Second International Summit on Human Genome Editing

November 27, 2018 – Hong Kong

Exploiting (Epi)-Genome Editing for Therapeutic Applications

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ISTITUTO SAN RAFFAELE TELETHON PER LA TERAPIA GENICA

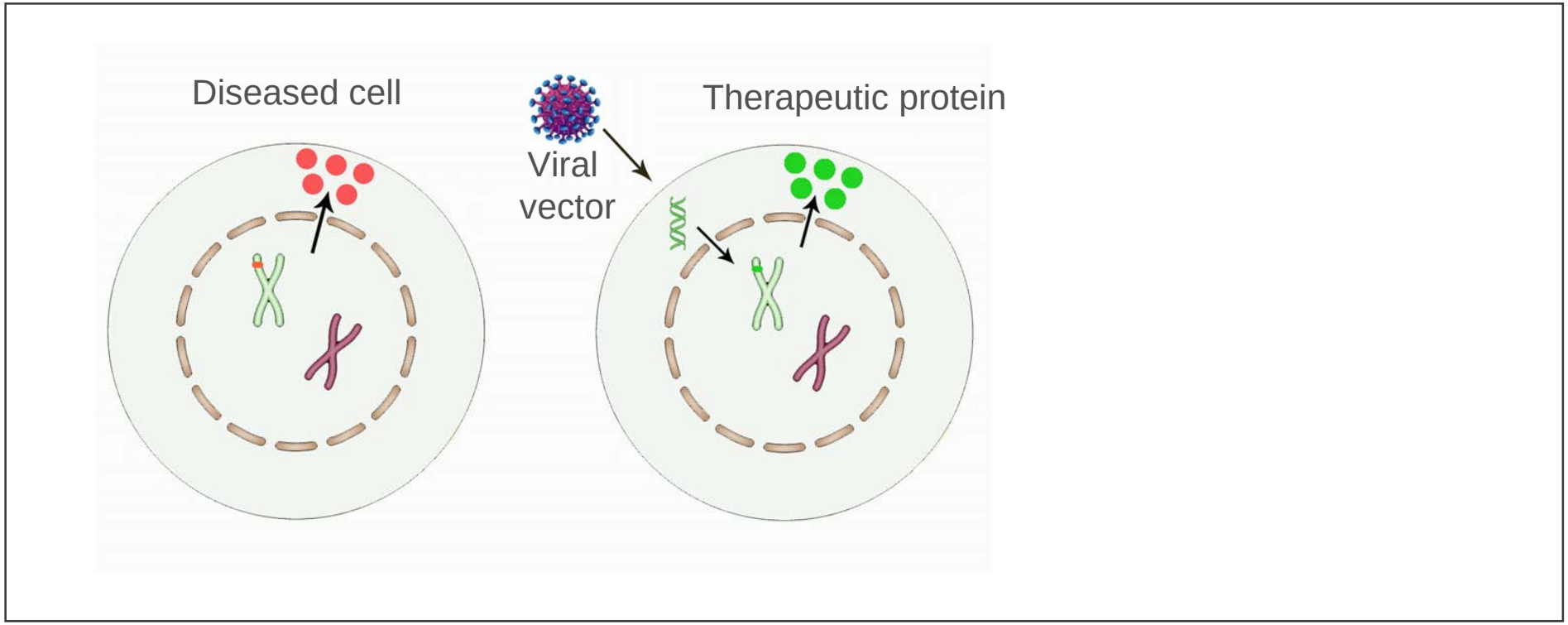
SR-Tiget

UNIVERSITÀ VITA-SALUTE SAN RAFFAELE UniSR QUID EST HOMO?

OSPEDALE SAN RAFFAELE

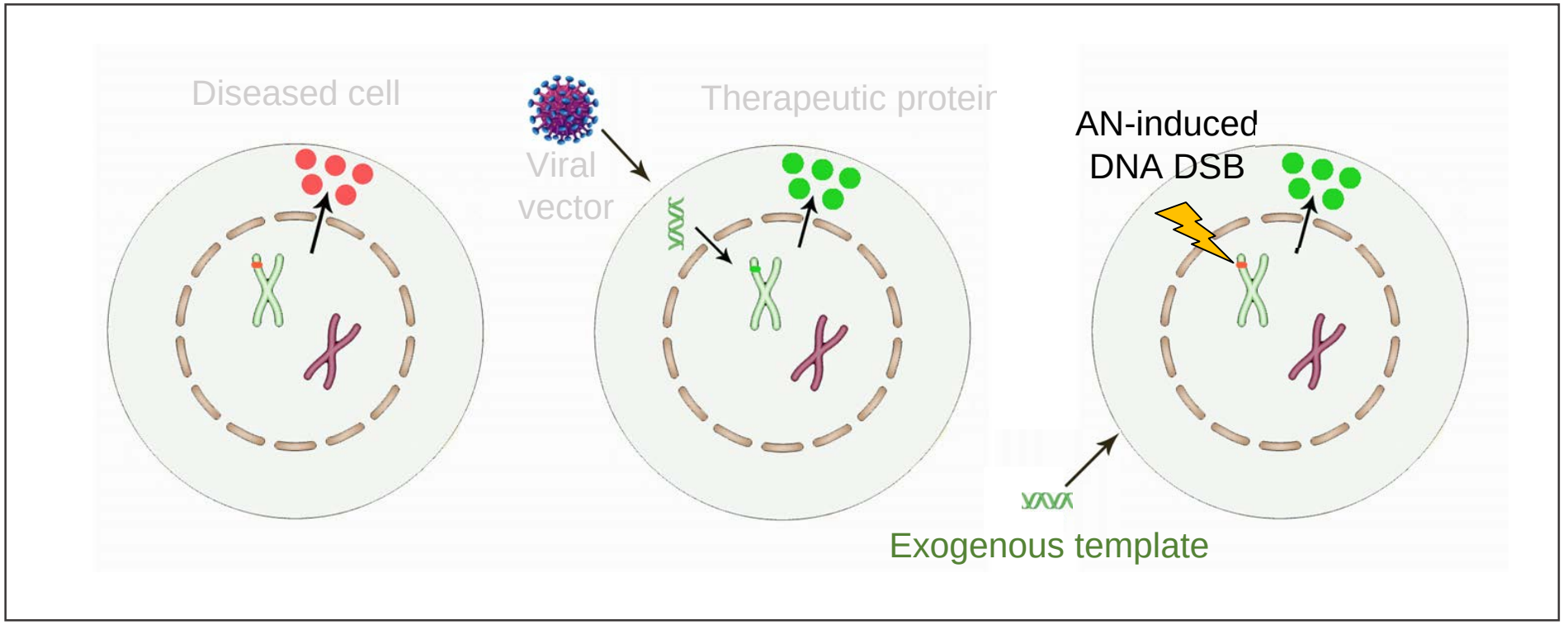
FONDAZIONE Telethon

Gene Transfer vs. Targeted Genome Editing



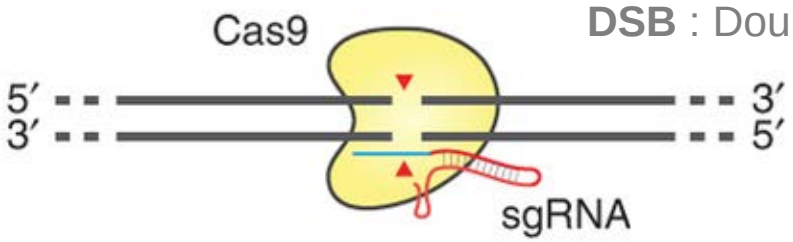
- *Gene replacement by viral-derived vectors*
  - Permanent (RV/LV) or long-term stable (AAV) modification of the cell
    - Effective & safe in several disease conditions
      - Risks of genotoxicity, unregulated transgene expression, vector immunogenicity

Gene Transfer vs. Targeted Genome Editing



- *Gene replacement by viral-derived vectors*
- *Targeted gene correction with Artificial Nucleases (AN)*

