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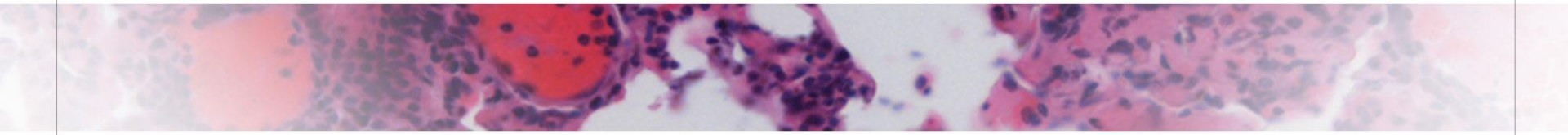

PKM2 Binds to the Regulatory Regions of GATA1 and STAT5 in β-Thalassemic Mouse Erythroblasts

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β-Thalassemia is characterized by chronic anemia and ineffective erythropoiesis linked to severe oxidation

- β-Thalassemia (β-Thal) is a hereditary red cell disorder that is prevalent worldwide and continues to impose a significant illness burden.
- In β-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 β-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.

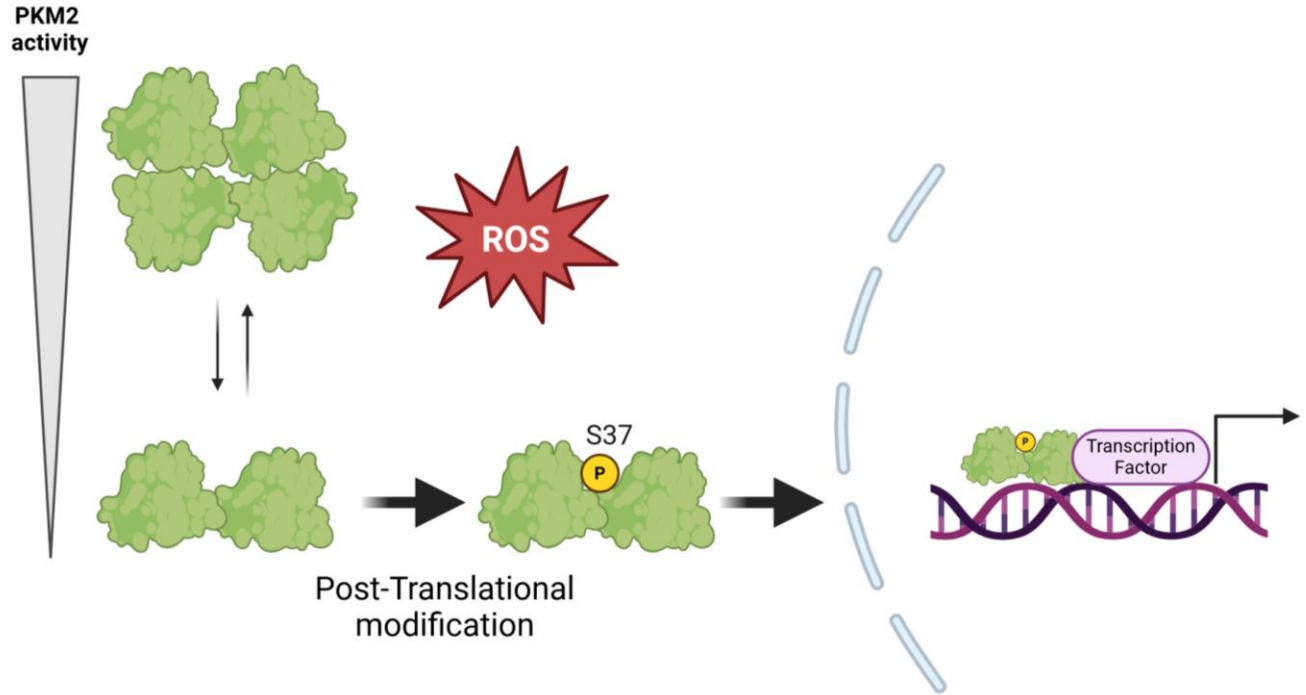
β-Thalassemic anemia benefits from the activation of pyruvate kinases (PKR-PKM2) by mitapivat

- In β-Thal mice, mitapivat improves anemia, decreases ineffective erythropoiesis, ameliorates EPO responsiveness and reduces the transfusion burden;

- In β-Thal human erythroid precursors, mitapivat
  - stabilizes and activates both PK isoforms;
  - metabolically reprograms β-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 β-Thalassemic erythropoiesis ???



