ERN-EuroBloodNet Topic on Focus on Inherited Platelet Function Disorders (IPFD)

webinar

HEALTH PROFESSIONALS

Inherited thrombocytopenias associated with platelet dysfunction, NBEAL2 and others

Focus on thrombocytopenias with platelet granule abnormalities, using NBEAL2 as a key example of alpha granule deficiency.

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18 March 2026

CRPP Centre de Référence Pathologies Plaquettaires
MHEMO La Filière des maladies rares de l'hémostase
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Disclosure for conflict of interest

No conflicts of interest

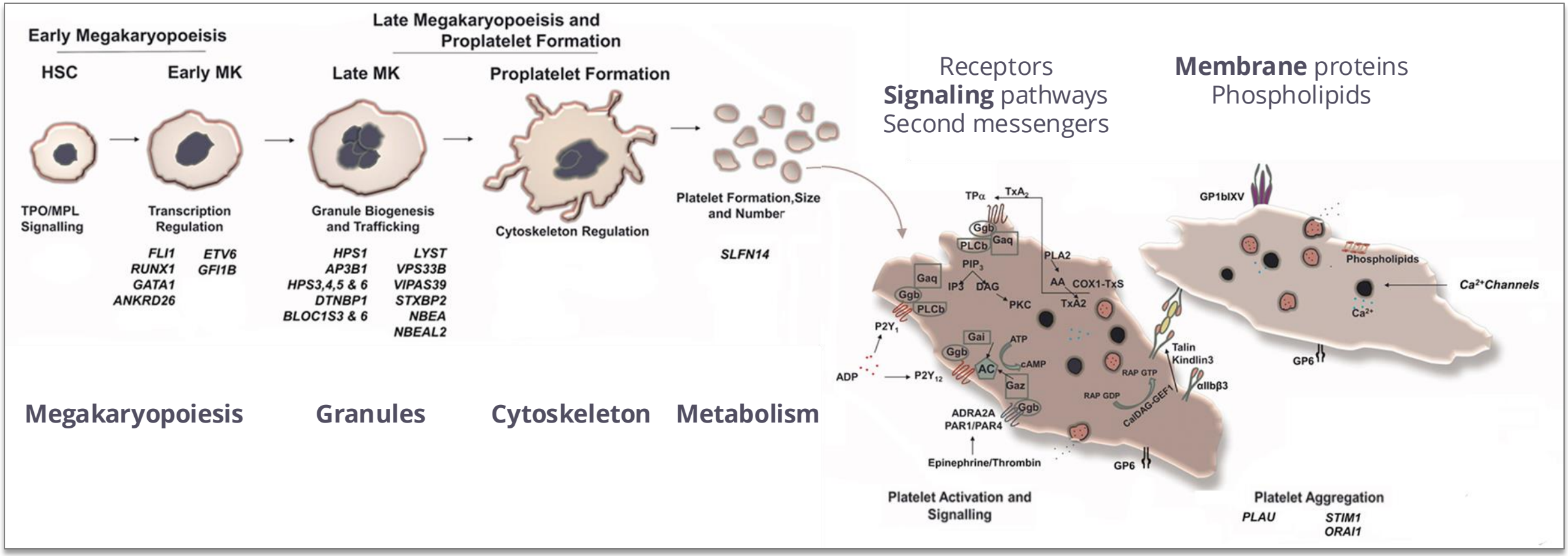
Inherited **platelet disorders, a rare breed of complexity**

- Highly **variable clinical presentation** from **mild bleeding to life-threatening**
  often underdiagnosed

- **Early diagnosis** is essential standardized bleeding assessment tools (ISTH-BAT)
  standardized laboratory evaluations (diagnostic and follow-up)
  because of hematological and/or extra-hematological consequences

- Major categories of inherited platelet disorders
  - Inherited thrombocytopenias (macro/normo/micro) → decreased platelet count
  - Inherited thrombopathies (receptor/signaling/granules/cytoskeleton) → functional defects
  - **Combined forms**

Rodriguez-Alen et al, Biomolecules 2025
Sanchez-Fuentes et al, Biomolecules 2025
Bourguignon et al, Crit Rev Clin Lab Sci 2022
Palma-Barqueros et al, Int J Mol Sci 2021



From Bourguignon 2022

01

Inherited thrombocytopenias associated with *platelet granule defects*

A heterogeneous spectrum

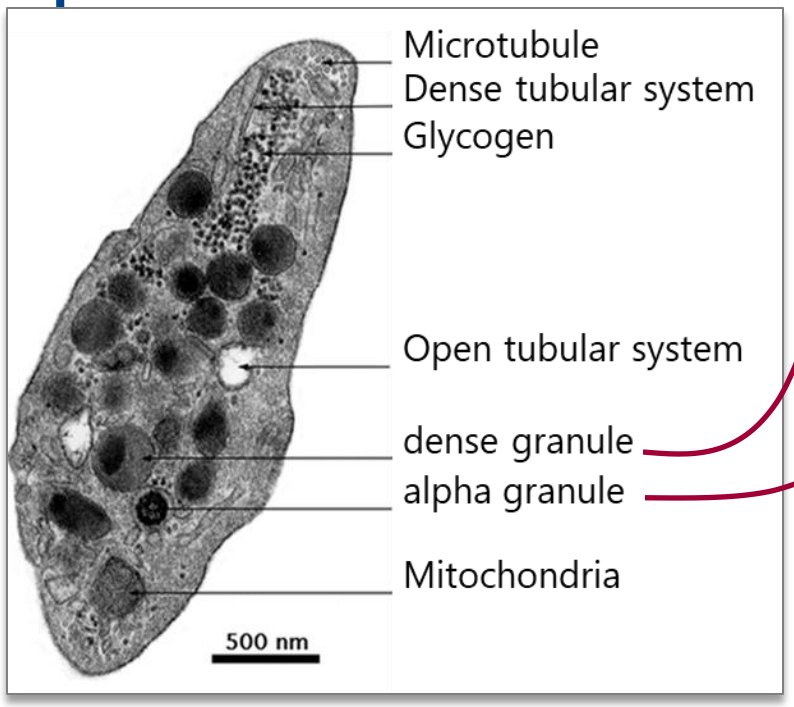
Inherited thrombocytopenias associated with platelet dysfunction, NBEAL2 and others…

- Some inherited platelet disorders are characterized by both **defective platelet production** and **platelet dysfunction**

- which involve transcription factors regulating **megakaryopoiesis** and/or proteins implied in **granule maturation, trafficking, cargo storage and/or secretion**

Intraplatelet vesicular trafficking: the long and winding road

- Platelet granule **biogenesis** is initiated in **megakaryocytes** and continues in circulating **platelets**



alpha granules
50-80 per platelet
>300 proteins derived from synthesis by megakaryocytes or plasma endocytosis
Adhesive proteins: VWF, fibrinogen, fibronectin, thrombospondin
Pro-angiogenic factors: PDGF, TGF1b, EGF, IGF
Metalloproteases (MMP2) and inhibitors (TIMPs)
Antifibrinolytics: PAI1, α2AP, TAFI, TPa
Coagulation (II, V, VII, XIII) and **anticoagulation factors** (antithrombin)

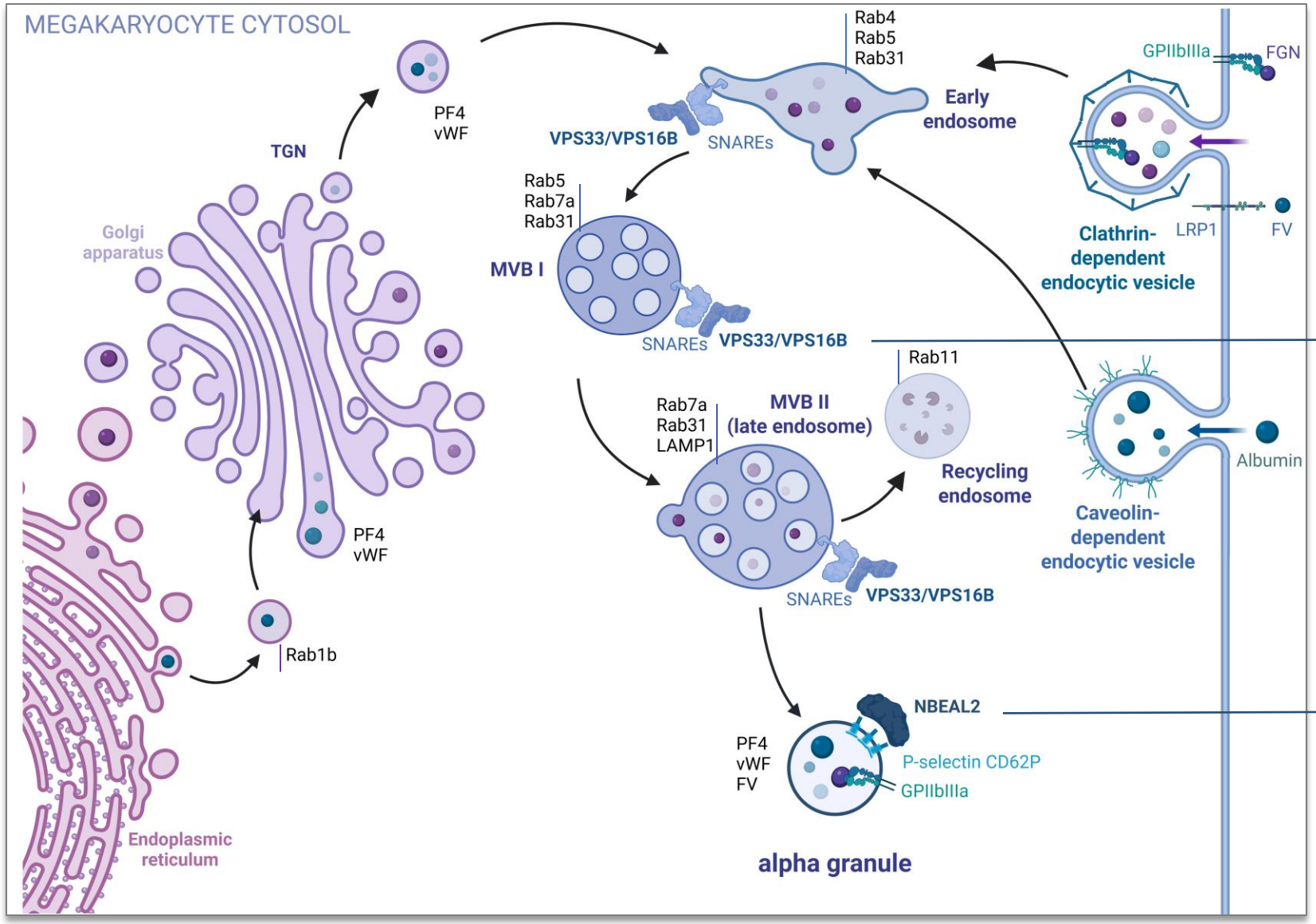
dense granules
3-8 per platelet, lysosome-related organelles (LRO), specific content
ADP (653mM) ATP (436mM) Serotonin (65mM)
Polyphosphates Ca^{2+} (2181mM)

