Supplementary Figure 1

Supplementary Figure 1: Representative images of the diabetic rat brain after intracerebral injection of a mixture of autologous blood and Evans blue. (a) Dorsal surface and (b) coronal section of a diabetic rat brain at 30 minutes after intracerebral injection of 50 µl of a mixture of autologous blood and Evans blue dye into the right hemisphere and autologous blood only into the left hemisphere. Scale bar, 2mm.
Supplementary Figure 2: Characteristics of Klkb1 knockout mice. (a) Real time PCR of total Klkb1 mRNA isolated from 2-month-old Klkb1+/+, Klkb1+/−, and Klkb1−/− mice for determination of total Klkb1 mRNA levels. n = 3 mice. (b) Protein levels of prekallikrein from 2-month-old Klkb1+/+, Klkb1+/−, and Klkb1−/− mice. n = 3 mice. (c) Body weight, (d) blood glucose, (e) prothrombin time (PT), and (f) tail bleeding time from 2-month-old Klkb1+/+, Klkb1+/−, and Klkb1−/− mice. n = 5–28 mice. * P < 0.05.
Supplementary Figure 3: aPTT and tail bleeding time from NDM, DM and DM rats with ASP-440 treatment. (a) aPTT from NDM (n = 7), DM (n = 13) and DM rats with ASP-440 treatment (n = 6). (b) Tail bleeding time from NDM (n = 26), DM (n = 26), and DM rats with ASP-440 treatment (n = 18). * P < 0.05; ** P < 0.01; *** P < 0.001.
Supplementary Figure 4: Blood glucose levels after insulin injection of rats with 4-weeks of STZ-induced diabetes. Blood glucose was measured before (pre-surgery) and after (post-surgery) the injection of PK. *** $P < 0.001$; $n = 13$ rats.
Supplementary Figure 5: Blood pressure and heart rate changes from NDM or DM rats following cerebral infusion of PK. Representative images of the blood pressure recording from (a) NDM and (b) DM rats following cerebral infusion of PK. (c) Mean blood pressure, (d) mean blood pressure changes, and (e) heart rate changes from NDM or DM rats following cerebral infusion of PK. *n* = 4–5 rats.
Supplementary Figure 6: Effects of BK antagonists or BK on hematoma expansion. (a) The ratio of hematoma area to hemisphere area of brains after intracerebral co-injections of autologous blood mixed with 2 µM [des-Arg10]-Hoe140 (Des-Hoe) and Hoe-140 (Hoe) into right hemisphere or blood only into left hemisphere of DM rats. $n = 7$ rats. (b) The ratio of hematoma area to hemisphere area of brains at 0.5 h following intracerebral injection of 5 µM BK or PBS from saline or glucose intraperitoneally injected rats. $n = 7–10$ rats.
Supplementary Figure 7: Effect of PK-induced plasmin activation on clot formation, clot lysis, and hematoma expansion. (a) Representative western blot and (b) densitometric quantitation of the activation of plasminogen by PK (160–640 nM) in the absence or presence of 25 mM glucose compared with the activation by 5 nM tPA. (c) Effects of 5 nM tPA or 320 nM PK on thrombin-mediated clot formation in the presence of 25 mM glucose. Data represent the mean of triplicate wells from 3 independent experiments. (d) Effects of tPA or PK on clot lysis in the presence of 25 mM glucose. Data represent the mean of triplicate wells from 3 independent experiments. (e) The ratio of surface hematoma area to hemisphere area of brains from NDM and DM rats at 30 min after intracerebral injection of PK, plasmin (3 µg), or tPA (1 µg). n = 6–8 rats. * P < 0.05.
Supplementary Figure 8: Hydrolytic activity of PK and effect of ASP-440 on PK-induced inhibition of collagen-stimulated platelet aggregation. (a) Hydrolytic activity of PK, deactivated PK (De-PK), and plasma prekallikrein against the fluorogenic substrate H-D-Val-Leu-Arg-AFC. (b) Effect of glucose on PK activity. (c) Effect of PK (160 nM) on collagen-stimulated aggregation of platelets from NDM and DM rats. (d) Effect of ASP-440 on PK-induced inhibition of collagen-stimulated platelet aggregation. (e) Effect of ASP-440 on PK activity. n = 3 independent experiments. ** P < 0.01; *** P < 0.001.
SUPPLEMENTARY METHODS

Generation of Klkb1−/− mice, genotyping and expression analysis. The Klkb1−/− mice were provided by Texas Institute for Genomic Medicine. We used genomic DNA from tail samples for PCR genotyping under the following conditions: denaturation at 94°C for 15 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 40 seconds, 30 cycles. We used the following two primers for genotyping of wild type-specific product: 5’CTTCCAGG TAGCTGCTTTCTACC 3’ (forward) and 5’TCACCCACAACCTTCACAGAAAGG 3’ (reverse) and for mutation-specific product: 5’CGCTGCTT GATGGATGGTAGGAG 3’ (forward) and 5’GCTAGACTAGTCTAGCTAGAGCGG 3’ (reverse).

