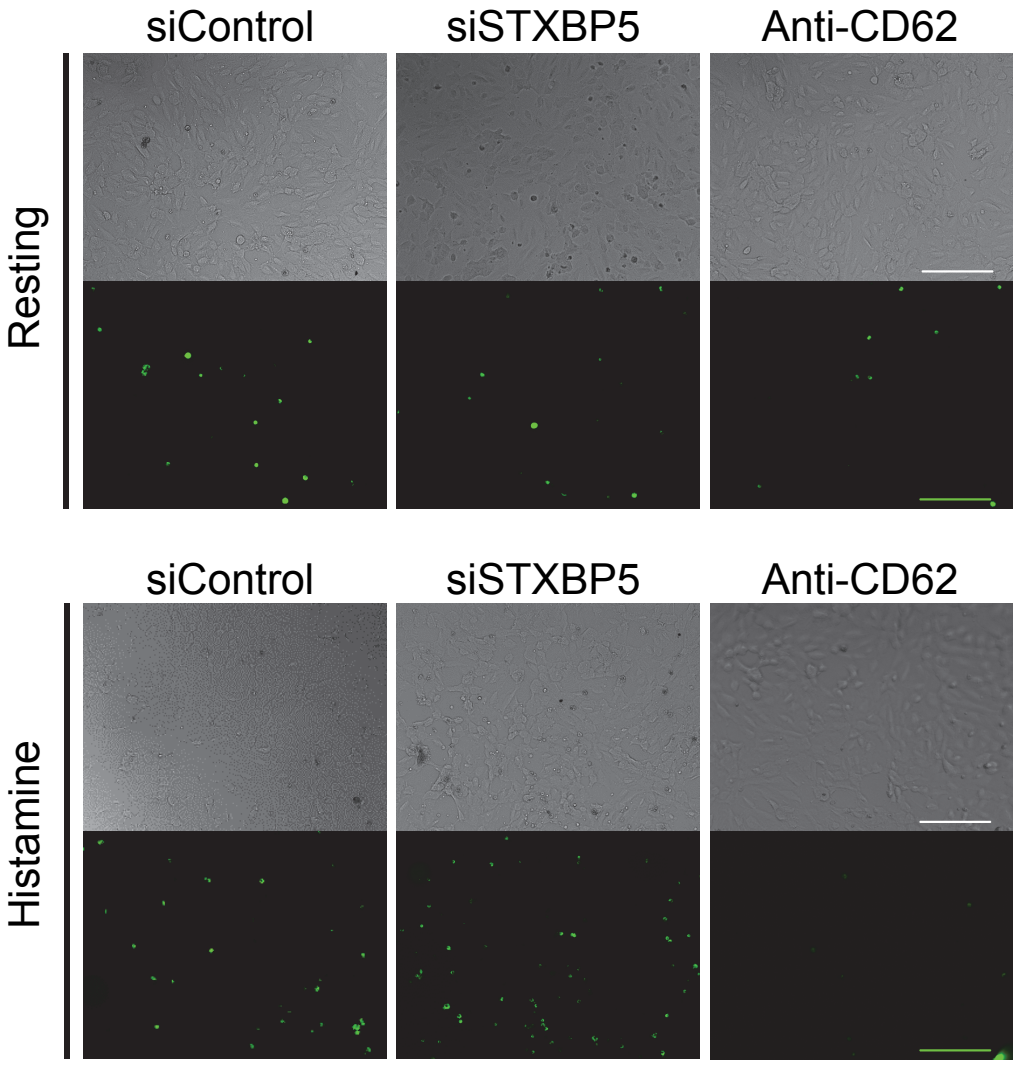
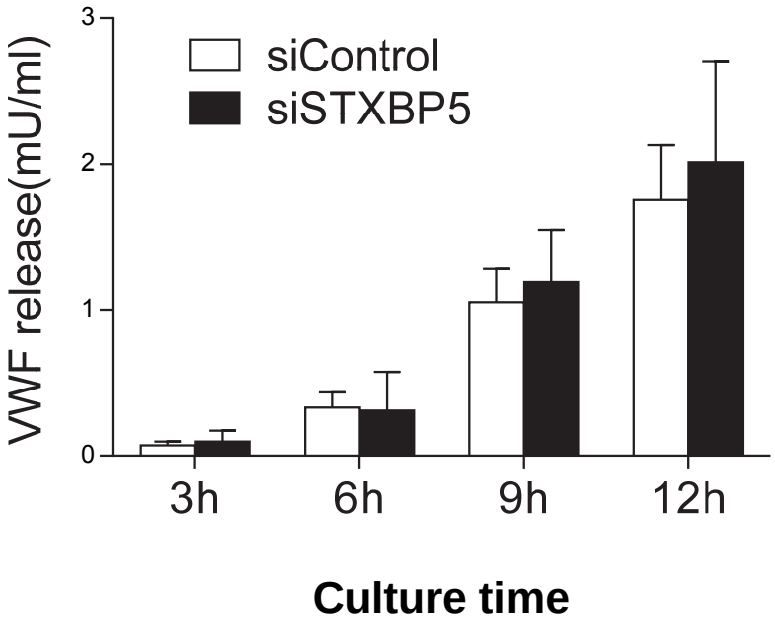
Supplemental Figure S1



