Supplementary Figures

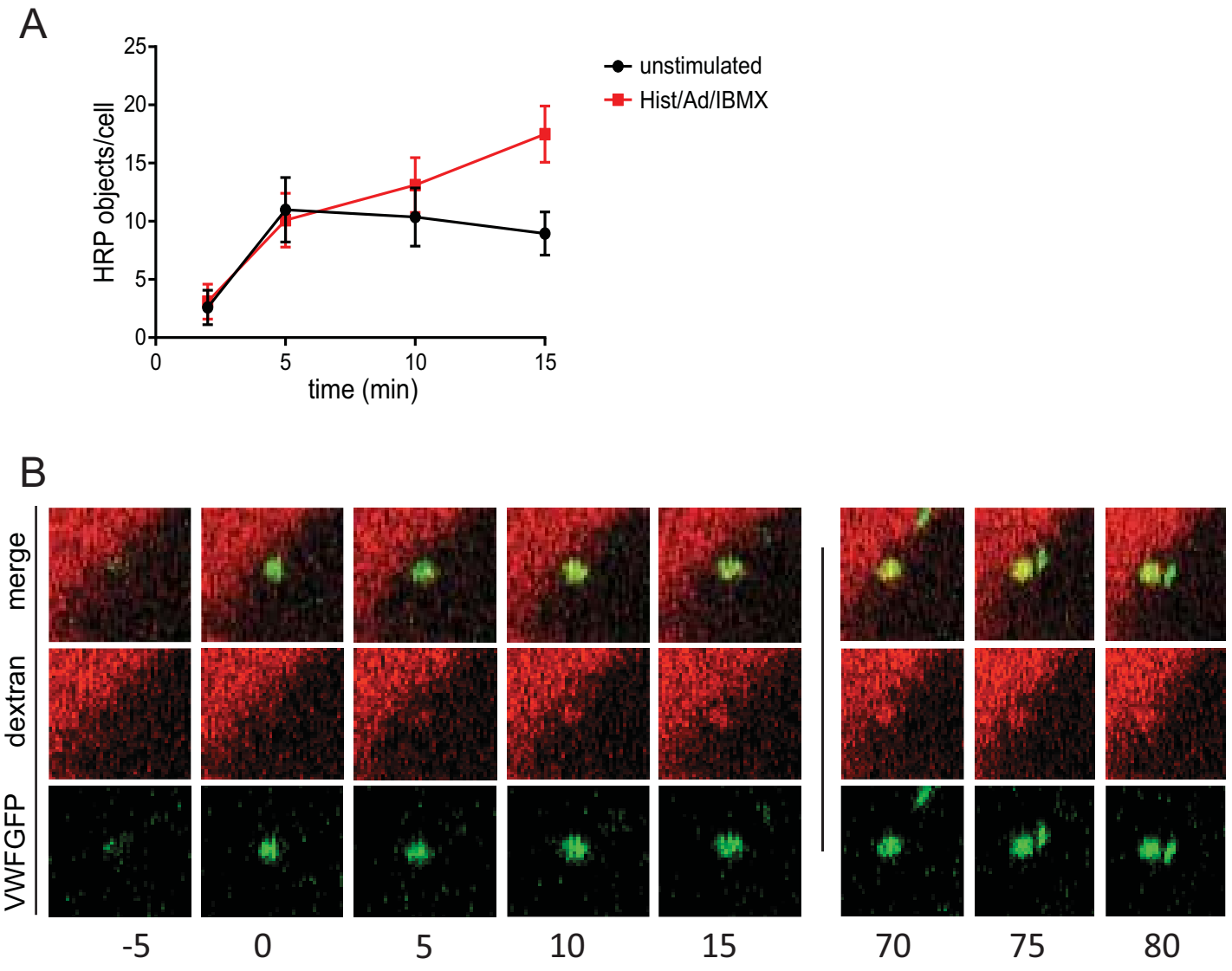

Figure S1. Live cell imaging of dextran incorporation into fused WPB

(A) HUVEC were incubated with HRP +/- a cocktail of Histamine (100µM), Adrenaline (10µM) and IBMX (100 µM) for 15 minutes. The number of HRP positive objects/cell was determined and plotted relative to time. (B) HUVECs expressing VWF-GFP (green) were imaged live in the presence of PMA (100ng/ml) and dextran-tetramethylrhodamine (red). One representative exocytic event is presented, which occurs at relative time 0 (time indicated underneath pictures in seconds). Images are single confocal planes. The fusion pore can be seen to fill with dextran, whereas a second WPB, which comes into focus and does not fuse, does not co-localise with dextran.

