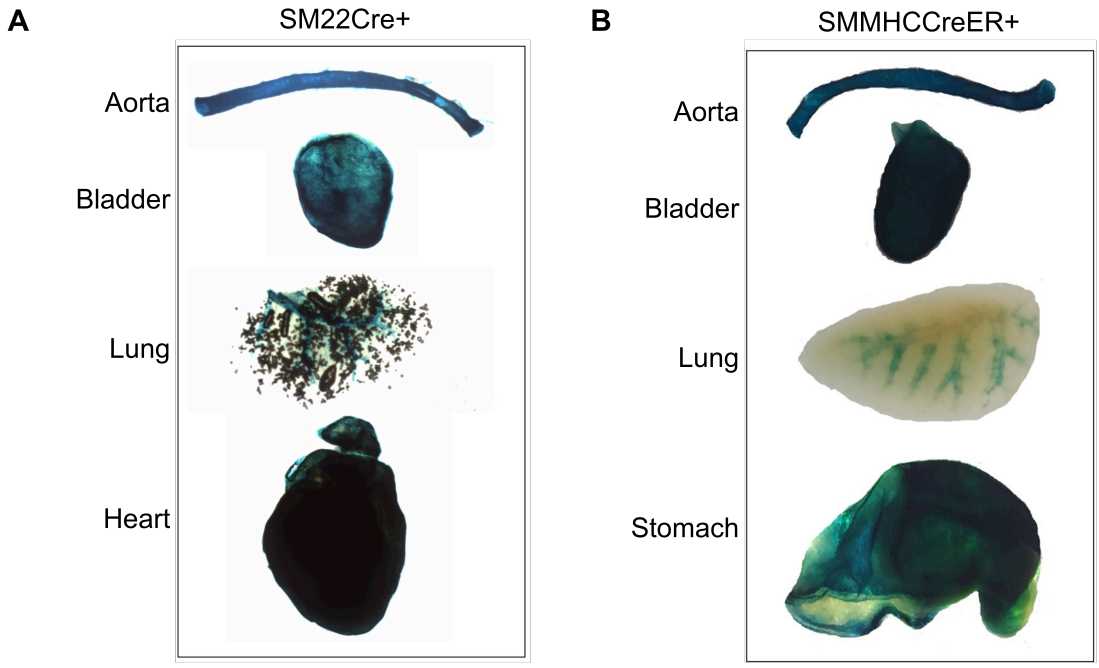
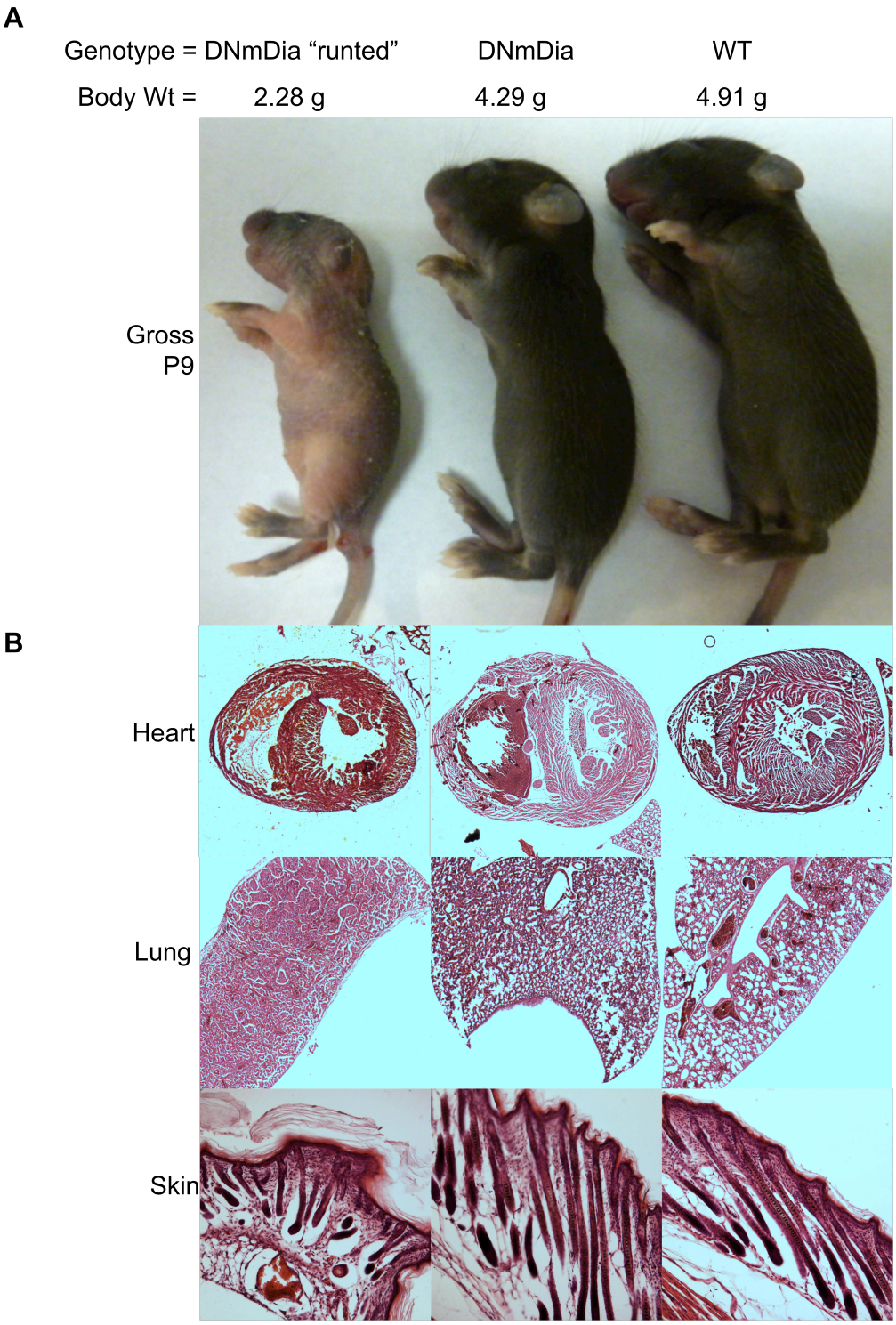
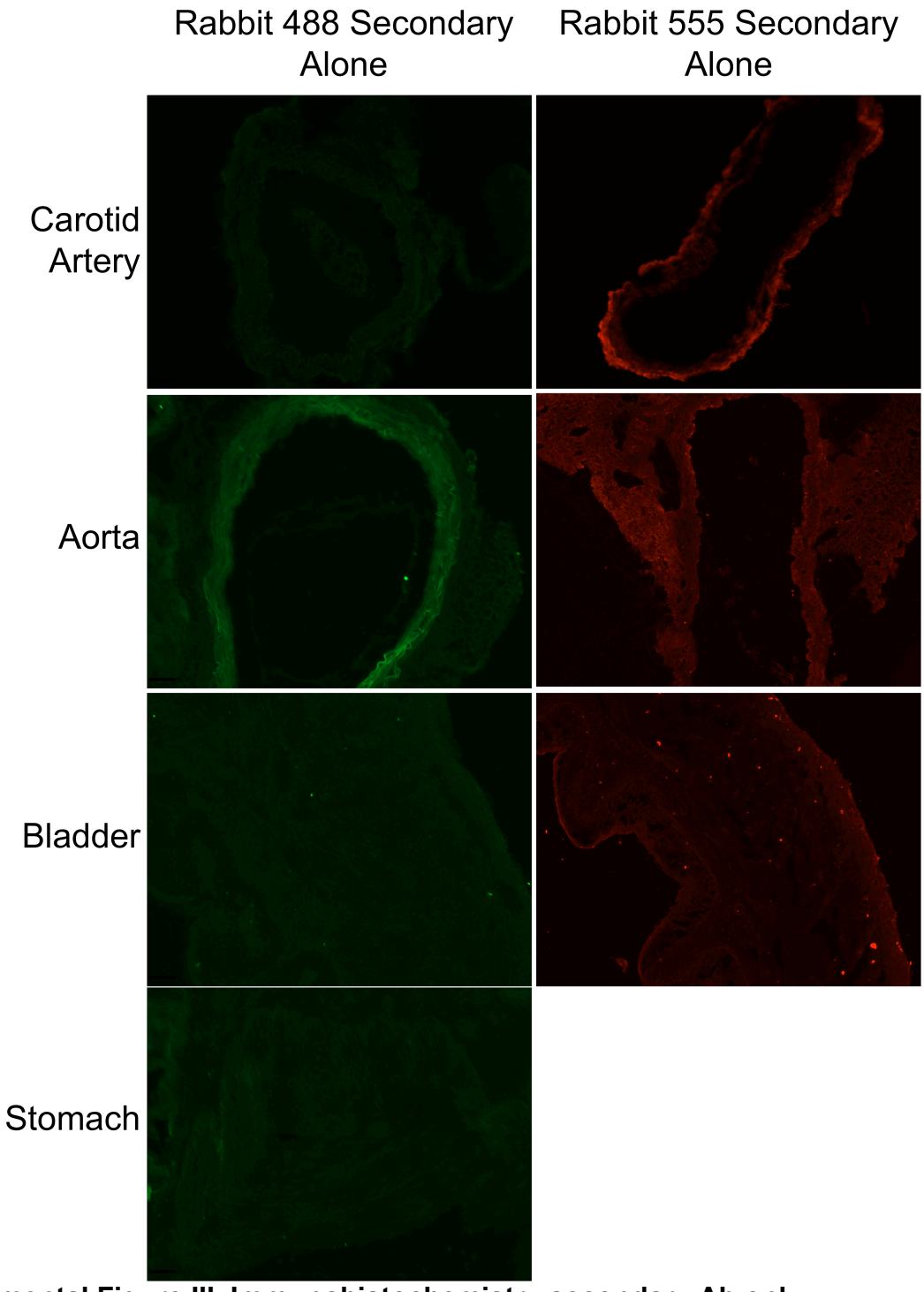
SUPPLEMENTAL FIGURES AND FIGURE LEGENDS



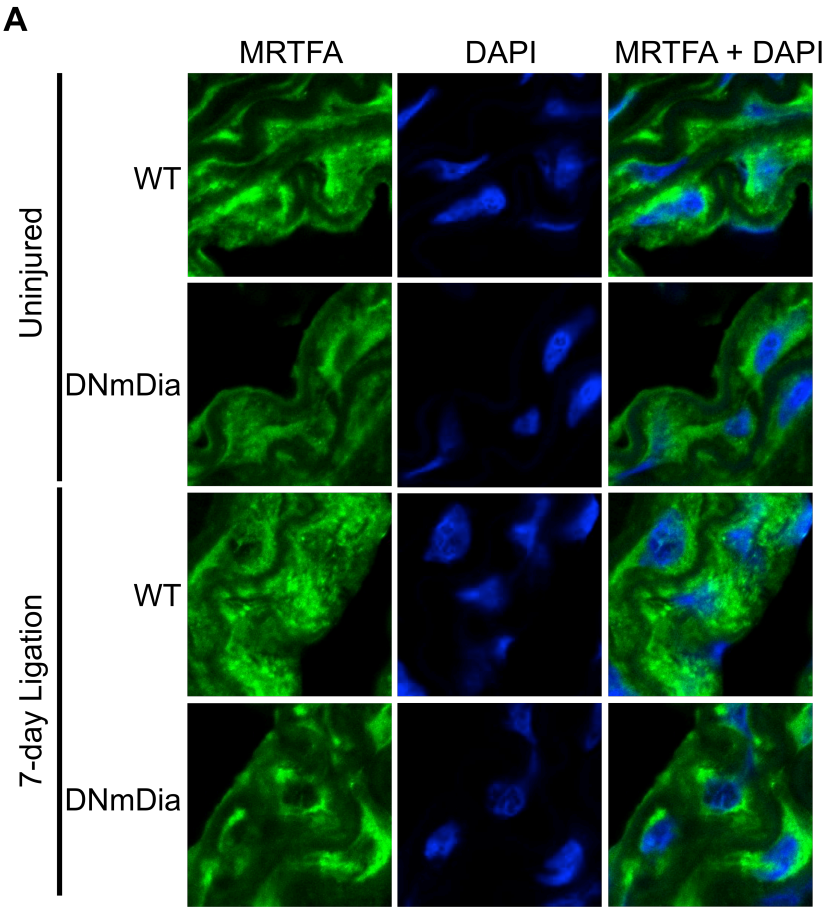
