Supplemental Figure 1. Differentiation marker in VSMC exposed to aldosterone. A marker of VSMC differentiation, SMC-22, was visualized after immunoblotting of cell lysates from VSMC exposed or not to aldosterone (10nmol/L) for 18h. The membranes were then stripped and reprobed with anti-β-actin. NS= no significant difference, when relative SMC-22 protein between VSMC exposed to aldosterone was compared to non exposed cells.

Supplemental Figure 2. Aldosterone has no enhancing effect on PDGF mediated signaling. Aldosterone (10nmol/L) was added for 18h then PDGF (10ng/ml) was added for the indicated times. Cell lysates were immunoblotted for pAkt and then reprobed using anti- β-actin antibody. For each data point the phosphorylated protein band intensities were quantified using scanning densitometry. The results are presented as the mean±SEM of three independent experiments. NS= not significant.

Supplemental Figure 3. Effect of aldosterone on IRS-1 abundance. VSMC were exposed to aldosterone (10nmol/L) or medium alone for 18h. IRS-1 protein was determined by immunoblotting cell lysates with an anti-IRS-1 antibody. The membranes were then stripped and reprobed with anti-β-actin. NS= no significant difference, when relative IRS-1 protein between VSMC exposed to aldosterone was compared to non exposed cells.

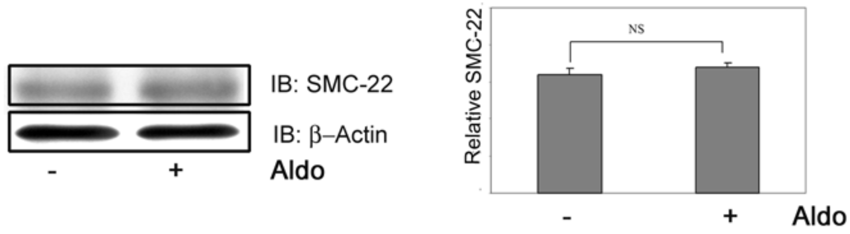
Supplemental Figure 4. The effect of actinomycin-D, eplerenone and antiβ-3 IgG on IGF-IR and IRS-1 phospho and total protein levels. VSMC were incubated with actinomycin-D or eplerenone or antiβ-3 IgG and then with IGF-I as indicated for Fig.6. IGF-IR (panels A-C) or IRS-1 (panels D-E) phospho and total protein levels were determined as described previously (Fig.6).

Supplemental Figure 5. The effect of NAC on IGF-IR and IRS-1 phosphorylation and protein. VSMC were incubated with NAC and aldosterone as described in Fig.7C and then IGF-I was added as in Fig.7D. The amount of IRS-1 phospho and total protein (panel A) was determined as described previously (Fig. 7E). The levels of IGF-IR phosphorylation and total protein (panel B) were determined as described in (Fig.7D).

Supplemental Figure 6. The enhancing action of aldosterone on IGF-I mediated AKT/MAPK signaling is inhibited by NAC. VSMC were incubated with NAC and aldosterone prior to stimulation with IGF-I and the levels of phospho AKT and MAPK were determined as described in Fig.8A.

Supplemental Figure 7. The enhancing action of aldosterone on IGF-I mediated signaling is inhibited by catalase. VSMC were incubated for 60minutes with or without catalase (50U/ml) and aldosterone (10nmol/L) for 18h before adding IGF-I (50ng/ml) for 5minutes. The levels of Akt and MAPK phosphorylation were assessed by immunoblotting. The blots were stripped and reprobed with anti-βactin. For each data point the phosphorylated protein band intensities were quantified using scanning densitometry. The results of three experiments are represented in graphs which show the ratio (mean±SEM) of phosphorylated to total protein. ***$p<0.001$, NS= not significant.

SUPPLEMENTAL Figure 1.



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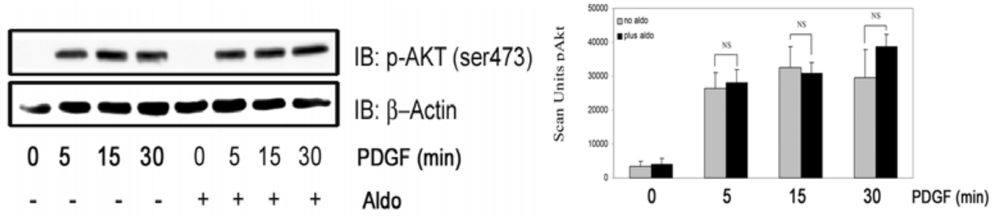

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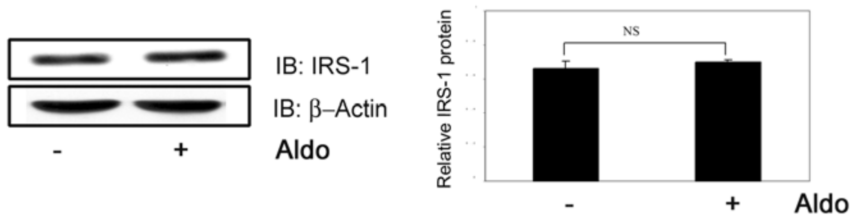

