

## Supplement Material

### **Inactivation of Nuclear Factor- $\kappa$ B inhibits vascular smooth muscle cell proliferation and neointima formation**

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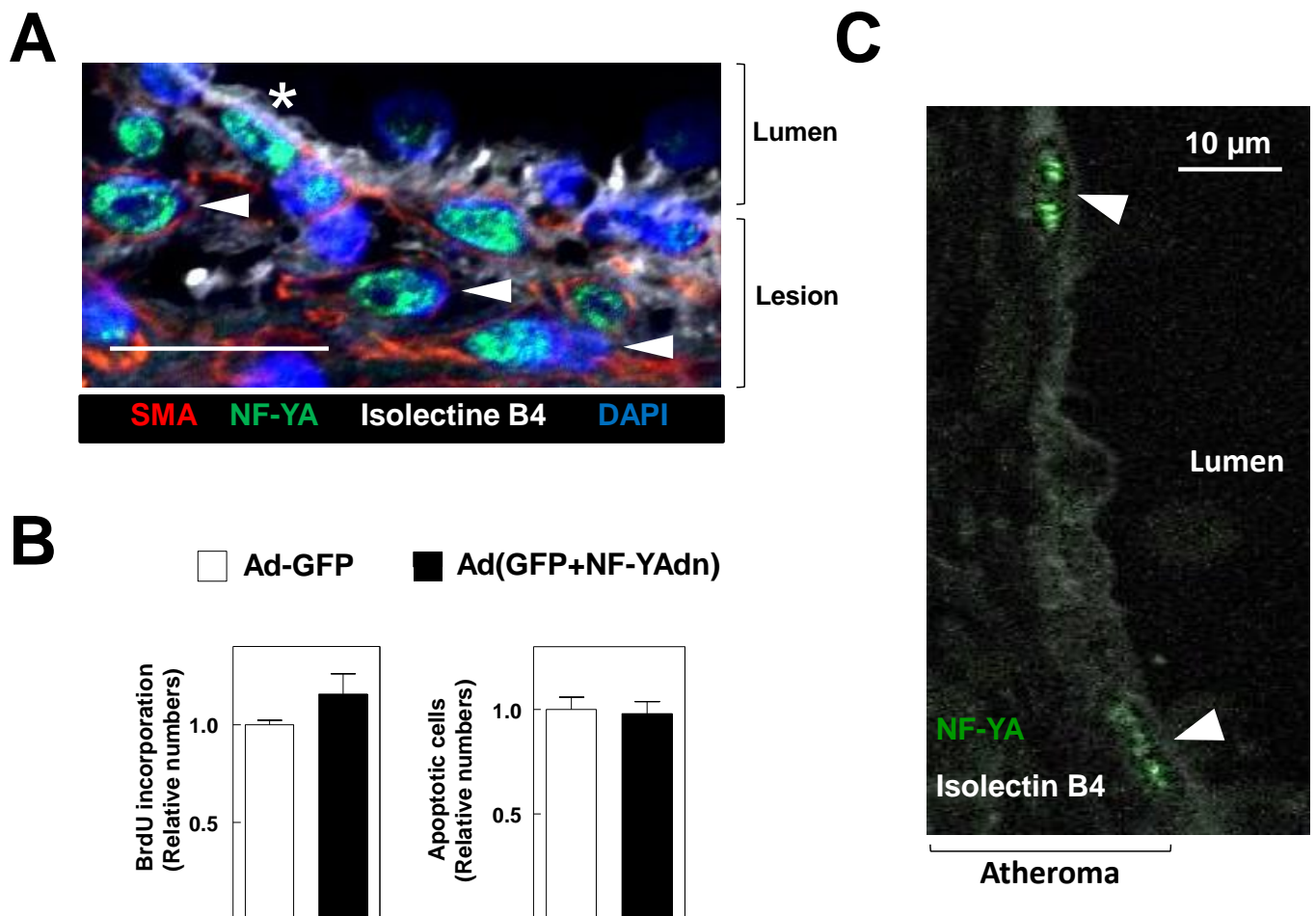
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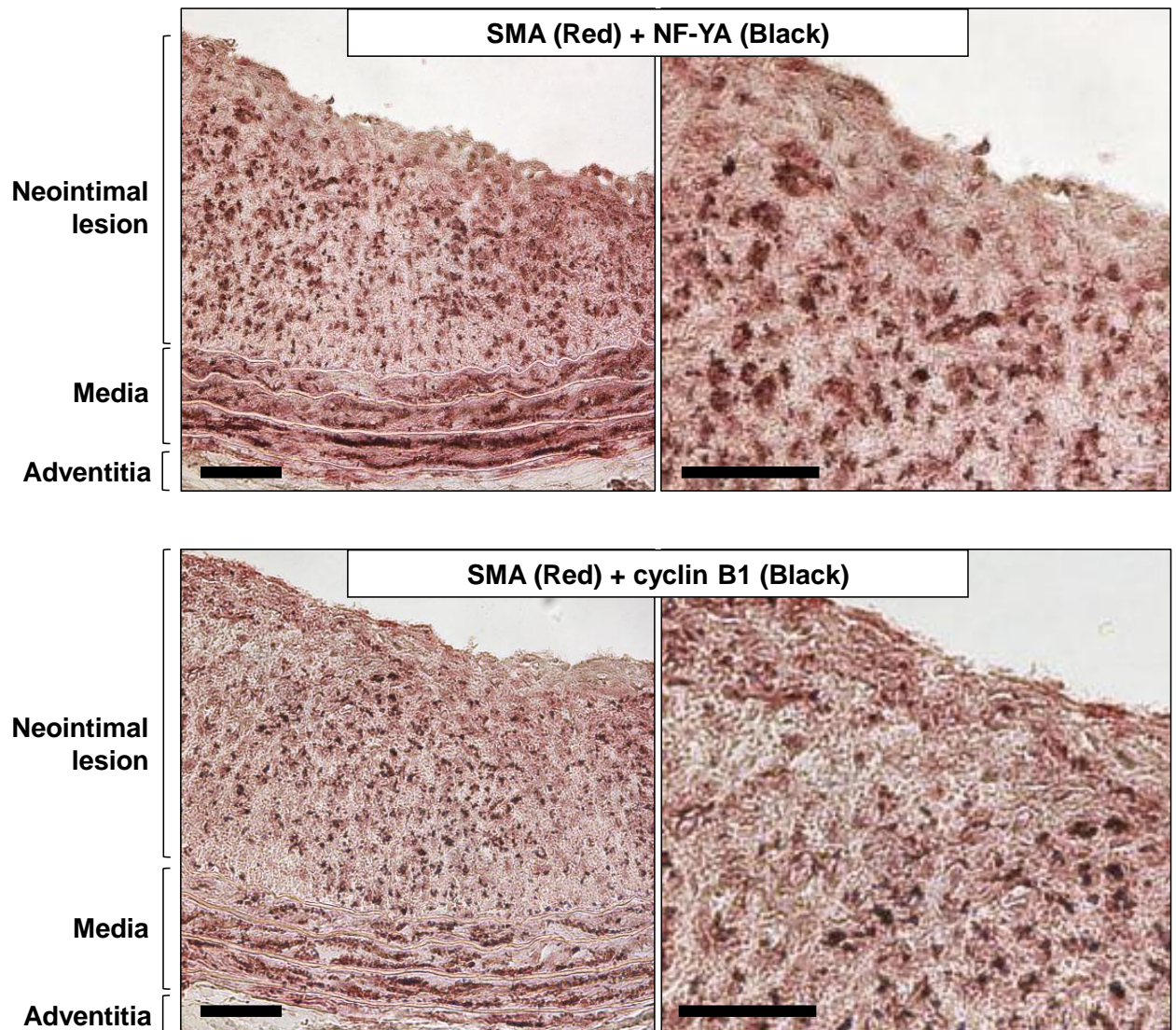
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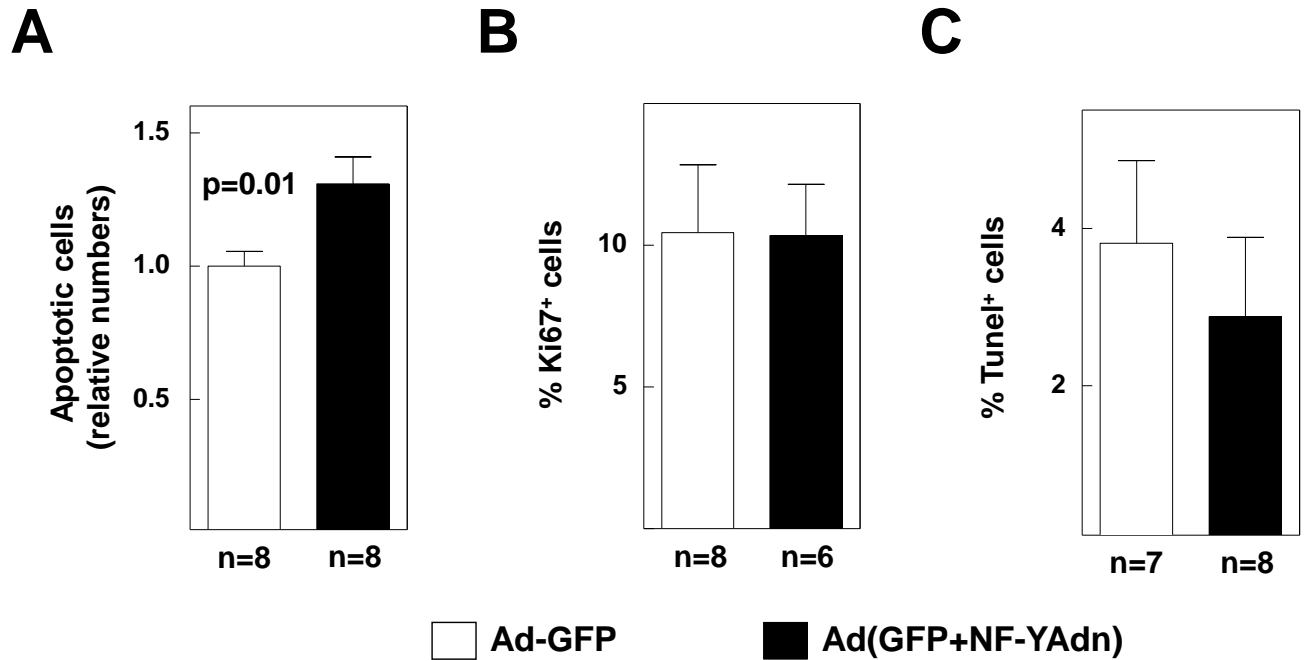
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**Supplemental Figure I. NF-YA is expressed in endothelium in vivo and its inactivation has no effect on endothelial cell proliferation and apoptosis in vitro.