

Supplementary information

Supplemental Methods

VWF and VWFpp ELISA

For VWF detection, a rabbit polyclonal anti-human VWF antibody (DAKO; 0.3 µg/well) was used as the capture antibody, and an HRP-conjugated rabbit polyclonal anti-human VWF antibody (DAKO; 0.5 µg/mL) for detection. For VWFpp a mouse monoclonal anti-human VWFpp antibody (CLB-Pro35; 1.0 µg/well) was used along with an HRP-conjugated mouse monoclonal anti-human VWFpp antibody (CLB-Pro14-3; 0.125 µg/mL). The TMB substrate kit (ThermoFisher) was used for detecting HRP activity. Blocking, washing, and detection steps were conducted in PBS supplemented with 0.1% Tween-20, 0.2% gelatin, and 1 mM EDTA. Concentrated conditioned media from HEK293s stably expressing human wildtype VWF and VWFpp1 was used as a standard.

Mass spectrometry

Cells were lysed in sodium deoxycholate buffer, incubated at 95°C for 5 minutes, sonicated and digested overnight using trypsin gold (Promega). Tryptic digests were transferred to an Evotip Pure (Evosep) and separated on a 15 cm×150 µm, 1.5 µm Performance Column (EV1137 from EvoSep) with an Evosep One liquid chromatography (LC) system (Evosep) with the 15 samples per day program. Peptides were ionized and electrosprayed into the Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher scientific, USA). Data was acquired in Data Independent Acquisition mode (DIA) as described previously². Raw mass spectrometry files were analyzed in DIANN (version 1.8), using the filtered human Swissprot database (2021.22.04) to detect proteins and peptides as described previously². Data was analyzed with R 4.2.3 and Rstudio (2022.07.02).

Live cell imaging of WPB exocytosis

ECFCs were nucleofected with 4 μg of the VWFpp-mStayGold expression vector and plated at 70% density in EGM-18 onto 35 mm diameter poly d-lysine-coated glass-bottomed culture dishes (MatTek Corp). Imaging was conducted 24 hours post-nucleofection. For intracellular $[\text{Ca}^{2+}]_i$ monitoring, cells were loaded with 0.25 μM Fura-2 for 15 minutes at 37°C. Prior to imaging, cells were transferred to a physiological saline solution (145 mM NaCl, 5 mM KCl, 1.2 mM CaCl_2 , 1 mM MgCl_2 , 1 mM NaH_2PO_4 , 10 mM glucose, 20 mM Na^+ -HEPES pH 7.4). Secretion was triggered by stimulation with 100 μM histamine. Images were acquired at 30 frames/second using the free imaging software Winfluor (Dr John Dempster; Strathclyde University; Winfluor). The imaging setup included an Olympus IX71 inverted fluorescence microscope equipped with an Olympus UPLSAPO x 100 1.40 NA objective, an OptoLED light source (Cairn Research, Faversham, UK), and an Ixon3 EMCCD camera (Andor, Belfast, UK), cooled to -68°C. The microscope was housed in a temperature-controlled box maintained at 37°C. Fura-2 was illuminated sequentially with 355 ± 5 nm and 380 ± 5 nm light and mStayGold with 470 ± 10 nm light. Analysis of individual exocytosis events was performed using FIJI software. The moment of fusion of mStayGold-labeled WPBs was identified as the first frame showing a shape change or loss of fluorescence. WPBs were counted manually using the FIJI pointpicker plugin (Philippe Thévenaz, Biomedical Imaging Group, Swiss Federal Institute of Technology, Lausanne, Switzerland). The timing of the calcium rise and exocytosis events was recorded.

Supplementary Table S1: Antibody reagents

Antigen	Species	Supplier	Catalogue number	Concentration used for IF (µg/mL)	Concentration used for WB (µg/mL)
Von Willebrand factor propeptide	Rabbit pAb	Prof. Tom Carter, SGUL ³	-	1.12	N/A
Von Willebrand factor	Rabbit pAb	DAKO	A0082	0.06	N/A
Von Willebrand factor	Mouse mAb	Sanquin	CLB-RAg20	3.94	N/A
Rab27A	Mouse mAb	Abnova	H00005873-M01	4.40	0.88
Rab3D	Rabbit pAb	Proteintech	12320-1-AP	6	0.24
VE-cadherin	Goat pAb	R&D Systems	AF938	0.80	N/A
alpha-tubulin	Mouse mAb	Sigma / Merck	T6199	N/A	0.2
Slp4-a	Rabbit pAb	Atlas	HPA001475	0.80	N/A

