Supplementary Material

KLF4 Regulates Abdominal Aortic Aneurysm Morphology and Deletion Attenuates Aortic Aneurysm Formation

Morgan Salmon[1], Ph.D.; William F. Johnston[1], M.D.; Andrew Woo[1], M.D.; Nicolas H. Pope, M.D.[1]; Gang Su[1], M.D.; Gilbert R. Upchurch[1,2], M.D.; Gary K. Owens[2], Ph.D; and Gorav Ailawadi[1,2,3], M.D.

[1]Department of Surgery, University of Virginia School of Medicine, Charlottesville, Virginia, USA
[2]The Robert M. Berne Cardiovascular Research Center, University of Virginia School of Medicine, Charlottesville, Virginia, USA.

[3]Address correspondence to: Gorav Ailawadi, University of Virginia, Department of Surgery, PO Box 800679, Charlottesville, Virginia 22908-1394, USA
Phone: (434)924-5052, Fax: (434) 244-7588; E-mail:gorav@virginia.edu

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Categories: Vascular Biology, Basic Science Research, CV surgery: aortic and vascular disease

Supplemental Figures

Supplemental Figure 1. KLF4 protein quantification and z-stacking confocal. A) Quantitation of immunohistochemical staining of Figure 1B. **B)** Confocal immunofluorescence staining of WT elastase perfused aortas 7 days following injury. White boxes show regions of overlap between KLF4 and SM-actin while circle indicate regions of overlap between KLF4 and Mac2. **C)** Z-stacking confocal immunofluorescence staining of WT aortas as mentioned in Supplemental Figure 1B.

Supplemental Figure 2. Heterozygous deletion of KLF4 results in attenuated aneurysm formation A) Immunohistochemistry was performed on ERTCre+/- KLF4 flx/wt mice and sample images are shown. **B**) Staining for neutrophils is shown in brown using an anti-neutrophil antibody, MMP2 stain is shown in brown using an anti-MMP2 antibody. Quantitation of immunohistochemistry from part A. * indicates p-value <0.05.

Supplemental Figure 3. Staining for KLF4 in the smooth muscle specific knock-out mouse model. A-C) Immunohistochemical and confocal staining for KLF4 (red), SM a-actin(green) and DAPI(blue)

Supplemental Figure 4. Smooth muscle specific knock-out of KLF4 results in attenuated aneurysm formation. A) Pictoral examples of dilated aortas 14 days following elastase perfusion. B). Neutrophil staining was visualized using an anti-neutrophil antibody, MMP2 staining was visualized using an antibody to anti-MMP2. Quantitation of staining from part B. * indicates significant staining over controls.

Supplemental Figure 5: KLF4 deletion results in decreased MCP1 staining. A and **B**) Immunohistochemical staining of MCP-1 in ERTCre+ KLF4 flx/wt, ERTCre- KLF4 flx/wt, and MYHCre+ KLF4 flx/flx, flx/wt and wt/wt mice using an anti-MCP1 antibody.

Supplemental figure 6. Knock-down of KLF4 modulates smooth muscle marker genes following IL1β treatment. Mouse abdominal aortic smooth muscle cells were plated and. 24 hours following

transfection cells were treated with IL1β for 24 hours and then harvested and RNA extracted. * indicates significant expression over control treated siRNA. Results are the average of three independent experiments performed in triplicate.

Supplemental Figure 7. Over-expression of KLF4 modulates smooth muscle marker genes with IL1β treatment. Mouse abdominal aortic smooth muscle cells were plated and infected with control or KLF4 adenovirus. 24 hours following transfection cells were treated with IL1β for 24 hours and then harvested and RNA extracted. * indicates significant expression over control treated siRNA. Results are the average of three independent experiments performed in triplicate.

Supplemental Figure 8. Smooth Muscle Marker gene expression following retinoic acid treatment. Mouse abdominal aortic smooth muscle cells were plated and treated with siKLF4. 24 hours following transfection cells were treated with Retinoic Acid for 24 hours and then harvested and RNA extracted. * indicates significant expression over control treated siRNA. Results are the average of three independent experiments performed in triplicate.

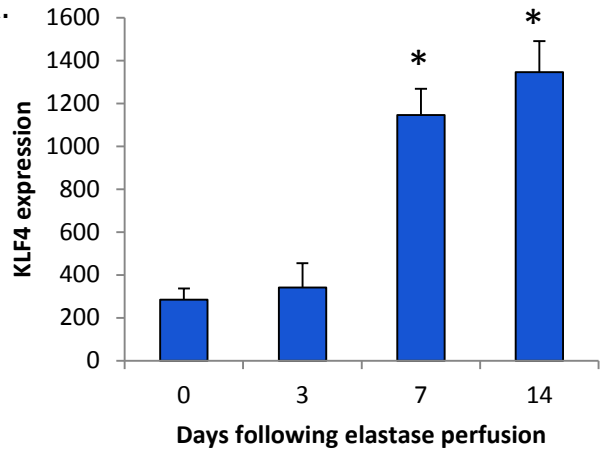
Supplemental Figure 9. Smooth Muscle Marker gene expression following TGFβ treatment. Mouse abdominal aortic smooth muscle cells were plated and treated with siKLF4. 24 hours following transfection cells were treated with TGFbeta for 24 hours and then harvested and RNA extracted. * indicates significant expression over control treated siRNA. Results are the average of three independent experiments performed in triplicate.

Supplemental Figure 10. Smooth Muscle Marker gene expression following phorbol ester treatment. Mouse abdominal aortic smooth muscle cells were plated and treated with siKLF4. 24 hours following transfection cells were treated with phorbol ester for 24 hours and then harvested and RNA extracted. * indicates significant expression over control treated siRNA. Results are the average of three independent experiments performed in triplicate.

Supplement Figure 11: KLF4 bind smooth muscle cell marker genes in vitro following elastin degradation product treatment. A-C) Aortic smooth muscle cells were plated and treated as mentioned previously with elastin degradation productions for 24 hours following serum starvation. ChIP assays were performed for KLF4 and then qPCRs were run priming for SM actin, SM22, and SM-MHC promoter. * indicates significant binding over controls. Results were performed three times in triplicate.

Supplemental Figure 12. KLF4 is protective against angiotensin II aneurysm formation A) Model Depicting the process of tamoxifen injections followed by Angiotensin II treatment for the ERTCre +/- KLF4 Flx/wt ApoE-/- mice. Kaplan-meier curves of ERTCre+/- KLF4 ApoE-/- flx/wt mice following Angiotensin II treatment. P-values indicate significant survival over WT controls. **B**) Model depicting the process of tamoxifen injections and angiotensin II treatment of MYHCre+ KLF4. Kaplan-meier curves depicting percent survival free from aneurysm rupture following Angiotensin II treatment. P-values indicate significant survival over WT controls.

A.



