Supplementary Material to Bird et al. “Effects of plasma kallikrein deficiency on haemostasis and thrombosis in mice: Murine ortholog of Fletcher trait” (Thromb Haemost 2012; 107.5)

Generation of plasma kallikrein-deficient (Klkb1 mutant) mice, genotyping, and expression analysis

Plasma kallikrein-deficient (Klkb1 mutant) mice were generated in collaboration with Lexicon Pharmaceuticals (The Woodlands, TX). The targeting strategy used to disrupt the Klkb1 locus is diagrammed in Supplemental Figure 1. The Klkb1 targeting vector was derived using the Lambda KOS system (1). The Lambda KOS phage library, arrayed into 96 superpools, was screened by PCR using exon 3-specific primers Klkb1-3 (5’-CGCTGCTTAGGATTGGTAGGAG-3’) and Klkb1-4 (5’-CCTGGAAGGATGGTCACGTTGTGG-3’). The PCR-positive phage superpools were plated and screened by filter hybridization using the 451 bp amplicon derived from primers Klkb1-3 and Klkb1-4 as a probe. One pKOS genomic clone, pKOS-59, was isolated from the library screen and confirmed by sequence and restriction analysis. Gene-specific arms (5’-TGGGATGTCCGTGGACTATAT TGACAGGTTAAACAGTTGAAACTG-3’) and (5’-AAAGCTGTACAGTTAAAGG CTTCTCCAGGAGTTAGATTGC-3’) were appended by PCR to a yeast selection cassette containing the URA3 marker. The yeast selection cassette and pKOS-59 were co-transformed into yeast, and clones that had undergone homologous recombination to replace an 854 bp region containing exons 3 and 4
with the yeast selection cassette were isolated. The yeast cassette was subsequently replaced with a LacZ/ Floxed Neo selection cassette to complete the Klkb1 targeting vector. The Not I linearized targeting vector was electroporated into strain 129 ES cells (Pro-Cre) that have the Protamine promoter driving Cre recombinase expression (2) allowing excision of the Neo selection cassette in the testes. G418/FIAU resistant ES cell clones were isolated, and correctly targeted clones were identified and confirmed by Southern analysis using a 550 bp 5’ external probe (13/14), generated by PCR using primers Klkb1-13 (5’-CCGCAGAGTCTCCT TAACTACATCT-3’) and Klkb1-14 (5’-CTCTGGAGTGTCATAAAGCAGCGTA-3’), and a 597 bp 3’ external probe (15/16), amplified by PCR using primers Klkb1-15 (5’-GCCCTCCAAGTGTTGTCCTAAA-3’) and Klkb1-16 (5’-GAACACTCTGAGAAACATGGCACTA-3’). Southern analysis using probe 13/14 detected a >40Kb wild type band and 9.1 Kb mutant band in Apa I digested genomic DNA while probe 15/16 detected a 8.5 Kb wild type band and 11.2 Kb mutant band in Hind III digested genomic DNA. Five targeted ES cell clones were identified and microinjected into C57BL/6 (albino) blastocysts to generate chimeric animals which were bred to C57BL/6 (albino) females, and the resulting heterozygous offspring were interbred to produce homozygous Klkb1 deficient mice. Genotypes were determined using polymerase chain reaction (PCR) performed on tail lysates to enable discrimination of Klkb1 wild type (WT), heterozygous (Het), and homozygous knockout (KO) mice.
Suppl. References


Suppl. Figure 1: Targeted disruption of the Klkb1 gene locus. A) Targeting strategy used to disrupt the Klkb1 locus. Homologous recombination (represented by X) between the targeting vector and the Klkb1 gene results in the replacement of exons 3 and 4 with the selection cassette. B) Southern hybridisation indicating proper gene targeting in the embryonic stem cell clones. Clones 1A9, 1A10, 1B9, and 1G5 were selected for blastocyst injections; Lex represents untransfected embryonic stem cell DNA.