CCEdit
Enhanced gene editing

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Coiled-coil mediated tethering of CRISPR/Cas and exonucleases for enhanced genome editing

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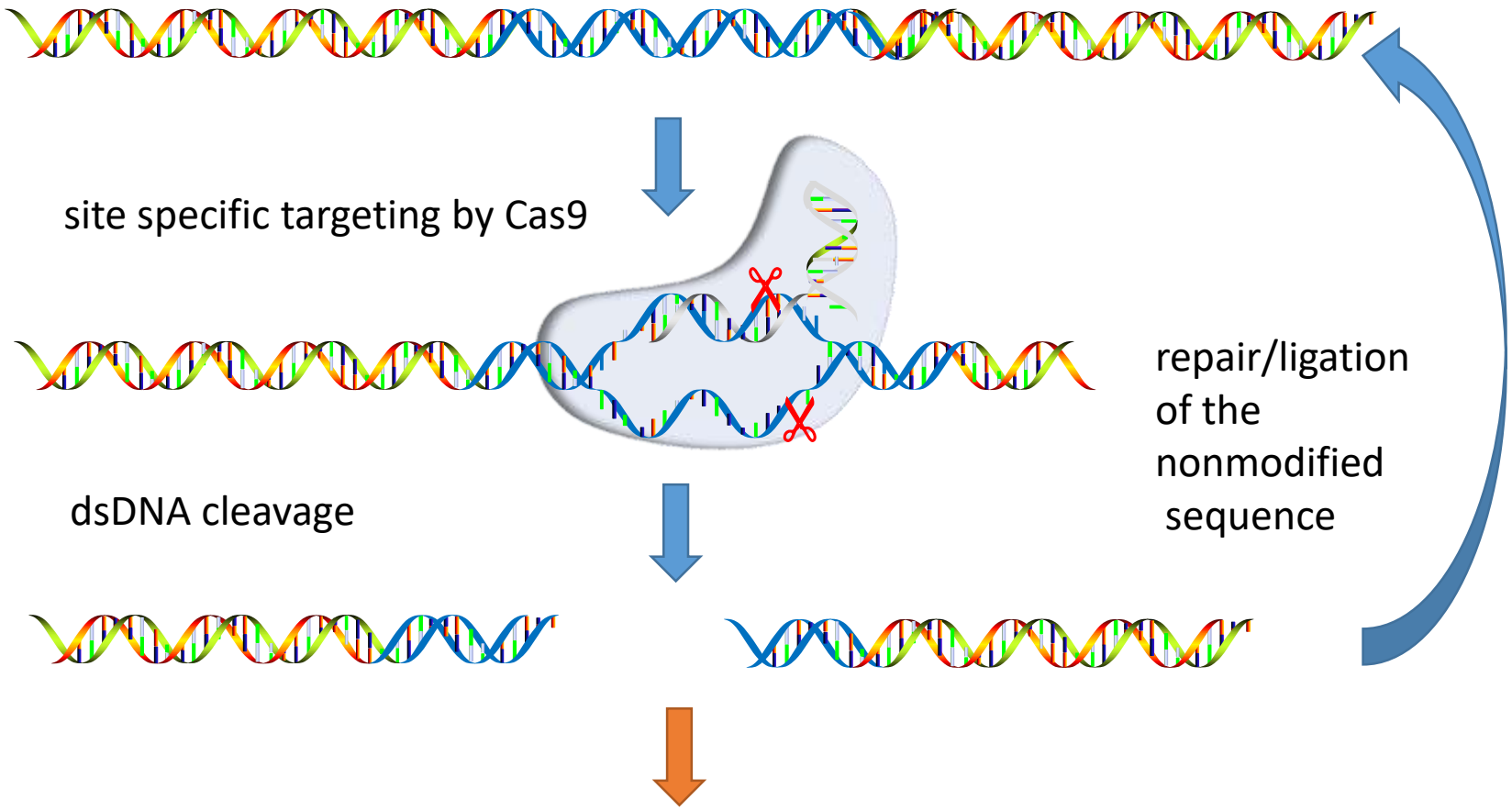
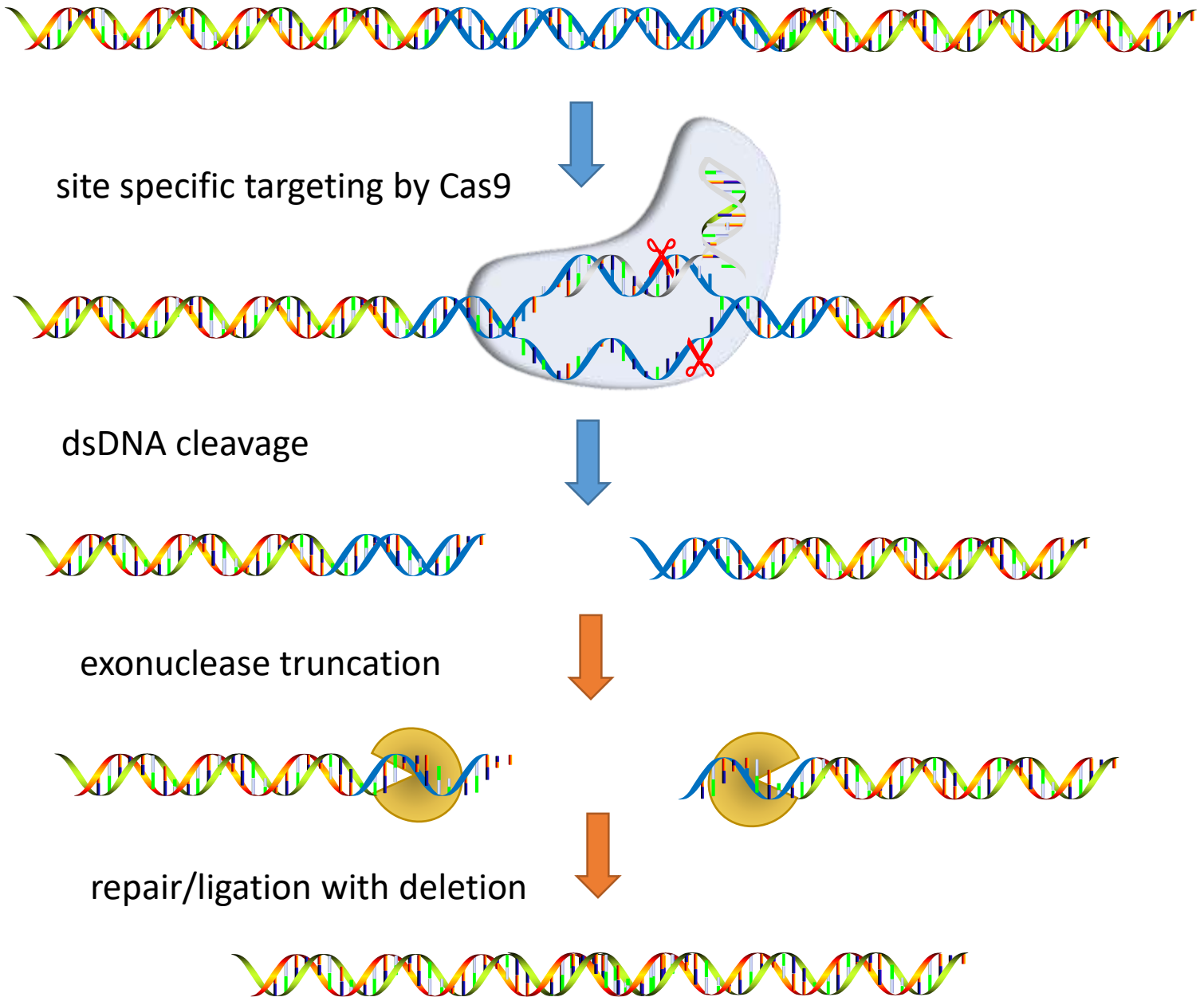
CRISPR revolution

