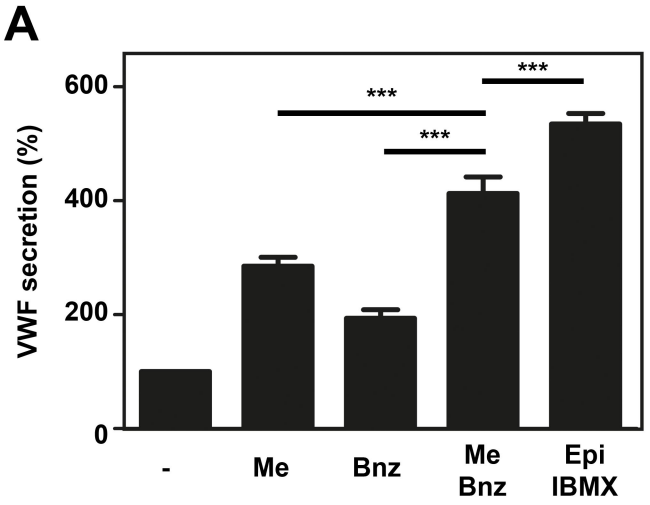
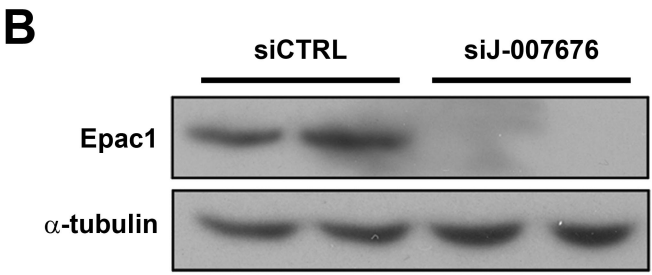
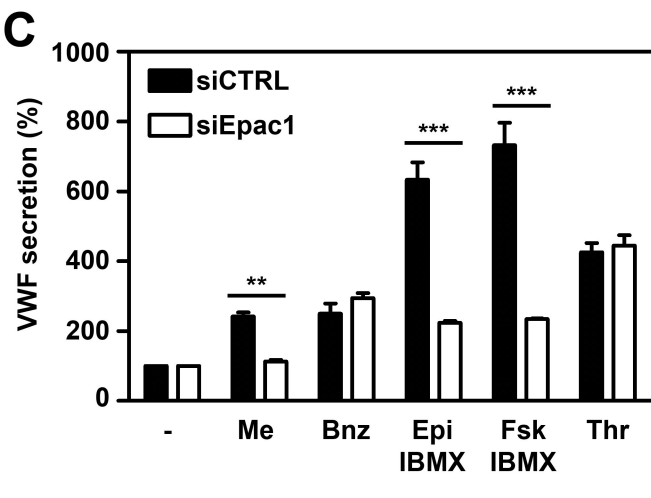
Supplemental Figure 1

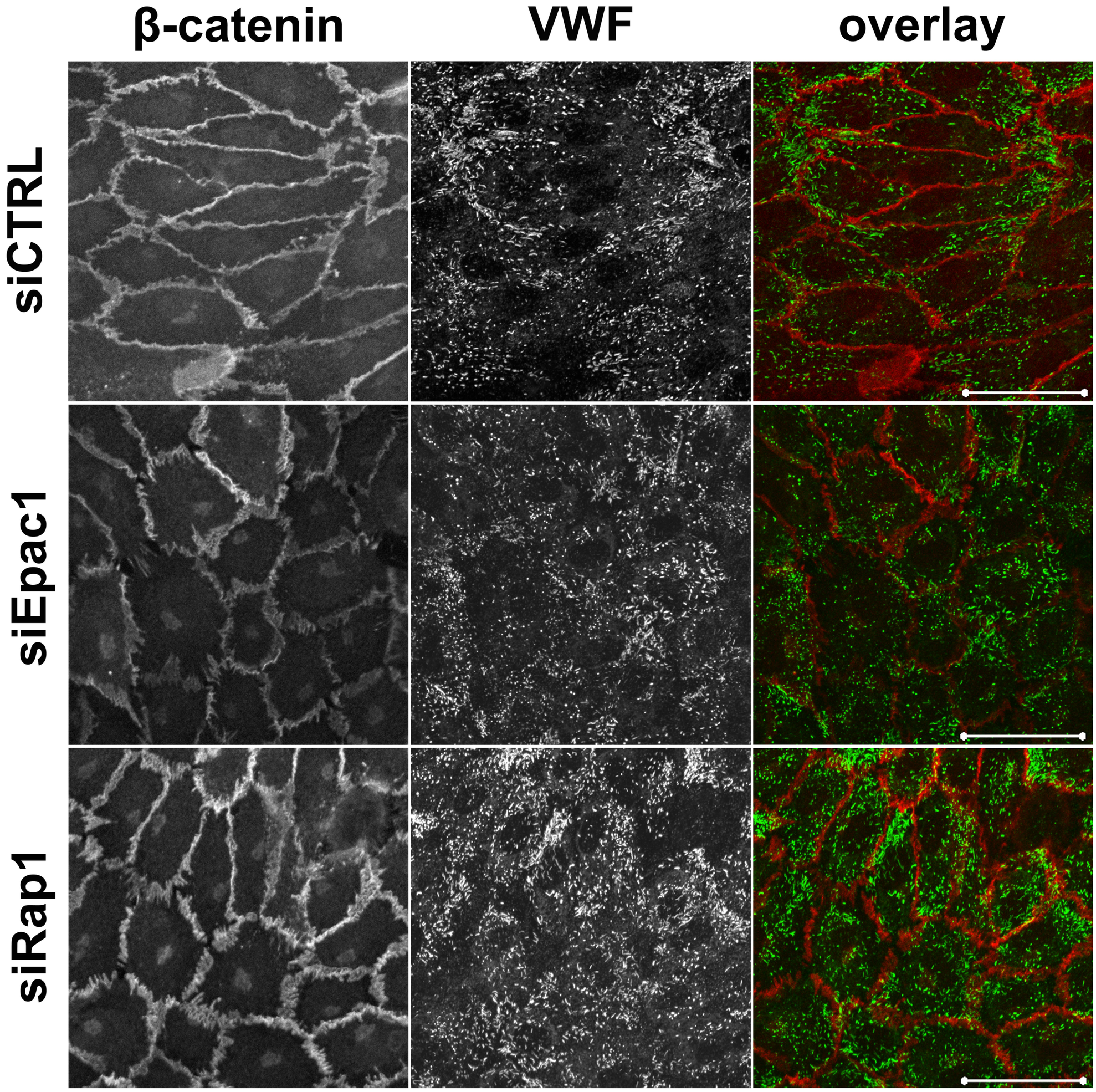






Supplemental Figure 2

A



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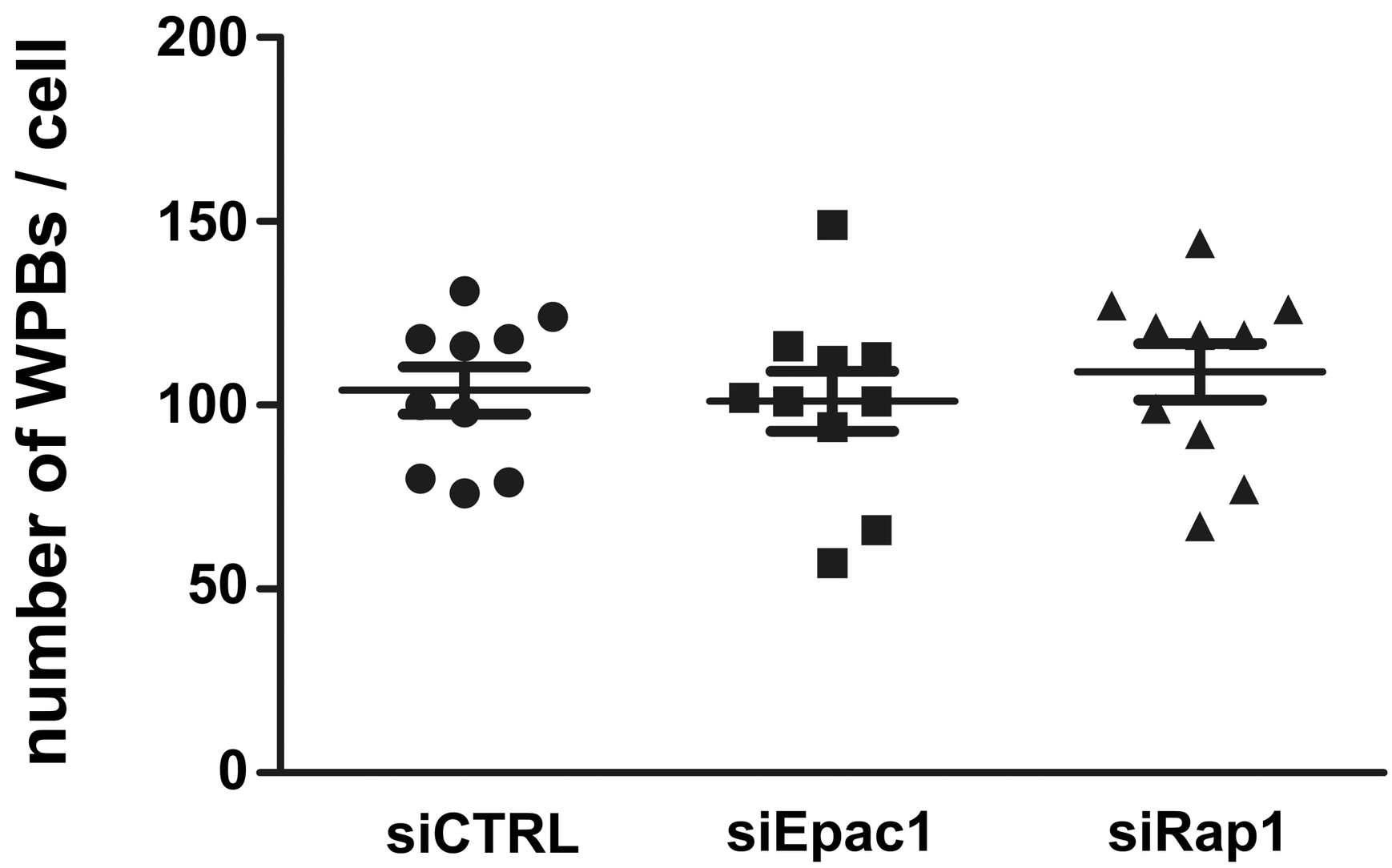
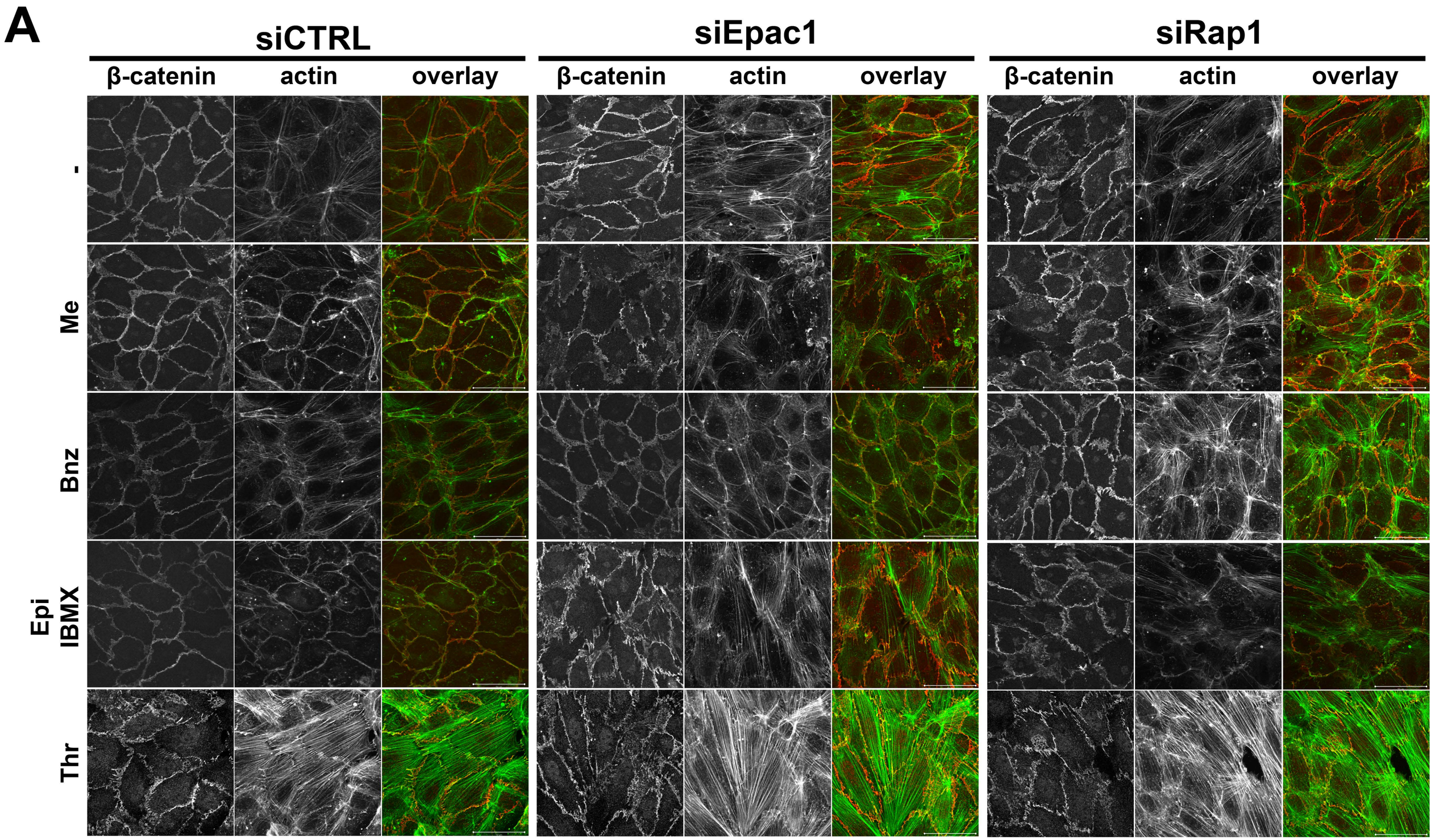
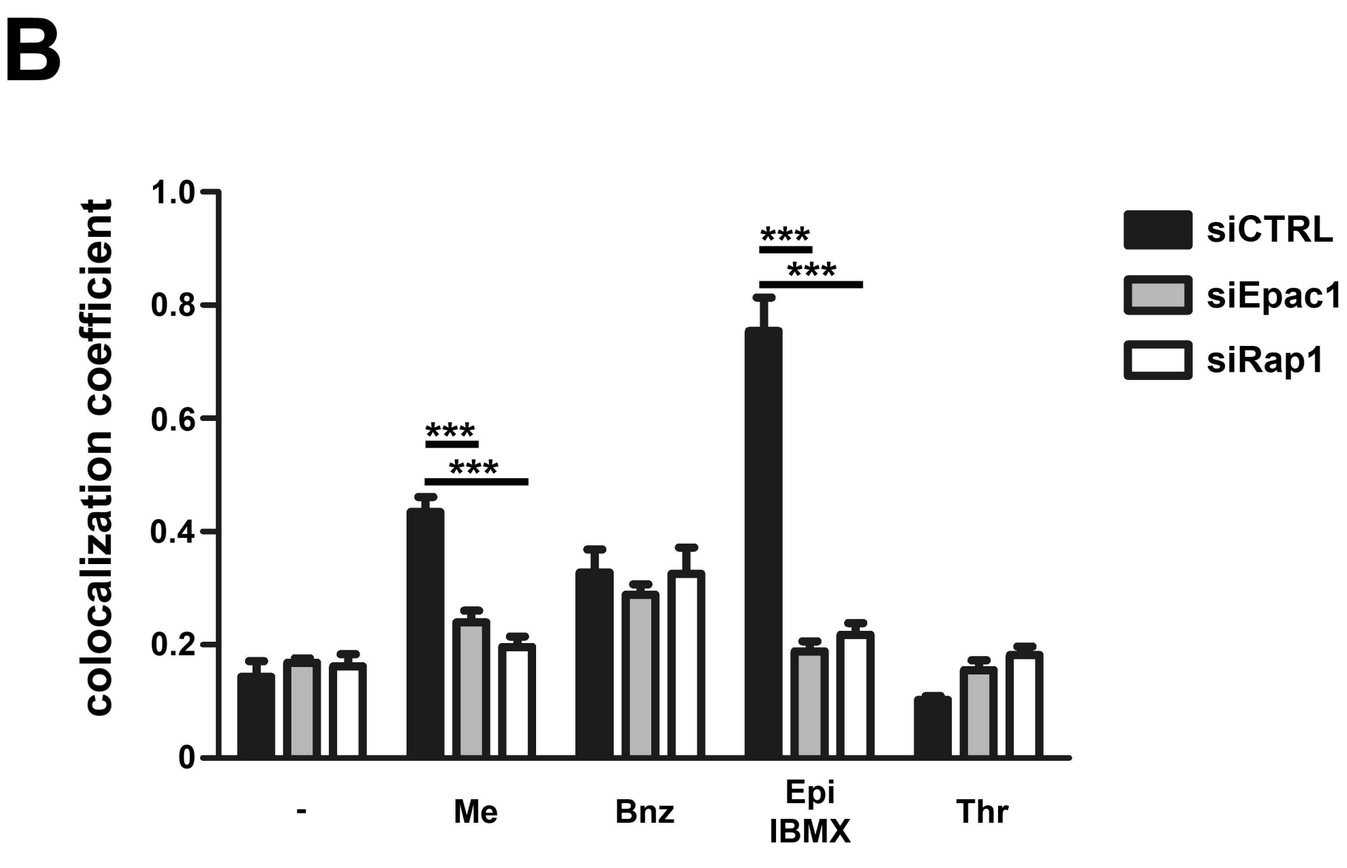
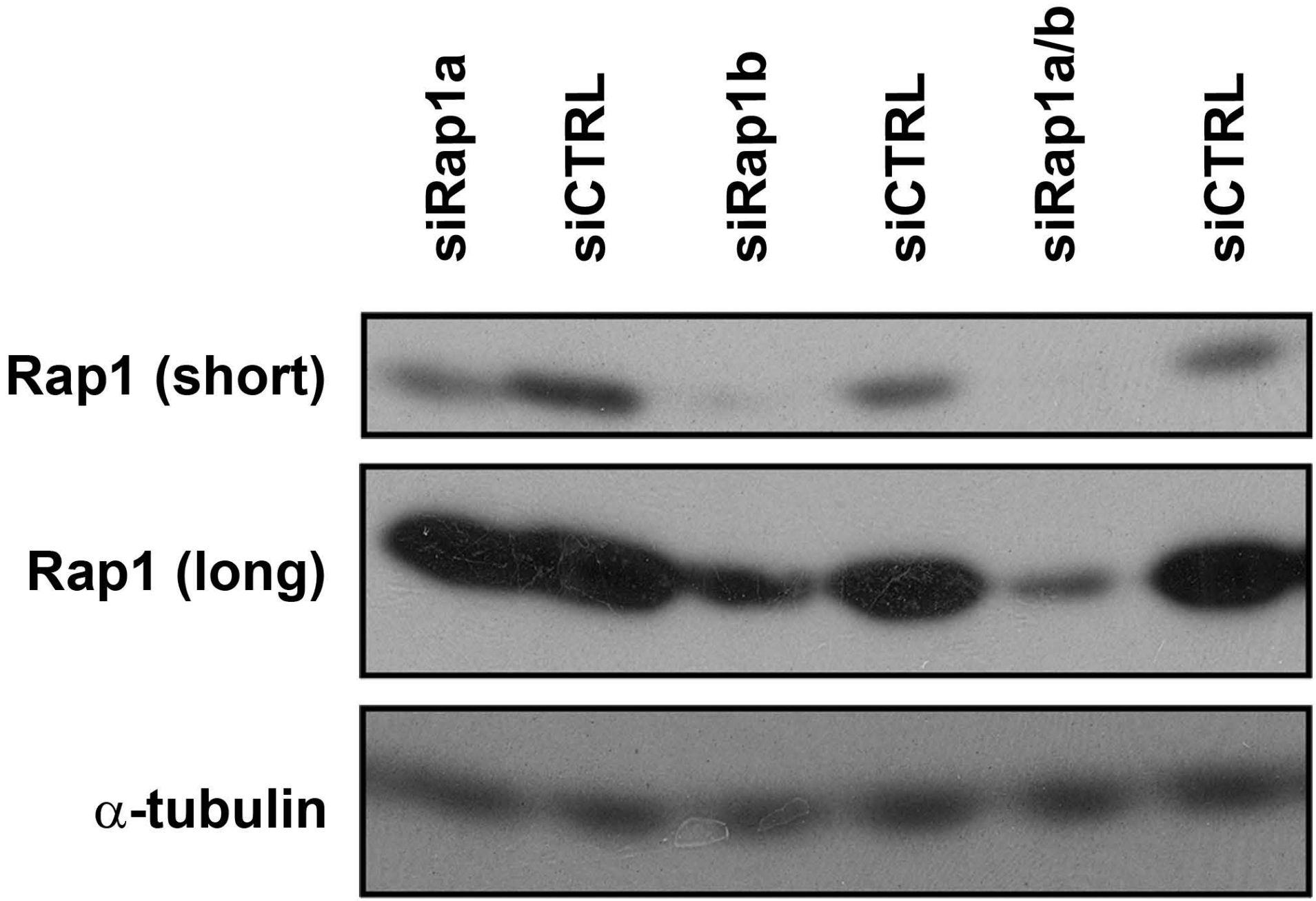


Supplemental Figure 3





Supplemental Figure 4



SUPPLEMENTAL FIGURE S1: Involvement of Epac in cAMP-mediated WPB release. (A) HUVECs were incubated with SF medium (-), supplemented with 1 μM Me-cAMP-AM (Me), 1 μM 6-Bnz-cAMP-AM (Bnz), 1 μM Me-cAMP-AM + 1 μM 6-Bnz-cAMP-AM (Me+Bnz) or 10 μM epinephrine and 100 μM IBMX (Epi). After 60 minutes the amount of VWF secreted in the medium was measured by ELISA. Basal VWF secretion (unstimulated) was set to 100%. (n=6, ***, $P<0.001$, 1-way ANOVA followed by Bonferroni post-hoc test.) Error bars show SEM. (B) HUVECs were transfected with a control siRNA (siCTRL) or a single siRNA targeting Epac1 (siJ-007676). Western blot analysis 72 hours post transfection showed downregulation of Epac1 expression. Levels of α-tubulin are shown as a protein loading control. (B) Control siRNA (siCTRL) and single Epac1 siRNA (siJ-007676) treated HUVECs were incubated for 60 minutes with 1 U/ml thrombin, 10 μM forskolin and 100 μM IBMX, 10 μM epinephrine and 100 μM IBMX, 1 μM Me-cAMP-AM, 1 μM 6-Bnz-cAMP-AM or SF-medium alone (unstimulated). The amount of VWF secreted in the medium. Unstimulated VWF secretion (unstimulated) is set to 100%. (n=3; ***, $P<0.001$; *, $P<0.05$; n.s., non significant; by 2-way ANOVA followed by Bonferroni post-hoc test for selected comparison.) Error bars show SEM.

SUPPLEMENTAL FIGURE S2: Number and distribution of WPBs at steady state is independent of Epac1 or Rap1. Endothelial cells were treated with pools of