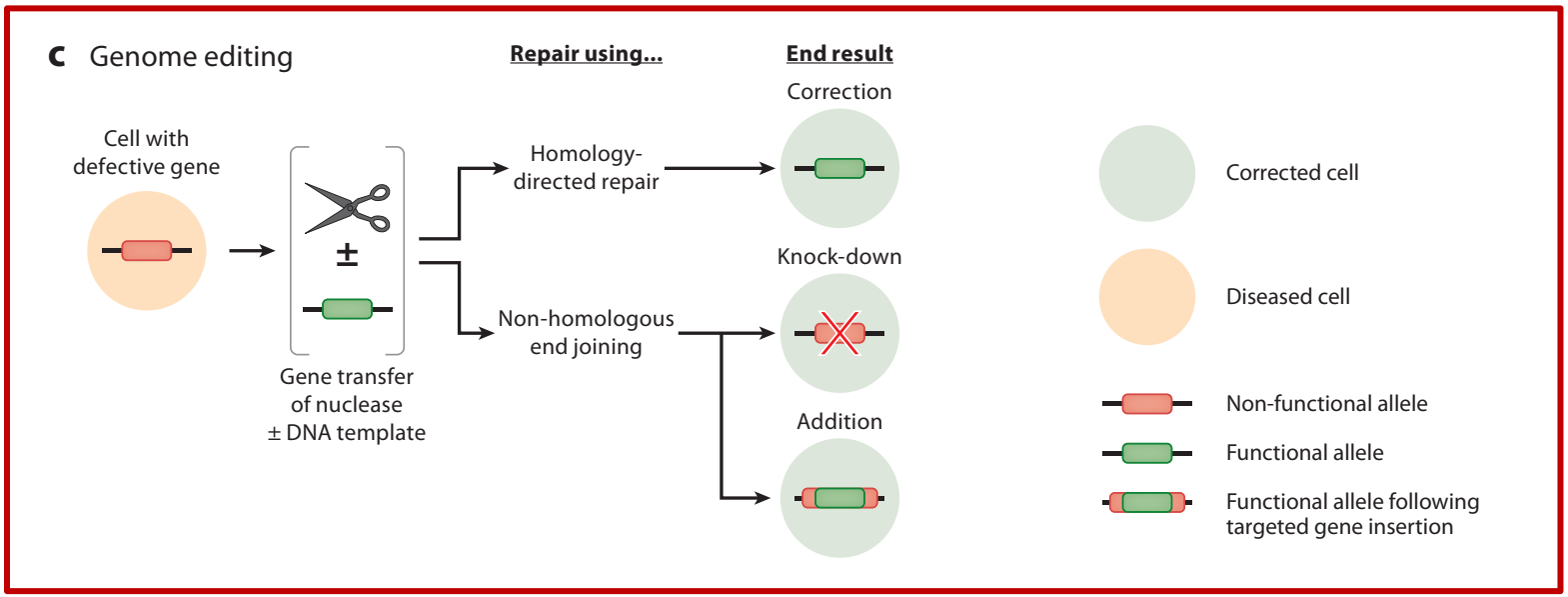
*long- term correction of the 'edited' gene high enough to be therapeutic*

# 2.0 gene therapy vs 3.0 gene therapy

2.0

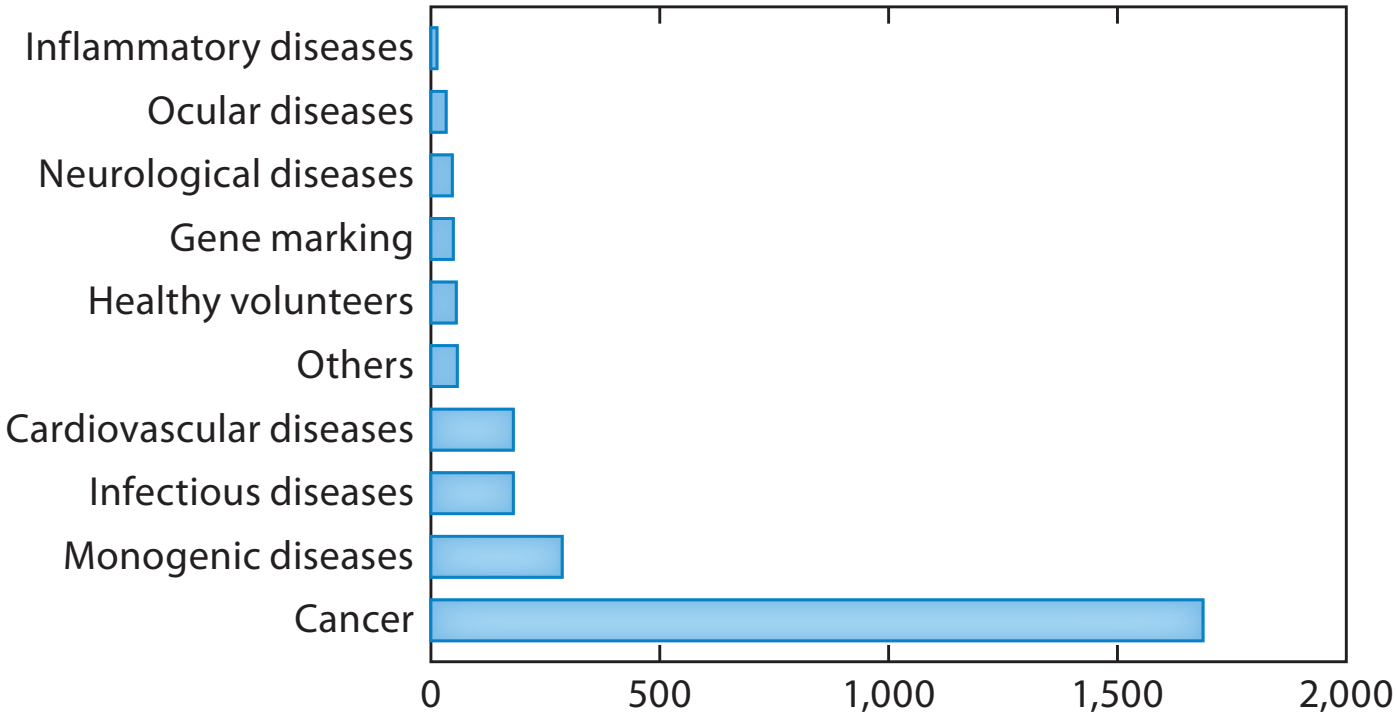



3.0

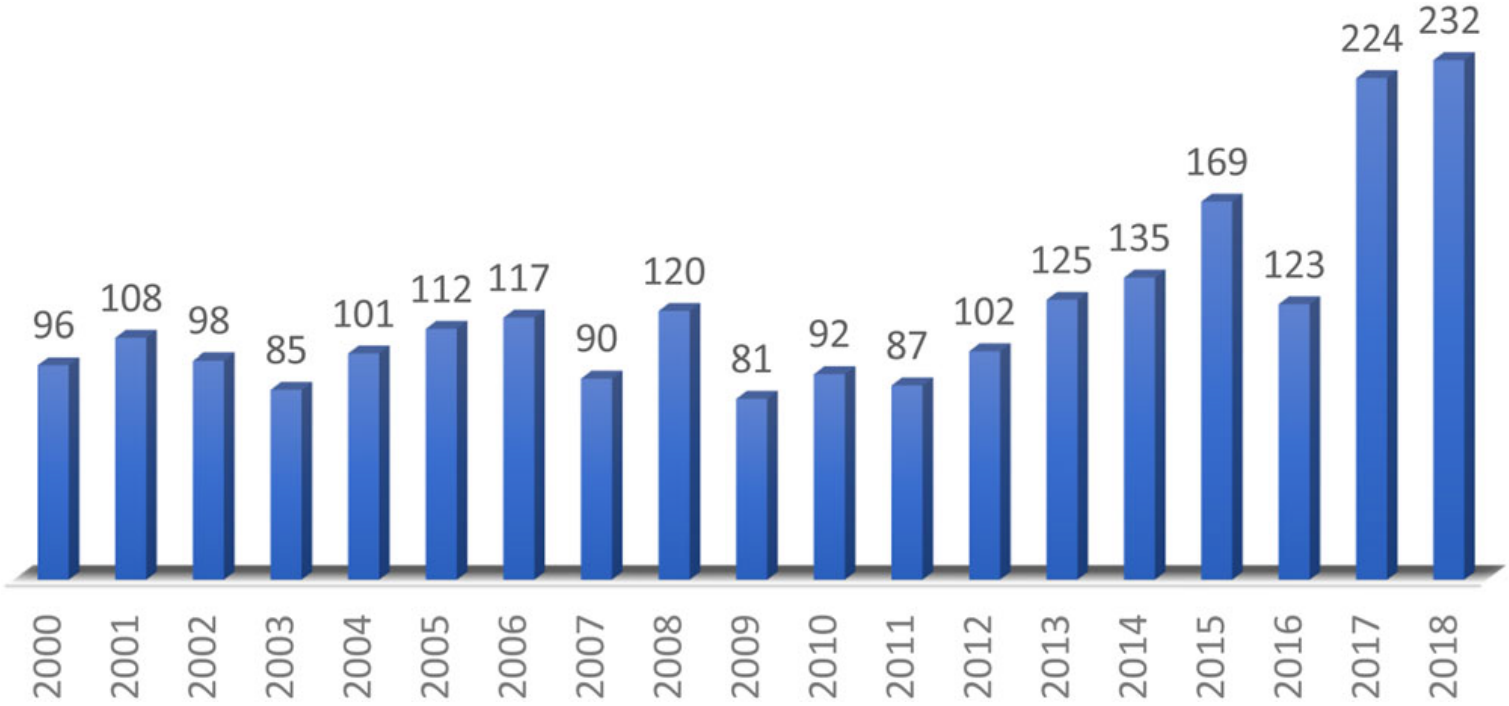



(Xavier M. Anguela and Katherine A. High, Annual Reviews of Medicine 2018)

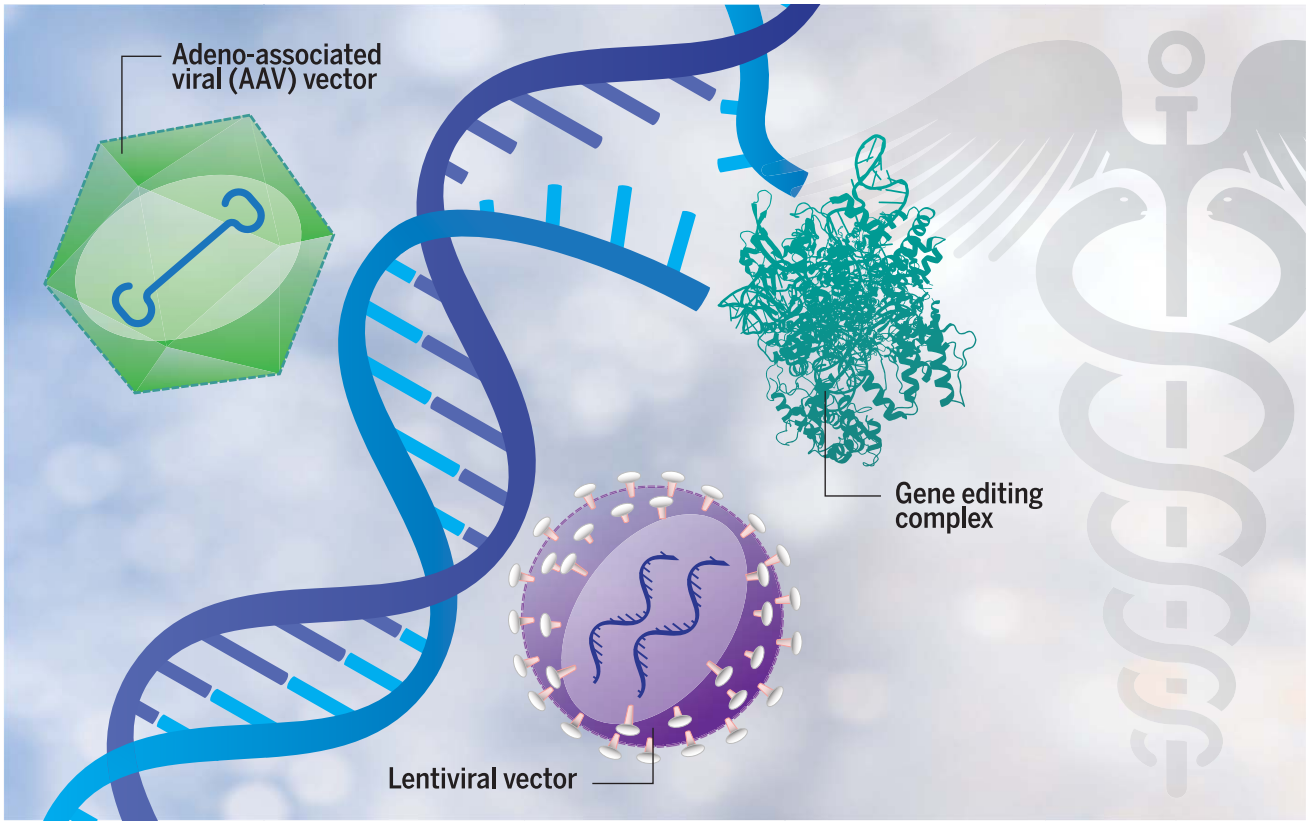
# Monogenic disease and cancer gene therapy




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




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

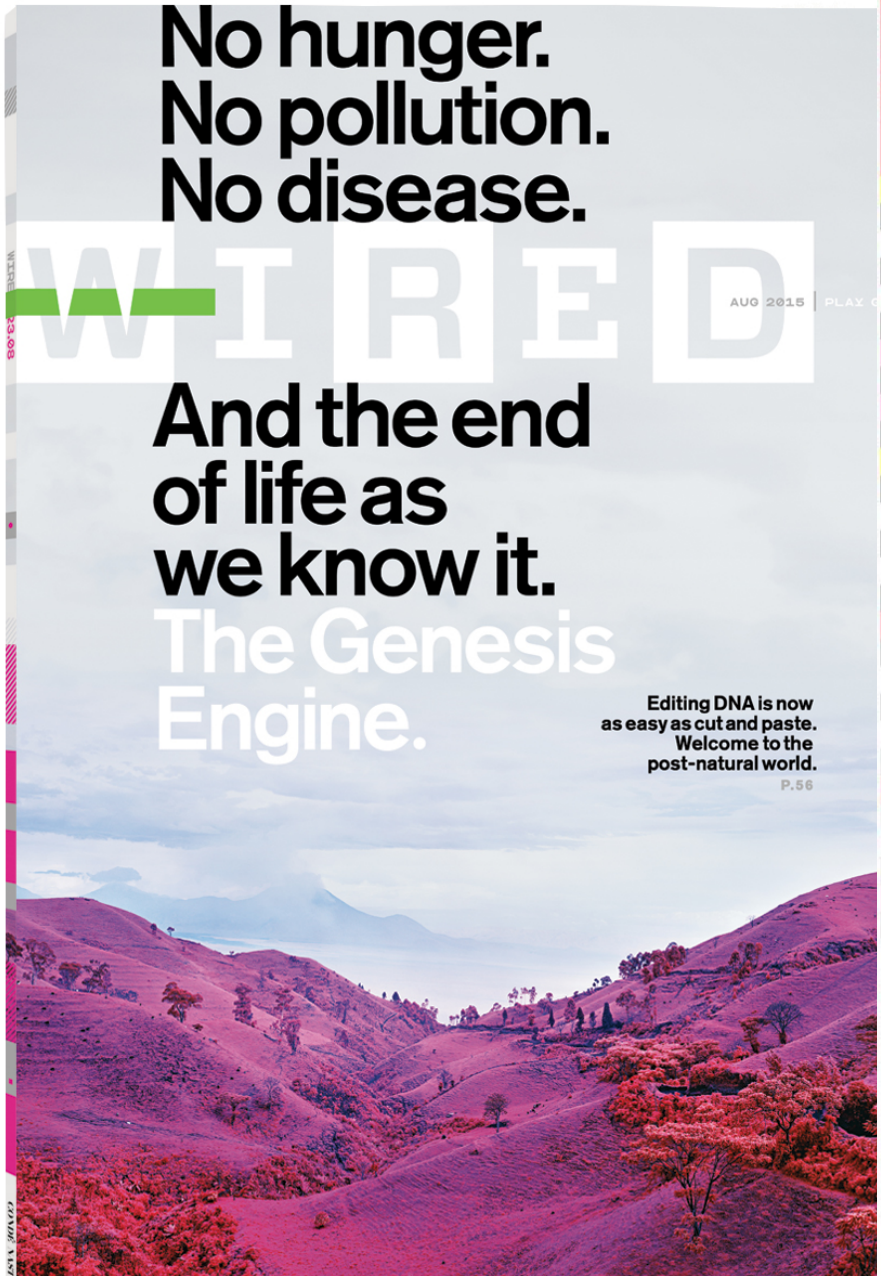



(Fazhan Wang et al., J Gene Med. 2019)

# Bubble boy

*CRISPR Revolution*

2015


No hunger.
No pollution.
No disease.
WIRED
AUG 2015
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 ho
irons or caustic chemicals
Meet Princess, who wa
engineered never to grow them

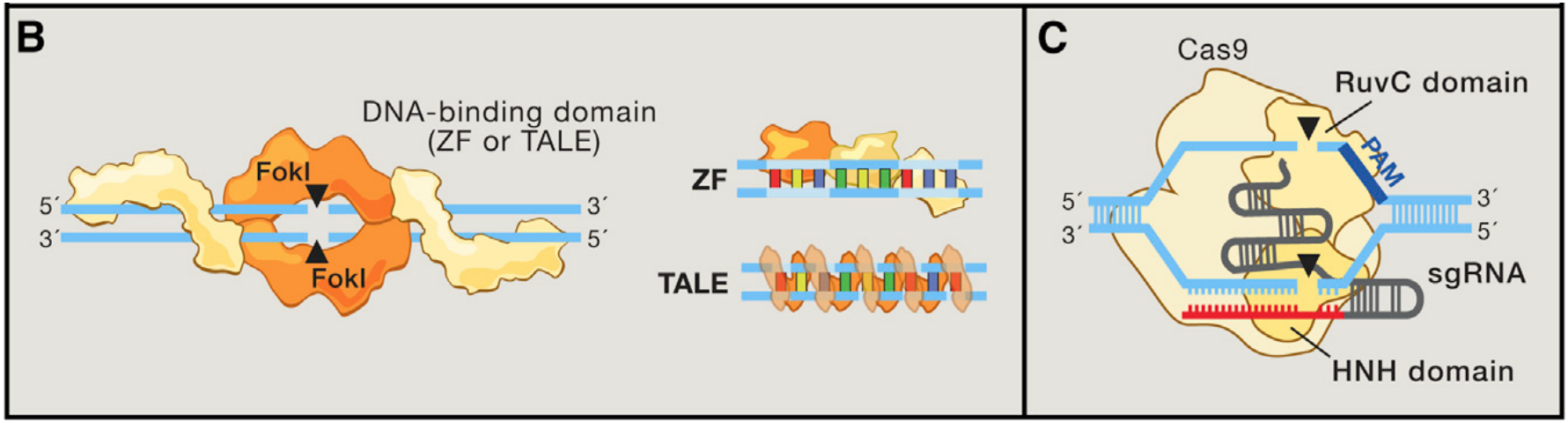
# CRISPR/Cas9 - It all started with yogurt


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

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

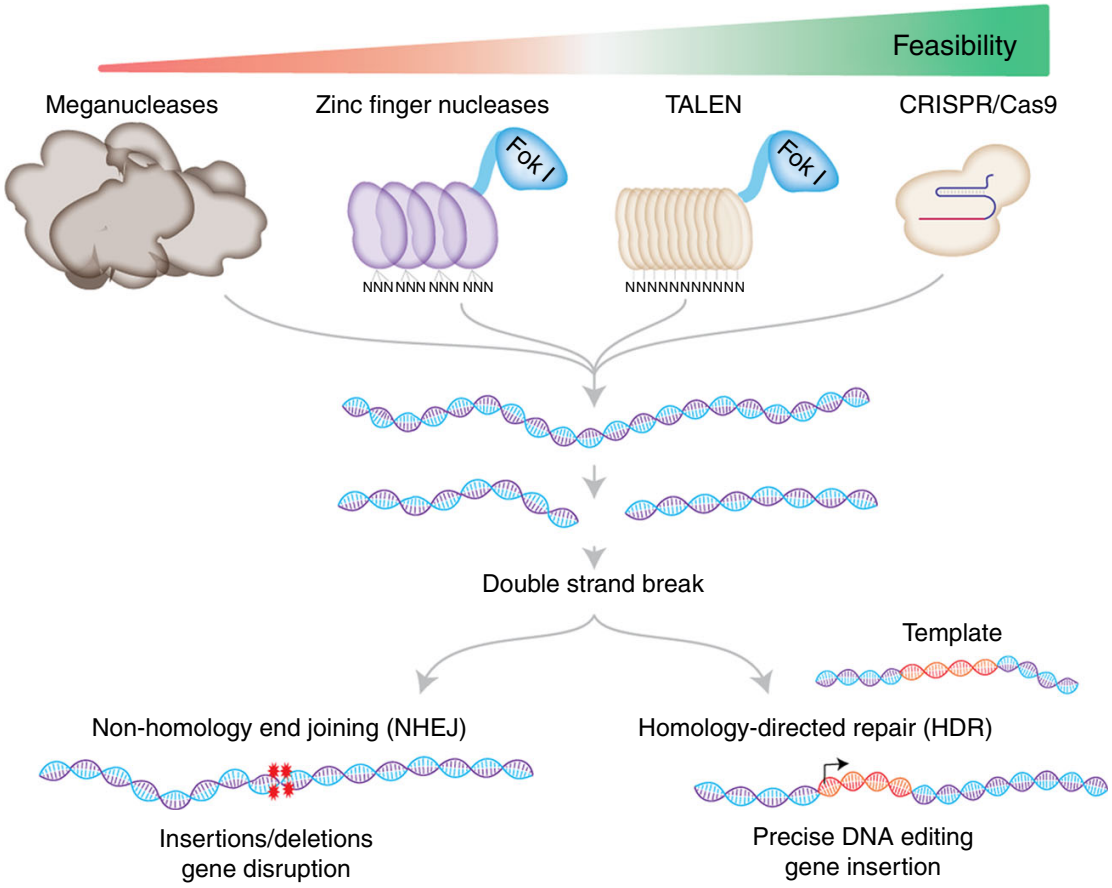



*(Hsu et al., Cell, 2014)*

| | 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)