Supplemental Files

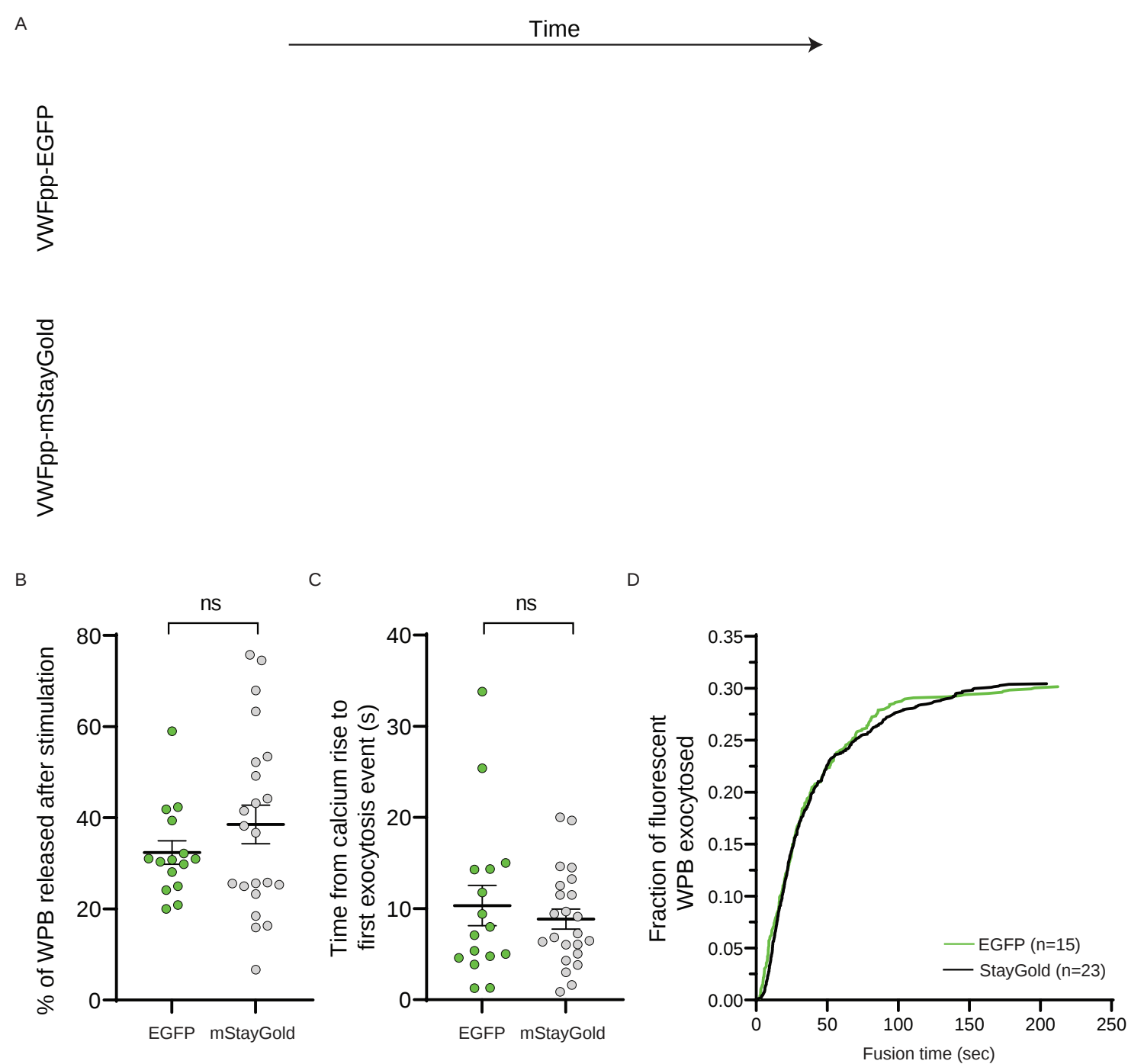
Supplemental File 1: Code for the CellProfiler pipeline used for quantification of Rab intensity on Weibel-Palade bodies displayed in Figure 3 of the original manuscript. The OrganelleProfiler pipeline can be found on the Clotterdam Github repository <https://github.com/Clotterdam/>

Supplemental File 2: Proteomics data.

References

1. Bierings R, van den Biggelaar M, Kragt A, et al. Efficiency of von Willebrand factor-mediated targeting of interleukin-8 into Weibel-Palade bodies. *J Thromb Haemost.* 2007;5(12):2512–2519.
2. Groten SA, Smit ER, van den Biggelaar M, Hoogendijk AJ. The proteomic landscape of in vitro cultured endothelial cells across vascular beds. *Commun. Biol.* 2024;7(1):989.
3. Hewlett L, Zupančič G, Mashanov G, et al. Temperature-dependence of weibel-palade body exocytosis and cell surface dispersal of von willebrand factor and its propolypeptide. *PLoS One.* 2011;6(11):e27314.