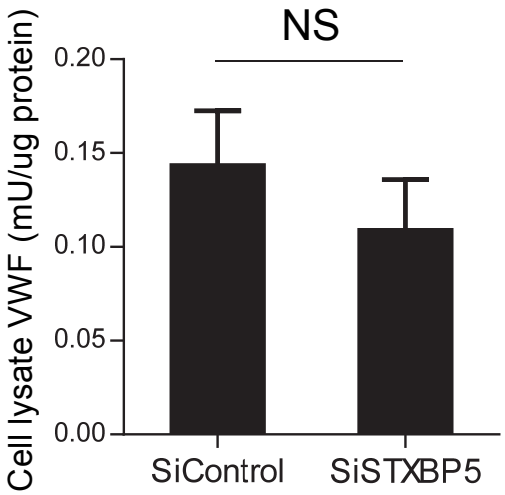
Supplemental Figure S1. Knockdown of STXBP5 in HUVEC increases P-selectin externalization as measured by HL-60 cell adherence after 10 μM histamine stimulation. HL-60 cells were labeled with calcein AM to allow visualization by a fluorescent microscope. HUVEC treated with an antibody to full-length P-selectin (Anti-CD62) serve as a negative control. Shown are representative bright-field and corresponding fluorescent images from multiple fields from 3 wells per transfection per treatment (10 × water immersion lens; scale bar = 150 μm).

Supplemental Figure S2



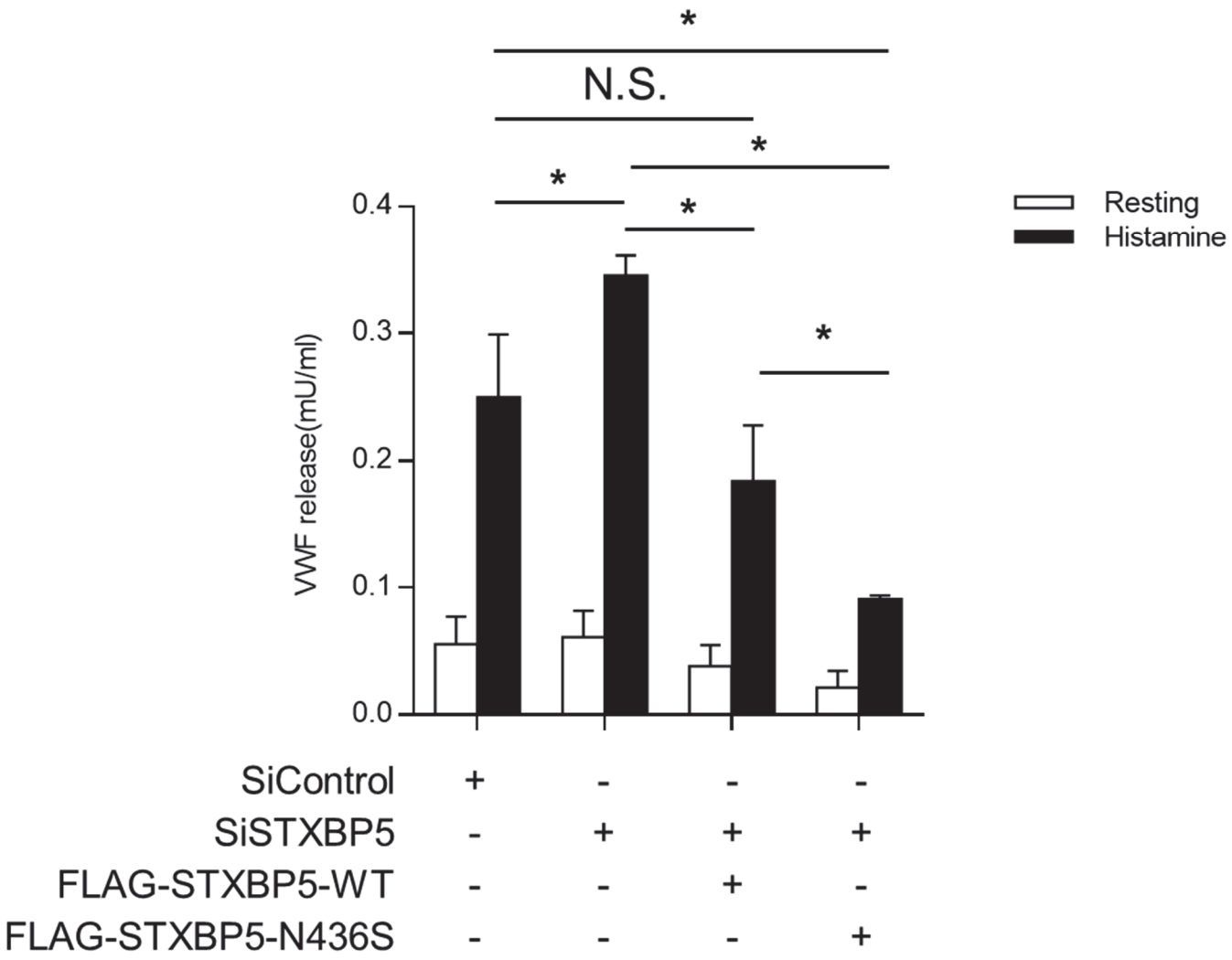
Supplemental Figure S2. STXBP5 and constitutive release of VWF. Constitutive release of VWF was measured in 3-h intervals over 12 h in resting HUVEC that were transfected with control siRNA or STXBP5 siRNA for 72 hours (n = 3 ± S.D. No significant difference between siControl and siSTXBP5 were detected).