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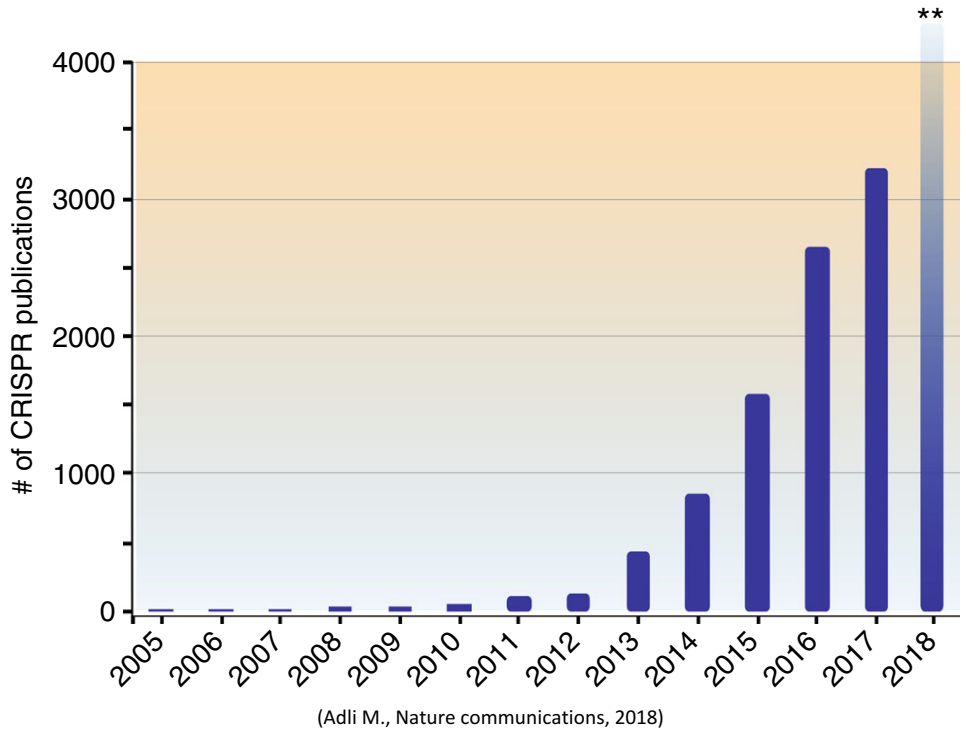
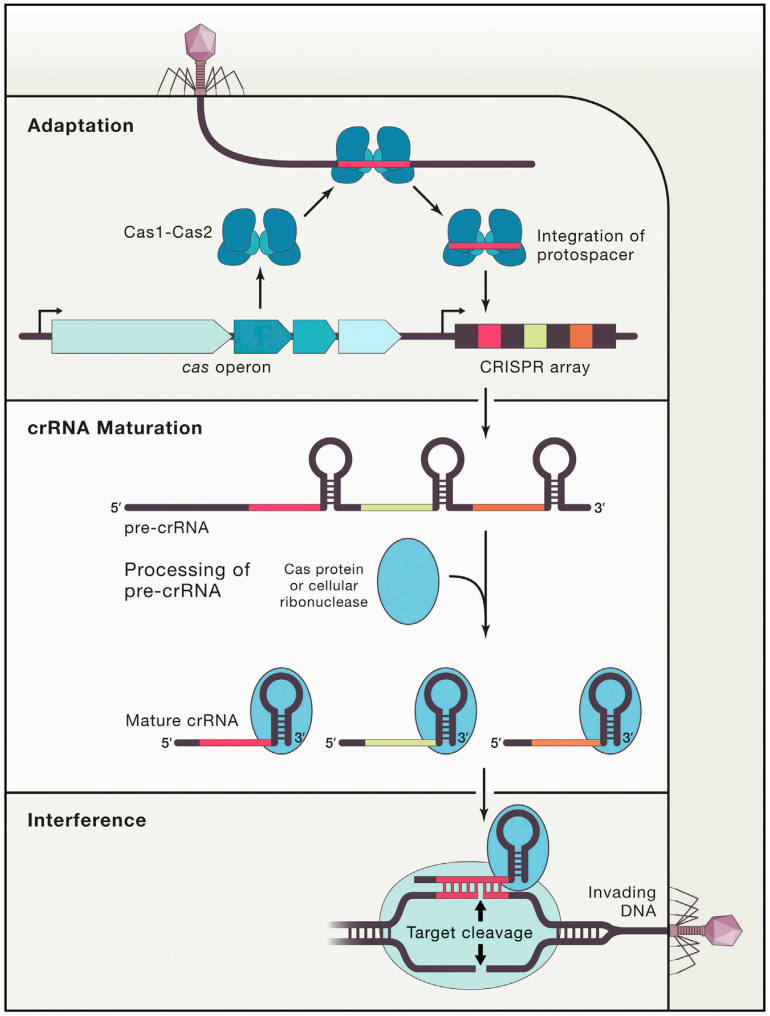



(Adli M., Nature communications, 2018)

*CRISPR/Cas9*

# CRISPR system in prokariotes is an adaptive immunity system



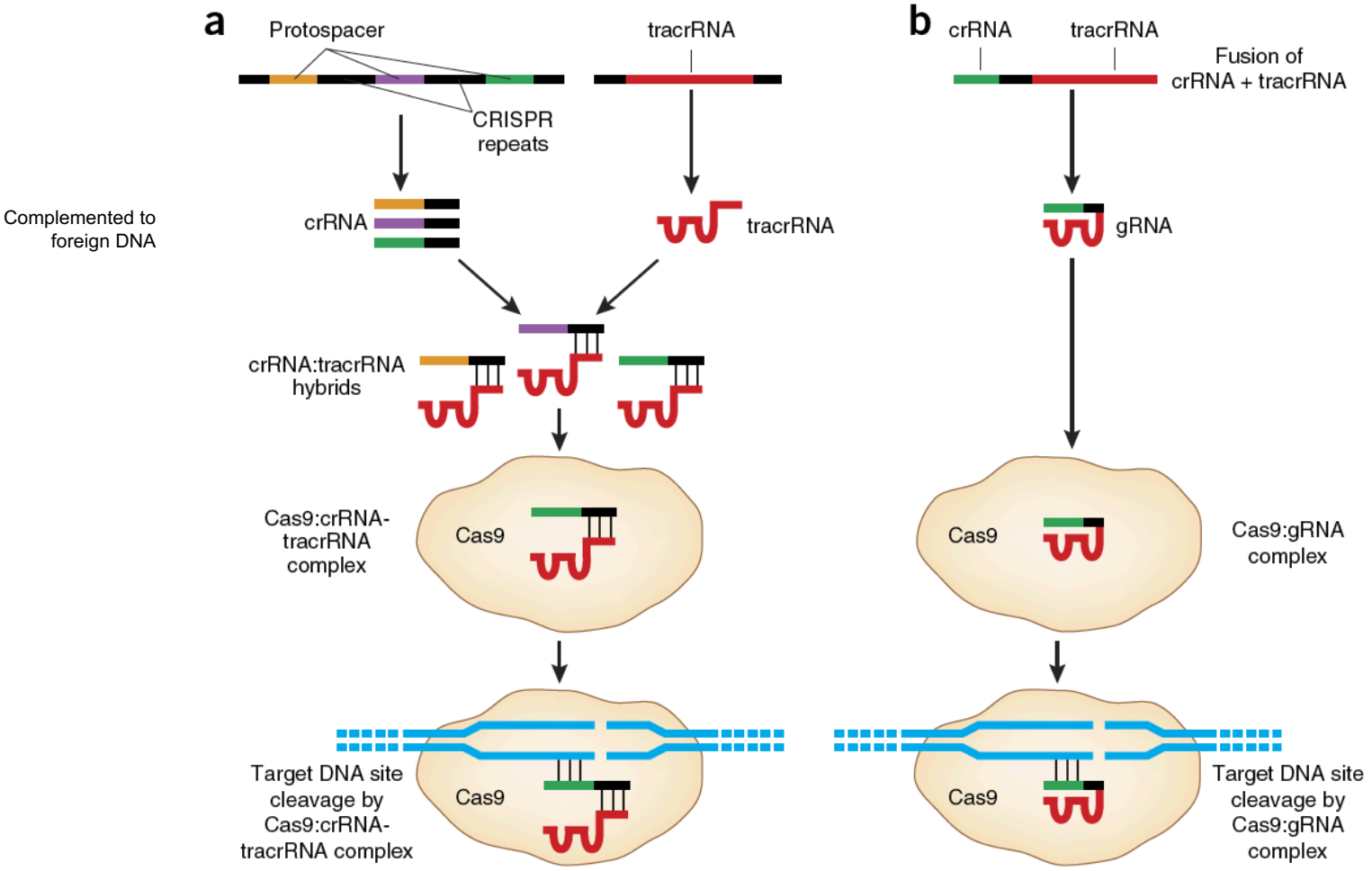

*(Hille F. et al., Cell, 2018)*

# 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

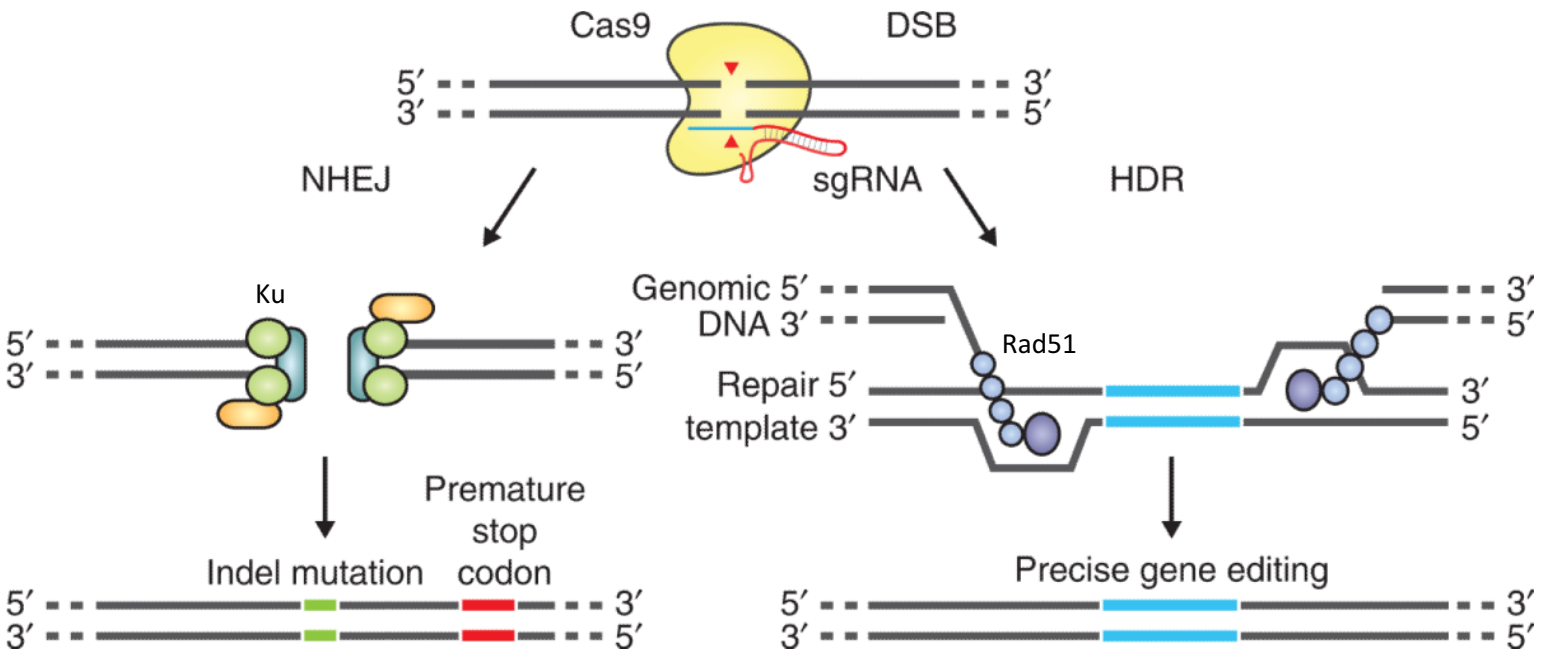
Naturally occurring CRISPR-Cas9 systems

Engineered CRISPR-Cas9 systems



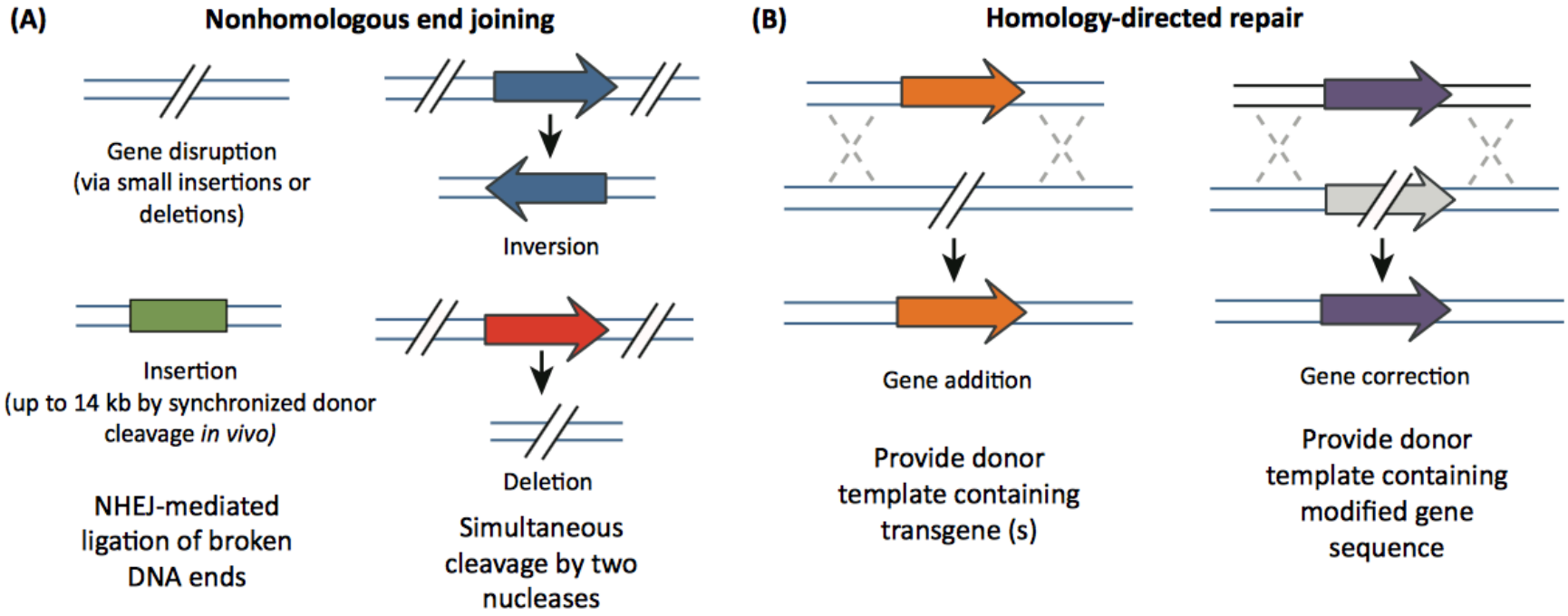

*(Sander D. and Joung K., nature biotechnology, 2014)*

# CRISPR/Cas9 Genome editing tool exploit endogenous DNA repair machinery




*(Ran et al, Nat Protoc. 2013)*

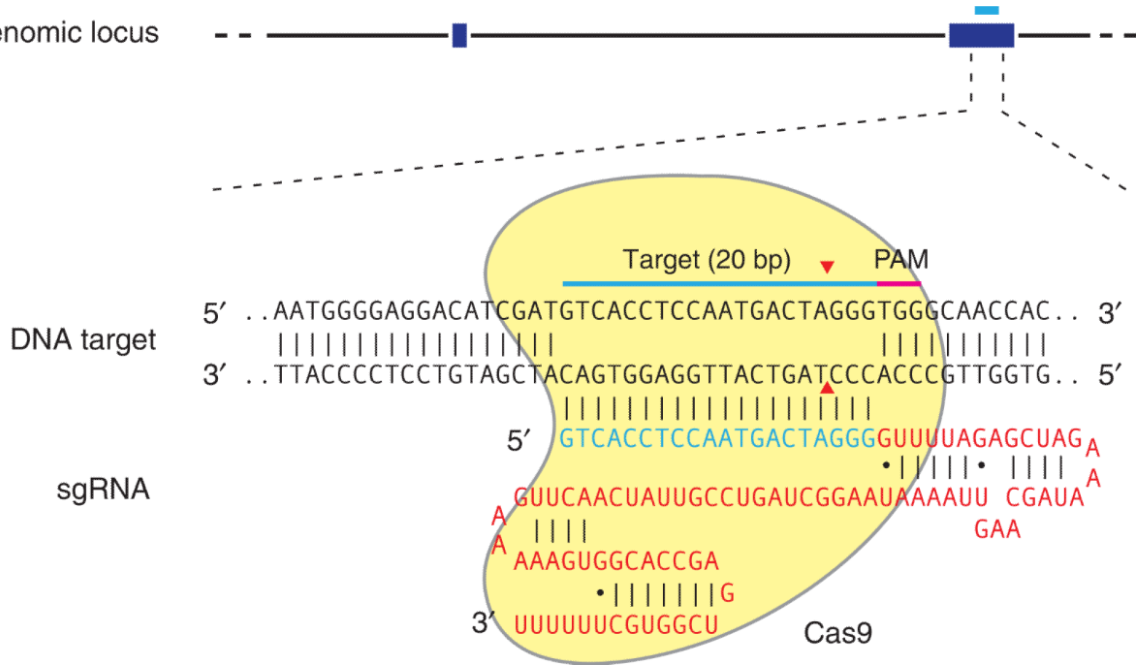
# CRISPR/Cas9 Genome editing tool exploit endogenous DNA repair machinery




*(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

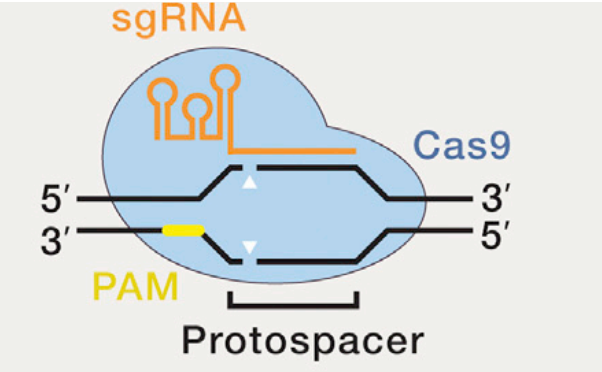
Genetic GPS




*(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



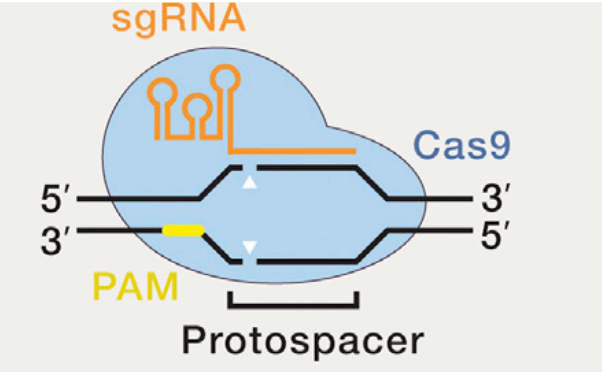

SpCas9:

- 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′-N…N-3′ / 3′-N…N-5′ ; 18 ; NGG-3′ / NCC-5′ ; 1 ; 17 ; 20 |
| St1Cas9 | 1121 | 5′-N…N-3′ / 3′-N…N-5′ ; 18 ; NNAGAAW-3′ / NNTCTTW-5′ ; 1 ; 17 ; 20 |
| St3Cas9 | 1409 | 5′-N…N-3′ / 3′-N…N-5′ ; 18 ; NGGNG-3′ / NCCNC-5′ ; 1 ; 17 ; 20 |
| NmCas9 | 1082 | 5′-N…N-3′ / 3′-N…N-5′ ; 22 ; NNNNGATT-3′ / NNNNCTAA-5′ ; 1 ; 21 ; 24 |
| SaCas9 | 1053 | 5′-N…N-3′ / 3′-N…N-5′ ; 19 ; NNGRRT-3′ / NNCYYA-5′ ; 1 ; 18 ; 21 |
| AsCpf1 / LbCpf1 | 1307 / 1228 | 5′-TTTN 3′-AAAN ; 24 ; 3′ ; 5′ ; 1 ; 19 |
| VQR SpCas9 | 1368 | 5′-N…N-3′ / 3′-N…N-5′ ; 18 ; NGA-3′ / NCT-5′ ; 1 ; 17 ; 20 |
| EQR SpCas9 | 1368 | 5′-N…N-3′ / 3′-N…N-5′ ; 18 ; NGAG-3′ / NCTC-5′ ; 1 ; 17 ; 20 |
| VRER SpCas9 | 1368 | 5′-N…N-3′ / 3′-N…N-5′ ; 18 ; NGCG-3′ / NCGC-5′ ; 1 ; 17 ; 20 |
| RHA FnCas9 | 1629 | 5′-N…N-3′ / 3′-N…N-5′ ; 18 ; YG-3′ / RC-5′ ; 1 ; 17 ; 20 |
| KKH SaCas9 | 1053 | 5′-N…N-3′ / 3′-N…N-5′ ; 19 ; NNNRRT-3′ / NNNYYA-5′ ; 1 ; 18 ; 21 |

# 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′ |
| 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′ |

(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

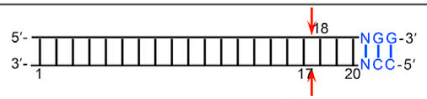
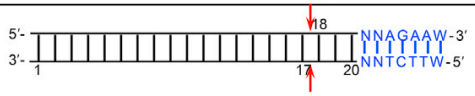
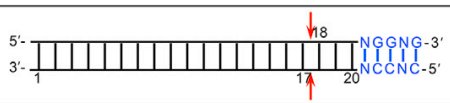
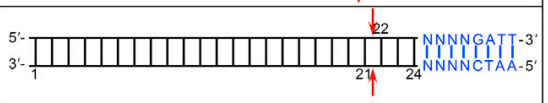
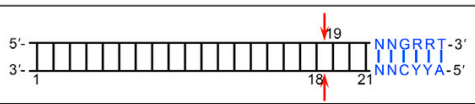
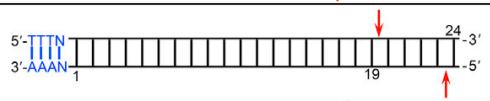
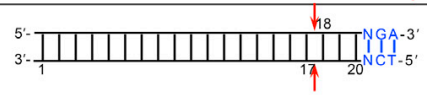
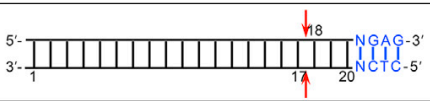
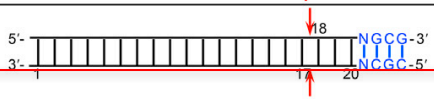
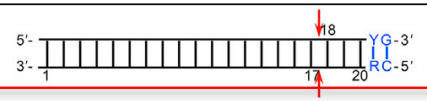
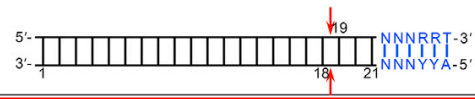
- Target design simplicity;
- Higly efficiency
- Fast (4 weeks for mice);

