

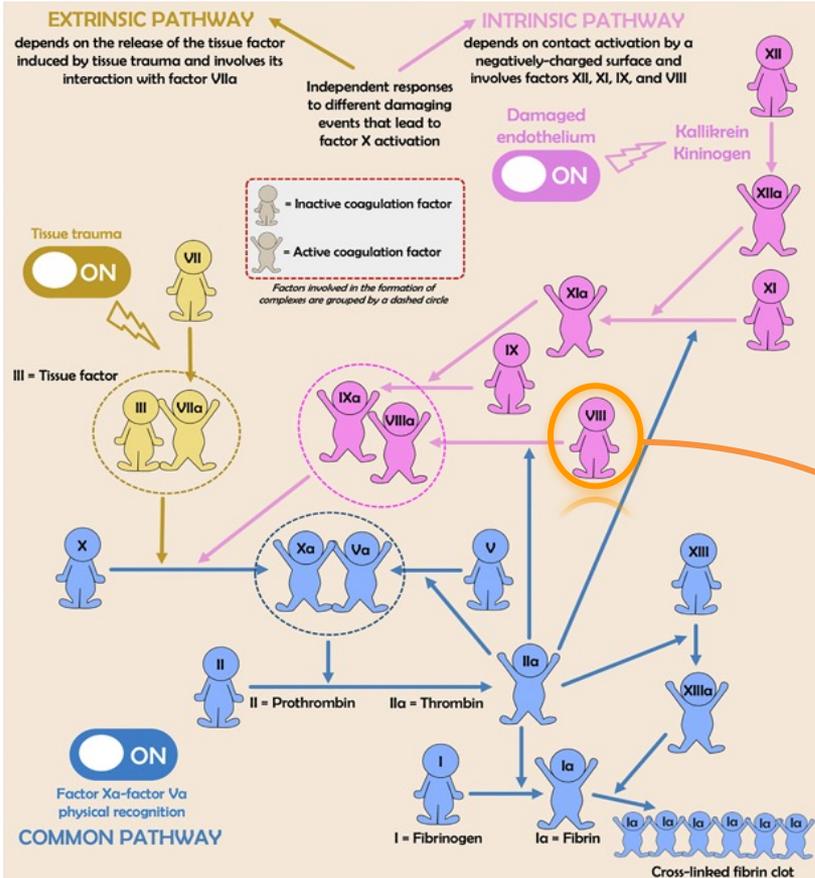


XIX CONGRESSO  
NAZIONALE  
SIES 2026

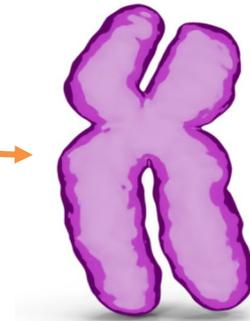
**CELLULE ENDOTELIALI SINUSOIDALI DERIVATE DA  
iPSCs PERSONALIZZATE: UNA STRATEGIA PER LA  
CORREZIONE FENOTIPICA DELL'EMOFILIA A**

Dott.ssa Andrea Benetti

Firenze | 4-6 marzo 2026  
Palazzo degli Affari



- Inherited X-linked congenital bleeding disorder.
- *F8* gene variations resulting in a deficiency/absence of FVIII.
- More than 2000 different *F8* gene variants are responsible for HA have been reported.
- The incidence of HA is about 1 in 5000 live male births.



**SEVERE HA: FVIII activity < 1%**

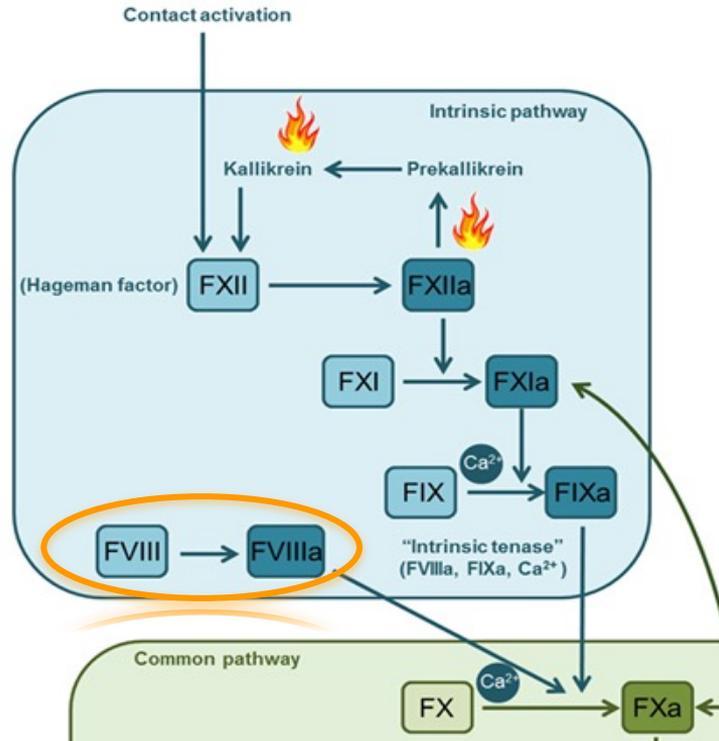
**MODERATE HA: FVIII activity 2-5%**

**MILD HA: FVIII activity 5 - 40%**

**HEALTHY SUBJECT: FVIII activity >50%**

- Clinical manifestations: prolonged clotting time and repeated bleeding since childhood.
- Prophylaxis: FVIII concentrates to maintain a factor level above 1 % of normal FVIII activity.
  - Regular prophylaxis: early childhood.

- Factor VIII is the cofactor of FIX and the binding form the tenase complex.
- Tenase complex activates Factor X that contributed to the thrombin formation starting the common pathway.

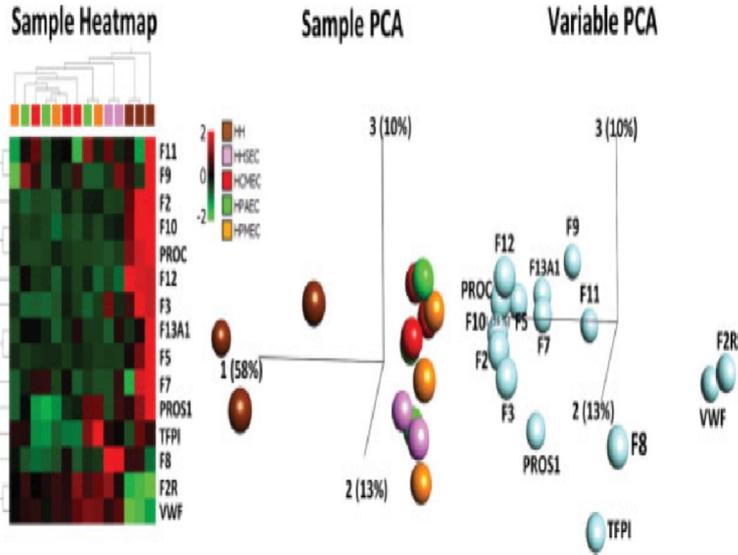


- **Gene:** *F8*
- **Location:** Chromosome Xq28
- **Size:** ~186 kb of genomic DNA
- **Exons:** 26
- **Encoded protein:** Coagulation Factor VIII
- **Synthesis:** Hepatocytes and sinusoidal endothelial cells

## «Molecular Profiling of Liver Sinusoidal Endothelial Cells in Comparison to Hepatocytes: Reflection on Which Cell Type Should Be the Target for Gene Therapy.»

