Supplemental Information

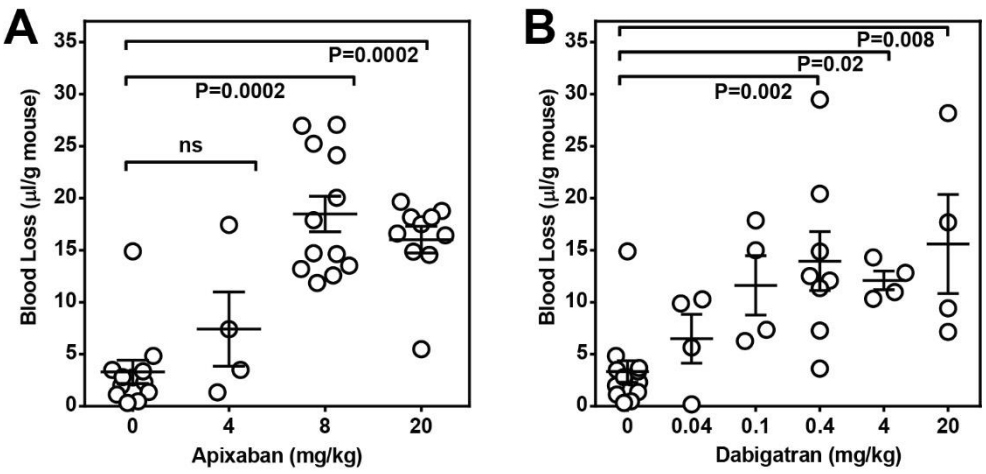
An engineered factor Va prevents bleeding induced by direct-acting oral anticoagulants by different mechanisms

Annette von Drygalski, Vikas Bhat, Andrew J. Gale, Patty M. Averell, Thomas J. Cramer, Darlene J. Elias, John H. Griffin, Laurent O. Mosnier.

Supplemental Table 1. Patient and sample characteristics

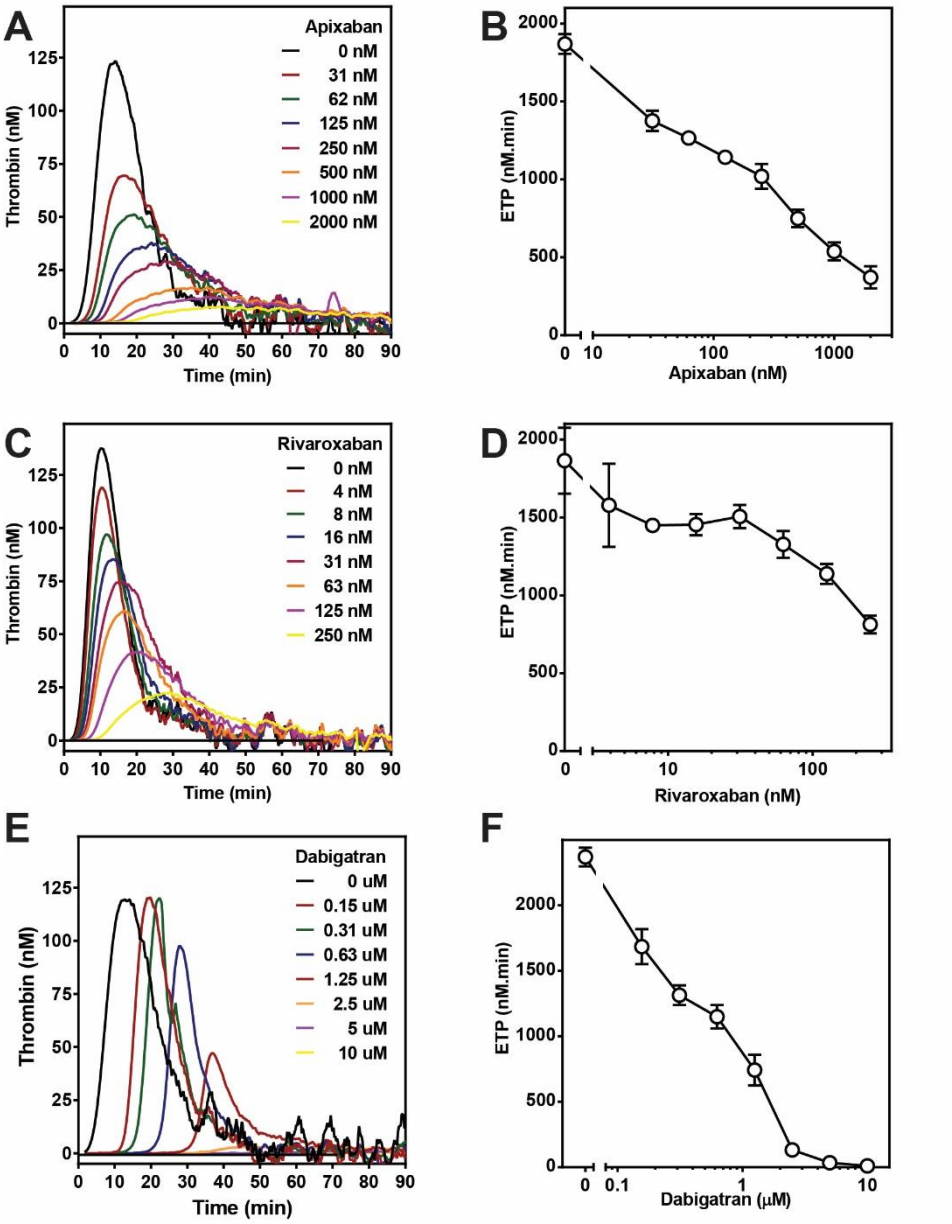
	n=	M / F	Rx dose (# patients)	Time to last dose (min ± SD)	Levels (ng/ml ± SD)
apixaban	10	7 / 3	2.5 bid (2) 5.0 bid (8)	263 ± 40	167 ± 47
rivaroxaban	10	9 / 1	15 mg/d (4) 20 mg/d (6)	228 ± 21	287 ± 144
dabigatran	10	6 / 4	75 mg/d (2) 150 bid (8)	250 ± 15	115 ± 105

