

von Willebrand factor binds to the surface of dendritic cells and modulates peptide presentation of factor VIII

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Supplementary Methods

Generation of immature dendritic cells

Blood was drawn from healthy HLA class II-typed volunteers after giving informed consent in accordance with Dutch regulations and after approval from the Sanquin Ethical Advisory Board in accordance with the Declaration of Helsinki. Depending on the amount of cells required, peripheral blood mononuclear cells were collected using Ficoll-Paque Plus from which monocytes were isolated using CD14⁺ magnetic beads according to manufacturer's protocol or, when more cells were needed, monocytes were isolated from monocyte apheresis products using centrifugal elutriation.¹ Monocytes were subsequently differentiated into immature dendritic cells (iDCs) by culturing them in Cellgro medium supplemented with 800 U/mL IL-4 and 1000 U/mL GM-CSF for 5 days.

Purification of FVIII and VWF

FVIII and VWF were purified as described previously.² Briefly, HEK293 stable cell lines expressing B-domain deleted FVIII or VWF were cultured in DMEM/F12 supplemented with 10% fetal calf serum. Proteins were purified from concentrated medium using immunoaffinity chromatography using Sepharose coupled with human monoclonal antibody VK34 or murine monoclonal antibody CLB-RAg20 for FVIII and VWF respectively. FVIII was purified further using Q sepharose ion-exchange. FVIII was stored in 50 mM Tris-HCl pH 7.4, 800 mM NaCl, 5 mM CaCl₂ and 50% glycerol and VWF was stored in 20 mM HEPES pH 7.4, 150 mM NaCl and 50% glycerol at -20°C until further use.

Purification of HLA-DR-bound peptides

HLA-DR/peptide complexes were purified as described previously.³⁻⁵ Five million monocyte-derived iDCs were incubated at 37°C with 25 nM FVIII, 250 nM VWF or FVIII/VWF complex at a ratio 1:5 or 1:10 obtained as described above. After 5 hours of incubation, cells were

matured overnight by addition of 1 µg/mL of LPS in the presence of 1% pooled human serum. Subsequently, cells were lysed (10 mM Tris-HCl pH 7.0; 4% NP40, complete protease inhibitor cocktail) and HLA-DR/peptide complexes were purified employing an anti MHC class II L243-coupled CNBr Sepharose 4B column that was prewashed with 10 mM Tris-HCl pH 7.0, protease inhibitor cocktail. After this incubation the column was washed 3 times with 10 mM Tris-HCl pH 7.0 containing protease inhibitors and subsequently 5 times with 10 mM Tris-HCl pH 7.0 only. Next peptides were eluted employing 10% acetic acid at 70 °C for 15 minutes. C18 Stage-tips equilibrated with 100% acetonitrile and washed with 1% formic acid in H₂O were prepared. After loading of the sample, C18 Sepharose was washed twice with 1% formic acid and 1% formic acid supplemented with 5% acetonitrile. Peptides were then eluted with 1% formic acid in 50% acetonitrile.

Mass spectrometry analysis of purified peptides

Mass spectrometry was used for peptide analysis as previously described.^{3,5} Eluted peptides were separated using columns filled with 1.9 µm C18 particles (New Objective type FS360-75-8-N-5-C20, Inc., Woburn, MA, USA) at a flow rate of 300 nl/min with a gradient from 0% to 80% (v/v) acetonitrile in 0.1 M acetic acid. Column eluate was directly sprayed into the Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific Inc., Bremen, Germany) using nanoelectrospray source with a spray voltage of 2.15 kV. Higher-energy Collisional Dissociation (HCD) was performed in top-speed mode with 3 second cycles (400-1500 m/z, resolving power 120.000). The mass spectrometer was calibrated on a regular basis as recommended by the manufacturer in order to ensure a high mass accuracy.

Characterization of peptides

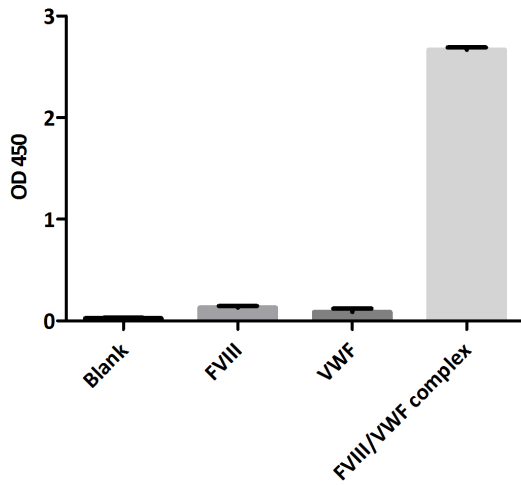
Peptides were identified using Proteome Discoverer 1.4 (Thermo Scientific, Bremen, Germany). Raw Xcalibur data files were screened against the UniprotKB non-redundant protein 25.H_sapiens.fase database with a mass deviation of 20 ppm, a fragment mass tolerance of 0.8 Da and a false positive discovery rate of 95%. All identified peptides were grouped and aligned for each donor after which core peptides were predicted using NetMHCII 2.2.²³ The core peptide with the highest predicted binding affinity was used to indicate the location of that specific group of identified peptides.

Statistical analysis

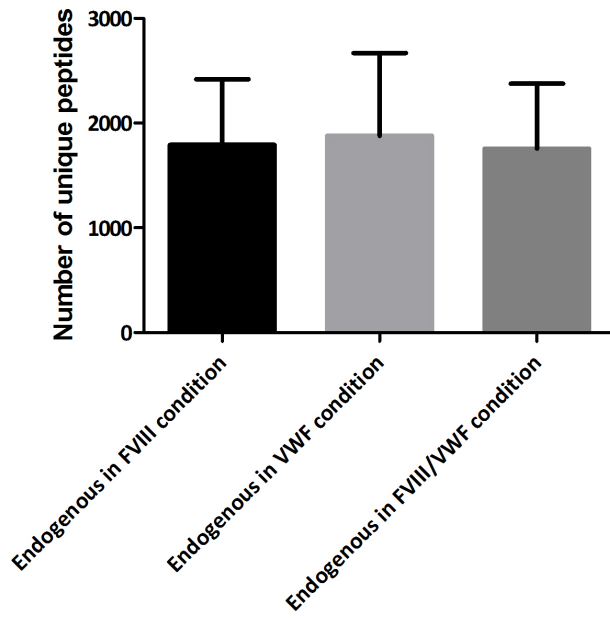
Statistical analysis was performed using Graphpad Prism 6 (GraphPad Software, Inc. La Jolla, CA, USA). Significance between groups of measured mean fluorescent intensities was assessed using an unpaired student's t-test or a one-way ANOVA with Bonferroni's multiple comparison correction.

1. Strasser EF, Eckstein R. Optimization of leukocyte collection and monocyte isolation for dendritic cell culture. *Transfus Med Rev.*2010;24(2):130–139.
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Supplementary results



Supplementary figure S1. FVIII/VWF complex detection using ELISA. FVIII (25 nM) and VWF (250 nM) were incubated separately or in complex for 30 minutes at 37°C in Cellgro medium. Samples were 100x pre diluted in conjugate buffer (50mM Tris, 150 mM NaCl, 0.2% Tween-20) containing 0.22µg/mL DAKO polyclonal anti VWF PO labeled antibody. Subsequently, samples were incubated on a 2.5µg/mL CLB-CAg9 pre-coated plate for 1 hour at 37°C to capture FVIII after which VWF was detected by peroxidase hydrolysis of TMB. A clear signal increase was observed in the complex condition, indicating stable interaction between FVIII and VWF in this condition.



Supplementary figure 2. Total number of unique peptides discovered. Raw mass spectrometry data was analyzed using Proteome discoverer which detects endogenous proteins. A comparable level of total number of peptides identified is shown, which indicates that similar levels of material was analyzed.

