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## PKM2 Binds to the Regulatory Regions of GATA1 and STAT5 in $\beta$ -Thalassemic Mouse Erythroblasts

Enrica Federti<sup>1</sup>, Silvia Pietrobono<sup>2</sup>, Veronica Riccardi<sup>1</sup>, Jacopo Ceolan<sup>1,3</sup>, Richard Pozzetto Huot<sup>1</sup>, Filippo Mazzi<sup>1,3</sup>, Tolunay Kavaz<sup>1</sup>, Immacolata Andolfo<sup>4</sup>, Roberta Russo<sup>4</sup>, Davide Melisi<sup>2</sup>, Achille Iolascon<sup>4</sup>, and Lucia De Franceschi<sup>1,3</sup>

<sup>1</sup> Department of Engineering for Innovation Medicine, University of Verona, Verona, Italy;

<sup>2</sup> Department of Medicine, University of Verona, Verona, Italy; Department of Medicine, University of Verona, Verona, Italy;

<sup>3</sup> Azienda Ospedaliera Universitaria di Verona, Verona, Italy;

<sup>4</sup> Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, CEINGE Biotechnologie Avanzate, Naples, Italy.

# **$\beta$ -Thalassemia is characterized by chronic anemia and ineffective erythropoiesis linked to severe oxidation**

- $\beta$ -Thalassemia ( $\beta$ -Thal) is a hereditary red cell disorder that is prevalent worldwide and continues to impose a significant illness burden.
- In  $\beta$ -Thal, reduced red cell survival and ineffective erythropoiesis are due to severe cellular oxidative stress, requiring sustained cellular energy.
- We recently demonstrated an up-regulation of pyruvate kinase R (PKR) and a persistent expression of PKM2 in both mouse and human  $\beta$ -Thal erythroid cells compared to healthy controls.

Tuo Y et al eClin Med 72: 102619, 2024; Taher AT et al Lancet 391: 155, 2018; Matte A et al JCI 131, 2021; Matte A et al Haematologica 108: 2535, 2023.



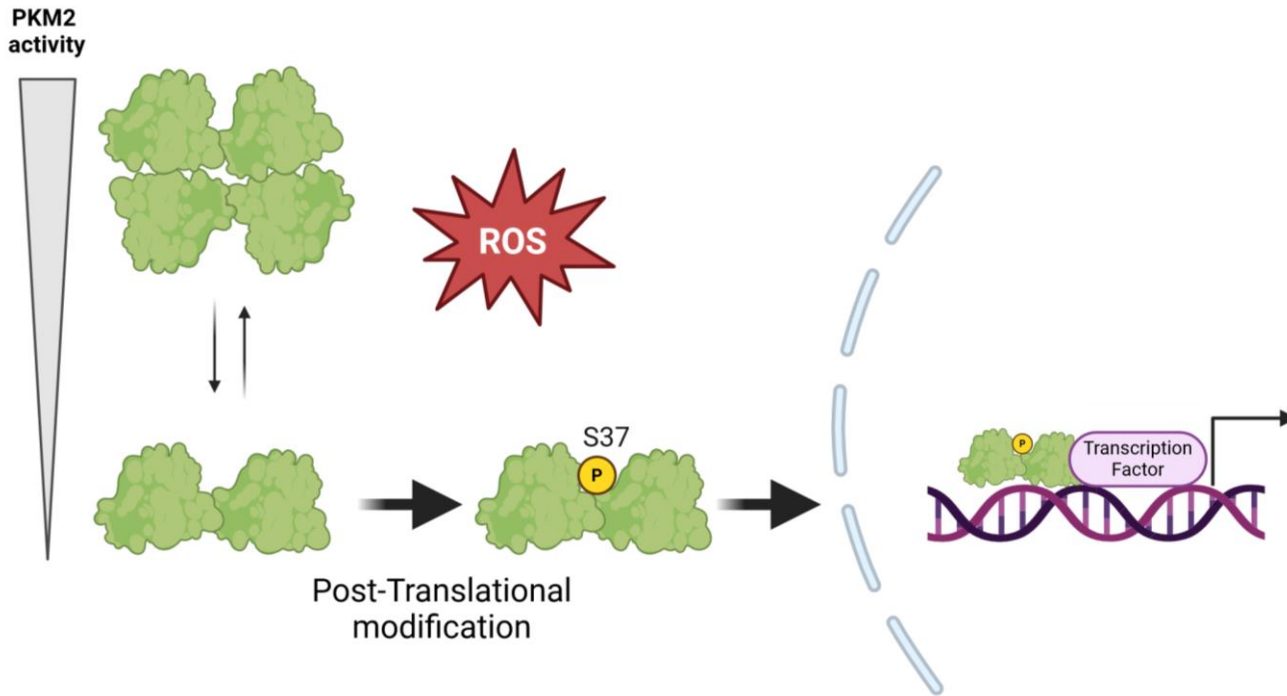
## **$\beta$ -Thalassemic anemia benefits from the activation of pyruvate kinases (PKR-PKM2) by mitapivat**

