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Improving the Safety of HSC Gene Therapy by Targeting/De-targeting Gene Transfer

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Improving the Safety of HSC Gene Therapy

- *Regulate transgene expression*
 - Ectopic or constitutive expression may cause toxicity
- *Alleviate risk of insertional mutagenesis*
 - Improve vector choice & design
- *Target integration to specific sites*

Targeting Gene Transfer

- *Transcriptional targeting*

Select lineage-specific transcriptional control elements

→ *Transgene expression directed to desired cell lineage*

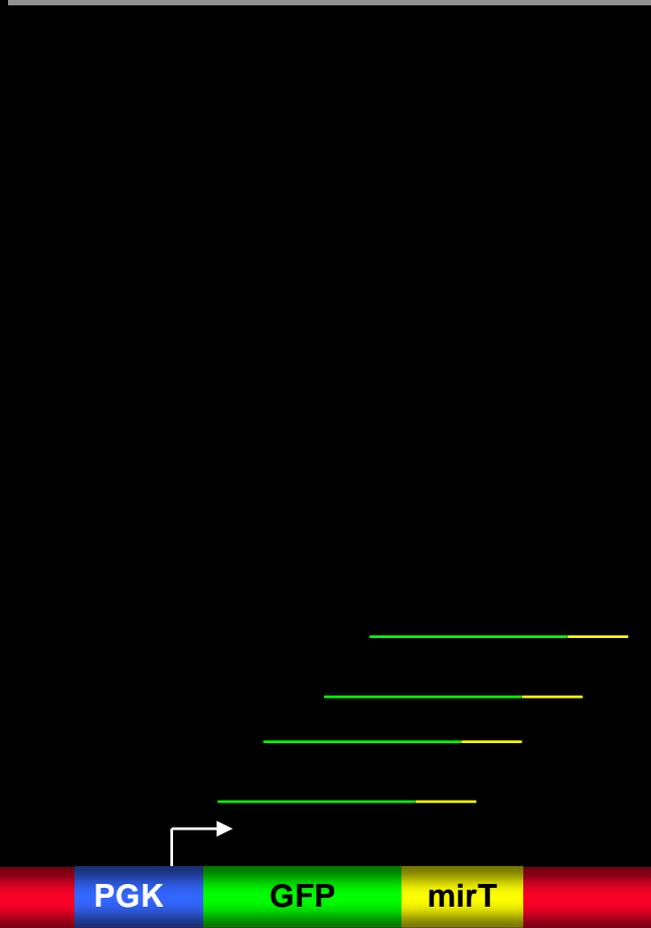


- *Post-transcriptional targeting*

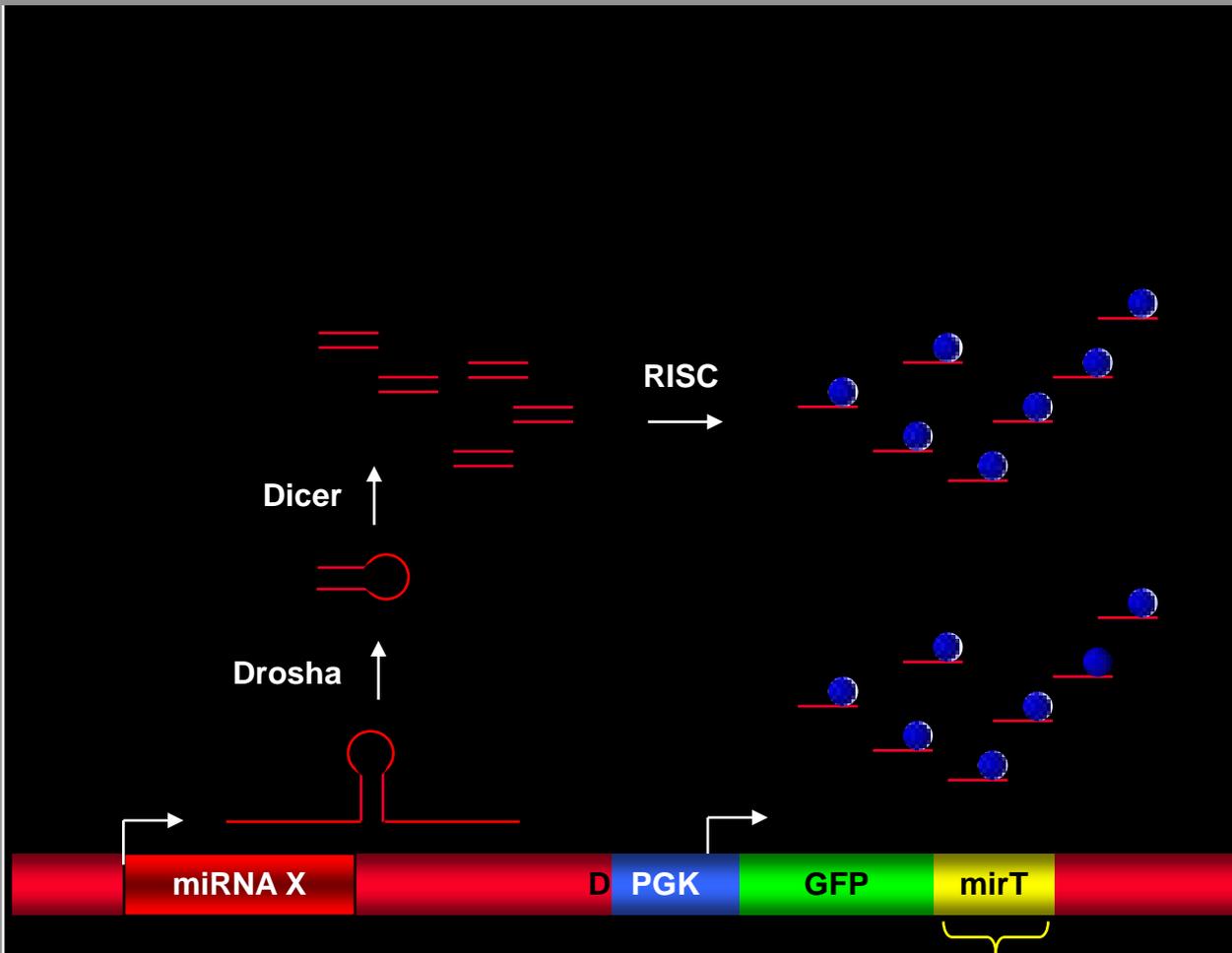
*Incorporate miRNA target sequences
into vector transcript*

