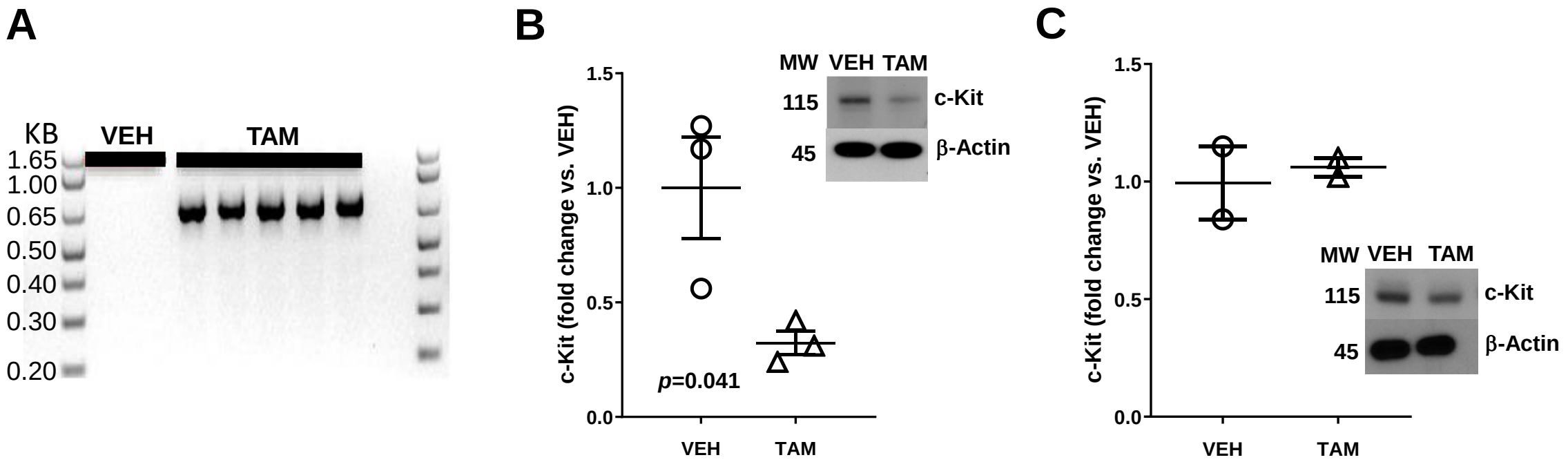
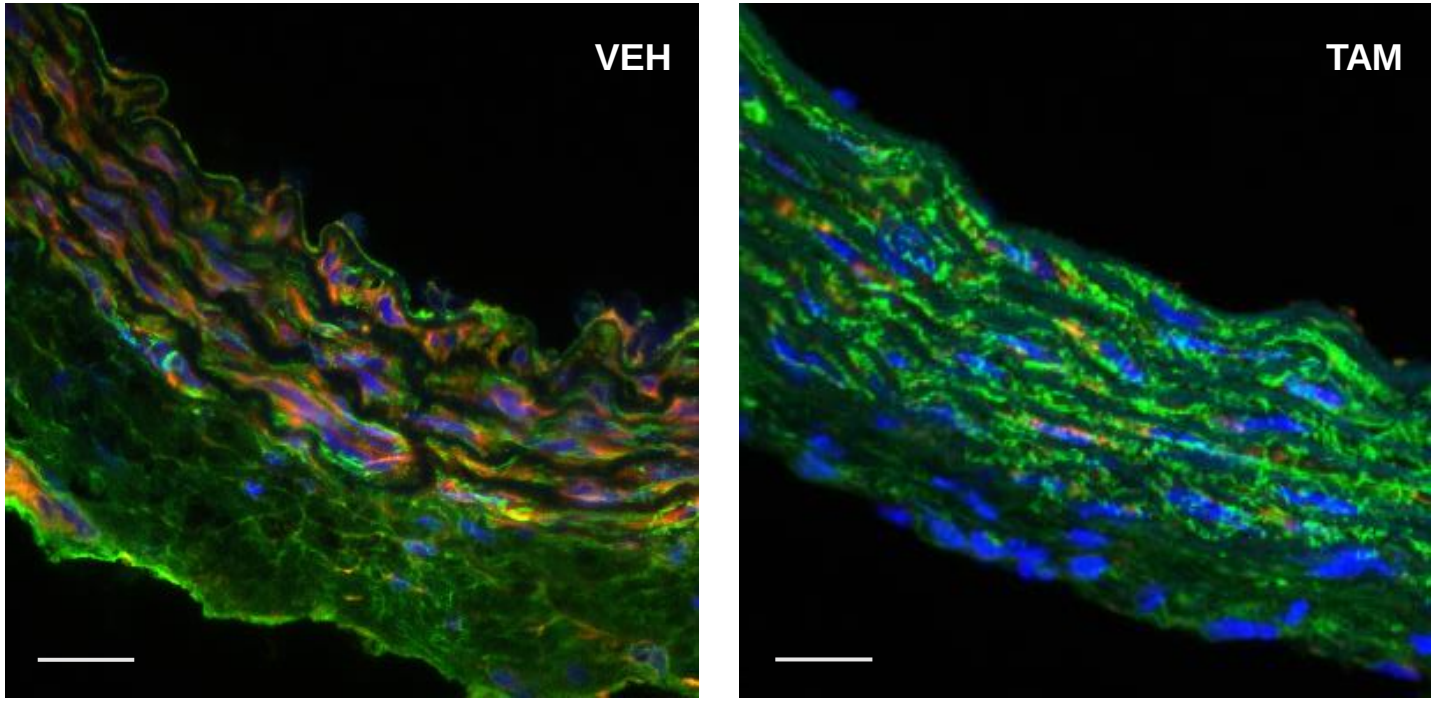
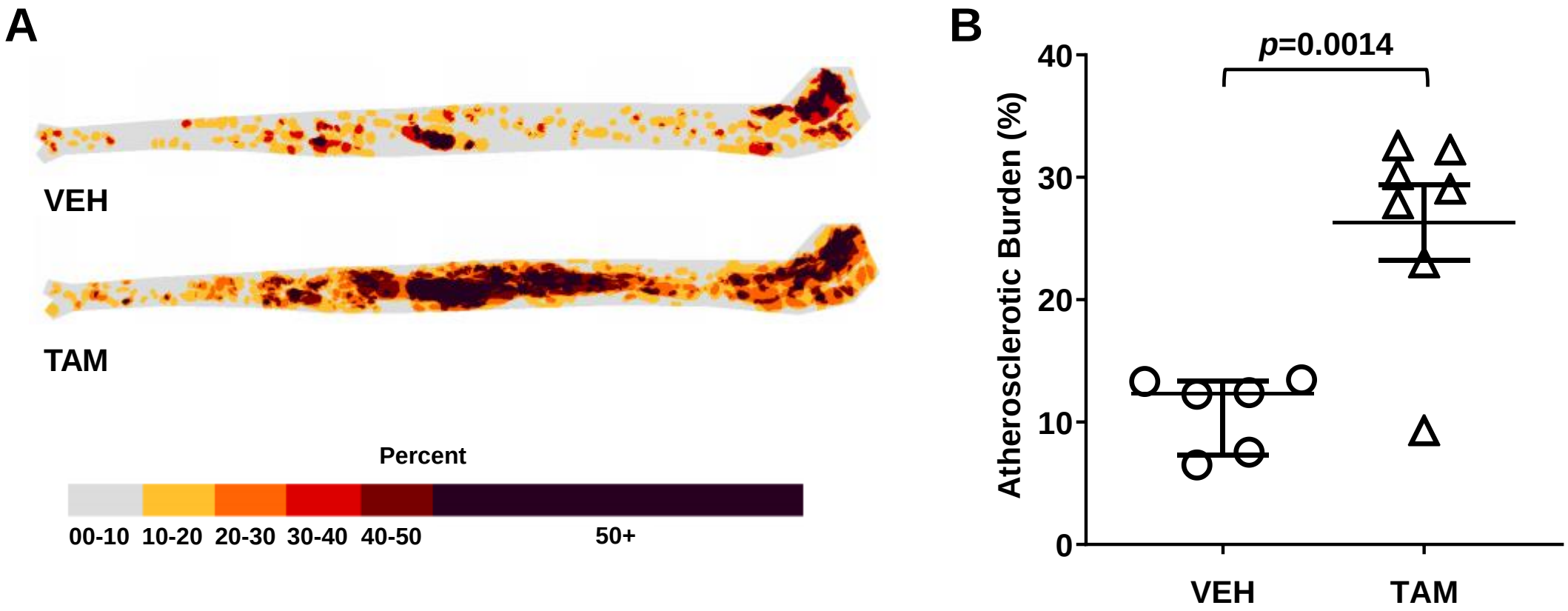