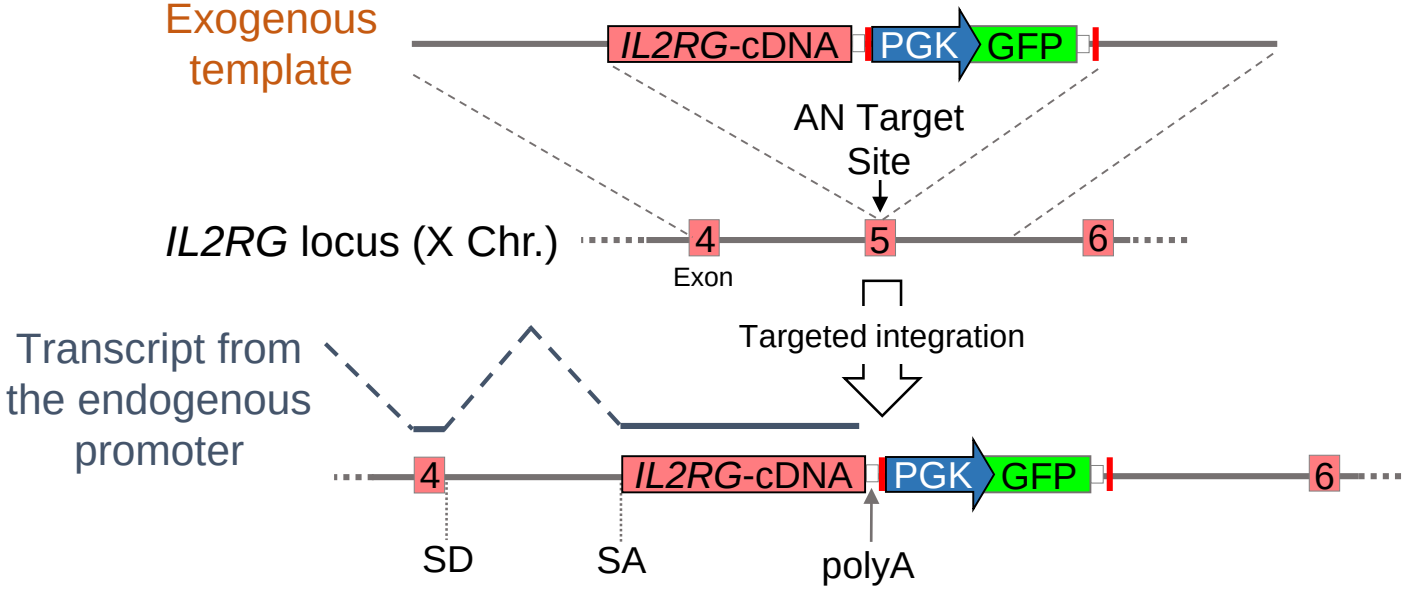
Targeted Genome Editing

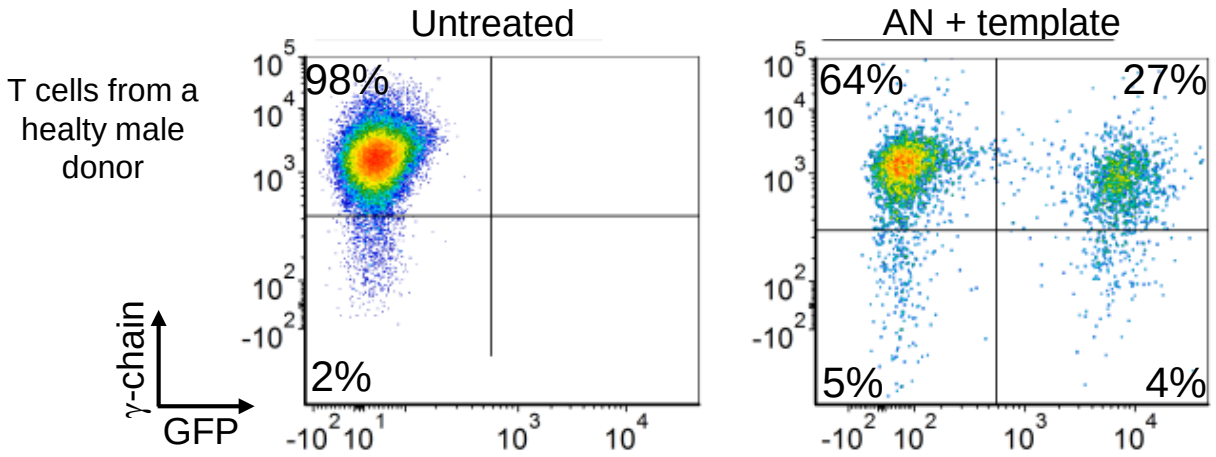


DSB : Double Strand Break

Adapted from Ann Ran et al., Nat Prot 2013

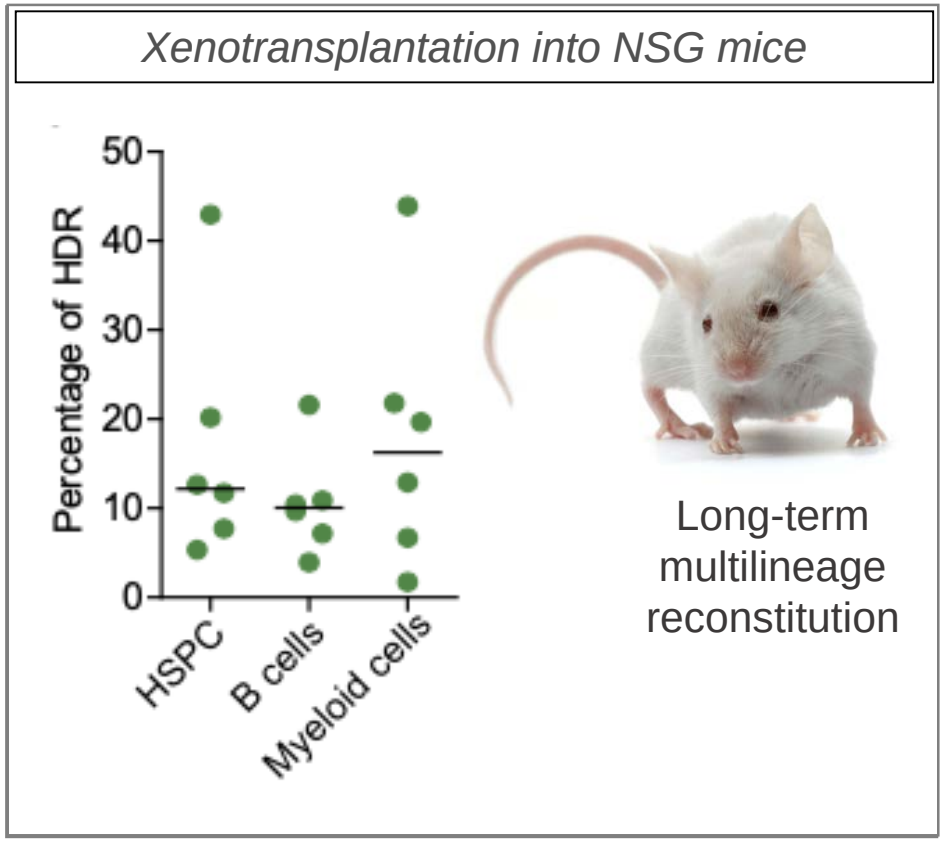
Gene Editing: "One Strategy to Fix Them All"





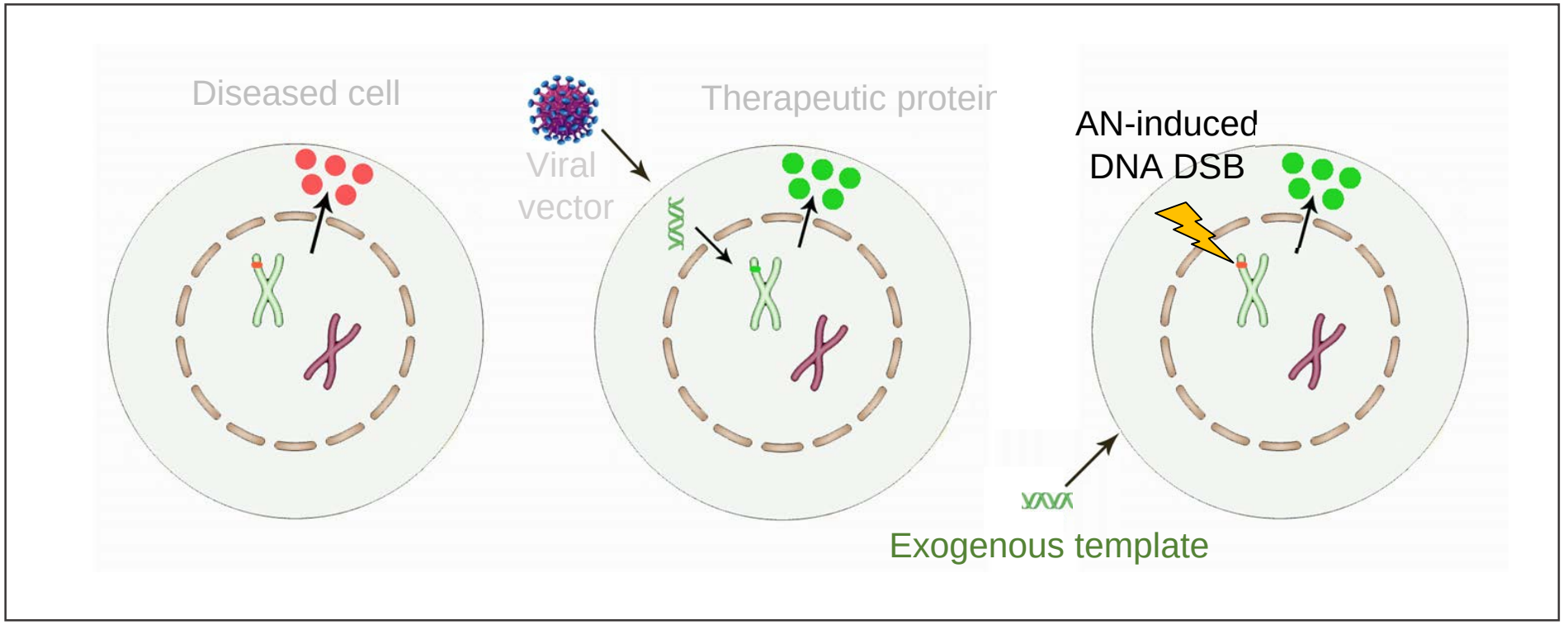
Lombardo *et al.*, Nat Biotech 2007; Lombardo *et al.*, Nat Meth 2011

CD34+ cells from Bone Marrow or Cord Blood (also from of a SCID-X1 patients)



