Webinars

Constitutional thrombocytopenia

EuroBloodNet

Pathogenesis of inherited thrombocytopenia

Speaker Hana Raslova

Research Director, co-leader of Team « From hematopoietic stem cell to megakaryocyte"

INSERM & Gustave Roussy Institute,

ERN-EuroBloodNet subnetwork

Villejuif – France

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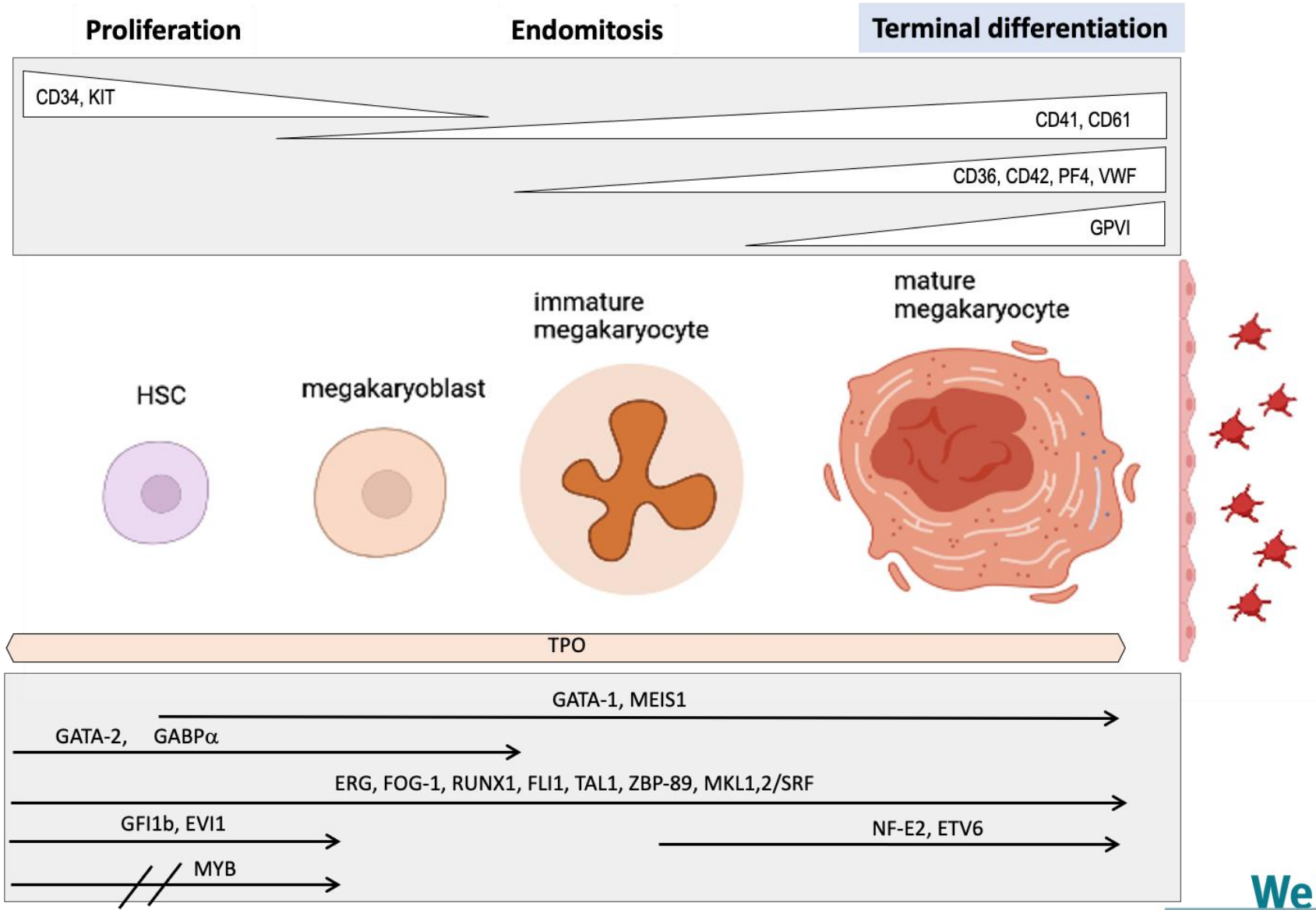
Conflicts of interest

No conflict of interest to declare

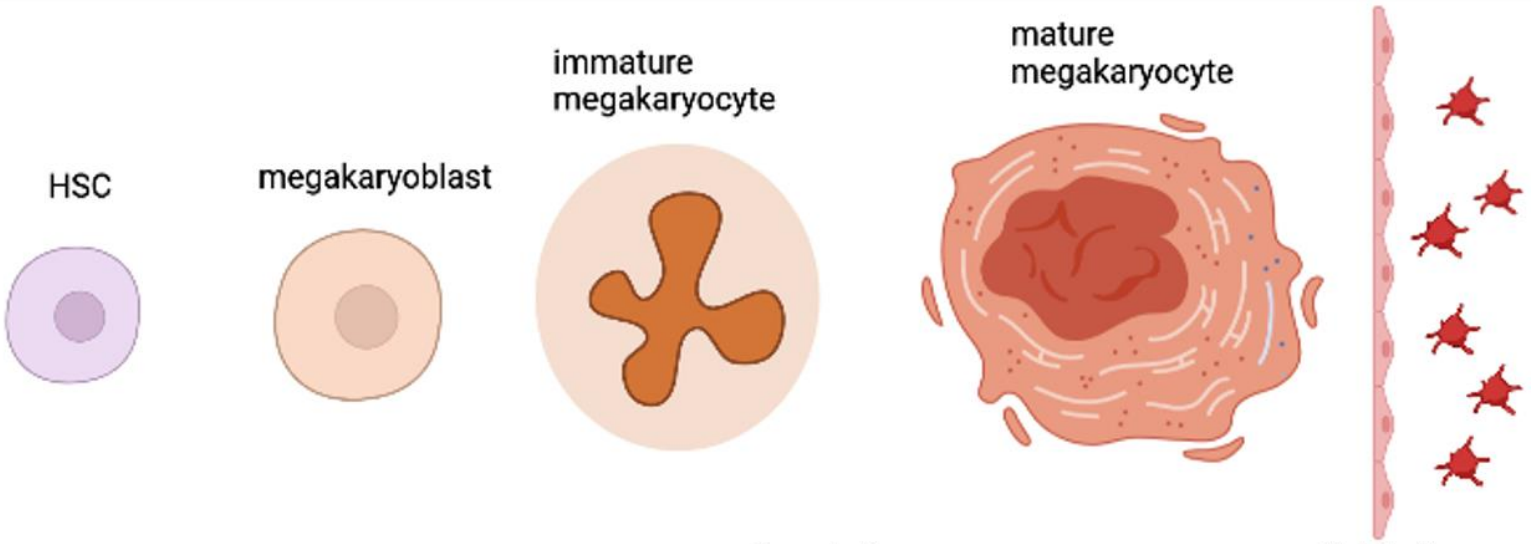
Learning objectives of the webinar

1. What are the different types of inherited thrombocytopenia
2. What is the purpose of a better understanding of the regulation of normal megakaryopoiesis
3. Why and how can we study the pathophysiological mechanismes of IT

Megakaryopoiesis



Inherited thrombocytopenias (IT)



TPO/MPL signaling

THPO
MPL
ANKRD26

Transcription factors

GATA1
FLI1
GFI1B
RUNX1
ETV6

Predisposition to leukemia

IKZF5
HOXA11
MECOM

Splicing factor

RBM8A

Metabolism granule biogenesis, ion channels

NBEAL2
KDSR
ABCG5
ABCG8
CYCS
STIM1
TRPM7

Cytoskeleton

MYH9
TUBB1
ACTN1
FLNA
TPM4
DIAPH1
CDC42
WAS
ARPC1B
FYB
WIPF1

receptor signaling

GP1BA
GP1BB
GP9
ITGA2B
ITGB3
SLNF14
PRKACG
VWF
SRC
PTPRJ
MPIG6B

Sialylation

GNE
SLC35A1
GALE

Large (giant) platelet size

Small platelet size

Normal platelet size

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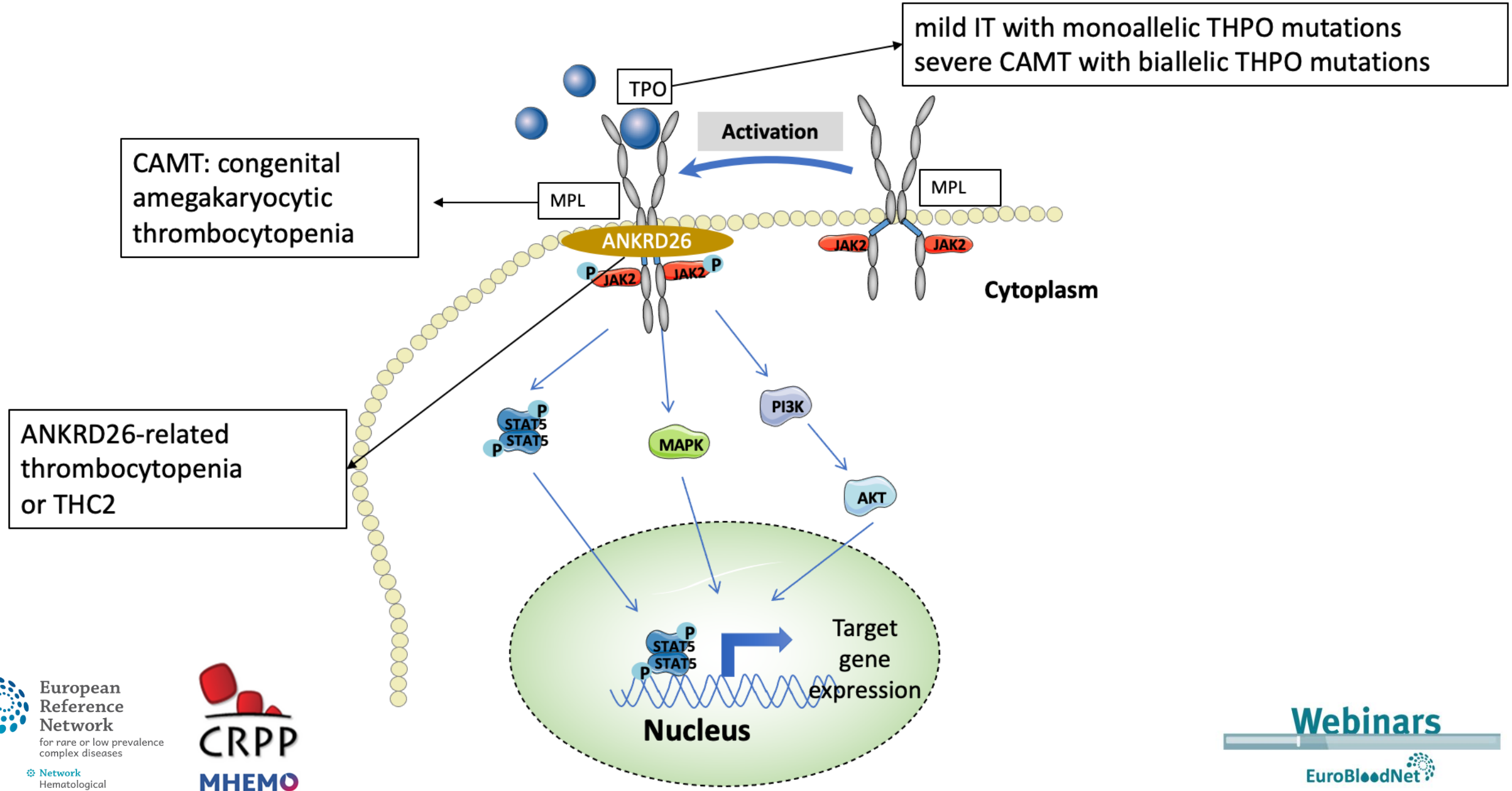
IT linked to the mutations in TPO/MPL signaling



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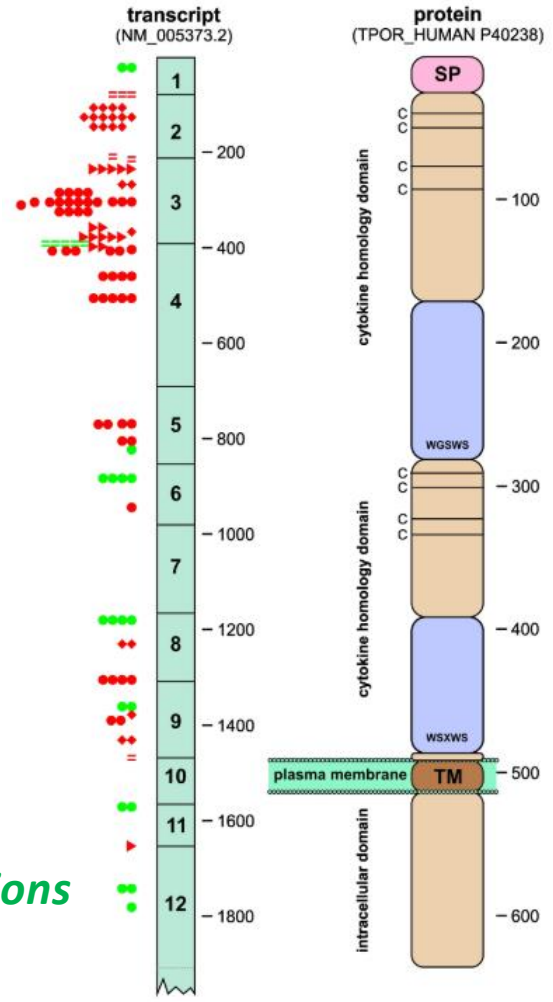
Congenital amegakaryocytic thrombocytopenia (CAMT)

- Mutations affecting the thrombopoietin receptor MPL
- Autosomal recessive inheritance
- Severe thrombocytopenia (platelet count <50 000/µL and TPO levels)
- Bone marrow failure disorder

- Type I: complete MPL loss of function, early progression to BMF
- Type II: receptor maintains residual function, delayed development of BMF



Green: less severe mutations

(Germeshausen, M., & Ballmaier, M., 2020)

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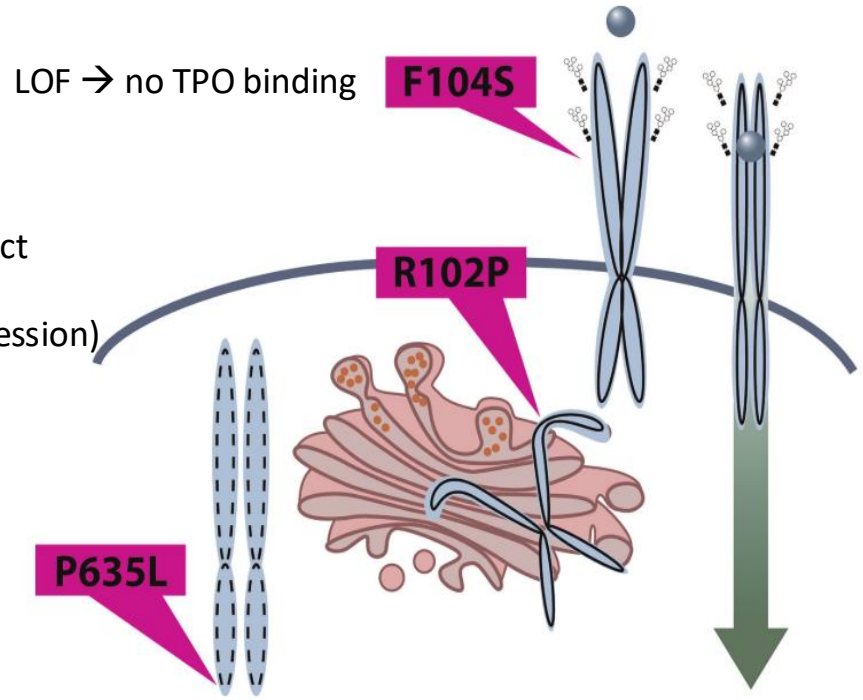
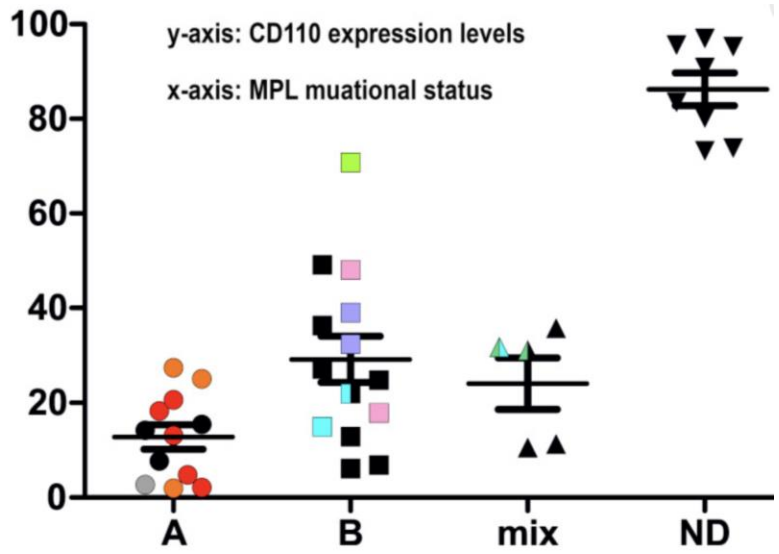
Congenital amegakaryocytic thrombocytopenia (CAMT)



