T1DM and Immune system evasion:

Evaluation of a stem cell-based approach

GENE THERAPY COURSE 2017/2018
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SAPIENZ
UNIVERSITÀ DI RO

Type 1 diabetes mellitus

Autoimmune disease that develops as a consequence of :

Genetic predisposition

Environmental factors

Stochastic events

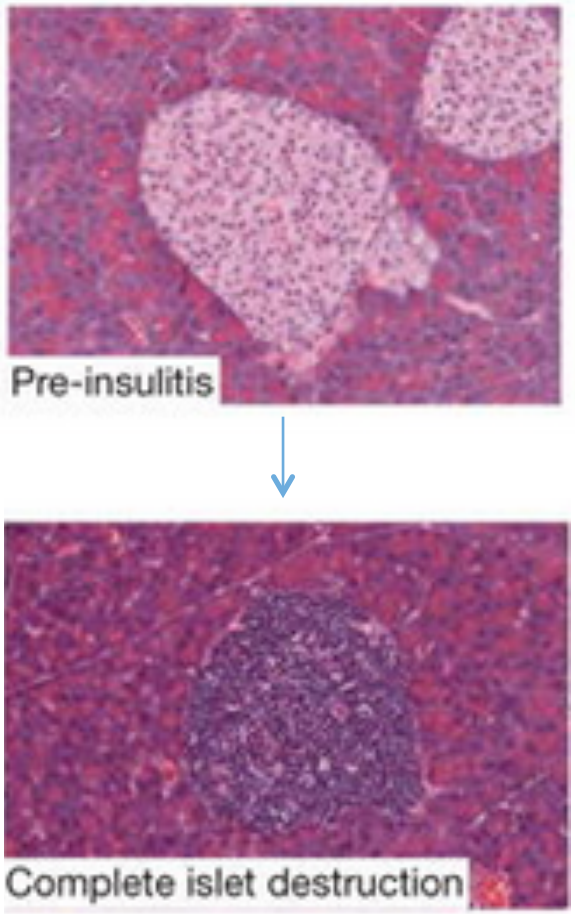
Characterized by an organ-specific immune destruction of insulin-producing β-cells

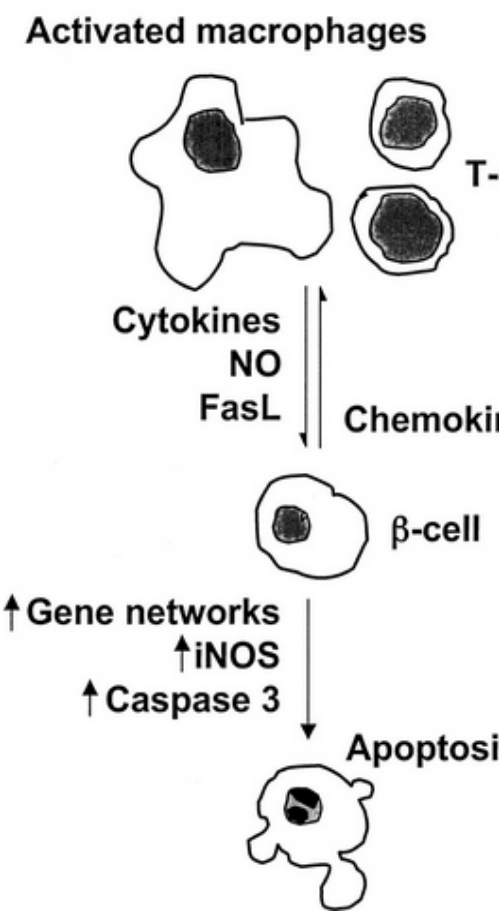
→ ABSOLUTE INSULIN DEFICIENCY

is associated with an increased risk of

heart disease, stroke, blindness, kidney failure.

There is still not a cure, hyperglycemia is taken under control by daily insulin administration.





J.A. Bluestone et al.,Nature (201

Overview of our strategy

NOD mouse

1

De-differentiation through Yamanaka's factors

IPS Cells

Lentiviral Infection

- IDO
- PDL1+CTLA4
- PDL1+CTLA4+IDO

Goals:

- Replacement of functional β-like cells
- Auto immune evasion

Differentiation in Pancreatic β -like cells: «Lineage-control Network» protocol

C-IPS Cells

3

β-like cells

Cells injection In Kidney and Liver

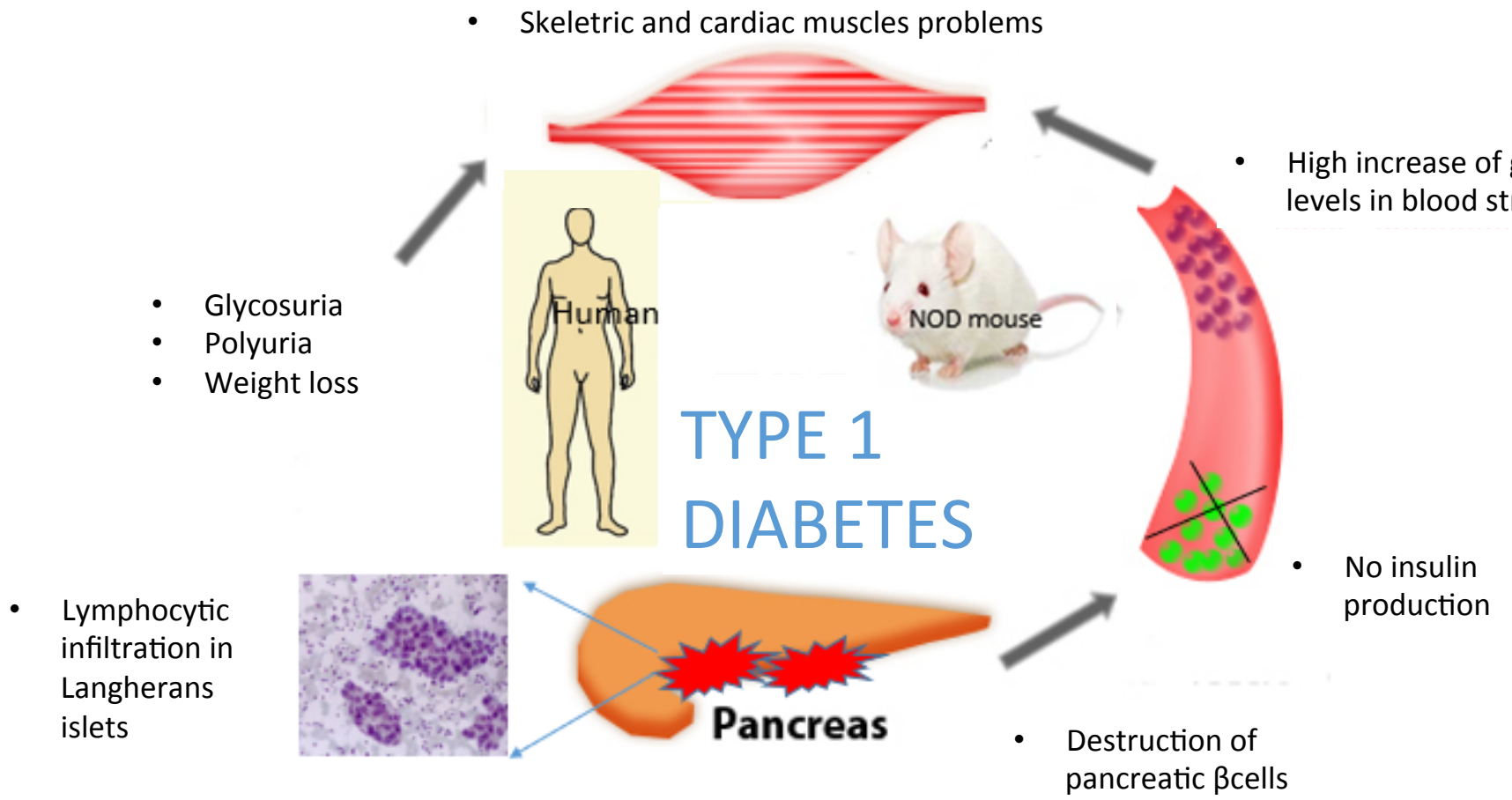
4

Nephrectomy Hepatectomy

In vivo results

NOD mouse

- **The Non-Obese Diabetic mouse model matches closely enough with T1DM patients**



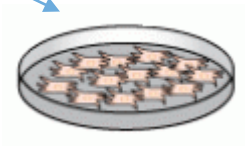
J.A.Pearson et al., *J.Autoimmu*

1 MAF-derived iPS cells and validation of pluripotency

NOD mouse

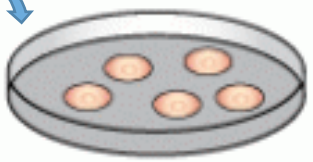
Oct4
Sox2
c-MYC
KLF4

aka's factors
ramming

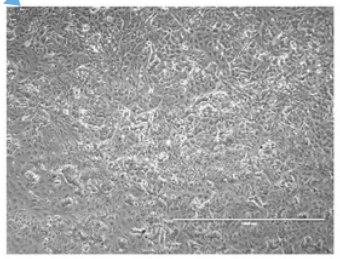


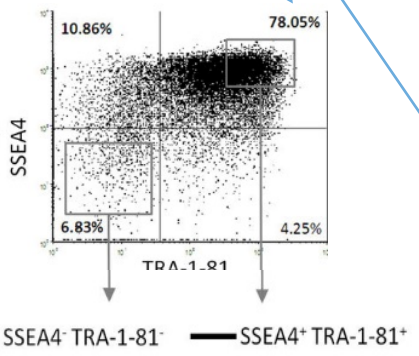
Somatic cells:
MAF (mouse Adult Fibroblast)

