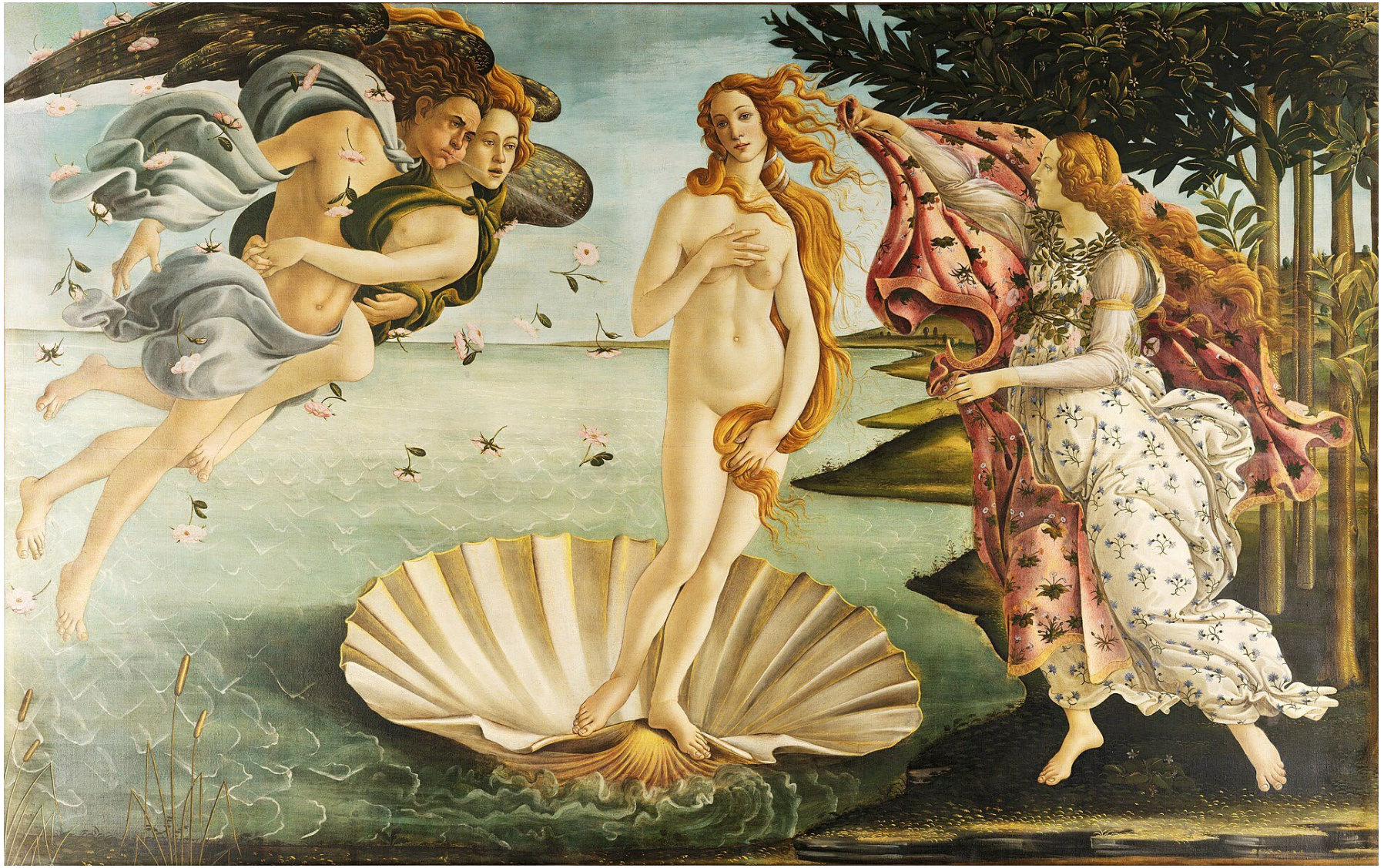
The Birth of Platelets



Ponlapat Rojnuckarin, MD PhD, Chulalongkorn University

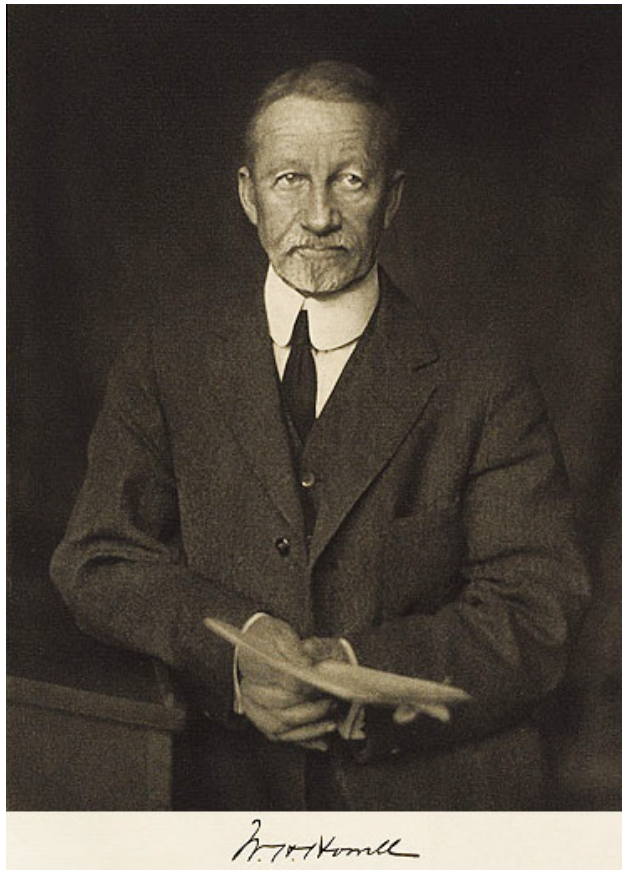
History

Giulio Bizzozero



1881 Platelets in Hemostasis

William Henry Howell



1890 termed Megakaryocyte

James Homer Wright



1906 Mk produce platelets

Discoveries of Thrombopoietin in 1994



Ken Kaushansky
+ ZymoGenetics Team



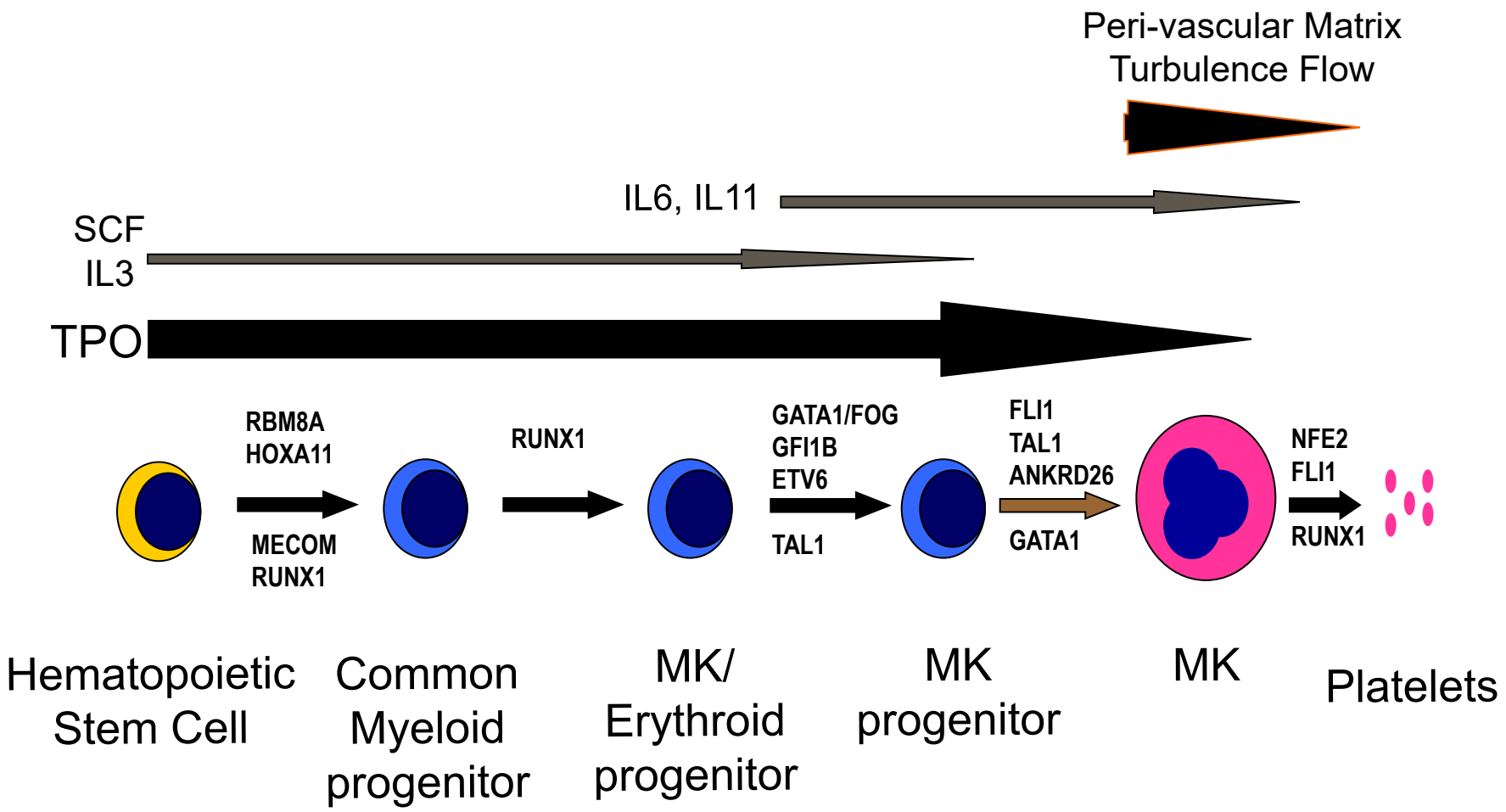
Fred de Sauvage
+Genentech Team

Amgen Team

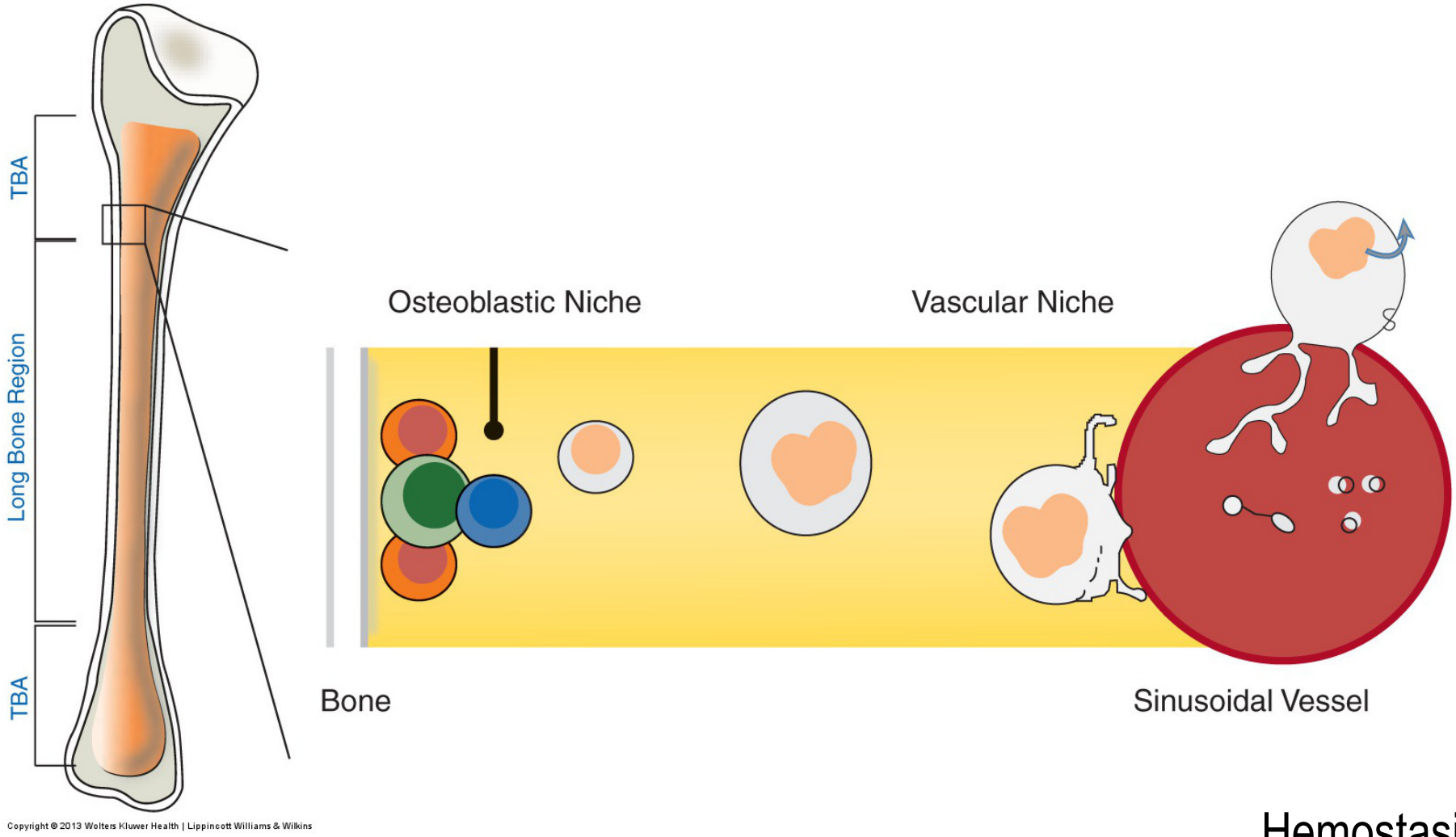
Kirin team

Nature 1994;369:565-8, Nature 1994;369:533–538, Cell 1994;77:1117–1124, FEBS Lett 1994;353:57–61

Control of Megakaryopoiesis by Extrinsic and Intrinsic factors



Proplatelet formation



Copyright © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins

Hemostasis and Thrombosis 2013

TPO or MPL mutation in mice and men





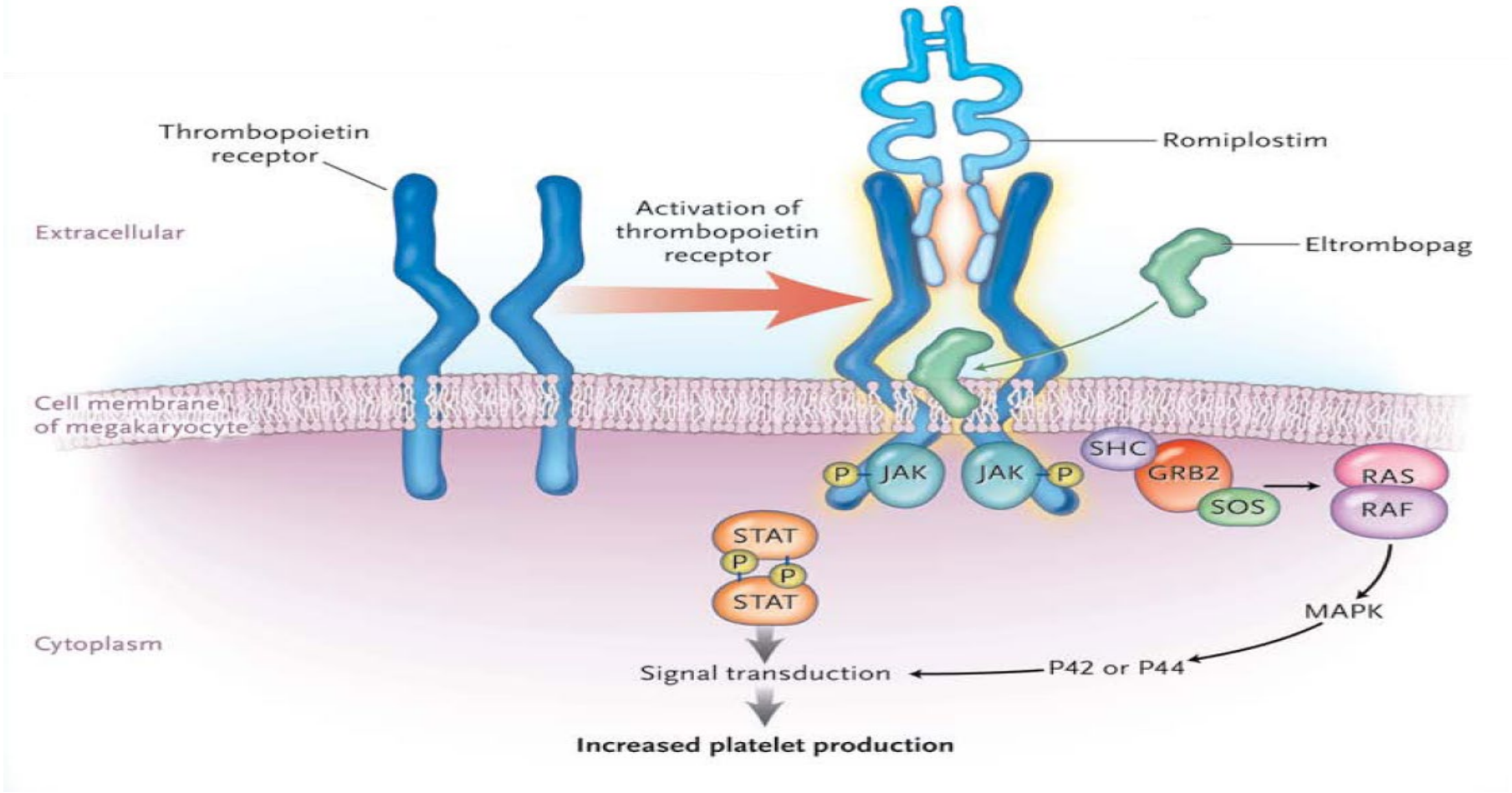
- Platelet ~100x 10^9/L (10% of normal)
- Decreased MK and MK progenitors
- Decreased erythroid and myeloid progenitors and Stem cells

Alexander WS 1996, Kimura S 1998, Solar GP 1998
N Engl J Med. 2012; 367:11-9.

- Congenital Amegakaryocytic Thrombocytopenia
- Congenital severe thrombocytopenia
- Absent megakaryocyte in Marrow
- Becomes Aplastic Anemia at 4-5 yrs
- MPL: Bone marrow transplantation
- TPO: TPO-RA

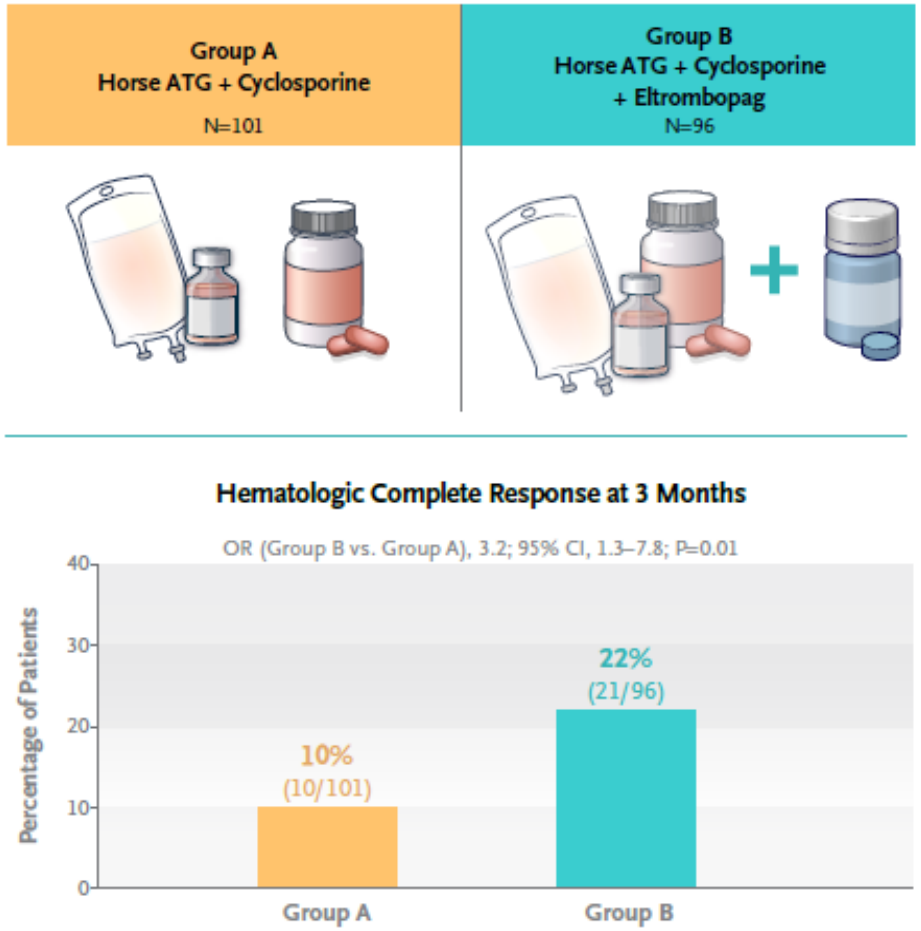
Br J Haematol 2000;110:441-8, Blood 2001;97:139-46
J Coll Physicians Surg Pak. 2014;24:285

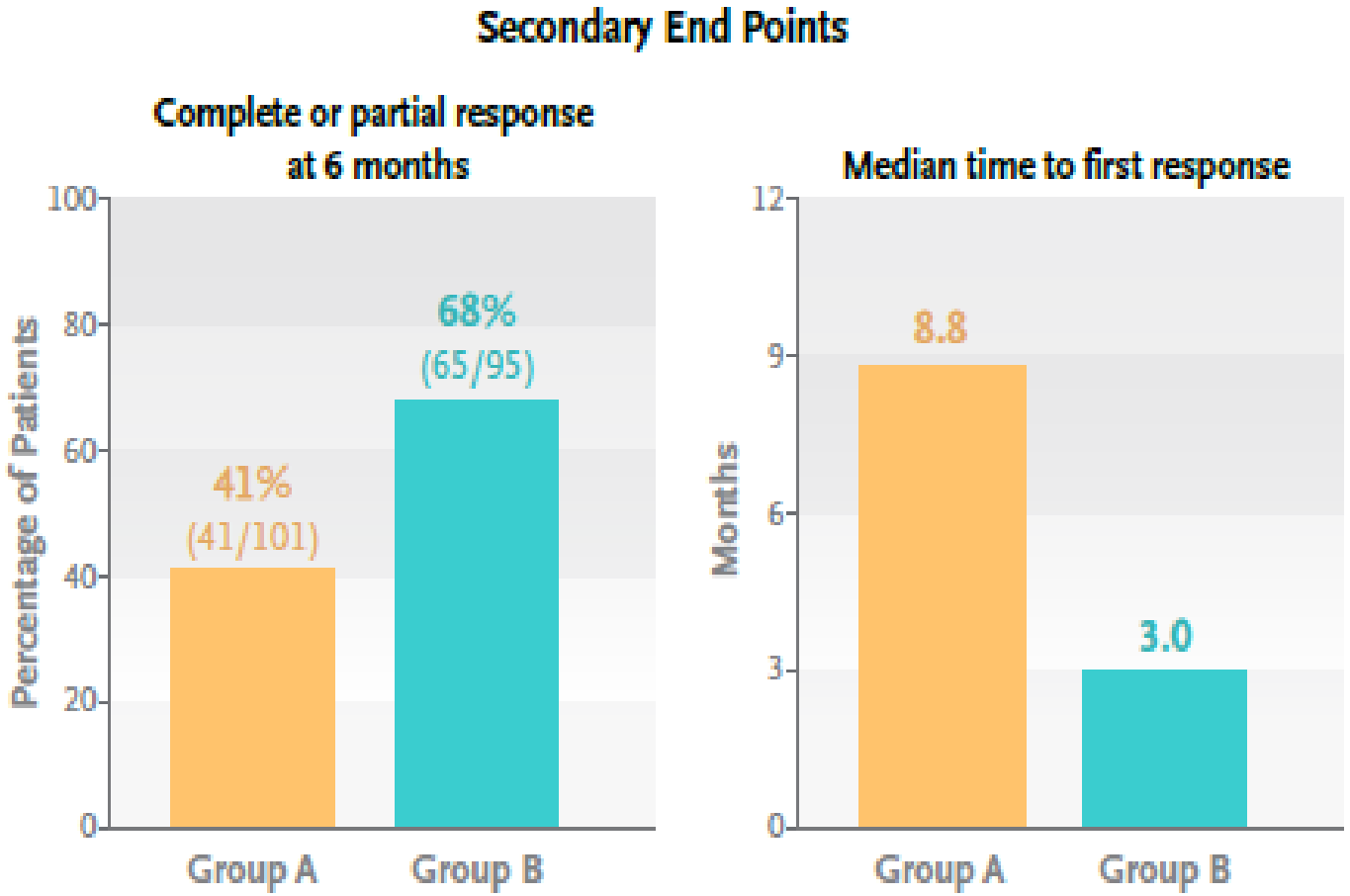
TPO receptor agonists for ITP



N Engl J Med 2011; 365: 734

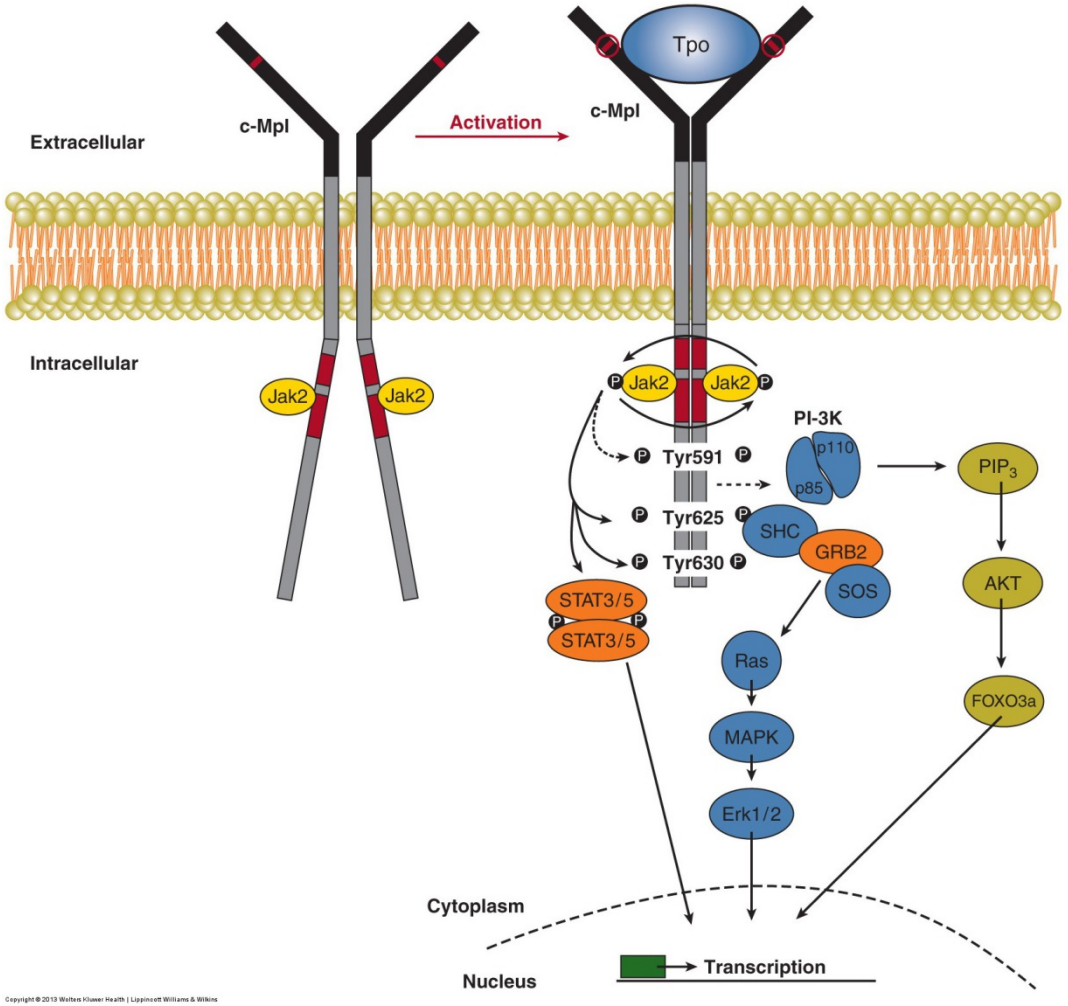
Thrombopoietin receptor agonist (eltrombopag) in aplastic anemia

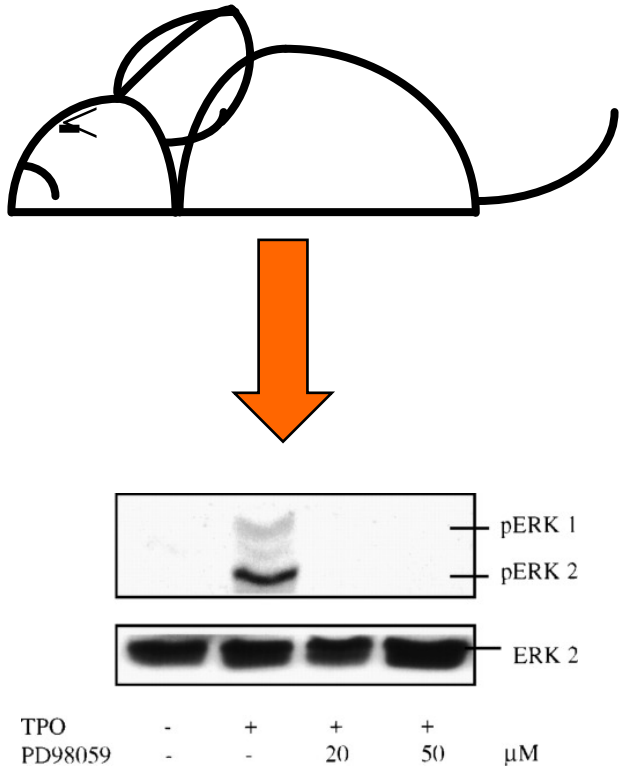


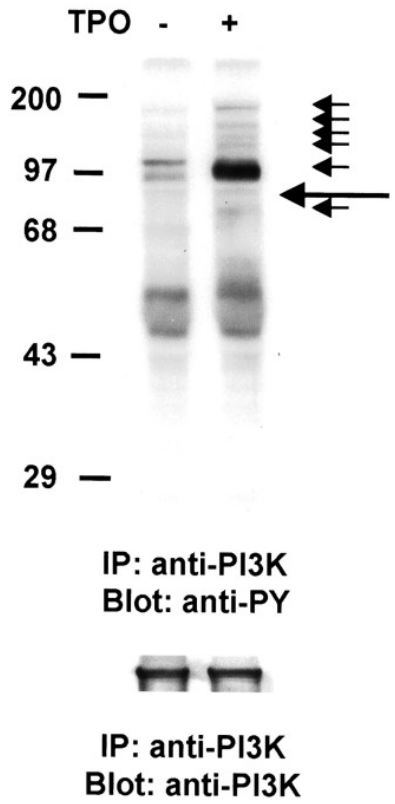


N Engl J Med. 2022 Jan 6;386(1):11-23

Thrombopoietin activates many signaling molecules.





