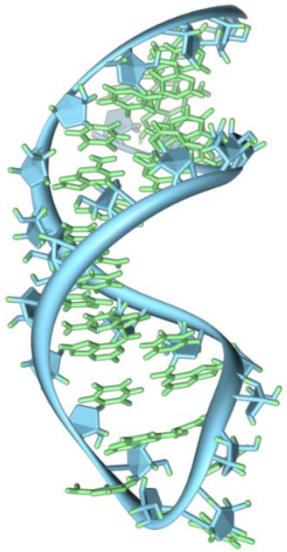
Joslin Diabetes

Harvard Medical School



Interrogating RNA modifications to gain new insights into metabolic disorders

Rohit N. Kulkarni MD PhD

Session I: Precision Medicine for Chronic Metabolic Diseases

Precision Workshop I: US and Kuwait

Feb 2-4, 2025

Beth Israel Lahey Health

Disclosures

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NIH National Institute of Diabetes and Digestive and Kidney Diseases

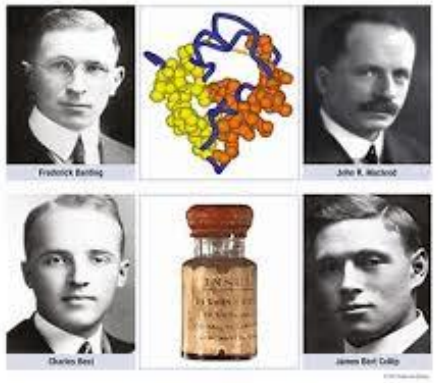
HiRN Human Islet Research Network

American Diabetes Association

nPOD Network for Pancreatic Organ Donors with Diabetes

103

INSULIN



No Cure yet

Diabetes:

Total: 38.4 M (11.6% of U.S. pop); **8.7 % in MA**

Diagnosed: 29.7 M

Undiagnosed: 8.7 M (22.8% of adult U.S.pop)

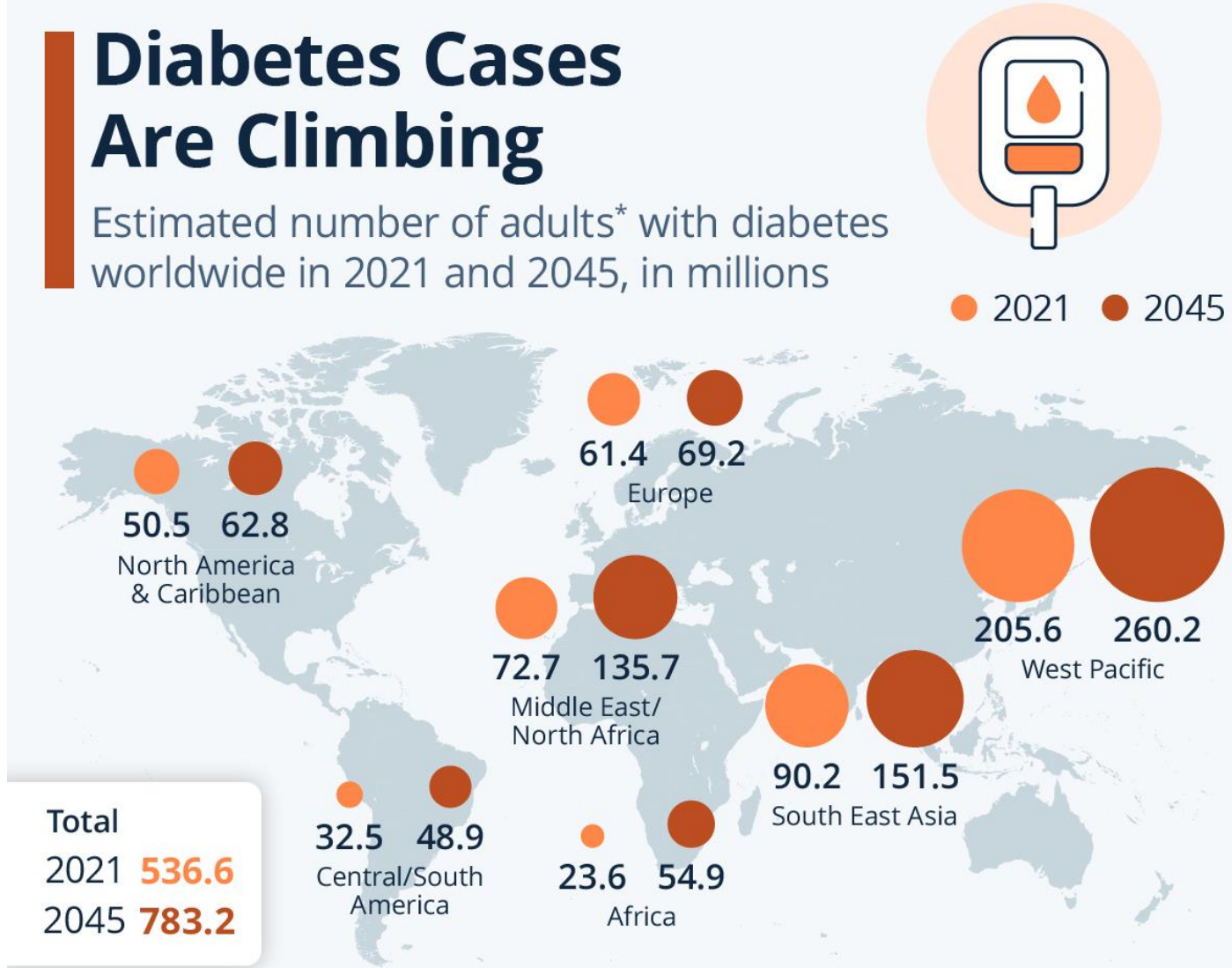
Pre-Diabetes:

Total: 97.6M 18 y/older (38.0% adult U.S. pop)

First patient to receive insulin

Diabetes Cases Are Climbing

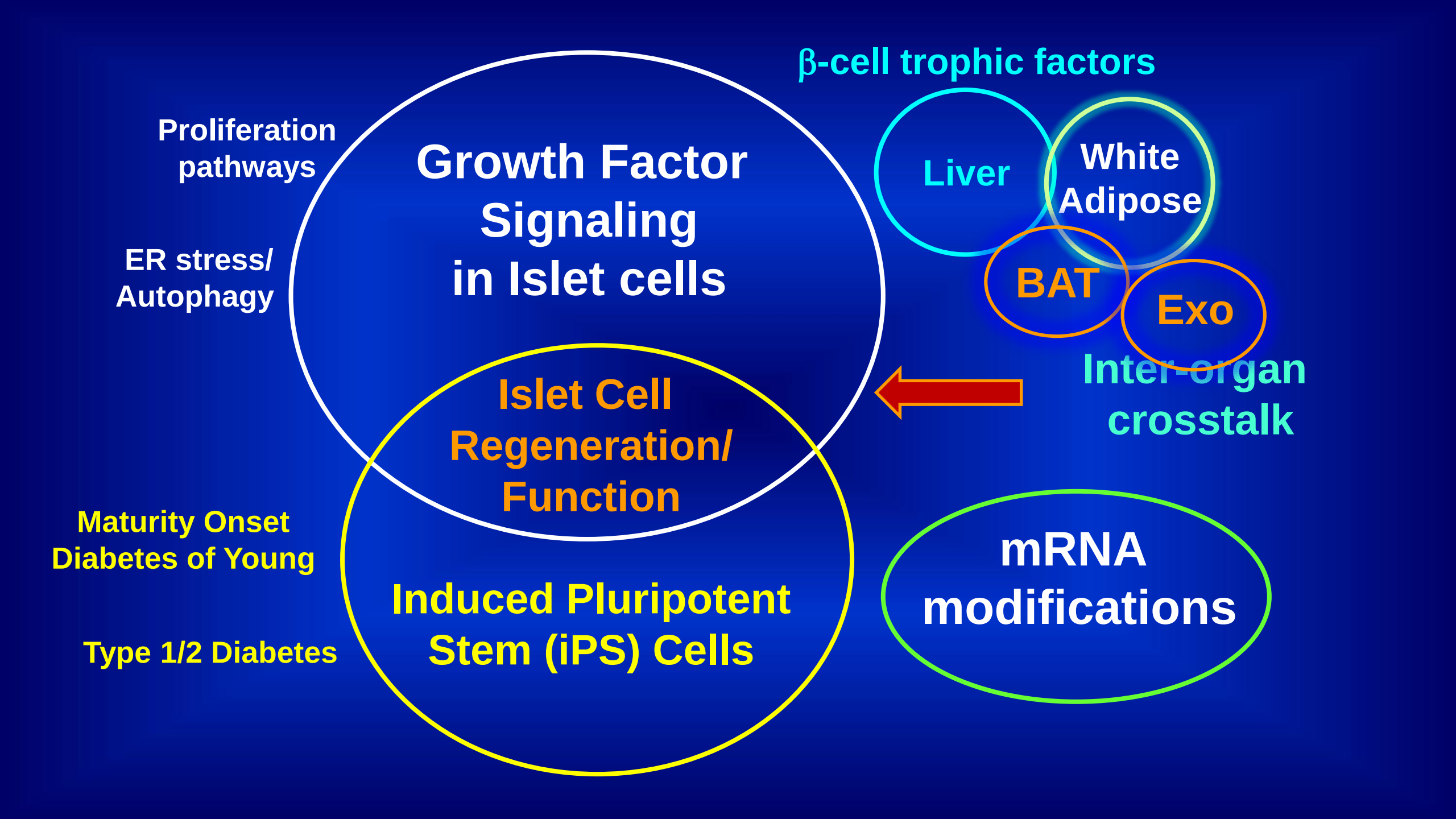
Estimated number of adults* with diabetes worldwide in 2021 and 2045, in millions

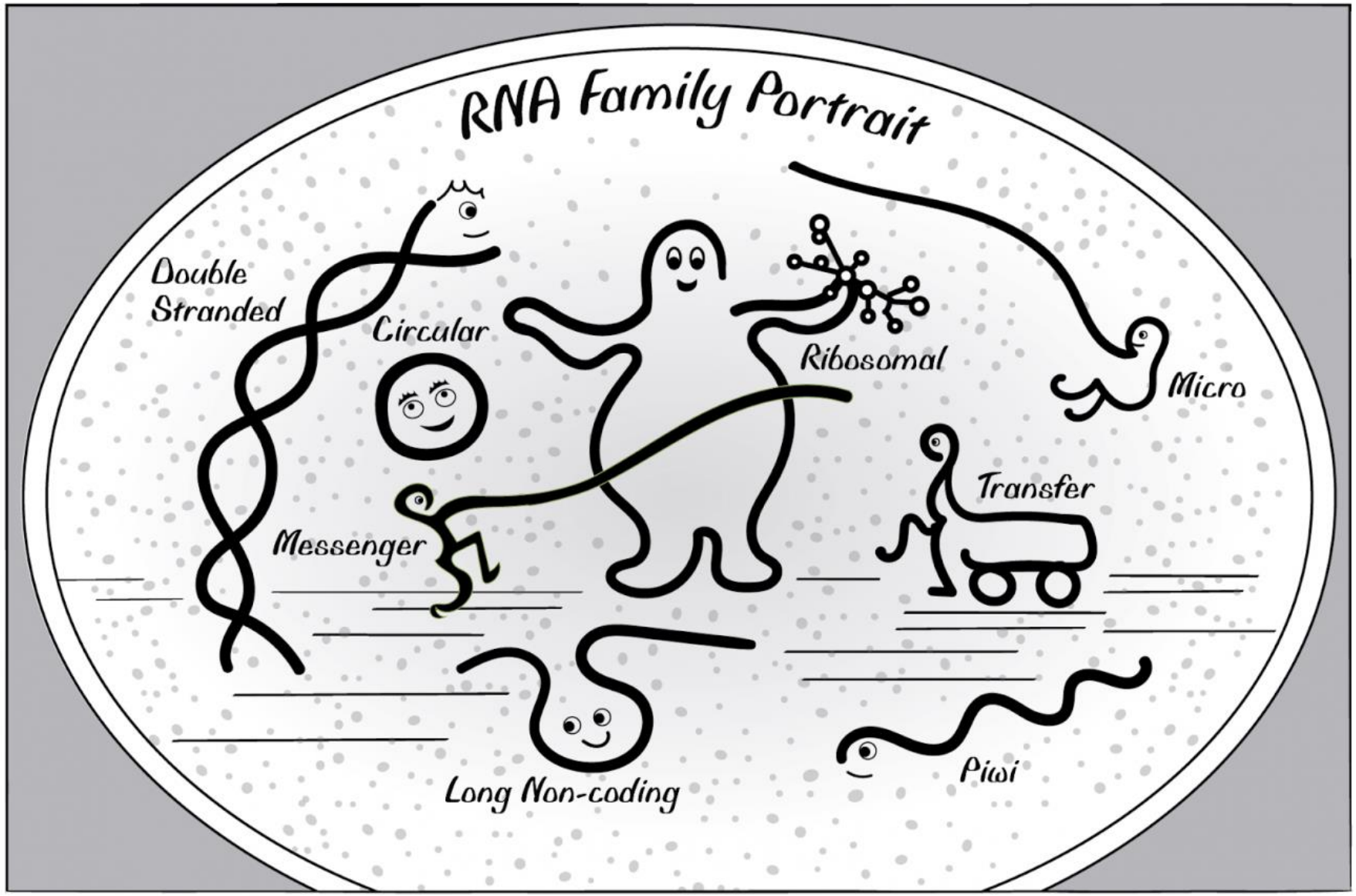


* aged 20 to 79

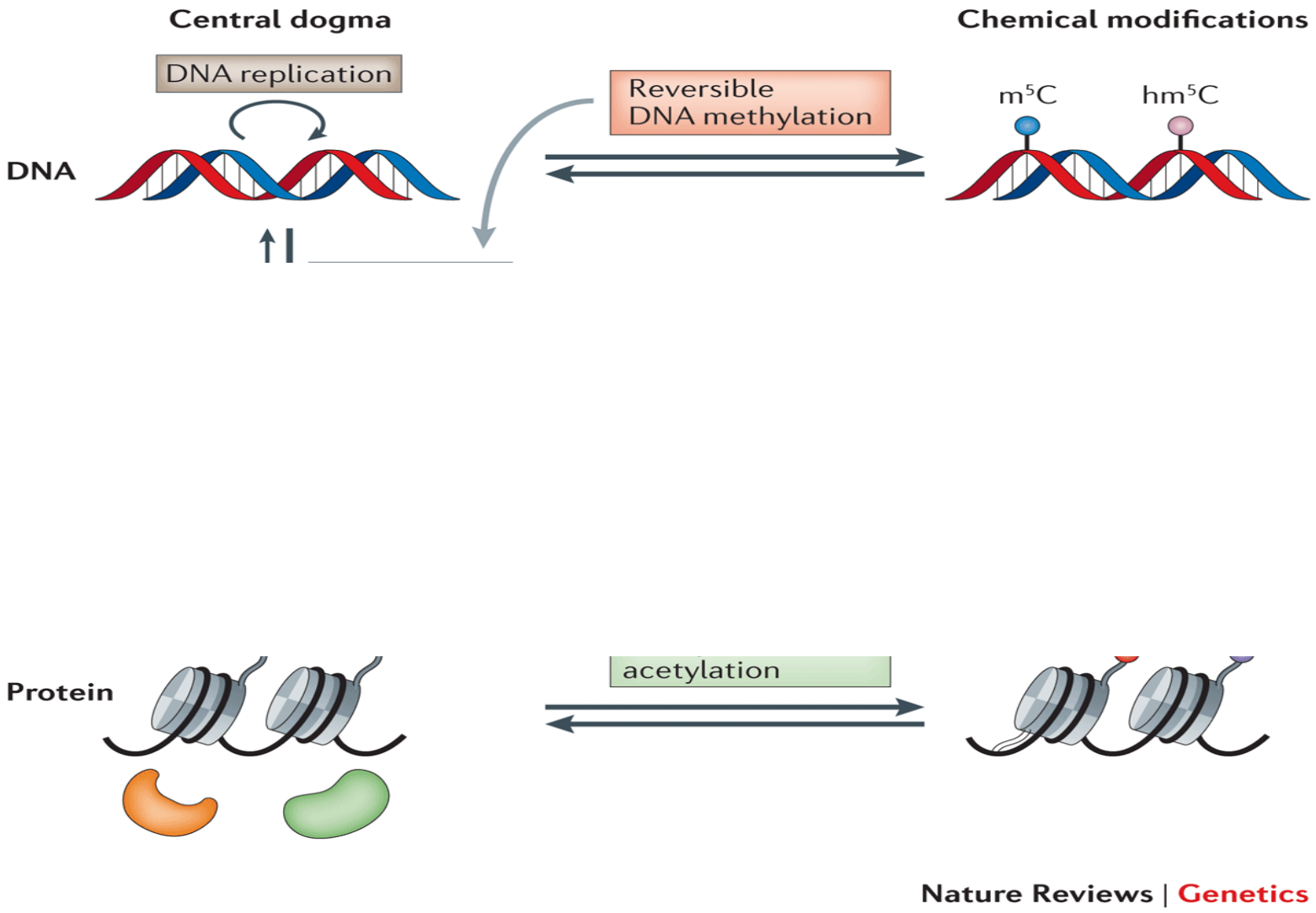
Source: International Diabetes Federation

statista


β-cell trophic factors
Liver
White Adipose
BAT
Exo
Inter-organ crosstalk
Proliferation pathways
ER stress/ Autophagy
Growth Factor Signaling in Islet cells
Islet Cell Regeneration/ Function
Maturity Onset Diabetes of Young
Type 1/2 Diabetes
Induced Pluripotent Stem (iPS) Cells
mRNA modifications


RNA Family Portrait
Double Stranded
Circular
Ribosomal
Micro
Transfer
Messenger
Long Non-coding
Piwi

