Supporting information

MiR-326 inhibits inflammation and promotes autophagy in silica-induced pulmonary fibrosis through targeting TNFSF14 and PTBP1

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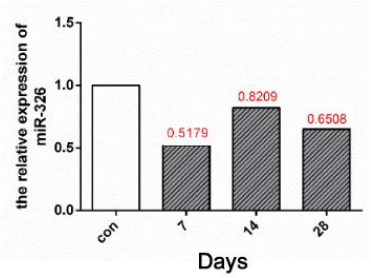

Figure S1. The expression of miR-326 in fibrotic lung tissues of mice.
The expression of miR-326 in mouse lung tissues of the SiO_2 group compared to the control group according to our miRNA microarray data.

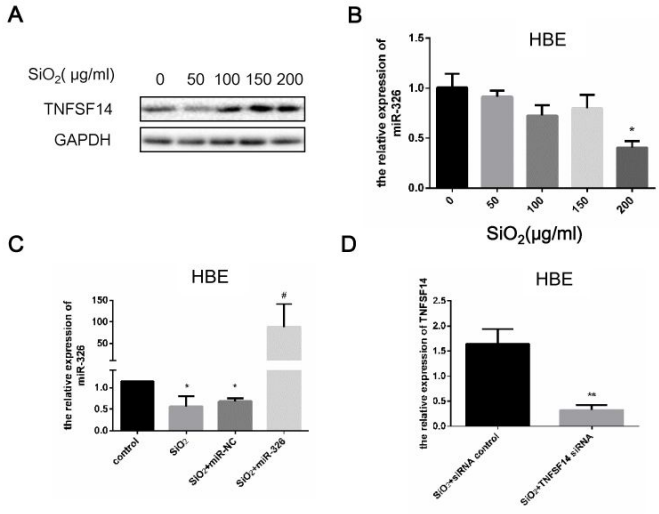

Figure S2. MiR-326 dampens lung inflammation by targeting TNFSF14 in HBE cells.
A: The HBE cells were exposed to SiO_2 (0, 50, 100, 150, 200μg/ml) for 24 h. The protein level of TNFSF14 was detected by western blot assay. **B:** The HBE cells were exposed to SiO_2 (0, 50, 100, 150, 200 μg/ml) for 24 h. qRT-PCR analysis was performed to detect miR-326 levels (mean ± SD, n=3), $^{*}P < 0.05$ compared with 0 μg/ml SiO_2 group. **C:** The HBE cells were transfected with miR-326 mimic. QRT-PCR was performed to detect miR-326 levels (mean ± SD, n=3), $^{*}P < 0.05$ compared with 0 μg/ml SiO_2 treated cells. $^{\#}P < 0.05$ compared with the SiO_2 + mimic-NC group. **D:** HBE cells were transfected with TNFSF14 siRNA. QRT-PCR was performed to detect TNFSF14 levels (mean ± SD, n=3), $^{**}P < 0.01$ compared with the SiO_2 + siRNA control group.

