

Expanded View Figures

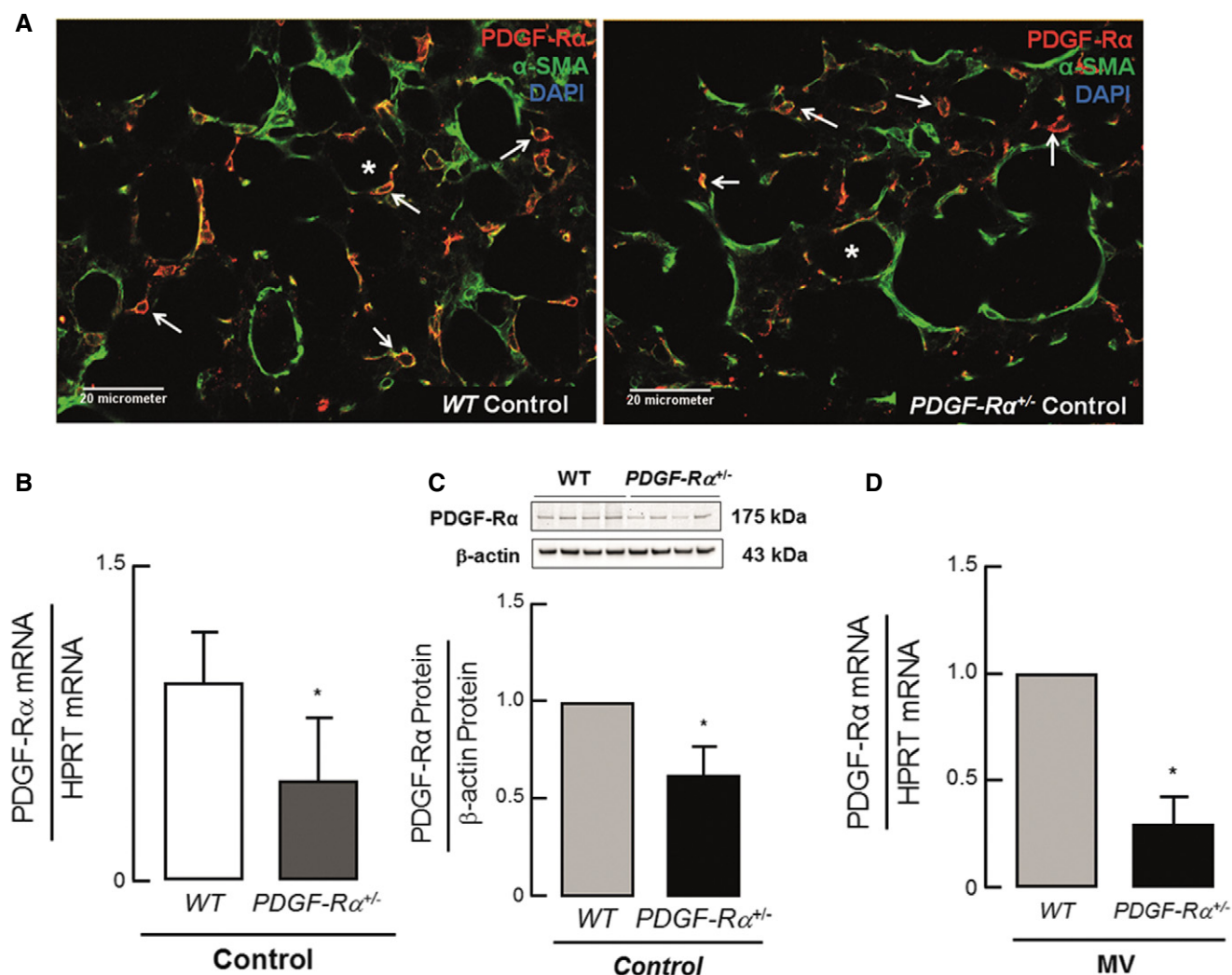


Figure EV1. Reduced PDGF-R α abundance in PDGF-R α haploinsufficient mice.

A Double staining for PDGF-R α (red) and α -SMA (green) showed a co-localization of these two proteins as well as a reduced number of double-positive cells (white arrows) in 5–8-day-old PDGF-R $\alpha^{+/-}$ mice (right panel) when compared to PDGF-R $\alpha^{+/+}$ (WT) littermates (left panel). * represents alveolar air space.