Lysosome/peroxysome, 1-3 per platelet,

+/- T granules

- Rare platelet disorders (Gray platelet syndrome and Hermansky-Pudlak syndrome) provided key evidence **for distinct platelet granule biogenesis pathways**

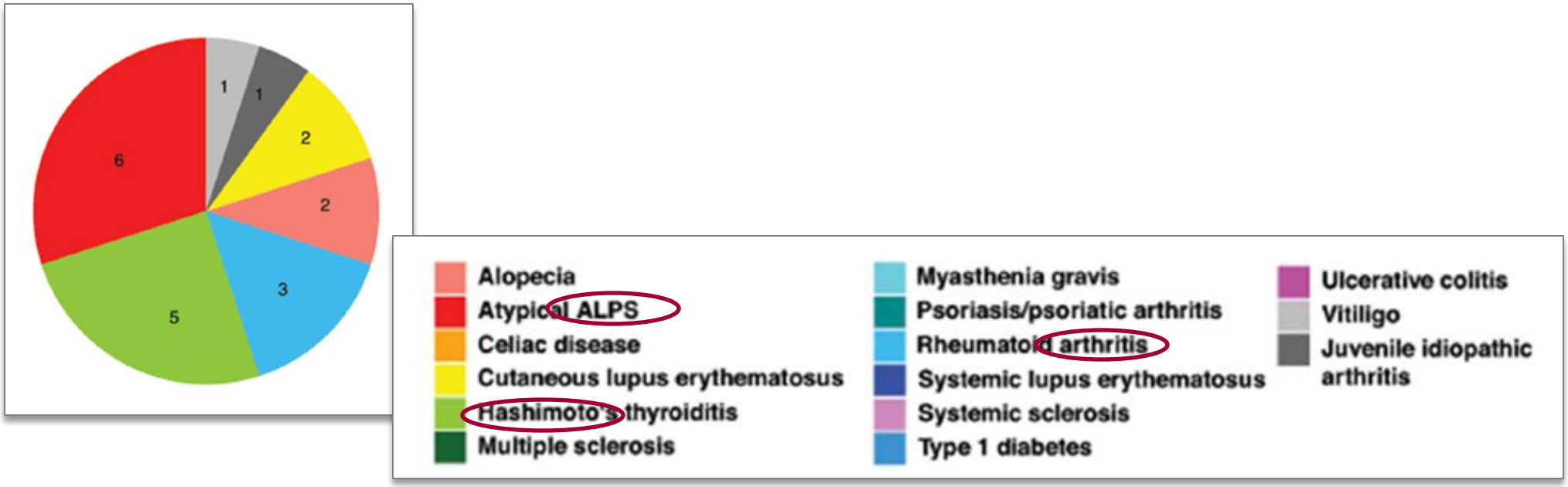
- **Alpha** granule biogenesis



Gray platelet syndrome, NBEAL2, Neurobeaching-like 2

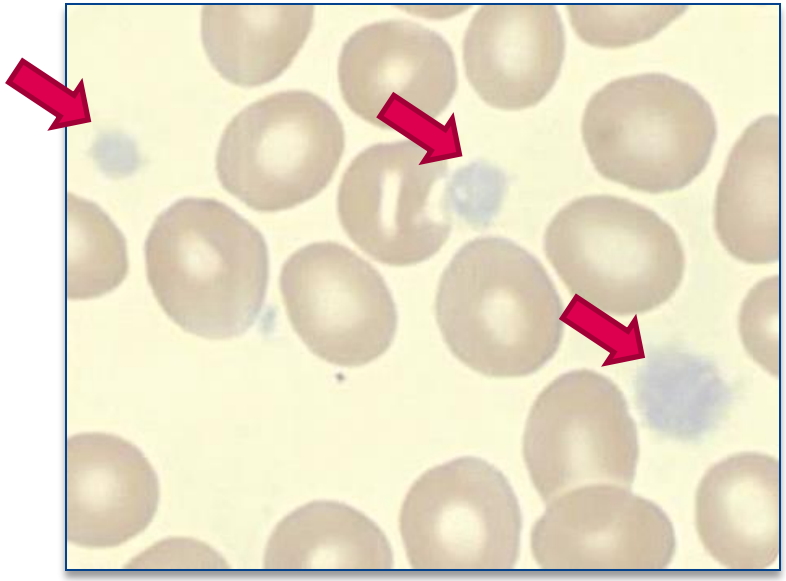
Autosomal recessive pattern

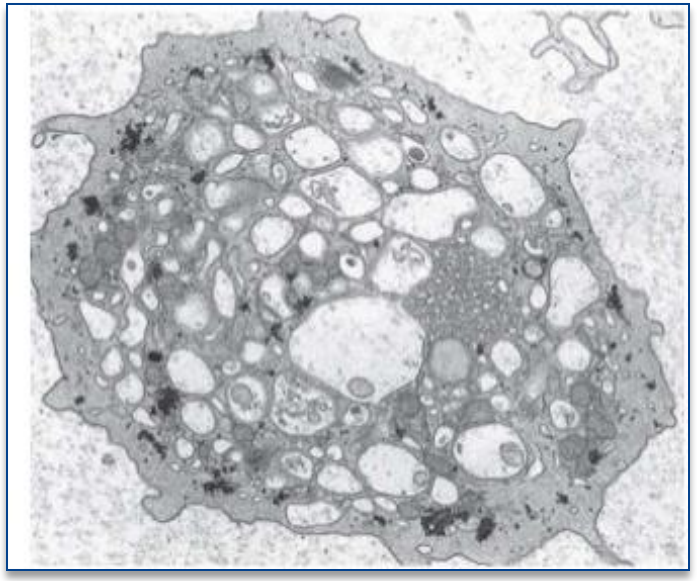
Autoimmune diseases (25%)



Collins et al, JTH

Gray platelet syndrome, NBEAL2, Neurobeaching-like 2





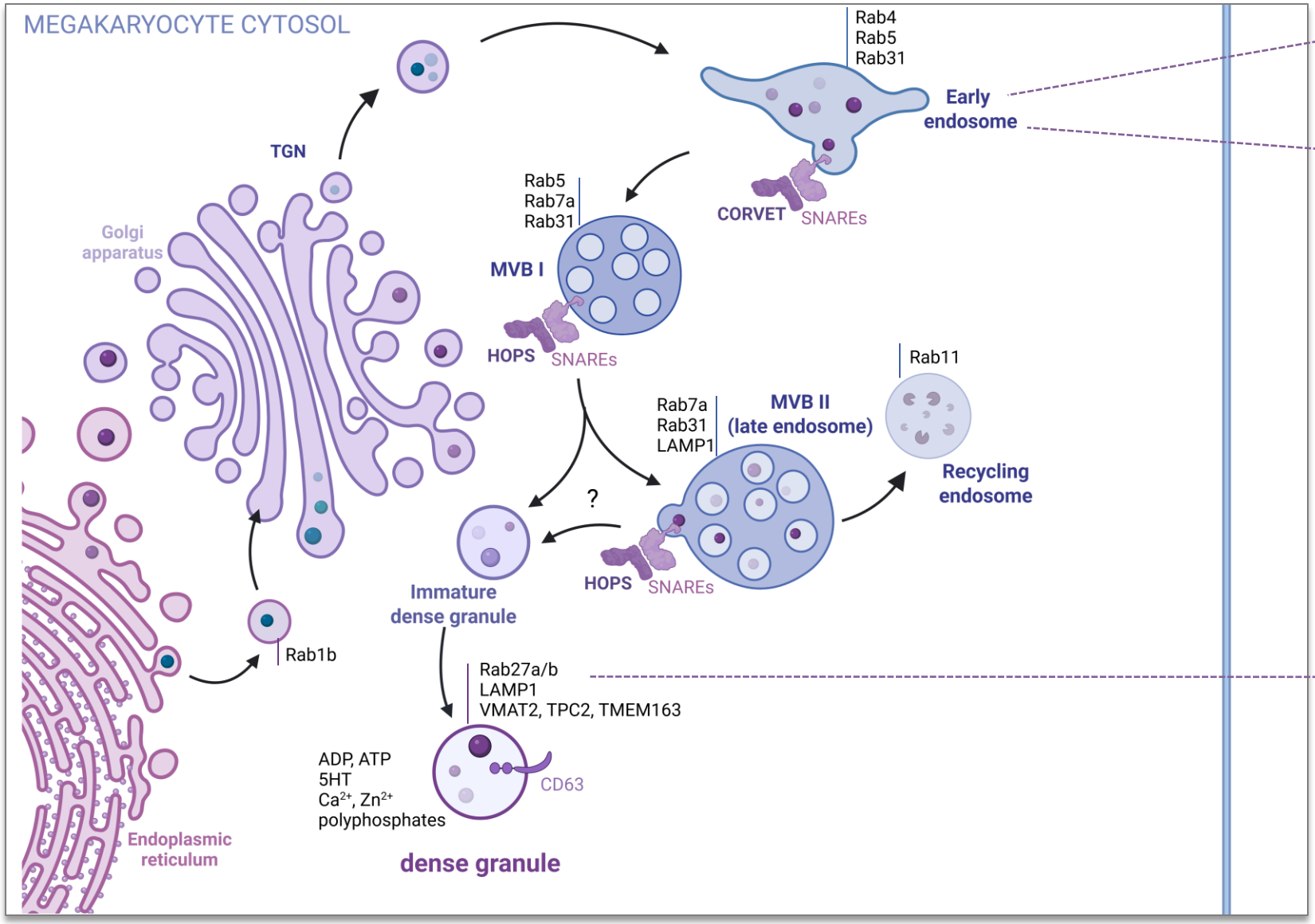
Required for **retention** of both endocytosed and synthesized cargo in maturing alpha granules