- In  $\beta$ -Thal mice, mitapivat improves anemia, decreases ineffective erythropoiesis, ameliorates EPO responsiveness and reduces the transfusion burden;
- In  $\beta$ -Thal human erythroid precursors, mitapivat
  - stabilizes and activates both PK isoforms;
  - metabolically reprograms  $\beta$ -Thal erythroblasts;
  - mitigates Cys-oxidative protein damage and decreases DNA-oxidation.

Kung C et al Blood 130: 1347, 2017; Matte A et al JCI 131, 2021; Matte A et al Haematologica 108: 2535, 2023; Siciliano A et al Blood Advances, under revision.



# May the role of PKM2 be extended beyond glycolytic pathway in $\beta$ -Thalassemic erythropoiesis ???



Zahara K et al Front Oncol 10: 159, 2020; Wang P et al Prot & Cell 2015; Gu J et al Cell Death & Disease 12:291, 2021; Nandi S et al JBC 295: 17425, 2020; Angiari S et al Cell Metabol 31: 391, 2020



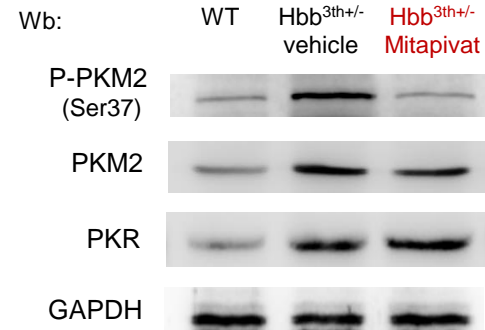
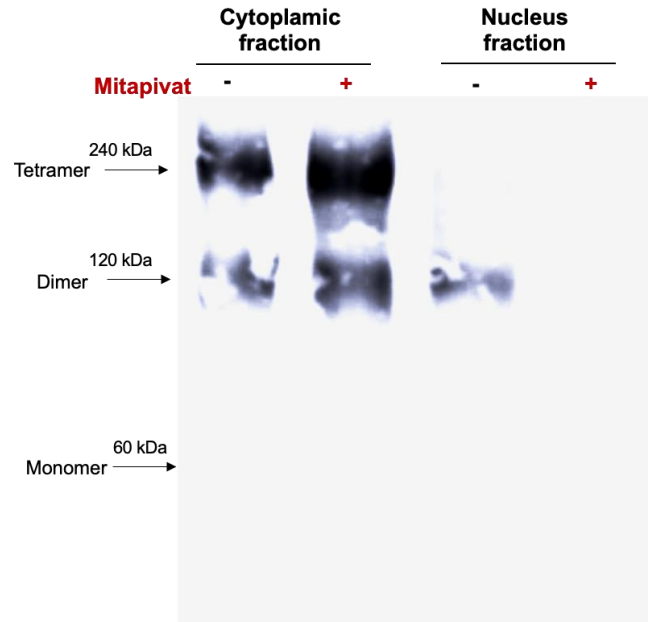
## Methods

- 4 months old female  $\beta$ -Thal ( $Hbb^{th3/+}$  mice) and WT mice (C57B6) were studied.
- Vehicle or Mitapivat (50 mg/kg, twice daily) was administered by gavage for 4 weeks to  $\beta$ -Thal mice.
- $CD44^+Ter119^+FSC^{High}$  cells were sorted from bone marrow and used for:
  - Western-blot (Wb) and/or Immunoprecipitation assay (IP);
  - Chromatin Immunoprecipitation (ChIP) assay;
  - RNA extraction and real-time qPCR.
- Plasma fibroblast growth factor-23 (FGF23), which has been linked to ineffective erythropoiesis, was determined by ELISA (Abcam, Cambridge, UK) according to the manufacturer's instruction.