FVIII

FVIII/VWF

A1

Peptide	Predicted Affinity (nM)	Start	Stop	Length
YGEVGTLLIIFKNQASRPYNIYPHG		473	498	26
YGEVGTLLIIFKNQASRPYNIYPHG		473	493	21
-GEVGTLLIIFKNQASRPYNIYPHG		474	498	25
-GEVGTLLIIFKNQASRPYNIYPHG		474	493	20
-GEVGTLLIIFKNQASRPYNIYPHG		474	492	19
-EVGDTLLIIFKNQASRPYNIYPHG		475	498	24
-EVGDTLLIIFKNQASRPYNIYPHG		475	493	19
-EVGDTLLIIFKNQASRPYNIYPHG		475	491	17
-EVGDTLLIIFKNQASRPYNIYPHG		475	492	18
-VGDFTLLIIFKNQASRPYNIYPHG		476	493	18
-VGDFTLLIIFKNQASRPYNIYPHG		476	492	17
-GDTLLIIFKNQASRPYNIYPHG		477	492	16
-DFTLLIIFKNQASRPYNIYPHG		478	493	16
-DFTLLIIFKNQASRPYNIYPHG		478	492	15
-DFTLLIIFKNQASRPYNIYPHG		478	491	14
-DFTLLIIFKNQASRPYNIYPHG		478	494	17
-----LLIIFKNQA-----	52.90-63.53	480	488	9
-----LIIFKNQAS-----	34.82-43.56	481	489	9
-----IIFKNQASR-----	30.53-88.86	482	490	9
-----IFKNQASRP-----	32.86-40.53	483	491	9
-----FKNQASRPY-----	19.96-24.75	484	492	9

WTVTVEDGPTKSD		532	544	13
--VTVEDGPTK--	8929.91-10362.12	534	542	9

YSSSFVNMERDLASGLIG		551	568	18
YSSSFVNMERDLASGLIG		551	565	15
-----YSSSFVNMER-----	169-333.79	552	560	9
-----YSSSFVNMERDLASGLIG		552	565	14
-----YSSSFVNMERDLASGLIG		552	569	18
-----YSSSFVNMERDLASGLIG		552	565	14
-----YSSSFVNMERDLASGLIG		552	566	15
-----YSSSFVNMERDLASGLIG		552	568	17
-----SSSFVNMERDLASGLIG		553	565	13
-----FVNMERDLA-----	185.82-787.09	555	563	9
-----VNMERDLAS-----	359.63-964.58	556	564	9
-----MERDLASGL-----	182.05-195.49	558	566	9

RGNQIMSDKRNIVL		581	594	14
-----GNQIMSDKRNIVL		582	594	13
-----TMSDKRNVI-----	228.71-1595.83	585	593	9

TENIQRFLENFAGVQLEDPE--		607	626	20
TENIQRFLENFAGVQLEDPEFQ		607	628	22
TENIQRFLENFAGVQLEDPEFQ		608	628	21
-ENIQRFLENFAGVQLEDPE--		608	626	19
-ENIQRFLENFAGVQLEDPE--		608	624	17
-ENIQRFLENFAGVQLEDPE--		608	623	16
-ENIQRFLENFAGVQLEDPEF--		608	627	20
-NIQRFLENFAGVQLEDPEF--		609	626	18
-NIQRFLENFAGVQLEDPEFQ		609	628	20
-NIQRFLENFAGVQLEDPE--		609	624	16
-IQRFLENFAGVQLEDPE--		609	623	15
-IQRFLENFAGVQLEDPEF--		610	626	17
-----IQRFLENFA-----	104.95-447.54	610	618	9

SFQKTRHYFIAAVERLWDYGMSSSPHVLRN		1709	1739	29
SFQKTRHYFIAAVERLWDYGMSSSPHVL--		1709	1737	31
-----YFIAAVERL-----	30.3	1717	1725	9
-----YGMSSSPHV-----	19.94-85.66	1728	1736	9

DNIMVTFRNQASRPYSFYS		1788	1806	19
DNIMVTFRNQASRPYSF--		1788	1804	17
DNIMVTFRNQASRPYS--		1788	1803	16
-----ImVTFRNQA-----	85.33-118.23	1790	1798	9
-----FRNQASRPY-----	41.81-105.22	1794	1802	9

FKENVRFHAINGYIM		1931	1945	15
-----YRFHAINGY--	106.8-248.71	1935	1943	9

LYISQFIIMYSLDGKK--		2115	2130	16
-YISQFIIMYSLDGKKW		2116	2131	16
-YISQFIIMYSLDGKKW		2116	2130	15
-ISQFIImYSLDGKKW		2117	2131	15
-ISQFIIMYSLDGKKW		2117	2130	14
-ISQFIIMYSLDGKKW		2117	2131	15
-----FIIMYSLDG--	74.23-117.71	2120	2128	9
-----ImYSLDGKK--	207.7-525.75	2122	2130	9

A2

SVAKKHKPTWVHYTAAEEDWDYAPLVLPDDR		392	424	33
-----WDYAPLVLA-----	244.96-403.15	412	420	9

YGEVGTLLIIFKNQASRPYNIYPHG		473	493	21
YGEVGTLLIIFKNQASRPYNIYPHG		473	498	26
-GEVGTLLIIFKNQASRPYNIYPHG		474	493	20
-EVGDTLLIIFKNQASRPYNIYPHG		475	493	19
-EVGDTLLIIFKNQASRPYNIYPHG		475	492	18
-EVGDTLLIIFKNQASRPYNIYPHG		475	491	17
-VGDFTLLIIFKNQASRPYNIYPHG		476	492	17
-VGDFTLLIIFKNQASRPYNIYPHG		476	493	18
-VGDFTLLIIFKNQASRPYNIYPHG		476	491	16
-GDTLLIIFKNQASRPYNIYPHG		477	493	17
-DFTLLIIFKNQASRPYNIYPHG		478	493	16
-DFTLLIIFKNQASRPYNIYPHG		478	492	15
-DFTLLIIFKNQASRPYNIYPHG		478	491	14
-DFTLLIIFKNQASRPYNIYPHG		478	497	20
-----LLIIFKNQA-----	52.90-63.53	480	488	9
-----LIIFKNQAS-----	34.82-43.56	481	489	9
-----IIFKNQASR-----	30.53-88.86	482	490	9
-----IFKNQASRP-----	32.86-40.53	483	491	9
-----FKNQASRPY-----	19.96-24.75	484	492	9

YSSSFVNMERDLASGLIG		551	568	18
-----YSSSFVNMER-----	169-333.79	552	560	9
-----YSSSFVNMERDLASGLIG		552	568	17
-----YSSSFVNMERDLASGLIG		552	569	18
-----YSSSFVNMERDLASGLIG		552	565	14
-----YSSSFVNMERDLASGLIG		552	566	15
-----FVNMERDLA-----	185.82-787.09	555	563	9
-----MERDLASGL-----	182.05-195.49	558	566	9

ESVDQRGNQIMSDKRNIVL		576	594	19
-----RGNQIMSDKRNIVL		581	594	14
-----IMSDKRNVI-----	228.71-1595.83	585	593	9

TENIQRFLENFAGVQLEDPEFQ		607	628	22
TENIQRFLENFAGVQLEDPE--		607	626	20
-ENIQRFLENFAGVQLEDPE--		608	626	19
-ENIQRFLENFAGVQLEDPEFQ		608	628	21
-ENIQRFLENFAGVQLEDPE--		608	623	16
-ENIQRFLENFAGVQLEDPE--		608	624	17
-ENIQRFLENFAGVQLEDPE--		608	622	15
-NIQRFLENFAGVQLEDPE--		609	626	18
-NIQRFLENFAGVQLEDPEFQ		609	628	20
-NIQRFLENFAGVQLEDPE--		609	622	14
-IQRFLENFAGVQLEDPE--		610	626	17
-IQRFLENFAGVQLEDPEFQ		610	628	19
-----IQRFLENFA-----	104.95-447.54	610	618	9

FSGYTFKHKRMVYEDT		672	686	15
-----YTFKHKRMVY--	115.86-1979.21	675	683	9

SFQKTRHYFIAAVERLWDYGMSSSPHVLRN		1709	1739	31
-----YGMSSSPHV-----	19.94-85.66	1728	1736	9

VEDNIMVTFRNQASRPY--		1786	1802	17
--DNIMVTFRNQASRPYSF		1788	1804	17
--DNIMVTFRNQASRPYS--		1788	1803	16
-----IMVTFRNQA-----	85.33-118.23	1790	1798	9
-----FRNQASRPY-----	41.81-105.22	1794	1802	9

KPNETKTYFWKQVHMAPT		1827	1845	19
-----FWKQVHMA--	106.8-248.71	1835	1843	9

YISQFIIMYSLDGKKW		2116	2131	16
-ISQFIIMYSLDGKKW		2117	2131	15
-ISQFIIMYSLDGKKW		2117	2130	14
-ISQFIImYSLDGKKW		2117	2130	14
-----ImYSLDGKK--	207.7-525.75	2122	2130	9

A3

C1

C2

Donor B
DRB1*13/13

A1

Peptide	Predicted Affinity (nM)	Start	Stop	Length
EKTQTLHKFILLFAVFDGKSMHSETK		206	232	27
EKTQTLHKFILLFAVFDGKSMHSETK		206	231	26
---TQTLHKFILLFAVFDGKSMHSETK		208	232	25
-----VFDEGKSMH-----	616.53-840.74	220	228	9
-----FDEGKSMHS-----	548.87	221	229	9

Peptide	Predicted Affinity (nM)	Start	Stop	Length
EKTQTLHKFILLFAVFDGKSMHSETK		206	232	27
EKTQTLHKFILLFAVFDGKSMHSETK		206	231	26
---TQTLHKFILLFAVFDGKSMHSETK		208	232	25
-----VFDEGKSMH-----	616.53-840.74	220	228	9
-----FDEGKSMHS-----	548.87	221	229	9