Associates with P-selectin to **stabilize** alpha granule cargo

→ **NBEAL2 loss** redirects cargo to Rab11-dependent recycling pathways thereby contributing to bone marrow **remodeling**

Lo et al., ATVB 2018 - Glembotsky et al., Journal of Blood Medicine

- **Dense** granule biogenesis



Hermansky-Pudlak Syndrome, BLOC/AP

Chediak-Higashi Syndrome, LYST

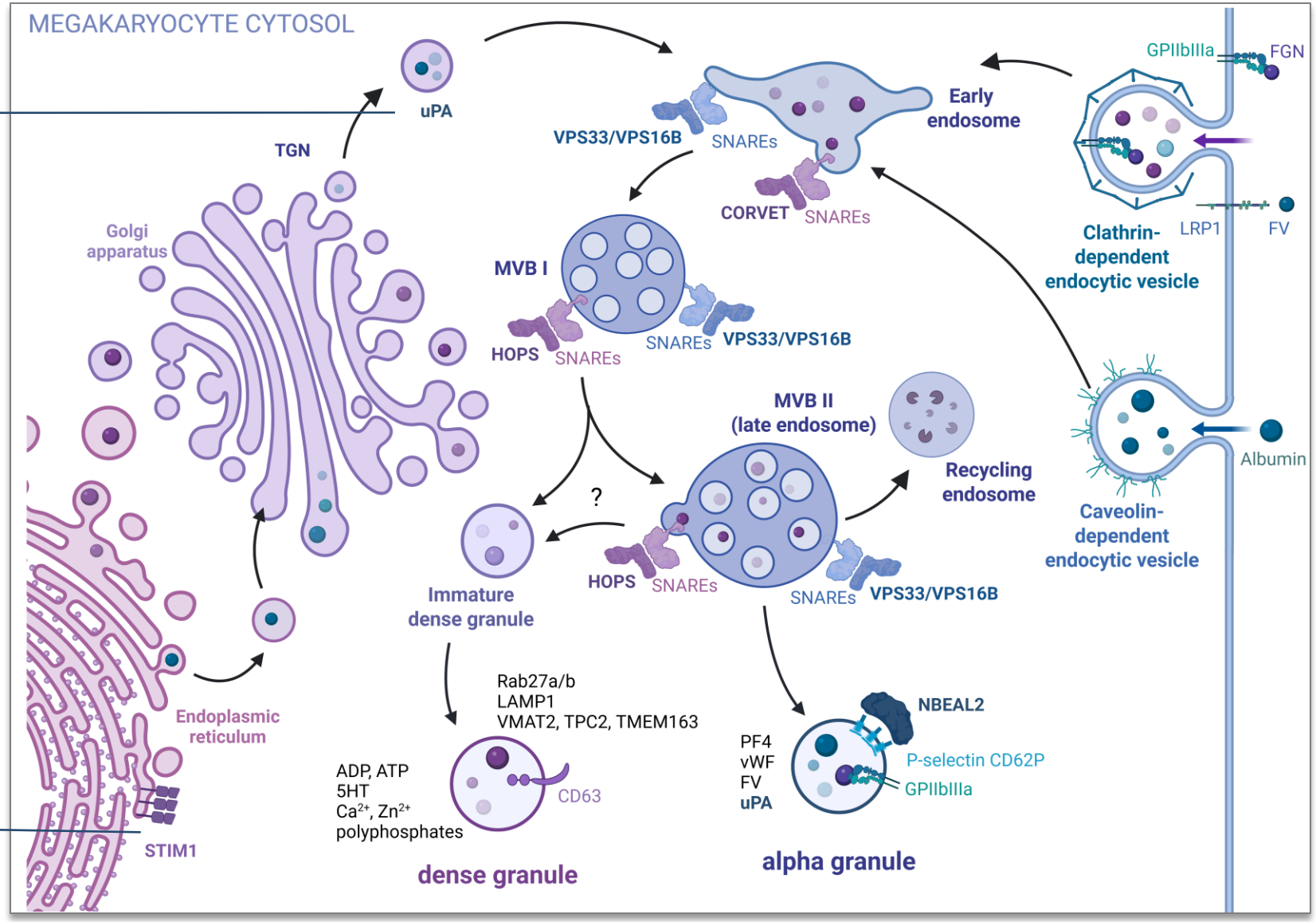
Griselli Syndrome, Rab27a

No thrombocytopenia

- **No specific** granule biogenesis or trafficking defect

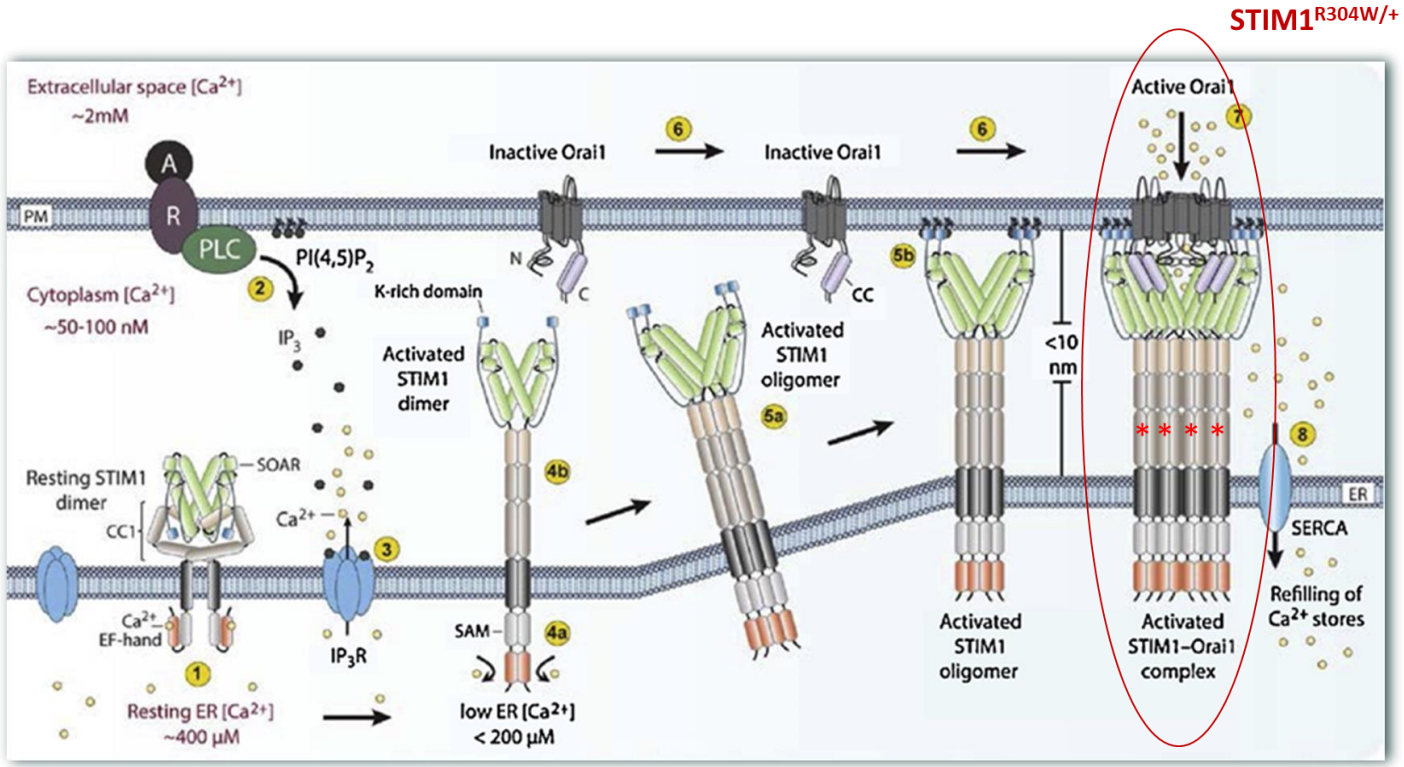
Quebec Syndrome, PLAU