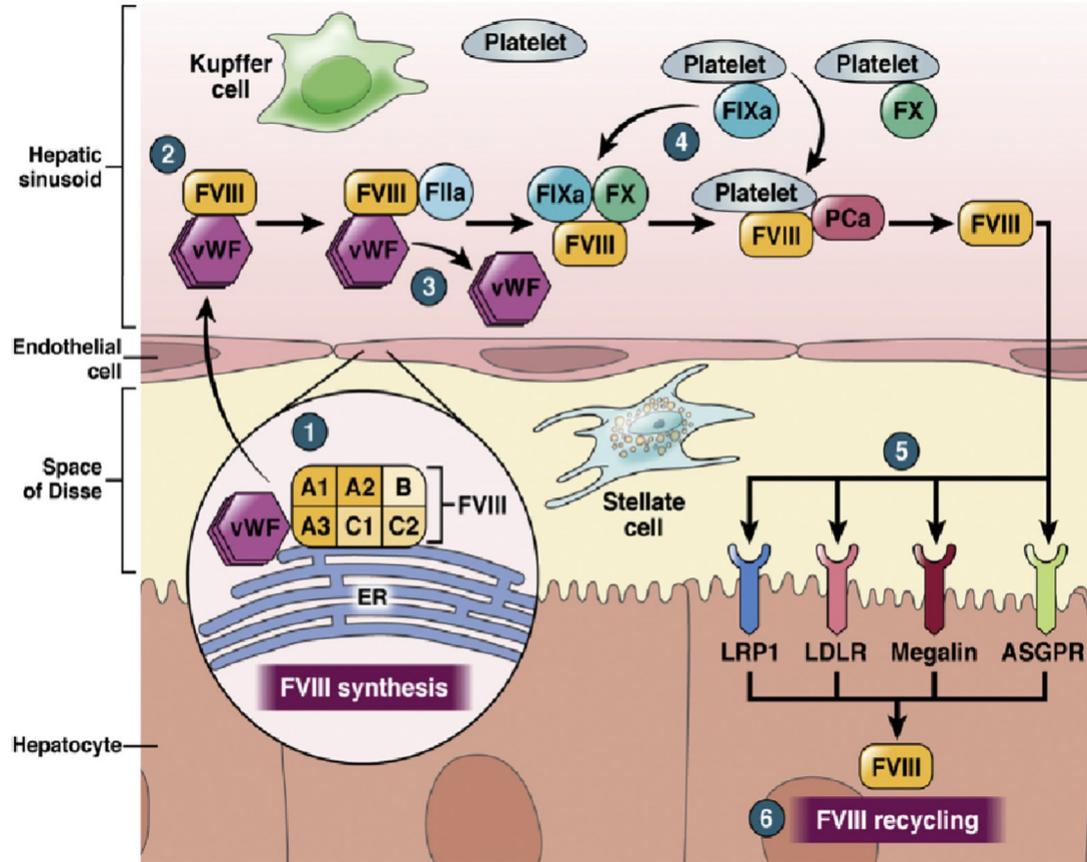
El-Maarri O, Jamil MA, Oldenburg J.

Hamostaseologie 2020



Expression of different coagulation factors in fetal endothelial cells, fetal liver sinusoidal endothelial cells, and hepatocytes.

Therefore, **optimizing gene therapy protocols is of particular importance**. Due to the specific characteristics of certain cell types, **targeting the natural FVIII-producing cells** may represent a promising future strategy for the treatment of haemophilia A.



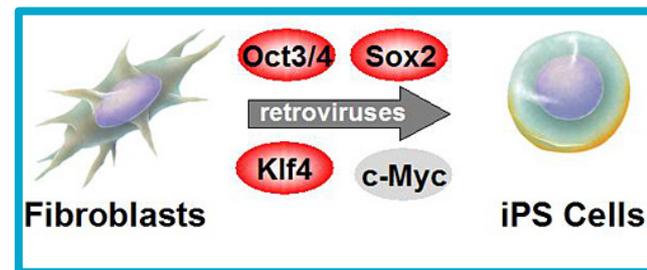
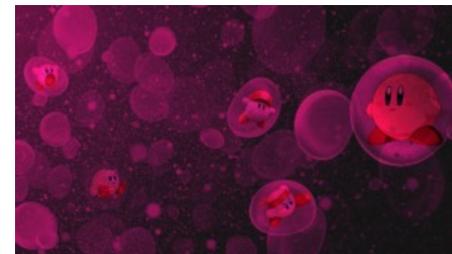
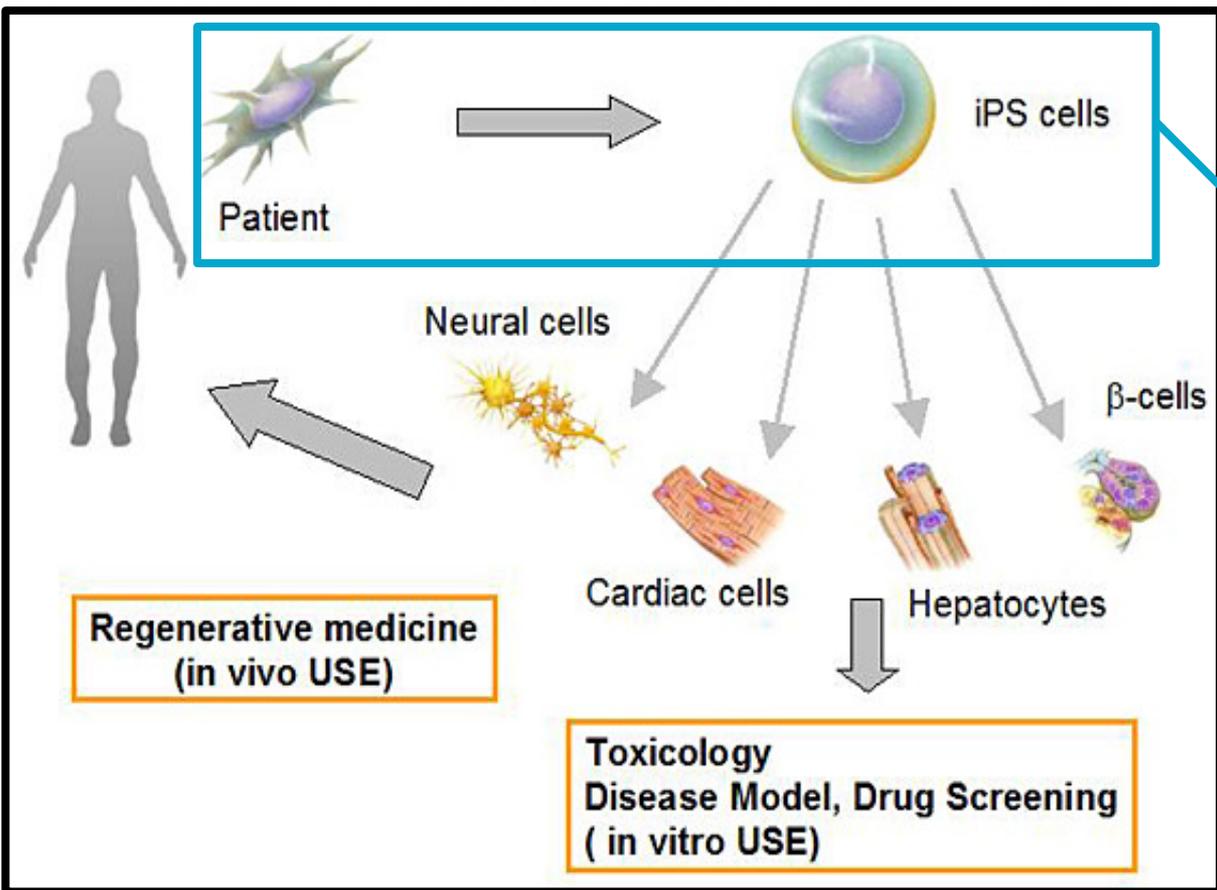
Liver sinusoidal endothelial cells are considered the chief source for the circulating FVIII.



Liver sinusoidal endothelium that normally produces the majority of circulating FVIII is an attractive therapeutic approach.

