

Platelet production in vitro: Opportunities for front line medicine

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THOR June 22

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Consulting for

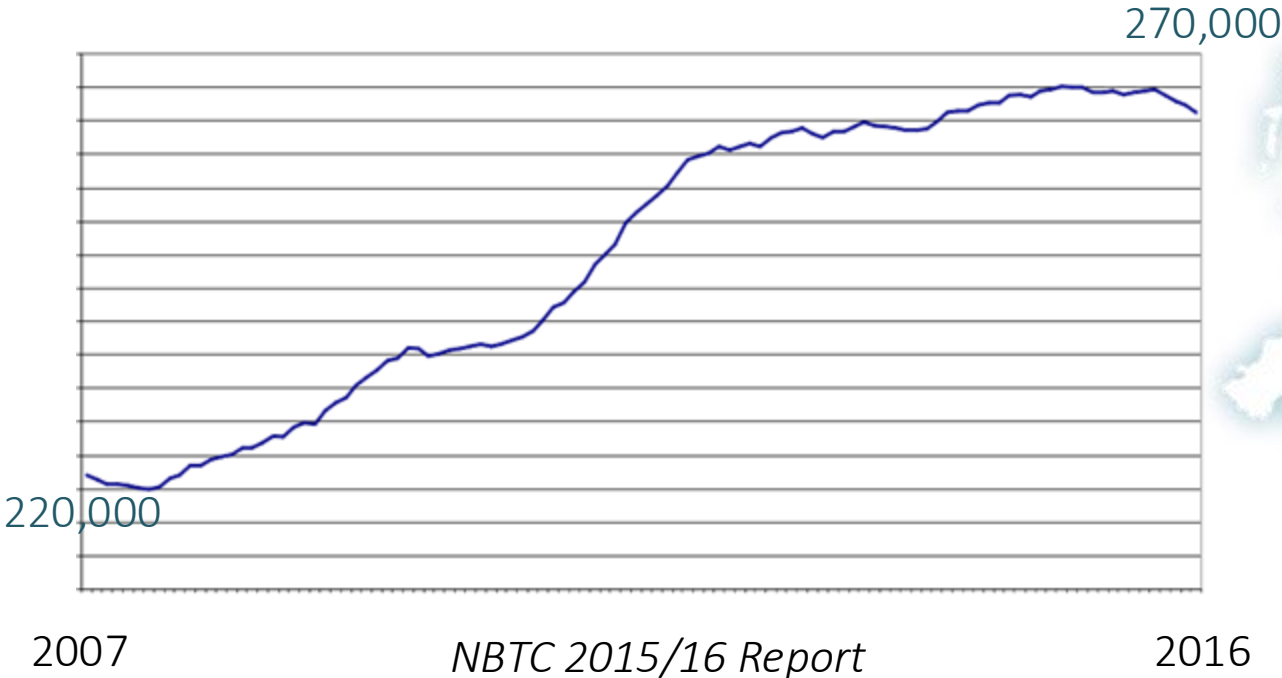
- BitBio (Cambridge, UK)
- Platelet Bio (Boston, USA)
- Xap Therapeutics (Cambridge, UK)
- RedC Biotech (Israel)

Platelets – transfusion needs

280,000 platelet units per year in the UK (£58M)

60% prophylactic (oncology, inherited deficiencies)

40% therapeutic (surgery, trauma)



*NBTC 2015/16 Report
Moving annual total issues to Hospitals*

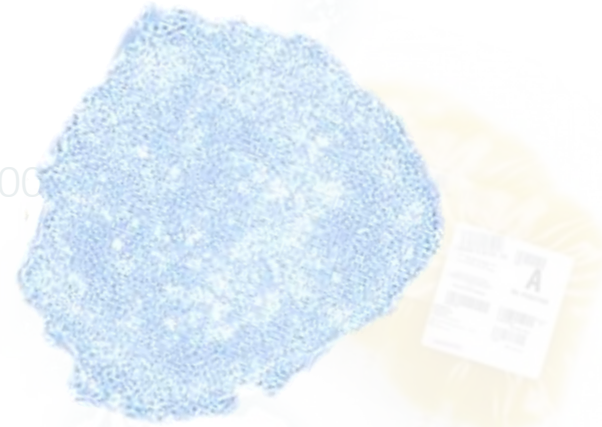


Platelets – Sources

280,000 platelet units per year in the UK (£58M) **Donors** **hPSC**

60% primary, inherited deficiencies
40% trauma

3E+11
platelets
per unit



Supply line with platelets
Biosafety management
(irradiation)
HLA universal platelets
Engineered platelets

Voluntary donation **dependency**
Human product **biosafety**
Platelet unit **shelf life (5-7 days)**
Rare groups
Alloimmunisation

15,000 HLA-matched units/year
(cost x2; restricted pool of recallable donors)



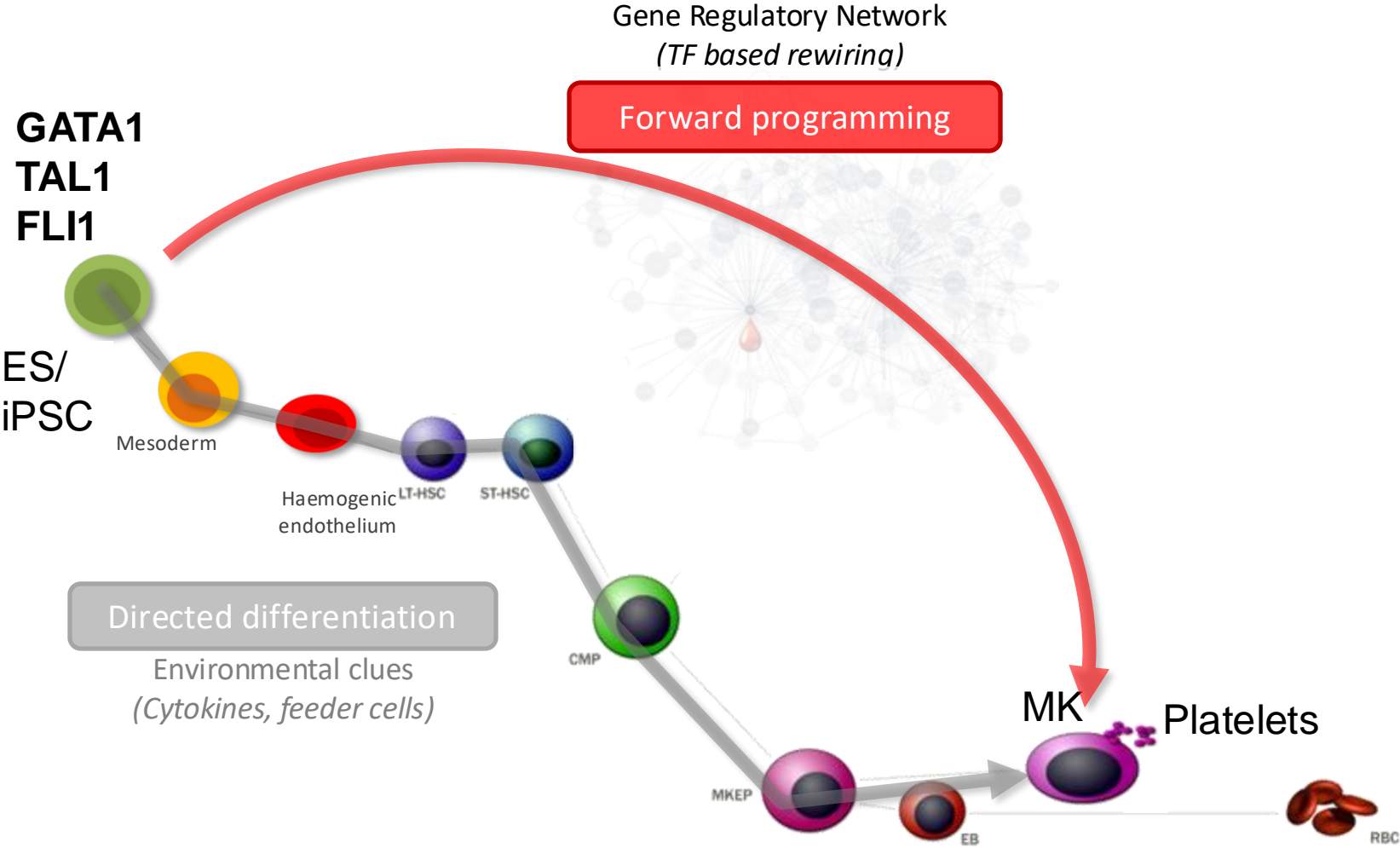
Platelets – the journey to the front line



Platelets have a shelf-life of 7 days, need to stay at room temperature and need constant agitation....

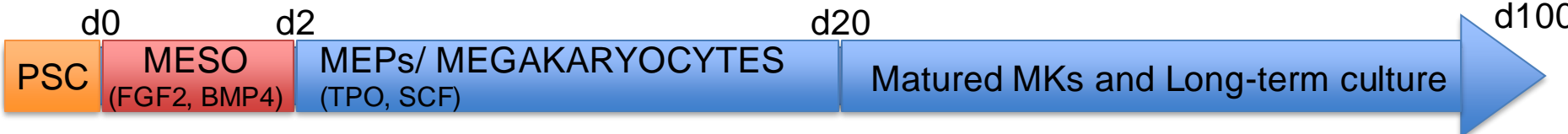
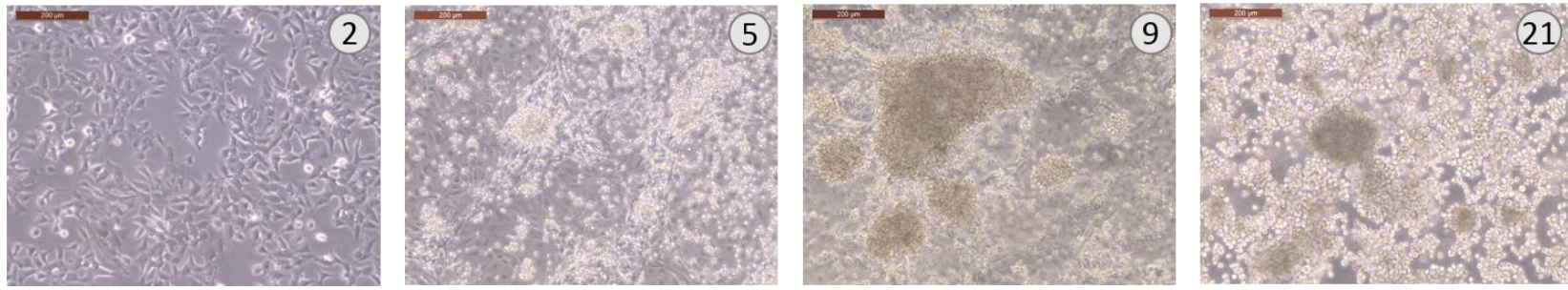
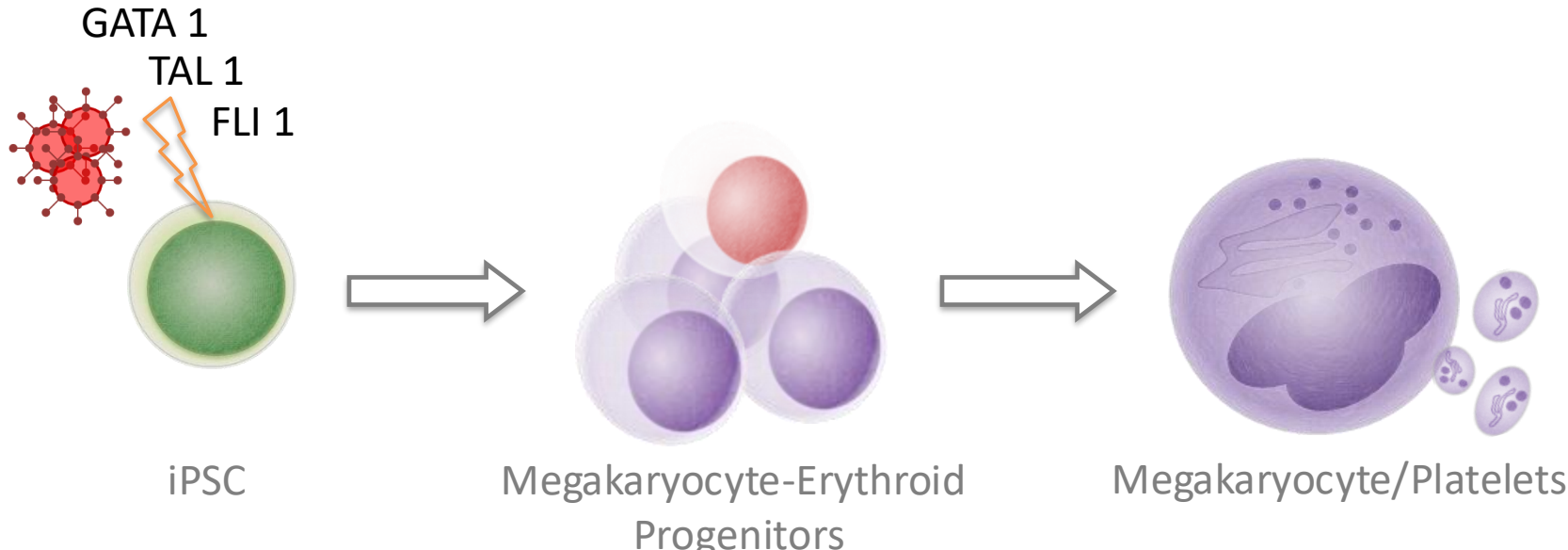
 Save a life
Give platelets