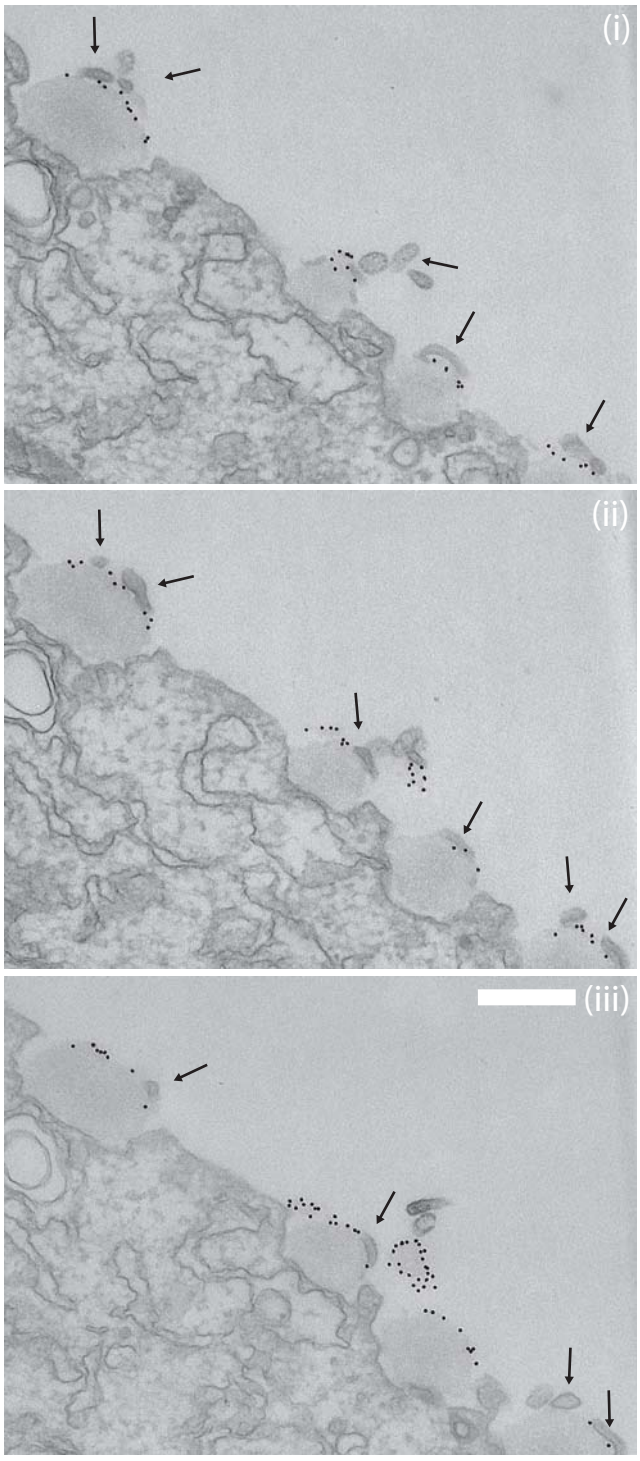

Figure S2. Multiple incomplete fusion events are apparent even in close proximity following actin depolymerisation

HUVEC were stimulated with PMA (100ng/ml) for 10 minutes in media supplemented with 1µM cytochalasin E (CCE). Cells were then fixed and external VWF labelled with anti-VWF antibodies before preparation for TEM. Serial sections are shown. Arrows show pieces of membrane still present around the fused exocytic structure, scale bar 500nm.

