

Application of TadA in DNA editing

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P68398 · TADA_ECOLI

Proteinⁱ | tRNA-specific adenosine deaminase

Geneⁱ | tadA

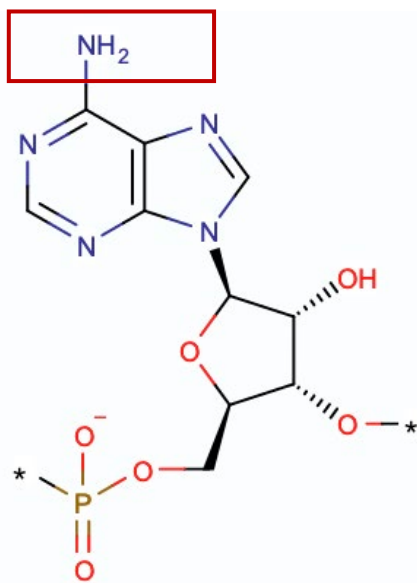
Statusⁱ |  UniProtKB reviewed (Swiss-Prot)

Organismⁱ | Escherichia coli (strain K12)

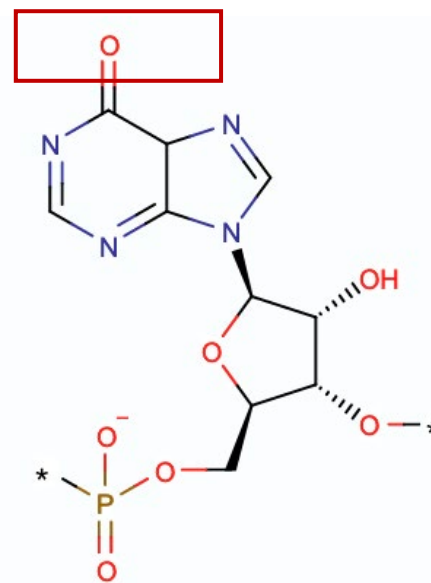
Amino acids | 167

Protein existenceⁱ | Evidence at protein level

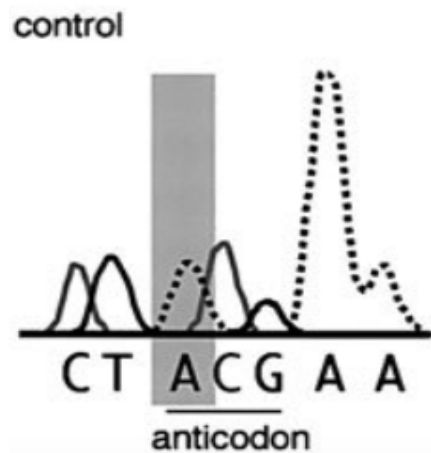
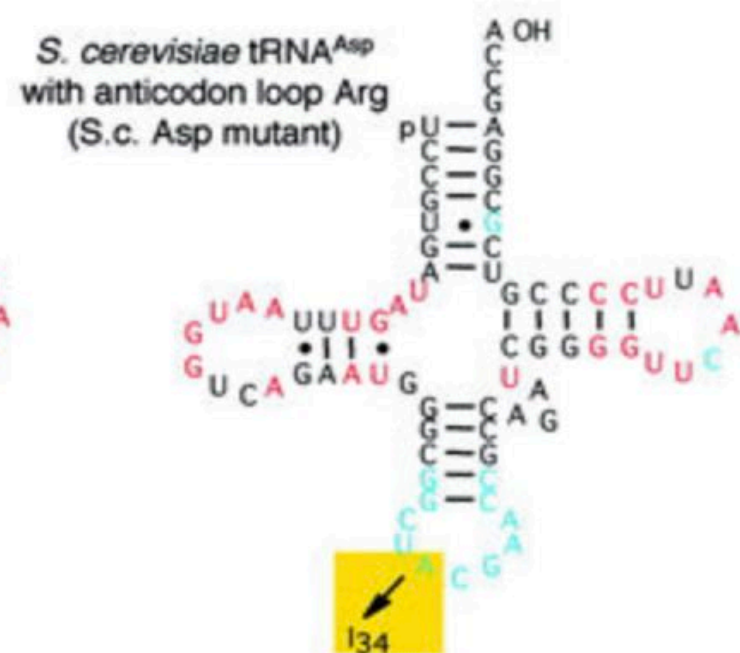
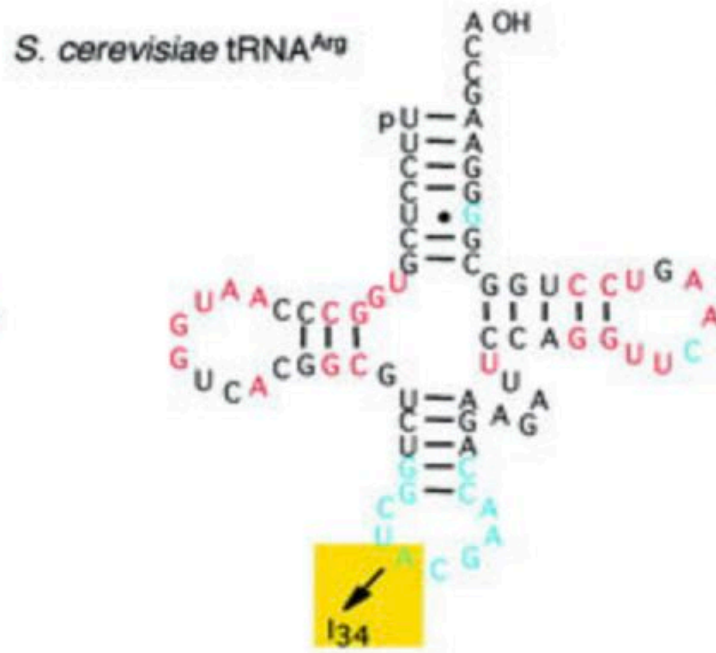
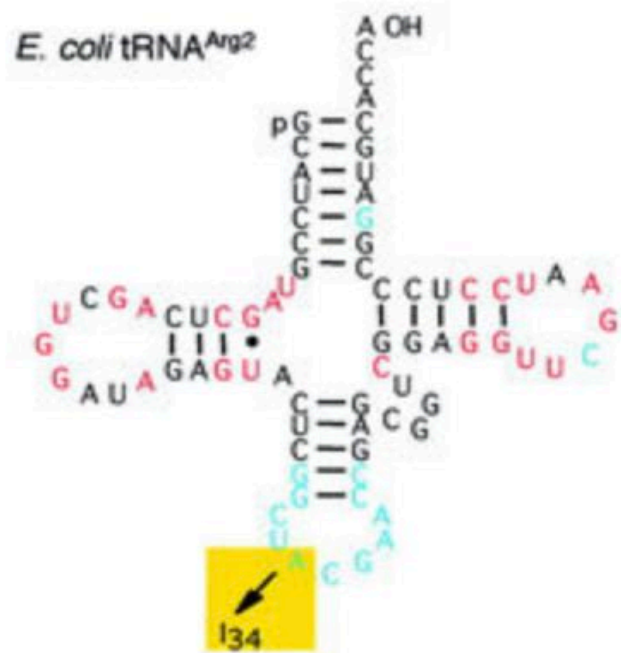
Annotation scoreⁱ |  5/5