From iPSCs to Megakaryocytes and Platelets



Moreau et al., *Nat. Commun* 2016

Forward Programming

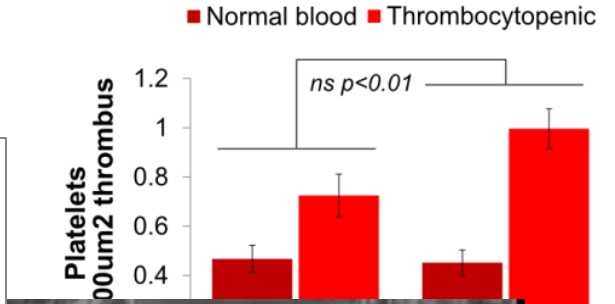
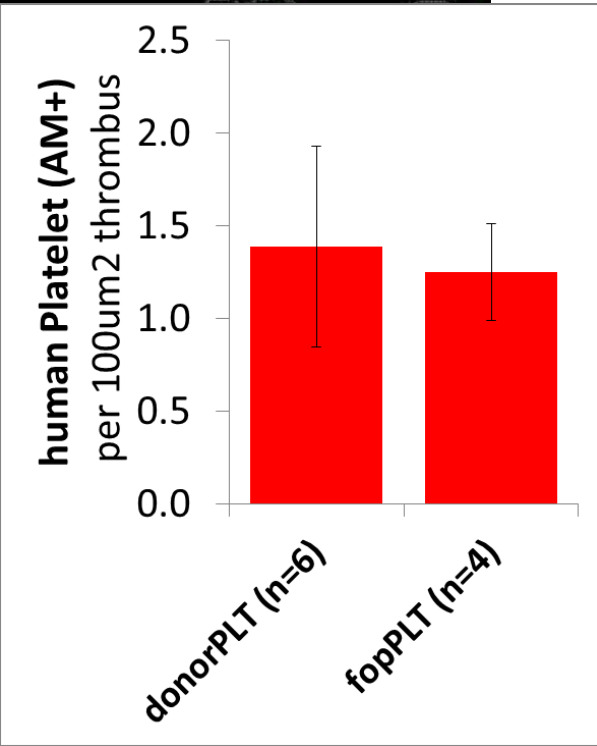
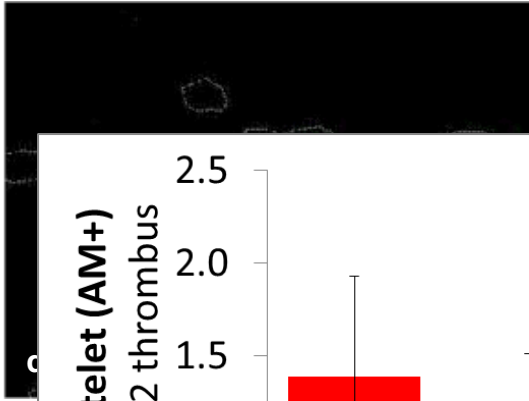
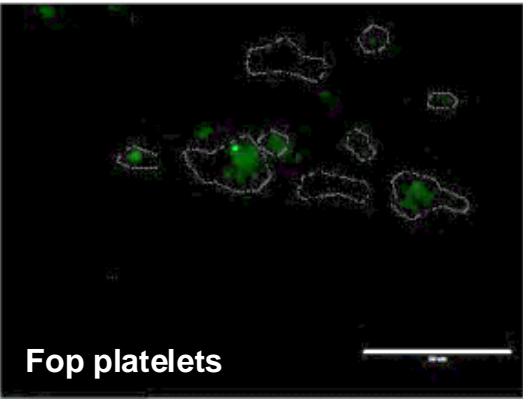


Moreau et al., *Nat. Commun* 2016

Platelet functionality

Thrombus formation *in vitro* (Collagen, flow chamber)

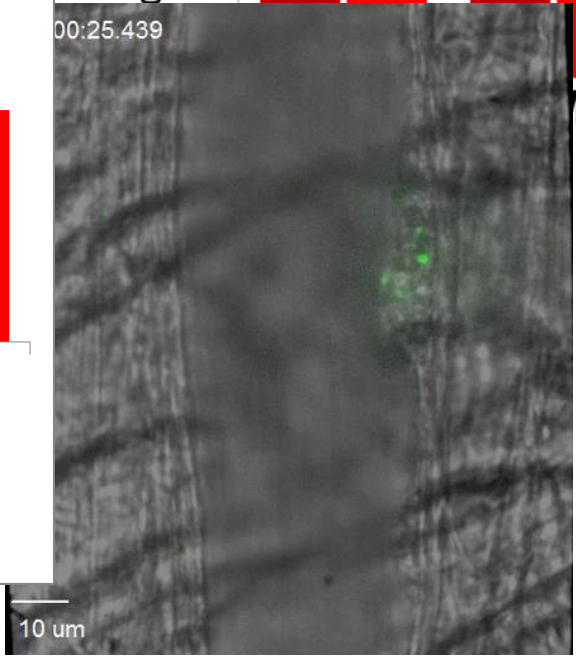
Spiked calceinAM platelets (1E+7/ml)



Thrombus formation *in vivo*



(DONOR or iPSC)



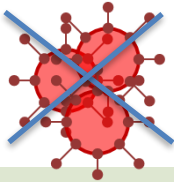
The real challenge

- Scaling up and manufacturing process
- Quality assurance and safety/ regulatory considerations
- Adoption by health providers and costs

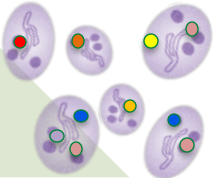
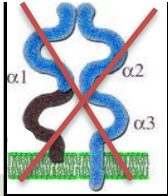
Moving towards transfusion medicine



GATA1
TAL1
FLI1



HLA Class 1

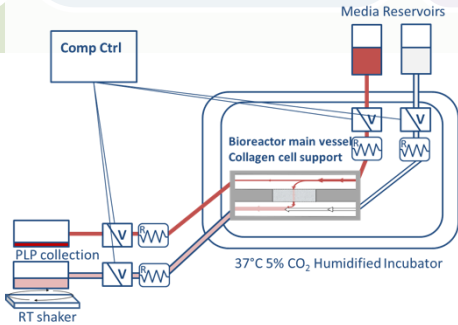


GMP hiPSC lines

Viral free MKFOP
inducible hiPSC lines

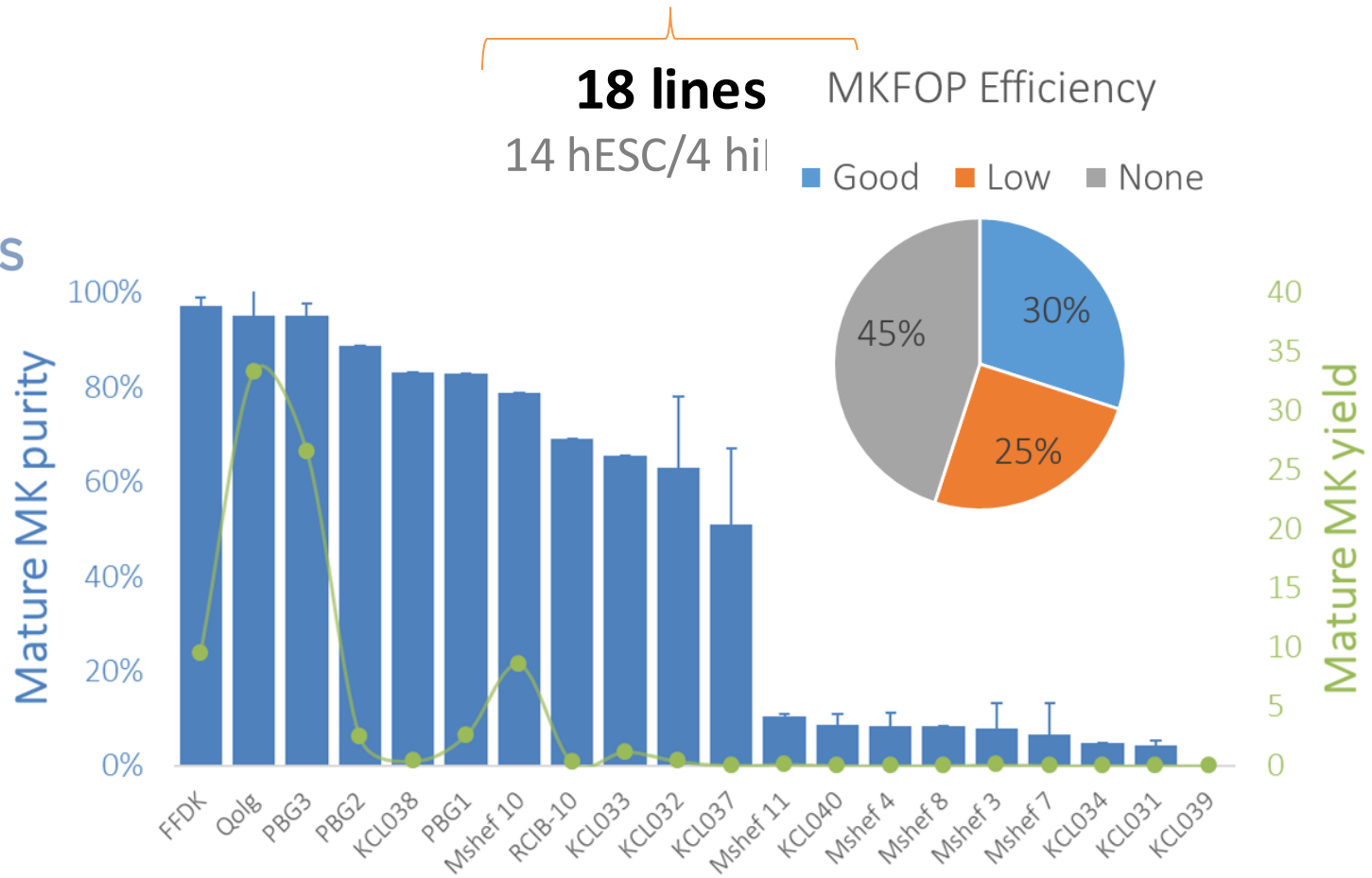
Platelet production
scaffold & bioreactors

Adding benefits
customised platelets for everyone



Identifying the seed material

Identify GMP-grade hPSC lines with high MK programming potential

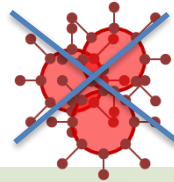


Evans et al, Blood Advances 2021

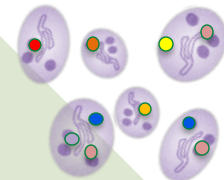
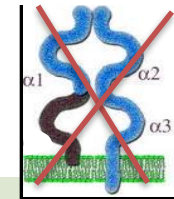
Moving towards transfusion medicine



GATA1
TAL1
FLI1



HLA Class 1



GMP hiPSC lines

UKSCB repository

Viral free MKFOP

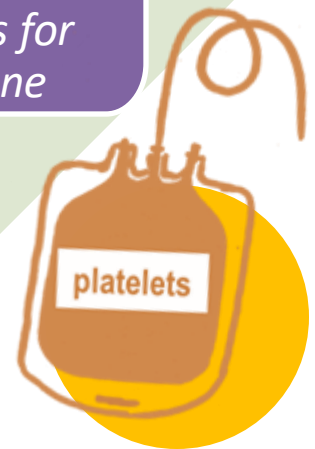
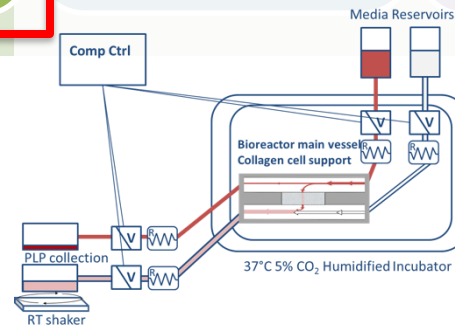
inducible hiPSC lines