Systemic hemostasis measurement. For measurement of aPTT and PT, we collected blood (0.5 ml from mice, 1 ml from rats) via the heart puncture from each animal and transferred the blood into tubes containing sodium citrate anticoagulant. We analyzed freshly prepared plasma samples for aPTT and PT using aPTT and PT reagent, and a Cascade M-4 Manual Coagulation Analyzers (Helena Laboratories) according to the manufacturer's instructions. We measured tail bleeding time by cutting the extremity of the tail (3 mm from the tip) with a surgical blade and immediately inserting the tail into the pre-warmed tube of saline (37 °C). The time to arrest of bleeding was noted.

Hemoglobin analysis. For the rat hematoma volume assay, we homogenized identical brain slices in a 5 mm anterior section of the hemisphere encompassing the injection site from each hemisphere in buffer consisting of: 150 mM NaCl, 10 mM Tris-HCl (pH 7.4), 10% glycerol, 1% Triton X-100 and protease inhibitors. We centrifuged the homogenate at 10,000 rpm for 15 min at 4 °C. To determine hemoglobin level, we mixed 100 µl of
protein lysate with 6 µl of 1% SDS (2.08 mM final concentration) in a 96-well plate and gently shaken. We determined the relative amount of hemoglobin spectrophotometrically at 540 nm. The total hemispheric hemoglobin content in mice was determined with QuantiChrom Hemoglobin Assay Kit (BioAssay Systems).

**Western blotting.** We measured the protein concentration of the brain lysate using the Bradford protein assay kit (Bio-Rad). 20 µg protein from each sample was separated by 4–20% SDS-PAGE and immunoblotted using primary antibodies against carbonic anhydrase-I (Abcam), plasminogen (Abcam) and plasma kallikrein 1B (Abcam). Results were visualized by enhanced chemiluminescence (Cell Signaling) and quantified using ImageQuant 5.0 (Molecular Dynamics).

**Arterial pressure measurement.** We anesthetized the rats with pentobarbital (50 mg/kg, ip), and implanted a polyethylene catheter into the right femoral artery. We recorded the arterial blood pressure with a transducer connected to a PowerLab system (ADInstruments Pty). Rats were subjected to intracerebral injection of 15 µg PK after 20 min of baseline blood pressure recording. Blood pressure was recorded for additional 30 min. We calculated mean arterial blood pressure and heart rate (beats per minute) using PowerLab Chart software (ADInstruments Pty).

**PK activity assay.** We used fluorogenic PK substrate (H-D-Val-Leu-Arg-AFC, Calbiochem) to quantify PK enzymatic activity. The reaction kinetics were performed in 100 µl HEPES (10 mM) buffer containing 137 mM NaCl, 4 mM KCl, 11 mM D-glucose, 0.5 mg/ml BSA, 50 µM zinc sulfate, and 0.4 mM PK substrate. We monitored PK activity by fluorescence (excitation 420 nm/emission 528 nm) at 37°C on a Synergy HT Multi-Detection microplate reader (BioTek Instruments).
Deactivation of PK was induced by incubation of PK in 20 mM 4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF, pH 7) for 1 hr at 25°C. We removed AEBSF with 3 washes of 4 mM sodium acetate-HCl/0.15 M NaCl (pH 5.3) through a 10 kD cut-off filter (Millipore). PK activity was not detected in the residue and the final filtrate did not inhibit PK activity.

**Plasminogen activation.** We incubated 2.2 μM human glu-type plasminogen (Sigma) with 5 nM tPA (Calbiochem) or 160–640 nM PK at 37°C in Tris buffer (50 mM Tris, 10 mM CaCl₂ and 0.01% Tween-20, pH 7.5). Plasminogen/plasmin was detected by Western blotting using an antibody against human plasminogen. We used reduced samples of human glu-type plasminogen and human plasmin as controls.

**Continuous assays of clot formation and lysis.** 50 μl normal human pooled plasma anticoagulated with citrate-phosphate-dextrose (Innovative Research) was combined with an equal volume of tris buffered saline containing 2 mM CaCl₂. We added glucose to bring the final exogenous glucose concentration to 5 mM or 25 mM. We added tPA (5nM) or PK (40–320 nM) to certain samples accordingly. We initiated the clotting by adding 25 μl of thrombin to give a 5 nM final concentration. The turbidity associated with the clot formation and dissolution was monitored at 340 nm every 60 sec at 25°C using a SpectraMax Spectrophotometric plate reader (Molecular Devices).