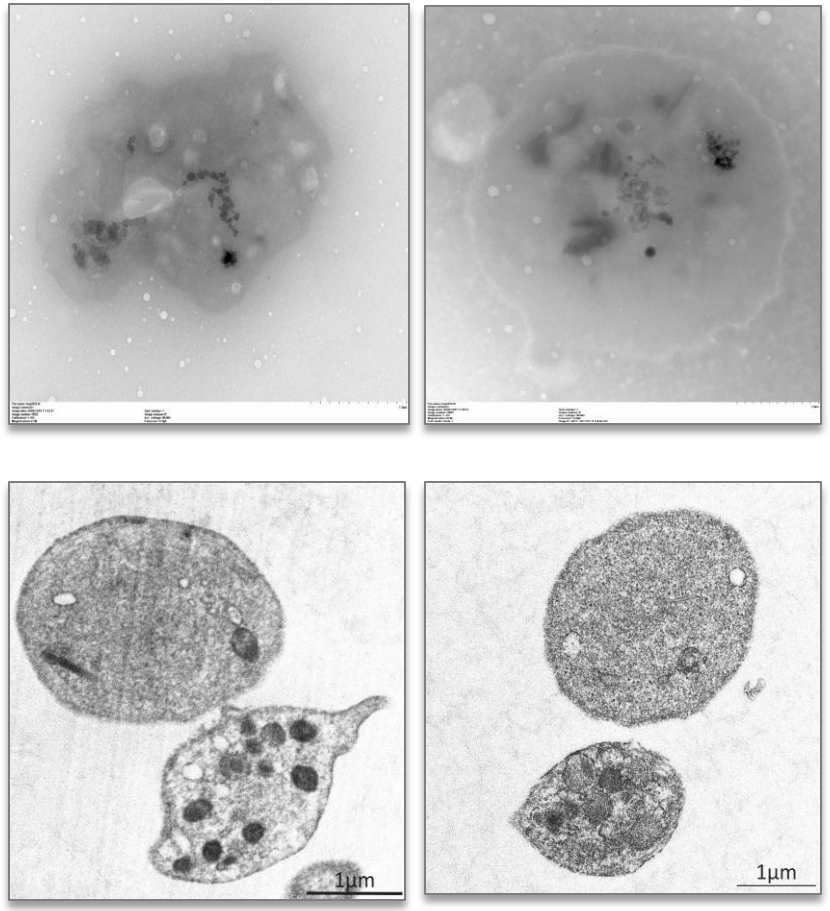
Stormorken Syndrome, STIM1



Stormorken syndrome, STIM1 → megakaryopoiesis

Autosomal **dominant**
STIM1 11p15; c.910C→T; p. R304W

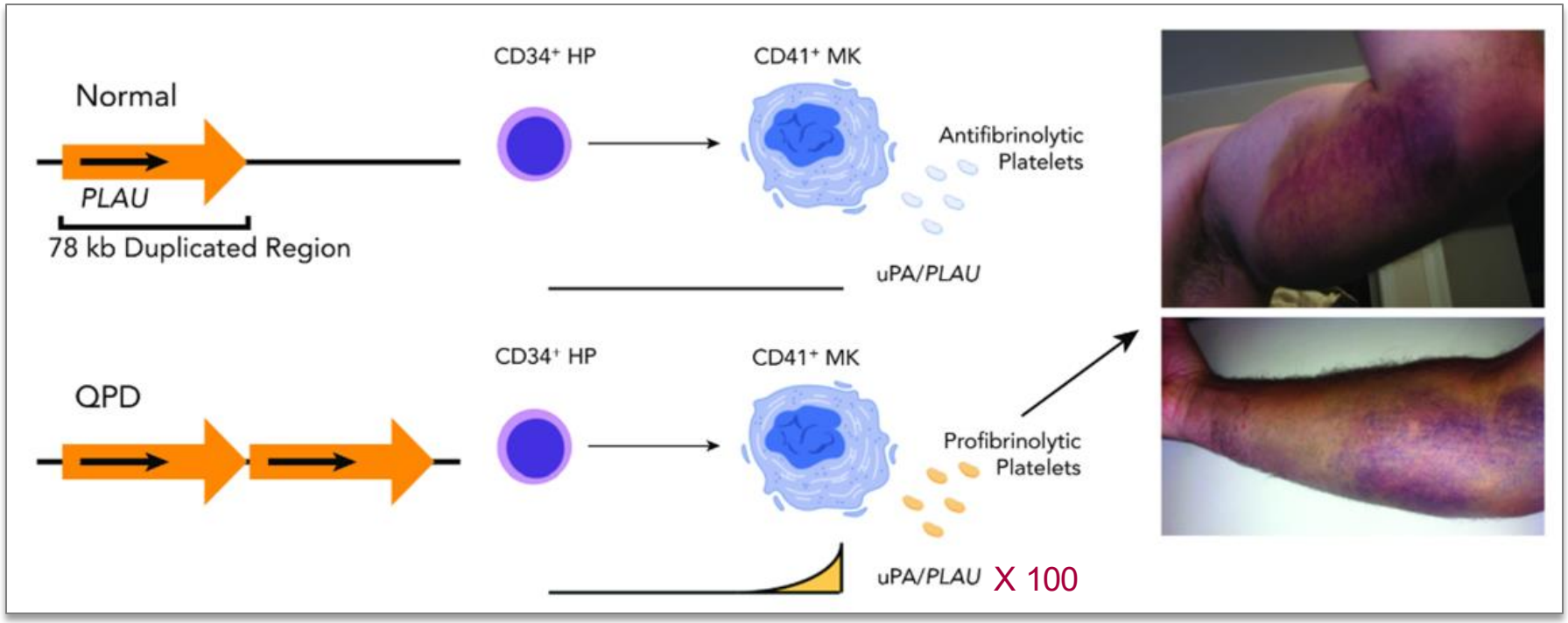




Bhardwaj et al. Cell Calcium

Quebec syndrome, PLAU → intraplatelet hyperfibrinolysis

Autosomal **dominant**
78 kb duplication-insertion of *PLAU* on 10q



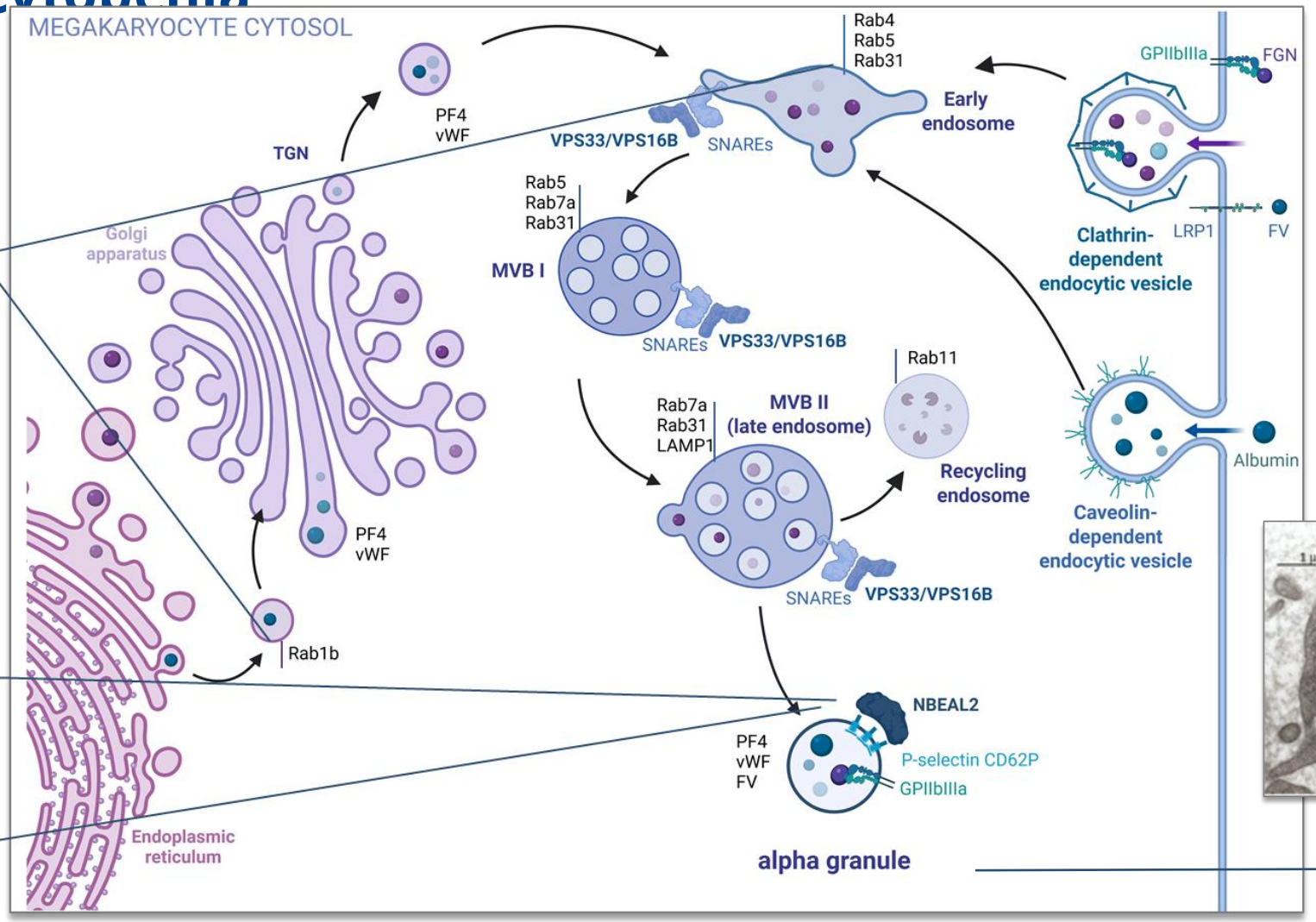
Liang et al, Blood 2020

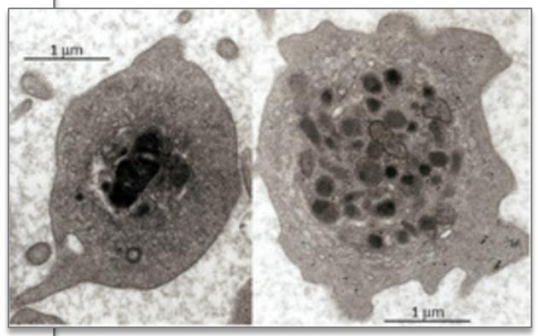
Germline mutations drive granule defects in hereditary thrombocytopenia