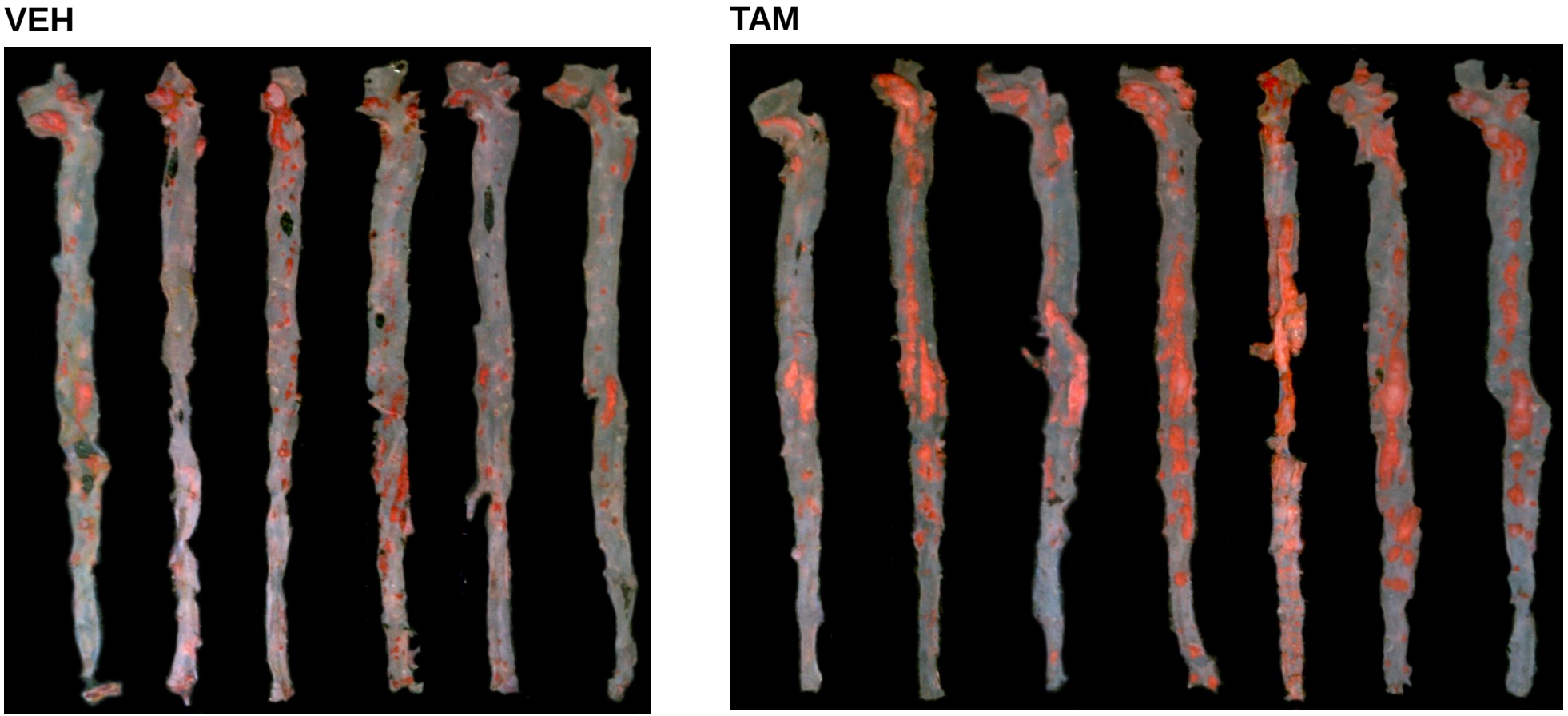
Supplementary Figure 1. **Characterization of the Kit^{SMC} transgenic mouse. (A)** Aortic PCR of Kit^{SMC} transgenic mice (Kit$^{lox66\text{-}71/\ lox66\text{-}71}$ Myh11-CreER^{T2} ApoE$^{-/-}$) injected intraperitoneally with either oil (VEH) or tamoxifen (TAM). PCR were performed using primers specific for the inverted (post-recombination) Kit$^{lox66\text{-}71}$ allele. Kit^{SMC} mice treated with TAM showed the expected 0.65 kb band, while control mice injected with oil showed no bands. **(B)** Tamoxifen (TAM) rich diet for 4 weeks led to a significant reduction of c-Kit protein in the aorta of conditional mouse as determined by IP-WB. **(C)** Protein expression of c-Kit in the spleen showed no significant differences between TAM- or vehicle-treated animals. Data are presented as mean ± S.E.M. c-Kit expression was normalized using β-actin levels, then standardized against expression in the vehicle. Groups were compared using a two-tailed t-test assuming unequal variance.