(Geddis, 2008)



A: Nonsense, frame shift mutations and mutations leading to a complete loss of a splice site: no MPL at cell surface

B/Mix: Missence mutations or compound heterozygous mutations: variable MPL expression on cell surface

(Germeshausen, M., & Ballmaier, M., 2020)

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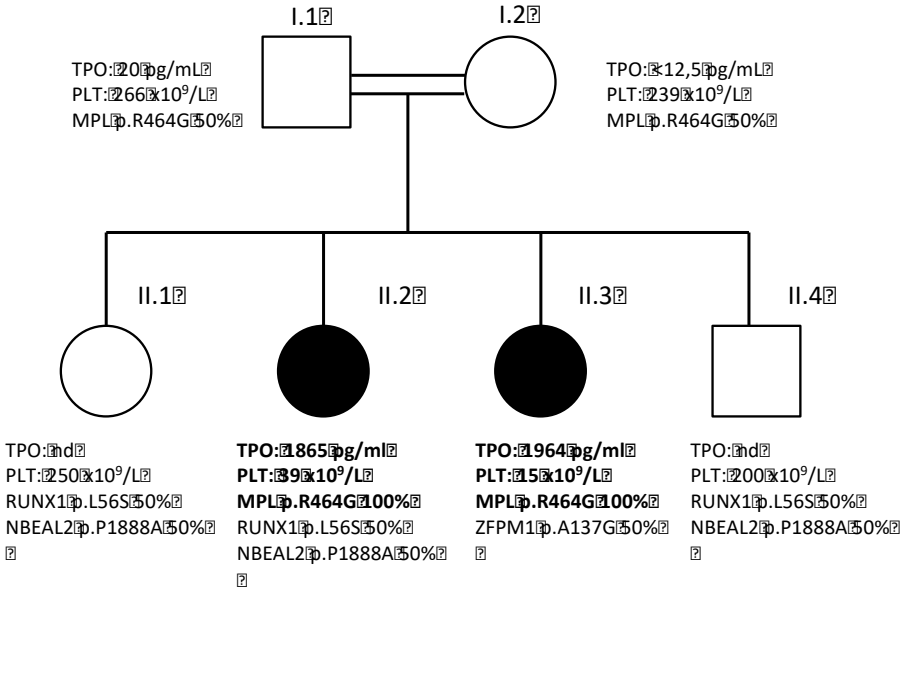
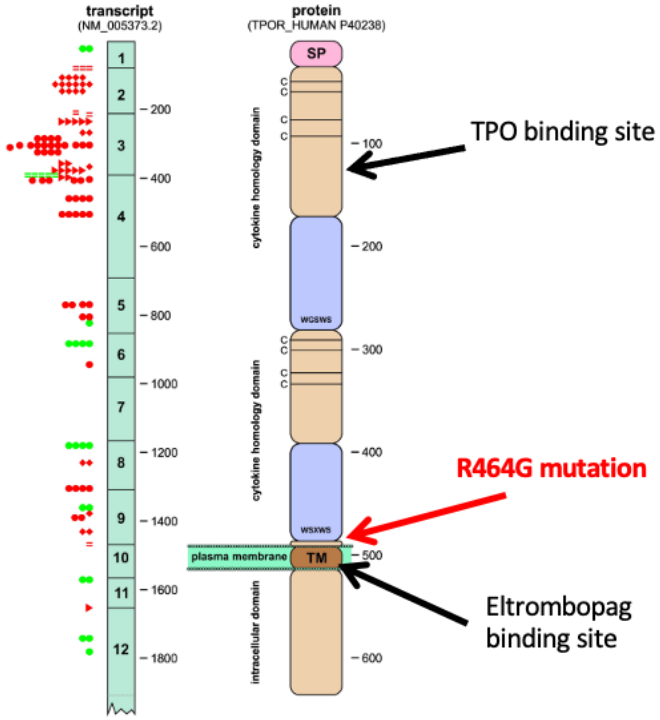
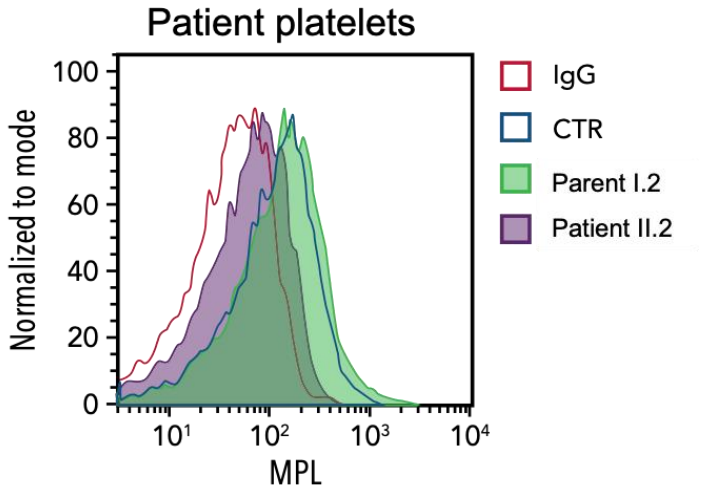
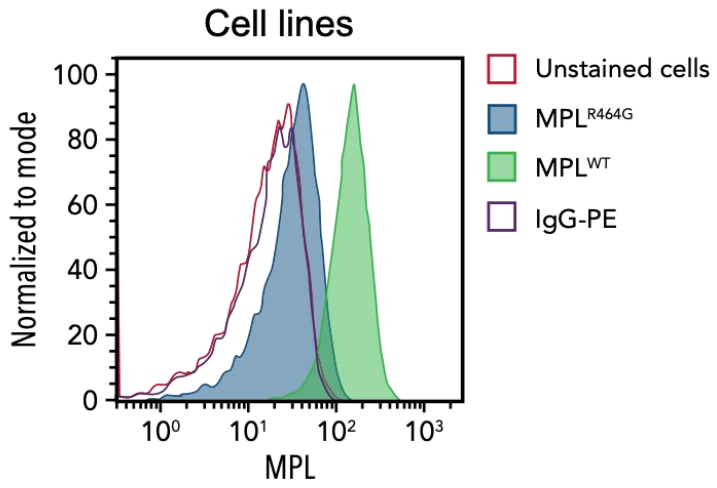
MPL^{R464G} is responsible for CAMT in a consanguineous pedigree









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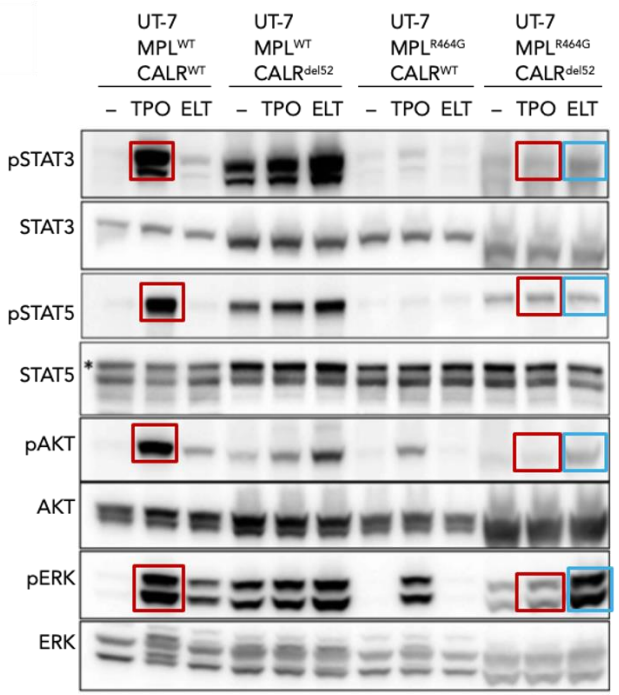
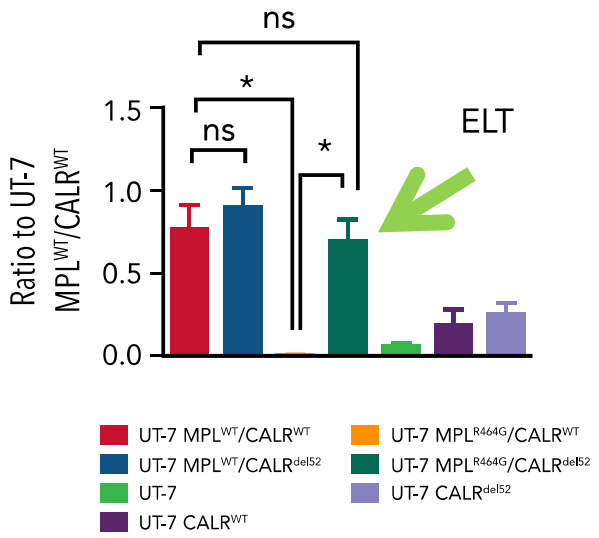
MPL ^{R464G} is an immature receptor retained in the endoplasmic reticulum

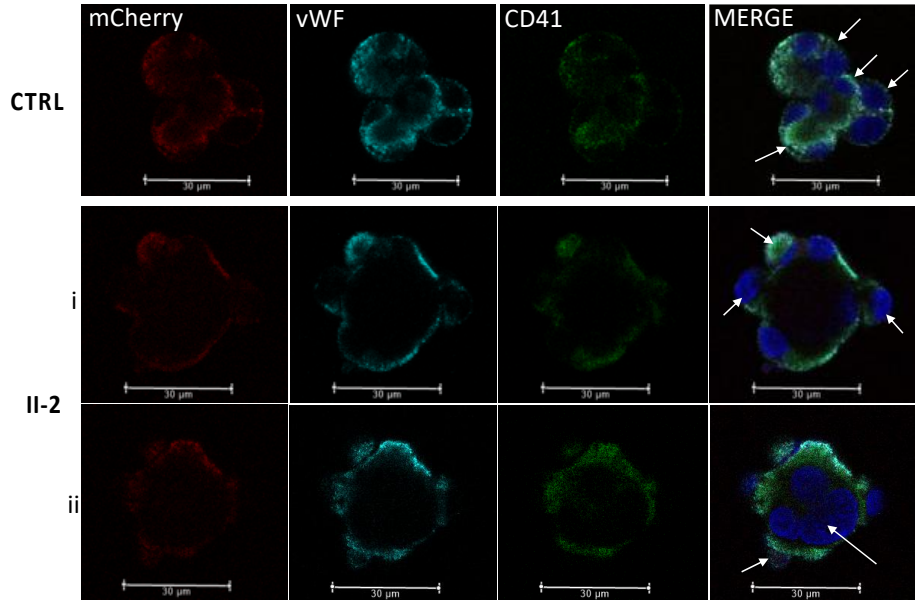
- MPL R464G is not glycosylated and is retained in ER
- only weekly responds to TPO but the synergy of TPO with ELT slightly improves the signaling
- overexpression of CALR^{del52} does not rescues its trafficking to cell surface
- **CALR^{del52} and ELT partially restore the signaling and cell proliferation**
- and more importantly **CALR^{del52} and ELT restore MK differentiation of patient CD34^{+} cells**





Overexpression of CALR ^{del52}

Overexpression of CALR ^{del52}/mCherry



Basso-Valenitna et al, Blood 2021

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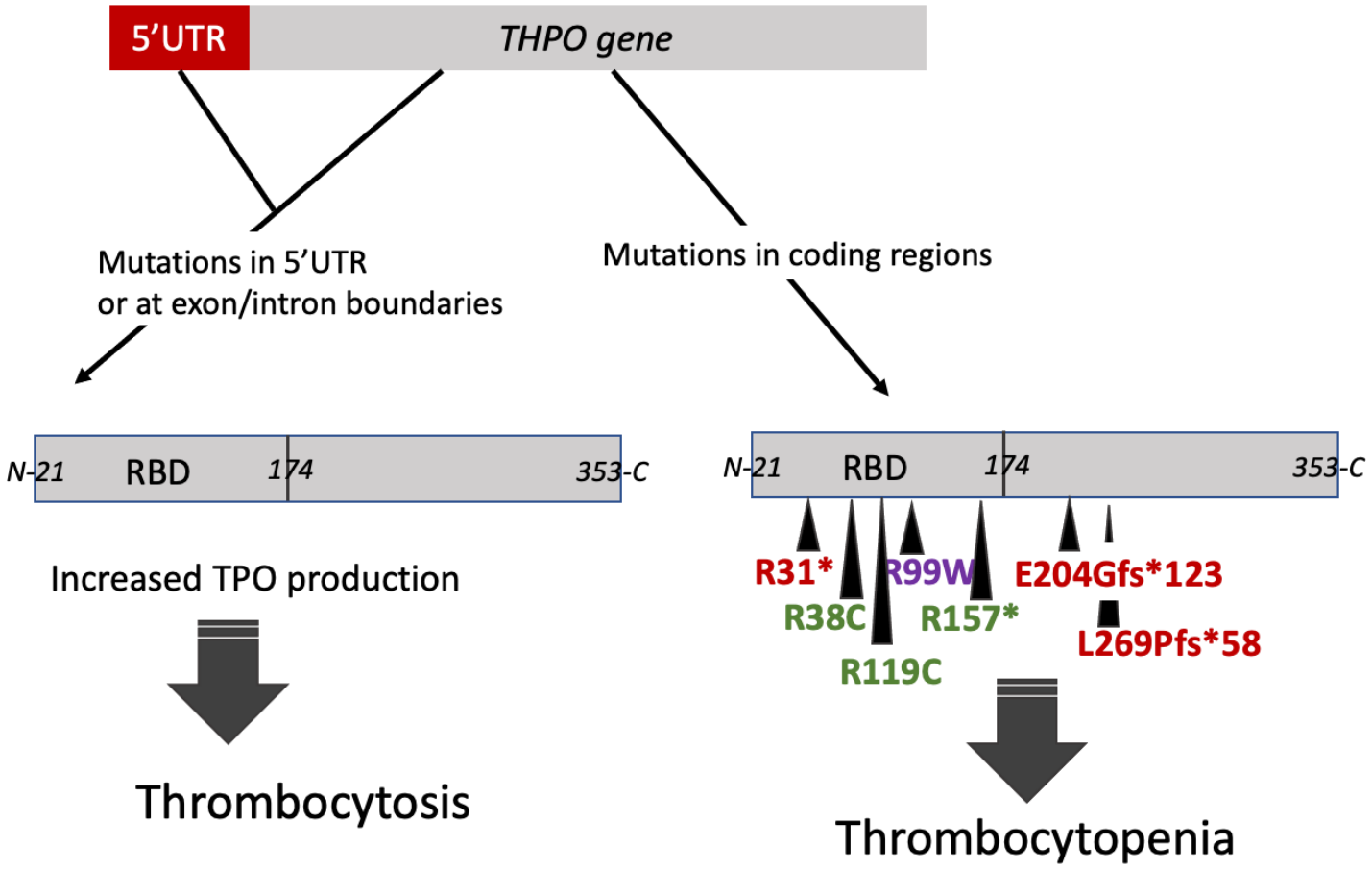
THPO linked thrombocytopenias



Adapted from Plo et al, 2017

Green: *homozygous mutations*
Red: *monoallelic mutations*
Purple: *homozygous in CAMT, heterozygous in mild IT*

RBD: receptor binding domain

R31*:
- Happloinsuficiency: reduced TPO levels; mild thrombocytopenia

R157*, R99W:
- Lack of TPO in plasma

R119C, R38C
- normal TPO level
- affects both secretion and receptor binding

E204Gfs*123, L269Pfs*58, R99W
- reduced TPO secretion due to mistrafficking to the secretory compartment: consequence of the loss of TPO C-terminal domain glycosylated residues or to the discrupted trafficking
- normal TPO plasma levels

