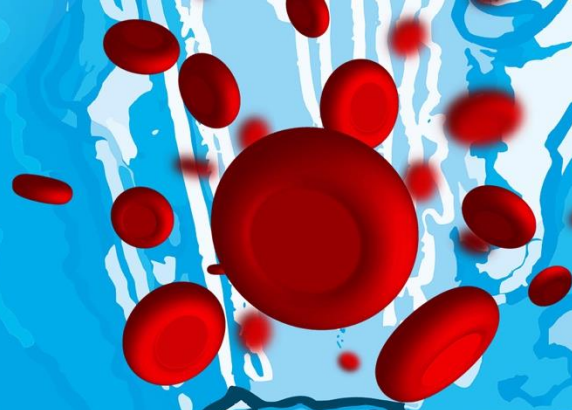




Meeting del CLUB DEL

**Multi-omics integration reveals
shared heme-mitochondrial stress in cellular models of CDA**

A. Nostroso, B.E. Rosato, R. Marra, V. Pennella, F.M. Esposito, V. D'Onofrio, A. Perrotta, V. Cerbone,
R. Kurita, Y. Nakamura, J. Haines, A. D'Alessandro, A. Iolascon, I. Andolfo, R. Russo

**Globulo
Rosso** 2026

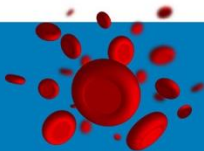
NAPOLI

28-29 SETTEMBRE 2026

Complesso dei SS. Marcellino e Festo

Disclosures

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other



Congenital Dyserythropoietic Anemias (CDAs)



Hereditary red blood cell defects



Hyporegenerative anemias



Enzyme disorders

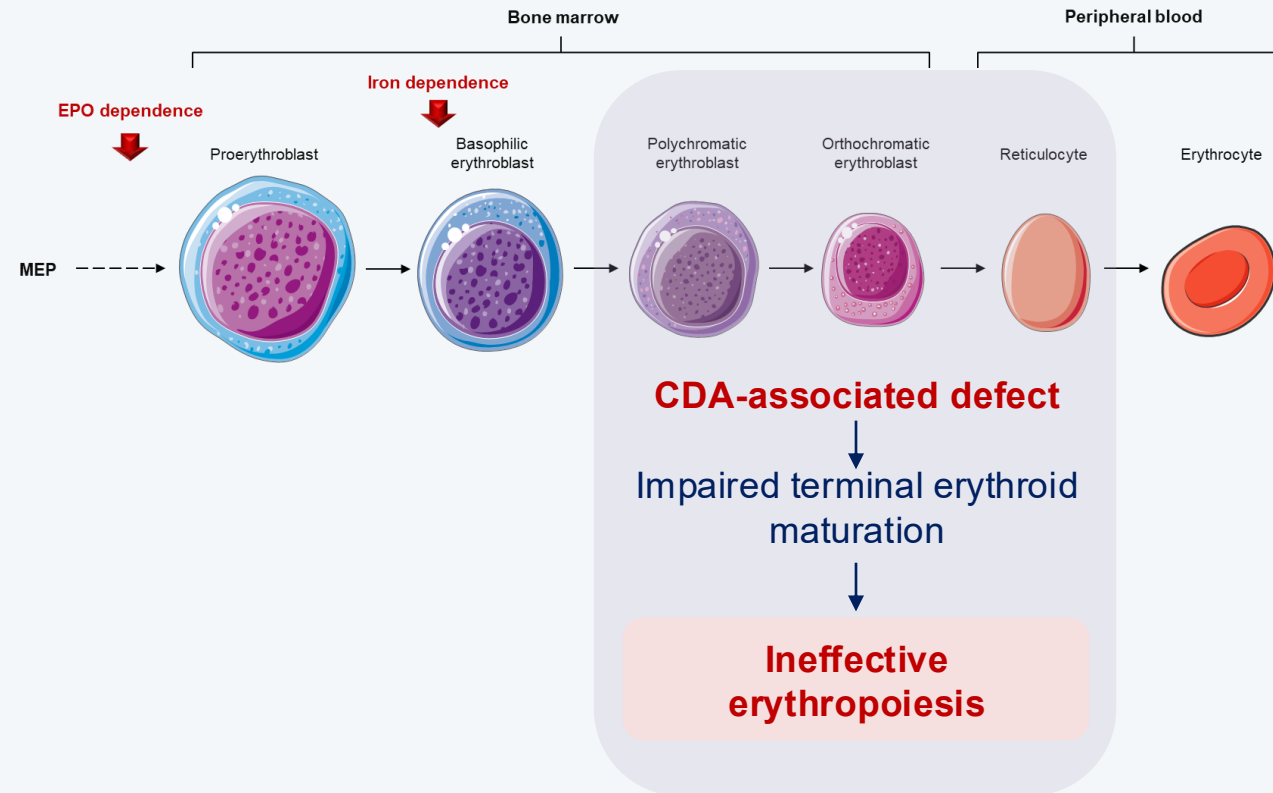


Red blood cell membrane defects



Hemoglobinopathies

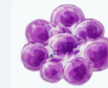
Terminal erythroid differentiation



Hallmark features of CDAs



Chronic anemia with inappropriately low reticulocyte count



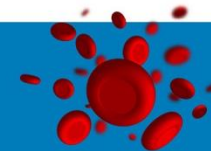
Erythroid hyperplasia with morphological abnormalities of late erythroblasts



