Supplementary Materials

Figure S1

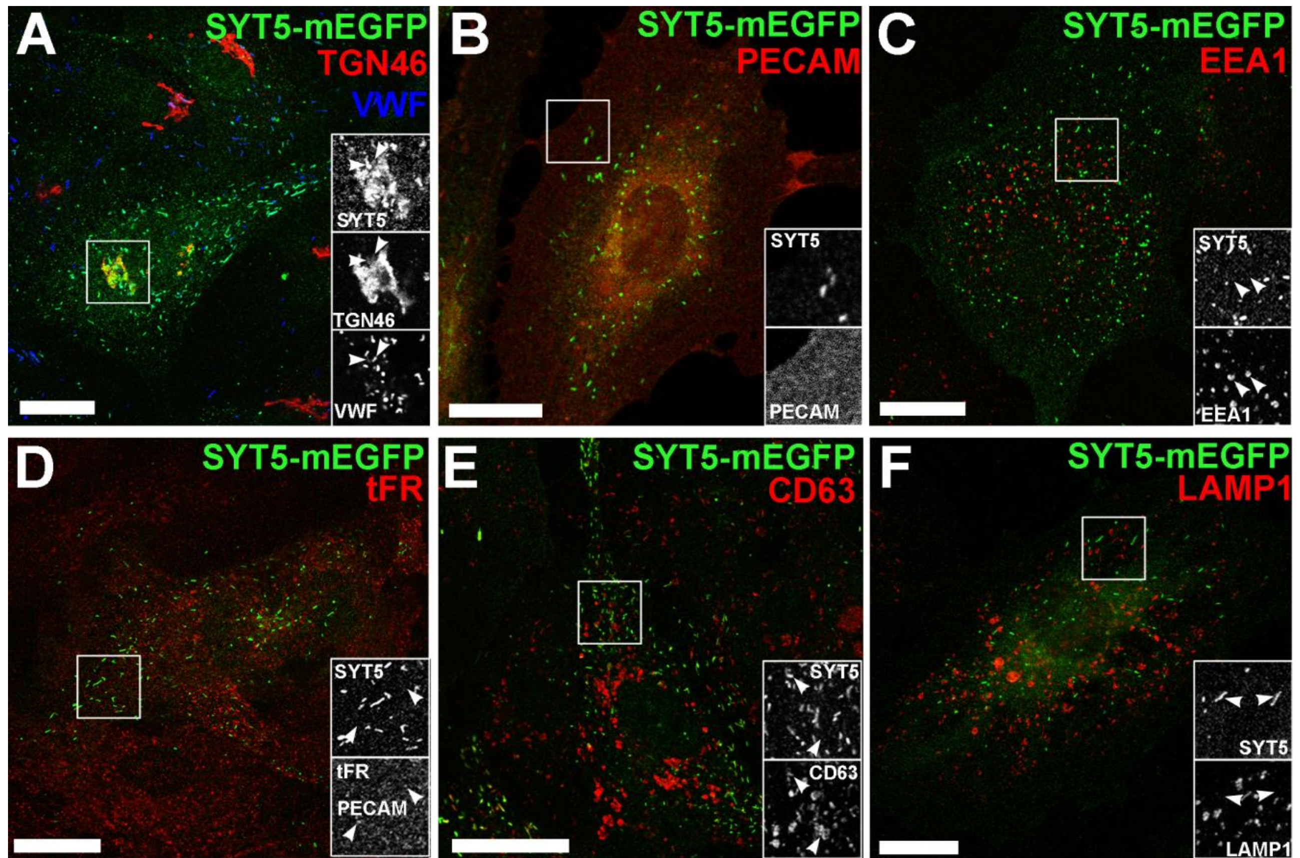

Figure S1. Subcellular distribution of SYT5-mEGFP. Confocal fluorescence images of single HUVEC 24 hours after Nucleofection™ with SYT5-mEGFP, and immuno-labeled with specific antibodies to GFP (sheep; green), VWF (top left panel only, rabbit; blue) and (A) TGN46, (B) PECAM, (C) tFR, (D) EEA1, (E) CD63 or (F) LAMP1 as indicated (red). Scale bars are 10µm. Inset panels show in greyscale regions indicated by white box.