- Fast and widely applicable genome editing
- Diverse biotechnological and therapeutic applications
- Most current applications are based on target gene inactivation
- Therapy:
  - 47 registered clinical trials (16 in China)
  - Diagnostics
  - Cancer, viral infections, gene therapy: sickle cell disease, transthyretin amyloidosis
- Any improvement in the efficiency could have large impact

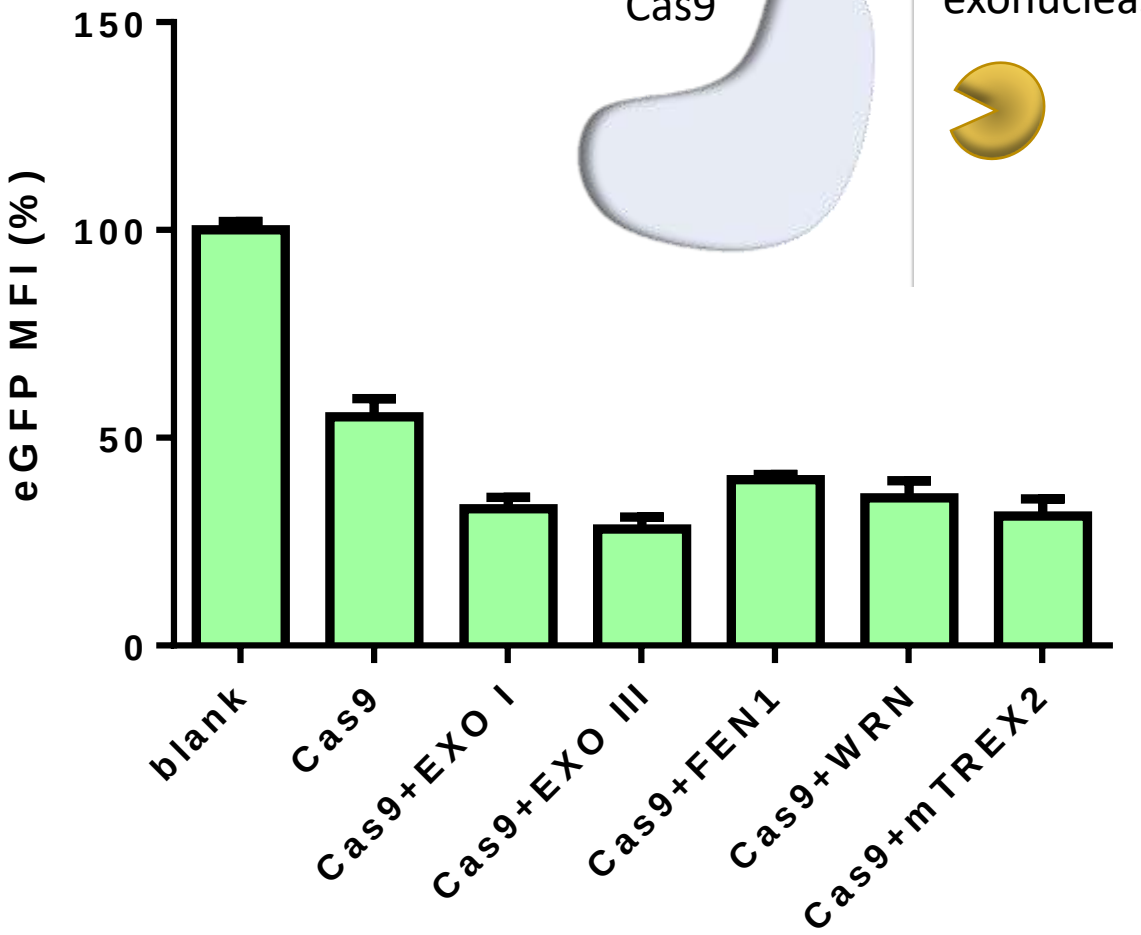
Conventional CRISPR-mediated gene inactivation

