Generation of HLA universal platelets for regenerative applications

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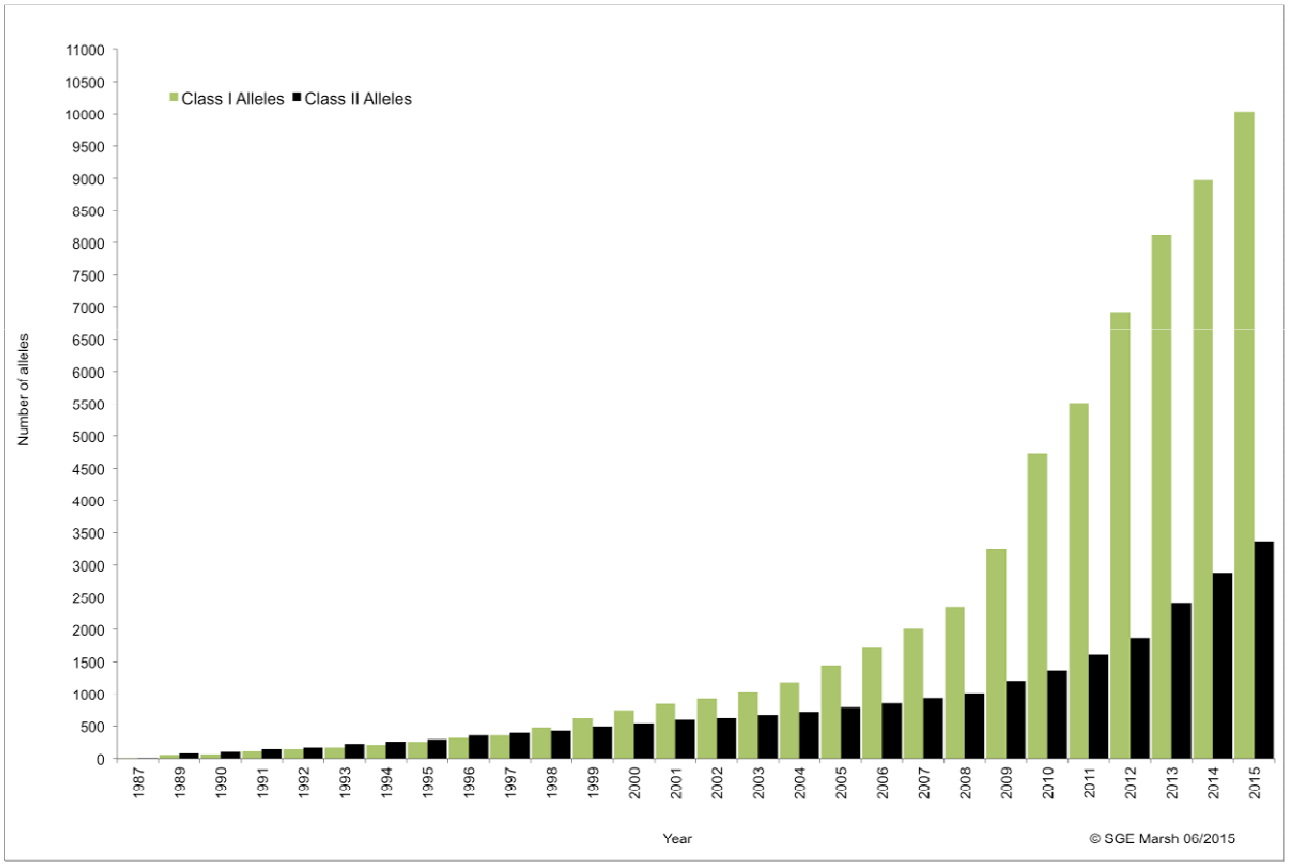
HLA loci are the most polymorphic of the entire human genome



