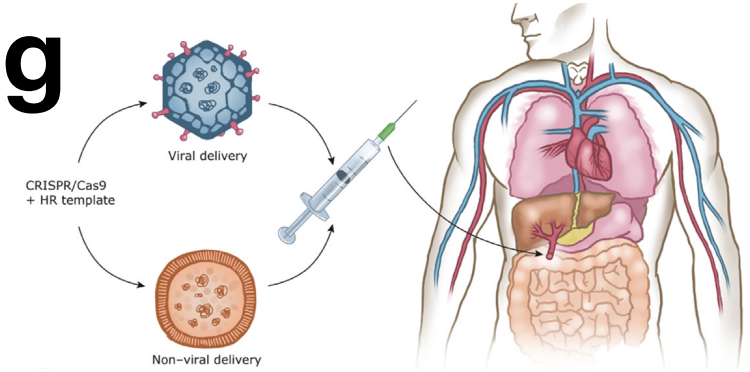
Universität Zürich[UZH]

SCHWANK LAB.org
Translational Genome Editing

@schwanklab

Correcting metabolic liver diseases by *in vivo* genome editing

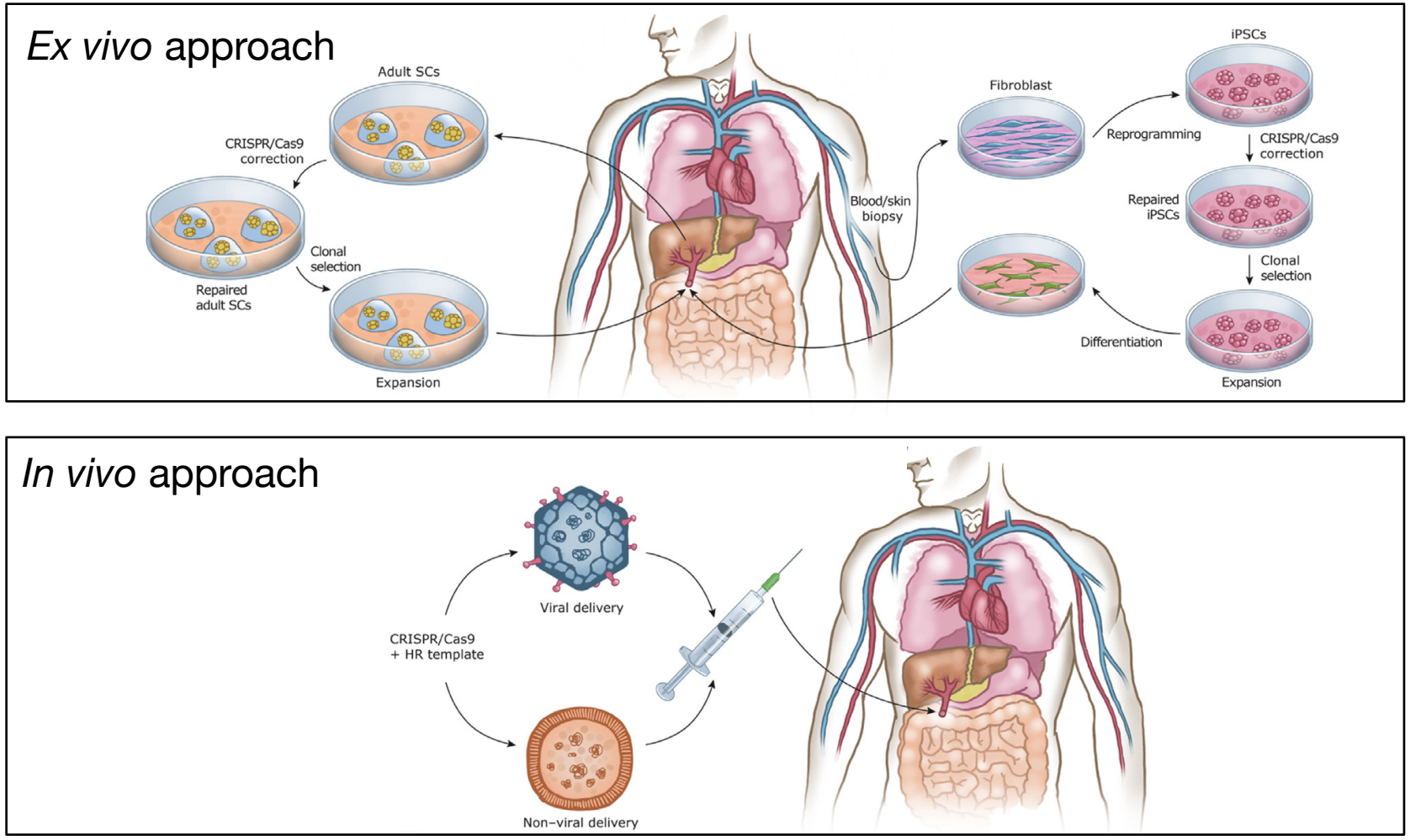


Gerald Schwank

Institute of Pharmacology and Toxicology, University of Zürich

Zürich – Nov. 12, 2021

Approaches for gene editing therapies



Savic and Schwank (2015) *Translational Research, 168, 15-21*

In vitro expansion and transplantation of hepatocytes

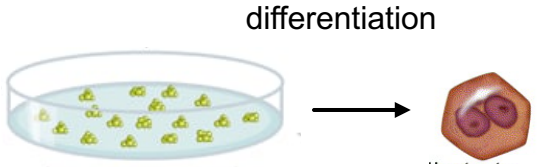
hepOrgs

Hu et al., Cell 2018

CLiPs

Katsuda et al., Cell Stem Cell 2017

iPSCs

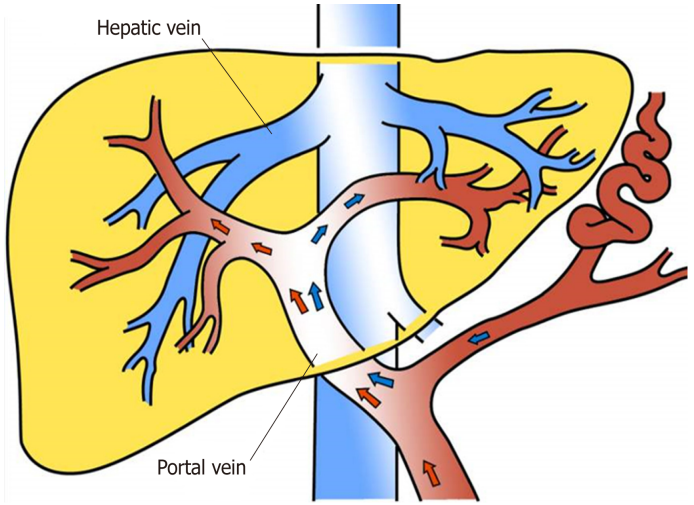


Takahashi et al., Cell 2007

Clinical trials for hepatocyte transplantation

Crigler-Najjar syndrome type 1 (CN-1), urea cycle defects and factor VII (fVII) deficiency:

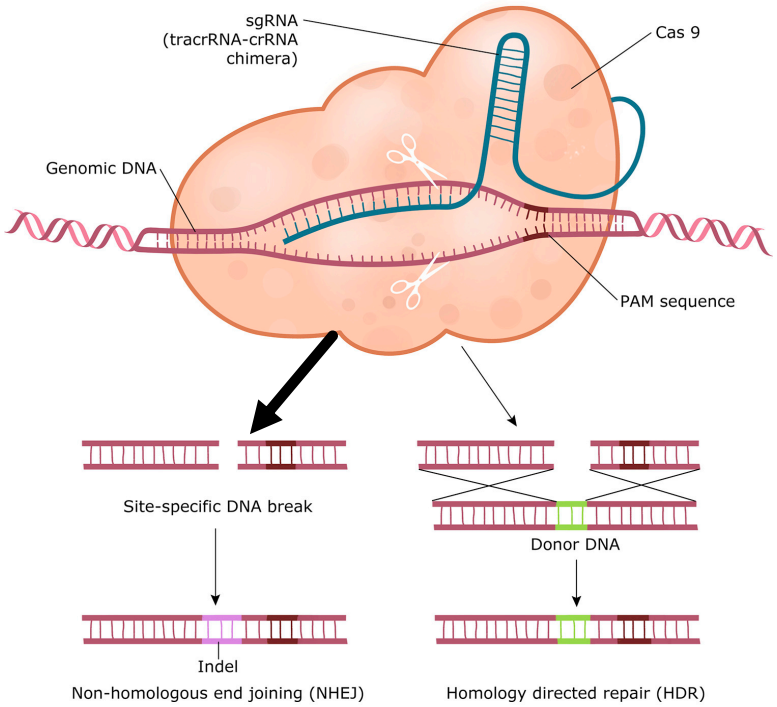
No long-term benefit observed!



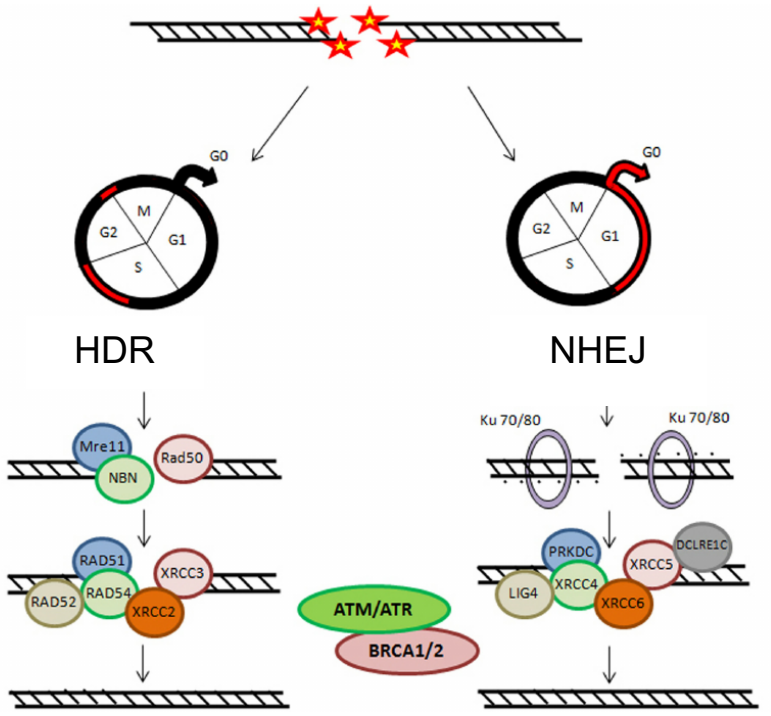
Lee et al., (*2018), J Mol Med; 96(6): 469–481*.

In non-dividing cells DNA double-stand breaks mainly induce indel mutations

NHEJ vs. HDR



HDR is inactive in postmitotic cells



DNA double strand breaks can induce complex DNA damage

nature medicine

LETTERS

https://doi.org/10.1038/s41591-018-0050-6

p53 inhibits CRISPR–Cas9 engineering in human pluripotent stem cells

Robert J. Ihry[1], Kathleen A. Worringer[1], Max R. Salick[1], Elizabeth Frias[2], Daniel Ho[1], Kraig Theriault[1], Sravya Kommineni[1], Julie Chen[3], Marie Sondey[4], Chaoyang Ye[5], Ranjit Randhawa[1], Tripti Kulkarni[1], Zinger Yang[2], Gregory McAllister[2], Carsten Russ[2], John Reece-Hoyes[2], William Forrester[2], Gregory R. Hoffman[2], Ricardo Dolmetsch[1] and Ajamete Kaykas[1]*

nature medicine

BRIEF COMMUNICATION

https://doi.org/10.1038/s41591-018-0049-z

CRISPR–Cas9 genome editing induces a p53-mediated DNA damage response

Emma Haapaniemi[1,2,4], Sandeep Botla[1,4], Jenna Persson[1], Bernhard Schmierer[1,5]* and Jussi Taipale[1,2,3,5]*

BRIEF COMMUNICATIONS ARISING

Inter-homologue repair in fertilized human eggs?