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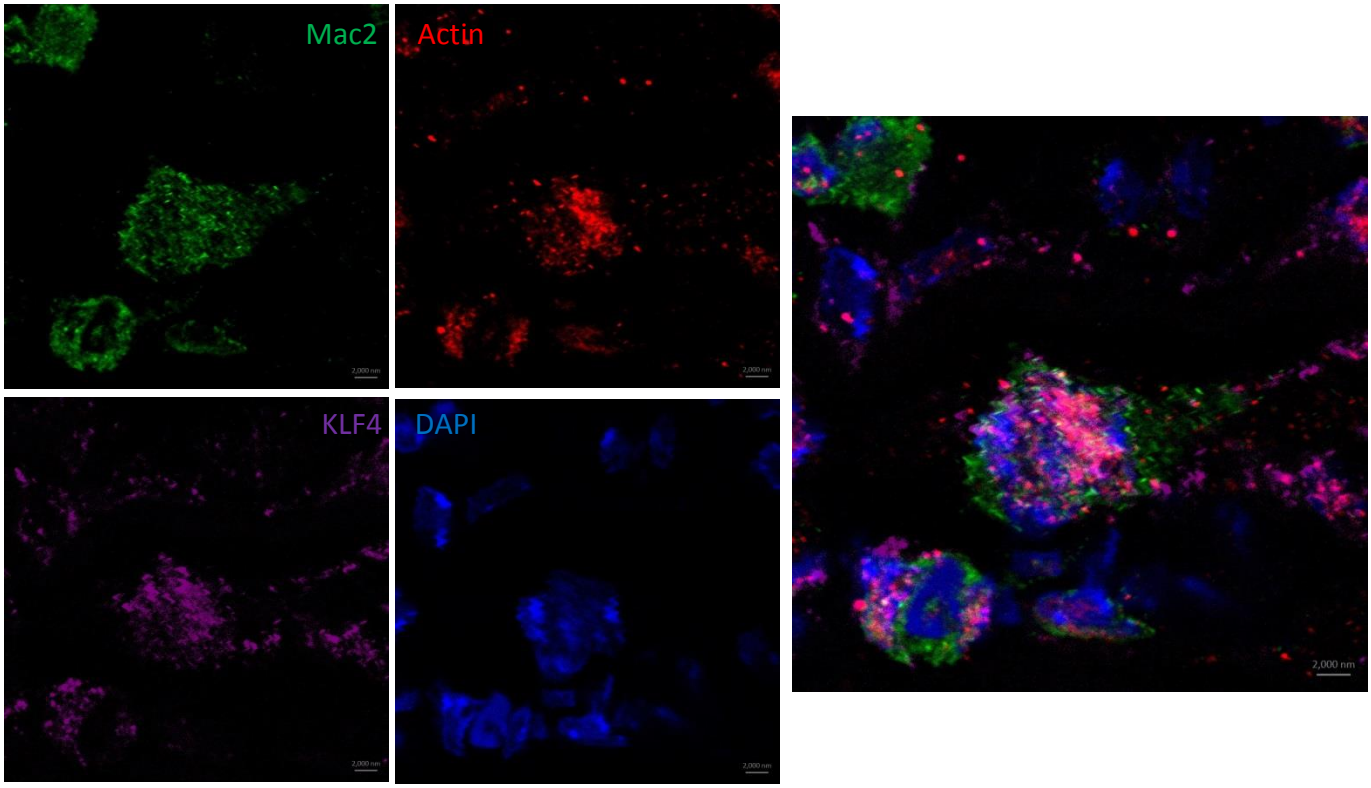
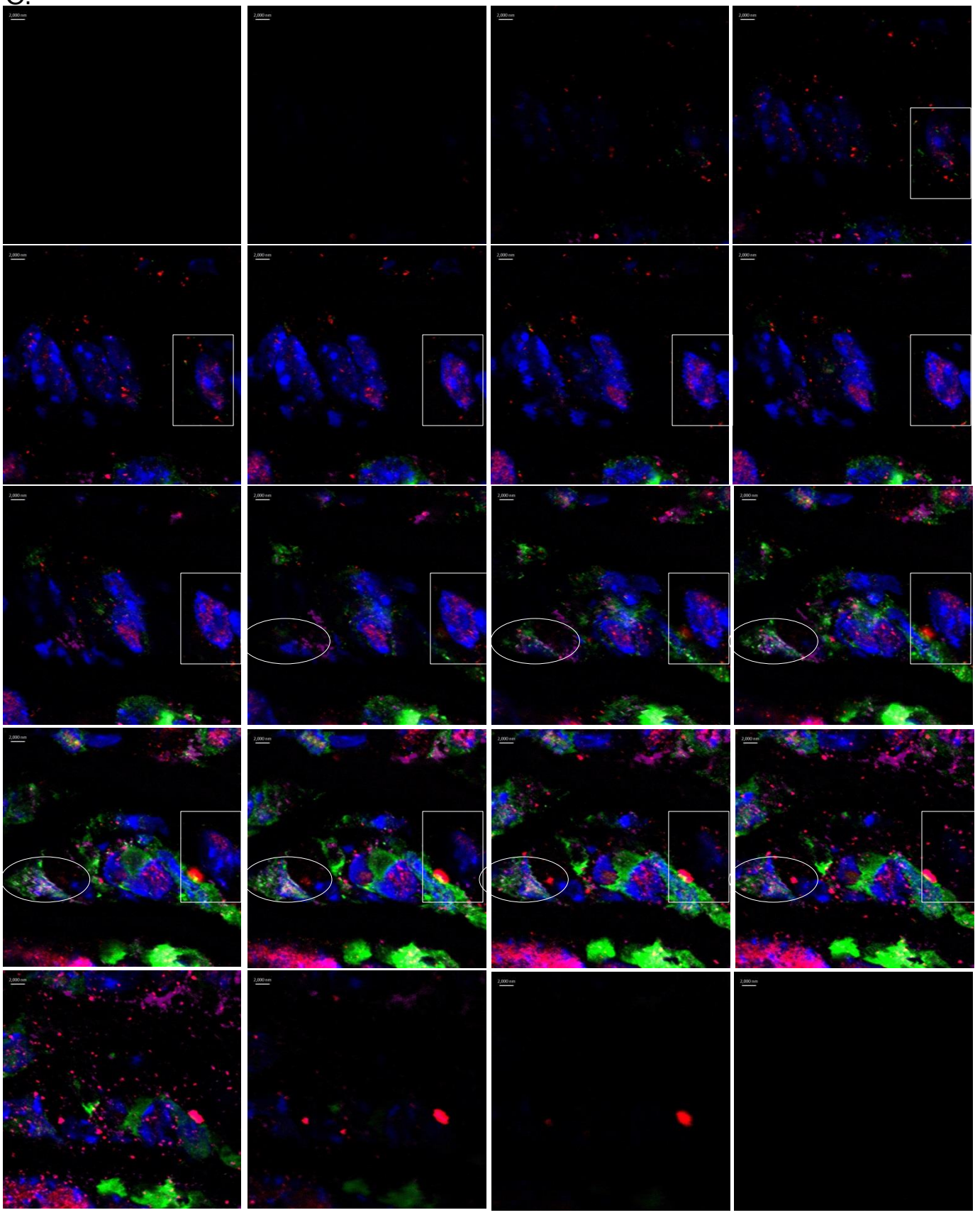
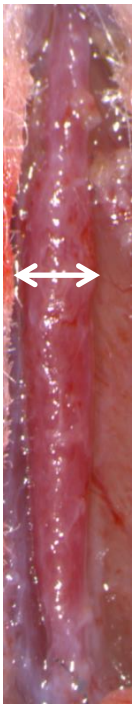


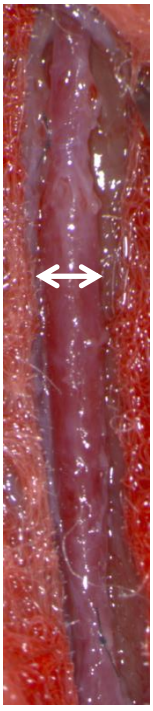
Supplementary Figure 1

C.



A. ERT Cre KLF4 heterozygous knockouts





ERT Cre- KFL4 flx/wt (Control)

ERT Cre+ KFL4 flx/wt

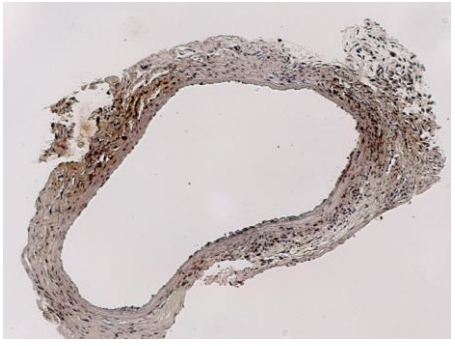
B.