siCTRL, siEpac1 and siRap1 oligos as described and were cultured for 72 hours. Cells were paraformaldehyde fixed and stained for β-catenin (red) and VWF (green). (A) Representative low power images are shown. Scale bar represents 50 μm. (B) For the conditions described above, numbers of WPBs per cell were counted as described (11) (n=10). No significant differences in numbers of WPBs per cell were observed by 1-way ANOVA. Error bars show SEM.

SUPPLEMENTAL FIGURE S3: Cytoskeletal reorganization in response to Me-cAMP-AM or epinephrine is dependent on Epac and Rap1. Endothelial cells were treated with pools of siCTRL, siEpac1 and siRap1 oligos as described and were cultured for 72 hours and treated with vehicle (-), 1 μM Me-cAMP-AM (Me), 1 μM 6-Bnz-cAMP-AM (Bnz), 10 μM Epinephrine and 100 μM IBMX (Epi+IBMX) or 1 U/ml thrombin (Thr). Cells were paraformaldehyde fixed and stained for β-catenin (red) and actin with Alexa 488-phalloidin (green). (A) Representative low power images are shown. Scale bar represents 50 μm. (B) For the conditions described above, the degree of colocalization (colocalization coefficient) between actin and β-catenin in 5 fields of view was determined using the Zen software package (Carl Zeiss B.V., Sliedrecht, Netherlands). Shown are the results of one experiment, representative of three independent experiments. ***, $P<0.001$ by 2-way ANOVA followed by Bonferroni post-hoc test for selected comparison. Error bars show SEM.

SUPPLEMENTAL FIGURE S4: Downregulation of Rap1 in endothelial cells. HUVECs were transfected with a control siRNA SMARTpool (siCTRL) or siRNA SMARTpool targeting Rap1a, Rap1b or a mixture of SMARTpool siRNA targeting Rap1a and Rap1b. Western blot analysis 72 hours post transfection showed downregulation of Rap1 expression. The shorter and longer exposure times illustrate the partial effect of siRap1a and siRap1b. Levels of α-tubulin are shown as a protein loading control.