CONS

- **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

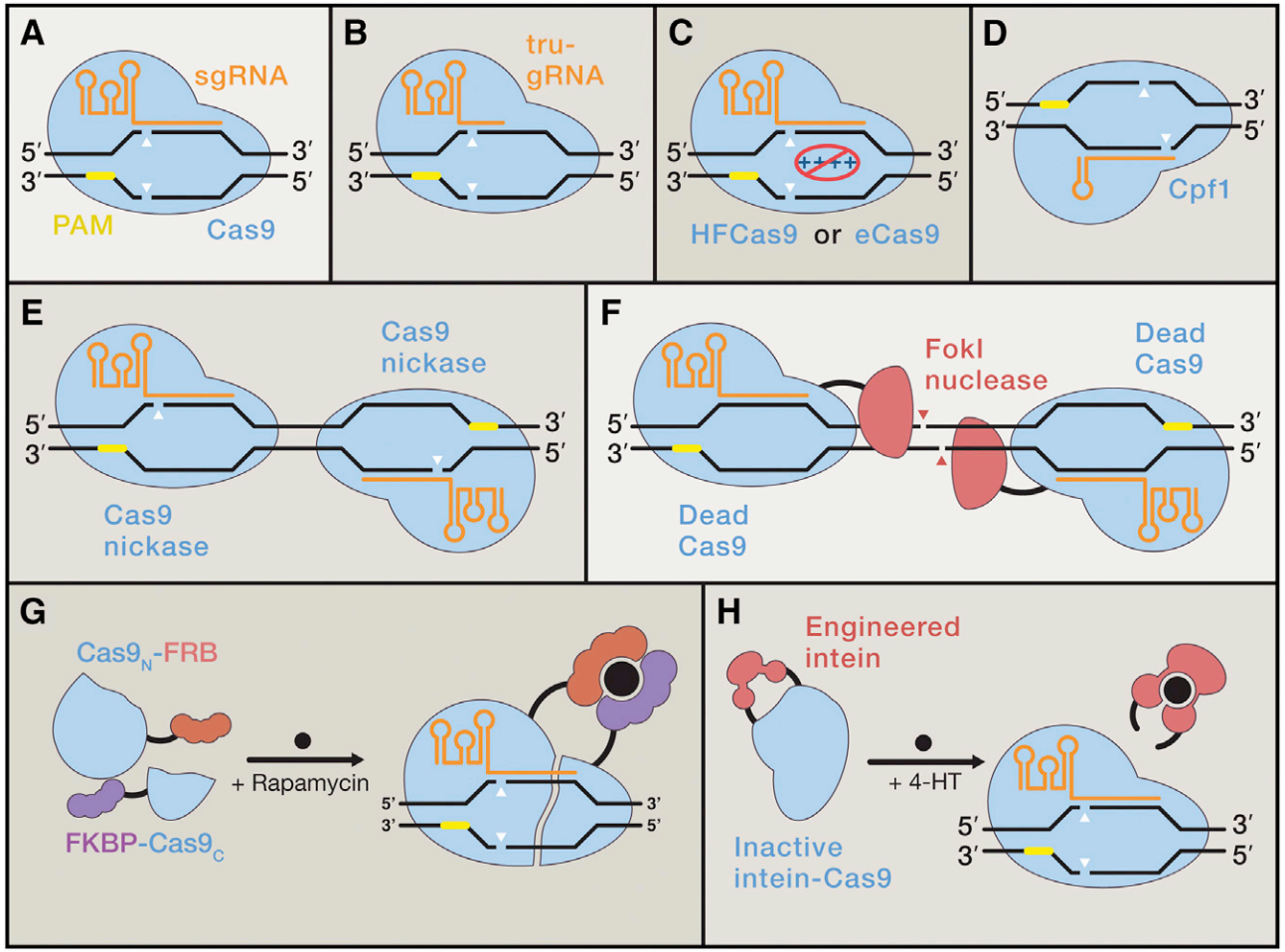
# *II - fidelity*

**How to check?**

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

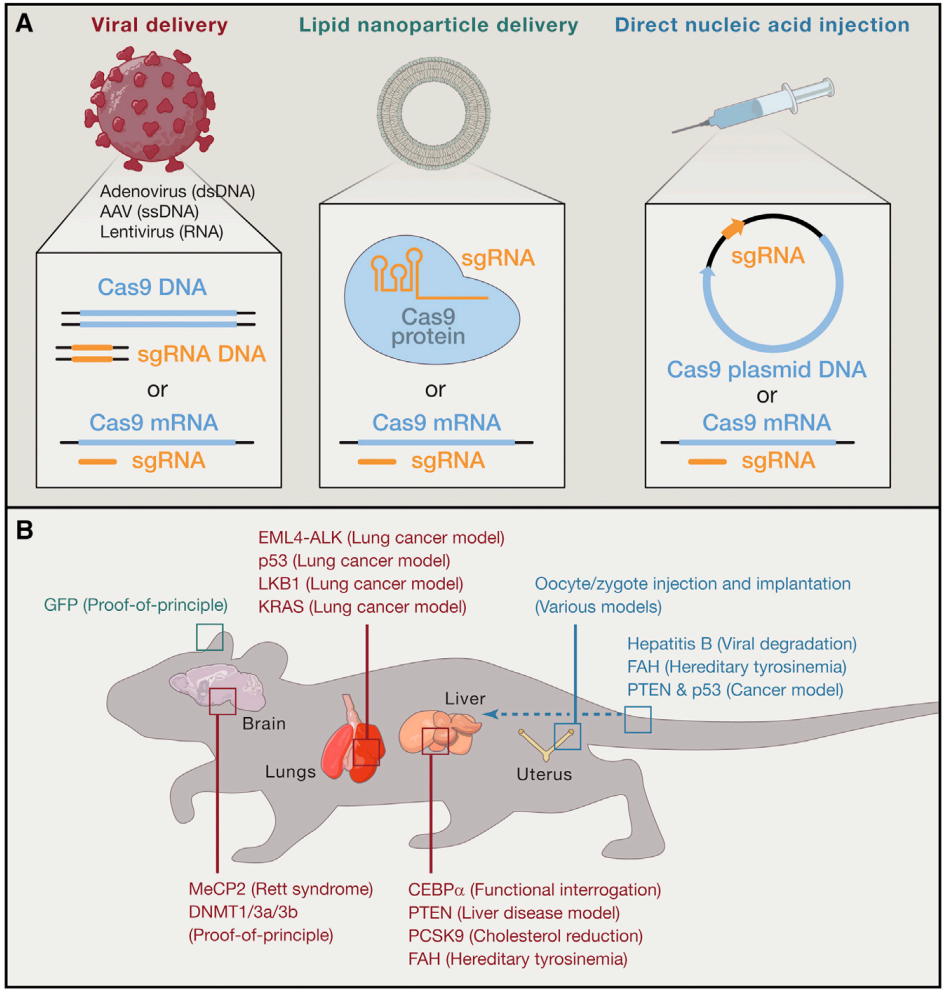
# II - Fidelity

## How to improve?



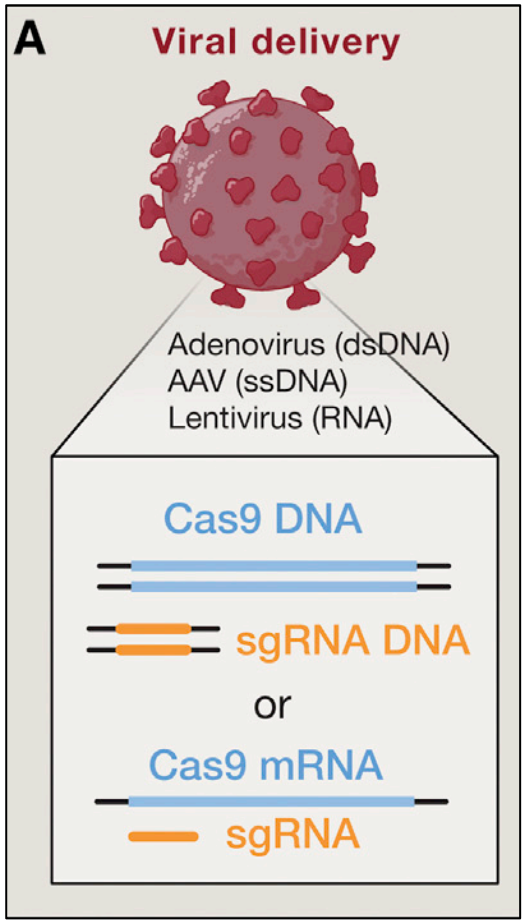

(Komor A.C. et al., Cell, 2017)

# 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

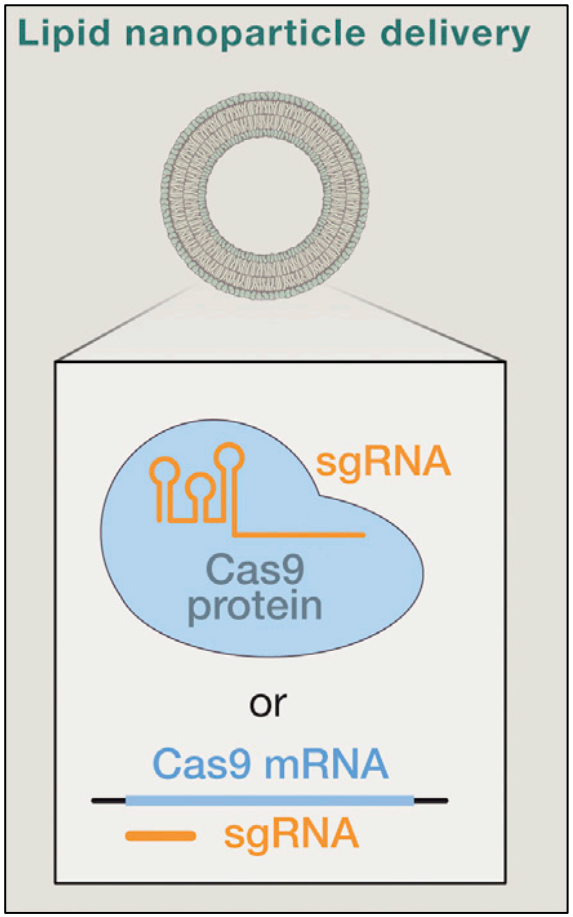
# III – delivery

**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]<br>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]<br>Cong et al.[45] |
| St3Cas9 | 1409 | NGGNG | 20 bp | ~ 3 bp 5′ of PAM | Gasiunas et al.[43]<br>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]<br>Kim et al. 2016 |
| LbCpf1 | 1228 | TTTV | 24 bp | 19/24 bp 3′ of PAM | Yamano et al.[50]<br>Kim et al. 2016 |
| Cas13 | Multiple orthologs | RNA targeting | 28 bp | | Abudayyeh et al. 2017 |

(Adli M., Nature communications, 2018)

# *III – delivery*

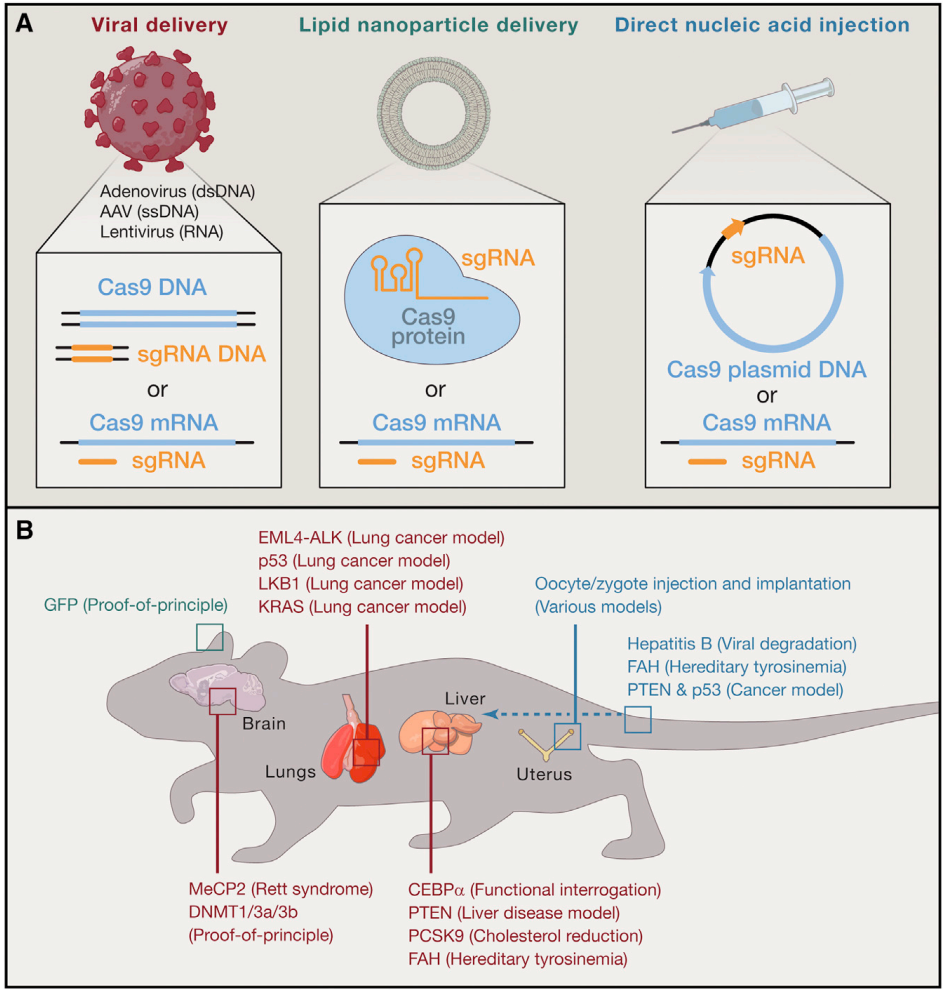



**Lipid nanoparticle delivery:**

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

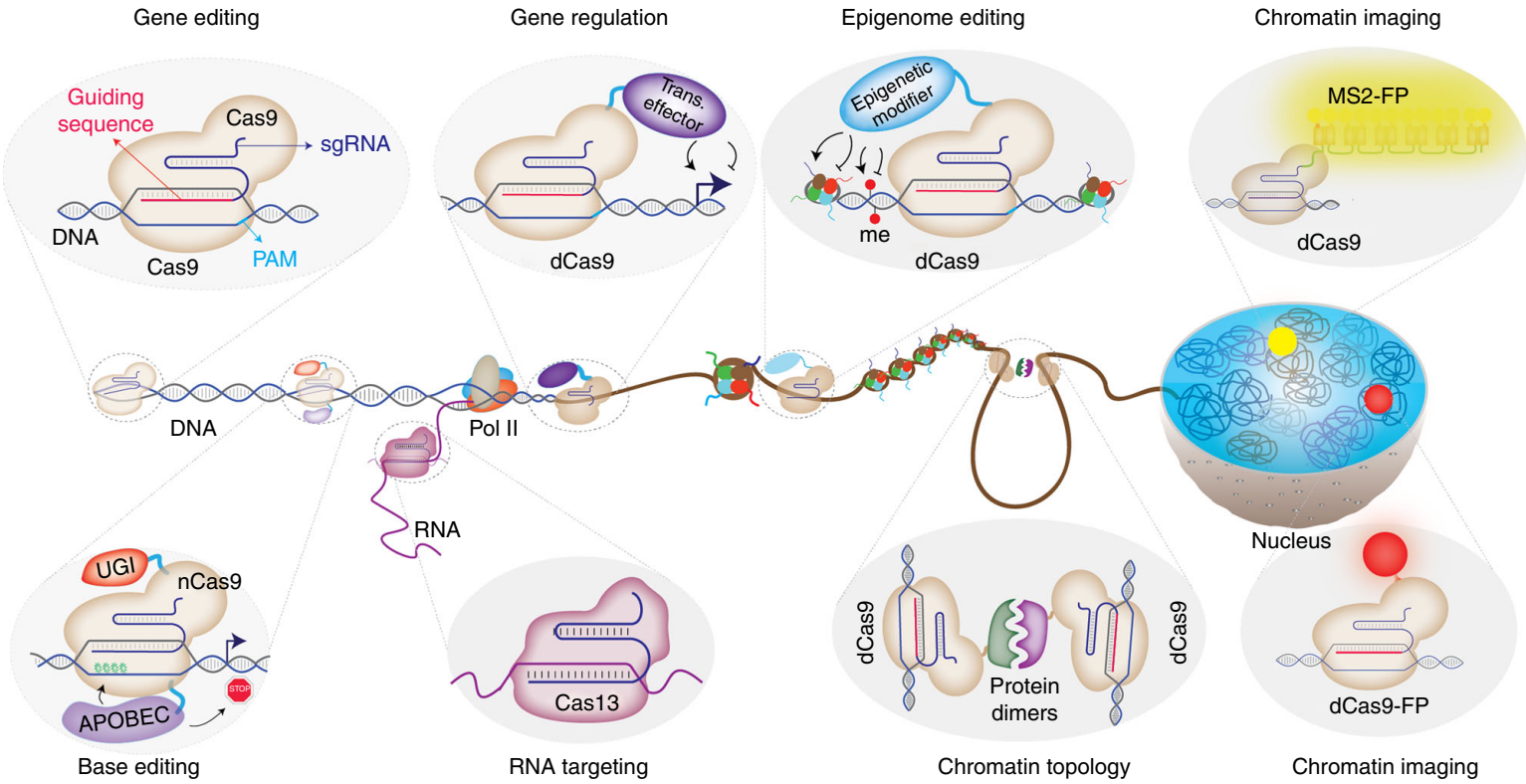
(Komor A.C. et al., Cell, 2017)

# III – delivery




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

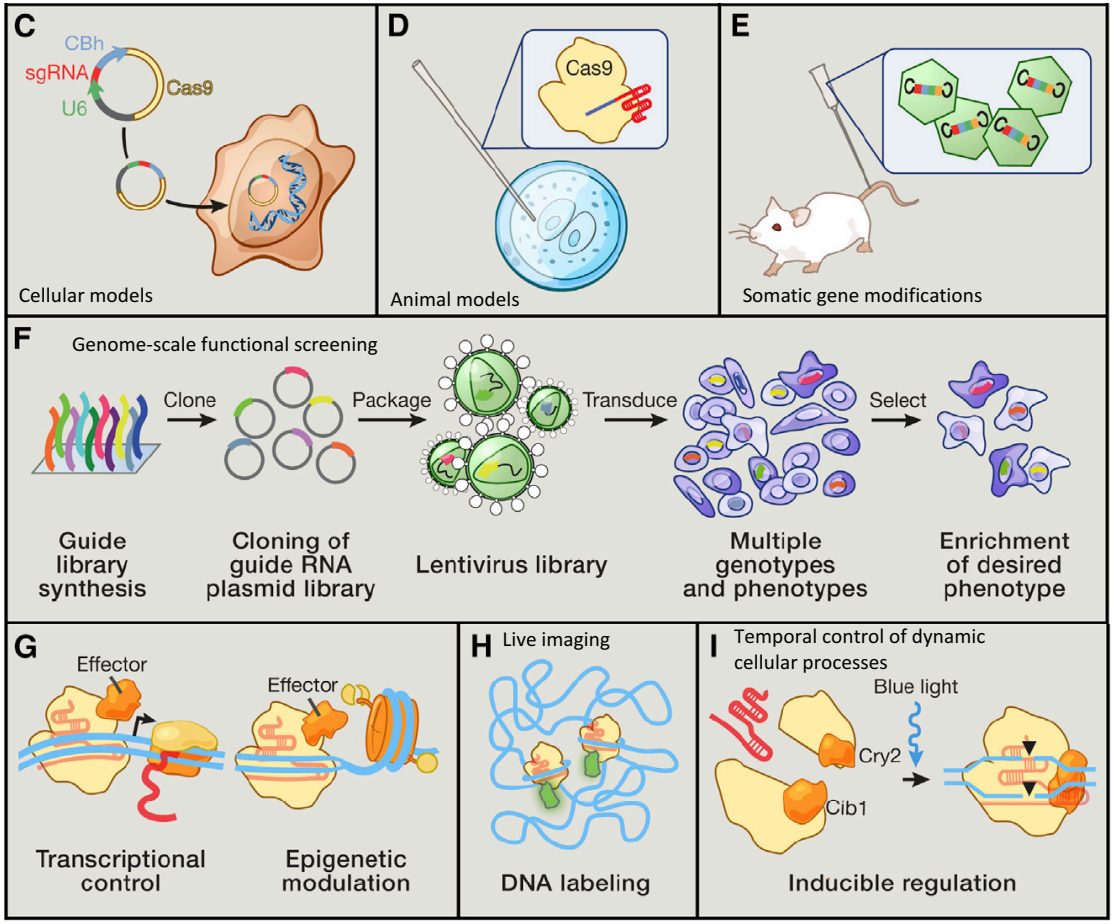
# CRISPR/Cas9 technologies beyond genome editing are based mainly on dead-Cas9




(Adli M., Nature communications, 2018)

# *CRISPR/Cas9*
# APPLICATIONS

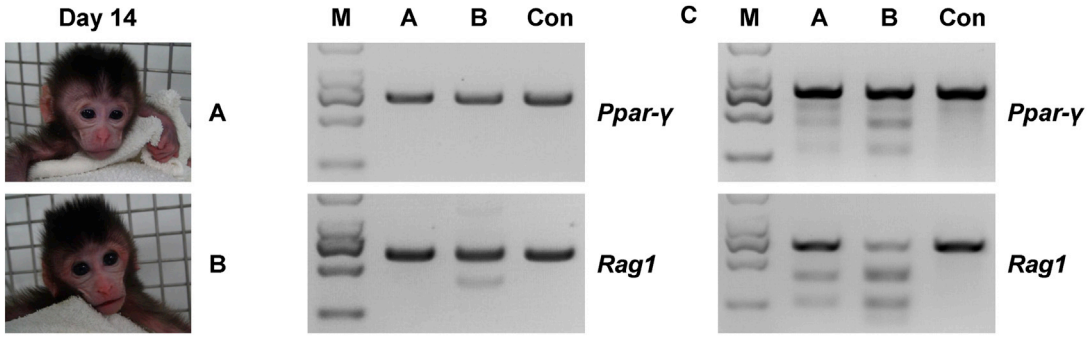
# CRISPR/Cas engineering is enabling a broad range of applications