A to I



TadA——大肠杆菌 tRNA-specific 腺苷脱氨酶

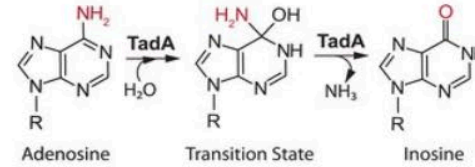


TadA——大肠杆菌 tRNA-specific 腺苷脱氨酶

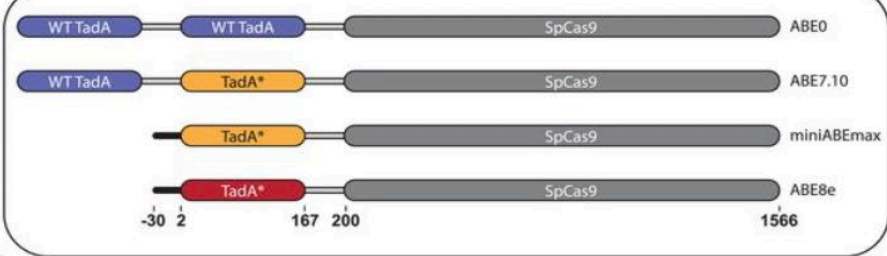
A



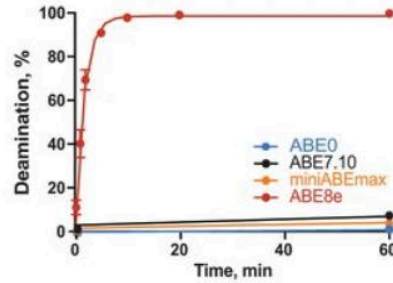
B



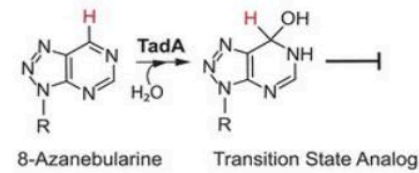
C



D

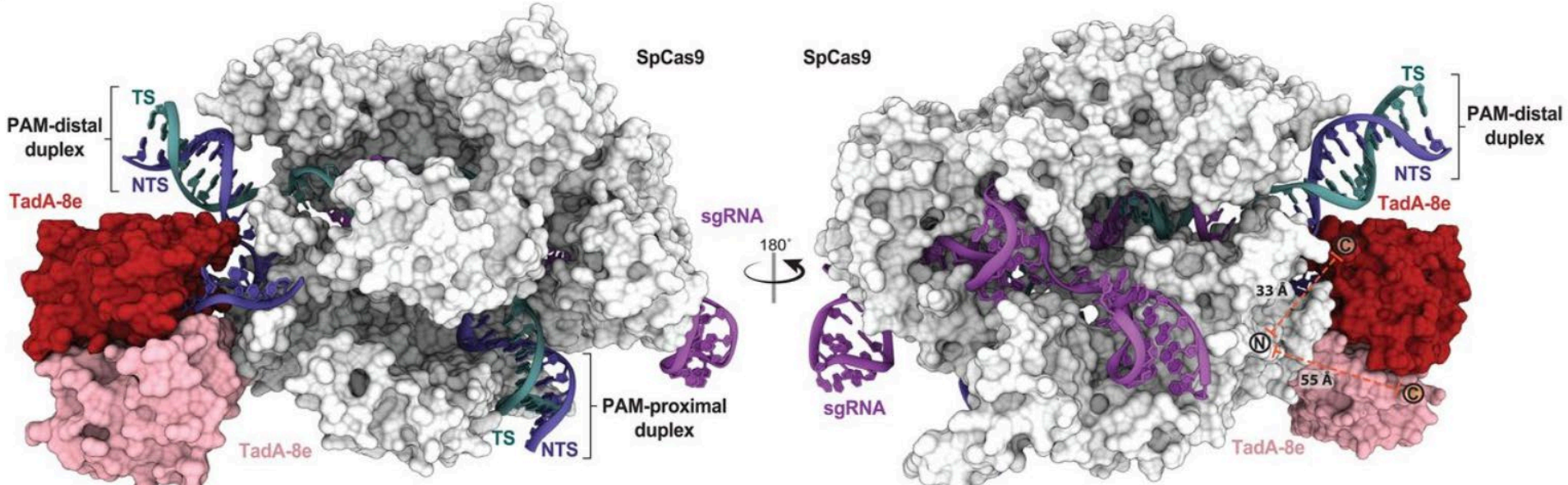


E



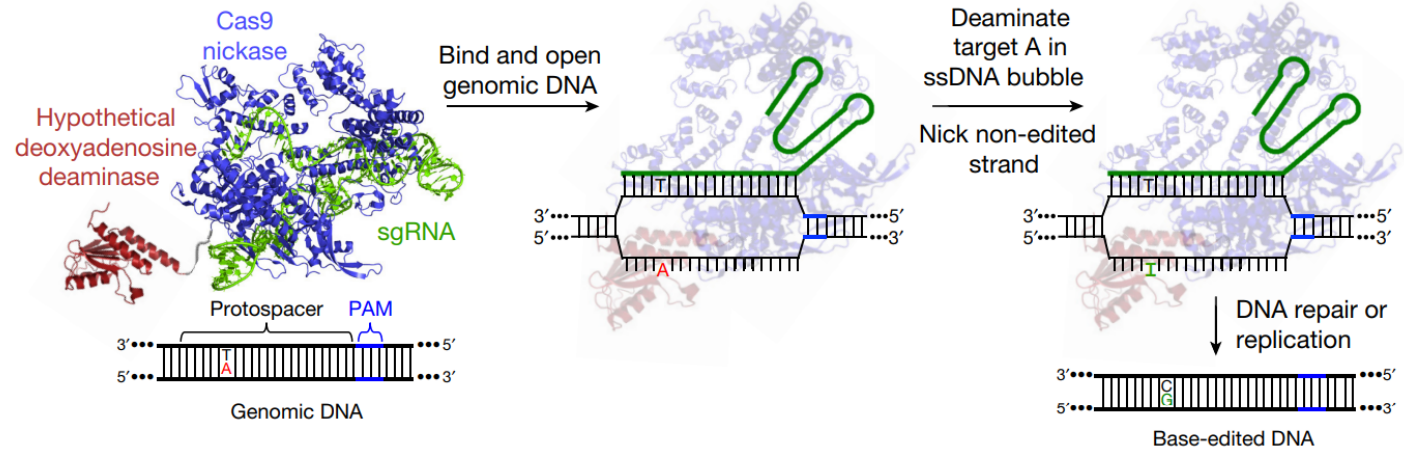
Cryo-EM structure of ABE8e in a substrate-bound state

