Online Supplement

Mechanistic Target of Rapamycin Complex 1 (mTORC1) Signaling in Endothelial and Smooth Muscle Cells is Required for Vascular Function

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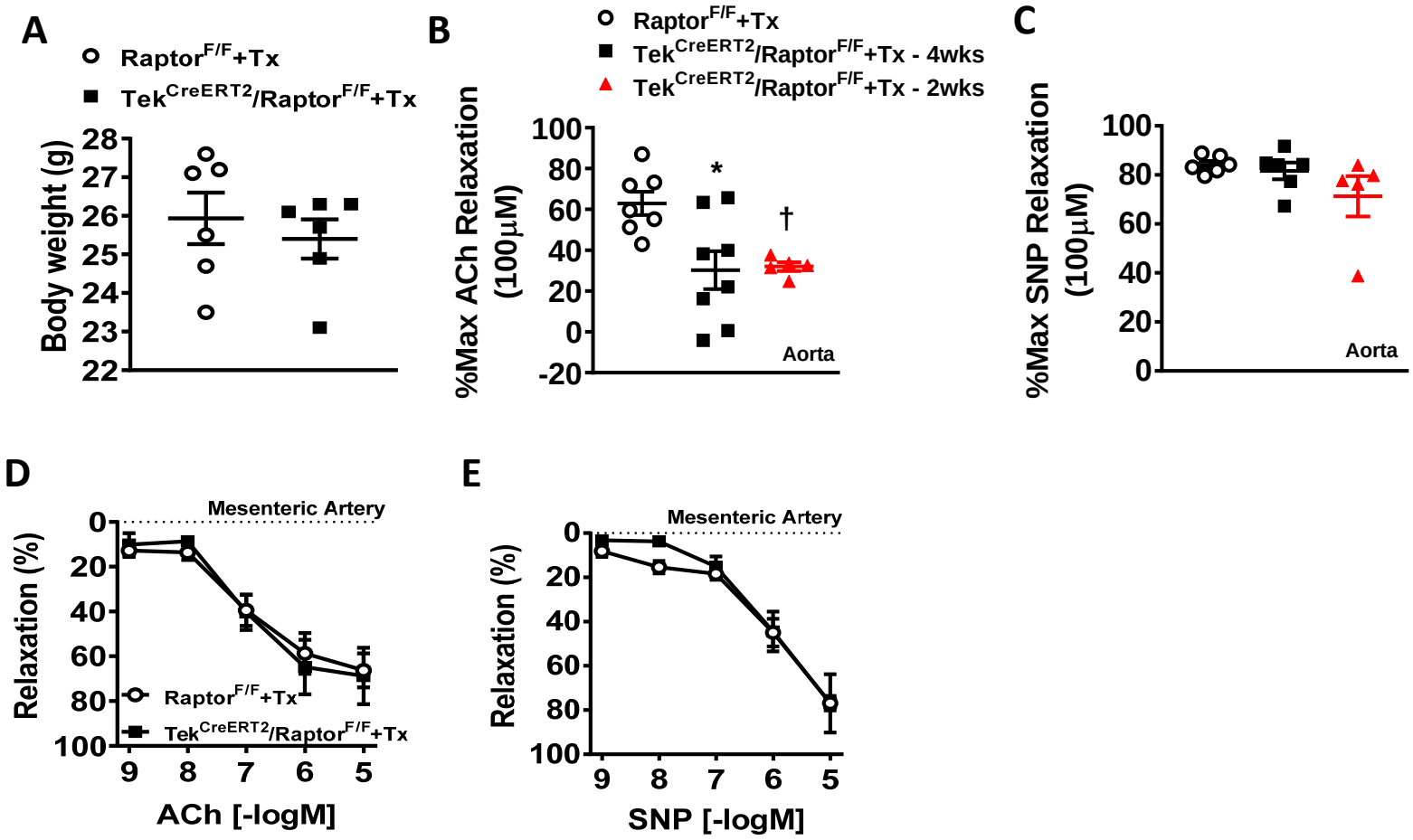

Figure S1 – Effects of conditional endothelial-specific Raptor deletion on body weight and vascular function. (**A**) Body weights of Tek^{CreERT2}/Raptor$^{F/F}$ and control male mice measured 4-week post-tamoxifen. (**B-C**) Aortic ring relaxation responses to ACh (acetylcholine; **B**) and SNP (sodium nitroprusside; **C**) of Tek^{CreERT2}/Raptor$^{F/F}$ and control male mice 2- and 4-week post-tamoxifen treatment. (**D-E**) Mesenteric artery relaxation responses to ACh (D) and SNP (E) of Tek^{CreERT2}/Raptor$^{F/F}$ and control male mice post-Tamoxifen treatment. n=7-8/group; * and †$p<0.05$ vs Raptor$^{F/F}$+Tx via one-way ANOVA.