*(Hsu et al., Cell, 2014)*

# CRISPR/Cas9 system can be used in other mammals?

*In vivo*



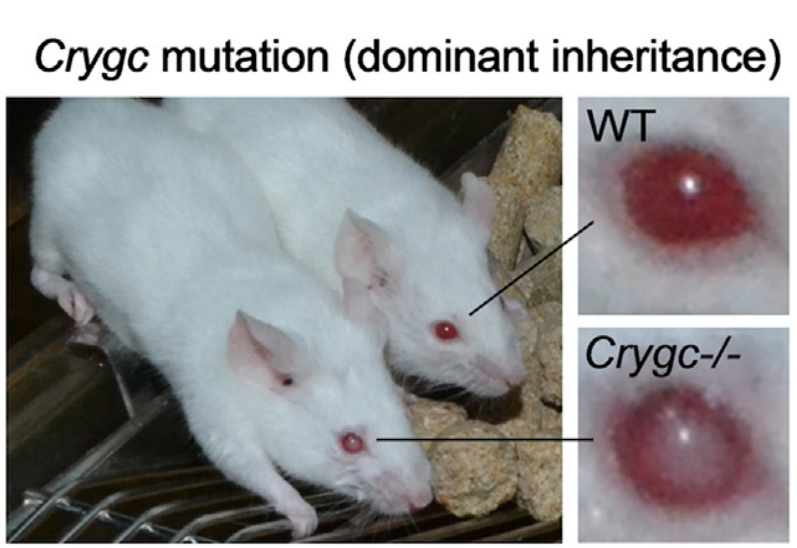

```
Ppar-γ ACTCCTTTGACATCAAGCCCTTCACTACTGTTGACTTCTCCAGCATTTCTGCTCCAC (WT)
  A    ACTCCTTTGACATCAAGCCCTTC^aCACTACTGTTGACTTCTCCAGCATTTCTGCTCCAC (+1,2/23)
  B    ACTCCTTTGACATCAAGCCCTTC^aCACTACTGTTGACTTCTCCAGCATTTCTGCTCCAC (+1,6/23)

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

*(Niu et al., Cell 2013)*

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

# Can CRISPR/Cas9 be used for correct genetic disorders?

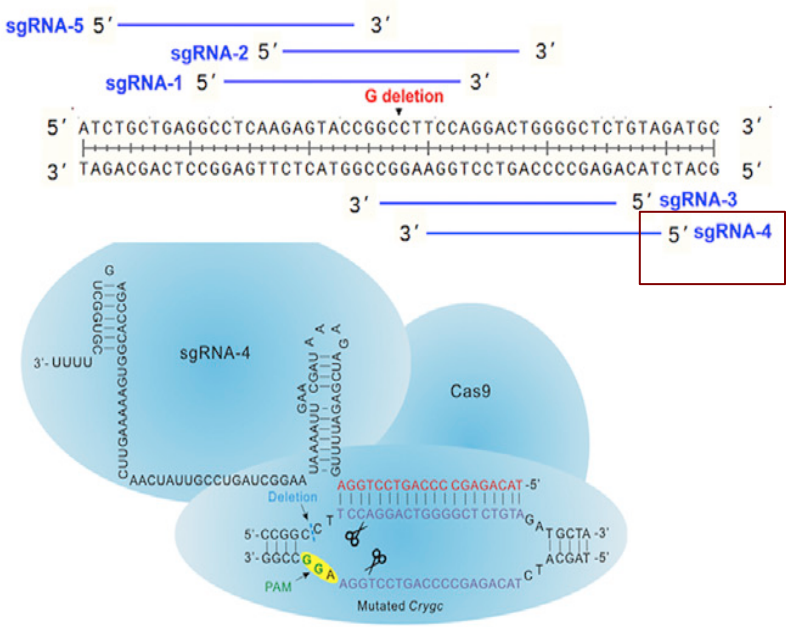



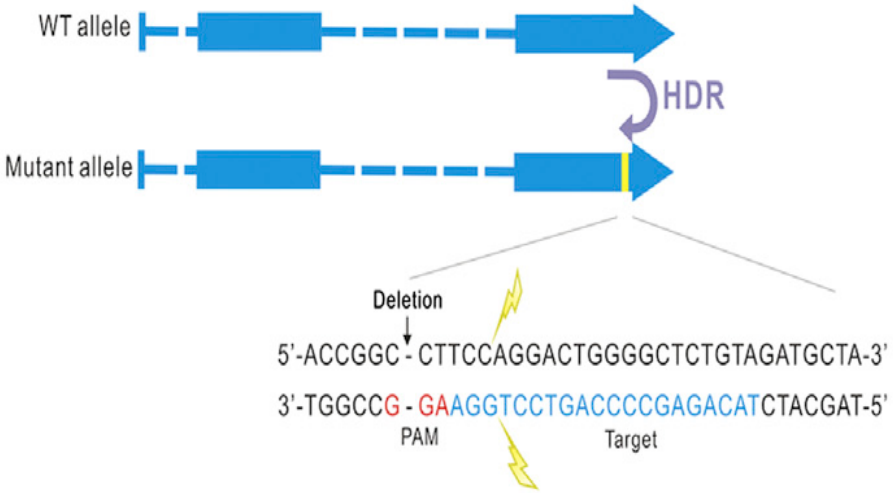

*(Wu et al., Cell 2013)*

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

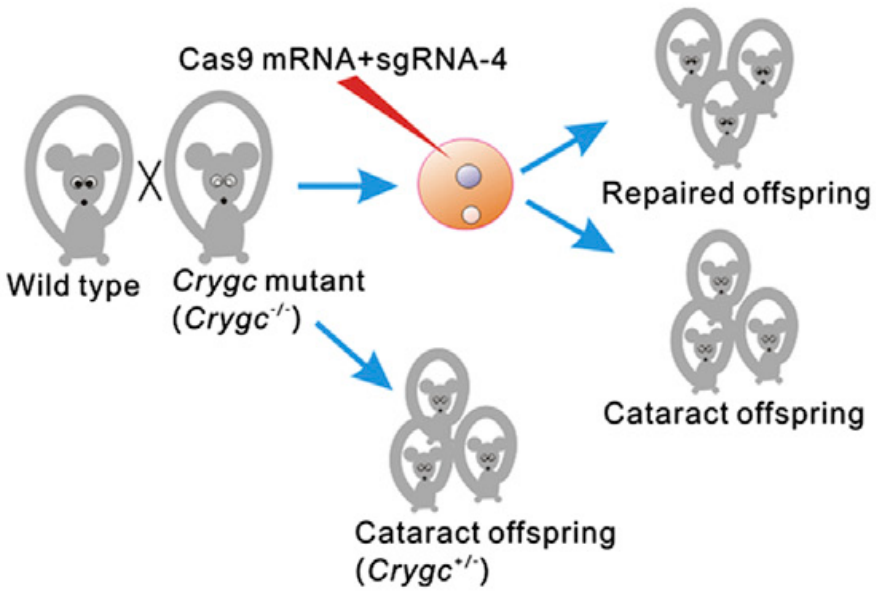
| sgRNA | E14 ESC clones | | m*Crygc* (*Crygc*$^{+/-}$) ESC clones | | |
|---|---|---|---|---|---|
| | Cleavage at 1 Allele/Total | Cleavage at 2 Alleles/Total | Cleavage at WT Allele/Total | Cleavage at Mutant Allele/Total | HDR-mediated Repair/Total |
| sgRNA-1 | 4/36 | 0/36 | 0/36 | 10/36 | 7/36 |
| 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 |

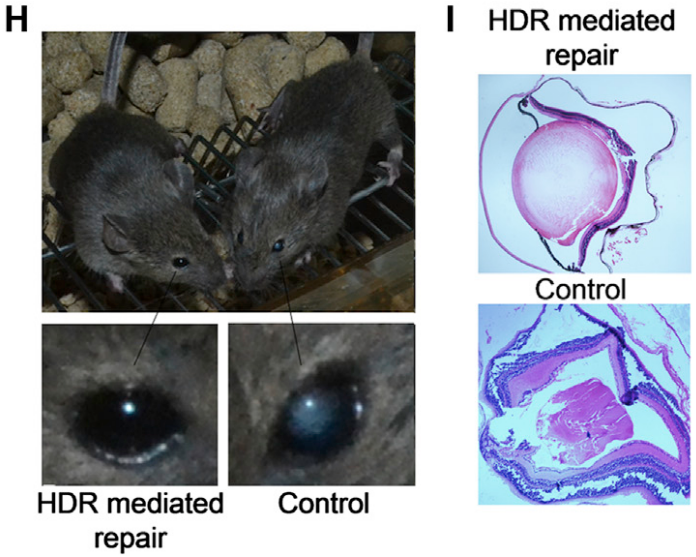
*(Wu et al., Cell 2013)*

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)*

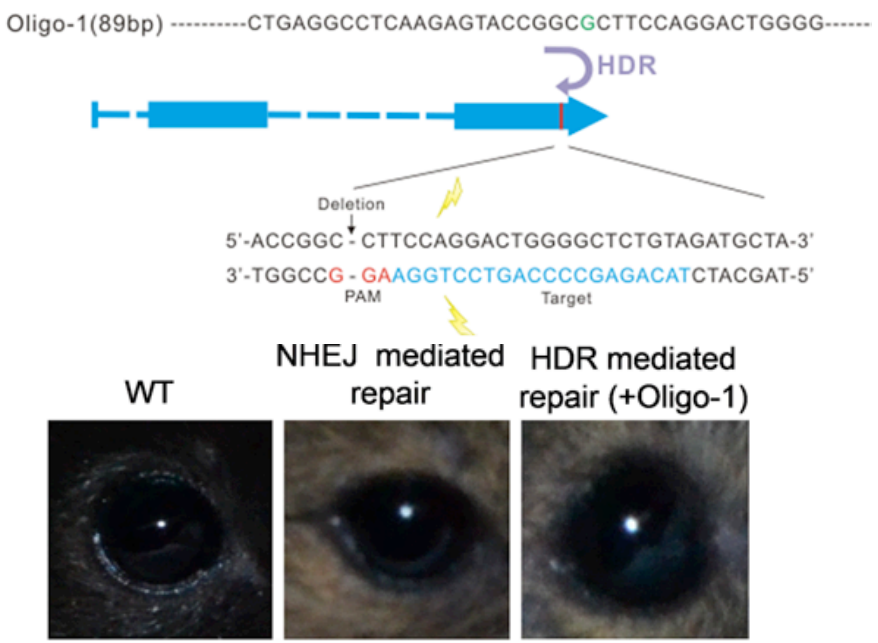
CRISPR/Cas9 system leads to gene correction via HDR using wt allele on the homologous chromosome

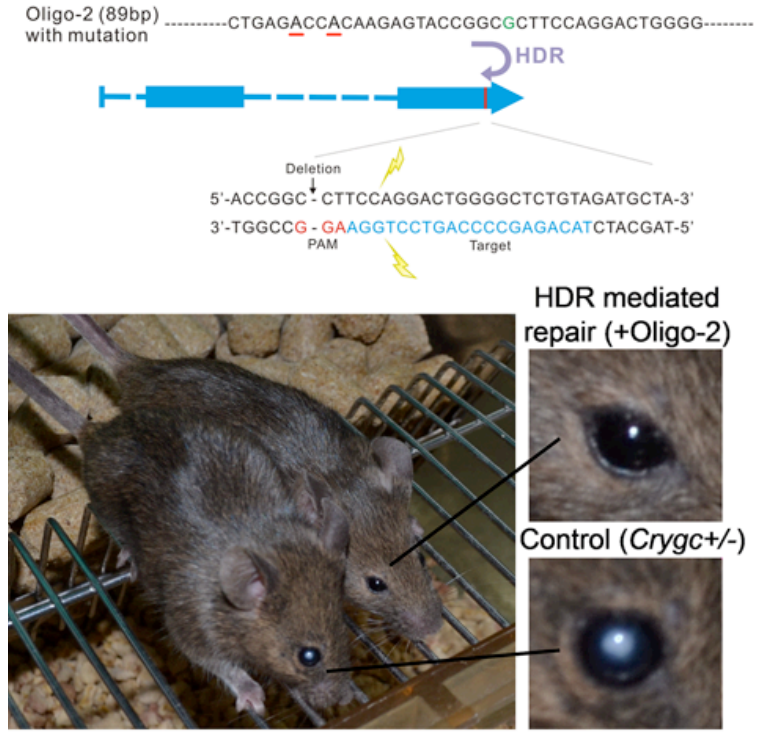
# Can CRISPR/Cas9 be used for correct genetic disorders?


*(Wu et al., Cell 2013)*

NHEJ events can lead to correct reading frame

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



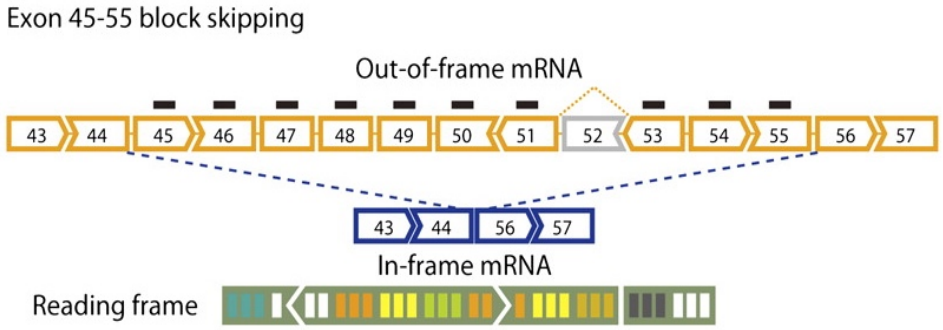



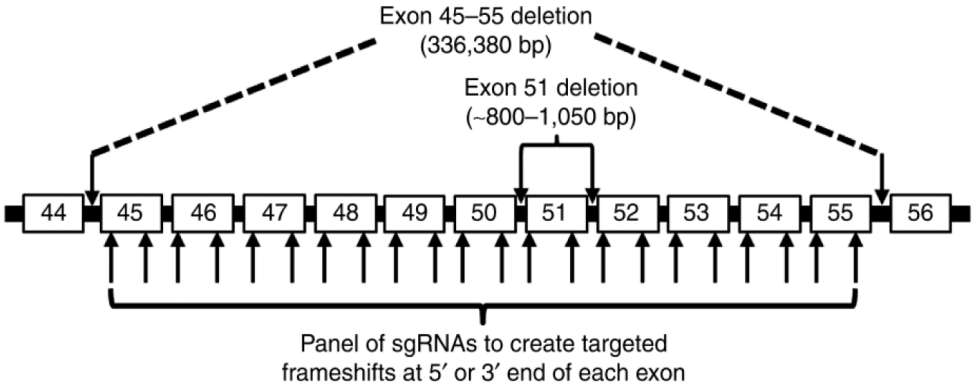

*(Wu et al., Cell 2013)*

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

# 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)

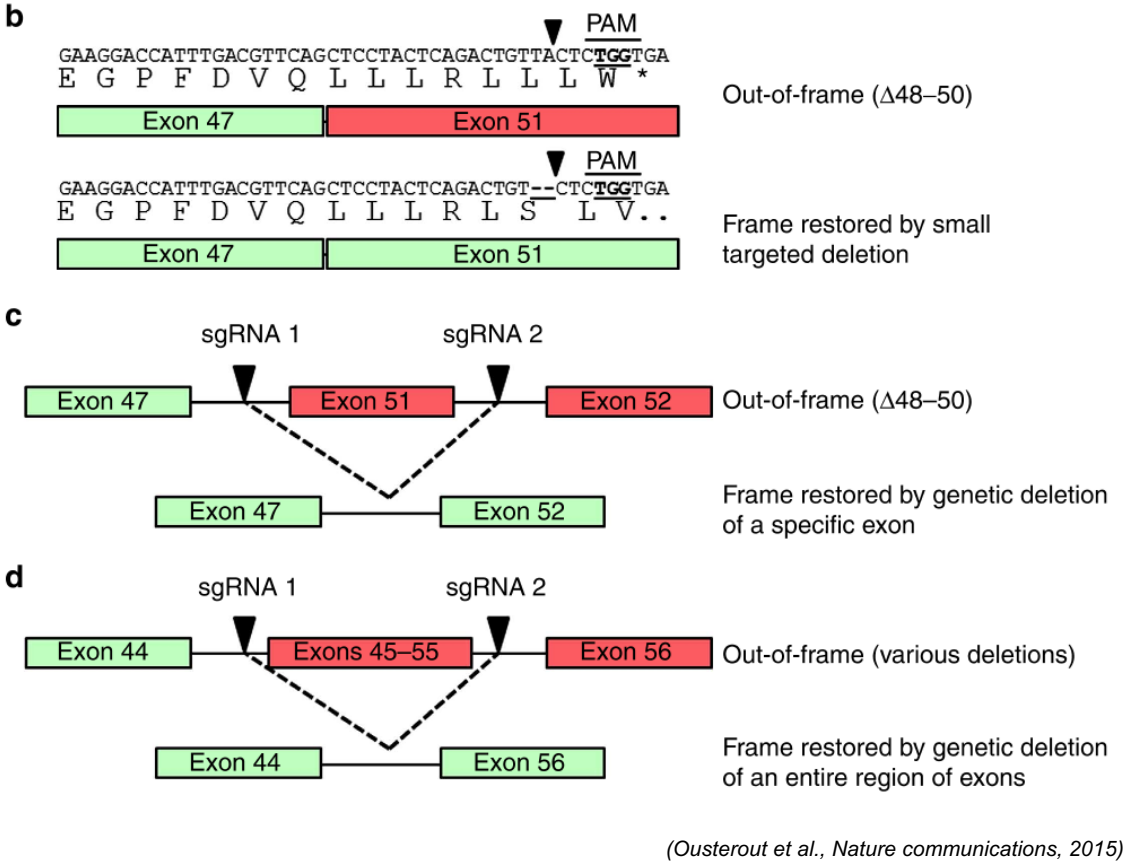



*(Ousterout et al. Nature communications 2015)*

