Supplementary Figure Legends

Fig.S1. Immunofluorescent co-stainings and quantitation of P-PERK and SMC marker α-SMA following targeted delivery of PERK inhibitor GSK2606414 in vivo.

Angioplastied rats were subject to targeted delivery of PERK inhibitor GSK2606414 using 2 distinct platforms as illustrated in Fig.1 and Fig.2. Carotid artery sections were then subject to immunofluorescent co-stainings of P-PERK and α-SMA in control versus targetedly treated groups.
A, D. Representative images of P-PERK and α-SMA co-stainings from animals treated with biomimetic nanocluster (A) and peri-carotid hydrogel (D) for targeted delivery of PERK inhibitor.
B, E. Quantitation of α-SMA fluorescent intensity per cell to demonstrate the efficacy of targetedly delivered PERK inhibitor in restoring α-SMA expression levels in vivo.
C, F. Quantitation of P-PERK fluorescent intensity per cell to validate the efficacy of the targetedly delivered PERK inhibitor. Mean ± SEM, n=4 rats; *p<0.05, **p<0.01, ***p<0.001. Unpaired Student's t-test (E, F) and One-way ANOVA with Bonferroni post hoc test (B, C).

Fig.S2. Quantitation of PERK pathway activation in balloon-injured rat carotid arteries

Quantitation of PERK pathway proteins in injured arteries following angioplasty as described in Fig.3. Rat common carotid arteries were harvested at days 3, 7, and 14 (3d-14d) for tissue homogenate immunoblotting analysis and immunofluorescent co-stainings of P-PERK and α-SMA.
A-C. Quantitation of protein level changes for PERK pathway proteins P-eIF2α, ATF4, and CHOP. Mean ± SEM, n=3 rats; *p<0.05, **p<0.01, ***p<0.001. One-way ANOVA with Bonferroni post hoc test.
D-F. Quantitation of P-PERK fluorescent intensity per cell and P-PERK positive cell percentage in intima and media layers. Mean ± SEM, n=4 rats; *p<0.05, **p<0.01, ***p<0.001. One-way ANOVA with Bonferroni post hoc test.

Fig.S3. In vivo adenovirus-mediated PERK gain-of-function in balloon-injured rat carotid arteries

Rat carotid arteries were injured with balloon angioplasty, and subsequently locally infused with adenoviruses overexpressing GFP (Ad-GFP) or PERK (Ad-PERK) as described in method section.
A. Adenovirus-infected rat carotid arteries were harvested at day 3 post injury for protein extraction and immunoblot analysis. In vivo infection efficiency was manifested by increased level of P-PERK and P-eIF2α.
B. Adenovirus-infected rat carotid arteries were harvested at day 14 post injury for RNA extraction and qPCR analysis. Mean ± SEM, n=6 rats; *p<0.05. Unpaired Student's t-test.

Fig.S4. PERK loss- and gain-of-function respectively rescues and exacerbates the reduction of SMC contractile markers at mRNA levels

To corroborate our findings on PERK's involvement in regulating SMC phenotypic switching, we evaluated the mRNA levels of additional SMC contractile markers including α-SMA, CNN1 (calponin), and MYH11 (smooth muscle myosin heavy chain). Experiments were performed as described in Fig.4.
A-C. Pharmacological blockade of PERK kinase activity. Quantitation of mRNA level changes

revealed effective restoration of PDGF-BB-induced reduction of α-SMA and CNN1 mRNA.
D-G. PERK genetic silencing. Consistent with α-SMA protein level change observed in Fig.4, PERK silencing with a different siRNA recapitulated the rescuing effect in multiple SMC contractile markers.
H-J. Adenovirus-mediated PERK gain-of-function. PERK overexpression reduced the mRNA levels of SMC contractile markers α-SMA and CNN1 at basal level.
All data are presented as mean ± SEM, n=6; *p<0.05, **p<0.01, ***p<0.001. One-way ANOVA with Bonferroni post hoc test.

Fig.S5. Effect of PERK inhibition in a murine $FeCl_3$-induced thrombosis model

Mouse carotid arteries were locally applied with $FeCl_3$-soaked patches to induce thrombosis formation. Mice were pre-treated with either vehicle or PERK inhibitor GSK2606414 (150 mg/kg) 4 hours prior to experiment.
A. Representative H&E staining of $FeCl_3$-injured carotid arteries from mice treated with vehicle or GSK2606414.
B. Heart rate was monitored during the procedure. Initial and terminal heart rates are shown here. n=15-16 mice.

Fig.S6. PERK inhibitors were identified with differential inhibition over SMC versus EC in a high-throughput phenotype screening

Human aortic SMCs and ECs were screened for their differential phenotypes to the PKIS 2 kinase inhibitor library as previously described.
A. Scheme of the high-throughput drug screening. Cell viability was selected as the phenotype readout of the assay.
B. Overview of the drug screening result.
C. Among the identified hits with differential inhibition over SMC versus EC, we discovered 3 compounds that were previously shown with selective inhibition to PERK. These 3 compounds were derivatives of the first-in-class PERK inhibitor GSK2606414.

Supplemental table

Table S1. Antibodies used in Western blotting and immunofluorescent staining

Antigen	Manufacturer	Catalog Number	Dilution Ratio	Application
P-PERK	Abcam	ab192591	1:100	Immunofluorescence
α-SMA	Cell Signaling Technology	48938s	1:100	Immunofluorescence
P-PERK	Santa Cruz	sc-32577	1:100	Western Blot
P-EIF2α	Cell Signaling Technology	3398	1:1000	Western Blot
P-STAT3	Cell Signaling Technology	4113	1:1000	Western Blot
P-P65	Cell Signaling Technology	3033	1:1000	Western Blot
MRTF-A	Cell Signaling Technology	14760	1:1000	Western Blot
CD142/Tissue Factor	Thermo Fisher	PA5-27278	1:500	Western Blot
CHOP	Santa Cruz	sc-8327	1:100	Western Blot
ATF4	Abcam	ab184909	1:1000	Western Blot
Cyclin D1	Abcam	ab134175	1:1000	Western Blot
α–SMA	Sigma-Aldrich	A2547	1:5000	Western Blot
β-actin	Sigma-Aldrich	A5441	1:5000	Western Blot

A

No Nanoclusters | With Nanoclusters

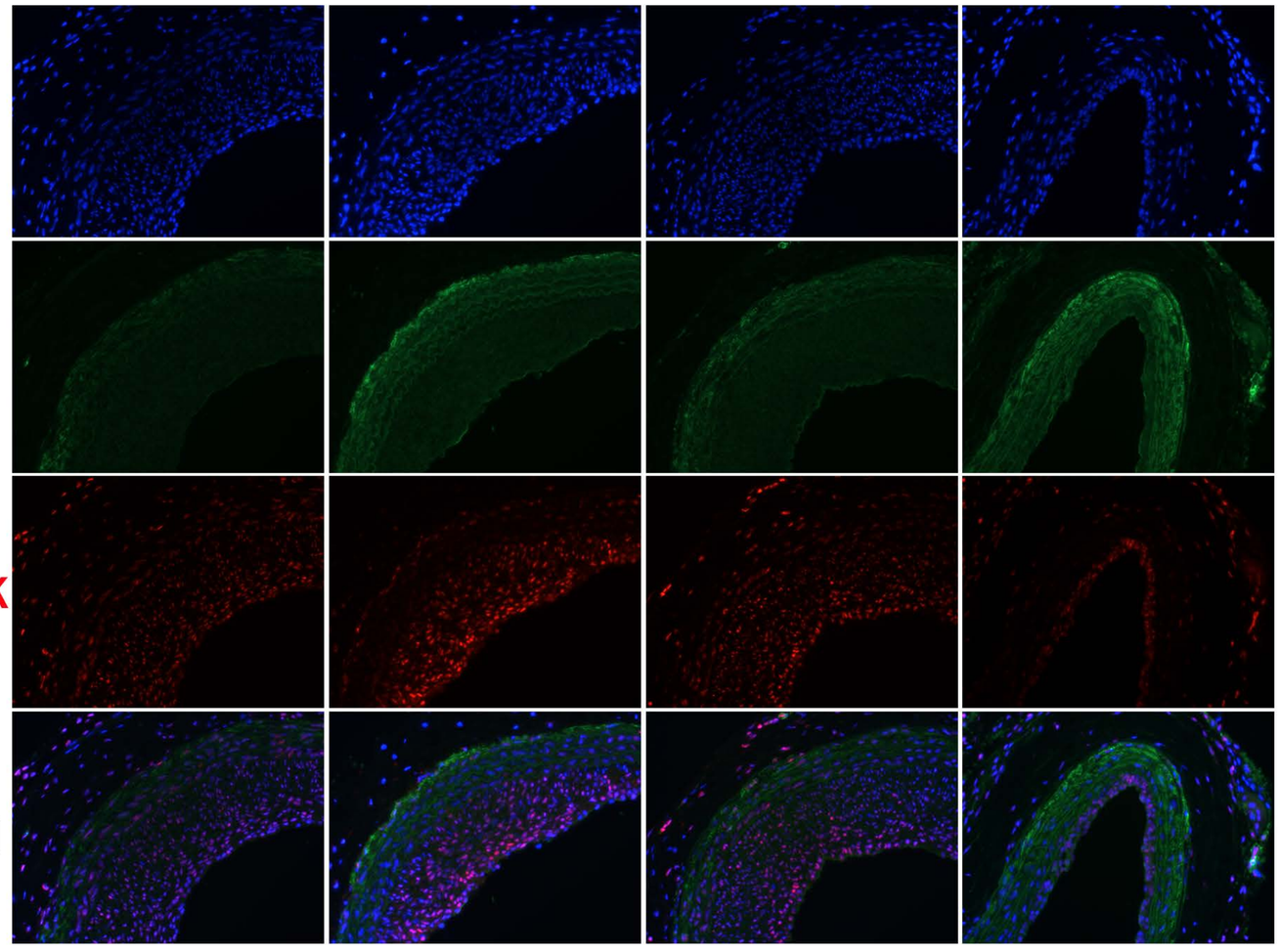
Vehicle | *GSK2606414* | *Vehicle* | *GSK2606414*