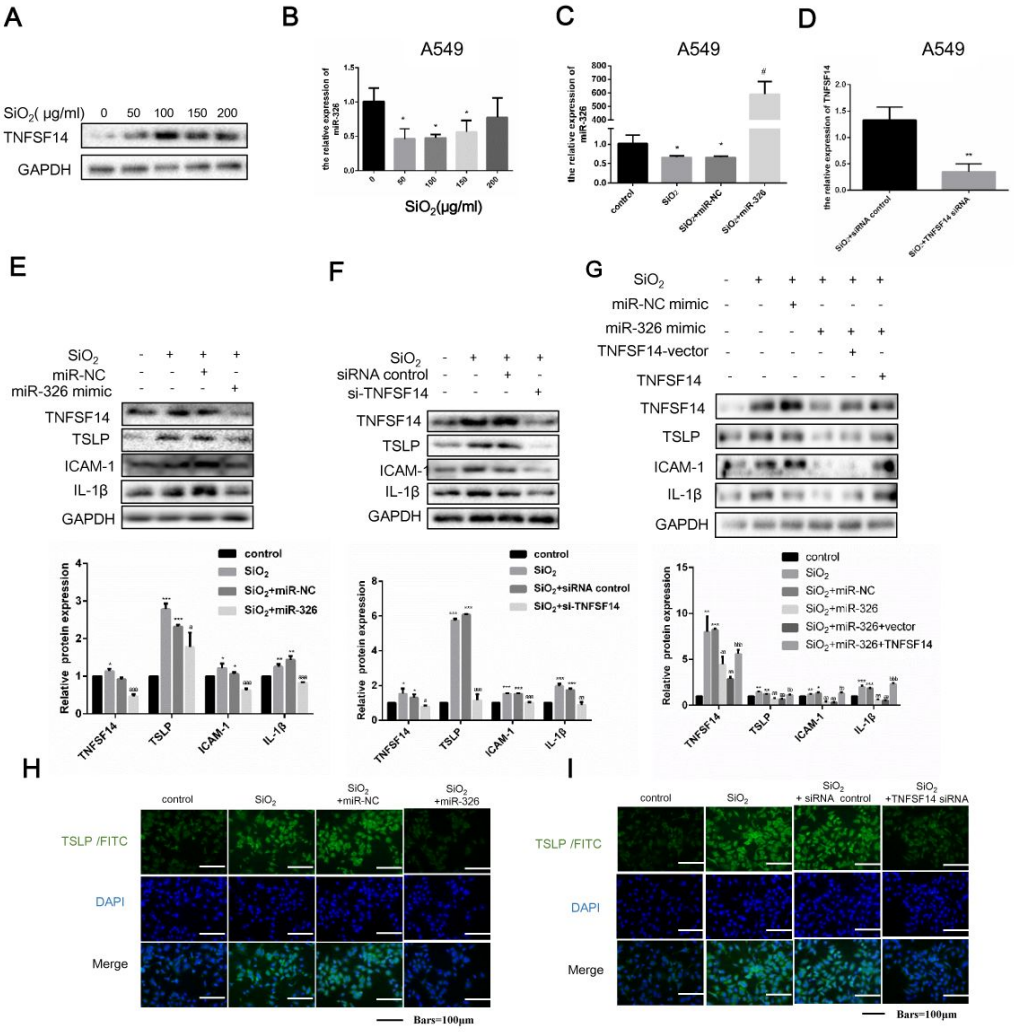

Figure S3. MiR-326 inhibits lung inflammation in mice by targeting TNFSF14 in A549 cells.

A: A549 were exposed to SiO_2 (0, 50, 100, 150, 200ug/ml) for 24 h. The protein level of TNFSF14 was detected by western blot assays. **B:** A549 cells were exposed to SiO_2 (0, 50, 100, 150, 200ug/ml) for 24 h. QRT-PCR was performed to detect miR-326 levels (mean ± SD, n=3), $^{*}P < 0.05$ compared with 0 μg/ml SiO_2 group. **C:** A549 cells were transfected with miR-326 mimic. QRT-PCR was performed to detect miR-326 levels (mean ± SD, n=3), $^{*}P < 0.05$ compared with 0 μg/ml SiO_2 treated cells. $^{\#}P < 0.05$ compared with the SiO_2+mimic-NC group. **D:** A549 cells were transfected with TNFSF14 siRNA. QRT-PCR was performed to detect TNFSF14 levels (mean ± SD, n=3), $^{**}P < 0.01$ compared with the SiO_2 plus siRNA control group. **E:** A549 cells were transfected with miR-326 mimic. Western blot were performed to determine TNFSF14, TSLP, ICAM-1 and IL-1β protein levels (mean ± SD, n=3), $^{*}P < 0.05$ compared with the SiO_2+mimic-NC group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group. $^{a}P < 0.05$, $^{aaa}P < 0.001$ compared with miR-NC group. **F:** A549 cells were transfected with TNFSF14 siRNA. Western blot were performed to determine TNFSF14, TSLP, ICAM-1 and IL-1β protein levels (mean ± SD, n=3), $^{**}P < 0.01$ compared with the SiO_2 plus siRNA control group. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with the control group. $^{a}P < 0.05$, $^{aa}P < 0.01$, $^{aaa}P < 0.001$ versus siRNA control group. **G:** A549 cells

were exposed to TNFSF14 plasmid/miR-326 mimic for 24 h. Western blot were performed to determine TNFSF14, TSLP, ICAM-1 and IL-1β protein levels (mean ± SD, n=3). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group. $^{aa}P < 0.01$ compared with SiO_2 + miR-NC group. $^{bb}P < 0.01$, $^{bbb}P < 0.001$ compared with SiO_2 + miR-326 + vector group. **H:** TSLP in A549 cells transfected with miR-326 mimic detected by immunofluorescence staining. Green for TSLP staining. Blue for nuclear DNA staining. **I:** TSLP in A549 cells transfected with TNFSF14 siRNA detected by immunofluorescence staining. Green means TSLP staining. Blue means nuclear DNA staining.