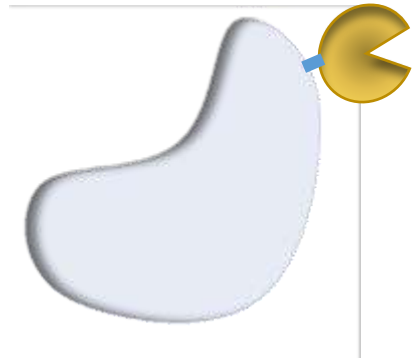


Exonuclease augmented gene inactivation



Coexpression with exonucleases increases the gene KO efficiency

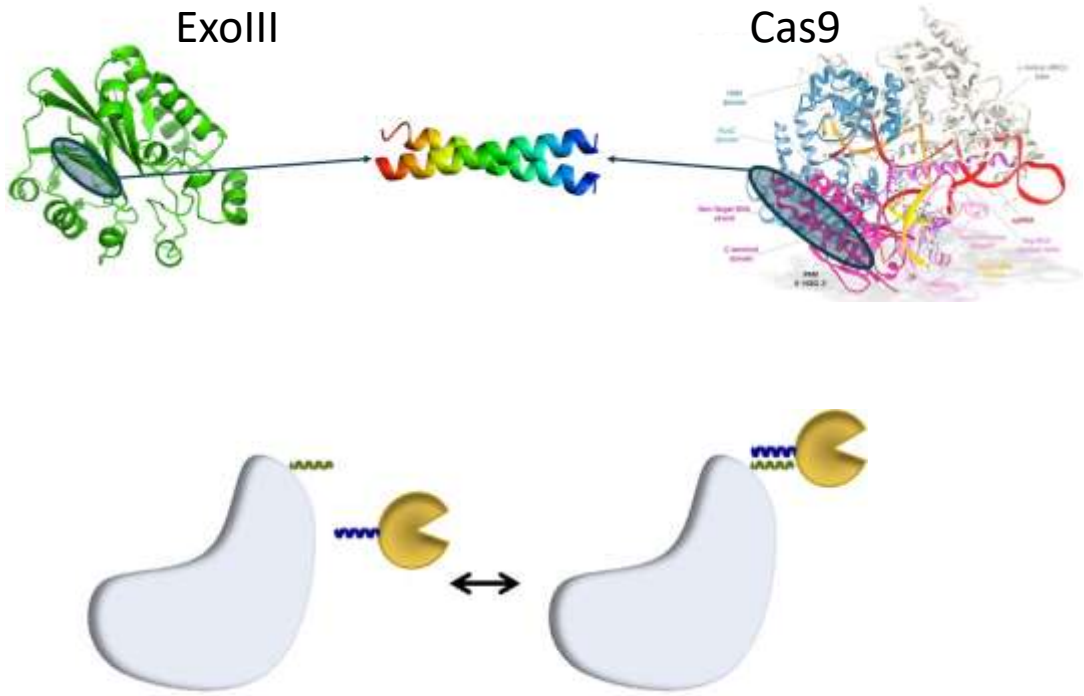




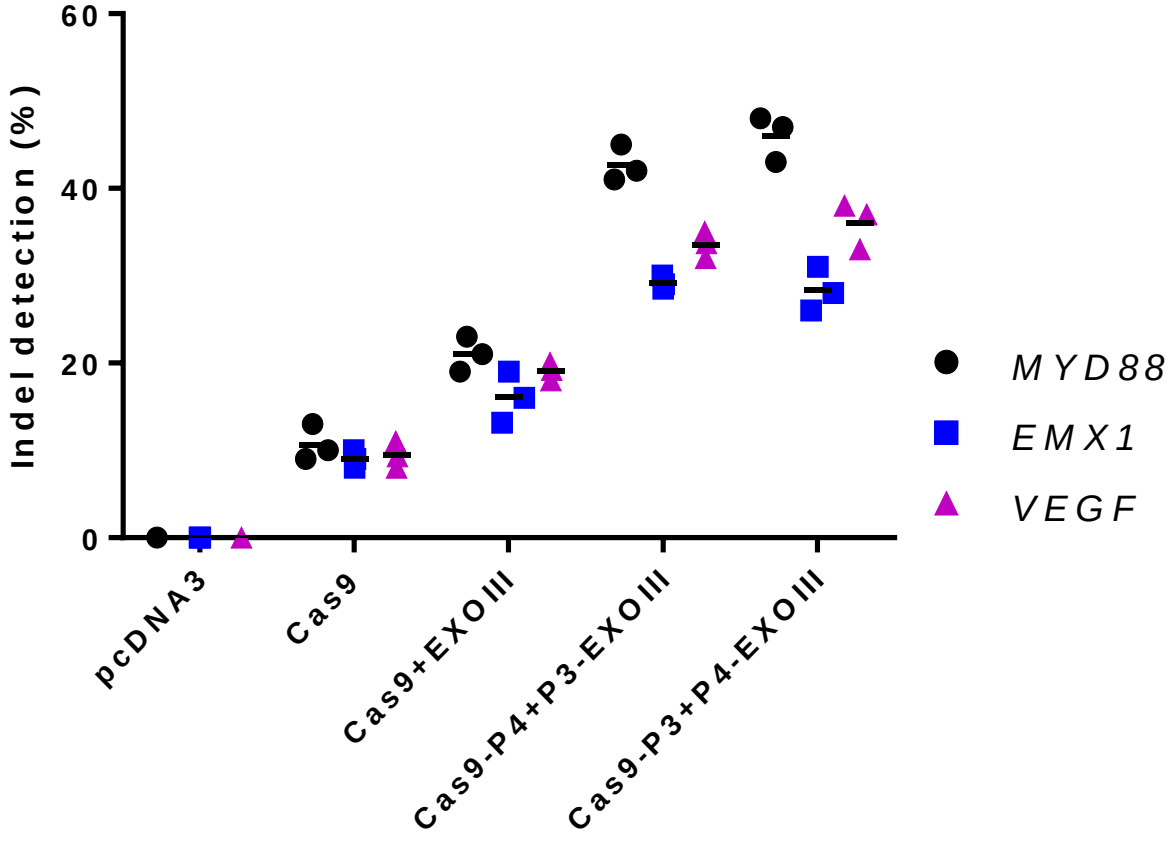
No improvement by tethered exonuclease

Coiled-coil-linker peptide-mediated targeting improves the efficiency of genome editing