Figure S2

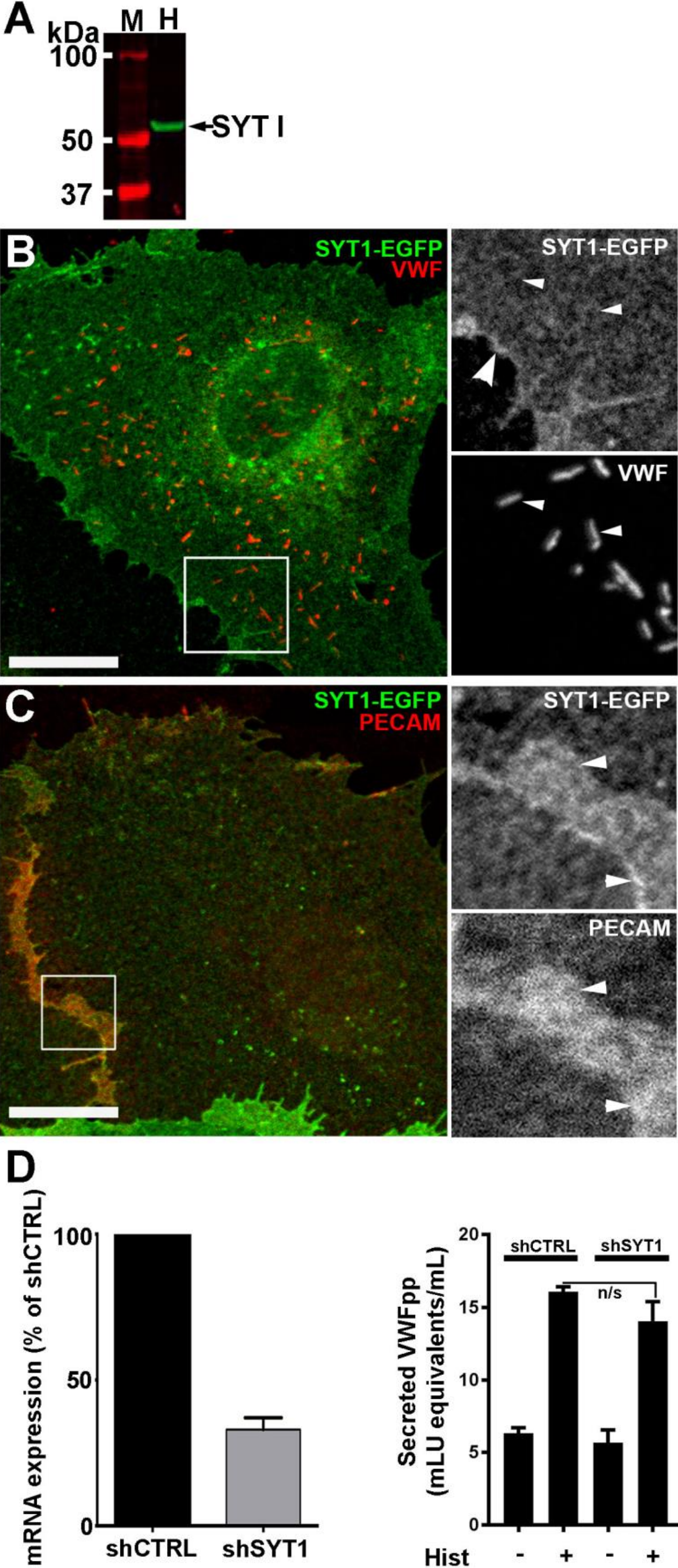

Figure S2 SYT1 is expressed in HUVEC but is not recruited to WPBs. (A) representative Western blot produced by probing HUVEC (H) lysate with the SYT1 primary antibody at a concentration of 1:200. The sizes of the marker (M) are shown on the left. A strong band at approximately 55 kDa represents SYT1 protein. (**B, C)**, Confocal fluorescence images of HUVEC 24 hours after Nucleofection™ with SYT1-mEGFP, and immuno-labeled with specific antibodies to (**B**) GFP (sheep; green), VWF (rabbit; red) and (**C**) GFP (sheep; green), PECAM (mouse; red) Scale bars are 10µm. Inset panels show in greyscale regions indicated by white box. (**D**) left; Quantification of shSYT1 mediated SYT1 mRNA depletion. Data is normalized to shCTRL and is mean±SEM of 3 independent experiments carried out in duplicate. Right; Histamine (100 µM) evoked VWFpp secretion in shCTRL or shSYT1 treated HUVEC. Experiment shown is mean ±sem and is representative of 3 independent experiments each carried out in triplicate.