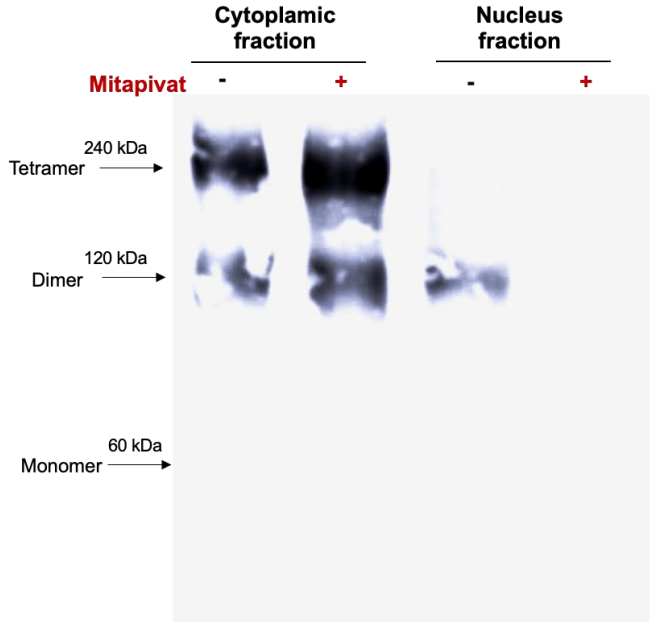
Zahara K et al Front Oncol 10: 159, 2020; Wang P et al Prot & Cell 2015; Gu J et al Cell Death & Disease 12:291, 2021; Nandi S et al JBC 295: 17425, 2020; Angiari S et al Cell Metabol 31: 391, 2020

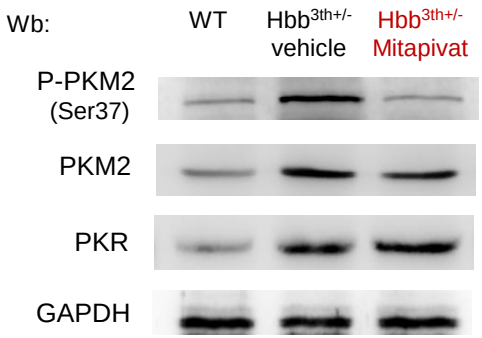
Methods

- 4 months old female β-Thal ($Hbb^{th3/+}$ mice) and WT mice (C57B6) were studied.
- Vehicle or Mitapivat (50 mg/kg, twice daily) was administrated by gavage for 4 weeks to β-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.

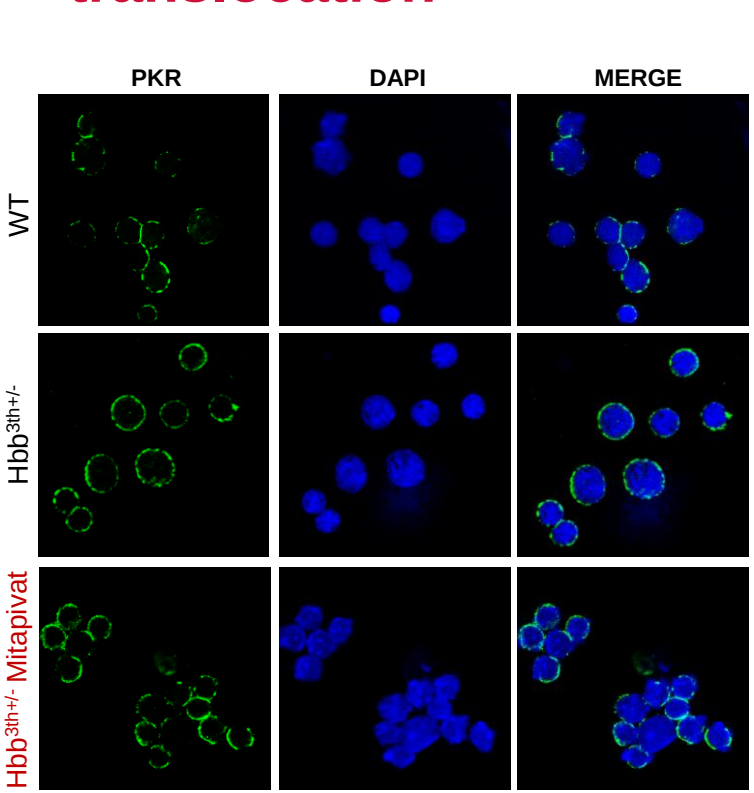
Matte A et al JCI 131, 2021; Matte A et al Haematologica 108: 2535; Mbiandjeu SCT et al13(4): 454,2024.
Weidner H et al JCI Insight 5: e137062, 2020; Aprile et al Scince Transl Med 15: eabq3679, 2023.