IMGT/HLA database

12.672 HLA alleles

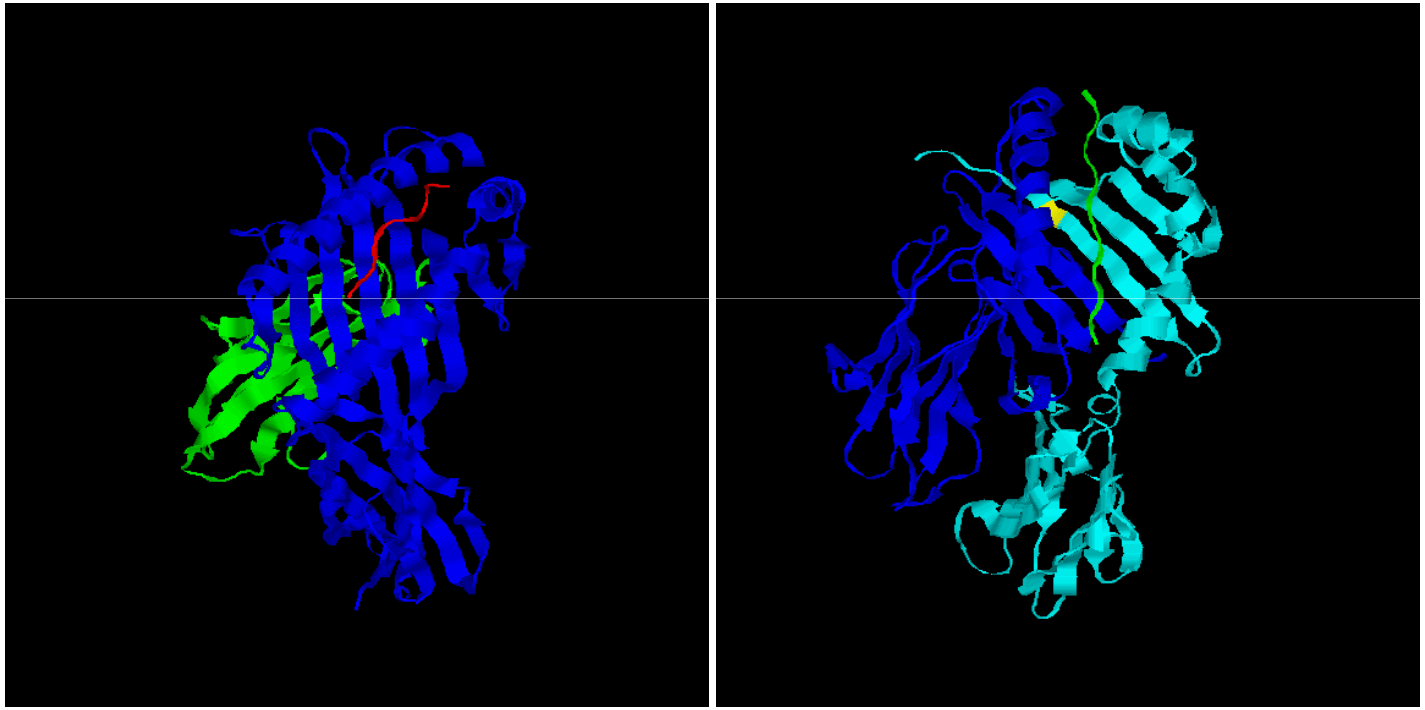
A mean of 450 new alleles/ Year

Structure of HLA class I and II molecules

HLA Class I

HLA Class II

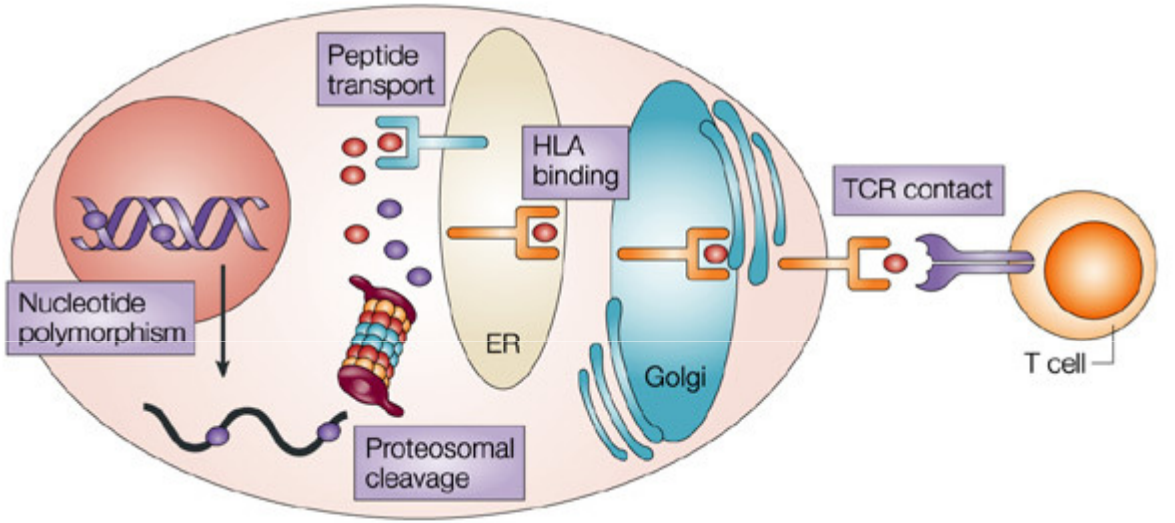
HLA-A
HLA-B
HLA-C



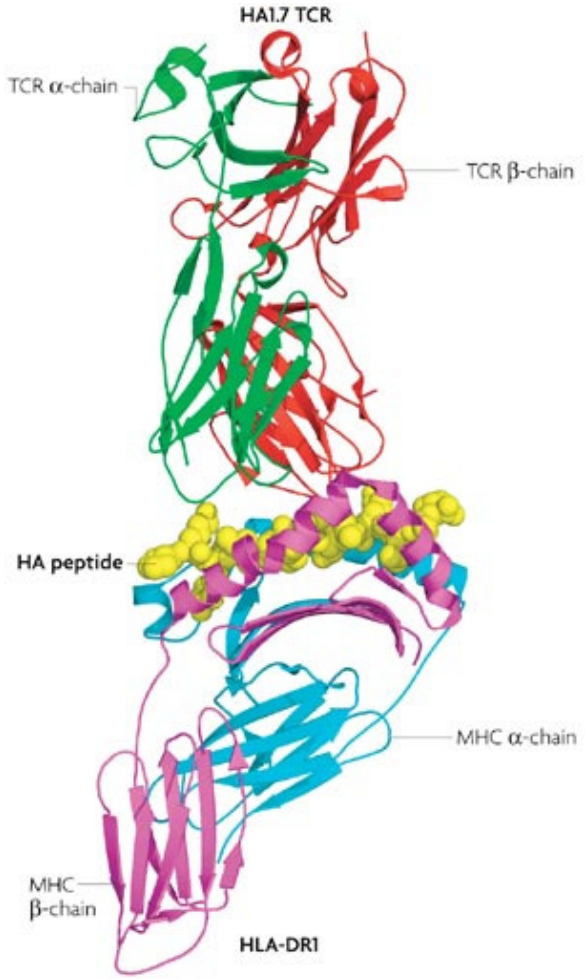
HLA-DR
HLA-DP
HLA-DQ

The function of HLA molecules

Antigen presentation



Nature Reviews | Cancer

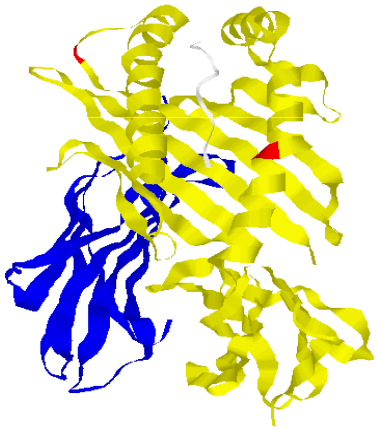


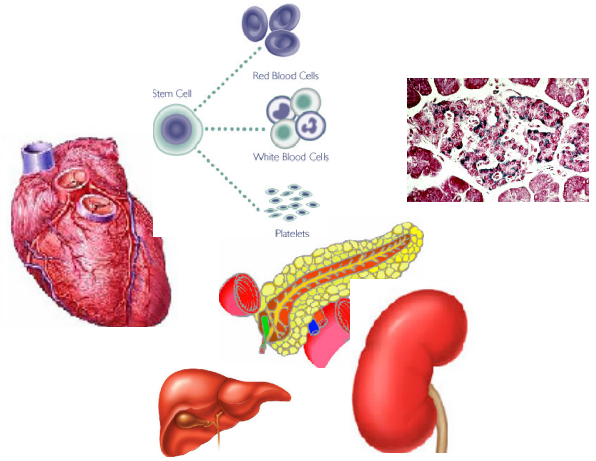
Nature Reviews | Immunology

Efficient immune response against pathogens and cancer cells

HLA variability is the major hurdle for transplantation

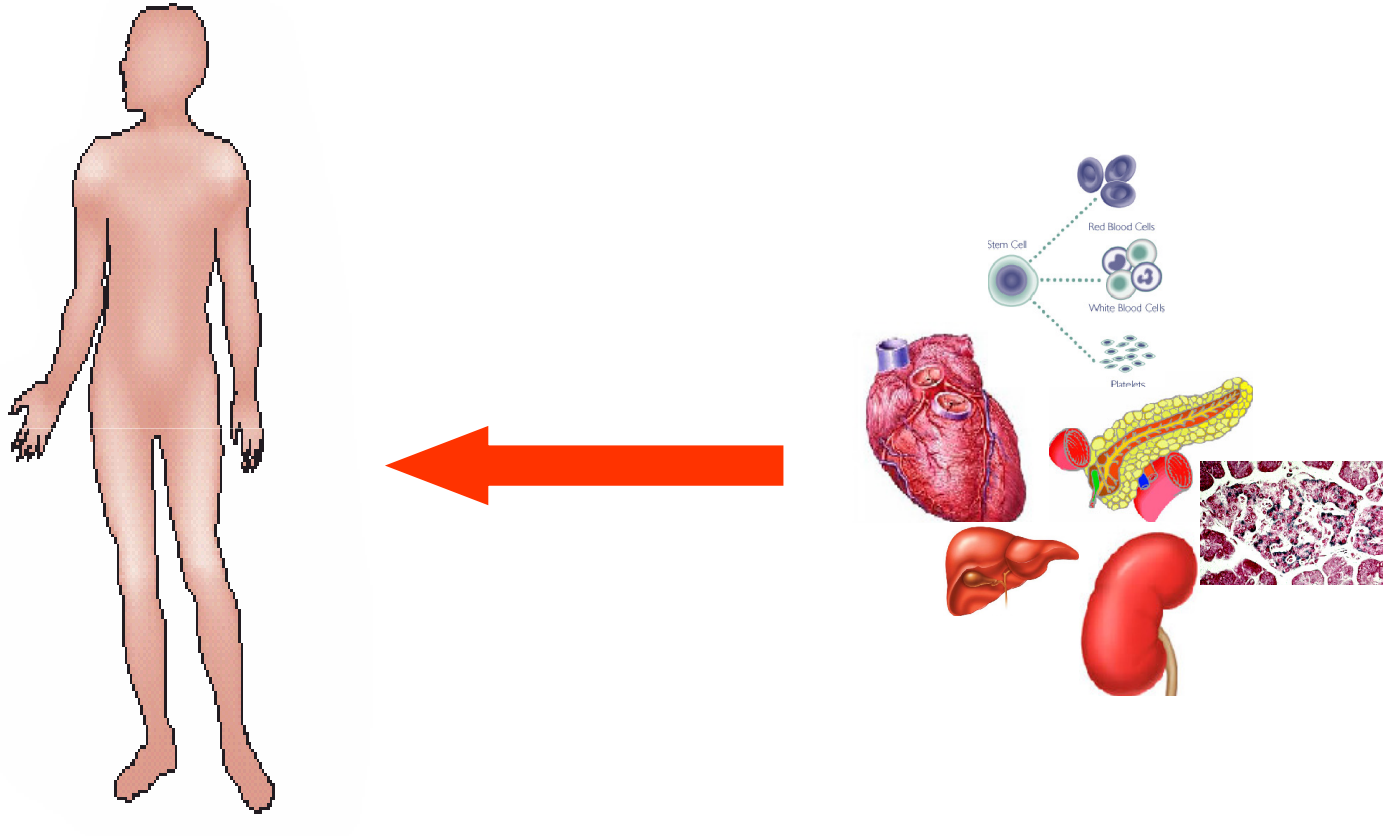
- HLA Antigens = Major Antigens
- Non-HLA Antigens = Minor Antigens = Presented Peptides





MHC/non-MHC Mismatch → Immune response (humoral/cellular) → Rejection

Standard Strategy to prevent rejection: Immunosuppression



Suppression of the recipient's immune system

Aim

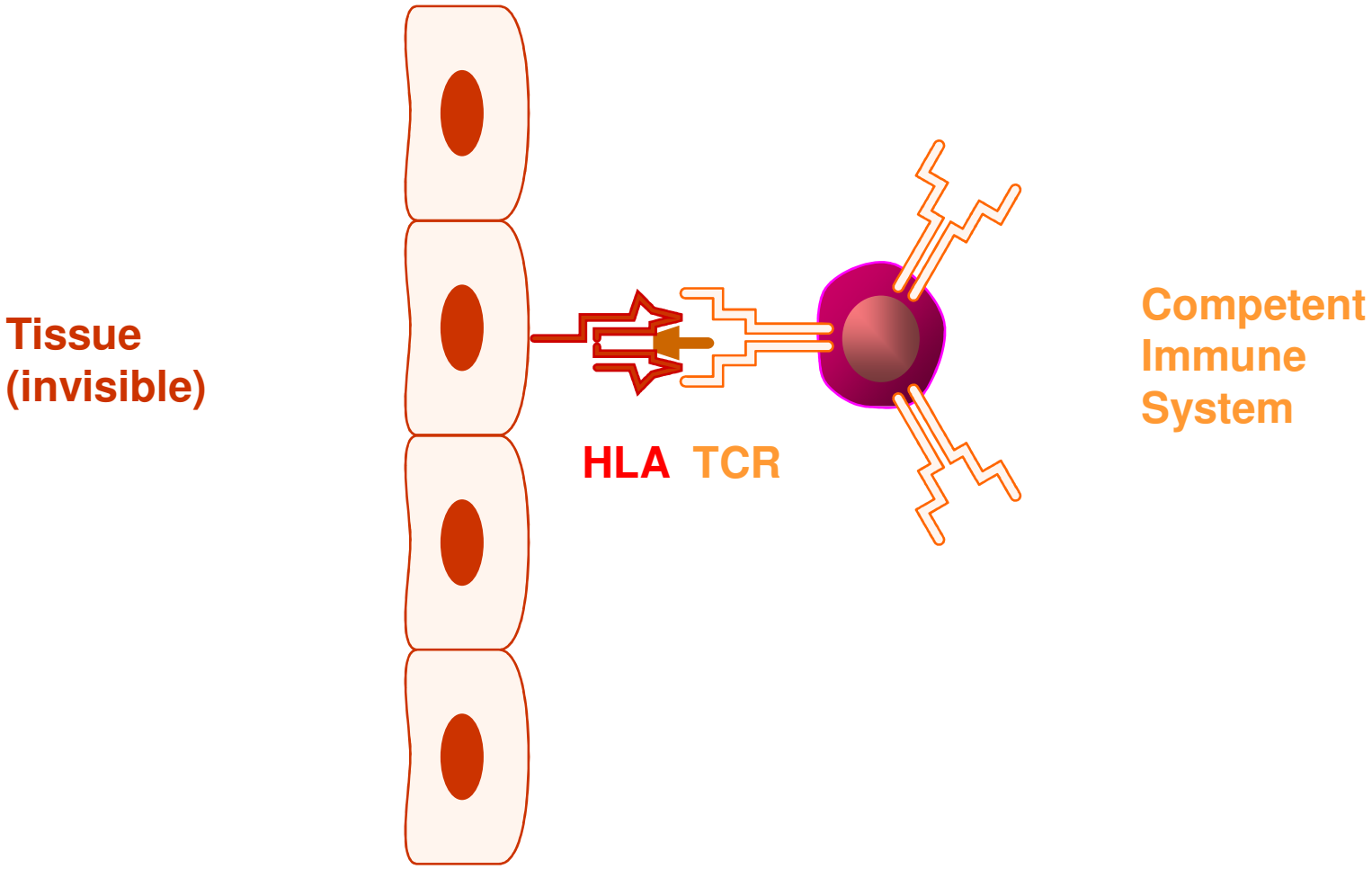
Control HLA expression in a allele-, gene- or class-specific way.