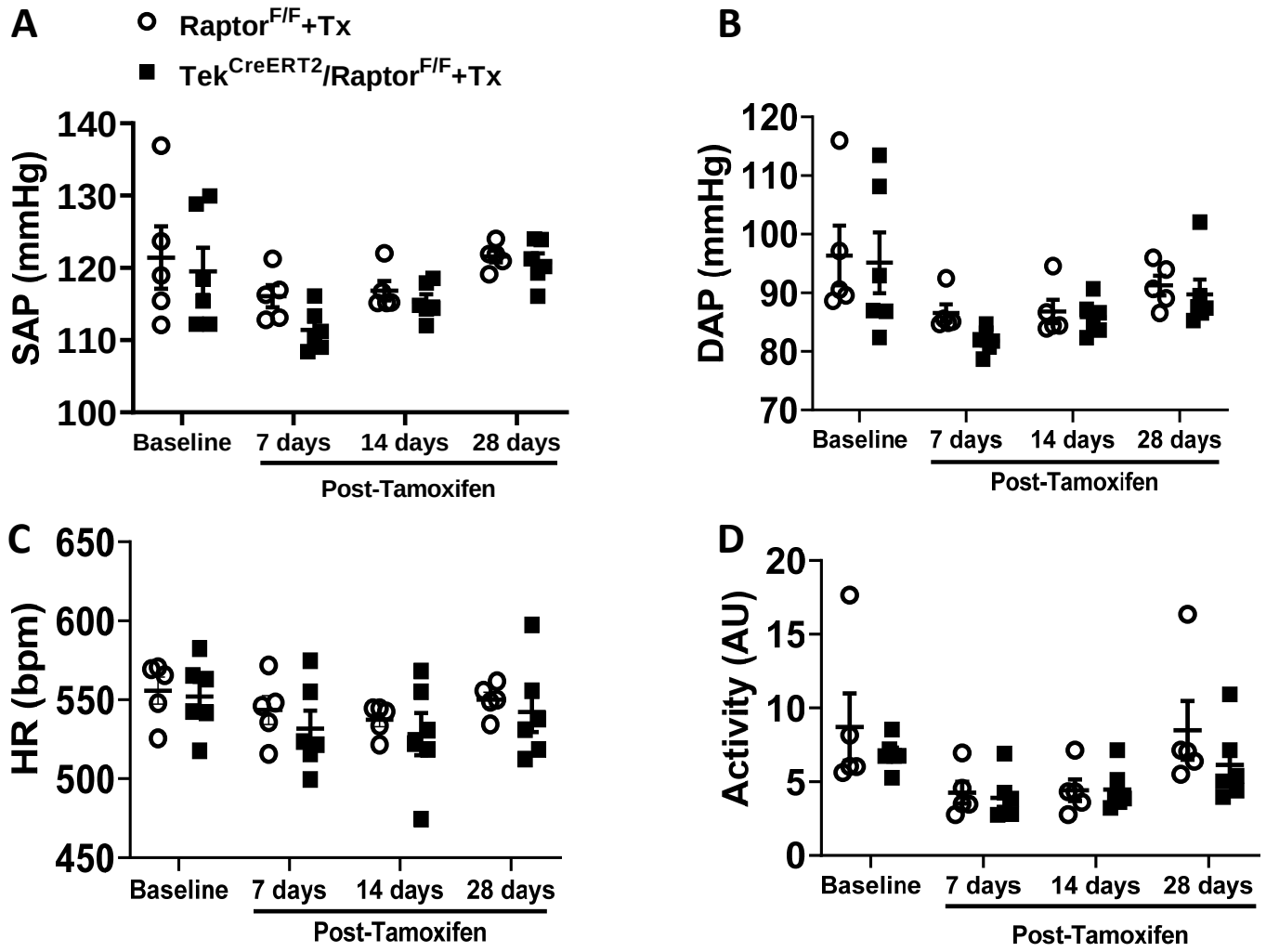

Figure S2 – Effects of conditional endothelial-specific Raptor deletion on hemodynamic parameters and activity. (**A-D**) Systolic (SAP, A), diastolic (DAP, B) blood pressure, heart rate (HR, C) and motor activity (D) of $Tek^{CreERT2}/Raptor^{F/F}$ and $Raptor^{F/F}$ control male mice at baseline and at different time points after tamoxifen treatment.