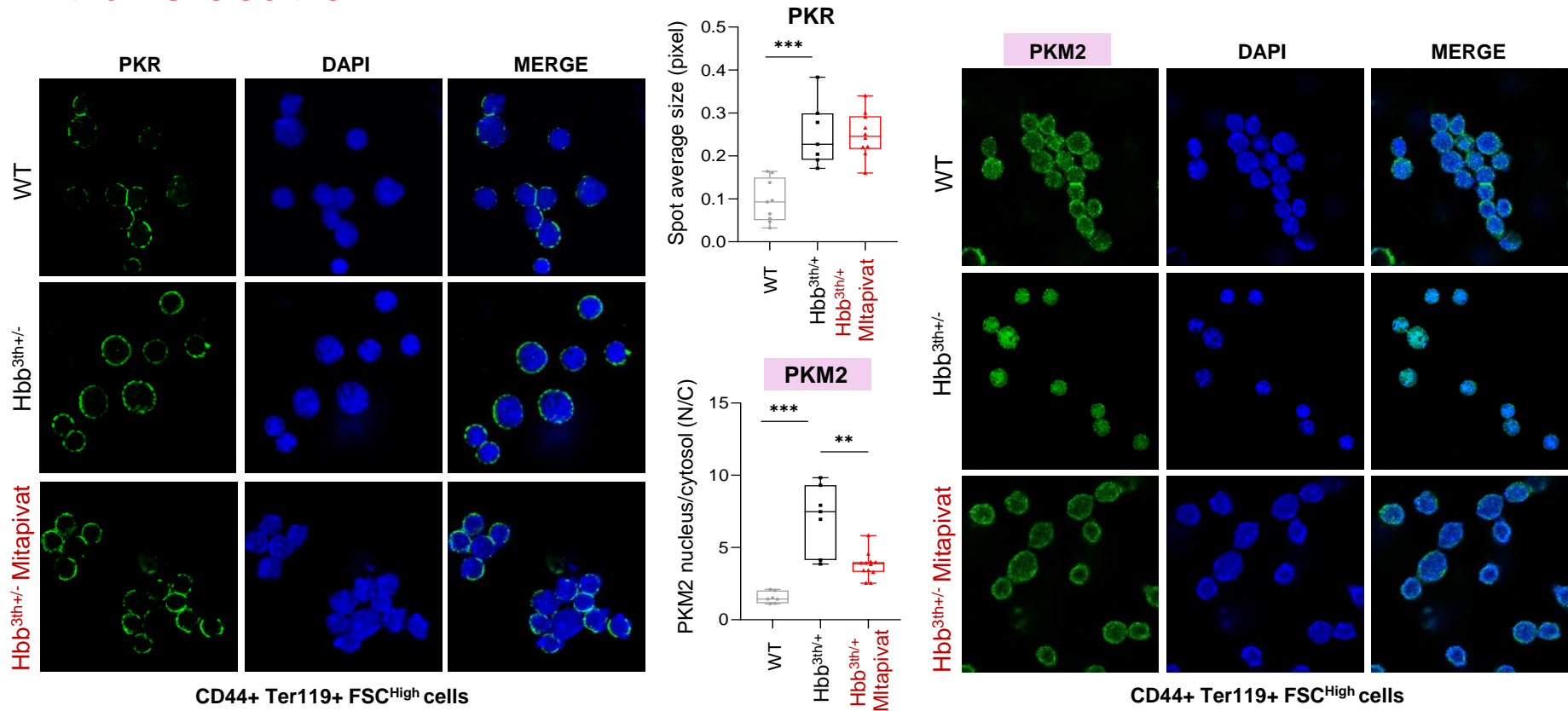
Matte A et al JCI 131, 2021; Matte A et al Haematologica 108: 2535; Mbiandjeu SCT et al JCI 133(4): 454, 2024.  
Weidner H et al JCI Insight 5: e137062, 2020; Aprile et al Science Transl Med 15: eabq3679, 2023.



