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Clearance receptor CLEC4M regulates FV levels

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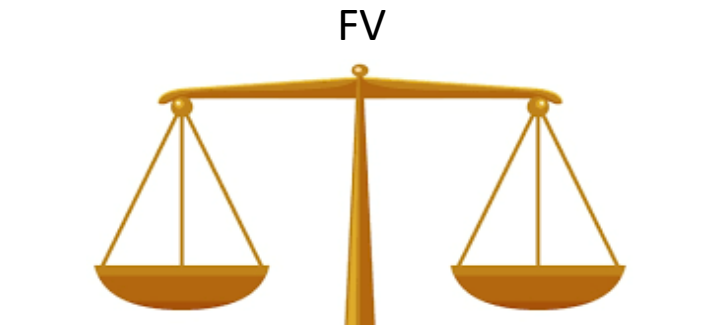
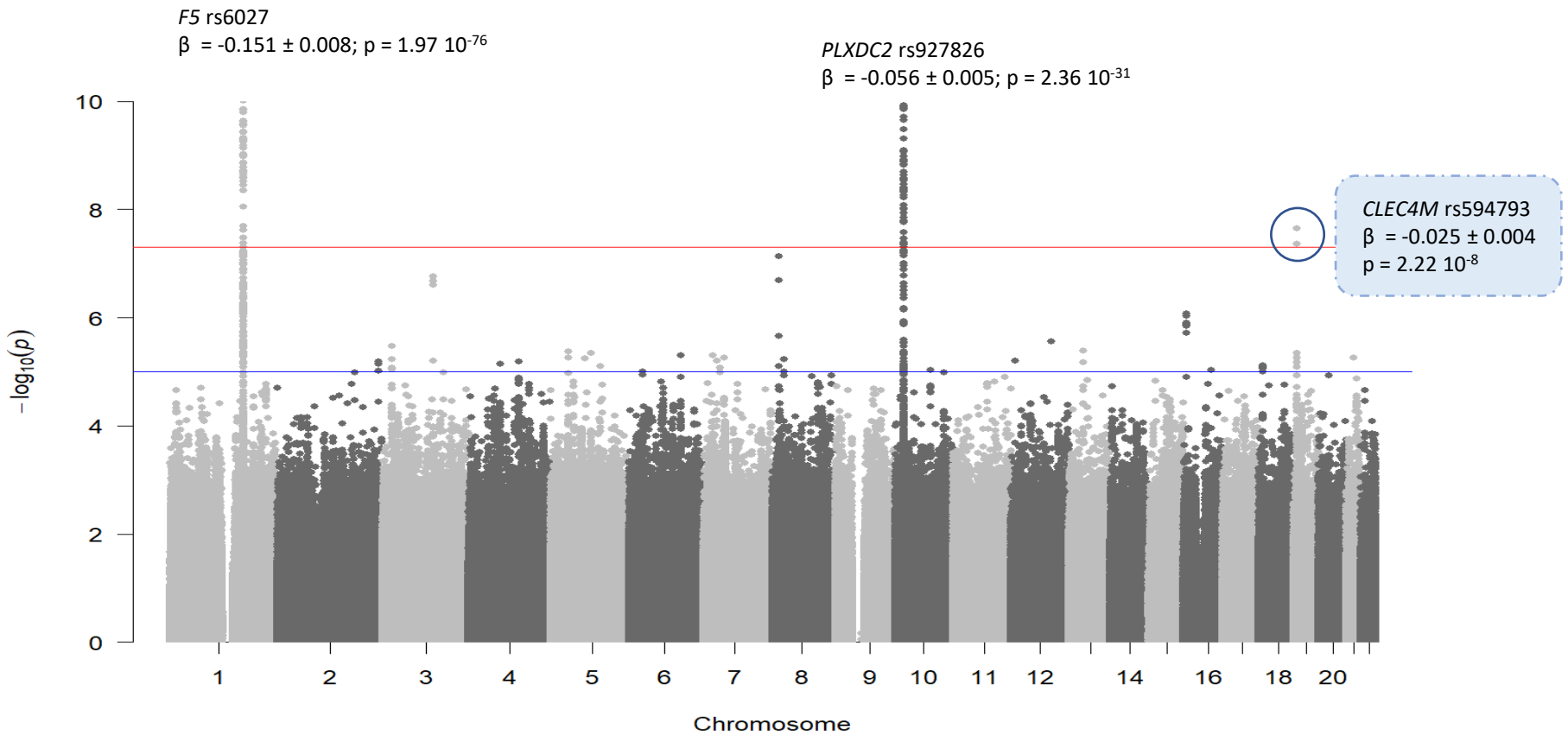
Factor V in coagulation

- *F5* gene on chromosome 1
- 330 kDa protein
- Produced in hepatocytes
- Circulates at ~25 nM in blood



Plasma FV levels associated with both venous thrombosis and bleeding

Genetic determinants of FV plasma levels (GWAS)



CLEC4M (rs594793) is associated with plasma FV levels

Courtesy: Pierre-Emmanuel Morange and David-Alexandre Trégouët (ISTH London 2022)

CLEC4M

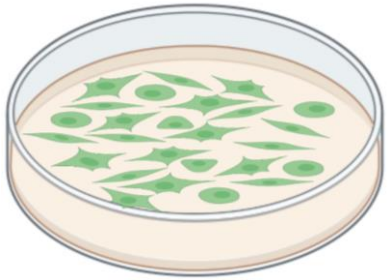
- C-type lectin
- Expressed in liver sinusoidal endothelial cells
- Pathogen recognition receptor (HIV, SARS-CoV)
- **Clearance receptor**: VWF and FVIII (Rydz *et al*; Blood, 2013, Swystun *et al;* JTH, 2019)

