SUPPLEMENTARY MATERIAL

Manuscript Title:

miR-4739 mediates pleural fibrosis by targeting bone morphogenetic protein 7

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Contents:

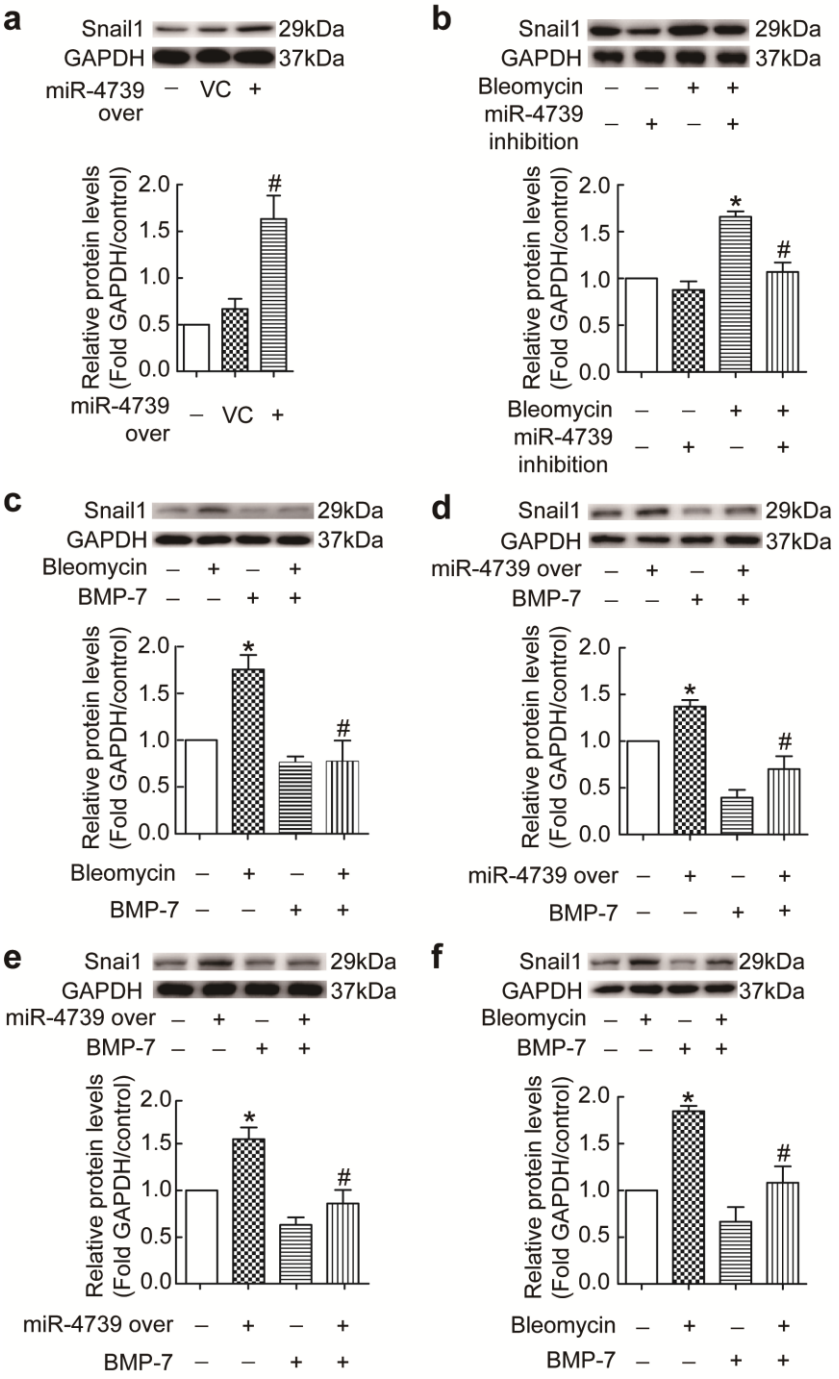

Fig. S1. Bleomycin induces increases in Snail1 expression via miR-4739/BMP-7 axis in PMCs. (a) Human PMCs were transducted with recombinant lentivirus vector containing miR-4739 or scrambled negative control (vector control) plasmids. After 96 h, protein level of Snail1 was measured. n=3, $^{\#}P<0.05$ versus vector control (Student's *t*-test). (b) Human PMCs were transducted with recombinant lentivirus vector containing siRNA (miR-4739