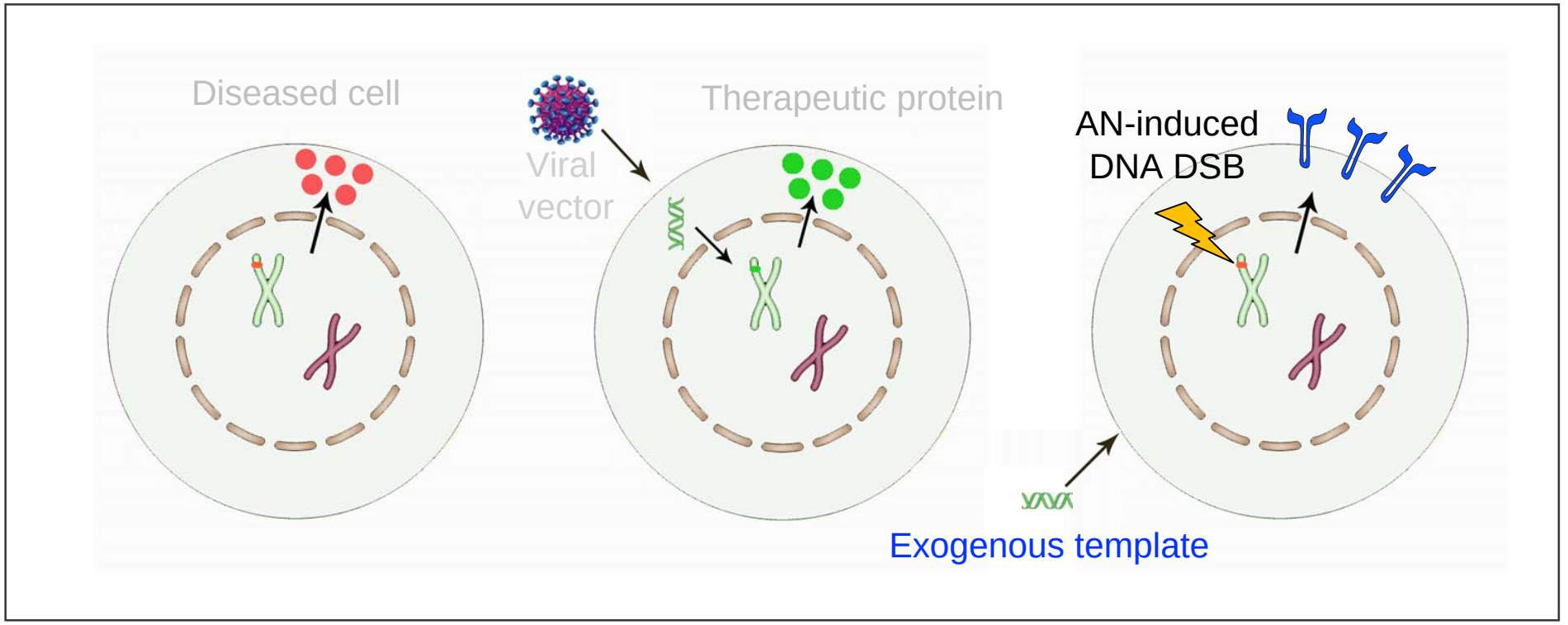
Genovese *et al.*, Nature 2014
Schiroli *et al.*, Sci Transl Med 2017

Gene Transfer vs. Targeted Genome Editing



- *Gene replacement by viral-derived vectors*
- *Targeted gene correction with Artificial Nucleases (AN)*
  - Reconstitute the gene & its physiological expression levels
  - Overcome the risk of random vector insertion
    - Improve efficiency / yield of edited cells & characterize specificity/immunogenicity

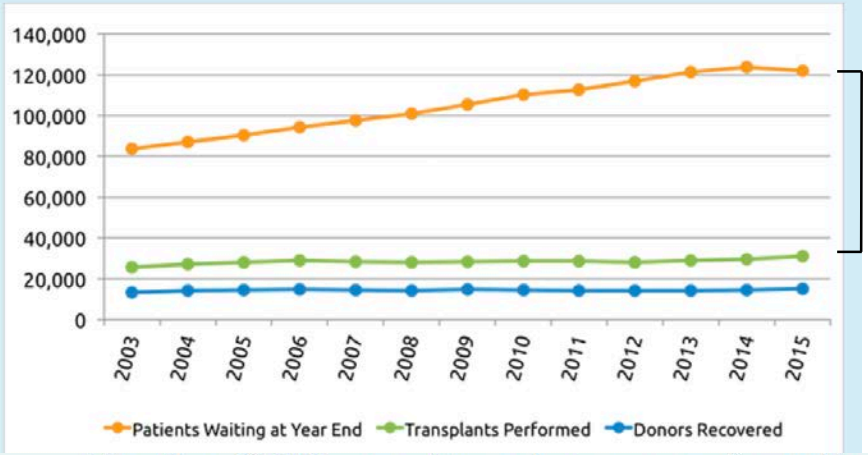
Expanding the Breadth of Targeted Genome Editing



- *Gene replacement by viral-derived vectors*
- *Targeted gene correction with Artificial Nucleases (AN)*
  - Reconstitute the gene & its physiological expression levels
  - Overcome the risk of random vector insertion
- *Exploit gene editing to confer novel biological properties to the cells*

Tissue & Organ Transplantation

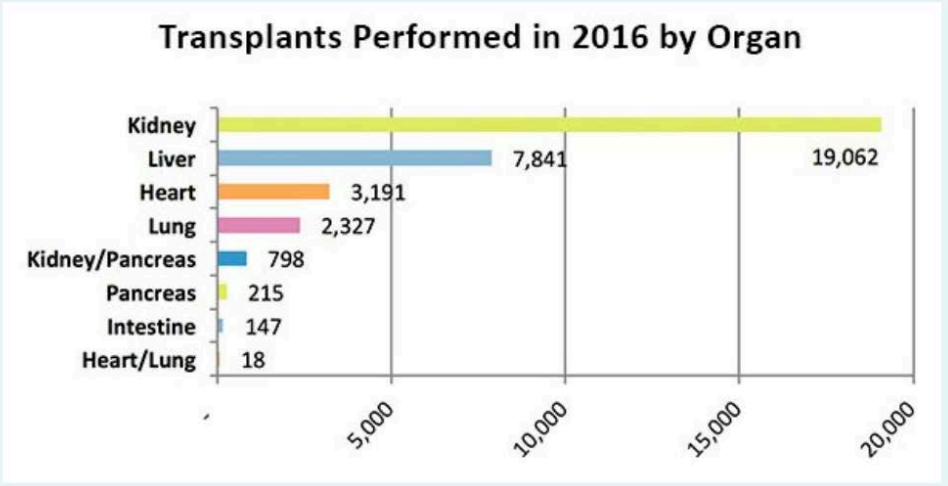
The organ shortage continues



Paucity of donors (HLA-matching)

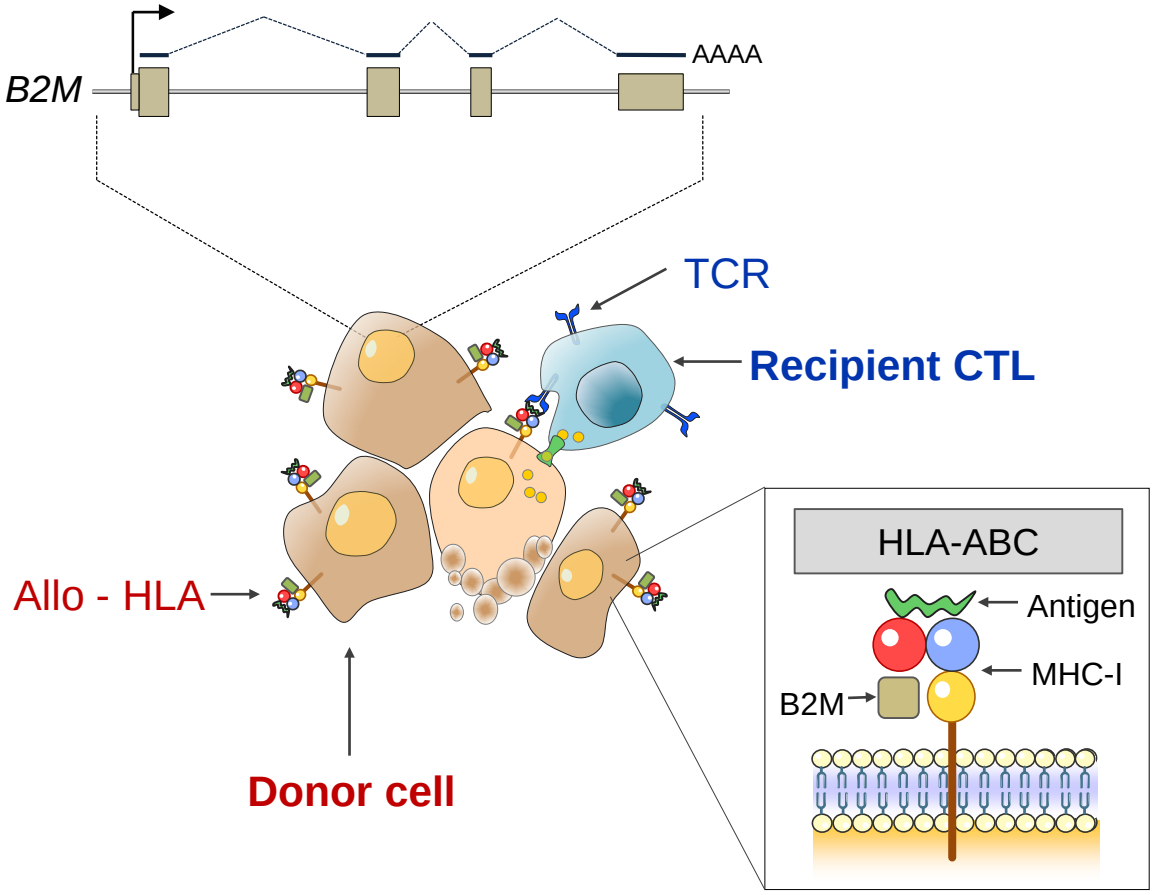
- More than 82,000 corneal transplants were performed in 2016.
- More than 1 million tissue transplants are performed each year.

