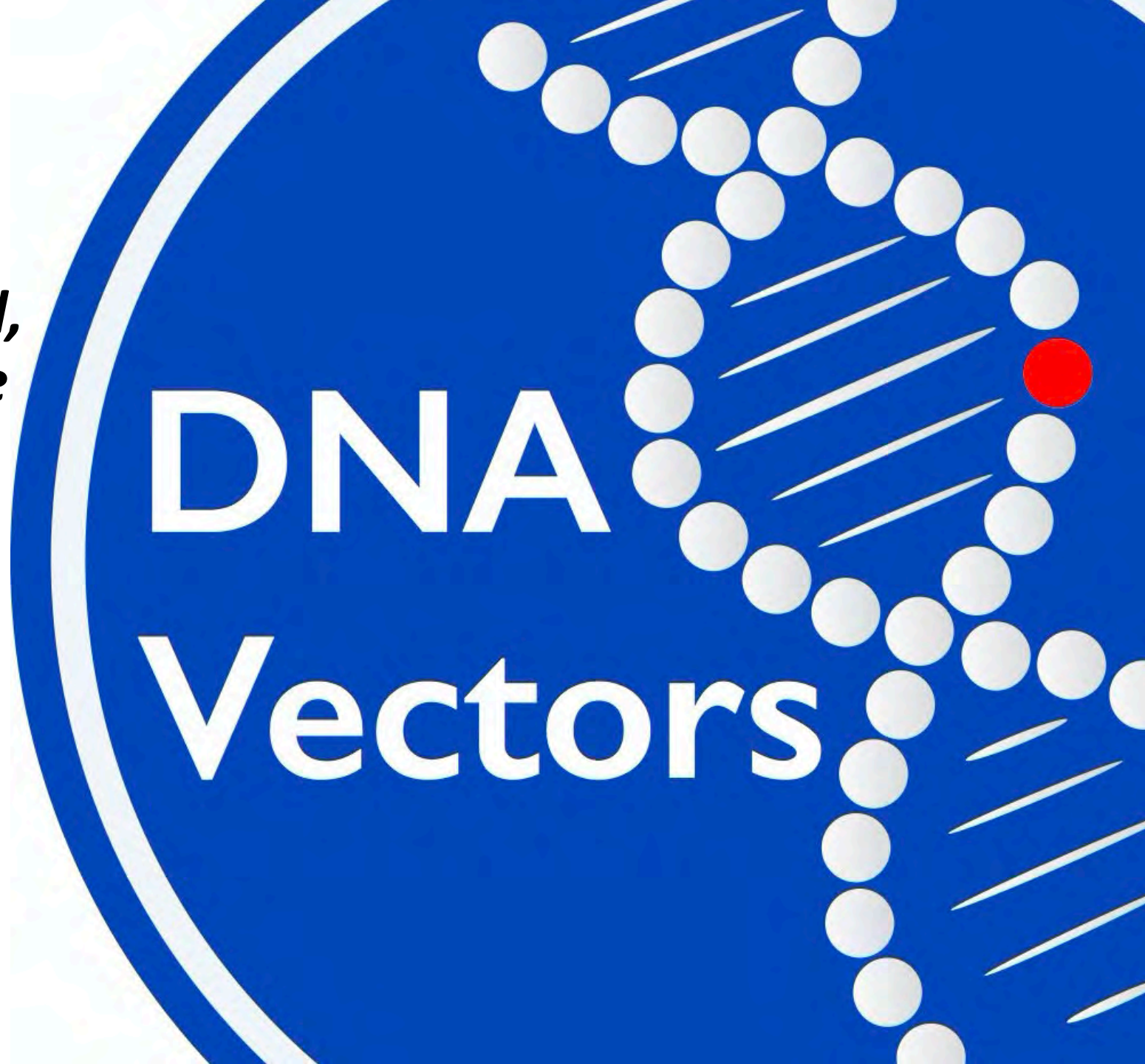


***Next-Generation DNA
NanoVectors -
An Alternative Vector
Platform for the Safe, Rapid,
and Persistent Manufacture
of Recombinant T cells for
Autologous T Cell
Immunotherapy***

Dr Richard Harbottle
r.harbottle@dkfz.de

DNA Vector Laboratory
DKFZ Heidelberg



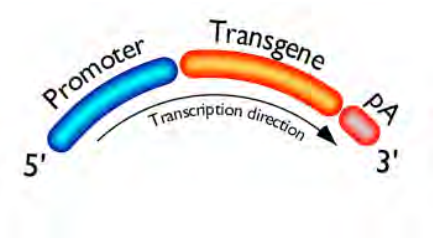
Where I started
working on
Non-Viral Gene
Therapy in 1991



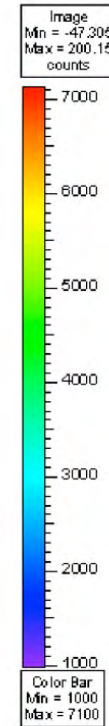
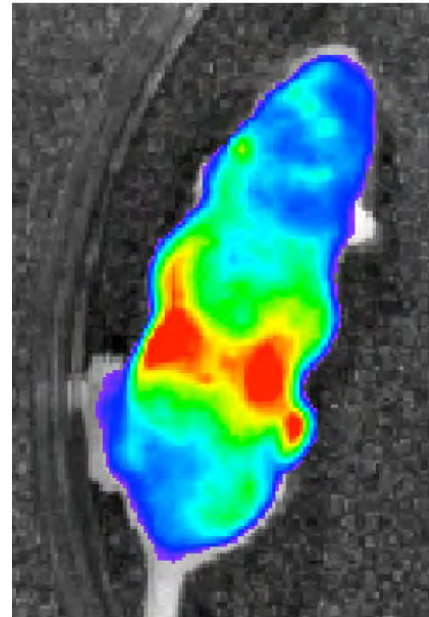
ST MARY'S HOSPITAL



Gene Delivery was improving but expression from Non-Viral Vectors was still transient



Wong SP, Argyros O, Howe SJ and Harbottle RP
Systemic gene transfer of non-viral plasmids to neonatal mice
Journal of Controlled Release 2011 Mar 30;150(3):298-306



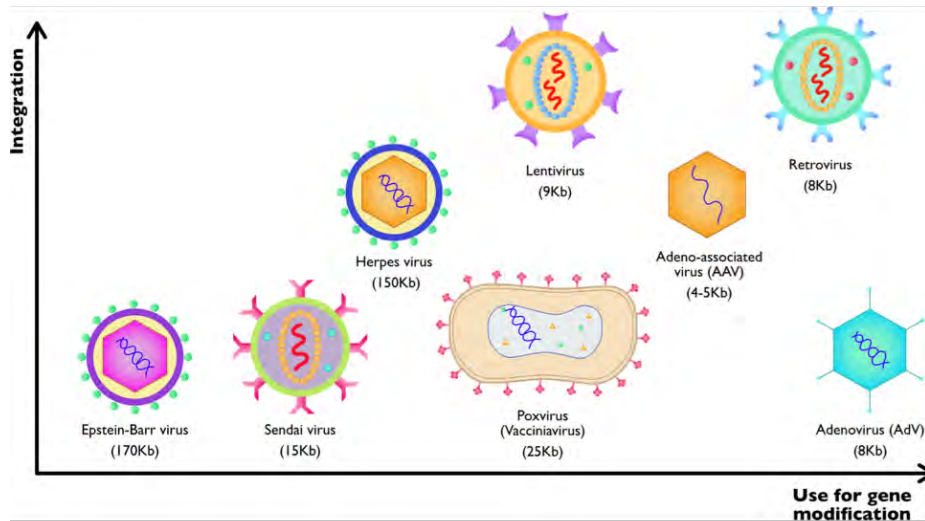


DNA
Vector
Research
DKFZ





Viral vectors



- Natural ability to infect cells
- High transduction efficiency
- Surpass all cellular barriers
- Safety concerns (Integration)
- Associated immune reactions
- Size limitation

Non-viral vectors

Transposons

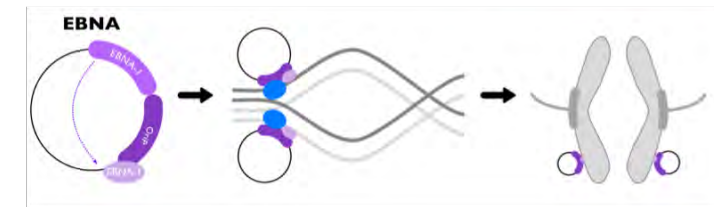
Excisable systems



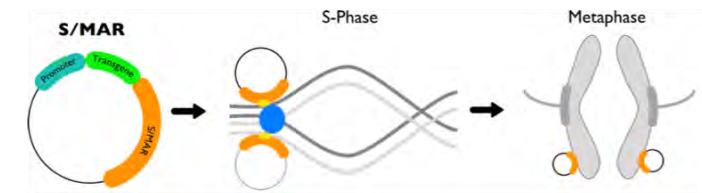
- Genomic “scar”

EBNA-based episomal vectors

- Gene dysregulation
- Proto-oncogene

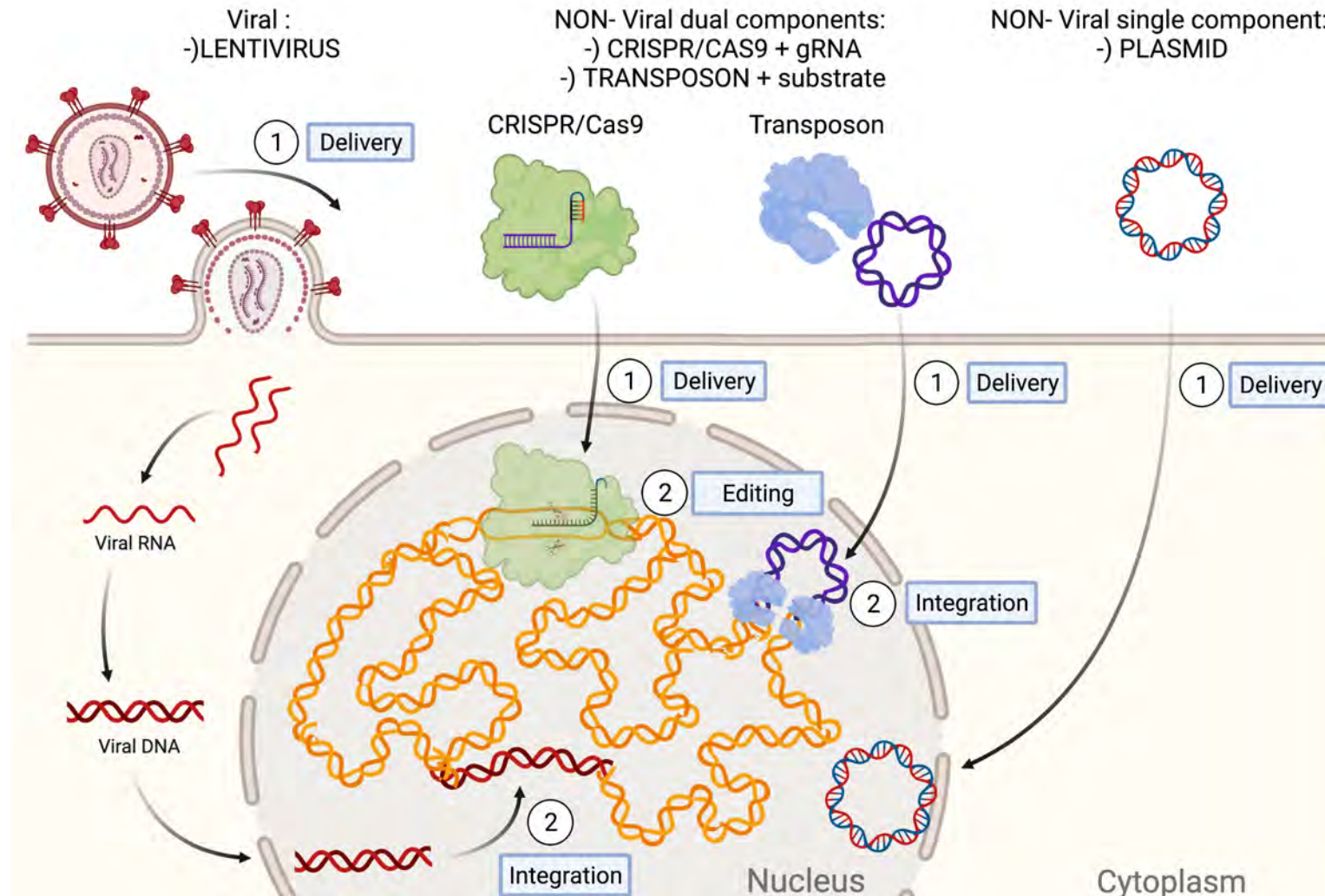


S/MAR episomal vectors



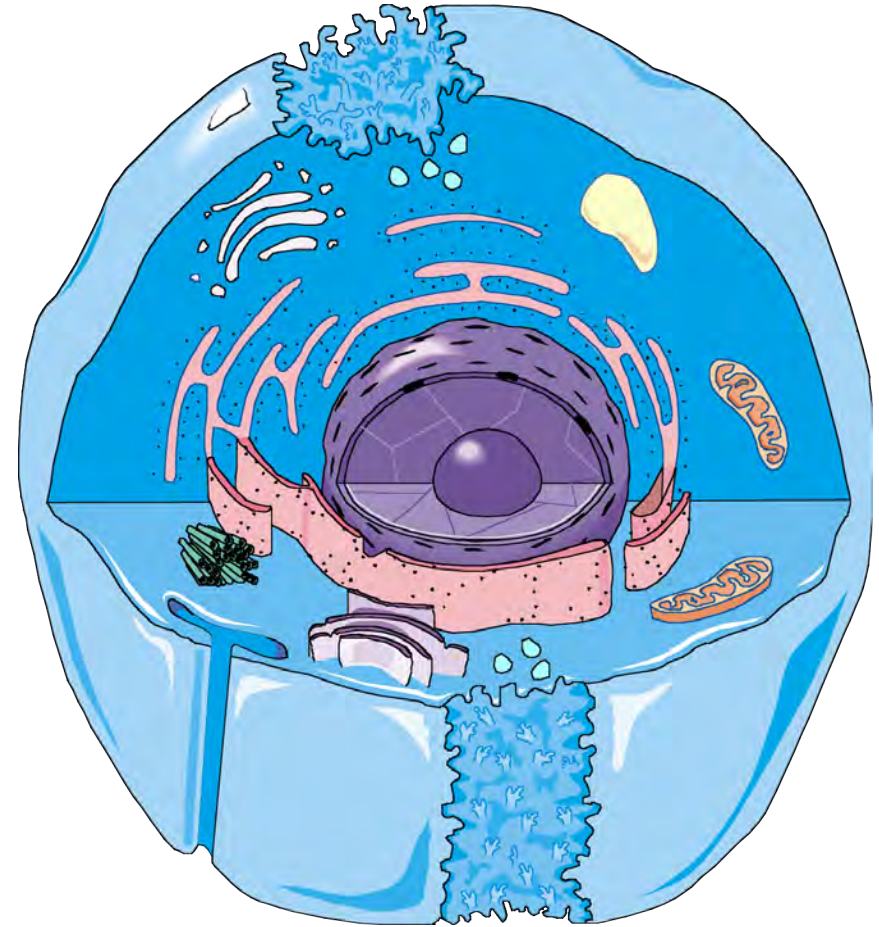
Persistent Genetic Modification of Cells