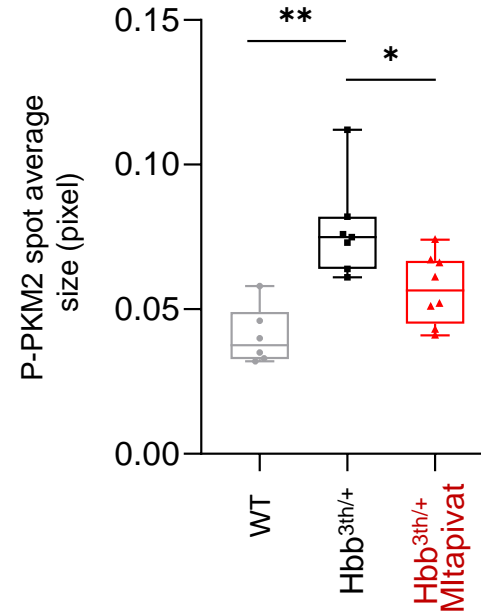
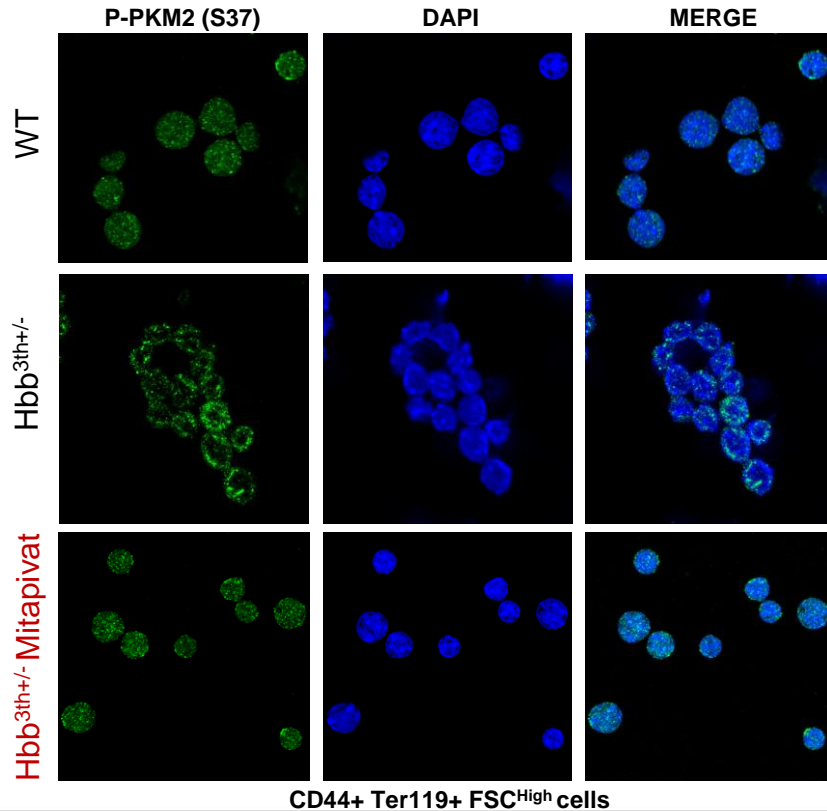
# In $\beta$ -Thal erythroid precursors, mitapivat promotes PKM2 dimer to tetramer conversion, preventing dimeric PKM2 nuclear localization with reduced Phospho-PKM2 (S37)