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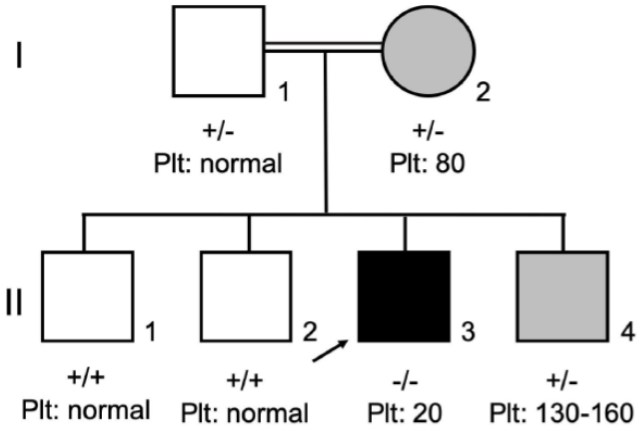
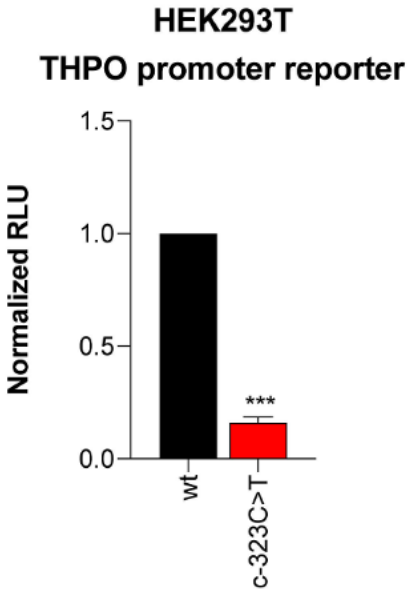
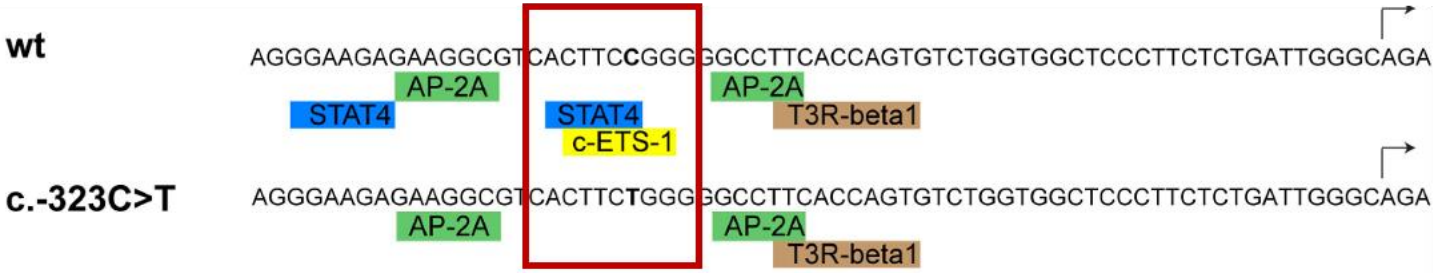
CAMT with 5'UTR mutation in THPO





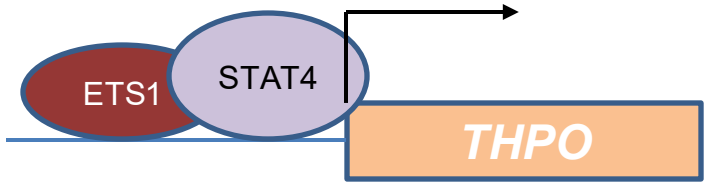
- decreased *THPO* expression

STAT4 and ETS1 putative binding sites



STAT4 and ETS1 regulates THPO expression

- Shown by luciferase assays,
- Chromatin immunoprecipitation



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Capaci et al, Haematologica, 2022

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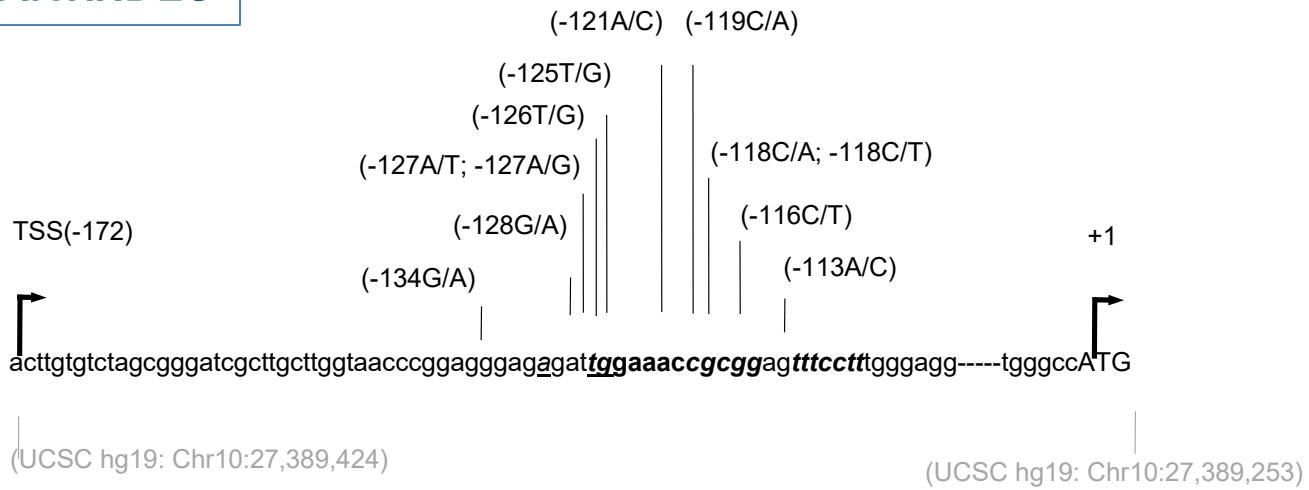
ANKRD26-related disease or thromocytopenia 2

- Autosomal dominant transmission
- Normal mean platelet volume
- Moderate thrombocytopenia
- Non syndromic

Predisposition to myeloid malignancies (AML, MDS, CMML, CML) (10%)

Mutations in 5'UTR region of *ANKRD26*



Pippucci et al, Am J Hum Genet, 2011

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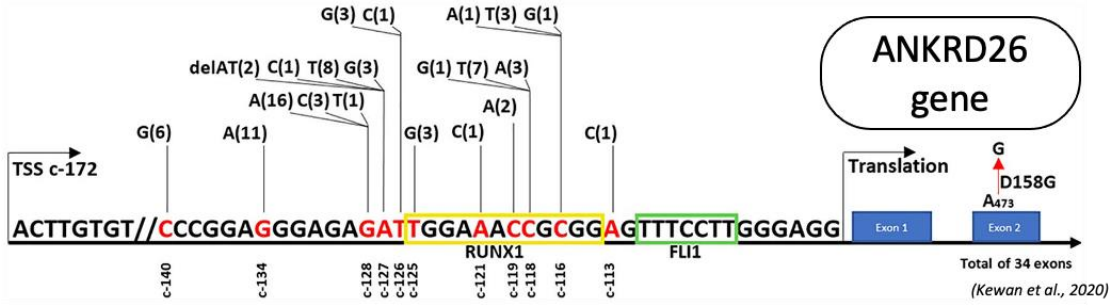
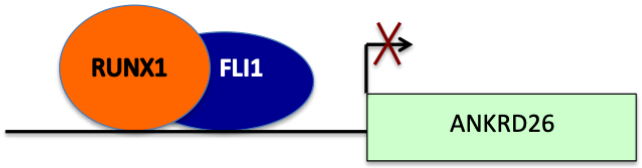
5'UTR mutations of *ANKRD26* gene lead to the disruption of RUNX1/FLI1 binding and ANKRD26 overexpression

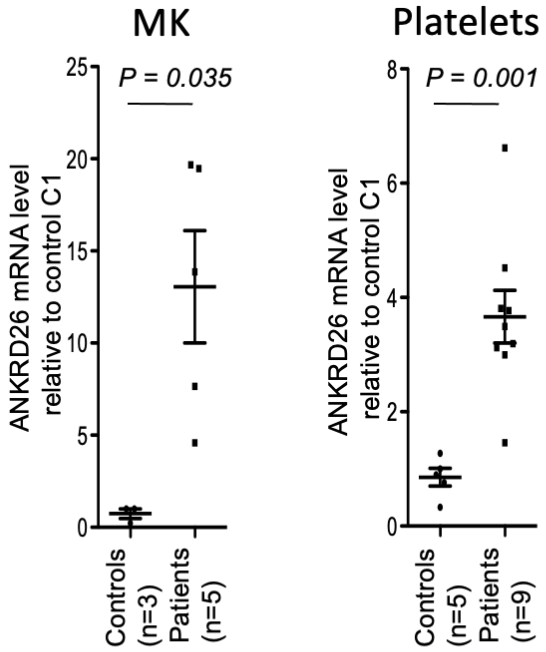


RUNX1 binding site

FLI1 binding site



- **RUNX1 and FLI1 are negative regulators of ANKRD26 during normal MK-poiesis**



- **Persistent expression of *ANKRD26* in MK and platelets from THC2 patients**

Bluteau&Balduini et al, JCI, 2014

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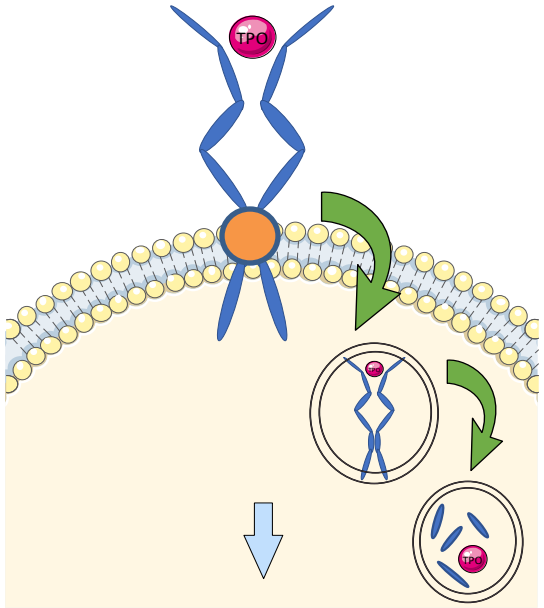
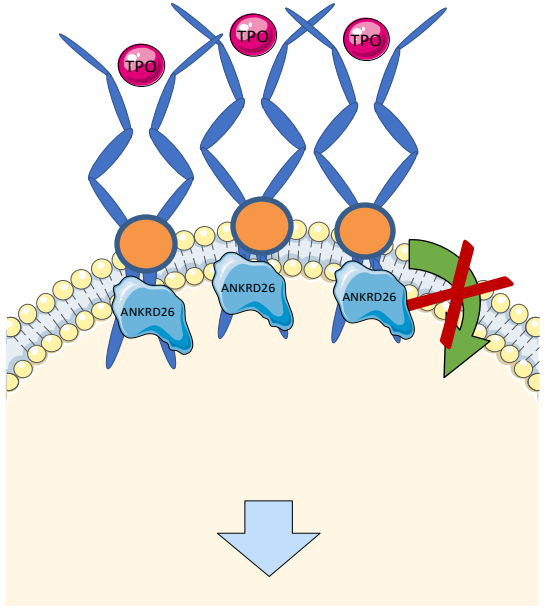
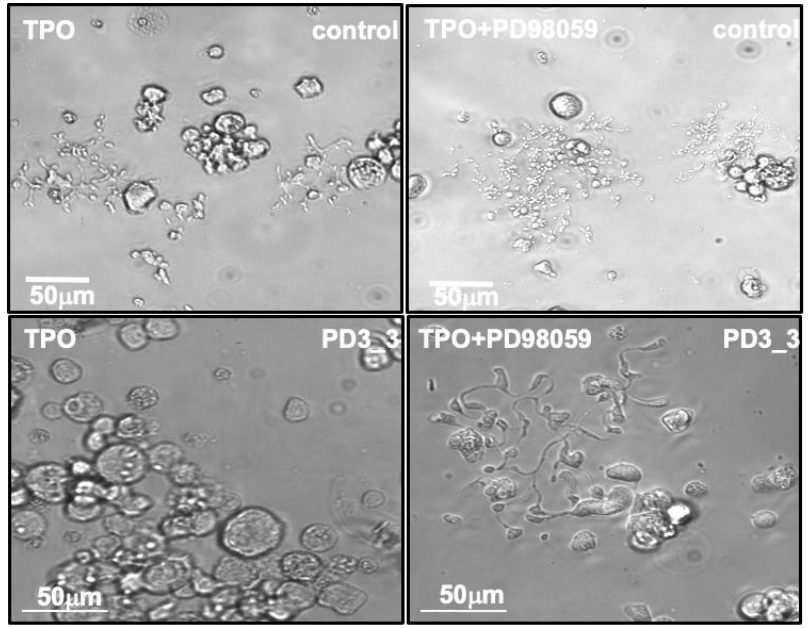
ANKRD26 interaction with MPL leads to overactivation of TPO/MPL signaling

NORMAL CONDITION



THC2

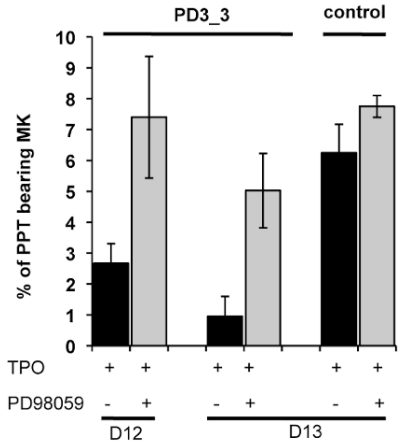




Normal activation of JAK/STAT, PI3K and MAPK pathways

- Signaling overactivation due to a defect of MPL internalization
- Hypersensitivity to TPO and ELT
- Defect in proplatelet formation

Basso-Valentina & Donada A et al, Haematologica 2023



Bluteau&Balduini et al, JCI, 2014

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IT linked to the mutations in transcription factors:

RUNX1, FLI1, GATA1, ETV6, GFI1B, HOXA11, MECOM, IKZF5

RUNX1

FPD/AML: Familial Platelet Disorder with predisposition to acute myelogenous leukemia

- Autosomal dominant transmission
- Normal platelet size
- Moderate thrombocytopenia
- Non syndromic
- Predisposition to myeloid malignancies (AML, MDS, CMML, rare T-ALL)
- α-granule storage and secretion defects

FLI1

Jacobsen Syndrome/
Paris-Trousseau thrombocytopenia, del11q23
FLI1-related thrombocytopenia: mutations

- Autosomal dominant transmission
- Slightly increased platelet size
- Moderate thrombocytopenia
- Syndromic
- Bleeding
- Fused and enlarged α-granules

GATA1

X-linked thrombocytopenia with dyserythropoietic anemia

- X-linked
- Slightly increased platelet size
- Moderate to severe thrombocytopenia
- Platelet dysfunction
- α-granule deficiency
- Congenital erythropoietic porphyria

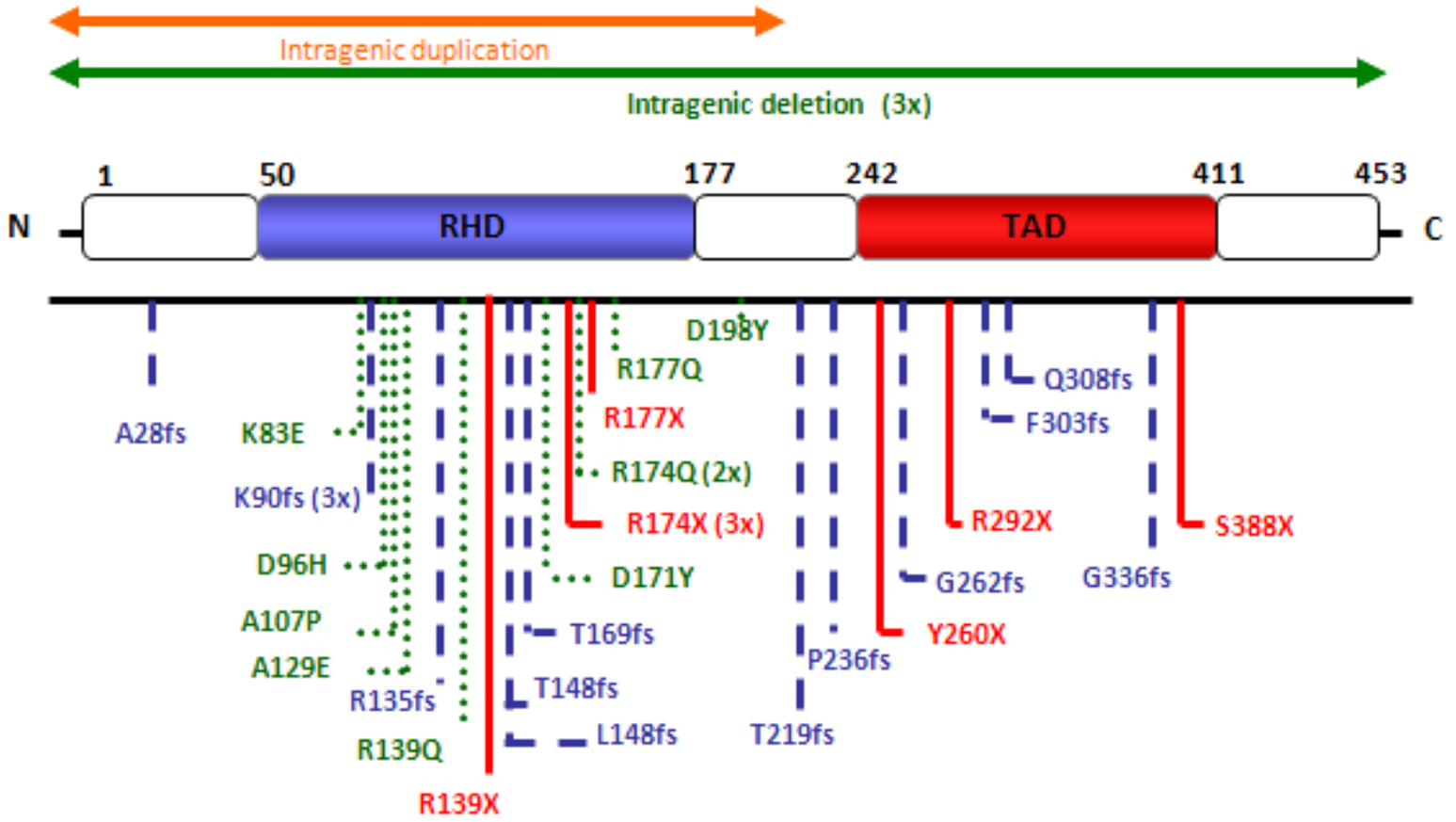
IT linked to the mutations in transcription factors:

RUNX1, FLI1, GATA1, ETV6, GFI1B, HOXA11, MECOM, IKZF5

ETV6	**ETV-6 related thrombocytopenia**	• Autosomal dominant transmission • Normal platelet size • Moderate to severe thrombocytopenia • Predisposition to lymphoid malignancies (pre B-ALL)
GFI1B	**GFI1B-related thrombocytopenia**	• Autosomal dominant transmission • Increased platelet size • Associated to anisocytosis and poikilocytosis • Moderate bleeding diathesis • α-granule deficiency in some cases
HOXA11/ MECOM (MDS1-EVI1)	**Radioulnar synostosis with amegakaryocytic thrombocytopenia (RUSAT1/RUSAT2)**	• Autosomal dominant transmission • Syndromic • B-cell deficiency • Progression to bone marrow aplasia
IKZF5	**IKZF5-related thrombocytopenia**	• Autosomal dominant transmission • Normal platelet size • Mild thrombocytopenia • Reduced quantity of α-granules

FPD/AML

RUNX1



Missense mutation
Framshift mutation
Nonsense mutation

Two types of *RUNX1* mutations: a) haploinsuficiency-like mutations
b) dominant negative-like mutations

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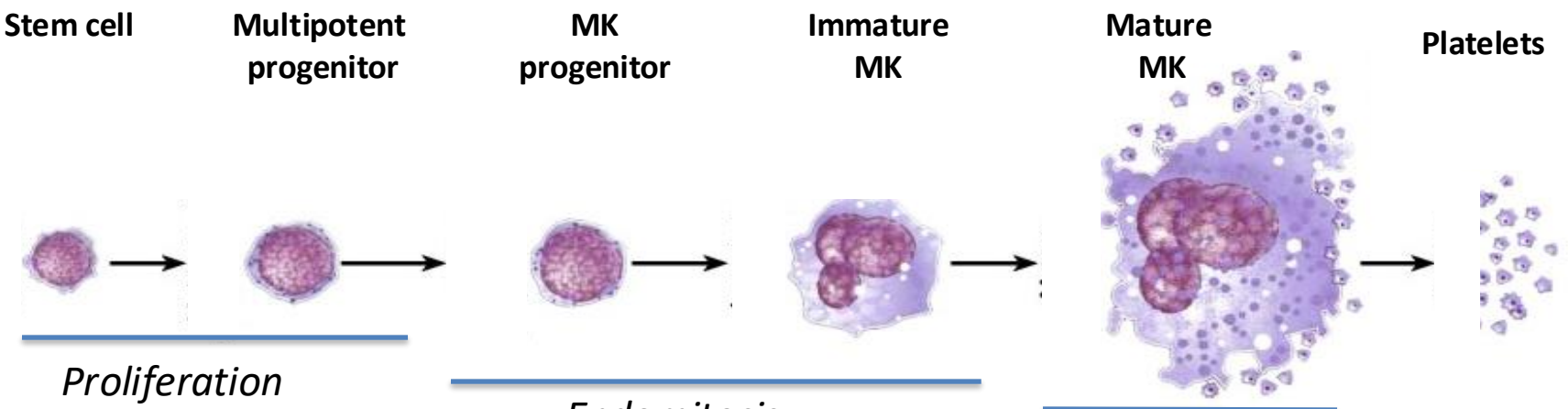
FPD/AML



Progenitor number
NR4A3 ↗
Bluteau et al, Blood, 2011
Antony-Debré et al
Blood, 2015

Endomitosis induction: ↘ MYH10

Endomitosis arrest: ↗ p19INK4D

Lordier et al, Nature Com, 2012
Bluteau et al, Blood, 2012
Gilles et al, Blood, 2008

Proplatelet formation
ANKRD26, ↘ MYL9, MYH9 ↗

Bluteau et al, Blood, 2012
Antony-Debré et al, Blood, 2012
Bluteau&Balduini et al, JCI, 2014

Platelet dysfunction
TREML1, ITGA2 ↗

Glembotsky&Sliwa et al,
Haematologica, 2019

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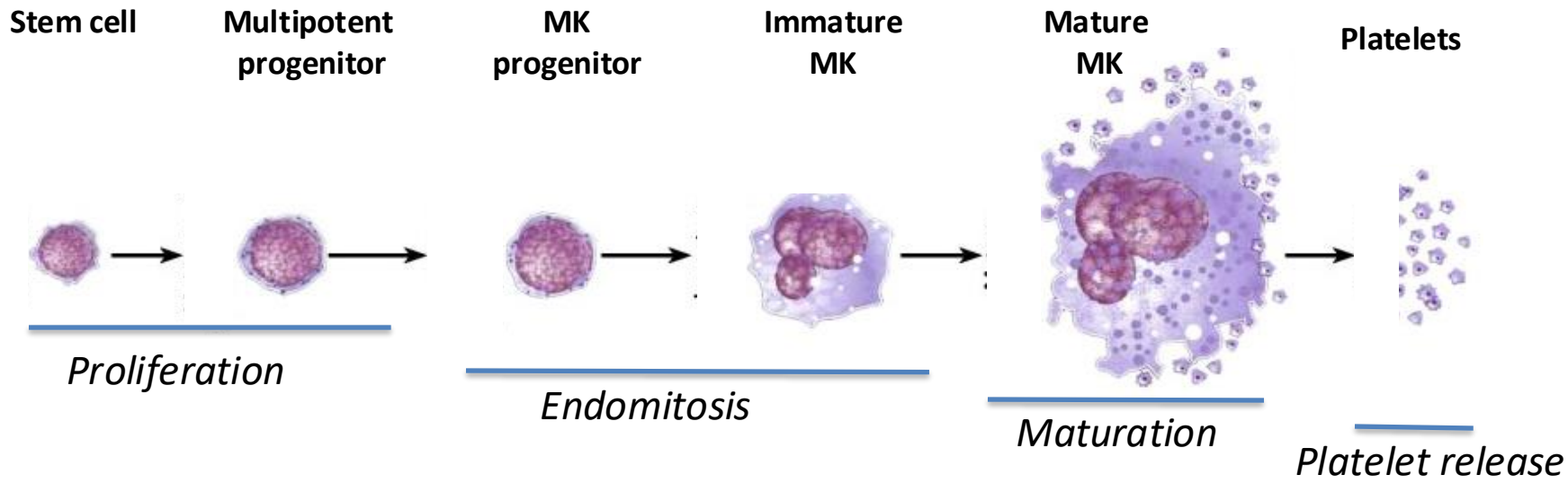
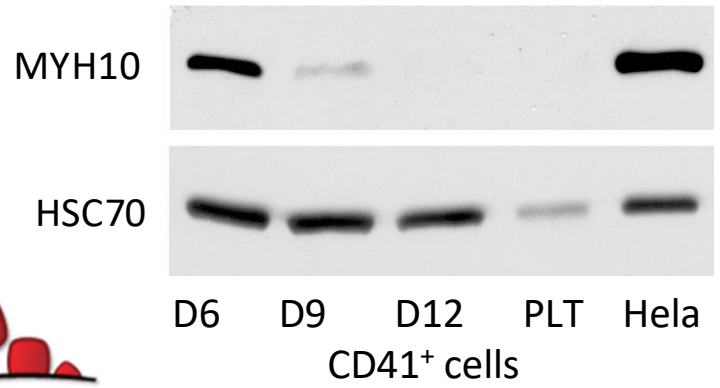
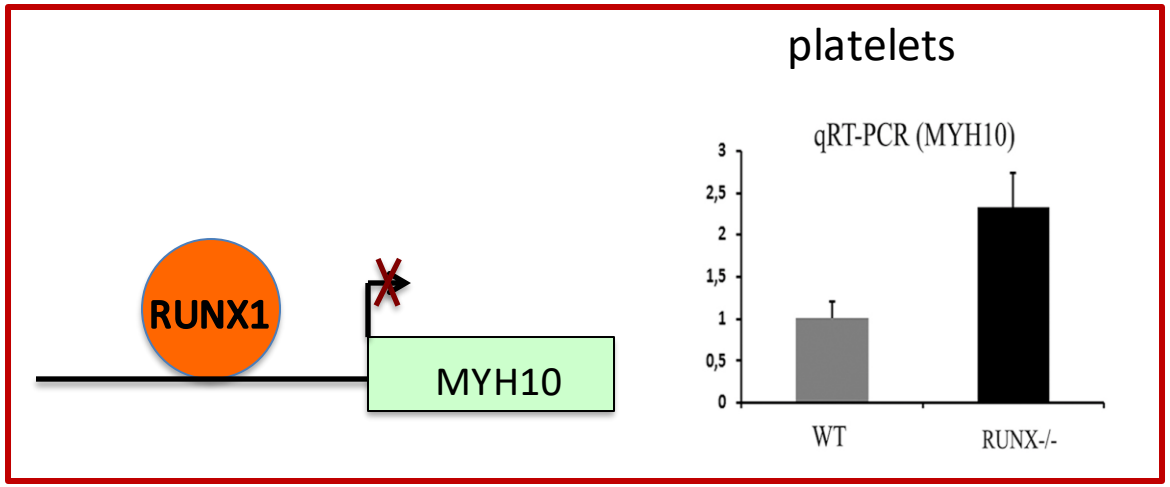
MYH10 silencing is necessary for megakaryocyte ploidization



