mRNA studies. RNA was isolated from kidneys, aorta, and human umbilical vein endothelial cells (HUVEC) using TriZOL (Invitrogen) and cDNA produced from DNAse treated RNA using Superscript III reverse transcriptase (Invitrogen). Real-time PCR analysis was performed using BioRad SYBR Green PCR Supermix and measurements were taken using a BioRad IQ5 real-time PCR machine. Concentrations of cDNA were quantified by subtracting gene of interest ct values from GAPDH ct values to obtain Δct values. An average Δct value ("Δct-WT", or 1.0-fold expression) was obtained from wild type samples and relative concentrations of each PRCP-depleted sample was obtained by subtracting ct values from Δct-WT to obtain $\Delta\Delta$ct values and were then applied to the formula $2^{-\Delta\Delta ct}$. Primer sequences for the various mRNA examined in this study are shown in Supplemental Table 1.

Immunoblot studies. Western blot analysis of kidney or HUVEC PRCP was performed on tissue or cell samples digested with RIPA buffer (1% NP-40, X% sodium deoxycholate, 0.1% SDS) containing protease inhibitors (Roche, Indianapolis, IN) and were loaded on a 4-15% gradient SDS-PAGE gel (BioRad). Protein was transferred to nitrocellulose blotting paper, blocked with 5% dried milk in PBS-Triton and rocked overnight at 4°C with 2 μg/mL goat anti-mouse TND20 antibody. After incubation and washing, the blotting paper was treated for 1 hour with an anti-goat HRP secondary antibody. Immunoblot for HUVEC thrombomodulin (Santa Cruz) was performed with 0.2 mg/ml mouse anti-human antibody.

Histologic studies. Hematoxylin and eosin (H&E) staining were performed on formalin fixed, paraffin embedded tissue. X gal staining of LacZ and immunofluorescence staining was performed on fresh-frozen, dried, 4 μm thick sections. For immunostaining studies, the slides were blocked for one hour at room temperature in 5% donkey serum/PBS and incubated at 4°C overnight in a humid chamber with primary antibody diluted in 1% donkey serum. After washing with PBS, slides were incubated with secondary antibody for 1 hr at room temperature protected from light. Photos were obtained using a Zeiss LSM 510

confocal microscope. Immunoperoxidase staining for PRCP was performed on frozen tissue using goat anti-TND20 mouse-PRCP as a primary antibody and anti-goat-HRP (Bio-Rad) as the secondary antibody. Tissue was then exposed to 3,3-diaminobenzidine (DAB) (Vector Laboratories, Burlingame, CA), dehydrated, and counterstained with hematoxylin. In immunofluorescent studies antibodies were incubated at the following concentrations: goat anti-PRCP (anti-TND20) at 2 μg/mL followed by a donkey anti-goat antibody labeled with Alex-594 or a donkey anti-goat antibody labeled with Alexa-488; rat anti-PECAM-1 antibody (CD31) (MEC 3.3.1, Santa Cruz Biochemicals, Santa Cruz, CA) at 4 μg/mL followed by donkey anti-rat antibody labeled with Alexa-594; rabbit anti-alpha-smooth muscle actin antibody (ab5694, Abcam, Cambridge, MA) at 0.4 μg/mL followed by donkey anti-rabbit antibody labeled with Alexa-594. Alexa-488-labeled lotus lectin was incubated with renal tissue at 4 μg/mL for 1 hour (Vector).

In vitro assays. After celiotomy, plasma was collected in 3.2% sodium citrate buffer from the inferior vena cava of mice anesthetized with isoflurane. Whole blood was centrifuged at 2000 xg for 15 minutes. Plasma bradykinin and angiotensin II measurement were performed using ELISA from Bachem or Cayman Chemicals (Ann Arbor MI) according to the manufacturer's instructions, respectively (15). Factor XII was measured using an APTT-based coagulant assay with factor XII deficient human plasma (King George, Inc., Overland Park, KS). Plasma prekallikrein was measured in acid treated plasma diluted 1:8 in 50 mM Tris-HCl, pH 7.9, 0.1% PEG and incubated with an equal volume of plasma prekallikrein activator in a 96 well plate at room temperature for 5 minutes. After incubation an equal volume of 2 mM S-2302 was added and the mixture was incubated at room temperature for 10 minutes. At the end of the reaction, 100 μL 50% acetic acid was added to each cuvette and the absorbance was read at 405 nm (Supplement Reference 1). Samples were compared against a standard curve of diluted normal pooled mouse plasma. Thrombin generation times (TGT) were performed as previously described (Supplement Reference 2). Both tissue factor- and contact activation-induced thrombin generation times were performed. The tissue factor-induced TGT was performed by incubating a 1:2 dilution of mouse plasma in 25 mM HEPES, 175 mM $NaCl_2$, containing 5 mg/mL bovine serum albumin, pH 7.7, ~3 pM tissue factor (3 μL of 1:60 dilution of