Supplemental Figure I. SMC-specific expression in our conditional models. β-gal stained SMC tissues of DNmDia^{+}/SM22Cre^{+}/Rosa^{+} **(A)** and tamoxifen-treated DNmDia^{+}/SMMHCCreER^{T2}/Rosa^{+} mice **(B)**.



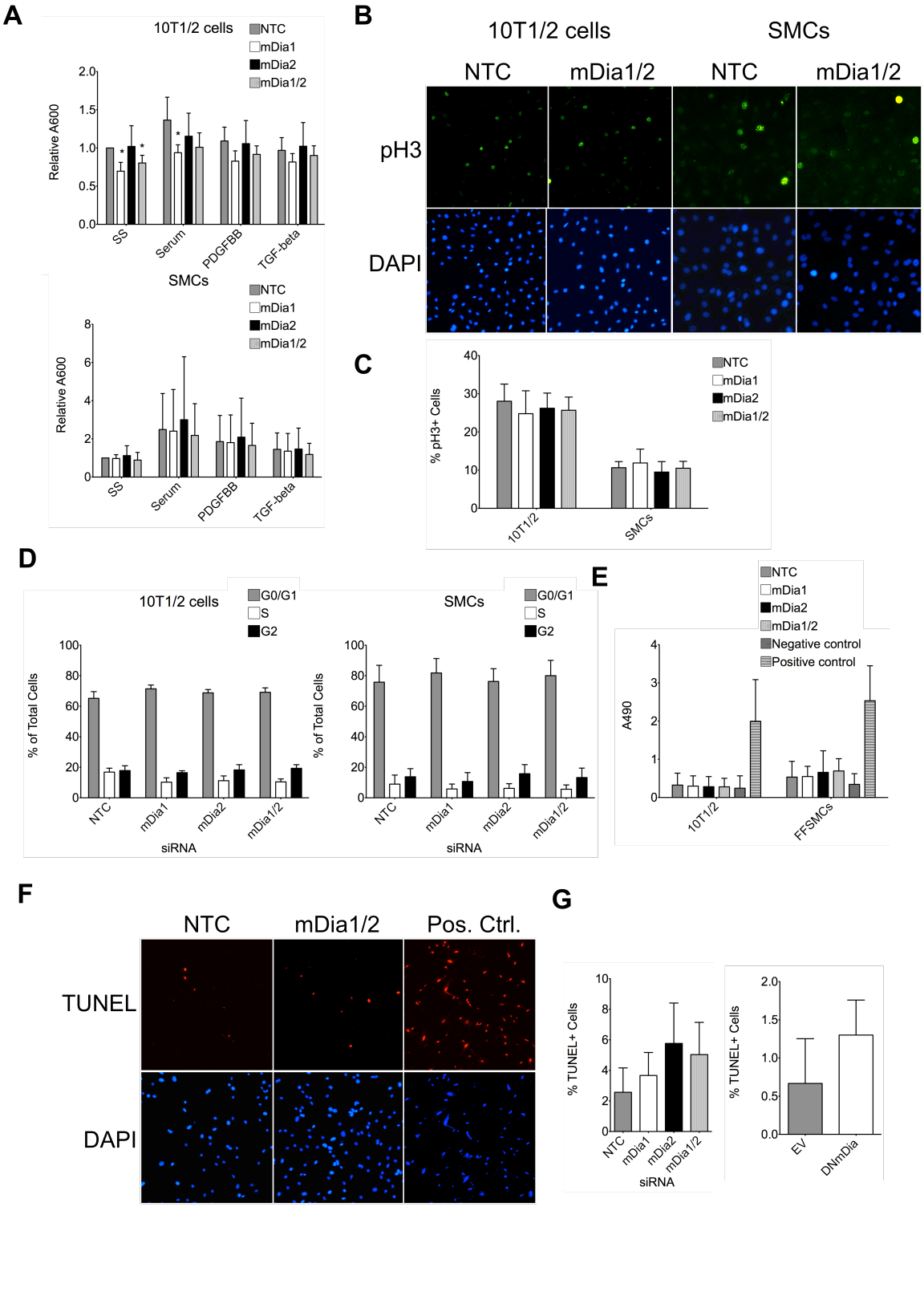
Supplemental Figure II. Some DNmDia exhibit a runted, hairless phenotype. **A)** Gross images of a runted DNmDia^{+}/SM22Cre^{+} mouse, a phenotypically normal