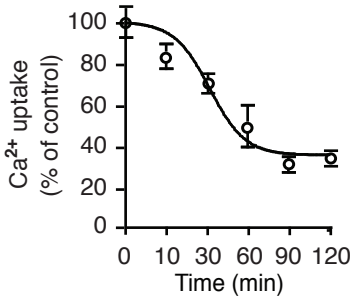
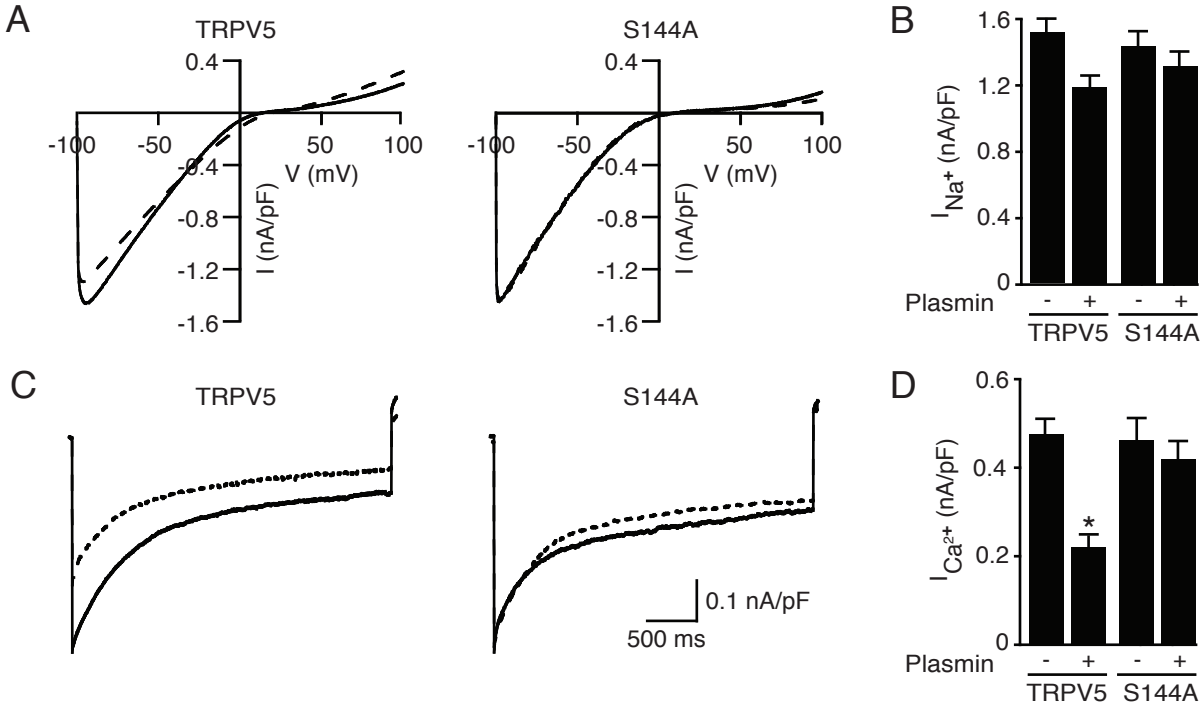
Suppl Fig.1



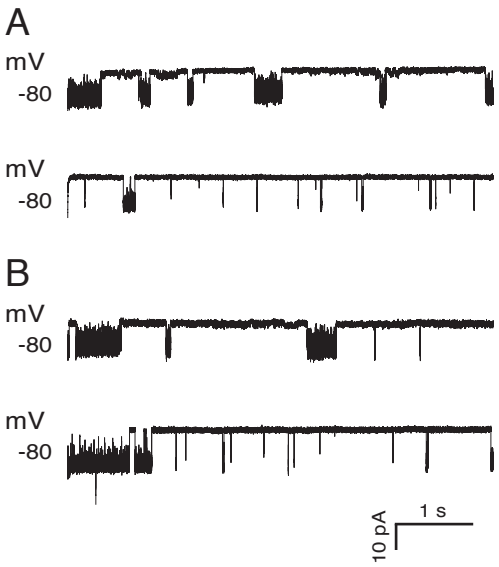
Supplemental Fig. 1 Plasmin inhibits TRPV5-mediated Ca^{2+} influx in HEK293 cells in time-dependent manner. TRPV5-expressing HEK293 cells were incubated with 10 nM plasmin for 1 h. BAPTA was added 30 min prior to the $^{45}Ca^{2+}$ experiments.