Tirthadipa Pradhan-Sundd et al. *Hepatology* 2021;12:1061–1072;  
Kumaran V et al. *J Thromb Haemost.* 2005 Sep;3(9):2022–31

# INDUCED PLURIPOTENT STEM CELLS (iPSCs)

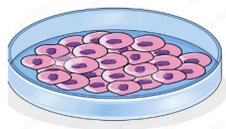




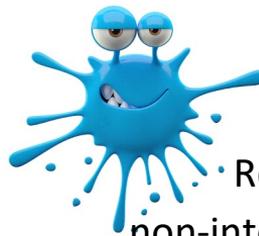
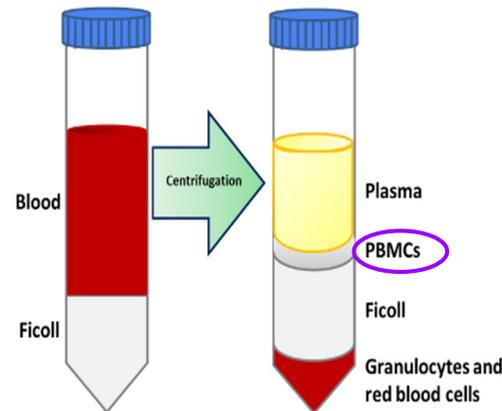
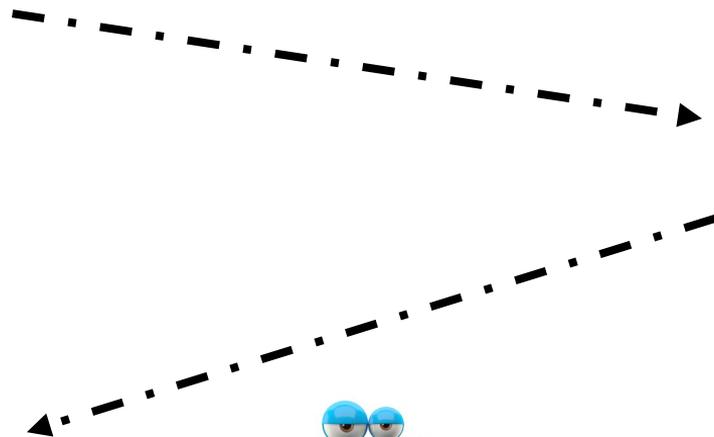
Restore **FVIII** levels to prevent spontaneous bleeding by **correcting FVIII variants** using **CRISPR-Cas9** base editing in **liver sinusoidal endothelial cells (LSECs)**.



Patients with *F8 variants* and *healthy controls* were enrolled



Induced Pluripotent Stem Cells (iPSCs)



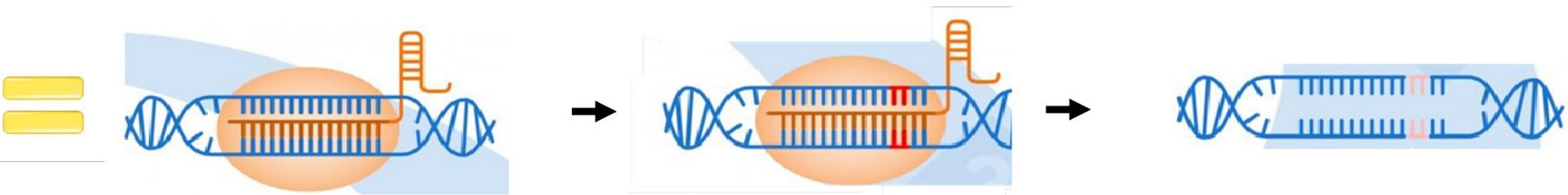
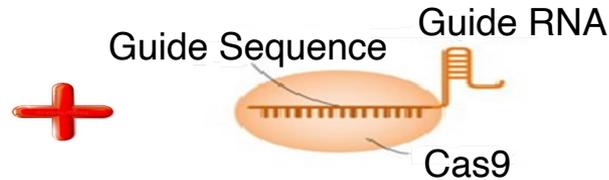
Reprogramming using a non-integrative Sendai virus system



Chromosome X, *F8* gene  
*Haemophillia* A patient



NM\_000132.4:c.6967C>T; NP\_000123.1:p.Arg2323Cys



A) Cas9 is integrated in the genome

B) Cas9 cleavage

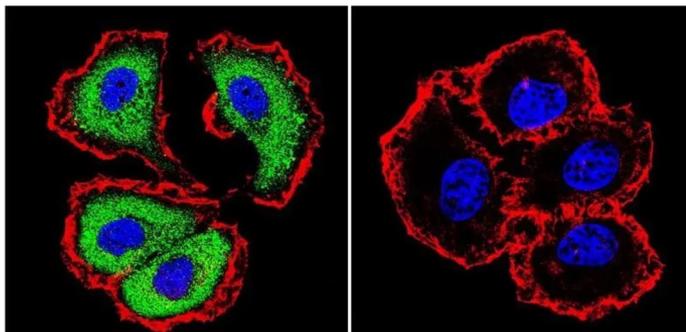
C) Correction and repairing



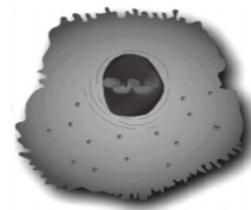
Liver sinusoidal endothelial cells (LSECs)



Specific Marker:  
**FCGR2B**



Hepatocyte-like cells (HLCs)

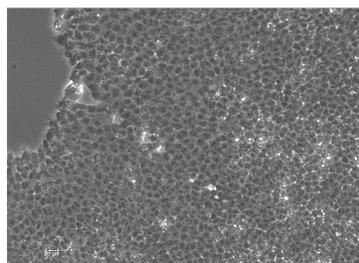
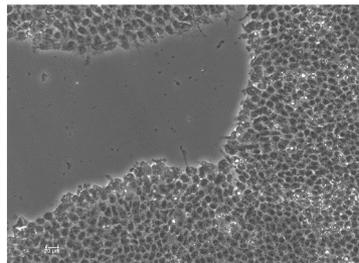
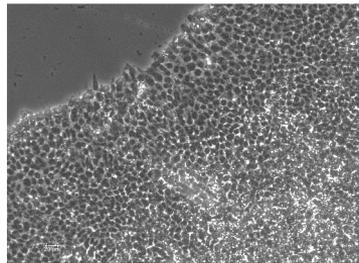


Specific Marker:  
**Cytokeratin 18**

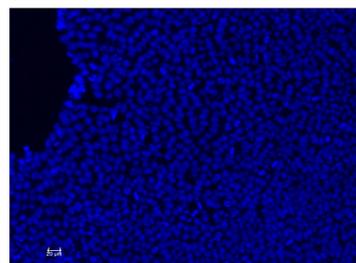
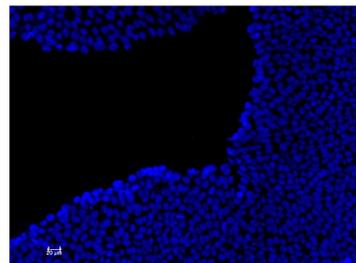
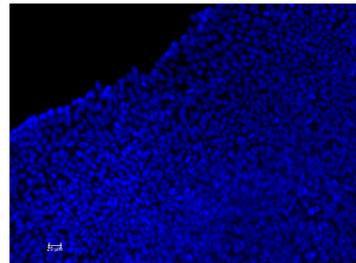
## iPSCs – PHASE CONTRAST



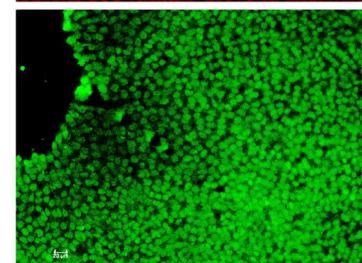
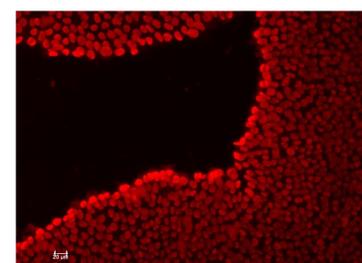
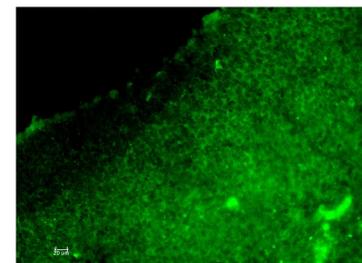
DIC