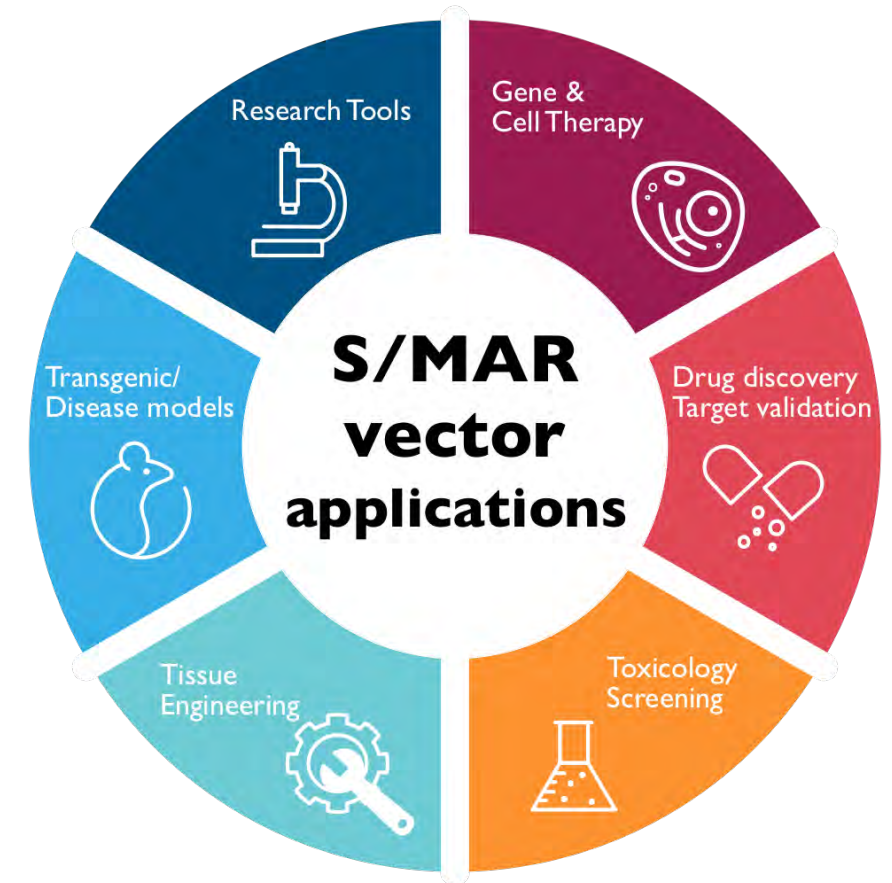
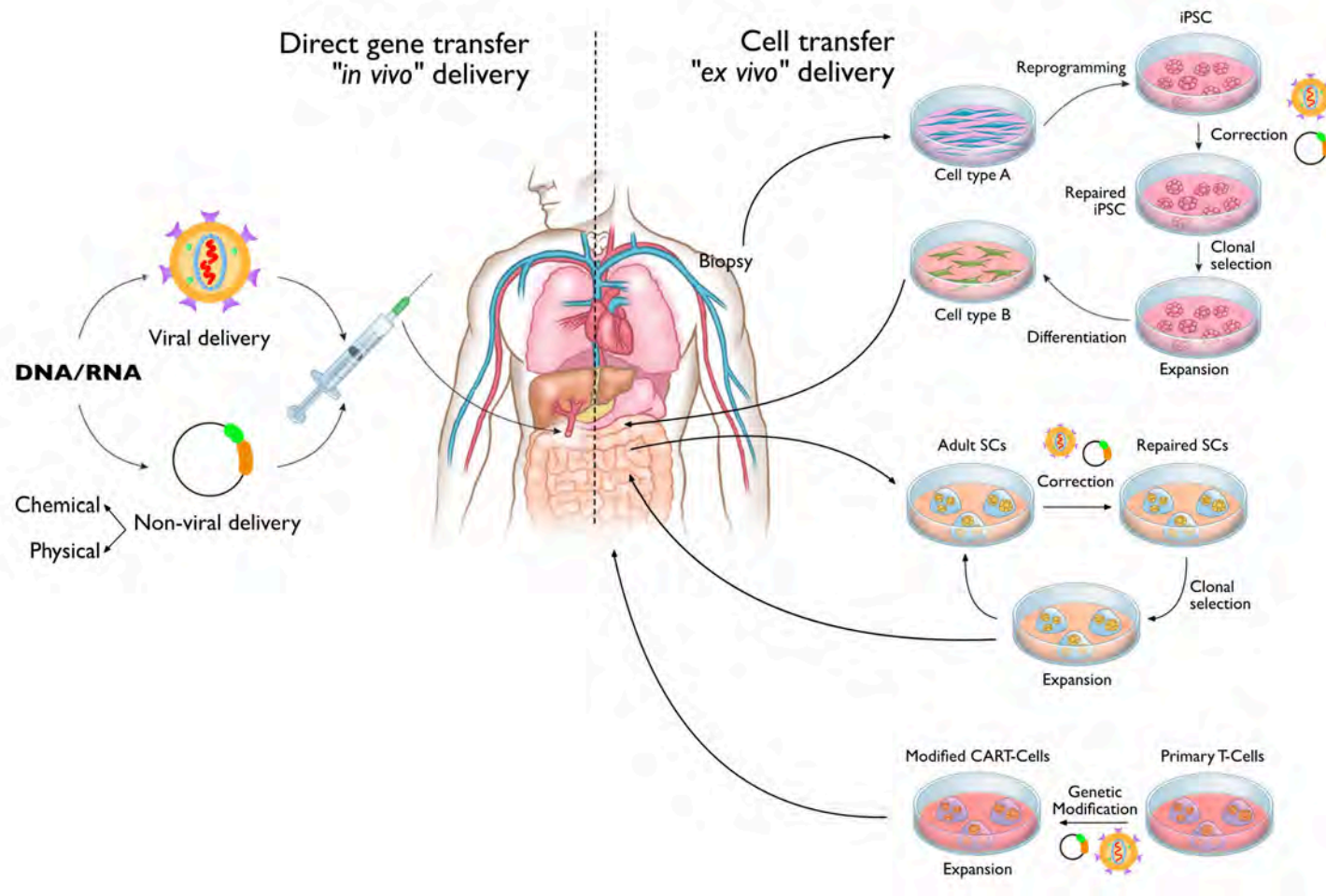
Developing a Safer and Versatile Universal Vector Platform



nS/MARt Vectors: A DNA Vector platform for efficient genetic modification of Human Cells

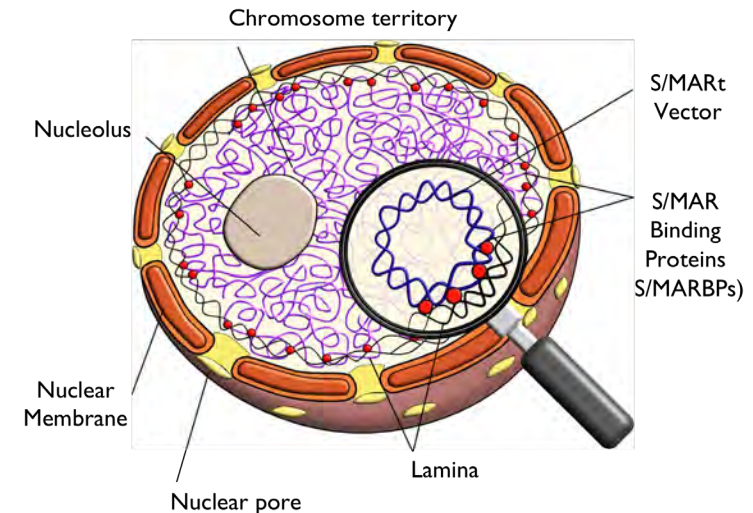
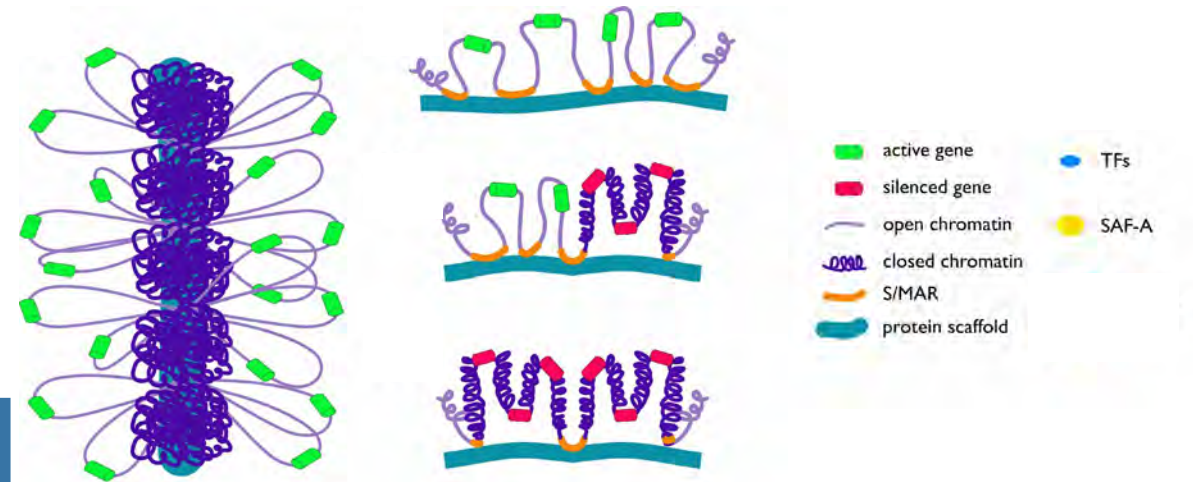
- Non-Viral, Non-Pathogenic
- Simple and Economical to Manufacture
- Efficient to Deliver
- Episomal
- Unlimited capacity
- Use *in vivo*, *in vitro* and *ex vivo*
- Mitotic Stability
- Persistent expression



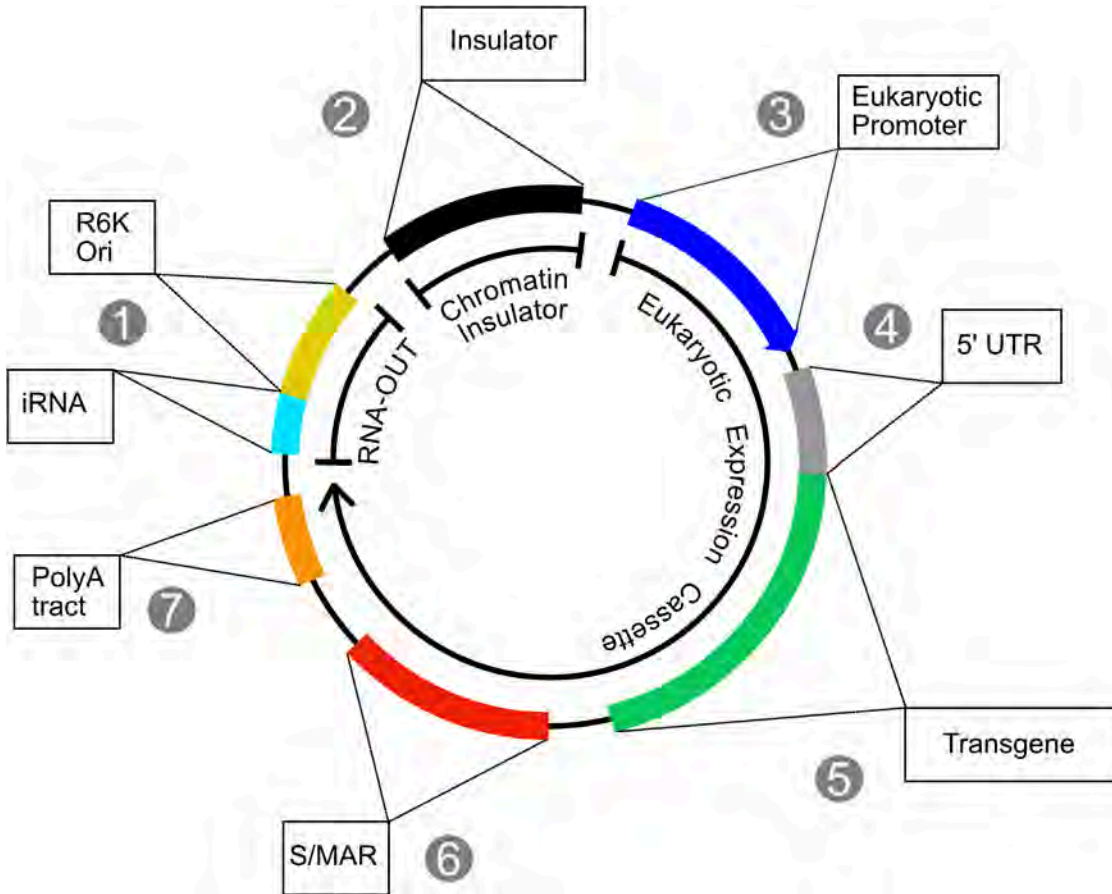


Scaffold Matrix Attachment Regions (S/MARs)

- AT-rich sequence found on gDNA
 - Involved in chromatin structure and attachment to nuclear matrix
 - Involved in the generation of transcription “bubbles”
- Tethers DNA Vectors to transcriptionally active nuclear sites
 - Drives episomal replication and maintenance when incorporated in DNA vectors
 - Mediates DNA Vector segregation and transmission during mitosis
 - Prevents epigenetic silencing and provides robust and persistent transgene expression