inhibition) or scrambled negative control (vector control) plasmids. Cells which were successfully transfected with lentivirus were employed for next experiments after 48 h. The cells were treated with or without bleomyin (0.2 μg/ml) for 24 h. Protein level of Snail1 was detected. n=3, **P*<0.05 versus vector control, $^{\#}P$<0.05 versus bleomycin group (ANOVA test). (c-d) Human PMCs were treated with bleomycin (0.2 μg/ml) or miR-4739 over-expression, and then exogenous BMP-7 was added (100 ng/ml). After 24 h, protein level of Snail1 was detected. n=3, **P*<0.05 versus control, $^{\#}P$<0.05 versus bleomycin group or miR-4739 over-expression group (ANOVA test). (e-f) Rat primary PMCs were treated with bleomycin (0.2 μg/ml) or miR-4739 over-expression, and then exogenous BMP-7 was added (100 ng/ml). After 24 h, protein level of Snail1 was detected. n=3, **P*<0.05 versus control, $^{\#}P$<0.05 versus bleomycin group or miR-4739 over-expression group (ANOVA test).

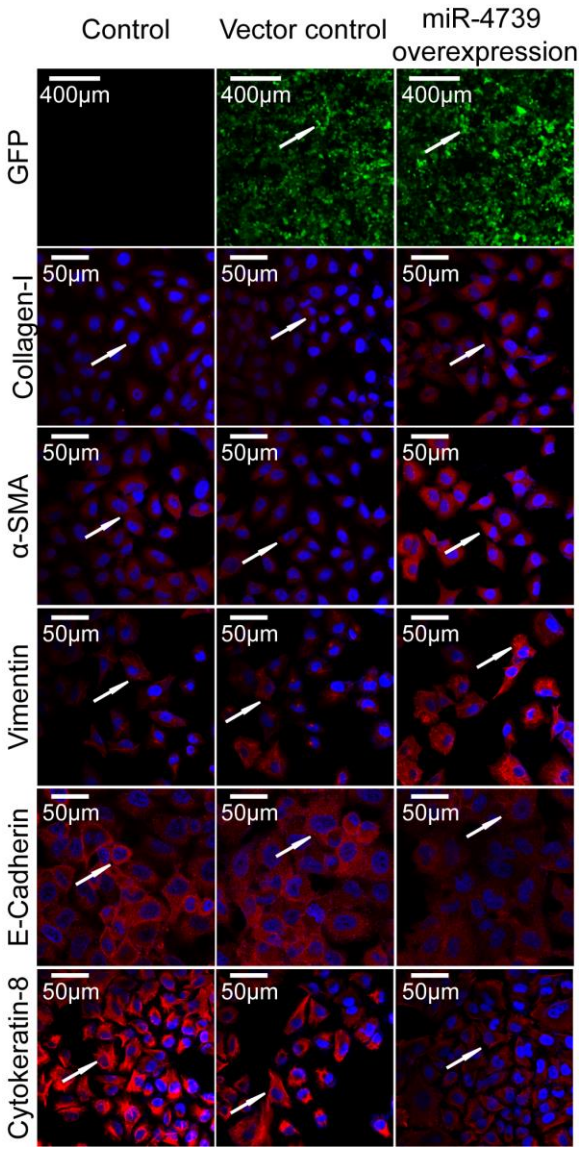

Fig. S2. Immunofluorescence staining of proteins in human PMCs treated with miR-4739 over-expression. Human PMCs were transducted with recombinant lentivirus vector containing miR-4739 or scrambled negative control (vector control) plasmids. After 72 h, proteins of collagen-I, α-SMA, vimentin, E-cadherin and cytokeratin-8 were investigated by immunofluorescence staining.