F



Programmable base editing of TadA in genomic DNA

| | <i>E. coli</i> TadA amino acid | | | | | | | | | | | | | TadA state | Linker 1 length | Linker 2 length | | |
|---------|--------------------------------|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|------------|-----------------|-----------------|----|----|
| | 23 | 36 | 48 | 51 | 84 | 106 | 108 | 123 | 142 | 146 | 147 | 152 | 155 | 156 | 157 | | | |
| ABE0.1 | W | H | P | R | L | A | D | H | A | S | D | R | E | I | K | Monomer | 16 | |
| ABE1.1 | W | H | P | R | L | A | N | H | A | S | D | R | E | I | K | Monomer | 16 | |
| ABE1.2 | W | H | P | R | L | V | N | H | A | S | D | R | E | I | K | Monomer | 16 | |
| ABE2.1 | W | H | P | R | L | V | N | H | A | S | Y | R | V | I | K | Monomer | 16 | |
| ABE2.9 | W | H | P | R | L | V | N | H | A | S | Y | R | V | I | K | Homodimer | 32 | 16 |
| ABE2.10 | W | H | P | R | L | V | N | H | A | S | Y | R | V | I | K | Heterodimer | 32 | 16 |
| ABE3.1 | W | H | P | R | F | V | N | Y | A | S | Y | R | V | F | K | Homodimer | 32 | 32 |
| ABE4.3 | W | H | P | R | F | V | N | Y | N | S | Y | R | V | F | K | Homodimer | 32 | 32 |
| ABE5.1 | W | L | P | L | F | V | N | Y | A | C | Y | R | V | F | N | Homodimer | 32 | 32 |
| ABE5.3 | W | L | P | L | F | V | N | Y | A | C | Y | R | V | F | N | Heterodimer | 32 | 32 |
| ABE6.3 | W | L | S | L | F | V | N | Y | A | C | Y | R | V | F | N | Heterodimer | 32 | 32 |
| ABE6.4 | W | L | S | L | F | V | N | Y | N | C | Y | R | V | F | N | Heterodimer | 32 | 32 |
| ABE7.4 | R | L | A | L | F | V | N | Y | A | C | Y | R | V | F | N | Heterodimer | 32 | 32 |
| ABE7.9 | L | L | A | L | F | V | N | Y | N | C | Y | R | V | F | N | Heterodimer | 32 | 32 |
| ABE7 | L | L | A | L | F | V | N | Y | N | C | Y | P | V | F | N | Heterodimer | 32 | 32 |
| ABE7. | L | L | A | L | F | V | N | Y | A | C | Y | P | V | F | N | Heterodimer | 32 | 32 |



Evolving a transfer RNA adenosine deaminase (TadA) to operate on DNA when fused to a catalytically impaired CRISPR–Cas9 mutant.

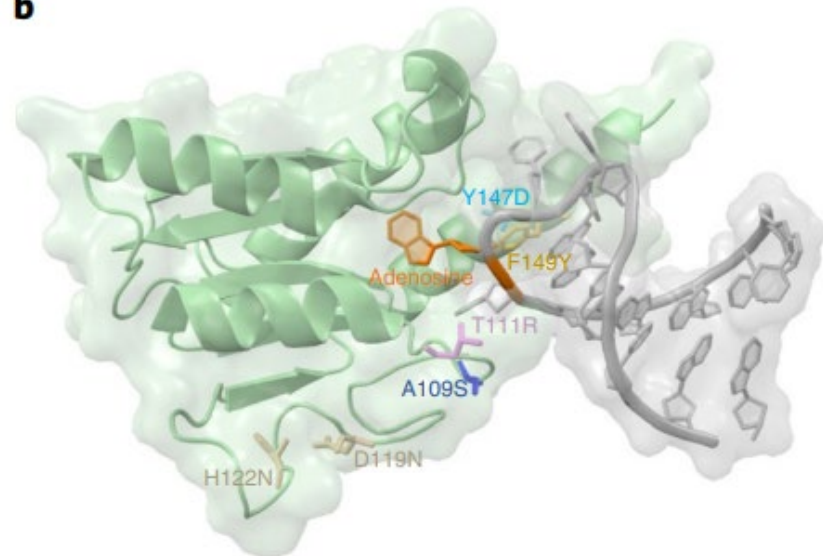
Directed evolution of adenine base editors with increased activity

TadA-8e

a

| Clone | TadA amino acid number | | | | | | | | | |
|--------------|------------------------|----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 26 | 88 | 109 | 111 | 119 | 122 | 147 | 149 | 166 | 167 |
| TadA-7.10 | R | V | A | T | D | H | Y | F | T | D |
| PANCE 1 | | | | R | | | | | | |
| PANCE 2 | | | S/T | R | | | | | | |
| TadA-8a | C | | S | R | N | N | D | Y | I | N |
| TadA-8b | | A | S | R | N | N | | Y | I | N |
| PACE TadA-8c | C | | S | R | N | N | | Y | I | N |
| TadA-8d | | A | | R | N | | | Y | | |
| TadA-8e | | | S | R | N | N | D | Y | I | N |