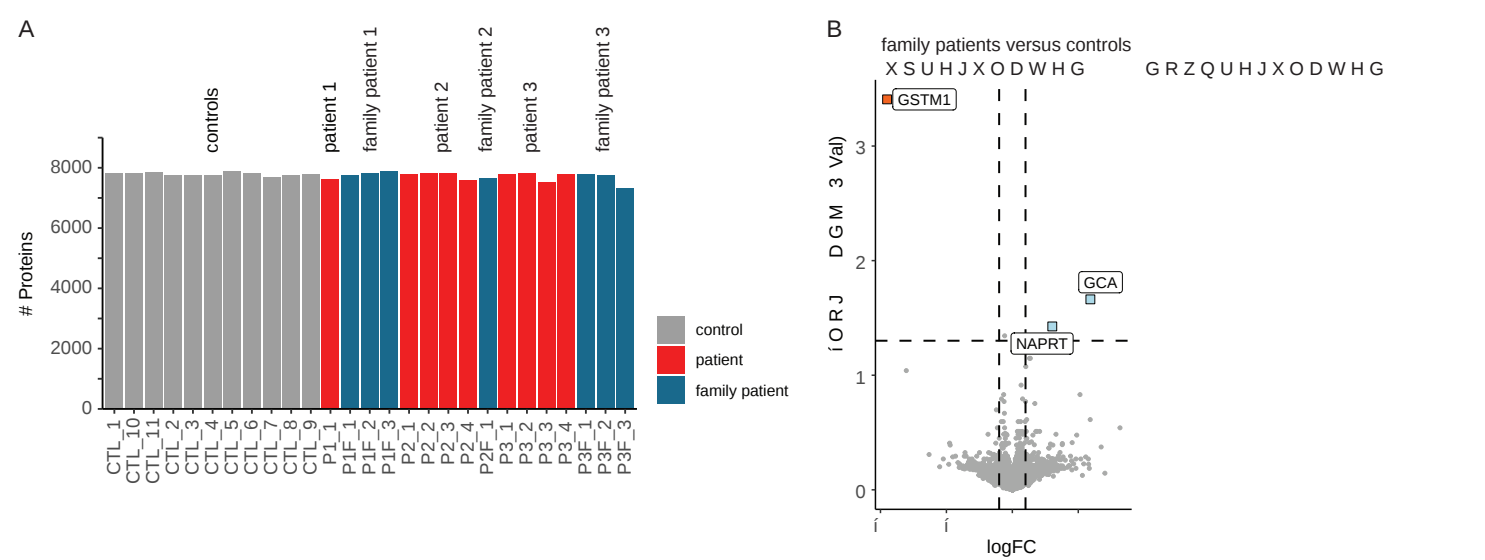
Supplemental Figure 1



Supplemental Figure 1. Comparison of VWFpp-EGFP and VWFpp-mStayGold for live imaging of WPB exocytosis in HUVEC.