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

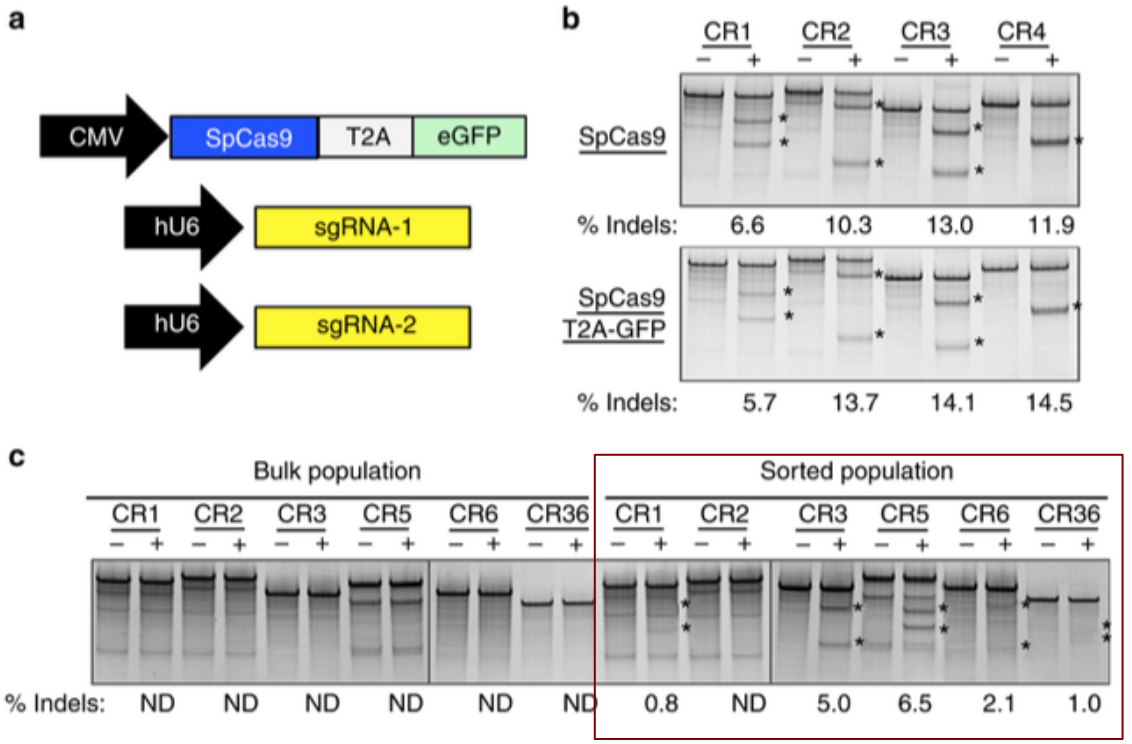
# Can CRISPR/Cas9 be used for gene therapy?




*(Ousterout et al., Nature communications, 2015)*

sgRNA are designed to restore dystrophin reading frame

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



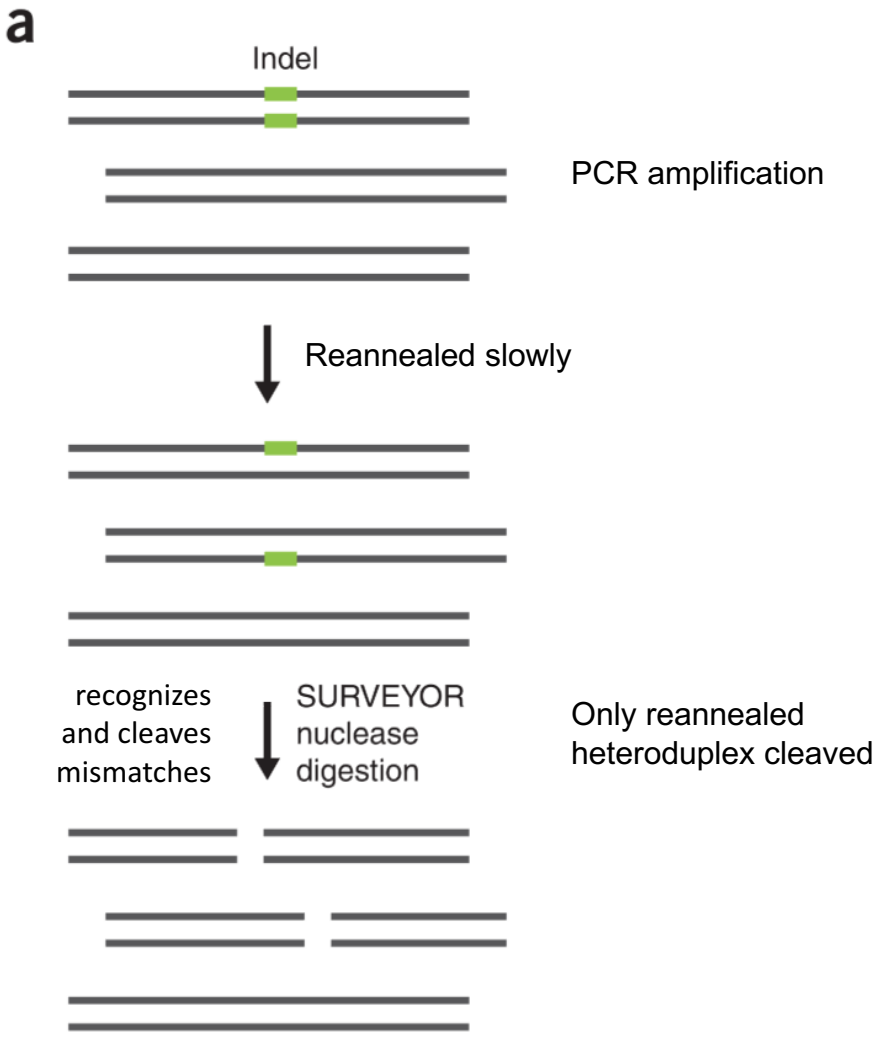

HEK293T cells

DMD myoblasts

*(Ousterout et al., Nature communications, 2015)*

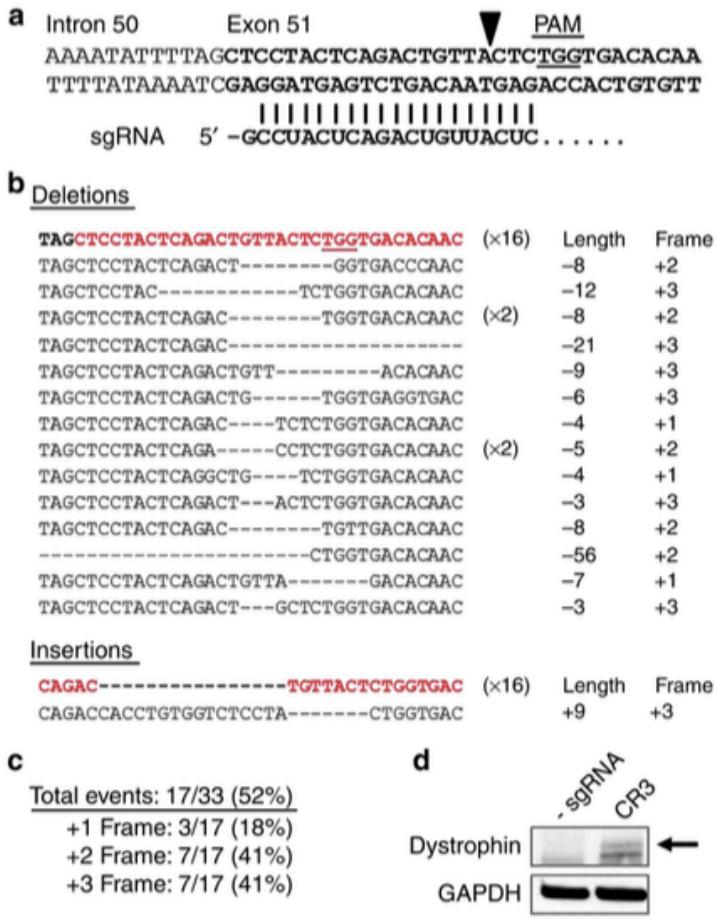
In DMD sorted cells there is detectable level of sgRNA activity

# SURVEYOR ASSAY




*(Adapted from Ran F.A. et al., Nature protocols, 2013 )*

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



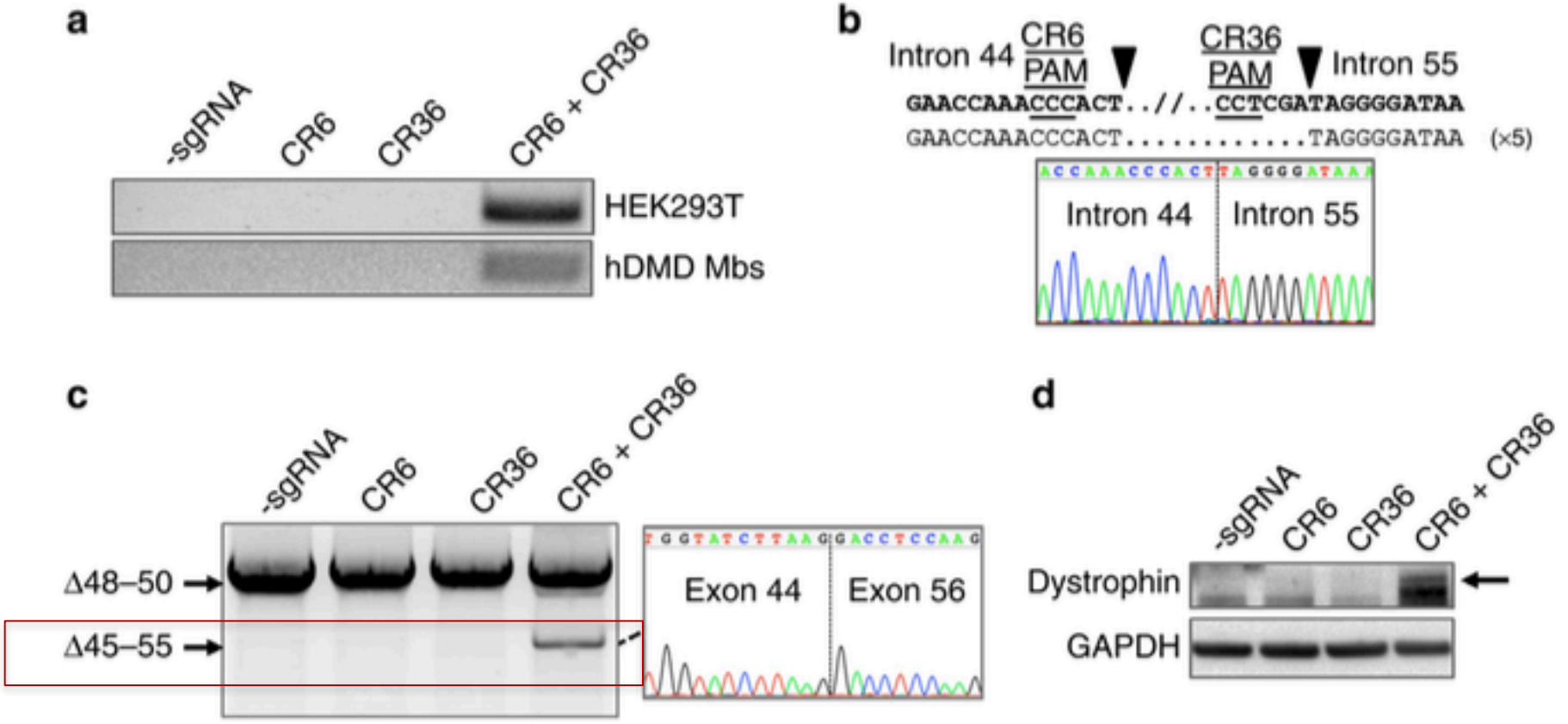

Sanger sequencing

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

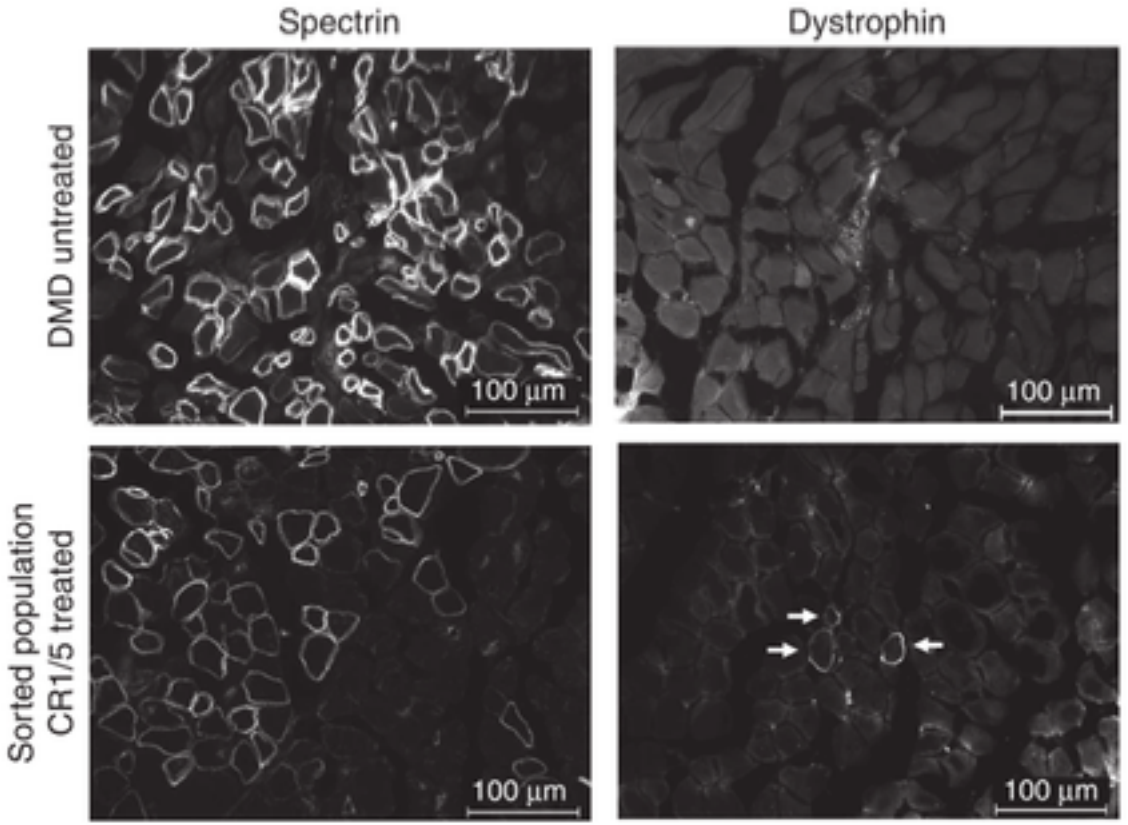
# Is it possible to develop a single method that can address different common patients deletions?




*(Ousterout et al., Nature communications, 2015)*

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

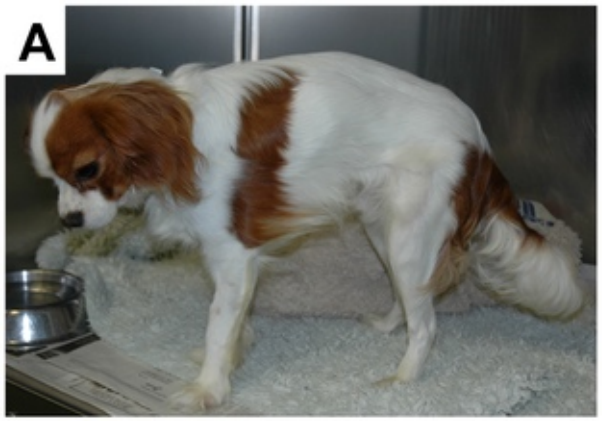
# Can CRISPR/Cas9 be used for correct DMD *in vivo*?



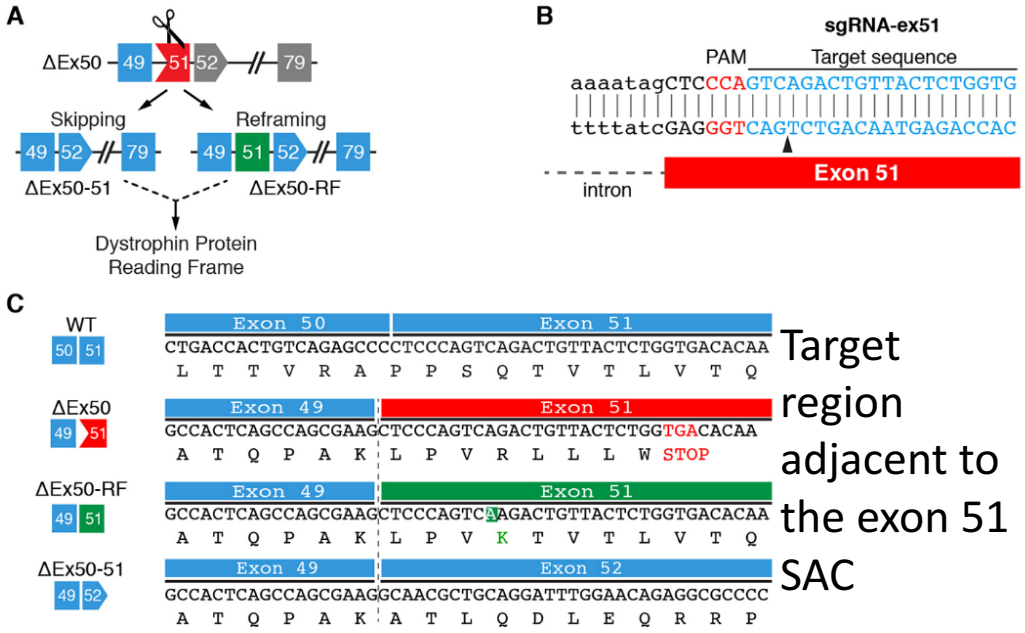

*(Ousterout et al., Nature communications, 2015)*

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

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



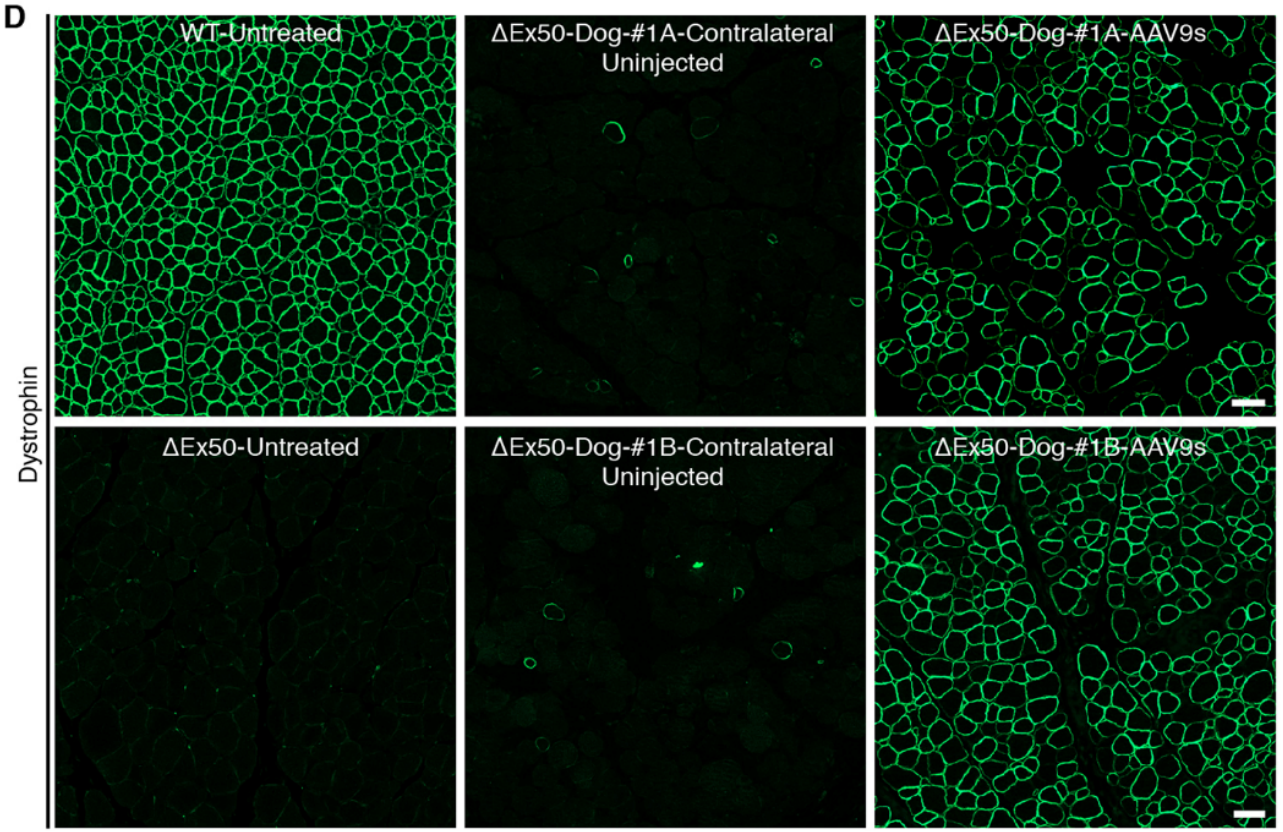

(Walmsley G.L., et al., PlosOne, 2010)




Target region adjacent to the exon 51 SAC

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

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




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

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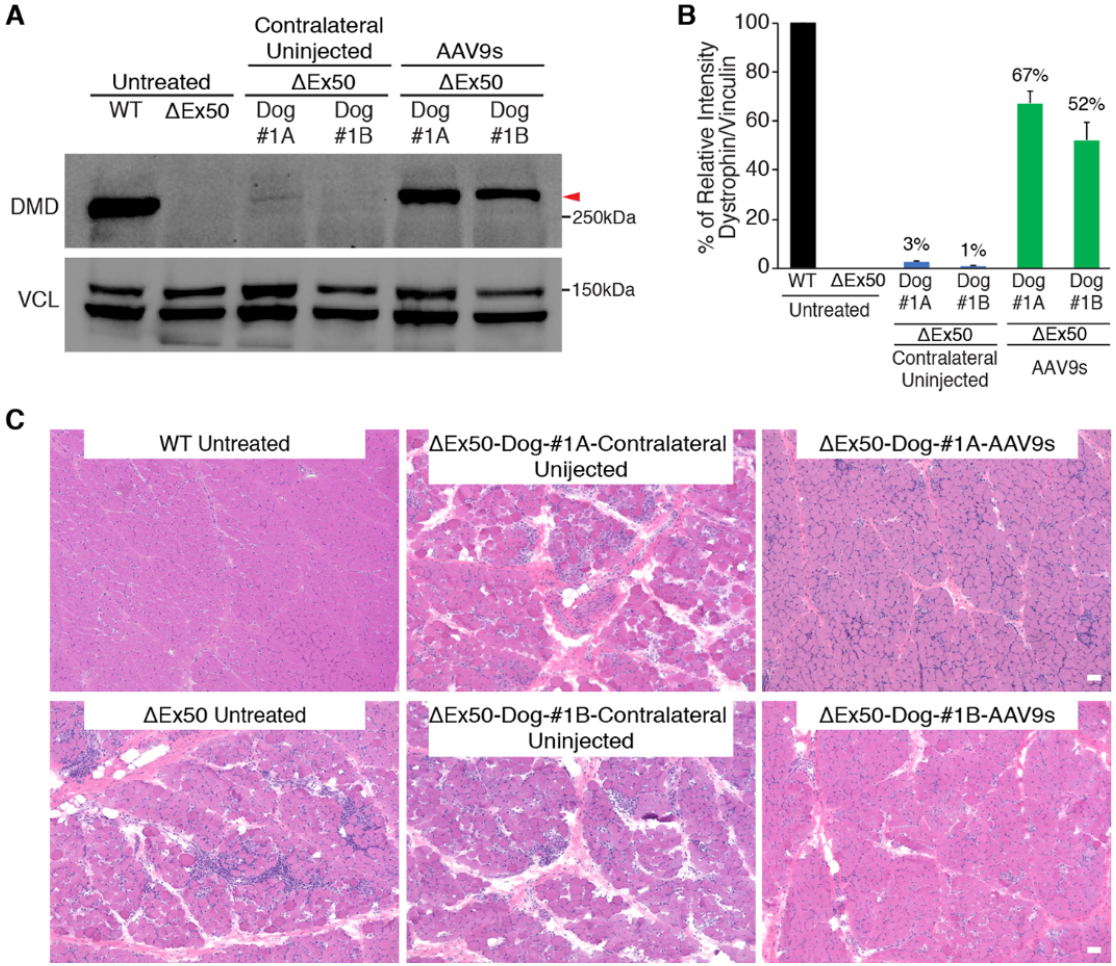


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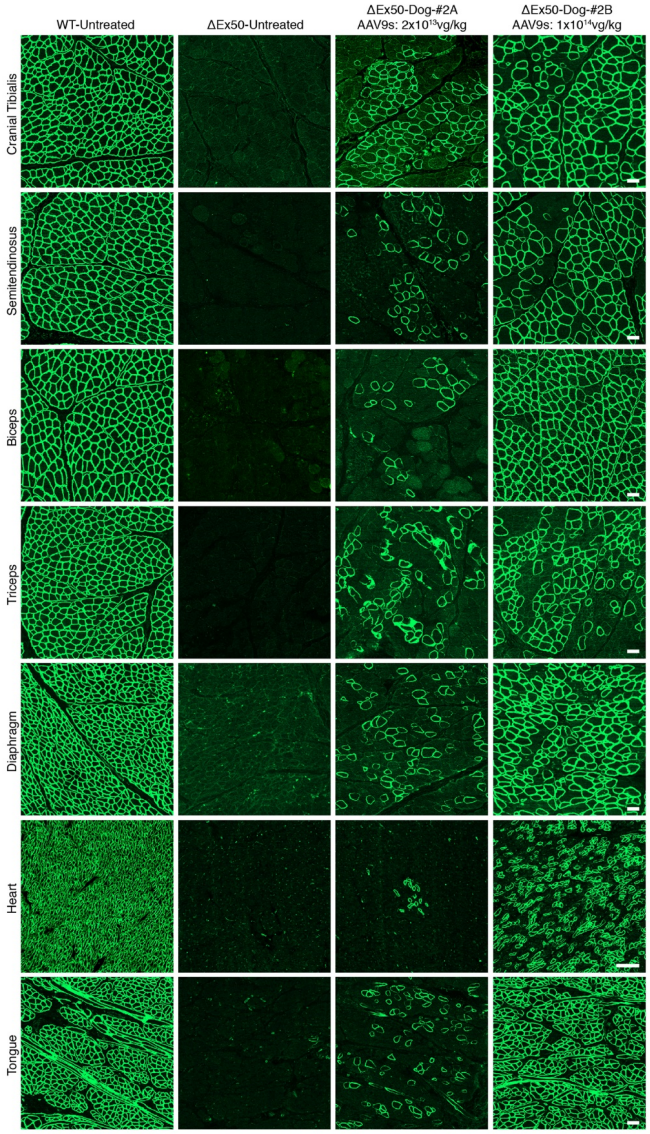
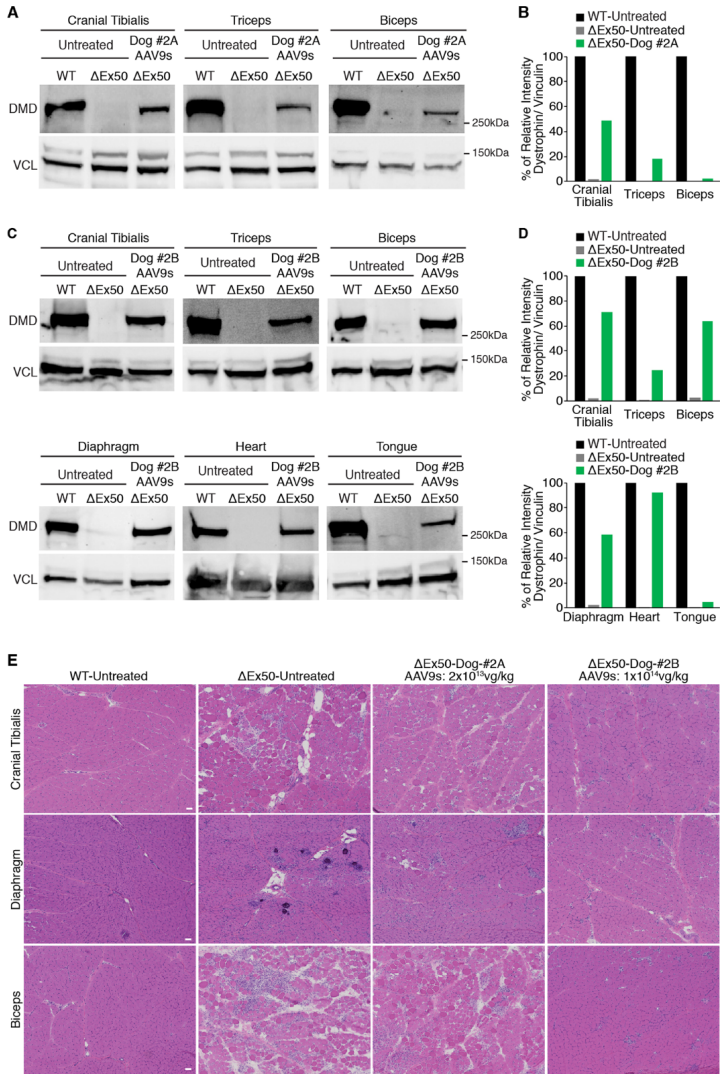
# Can CRISPR/Cas9 be used for correct DMD *in vivo*?



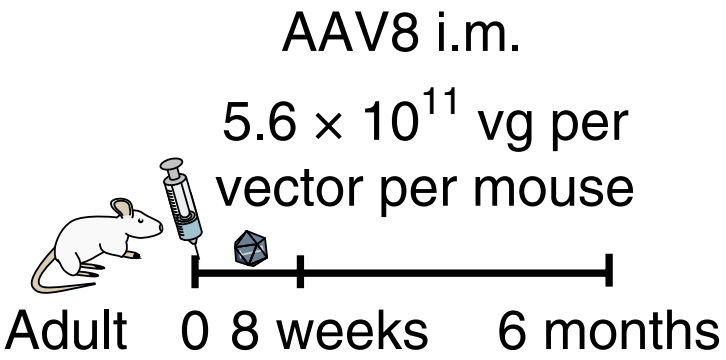

