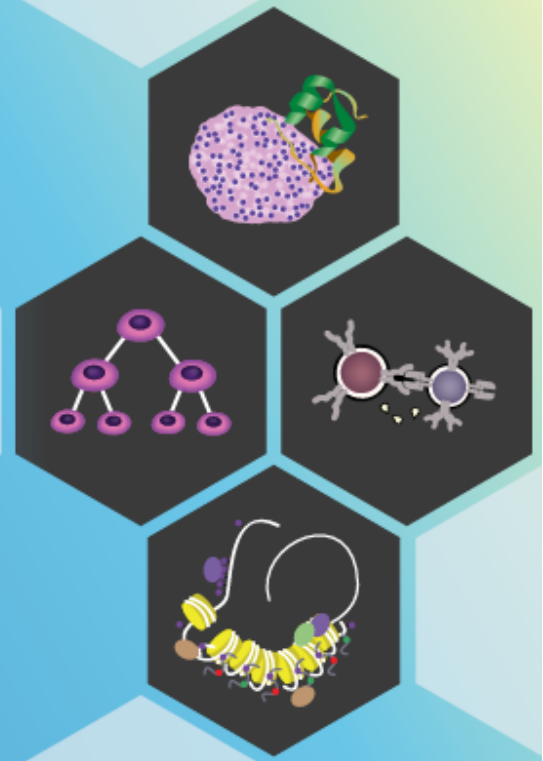
2024 RACHMIEL LEVINE-ARTHUR RIGGS

Diabetes Research Symposium

Stem Cell-Based Therapies for Diabetes



Matthias Hebrok, PhD

Center for Organoid Systems and Tissue Engineering (COS)

Technical University Munich (TUM)

Institute for Diabetes and Organoid Technology (IDOT)

Helmholtz Center, Munich

Disclaimer

This is a Non-CME Accredited Presentation.

Matthias Hebrok - Disclosures

Minutia Inc: Co-Founder, SAB member, Stock/Option Holder

Encellin: Stock Holder

Thymmune Therapeutics: Stock Option Holder

CV Next: Consultant, Stock Holder

Center for Organoid Systems (COS), Technical University Munich

Personalized medicine for Cancer

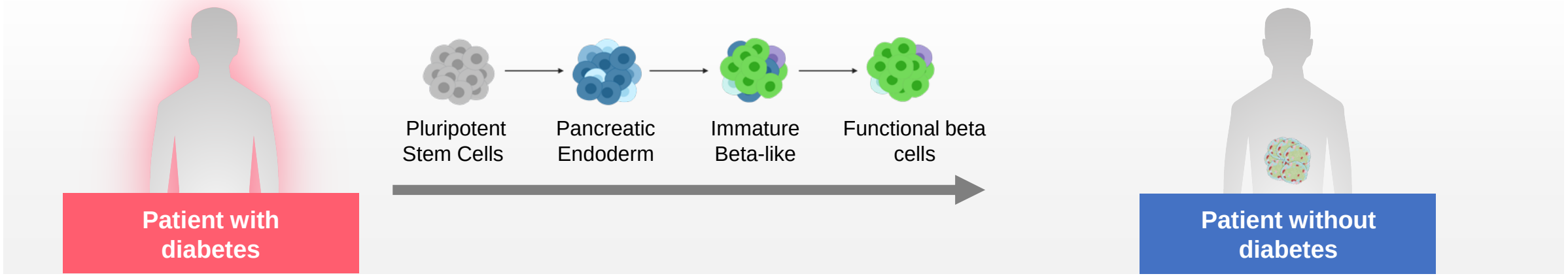
Integration with next generation technologies:
- microfluidics – organ(s) on a chip
- nanotechnology
- AI

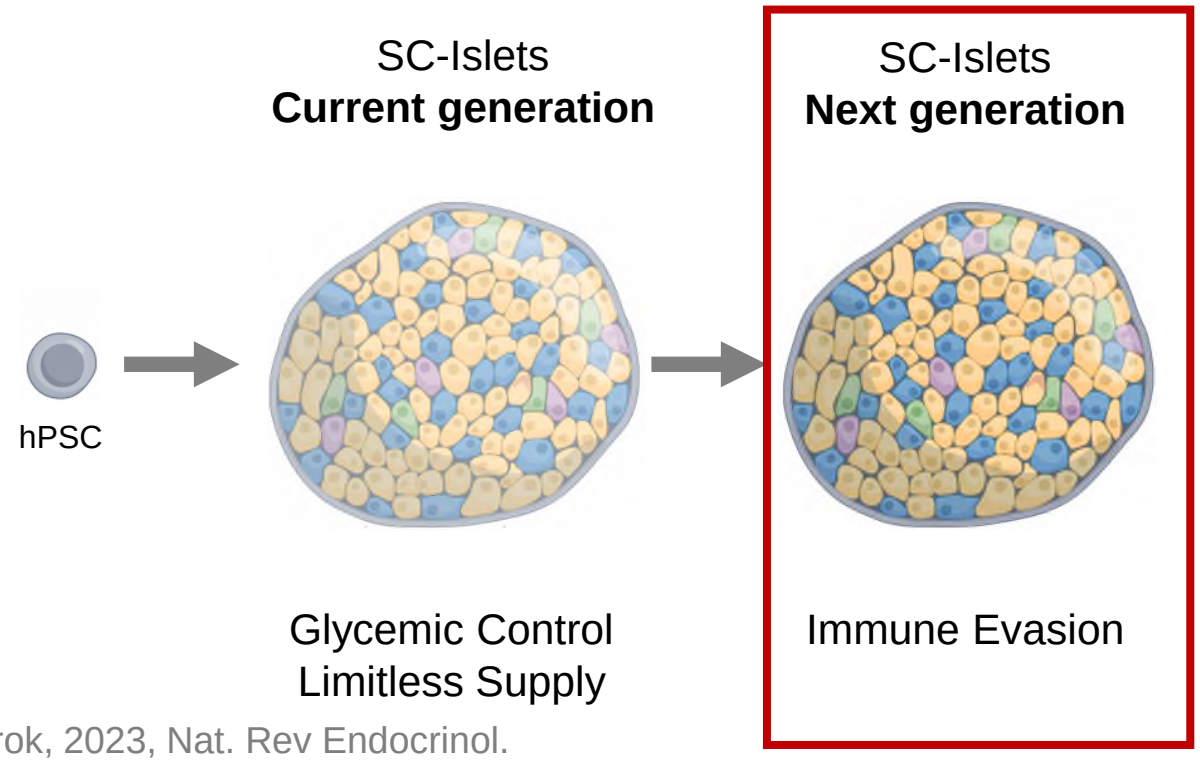
Identify and exploit Human-specific properties for therapies

Generate novel cells with superior functions for:
- drug screening
- cell therapy

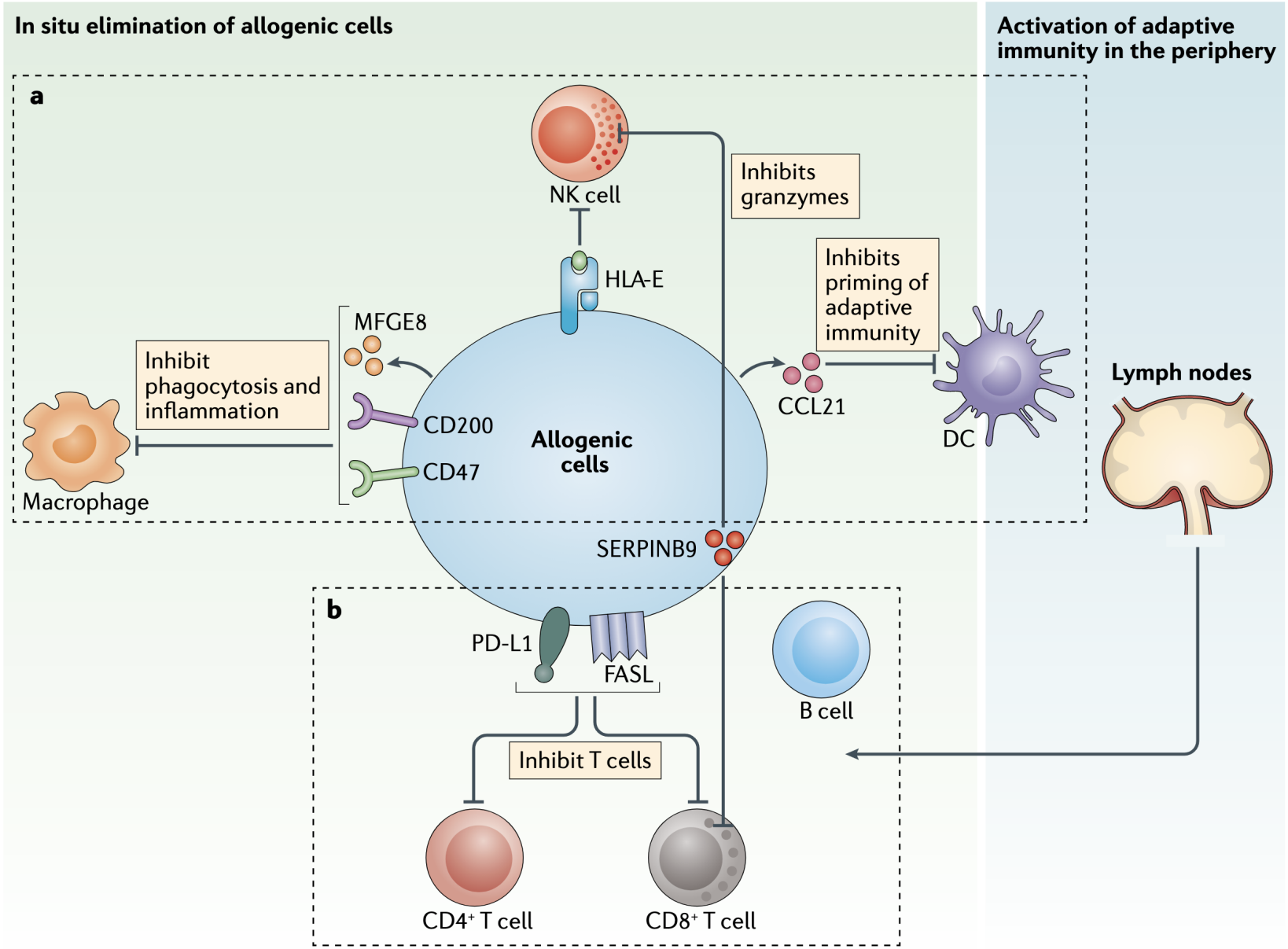
Institute for Diabetes and Organoid Technology, Helmholtz Munich

Stem cell-derived islets for diabetes therapy





Cochrane & Hebrok, 2023, Nat. Rev Endocrinol.



Lanza, R., Russell, D. W. and Nagy, A. (2019). *Nat Rev Immunol* 19, 723–733.

Barkal, A. A., et al. (2019). CD24 signalling through macrophage Siglec-10 is a new target for cancer immunotherapy. *Nature* 572, 392–396.

Deuse, T., et al. (2019). Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat Biotechnol* 37, 252–258 (2019) .

Gerace, D., et al. (2023). Engineering human stem cell-derived islets to evade immune rejection and promote localized immune tolerance. *Cell Reports Medicine* 100879.

Gravina, A., et al. (2023). Protection of cell therapeutics from antibody-mediated killing by CD64 overexpression. *Nat Biotechnol* 1–11.

Parent, A. V., et al. (2021). Selective deletion of human leukocyte antigens protects stem cell-derived islets from immune rejection. *Cell Rep* 36, 109538.

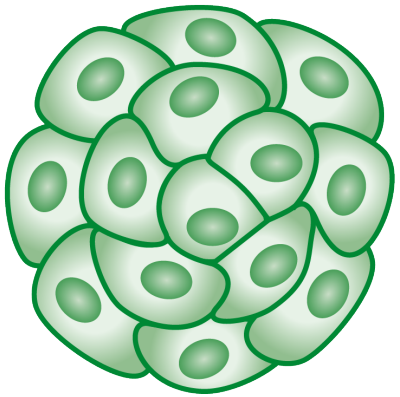
Hu, X. *et al.* Human hypoimmune primary pancreatic islets avoid rejection and autoimmunity and alleviate diabetes in allogeneic humanized mice. *Sci Transl Med* **15**, eadg5794 (2023).