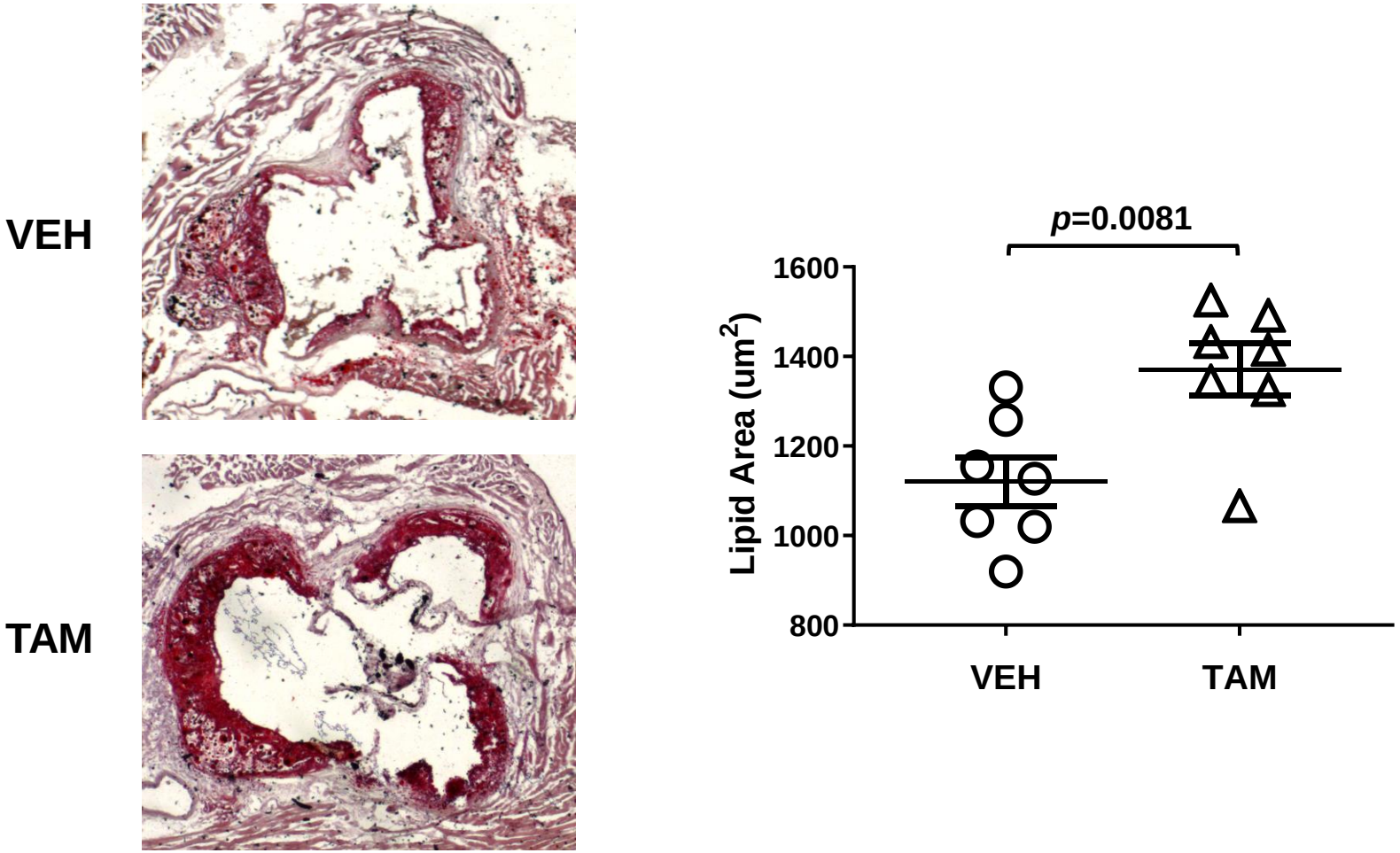
Supplementary Figure 2. **Significant reduction of c-Kit protein in the tunica media of conditional mice (Kit^{SMC}) after tamoxifen-rich diet.** Confocal microscopy images of aortas from Kit^{SMC} mice ($Kit^{lox66\text{-}71/\ lox66\text{-}71}$ Myh11-$CreER^{T2}$ $ApoE^{-/-}$) after feeding a tamoxifen chow (TAM) or normal chow (VEH) for 4 weeks. SMC were identified with an antibody against smooth muscle actin (SMA). Double positive cells for c-Kit (red) and SMA (green) in the media of vehicle treated mice appear yellow after merging color images. Nuclei were counterstained with DAPI (blue). Scale bars: 100 µm.



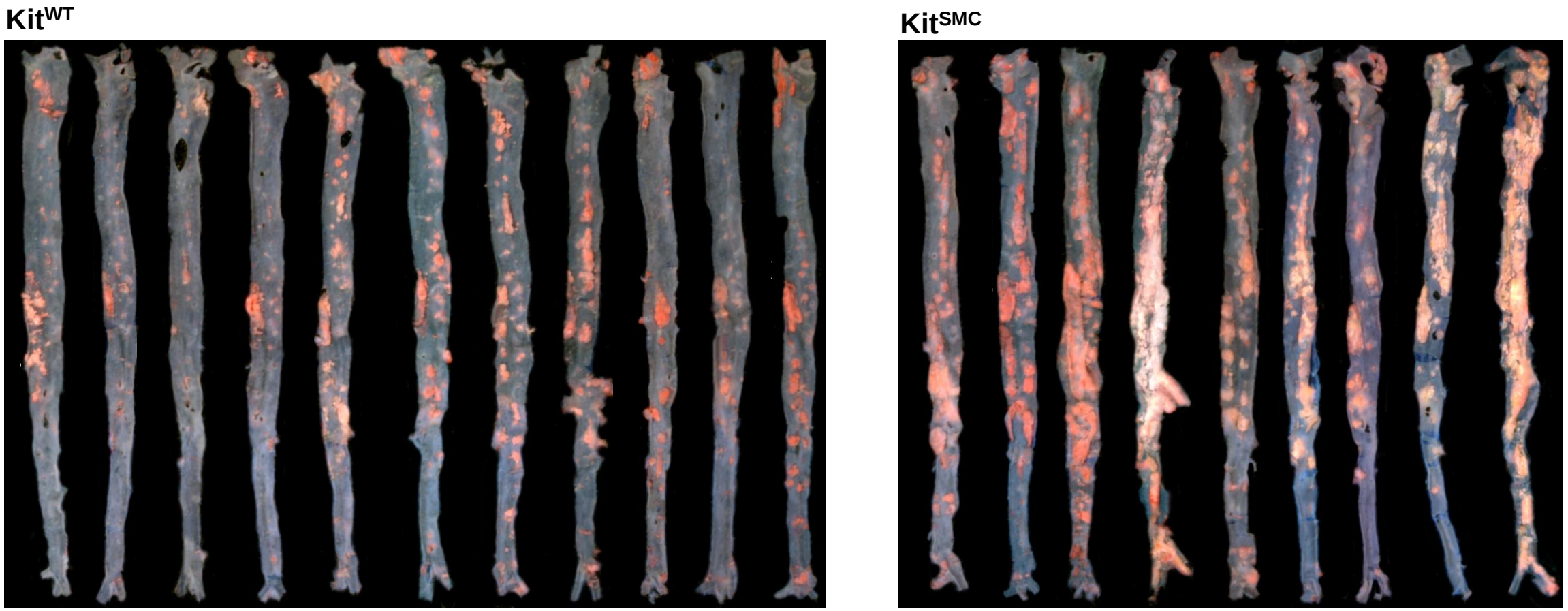
Supplementary Figure 3. **c-Kit inactivation in vascular SMC increases atherosclerosis in hyperlipidemic mice. (A)** Heat map of plaque occurrence in whole aortas from Kit^{SMC} (*Kit*$^{lox66\text{-}71/\ lox66\text{-}71}$ *Myh11-CreER*^{T2} *ApoE*$^{-/-}$) mice after feeding normal chow (VEH, n=6) or tamoxifen (TAM, n=7) rich diet for 4 weeks, followed by 16 weeks of high fat diet. **(B)** Quantification of percent atherosclerosis burden in the aorta. Error bars indicate the groups' median ± interquartile range. Groups were compared using the Mann-Whitney test.



