Brd4-p300 Inhibition Down-regulates Nox4 and Accelerates Lung Fibrosis Resolution in Aged Mice

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Supplementary Materials:

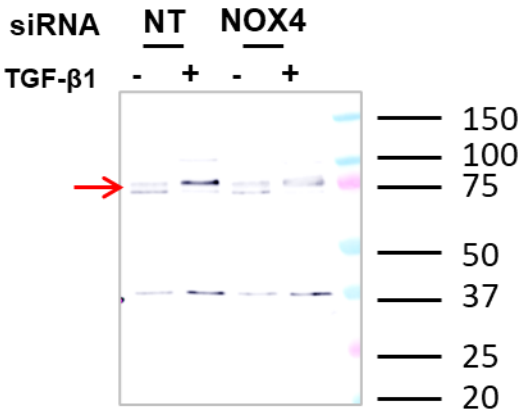


Fig. S1. Validation of Nox4 antibody from R&D systems AF8158.

IMR90 cells are transfected with siRNA NT or Nox4, then treated with or without TGF-β1 at 2ng/ml for 24h. Cell lysate were subject to western blot, and Nox4 signal was detected with Nox4 antibody from R&D system AF8158 (red arrow). The up-regulation of Nox4 by TGF-β1 in this cell line have been reported (5), and was used to validate the antibody.

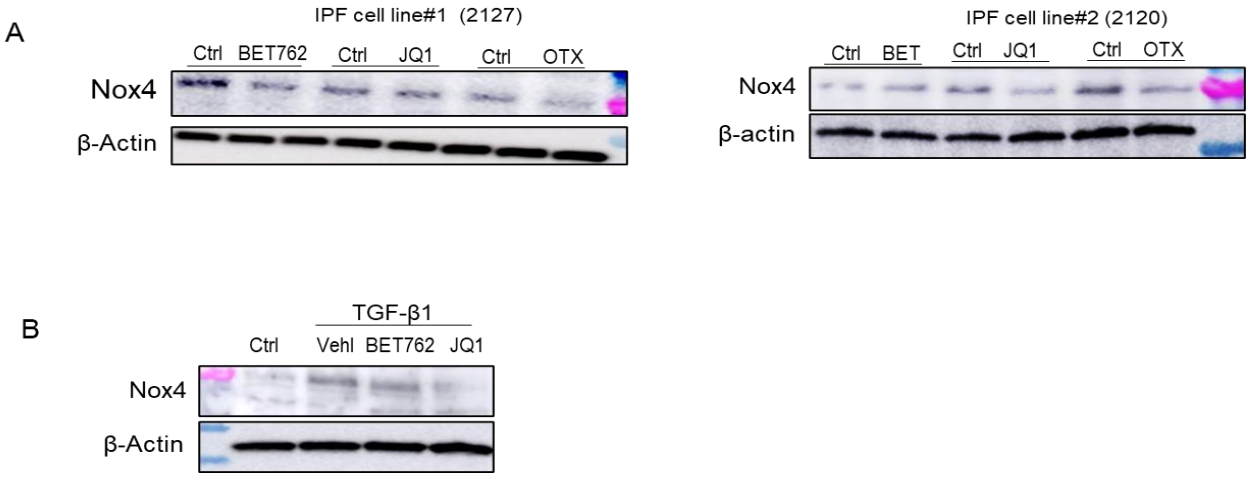
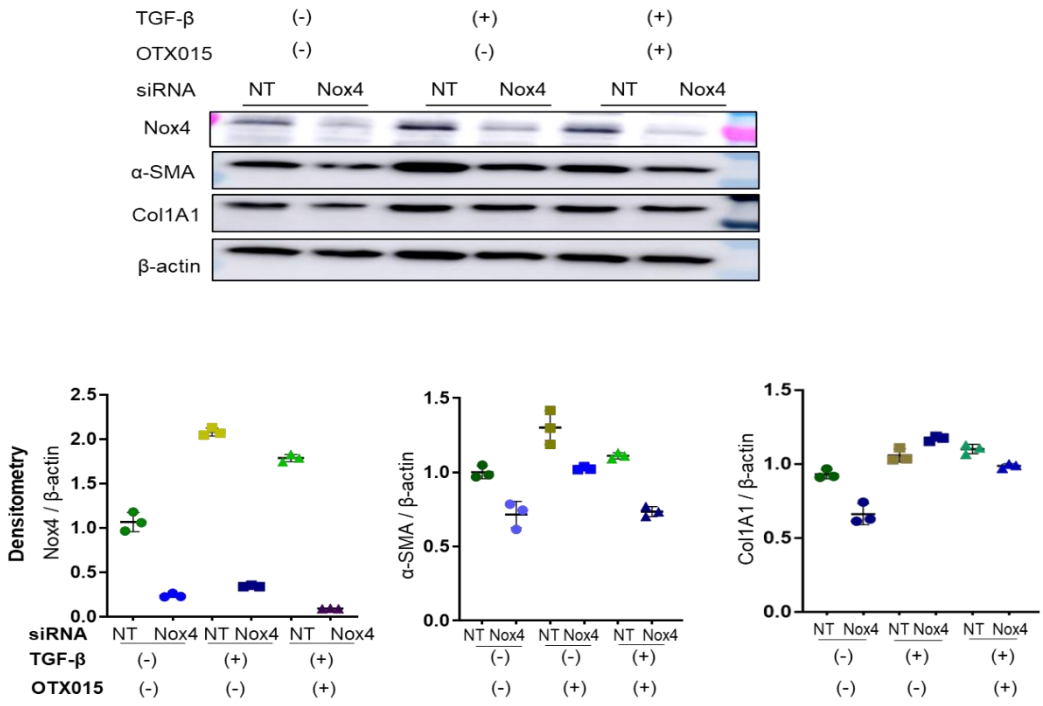