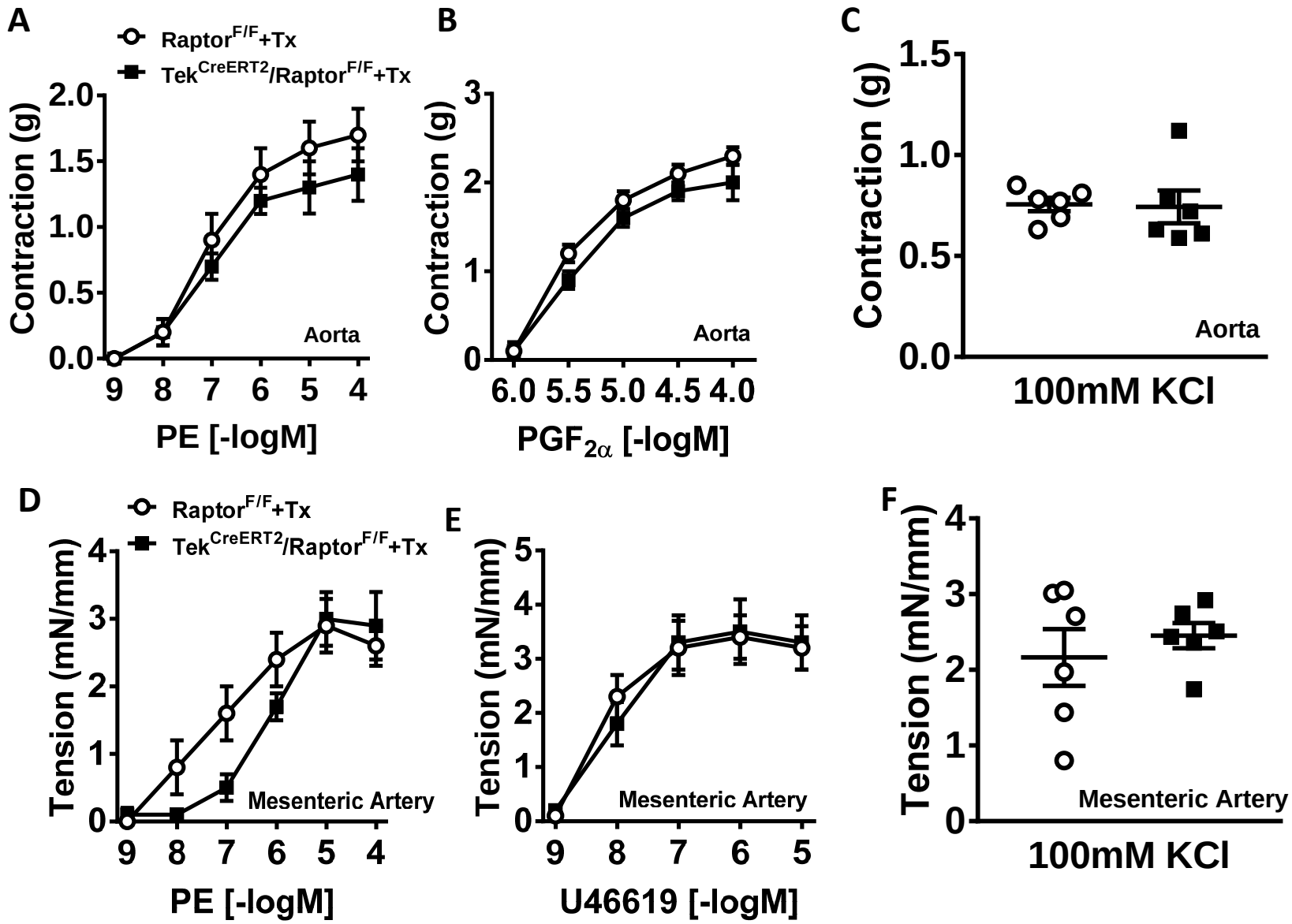

Figure S3 – Conditional endothelial-specific Raptor deletion does not alter contractile function in aortic and mesenteric arterial rings. Contractile responses evoked by PE (phenylephrine; **A**,**D**), PGF$_{2\alpha}$ (**B**), U46619 (**E**), and 100mM KCl (**C**,**F**) in aortic and mesenteric arterial ring of tamoxifen-treated Tek^{CreERT2}/Raptor$^{F/F}$ and control male mice. n=6/group.

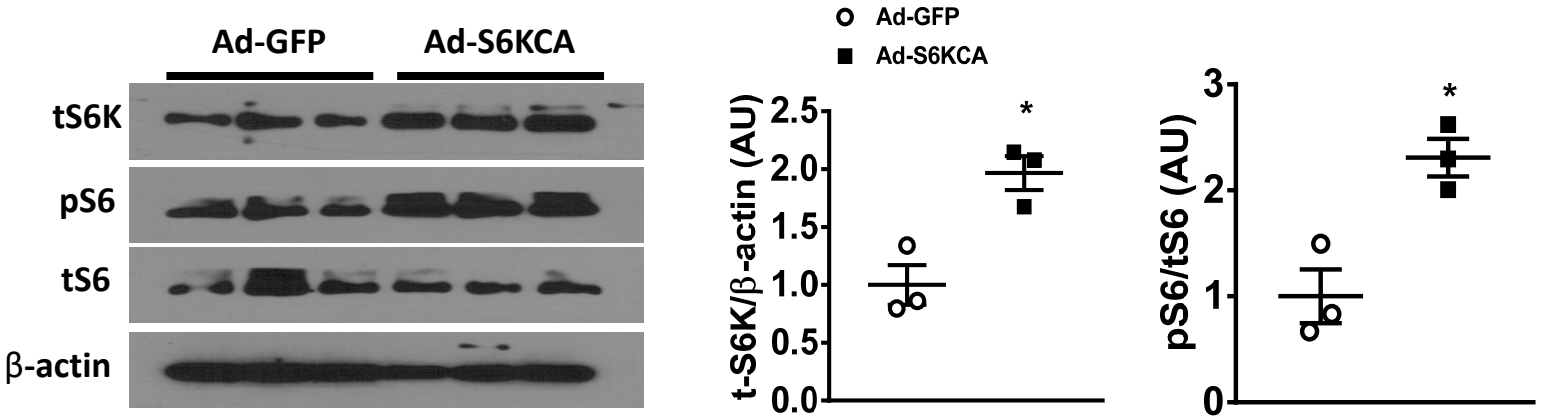

Figure S4 – Validation of adenoviral-mediated restoration of mTORC1 signaling in aortic rings of conditional endothelial specific Raptor deleted mice. Representative western blot images and quantification of tS6K (total S6K), pS6 (phospho-S6) tS6 (total S6) relative to β-actin in aortic lysates from tamoxifen treated endothelial-specific conditional Raptor knockout ($Tek^{CreERT2}/Raptor^{F/F}$) male mice at 4 weeks post-tamoxifen and cultured for 24hrs with Ad-GFP or Ad-S6KCA. *$p<0.05$ via unpaired t-test.

