

YMTHE, Volume 25

Supplemental Information

miR-18a-5p Inhibits Sub-pleural Pulmonary

Fibrosis by Targeting TGF- β Receptor II

Qian Zhang, Hong Ye, Fei Xiang, Lin-Jie Song, Li-Ling Zhou, Peng-Cheng Cai, Jian-Chu Zhang, Fan Yu, Huan-Zhong Shi, Yunchao Su, Jian-Bao Xin, and Wan-Li Ma

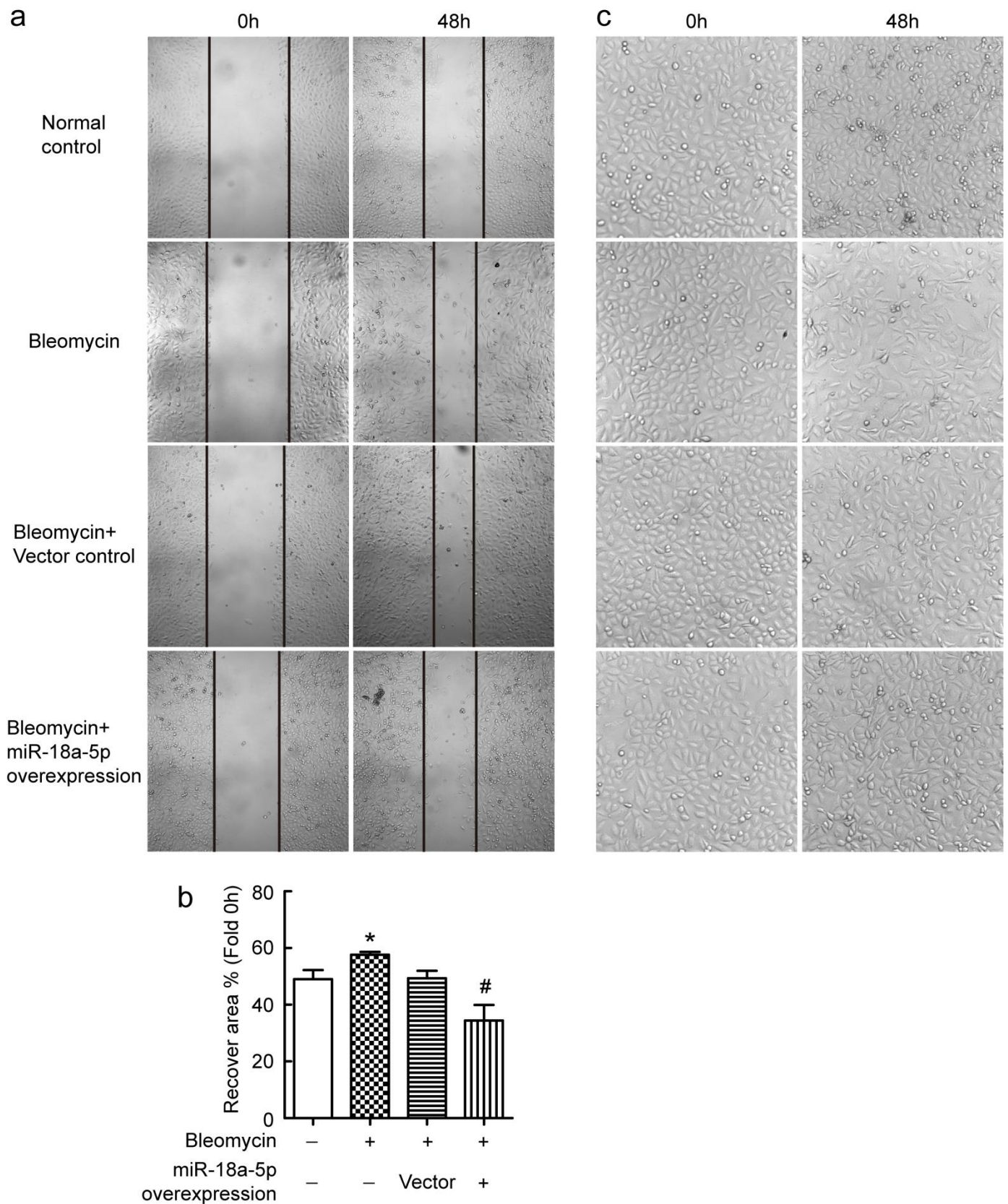


Figure S1 Over-expression of miR-18a-5p prevents bleomycin-induced cell migration and morphological changes of PMCs. PMCs were transduced with recombinant lentivirus vector encoding miR-18a-5p or scrambled negative control RNAs (vector control). PMC monolayers were then subjected to migration assay as described in the Methods. **(a)** Representative images of PMC migration into the wound areas (indicated

by vertical lines). **(b)** Bar graph depicting percentages of wound closure after 48 h. $n=5$, $*P<0.05$ versus control. $^{\#}P<0.05$ versus vector control. **(c)** Representative images showing PMC morphologic changes.