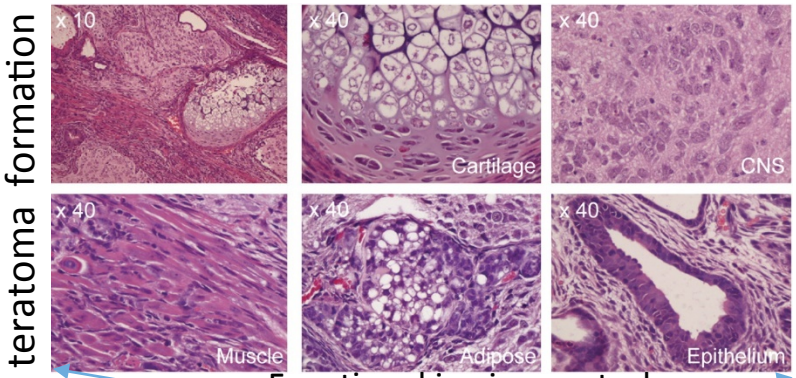
micin
ction



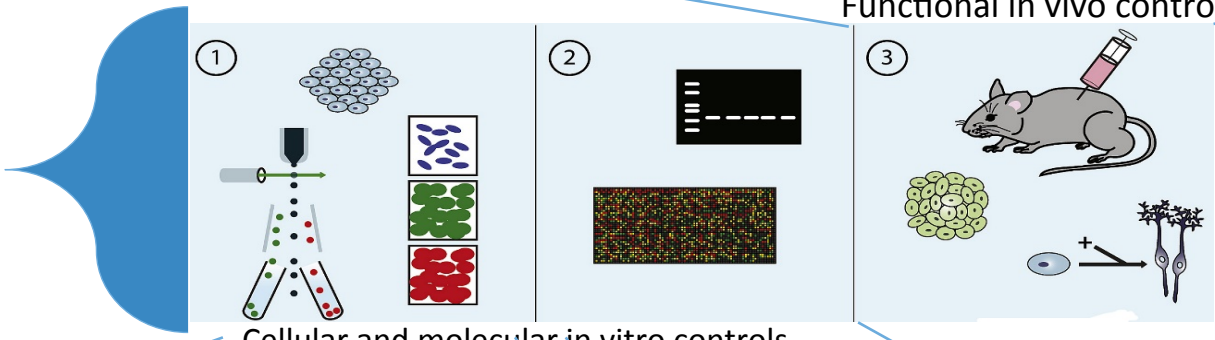
NOD- iPScells



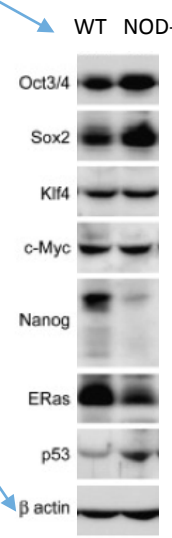




Functional in vivo control

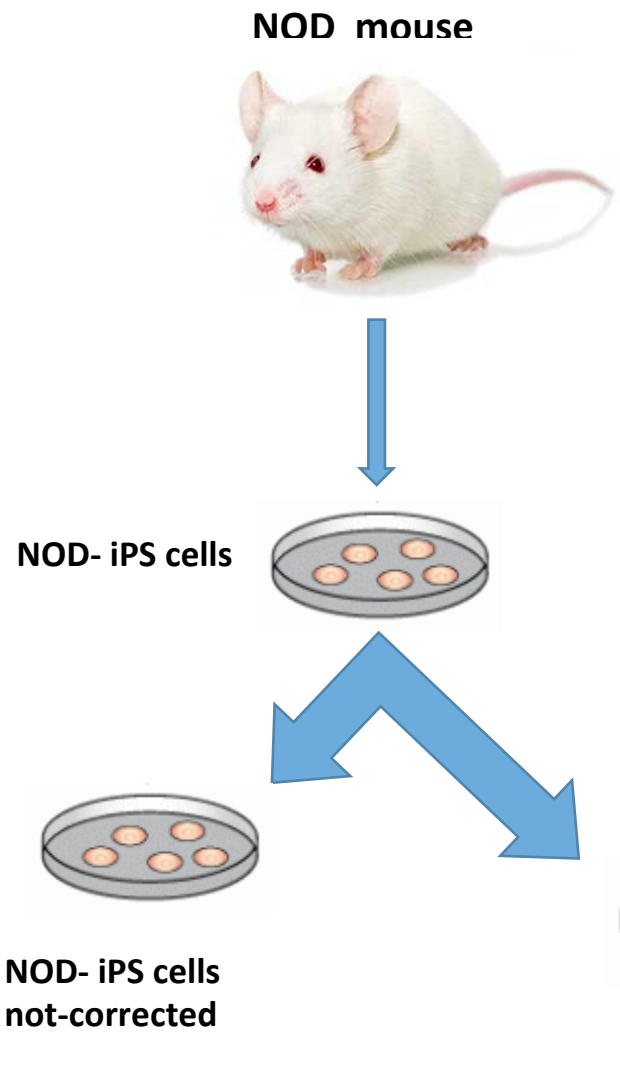


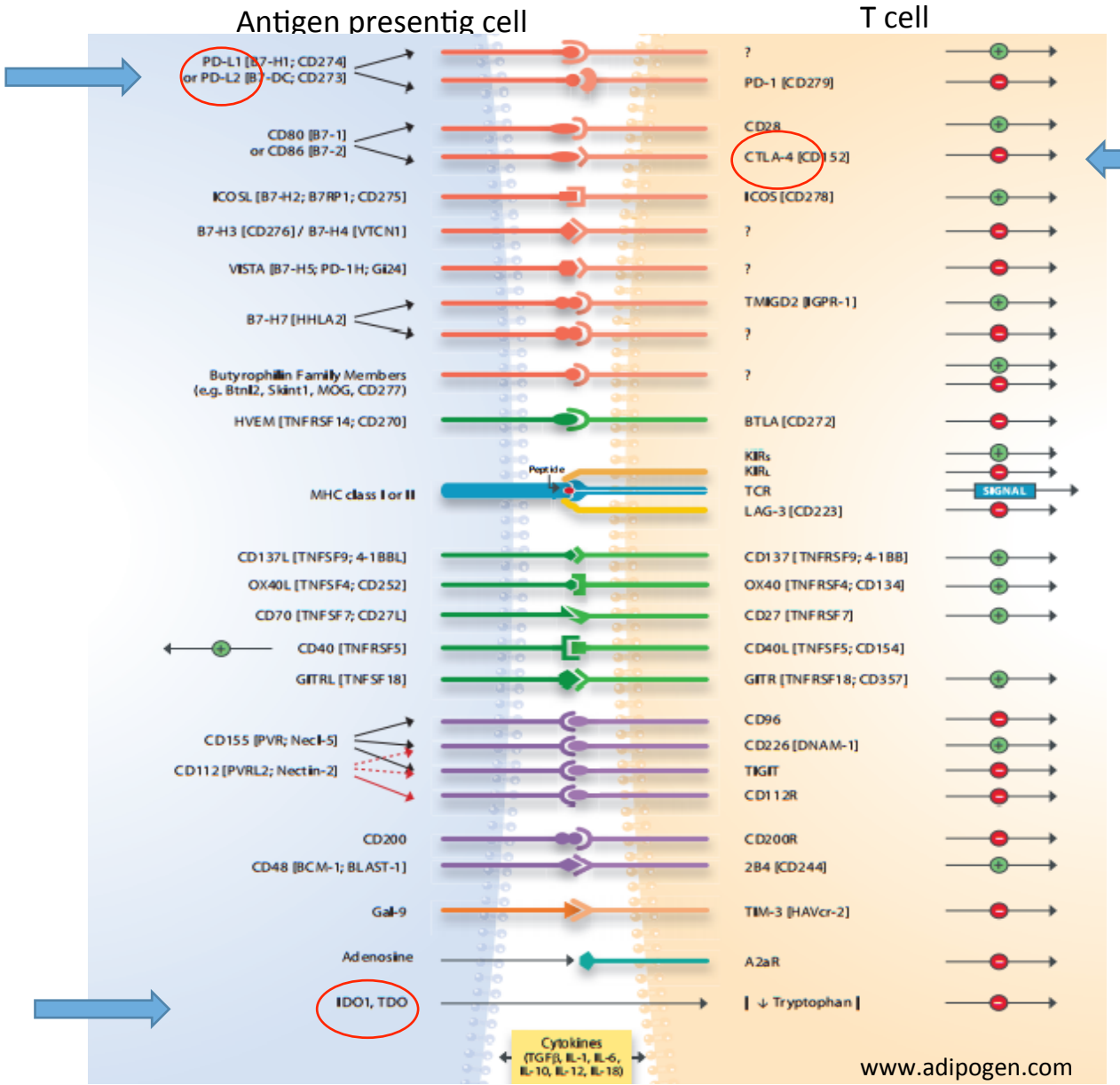
Cellular and molecular in vitro controls



K.Takahashi et al., C

What are we going to do with MAF-derived iPScells?



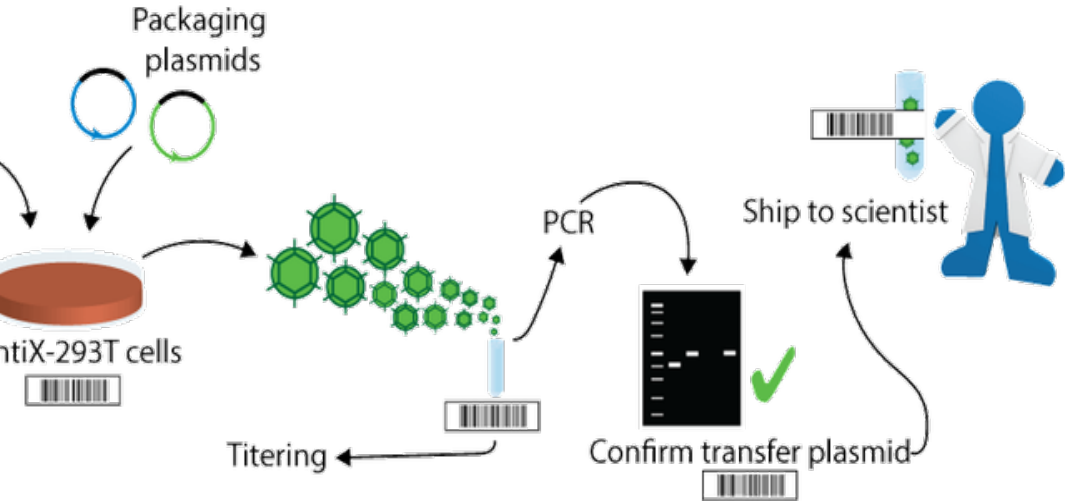


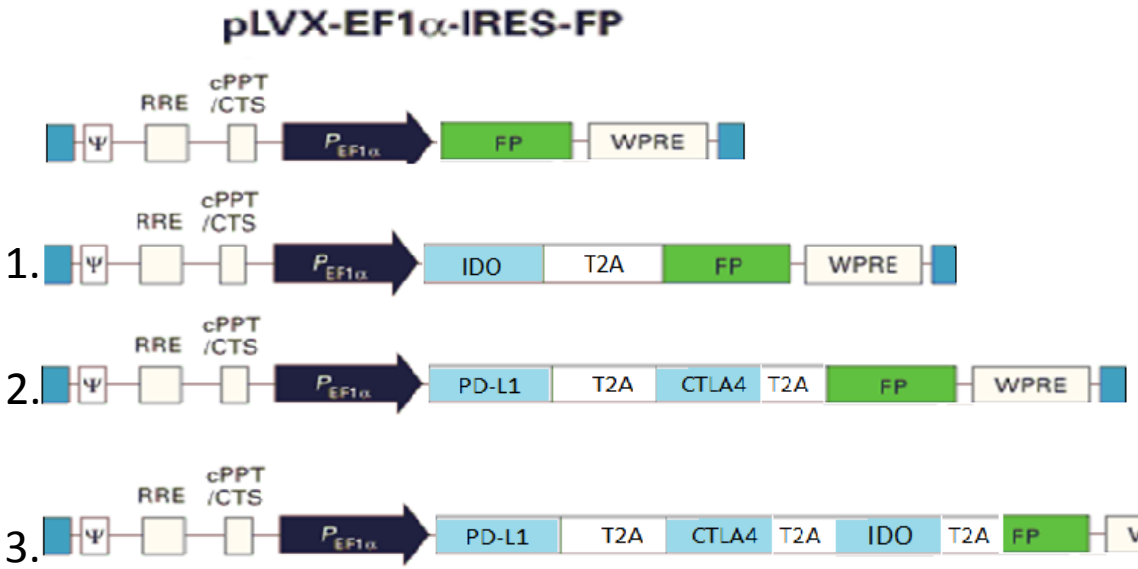
2

How to correct iPSC?