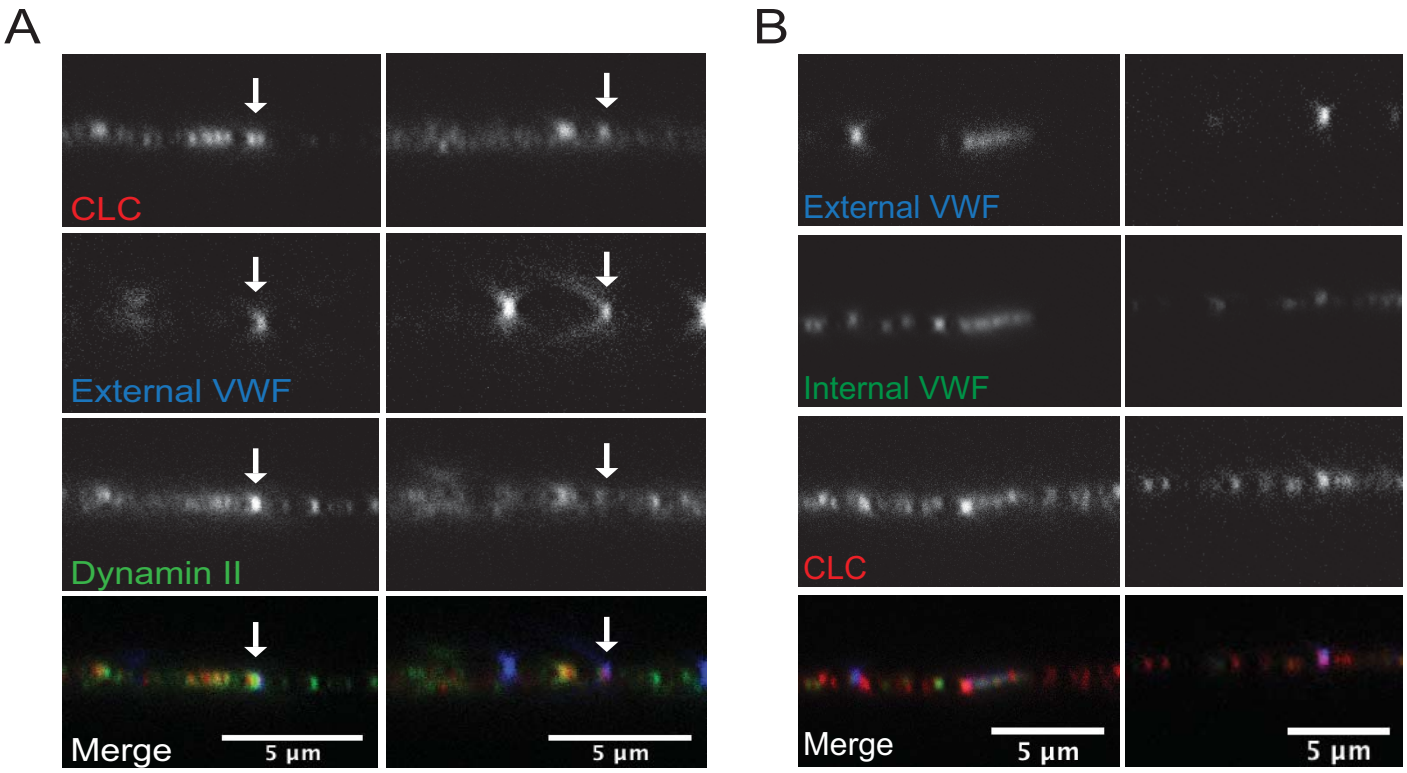

Figure S3. Clathrin and sometimes dynamin II co-localise with sites of string release

(A) Confluent HUVECs were stimulated with 100ng/ml PMA for 5 minutes at 37^0C then fixed and stained for external VWF (blue), dynamin II (green) and CLC (red). Cells were then imaged by confocal microscopy in the longitudinal plane to examine co-localisation between exocytosed VWF and clathrin at the exocytic pore. Single plane images are presented. Scale bar 5 µm. (B) Confluent HUVECs were stimulated with 100ng/ml PMA for 5 minutes at 37^0C then fixed and stained for external VWF (blue), total VWF (green) and CLC (red). Cells were then imaged by confocal microscopy in the longitudinal plane to examine co-localisation between exocytosed VWF and clathrin at the exocytic pore. Single plane images are presented. Scale bar 5 µm.