Refining S/MAR DNA vectors

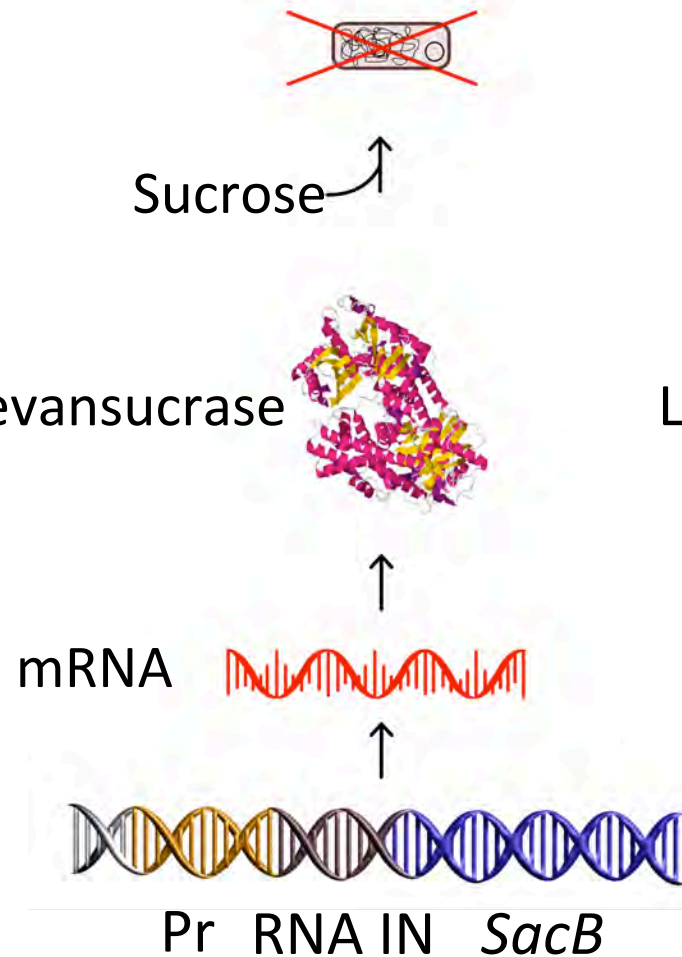


S/MAR vectors are multi-component systems

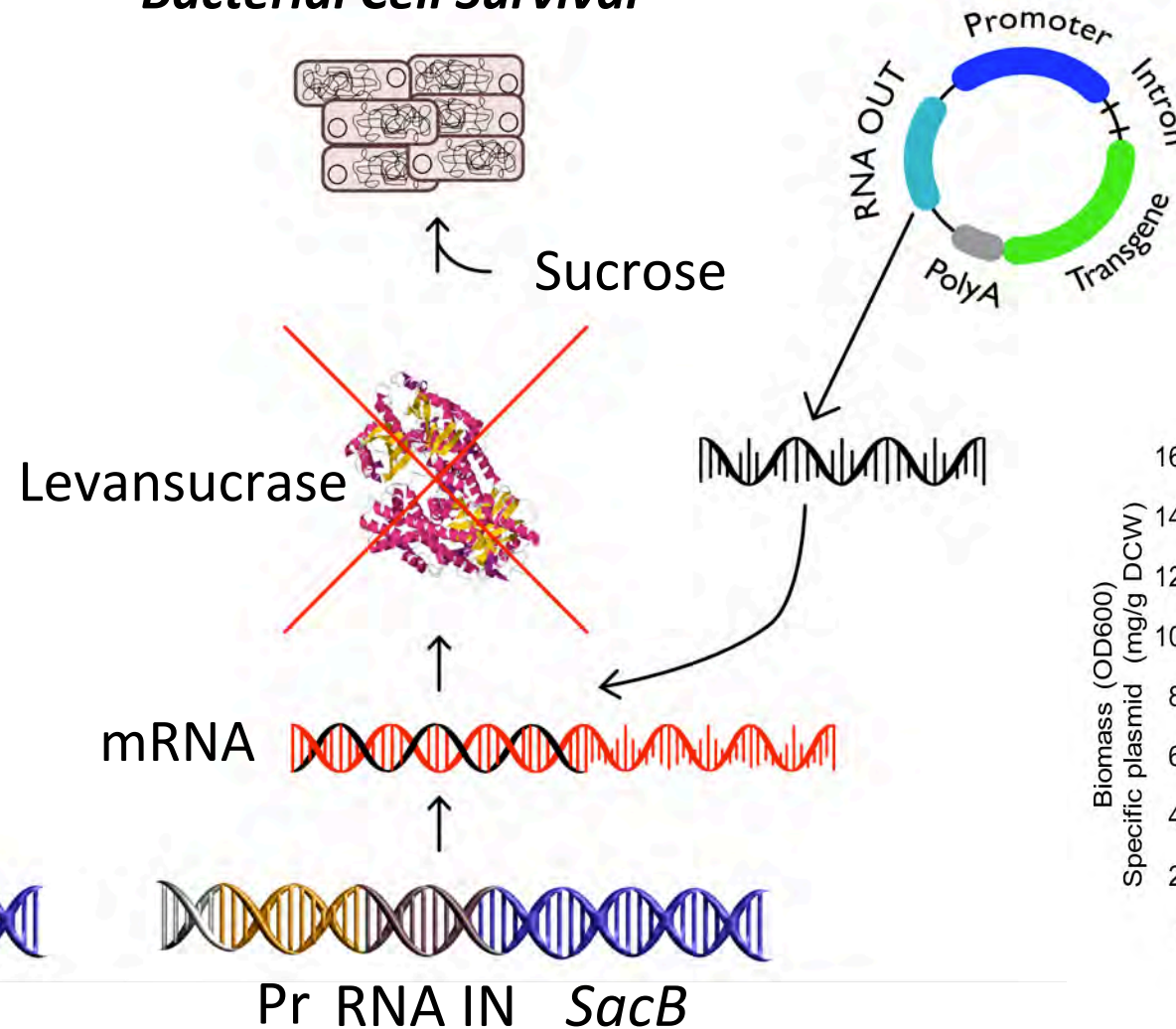
- 1) Bacterial Backbone (essential for production but determines the overall immunogenicity of the vector in Human cells)
- 2) *Cis-acting* sequences (insulators and enhancers, which regulate expression of the vector contents)
- 3) Cell and Application appropriate promoter
- 4) 5' and 3' UTRs (which determine the mRNA stability of the vector payload)
- 5) Cell and Application optimized gene
- 6) S/MAR sequence (which acts as a substrate for transcription factor binding tethering the vector to the nuclear lamina, and a replication origin due to AT richness)