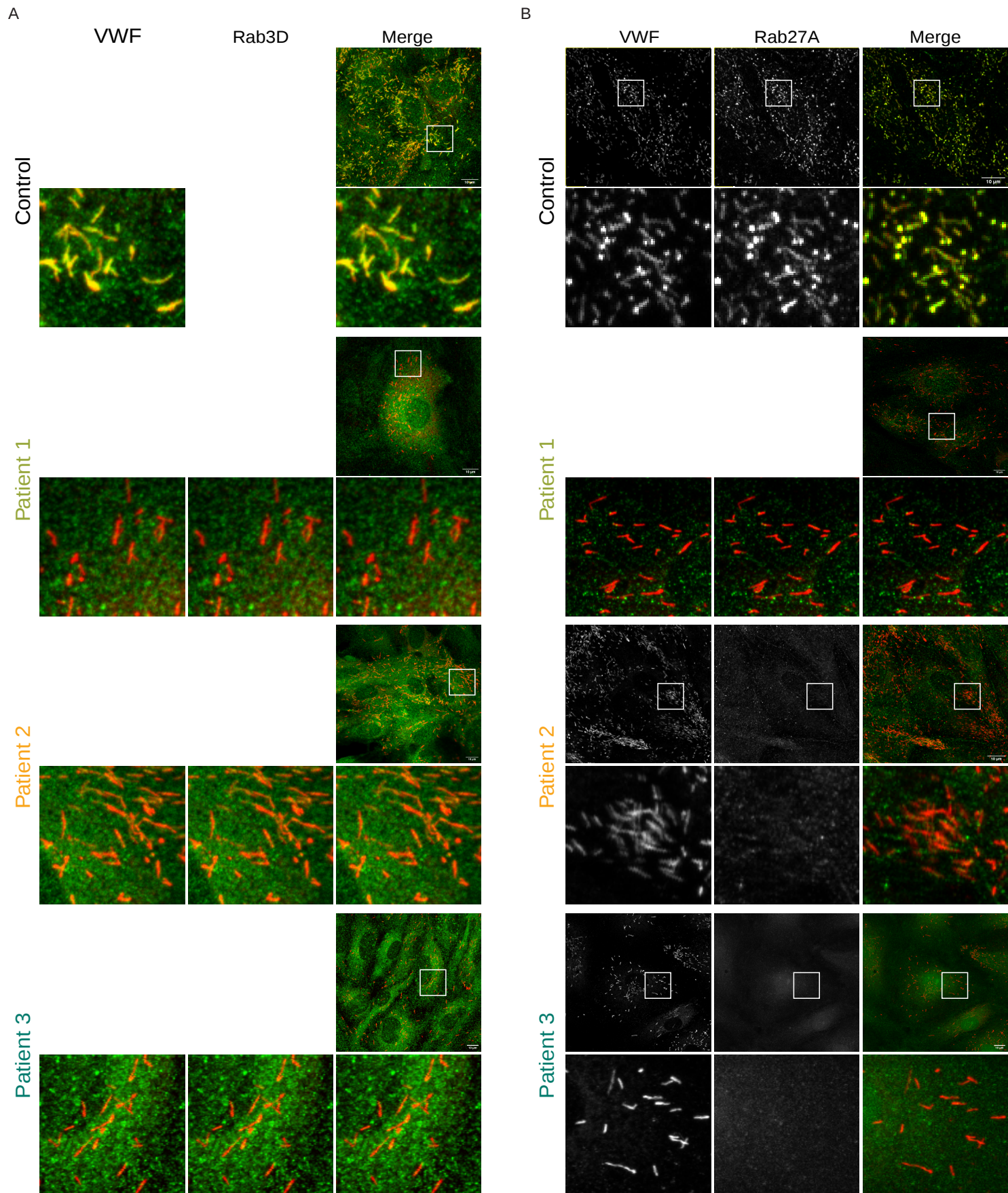
(A) HUVECs were transfected with VWFpp-EGFP (top panel) or VWFpp-mStayGold (bottom panel). After 24 hours, cells were stimulated with 100 nM Bay K 8646. (B) Quantification of the percentage of WPB released after stimulation. (C) Quantification of the time from calcium rise to first exocytosis event. (D) Quantification of the fraction of fluorescent WPB exocytosed over time.

Supplemental Figure 2



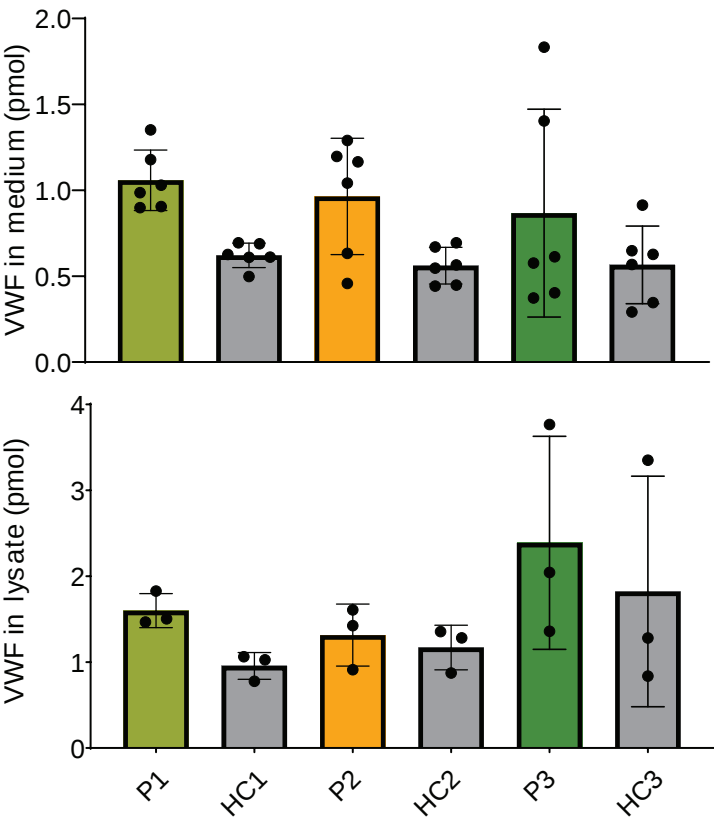
Supplemental Figure 2 \$ Number of proteins X D Q V protein \$ Volcano plot of patient family members versus controls (1) & V' R W W H G protein U H D Q G patient family member E O X H p-value OR J D Q G logFC change & > W K U H V 6 R S G Q G L G R W Q E O X H Q S U H J X O D W H G protein 3 U R W H L Q V R I L Q W H U H V W D U H O D E H O H G