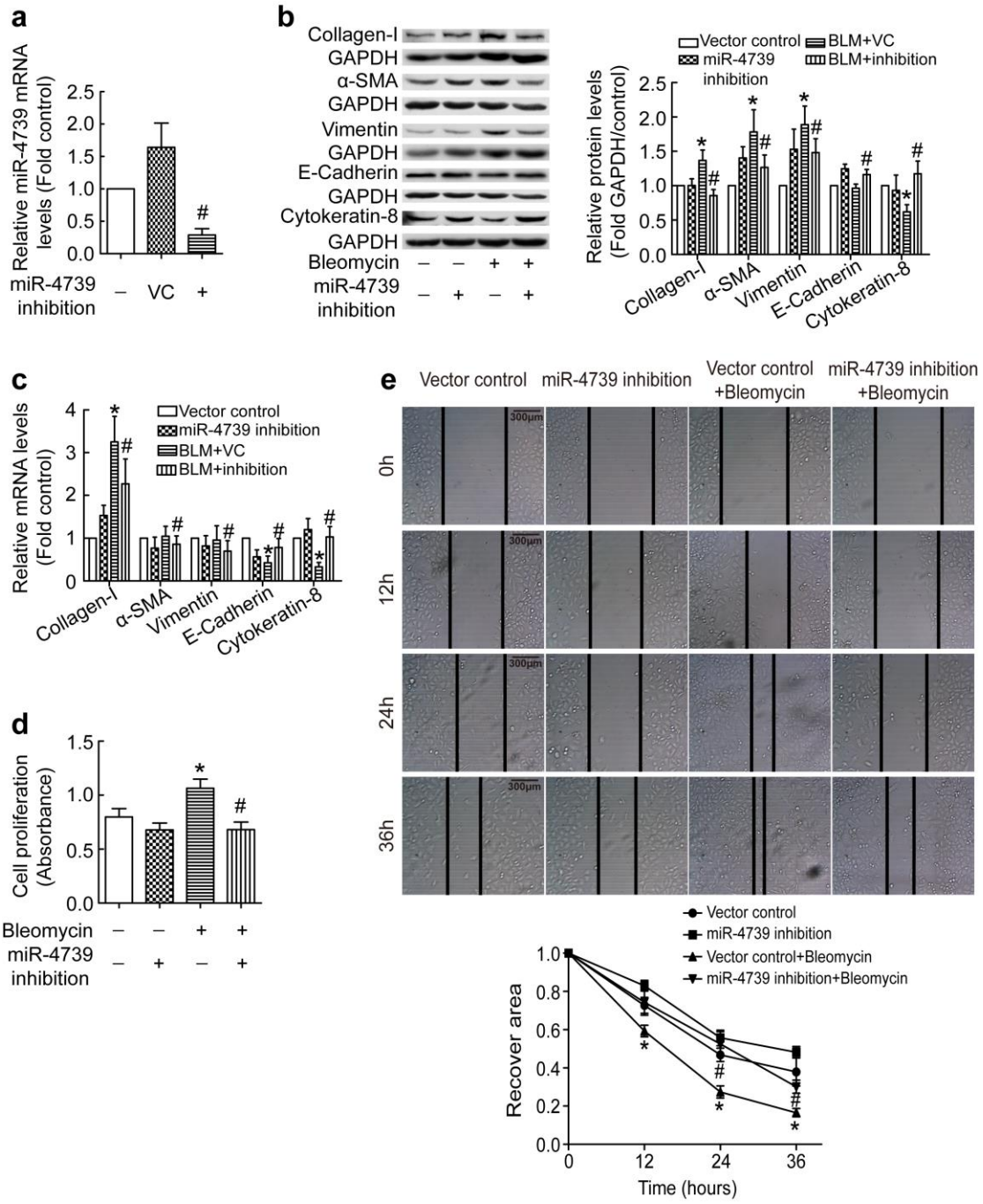

Fig. S3. miR-4739 inhibition prevents MMT, proliferation and migration of PMCs. Human PMCs were transducted with recombinant lentivirus vector containing siRNA (miR-4739 inhibition) or scrambled negative control (vector control) plasmids. Cells which were successfully transfected with lentivirus were employed for next experiments after 48 h. The cells were treated with or without bleomyin (0.2 μg/ml) for 24 h. The levels of mRNAs, proteins, cell proliferation and migration of PMCs were detected as descriptions in the

Methods. (a) Changes in relative miR-4739 mRNA levels in PMCs . n=6, $^{\#}P<0.05$ versus vector control (Student's *t*-test). (b) Changes in relative protein levels of collagen-I (n=8), α-SMA (n=10), vimentin (n=5), E-cadherin (n=13) and cytokeratin-8 (n=4) were detected by Western blotting. $^{*}P<0.05$ versus vector control, $^{\#}P<0.05$ versus bleomycin group (ANOVA test). (c) Changes in relative mRNA levels of collagen-I(n=5), α-SMA (n=5), vimentin (n=6), E-cadherin (n=4) and cytokeratin-8 (n=7). $^{*}P<0.05$ versus vector control, $^{\#}P<0.05$ versus bleomycin group (ANOVA test). (d) Changes in cell proliferation determined by CCK-8 kit. n=12, $^{*}P<0.05$ versus vector control; $^{\#}P<0.05$ versus bleomycin group (ANOVA test). (e) Representative image of cell migration and line graph depicting changes in recovery area in percentage of the initial scratched area. n=5, $^{*}P<0.05$ versus vector control, $^{\#}P<0.05$ versus bleomycin group (ANOVA test).