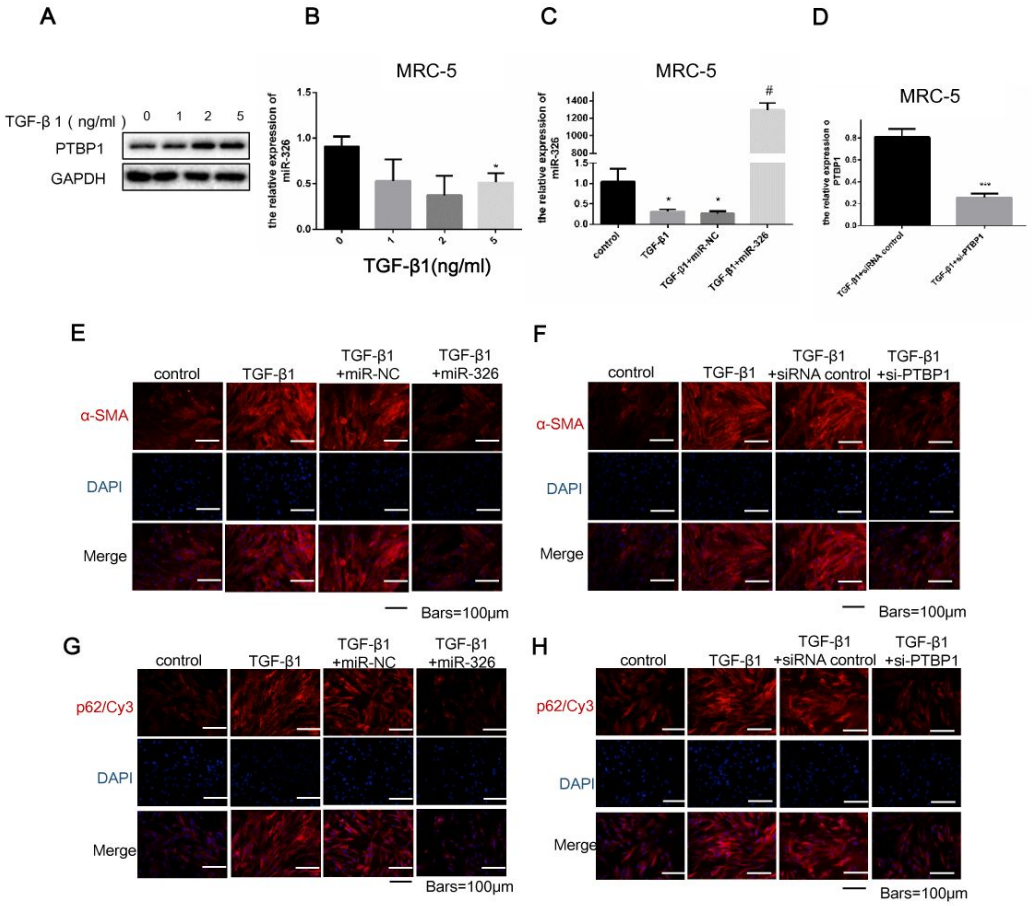

Figure S4. MiR-326 regulates autophagy by targeting PTBP1 in MRC-5 cells.

A: The MRC-5 cells were exposed to TGF-β1 (0, 1, 2, 5ng/ml) for 48h. Western blot were performed to determine the expression of PTBP1. **B:** MRC-5 cells were exposed to TGF-β1 (0, 1, 2, 5 ng/ml) for 48 h. QRT-PCR analysis was performed to detect miR-326 levels (mean ± SD, n=3), $^{*}P < 0.05$ compared with 0 ng/ml TGF-β1 treated cells. **C:** The MRC-5 cells were transfected with miR-326 mimic. MiR-326 levels were detected via qRT-PCR (mean ± SD, n=3), $^{*}P < 0.05$ compared with 0 ng/ml TGF-β1 treated cells. $^{\#}P < 0.05$ compared with the TGF-β1 plus mimic-NC group. **D:** MRC-5 cells were transfected with PTBP1 siRNA. PTBP1 levels were detected via qRT-PCR (mean ± SD, n=3), $^{***}P < 0.001$ compared with the TGF-β1 + siRNA control group. **E:** MRC-5 cells were transfected with miR-326 mimic. α-SMA were detected via Immunofluorescence staining. Red means α-SMA. Blue means nuclear DNA. **F:** MRC-5 cells were transfected with PTBP1 siRNA. α-SMA were detected via Immunofluorescence staining. Red means α-SMA staining. Blue means nuclear DNA staining.**G:** The MRC-5 cells were transfected with miR-326 mimic. The expression of p62 was detected by immunofluorescence staining. Red represents p62 staining, bars=100μm; blue represents DAPI. **H:** The MRC-5 cells were transfected with PTBP1 siRNA. The expression of p62 was detected by immunofluorescence staining. Red represents p62 staining, bars=100μm. The blue staining represents DAPI.