Supplemental Figure 1



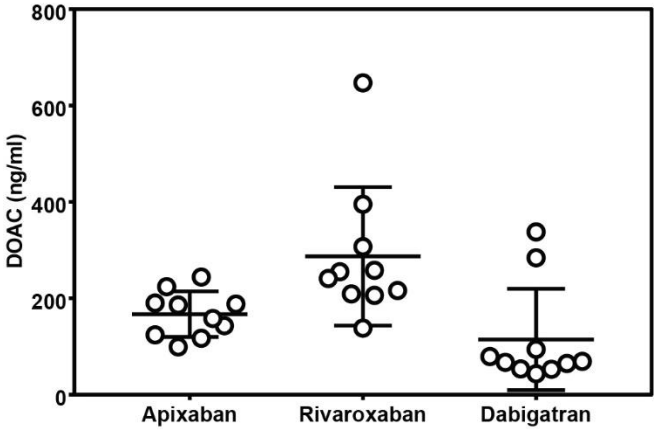
Supplemental Figure 1. Titration of apixaban and dabigatran in wild-type BalbC mice to induce bleeding. Bleeding induce by titration of (A) apixaban and (B) dabigatran by intravenous tail vein injection into wild-type BalbC mice. Bleeding was measured for 20 minutes after tail clip. Blood loss was expressed in µl blood per gram (g) mouse. ns; non-significant. Error bars represent standard error of the mean (n=4-12). P-values were determined by Kruskal-Wallis followed by a two-tailed Mann-Whitney test and values ≤ 0.05 were considered statistically significant.

