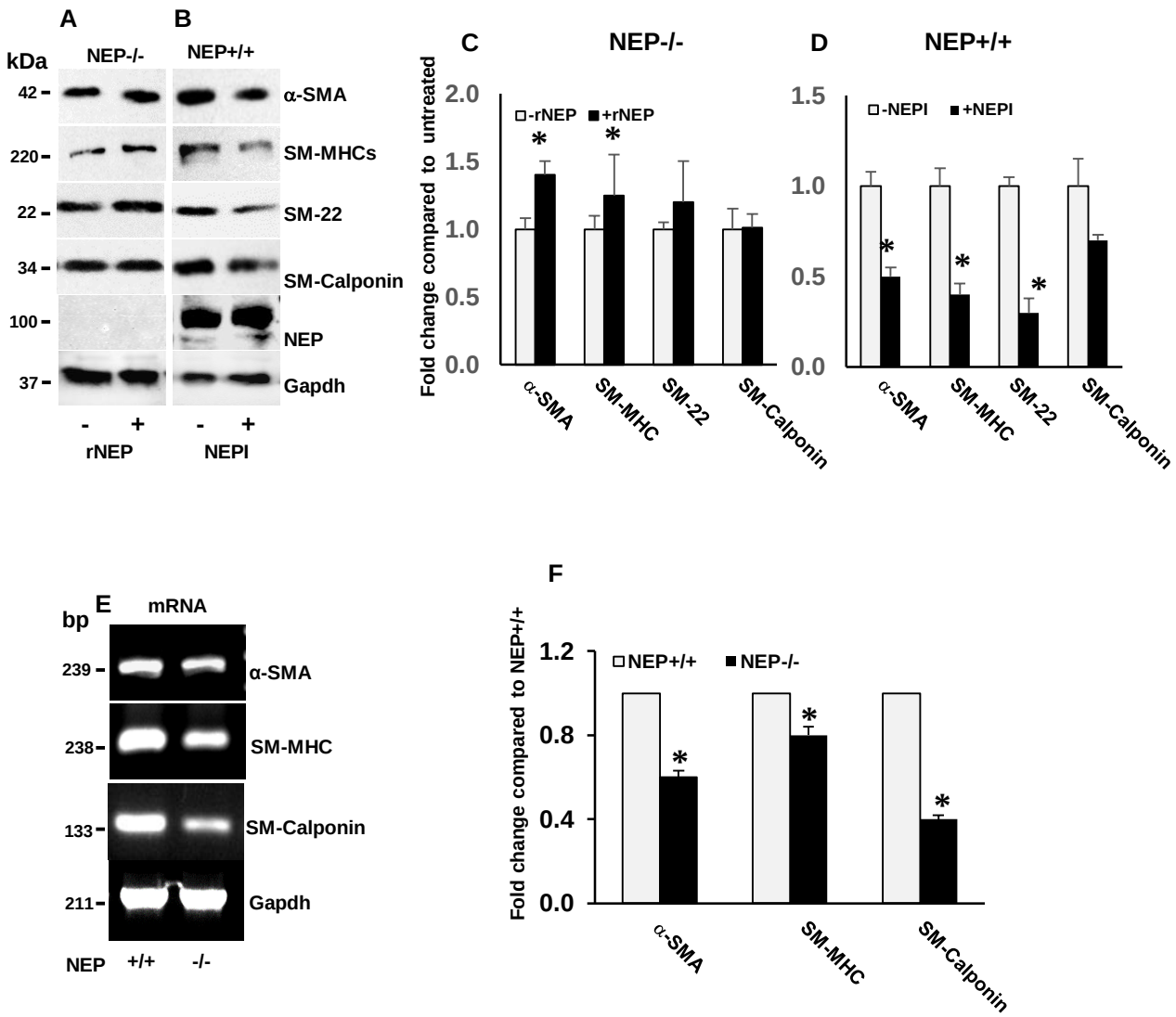
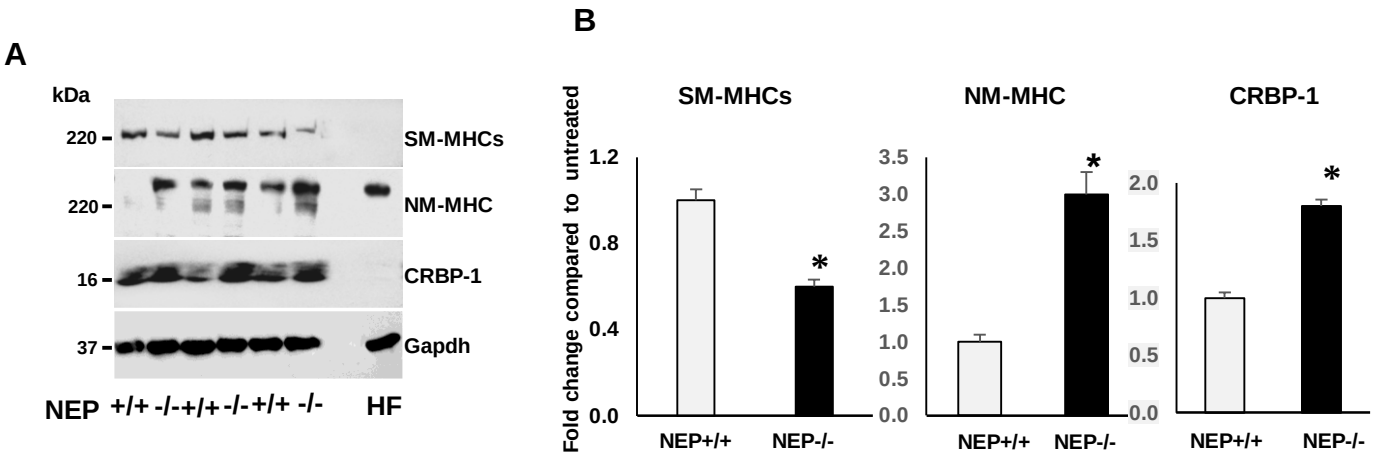
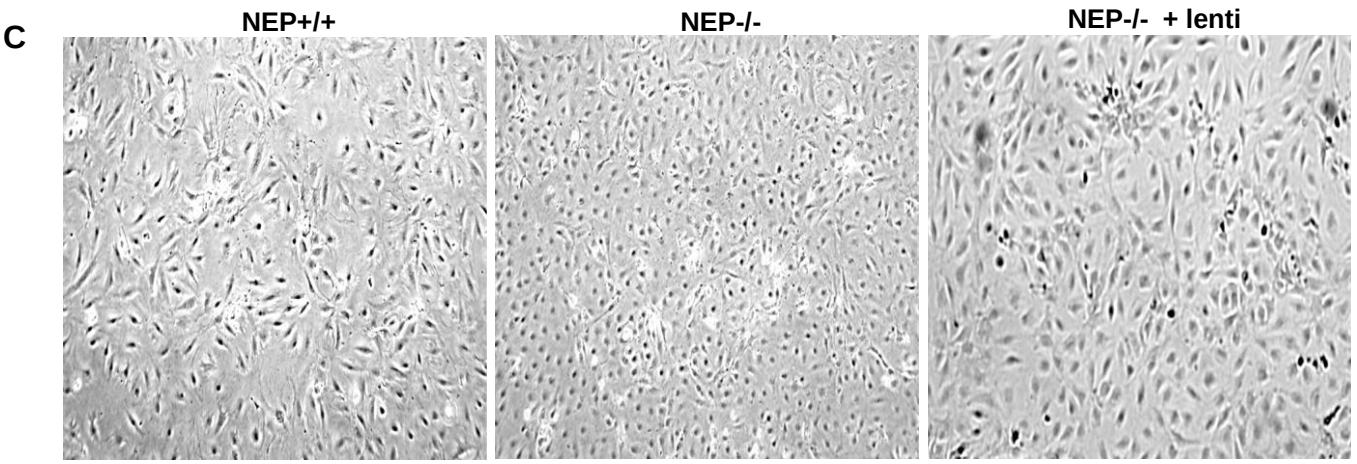
**Supplement Figure I**




