Supplemental Methods and Figures:

Maintaining extraembryonic expression allows generation of mice with severe Tissue Factor Pathway Inhibitor deficiency

Running title: TFPI Kunitz1 deficient mice

Michelle M. Castillo[1,4*], Qiuhui Yang[1*], Min Zhan[1*], Amy Y. Pan[2], Michael W. Lawlor[1], Alan E. Mast[3,4] and Rashmi Sood[1,4]

[1]Division of Pediatric Pathology, Department of Pathology, Medical College of Wisconsin, Milwaukee, WI, USA;
[2]Quantitative Health Sciences, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI, USA;
[3]Blood Research Institute, BloodCenter of Wisconsin, Milwaukee, WI, USA;
[4]Department of Cell Biology, Neurobiology & Anatomy, Medical College of Wisconsin, Milwaukee, WI, USA;
*These authors have contributed equally.

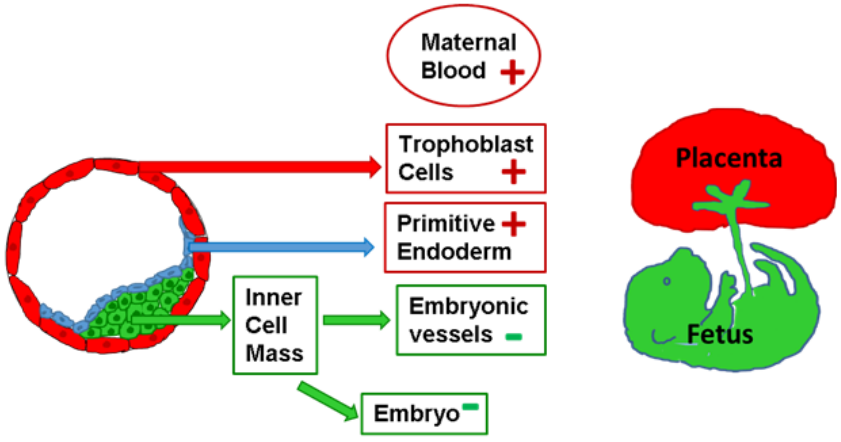
Corresponding Author:
Rashmi Sood
Medical College of Wisconsin
8701 Watertown Plank Road
Milwaukee WI 53226
Phone: 414 955 2451
E-mail: rsood@mcw.edu

SUPPLEMENTARY METHODS:

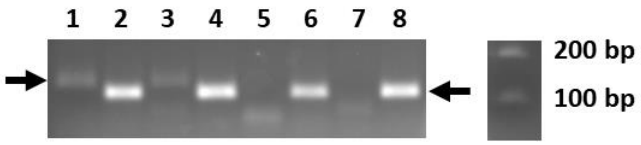
For fXa generation assay, 10 µl of 1:10 dilution of citrated mouse plasma was incubated with 25mM Hepes pH 7.2 buffer containing 5mM $CaCl_2$, 0.005% BSA, phospholipids (rabbit brain cephalin), 10pM fVIIa (Enzyme Research, South Bend, IN), and 1:4000 dilution of TF (RecombiPlasTin 2G, Instrumentation Laboratory, Bedford, MA) in an 80µl reaction volume at room temperature for 20 minutes. FXa generation was monitored at 405 nm in the kinetic mode for 1 hour immediately after adding 10µl each of 200 nMolar Human fX (Enzyme Research laboratories, South Bend, IN) and 2.5 mMolar Spectrozyme Xa, (Sekisui Diagnostics LLC, Stamford, CT).

SUPPLEMENTARY FIGURES:

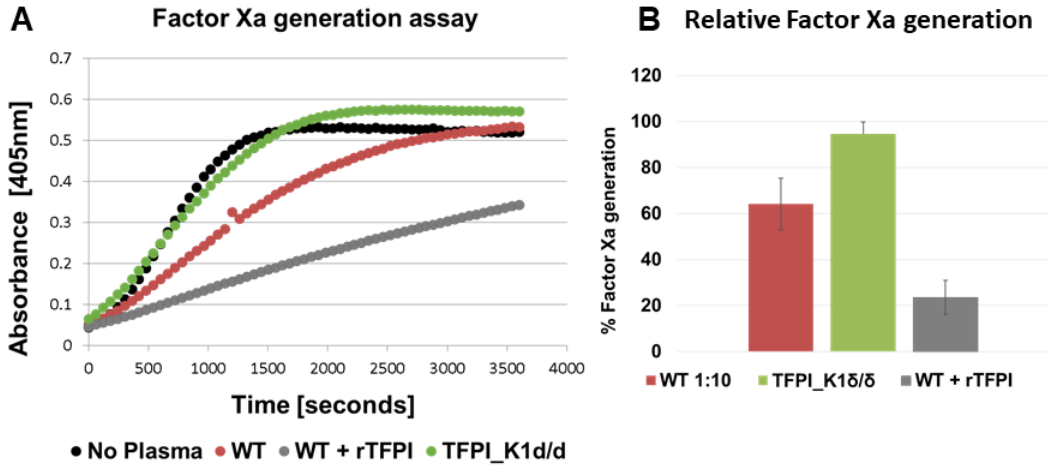
Supplementary Figure 1: Meox2Cre-mediated excision of floxed genes removes expression from the embryo and feto-placental circulation but maintains expression in trophoblast cells of the placenta. Meox2 is expressed in cells of the inner cell mass soon after differentiation of trophoblast cell lineage and the primitive endoderm. This is expected to result in Meox2Cre-mediated deletion of TFPI exon 4 in the embryo and embryonic vessels in the placenta. Derivatives of the trophoblast cells and primitive endoderm are expected to continue to express full length TFPI. We have confirmed this excision pattern by using a double fluorescent Cre recombinase reporter mouse (VanMens *et al* Blood Advances 2017;1:1148-1158).