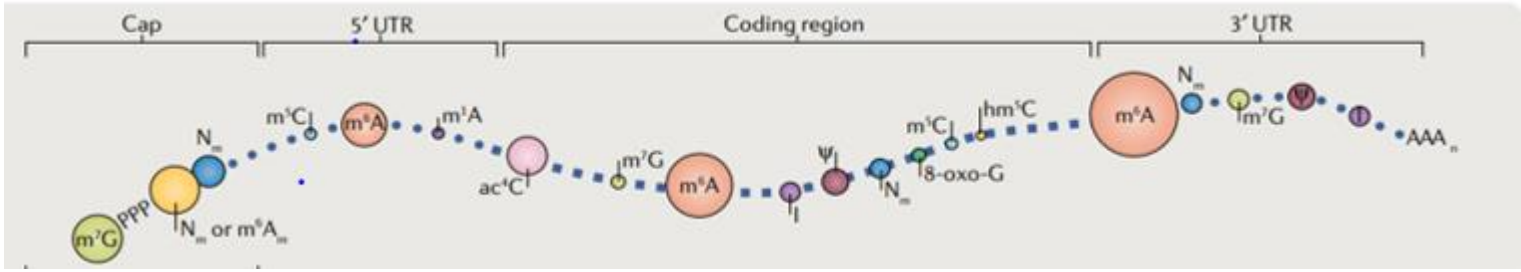
Rationale for Interrogating Dynamic RNA Modifications



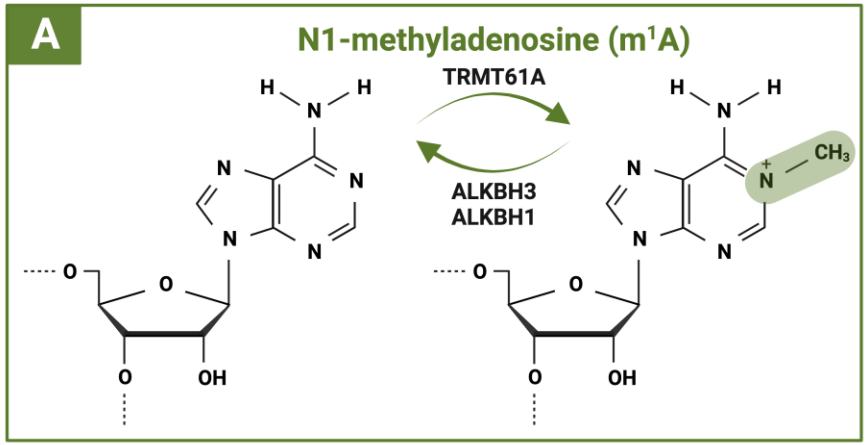
mRNA ≠ Protein

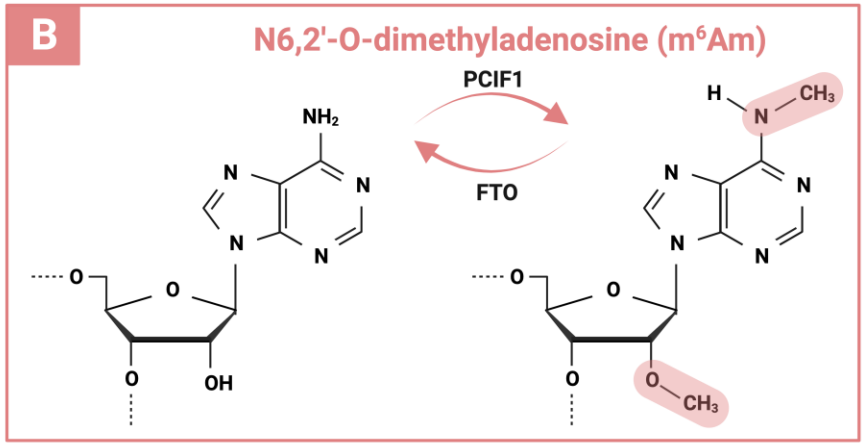
mRNA regulation of protein – 40%

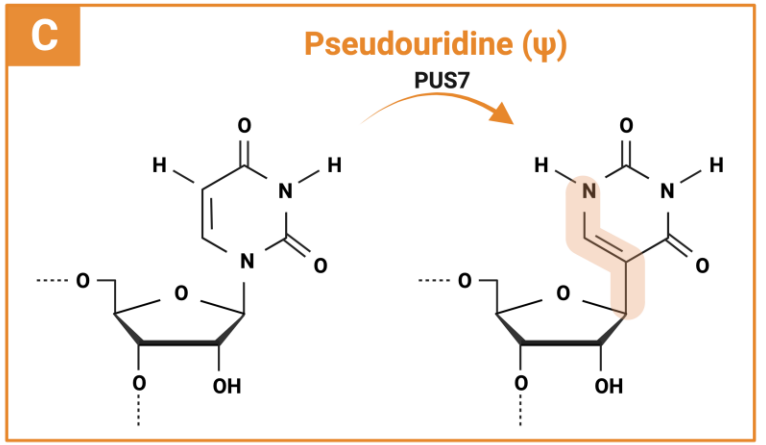
Most common chemical modifications in mRNAs

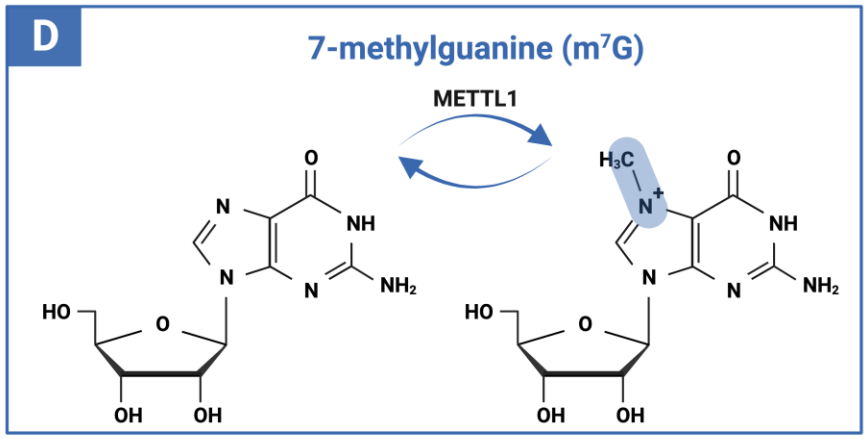


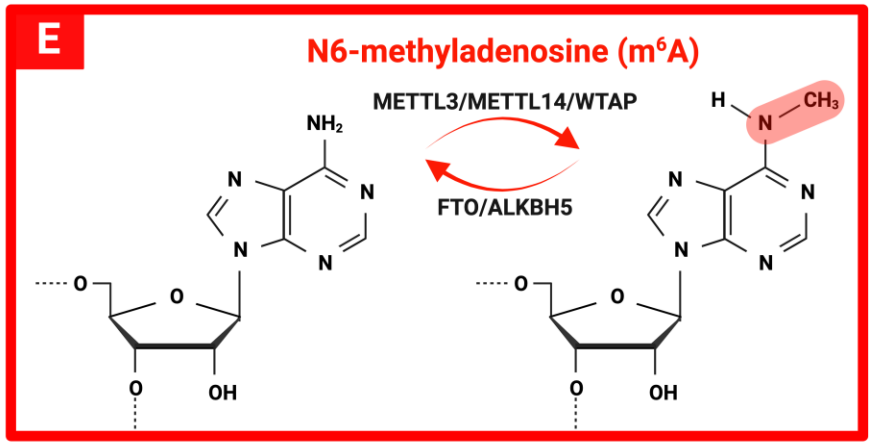
mRNA
tRNA
Long non-coding RNA
Ribosomal RNA

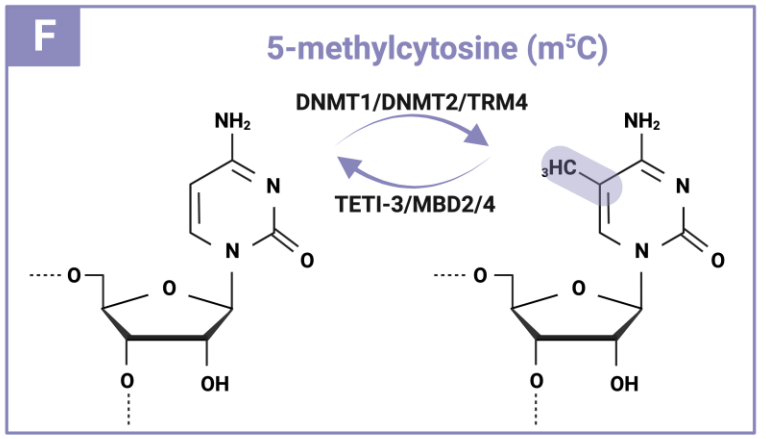








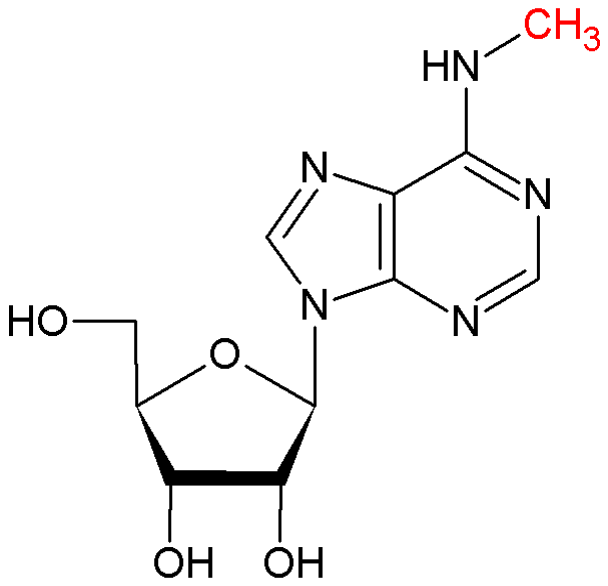


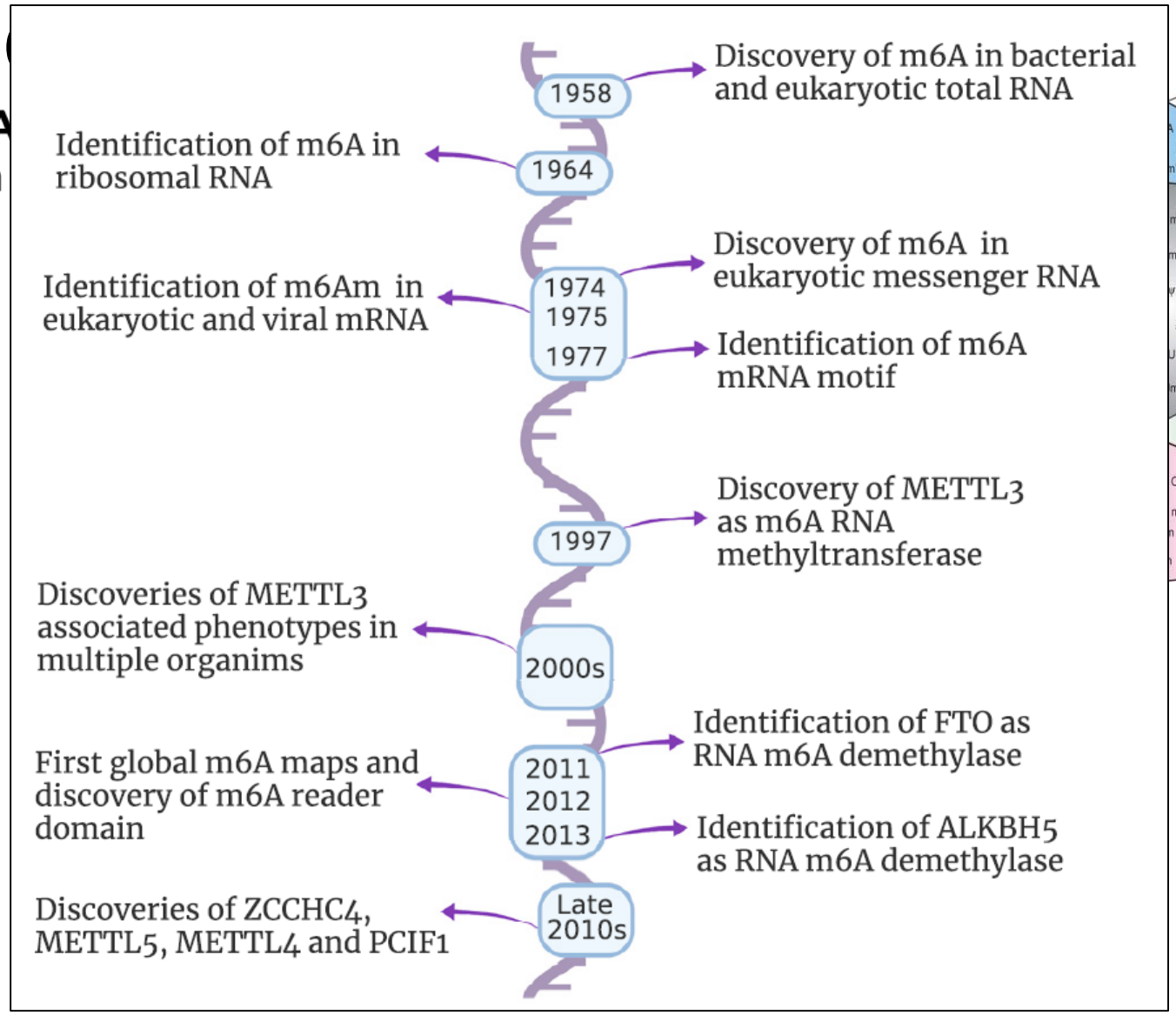


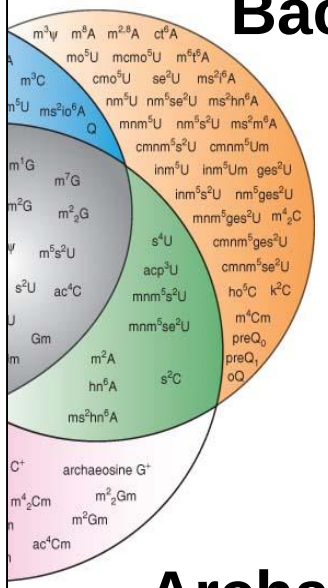
N^6-methyladenosine - highly conserved

Discovery: Fritz Rottman (

Mammals: Amount of m^6A is 0.1-0.4% of adenines in ~ 3-5 m^6A sites/mRNA



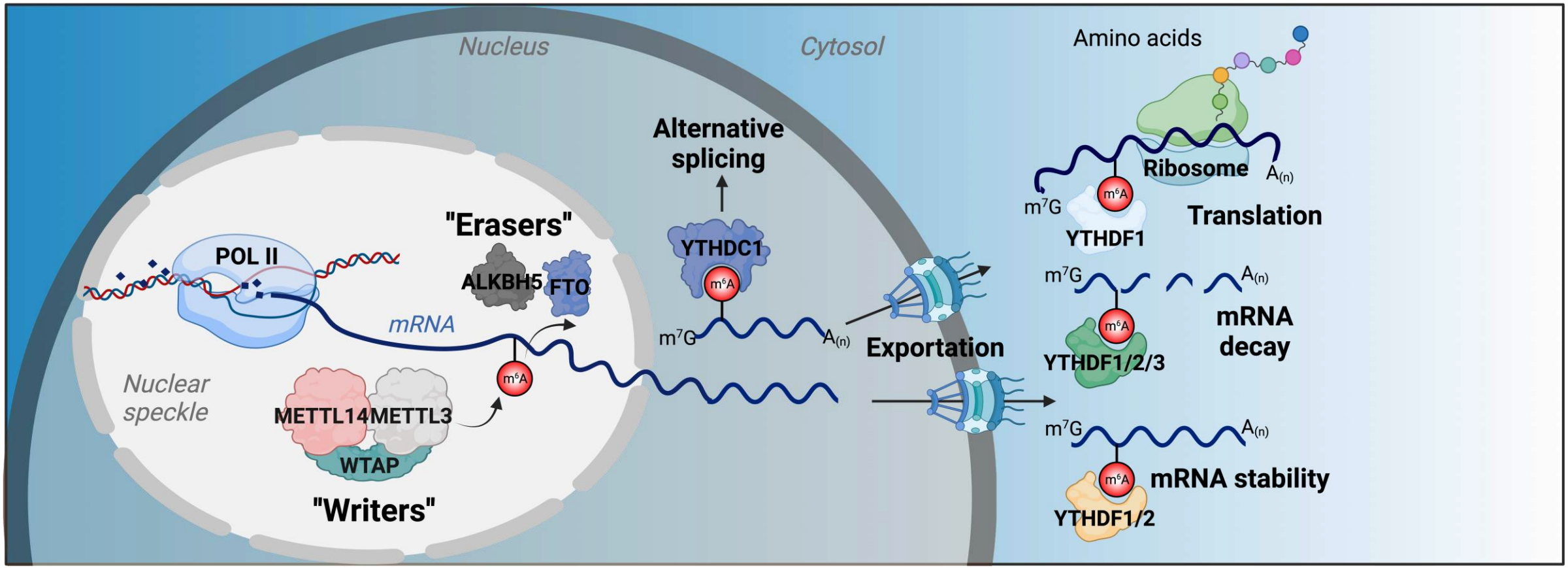




Bacteria

Archaea

Methylation, demethylation and recognition of m^6A on mRNA



Mettl3, 14: Methyl transferase like proteins; **WTAP:** Wilm's tumor-1 associating protein
Alkbh5: alkB homolg 5 demethylase; **FTO:** fat mass and obesity associated protein
YTHDC1: YTH domain containing N6-methyadenosine RNA binding protein C1
YTHDF1/2/3: YTH domain containing N6-methyadenosine RNA binding protein F1; **m^7G**: 7-methyguanosine

Dario DeJesus PhD

K99/R00 Awardee

THE UNIVERSITY OF CHICAGO

Sevim Kahraman PhD

Boehringer Ingelheim

nature metabolism

LETTERS

https://doi.org/10.1038/s42255-019-0089-9

m^{6}A mRNA methylation regulates human β-cell biology in physiological states and in type 2 diabetes

Dario F. De Jesus[1,5], Zijie Zhang[2,3,5], Sevim Kahraman[1,5], Natalie K. Brown[1], Mengjie Chen[4], Jiang Hu[1], Manoj K. Gupta[1], Chuan He[2,3*] and Rohit N. Kulkarni[1*]