RUNX1 haploinsufficiency

Pseudo-Gray Platelet Syndrome, GFI1B

X-linked thrombocytopenia and dyserythropoiesis, GATA1

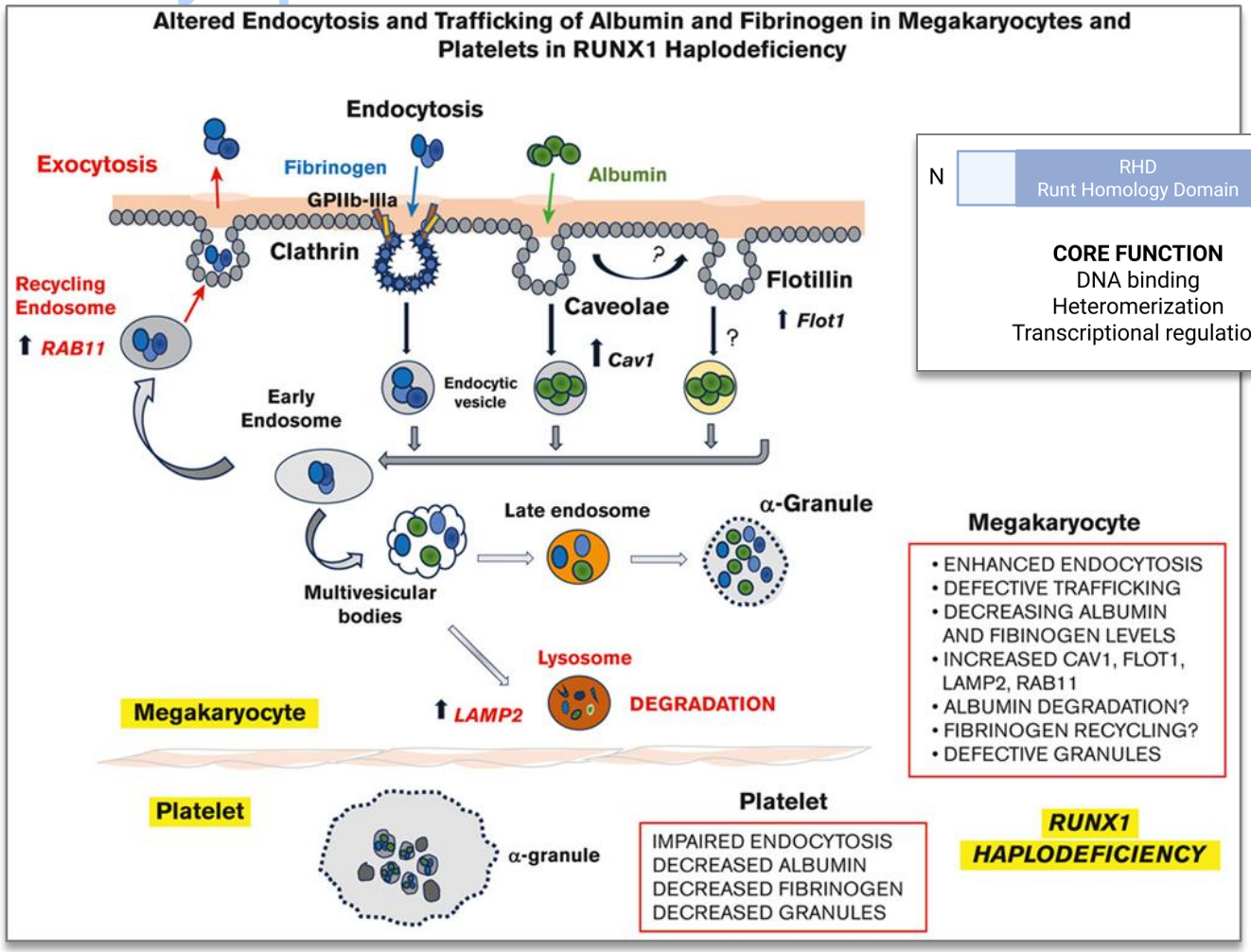


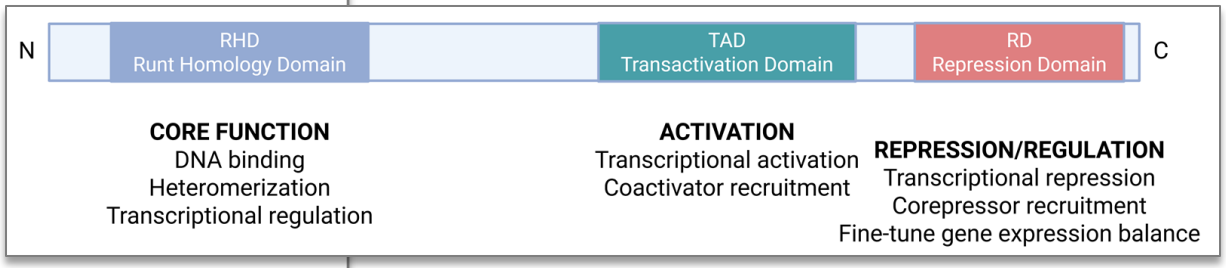


Paris-Trousseau Syndrome, Fli1

Germline mutations drive granule defects in hereditary thrombocytopenia

- **RUNX1**





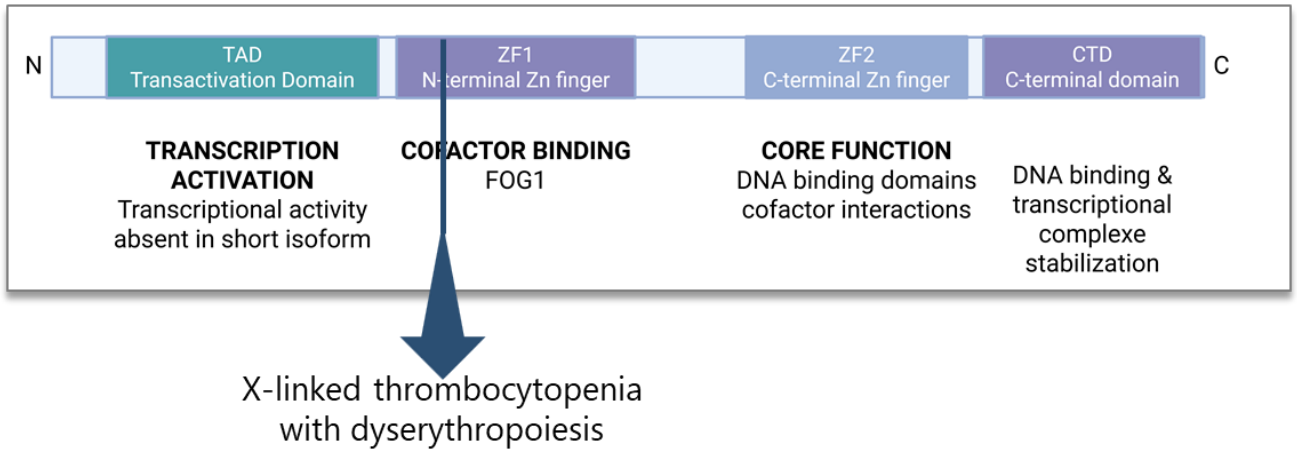
Rab1b
Rab31

No evidence supporting an association between a specific RUNX1 mutation and a distinct granule defect

Del Carpio-Cano et al, Blood Advances 2024

Germline mutations drive granule defects in hereditary thrombocytopenia

- **GATA1** important for NBEAL2 expression
  distal enhancer located about 31 kb upstream of the *NBEAL2* gene



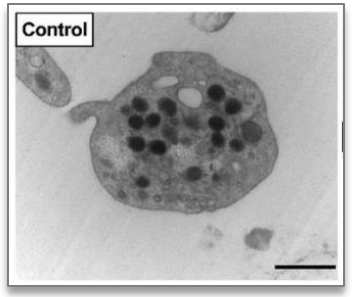
« Gray Platelet Syndrome » → pseudo-Gray Platelet Syndrome

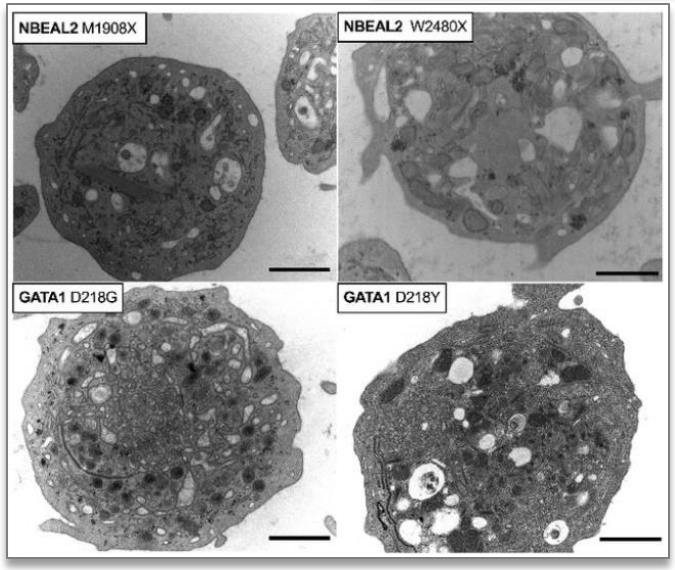
Wijgaerts et al, Haematologica 2017

X-linked thrombocytopenia with dyserythropoiesis, GATA1 Pseudo-Gray Platelet Syndrome