Supplement Figure I: Knockdown of NEP decreases SM-proteins and mRNA in PASMCs. Panel A and C show the effect of rNEP on levels of SM-proteins in NEP-/- PASMCs and Panel B and D show effect of NEP inhibitor, phosphoramidon (10μM)). Levels of mRNA for SM-genes (α-actin, myosin, and calponin) were assessed by semi quantitative RT-PCR shown in Panel E, and average levels from 6 different isolates is shown in Panel F. Gapdh was used as loading control. (*) represents p≤ 0.05 for comparison between NEP+/+ and NEP -/- PASMC or treated versus untreated.

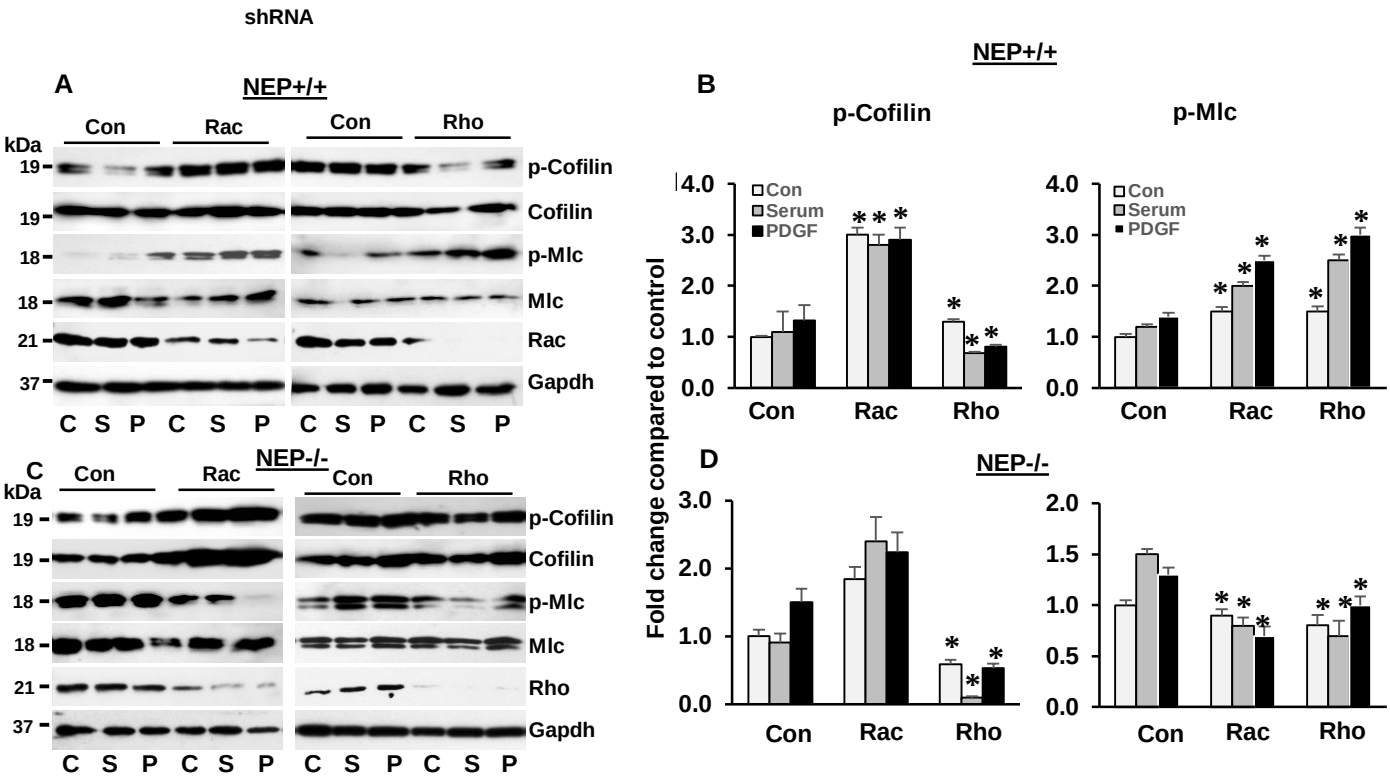
**Supplement Figure II**



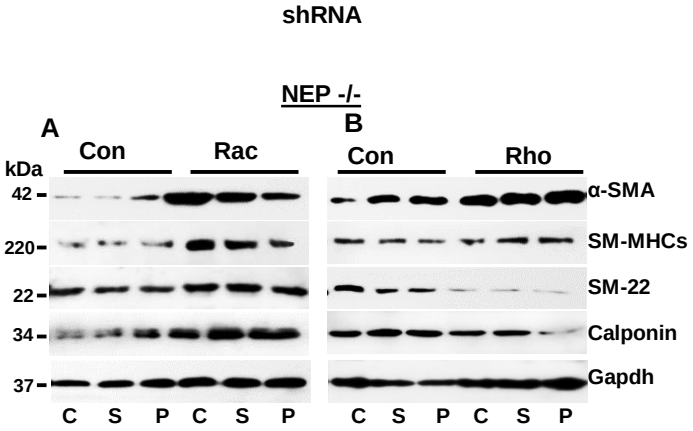

Supplement Figure II: Increase in NM-myosin in NEP-/- PASMCs. To further characterize the phenotype of NEP-/- PASMCs we probed cell lysates from NEP+/+ and -/- PASMCs with antibodies to SM-myosin, NM-myosin and CRBP; see Panel A. Panel B shows average levels from 6 different isolates