b



TadA-8.20

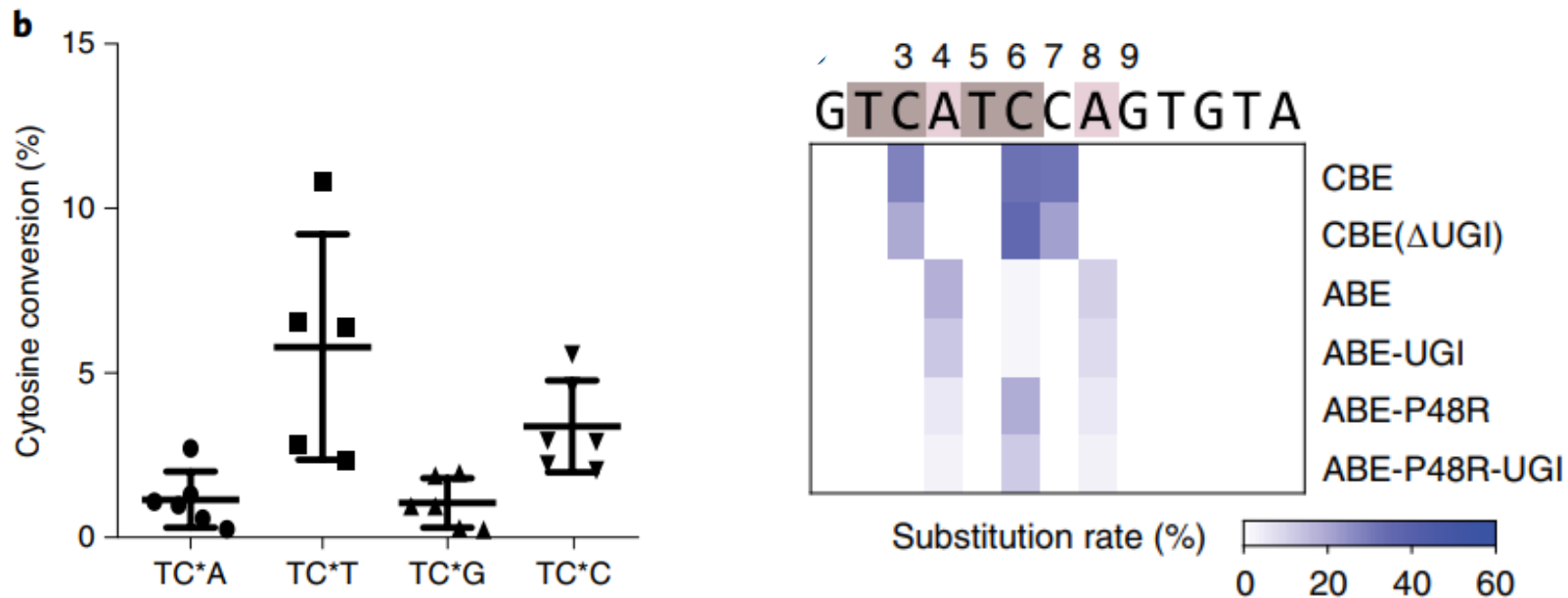
| | residue identity in evolved TadA | | | | | | | | | | | | | | | | | |
|-----------|----------------------------------|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 23 | 36 | 48 | 51 | 76 | 82 | 84 | 106 | 108 | 123 | 146 | 147 | 152 | 154 | 155 | 156 | 157 | 166 |
| ABE7.10 | R | L | A | L | I | V | F | V | N | Y | C | Y | P | Q | V | F | N | T |
| ABE8.1-m | | | | | | | | | | | | | T | | | | | |
| ABE8.2-m | | | | | | | | | | | | | R | | | | | |
| ABE8.3-m | | | | | | | | | | | | | | | S | | | |
| ABE8.4-m | | | | | | | | | | H | | | | | | | | |
| ABE8.5-m | | | | | S | | | | | | | | | | | | | |
| ABE8.6-m | | | | | | | | | | | | | | | | | | R |
| ABE8.7-m | | | | | | | | | | | | | | | | | | R |
| ABE8.8-m | | | | | | | | | | H | | R | | R | | | | |
| ABE8.9-m | | | | | Y | | | | | | | R | | R | | | | |
| ABE8.10-m | | | | | | | | | | | | R | | R | | | | R |
| ABE8.11-m | | | | | | | | | | | | T | | R | | | | |
| ABE8.12-m | | | | | | | | | | | | T | | S | | | | |
| ABE8.13-m | | | | | Y | | | | | H | | R | | R | | | | |
| ABE8.14-m | | | | | Y | S | | | | | | | | | | | | |
| ABE8.15-m | | | | | | S | | | | | | R | | | | | | |
| ABE8.16-m | | | | | | S | | | | H | | R | | | | | | |
| ABE8.17-m | | | | | | S | | | | | | | | | | | | R |
| ABE8.18-m | | | | | | S | | | | H | | | | R | | | | |
| ABE8.19-m | | | | | | S | | | | H | | R | | R | | | | |
| ABE8.20-m | | | | | Y | S | | | | H | | R | | R | | | | |
| ABE8.1-d | | | | | | | | | | | | | | T | | | | |
| ABE8.2-d | | | | | | | | | | | | | | R | | | | |
| ABE8.3-d | | | | | | | | | | | | | | | | | | |
| ABE8.4-d | | | | | | | | | | H | | | | | | | | |
| ABE8.5-d | | | | | | S | | | | | | | | | | | | |
| ABE8.6-d | | | | | | | | | | | | | | | | | | R |
| ABE8.7-d | | | | | | | | | | | | | | | | | | R |
| ABE8.8-d | | | | | | | | | | H | | R | | R | | | | |
| ABE8.9-d | | | | | Y | | | | | | | R | | R | | | | |
| ABE8.10-d | | | | | | | | | | | | R | | R | | | | R |
| ABE8.11-d | | | | | | | | | | | | T | | R | | | | |
| ABE8.12-d | | | | | | | | | | | | T | | S | | | | |
| ABE8.13-d | | | | | Y | | | | | H | | R | | R | | | | |
| ABE8.14-d | | | | | Y | S | | | | | | | | | | | | |
| ABE8.15-d | | | | | | S | | | | | | R | | | | | | |
| ABE8.16-d | | | | | | S | | | | H | | R | | | | | | |
| ABE8.17-d | | | | | | S | | | | | | | | | | | | R |
| ABE8.18-d | | | | | | S | | | | H | | | | R | | | | |
| ABE8.19-d | | | | | | S | | | | H | | R | | R | | | | |
| ABE8.20-d | | | | | Y | S | | | | H | | R | | R | | | | |

TadA displayed increased cytosine deamination activity

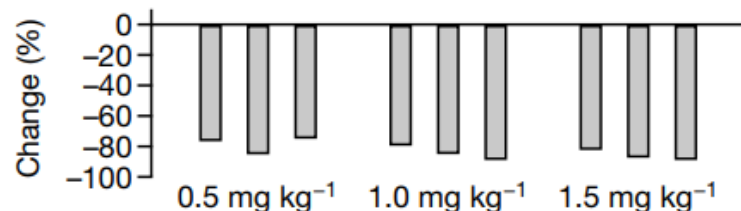
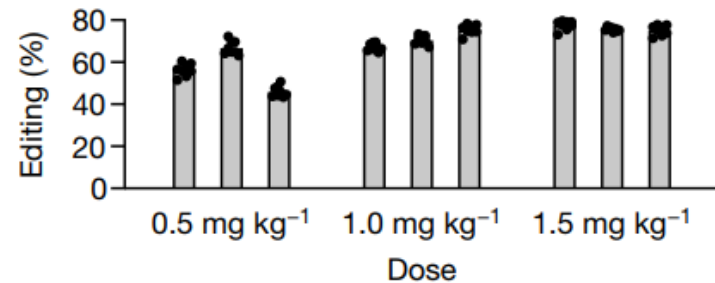
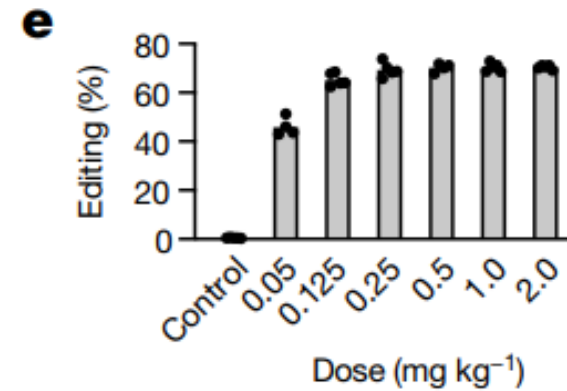
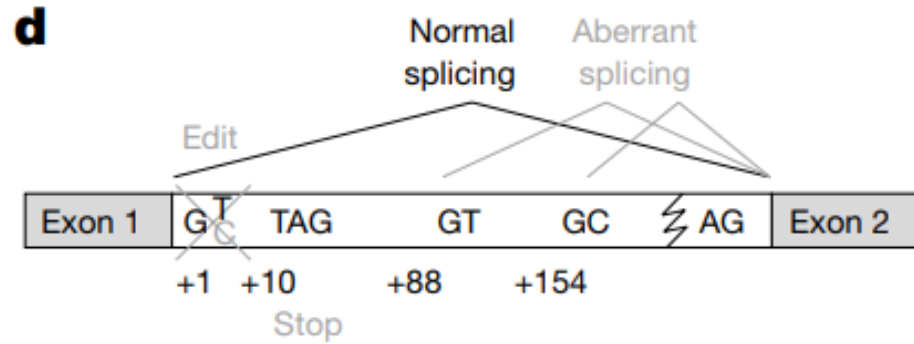
ABE7.10