Generating an immune protected environment

Wendell Lim, Nishith Reddy (UCSF)

Hasna Maachi, PhD
MRI/TUM/Helmholtz

Islet transplant



Generating an immune protected environment

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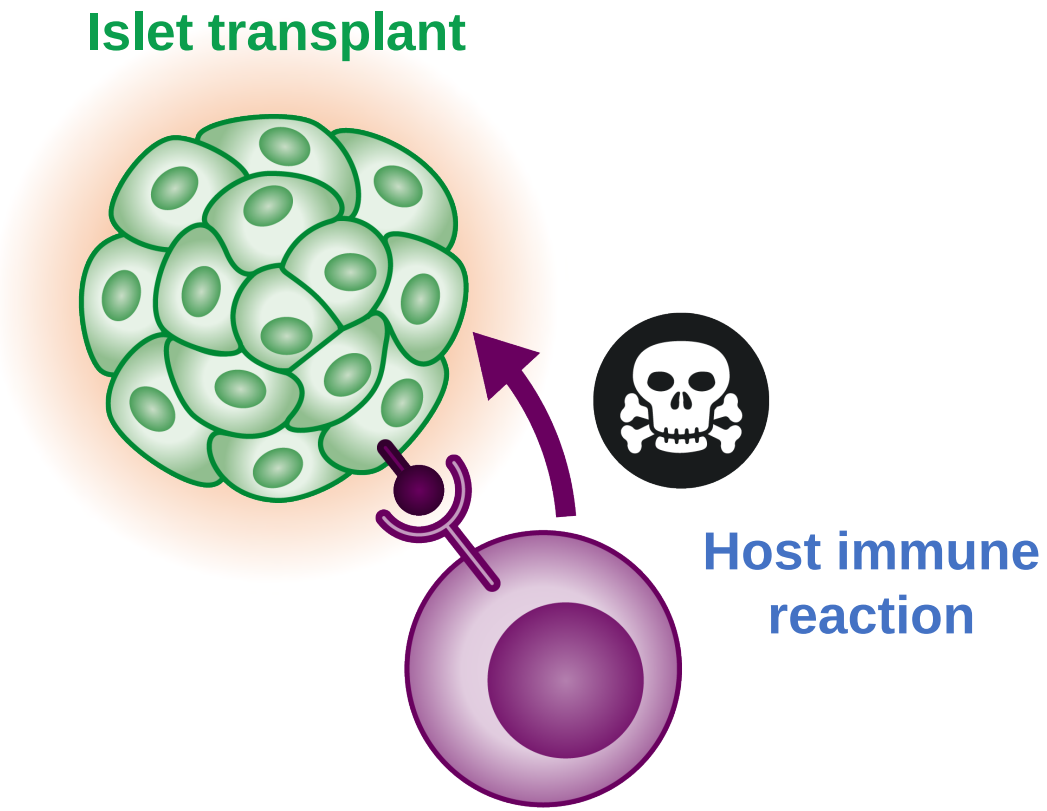
MRI/TUM/Helmholtz



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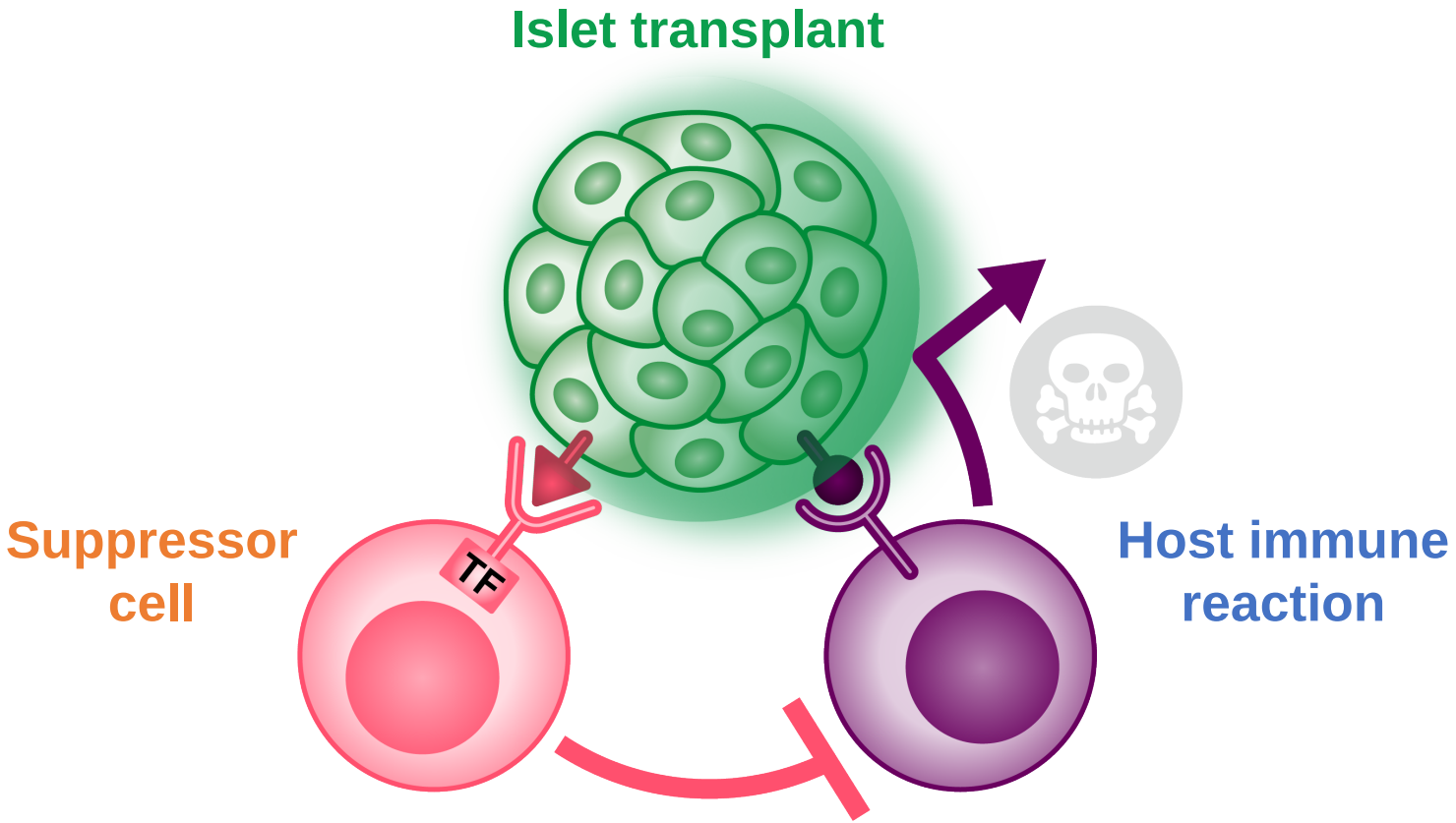

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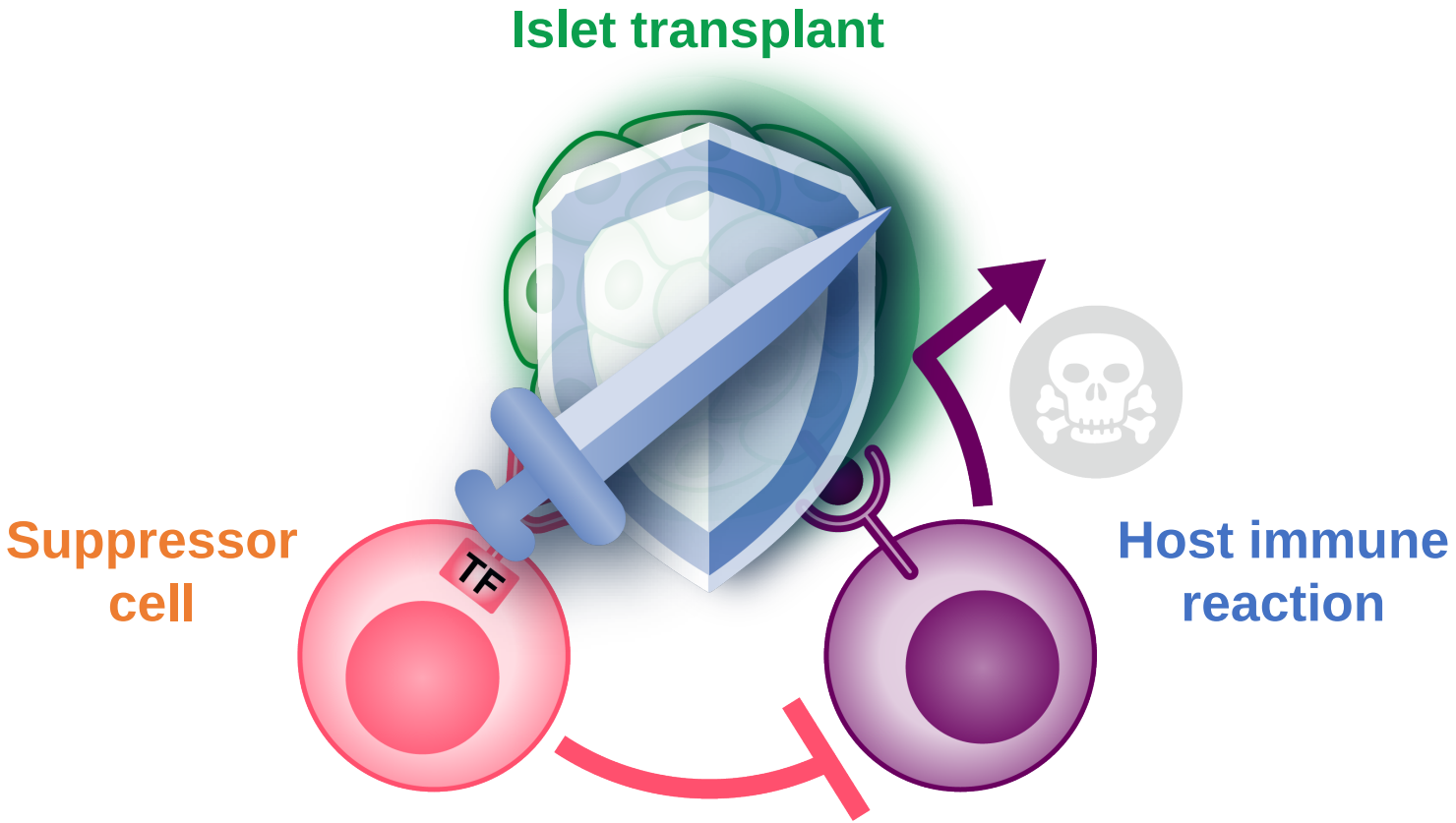
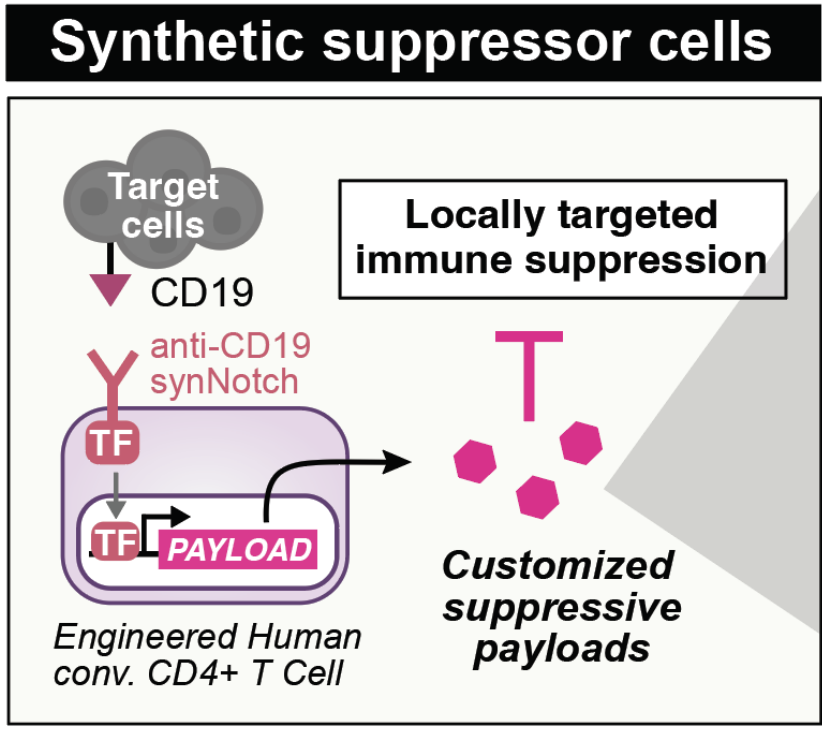
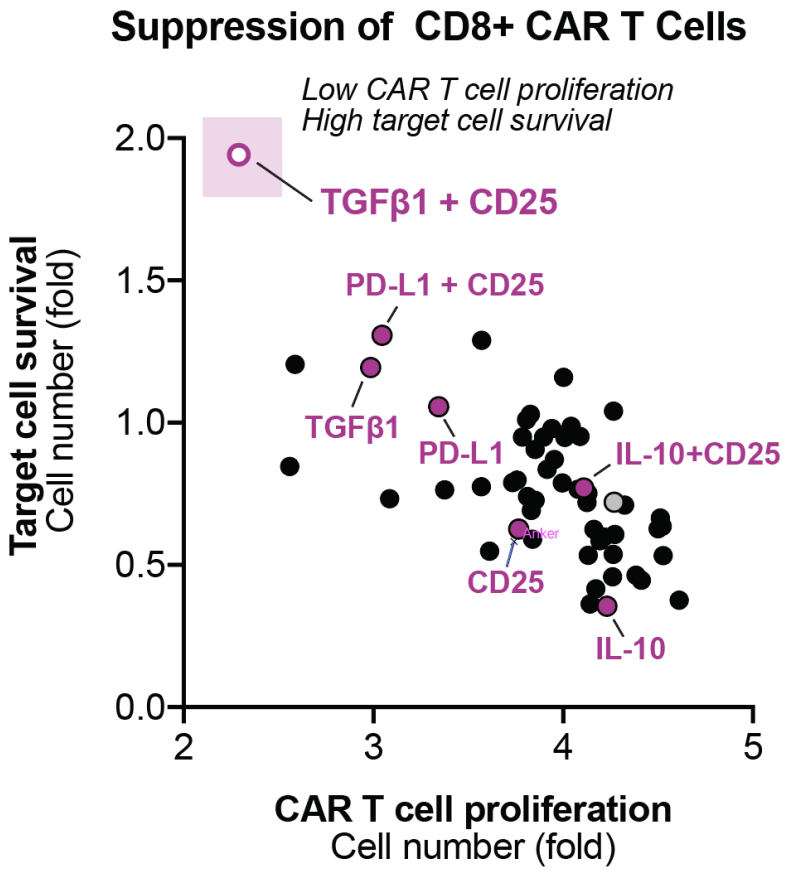
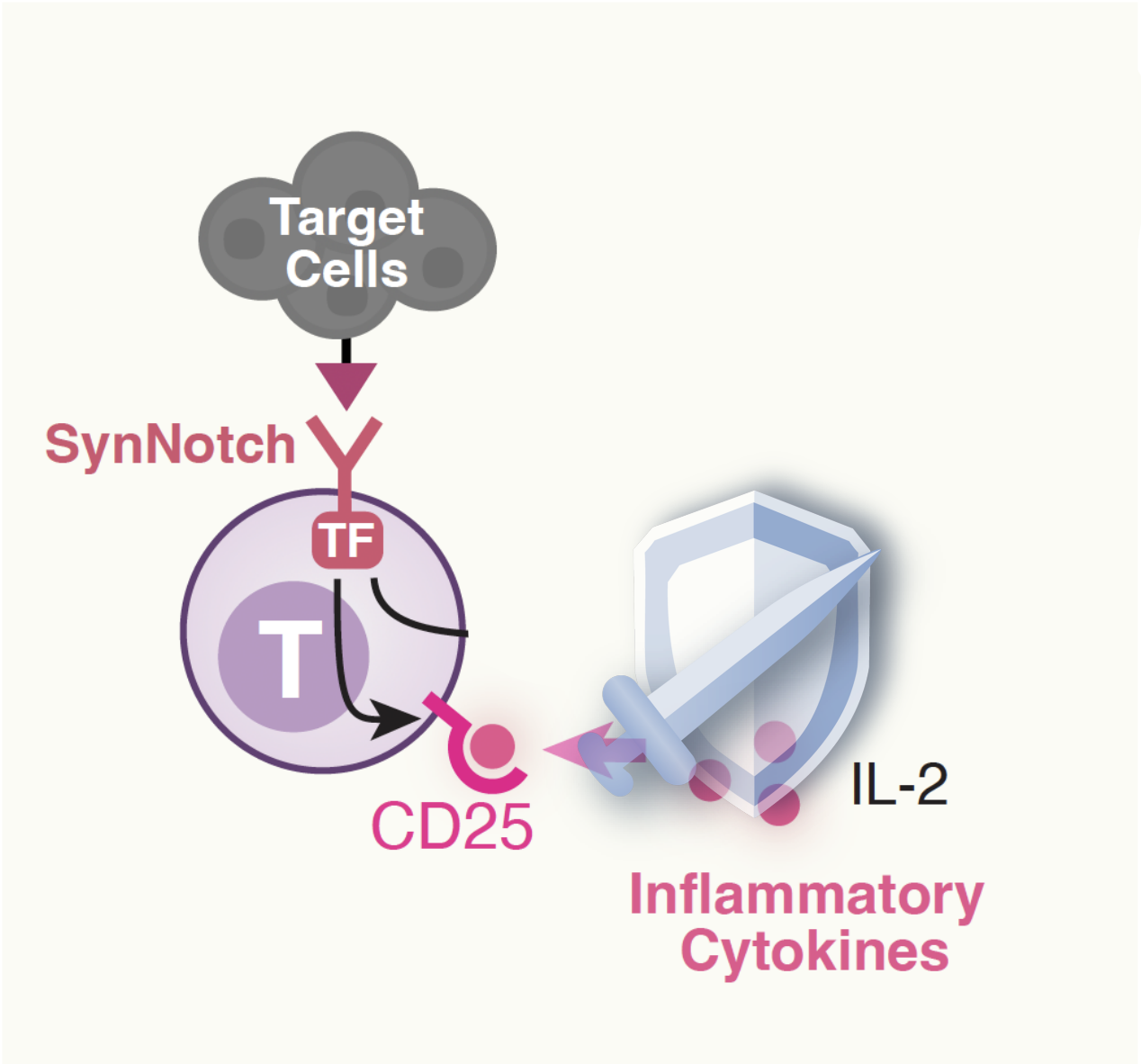


