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Supplementary Materials for

PKM2 promotes pulmonary fibrosis by stabilizing TGF-β1 receptor I and enhancing TGF-β1 signaling

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Figs. S1 to S13

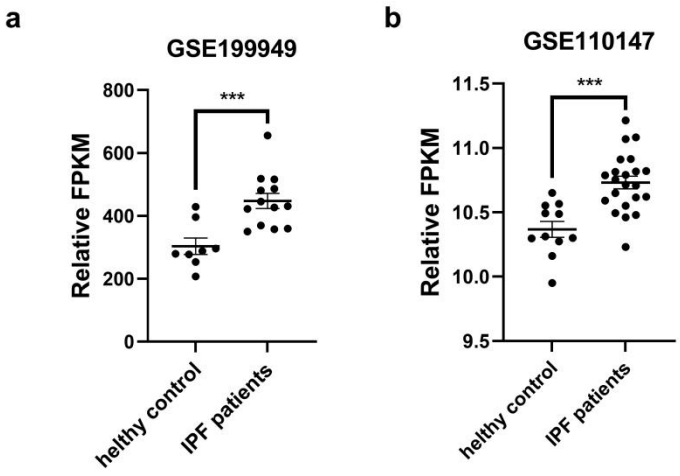

Fig. S1. Elevation of PKM2 mRNA level in IPF patients. (a) *PKM2* expression in IPF lung tissues was examined in a published GEO dataset (GSE199949) of IPF lungs. Microarray analysis of *PKM2* mRNA in lung samples of IPF patients (n = 13) compared with healthy control (n = 8). Study was organized by University of Viginia, USA. (b) *PKM2* expression in IPF lung tissues was examined in a published GEO dataset (GSE110147) of IPF lungs. Microarray analysis of *PKM2* mRNA in lung samples of IPF patients (n = 22) compared with healthy control (n = 11). Study was organized by Western University, Canada.

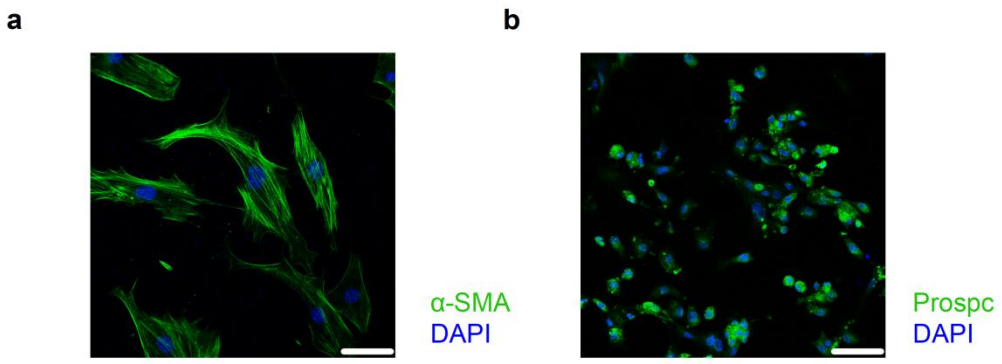

Fig. S2. Characterization of fibroblast and AT2 using immunostaining. (a) Primary lung fibroblasts were isolated and stained with antibodies against α-SMA (green). (b) Primary AT2 cells were isolated and stained with antibodies against Prospc (green). Scale bars, 50 μm.