Platelet production

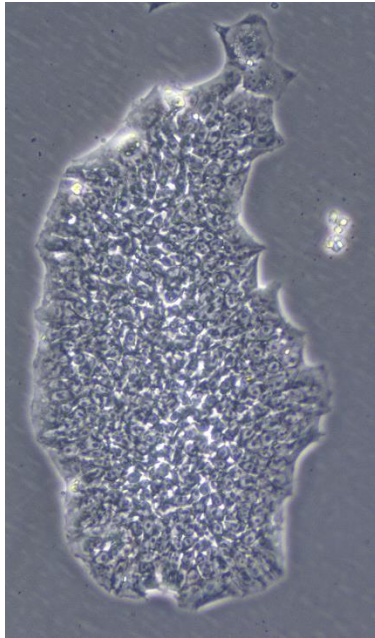
scaffold & bioreactors

Adding benefits

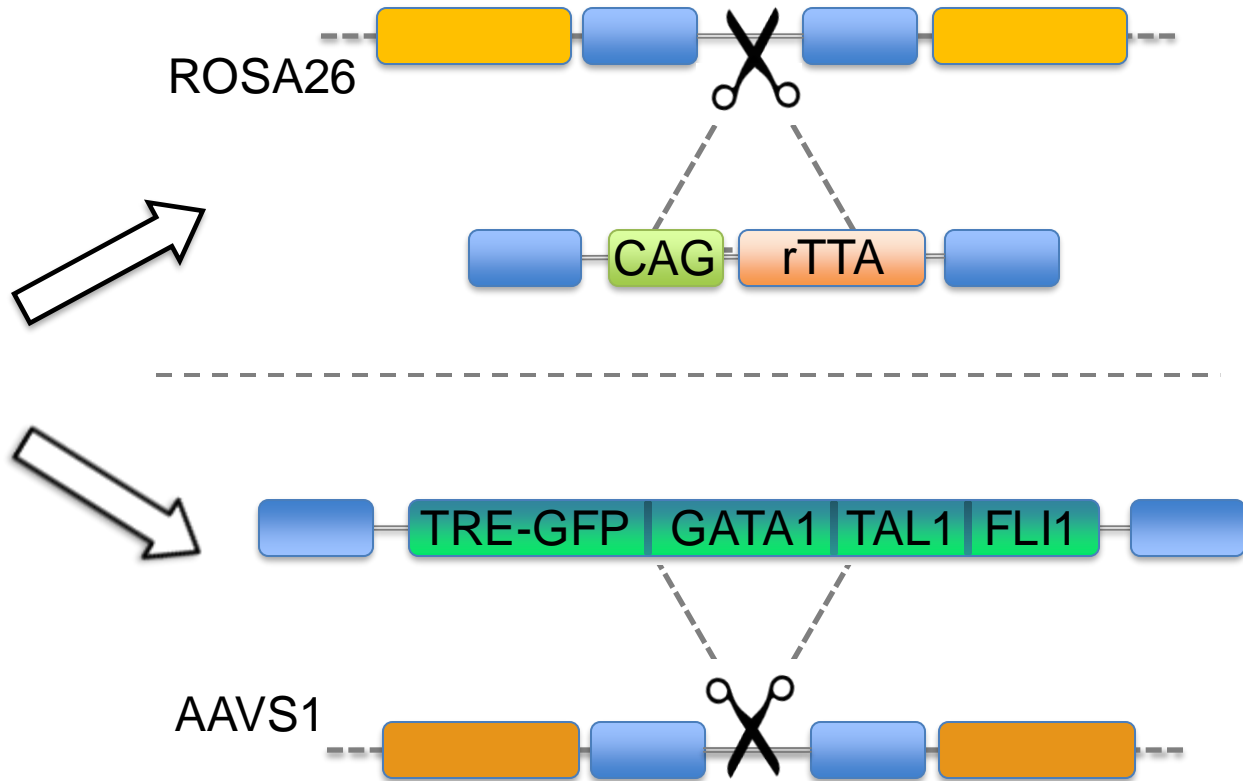
customised platelets for everyone



Inducible FOP – optimized TET-ON system



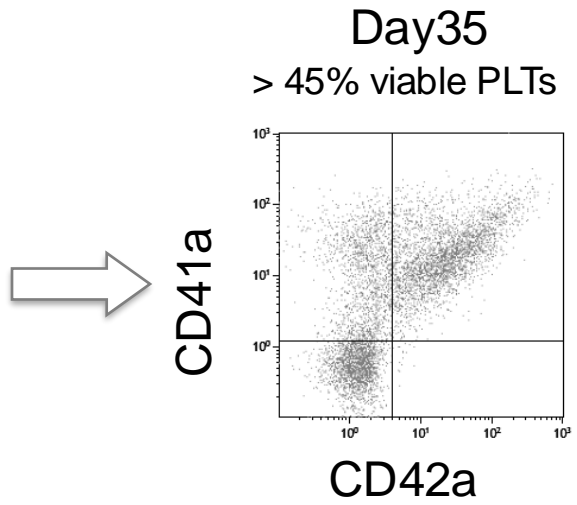
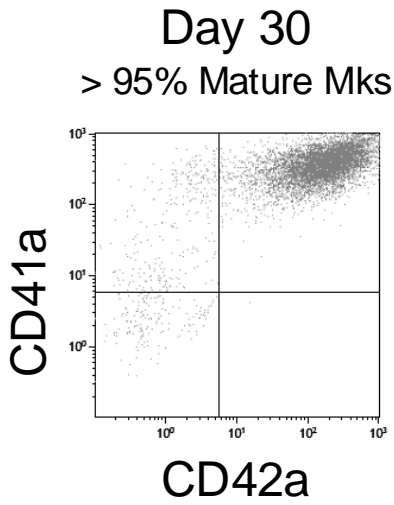
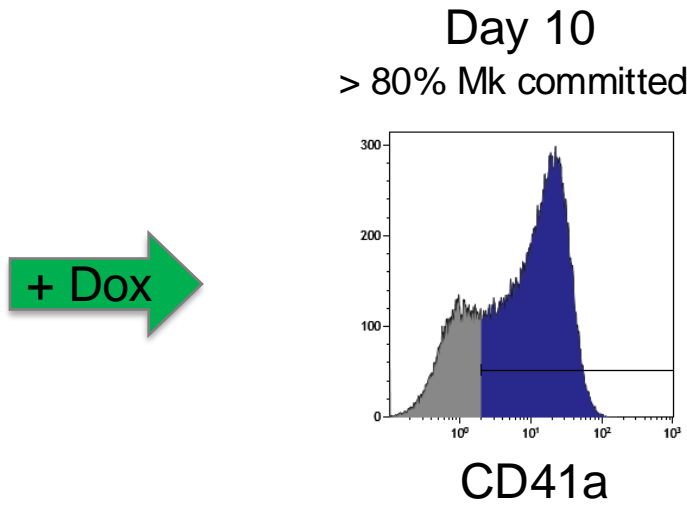
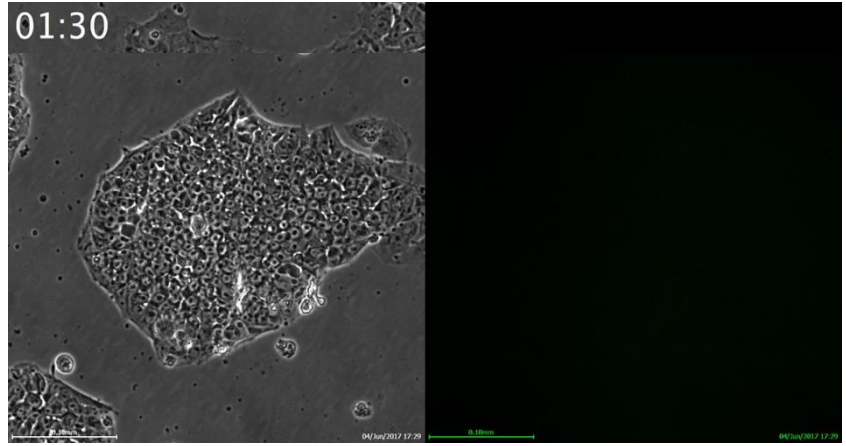
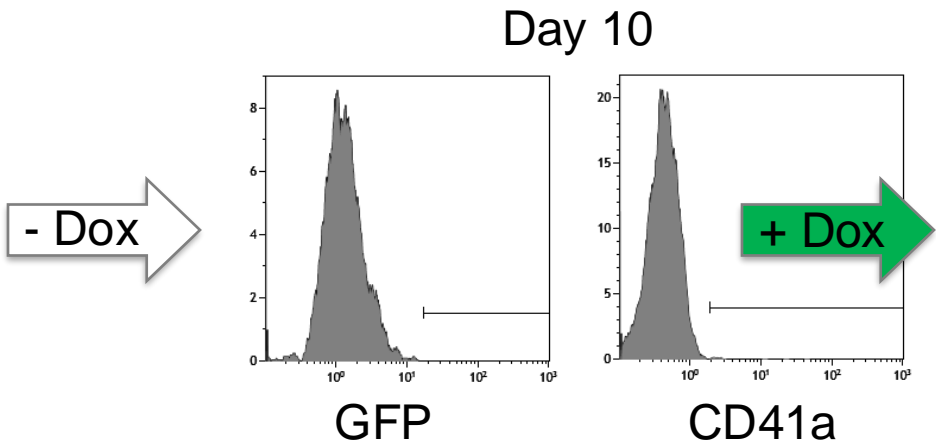
hiPSCs



rTTA=reverse tetracycline transactivator
TRE= tetracycline responsive promotor element

OPTi-OX system
Pawlowski et al., *Stem Cell Reports* 2017
Evans et al, *Blood Advances* 2021

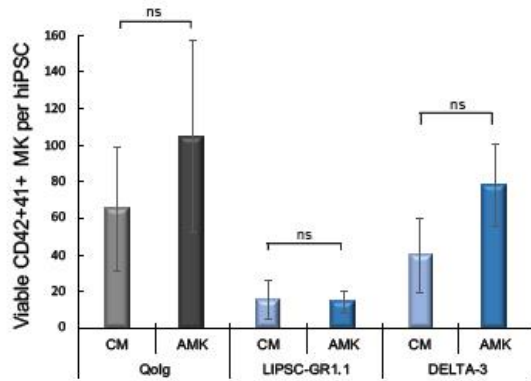
Mks and Platelets from inducible FOP



Evans et al, Blood Advances 2021

Optimising media for MK culture

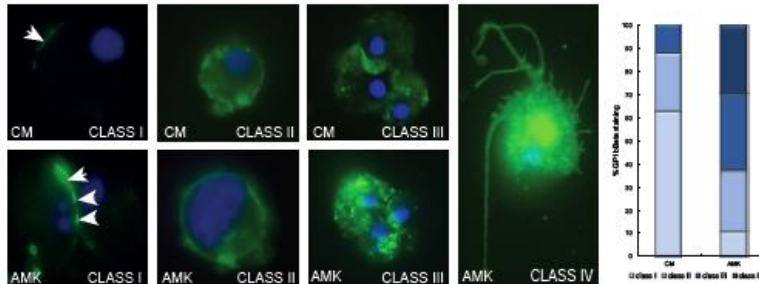
A



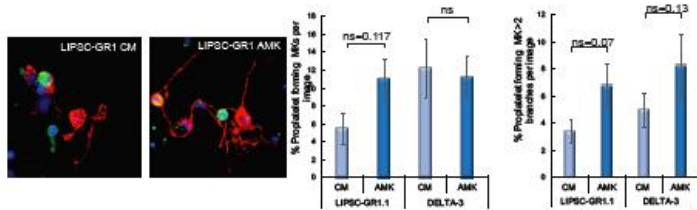
AMK: bespoke culture medium with individual compounds available at GMP grade

Component	Company	Cat. No.	GMP Compliant	ml	Concentration
Basal Media IMDM w/o phenol red	ThermoFisher Scientific (Gibco™)	21056 (500ml)	✓	500	1x
Chemically defined lipid concentrate	ThermoFisher Scientific (Gibco™)	11905-031 (100ml)		5	1x
Insulin-Transferrin-Selenium (ITS -G) (100X)	ThermoFisher Scientific (Gibco™)	41400-045 (10ml)	✓	5	1x
2-Mercaptoethanol	ThermoFisher Scientific (Gibco™)	21985-023 (50ml) @55mM	✓	0.5	55µM
Bovine Serum Albumin (BSA) 30%	Biosera	SA-296 (1000ml)	BSE Free	8.4	0.5%

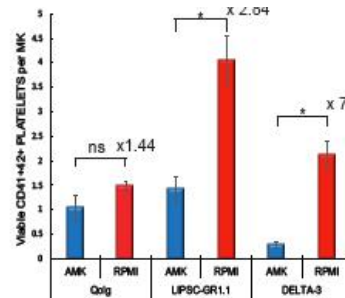
D



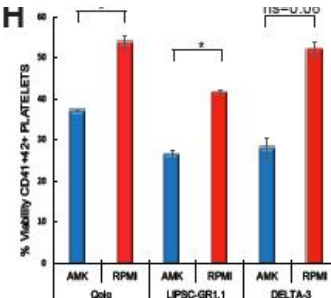
F



G

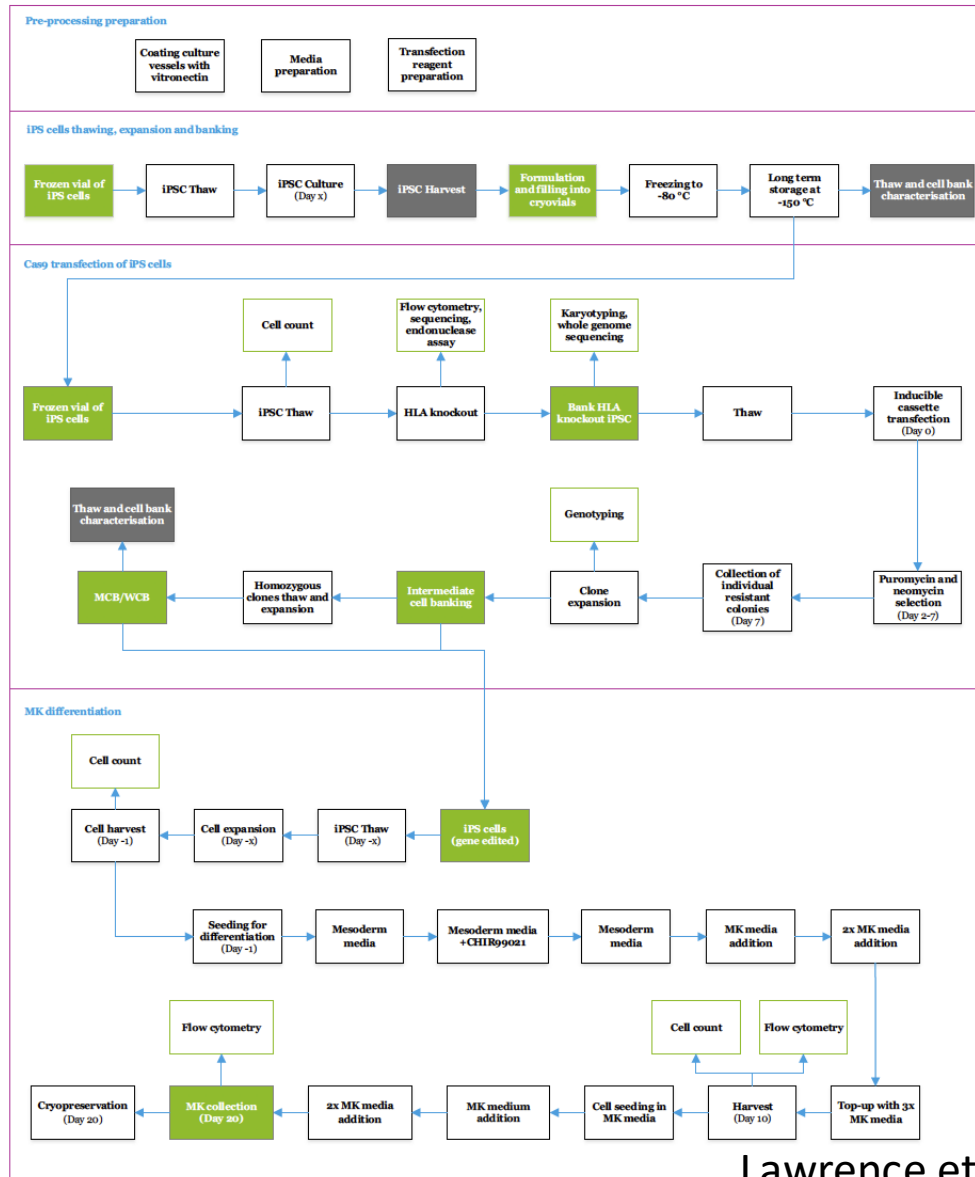


H



Transitioning to GMP manufacturing

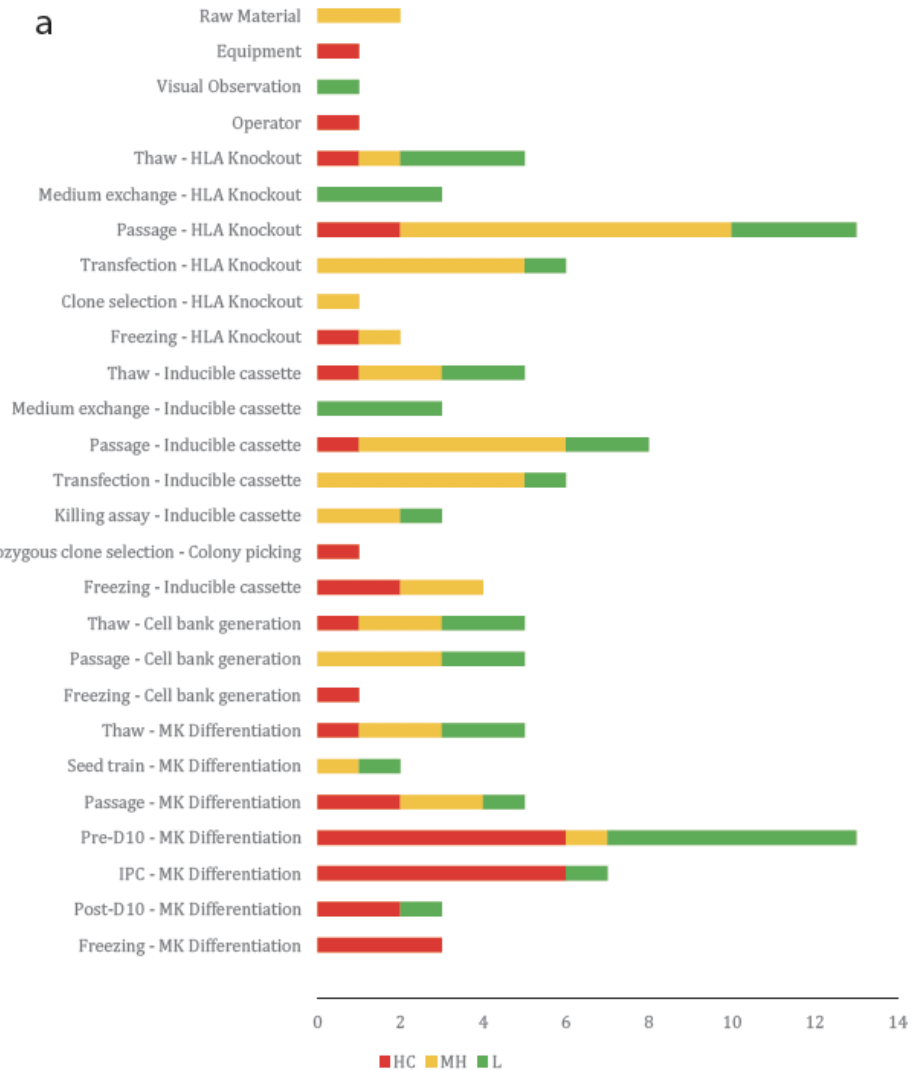
Simplified Process Flow Diagram (PFD)