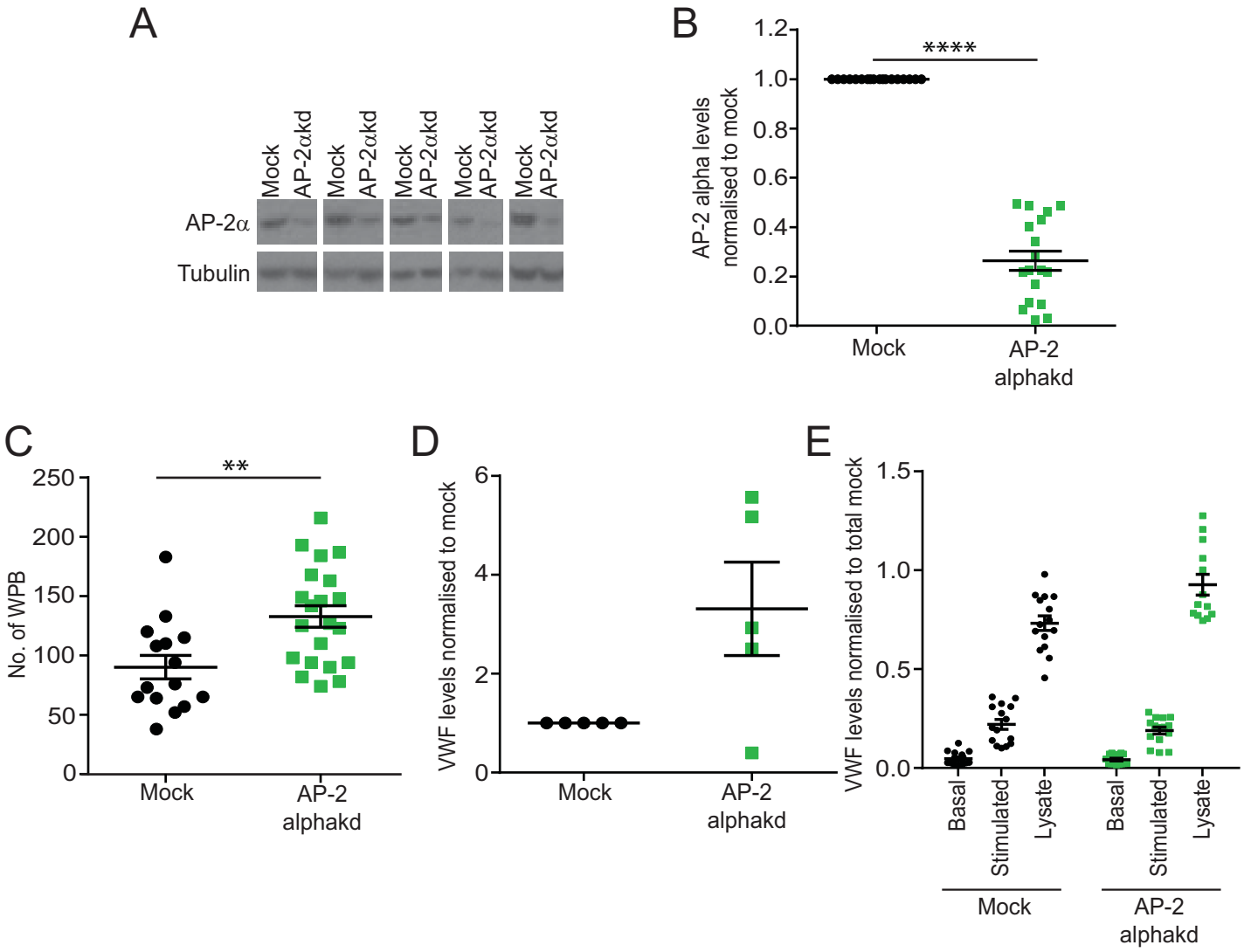

Figure S4. siRNA mediated knockdown of AP-2 alpha increases the number of WPB in endothelial cells.

(A-E) HUVEC were transfected with 300 pmol siRNA targeting AP-2 alpha for one or two rounds. (A & B) The extent of kd was determined by SDS PAGE and western blot. (A) Examples of western blots from 5 separate experiments showing AP-2 kd vs. the loading control β-tubulin. (B) Quantification of kd normalised to β-tubulin and relative to the mock, kd efficiency was on average 74% over 19

experiments. Error bars show SEM and statistics shown derived from unpaired student's T test on raw data (P=<0.0001). (C) At the second round of AP-2 alpha kd transfection 5μg GFP-VWF was incorporated and the cells were imaged live on a scanning confocal microscope. Images were taken and the number of WPB determined using Fiji and an arbitrary threshold. The number of WPB following AP-2 alpha kd was significantly increased. Error bars show SEM and statistics shown derived from unpaired student's T test (P=<0.005) n=5 experiments. (D) Quantification of VWF levels normalised to β-tubulin and relative to the mock, error bars show SEM n=5 experiments. (E) VWF levels as determined by ELISA. The amount of unreleased VWF in lysates was higher in AP-2 alpha kd cells compared to mock treated controls, error bars show SEM for n=5 experiments.

Table S1 – Co-localisation between internalised fluid-phase HRP and other proteins

SD = standard deviation. Data is the mean of 8-12 fields of view

Marker		**Unstimulated cells**	**Stimulated cells**	**Student's t-test**
EEA1	tM1	0.182 (SD = 0.046)	0.161 (SD = 0.038)	P = 0.285
	tM2	0.166 (SD = 0.038)	0.147 (SD = 0.063)	P = 0.278
P-selectin	tM1	0.195 (SD = 0.137)	0.207 (SD = 0.091)	P = 0.745
	tM2	0.03 (SD = 0.054)	0.111 (SD = 0.068)	P = 0.000063 ***
Transferrin	tM1	0.162 (SD = 0.037)	0.117 (SD = 0.117)	P = 0.421
	tM2	0.162 (SD = 0.030)	0.175 (SD = 0.063)	P = 0.261