Supplemental Figure 2



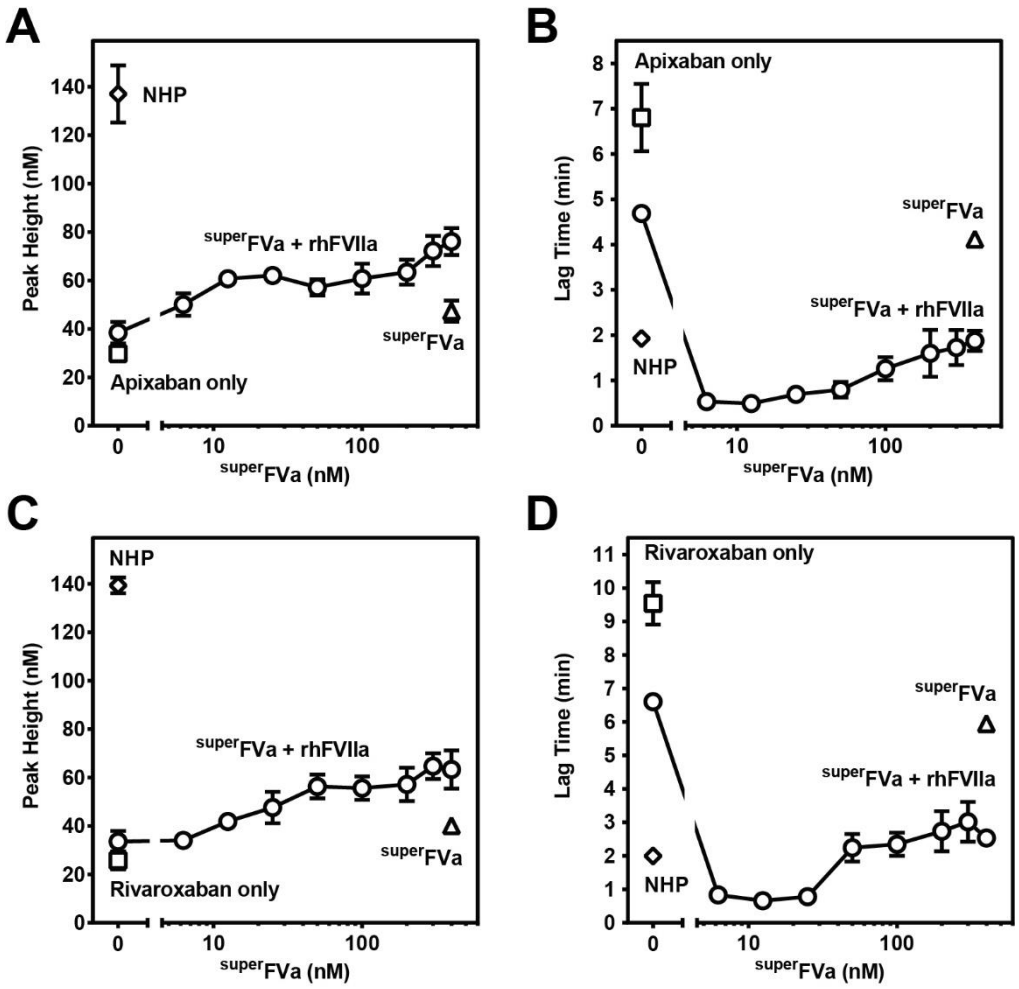
Supplemental Figure 2. Dose-dependent inhibition of thrombin generation and ETP in NHP by DOACs. Thrombin generation was measured in NHP in the presence of an increasing concentrations of (A/B) apixaban, (C/D) rivaroxaban, and (E/F) dabigatran. (A/C/E) Representative graphs showing the change in thrombin generation curves as the result of increasing concentrations DOACs. (B/D/F) Inhibition of ETP in NHP by increasing concentrations of DOACs. DOAC; Direct oral anticoagulant; Endogenous Thrombin Potential; ETP. Normal human plasma; NHP. Error bars represent standard error of the mean (n≥3).

