Supplemental Methods

Linkage Disequilibrium (LD) Analysis.

To determine the LD between CLEC4M VNTR alleles and the rs868875, we generated 6 genotype variables, each indicating the presence of each of 5 VNTR alleles. LD analysis between CLEC4M and its homologue, DC-SIGN (or CD209) was performed using the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/index.html.en, release #28) for the CEU European population and Haploview (Version 4.2). (Barrett et al. Bioinformatics. 2005)

Cell culture, transfection, stable cell lines

The CLEC4M -7 allele cDNA (IMAGE, Bethesda, MD, USA) was cloned into the pCI-neo (Promega, Madison, WI, USA). Expression vectors for the 4, 6 and 9 VNTR alleles were generated by site directed mutagenesis using the QuikChange II Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA, USA). CLEC4M -7, -4, -6, and -9 VNTR expression vectors were transfected into HEK293 11654 cells using lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were maintained in αMEM supplemented with 10% FBS (Gibco, Life Technologies, Carlsbad, CA, USA), 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine, 250 ng/mL fungizone, 100 µg/mL hygromycin. Stable cell lines were created with CLEC4M (VNTR 7) and pCIneo (control) vectors through selection with geneticin (500 µg/ml). Flow cytometry using nti-hDC-SIGNR FAB162P (R&D) or IgG2B isotype Control IC0041P (R&D) antibodies regularly demonstrated > 90 % of stable cells expressed CLEC4M.

ELISA quantification of VWF in cell lysate

293 cells expressing CLEC4M or pCIneo (control) were grown on plates coated with poly-L-lysine (100 µg/ml) for 1.5 hours, and washed twice with HBSS. Cells were exposed to Humate-P (CSL Behring, King of Prussia, PA, USA) in binding buffer (10 mM HEPES pH4, 135 mM NaCl, 10 mM KCl, 5 mM $CaCl_2$, 2 mM $MgSO_4$). For inhibition studies, cells were preincubated with mannan (100 mg/mL) (Sigma Aldrich, St. Louis, MO, USA) for 30 minutes. Cells were washed 3x in ice cold PBS and lysed in 100 mM potassium phosphate, 0.2% triton X-100 and 500 uM dTT. VWF levels in cell lysates were quantified by ELISA using rabbit anti-human VWF antibodies A0082 and P0226 (Dako, Glostrup, Denmark) and normalized to total protein concentration as determined by a BioRad (Hercules, CA, USA) protein assay.

Immunofluorescence

Cells were grown on glass coverslips treated with poly-L-lysine, washed with HBSS and exposed to Humate P in binding buffer. Cells were washed and fixed with BD cytofix/cytoperm (BD, Franklin Lakes, NJ, USA) for 20 minutes. Cells were permeabilized with PBS+1% Triton X-100 for 10 minutes on ice, washed, and blocked with Dako protein block (Dako) for 20 minutes. Cells were washed, and exposed to anti-CLEC4M antibody 120604 IgG2B (R&D, Minneapolis, MN USA), anti-VWF (Dako), early endosomal antigen 1 (Santa Cruz, Santa Cruz, CA, USA) or matched isotype control antibodies overnight. Cells were washed, and exposed to appropriate secondary

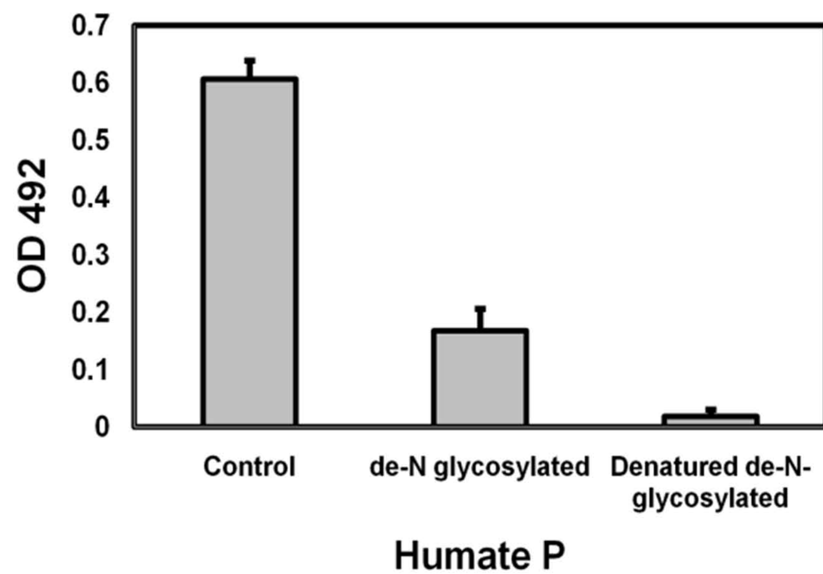
antibodies for 1 hour in the dark. Cells were washed and incubated with DAPI (Sigma Aldrich, 0.8 μg/ml) for 10 minutes, mounted on slides, and imaged with a Quorum Wave FX Spinning Disc confocal microscope and Hamamastsu Orca high resolution camera. Images were analyzed using ImageJ software (NIH).