In a clinical setting, this would allow to

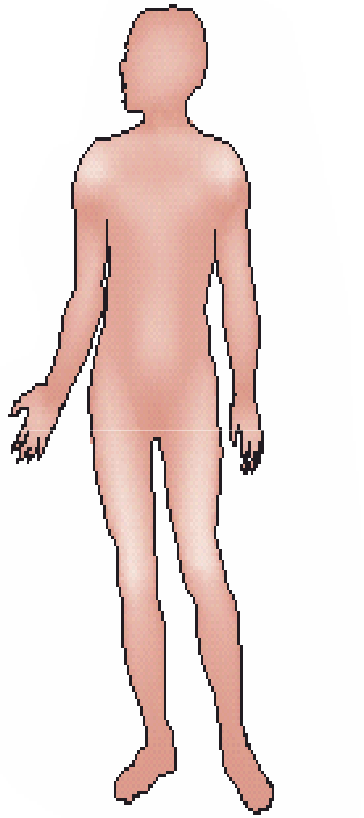
1. **develop tissues with low or no immunogenicity.**

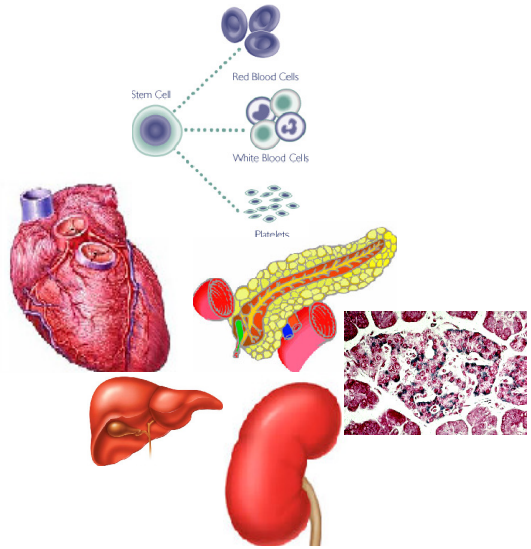
2. **decrease the risk of HLA-mediated post-transplant tissue/organ failure.**

Universal cells and tissues



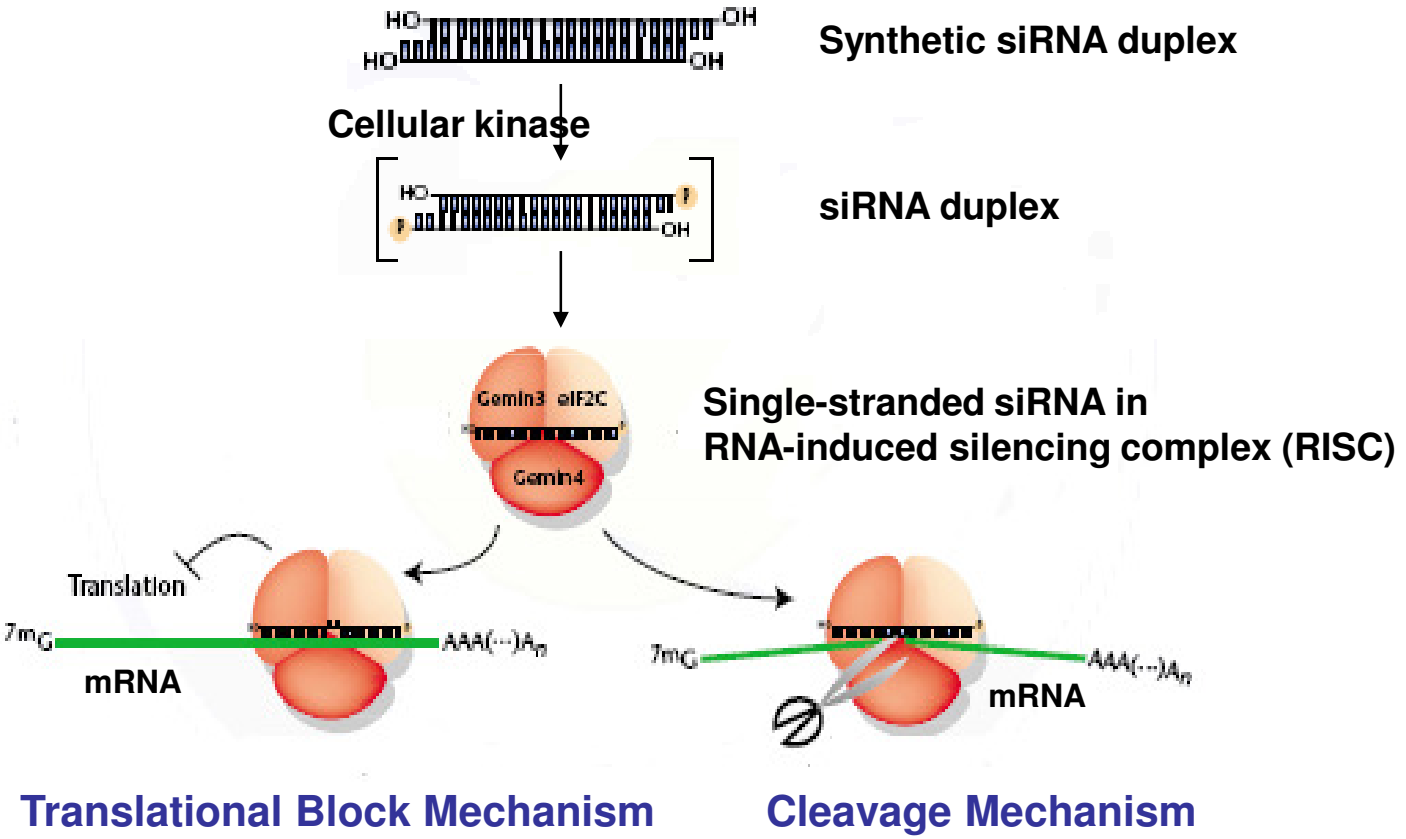
Alternative Strategy to prevent rejection: Reducing the organ‘s immunogenicity





Reducing the organ’s Immunogenicity

Gene silencing by RNA interference (RNAi):
Small interfering RNA (siRNA)

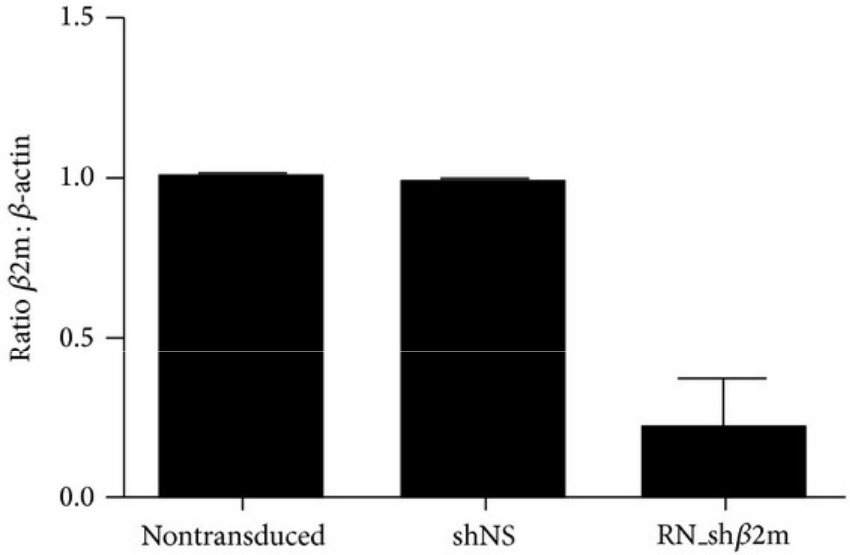


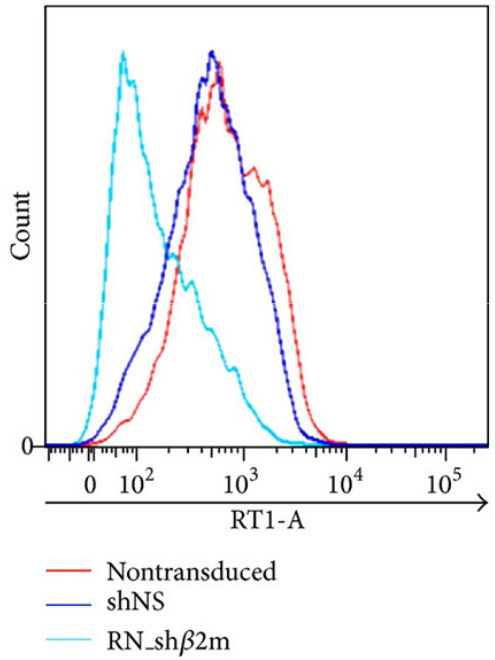
Andrew Fire
Stanford University

Craig Mello
University of Massachusetts

Nobel Prize 2006

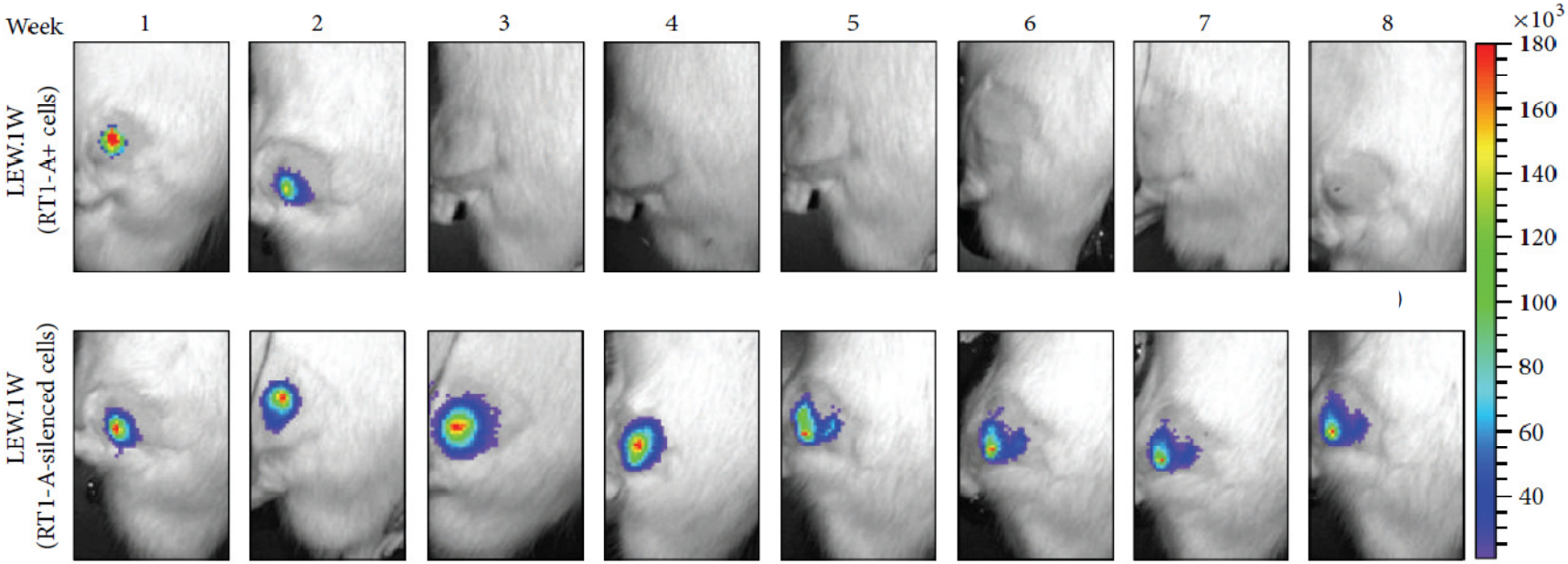
Silencing of β2-microglobulin in rat Lew fibroblasts





