## Supplementary Material

***Antibodies.*** The following were used: mouse anti-human TBM monoclonal (clone IA4) (gift of Dr Phil Bird, Monash University, Australia); peroxidase-labelled sheep anti-FITC (POD) (Roche, Dee Why, Australia), rabbit anti-human von Willebrand factor (vWF) (DakoCytomation, Hamburg, Germany); TCR α/ß clone R73 (AbD Serotec, Kidlington, UK).

***Histopathology.*** Fresh-frozen tissue sections (4µm) were stained with mouse anti-hTBM-FITC (1:100) followed by sheep anti-FITC POD (1:250). vWF staining was performed on paraffin-embedded sections, which were incubated with the primary antibody followed by DAKO Envision$^{+}$ goat anti-rabbit HRP. Slides were developed and counterstained with Ehrlich's haematoxylin (Sigma-Aldrich, Sydney, Australia).

***Statistical analysis.*** Quantitative data were summarized by mean and standard error of the mean (SEM). Statistical analyses were performed with Microsoft Excel and Graphpad Prism 5 (Graphpad Software, Inc., CA, USA) using the unpaired t-test and the Wilcoxon signed rank test. The two-tailed p value was calculated; $p < 0.05$ was regarded as significant.

***Ethical approvals:*** All experiments were approved by and conducted under the supervision of the Animal Ethics Committee of St. Vincent's Hospital Melbourne.

### Full blood counts and clotting studies

Platelet counts and coagulation parameters were measured using EDTA and citrate treated blood respectively on a full blood count (FBC) analyser (Sysmex, SE-9000, TOA Medical Electronics Co Ltd, Kobe, Japan).

**Measurement of renal function.**
Whole blood was collected via an Inferior vena cava puncture and analysed using an Olympus AU 2700 (Integrated Science, Chatswood, NSW, Australia). Creatinine and urea are expressed in mmol/l.Platelet and whole blood aggregation.

**Whole blood and washed platelet aggregation.**
***Preparation of whole blood.*** 500 µl of citrated venous blood (1:10 v/v) was drawn from the inferior vena cava (IVC) of anaesthetised mice. The blood was diluted 1:2 with normal saline to which 2 mM $CaCl_2$ and 4 units /ml collagen was added. Impedance aggregation was determined using an aggregometer, (Chrono-log, model number 570VS, Chrono-log, Haverton PA). Platelet aggregation is detected by passing a very small electric current between two electrodes immersed in whole blood, and measuring the impedance between the electrodes. During the initial contact with the sample a monolayer of platelets forms on the electrodes, when the agonist is added there is gradual aggregation at the electrodes increasing the impedance.

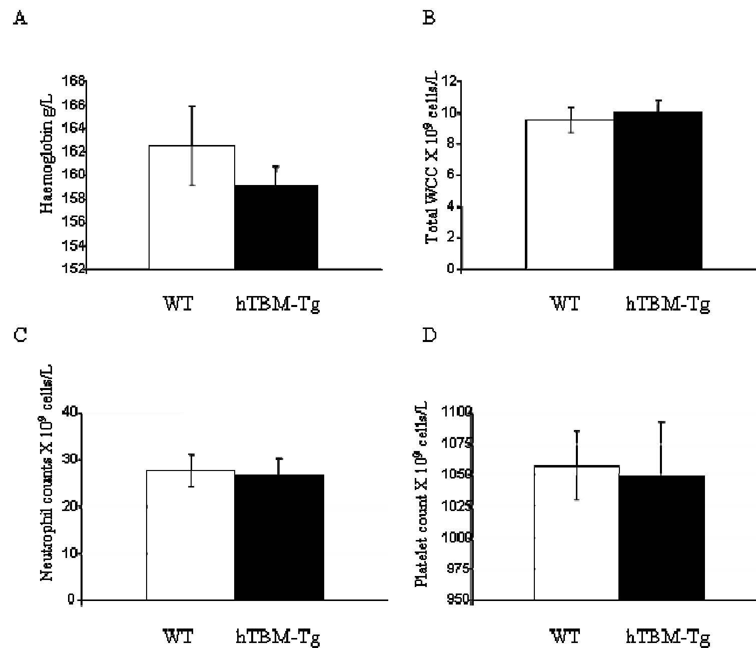
***Preparation of washed platelets.*** Blood was collected via the IVC into acid-citrate dextrose (90 mM sodium citrate, 7 mM citric acid, pH 4.6, 140 mM dextrose, 70 mM theophylline) as anticoagulant (9:1 ratio). 200 microlitres of platelet wash buffer (43 mM Na2HPO4, 24.3 mM NaH2PO4.2H20, 4.3 mM K2HPO4, 113 mM NaCl, 5.5 mM glucose) was added and the mixture was centrifuged at 200 g for 2 minutes at 37C without brakes. The supernatant (platelet-rich plasma, PRP) was removed and stored at 37C. The process was repeated. The PRP was then centrifuged at 2000 g for 2 minutes with brakes and the platelet pellet resuspended in 550µl of Tyrodes buffer (137 mM NaCl, 2 mM KCl, 12 mM NaHCO3, 0.3 mM NaH2Po4, 1mM