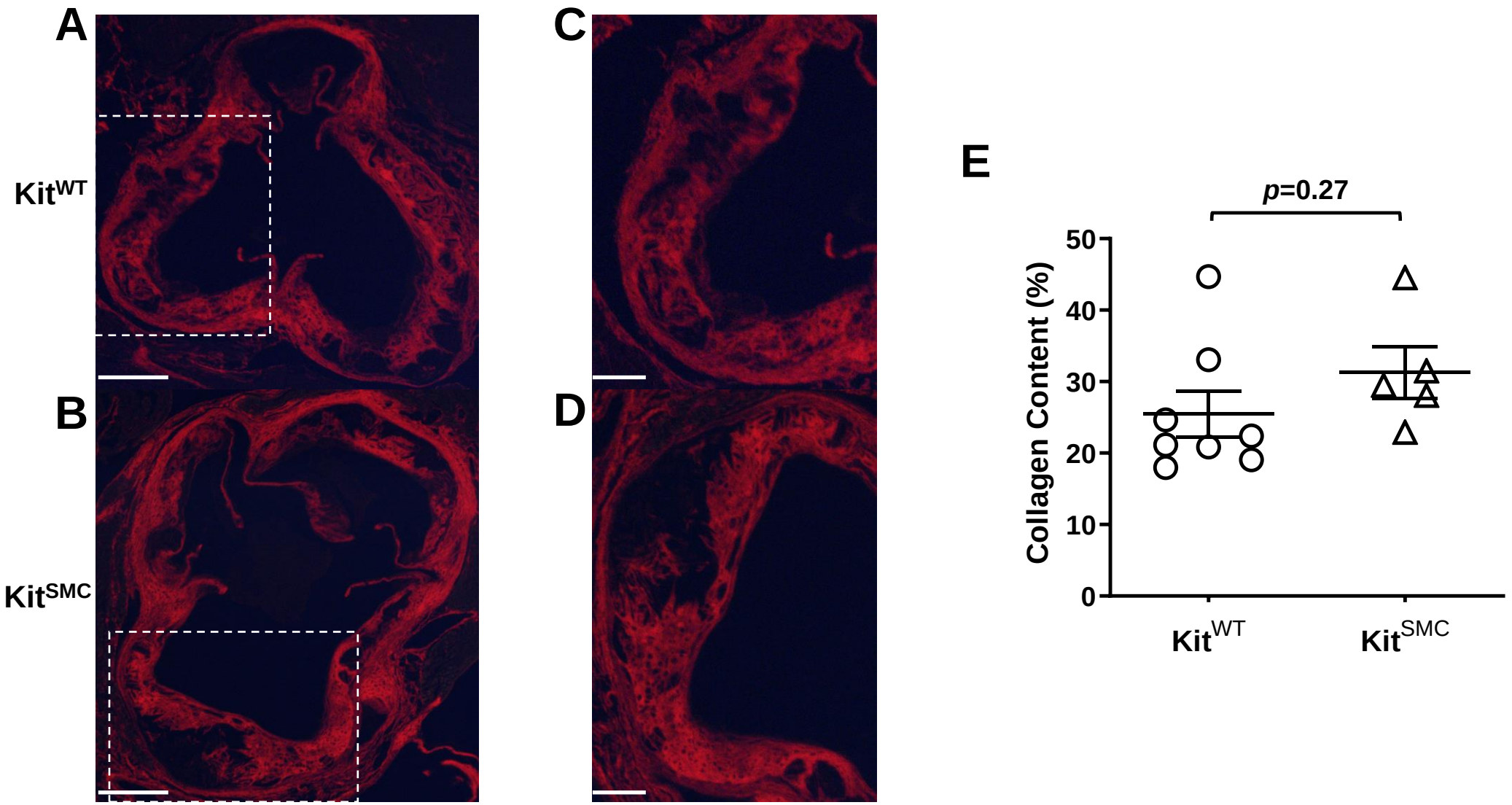
Supplementary Figure 4. Complete images of Oil Red-O stained aortas from vehicle- and tamoxifen treated Kit^{SMC} mice. Complete images of Oil Red-O stained aortas from Kit^{SMC} mice (Kit$^{lox66\text{-}71/\ lox66\text{-}71}$ Myh11-CreER^{T2} ApoE$^{-/-}$) fed tamoxifen (TAM)-rich chow (n=7) or normal chow (VEH, n=6) for 4 weeks, followed by high fat diet for 16 weeks.



