

Syntaxin 5 determines Weibel-Palade body size and von Willebrand factor secretion by controlling Golgi architecture

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Supplementary Methods

Cell culture, transfection and lentiviral transduction

Pooled, cryo-preserved primary human umbilical vein endothelial cells (HUVECs) obtained from Promocell (Heidelberg, Germany) were cultured in endothelial cell growth medium (EGM-2, Promocell) supplemented with 18% fetal calf serum (FCS, Bodinco, Alkmaar, The Netherlands), 100 U/mL penicillin, and 100 ug/mL streptomycin (P/S, Gibco), henceforth this medium will be referred to as EGM-18. Lentiviral transduction was performed as previously described.¹ Lentiviral vectors were produced in human embryonic kidney 293T (HEK293T) cells (ATCC) using a 3rd generation lentiviral packaging system. Transduced HUVECs were used for experiments at passage 5.

DNA constructs

The lentiviral LVX-mEGFP-LIC vector has been described.² A synthetic human SEC22B fragment was generated by gene synthesis containing all 214 codons of the SEC22B coding sequence, flanked by *Bsr*GI and *Not*I sites, to facilitate cloning in frame behind mEGFP in LVX-mEGFP-LIC. The non-targeting, SEC22B-targeting, and STX5-targeting pLKO.1-puro shRNA vectors were obtained from the MISSION® shRNA library developed by TRC at the Broad Institute (**Table S2**).

Mass spectrometry analysis

SEC22B interactome

HUVECs were seeded in 3 separate wells of 6-well culture plates and lentivirally transduced with mEGFP or mEGFP-SEC22B to enable sample preparation in triplicate for each pulldown condition. After 3 days of confluency, cells were rinsed 2x in PBS and subsequently lysed in mass spectrometry (MS) grade lysis buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5% NP40 (v/v)) supplemented with Halt protease and phosphatase inhibitor cocktail (Thermo Scientific). Lysates were centrifuged for 10 minutes at 16,000g and supernatants

were transferred to fresh tubes. Cleared lysates were incubated with GFP-Trap magnetic agarose beads or control (CTRL) beads (ChromoTEK) by rotation for 2 hours at room temperature (RT). For interactome analysis by MS, beads were collected on a magnetic stand and were washed 3 times with wash buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5 mM EDTA) supplemented with Halt protease and phosphatase inhibitor cocktail (Thermo Scientific, Breda, The Netherlands) and two times with 1 ml PBS. Sample preparation, data acquisition and data analysis were performed according to.³

shSEC22B and shSTX5 whole proteome

HUVECs were seeded in 3 separate wells of 6-well plates and lentivirally transduced with shRNAs targeting SEC22B as described,⁴ STX5, or non-targeting control shRNA. Untransduced cells were also included. After reaching confluency cells were lysed, samples were processed for mass spectrometry and analyzed as previously described in.⁵

MS Data analysis

MS raw files were processed with MaxQuant 1.6.2.10 using the human Uniprot database (downloaded February 2019).⁶ MaxQuant output tables were analyzed using R/Bioconductor (version 4.1.0/3.13),⁷ 'reverse', 'potential contaminants' and 'only identified by site' peptides were filtered out and label free quantification values were log₂ transformed. Proteins quantified in 2/3 of an experimental group were selected for further analysis. Missing values were imputed by a normal distribution (width=0.3, shift = 1.8), assuming these proteins were close to the detection limit. Statistical analyses were performed using moderated t-tests in the LIMMA package.⁸ A Benjamini-Hochberg adjusted P value <0.05 and absolute log₂ fold change >1 was considered statistically significant and relevant. SNARE-complex annotation were obtained from GO-term 'SNARE complex' (GO:0031201). Overrepresentation analyses were performed using clusterprofiler.⁹ Protein interactions with STRING-DB combined scores >0.9 were visualized in Cytoscape v3.8.2. The .raw MS files and search/identification files obtained

with MaxQuant have been deposited in the ProteomeXchange Consortium¹⁰ via the PRIDE partner repository with the dataset identifier PXD027516.

Fluorescence microscopy

Immunofluorescence (IF) staining and imaging was performed as previously described⁵ using a Leica SP8 confocal microscope (Leica, Wetzlar, Germany). Super resolution imaging of ER and Golgi stainings was performed on a Zeiss Airyscan (Zeiss, Breda, The Netherlands). All antibodies used for IF stainings have been specified in Table S1. WPBs were visualized using mouse monoclonal anti-VWF CLB-Rag20¹¹ or rabbit polyclonal anti-VWF (DAKO, A0058) as indicated in Figure legends. Nuclei were stained using Hoechst nuclear dye (Life Technologies; H-1399). Images were processed and analyzed using ImageJ (<https://imagej.nih.gov/ij/>). WPB length (major axis of cigar-shaped VWF positive structures) and TGN area (periphery of TGN46 staining) were measured in pixels and automatically converted to actual size in ImageJ.

VWF string assay

ECs were first transduced with shCTRL or shSTX5 and 75,000 cells per channel were seeded on day 0 in gelatin-coated 6-channel μ -slides VI 0.4 (#80606, IBIDI). The growth medium was replaced every morning and afternoon. After 4 days the VWF string assay was performed under flow conditions using 2.5 dynes/cm² and at 37°C. The cells were starved for 5 minutes using M-199 medium (Gibco) supplemented with 1% bovine serum albumin (BSA, Fraction V) and subsequently stimulated with 100 μ M histamine (Sigma-Aldrich; H7125) added to the starvation medium for 5 minutes. To visualize VWF strings, an Alexa Fluor 488 (AF488)-conjugated anti-VWF antibody (DAKO), prepared using the Alexa Fluor 488 protein labeling kit (ThermoFisher Scientific, A10235), was added at 2 μ M concentration to the medium for 5-10 minutes. Live imaging was performed on an Axiovert widefield microscope (Zeiss) using a 10x objective. Unstimulated conditions were fixed with 4% PFA for 5 minutes at RT. IF stainings for VWF, TGN46, Vascular Endothelial (VE) cadherin, and Hoechst, and confocal imaging were performed as previously described.⁵

Western blotting and VWF multimer analysis

Western blotting was performed as described.⁴ For VWF multimer analysis EC media and lysates, prepared as described in the “secretion assay” section above, were loaded on 0.9% ultrapure agarose gels run at 75 V for 5 hours. VWF was reduced using β -mercaptoethanol and transferred onto a nitrocellulose membrane (GE Healthcare) overnight by capillary suction. After blocking for 1 hour in 10% non-fat dry milk (Blotto, Chem Cruz) in PBS-0.05% Tween-20 (Sigma) and incubation with VWF-HRP (DAKO, #P0226, 1:1000) for 3 hours, the blot was visualized by enhanced chemiluminescence according to manufacturer’s protocol (Supersignal West Pico Plus chemiluminescent substrate, ThermoFisher Scientific). VWF multimers profile plots were generated in ImageJ (Analyze>Profile plot). Relative amounts of different VWF multimer sizes were quantified as the area under the curve (AUC), as a percentage of the total AUC of the plot (Analyze>Gels>Plot lanes).

Electron microscopy

Transmission electron microscopy

ECs were processed for transmission electron microscopy essentially as described.⁴ Briefly, HUVECs cultured in 10 cm petridishes were fixed (TEM: 1,5% glutaraldehyde (GA), 0,1 M cacodylate buffer, pH 7.4;) for 2 hours at RT, washed three times (0,1 M cacodylate), scraped and pelleted in 2% agar solution. Solidified pellets were cut into 1 x 1 mm³ pieces, dehydrated in an ethanol series followed by a series of EPON (LX11, Leadd) to pure EPON. After polymerizing for 2 days at 70°C, ultrathin sections (80 μ m) were cut parallel to the surface of the beam capsules using a Reichert Ultracut S (Leica Microsystems) and contrasted with uranylacetate and lead citrate. Sections were examined on a Fei Tecnai Twin transmission electron microscope (FEI, Eindhoven, Netherlands) at 120 kV, with a Gatan Oneview camera (Gatan, Pleasanton) on binning 2. Overlapping images were stitched together as previously described.¹² Image analysis was performed using Aperio Imagescope (Leica) and colored overlays were made in Adobe Illustrator.