NanoVectors : Next-Generation “Minicircles” with Antibiotic-free selection

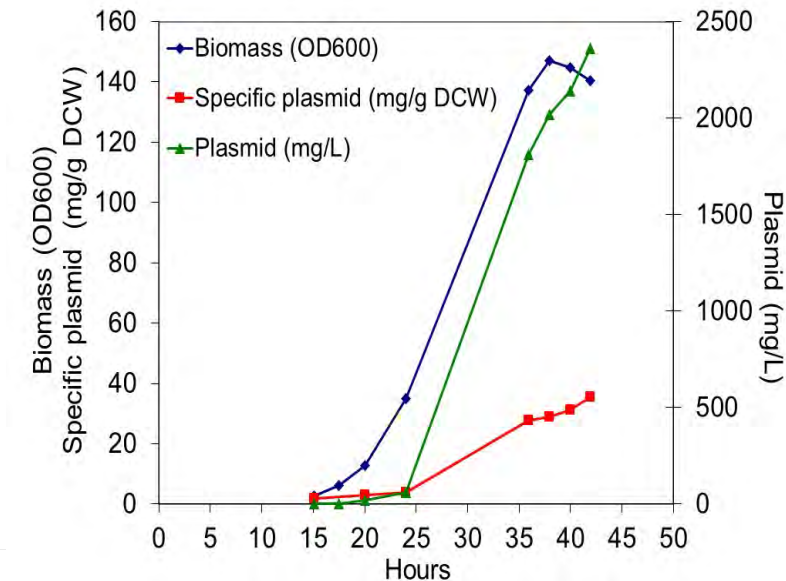
Bacterial Cell Death



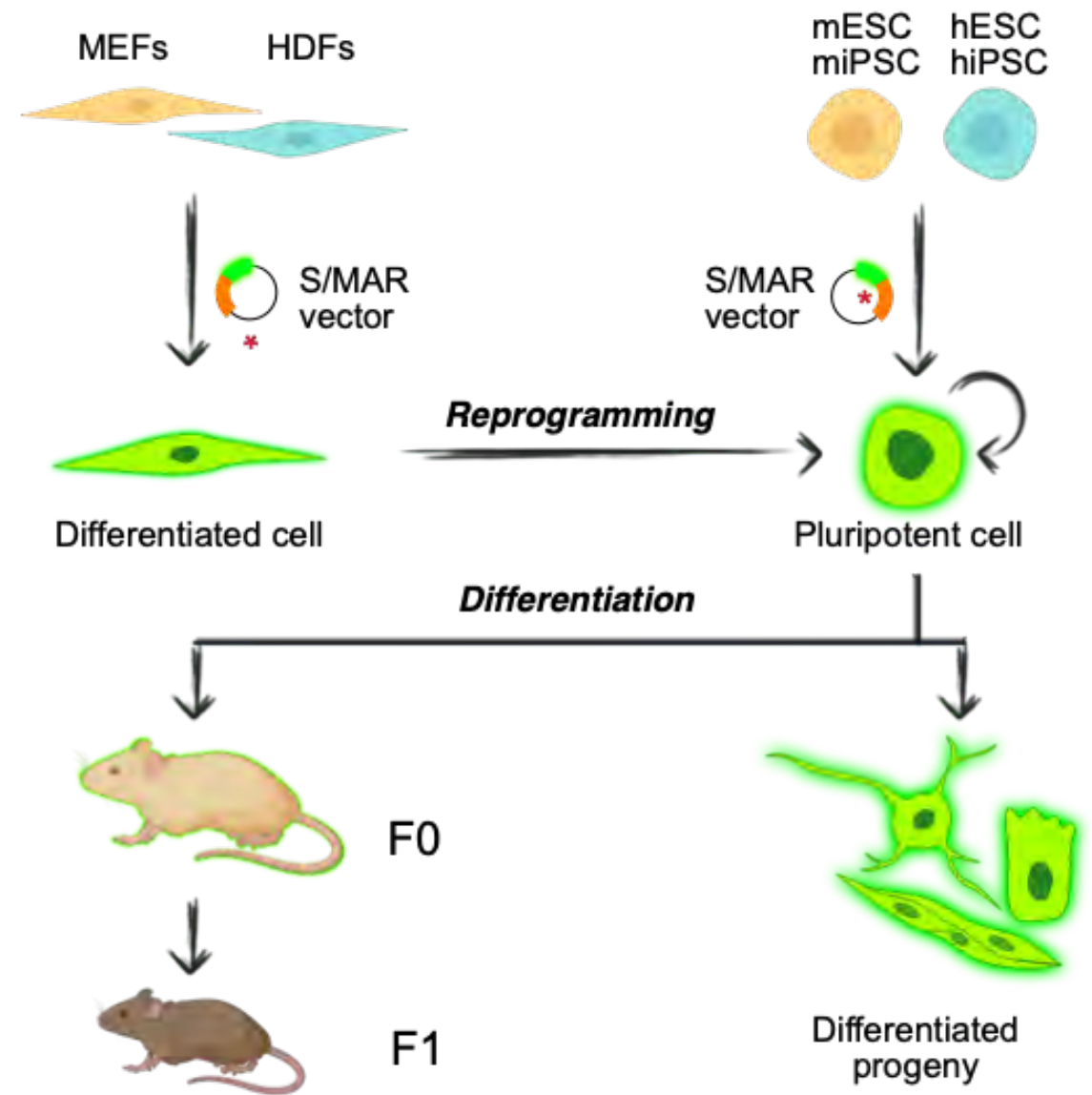
Bacterial Cell Survival



HyperGro™ for large
scale DNA manufacturing

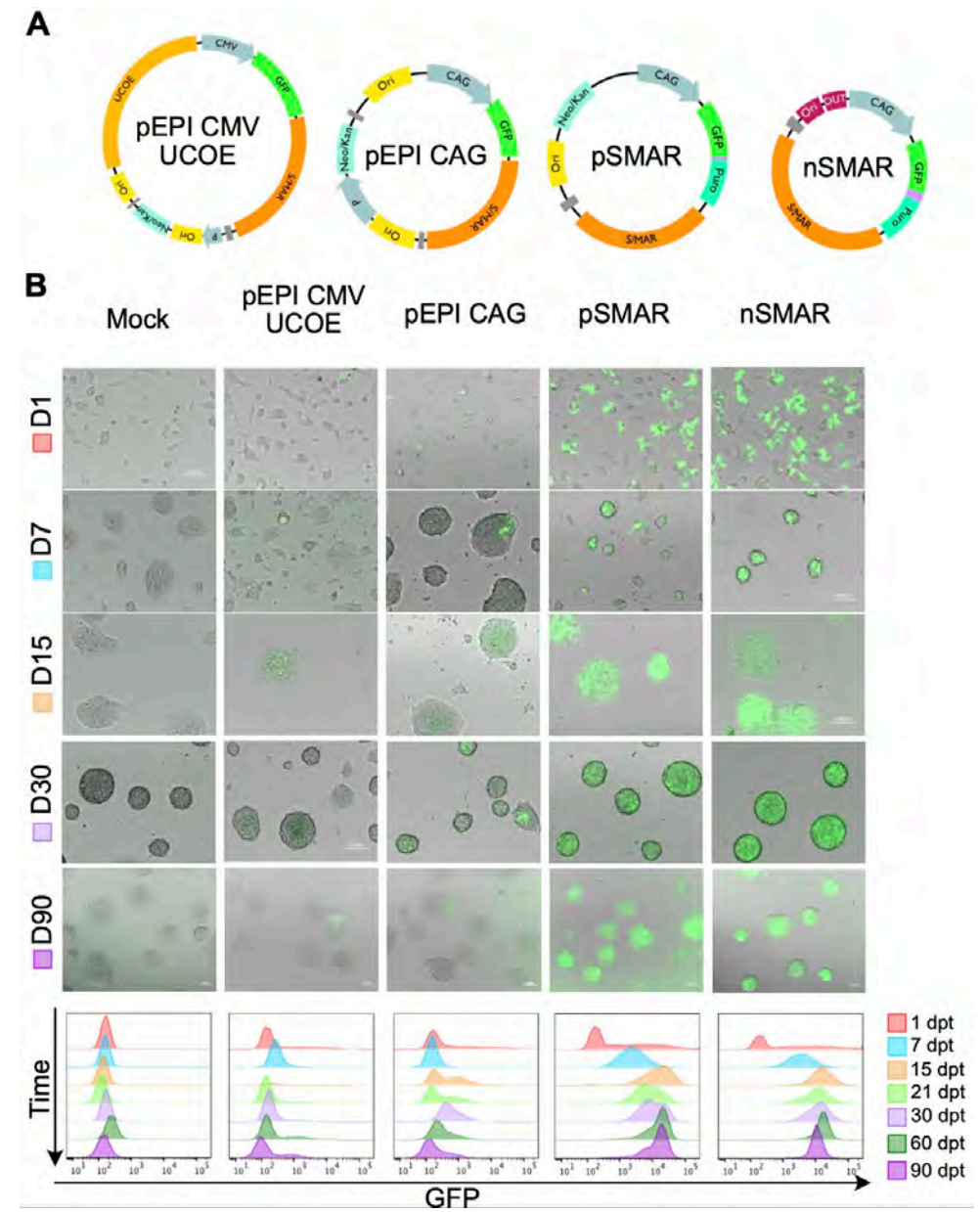


Genetic Modification of Stem Cells with *nS/MARt* DNA Vectors

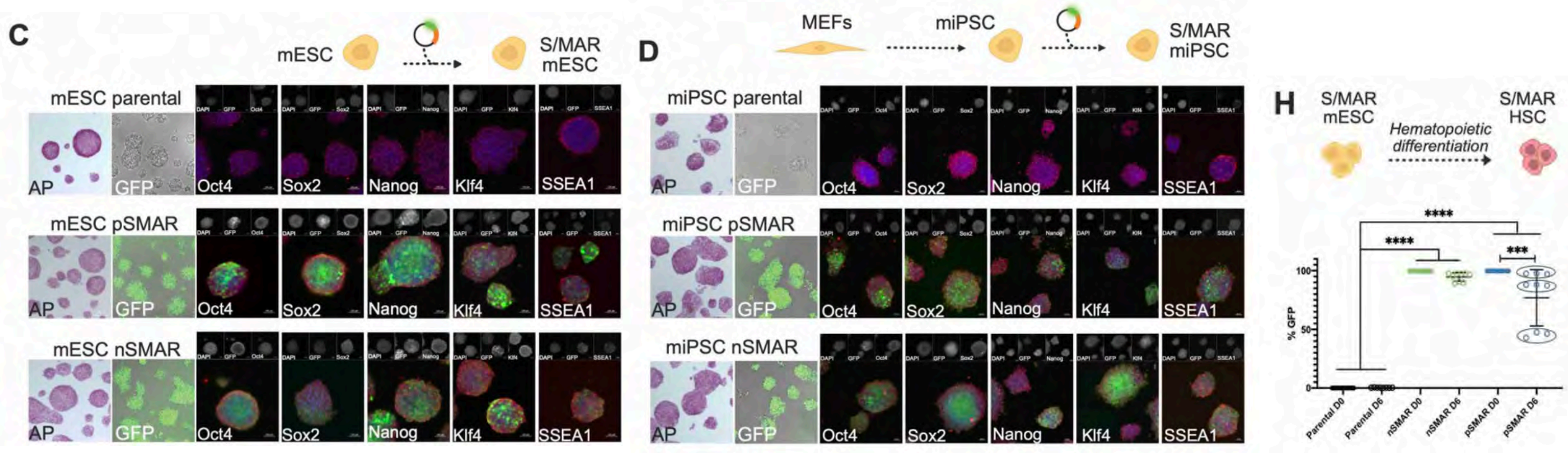


Generation of Genetically Modified Mouse Embryonic Stem Cells with nS/MARt DNA Vectors

nS/MARt-GFP vectors were used to engineer mESC and the transgene expression was found to be stable for at least 90 days.

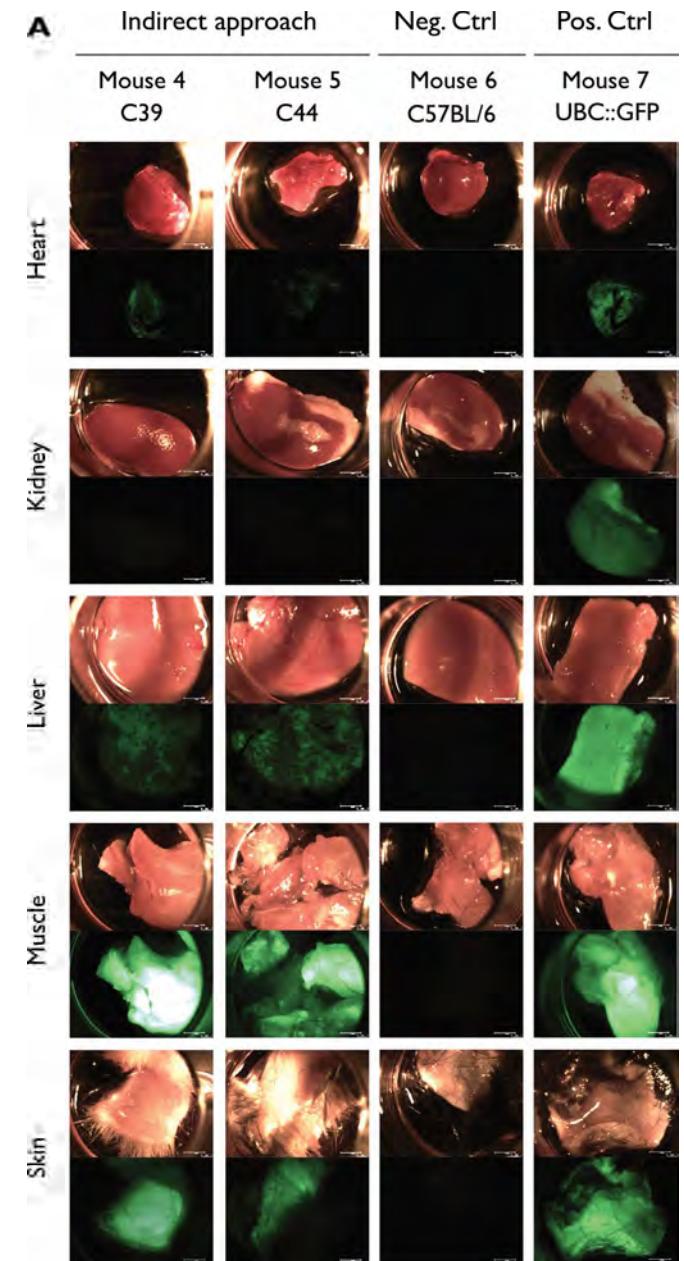
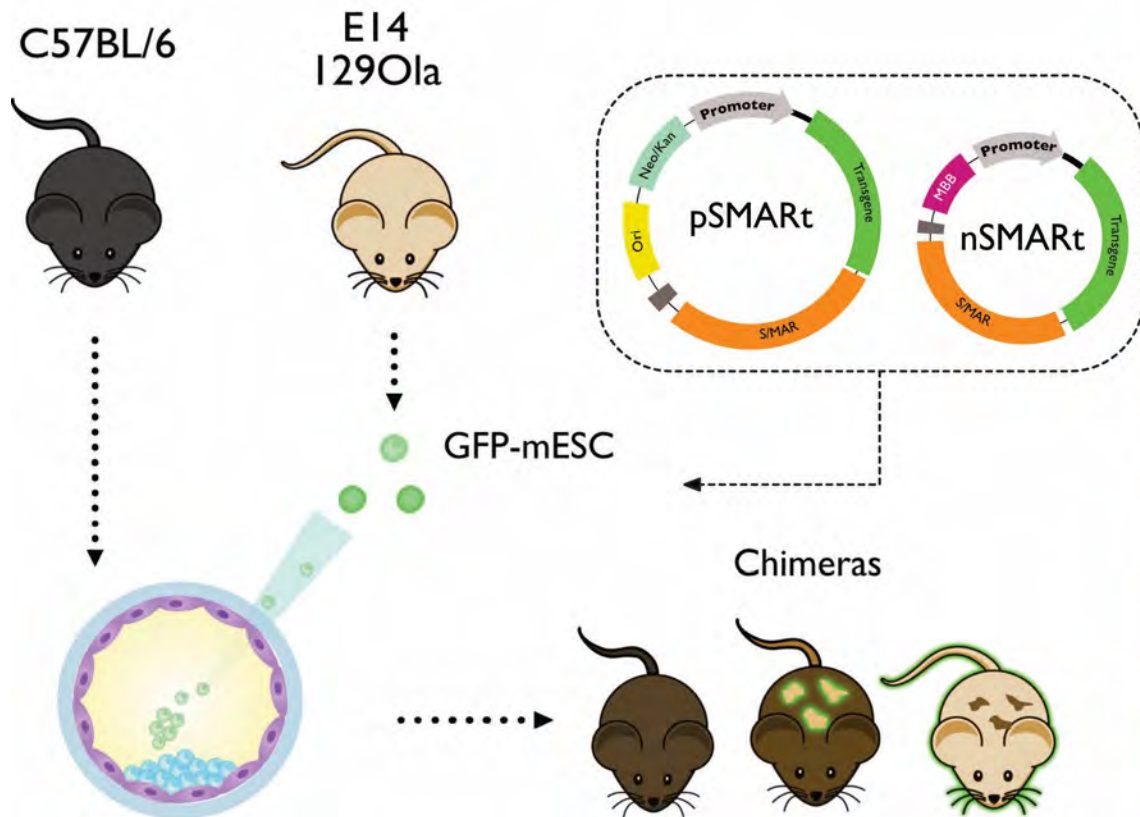


Generation of Genetically Modified Mouse Embryonic Stem Cells with *nS/MARt* DNA Vectors

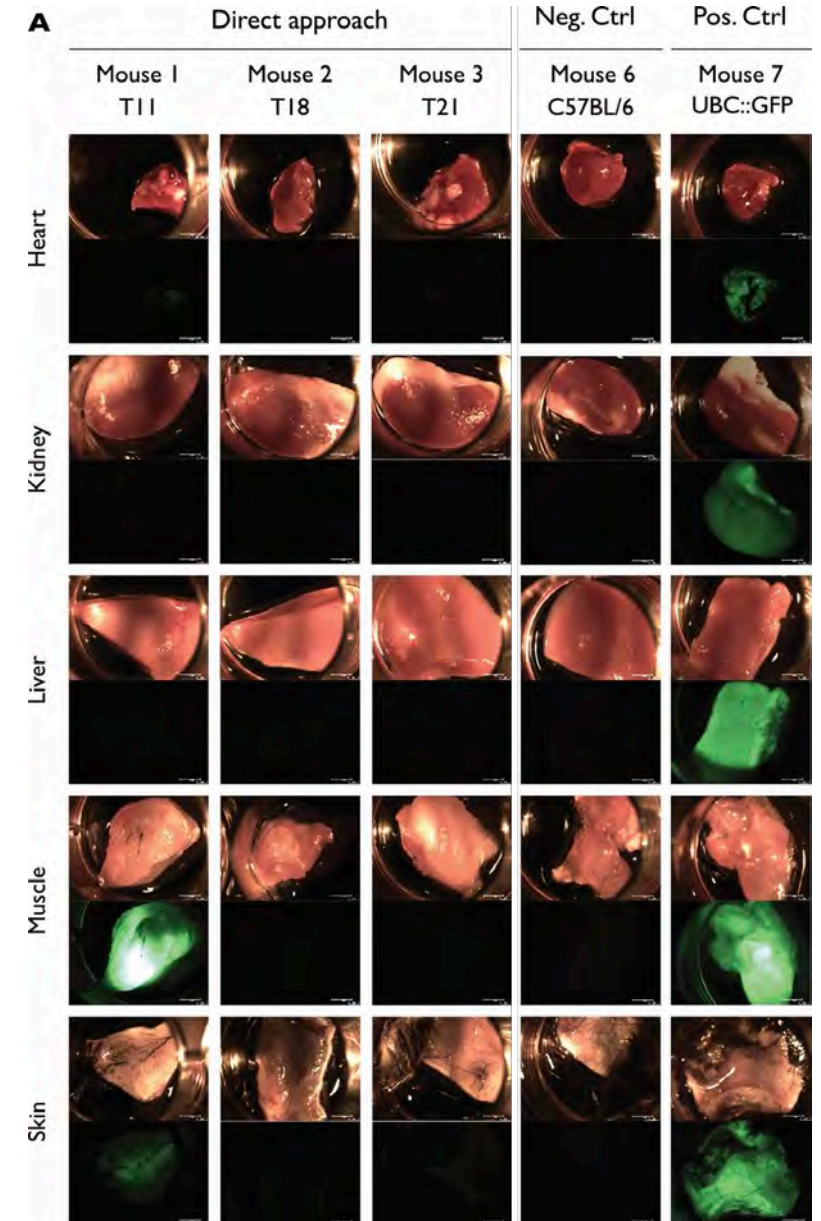
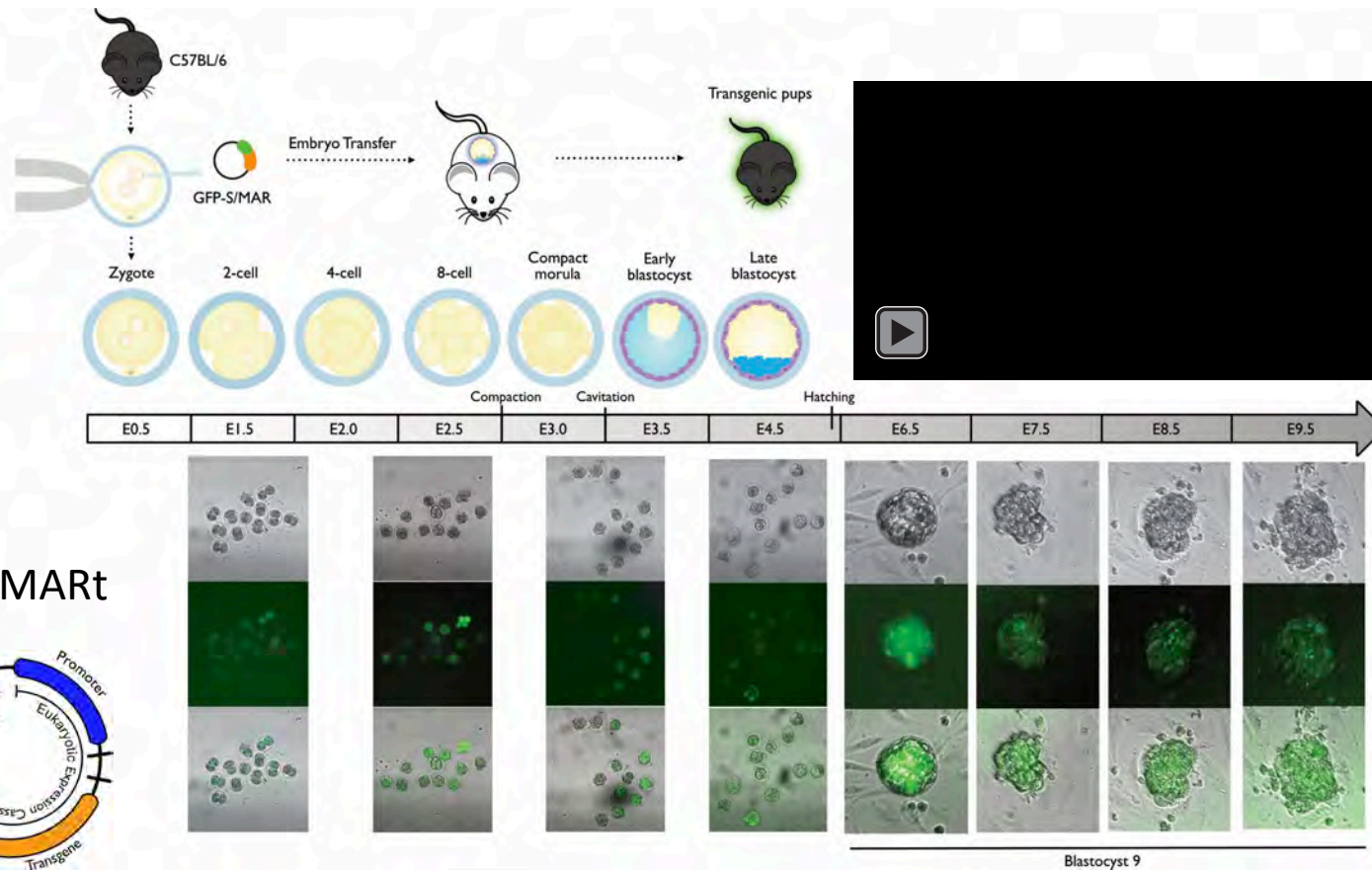


nS/MARt vectors don't affect the pluripotency of Mouse Embryonic Stem Cells (mESC) and induced Pluripotent Stem Cells (miPSC) stably modified to express GFP and provide long-term expression during hematopoietic differentiation