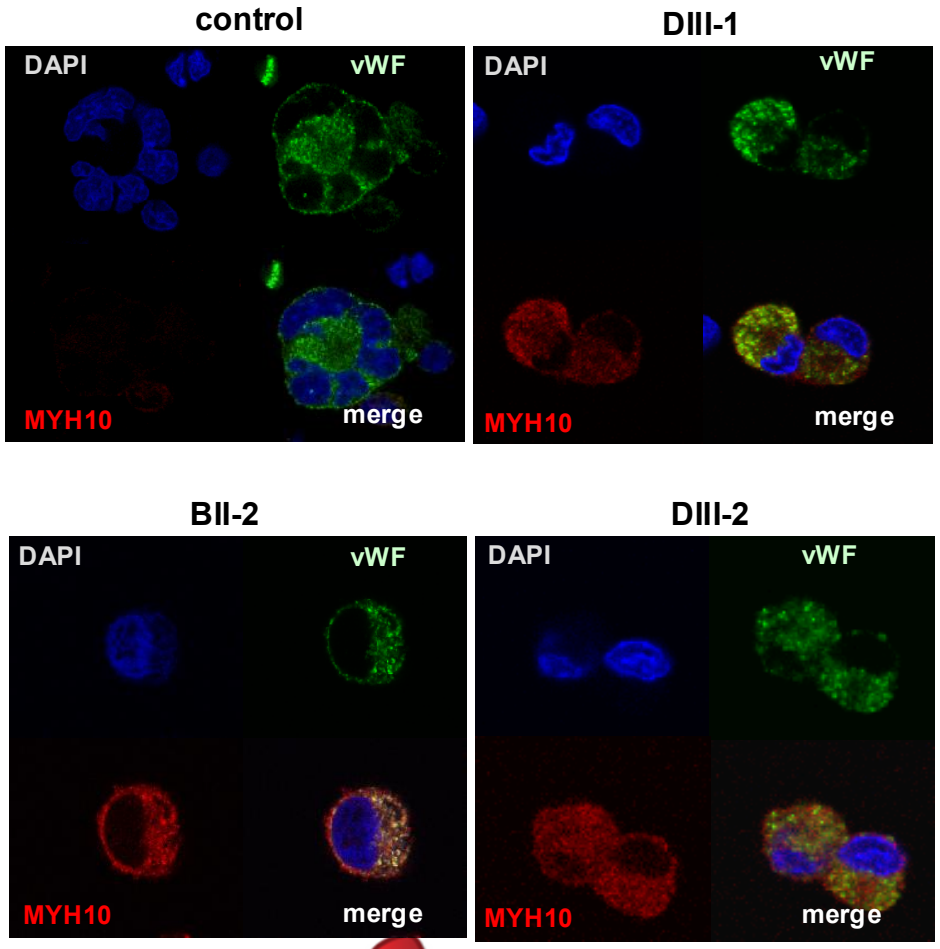




Lordier et al, Nat Com, 2012

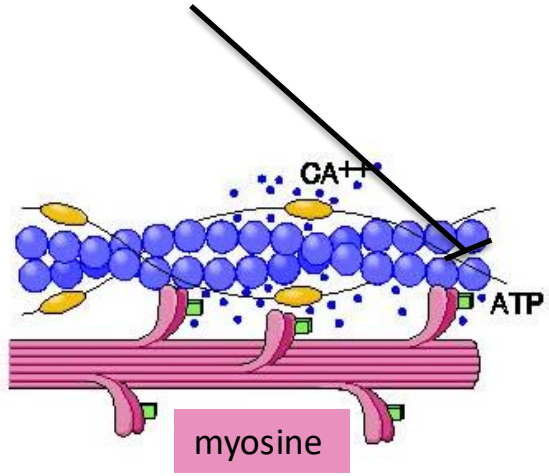
MYH10 persists in mature FPD/AML MKs

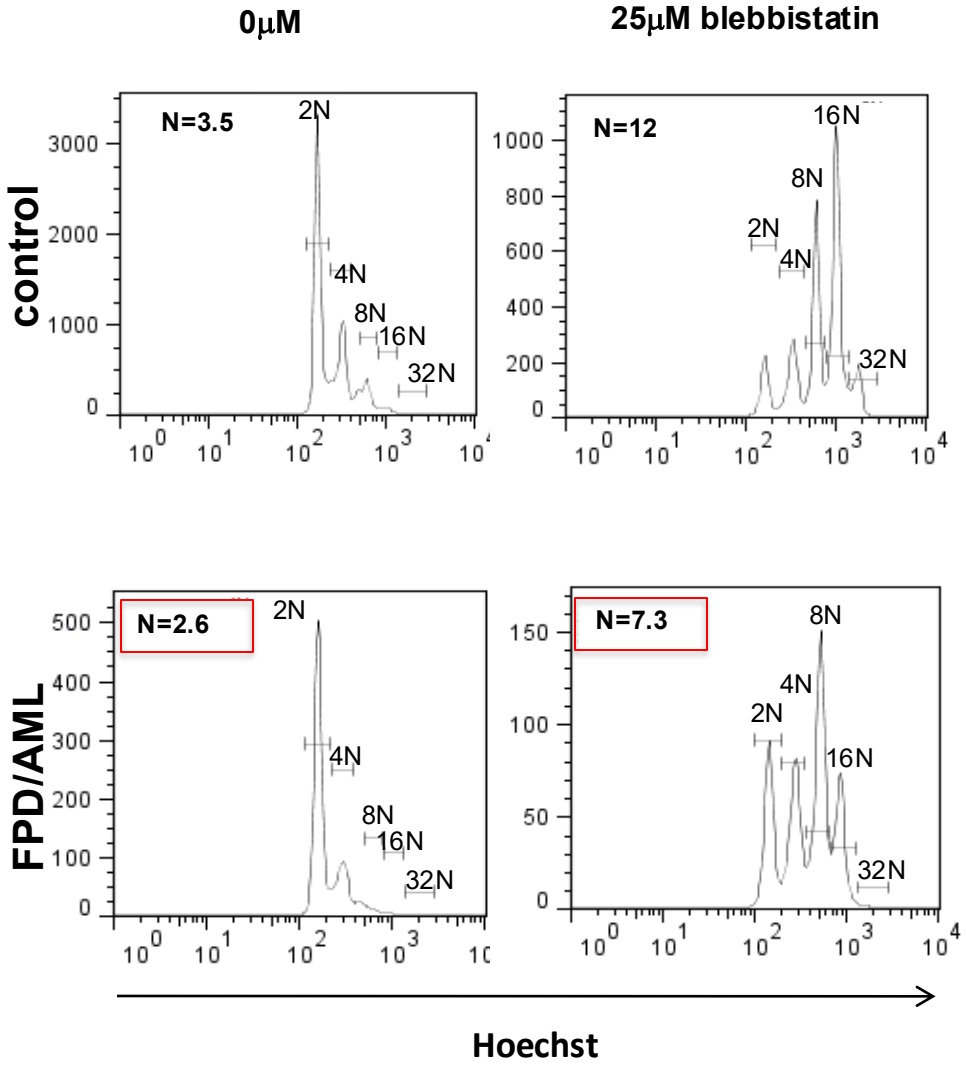
Mature megakaryocytes



Blebbistatin

Chemical inhibitor specific of ATPase activity of myosin II





Bluteau et al, Blood 2012

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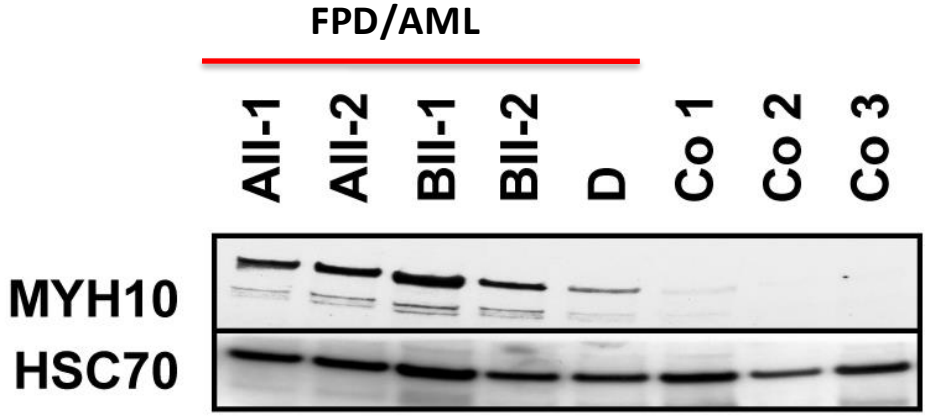
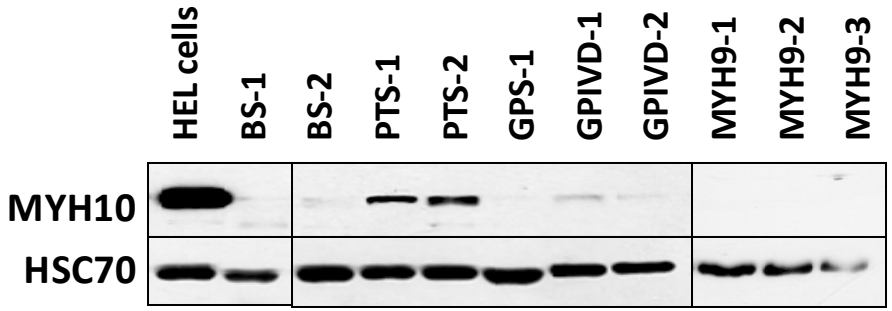
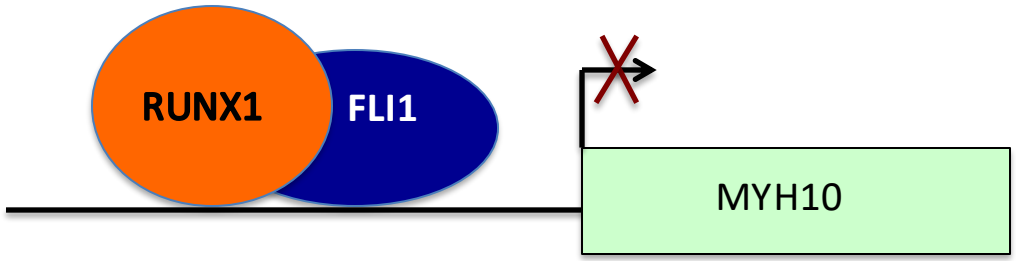
MYH10 also persists in platelets of FPD/AML patients





BS: Bernard Soulier (GPIb-V-IX)
PTS: Paris Trousseau Syndrome (*Fli-1* deletion)
GPS: Gray Platelet Syndrome (NBLEA2)
GPIVD: Moderate thrombocytopenia associated with a GPIV defect
MYH9: MYH9 Syndrome

Presence of MYH10 represents a marker of *RUNX1* and *FLI1* genetic alterations



Antony-Debré et al, Blood, 2012

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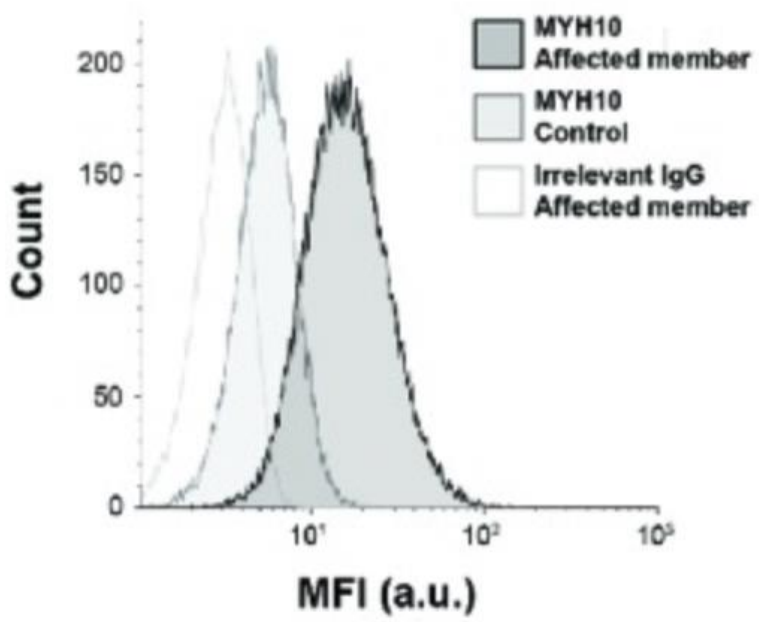
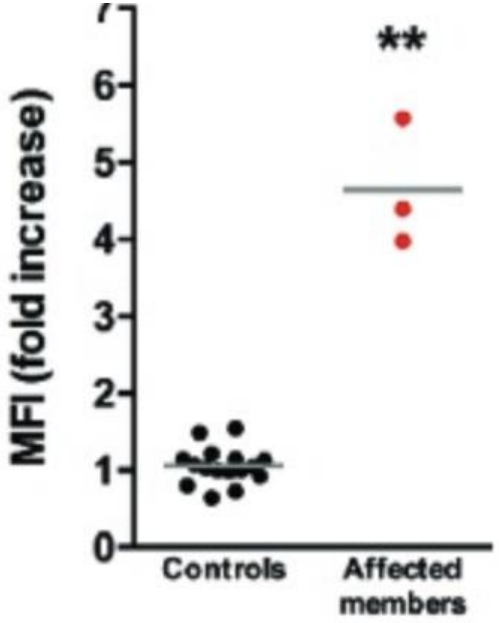
Inherited macrothrombocytopenia with point mutations in *FLI1*

2 FLI1 variants : c.1010G>A and c.1033A>G identified in two families

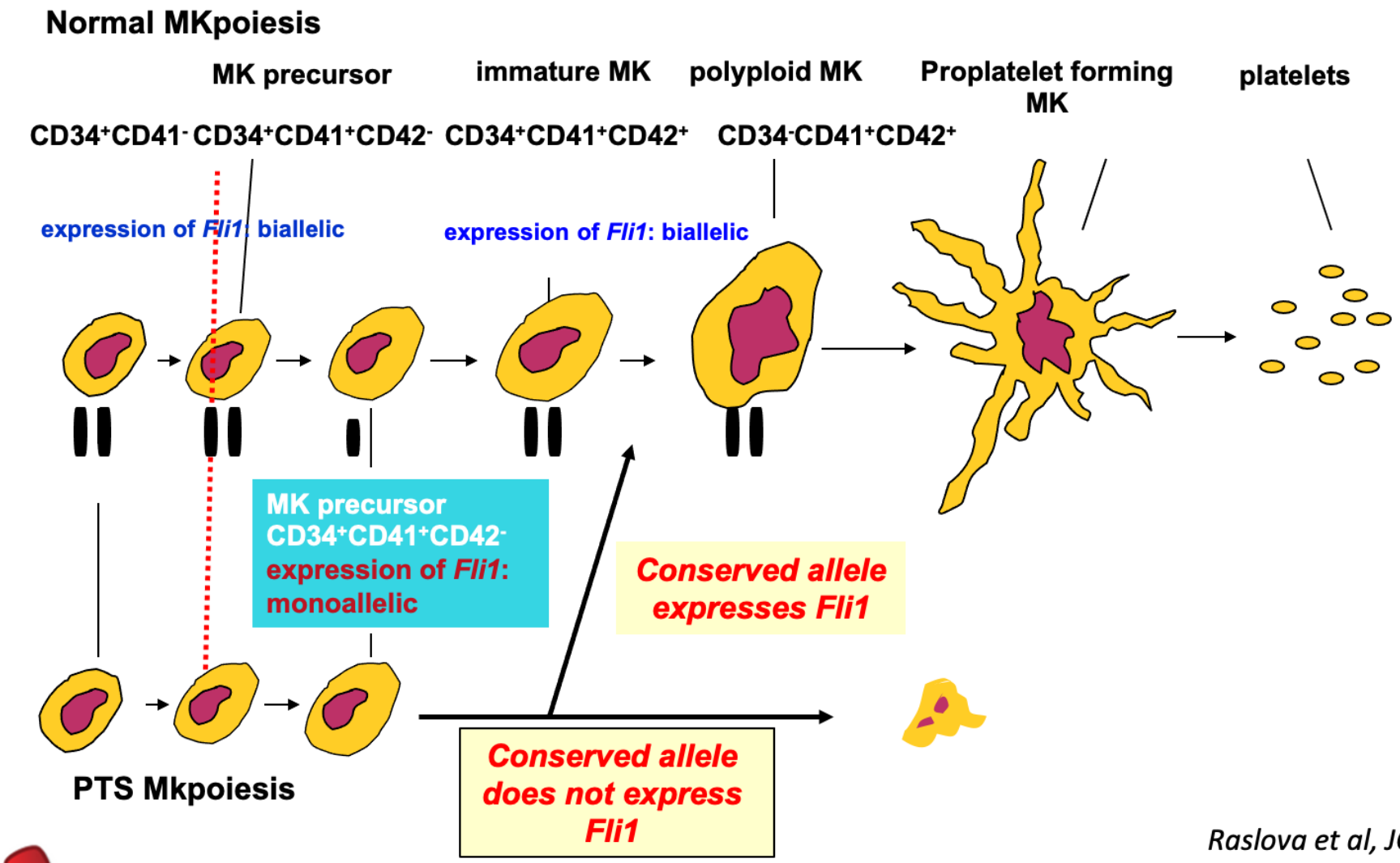




Analysis of MYH10 by FACS has confirmed a functionality of these 2 variants

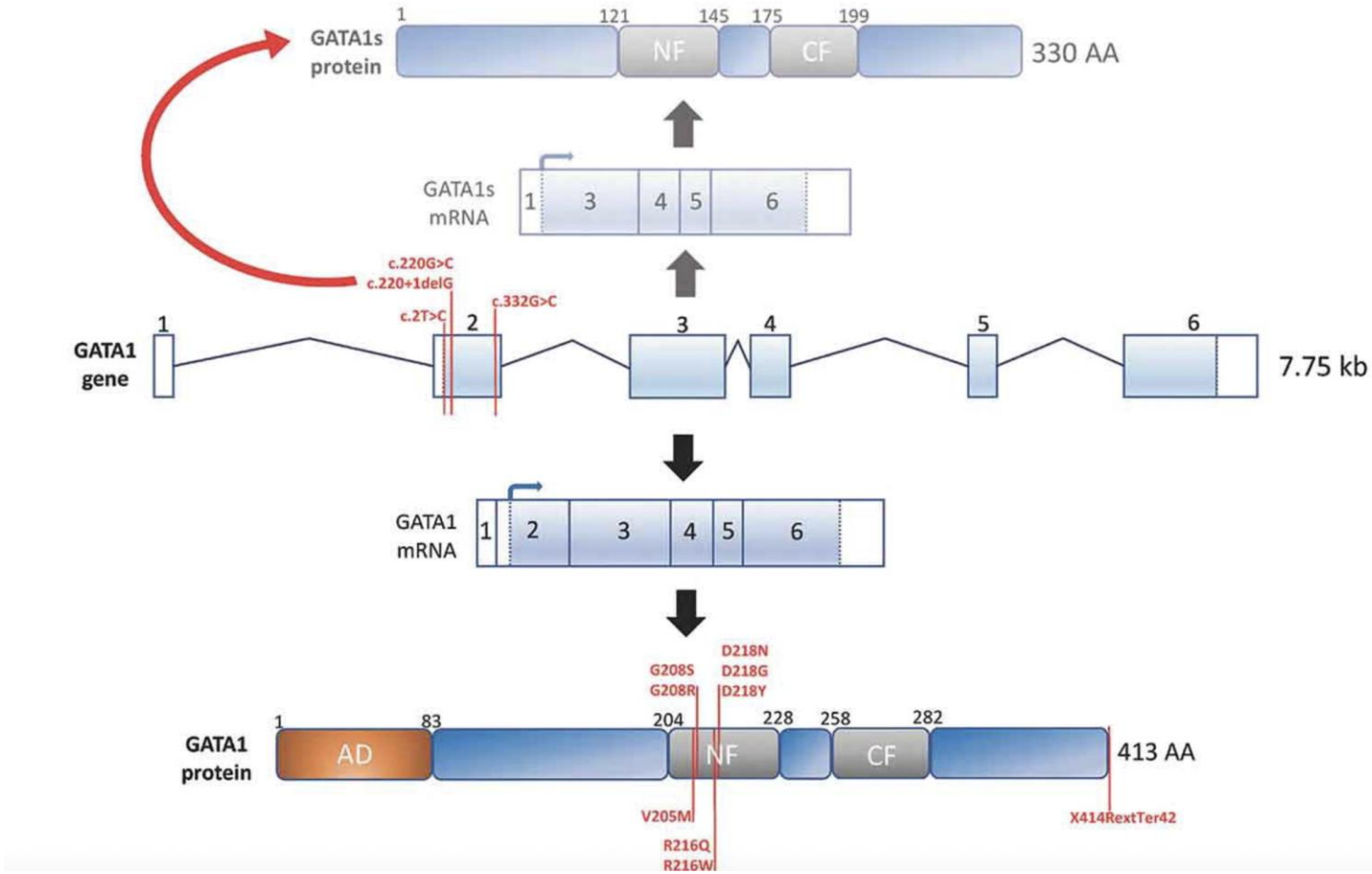
Saultier et al, Haematologica, 2017

Jacobsen syndrome / Paris-Trousseau Thrombocytopenia



Raslova et al, JCI, 2004

GATA1 mutations leading to AMKL or thrombocytopenias associated or not to anemias



Freson et al, Platelets 2017

GATA1S: acute megakaryoblastic leukemia

NF mutants

V205M, G208R, and D218Y:severe dyserythropoietic anemia with macrothrombocytopenia (DNA binding)

G208S, D218G, and D218N: macrothrombocytopenia with mild dyserythropoietic features (DNA binding)

R216Q: macrothrombocytopenia with beta-thalassemia (FOG-1 binding)

R216W: macrothrombocytopenia with congenital erythropoietic porphyria (FOG-1 binding)

X414R: removes Stop codon: mild thrombocytopenia

One of GATA1 targets: *NBEAL2* mutated in Gray Platelet Syndrome

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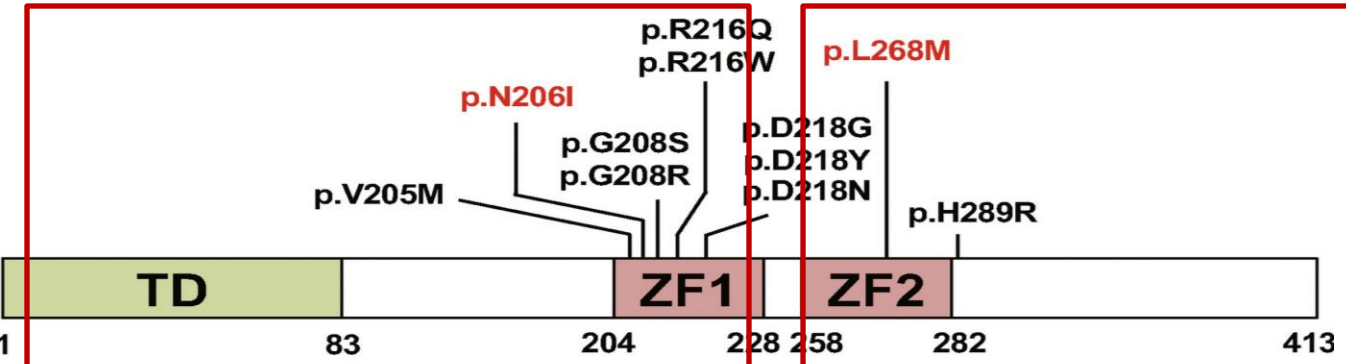
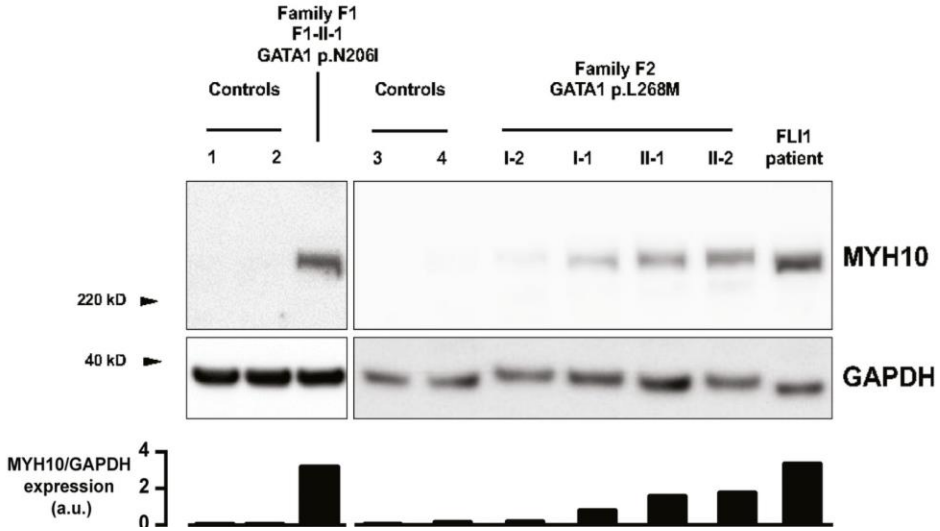
MYH10: biomarker of IT with *GATA1* mutations