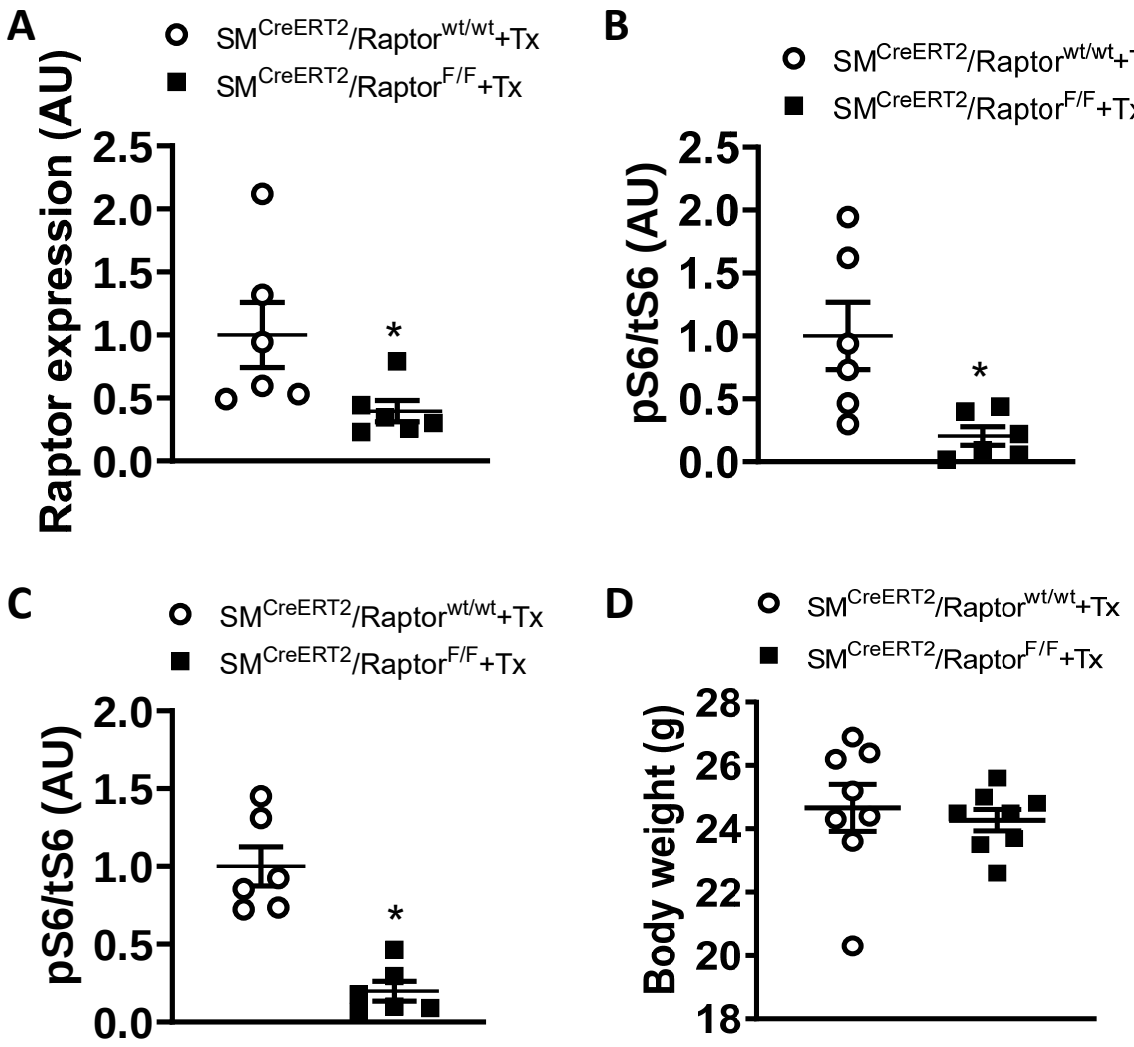

Figure S5 – Smooth muscle-specific deletion of Raptor decrease mesenteric Raptor expression and inhibit mTORC1 signaling without altering body weight. (**A-B**) Reduced Raptor protein expression (A) and phosphorylated levels of S6 protein (B) in the mesenteric artery of tamoxifen-treated $SM^{CreERT2}/Raptor^{F/F}$ male mice. (**C**) Reduced phospho-S6 levels in the aorta of tamoxifen-treated $SM^{CreERT2}/Raptor^{F/F}$ male mice. (**D**) Normal body weight, measured 4-week post-tamoxifen, of $SM^{CreERT2}/Raptor^{F/F}$ mice relative to controls. *$p<0.05$ via unpaired t-test.

