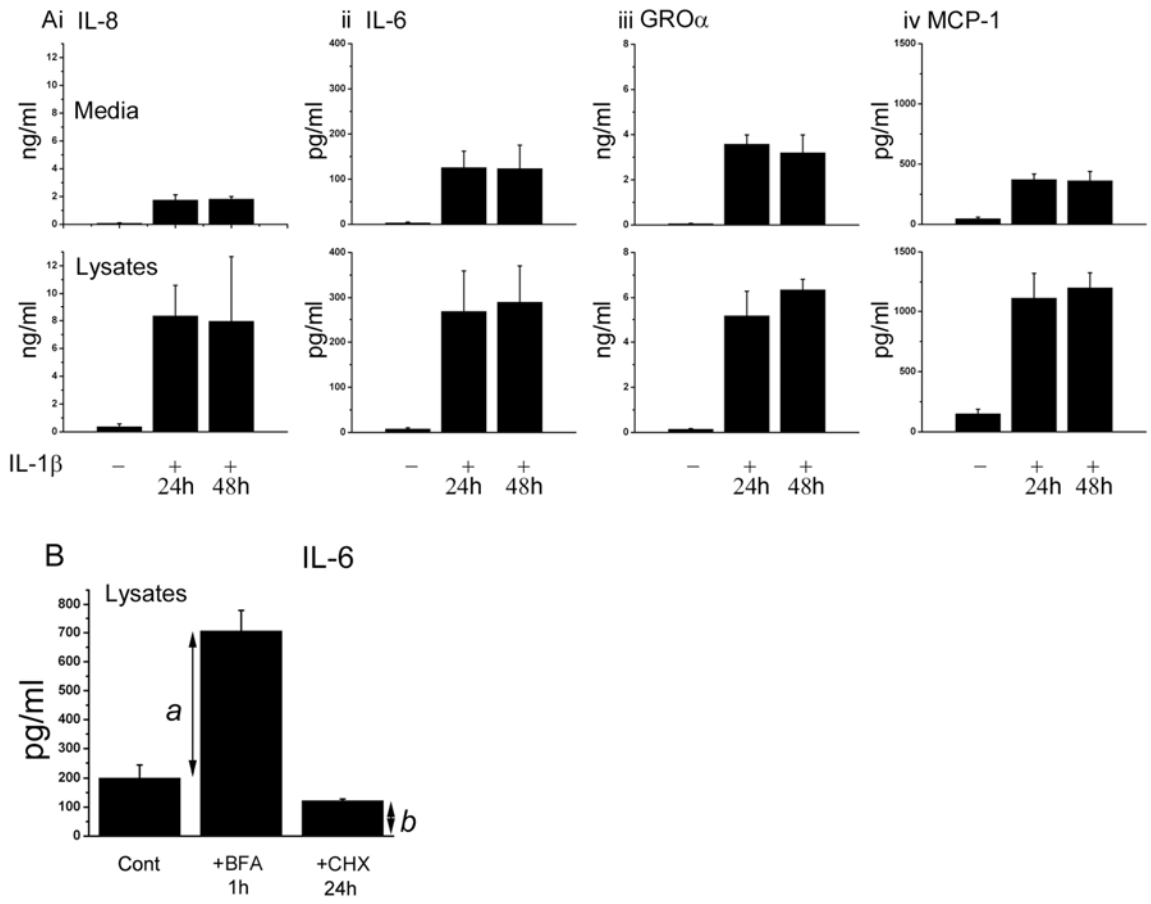


Figure S1. IL-1β upregulation of cytokine expression in HUVEC

Panel A shows the levels of IL-8 (i) IL-6 (ii) GROa (iii) and MCP-1 (iv) in Media (measured over a 10 minute time period) and in Lysates of cells in vehicle control (24hrs; - IL-1β) or IL-1β treated cells (1ng/ml, 24 or 48hrs as indicated). Data for Media or Lysate levels in vehicle control treated cells at 48hrs was not different from that at 24hrs (not shown). Data is the mean of 3-4 separate experiments. Panel B: Left column shows IL-6 levels in lysates from cells exposed to 1ng/ml IL-1β for 24hrs. Middle column shows the increase in IL-6 levels in the cell lysates of IL-1β-treated cells (1ng/ml, 24hrs) following a 1hr treatment with 5μM BFA. Right column shows the IL-6 levels in lysates from IL-1β-treated cells exposed to 5μM CHX for 24hrs. IL-1β was included in the media during the 24hr CHX treatment. Data shown is an individual experiment carried out in triplicate and is representative of three replicate experiments. The amount of material synthesized, at steady state expression, during a 1hr time period is denoted by ***a*** (see methods). The amount of that material remaining within cells after 24hr exposure to CHX (***b***) is defined as the stored material (see methods).

