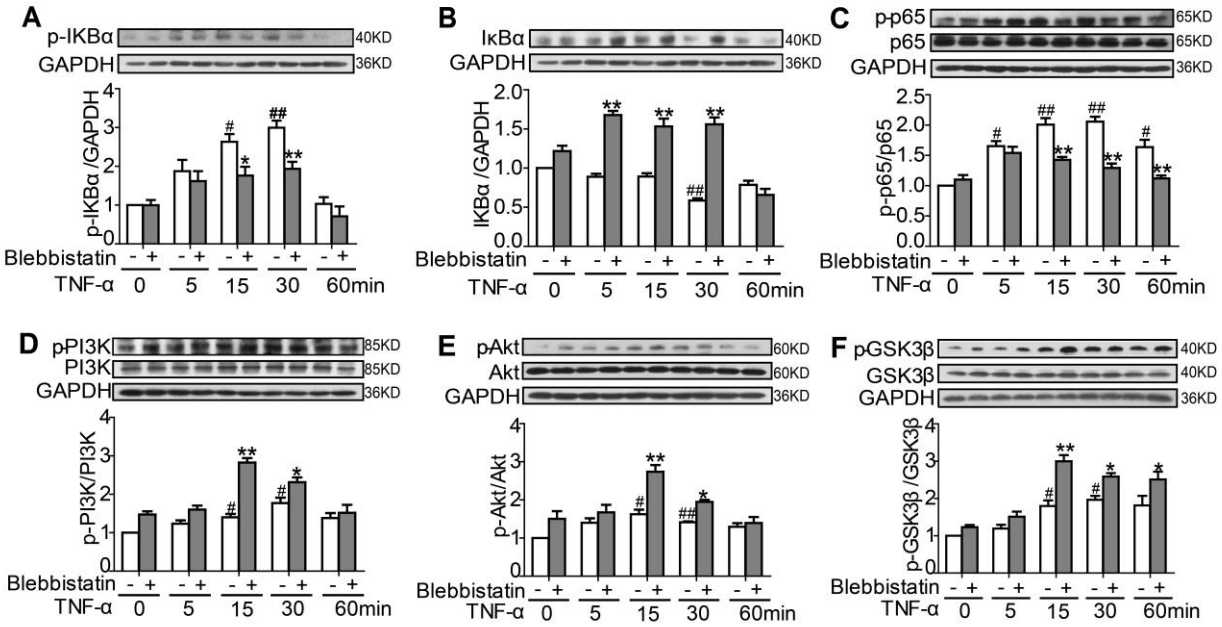
Supplementary Material to Zhai et al. “NMMHC IIA inhibition impedes tissue factor expression and venous thrombosis via Akt/GSK3β-NF-κB signalling pathways in the endothelium” (Thromb Haemost 2015; 114.1)

Supplementary figure 1.tif



Suppl. Figure 1: Blebbistatin downregulated NF-κB pathway and activated Akt/GSK3β signaling pathway induced by TNF-α in the endothelial cells. EA.hy926 endothelial cells were treated with TNF-α (10 ng/ml) with or without blebbistatin (1 μM) for the indicated time. **A-C**) Total and phosphorylated forms of IκBα and p65 were examined by western blotting. $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs the unstimulated group at 0 min; $^{*}P<0.05$, $^{**}P<0.01$ vs TNF-α stimulated group at certain time. **D-F**) PI3K, Akt, GSK3β and their phosphorylated forms were examined by western blotting. $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs the unstimulated group at 0 min; $^{*}P<0.05$, $^{**}P<0.01$ vs TNF-α model group at certain time.

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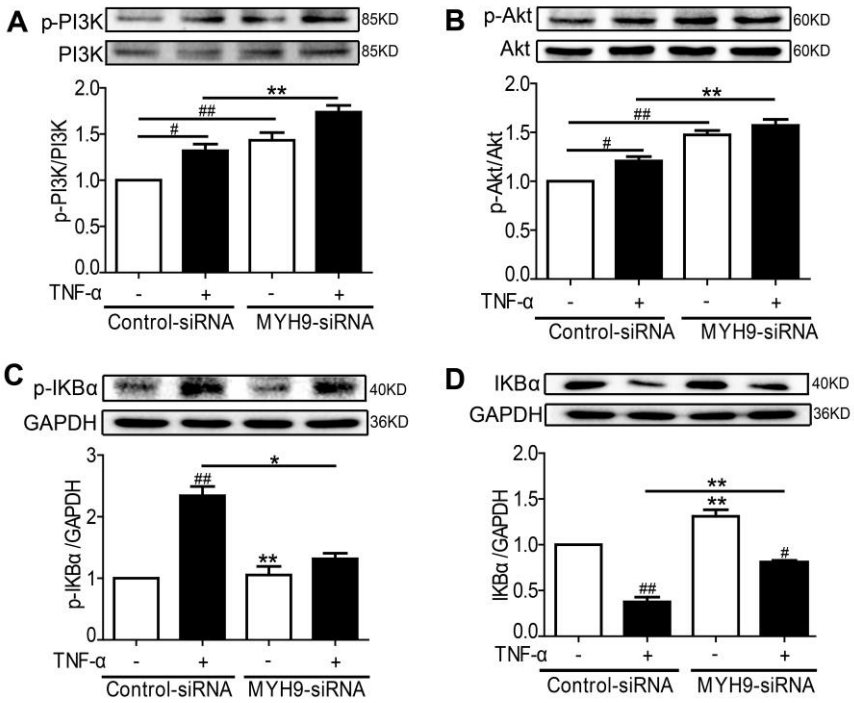
Supplementary figure 2.tif