B, C (B) Quantitative RT–PCR and (C) immunoblot analysis showing reduced PDGF-R α protein and mRNA expression in lungs of PDGF-R $\alpha^{+/-}$ newborn mice when compared to WT littermates ($n = 4$ /group).

D Reduced PDGF-R α transcript in newborn PDGF-R $\alpha^{+/-}$ mice upon MV-O₂ for 8 h when compared to WT littermates ($n = 3$ mice/group).

Data information: In (B–D), the data are presented as mean \pm SD. * $P < 0.05$. Statistical test in (B–D) is two-tailed unpaired Student's t -test or Mann–Whitney test.

Figure EV2. Effect of TGF- β in combination with mechanical stretch on PDGF-R α signaling and functional properties of fibroblasts.

- A No change in migration of myofibroblasts (MFBs) from newborn WT mice with mechanical stretch ($n = 5$ mice/group).
- B–D Analysis of proliferation (Cell Titer Glo) exhibited no change upon TGF- β (B) or stretch (C) in mouse myofibroblasts (MFBs) from WT mice, while an increase in proliferation was observed when mouse myofibroblasts stretched in the presence of TGF- β were subjected to an additional dose of TGF- β (D) ($n = 9$ mice/group).
- E, F Immunoblot analysis showing increased proliferation markers like PCNA (E) levels with mechanical stretch and PI3K (F) upon additional TGF- β incubation on stretched myofibroblasts (MFBs) from newborn WT mice ($n = 6$ mice/group).

Data information: Values here are normalized to respective controls. Data are presented as mean \pm SD. Statistical analysis in (A–C, E) is two-tailed unpaired Student's t -test and in (D, F) is ordinary one-way ANOVA with Bonferroni's correction. $**P < 0.01$, $*P < 0.05$ (P -value range = 0.0080–0.0232). C, un-stretched untreated control; S, stretched myofibroblasts (24 h); Th1, un-stretched myofibroblasts subjected to 5 ng/ml TGF- β (24 h); S+Th1, myofibroblasts stretched in parallel to TGF- β application (5 ng/ml) (24 h); S+Th2, myofibroblasts stretched for 24 h followed by incubation with TGF- β (5 ng/ml) for 24 h.

