SUPPLEMENTAL MATERIAL

Histology

Paraffin sections were deparaffinized, rehydrated, and then incubated with 3% H_2O_2 to block endogenous peroxidase activity for immunohistochemistry. After antigen-retrieval and blocking non-specific IgG binding, anti- TN-C rabbit polyclonal antibody (Chemicon International Inc., Temecula, CA) was applied followed by incubation with biotinylated species-specific secondary antibodies in conjunction with a Vectastain Elite ABC kit (Vector Laboratory Inc, Burlingame, CA). Sections were exposed to diaminobenzidine (DAB) (Vector Laboratory Inc, Burlingame, CA), prior to counter-staining with hematoxylin to visualize antigens. Fluorescence conjugated anti-rabbit IgG (Invitrogen, Carlsbad, CA) was applied to the fixed cells following primary antibody incubation for detection of TN-C in SMC. Nuclei were stained with DAPI (KPL Inc., Gaithersburg, Maryland) and mounted with fluorescent mounting media (KPL Inc). The same exposure time for fluorescence microscope imaging was used in all groups for comparison. Staining of mPGES-1 was carried out as described above with the corresponding primary antibody (Cayman Chemicals, Ann Arbor, MI, Cat. 160140). Rabbit Antibody Enhancer and Polymer-HRP for rabbit (Golden Bridge International, Mukilteo, WA, Cat. 40-6) was used as secondary antibody. Non-immunized rabbit IgG was used for negative control staining.

Supplemental Figure 1. mPGES-1 expression in injured artery. A cross section from an artery, harvested four weeks after the injury, was stained for mPGES-1(demarked by brown). N denotes neointima; M denotes media; ▸ indicates internal elastin; ◂ indicates external elastin. Scale bar denotes 10 μm.

Supplemental Figure 2. Cellular infiltration/migration in subendothelium. Wire injury induced cell infiltration/migration to the subendothelial space (as indicated by arrow head) one week after the injury. Subendothelial cell number (right panel) was quantified on H&E stained sections across the injured arteries (average of 8 levels). (*: $p<0.05$, n=5).

Supplemental Figure 3. Consequences of mPGES-1 deletion on urinary metabolites of PGD_2 and TxA_2 in vascular remodeling. Urinary metabolites of PGD_2 (A) and TxB_2 (B), 11,15-dioxo-9alpha-hydroxy-2,3,4,5-tetranorprostan-1,20-dioic acid (tetranor PGDM) and 2,3 dinor - TxB_2 (Tx-M), respectively, were compared between genotypes at baseline, day 1 and day 14 post injury. (t-test with Bonferroni correction. *: $p<0.05$; **: $p < 0.01$. n=14-16 per group)

Supplemental Figure 4. Impaired adhesion and spreading in mPGES-$1^{-/-}$ VSMCs. VSMC motility was examined for 24 hours after plating cells on collagen thin films, as detailed in methods. The percentage of spreading cells are plotted for WT (left panel) and KO (right panel) VSMCs. Each curve corresponds to a single observation field under the microscope over the 24-hour observation period. Seven and eight movies were recorded from two animals of each genotype. Representative movies are shown in online supplemental data.