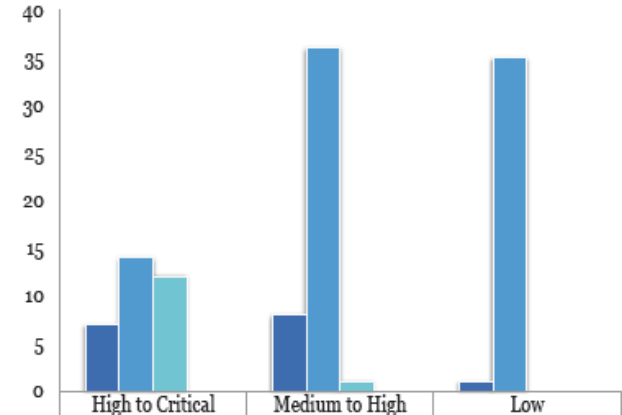
Transitioning to GMP manufacturing

Figure 3

a

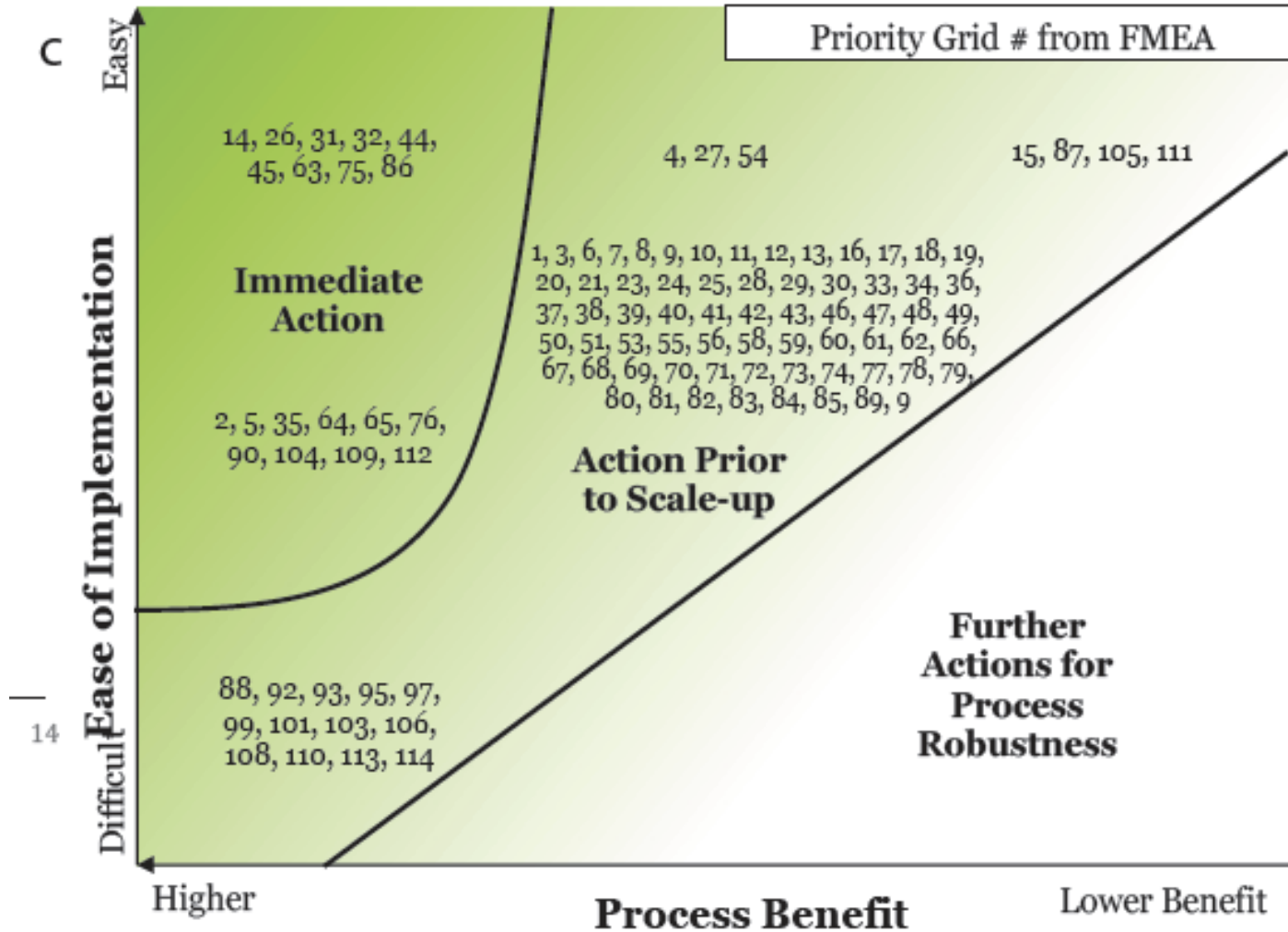


b



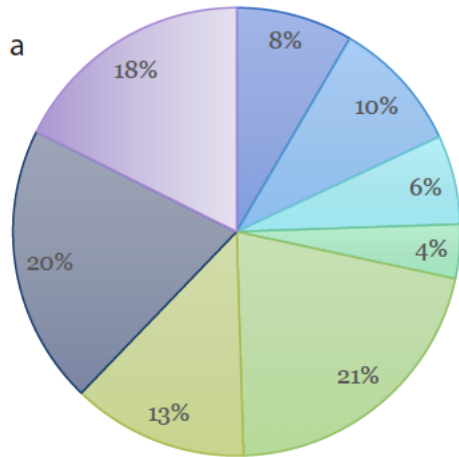
	High to Critical	Medium to High	Low
Easy (Documentation, off the shelf kit)	7	8	1
Medium (Experimentation)	14	36	35
Difficult (Extensive experimentation, Equipment/Material build)	12	1	0
No further action for this phase	0	0	0

Transitioning to GMP manufacturing

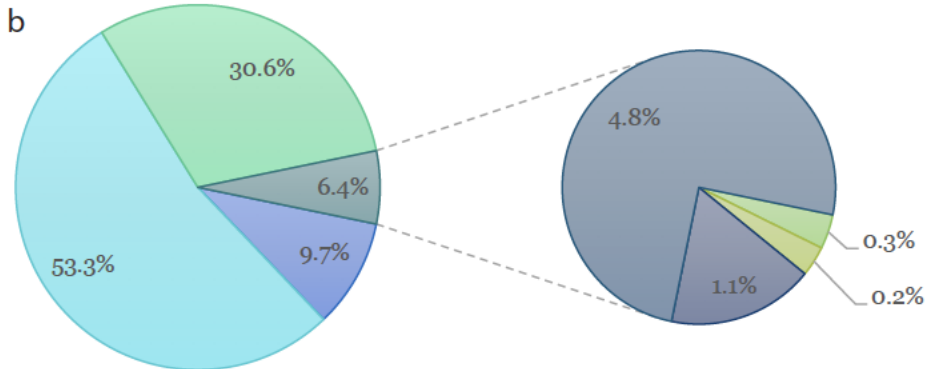


Transitioning to GMP manufacturing

Figure 4



■ 6 well plates ■ BMP4 ■ E6 ■ E8 ■ SCF ■ TPO ■ Vitronectin VTN-N ■ Other



■ Total cleanroom space ■ Renewals
 ■ Equipment depreciation and maintenance ■ Operators
 ■ Logistics, support and maintenance ■ QC
 ■ QA and QP ■ Recruitment costs

Current estimated
production costs of 1 unit of
platelets

£150,000 or 170,000 euros

...if we produce 100 functional
platelets per MK....

Moving towards transfusion medicine



GATA 1
TAL 1
FLI 1

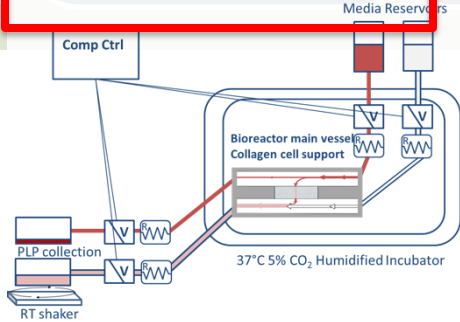
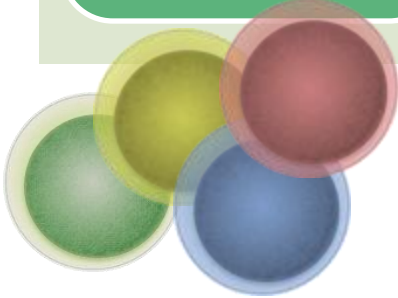
HLA Class 1

GMP hiPSC lines
UKSCB repository

Viral free MKFOP
inducible hiPSC lines

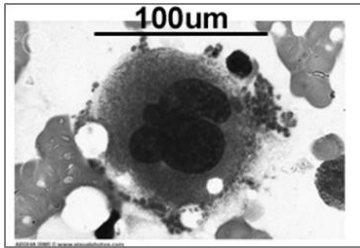
Platelet production
scaffold & bioreactors

Adding benefits
customised platelets for everyone



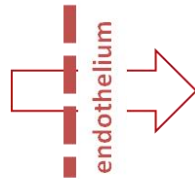
Platelets: defining a cell

Megakaryocytes

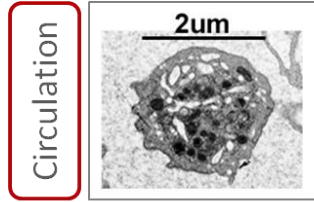


<1% blood cells

Bone marrow

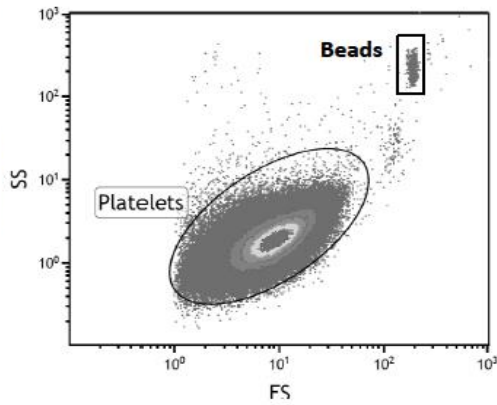


Platelets

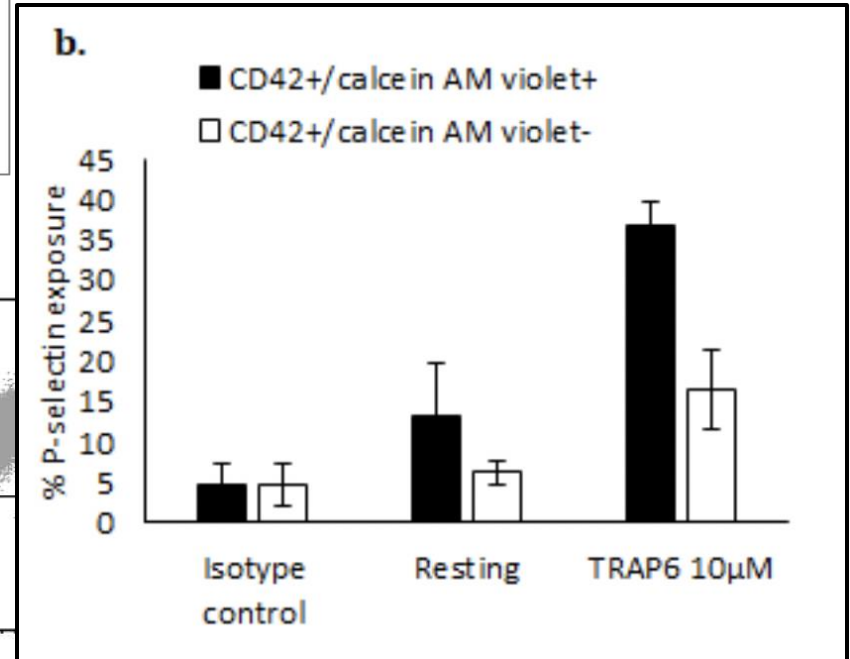
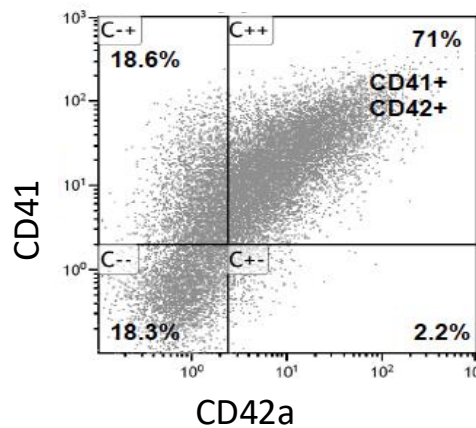
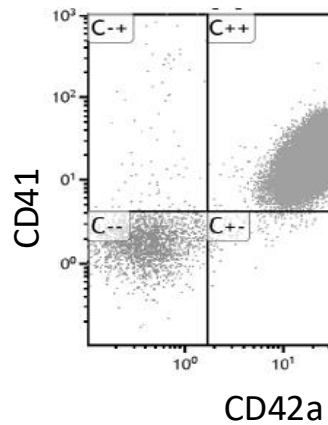
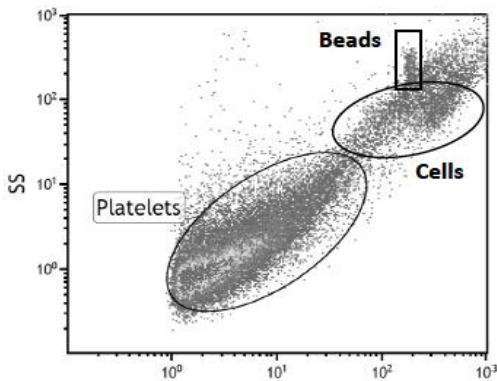


200,000 / μ L

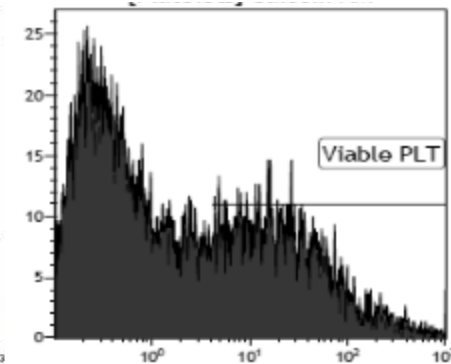
Donor-derived platelets



In vitro-derived platelets



Calcein-AM



Calcein-AM

Platelets: defining a cell

Defining the cell we are going to give to patients in a central pillar of product safety and efficacy.



**World Health
Organization**

has agreed that a “standard” should be made against which academic and commercial venture that are making platelets for clinical use should test their product.

This will be a lyophilized platelet prep, stained with calcein AM to test viability and expressing by CD41 and CD42b for flow cytometry.