Testing of SynNotch induced payloads

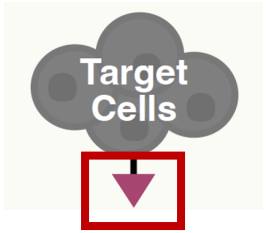




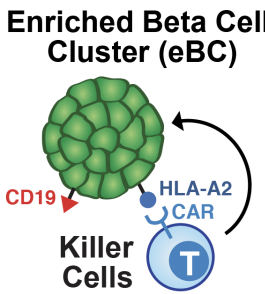
Synthetic Suppressor Cells



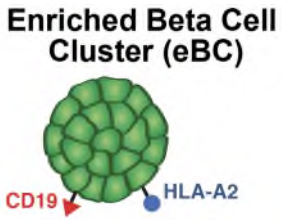
T cell killing of hESC-derived beta cells *in vitro*

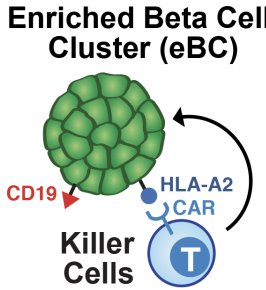


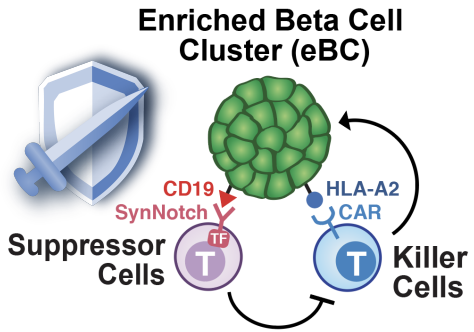
Synthetic suppressor cells can protect eBCs expressing CD19 from T cell mediated killing *in vitro*



Synthetic suppressor cells protect ES-derived β-like cells from T cell killing *in vitro*

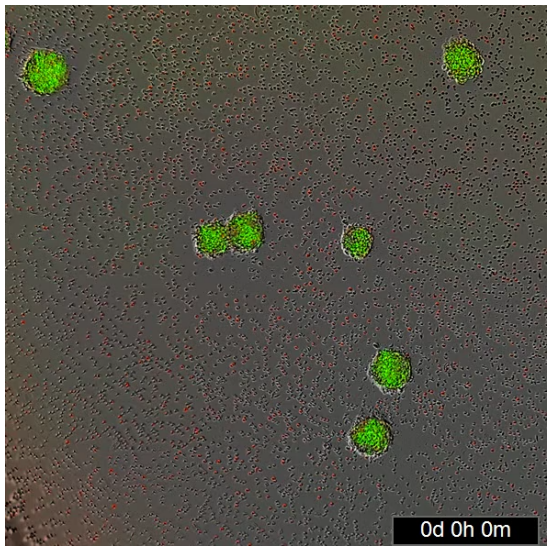




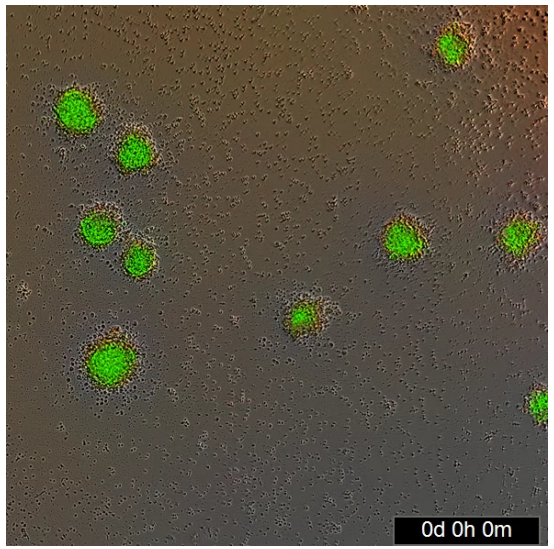


UNTRANSDUCED, No CAR

NO SUPPRESSOR, CAR

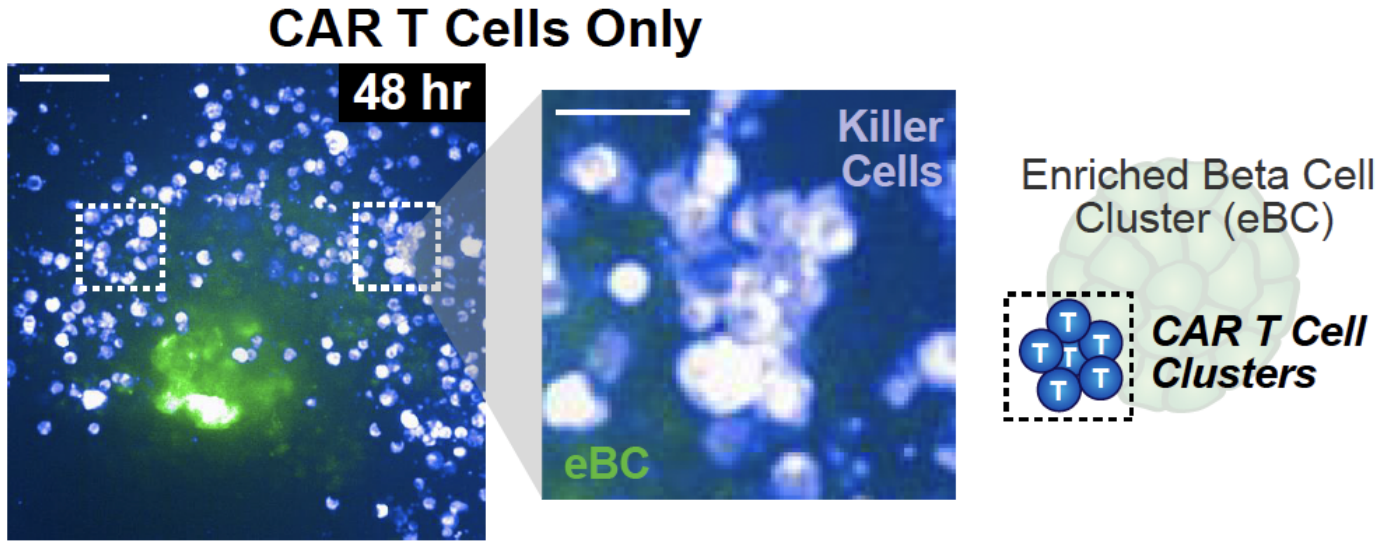


SUPPRESSOR, CAR

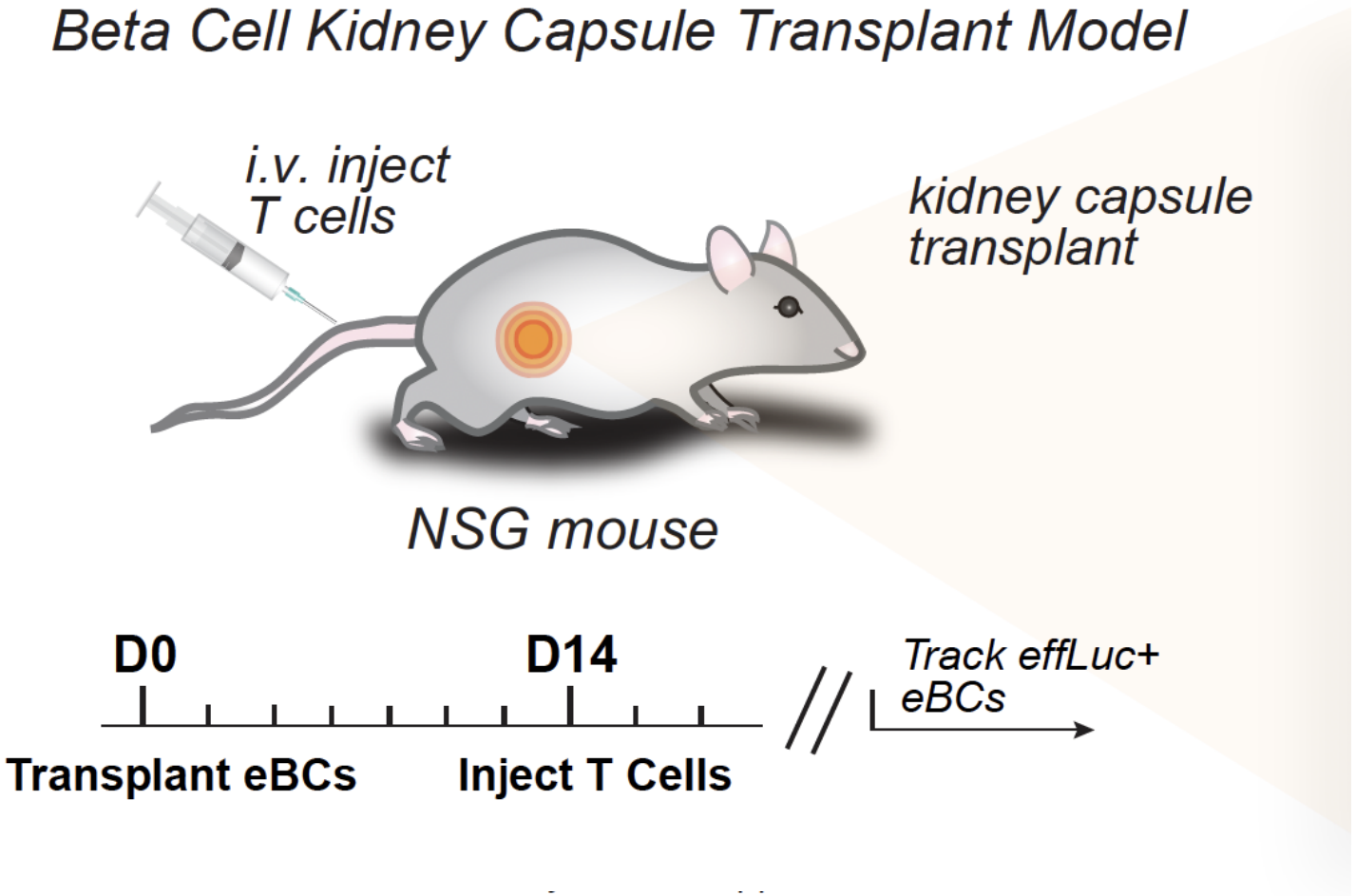


CD19+ β-like Cells (INS → GFP): 50K
Killer T cells, anti-HLA-A2 CAR (mCherry): **10K**
Suppressor Cells, anti-CD19 SynNotch: **20K**