→ *De-target* from unwanted cell types

Regulating Transgenes by Endogenous miRNAs



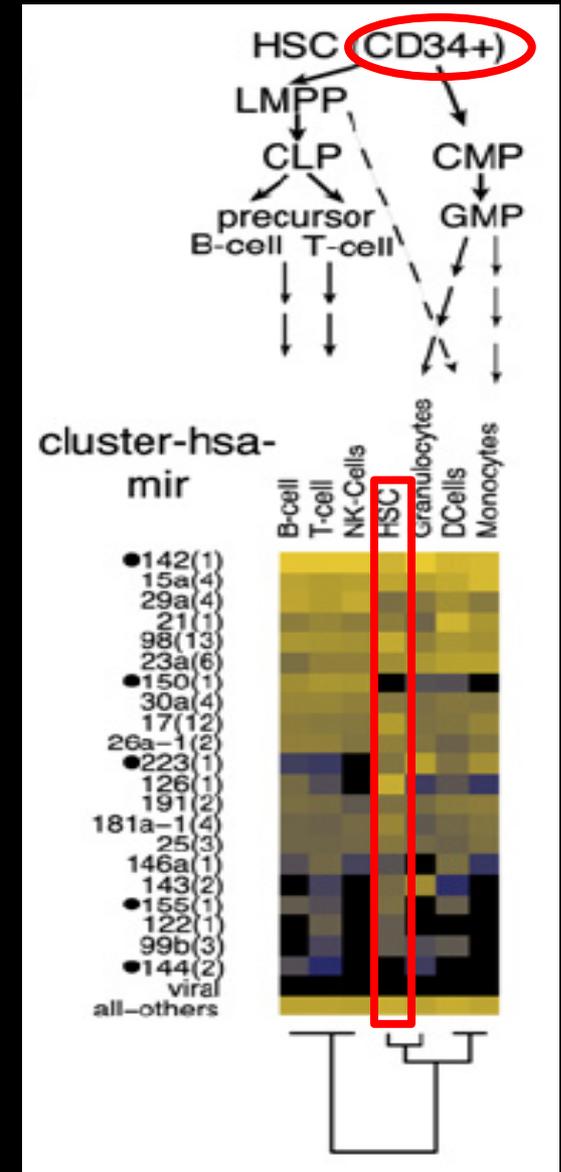
Mature Cells



Stem/Progenitor Cells

miRNAs in Hematopoietic Stem Cells

- Little is known of miRNAs expressed in hematopoietic stem/progenitor cells (HSPC)
- Expression profile data
 - bulk vs. pure populations
 - in rare cell populations
- Functional activity
 - expression level vs. activity
 - *in vivo* studies

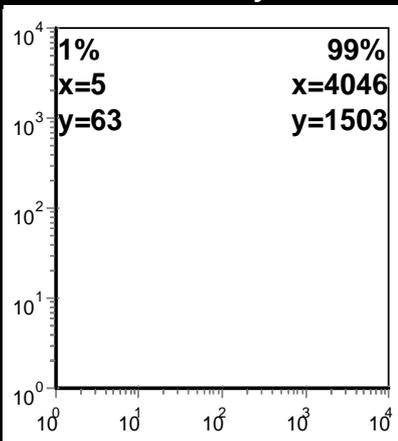


Bidirectional miRNA-Regulated Vectors

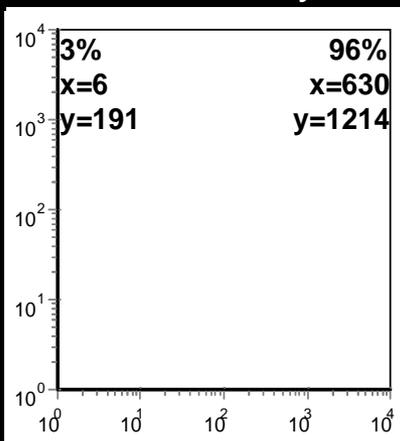


*Amendola et al,
Nat Biotech 2005*

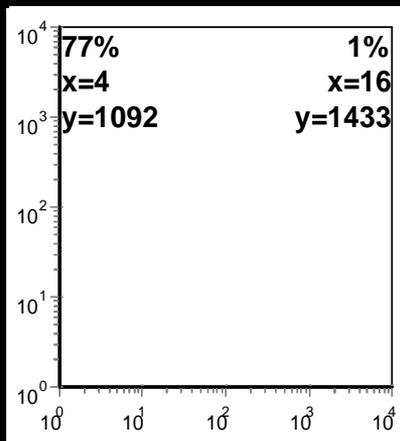
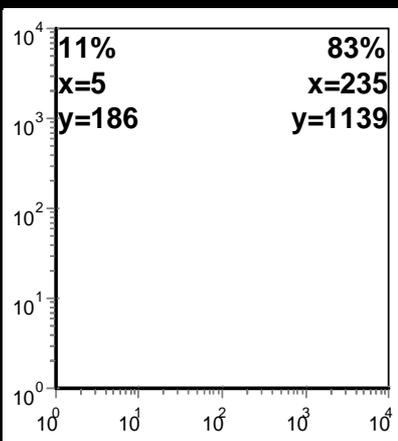
293T Kidney Cells