lentiviral vector mediated gene therapy:

ycystronic transfer plasmid with a constitutive promoter containing the transgenes separated by a self-cleavable T2A peptide linke
- IDO
- PD-L1 + CTLA4
- PD-L1 + CTLA4 + IDO





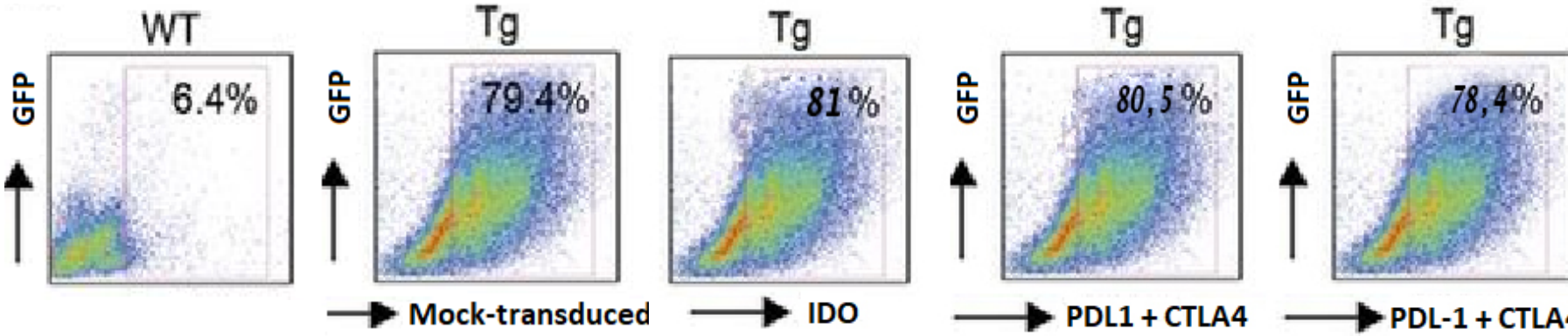
Vector taken from www.Addgene.o

M. B. Nasr et al. *Science Translational Medicine*

Trasduction efficiency

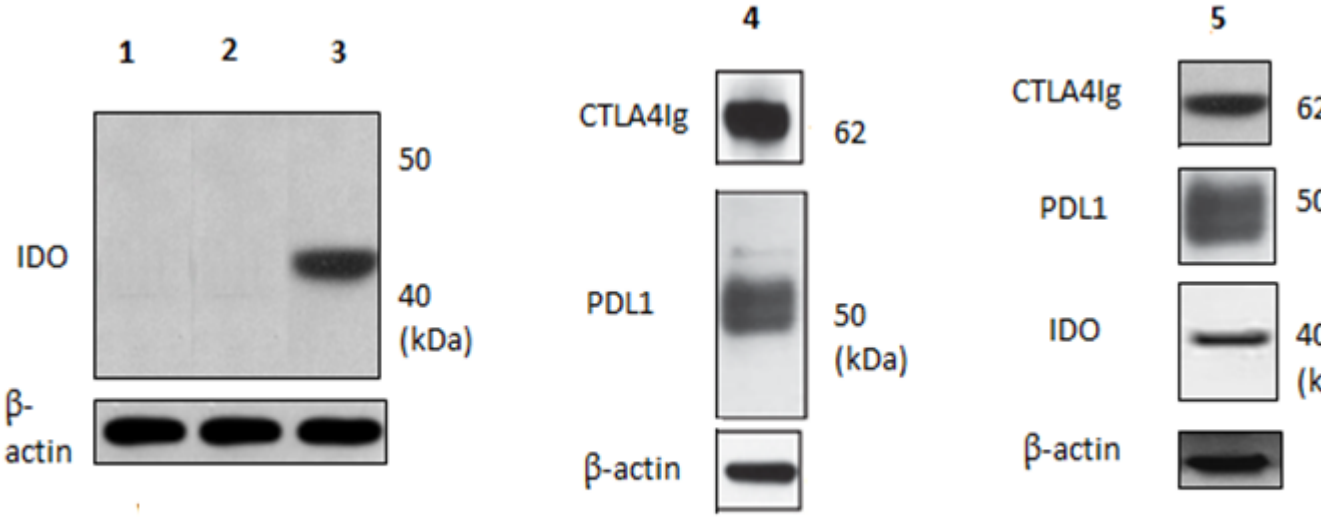
FACS analysis:

good transduction
Efficiency.



roteic expression evaluation through
estern Blot analysis:

- Mock-transduced cells
- Untreated cells
- IDO
- PDL-1 + CTLA4
- PDL-1 + CTLA4 + IDO

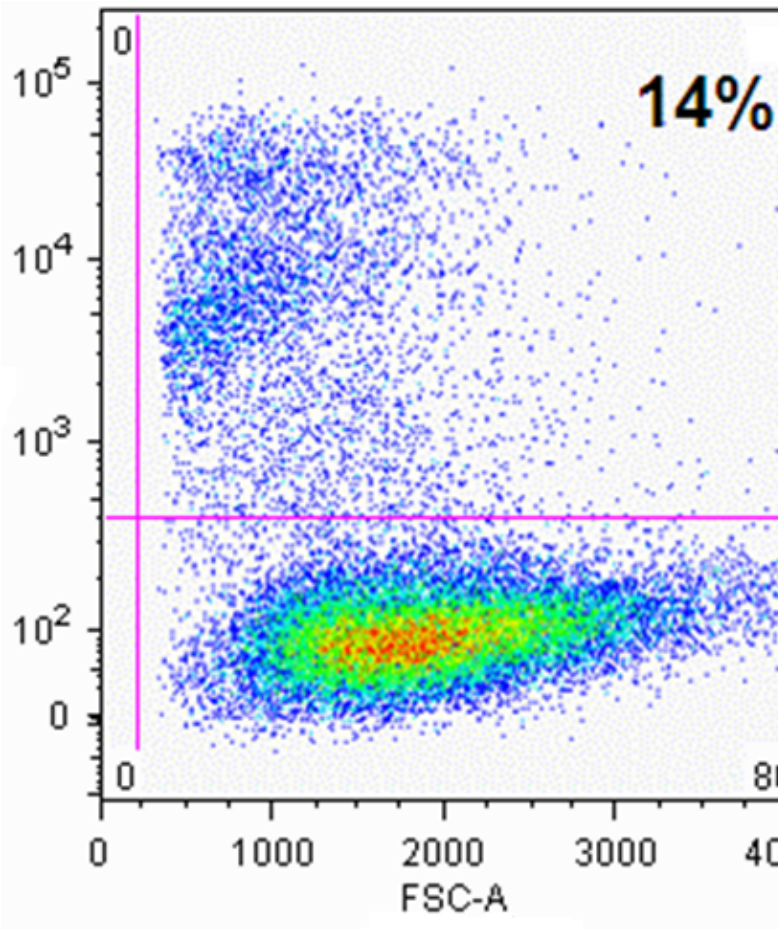


Western Blot anti-PDL-1, anti-CTLA4Ig, anti-IDO

Why a lentiviral vector?

ny advantages:

strong and constitutive expression
ong-term efficacy
contains up to 7,5-8,5 kb
Random insertion sites ➡ lower risk of genotoxicity
seen by PCR and sequencing of the amplicon



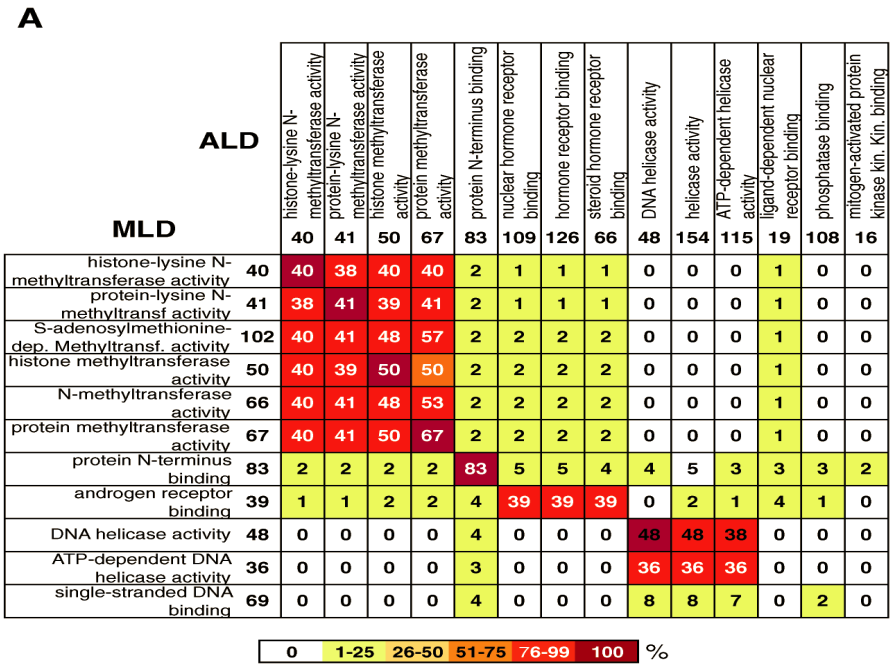
XICITY EVALUATION:

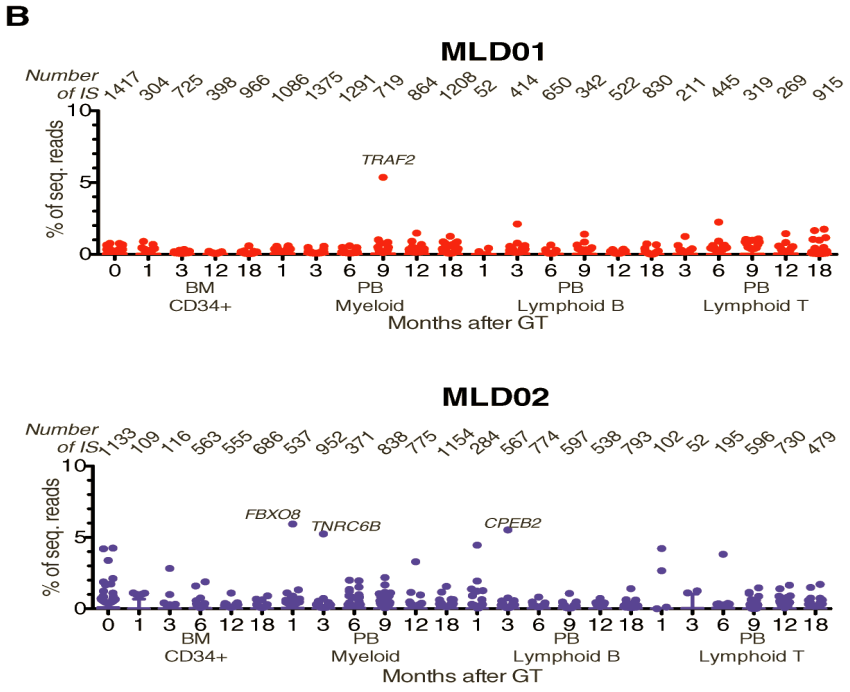
ell viability analysis:

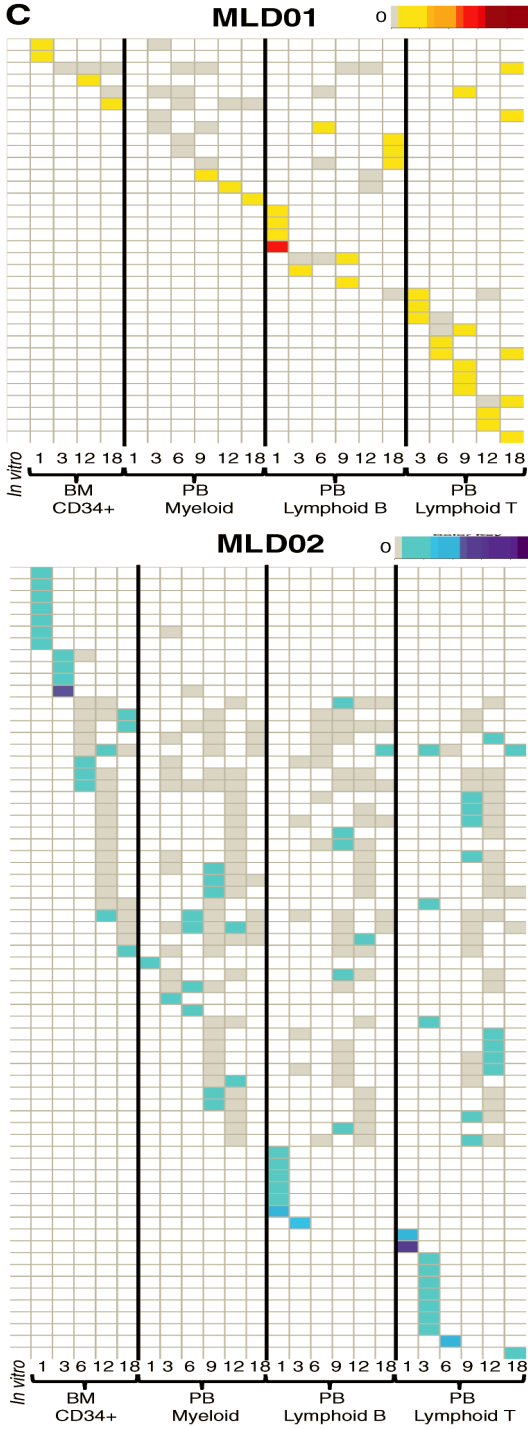
igh percentage of viable cells obtained.

Safety evaluation

genomic integration
ofile

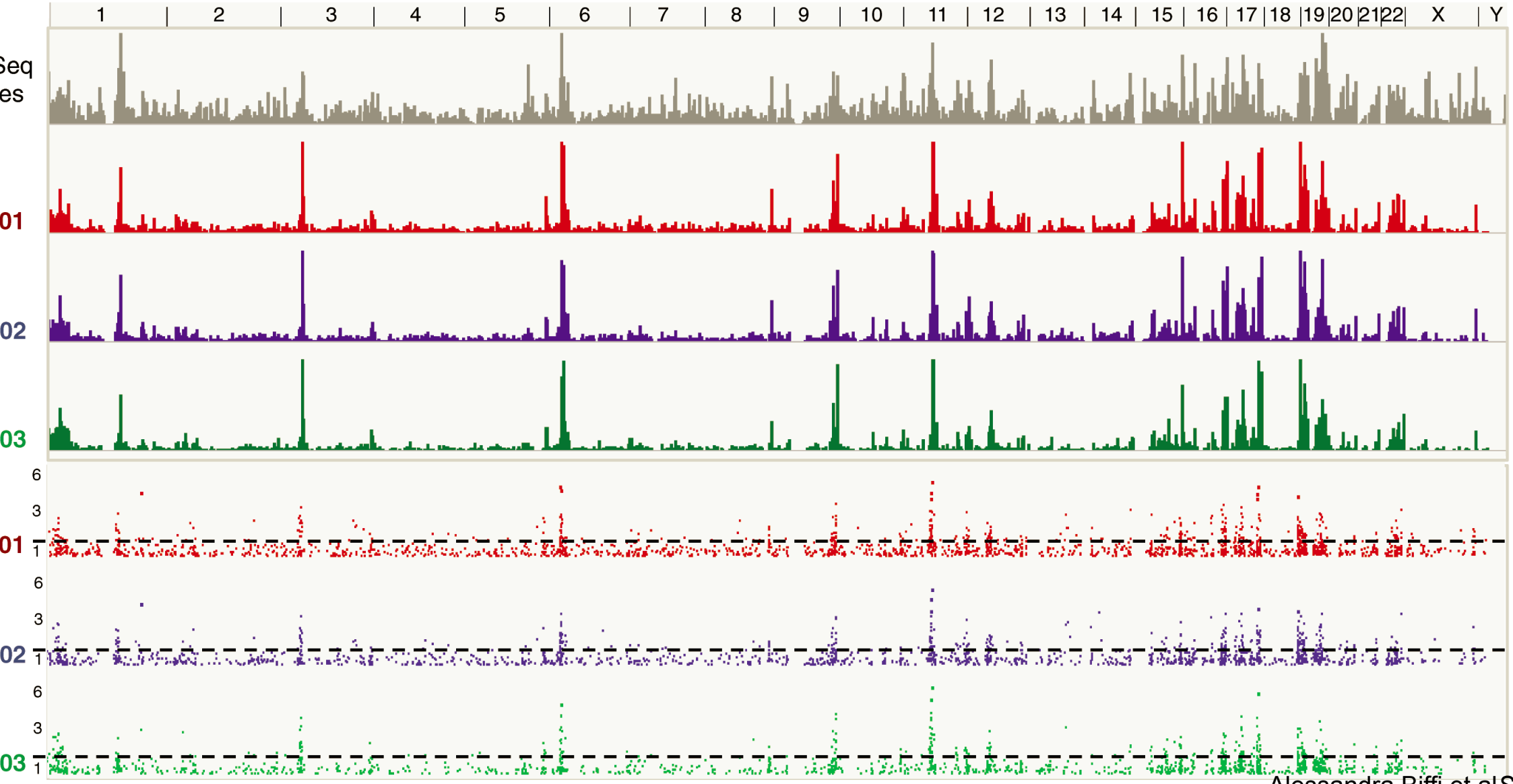






ndra Biffi et al., *Science* **341** (2013)

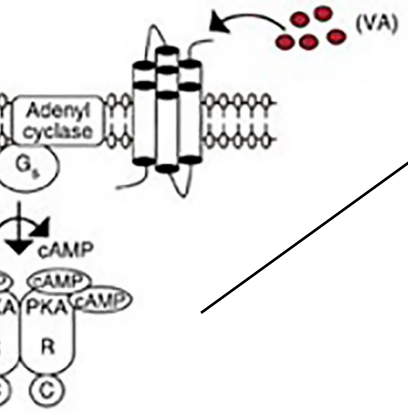
Common insertion site analysis.

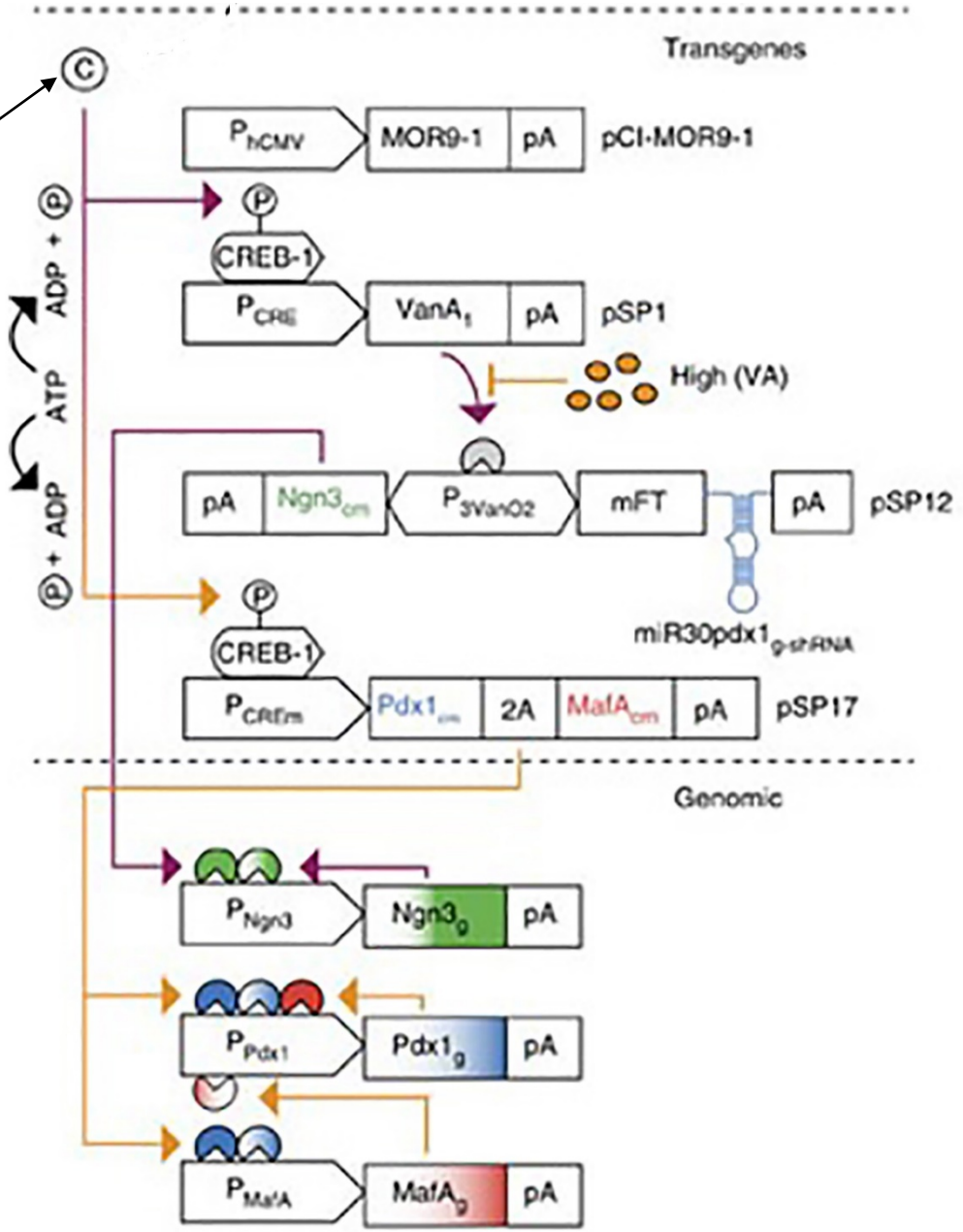


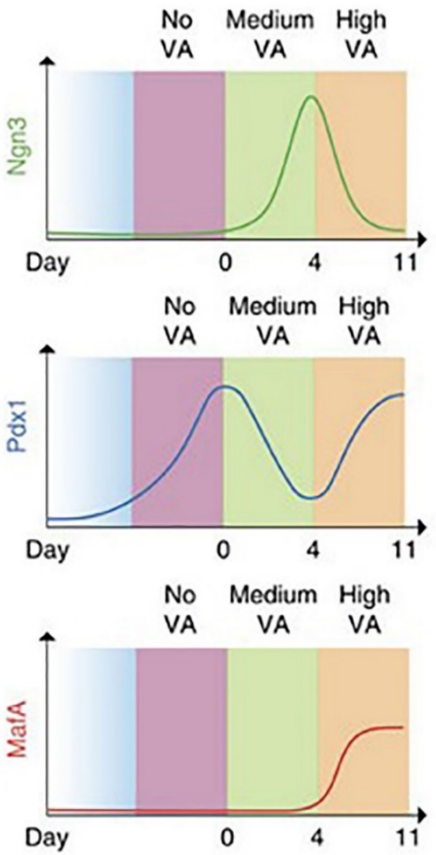
Alessandra Biffi et alS
341.(2013)