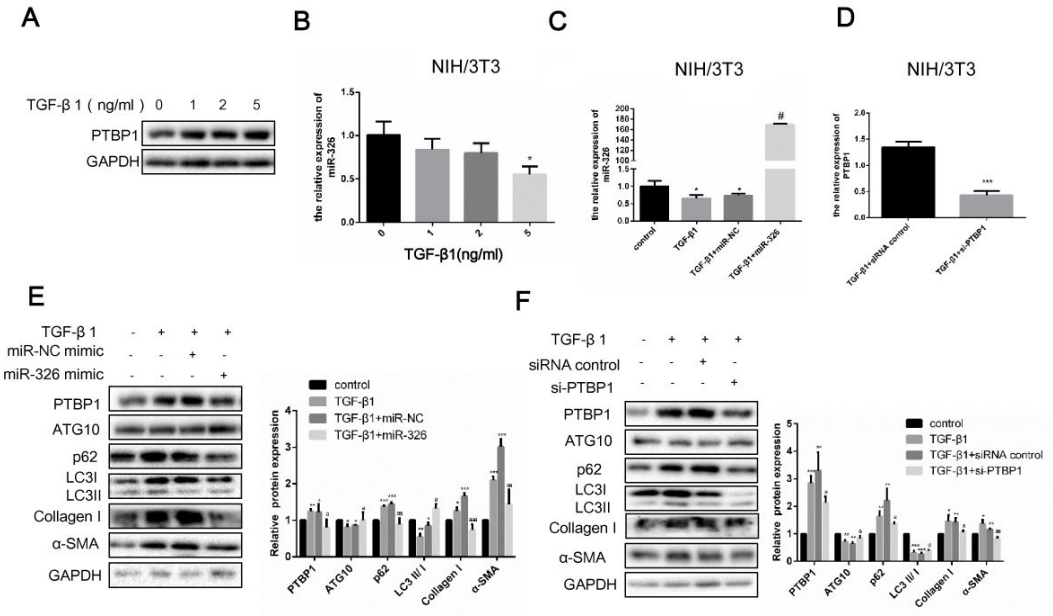

Figure S5. MiR-326 regulates autophagy by targeting PTBP1 in NIH/3T3 cells.

A: NIH/3T3 cells were exposed to TGF-β1 (0, 1, 2, 5 ng/ml) for 48 h. Western blot were performed to determine PTBP1 expression. **B:** NIH/3T3 cells were exposed to TGF-β1 (0, 1, 2, 5 ng/ml) for 48 h. MiR-326 levels were detected via qRT-PCR (mean ± SD, n=3), $^{*}P <$ 0.05 compared with 0 ng/ml TGF-β1 treated group. **C:** NIH/3T3 cells were transfected with miR-326 mimic. MiR-326 levels were detected via qRT-PCR (mean ± SD, n=3), $^{*}P < 0.05$ compared with 0 ng/ml TGF-β1 group. $^{\#}P < 0.05$ compared with the TGF-β1+ mimic-NC group. **D:** NIH/3T3 cells were transfected with PTBP1 siRNA. PTBP1 levels were detected via qRT-PCR (mean ± SD, n=3), $^{***}P < 0.001$ compared with the TGF-β1 + siRNA control group. **E:** NIH/3T3 cells were treated with miR-326 mimic. Western blot analysis were performed to determine PTBP1, ATG10, p62, LC3 I, LC3 II, collagen I and α-SMA protein expression (mean ± SD, n=3), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group. $^{a}P < 0.05$, $^{aa}P < 0.01$, $^{aaa}P < 0.001$ compared with TGF-β1+ miR-NC group. **F:** NIH/3T3 cells were treated with PTBP1 siRNA. PTBP1, ATG10, p62, LC3 I, LC3 II, collagen I and α-SMA protein expression were measured by western blot analysis (mean ± SD, n=3), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group. $^{a}P < 0.05$, ^{aa}P < 0.01 compared with TGF-β1 + siRNA control group.

