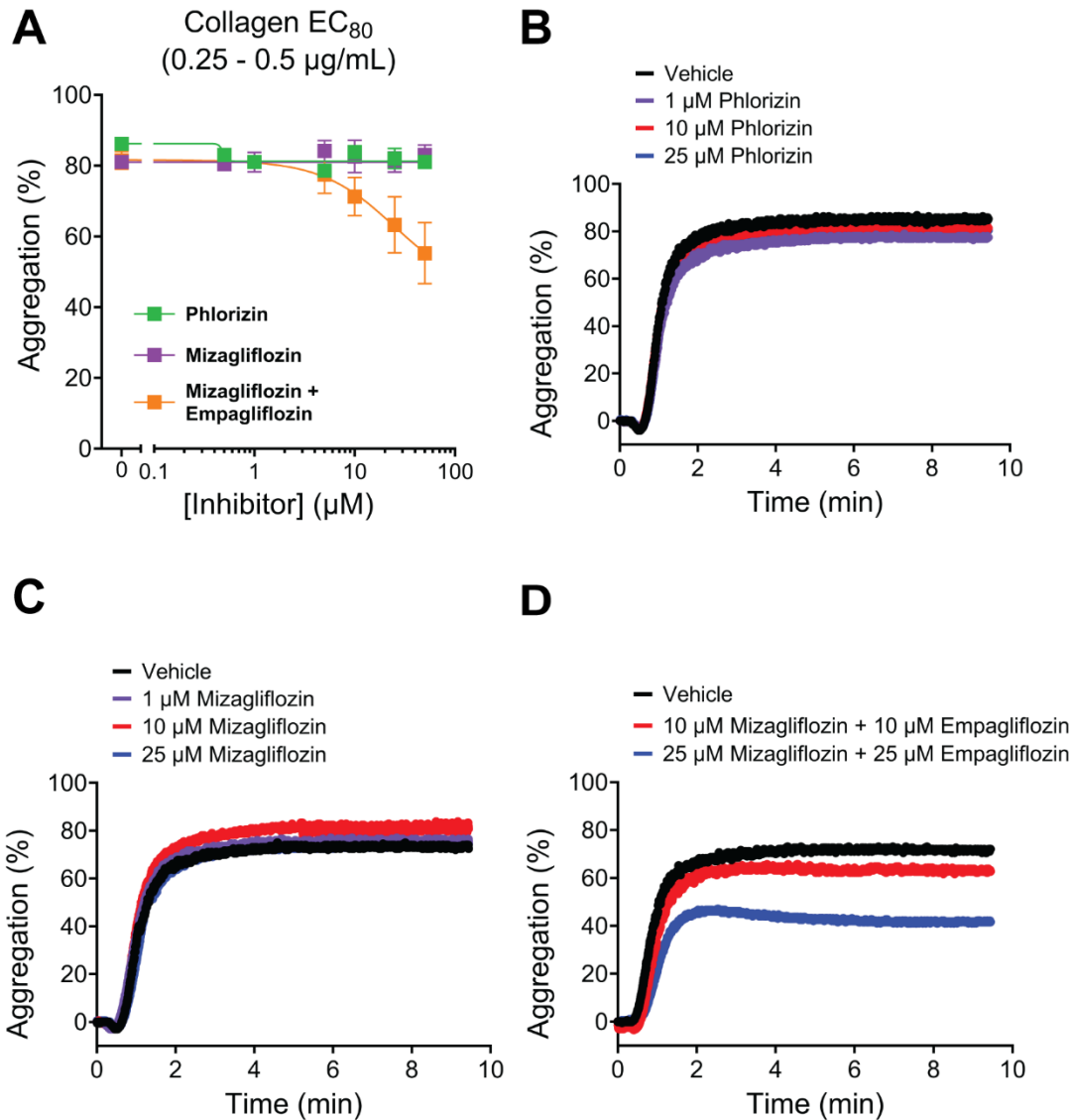


Supplemental Figures

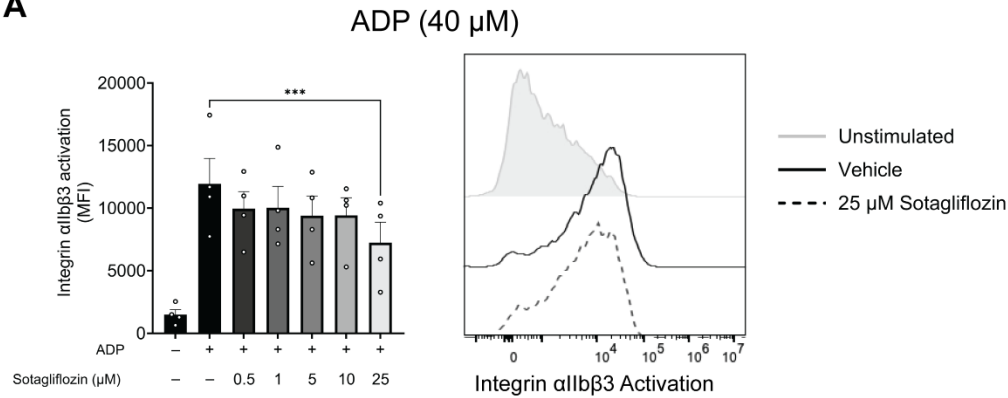
Supplemental Figure S1



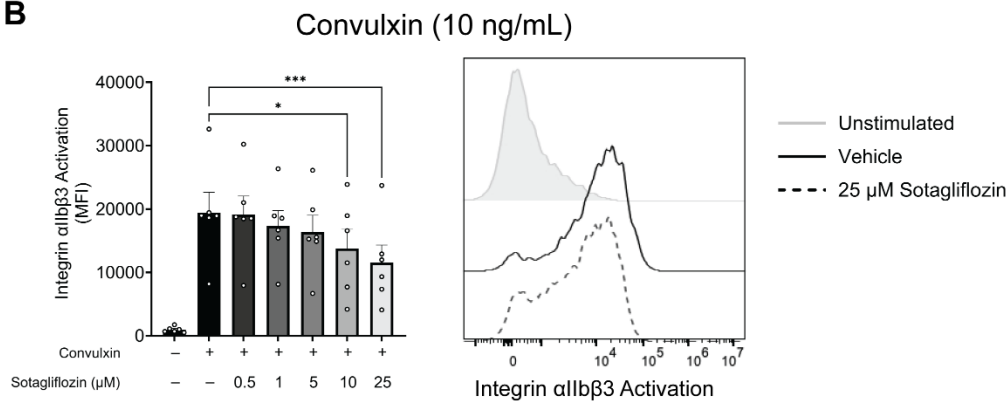
Supplemental Figure S1: Mizagliflozin and empagliflozin together inhibit platelet aggregation, while phlorizin and mizagliflozin alone have no effect. Washed human platelets were incubated with vehicle control (DMSO) or increasing concentrations (0.5 – 25 µM) of either phlorizin, mizagliflozin, or equal concentrations of both mizagliflozin and empagliflozin for 10 minutes and then stimulated with an EC₈₀ concentration of collagen (**A**; n = 4 – 5). One-way ANOVA with Dunnett's multiple comparisons; all conditions are compared to the vehicle control condition. Data represents mean ± SEM. Representative tracings for collagen with phlorizin (**B**), mizagliflozin (**C**), and mizagliflozin with empagliflozin (**D**) illustrate the dynamics of platelet aggregation with each agonist.