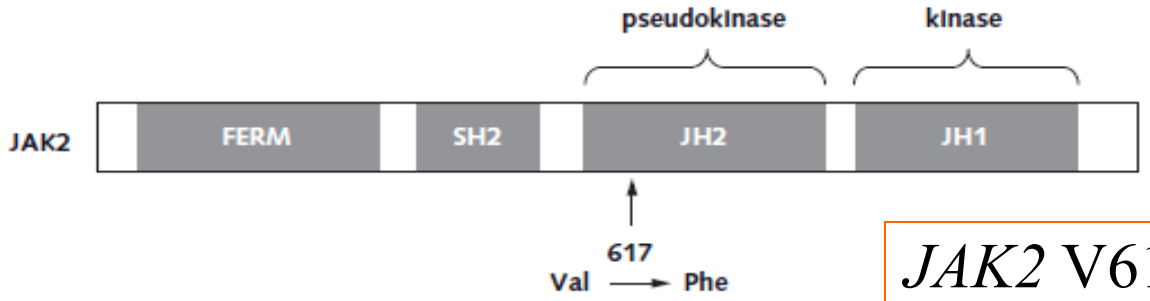


Blood. 1999; 94: 1273
J Biol Chem. 2001;276: 2494

Janus

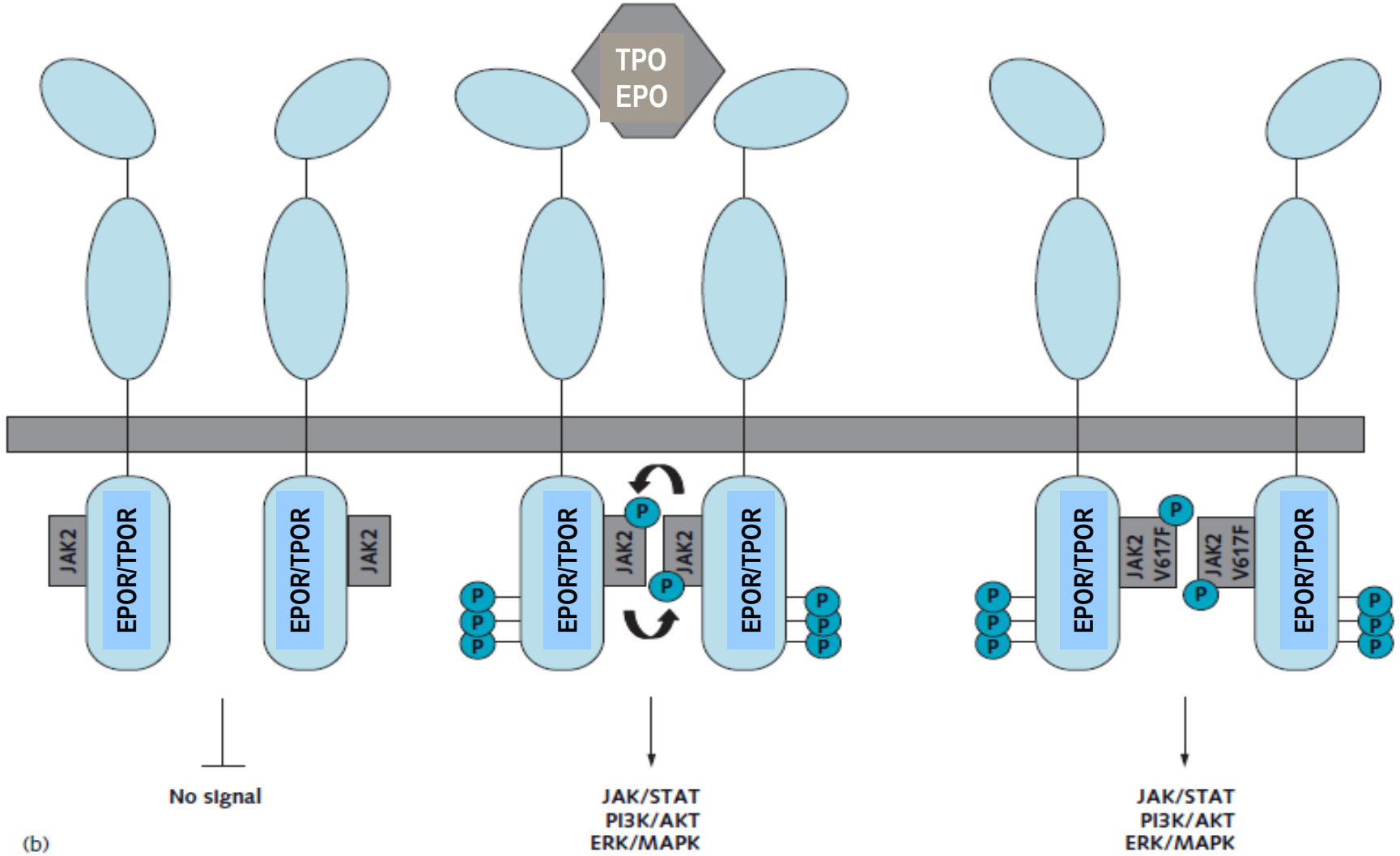


(a)



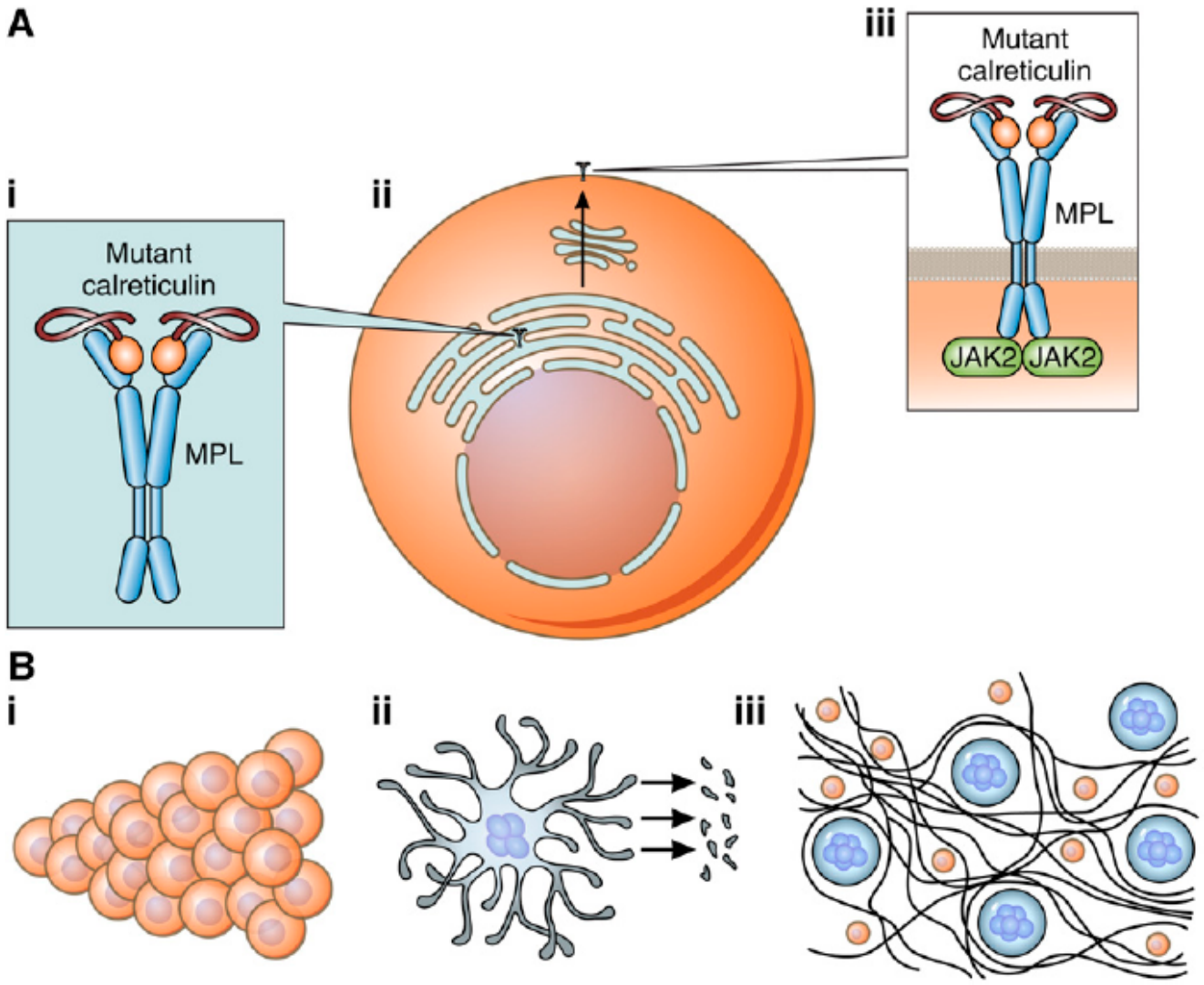
JAK2 V617F Mutation

EPO-R: PV
TPO-R: ET



(b)

CALR binds and activates TPO receptor

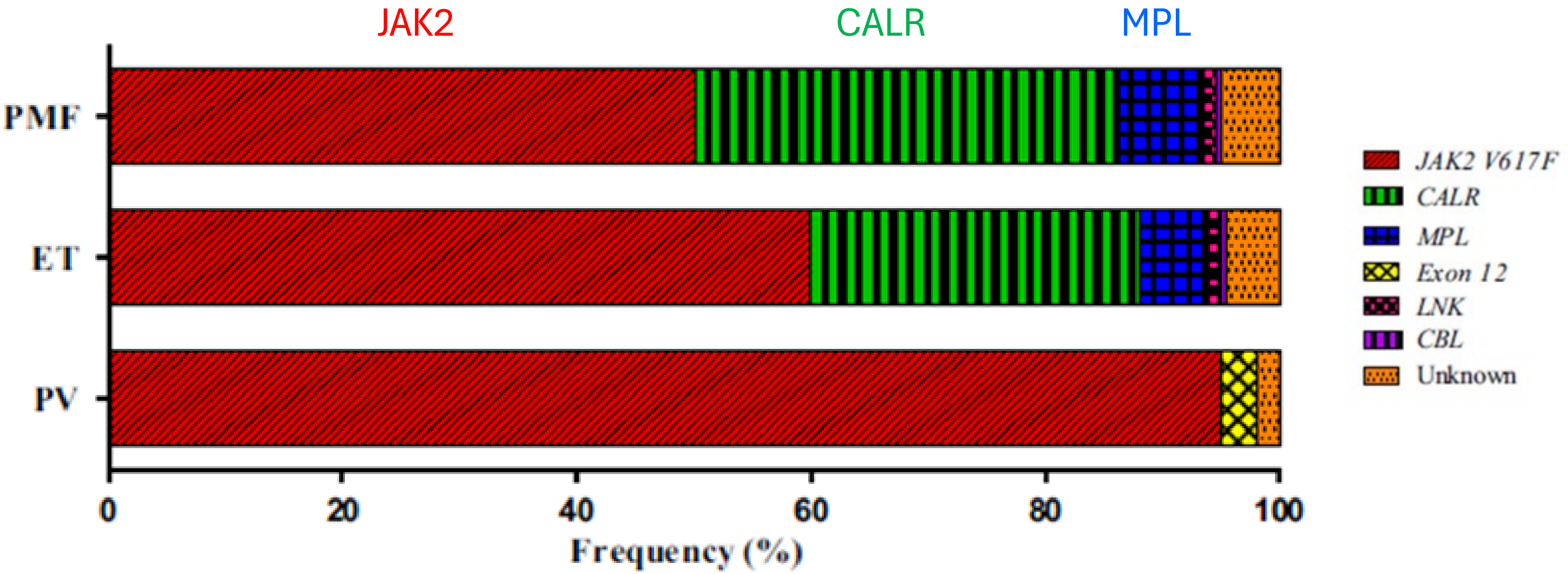


CALR is discovered by Whole exome sequencing of JAK2-negative MPN

CALR/MPL mutations
Only ET/MF, Not PV

Blood 2016; 127: 1219
Blood 2016; 127: 1317
Blood 2016; 127: 1325
Blood 2016; 127: 1307

Disease-defining Driver Mutations

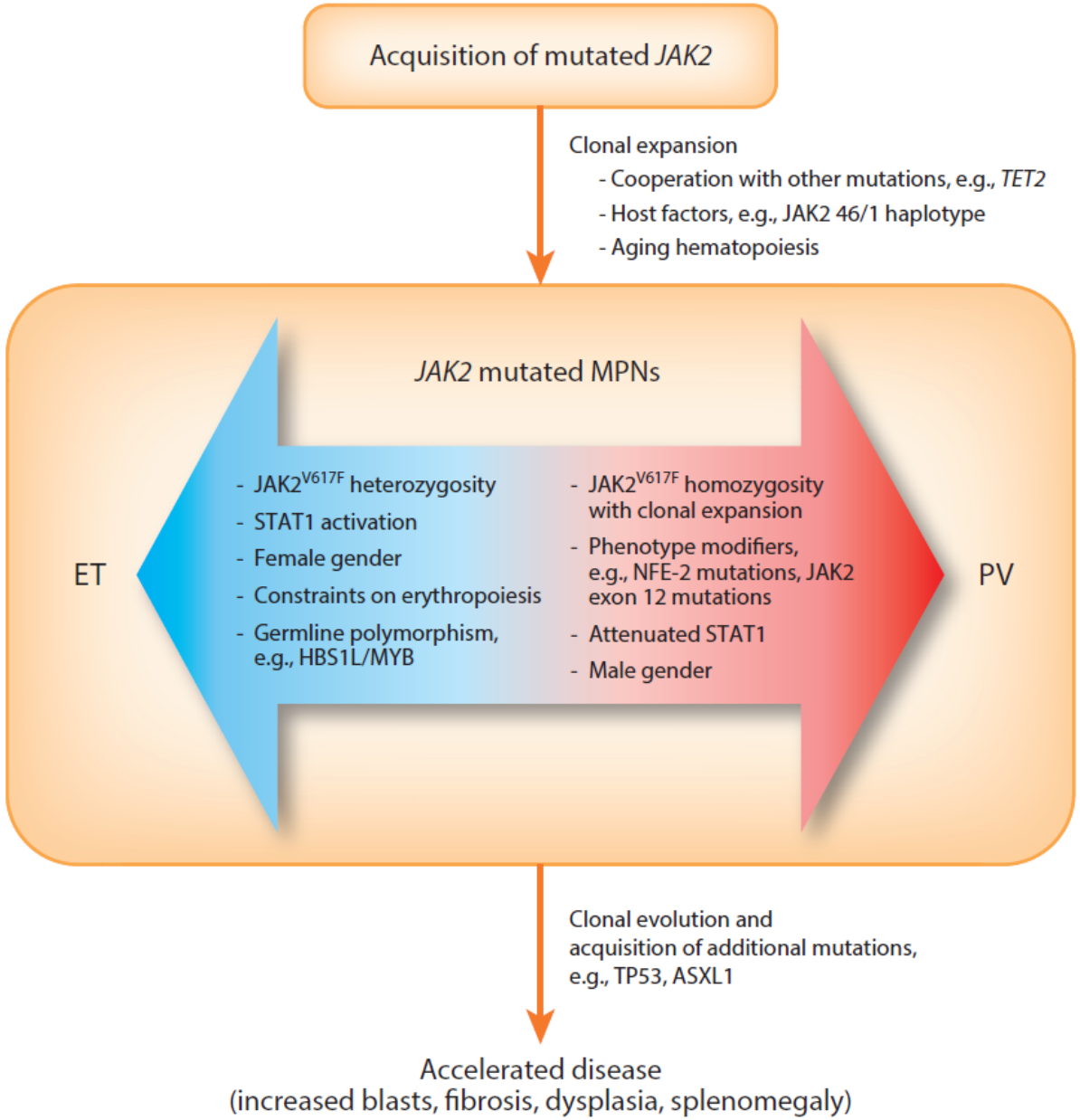


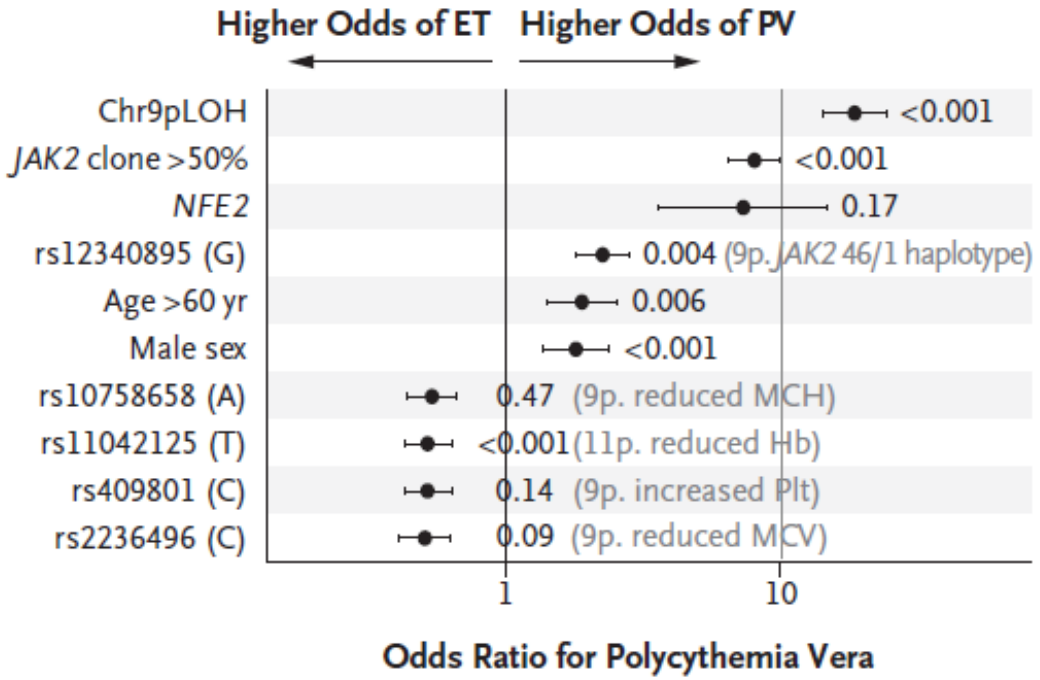
5-10% Triple negative MPN

LNK: Negative regulator of JAK signaling
CBL: E3 Ub ligase for mutant kinases

Blood. 2017;129: 667-679

ET vs. PV

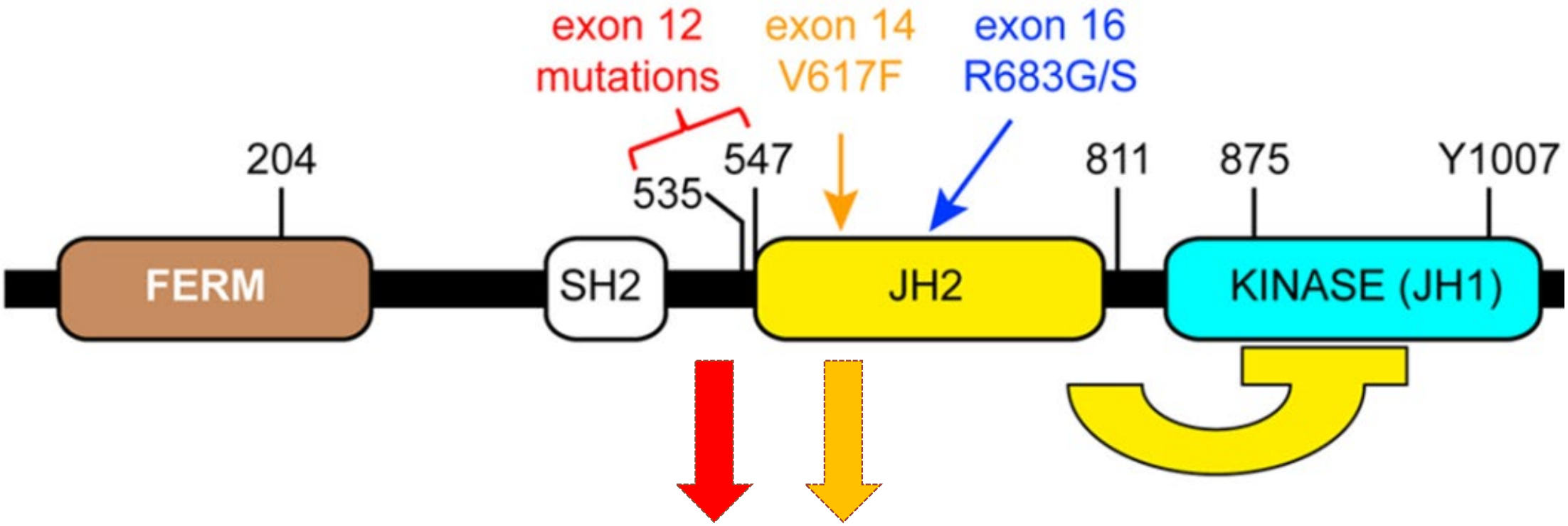




Ann Rev Pathol Mech Dis 2016; 11: 101
N Engl J Med. 2018; 379: 1416-1430.

Why JAK2V617F causes thrombocytosis, while JAK2 exon12 mutations cause erythrocytosis?

Signal differences?



Erythrocytosis

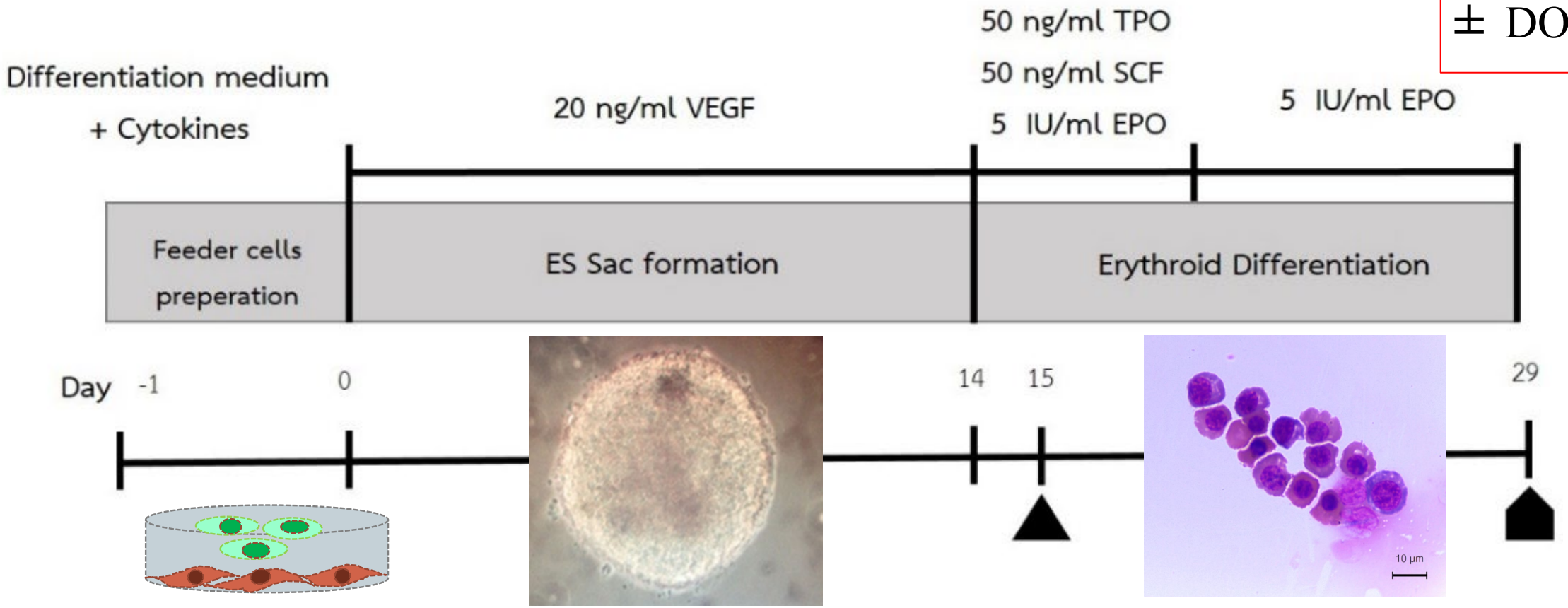
Heterozygous (Containing normal JAK2): ET
Homozygous (No normal JAK2): PV

iPS cell model for JAK2V617F vs. JAK2Exon12

Human Induced Pluripotent Stem cells (*OCT2/SOX2/KLF4/CMYC* reprogramming)

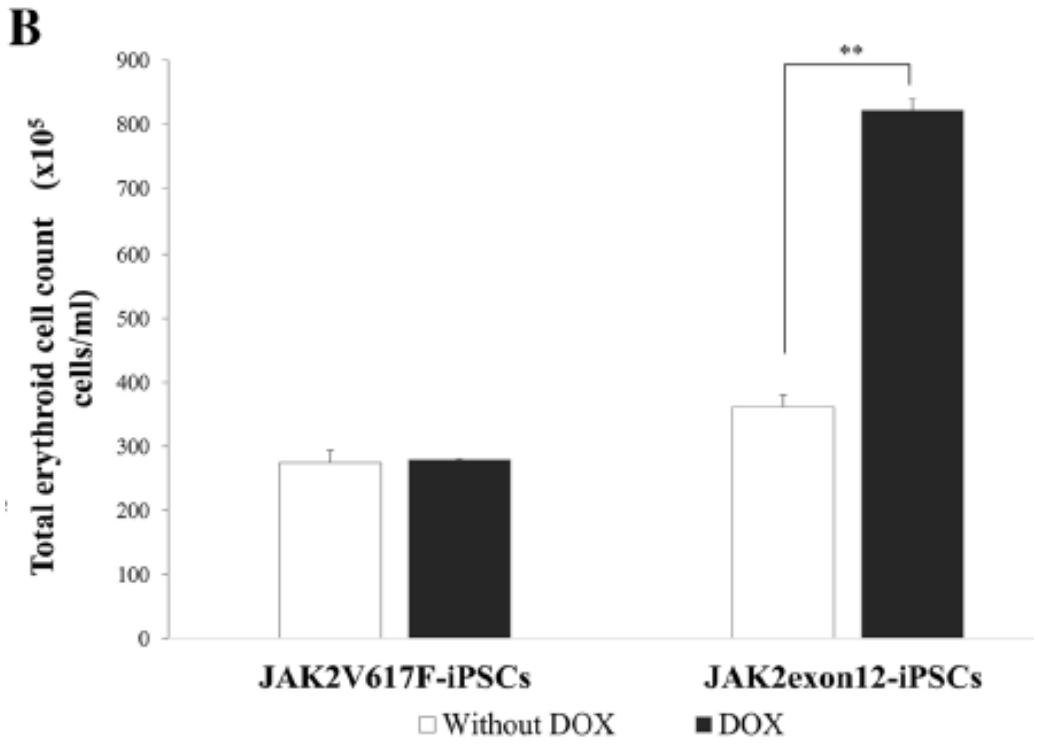
Retroviral transduced with JAK2 mutants under Doxycycline-inducible system

± DOX

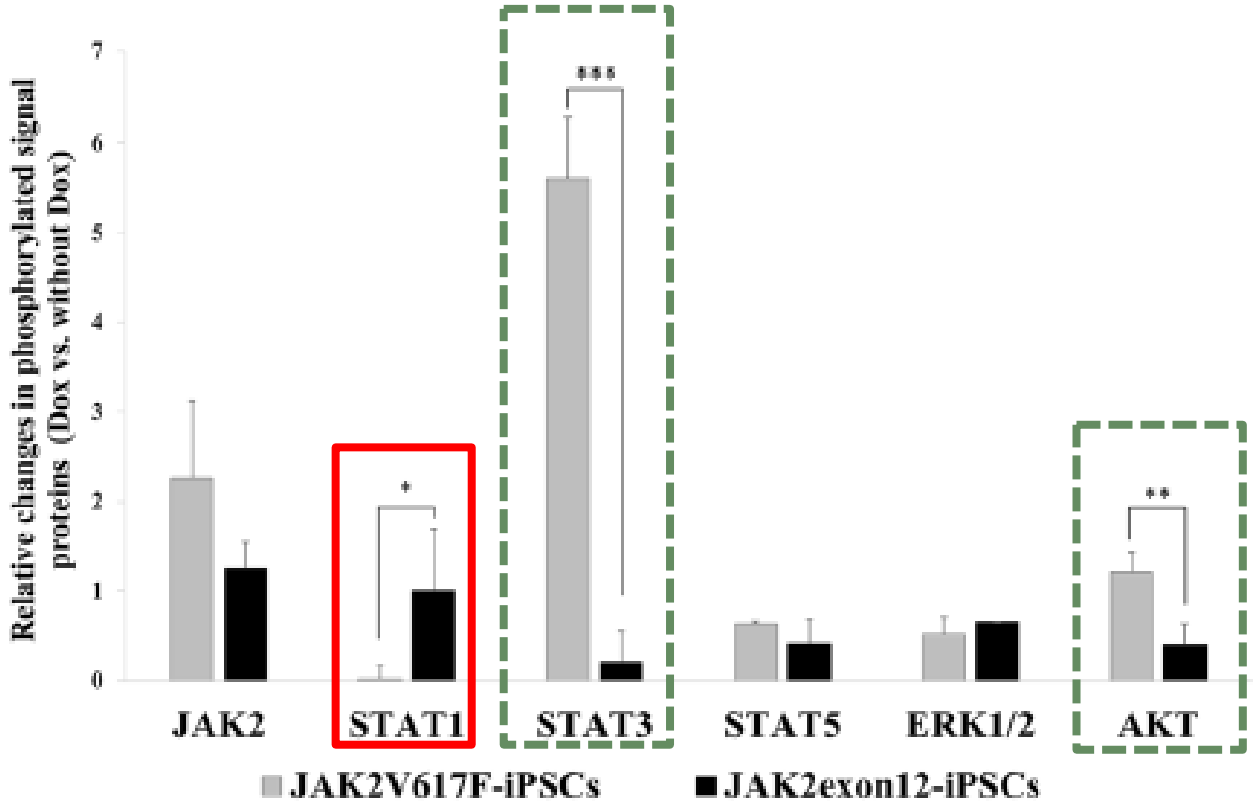


Nungruthi Nilsri et al. Sci Rep. 2021;11:5255

Distinct signaling of JAK2Exon12 vs. JAK2V617F in **Erythroid cells**



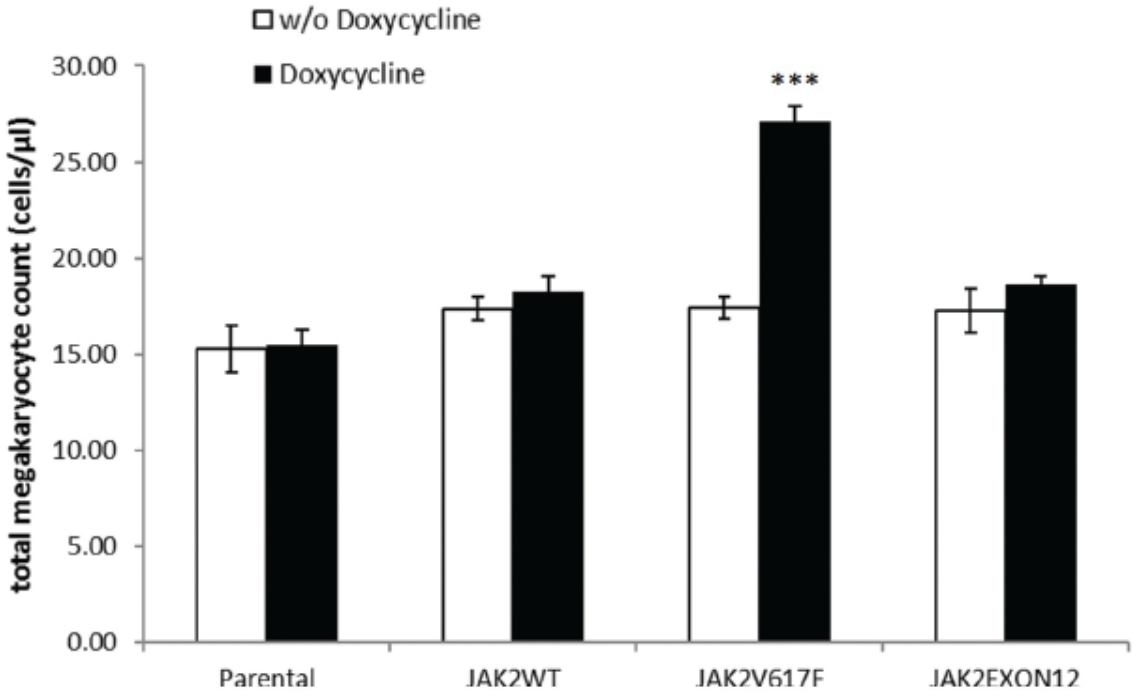
Total erythroid cells are increased
By JAK2 exon 12 mutation