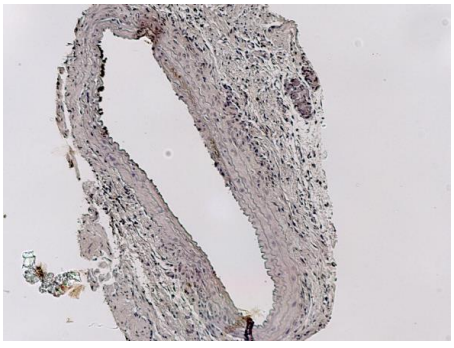
ERTCre- KFL4 flx/wt

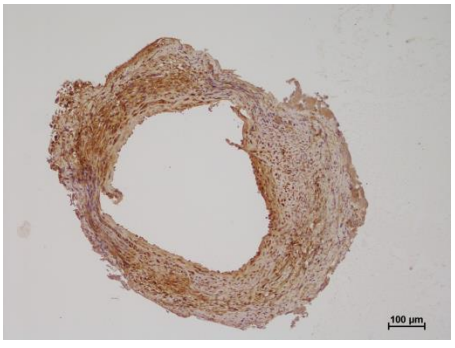
ERTCre+ KFL4 flx/wt

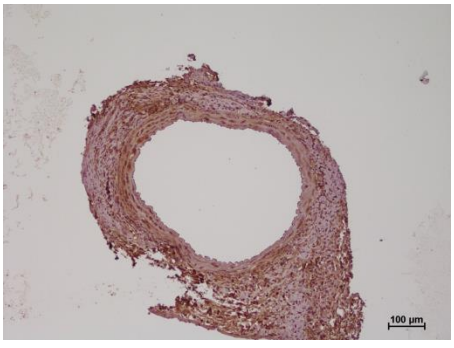
Neutrophil

MMP2

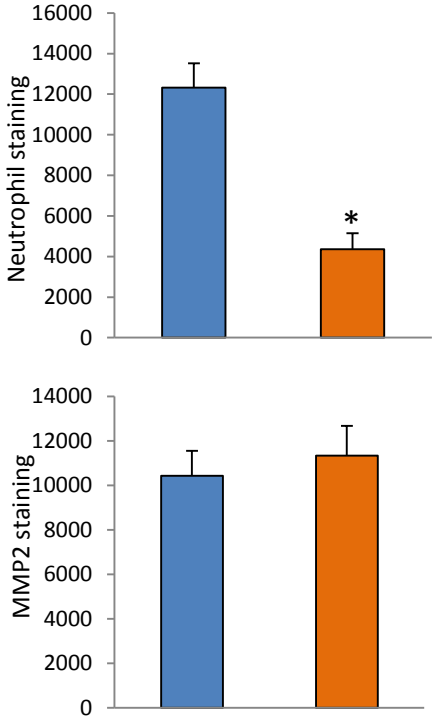




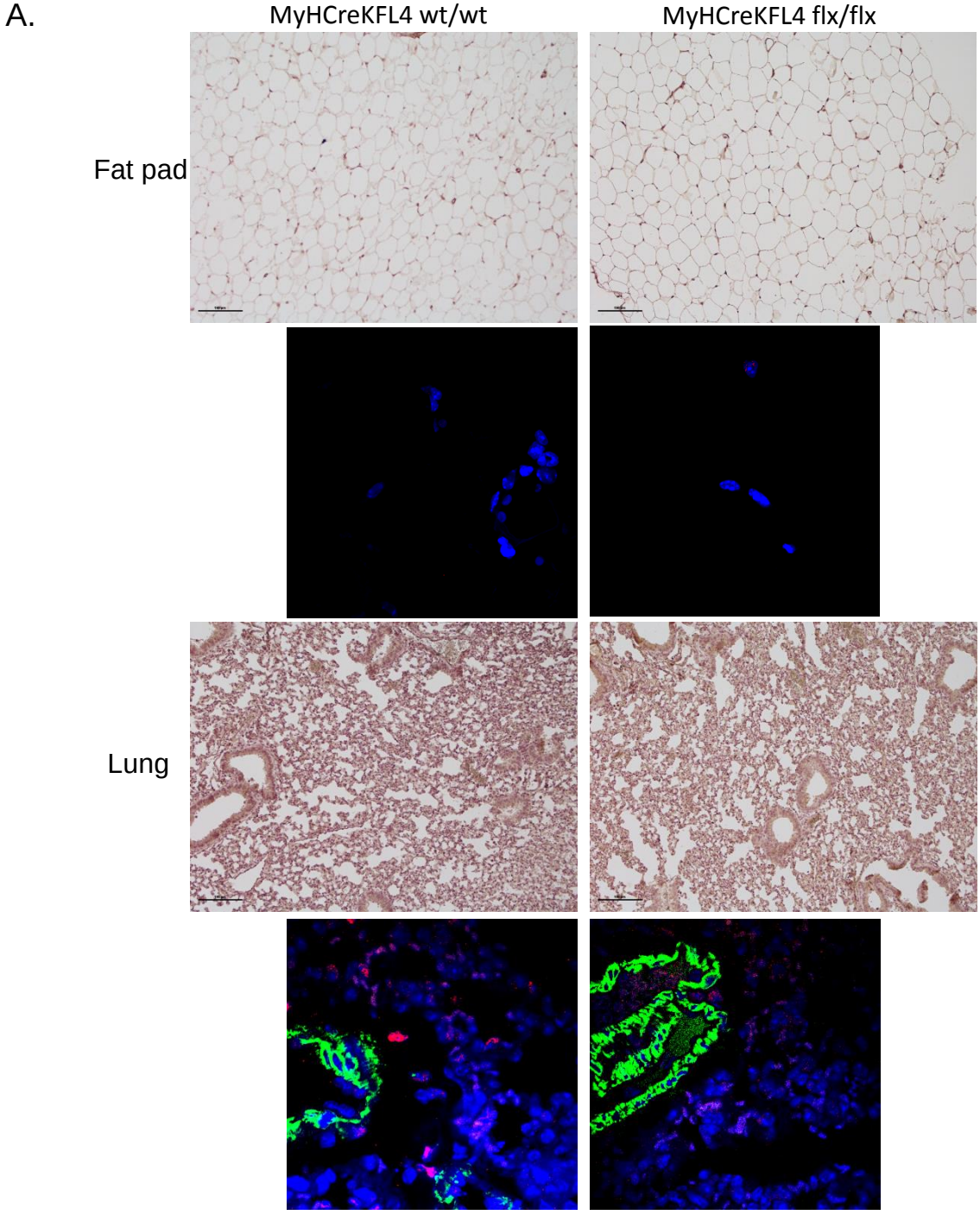




Histologic Grading

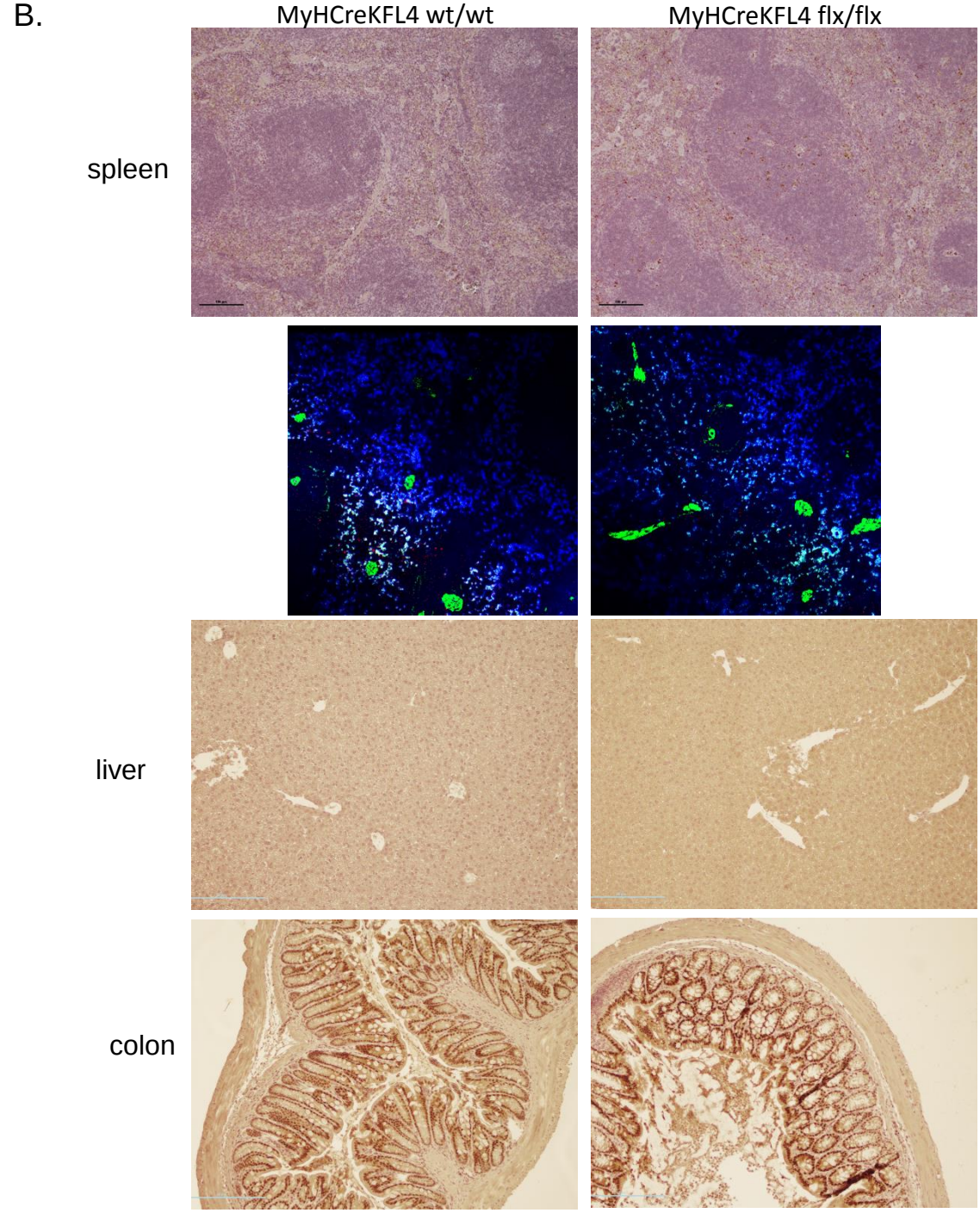


Supplementary Figure 2