3

«Lineage-control network» protocol

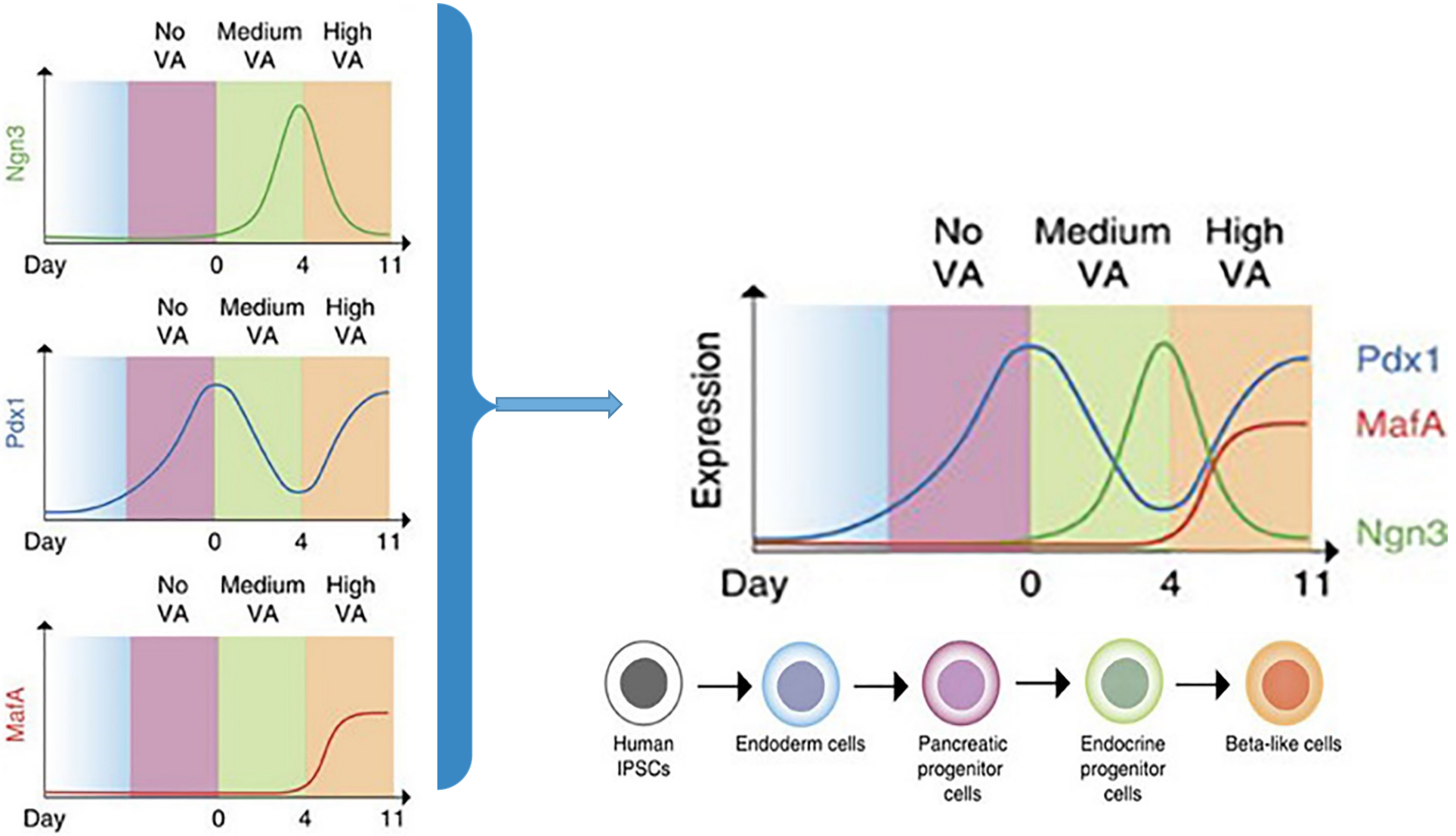






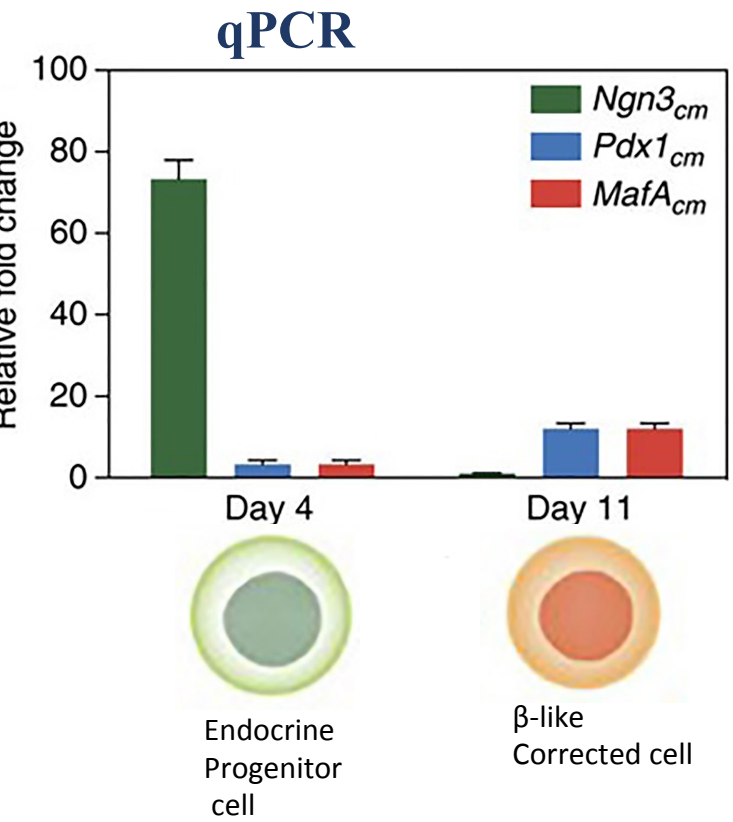
P.Saxena et al., *Nature Communication* (20

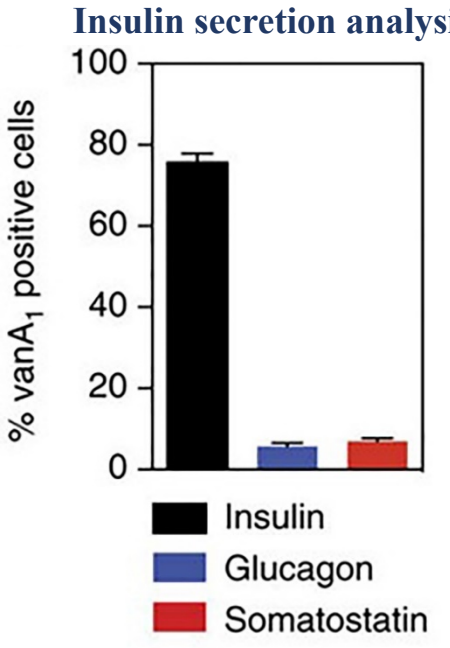
«Lineage-control network» protocol

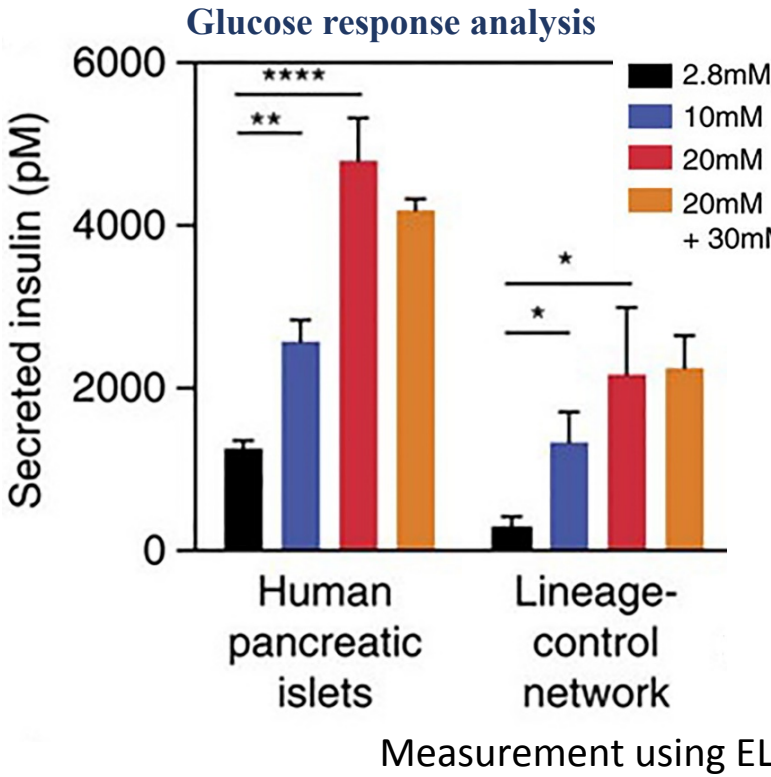


P.Saxena et al., *Nature Communication* (20

Are the iPSC-derived β-like corrected cells good enough in vitro?