U937 Monocytes



ΔLNGFR



GFP →

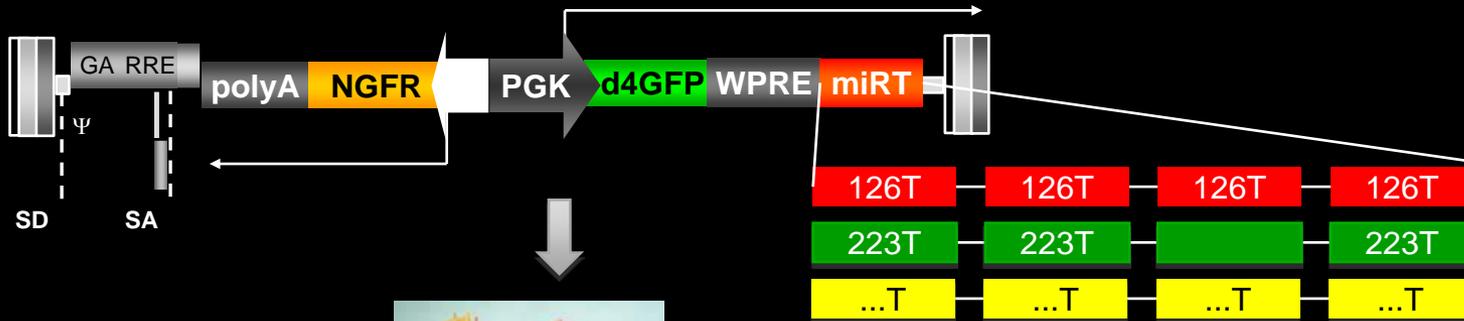


Bd.LV.miRT

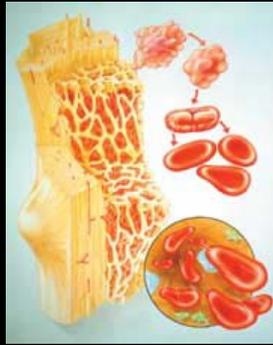
Brown et al, Nat Med 2006

Brown, Gentner et al, Nat Biotech 2007

Profiling miRNAs in Mouse HSPC



Ex vivo HS/PC transduction

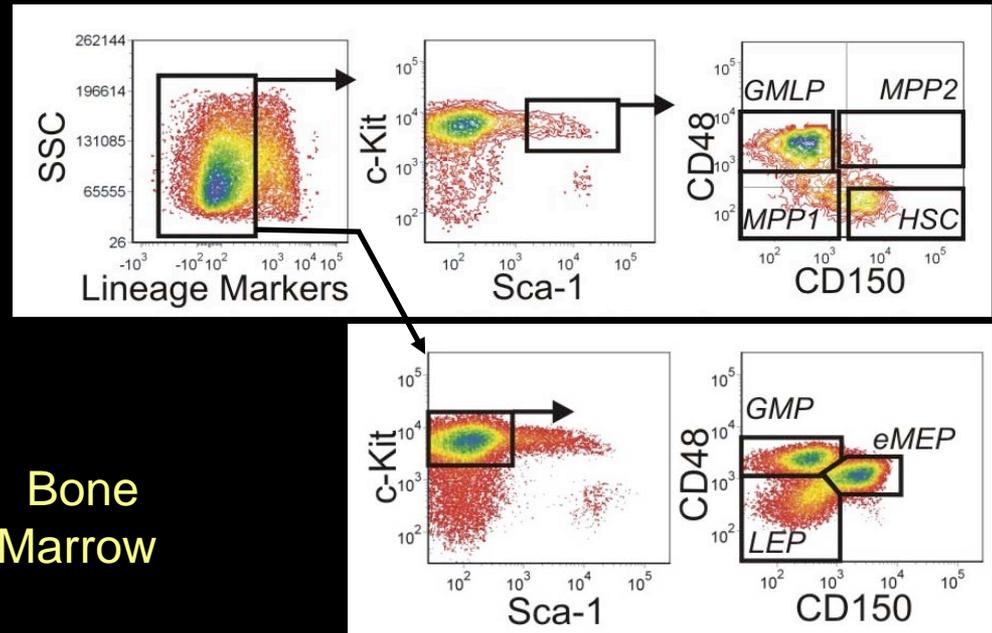