ARISING FROM H. Ma et al. *Nature* **548**, 413–419 (2017); https://doi.org/10.1038/nature23305

LETTERS

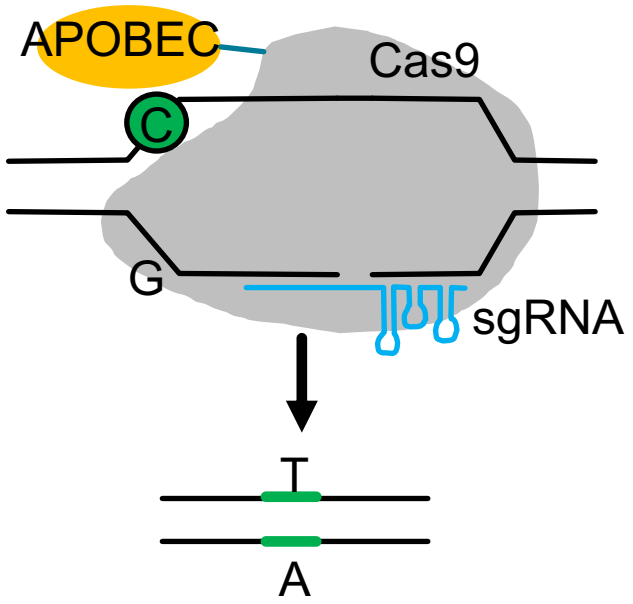
nature biotechnology

Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements

Michael Kosicki, Kärt Tomberg & Allan Bradley

Base editors can install/correct transition mutations without inducing DNA double-strand breaks

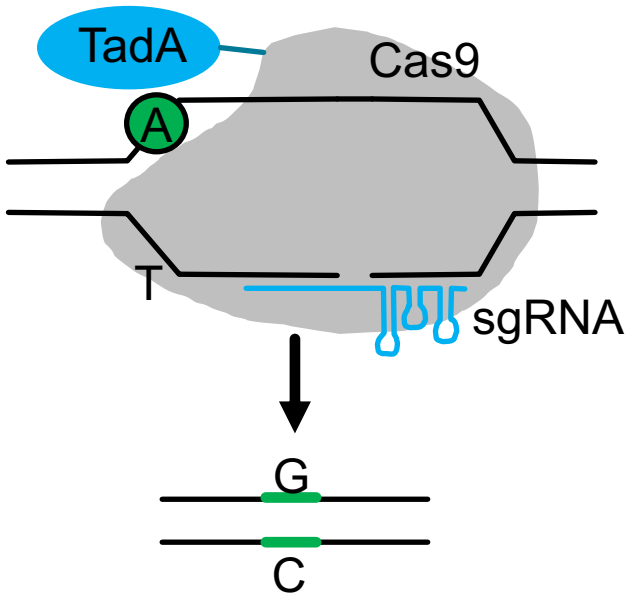
Cytidine Base Editor



C•G to T•A conversion

Komor et al. (2016), Nature; 533, pages420–424.

Adenine Base Editor



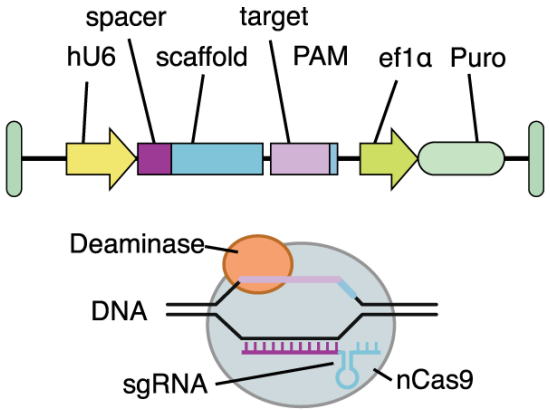
A•T to G•C conversion

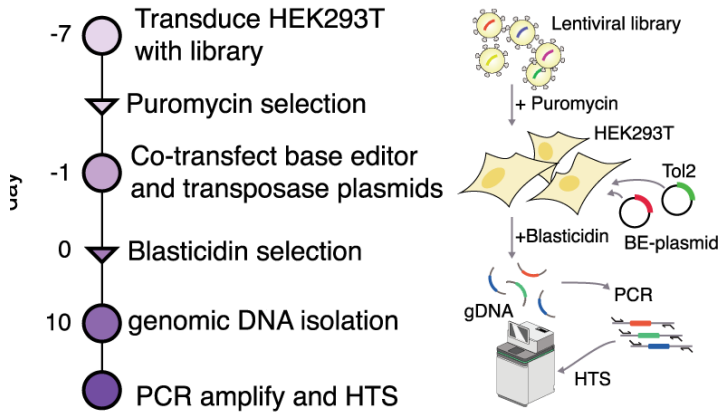
Gaudelli et al. (2017), Nature; 551(7681):464-471.

Limitations:

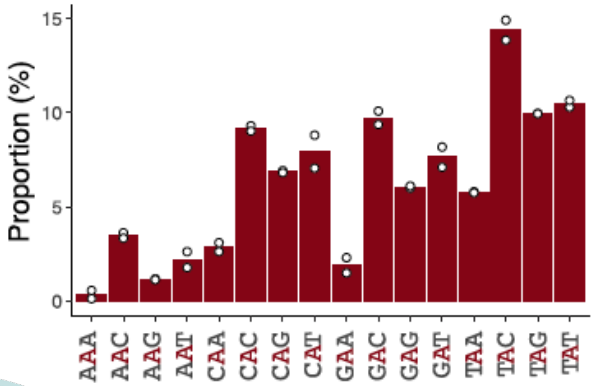
- Transition mutations
- PAM availability
- Bystander edits

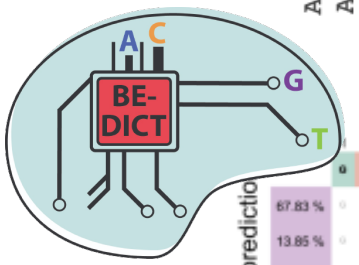
Predicting base editing efficiencies



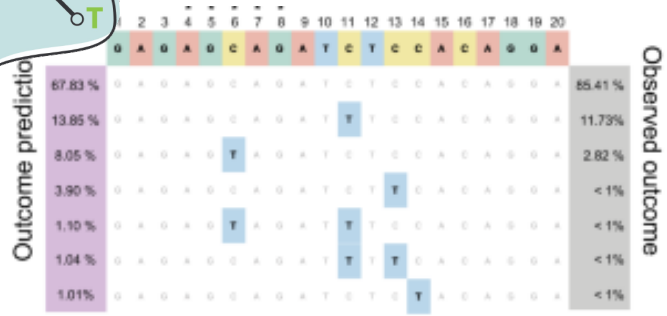


ABE-max trinucleotide motifs



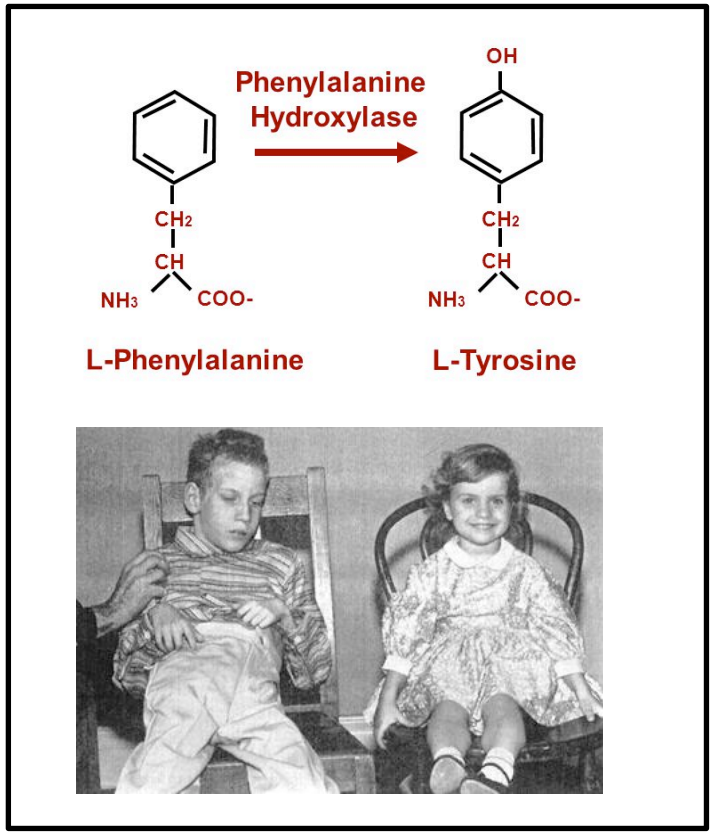


www.be-dict.org

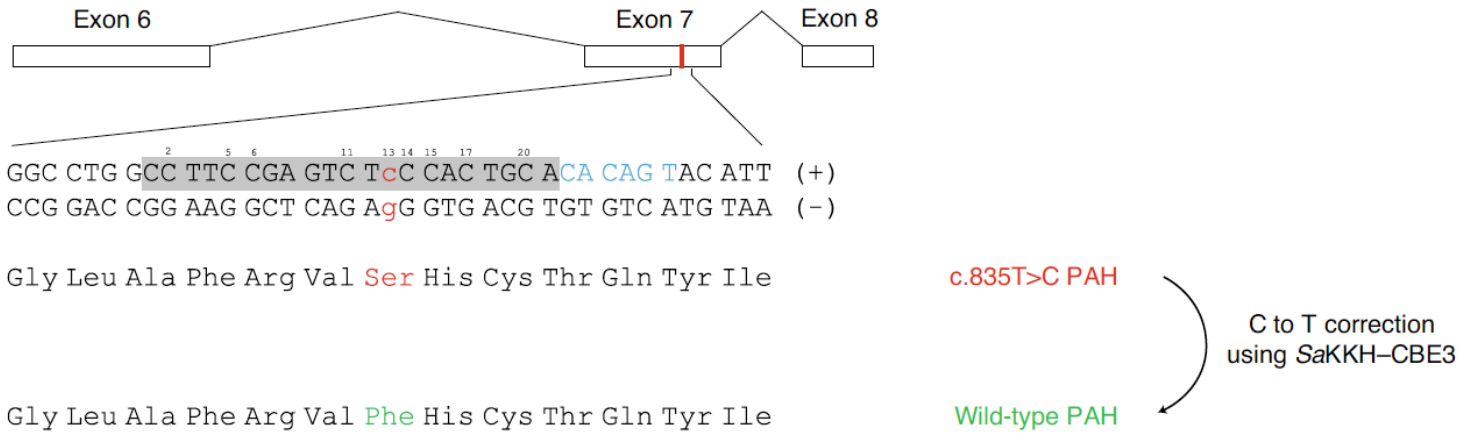


Marquart et al. *(2021), Nat Commun. Aug 25;12(1):5114.*

The *Pah*^{enu} mouse model for Phenylketonuria

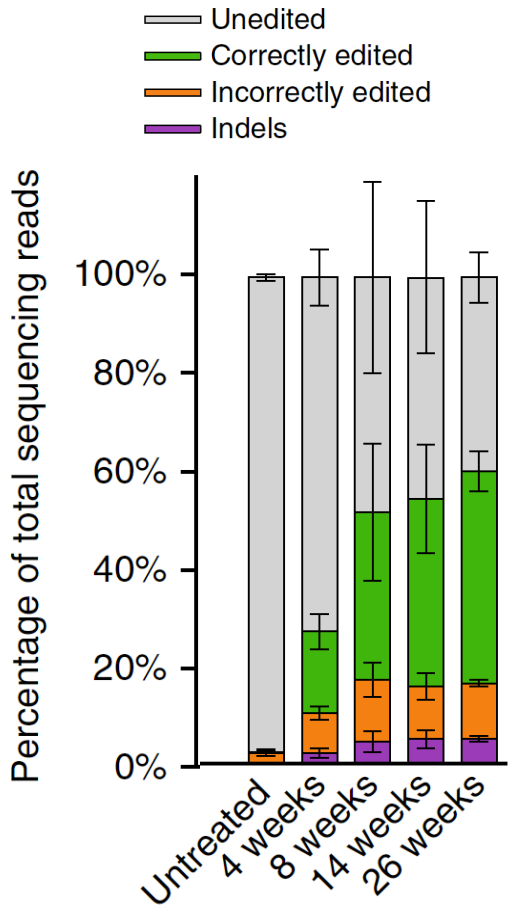


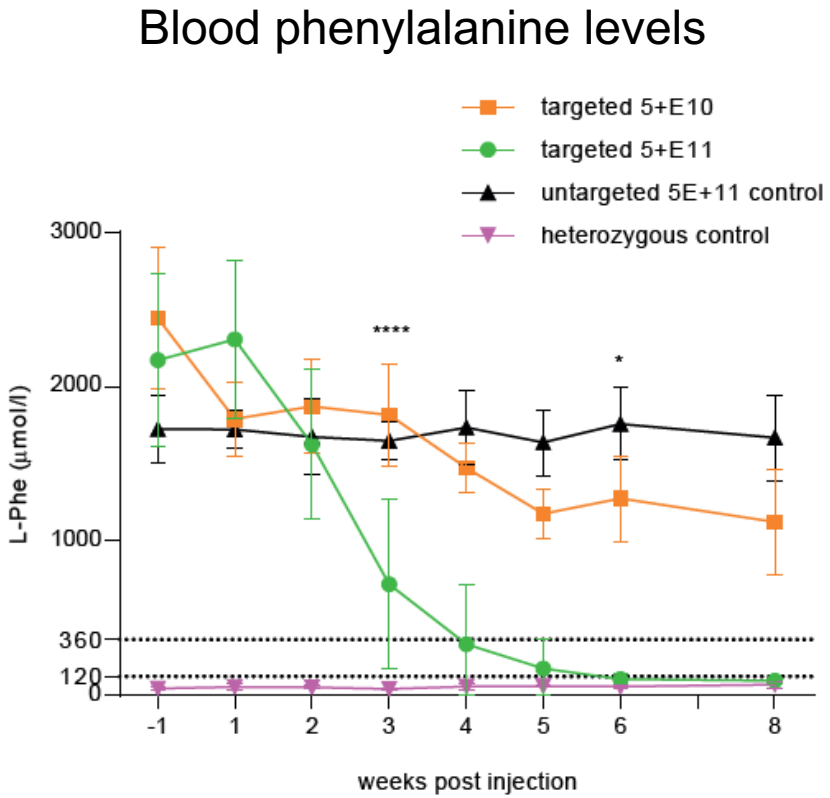
- PAH deficiency leads to excess amounts of Phe in the blood, causing damage of the central nervous system
- The pathogenic mutation is a T>C mutation that can be targeted by cytidine base editors