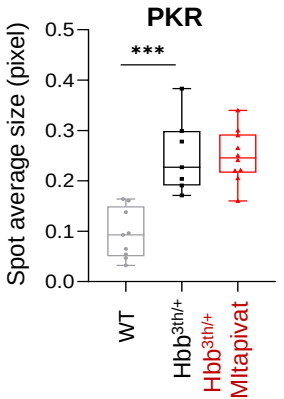
In β-Thal erythroid precursors, mitapivat promotes PKM2 dimer to tetramer conversion, preventing dimeric PKM2 nuclear localization with reduced Phospho-PKM2 (S37)

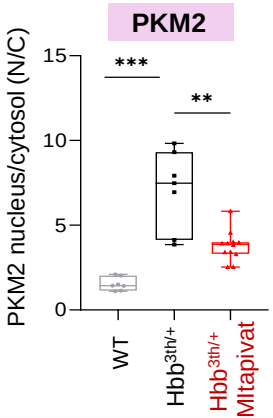


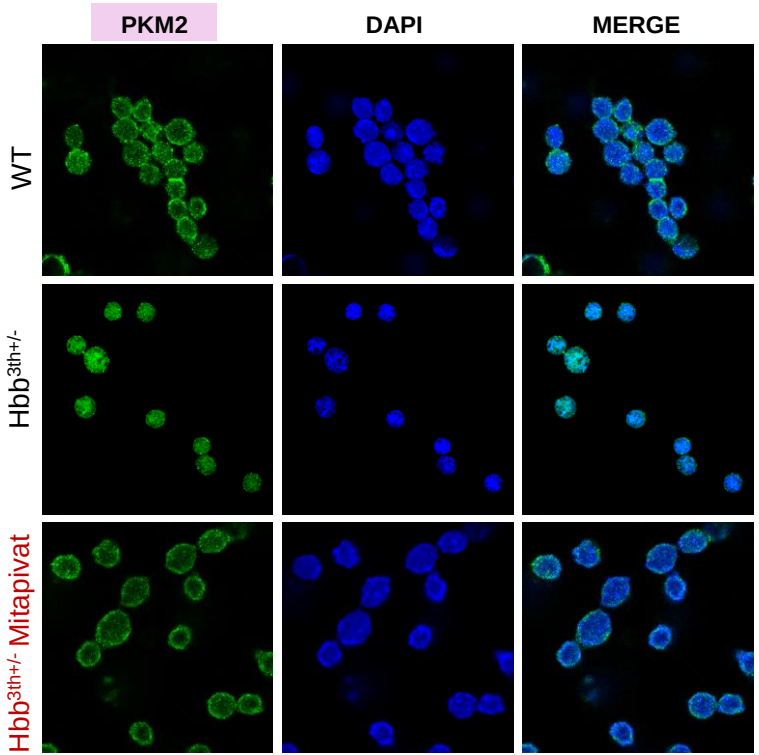


In β-Thal erythroblasts, mitapivat reduces PKM2 nuclear translocation

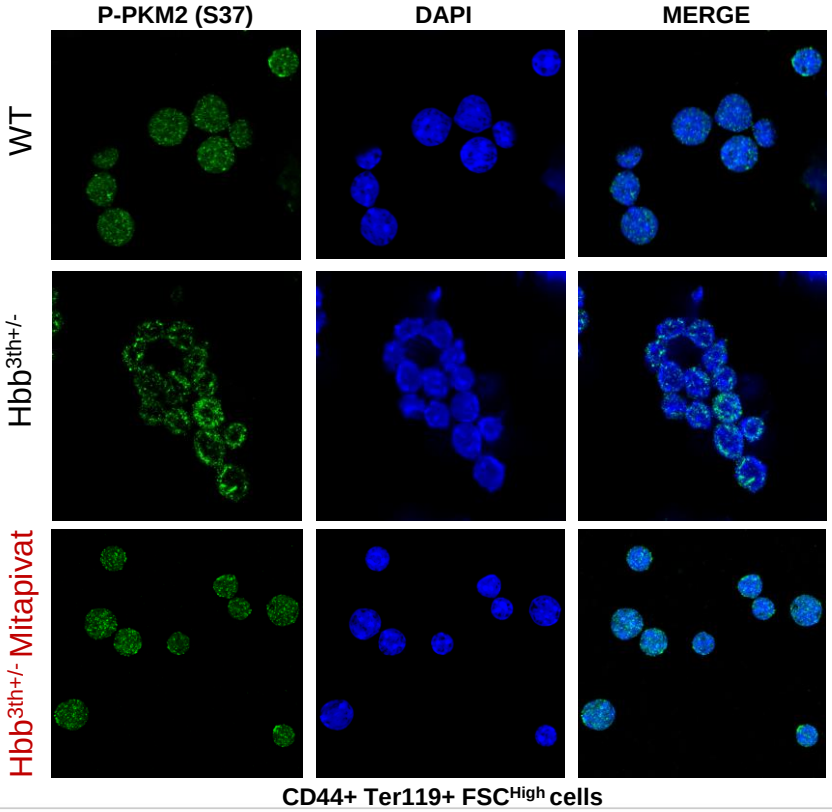


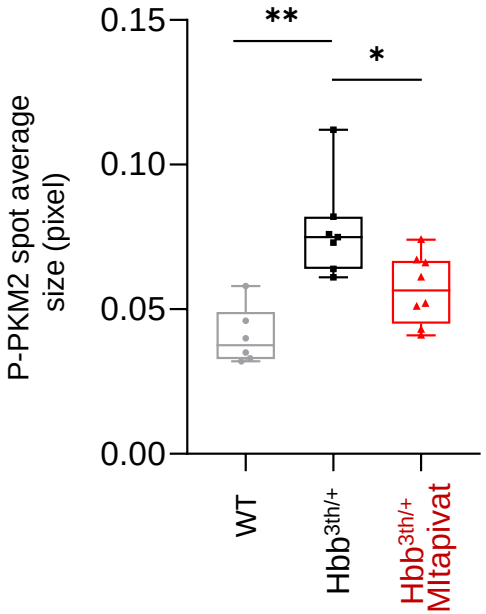