DAPI

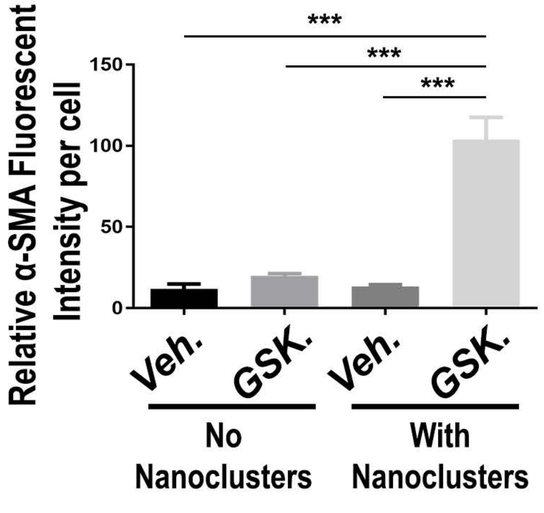
α-SMA

P-PERK

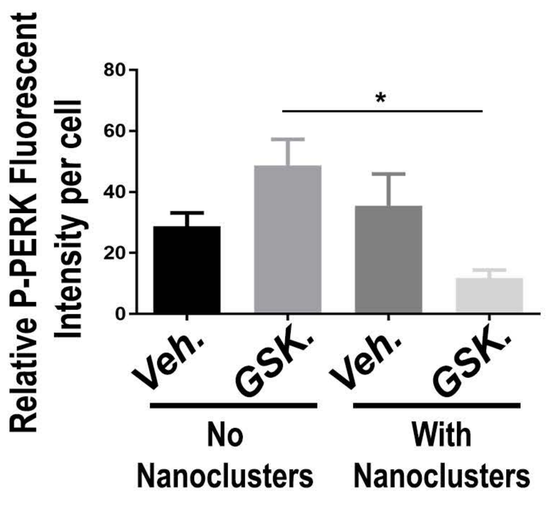
Merged



B



C



D

Peri-carotid Hydrogel

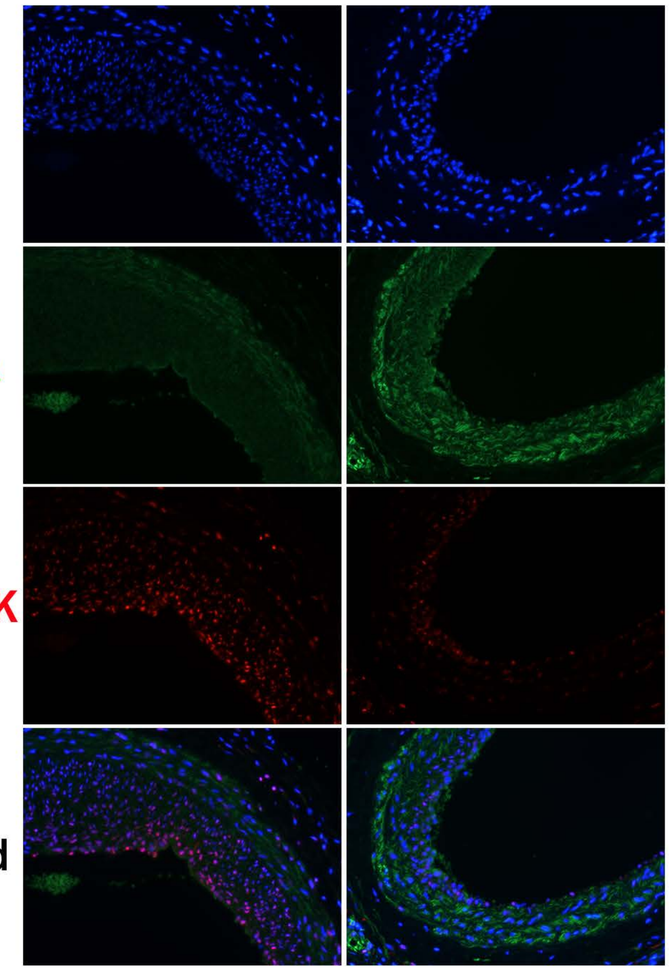
Vehicle | *GSK2606414*

DAPI

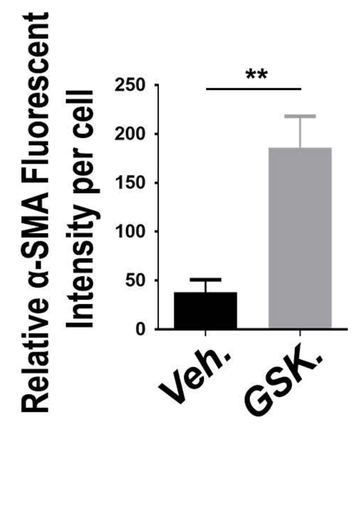