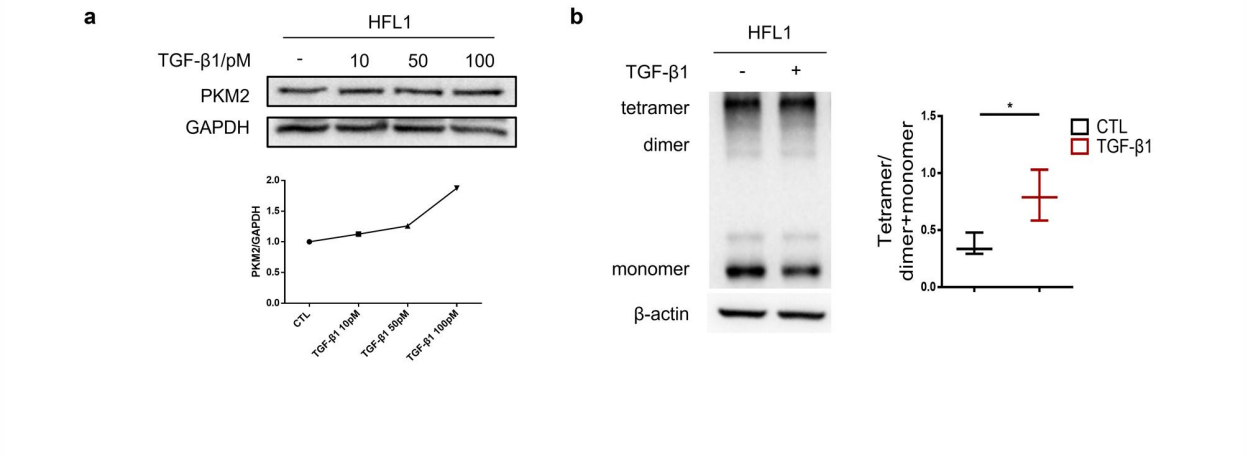

Fig. S3. Alteration of PKM2 in TGF-β1-stimulated HFL1 cells. (a) HFL1 cells were treated with increasing concentrations of TGF-β1 for 24 h and subjected to western blot analysis. (b) HFL1 cells were treated with TGF-β1 (5 ng/mL) for 24 h, then cross-linked and subjected to western blot analysis. Representative result is shown, data was replicated for 3 times. The data are represented as the means ± SD. **P* < 0.05, Student's *t* test.

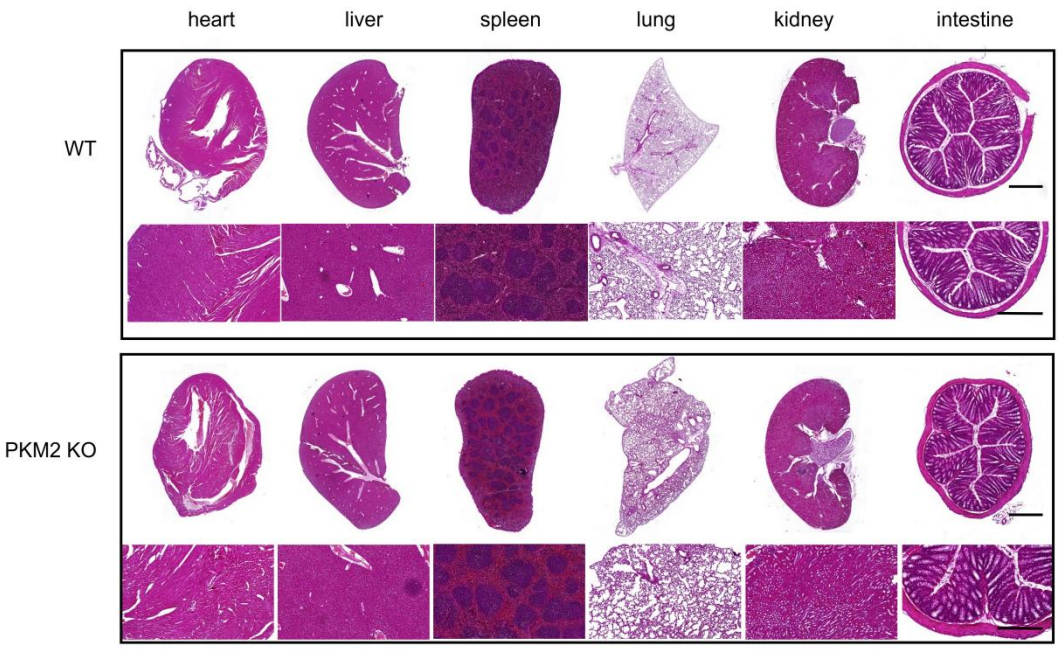

Fig. S4. No obvious lesions are found in PKM2 KO mice. 12 week-old WT and PKM2-KO mice were sacrificed and main tissues including heart, liver, spleen, lung, kidney and intestine were separated to perform pathological examination. Scale bars, 2 mm in the top panel and 100 μm in the bottom panel.