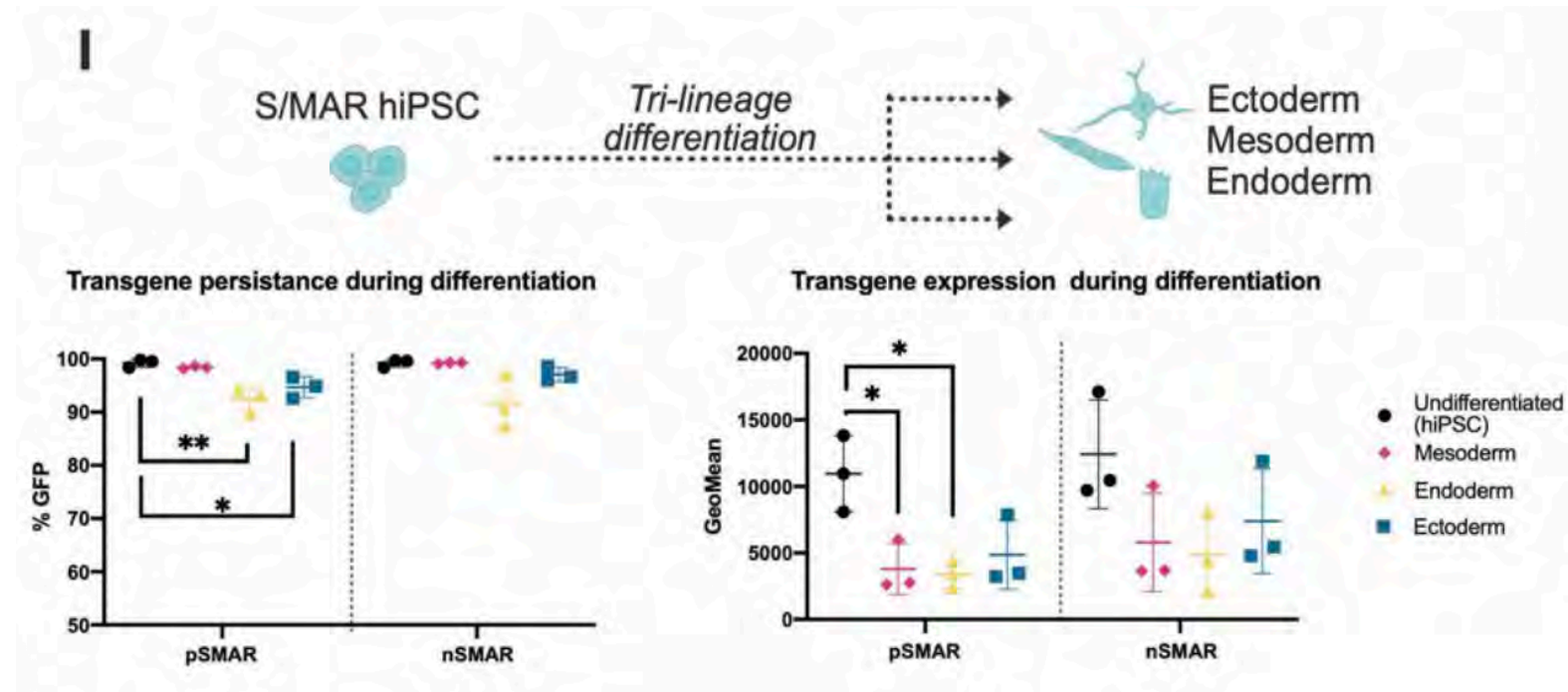
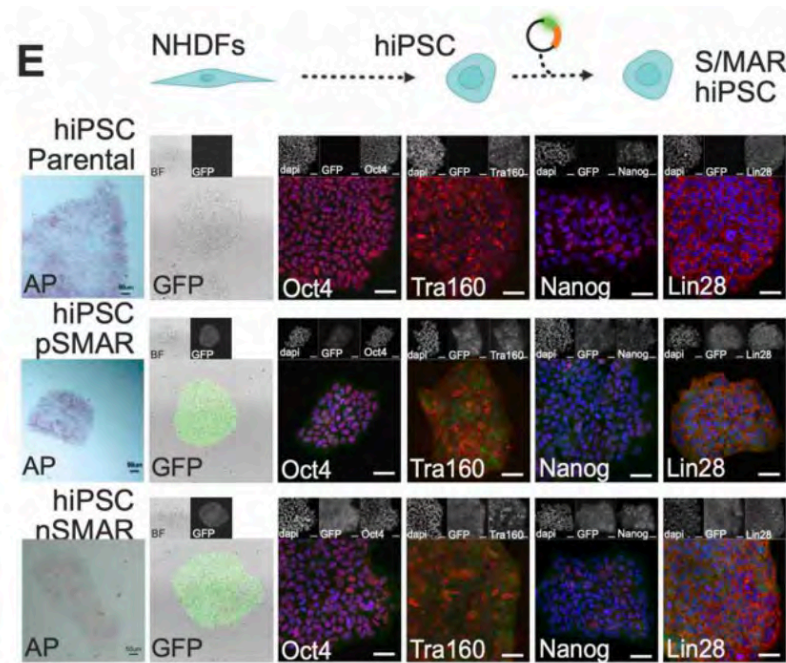
Genetically Modified Stem cells can be used to generate Chimeric Mice



nS/MARt pronuclear injection

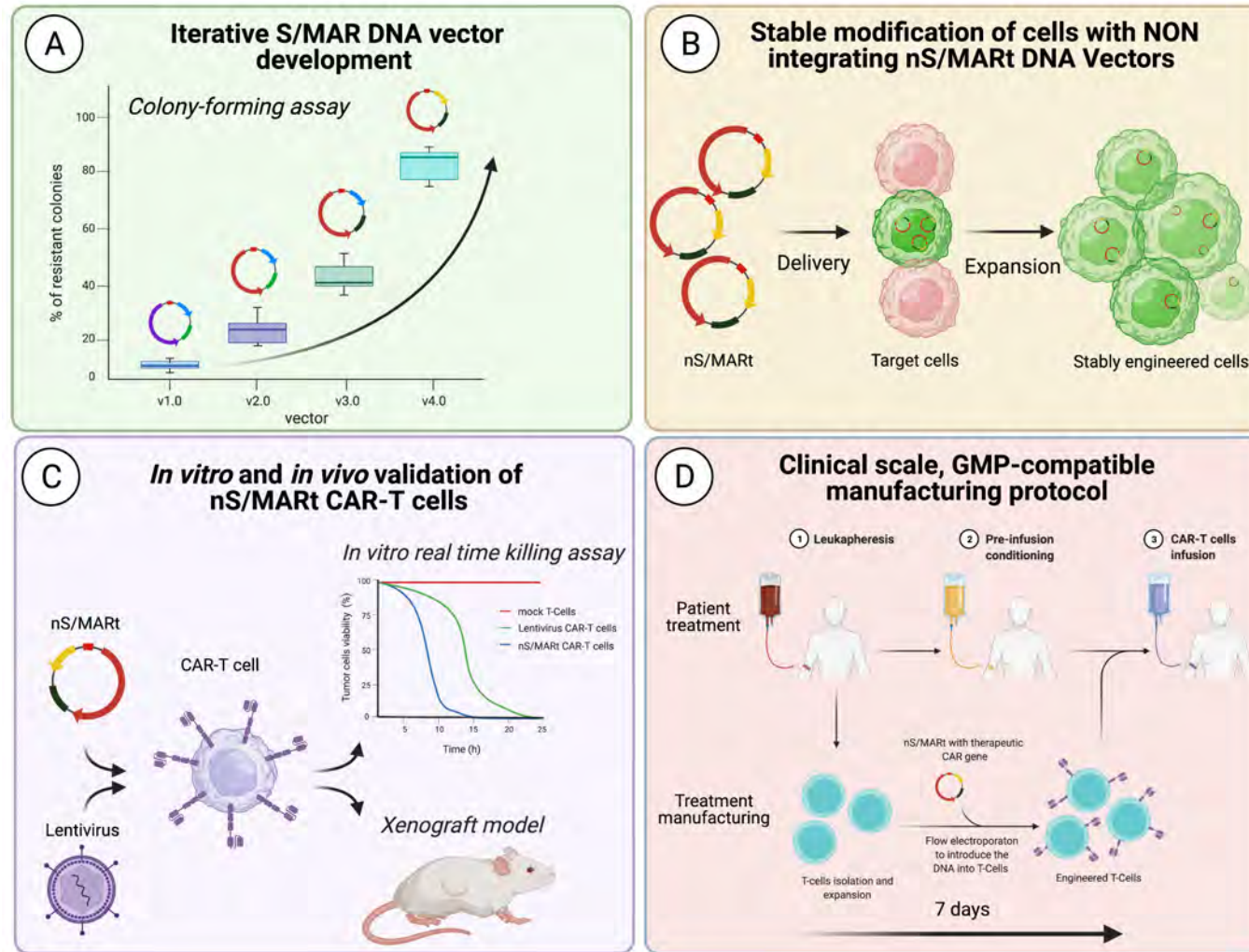


Generation of Genetically Modified Human Stem Cells with nS/MARt DNA Vectors



nS/MARt vectors can efficiently be used to engineer human induced Pluripotent Stem Cell (hiPSC) and the expression of the transgene doesn't change during tri-lineage differentiation

A non-viral, non-integrating DNA Nanovector platform for the safe, rapid and persistent manufacture of recombinant T-Cells



**Matthias Bozza^{1†}, Alice de Roia¹,
Margareta Correia², Aileen Berger^{3,6},
Alexandra Tuch^{3,6}, Andreas Schmidt⁴,
Inka Zörnig^{5,6}, Dirk Jäger^{3,5,6}, Patrick
Schmidt^{5,6,7,†,‡} & Richard P Harbottle^{1,‡,§}**

¹ DNA Vector Laboratory, DKFZ Heidelberg, Im Neuenheimer Feld 242, Heidelberg, Germany.

² Cancer Biology and Epigenetics Group, Research Centre of Portuguese Oncology Institute of Porto, 4200-072 Porto, Portugal

³ Clinical Cooperation Unit Applied Tumorimmunity, DKFZ Heidelberg, Im Neuenheimer Feld 460, Heidelberg, Germany

⁴ Proteona, 2 Jurong East St 21, 609601, Singapore

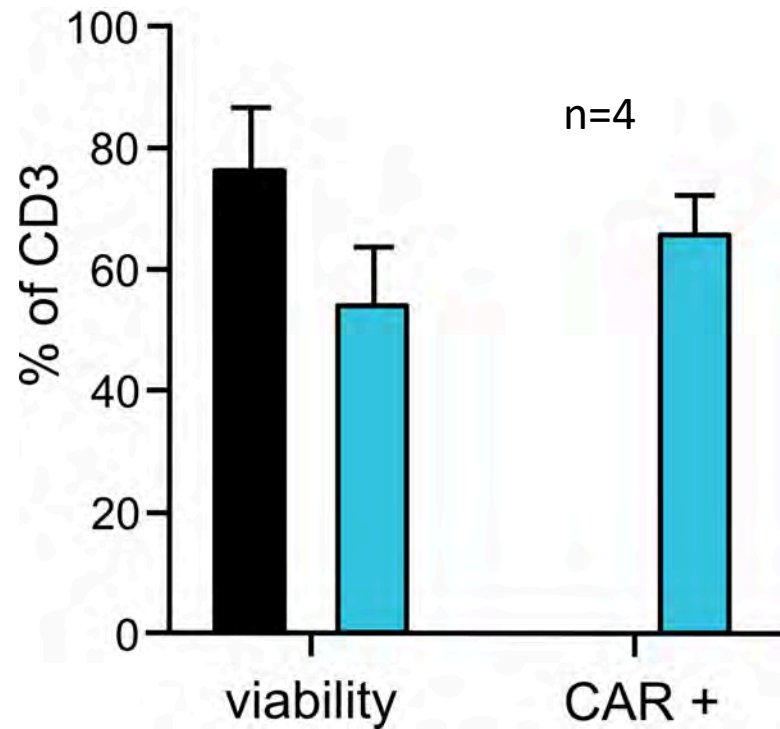
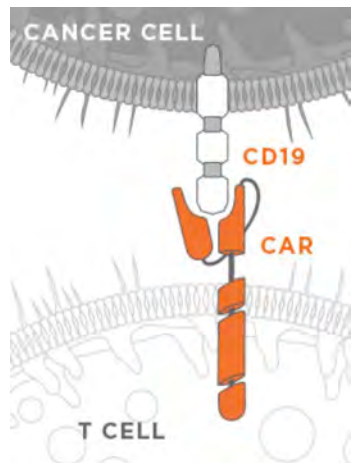
⁵ Department Medical Oncology, University Hospital Heidelberg, Im Neuenheimer Feld 460, Heidelberg, Germany

⁶ National Center for Tumor Diseases, Medical Oncology, Im Neuenheimer Feld 460, Heidelberg, Germany