α-SMA

P-PERK

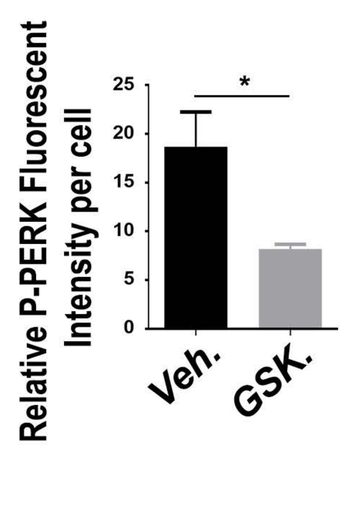
Merged

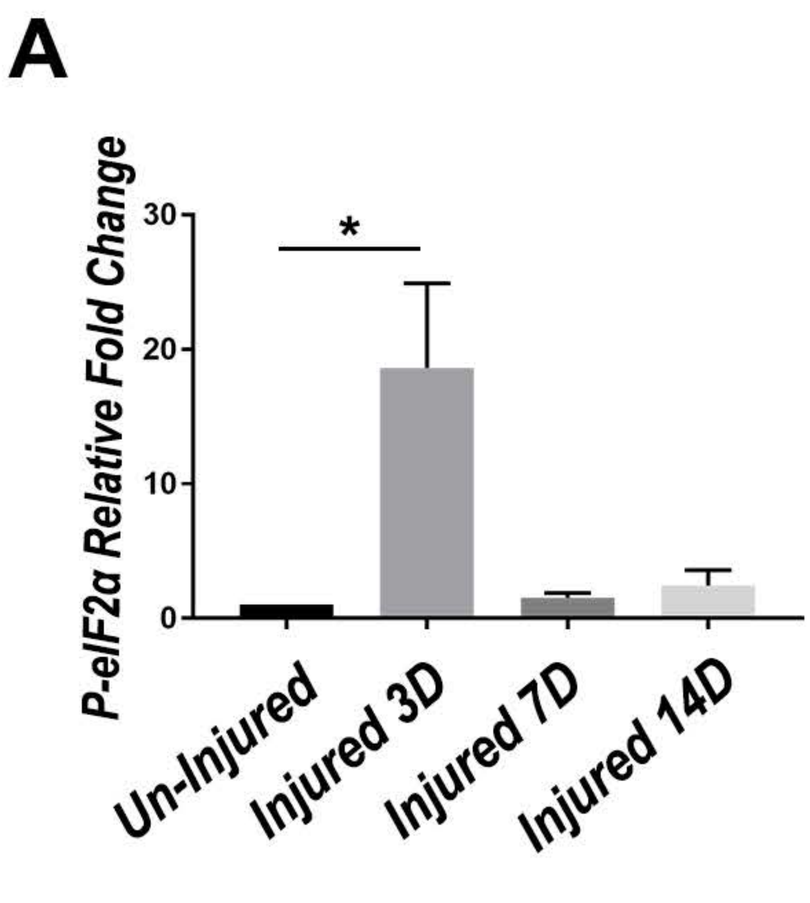


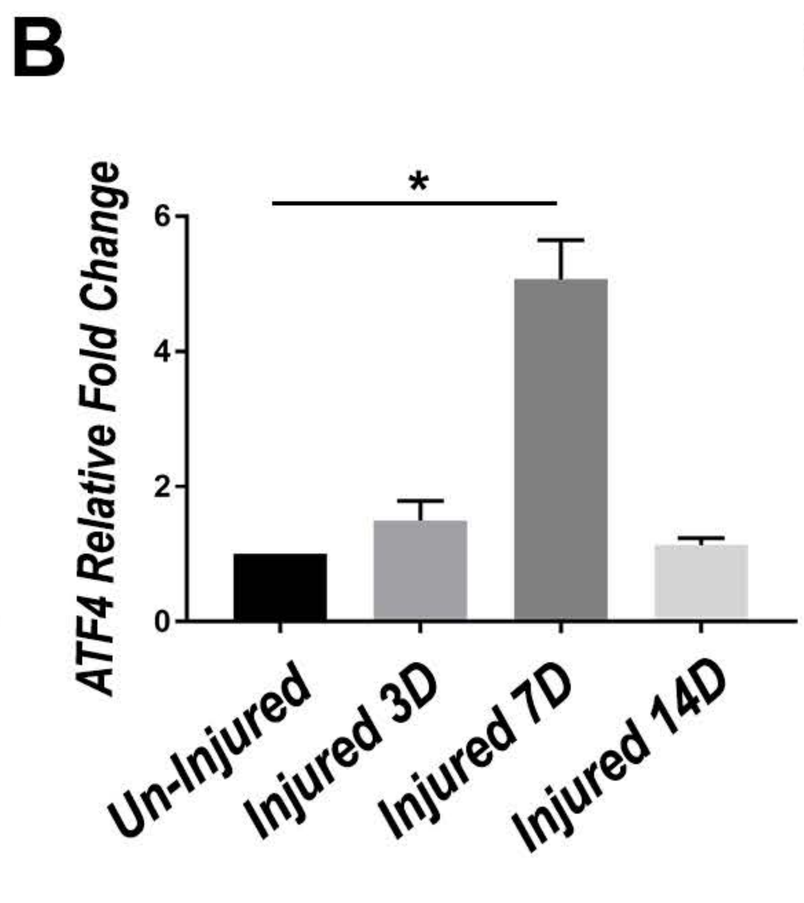
E

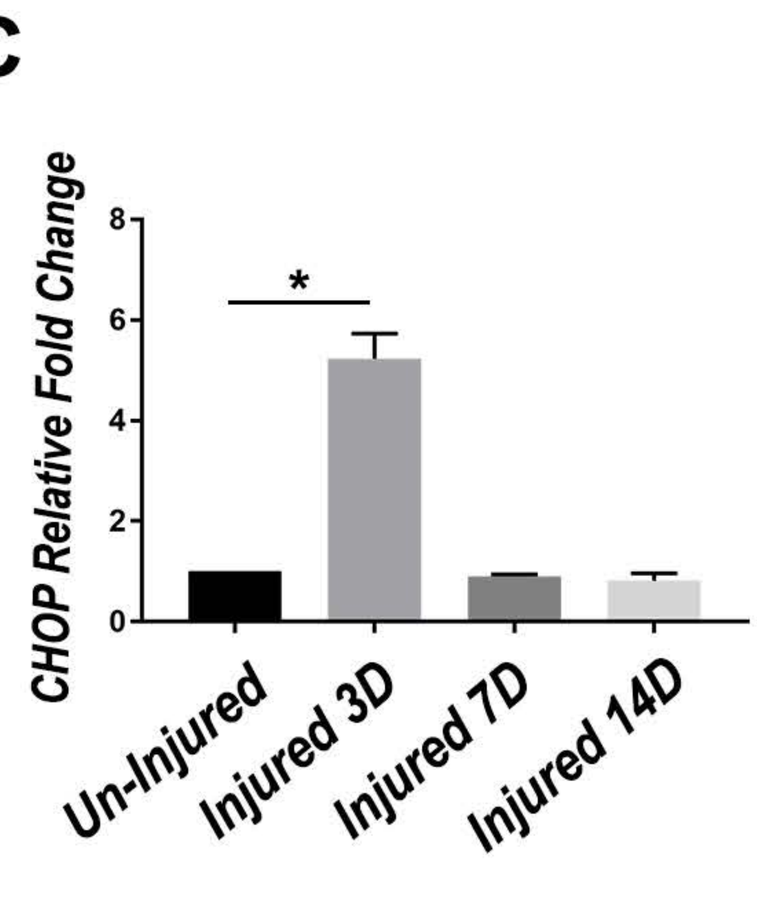


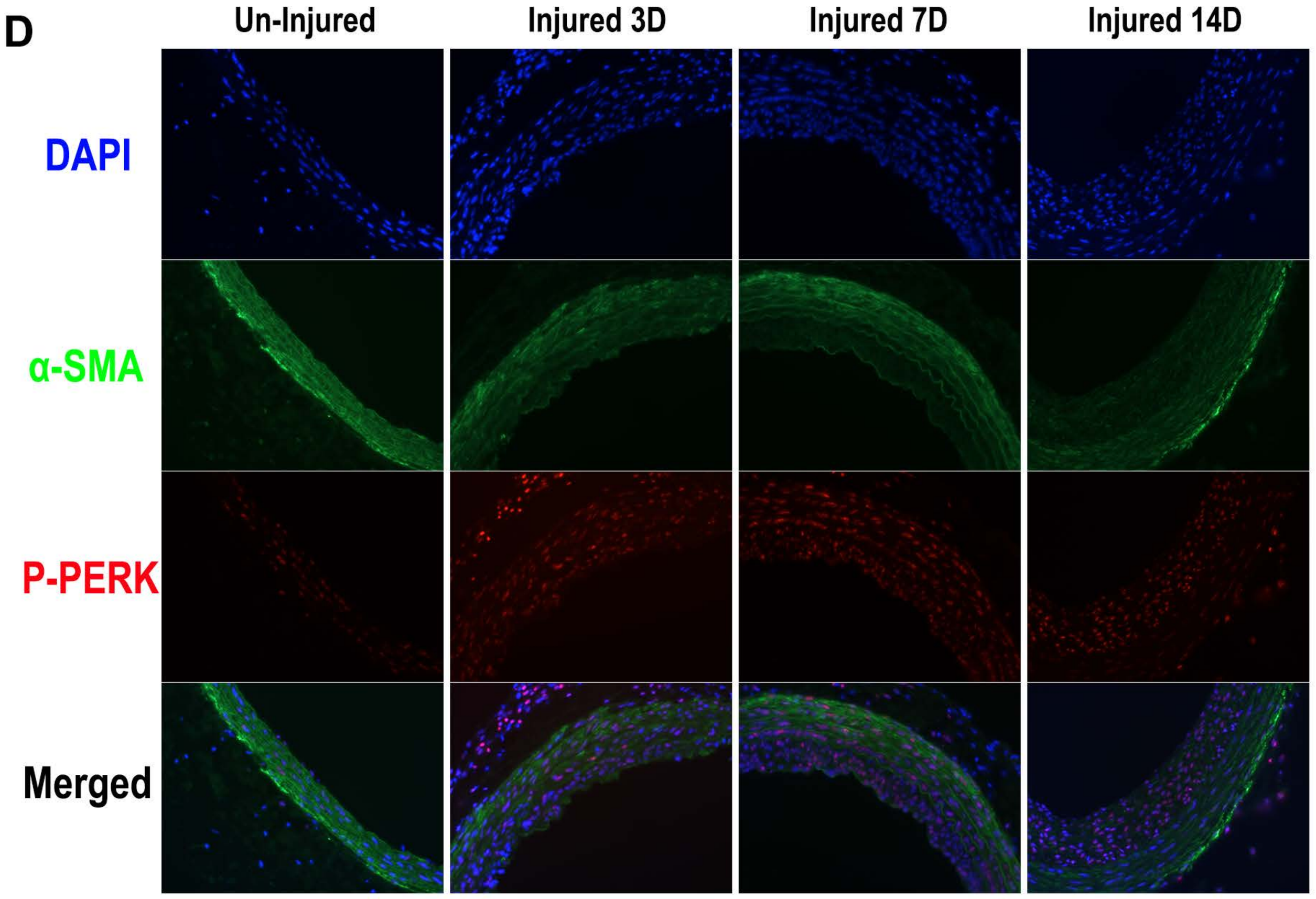
F