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Article | 25 September 2024 |

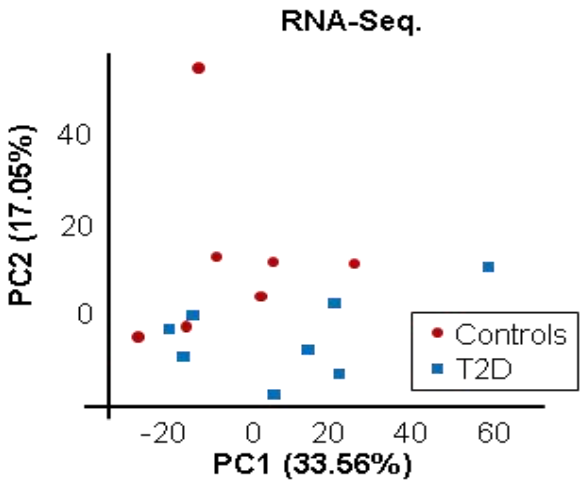
TRANSPARENT PROCESS

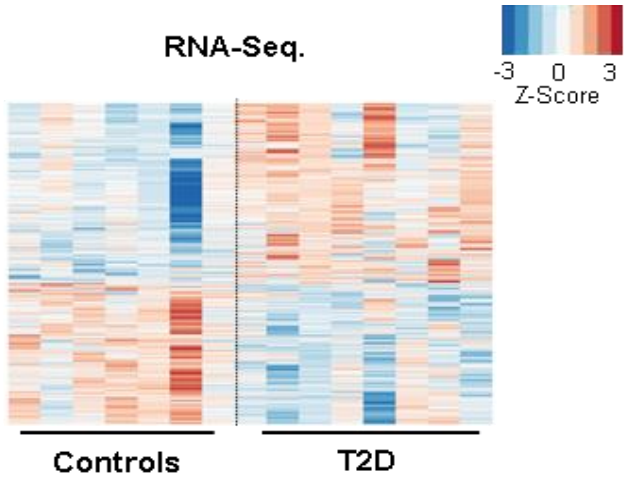
m^{6}A mRNA methylation by METTL14 regulates early pancreatic cell differentiation

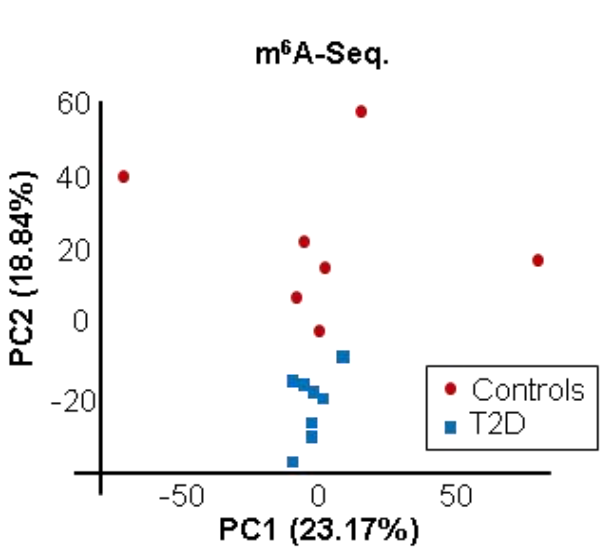
Sevim Kahraman, Dario F De Jesus, Jiangbo Wei, Natalie K Brown, Zhongyu Zou, Jiang Hu, Mehdi Pirouz, Richard I Gregory, Chuan He, and Rohit N Kulkarni | AUTHOR INFORMATION

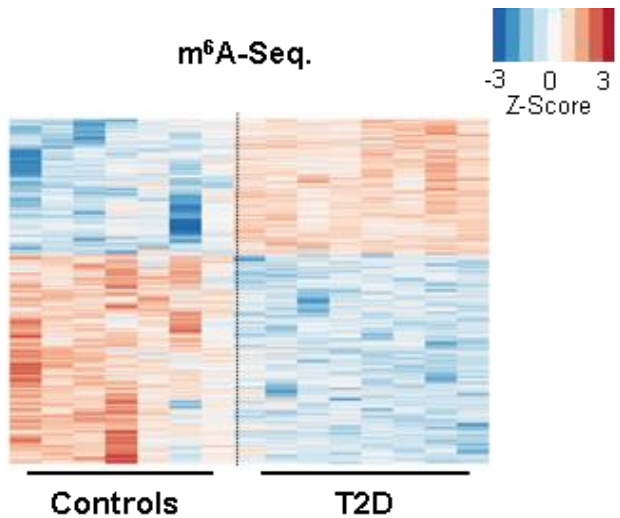
EMBO J (2024) | https://doi.org/10.1038/s44318-024-00213-2

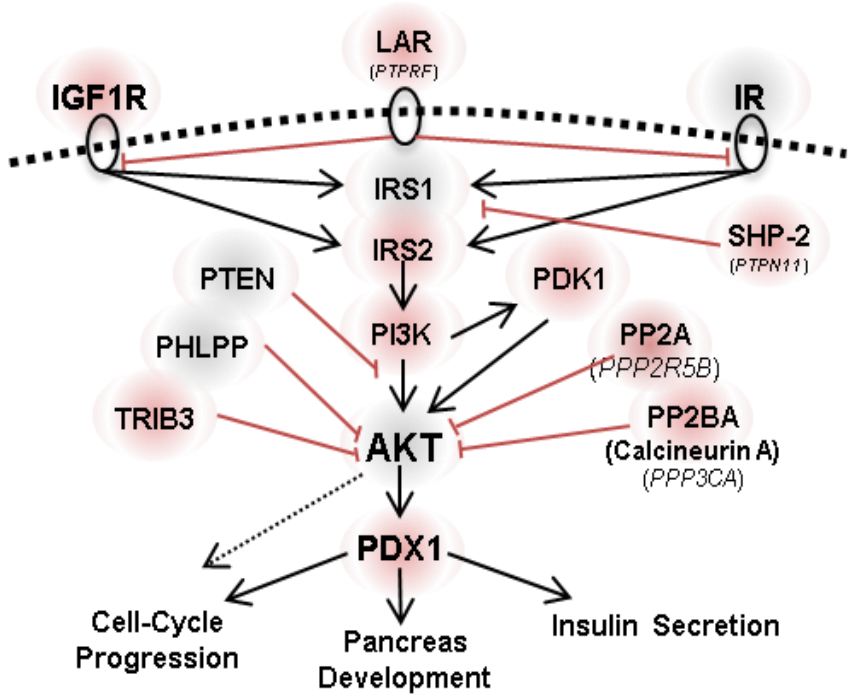
m⁶A Methylome Segregates Disease Status Better than Transcriptome

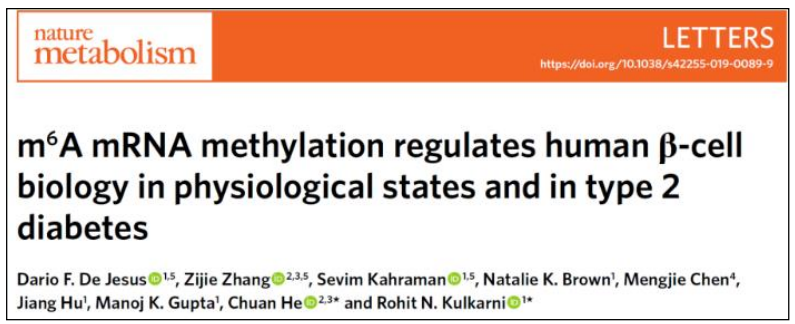












nature metabolism LETTERS

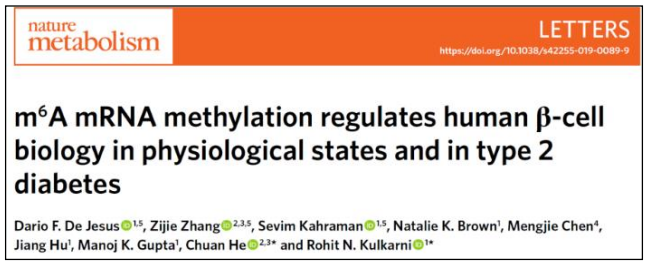
https://doi.org/10.1038/s42255-019-0089-9

m⁶A mRNA methylation regulates human β-cell biology in physiological states and in type 2 diabetes

Dario F. De Jesus[1,5], Zijie Zhang[2,3,5], Sevim Kahraman[1,5], Natalie K. Brown[1], Mengjie Chen[4], Jiang Hu[1], Manoj K. Gupta[1], Chuan He[2,3*] and Rohit N. Kulkarni[1*]

Immune and Antigen-presenting Pathways are Upregulated in Mettl14 Deficient C57BL/6N Mouse β-cells

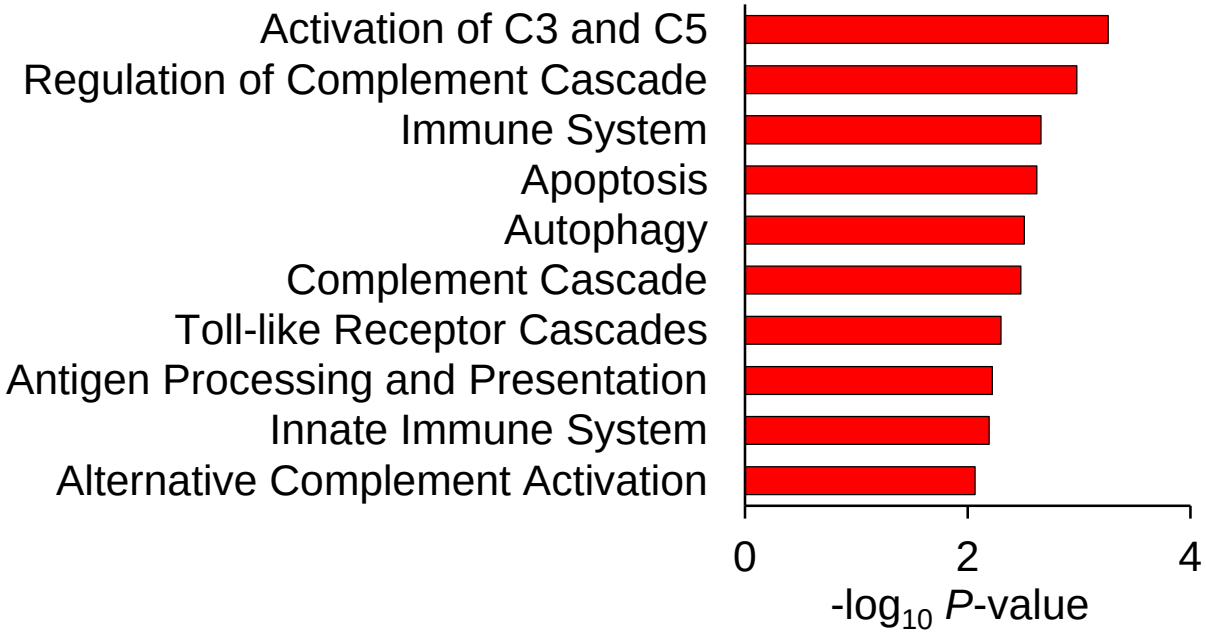
Differentially Expressed Genes
Mettl14 KO Vs Control
(FDR<0.10)



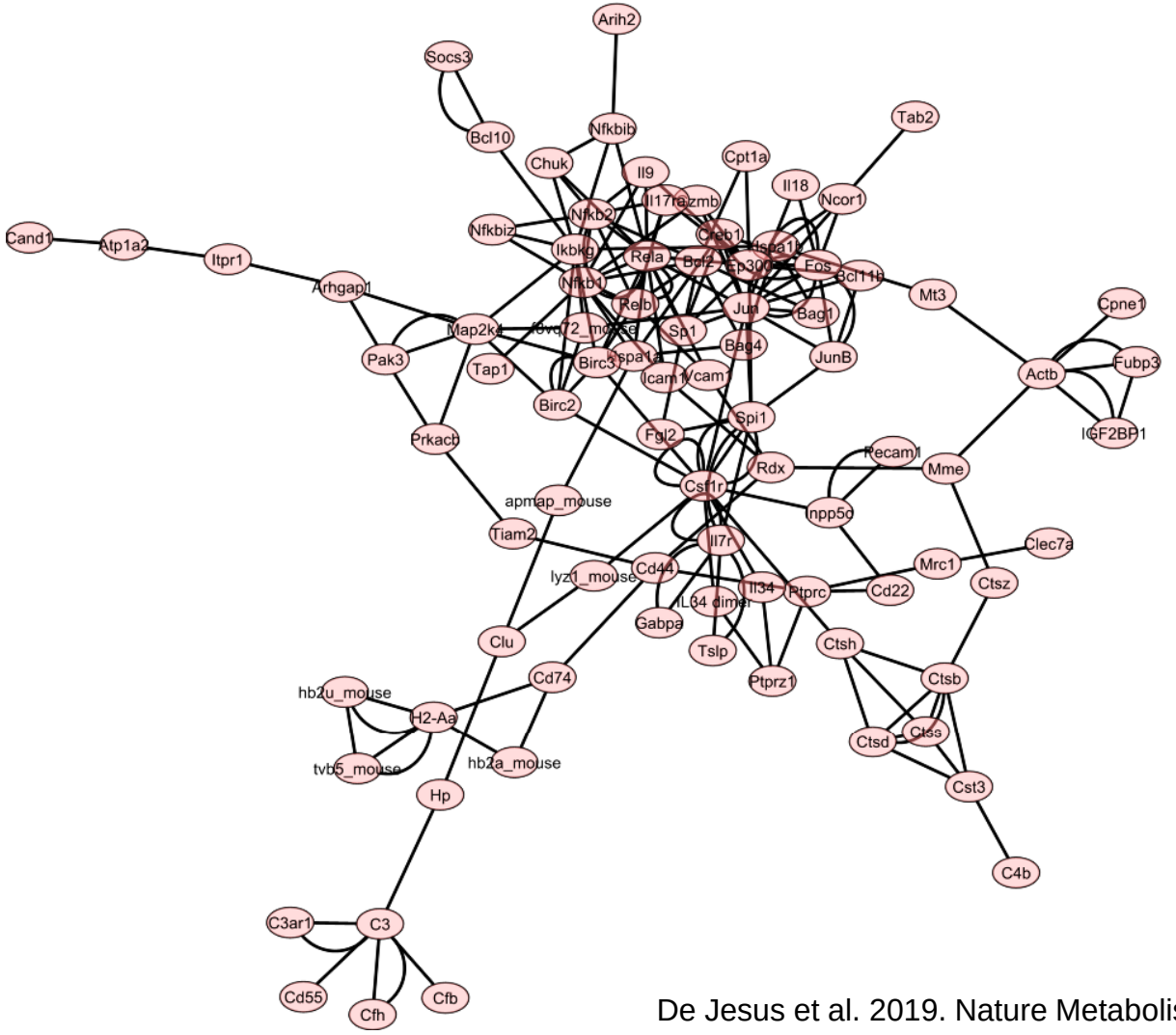
Down	Up
708	761

Unchanged
22971

Enriched Pathways – Upregulated Genes
Mettl14 KO Vs Control



Induced Gene Network
Genes Associated with Immune System and Antigen Presentation



De Jesus et al. 2019. Nature Metabolism

nature cell biology

Article

https://doi.org/10.1038/s41556-024-01368-0

Redox regulation of m^{6}A methyltransferase METTL3 in β-cells controls the innate immune response in type 1 diabetes

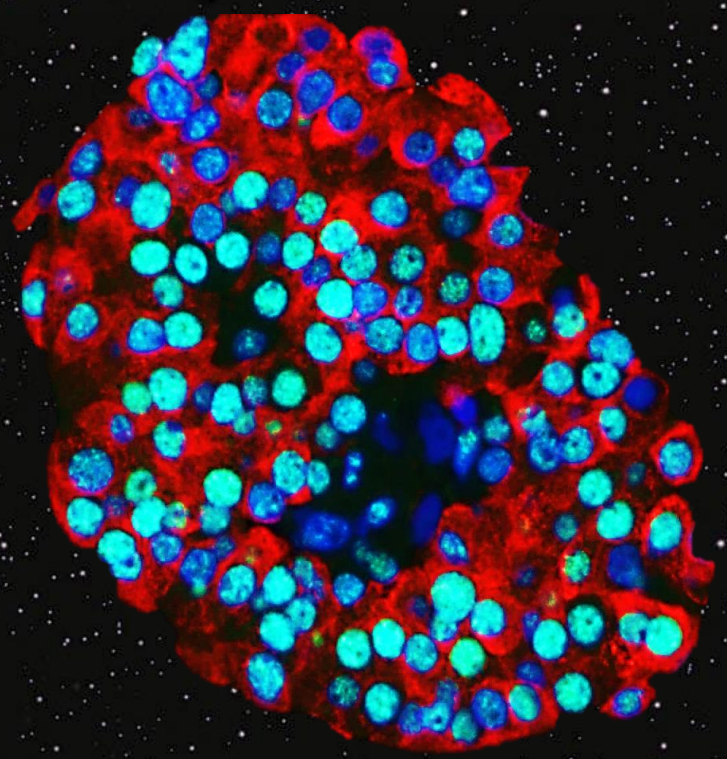
News & Views | Published: 26 February 2024