Supplemental Figure S2

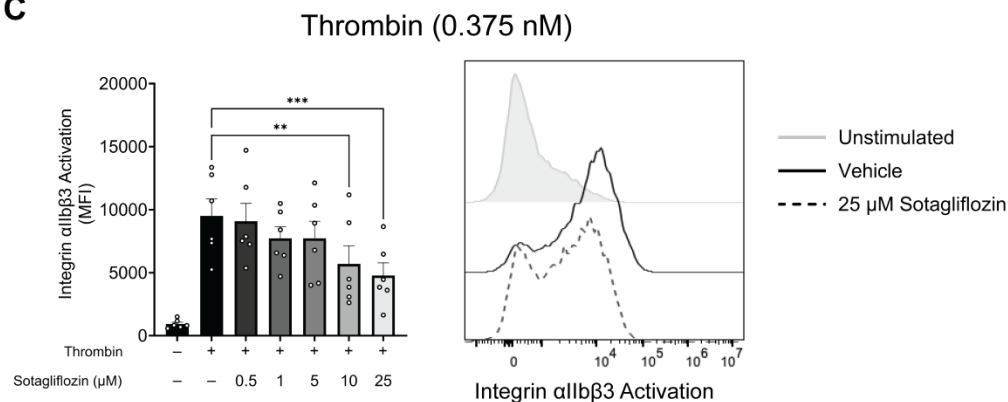
A



B



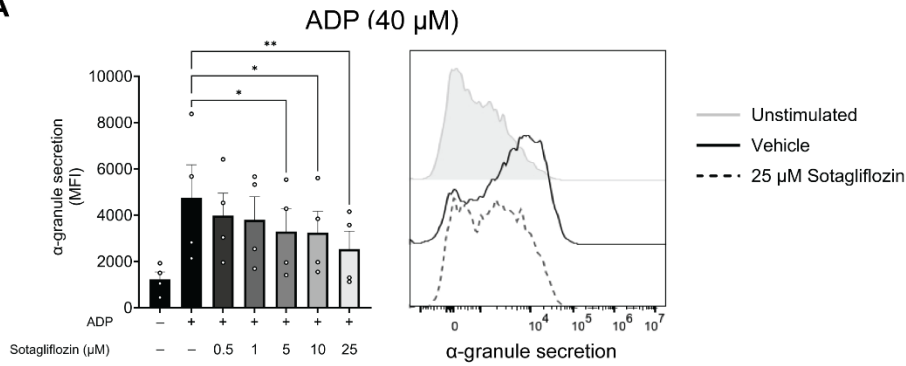
C



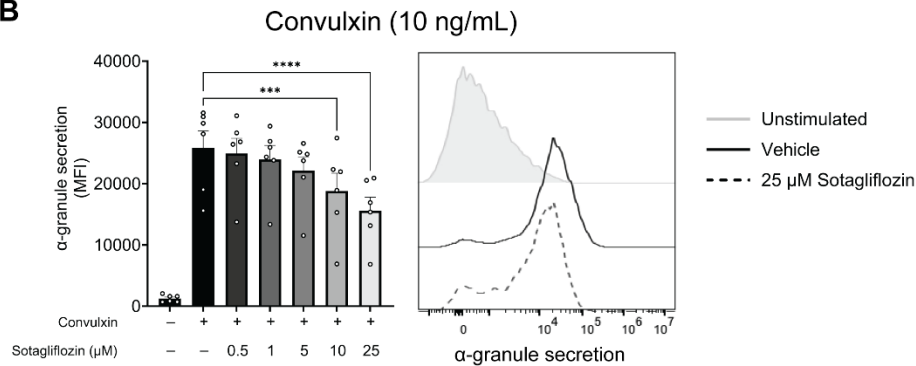
Supplemental Figure S2: Sotagliflozin attenuates ADP-, convulxin-, and thrombin-induced integrin α IIb β 3 activation. Platelet activation was analyzed by measuring integrin α IIb β 3 activation in washed human platelets treated with vehicle (DMSO) or increasing concentrations of sotagliflozin (0.5, 1, 5, 10 and 25 μ M) for 10 minutes prior to stimulation with 40 μ M ADP (**A**; n=4), 10 ng/mL convulxin (**B**; n=6) or 0.375 nM thrombin (**C**; n=6). One-way ANOVA with Dunnett's multiple comparisons; all conditions are compared to the vehicle control condition. Asterisks denote statistical differences between vehicle and treated groups: *P < 0.05, **P < 0.01, ***P < 0.001. Data represent mean $\pm$ SEM. Rightward shifts in the histograms demonstrate increases in integrin α IIb β 3 activation.