Transplant into conditioned mice

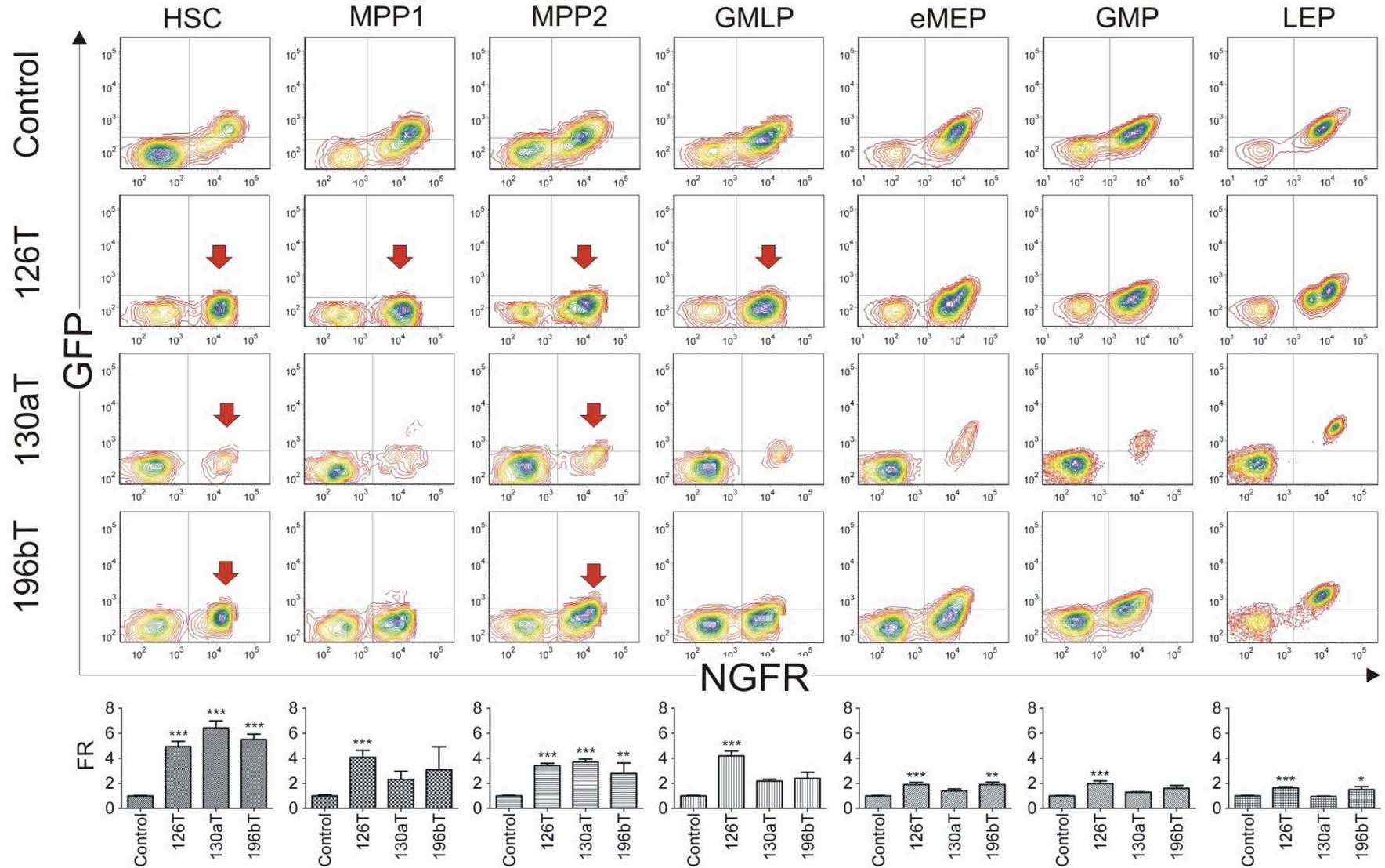


Bone Marrow

Identify HS/PC by immunophenotyping



miRNAs Specific for HSC / Early Progenitors



miRNAs in Human HSPC and LV Regulation

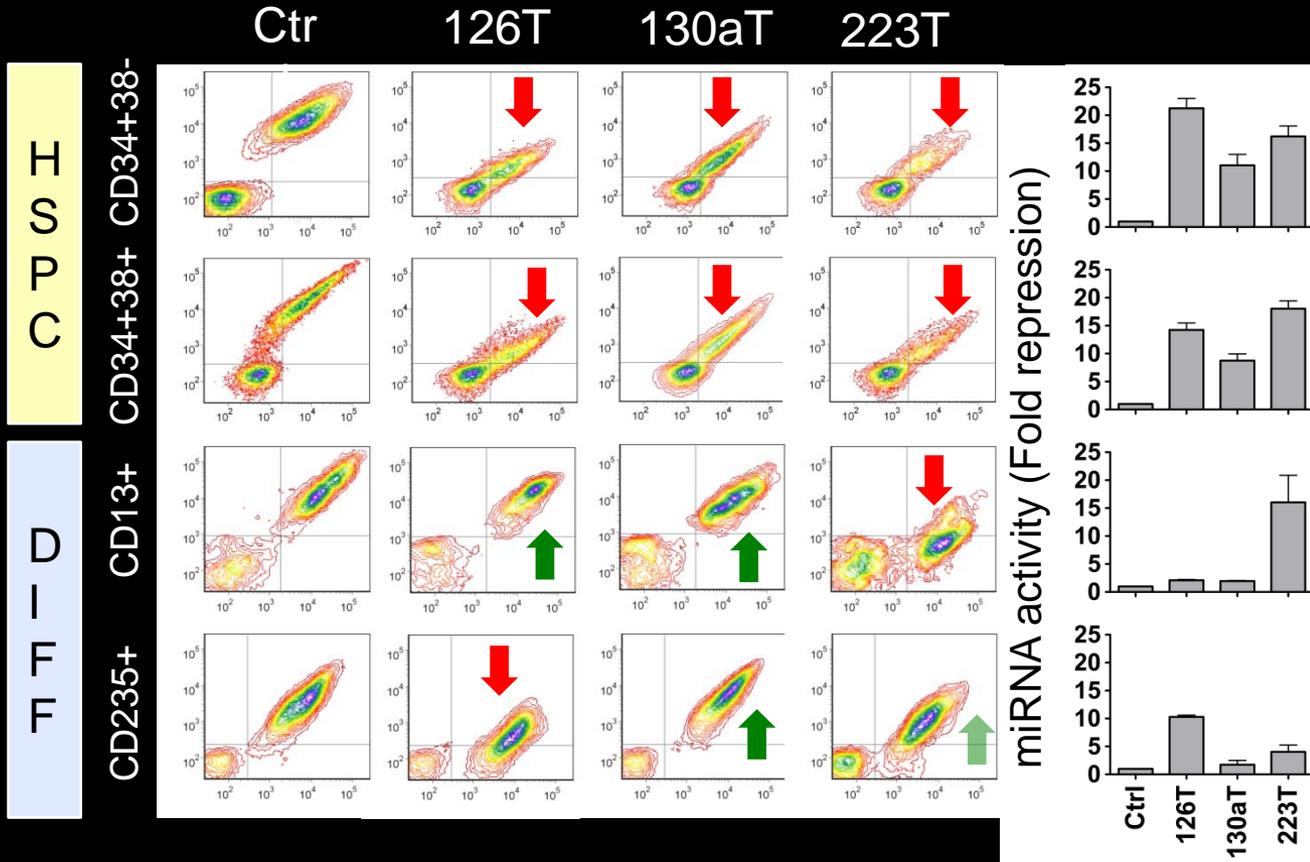


Transduce human HSPC
(CD34+ cells from cord
blood or bone marrow)