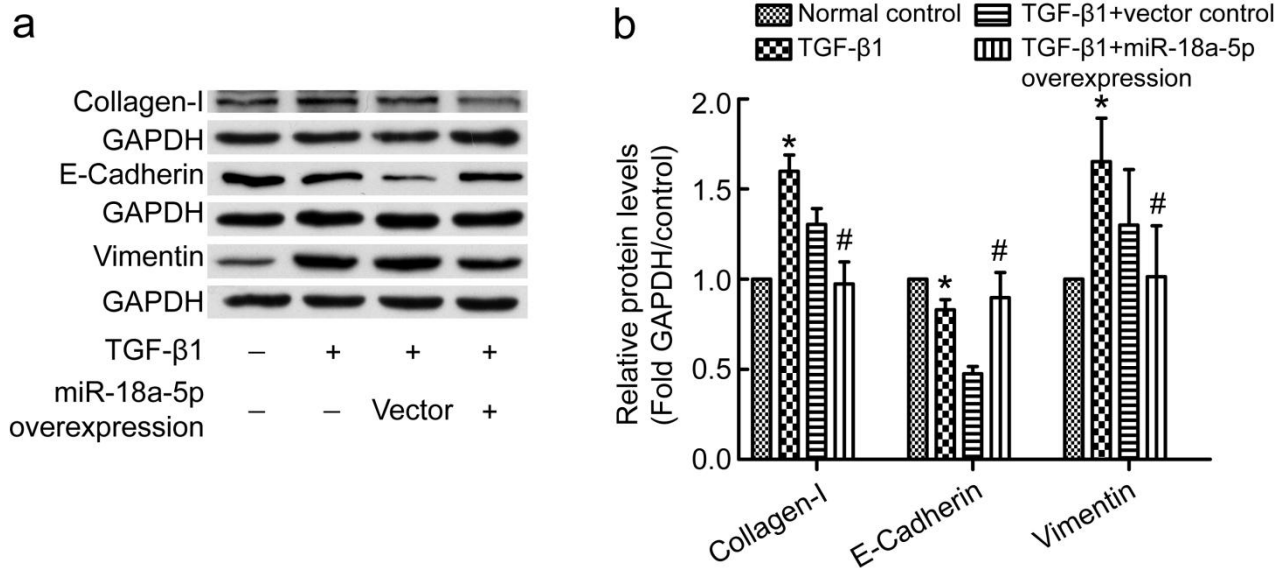


Figure S2 over-expression of miR-18a-5p prevents TGF-β1-induced EMT in primary PMCs. Primary PMCs were isolated and cultured as described as the Methods. Primary PMCs were transduced with recombinant lentivirus encoding miR-18a-5p or scrambled negative control RNAs (vector control). The cells were treated with TGF-β1 (5 ng/ml) for 48 h, after which protein levels were measured by western blotting. **(a)** Representative image of immunoblots. **(b)** Bar graphs depicting changes in relative levels of collagen-I (n=3), E-cadherin (n=5), and vimentin (n=5). The density values of blots were normalized to the GAPDH and controls. * $P < 0.05$ versus control. # $P < 0.05$ versus vector control.