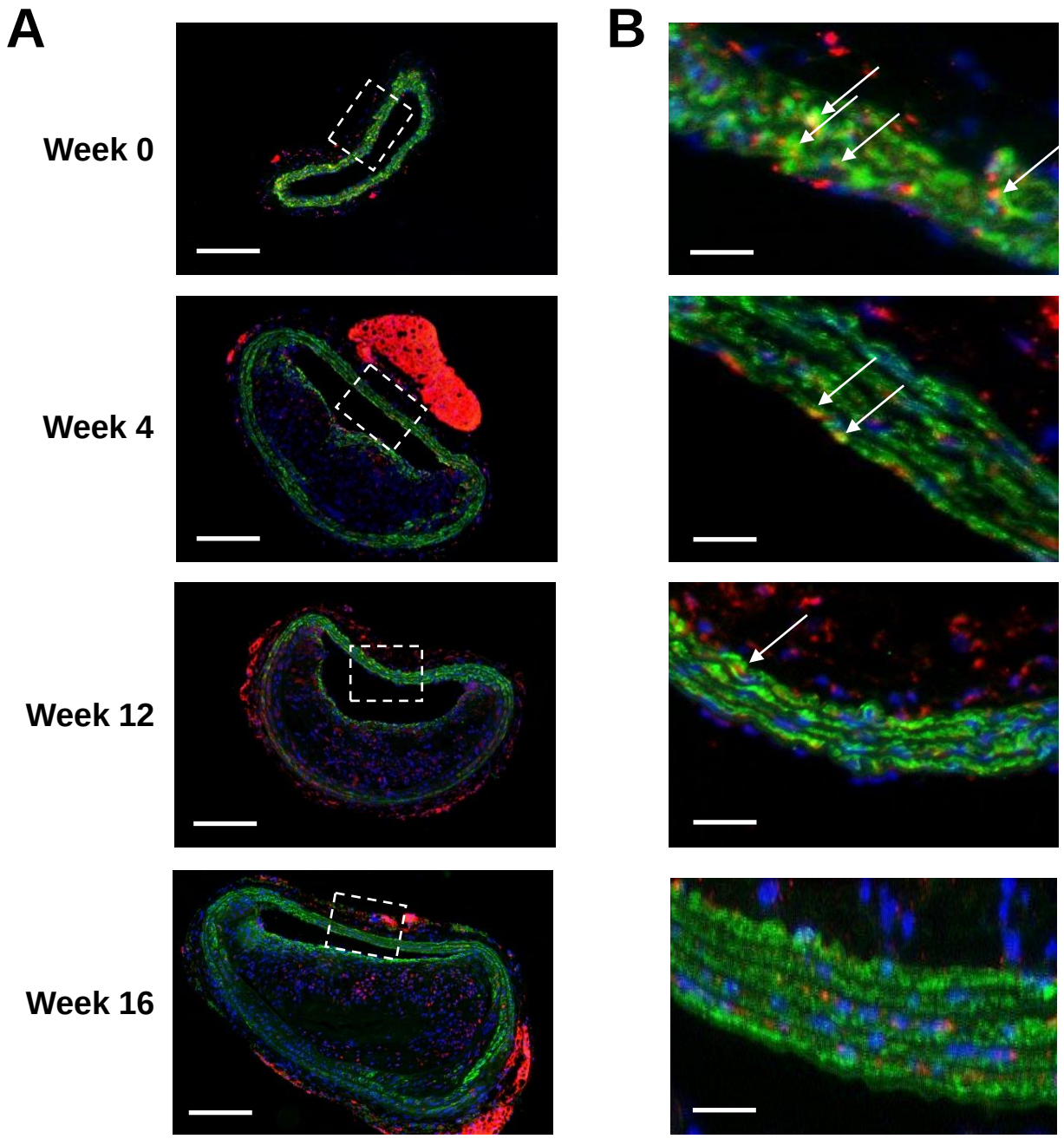
Supplementary Figure 5. **c-Kit inactivation in vascular SMC increases cholesterol content in lesions. (A)** Representative Oil Red-O staining of aortic valves from Kit^{SMC} (*Kit*$^{lox66\text{-}71/\,lox66\text{-}71}$ *Myh11-CreER*^{T2} *ApoE*$^{-/-}$) mice after feeding normal chow (VEH) or tamoxifen (TAM) rich diet for 4 weeks, followed by 16 weeks of high fat diet (n=7 per group). **(B)** Quantification of lipid positive area in aortic valves. Data are presented as mean ± S.E.M. and compared using a two-tailed Student's t-test.

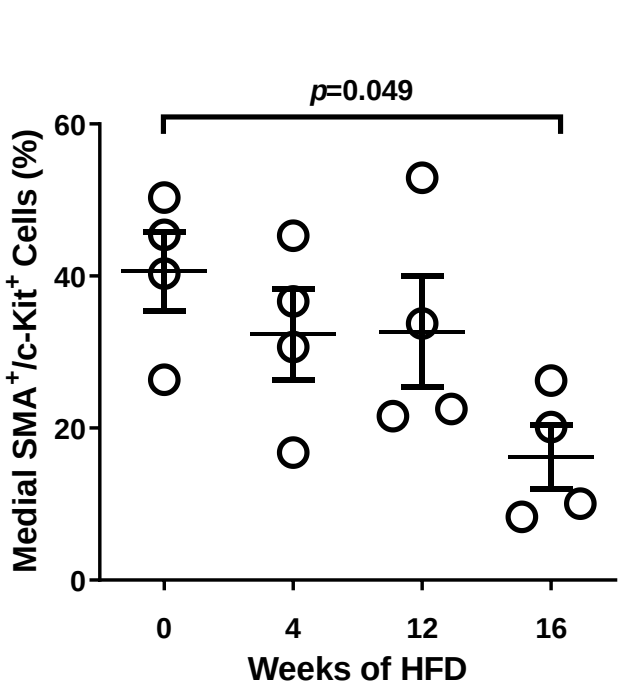


Supplementary Figure 6. Complete images of Oil Red-O stained aortas from tamoxifen-treated Kit^{WT} and Kit^{SMC} mice. Complete images of Oil Red-O stained aortas from Kit^{SMC} (Kit$^{lox66\text{-}71/\ lox66\text{-}71}$ Myh11-CreER^{T2} ApoE$^{-/-}$, n=9) and Kit^{WT} mice (Kit$^{+/+}$ Myh11 CreER^{T2} ApoE$^{-/-}$, n=11) fed tamoxifen-rich chow for 4 weeks, followed by high fat diet for 16 weeks.