Supplemental Figure S3



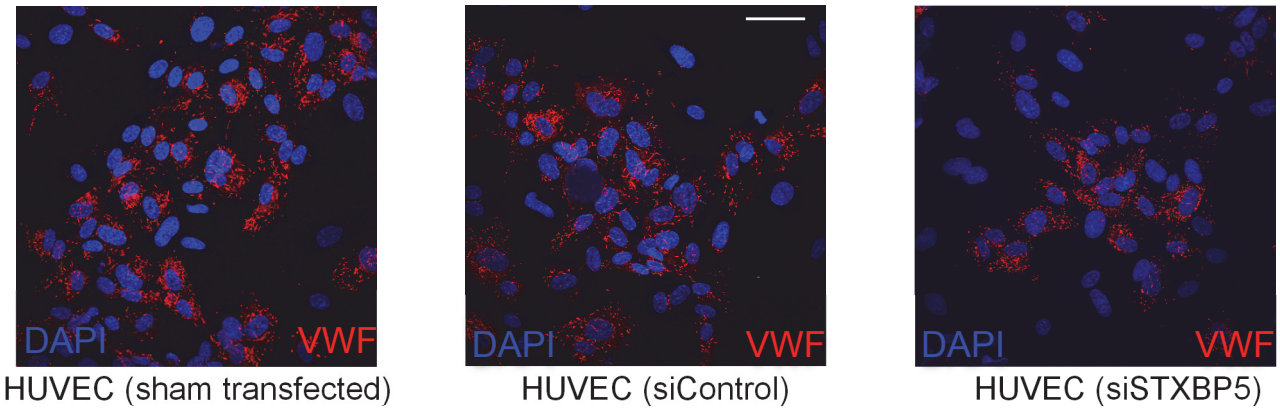
Supplemental Figure S3. STXBP5 does not affect HUVEC content of VWF. Total protein content in lysates of HUVEC transfected with siControl or siSTXBP5 was measured and normalized by bicinchoninic acid assay, and lysates contain 1 μg total protein were measured VWF content by ELISA (n = 4 ± S.D. No significant difference was detected).

Supplemental Figure S4

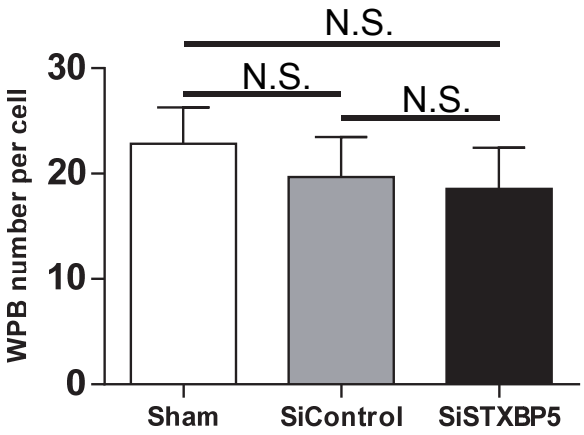


Supplemental Figure S4. STXBP5 SNP and release of VWF. HUVEC were transfected with siSTXBP5 to knock down endogenous STXBP5 expression, and some co-transfected with an expression vector for Stxbp5(WT) or Stxbp5(N436S). Release of VWF was measured after treatment with media or histamine (n = 4 ± S.D. * P<0.05).

Supplemental Figure S5

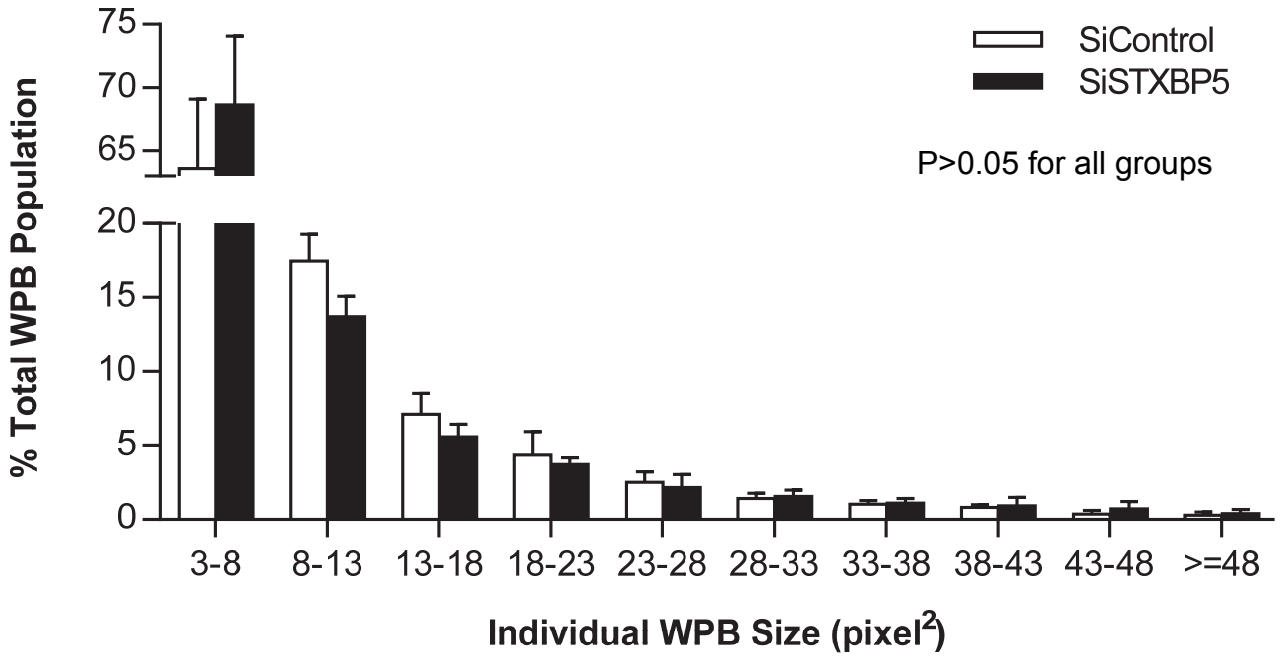


HUVEC (sham transfected) HUVEC (siControl) HUVEC (siSTXBP5)