Hypothesis

CLEC4M acts as a clearance receptor for FV

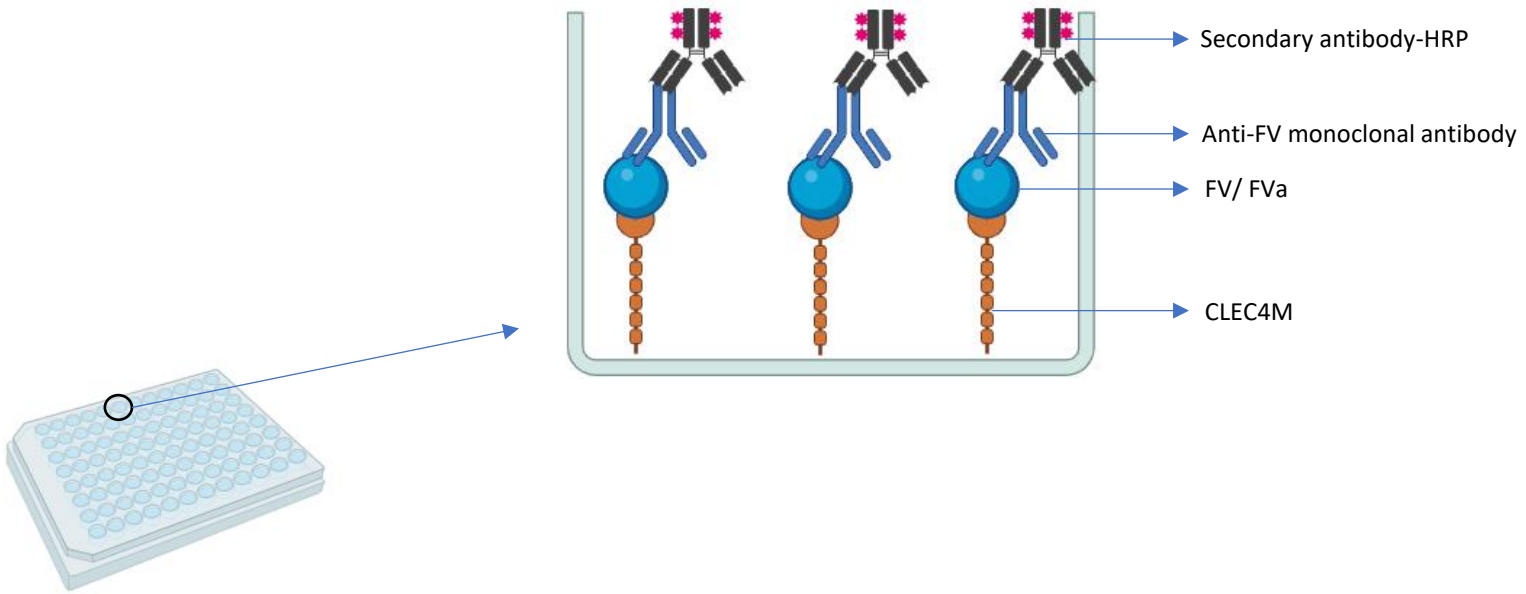
Methods to study CLEC4M-FV interaction

1. Solid-phase assays to determine FV binding to CLEC4M

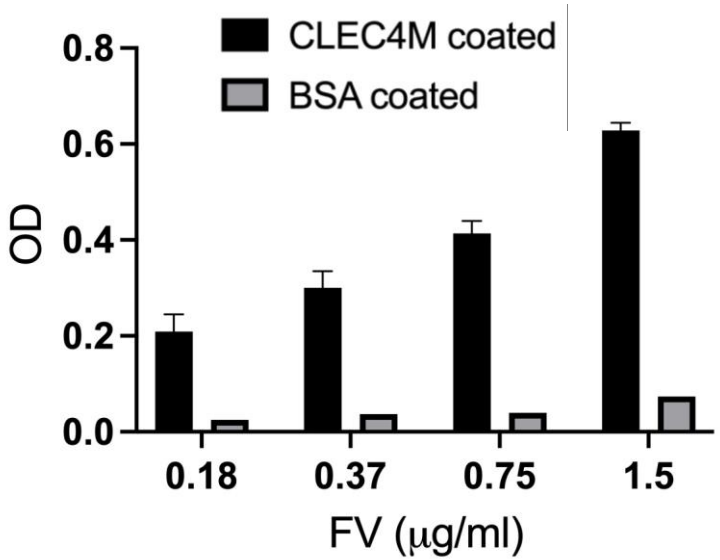


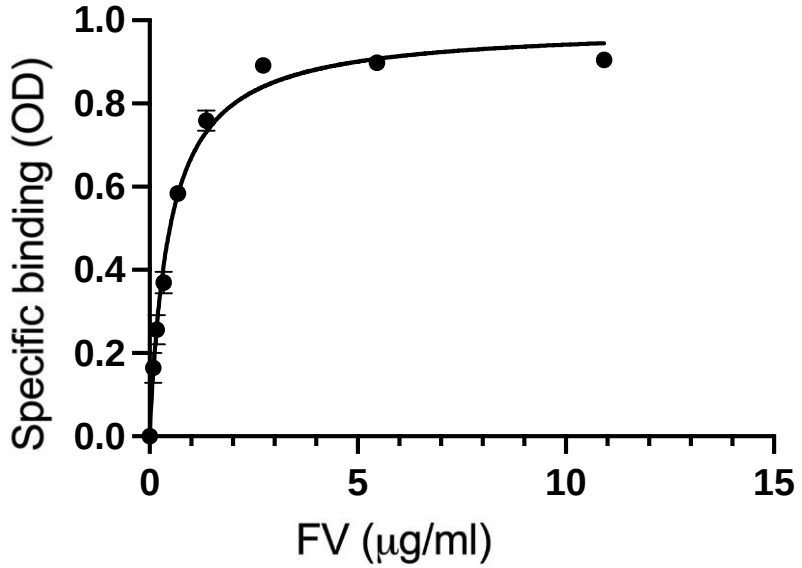
2. Cell models to investigate binding and internalization of FV by CLEC4M