Supplemental Figure S3

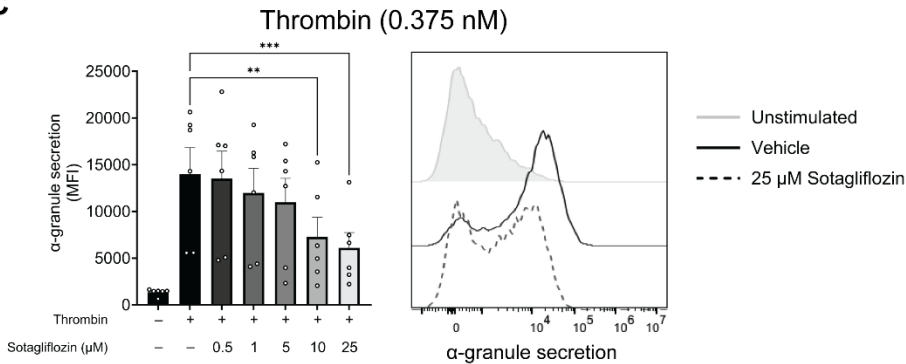
A



B

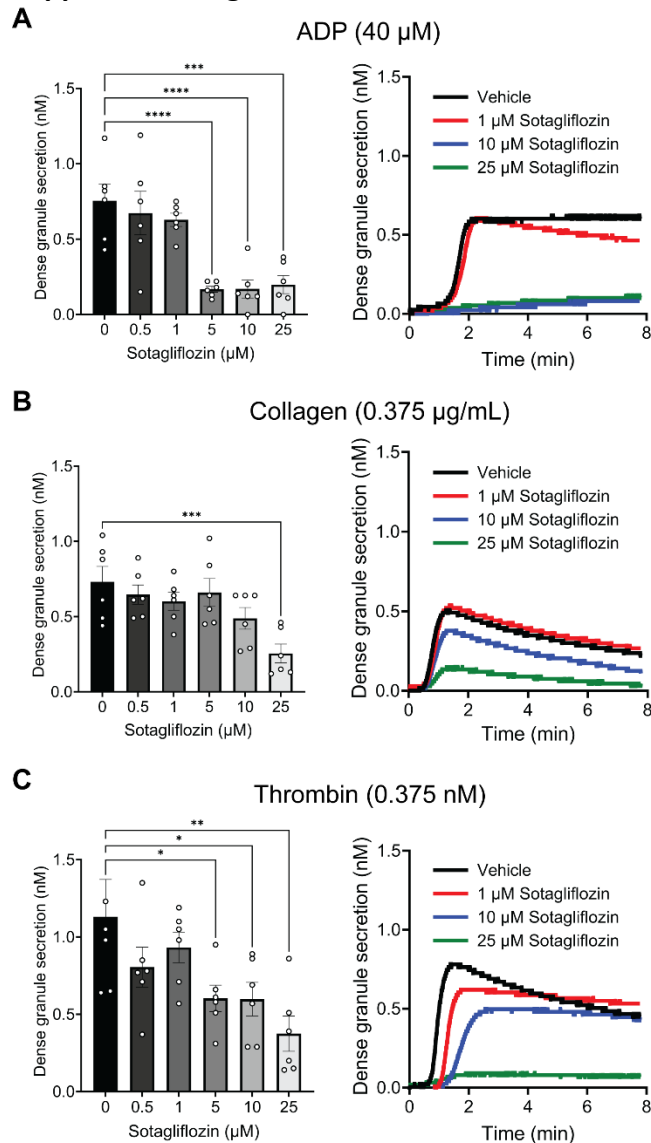


C



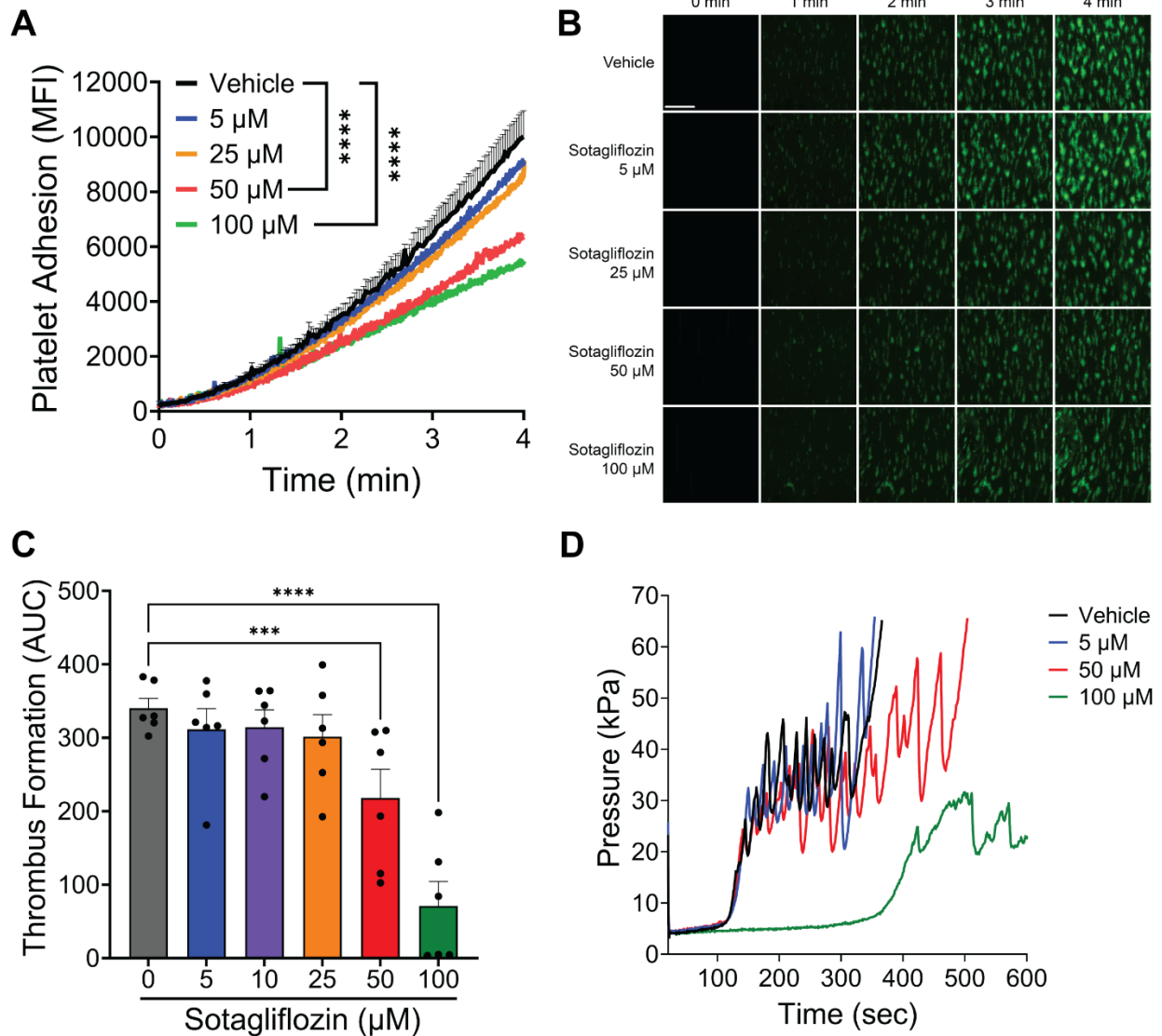
Supplemental Figure S3: Sotagliflozin decreases α -granule secretion in platelets activated by ADP, convulxin, and thrombin. Platelet α -granule secretion was assessed in platelets treated with vehicle (DMSO) or increasing concentrations of sotagliflozin (0.5, 1, 5, 10 and 25 μ M) for 10 minutes prior to stimulation with agonist. α -granule secretion was determined by measuring P-selectin expression on the surface of the platelet. The effects of sotagliflozin on ADP-stimulated α -granule secretion were assessed using 40 μ M ADP (**A**; n=7). Platelets stimulated with 10 ng/mL convulxin following incubation with sotagliflozin were probed for α -granule secretion (**B**; n=6). To determine the effects of sotagliflozin in the presence of thrombin, platelets incubated with sotagliflozin were assessed for α -granule secretion (**C**; n=6) following stimulation with 0.375 nM thrombin. One-way ANOVA with Dunnett's multiple comparisons; all conditions are compared to the vehicle control condition. Asterisks denote statistical differences between vehicle and treated groups: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data represent mean $\pm$ SEM.

