Essential Role of Smooth Muscle STIM1 in Hypertension and Cardiovascular Dysfunction

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Short title: STIM1 and cardiovascular dysfunction

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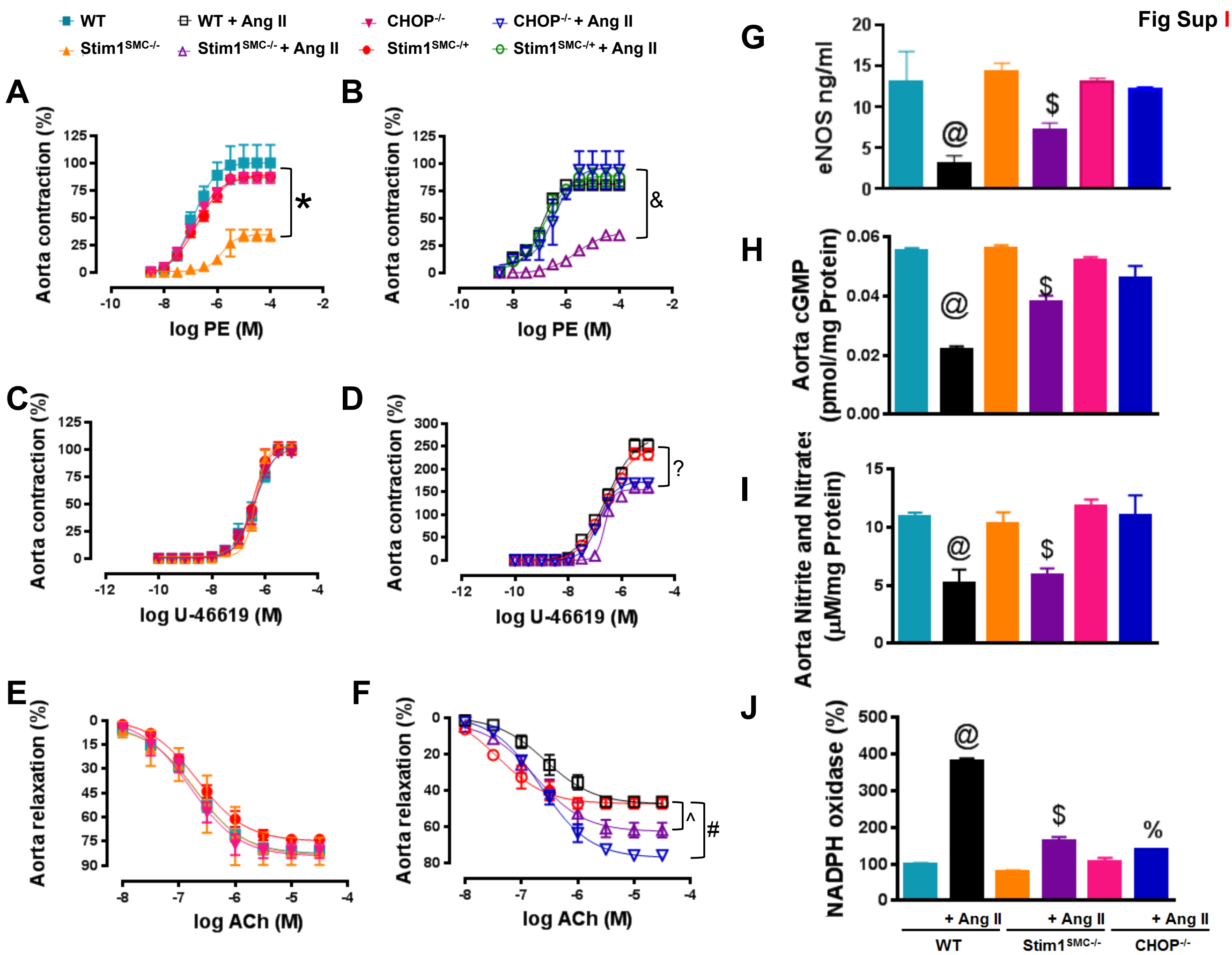

Figure Supp I.- STIM1 and CHOP mediate hypertension-induced cardiac damage and fibrosis. Wire Myograph vascular reactivity showing Contraction response to phenylephrine **(A, B, n=5-6)** and thromboxaneA2 analogue U-46619 **(C, D, n=5),** and endothelial-dependent relaxation in response to acetylcholine **(E, F, n=5)** in thoracic aorta from in mesenteric resistance arteries from WT, heterozygous (Stim1$^{SMC-/+}$) and homozygous Stim1 knockout specifically in smooth muscle (Stim1$^{SMC-/-}$), and CHOP knockout (CHOP$^{-/-}$) mice infused with saline or Ang II. cGMP levels were determined using a sandwich enzyme-linked immunosorbent assay **(G, n=3)** Elisa showing eNOS levels **(I, n=3),**and nitrites/nitrate levels were determined using the Griess reaction **(H, n=3),** and NADPH oxidase using lucigenin chemiluminescence assays **(J, n=5),** in thoracic aortas from WT, Stim1$^{SMC-/-}$ and CHOP$^{-/-}$ mice infused with saline or Ang II.

Two-way repeated measured ANOVA followed by *Tukey's Post-Hoc test* was applied for figures (A, B, C, D, E, F). One-way ANOVA followed by *Bonferroni Post-Hoc test* was applied for figures (G, H, I, J).