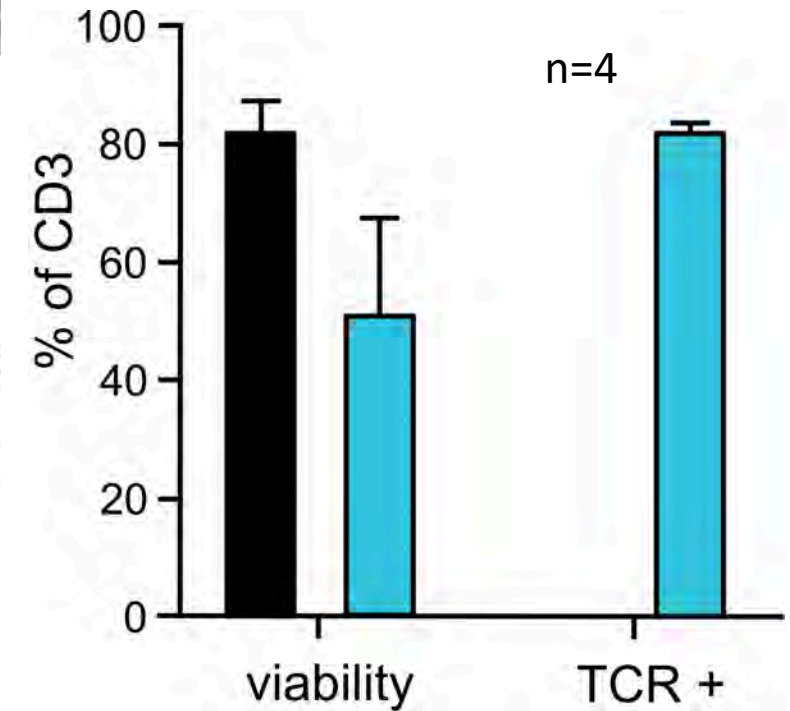
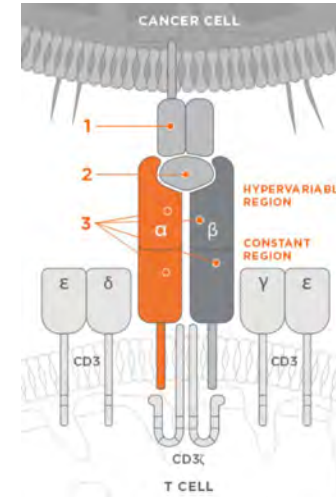
⁷ GMP & T cell Therapy Unit, DKFZ Heidelberg, Im Neuenheimer Feld 280, Heidelberg, Germany

nS/MARt can be efficiently delivered to primary human T-Cells by electroporation

nS/MARt CAR-T cells

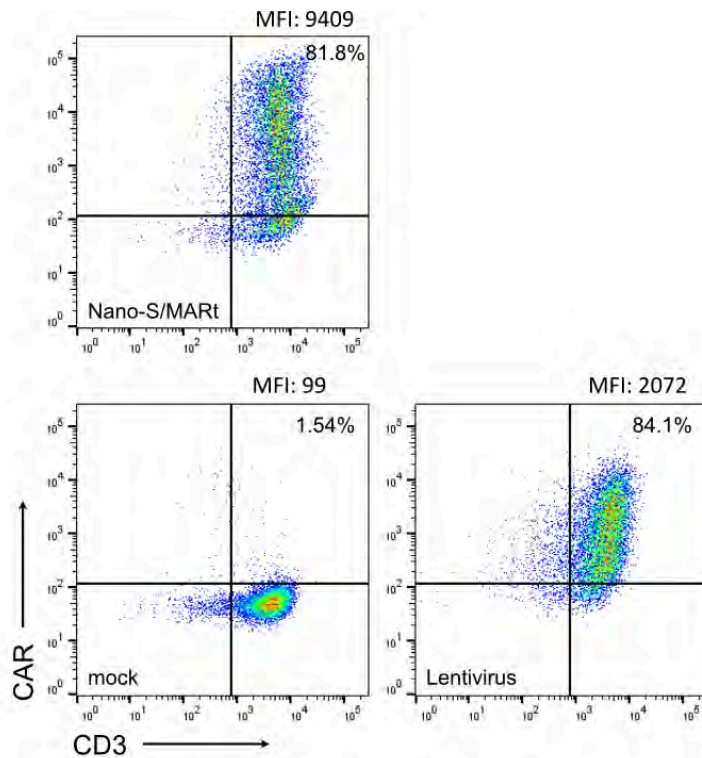


nS/MARt TCR-T cells

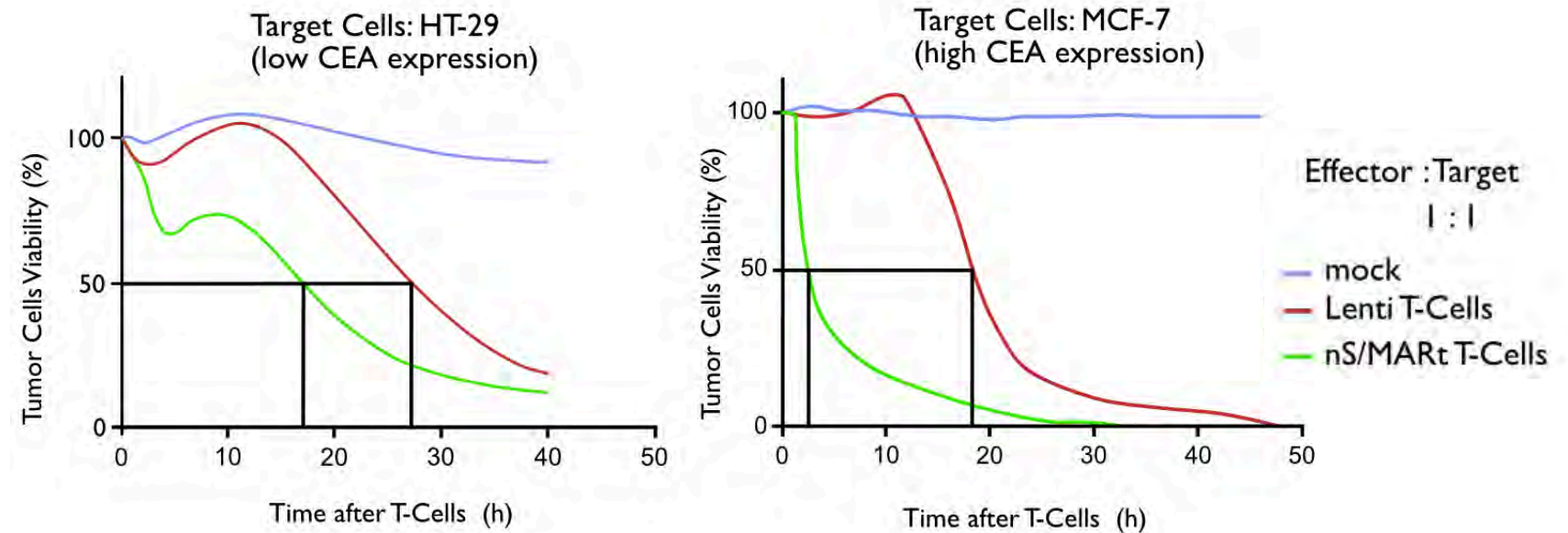


nS/MARt T-Cells efficiently target and kill tumor cells in vitro

Efficacy of delivery



Real-Time killing assay

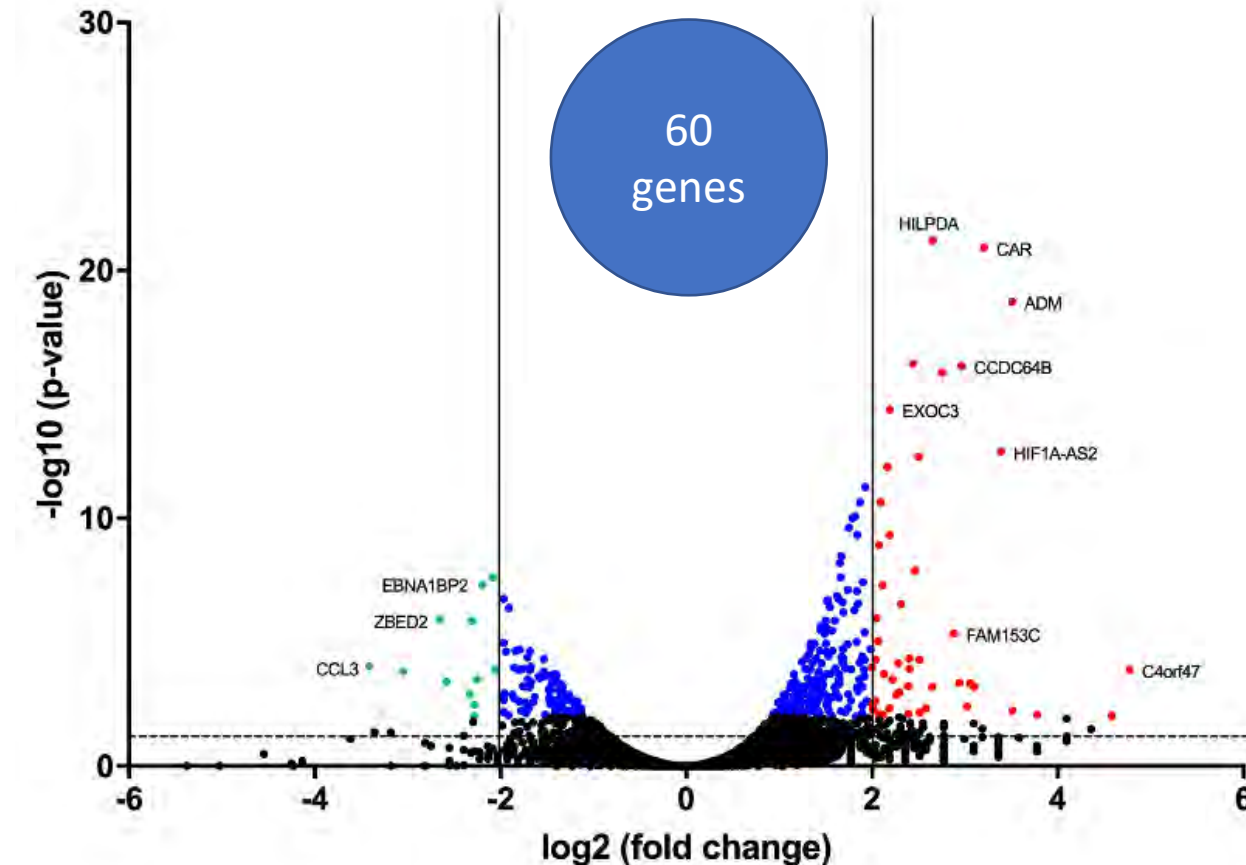


nS/MARt T-Cells provide potent anti-tumor activity

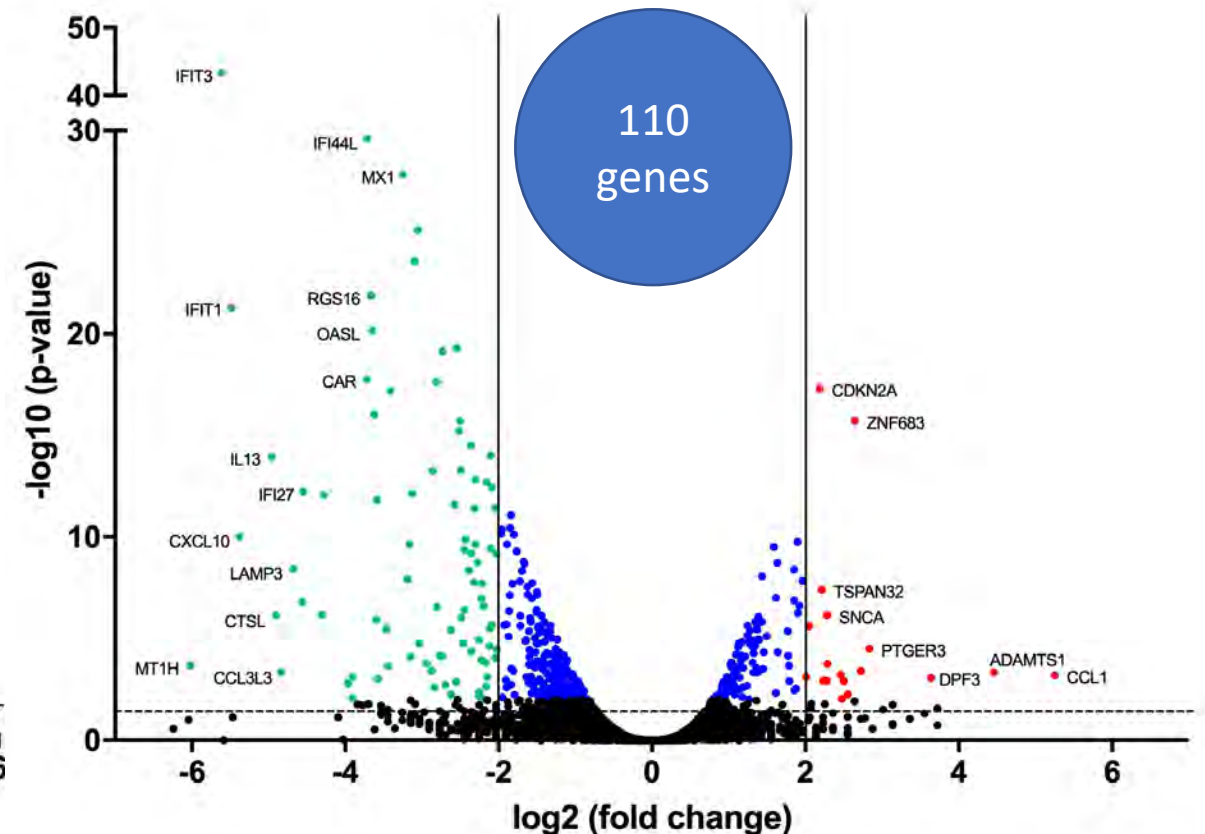
nS/MARt do not impact cells' homeostasis