Figueiredo et al. BioMed Res Int, 2013

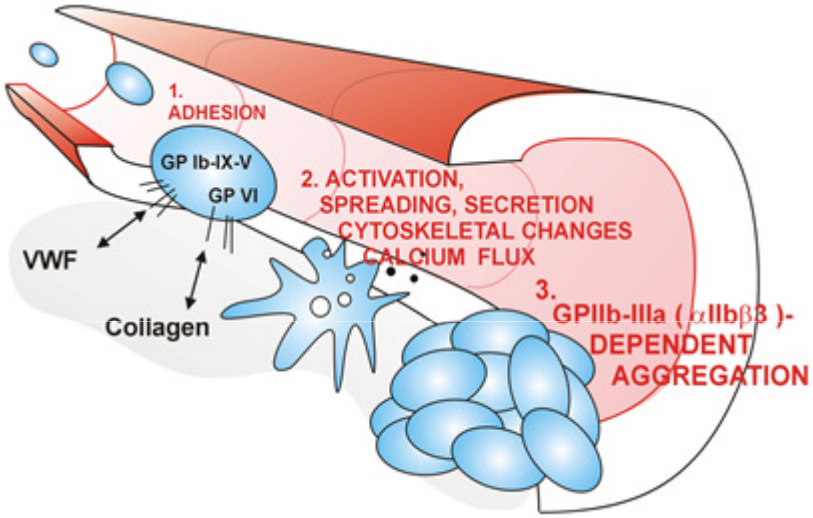
MHC silencing prolongs graft survival after allogeneic transplantation



Figueiredo et al. BioMed Res Int, 2013

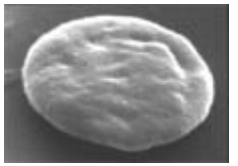
Role of platelets

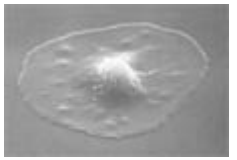
- Maintain Haemostasis
- Secretory functions
- Modulation of immune responses
- Regulate proliferation



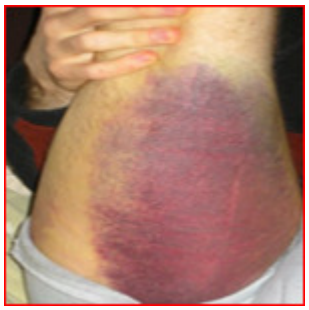
Application of platelets in regenerative medicine

- Treatment of thrombocytopenia
- Wound healing
- Tissue remodelling
- Drug carriers





Thrombocytopenia



Characteristics

- Low platelet number
- Major bleeding complications

HLA-universal platelets

Treatment options

TPO receptor agonists

Allogeneic platelets

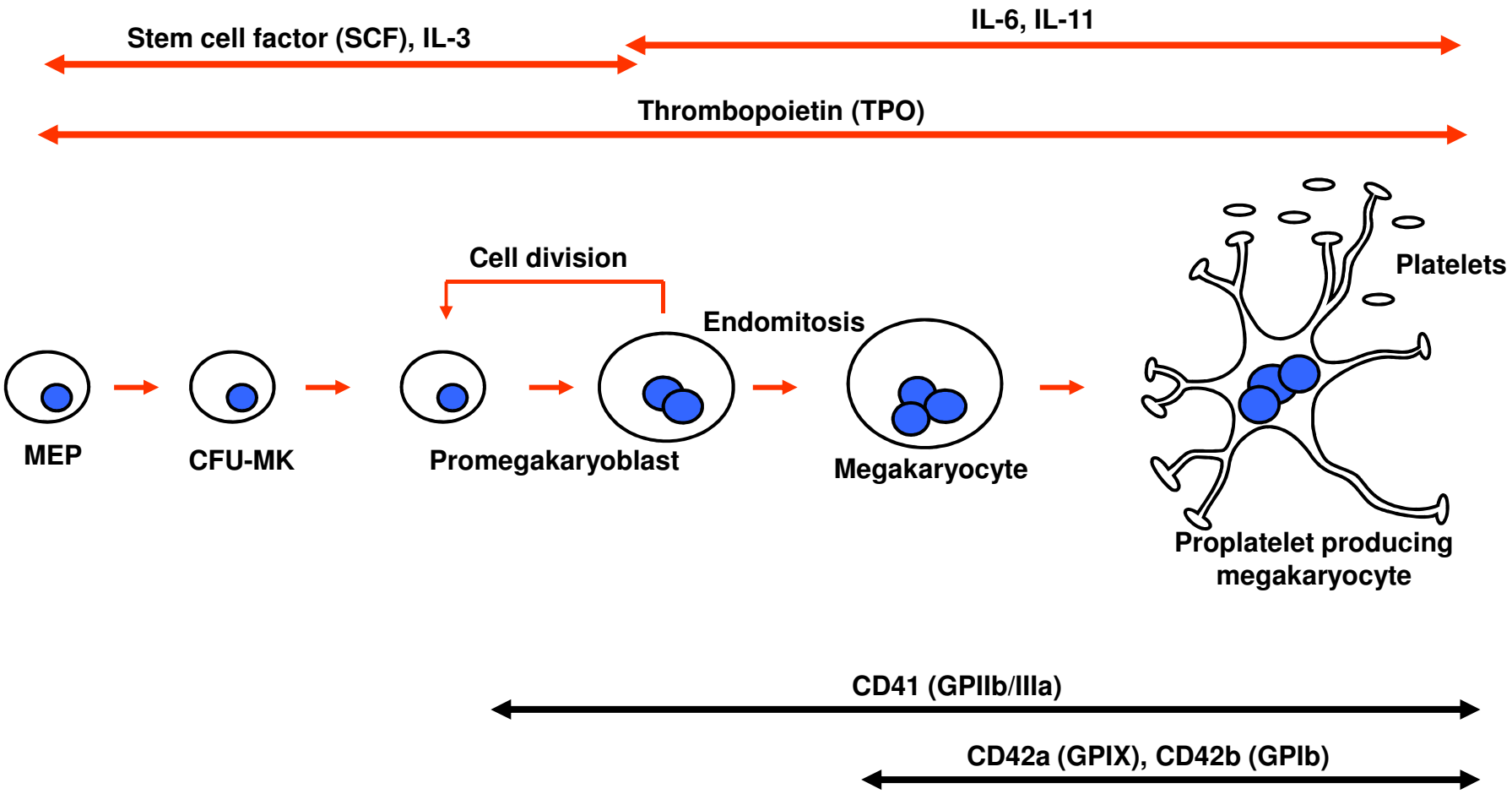
***Ex-vivo* induced megakaryocyte progenitors**

Alloimmunization against HLA class I antigens

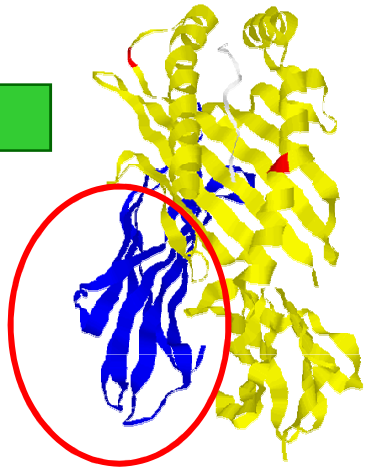
→ Platelet refractoriness due to HLA antibodies

