

Expanded View Figures

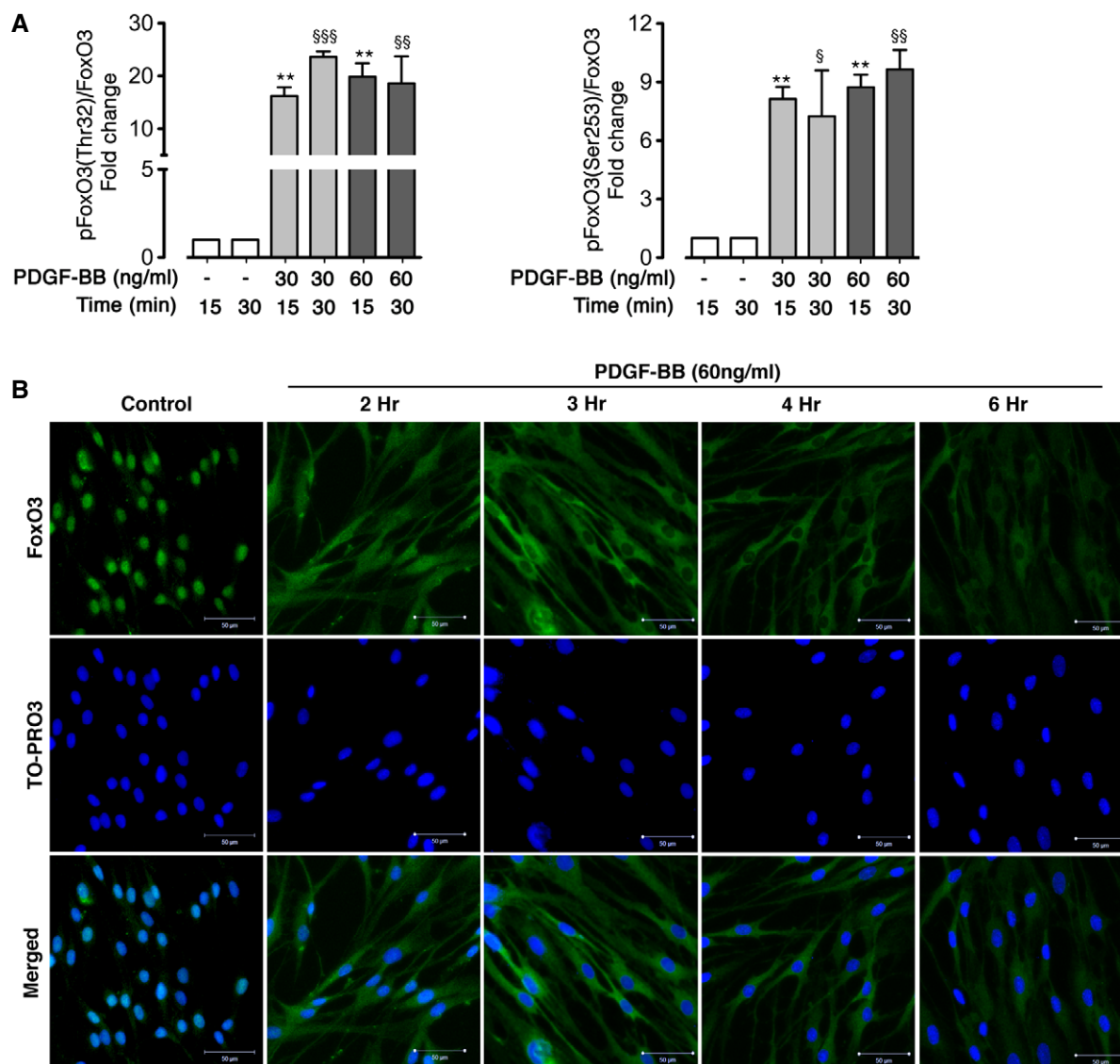


Figure EV1. PDGF induces FoxO3 phosphorylation and nuclear exclusion of FoxO3.

