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Suppl Figure II

A

WT

Akt2^-/-

Vehicle

Rapamycin

Vehicle

Rapamycin

% Ki67 Positive Cells

Vehicle Rap.

B

Control

SM-Akt2^-/-

Vehicle

Rapamycin

Vehicle

Rapamycin

% Ki67 Positive Cells

Vehicle Rap.

C

Control

SM-Akt2^-/-

Ki67 Positive Cells

Vehicle Rap.
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Suppl Figure V

A

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B

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Suppl Figure VII

A

Wild Type

Akt2−/−

Lumen Area

WT

Akt2−/−

Medial Area

ns

Control

SM-Akt1−/−

B

Lumen Area

ns

Control

SM-Akt1−/−

Medial Area

ns

Control

SM-Akt1−/−

C

Control

SM-Akt2−/−

Lumen Area

*  ns

Control

SM-Akt2−/−

Medial Area

ns
Supplemental Figure VII. Morphometry of Akt2−/−, SM-Akt1−/−, and SM-Akt2−/− femoral arteries at baseline, relative to Controls. Sections of naive femoral arteries from (A) WT or Akt2−/−, (B) Control or SM-Akt1−/−, and (C) Control or SM-Akt2−/− were stained for EVG; vessel lumen and medial area were quantified. Data are presented as mean ±SEM; *P<0.05.
Supplemental Figure VIII. Contractile protein expression in WT, Akt1−/−, and Akt2−/− femoral arteries at baseline. Sections of naive femoral arteries from mice of the indicated genotypes were immunofluorescently stained for MYH11 or ACTA2. Representative micrographs are shown.
Suppl Figure IX

A

![Western Blot Image]

**siCont.**  siAKT1  siAKT2  siAKT1+2

Vehicle  Rapamycin

Protein/GAPDH

n=3, 200 cells/sample

B

![Cell Images]

siCont.  siAKT1  siAKT2  siAKT1+2

Vehicle  Rapamycin

n=3, 200 cells/sample

C

![Graph A]

WT  Akt1^−/−  Akt2^−/−

Cell Index

Hours

D

![Graph B]

WT  Akt1^−/−  Akt2^−/−

Cell Index

Hours
Supplemental Figure IX. Opposing effects of AKT isoforms on SMC differentiated morphology and proliferation. hCASMCs were transfected with either control siRNA or siRNA against AKT1, AKT2 or both for 48hrs. (A) Representative western blot (left) and quantitation (right) of 3 independent experiments are shown to indicate efficient knockdown. (B) Knockdown cells were treated with ethanol (vehicle) or 50nM rapamycin for 24 hours. Representative photographs were taken under phase contrast microscopy to assess cell morphology as quantitated in Figure 2A. Insets show higher power. (C-D) Cell proliferation was measured using the xCELLigence system. 2,000 aortic SMCs isolated from WT, Akt1−/− and Akt2−/− mice cells were plated per well (n=3 biological replicates were each assayed in duplicate). After starvation for 8-12 hours, proliferation in 10% FBS medium was recorded at the indicated times. (D) SMC were plated and measured as in C but were pre-treated with 50nM rapamycin for 24 hours. (n=3). Data are presented as mean ± SEM; *P<0.05, **P<0.01, ***P<0.001.
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Supplemental Figure XI. Opposing effects of AKT isoforms on PAK1 phosphorylation and its association with RAC1. hCASMCs were transfected with control siRNA or siRNA against AKT1 or AKT2 for 48hrs, then treated with vehicle or PDGF-BB for 10min. Lysates were subjected to western blots. Active RAC1 in lysates was measured by pulldown with a GST-PAK1/PBD fusion protein followed by immunoblotting for RAC1 and normalized to a western blot of total RAC1 in input. Data are presented as mean ± SEM; *P<0.05, **P<0.01, ***P<0.001.
Supplemental Figure XII. Opposing effects of AKT isoforms on hAoSMC proliferation and differentiation in vitro. (A-B) hAoSMCs were transfected with either control siRNA or siRNA against AKT1, AKT2 (Dharmacon) or both for 48hrs. (A) 60,000 cells were plated per well in 6-well plates (n=3 biological replicates) in 10% FBS medium. Cells in each well were trypsinized and counted using a Cellometer (Nexcelom) after 72 hours. Cell proliferation was presented as the fold change of cell number normalized to input. (B) qPCR for indicated mRNAs normalized to GAPDH mRNA expression is shown (n=3). Data are presented as mean ±SEM; *P<0.05, ***P<0.001.
Suppl Figure XIII

A

-2000

-1561 to -1396
-1363 to -1163

#1

#2

:primer

+1

MYOCD

B

FOXO4 Primer #1

Fold Enrichment

0.0

0.5

1.0

1.5

Vehicle
Rapamycin

ns

FOXO4 Primer #2

Fold Enrichment

0.0

0.5

1.0

1.5

Vehicle
Rapamycin

ns

C

Truncated Myocd-promoter Luciferase

-654

-1664

TGTCTTC

Full length Myocd-promoter luciferase

-1036

-771

TGGTGTA

MYOCD
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Suppl Figure XIV

A

Rapamycin

\[ \text{AKT1} \]

\[ \text{AKT2} \]

proliferation, migration

contractile protein expression

B

\[ \text{AKT2} \]

\[ \text{FOXO4} \]

\[ \text{FOXO4} \]

\[ \text{MYOCD} \]

\[ \text{MYH11, TAGLN, ACTA2, etc.} \]

\[ \text{SRF} \]

\[ \text{CARG} \]
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SUPPLEMENTAL MATERIAL

Opposing actions of AKT isoforms in vascular smooth muscle injury and therapeutic response

Jin: Akt isoforms in injury response

Supplemental Figure Legends

Supplemental Figure I. SM-specific Akt2−/− mice exhibit severe intimal hyperplasia after vascular injury. (A-B) Akt2flox/flox mice (Control) or SM-Akt2−/− mice were subjected to femoral artery wire injury. EVG-stained cryosections (left) and quantitation of intima/media (right) are shown at (A) 21, and (B) 7 days post-injury. Scale bars = 100 µm. Data are presented as mean ± SEM; **P<0.01, ***P<0.001.

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