	NBEAL2 *MIM13909*		GATA1 *MIM300367/314050*	
Mode of inheritance	Autosomal recessive		X-linked	
Genetic defect	Homozygous c.7440G>A	Homozygous c.5721-1G>C	c.653A>G	c.652G>T
Protein defect	W2480X	Predicted M1908X	D218G	D218Y
Dyserythropoiesis	No		Yes	Yes + anemia
Myelofibrosis	Yes	No	No	Yes
Macrothrombocytopenia	Yes		Mild TP	Severe TP
α-granule defect as determined by EM	Yes		Yes	
Megakaryocytic emperipolesis	Yes	ND	Yes	
Plt count, x 10^9/L*	40	55	53	8
MPV, fL	> Max value	> Max value	> Max value	> Max value
Bleeding severity	Mild to moderate		Mild	Severe
Defective platelet aggregation	ND	Performed at low plt count in PRP: Impaired ADP, collagen, arachidonic acid, and ristocetin-induced plt aggregration	Performed at low plt count in PRP: Impaired collagen, and ristocetin- induced plt aggregation	ND

Normal platelet count should be between 150-400 x 10^9/L. ND: not determined; EM: electron microscopy; Plt: platelet; MPV: mean platelet volume; PRP: platelet rich plasma; ADP: adenosine diphosphate; TP: thrombocytopenia.

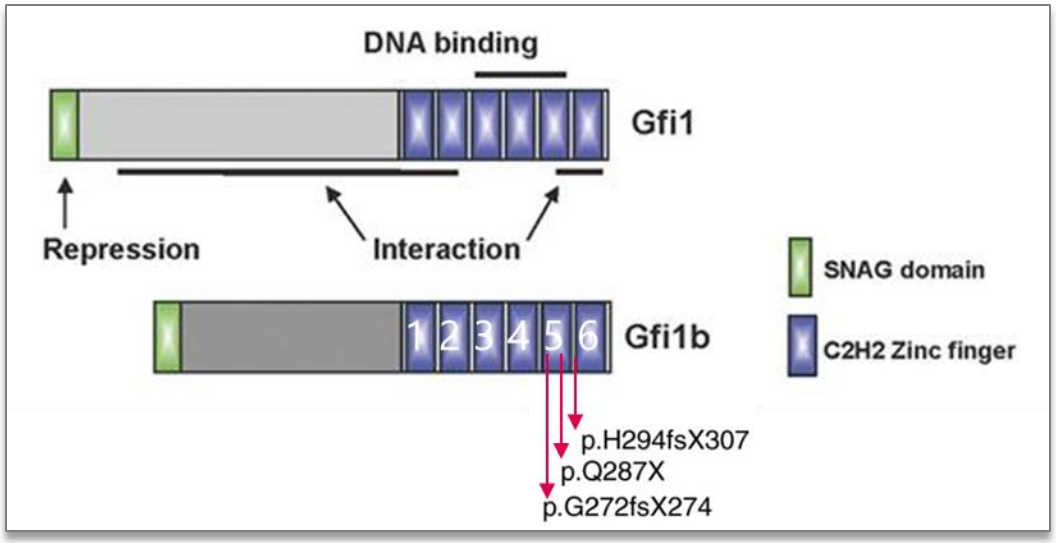


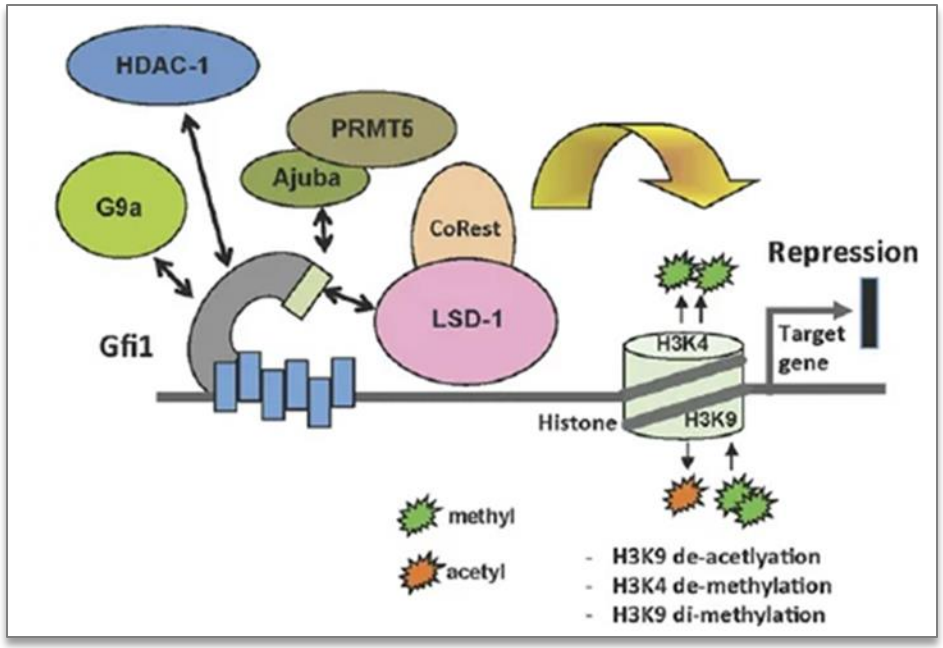


Wijgaerts et al, Haematologica 2017

Germline mutations drive granule defects in hereditary thrombocytopenia

- **GFI1B** regulates megakaryocyte (MK) differentiation by recruiting a corepressor complex
  Dominant-negative GFI1B variants sequester LSD1 → transcriptional deregulation
  Increase in MK **proliferation** and maturation defect → **secondary** granule loss

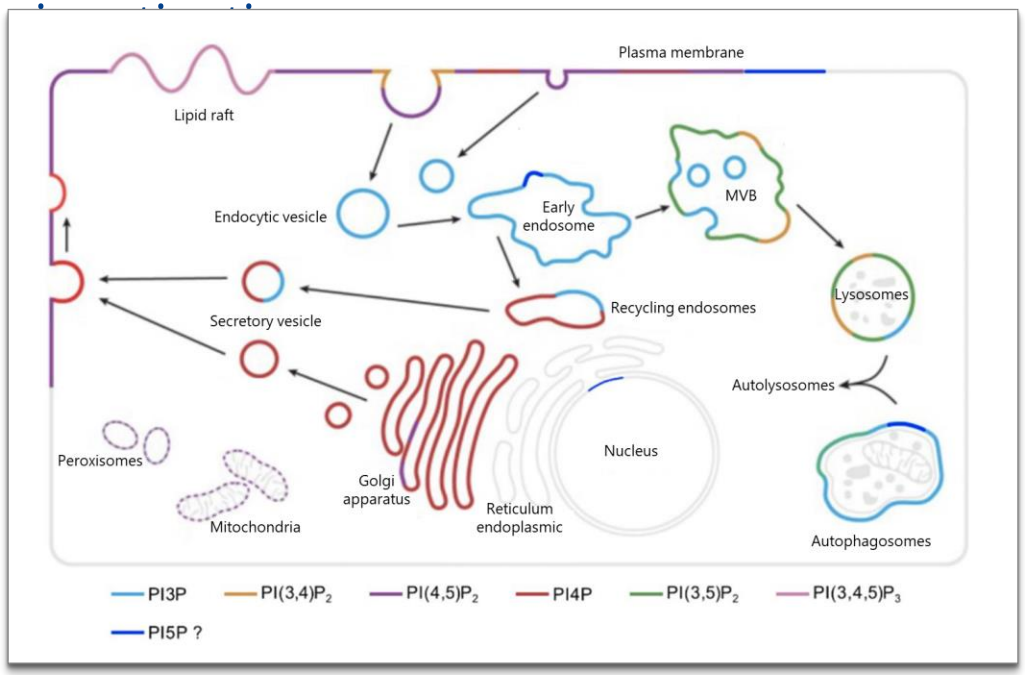


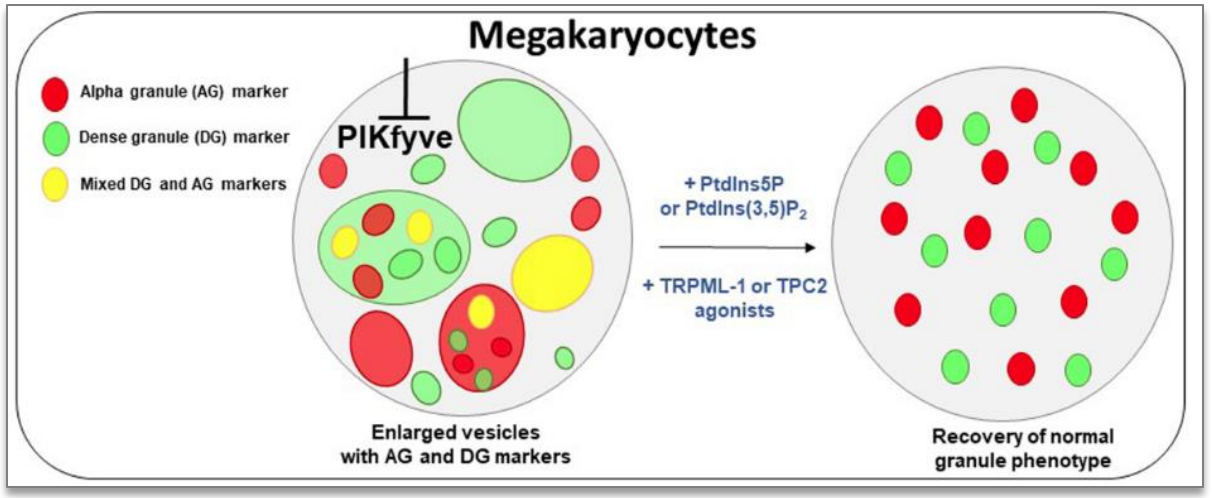


From Möröy et al, Blood 2015 - Oorschot et al, Haematologica 2019

Platelet membranes: fluidity and dynamics

- Beyond protein specificities, these organelles also exhibit a **distinct lipid membrane identity**
- **PtdIns** → cell signaling, cytoskeletal remodeling, DNA synthesis and cell cycle, intracellular trafficking
- PIKFyve PtdIns(3,5)P2 production → loss of alpha and dense granules identity upon