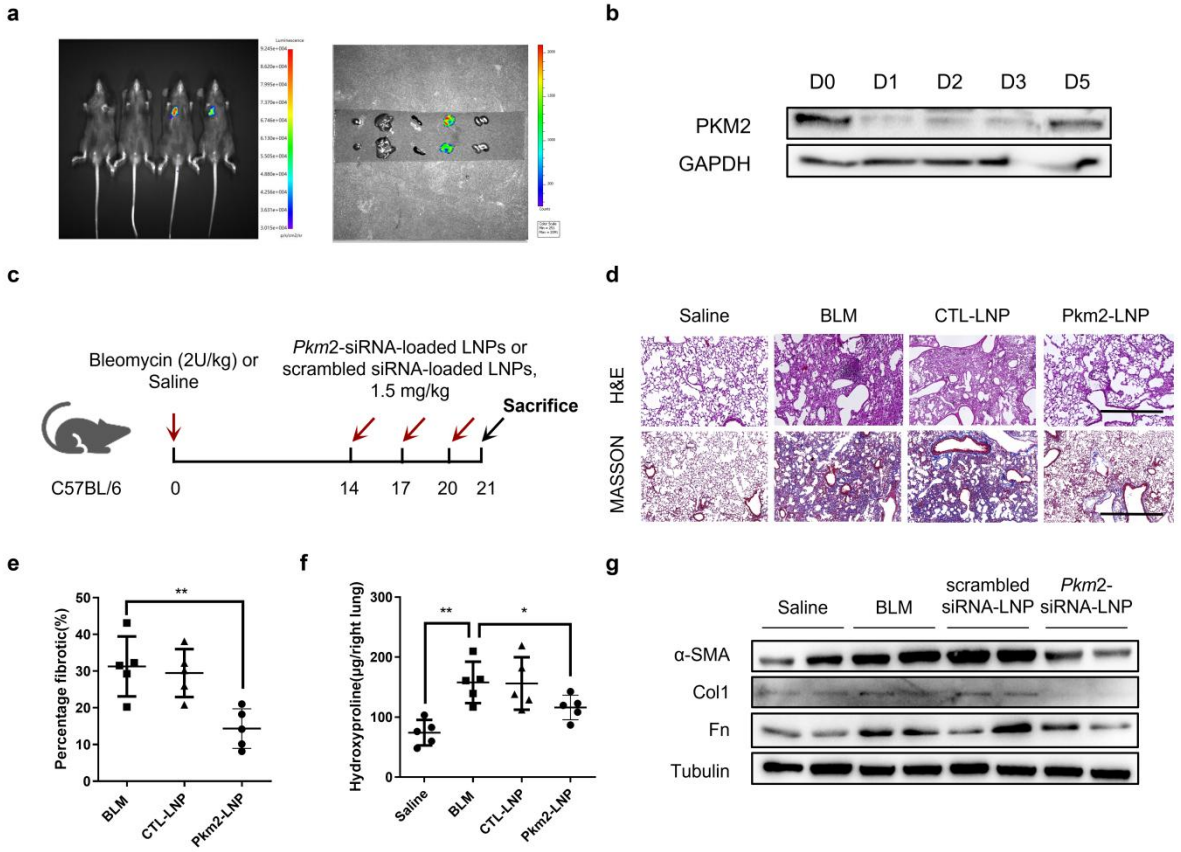

Fig. S5. *Pkm2*-siRNA-loaded lung specific LNPs protected mice from BLM-induced lung injury and fibrosis. (a) Representative in vivo images of the mice (left panel) and major organs (right panel) administrated with luciferase mRNA-loaded LNPs. In the left panel, the former two mice were administrated with LNPs without loading luciferase mRNA, the latter two mice were administrated with LNPs loaded with luciferase mRNA. The latter two mice were further sacrificed to obtain major tissues, and the florescence scanning image was presented in the right panel. (b) PKM2 expression changes in the lungs from liposome administrated mice (n = 3 for each group). (c) Schema of intervention-study design using BLM-induced C57BL/6 mice treated with *Pkm2*-siRNA-LNPs by intravenously injected at day 14, 17 and 20 after surgery. Mice were sacrificed at day 21. (d) H&E staining (up panel) and Masson's staining (bottom panel) of lung sections from control and *Pkm2*-siRNA-LNPs mice. Scale bars, 100 μm. (e) Quantification of the severity of fibrosis. The fibrotic area is presented as percentage (n = 5 per group). (f) Hydroxyproline content in lung tissues (n = 5 per group). (g) Representative results for western