normalized to Gapdh. Panel C shows representative light microscopy images (taken at 4x magnification using a Nikon microscope) for NEP+/+, NEP-/-, and NEP-/- treated with NEP-lentivirus PASMCs. (*) represents $p \leq 0.05$ for comparison between NEP+/+ and NEP -/- PASMC.

## Supplement Figure III



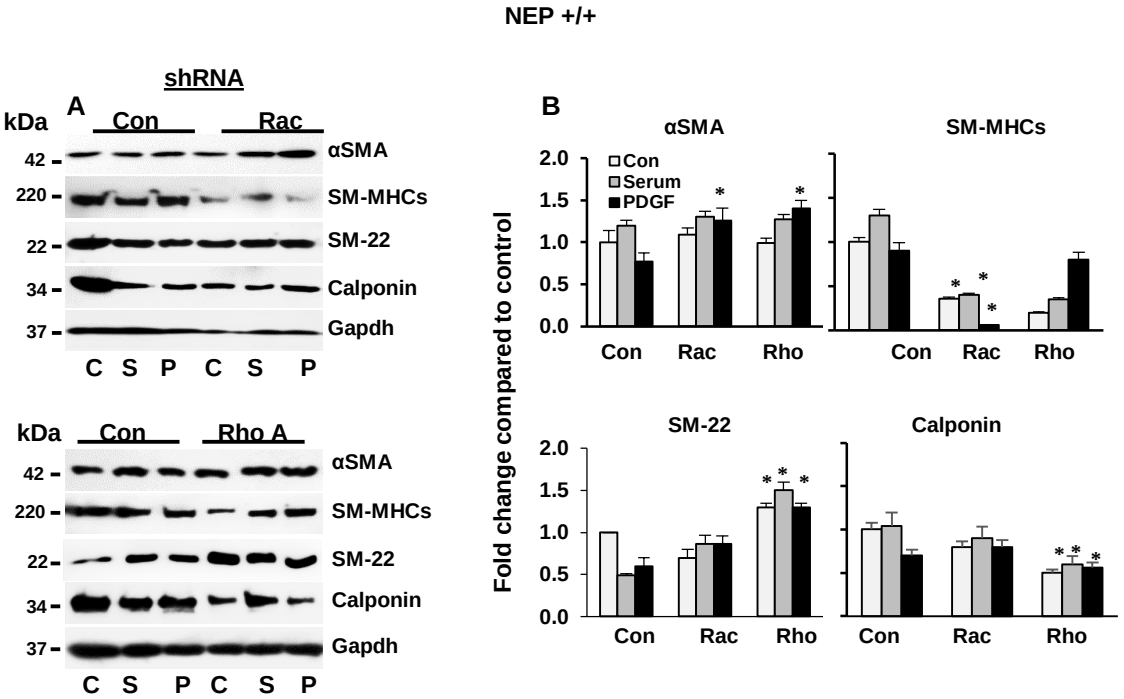

Supplement Figure III: ShRNA to Rac and RhoA inhibit phosphorylation of Cofilin and Mlc. NEP+/+ and -/- PASMCs were treated with control shRNA, or shRNA to Rac or Rho, and selected with puromycin and treated with serum 0.2% and PDGF (10ng/ml). Panel A shows the effect of shRNAs on phospho and total cofilin and Mlc levels in NEP+/+ PASMCs and Panel B the average effects on p-cofilin and p-Mlc levels from 3 different infections. Panels C and D show effects on NEP-/- PASMCs. (*) represents p≤ 0.05 for comparison between control and shRNA treated for NEP+/+ and NEP -/- PASMCs. (C=con, S=serum, P=PDGF).