Iron overload due to ineffective erythropoiesis



Extramedullary hematopoiesis
(Jaundice, splenomegaly, gallstones)



CDA I and CDA II arise from distinct molecular defects



CDA I (type Ia/Ib)



Genes

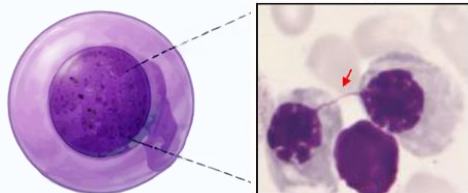
CDAN1 (15q15.2)

CDIN1 (15q14)

Altered chromatin organization and nucleolar function



Defective histone chaperone activity (e.g. Asf1 complex)



internuclear chromatin bridge



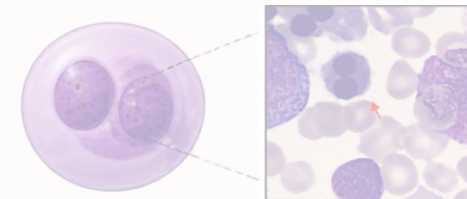
Genes

SEC23B (20p11.23)

Impaired COPII-dependent ER-to-Golgi trafficking

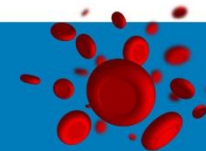


Abnormal processing and maturation of erythroid membrane proteins



binucleate erythroid precursors

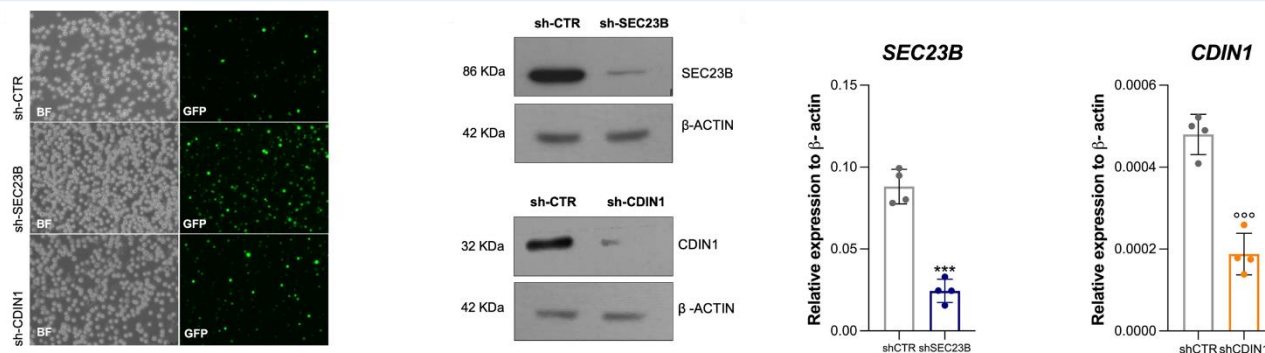
Distinct molecular defects
shared phenotype: **ineffective erythropoiesis**



HUDEP-2 models recapitulate altered erythroid differentiation in CDA

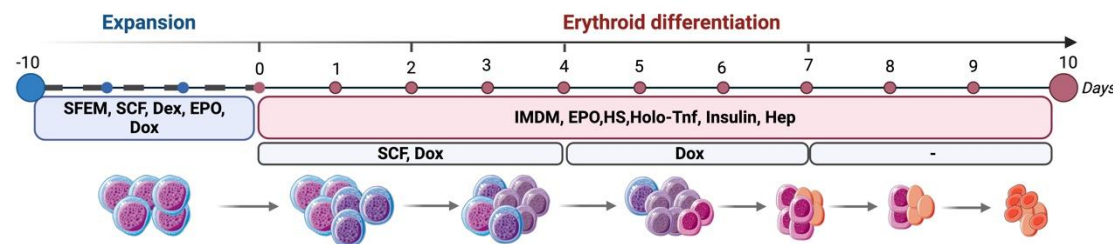
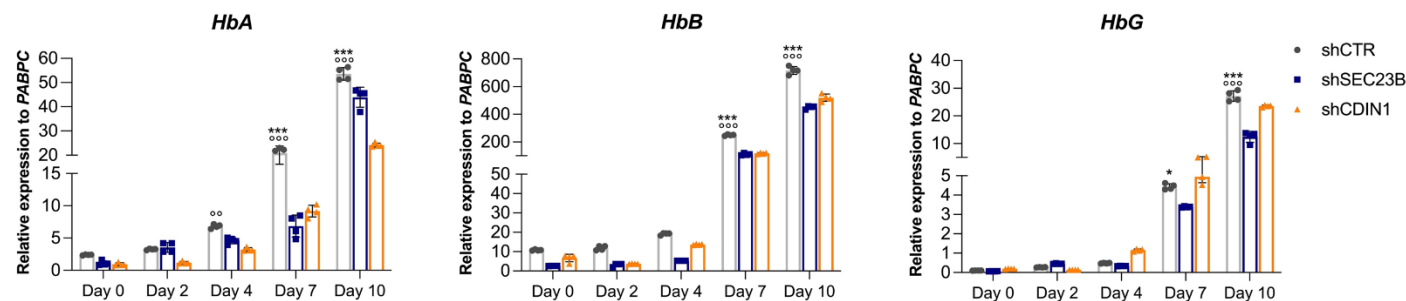