blot analysis of α-SMA, Col1 and Fn in lung tissues (n = 2 of western blot with n = 5 mice per group). The data are represented as the means ± SEM. **P* < 0.05 and ****P* < 0.001, Student's *t* test.

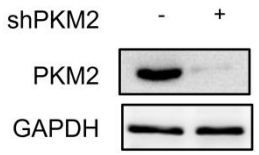

Fig. S6. PKM2 shRNA decrease endogenous PKM2 expression. 293T cells transfected with the shPKM2 for 48 h and subjected to western blot analysis.

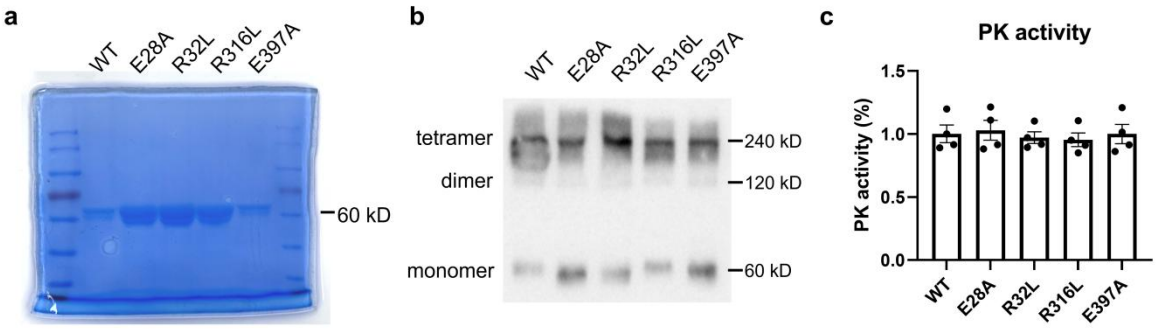

Fig. S7. PKM2 mutants remain tetrameric form and pyruvate kinase activity. 293T cells transfected with Flag-PKM2 (WT, E28A, R32L, R316L, E397A) for 48 h, and protein was purified using Anti-Flag Affinity Gel (Beyotime). (a) Purified Flag-PKM2 mutants were analyzed using SDS-PAGE. (b) Purified Flag-PKM2 mutants were cross-linked using DSS and detected by western blot analysis. (c) PK activity assays of purified PKM2 mutants. The data are represented as the means ± SEM.

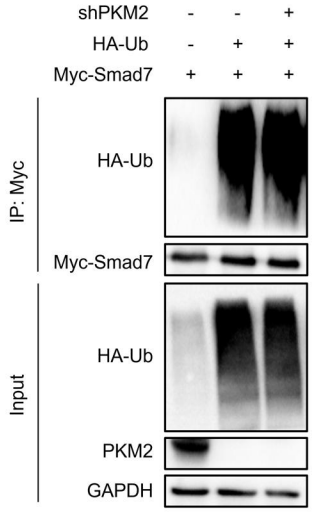

Fig. S8. PKM2 knockdown did not alter the ubiquitination of Smad7. Immunoprecipitation with anti-Myc antibody in 293T cells transfected with Myc-Smad7, HA-Ubiquitin and shPKM2. Cells were treated with MG132 (5 μM) for 4 h before harvested. Western blot analysis of the indicated proteins is shown.