*p<0.05 between STIM$^{SMC-/-}$ vs WT, STIM$^{SMC-/+}$, and CHOP$^{-/-}$. $^{\&}$p<0.05 between STIM$^{SMC-/-}$ + AngII vs WT + Ang II, STIM$^{SMC-/+}$ + Ang II, CHOP$^{-/-}$ + Ang II. $^{?}$p<0.05 between STIM$^{SMC-/-}$ + AngII, CHOP$^{-/-}$ + Ang II VS WT + Ang II, STIM$^{SMC-/+}$ + Ang II. $^{\wedge}$p<0.05 between WT + Ang II, STIM$^{SMC-/+}$ + Ang II vs STIM$^{SMC-/-}$ + AngII. $^{\#}$p<0.05 between WT + Ang II, STIM$^{SMC-/+}$ + Ang II vs STIM$^{SMC-/-}$ + AngII vs CHOP$^{-/-}$ + Ang II. $^{@}p<0.05$ between WT + Ang II vs WT, STIM$^{SMC-/-}$, STIM$^{SMC-/-}$ + AngII, CHOP$^{-/-}$, CHOP$^{-/-}$ + Ang II. $^{\$}p<0.05$ between STIM$^{SMC-/-}$ vs STIM$^{SMC-/-}$ + AngII. $^{\%}p<0.05$ between CHOP$^{-/-}$ vs CHOP$^{-/-}$ + Ang II.

Fig Sup II

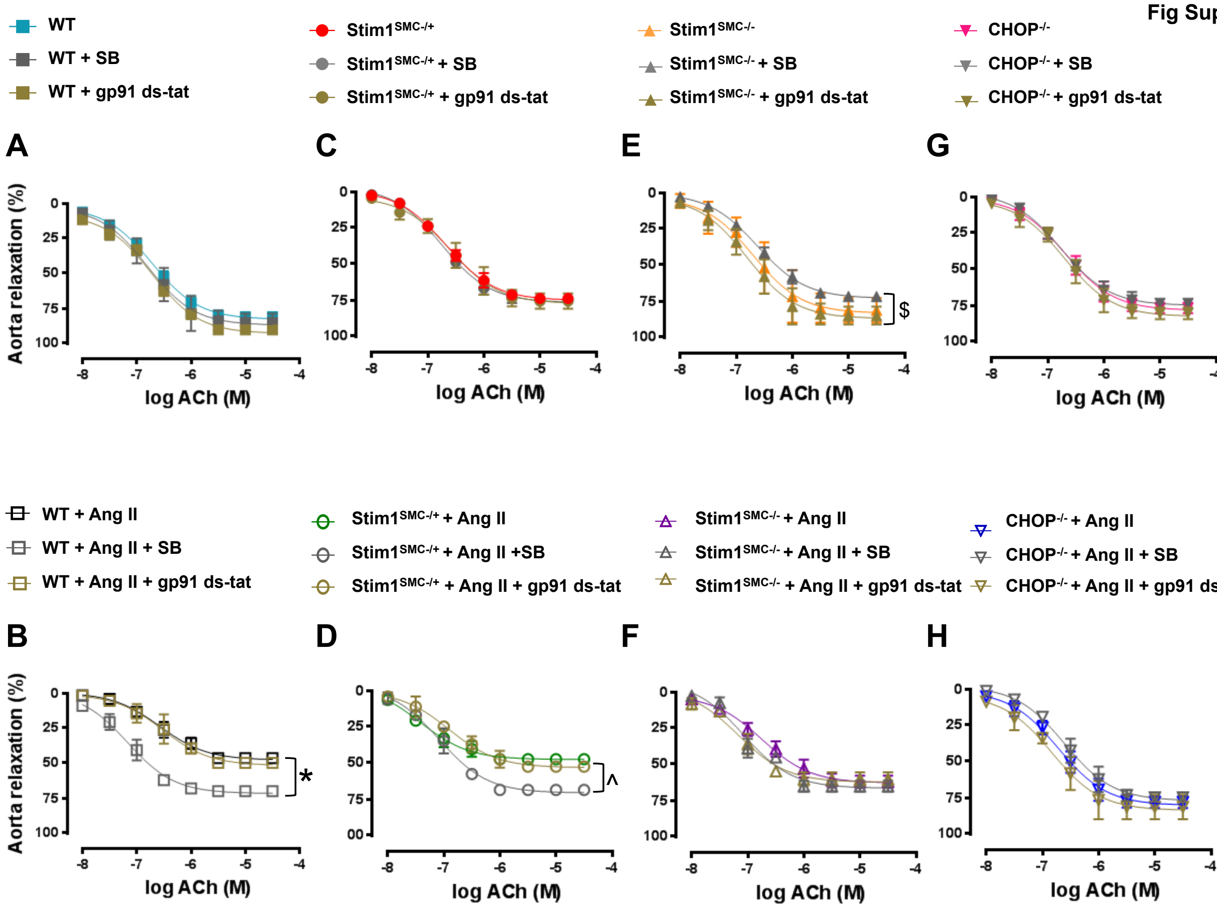

Figure Supp. II.- STIM1 and CHOP regulate hypertension-induced vascular damage. Wire Myograph vascular reactivity showing endothelial-dependent relaxation in response to acetylcholine before and after incubation with TGF-β inhibitor (SB431542) and NADPH oxidase inhibitor (gp91 ds-tat) in thoracic aorta from:

- WT mice infused with saline or AngII **(A, B, n=5)**
- Heterozygous Stim1 knockout specifically in SMC (Stim1$^{SMC-/+}$) mice infused with saline or AngII **(C, D, n=5)**
- Homozygous Stim1 knockout specifically in SMC (Stim1$^{SMC-/-}$) mice infused with saline or AngII **(E, F)**
- CHOP knockout (CHOP$^{-/-}$) mice infused with saline or AngII **(G, H, n=5)**

Two-way repeated measured ANOVA followed by *Tukey's Post-Hoc test* was applied for figures (A, B, C, D, E, F, G, H).