MgCl2.6H20, 5.5 mM glucose, 5 mM Hepes, pH 7.3). The platelets were allowed to recover for 30 minutes at 37C.

Washed platelets as obtained above were then counted, and standardised so that $2x10^5$ washed platelets/μl of Tyrodes buffer were reconstituted with fibrinogen, and aggregation was by optical (turbidometric) aggregation on an aggrometer (Chrono-log, model number 570VS Chrono-log, Haverton PA). Dual beam infra-red light sources and photodiode detectors are used to determine the difference in light transmission between platelet rich plasma (PRP) and platelet poor plasma (PPP).

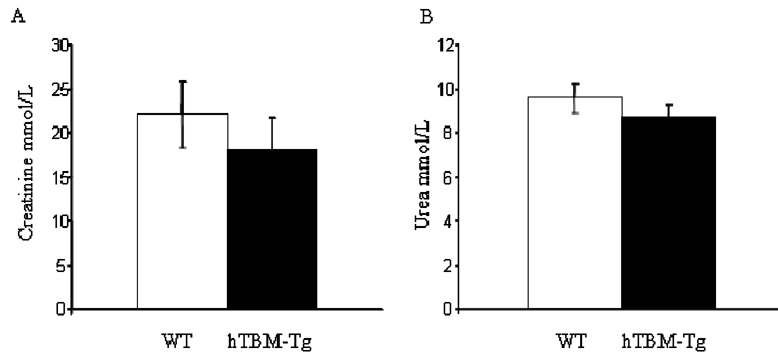
**P selectin staining.**

Washed platelets (obtained as described above) were stained with P selectin (CD62P) (BD Pharmingen, San Diego, California) on ice for 30 minutes and analysed using a FACScalibur flow cytometer (Becton Dickinson, NJ).

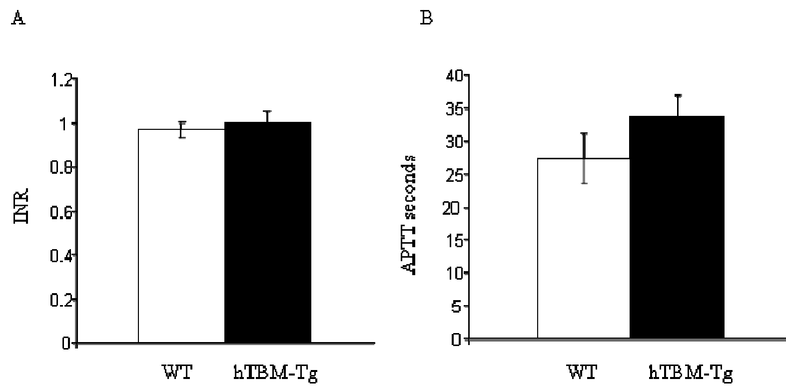



Supplementary figure 1) Haematological testing. A) Haemoglobin, B) WCC, C) Neutrophil count, D) Platelet count.