NUCLEI



FLUORESCENCE

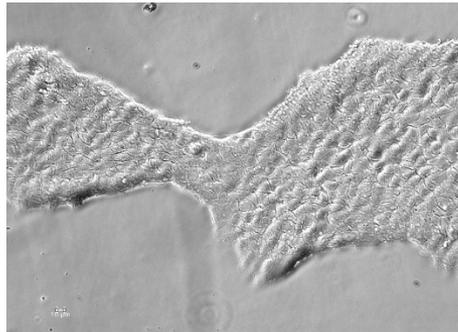


Nanog

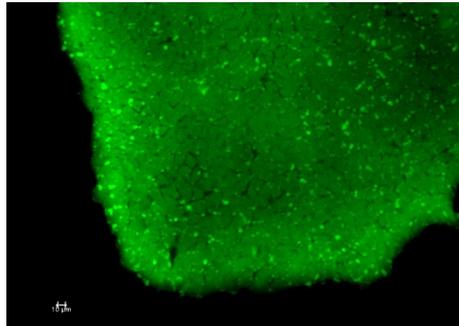
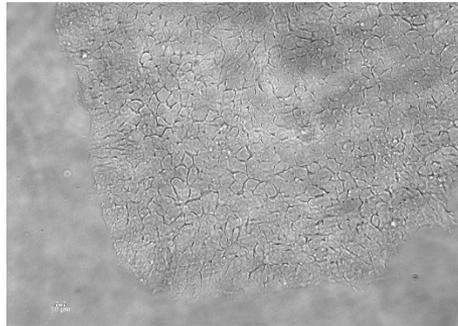
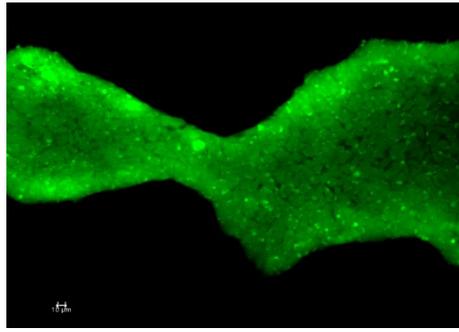
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Sox2

DIC



AP FLUORESCENCE

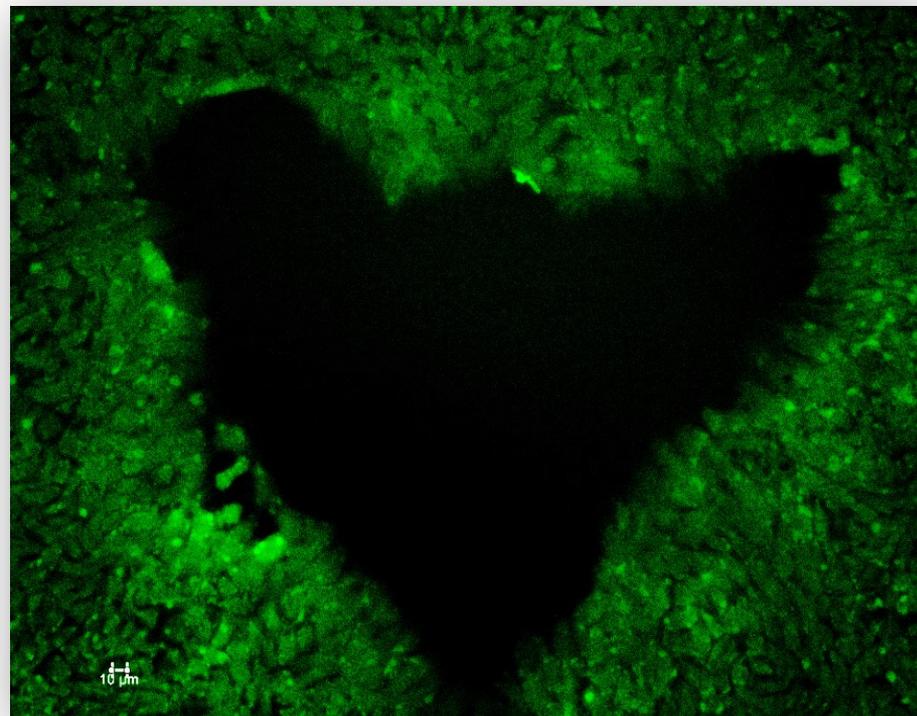


Healthy control-derived iPSCs clones expressed the **pluripotency** marker alkaline phosphatase (AP).

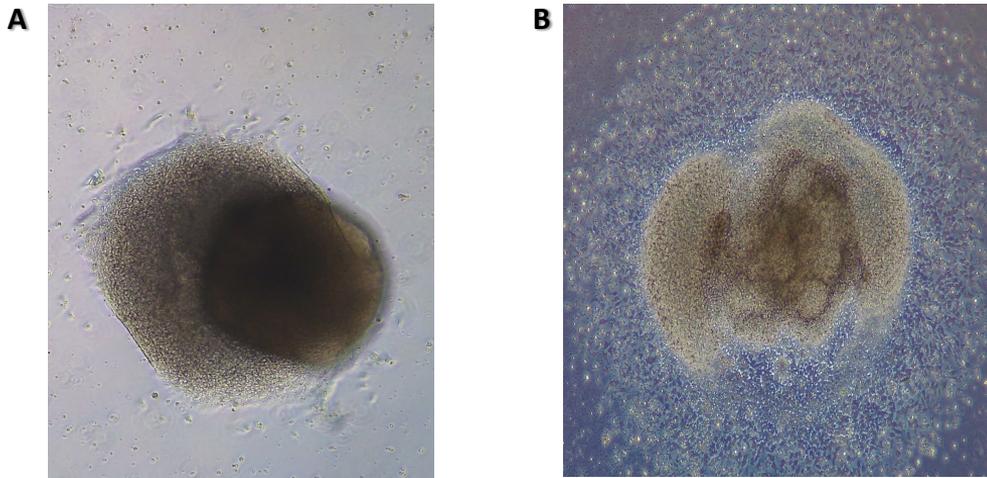
Alkaline phosphatase (green fluorescence) in iPSC cells. Bright-field DIC. DMI6000CS microscope, 20× objective.



Overlay of fluorescence and DIC.  
DMI6000CS microscope, 20× objective.

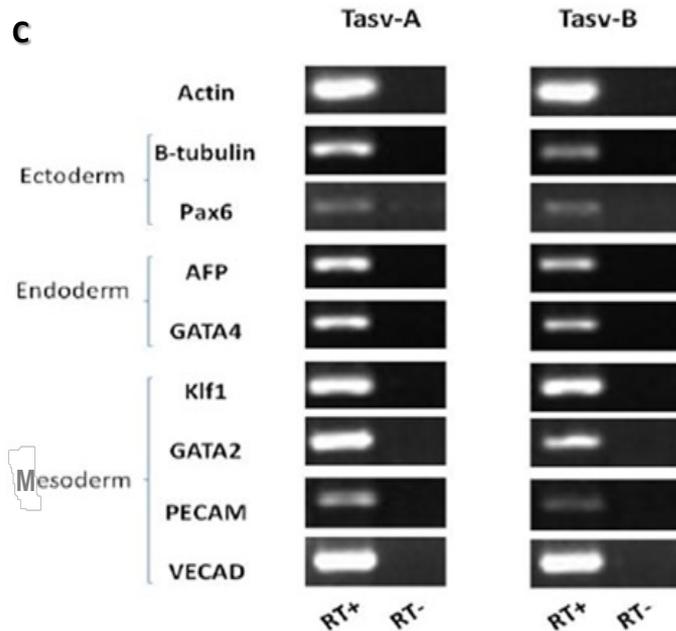


Alkaline phosphatase (green fluorescence) in iPSC cells. Bright-field  
DIC. DMI6000CS microscope, 20× objective.



Embryoid bodies (EBs) test performed on iPSCs clones:

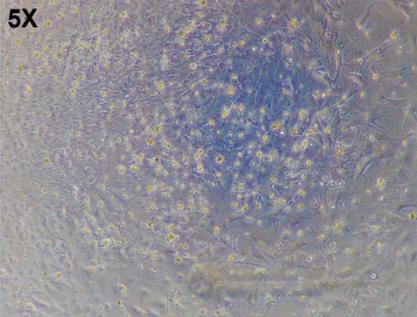
- A. EBs in suspension for the first 7 days.
- B. EBs after adhesion on gelatin-coated surfaces.