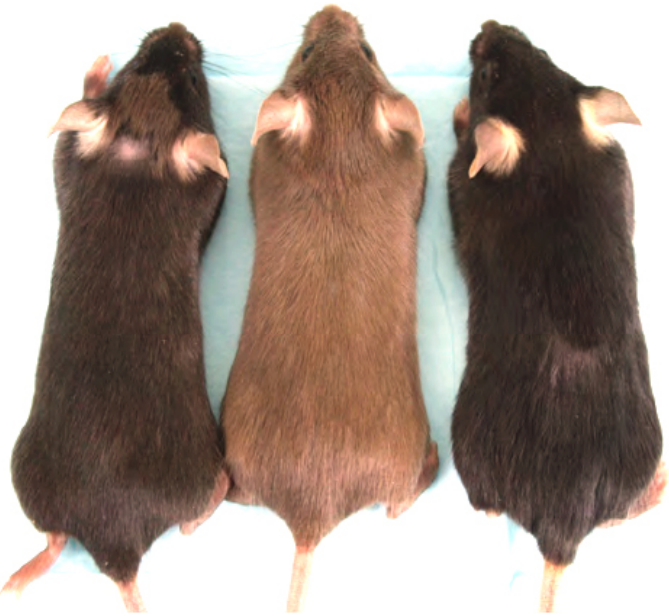


AAV-mediated base editor delivery restores physiological blood phenylalanine levels

Targeting the *Pah*^{enu} mouse model with *Sa*KKH-CBE3



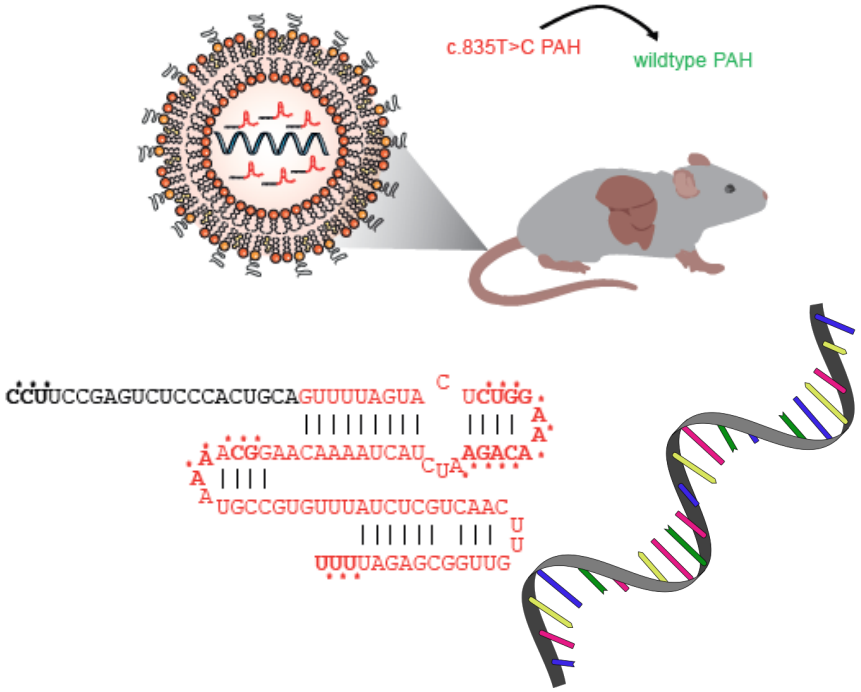


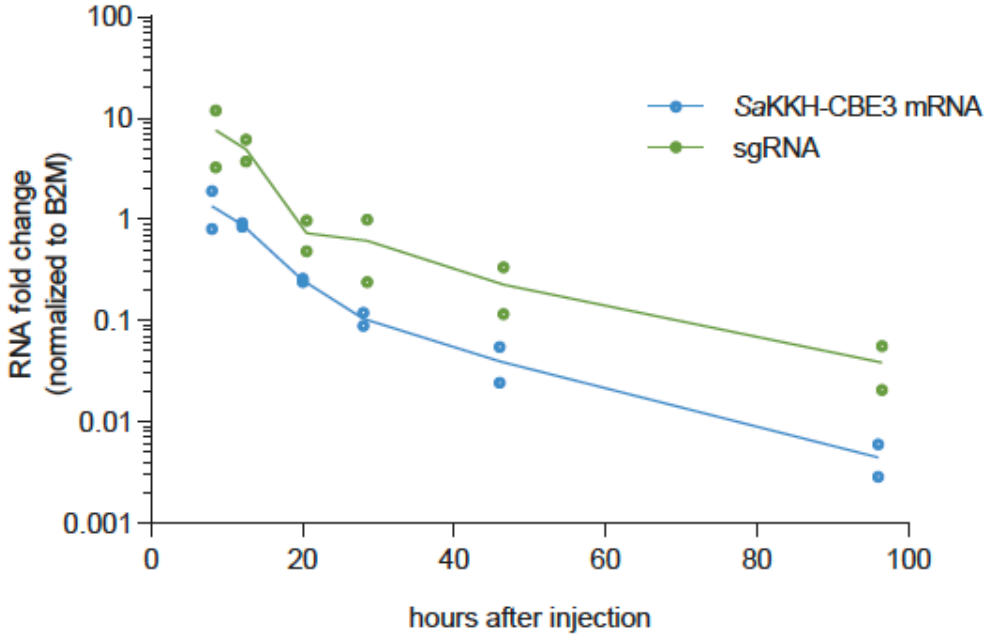


PAH^{enu2} treated PAH^{enu2} C567Bl/6

Villiger et al. (2018) *Nat Med. Oct;24(10):1519-1525.*

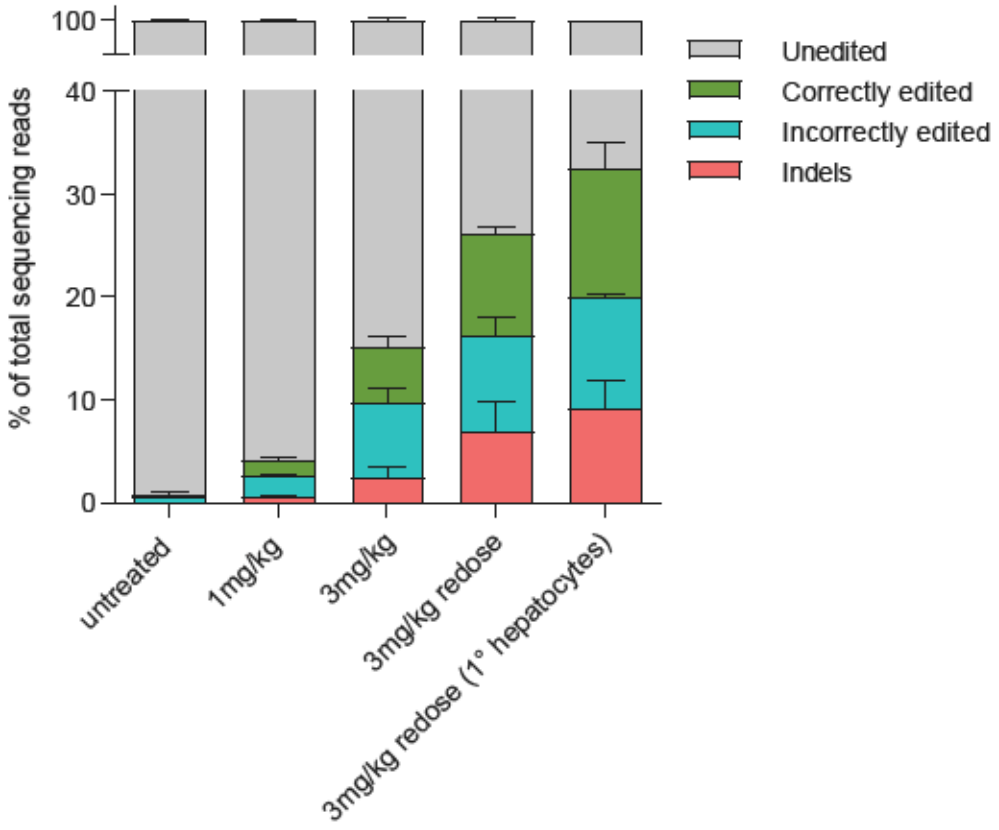
In vivo cytidine base editing using RNA encapsulated in lipid nanoparticles

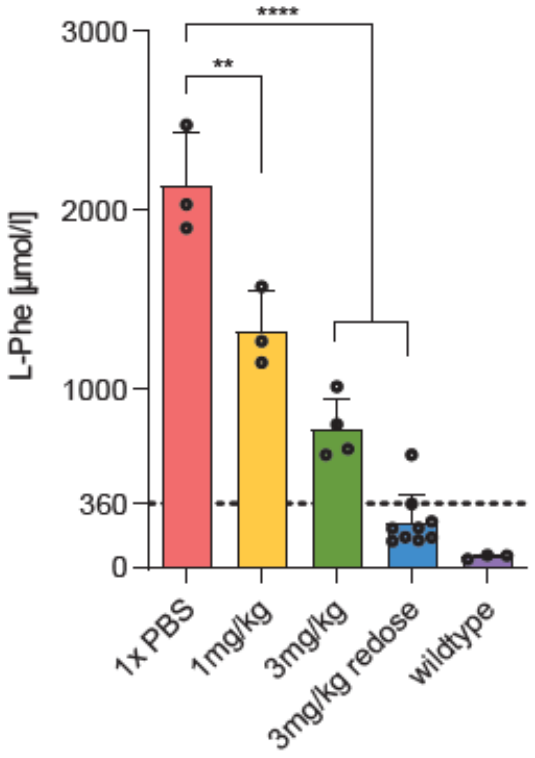


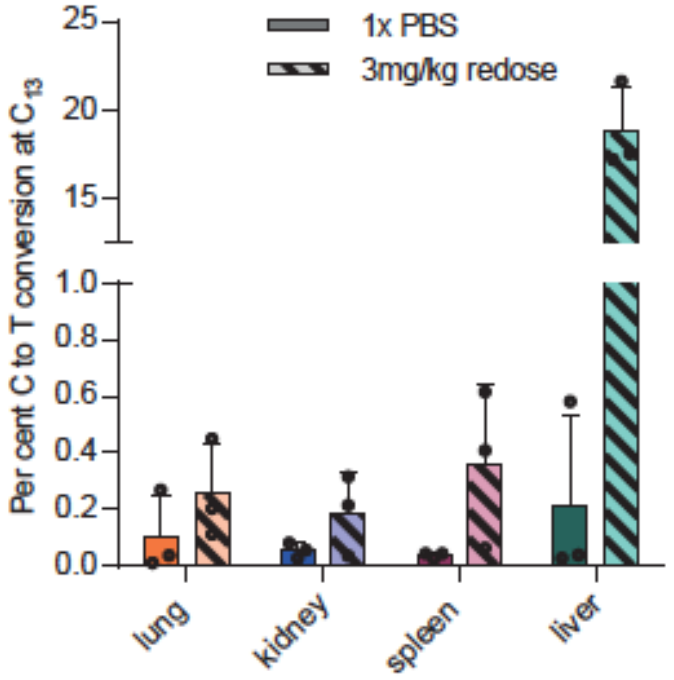


Villiger and Rothgangl et al. (2021) *Nat Biomed Eng. Feb;5(2):179-189.*

In vivo cytidine base editing using RNA encapsulated in lipid nanoparticles







Villiger and Rothgangl et al. (2021) *Nat Biomed Eng. Feb;5(2):179-189.*

Base editors can generate sgRNA-independent off-target mutations on RNA and DNA

LETTER

https://doi.org/10.1038/s41586-019-1161-z

Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors

Julian Grünewald[1,2,3,4], Ronghao Zhou[1,2,3], Sara P. Garcia[1,6], Sowmya Iyer[1,6], Caleb A. Lareau[1,5,6], Martin J. Aryee[1,2,3,4,5] & J. Keith Joung[1,2,3,4]*