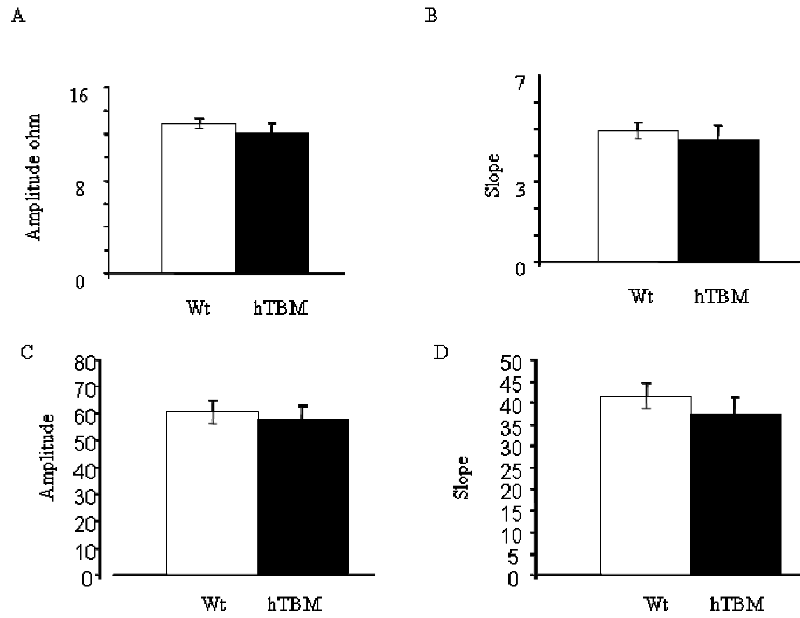
Results presented as mean ± SEM, N=6 per group.



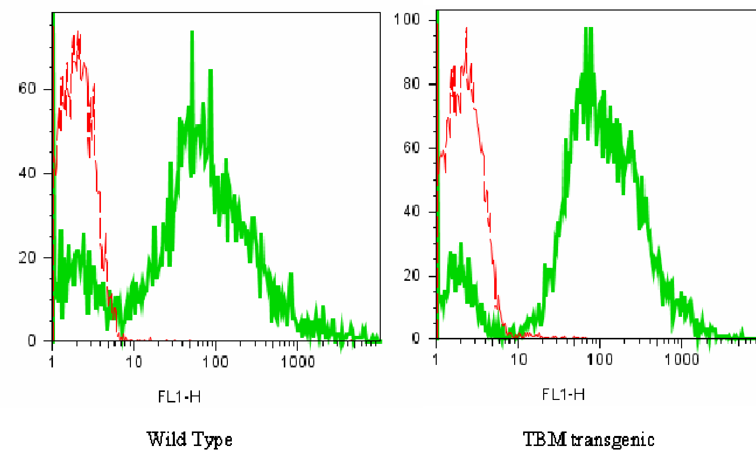

Supplementary figure 2) Renal function as assessed by A) creatinine B) urea. Results presented as mean ± SEM, N=6 per group.




Supplementary figure 3) A) INR and B) APTT. Results presented as mean ± SEM, N=6 per group.




Supplementary figure 4) Whole blood (A&B) and washed platelet (C &D) aggrometry response to collagen (4units/ml). The mean of experiments demonstrated no difference in amplitude or slope to time to clot in whole blood (A & B respectively) or washed platelets (C & D respectively). Results presented as mean ± SEM, N=6 per group.




Supplementary figure 5) Washed platelets were obtained. P selectin expression post high dose thrombin stimulation (1U/ml) (green) and in resting platelets (red) from wild type and TBM transgenic mice fail to demonstrate any difference in expression.