**Supplement Figure IV**



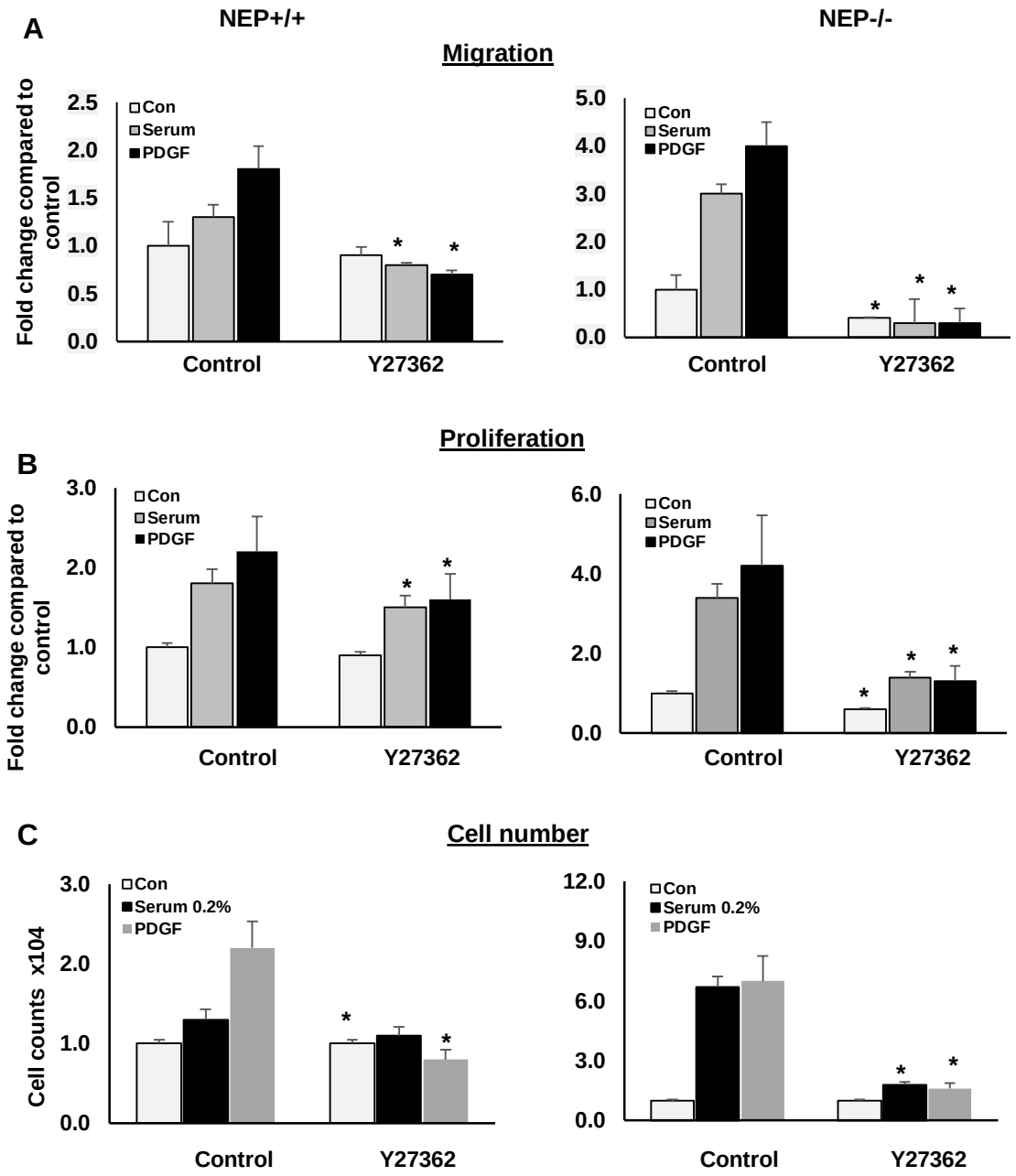

Supplement Figure IV: ShRNA to Rac, and RhoA, restores SM-protein expression NEP-/- PASMCs. NEP-/- PASMCs were infected with control shRNA or shRNA to Rac or Rho and selected with puromycin. Cells were treated with serum (0.2%) or PDGF(10ng/ml) for 24h and lysates were analyzed for SM-proteins. Panel A shows effect of Rac shRNA and Panel B effect of Rho shRNA on SM-proteins levels from 3 different isolates (C=con, S=serum, P=PDGF).

**Supplement Figure V**



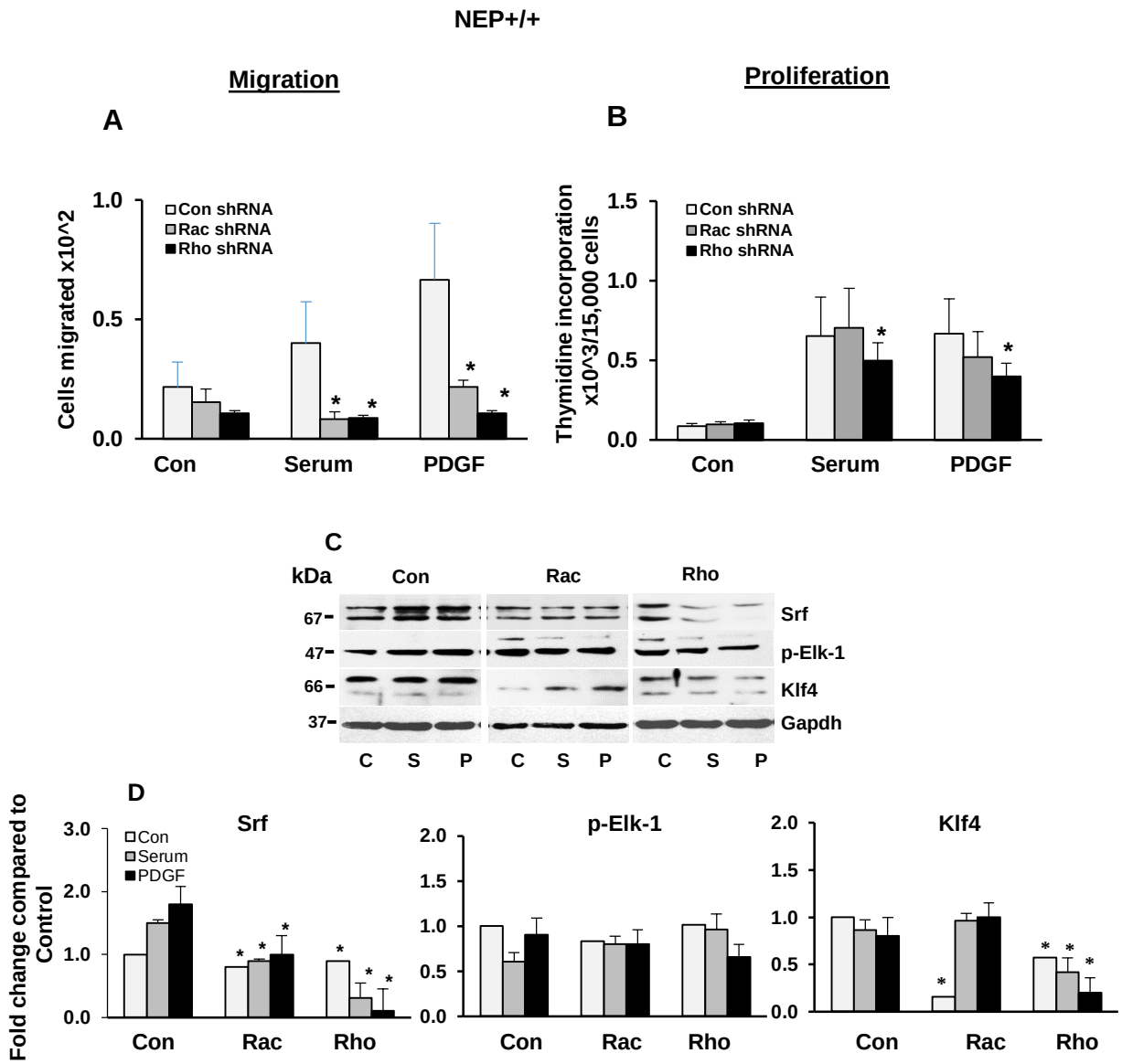

Supplement Figure V: Effect of shRNA to Rac and RhoA on SM-proteins and transcription factors in NEP+/+ PASMCs. NEP+/+ PASMCs treated with control shRNA, or shRNA to Rac Rho, and selected with puromycin. PASMCs were treated with serum or PDGF for 24h and lysates analyzed for SM-proteins and transcription factors. Panel A shows the effect of shRNAs on SM-proteins in NEP+/+ PASMCs treated with serum and PDGF. Graphical representation of the average effects of the shRNAs from 3 different infections on SM-proteins is shown in Panel B. (*) represents $p \leq 0.05$ for comparisons between control and shRNA treatment.