A2

SVAKKHKPTWVHYIAAEEEDWDYAPLVLPDDRYS		392	426	35
SVAKKHKPTWVHYIAAEEEDWDYAPLVLPDDRYS		392	424	33
-----WDYAPLVLA-----	403.15	412	420	9
-----LVLPDDRYS-----	136.52	417	425	9

LPGLIGCHRKSVYWH		261	275	15
---LIGCHRKSV---	689.67	264	272	9

SVAKKHKPTWVHYIAAEEEDWDYAPLVLPDDRYS		392	426	35
SVAKKHKPTWVHYIAAEEEDWDYAPLVLPDDRYS		392	427	36
-----WDYAPLVLA-----	403.15	412	420	9
-----LVLPDDRYS-----	136.52	417	425	9

EVGDTLLIFKNQASRPY--		474	493	20
---EVGDTLLIFKNQASRPY---		475	492	18
-----EVGDTLLIFKNQASRP-----		475	491	17
-----EVGDTLLIFKNQASRPY--		475	493	19
-----VGDTLIFKNQASRPY--		476	493	18
-----VGDTLIFKNQASRPYNI		476	494	19
-----VGDTLIFKNQASRPY---		476	492	17
-----VGDTLIFKNQASRP---		476	491	16
-----GDTLLIFKNQASRPY--		477	493	17
-----GDTLLIFKNQASRPY---		477	492	16
-----DTLLIFKNQASRPY--		478	493	16
-----DTLLIFKNQASRPY---		478	492	15
-----DTLLIFKNQASRPYNI		478	494	17
-----DTLLIFKNQASRPYNIYPHG		478	498	21
-----IFKNQASRP-----	38.39-88.86	482	490	9
-----IFKNQASRP-----	40.53	483	491	9

EVGDTLLIFKNQASRPY--		475	492	18
---EVGDTLLIFKNQASRPY---		475	493	19
-----EVGDTLLIFKNQASRP---		475	491	17
-----VGDTLIFKNQASRPY--		476	492	17
-----VGDTLIFKNQASRPYNI		476	494	19
-----VGDTLIFKNQASRPY---		476	493	18
-----VGDTLIFKNQASRP---		476	491	16
-----GDTLLIFKNQASRPY--		477	493	17
-----GDTLLIFKNQASRPY---		477	492	16
-----DTLLIFKNQASRPY--		478	493	16
-----DTLLIFKNQASRPY---		478	492	15
-----DTLLIFKNQASRPYNI		478	494	17
-----DTLLIFKNQASRPYNIYPHG		478	498	21
-----IFKNQASRP-----	38.39-88.86	482	490	9
-----IFKNQASRP-----	40.53	483	491	9

WTVTVEDGPTKSD		532	544	13
---WTVTVEDGPTK---	8929.91	534	542	9

WTVTVEDGPTKSD		532	544	13
---WTVTVEDGPTK---	8929.91	534	542	9

RGNQIMSDKRNVL		581	594	14
---RGNQIMSDKRNVL---		581	594	14
-----GNQIMSDKRNVL---		582	594	13
-----IMSDKRNVI-----	135.55-228.71	585	593	9

ESVDQRGNQIMSDKRNVL		576	594	19
---ESVDQRGNQIMSDKRNVL---		581	594	14
-----GNQIMSDKRNVL---		581	595	15
-----GNQIMSDKRNVL---		582	594	13
-----IMSDKRNVI-----	135.55-228.71	585	593	9

PPSMPVHYDQLDTLFGKSSPLTESGGPLSLEENNDKLESG		925	970	46
---PPSMPVHYDQLDTLFGKSSPL---	51.36	940	948	9

A3

SFQKTRHYFIAAVERLWDYGMSSSPHVL		1709	1737	29
-----YGMSSSPHV-----	85.66	1728	1736	9

SFQKTRHYFIAAVERLWDYGMSSSPHVL		1709	1739	31
-----YGMSSSPHV-----	85.66	1728	1736	9

AEVEDNIMVFRNQASRPYS---		1784	1803	20
---AEVEDNIMVFRNQASRPYS---		1785	1802	18
-----VEDNIMVFRNQASRPYS---		1786	1802	17
-----EDNIMVFRNQASRPYS---		1787	1802	16
-----EDNIMVFRNQASRPYS---		1787	1802	16
-----DNIMVFRNQASRPYS---		1788	1802	15
-----DNIMVFRNQASRPYS---		1788	1803	16
-----DNIMVFRNQASRPYS---		1788	1803	16
-----DNIMVFRNQASRPYSFYS		1788	1806	19
-----IMVFRNQA-----	102.56-124.13	1790	1798	9
-----MVFRNQASRPYSFYS		1791	1806	16
-----MVFRNQASRPYSF---		1791	1804	14
-----FRNQASRPY-----	100.53-255.06	1794	1802	9

AEVEDNIMVFRNQASRPY--		1784	1802	19
---AEVEDNIMVFRNQASRPY---		1786	1802	17
-----DNIMVFRNQASRPY---		1788	1802	15
-----IMVFRNQA-----	102.56-124.13	1790	1798	9
-----MVFRNQASRPYSF		1791	1804	14
-----FRNQASRPY-----	100.53-255.06	1794	1802	9

PPSMPVHYDQLDTLFGKSSPLTESGGPLSLEENNDKLESG		925	970	46
---PPSMPVHYDQLDTLFGKSSPL---	51.36	940	948	9

-----NQASRPYSFYSLLISYEEDQRQGAEP		1796	1822	27
-----SFYSSLISY-----	1003.1	1803	1811	9

-----NQASRPYSFYSLLISYEEDQRQGAEP		1796	1822	27
-----SFYSSLISY-----	1003.1	1803	1811	9

RPNETKTYFWKVQHMAPT		1827	1845	19
---RPNETKTYFWKVQHMAPT---	635.68	1835	1843	9

ETKTYFWKVQHMAPT		1830	1845	16
---ETKTYFWKVQHMAPT---	698.49	1834	1842	9

KENYRFHAINGYIMDT-----		1932	1947	16
---KENYRFHAINGYIMDT---		1933	1947	15
-----ENYRFHAINGYIM-----		1933	1945	13
-----YRFHAINGYI-----	225.09-265.92	1936	1944	9

KENYRFHAINGYIM--		1932	1945	14
---KENYRFHAINGYIM---		1933	1945	13
-----ENYRFHAINGYIMDT		1933	1947	15
-----YRFHAINGYI-----	279.46-352.09	1935	1943	9
-----YRFHAINGYI-----	225.09-265.92	1936	1944	9

-----PHAINGYIMDTLPGLVMAQDQR		1937	1958	22
---PHAINGYIMDTLPGLVMAQDQR---		1937	1957	21
-----PHAINGYIMDTLPGLVMAQDQRI		1937	1959	23
-----IMDTLPGLV-----	85.68-86.37	1944	1952	9
-----LVMAQDQRI-----	41.91	1951	1959	9

-----PHAINGYIMDTLPGLVMAQDQR		1937	1958	22
---PHAINGYIMDTLPGLVMAQDQR---		1937	1957	21
-----IMDTLPGLV-----	85.68-86.37	1944	1952	9