Immuno electron microscopy

After fixation (2% PFA, 0.2% GA, 0.1 M PHEM) for 2 hours at RT HUVECs were rinsed with 0.1M PHEM buffer, scraped and pelleted in 10% gelatin. Pieces of the pellet (~0.5-1 mm³) were cryoprotected by immersion in PBS / 2.3 M sucrose solution for 1 hour, mounted on a stub, and snapfrozen in liquid nitrogen. Ultrathin cryosections (90 nm) made with a Leica EM Ultracryotome were collected on grids and incubated with rabbit-anti-VWF (DAKO, 1:1000) followed by 10 nm Protein A gold particles (1:300) in 1% BSA in PBS as described.¹³ The sections were embedded (methylcellulose, 0.3 % uranylacetate solution) and examined as described above.

Data statistical analysis

Student's t-test or one-way ANOVA with Dunnett's multiple comparisons test comparing to shCTRL were performed using GraphPad Prism 8 (Graphpad, La Jolla, CA, USA). Significance values are specified in the Figures and in Figure legends. Data are shown as mean \pm standard error of the mean (SEM), median \pm interquartile range (IR), or as contingency-stacked bar graphs.

Supplementary References

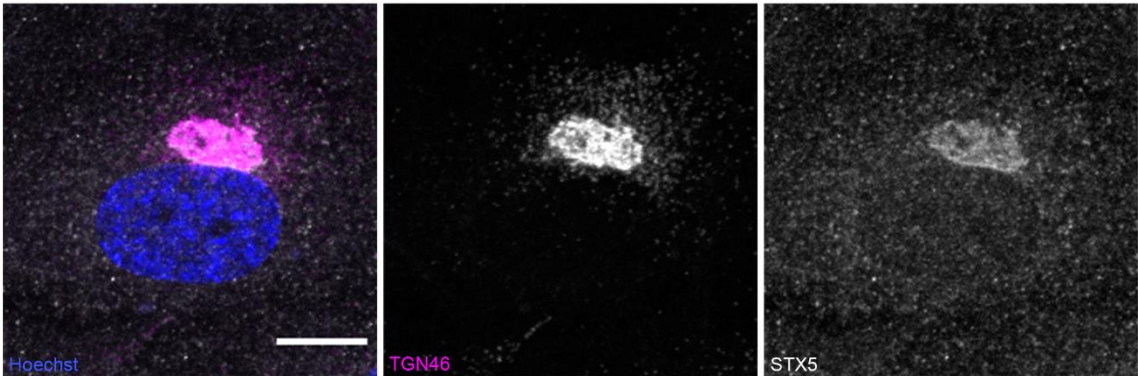
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Supplementary Figures

A



B

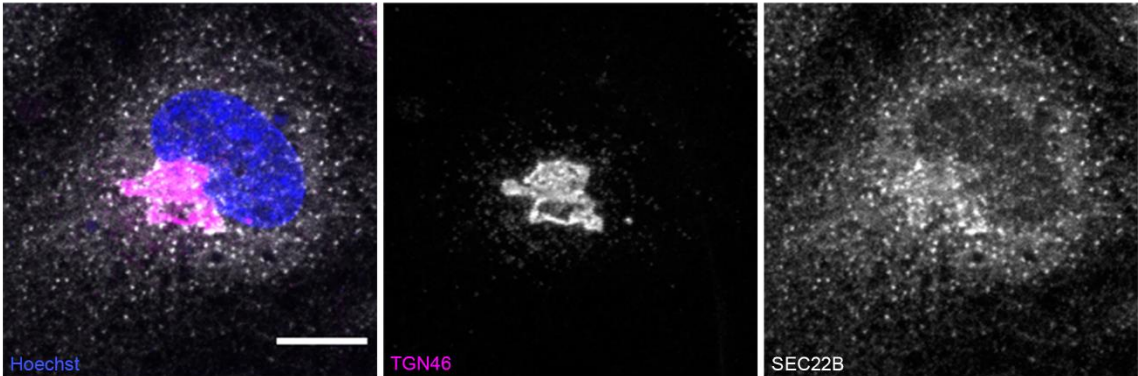


Figure S1. Endogenous SEC22B and STX5 localization. Immunofluorescent stainings of (A) STX5 and (B) SEC22B (both in gray) in HUVECs combined with *trans*-Golgi marker TGN46 (magenta) and nuclear staining Hoechst (blue). Scale bars indicate 10 μ m.

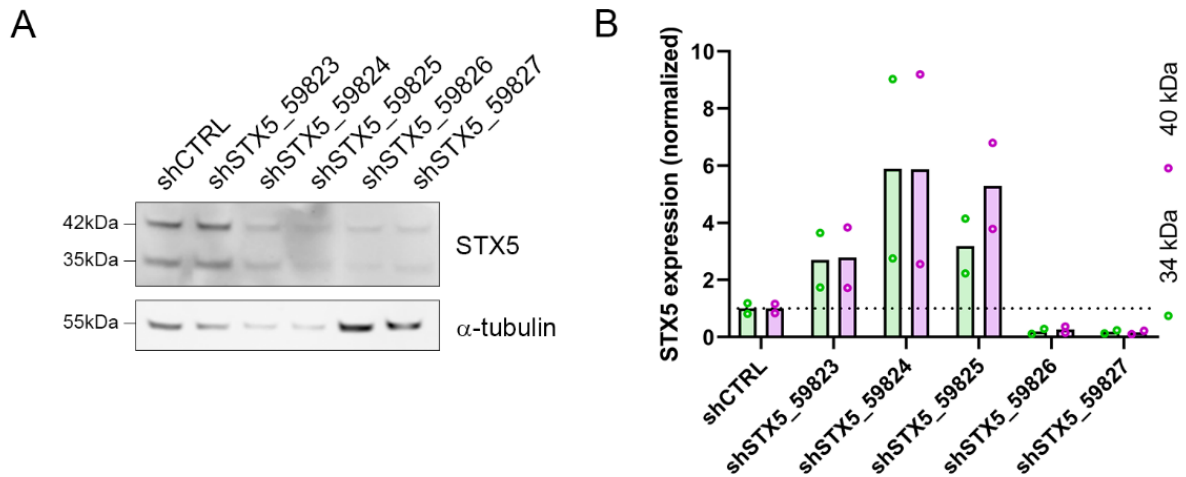


Figure S2. STX5 knockdown efficiency of single shRNAs. A) Western blot analysis of STX5 in shCTRL and shSTX5-transduced HUVECs (α -tubulin as loading control). B) Densitometry analysis of 34 and 40 kDa STX5 isoforms normalized to α -tubulin expression (mean, n=2 technical replicates).