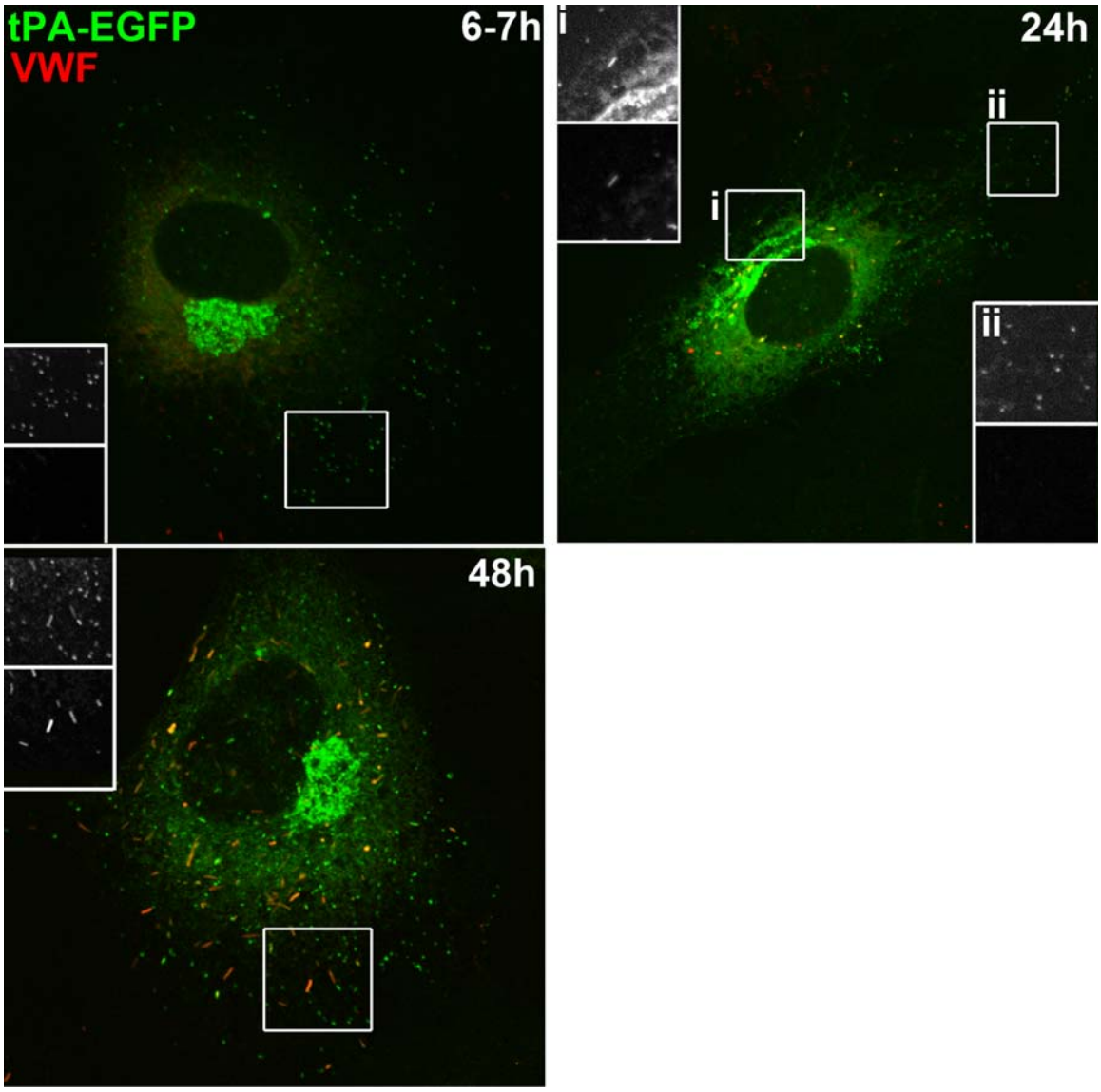


Figure S2. tPA-EGFP expression in HUVEC

Representative examples of individual HUVEC at 6-7hrs, 24hrs and 48hrs post-Nucleofection with tPA-EGFP (green and top panels in grey scale inserts) and fixed and stained with a specific antibody to VWF (red and bottom panels in grey scale inserts). Regions from which the grey scale inserts are taken are indicated by white boxes on the colour image.

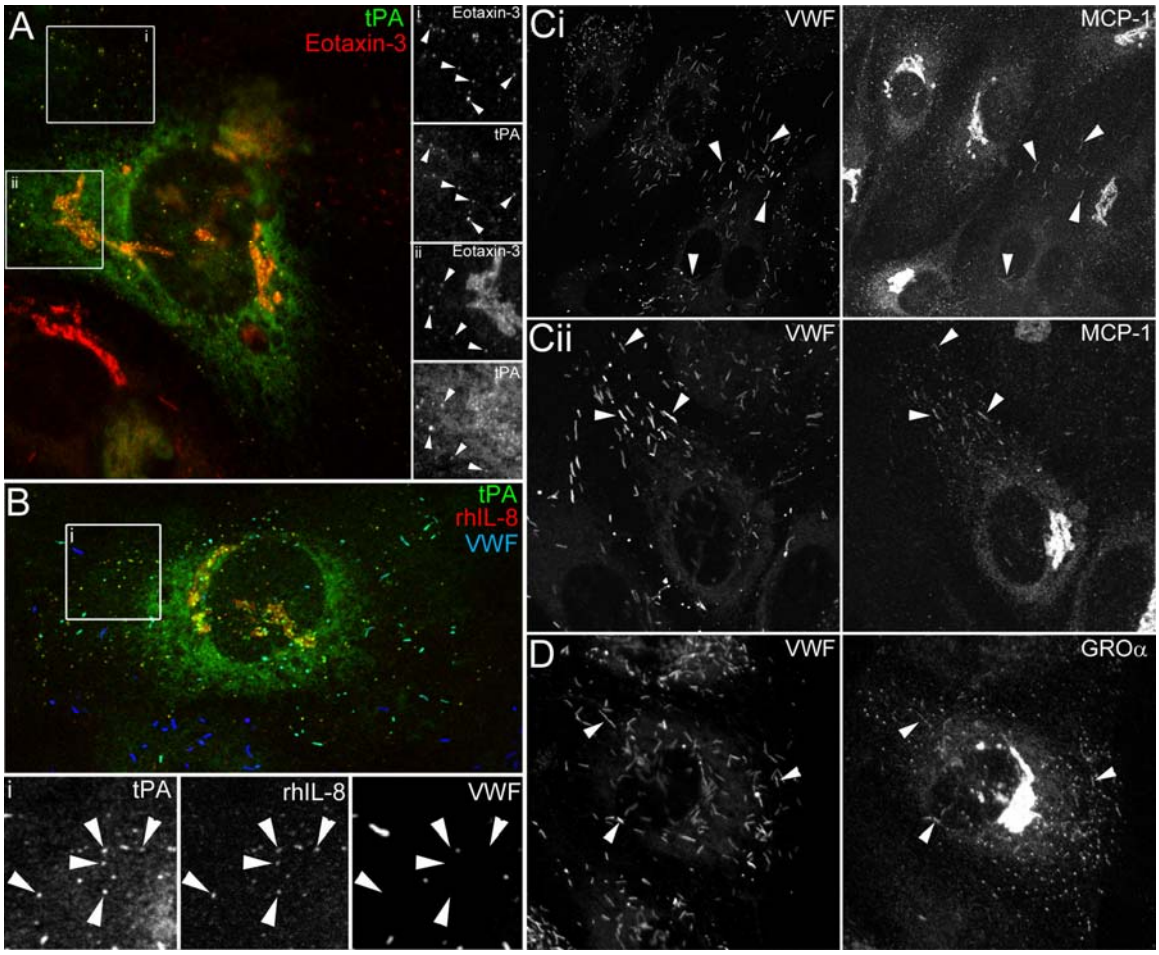
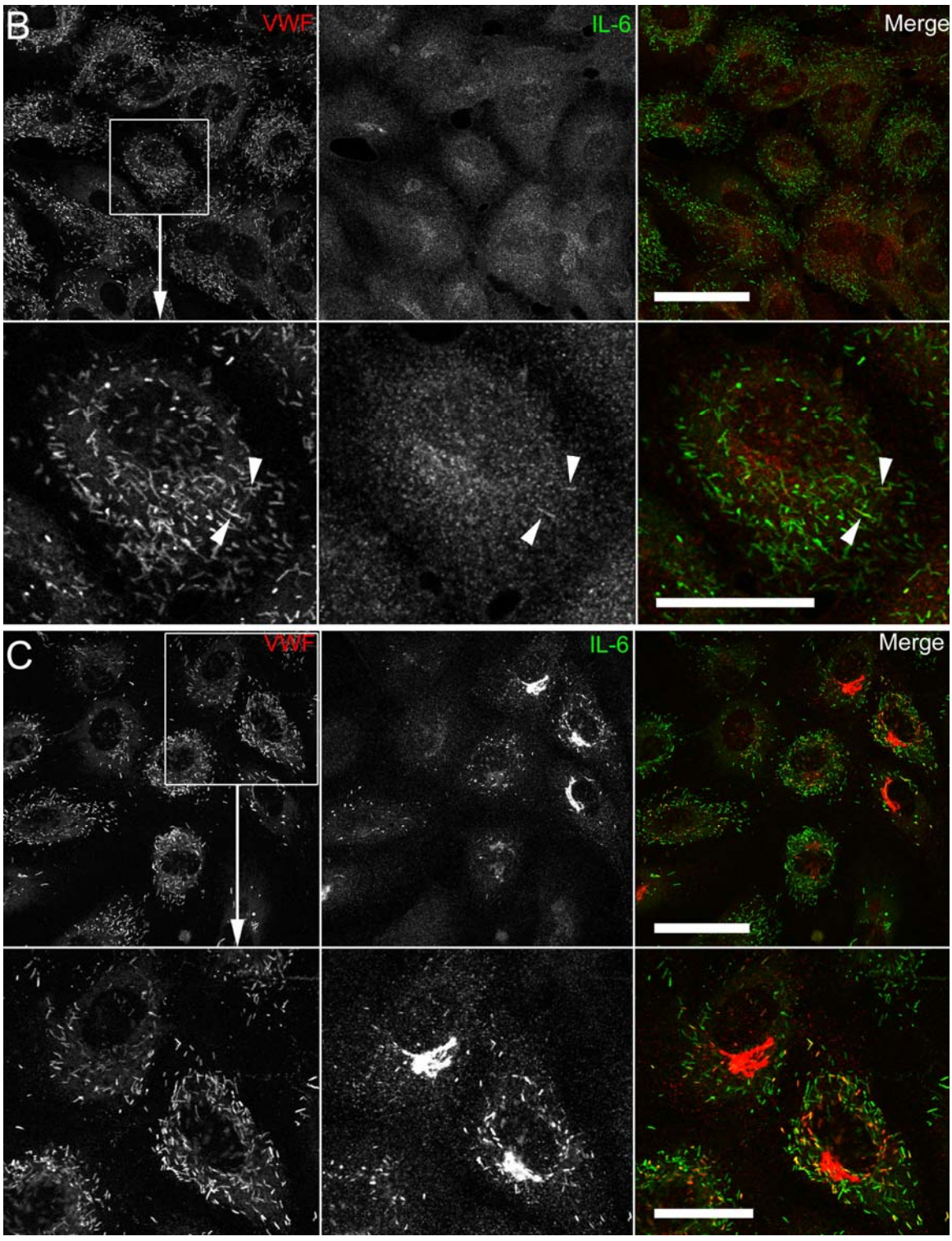