# In $\beta$ -Thal erythroblasts, mitapivat reduces PKM2 nuclear translocation



# In $\beta$ -Thal erythroblasts, mitapivat decreases phospho-PKM2 nuclear localization



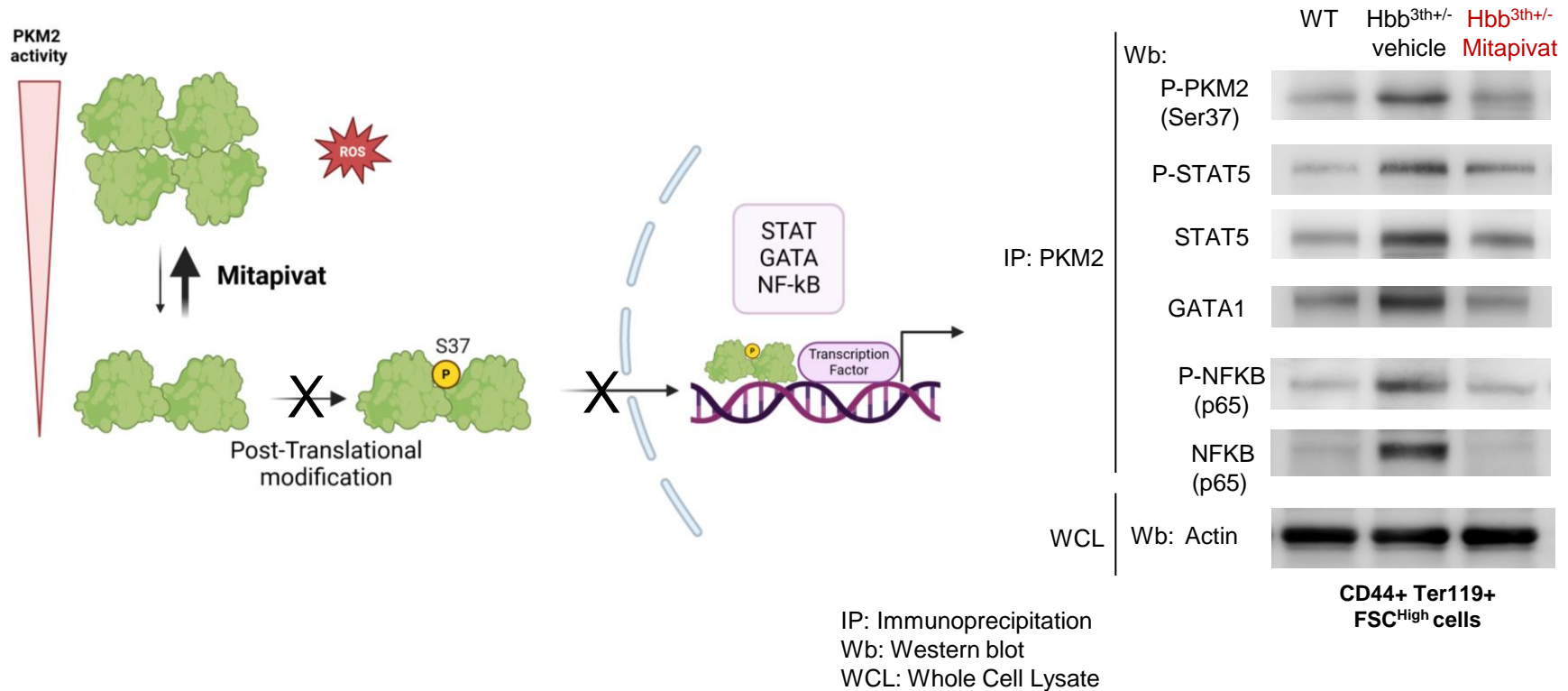


# Phospho-PKM2 and transcriptional factors: STAT, GATA and NF- $\kappa$ B p65

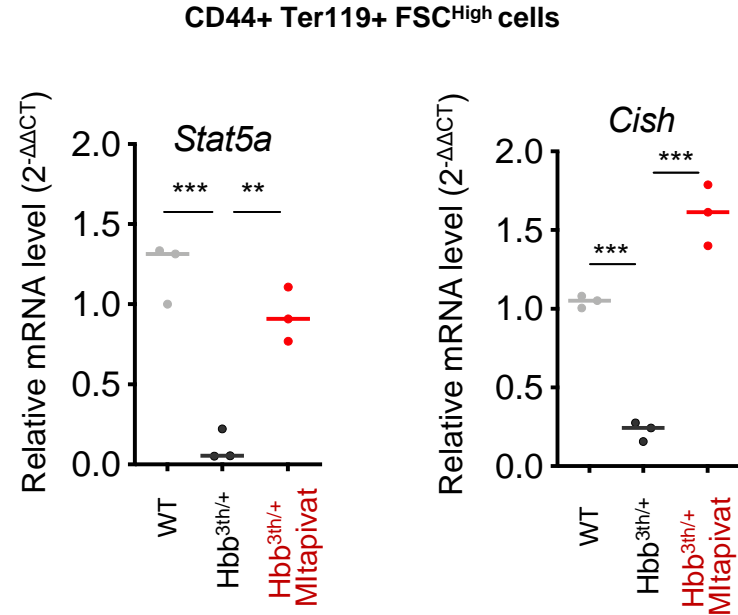
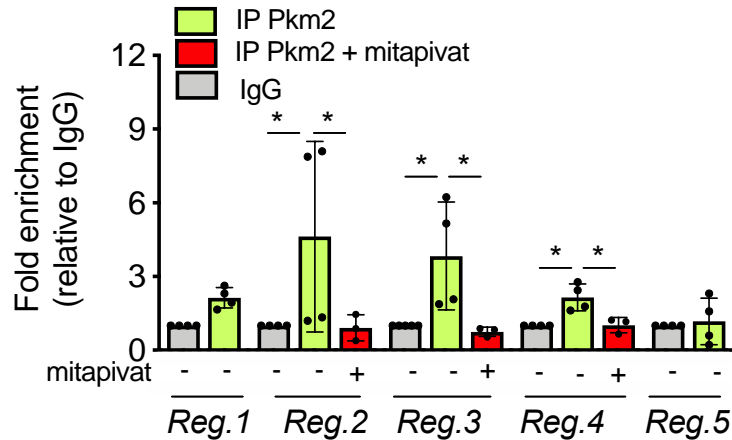
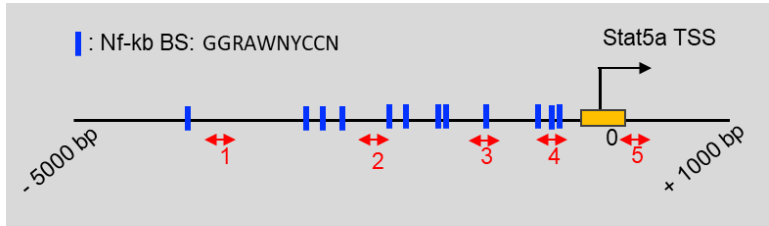
- A functional crosstalk between metabolic inactive dimeric PKM2 and transcriptional factors has been reported in different cell- and animal-based models.
- The following transcriptional factors has been shown to interact with phospho-PKM2 (S37):
  - **STAT3** (Gao X et al Mol Cell 45: 598, 2012; Gui DY et al Sci Signal 6: pe7, 2013; Yang P et al Cell Signal 26: 1853, 2014; Zahara K et al Front Oncol 10: 159, 2020)
  - **GATA4, 6** (Lorenzana-Carrillo MA et al. Sci. Transl Med 14: eabm:3565, 2022) and **GATA1** (Te Ling et al # 940, Session 101, ASH meeting 2024)
  - **NF- $\kappa$ B (p65)** (Gu J et al Cell Death & Disease 12:291, 2021)



