

Supplementary Figure S1

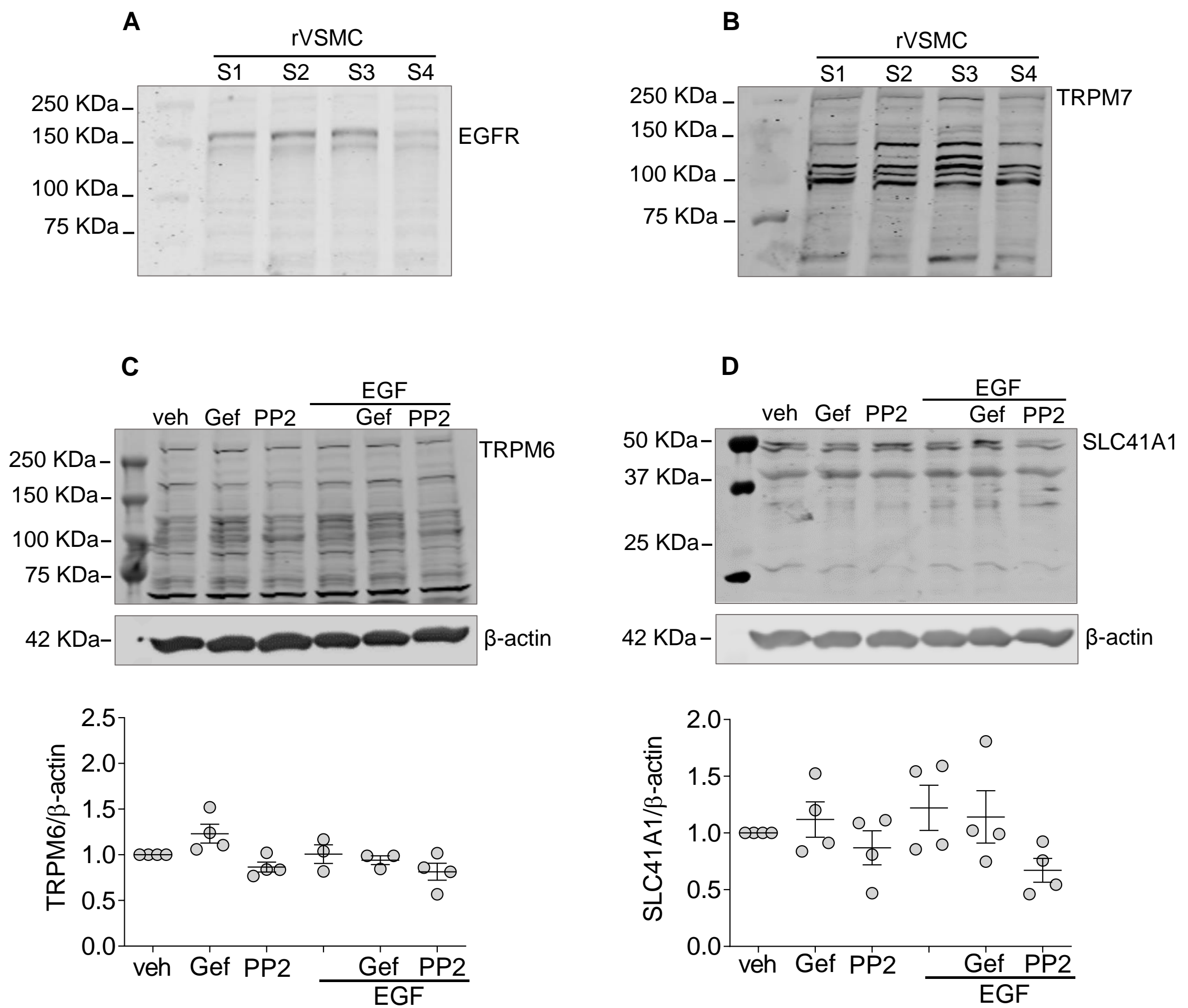