In vitro production of Platelets to treat platelet transfusion refractoriness

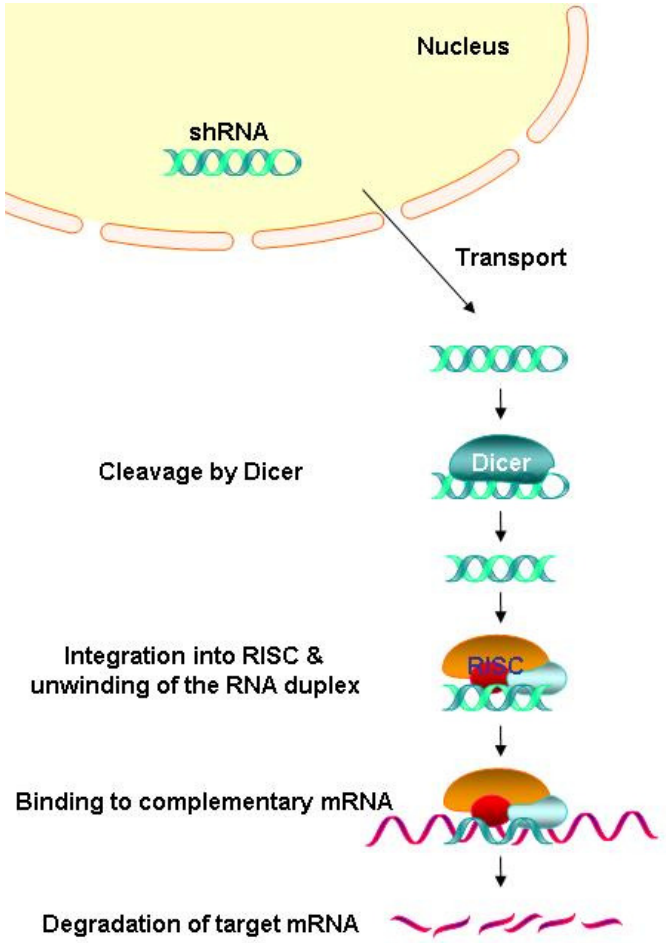
Thrombopoiesis



Silencing HLA expression

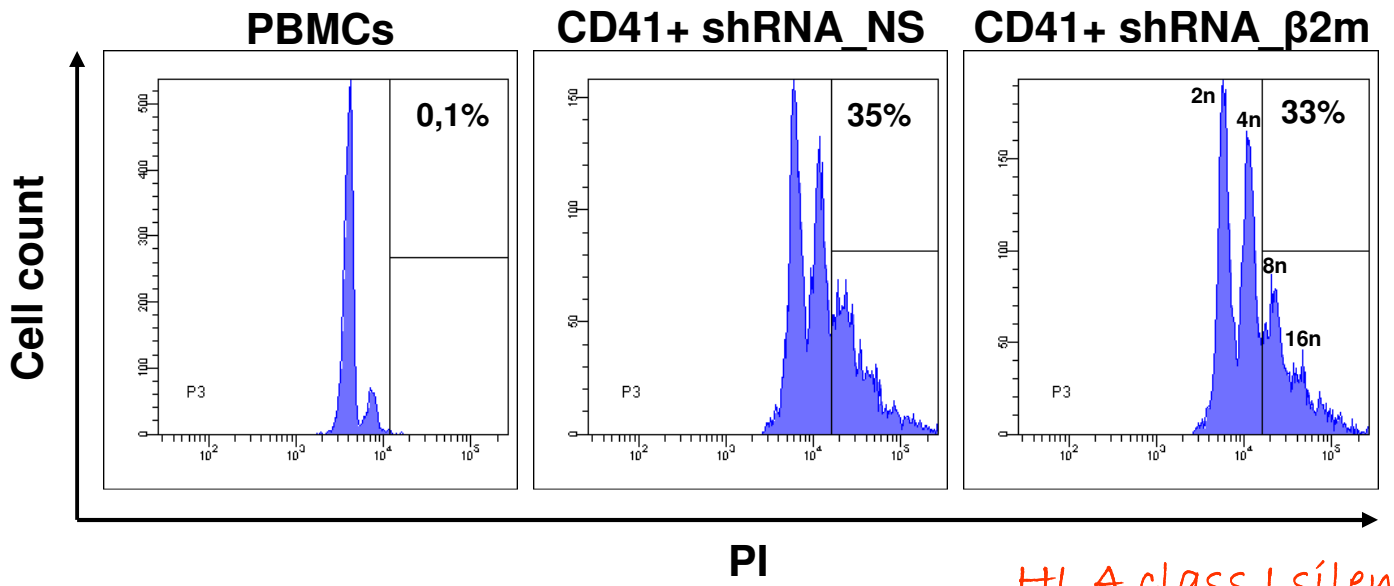


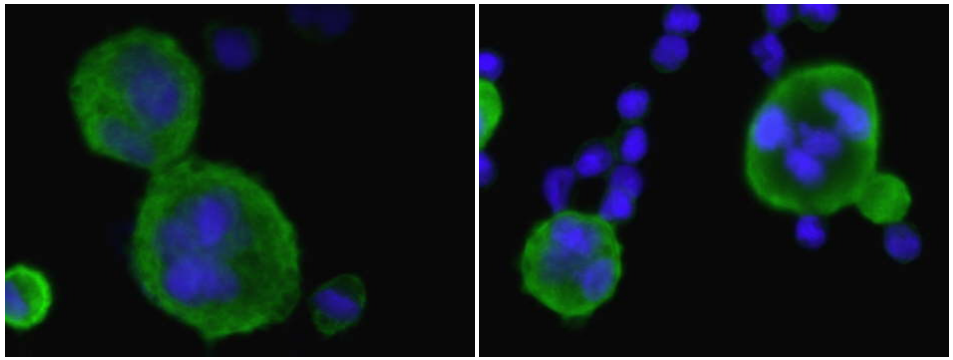
HLA class I-specific silencing



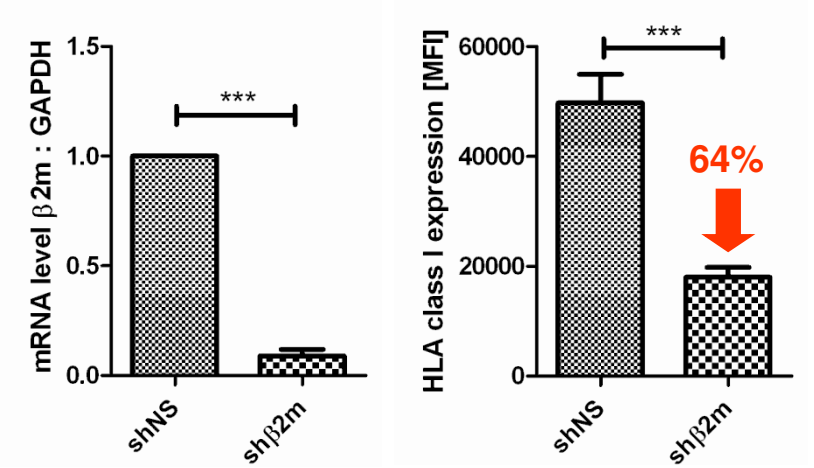
Figueiredo et al, J mol Med, 2006
Figueiredo et al, Transfusion, 2007
Figueiredo et al, BioMed Res Int, 2013
Wiegmann & Figueiredo et al, Biomaterials, 2014