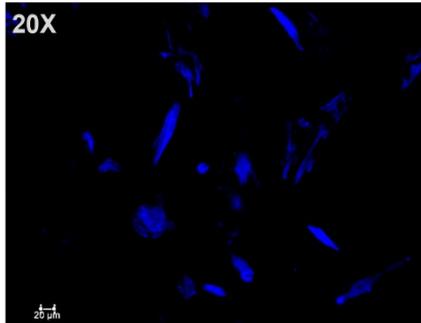
C. Healthy control-derived iPSCs clones express all three germ layers differentiation markers after embryoid bodies differentiation test.

## HEPATOCYTE-LIKE CELLS (HLCs)

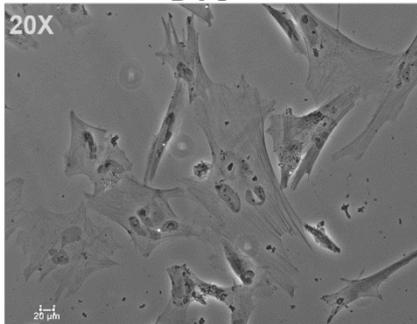
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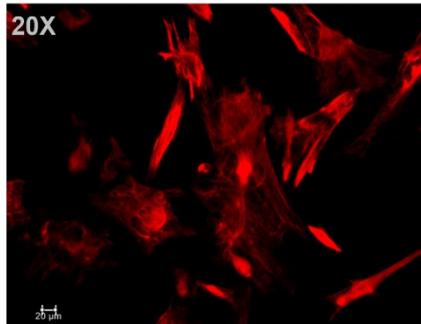
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DIC

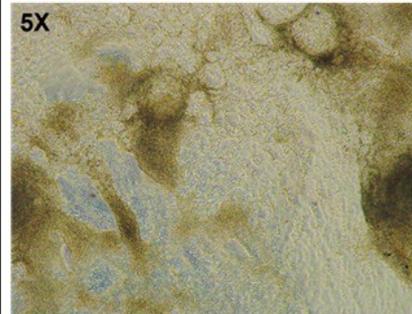


CYTOKERATIN 18

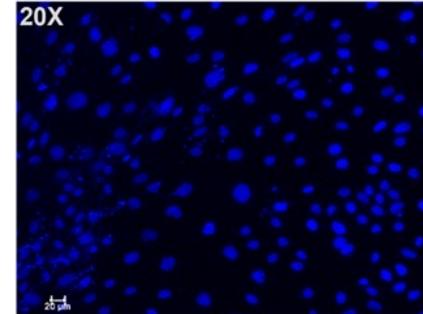


## LIVER SINUSOIDAL ENDOTHELIAL CELLS (LSECs)

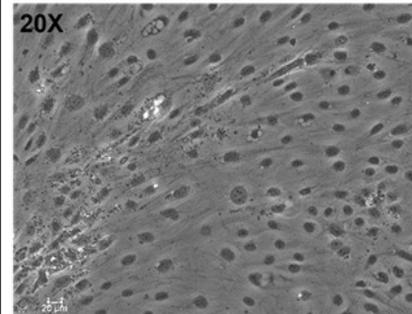
PHASE CONTRAST



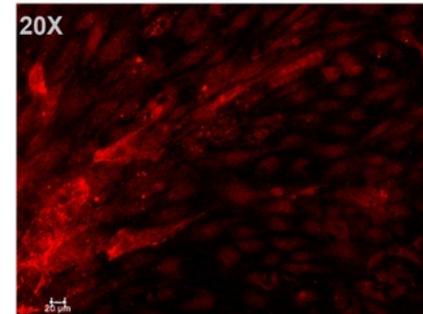
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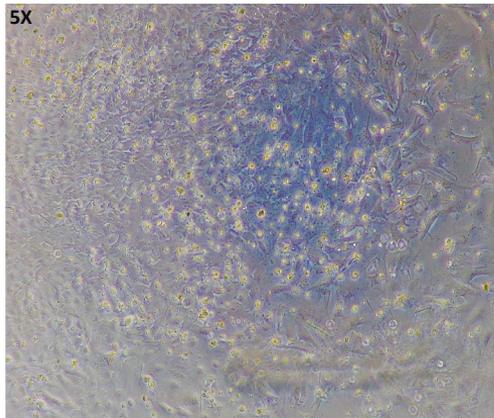
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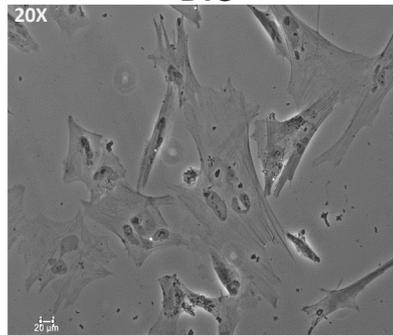
FCGR2B



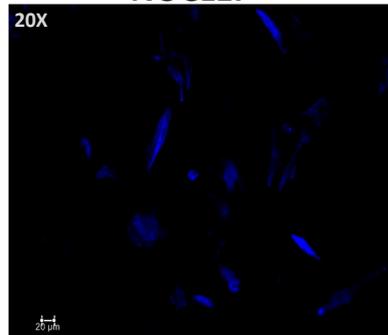
## HLCs – PHASE CONTRAST



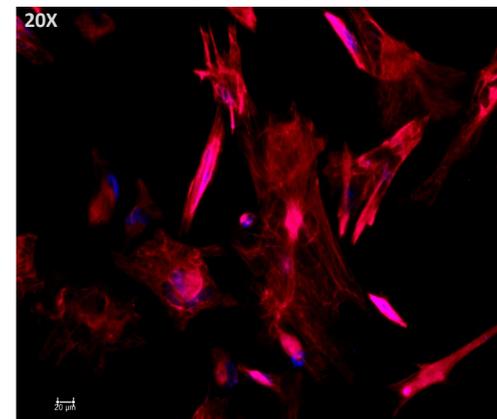
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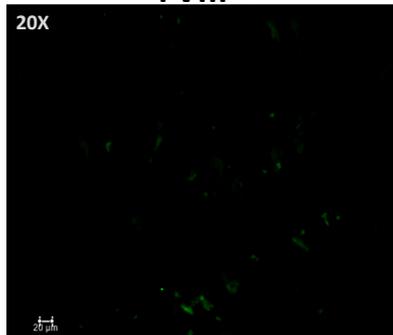
## NUCLEI