**Supplement Figure VI**



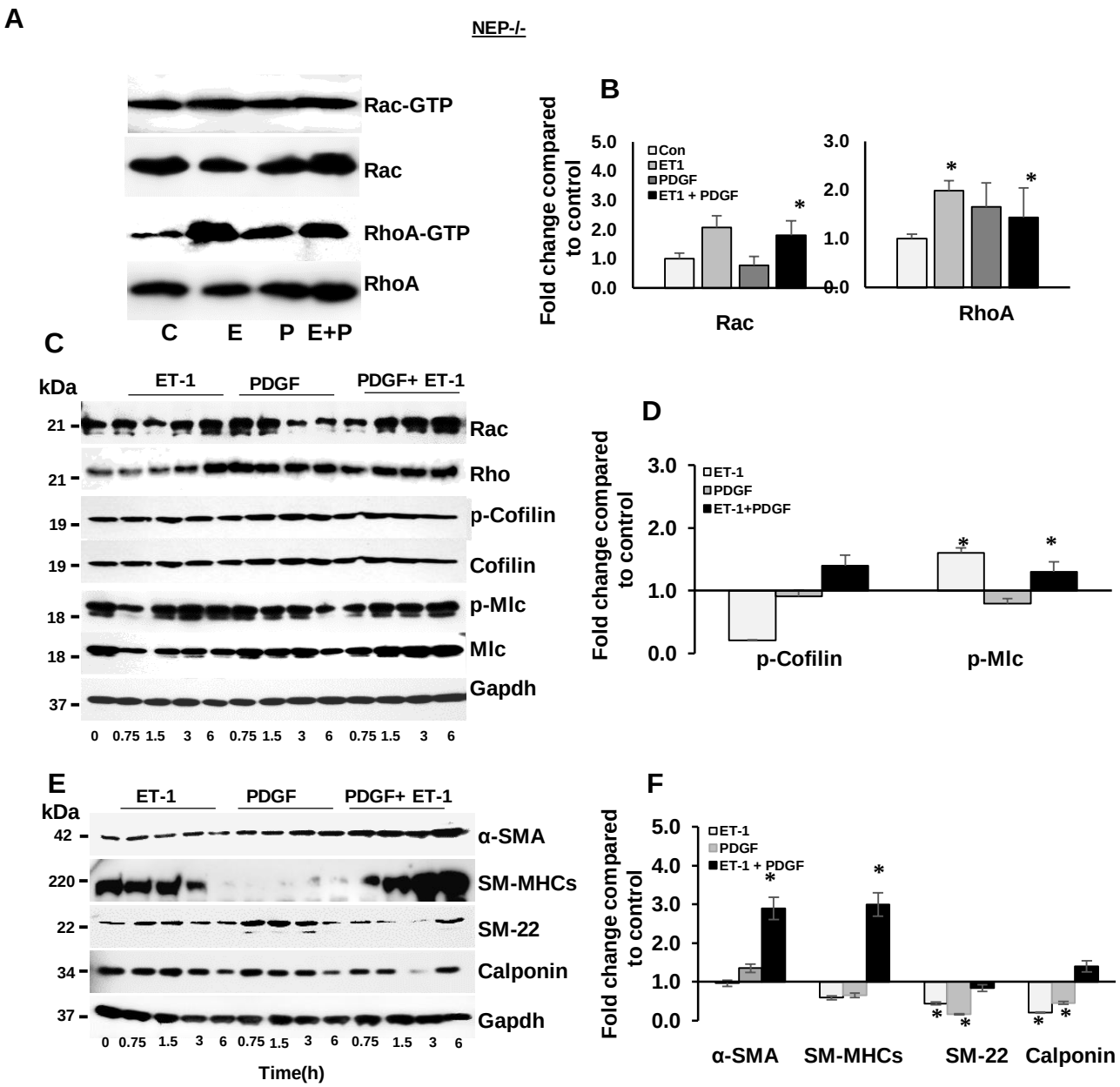

Supplement Figure VI: Rho kinase (Rock) inhibitor Y27362, attenuates migration in NEP+/+ and -/-PASMCs. NEP+/+ and -/- PASMCs were treated with the Rock inhibitor Y27362 (10μM). Migration, proliferation and cell numbers were measured in cells treated with serum (0.2%) or PDGF(10ng/ml) for 24h. Panel A shows effect of shRNA on migration and Panel B on proliferation and Panel C on cell numbers. NEP+/+ and -/- PASMCs from 3 different isolates. (*) represents $p \leq 0.05$ for comparison between control and treated PASMC.