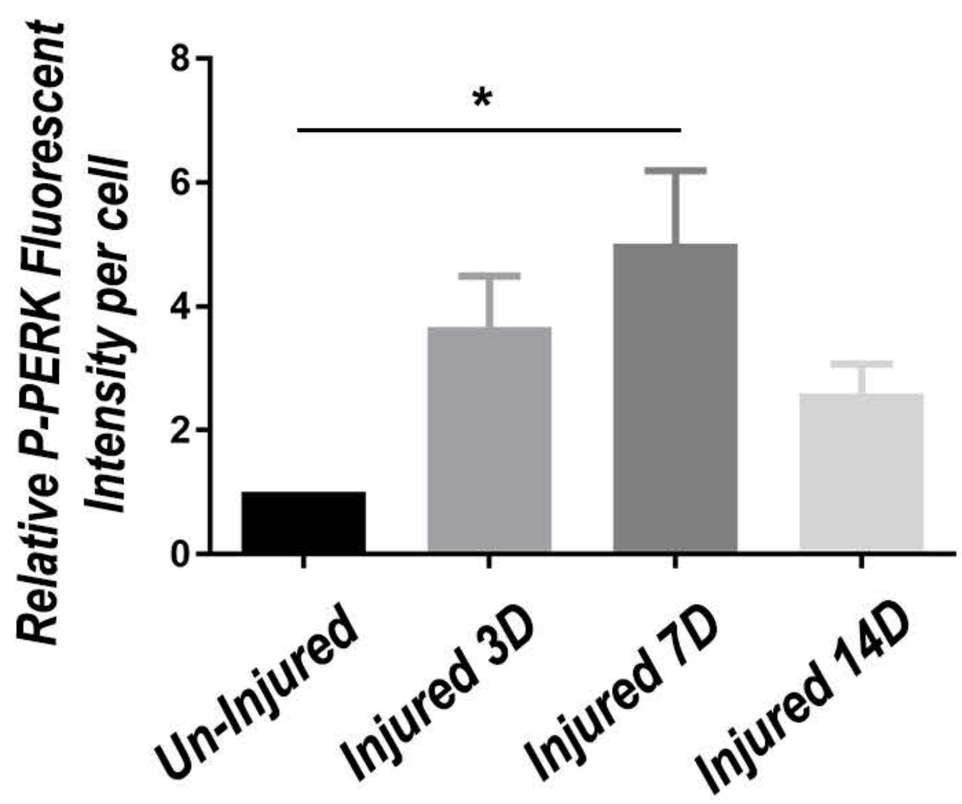


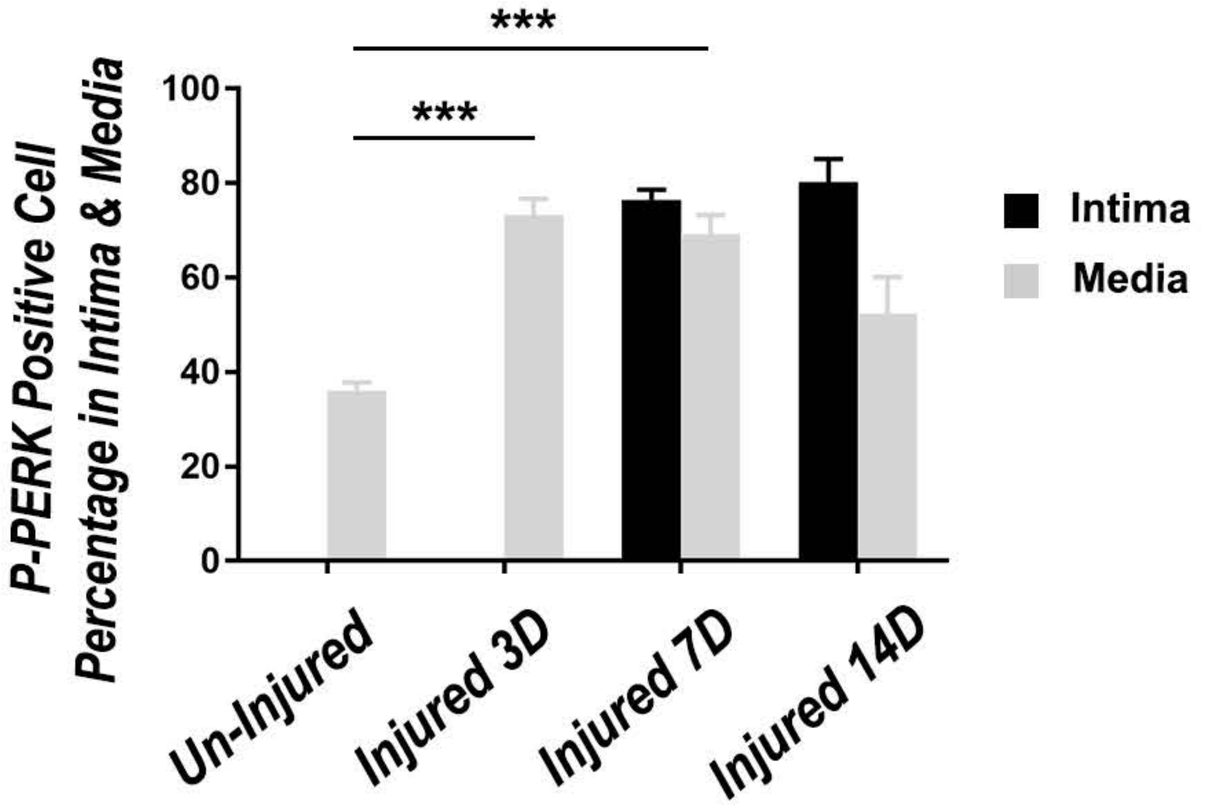

A
P-eIF2α Relative Fold Change
30
20
10
0
*
Un-Injured
Injured 3D
Injured 7D
Injured 14D


B
ATF4 Relative Fold Change
6
4
2
0
*
Un-Injured
Injured 3D
Injured 7D
Injured 14D