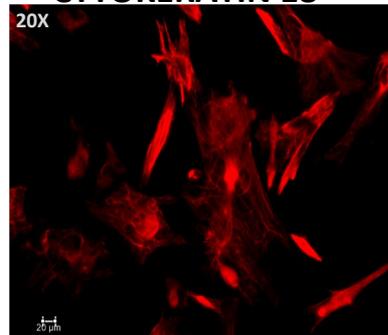
## OVERLAY OF FLUORESCENCES



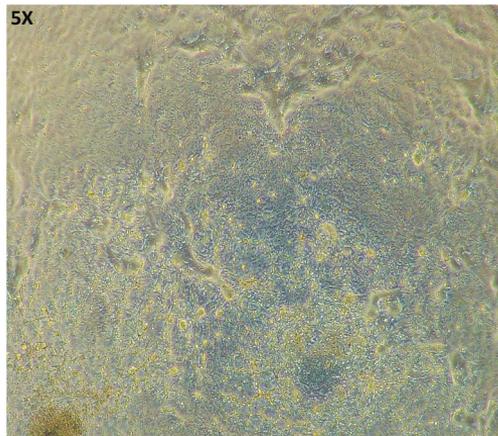
## FVIII



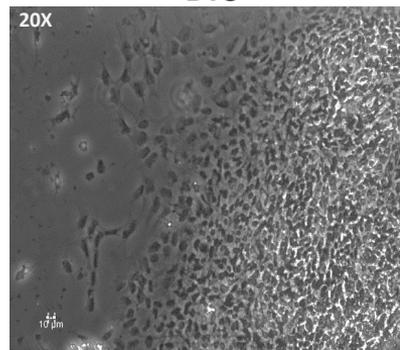
## CYTOKERATIN 18



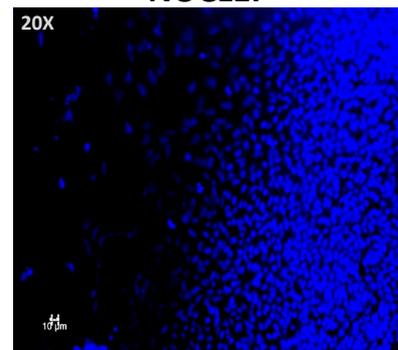
## LSECs – PHASE CONTRAST



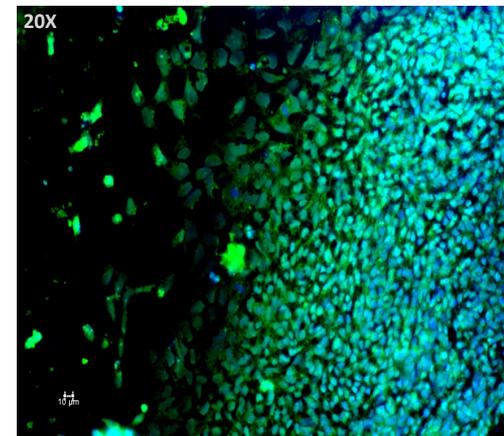
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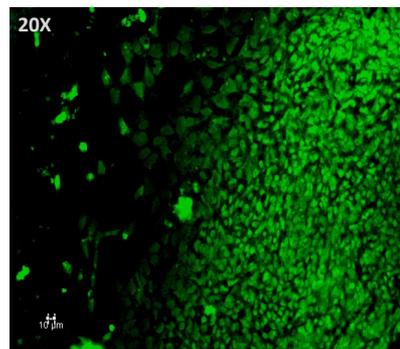
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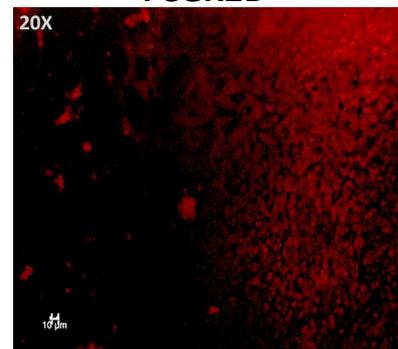
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## FVIII



## FCGR2B







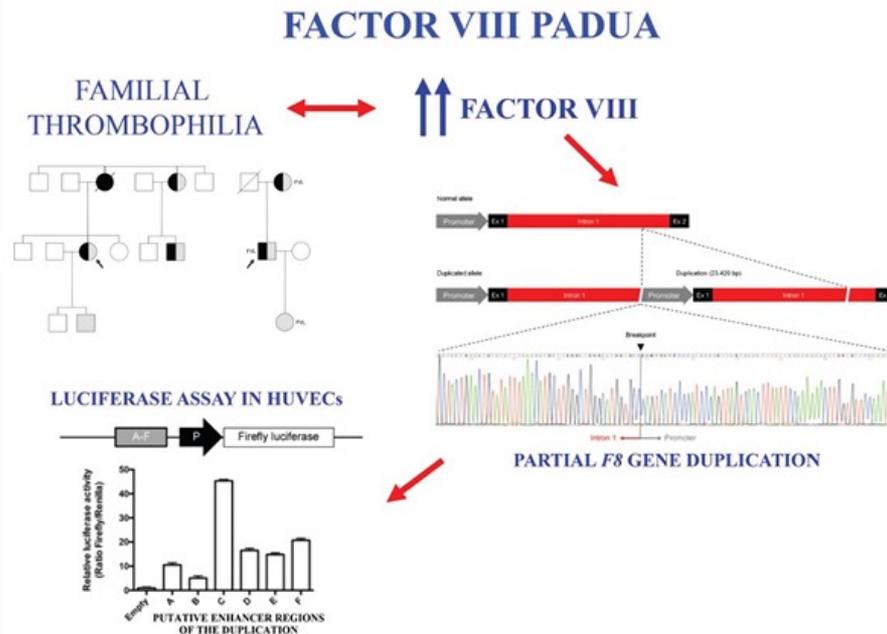
**blood** Regular Article

**THROMBOSIS AND HEMOSTASIS**

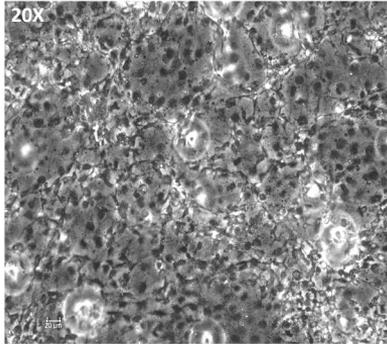
**Partial *F8* gene duplication (factor VIII Padua) associated with high factor VIII levels and familial thrombophilia**

Paolo Simioni,<sup>1</sup> Stefano Cagnin,<sup>2,4</sup> Francesca Sartorello,<sup>1</sup> Gabriele Sales,<sup>2</sup> Luca Pagani,<sup>2,5</sup> Cristiana Bulato,<sup>1</sup> Sabrina Gavasso,<sup>1</sup> Francesca Nuzzo,<sup>6</sup> Francesco Chemello,<sup>2</sup> Claudia M. Radu,<sup>1</sup> Daniela Tormene,<sup>1</sup> Luca Spiezia,<sup>1</sup> Tilman M. Hackeng,<sup>6</sup> Elena Campello,<sup>1</sup> and Elisabetta Castoldi<sup>6</sup>

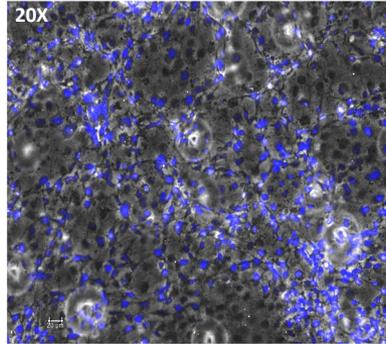
<sup>1</sup>General Internal Medicine and Thrombotic and Hemorrhagic Diseases Unit, Department of Medicine, University of Padua Medical School, Padua, Italy; <sup>2</sup>Department of Biology, <sup>3</sup>Centro di Ricerca Interdipartimentale per le Biotecnologie Innovative, and <sup>4</sup>Centro di Miologia, University of Padua, Padua, Italy; <sup>5</sup>Estonian Biocentre, Institute of Genomics, University of Tartu, Tartu, Estonia; and <sup>6</sup>Department of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands



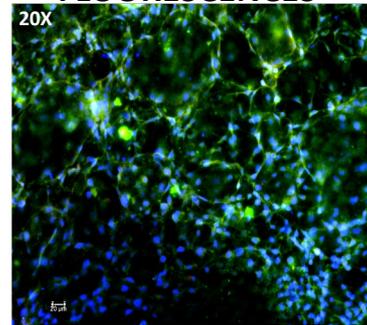
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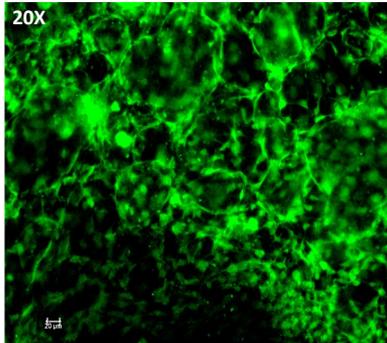
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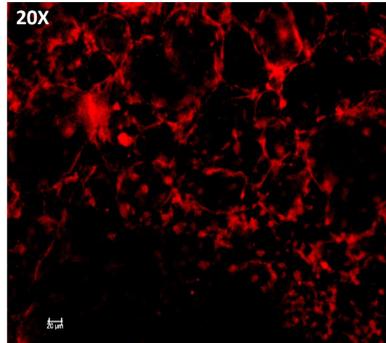
OVERLAY OF  
FLUORESCENCES