** (A). Confocal immunofluorescence microscopy of neointimal lesion (14 days post-angioplasty) to visualize isolectin B4 (white signal, endothelial cells), SMA (red signal, VSMCs), NF-YA (green signal), and nuclei (blue signal, DAPI). Arrowheads and asterisks point NF-YA/SMA and NF-YA/isolectin B4-positive cells, respectively. (B) Primary mouse aortic endothelial cells were infected with AdGFP (encoding GFP) or Ad(GFP+NF-YAdn) (which gives rise to a bicistronic mRNA encoding both GFP and NF-YA13m29 dominant-negative mutant). For proliferation studies, cells were starved and stimulated with medium containing 10% FBS, 0.1 mg/ml heparin, 50  $\mu$ g/ml ECGF and 50  $\mu$ M BrdU. Incorporation of BrdU was quantified 1 day after stimulation (n=7 replicates from 2 independent experiments). For apoptosis studies, infected cells growing asynchronously were analyzed by supravital incubation with propidium iodide (n=6 replicates from 2 independent experiments). (C) Confocal immunofluorescence microscopy of aortic atheroma from 2 month fat-fed apoE-KO mice to visualize NF-YA and isolectin B4 (endothelium). Arrowheads point to NF-YA/isolectin B4-positive cells.