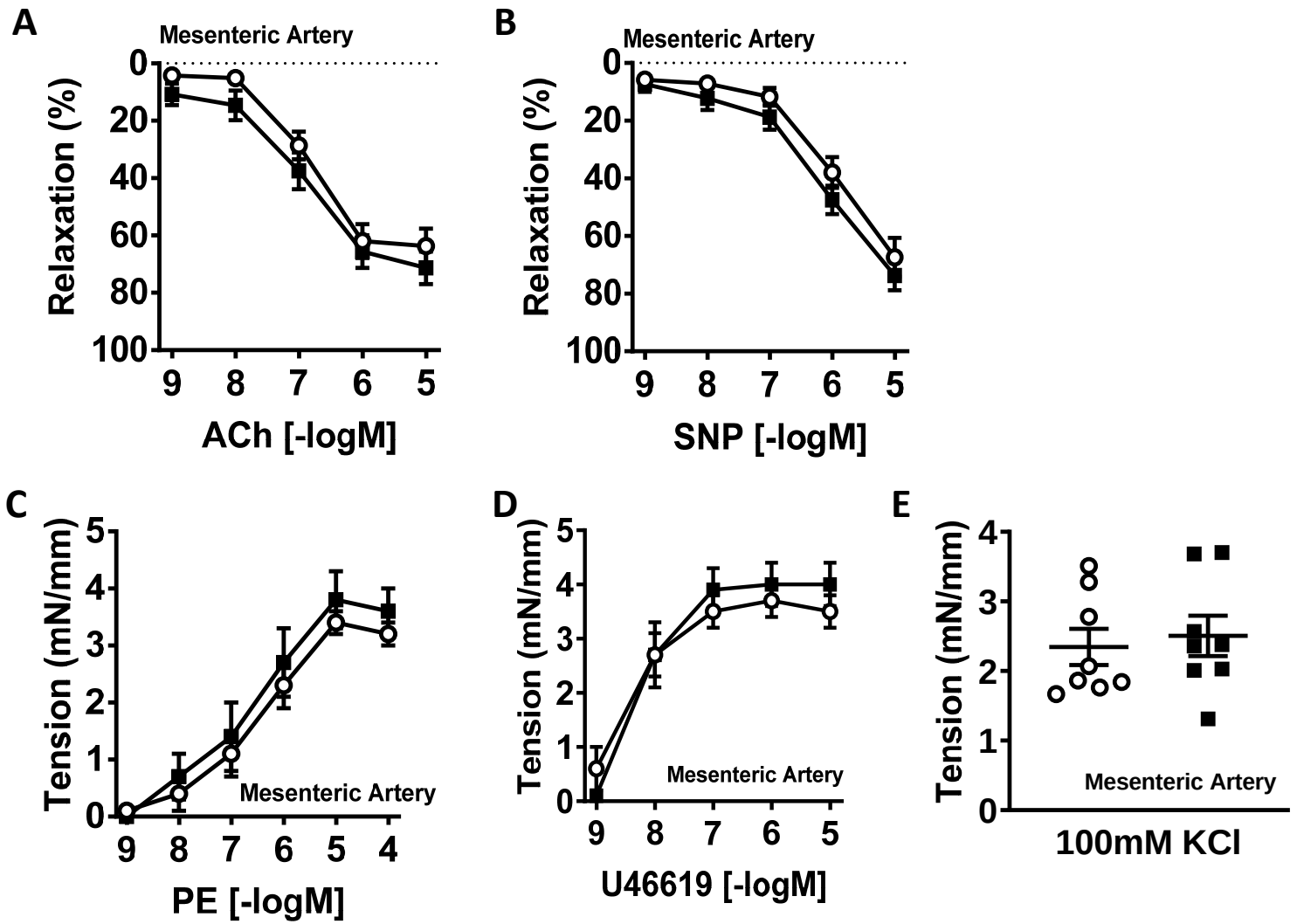

Figure S6 – Smooth muscle-specific deletion of Raptor does not alter mesenteric vascular function. (**A-B)** Relaxation responses to ACh (acetylcholine, A) and SNP (sodium nitroprusside, B) of mesenteric artery from $SM^{CreERT2}/Raptor^{F/F}$ and control male mice post-tamoxifen treatment (n=8/group). (**C-E**) Contractile responses to PE (phenylephrine, **C**), U46619 (**D**) and KCl (**E**) in mesenteric arterial rings isolated from tamoxifen treated $SM^{CreERT2}/Raptor^{F/F}$ mice and controls. n=8/group.