Single Cells analysis of CAR T-Cells made with Lentivirus and nS/MARt vectors in comparison to naive cells

nS/MARt CAR T-Cells

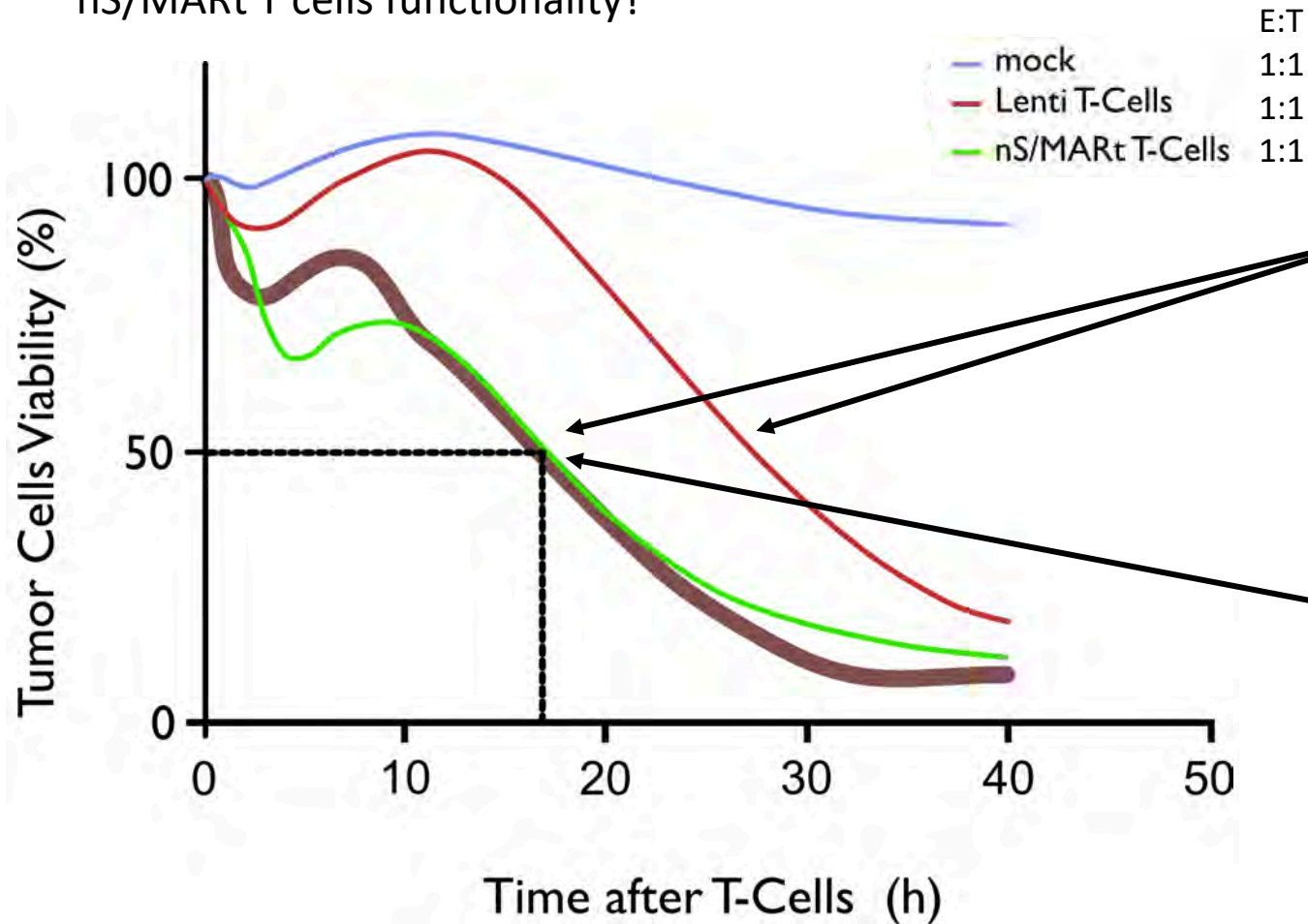


Lenti CAR T-Cells



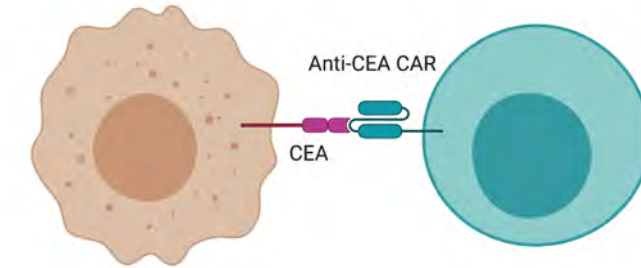
nS/MARt T-Cells provide potent anti-tumor activity

How many Lenti CAR T-cells are necessary to match nS/MARt T cells functionality?



CEA+ cancer cell

CAR-T cell



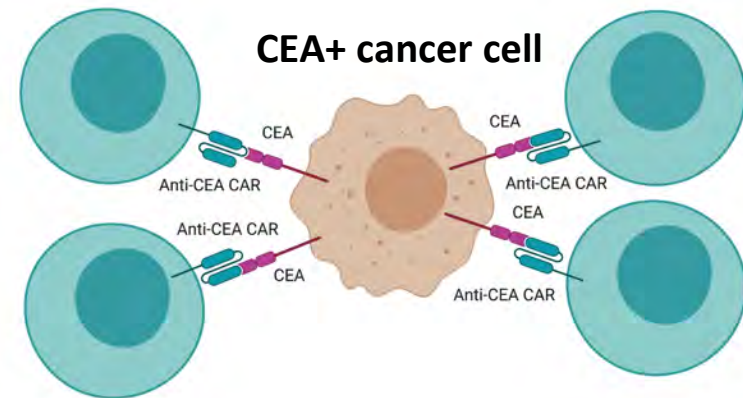
E:T nS/MARt
1:1



CAR-T cell

CEA+ cancer cell

CAR-T cell



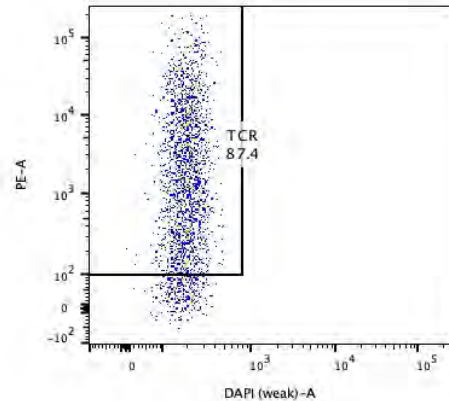
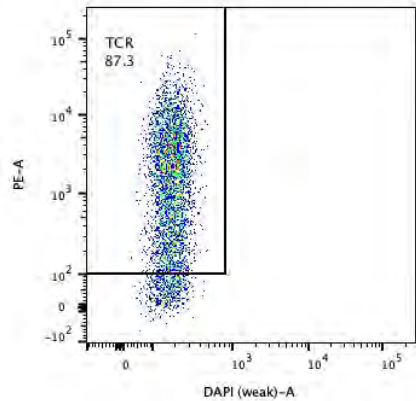
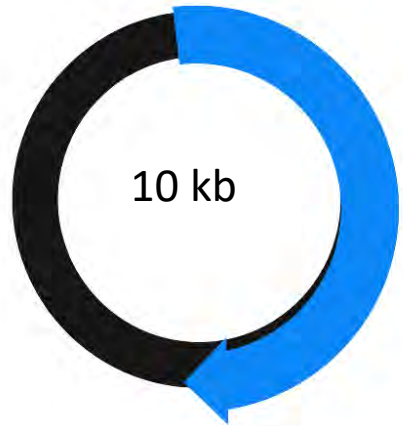
E:T Lenti
4:1



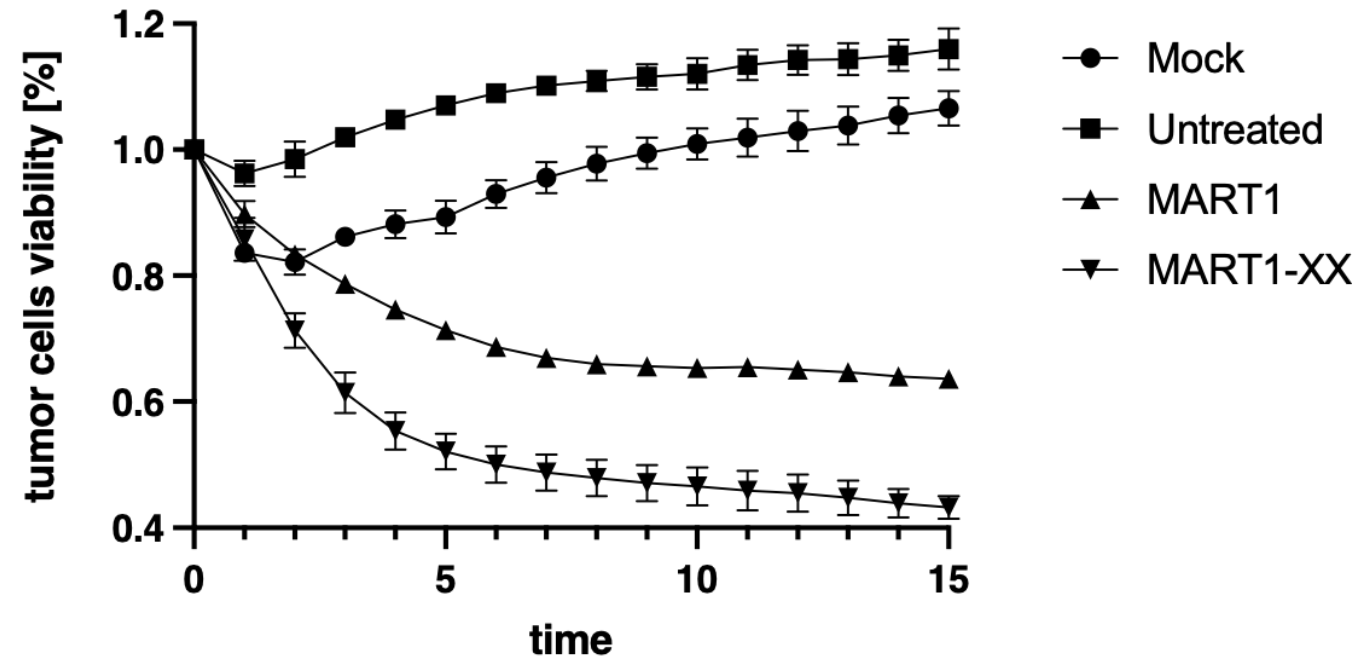
nS/MARt vectors have a high Cargo capacity

nS/MARt-MART1

nS/MARt-MART1-XX

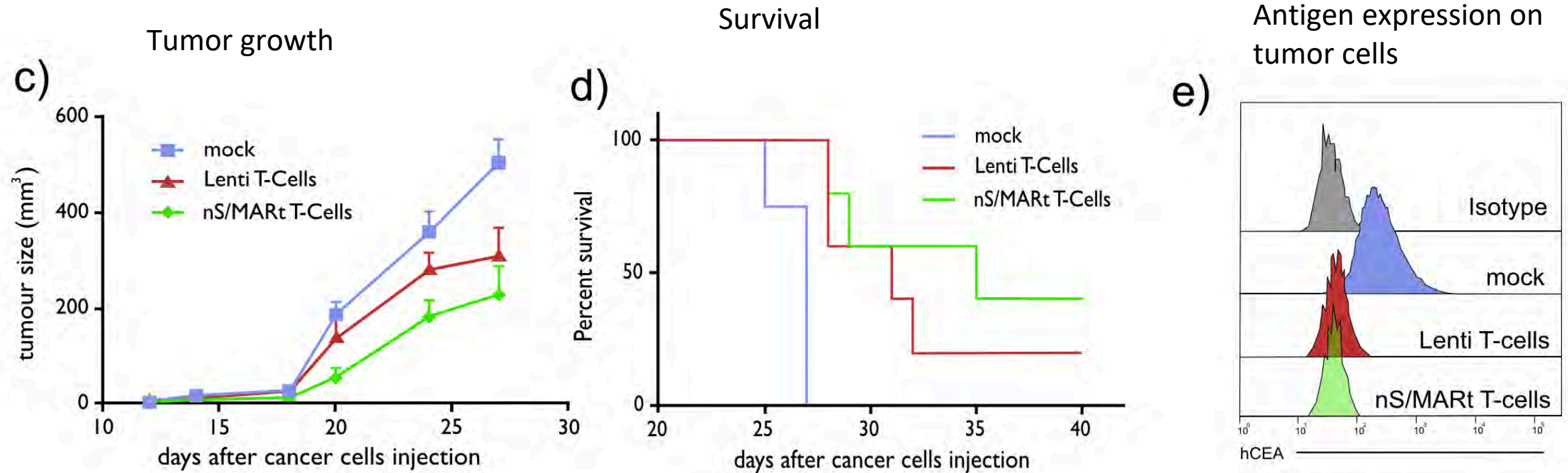


Real-time killing assay



Unpublished data

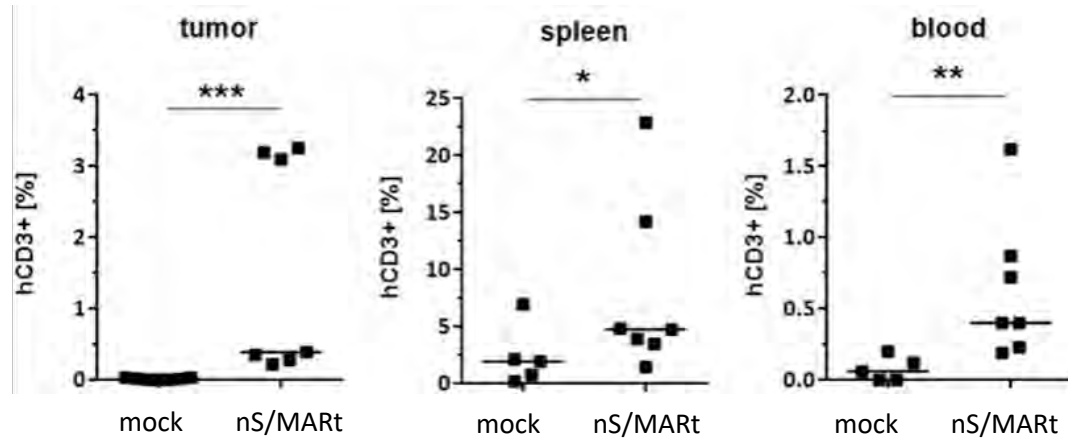
nS/MARt T-Cells efficiently target and kill tumor cells in xenograft models



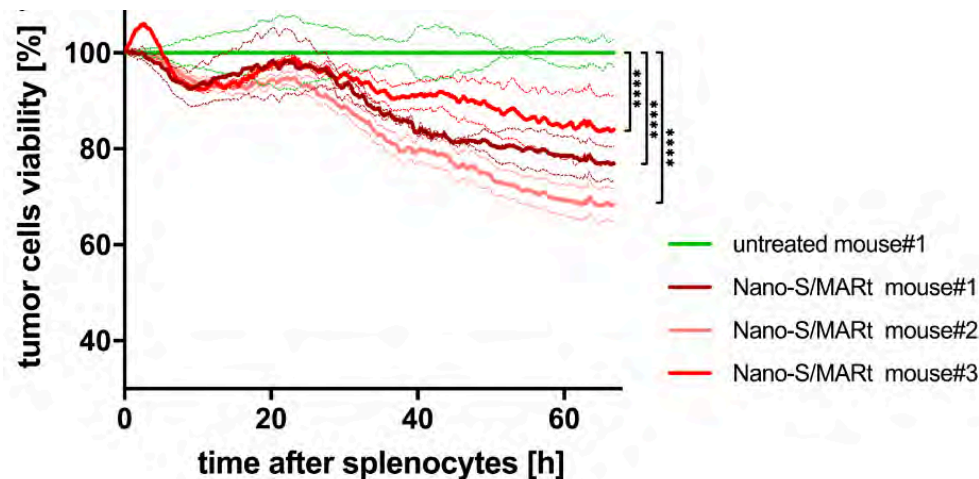
nS/MARt T-Cells delay tumor growth in vivo