Figure S3 Eotaxin-3 and Il-8 in endogenous tPA-punta and the immunolocalisation of MCP-1 and GROα in WPBs

Panel A shows endogenous tPA immunoreactivity (green and lower panels of grey scale images) and endogenous eotaxin-3 immunoreativity (red and upper panels in grey scale images) in a cell pre-treated with Na-butyrate (3mM, 24hrs) and then subsequently with Na-butyrate and rhIL-4 20nm/ml) together for a further 24hrs. Grey scale images were taken from regions indicated by the white boxes. Arrow heads indicate puncta positive for tPA and eotaxin-3. Panels B and ii show endogenous MCP-1 immunoreactivity (right panels) and endogenous VWF immunoreativity (left panels) in cells pre-treated with IL-1β (1ng/ml, 24hrs). Arrow heads indicate MCP-1 positive WPBs. Panel Biii shows endogenous GROα immunoreactivity in cells pre-treated with IL-1β (1ng/ml, 24hrs). Arrow heads indicate GROα positive WPBs.


B
VWF
IL-6
Merge
C
VWF
IL-6
Merge

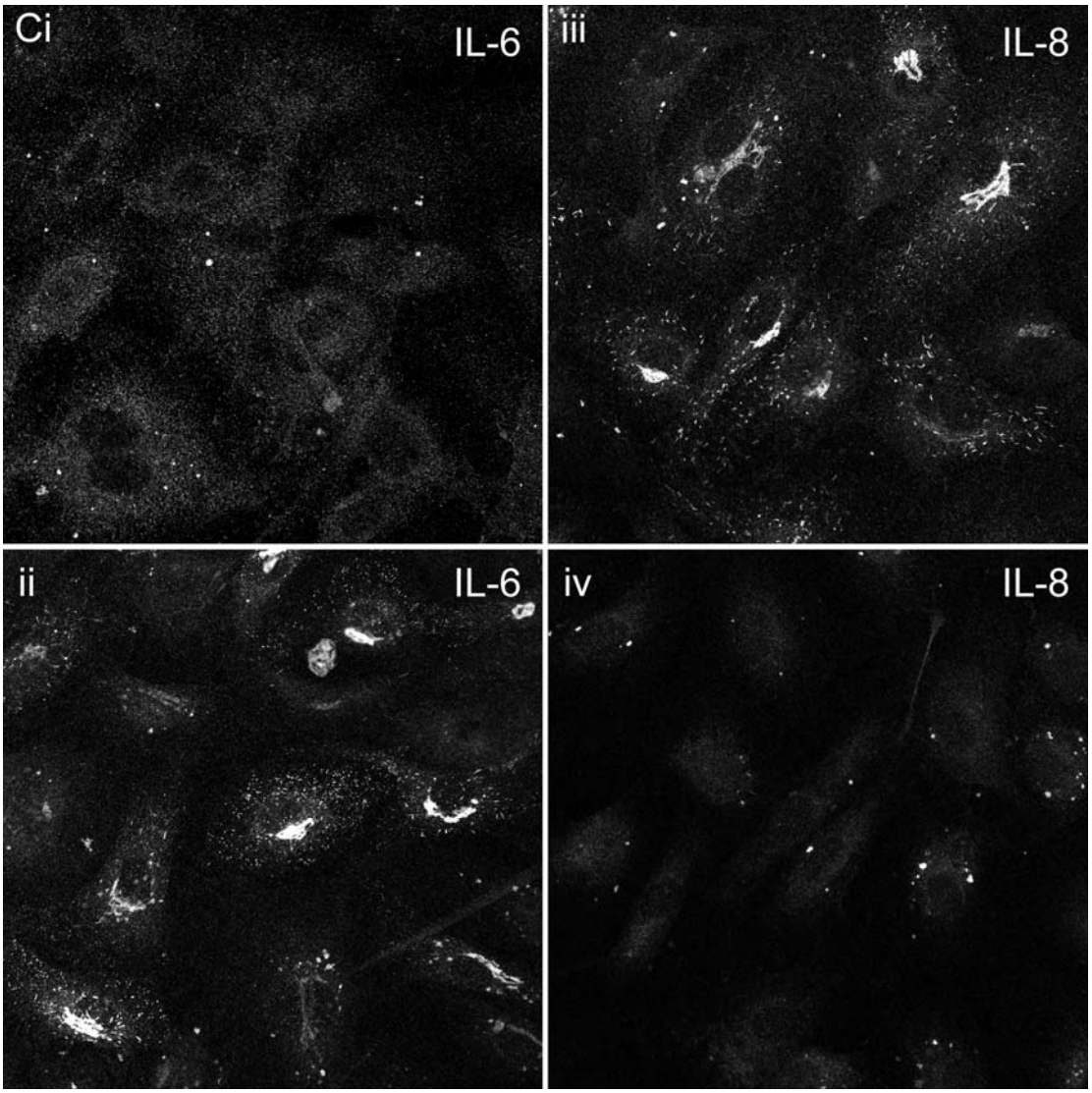
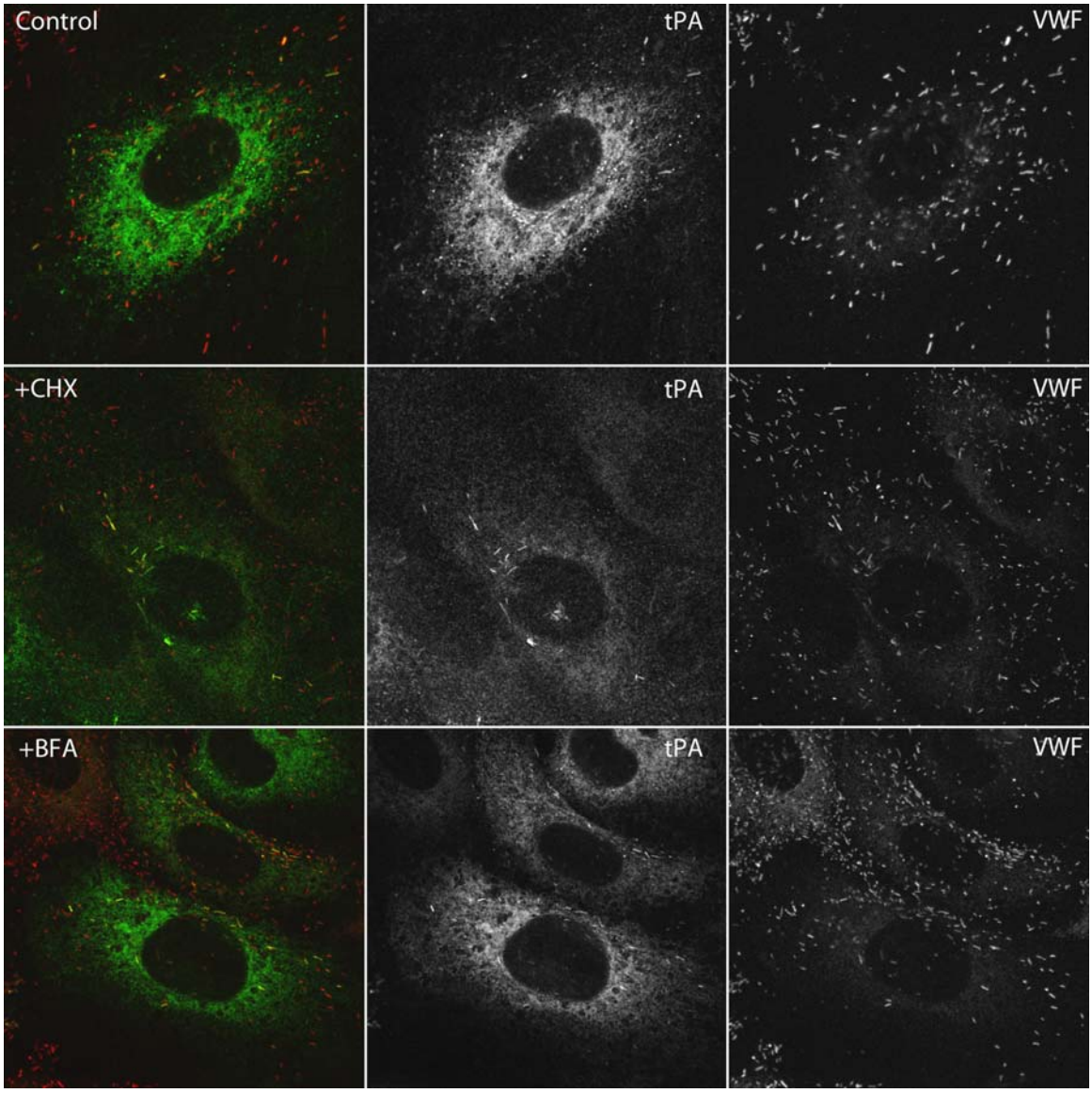