*p<0.05 between WT + Ang II + gp91 ds-tat, WT + Ang II vs WT + Ang II + SB. $^{\wedge}$p<0.05 between STIM$^{SMC-/+}$ + Ang II + gp91 ds-tat, STIM$^{SMC-/+}$ + Ang II vs STIM$^{SMC-/+}$ + Ang II + SB.

Fig Sup III

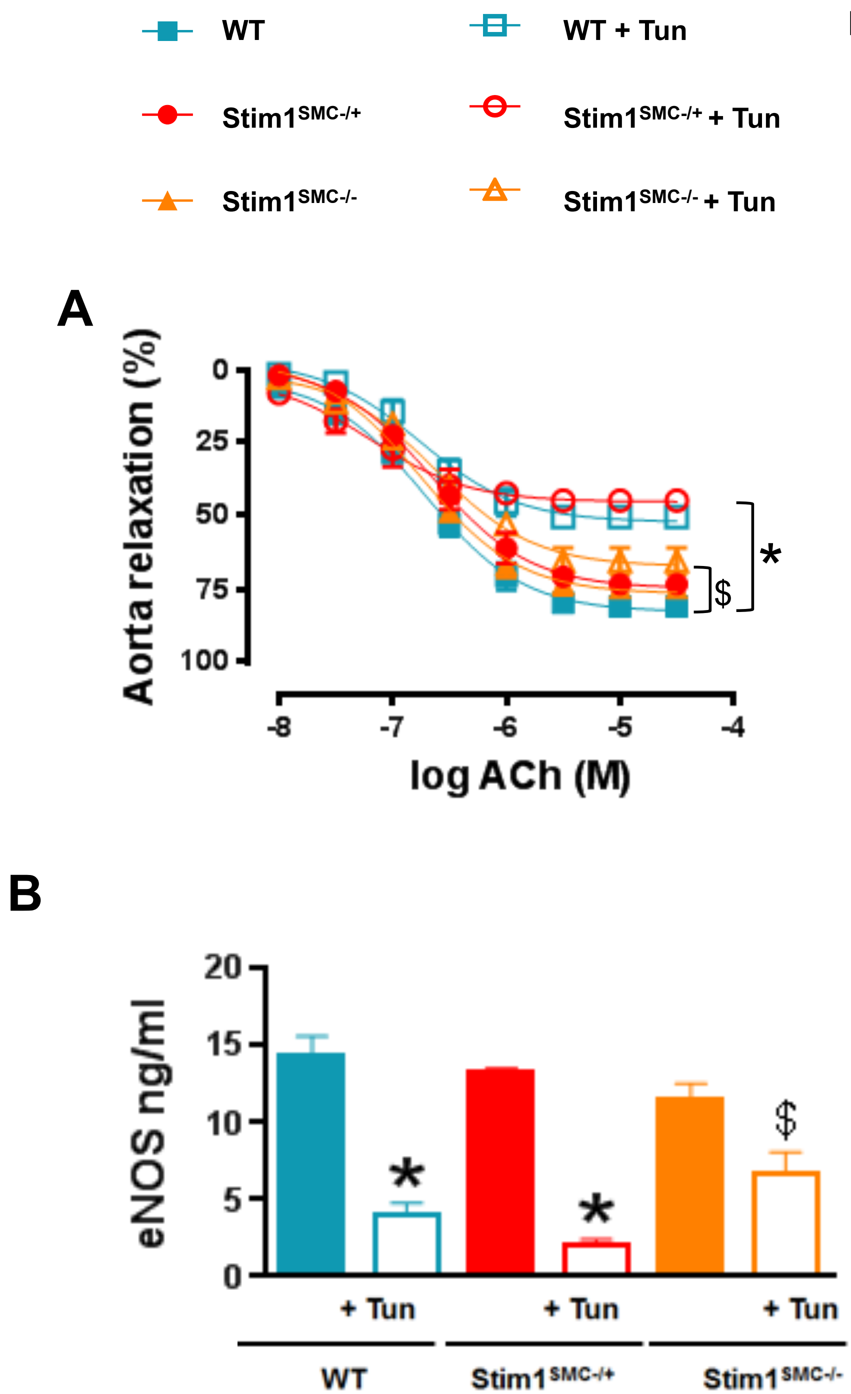

Figure Supp. III.- Effect of Tunicamycin-induced ER stress on vascular reactivity in STIM1 knockout mice. Wire Myograph vascular reactivity showing endothelial-dependent relaxation in response to acetylcholine **(A, n=5)** and eNOS levels **(B, n=3)** in thoracic aorta from WT, heterozygous (Stim1$^{SMC-/+}$) and homozygous Stim1 knockout specifically in SMC (Stim1$^{SMC-/-}$) mice treated with saline or Tunicamycin.