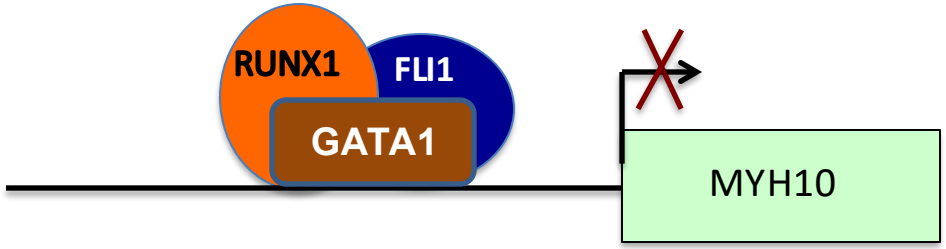
3 patients from 2 families with new GATA1 variants: **p.N206I; p.L268M**



Severe thrombocytopenia
Bleeding
No anemia, mild dyserythropoiesis
Normal platelet size

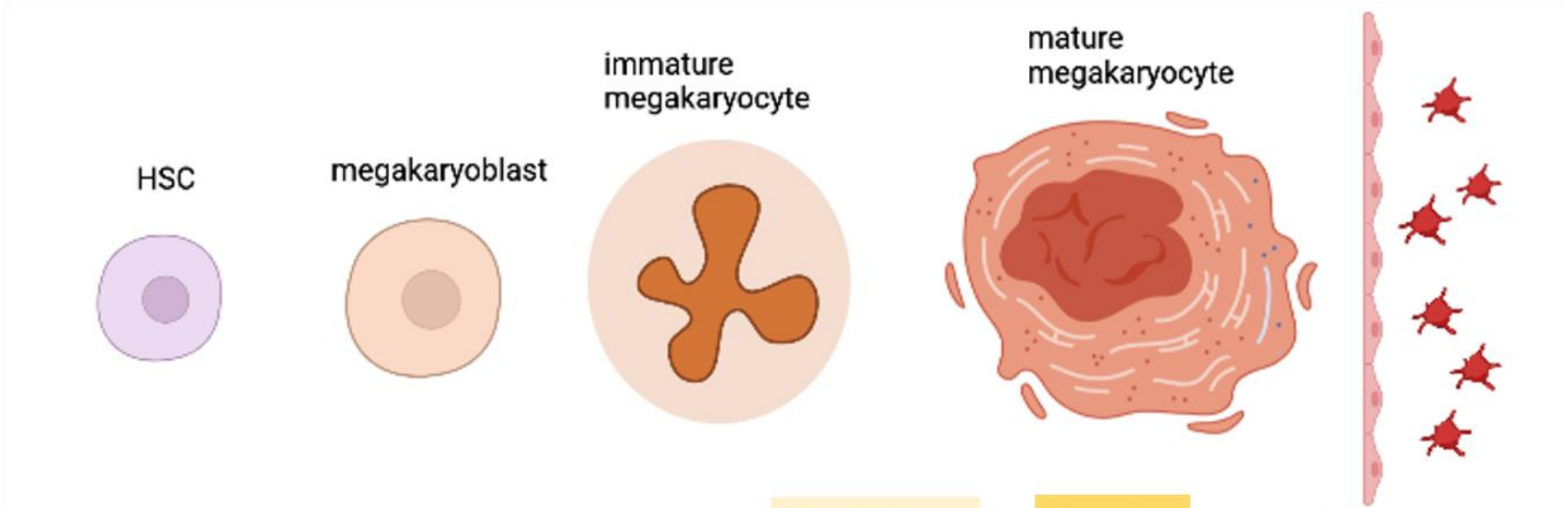
Initial absence of thrombocytopenia which developed over time
Mucocutaneous bleeding
Defects in platelet aggregation
Normal platelet size





Saultier et al, JTH 2021

IT linked to the mutations in cytoskeleton regulators and signaling



TPO/MPL signaling

THPO
MPL
ANKRD26

Transcription factors

GATA1
FLI1
GFI1B
RUNX1
ETV6
IKZF5
HOXA11
MECOM

Splicing factor

RBM8A

Metabolism granule biogenesis, ion channels

NBEAL2
KDSR
ABCG5
ABCG8
CYCS
STIM1
TRPM7

Cytoskeleton

MYH9
TUBB1
ACTN1
FLNA
TPM4
DIAPH1
CDC42
WAS
ARPC1B
FYB
WIPF1

Receptor signaling

GP1BA
GP1BB
GP9
ITGA2B
ITGB3
SLNF14
PRKACG
VWF
SRC
PTPRJ
MPIG6B

Sialylation

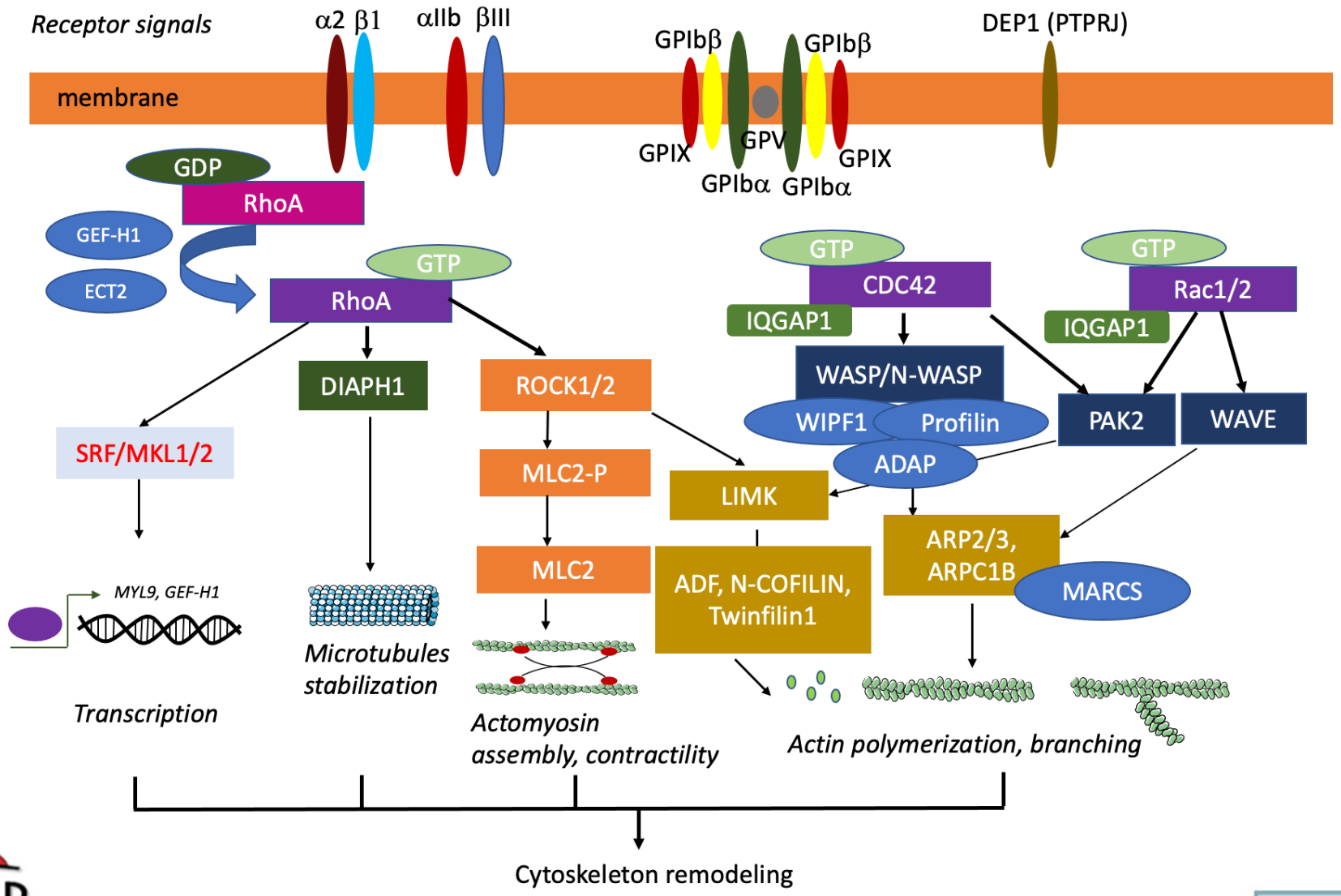
GNE
SLC35A1
GALE

Large (giant) platelet size

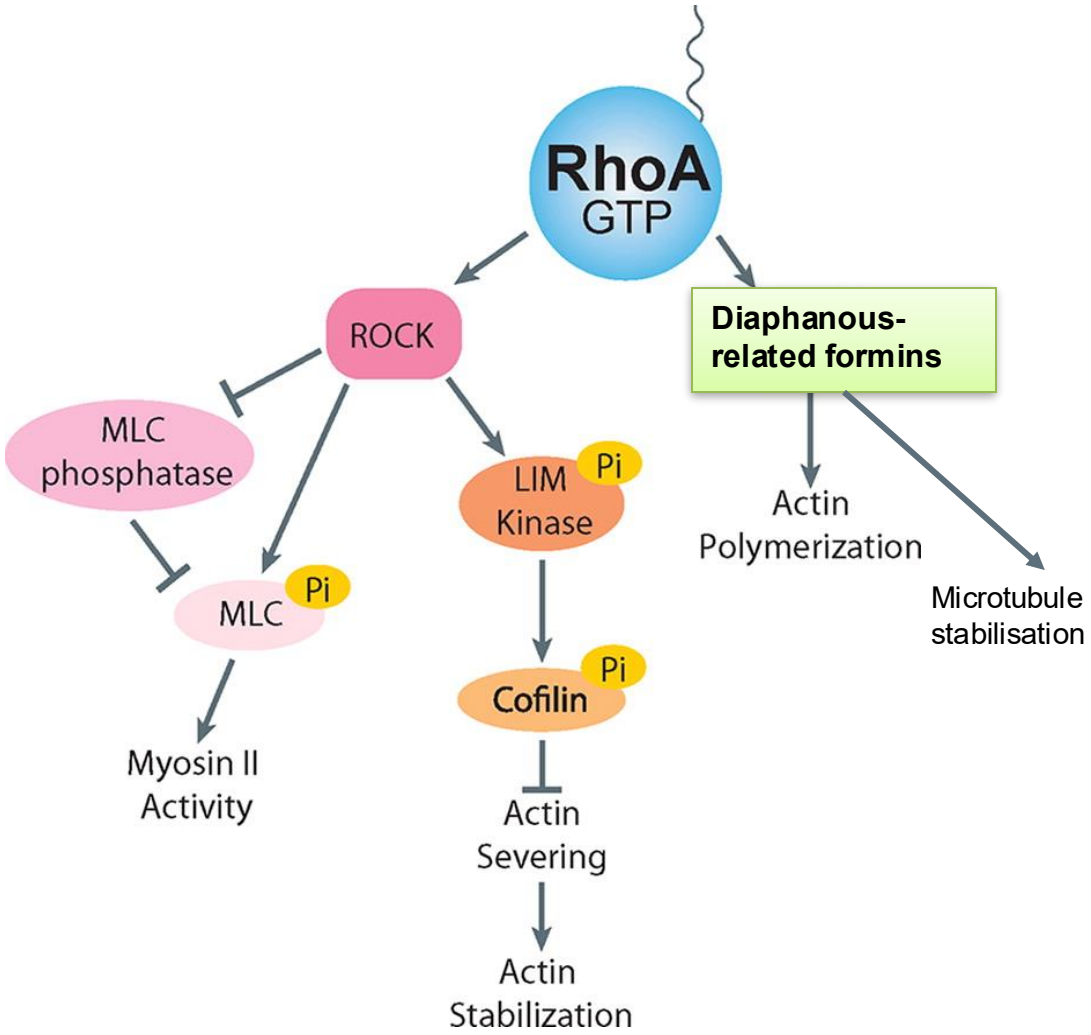
Small platelet size

Normal platelet size

GP receptor signaling



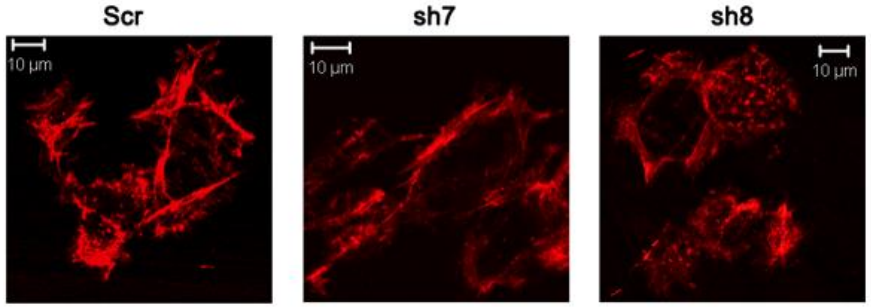
RhoA effectors

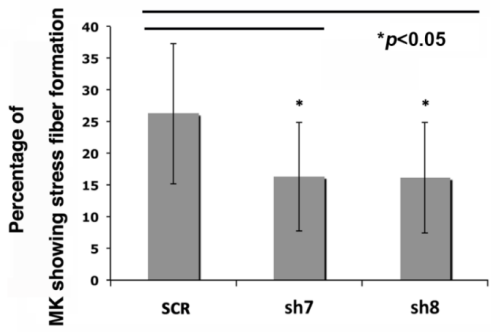


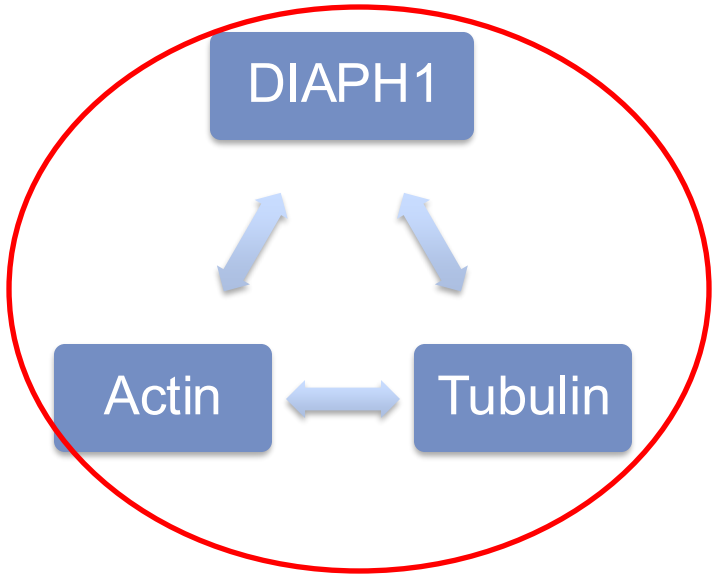
DIAPH1 negatively regulates proplatelet formation

- **DIAPH1 inhibition decreases stress fiber formation**

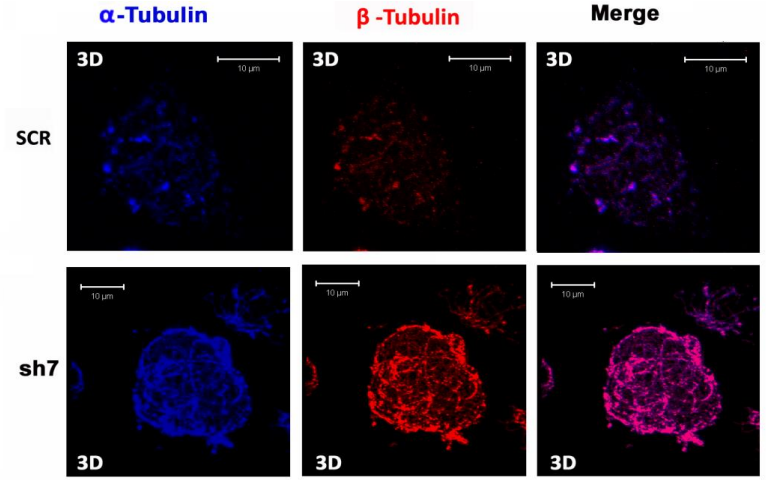
- adhesion on collagen I



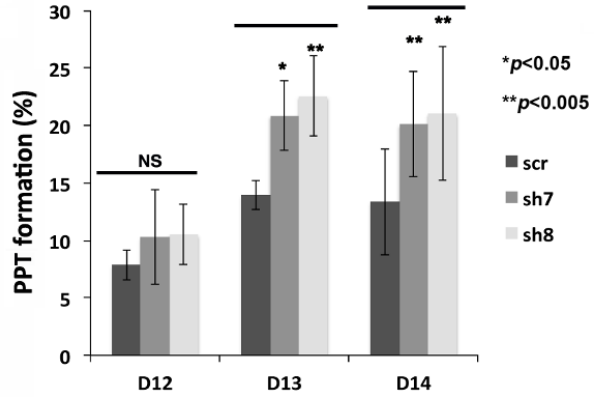




- **DIAPH1 inhibition accelerates microtubule elongation**



5 µM Nocodazole; 5 min



DIAPH1 could control proplatelet formation by regulating the balance between actomyosin complex and microtubule system.