DNmDia^{+}/SM22Cre^{+} mouse, and a wildtype littermate at P9. **B)** Representative H&E sections from the heart, lungs, and skin of the above animals. Note the thin ventricular wall, congested lungs, and degenerative hair follicles in the runted, hairless DNmDia^{+}/SM22Cre^{+} mouse.



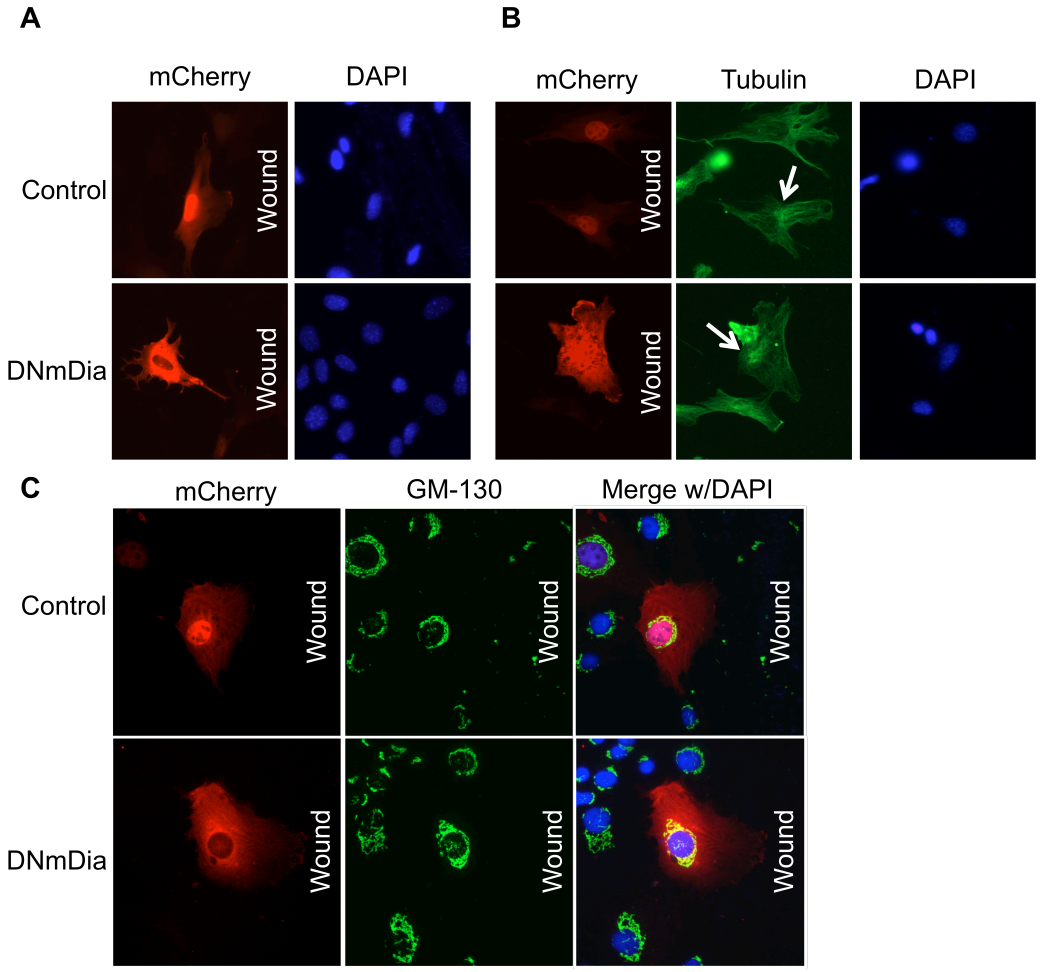
Supplemental Figure III. Immunohistochemistry secondary Ab only.



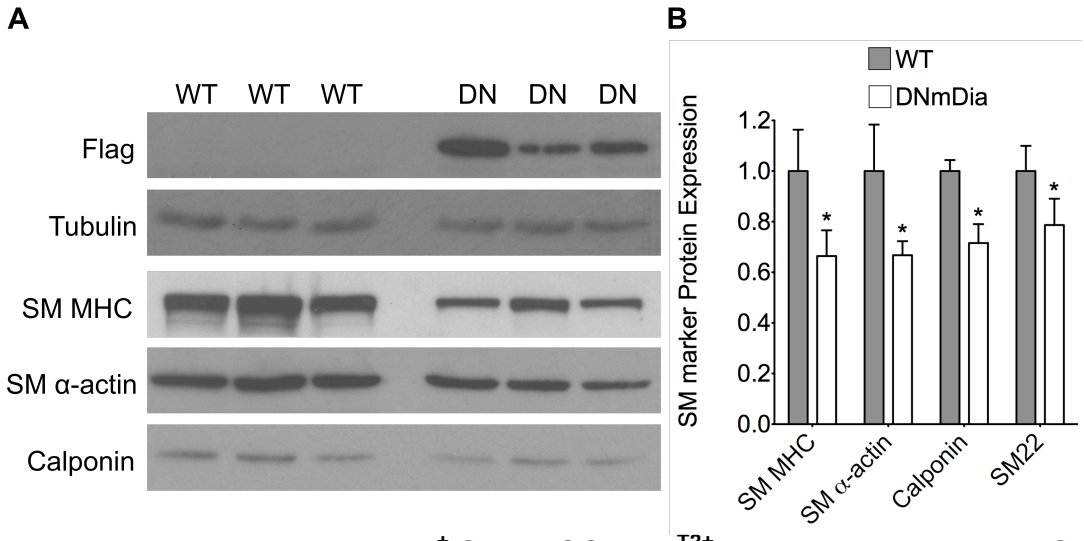
Supplemental Figure IV. Carotid artery ligation imaging. A) Representative images of MRTF-A localization, quantified in Figure 3D. **B)** Representative images of Masson's Trichrome stain.