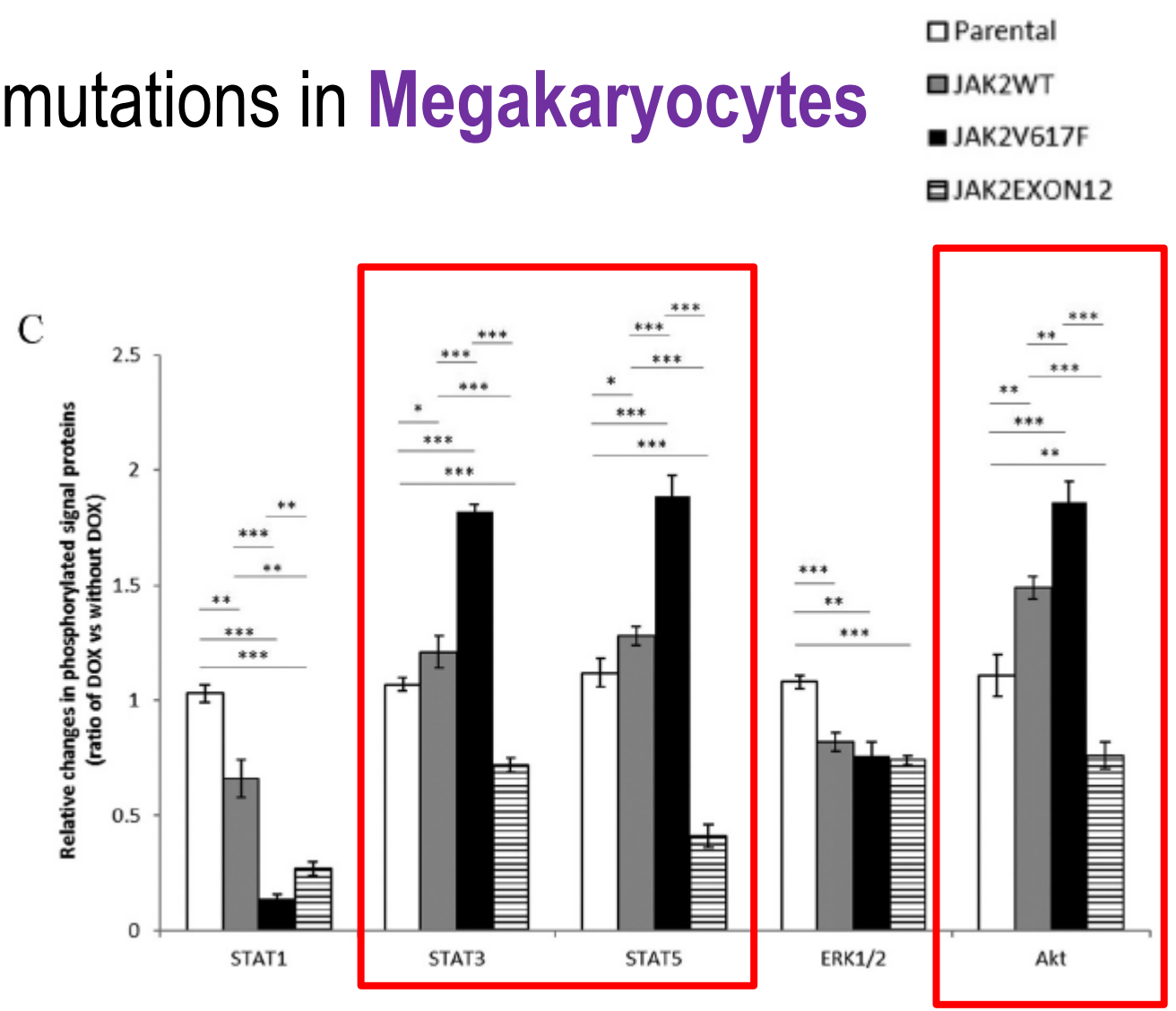
Protein phosphorylation

Nungruthi Nilsri et al. Sci Rep. 2021;11:5255

Distinct signaling of JAK2 mutations in **Megakaryocytes**



Total Megakaryocytes are increased
By JAK2V617F

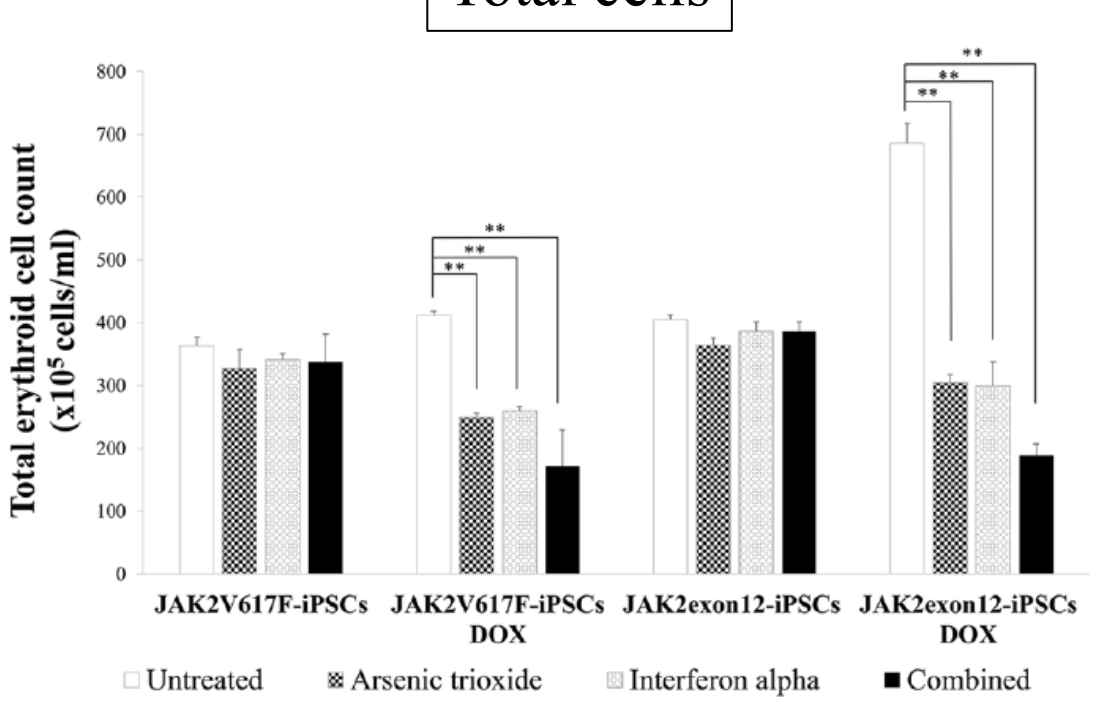


Protein phosphorylation

Jaturawat Pawinwongchai et al. Platelets. 2022; 33(5):700-708

Drug screening: Arsenic trioxide and Interferon target mutant JAKs

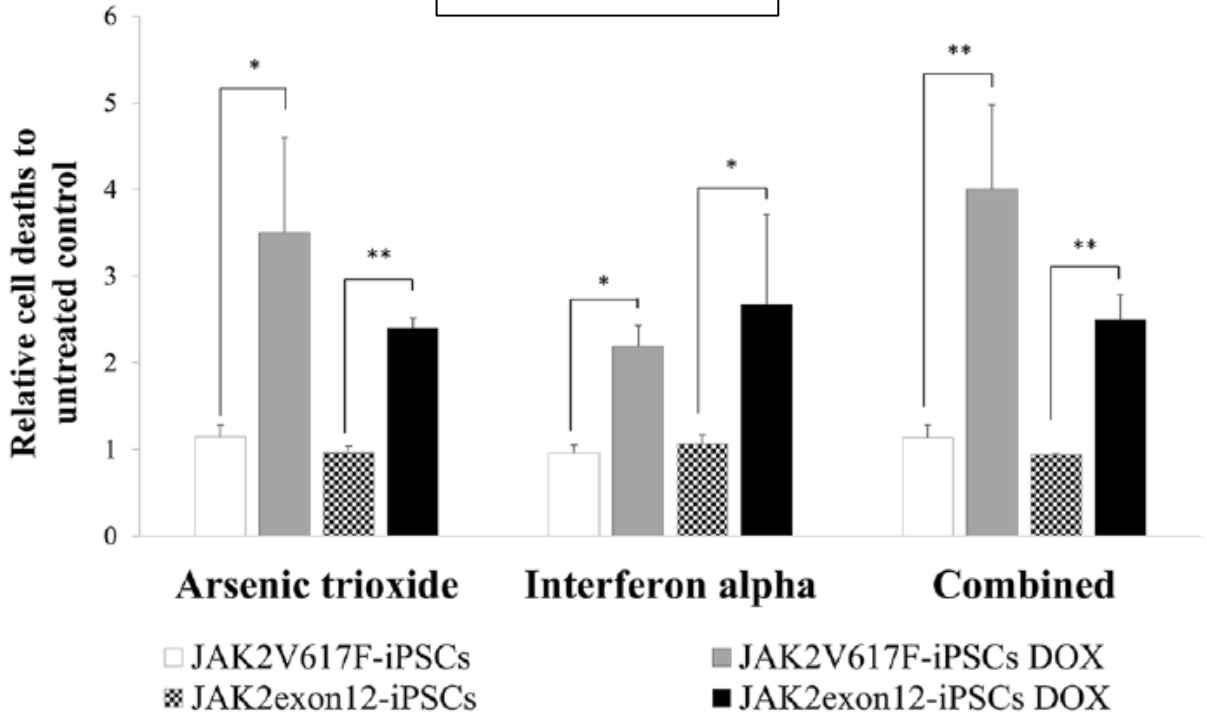
Total cells



Total erythroid cells

D

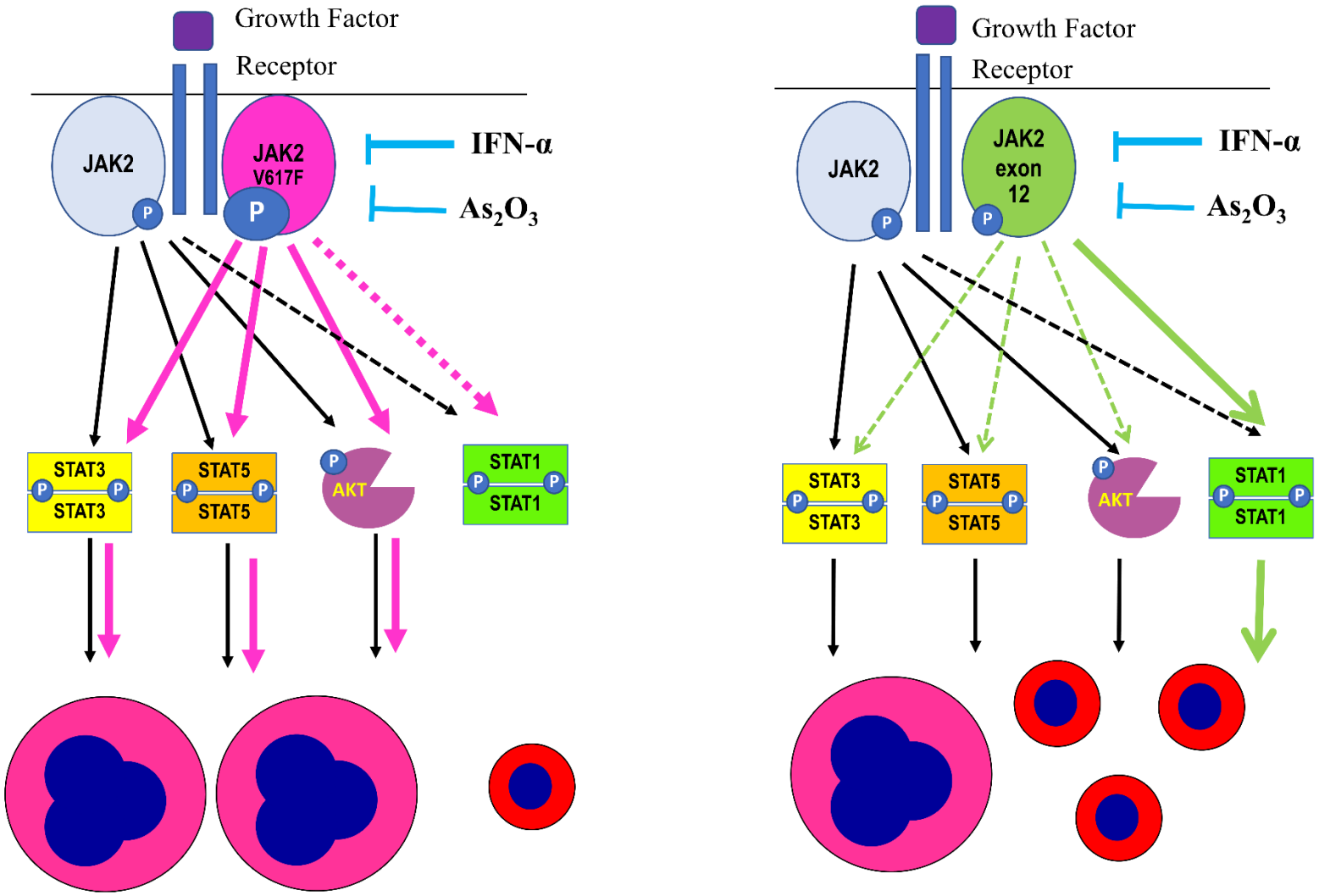
Cell Deaths



Cell Deaths

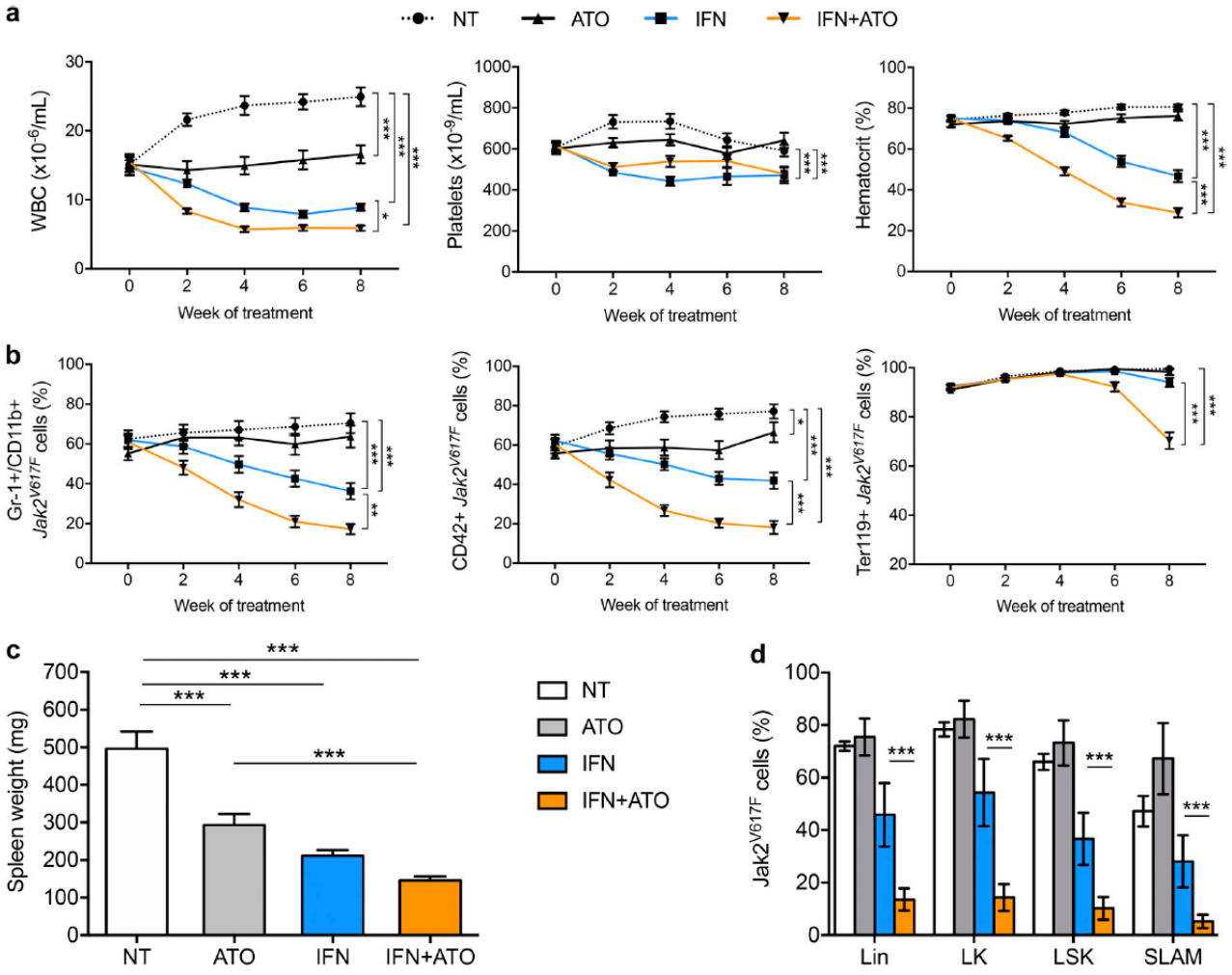
Nungruthi Nilsri et al. Sci Rep. 2021;11:5255

Molecular Mechanisms of JAK2 mutations



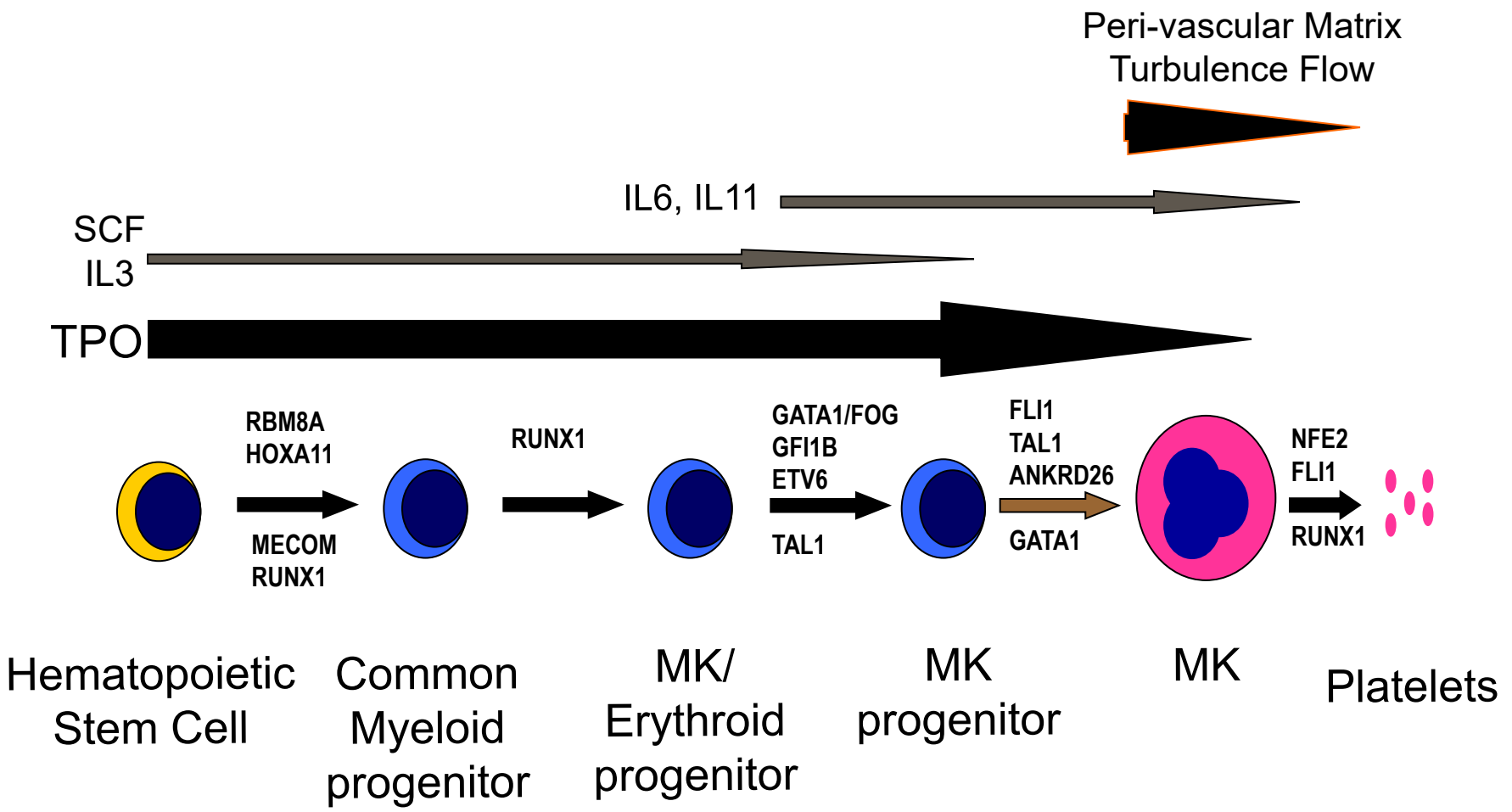
Nungruthi Nilsri et al. Sci Rep. 2021;11:5255, Jaturawat Pawinwongchai et al. Platelets. 2022; 33:700-708

IFN + ATO inhibited a JAK2V617F+ mouse model



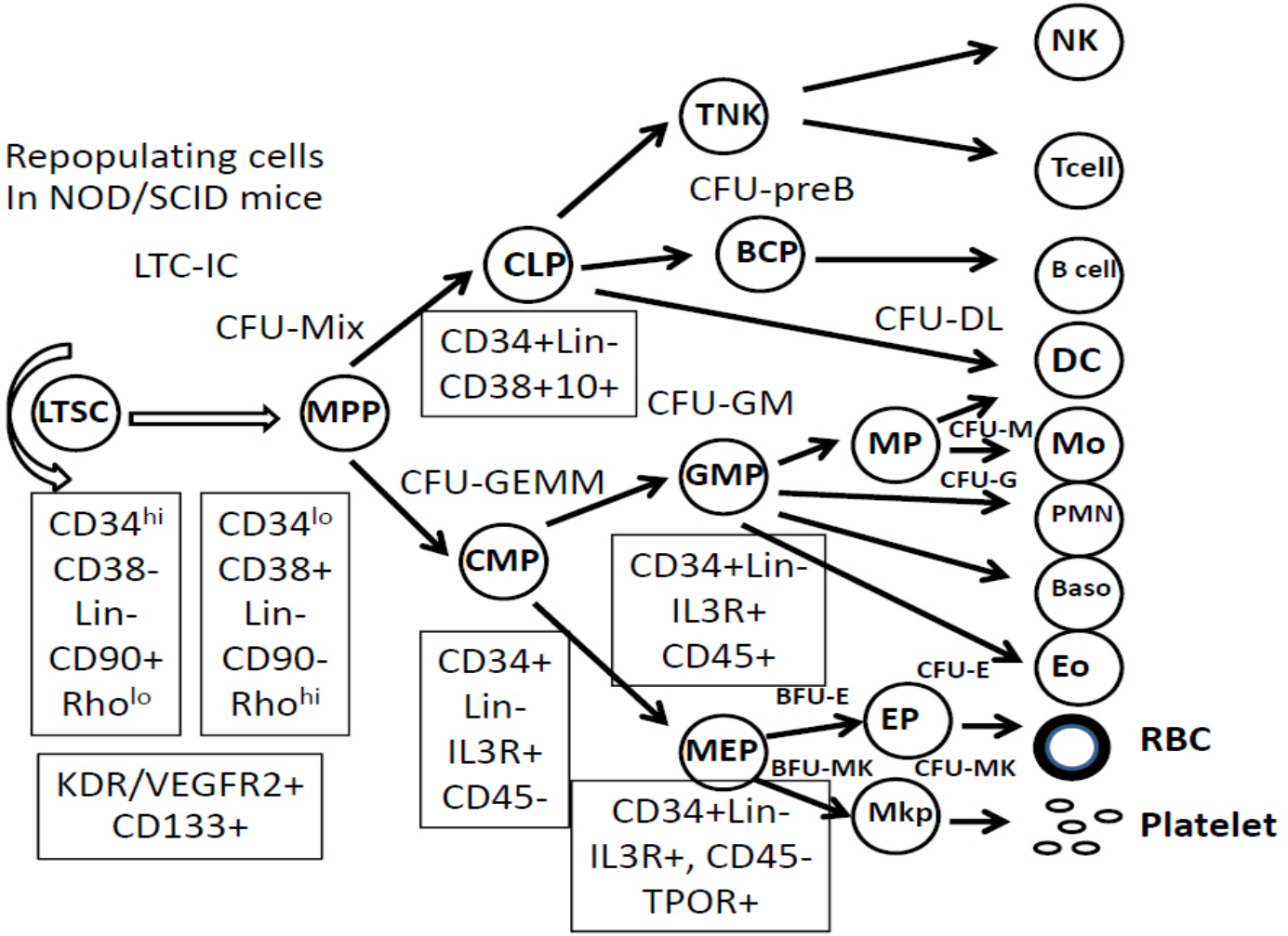
J. Exp. Med. 2020 Vol. 218 No. 2 e20201268

Control of Megakaryopoiesis by Extrinsic and Intrinsic factors

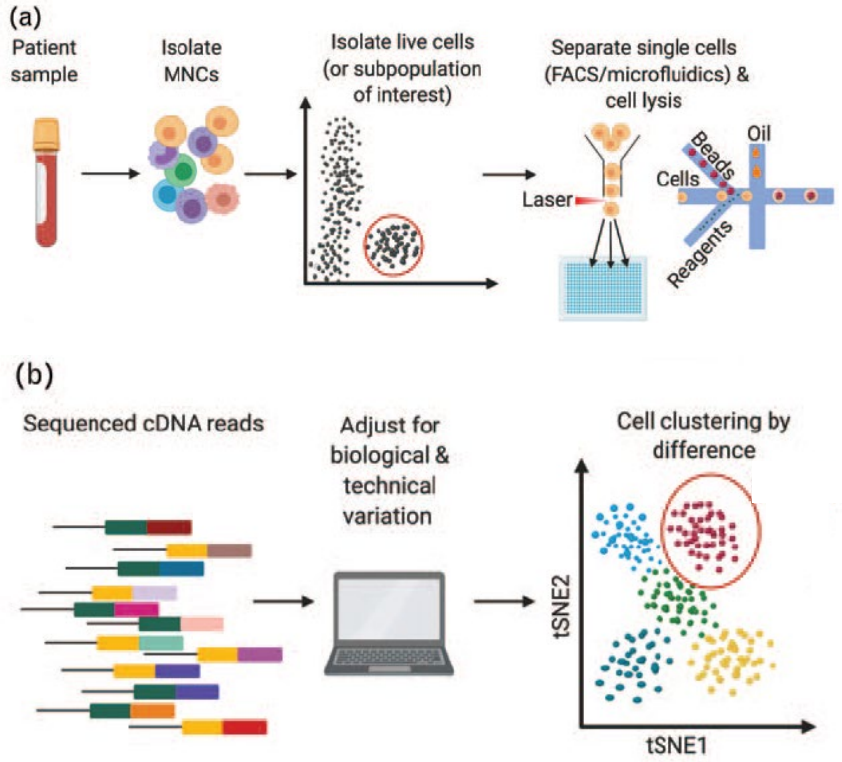


Transcription factor mutations: Hereditary Thrombocytopenia

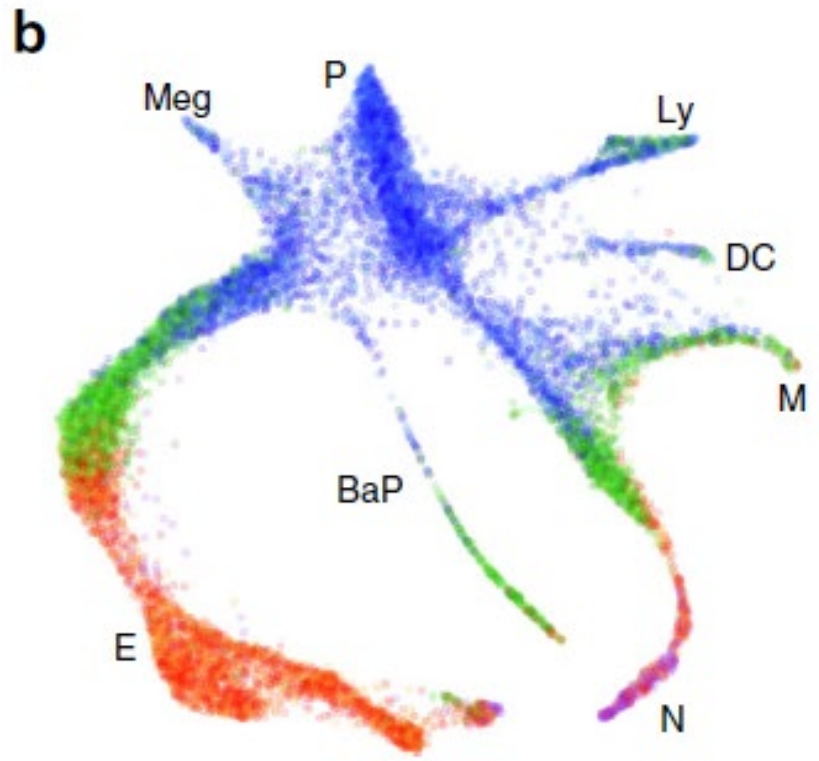
Hematopoietic Hierarchy
Cell identification by surface markers