Pan et al, Blood, 2014

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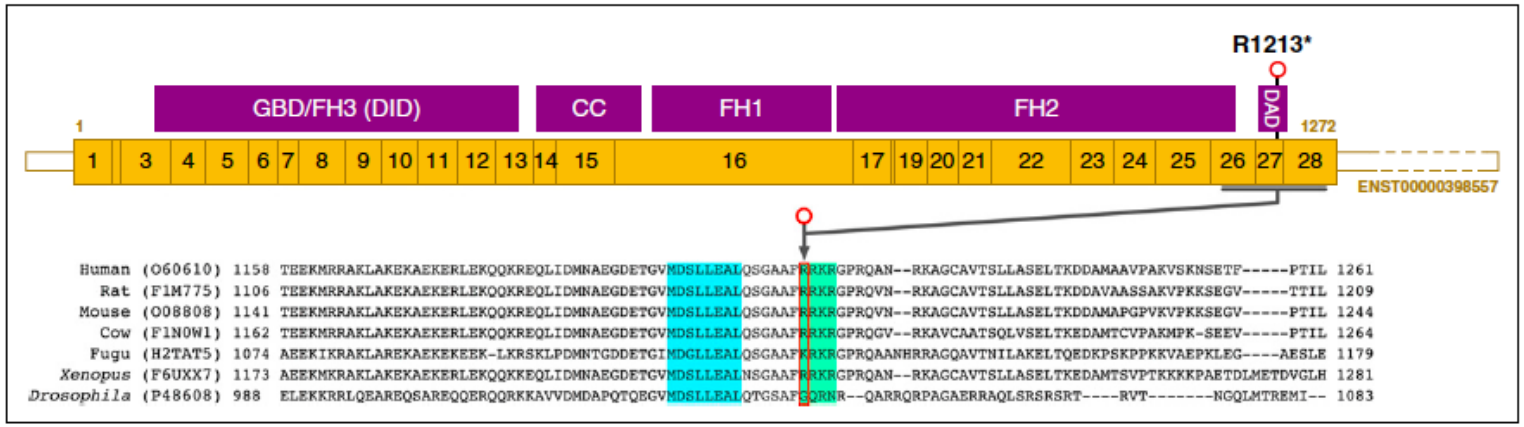
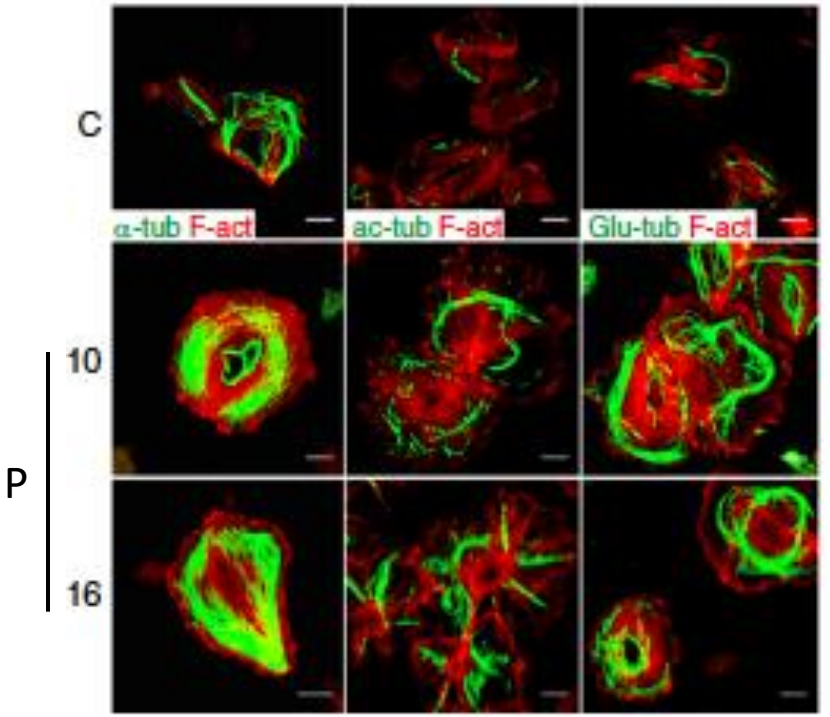
DIAPH1 and macrothrombocytopenia

- DIAPH1 R1213* variant predicting partial truncation of the DIAPH1 diaphanous autoregulatory domain was reported in 2 unrelated pedigrees with macrothrombocytopenia and sensorineural hearing loss

- increased filamentous actin and stable microtubules, indicating constitutive activation of DIAPH1





Stritt et al, Blood, 2016

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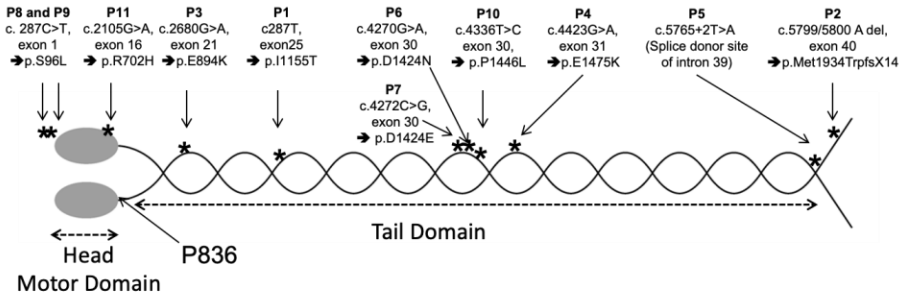
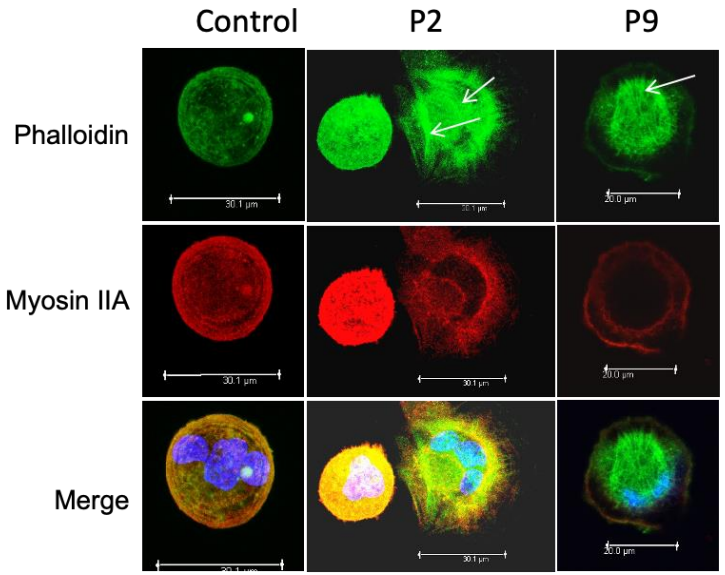
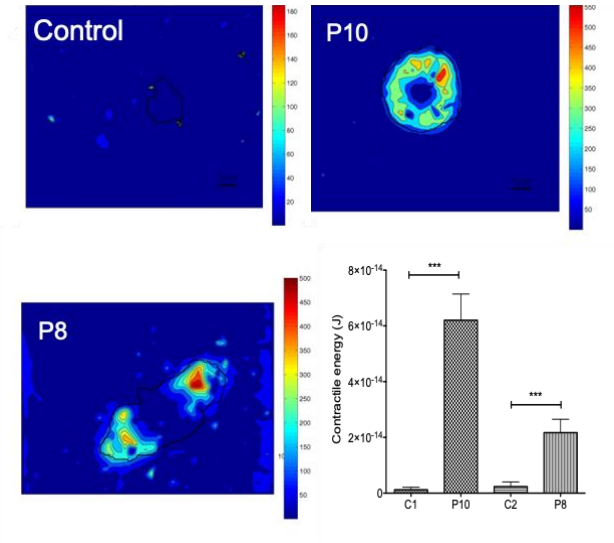
MYH9-related disease

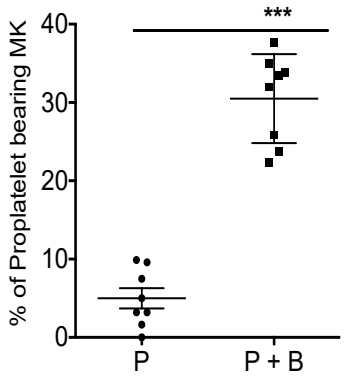


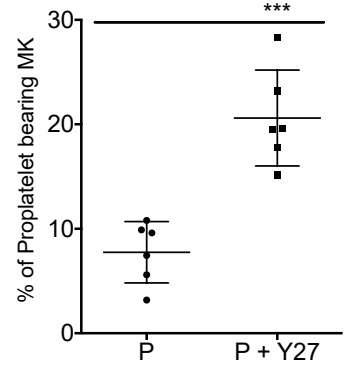


Traction force microscopy



Inhibition of MYH9 ATPase activity and ROCK1/2





restores proplatelet formation

Chen Y et al, JTH, 2013

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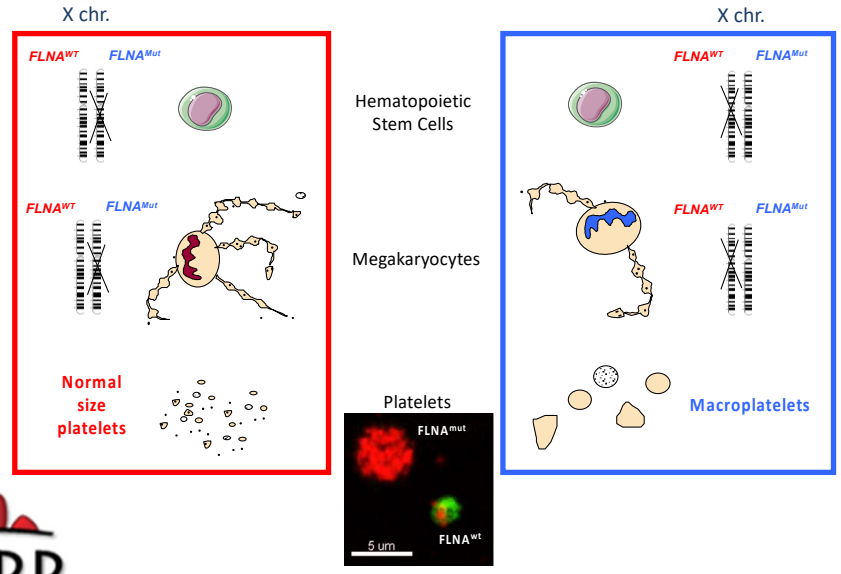
Filaminopathy A

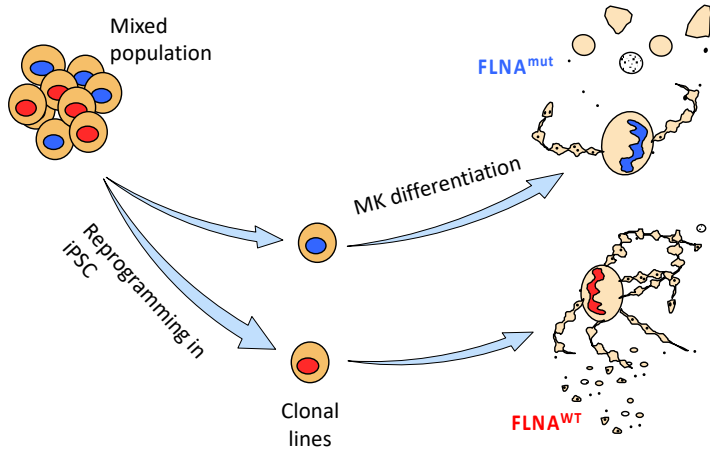
- X-linked – Hemizygosis is lethal
- Female patients: heterozygous mutations/deletions in *FLNA* gene localized on chromosome Xq28 encoding for cytoskeleton binding protein
- Multiple clinical manifestations including **macrothrombocytopenia**

Working hypothesis: random X inactivation during embryogenesis



Nurden et al, Blood, 2011

Isogenic iPSC model



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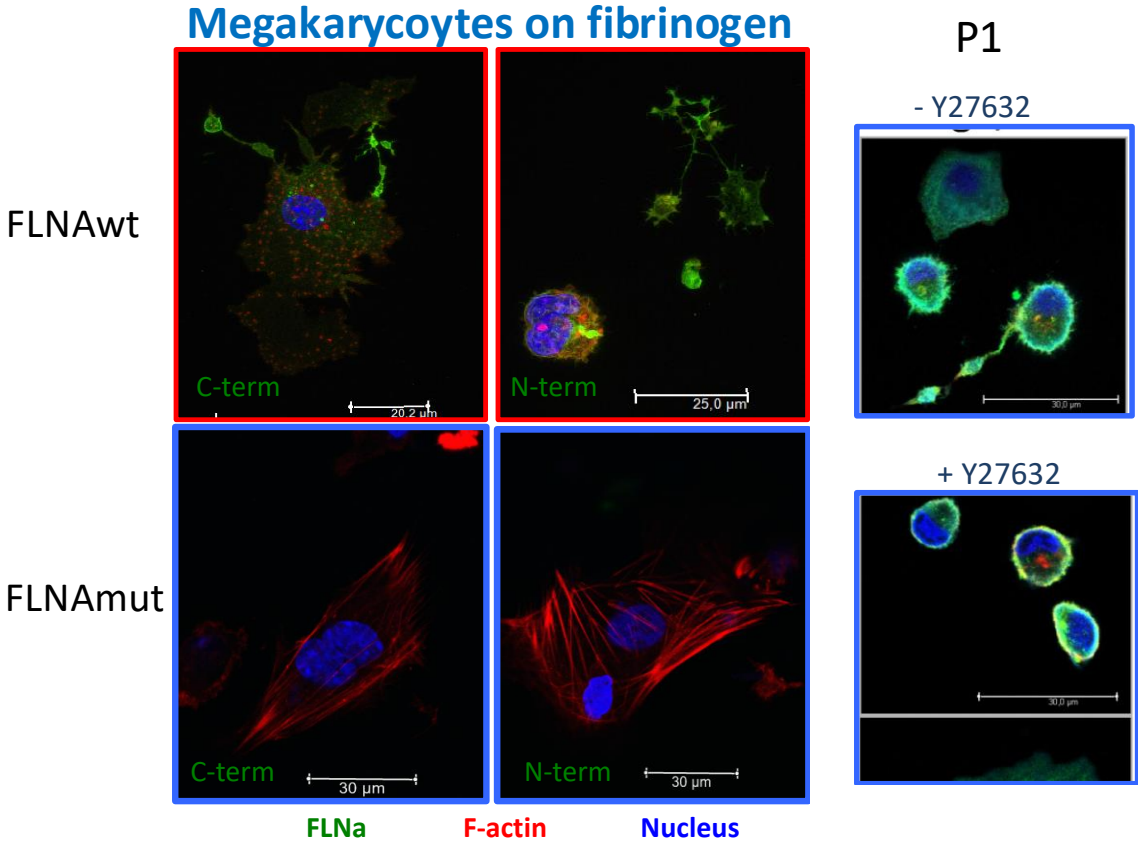
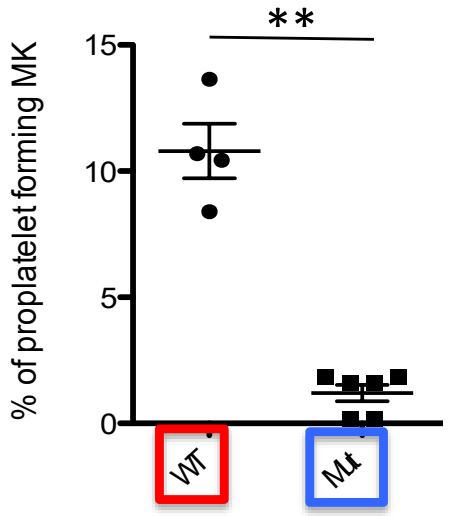
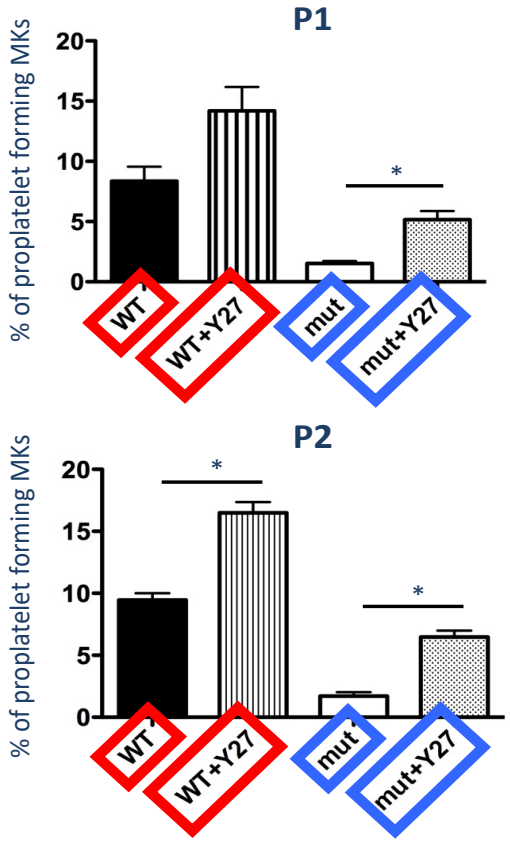
Inactivation of RhoA pathway: necessary for platelet generation