Suppl Fig. 2



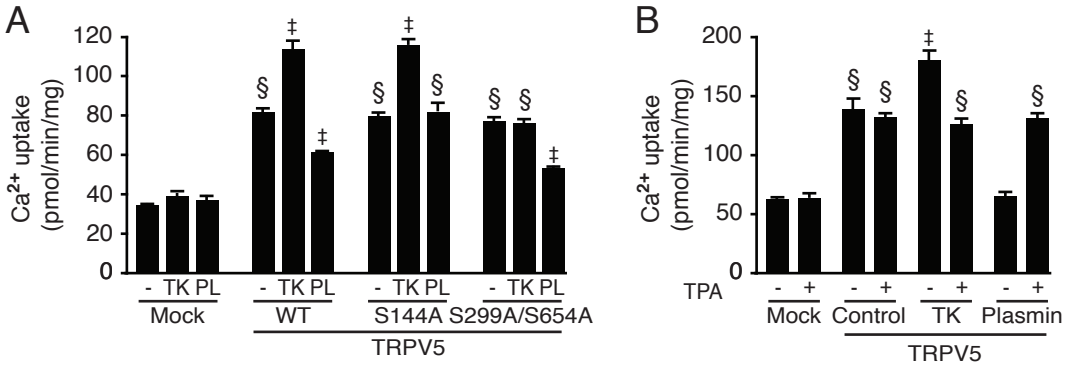
Supplemental Fig. 2 Plasmin-mediated inhibition of TRPV5 Ca^{2+} currents in HEK 293 cells in the presence of EGTA in the intracellular pipette. A, representative current traces (I/V) obtained from TRPV5 incubated with plasmin. Voltage ramps were applied in presence of EDTA until currents reached steady state (solid line, control ;and dotted line, plasmin-treated). B, averaged Na^+ current density at -80 mV in the presence of EDTA for TRPV5 wild type and TRPV5-S144A mutant. No significant differences in the Na^+ currents were observed in those conditions. C, representative TRPV5 Ca^{2+} currents evoked by application of a hyperpolarizing voltage step to -100 mV in presence of 10 mM Ca^{2+} . Plasmin promotes a significant reduction in the peak of Ca^{2+} currents without affecting the inactivation kinetics. D, averaged density of the Ca^{2+}-mediated peak currents shows that mutation of the S144 to A abolishes effect of plasmin on Ca^{2+} currents. *P<0.05 versus a non-plasmin condition (-).

Suppl Fig. 3



Supplemental Fig. 3 Plasmin-mediated inhibition of TRPV5 single channel activity. Representative traces of cell-attached single-channel recordings from HEK293 cells expressing TRPV5-WT (A) or TRPV5-S144A (B) in control (upper panel) and after 1-h incubation with 10 nM plasmin (lower panel). The recordings were made for 10 s intervals using a holding potential of -80 mV.

Suppl Fig. 4



Supplemental Fig. 4 Tissue kallekrein (TK) and plasmin differentially affect PKC phosphorylation sites of TRPV5. TRPV5-mediated Ca^{2+} influx experimentss in HEK293 cells expressing wild-type, S144A mutant, or S299A/S654 double mutant were performed after 1-h incubation with and without TK or plasmin (A). TRPV5-transfected cells were pre-incubated with 1 μM TPA to downregulate DAG-dependent PKCs prior to investigating effects of TK and plasmin (B). §$P<0.05$ versus mock. ‡$P<0.05$ versus respective non-treated (-).