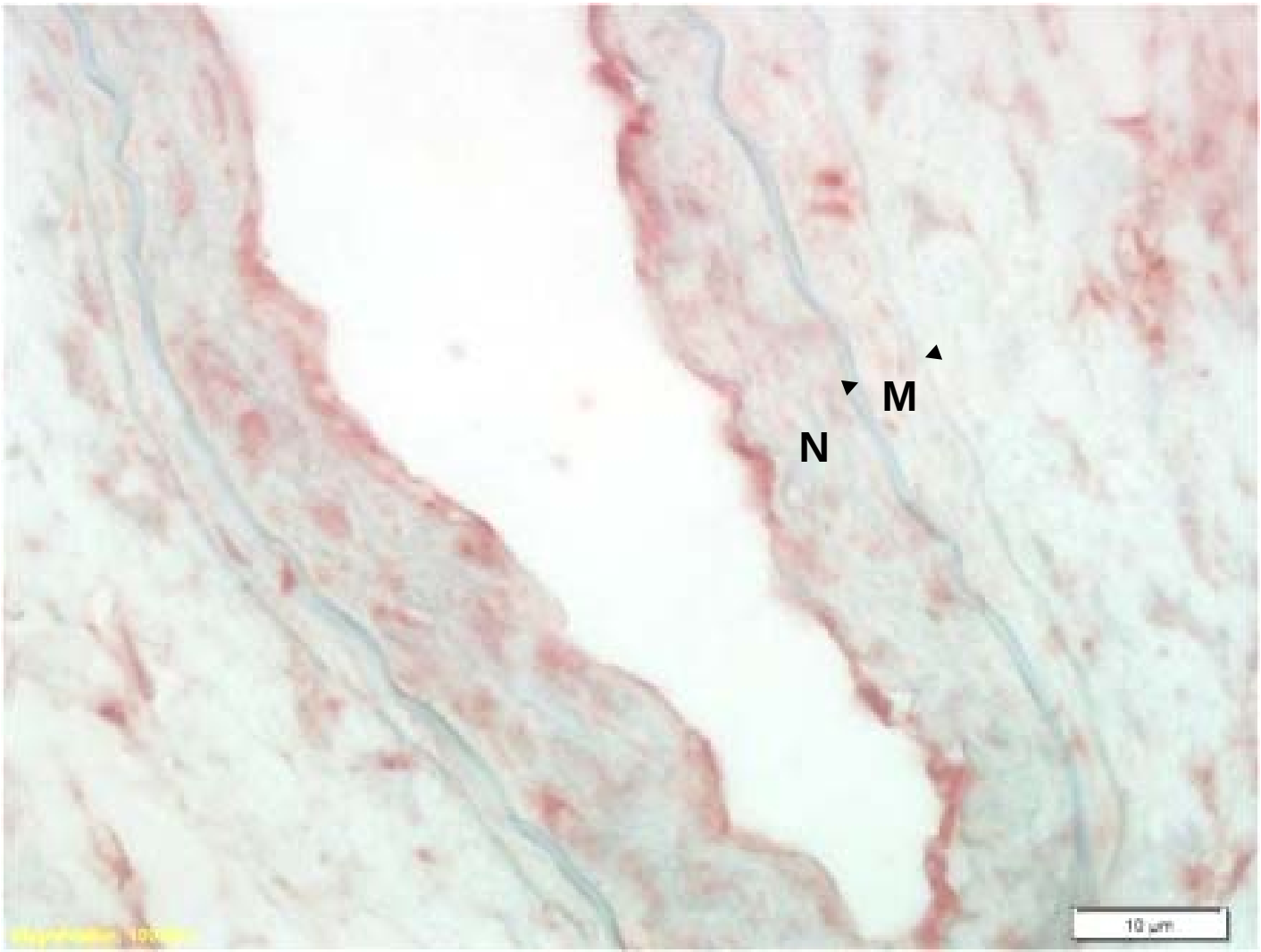
Supplemental Figure 5. TN-C knockdown in VSMCs.

A: Staining of TN-C expression (green) in cultured VSMCs 60 hours after transfection. Nuclei shown in blue by DAPI staining. B: TN-C mRNA expression was analyzed 48 hours after transfection by quantitative PCR. Expression of TN-C mRNA was normalized to 18S rRNA and presented as percentage change relative to control siRNA. Data are representative of three independent experiments.