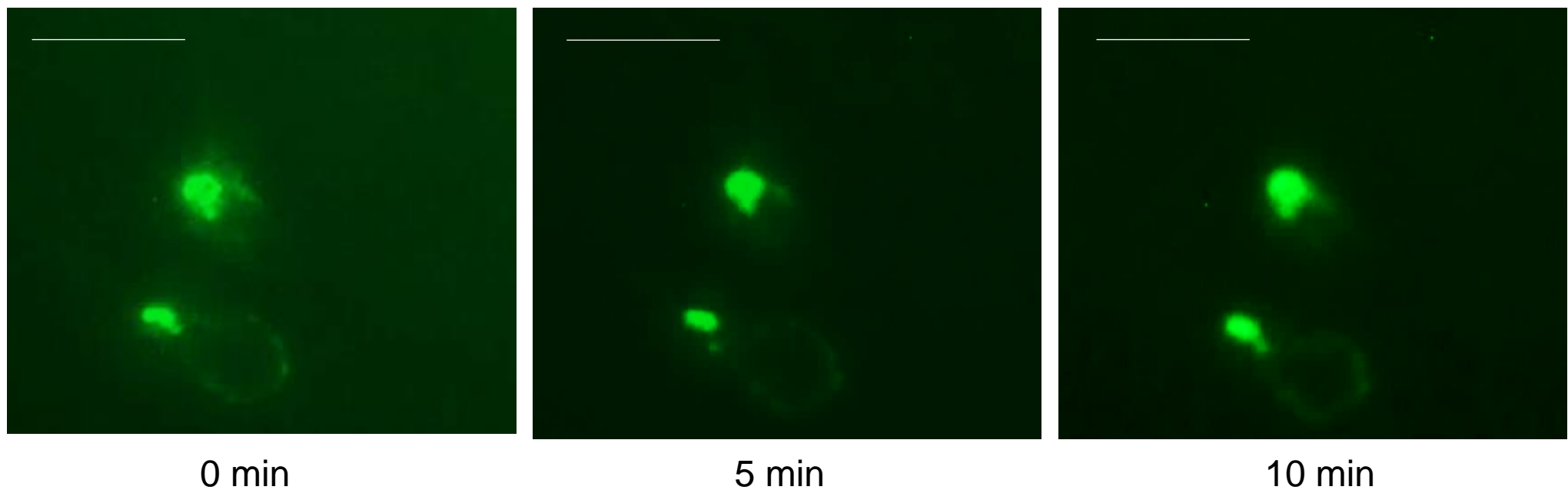