Two-way repeated measured ANOVA followed by *Tukey's Post-Hoc test* was applied for figure (A). One-way ANOVA followed by *Bonferroni Post-Hoc test* was applied for figure (B).

$^{\$}p<0.05$ between STIM$^{SMC-/+}$ + Tunica vs WT, STIM$^{SMC-/-}$, STIM$^{SMC-/+}$. $^{*}p<0.05$ between STIM$^{SMC-/+}$ + Tunica, WT + Tunica vs STIM$^{SMC-/+}$ + Tunica, WT, STIM$^{SMC-/-}$, STIM$^{SMC-/+}$.. $^{\%}p<0.05$ between STIM$^{SMC-/-}$ + Tunica vs STIM$^{SMC-/+}$ + Tunica and WT + Tunica.

Fig Sup IV

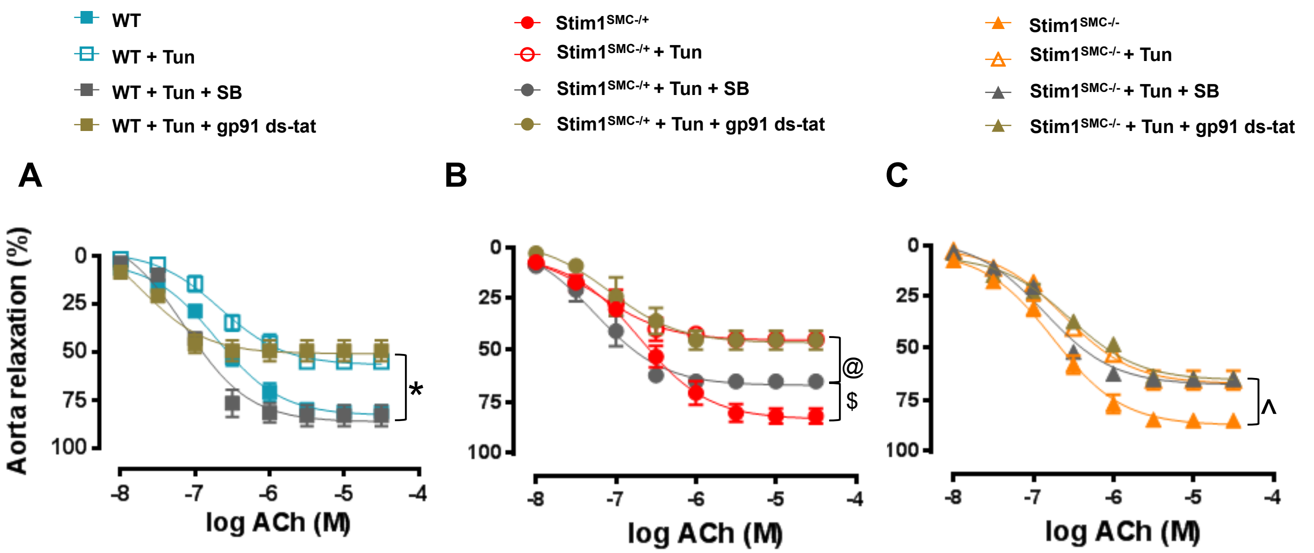

Figure Supp. IV.- Effect of inhibition of NADPH oxidase and TGF-β signaling on Vascular reactivity in tunicamycin-treated STIM1 knockout mice. Wire Myograph vascular reactivity showing endothelial-dependent relaxation in response to acetylcholine before and after incubation with TGF-β inhibitor (SB431542) and NADPH oxidase inhibitor (gp91 ds-tat) in thoracic aorta from:

- WT mice treated with saline or Tunicamycin **(A, n=5)**
- Heterozygous Stim1 knockout specifically in SMC ($Stim1^{SMC-/+}$) mice treated with saline or Tunicamycin **(B, n=5)**
- Homozygous Stim1 knockout specifically in SMC ($Stim1^{SMC-/-}$) mice treated with saline or Tunicamycin **(C, n=5)**

Two-way repeated measured ANOVA followed by Tukey's Post-Hoc test was applied for figures (A, B, C)

$^{*}p<0.05$ between WT + Tunica, WT + Tunica + gp91 ds-tat vs WT + Tunica + SB. $^{\$}p<0.05$ between $STIM^{SMC-/+}$ vs $STIM^{SMC-/+}$ + Tunica + SB. $^{@}p<0.05$ between $STIM^{SMC-/+}$ + Tunica + SB vs $STIM^{SMC-/+}$ + Tunica, $STIM^{SMC-/+}$ + Tunica + gp91 ds-tat. $^{\wedge}p<0.05$ between $STIM^{SMC-/-}$ vs $STIM^{SMC-/-}$ + Tunica, $STIM^{SMC-/-}$ + Tunica + gp91 ds-tat, $STIM^{SMC-/-}$ + $STIM^{SMC-/-}$ + Tunica + SB.