Polyploidy of differentiated megakaryocytes

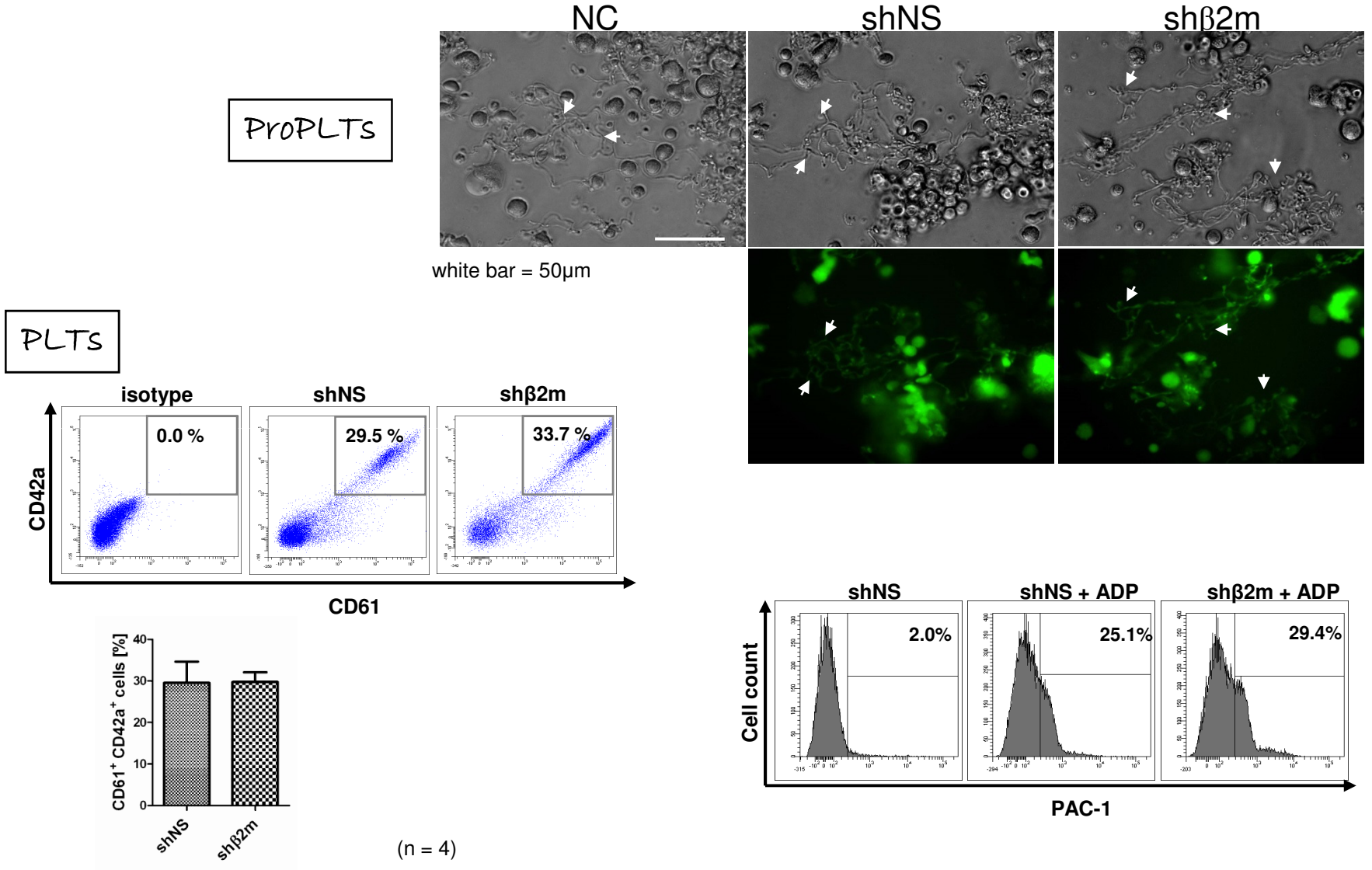




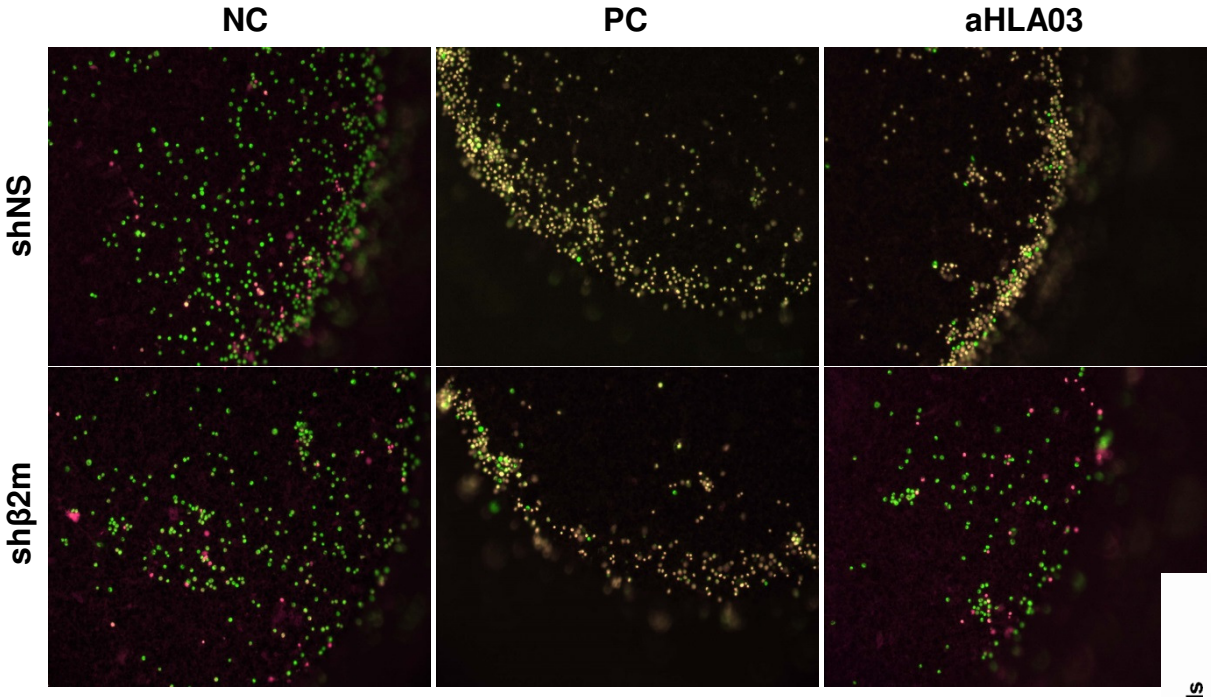
HLA class I silencing

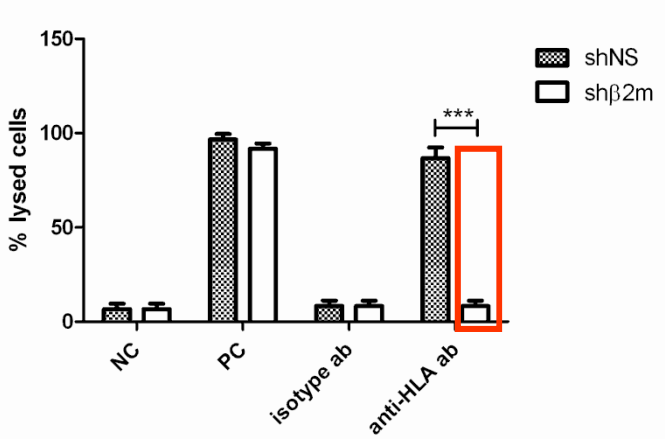


ProPLT/ PLT-production by differentiated MKs



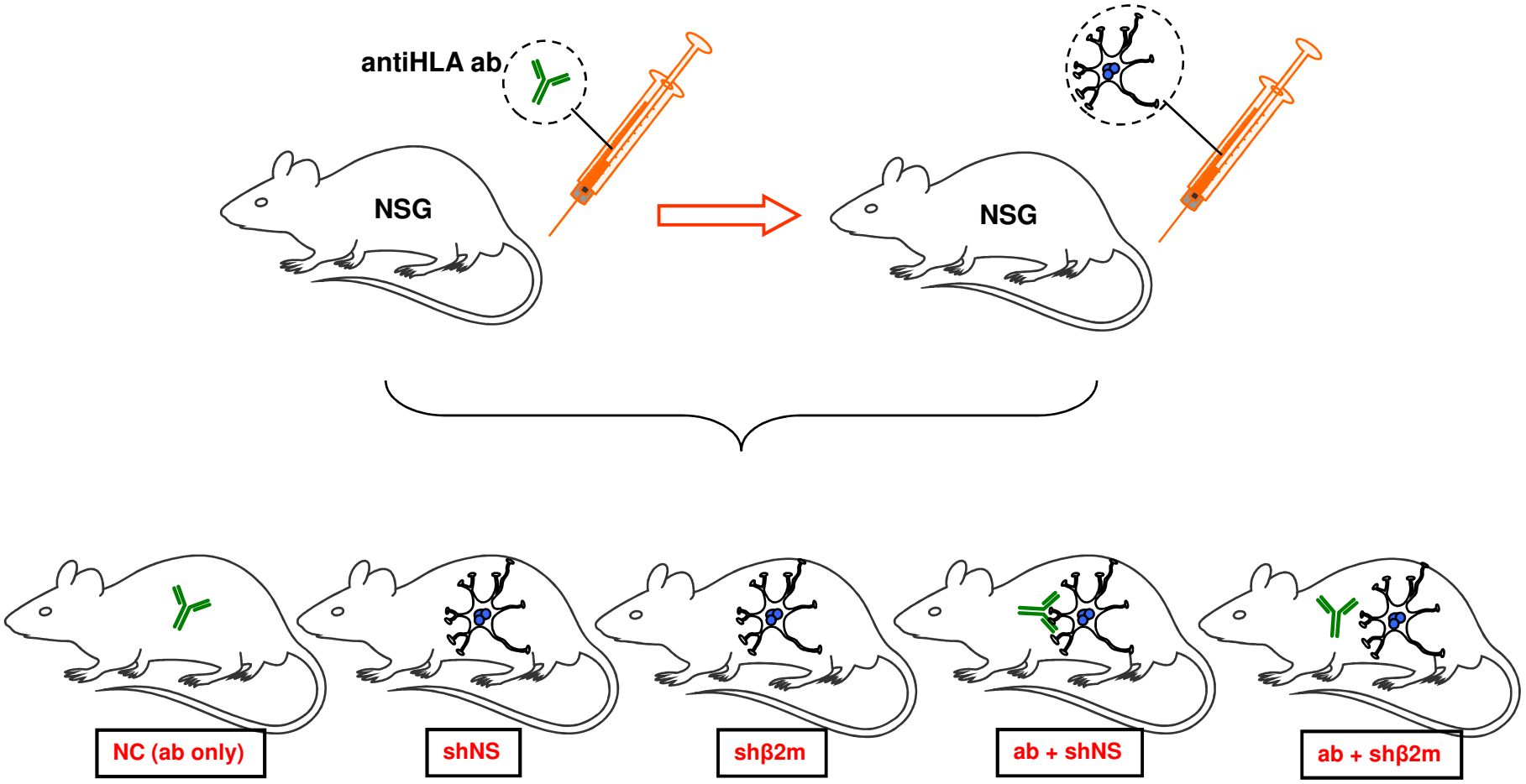
HLA-universal MKs protected from antibody-mediated cytotoxicity





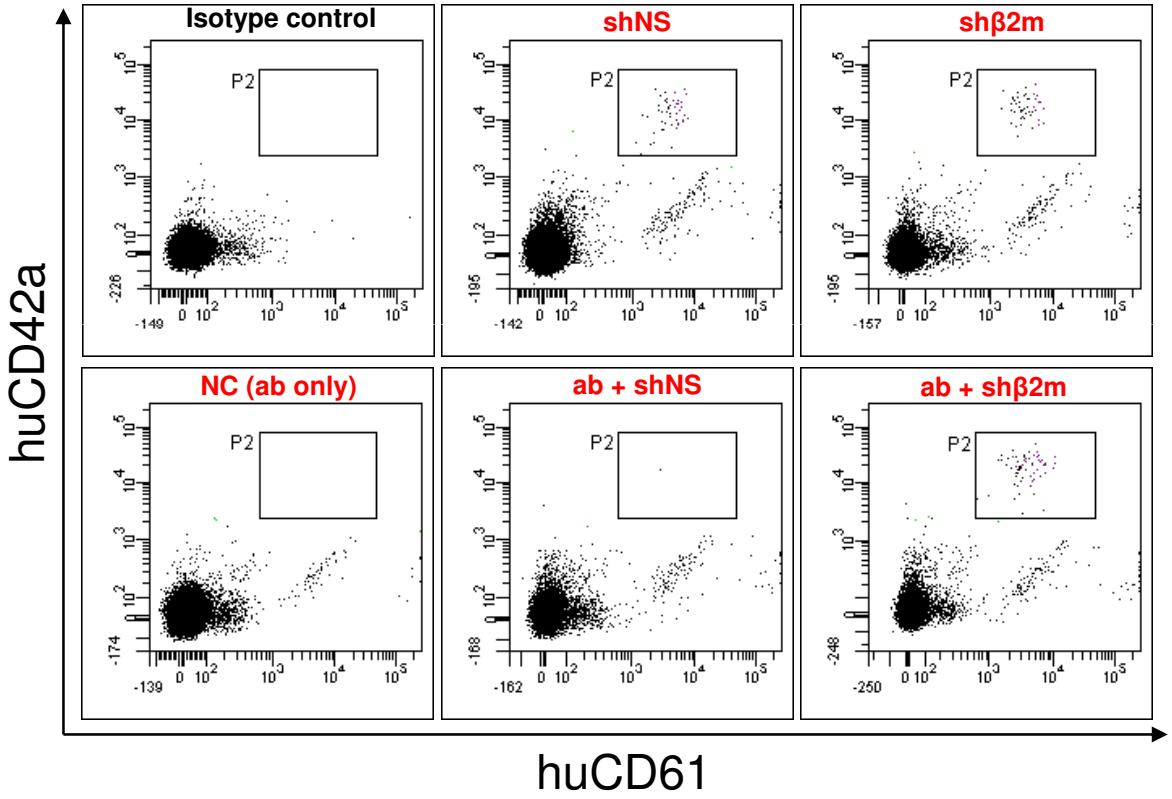
(n = 4, ***p<0.001)

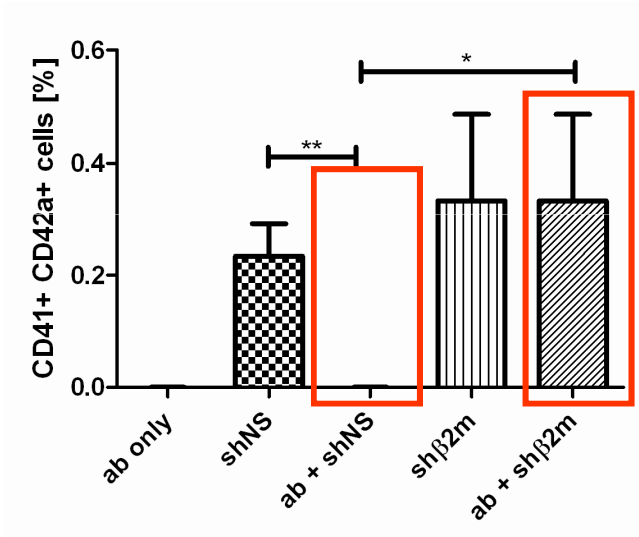
HLA-universal MKs & PLTs prevent PLT refractoriness in a mouse model



HLA-universal PLTs prevent PLT refractoriness in a mouse model

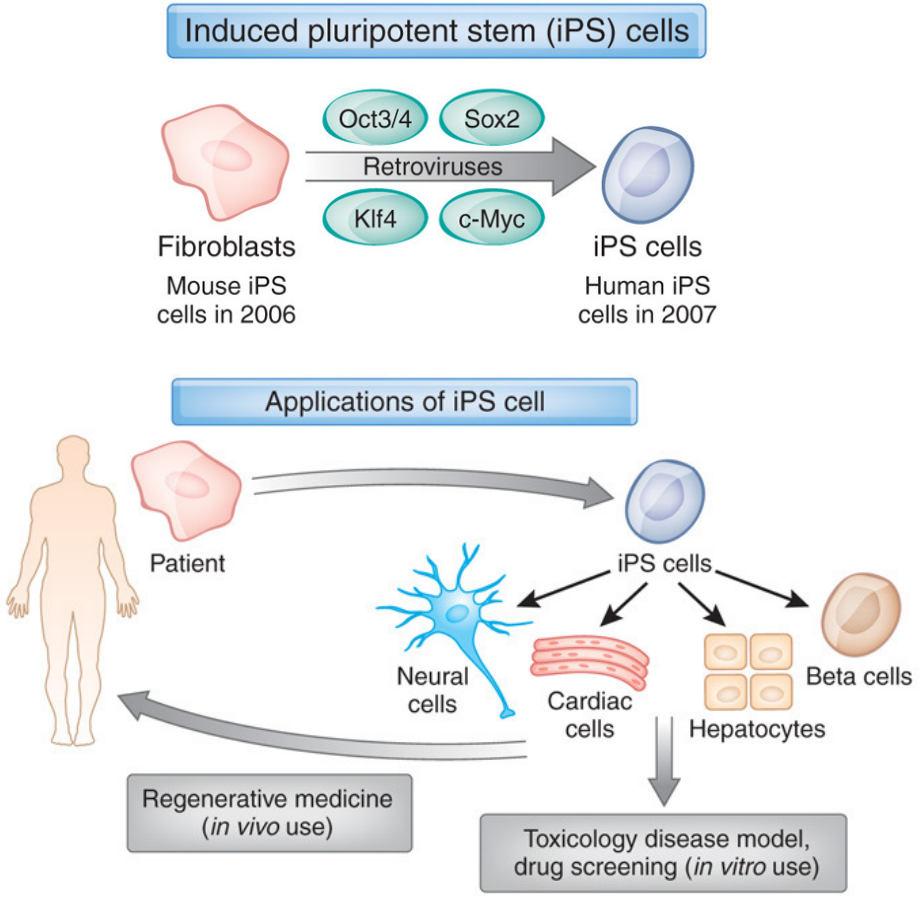
Human PLTs in the circulation of NSG mice





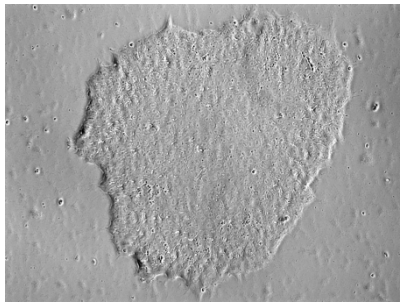
(n = 3, *p<0.05, **p<0.01)

Induced pluripotent Stem (iPS) cells as unilimited cell sources

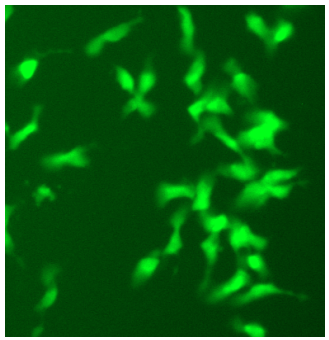


Yamanaka S. Nature Medicine 2009.