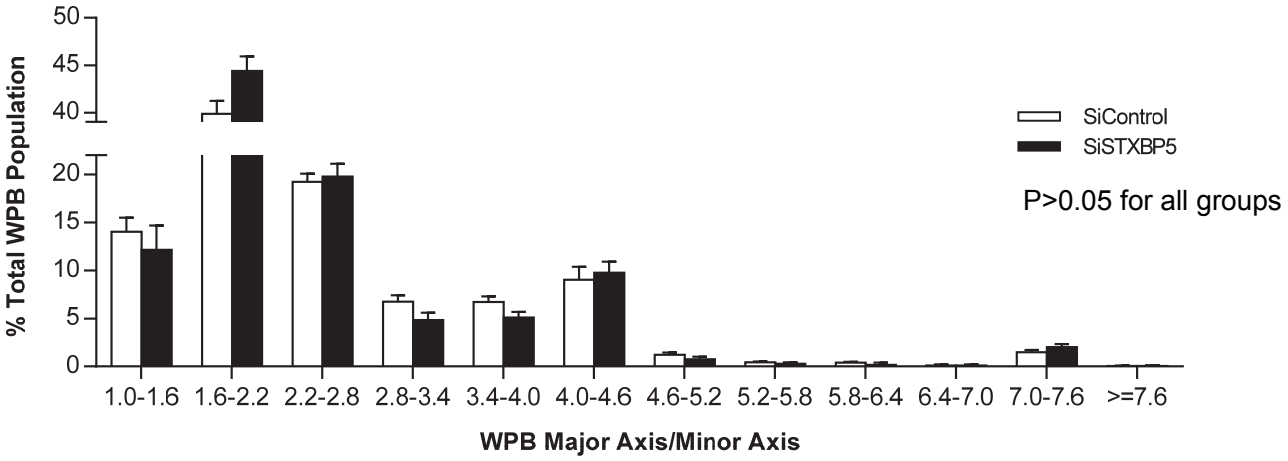
Supplemental Figure S5. Knockdown of STXBP5 does not affect the number of WPB granules per cell. HUVEC were transfected with siSTXBP5 or siControl, or sham transfected, and the number of WPB was counted by ImagePro Plus (n = 3 replicate plates ± S.D.; granules were counted in over 240 cells per plate. No significant difference were detected by one way ANOVA. Scale bar = 50 μm).

Supplemental Figure S6

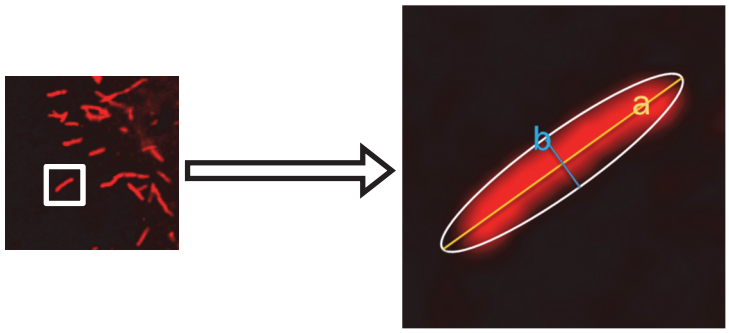


Supplemental Figure S6. Knockdown of STXBP5 does not affect the size of WPB granules. HUVEC were transfected with siSTXBP5 or siControl, and the size of individual WPB was measured by ImagePro Plus (n = 3 replicate plates ± S.D.; granules were counted in over 240 cells per plate. No significant difference between siControl and siSTXBP5).