**Supplement Figure VII**



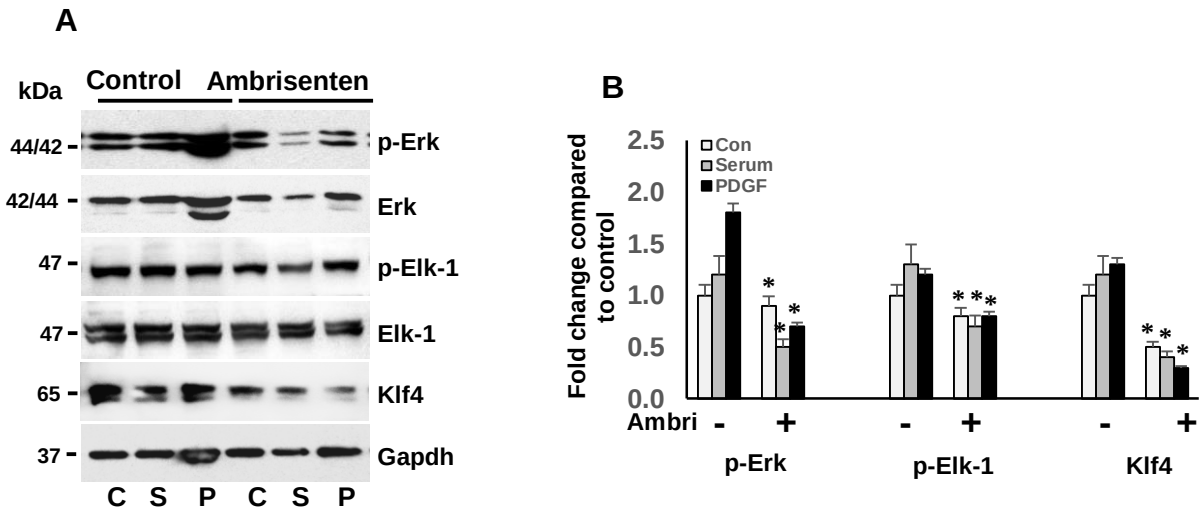

Supplement Figure VII: Effect of ShRNA to Rac and RhoA on migration. proliferation and transcription factor levels in NEP +/+ PASMCs. NEP+/+ PASMCs were infected with either control shRNA or shRNA to Rac or Rho and selected with puromycin. Migration and proliferation are measured in cells treated with serum (0.2%) or PDGF(10ng/ml) for 24h. Panel A shows effect of shRNA on migration and Panel B on proliferation of NEP+/+ PASMCs from 3 different isolates. Panel C shows effect of the shRNA on levels of Srf, p-Elk-1 and Klf4 in NEP+/+ PASMCs and Panel D graphical representation levels from 3 different isolates NORMALIZED TO Gapdh. (*) represents p≤ 0.05 for comparison between control and treated PASMC.

**Supplement Figure VIII**



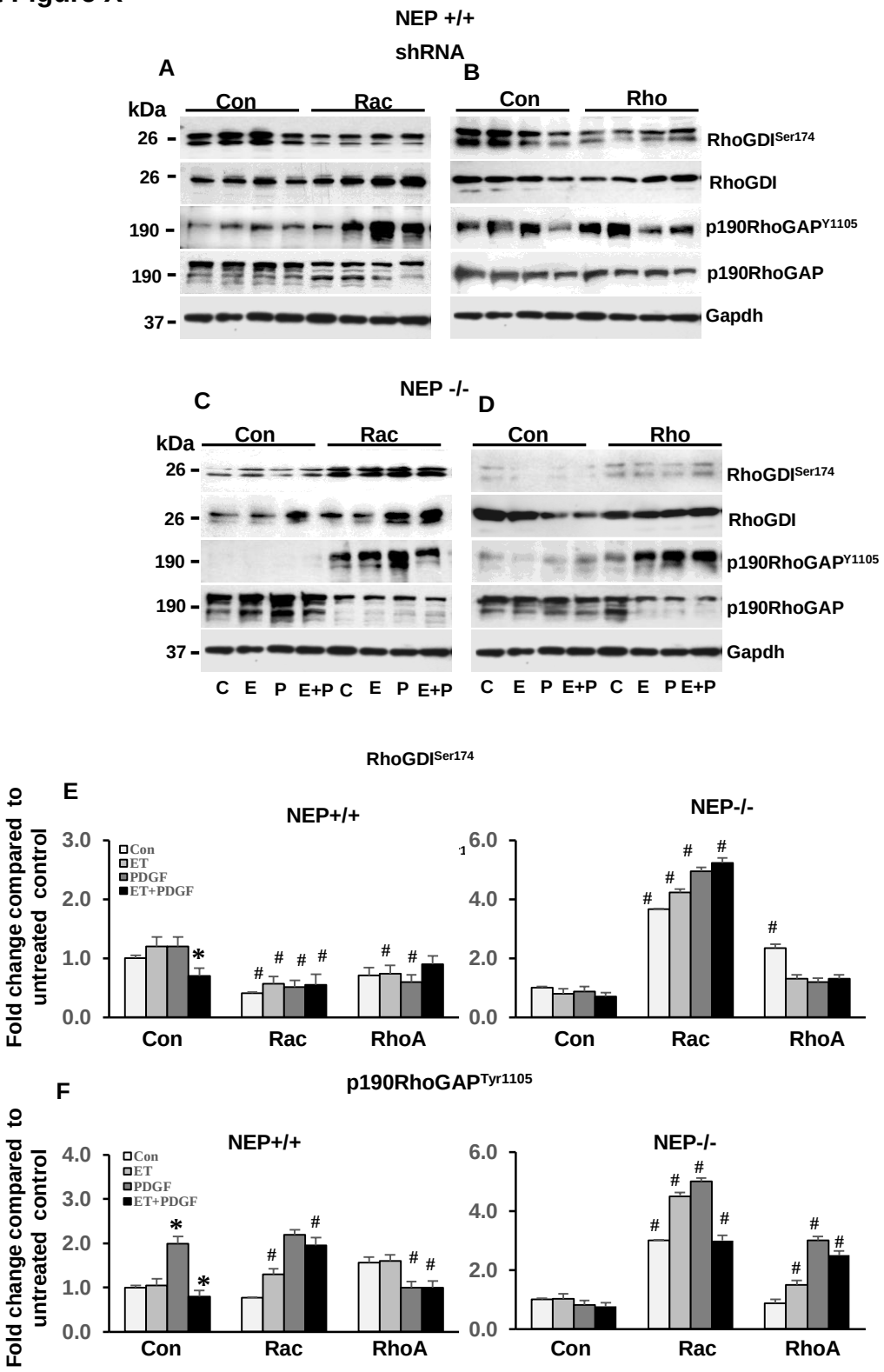

Supplement Figure VIII: Treatment of NEP-/- PASMCs with the NEP substrate, ET-1, and PDGF enhances the null phenotype. NEP-/- PASMCs were treated with PDGF (10 ng/ml) in the absence or presence of ET-1(100 nM) for 24h and GTP bound Rac and Rho levels are shown in Panel A. Graphical representation of average levels from 6 paired isolates is shown in Panel B. A time course with the agonists (0.75, 1.5, 3, and 6h) was analyzed for levels of phospho and total cofilin and Mlc shown in Panels C and D. Levels of SM-proteins are shown in Panel E and F. * p≤ 0.05 for comparisons between control and treated (n=3).