Generation of HUDEP-2 CDA models

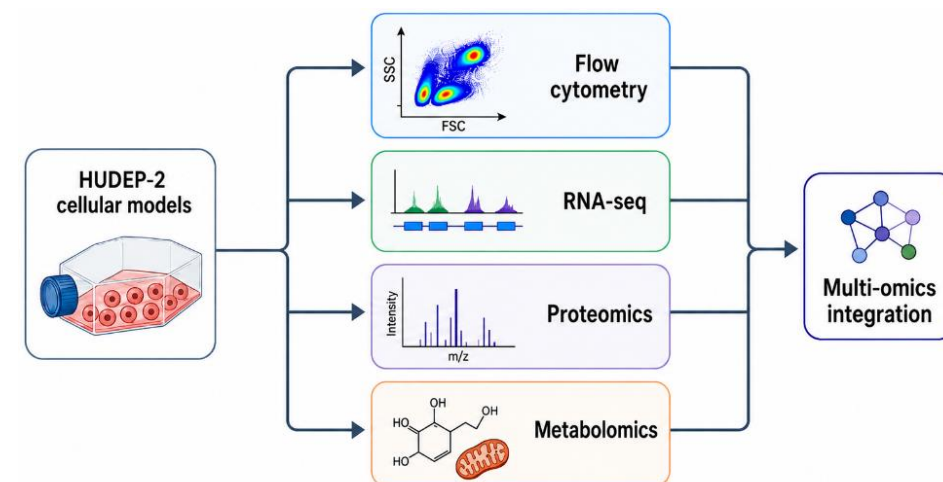


Distinct *CDIN1* and *SEC23B* deficiencies converge on **impaired erythroid differentiation** and altered globin maturation.

Impaired erythroid differentiation and globin expression

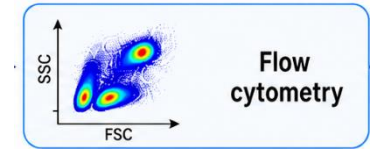
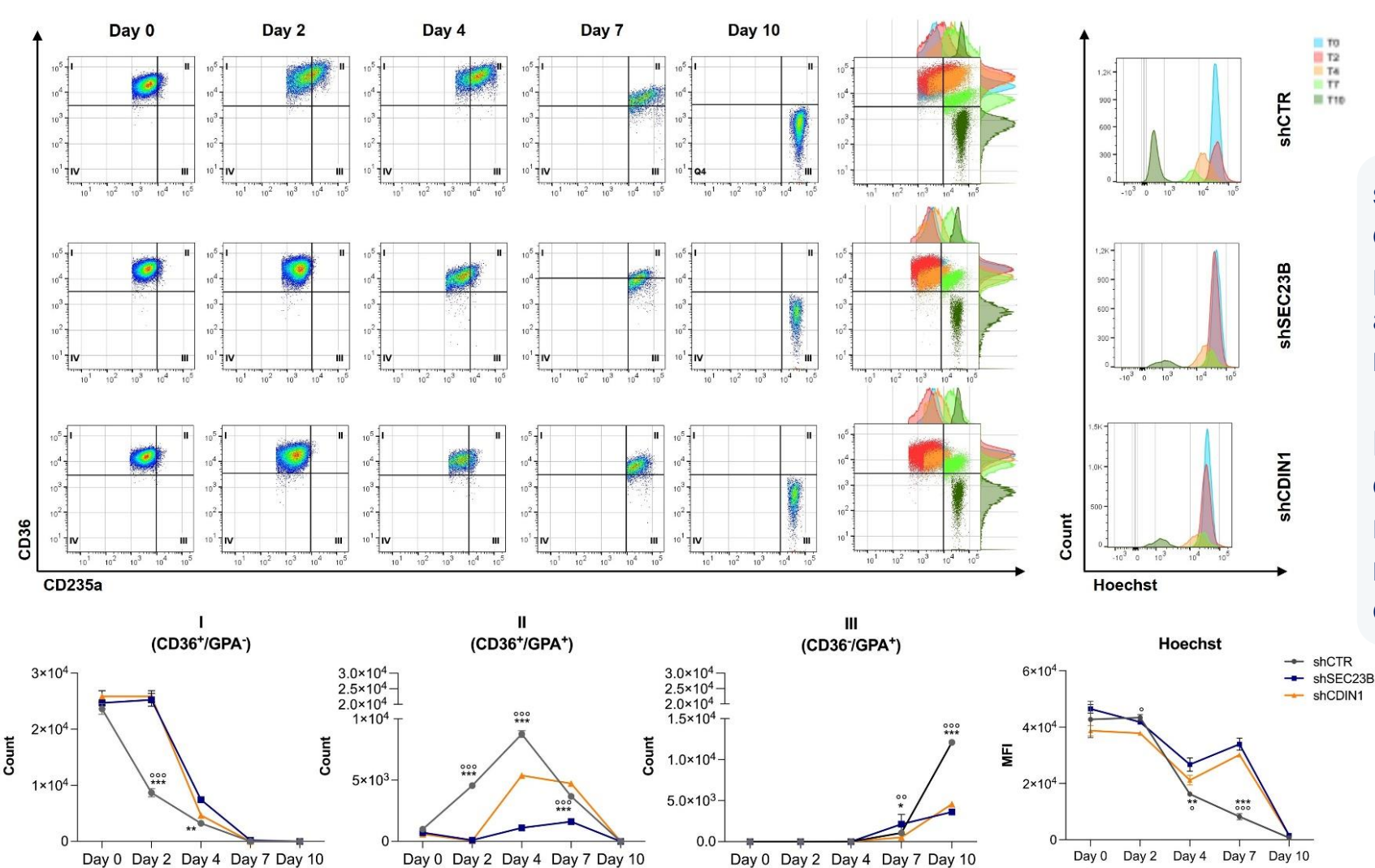


Multi-omics characterization



p<0.05, *p<0.01 shSEC23B vs sh-CTR; **p<0.05, ***p<0.01 shCDIN1 vs sh-CTR; Student's t-test

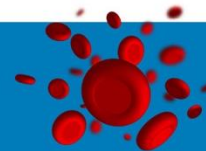
CDA models show delayed erythroid and nuclear maturation



shSEC23B and shCDIN1 cells showed delayed erythroid maturation, with **persistence of immature populations** and **reduced progression toward the mature CD36⁻/GPA⁺ compartment**.