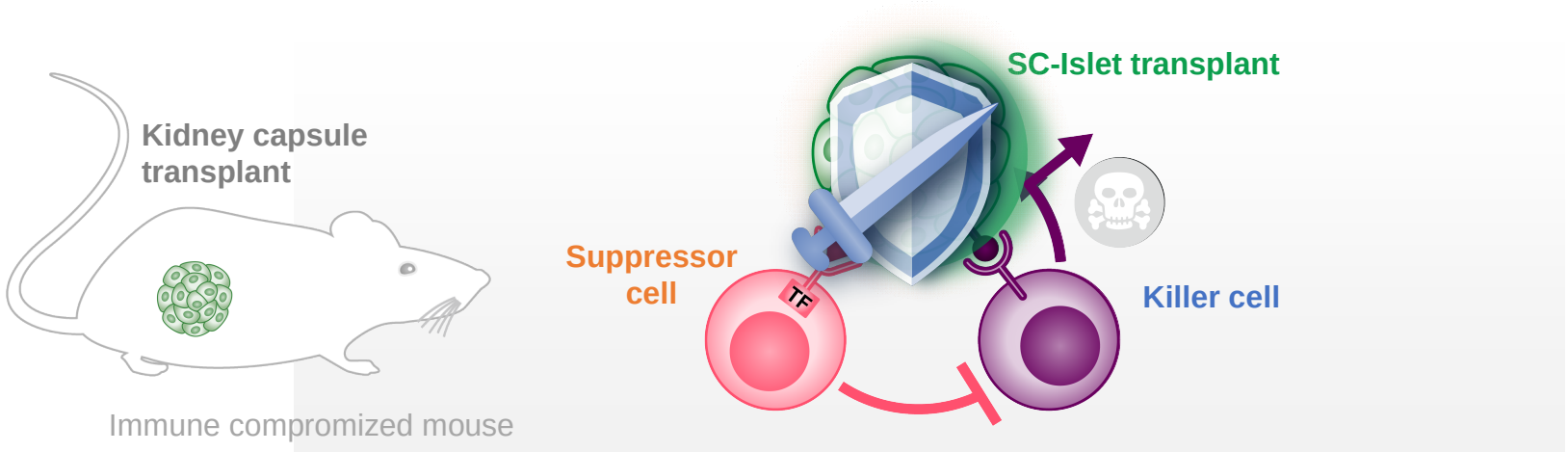
Spatial organization of T cells

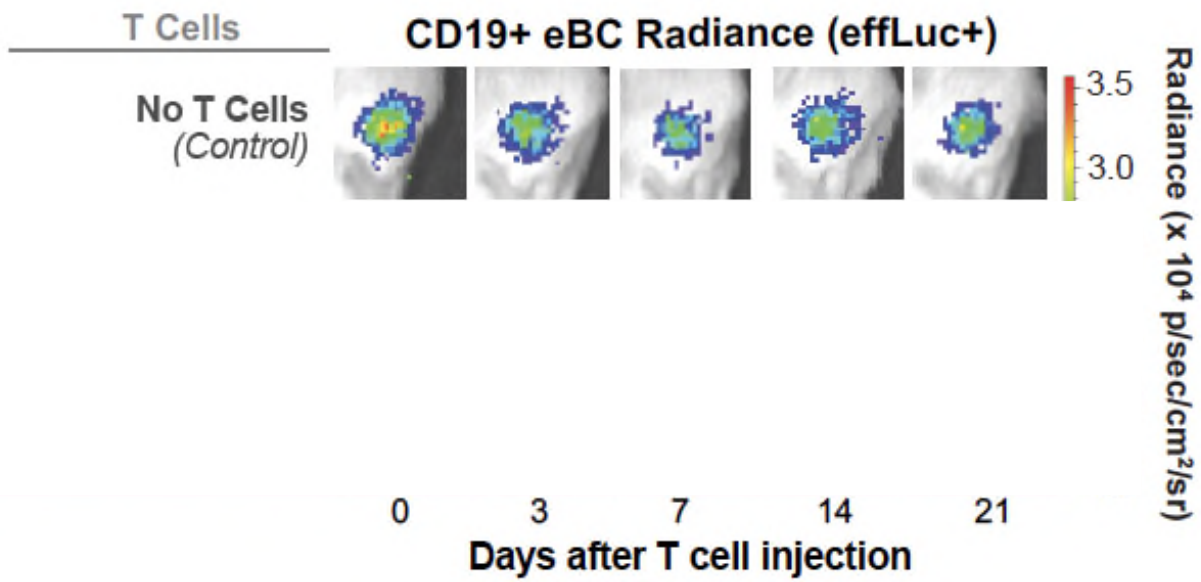


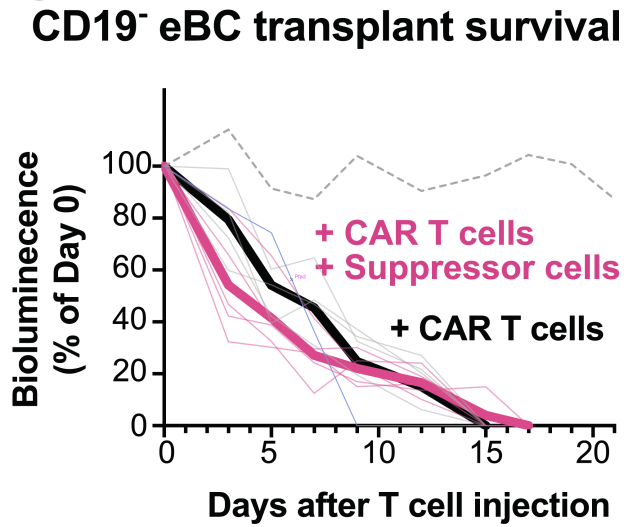
Protection of hESC-derived beta cells *in vivo*

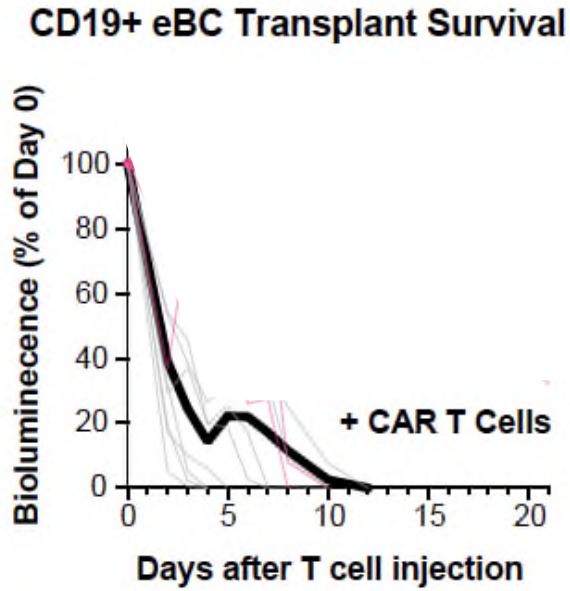


Synthetic suppressor cells protect transplanted SC beta cells from T cell mediated killing *in vivo*









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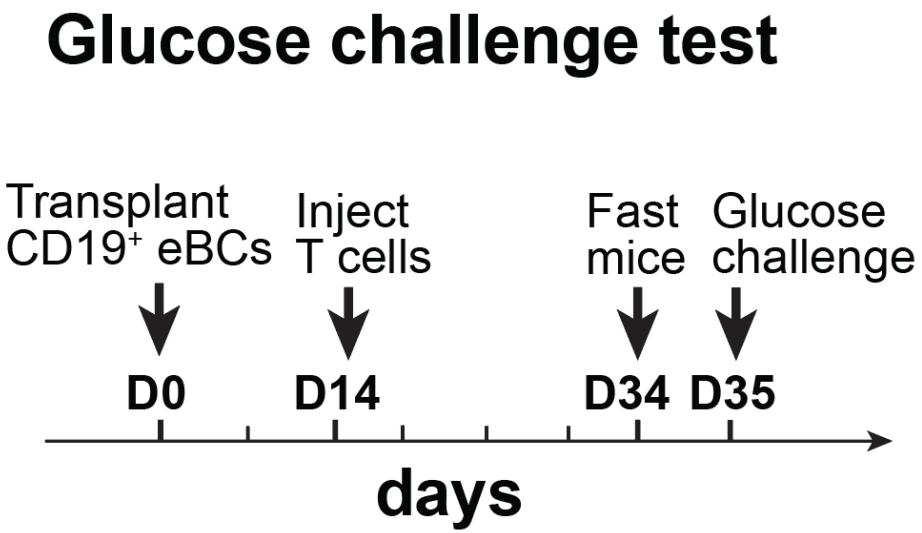
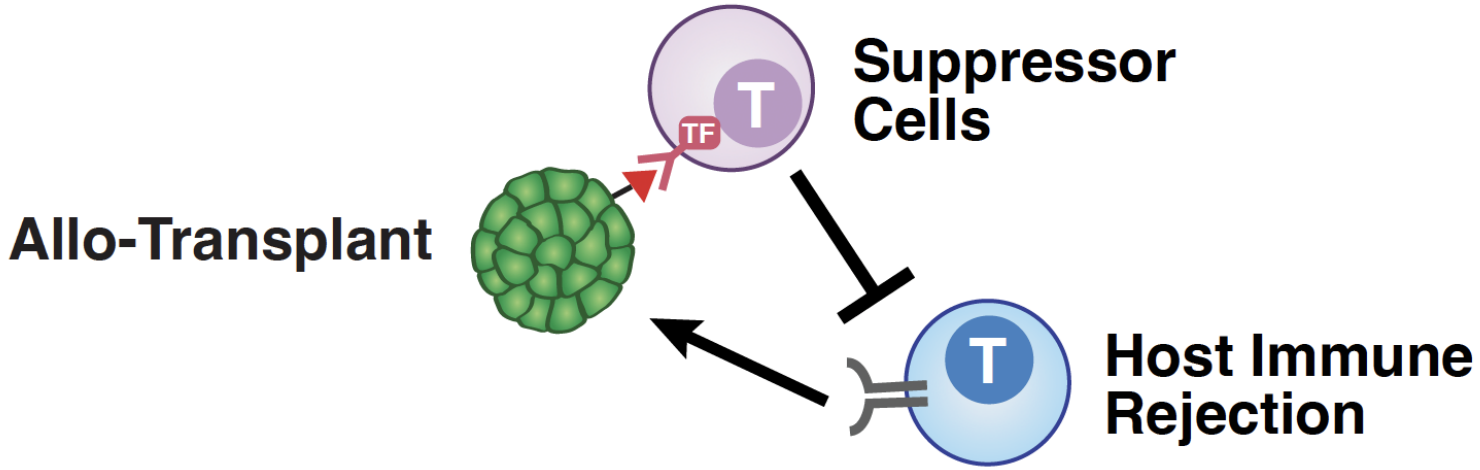
Protected transplanted SC beta cells remain functional *in vivo*



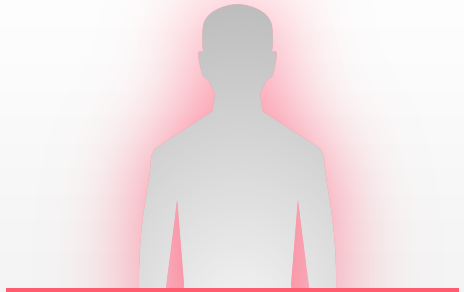
Summary I



- Generation of suppressor cells capable of **recognizing** a **specific** beta cell antigen
- Targeted **local immune suppression** prevents CD8 CAR T cells mediated beta cell killing *in vitro* and *in vivo*

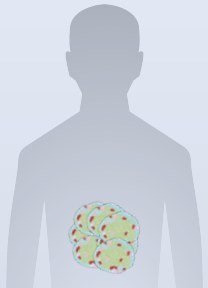
Designer suppressor cells generate a protective barrier for stem cell transplants

Stem cell-derived islets for diabetes therapy



Pluripotent Stem Cells → Pancreatic Endoderm → Immature Beta-like → Functional beta cells