LETTER

https://doi.org/10.1038/s41586-019-1314-0

Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis

Changyang Zhou[1,2,9], Yidi Sun[2,3,4,9], Rui Yan[5,9], Yajing Liu[2,6,9], Erwei Zuo[1,7,9], Chan Gu[5], Linxiao Han[1], Yu Wei[1], Xinde Hu[1,2], Rong Zeng[3,6], Yixue Li[5,6,8]*, Haibo Zhou[1]*, Fan Guo[5]* & Hui Yang[1]*

Science

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

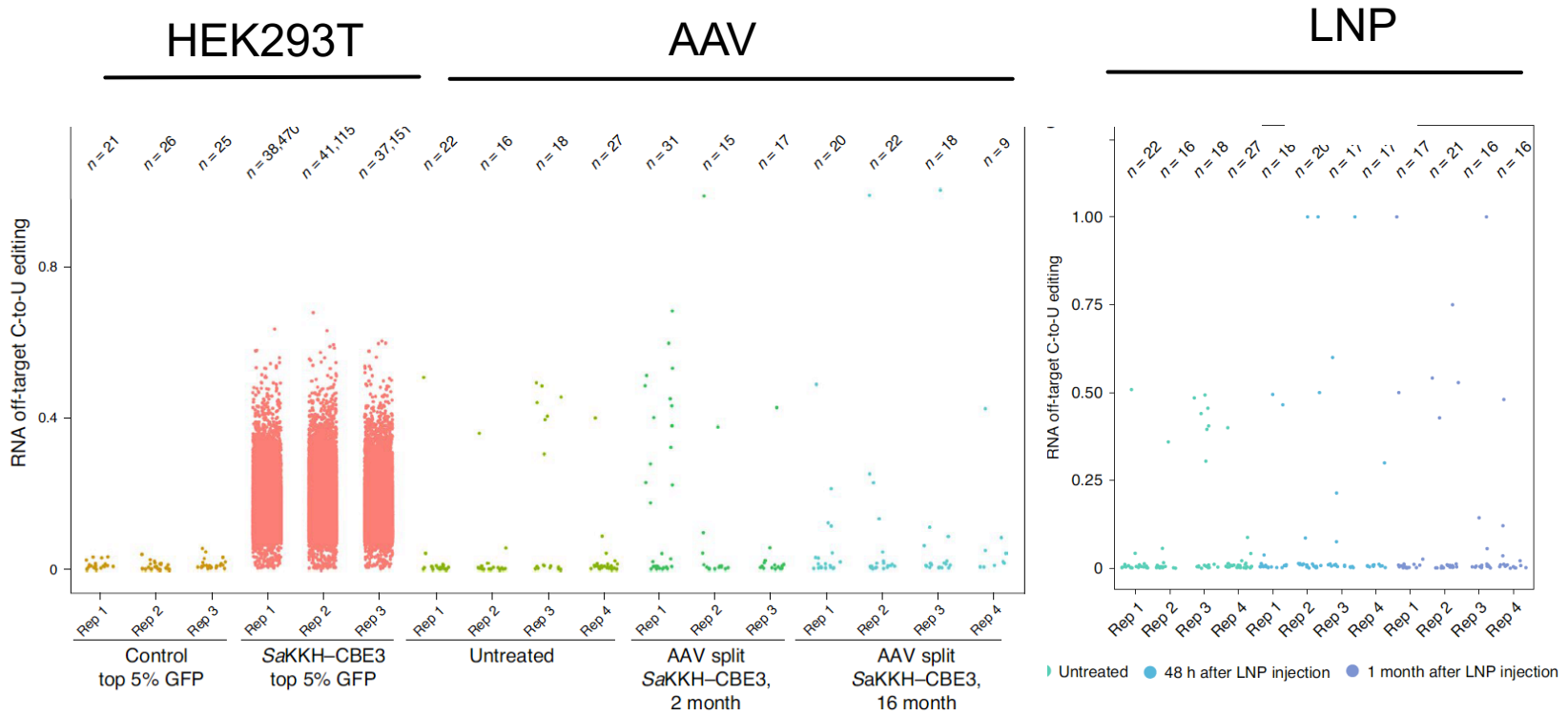
Erwei Zuo[1,2]*, Yidi Sun[3]*, Wu Wei[3,4,5]*, Tanglong Yuan[2]*, Wenqin Ying[1], Hao Sun[6], Liyun Yuan[3], Lars M. Steinmetz[4,7,8]†, Yixue Li[3,9,10]†, Hui Yang[1]†

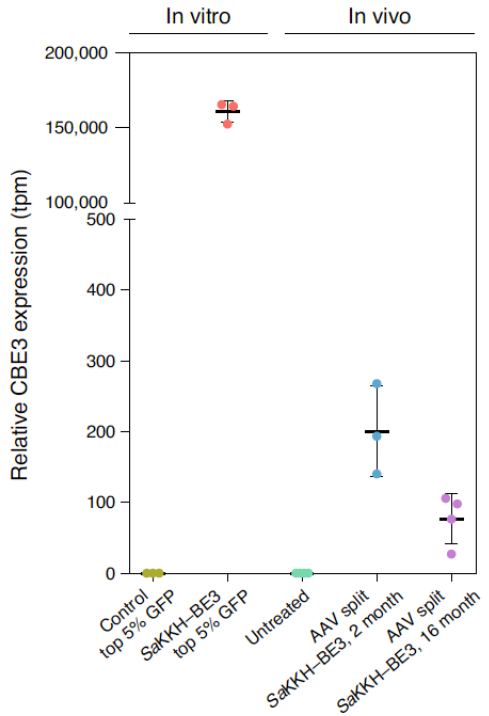
BIOTECHNOLOGY

Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice

Shuai Jin[1,2]*, Yuan Zong[1,2]*, Qiang Gao[3]*, Zixu Zhu[1,2], Yanpeng Wang[1], Peng Qin[4], Chengzhi Liang[2,3], Daowen Wang[1,2], Jin-Long Qiu[5], Feng Zhang[6], Caixia Gao[1,2]†

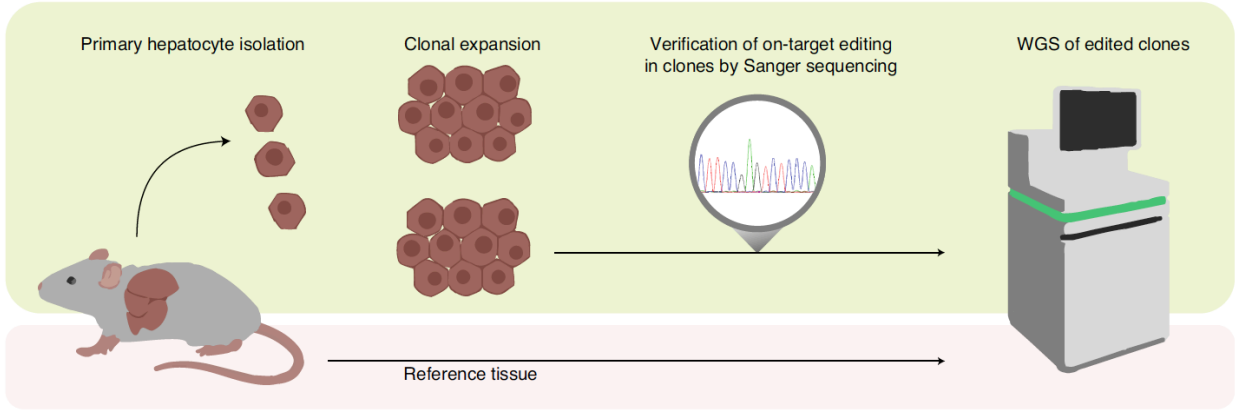
In vivo cytidine base editing did not induced RNA off-target deamination

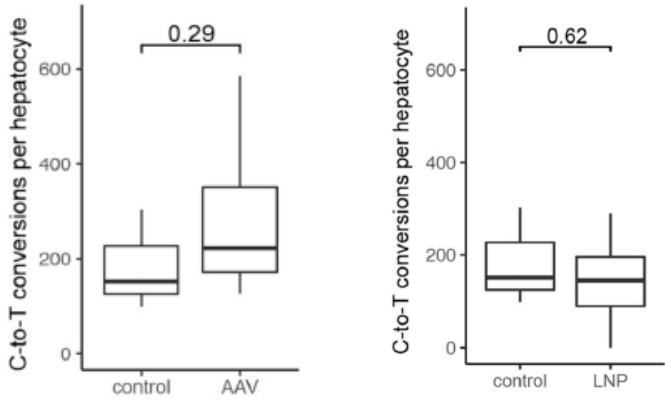




Villiger and Rothgangl et al. (2021) *Nat Biomed Eng. Feb;5(2):179-189.*

In vivo cytidine base editing did not induced DNA off-target deamination





	28_1	28_2	28_3
SBS2	0.19	0.17	0.32
rAPOBEC1	0.24	0.20	0.38

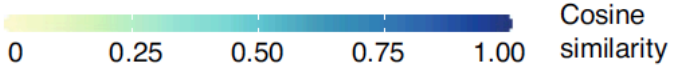
control

	341_10	341_14	341_2	341_4	341_5	341_7	341_8	344_18	344_26	344_27	344_36
SBS2	0.20	0.10	0.14	0.08	0.05	0.10	0.14	0.05	0.07	0.10	0.16
rAPOBEC1	0.24	0.14	0.19	0.12	0.08	0.15	0.18	0.03	0.08	0.12	0.17

AAV treated

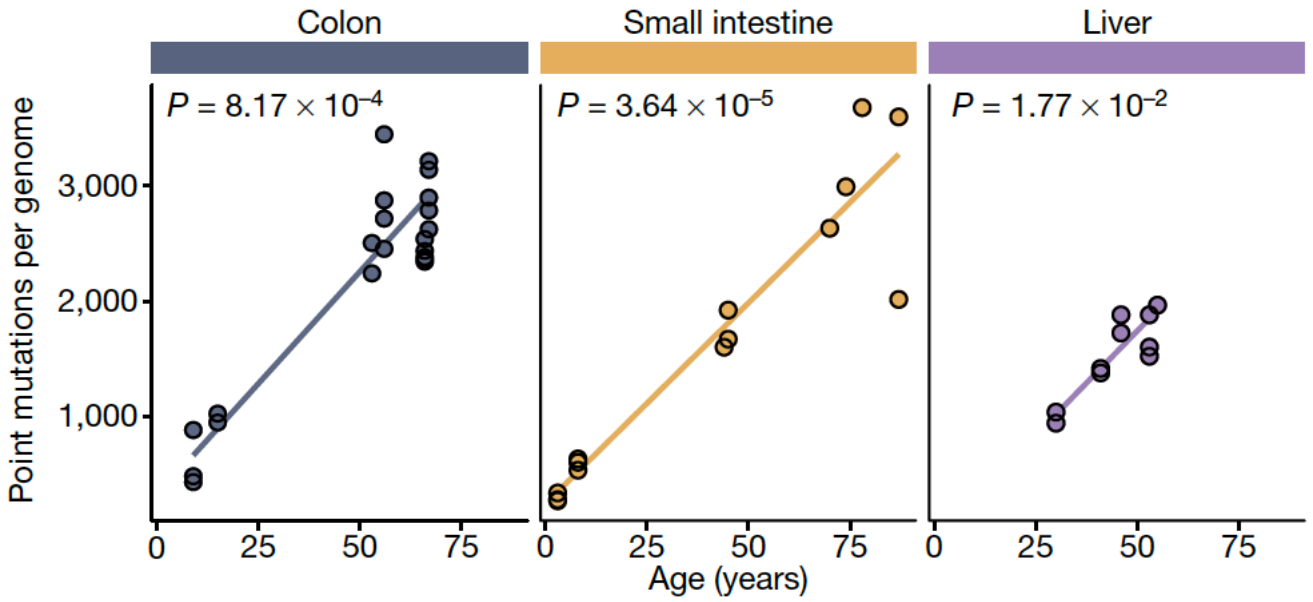
	362_5	362_6	362_7	362_8	362_9	324_1_A2	324_1_A3	324_1_A4	324_1_B1	324_1_C5	324_1_C7	324_1_D11	324_2_B5	324_2_B7	324_2_F8	329_1_F12	329_1
SBS2	0.05	0.18	0.10	0.10	0.12	0.20	0.04	0.09	0.04	0.13	0.07	0.19	0.12	0.10	0.15	0.13	0.06
rAPOBEC1	0.06	0.23	0.13	0.13	0.15	0.14	0.05	0.11	0.05	0.14	0.09	0.24	0.16	0.13	0.17	0.14	0.06