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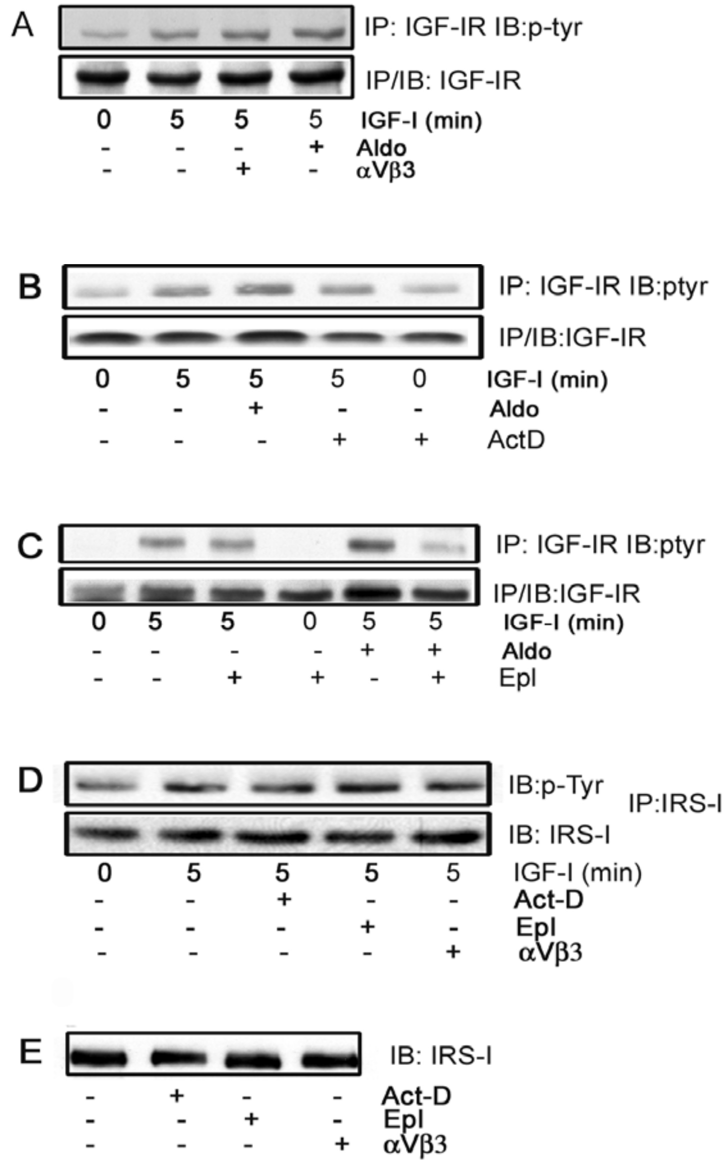

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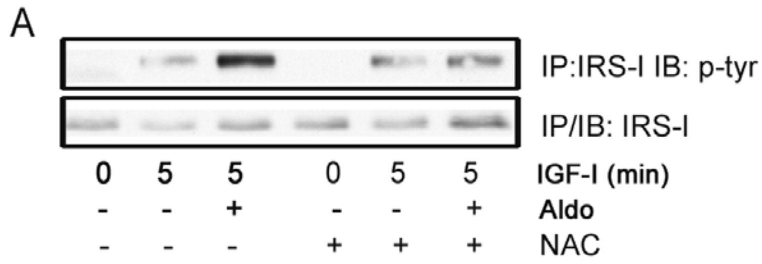

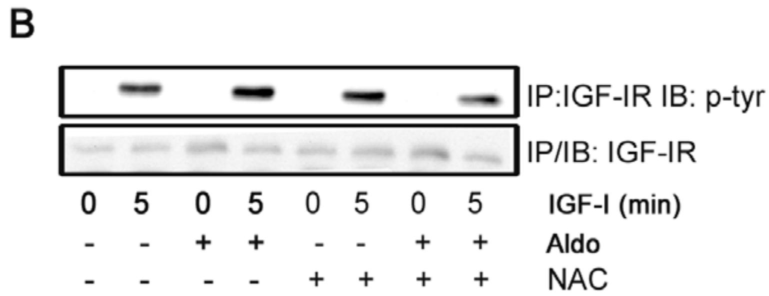

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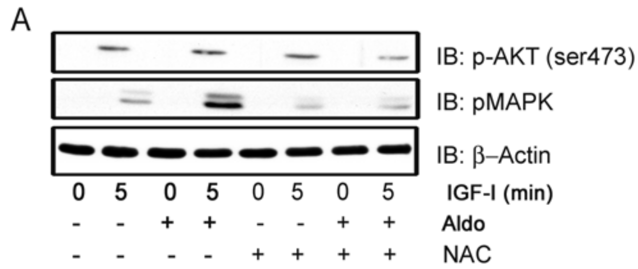

SUPPLEMENTAL Figure 7.