**Supplementary Material to Mahajan et al. “A novel function of FoxO transcription factors in thrombin-stimulated vascular smooth muscle cell proliferation” (Thromb Haemost 2012; 108.1)**

**Supplemental Figure 1.** No effects of activating peptides for PAR-3 or PAR-4 on FoxO1 and FoxO3 phosphorylation (5-60 min) in human saphenous vein SMC. Cells were grown to 80-85% confluence, serum-deprived for 48 h then incubated with PAR-3-AP (200 µM) or PAR-4-AP (200 µM) for the indicated time periods. Representative immunoblots (upper panel) and densitometric analysis (bottom panel) of phosphorylated FoxO1 and FoxO3 normalized to total protein (A-D) respectively from independent experiments (mean ± SEM, n=4; NS: not significant).

**Supplemental Figure 2.** Extended time-course (up to 24 h) studies in thrombin-stimulated phosphorylation of FoxO1 and FoxO3. Cells were grown to 80-85% confluence, serum-deprived for 48 h then stimulated with thrombin (3 U/ml) for the indicated time periods. Representative immunoblots (upper panel) and densitometric analysis (bottom panel) of phosphorylated FoxO1 and FoxO3 normalized to total protein was sustained to 1 h and 2 h respectively (A-B). Phosphorylation of FoxO4 was not influenced upon thrombin stimulation (5-60 min) (C). Data represented as densitometric analysis from independent experiments (mean ± SEM, n=3; *P <0.05 vs. control; NS: not significant).

**Supplemental Figure 3.** Thrombin activates ERK1/2, AKT, and p38MAPK in human saphenous vein SMC. After 48 h of serum withdrawal cells were stimulated with thrombin (3 U/ml) for the indicated time periods. Phosphorylated ERK1/2, AKT, and p38 MAPK (A-C, respectively) was detected by immunoblot analysis. Where indicated, cells were pretreated (30 min) with inhibitors of MEK1/2 (PD98059, 20 µM) or p38 MAPK (SB204294, 30 µM) prior to stimulation with thrombin (3 U/ml) or PAR-1-AP (200 µM) (E-D). Membranes were stripped and reprobed for total protein for normalisation. The histograms show densitometric analysis from independent experiments (mean ± SEM, n=3; *P <0.05 vs. control; #P <0.05 stimuli vs. stimuli + inhibitor).

**Supplemental Figure 4.** ERK1/2 and p38 MAPK inhibition does not affect phosphorylation of FoxO factors in human vascular SMC. Cells were grown to confluence, deprived of serum for 48 h then pretreated (30 min) with inhibitors of MEK1/2 (PD98059, 20 µM) or p38 MAPK (SB203580, 30 µM) prior to incubation with thrombin (3 U/ml) or PAR-1-AP (200 µM). Representative immunoblots (upper panel) and densitometry data (bottom panel) for phospho-FoxO1 (A, C) and phospho-FoxO3 (B, D) normalized to total protein were obtained from independent experiments (mean ± SEM, n=3; *P <0.05 vs. control).

**Supplemental Figure 5.** FoxO expression and phosphorylation in presence of FoxO siRNA and thrombin. Human vascular SMC transfected with scrambled control siRNA or specific siRNA against FoxO1 (A) or FoxO3 (B) prior to serum deprivation (48 h) and stimulation with thrombin for 24 h. Representative blots at the top of each graph indicate phosphorylation and total amounts of the respective proteins. The bars in the graphs show densitometric analysis (mean ± SEM, n=3; *P <0.05 vs. control).
Supplemental Figure 2 (A-C)

A  
FoxO1  
Phospho  
82kDa  
76-82kDa  
Total  
B  
FoxO3  
Phospho  
97kDa  
82-97kDa  
Total  
C  
Foxo4  
Phospho  
70kDa  
65kDa  
Total  

Phospho vs total FoxO1  
(min)  (h)  Thrombin  

Phospho vs total FoxO3  
(min)  (h)  Thrombin  

Phospho vs total FoxO4  
(min)  (h)  Thrombin  

Con 5 10 15 30 60 (min)  Thrombin  

NS
Supplemental Figure 5 (A-B)

A

B

Phospho vs total FoxO1
(fold control)

Phospho vs total FoxO3
(fold control)