C1

DGHQNTLFFQNGKVK		2286	2300	15
---DGHQNTLFFQNGKVK---	1140.08	2291	2299	9

C2

Donor C
DRB1*10/15

FVIII

FVIII/VWF

Peptide	Predicted Affinity (nM)	Start	Stop	Length
ATRRYYLGAVELSWMQSDLGELPVDARFPPRPVK		20	55	36
-----YVLGAVELS-----	86.64	24	32	9
-----VDARFPPRV-----	105.65	45	53	9
VEFTDHLFNIAPRPPFWMG		71	89	19
---EFTDHLFNIAPRPPFWMG---		72	89	18
---FTDHLFNIAPRPPFWMG---		73	89	17
---TDHLFNIAPRPPFWMG---		74	89	16
-----FNIAPRPP-----	211.14-339.22	78	86	9
DTVVITLKNMASPEVFS		101	116	16
-----VITLKNMAS-----	29.08-64.39	104	112	9
EDDKVFPFGGSHTYVWQVLKENGPM		143	166	24
-----FPGGSHTYVWQVLKENGPM---		148	166	19
-----GSHTYVWQVLKENGPM---		151	166	16
-----TVWQVLKE-----	1127.12	154	162	9
-----YVWQVLKEN---	584.05-1514.22	155	163	9
EKTQTLHKFILLFAVFDGKSWHSETK		206	232	27
EKTQTLHKFILLFAVFDGKSWH---		206	228	23
---TQTLHKFILLFAVFDGKSWHSETK---		208	232	25
-----LHKFILLFA-----	221.02	211	219	9
-----LFAVFDGK-----	584.5	217	225	9
-----FDEGKSWHS---	130.44-496.08	221	229	9
DDNSPSFQIRSVAKKHKP		380	399	20
DDNSPSFQIR-----		380	391	12
DDNSPSFPI-----	9851.22	380	388	9
NSPSFQIRSVAKKHKP		383	399	17
NSPSFQIR-----	11888.32	383	391	9
-SPSFQIRSVAKKHKP		384	399	16
-SPSFQIRSVAKK---		384	396	13
-----FIQIRSVAK-----	26.67-83.82	387	395	9
SVAKKHKPTWVHYIAAEEEDWDYAPLVLPAPDRSY-		392	426	35
SVAKKHKPTWVHYIAAEEEDWDYAPLVLPAPDR---		392	424	33
SVAKKHKPTWVHYIAAEEEDWDYAPLVLPAPDRSYK		392	427	36
---KHKPTWVHYIAAEEEDWDYAPLVLPAPDR---		396	424	29
-----NDYAPLVLA-----	95.18-330.96	412	420	9
-----YAPLVLPAPDR---	81.95	414	422	9
-----LVLPAPDRS---	61.99-70.32	417	425	9
TREATQHSGLGPLLYGEVGDLLIIIFKNQASRPYNIYPHG		457	498	42
-----LLYGEVGDLLIIIFKNQASRPYNIYPHG---		471	498	28
-----YGEVGDLLIIIFKNQASRPYNIYPHG---		473	498	26
-----YGEVGDLLIIIFKNQASRPYNIYPHG---		473	492	20
-----GEVGDLLIIIFKNQASRPYNIYPHG---		474	494	21
-----GEVGDLLIIIFKNQASRPYNIYPHG---		474	493	20
-----GEVGDLLIIIFKNQASRPYNIYPHG---		474	492	19
-----GEVGDLLIIIFKNQASRPYNIYPHG---		474	498	25
-----EVGDLLIIIFKNQASRPYNIYPHG---		475	493	19
-----EVGDLLIIIFKNQASRPYNIYPHG---		475	492	18
-----EVGDLLIIIFKNQASRPYNIYPHG---		475	494	20
-----EVGDLLIIIFKNQASRPYNIYPHG---		475	498	24
-----VGDLLIIIFKNQASRPYNIYPHG---		475	491	17
-----VGDLLIIIFKNQASRPYNIYPHG---		475	497	23
-----VGDLLIIIFKNQASRPYNIYPHG---		476	492	17
-----VGDLLIIIFKNQASRPYNIYPHG---		476	493	18
-----VGDLLIIIFKNQASRPYNIYPHG---		476	494	19
-----VGDLLIIIFKNQASRPYNIYPHG---		476	498	23
-----GDTLLIIIFKNQASRPYNIYPHG---		477	492	16
-----GDTLLIIIFKNQASRPYNIYPHG---		477	493	17
-----GDTLLIIIFKNQASRPYNIYPHG---		477	491	15
-----DTLLIIIFKNQASRPYNIYPHG---		478	492	15
-----DTLLIIIFKNQASRPYNIYPHG---		478	493	16
-----DTLLIIIFKNQASRPYNIYPHG---		478	494	17
-----DTLLIIIFKNQASRPYNIYPHG---		478	491	14
-----DTLLIIIFKNQASRPYNIYPHG---		478	498	21
-----TLLIIIFKNQASRPYNIYPHG---		479	492	14
-----TLLIIIFKNQASRPYNIYPHG---		479	494	16
-----LIIIFKNQAS-----	26.52-110.49	481	489	9
-----IFKNQASRP-----	9.02-51.87	483	491	9
-----FKNQASRPY-----	6.19-26.85	484	492	9
WTVTVEDGPTKSD		532	544	13
---VTVEDGPTK---	10453.55-12418.8	534	542	9
GNQIMSKRNVILFSVFDENRSWYLTENIQR		582	612	31
-----FDENRSWYL-----	61.21-102.97	598	606	9

Peptide	Predicted Affinity (nM)	Start	Stop	Length
TDHLFNIAPRPPFWMG		74	89	16
----FNIAPRPP----	231.57-310.76	78	86	9
FPGGSHTYVWQVLKENGPM		148	166	19
-----GSHTYVWQVLKENGPM---		151	166	16
-----YVWQVLKEN---	584.05-1514.22	155	163	9
EKTQTLHKFILLFAVFDGKSWHSETK		206	232	27
EKTQTLHKFILLFAVFDGKSWHSET-		206	231	26
---TQTLHKFILLFAVFDGKSWHSETK---		208	232	25
-----FAVFDGKS-----	372.63	218	226	9
-----FDEGKSWHS---	130.44-496.08	221	229	9
DLTDEMDVVR		368	379	12
---DSEMDVVR---	9354.48-11457	371	379	9
DDNSPSFQIR		380	391	12
DDNSPSFI---	9851.22	380	388	9
DNSPSFQIRSVAKKHKP		382	399	18
-NSPSFQIRSVAKKHKP		383	399	17
-NSPSFQIR-----	11888.32	383	391	9
-SPSFQIRSVAKKHKP		384	399	16
-SPSFQIRSVAKK---		384	396	13
-----FIQIRSVAK-----	26.67-83.82	387	395	9
SVAKKHKPTWVHYIAAEEEDWDYAPLVLPAPDRSYK		392	427	36
SVAKKHKPTWVHYIAAEEEDWDYAPLVLPAPDRSY-		392	426	35
SVAKKHKPTWVHYIAAEEEDWDYAPLVLPAPDR---		392	424	33
---KHKPTWVHYIAAEEEDWDYAPLVLPAPDR---		396	424	29
-----HPKTWVHYIAAEEEDWDYAPLVLPAPDR---		397	424	28
-----NDYAPLVLA-----	95.18-330.96	412	420	9
-----YAPLVLPAPDR---		414	424	11
-----LVLPAPDRS---	61.99-70.32	417	425	9
YGEVGDLLIIIFKNQASRPYNIYPHG		473	498	26
YGEVGDLLIIIFKNQASRPY-----		473	492	20
---GEVGDLLIIIFKNQASRPYNIYPHG---		474	493	20
---GEVGDLLIIIFKNQASRPYNIYPHG---		474	494	21
---GEVGDLLIIIFKNQASRPYNIYPHG---		474	492	19
---GEVGDLLIIIFKNQASRPYNIYPHG---		474	498	25
---EVGDLLIIIFKNQASRPYNIYPHG---		475	493	19
---EVGDLLIIIFKNQASRPYNIYPHG---		475	492	18
---EVGDLLIIIFKNQASRPYNIYPHG---		475	494	20
---EVGDLLIIIFKNQASRPYNIYPHG---		475	498	24
---EVGDLLIIIFKNQASRPYNIYPHG---		475	491	17
---VGDLLIIIFKNQASRPYNIYPHG---		476	493	18
---VGDLLIIIFKNQASRPYNIYPHG---		476	492	17
---VGDLLIIIFKNQASRPYNIYPHG---		476	494	19
---VGDLLIIIFKNQASRPYNIYPHG---		476	498	23
---GDTLLIIIFKNQASRPYNIYPHG---		477	493	17
---GDTLLIIIFKNQASRPYNIYPHG---		477	492	16
---GDTLLIIIFKNQASRPYNIYPHG---		477	491	15
---DTLLIIIFKNQASRPYNIYPHG---		478	492	15
---DTLLIIIFKNQASRPYNIYPHG---		478	493	16
---DTLLIIIFKNQASRPYNIYPHG---		478	494	17
---DTLLIIIFKNQASRPYNIYPHG---		478	491	14
---DTLLIIIFKNQASRPYNIYPHG---		478	498	21
---TLLIIIFKNQASRPYNIYPHG---		479	492	14
---TLLIIIFKNQASRPYNIYPHG---		479	494	16
---LIIIFKNQAS-----	26.52-110.49	481	489	9
---IFKNQASRP-----	9.02-51.87	483	491	9
---FKNQASRPY-----	6.19-26.85	484	492	9
YKWTVTVEDGPTKSD		530	544	15
---WTVTVEDGPTK---	10453.55-12418.8	532	544	13
---VTVEDGPTK---	10453.55-12418.8	534	542	9
GNQIMSKRNVILFSVFDENRSWYLTENIQR		582	612	31
GNQIMSKRNVILFSVFDENRSWYLTENIQ-----		582	611	30
---IMSKRNVILFSVFDENRSWYLTENIQ---		585	612	28
---RNVILFSVFDENRSWYLTENIQ---		590	612	23
-----FDENRSWYL-----	61.21-102.97	598	606	9

A1

A2

DENRSWLTENIQRFLENFA	599	-	618	20	
-ENRSWLTENIQRFLENFA	600	-	618	19	
-----IQRFLENFA	91.84-126.21	610	-	618	9

DENRSWLTENIQRFLENFA	599	-	618	20	
-ENRSWLTENIQRFLENFA	600	-	617	18	
-----WLTENIQRFLENFA	272.72-342.77	604	-	612	9
-----IQRFLENFA	91.84-126.21	610	-	618	9