In β-Thal erythroblasts, mitapivat decreases phospho-PKM2 nuclear localization



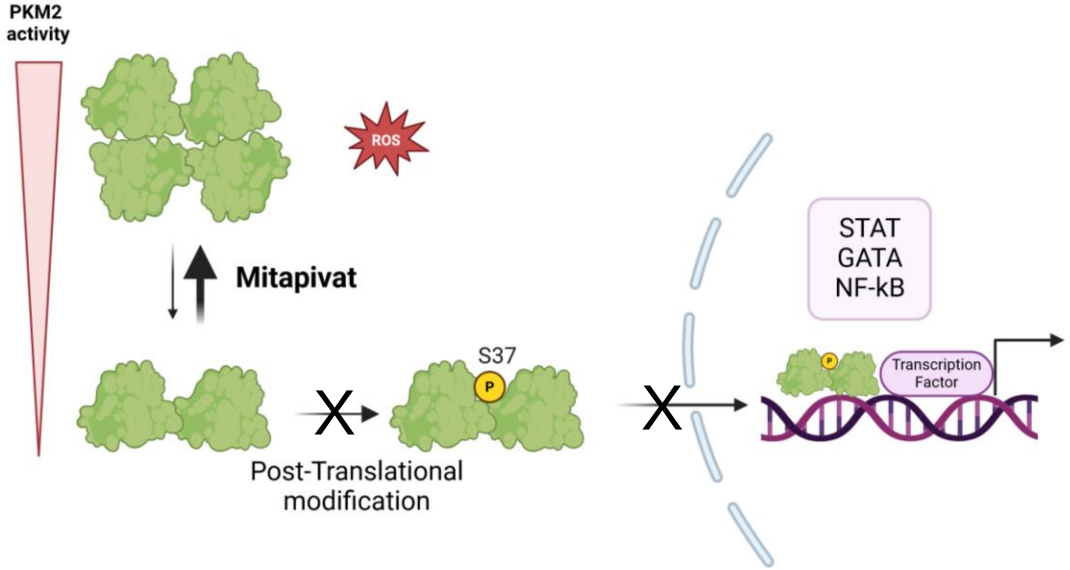


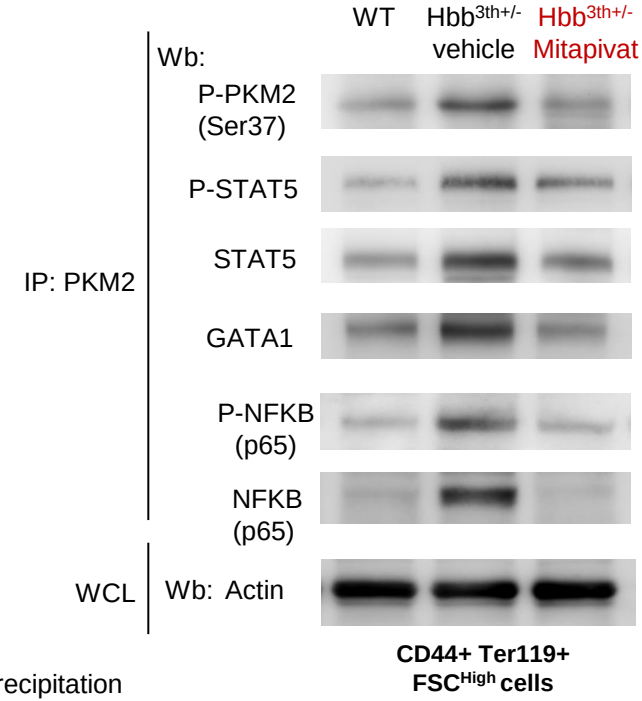
Phospho-PKM2 and transcriptional factors: STAT, GATA and NF-kB p65

- 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-kB (p65)** (Gu J et al Cell Death & Disease 12:291, 2021)

In β-Thal erythroblasts, mitapivat decreases PKM2 interaction with active STAT5, GATA1 and active NF-kB