Patient with diabetes



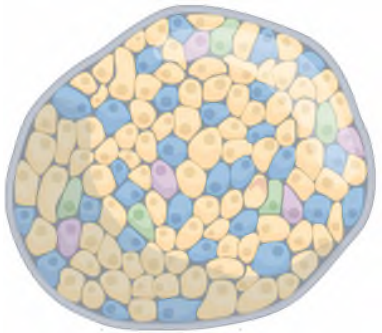
Patient without diabetes

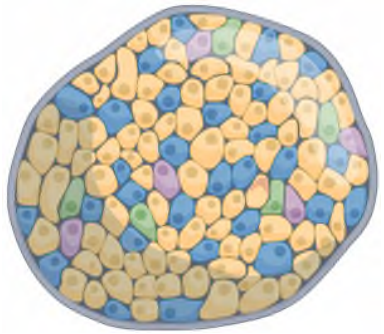
SC-Islets
Current generation

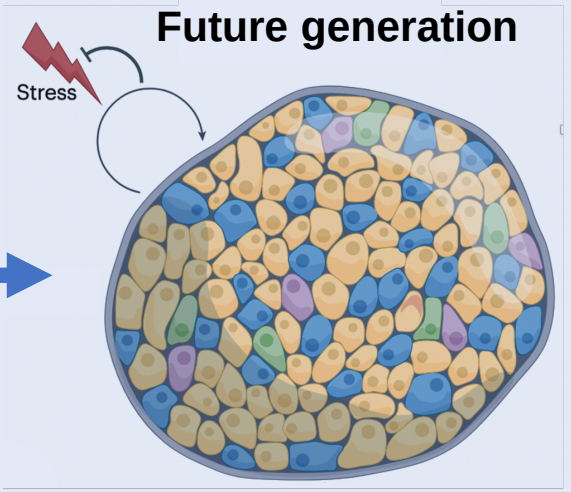
SC-Islets
Next generation

SC-Islets
Future generation

hPSC





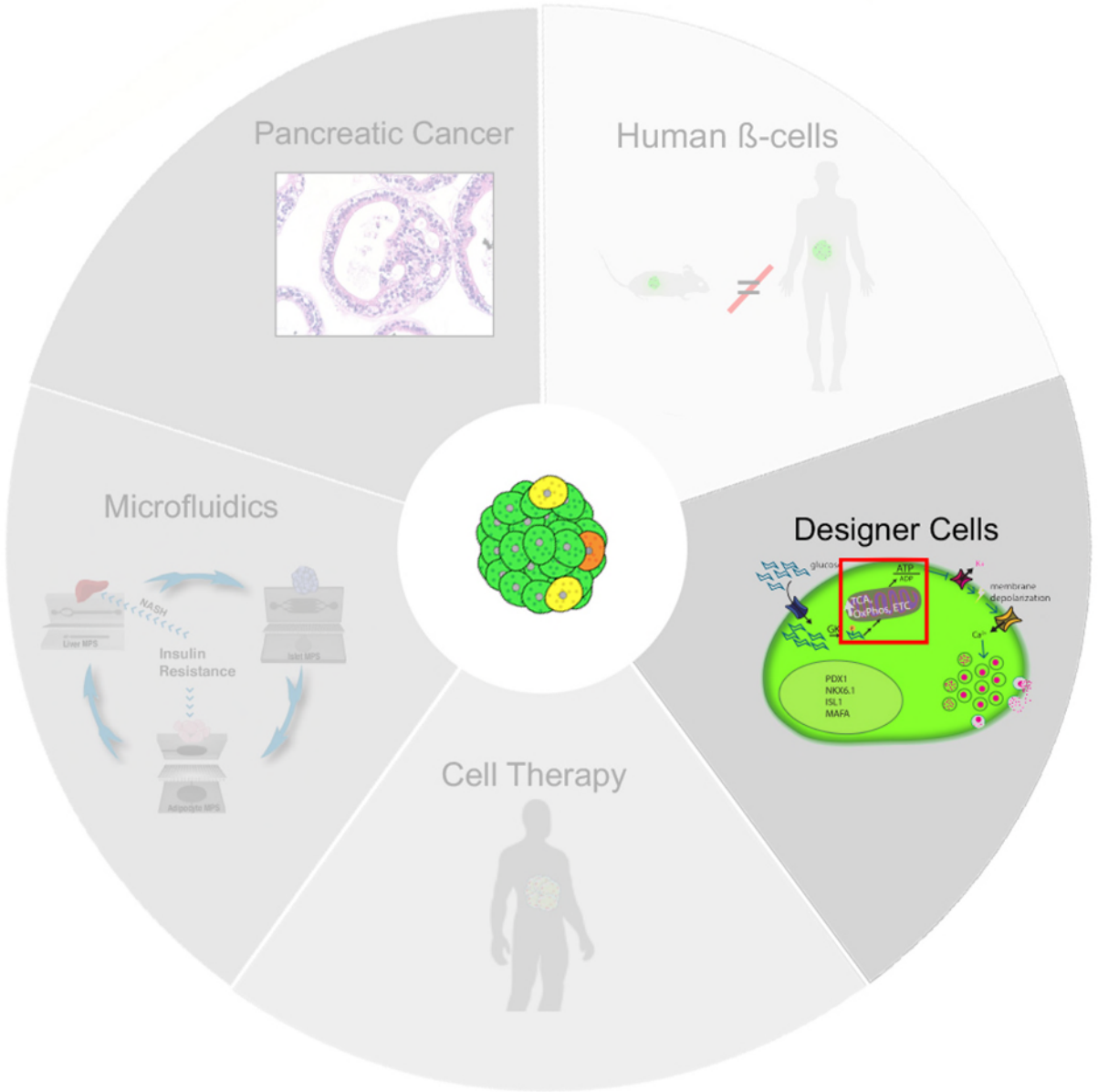


Glycemic Control
Limitless Supply

Immune Evasion

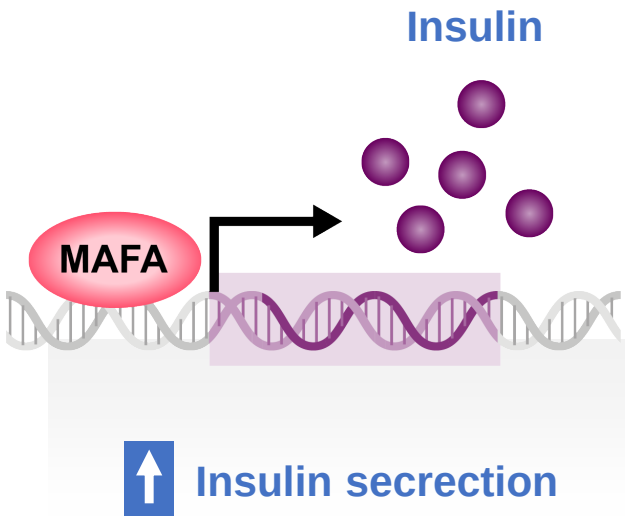
Enhanced Longevity, Optimized Metabolism, Self-Healing

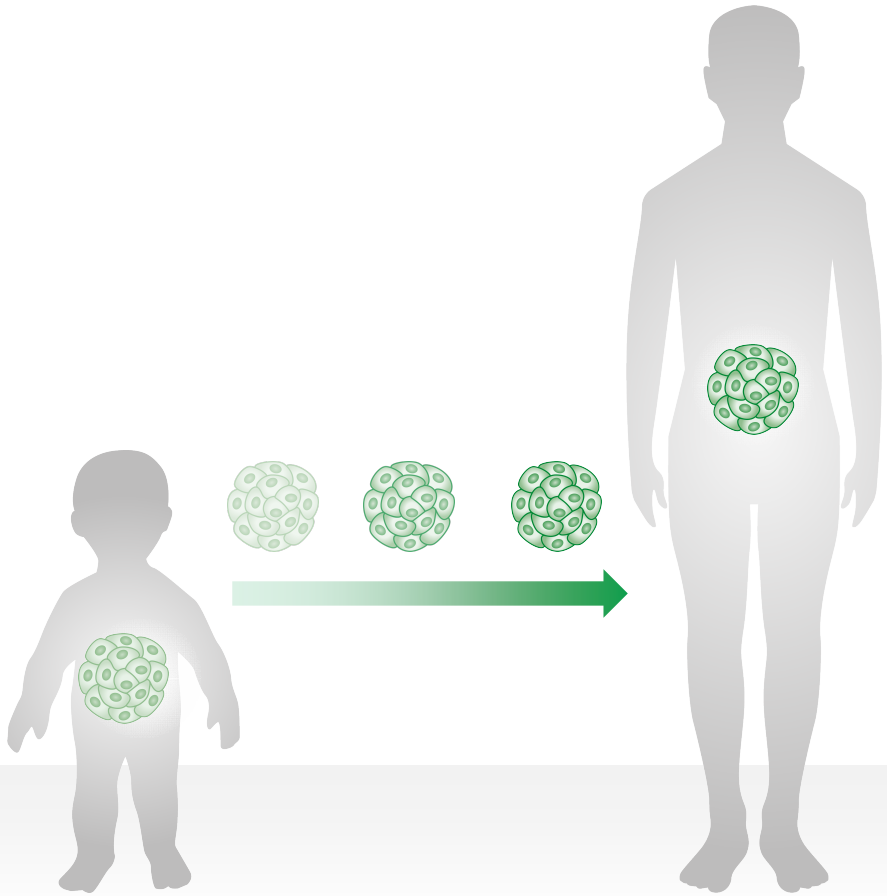
Optimizing beta cell function



Veronica Cochrane, PhD
Helmholtz Munich/UCSF

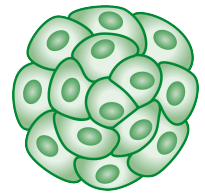
MAFA – a critical regulator of beta cell function

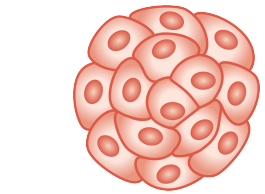




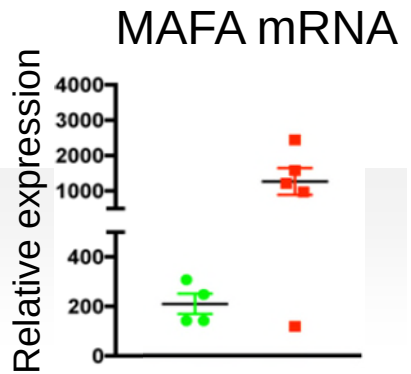
Sc-β cell (d27)