FLPNPAGVQLED	613	-	624	12	
FLPNPAGVQ---	3201.73-7844.2	613	-	621	9

FLPNPAGVQLED	613	-	624	12	
FLPNPAGVQ---	3201.73-7844.2	613	-	621	9

SFQKKTRHYFIAAVERLWDYGMSSSPHVLRN	1709	-	1739	31	
SFQKKTRHYFIAAVERLWDYGMSSSPHVL--	1709	-	1737	29	
SFQKKTRHYFIAAVERLWDYGMSSSPHVL--	1709	-	1738	30	
---KTRHYFIAAVERLWDYGMSSSPHVL--	1712	-	1729	18	
---KTRHYFIAAVERLWDYGMSSSPHVL--	1713	-	1727	15	
---KTRHYFIAAVERLWDYGMSSSPHVL--	1713	-	1729	17	
---TRHYFIAAVERLWDYGMSSSPHVL--	1714	-	1739	26	
---TRHYFIAAVERLWDYGMSSSPHVL--	1714	-	1737	24	
---TRHYFIAAVERLWDYGMSSSPHVL--	1714	-	1735	22	
---TRHYFIAAVERLWDYGMSSSPHVL--	1714	-	1729	16	
---YFIAAVERLWDYGMSSSPHVL--	29.75-156.95	1717	-	1725	9
---YGMSSSPHVL--	1728	-	1739	12	
---YGMSSSPHVL--	1728	-	1737	10	
---YGMSSSPHVL--	15.05-4029.46	1728	-	1736	9

SFQKKTRHYFIAAVERLWDYGMSSSPHVL--	1709	-	1737	29	
SFQKKTRHYFIAAVERLWDYGMSSSPHVL--	1709	-	1739	31	
SFQKKTRHYFIAAVERLWDYGMSSSPHVL--	1709	-	1737	29	
SFQKKTRHYFIAAVERLWDYGMSSSPHVL--	1709	-	1738	30	
SFQKKTRHYFIAAVERLWDYGMSSSPHVL--	1709	-	1739	31	
---KTRHYFIAAVERLWDYGMSSSPHVL--	1713	-	1727	15	
---KTRHYFIAAVERLWDYGMSSSPHVL--	1713	-	1729	17	
---TRHYFIAAVERLWDYGMSSSPHVL--	1714	-	1737	24	
---YFIAAVERLWDYGMSSSPHVL--	29.75-156.95	1717	-	1725	9
---YGMSSSPHVL--	1728	-	1739	12	
---YGMSSSPHVL--	15.05-4029.46	1728	-	1736	9

NRAQSGSVQFQKVVQFQFTD	1739	-	1759	21	
AQSGSVQFQKVVQFQFTD--	1741	-	1759	19	
---QKVVQFQFTD--	1850.81-2074.34	1748	-	1756	9
---EKVVQFQFTD--	900.96-1120.28	1749	-	1757	9

LGPIYRAEVEDNIM	1778	-	1791	14	
-GPIYRAEVEDNIm	1779	-	1791	13	
---GPIYRAEVEDNIM	1779	-	1791	13	
---YIRAEVEDN--	1418.01-6720.55	1781	-	1789	9
VEDNIMVTFRNQASRPY-	1786	-	1802	17	
VEDNIMVTFRNQASRPYS	1786	-	1803	18	
-EDNIMVTFRNQASRPY-	1787	-	1802	16	
---DNIMVTFRNQASRPY-	1788	-	1802	15	
---MVTFRNQAS--	99.58-265.52	1791	-	1799	9
---FRNQASRPY-	75.84-93.88	1794	-	1802	9

GLLGPYIRAEVEDNIM	1776	-	1791	16	
GLLGPYIRA-----	2367.41	1776	-	1784	9
---LLGPYIRAEVEDNIM	1777	-	1791	15	
---LGPYIRAEVEDNIm	1778	-	1791	14	
---LGPYIRAEV-----	3046.9-3374.27	1778	-	1786	9
---GPYIRAEVEDNIm	1779	-	1791	13	
---YIRAEVEDN--	1418.01-6720.55	1781	-	1789	9

AEVEDNIMVTFRNQASRPYS	1784	-	1803	20	
-EVEDNIMVTFRNQASRPY-	1785	-	1802	18	
---VEDNIMVTFRNQASRPY-	1786	-	1802	17	
---VEDNIMVTFRNQASRPYS	1786	-	1803	18	
---EDNImVTFRNQASRPY-	1787	-	1802	16	
---DNIMVTFRNQASRPYS	1788	-	1803	16	
---DNIMVTFRNQASRPY-	1788	-	1802	15	
---ImVTFRNQA-----	105.69-198.65	1790	-	1798	9
---MVTFRNQAS--	99.58-265.52	1791	-	1799	9
---FRNQASRPY-	75.84-93.88	1794	-	1802	9

NQASRPYSFYSSLI SYEEDQQAEPFR	1796	-	1822	27	
---YSFYSSLI-----	107.11-128.36	1802	-	1810	9

DETKSWYFTENMERNCRAPC	1903	-	1922	20	
---WYFTENMER-----	785.48	1908	-	1916	9

NQASRPYSFYSSLI SYEEDQQAEPFR	1796	-	1822	27	
---YSFYSSLI-----	107.11-128.36	1802	-	1810	9

IFDETKSWYFTENMERNCRAPCNIQMEDPT	1901	-	1930	30	
---WYFTENMER-----	785.48	1908	-	1916	9
---YFTENMERN-----	746.53	1909	-	1917	9

IRWYLLSMGSENHISHPGSHVFTVR	1959	-	1985	27	
---YLLSMGSNE-----	59.45	1962	-	1970	9
---SIHPSGHVF---	31.78	1974	-	1982	9

IRWYLLSMGSENHISHPGSHVFTVR	1959	-	1985	27	
---YLLSMGSNE-----	59.45	1962	-	1970	9
---SIHPSGHVF---	31.78	1974	-	1982	9

KEPFSWIKVDLLAPMIHGIKT	2084	-	2105	22	
KEPFSWIKVDLLAPMIH---	2084	-	2101	18	
KEPFSWIKVDLLAPMIH---	2084	-	2102	19	
KEPFSWIKVDLLAPMIHGIKT	2084	-	2105	22	
KEPFSWIKVDLLAPMIH---	2084	-	2098	15	
---EPFSWIKVDLLAPMIH---	2085	-	2098	14	
---FSWIKVDLL-----	162.41-298.76	2087	-	2095	9
---IKVDLLAPMI-----	80.31-106.2	2090	-	2098	9
---KVDLLAPMI-----	81.16-108.82	2091	-	2099	9

KEPFSWIKVDLLAPMIHGIKT	2084	-	2105	22	
KEPFSWIKVDLLAPMIH---	2084	-	2098	15	
KEPFSWIKVDLLAPMIH---	2084	-	2097	14	
KEPFSWIKVDLLAPMIH---	2084	-	2102	19	
---EPFSWIKVDLLAPMIH---	2085	-	2098	14	
---FSWIKVDLL-----	162.41-298.76	2087	-	2095	9
---IKVDLLAPMI-----	80.31-106.2	2090	-	2098	9

WTLFPQNGKVKVFG	2290	-	2304	15	
---LFPQNGKVKR---	104.34	2292	-	2300	9

A3

C1

C2

Donor D

DRB1*04/07

FVIII

FVIII/VWF

A1

Peptide	Predicted Affinity (nM)	Start	Stop	Length
AEVYDTVVITLKNMASHFVS		97	116	20
-EYDTVVITLKNMASHFVS		98	116	19
--VYDTVVITLKNMASHFVS		99	116	18
---YDTVVITLKNMASHFVS		99	116	18
----YDTVVITLKNMASHFVS		100	116	17
-----YDTVVITLKNMASHFVS		100	116	17
-----DTVVITLKNMASHFV-		101	115	15
-----DTVVITLKNMASHFVS		101	116	16
-----DTVVITLKNMASHFVS		101	116	16
-----VITLKNMAS----	28.63-47.15	104	112	9
-----LKNMASHFV-	30.33-58.62	107	115	9

FDEGKSWHSETKNSLMQDRDAASARAWPK		221	249	29
-DEGKSWHSETKNSLMQDRDAASARAWPK		222	249	28
---TKNSLMQDRDAASARAWP-		231	248	18
-----LMQDRDAAS----	162.98-2470.2	235	243	9
-----AASARAWPK	195.28-245.53	241	249	9

KSVYWHVIGMGTPE		270	284	15
---YWHVIGMT---	447.27-781.92	273	281	9

SVAKKHKPTWVHYIAAEEEDWDYAPLVLPAPDRSY		392	426	35
SVAKKHKPTWVHYIAAEEEDWDYAPLVLPAPDR--		392	424	33
-----WDYAPLVLA----	59.3-109.1	412	420	9
-----LVLAPDRS-	65.18	417	425	9