| <i>FANCF</i> | G | G | A | A4 | T | C6 | C | C | T | T | C | T | G | C | A | G | C | A | C | C | T | G | G |
|--------------|------|-----|------|------|-----|------|------|------|------|-----|-----|-----|-----|------|-----|------|------|-----|------|------|------|------|------|
| A | 0 | 0 | 99.3 | 87.4 | 0 | 0.8 | 0.1 | 0.1 | 0.1 | 0 | 0 | 0 | 0 | 0.1 | 100 | 0.1 | 0.1 | 100 | 0.1 | 0.1 | 0.1 | 0 | 0.1 |
| T | 0 | 0 | 0 | 0 | 100 | 1.6 | 0 | 0.1 | 99.9 | 100 | 0 | 100 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 99.8 | 0 | 0 |
| G | 99.9 | 100 | 0.7 | 12.6 | 0 | 8.5 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 99.9 | 0 | 0 | 0 | 0 | 0.1 | 99.9 | 99.9 |
| C | 0 | 0 | 0 | 0.1 | 0 | 89.1 | 99.9 | 99.8 | 0 | 0 | 100 | 0 | 0 | 99.9 | 0 | 0 | 99.9 | 0 | 99.9 | 99.9 | 0.1 | 0 | 0 |

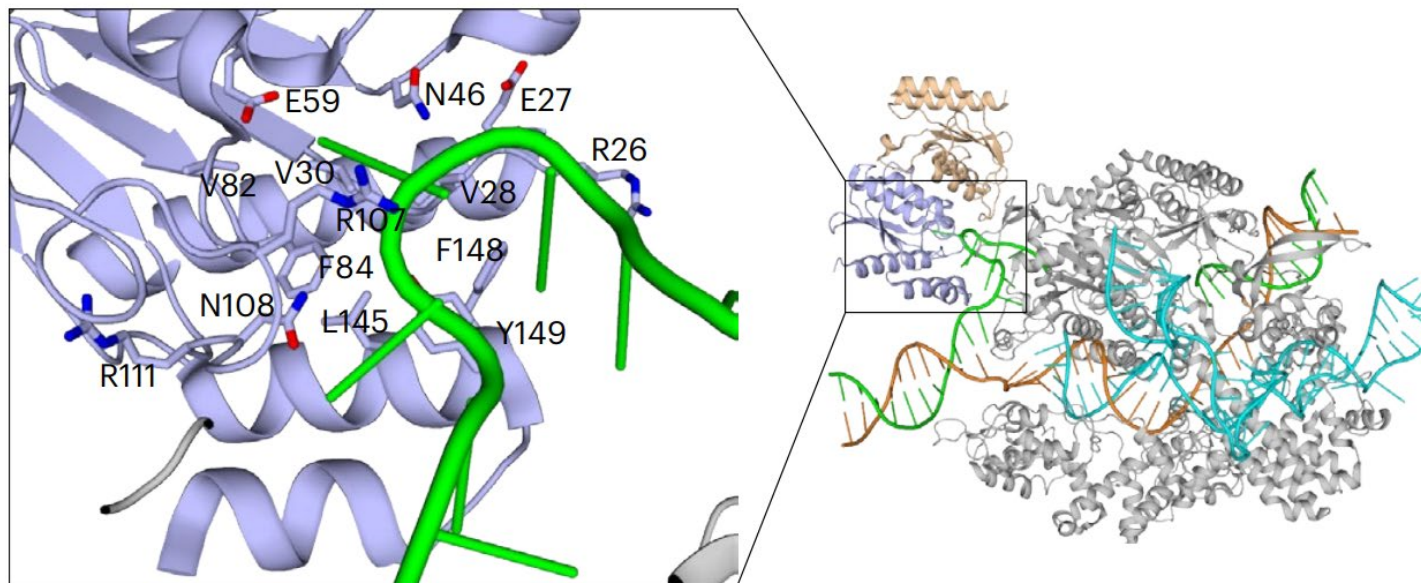
| <i>RNF2</i> | G | T | C | A4 | T | C6 | T | T | A | G | T | C | A | T | T | A | C | C | T | G | A | G | G |
|-------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| A | 0.1 | 0.1 | 0 | 56.7 | 0 | 0.1 | 0 | 0 | 91.0 | 0 | 0 | 0.1 | 99.9 | 0 | 0 | 99.9 | 0.1 | 0.1 | 0.1 | 0 | 99.9 | 0 | 0 |
| T | 0 | 99.9 | 0.1 | 0 | 99.9 | 1.4 | 99.9 | 99.9 | 0 | 0 | 99.9 | 0.1 | 0 | 99.9 | 99.9 | 0 | 0 | 0 | 99.8 | 0 | 0 | 0 | 0 |
| G | 99.9 | 0 | 0 | 43.2 | 0 | 8.6 | 0 | 0 | 8.9 | 99.9 | 0 | 0 | 0.1 | 0 | 0 | 0 | 0 | 0 | 0 | 99.9 | 0.1 | 99.9 | 99.9 |
| C | 0 | 0 | 99.8 | 0 | 0 | 89.8 | 0 | 0 | 0.1 | 0 | 0 | 99.8 | 0 | 0 | 0 | 0 | 99.9 | 99.8 | 0.1 | 0 | 0 | 0 | 0 |