Supplemental Figure 3



Supplemental Figure 3. Rab3D and Rab27A do not localize to WPB in MADD patient ECFCs. Endogenous Rab3D (A) and Rab27A (B) colocalize with VWF in healthy control ECFCs but not MADD patient ECFCs. Zoom ins of LQGLFDWHG DUHDV DUH VKRZQ EHORIZ HDFK RYHUYLHZ LPDJH 5HG 9:) *UHHQ 5D

Supplemental Figure 4



Supplemental Figure 4. Basal VWF levels in ECFCs of MADD patients and healthy controls. ELISA measurements of VWF secreted over 24 hours (basal VWF secretion; top panel) and in the ECFC lysates at time of secretion assay (bottom panel) for MADD and healthy control ECFCs.

3URFHVV DV ' " 1R
5HODWLYH SL[HO VSDFLQJ LQ ;
5HODWLYH SL[HO VSDFLQJ LQ <
5HODWLYH SL[HO VSDFLQJ LQ =
6HOHFW WKH UXOH FULWHULD DQG ILOH GRHV FRQWDLQ
1DPH WR DVVLJQ WKHVV LPDJHV '1\$
1DPH WR DVVLJQ WKHVV REMHFWV &HOO
6HOHFW WKH LPDJH W\SH *UD\VFDOH LPDJH
6HW LQWHQVLW\ UDQJH IURP ,PDJH PHWDGDWD
0D[LPXP LQWHQVLW\

*URXSV >PRGXOHBQXP _VYQBYHUVLRQ 8QNQRZQ _YDULDEOHBUHY
ZBZLQGRZ)DOVH_QRWHV > 7KH *URXSV PRGXOH RSWLRQDOO\ DOO
\RXU OLVW RI LPDJHV LQWR LPDJH VXEHWV JURXSV ZKLFK ZLOO
LQGHSHQGHW\ RI HDFK RWKHU JURXSV LQFOXGH VFUHHQLQJ
EDWFKHV PLFURWLWHU SODVMM PLYHHV
HWF @_EDWFKBVWDWH DUUD\ >@ GW\SH XLQW _HQDEOHG 7UXH_
'R \RX ZDQW WR JURXS \RXU LPDJHV" 1R
JURXSLQJ PHWDGDWD FRXQW
0HWDGDWD FDWHJRU\ 6HULHV

&RORU7R*UD\ >PRGXOHBQXP _VYQBYHUVLRQ 8QNQRZQ _YDULDEO
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QHGHGH IRU REMHFW UHFRJQLWLRQ 0RGXOH IURP /DDQ HW D
@_EDWFKBVWDWH DUUD\ >@
GW\SH XLQW _HQDEOHG ZDQWVBSDXVH)DOVH@
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&RQYHUVLRQ PHWKRG 6SOLW
,PDJH W\SH &KDQQHOV
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IURP /DDQ HW DO @ _EDWFKBVWDWH DUUD\ >@
GWISH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
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&DOFXODWH DUWLIDFW GLDPHWHU DXWRPDWLFDOO\ " 1R
7\SLFDO DUWLIDFW GLDPHWHU
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GW\SH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
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1DPH WKH RXWSXW LPDJH 0DVNHGB2UJDQHOOH
8VH REMHFW RU DQ LPDJH DV D PDVN" 2EMHFWV
6HOHFW REMHFW IRU PDVN &HOVB)LOWHUHG
6HOHFW LPDJH IRU PDVN 1RQH
,QYHUW WKH PDVN" 1R

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GW\SH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
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'HFD\
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6SHHG DQG DFFXUDF\)DVW
5HVFDOLQJ PHWKRG 7XEHQHVV

(QKDQFH2U6XSSUHV)HDWXUHV >PRGXOHBQXP _VYQBYHUVLRQ 8
HYLVLRQBQXPEHU _VKRZBZLQGRZ)DOVH_QRWHV > IXUWKHU HQKD
0RGXOH IURP /DDQ HW DO @_EDWFKBVWDWH DUUD\ >@