A further standard expressing P-selectin and activated $\alpha\text{IIb}\beta\text{3}$ may come subsequently

Bouet et al., Bull Acad Natl Med. 2020

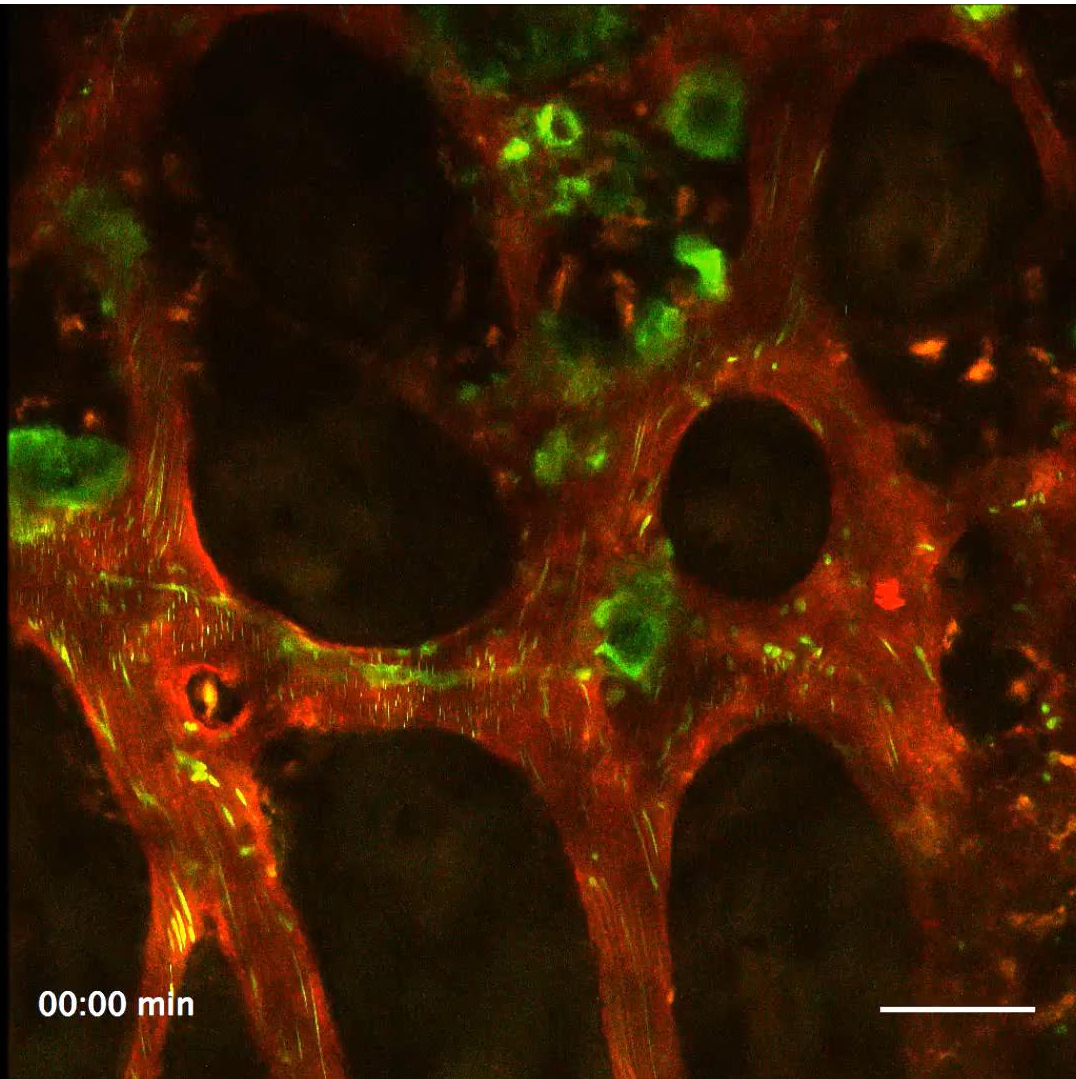
Mookerjee et al., Platelets 2020

Proplatelet formation

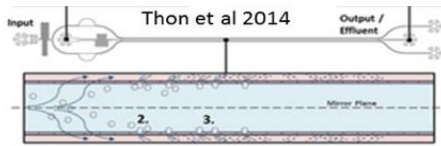
12600.013



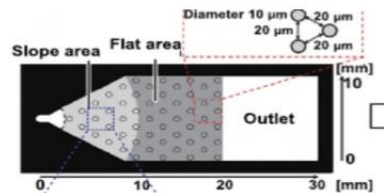
Proplatelet formation



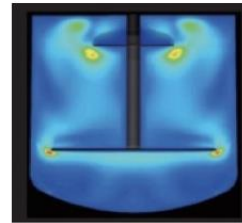
Bioreactors for platelet production....choices choices...



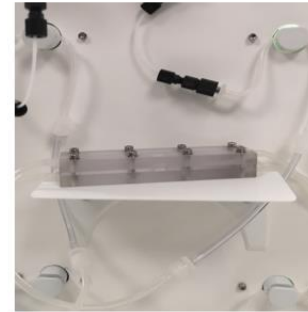
Thon *et al* 2014
(Boston MA)



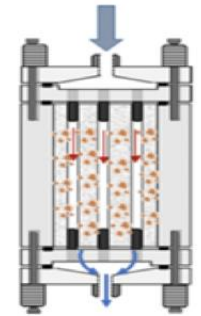
Six *et al* 2019
(Ghent/Paris/PlatOD)



Ito *et al* 2018
(Kyoto/Tokyo)



Shepherd *et al* 2018
(Cambridge)



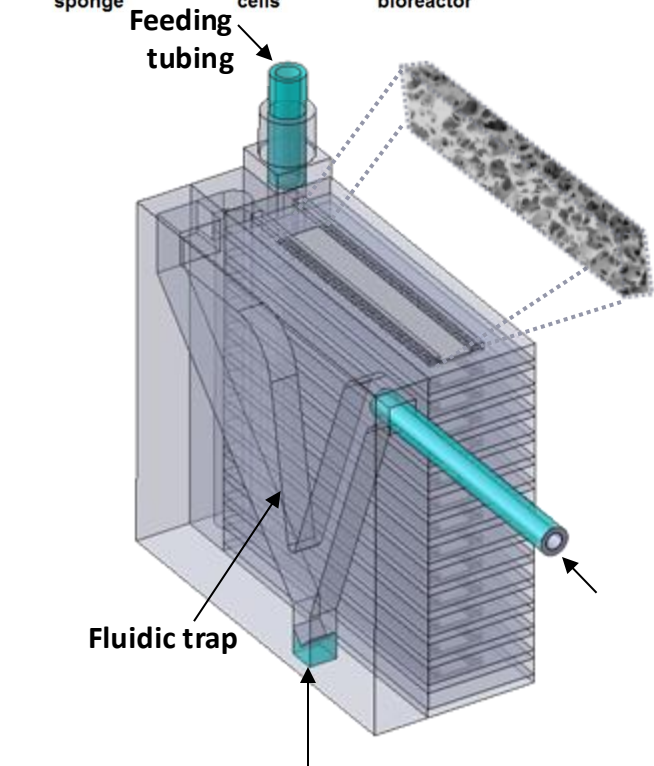
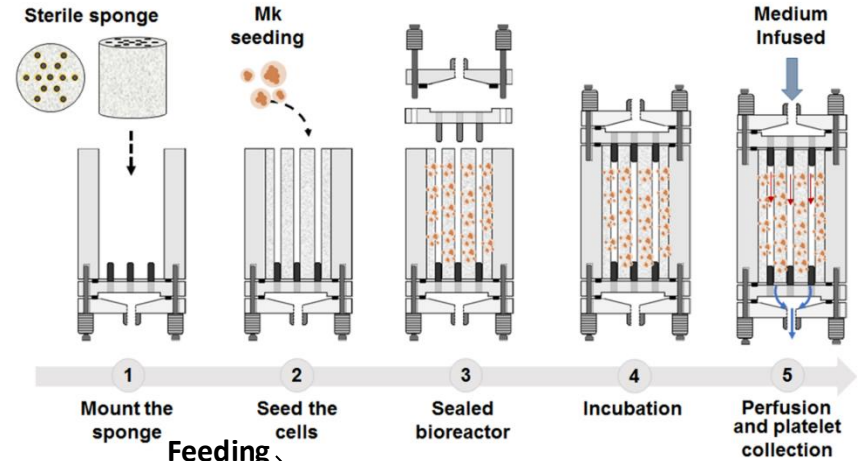
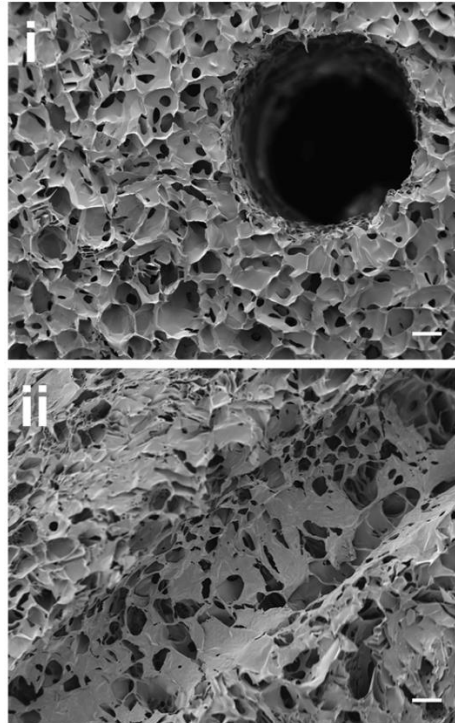
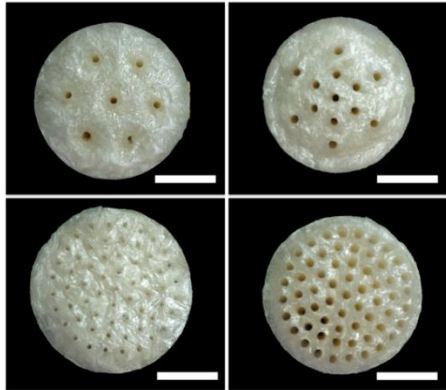
Tozzi et al 2018
(Pavia/Tufts)

Platelet
extraction



MK culture
& Bone marrow similar

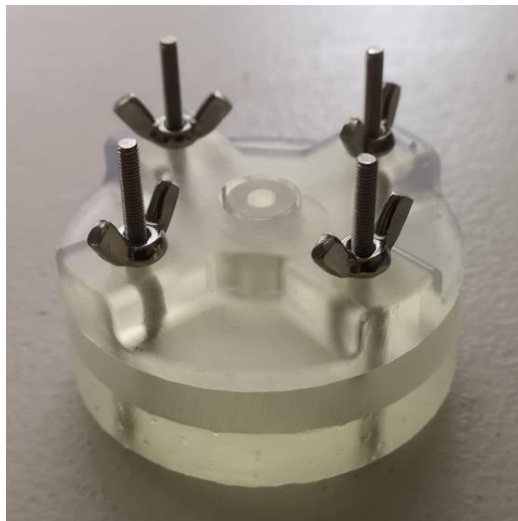
From 2D to 3D bioreactors



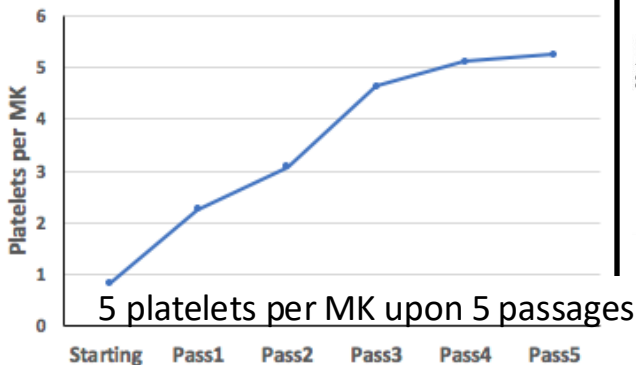
Platelet drainage
(platelet-rich medium:
 1×10^{10} in 200 mL)

Bioreactors for platelet production...and final product prep

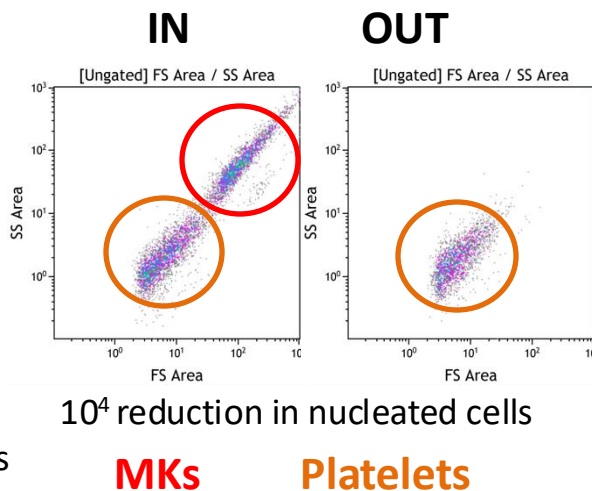
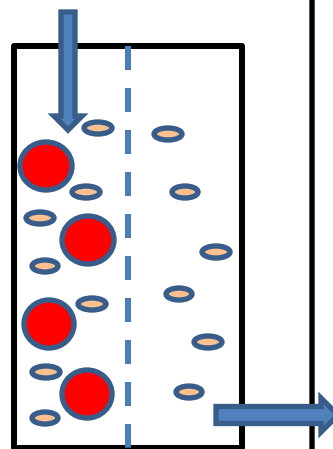
(1) Platelets release



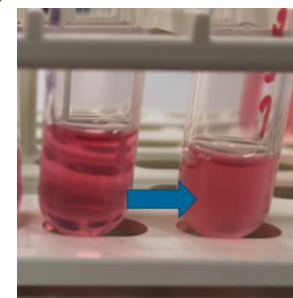
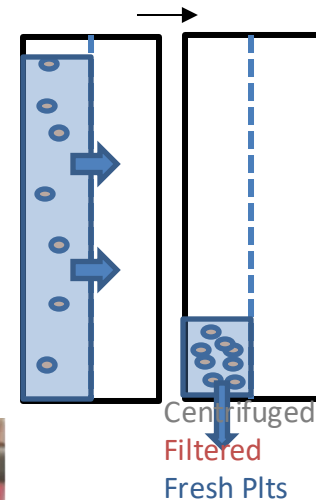
Bioreactor that triggers platelet release by cultured MKs