A
10T1/2 cells
NTC
mDia1
mDia2
mDia1/2
Relative A600
SS
Serum
PDGFBB
TGF-beta
SMCs
B
10T1/2 cells
SMCs
NTC
mDia1/2
pH3
DAPI
C
% pH3+ Cells
10T1/2
SMCs
D
G0/G1
S
G2
% of Total Cells
siRNA
E
Negative control
Positive control
A490
FFSMCs
F
Pos. Ctrl.
TUNEL
G
% TUNEL+ Cells
EV
DNmDia

Supplemental Figure V. Inhibition of mDia signaling does not alter cell cycle, death, or proliferation. siRNA-mediated knockdown of mDia1 and/or mDia2 was performed in 10T1/2 or mouse SMCs. **A)** 48-hr post-knockdown, cells were subjected to indicated media conditions for 24 hr, followed by colorimetric tetrazolium MTT assay. Absorbance at 600nm corresponds to active cell proliferation. N = 3 separate experiments. **B)** 48-hr post knockdown, cells were fixed, permeabilized, and probed for phospho-histone H3. **C)** Quantification of pH3+ cells from three separate experiments with over 100 cells counted per condition. **D)** 48-hr post-knockdown, cells were harvested, briefly stained with DAPI, and subjected to cell cycle analysis by flow cytometry. 50,000+ cells cells were analyzed in three separate experiments. **E)** 48-hr post-knockdown, cells were split and re-plated in 1% serum media. After 6hr, media was removed from cells and subjected to colorimetric cytotoxicity assay based upon lactate dehydrogenase activity in the media, which occurs upon plasma membrane rupture, and is measured by absorbance at 490 nm. Negative control = untransfected cells. Positive control = cells treated with 1% Triton X-100. N = 3 separate experiments. **F)** 48-hr post-knockdown, SMCs were fixed, permabilized, and stained for TUNEL+ cells. Positive control = cells subjected to UV light for 4 hr. **G)** Quantification of TUNEL+ cells from siRNA-knockdown SMCs and DNmDia-overexpressing 10T1/2 cells. Three separate experiments with over 100 cells counted per condition. * $p < 0.05$.



Supplemental Figure VI. DNmDia expression inhibited cellular orientation. 10T1/2 cells were transfected with mCherry vector or mCherry-DNmDia, subjected to an 8-hr scratch wound, and fixed. **A)** Representative images of lamellipodia formation in response to indicated scratch wound. **B)** Cells were probed with α-Tubulin to mark MTOC, marked by arrows. For DNmDia cell, note the MTOC location at the back end of the nucleus. **C)** Primary rat aortic SMCs were transfected with mCherry empty vector or mCherry-DNmDia, subjected to 8-hr scratch wound, fixed, and stained for Golgi marker (GM)-130. Representative images are shown with the scratch oriented to the right.



Supplemental Figure VII. DNmDia^{+}/SMMHCCreER^{T2+} mice exhibit reduced SM marker expression in the bladder. A) Western blot of SM marker expression and DNmDia expression (Flag) in control (WT) and DNmDia^{+}/SMMHCCreER^{T2+} (DN) mice. Note the variable transgene expression. **B)** SM marker expression was quantified using ImageJ in 9 controls and 9 DNmDia mice, normalized to tubulin load, and expressed relative to values in control animals, set to 1. *$p < 0.05$.

VIDEO FILE LEGEND

DNmDia expression prevents directional migration in a scratch wound assay. Confluent cultures of 10T1/2 cells expressing GFP-LifeAct and either mCherry or mCherry-DNmDia were scraped with a P1000 pipette tip and then placed on an inverted microscope equipped with a heated, humidified, and O_2/CO_2 perfused stage. Pictures taken every 5 minutes for 24 hr were assembled into movies using Quicktime. Green channel (LifeAct) movie is shown for better view of cytoskeletal movement. Representative movies for each construct are included. Note that scratch wound is located on the right. 1 sec of movie = 1 hr of live cell imaging.

Video I. Control

Video II. DNmDia