Supplemental Figure S7



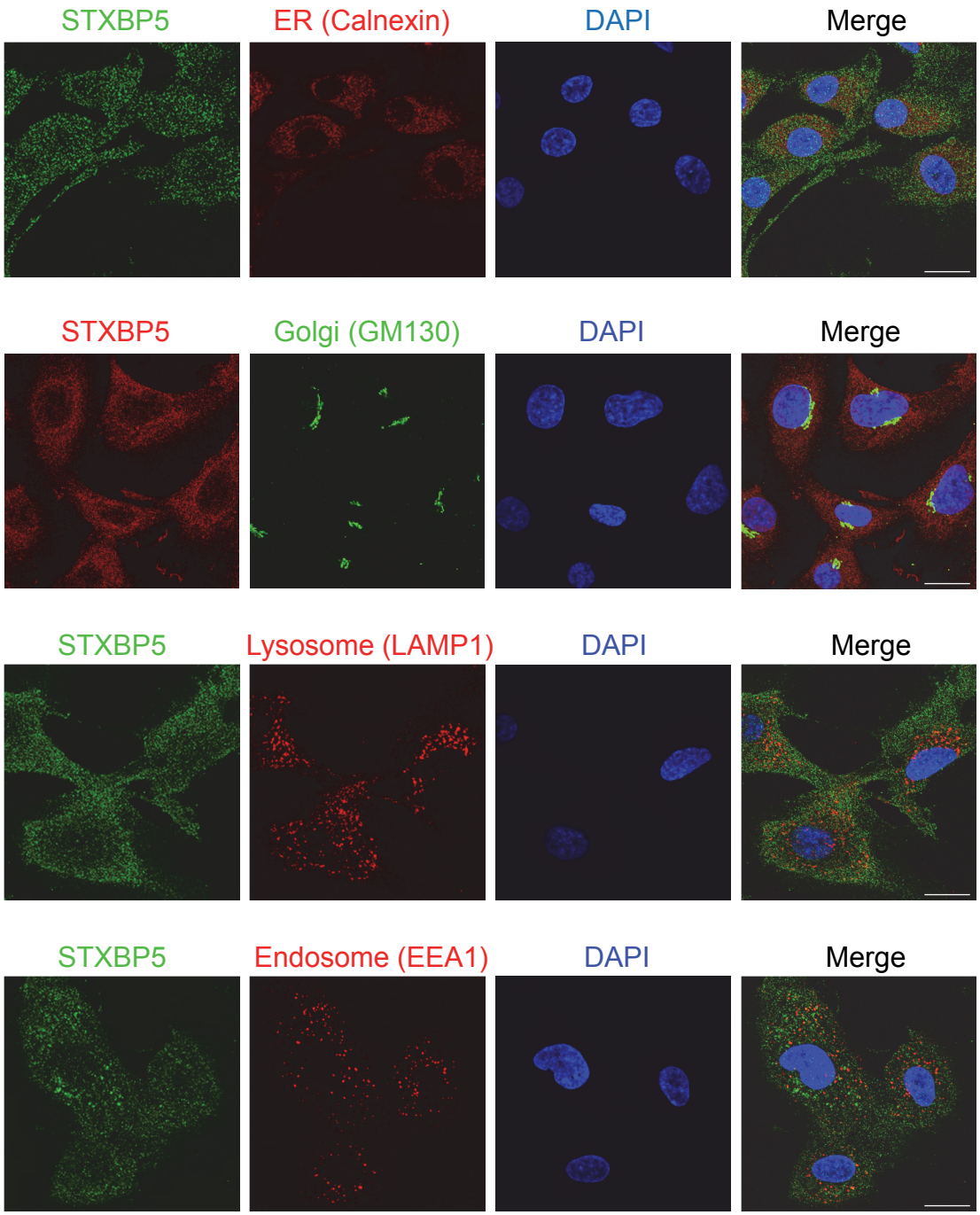
Aspect ratio: Ratio between the **major axis** and the **minor axis** of the ellipse equivalent to the object.



Aspect Ratio= a/b

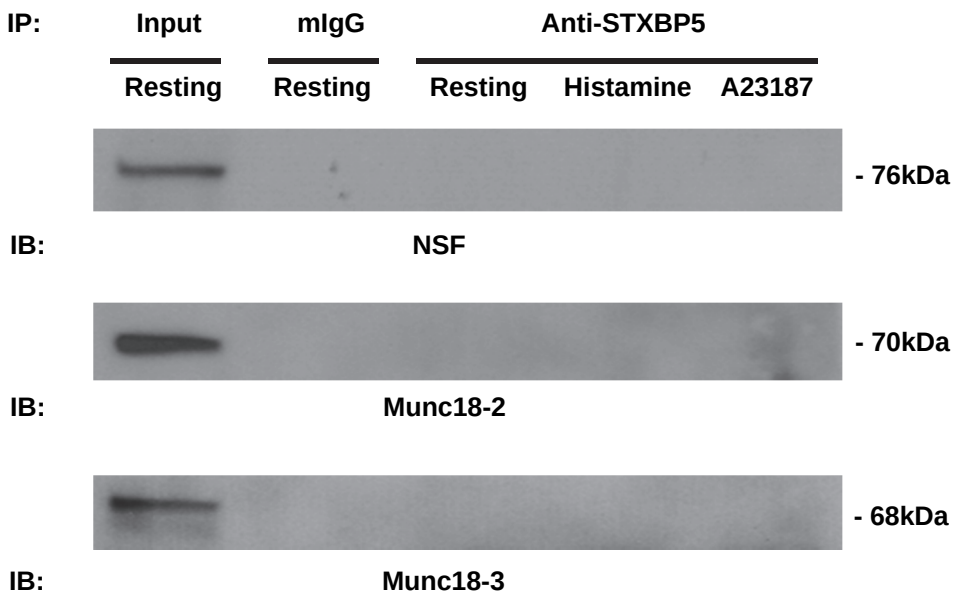
Supplemental Figure S7. Knockdown of STXBP5 does not affect the shape of WPB granules. HUVEC were transfected with siSTXBP5 or siControl. The aspect ratio of individual WPB was measured by ImagePro Plus, and its distribution was plotted as histogram (n = 3 replicate plates ± S.D.; granules were counted in over 240 cells per plate. No significant difference between siControl and siSTXBP5). Shown at the lower panel is one representative image of WPB and one example of measurement.