Identification by Single cell RNA seq



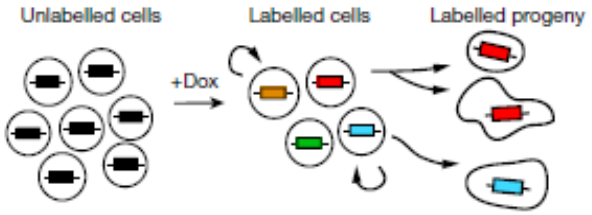
Curr Opin Oncol 2020; 32: 139

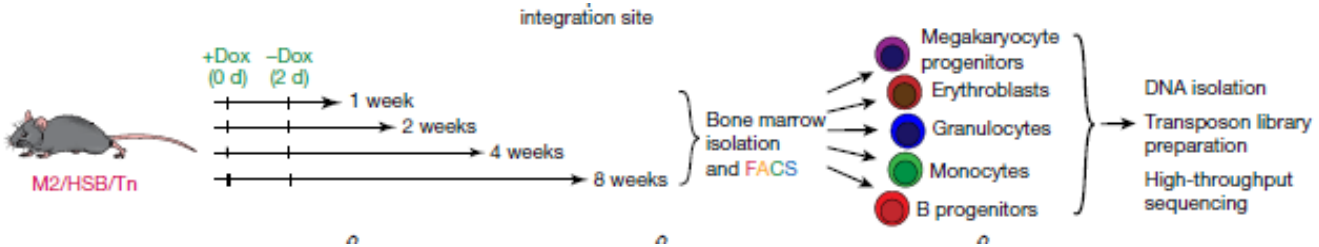


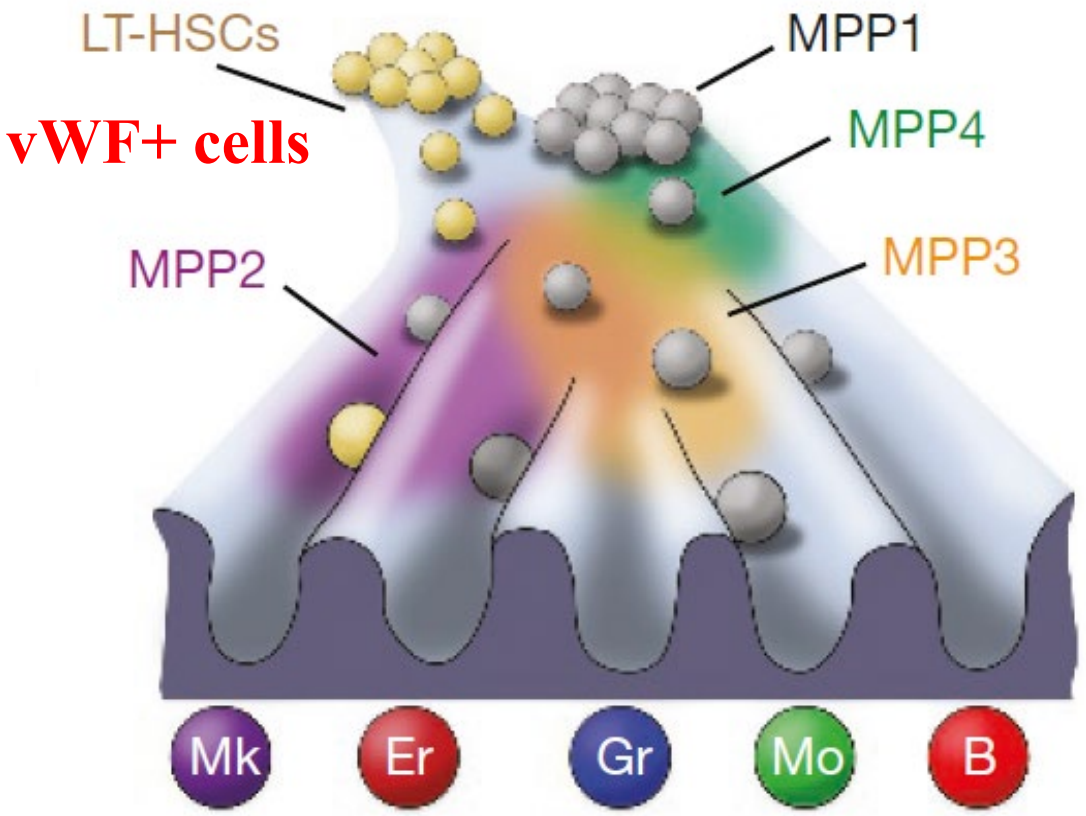
Nat Commun. 2019 Jun 3;10(1):2395

MKs are derived directly from stem cells

In vivo labelling in mice

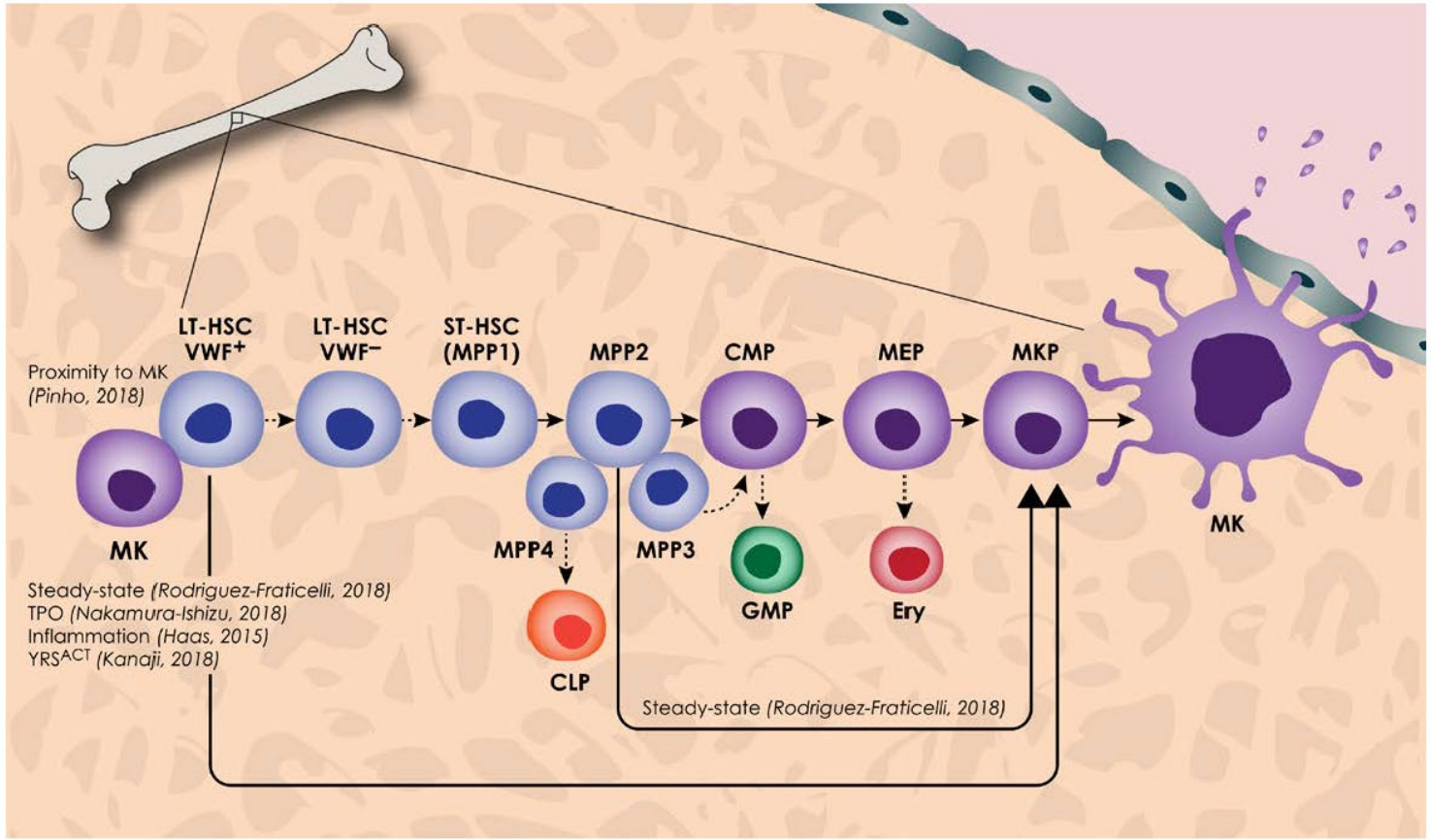






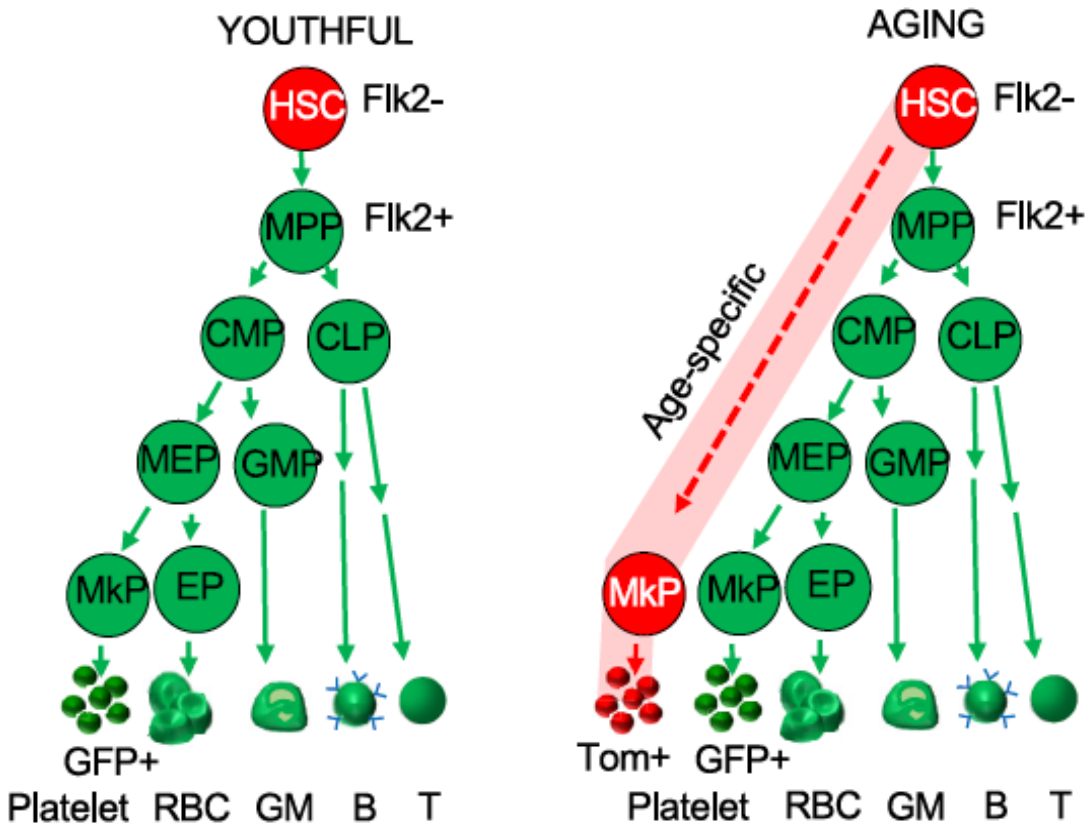
Nature 2018; 553: 212

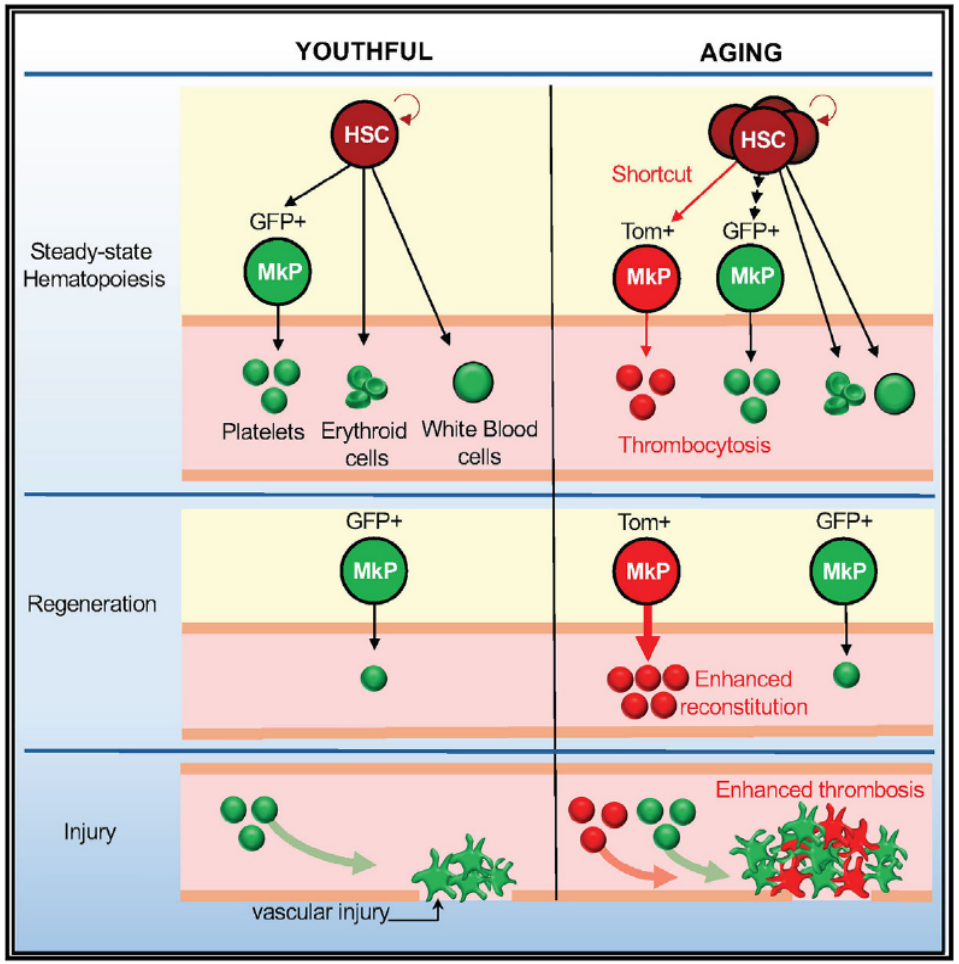
New Hematopoietic Hierarchy



Arterioscler Thromb Vasc Biol. 2019;39:1288

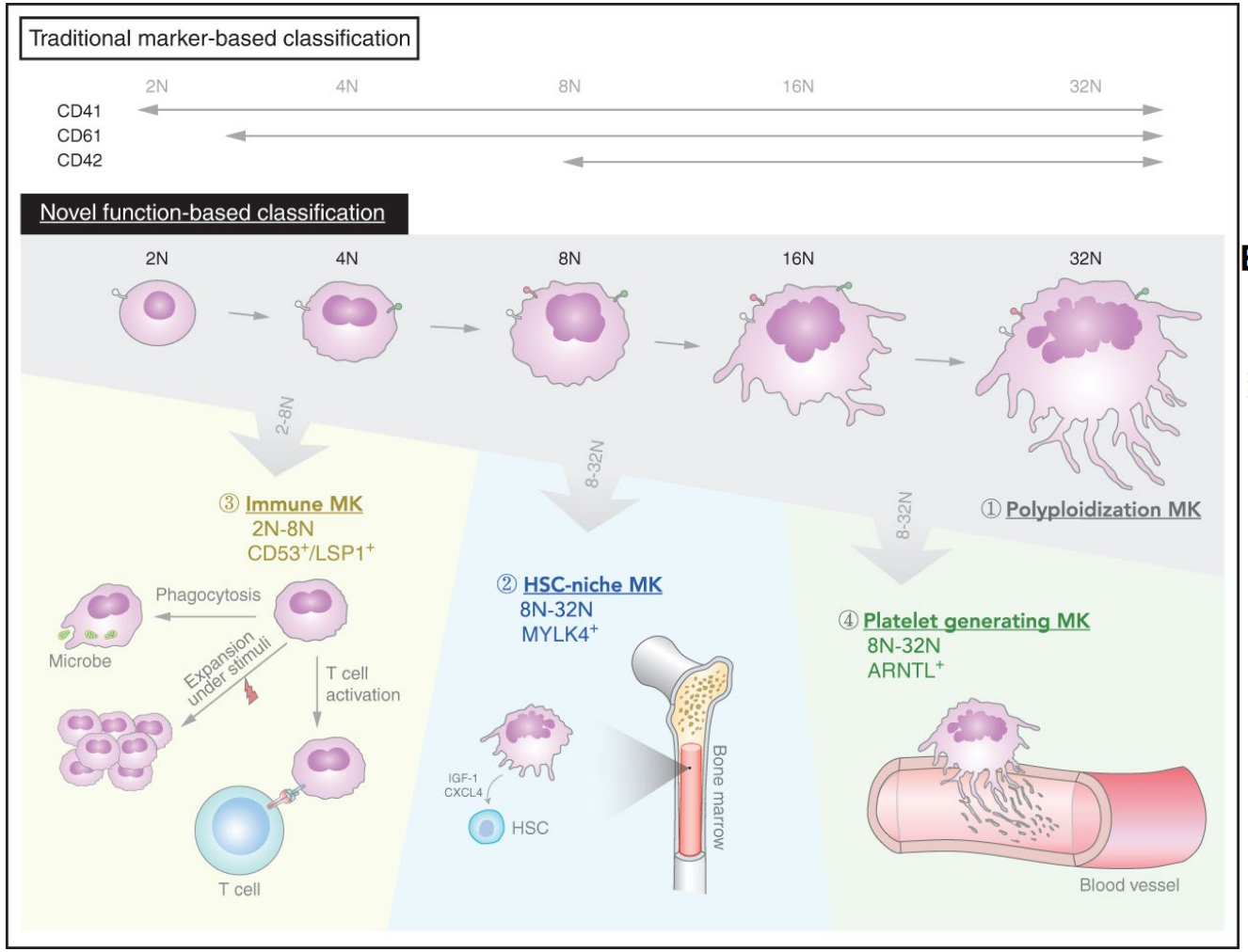
Age-related thrombosis: MK from HSC

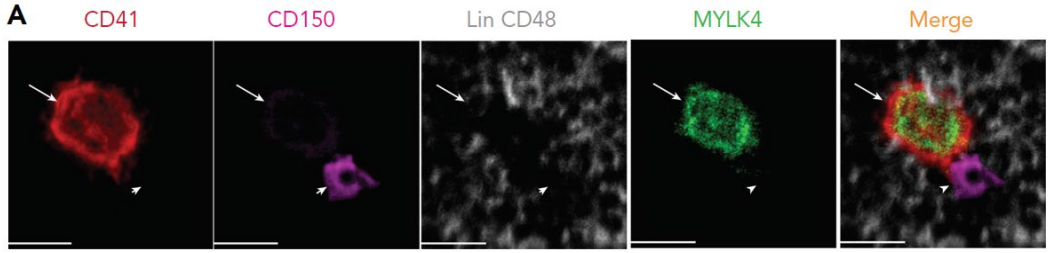


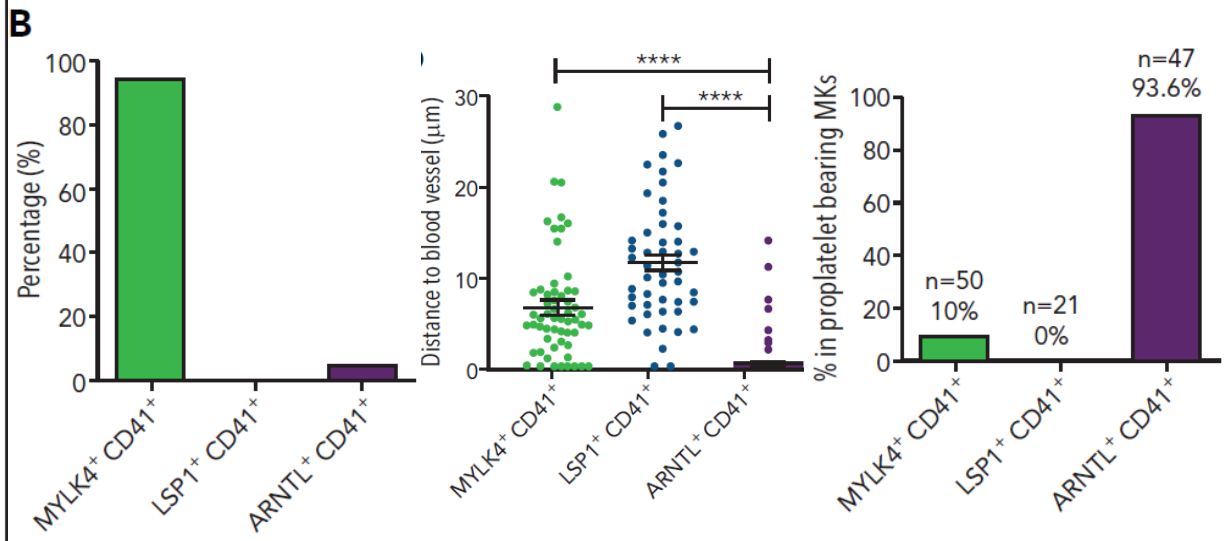


Cell 2024;187(12):3090

MK subpopulations by single-cell analysis







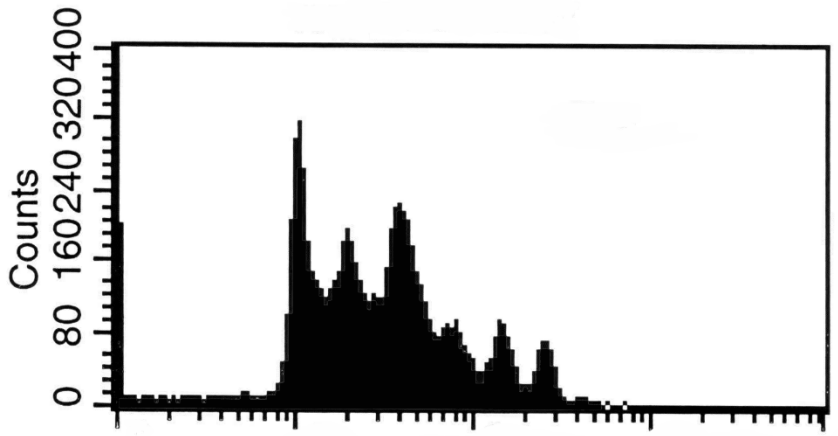
HSC contact Vessel contact Proplatelet

MK **HSC-niche** **Immunity** **Platelet-forming**

Blood. 2021; 138: 1211

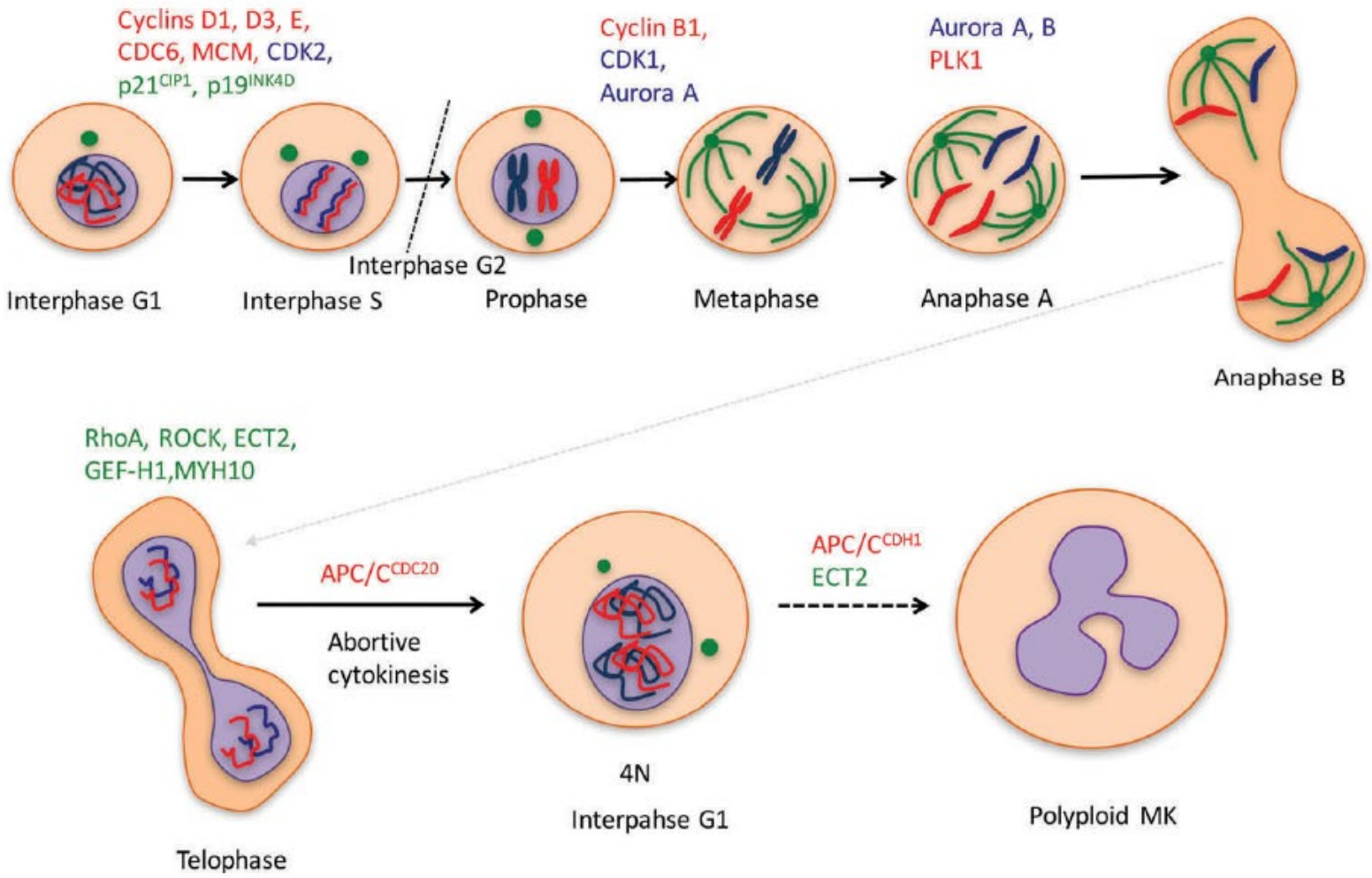
Endomitosis

- Unique to megakaryocytes
- Increased DNA content: 2N, 4N, 8N, 16N, 32N, 64N and up to 124N
- Increased protein production: cellular enlargement
- More efficient platelet production?
- MDS, cord blood and CML MKs have low ploidy (Not necessary ↓platelet)



Flow cytometry
Log DNA content

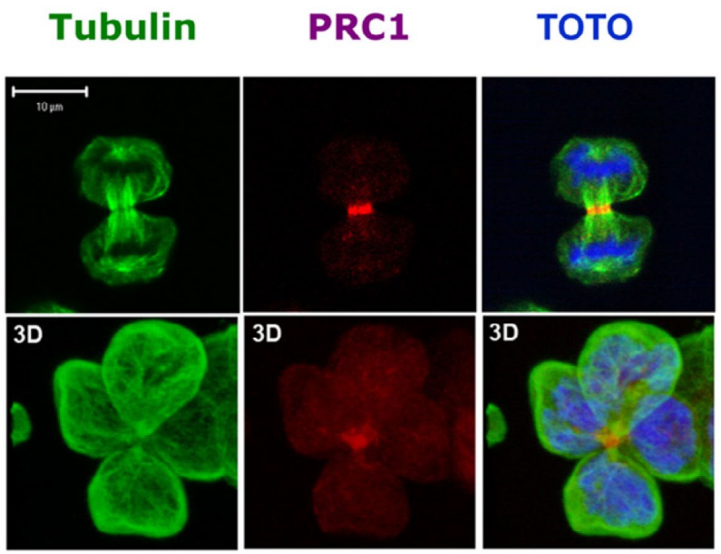
Endomitosis: No cytokinesis



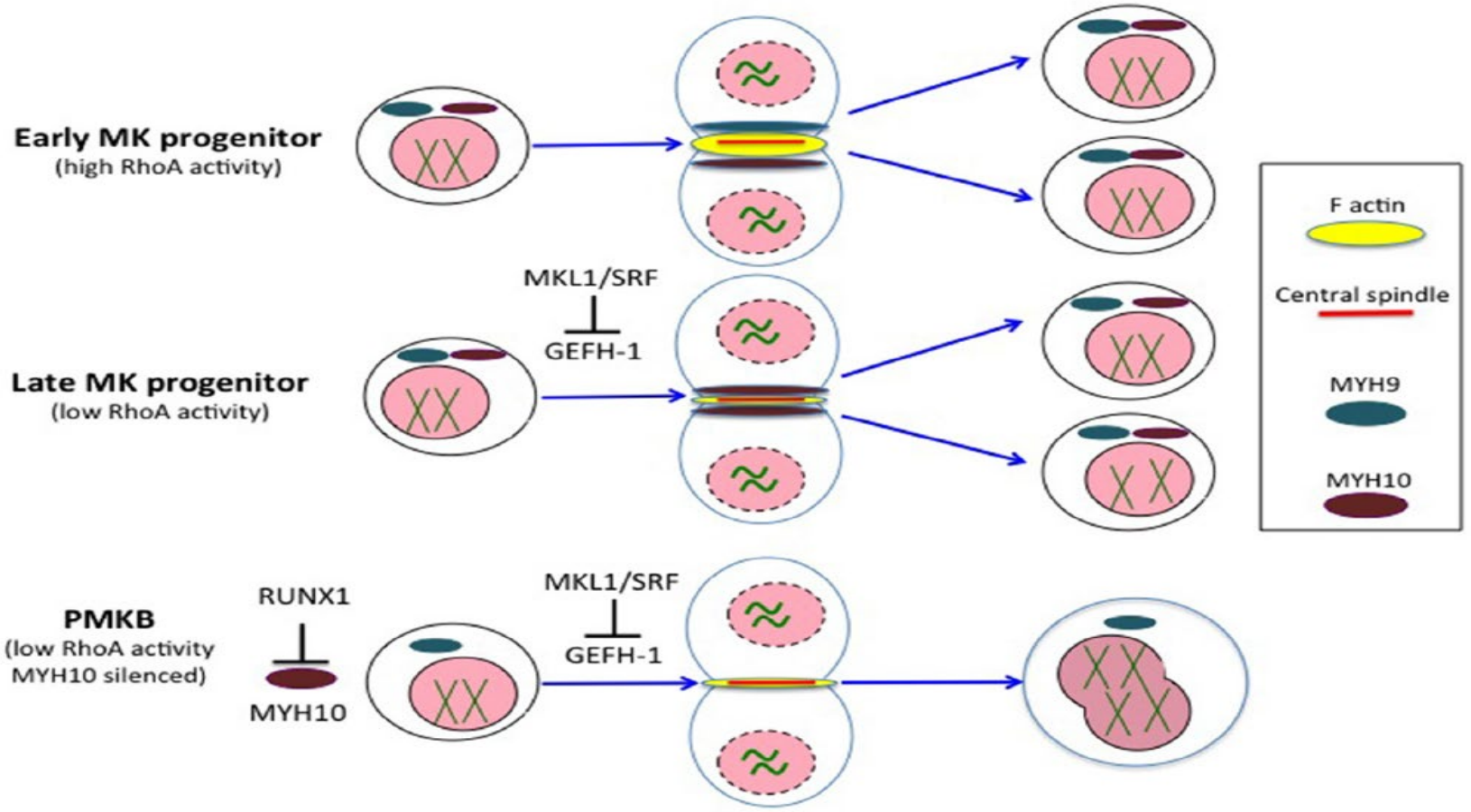
Platelets 2020; 31: 707

Endomitosis: ↓RhoA and ↓ MYH10 (RUNX1)

Mitosis

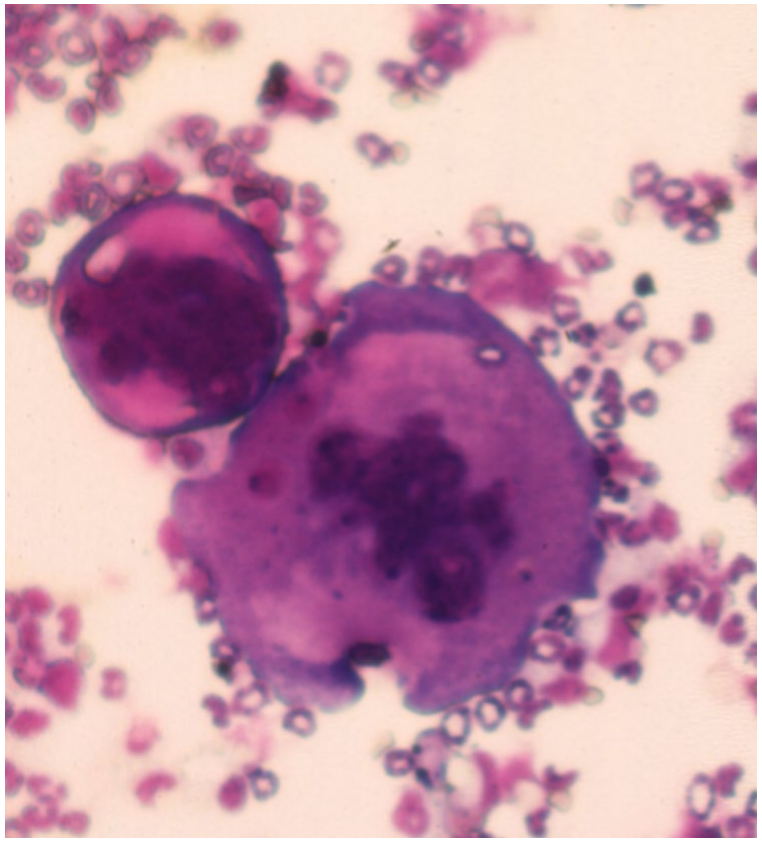


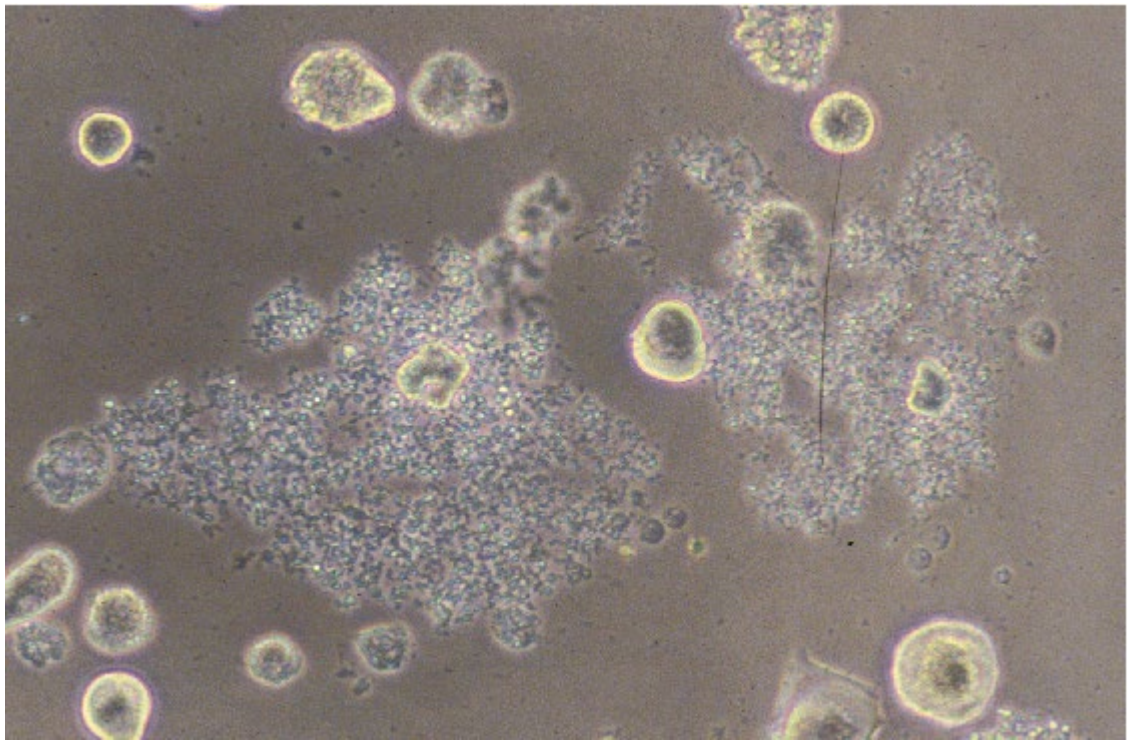
Endomitosis



Exp Hematol 2018; 57: 1-13

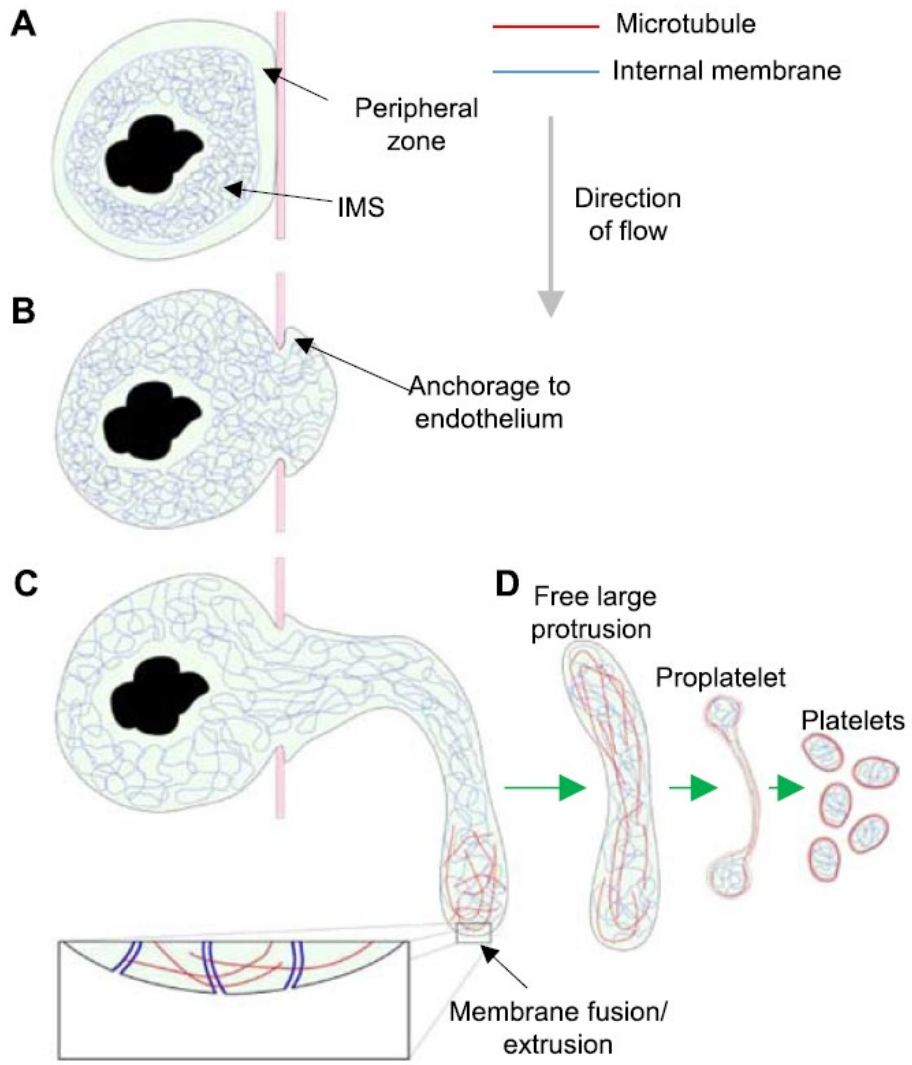
Proplatelet formation: Thin and branching