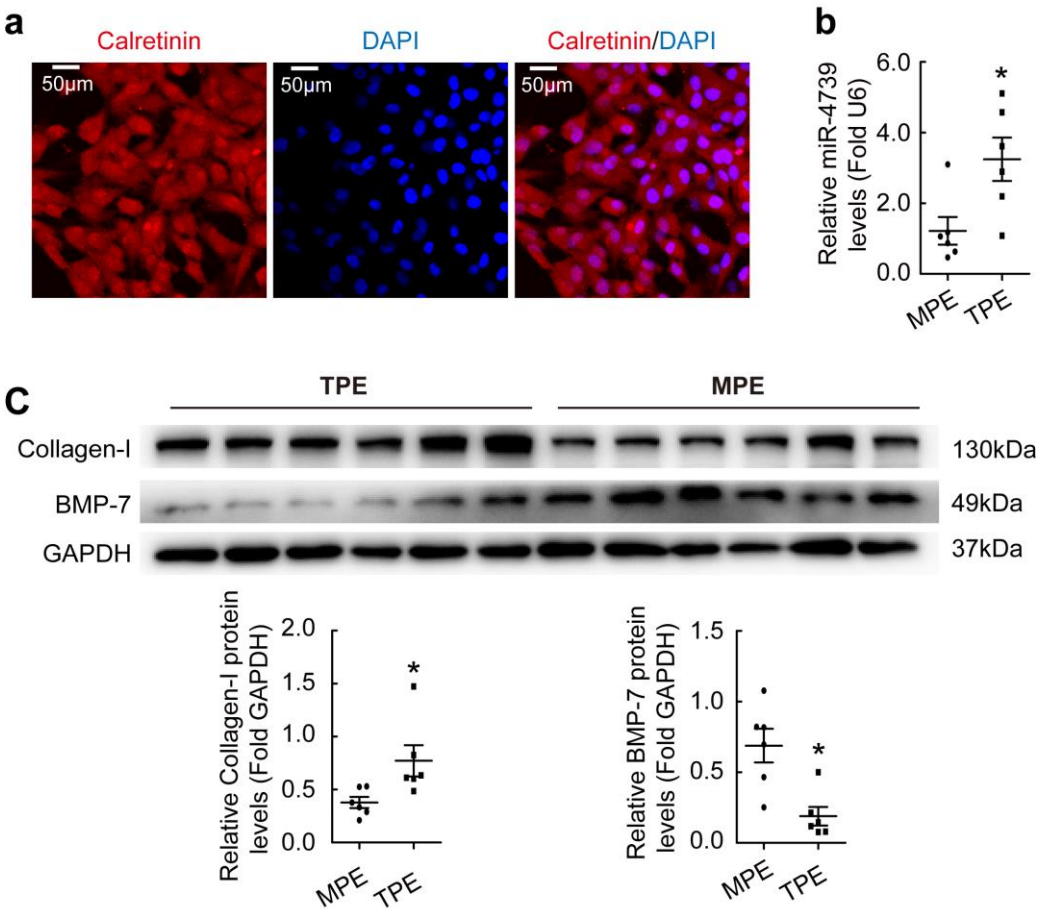

Fig. S4. miR-4739 is high but BMP-7 is low expressed in PMCs from TPE patients with pleural fibrosis. TPE: tuberculous pleural effusion. MPE: malignant pleural effusion. (a) Identification of PMCs from pleural effusion. The marker of PMCs, calretinin was investigated by immunofluorescence staining. (b) Relative miR-4739 levels in PMCs were determined by RT-qPCR. n=12, $^{*}P<0.05$ versus MPE group (Student's *t*-test). (c) Relative collagen-I and BMP-7 protein levels in PMCs were determined by Western blotting. n=12, $^{*}P<0.05$ versus MPE group (Student's *t*-test).