GEVGDLLIIFKNQASRPYNI----		474	493	20
-EVGDLLIIFKNQASRPYNI----		475	492	18
---EVGDLLIIFKNQASRPYNI----		475	493	19
----EVGDLLIIFKNQASRPYNI----		475	494	20
-----EVGDLLIIFKNQASRPYNIYPHG		475	498	24
-----VGDLLIIFKNQASRPYNI----		476	492	17
-----VGDLLIIFKNQASRPYNI----		476	494	19
-----VGDLLIIFKNQASRPYNI----		477	493	17
-----VGDLLIIFKNQASRPYNI----		478	493	16
-----VGDLLIIFKNQASRPYNI----		478	492	15
-----LIIIFKNQAS----	41.15-61.85	481	489	9
-----IFKNQASRP----	27.4-31.67	483	491	9
-----FKNQASRPY----	18.32-64.07	484	492	9

A2

ENRSWYLTENIQRF		600	613	14
---WYLTENIQR-	159.45-256.44	604	612	9

EDPEFQASNIMHSING		623	638	16
---FQASNIMHS---	95.42-126.34	627	635	9

EDISAYLLSKNNAIEPR		743	759	17
-DISAYLLSKNNAIEPR		744	759	16
--ISAYLLSKNNAIEPR		745	759	15
---ISAYLLSKNNAIE--		745	757	13
----AYLLSKNNAIEPR		747	759	13
-----YLLSKNNAIEPR		748	759	12
-----YLLSKNNAI----	20.61-131.38	748	756	9

SFQKTRHYFIAAVERLDYGMSSSPHVL--		1709	1737	29
---KTRHYFIAAVERLDW----		1713	1727	15
----KTRHYFIAAVERLDYG----		1713	1729	17
-----TRHYFIAAVERLDYGMSSSPHVL--		1714	1737	24
-----YFIAAVERL----	10.07-134.58	1717	1725	9
-----IAAVERLDYGMSSSPHVL--		1719	1737	19
-----AVERLDYGMSSSPHVL--		1721	1737	17
-----AVERLDYGMSSSPH--		1721	1735	15
-----VERLDYGMSSSPHVLRN		1722	1739	18
-----VERLDYGMSSSPHVLR-		1722	1738	17
-----VERLDYGMSSSPHVL--		1722	1737	16
-----VERLDYGMSSSPHVL--		1722	1736	15
-----VERLDYGMSSSPHVLR-		1722	1738	17
-----VERLDYGM--	1727.36	1722	1730	9
-----ERLDYGMSSSPHVLR-		1723	1738	16
-----RLDYGMSSSPHVLR-		1724	1738	15
-----RLDYGMSSSPHVLRN		1724	1739	16
-----RLDYGMSSSPHVL--		1724	1737	14
-----LDYGMSSSPHVLR-		1725	1738	14
-----LDYGMSSSPHVL--		1725	1736	12
-----WDYGMSSSPHVL--		1726	1736	11
-----WDYGMSSSPHVL--		1726	1737	12
-----DYGMSSSPHVL--		1727	1736	10
-----YGMSSSPHVL--	17.94-1164.76	1728	1736	9

VEDNIMVTRFNQASRPY		1786	1802	17
-EDNIMVTRFNQASRPY		1787	1802	16
---MVTRFNQAS---	92.03-97.49	1791	1799	9
-----FRNQASRPY	92.58-96.5	1794	1802	9

Peptide	Predicted Affinity (nM)	Start	Stop	Length
AEVYDTVVITLKNMASHFVS		97	116	20
-EYDTVVITLKNMASHFVS		98	116	19
--VYDTVVITLKNMASHFVS		99	116	18
---YDTVVITLKNMASHFV-		99	115	17
----DTVVITLKNMASHFVS		101	116	16
-----DTVVITLKNMASHFV-		101	115	15
-----VITLKNMAS----	28.63-47.15	104	112	9
-----LKNMASHFV-	30.33-58.62	107	115	9

TKNSLMQDRDAASARAWP		231	248	18
-KNSLMQDRDAASARA--		232	246	15
---LMQDRDAAS----	162.98-2470.2	235	243	9

KSVYWHVIGMGTPE		270	284	15
---YWHVIGMT---	447.27-781.92	273	281	9

SVAKKHKPTWVHYIAAEEEDWDYAPLVLPAPDRSYK		392	427	36
SVAKKHKPTWVHYIAAEEEDWDYAPLVLPAPDRSY-		392	426	35
SVAKKHKPTWVHYIAAEEEDWDYAPLVLPAPDR--		392	424	33
-----WDYAPLVLA----	59.3-109.1	412	420	9
-----LVLAPDRS--	65.18	417	425	9

GEVGDLLIIFKNQASRPYNIYPHG		474	498	25
-EVGDLLIIFKNQASRPYNI----		475	493	19
---EVGDLLIIFKNQASRPYNI----		475	494	20
----EVGDLLIIFKNQASRPYNI----		475	492	18
-----EVGDLLIIFKNQASRP----		475	491	17
-----EVGDLLIIFKNQASRPYNIYPHG		475	498	24
-----VGDLLIIFKNQASRPYNI----		476	493	18
-----VGDLLIIFKNQASRPYNI----		476	494	19
-----VGDLLIIFKNQASRPYNI----		476	492	17
-----VGDLLIIFKNQASRPYNI----		477	493	17
-----VGDLLIIFKNQASRPYNI----		478	492	16
-----VGDLLIIFKNQASRPYNI----		478	493	17
-----DTLLIIFKNQASRPYNI----		478	493	16
-----DTLLIIFKNQASRPYNI----		478	492	15
-----LIIIFKNQAS----	41.15-61.85	481	489	9
-----IFKNQASRP----	27.4-31.67	483	491	9
-----FKNQASRPY----	18.32-64.07	484	492	9

SVFDENRSWYLTENIQRFLPNPAG		596	619	24
---DENRSWYLTENIQRFLPNPA-		599	618	20
-----ENRSWYLTENIQRFL----		600	614	15
-----WYLTENIQR-	159.45-256.44	604	612	9
-----IQRFLPNPA-	38.15-99.99	610	618	9

DPEFQASNIMHSING		624	638	15
---FQASNIMHS---	95.42-126.34	627	635	9

EDISAYLLSKNNAIEPR		743	759	17
EDISAYLLSKNNAIE--		743	757	15
EDISAYLLSKNNAIEP-		743	758	16
-DISAYLLSKNNAIEPR		744	759	16
---DISAYLLSKNNAIE--		744	757	14
----ISAYLLSKNNAIEPR		745	759	15
-----ISAYLLSKNNAIE--		745	757	13
-----ISAYLLSKNNAIEP-		745	758	14
-----SAYLLSKNNAIEPR		746	759	14
-----YLLSKNNAIEPR		748	759	12
-----YLLSKNNAI----	20.61-131.38	748	756	9

SFQKTRHYFIAAVERLDYGMSSSPHVL--		1709	1737	29
---SFQKTRHYFIAAVERLDW----		1709	1738	30
----TRHYFIAAVERLDYGMSSSPHVL--		1714	1737	24
-----YFIAAVERL----	10.07-134.58	1717	1725	9
-----VERLDYGMSSSPHVL--		1722	1737	16
-----VERLDYGMSSSPHVL--		1722	1738	17
-----LWDYGMSSSPHVL--		1725	1737	13
-----LWDYGMSSSPHVLRN		1725	1739	15
-----LWDYGMSSSPHVL--		1726	1736	11
-----DYGMSSSPHVL--		1727	1736	10
-----YGMSSSPHVL--	1727.36	1728	1736	9

VEDNIMVTRFNQASRPY		1786	1802	17
---MVTRFNQAS---	92.03-97.49	1791	1799	9
-----FRNQASRPY	92.58-96.5	1794	1802	9

A3

KAWAYFSDVLEKDVH	1852 - 1867	16	
---YFSDVLEK---	844.15-933.03	1856 - 1864	9

FTIFDETKSWYFTEN	1899 - 1913	15	
FTIFDETKSWYFTE--	1899 - 1912	14	
FTIFDETKSWYFT--	1899 - 1911	13	
FTIFDETKS-----	972.74-1070.2	1899 - 1907	9
TIFDETKSWYFTENMERN	1900 - 1917	18	
TIFDETKSWYFTEN----	1900 - 1913	14	
TIFDETKSWYFTE-----	1900 - 1912	13	
TIFDETKSWYFTENmERN	1900 - 1917	18	
TIFDETKSWYFTENME--	1900 - 1915	16	
TIFDETKSWYFTENm---	1900 - 1914	15	
TIFDETKSWYFTENmE--	1900 - 1915	16	
IFDETKSWYFTENMERN-	1901 - 1917	17	
IFDETKSWYFTENmE---	1901 - 1915	15	
IFDETKSWYFTE-----	1901 - 1912	12	
IFDETKSWYFTENME--	1901 - 1915	15	
FDETKSWYFTENMERN--	1902 - 1917	16	
FDETKSWYFTE-----	1902 - 1912	11	
FDETKSWYFTENM----	1902 - 1914	13	
FDETKSWYFTENM-----	1902 - 1914	13	
FDETKSWYF-----	370.14-6469	1902 - 1910	9
DETKSWYFTENMERN---	1903 - 1917	15	
----WYFTENMER----	1053-1255	1908 - 1916	9