1. Solid-phase assays



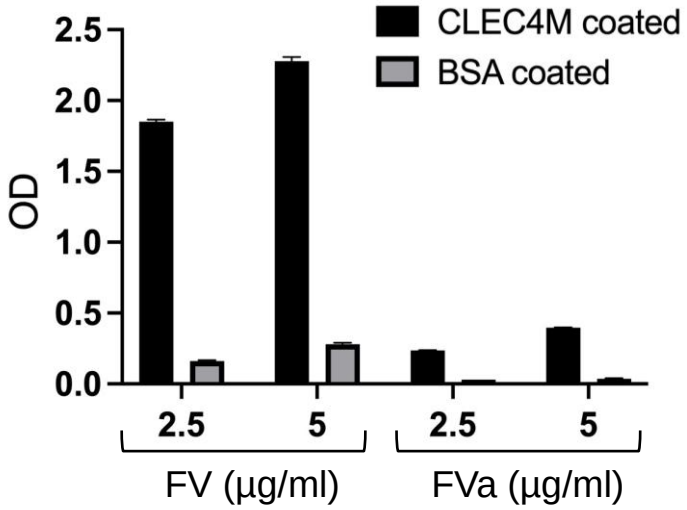
Results: Solid-phase assays



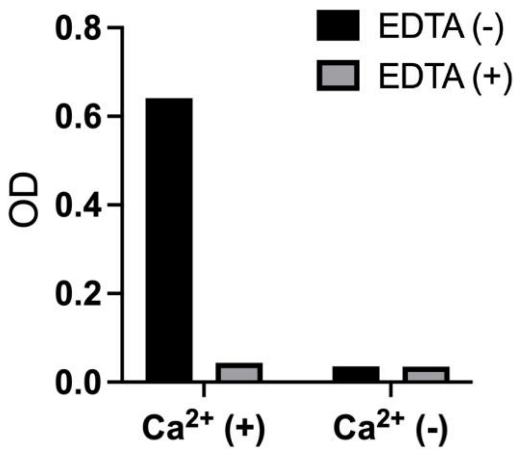


- FV binds to CLEC4M in a dose-dependent manner with a K_d of 0.47 µg/ml **(1.4 nM)**

Results: Solid-phase assays

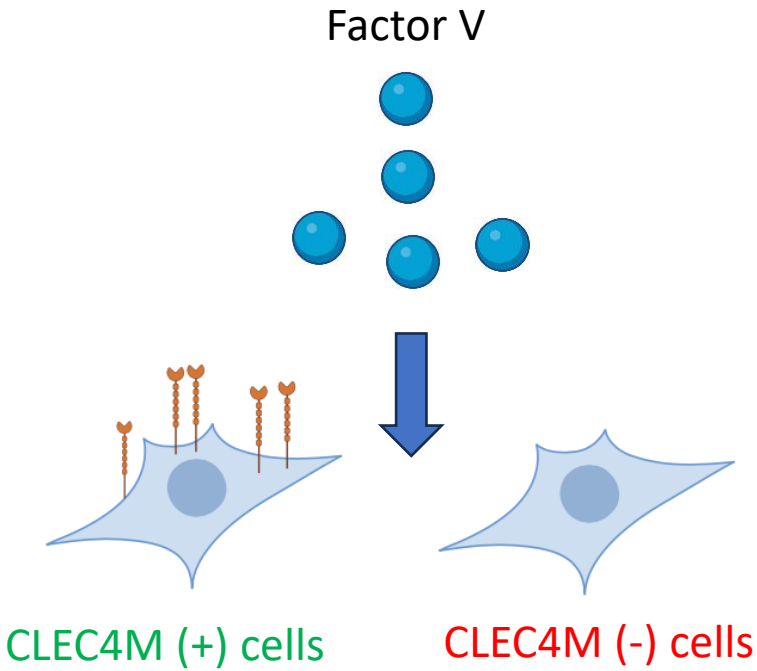


- FV binds to CLEC4M with **higher affinity** than FVa



- FV binding to CLEC4M is **Ca^{2+}-dependent**

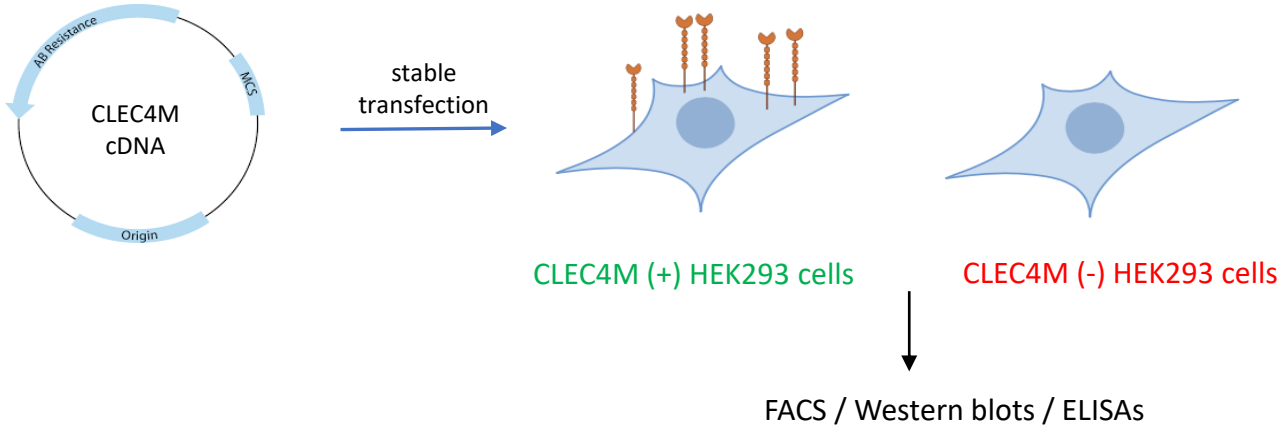
2. FV binding and internalization by CLEC4M