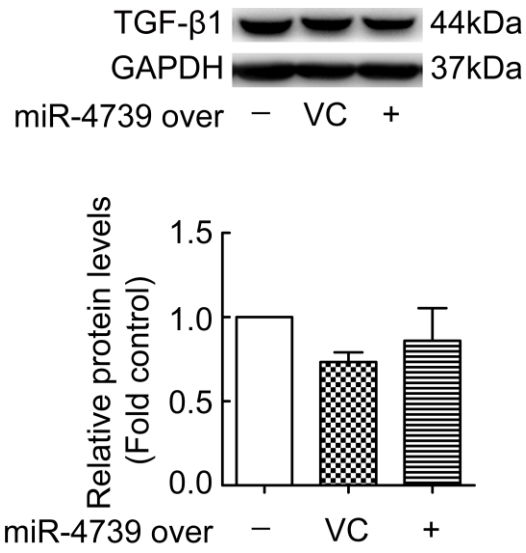

Fig. S5. miR-4739 over-expression does not change protein levels of TGF-β1 in PMCs. Human PMCs were transducted with recombinant lentivirus vector containing miR-4739 or scrambled negative control (vector control) plasmids. Cells which were successfully transfected with lentivirus were employed for next experiments after 72 h. The protein levels of TGF-β1 were measured by Western blot analysis. A representative image of immunoblots was presented. The bar graphs were depicting changes in relative density of TGF-β1. n=6 (Student's *t*-test).

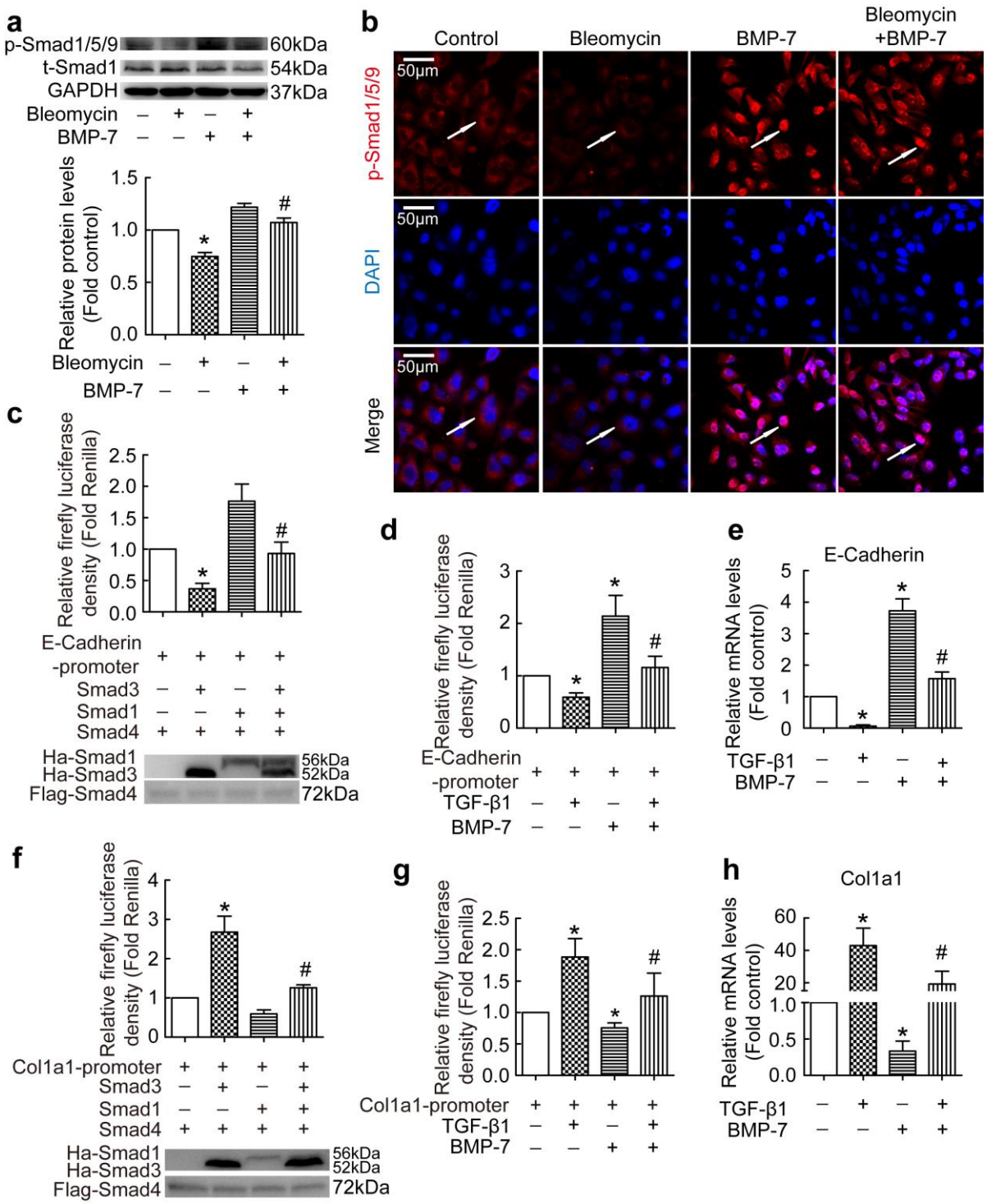

Fig. S6. BMP-7-Smad1/5/9 signaling regulates the promoter activity of E-cadherin and collagen-I genes. Human PMCs were treated by bleomycin (0.2 μg/ml) with or without exogenous BMP-7 (100 ng/ml) for 24 h. (a) Changes of p-Smad1/5/9 protein measured by