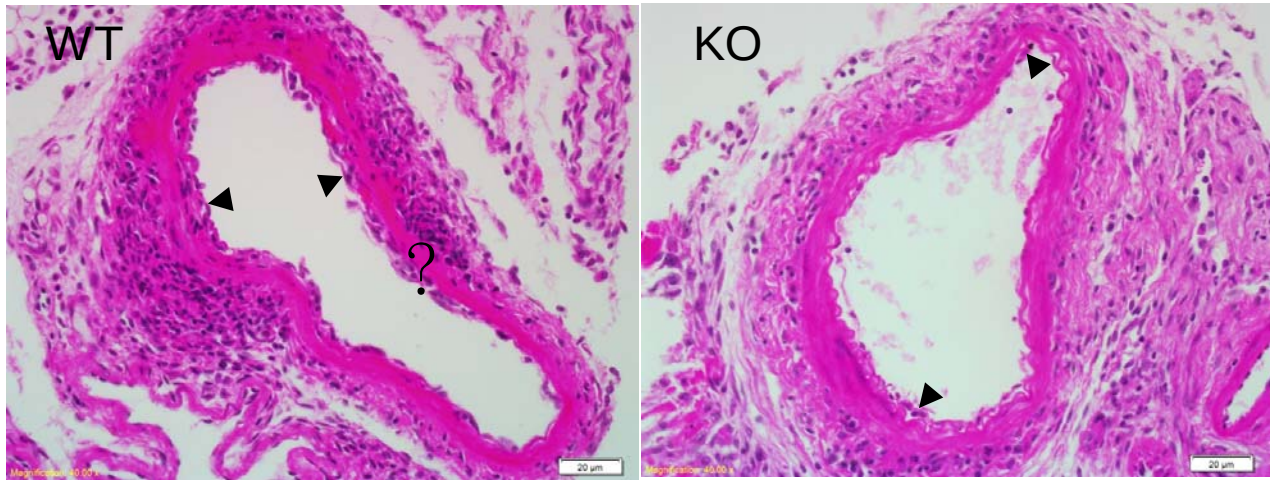
Supplemental Figure 6. Masson's trichrome staining of wire-injured arteries.
Remodeled arteries four weeks after wire-injury (right panels) and non-injured control arteries (left panels) from WT (upper panels) and mPGES-1 KO (lower panels) were stained by Trichrome. Blue: collagen, mucin; Black: nuclei; Red: cytoplasm, keratin, muscle fibers. Bar=20 μm.

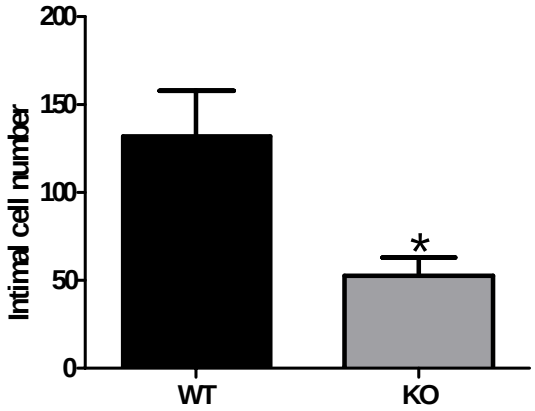
Supplemental Figure 7. Knock-down of TN-C reduces S phase entry in VSMCs.
Mouse SMCs transfected with control or TN-C siRNA (100 nM) for 60 hours were serum starved for 48 h and stimulated with 2% FBS for 48 h in the presence of BrdU. The data are presented as mean ± SD of three independent experiments. (*: $p<0.05$, n=3).