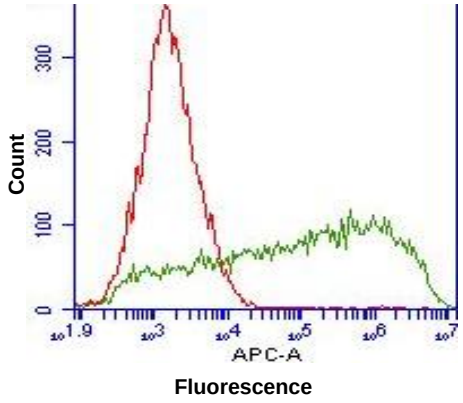
CLEC4M stable expression by HEK293 cells

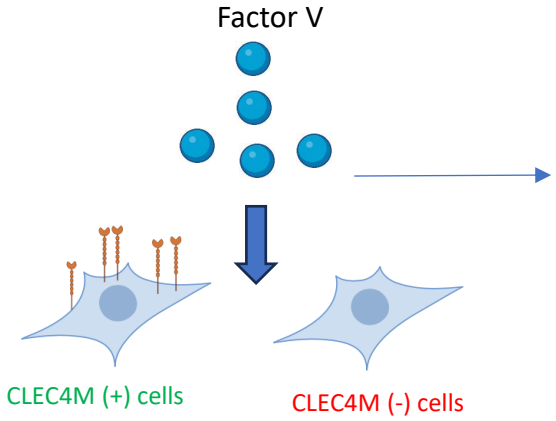
- Liver sinusoidal endothelial cells rapidly lose their phenotype in culture
- Commercial primary liver sinusoidal endothelial cells do not express CLEC4M

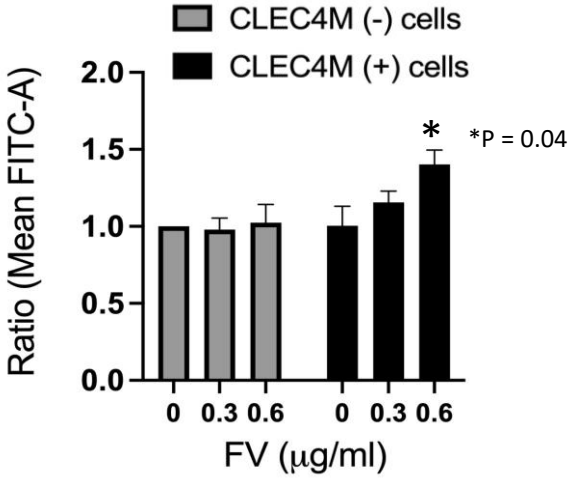
=> HEK293 cells stably expressing CLEC4M were generated



CLEC4M stable cell line and FV surface binding

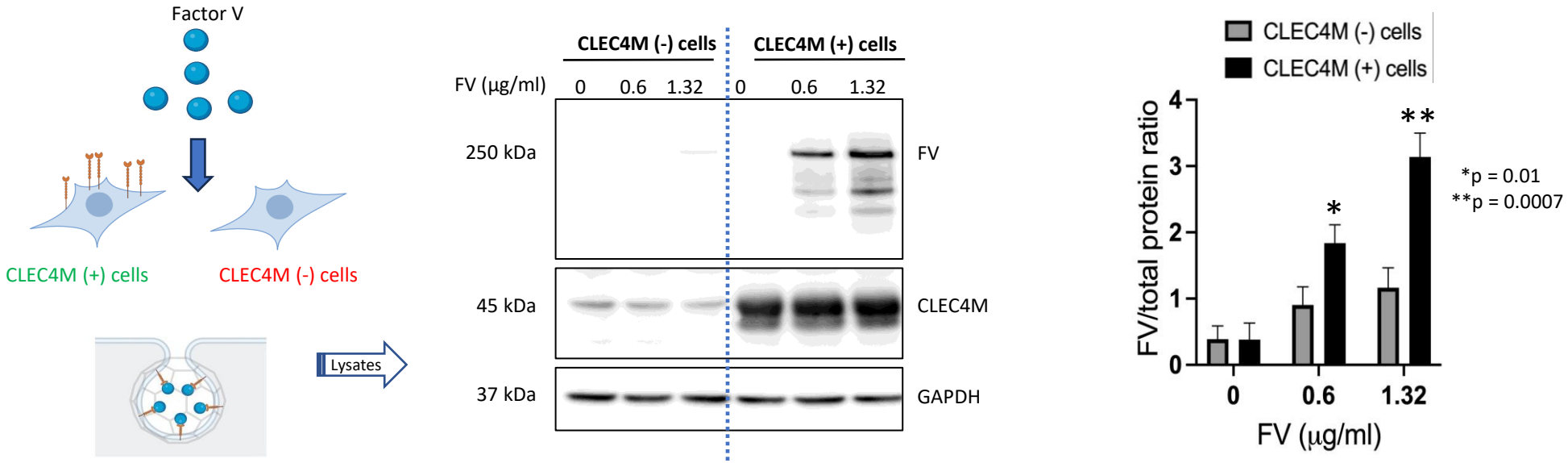






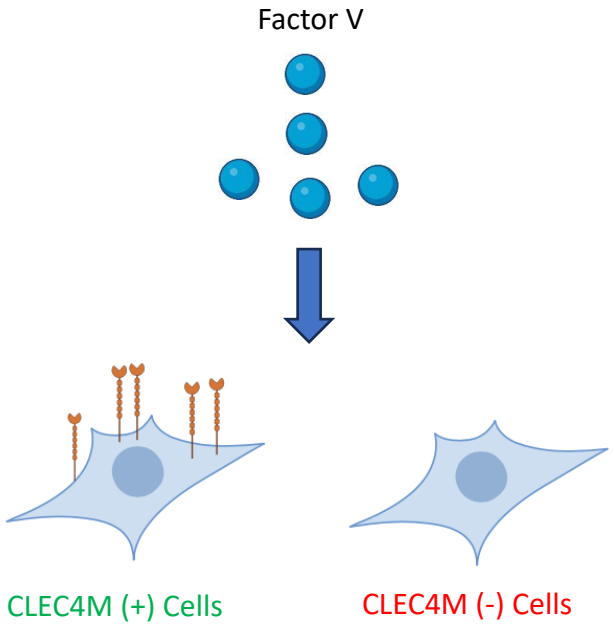
- Stable expression of CLEC4M receptors
- CLEC4M receptors **enhance FV binding** to the cell surface