Supplemental Figure S8



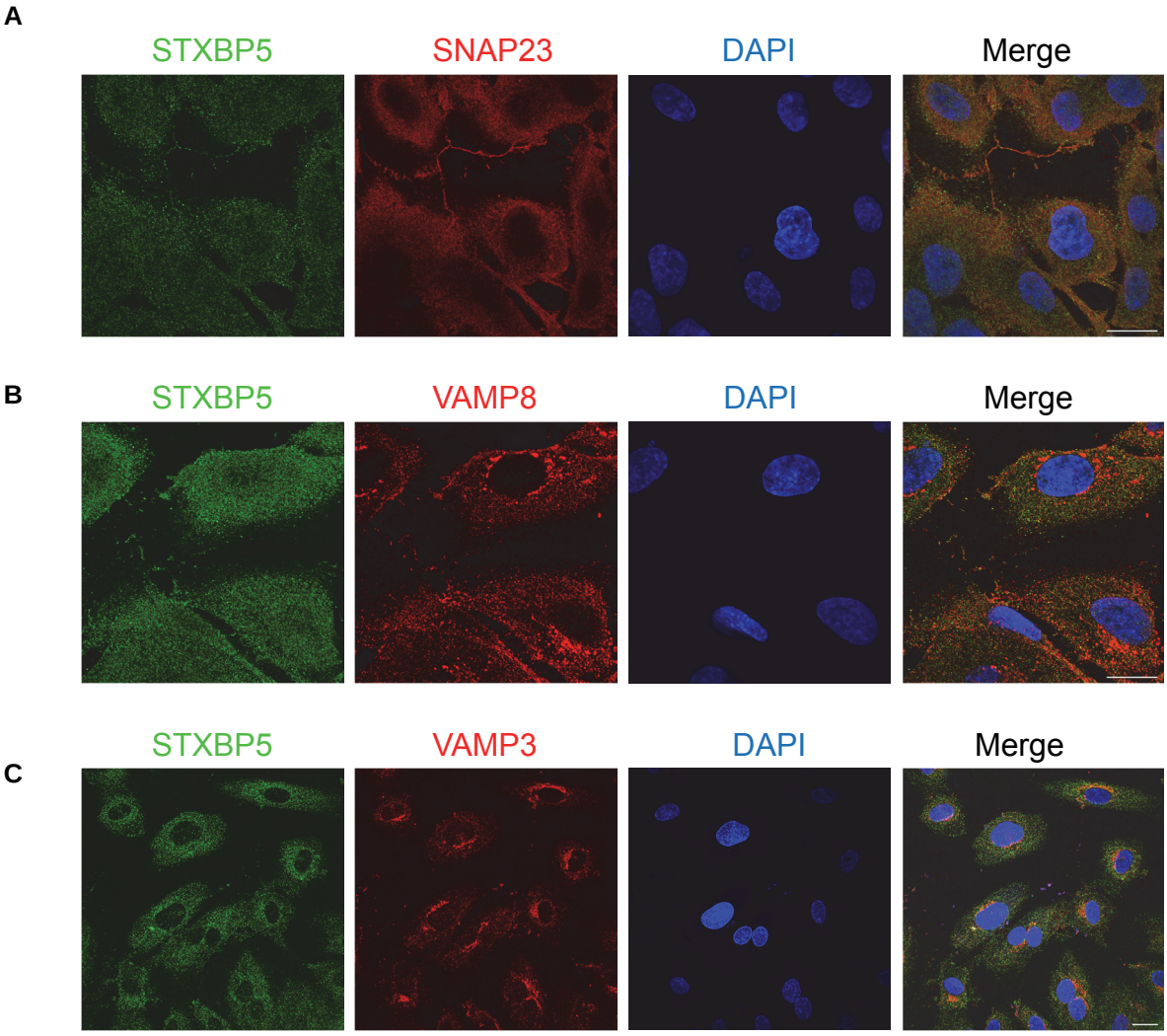
Supplemental Figure S8. STXBP5 does not co-localize with markers for ER, Golgi, lysosomes, or endosomes. Confocal microscopy was used to localize STXBP5, markers for endoplasmic reticulum, Golgi apparatus, lysosome, or early endosome. STXBP5 does not co-localize with these organelle markers. Scale bar = 20 µm.