(Amoasii L. et al., Science, 2018)

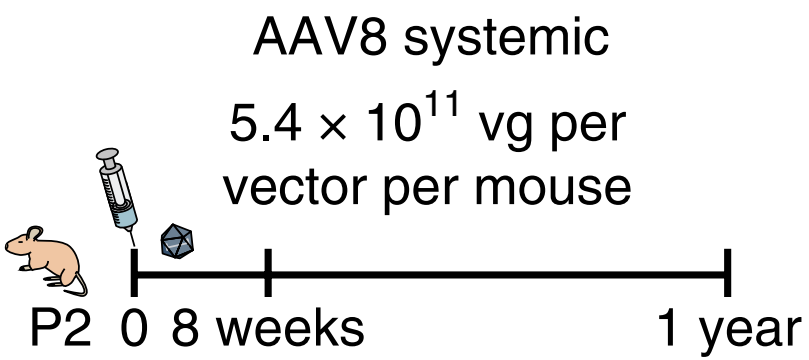
# CRISPR/Cas9 can be used for correct DMD *in vivo*?




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

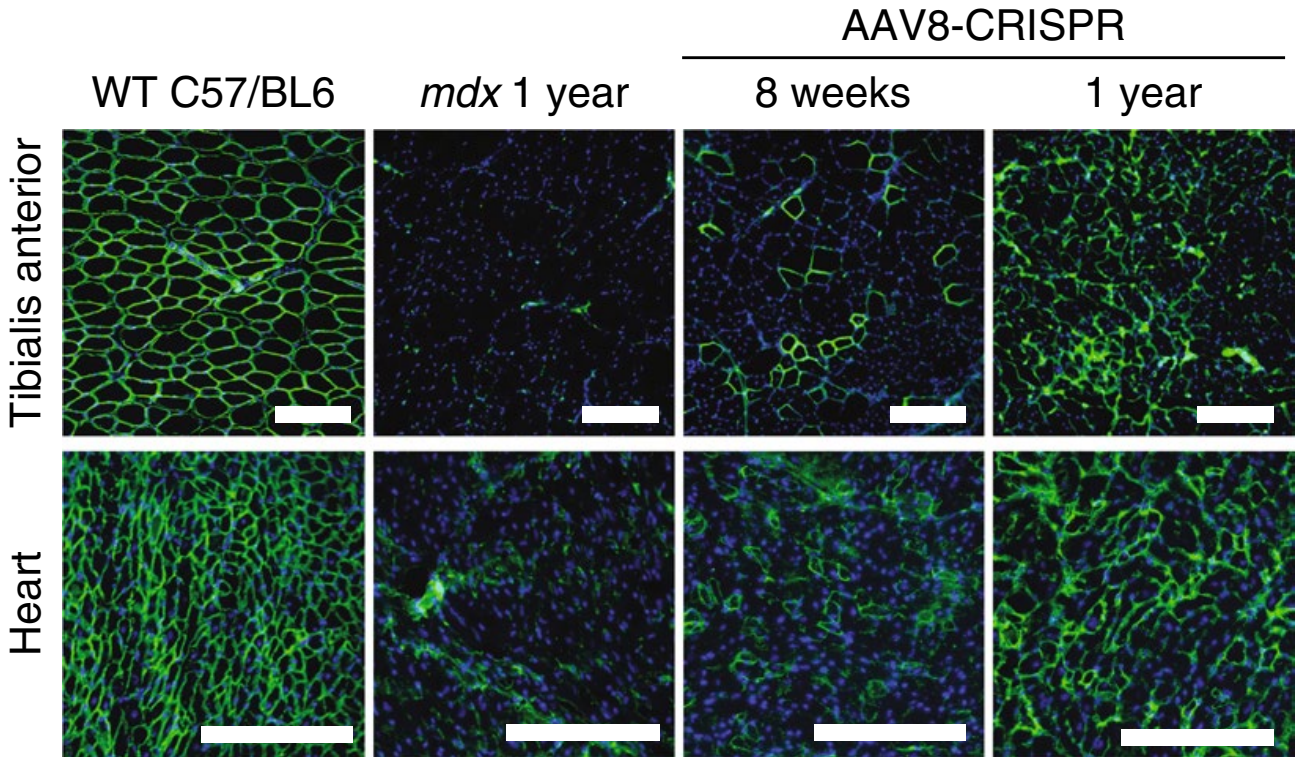
# Can CRISPR/Cas9 sustain long term dystrophin expression?




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

# Can CRISPR/Cas9 sustain long term dystrophin expression?




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

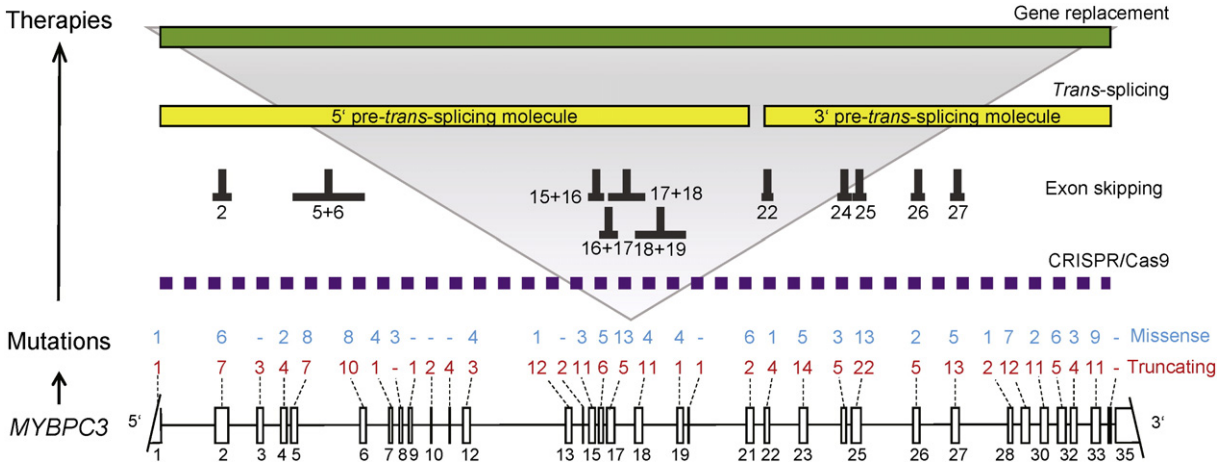
# ‘Gene editing has to learn from 1.0 gene therapy’
# Ronald Cohn, Hospital for Sick Children, Toronto

at XVII Conferenza internazionale sulla distrofia muscolare
di Duchenne e Becker,
Rome, February 15-17

*CRISPR/Cas9 correction in human embryos?*

# CRISPR/Cas9 correction in human embryos?

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

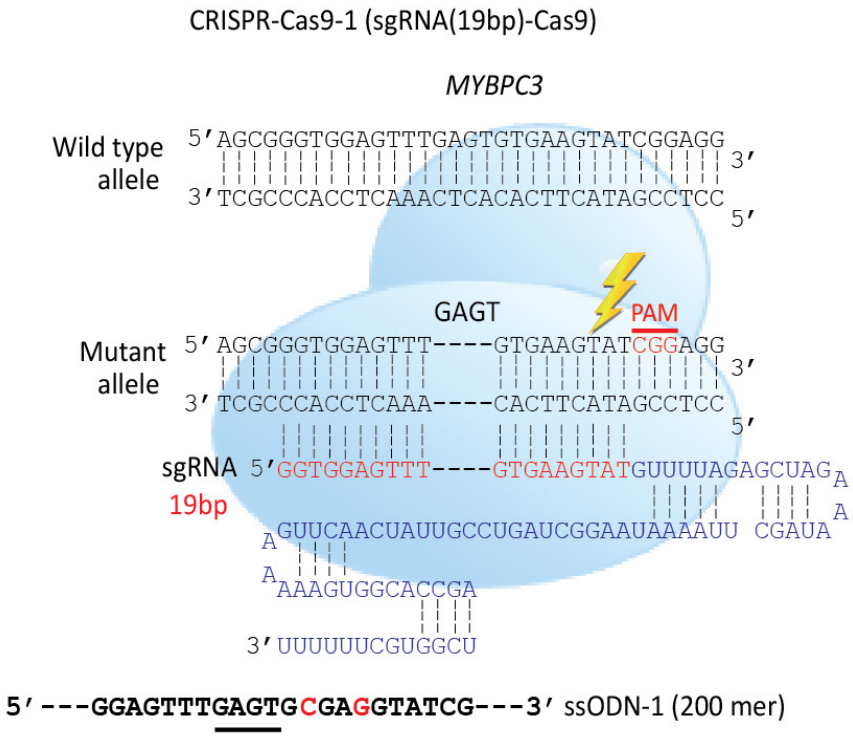



(Carrier L. et al., Gene review, 2015)

Heart failure in healthy individuals
Mostly autosomal dominant

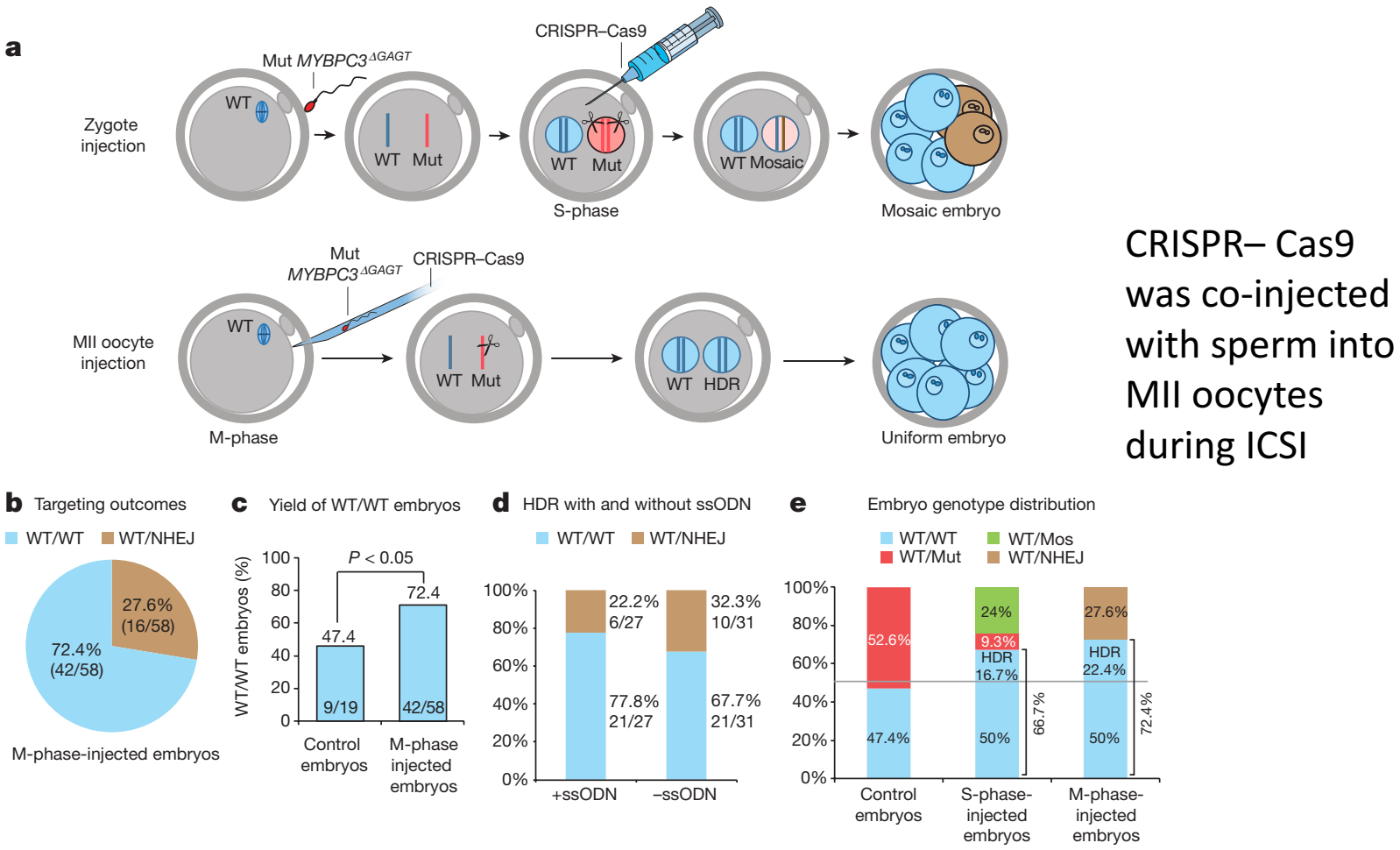
# CRISPR/Cas9 correction in human embryos?

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




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

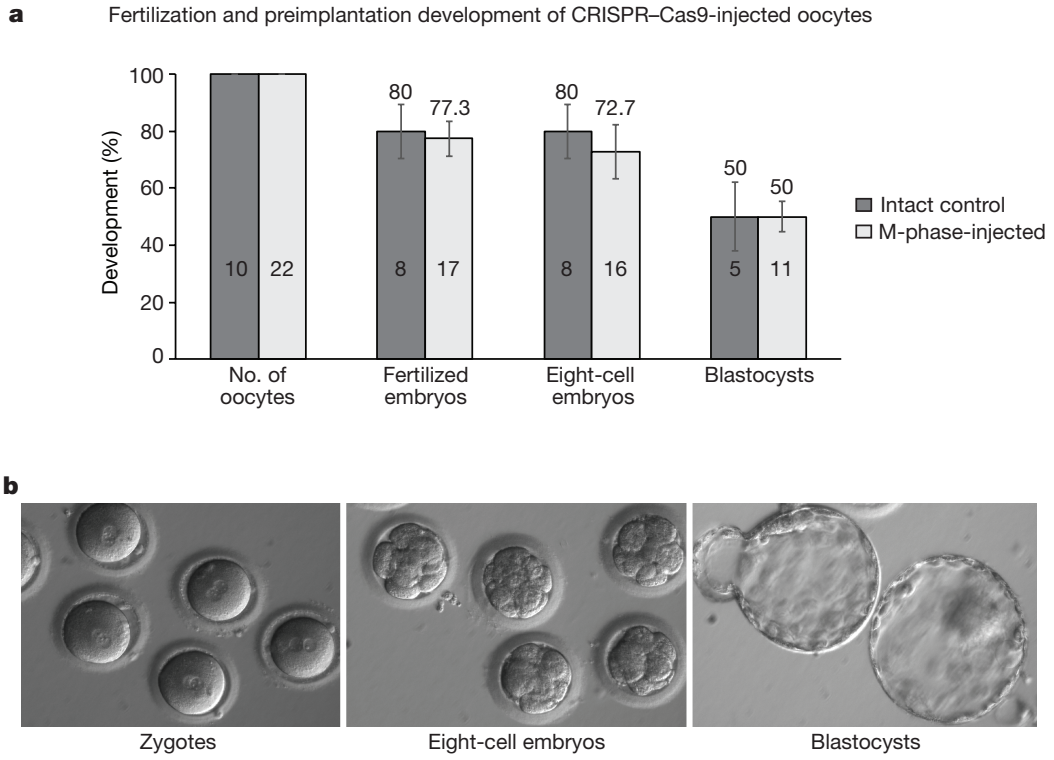
# CRISPR/Cas9 correction in human embryos?




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 |

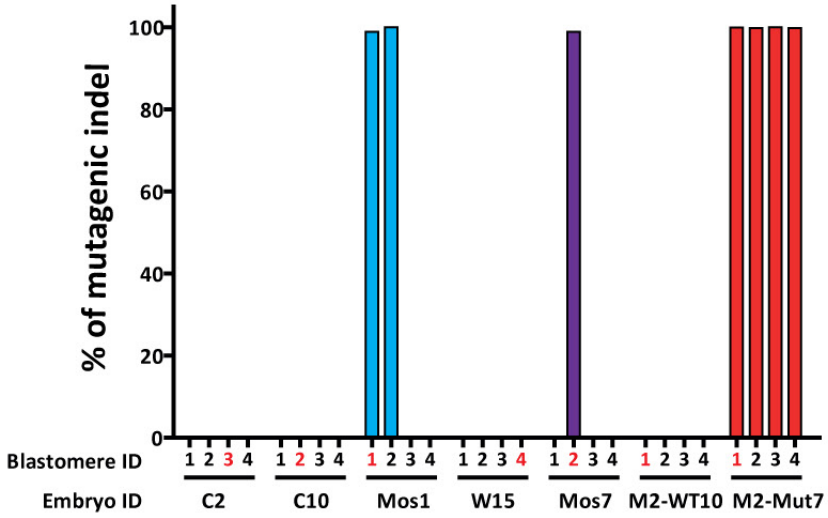
Corrected ES from blastocysts

(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

**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

# 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.*

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

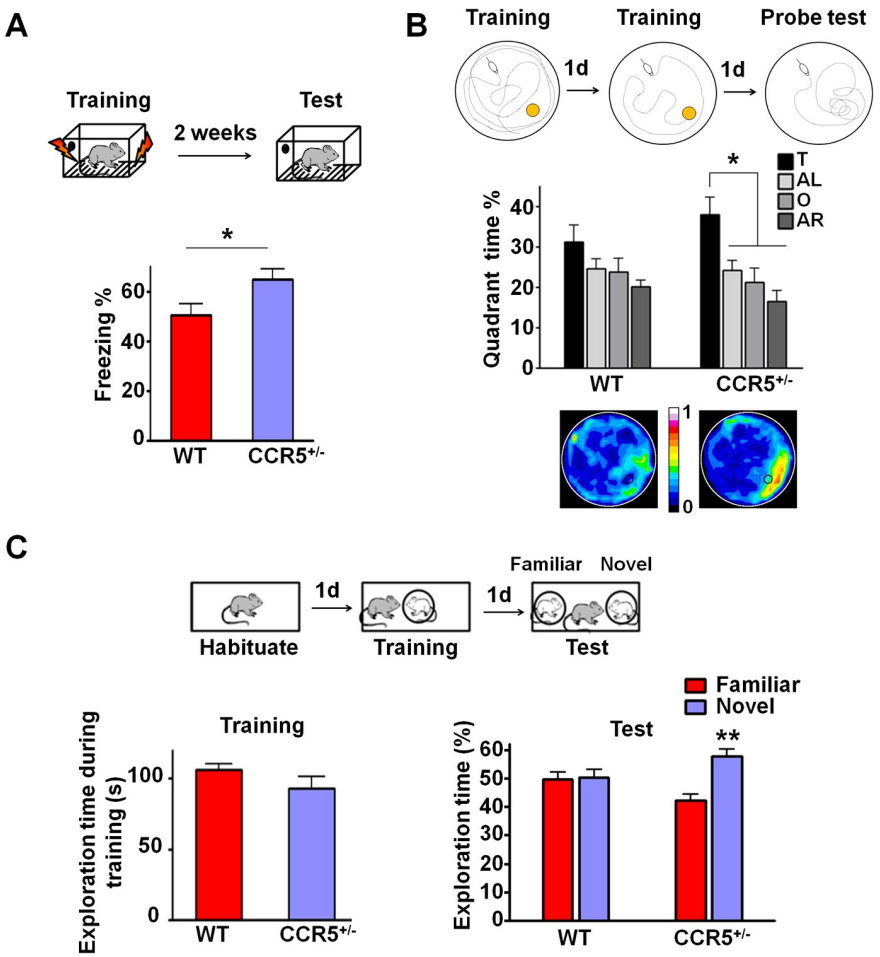
**NEWS** · 12 DECEMBER 2018 nature

# 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.*

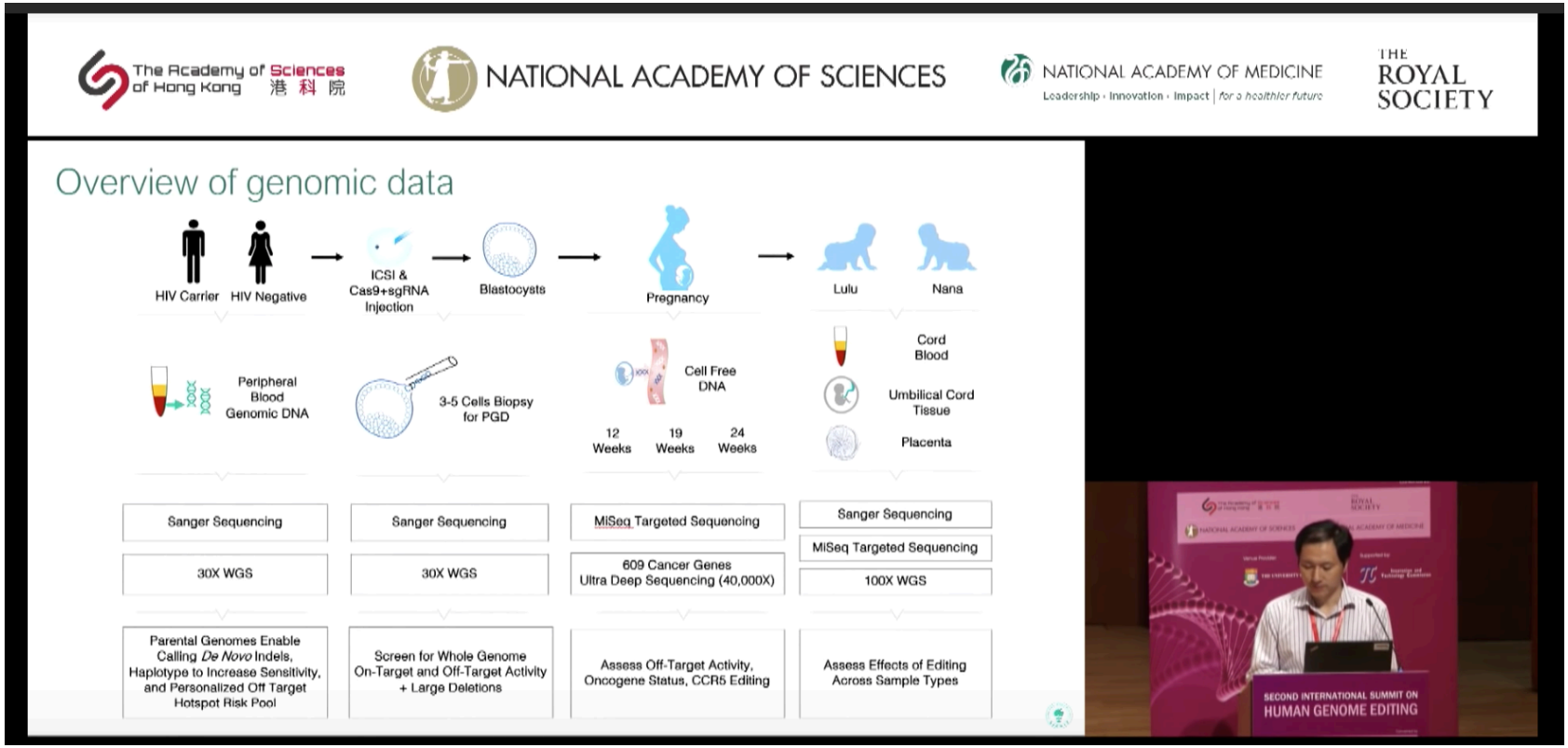
## Brain enhancement?

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



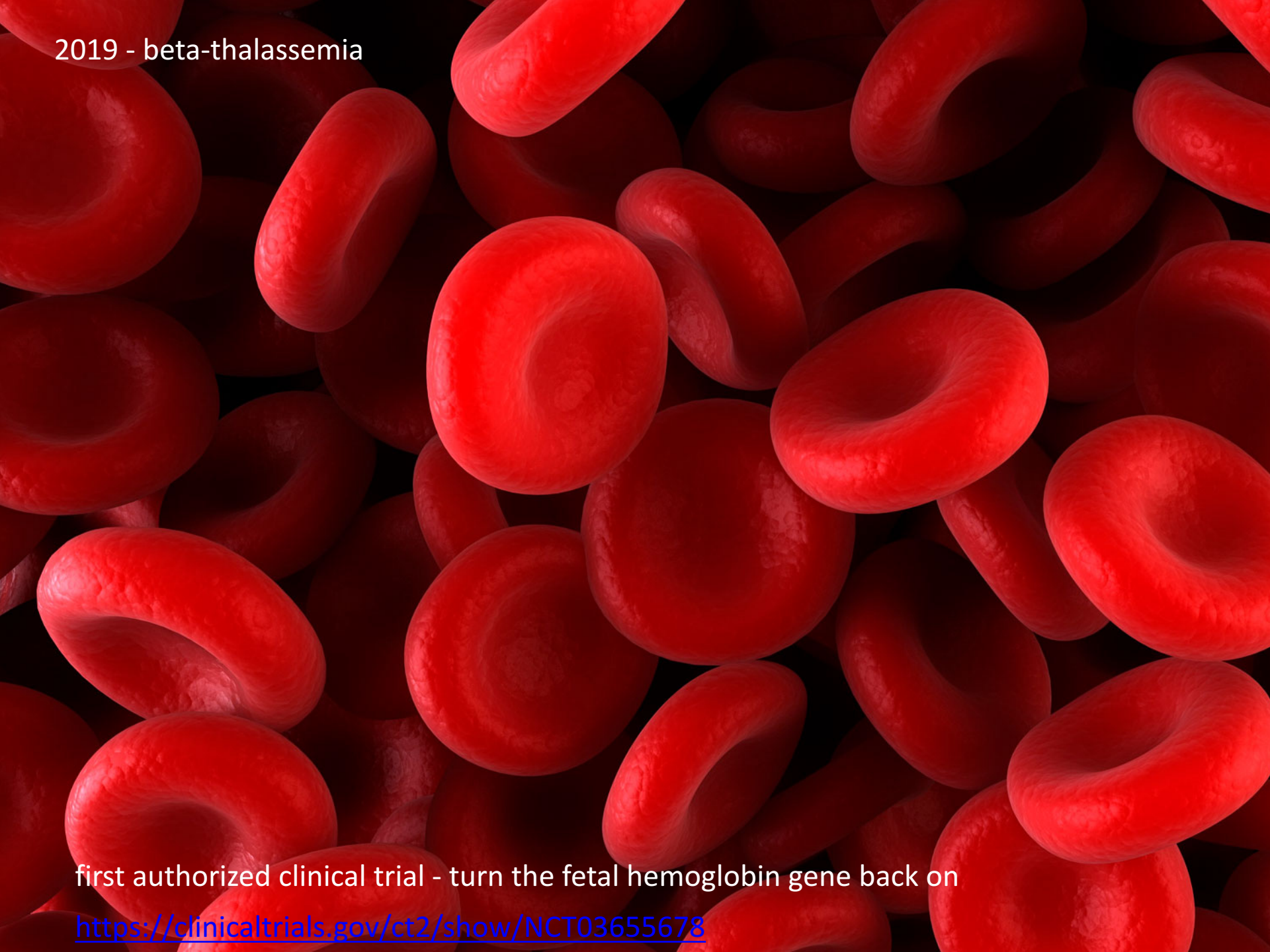