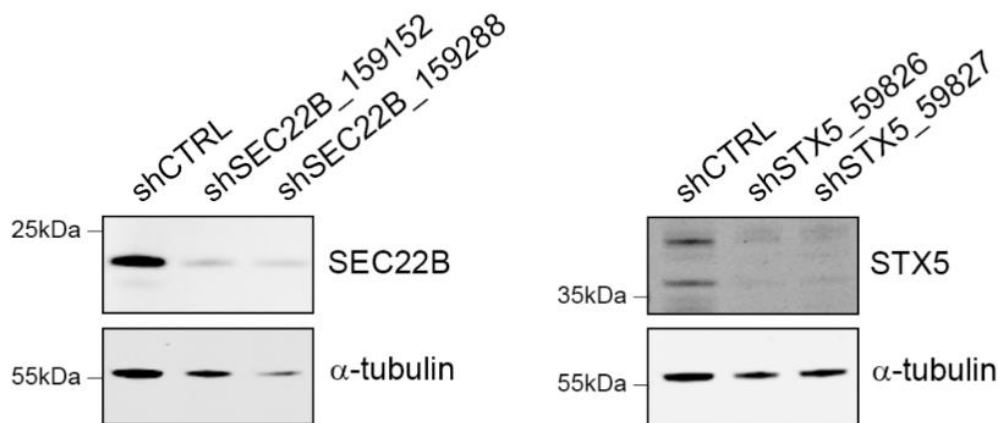


Figure S3. Efficient STX5 and SEC22B knockdown. Western blot analysis to confirm SEC22B and STX5 knockdown in SEC22B, respective STX5 shRNA-transduced HUVECs compared to shCTRL (α -tubulin as loading control).

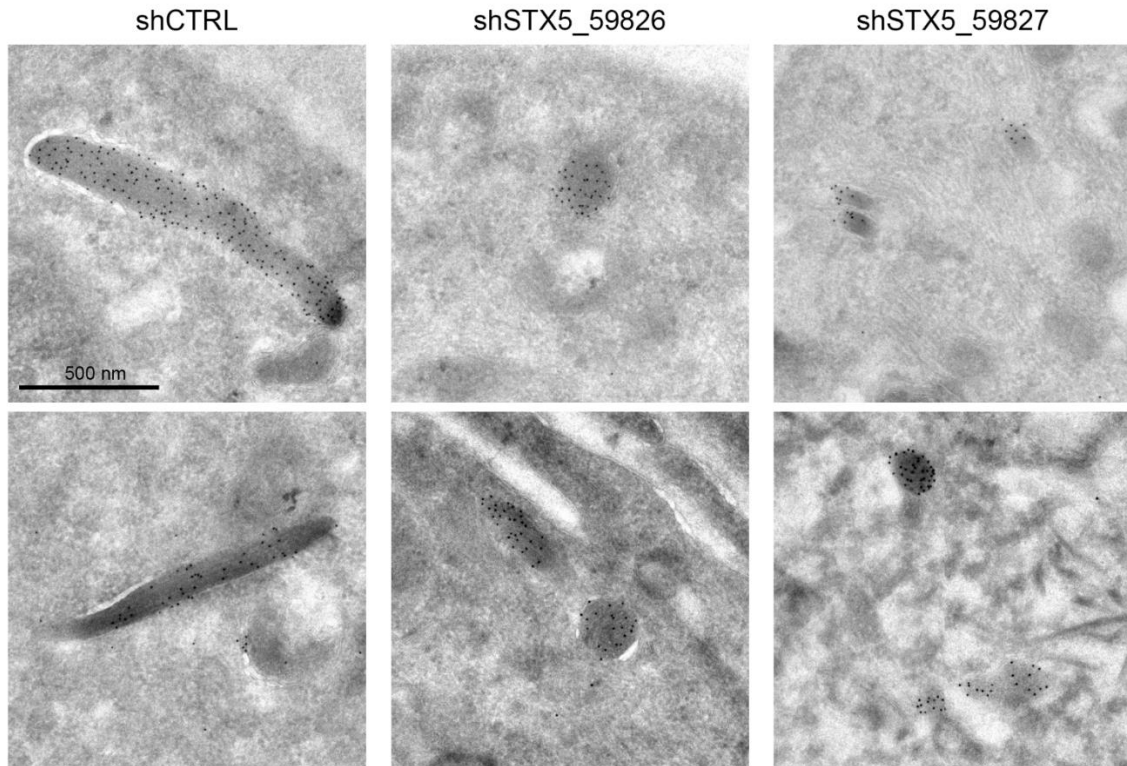


Figure S4. VWF positive structures in STX5 depleted cells resemble WPBs. Electron microscopy images of structures containing VWF immuno-gold labeling in shCTRL and shSTX5-transduced HUVECs. (scale bar: 500 nm).

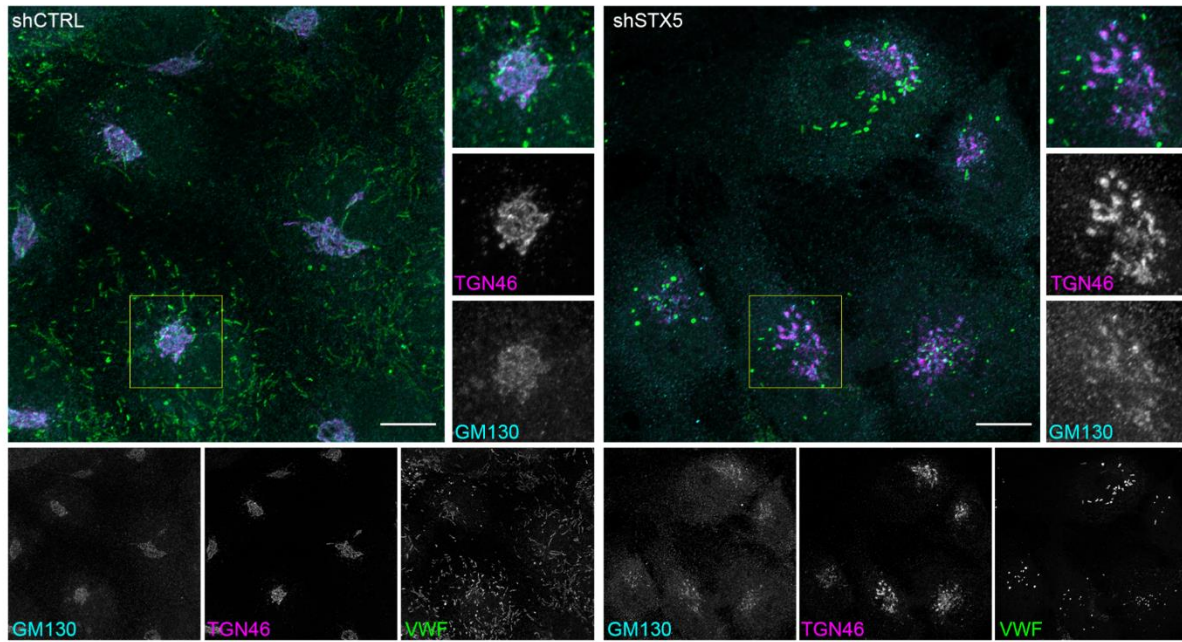


Figure S5. STX5 knockdown causes fragmentation of *cis*-Golgi. Immunofluorescent stainings of GM130 (cyan), TGN46 (magenta), and VWF (green) in shCTRL and shSTX5 transduced HUVECs (scale bar: 10 μ m). Individual channels are shown below in gray scale. Boxed areas are magnified on the right.