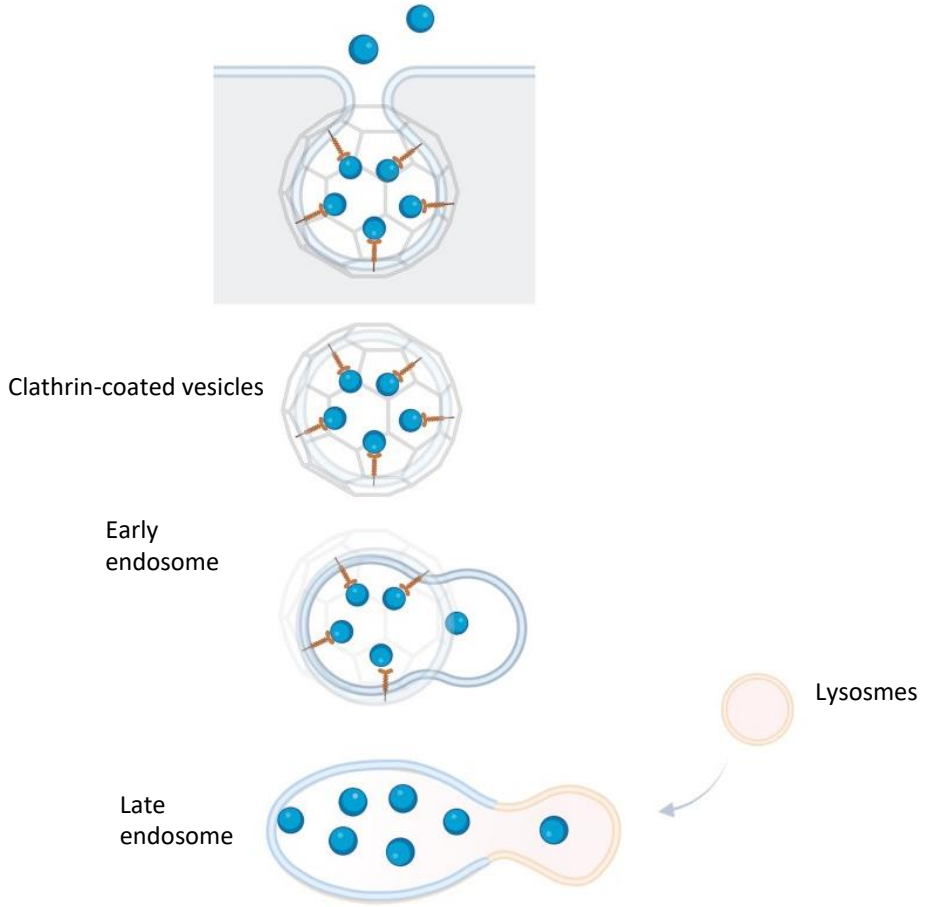
FV binding/internalization by CLEC4M



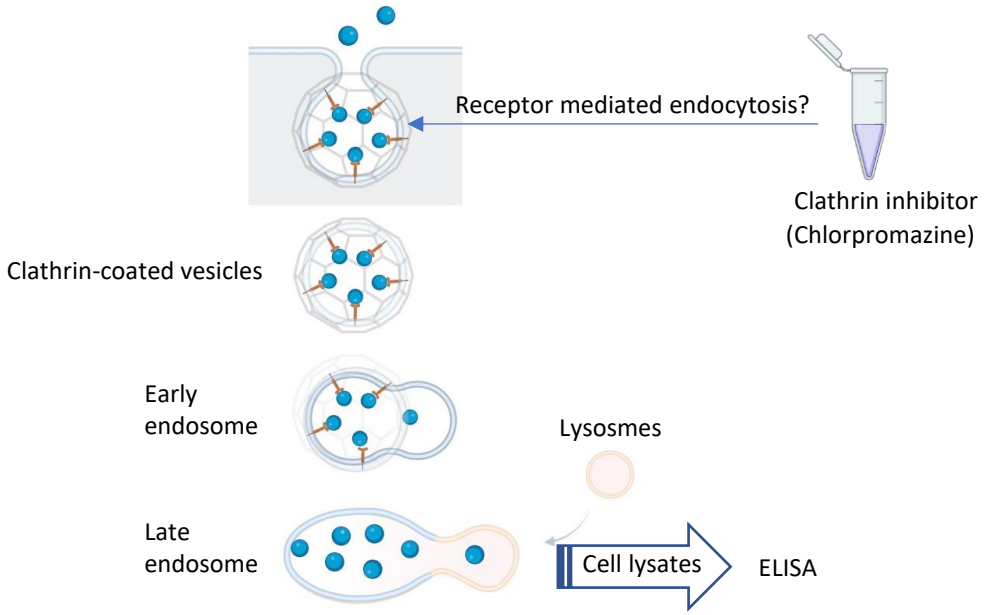
- **More FV detected** (~2x) in **CLEC4M (+) cells** as compared to CLEC4M (-) cells