GW\SH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
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1DPH WKH RXWSXW LPDJH 1HXULWHVB2UJDQHOOH
6HOHFW WKH RSHUDWLRQ (QKDQFH
)HDWXUH VL]H
)HDWXUH W\SH 1HXULWHV
5DQJH RI KROH VL]HV
6PRRWKLQJ VFDOH
6KHU DQJOH
'HFD\
(QKDQFHPHQW PHWKRG /LQH VWUXFWXUHV

6SHHG DQG DFFXUDF\)DVW
5HVFDOH UHVXOW LPDJH <HV

,GHQWLI\3ULPDU\2EMHFWV >PRGXOHBQXP _VYQBYHUVLRQ 8QNQR
VLRQBQXPEHU _VKRZBZLQGRZ)DOVH_QRWHV > 8VLQJ WKH WKUHV
:3% DV REMHFWV ILQHWXQH WR LQFOXGH ORQJ WKLQ :3% DQG V
FOXVWHUHG WRJHWKHU 0/DOXQHHW UDRP

@_EDWFKBVWDWH DUUD\ >@
GWISH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
6HOHFW WKH LQSXW LPDJH 1HXULWHVB2UJDQHOOH
1DPH WKH SULPDU\ REMHFWV WR EH LGHQWLILHG 2UJDQHOOHB
7\SLFDO GLDPHWHU RI REMHFWV LQ SL[HO XQLWV 0LQ 0D[
'LVFDUG REMHFWV RXWVLGH WKH GLDPHWHU UDQJH" <HV
'LVFDUG REMHFWV WRXFKLQJ WKH ERUGHU RI WKH LPDJH" <HV
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6L]H RI VPRRWKLQJ ILOWHU
6XSSUHVV ORFDO PD[LPD WKDW DUH FORVHU WKDQ WKLV PLQL
GLVWDQFH

6SHHG XS E\ XVLQJ 0RZHU WKLRQ LPDJH WR ILQG ORFDO PD[LPD
)LOO KROHV LQ LGHQWLILHG REMHFWV" 1HYHU
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PD[LPD" 1R

+DQGOLQJ RI REMHFWV LI H[FHVVLYH QXPEHU RI REMHFWV
LGHQWLILHG &RQWLQXH
0D[LXP QXPEHU RI REMHFWV
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/RJ WUDQVIRUP EHIRUH WKUHVKROGLQJ" 1R
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RI GHYLDWLRQV
7KUHVKROGLQJ PHWKRG 2WVX

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LRQBQXPEHU _VKRZBZLQGRZ)DOVH_QRWHV > H[SDQG WKH :3% RE
7KLW LV SHUIRUPHG EHFDXVH WKH :3% REMHFW LV PDGH EDVHG RQ
ZKLFK LV UHWDLQHG LQVLGH WKH :3% SURWHQ RI LQWHUHV LV SUH
RQ WKH PHPEUDQH RI WKH :3% WKUHIRUH WKH :3% REMHFW LV H
DOO RUJDQHOH FRQWHQW VLJQDO SUHVHQW LQ DQG RQ WKH :3%
IURP /DDQ HW DO @_EDWFKBVWDWH DUUD\ >@

GWISH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
6HOHFW WKH LQSXW REMHFWV 2UJDQHOOHB2EMHFW
1DPH WKH RXWSXW REMHFWV ([SDQGHGB2UJDQHOOHB2EMHFW

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)LOO KROHV LQ REMHFWV VR WKDW DOO REMHFWV VKULQN WR
SRLQW" 1R
([SDQG RU VKULQN PHDVXUHPHQW 1RQH

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XPEHU _VKRZBZLQGRZ)DOVH_QRWHV > UHVFDOH 2UJDQHOOHFRQW
HDVLHU FRQWURO LQ WKH RYHUOD\ 0RGXOH IURP /DDQ HW DO
@_EDWFKBVWDWH DUUD\ >@

GWISH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
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5HVFDOLQJ PHWKRG 6WUHWFK HDFK LPDJH WR XVH WKH IXOO L
0HWKRG WR FDOFXODWH WKH PLQLXP LQWHQVLW\ &XVWRP
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'LYLVRU YDOXH
'LYLVRU PHDVXUHPHQW 1RQH

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_VKRZBZLQGRZ)DOVH_QRWHV > 0DVN WKH 2UJDQHOOHFRQWHQW L
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VKRZQ ZKHUH :3% ZHUH LGHQWLILHG DV R 0RGXOH IURP /DDQ HW
DO @_EDWFKBVWDWH DUUD\ >@