Figure S1. Expression of EGFR and Mg²⁺ transporters in EGF-stimulated rVSMCs. (A, B) Representative immunoblots demonstrating expression of EGFR and TRPM7 in rVSMCs. Sample S1-S4 are rVSMCs derived from 4 independent WKY rats. (C-E) rVSMCs were treated with EGF (50 ng/ml, 24 h) in the presence and absence of gefitinib (Gef, 1 μM) and PP2 (10 μM) and protein expression was assessed by immunoblotting. (C) TRPM6, and (D) SLC41A1. Results represent the mean ± SEM of 4 to 6 independent experiments.

Supplemental Figure S2

A



B

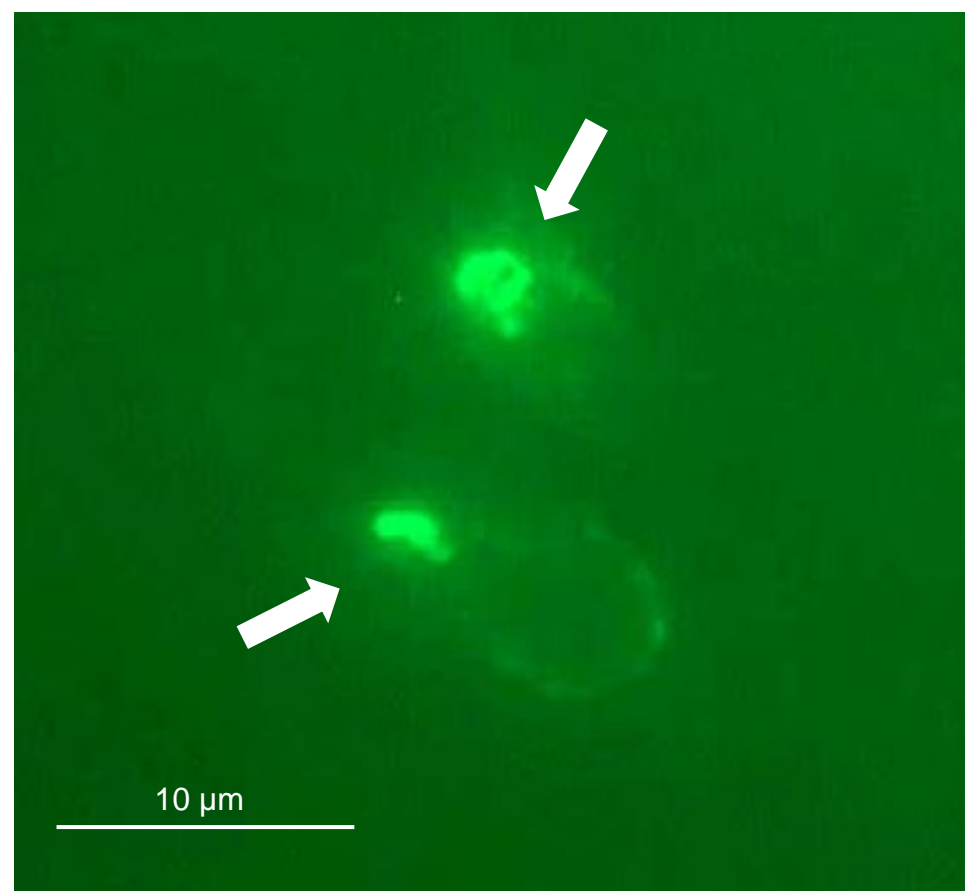


Figure S2. EGF/EGFR does not influence TRPM7 trafficking in HEK-293 cells. HEK-293 cells overexpressing mTRPM7-YFP were visualized by an inverted epifluorescence microscope (Axio Observer Z1 Live-Cell imaging system, ZEISS). TRPM7 movements were recorded after cells were treated with EGF (50 ng/ml) for 10 min. **(A)** shows TRPM7 intracellular location at different time point (0 min, 5min and 10 min) after EGF treatment. **(B)** Shows intracellular TRPM7 movement after EGF stimulation. Images were acquired using 40 x magnification length. Scale bar = 10 µm. Representative images were from 2 independent experiments.