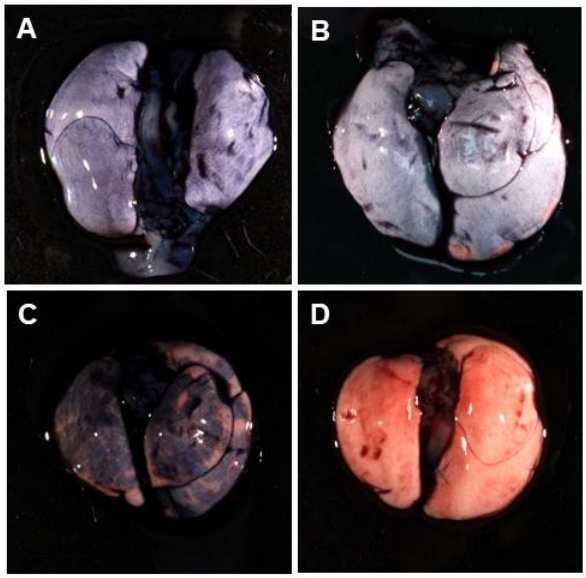
Supplementary Figure 2: Gel electrophoresis of real time PCR amplification products. End point amplification products from real time qPCR on RNA extracted from organs of TFPI_K1$^{\delta/\delta}$ mice with estimated 2% residual full-length expression (lanes 1, 2, 3, 4) and 1% residual full-length expression (lanes 5, 6, 7, 8) are shown. RNA from liver (lanes 1, 2, 5 and 6) or Kidney (lanes 3, 4, 7 and 8) was reverse transcribed and amplified with primers encompassing exon 4-5 (lanes 1, 3, 5 and 7) or exon 7-8 (lanes 2, 4, 6 and 8) to obtain 123 bp and 102 bp products, respectively. Estimated 2% residual full-length expression corresponded to a faint end point product with exon 4-5 encompassing primers. Exon 6-7 encompassing primers used as control amplified a product in all samples.