Supplemental Figure 1



Supplemental Figure 2





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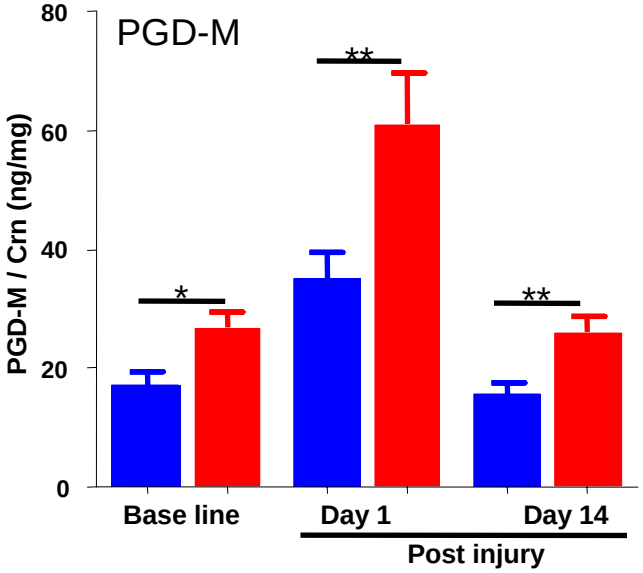
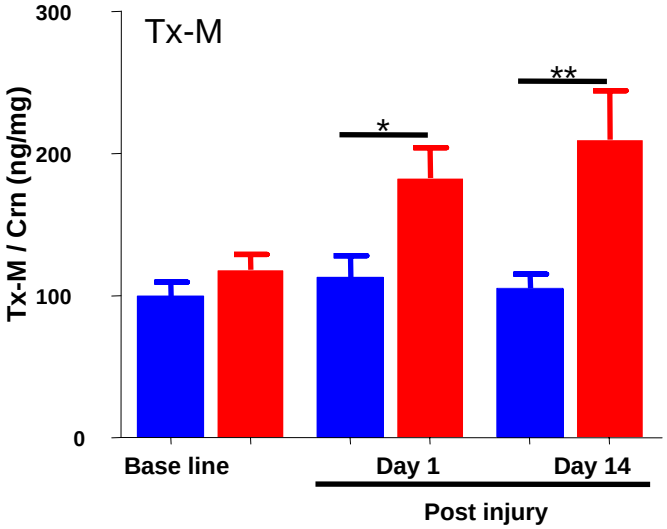
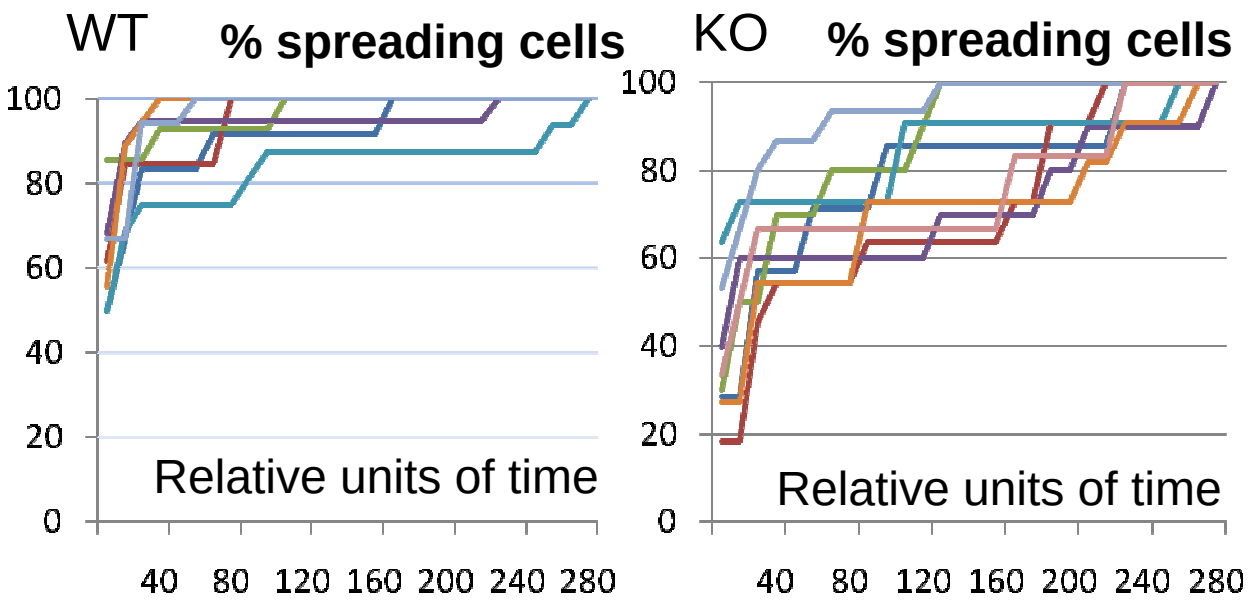
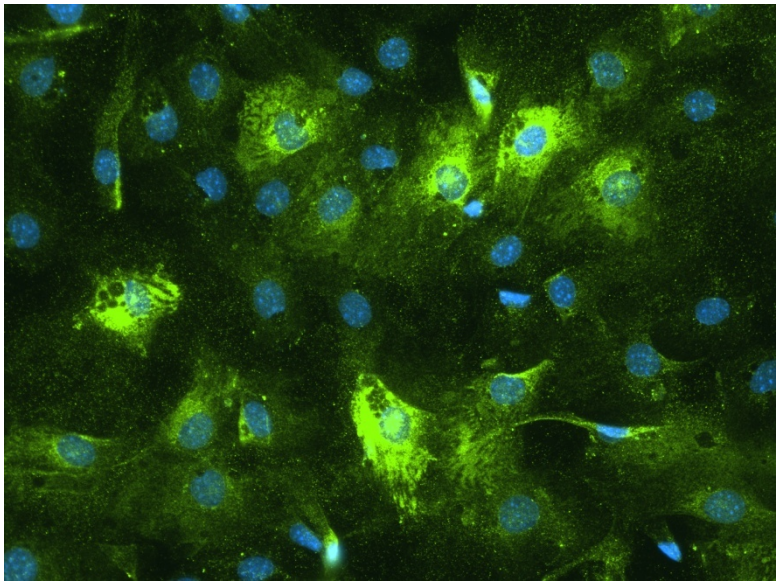