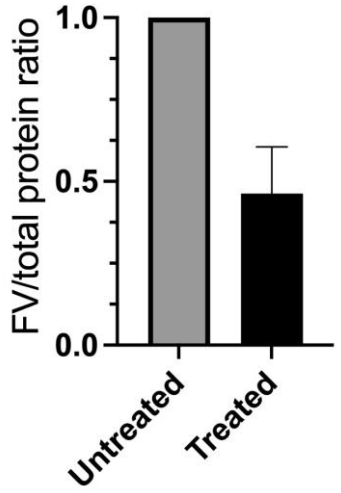
FV internalization by CLEC4M





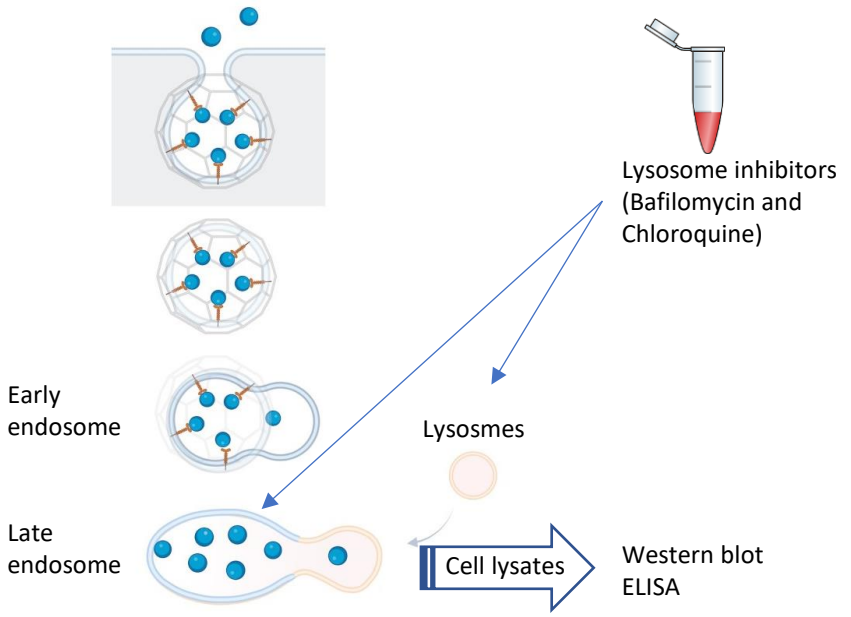
FV internalization by CLEC4M

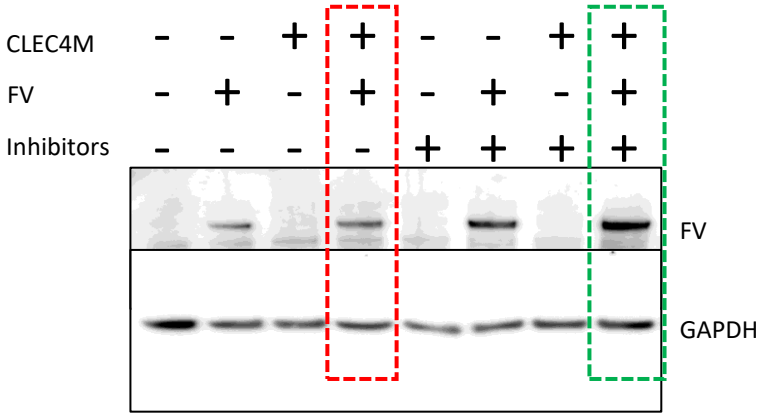


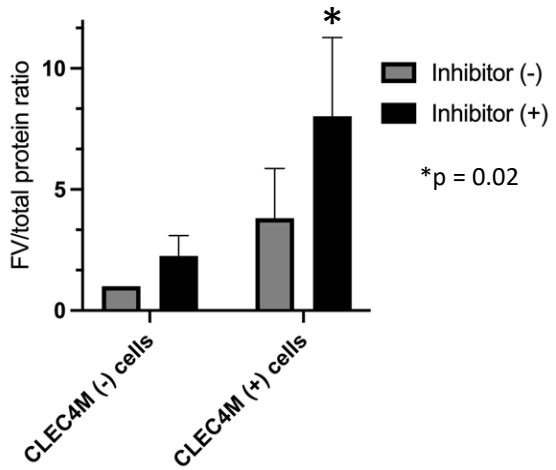


50 % less FV discovered in lysates of treated cells

FV internalization by CLEC4M







- Lysosomal inhibitors increased FV detection

Regulation of FV levels

Regulation by production

Regulation by clearance

Conclusions

- FV and FVa bind to CLEC4M with different affinities
- CLEC4M facilitates internalization of FV, which is partially trafficked through clathrin-coated pits into the cells and then degraded in lysosomes
- Our findings provide new insights into FV biology and may suggest novel therapeutic strategies for FV-related disorders