A Densitometry quantitation ratio of p-FoxO3 (Thr32) (left panel) and p-FoxO3 (Ser253) (right panel) in serum-starved (48 h) N-HLF ($n = 3$) that were stimulated without/with PDGF-BB as indicated. Quantification is represented as a fold change to control (time corresponded non-stimulated cells). Data were analyzed using repeated-measures ANOVA, ** $P < 0.01$ versus control 15 min, \$ $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus control 30 min.

B ICC of FoxO3 in N-HLF that was serum-starved for 48 h, and stimulated with PDGF-BB (60 ng/ml) as indicated. Control image panel represents cells that were left non-stimulated for 6 h. TO-PRO3 (blue) was used to label nuclei. FoxO3 and TO-PRO3 images were overlaid to visualize nuclear and cytoplasmic localization of FoxO3. Images are representative of $n = 3$. Scale bar = 50 μm .

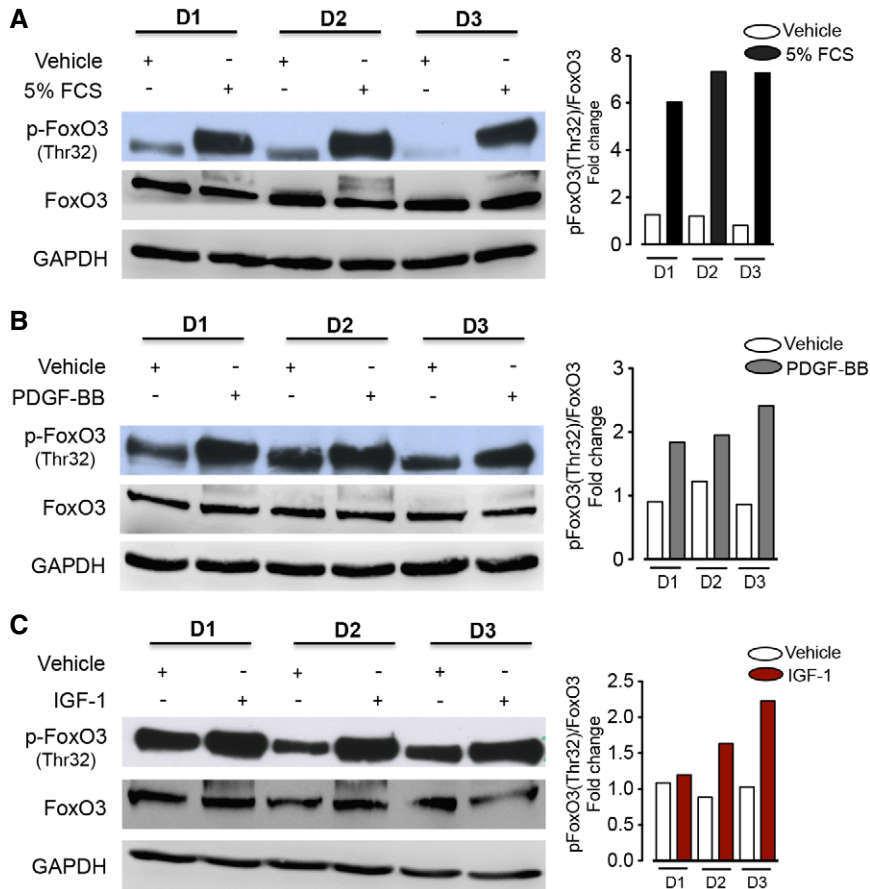


Figure EV2. Different N-HLFs respond in similar manner to various growth factors.

A–C Western blots of p-FoxO3 (Thr32), FoxO3, and GAPDH in serum-starved (48 h) N-HLF ($n = 3$) that was stimulated with 5% FCS (A), PDGF-BB (B), or IGF-1 (C) as indicated. Densitometry quantified data of p-FoxO3 (Thr32) to FoxO3 expression ratios, represented as a fold change to non-stimulated cells.