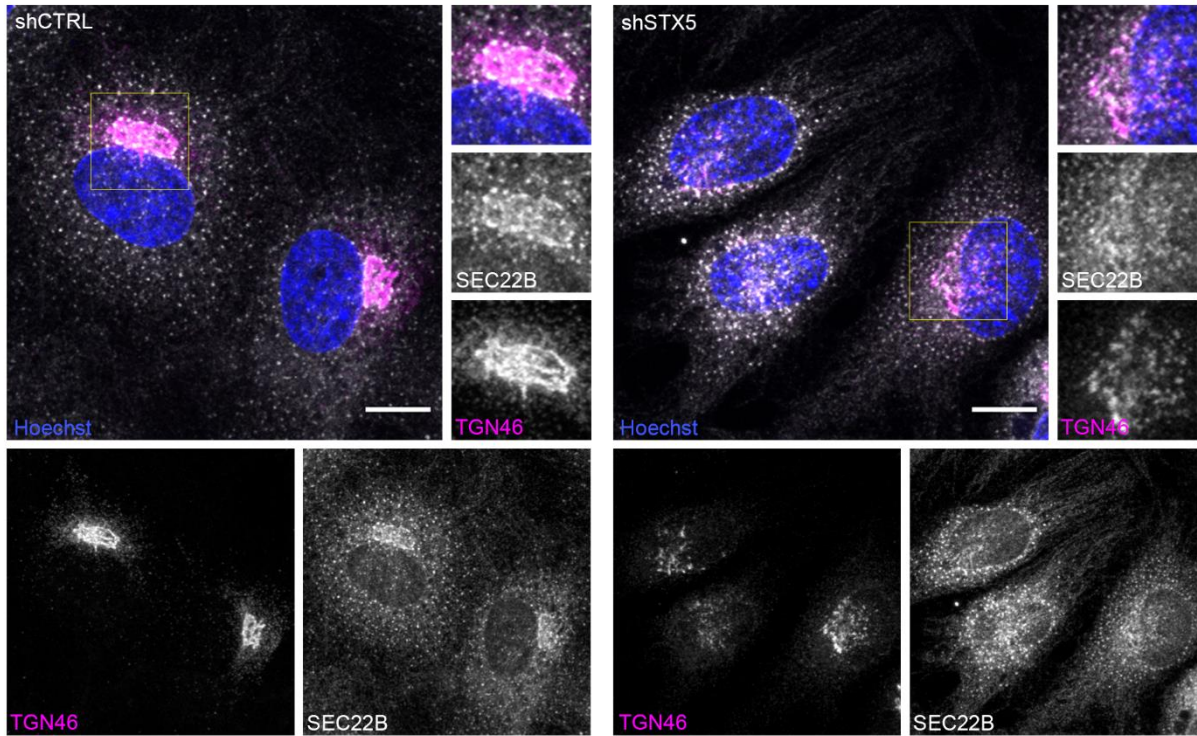


Figure S6. SEC22B localization is dispersed in STX5 depleted cells. Immunofluorescent stainings of TGN46 (magenta), SEC22B (gray), and nuclei (Hoechst, blue) in shCTRL and shSTX5 transduced HUVECs (scale bar: 10 μ m). Individual channels are shown below in gray scale. Boxed areas are magnified on the right.

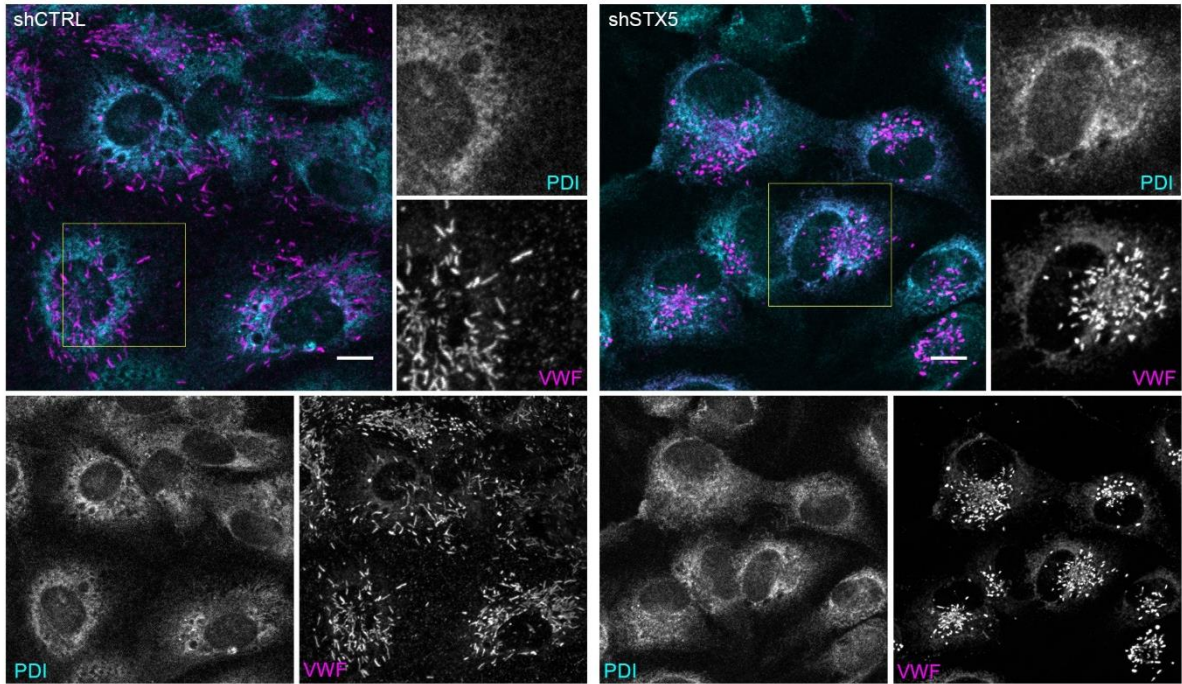


Figure S7. VWF retention in rough ER. Immunofluorescent staining of VWF (magenta) and PDI (cyan) in shCTRL and shSTX5 (scale bar: 10 μ m). Individual channels are shown below and boxed area are magnified on the right.



Figure S8. ER dilation upon STX5 knockdown. Transmission electron microscopy image of a shSTX5 cell with dilated rER sheets highlighted in yellow (scale bar: 1 μ m).

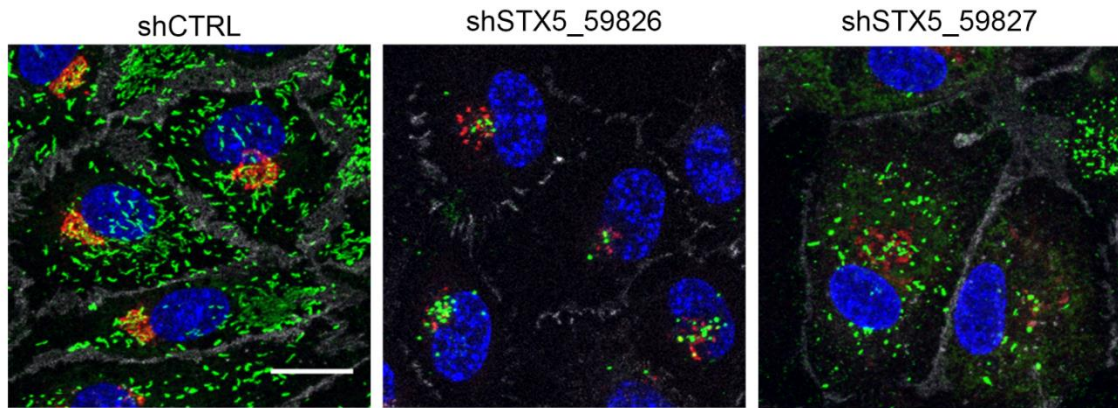


Figure S9. WPB content in unstimulated cells cultured for VWF string assay. Immunofluorescent stainings of VWF (green), vascular endothelial (VE) cadherin (gray), TGN46 (red), and nuclei (Hoechst, blue) in shCTRL and shSTX5 transduced HUVECs fixed in IBIDI channel (scale bar: 20 μ m).