Differentiation of iPSCs toward HLA-universal PLTs

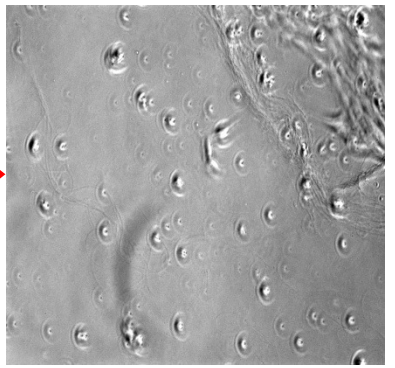


iPSC colony



Silencing HLA expression
iPSC cultured in monolayer

Orbital shaker



Megakaryocyte and
Platelet formation

Xenofree conditions

Mesoderm

BMP-4
VEGF

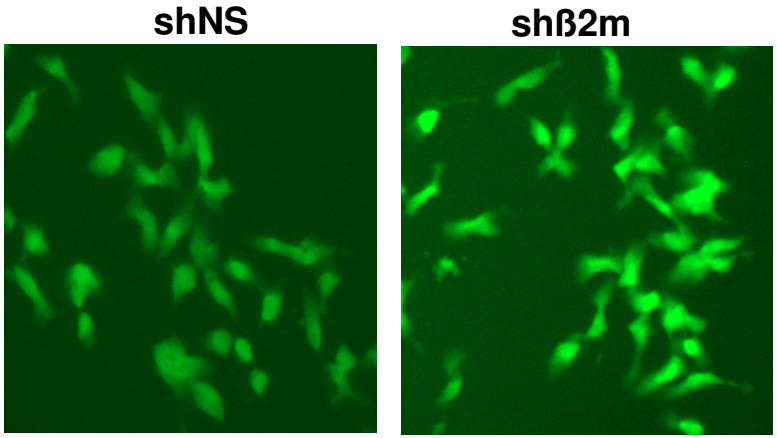
Hematopoiesis/MK

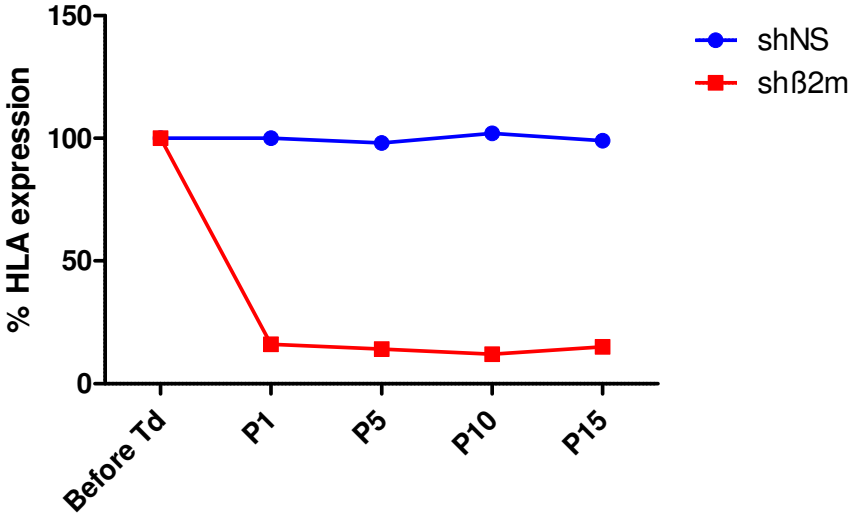
SCF
IL-3
TPO

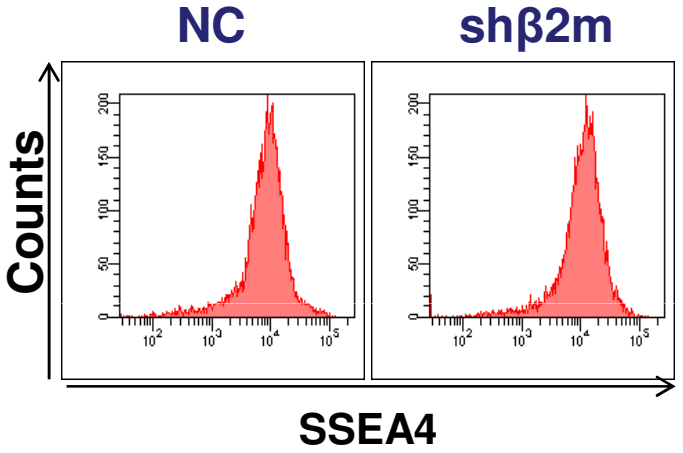
Megakaryocytes

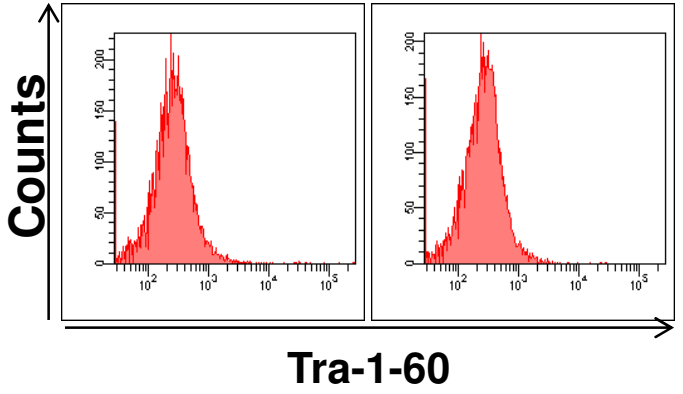
TPO

Generation of HLA-universal iPSC lines

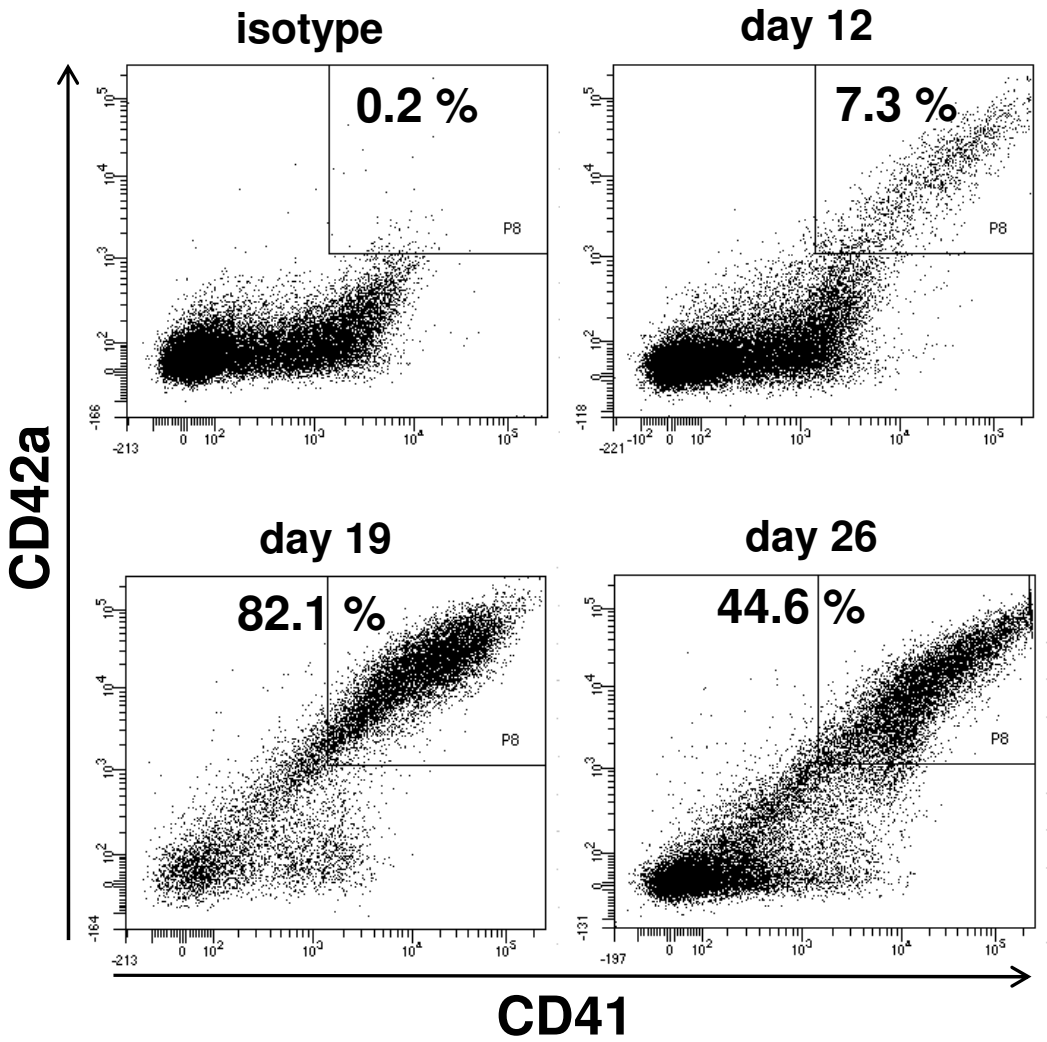


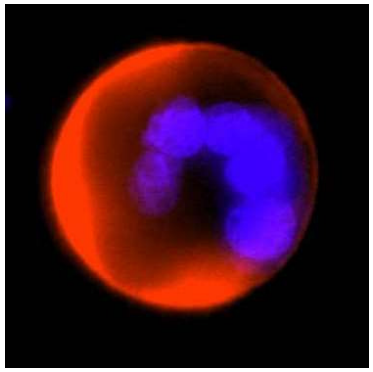


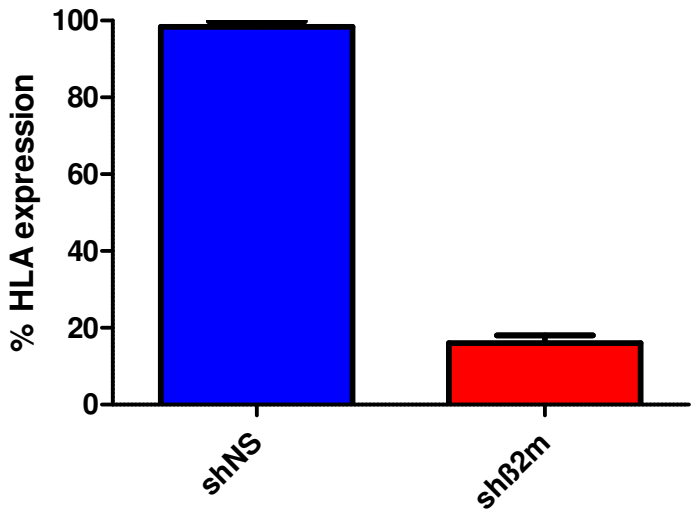




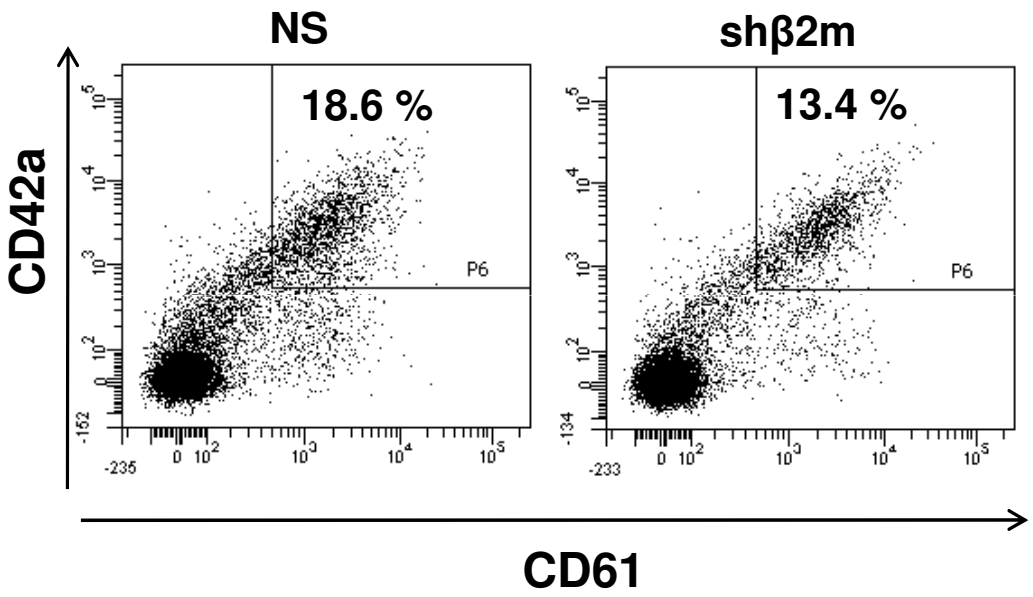
Generation of HLA-universal Megakaryocytes

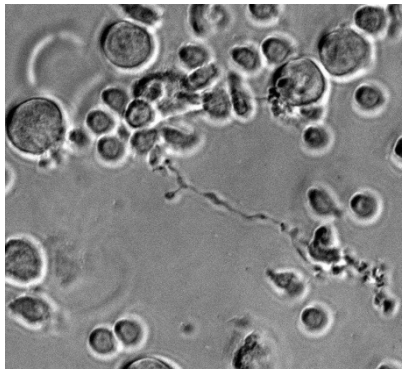


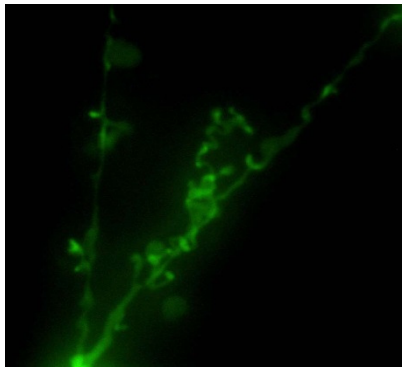


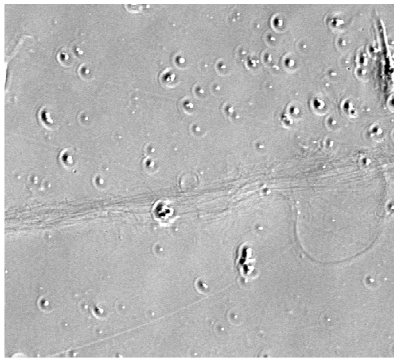


Differentiation of Platelets