Figure EV3. Foxo3 knockout (global- and fibroblast-specific) influences immune cell composition in bleomycin-instilled mice lungs.

A–D Immunofluorescence staining was performed on WT, *Foxo3*^{-/-} and *Foxo3*^{fb}^{-/-} mice lung sections (saline- and bleomycin-instilled) using CD68 (A), CD45 (B), and CD3 (C) antibodies. Representative pictographs depicting CD68 (A), CD45 (B), and CD3 (C) staining in green from mice ($n = 3$) in each group. DAPI was used as a nuclear stain. Scale bar = 50 μ m. Fluorescence intensities of CD3-stained sections ($n = 5/6$ per group) were quantified using ImageJ software and normalized to DAPI intensity. Data are expressed as mean \pm SEM and were analyzed using repeated-measures one-way ANOVA, *** $P < 0.001$ versus WT saline group and ^{§§} $P < 0.01$ versus WT bleomycin group. m1, m2, and m3 represent three different mice evaluated in each group.

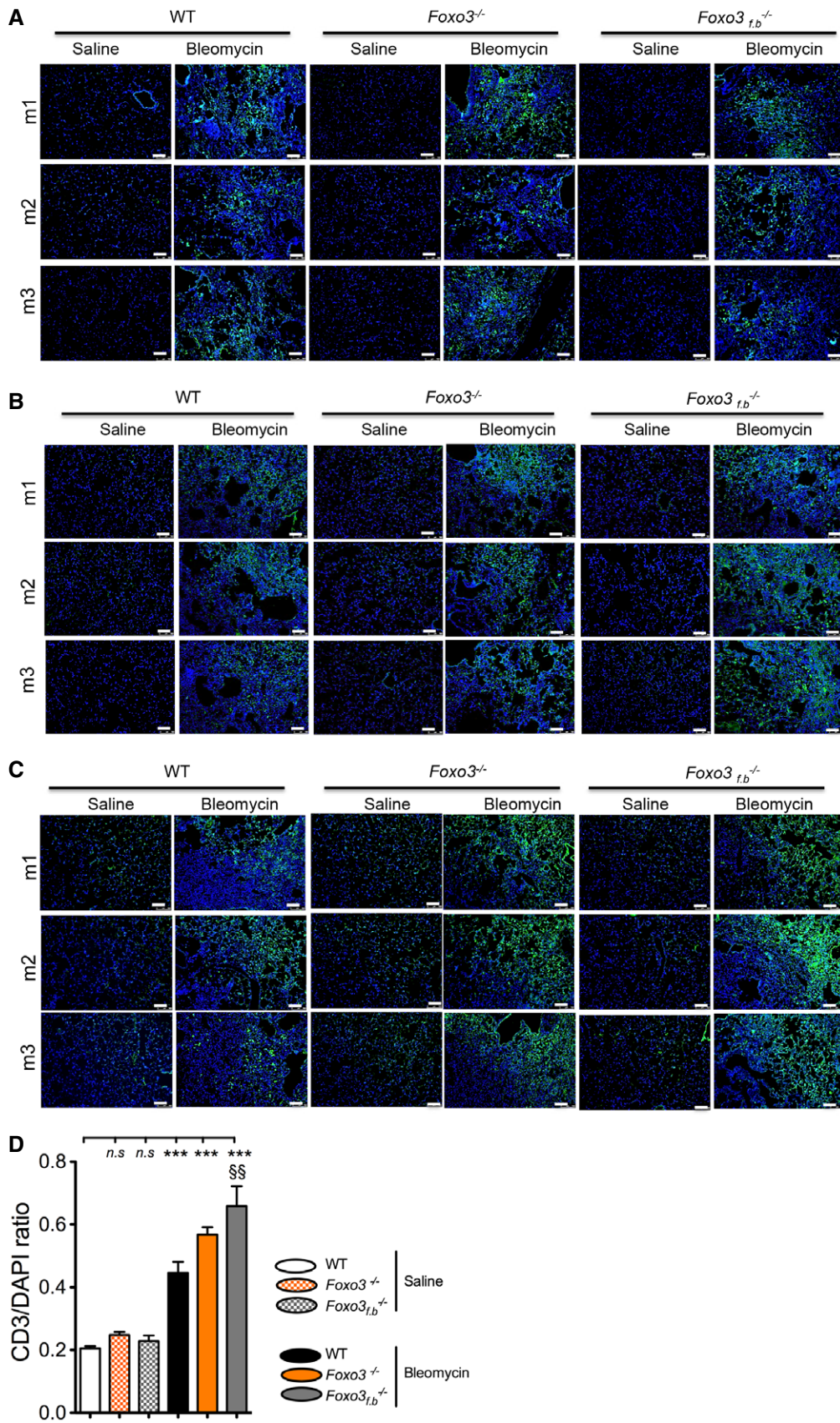


Figure EV3.