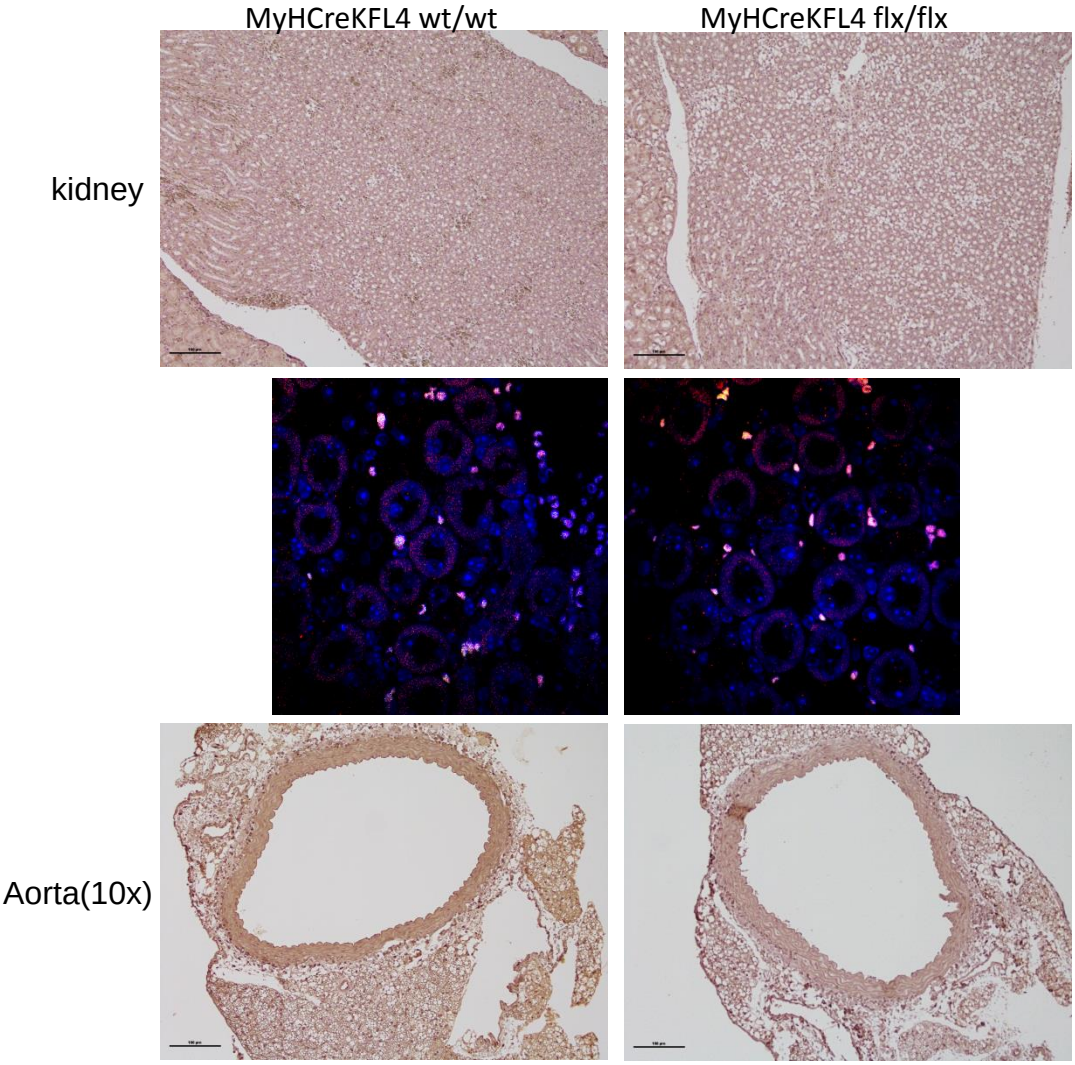
Supplementary Figure 3

B.



Supplementary Figure 3

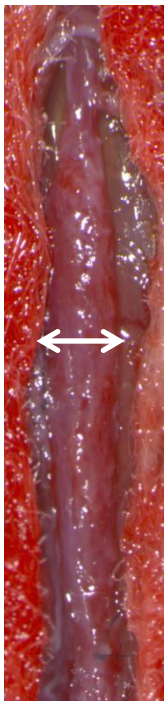
C.



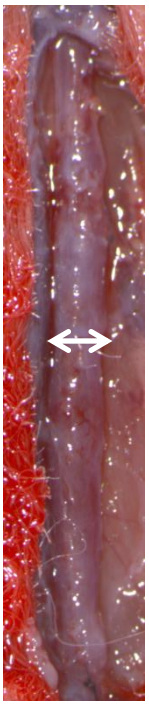
Supplementary Figure 3

A.

MyH Cre KLF4 knockouts



MyHCre+ KFL4 wt/wt



MyHCre+ KFL4 flx/wt

MyHCre+ KFL4 flx/flx

B.

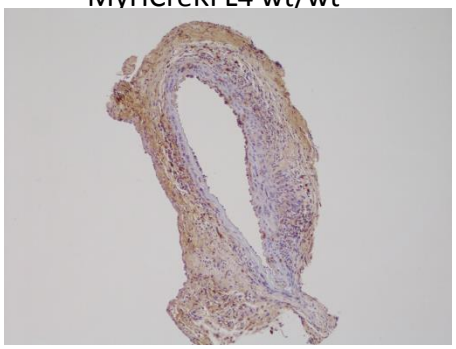
(Control)