LNP treated



Villiger and Rothgangl et al. (2021) *Nat Biomed Eng. Feb;5(2):179-189.*

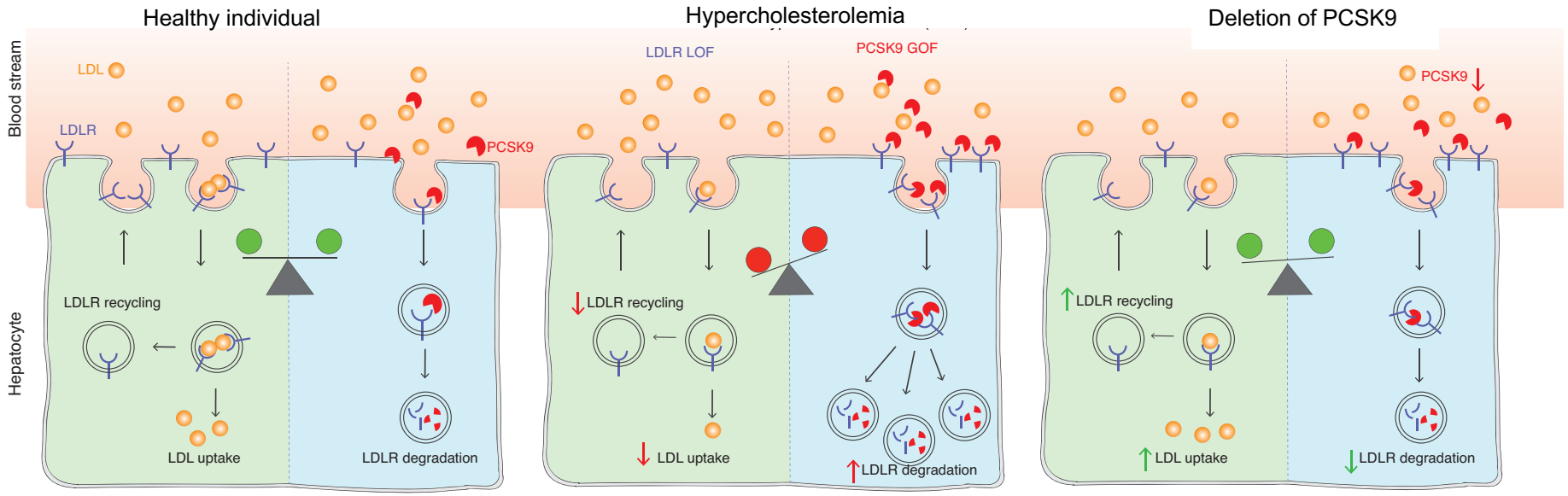
SNPs are well tolerated in humans



Blokzijl et al. (2016) *Nature. Oct 13;538(7624):260-264.*

Targeting *PCSK9* via adenine base editing

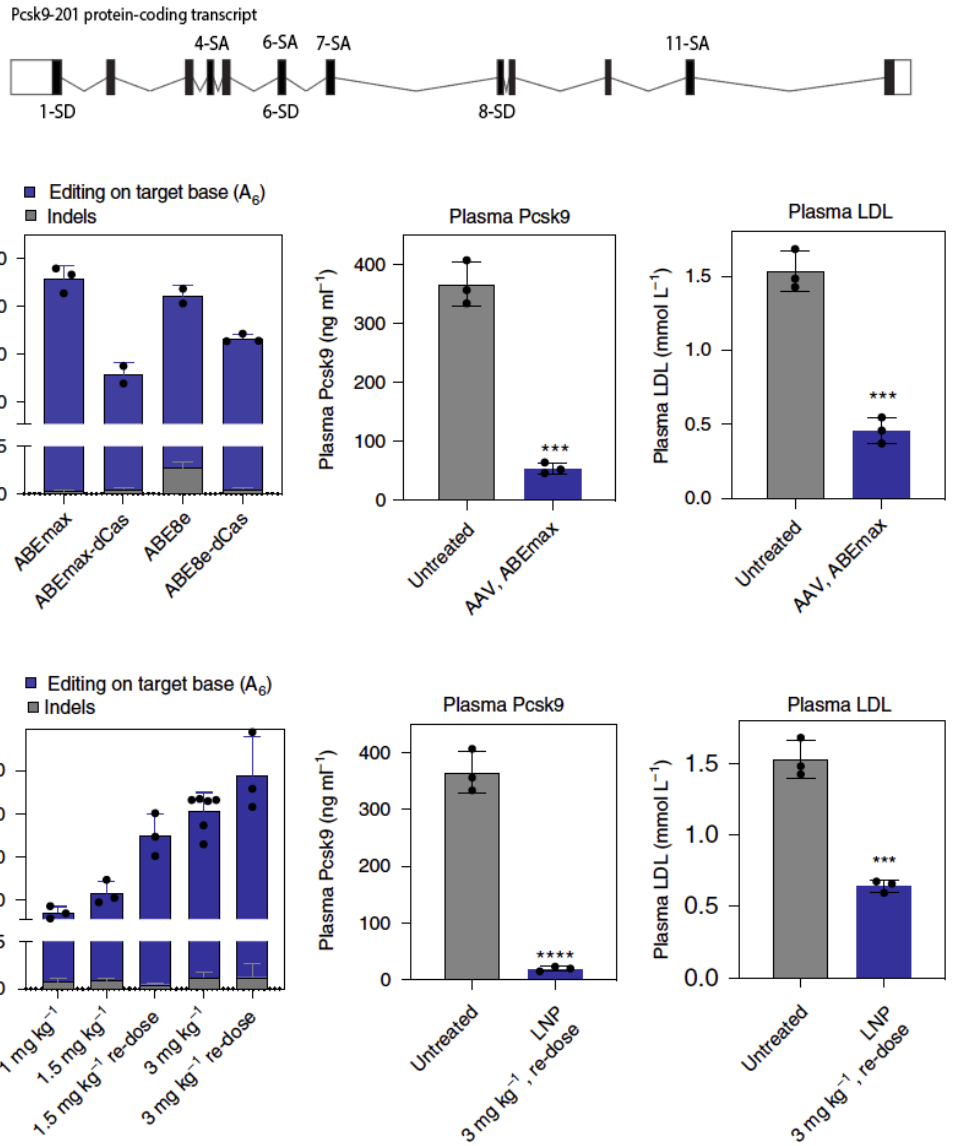
PCSK9 is a negative regulator of LDL receptor