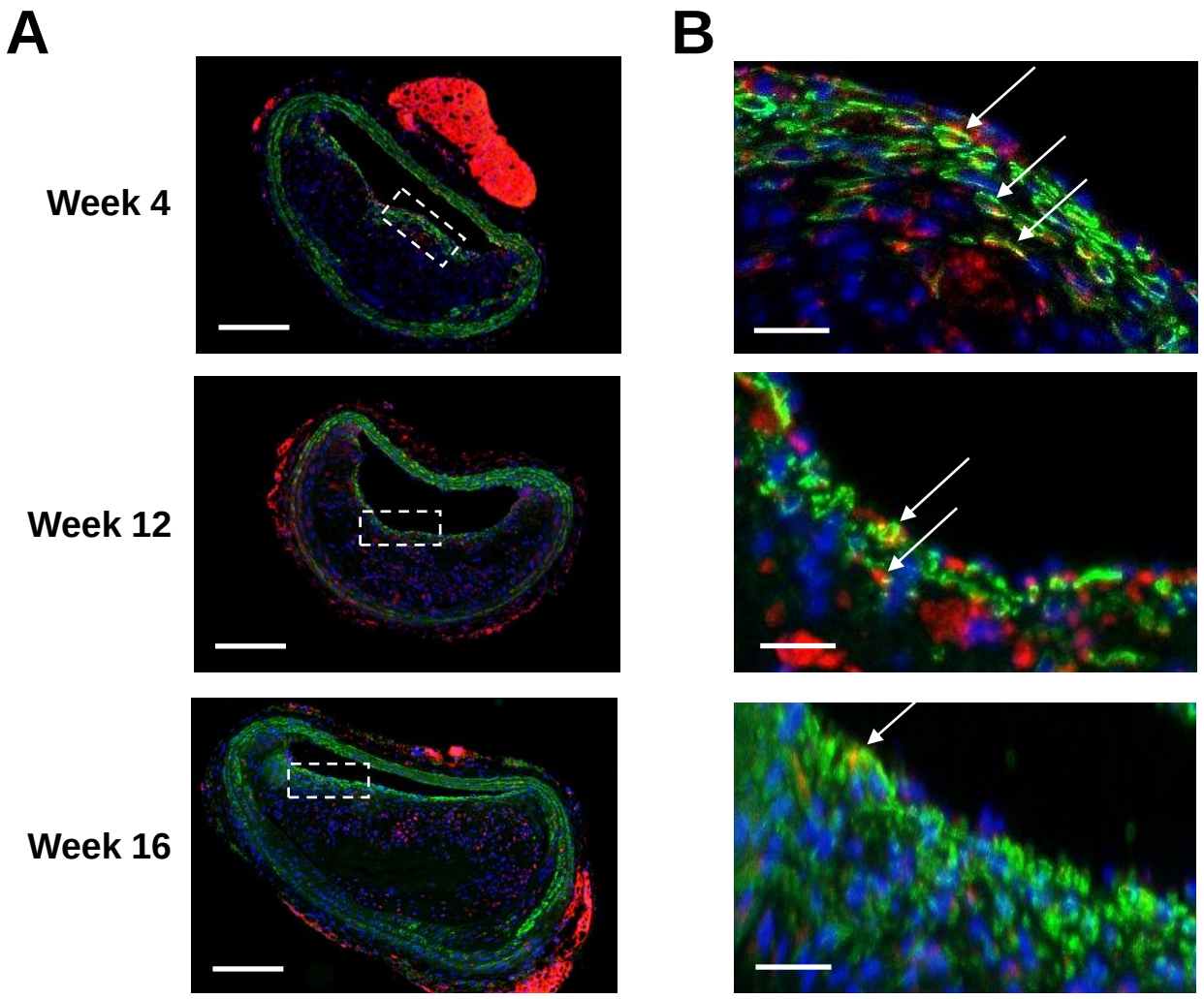


Supplementary Figure 7. c-Kit inactivation in vascular SMC does not modify collagen content in lesions. (A-B) Representative picrosirius red staining of the aortic valve in Kit^{WT} (*Kit$^{+/+}$ Myh11-CreER^{T2} ApoE$^{-/-}$*, n=8) and Kit^{SMC} mice (*Kit$^{66/71\ /\ 66/71}$ Myh11-CreER^{T2} ApoE$^{-/-}$*, n=5) after feeding tamoxifen-rich diet for 4 weeks, followed by 16 weeks of high fat diet. Scale bars=100 µm. **(C-D)** Zoomed images of boxed areas in **A** and **B**, respectively. Scale bars=50 µm. **(E)** Quantification of collagen content as percent of total lesion area. Data are presented as mean ± S.E.M. and compared using a two-tailed Student's t-test.