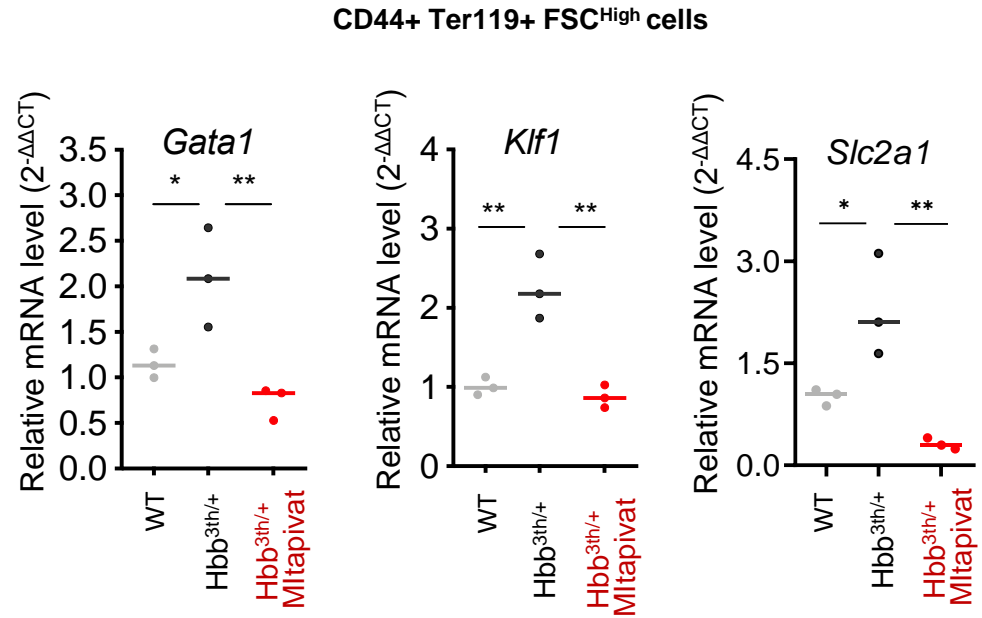
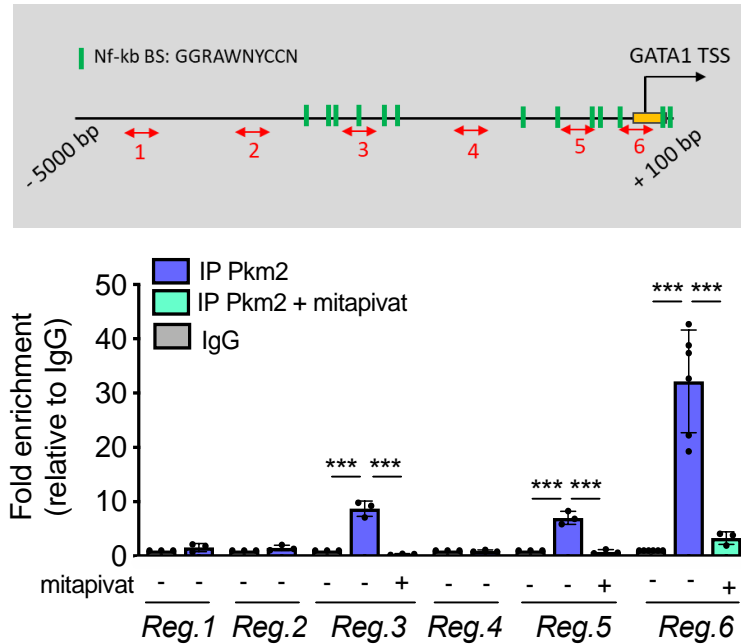
# In $\beta$ -Thal erythroblasts, mitapivat decreases PKM2 interaction with active STAT5, GATA1 and active NF-kB