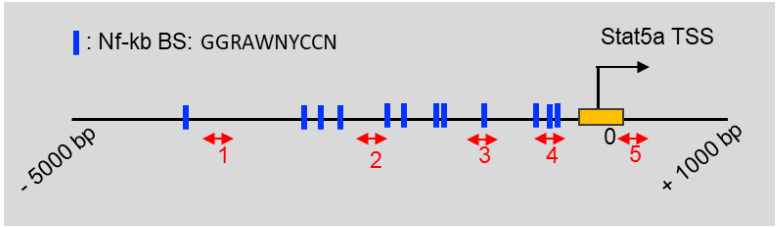


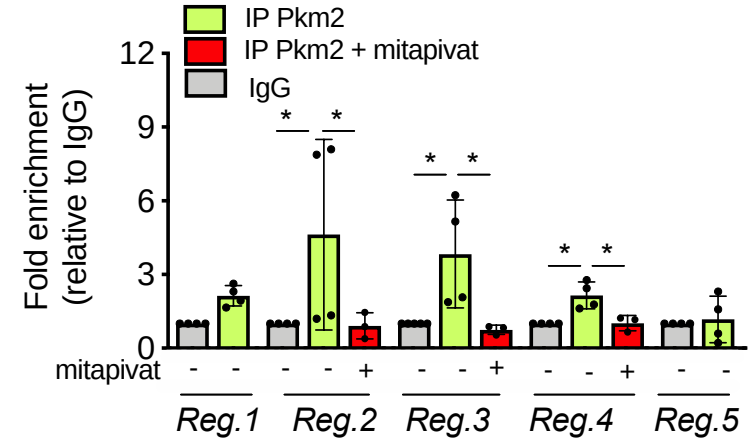


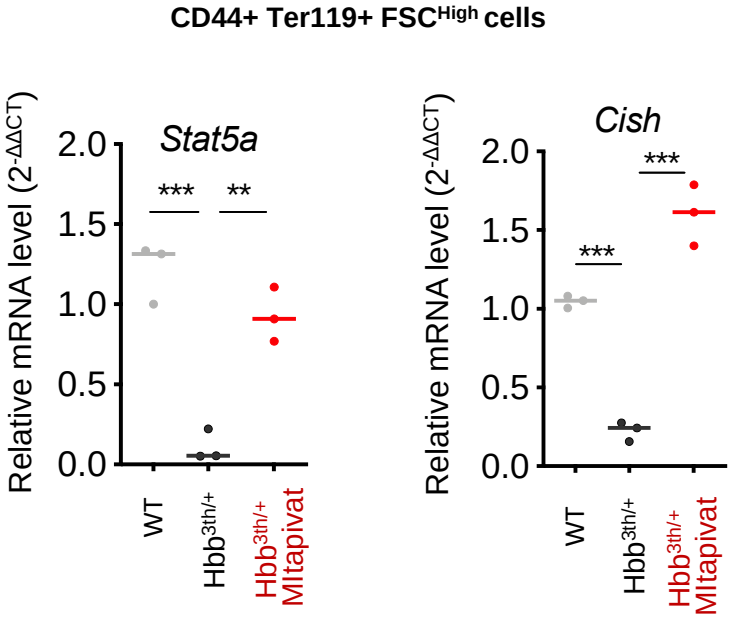
CD44+ Ter119+ FSC^{High} cells

IP: Immunoprecipitation
Wb: Western blot
WCL: Whole Cell Lysate

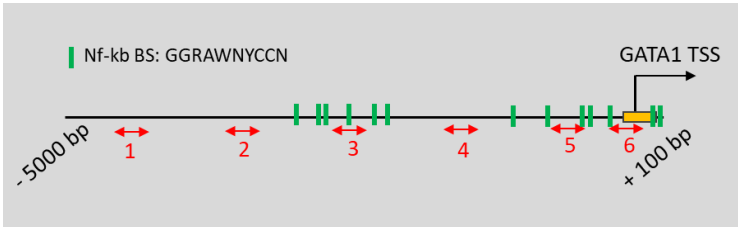
In β-Thal erythroblasts, mitapivat prevented PKM2 binding to STAT5 promoter region as STAT5-repressor, resulting in increased EPO responsiveness