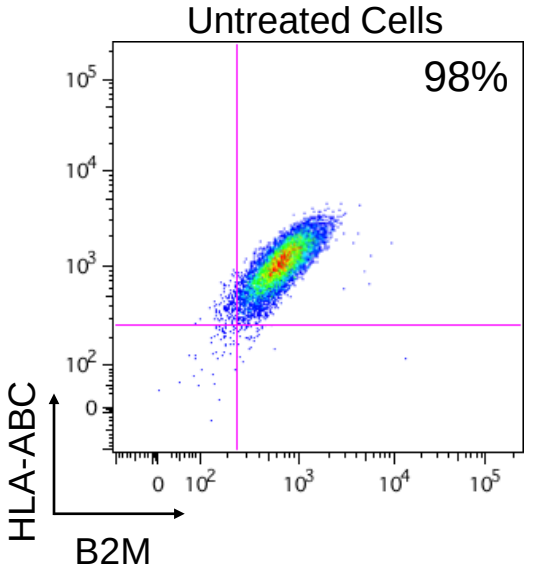
World Health Organization



Generation of Universal Donor Cells

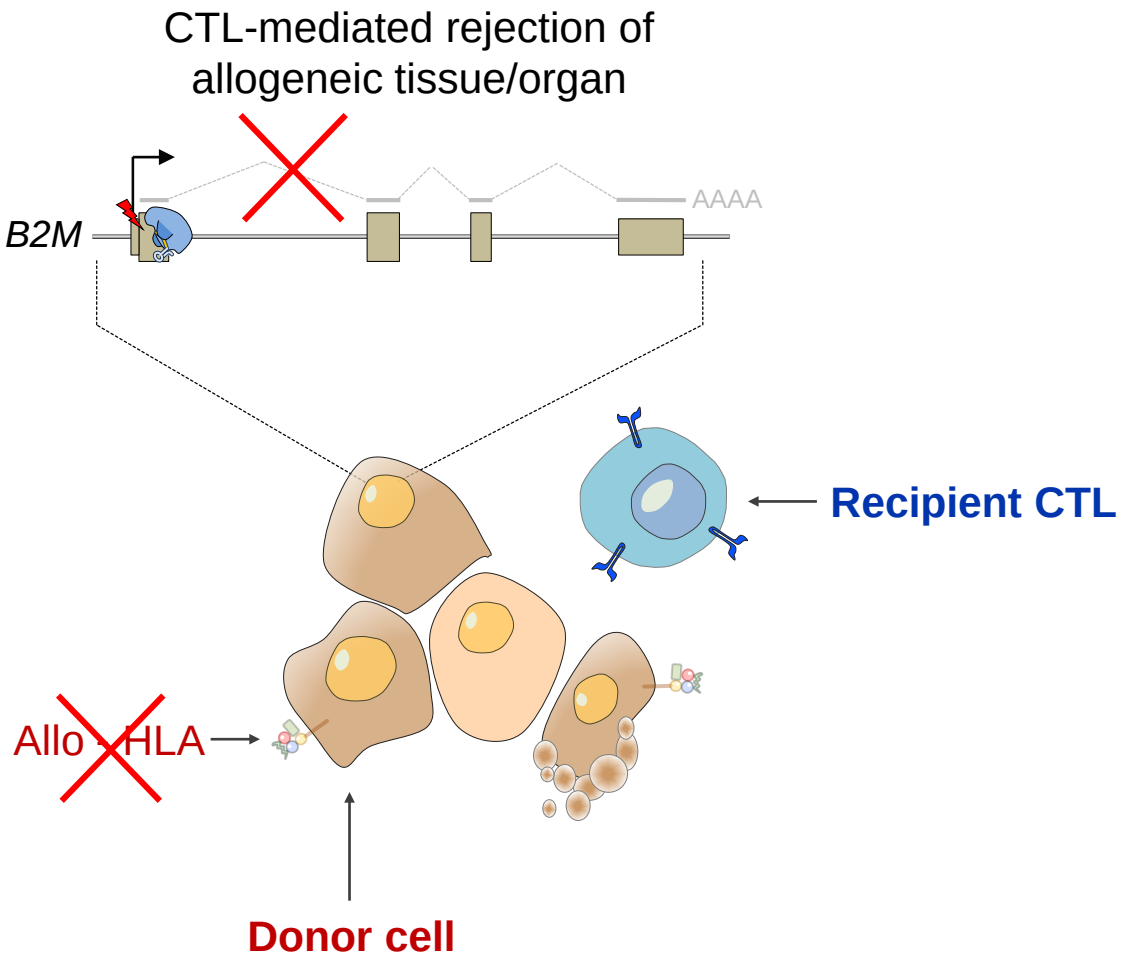
CTL-mediated rejection of allogeneic tissue/organ