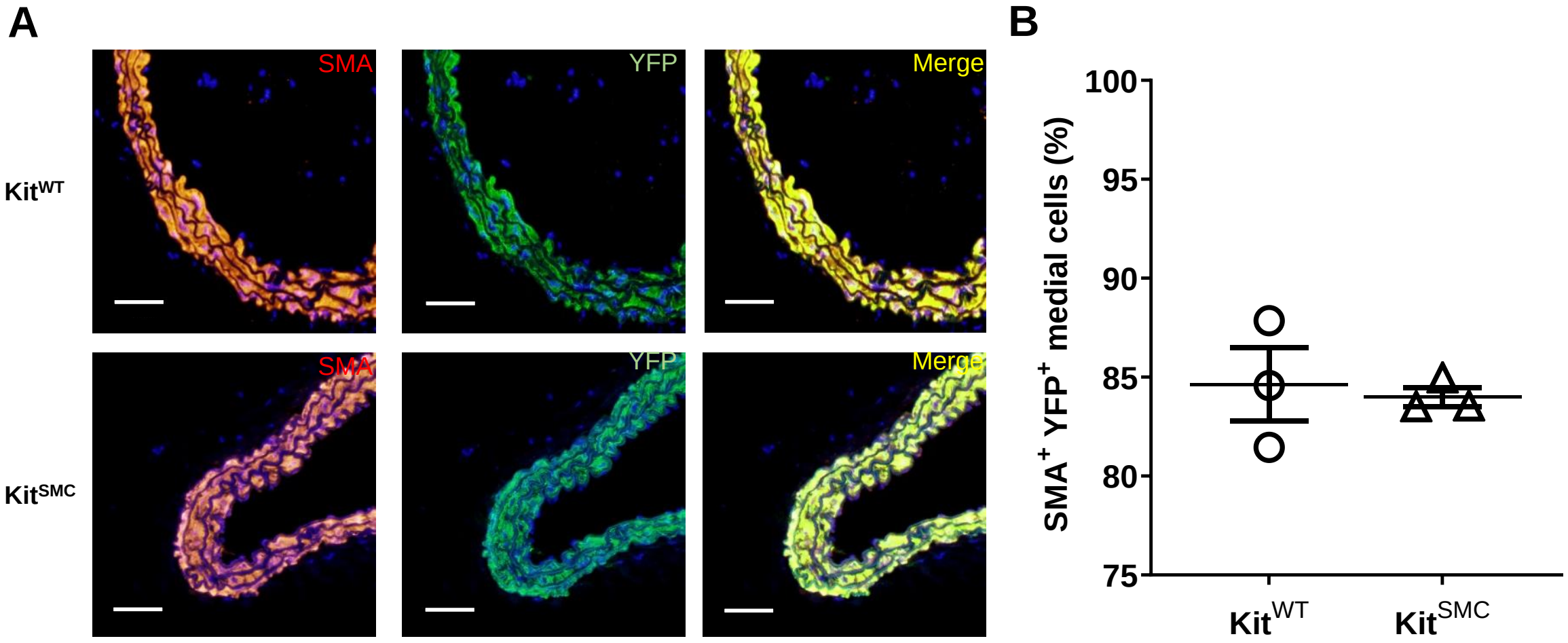




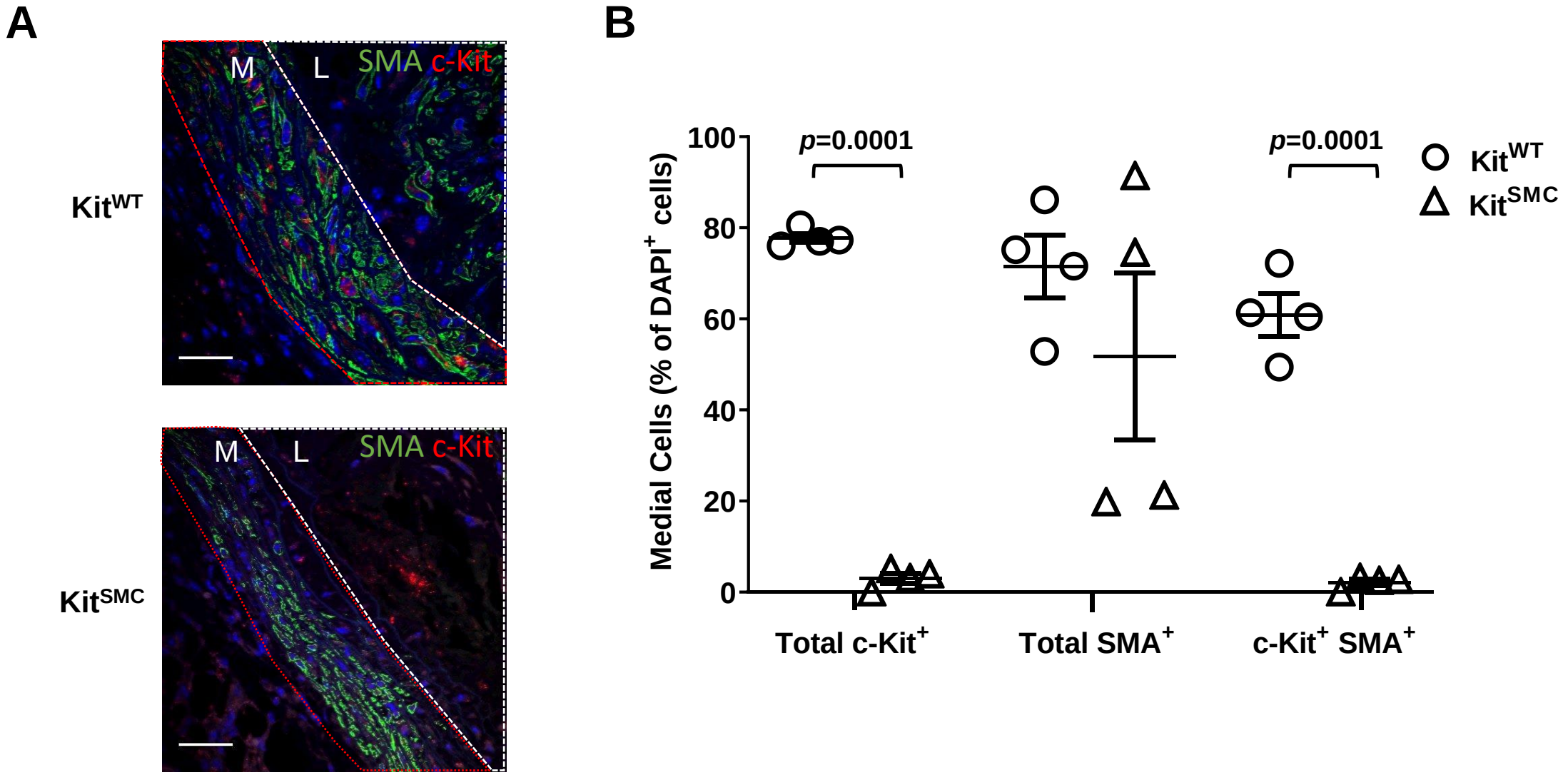
Supplementary Figure 8. c-Kit expression over time in hyperlipidemic mice (A) Representative merged immunofluorescent images of carotid arteries from Kit^{WT} (*Kit*$^{+/+}$ *Myh11-CreER*^{T2} *ApoE*$^{-/-}$) mice after high fat diet for 0, 4, 12, and 16 weeks (n=4 per time point). Scale bars=100 µm. **(B)** Zoomed images of the media in the boxed areas in **A**. Sections were stained for c-Kit (red) and SMA (green). Nuclei were counterstained with DAPI (blue). Scale bars=20 µm. **(C)** Quantification of double positive SMA^+/$c\text{-}Kit^+$ cells in the carotid media as percentage of total $DAPI^+$ cells. Data are presented as mean ± S.E.M. Groups were compared using a one-way ANOVA followed by Tukey's multiple comparisons test.



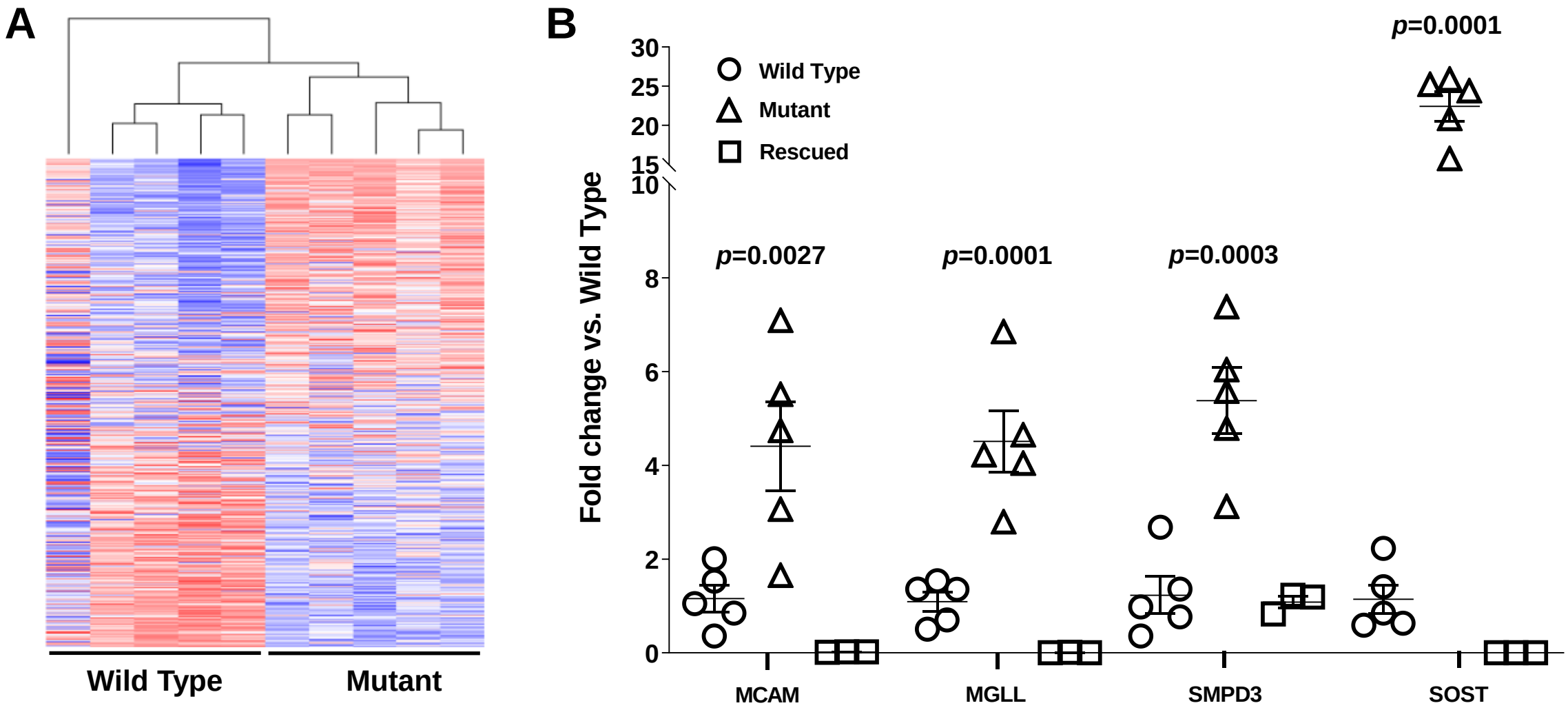
Supplementary Figure 9. c-Kit expression over time in hyperlipidemic mice (A) Representative merged immunofluorescent images of carotid arteries from Kit^{WT} (*$Kit^{+/+}$ Myh11-CreER^{T2} ApoE$^{-/-}$*) mice after high fat diet for 4, 12, and 16 weeks (n=4 per time point). Scale bars=100 µm. **(B)** Zoomed images of the lesion caps in the boxed areas in **A**. Sections were stained for c-Kit (red) and SMA (green). Nuclei were counterstained with DAPI (blue). Scale bars=20 µm. **(C)** Quantification of double positive $SMA^+/c\text{-}Kit^+$ cells in the carotid plaque cap as percentage of total $DAPI^+$ cells. Data are presented as mean ± S.E.M. Groups were compared using a one-way ANOVA followed by Tukey's multiple comparisons test.