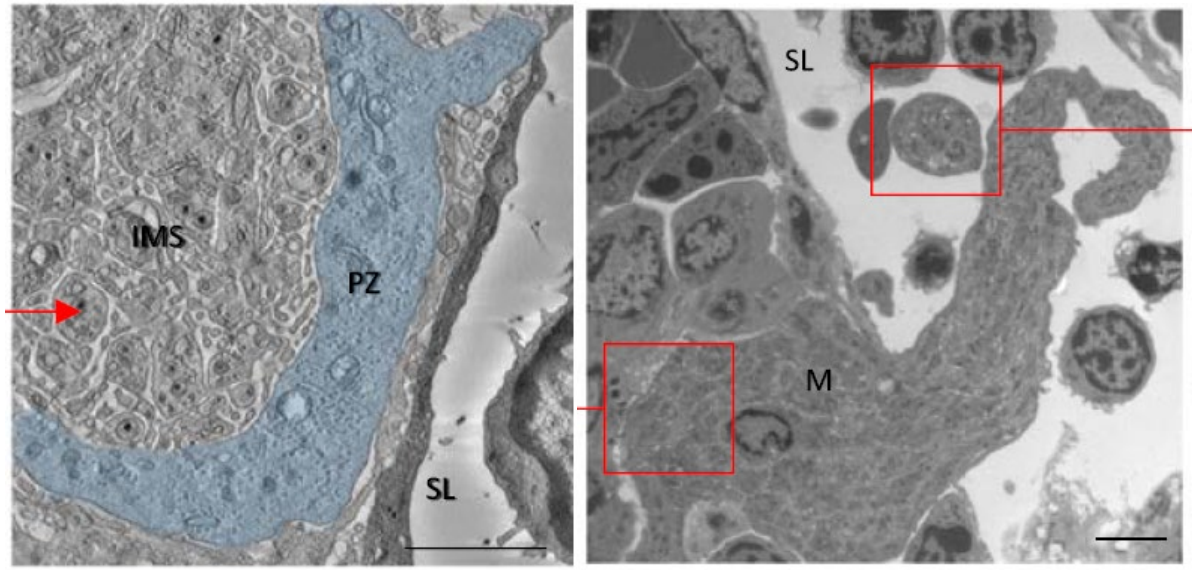




Rojnuckarin and Kaushansky. Blood. 2001;97:154-161

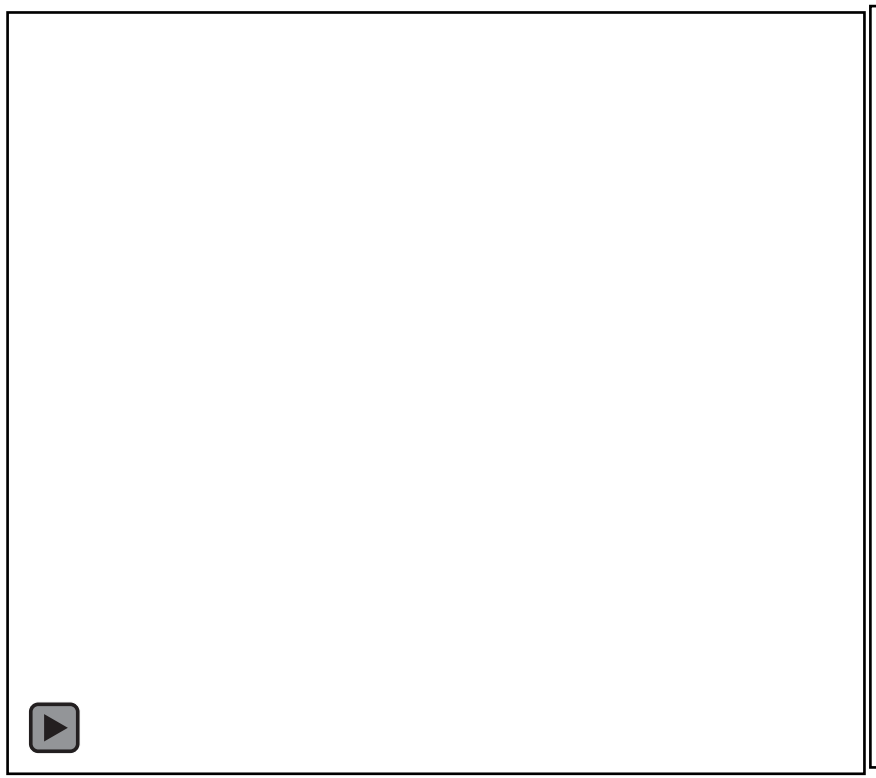
Large Protrusions *in vivo*

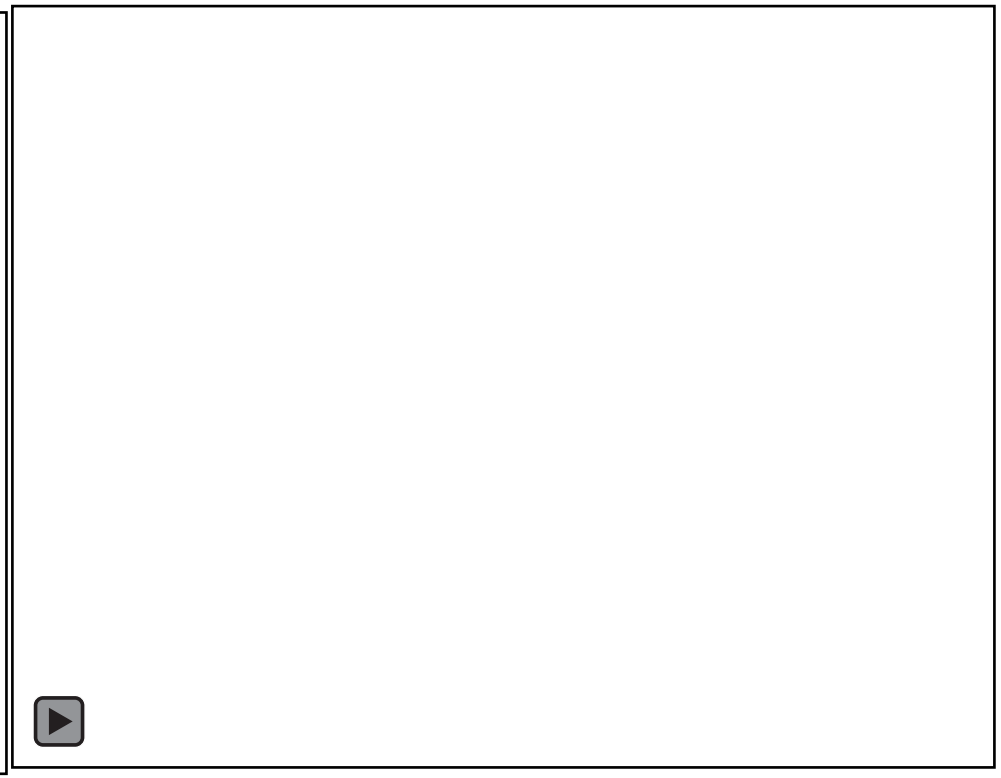




Brown et al. Life Sci Alliance. 2018;1(2):e201800061.

Megakaryocyte rupture
Intravital visualization in mice





MK: Green fluorescence protein (GFP)
Blood flow: Dextran (red)
Nucleus: Hoechst 33342 (blue)

Nishimura et al. J Cell Biol 2015; 209: 453-66

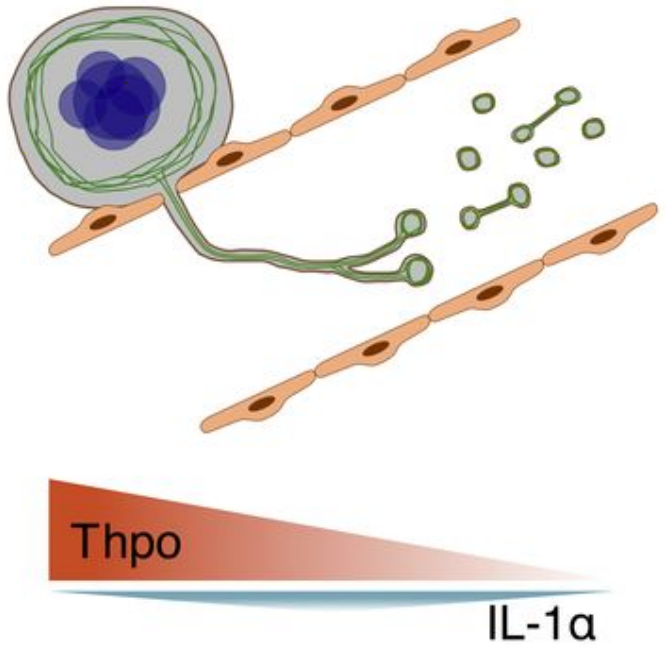
Megakaryocyte rupture

Normal physiology

Continuous platelet production/clearance

- Low IL-1α levels
- Thpo signals via megakaryocytic c-Mpl
- Inhibition of Caspase-3 activation
- Normal β1-tubulin

→ Slow release of a few normally sized platelets

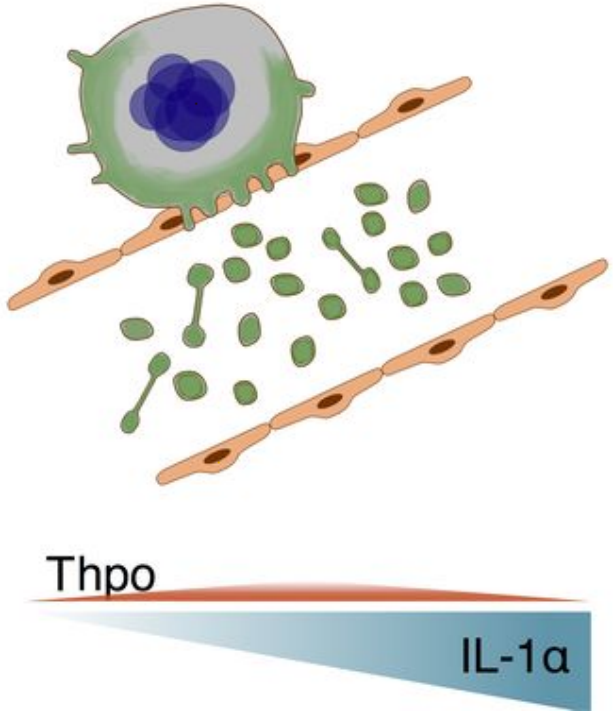


Acute platelet needs

Platelet consumption/ Inflammation

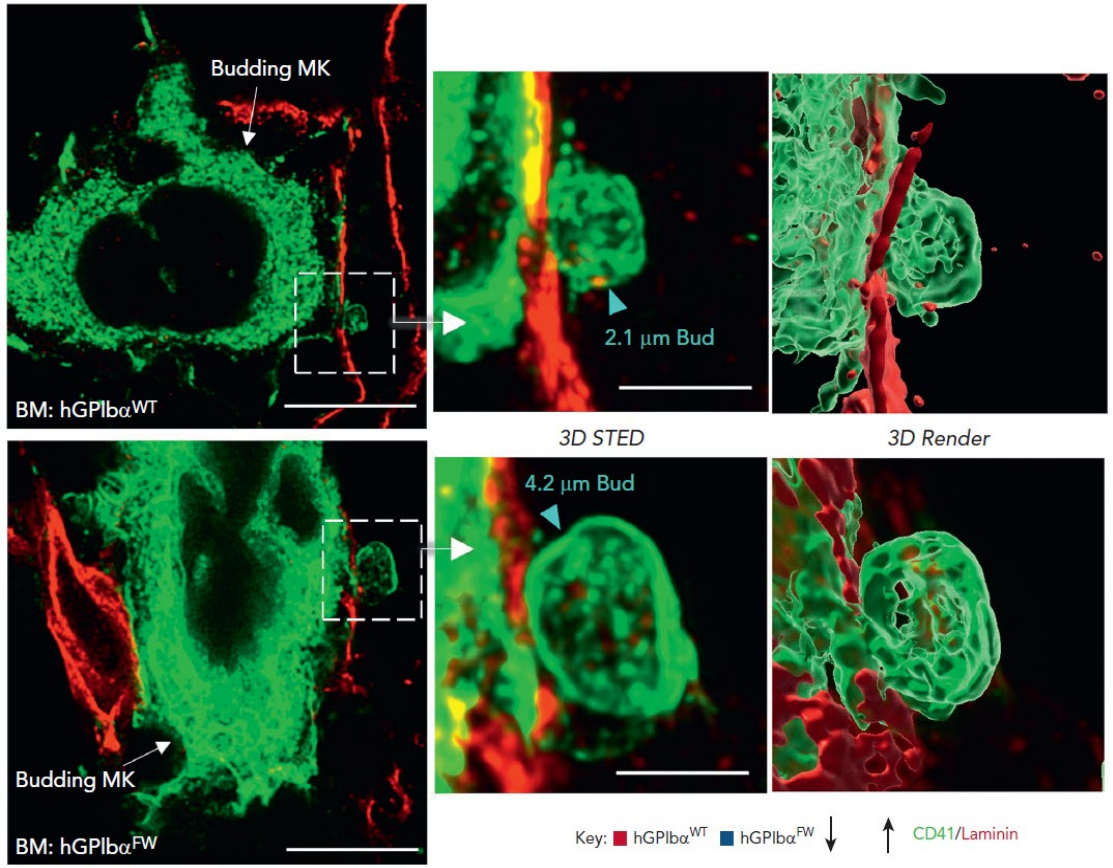
- High IL-1α levels
- IL-1α signals via megakaryocytic IL-1R1
- Activation of Caspase-3
- Deregulated β1-tubulin

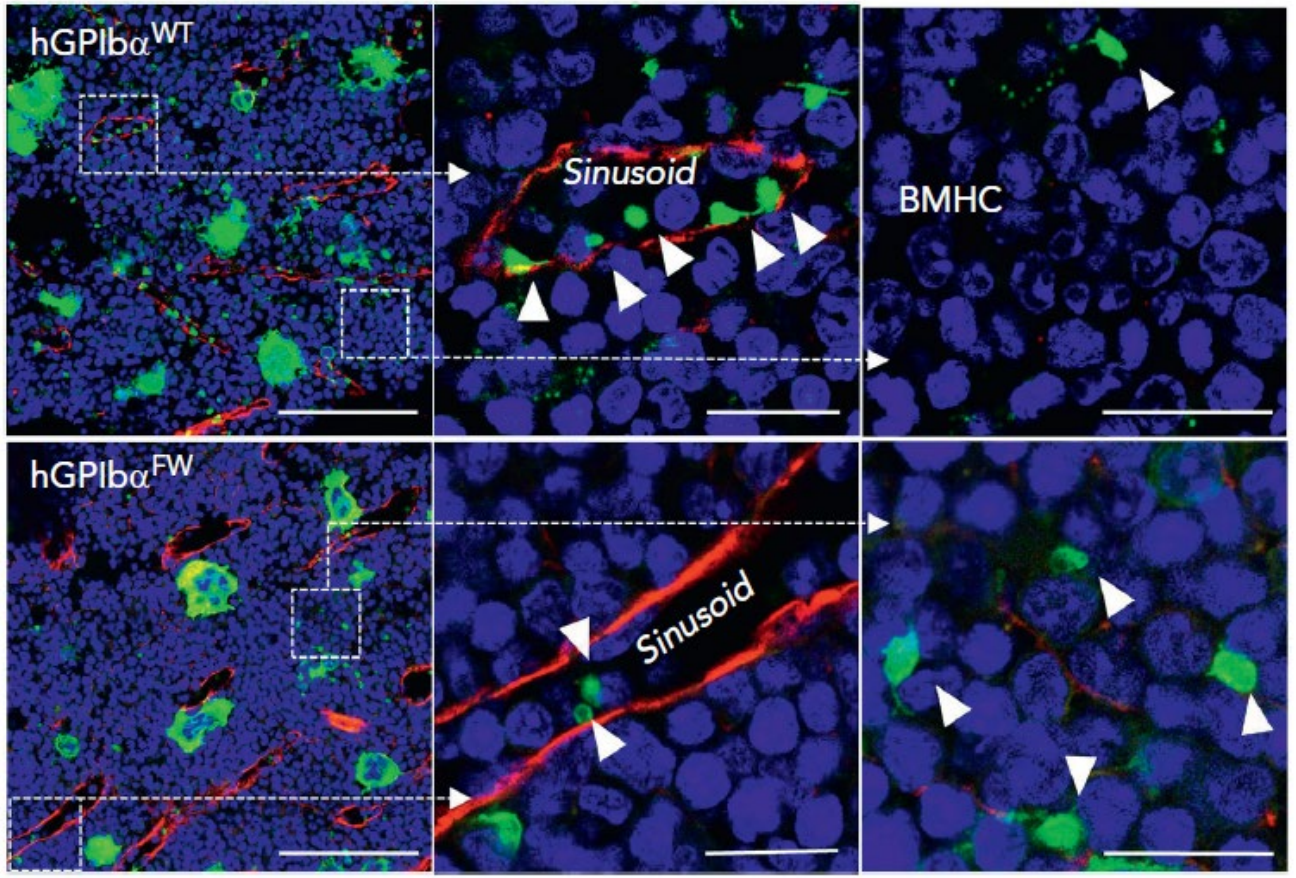
→ Fast release of a large number of enlarged platelets



Nishimura et al. J Cell Biol 2015; 209: 453-66, Nieswandt et al. J Cell Biol 2015; 209:327-8

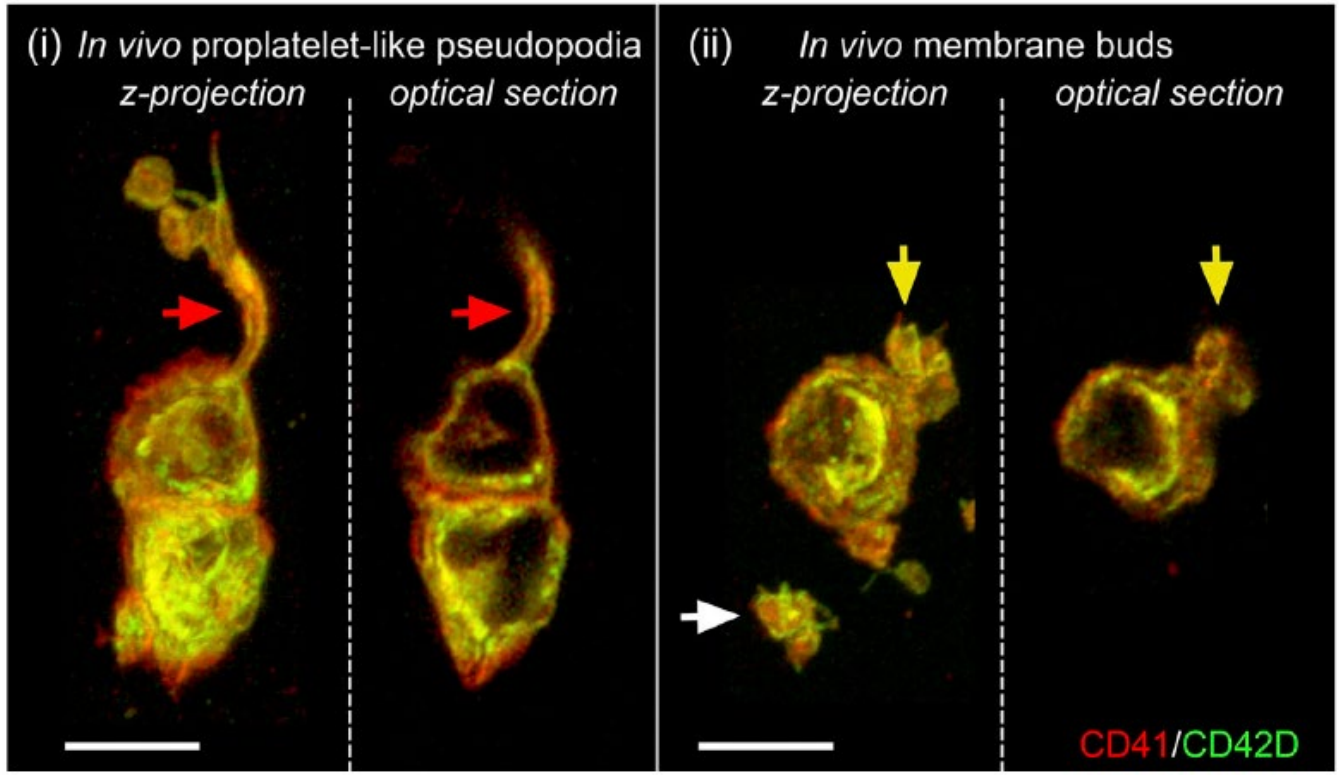
MK Budding in vivo

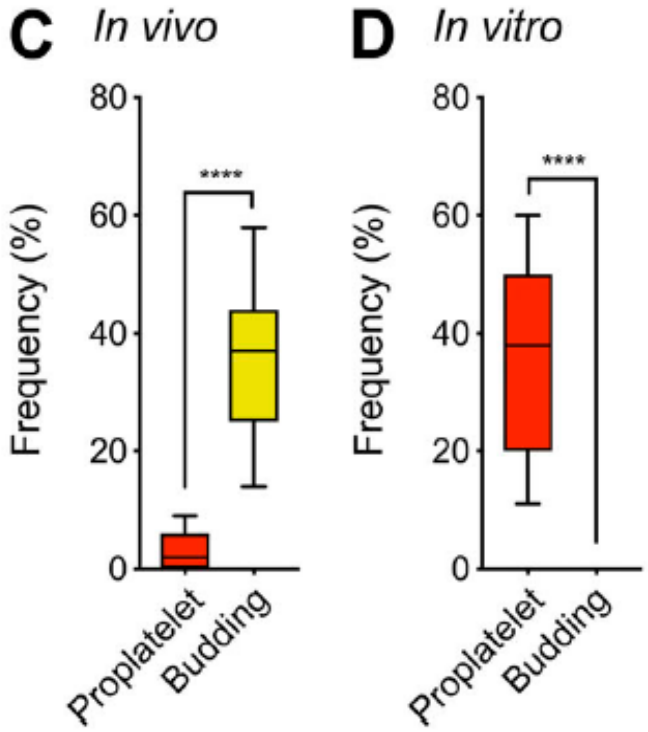




Ellis et al. Blood 2024; 143 (4): 342

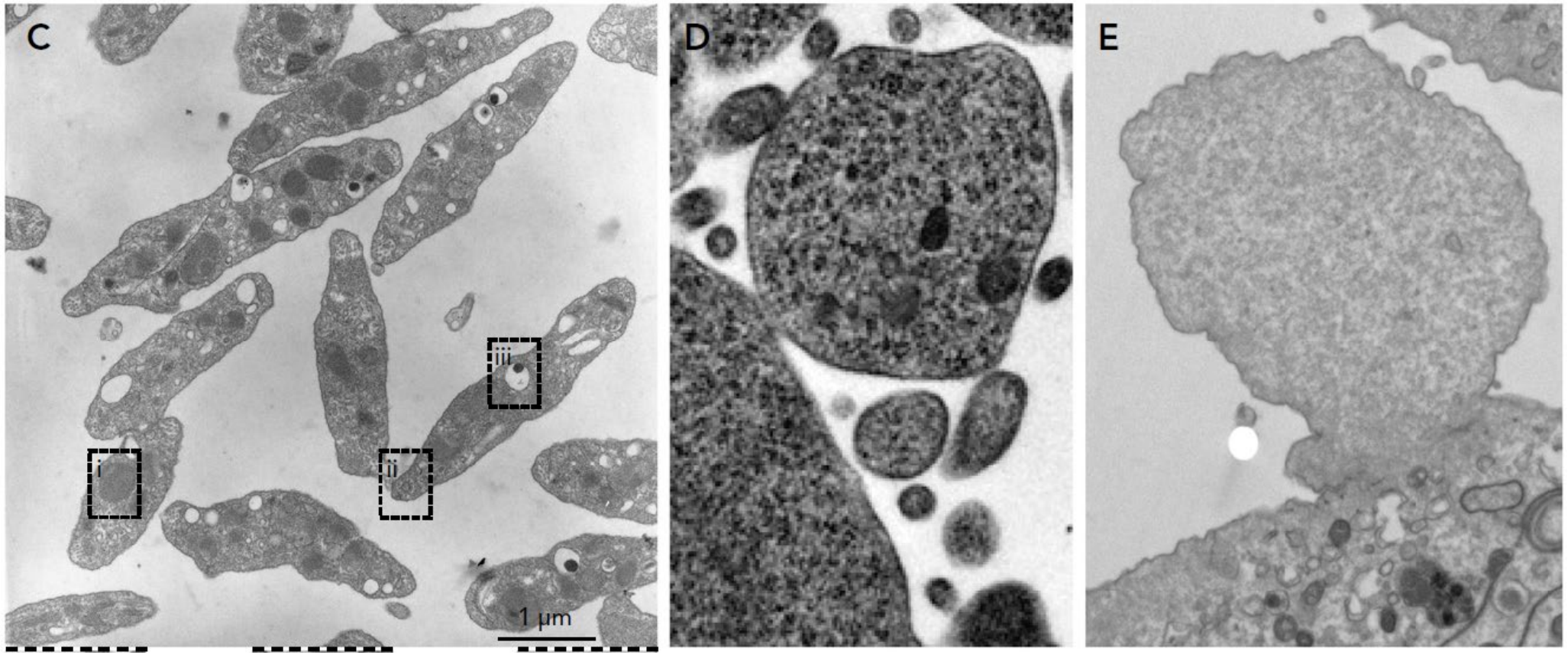
MK budding is more than proplatelets
Intravital visualization in mice