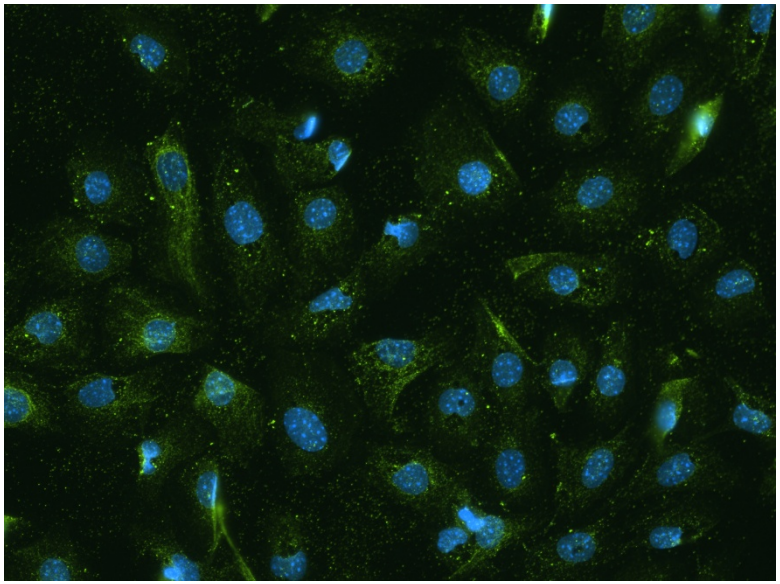
Supplemental Figure 4



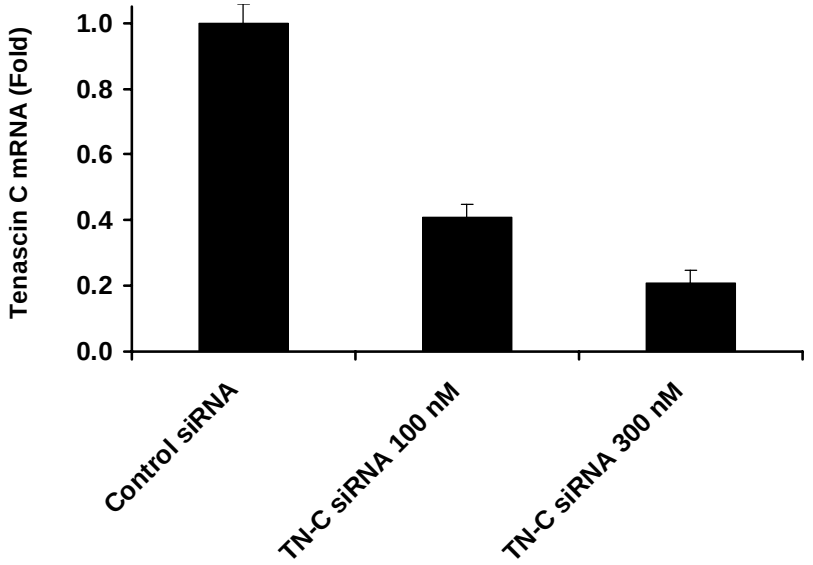
Supplemental Figure 5



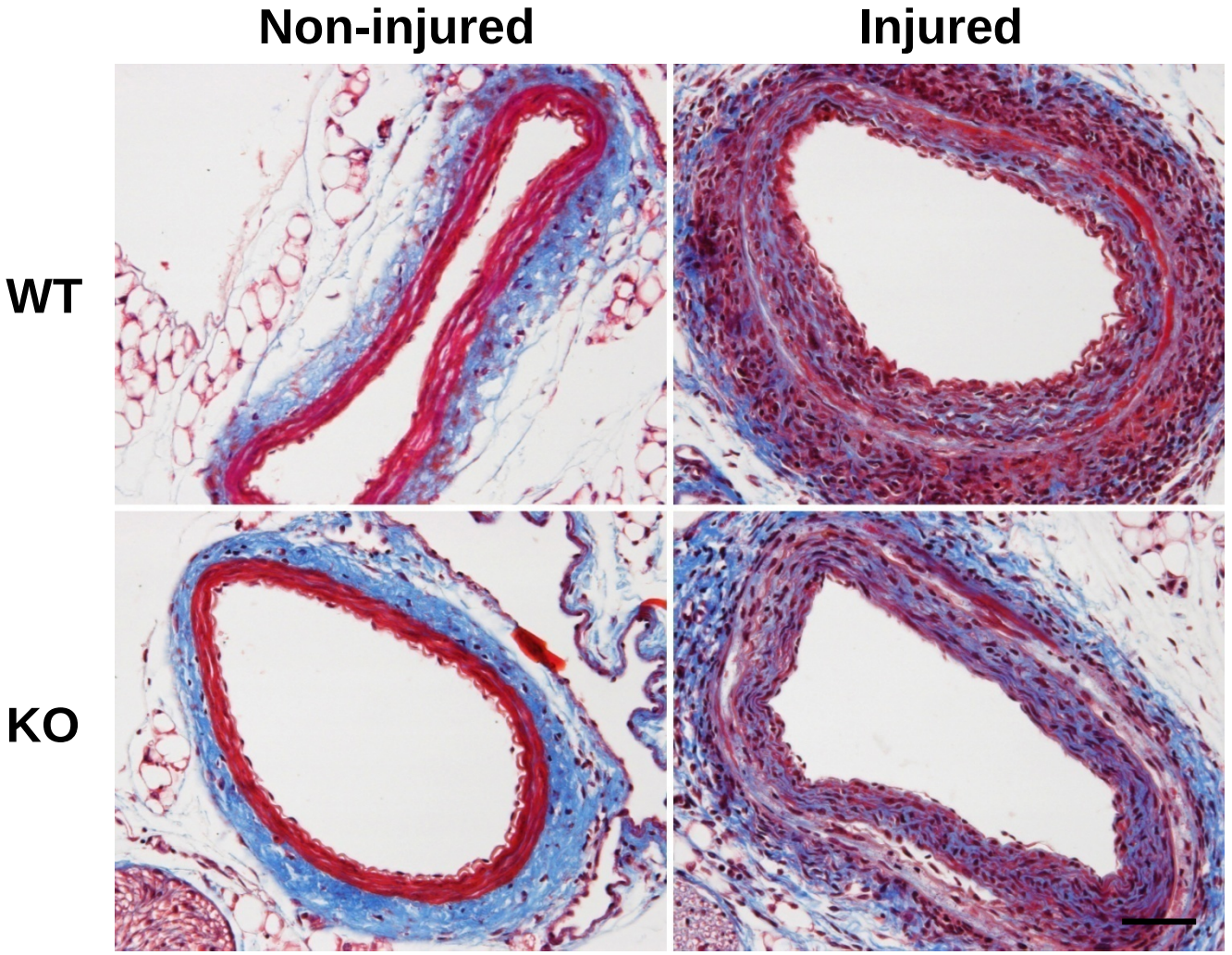
Control siRNA