Fig Sup V

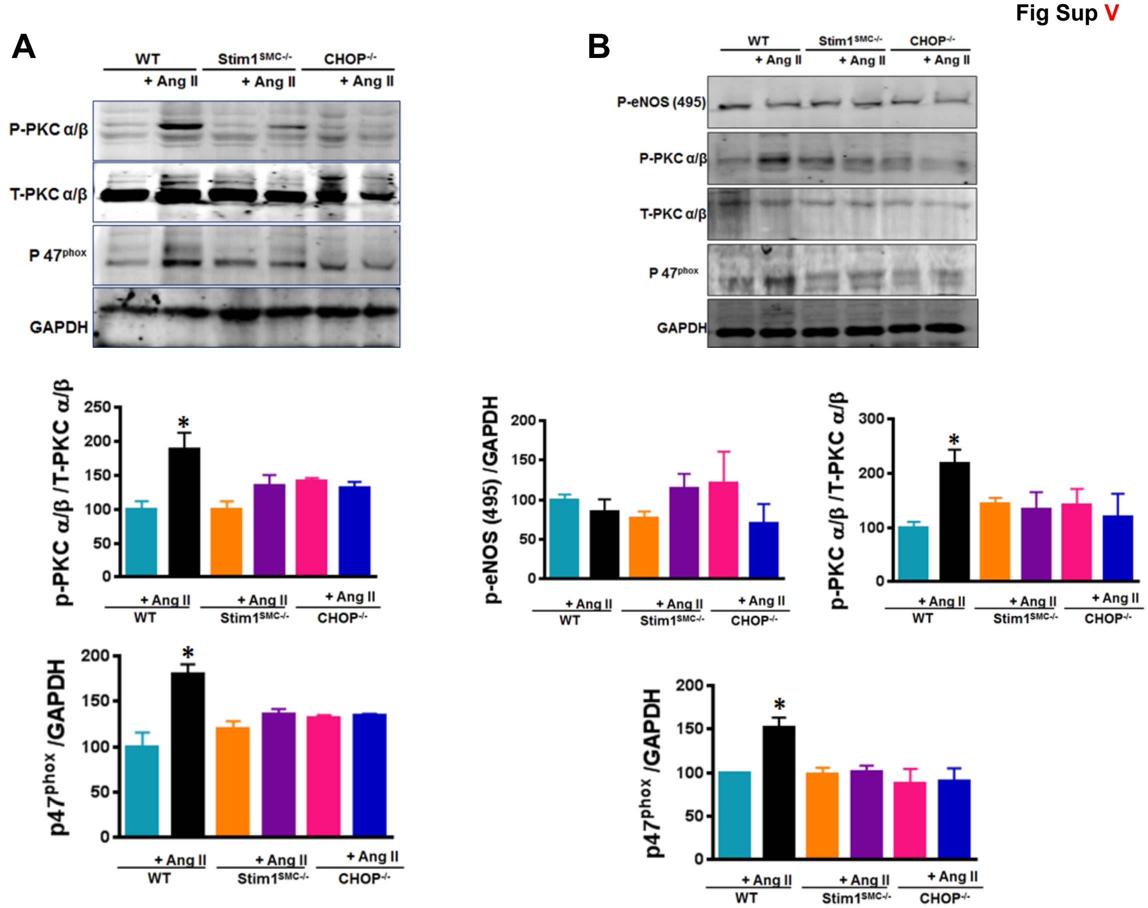

Figure Supp. V.- Effect of STIM1 and CHOP deletion on hypertension-induced uncoupling eNOS and NADPH oxidase. Western blot analysis and quantification for uncoupled eNOS markers (eNOS T495, P and T-PKCα/β) and NADPH oxidase subunit p47 phox in mesenteric arteries (MRA, **A, n=4**) and thoracic aorta **(B, n=4)** from:

- WT mice infused with saline or Angiotensin II
- CHOP knockout mice infused with saline or Angiotensin II.
- Homozygous Stim1 knockout specifically in SMC (Stim1$^{SMC-/-}$) mice infused with saline or Angiotensin II.

One-way ANOVA followed by *Bonferroni Post-Hoc test* was applied for figures (A, B).

*p<0.05 between WT + Ang II vs WT, CHOP$^{-/-}$, CHOP$^{-/-}$ + Ang II, STIM$^{SMC-/-}$ and STIM$^{SMC-/-}$ + Ang II.