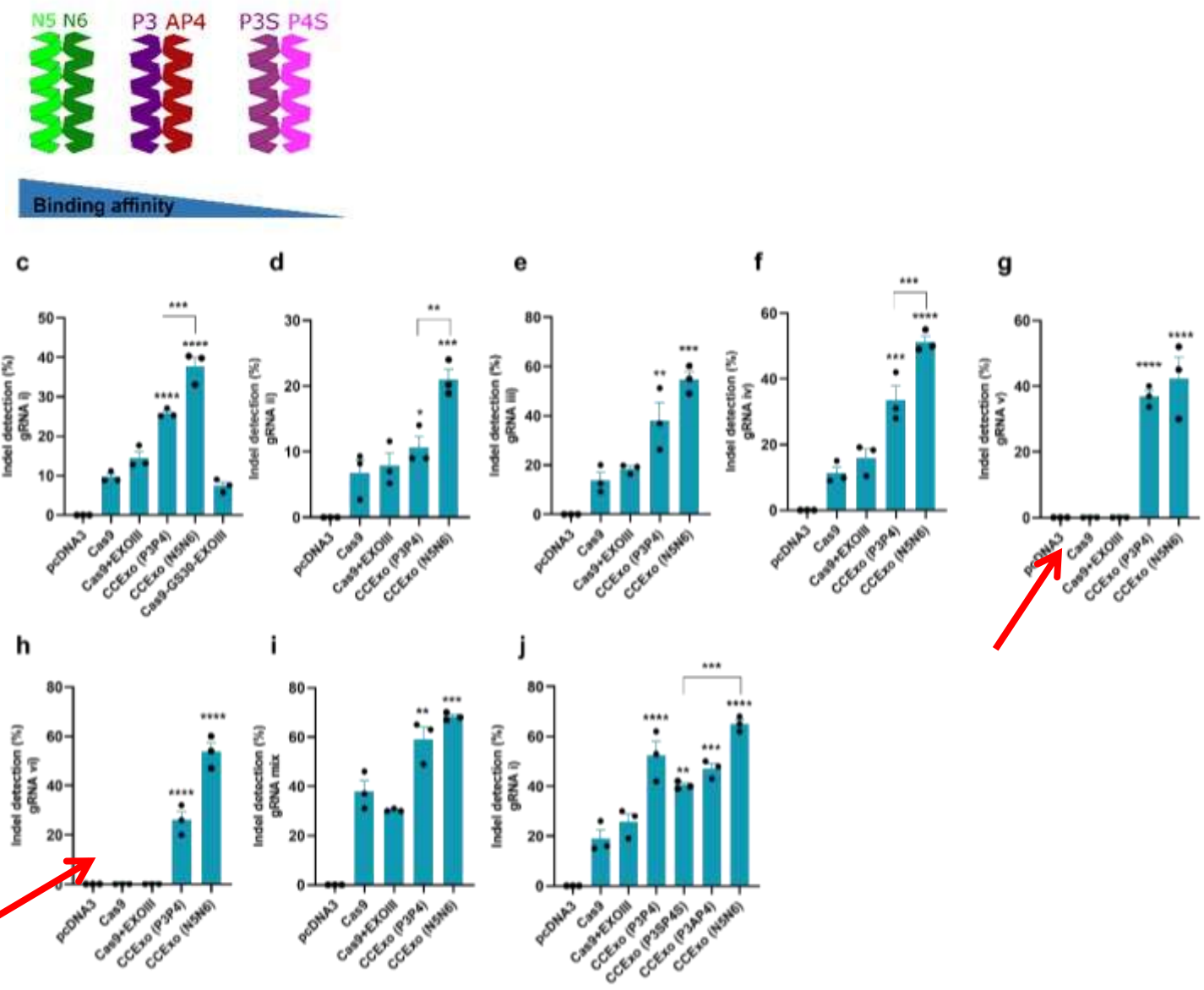
Exonuclease	GS 10AA linker	P3	P4	GS 10AA linker	Cas9 or its variants