Metabolism

METTL3 restrains autoimmunity in β-cells

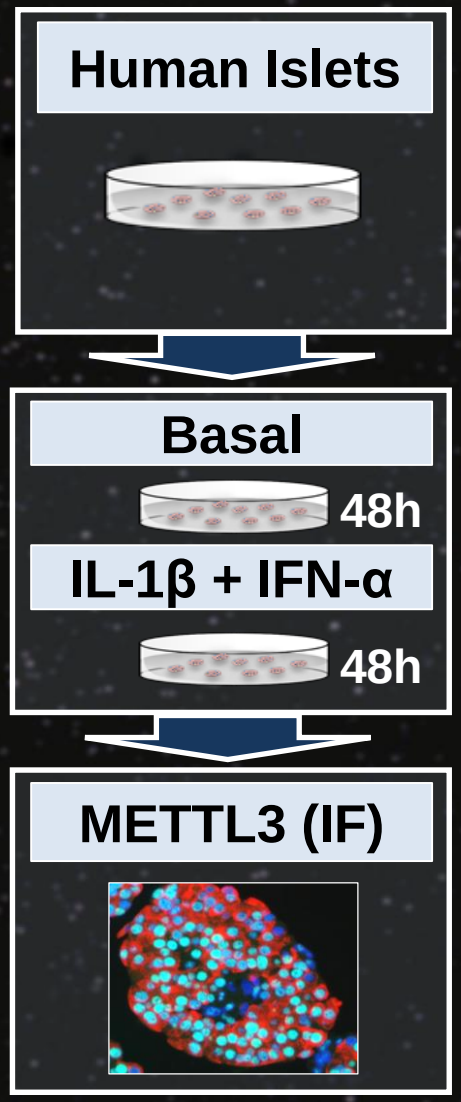
Balasubramanian Krishnamurthy & Helen E. Thomas

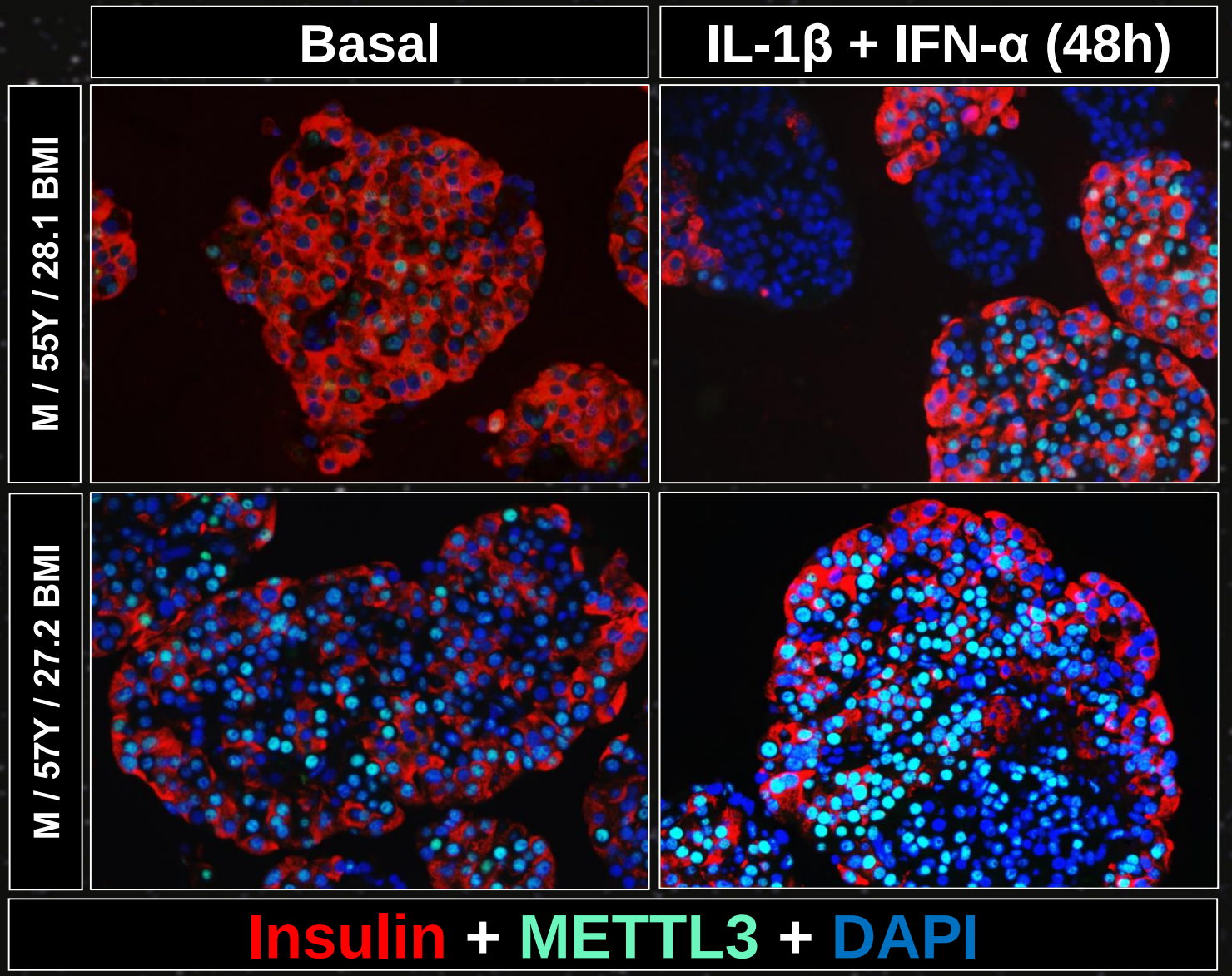
Nature Cell Biology **26**, 321–322 (2024) | Cite this article



METTL3 Dynamics *in T1D* Progression

Proinflammatory Cytokines (IL-1β, IFN-α) Increase METTL3 Protein Levels in Human β-Cells





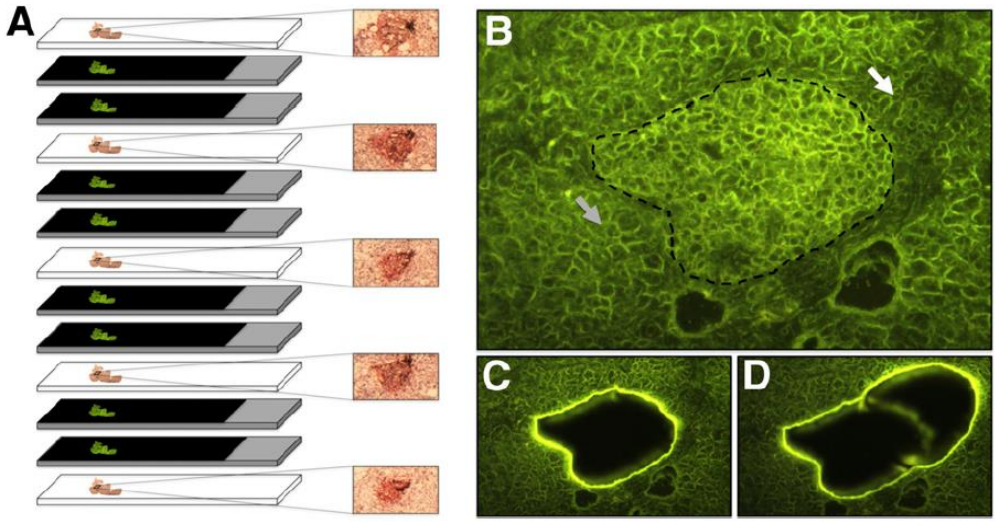
Innate Immune Genes Are Upregulated in Insulitic Islets During T1D Onset

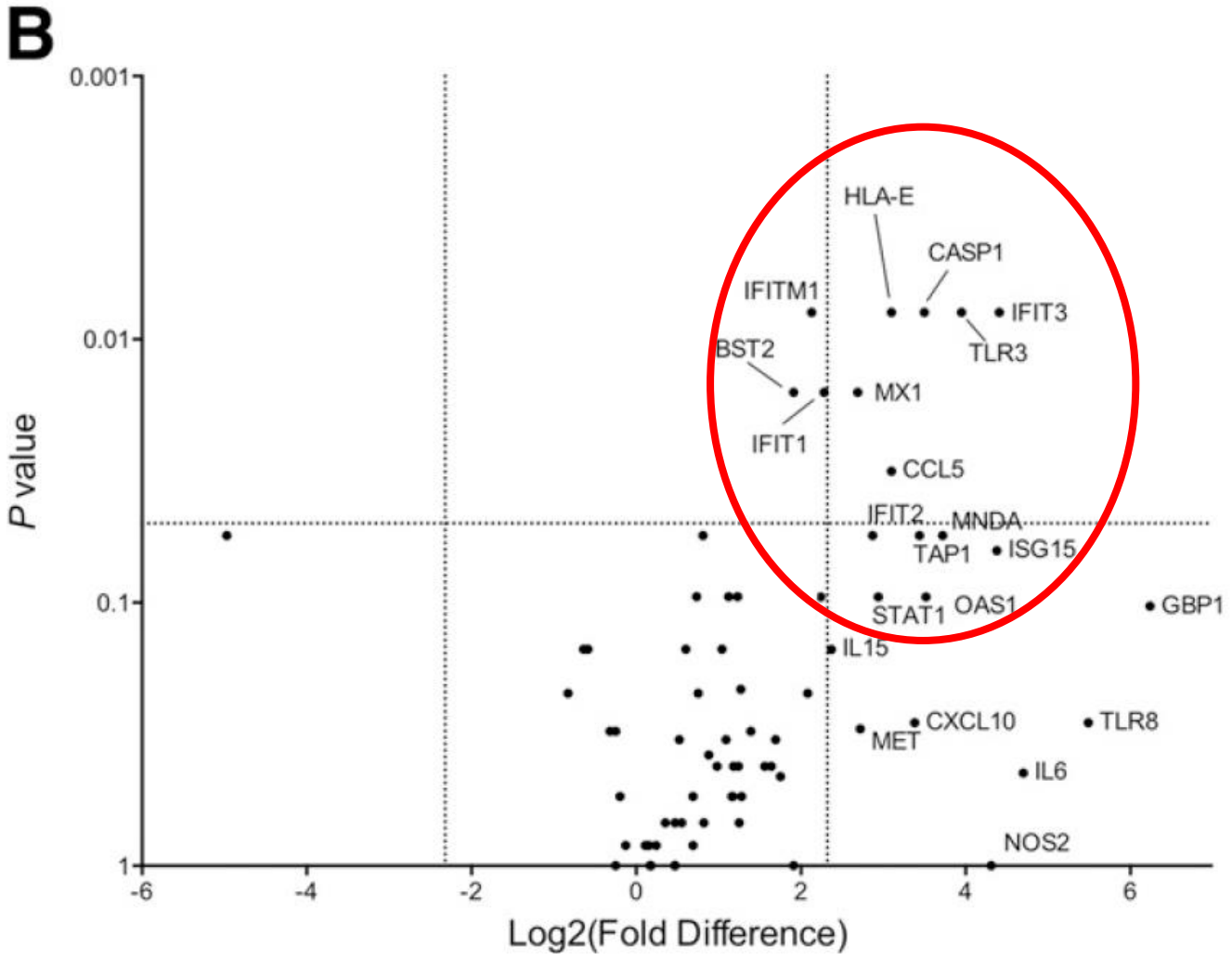
3104 Diabetes Volume 65, October 2016

Marcus Lundberg,[1] Lars Krogvold,[2,3] Enida Kuric,[1] Knut Dahl-Jørgensen,[2,3] and Oskar Skog[1]

Expression of Interferon-Stimulated Genes in Insulitic Pancreatic Islets of Patients Recently Diagnosed With Type 1 Diabetes

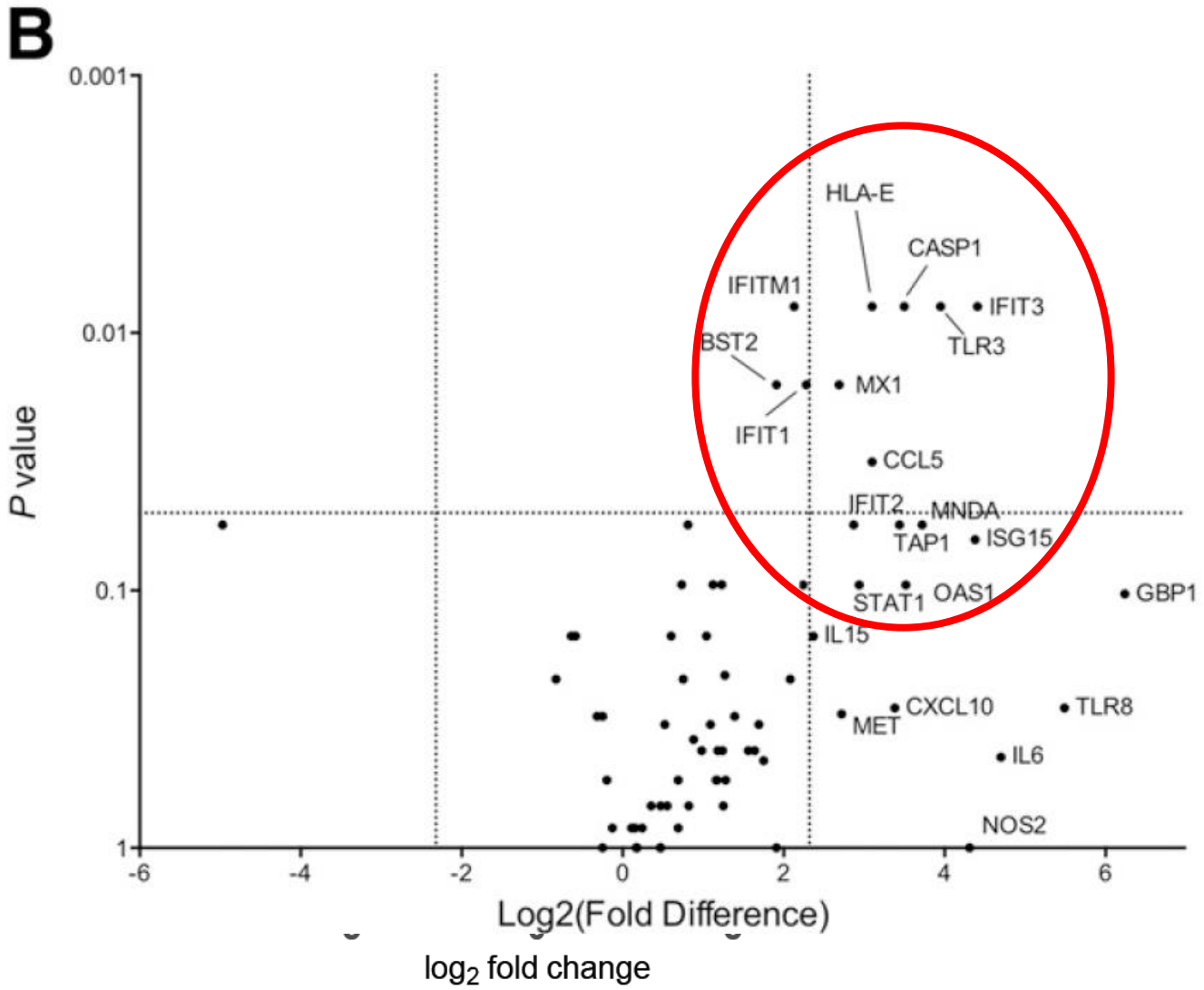
Diabetes 2016;65:3104–3110 | DOI: 10.2337/db16-0616



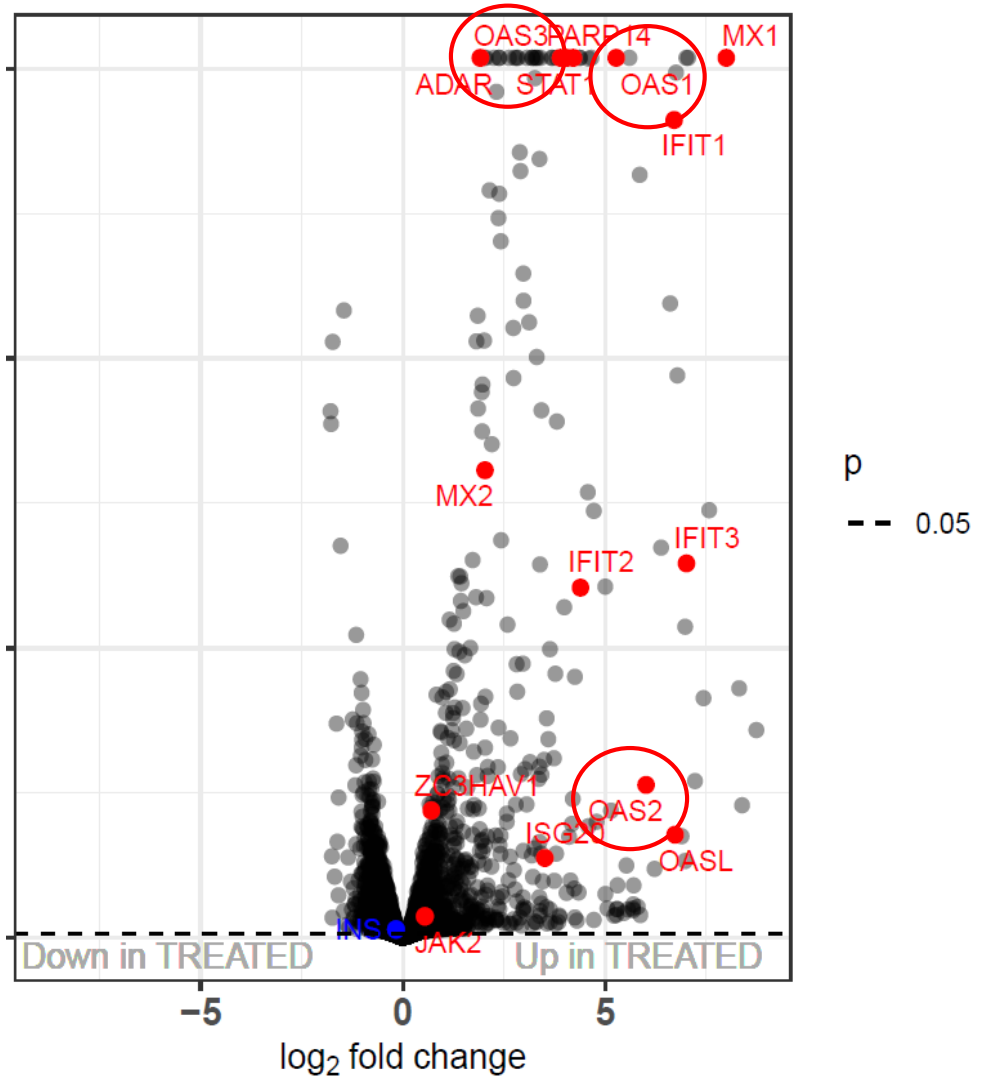


Freshly frozen and cultured pancreatic tissue **from living** patients with recent-onset T1D collected within the Diabetes Virus Detection (DiViD) study.