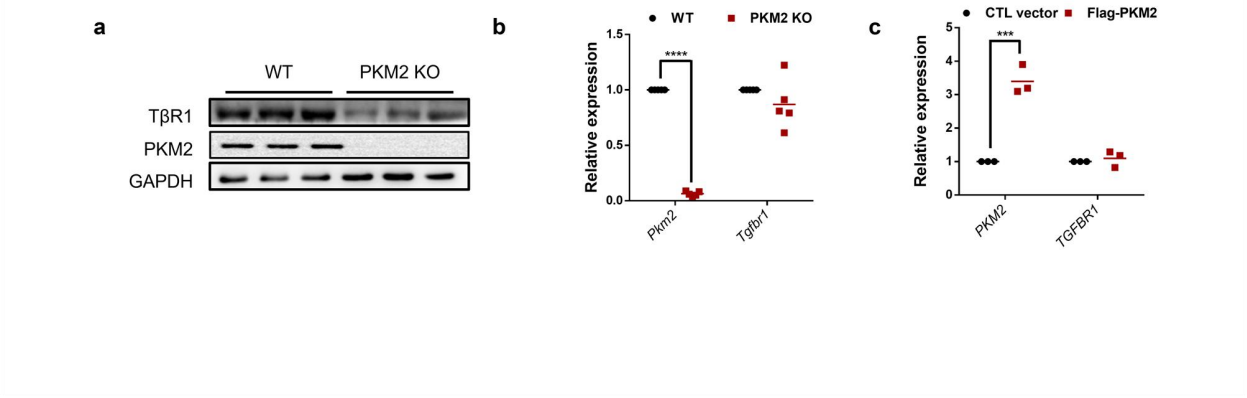

Fig. S9. PKM2 stabilizes TβR1. (a-b) Protein levels of TβR1 decreased in PKM2-KO group while mRNA levels showed no significant change. (a) Primary lung fibroblasts of WT and PKM2-KO mice were isolated and subjected to western blot (n = 3 per group). (b) Primary lung fibroblasts of WT and PKM2-KO mice were isolated and subjected to q-RT PCR analysis. (n = 5 per group. (c) q-RT PCR analysis of *TGFBR1* mRNA expression in 293T cells transfected with Flag-PKM2. *GAPDH* mRNA levels were used as an internal normalization control (n = 3 per group). The data are represented as the means ± SEM. ***$P < 0.001$ and ****$P < 0.0001$, Student's *t* test.

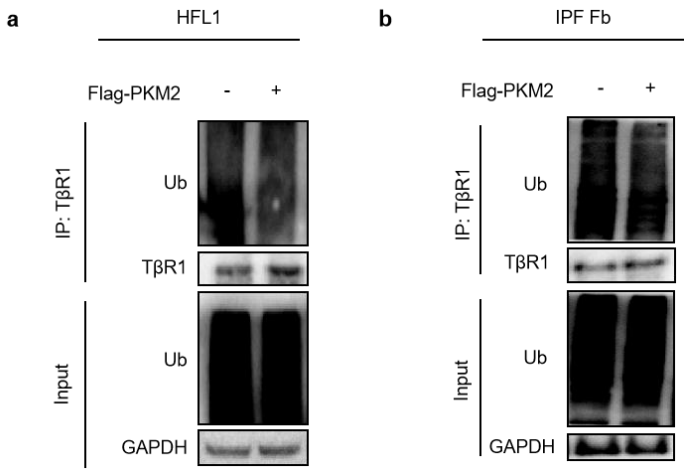

Fig. S10. Overexpression of PKM2 decreased endogenous TβR1ubiquitination. (a-b) HFL1 cells (a) or IPF Fb (b) were transfected with Flag-PKM2 or control vector for 48 h, then immunoprecipitated with anti-TβR1 antibody. Western blot analysis of indicated proteins is shown.