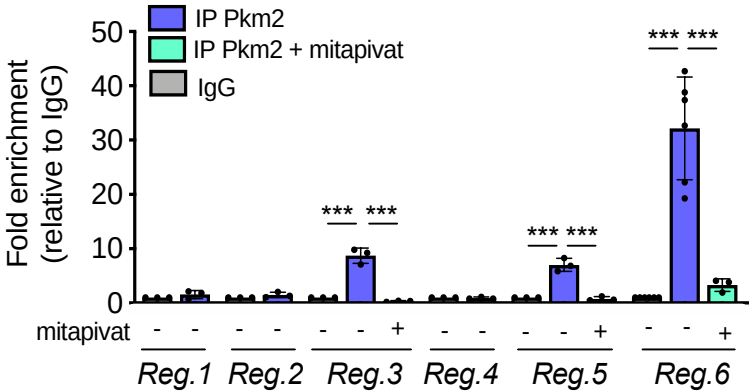




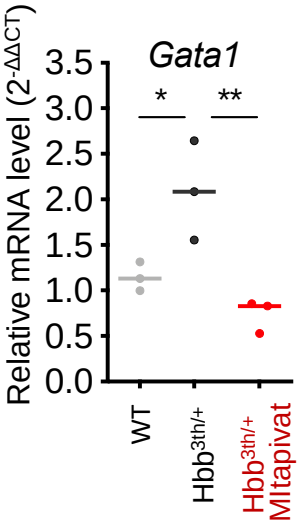


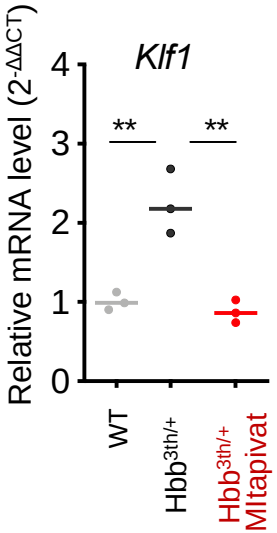
In β-Thal erythroblasts, mitapivat prevented PKM2 binding to GATA1 promoter region as GATA1-enhancer, normalizing the homeostatic control mechanism during erythroid maturation