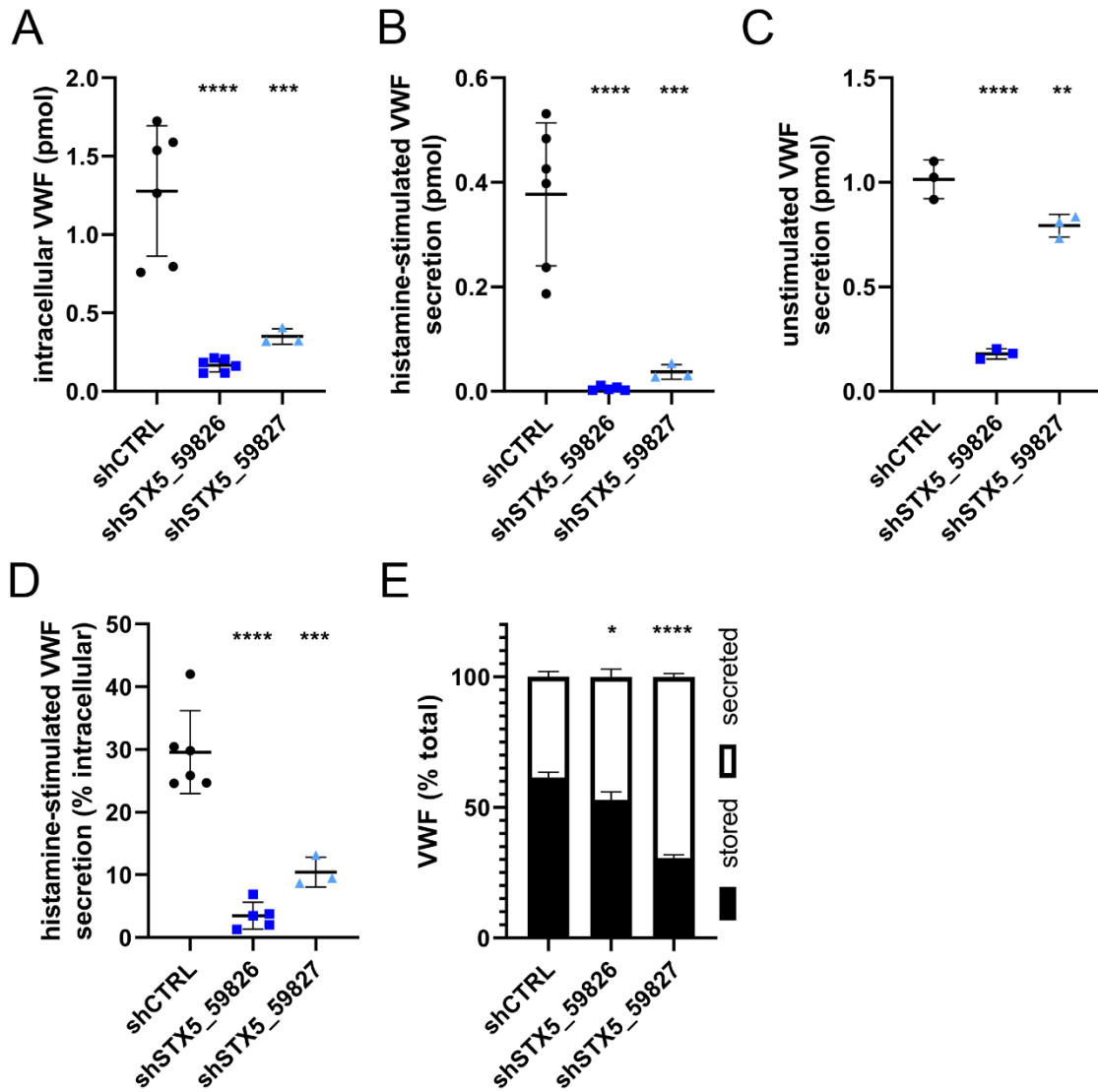


Figure S10. Reduced VWF secretion upon STX5 knockdown. A) Intracellular VWF levels in picomoles (pmol) as measured by ELISA in shCTRL and shSTX5 cells B) 1-hour histamine-induced VWF secretion pmol and C) as a percentage of intracellular VWF (of unstimulated cells). D) 48-hour unstimulated VWF release in pmol and E) calculated proportions of secreted versus stored VWF (mean±SEM, n=3-6, one way ANOVA, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).

Supplementary Tables

Table S1. Antibody list.

Target	Species (isotype)	Company/article	Clone/Cat#	Application [dilution/conc]
STX5	Rabbit	Synaptic Systems	110 053	IF [1:100] WB [1:1000]
SEC22B	Rabbit	Synaptic Systems	186 003	IF [1:100]
TGN46	Sheep	Bio-rad	AHP500GT	IF [1:500]
GM130	Rabbit	Cell signaling	D6B1	IF [1:1000]
Calnexin	Rabbit	Abcam	ab22595	IF [1:100]
PDIA3	Mouse IgG1	Sigma-Aldrich	CL2444	IF [1:1000]
VE cadherin-AF647	Mouse IgG1	BD Pharmigen	55-7H1	IF [1:200]
Ang-2	Goat	R&D Systems	AF623	IF [1:200]
CD63	Mouse IgG1	CLB	CLB-gran/12	IF [1:100]
CD62P-AF488	Mouse IgG1	AbD Serotec	AK-6	IF [1:100]
Rab27A	Rabbit	Bierings et al. ¹⁴	B2423	IF [1:50]
VWF	Rabbit	DAKO	A0085	IF [1:5000] WB [1:5000] ELISA [6 µg/ml]
VWF	Mouse IgG2B	CLB	CLB-Rag20	IF [1:5000]
VWF-HRP	Rabbit	DAKO	A0085	ELISA [2 µg/ml]
α-tubulin	Mouse IgG1	Sigma	DM1A	WB [1:10000]
GFP	Mouse IgG1	Clontech	JL-8	WB [1:2500]

Fluorophore-conjugated secondary antibodies (Molecular Probes) used for immunofluorescence microscopy were purchased from Invitrogen. Secondary antibodies conjugated with infrared dyes (680LT and 800CW) used for immunoblotting were from LI-COR.

Table S2. pLKO-shRNA sequences.

Target	TRCN	Short hairpin sequence (5'→3')
Non-targeting	shCTRL-C002	CAACAAGATGAAGAGCACCAA
SEC22B	TRCN0000159152	GCCATCAATGAGATTTAACTT
SEC22B	TRCN0000159288	GCCACAATTTGCTAACATTTA
STX5	TRCN0000059823	CCAGACAAATAAGCCAGCTTT
STX5	TRCN0000059824	GCAGTCGAAACTGGCTTCTAT
STX5	TRCN0000059825	CCATTCAGAGATCCTCAAGTA
STX5	TRCN0000059826	CCTTAGCAACACATTTGCCAA
STX5	TRCN0000059827	GCAGAACATTGAGTCGACAA

ELMO1	28,23196689	28,30708592	27,92682694	29,27659674	29,26228381	29,28786647	0,038459149
COG8	23,77776098	23,5843992	23,24190869	24,18108976	24,90554713	24,87591167	0,049045448
SFXN2	24,09192409	24,34840831	24,21044543	25,12206491	25,67656382	25,1669127	0,032313466
CTNNA1	25,35039942	25,32433247	25,91179956	26,91239362	26,0535993	26,91182242	0,021385268
HM13	NA	NA	NA	22,70186906	22,72014988	22,51542458	0,041040287
HYOU1	22,96171039	22,40914229	22,58836237	23,31824158	23,97971755	23,92858994	0,016791221
SLC25A12	27,04666425	26,97585339	26,83134842	27,68992505	28,39737982	28,02226324	0,036946623
WDR61	24,33449535	24,17584677	24,29691117	25,47342409	25,44774134	25,14156772	0,003649987
GALK1	24,80459427	24,70197479	24,46369848	25,54474658	25,95881111	25,6748681	0,029597961
SH3BP4	26,11812855	26,00945243	26,1176729	27,18196174	27,17111937	27,07856993	0,005254074
OXA1L	24,8146517	25,60902043	25,37931305	26,28211886	26,46163931	26,23128945	0,028960271
GEMIN5	25,56104177	25,70888307	25,79116547	26,88180367	26,62016858	26,71764212	0,014994316
TIMM50	26,64718743	26,35368381	26,62044828	27,26865521	27,8754899	27,62373071	0,022436251
NDUFAF4	25,98561388	24,91756985	25,20172371	26,24758756	26,70147251	26,27478094	0,033062078
EIF4A1	28,7362159	28,782318	29,0589697	29,9433549	29,86973672	29,83018507	0,026806268
THBS1	30,89489819	30,85630686	30,62400972	31,7779992	31,82937622	31,81989325	0,00469906
ACBD5	NA	NA	NA	22,35987179	22,56818159	22,32077742	0,010883419
UNC45A	29,19387487	29,2420223	29,47875231	30,51163866	30,35877664	30,04557113	0,040459904