GWISH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
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1DPH WKH RXWSXW LPDJH 2&B,QB2UJDQHOOHB0DVN
8VH REMHFWV RU DQ LPDJH DV D PDVN" 2EMHFW
6HOHFW REMHFW IRU PDVN ([SDQGHGB2UJDQHOOHB2EMHFW
6HOHFW LPDJH IRU PDVN 1RQH
,QYHUW WKH PDVN" 1R

,GHQWL\3ULPDU\2EMHFWV >PRGXOHBQXP _VYQBYHUVLRQ 8QNQR
VLRQBQXPEHU _VKRZBZLQGRZ)DOVH_QRWHV > 0HDVXUH 2UJDQH
LQ :3% DV REMHFWV VR WKH\ FDQ EH PHDVXUH 0RGXOH IU
DO @_EDWFKBVWDWH DUUD\ >@

GWISH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
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1DPH WKH SULPDU\ REMHFWV WR EH LGHQWLILHG 2&B,QB2UJDQ
7\SLFDO GLDPHWHU RI REMHFWV LQ SL[HO XQLWV 0LQ 0D[
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%DFNJURXQG 6HHG @_EDWFKBVWDWH DUUD\ >@
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HU _VKRZBZLQGRZ)DOVH_QRWHV > HVWDEOLVK D UHODWLRQVKLS
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HW DO @_EDWFKBVWDWH DUUD\ >@
GWISH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
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&KLOG REMHFWV 2&B2XWB2UJDQHOOHB2EMHFW
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3DUHQW QDPH 1RQH

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HU _VKRZBZLQGRZ)DOVH_QRWHV > UHODWH &HOOV WR :3%V WR P
GLVWDQFH EHWZHHQ WKH :3% DQG WKH FHOO PHPEUDQH 0RG
DO @ _EDWFKBVWDWH DUUD\ >@
GW\SHLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
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'LVSOD\ REMHFW RU LPDJH PHDVXUHPHQWV" 2EMHFW
6HOHFW WKH LQSXW REMHFWV &HOOVB)LOWHUHG
0HDVXUHPHQW WR GLVSOD\ 1XPEHUB2EMHFWB1XPEHU
6HOHFW WKH LPDJH RQ ZKLFK WR GLVSOD\ WKH PHDVXUHPHQW
7H[W FRORU '())
1DPH WKH RXWSXW LPDJH WKDW KDV WKH PHDVXUHPHQWV
GLVSOD\HG 2YHUOD\B1XPEHUHG
)RQW VLJH SRLQWV
1XPEHU RI GHFLPDOV
,PDJH HOHPHQWV WR VDYH ,PDJH
\$QQRWDWLRQ RIIVHW LQ SL[HOV
'LVSOD\ PRGH 7H[W
&RORU PDS 'HIDXOW
'LVSOD\ EDFNJURXQG LPDJH" <HV
&RORU PDS VFDOH 8VH WKLW LPDJH V PHDVXUHPHQW UDQJH
&RORU PDS UDQJH
)RQW \$GREH 'HYDQDJDL
8VH VFLHQWLILF QRWDWLRQ" 1R
)RQW ZHLJKW QRUPDO

6DYH,PDJHV >PRGXOHBQXP _VYQBYHUVLRQ 8QNQRZQ _YDULDEOH
_VKRZBZLQGRZ)DOVH_QRWHV > RYHUOD\ PDGH RQ 2UJDQHOOHFF
KHUH 0RGXOH IURP /DDQ HW DO @_EDWFKBVWDWH DU
GW\SH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH
6HOHFW WKH W\SH RI LPDJH WR VDYH ,PDJH
6HOHFW WKH LPDJH WR VDYH 2YHUOD\B1XPEHUHG
6HOHFW PHWKRG IRU FRQVWUXFWLQJ ILOH QDPHV)URP LPDJH
6HOHFW LPDJH QDPH IRU ILOH SUHIL[2ULJLQDO
(QWHU VLQJOH ILOH QDPH 2ULJ%OXH
1XPEHU RI GLJLWV
\$SSHQG D VXIIL[WR WKH LPDJH ILOH QDPH" 1R
7H[W WR DSSHQG WR WKH LPDJH QDPH
6DYHG ILOH IRUPDW SQJ
2XWSXW ILOH ORFDWLRQ (OVH?RQURFDO(
GDW\$'??&HOOSURILOHU DQD&V\$.2B*)3B'\$3,
,PDJH ELW GH\$WW LQWHJHU
2YHUZULWH H[LVWLQJ ILOHV ZLWKRXW ZDUQLQJ" <HV
:KHQ WR VDYH (YHU\ F\FOH
5HFRUG WKH ILOH DQG SDWK LQIRUPDWLRQ WR WKH VDYHG LPD
&UHDWH VXEIROGHUV LQ WKH RXWSXW IROGHU" 1R
%DVH LPDJH IROGHU (OVHZKHUH _
+RZ WR VDYH WKH VHULHV 7 7LPH
6DYH ZLWK ORVVOHVV FRPSUHVVLRQ" <HV