Deglycosylation of VWF

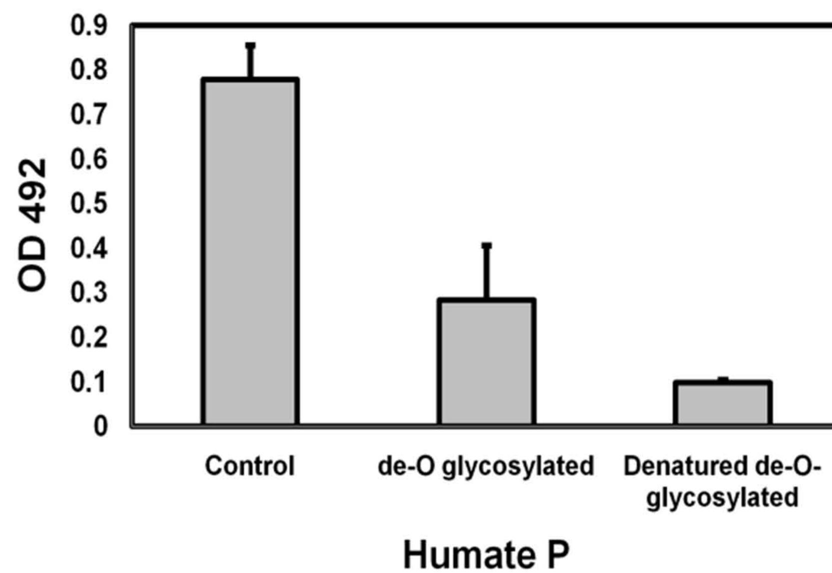
Humate-P was de-N- and de-O-glycosylated under non-denaturing conditions as previously reported (McKinnon et al. Blood. 2008; McGrath et al. Blood. 2010). De-N and de-O Humate-P was confirmed with a concanavalin A (Vector Labs, Burlingame, CA, USA) and peanut agglutinin binding assays as previously described (McKinnon et al. Blood. 2008; van Schooten et al. Blood. 2007.)

Supplement. (A) Binding of concanavalin A-biotin to de-N-glycosylated Humate-P. (B) Binding of peanut agglutinin-biotin to de-O-glycosylated Humate-P. (C) Binding of rhADAMTS13 to CLEC4M and DC-SIGN. (D) Amount of VWF in the cell lysate of CLEC4M variant transfected cells relative to expression of CLEC4M. (E) Linkage disequilibrium between CLEC4M (CD299) and its homologue, DC-SIGN (CD209). (F) Plasma Factor X levels in CLEC4M transfected mice relative to control mice.