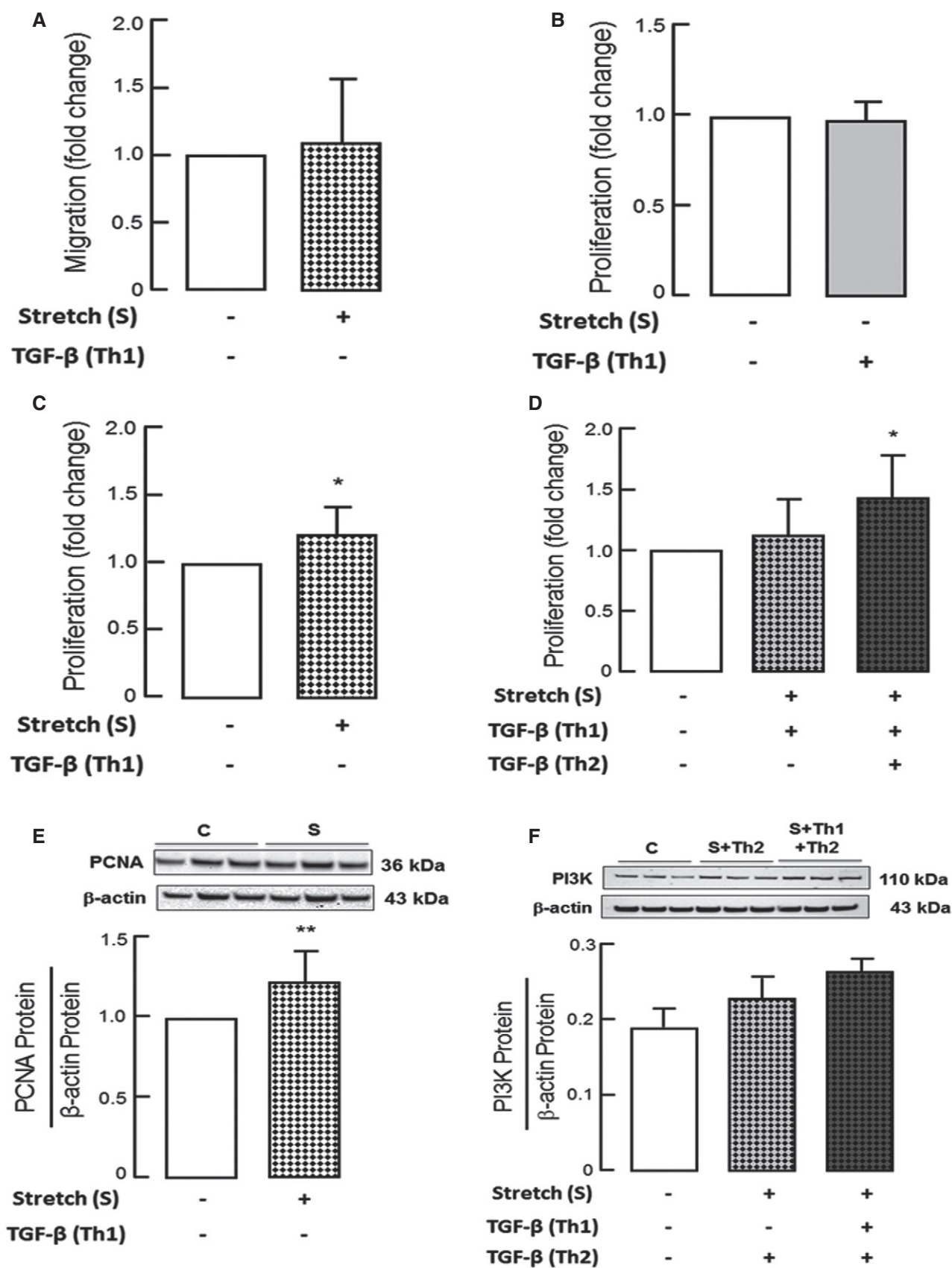


Figure EV2.

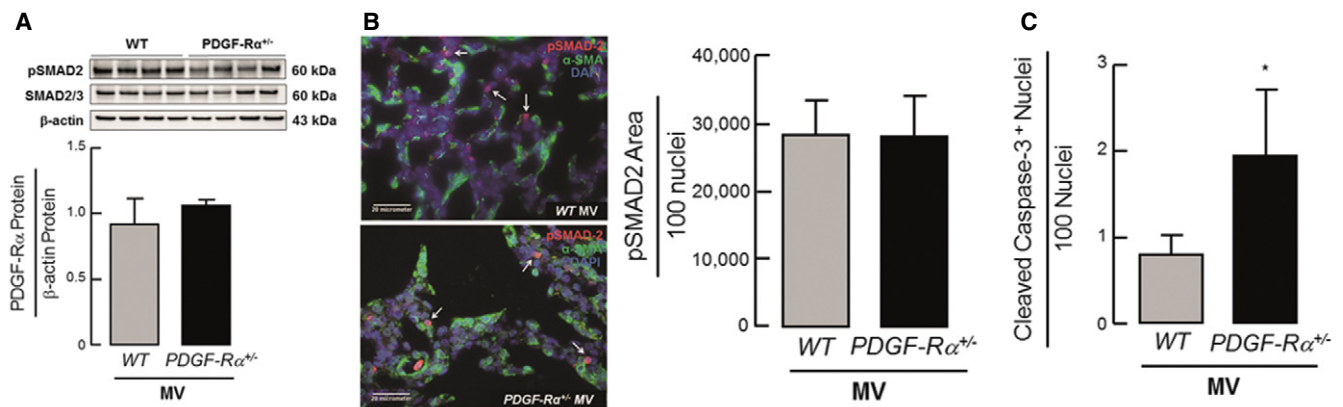


Figure EV3. Similar TGF- β activation with increased apoptosis in lungs of newborn PDGF-R α haploinsufficient mice after MV-O₂ for 8 h.

A Immunoblot analysis showing similar pSMAD levels in whole lung homogenate of newborn WT as well as PDGF-R $\alpha^{+/-}$ mice ($n = 3$ mice/group).
 B Representative immunofluorescence image showing similar pSMAD levels (red) in both newborn WT (upper panel) and PDGF-R $\alpha^{+/-}$ mice (lower panel). α -SMA is in green, and nucleus is stained with DAPI (blue). Quantification of the image showing pSMAD-2 area to 100 nuclei ($n = 3$ –4 mice/group, 4 sections/mice, 10 images/section).
 C Increased apoptosis quantified by cleaved caspase-3⁺ nuclei normalized to 100 nuclei in newborn PDGF-R $\alpha^{+/-}$ compared to WT mice ($n = 4$ mice/group).
 Data information: In (A–C), data are presented as mean \pm SD. Statistical test is two-tailed unpaired Student's t -test (* $P = 0.0159$).