Acknowledgements

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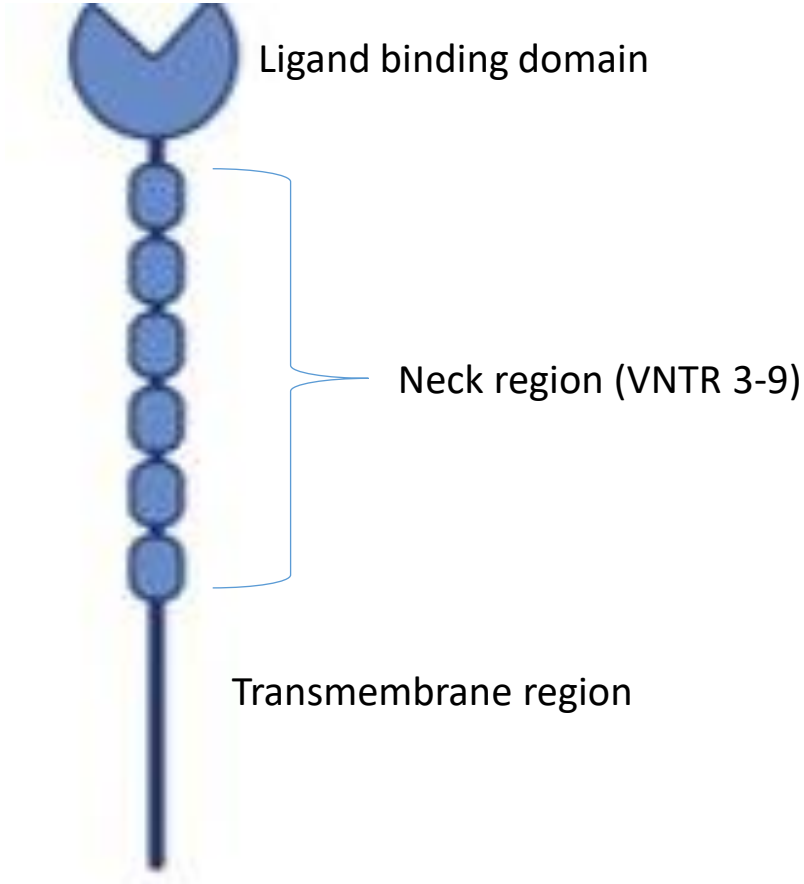
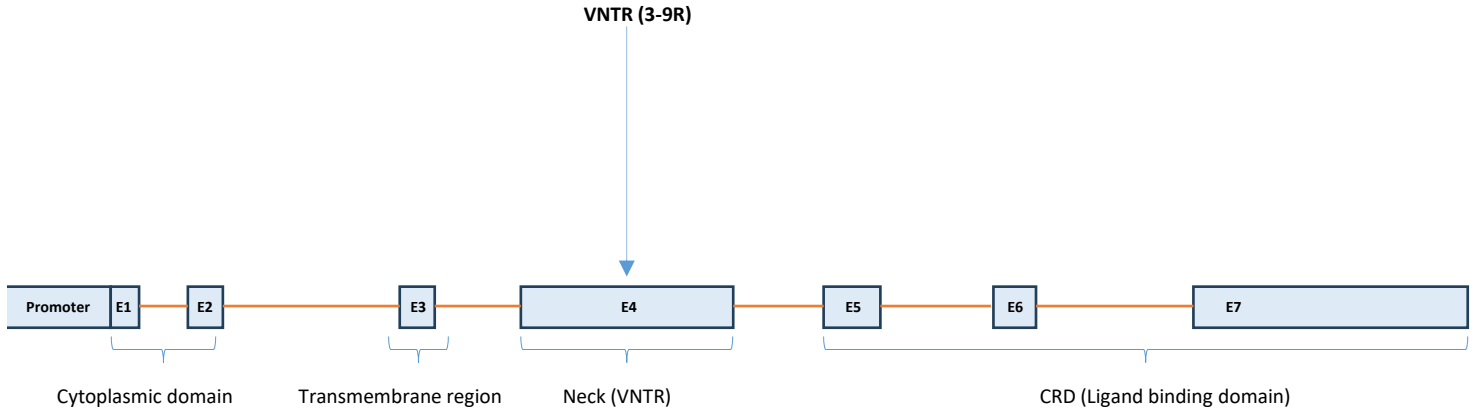
Investigators of FV GWAS