Potts et al J. Exp. Med. 2020; 217 (9): e20191206

MK buds are NOT platelets

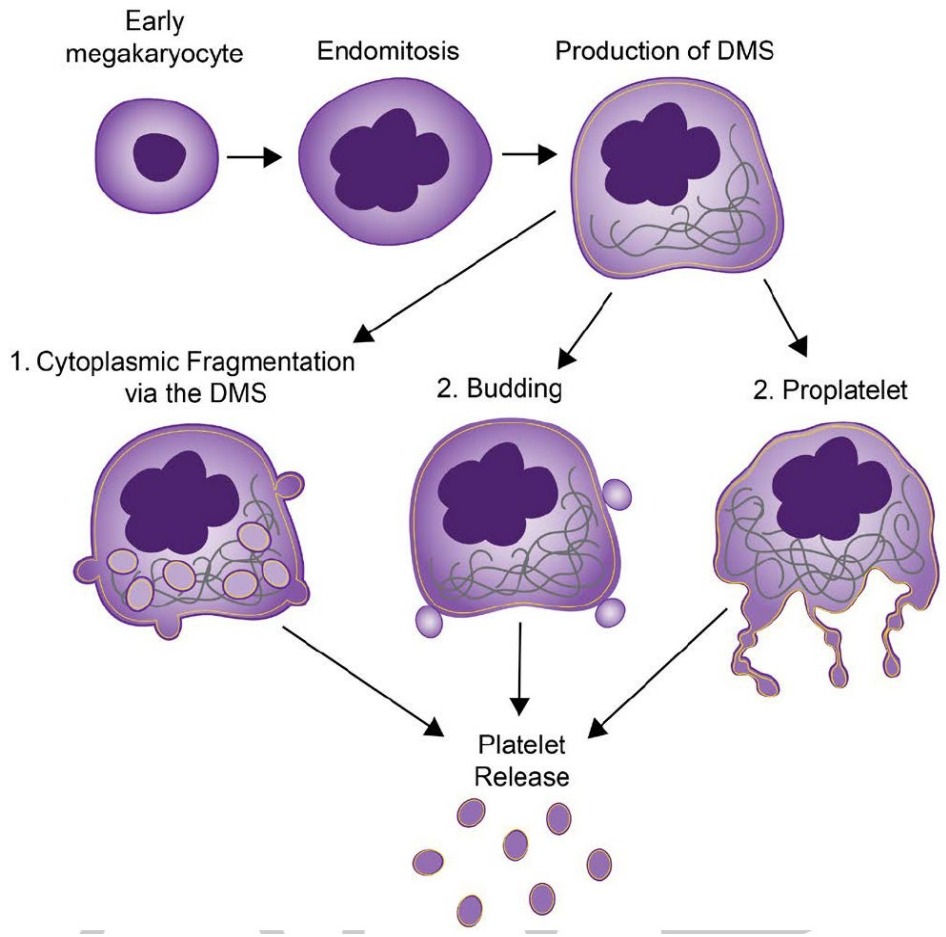


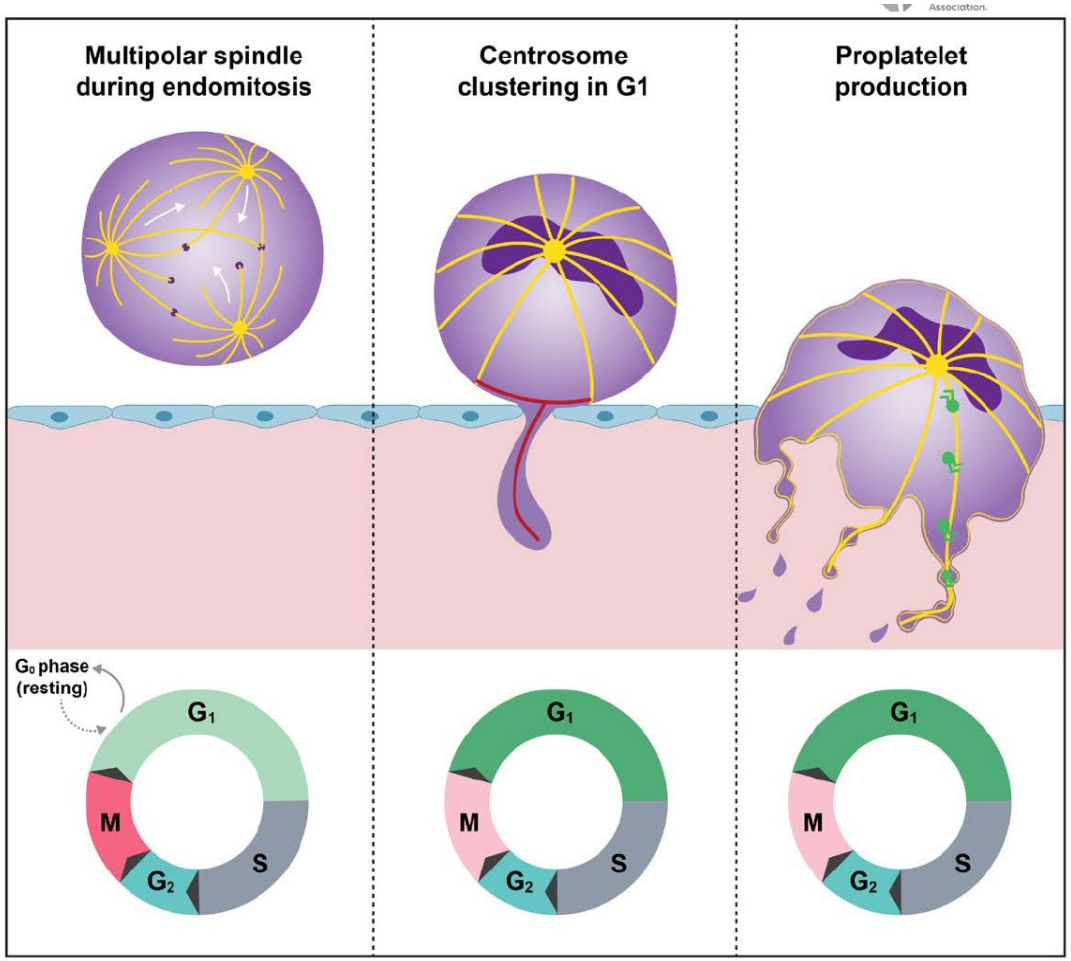
Platelets

MK Buds

Italiano et al. Blood. 2021 Nov 18;138(20):1998-2001

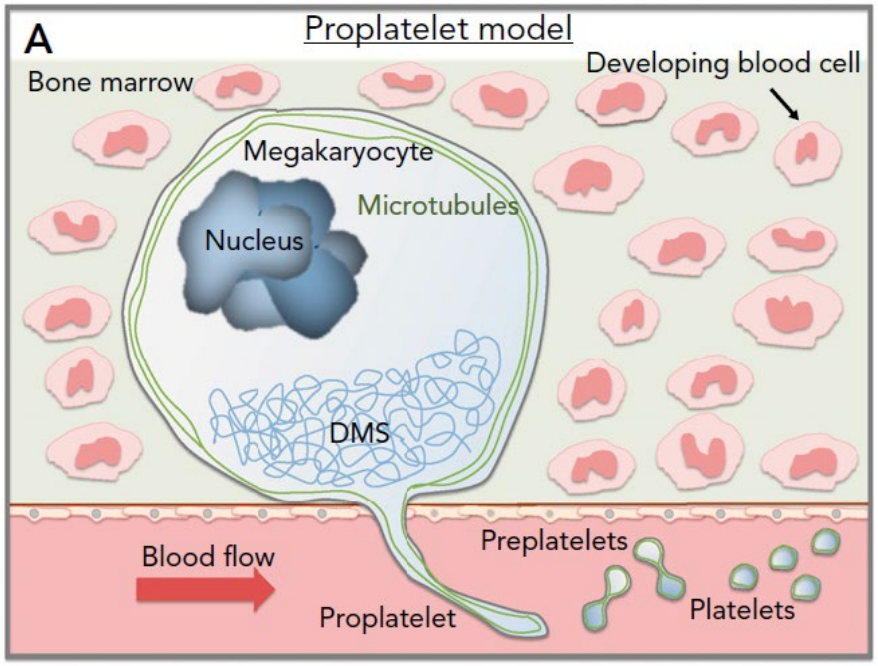
Platelet formation from MKs



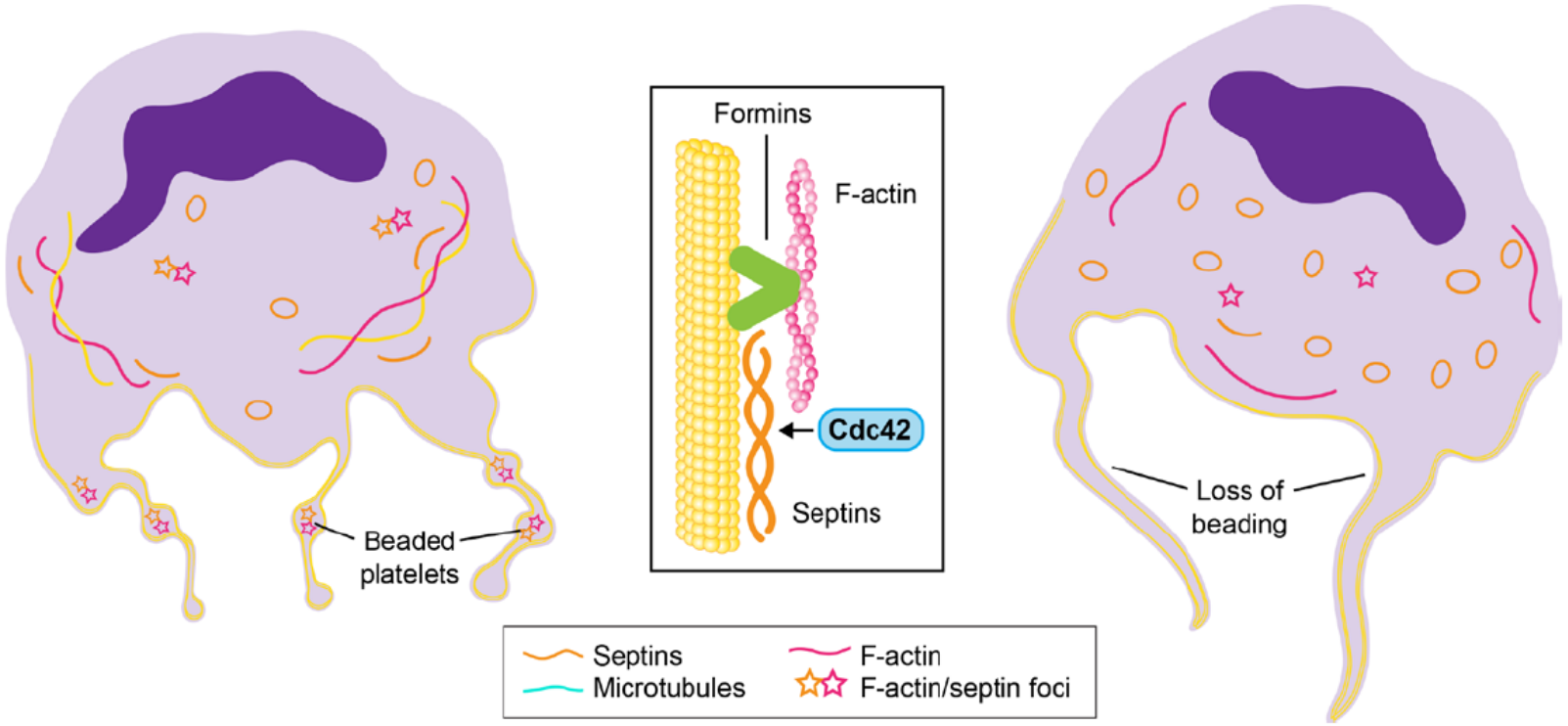


Italiano et al. Arterioscler Thromb Vasc Biol. 2025;45:00–00. DOI: 10.1161/ATVBAHA.124.320392

Actin and Proplatelets



Blood. 2021;138:1998

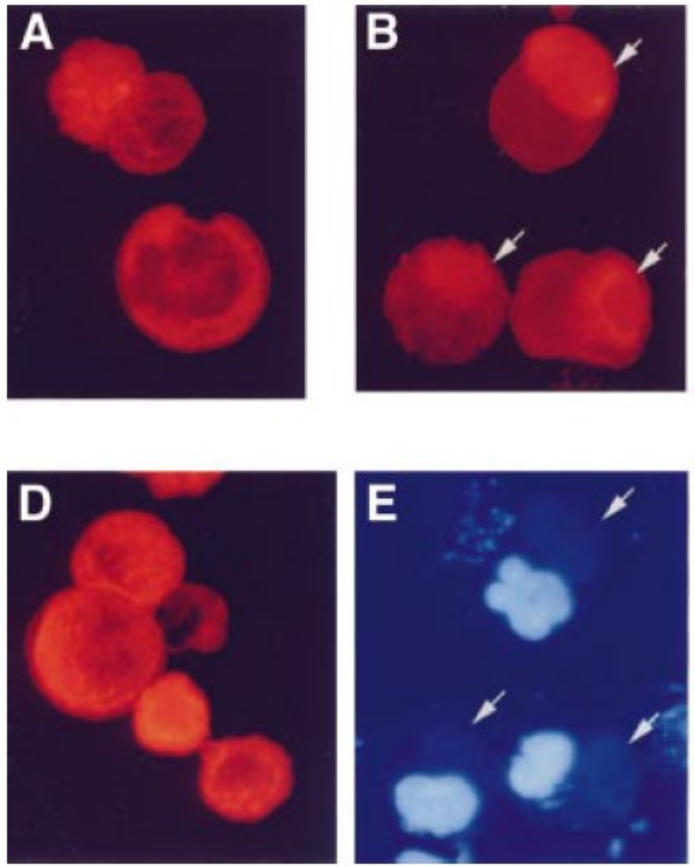


Italiano et al. Arterioscler Thromb Vasc Biol. 2025;45:00–00. DOI: 10.1161/ATVBAHA.124.320392

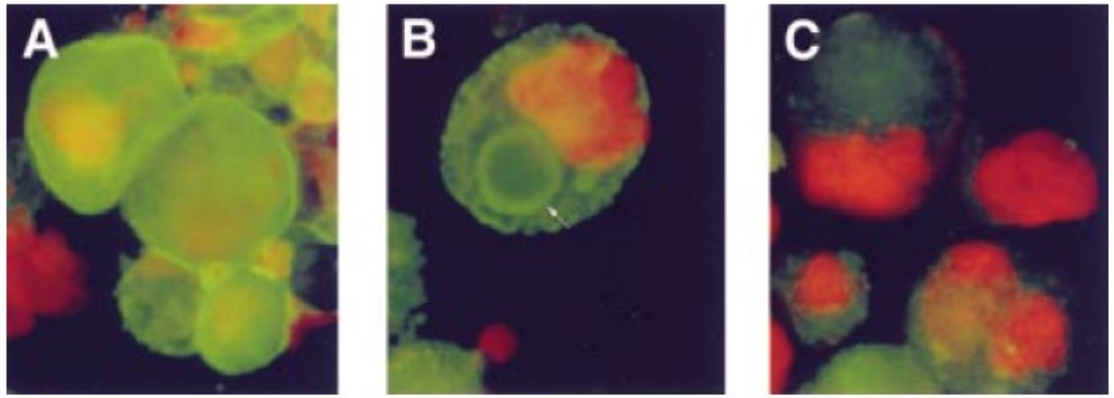
Actin organization in Mks

Plasma

F-Actin
Nucleus



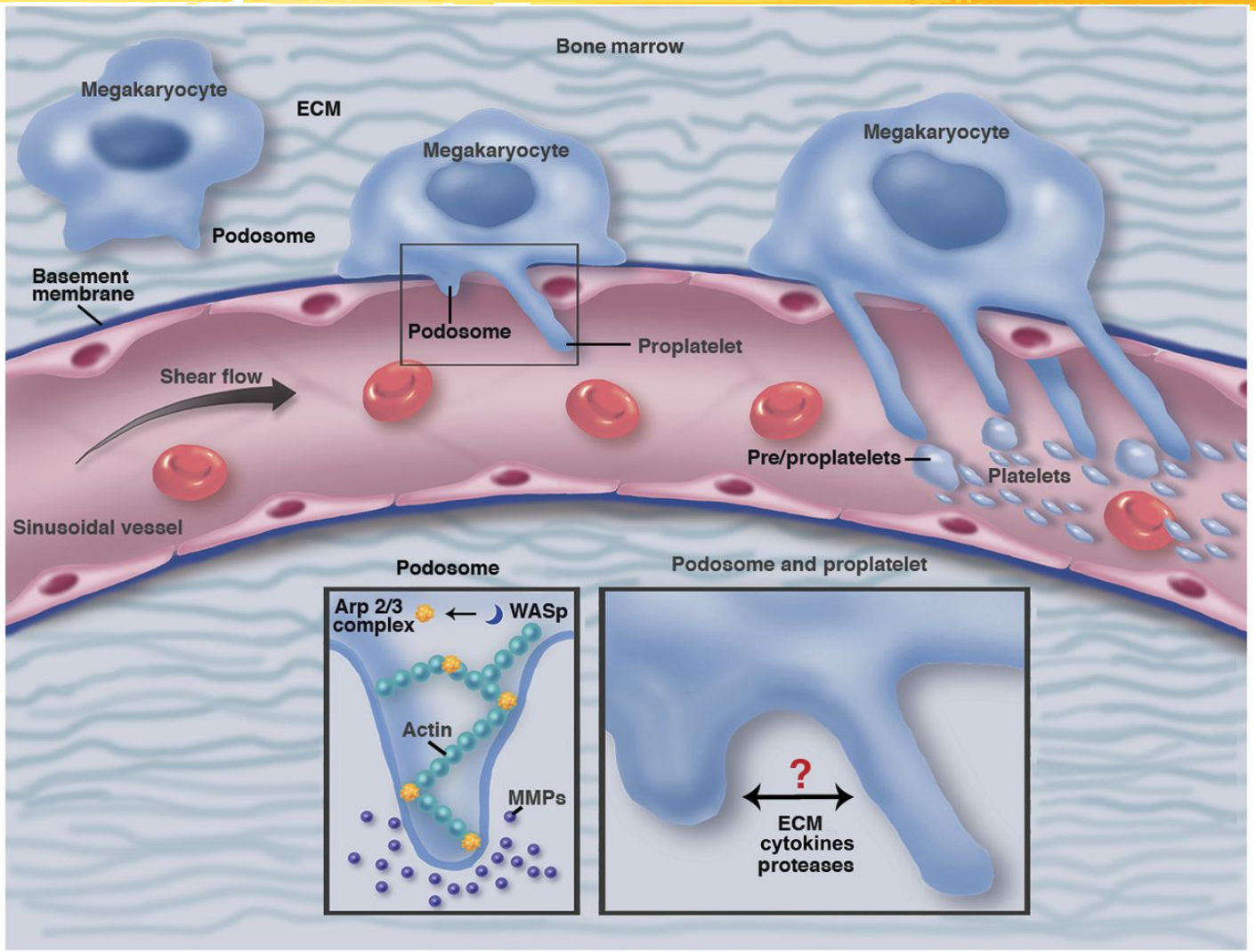
Plasma Plasma+PMA



Nucleus
PKC

Rojnuckarin and Kaushansky. Blood. 2001;97:154-161

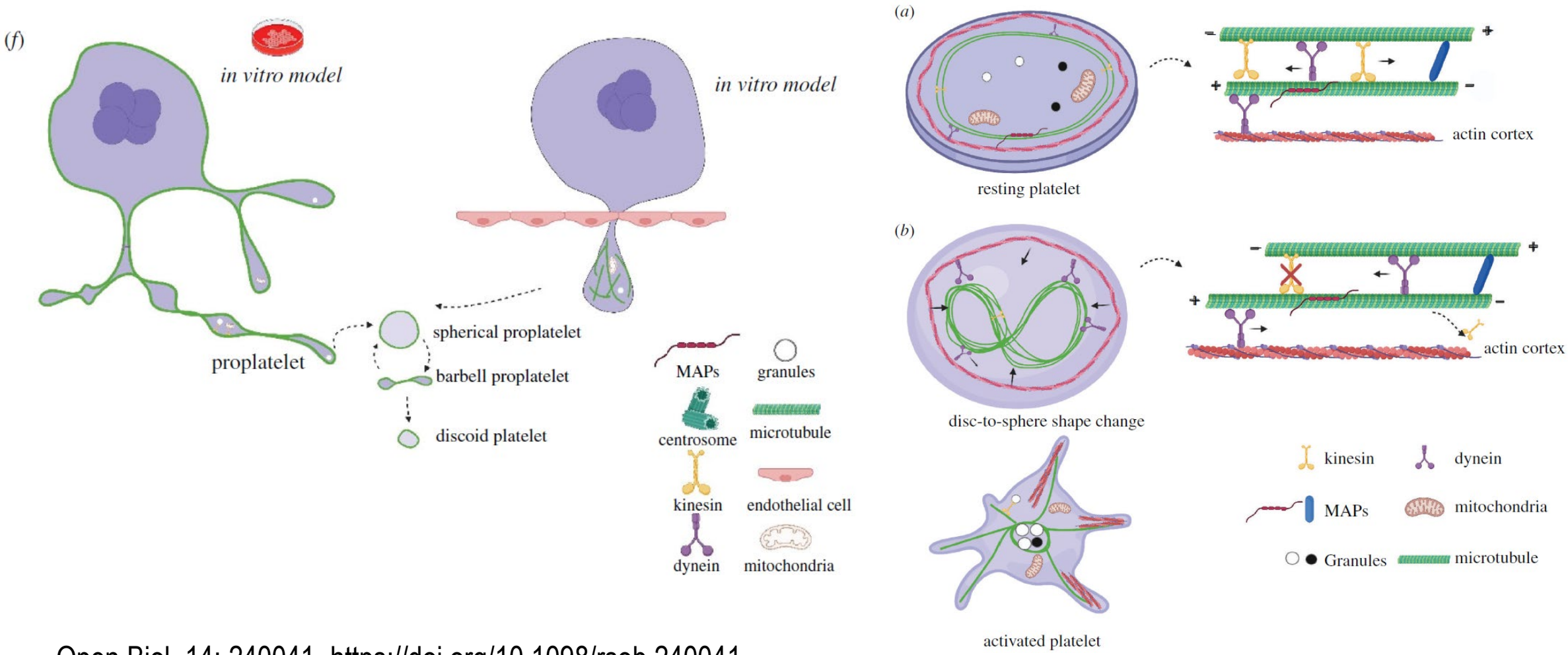
MK actin-rich podosomes



Podosome
Defect in
WASP
XLT

Blood 2013
121: 2379

Tubulin in MKs and Platelets

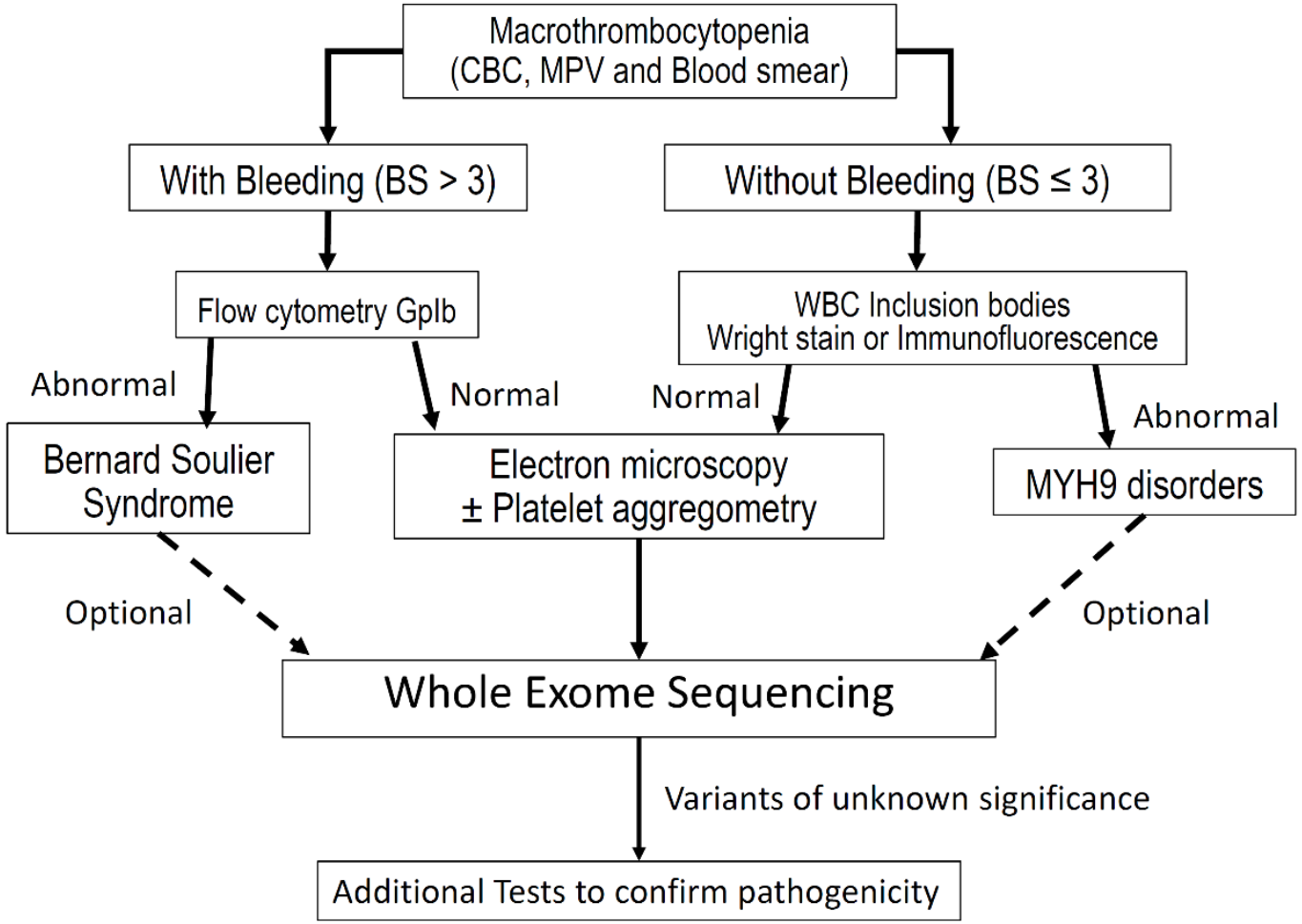


Open Biol. 14: 240041. https://doi.org/10.1098/rsob.240041

Cytoskeletal system: Hereditary thrombocytopenia with abnormal-sized platelets

- **Macrothrombocytopenia**
  - Bernard Soulier syndrome (GpIb or Gp IX)
  - *MYH9*-related disorders (May Hegglin anomaly)
  - Filamin A (*FLNA*) mutations (GpIb-Actin link)
  - Actinin-1 (*ACTN1*) mutations (Actin control)
  - β1 tubulin (*TUBB1*) mutations
- **Microthrombocytopenia**
  - Wiskott Aldrich syndrome or X-linked thrombocytopenia (WASP)

Approach to Macrothrombocytopenia

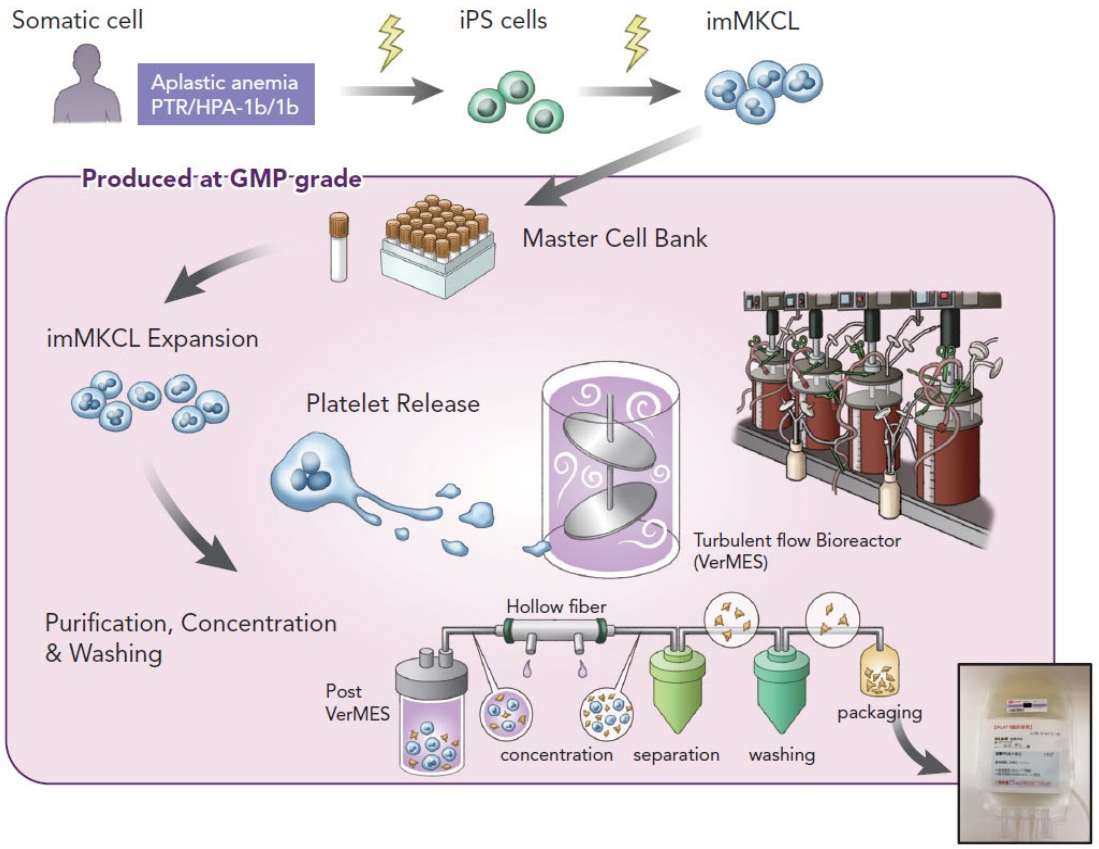


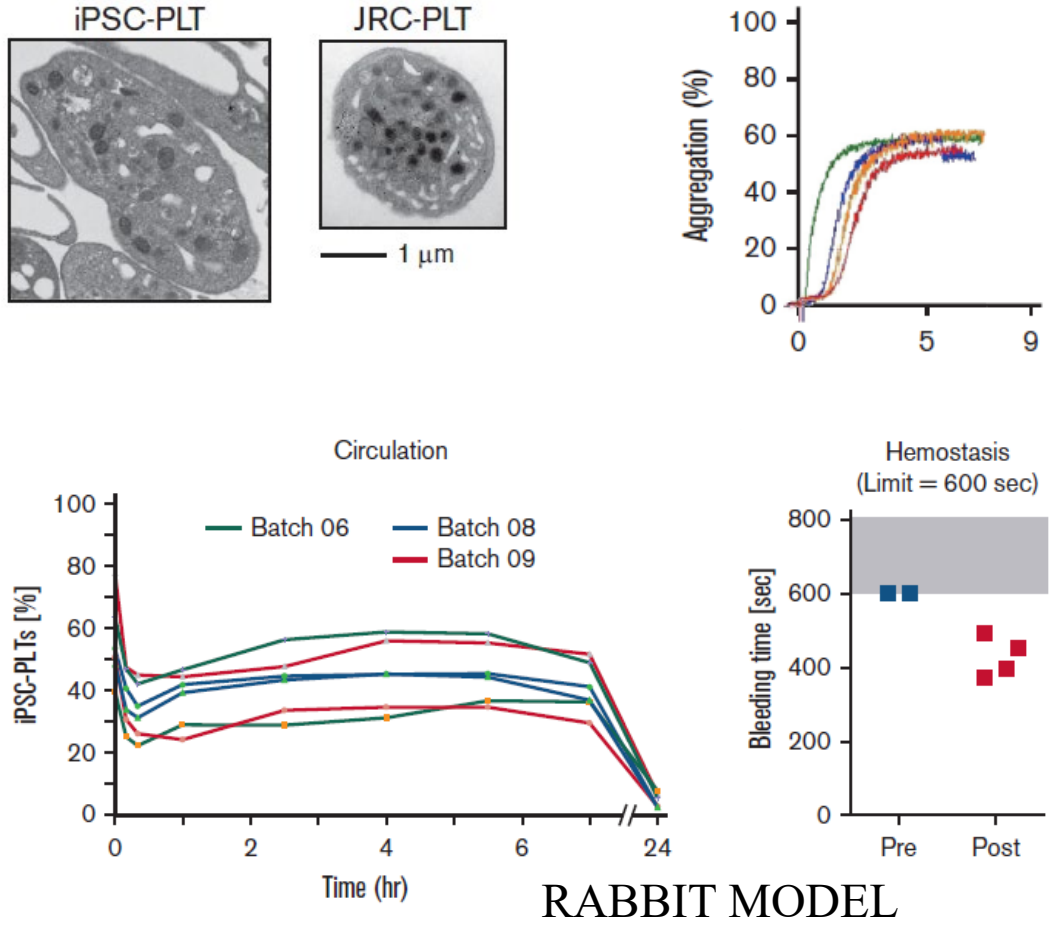
Medicine (Baltimore). 2020; 99 (47): e23275.

Benefits of Hereditary Thrombocytopenia Diagnosis

- Avoid unnecessary treatments of ITP and MDS.
- Guide the treatment of amegakaryocytic thrombocytopenia (Thrombocytopenia → Aplastic anemia)
  - *MPL* mutations: Rx Allogeneic BMT
  - *THPO* mutations: Rx TPO-RA
- Screening for future abnormalities: Eye, Ear, Urine examination, LFT in *MYH9* disorders, May give ACEI
- Genetic predisposition to hematological malignancy
  - BMT Donor selection
  - Couple counseling for future child

First iPSC-derived platelet transfusion in human



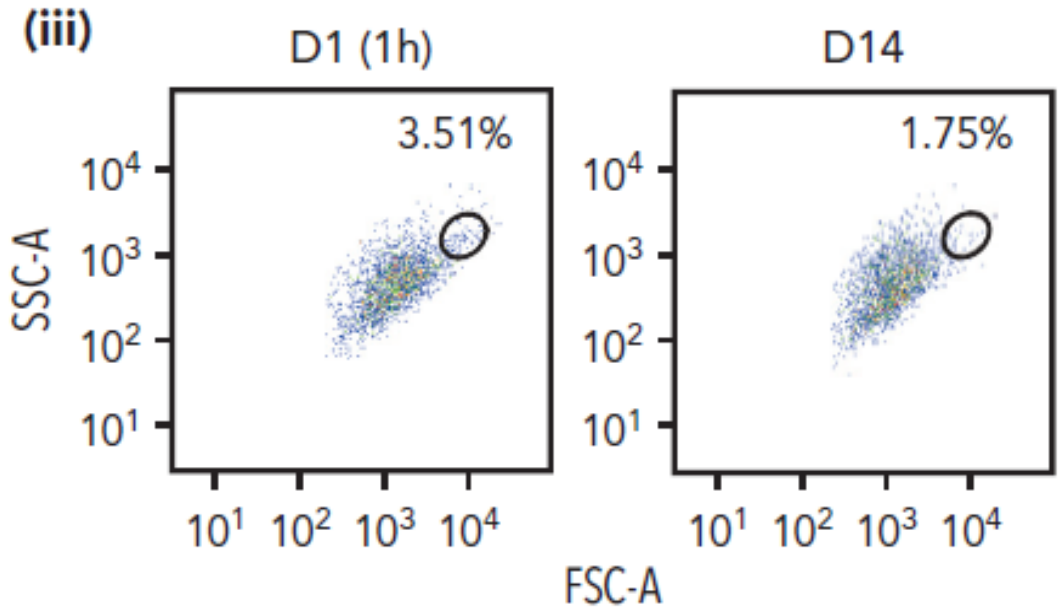


RABBIT MODEL

Sugimoto et al. iPLAT trial, Blood 2022; 140: 2398 and Blood Adv 2022; 6: 6056

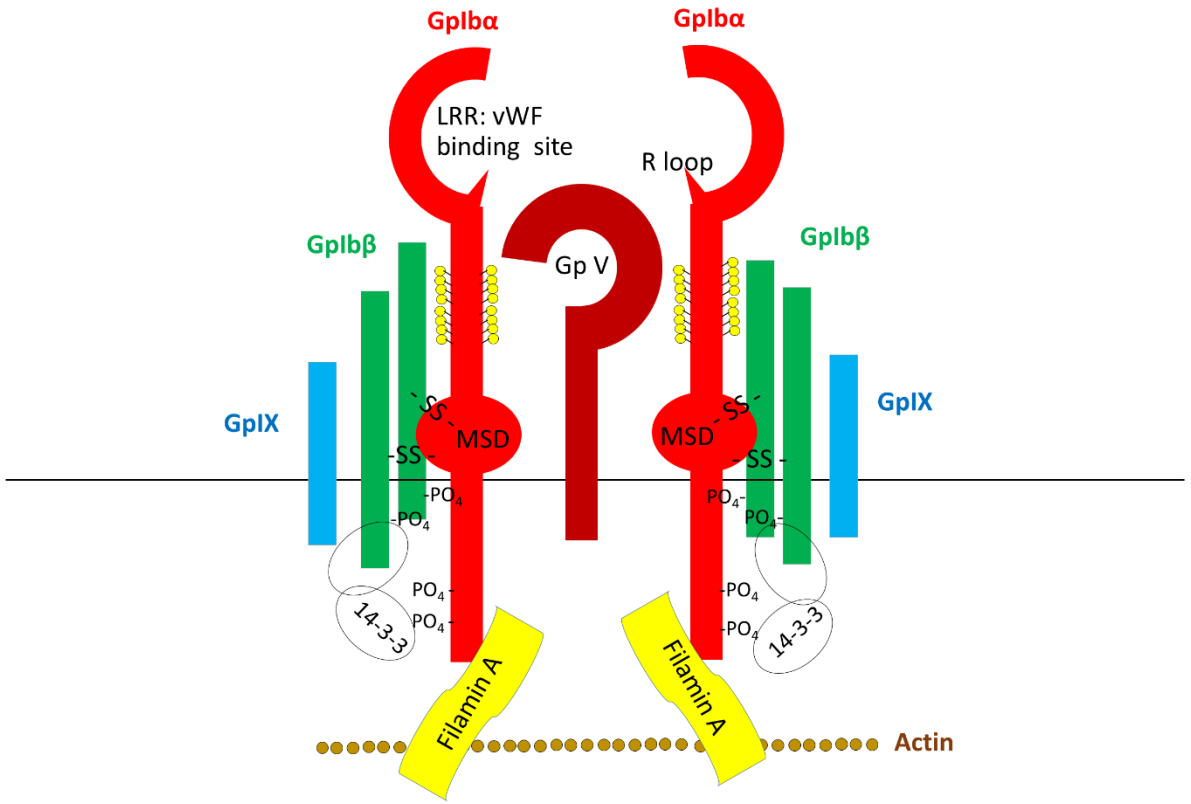
First iPSC-derived platelet transfusion in human

	Cohort 1	Cohort 2	Cohort 3
Date of transfusion	2019.5.22	2019.8.21	2020.1.22
Transfusion dose	0.5 JPN Units 0.1×10^{11}	1.5 JPN Units 0.3×10^{11}	5 JPN Units 1.0×10^{11}
PLT-pre [$\times 10^3/\mu L$]	93	94	92
PLT-1h [$\times 10^3/\mu L$]	90	92	92
PLT-24h [$\times 10^3/\mu L$]	93	97	92
PLT-48h [$\times 10^3/\mu L$]	96	101	100
CCI-1h [$\times 10^3/\mu L$]	–47.5	–10.8	0
CCI-24h [$\times 10^3/\mu L$]	0	16.2	0
Adverse events	None	None	None



Sugimoto et al. iPLAT trial, Blood 2022; 140: 2398

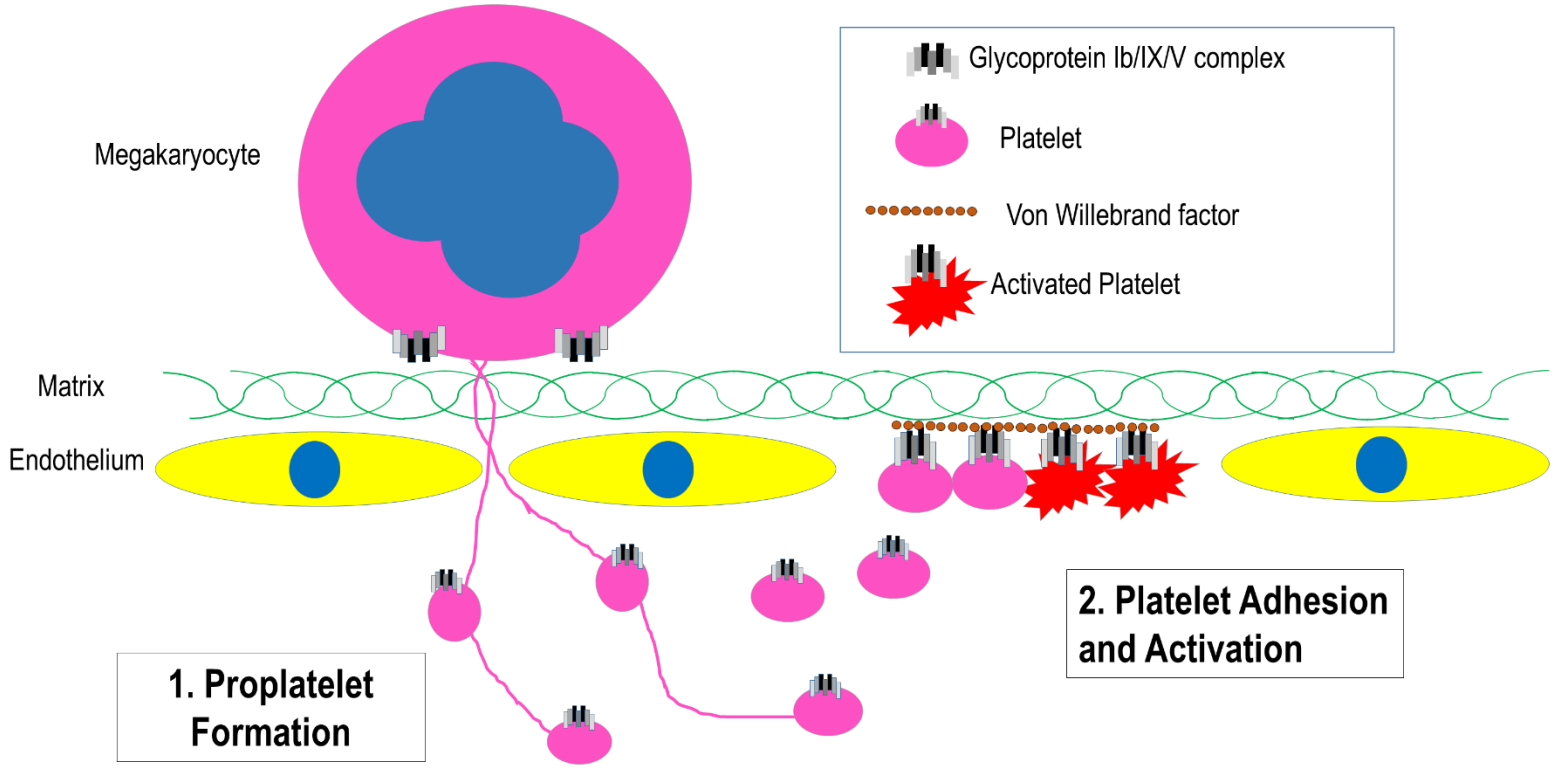
Glycoprotein Ib/IX/V complex



Bernard Soulier
Mutations in
either
GP1BA
GP1BB
GP9

Rojnuckarin P. Recent Advances in iPSC Disease Modeling, Academic Press 2020; p. 187-199.