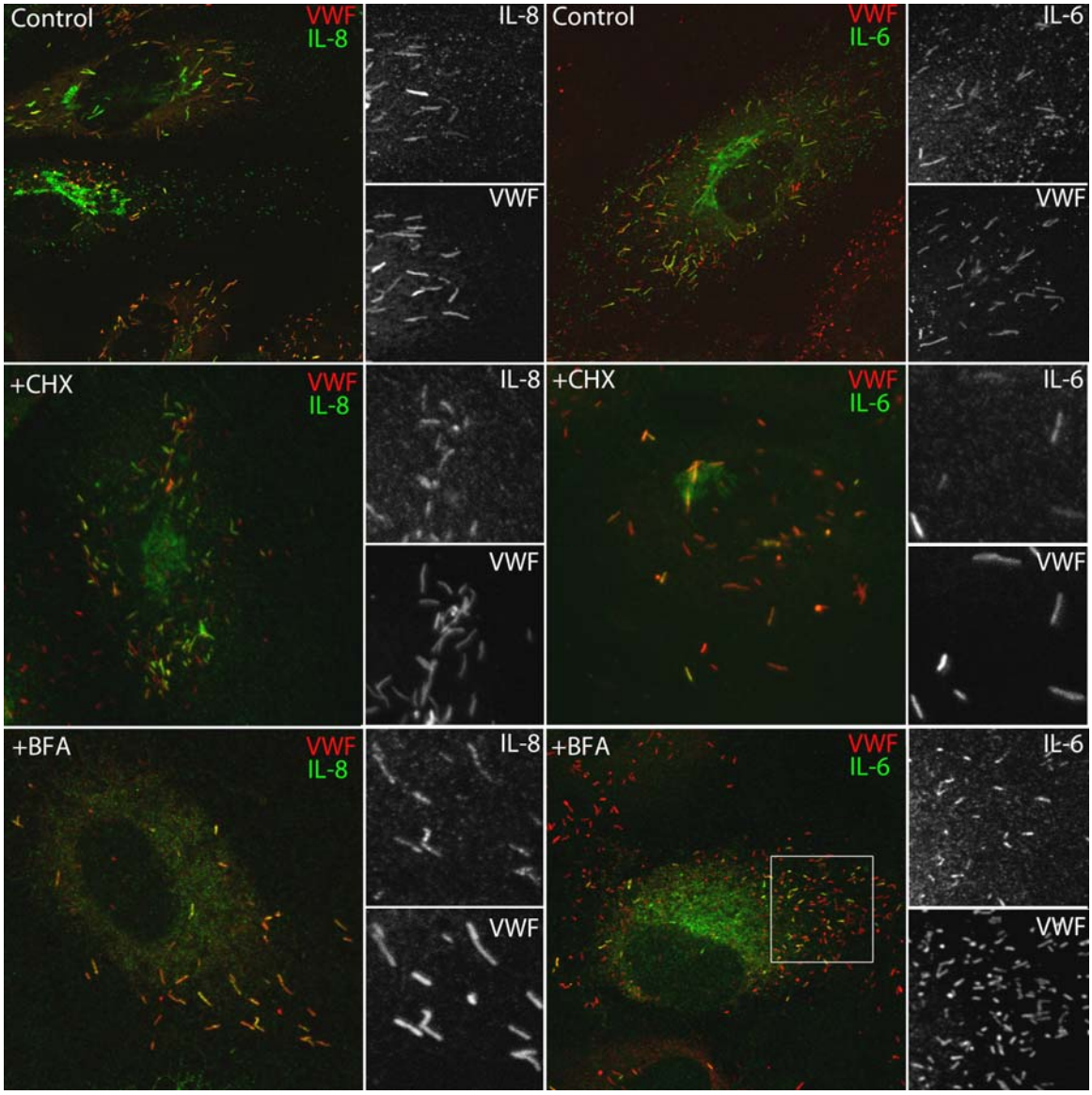
Figure S4 Subcellular localisation of IL-6 in IL-1β treated HUVEC