Supplement A



Supplement B



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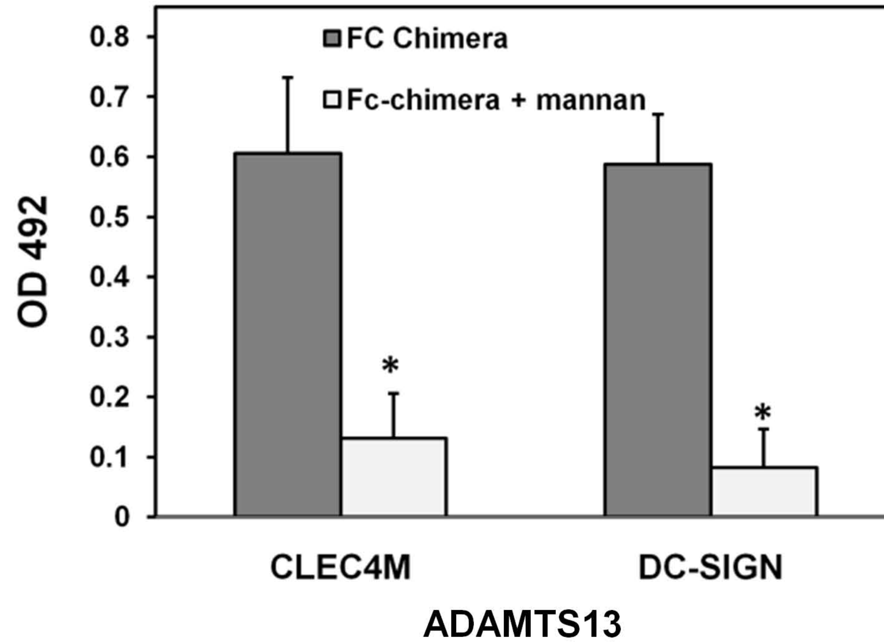

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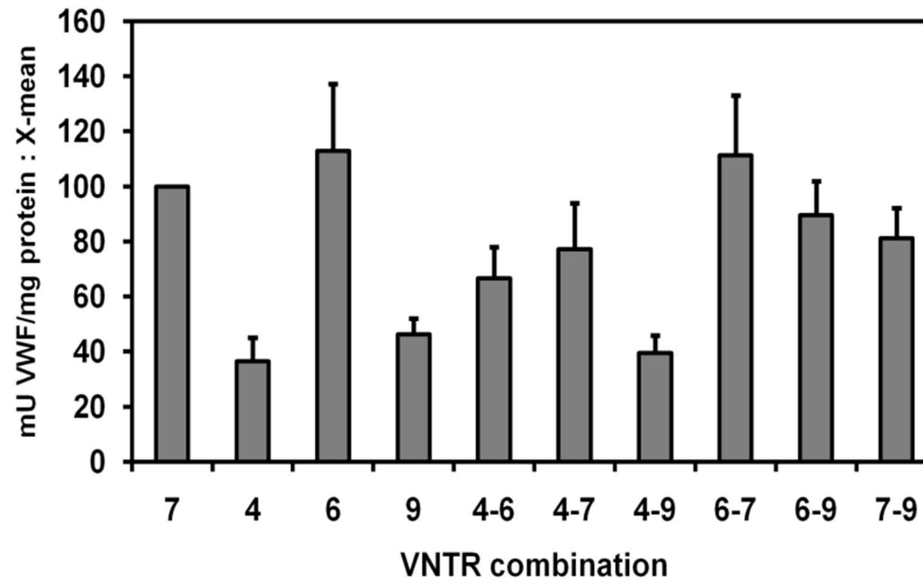