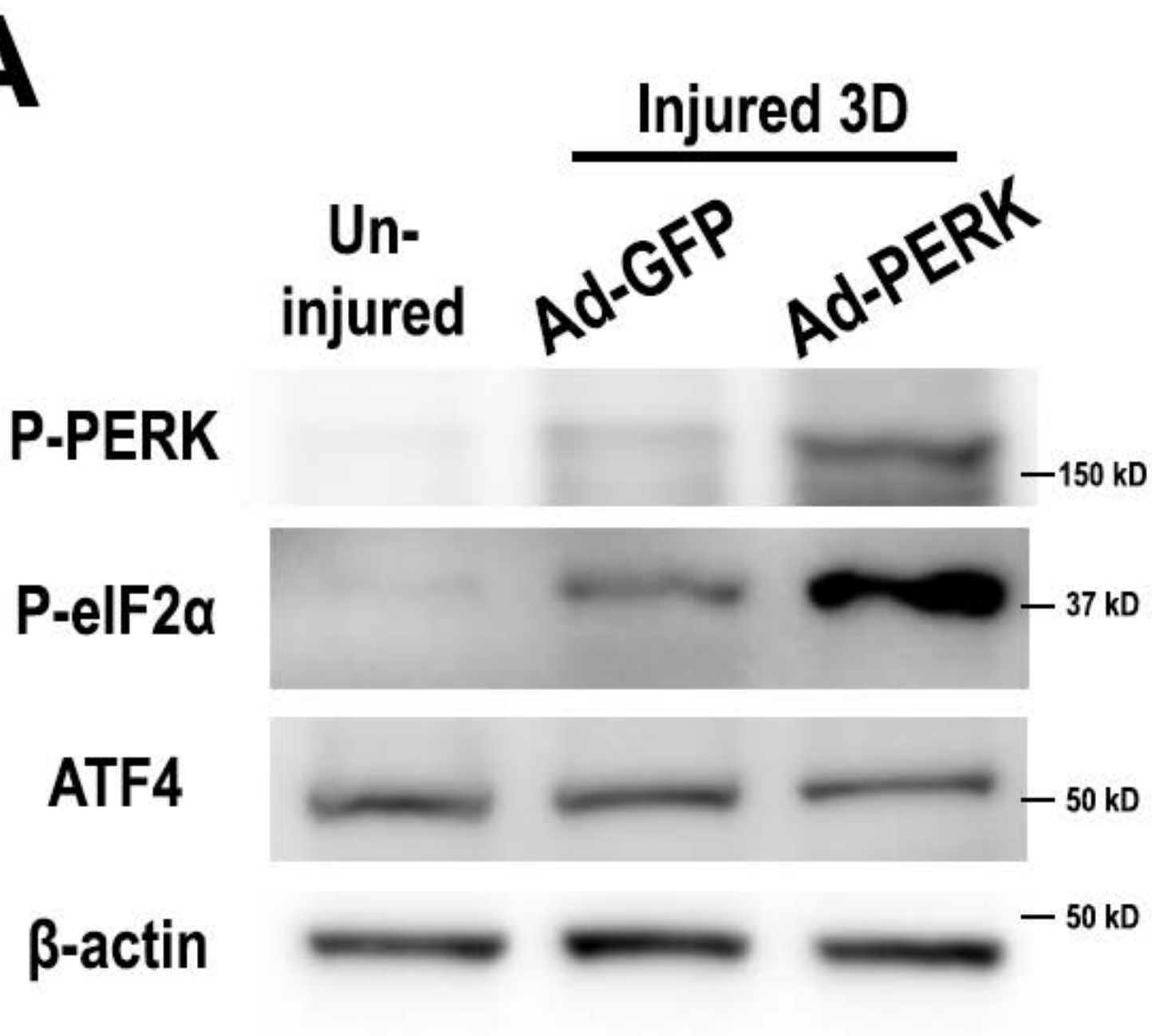

C
CHOP Relative Fold Change
8
6
4
2
0
*
Un-Injured
Injured 3D
Injured 7D
Injured 14D


D
Un-Injured
Injured 3D
Injured 7D
Injured 14D
DAPI
α-SMA
P-PERK
Merged

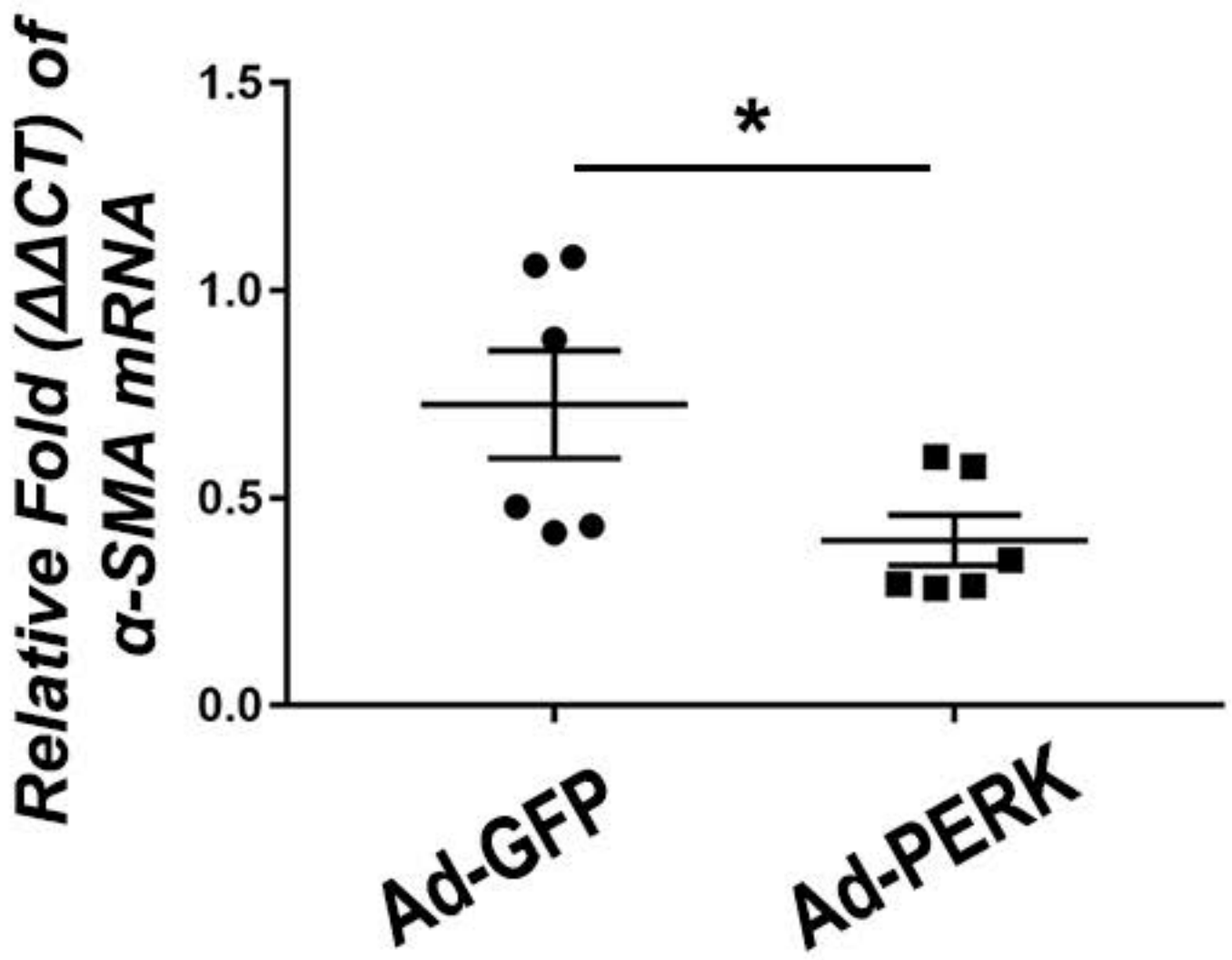

E
Relative P-PERK Fluorescent Intensity per cell
8
6
4
2
0
*
Un-Injured
Injured 3D
Injured 7D
Injured 14D


F
P-PERK Positive Cell Percentage in Intima & Media
100
80
60
40
20
0
***
***
Un-Injured
Injured 3D
Injured 7D
Injured 14D
Intima
Media

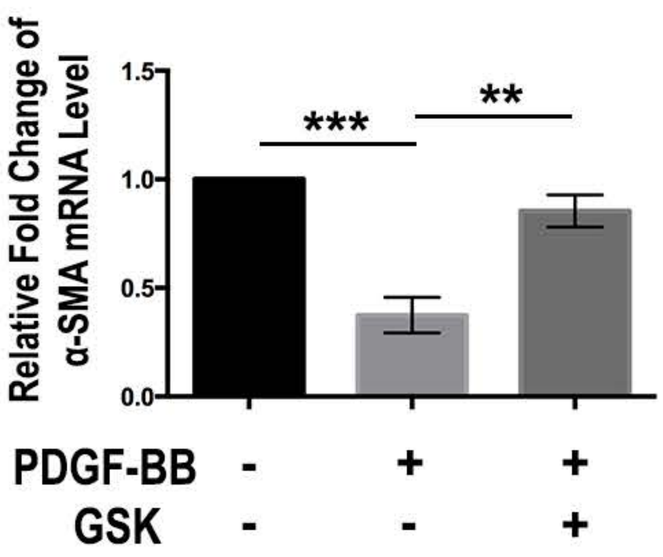
A


Injured 3D
Un-injured
Ad-GFP
Ad-PERK
P-PERK
P-eIF2α
ATF4
β-actin
150 kD
37 kD
50 kD
50 kD

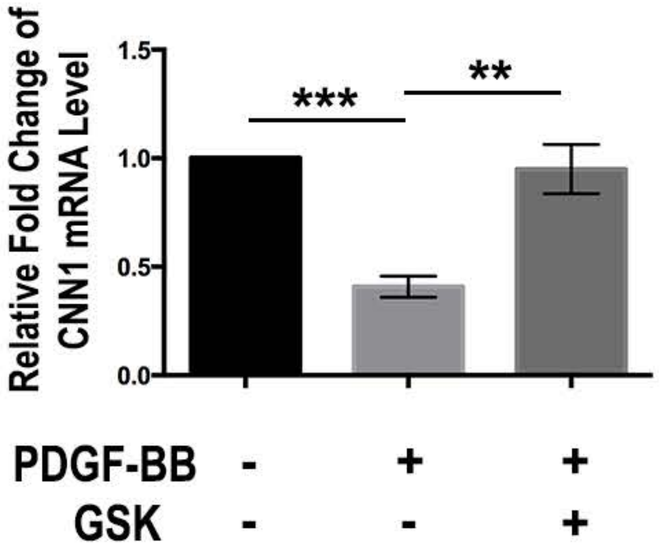
B


Relative Fold (ΔΔCT) of α-SMA mRNA
1.5
1.0
0.5
0.0
*
Ad-GFP
Ad-PERK

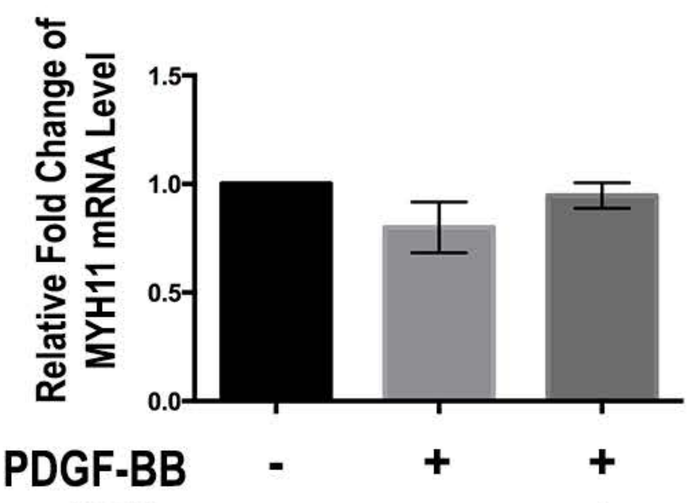
A


Relative Fold Change of α-SMA mRNA Level
1.5
1.0
0.5
0.0
***
**
PDGF-BB - + +
GSK - - +