Proinflammatory Cytokines (IL-1β, IFN-α) Treatment Recapitulate the Transcriptomic Make-Up of T1D Onset in Human β-Cells

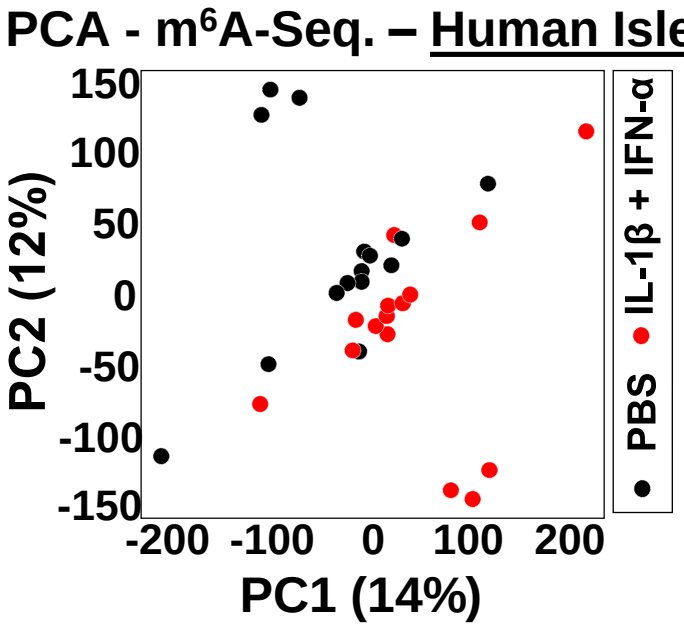


Human Islets
RNA-Seq. (IL-1β + IFN-α)

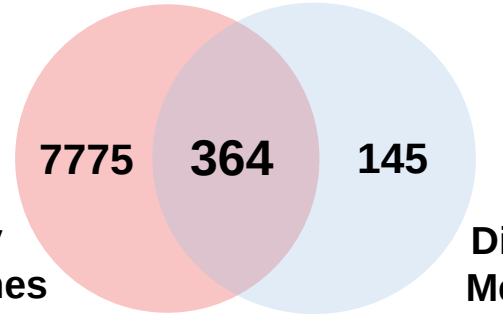


EndoC-βH1 Cells
RNA-Seq. (IL-1β + IFN-α)

m^{6}A-Decorated Genes in Cytokine-Treated Human Islets Reveal Enrichment in OAS Antiviral Innate Immune Response



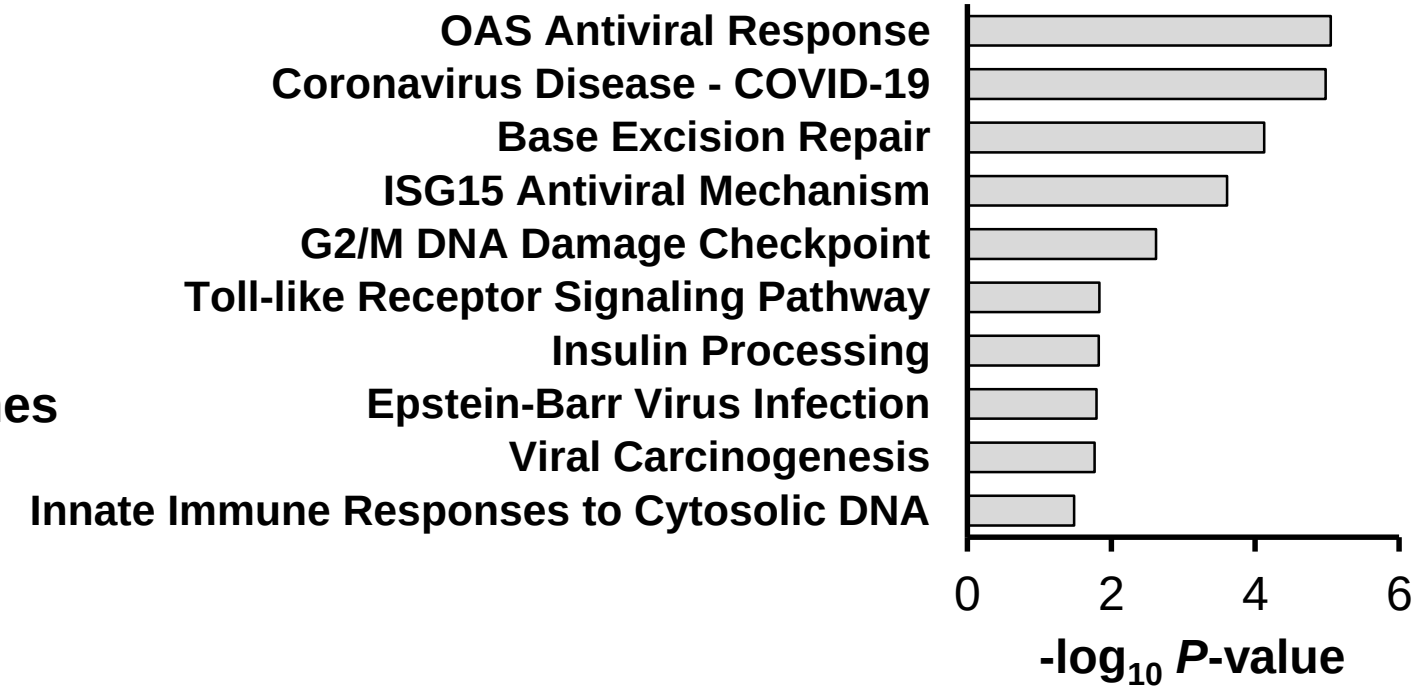
Intersect Differentially Expressed and m^{6}A Methylated Genes (IL-1β + IFN-α Vs PBS)



Differentially Expressed Genes (FDR<0.01)

Differentially m^{6}A Methylated Genes (FDR<0.05)

Enriched Pathways – Commonly Differentially Expressed and m^{6}A Methylated Genes
(IL-1β + IFN-α Vs PBS)



OAS gene: 2'-5'-oligoadenylate synthetase (dsRNA sensors)

DeJesus DF, et al. ***Nature Cell Biology,*** 2024

OAS Genes Are Genetically Linked to T1D and Its Upregulation Results in Increased β-Cell Apoptosis

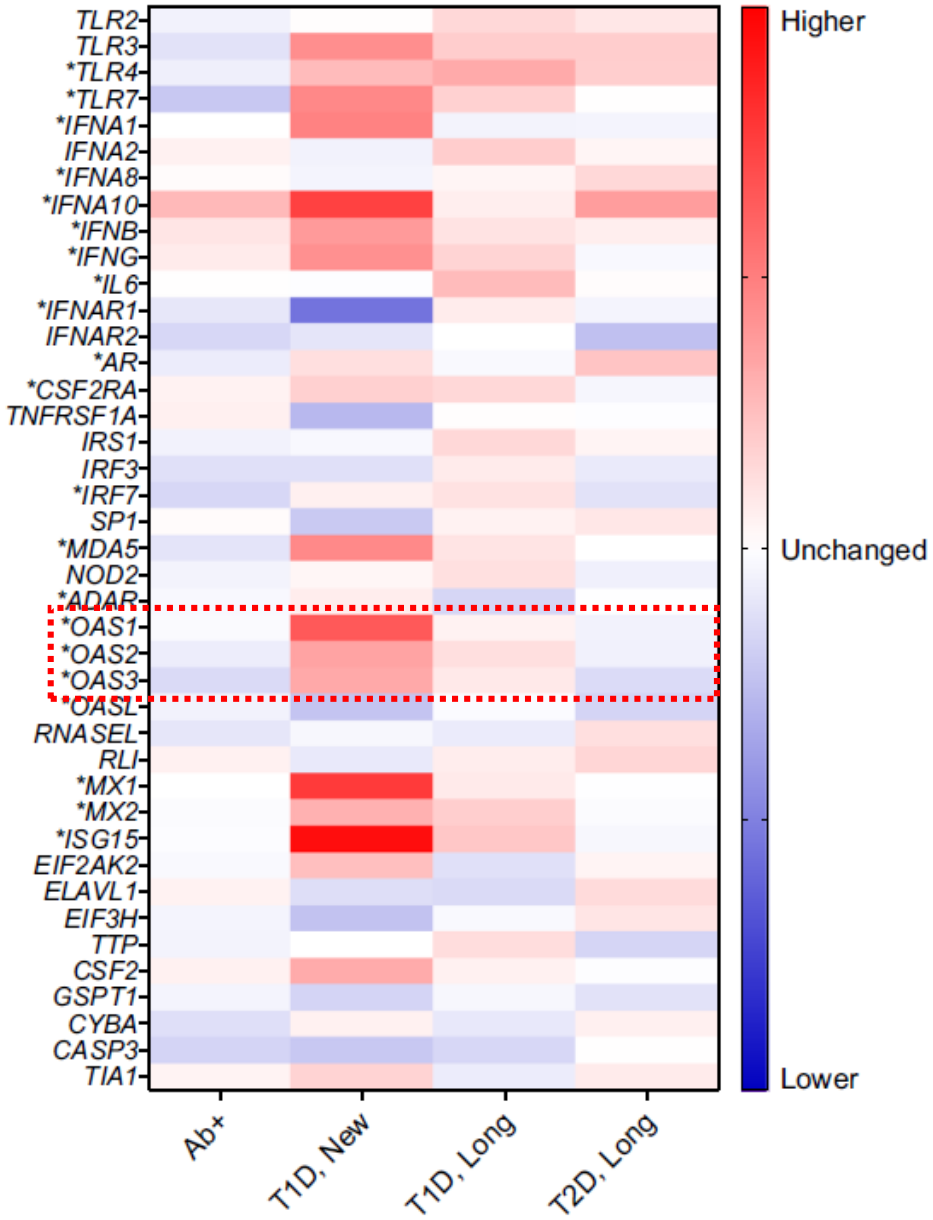
Diabetologia (2021) 64:1805–1815
https://doi.org/10.1007/s00125-021-05469-5

ARTICLE

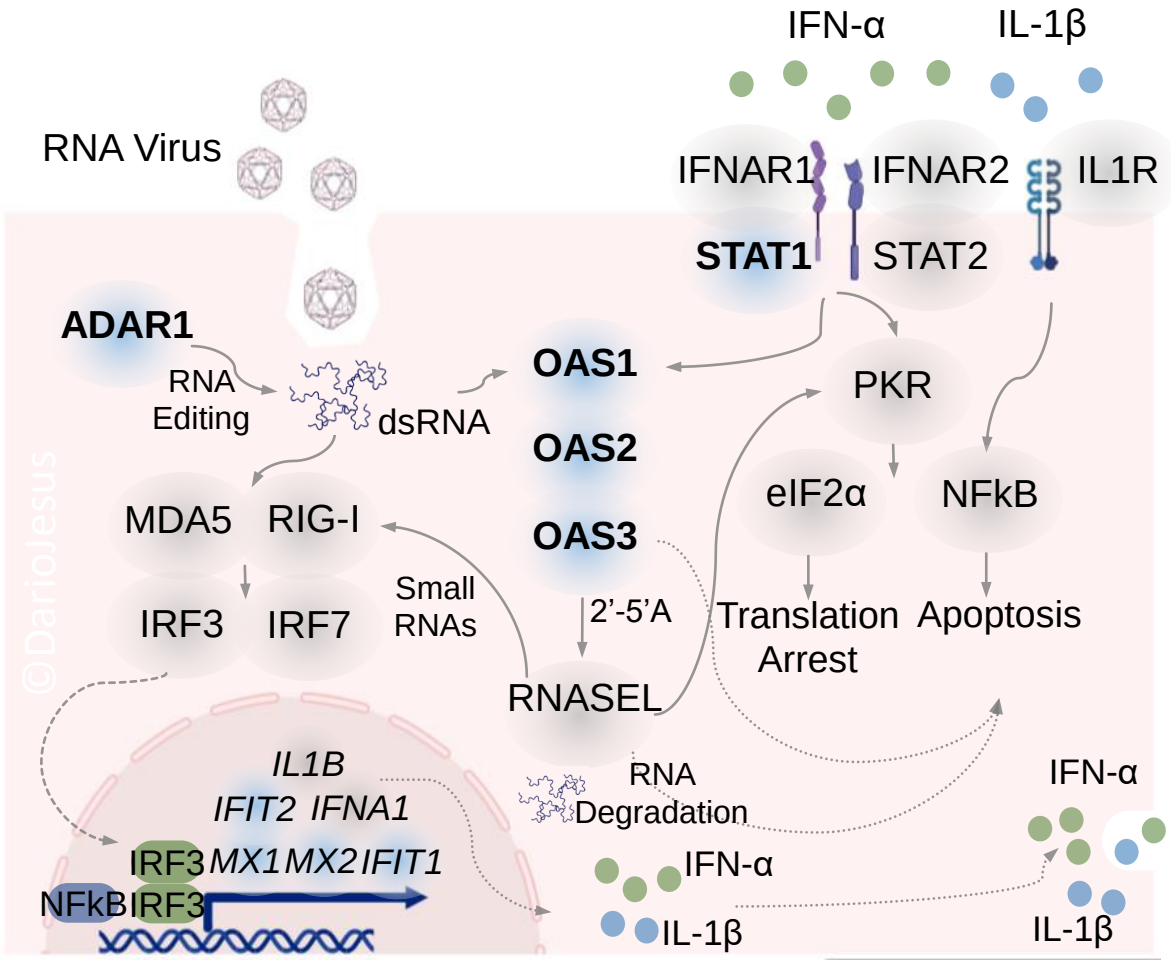
Genetic predisposition in the 2′-5′A pathway in the development of type 1 diabetes: potential contribution to dysregulation of innate antiviral immunity

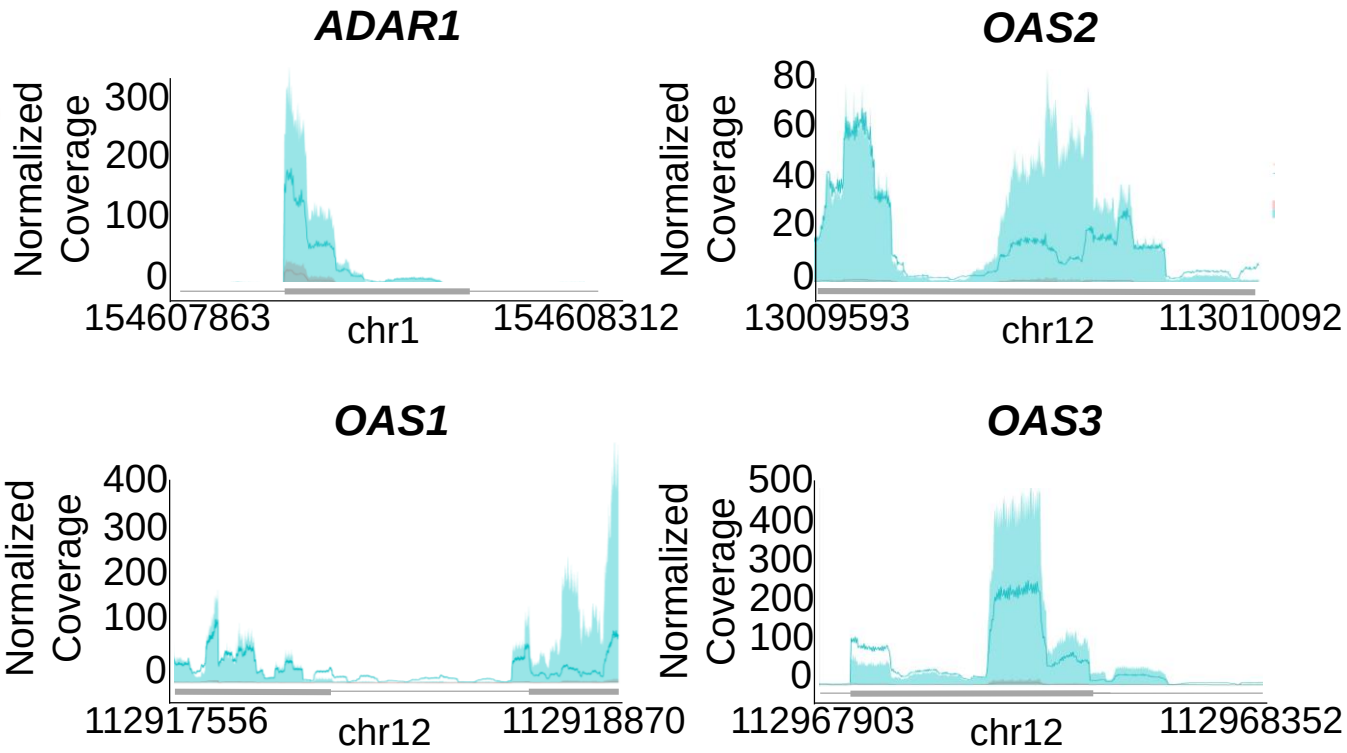
Kristina Pedersen[1] · Martin Haupt-Jorgensen[1] · Lars Krogvold[2,3] · Simranjeet Kaur[4] · Ivan C. Gerling[5] · Flemming Pociot[4,6] · Knut Dahl-Jørgensen[2,7] · Karsten Buschard[1]

Received: 9 October 2020 / Accepted: 4 March 2021 / Published online: 11 May 2021



Human Islets Treated with Cytokines Present Hypermethylation of 2'-5'-Oligoadenylate Synthetase (OAS) Genes