Supplemental Figure 3



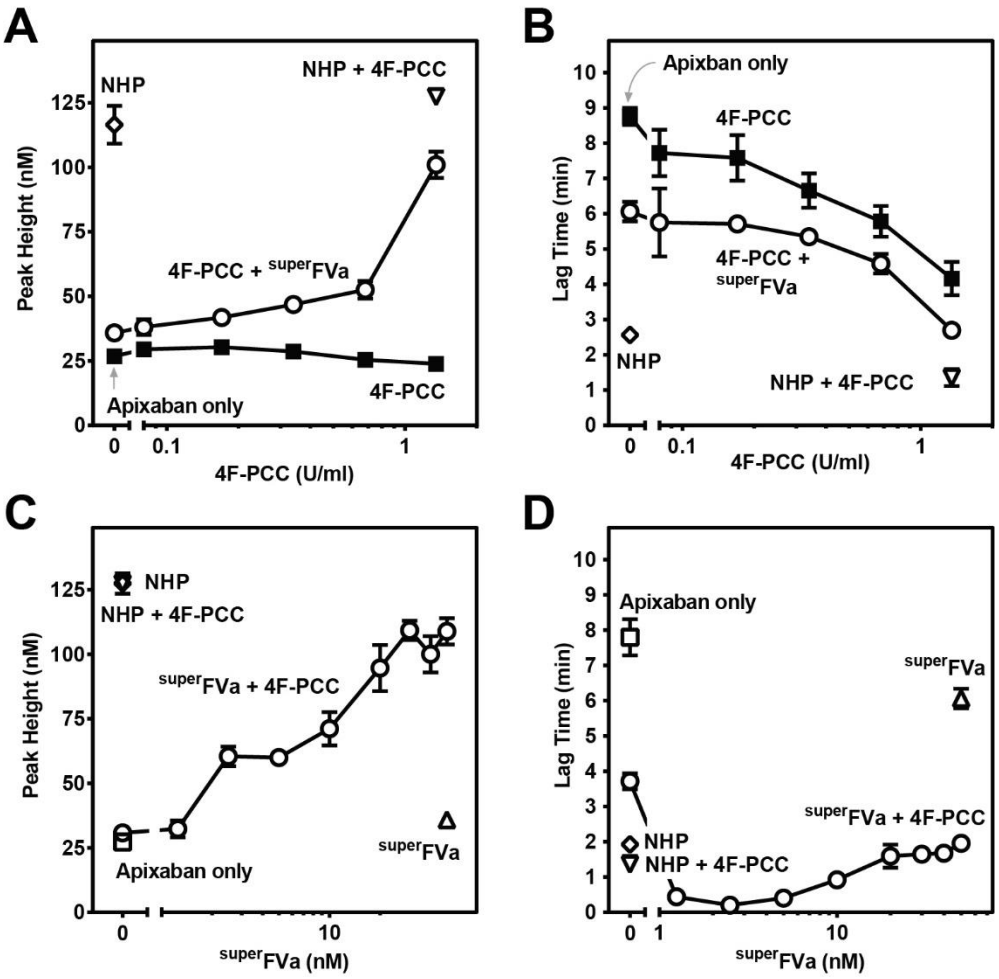
Supplemental Figure 3. Plasma Concentrations of DOACs in samples collected from patients on routine anti-coagulation. Blood drawn from patients during routine clinic visits through the Scripps Anticoagulation Service, and plasma was stored at -80ºC for further experiments. FXa DOAC levels were determined using the chromogenic DiXal kit and thrombin DOAC levels were determined using the clotting-based Hemoclot Thrombin-inhibitor kit. All DOACs were calibrated with the appropriate commercial 3-level reference plasmas.