(Zhou M., Elife, 2016)

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



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

2019 - beta-thalassemia

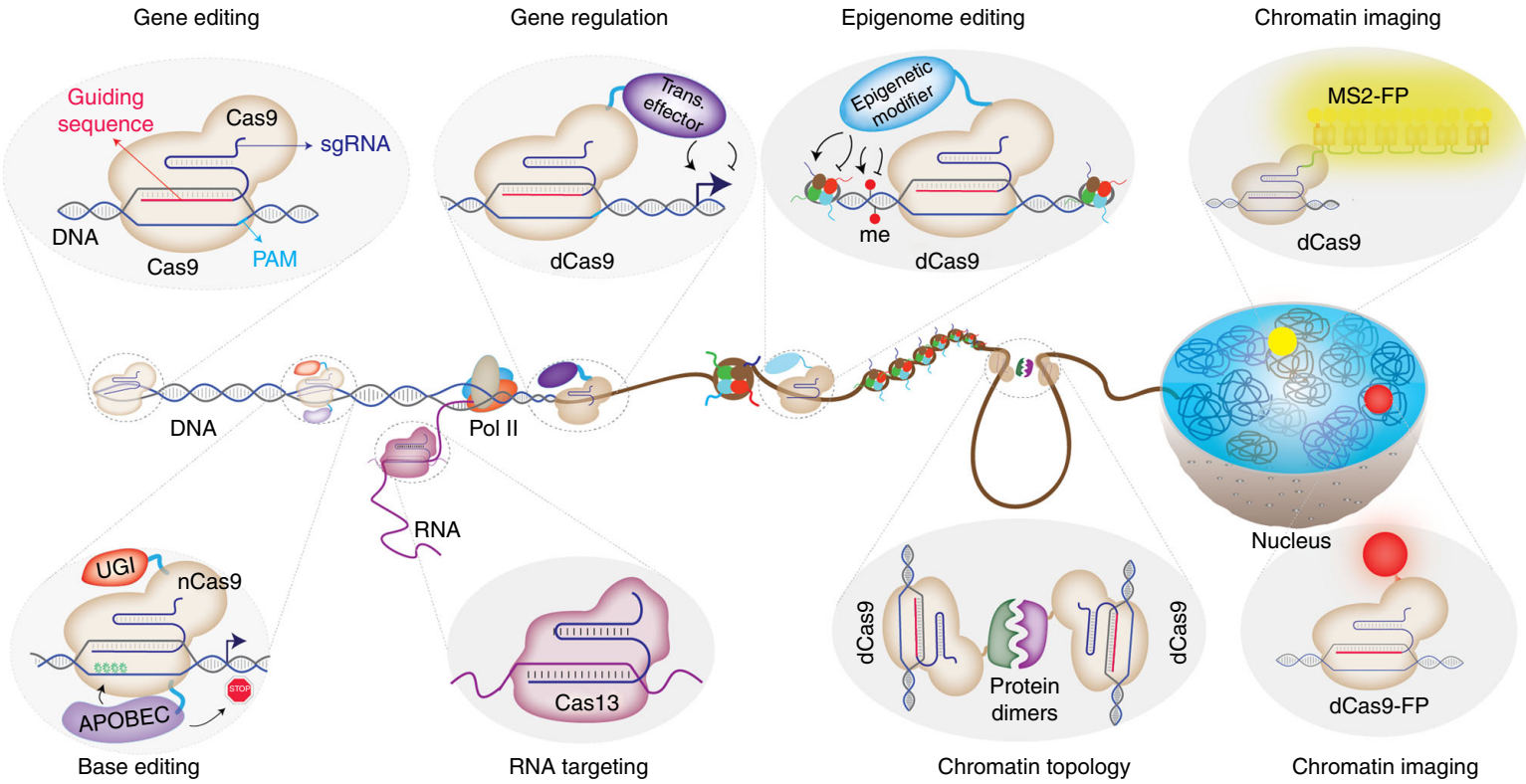


first authorized clinical trial - turn the fetal hemoglobin gene back on

https://clinicaltrials.gov/ct2/show/NCT03655678

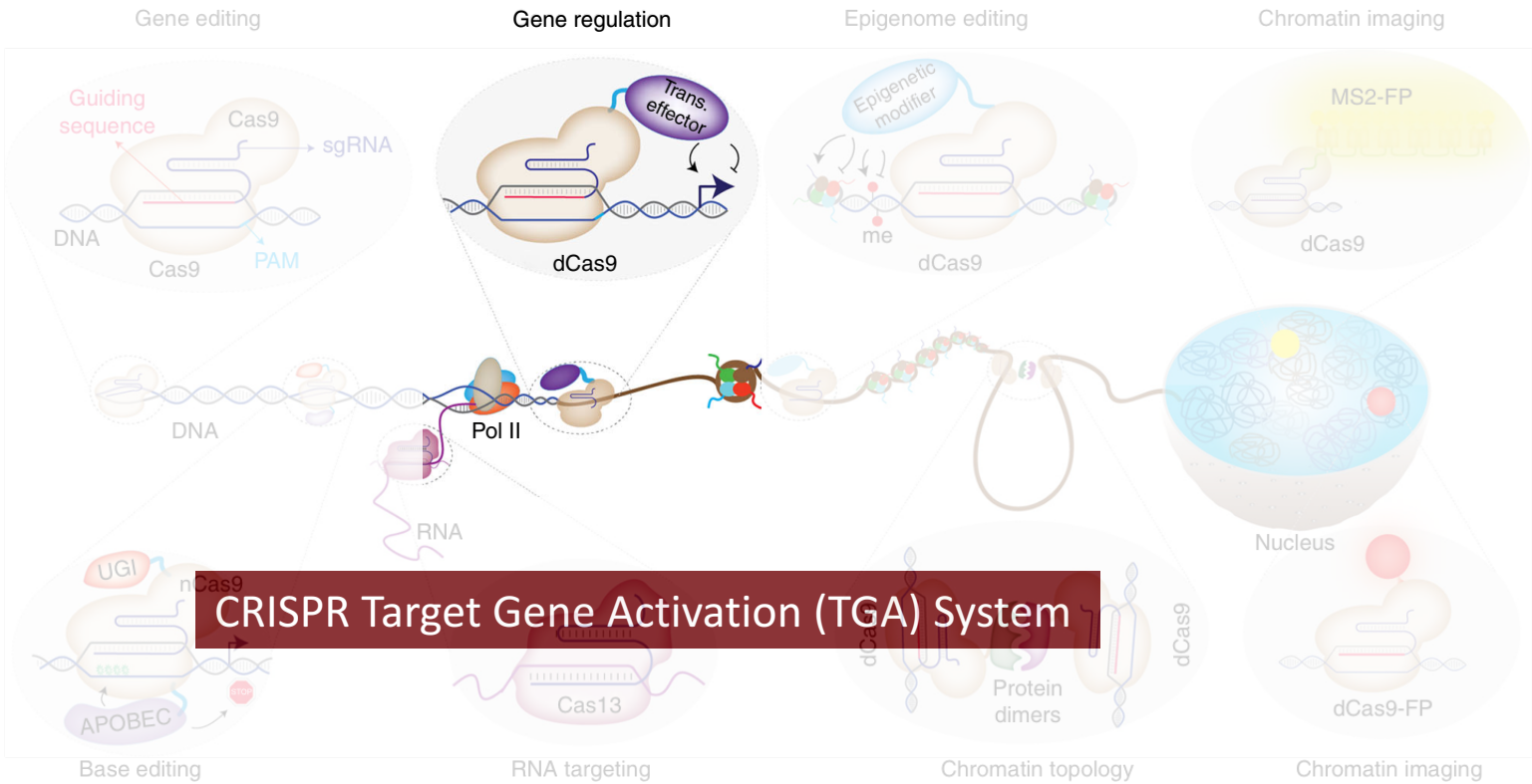
*Can we use CRISPR without permanently modify the genome?*

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




(Adli M., Nature communications, 2018)

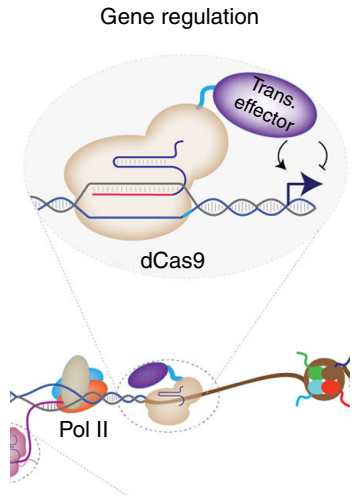
# CRISPR/Cas9 technologies beyond genome editing are based mainly on dead-Cas9




CRISPR Target Gene Activation (TGA) System

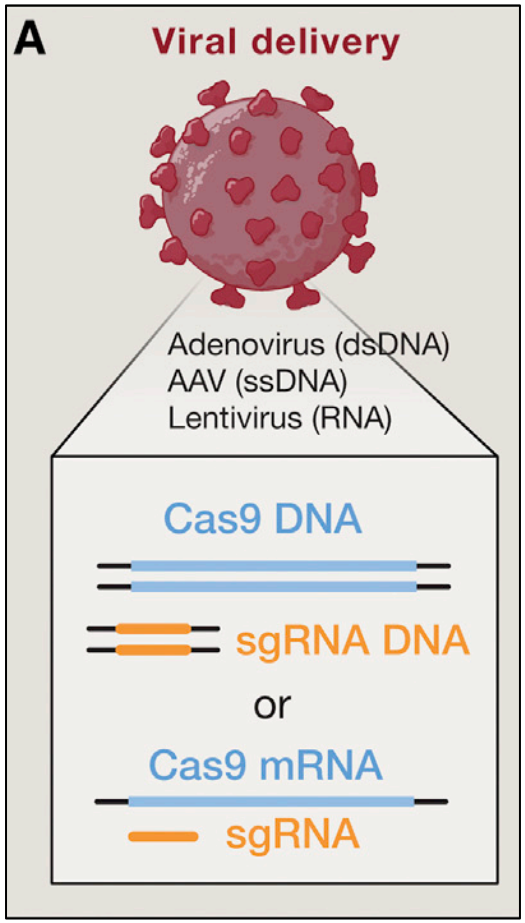
(Adli M., Nature communications, 2018)

# Limits of CRISPR TGA system *in vivo*




(Adli M., Nature communications, 2018)

- Insufficient transduction of the Cas9 fusion protein
- Low level of *in vivo* TGA
- Size (sequences of dCas9/gRNA and co-transcriptional activator)
- Not yet able to induce a physiologically relevant phenotype in a postnatal mammal



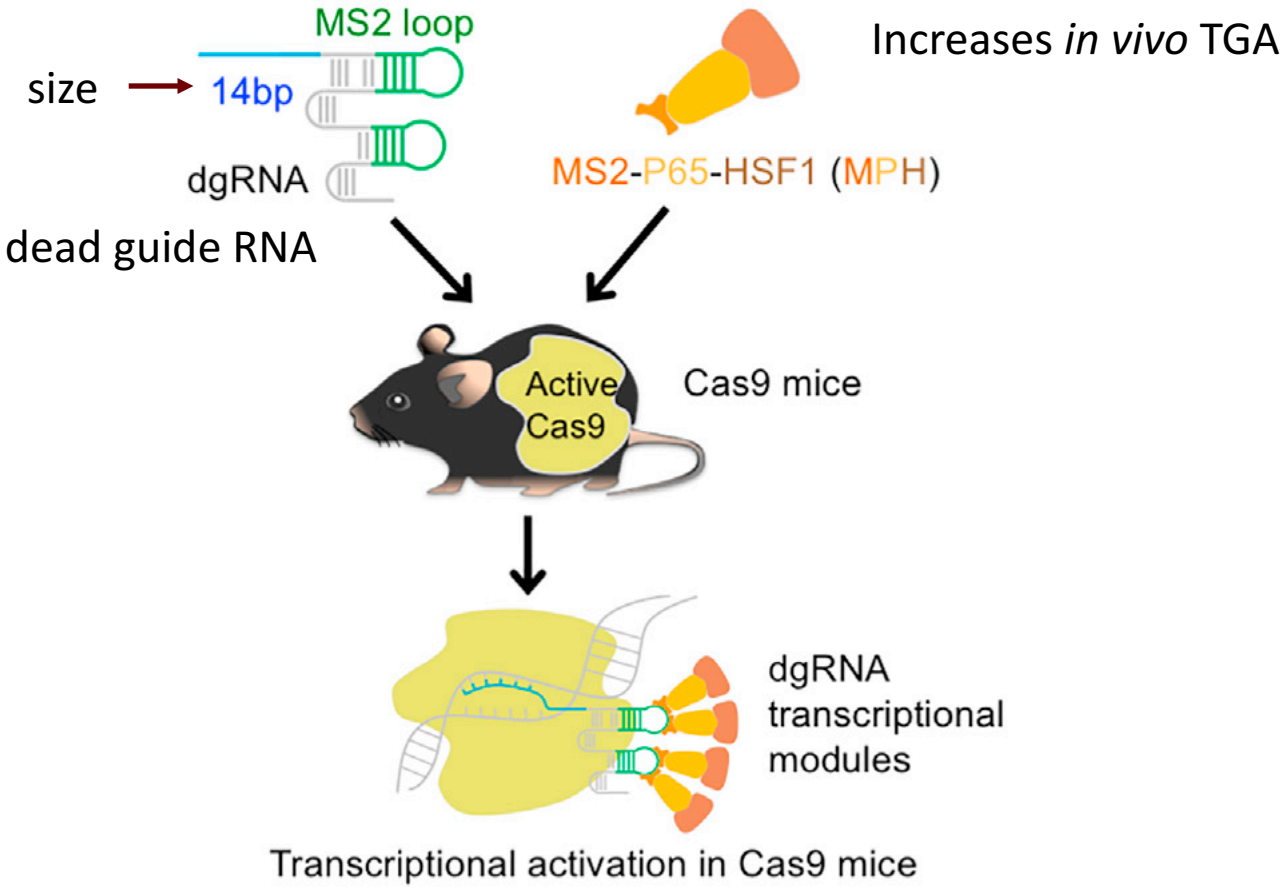

(Komor A.C. et al., Cell, 2017)

**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

# Is it possible to increase the TGA efficiency?

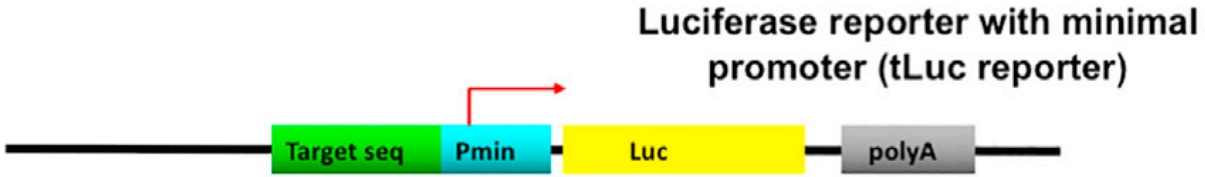


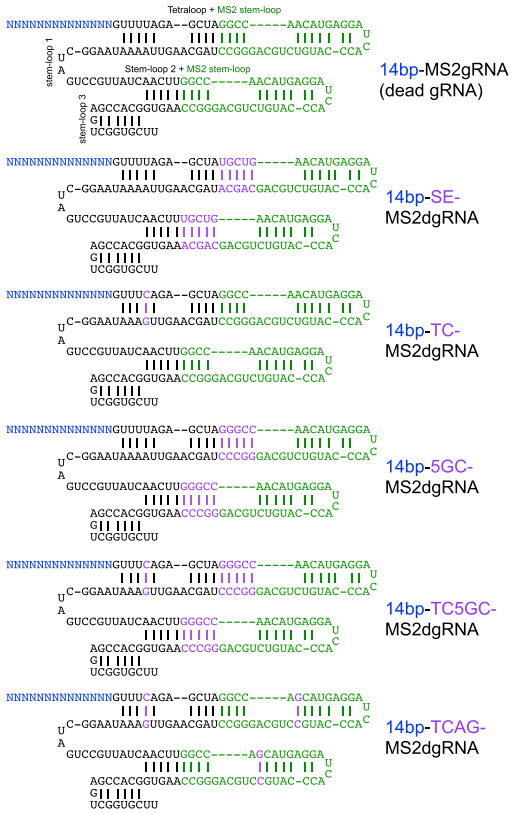

(Liao H.K., et al, Cell, Oct 2018)

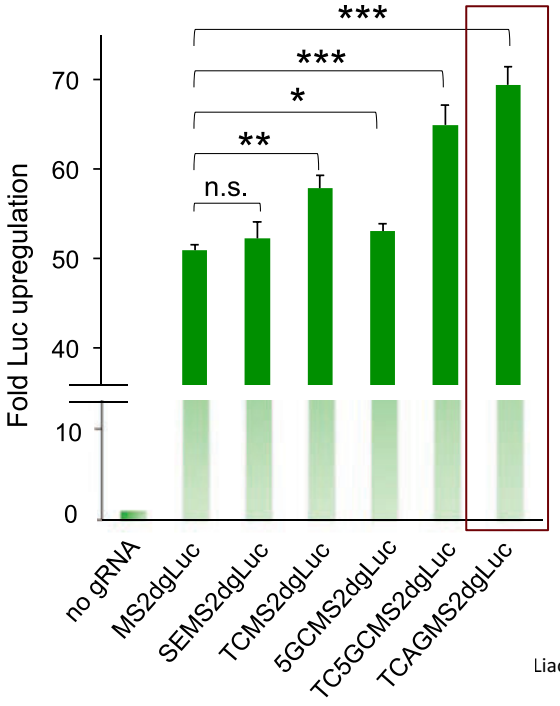
Adapted module with dead guide RNA (Cas9/MS2dgRNA)

# Is it possible to increase the TGA efficiency?

**Optimzed scaffold** by changing **G:C** and/or **shortening** repetitive sequences



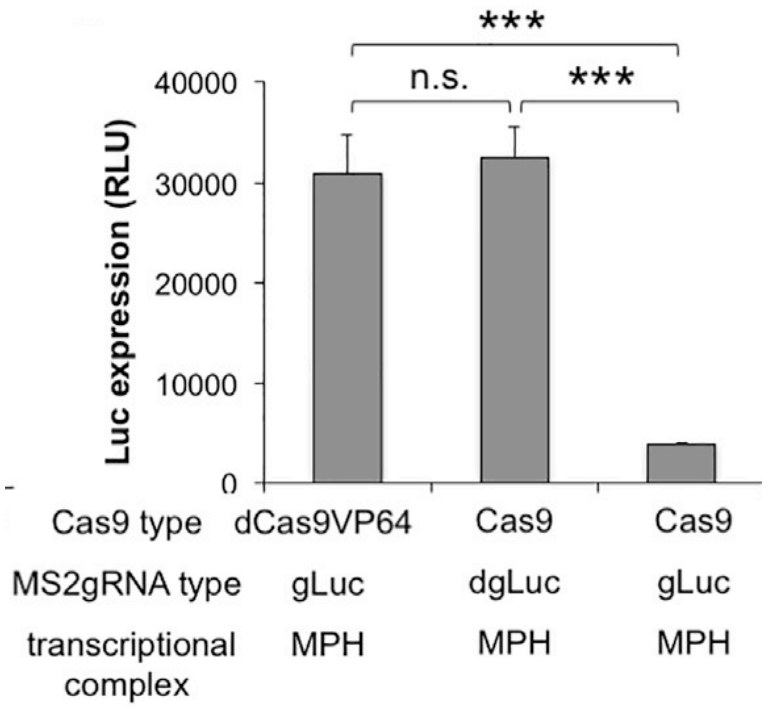



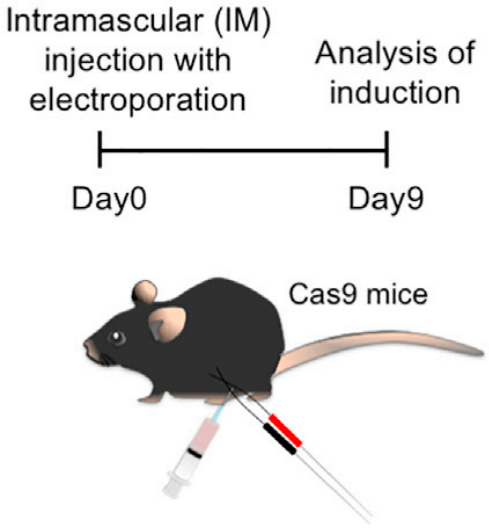

Liao H.K., et al, Cell, Oct 2018)

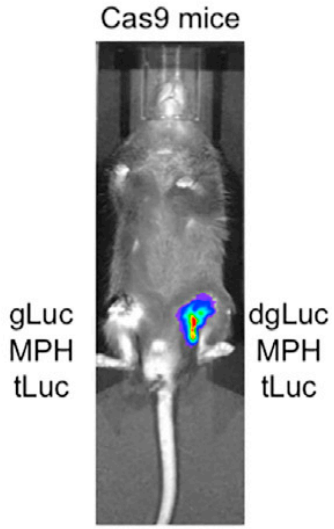
Cas9/MS2dgRNA shows high levels of TGA

# Is it possible to increase the TGA efficiency?




(Liao H.K., et al, Cell, Oct 2018)

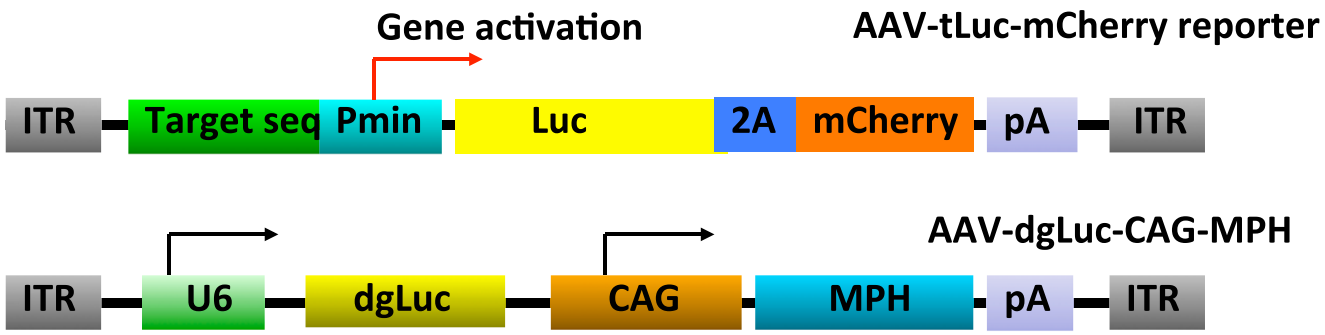