Western blotting. n=7, *P<0.05 versus control, $^{\#}P$<0.05 versus bleomycin group (ANOVA test). (b) Changes of p-Smad1/5/9 protein were investigated by immunofluorescence staining. (c) HEK 293T cells were used to detect the promoter activity of E-cadherin. All groups were transfected with Smad4 expression vector and a control renilla vector. n=3, *P<0.05 versus control group, $^{\#}P$<0.05 versus Smad3 group (ANOVA test). (d-e) Human PMCs were transfected with E-cadherin promoter plasmid or empty control vector. After 24 h, TGF-β1 (0.5 ng/ml) or BMP-7 (100 ng/ml) was added. The E-cadherin promoter activity was presented as d. All groups were transfected with a control renilla expression. n=5, *P<0.05 versus control group; $^{\#}P$<0.05 versus TGF-β1 group (ANOVA test). The relative mRNA levels of E-cadherin were detected by RT-qPCR as e**.** n=3, *P<0.05 versus control group; $^{\#}P$<0.05 versus TGF-β1 group (ANOVA test). (f) HEK 293T cells were used to detect the promoter activity of collagen-I (Col1a1). All groups were transfected with Smad4 expression vector and a control renilla vector. n=5, *P<0.05 versus control group, $^{\#}P$<0.05 versus Smad3 group (ANOVA test). (g-h) Human PMCs were transfected with Col1a1 promoter plasmid or empty control vector. After 24 h, TGF-β1 (0.5 ng/ml) or BMP-7 (100 ng/ml) was added. The Col1a1 promoter activity was presented as g. All groups were transfected with a control renilla expression. n=5, *P<0.05 versus control group, $^{\#}P$<0.05 versus TGF-β1 group (ANOVA test). The relative mRNA levels of Col1a1 were detected by RT-qPCR as h**.** n=3, *P<0.05 versus control group; $^{\#}P$<0.05 versus TGF-β1 group (ANOVA test).