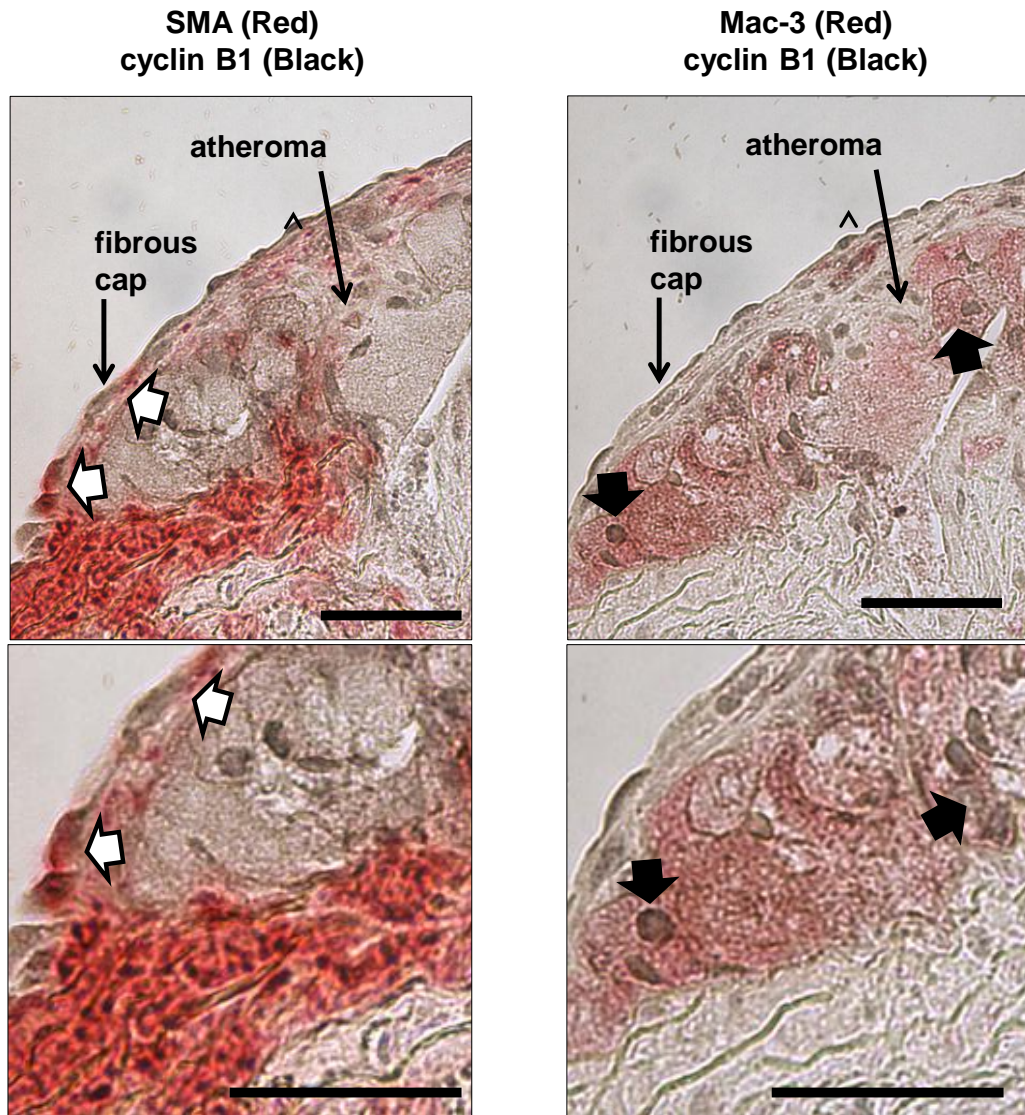


**Supplemental Figure II. NF-YA and cyclin B1 expression in neointimal VSMCs in balloon-injured rat carotid artery.** Representative examples of immunohistochemical analysis of consecutive sections of injured artery (18 days post-angioplasty) to visualize NF-YA (black)/SMA (red) or cyclin B1 (black)/SMA (red). The images in the right are high-power views of the neointimal lesions shown in the left. Bar: 50  $\mu$ m.