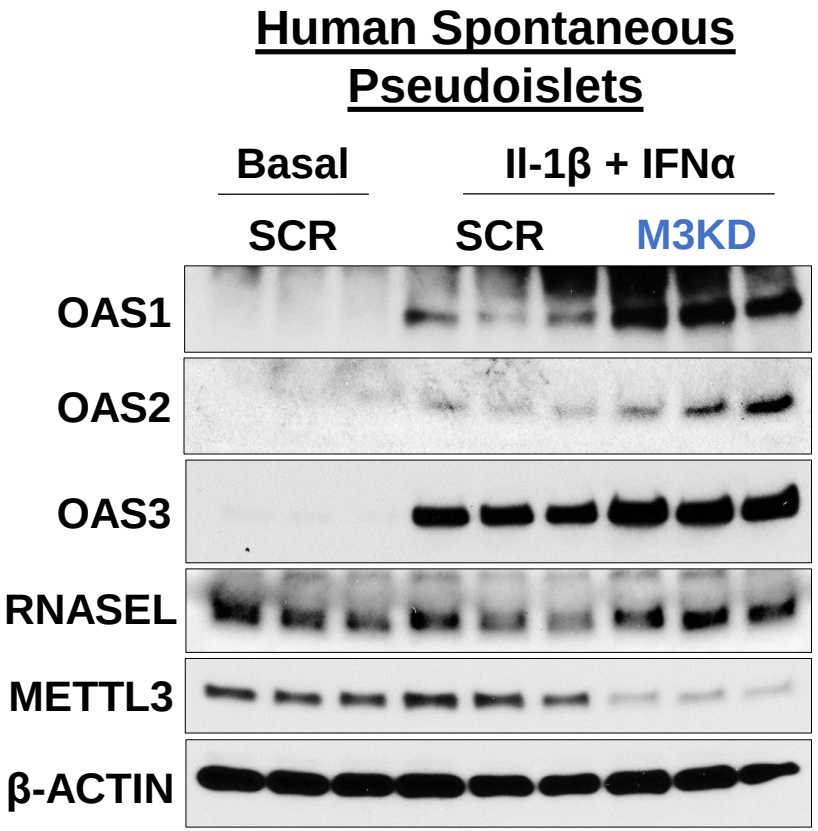




OAS gene: 2'-5'-oligoadenylate synthetase (dsRNA sensors)

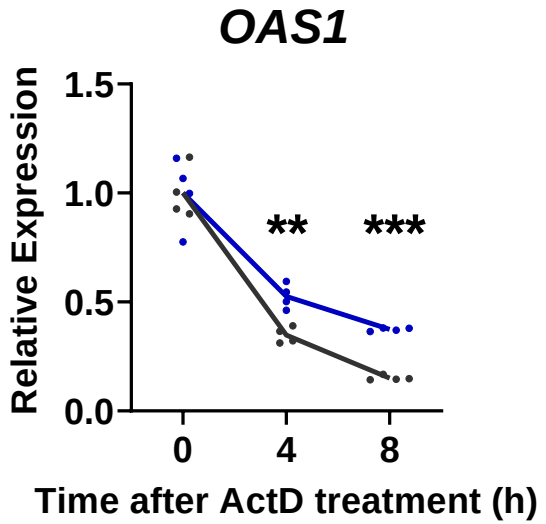
ADAR1: adenosine deaminase acting on RNA

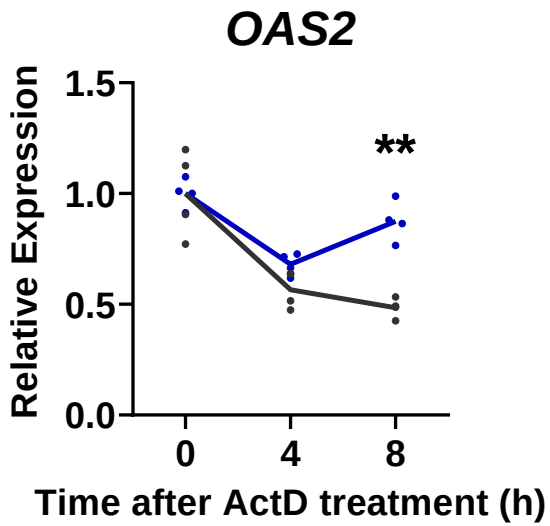
METTL3 Downregulation Leads to Enhanced Expression of OAS Proteins due to Decreased mRNA Decay in Human β-Cells

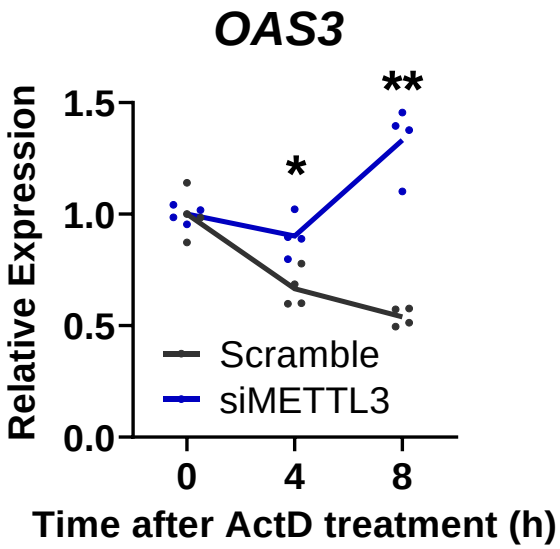


SCR: Scramble

M3KD: METTL3 Knock-Down







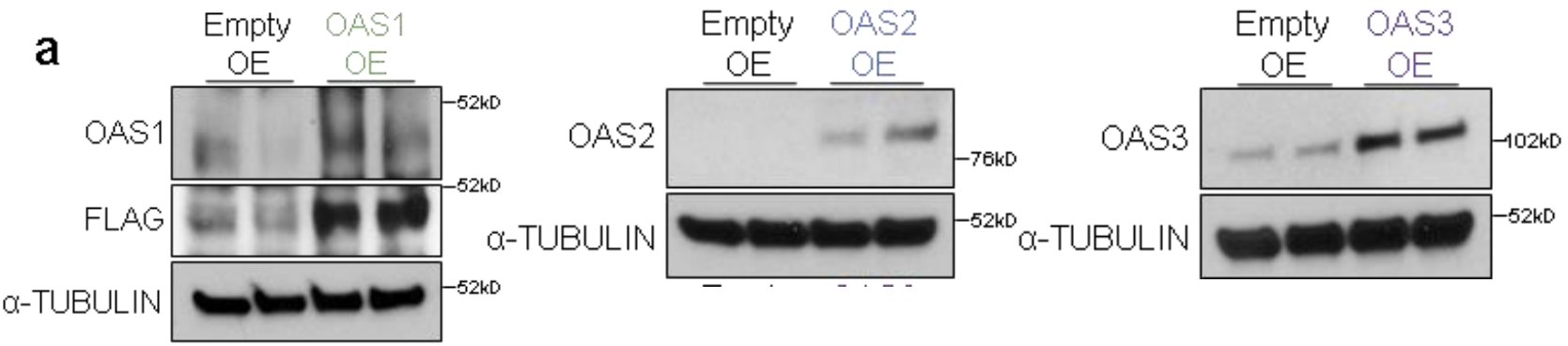
Conversely, Mettl3OE enhances decay of OAS genes

m^{6}A regulation of OAS genes is via readers YTHDF1/3

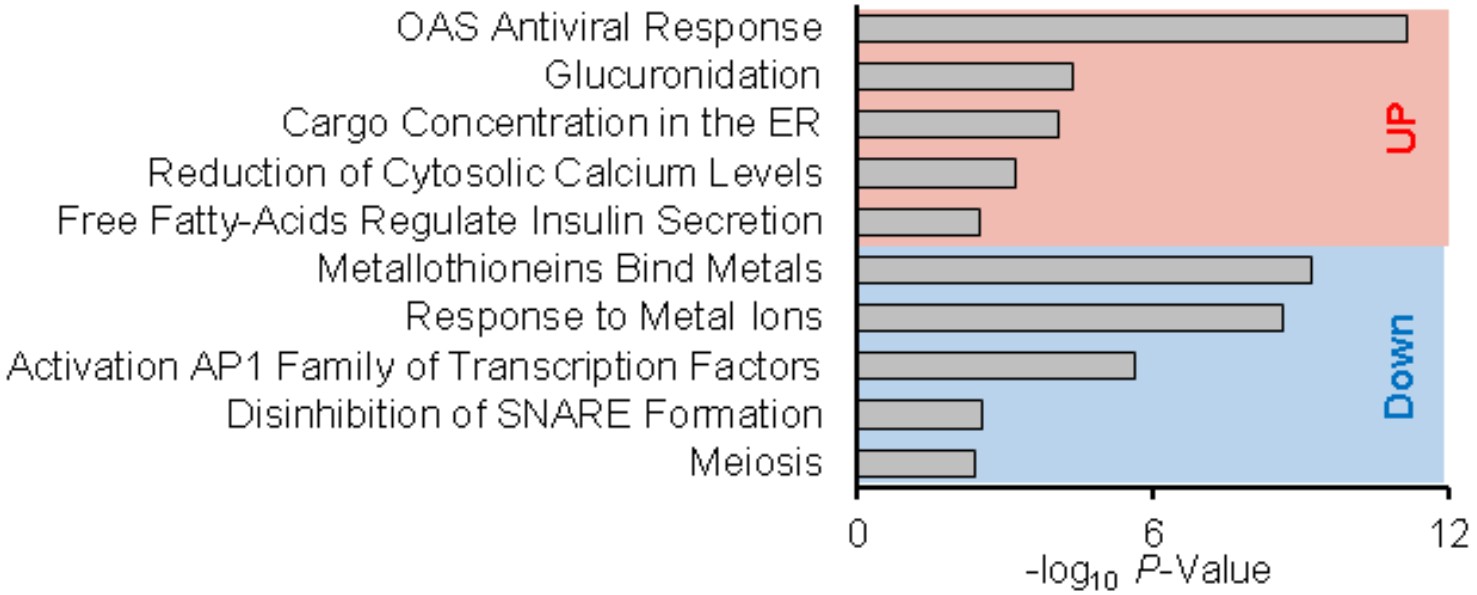
ActD: Actinomycin *(Inhibits Transcription)*

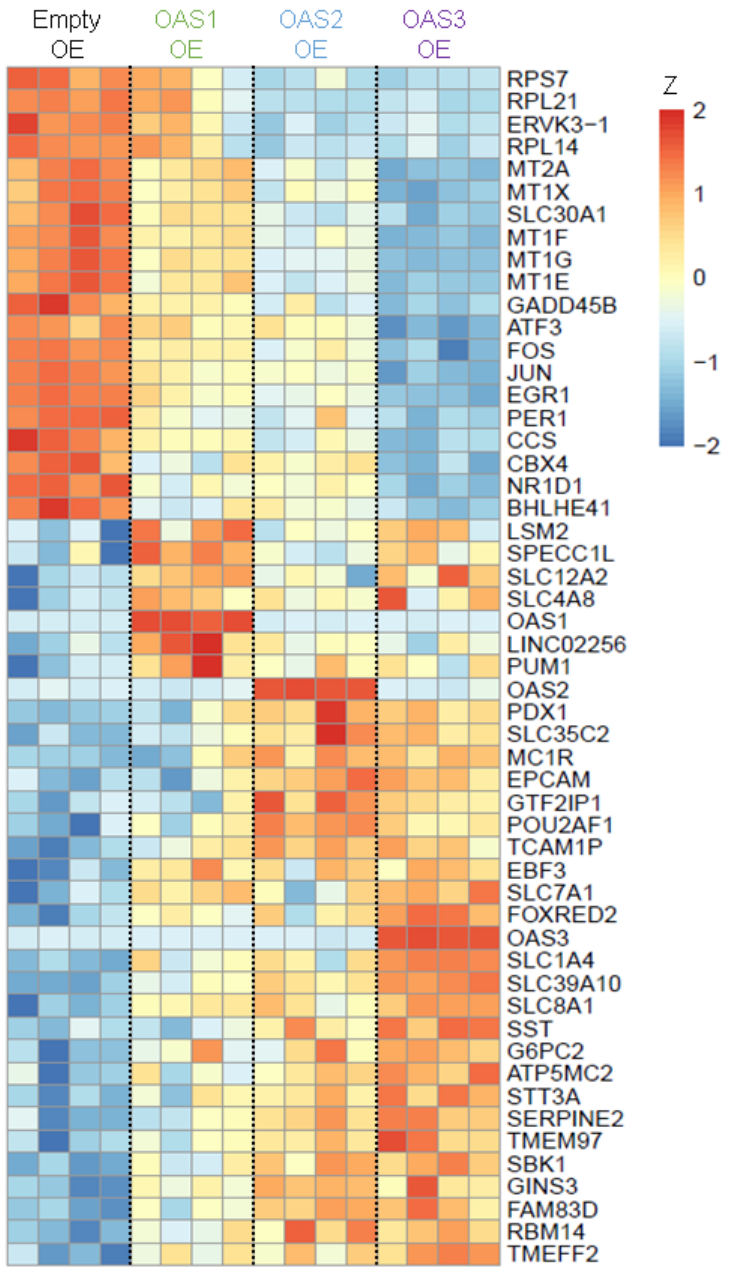
How is METTL3 Regulated ?

OAS Upregulation Impacts β-Cell Antioxidant Response



Enriched Pathways
OAS OE Versus Empty
(FDR<0.05)





ER Stress and ROS - Triggers For β-Cell Dysfunction and Autoimmunity

COMMENTARY

ER Stress as a Trigger for β-Cell Dysfunction and Autoimmunity in Type 1 Diabetes

Bryan O'Sullivan-Murphy and Fumihiko Urano

ORIGINAL ARTICLE

Islet β-Cell Endoplasmic Reticulum Stress Precedes the Onset of Type 1 Diabetes in the Nonobese Diabetic Mouse Model

Sarah A. Tersey,[1,2] Yurika Nishiki,[1,2] Andrew T. Templin,[3] Susanne M. Cabrera,[1,2] Natalie D. Stull,[1,2] Stephanie C. Colvin,[1,2] Carmella Evans-Molina,[2,3,4] Jenna L. Rickus,[5,6,7] Bernhard Maier,[1,2] and Raghavendra G. Mirmira[1,2,3,4]

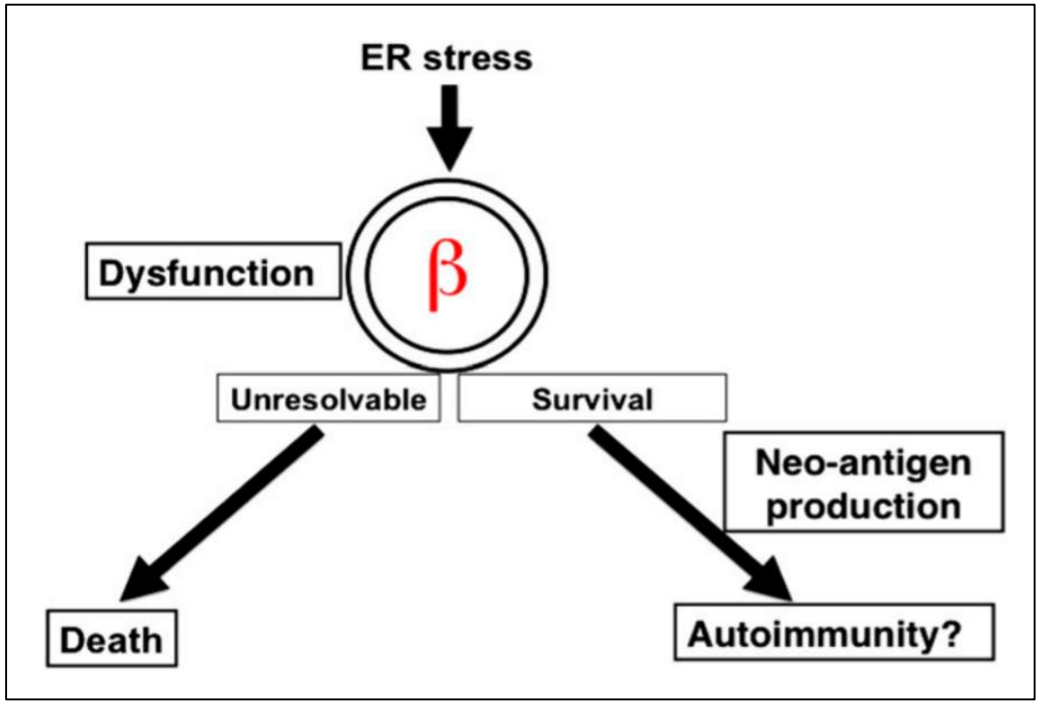
Balzano-Nogueira *et al. Genome Biology* (2021) 22:39
https://doi.org/10.1186/s13059-021-02262-w