Top panels in A and B show endogenous VWF immunoreactivity (left and green in colour merged images) and endogenous IL-6 immunoreactivity (middle and red in colour merge panels) in cells pre-treated with vehicle control (A) or IL-1β (1ng/ml) for 24hrs (B). Lower panels in A and B show on an expanded scale the regions indicated by the white boxes. Scale bars are 20μm. Arrow heads in A (lower panels) indicate IL-6 positive WPBs in vehicle control treated cells. Panel C shows the specificity of IL-6 (and IL-8) immuno-staining. Staining obtained with the primary goat anti-human IL-6 specific antibody in IL-1β treated cells was completely blocked by pre-absorption of the primary antibody with rhIL-6 (at a 6 fold molar excess, 24hrs; Ci) but was unaffected by incubation with rhIL-8 (6 fold molar excess; Cii). Pre-absorption of the primary goat anti-human IL-8 antibody with rhIL-8 (Civ) completely blocked staining, while rhIL-6 had no effect on staining (Ciii).

A



B


Control
VWF
IL-8
IL-8
VWF
Control
VWF
IL-6
IL-6
VWF
+CHX
VWF
IL-8
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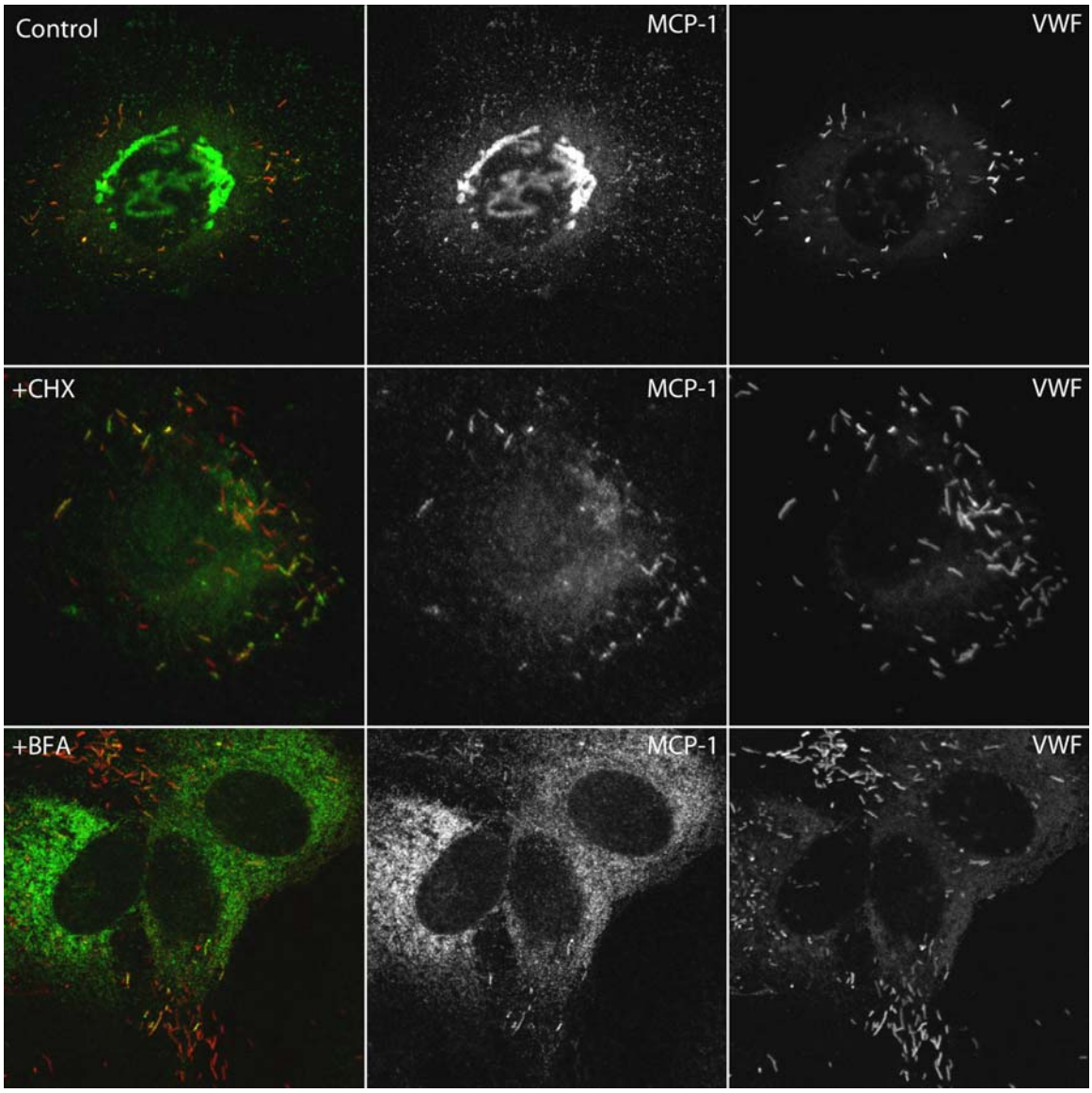
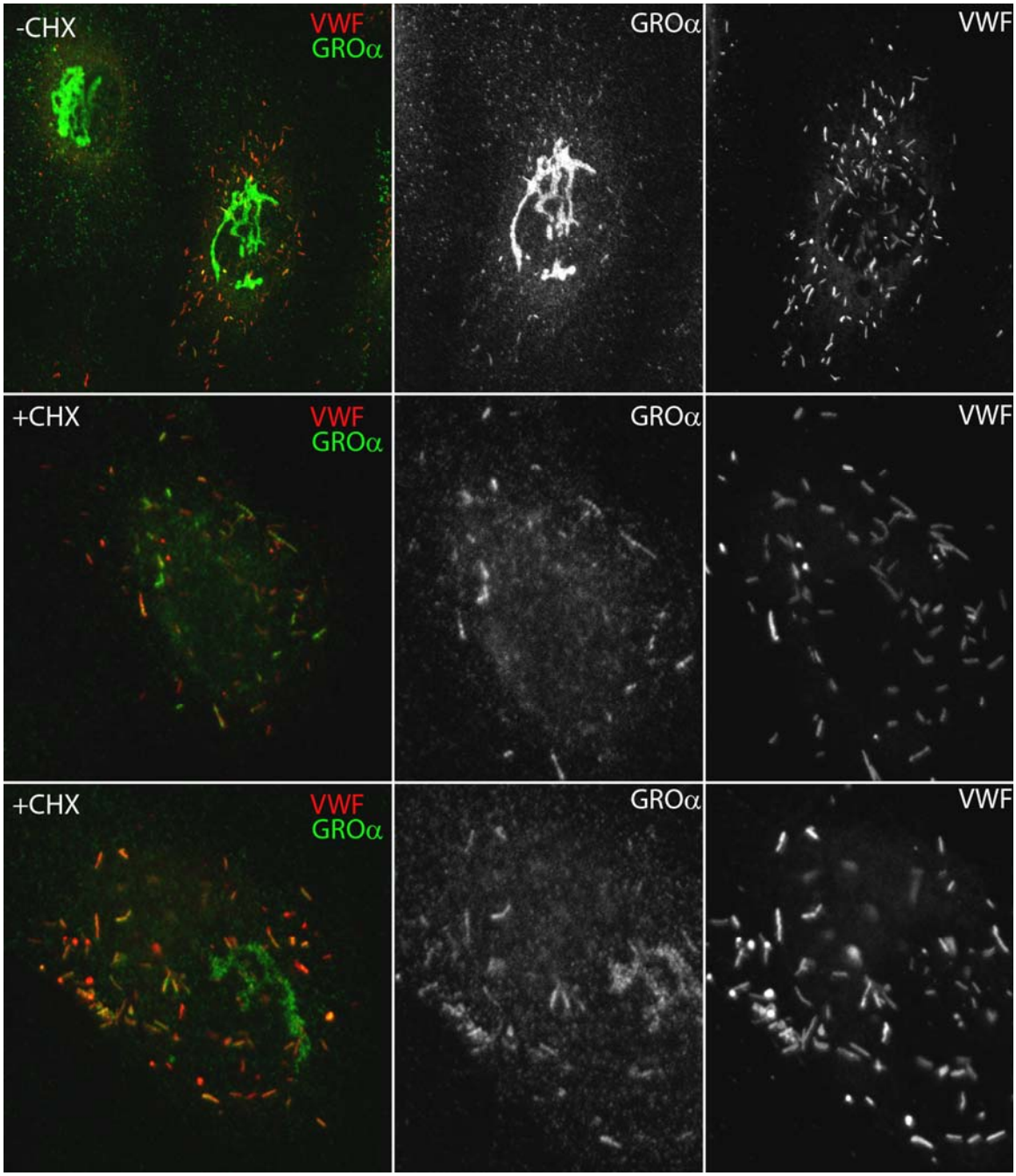
VWF
+CHX
VWF
IL-6
IL-6
VWF
+BFA
VWF
IL-8
IL-8
VWF
+BFA
VWF
IL-6
IL-6
VWF