Inactivating PCSK9 *in vivo* reduces LDL cholesterol

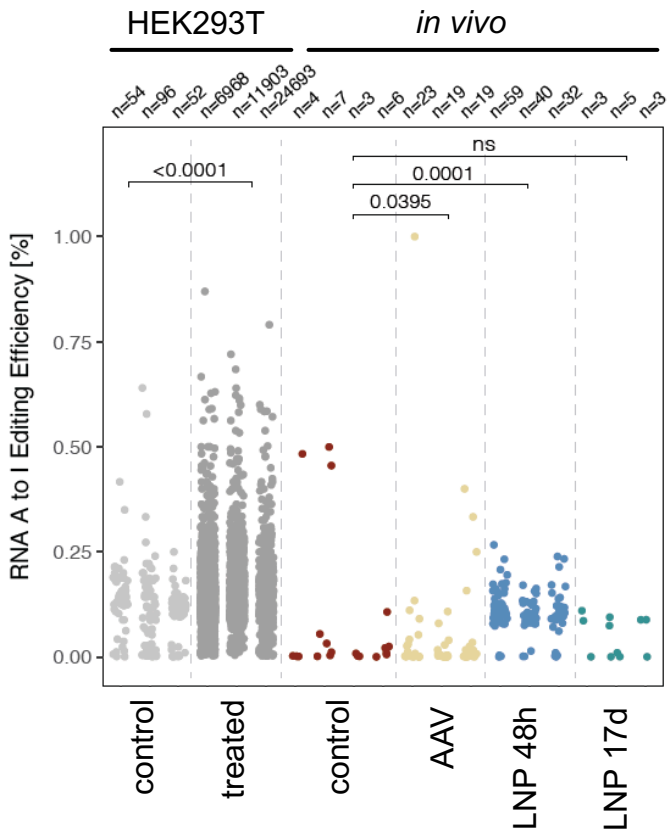
AAV-treated mice

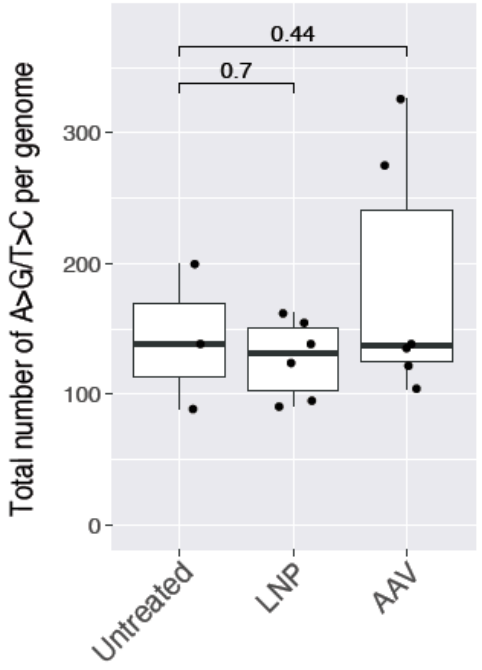
LNP-treated mice

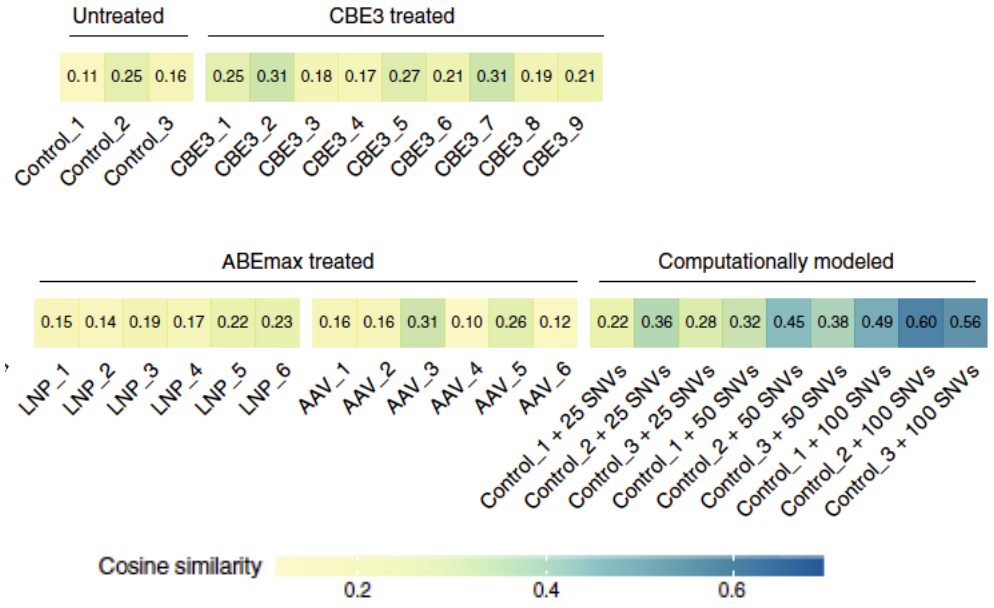


Rothgangl et al. (2021) *Nature Biotechnology, volume 39, pages (949–957)*

In vivo adenine base editing did not induced RNA and DNA off-target deamination

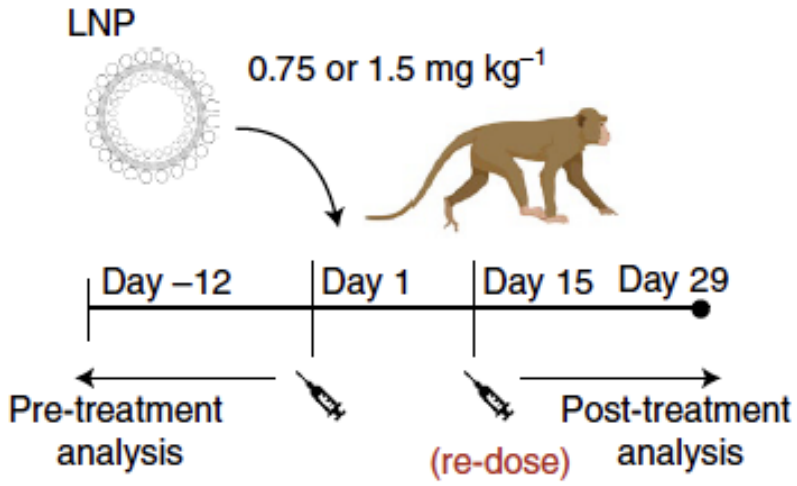


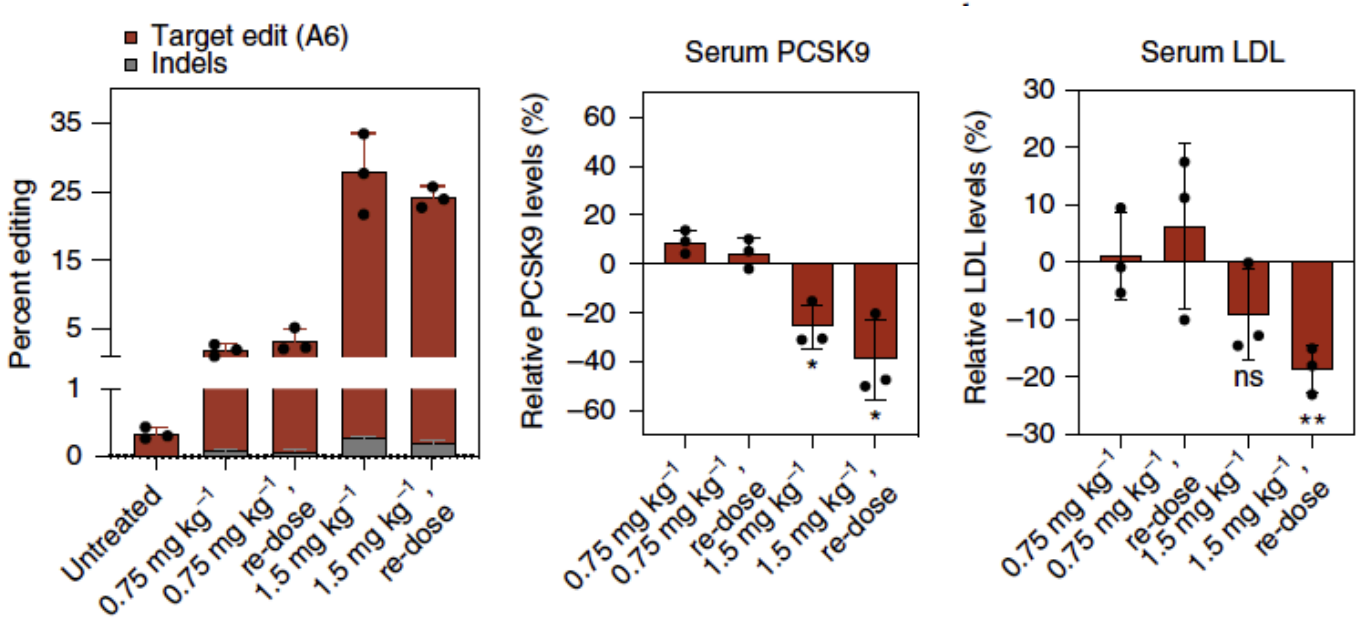




Rothgangl et al. (2021) *Nature Biotechnology, volume 39, pages (949–957)*

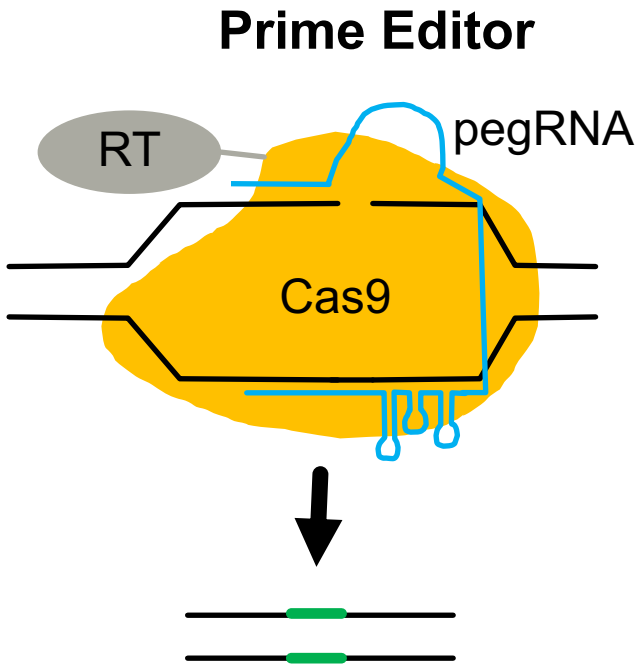
LNP-mediated adenine base editing of *PCSK9* in primates

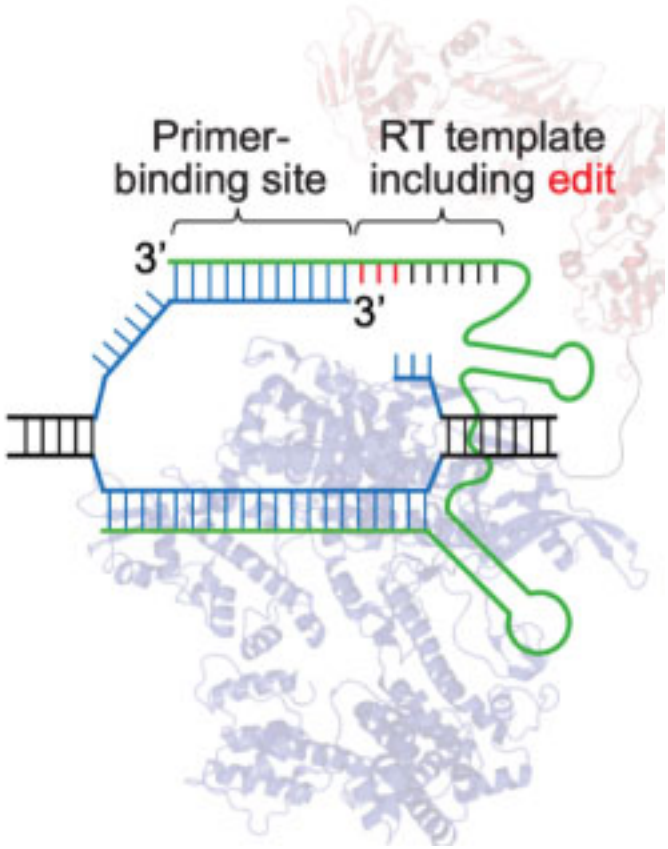




Rothgangl et al. (2021) *Nature Biotechnology, volume 39, pages (949–957)*

Prime editors: A highly versatile DNA double strand break-independent genome editor

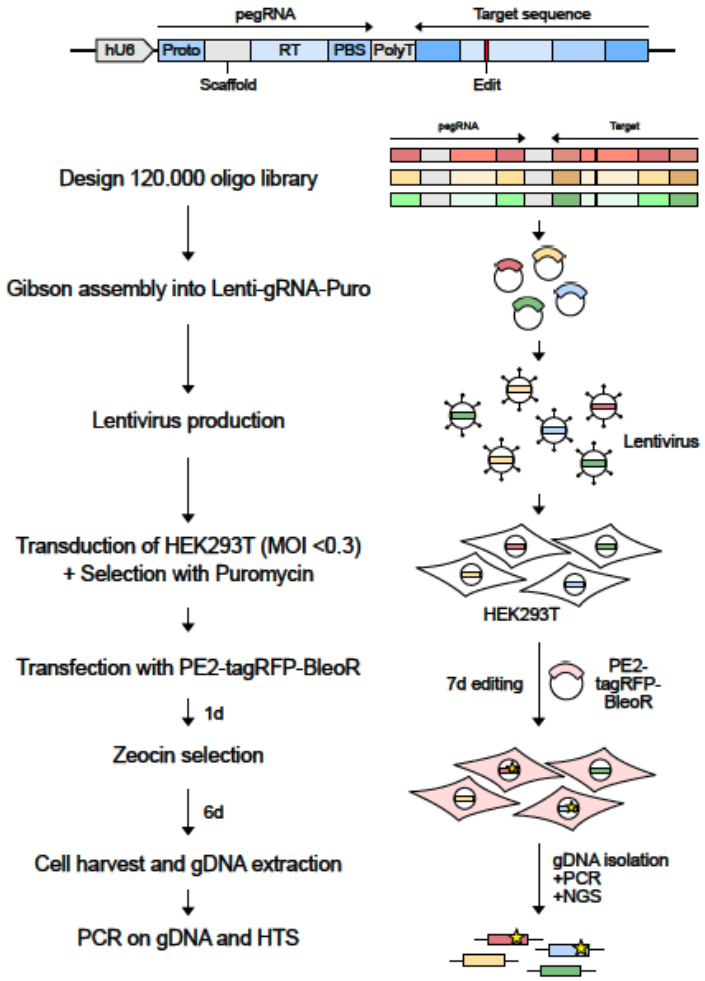


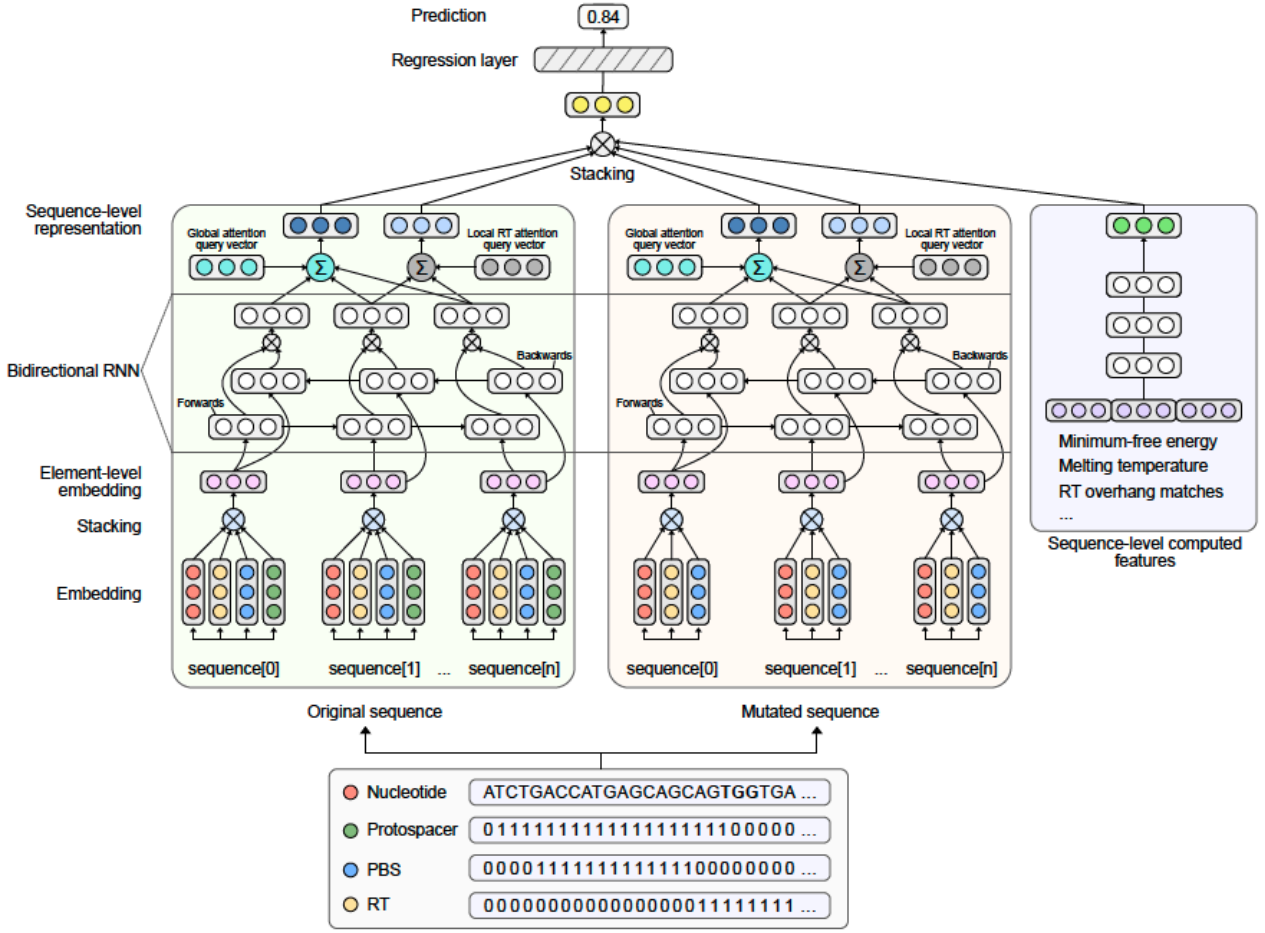


Desired edit (transition, transversion, deletion, insertion)