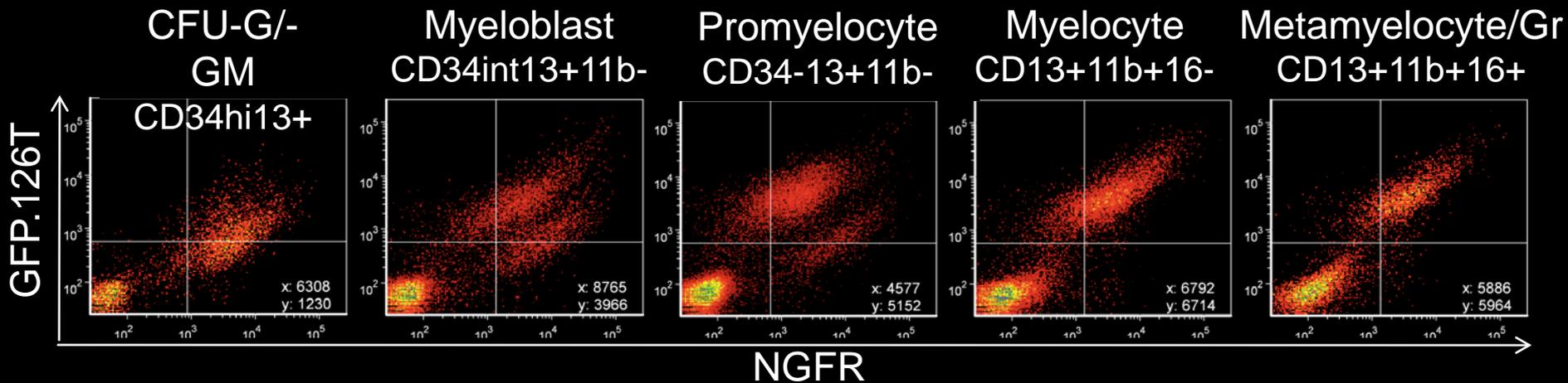
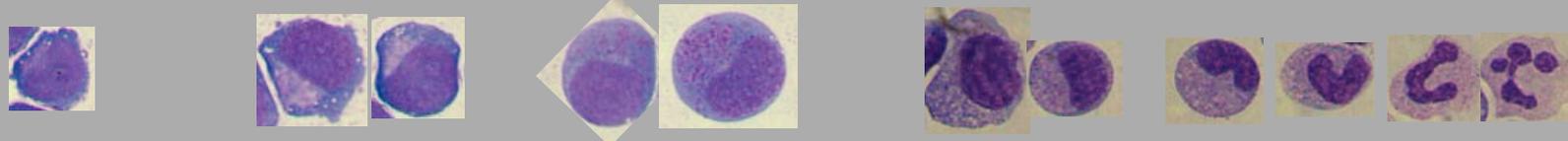
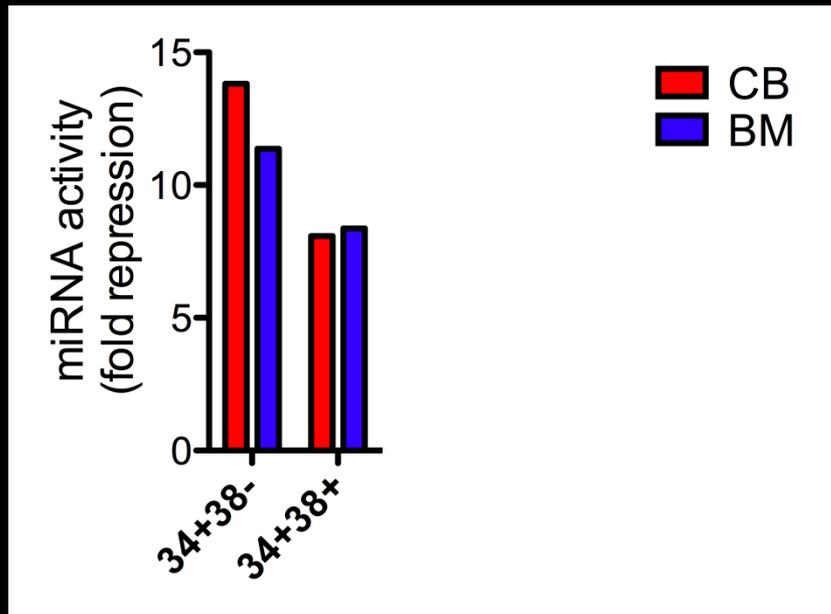
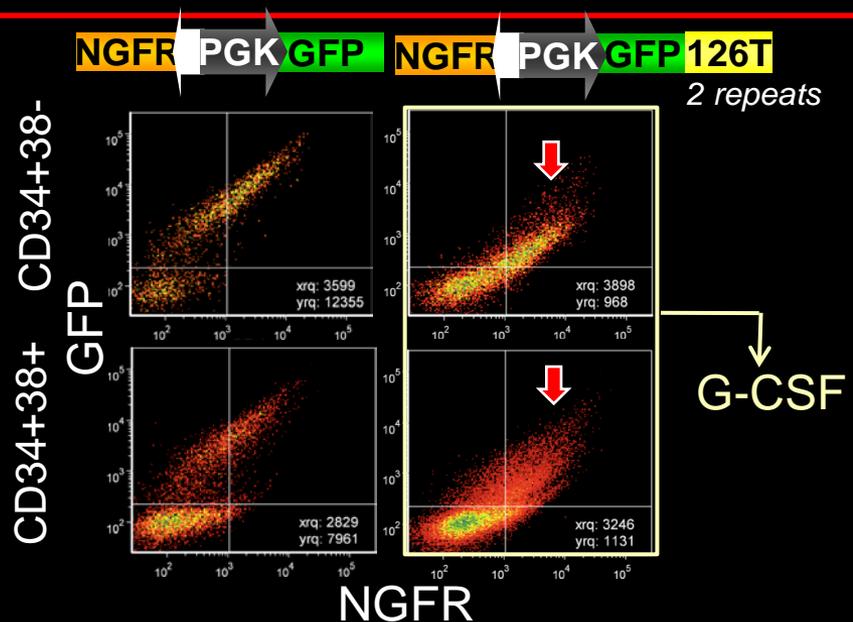
Read miRNA activity

In HSPC
(2 days post
transduction)

After DIFF
(*in vitro*
culture)



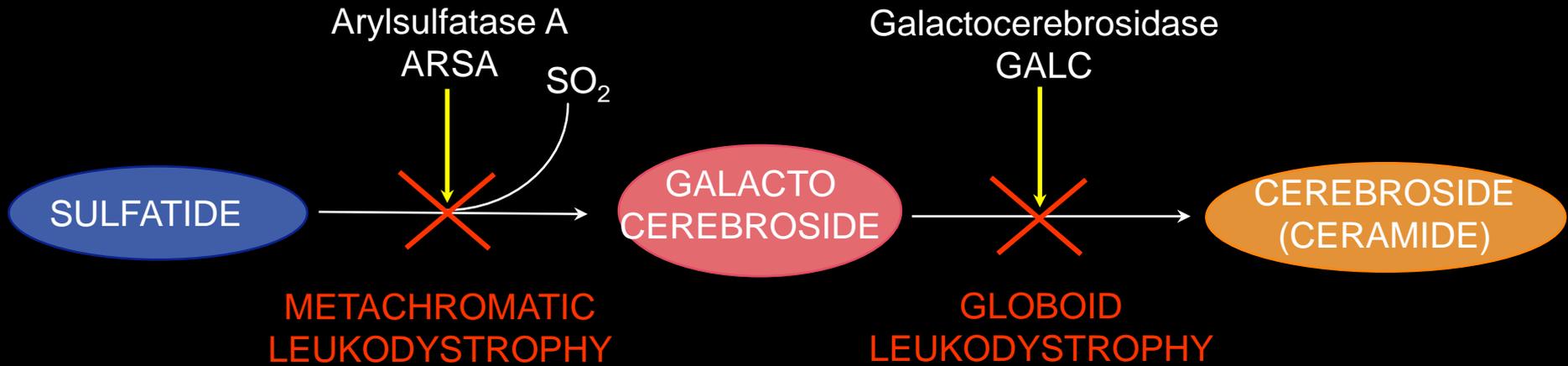
miR-126 in Human HSP & Myeloid Cells



Summary: miRNAs in HS/PC

- *Quantitative functional profile of miRNAs*
 - miRNAs specifically active in human and murine HSPC identified
- *Exploit them to*
 - Improve the safety of HSC gene therapy