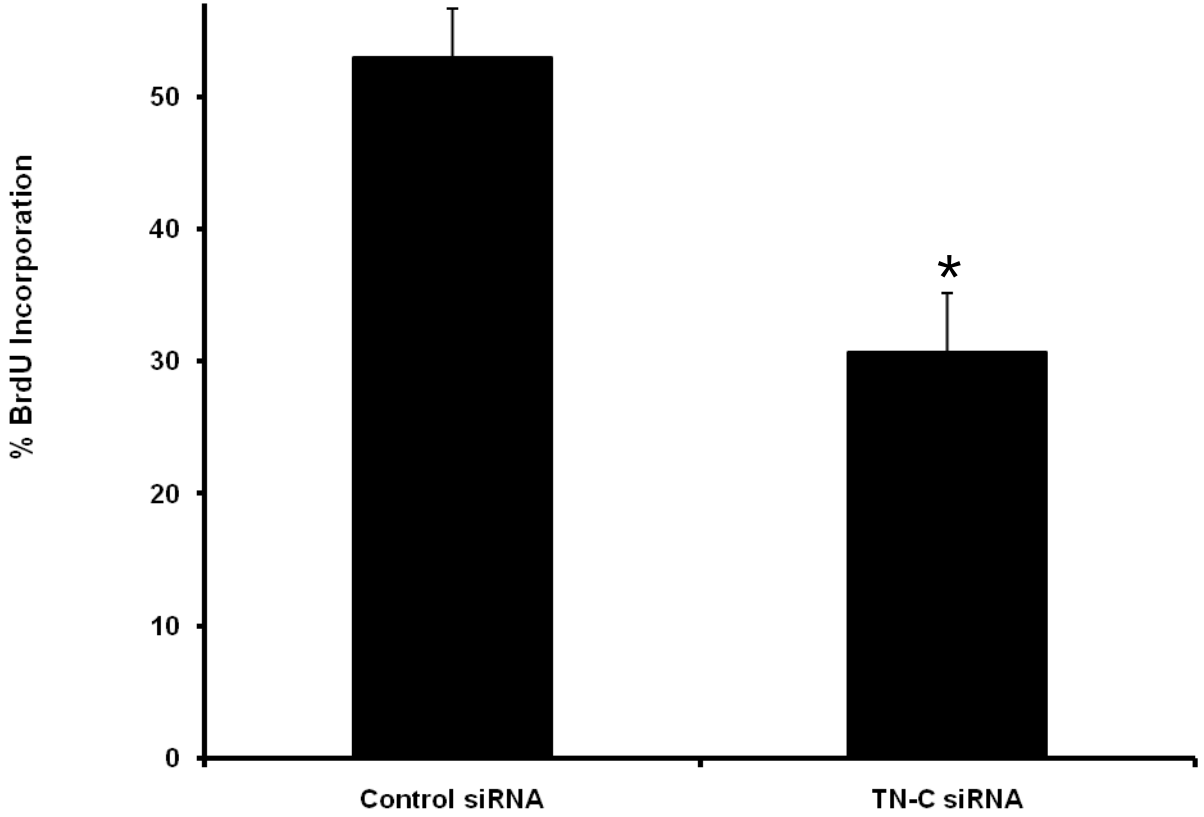
TN-C siRNA



Supplemental Figure 6



Supplemental Figure 7



Supplemental video data

VSMC motility was examined for 24 hours after plating VSMCs on collagen thin films, as detailed in methods. Representative movies for WT and in mPGES-1 KO VSMCs are shown in online Supplementary video file WT and KO respectively. VSMC velocity was decreased in mPGES-1 KOs.