Anzalone et al. (2019), Nature; 576, pages149–157

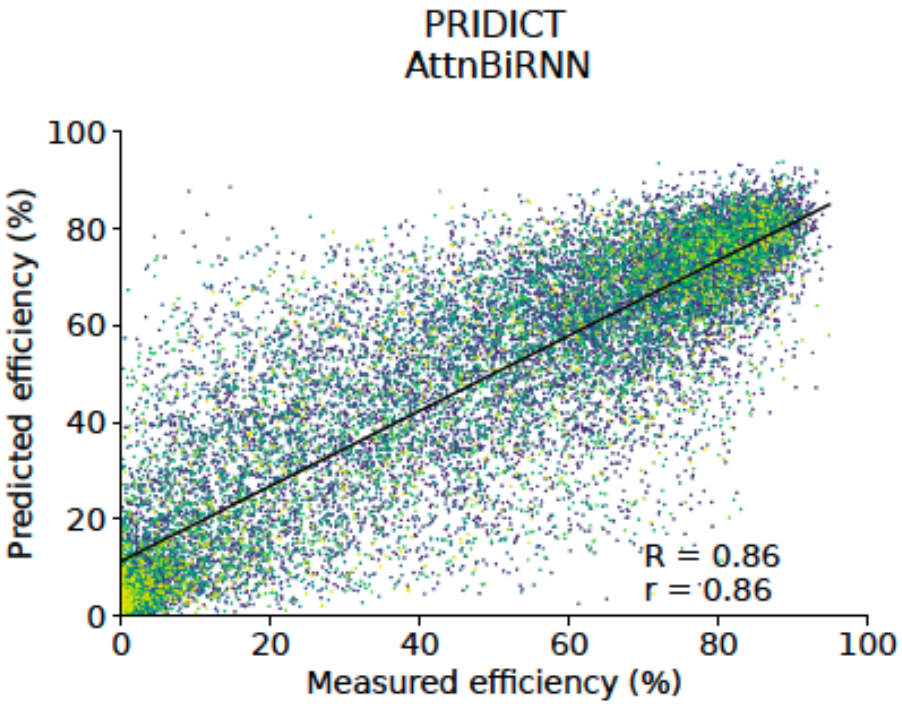
PRIDICT: Predicting prime editing efficiencies

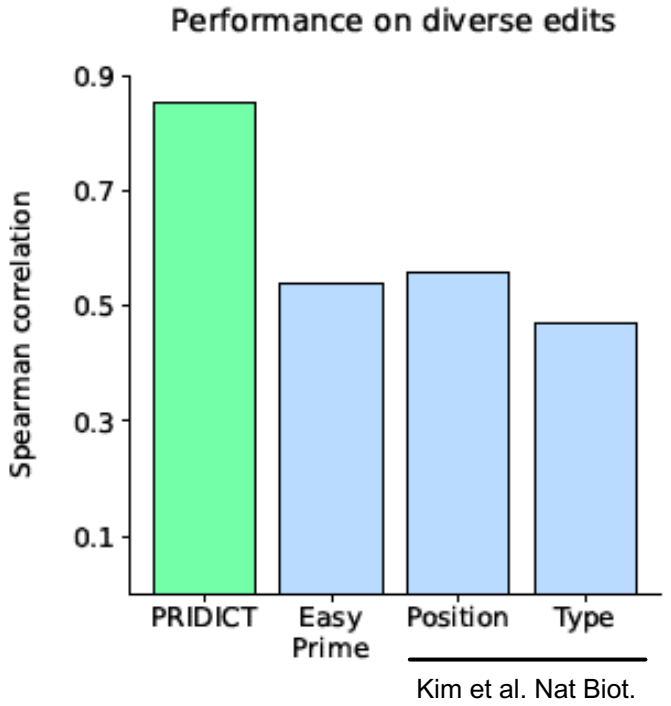




Mathis and Allam *et al., unpublished*

PRIDICT: Predicting prime editing efficiencies



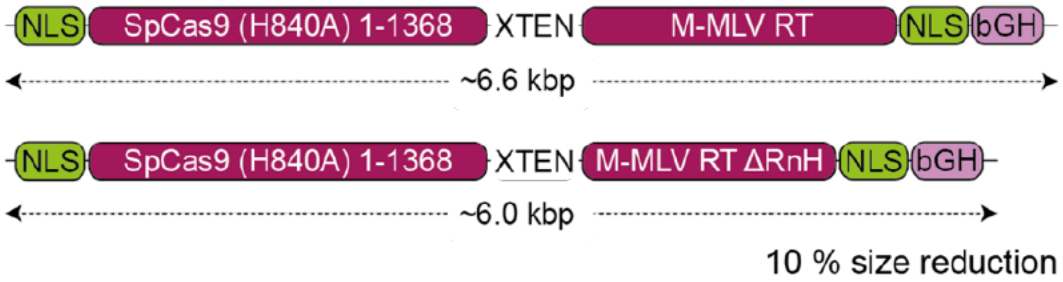


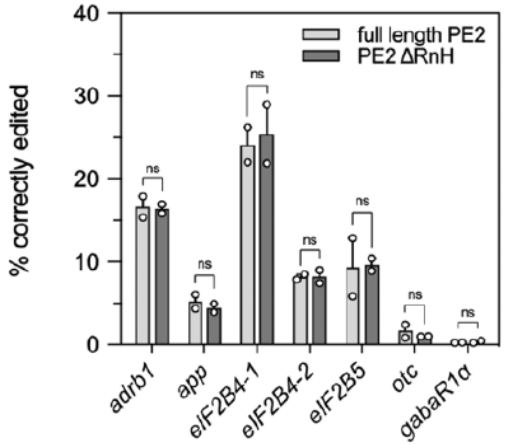
Kim et al. Nat Biot.

Mathis and Allam *et al., unpublished*

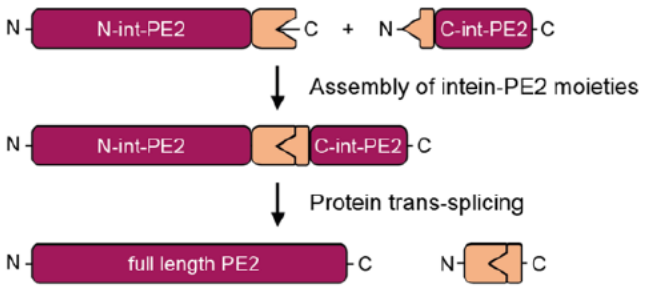
Generation of size-optimized prime editors

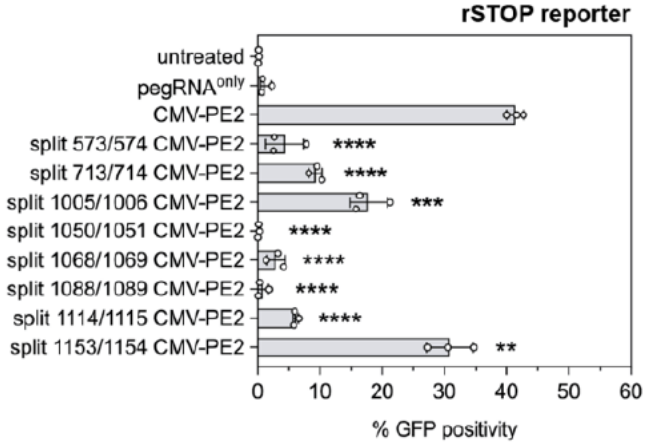
PE2 Δ RnH





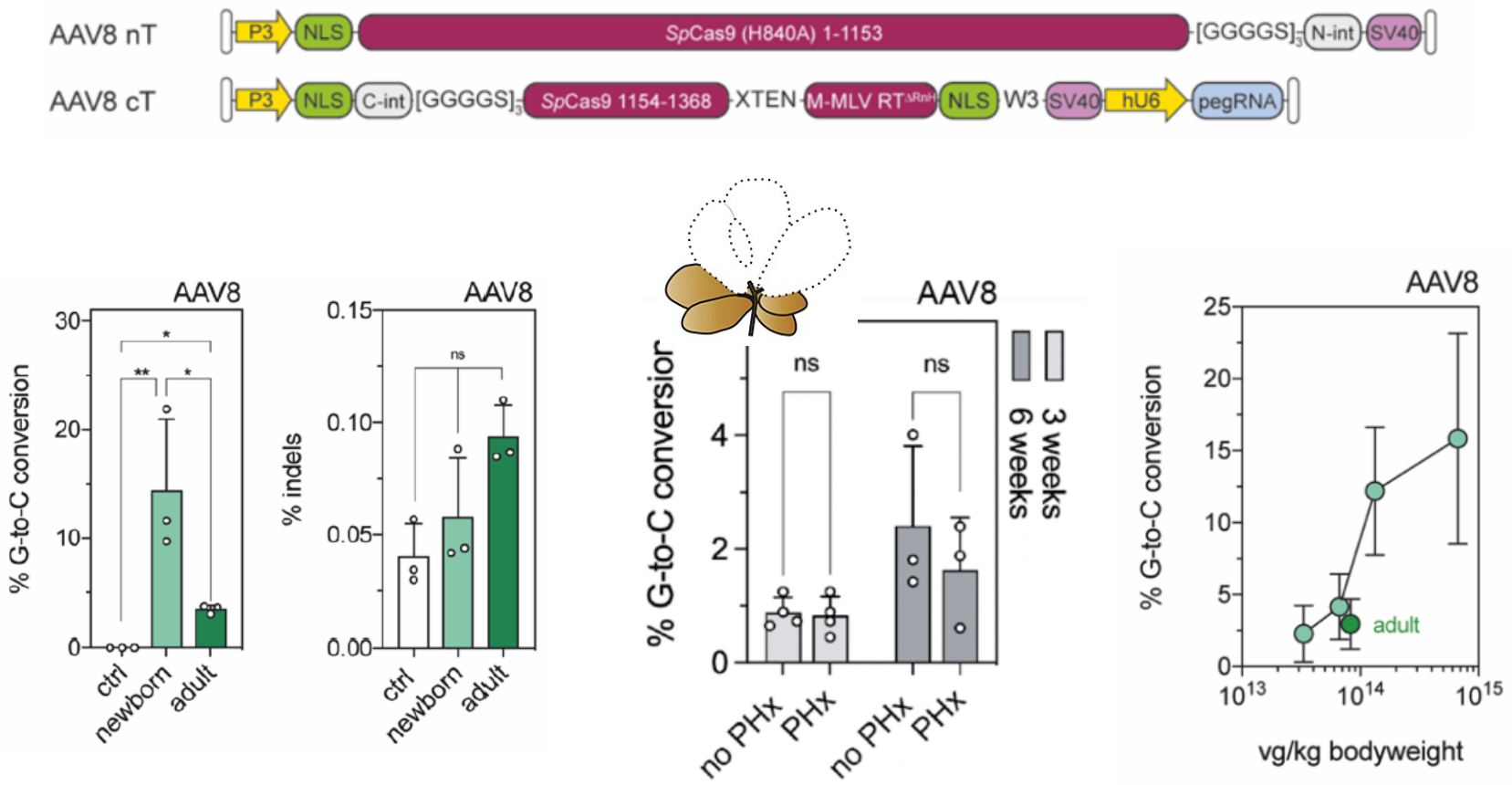
split PE2





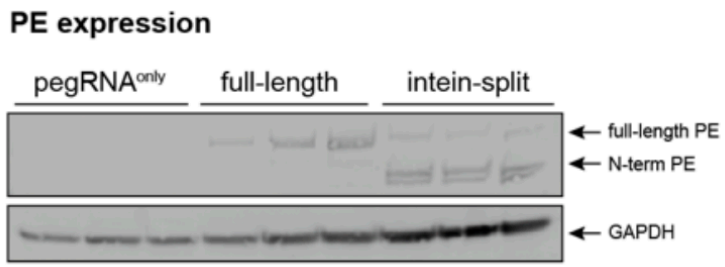
Boeck, Rothgangl, Villiger et al. (2021) bioRxiv, https://doi.org/10.1101/2021.08.17.456632