Genome Biology

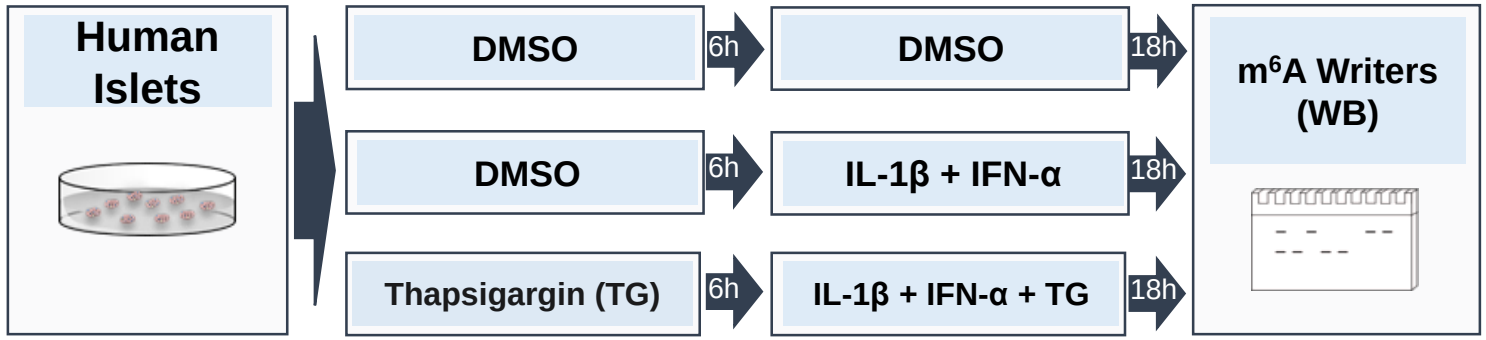
RESEARCH Open Access

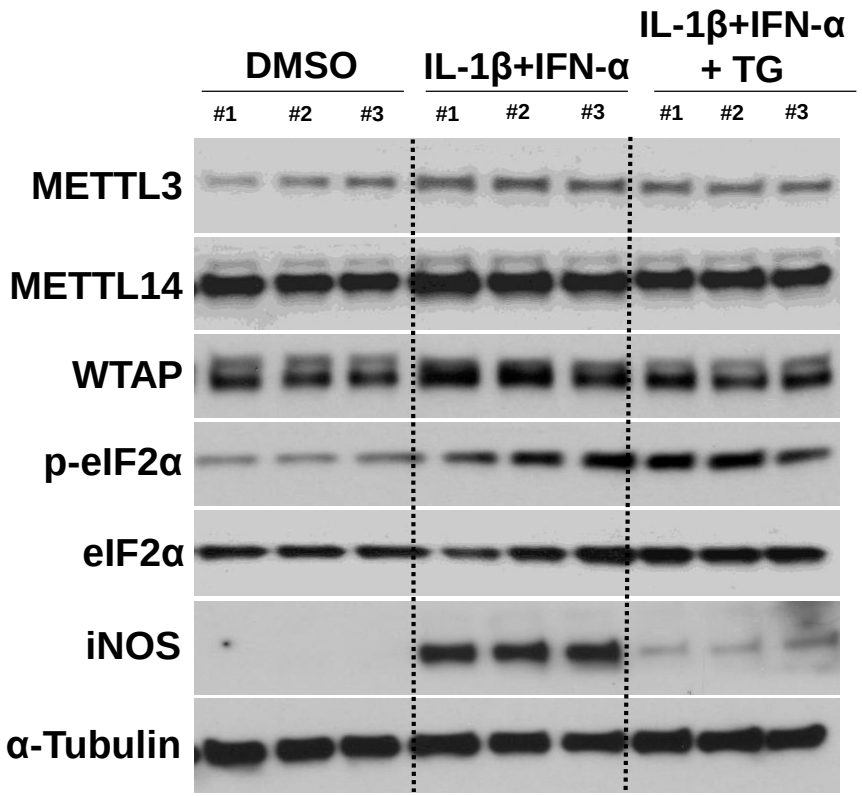
Integrative analyses of TEDDY Omics data reveal lipid metabolism abnormalities, increased intracellular ROS and heightened inflammation prior to autoimmunity for type 1 diabetes

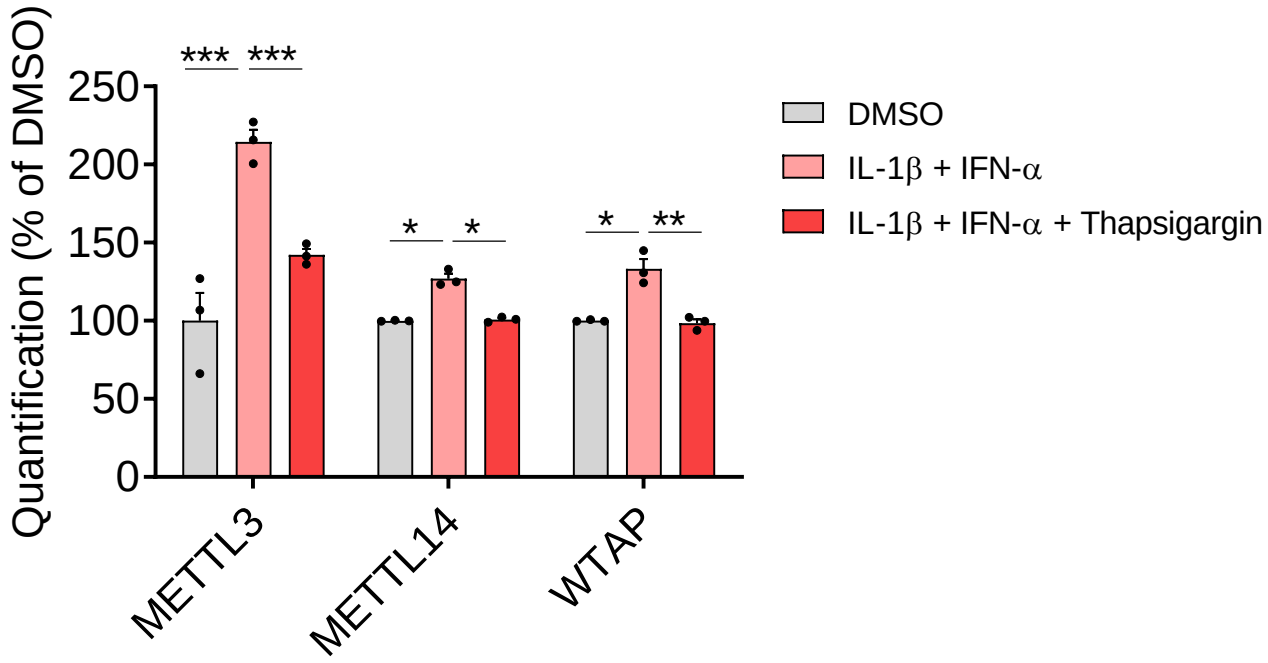
Leandro Balzano-Nogueira[1], Ricardo Ramirez[1], Tatyana Zamkovaya[1], Jordan Dailey[1], Alexandria N. Ardissone[1], Srikar Chamala[2], Joan Serrano-Quílez[3], Teresa Rubio[4], Michael J. Haller[5], Patrick Concannon[2,6], Mark A. Atkinson[5], Desmond A. Schatz[5], Eric W. Triplett[1] and Ana Conesa[1,6*]



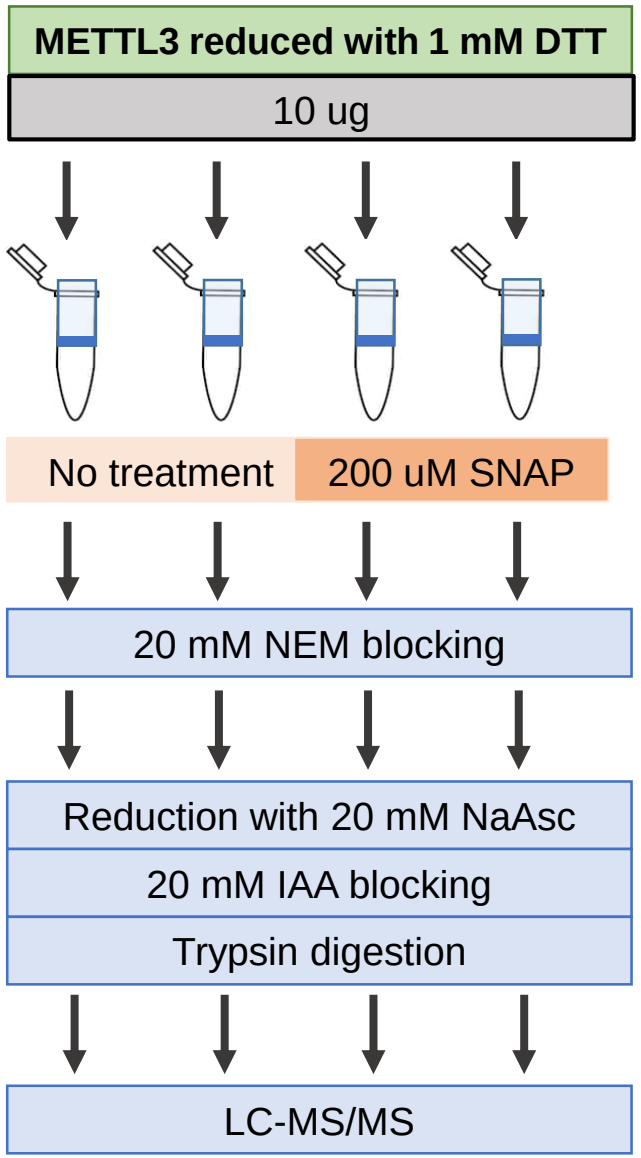
ER Stress Blocks Upregulation of m^{6}A Writers Upon Cytokine Stimulation

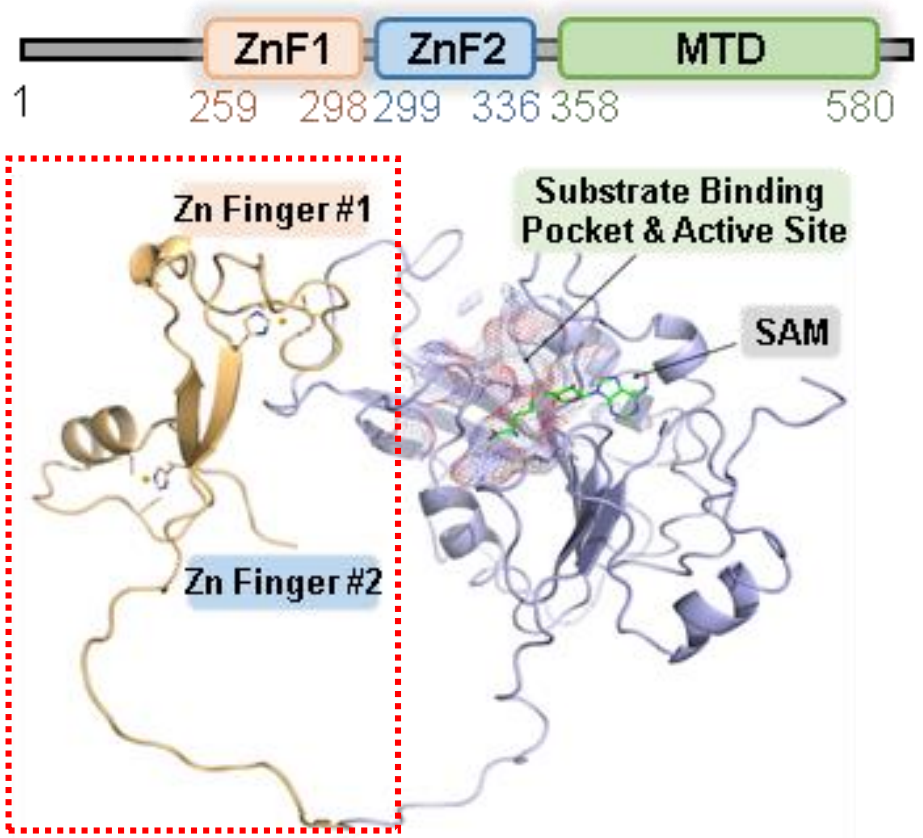


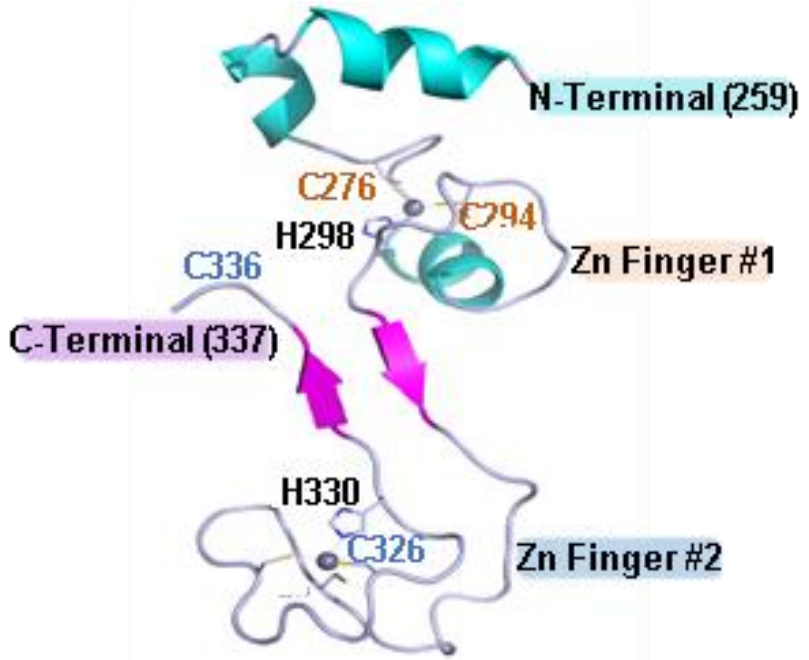




Identification of Redox-Sensitive METTL3 Cysteines



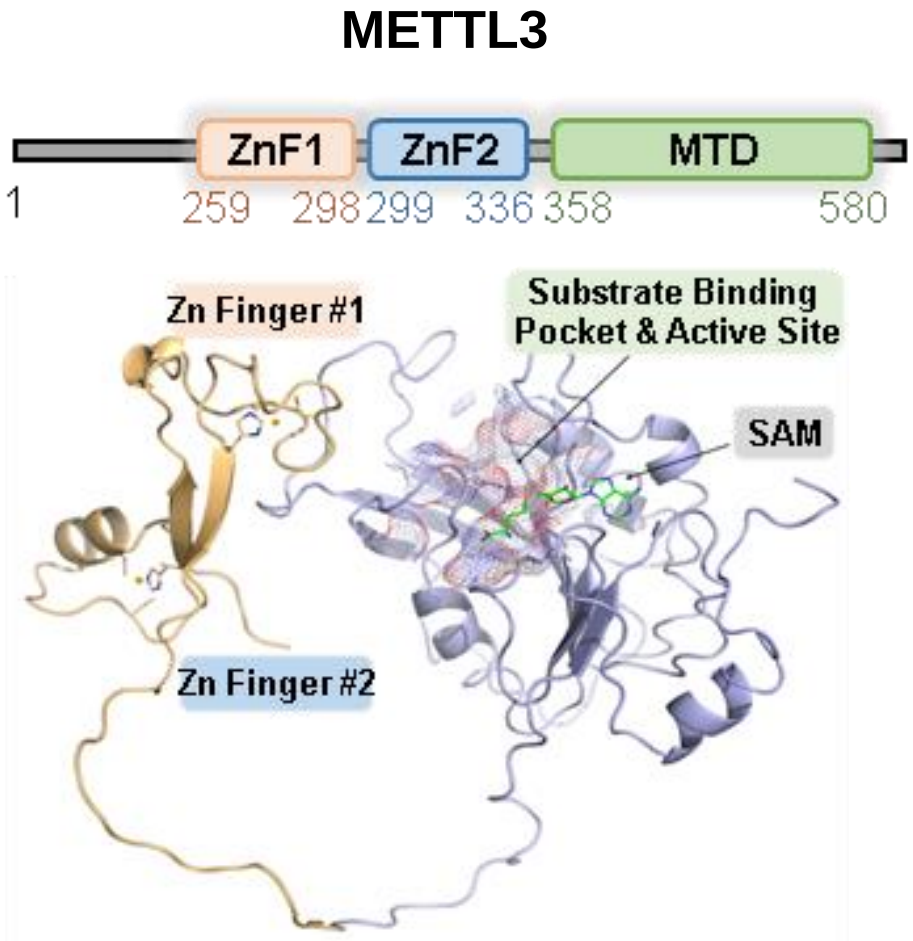


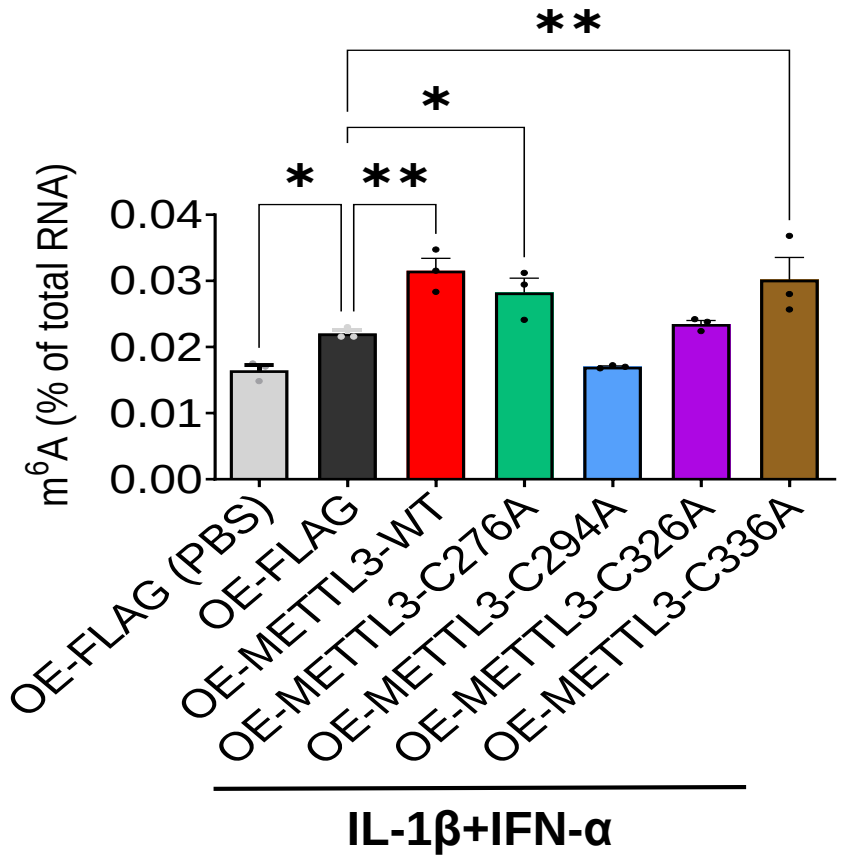


(Collaboration: Sirano Dhe-Phagnanon PhD; DFCI)

Recombinant METTL3 |Q86U44|MTA70_HUMAN

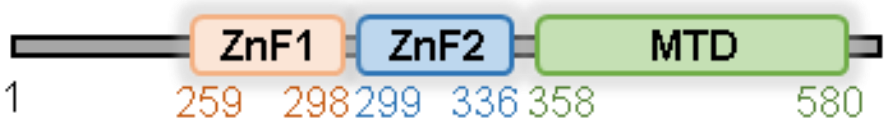
Point Mutations in Cysteines (294 and 326) Render METTL3 Methyltransferase Inactive