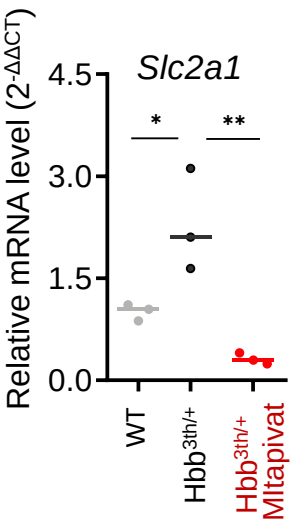




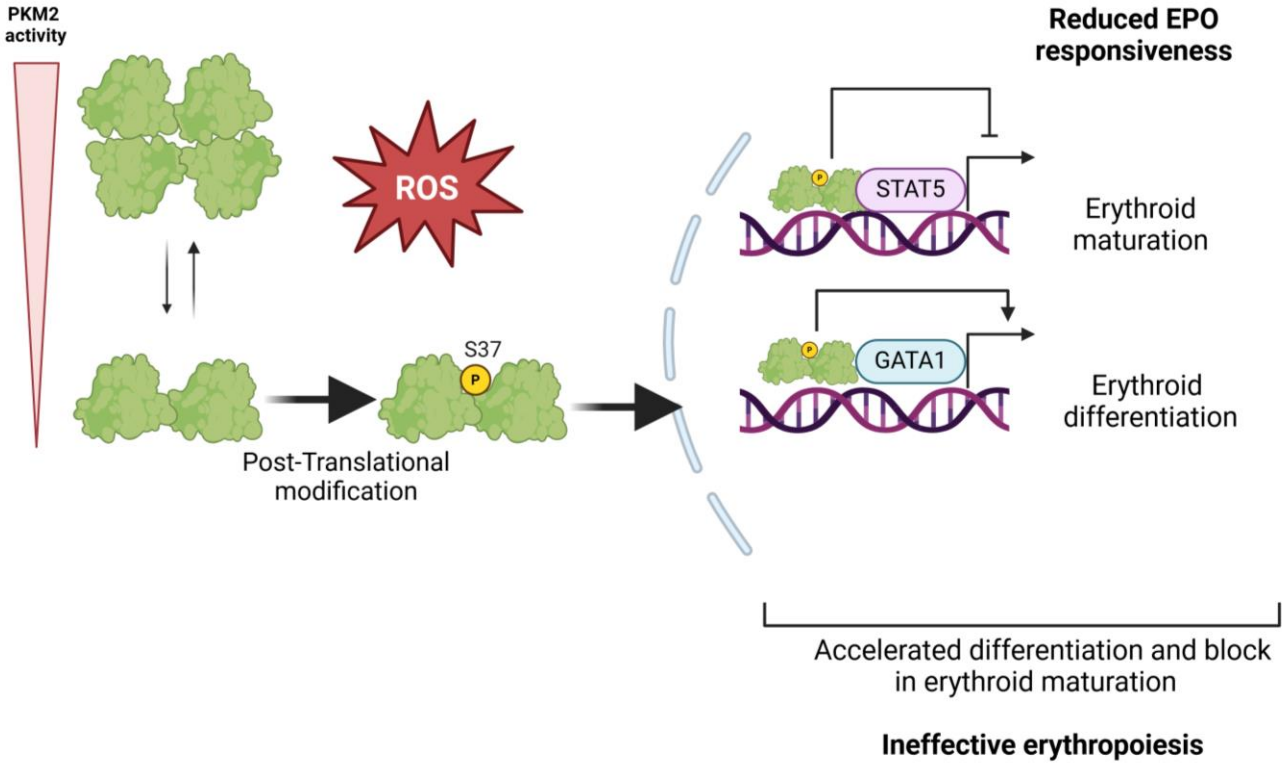
CD44+ Ter119+ FSC^{High} cells



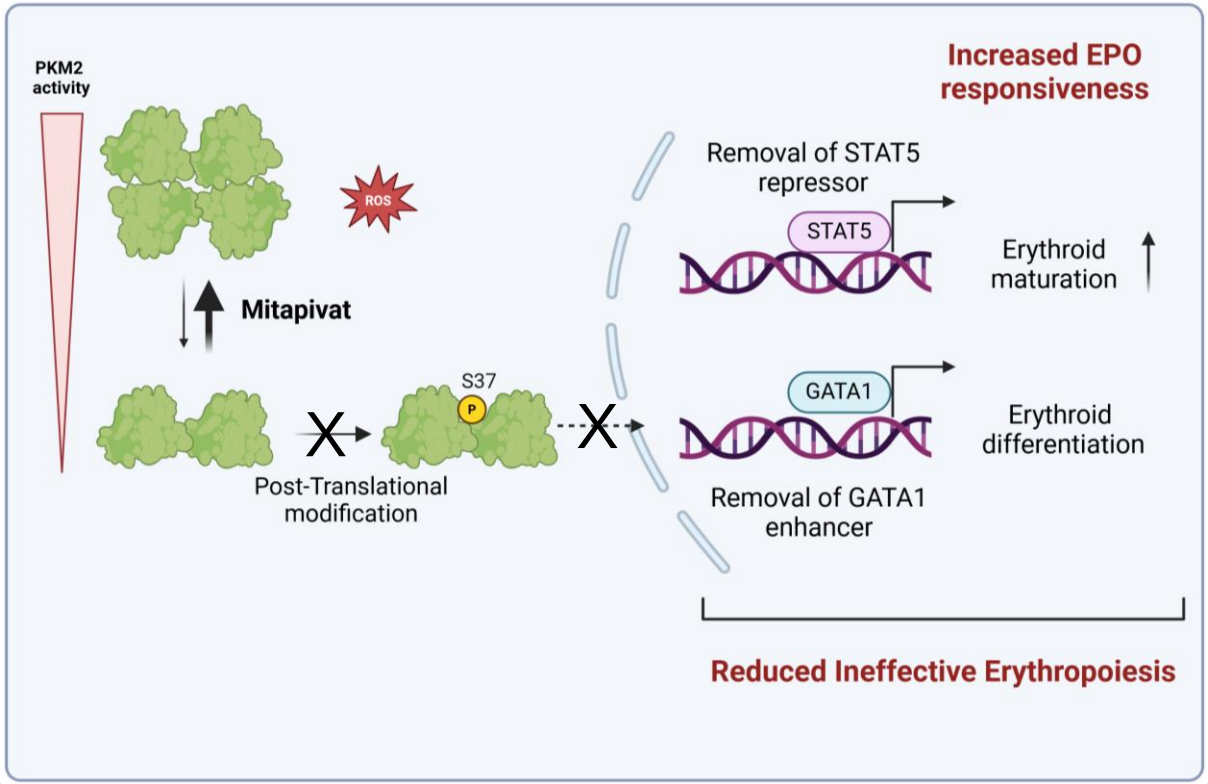


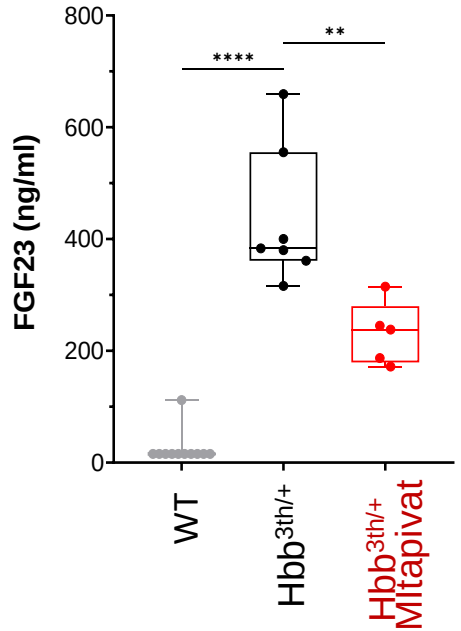


Phospho-PKM2 participates to β-Thalassemic ineffective erythropoiesis



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





Conclusions

- β-Thal mouse erythroblasts displayed increased amounts of p-PKM2 (S37), with nuclear localization when compared to healthy cells.

- In β-Thal mouse erythroblasts, p-PKM2 acts as STAT5 suppressor, and as GATA1 enhancer, contributing to ineffective erythropoiesis.

- In β-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.

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