Human islets

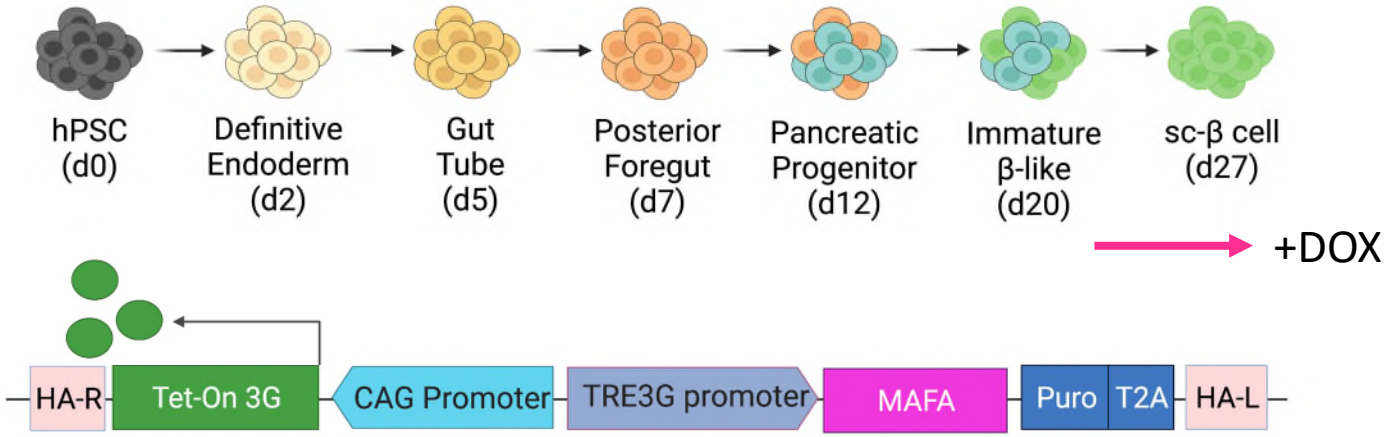


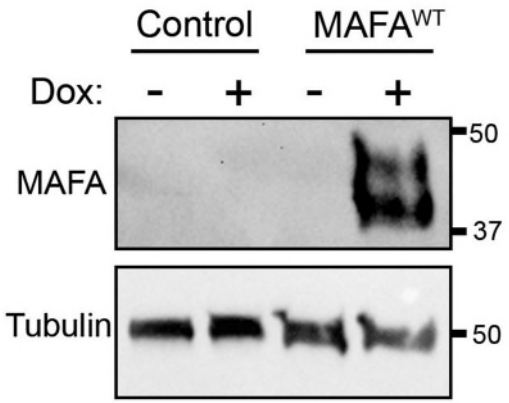


MAFA mRNA

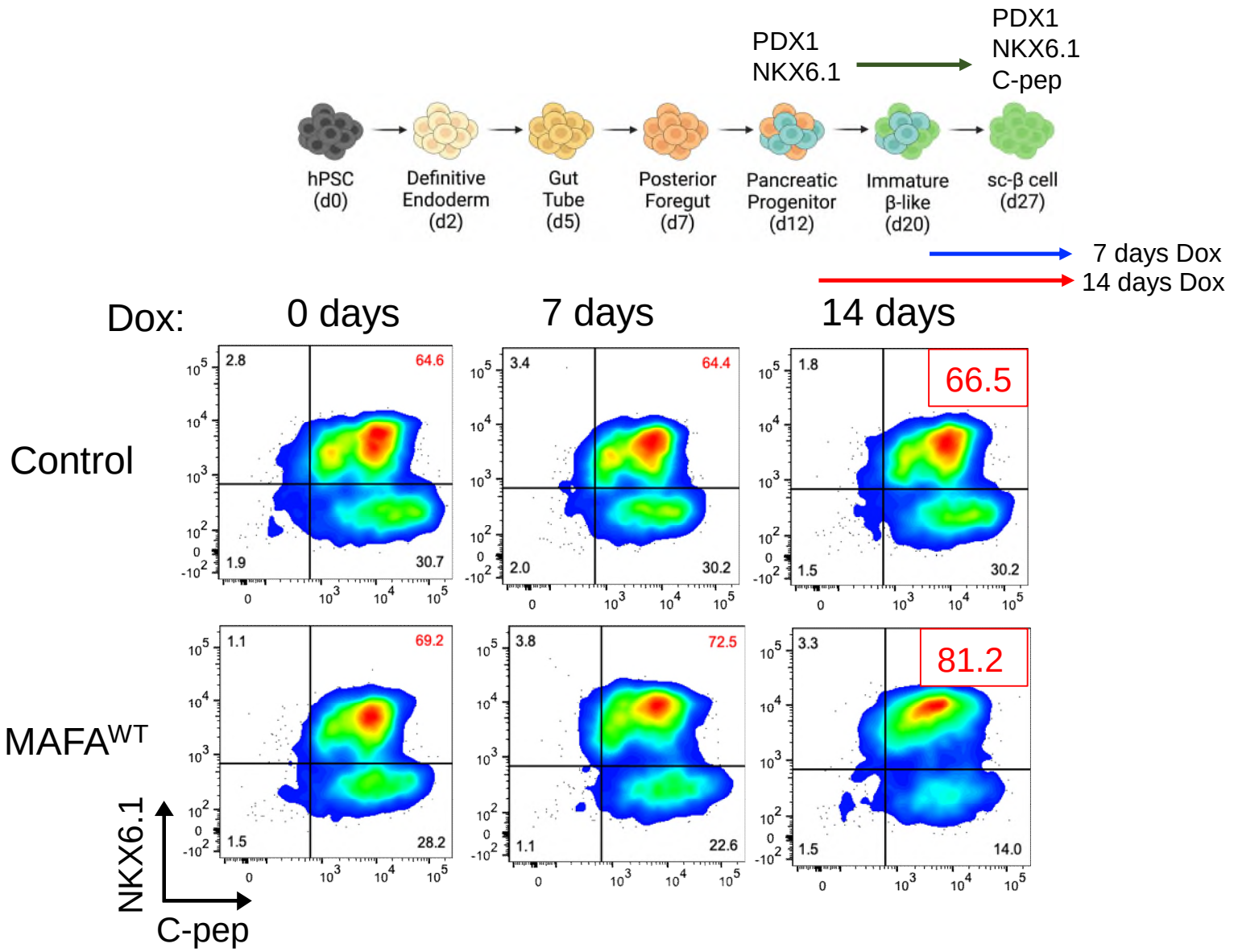


Generating MAFA overexpression cells

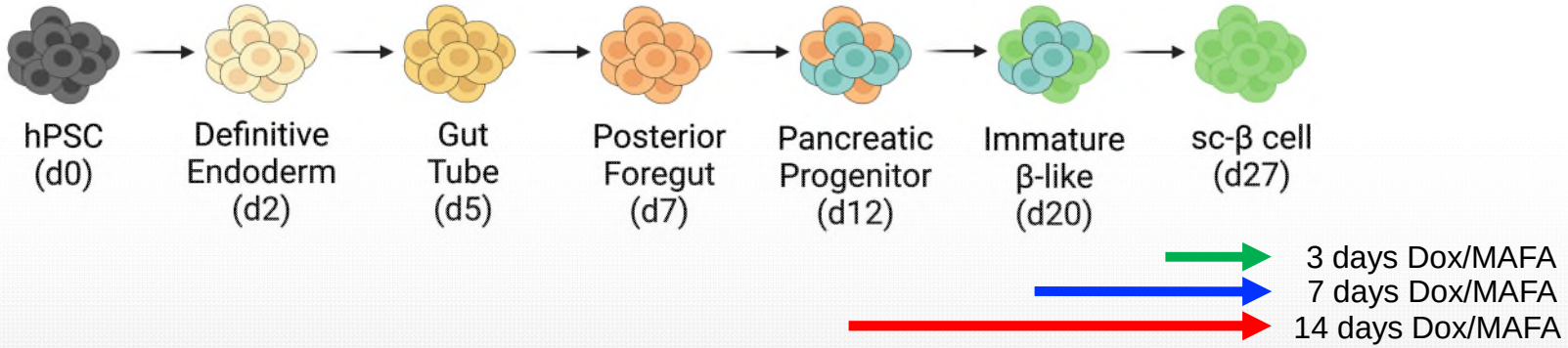




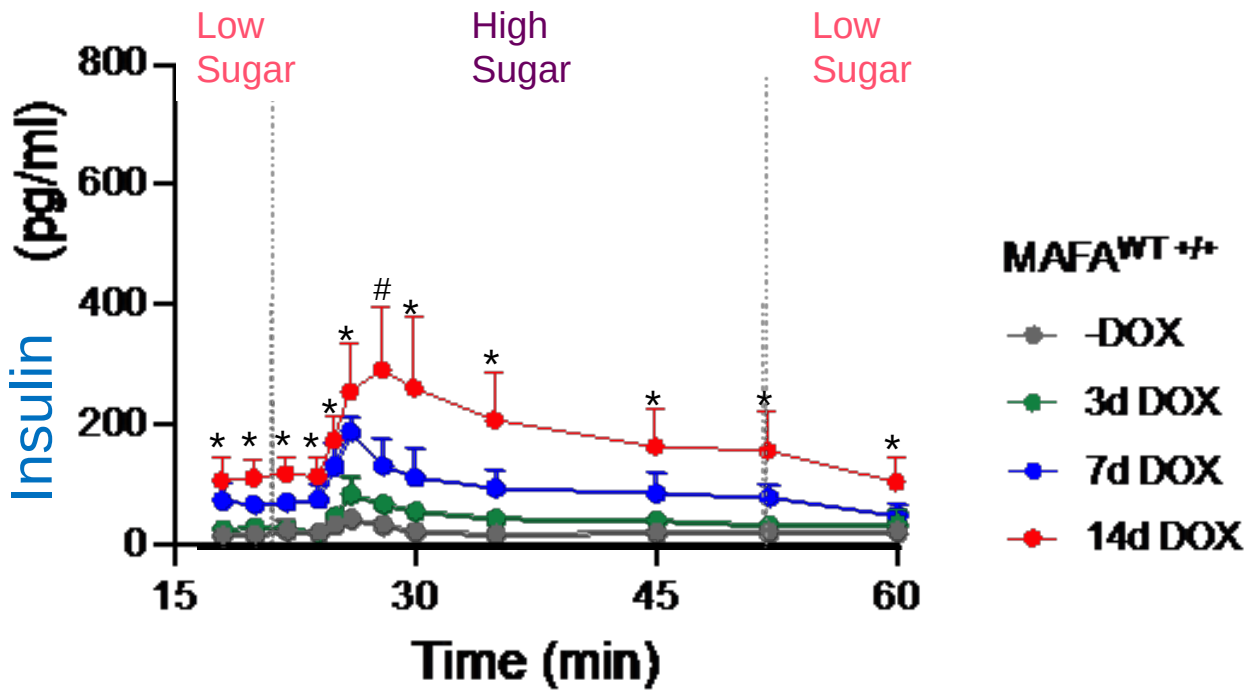
MAFA overexpression increases beta cell markers



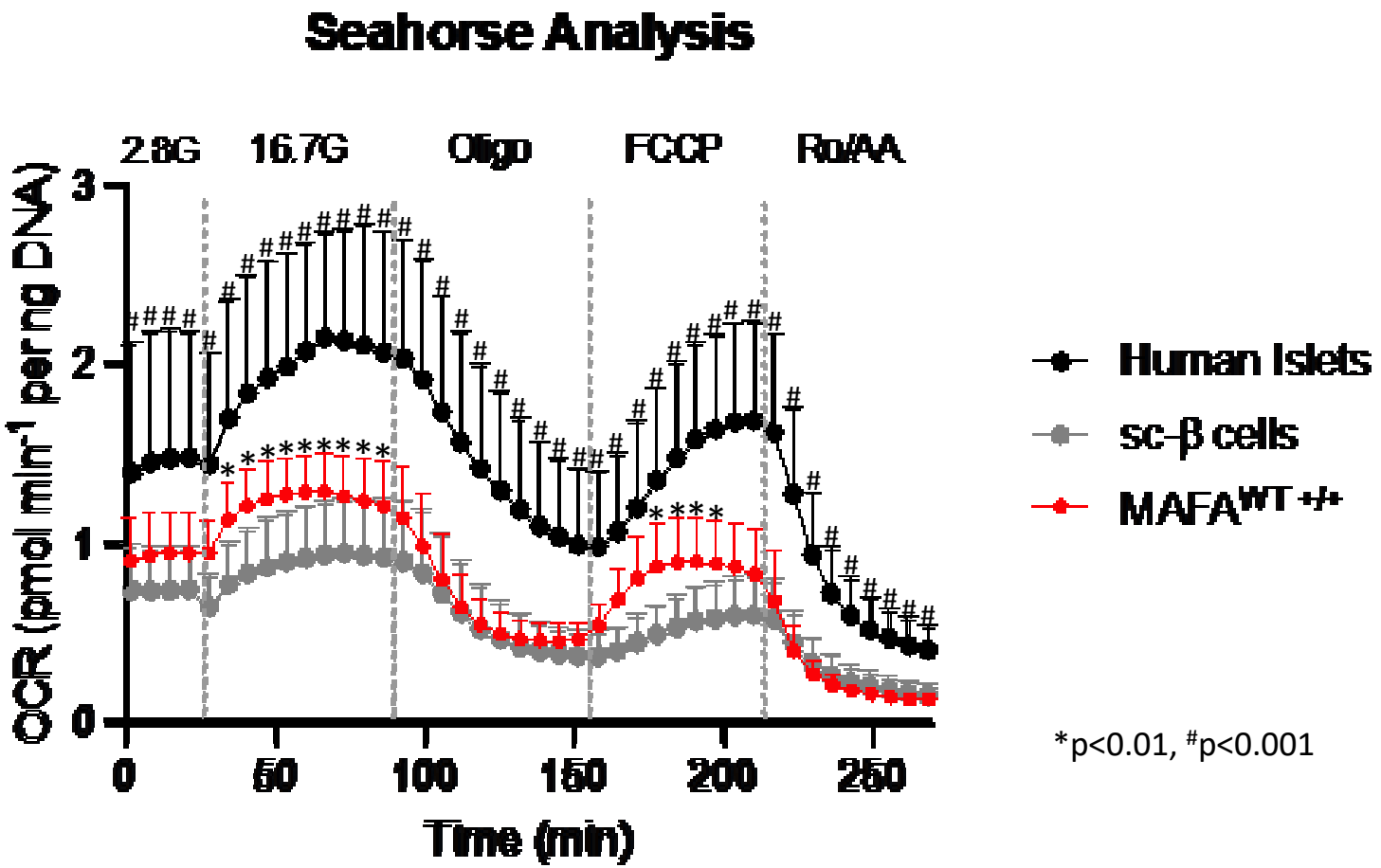
MAFA overexpression enhances sc-islet functionality



Insulin Secretion Assay

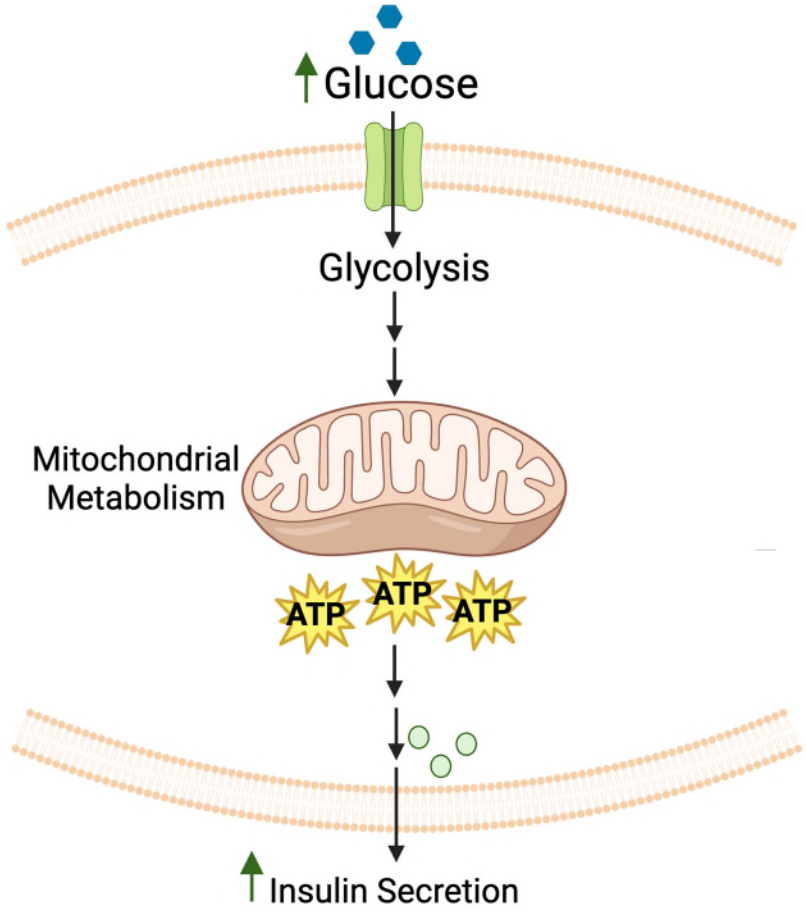


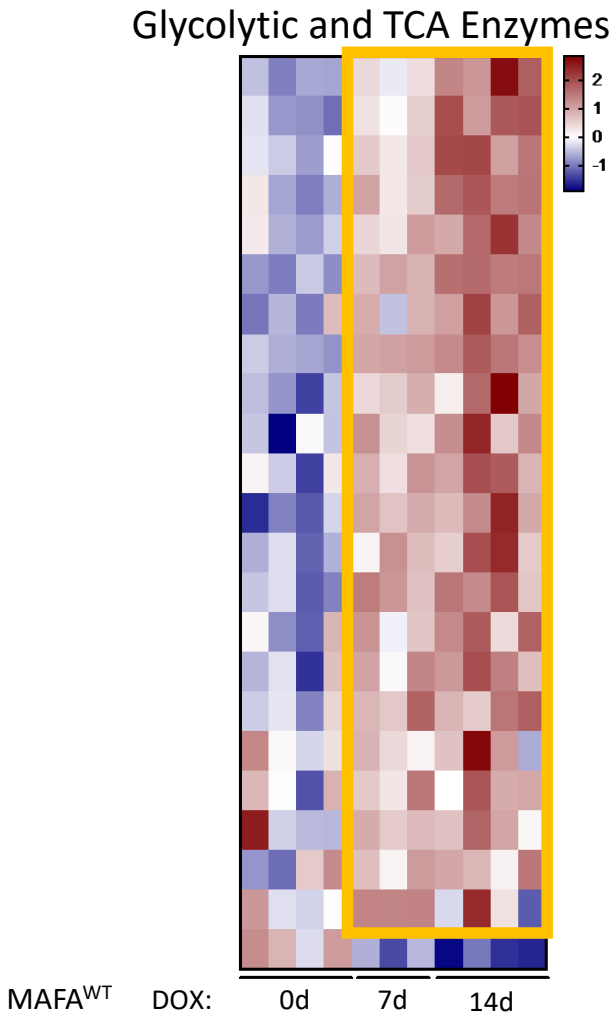
MAFA enhances mitochondrial metabolic activity