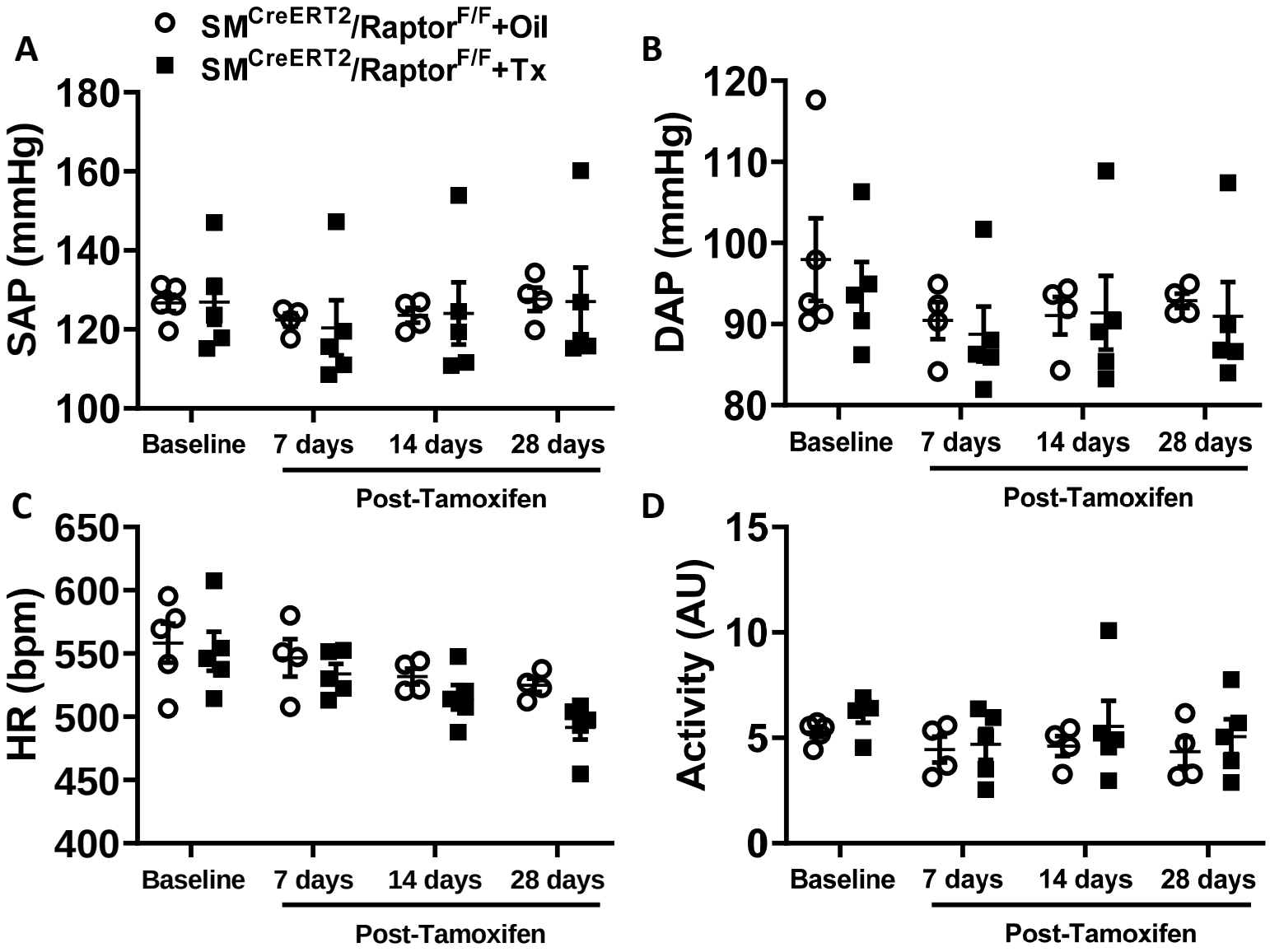

Figure S7 – Effects of conditional smooth muscle-specific Raptor deletion on hemodynamic parameters and activity. (**A-D**) Systolic (SAP, A), diastolic (DAP, B) blood pressure, heart rate (HR, C) and motor activity (D) of $SM^{CreERT2}/Raptor^{F/F}$ male mice at baseline and at different time points after tamoxifen or vehicle (oil) treatments.