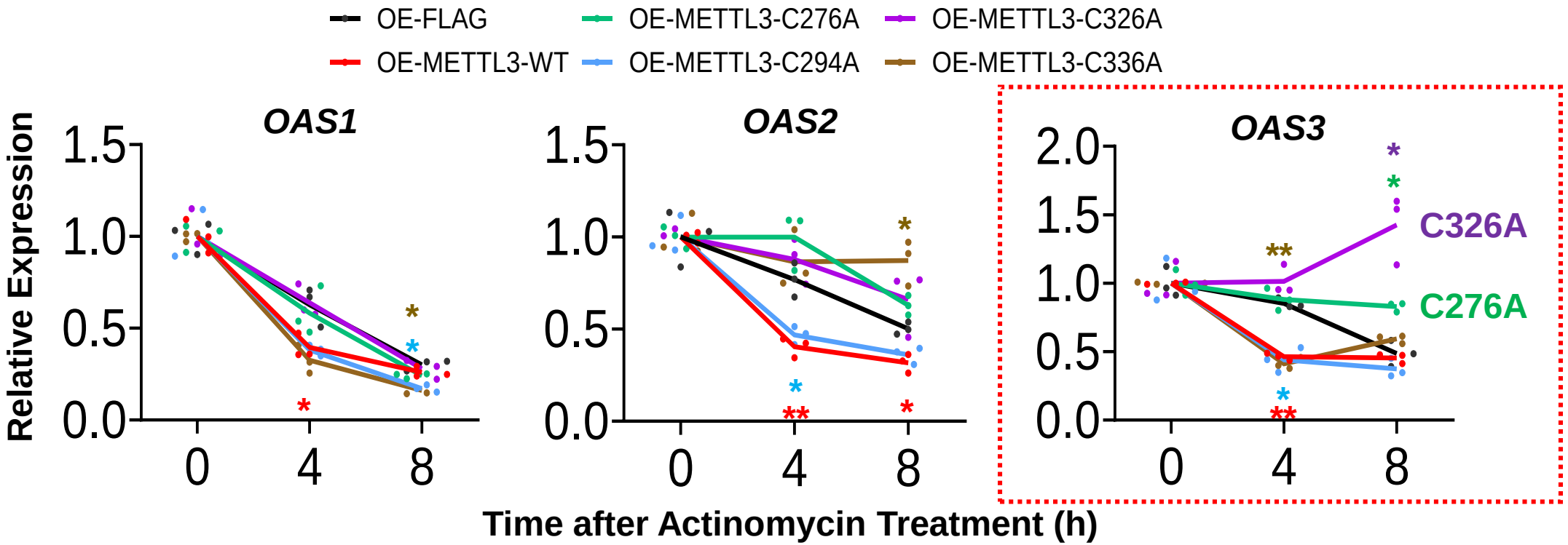




EndoC-βH1 Cells

Point Mutations in Cysteines (276 and 326) Impact METTL3 Regulation of OAS mRNA Decay



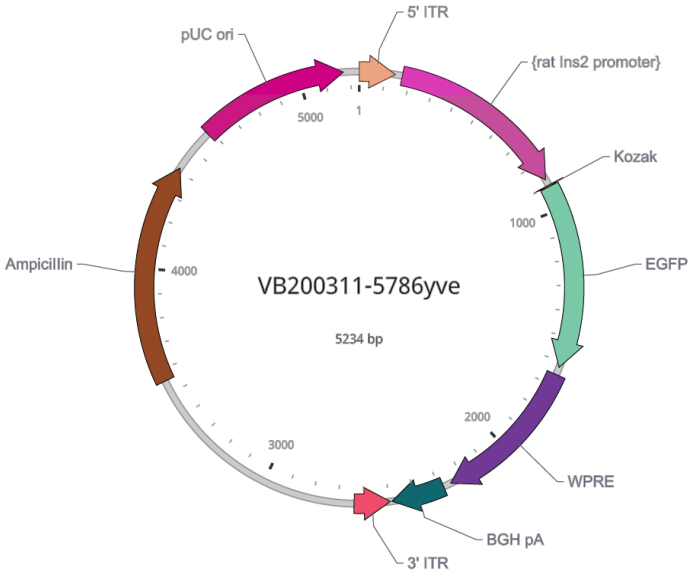


DeJesus DF, et al. ***Nature Cell Biology*** 2024

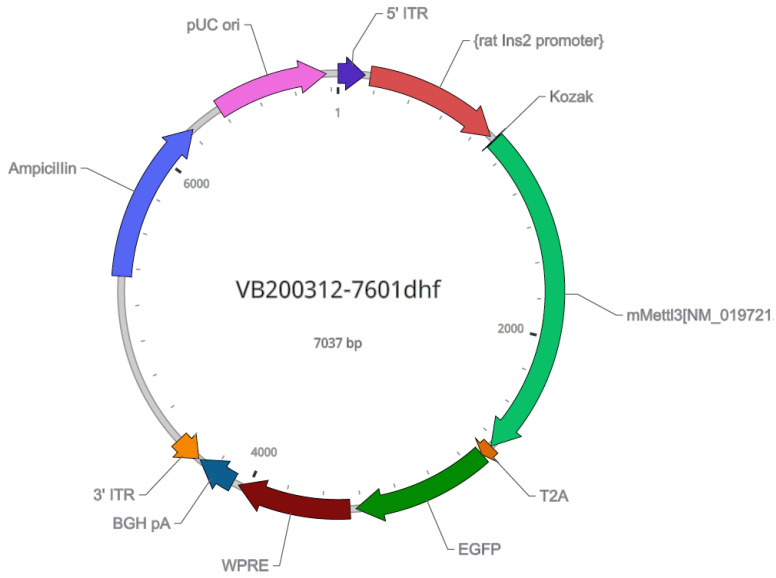
EndoC-βH1 Cells

Can METTL3 be Targeted ?

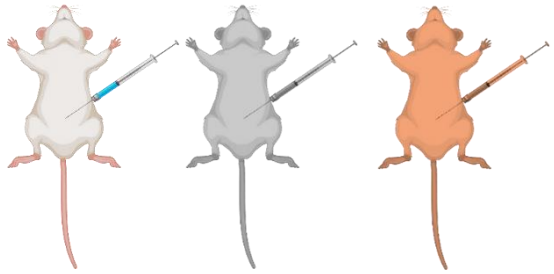
In Vivo AAV8-RIP2 Mediated Overexpression of Mettl3 in NOD β-Cells



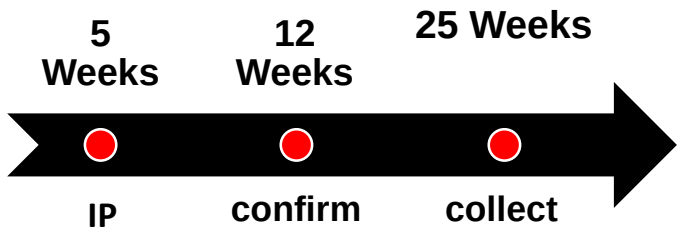
RIP2-eGFP



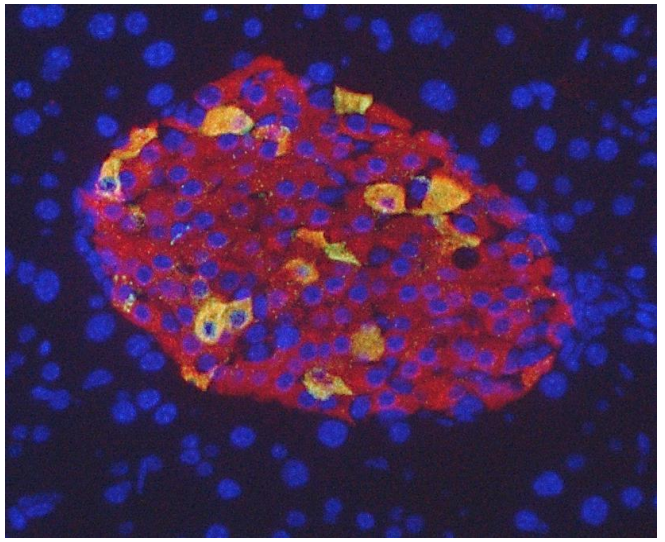
RIP2-Mettl3-eGFP



PBS eGFP Mettl3-eGFP

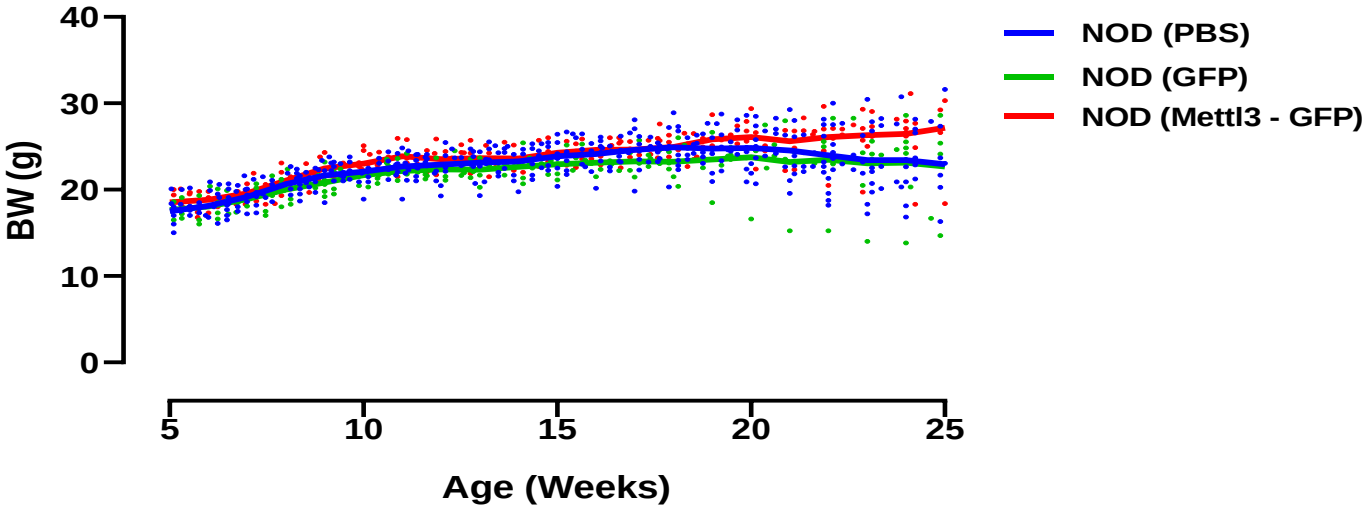


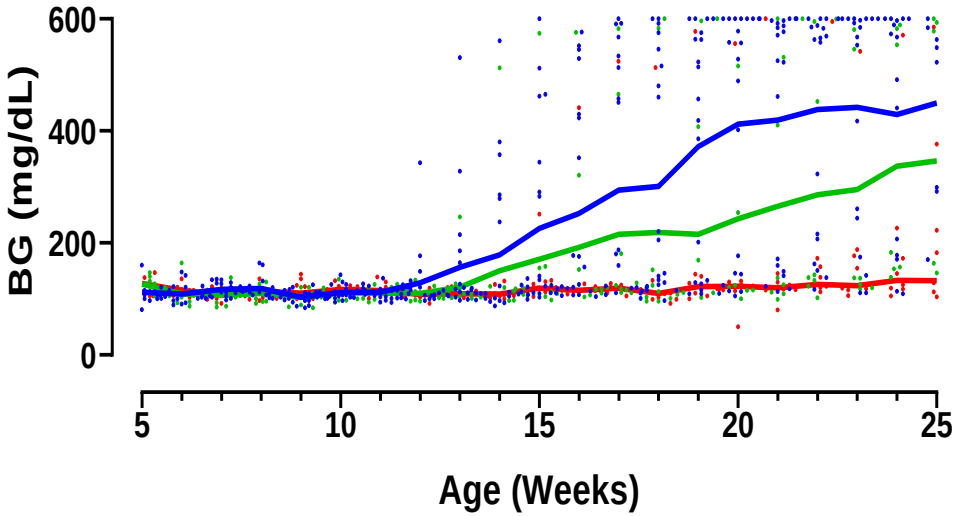
NOD (8 Weeks Post-IP)

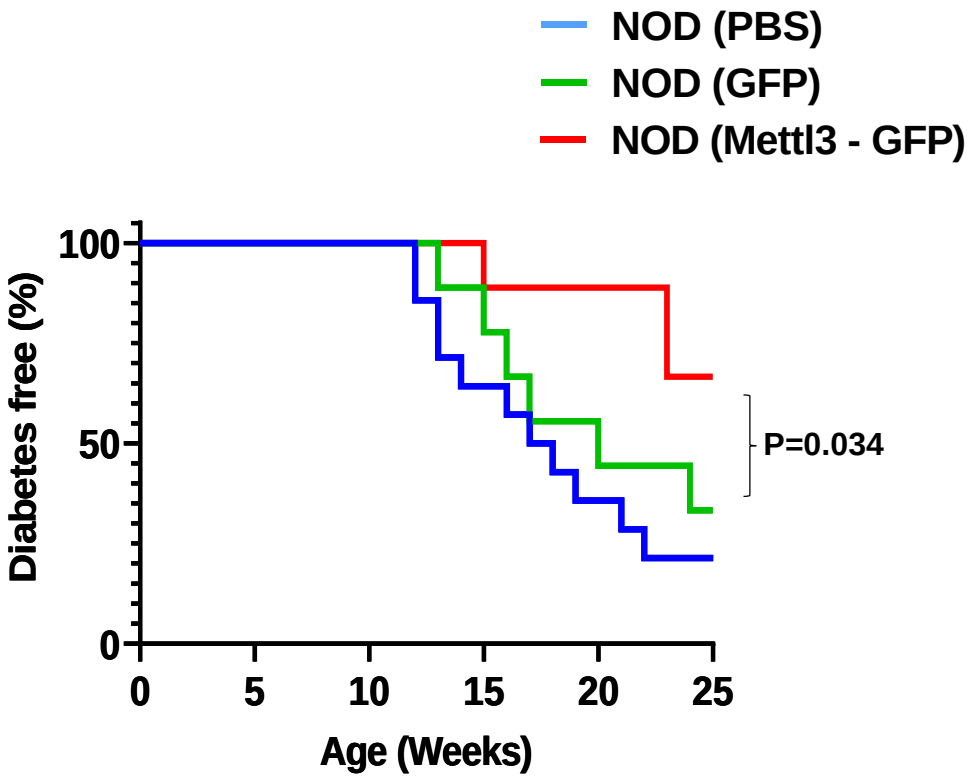


Insulin + eGFP + DAPI

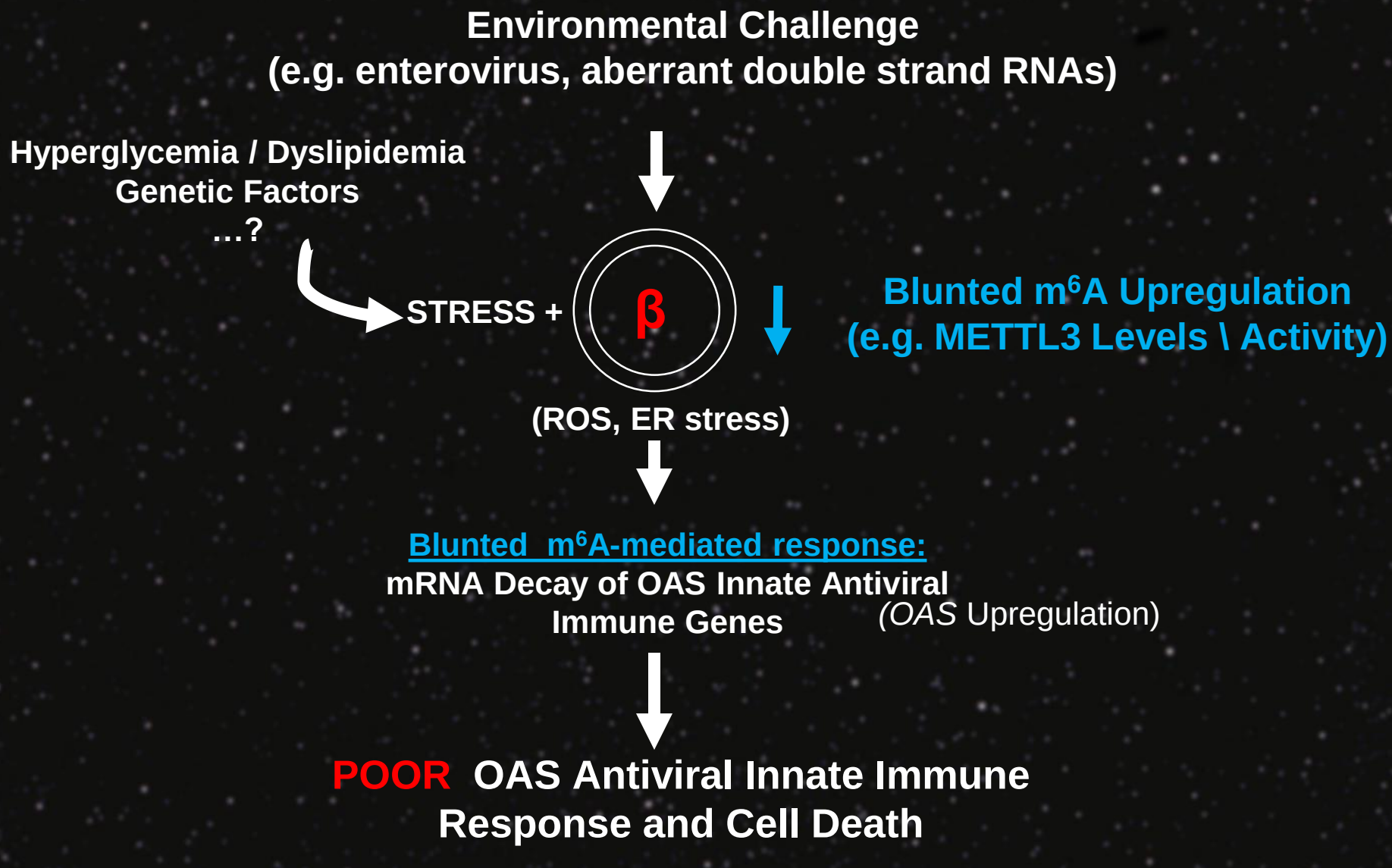
In Vivo AAV8-Mediated Overexpression of Mettl3 in NOD β-Cells Delays T1D Progression







m⁶A is a Novel Safeguard Mechanism in β-cells: Regulating Magnitude and Duration of Innate Antiviral Immune Response



Thank You