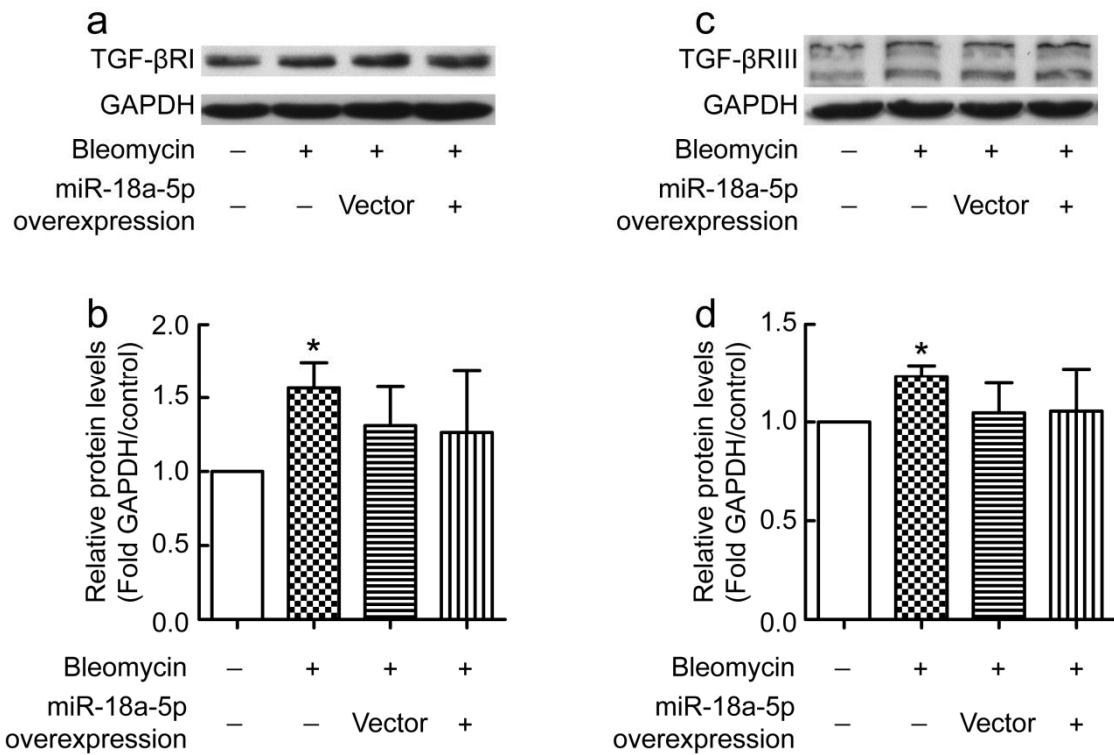


Figure S3 miR-18a-5p does not affect expression of TGF-βRI and TGF-βRIII proteins in PMCs. PMCs were transduced with recombinant lentivirus encoding miR-18a-5p or scrambled negative control RNAs (vector control). The cells were treated with or without bleomycin (0.2 μg/ml) for 48 h, after which protein levels of TGF-βRI and TGF-βRIII were measured by western blotting. **(a, c)** Representative images of immunoblots. **(b, d)** Bar graphs depicting changes in relative protein expression according to a and c. The density values of blots were normalized to the GAPDH and controls. n=4, **P*<0.05 versus control.

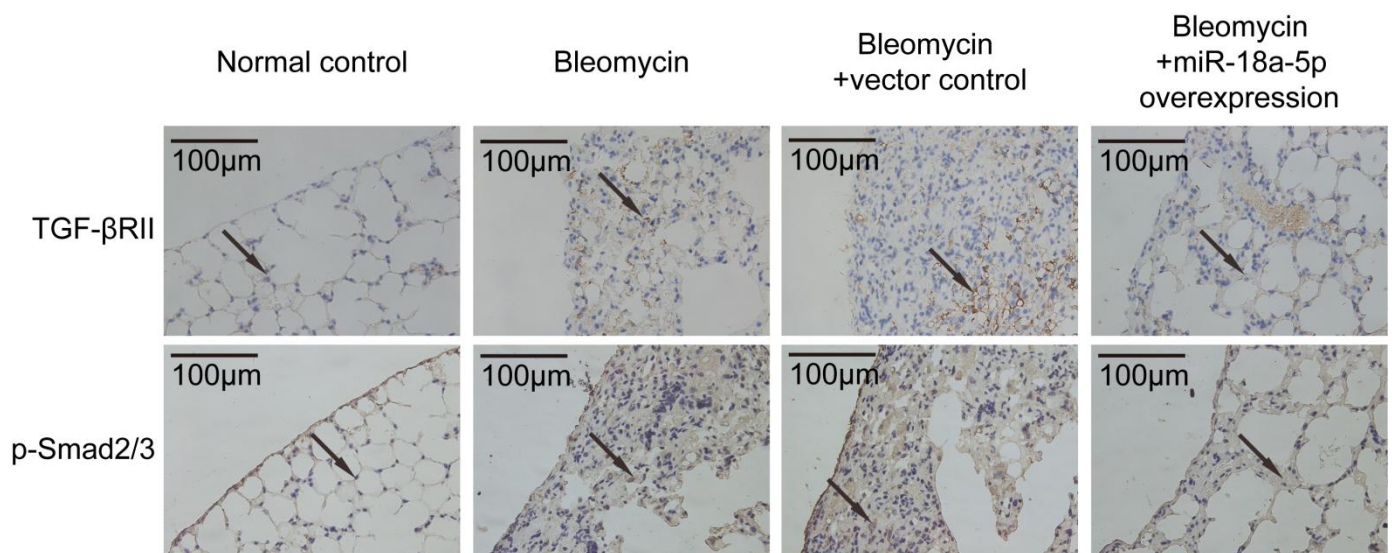


Figure S4 miR-18a-5p over-expression prevents increases of TGF- β RII and p-Smad2/3 *in vivo*. C57BL/6 mice were treated with bleomycin (50 mg/kg) by intraperitoneal injection at days 1, 5, 8, 11 and 15. miR-18a-5p over-expression or control lentivirus were intraperitoneal injected at a dose of 2×10^6 TU on days 24, 26 and 28. All mice were euthanized at day 40, and then lung tissues were taken for immunohistochemistry staining of TGF- β RII and p-Smad2/3.