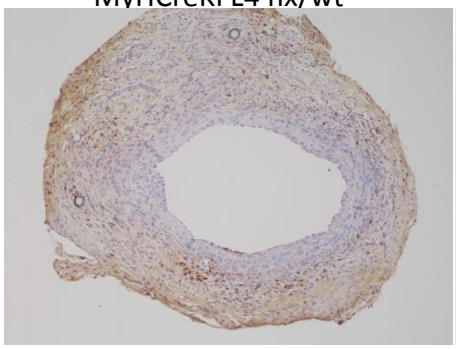
MyHCreKFL4 wt/wt

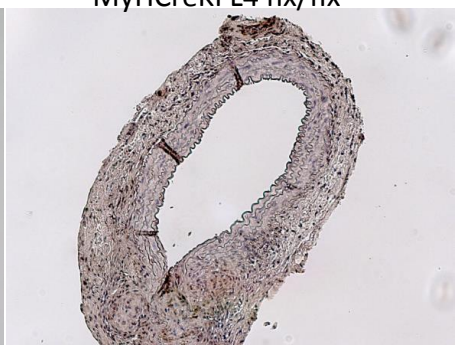
MyHCreKFL4 flx/wt

MyHCreKFL4 flx/flx

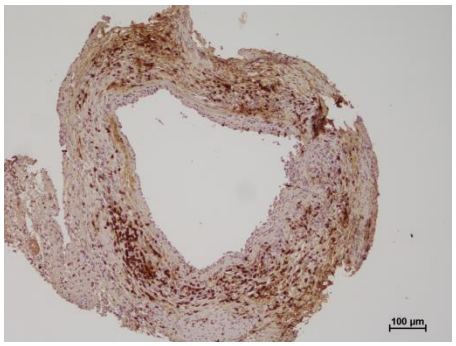
Neutrophil

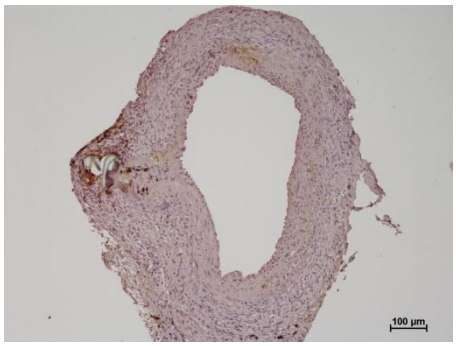


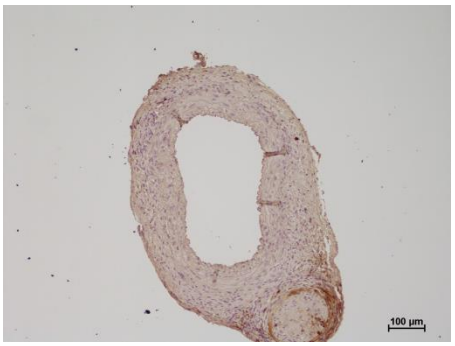




MMP2







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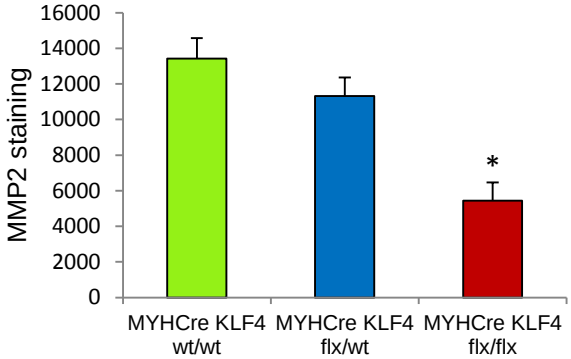
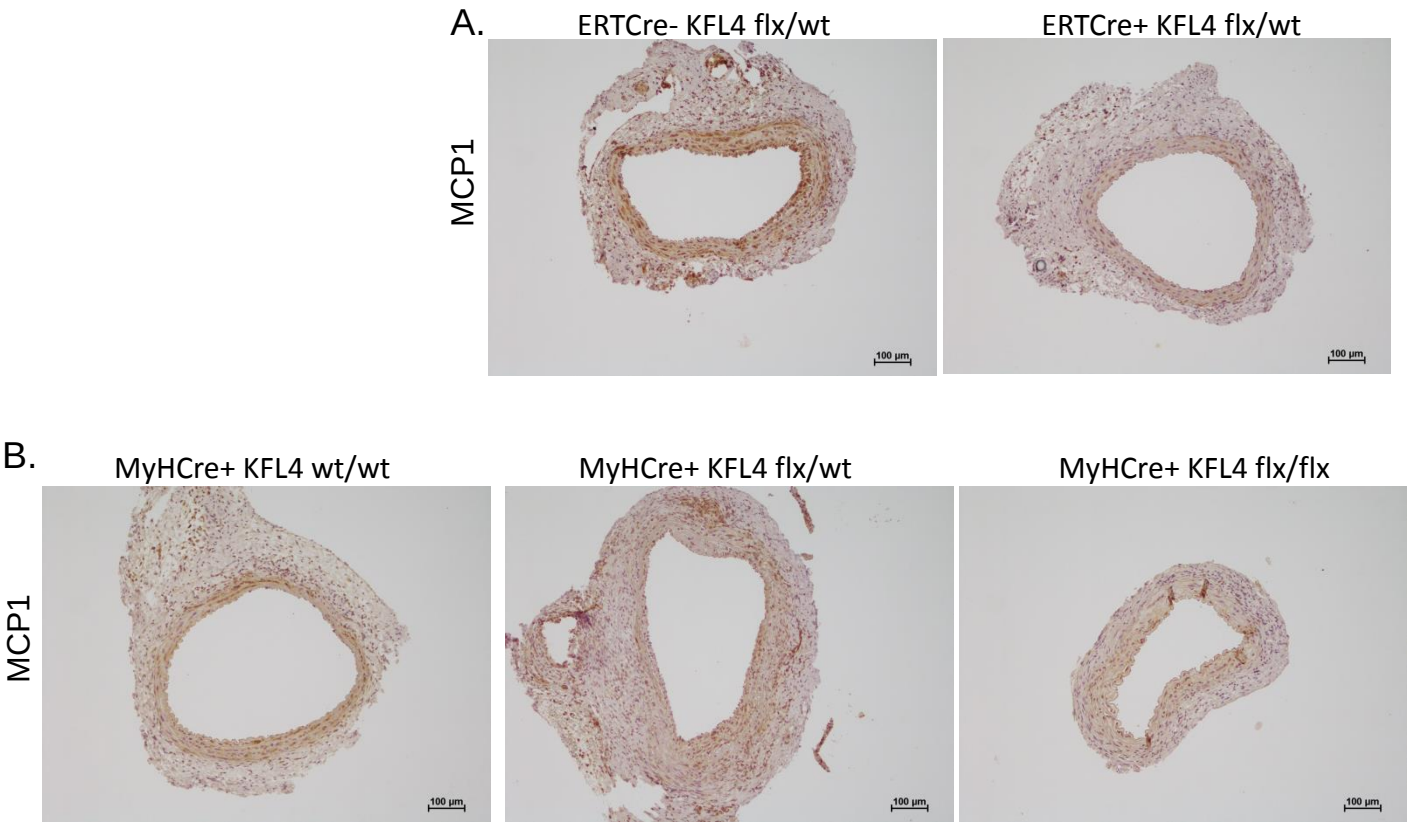
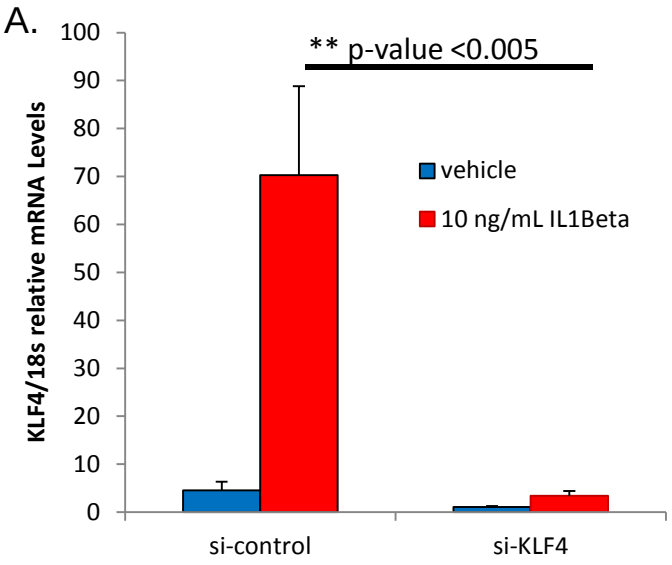
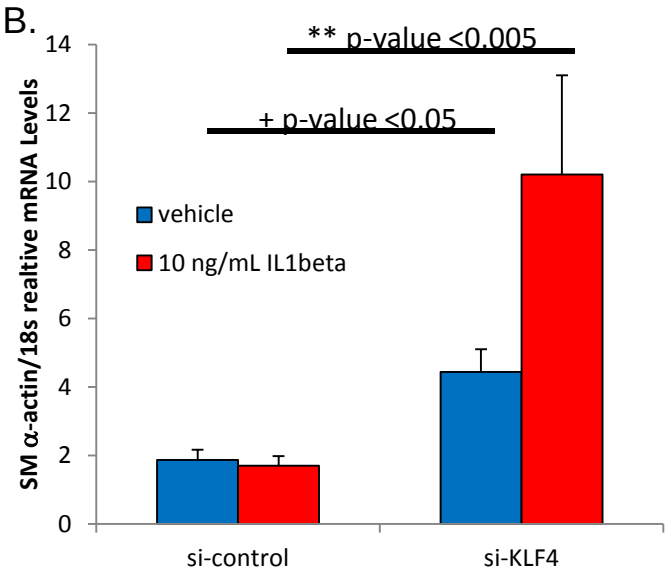