Suppl. Figure 2: NMMHC IIA specific knock-down facilitated TNF-α induced PI3K/Akt activation and attenuated NF-κB in the endothelial cells. At 48 h post-transfection, EA.hy926 endothelial cells were treated with 10 ng/ml TNF-α for 15 min or 30 min. **A-B**) Total and phosphorylated PI3K, Akt were analyzed by western blotting. **C-D**) Total and phosphorylated IκBα were analyzed by western blotting. Densitometry quantification represents data from 3 individual experiments. $^{\#\#}P<0.01$ vs control-siRNA group; $^{**}P<0.01$ vs TNF-α stimulation group.

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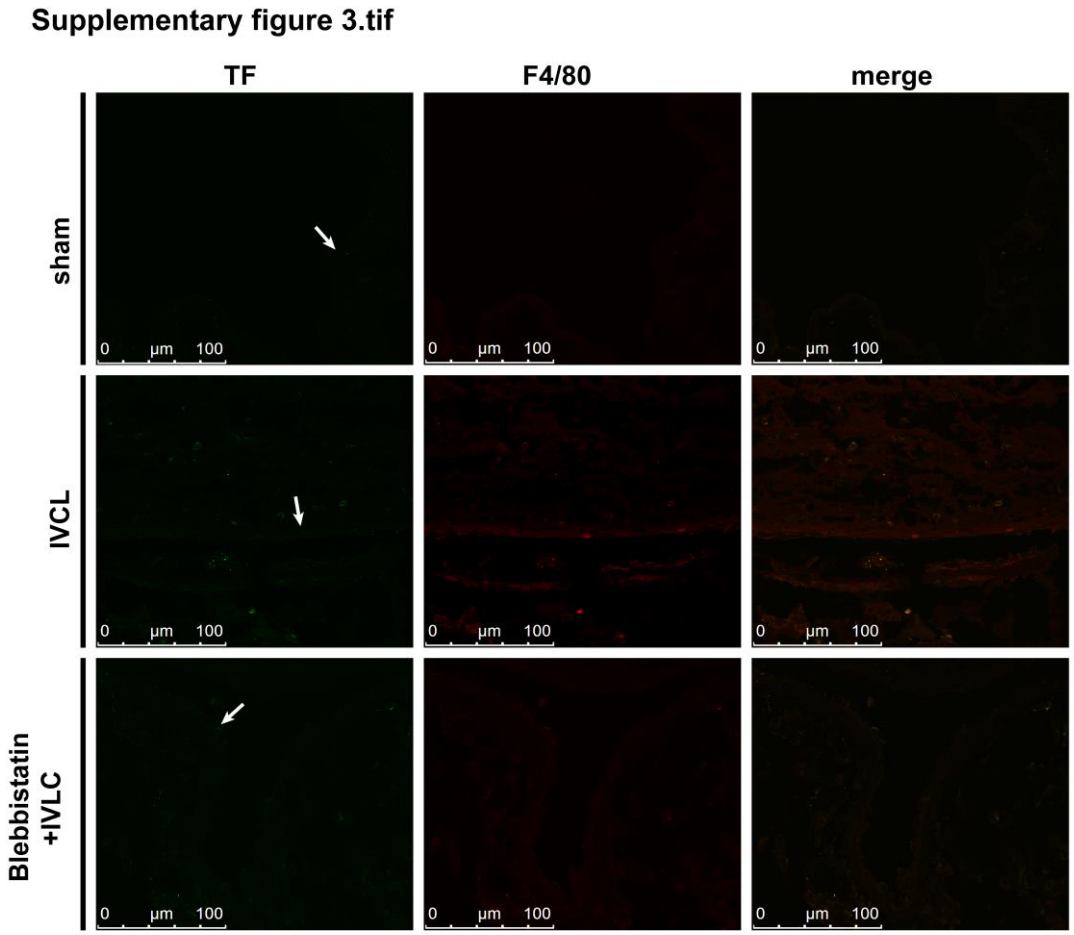

Suppl. Figure 3: Double staining for TF and a macrophage marker. IVC sections were immunostained with a combination of anti-TF pAbs (green) and anti-F4/80 pAbs (red). Images were digitally merged. Representative results are shown. Bar, 100 µm. (arrows indicate vessel wall).