Patent pending



Effect of coiled-coil peptide tether selection

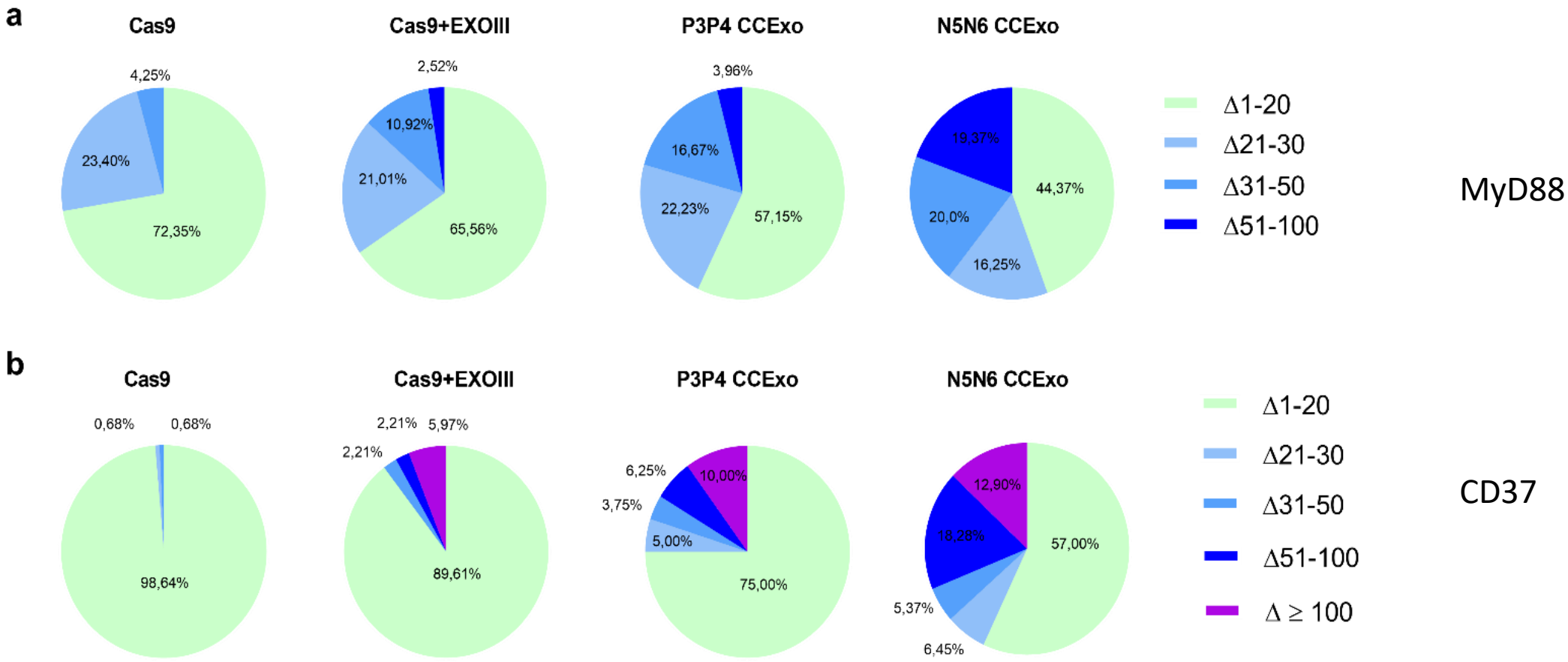


Designed coiled coil set with different orientations and tunable affinity

Increased indel efficiency

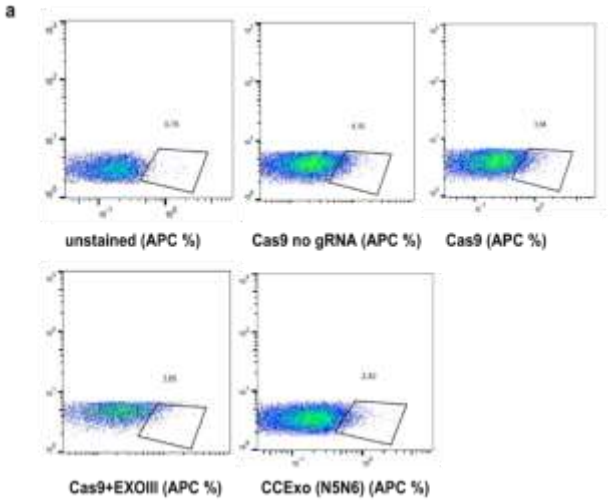
Indel formation in cases where Cas9/gRNA alone were not effective