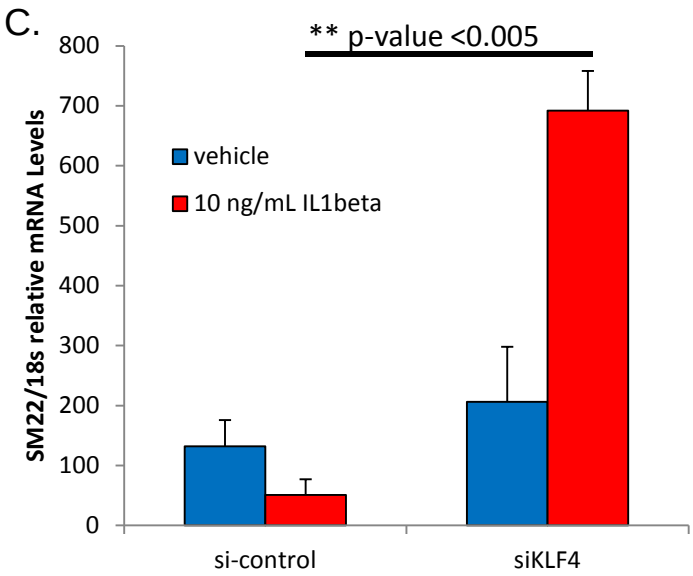
Supplementary Figure 4

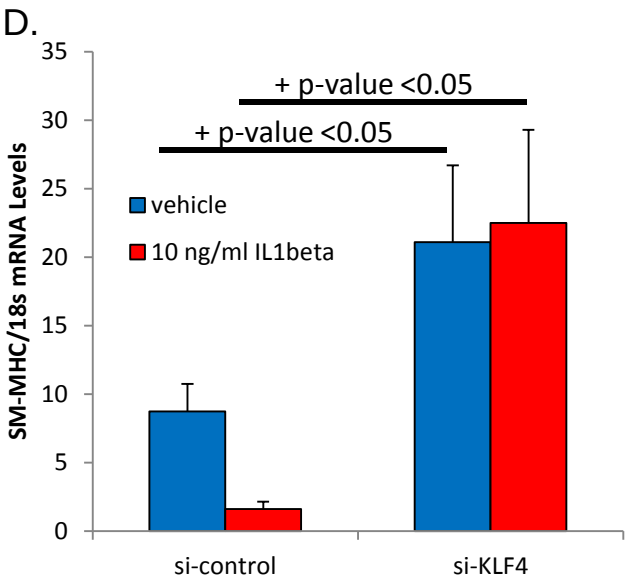


Supplementary Figure 5

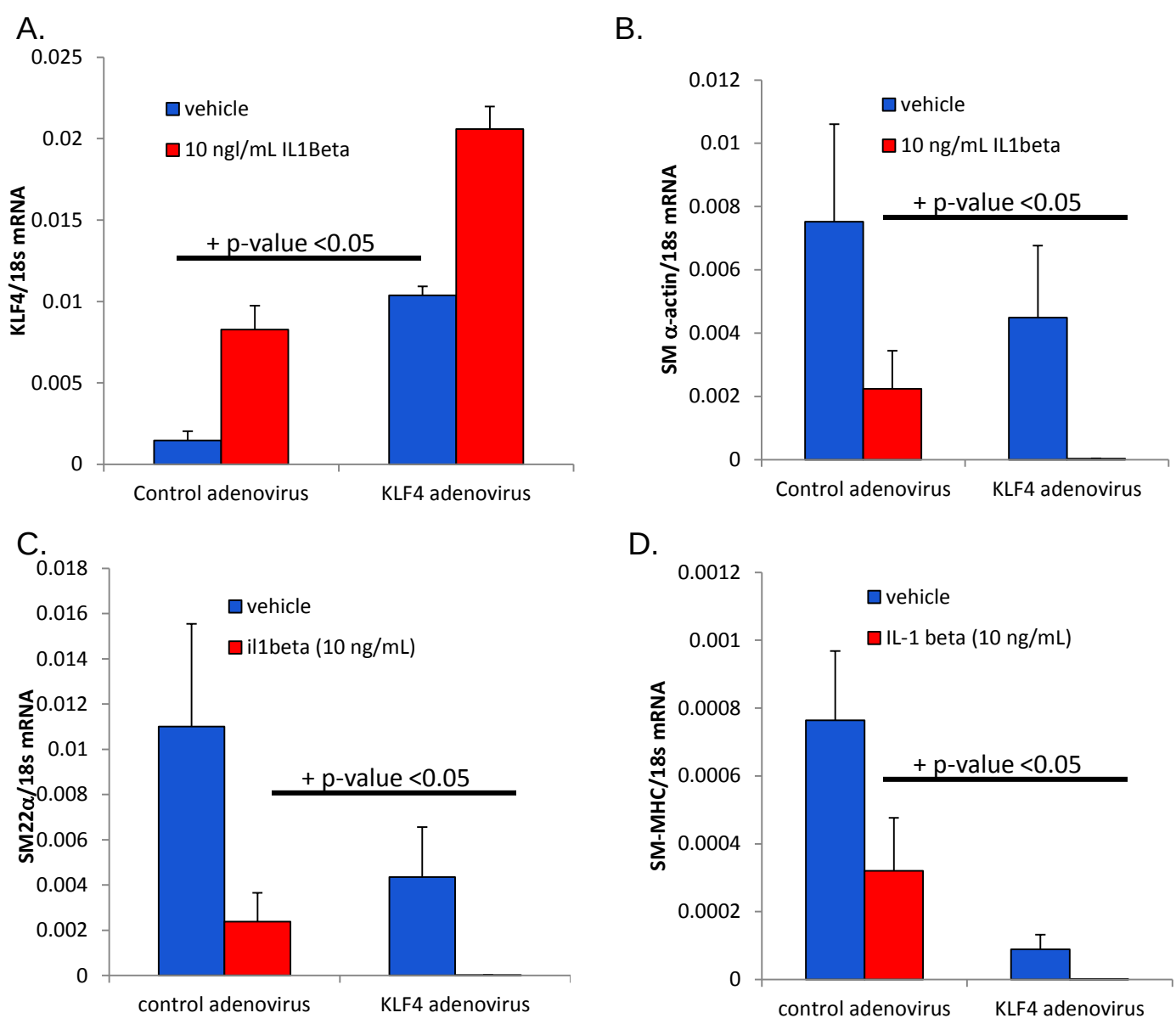




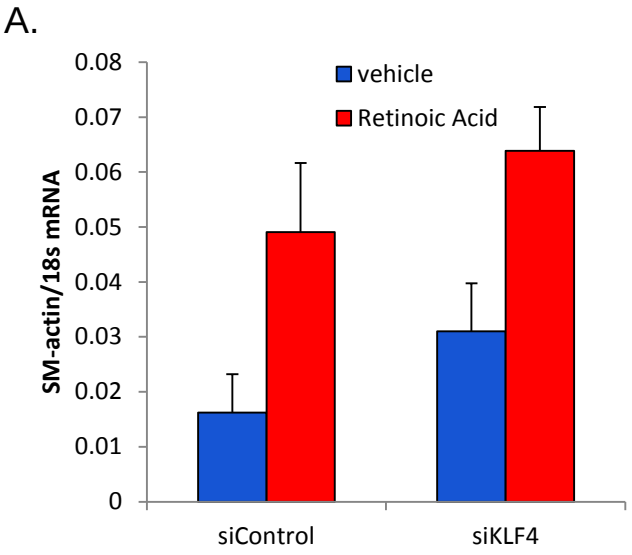


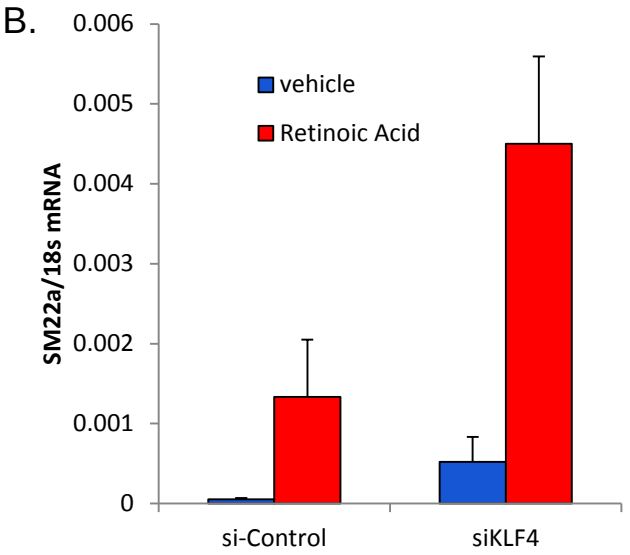


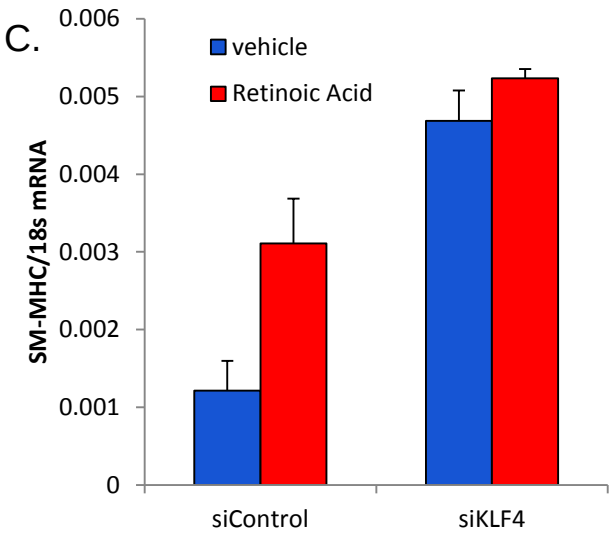
Supplementary Figure 6