CLEC4M

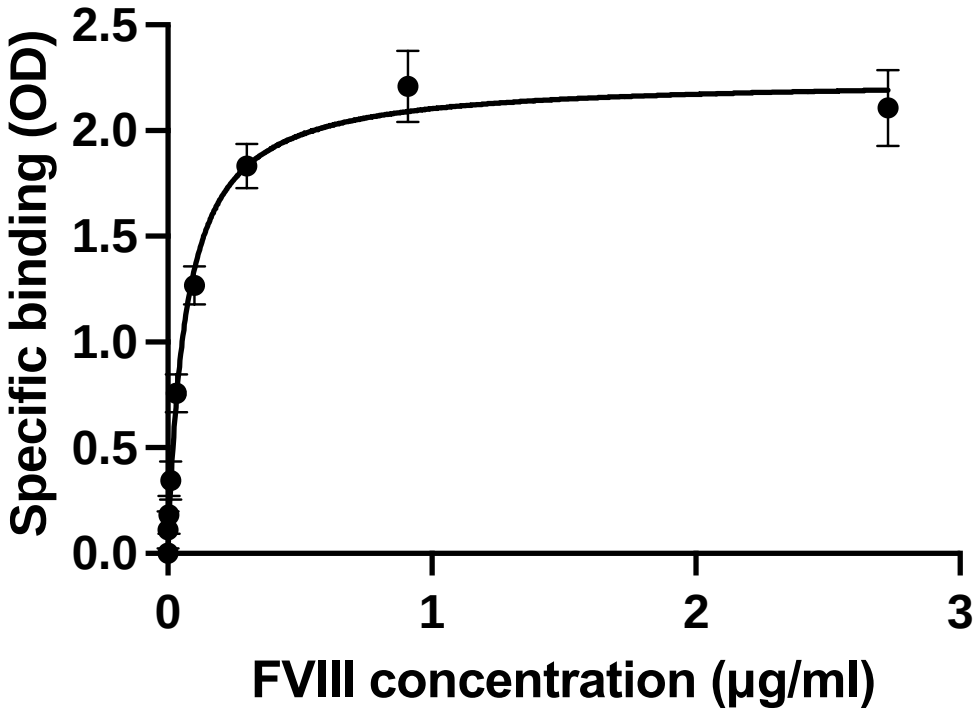


CLEC4M protein

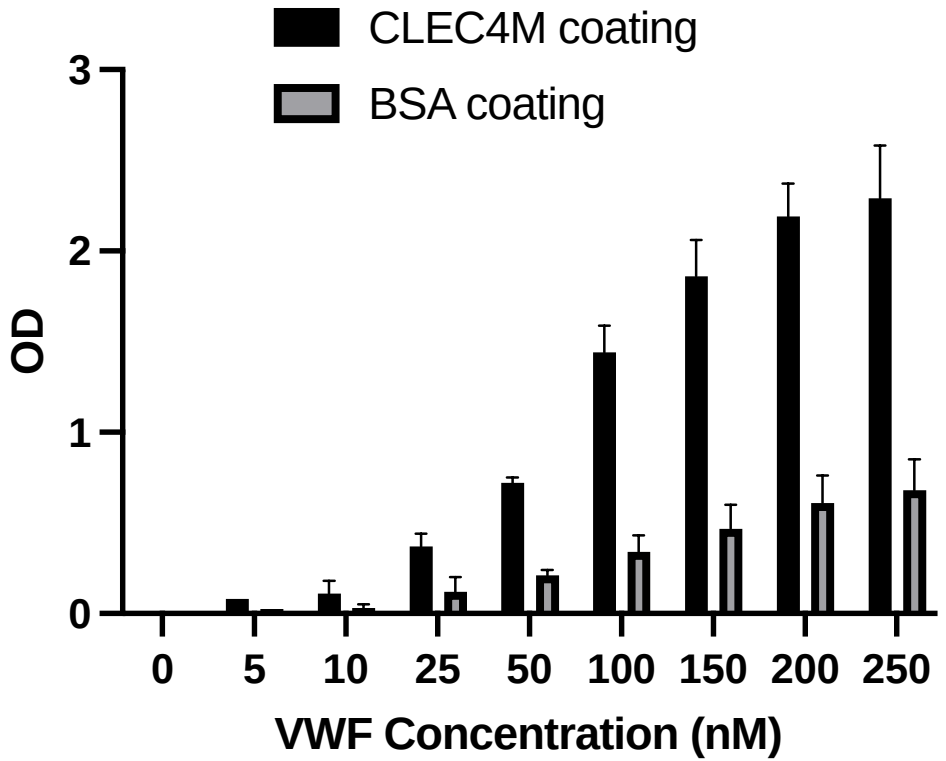


CLEC4M gene

FVIII and VWF binding to CLEC4M



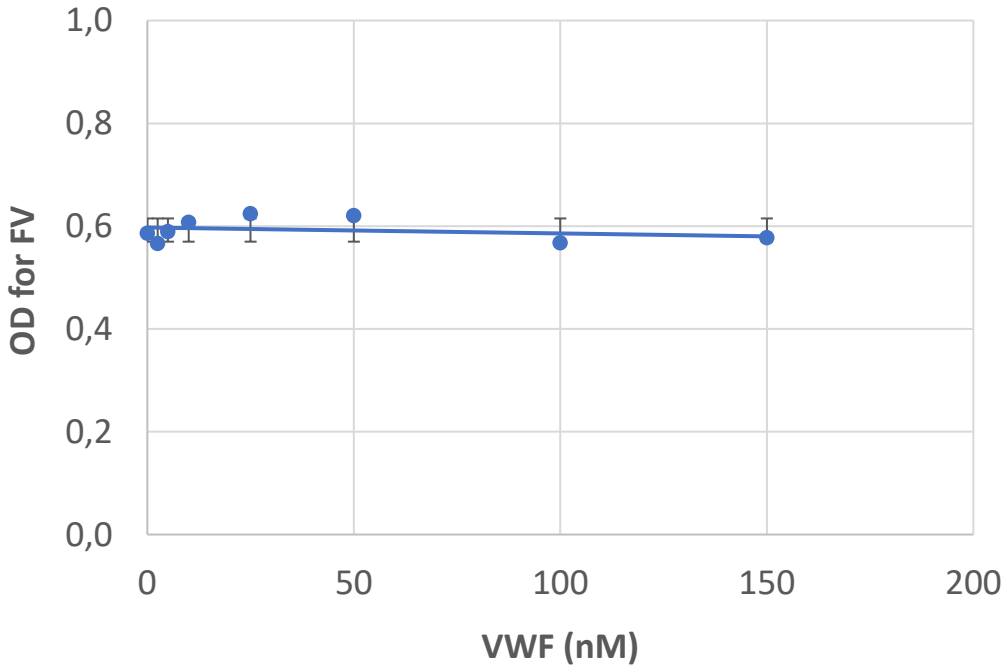
Binding of FVIII **(Elocta)** to immobilized CLEC4M.
K_d = 0.06 µg/ml or 0.2 nM



Binding of VWF **(Haemate P**, contains FVIII**)**
to immobilized CLEC4M.
K_d = above physiological plasma VWF concentration

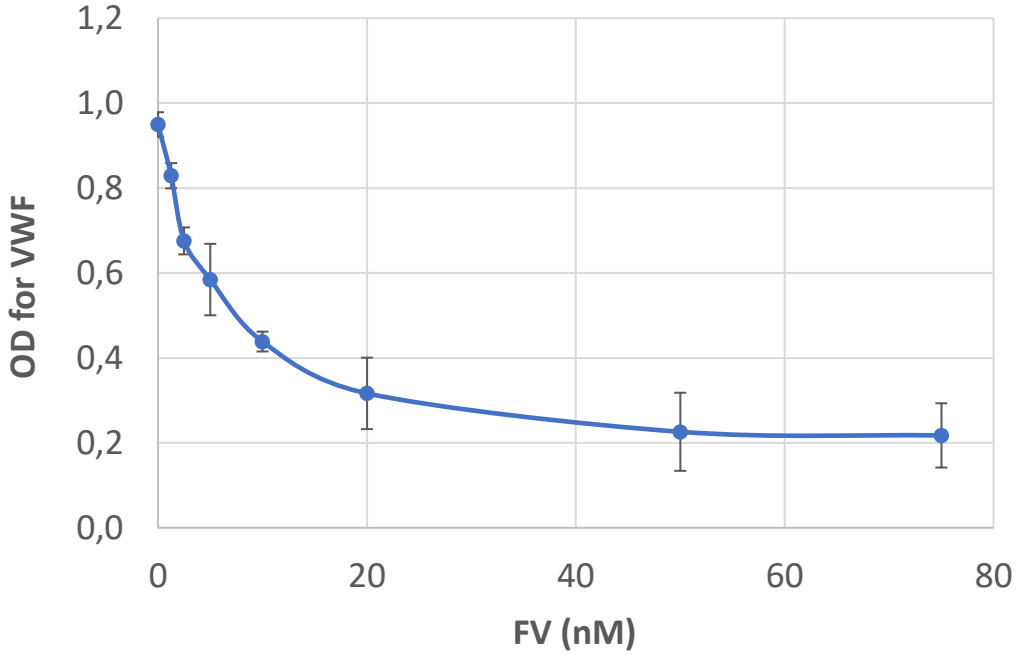
FV and VWF competition to CLEC4M

Binding of 2.5 nM FV to CLEC4M
in the presence of 0-150 nM VWF



No inhibition of FV binding by VWF

Binding of 50 nM VWF to CLEC4M
in the presence of 0-75 nM FV



Inhibition of VWF binding by FV