Supplemental Figure S9



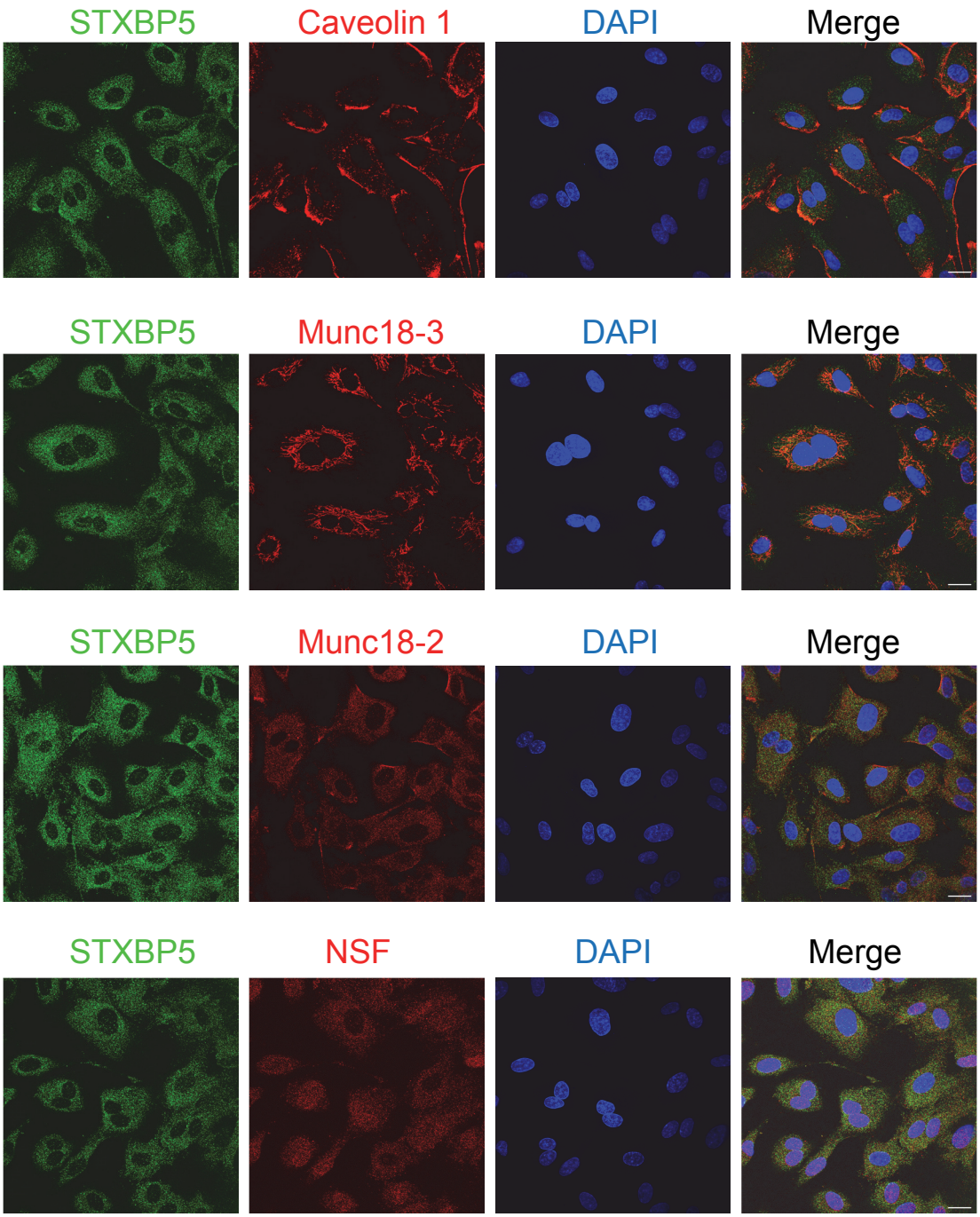
Supplemental Figure S9. STXBP5 does not interact with NSF and Munc family members. Lysates of resting or stimulated HUVEC were precipitated with antibody to STXBP5 or IgG, and precipitants were immunoblotted with antibody to NSF or Munc18-2 or Munc18-3.

Supplemental Figure S10



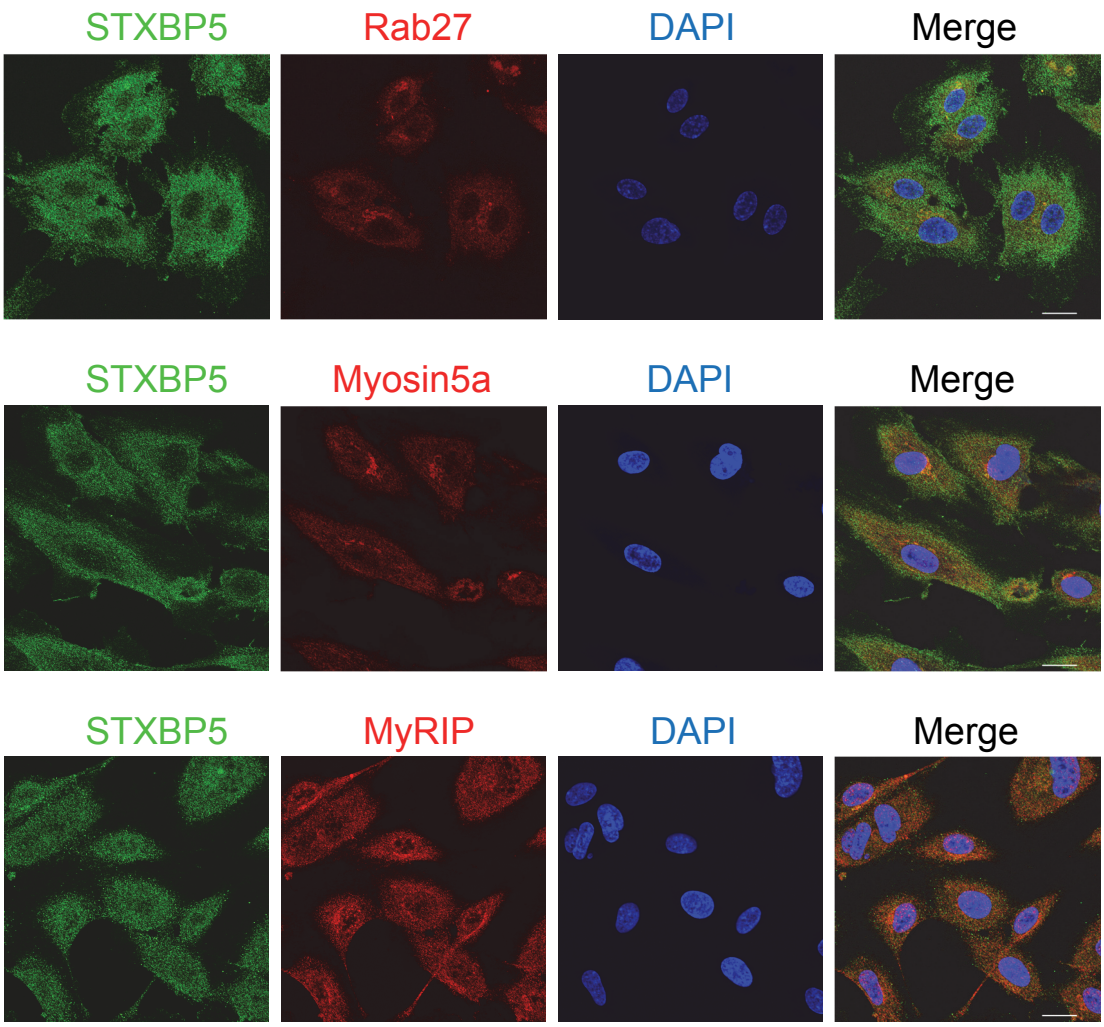
Supplemental Figure S10. STXBP5 does not co-localize with SNAP23, VAMP8, or VAMP3. Confocal microscopy was used to localize STXBP5 (green), SNAP23, VAMP8, VAMP3 (red), and DNA (blue) in HUVEC. STXBP5 does not co-localize with these SNAREs. Scale bar = 20 μm.