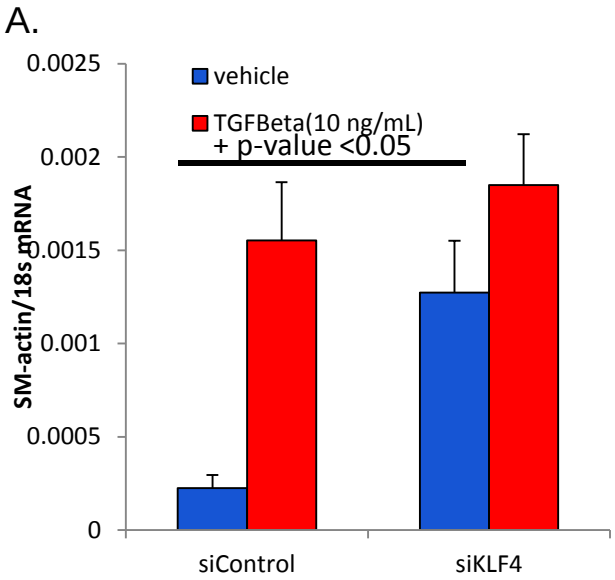
Supplementary Figure 7

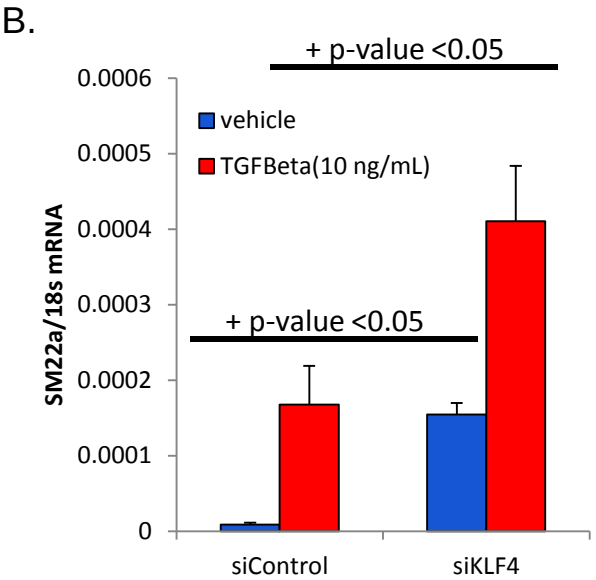


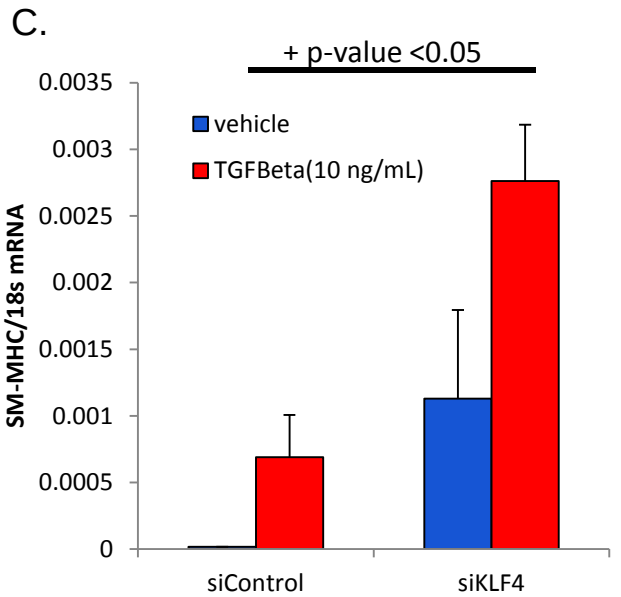




Supplementary Figure 8

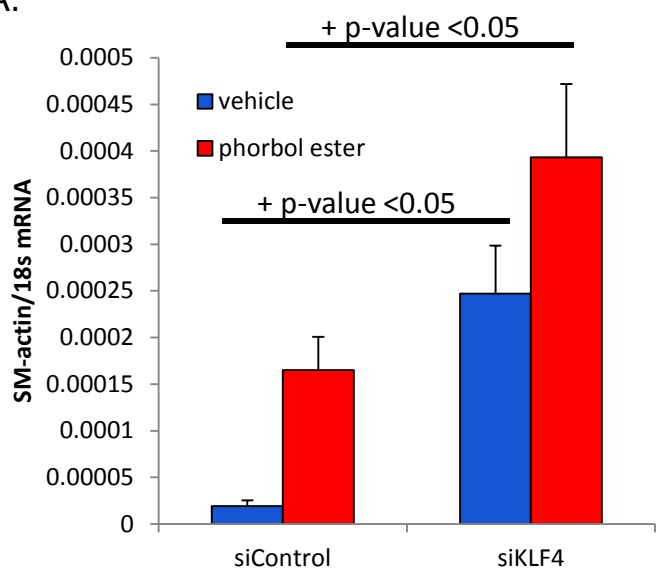






Supplementary Figure 9

A.



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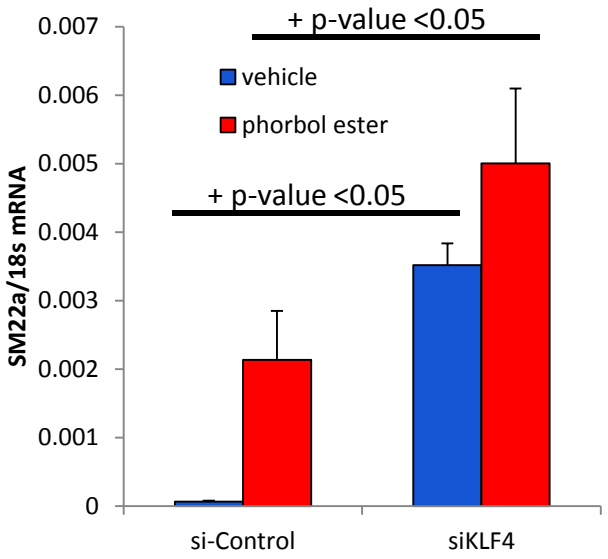