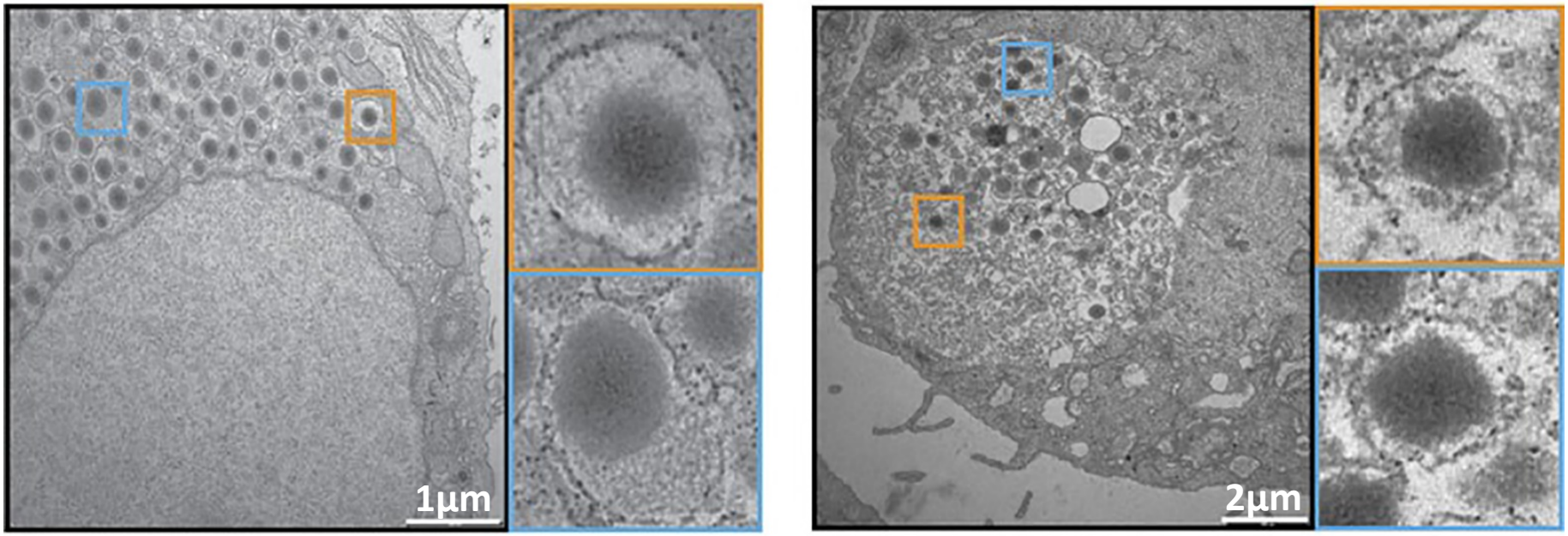
Measurement using EL

- In vitro is shown a good transcription profile for the pancreatin key genes
- In vitro the lineage-control network-derived β-like cells are insulin-secreting and glucose responsive

P.Saxena et al., *Nature Communication* (20

Are the iPSC-derived β-like corrected cells good enough in vivo?

Trasmission-electron micrographs



Human pancreatic islets

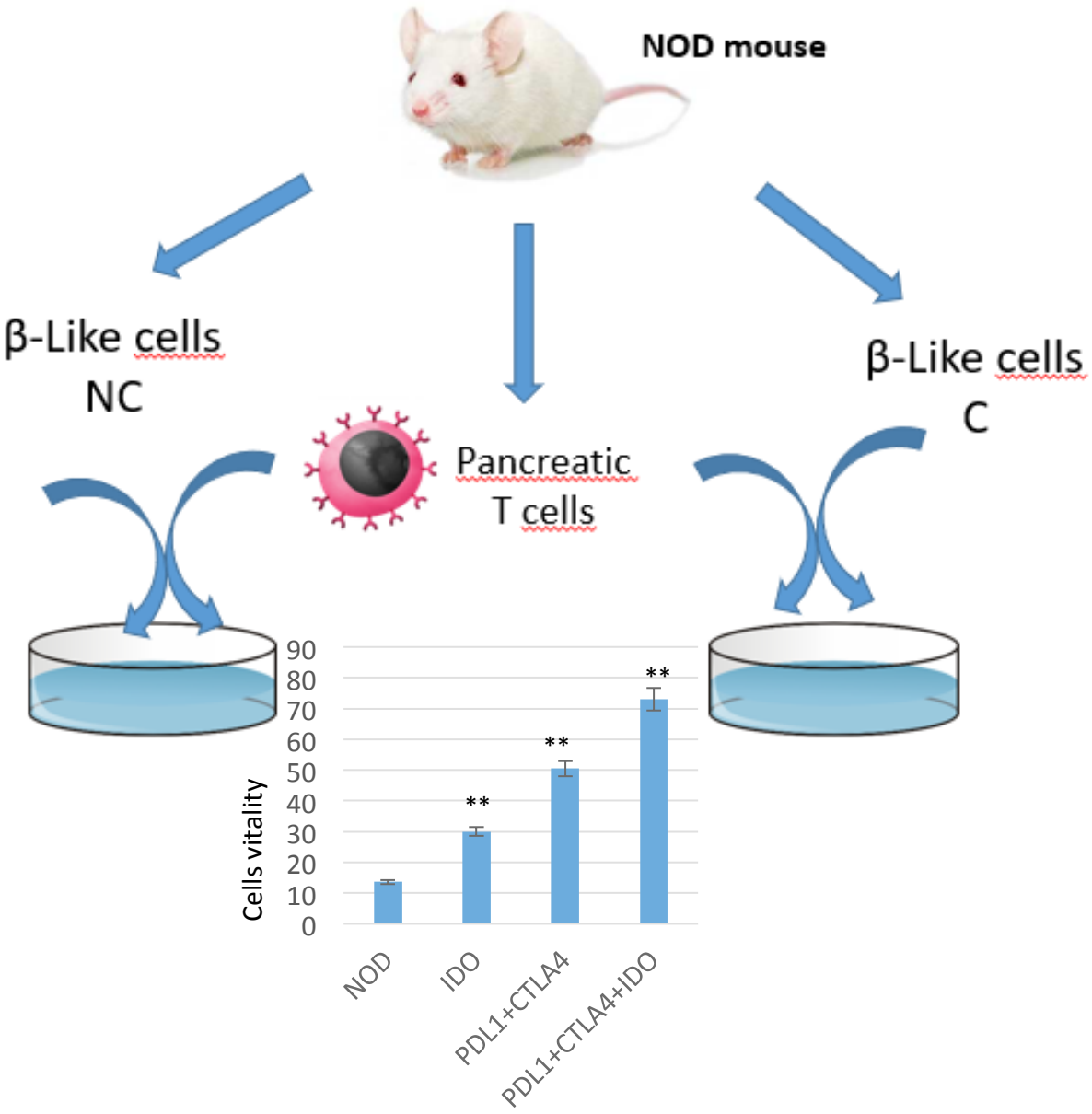
Lineage-control network

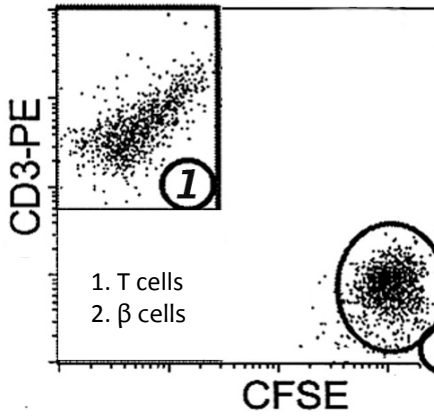
In vivo analysis demonstrate that the iPSC network-derived β-like cells:

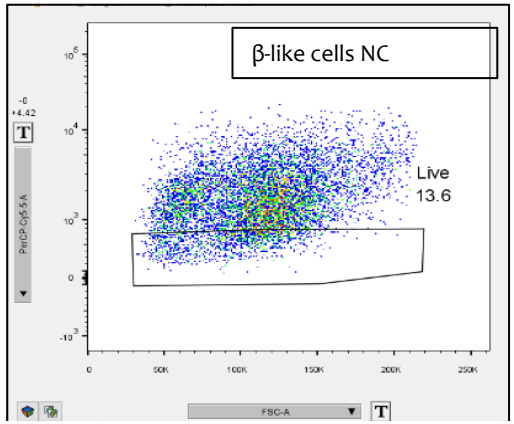
- show the typical insulin-storage vescicles that are found in mature pancreatic beta cells.

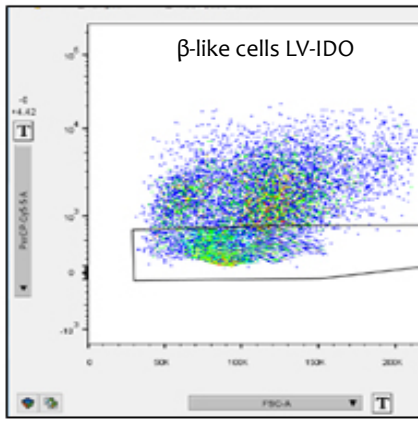
P.Saxena et al., *Nature Communication* (20

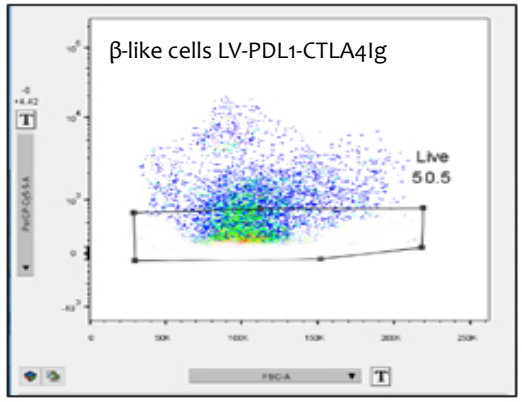
uld our β-like corrected cells escape the immune system?