Supplemental Figure S3

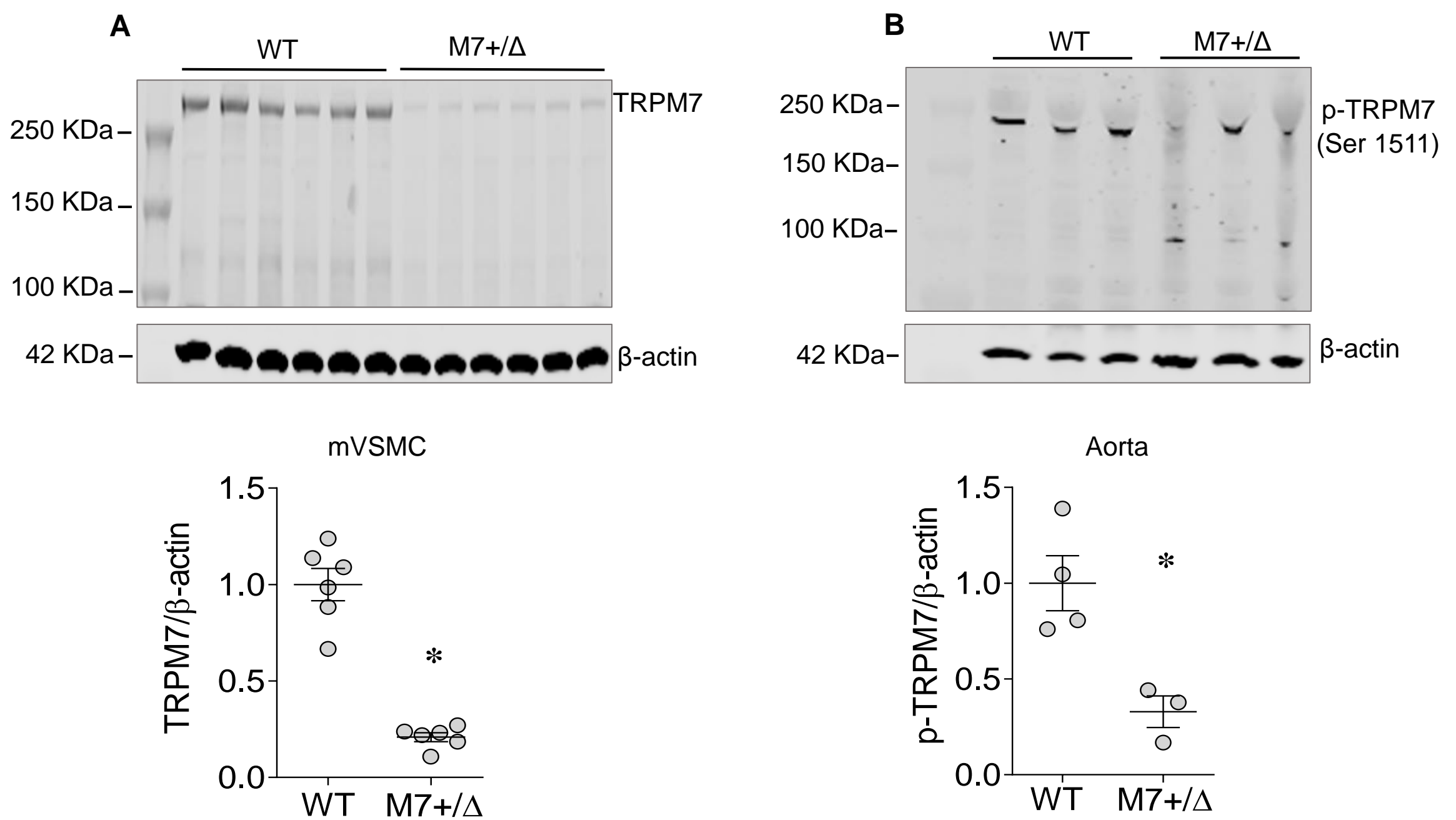


Figure S3. TRPM7^{+/ Δ kinase} mice display reduced expression and phosphorylation of TRPM7. (A) TRPM7 expression in mVSMCs derived from WT and TRPM7^{+/ Δ kinase} mice (M7+/ Δ) and (B) TRPM7 phosphorylation at serine 1511 residue (Ser 1511) in aortic tissues from WT and TRPM7^{+/ Δ kinase} mice were examined by immunoblotting. Results represent the mean \pm SEM of 3 to 6 independent experiments. * $P < 0.05$ compared to WT.