In vivo base editing lowers cholesterol in primates



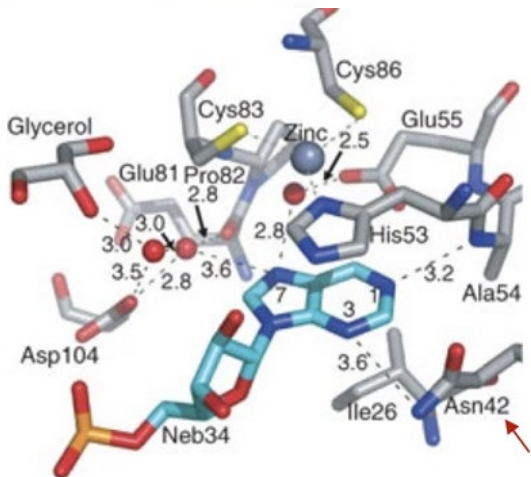
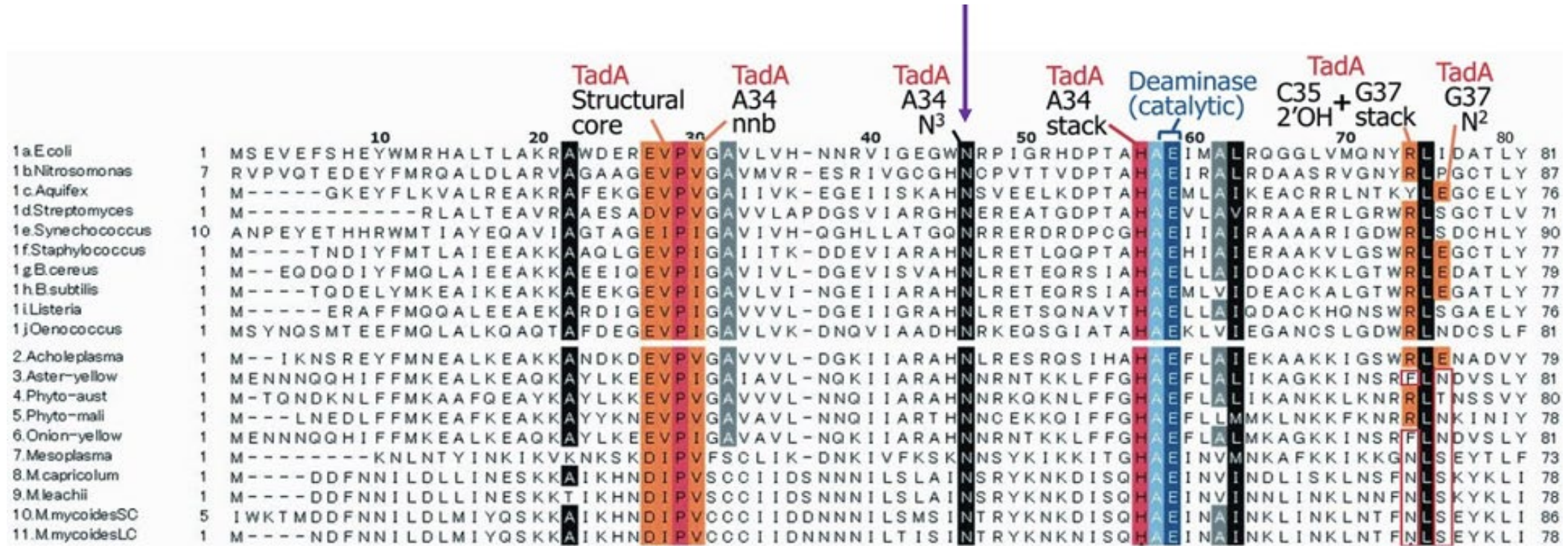
TadA&LNP achieve in vivo editing to prevent diseases