Supplemental Figure S4



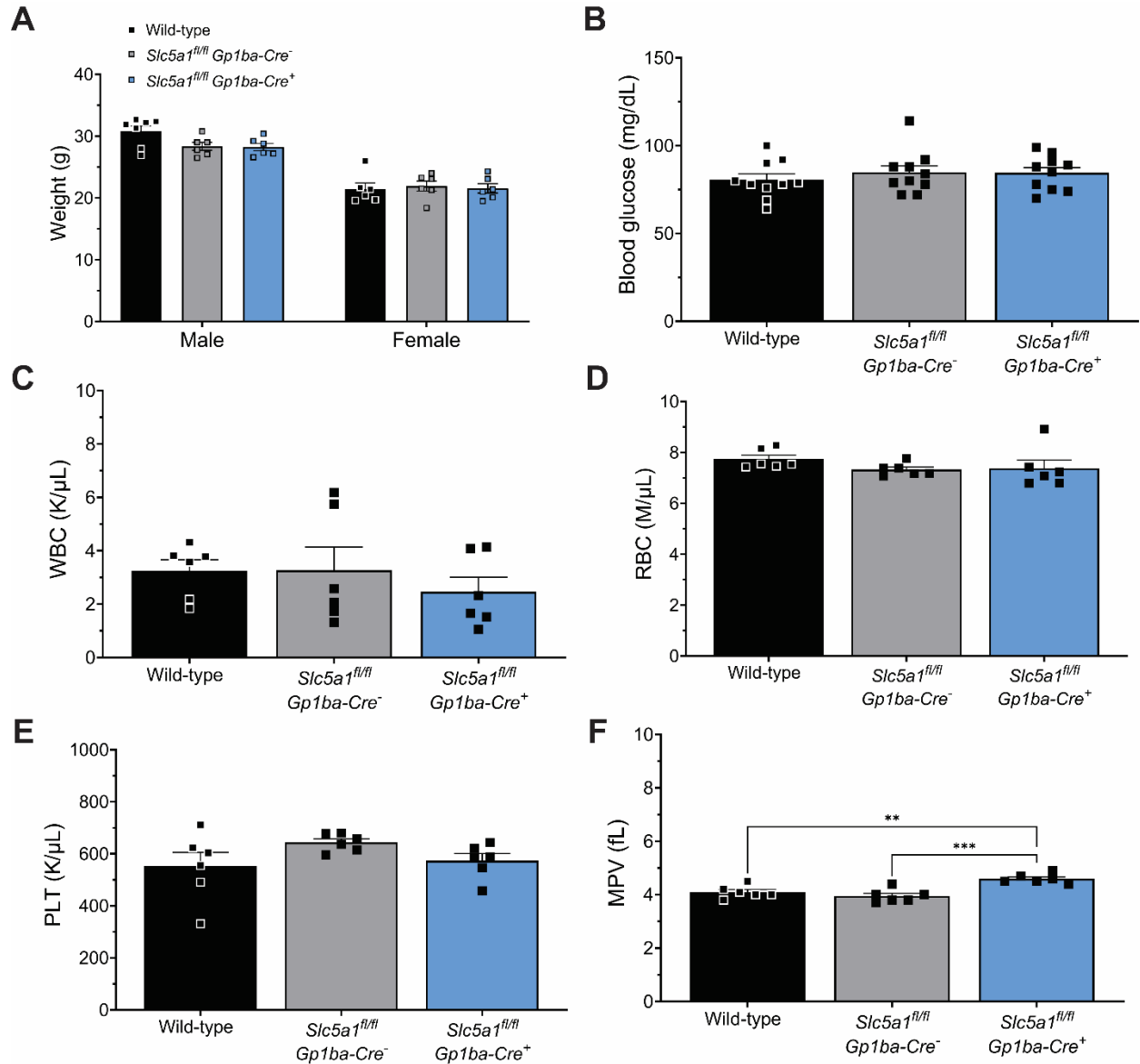
Supplemental Figure S4: ADP, collagen, and thrombin-induced dense granule secretion is inhibited by sotagliflozin. Platelet dense granule secretion was assessed through detection of ATP release using luciferin-luciferase reagent in platelets treated with vehicle (DMSO) or increasing concentrations of sotagliflozin (0.5, 1, 5, 10 and 25 μ M) for 10 minutes prior to stimulation with agonist. The effects of sotagliflozin on ADP-stimulated dense granule secretion were assessed using 40 μ M ADP, measuring ATP release (**A**; n=6). Platelets were stimulated with 0.375 μ g/mL collagen following treatment with sotagliflozin to quantify dense granule secretion (**B**; n=6). To determine the effects of sotagliflozin in the presence of thrombin, platelets incubated with sotagliflozin were assessed dense granule secretion following stimulation with 0.375 nM thrombin (**C**; n=6). One-way ANOVA with Dunnett's multiple comparisons; all conditions are compared to the vehicle control condition. Asterisks denote statistical differences between vehicle and treated groups: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data represent mean $\pm$ SEM.