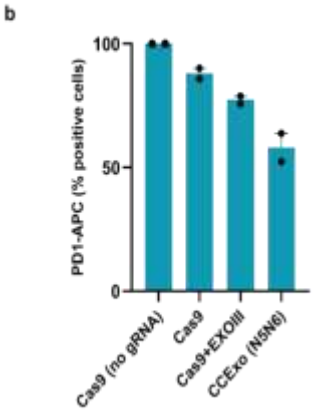
CCexo increases deletion length



Applications of CCExo

- Already tested in several primary human cells and cell lines
- More efficient generation of KO cell lines
- Generation of KO in plants
- Therapeutic inactivation of selected genes (e.g. PD-1, TRACA in CAR T cell therapy)
- Therapeutic killing of cancer cells with characteristic genomic translocations

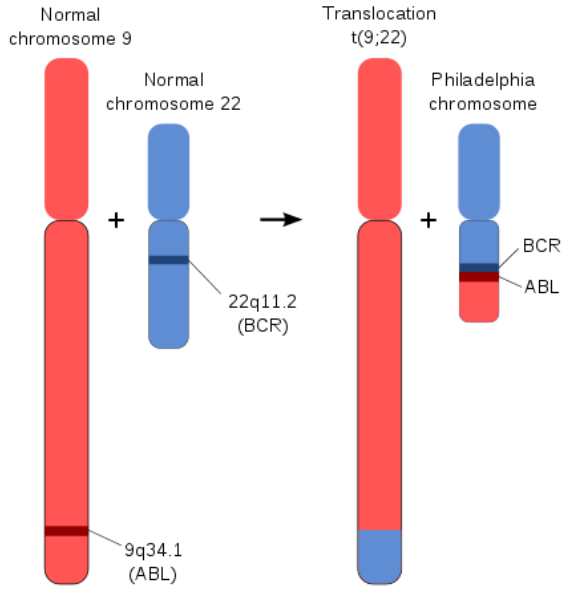


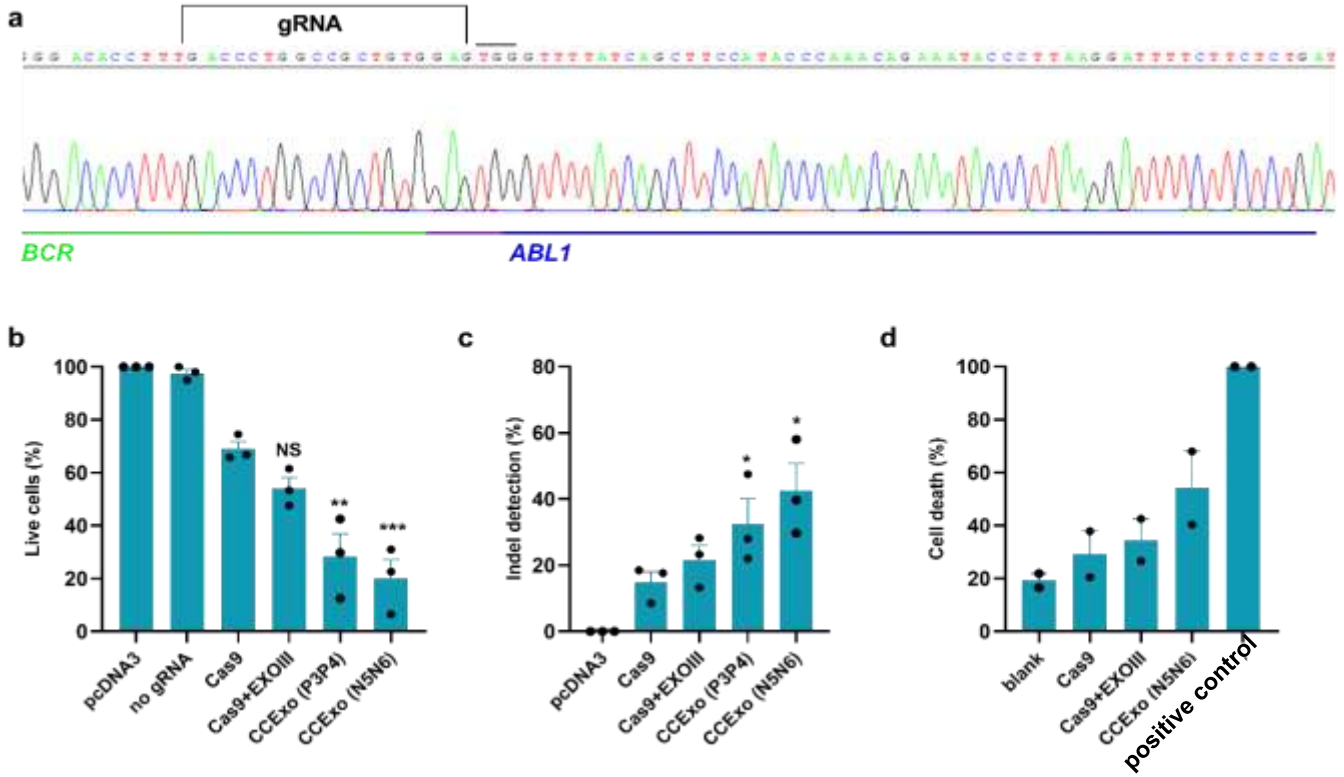


Potential of Cas-CC-EXO for anticancer therapy

Chronic myeloid leukemia (CML) is characterized by **the genetic translocation** between the t(9;22)(q34;q11.2), resulting in the **formation of Philadelphia fusion chromosome, coding for BCR-ABL1 oncoprotein**.

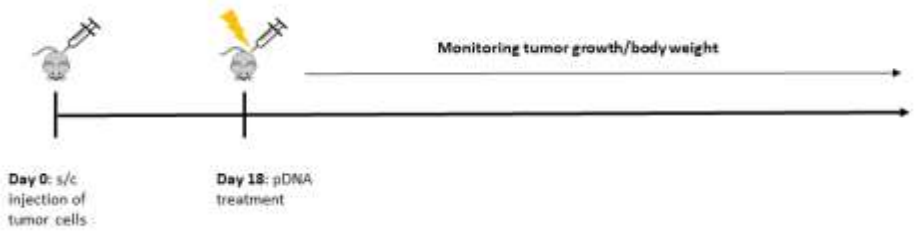
BCR-ABL1 editing in human CML cells via CCExo

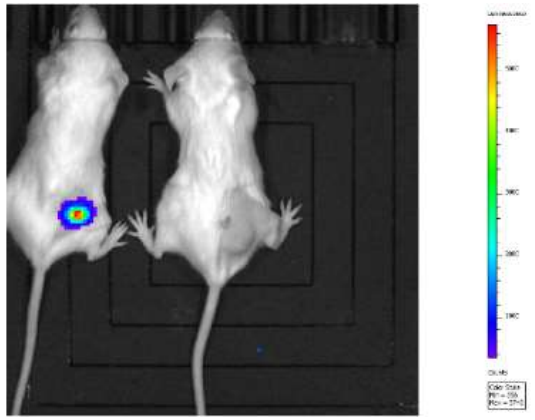


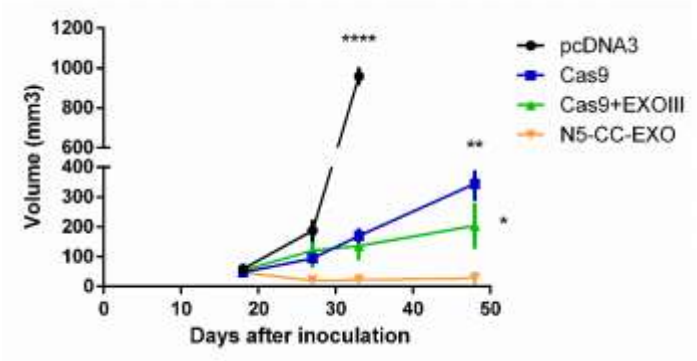