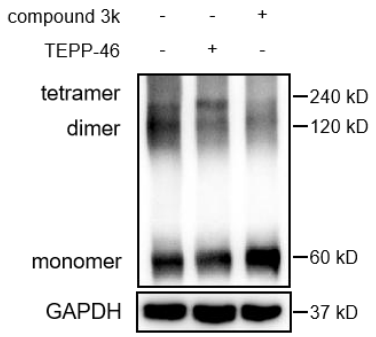

Fig. S11. Effect of TEPP-46 and compound 3k in regulating PKM2 conformation. HFL1 cells were treated with TEPP-46 (10 μM) or compound 3k (1 μM) for 24 h, then cross-linked using DSS and subjected to western blot analysis.

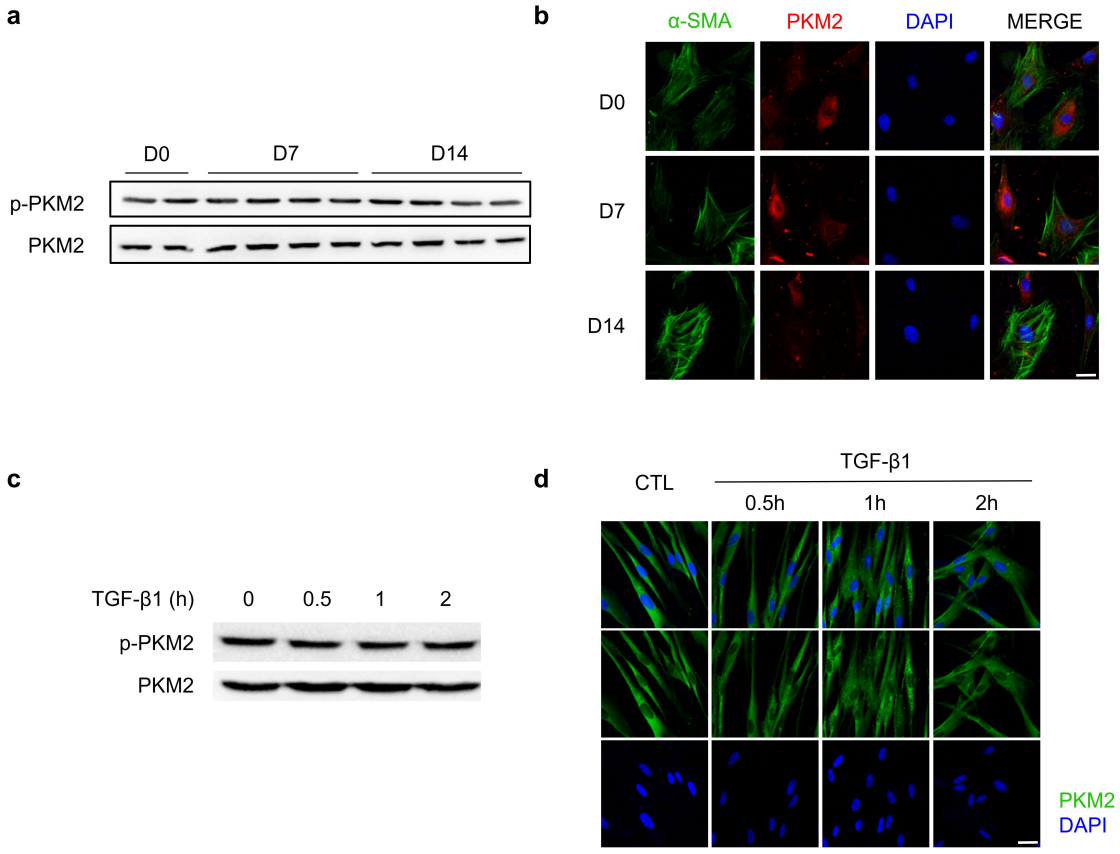

Fig. S12. Phosphorylation and nuclear translocation of PKM2 showed no obvious change in fibrotic fibroblasts. (a) On post-BLM day 7 and 14, primary lung fibroblasts were isolated and subjected to western blot analysis. Level of p-PKM2 (Tyr 105) had no obvious change. (b) On post-BLM day 7 and 14, primary lung fibroblasts were isolated and stained with antibodies against α-SMA (green) and PKM2 (red). No obvious nuclear translocation of PKM2 was found. Scale bars, 25 μm. (c) HFL1 cells were stimulated with TGF-β1 for 0.5, 