C



D


-CHX
VWF
GROα
GROα
VWF
+CHX
VWF
GROα
GROα
VWF
+CHX
VWF
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GROα
VWF

E

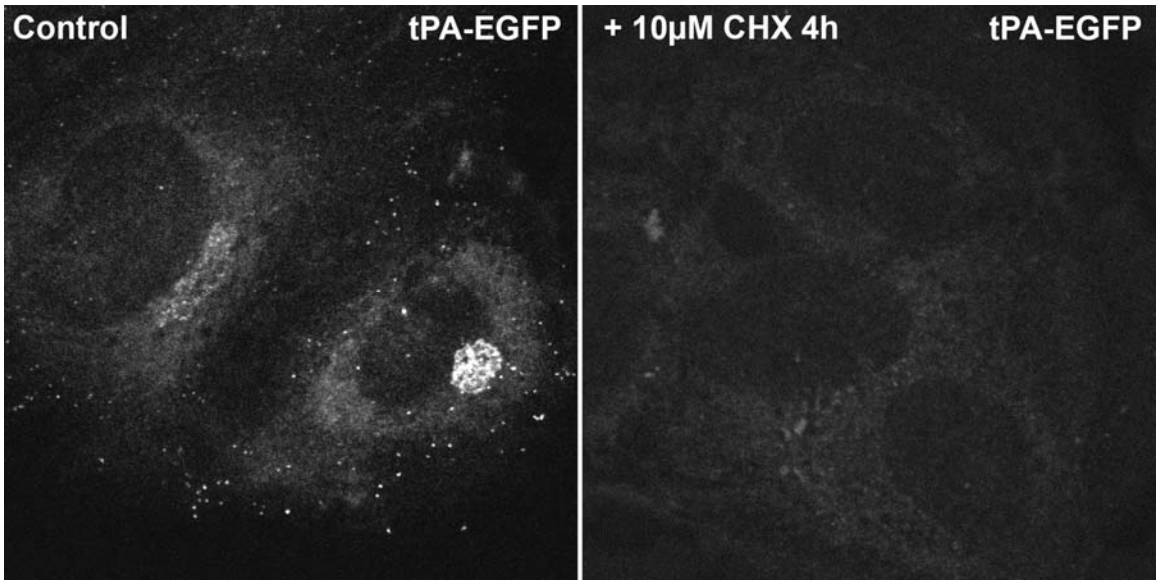


Figure S5. Subcellular localization of endogenous tPA and cytokines in WPBs of Na-Butyrate or rhIL-1B treated HUVEC following CHX or BFA treatment, and the effect of CHX treatment on tPA-EGFP puncta

Top panels in A (Control) show an example of a HUVEC pre-treated with Na-Butyrate (3mM, 24hrs) and stained with specific antibodies against endogenous tPA (green in colour image) and VWF (red in colour image) as indicated. The middle and lower panels show images of Na-Butyrate treated HUVEC following exposure to CHX (5μM, 24hrs) or BFA (5μM, 1hr) as indicated. Top panels in B-D (Control) show cells pre-treated with rhIL-1β (1ng/ml 24hrs) and stained for endogenous IL-8 or IL-6 (B), MCP-1 (C) or GROα (D), (green in colour merged images) as indicated. Cells were counter stained for endogenous VWF (red in colour merged images). Middle or lower panels in B-D show images of rhIL-1β treated HUVEC following exposure to CHX (5μM, 24hrs; B-D) or BFA (5μM, 1hr; B & C) as indicated. Panel E shows cells Nucleofection with tPA-EGFP and treated either with vehicle (control; left panel) or CHX (right panel). Cells were treated with CHX or vehicle 6hrs post Nucleofection for 4hrs.