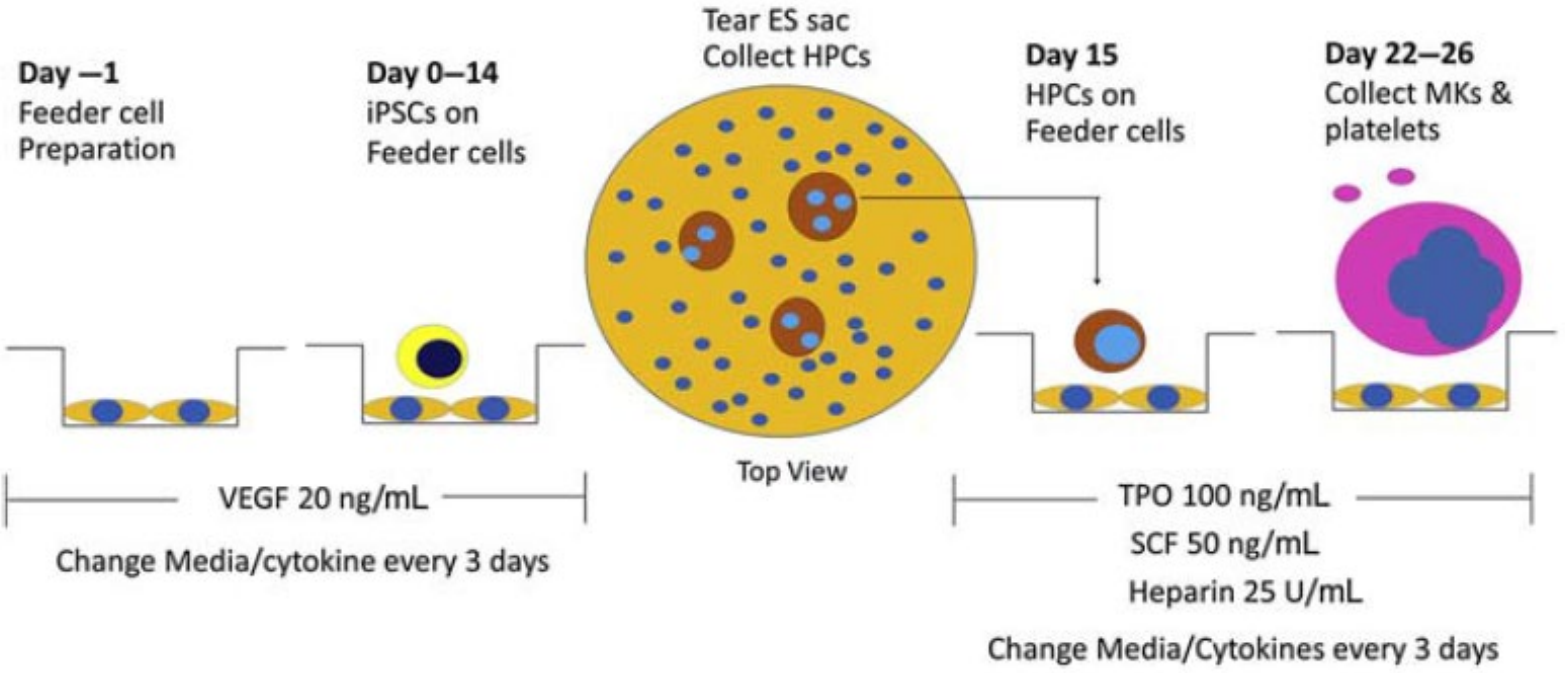
Glycoprotein Ib complex : Dual function

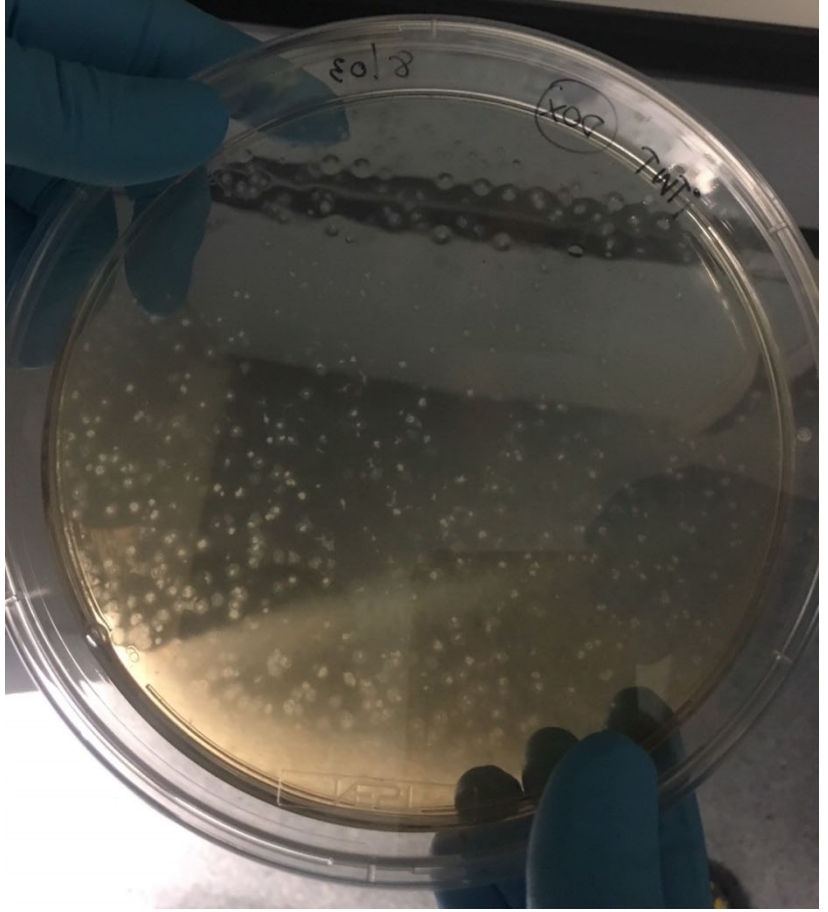


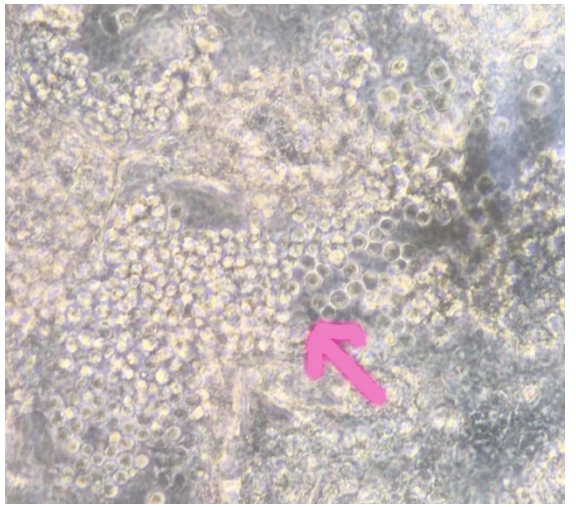
Rojnuckarin P. Recent Advances in iPSC Disease Modeling, Academic Press 2020; p. 187-199.

Induced Pluripotent stem cell model

iPS from patients or Transduced iPS



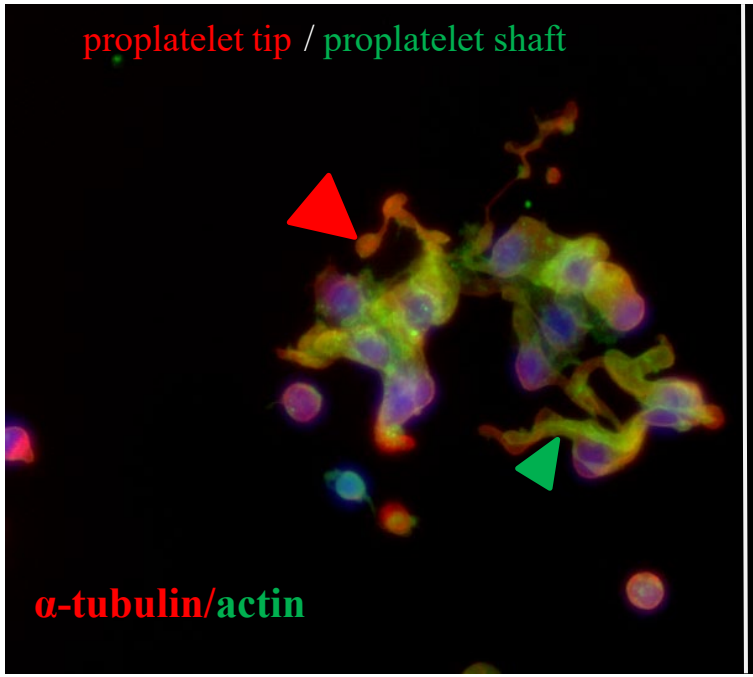




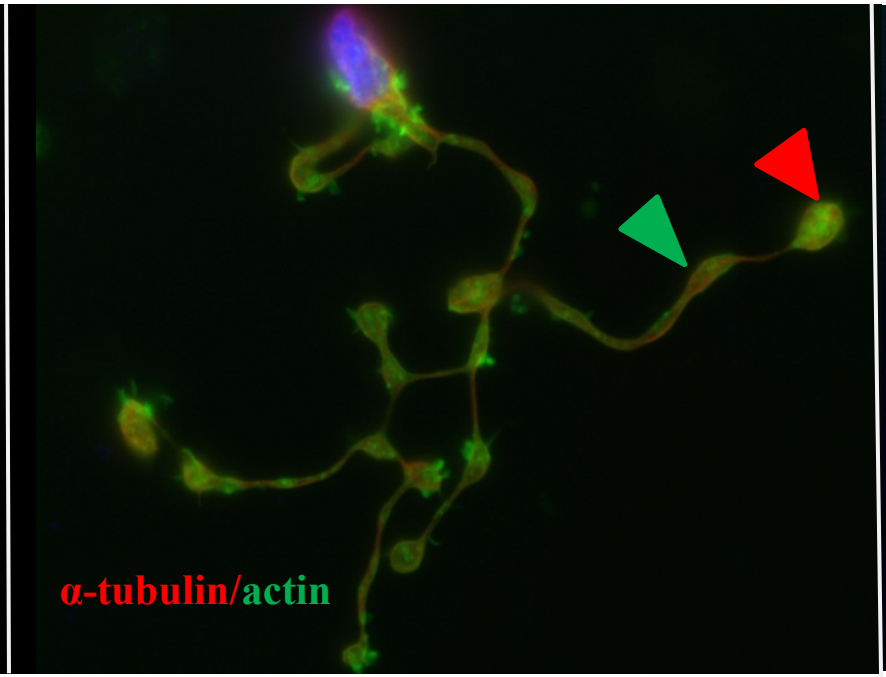
Rojnuckarin P. Recent Advances in iPSC Disease Modeling, Academic Press 2020; p. 187-199.

Proplatelet formation from Bernard Soulier Syndrome

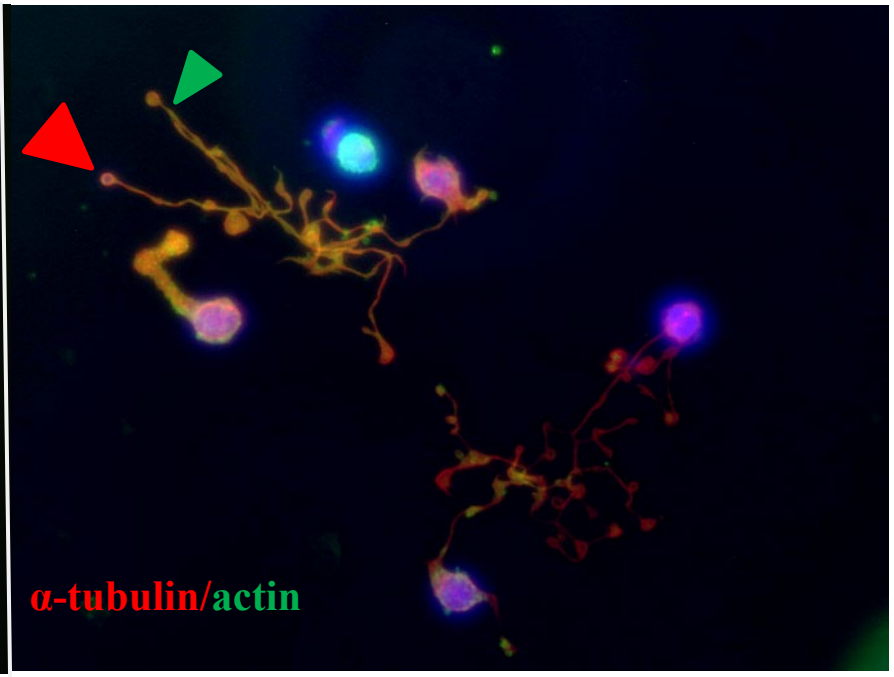
BSS-A iPSC-derived proplatelet



BSS-B iPSC-derived proplatelet



Normal iPSC-derived proplatelet



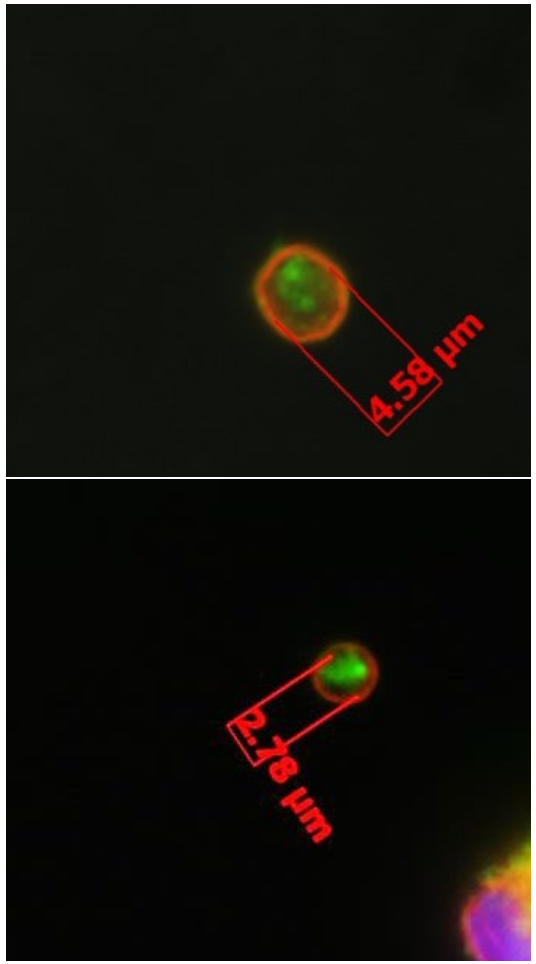
Thick proplatelets

Mekchay et al. Thromb Haemost. 2019;119:1461

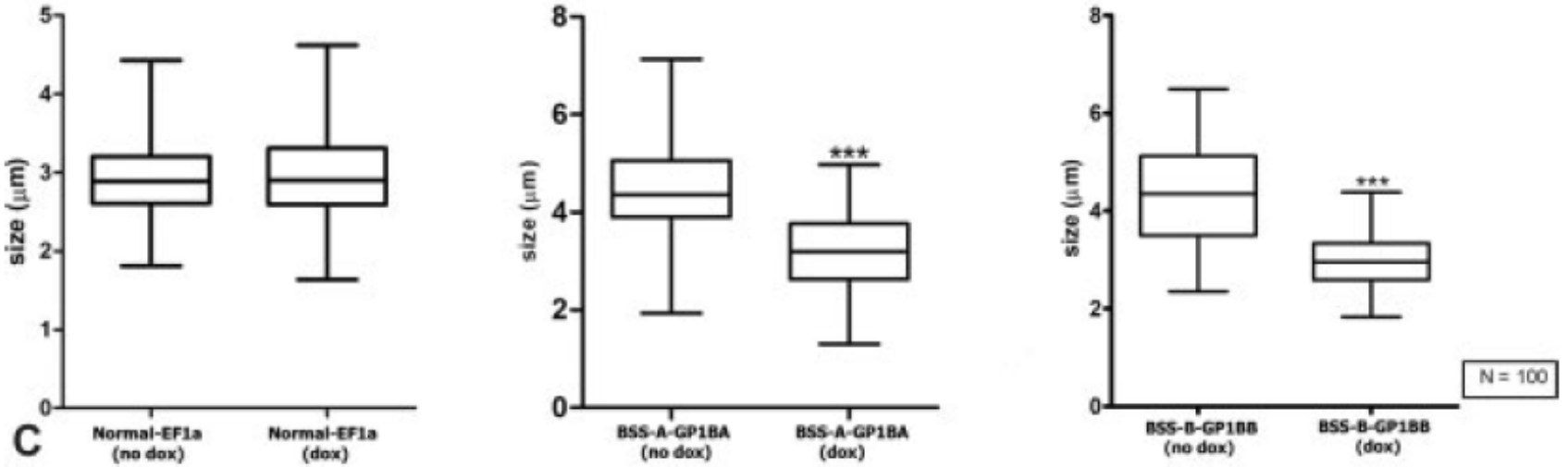
iPSC-derived platelets from Bernard Soulier patients

BSS

WT



iPS-derived Platelet sizes can be corrected by retroviral transduction of *GP1BA* or *GP1BB* genes