GO:0042470	CC	melanosome	24/765	48/2446	4,10258E+14	BSG/STX3/TPP1/SLC3A2/RAB35/RAB27A/RAB29/SEC22B/TFRC/RPN1/ATP1A1/TGB1/HSP90A1/CNP/SLC2A1/RAB5A/STOM/TMED1	24
GO:0048770	CC	pigment granule	24/765	48/2446	4,10258E+14	BSG/STX3/TPP1/SLC3A2/RAB35/RAB27A/RAB29/SEC22B/TFRC/RPN1/ATP1A1/TGB1/HSP90A1/CNP/SLC2A1/RAB5A/STOM/TMED1	24
GO:0005778	CC	peroxisomal membrane	14/765	24/2446	4,41353E+14	PEX1/PEXS/ACBD5/ACSL1/MGST1/ALDH3A2/ACSL4/ACSL3/ABCD3/ABCD1/MAP2K2/PEX6/MAVS/ATAD1	14
GO:0031903	CC	microbody membrane	14/765	24/2446	4,41353E+14	PEX1/PEXS/ACBD5/ACSL1/MGST1/ALDH3A2/ACSL4/ACSL3/ABCD3/ABCD1/MAP2K2/PEX6/MAVS/ATAD1	14
GO:0030672	CC	synaptic vesicle membrane	8/765	11/2446	4,53968E+14	SCAMP1/STX12/RAB35/STX10/STX6/VAMP4/RAB5A/VAMP3	8
GO:0099501	CC	exocytic vesicle membrane	8/765	11/2446	4,53968E+14	SCAMP1/STX12/RAB35/STX10/STX6/VAMP4/RAB5A/VAMP3	8
GO:0005769	CC	early endosome	42/765	96/2446	4,68937E+14	TM9SF4/CLN6/STX12/VPS8/WLS/RFTN1/GPR107/MGRN1/RAB29/STX7/STX6/ATP9A/SNX4/TFRC/APP/IGF2R/HLA-E/GJA1/PTPN1/RJ	42

Table S5. Differentially regulated proteins in shSEC22B and shSTX5 endothelial cells.