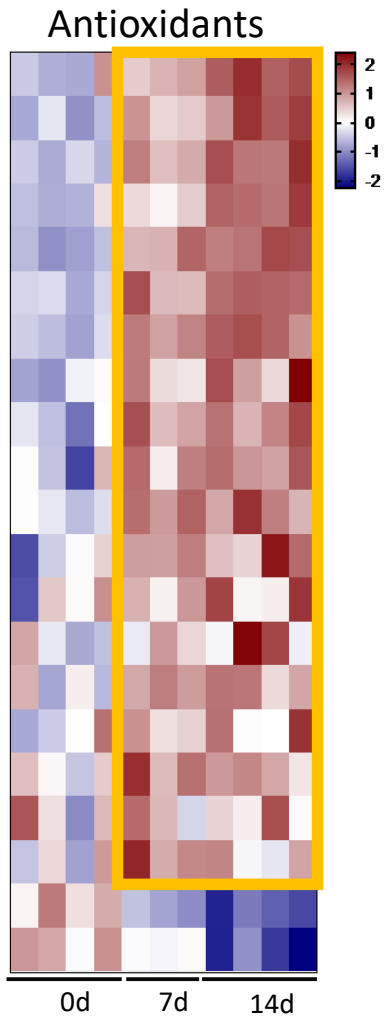


*p<0.01, #p<0.001

MAFA remodels SC-islet metabolic signature







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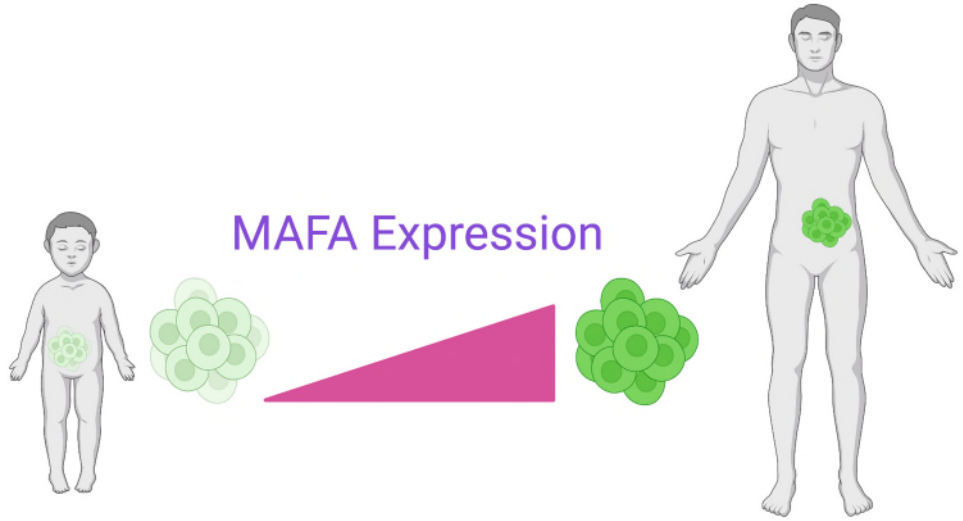
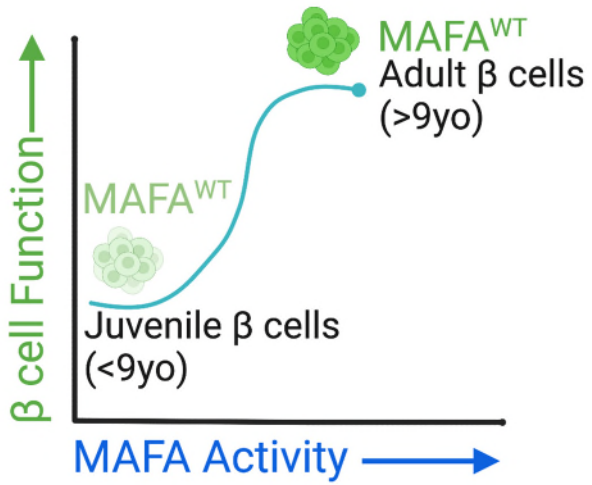
MAFA sc-Beta Cell - Conclusions

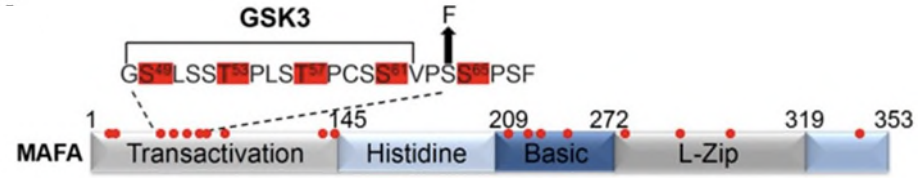
- Increasing MAFA expression in sc-beta cells increases beta cell identity markers
- MAFA enhances sc-beta functionality
- MAFA increases sc-beta cell metabolic activity



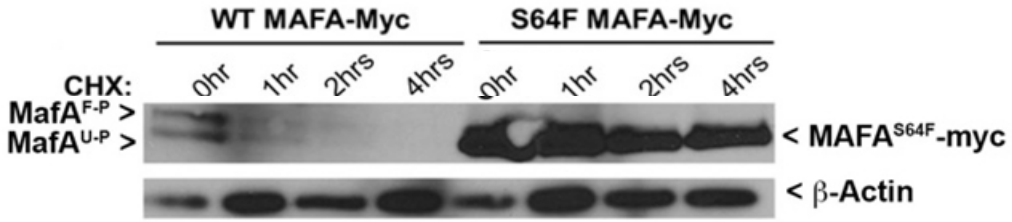


The dark side of MAFA: human MAFA mutant S64F

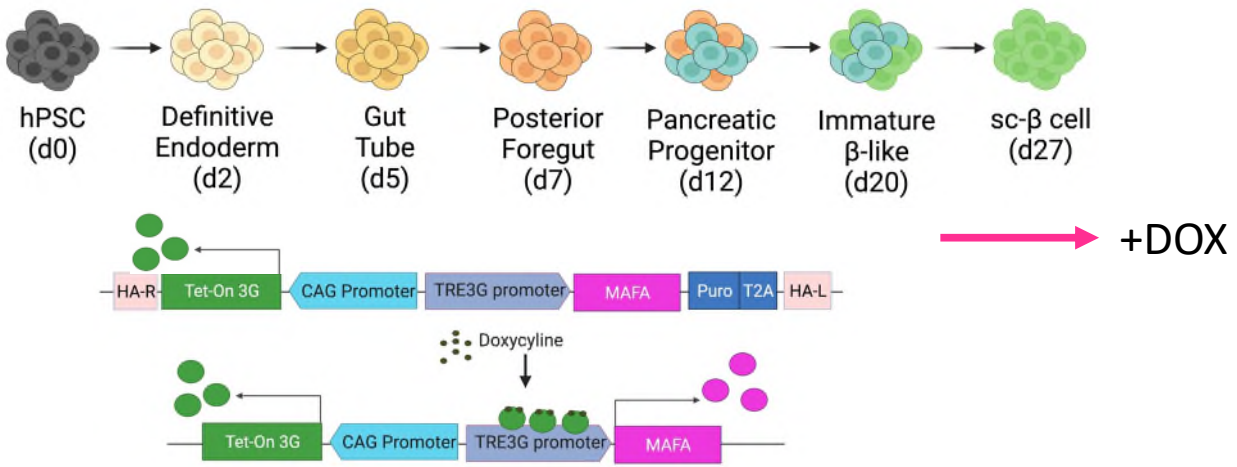
- MAFA phosphorylation regulates transcriptional activity, DNA binding, and protein degradation

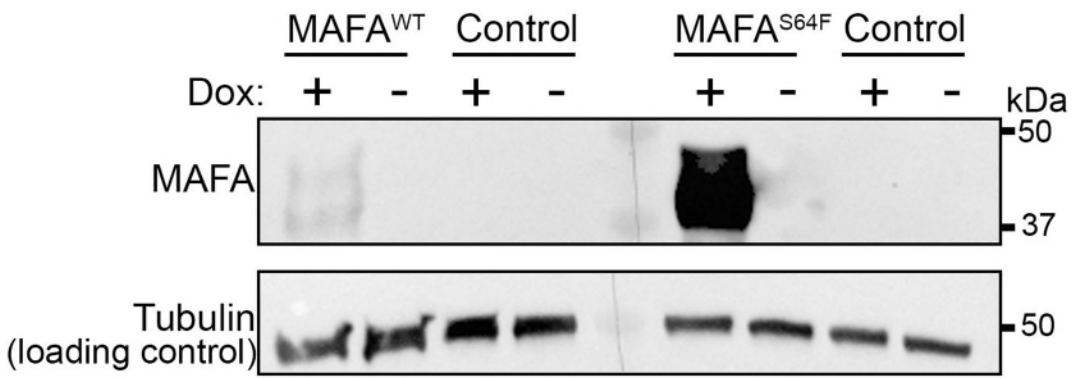


Lacovazzo, D., et al., *PNAS* (2018)