stock Innovin), and 0.42 mM Z-Gly-Gly-Arg-AMC. The reaction was initiated with the injection of calcium chloride, final concentration 16 mM. Substrate hydrolysis was measured on a fluorescent plate reader (NOVOstar, BMG Labtech). Contact activation-induced thrombin generation was performed similarly, except, APTT reagent (Siemans) was substituted for the tissue factor containing reagent. The TGT data was expressed as an arbitrary rate of fluorescent accumulation as determined by the second derivative of the raw fluorescent values. The lag time, peak height, and total area under the curve were calculated using Prism by Graphpad, San Diego, CA.

Investigations determined if PFRCK is a better inhibitor of plasma kallikrein inhibitor than factor XIIa or if SBTI is a better plasma kallikrein inhibitor than factor XIa. In these experiments 25 mM Tris, 0.15 M NaCl, pH 7.4 with 0.05% polyethylene glycol (MW: 8000 kd) was incubated in the absence or presence of increasing concentrations of PFRCK (0.1 nM to 100 μM) and 0.5 mM S-2302. The reaction was started by the addition of 2 nM plasma kallikrein or factor XIIa. In other experiments, 25 mM Tris, 0.15 M NaCl, pH 7.4 with 0.05% polyethylene glycol (MW: 8000 kd) was incubated in the absence or presence of increasing concentrations of SBTI (1.0 pM to 100 μM) and 0.5 mM S-2302 for plasma kallikrein or 0.5 mM S-2366 for factor XIa. The reaction was started by the addition of 5 nM plasma kallikrein or factor XIa, respectively. In all experiments, the initial rate of substrate hydrolysis at 405 nm was determined and the IC_{50} of inhibition of each enzyme in the presence of each inhibitor was determined.

Supplement Reference List.

1. De La Cadena RA, Scott CF, Colman RW. Evaluation of a microassay for human plasma prekallikrein. *J Lab Clin Med*. 1987; 109:601-607.

2. Tchaikovski SN, Van Vlijmen BJM, Rosing J, Tans G. Development of a calibrated automated thrombography based thrombin generation test in mouse plasma. *J Thromb Haemost.* 2007; 5:2079-2086.

Figure S1. *Immunohistochemical characterization of mouse renal PRCP.* Panel A. Immunofluorescent staining of mouse renal cortex frozen sections with anti-PRCP followed by an anti-goat secondary antibody labeled with Alexa-594 (left panel) and the same vessel is co-stained with an Alexa-488 tagged Lotus Lectin glycoprotein. The immunofluorescent staining with both labels is merged in the right panel. Panel A was photographed on a Zeiss LSM510 confocal microscope aperture 40X/1.3 oil immersion. Panel B. Immunoperoxidase/DAB staining was performed on a frozen section of mouse renal cortex using a goat anti-PRCP antibody (TND20) to determine the presence of PRCP in renal tubules and glomeruli. Panel C-E. Tissue sections from various organs were stained for LacZ with X-Gal to determine the presence of the CD4TM-beta-geo transgene in place of the *prcp* gene. Blue LacZ positive staining (top panels) indicates where there is endogenous expression of PRCP would be in wild type mice. Sections of $PRCP^{gt/gt}$ tissue were taken from brain (C), small bowel (D) and testes (E). The bottom panels demonstrate the same sections from wild type mice that have no LacZ staining. Panels B-E were photographed on an Olympus BH-2, aperture: 0.30 160/0.37.

Figure S2. *Pharmacologic Inhibition of PRCP and plasma kallikrein in 129svj mice.* The time to carotid artery occlusion on the Rose Bengal assay was determined in 129svj mice that were untreated (n=9) (WT-open bar graph) or treated (n=5) with the inhibitor to the PRCP, Z-Pro-Prolinal (ZPP), or plasma kallikrein inhibitors - soybean trypsin inhibitor (SBTI) (n=5), Pro-Phe-Arg-Chloromethylketone (PFRCK) (n=5), or PKSI 527 (PKSI) (n=5). The data represent the mean±SEM of 5 to7 wild type mice. One way ANOVA was used to determine differences among groups. The asterisk indicates a p value < 0.05 from wild type mice.