Figure S3

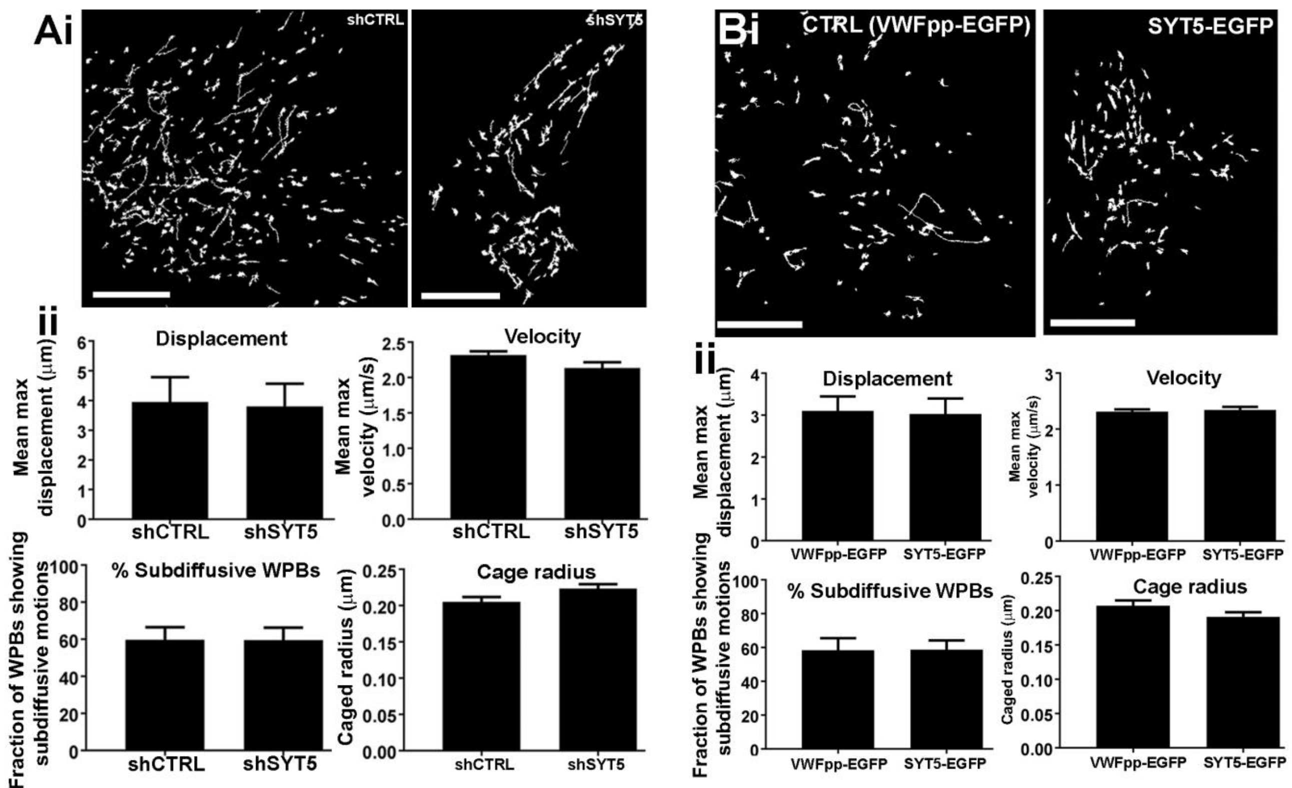

Figure S3. SYT5 depletion (A) or SYT5-EGFP overexpression (B) does not alter WPB trafficking close to the plasma membrane. (Ai). Representative X-Y trajectories of individual WPBs in single HUVEC expressing VWFpp-EGFP and following lentiviral transduction with shCTRL (**left**) or shSYT5 (**right**). Trajectories were determined here and elsewhere from TIRFM videos using the ASPT function of GMimPro software as described previously (Conte et al., 2016). Number of cells imaged and trajectories detected were: shCTRL, n=6 cells, 890 trajectories; shSYT5 n=6 cells, 740 trajectories. (**Aii).** Parameters determined from detected trajectories of long range (top panels) or short range (lower panels) WPB movements. Number of WPBs analysed for short range movements were: shCTRL, 135 trajectories; shSYT5, 149 trajectories. **(Bi).** Representative X-Y trajectories of individual WPBs in single HUVEC expressing VWFpp-EGFP (**i**) (control, n=7 cells, 693 trajectories) or SYT5-EGFP (**ii**) (n=7 cells, 735 trajectories) after lentiviral transduction. (**Aii).** Parameters determined from detected trajectories of long range (top panels) or short range (lower panels) WPB movements. Number of WPBs analysed for short range movements were: control, 76 trajectories; SYT5-EGFP, 103 trajectories.