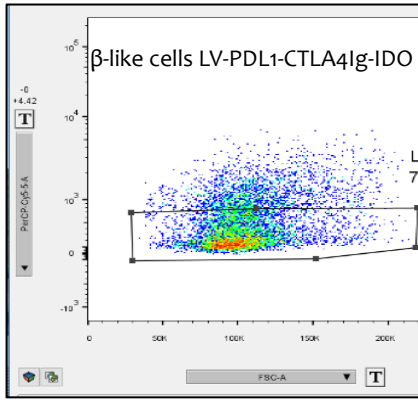










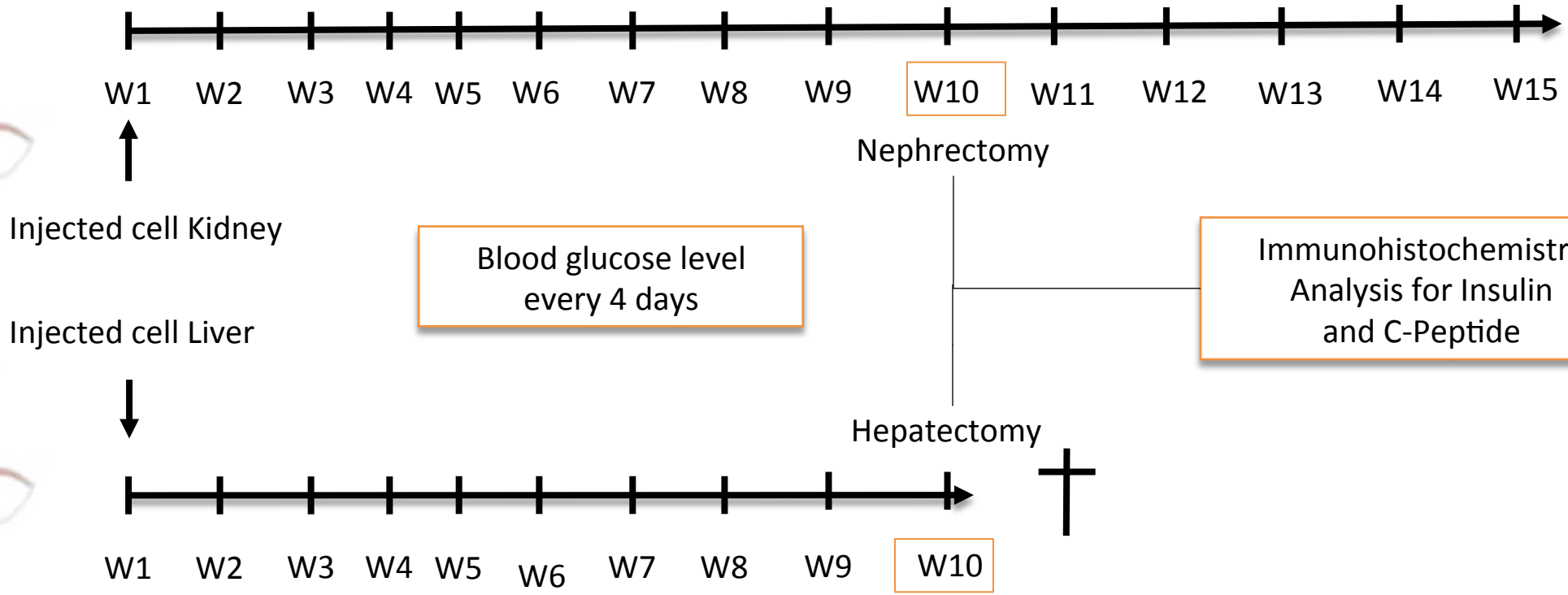


4 In vivo experiments

IOD mice
weeks old

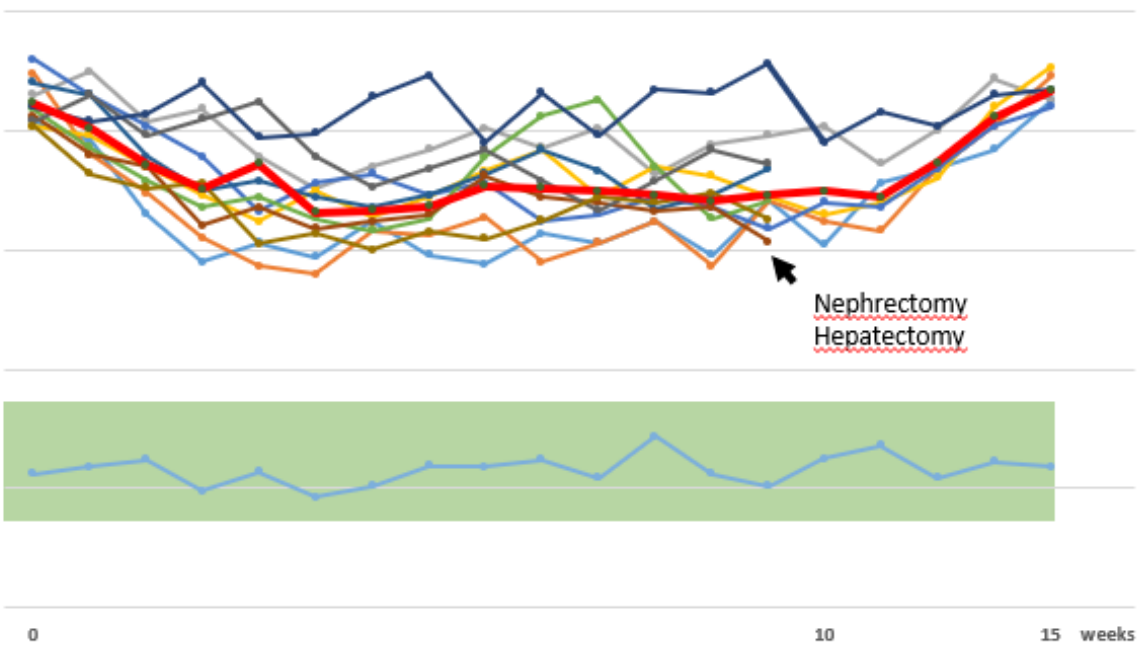
X15

IOD mice
weeks old

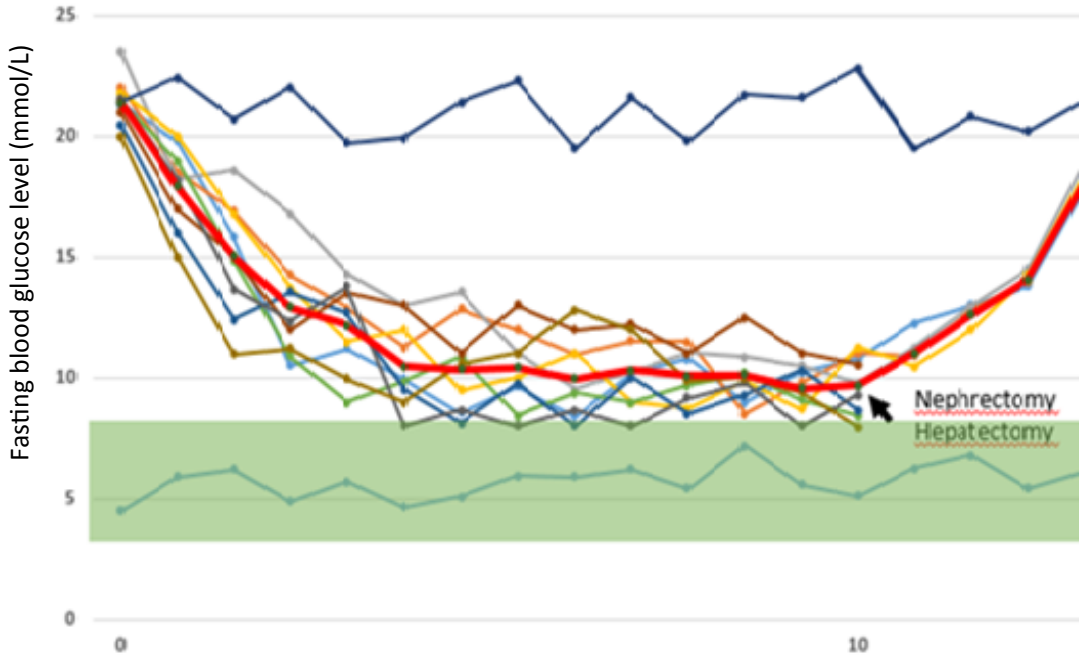


Same experiments in parallel have been done with injection of differentiated beta like cells without correction.