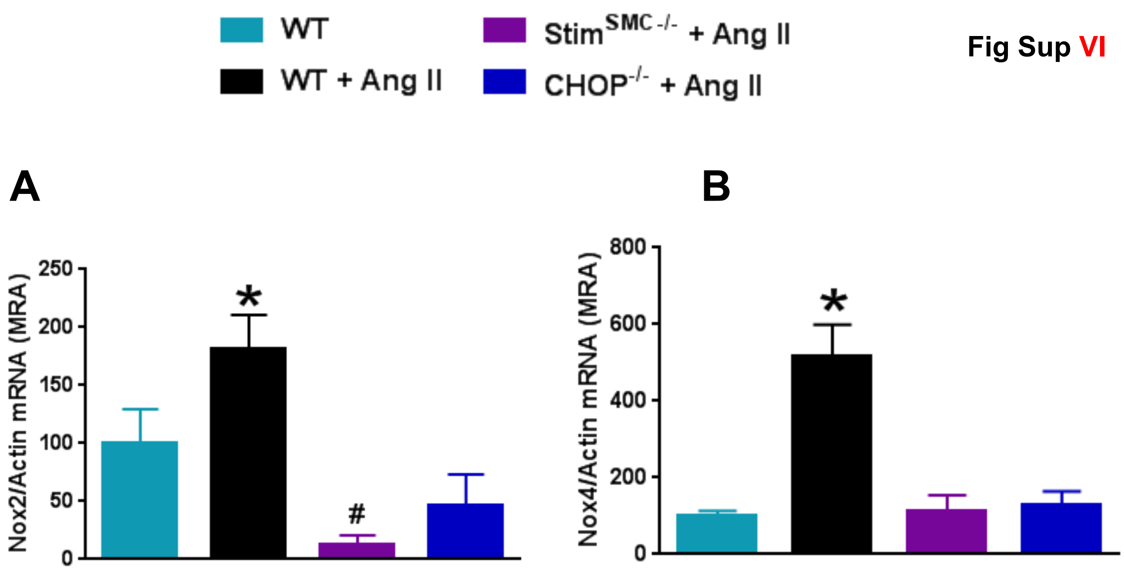

Figure Supp. VI.- Effect of STIM1 and CHOP deletion on NADPH oxidase subunits during hypertension. Real Time PCR showing the mRNA expression of Nox1 **(A, n=3-4)**, Nox2 **(B, n=3-4)** and Nox4 **(C, n=3-4)** in mesenteric arteries (MRA) from:

- WT mice infused with saline or Angiotensin II
- CHOP knockout mice infused with Angiotensin II.
- Homozygous Stim1 knockout specifically in SMC (Stim1$^{SMC-/-}$) mice infused with Angiotensin II.

One-way ANOVA followed by *Bonferroni Post-Hoc test* was applied for figures (A, B).

$^{*}p<0.05$ between WT + Ang II vs WT, CHOP$^{-/-}$ + Ang II and STIM$^{SMC-/-}$ + Ang II. $^{\#}p<0.05$ between STIM$^{SMC-/-}$ + Ang II vs WT and CHOP$^{-/-}$ + Ang II.

Fig Sup VII

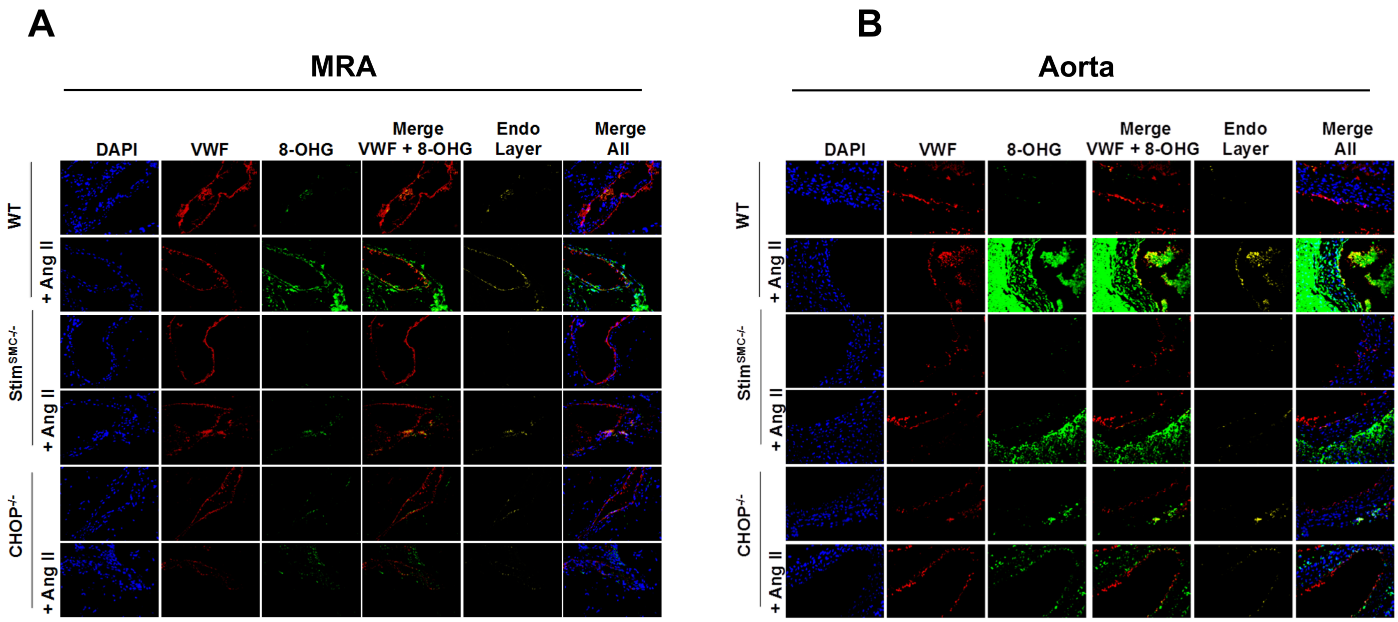

Figure Supp. VII.- Effect of STIM1 and CHOP deletion on oxidative stress. Immunostaining showing the expression of 8-hydroxy-2-deoxyguanosine (8-OHD) in mesenteric arteries (MRA, **A, n=3**) and thoracic aorta **(B, n=3)** from:

- WT mice infused with saline or Angiotensin II
- CHOP knockout mice infused with saline or Angiotensin II.
- Homozygous Stim1 knockout specifically in SMC (Stim1$^{SMC-/-}$) mice infused with saline or Angiotensin II.