Figure S4

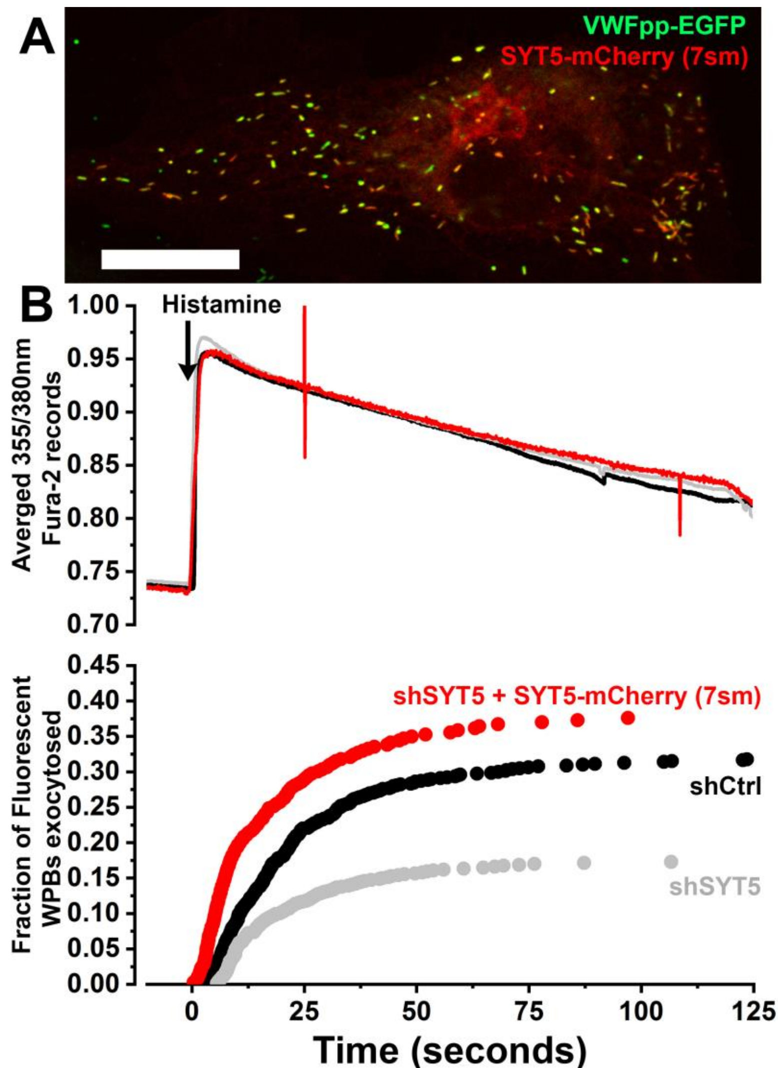

Figure S4. Overexpression of SYT5-mCherry (7sm) prevents shSYT5 mediated reduction in histamine-evoked WPB exocytosis in HUVEC. (A). Confocal fluorescence image of a HUVEC co-expressing VWFpp-EGFP (green) and SYT5-mCherry (7sm) (red) 24 hours post transfection. Scale bar is 10μm. (**B**) top panel shows averaged 355nm/380nm Fura-2 fluorescence ratios from shControl treated HUVEC (black, 10 cells), shSYT5 treated HUVEC (grey, 9 cells) and shSYT5 treated cells Nucleofected™ with SYT5-mCherry (7sm) (red, n=12 cells, 24 hrs post transfection). For clarity the ±95% confidence limits for the mean fluorescence ratios have been omitted. Histamine (100μM). Was added at the arrow. Lower panel in B shows cumulative plots of histamine-evoked WPB fusion times scaled by the mean fraction of WPBs that underwent exocytosis and colour coded as in (**A**). The mean (±SEM) maximal rates of WPB exocytosis in response to histamine were; shCtrl; 2.3 ± 0.3 WPBs/second, n=13 cells, shSYT5; 1.4 ± 0.2

WPBs/second, n=16 cells, and shSYT5 + SYT5-mCherry (7sm) 6.4 ± 1.1 WPBs/second, n=12 cells. The fraction of fluorescent WPBs that underwent exocytosis were; shCtrl; black trace 32.4 ± 1.4%, 136 fusion events, n=13 cells, shSYT5; grey trace,17.3 ± 2.9%, 129 fusion events, n=16 cells, and shSYT5 + SYT5-mCherry (7sm); red trace, 37.5 ± 3.1%, 158 fusion events, n=12 cells.

Supplementary Tables

Table S1. Antibody reagents.

Antigen	Manufacturer	Catalogue Number	Host Species	Optimum Dilution for ICC	Optimum Dilution for Western Blotting