ENYRFHAINGYImDTLPG	1933 - 1950	18	
ENYRFHAINGYIM----	1933 - 1945	13	
ENYRFHAINGYIMDTLPG	1933 - 1950	18	
--YRFHAINGY-----	169.12-188.02	1935 - 1943	9
---RFHAINGYI-----	142.71	1936 - 1944	9
----FHAINGYIm-----	35.9-62.78	1937 - 1945	9

SGHIRDFQITASGQYGWAPK	2048 - 2068	21	
---RDPQITASGQYGWAPK	2052 - 2068	17	
-----PQITASQY-----	35.9-62.78	2054 - 2062	9

KEPFSWIKVDLLAPM---	2084 - 2098	15	
KEPFSWIKVDLLAPm---	2084 - 2098	15	
KEPFSWIKVDLLAPMIHG	2084 - 2102	19	
---FSWIKVDLL-----	52.1-180.15	2087 - 2095	9
----IKVDLLAPM----	110.67	2090 - 2098	9

YISQFIImYSLDGKKWQT	2116 - 2133	18	
YISQFIIMYSLDGKKW--	2116 - 2131	16	
YISQFIIMYSLDGKKWQT	2116 - 2133	18	
-ISQFIIMYSLDGKKW--	2117 - 2131	15	
-ISQFIImYSLDGKKW--	2117 - 2131	15	
-ISQFIIMYSLDGKK---	2117 - 2130	14	
-ISQFIIMYSLDGKKWQT	2117 - 2133	17	
-ISQFIImYSLDGKKWQ-	2117 - 2132	16	
-ISQFIIMYSLDGKKWQ-	2117 - 2132	16	
-ISQFIImYSLDGKKWQT	2117 - 2133	17	
-ISQFIImYSLDGKK---	2117 - 2130	14	
---FIIMYSLDG-----	115.16-329.01	2120 - 2128	9
----IMYSLDGKK---	122.61-141.95	2122 - 2130	9

YVKEFLISSQDGHQW-	2275 - 2290	16	
YVKEFLISSQDGHQWT	2275 - 2291	17	
YVKEFLISSQDGHQ--	2275 - 2289	15	
--VKEFLISSQDGHQW-	2276 - 2290	15	
--KEFLISSQDGHQWT	2277 - 2291	15	
--KEFLISSQDGHQW-	2277 - 2290	14	
--KEFLISSQDGHQ--	2277 - 2289	13	
----FLISSQDG-----	69.01-204.3	2279 - 2287	9

TIFDETKSWYFTE--	1900 - 1912	13	
TIFDETKSWYFTEN--	1900 - 1913	14	
-IFDETKSWYFTEN--	1901 - 1913	13	
-IFDETKSWYFTE--	1901 - 1912	12	
--FDETKSWYFTEN--	1902 - 1913	12	
--FDETKSWYFTENM	1902 - 1914	13	
----FDETKSWYF----	844.15-933.03	1902 - 1910	9

ISQFIIMYSLDGKKW-	2117 - 2131	15	
ISQFIIMYSLDGKKWQ	2117 - 2132	16	
ISQFIIMYSLDGKKWQ	2117 - 2130	14	
---FIImYSLDG-----	115.16-329.01	2120 - 2128	9
----ImYSLDGKK--	122.61-141.95	2122 - 2130	9

YVKEFLISSQDGHQW	2275 - 2290	16	
YVKEFLISSQDGHQ-	2275 - 2289	15	
--KEFLISSQDGHQW	2277 - 2290	14	
--KEFLISSQDGHQ-	2277 - 2289	13	
----FLISSQDG---	69.01-204.3	2279 - 2287	9

C1

C2

Donor E
DRB1*07/15

FVIII

FVIII/VWF

A1

Peptide	Predicted Affinity (nM)	Start	Stop	Length
DTVVITLKNMASHPVSLHA		101	119	19
DTVVITLKNMASHPV---		101	116	16
---VITLKNMAS-----	64.39	104	112	9
-----LKNMASHPV----	22.92-49.63	107	115	9
EKTQTLKHFILLFAVFDGKSWHSETK		206	232	27
-----FDEGKSWHS---	130.44-160.65	221	229	9
RKSVYWHVIGMGTTFE--		269	284	16
RKSVYWHVIGMGTTFEVH		269	286	18
--KSVYWHVIGMGT-----		270	281	12
---VYWHVIGMG-----	467.66-1088.45	272	280	9
----YWHVIGMGT-----	326.57-930.85	273	281	9
DDNSPSFIQIRSVAKKHFK		381	399	19
---SPSFIQIRSVAKKHFK		384	399	16
-----FIQIRSVAR-----	24.76-58.01	387	395	9
SVAKKHFKTWVHYIAAEEEDWDYAPLVLPAPDRSY		392	426	35
SVAKKHFKTWVHYIAAEEEDWDYAPLVLPAPDR--		392	424	33
-----WDYAPLVLA-----	59.3-142.7	412	420	9

Peptide	Predicted Affinity (nM)	Start	Stop	Length
RSVYWHVIGMGTTFE		270	284	15
---YWHVIGMGT-----	326.57-930.85	273	281	9
DDNSPSFIQIRSVAKKHFK		380	399	20
---NSPSFIQIRSVAKKHFK		383	399	17
-----SPSFIQIRSVAKKHFK		384	399	16
-----FIQIRSVAR-----	24.76-58.01	387	395	9

A2

YGEVGDLLIFKQASRPYNIYPHG		473	498	26
--GEVGDLLIFKQASRPYNI---		474	494	21
---GEVGDLLIFKQASRPYNIYPHG		474	498	25
----EVGDLLIFKQASRPYNI----		475	494	20
-----EVGDLLIFKQASRPYNI-----		475	493	19
-----EVGDLLIFKQASRPYNIYPHG		475	498	24
-----EVGDLLIFKQASRPYNI-----		475	492	18
-----EVGDLLIFKQASRPYNI-----		475	491	17
-----VGDLLIFKQASRPYNI-----		476	494	19
-----VGDLLIFKQASRPYNI-----		476	493	18
-----VGDLLIFKQASRPYNI-----		476	492	17
-----GDTLLIFKQASRPYNI-----		477	493	17
-----GDTLLIFKQASRPYNI-----		477	492	16
-----DTLIIKFNQASRPYNI-----		478	494	17
-----DTLLIFKFNQASRPYNI-----		478	492	15
-----DTLLIFKFNQASRPYNI-----		478	493	16
-----DTLLIFKFNQASRPYNI-----		478	491	14
-----DTLLIFKFNQASRPYNIYPHG		478	498	21
-----TLLIFKFNQASRPYNI-----		479	492	14
-----TLLIFKFNQASRPYNIYPHG		479	498	20
-----LLIFKFNQASRPYNI-----		480	492	13
-----LIFKFNQASRPYNI-----		481	492	12
-----LIFKFNQASRPYNI-----	26.52-162.17	481	489	9
-----LIFKFNQASRPYNI-----	14.81-64.07	484	492	9
WTVTVDGPTKSD		532	544	13
--VTVEDGPTK--	12418-12435	534	542	9
ENRSWYLTENIQRF----		600	613	14
ENRSWYLTENIQRF----		600	614	15
ENRSWYLTENIQRFLENP-		600	617	18
ENRSWYLTENIQRFLENP-		600	618	19
-----WYLTENIQRF-----	94.6-507.81	604	612	9
-----IQRFLENP-----	68.5-95.7	610	618	9
EDISAYLLSKNNAIEPR		743	759	17
-DISAYLLSKNNAIEPR		744	759	16
--DISAYLLSKNNAIEPR		744	757	14
---ISAYLLSKNNAIEPR		745	759	15
----ISAYLLSKNNAIEPR		745	757	13
-----SAYLLSKNNAIEPR		746	759	14
-----YLLSKNNAIEPR	20.02-118.82	748	756	9
SFQKTRHYFIAAVERLWDYGMSSSPHVL---		1709	1737	29
---KTRHYFIAAVERLW-----		1712	1726	15
----KTRHYFIAAVERLW----		1713	1727	15
-----KTRHYFIAAVERLW-----		1713	1726	14
-----YFIAAVERL-----	10.07-97.85	1717	1725	9
-----AAVERLWDYGMSSSPHVL---		1720	1738	19
-----VERLWDYGMSSSPHVL---		1722	1738	17
-----VERLWDYGMSSSPHVL---		1722	1737	16
-----VERLWDYGMSSSPHVLNRN		1722	1739	18
-----VERLWDYGMSSSPHVL---		1722	1736	15
-----VERLWDYGMSSSPHVL---		1722	1737	16
-----RLWDYGMSSSPHVL---		1724	1738	15
-----RLWDYGMSSSPHVL---		1724	1736	13
-----LWDYGMSSSPHVL---		1725	1738	14
-----LWDYGMSSSPHVLNRN		1725	1739	15
-----LWDYGMSSSPHVL---		1725	1737	13
-----LWDYGMSSSPHVLNRN		1725	1740	16
-----WDYGMSSSPHVL---		1726	1736	11
-----WDYGMSSSPHVL---		1726	1737	12
-----DYGMSSSPHVL---		1727	1736	10
-----YGMSSSPHVL---	33.64-5819.81	1728	1736	9