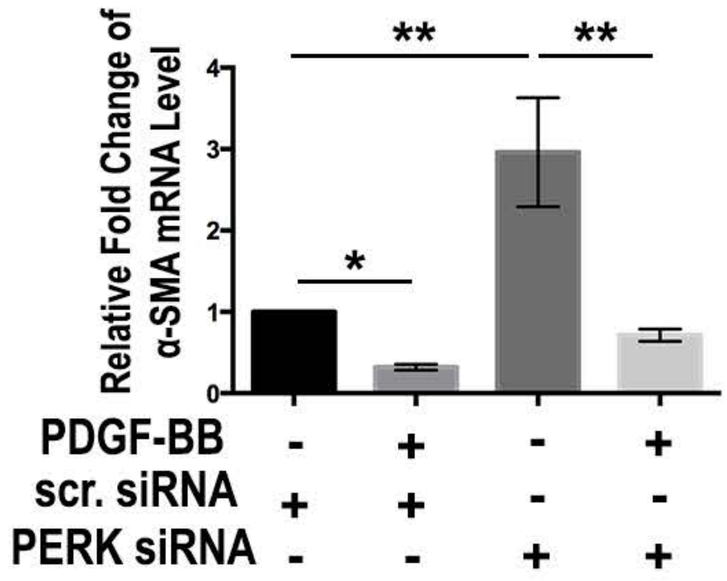
B


Relative Fold Change of CNN1 mRNA Level
1.5
1.0
0.5
0.0
***
**
PDGF-BB - + +
GSK - - +

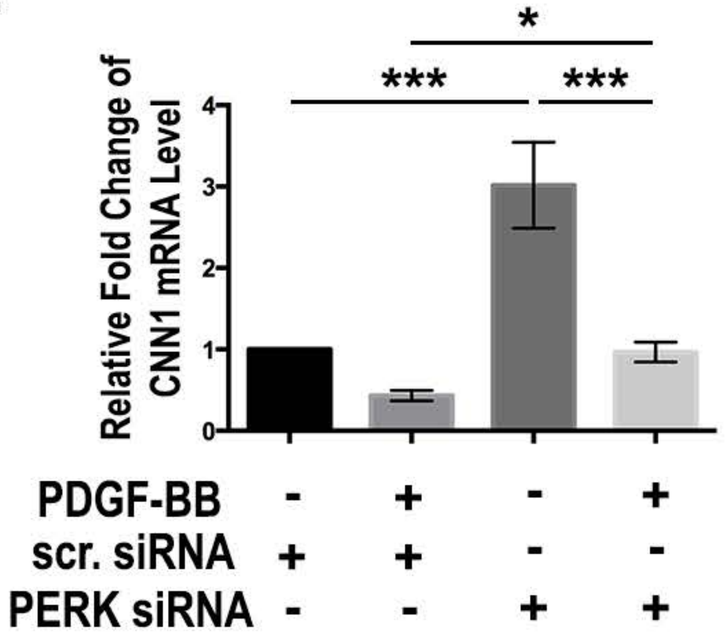
C


Relative Fold Change of MYH11 mRNA Level
1.5
1.0
0.5
0.0
PDGF-BB - + +
GSK - - +

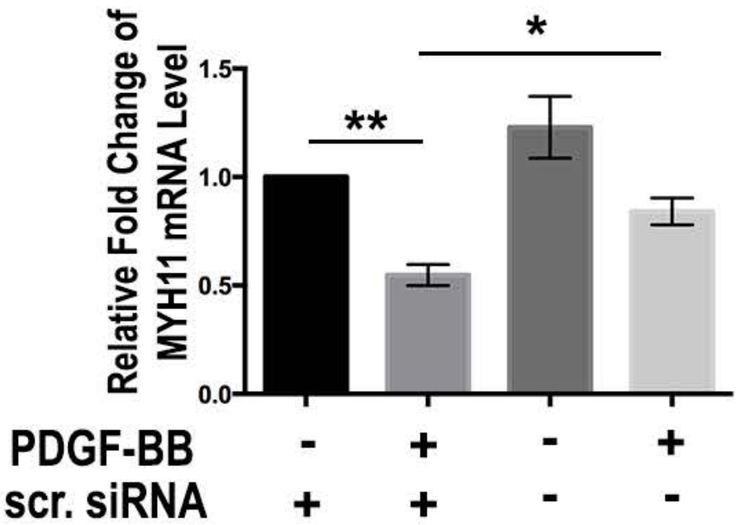
D


Relative Fold Change of α-SMA mRNA Level
4
3
2
1
0
**
**
*
PDGF-BB - + - +
scr. siRNA + + - -
PERK siRNA - - + +

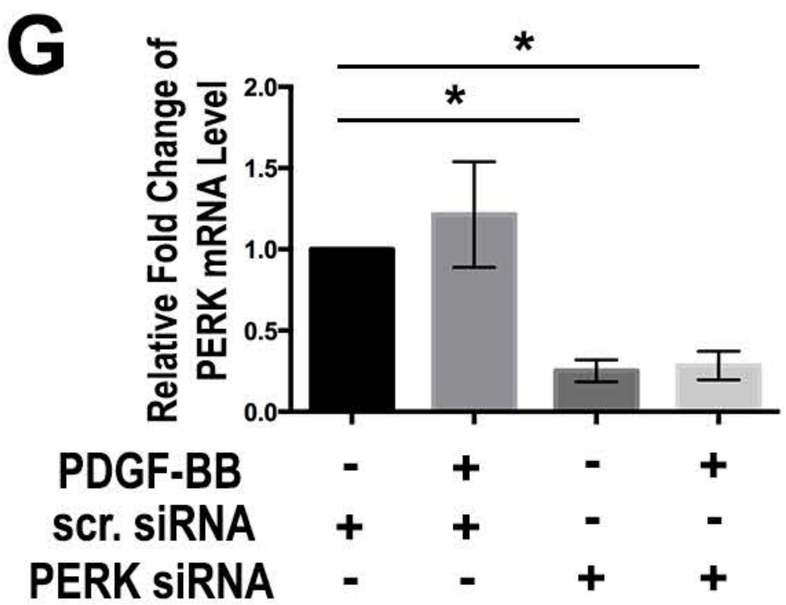
E


Relative Fold Change of CNN1 mRNA Level
4
3
2
1
0
***
***
*
PDGF-BB - + - +
scr. siRNA + + - -
PERK siRNA - - + +

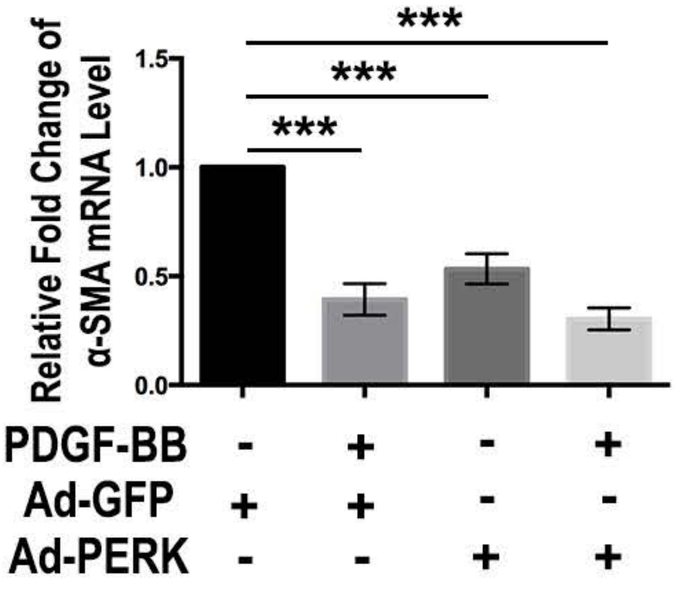
F


Relative Fold Change of MYH11 mRNA Level
1.5
1.0
0.5
0.0
**
*
PDGF-BB - + - +
scr. siRNA + + - -
PERK siRNA - - + +