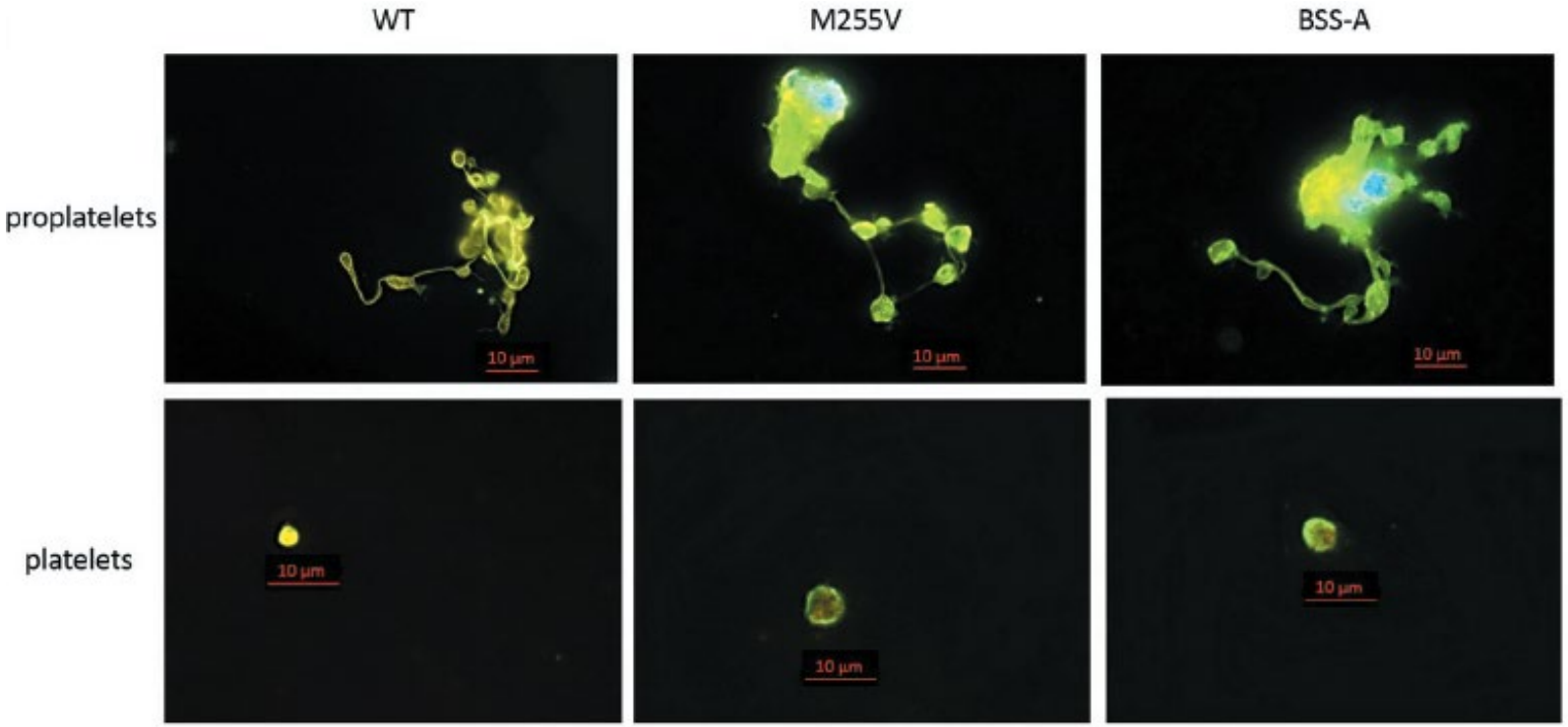


Mekchay et al. Thromb Haemost. 2019; 119: 1461

Roles of GPIb in proplatelet formation

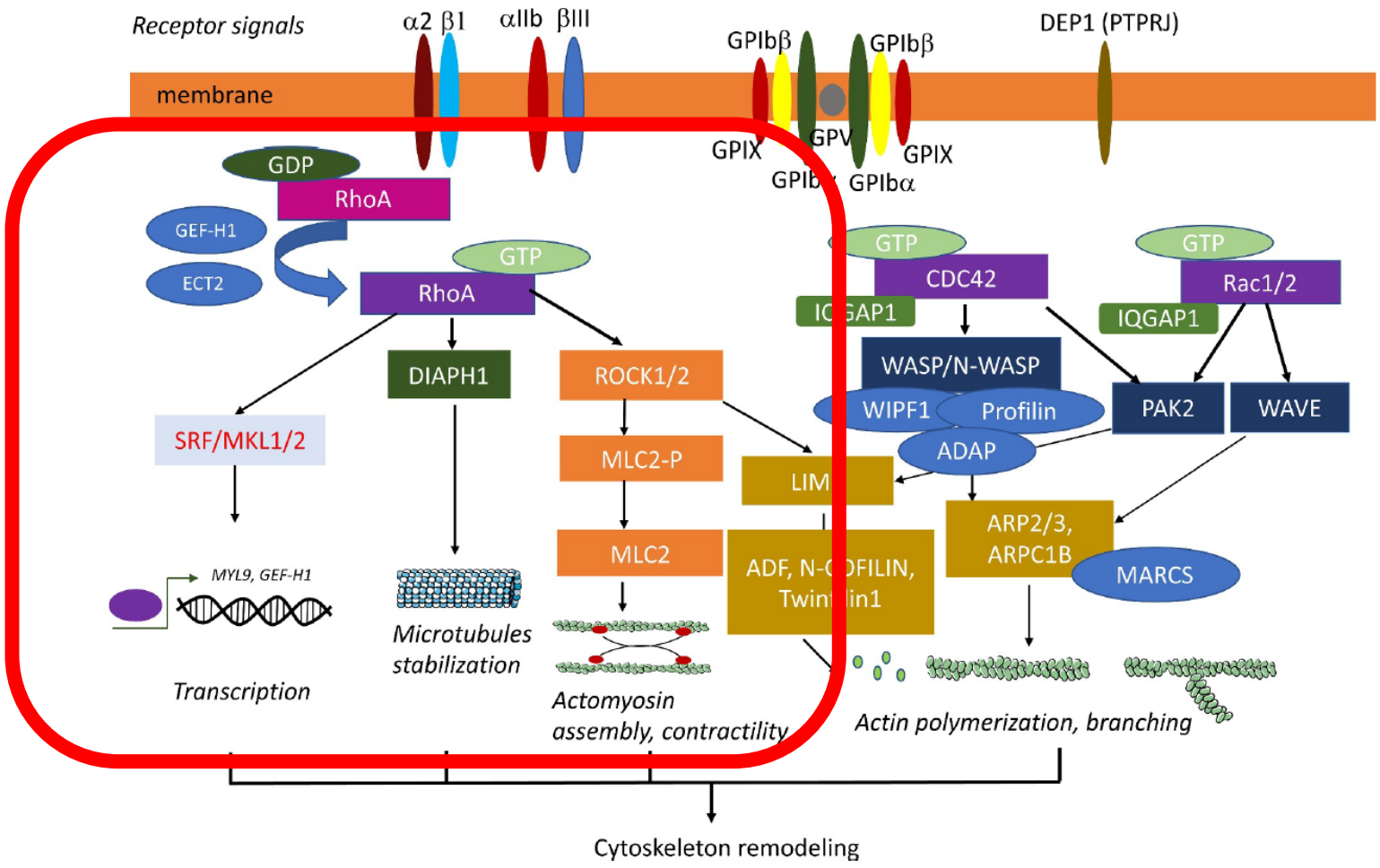
Hyperactive GPIb

Hypoactive GPIb



Pawinwongchai J et al. Platelets. 2021; 32: 1073

Rho GTPases control cytoskeleton



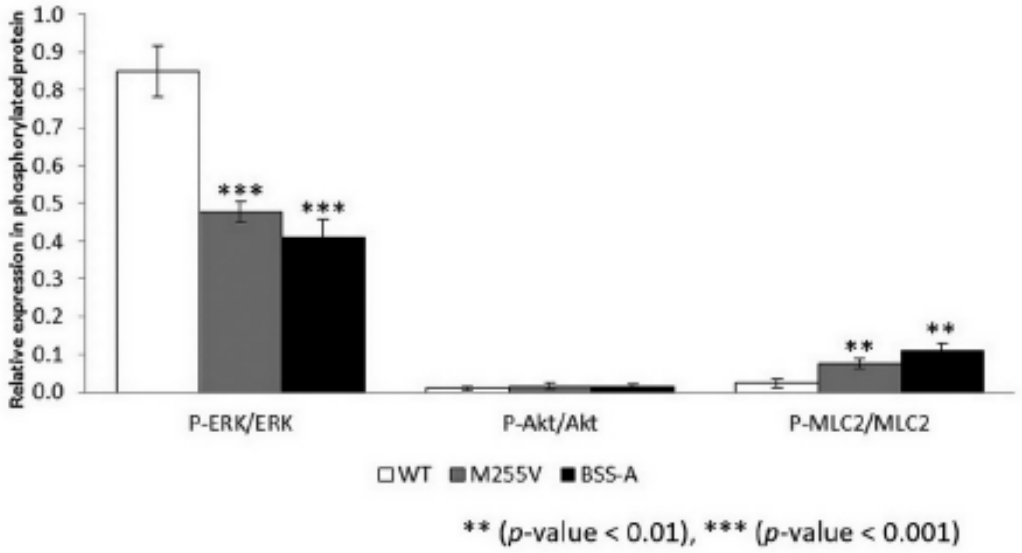
Small GTPases, 12:5-6, 399-415,

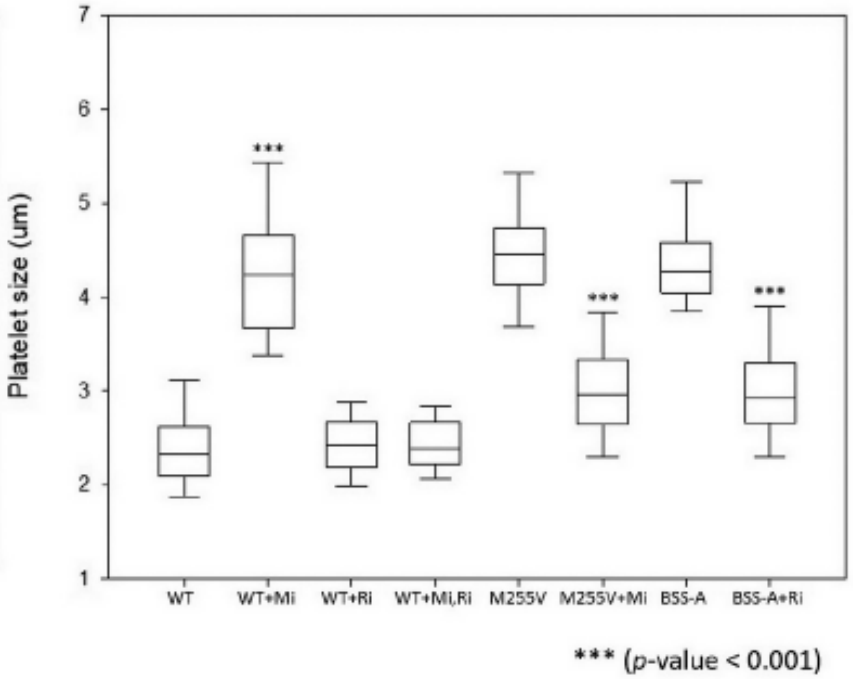
Roles of GPIb in proplatelet formation

M255V: Hyperactive GPIb

BSS: Hypoactive GPIb

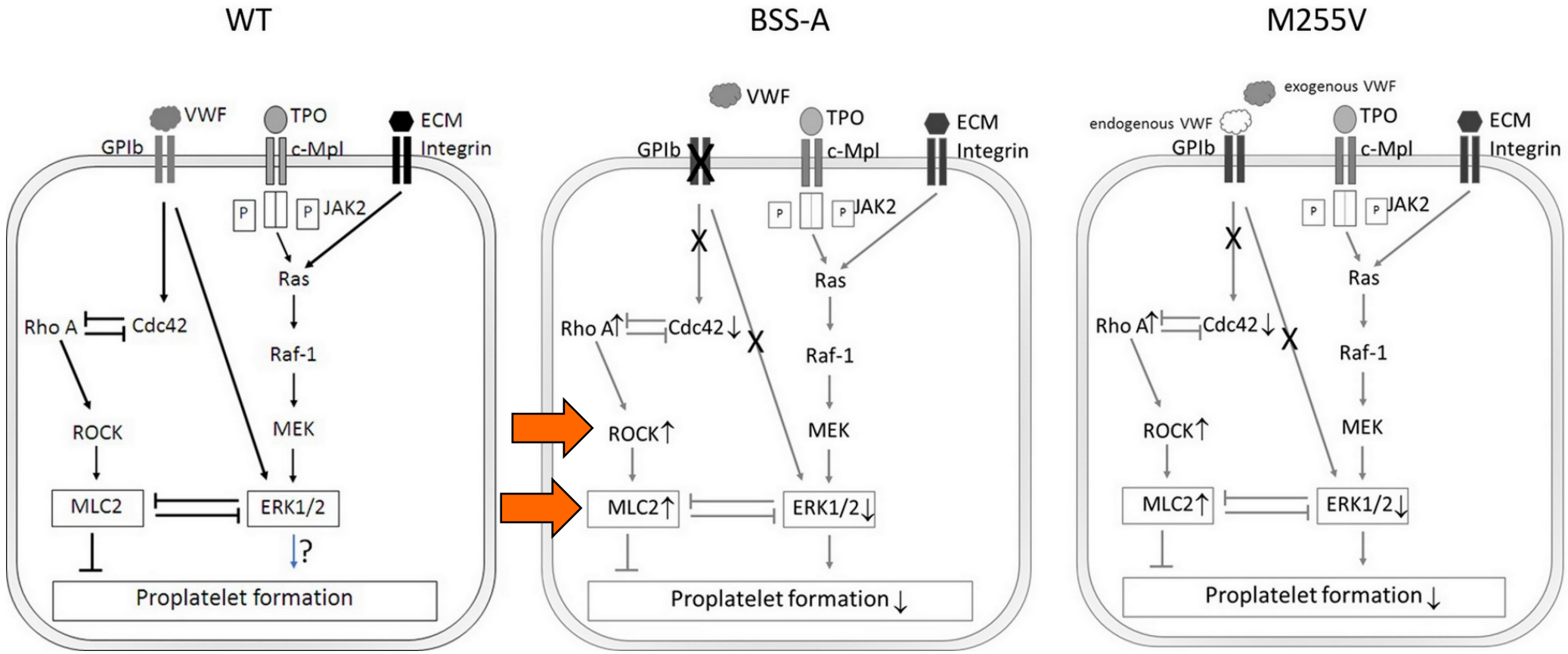
Mi: MAPK inhibitor
Ri: ROCK inhibitor





Pawinwongchai J et al. Platelets. 2021; 32: 1073

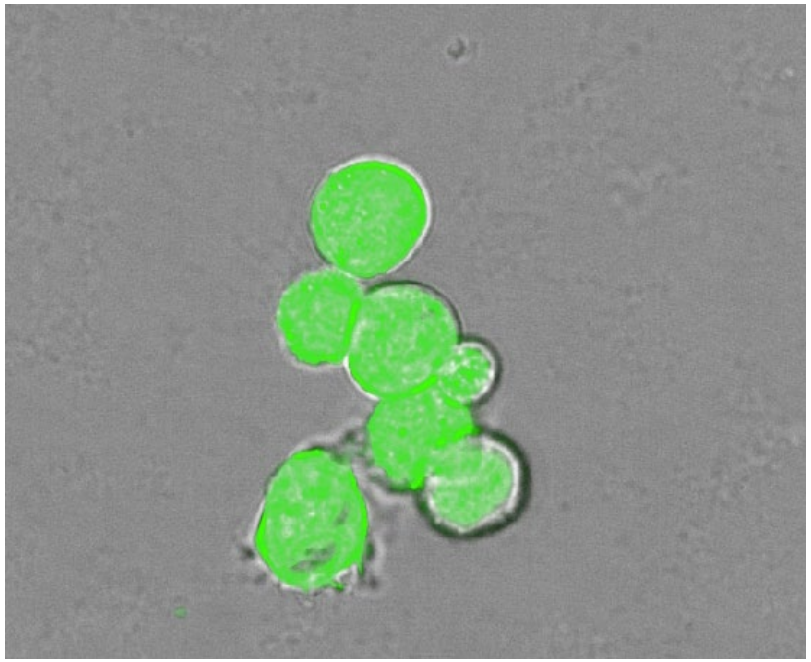
Roles of GPIb in proplatelet formation



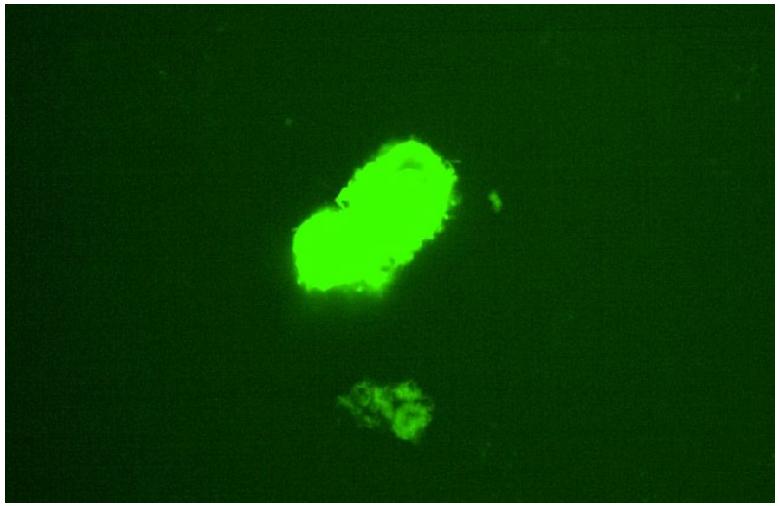
Potential targets

Pawinwongchai J et al. Platelets. 2021; 32: 1073

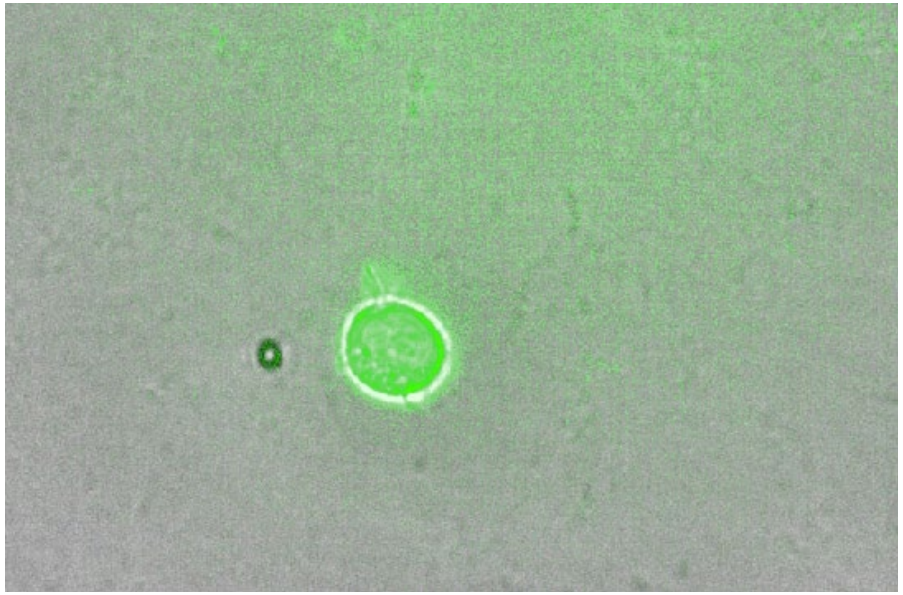
Platelet formation in Tubulin-GFP MKs



Proplatelet formation



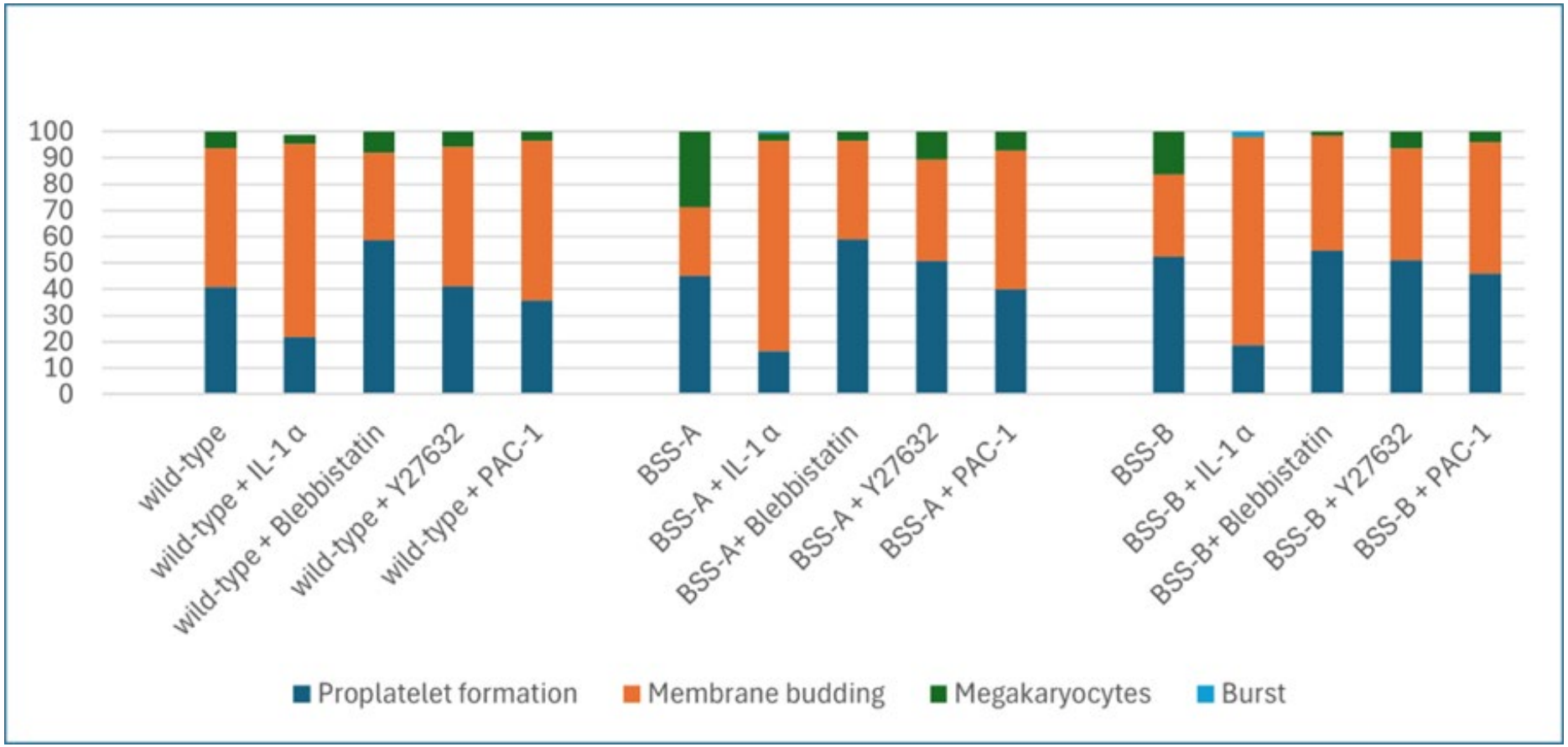
Budding



Rupture

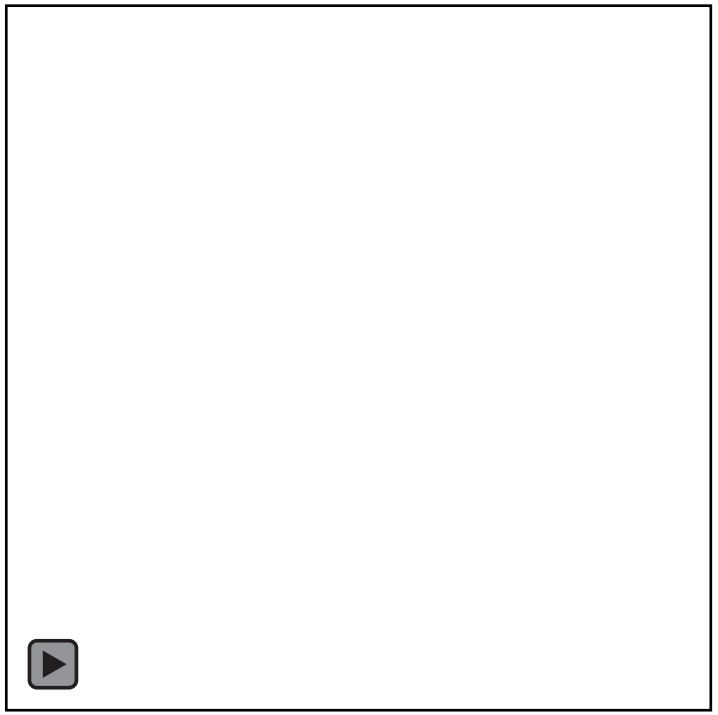
Dr Ponthip Mekchay

IL-1α increased Mk budding

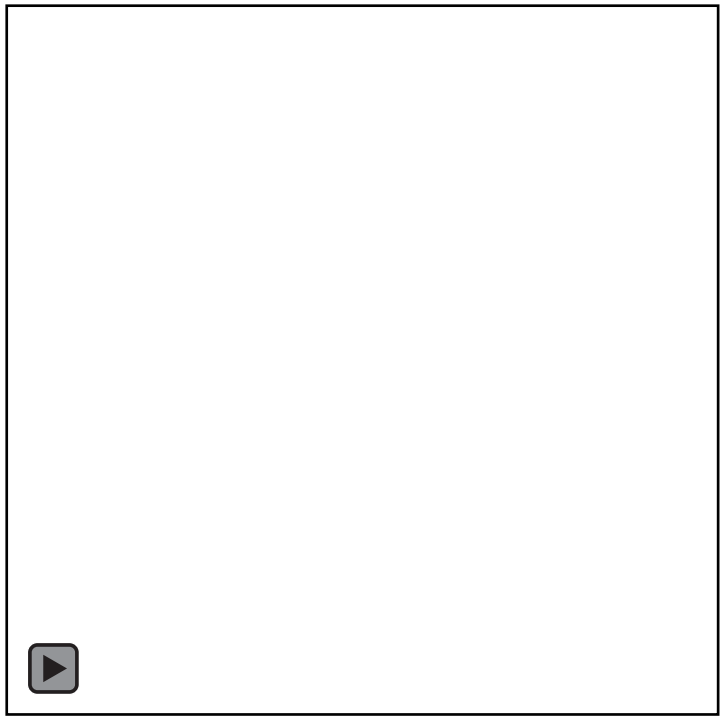


Mekchay et al ISTH 2024

Thick proplatelets in Bernard Soulier cells



Wild-type

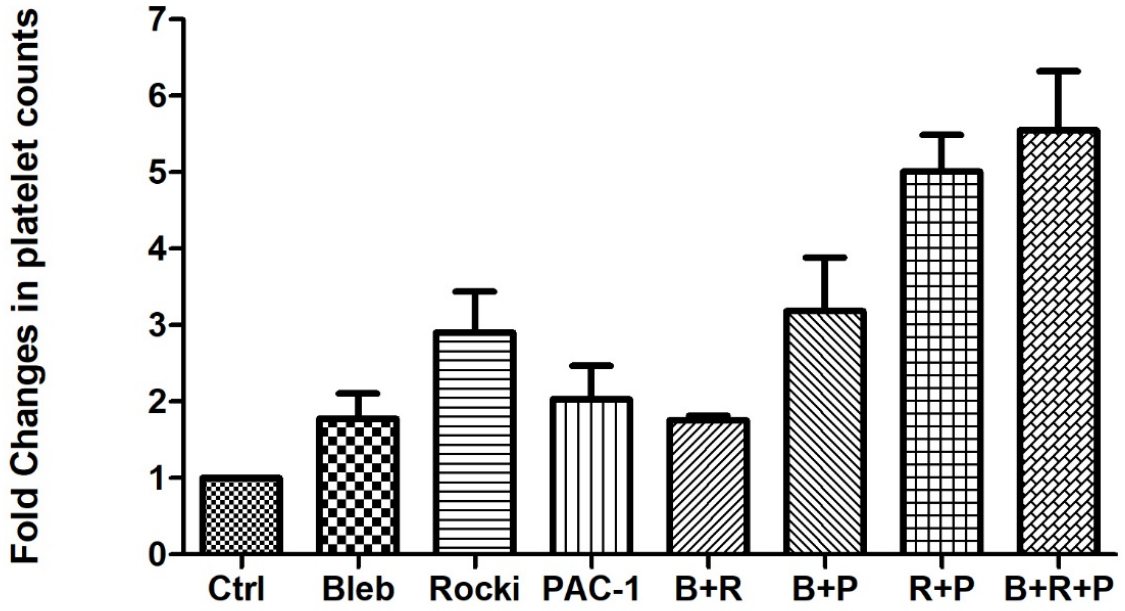


Bernard Soulier MK

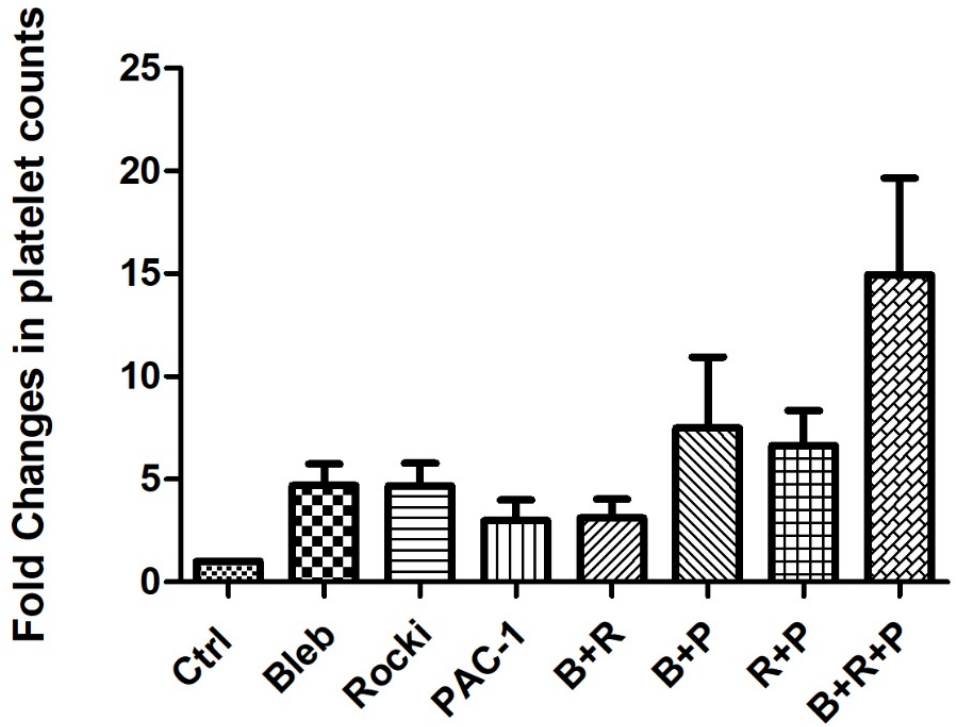
Makchay et al. Thrombosis and Haemostasis 2025

Increase platelet yields by ROCK and Myosin II inhibitors

WT



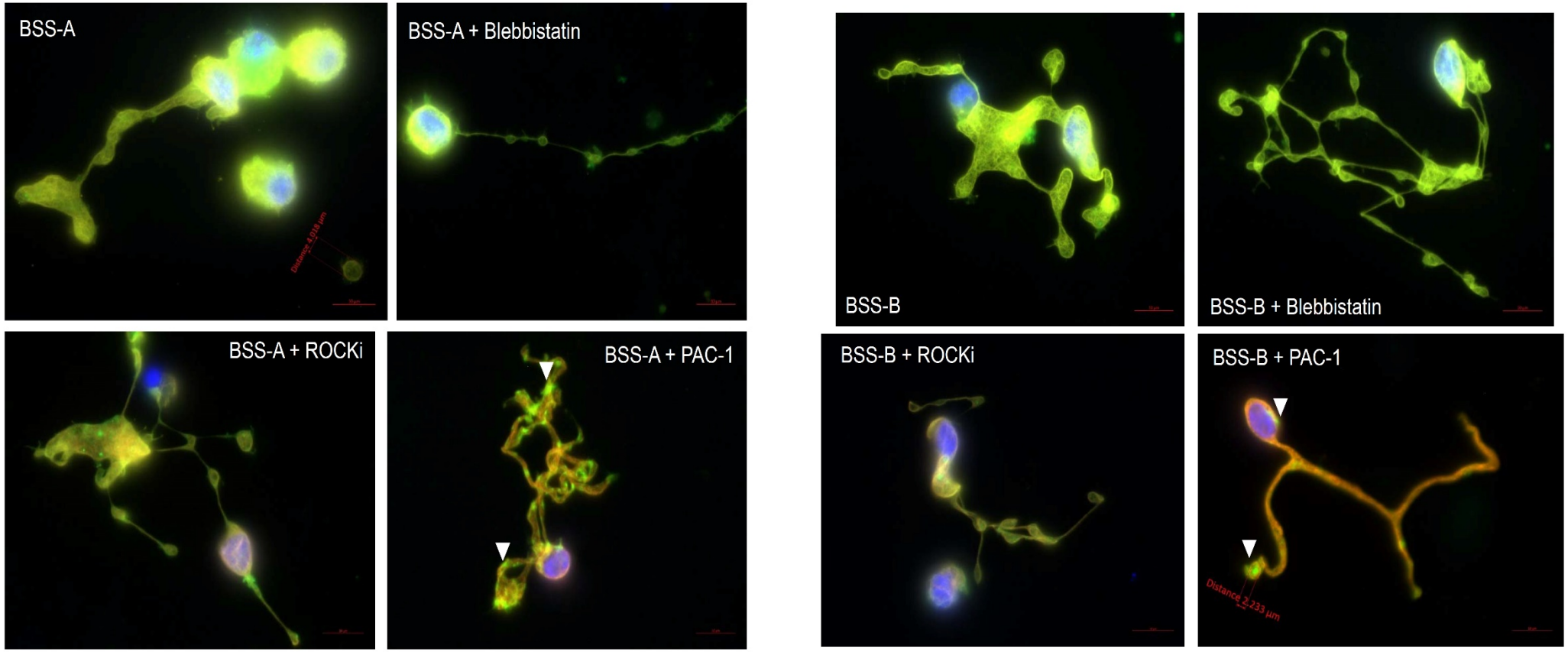
BSS-A



Blebbistatin (Myosin inhibitor)
ROCK inhibitor
PAC1: Caspase 3 activator

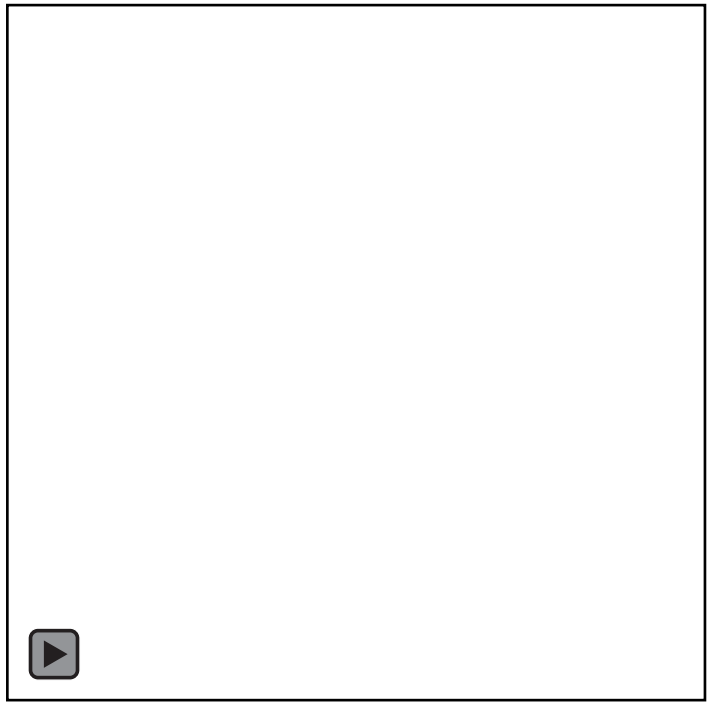
Makchay et al. Thrombosis and Haemostasis 2025

Reversal of BSS phenotypes by ROCK and Myosin II inhibitors

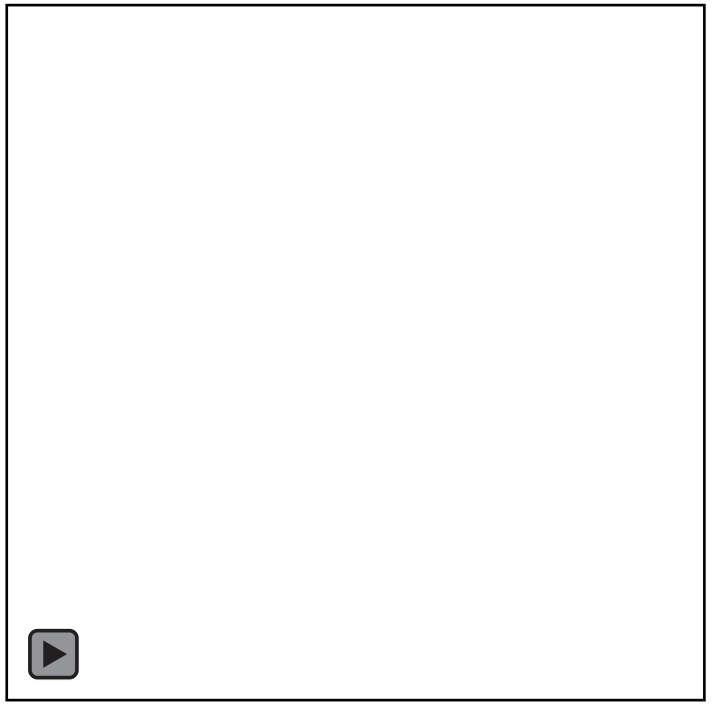


Makchay et al. Thrombosis and Haemostasis 2025

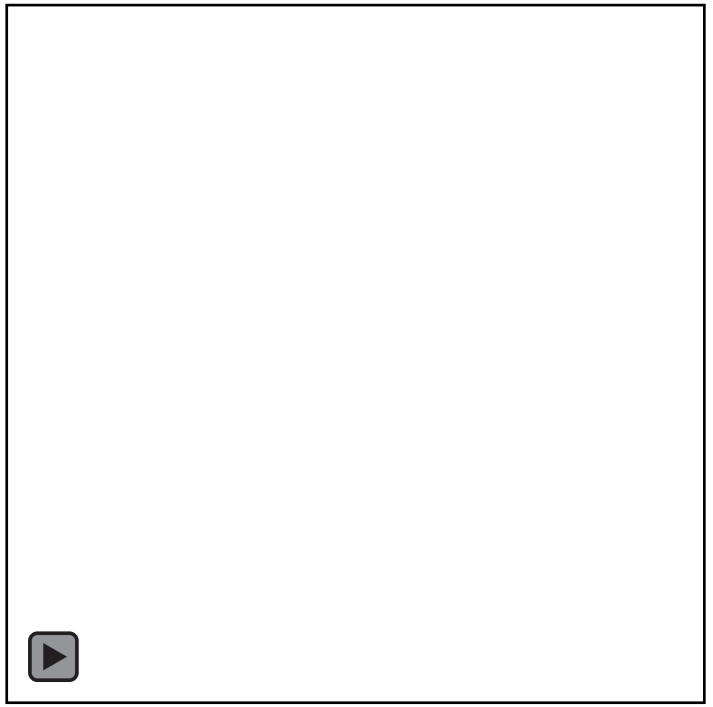
Reversal of BSS phenotypes by ROCK and Myosin II inhibitors



BSS MK

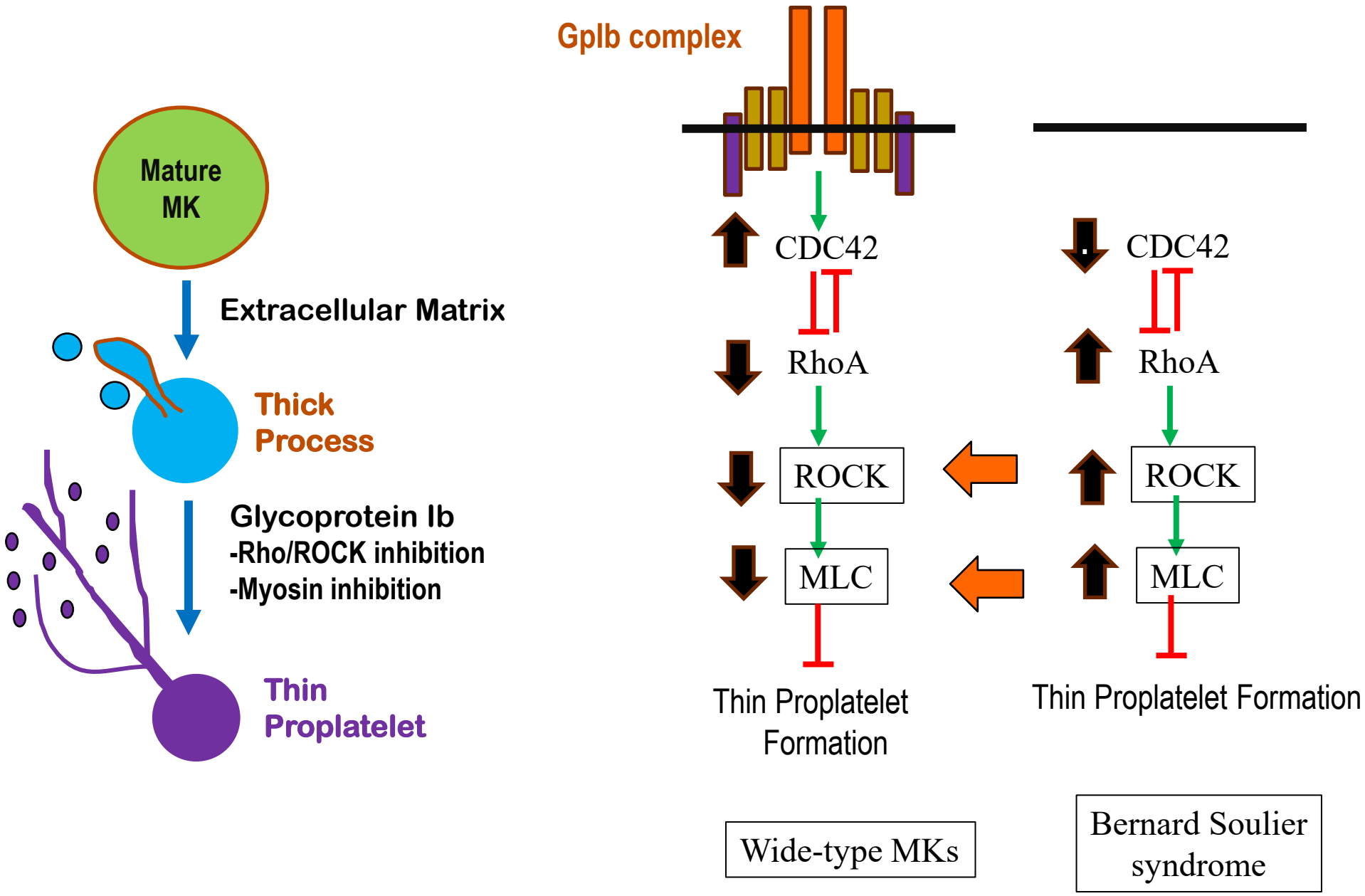


BSS MK + ROCKi



BSS MK + Myosin inhibitor

Makchay et al. Thrombosis and Haemostasis 2025



Mekchay et al. Thromb Haemost. 2024 Dec 18. doi: 10.1055/a-2474-5644

Conclusions

- Advance technology: IPSCs, Single-cell sequencing, Genetic manipulation led to deeper insights in MK development
- MKs can develop directly from HSC and modulate HSC properties
- iPSC models for MPNs may lead to better target therapy
- Understanding of platelet formation mechanisms may be helpful to treat thrombocytopenia (Disease model and Drug screening) and production of platelets for transfusion in the future

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