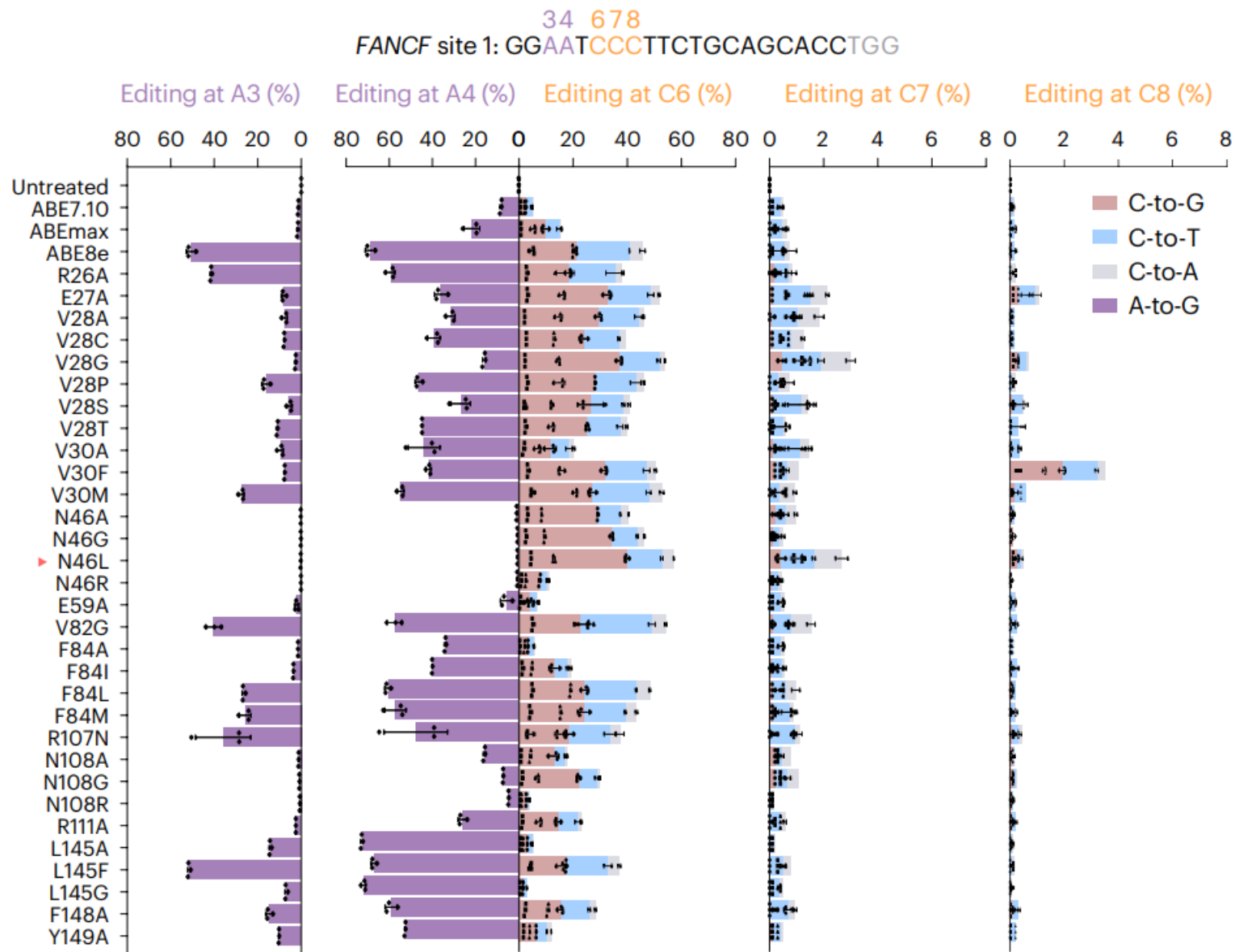
将腺嘌呤脱氨酶TadA进行突变，使其能够用于胞嘧啶转换。



TadA蛋白序列对比分析



- N46在进化中保守
- N46在催化中心，和底物腺嘌呤有氢键相互作用

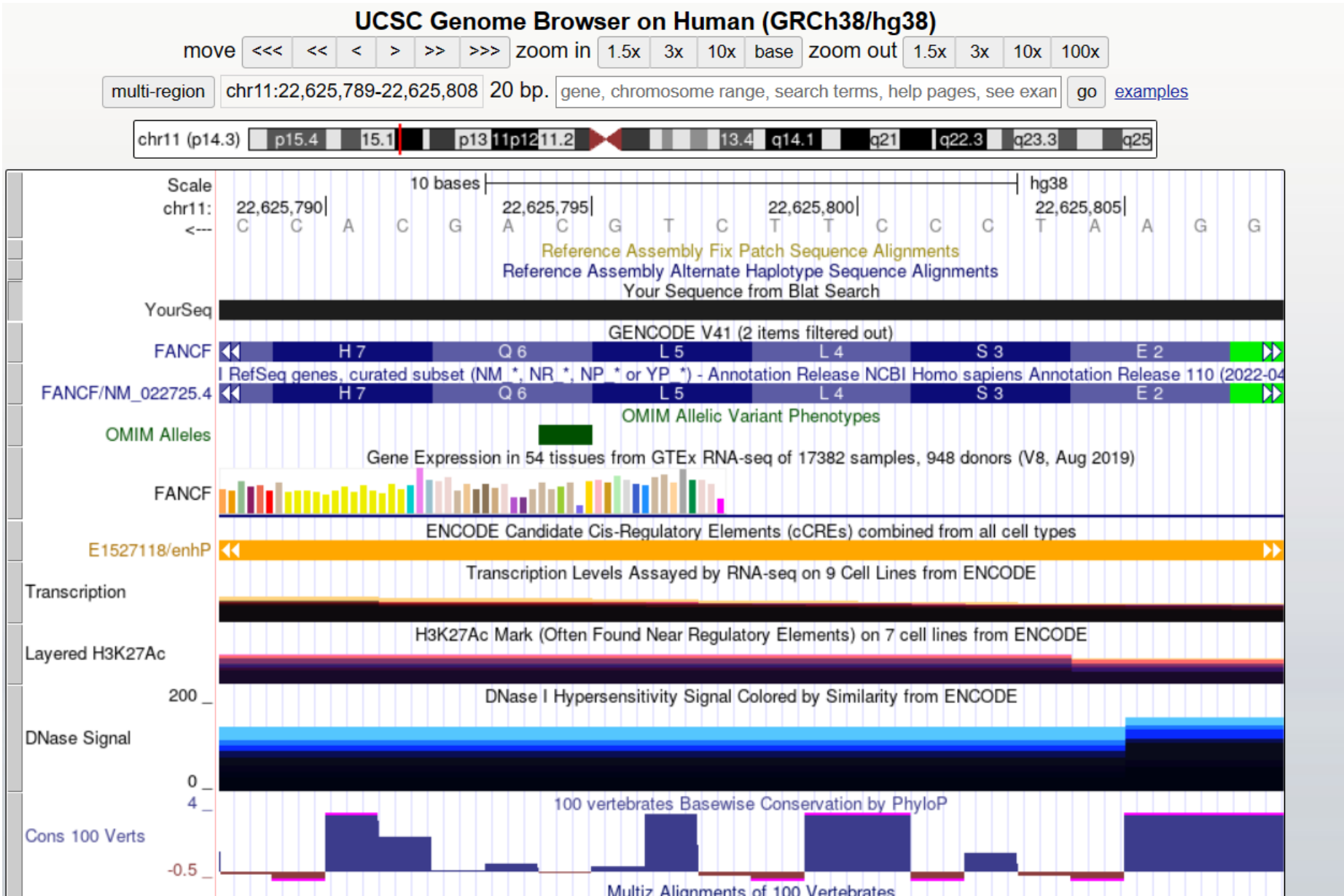


N46L可以消除TadA固有的腺嘌呤脱氨酶活性而展现高效的胞嘧啶编辑活性

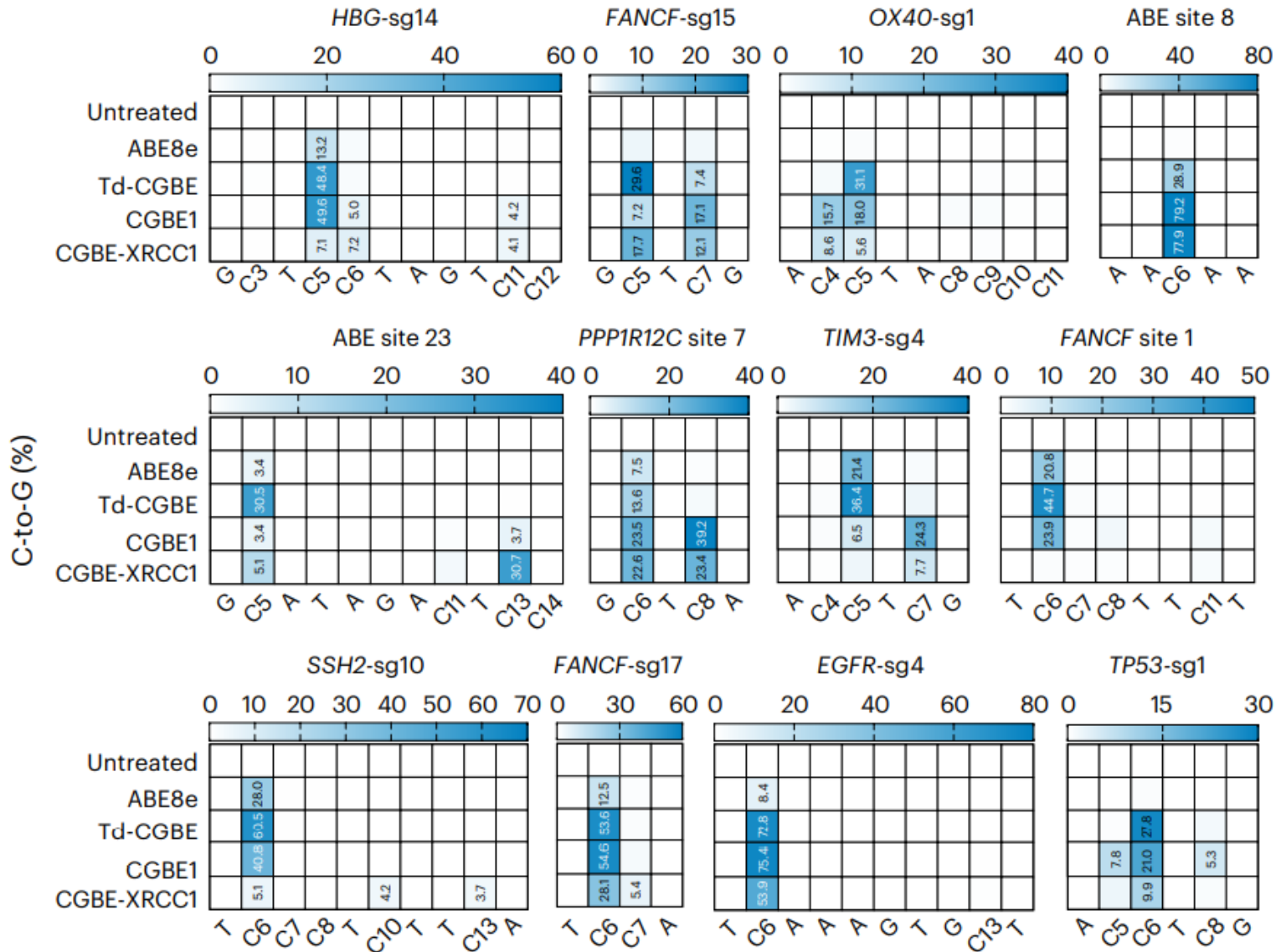
Target protospacer sequence

| sgRNA | Target site sequence (5'-3') | PAM | oligo | |
|---------------------|------------------------------|-----|----------|---------------------------|
| <i>FANCF</i> site 1 | GGAATCCCTTCTGCAGCACC | TGG | Oligo-up | CACCGGAATCCCTTCTGCAGCACC |
| | | | Oligo-dn | AAACGGTGCTGCAGAAGGGATTCC |
| <i>PCSK9</i> -sg3 | CCTCTTCACCTGCTCCTGAG | GGG | Oligo-up | CACCGCCTCTTCACCTGCTCCTGAG |
| | | | Oligo-dn | AAACCTCAGGAGCAGGTGAAGAGGC |
| <i>HBG</i> -sg11 | CTTCATCCCTAGCCAGCCGC | CGG | Oligo-up | CACCGCTTCATCCCTAGCCAGCCGC |