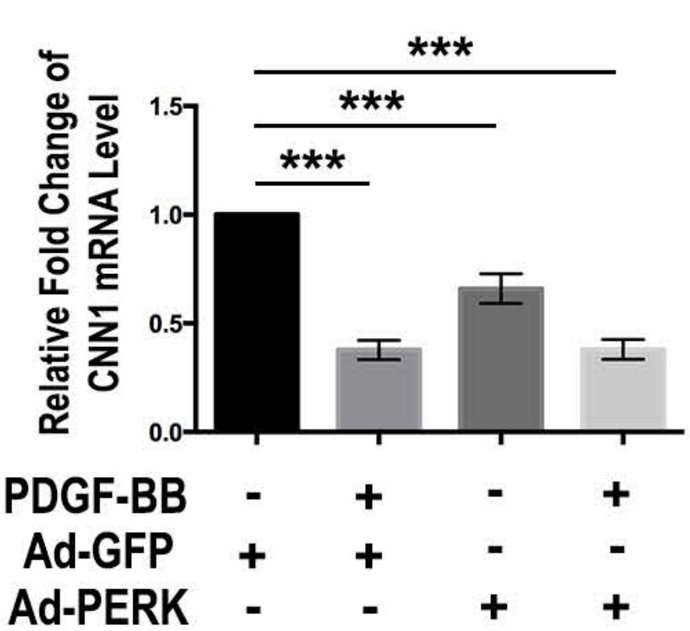
G


Relative Fold Change of PERK mRNA Level
2.0
1.5
1.0
0.5
0.0
*
*
PDGF-BB - + - +
scr. siRNA + + - -
PERK siRNA - - + +

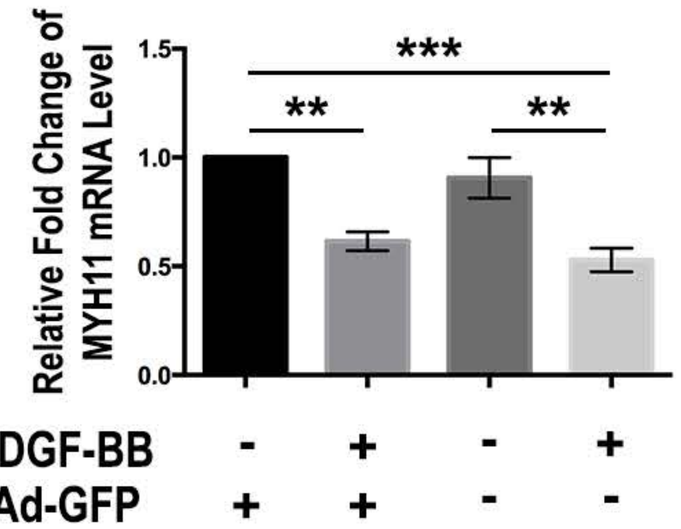
H


Relative Fold Change of α-SMA mRNA Level
1.5
1.0
0.5
0.0
***
***
***
PDGF-BB - + - +
Ad-GFP + + - -
Ad-PERK - - + +

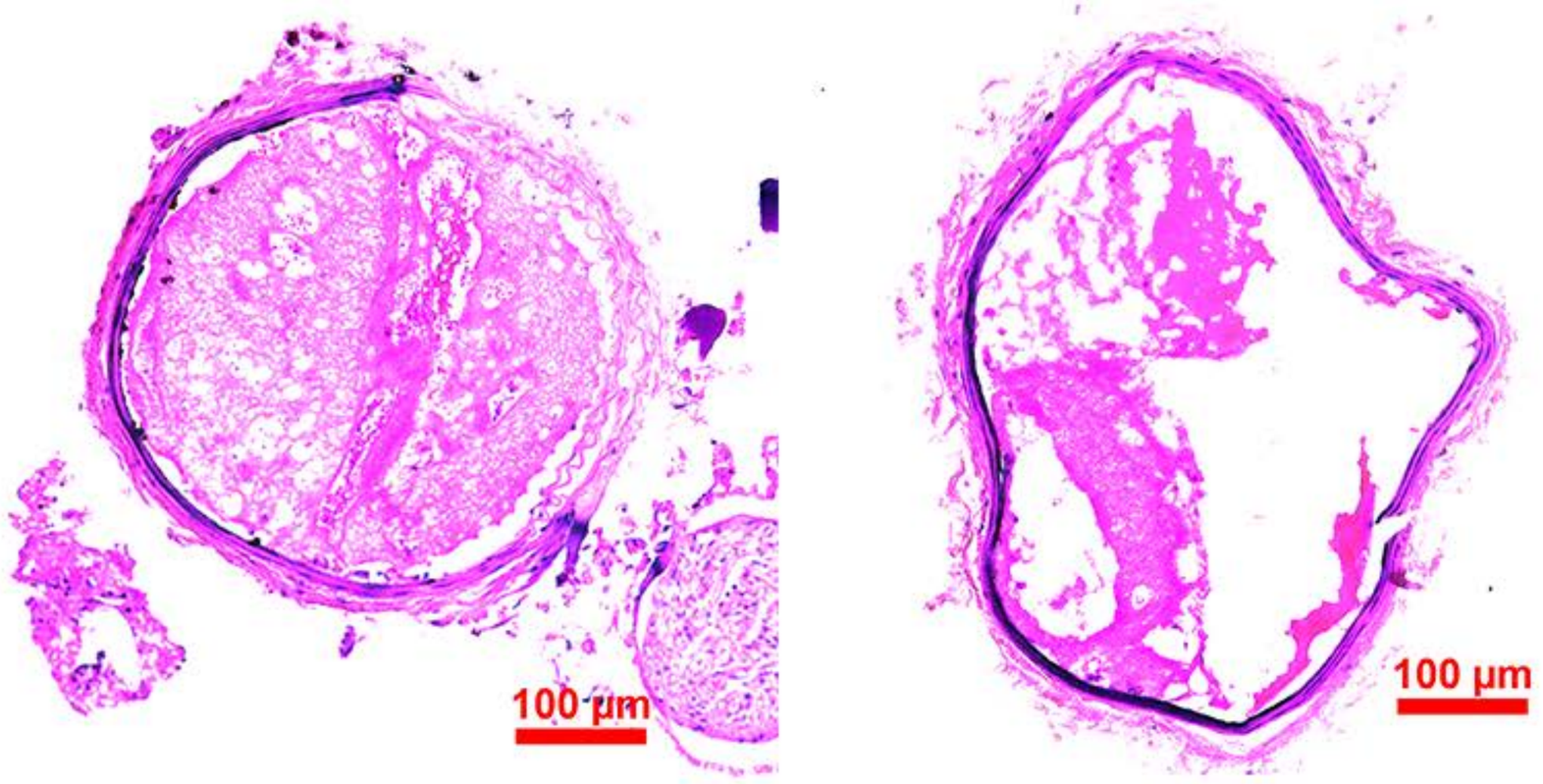
I


Relative Fold Change of CNN1 mRNA Level
1.5
1.0
0.5
0.0
***
***
***
PDGF-BB - + - +
Ad-GFP + + - -
Ad-PERK - - + +