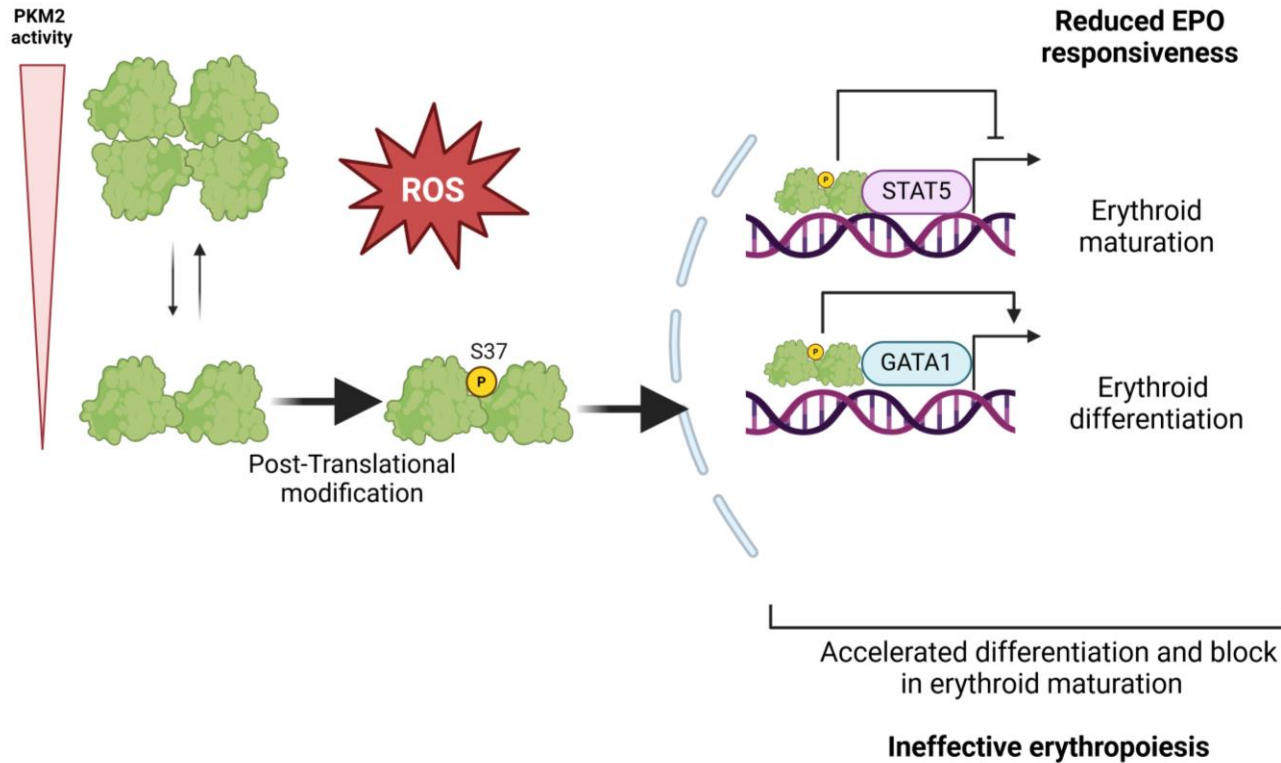
# In $\beta$ -Thal erythroblasts, mitapivat prevented PKM2 binding to STAT5 promoter region as STAT5-repressor, resulting in increased EPO responsiveness