VWF	DAKO	A0082	Rabbit	1:10000	N/A*
VWF	Serotec	AHP062	Sheep	1:10000	N/A
VWF	Serotec	MCA127	Mouse	1:100	N/A
Tubulin	Sigma-Aldrich	T9026	Mouse	N/A	1:5000
LAMP1	DSHB**	H4B4	Mouse	1:50	N/A
CD63	DSHB	H5C6	Mouse	1:200	N/A
EEA-1	BD Transduction Laboratories	610456/7	Mouse	1:100	N/A
TGN-46	Serotec	AHP500	Sheep	1:300	N/A
PDI	Stressgen	SPA-891	Mouse	1:500	N/A
tfR	Invitrogen	13-6800	Mouse	1:200	N/A
GFP	Molecular Probes	A-11122	Rabbit	1:300	Variable
GFP	Biogenesis	4745-1051	Sheep	1:250	Variable
GFP	Roche	11814460 001	Mouse	1:500	N/A
PECAM	DSHB	PSB1	Mouse	1:10	N/A
SYT1	Synaptic Systems	105011	Mouse	1:200***	1:500
SYT5	Abcam®	ab116452	Rabbit	1:100	N/A
SYT 5	Abcam®	ab140432	Goat	N/D	1:300

Fluorophore- or horseradish peroxidase–coupled secondary Abs were from Jackson ImmunoResearch Europe (Newmarket, UK). Infrared dye secondary Ab were from LI-COR

Biosciences UK Ltd (Cambridge, UK). * N/A = Not Applicable. Western blotting or ICC was not performed for this antigen. ** DSHB = Developmental Studies Hybridoma Bank. *** Unless otherwise stated, the dilutions of the SYT antibodies for ICC were determined in HEK cells expressing fluorescent constructs of the SYT proteins.

Table S2. Primers sequence used for LIC.

SYT5	Forward Primer	Reverse Primer
	GCAGGGGCGCAACAGACCCCGG TATGTTCCCGGAGCCCCCAAC	CCACCAGGCCGGCCAGCACCCGG TCCGGGCGCAGGCAGCAGCCTCA C

Table S3. Synaptotagmin 5 shRNA used in this study

Synaptotagmin	shRNA clone MISSION® Library	shRNA target sequence
SYT5	TRCN0000000959	CCAGAGTTACATAGACAAGGT