Figure S3. *Vascular gene expression in $PRCP^{gt/gt}$ kidneys.* Total renal mRNA was reverse transcribed to cDNA from wild type and $PRCP^{gt/gt}$ mice. qPCR was performed to measure mouse KLF2,

KLF4, eNOS and TM mRNA levels. The data represent mean±SEM of PCR studies from 4 samples in each group.

Figure S4. *PRCP gene expression in siRNA knockdown HUVEC and PRCP$^{gt/gt}$ MEF.* Panel A: total HUVEC mRNA was reverse transcribed to cDNA from control and PRCP siRNA-treated cells. qPCR was performed to measure human PRCP mRNA. Panel B: mouse PRCP was measured in murine embryonic fibroblasts from wild type and PRCP$^{gt/gt}$ embryos. The data represent mean±SEM of PCR studies from 3 samples in each group. An asterisk on any graph indicates $p<0.05$. The numbers on top of the gt/gt column indicate % mRNA in this tissue versus control (WT).

Table S1. Primer sequences

Primer Name	Sequence
Mouse eNOS	Forward – 5'-TCTACCGGGACGAGGTACTG-3' Reverse – 5'-CTGTCCTCAGGAGGTCTTGC-3'
Human eNOS	Forward – 5'-ATGTTTGTCTGCGGCGATGTTAC-3' Reverse – 5'-ATGCGGCTTGTCACCTCCTG-3'
Mouse GAPDH	Forward – 5'-TGTGTCCGTCGTGGATCTGA-3' Reverse – 5'-CCTGCTTCACCACCTTCTTGA-3'
Human GAPDH	Forward – 5'-GAGTCAACGGATTTGGCTGT-3' Reverse - 5'-TTGATTTTGGAGGGATCTCG-3'
Mouse PRCP	Forward – 5'-TCAAGGTCAACCTGTCATCG-3' Reverse – 5'-GGAACCATACCATCAAGCTG-3'
Human PRCP	Forward – 5'-GTGGCTGAGGAACTGAAAGC-3' Reverse – 5'-TGTCACCAAAGGGGAGAGAC-3'
Mouse KLF2	Forward – 5'-GGAGTTAGACTTCAGGCTGTG-3' Reverse – 5'GTTGTTTAGGTCCTCATCCGTG-3'
Mouse KLF4	Forward – 5'-GTGCCCCGACTAACCGTTG-3' Reverse – 5'-GTCGTTGAACTCCTCGGTCT-3'
Mouse TM	Forward – 5'-ATGCGTGGAGCATGAGTG-3' Reverse – 5'-CTGGCATCGAGGAAGGTC-3'