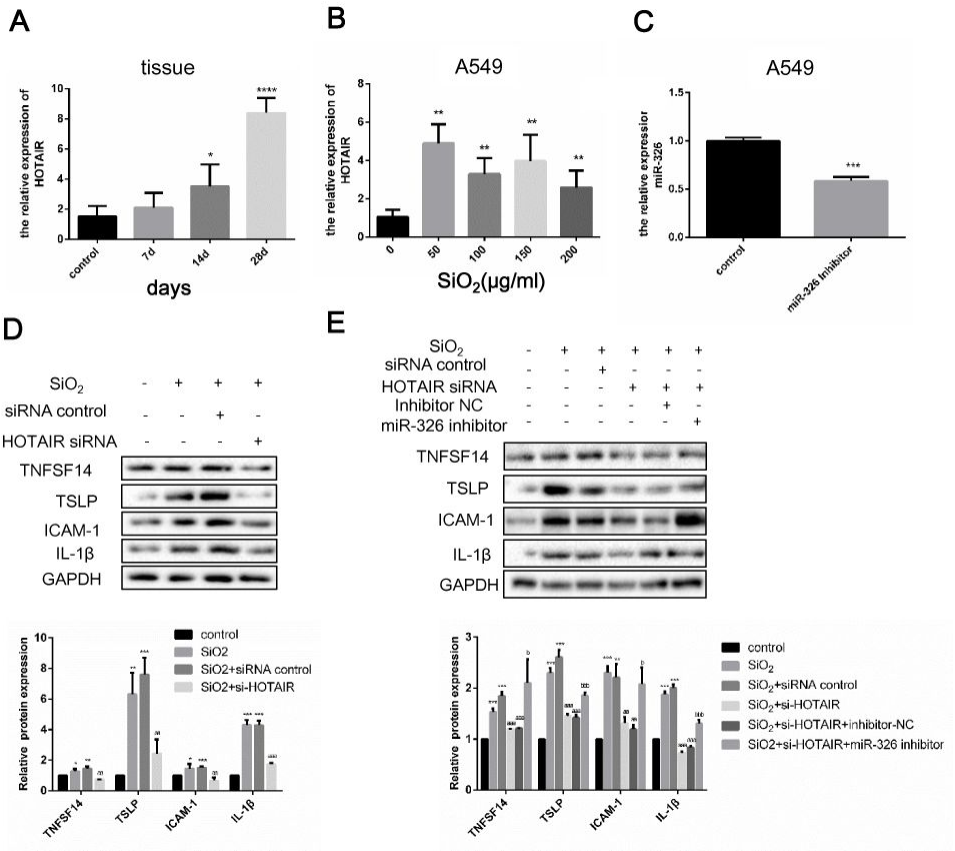

Figure S6. LncRNA HOTAIR facilitates inflammation via sponging miR-326 in A549 cells.

A: The levels of miR-326 on day 7, 14 and 28 groups compared to the saline group of mice. GAPDH was functioned as the internal control. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with the control group. **B:** A549 cells were treated with SiO_2 (0, 50, 100, 150, 200ug/ml) for 24 h. The expression of lncRNA HOTAIR were detected via qRT-PCR (mean ± SD, n=3), $^{**}P < 0.01$ compared with 0 μg/ml SiO_2 group. **C:** The A549 cells were exposed to miR-326 inhibitor. The expression of miR-326 were detected by qRT-PCR (mean ± SD, n=3), $^{***}P < 0.001$ compared with the control group. **D:** A549 cells were exposed to lncRNA HOTAIR siRNA. Western blot analysis were performed to determine TNFSF14, TSLP, ICAM-1 and IL-1β expression (mean ± SD, n=3), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group. $^{aa}P < 0.01$, $^{aaa}P < 0.001$ compared with SiO_2 plus HOTAIR siRNA control group. **E:** A549 cells were exposed to lncRNA HOTAIR siRNA/ miR-326 inhibitor for 24 h. Western blot analysis were performed to determine the protein expression of TNFSF14, TSLP, ICAM-1 and IL-1β (mean ± SD, n=3). $^{**}P < 0.01$, $^{***}P < 0.001$ compared with the control group. $^{aa}P < 0.01$, $^{aaa}P < 0.001$ compared with SiO_2 + siRNA control group. $^{b}P < 0.05$, $^{bbb}P < 0.001$ compared with SiO_2 + HOTAIR siRNA+ miR-326 inhibitor NC group.