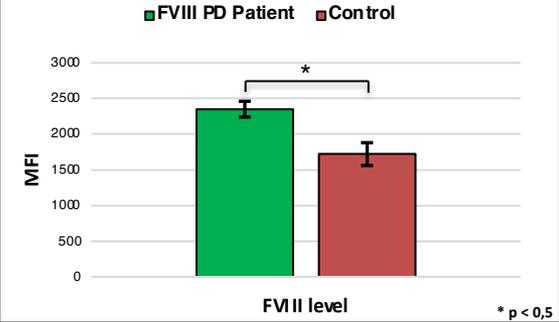
FVIII



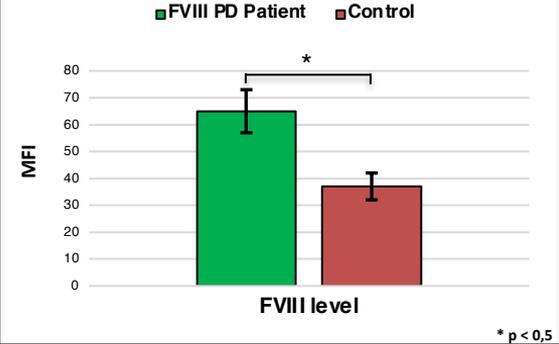
FCGR2B



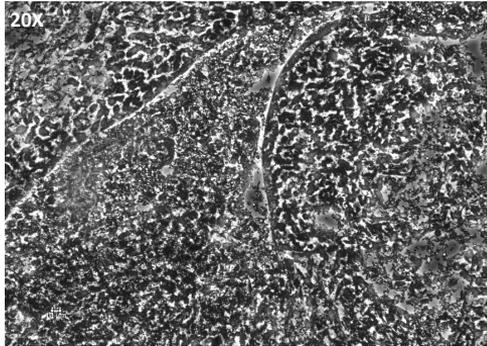
Immunofluorescence MFI quantification of FVIII expression



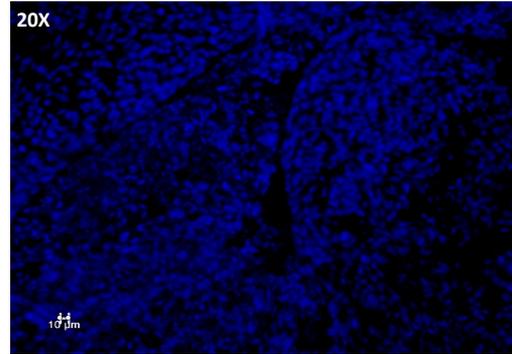
Immunofluorescence MFI quantification of FVIII expression



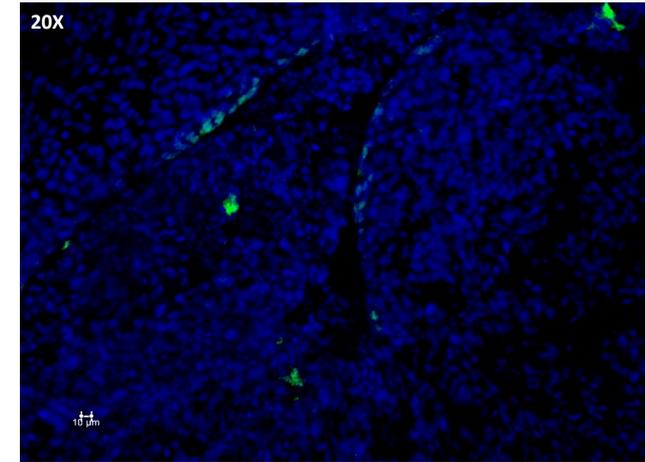
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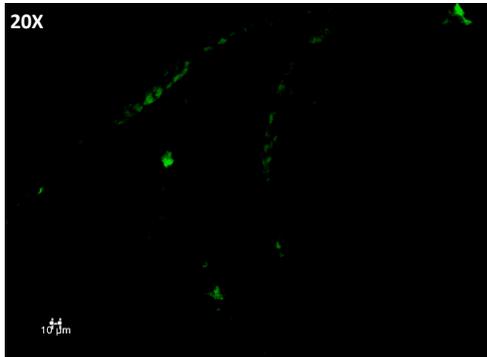
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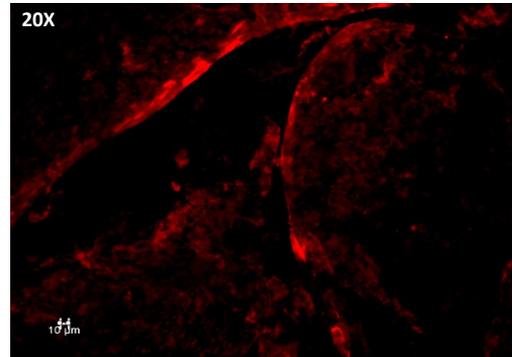
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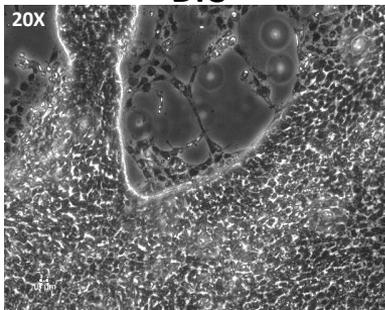
FVIII



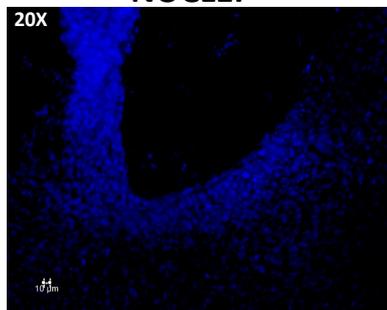
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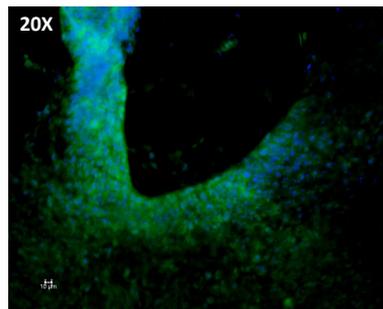
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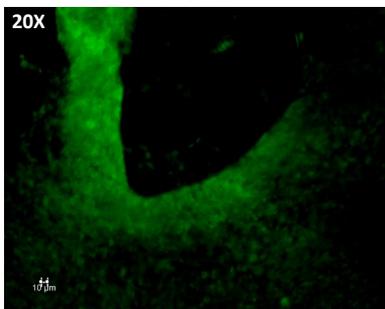
NUCLEI



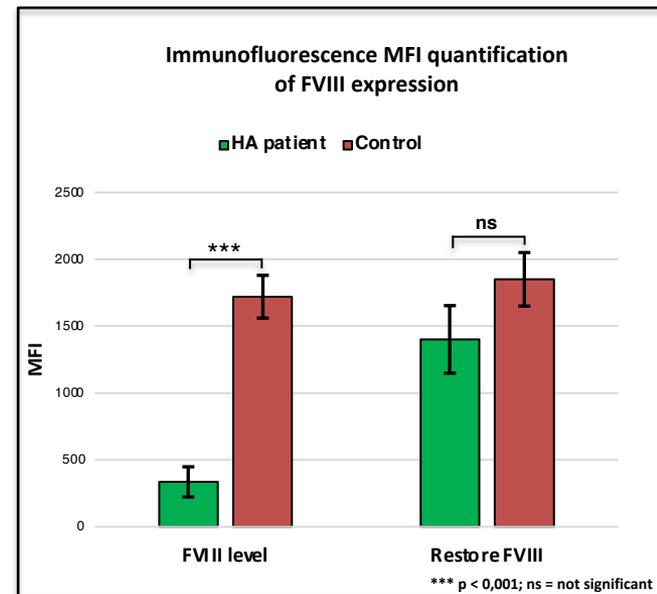
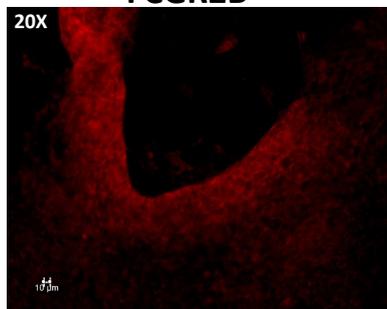
OVERLAY OF FLUORESCENCES