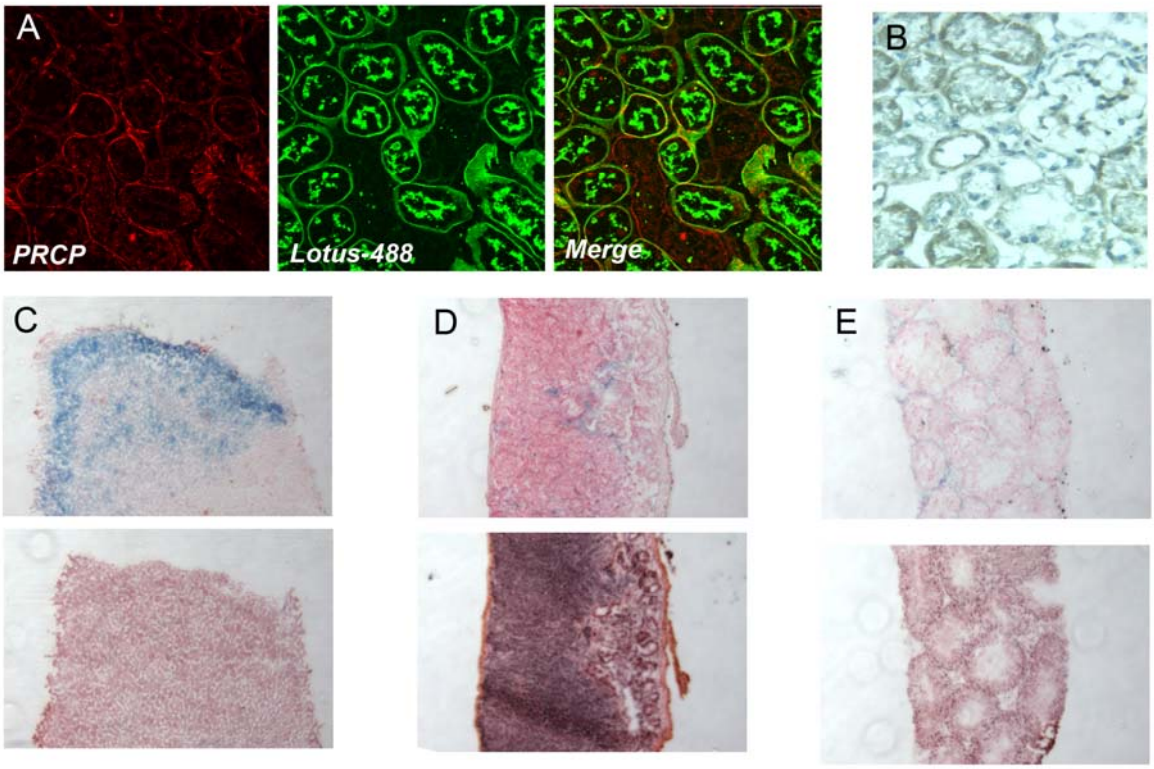


Figure S1

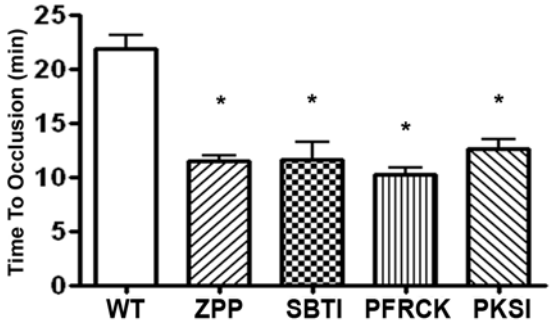
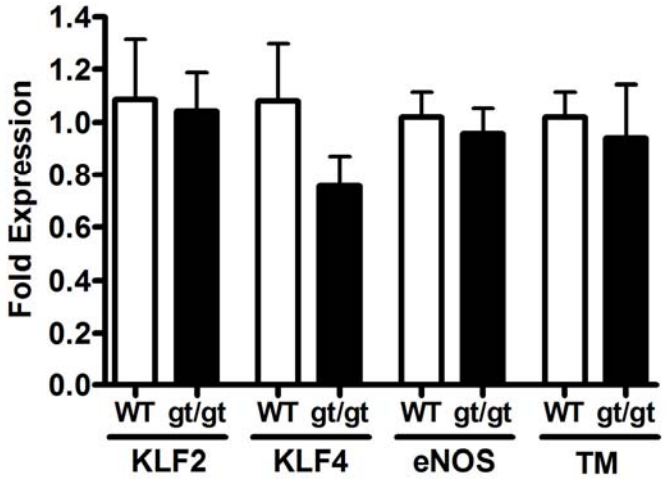


Figure S2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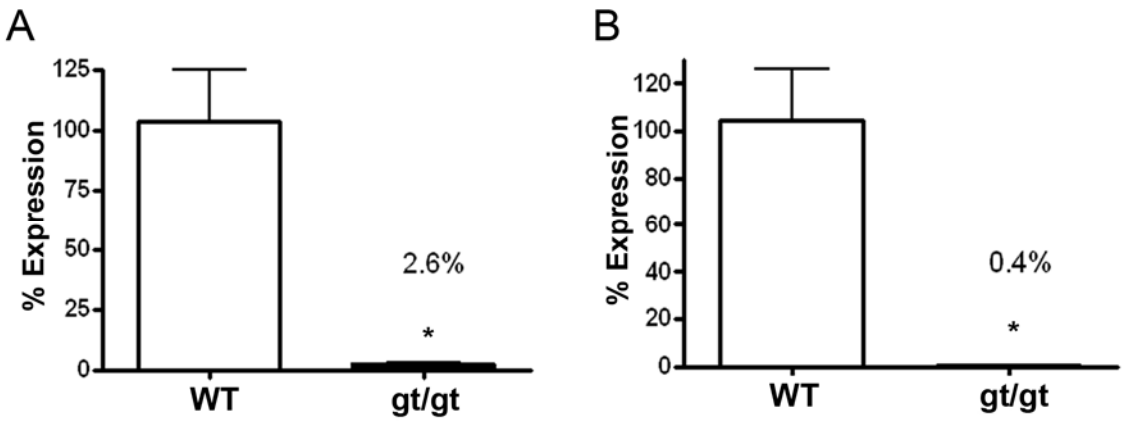
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WT gt/gt WT gt/gt WT gt/gt WT gt/gt
KLF2
KLF4
eNOS
TM

Figure S3


A
% Expression
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100
75
50
25
0
2.6%
*
WT
gt/gt
B
% Expression
120
100
80
60
40
20
0
0.4%
*
WT
gt/gt

Figure S4