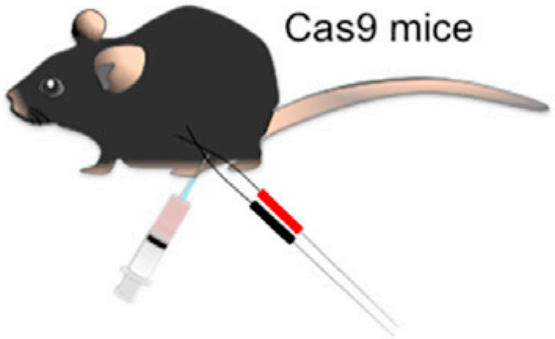

Cas9/MS2dgRNA TGA efficiency is comparable to dCas9VP64 but is smaller and works *in vivo*

# Is it possible to use MS2dgRNA for TGA of a *reporter gene in vivo* with AAV?

AAV9




(Liao H.K., et al, Cell, Oct 2018)



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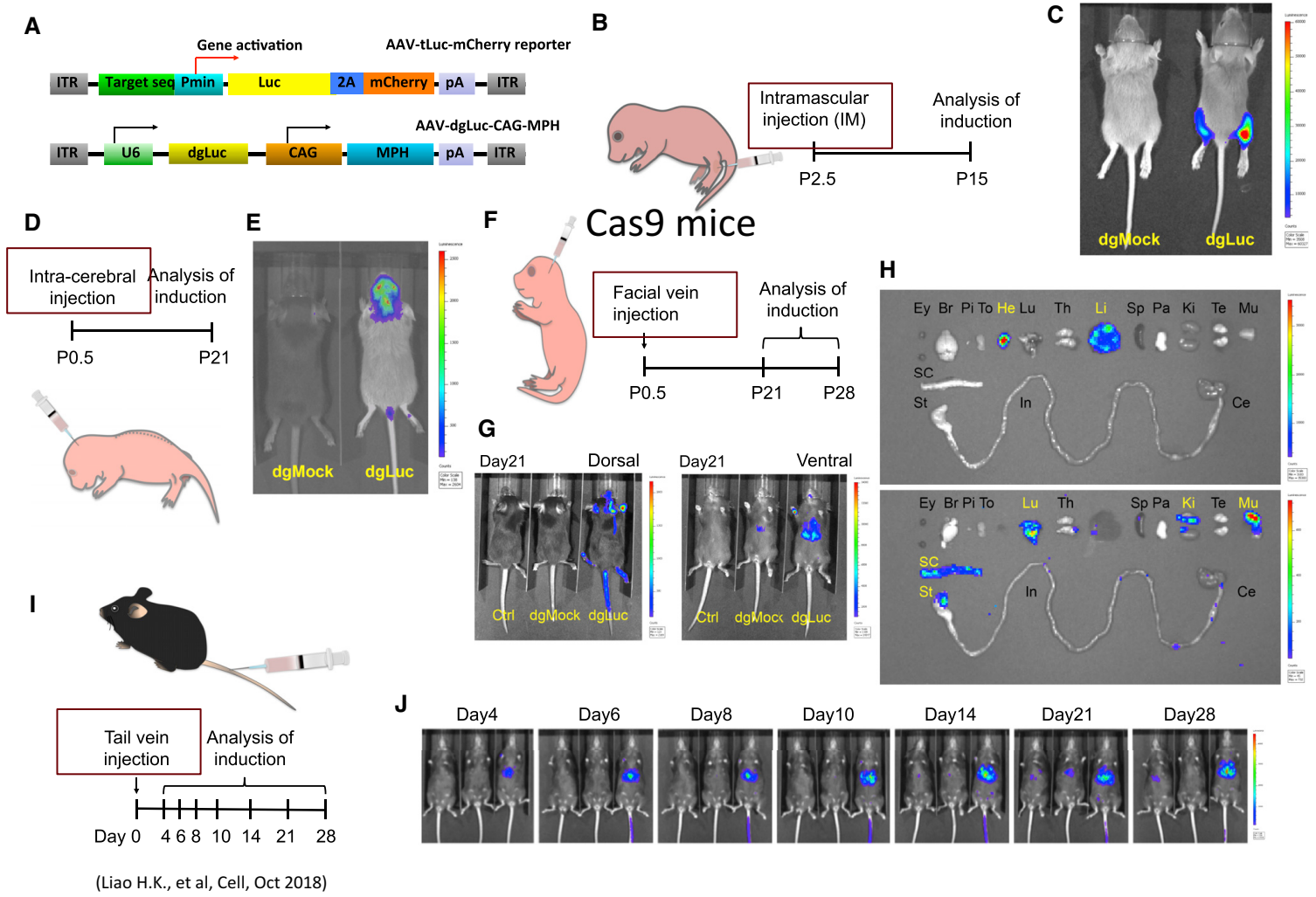
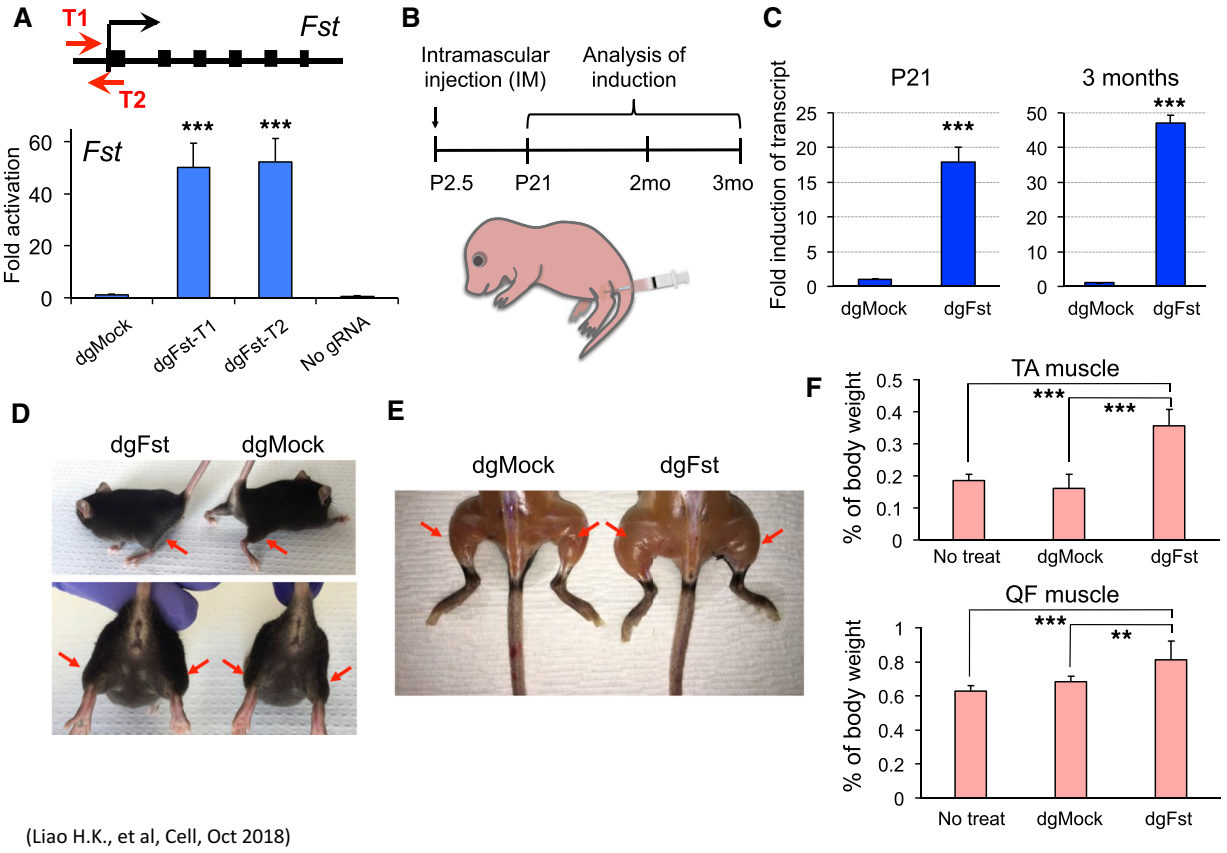



(Liao H.K., et al, Cell, Oct 2018)

AAV9-MS2dgRNAs induces transcription of a reporter gene *in vivo*

# Is it possible to use MS2dgRNA for TGA of an endogenous gene and cause a phenotype?

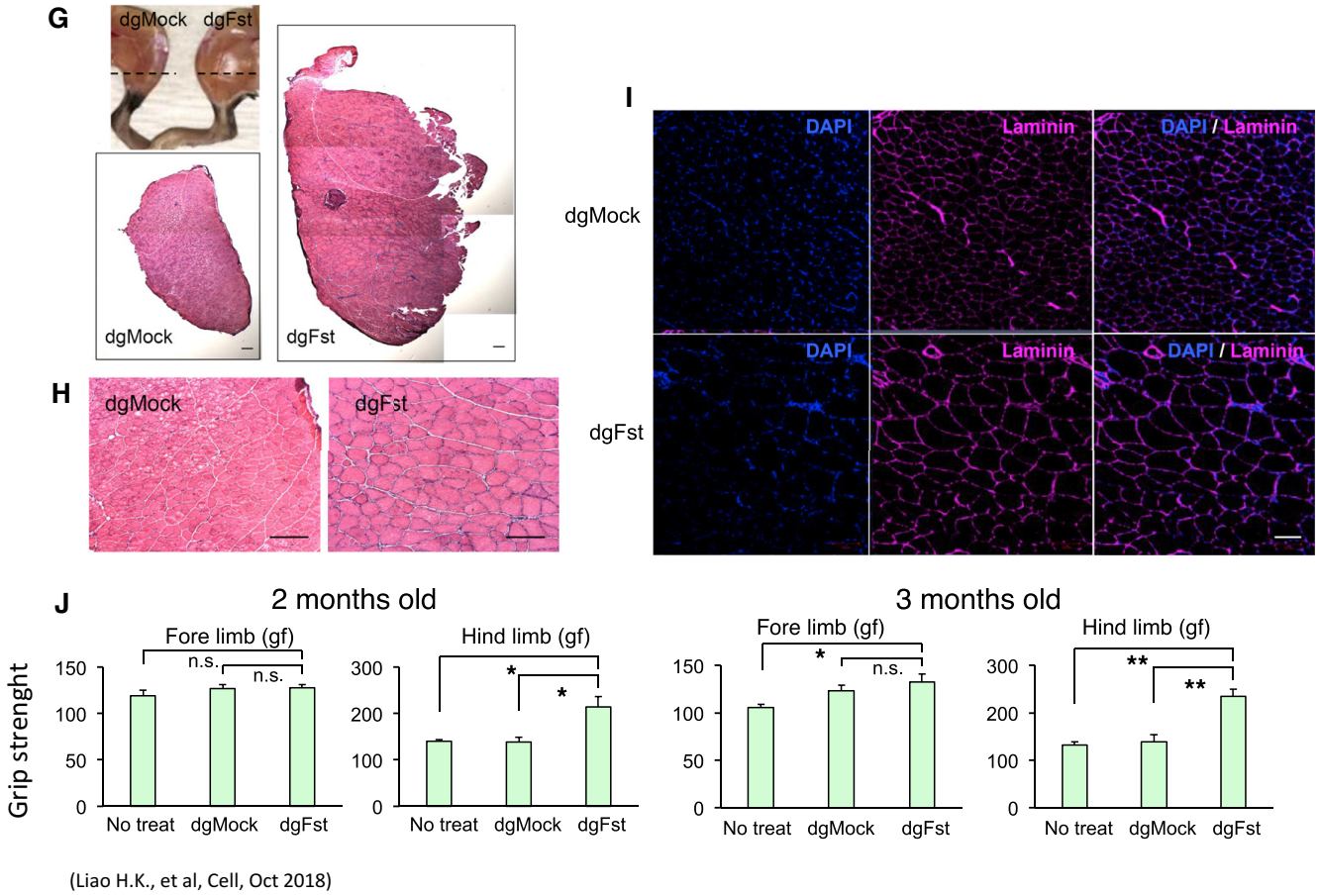
## Follistatin o/e increases muscle mass




(Liao H.K., et al, Cell, Oct 2018)

AAV9-MS2dgRNAs induces transcription of FST and induces muscle mass increase

# Is it possible to use MS2dgRNA for TGA of an endogenous gene and cause a phenotype?



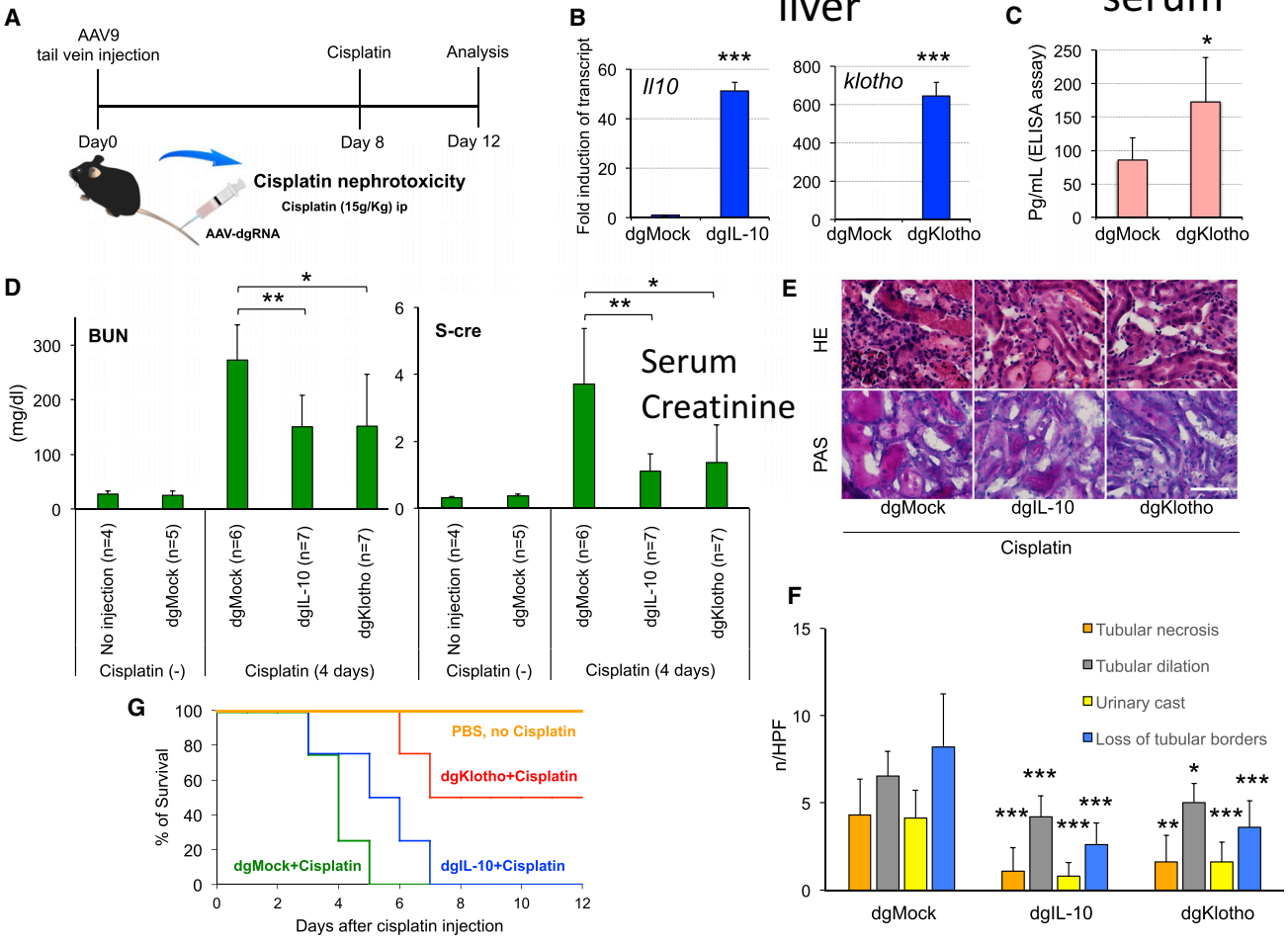

(Liao H.K., et al, Cell, Oct 2018)

AAV9-MS2dgRNAs induces sustained phenotypic changes after 3 months

# Is it possible to use MS2dgRNA for ameliorate mouse models of human diseases?

Acute kidney injury

Blood Urea Nitrogen

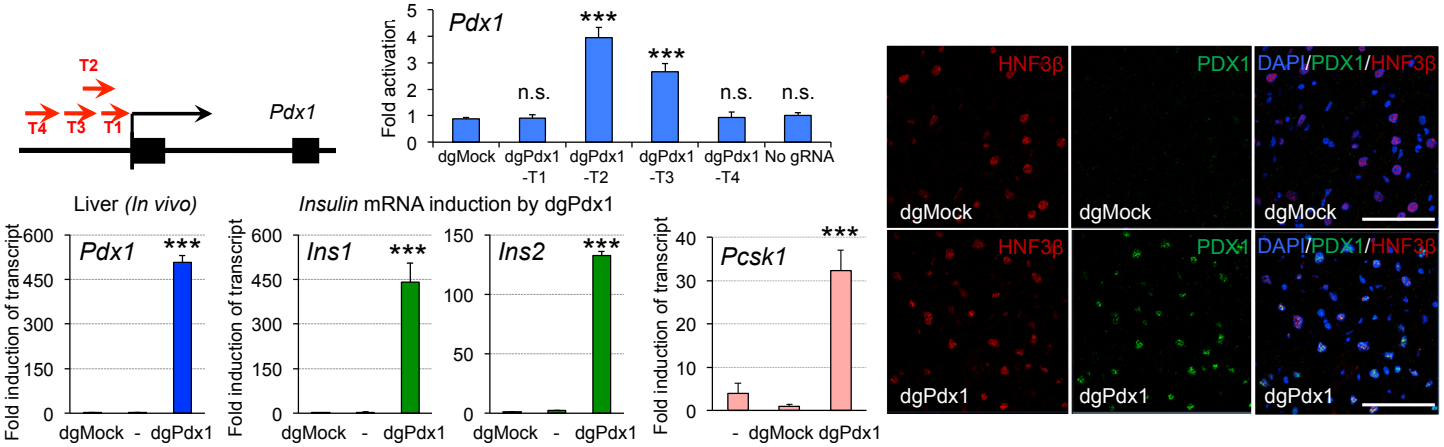


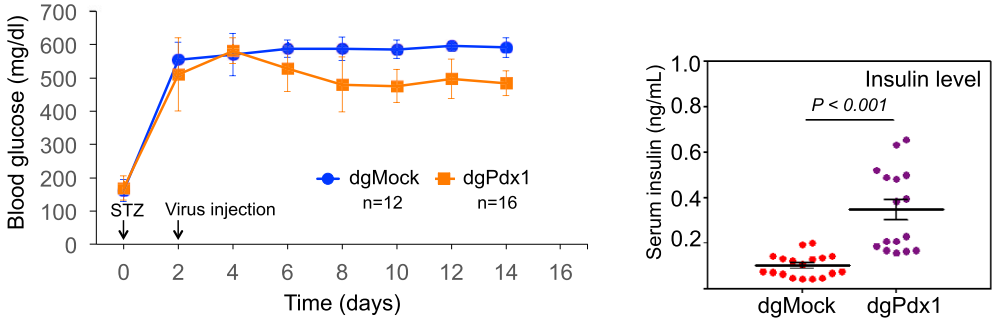

(Liao H.K., et al, Cell, Oct 2018)

TGA of Klotho/IL-10 is sufficient to provide a prophylactic interventions

# Is it possible to use TGA for *in vivo* cell transdifferentiation?

Pdx1 can transdifferentiate hepatocytes into pancreatic β-like insulin producing cell

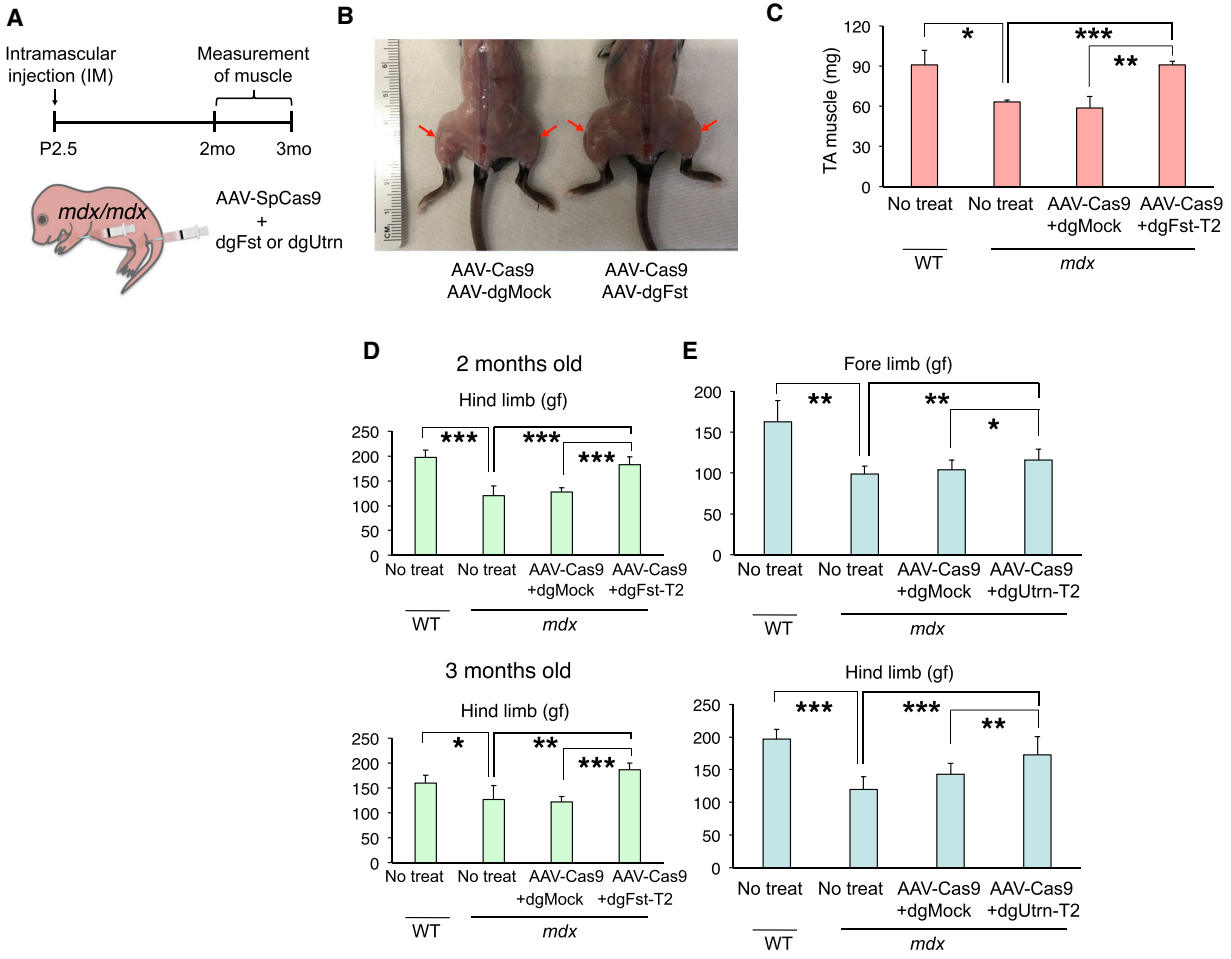



(Liao H.K., et al, Cell, Oct 2018)

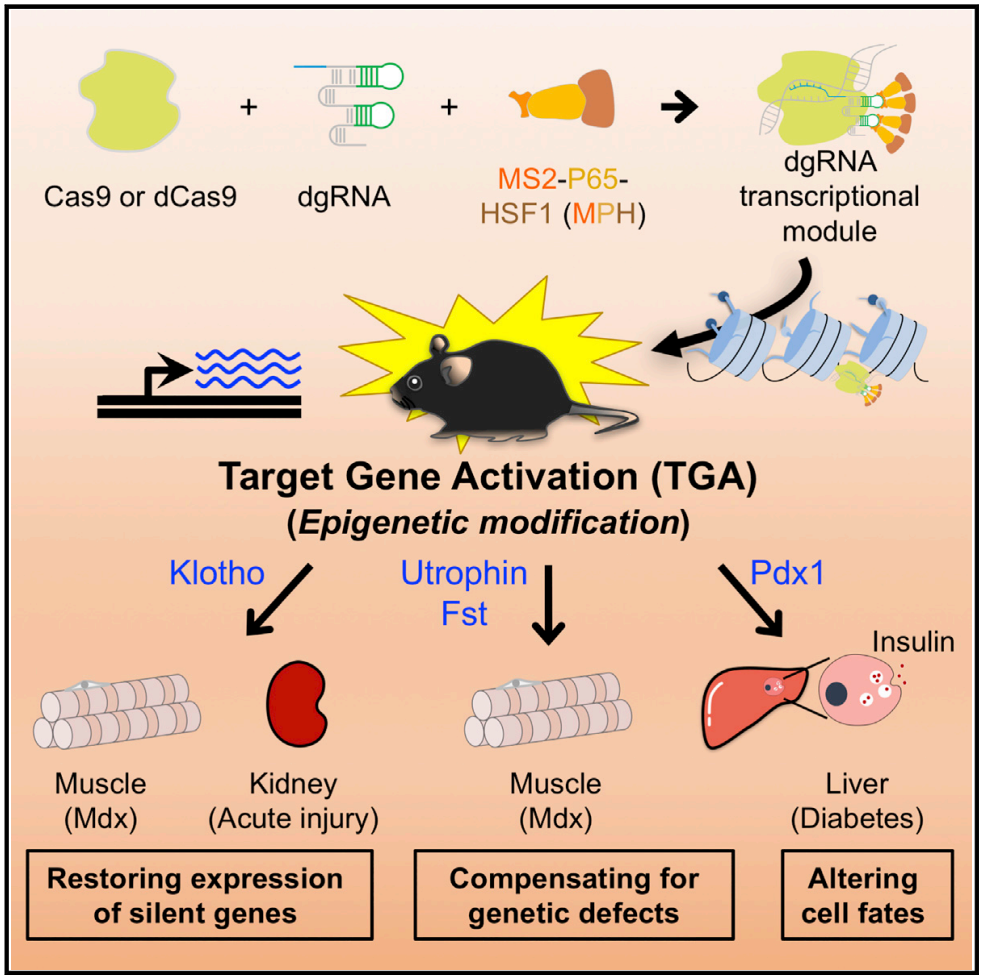
TGA of Pdx1 is capable of transforming liver cells into insulin-secreting cells *in vivo*

# Is the TGA system working in no-Cas9 expressing mice?




(Liao H.K., et al, Cell, Oct 2018)

TGA of AAV-Cas9 + AAV-dgRNA ameliorates Dystrophic phenotypes of *Mdx* Mice

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