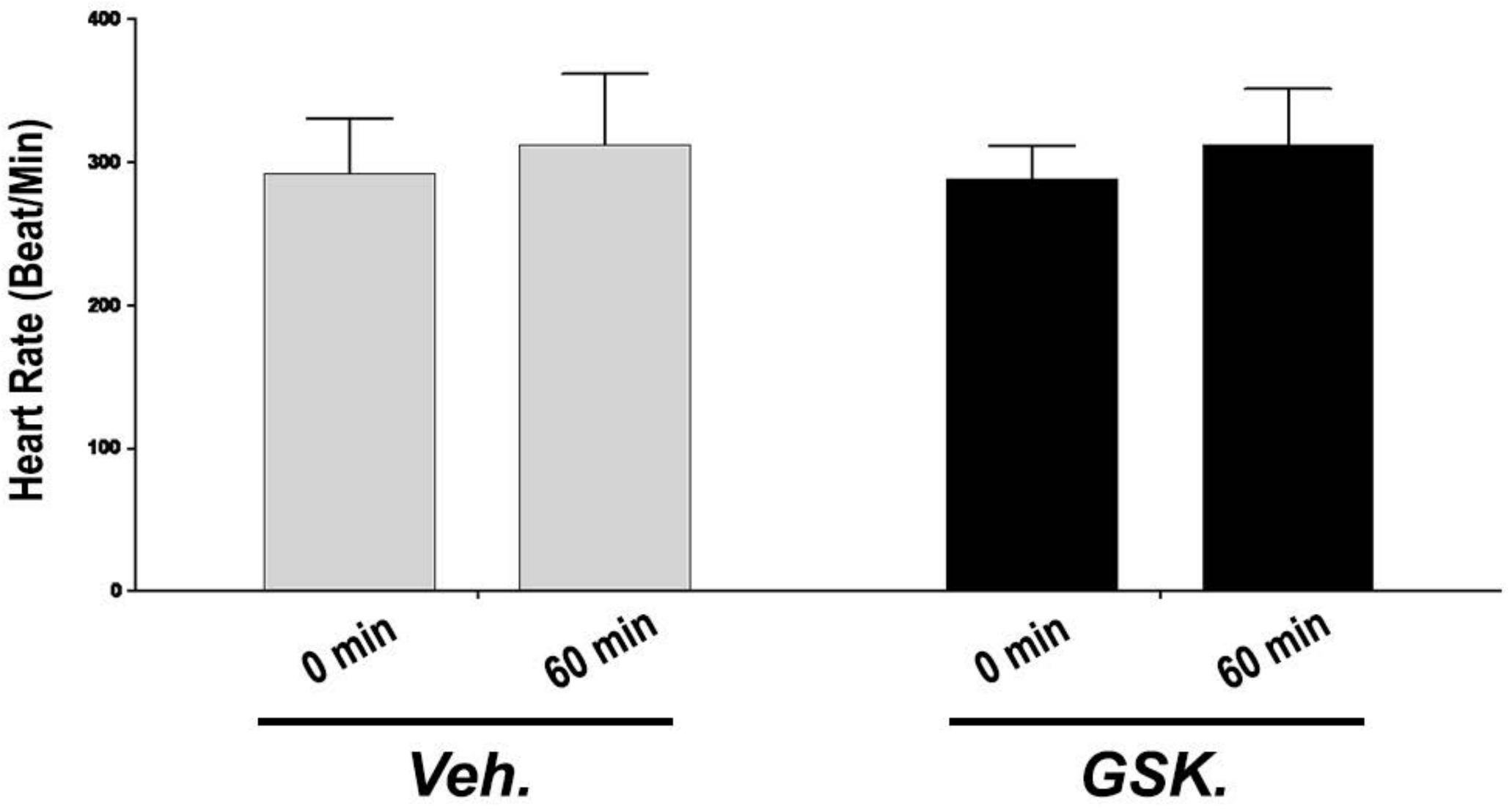
J


Relative Fold Change of MYH11 mRNA Level
1.5
1.0
0.5
0.0
***
**
**
PDGF-BB - + - +
Ad-GFP + + - -
Ad-PERK - - + +

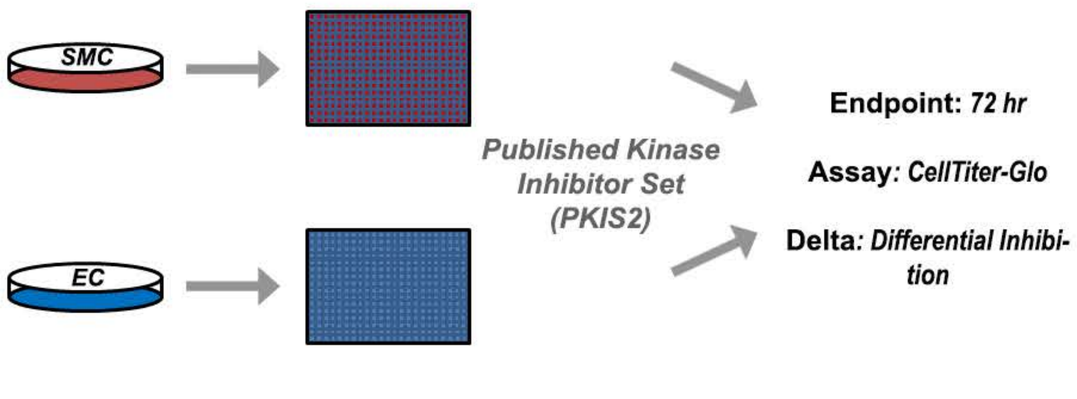
A


Veh.
GSK.
100 μm
100 μm

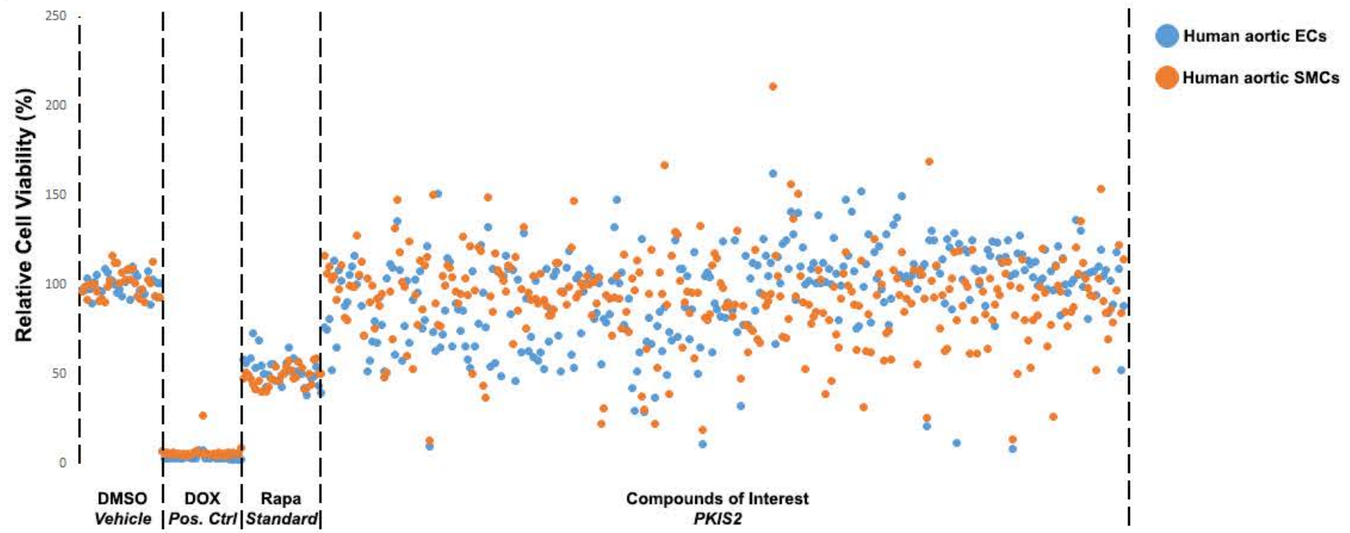
B


Heart Rate (Beat/Min)
400
300
200
100
0
0 min
60 min
0 min
60 min
Veh.
GSK.

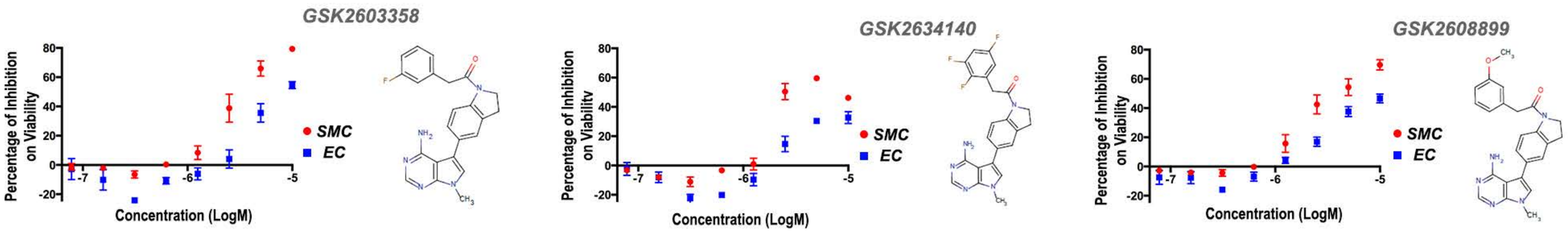
A


SMC
EC
Published Kinase Inhibitor Set (PKIS2)
Endpoint: 72 hr
Assay: CellTiter-Glo
Delta: Differential Inhibition

B


Relative Cell Viability (%)
250
200
150
100
50
0
Human aortic ECs
Human aortic SMCs
DMSO
Vehicle
DOX
Pos. Ctrl
Rapa
Standard
Compounds of Interest
PKIS2

C


GSK2603358
GSK2634140
GSK2608899
Percentage of Inhibition on Viability
Concentration (LogM)
SMC
EC