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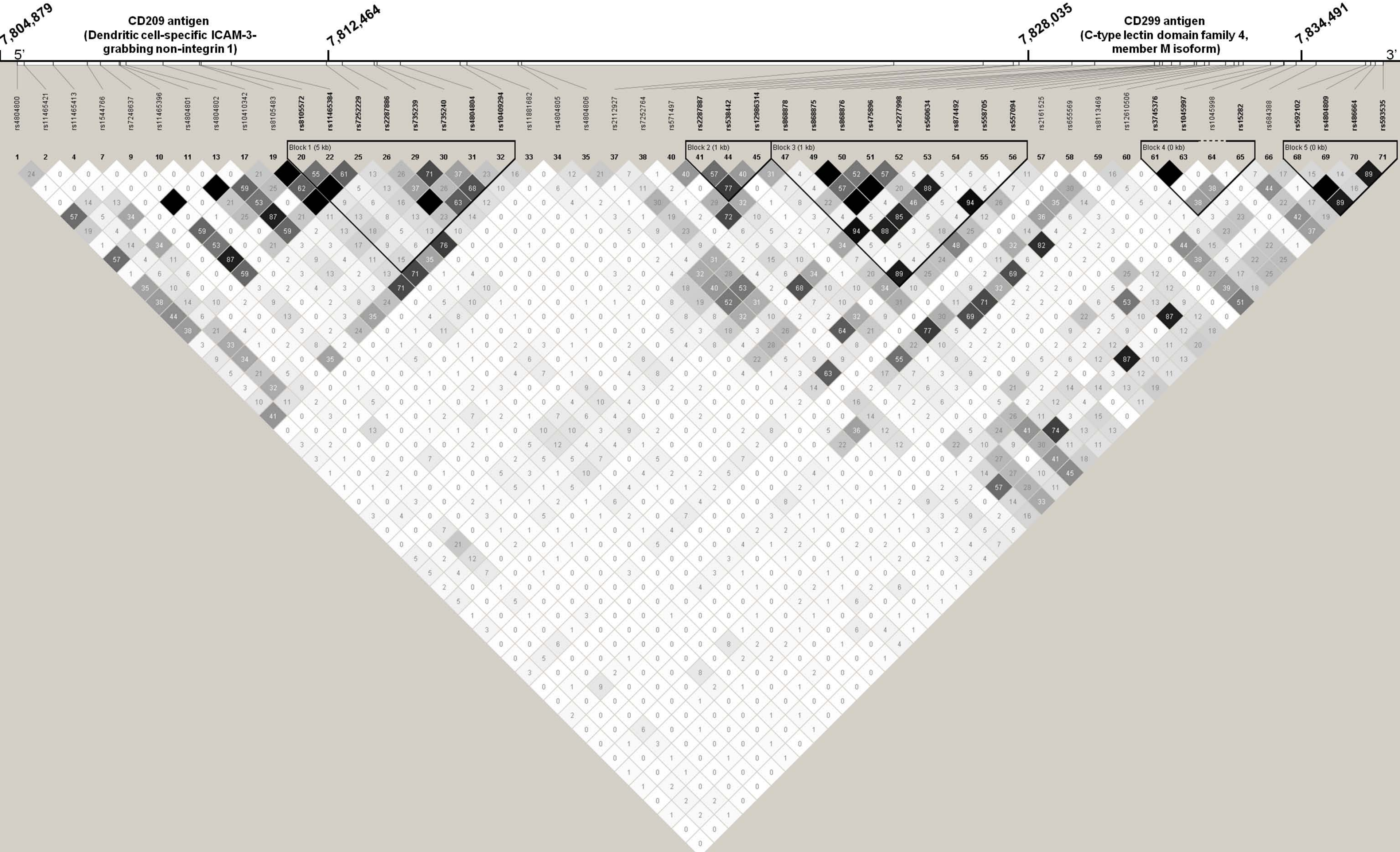

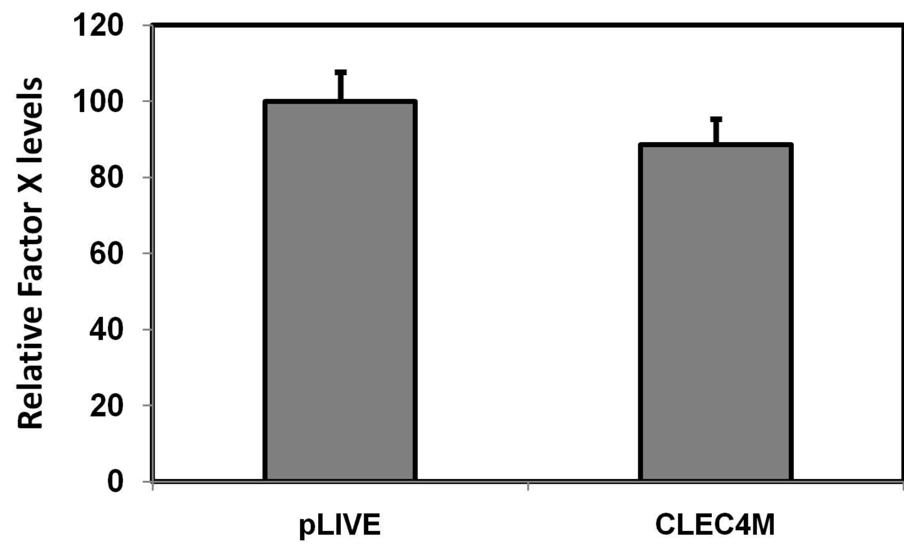

Relative Factor X levels
120
100
80
60
40
20
0
pLIVE
CLEC4M