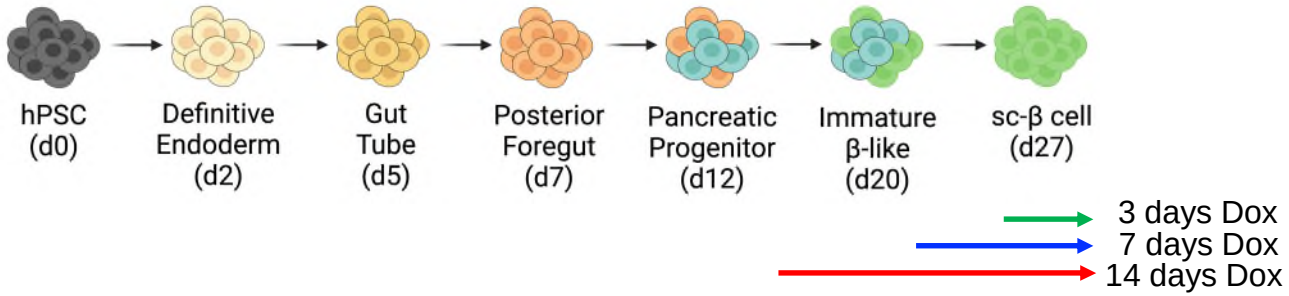


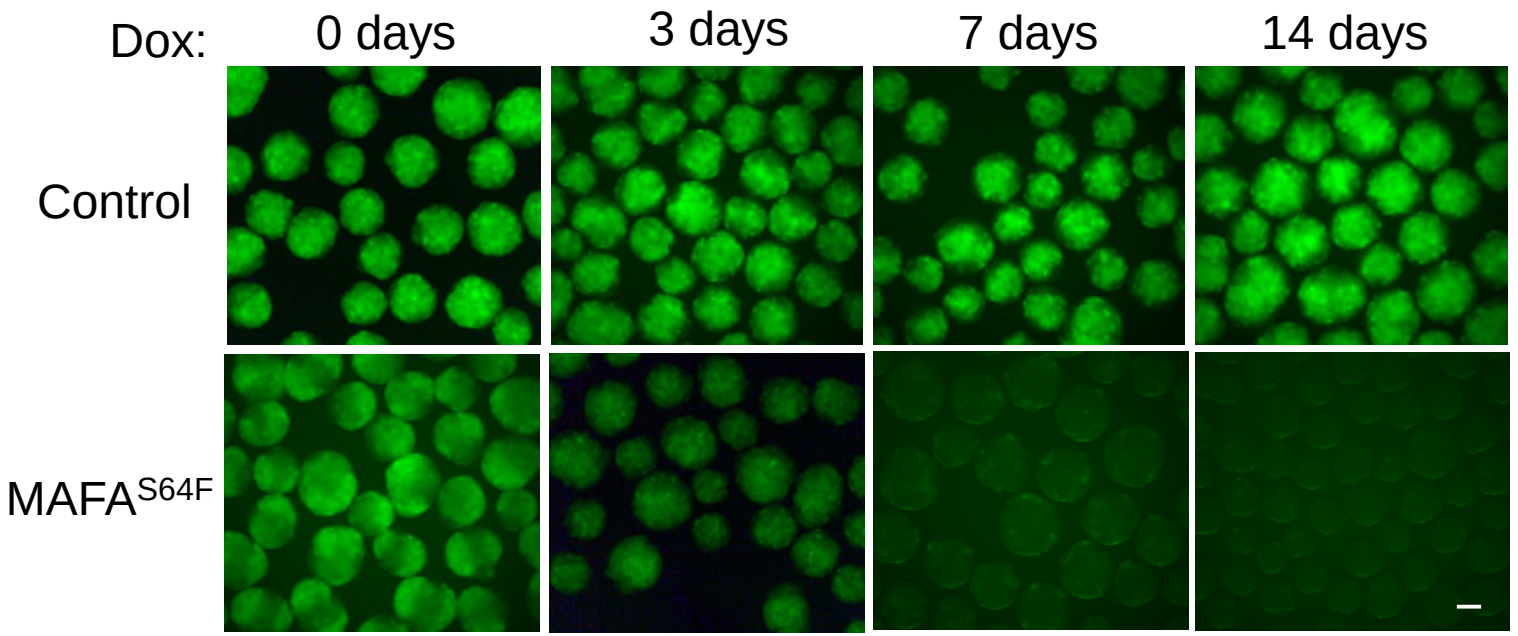
MAFA^{S64F} overexpression cells



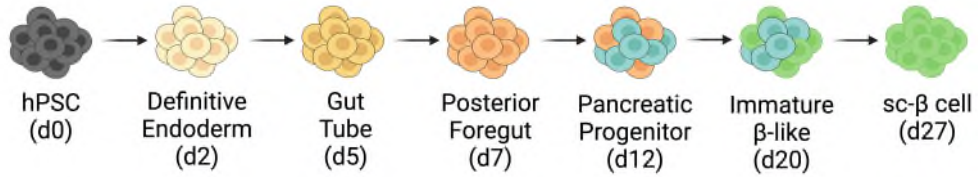


MAFA overexpression in sc-beta cells



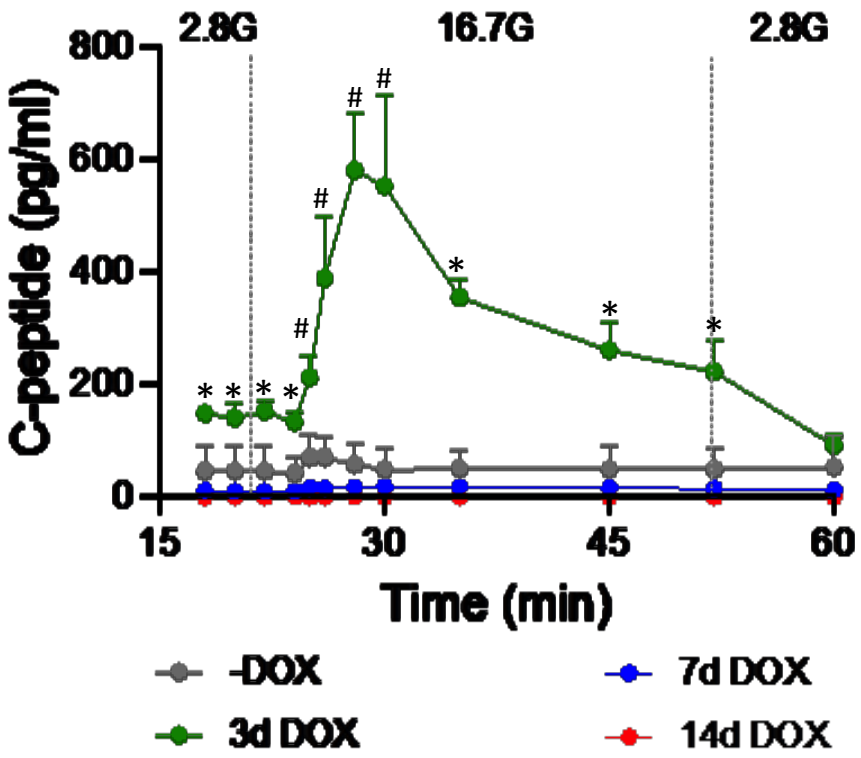


MAFA^{S64F} transiently improves sc-beta cell function



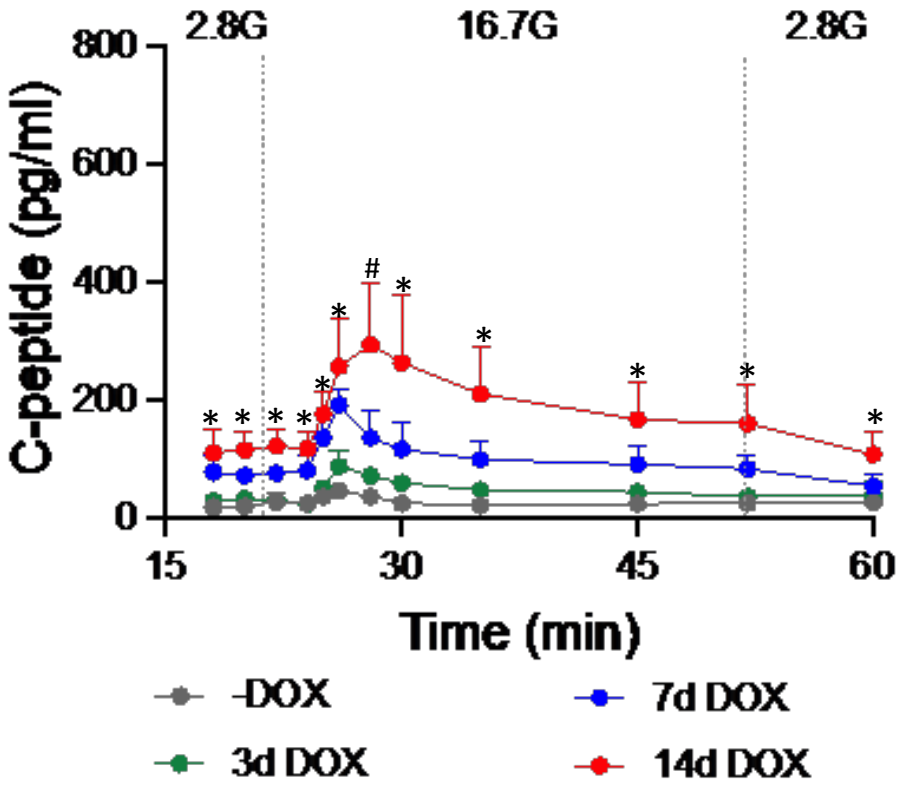
3 days Dox
7 days Dox
14 days Dox

MAFA^{S64F}



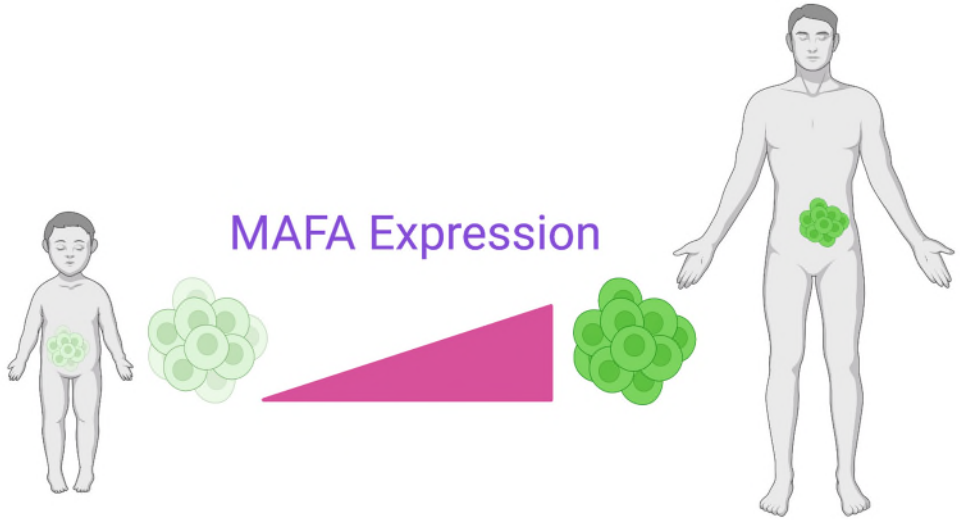
*p<0.001, #p<0.0001

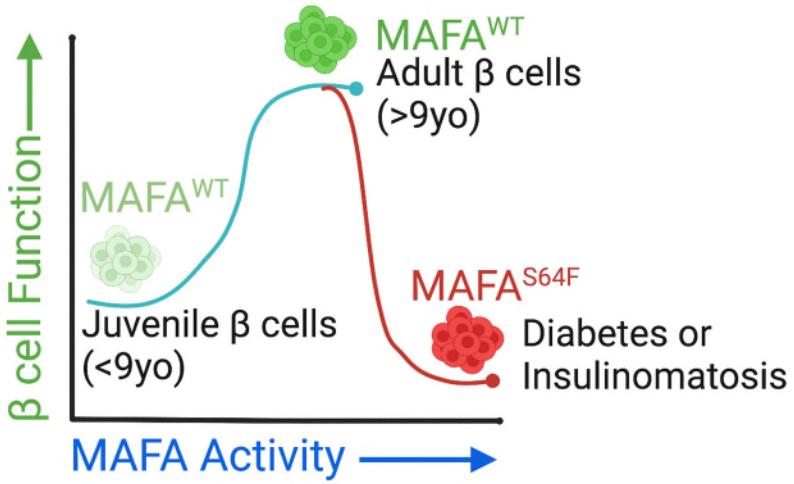
MAFA^{WT}



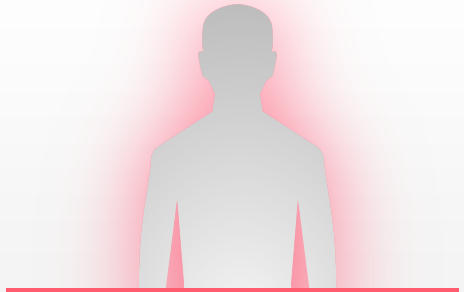
Summary II

- Sc-beta cells can be used to study and optimize human beta cell health and function
  - $MAFA^{WT}$ improves beta cell GSIS response via modulation of metabolism
- Sc-beta cells are valuable for studying disease pathology
  - Investigating $MAFA^{S64F}$ transient increase in functionality followed by a loss of function



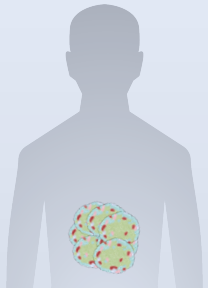


Stem cell-derived islets for diabetes therapy



Pluripotent Stem Cells → Pancreatic Endoderm → Immature Beta-like → Functional beta cells

Patient with diabetes



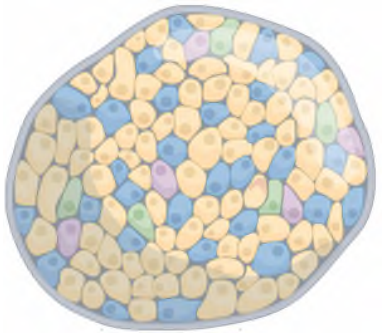
Patient without diabetes

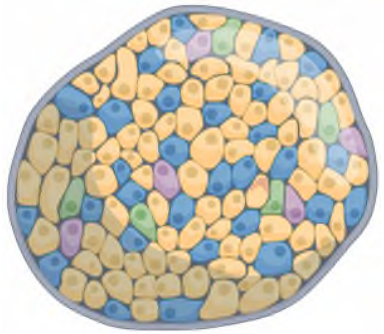
SC-Islets
Current generation

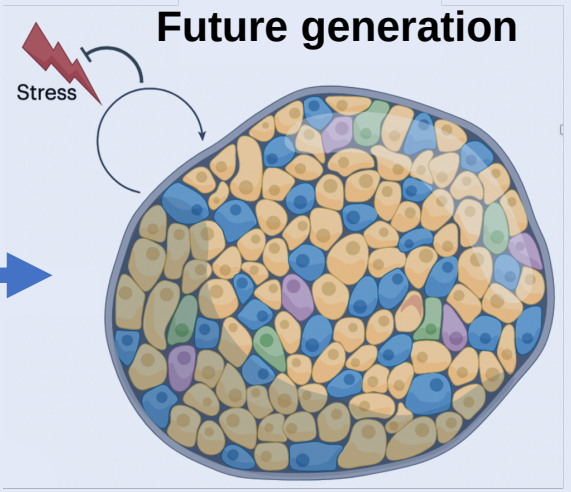
SC-Islets
Next generation

SC-Islets
Future generation

hPSC







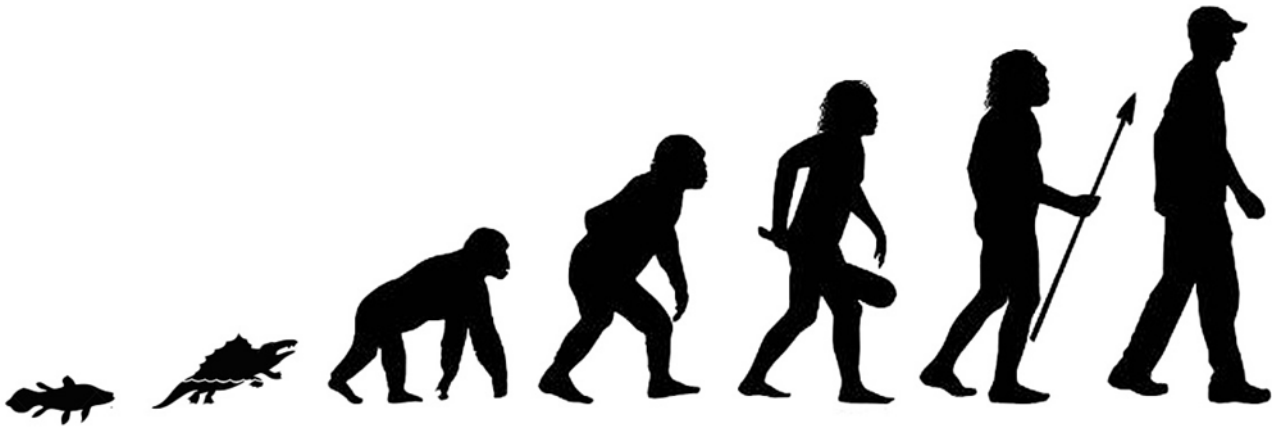
Glycemic Control
Limitless Supply

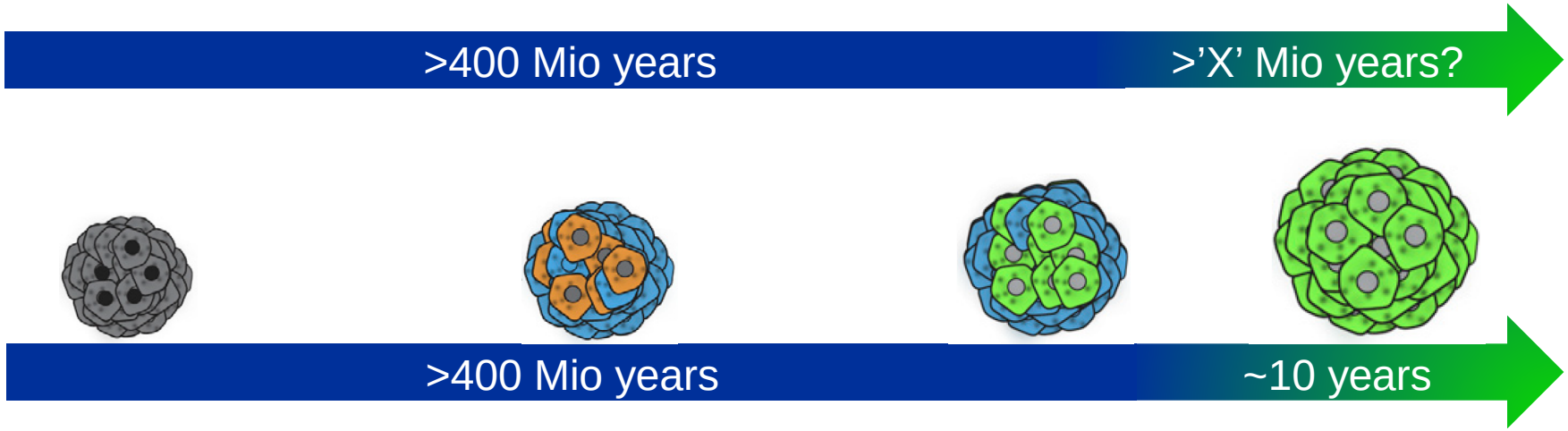
Immune Evasion

Enhanced Longevity, Optimized Metabolism, Self-Healing

The Overarching Goal:

Accelerating evolution to design superior beta/islet cells





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Bayerische Spitzenprofessur

NIH National Institute of Diabetes and Digestive and Kidney Diseases

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.

DZD Deutsches Zentrum für Diabetesforschung