**Supplement Figure IX**



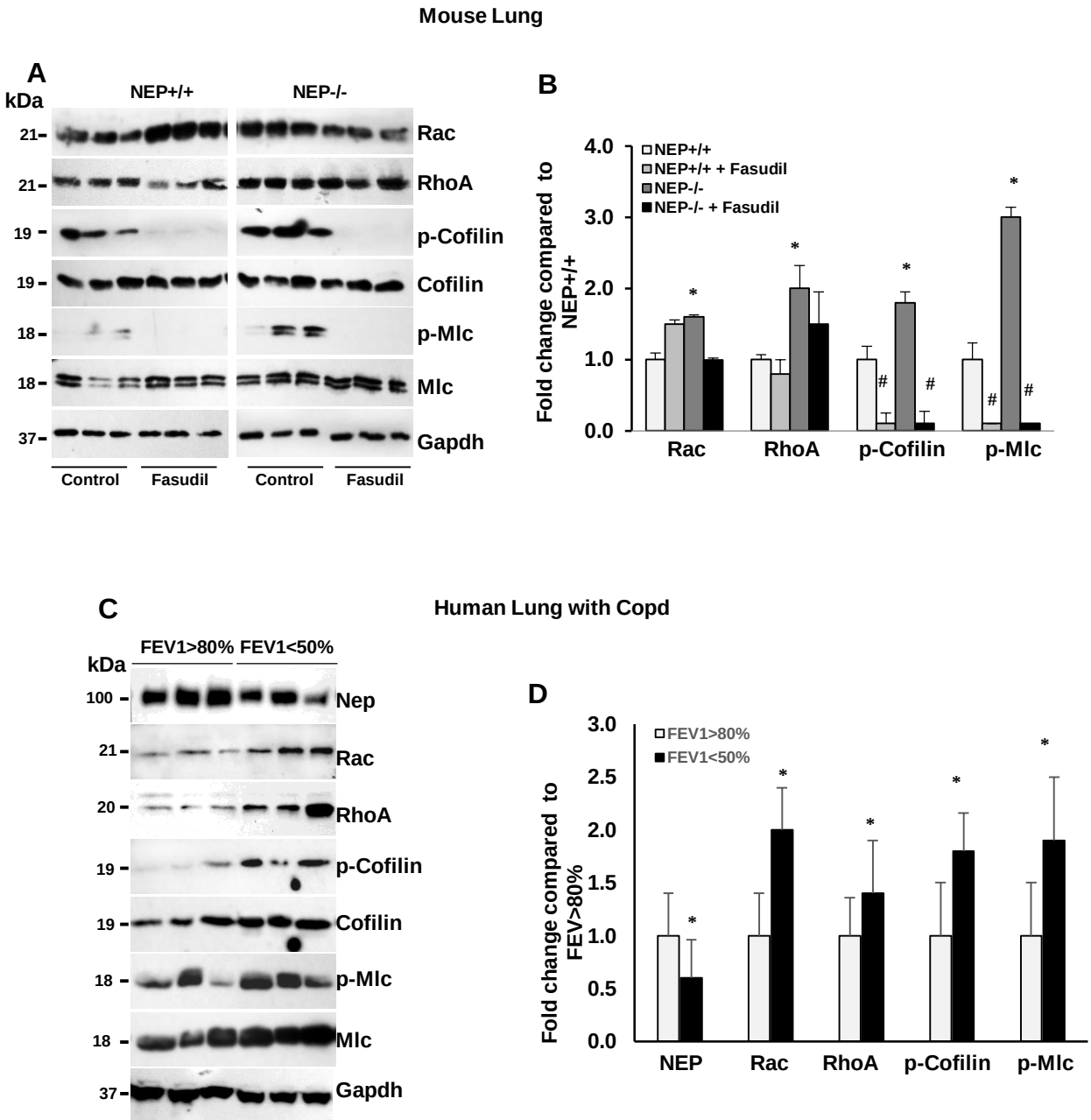

Supplement Figure IX An ETRA antagonist, Ambrisentan reduces p-Erk, p-Elk-1 and Klf4 levels in NEP-/- PASMCs. NEP -/- PASMCs were treated with serum 0.2% in the presence or absence of Ambrisentan (1µM). Levels of p-Erk, p-Elk-1, and Klf4 were assessed by Western blotting. Panel A shows the Western blot, and Panel B shows fold change after Ambrisentan treatment from 3 different isolates normalized to Gapdh. (*) represents $p \leq 0.05$ for comparison between control and inhibitor treated cells.

**Supplement Figure X**



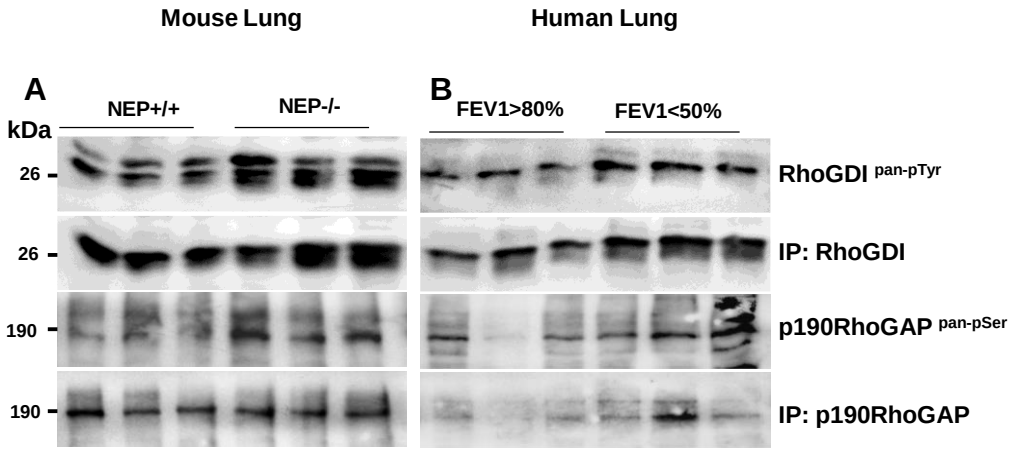

Supplement Figure X: Decreased levels of RhoGDI $^{pSer174}$ and p190 RhoGAP $^{pY1105}$ in NEP-/- PASMCs. NEP+/+ and -/- PASMCs were infected with either control shRNA or shRNA to Rac or Rho and selected with puromycin. PASMCs were treated with PDGF (10 ng/ml) in the presence or absence ET-1(100 nM) for 6h and lysates probed with antibodies to phospho and total RhoGDI and p190 RhoGap.. (C=con, E=ET1, P=PDGF). Panels A and B show representative Western blots for the effect of Rac and Rho shRNA in NEP+/+ and Panels C and D in NEP-/- PASMCs. Panel E and F show fold change compared to control after shRNA treatment from 3 different isolates normalized to Gapdh. (*) represents p≤ 0.05 for comparison between control and shRNA treated cells.