shCTRL A	shCTRL B	shSEC22B			shSEC22B			shSEC22B			shSTX5			shSTX5			Untransduced A	Untransduced B	Untransduced C	Untransduced D	Gene name	Correlation cluster	Regulated by
		159152 A	159152 B	159152 C	159288 A	159288 B	159288 C	59826 A	59826 B	59826 C	59827 A	59827 B	59827 C										
30.23008943	30.38655934	30.14479433	29.57287977	29.94893377	29.6563754	30.24524524	30.21754868	29.94251621	28.3301413	28.4172369	28.5327451	29.3750923	29.0812649	29.2628662	30.2067619	29.9795104	29.99293976	ACAT1	1	STX5			
27.34053512	27.247117103	27.51209096	27.24445157	27.47351699	27.09727956	26.48319225	NA	26.78144276	NA	NA	26.6499302	NA	NA	NA	NA	NA	26.75506319	CD33	1	STX5			
25.47540467	25.61837721	25.84340393	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	26.05465532	25.48105293	26.18012238	CREB1	1	SEC22B, STX5			
30.26472392	30.32635278	30.26852137	27.14180156	26.81784483	27.3900162	31.36202112	30.90591145	31.1369342	29.2740878	28.7231548	28.8434698	27.7101174	27.5546453	27.6247767	30.74518945	31.03091638	30.77266111	EFEMP1	1	STX5			
35.47311898	35.10968873	35.34314376	33.92768893	33.50403196	33.59890191	36.90477264	36.94251184	37.17849469	34.1093774	33.6472089	33.7954644	33.65105459	33.2716968	33.2479915	35.1943608	35.14910981	35.08079667	FN1	1	STX5			
28.81231531	28.4909839	28.36765543	28.08542456	26.57022169	27.02104553	27.03611491	27.3786518	27.59704662	26.9813082	26.920594	27.3281734	26.3464652	26.4090516	26.5085139	28.67033641	28.40944824	28.58373236	GRN	1	SEC22B, STX5			
26.32697417	26.46151442	26.2247551	NA	NA	24.49234465	31.94363436	31.93266004	31.99742223	24.8451761	25.2486917	24.0279148	NA	NA	NA	27.22370611	27.71279712	27.80636554	IGFBP7	1	STX5			
29.30014051	28.99023682	29.12045408	28.15450834	28.04207798	28.01179984	30.03249367	30.39130755	30.17080907	28.0818236	27.4581069	27.8037201	25.92241	25.9329655	26.1534843	28.47908009	28.69252132	28.46349581	LTBP1	1	STX5			
26.32890949	26.36688039	26.52169149	NA	NA	NA	27.4540324	27.2654395	27.57195813	NA	NA	NA	24.9483769	NA	NA	26.8062426	26.63159207	26.57369249	LTBP2	1	STX5			
28.06686743	27.78044185	27.92212641	26.53506139	26.82262315	26.31342295	28.29824049	27.91098082	28.18517972	26.7610376	26.5333096	26.4451225	26.8598583	26.7960024	26.6205881	27.43205197	28.01105335	27.66044143	MAOA	1	STX5			
30.81191738	30.76864455	30.75827593	29.33212785	29.32425091	29.63709729	30.41780025	30.39519511	30.40446245	28.4027154	28.2655289	28.1147077	29.7845506	29.9215307	29.5615626	31.10017801	30.88554671	30.80470504	MMRN2	1	STX5			
25.64839489	26.49596198	26.10860932	25.30949051	NA	25.56508697	25.38162506	24.90504209	NA	NA	NA	NA	25.5858036	NA	NA	25.57441452	25.59089378	25.83093758	MTFR1L	1	STX5			
28.05346466	28.24624415	28.21618449	27.9686762	27.5896372	27.65724157	27.75206668	27.81200922	28.13775267	26.6142823	26.6281189	26.4426254	27.0034599	26.8075943	26.9667526	27.69869365	27.3711086	27.59077959	NIPSNAP1	1	STX5			
25.30758106	25.03066393	25.10976531	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	25.88201365	25.30260462	25.9559907	FN2A	1	STX5			
25.20254595	25.53747768	25.61647144	25.4567918	25.40801507	25.412383	25.85357608	25.84302048	26.11628526	24.9937335	NA	24.6481754	NA	NA	NA	25.96974133	26.03309259	26.09105648	PCDH10	1	STX5			
25.36359259	25.48726204	25.37471104	25.76759367	25.35444068	25.47385758	25.40447869	25.02791477	25.15093073	NA	NA	24.6003685	24.496922	24.9685993	NA	24.92474474	25.04789173	24.9812429	PIP5K1C	1	STX5			
28.22999778	28.03773889	28.17944862	27.37285284	27.39607348	29.25745863	26.9760993	26.85263357	26.7490639	26.7261144	26.7774349	26.2804206	26.1567856	26.4819922	26.8050267	28.28330289	28.42930068	PYCR1	1	STX5				
25.88310974	25.77386682	26.07632877	25.88683487	26.07742941	25.77484898	27.44007645	26.82310927	26.61225409	24.2971211	NA	24.6805664	NA	NA	NA	26.5927766	26.74265421	26.28714872	PYOXC2	1	STX5			
24.92474474	25.52880176	25.68461145	NA	NA	NA	NA	24.79436708	NA	NA	NA	NA	NA	NA	NA	24.54073253	24.93701347	NA	QSX2	1	STX5			
25.84496067	26.17420918	25.62731189	25.36101716	NA	25.54008215	26.02455821	26.32993604	25.83414869	NA	NA	24.8892046	NA	NA	NA	26.02312032	25.66383777	25.66955271	RIF1	1	STX5			
30.85549025	31.06618741	30.75771978	31.43966044	31.27169684	31.36416266	30.62121718	30.45454235	30.49667065	29.6001522	29.5743423	29.4256441	29.2104594	29.2373567	29.3695911	30.16734349	30.11458357	30.24547194	SH3BGR1	1	STX5			
26.43264142	26.3948477	25.66133884	25.43096803	25.81984911	25.75608187	NA	NA	NA	NA	NA	NA	24.4008034	NA	NA	25.94937915	26.04693481	25.7464522	SYNM	1	STX5			
25.91754709	25.37607	25.55496725	NA	NA	NA	NA	NA	NA	NA	NA	24.607836	NA	NA	NA	NA	25.52886136	25.02808345	25.24796777	TACSTD2	1	SEC22B, STX5		
NA	NA	NA	26.78094239	26.4322591	26.52602754	NA	NA	NA	NA	NA	NA	26.6758642	26.4562121	26.1908411	NA	NA	NA	AFAP1L1	2	SEC22B			
NA	NA	NA	26.71161615	26.80636554	26.5567806	26.79012155	26.05355788	NA	25.7594638	25.7243198	25.6999911	26.2406174	26.095027	25.8210664	NA	NA	NA	AGO1	2	SEC22B, STX5			
NA	NA	NA	NA	26.38066768	26.95925345	25.7665831	25.74995901	26.04393509	NA	NA	NA	25.9040315	NA	NA	NA	NA	NA	NA	AGPAT1	2	SEC22B		
28.84828405	29.13560166	28.97254317	29.85196609	30.14297021	30.05452595	30.16088608	30.306608	30.09471504	29.1713819	29.6102919	29.3922088	29.5156667	29.3602637	29.321974	28.94519831	29.29756286	29.11063169	AK3	2	SEC22B			
26.27923423	26.03795877	26.25880682	27.02886332	27.89029527	27.54752919	27.3378161	27.3069556	27.97185829	26.6512996	25.893053	26.7219497	27.8463722	27.7863123	27.8698893	NA	26.30293576	26.39067227	APOC3	2	SEC22B			
NA	NA	NA	NA	25.99392777	NA	25.53198701	25.60002813	24.66394632	26.458326	26.9721323	26.7439384	25.7485522	25.8411017	25.2630588	25.18196174	NA	25.80769256	APPL2	2	SEC22B, STX5			
29.33287478	29.22953946	27.91337086	30.81596807	30.45032006	30.49018061	30.5995671	30.77462594	30.71947113	29.9145621	30.0905569	30.1305047	30.7049518	30.1836426	30.1781904	30.53296796	30.02194568	30.33031226	ARPC3	2	SEC22B			
NA	NA	NA	27.7979358	27.44442752	27.0095922	26.92592201	NA	NA	NA	NA	27.8186064	27.7002822	27.6351953	NA	NA	NA	NA	ATP6AP2	2	SEC22B			
27.39140999	25.81999524	26.15216939	29.04609177	29.97541611	29.14011997	28.96375208	28.51634321	28.90592579	28.0694819	28.4083553	28.4378563	28.0130786	28.2921523	27.839721	26.60978136	26.30975069	26.63878964	BICD2	2	SEC22B, STX5			
25.29263506	NA	25.47707367	26.90138676	26.71135357	26.85174275	27.18876781	27.28859266	26.65526364	27.0051746	27.2644555	26.8964292	27.3028661	27.4669995	27.2991144	27.67485463	27.92223985	27.68618888	BIN1	2	SEC22B, STX5			
NA	25.18570905	24.8811035	NA	25.87112428	25.81369803	25.55042388	25.9789911	25.09663635	26.0100503	26.2004146	26.064126	25.7366288	25.7421659	25.5143955	NA	25.15915563	NA	BNIP1	2	STX5			
24.39185244	24.64921758	NA	25.76817444	25.95251518	26.02780934	25.77210733	25.74783547	26.10795119	26.1028154	26.0830215	26.1389148	24.9367889	25.2087351	25.1152536	25.98997166	25.69763362	25.88778789	BRD4	2	SEC22B			
26.30535596	26.5830828	26.39275331	27.72929884	27.76407888	27.70272786	27.74406679	27.73918109	28.31704275	28.2673162	27.9691155	28.1575472	27.0437444	26.9496018	26.7781872	26.71133216	27.56000506	27.39737892	CBX1	2	SEC22B			
30.6462434	30.61745617	30.58530935	31.7876828	31.73976857	31.81130496	32.01733976	31.98243753	31.83424516	32.0443013	31.6486452	32.0638434	30.9174816	30.729068	30.9988685	30.46626053	30.67109605	30.48577367	CD9	2	SEC22B			
NA	NA	NA	25.51337172	24.8381225	25.33101313	25.06755483	26.08107183	NA	25.0703011	24.7958388	25.336063	25.1454602	25.2991493	27.23708316	26.96422046	26.82711356	CDH11	2	SEC22B				
NA	NA	NA	28.49533685	28.3600125	26.85174275	27.19131183	28.62968284	28.07423727	27.6707416	27.6753932	27.5832661	28.2582677	28.7286815	29.0962744	28.0403021	28.63554134	27.57036647	CFDP1	2	SEC22B, STX5			
NA	NA	NA	25.84677872	25.73127759	25.79317661	25.76564768	25.68955187	NA	NA	NA	NA	NA	25.5630221	25.7411631	25.3757718	NA	NA	CHMP4A	2	SEC22B			
27.73130349	28.15822159	27.96515676	28.94218059	29.25421783	28.78861649	29.31170491	29.34747931	29.53963854	28.2826403	28.225454	27.9913997	28.6470502	28.9561542	28.9657109	29.02427286	28.9054381	28.93691241	CIRBP	2	SEC22B			