Fig Sup VIII

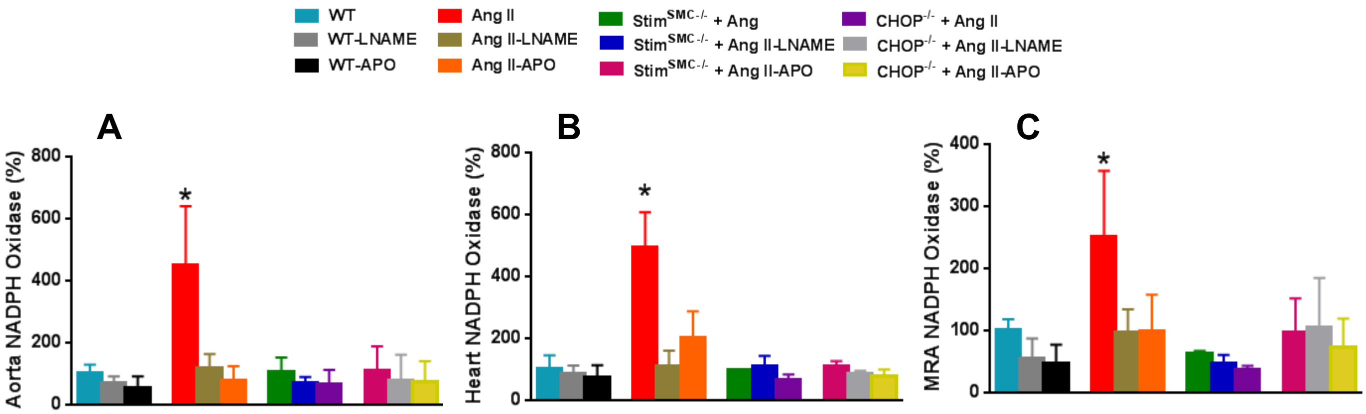

Figure Supp. VIII.- Effect of STIM1 and CHOP deletion on NADPH oxidase activity during hypertension. Luminometer showing the NADPH oxidase activity in the presence and absence of L-NAME and Apocynin in mesenteric arteries (MRA, **A, n=4**), heart **(B, n=4)** and thoracic aorta **(C, n=4)** from:

- WT mice infused with saline or Angiotensin II
- CHOP knockout mice infused with Angiotensin II.
- Homozygous Stim1 knockout specifically in SMC (Stim1$^{SMC-/-}$) mice infused with Angiotensin II.

One-way ANOVA followed by *Bonferroni Post-Hoc test* was applied for figures (A, B, C)

$^{*}p<0.05$ between WT + Ang II vs WT ± L-NAME or Apocynin, WT + Ang II ± L-NAME or Apocynin, STIM$^{SMC-/-}$ + Ang II± L-NAME or Apocynin and CHOP$^{-/-}$ + Ang II ± L-NAME or Apocynin.

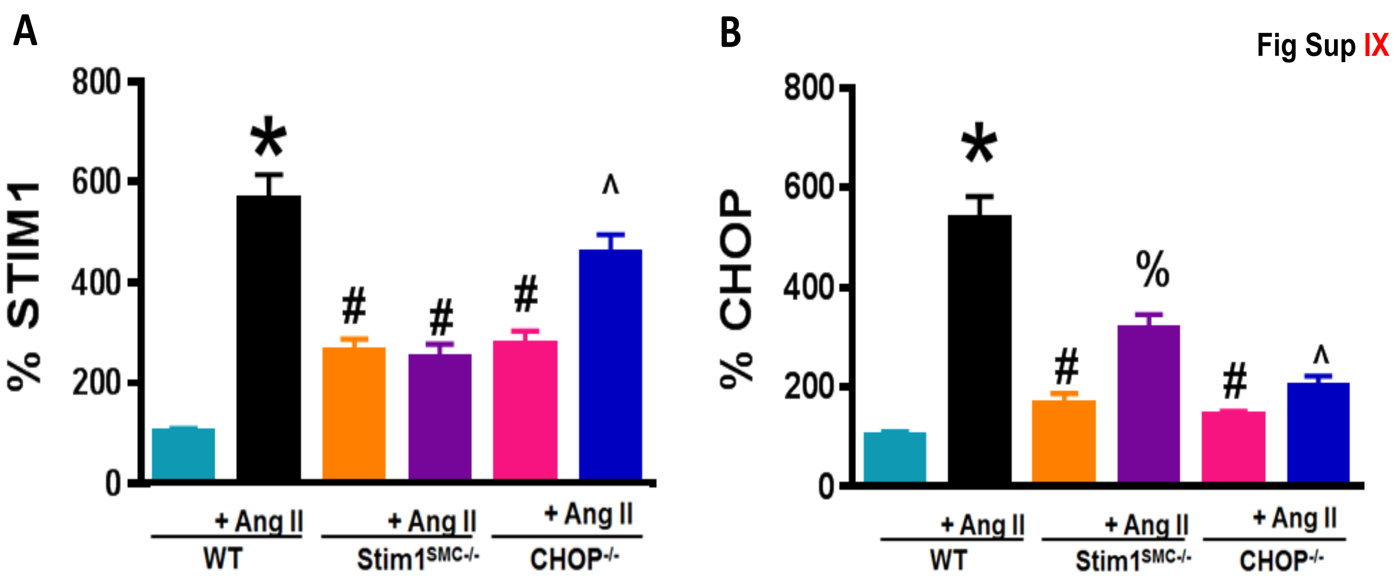

Figure Supp. IX.- Effect of Angiotensin II on STIM1 and CHOP in heart. Quantification of STIM1 **(A)** and CHOP **(B)** in heart (n=4) from:

- WT mice infused with saline or Angiotensin II
- CHOP knockout mice infused with Angiotensin II.
- Homozygous Stim1 knockout specifically in SMC ($Stim1^{SMC-/-}$) mice infused with Angiotensin II.