**Supplement Figure XI**



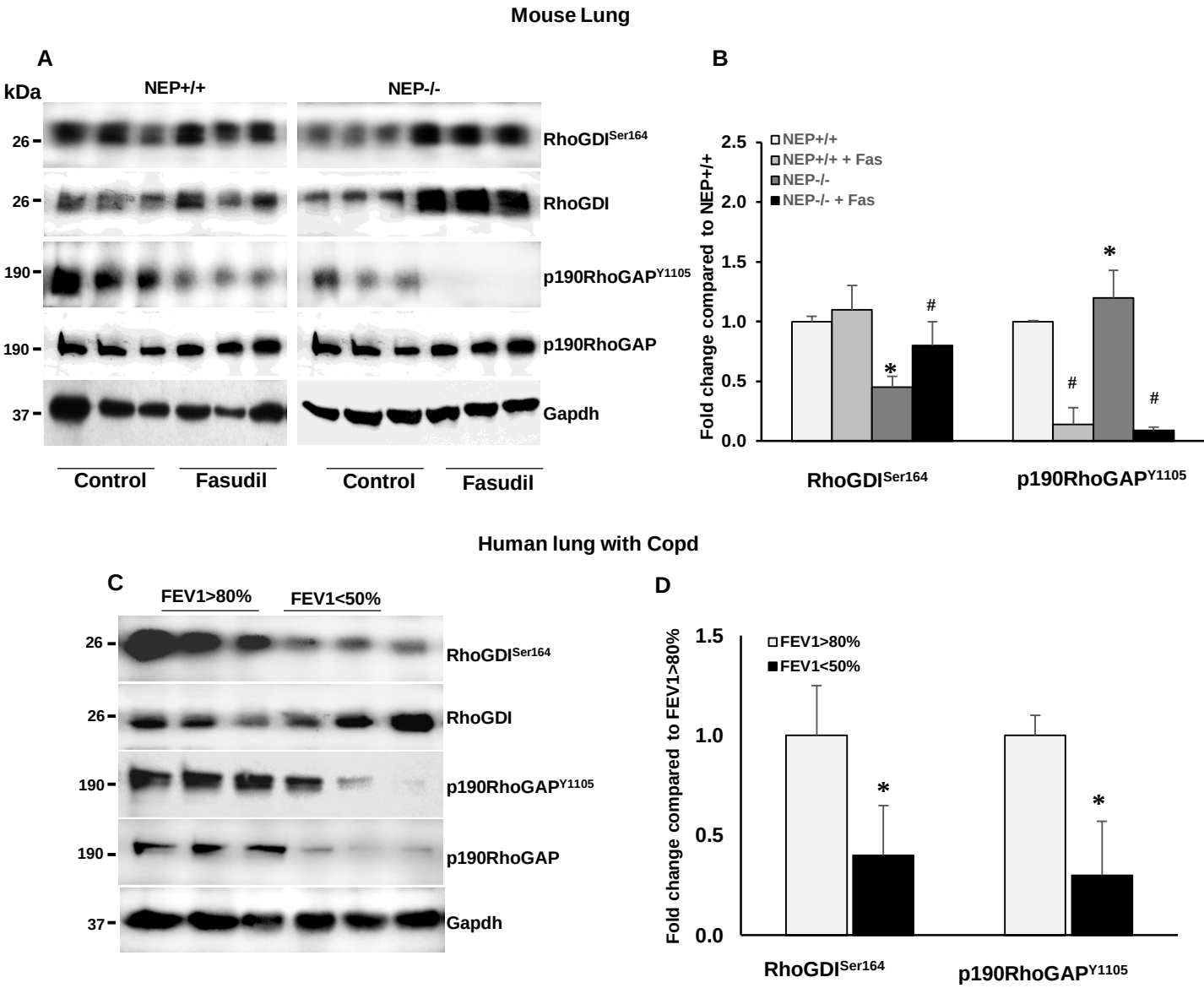

Supplement Figure XI: Increased activation of Rho GTPases and downstream effectors in lungs obtained from NEP-/- mice and Copd patients with FEV1 <50%. Lung lysates from NEP +/+ and -/- mice, and from Copd patients with FEV1 >80%, and <50% were probed for levels of Rac Rho, phospho and total -Cofilin and –Mlc. Panel A, shows levels by Western blot in NEP+/+ and -/- mice treated with fasudil and average expression from 6 different paired isolates is shown in Panel B. Panel C shows levels in samples from Copd lungs with FEV1 >80% and < 50% . Average levels from 6 different isolates normalized to Gapdh is shown in Panel D. (*)represents $p \leq 0.05$ for comparison between NEP+/+ to -/- mouse lung and Copd lung with FEV1<50% compared to FEV< 80%.

**Supplement Figure XII**




Supplement Figure XII: Increased RhoGDI $^{pTyr}$ and p190RhoGAP $^{pSer}$ levels in NEP-/- lungs and human Copd lungs . Lysates were prepared from lungs of NEP+/+ and -/- mice and human Copd with FEV1 >80% and FEV1< 50%. Lysates were immunoprecipitated with antibodies to RhoGDI and p190RhoGAP separated on SDS-PAGE and transferred to nitrocellulose, and probed with antibodies to Pan p-Tyr and, p-Ser and total RhoGDI and p190RhoGAP. Panel A shows representative Western blot from 3 different isolates of NEP+/+ and -/- mouse lungs. Panel B show results from Copd lungs.

**Supplement Figure XIII**




Supplement Figure XIII: Decreased Serine phosphorylation of RhoGDI and tyrosine phosphorylation of p190 RhoGAP in lungs from NEP-/- mice and humans with copd (FEV1<50%) . NEP+/+ and -/- mice were treated with fasudil 50mg/Kg) for 7d. Lung lysates from NEP+/+ and -/- mice and from humans with copd were probed for phospho and total RhoGDI and p190 RhoGAP. Panels A and B show representative Western blot and average fold change in NEP-/- mice compared to NEP+/+. Panel E and F show fold change in human Copd with FEV1<50% compared to FEV1>80% from 6 different isolates normalized to Gapdh.. (*)represents p≤ 0.05 for comparison between NEP+/+ to -/- mice and Copd with FEV1<50% compared to FEV< 80% (#) for comparisons between control and fasudil treatment.