Hoechst staining revealed **delayed loss** of nuclear signal in both knockdown models, supporting **defective nuclear maturation** during terminal erythroid differentiation.

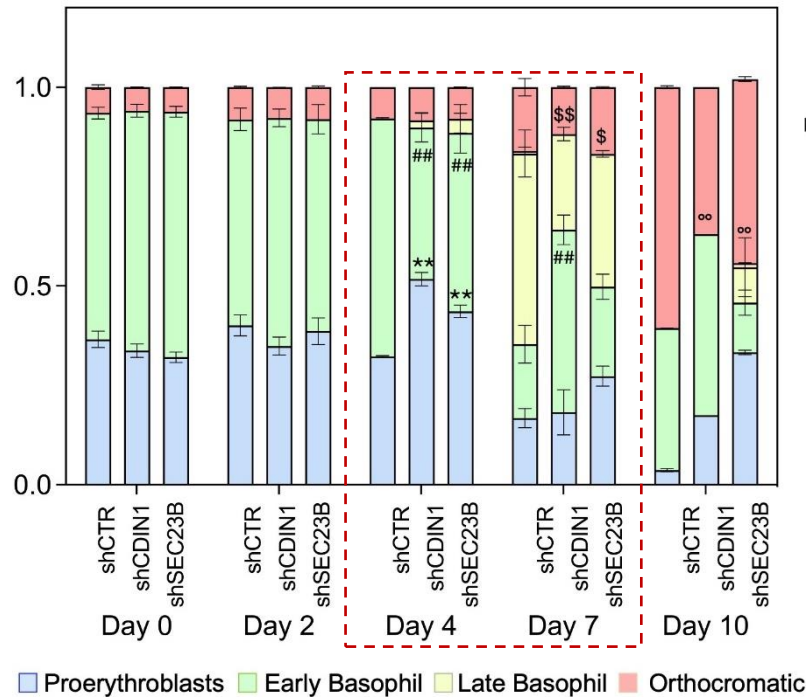
* p<0.05, **p<0.01, ***p<0.001 shSEC23B vs sh-CTR; *p<0.05 **p<0.01, ***p<0.001 shCDIN1 vs sh-CTR; two-way ANOVA



SEC23B and CDIN1 converge on a shared heme-stress response program



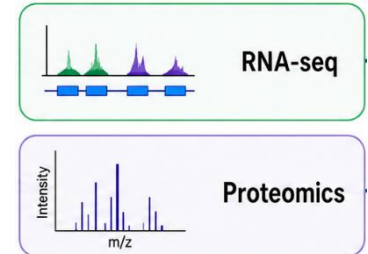
Deconvolution analysis



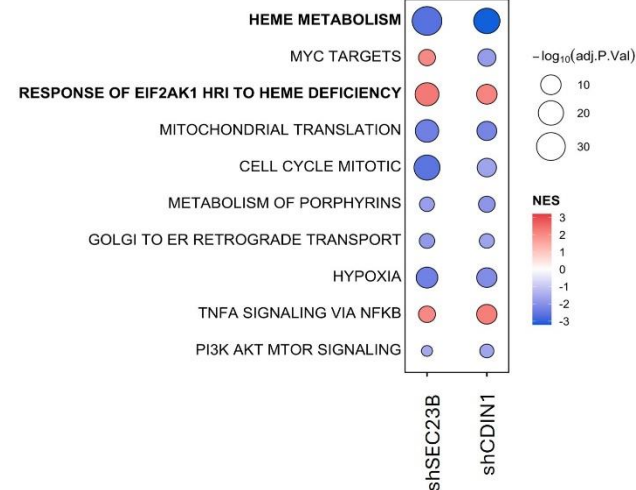
DAY 4 → DAY 7
RNA-PROTEIN MULTI-OMIC INTEGRATION
Differential analysis and pathway enrichment

Both CDA models showed a clear shift toward **less mature** erythroid populations.

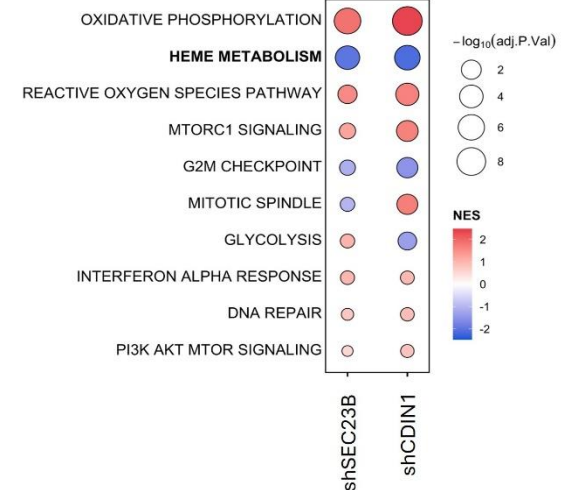
The most pronounced divergence from shCTR occurred during the transition from **days 4-7**.