Fig. S2. Nox4 protein level expression in primary IPF fibroblasts or IMR-90 pretreated with various BET inhibitors stimulated with TGF-β1. **A.** Primary IPF fibroblasts from different individuals were treated with different BET inhibitors, as indicated in the methods. The whole cell lysates were collected, and subjected to western blots. Nox4 and β-actin expression were detected. Different response to inhibitor I-BET762 (BET762 or BET in figure) in IPF cell line 1 and 2. **B.** IMR90 fibroblasts were pretreated with vehicle or various inhibitor for 2 h before treated with or without TGF-β1 at 2 ng/ml for 48 h. Then whole cell lysate was collected and subjected to western blots, to detect the Nox4 and β-actin expression.

A



B

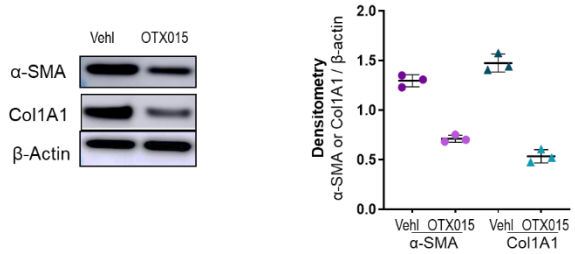


Fig. S3. A. Lung fibroblasts transfected with siRNA NT or Nox4, and subjected to TGF-β1 (2ng/ml) for 48 h with or without pre-treatment with OTX015 (0.5 μM) for 2 h. The whole cell lysate was collected, and subject to WB. The protein level expression of Nox4, α-SMA and Col1A1 were examined, and β-actin was used as loading control. Densitometry of Nox4, α-SMA or collagen I (ratio to β-actin, as mean ± SD) is shown at the lower panel. **B. IPF fibroblasts treated with OTX015** as in Figure 1D, also demonstrated down-regulation of α-SMA and Collagen I by western blots; densitometry (ratio to β-actin, as mean ± SD) is shown at the right.

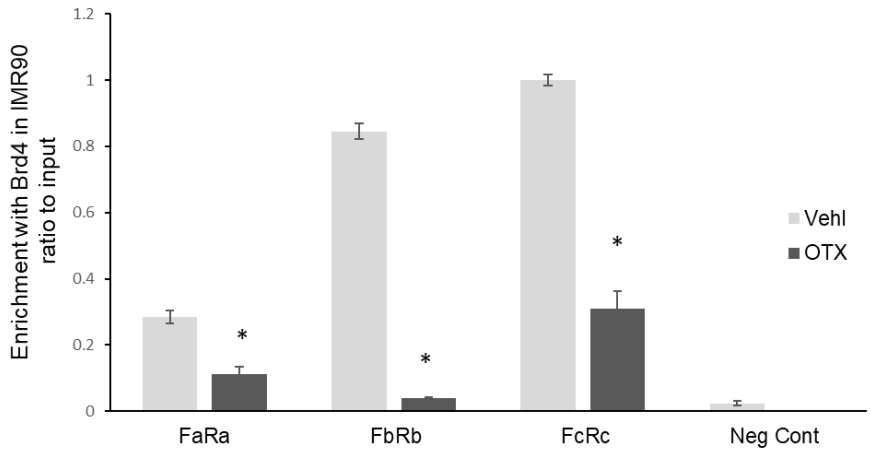


Fig. S4. ChIP assays with IMR90 treated with OTX015 alone as indicated in Figure 4. The cells were treated the same way as indicated in Figure 4. These cells were in serum free medium overnight, and then OTX015 was added at 0.5 μM for 48 h before collected for ChIP assays. Representative pull-down with Brd4 antibody. Negative control (Neg Cont) is IgG pull down. Quantitative real-time PCR was done with the same sets of Nox4 promoter primers shown in Figure 3A.