([SRUW7R'DWDEDVH >PRGXOHBQXP _VYQBYHUVLRQ 8QNQRZQ _YD
XPEHU _VKRZBZLQGRZ)DOVH_QRWHV > 0RGXOH IURP /DDQ HW DO
@_EDWFKBVWDWH DUUD\ >@
GW\SH XLQW _HQDEOHG 7UXH_ZDQWVBSDXVH)DOVH@
'DWDEDVH W\SH 64/LWH
'DWDEDVH QDPH 'HIDXOW'%
\$GG D SUHIL[WR WDEOH QDPHV" 1R
7DEOH SUHIL[0\([SWB
2XWSXW ILOH ORFDWLRQ (OVH?RQURFDO(
GDW\$'??&HOOSURILOHU DQD&V\$.2B*)3B'\$3,
&UHDWH D &HOO3URILOHU \$QDO\W SURSHUWLHV ILOH" 1R
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3DVVZRUG
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&DOFXODWH WKPHSHUWDQGUG GHYLDWLRQ YDOXH RI REMHFW
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PHDVXUHPHQW" 1R
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&UHDWH RQH WDEOH SHU REMHFW D VLQJOH REMHFW WDEOH F
YLHZ" 6LQJOH REMHFW WDEOH
(QWHU DQ LPDJH XUSHQG LI \RX SODQ WR DFFHVV \RXU I
:ULWH LPDJH WKXPEQDLOV GLUHFWO\ WR WKH GDWDEDVH" 1R
6HOHFW WKH LPDJHV IRU ZKLFK \RX ZDQW WR VDYH WKXPEQDL

\$XWRFDOH WKXPEQDLO SL[HO LQWHQVLWLHV" <HV
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6HOHFW WKH SODWH PHWDGDWD 1RQH
6HOHFW WKH ZHOO PHWDGDWD 1RQH
,QFOXGH LQIRUPDWLRQ IRU DOO LPDJHV XVLQJ GHIDXOW YDOX
3URSHUWLHV LPDJH JURXS FRXQW
3URSHUWLHV JURXS ILHOG FRXQW
3URSHUWLHV ILOWHU ILHOG FRXQW
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:KLFK REMHFWV VKRXOG EH XVHG IRU ORFDWLRQV" 1RQH
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&HOO3URILOHU \$QDO\W
([SRUW REMHFW UHODWLRQVKLSV" <HV
2YHUZULWH ZLWKRXW ZDUQLQJ" 1HYHU
\$FFHVV &HOO3URILOHU \$QDO\W LPDJHV YLD 85/" 1R
6HOHFW WKH FODVVLILFDWLRQ W\SH 2EMHFW
6HOHFW DQ LPDJH WR LQFOXGH 1RQH
8VH WKH LPDJH QDPH IRU WKH GLVSOD\ " <HV
,PDJH QDPH &KDQQHO
&KDQQHO FRORU UHG
'R \RX ZDQW WR DGG JURXS ILHOGV" 1R
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(QWHU WKHLB\H FROXPQV ZKLFK GHILQH WKH JURXS VHSDUDV
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'R \RX ZDQW WR DGG ILOWHU ILHOGV" 1R
\$XWRPDWLFDOO\ FUHDWH D ILOWHU IRU HDFK SODWH" 1R
&UHDWH D &HOO3URILOHU \$QDO\W ZRUNVSDFH ILOH" 1R
6HOHFW WKH PHDVXUHPHQW GLVSOD\ WRRO 6FDWWHU3ORW
7\SH RI PHDVXUHPHQW WR SOD\WR,QDWH ;
(QWHU WKH REMHFW QDPH 1RQH
6HOHFW W\FLY PHDVXUHPHQW 1RQH
6HOHFW W\FLY LQGH[,PDJH1XPEHU
7\SH RI PHDVXUHPHQW WR SOD\WR,QDWH <
(QWHU WKH REMHFW QDPH 1RQH
6HOHFW W\FLY PHDVXUHPHQW 1RQH
6HOHFW W\FLY LQGH[,PDJH1XPEHU