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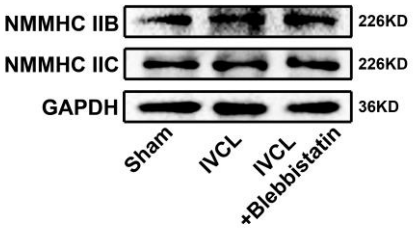
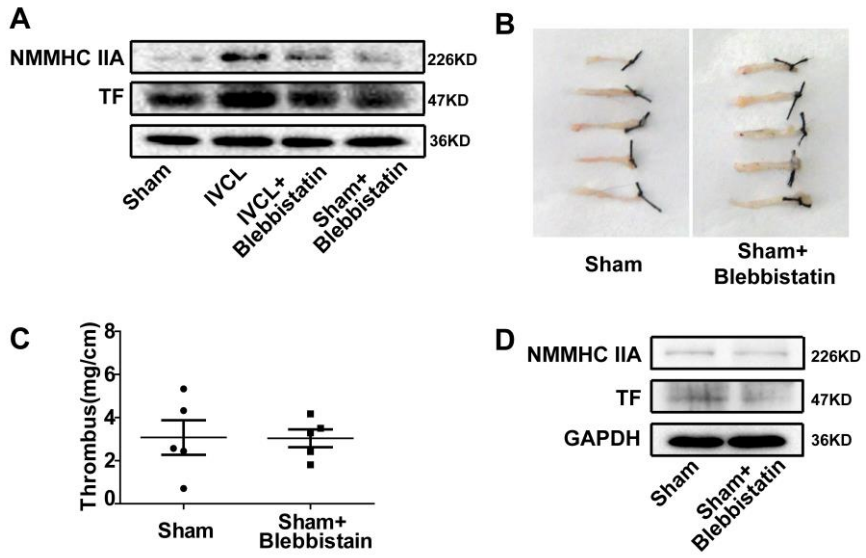
Supplementary figure 4.tif



Suppl. Figure 4: Blebbistatin barely affected NMMHC IIB and NMMHC IIC expression in DVT mice. At 48 h after IVCL, IVCs were harvested. NMMHC IIB and NMMHC IIC were analyzed by western blotting.

Supplementary Material to Zhai et al. “NMMHC IIA inhibition impedes tissue factor expression and venous thrombosis via Akt/GSK3β-NF-κB signalling pathways in the endothelium” (Thromb Haemost 2015; 114.1)

Supplementary figure 5 . tif



Suppl. Figure 5: Blebbistatin pretreatment alone had no obvious difference from the sham group in DVT mice. At 48 h after IVCL, IVCs were harvested. **A)** TF and NMMHC IIA were analyzed by western blotting. **B-C)** The thrombosed inferior vena cava was weighed and the length of thrombus was measured. The size of the thrombus was quantified as mg/cm. Five representative thrombosed inferior vena cava in each group and the quantitative data of thrombosed inferior vena cava are shown. **D)** TF and NMMHC IIA were analyzed by western blotting.

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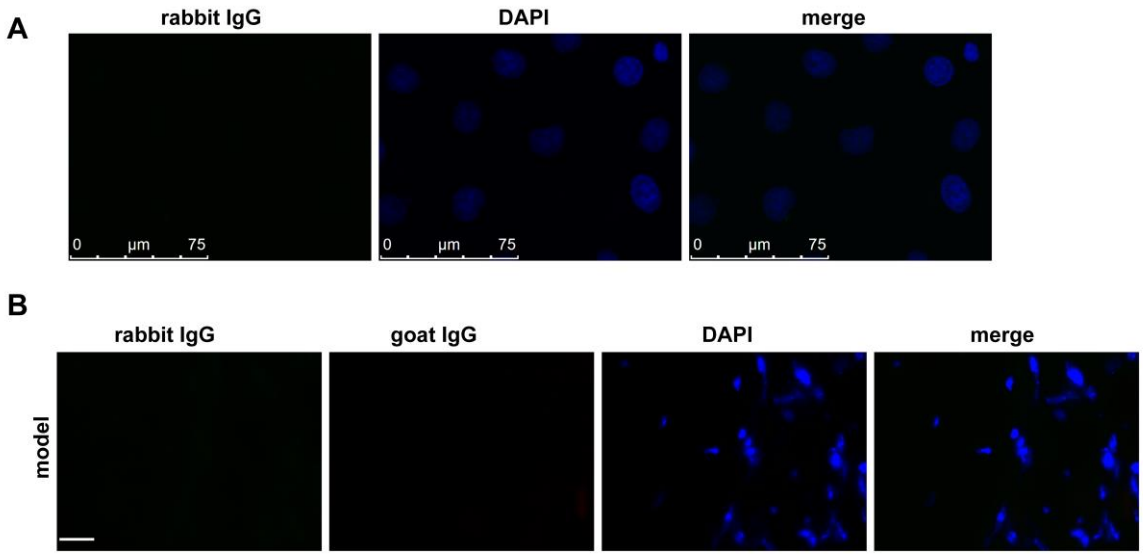
Supplementary figure 6.tif



Suppl. Figure 6: Staining controls. A) Staining controls for endothelial cells; **B**) Staining controls for IVC sections. Bar, 75 µm.