| | | | Oligo-dn | AAACGCGGCTGGCTAGGGATGAAGC |

寻找合适的内源靶点



在靶点测试碱基编辑器活性



**Over 5000 kinds of
monogenic diseases**

Cystic fibrosis 囊性纤维化

Huntington's chorea 亨廷顿舞蹈病

Duchenne muscular dystrophy (DMD) 杜氏肌营养不良症

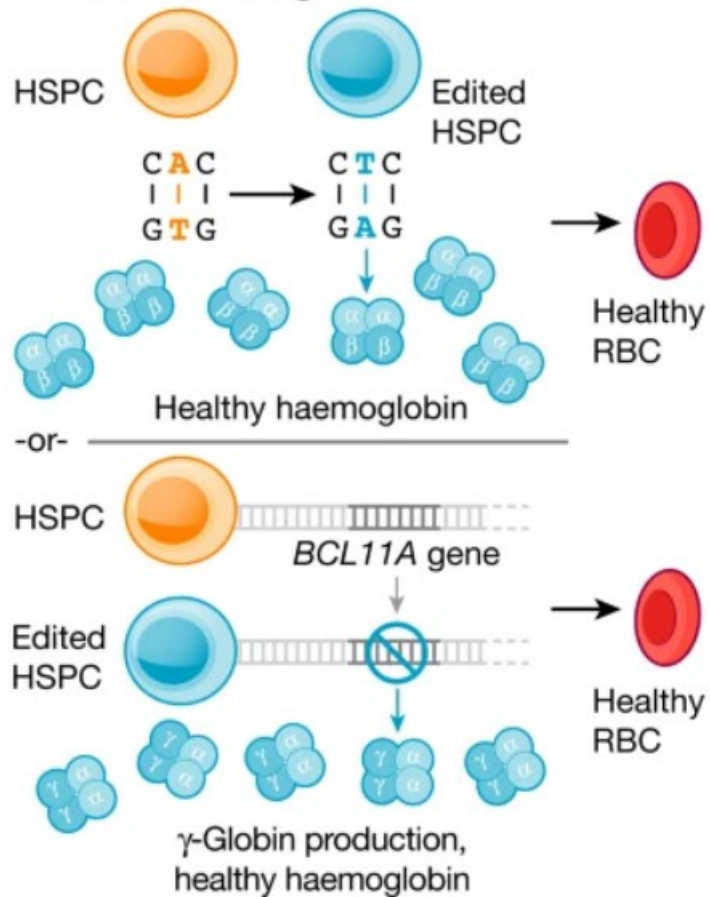
sickle cell anaemia 镰状细胞性贫血

**At least 250 million people
are affected globally**

Ex vivo editing

sickle cell anaemia 镰状细胞性贫血

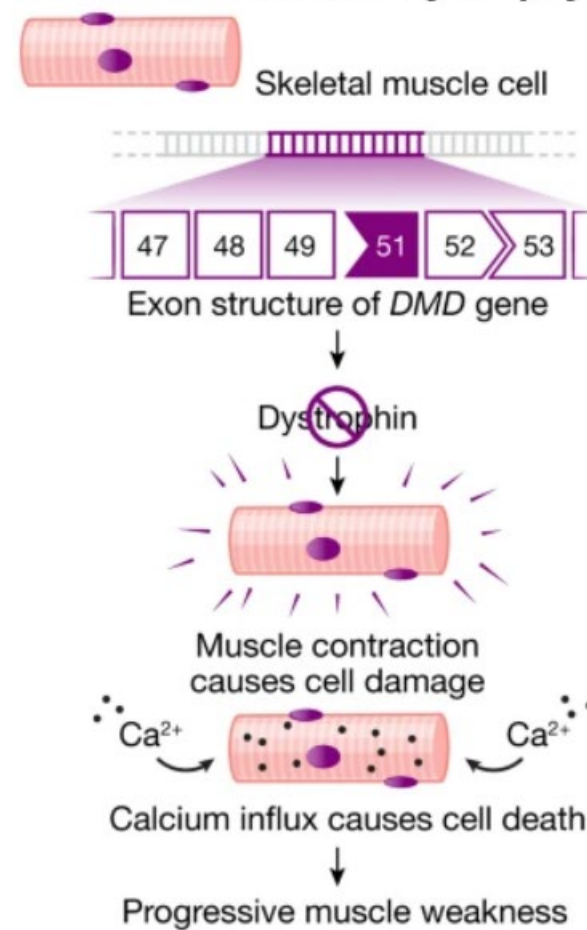
Blood cell editing



In vivo editing

Duchenne muscular dystrophy (DMD) 杜氏肌营养不良症

Duchenne muscular dystrophy



- 在有关TadA的这部分研究中，应用了课程学习了解到的方法，网站和软件，如 uniprot，序列比对blast，UCSC等等。
- 通过这些网站与软件，我们对于TadA蛋白的功能和结构有了比较清晰的认知，并对其发展方向和应用方向有了了解。
- 通过课上所学的知识，我们对于如何研究蛋白有了一定的研究思路，可以通过其结构与序列对其功能进行预测和改造

Thanks for listening!



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