Supplemental Figure S5



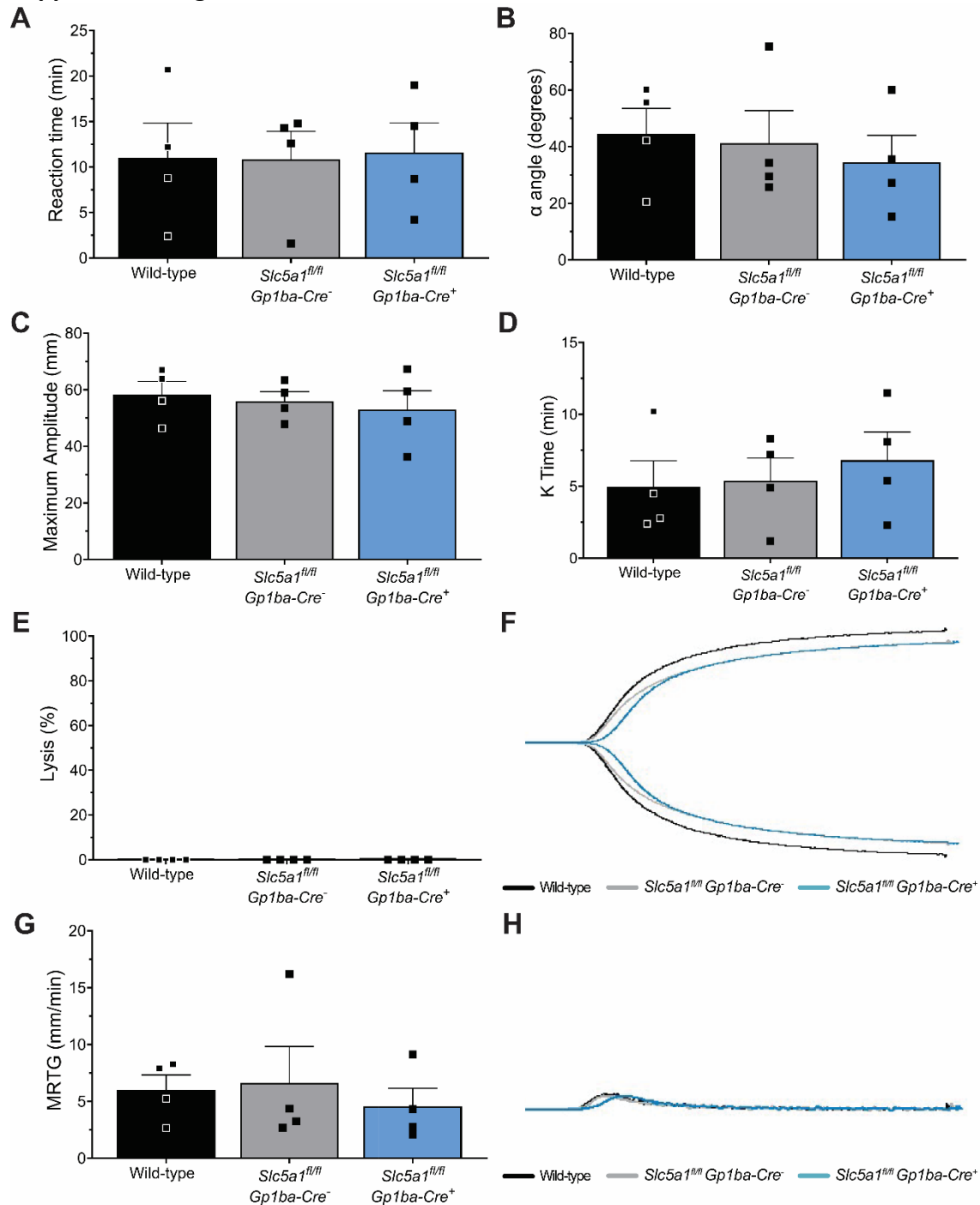
Supplemental Figure S5: Sotagliflozin dose-dependently inhibits platelet adhesion and thrombus formation under high shear in whole blood. Sodium-citrated human whole blood treated with vehicle (DMSO) or increasing concentrations of sotagliflozin (5, 25, 50 and 100 μ M) for 10 minutes and stained with DiOC₆ was perfused through a collagen-coated chamber at arterial shear (1800/s). Platelet adhesion was visualized by fluorescence and quantified as MFI (**A** and **B**; $n=5$). Scale bar (**B**; top left panel) represents 100 μ m. Two-way ANOVA comparing each condition to the vehicle control condition. Data represent mean $\pm$ SEM. Human whole blood anticoagulated with BAPA was treated with vehicle (DMSO) or increasing concentrations of sotagliflozin (5, 25, 50 and 100 μ M) for 10 minutes and perfused on a collagen-coated PL chip at arterial shear. In the T-TAS, pressure was monitored over time as a measurement of thrombus formation, which can be quantified and compared using the AUC (**C** and **D**; $n=6$). One-way ANOVA with Dunnett's multiple comparisons; all conditions are compared to the vehicle control condition. Asterisks denote statistical differences between vehicle and treated groups: *** $P < 0.001$, **** $P < 0.0001$. Data represent mean $\pm$ SEM.