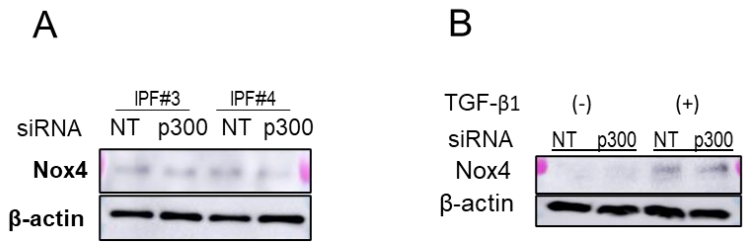


Fig. S5. Protein levels of Nox4 in cells transfected with non-targeting (NT) siRNA or p300 siRNA, with or without TGF-β1 treatment. A. IPF fibroblasts from two different individuals were transfected with siRNA NT or p300 as indicated in Fig. 5. Representative western blots demonstrated the levels of Nox4 and β-actin. **B.** Non-IPF fibroblasts IMR90 with siRNA NT or p300 knockdown, then treated with TGF-β1 as in Fig 5D, protein levels of Nox4 and β-actin by WB.

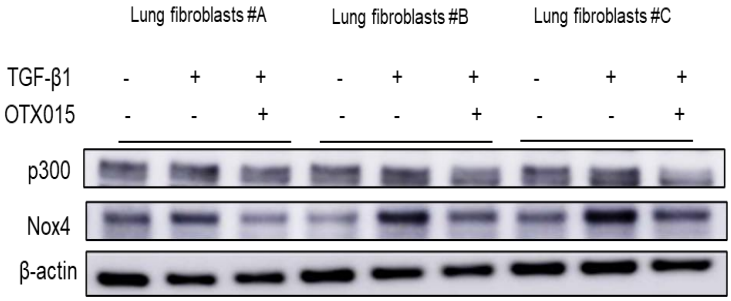


Fig. S6. Human lung fibroblasts (from 3 different non-IPF individuals) **were treated with TGF-β1** (2ng/ml) for 48 h with or without pretreatment with OTX015 (0.5 μM) for 2 h as indicated in Figure 6A. Whole cell lysate were collected, and subjected to WB, protein levels of p300, and Nox4 were examined, and β-actin was used as a loading control.

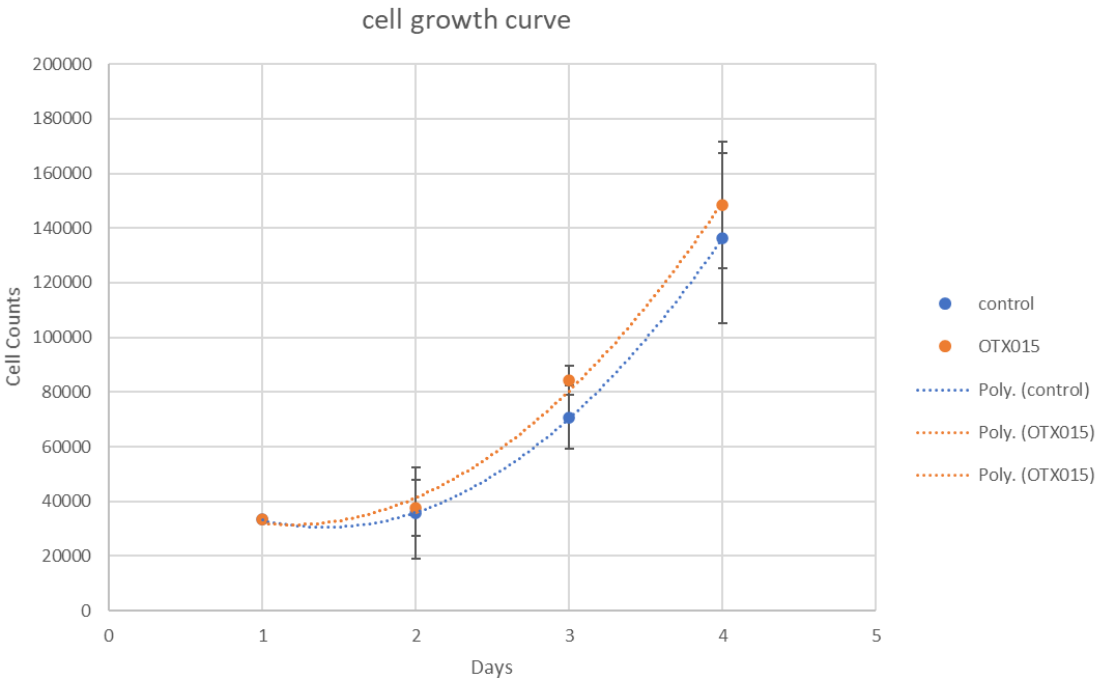


Fig. S7. Cell growth curve of lung fibroblasts with or without OTX015 (0.5 μM) for 3 days. Lung fibroblasts seeded in culture dishes at about 33000 on day 1, and the number of cells were counted each day from day 2 to day 4. The numbers of control cells are in blue dots, with vehicle only, while orange dots are the ones with OTX015 (0.5 μM). The cell numbers are average of at least 3-dish counts, and the error bars are standard deviation.