Gene Therapy of Leukodystrophies



Transplantation of autologous LV-transduced, enzyme over-expressing HSC

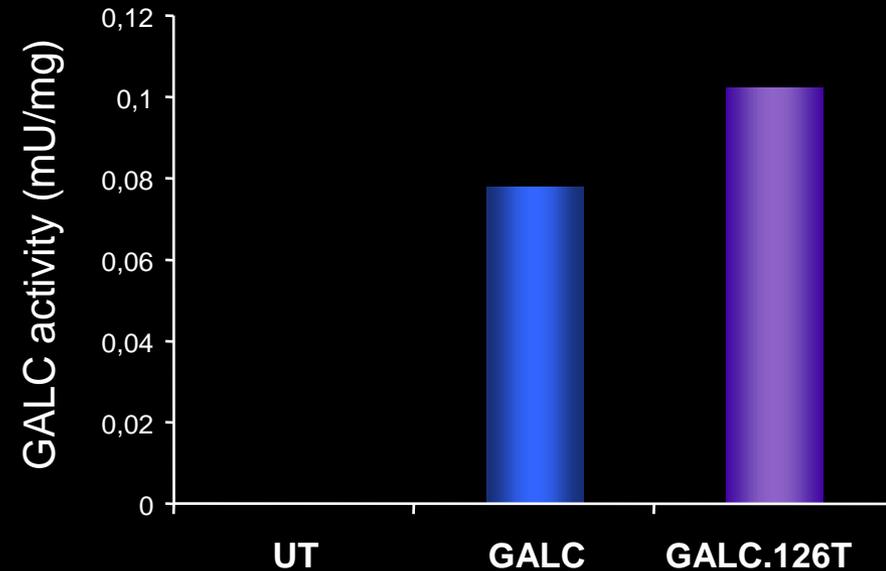
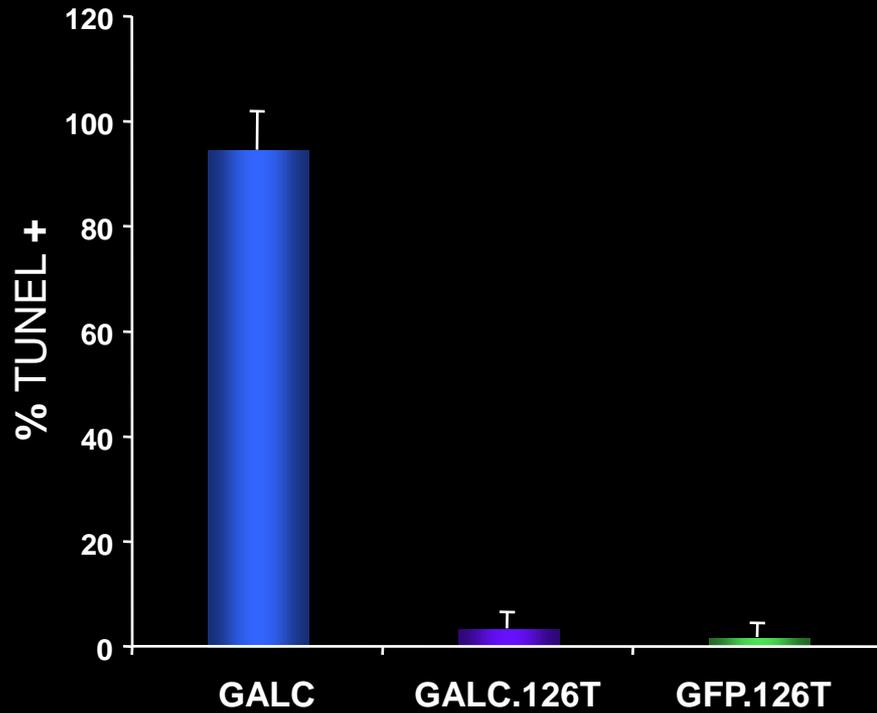
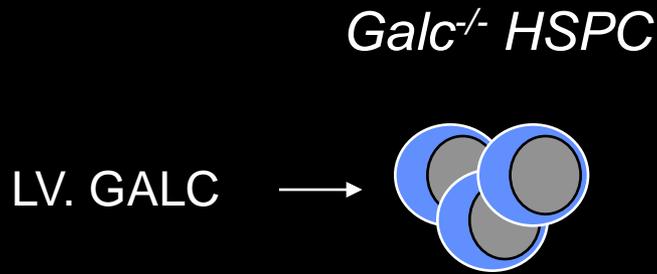
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Toxicity of GALC over-expression in HSPC but *NOT* in differentiated progeny

↓

De-target expression from HSPC

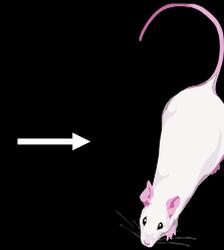
GALC Expression in HSPC is Limited by Toxicity