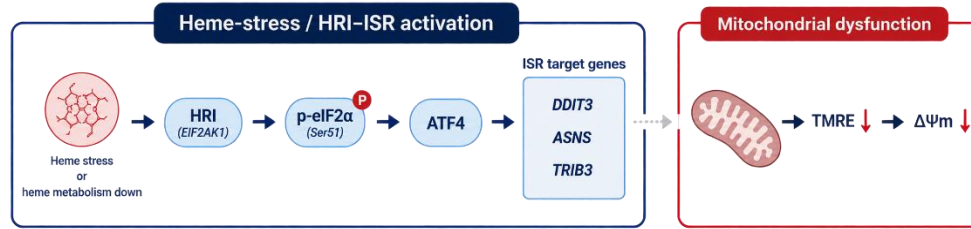
Transcriptomics



Proteomics

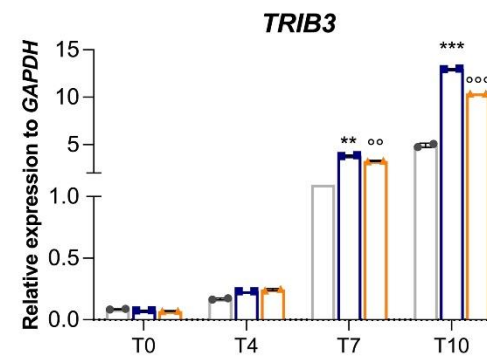
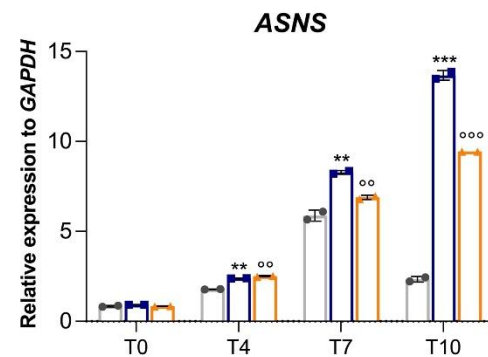
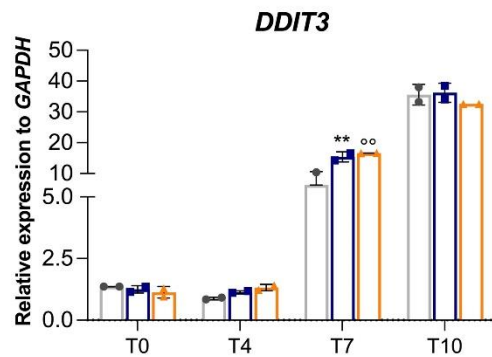
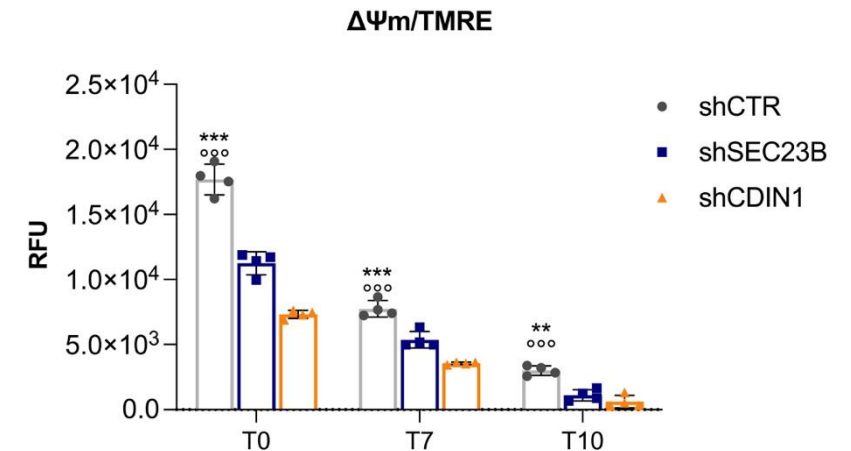
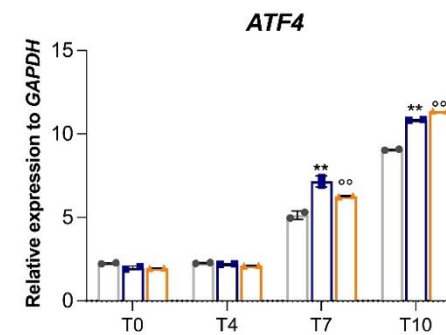
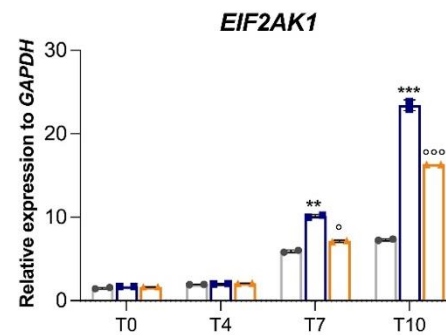
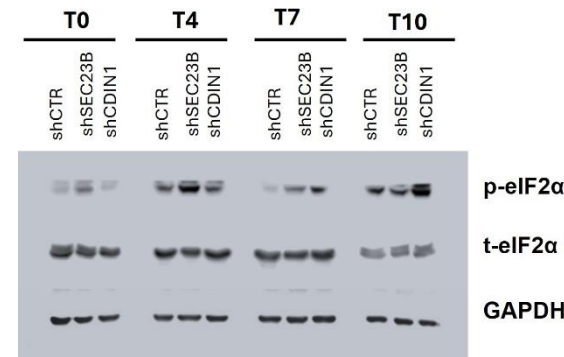


Targeted validation of HRI-ISR activation and mitochondrial dysfunction in CDA models



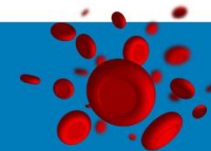
EIF2AK1 expression and **eIF2α phosphorylation** increased during erythroid differentiation in both CDA models.