- Increased stress fiber formation linked to the increased RhoA activation



- A deep defect in proplatelet formation in absence of FLNA: restored after RhoA/ROCK inhibition





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Donada et al. Blood 2019

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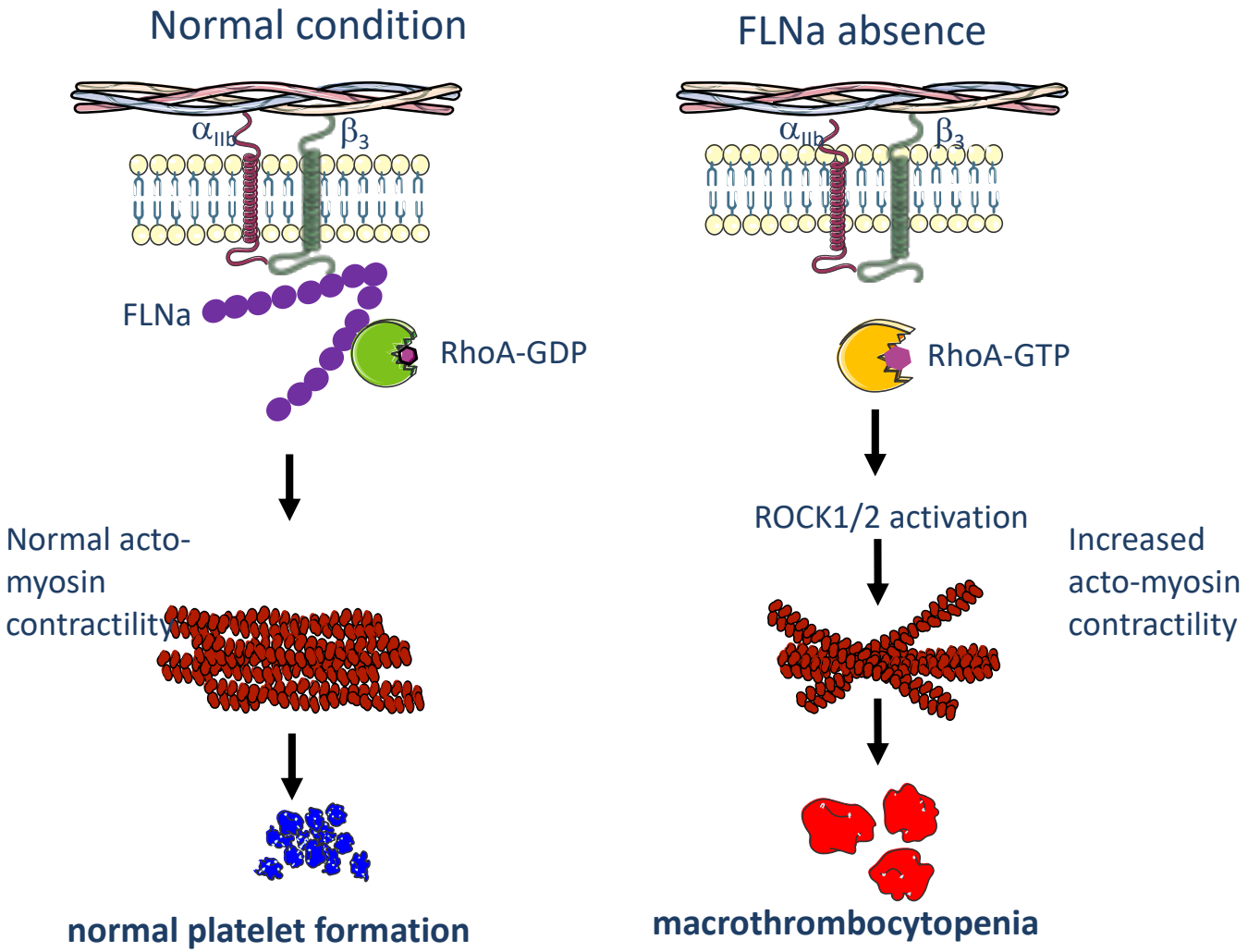
Physiopathological mechanism

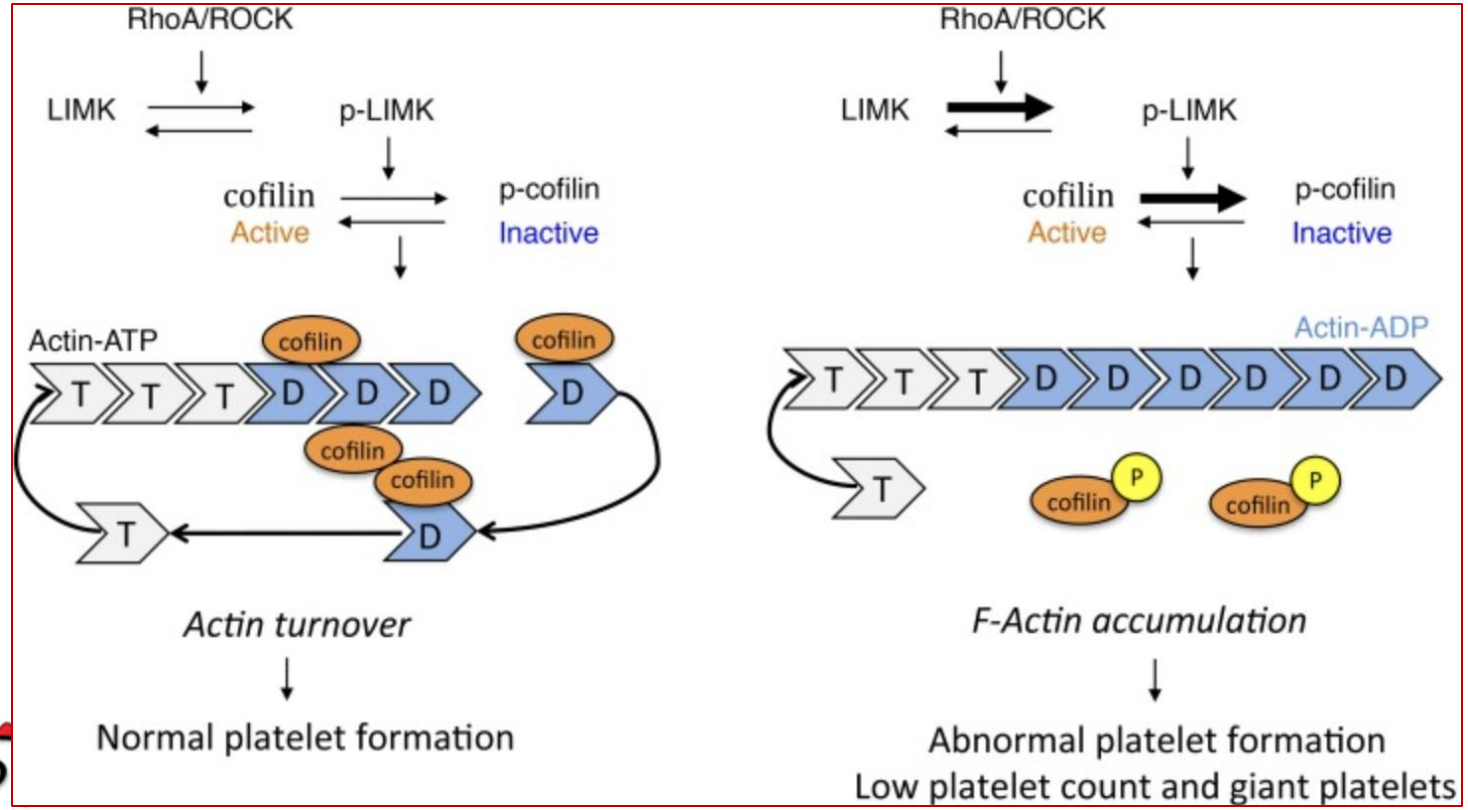


Donada et al. Blood 2019

Von Willebrand disease-type 2B

VWD-type 2B disease:

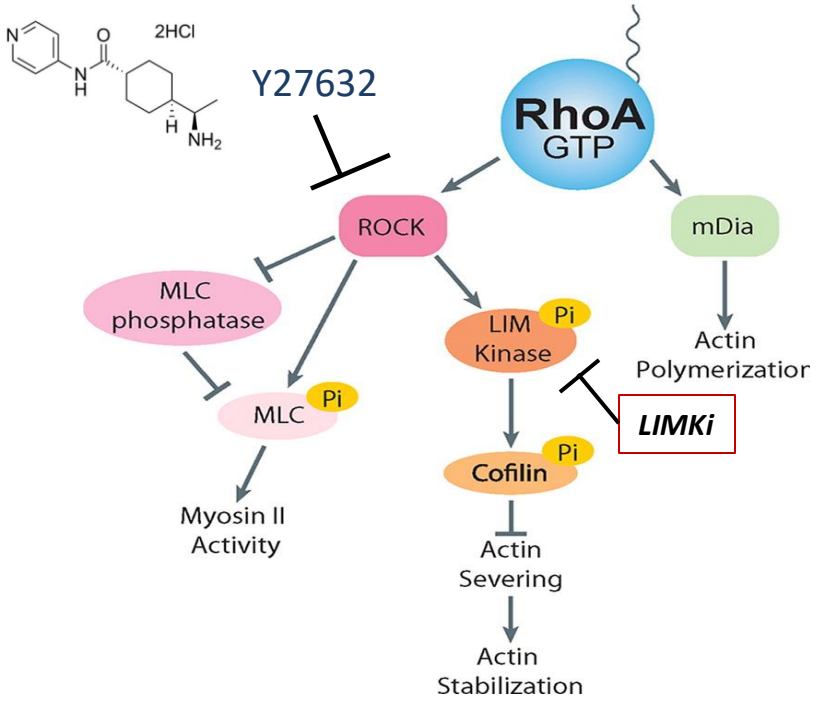
- gain-of function mutation of von Willebrand factor (vWF) p.V1316M
- variable extents of bleeding
- severe macrothrombocytopenia
- thrombopathy



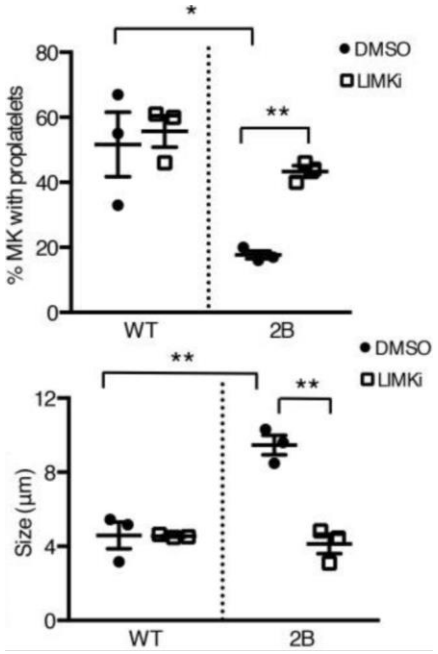
Kauskot A et al., JCI Insight, 2016

Von Willebrand disease-type 2B

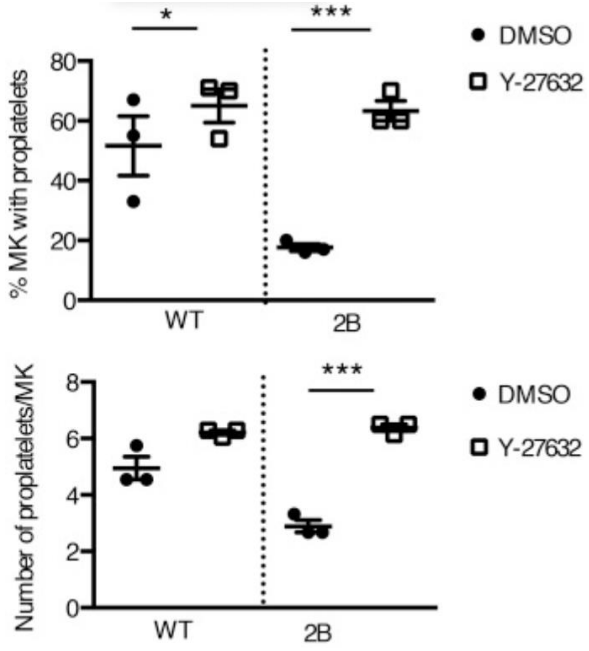
Y-27632: ROCK1/2 inhibitor



LIMK inhibition



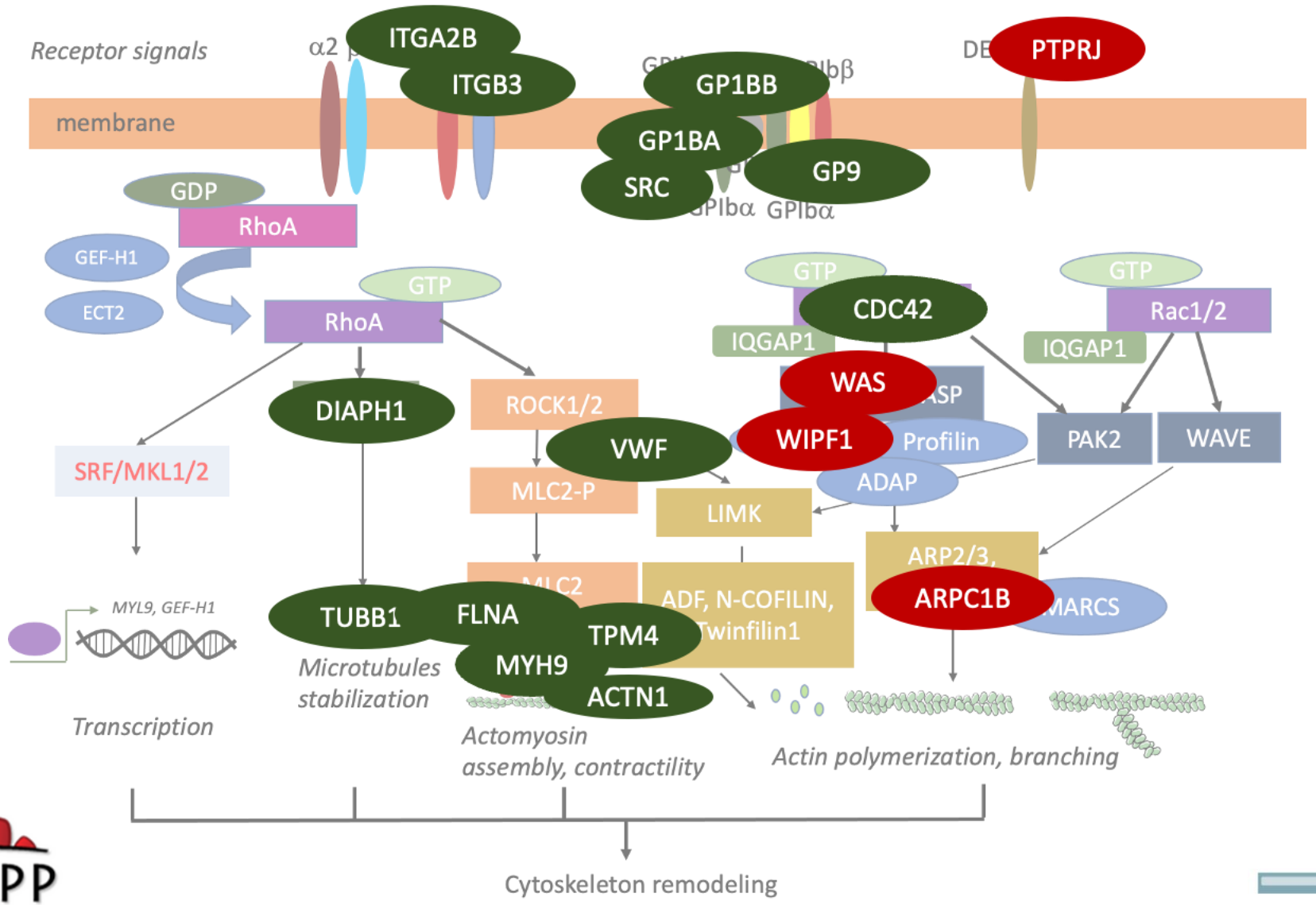
ROCK1/2 inhibition



Kauskot A et al., JCI Insight, 2016

Kauskot A et al., JCI Insight, 2016

GP receptor signaling



Take home messages

1. To incriminate each new mutated gene as responsible of IT, it is necessary to study its function in normal megakaryopoiesis
2. Study of pathophysiological mechanisms leads to the identification of new biomarkers that could be used for the determination of the variant pathogeneicity
3. Study of pathophysiological mechanisms permit to identify new deregulated pathways that could be targeted: the RhoA pathway seems to represent a good therapeutic target to treat macrothrombocytopenia
4. The use of 3D-bioreactor is helpful in predicting a drug response and could be used in personalized medecine

Overall, a better knowledge of pathophysiological mechansims leads to improved patient care

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