GLD Gene Therapy by LV.126T

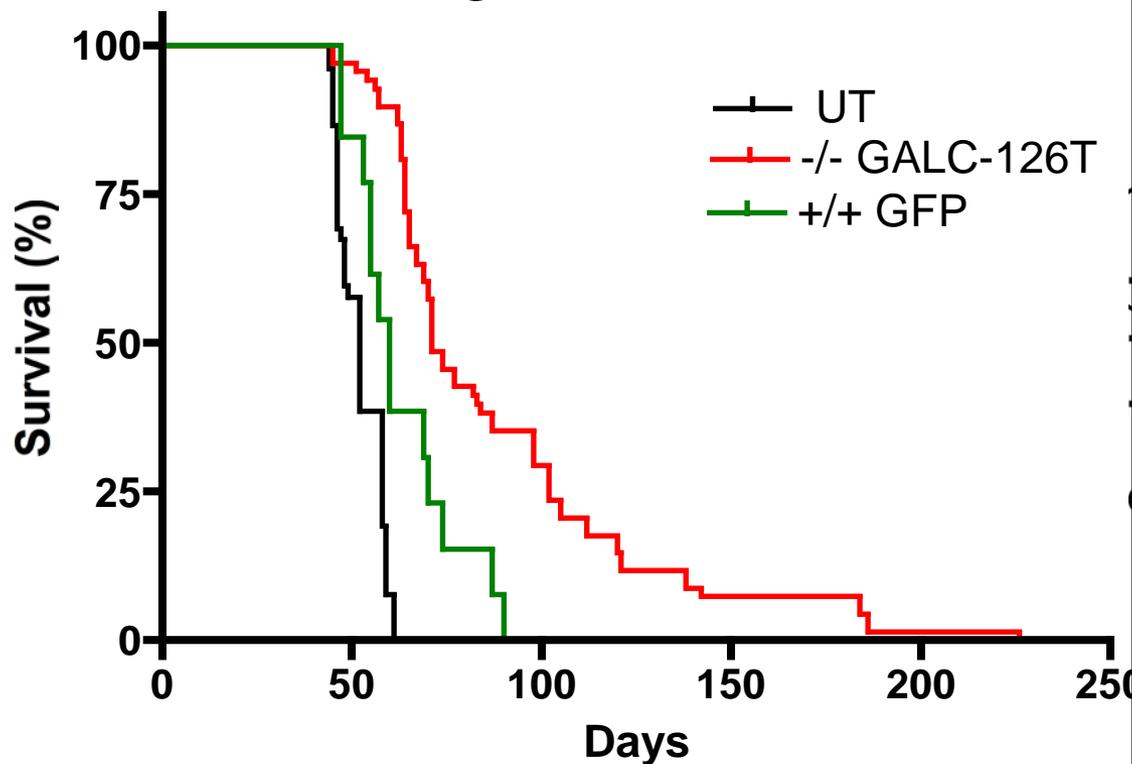
Wild-type HSPC  + LV.GFP

Galc^{-/-} HSPC  + LV.GALC.126T



Transplant
into neonate
GLD mice

Prolonged survival



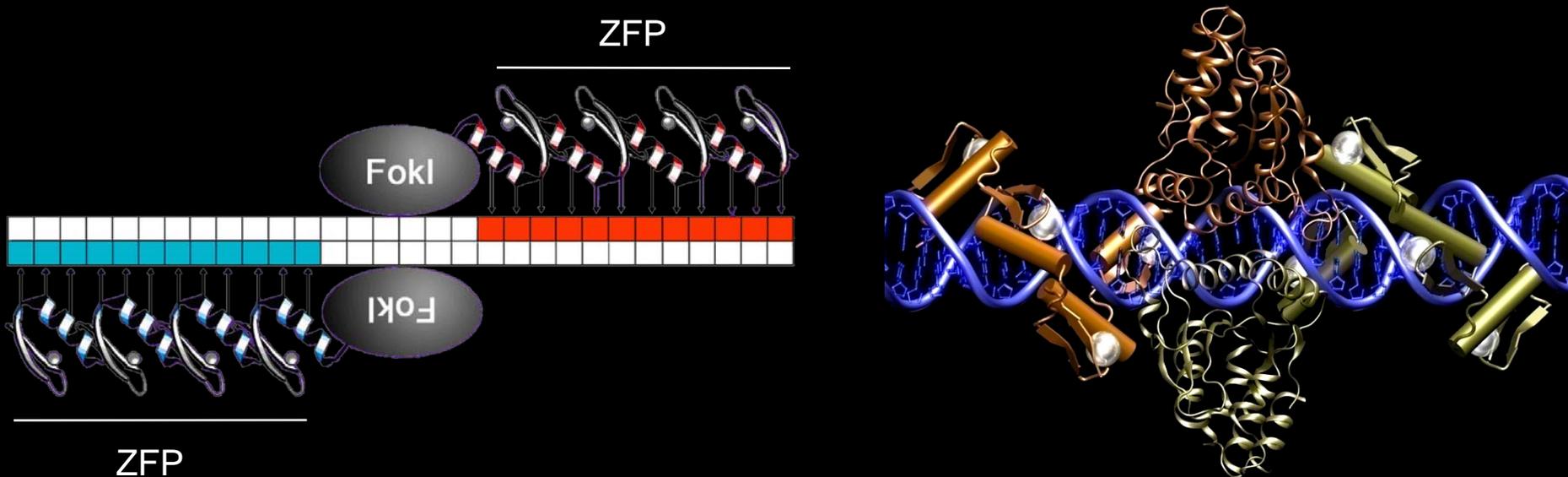
Summary: miR-126 LV & GLD Gene Therapy

- *miR-126 regulated LV*
 - Protect human and mouse HSPC from GALC toxicity
 - Enable HSC-based gene therapy of GLD in mouse model
 - Can be broadly used to improve safety of HSC gene therapy

Site-Specific Gene Insertion

- *Minimizes risk of insertional mutagenesis*
- *Insertion downstream endogenous promoter*
 - Restores function and endogenous expression control (gene correction)
 - Most mutations, including insertions and deletions can be corrected
- *Insertion into a safe genomic site*
 - Robust predictable expression without perturbing endogenous transcription