28.04072015	27.71423923	27.69849495	NA	25.70790948	26.04859862	27.16298178	26.13662868	NA	28.444072	28.0521175	28.4857737	27.7235128	27.2164158	27.7705203	28.29469583	28.21771044	28.35068605	SNRPC	3	SEC22B	
28.9643031	28.90339948	28.59985083	NA	NA	NA	26.11457861	26.20267672	NA	26.9919403	27.2729317	26.9238838	26.3316624	26.3124192	25.4535615	27.45159787	27.59903497	26.90058088	SORBS2	3	SEC22B, STX5	
31.75585114	31.83492324	31.44997585	29.48275771	29.2657748	29.54333113	30.20314832	30.49609917	30.18742332	29.4272837	29.4349173	29.4617798	31.6487309	31.7801915	31.7846052	30.48941515	30.64581409	30.58190232	SPARC	3	SEC22B	
27.87689535	27.6953121	28.08527259	NA	NA	25.6278963	26.089845	26.01627068	26.69232178	26.784691	27.0859817	27.4774597	27.0915408	26.5317937	27.1191186	27.16269373	27.76894421	27.75059802	SRSF6	3	SEC22B	
26.20235912	26.19964734	26.10837003	25.1226574	NA	25.33459764	NA	25.07361434	NA	25.7370416	26.2387056	25.6972624	26.2367366	26.3377821	26.193419	27.16086808	27.29386311	26.88599755	STAU2	3	SEC22B	
29.83421501	29.73280808	30.035512	25.17470446	26.10892831	25.0847659	26.03065333	25.53165988	26.02295106	28.3120729	28.3806347	28.4094887	29.3859707	29.0731138	29.3889494	29.26420938	29.61407164	29.19608154	TFRC	3	SEC22B	
33.58542128	33.3353795	33.8202167	28.71315778	28.58401921	28.84628257	33.03467603	32.89008504	33.08679791	30.8418365	30.415888	30.4998097	31.5243993	31.508478	31.6026231	33.17328365	33.13781984	33.1682854	THBS1	3	STX5	
27.19826143	27.10209457	26.88471738	25.56563903	26.36721383	24.89642923	NA	24.38396632	NA	25.2982755	25.1558404	25.2198164	26.8513853	26.6056902	26.511322	27.31094694	27.0215751	27.07551293	TIE1	3	SEC22B	
26.11676117	25.79585378	25.4652565	NA	NA	NA	25.14117789	24.88604408	NA	25.7157323	25.4535301	25.0992476	26.5957669	25.6387068	25.781968	25.7456575	26.86997199	26.39445524	TRIM22	3	SEC22B	
27.46629943	27.52663952	27.9290868	25.72507434	NA	26.03120042	NA	NA	NA	27.3056517	27.3062603	27.0708129	26.4404725	26.5582559	26.5314368	27.29570322	27.37815565	27.48684791	TRIM47	3	SEC22B	
26.89122282	27.42934059	27.5555232	NA	NA	NA	25.03132664	25.68217507	25.79382157	26.3882597	26.5305887	26.3439436	26.7341496	26.9298769	26.77505	27.0820267	27.41804162	27.1284903	TSNAX:DISC1	3	SEC22B	
29.03915183	28.95986144	28.94198478	27.61456315	27.59042269	27.67667142	27.06074853	27.35194991	26.76268576	27.2502469	27.3633587	27.465599	28.8670578	28.9232321	28.9726253	28.81729544	28.73489249	28.71230517	TST	3	SEC22B	
25.8362935	25.96778615	26.37999052	NA	24.23622563	24.02943215	25.22195611	25.43205197	NA	24.9431537	25.1772924	25.2711336	25.5049734	25.4768574	25.4126737	25.40691291	25.96941199	26.33596083	UAP1L1	3	SEC22B	
32.65731825	32.52318441	32.51451026	31.1016188	30.93368035	30.97240622	31.19876491	31.46372965	31.12028103	31.8700088	31.8440463	31.870707	31.7320236	31.6133341	31.6471016	32.02785546	32.33306145	32.25141001	UCHL1	3	SEC22B	
28.66444826	28.23260742	28.54333113	NA	25.89746826	26.36396014	NA	27.21604569	26.59576694	26.8329182	27.3014712	26.7771841	28.2451319	28.4086792	28.4688258	28.46995153	28.61999374	28.05765351	VAMP3	3	SEC22B	
27.72194965	27.44994634	27.39124608	NA	NA	NA	NA	NA	25.8393245	NA	26.2020228	27.1150551	26.9765091	26.834004	27.77379318	27.84128168	27.68739085	VAMP5	3	SEC22B		
35.41808938	35.35635816	35.36169439	32.75787871	32.72419621	32.64254715	33.52647629	33.44418559	33.68217089	32.6625057	32.4796198	32.5970599	33.3494211	33.5812738	33.5813861	35.30198354	35.26027855	35.27648859	VWF	3	SEC22B, STX5	
26.68618888	27.13858289	27.43356494	NA	NA	NA	NA	NA	NA	NA	NA	27.102395	27.5922063	27.128392	26.0898248	24.87965541	25.8159467	ZC3HAV1L	3	SEC22B		
32.24872677	32.24612349	32.16104834	30.92634627	30.99745588	31.24541527	30.53741844	31.217722	31.42947029	33.2353493	33.134121	33.1001623	32.1907528	32.1972715	31.985223	30.59894627	30.86348796	30.92500235	AKAP12	4	SEC22B	
25.61506853	25.78977341	25.60320163	NA	NA	23.70893568	23.94154271	NA	24.21697079	25.3365057	24.9905344	25.2325342	26.2232458	25.977623	26.3038939	NA	NA	NA	BNIP3	4	SEC22B	
NA	NA	NA	NA	24.81811872	NA	NA	NA	NA	NA	NA	NA	26.1907846	26.4842223	26.5371666	27.652531	27.19413327	27.70464182	CGNL1	4	STX5	
28.00099148	27.57036647	27.34798611	25.29498498	24.79585378	25.52109239	NA	NA	NA	28.9757168	28.8833487	28.8051357	28.3743877	28.4111872	28.5649998	NA	NA	NA	ESM1	4	SEC22B	
NA	NA	24.57871036	24.67986878	NA	NA	NA	NA	NA	25.1672956	25.1631546	25.285033	24.8664153	25.4931086	25.0544069	NA	NA	NA	FARP1	4	STX5	
25.61633121	25.45327892	25.38053558	NA	NA	NA	26.111193527	25.8540281	26.0548209	27.6097814	27.4998287	27.6675645	27.2409994	27.1916883	27.1631738	26.30789369	26.19844879	26.43273698	FSTL1	4	STX5	
30.2724201	30.36191657	30.22054996	30.94118727	30.89113589	31.02207801	29.53473125	29.35205518	29.49594674	31.6267534	31.5669693	31.7202461	32.0928264	32.0569497	32.1496171	31.08853646	31.12896958	31.16883829	IGF2BP3	4	STX5	
24.7280625	25.18744692	24.92261416	NA	NA	NA	25.53219515	25.85293349	25.46600375	26.628397	26.6772093	26.1701453	26.0371838	26.0981836	26.0857386	25.6975806	25.60087886	25.26931534	LMO7	4	STX5	
29.39065177	29.57535262	29.49300927	29.20263468	29.17776286	29.03456781	28.10529567	28.25435301	28.30029327	30.5723558	30.6902915	30.6192067	30.9388061	30.968429	30.8846737	27.92320379	27.89775675	27.81268253	MMP1	4	STX5	
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	27.2896483	27.1814879	27.2441793	NA	NA	NA	MYCT1	4	STX5	
NA	NA	NA	26.52157169	NA	NA	NA	NA	NA	26.5783073	NA	26.2736966	26.3766662	25.8997745	26.2174886	NA	NA	NA	QXR1	4	STX5	
NA	NA	NA	NA	25.87321485	NA	NA	NA	NA	25.2859855	25.6354168	25.8991522	26.1053556	25.9749349	26.2401988	NA	NA	25.23611612	NA	PDPK1:PDPK2P	4	STX5
24.9791942	NA	NA	25.87602881	25.78591337	26.15749898	24.43879236	NA	24.46687505	25.8736842	26.0397584	25.9066484	27.4752191	27.395665	27.3562388	25.67591806	25.88000589	25.84484098	RALGAPA2	4	STX5	
26.22171647	27.16432525	26.78556422	28.36185384	28.49121333	28.34726809	NA	24.60715871	NA	28.3066949	28.5262291	28.4599836	30.3088876	30.1821039	30.1144595	25.48646434	24.86240051	25.6164434	SLC12A2	4	STX5	
26.66261601	26.42447925	26.75951459	28.09898672	28.07183598	28.10559541	26.211764	26.45614946	26.01047723	28.425464	28.4057612	28.2814027	28.8120398	28.2633813	28.6582976	26.71685755	26.44733147	26.56293482	STK10	4	STX5	