Supplemental Figure S6



Supplemental Figure S6. Weight, blood glucose, and blood cell counts are not altered in *Slc5a1^{fl/fl} Gp1ba-Cre⁺* mice. WT mice, *Slc5a1^{fl/fl} Gp1ba-Cre⁻*, *Slc5a1^{fl/fl} Gp1ba-Cre⁺* were weighed (A; n= 6-7) and blood glucose was assessed following 6 hours of fasting (B; n=10). Citrated whole blood from WT, *Slc5a1^{fl/fl} Gp1ba-Cre⁻*, *Slc5a1^{fl/fl} Gp1ba-Cre⁺* mice was assessed via complete blood count to analyze WBC (C; n=6), RBC (D; n=6), platelet (PLT) (E; n=6), and mean platelet volume (MPV) (F; n=6). Data represent mean $\pm$ SEM. One-way ANOVA with Dunnett's multiple comparisons; each condition was compared to all other conditions. Asterisks denote statistical differences between vehicle and treated groups: **P < 0.01; ***P < 0.001.

Supplemental Figure S7



Supplemental Figure S7. Coagulation parameters are not altered in *Slc5a1^{fl/fl} Gp1ba-Cre⁺* mice. Coagulation parameters were assessed via TEG in citrated whole blood from WT, *Slc5a1^{fl/fl} Gp1ba-Cre⁻*, *Slc5a1^{fl/fl} Gp1ba-Cre⁺* mice. Reaction (R) time: time to initiation of formation of the fibrin clot (in min) (C; n=4). α -angle: rate of clot formation (in degrees) (D; n=4). Maximum amplitude: maximum strength of the clot (in mm) (E; n=4). K time: time required for the clot to reach a strength of 20 mm (in min) (F; n=4). Clot lysis: percentage of lysis (calculated from the maximum clot strength) after 30 minutes (G; n=4). Representative TEG tracing demonstrates the dynamics

of clot formation (**H**). MRTG, (measured using a velocity curve graphing strength over time (in mm/min) (**I**; n=4). Representative thrombin formation tracing shows peak of thrombin formation (**J**). Data represent mean $\pm$ SEM. One-way ANOVA with Dunnett's multiple comparisons; each condition was compared to all other conditions.