Supplemental Figure 4



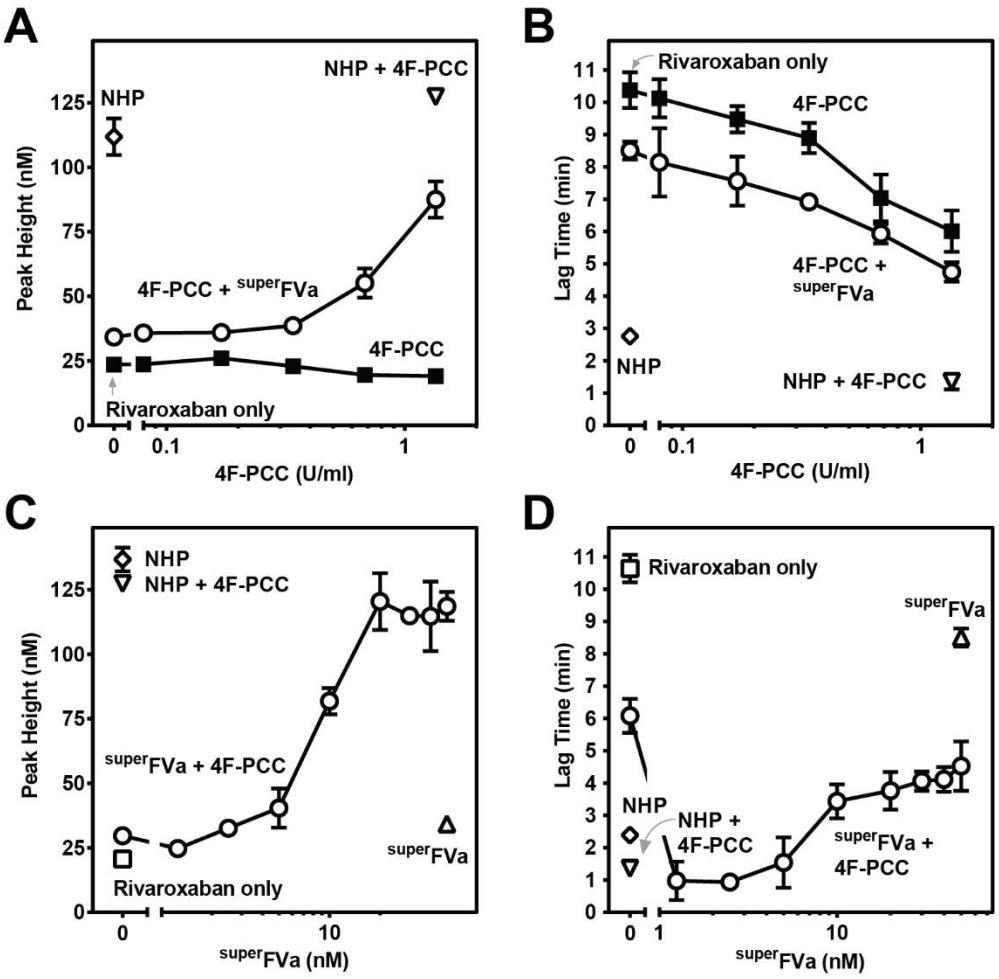
Supplemental Figure 4. Effects of ^{super}FVa and rhFVIIa on thrombin generation in NHP spiked with FXa inhibitors. Thrombin generation was measured in NHP in the presence of the FXa inhibitors, apixaban (A, B) or rivaroxaban (C, D). Thrombin generation was determined with increasing concentrations of ^{super}FVa in the absence (△) or presence (○) of rhFVIIa (40 nM) in NHP spiked with apixaban (200 nM) (A, B) or rivaroxaban (200 nM) (C, D). Shown are peak height (A, C) and lag time (B, D) matching the ETP shown in Figure 4 in the main manuscript. Additional controls shown are NHP with (□) and without (◇) apixaban or rivaroxaban. Normal human plasma, NHP. Error bars represent standard error of the mean (n≥3).

Supplemental Figure 5



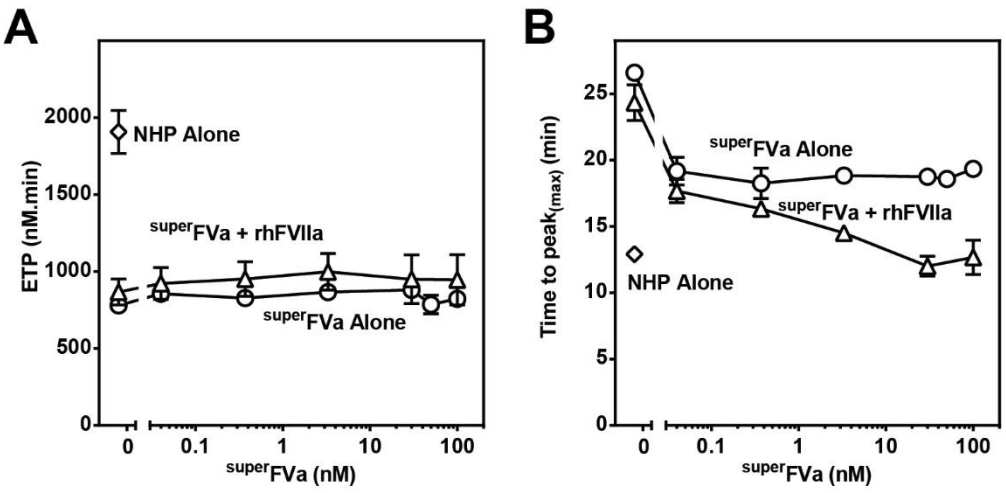
Supplemental Figure 5. Effects of ^{super}FVa and 4F-PCC on thrombin generation in NHP spiked with apixaban. Thrombin generation was measured in NHP in the presence of the FXa inhibitor, apixaban (200 nM). (A, B) Thrombin generation was determined with increasing concentrations of 4F-PCC in the absence (■) or presence (○) of ^{super}FVa (50 nM). (C, D) Thrombin generation was determined with increasing concentrations of ^{super}FVa in the absence (△) or presence (○) of 4F-PCC (1.35 U/ml). Shown are peak height (A, C) and lag time (B, D) matching the ETP shown in Figure 5B and 5C in the main manuscript. Additional controls shown are NHP with (□) and without (◊) apixaban or NHP with 1.35 U/ml 4F-PCC (▽). Normal human plasma, NHP. Error bars represent standard error of the mean (n≥3).