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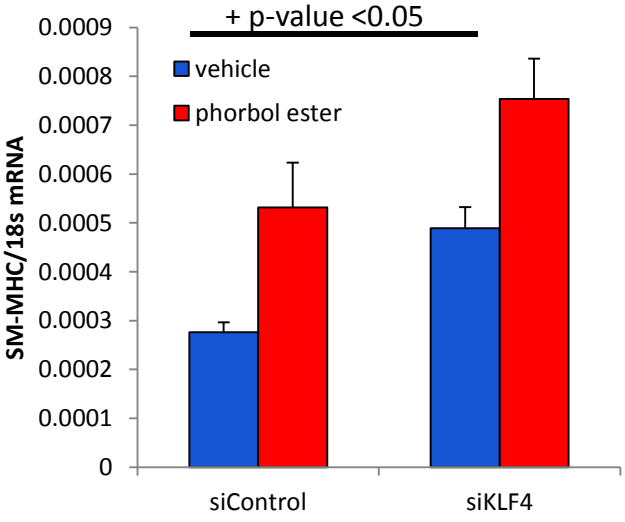
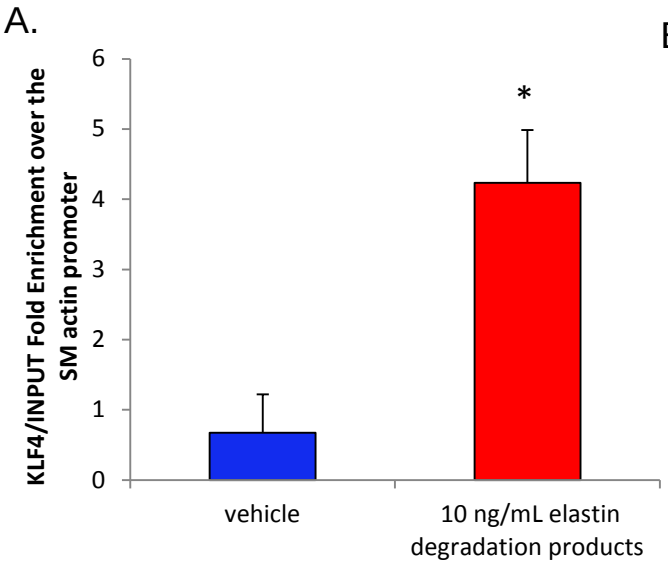
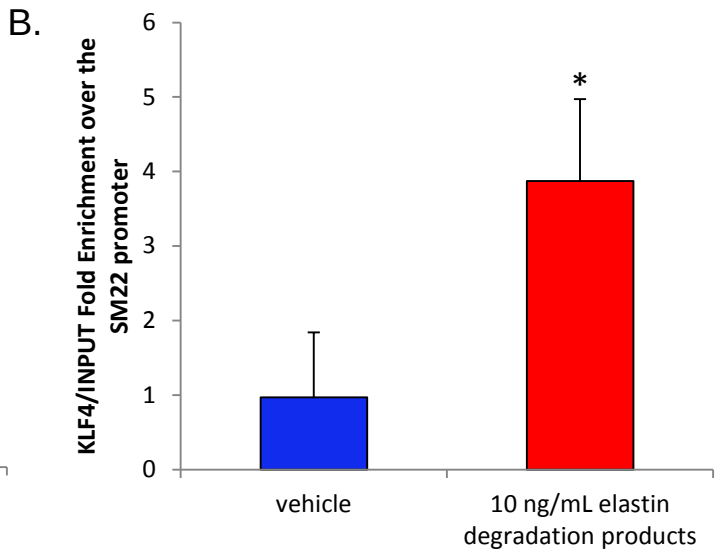
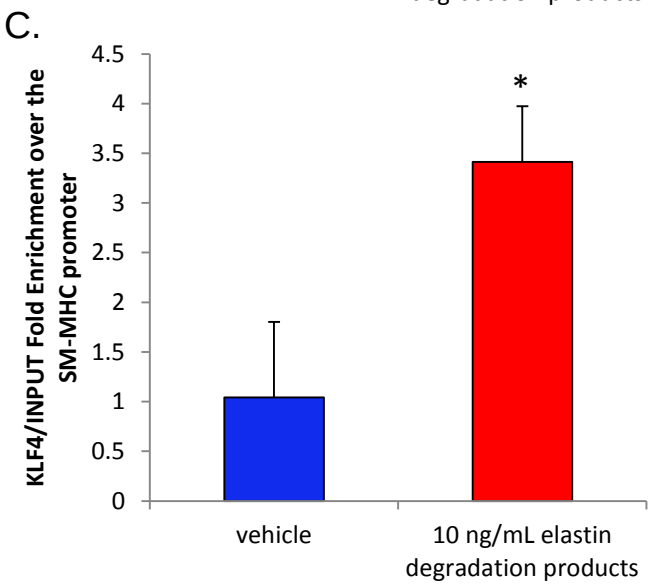


Supplementary Figure 10

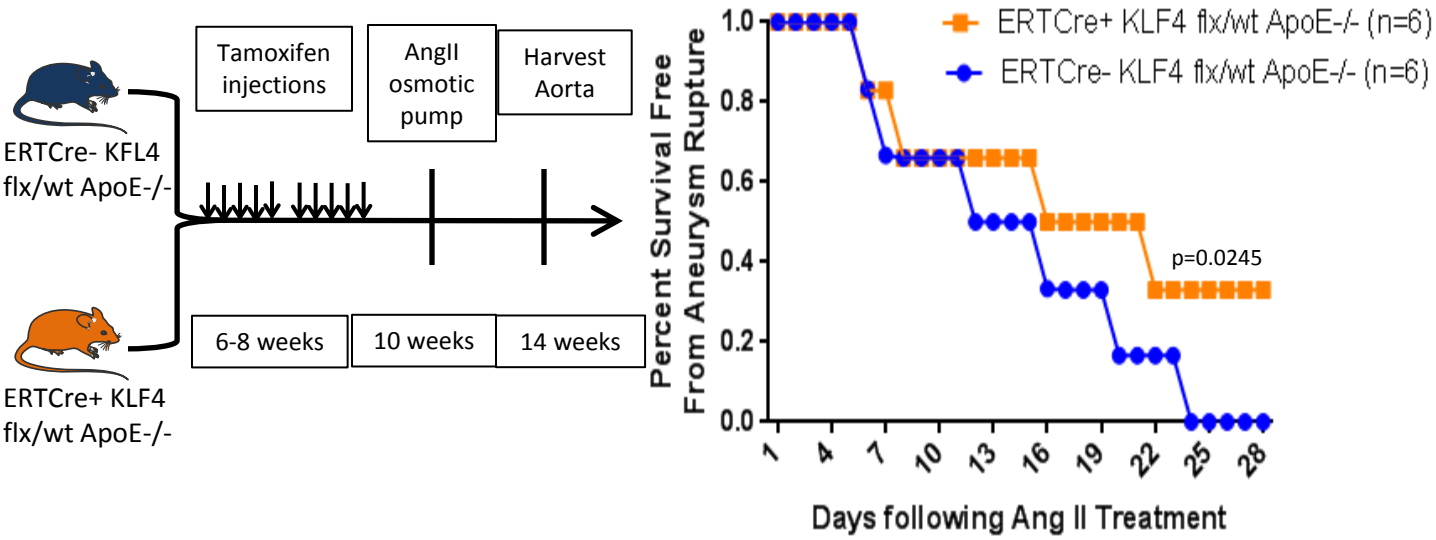






Supplementary Figure 11

A.



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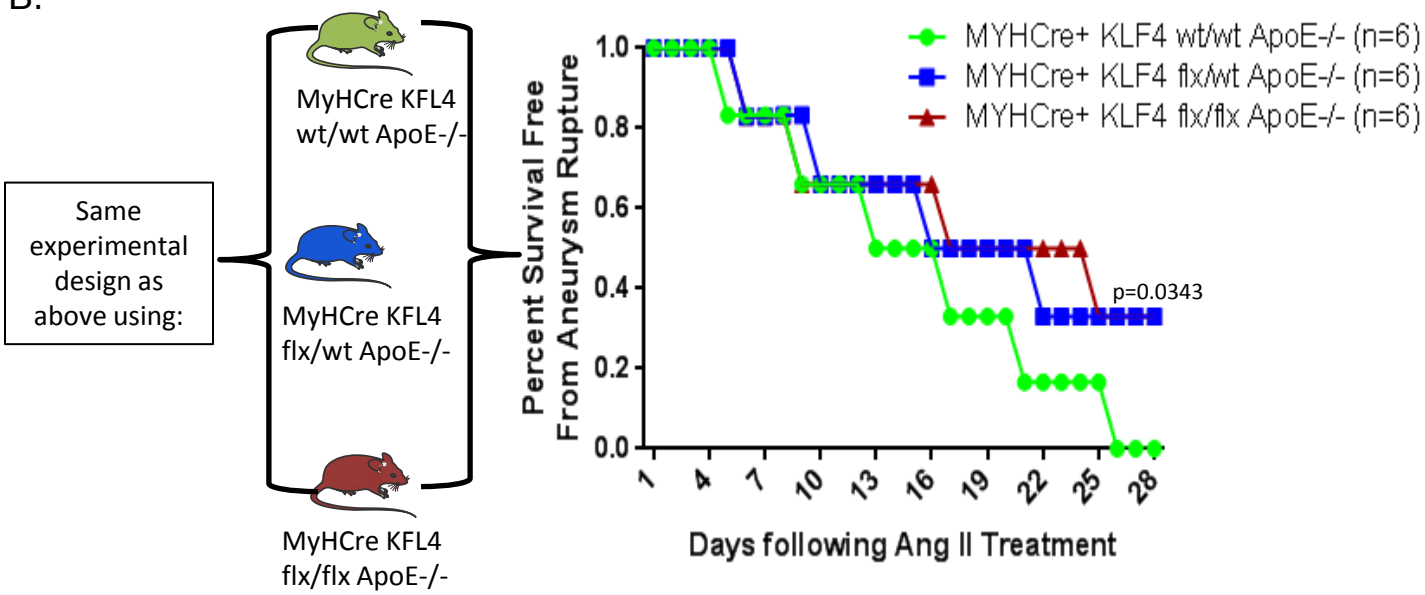

Supplementary Figure 12