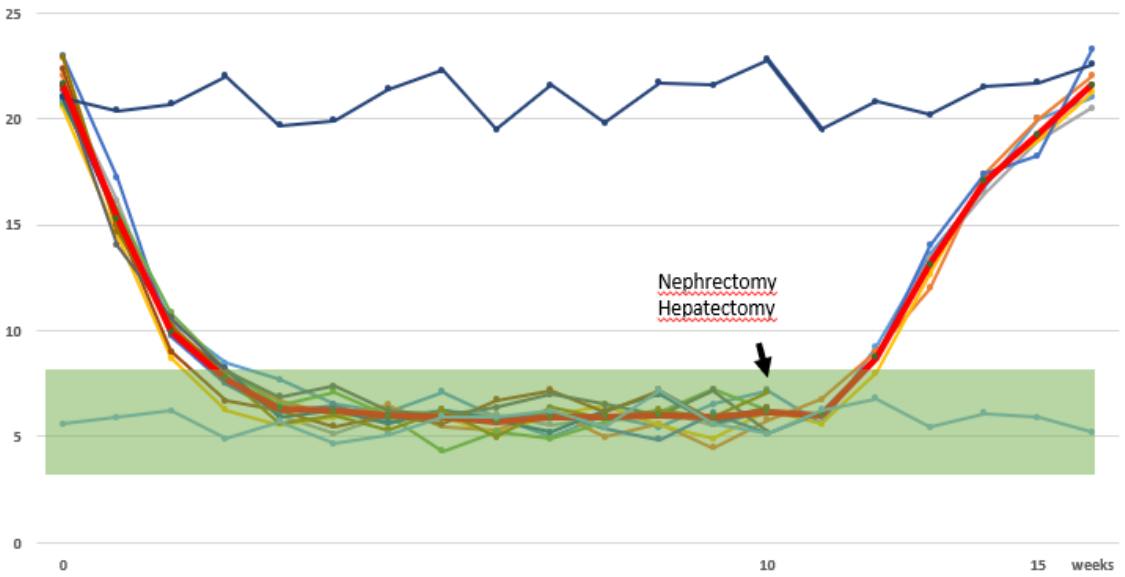
LV-IDO

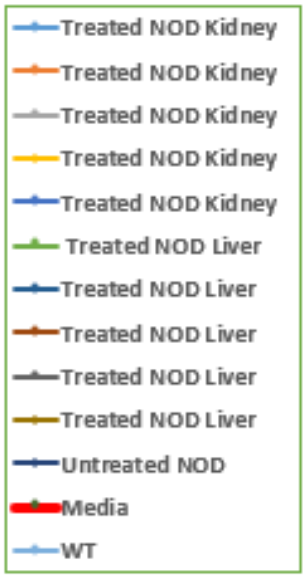


LV-PDL1+CTLA4Ig



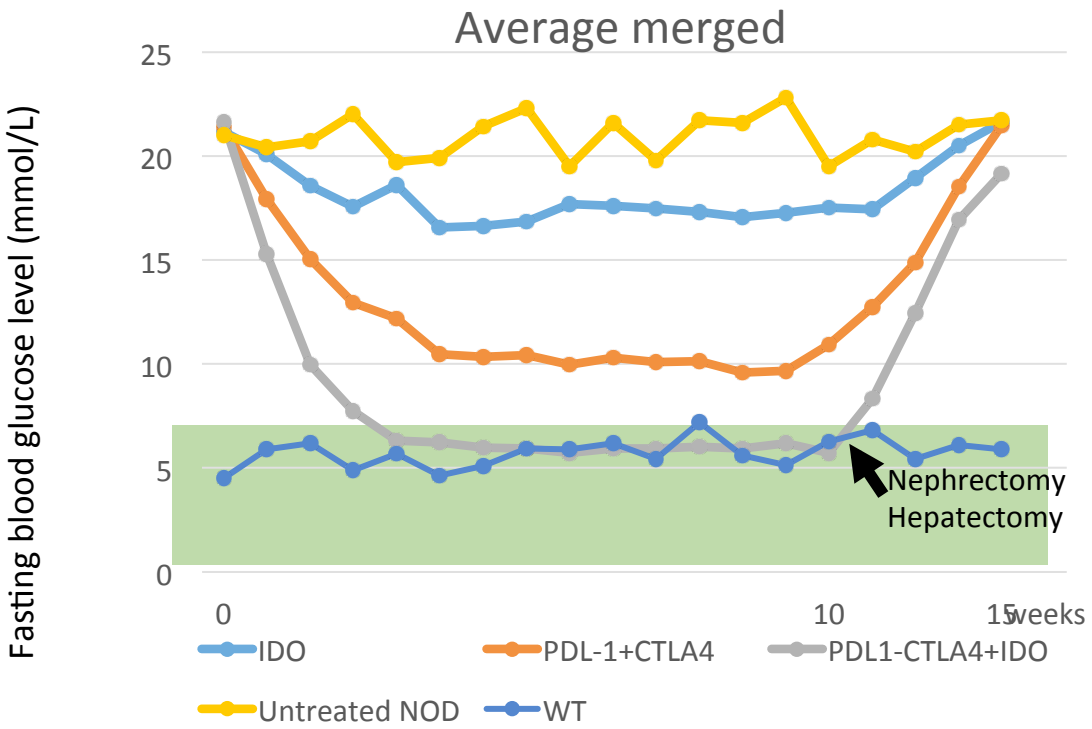
LV-PDL1+CTLA4Ig+IDO

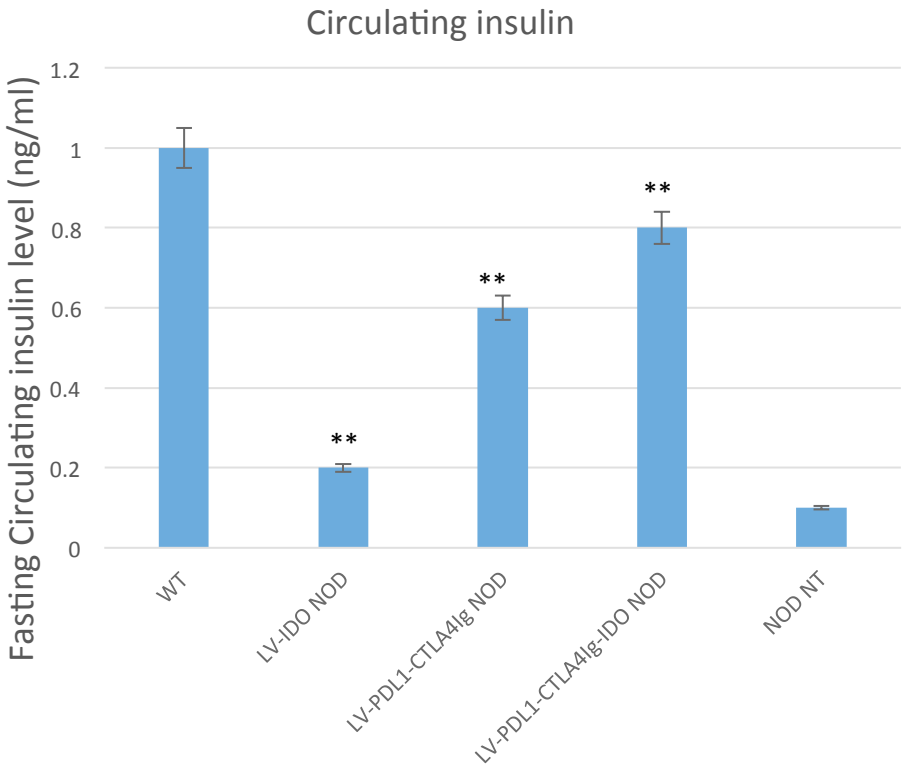




Using LV-PDL1-CTLA4-IDO expression we
obtained glucose levels within safety ran
resembling to WT

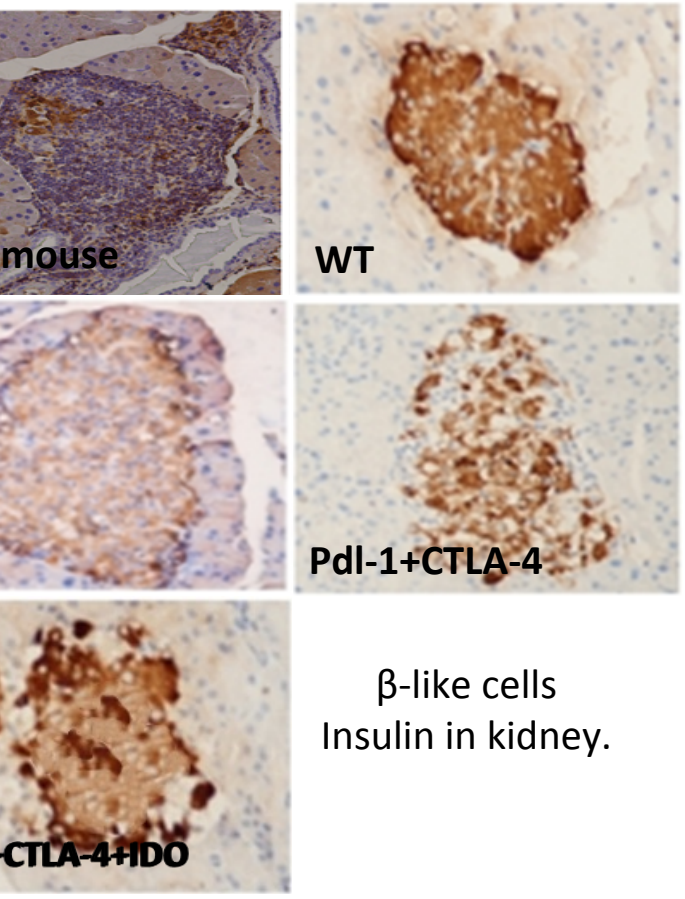
Average blood glucose and circulating insulin levels



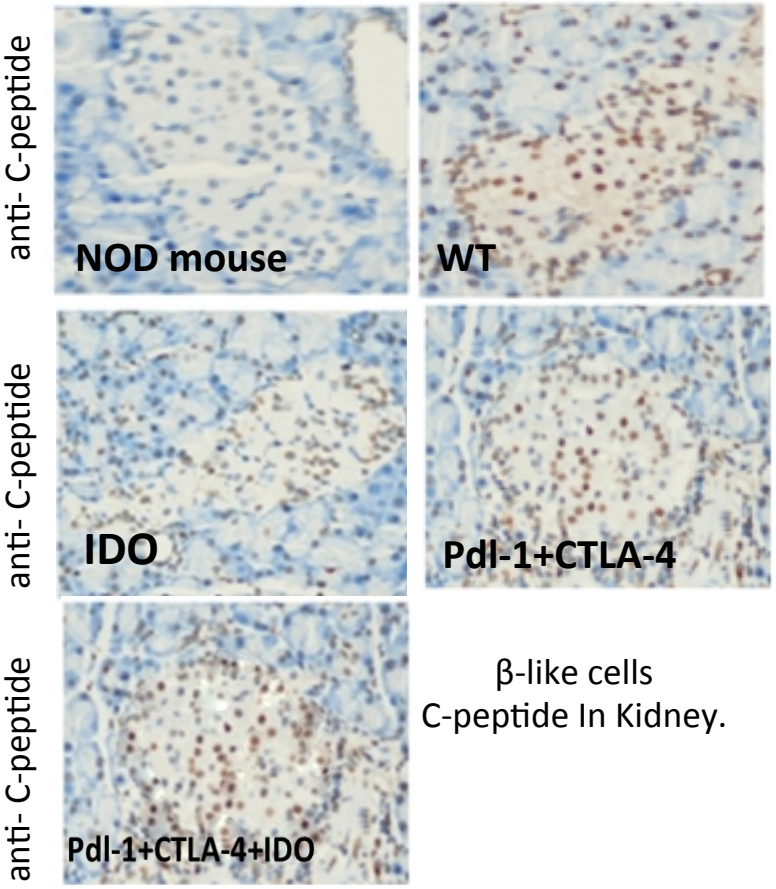


Measurement using ELISA test.

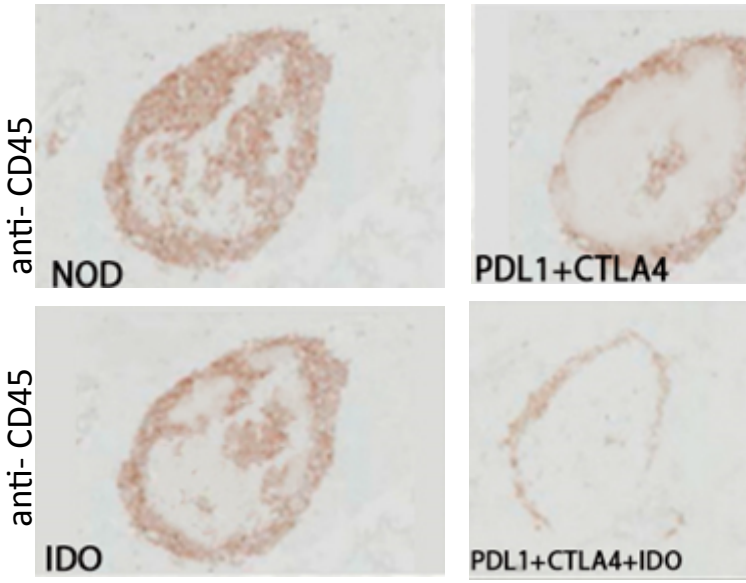
In vivo immune staining



β-like cells
Insulin in kidney.



β-like cells
C-peptide In Kidney.



β-like cells leukocyte common antigen
expression in Kidney

We obtained the same results from β-like cells in the liver

Pitfalls and ameliorations

- Induced pluripotent stem cells stability, efficiency and safety could be ameliorated using miRNAs instead of Yamanaka's factors or using Nanog and Lin28 instead of c-Myc
- NOD mouse can be humanized.
- According to our studies we suggest to go ahead with experimantation in non-human Primates.
- Challenges still remain for non-human Primates β-like cells differentiation.
- Suicide genes could be a way to enhance the safety of ex vivo gene therapy, by eliminating the transduced cells at the site of implantation.

F. Alaee et al., 2014 *Gene Therapy*

Costs and Time

Time of work: 5 years, 70 000/80 000 $

- WT mouse+ NOD mouse: 26$ (x51 WT mouse) + 44$ (x50 NOD mouse)
- Stabulation for the mice: about 500$/month
- Culture dishes (Sigma-Aldrich): 119$
- Yamanaka's factors plasmid: 65$
- Lipofectamine LT Reagent with Plus Reagent (Invitrogen) 0,75ml: 400€
- qPCR (miScript SYBR Green PCR Kit- QIAGEN): 451$
- Toxicity assay: 400$
- Taq PCR Core kit (QIAGEN): 171$
- Lentivirus (1ml at titer >1x10^6 TU/ml) and plasmid (Addgene): 250$ (x5)
- Next Generation Sequencing: 1500-3000€
- FACS antibodies: 200-300$/each + respective controls
- Immunohistochemistry antibodies: 200-300$/antibody + secondary antibody
- ELISA assay kit (biorbyt): 580$/plate
- Western blot antibodies: 300-400$/antibody + secondary antibody
- Supplementary costs including routine lab experiments are not evaluable

References

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