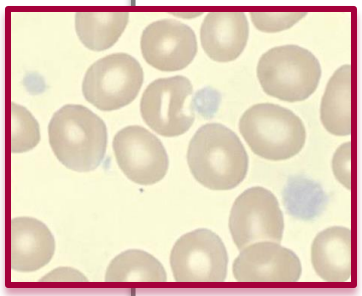


Valet et al. Res Pract Thromb Haemost 2020 - Caux et al. ATVB 2022

02

Beyond genetics: investigating platelet granule defects

Clinical presentation guides genetic testing; functional assays refine gene panel selection



Sanchez-Fuentes et al, Biomolecules 2025

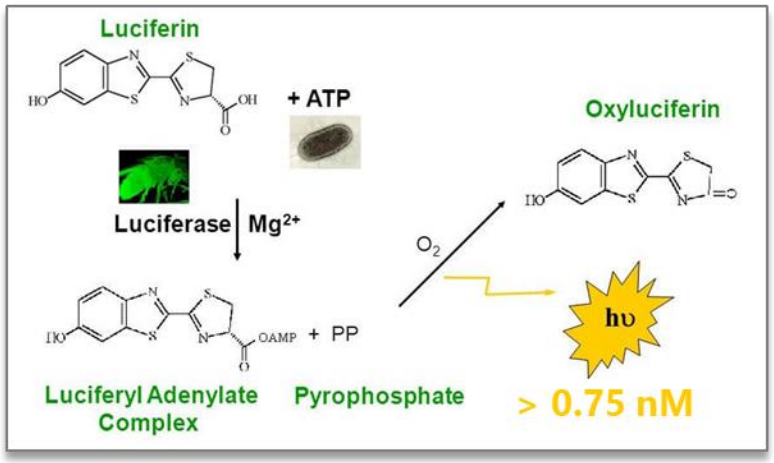
First clues: suspecting a platelet granule defect

- **Light Transmission Aggregometry (LTA)**
  - on platelet-rich plasma (PRP)
  - +/- low-dose agonists

  → Reversibility ? Aggregation intensity ?

ADP 2.5 – 5 – 10 µM
Collagen 3.3 µg/mL
Epinephrin 5 µM
Ristocetin 0.5 – 1.25 mg/mL
AA 1 mM

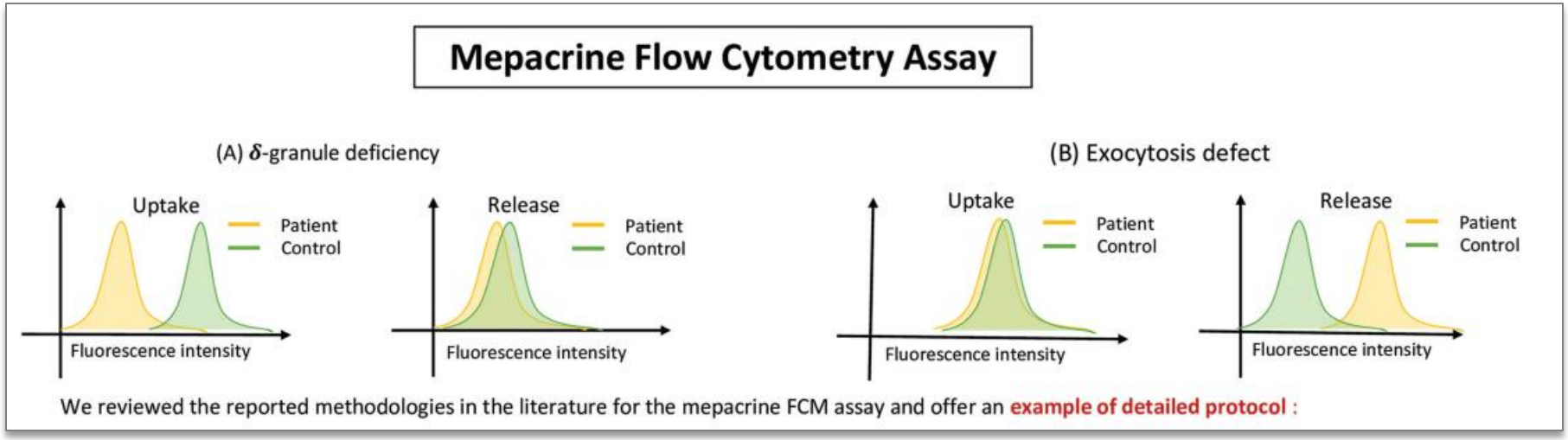
- **Luminoaggregometry**



- Agonists ADP 10 µM Collagen 3.3 µg/mL
  TRAP 25 µM TxA2 5 µM
- Dense granules only
- No distinction: secretion *versus* content defects

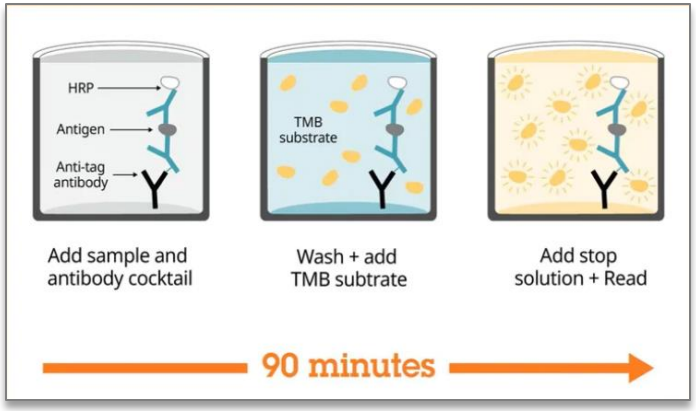
Confirmatory investigations

- **Mepacrine tests**
  - In living cells
  - Dense granules only



  - Distinction secretion *vs* content defect
  - **Avoid** DAPI and Thiazole Orange (TO) staining → readily accessible but **non-specific** (strong poly/pyrophosphate stainings)

- **ELISA tests** for 5HT, PF4, cytokines, etc…
  - Sandwich ELISA, HRP, TMP, DO measure



Khourssaji *et al.*, Thromb Haemost

Confirmatory investigations

- **Flow cytometry** assessment of platelet **alpha (CD62P)** and **dense (CD63)** granule secretion under **static conditions**
  - ADP 10 µM
  - TRAP14 50 µM
  - TxA2 analog U46619 5 µM
  - CRP-A 5 µg/mL