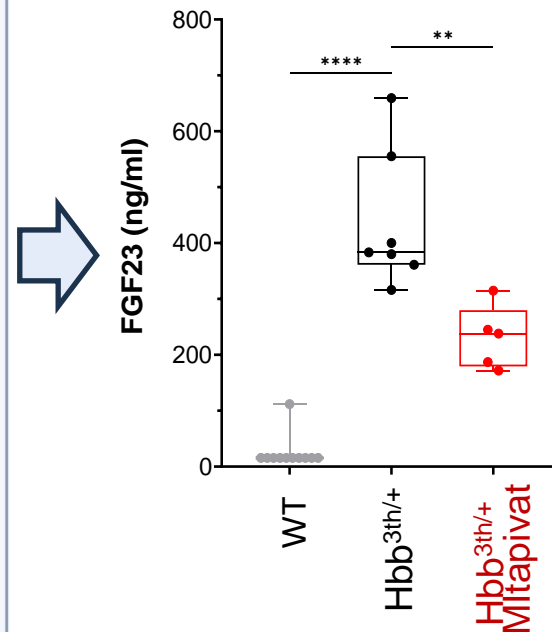
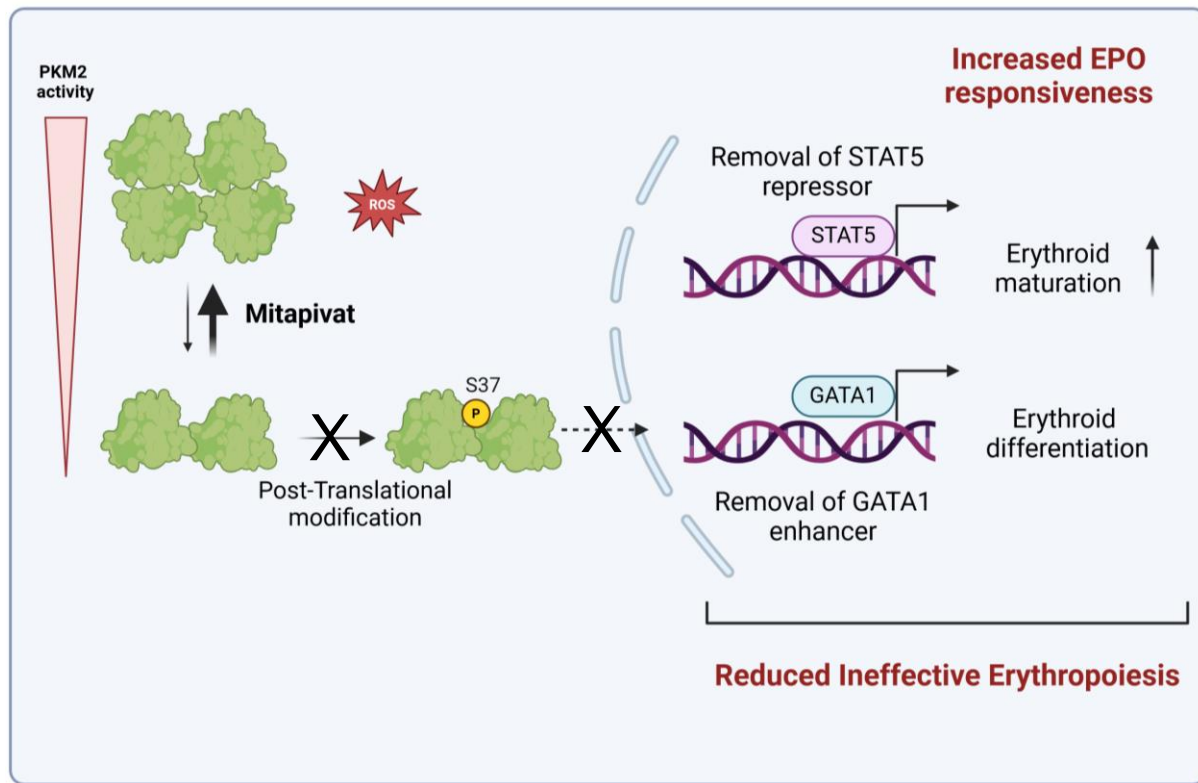
# In $\beta$ -Thal erythroblasts, mitapivat prevented PKM2 binding to GATA1 promoter region as GATA1-enhancer, normalizing the homeostatic control mechanism during erythroid maturation



# Phospho-PKM2 participates to $\beta$ -Thalassemic ineffective erythropoiesis



# Mitapivat prevented PKM2 nuclear translocation, contributing to the amelioration of ineffective erythropoiesis and the reduction of plasma FGF23



# Conclusions

- $\beta$ -Thal mouse erythroblasts displayed increased amounts of p-PKM2 (S37), with nuclear localization when compared to healthy cells.
- In  $\beta$ -Thal mouse erythroblasts, p-PKM2 acts as STAT5 suppressor, and as GATA1 enhancer, contributing to ineffective erythropoiesis.
- In  $\beta$ -Thal mouse erythroblasts, mitapivat:
  - promotes PKM2 dimer to tetramer conversion and reduces p-PKM2 nuclear localization
  - reverts STAT5 transcriptional repression and prevents GATA1 upregulation, supporting the improvement of ineffective erythropoiesis as corroborated by the reduction in FGF23 levels.



# Investigators and Acknowledgments

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Immacolata Andolfo