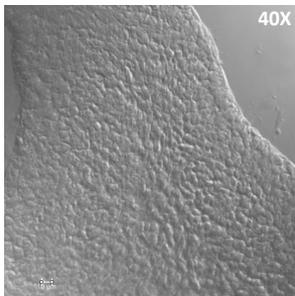
FVIII



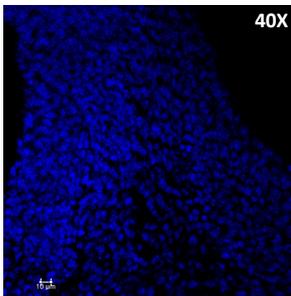
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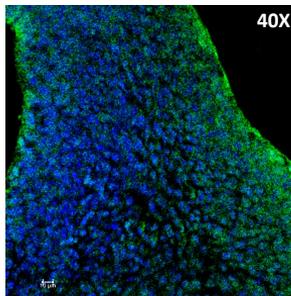
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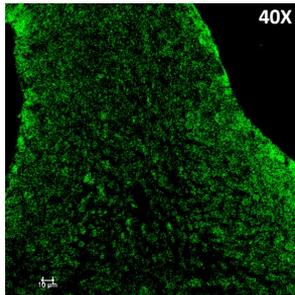
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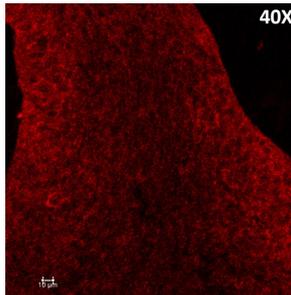
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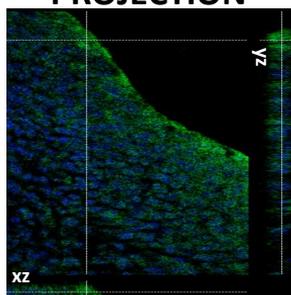
FVIII



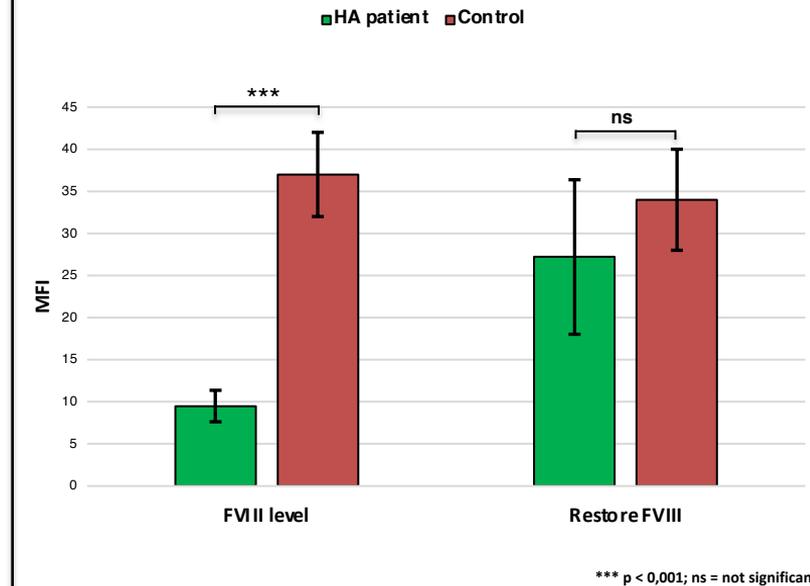
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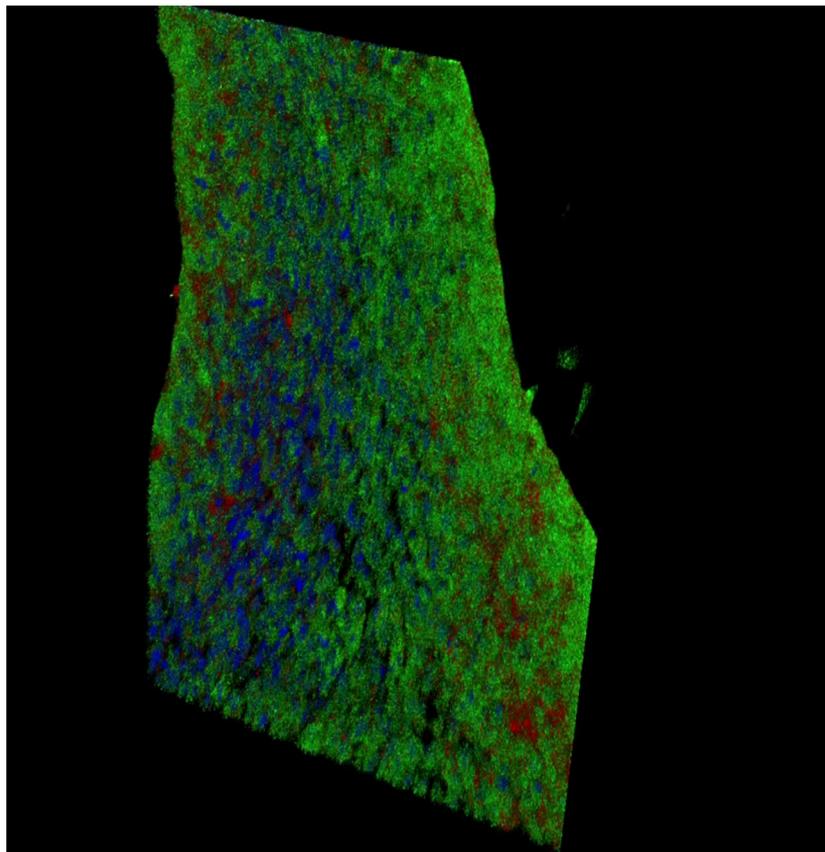


ORTHOGONAL PROJECTION



Confocal microscopy MFI quantification of FVIII expression

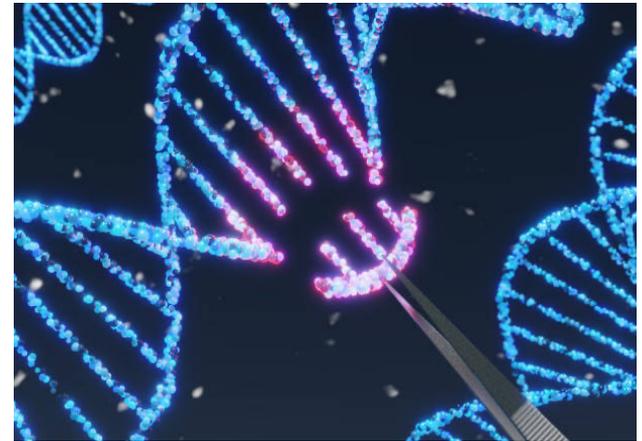




- ❖ Generation of **patient-specific iPSCs** from *Haemophilia A* individuals carrying different *F8* gene variants, and their *in vitro* differentiation into LSECs and HLCs.
- ❖ **CRISPR-Cas9 mediated gene correction** has demonstrated that specific pathogenic variants can be effectively **repaired**, resulting in restoration of FVIII expression.



- ❖ **LSECs are a useful *ex vivo* model** for evaluating FVIII restoration in patients with *Haemophilia A*.



- ❖ Our preliminary immunofluorescence data indicate FVIII expression levels, which will need to be further validated by **real-time PCR** and **western blot**.



- ❖ The successful reprogramming and differentiation protocols demonstrate the feasibility of creating an *in vitro (EX VIVO)* model for *Haemophilia A* patients.



- ❖ Ultimate goal of translating this technology into *in vivo* minimally invasive and personalized therapies.

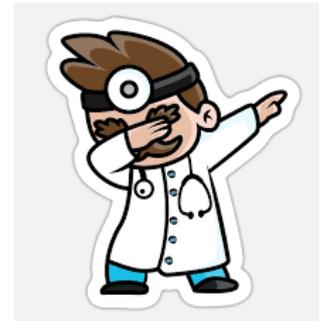


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