One-way ANOVA followed by *Bonferroni Post-Hoc test* was applied for figures (A, B).

*$p<0.05$ between WT+ Ang II vs WT, $STIM^{SMC-/-}$, $STIM^{SMC-/-}$ + Ang II, $CHOP^{-/-}$ and $CHOP^{-/-}$ + Ang II.

$^{\#}p<0.05$ between WT vs $STIM^{SMC-/-}$, $STIM^{SMC-/-}$ + Ang II, $CHOP^{-/-}$.

^$p<0.05$ between $CHOP^{-/-}$ + Ang II vs WT, $STIM^{SMC-/-}$, $STIM^{SMC-/-}$ + Ang II, $CHOP^{-/-}$.

$^{\%}p<0.05$ between $STIM^{SMC-/-}$ + Ang II vs WT, $STIM^{SMC-/-}$, $CHOP^{-/-}$ and $CHOP^{-/-}$ + Ang II.

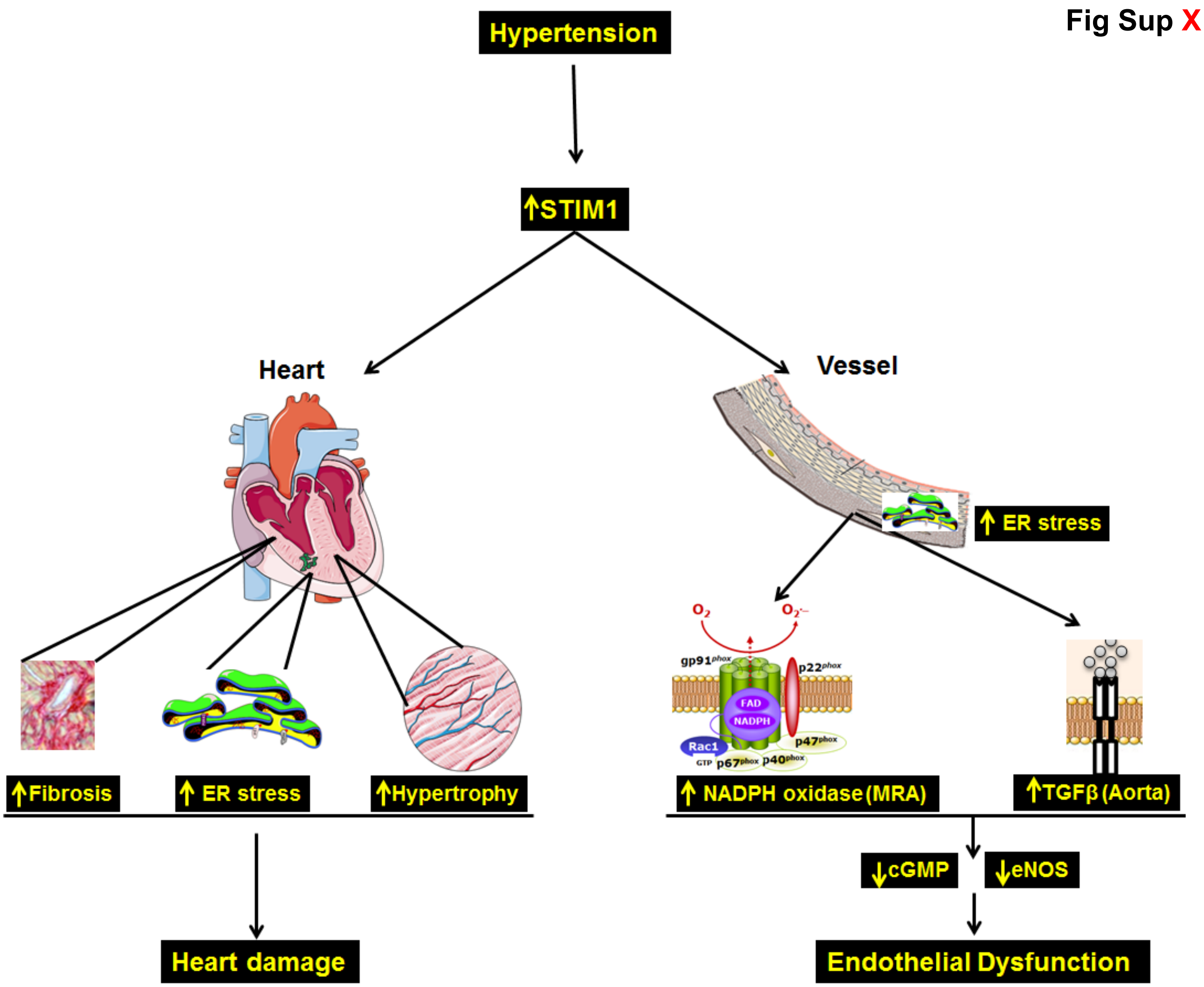

Figure Supp. 9.- schematic summarizing the main findings.