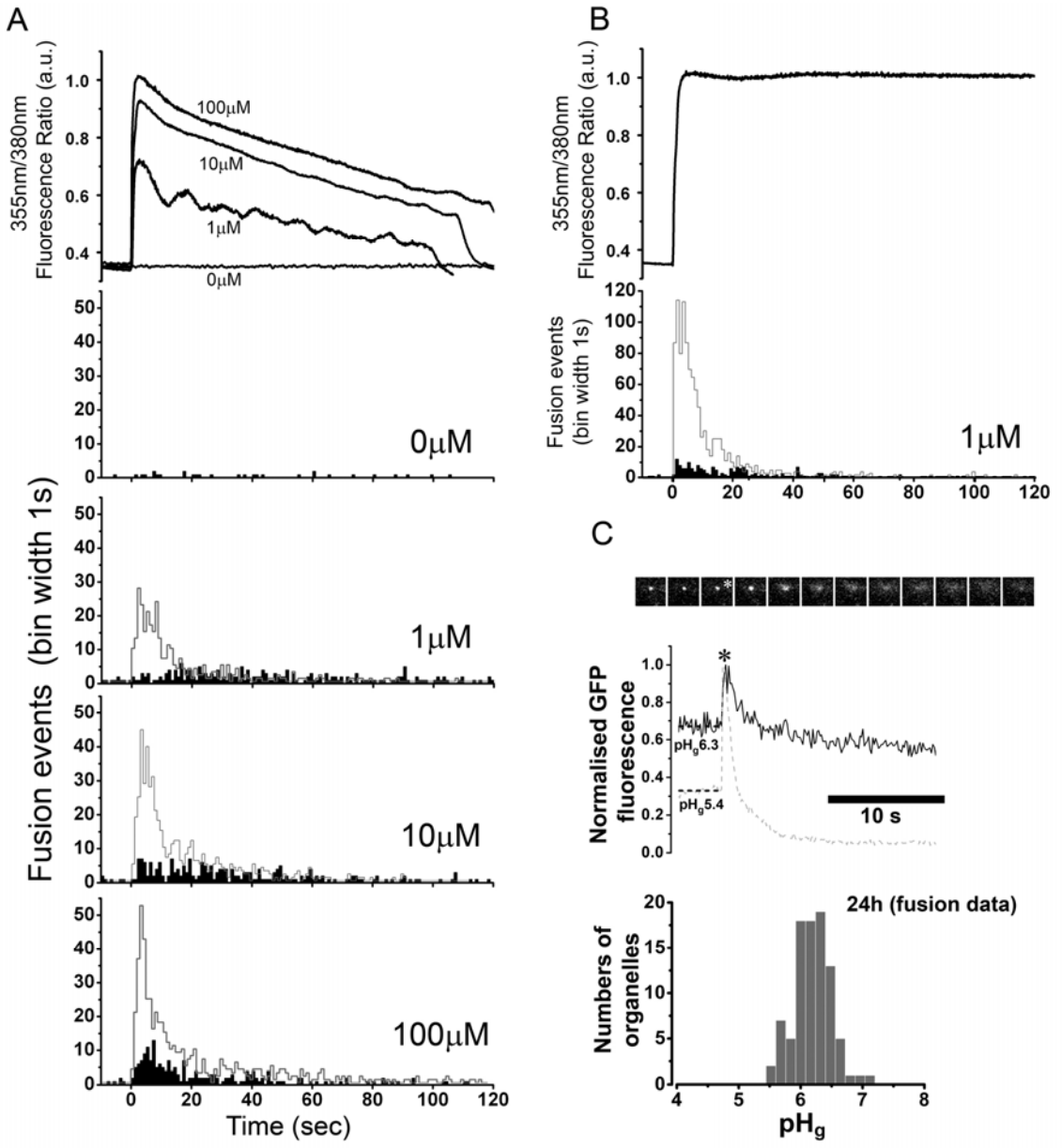
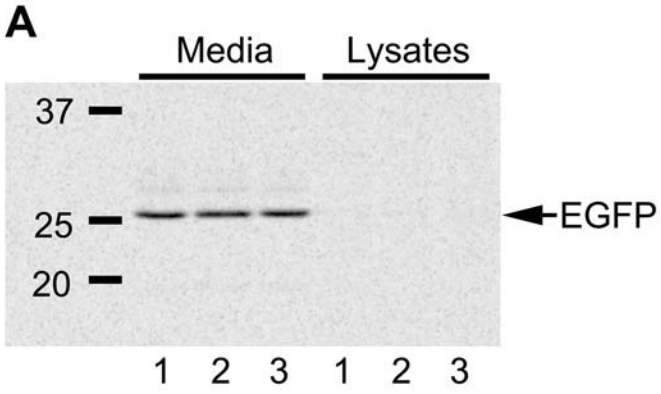


Figure S6. Unstimulated and stimulated exocytosis of the tPA-organelle

Cells co-expressing tPA-EGFP and proregion-mRFP, 24h post Nucleofection, were studied; proregion-mRFP fluorescence was used to identify and therefore exclude WPBs from analysis. Top panel in A shows representative examples of the changes in 355nm/380nm fura-2 fluorescence ratios in single HUVEC stimulated with either 0 (control) or 1, 10 or 100μM Histamine. The abrupt increase in the fura-2 fluorescence traces are aligned at time=0s. Lower panels in A show histograms of the times for tPA-EGFP granule fusion (solid black histograms). For comparison data for WPB fusion (from separate experiments using proregion-EGFP expressing cells) with histamine (A)

or ionomycin (B) is shown as open histograms (grey lines; 15 to 24 cells at each concentration). Fusion of tPA-organelles but not WPBs was observed in the absence of histamine. Approximately 50% of cells showed no stimulated secretion of tPA-organelles in response to histamine or ionomycin (1μM; 16/26 cells, 10μM; 18/33 cells, 100μM 15/24 cells, ionomycin 13/26 cells). The extent of degranulation was difficult to determine but was estimated to be 1-5% of granules (1-100μM histamine). WPB fusion was seen in all cells studied with a mean maximal extent of degranulation of 46±12% of fluorescent WPBs (100μM histamine). Because of unstimulated fusion it was not possible to determine the exact delay between histamine-evoked increases in $[Ca^{2+}]_i$ and the onset of stimulated tPA-organelle fusion. However, the mean delay between the increase in $[Ca^{2+}]_i$ and the first detected fusion event were 8.5±7.0s, 5.5±5.0s and 4.4±3.4s for 1μM, 10μM and 100μM histamine respectively (n=15-18 cells at each concentration). These delays were not significantly different from one another. Delays for WPB exocytosis were 4.2±3.7s, 2.0±1.0s and 1.6±0.8s respectively for 1-100μM histamine. The maximal rates of tPA-organelle fusion were 0.6±0.6, 0.6±0.5 and 2.5±2.4 organelles per second at 1μM, 10μM and 100μM histamine respectively. The maximum rate of WPB exocytosis was 2.4±1.9 WPB/s (100μM histamine). Panel B summarizes (as in panel A) ionomycin-evoked fusion of tPA-organelles. The mean interval between the increase in $[Ca^{2+}]_i$ and the first fusion event was 3.08±2.07s (n=13 cells) and the mean maximal rate of fusion was 2.36±1.92 organelles^{-1} (n=9 cells). Estimates of the extent of ionomycin-evoked tPA-EGFP organelle degranulation were similar to that for 100μM histamine (<5%). The maximum rate and extent of ionomycin-evoked WPB exocytosis was 11.4±3.7 WPB/s and 68±15.7% of fluorescent granules respectively. Panel C Top panel shows a montage of images from a time lapse movie of a single tPA-EGFP organelle undergoing exocytosis (images were acquired at 10 frames per second and are displayed with 2 frame averaging). The time course for the increase in fluorescence of this organelle is shown in the middle panel (black trace). The time point marked by the black asterisk corresponds to the frame in the image montage marked by the white asterisk. The grey trace in the middle panel shows a representative example of the time course for the increase in fluorescence within a tPA-EGFP containing WPB. Indicated on the traces are the pre-fusion resting pH values for these two different organelles calculated from the fluorescence signals as described in *Materials and Methods*. Lower panel in C summarizes the distribution of intra-granule pH for tPA-EGFP containing puncta calculated from fusion events as described in *Materials and Methods*. The mean pre-fusion pH was determined as pH6.26±0.40 (n=90 organelles, 16 cells).