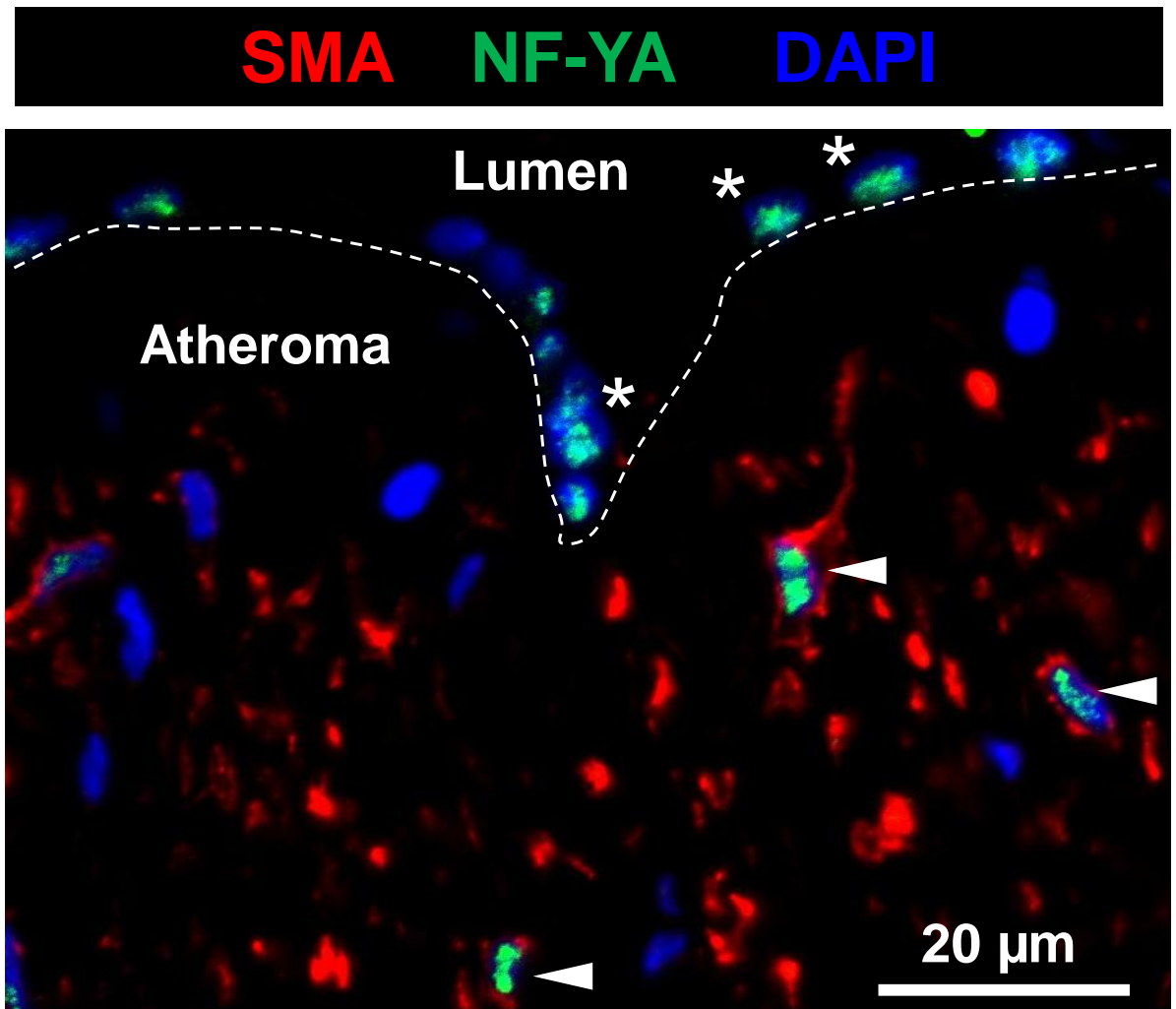


**Supplemental Figure III. Effect of NF-Y inactivation on VSMC apoptosis in vitro and neointimal cell proliferation and apoptosis in a mouse femoral artery injury model.** (A) Primary rat VSMCs were infected with AdGFP (encoding GFP) or Ad(GFP+NF-YAdn) (which gives rise to a bicistronic mRNA encoding both GFP and NF-YA13m29 dominant-negative mutant). Apoptosis of serum-starved rat VSMCs stimulated with 20% FBS was analyzed by flow cytometry after propidium iodide staining (n=8 replicates from 2 independent experiments). (B, C) Immunohistochemical analysis of neointimal cell proliferation (Ki67 staining) and apoptosis (TUNEL staining) in mouse femoral artery 9 days post-injury.





**Supplemental Figure IV. Cyclin B1 expression in neointimal VSMCs and macrophages in mouse atherosclerotic plaque.** Representative examples of immunohistochemical analysis of aorta from apoE-KO mice fed high fat diet for 2 months to detect simultaneously SMA and cyclin B1 or Mac-3 (red)/cyclin B1 in consecutive sections. SMA and Mac-3 show red cytoplasmic signal and cyclin B1 is visualized as brown nuclear signal. White and black arrows point to cyclin B1/SMA-positive and cyclin B1/Mac-3-positive cells, respectively. Bar: 50  $\mu$ m.



**Supplemental Figure V. Expression of NF-YA in human atherosclerotic coronary artery.**

Confocal immunofluorescence microscopy to visualize SMA (red signal), NF-YA (green signal) and nuclei (DAPI, blue signal). Arrowheads point to cells co-expressing NF-YA and SMA. Asterisks point to SMA-negative NF-YA-immunoreactive cells at the luminal border.