Supplemental Figure S11



Supplemental Figure S11. STXBP5 does not co-localize with Caveolin-1, Munc18-3, Munc18-2, or NSF. Confocal microscopy was used to localize STXBP5 (green), Caveolin-1, Munc18-3, Munc18-2, NSF (red), and DNA (blue) in HUVEC. STXBP5 does not co-localize with these proteins in HUVEC. Scale bar = 20 μm.

Supplemental Figure S12



Supplemental Figure S12. STXBP5 does not co-localize with Rab27, Myosin5a or MyRIP. Confocal microscopy was used to localize STXBP5 (green), Rab27, Myosin5a, MyRIP (red), and DNA (blue) in HUVEC. STXBP5 does not co-localize with these proteins in HUVEC. Scale bar = 20 μm.

Supplemental Table 1. Antibodies used for this study

Antibody Name	Species Immunized	Cat #	Source
Anti-MYRIP antibody	Goat	ab10149	Abcam
Anti-Syntaxin 4 antibody	Rabbit	ab96382	Abcam
Anti-Von Willebrand Factor antibody	Rabbit	ab6994	Abcam
FITC Anti-Mouse CD62P	Rat	553744	BD Pharmingen
Purified Mouse Anti-GM130	Mouse	610822	BD Transduction Laboratories
Polyclonal Rabbit Anti-Caveolin	Rabbit	610059	BD Transduction Laboratories
Purified Mouse Anti-Syntaxin 4	Mouse	610440	BD Transduction Laboratories
Calnexin (C5C9)	Rabbit	2679P	Cell Signaling
EEA1 (C45B10)	Rabbit	3288P	Cell Signaling
LAMP1 (D2D11) XP®	Rabbit	9091P	Cell Signaling
Integrin alphaIIbbeta3 (GPIIb/IIIa, CD41/CD61), clone JON/A	Rat	M023-2	Emfret
Anti - GPIbbeta derivative	Rat	X488	Emfret
Cy3-AffiniPure Bovine Anti-Goat IgG (H+L)	Bovine	805-165-180	Jackson ImmunoResearch Laboratories
Alexa Fluor® 488 Goat Anti-Rabbit IgG (H+L) Antibody, highly cross-adsorbed	Goat	A-11034	Molecular Probes
Alexa Fluor® 488 Goat Anti-Mouse IgG (H+L) Antibody, highly cross-adsorbed	Goat	A-11029	Molecular Probes
Alexa Fluor® 594 Goat Anti-Mouse IgG (H+L) Antibody, highly cross-adsorbed	Goat	A-11032	Molecular Probes
Alexa Fluor® 594 Goat Anti-Rabbit IgG (H+L) Antibody, highly cross-adsorbed	Goat	A-11037	Molecular Probes
Alexa Fluor® 680 Donkey Anti-Sheep IgG (H+L)	Donkey	A-21102	Molecular Probes
Rab27a Affinity Purified Polyclonal Ab	Sheep	AF7245	R&D Systems
Human VAMP-8 Antibody	Goat	AF5354	R&D Systems
Goat IgG Horseradish Peroxidase-conjugated Antibody	Donkey	HAF109	R&D Systems
Mouse IgG1 Isotype Control (Clone 11711)	Mouse	MAB002	R&D Systems
SNAP 23 Antibody (H-50)	Rabbit	sc-50371	Santa Cruz
VAMP-1/2/3 Antibody (FL-118)	Rabbit	sc-13992	Santa Cruz
Syntaxin 4 Antibody (H-16)	Goat	sc-14455	Santa Cruz
Tomosyn Antibody (15)	Mouse	sc-136105	Santa Cruz
Tomosyn Antibody (H-55)	Rabbit	sc-98350	Santa Cruz
NSF Antibody (H-300)	Rabbit	sc-15339	Santa Cruz
P-Selectin (AK4)	Mouse	sc-19996	Santa Cruz
β-Actin (C4)	Mouse	sc-47778	Santa Cruz
GAPDH (FL-335)	Rabbit	sc-25778	Santa Cruz
Anti-Myosin Va (LF-18) antibody	Rabbit	M4812-.2ML	SIGMA
Syntaxin 11	Rabbit	110 113	Synaptic Systems GmbH