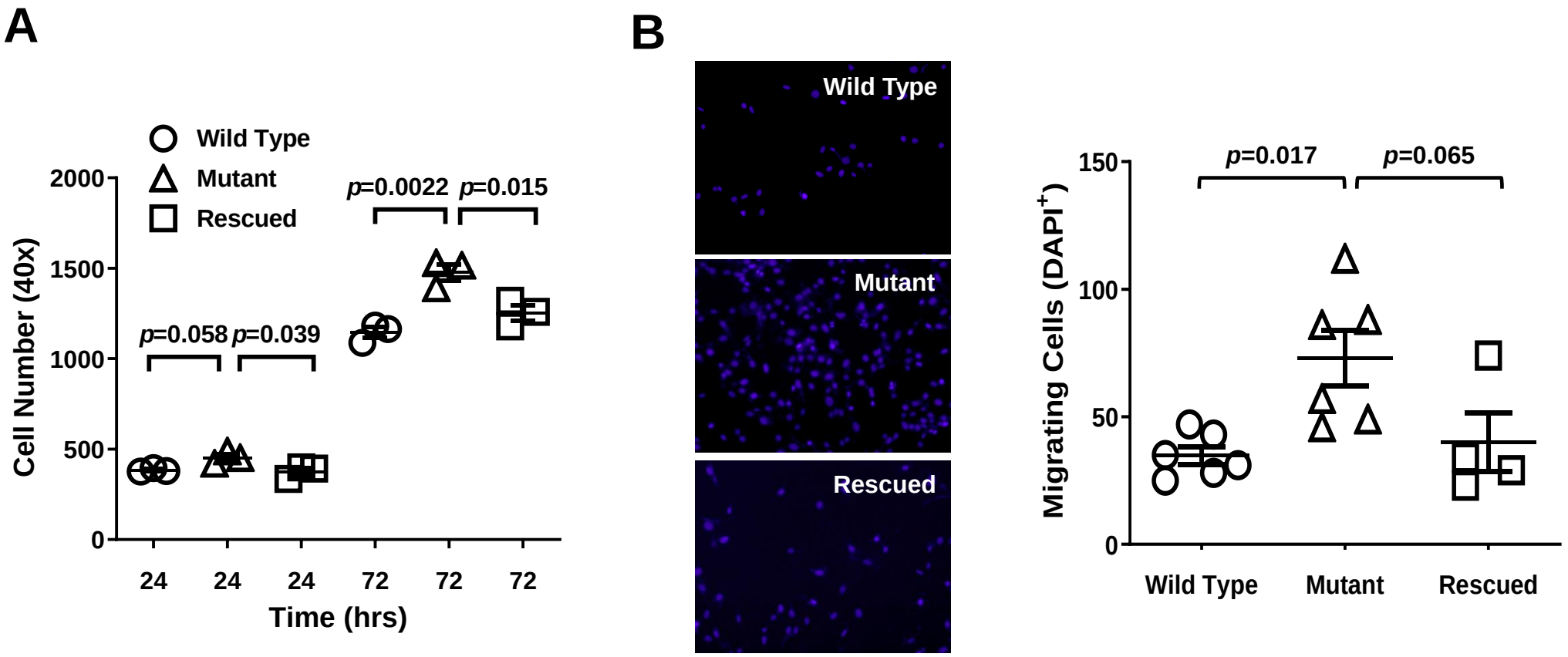
Supplementary Figure 10. No significant differences in SMA$^+$ YFP$^+$ expression in Kit^{SMC} eYFP and Kit^{WT} eYFP transgenic mice. (A) Representative immunofluorescent images of carotid arteries from Kit^{WT} (Kit$^{+/+}$ Myh11-CreER^{T2} eYFP ApoE$^{-/-}$, n=3) and Kit^{SMC} (Kit$^{lox66\text{-}71/\ lox66\text{-}71}$ Myh11-CreER^{T2} eYFP ApoE$^{-/-}$, n=3) mice after feeding tamoxifen-rich diet for 4 week. Sections were stained for SMA (red) and YFP (green). Nuclei were counterstained with DAPI (blue). **(B)** Quantification of medial SMA$^+$ YFP$^+$ double positive cells as percentage of DAPI$^+$ cells in both experimental groups. Data are presented as mean ± S.E.M. and compared using a two-tailed Student's t-test.



Supplementary Figure 11. c-Kit expression in the media of tamoxifen-treated Kit^{SMC} eYFP and Kit^{WT} eYFP mice. (A) Representative merged immunofluorescent images of the aortic sinus media from Kit^{WT} (Kit$^{+/+}$ Myh11-CreER^{T2} eYFP ApoE$^{-/-}$, n=4) and Kit^{SMC} (Kit$^{lox66\text{-}71/\ lox66\text{-}71}$ Myh11-CreER^{T2} eYFP ApoE$^{-/-}$, n=4) mice after tamoxifen-rich diet for 4 weeks, followed by high fat diet for 16 weeks. Sections were stained for c-Kit (red) and SMA (green). Nuclei were counterstained with DAPI (blue). The media (M) is outlined in red, while the lesion (L) is outlined in white. **(B)** Quantification of cell populations in the aortic sinus media as percentage of total DAPI^{+} cells in both experimental groups. Data are presented as mean ± S.E.M. Groups were compared using a two-tailed Student's t-test.



Supplemental Figure 12. Loss of c-Kit activity in primary SMC upregulates genes associated with foam cell formation. (A) Heatmap of 12,891 differentially expressed genes (FDR<0.05) in c-Kit deficient (mutant [Kit^{Mut}], n=5) vs. wild type (n=5) primary SMC as detected by bulk RNA sequencing. Upregulated genes are in red while downregulated genes are in blue. **(B)** qRT-PCR of select differentially expressed genes associated with SMC migration and lipid metabolism in wild type, mutant (Kit^{Mut}), and rescued Kit^{Mut} cells after transduction with a c-Kit expressing lentivirus. c-Kit expression was normalized using GAPDH levels. Data are presented as mean fold change ± S.E.M vs. wild type and compared using a one-way ANOVA followed by Tukey's multiple comparisons test.



Supplementary Figure 13. c-Kit deficiency increases proliferation and migration of primary SMC. (A) Wild type and c-Kit deficient (Kit^{Mut}) SMCs were seeded in 6-well plates. After synchronization with serum deprived medium overnight, cells were trypsinized and counted consecutively for 3 days. Results represent three independent experiments using primary cells from different wild type and Kit^{Mut} mice. **(B)** Representative images of Boyden chamber SMC migration of wild type, c-Kit mutant (Kit^{Mut}) and rescued SMC after exposure to PDGFB stimulation, along with the quantification of number of migrating cells. Each point represents independent experiments. Data are presented as mean ± S.E.M. Groups were compared using a one-way ANOVA followed by Tukey's multiple comparisons test.