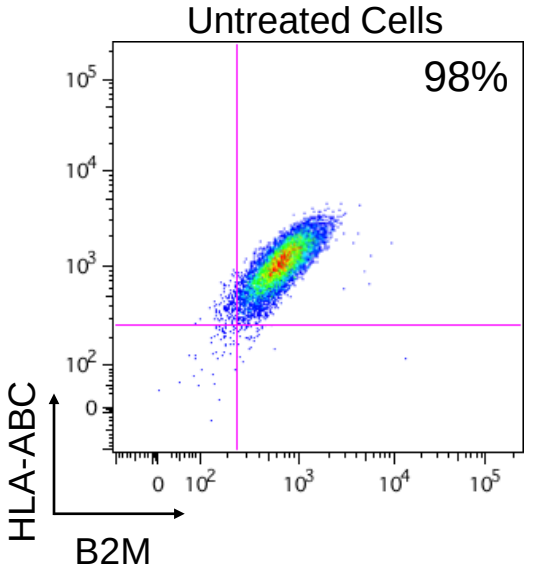


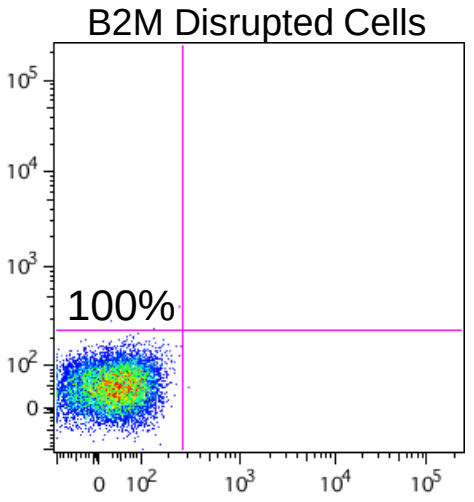


Clinical-grade iPSC cell line

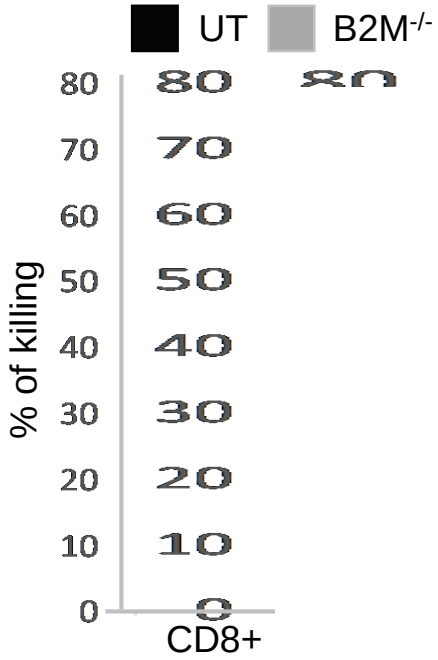
Generation of Universal Donor Cells





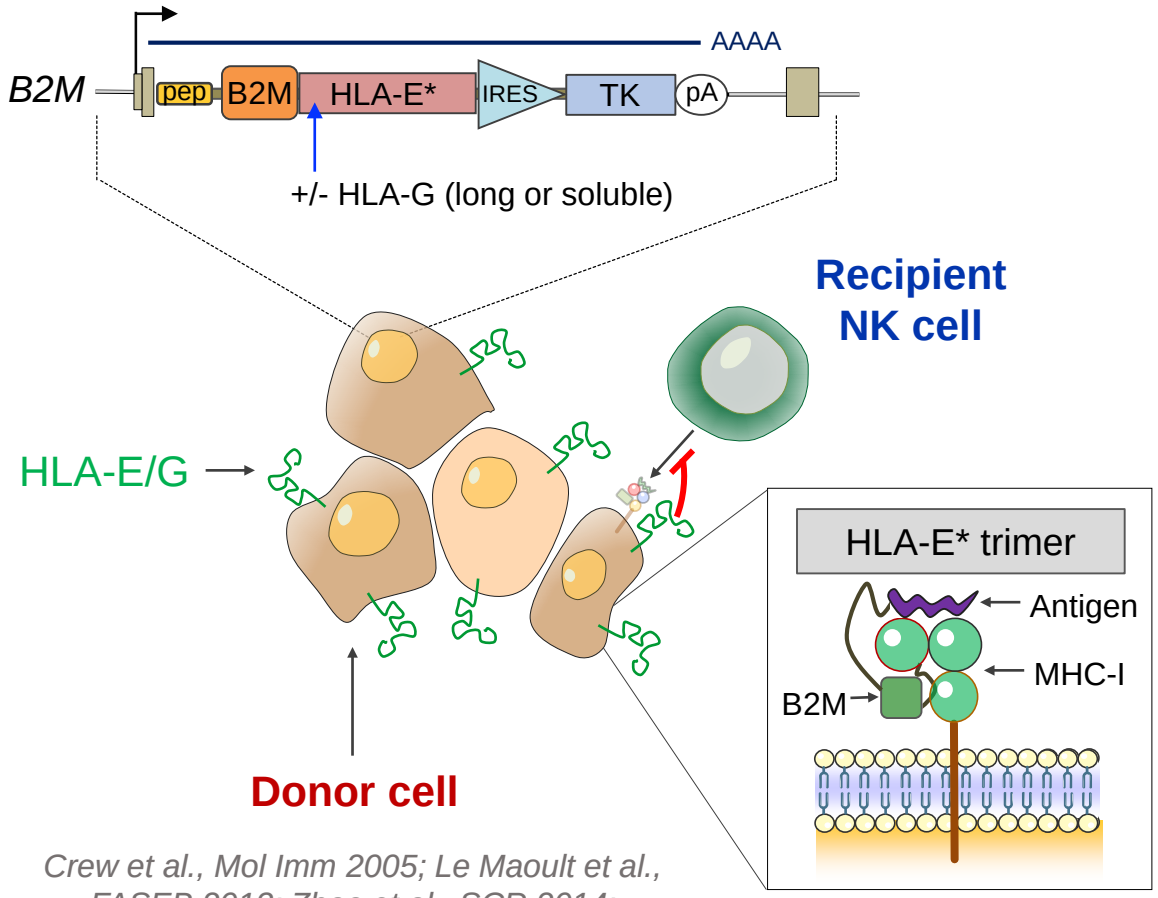


Clinical-grade iPSC cell line

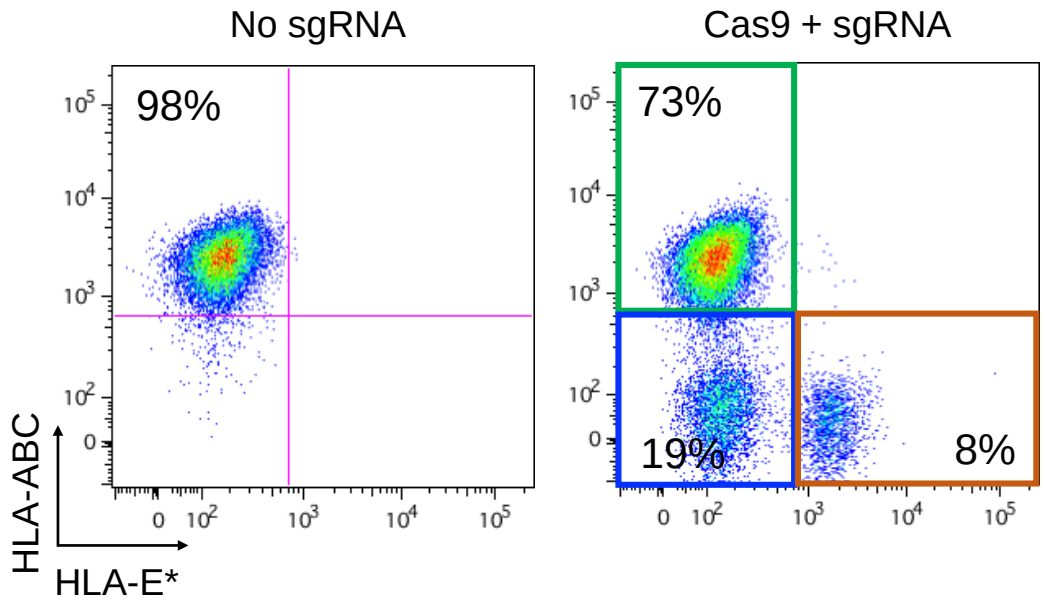


Generation of Universal Donor Cells

B2M–mediated expression of
a single chain HLA-E trimer linked to TK



Crew et al., Mol Imm 2005; Le Maoult et al., FASEB 2013; Zhao et al., SCR 2014; Gornalusse et al., Nat Biot 2017

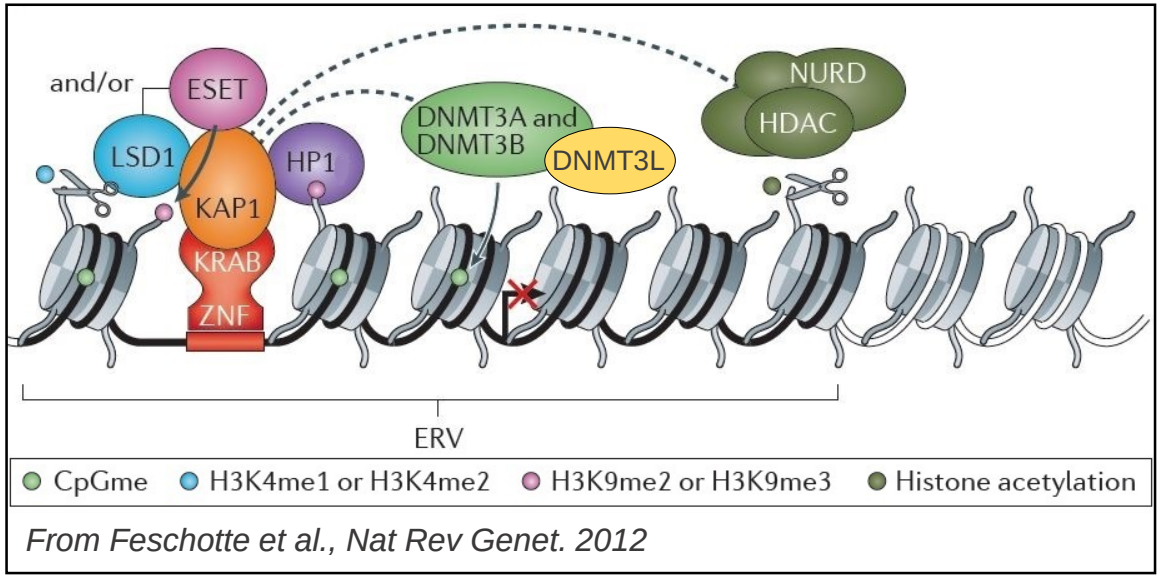


Therapeutic Gene Silencing

- The scope of gene therapy goes beyond gene replacement → gene silencing
  - *Diseases caused by dominant-negative/gain-of-function mutations & repeat expansion disorders:*
    - *Hypercholesterolemia*
    - *Spinocerebellar ataxia type 1 (SCA1) …*
  - *Infectious diseases:*
    - *HBV*
    - *HIV*
  - *Others:*
    - *Hemoglobinopathies*
- To silence gene expression:
  1. *RNA interference (si/shRNA)*
     - *Stable expression/delivery of the RNAi molecules*
  2. *Gene inactivation (Artificial Nucleases / Base Editors)*
     - *For AN, induction of the DNA Damage Response (DDR)*
  3. *Targeted epigenetic silencing*

Epi-editing: Background

Permanent epigenetic silencing of Endogenous Retroviruses (ERV)

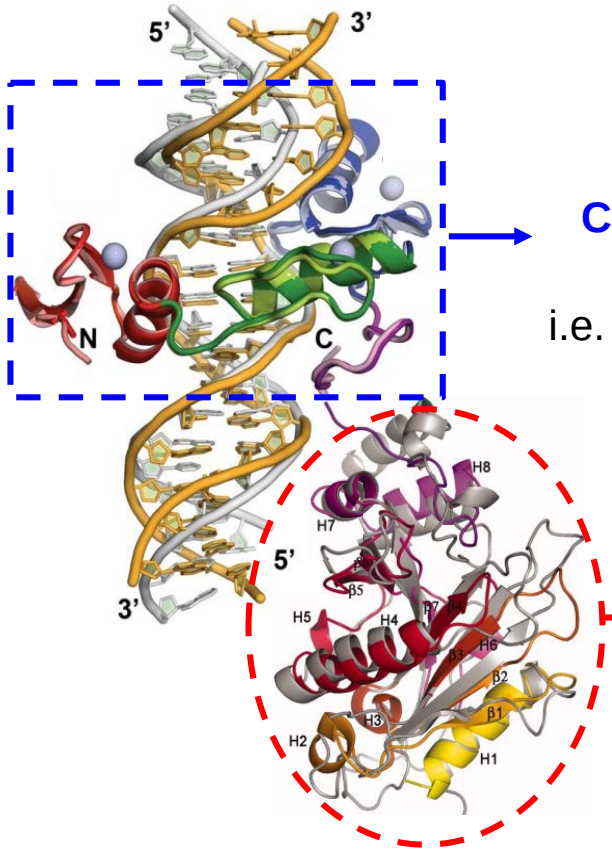


From Feschotte et al., Nat Rev Genet. 2012

1. *Targeting by KRAB-ZFPs* → *Initiator*
2. *Recruitment of an epigenetic repressive complex*
3. *De novo DNA methylation (DNMT3A)* → *Final locker*