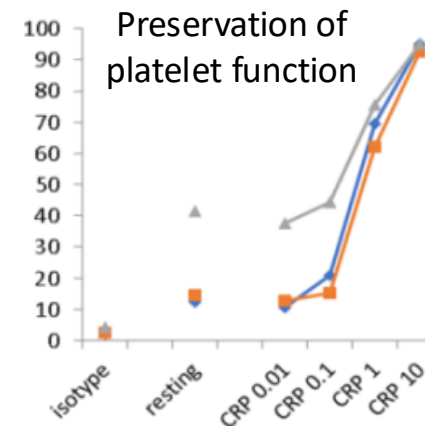
(2) Nucleated cells removal



(3) Platelets concentration



50x concentration

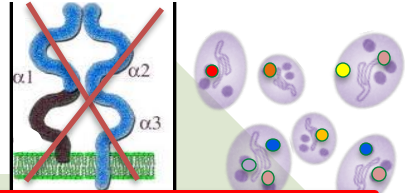


Moving towards transfusion medicine



GATA 1
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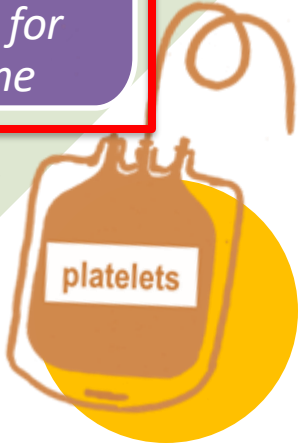
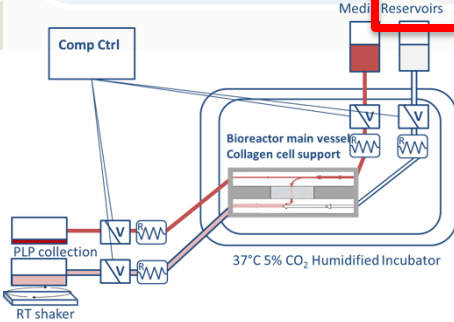


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UKSCB repository

Viral free MKFOP
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Adding benefits
customised platelets for everyone



Supercharged platelets

Table 2. Summary of 81 proteins from the thrombin-activated platelet releasate identified using MudPIT

Name	Accession no.	Known platelet protein	Known to be released/exocytosed	Function	mRNA rank in platelets
PROTEINS IN THE PLATELET RELEASATE KNOWN TO BE RELEASED FROM PLATELETS					
Thrombospondin 1	TSP1_HUMAN	Yes	From platelet α -granules	On secretion, can bind α _v β ₃ , α _v β ₅ and GPIV. Can potentiate aggregation	213
SECRETORY PROTEINS IN THE PLATELET RELEASATE NOT PREVIOUSLY IDENTIFIED IN PLATELETS					
Vitamin D-binding protein	VTDB_HUMAN	No	From liver to plasma	Carries vitamin D sterols. Prevents actin polymerization. Has T lymphocyte surface association.	—
β ₂ -microglobulin	B2MG_HUMAN	No	Exosomes from dendritic cells, B cells, enterocytes, tumor cells, and T cells	Is the β chain of the major histocompatibility complex (MHC) class I molecule.	3
Hemoglobin α chain	HBA_HUMAN	No	Exosomes from dendritic cells and phagosomes in macrophages	Oxygen transport. Potentiates platelet aggregation through thromboxane receptor.	21
Fibrinogen	F1MN_HUMAN	Yes	From kidney into plasma	Dissolves fibrin in blood clots, proteolytic factor in tissue remodeling, tumor invasion, and inflammation.	—
Serotransferrin	TRFE_HUMAN	Yes	From liver into plasma	Precursor to macrophage activators of phagocytosis (MAPP), which enhance leukocyte phagocytosis via the Fc γ RIII receptor. Involved in final stage of glycolysis. Presented as an autoantigen by dendritic cells.	—
Pyruvate kinase, M2 isozyme	KFY2_MOUSE	Yes	B-cell exosomes	Major cytoskeletal protein.	—
Actin, apric smooth muscle	ACTA_HUMAN	Yes	Exosomes from B cells, dendritic cells, enterocytes, and mastocytes	Major cytoskeletal protein. External function unknown.	11
Actin	ACTB_HUMAN	Yes	Exosomes from B cells, dendritic cells, enterocytes, and mastocytes	Major cytoskeletal protein. External function unknown.	11
14-3-3 protein (β)	1432_MOUSE	Yes	Exosomes from dendritic cells and phagosomes in macrophages	External function unknown. Involved intracellularly in signal transduction, however, may have a role in regulating exocytosis.	63
Hemopexin	HBMO_HUMAN	No	From liver to plasma	Haem-binding protein with metalloprotease domains.	—
Hemoglobin β chain	HBB_HUMAN	No	From liver to plasma, and phagosomes from macrophages	Oxygen transport.	9
Peptidyl-prolyl-cis isomerase A (cyclophilin A)	CYPA_MOUSE	No	From smooth muscle cells	Cellular protein with isomerase activity. Secreted vascular smooth muscle cell growth factor.	110
Cajalmin	CALU_MOUSE	No	From many cells, including fibroblast and CD8+ cells	An inhibitor of the vitamin K epoxide reductase-warfarin interaction.	1816

continued

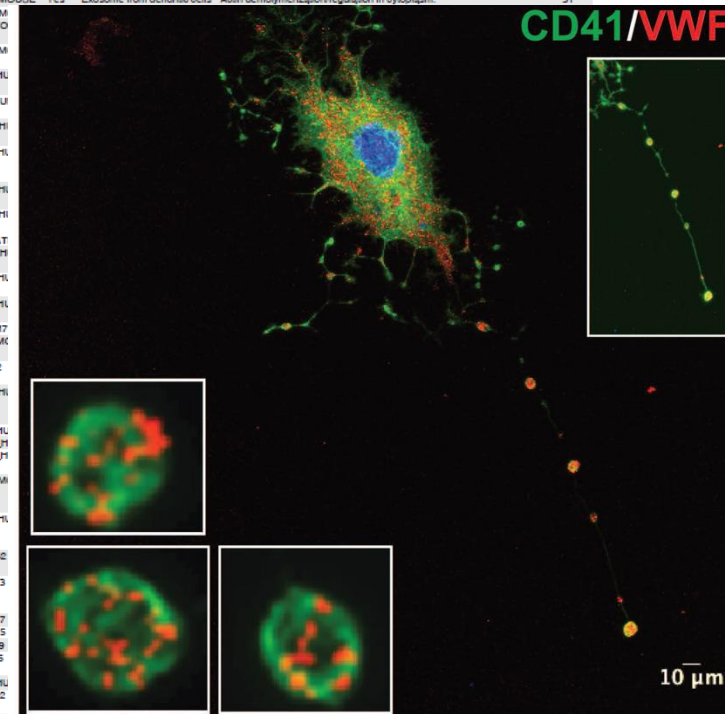
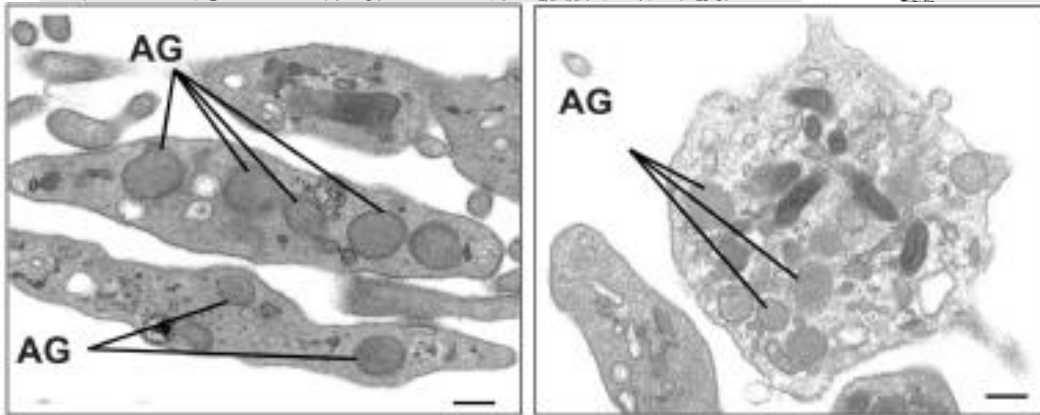
Table 2. Summary of 81 proteins from the thrombin-activated platelet releasate identified using MudPIT (continued)

Name	Accession no.	Known platelet protein	Known to be released/exocytosed	Function	mRNA rank in platelets
Adenylyl cyclase-associated protein 1 (CAP 1)	CAP1_MOUSE	No	Phagosomes from macrophages	Contains a WH2 actin-binding domain (as β -thymosin 4). Known to regulate actin dynamics. May mediate endocytosis.	174
A1_HUMAN	A1_HUMAN	Yes	Exosomes from dendritic cells and phagosomes from macrophages	Cytoskeletal protein involved in microtubule formation.	33
A1_HUMAN	A1_HUMAN	Yes	From liver to plasma, from monocytes and exosomes of dendritic cells	Role in high-density lipoprotein binding to platelets.	—
β _2_HUMAN	β _2_HUMAN	No	From liver cells and monocytes	Activator of the complement system. Cleaved to α , β , and γ chains normally prior to secretion and is a mediator of the local inflammatory response.	—
TY_HUMAN	TY_HUMAN	No	From choroid plexus into cerebrospinal fluid (CSF)	Thyroid hormone-binding protein secreted from the choroid plexus and the liver into CSF and plasma, respectively.	—
FL_MOUSE	FL_MOUSE	Yes	Exosome from dendritic cells	Actin demolymerization/regulation in cytosol.	31
O1_M_3_MO	O1_M_3_MO	—	—	—	—
K1_M	K1_M	—	—	—	—
IG_HU	IG_HU	—	—	—	—
L_HU	L_HU	—	—	—	—
MB_HU	MB_HU	—	—	—	—
P2_HU	P2_HU	—	—	—	—
PH_HU	PH_HU	—	—	—	—
LS_HU	LS_HU	—	—	—	—
AAAT	AAAT	—	—	—	—
LM_HU	LM_HU	—	—	—	—
Fleckstrin	FLEK_HU	—	—	—	—
Nidogen	NIDO_HU	—	—	—	—
Fibrinogen-type protein	QBVCM7	—	—	—	—
Rho GDP-dissociation inhibitor 2	GDIS2_MOUSE	—	—	—	—
Rho GTPase activating protein	QR25_HU	—	—	—	—
Transgelin	TAGE2_HU	—	—	—	—
Vinculin	VINC_HU	—	—	—	—
WD-repeat protein	WDR1_HU	—	—	—	—
Superoxide dismutase (SOD)	SODC_HU	—	—	—	—
78-kDa glucose-related protein	GR78_MOUSE	—	—	—	—
Bromodomain and PHD finger-containing protein 3 (fragment)	BRF3_HU	—	—	—	—
Titin	QBW242	—	—	—	—
Similar to hepatocellular carcinoma-associated antigen 59	Q9JWJ3	—	—	—	—
FKS330	QBXYX7	—	—	—	—
MNA-binding protein	Q9UC35	—	—	—	—
Hypothetical protein	Q9BTV9	—	—	—	—
Intracellular hyaluronan-binding protein p57	Q9UK55	—	—	—	—
Hypothetical protein	Y580_HU	—	—	—	—
Filamin fragment (hypothetical 54-kDa protein)	Q99KQ2	—	—	—	—
Filamin	FLNA_HU	—	—	Substrate for caspase-3.	—
Talin	TALL_HUMAN	Yes	No evidence	Actin-binding protein that binds to integrin- β 3 domain.	17
Zyxin	ZYX_HUMAN	Yes	No evidence	Associates with the actin cytoskeleton near adhesion plaques. Binds α actinin and VASP.	145

Eighty-one proteins were identified using MudPIT from the thrombin-stimulated platelet supernatant fraction. Spectra were identified using the SEQUEST program and a composite mouse and human database (NCBI July 2002 release) in 3 replicate experiments. Information on their functions and whether they are secretory proteins are provided. Also indicated is whether these proteins have a corresponding platelet mRNA. The rank of abundance of the message is denoted numerically in the last column.

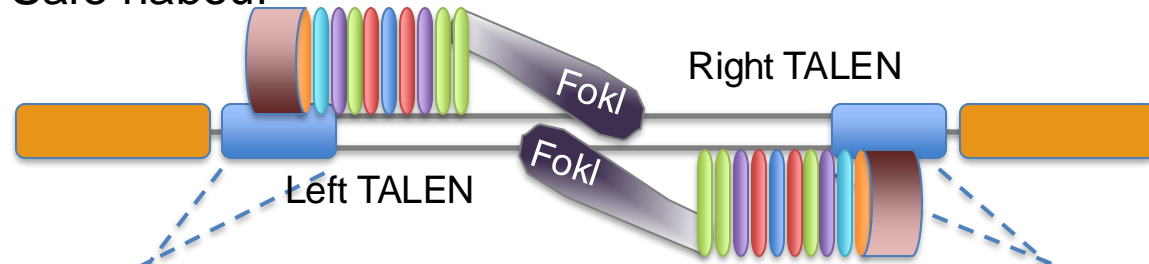
— Indicates levels below the threshold for detection on the Affymetrix microarray; “,” not present on Affymetrix microarray.

Control

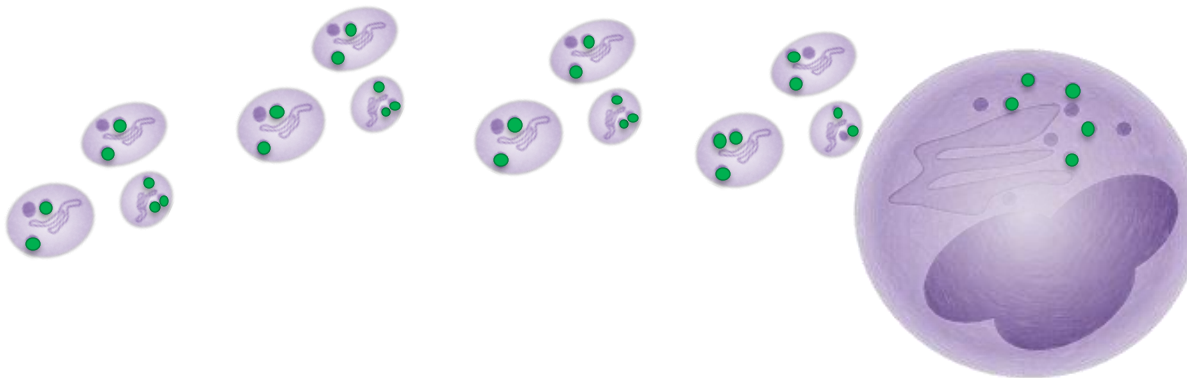
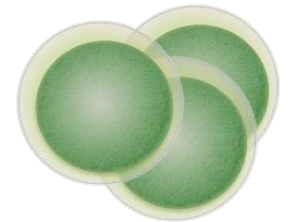