ATF4 and downstream **ISR-responsive genes** were induced, supporting activation of the stress response program.



Reduced **TMRE fluorescence** indicated loss of mitochondrial membrane potential (ΔΨm) in shSEC23B and shCDIN1 cells.

* p<0.05, **p<0.01, ***p<0.001 shSEC23B vs sh-CTR; *p<0.05 **p<0.01, ***p<0.001 shCDIN1 vs sh-CTR; two-way ANOVA



Cell-intrinsic metabolic stress in CDA erythroid models

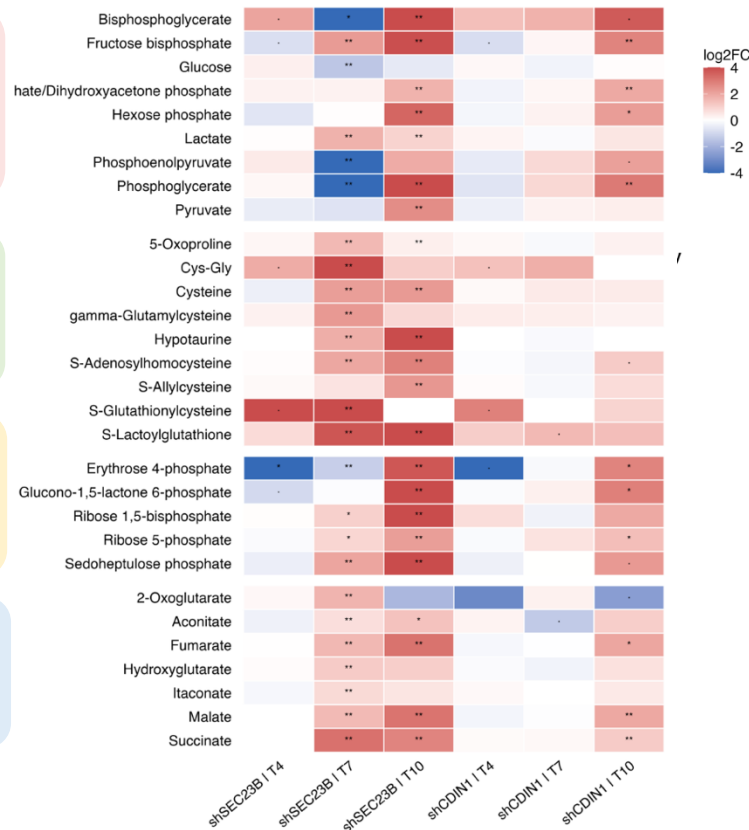


Glycolysis

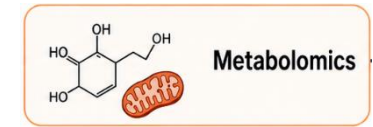
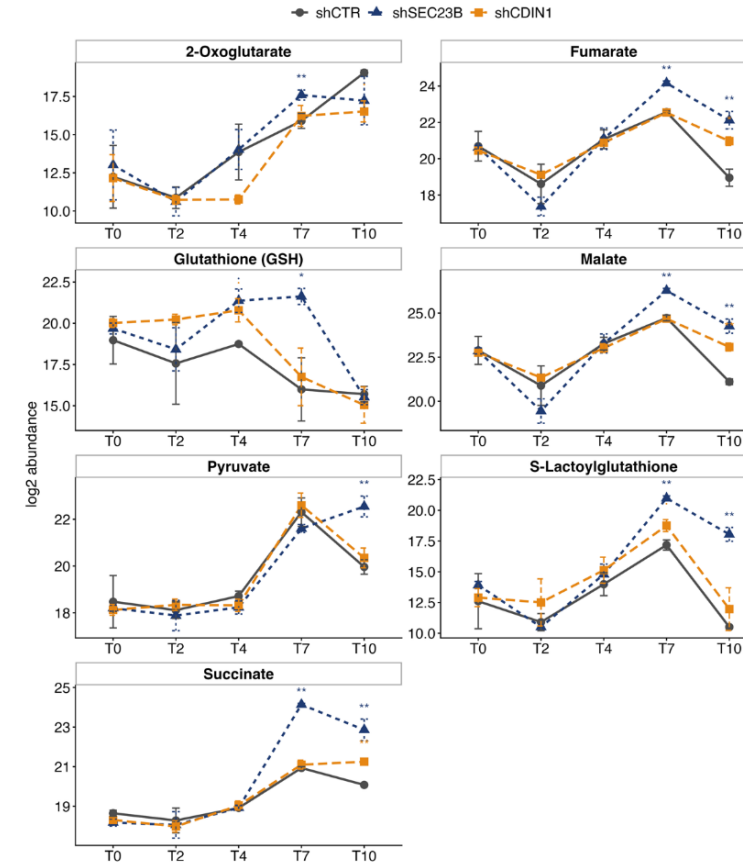
GSH/redox homeostasis