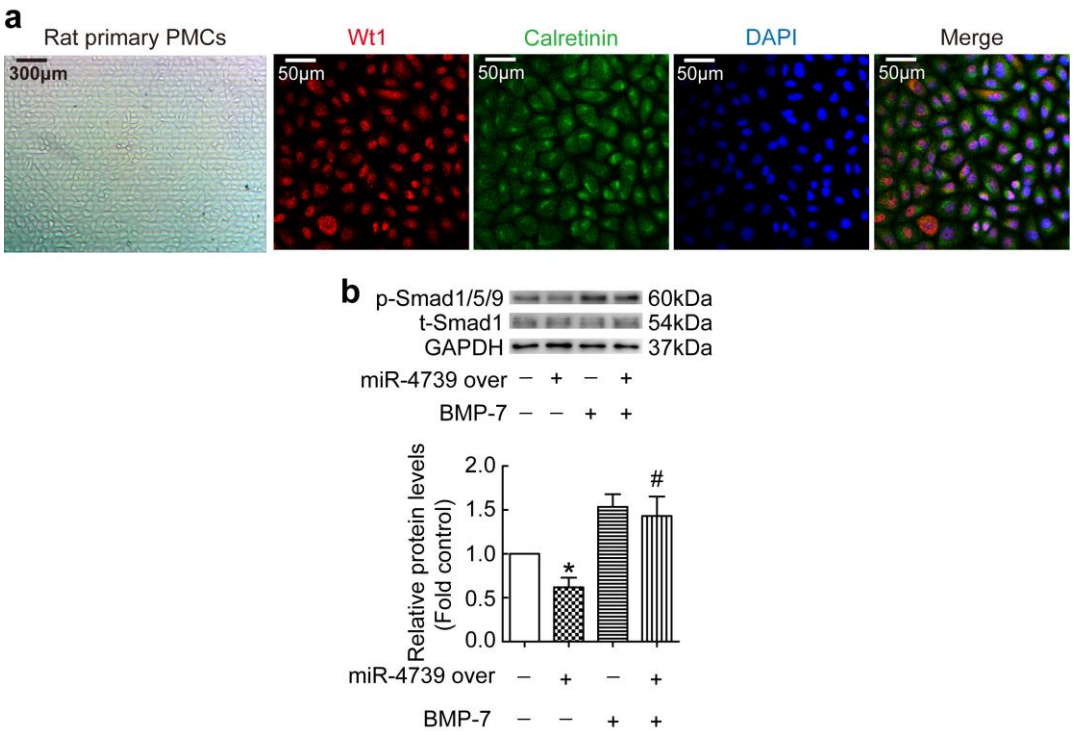

Fig. S7. BMP-7 restores miR-4739 over-expression induced decreases in p-Smad1/5/9. (a) Identification of rat primary PMCs. Wt1 and calretinin, the two markers of PMCs were investigated by immunofluorescence staining. (b) Rat primary PMCs were transducted with recombinant lentivirus vector containing miR-4739 or scrambled negative control (vector control) plasmids. Cells which were successfully transfected with lentivirus were employed for next experiments after 72 h. The cells were treated with or without BMP-7 (0.1 μg/ml) for 24 h. Changes of protein levels of p-Smad1/5/9 were measured by Western blotting. n=4, *P<0.05 versus vector control; $^{\#}P$<0.05 versus miR-4739 over-expression group (ANOVA test).