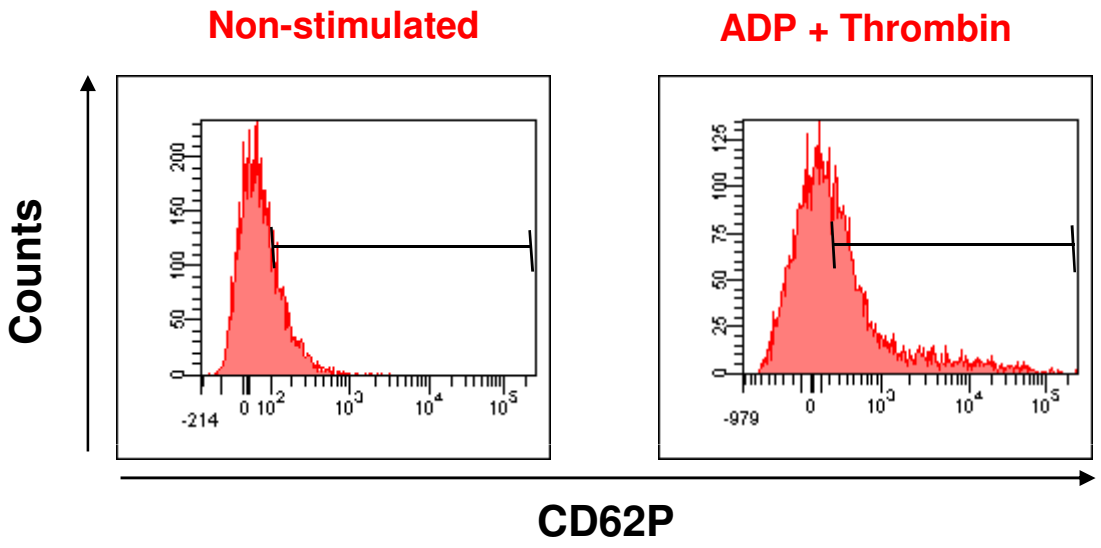


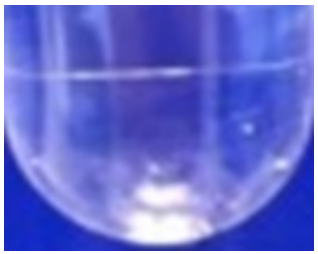


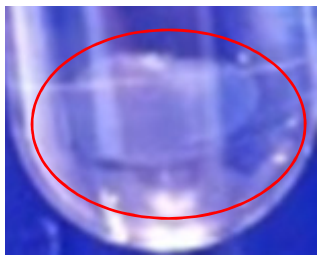




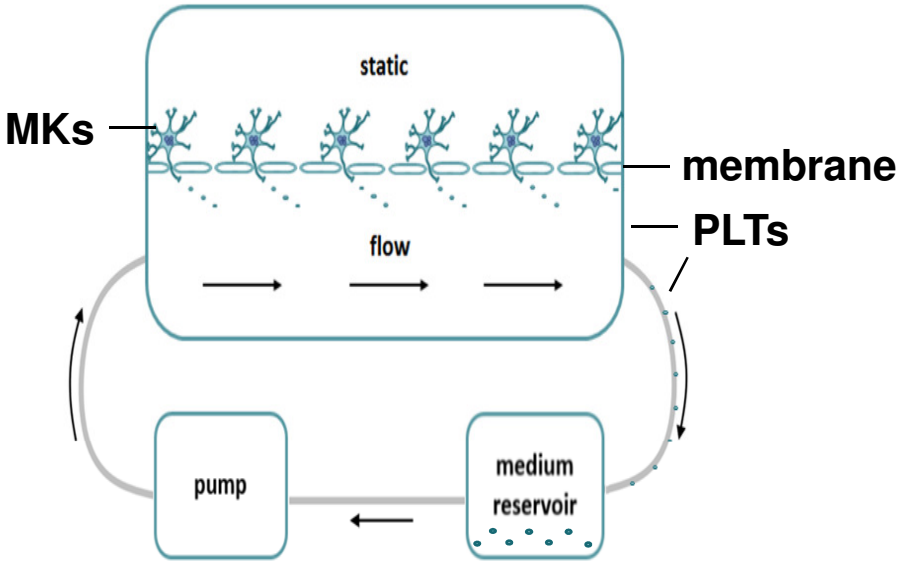
iPSC-derived Platelets respond to external stimuli

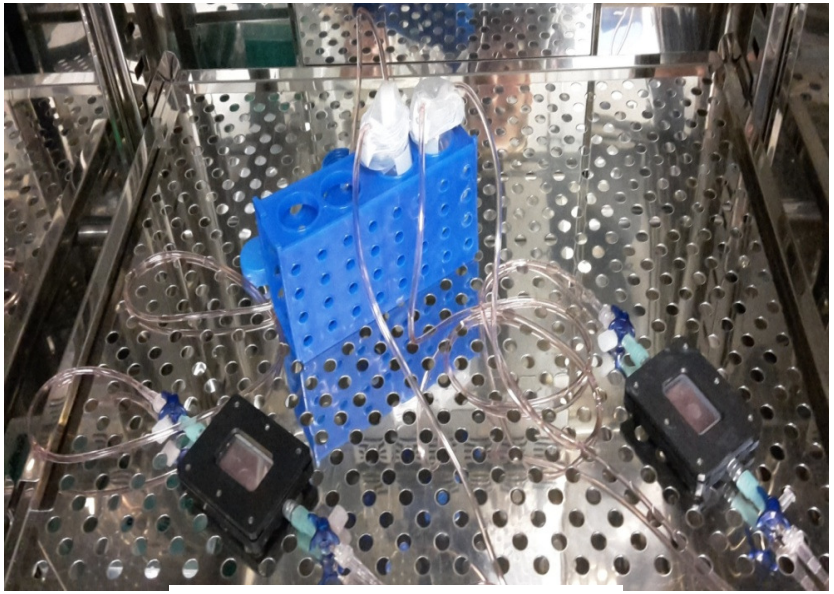


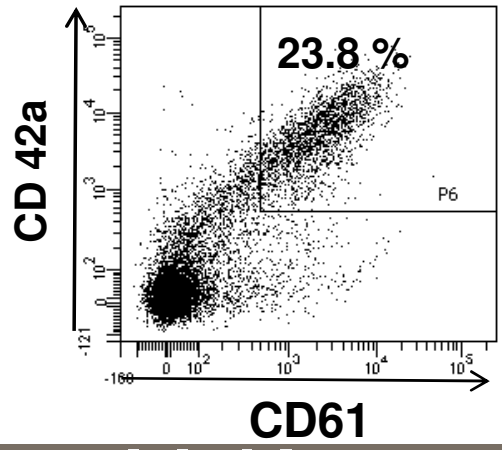




Large-scale production of platelets in bioreactors







Summary

- The generation of *in vitro* pharmed genetically modified platelets is feasible.
- IPSC serve as an unlimited cell source for the large-scale production of platelets.
- Universal platelets bring the concept of Universal cells one step closer to reality.

Acknowledgements

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Stefanie Wunderlich
Lena Engels

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rebirth

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MHH Medizinische Hochschule Hannover