Pentose phosphate pathway

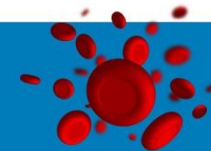
TCA/heme-linked carbon



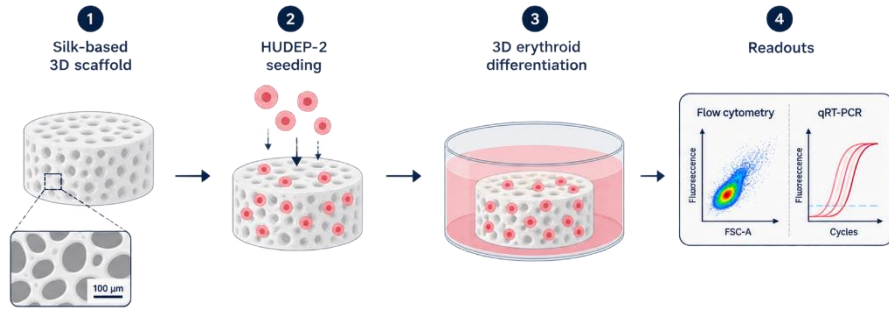
Differential metabolomics highlights coordinated alterations in **glycolysis, metabolism, GSH/redox homeostasis, and TCA/heme-linked carbon metabolism.**



Mitochondrial-associated metabolites, including **succinate, fumarate, malate, and 2-oxoglutarate**, showed differential dynamics during late erythroid differentiation.

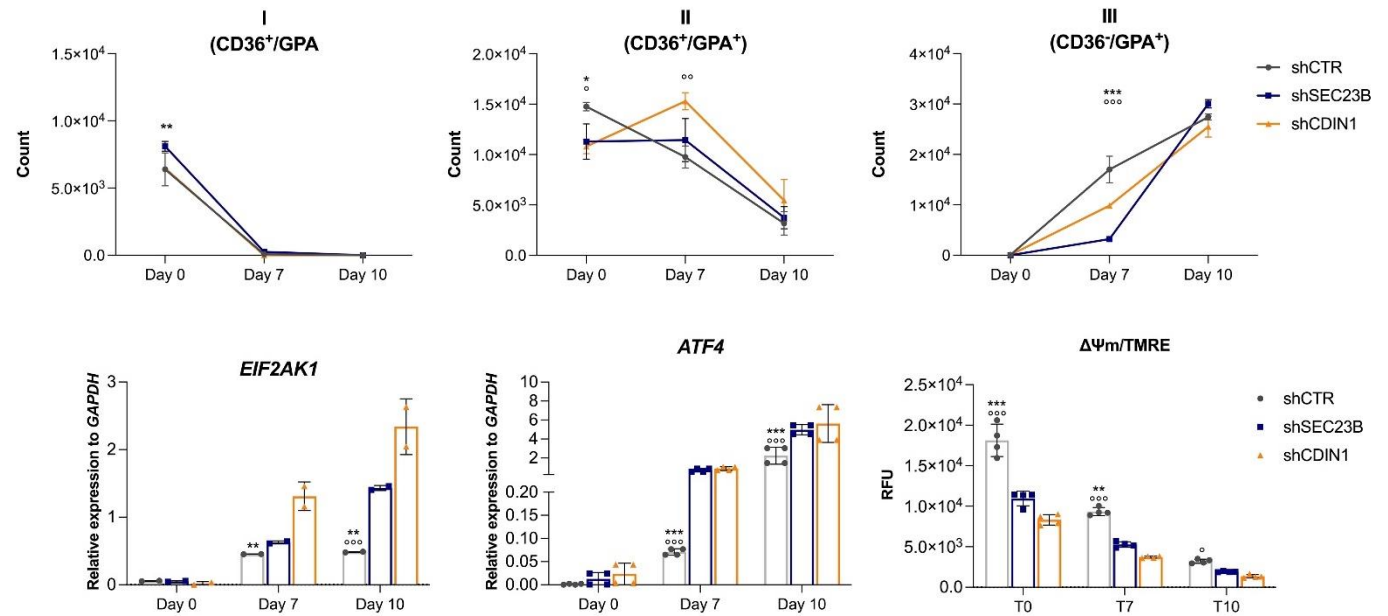
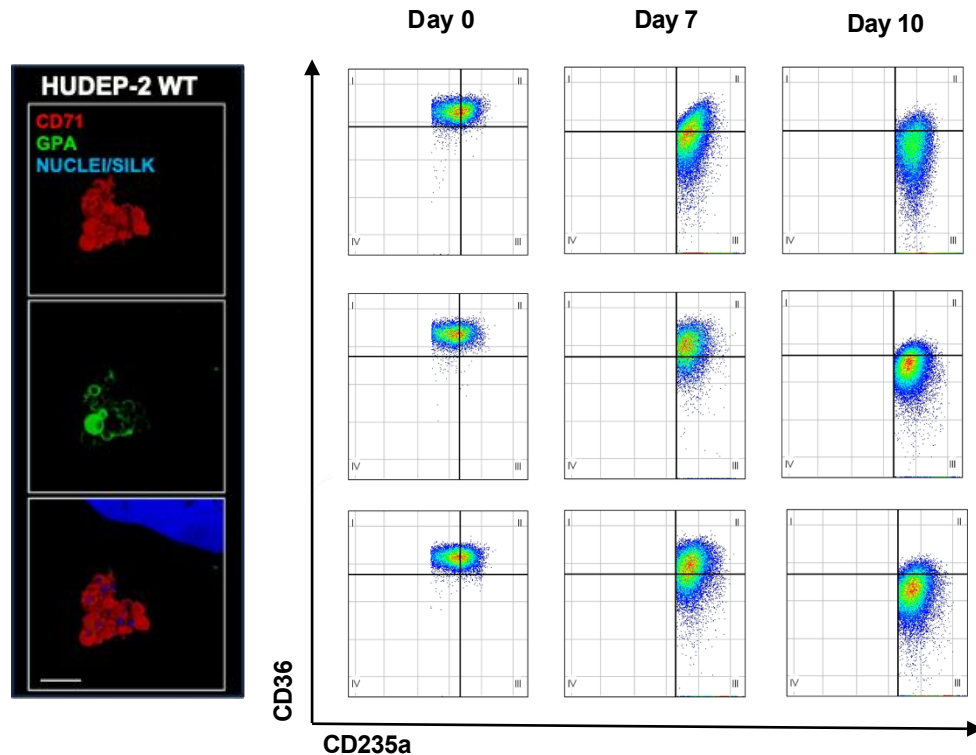