YGEVGDLLIFKQASRPYNIYPHG		473	492	20
--GEVGDLLIFKQASRPYNI---		474	493	20
---GEVGDLLIFKQASRPYNIYPHG		474	494	21
----EVGDLLIFKQASRPYNI----		474	492	19
-----EVGDLLIFKQASRPYNI-----		474	498	25
-----EVGDLLIFKQASRPYNIYPHG		475	494	20
-----EVGDLLIFKQASRPYNI-----		475	492	18
-----EVGDLLIFKQASRPYNI-----		475	491	17
-----VGDLLIFKQASRPYNI-----		475	493	19
-----VGDLLIFKQASRPYNI-----		476	493	18
-----VGDLLIFKQASRPYNI-----		476	494	19
-----GDTLLIFKQASRPYNI-----		476	492	17
-----GDTLLIFKQASRPYNI-----		477	492	16
-----GDTLLIFKQASRPYNI-----		477	494	18
-----GDTLLIFKQASRPYNI-----		477	493	17
-----DTLLIFKFNQASRPYNI-----		478	493	16
-----DTLLIFKFNQASRPYNI-----		478	492	15
-----DTLLIFKFNQASRPYNIYPHG		478	494	17
-----TLLIFKFNQASRPYNI-----		478	491	14
-----TLLIFKFNQASRPYNIYPHG		479	492	14
-----LLIFKFNQASRPYNI-----	26.52-162.17	481	489	9
-----LIFKFNQASRPYNI-----	14.81-64.07	484	492	9
WTVTVDGPTKSD		532	544	13
--VTVEDGPTK--	12418.84-12435.51	534	542	9
ENRSWYLTENIQRFLENPAG		599	619	21
DENRSWYLTENIQRFLENP-		599	618	20
DENRSWYLTENIQRFLENP-		599	614	16
-----WYLTENIQRF-----	94.6-507.81	604	612	9
-----IQRFLENP-----	68.5-95.7	610	618	9
EDISAYLLSKNNAIEPR		743	759	17
EDISAYLLSKNNAIEPR		743	758	16
-DISAYLLSKNNAIEPR		744	758	15
--DISAYLLSKNNAIEPR		744	759	16
---DISAYLLSKNNAIEPR		744	757	14
----ISAYLLSKNNAIEPR		745	759	15
-----SAYLLSKNNAIEPR		746	759	14
-----YLLSKNNAIEPR	20.02-118.82	748	756	9
SFQKTRHYFIAAVERLWDYGMSSSPHVL---		1709	1737	29
---YFIAAVERL-----	10.07-97.85	1717	1725	9
-----WDYGMSSSPHVL---		1726	1736	11
-----YGMSSSPHVL---	33.64-5819.81	1728	1736	9

A3	AEVEDNIMVTFRNQASRPYS- 1784 - 1803 20	VEDNIMVTFRNQASRPY- 1786 - 1802 17
	--VEDNIMVTFRNQASRPYSF 1786 - 1804 19	-EDNIMVTFRNQASRPY- 1787 - 1802 16
	--VEDNIMVTFRNQASRPYS- 1786 - 1803 18	-EDNIMVTFRNQASRPYS 1787 - 1803 17
	--VEDNIMVTFRNQASRPY-- 1786 - 1802 17	--DNIMVTFRNQASRPY- 1788 - 1802 15
	---EDNIMVTFRNQASRPY-- 1787 - 1802 16	---DNIMVTFRNQASRPYS 1788 - 1803 16
	----DNIMVTFRNQASRP- 1788 - 1801 14	----IMVTFRNQA----- 105.69-305.52 1790 - 1798 9
	----DNIMVTFRNQASRPYS- 1788 - 1803 16	----MVTFRNQAS----- 103.88-107.36 1791 - 1799 9
	----DNIMVTFRNQASRPYSF 1788 - 1804 17	-----FRNQASRPY- 31.9-96.5 1794 - 1802 9
	-----IMVTFRNQA----- 105.69-305.52 1790 - 1798 9	
	-----MVTFRNQAS----- 103.88-107.36 1791 - 1799 9	
-----FRNQASRPY-- 31.9-96.5 1794 - 1802 9		
IGPLLVCHTNTLNPA 1871 - 1885 15		
---LLVCHTNTL--- 366.5-614.07 1874 - 1882 9		
FTIFDETKSWYFTEN--- 1899 - 1913 15	FFTIFDETKSWYFTEN 1898 - 1913 16	
FTIFDETKSWYFTE---- 1899 - 1912 14	-FTIFDETKSWYFTEN 1899 - 1913 15	
-TIFDETKSWYFTE---- 1900 - 1912 13	--TIFDETKSWYFTE- 1900 - 1912 13	
-TIFDETKSWYFTENMERN 1900 - 1917 18	---IFDETKSWYFTEN- 1901 - 1913 13	
-TIFDETKSWYFTENME-- 1900 - 1915 16	----FDETKSWYFTE- 1902 - 1912 11	
-TIFDETKSWYF----- 1900 - 1910 11	----FDETKSWYF--- 314.07-4594.08 1902 - 1910 9	
-TIFDETKSWYFTENM--- 1900 - 1914 15		
-TIFDETKSWYFTEN---- 1900 - 1913 14		
---IFDETKSWYFTENM--- 1901 - 1914 14		
---IFDETKSWYFTENMERN 1901 - 1917 17		
---IFDETKSWYFTE---- 1901 - 1912 12		
---IFDETKSWYFTENME-- 1901 - 1915 15		
---FDETKSWYFTENM--- 1902 - 1914 13		
---FDETKSWYFTENMERN 1902 - 1917 16		
---FDETKSWYFTE---- 1902 - 1912 11		
---FDETKSWYFTEN---- 1902 - 1913 12		
---FDETKSWYF----- 1902 - 1910 9		
----DETKSWYFTENMERN 1903 - 1917 15		
----ETKSWYFTENMERN 1904 - 1917 14		
----WYFTENMER- 670.98-2190.7 1908 - 1916 9		
ENYRFHAINGYIMDT 1933 - 1947 15		
---RFHAINGYI--- 64.18 1936 - 1944 9		
----FHAINGYIM-- 26.7 1937 - 1945 9		
DTLPLGLVMAQDQRI 1946 - 1960 15		
---LVMAQDQRI- 249.74-594.36 1951 - 1959 9		
RDFQITASGQYGGW 2052 - 2065 14		
--FQITASGQY--- 164.05-1410.56 2054 - 2062 9		
APKRLARLHYSGSINAWS 2066 - 2082 17		
---LARLHYSGSINAWS 2069 - 2082 14		
---LARLHYSGSINA-- 2069 - 2080 12		
---LARLHYSGSINAW- 2069 - 2081 13		
----LHYSGSINA-- 51.21-729.77 2072 - 2080 9		
ISQFIMYSLDGKK 2117 - 2130 14		
---FIMYSLDG-- 74.31-161.14 2120 - 2128 9		
YVKEFLISSQDGHQW 2275 - 2290 16	YVKEFLISSQDGHQW 2275 - 2290 16	
YVKEFLISSQDGHQ-- 2275 - 2289 15	---FLISSQDG--- 118.58-987.24 2279 - 2287 9	
--KEFLISSQDGHQW- 2277 - 2290 14		
--KEFLISSQDGHQWT 2277 - 2291 15		
----FLISSQDG--- 118.58-987.24 2279 - 2287 9		
HPQSWVHQIALRMEVLG 2328 - 2344 17		
----WVHQIALRM--- 25.91-61.67 2332 - 2340 9		

Supplementary Figure S3. Overview identified peptides. Monocyte-derived dendritic cells from donors A, B, C, D and E were pulsed with FVIII (left half of the table) or with FVIII/VWF complex (right half of table). FVIII-derived peptides were selected and corresponding HLA-DRB1 genotype specific core peptides were predicted using NetMHCpan 2.8. The data is displayed for each donor separately. All identified FVIII-derived peptides were grouped according to their position within the protein. Bold peptides represent predicted core peptides with their predicted affinities. For each cluster of peptides the highest affinity core peptide was used as a representative marker for the peptides identified as shown in Figure 4 and Figure 5. Green clusters indicate peptides which were identified from cells which were pulsed with FVIII alone, while yellow clusters represent the identification of peptides after pulsing cells with FVIII/VWF complex which are found in both conditions. Clusters of red colored peptides indicate peptides only identified when cells are pulsed with FVIII/VWF and not when pulsed with FVIII only.