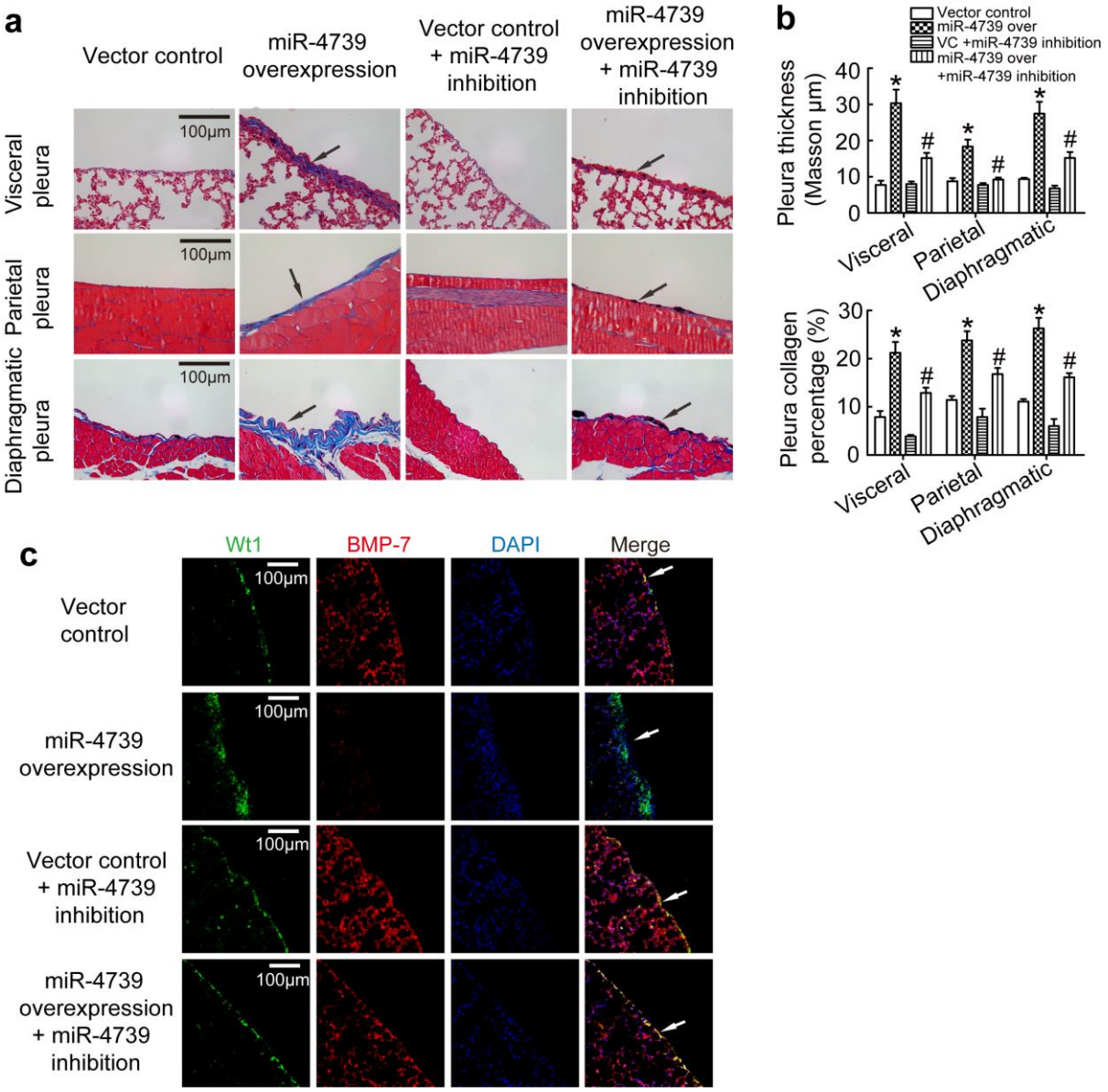

Fig. S8. Deletion miR-4739 reversed pleural fibrosis induced by miR-4739 over-expression *in vivo*. (a-b) C57BL/6 mice were treated with miR-4739 lentivirus plasmids or vector control lentivirus plasmids with carbon particles by intrapleural injection at days 1, 5 and 8. At days 11, 14 and 17, mice were respectively intraperitoneally injected with control lentivirus (2×10^6 TU/mouse) or miR-4739 inhibition lentivirus. All mice were euthanized at day 21, and then tissues were taken for histological staining. (a) Masson's trichrome staining of visceral pleura, parietal pleura and diaphragmatic pleura. (b) Bar graphs depicting changes of pleura

thickness and collagen percentage. n=3, $^{*}P<0.05$ versus vector control, $^{\#}P<0.05$ versus miR-4739 over-expression (ANOVA test). (c) BMP-7 levels of PMCs in mice were measured by immunofluorescence staining.

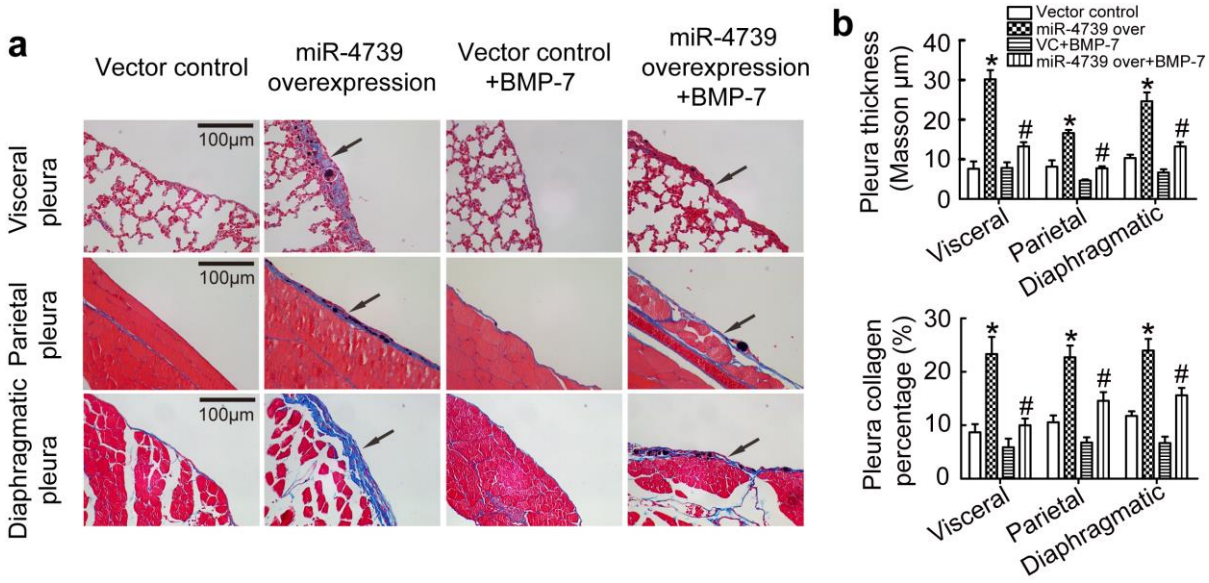

Fig. S9. BMP-7 reversed pleural fibrosis induced by miR-4739 over-expression *in vivo*. (a-b) C57BL/6 mice were treated with miR-4739 lentivirus plasmids or vector control lentivirus plasmids with carbon particles by intrapleural injection at days 1, 5 and 8. At days 11, 14 and 17, mice were respectively intraperitoneally injected with BMP-7 (150 μg/kg). All mice were euthanized at day 21, and then tissues were taken for histological staining. (a) Masson's trichrome staining of visceral pleura, parietal pleura and diaphragmatic pleura. (b) Bar graphs depicting changes of pleura thickness and collagen percentage. n=3, $^{*}P<0.05$ versus vector control, $^{\#}P<0.05$ versus miR-4739 over-expression (ANOVA test).