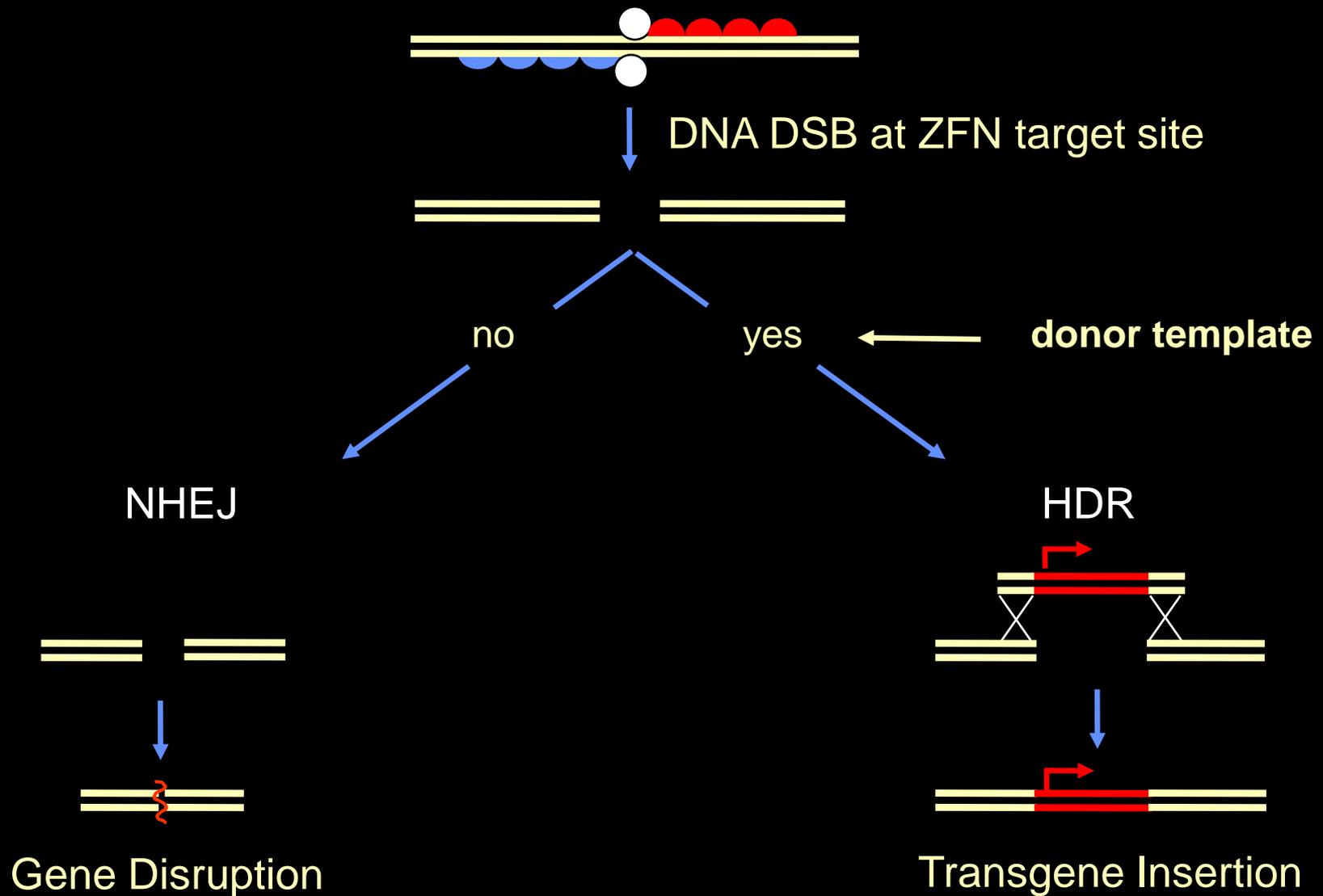
Designed Zinc Finger Nuclease (ZFN)



- Contains two domains:
 - Nuclease domain of FokI restriction enzyme
 - Engineered Zinc Finger Protein (ZFP)
- Cleaves DNA as a dimer
- Can be engineered to cleave a pre-determined sequence

*Kim & Chandrasegaran, PNAS USA 1996; Porteus & Baltimore, Science 2003
Bibikova & Carroll, Science 2003; Urnov et al. Nature 2005*

Exploiting ZFNs for Gene Targeting



Efficiency of Targeted Integration in Human Cells

<i>Cell Type</i>	<i>Target Site</i>	<i>% Transgene Positive</i>
Cell Lines (several types)	<i>ILR2G, CCR5, AAVS1</i>	up to 60%
Primary Fibroblasts	<i>CCR5, AAVS1</i>	up to 11%
Primary Keratinocytes	<i>CCR5</i>	up to 5%
Primary Lymphocytes	<i>CCR5, AAVS1</i>	up to 5%
Hematopoietic Stem/Progenitors	<i>ILR2G, CCR5, AAVS1</i>	up to 0.3%
ES Cell Lines	<i>ILR2G, CCR5</i>	up to 5%
iPS Cells	<i>CCR5, AAVS1</i>	up to 1%
Neuronal Stem Cells	<i>AAVS1</i>	up to 15%

Summary: Gene Correction

- *Knock-in of IL2RG cDNA downstream endogenous promoter*
 - normal γ -c expression
- *Gene correction coupled to selectable marker*
 - in primary SCID-X1 patient's fibroblasts
- *Reprogramming by single copy excisable LV*
 - Vector- and Reprogramming Factors-free, gene corrected iPSC

Insertion into a Safe Genomic Site?

- *Choice of site not obvious*
 - Gene desert vs. inter- or intra-genic
 - Info available mostly for transcribed genome
- *Knock-out of target locus must be well tolerated*
 - Insertion may inactivate target gene
 - ZFNs may disrupt both alleles
 - Candidate Loci: **CCR5, AAVS1**
- *Robust & predictable transgene expression*
- *Lack of interference with expression of the flanking genes*

Summary: Assessing Safe Insertions in the Genome

- *Transcriptional perturbation*
 - Upregulation of flanking genes depends on type - but not strength - of exogenous promoter
 - varies with the targeted locus
 - interference with transcription of targeted gene can be prevented by vector engineering
- *AAVS1 has desirable features*
 - No deregulation of flanking genes even with strong promoter
 - Stable robust expression

Contributors: HSC miRNAs & GLD Gene Therapy



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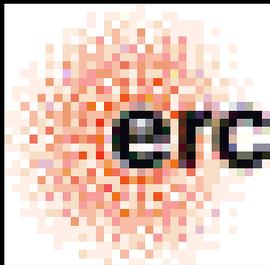
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**European Research
Council**
Advanced Grant



Contributors: Site-Specific Integration



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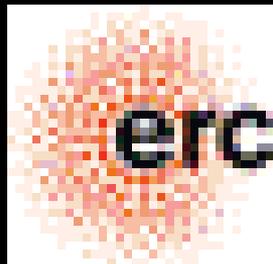
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Margherita Neri



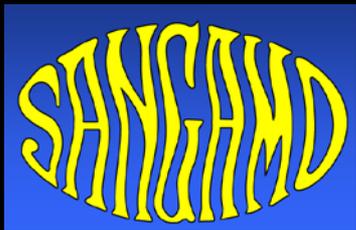
European Research
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