Supercharged platelets

Safe harbour



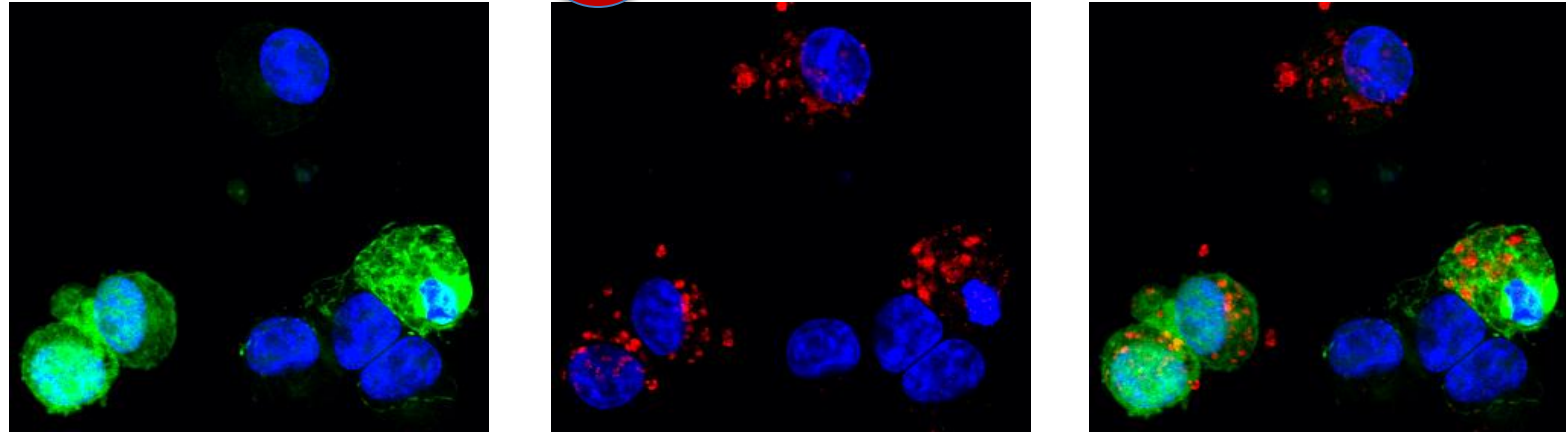
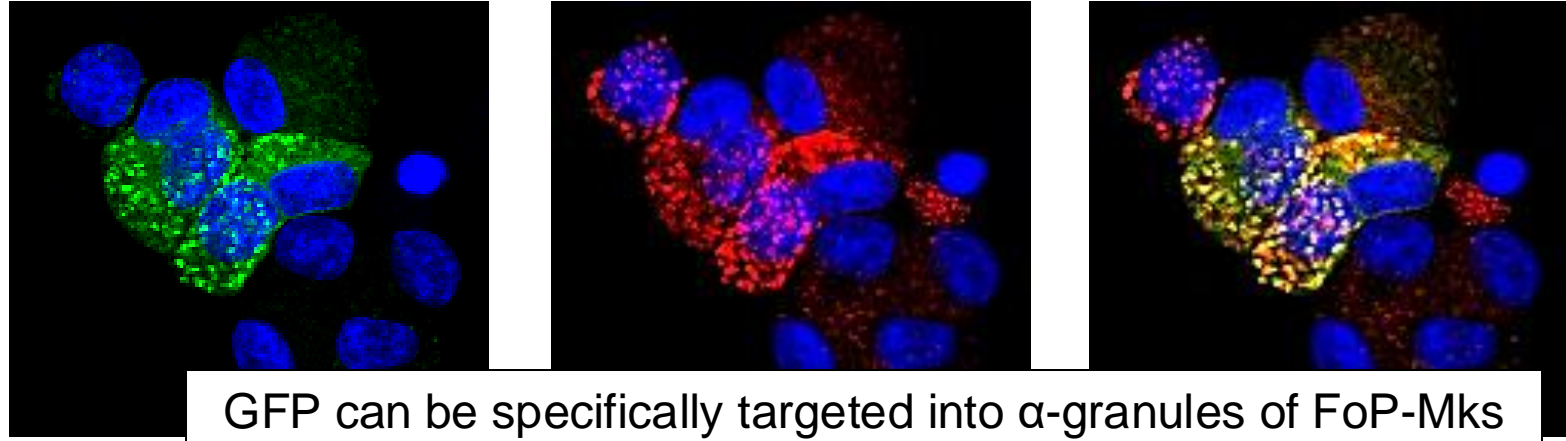
iPSC



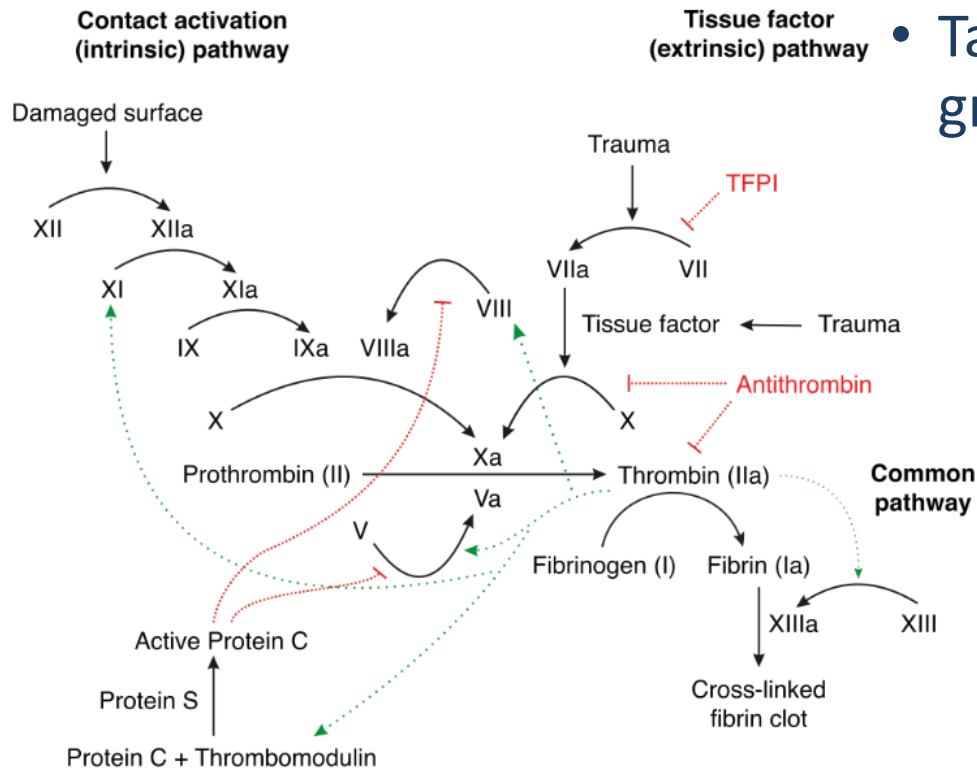
Supercharged platelets



THBS
GFP
DAPI



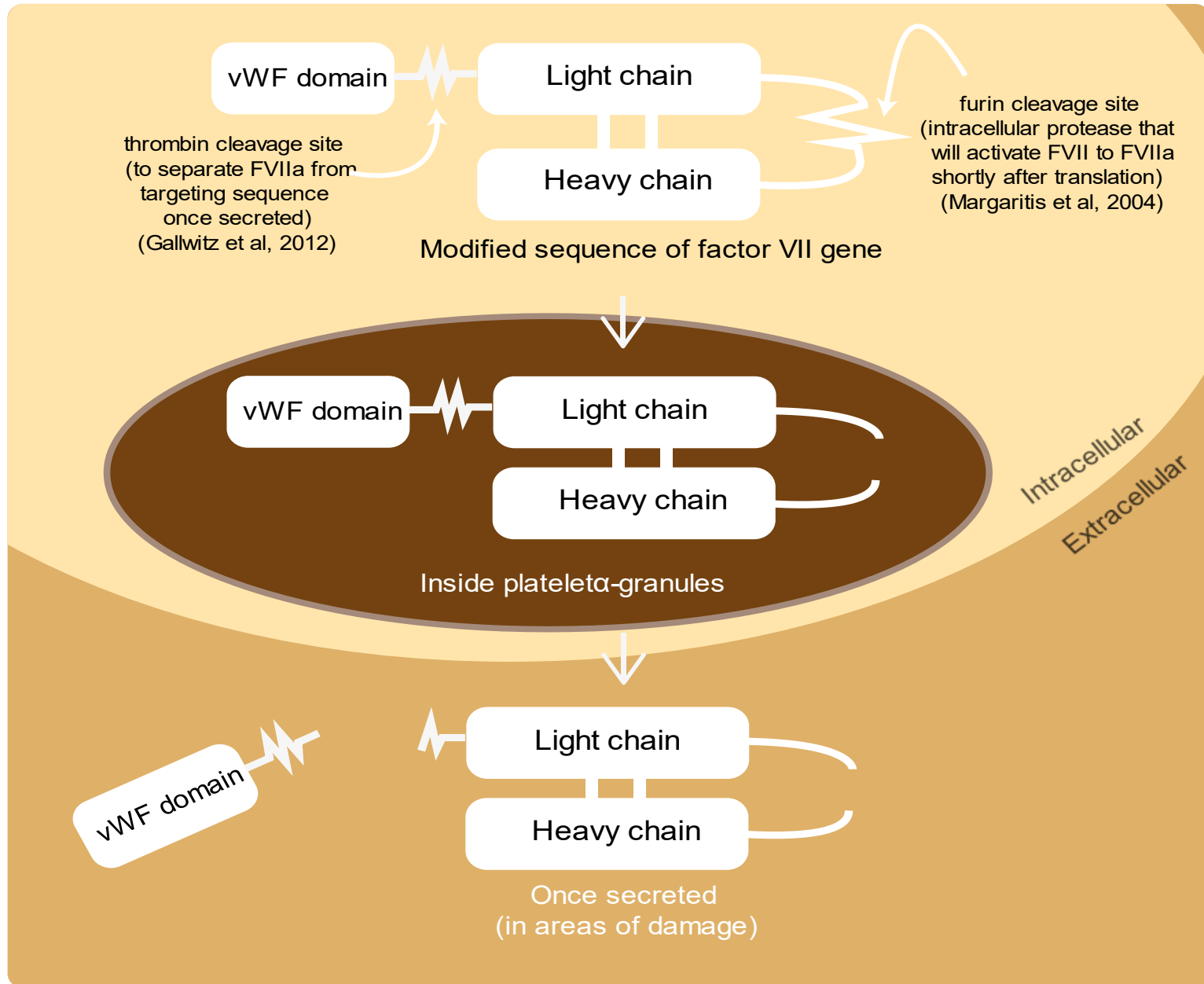
Supercharged platelets



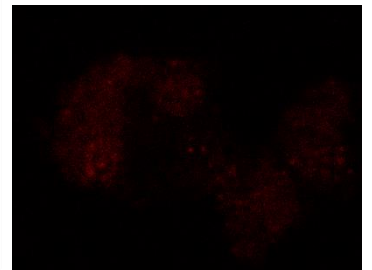
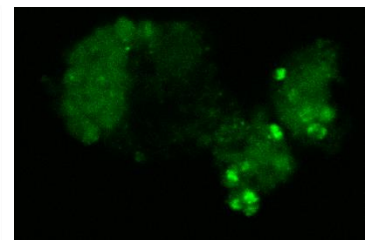
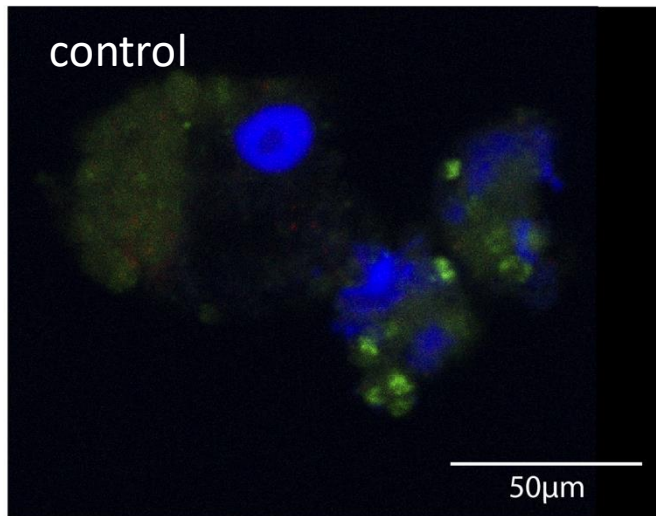
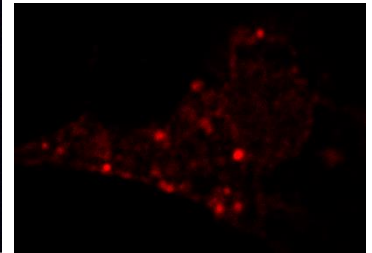
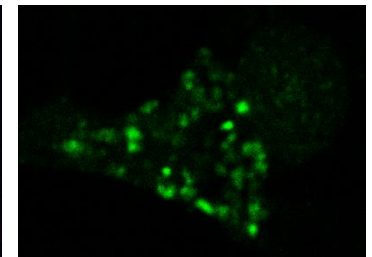
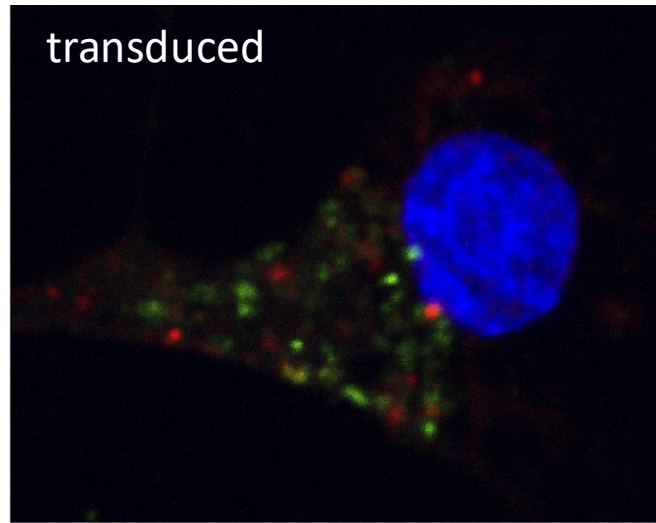
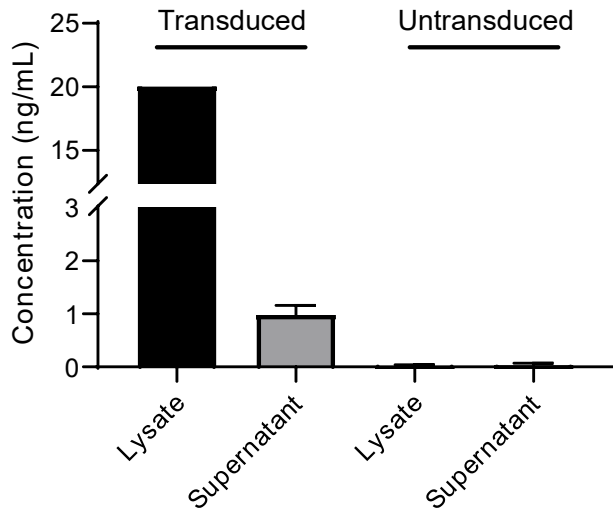
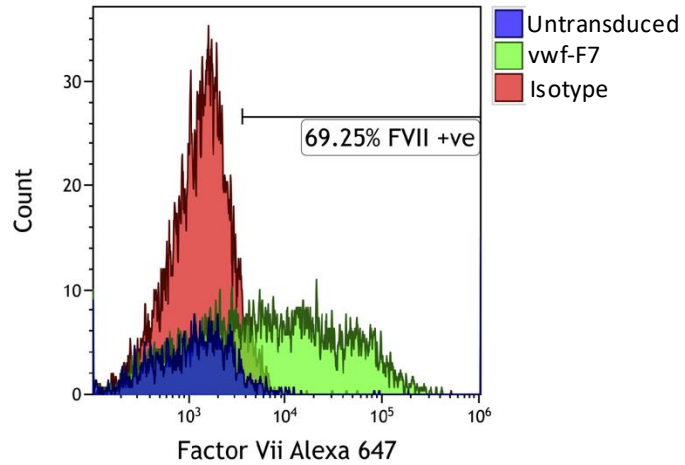
- Targeting FVIIa to the platelet α -granules:

- Novoseven is an IV injectable recombinant FVIIa with proven benefit for protracted bleeding
- Packaging into the platelet granules would remove thrombotic side-effects inherent to high systemic concentrations when using Novosevem

Supercharged platelets



Supercharged platelets

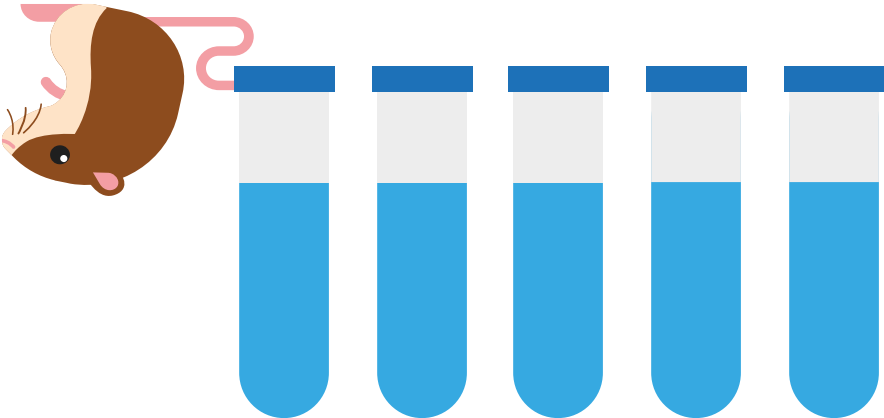
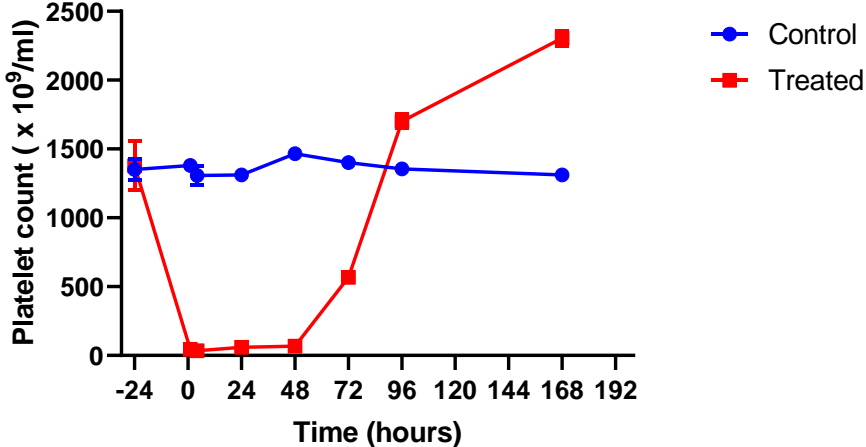


FVII DAPI THBS

Mouse model of haemostasis

Day -1
Weights taken
Baseline bleeds

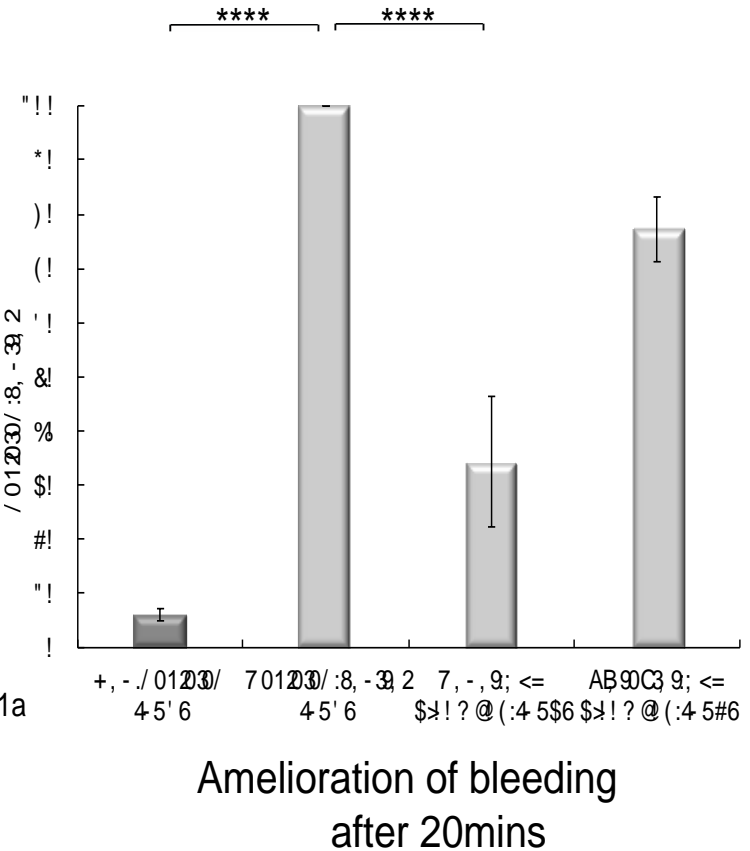
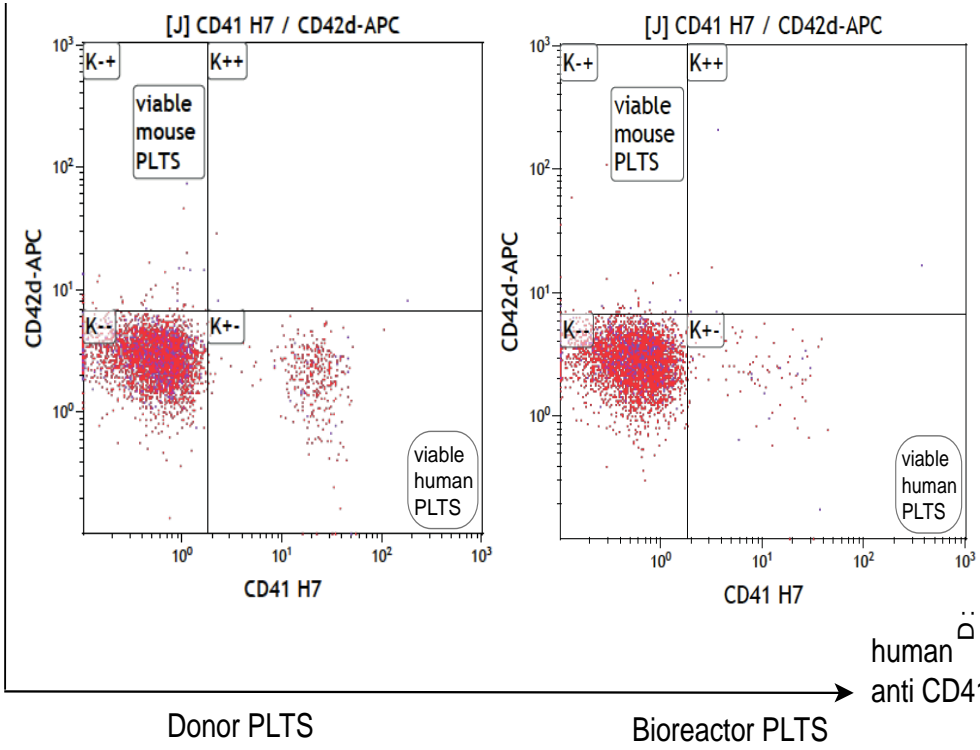
Day 0
IP injected with anti-GP1b antibody



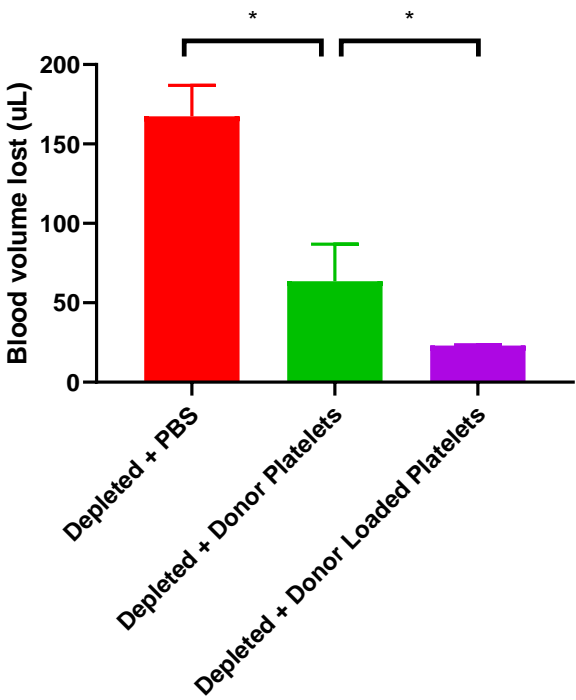
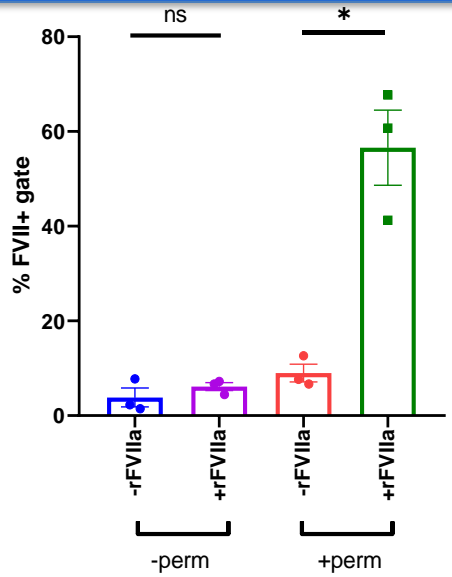
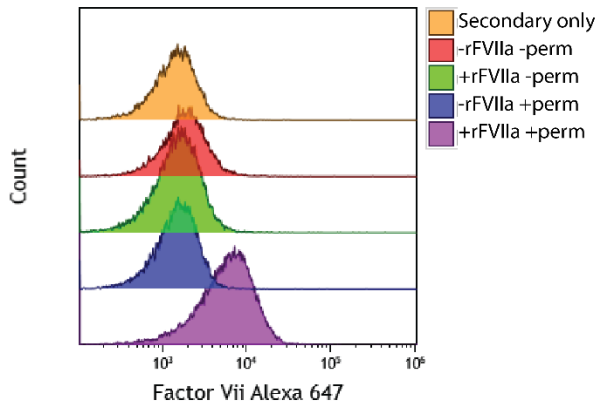
Day 2

- IV transfusion
- Tail transection 2mm, bled in tube with 40ml saline for 4mins (20mins total)

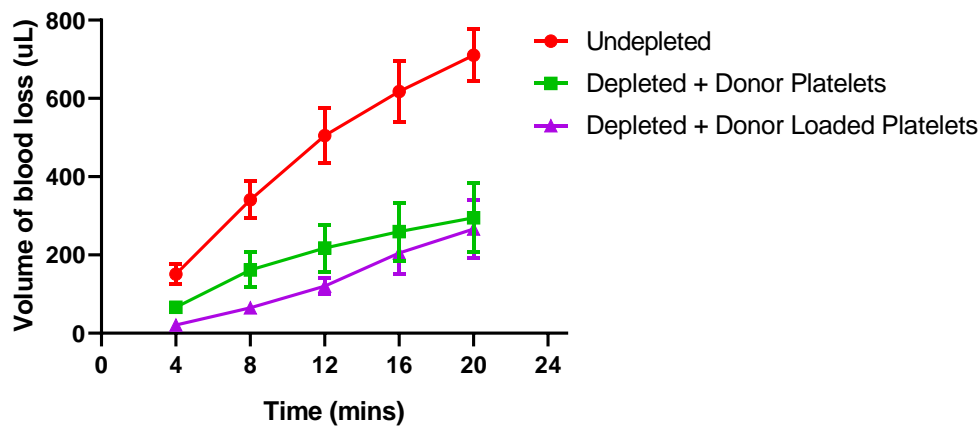
Mouse model of haemostasis



Platelets loaded with FVIIa show increase haemostasis in vivo



Cumulative blood loss over time



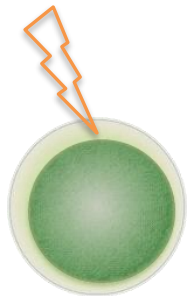
Platelet that can be cryopreserved

Upon cooling/freezing platelets

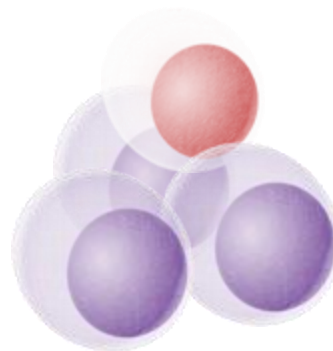
- Lose surface receptors due to cleavage driven by neuraminidases
- Lose CD42 expression (vWf receptor) due to cleavage at the hinge domain

- Shorter survival in circulation
- Loss of potency

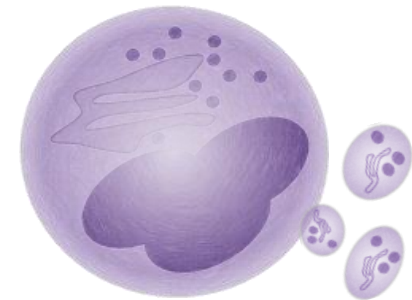
Gene editing by CRISPR/Cas9 to create a **Neu1^{-/-} Neu3^{-/-} GP1BA Δ** cell line



iPSC



Megakaryocyte-Erythroid
Progenitors



Megakaryocyte/Platelets

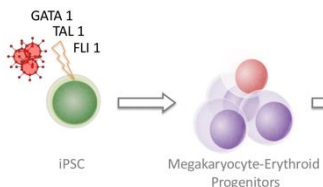
**Cryopreservable/Lyophilised
platelets**

Platelets made in vitro – the journey to the front line



Platelets made in vitro – the journey to the front line_truly disruptive

iPSC banks
MK production



Diluent that is temperature stable/does not freeze

Not in glass containers

Easy to reconstitute

Affordable

Lypophilised supercharged universal platelets



The Ghevaert Group



NHSBT/ Haematology Cambridge

Amanda Evans

Holly Foster

Dan Howard

Amie Waller

Moyra Lawrence

Winnie Lau

Nina Herbert

Souradip Mookerjee

James Warland

Rebecca Mc Donald

Samantha Mason

Adam Pullen

CSCI

Irina Mohorianu

Susanne Bornelov

Arash Shasavari

Mark Kotter

James Baye

Marion Perrin

Maike Steindel

Paula Jimenez

Material Sciences

Ruth Cameron

Serena Best

Maike Paramor

Christopher Penfold

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