Supplemental Figure S4

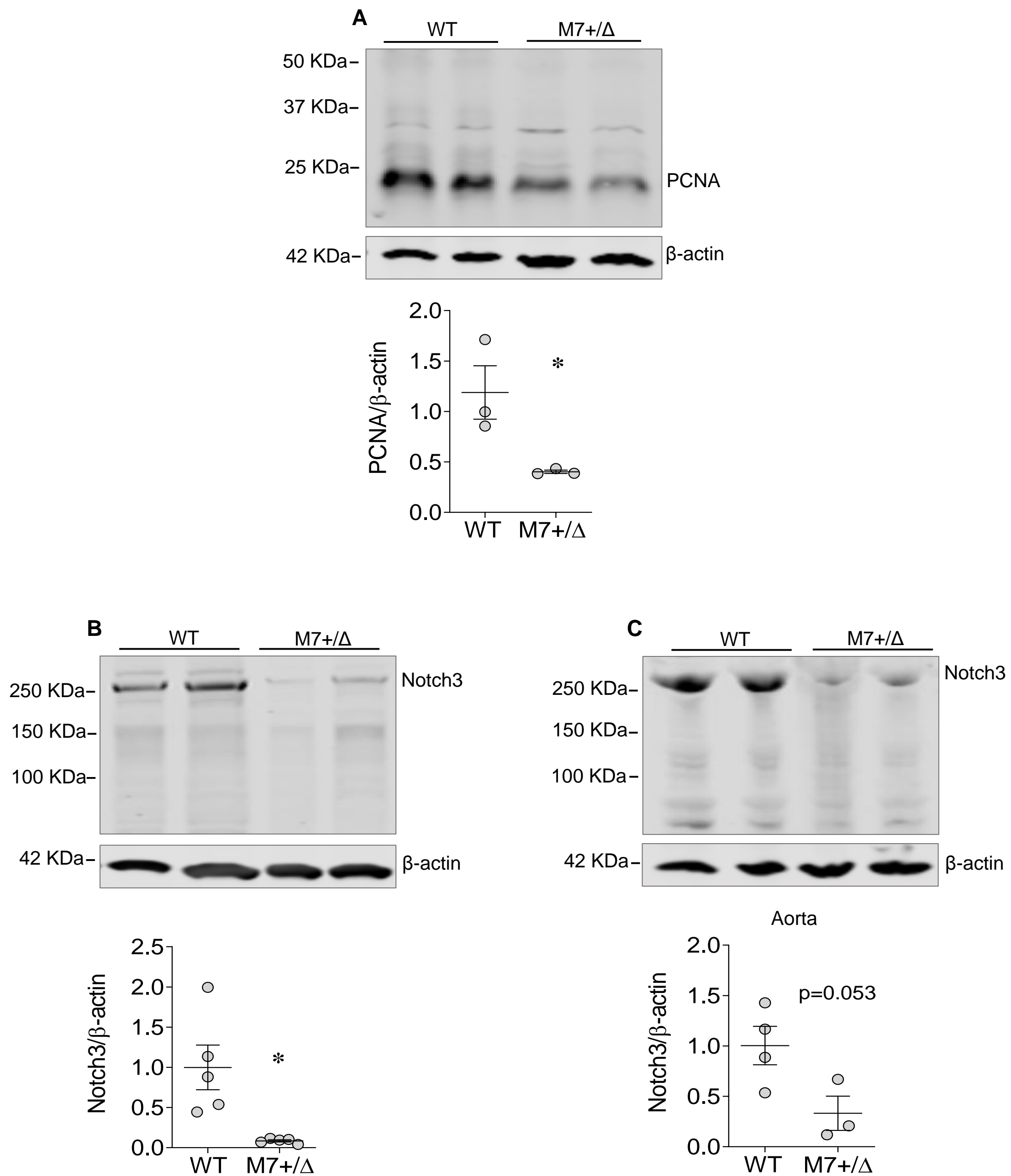


Figure S4. Expression of PCNA and Notch3 is reduced in the vasculature of TRPM7^{+/-}Δ^{kinase} mice. A) PCNA expression in aortic tissues from WT and TRPM7^{+/-}Δ^{kinase} mice (M7+/Δ). **(B)** Expression of Notch3, a VSMC-specific Notch isoform that regulates differentiation, survival and growth, in mVSMCs and **(C)** aortic tissues derived from WT and TRPM7^{+/-}Δ^{kinase} mice assessed by immunoblotting. Protein of interest expressed relative to β-actin. Results represent the mean±SEM of 3 to 5 independent experiments. * *P*<0.05 compared to WT.