Bozza et al. (2021) Science Advances

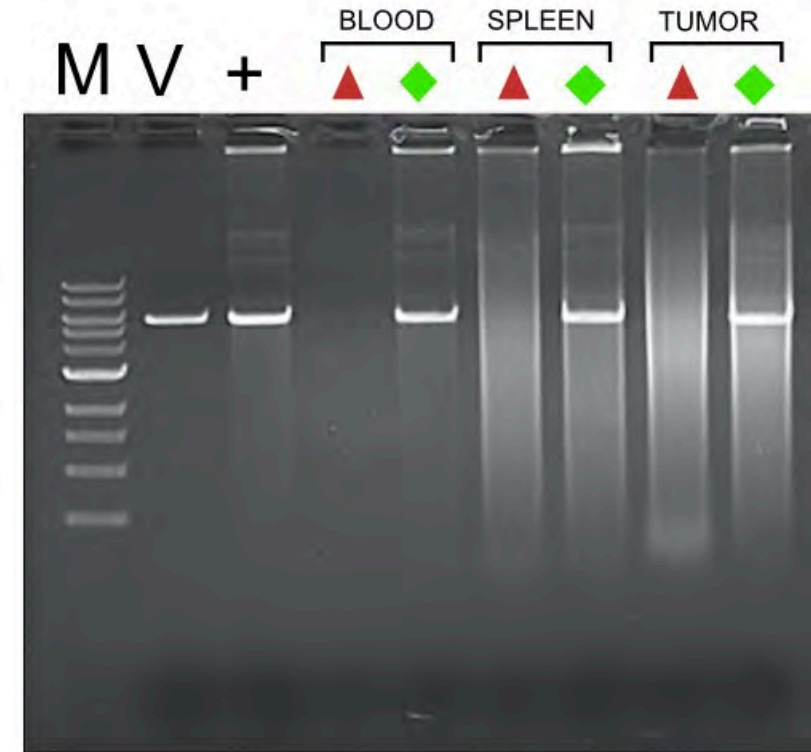
nS/MARt T-Cells retain long-term anti-tumor activity, the vectors remain episomal



Real-Time killing assay with T-Cells retrieved from spleens



Rolling Circle Amplification (RCA)

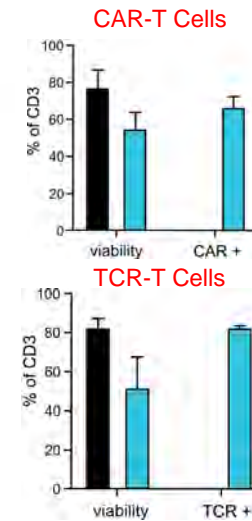
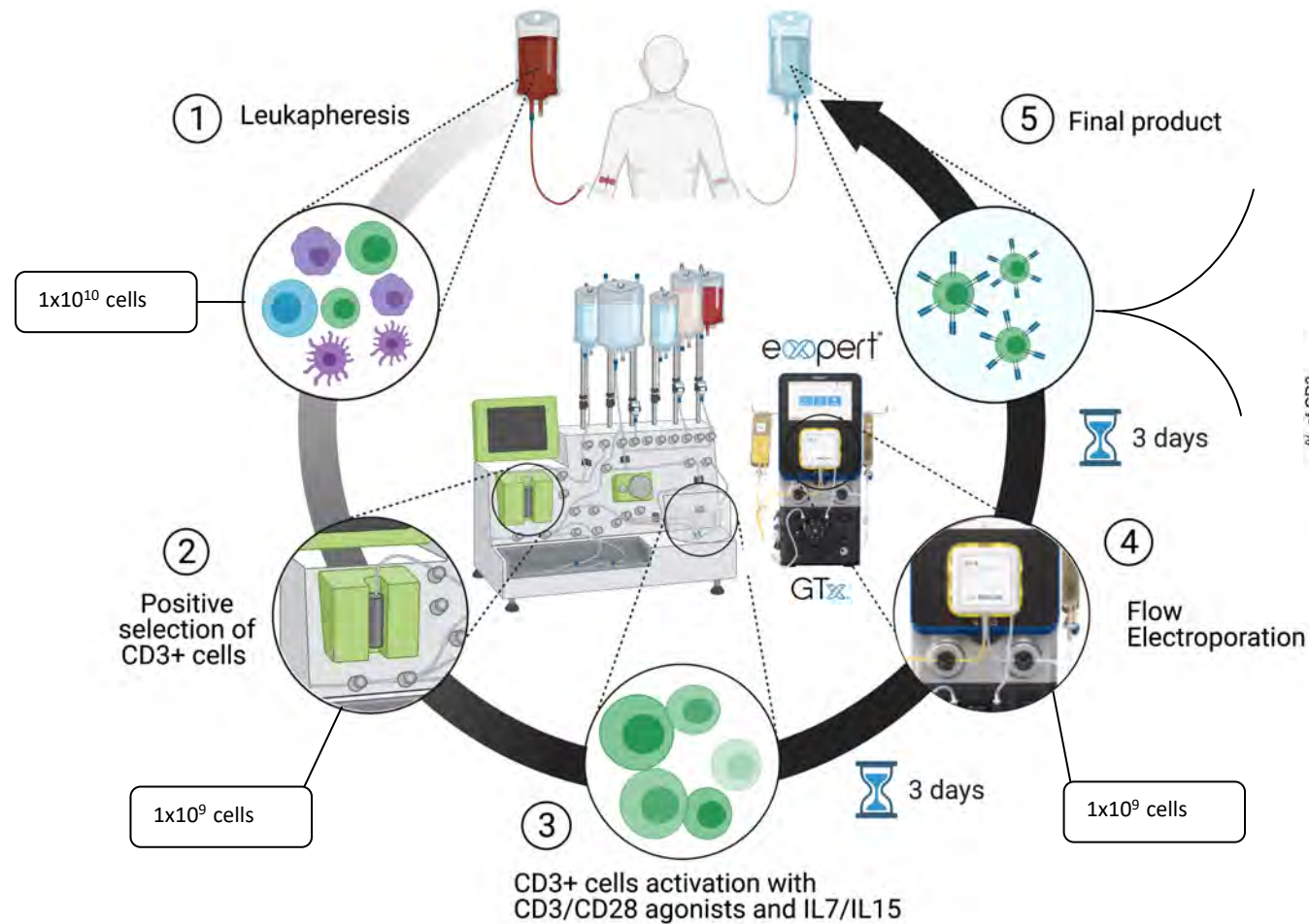


▲ Lenti CAR T-Cells ◆ nS/MARt CAR T-Cells

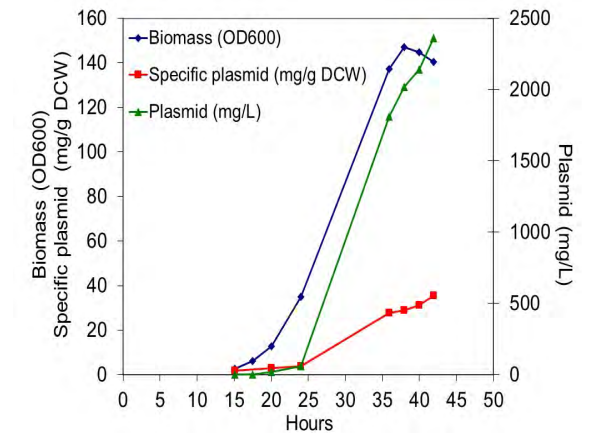
M=DNA marker , V=Vector control

+ = RCA on purified plasmid DNA

nS/MARt based clinical scale GMP-compatible manufacturing protocol

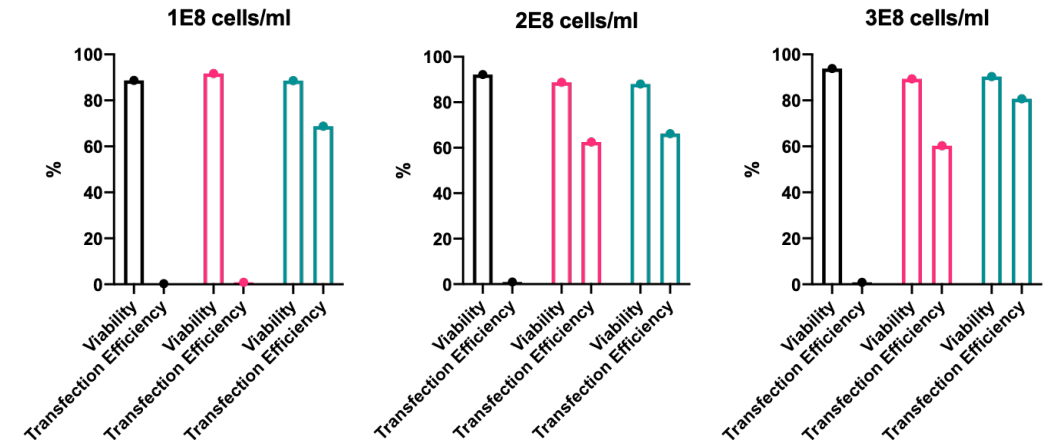


Large-scale DNA manufacturing

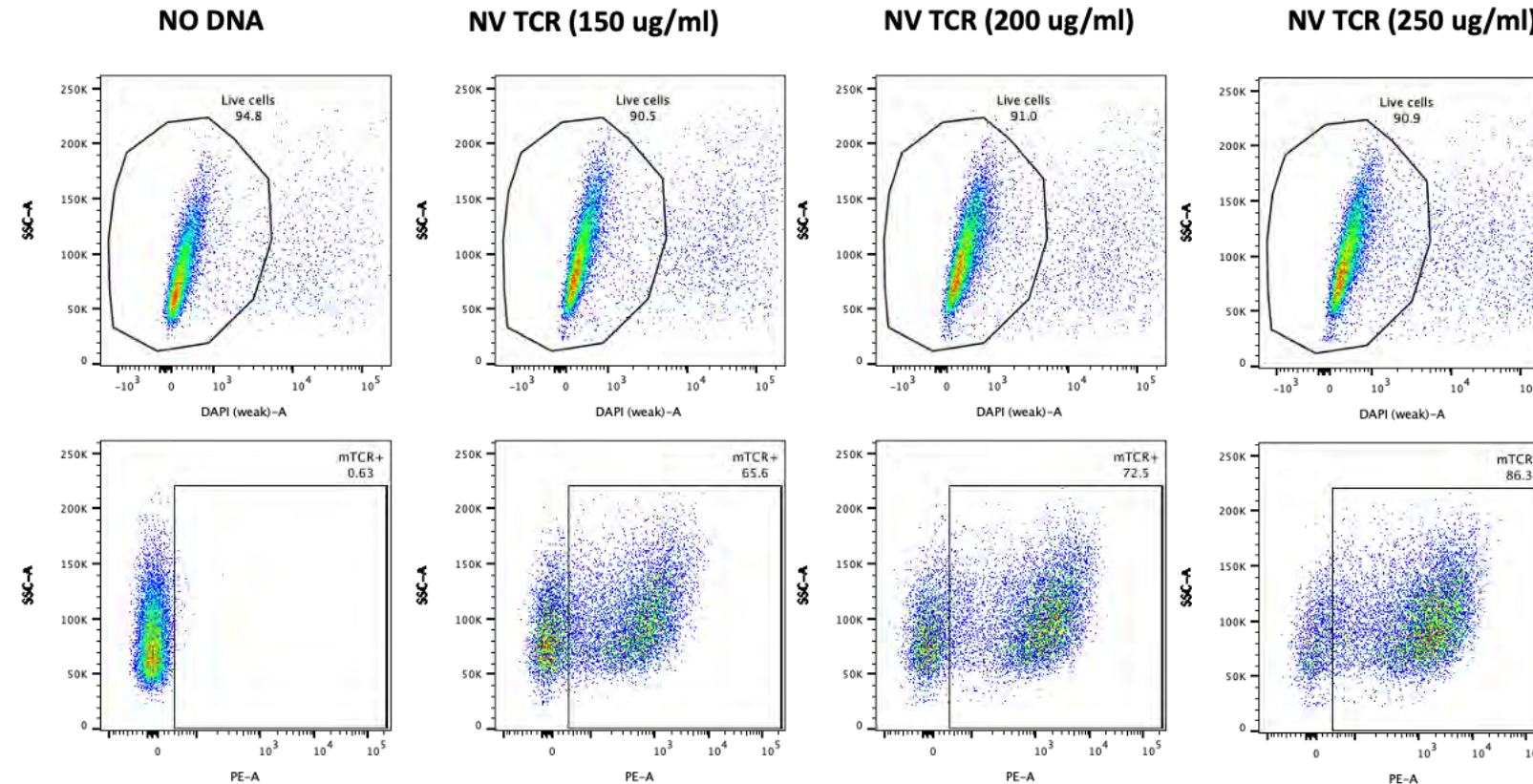
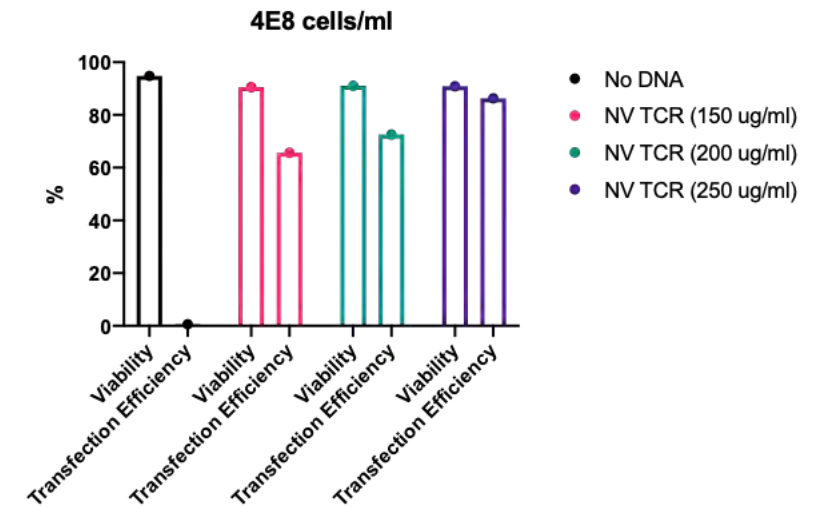


~ 2g/L of pure supercoiled DNA
1 L = 3500 patients

Safe and Efficient nS/MARt based Scalable GMP-compatible manufacturing protocol using MaxCyte Expert GTx electroporation Platform



⁸
4x10 Cells
Over 90% Viability
Over 80% TCR +

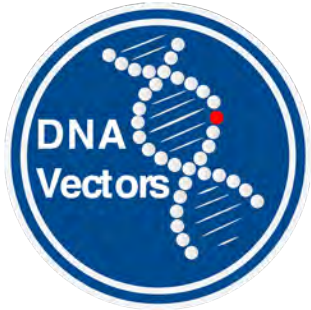


Nano-S/MARt DNA Vectors

Take home

- Best in class DNA Vector that combine mitotic stability with low immunogenicity
- Efficiently produced in bacterial cells at high yield and purity without post-processing
- Provide robust transgene expression in every cell and model system available
- Efficacy comparable to state-of-the-art clinically utilized vectors with short, efficient and economical manufacturing time

THANK YOU



HD Collaborators

Dr Edward Green
Prof Michael Platten

Prof Dirk Jäger
Dr Patrick Schmidt

Prof Rienk Offringa
Dr Mick Milsom

Dr Barbera Leuchs
Prof Stefan Eichmüller

DNA Vector Lab

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Manuela Urban
Alice De Roia
Julia Peterson
Luisa Burger
Anna Hartley

Annabel Grewenig



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German Cancer Research Center (DKFZ)
Heidelberg University Medical Center
Hospital for Thoracic Diseases
German Cancer Aid