Supplemental Figure 6



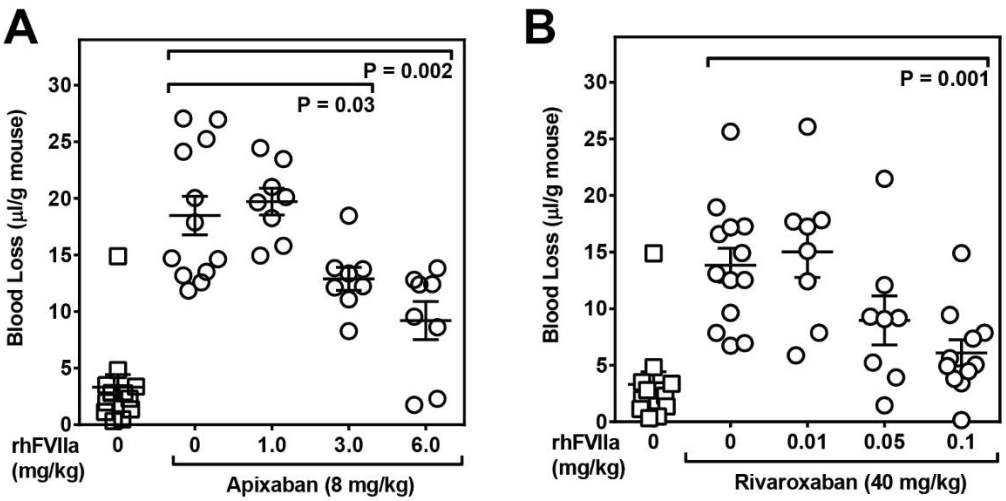
Supplemental Figure 6. Effects of ^{super}FVa and 4F-PCC on thrombin generation in NHP spiked with rivaroxaban. Thrombin generation was measured in NHP in the presence of the FXa inhibitor, rivaroxaban (200 nM). (A, B) Thrombin generation was determined with increasing concentrations of 4F-PCC in the absence (■) or presence (○) of ^{super}FVa (50 nM). (C, D) Thrombin generation was determined with increasing concentrations of ^{super}FVa in the absence (△) or presence (○) of 4F-PCC (1.35 U/ml). Shown are peak height (A, C) and lag time (B, D) matching the ETP shown in Figure 5E and 5F in the main manuscript. Additional controls shown are NHP with (□) and without (◊) rivaroxaban or NHP with 1.35 U/ml 4F-PCC (▽). Normal human plasma, NHP. Error bars represent standard error of the mean (n≥3).

Supplemental Figure 7



Supplemental Figure 7. Effects of ^{super}FVa and rhFVIIa on ETP of NHP thrombin generation in NHP spiked with dabigatran. Thrombin generation was measured in NHP in the presence of the thrombin inhibitor, dabigatran. (A) Effect of increasing concentrations of ^{super}FVa (0-100 nM) in the absence (○) or presence (△) of rhFVIIa (40 nM) on the ETP in NHP spiked with dabigatran (1 µM). (B) Effects of increasing concentrations of ^{super}FVa (0-100 nM) in the absence (○) or presence (△) of rhFVIIa (40 nM) on the time to peak of thrombin generation in NHP spiked with dabigatran (1 µM). NHP without dabigatran (◊) is shown as control. Endogenous Thrombin Potential, ETP; Normal human plasma, NHP. Error bars represent standard error of the mean (n≥3).

Supplemental Figure 8



Supplemental Figure 8. Reversal of FXa inhibitor-induced bleeding with rhFVIIa in wild-type BalbC mice. Mice were treated with apixaban (8 mg/kg) or rivaroxaban (40 mg/kg) by intravenous tail vein injection and bleeding was measured for 20 minutes after tail clip. Blood loss was expressed in µl blood per gram (g) mouse. Error bars represent SEM (n= 8-12 per group). P-values were determined by Kruskal-Wallis followed by a two-tailed Mann-Whitney test and values ≤ 0.05 were considered statistically significant.