Supplemental Figure S5

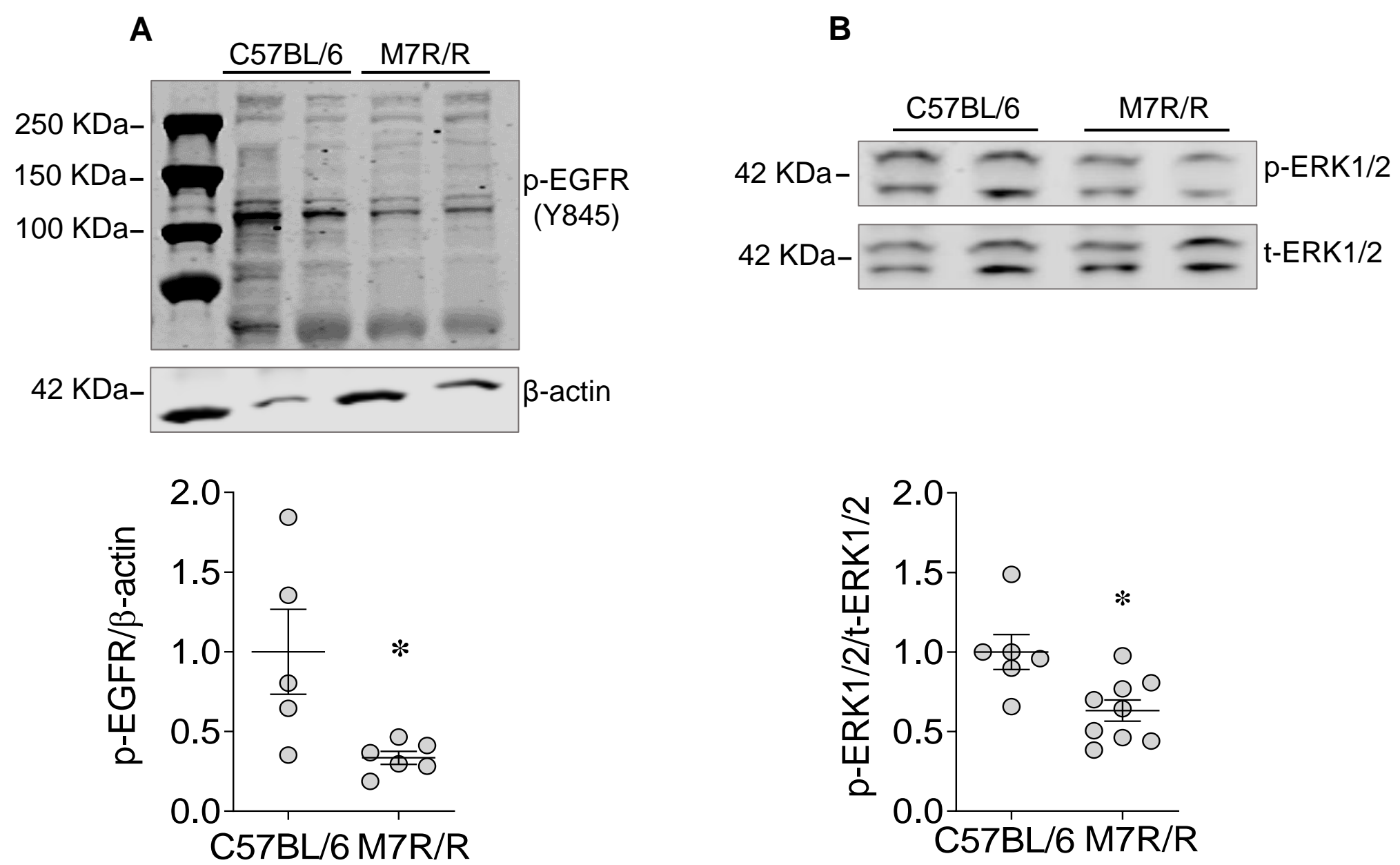


Figure S5. Phosphorylation of EGFR and ERK1/2 in TRPM7-kinase dead mice. (A) Phosphorylation of EGFR at tyrosine 845 (Y845) and (B) Phosphorylation of ERK1/2 in aortic tissues from C57BL/6 control mice and TRPM7-kinase dead (M7^{R/R}) mice was examined by immunoblotting. Results represent the mean \pm SEM of 5 to 9 independent experiments. * P <0.05 compared to C57BL/6 mice.