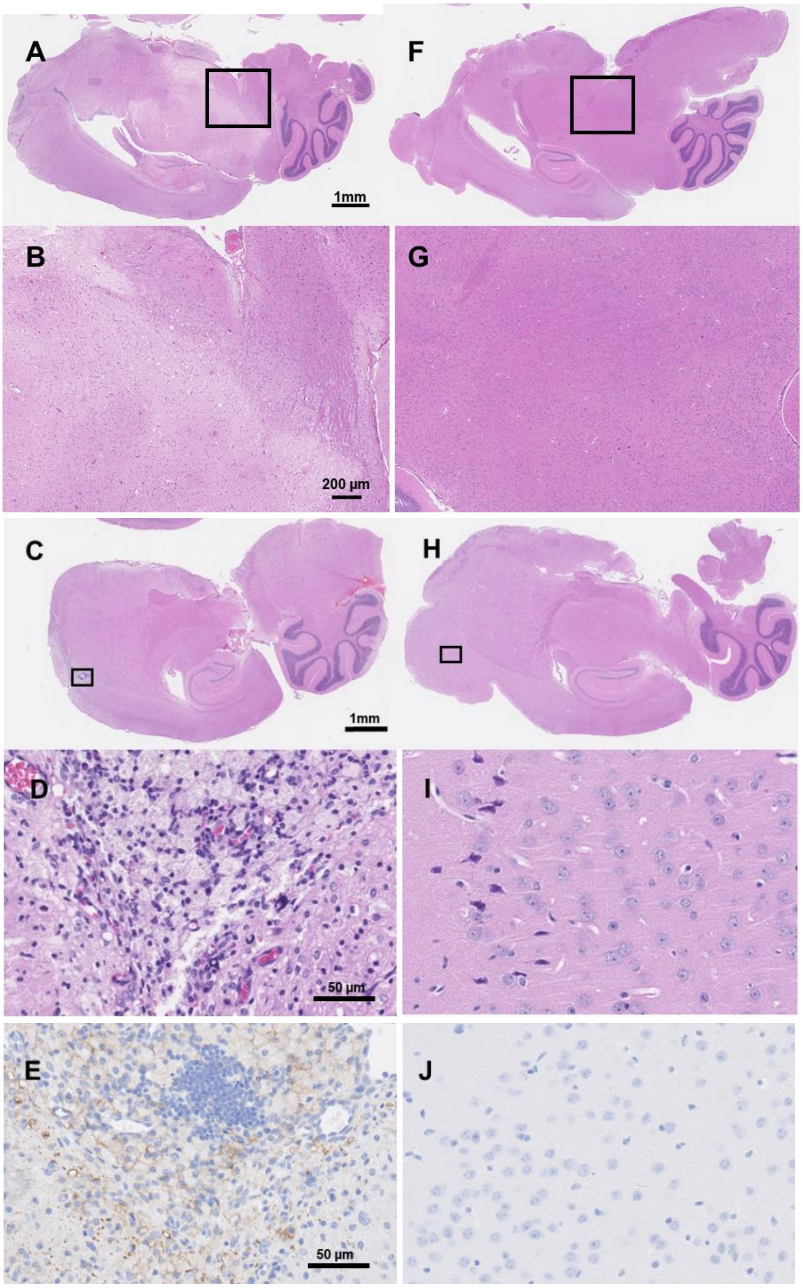
Supplementary Figure 3: Significantly reduced ability of TFPI_K1$^{\delta/\delta}$ plasma to inhibit FXa generation. Plasma from wild type or TFPI_K1$^{\delta/\delta}$ mice was incubated for 20 minutes at RT with TF and fVIIa in the presence of $CaCl_2$ and phospholipids. FX was added and fXa generation was measured with a chromogenic substrate. (A) Kinetic views of representative fXa generation assays are shown. The rate of fXa generation in the presence of TFPI K1 deficient plasma (green trace) is similar to the rate without added plasma (black trace) and significantly higher than the rate in the presence of wild type plasma (red trace). FXa generation rate falls further down if 18.8 pMoles/L recombinant TFPI is added to the reaction mix. (B) Pooled data for fXa generation rate relative to samples with no added plasma (assigned as 100%) are shown. Rate of fXa generation is significantly reduced in the presence of plasma from wild type C57Bl6 mice (64.2 ± 11.2% of no plasma control), while plasma from TFPI K1 deficient mice had minimal effect (94.6 ± 5.1% of no plasma control). Addition of rTFPI to wildtype plasma further reduced fXa generation (23.5 ± 7.4% of no plasma control).



Supplementary Figure 4: Compromised lung perfusion in TFPI_K1$^{\delta/\delta}$ mice compared to wild type controls upon equivalent challenge with Tissue Factor. A previously described Evan's blue perfusion assay and scoring system was used as an independent measure of lung vascular occlusion (Weiss *et al*. Blood 2002;100:3240-3244). 100% perfused lungs turned blue (panel A; wild type lungs; score 0; no perfusion defect), while in the complete absence of perfusion the lungs remained pink (panel D; TFPI_ K1$^{\delta/\delta}$ lungs; score 4; no perfusion). Examples of score 1 (panel B; wild type lung) and score 2 (panel C; TFPI_ K1$^{\delta/\delta}$ lung) are shown. Scoring data for all animals tested is shown in Figure 4D.



Supplementary Figure 5: A second case of TFPI_ K1$^{\delta/\delta}$ mouse with ≤1% residual full length TFPI and large infarcted lesions in the brain. Sagittal sections and corresponding enlarged views of brains from TFPI K1 deficient (A through E) and wild type control (F through J) mice are shown. A and B show a large early lesion, prior to macrophage infiltration and glial scarring. C through E show a more chronic lesion with immune cell involvement. E and K are immunostained with an antibody that recognizes the macrophage marker, F4/80. All other sections are stained with hematoxylin and eosin.



Supplementary Figure 6: A case of enlarged heart and organized thrombus in a TFPI_K1$^{\delta/\delta}$ mouse with ≤1% residual full length TFPI. Transverse sections of murine hearts from a case of TFPI K1 deficient (A, B, C) and wild type control (D, E, F) mice are either stained with hematoxylin & eosin (A, B, D, E) or immunostained with an antibody that recognizes neutrophil marker, LY-6G (C, F)