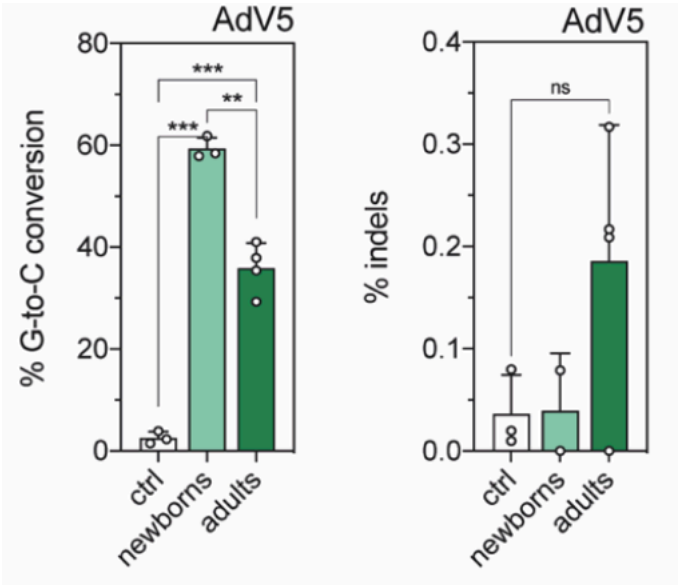
Installing a G-to-C edit in *Dnmt1* via AAV-mediated prime editing

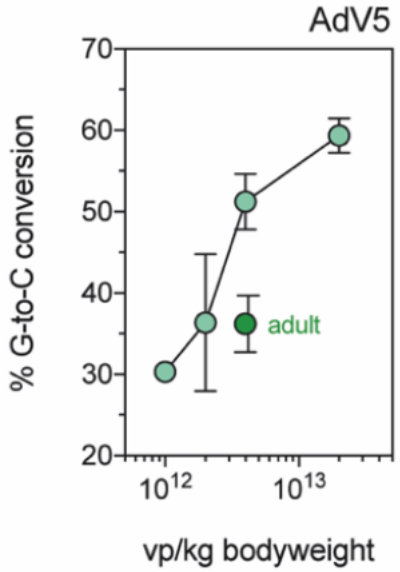


Boeck, Rothgangl, Villiger et al. (2021) bioRxiv, https://doi.org/10.1101/2021.08.17.456632

Installing a G-to-C edit in *Dnmt1* via AdV5-mediated prime editing

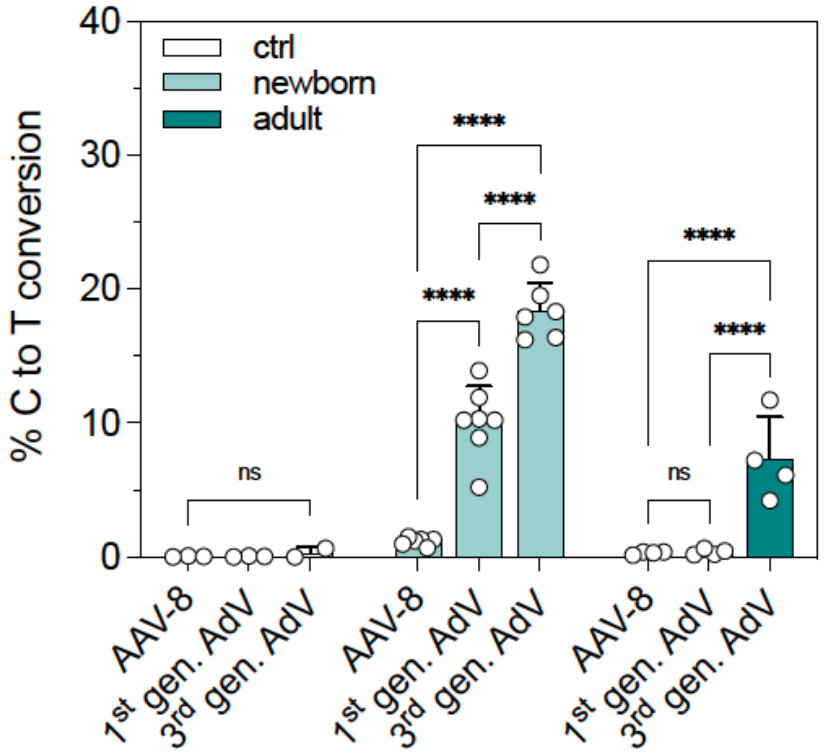


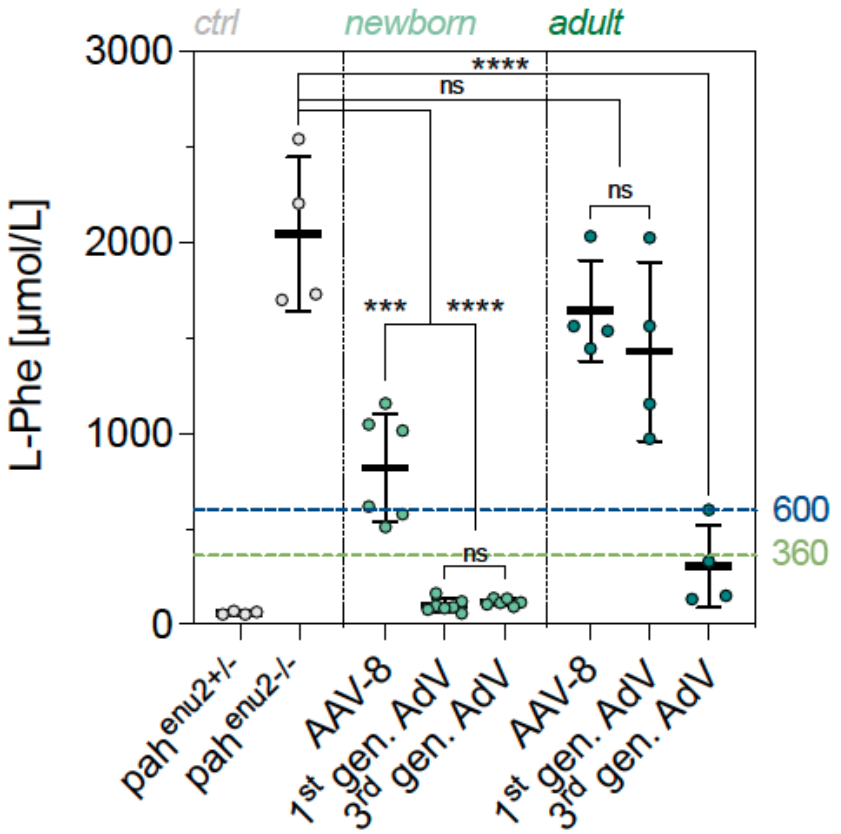




Boeck, Rothgangl, Villiger et al. (2021) bioRxiv, https://doi.org/10.1101/2021.08.17.456632

Correction of the *Pah*^{enu} mutation via AdV5-mediated prime editing

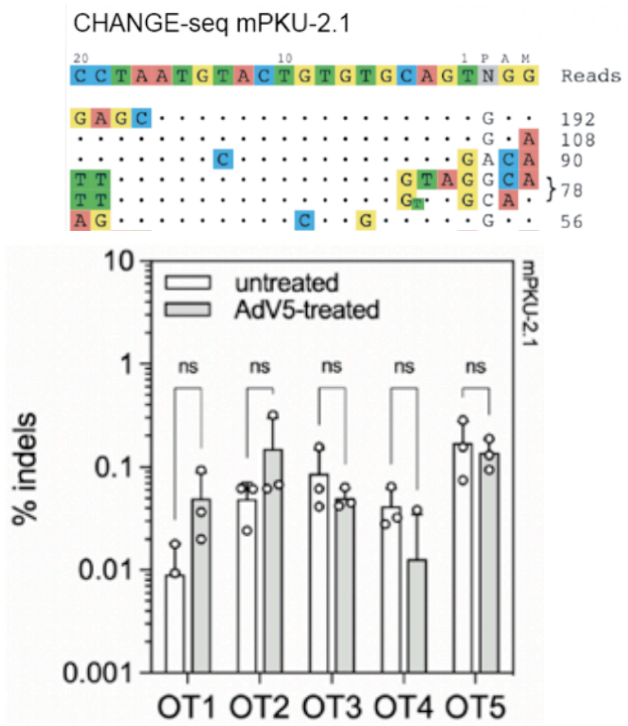




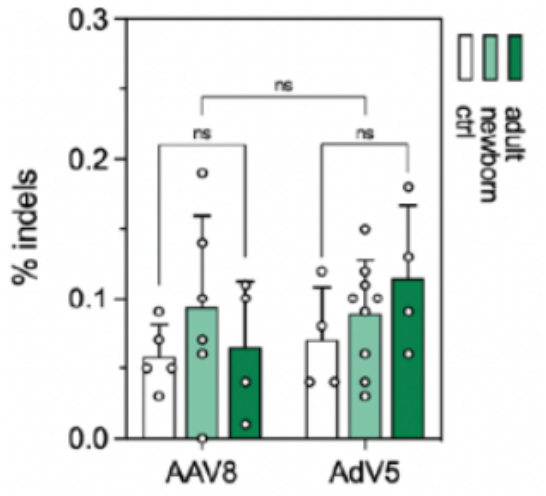
Boeck, Rothgangl, Villiger et al. (2021) bioRxiv, https://doi.org/10.1101/2021.08.17.456632

In vivo prime editing is highly precise

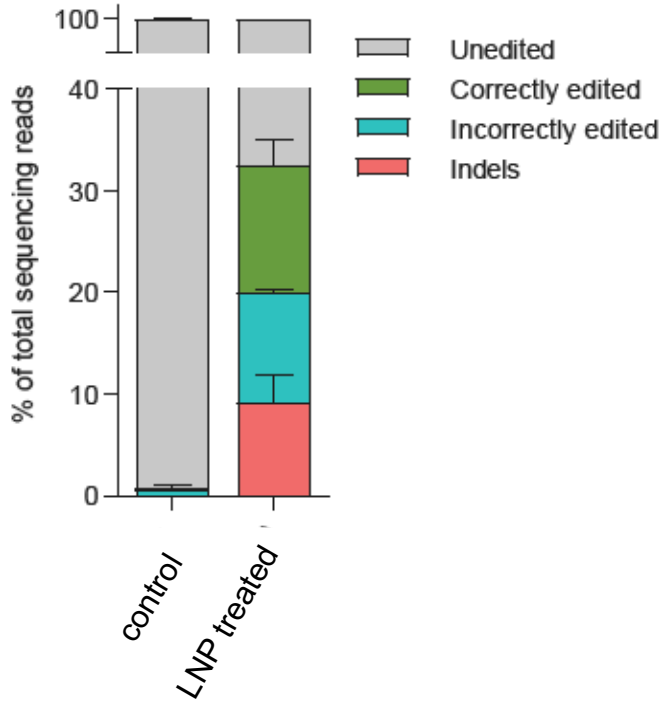
Prime editing off targets



Prime editing bystanders



Cytidine base editing bystanders



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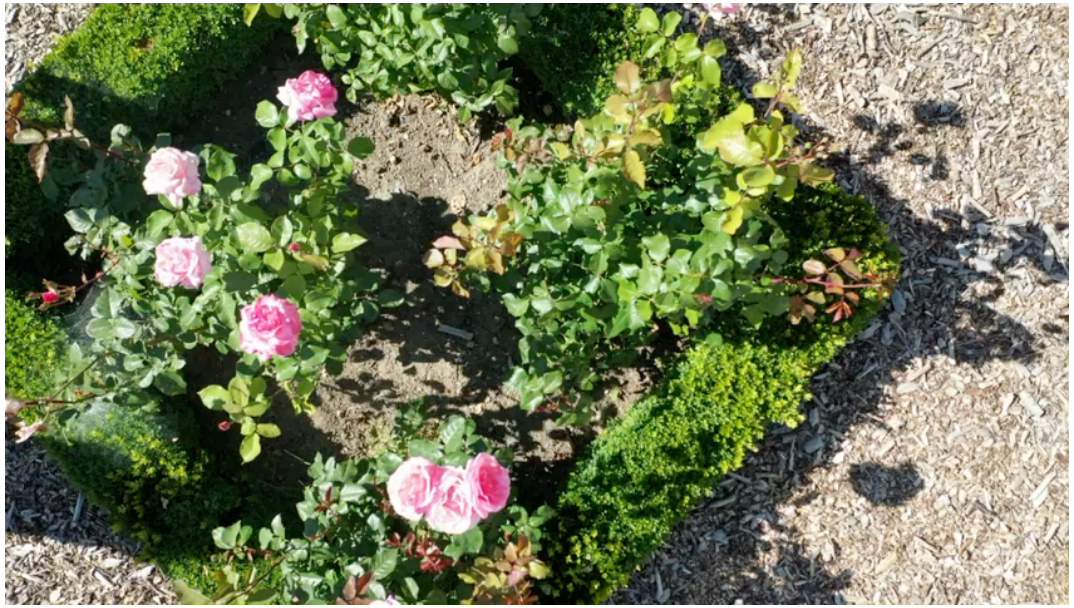

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