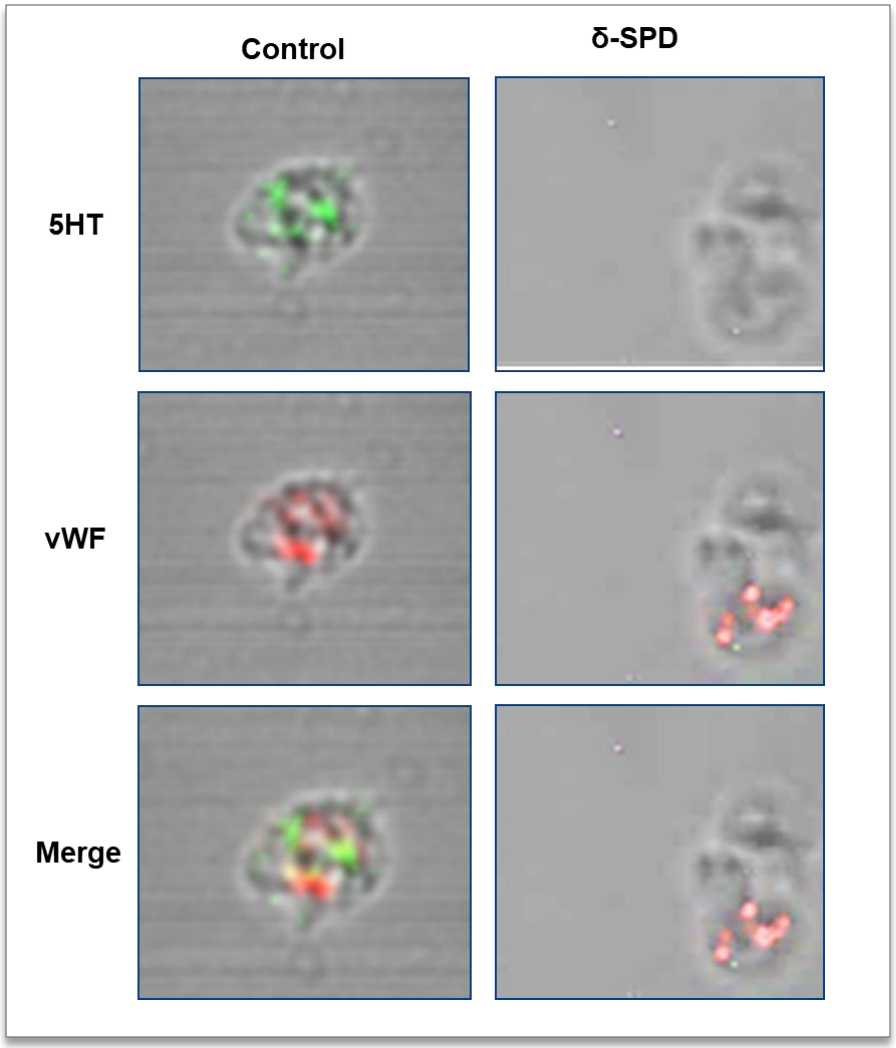
  → **Quantitative** assessment

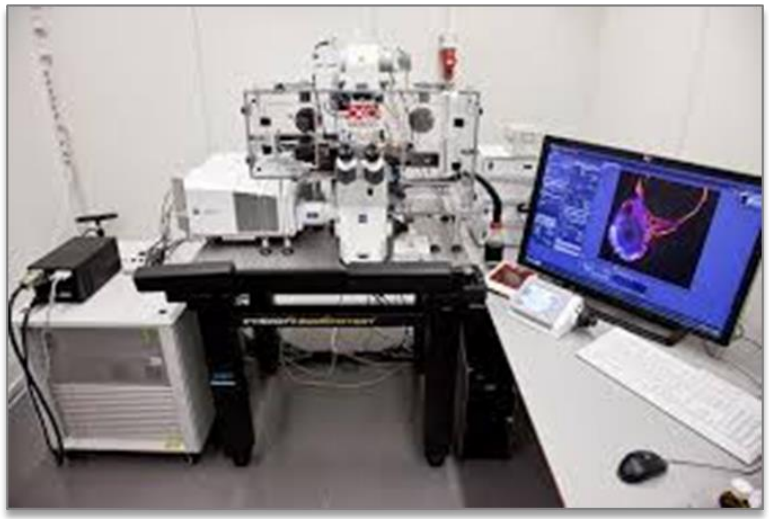
  → **Differentiate** alpha and dense granule defects

  → **Basal** granule content assessable after platelet permeabilization

- **Western blot analysis** of proteins involved in vesicular trafficking or membrane fusion (SNARE, VAMP, etc…)

Super-resolution characterization: confocal microscopy

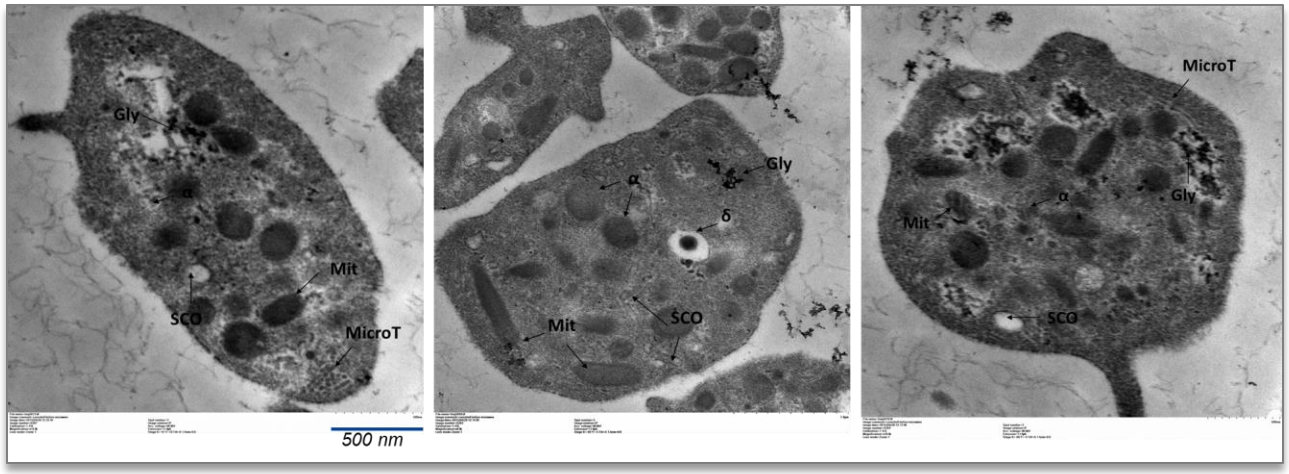


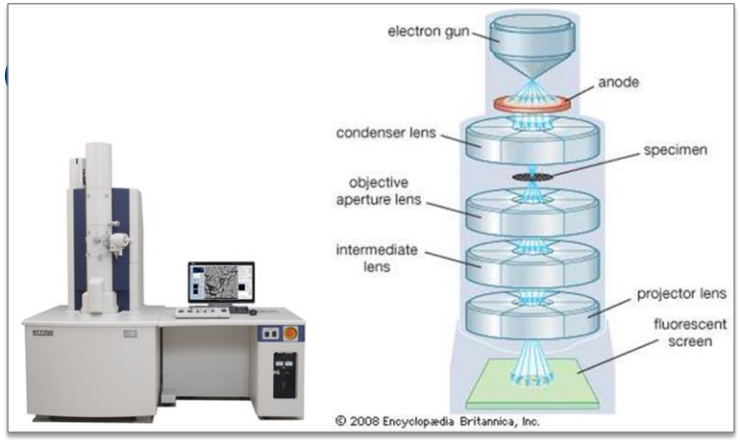


LSM900 ZEISS Microscopy

Ultrastructural characterization: electron microsc

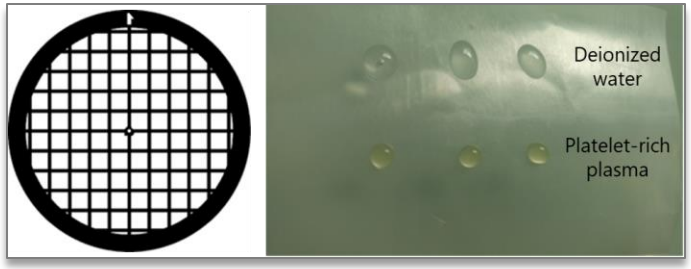
- **Transmission Electron Microscopy (TEM)**

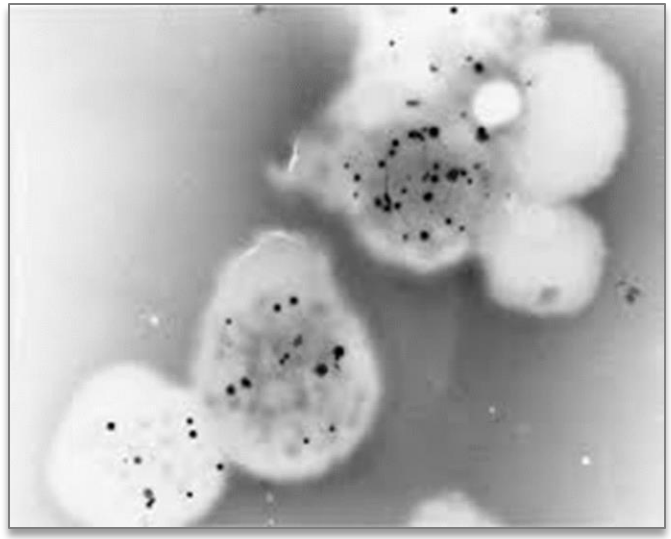




HITACHI HT7700 120kV, High-Contrast/High-Resolution (CMEAB Toulouse)

- **Whole Mount,** 300-mesh copper grid coated with a formvar film





From bedside to bench: dissecting the mechanisms of platelet granule disorders

- ***In vitro* models** — $CD34^+$ hematopoieic progenitors → MK differentiation
  CRISPR/Cas9 gene editing in iMKCL
  integrative approaches (single-cell RNA sequencing, multiomics)
- ***Ex vivo* models** — patient platelets or circulating megakaryocytes
  platelet functional assays (secretion, content, trafficking)
- ***In vivo* models** — conditional murine MK-specific knock-out/in models

→ Combine **functional and mechanistic** analyses

Clinical management: from diagnosis to long-term follow-up

Bleeding tendency minimal-absent to mild-moderate
Associated with systemic diseases/predisposition
other hematologic complications
extra-hematologic: cardiac, renal, hepatic, immunological, neurological, auditory, visual, skeletal

Management mainly supportive
Tranexamic acid
Desmopressin
Platelet transfusion TPO-Receptor Agonists may be considered on a case-by-case basis

Variable thrombocytopenia severity
Platelet morphology and size
Typical gray appearance: absence or marked reduction of alpha granules
Bone marrow biopsy: fibrosis, emperipolesis

STICKY MESSAGES

Laboratories investigations for patients

✓ Initial orientation: peripheral blood smear and platelet count (mean platelet volume)
✓ Functional investigations guide specific gene panels for NGS
✓ NGS panels evolve, reassess if needed

Risks → long term follow-up of patients

✓ Bone marrow **fibrosis**
✓ **Leukemic transformation**

Back to the bench: use *in vitro* and *ex vivo* models to dissect disease mechanisms

THANK YOU

CRPP Centre de Référence Pathologies Plaquettaires

MHEMO La Filière des maladies rares de l'hémostase

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https://mhemo.fr/

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