Silk-based 3D culture recapitulates delayed erythroid maturation in CDA models



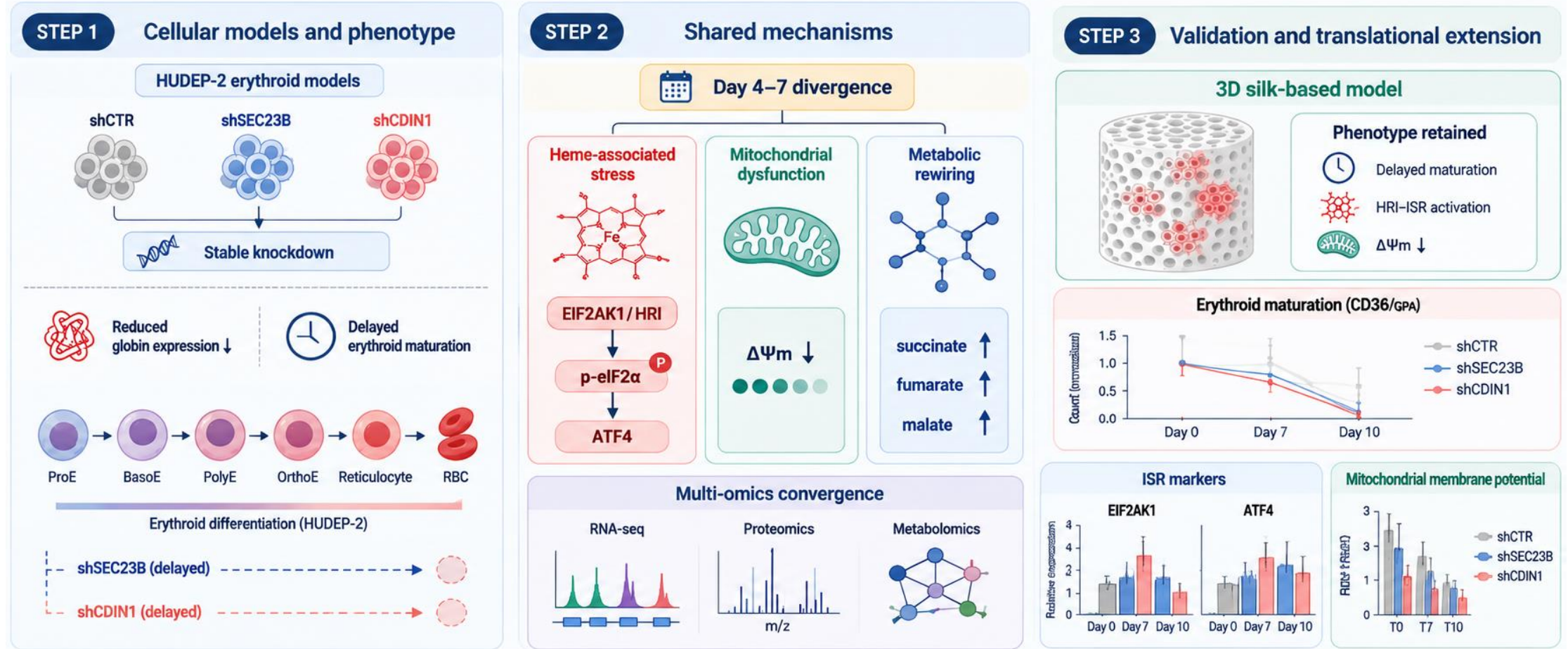
Both shSEC23B and shCDIN1 cells retained altered erythroid maturation kinetics in 3D, with **delayed progression** toward the CD36⁺/GPA⁺ compartment.

In addition to the maturation defect, 3D CDA models exhibited increased **EIF2AK1** and **ATF4** expression and reduced **mitochondrial membrane potential**.

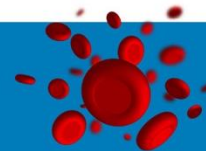


* p<0.05, **p<0.01, ***p<0.001 shSEC23B vs sh-CTR; *p<0.05 **p<0.01, ***p<0.001 shCDIN1 vs sh-CTR; two-way ANOVA

Conclusions



Distinct genetic defects converge on a shared stress-metabolic axis in CDA



Acknowledgments



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Chiara Maria