➢ Selected domains: **KRAB**, **DNMT3A** & **DNMT3L**

Engineered Transcriptional Repressors



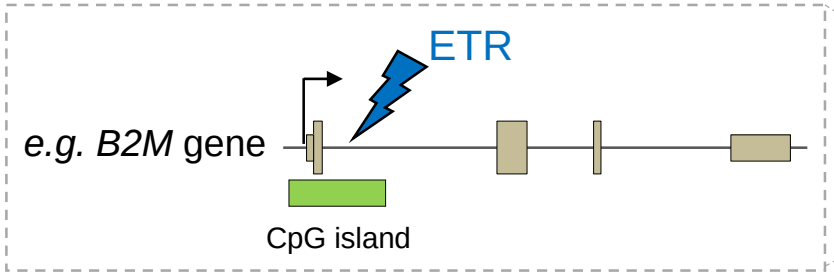
Custom-made DNA Binding Domain

i.e. ZFP / TALE / dCas9

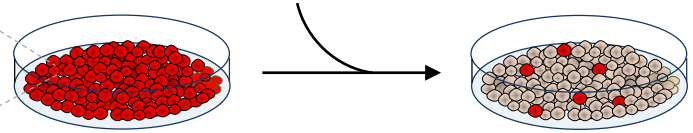
Effector Domain

from a naturally occurring transcriptional repressor

Epi-editing: Efficiency & Mode of Action

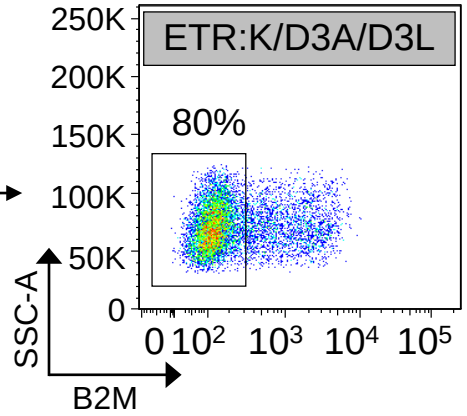


Transient expression of the ETRs

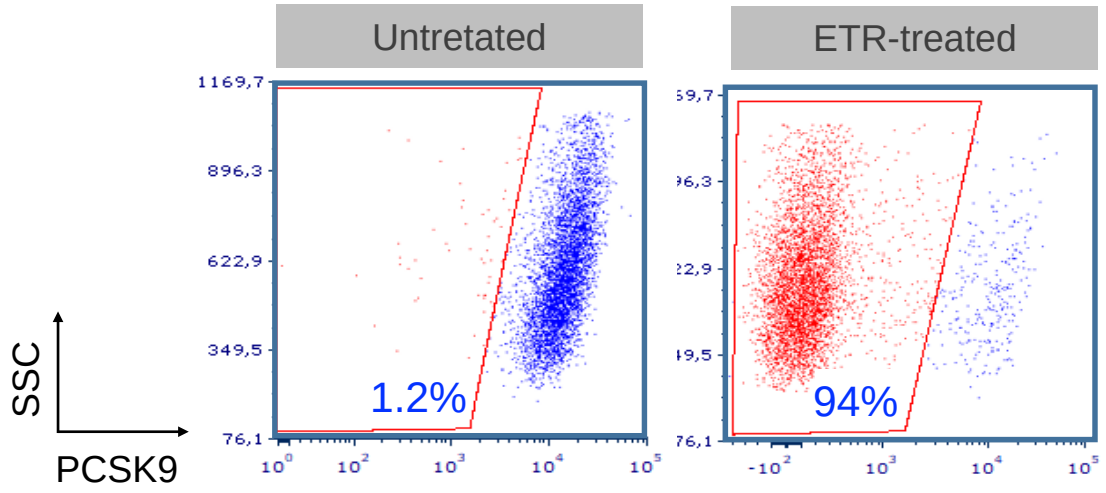


Human or mouse cells

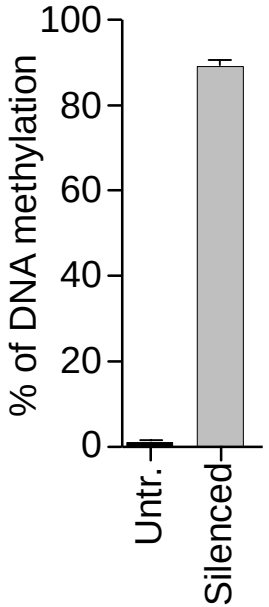
Flow cytometry



- *PCSK9* gene
  - GOF mutations cause Familial Hypercholesterolemia
  - LOF mutations protect from Coronary Heart Diseases

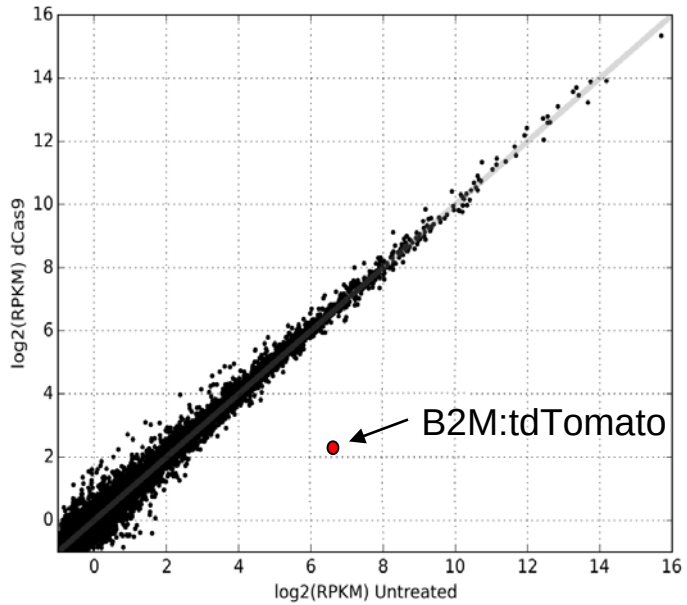


DNA methylation

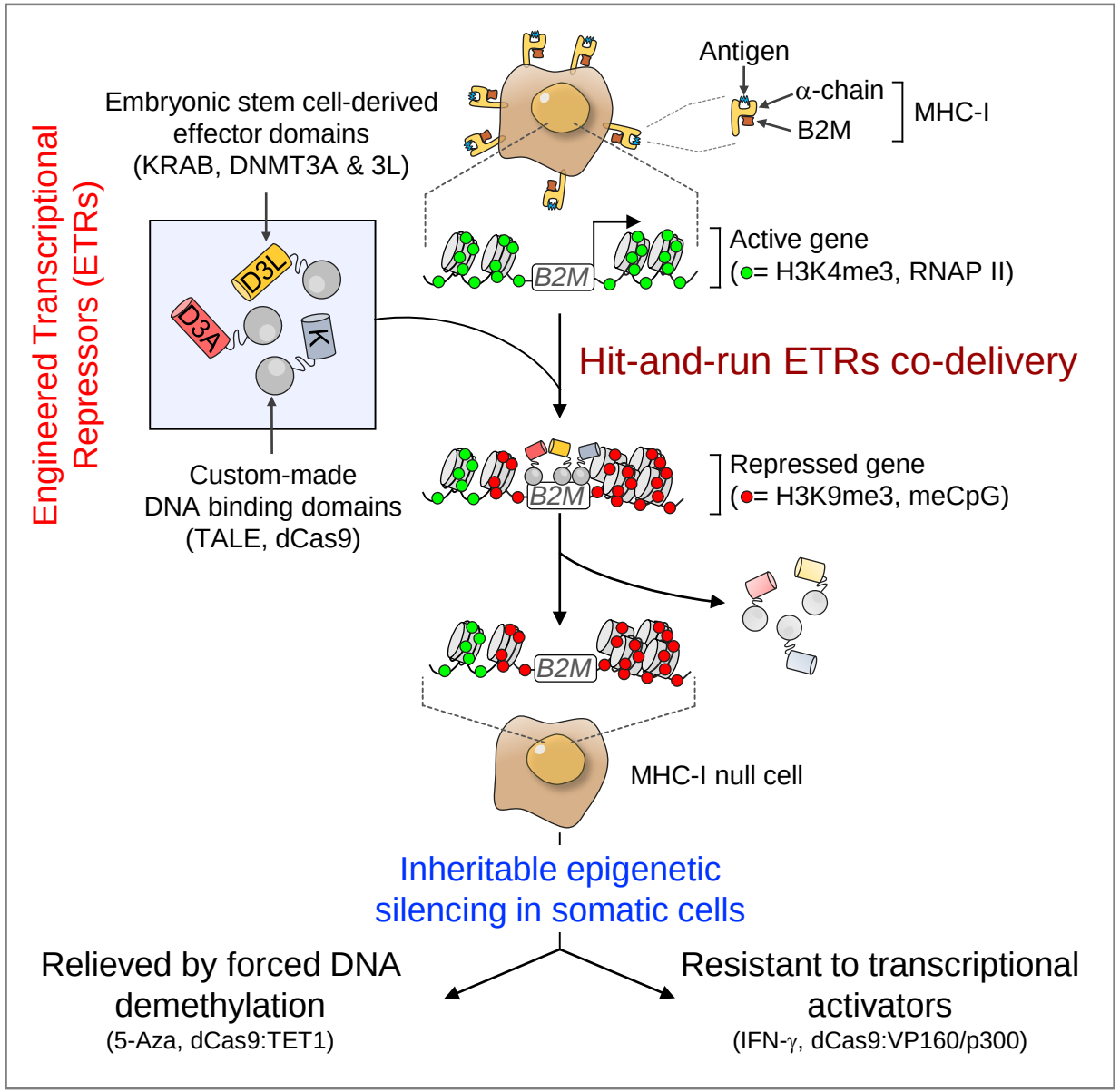


Specificity

Mock-treated vs dCas9



Targeted Epigenetic Silencing: Summary



- Repurposed the ERV's silencing machinery
  - Programmable & targeted (ETRs)
  - Combinatorial (embryonic-restricted *complex*)
    - Inheritable by DNA methylation
  - Transient ETR delivery (hit-and-run)
  - Amenable to multiplexing
    - ➢ Effective against multi-copy genes
  - Resistant to external stimuli
  - ❖ No activation of the DDR
  - ❖ Potentially reversible by pharma. treatments

Amabile et al., Cell 2016

Acknowledgments

- L. Piemonti
- V. Sordi
- R. Chimenti
- S. Pellegrini
- P. Monti

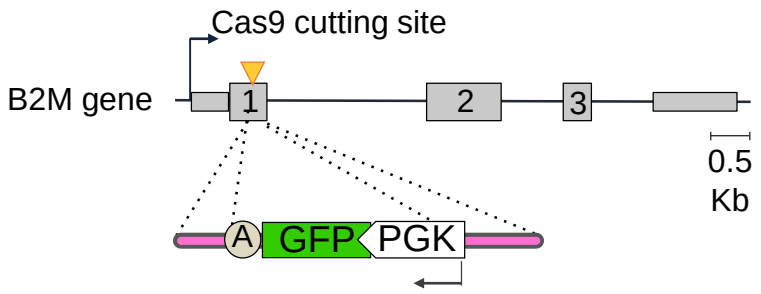
- L. Naldini
- P. Genovese

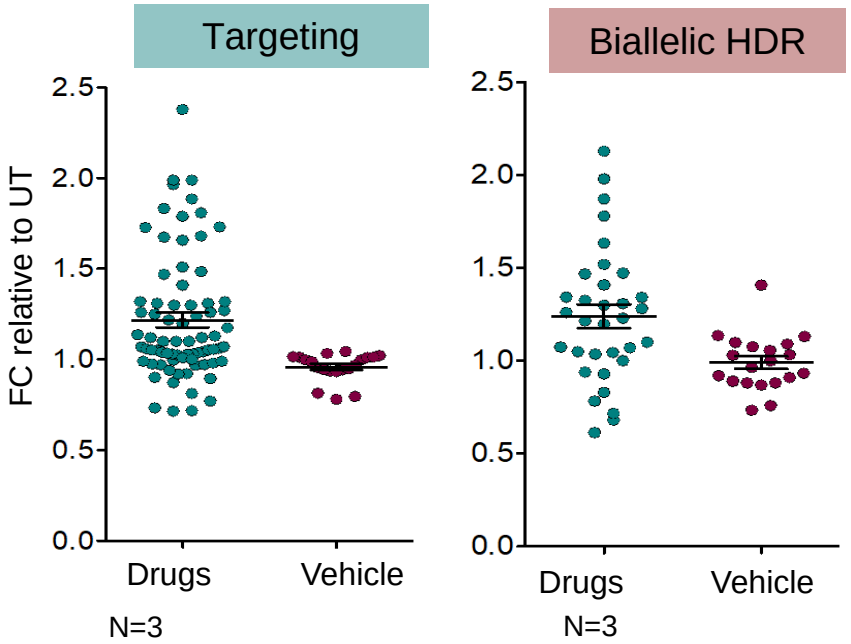
- E. Montini
- A. Calabria
- G. Spinozzi
- F. Benedicenti