A
Media
Lysates
37
25
20
EGFP
1 2 3 1 2 3

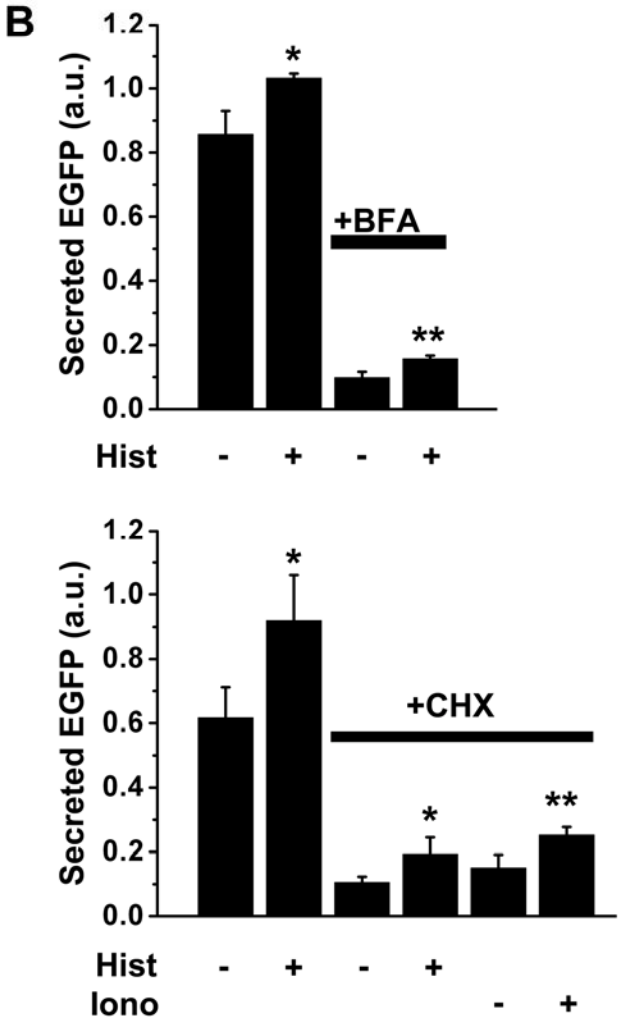

B
Secreted EGFP (a.u.)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
**
+BFA
Hist - + - +
Secreted EGFP (a.u.)
*
*
**
+CHX
Hist - + - +
Iono - +

Figure S7. **Pulse chase analysis of lumEGFP storage and BFA and CHX-insensitive stimulated secretion of LumEGFP in HUVEC**. Panel A shows an example of a pulse chase experiment to determine a storage efficiency of lumEGFP in HUVEC. Cells were pulsed for 1hr with 50μCi/ml Expre^{35}S^{35}S protein labeling mix and then chased for 24hrs. Media and lysates were collected from three individual samples (1-3 as indicated) and EGFP immunoprecipitated. Samples were run on a 12% gel and exposed to a storage phosphor plate. Upper panel in B shows histamine stimulated secretion of EGFP in the absence (BFA vehicle, 1hr) or following a BFA treatment (1hr) as indicated. Cells were Nuclefected with lumEGFP 24hrs prior to experiments. The lower panels in B show histamine or ionomycin-stimulated secretion of EGFP in control (CHX vehicle for 24hr) or CHX treated (24hrs) cells as indicated. Data shown is an individual experiment, carried out in triplicate, and is representative of three. * $P<0.05$, **$P<0.001$.

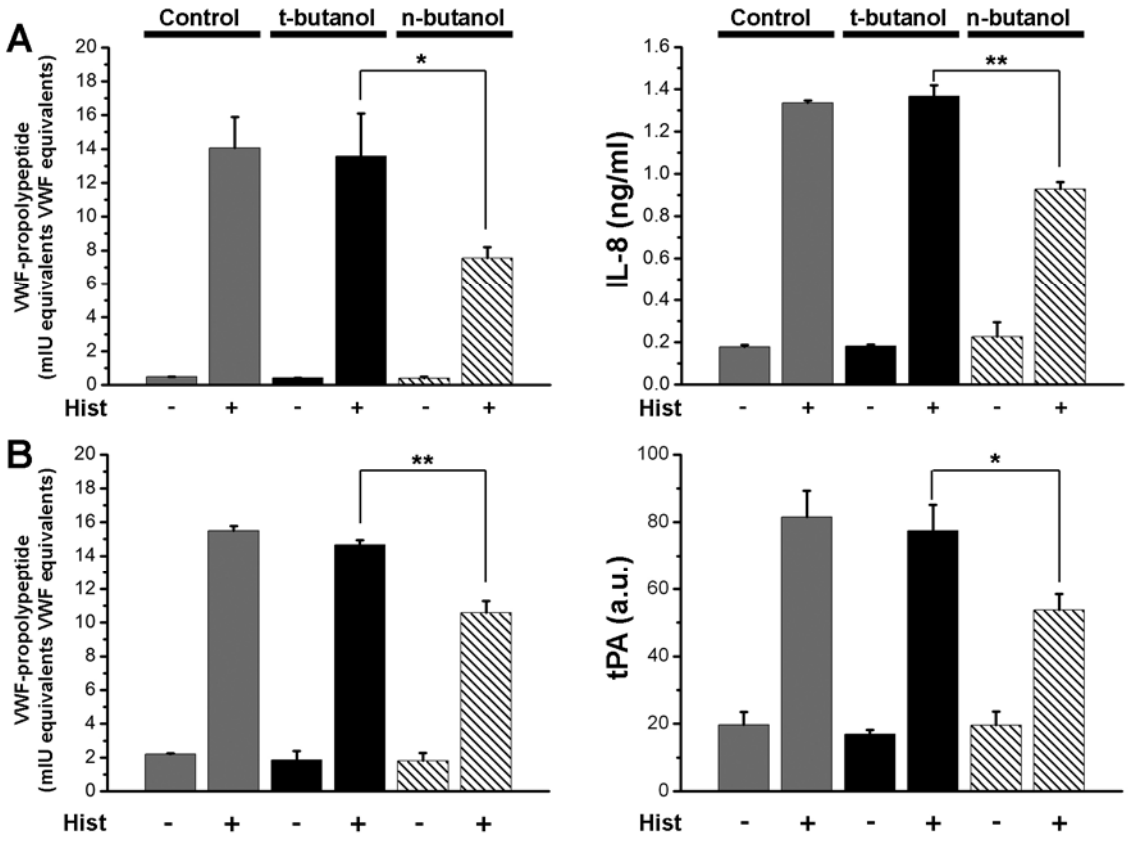


Figure S8. **Histamine-evoked secretion of IL-8 or tPA in BFA treated cells is sensitive to inhibition by n-butanol.** Solid grey bars in panel A show secretion (media samples) of the WPB specific protein VWF-propolypeptide (left) or IL-8 (right) in the absence (-; vehical) or presence (+) of 100μM histamine (as indicated) in IL-1β (1ng/ml, 24hrs) treated cells exposed to BFA (5μM, 1 hr). Stimulation period was 10 minutes. Solid black and open hatched bars indicate equivalent data from cells exposed to 25mM t-butanol or 25mM n-butanol (added 10 minutes prior to vehicle or histamine addition and maintained during the 10 minute stimulation period). Panel B shows similar data for histamine stimulated secretion of VWF-propolypeptide (left) or tPA (right) in cells exposed to Na-butyrate (3mM, 24hrs). VWF-polypeptide was assayed by specifc ELISA as previously described {Babich, 2009 #10}. Tert-butanol (Ultra pure 99.5%) was from Sigma, UK, n-butanol (99.5% pure) was from Fisher Scientific (UK). The experiment shown (means ± s.e.) is representative of two separate experiments, each carried out in triplicate. * $P<0.05$, ** $P<0.005$, two sample independent t-Test (Origin 7.5, Origin Lab, Northampton, MA).