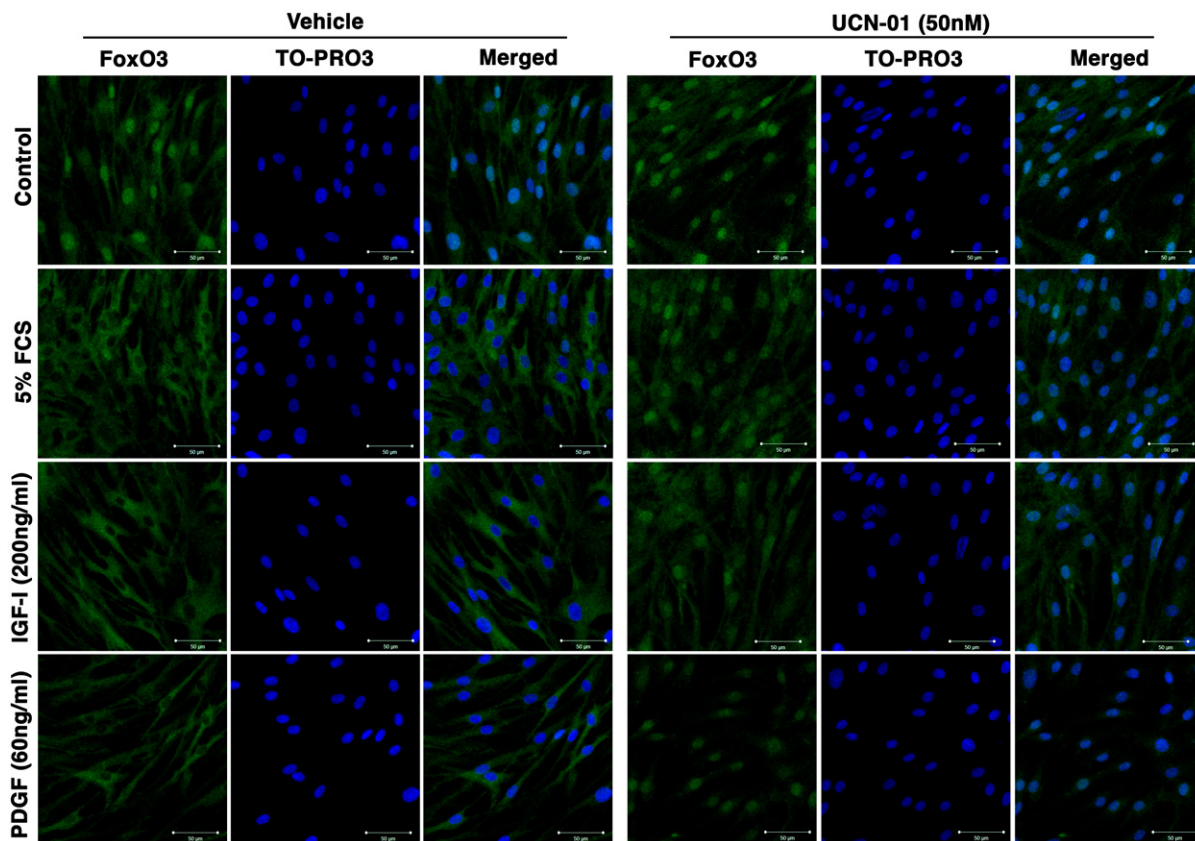


Figure EV4. UCN-01 inhibits FoxO3 nuclear exclusion of FCS-, IGF-1- or PDGF-BB- stimulated IPF-HLF.

Serum-starved (48 h) IPF-HLF were stimulated with 5% FCS or PDGF-BB (60 ng/ml) or IGF-1 (200 ng/ml) and treated with 50 nM UCN-01 or vehicle control (DMSO). After 6 h of treatment, ICC assessed FoxO3 cellular localization. Control panel represents cells that were treated for 6 h with 50 nM UCN-01 or vehicle control (DMSO). TO-PRO3 (blue) was used to label nuclei. FoxO3 and TO-PRO3 images were overlaid to visualize nuclear and cytoplasmic localization of FoxO3. Scale bar = 50 µm. Images are representative of *n* = 3.

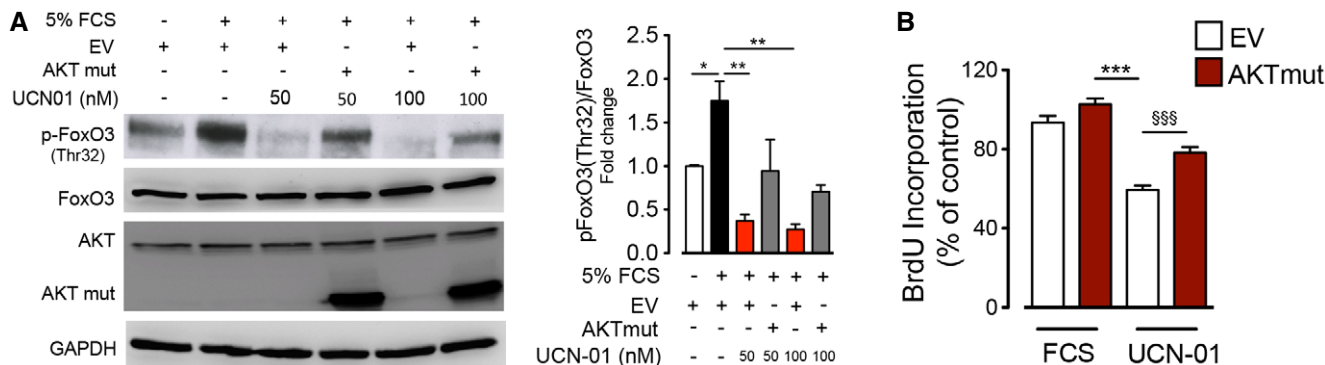


Figure EV5. Anti-proliferative effects of UCN-01 are mediated via FoxO3.

A, B N-HLF was transfected with empty vector (EV) or AKT mutant (AKT mut) plasmid. 6 h after transfection, cells were serum-starved for 36 h and then stimulated with 5% FCS in the presence or absence of UCN-01. From the above-treated samples, after 24 h, Western blots [p-FoxO3 (Thr32), FoxO3, AKT, AKT mut, GAPDH] and cell proliferation measurements (BrdU incorporation) were performed. Data represent percentage of control, EV non-stimulated cells (*n* = 3). Data are expressed as mean ± SEM and were analyzed using one-way ANOVA, **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 versus 5% FCS-EV, and §§§*P* < 0.001 versus UCN-01-EV.