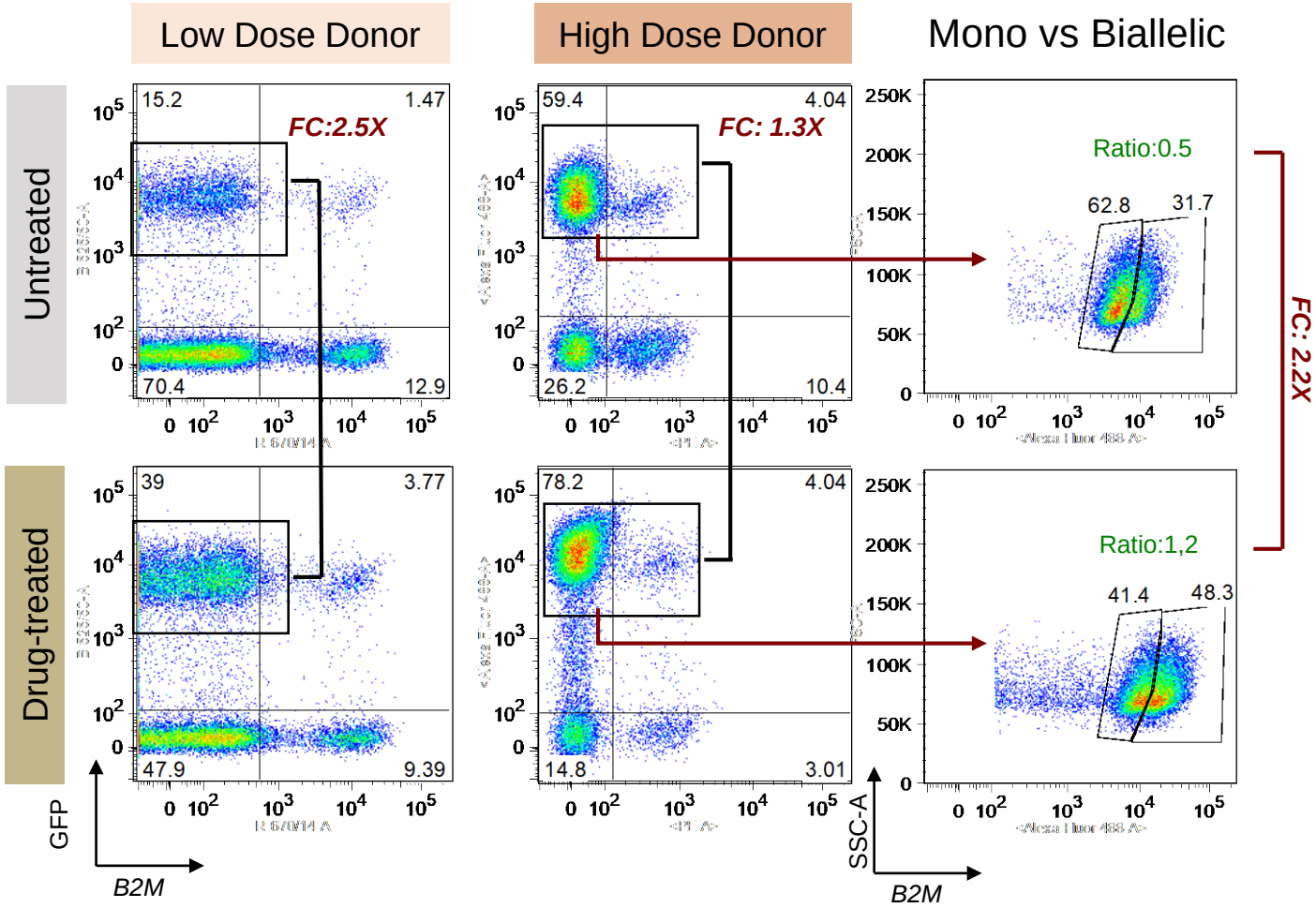
Alessandro Migliara

Enhancing Gene Editing in Human T cells

- Screening performed in an *ad hoc* developed HDR/NHEJ reporter cell line
- ~ 30 also active on T cells