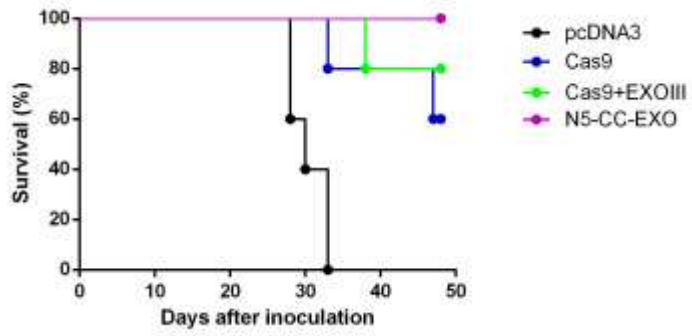
In vivo demonstration of Cas-CC-EXO for anticancer therapy

Targeting BCR-ABL translocation, animals with tumors showed improved survival and drastic reduction in tumor size.









CCExo technology

- CCexo technology increases the efficiency of genomic deletions
- The mechanism is most likely due to the dynamic recruitment and release of the exonuclease to the cleavage site
- Potential therapeutic applications for elimination of proviral insertions inactivation of cells with characteristic (cancer) translocations (adapted to each patient based on the sequence)

Current status

- Testing different delivery methods
- Demonstration on plants
- Implementation for gene KO as cancer immunotherapy supporting technology
- Formation of a startup, discussions with potential industrial partners

https://ccedit.si/

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