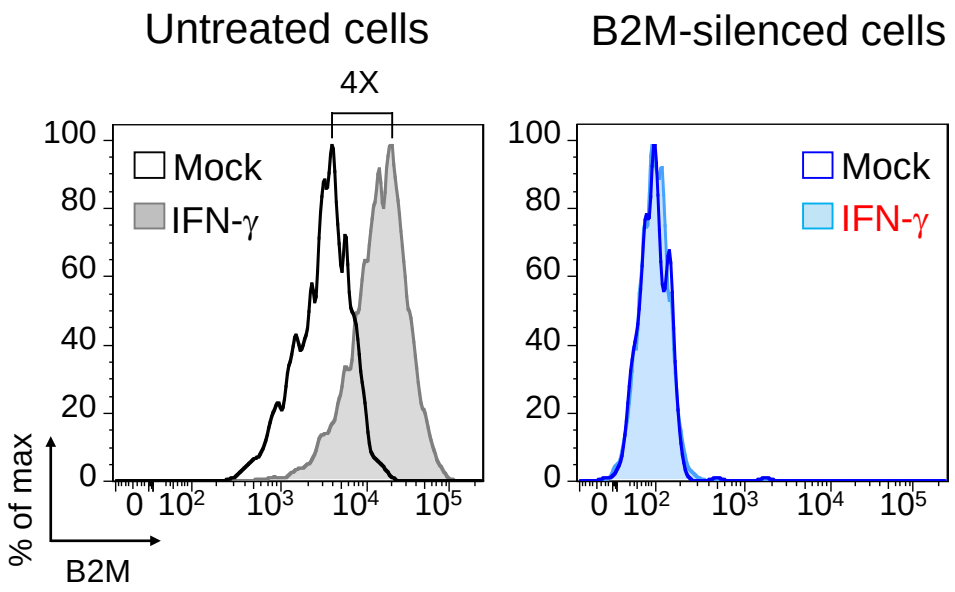


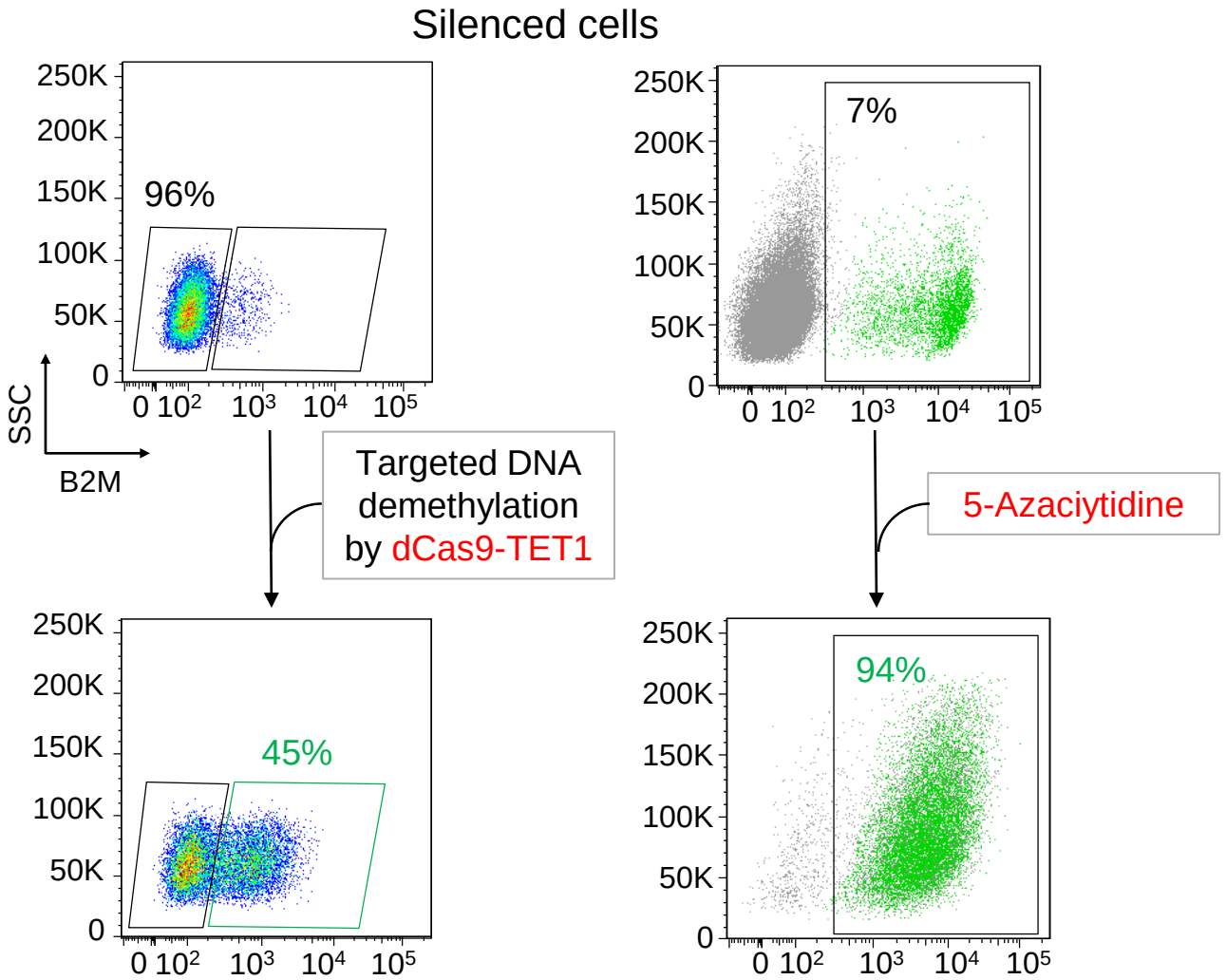




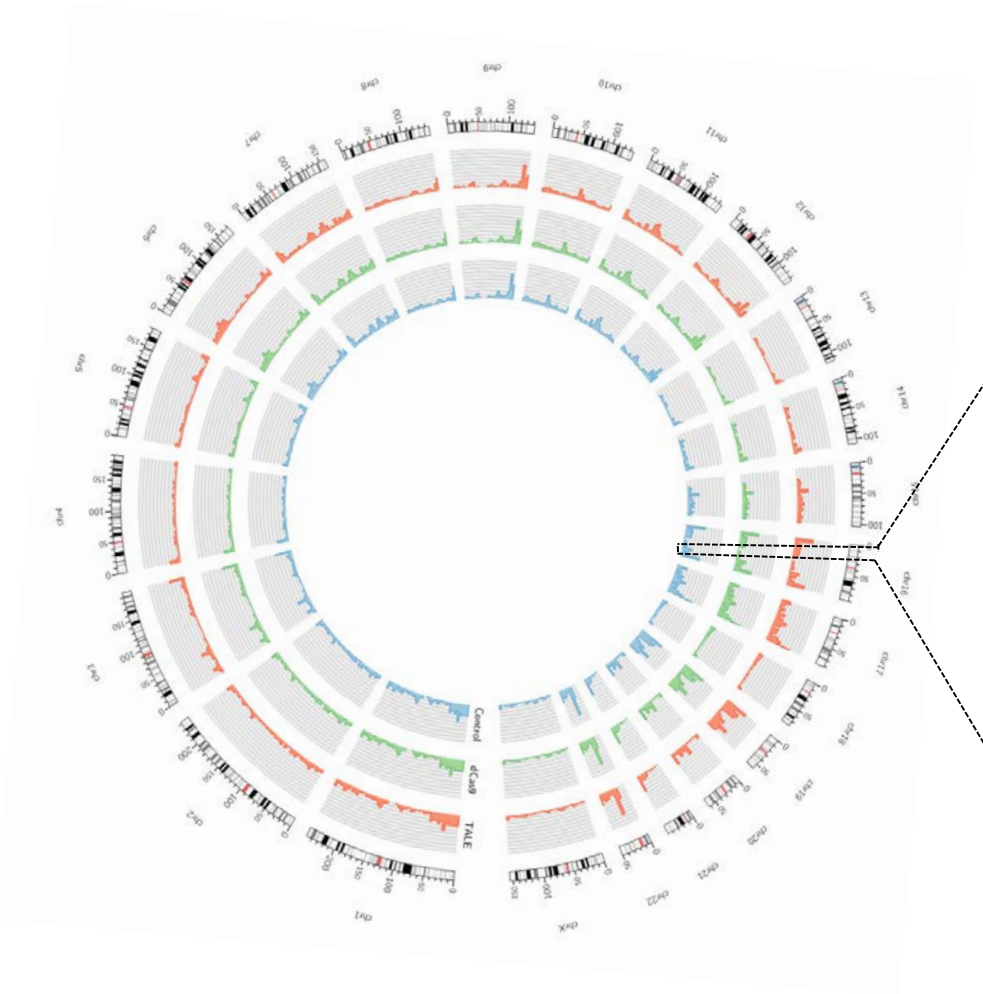
Epi-editing: Resistant to External Stimuli & Reversible

Flow cytometry analysis of cell treated as indicated

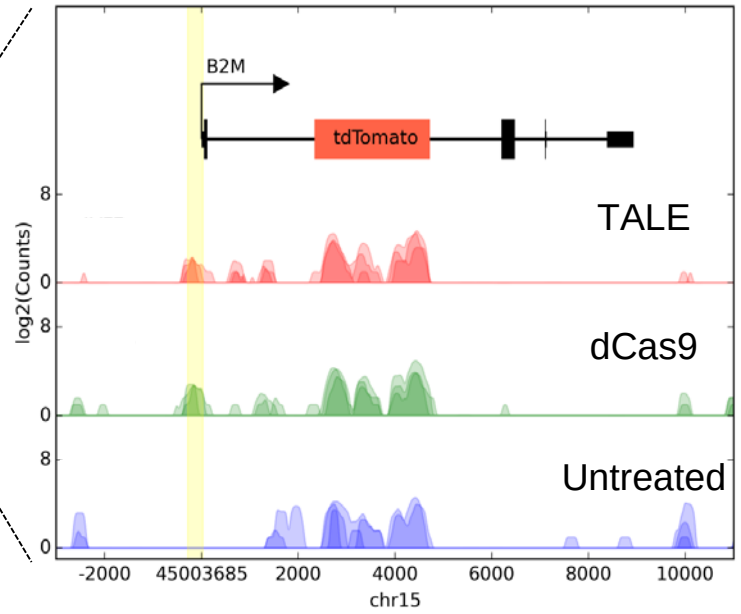




Epi-editing: Specificity

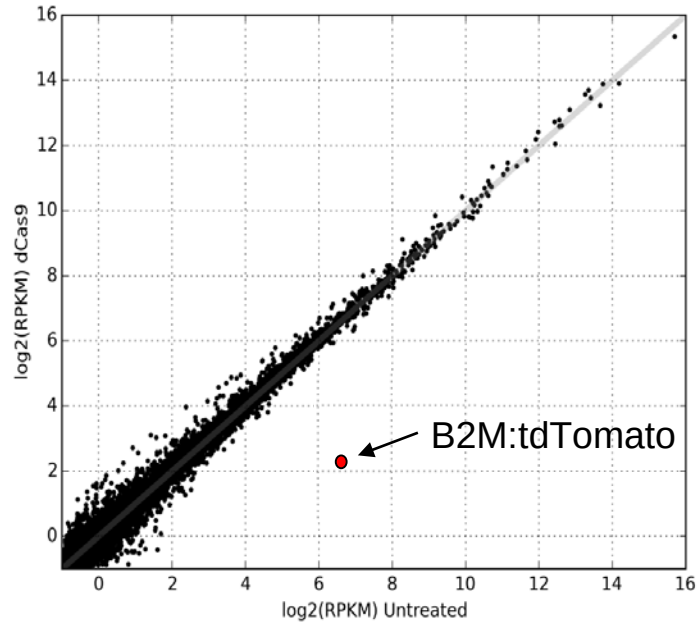


Genome-wide DNA methylation analysis

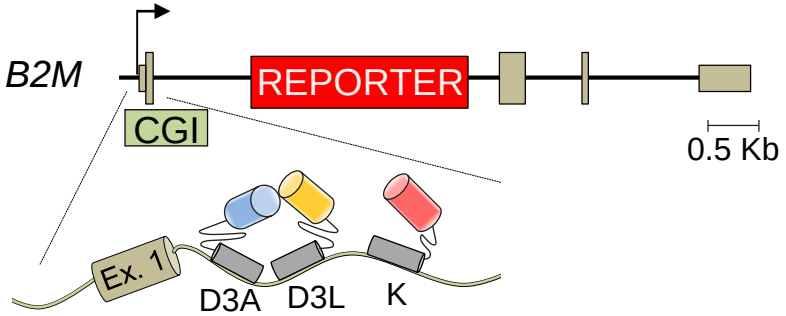


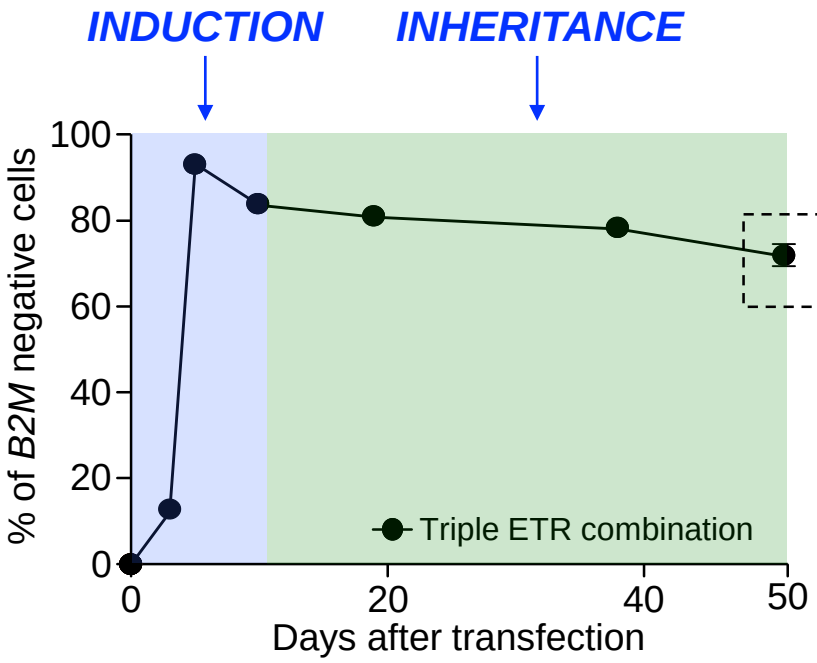
Transcriptomic analysis

Mock-treated vs dCas9

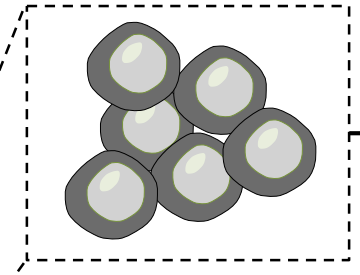


Experimental Layout: Inheritance

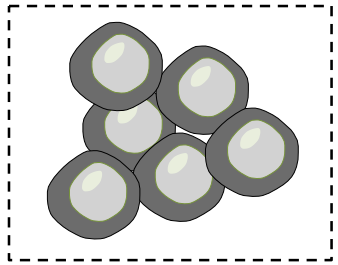


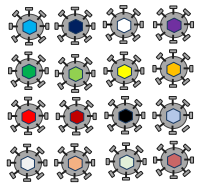


B2M silenced cells

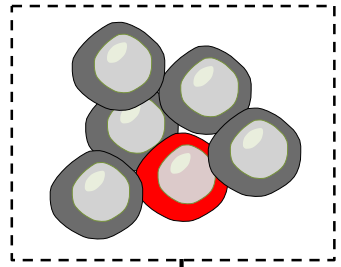


Clonal derivate with a drug-inducible Cas9





Genome-scale or focused CRISPR LV libraries



tdTomato enrichment

NGS analysis

Identification of a gene(s) crucial for silencing maintenance