1, 2 h and subjected to western blot analysis. Level of p-PKM2 (Tyr 105) had no obvious change. (d) HFL1 cells were stimulated with TGF-β1 for 0.5, 1, 2 h and stained with antibody against PKM2 (green). No obvious nuclear translocation of PKM2 was found. Scale bars, 25 μm.

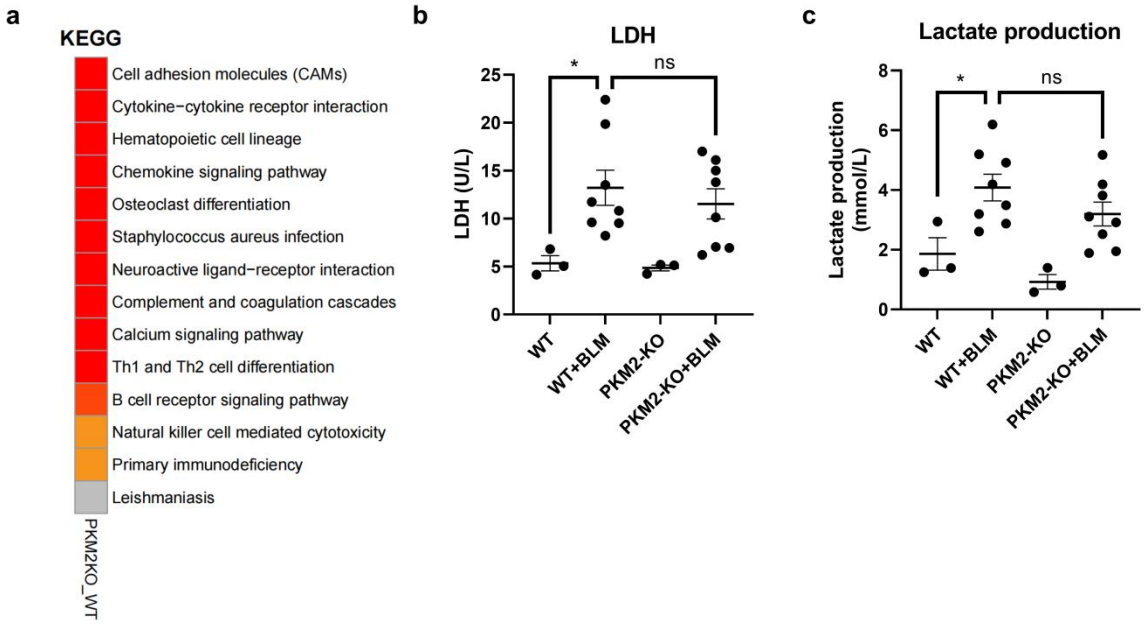

Fig. S13. PKM2 knockout did not affect glycolysis related signals during pulmonary fibrosis . (a) KEGG pathway enrichment analysis of the differential genes between WT+BLM and PKM2-KO+BLM group. The most significant enriched signals did not include metabolic related pathways. (b) Lactate dehydrogenase (LDH) enzymatic activity was assessed using a commercially available LDH Assay Kit (Beyotime, C0016) according to manufacturer's instructions. 30 μg tissue lysate was used for testing. (c) Lactic acid production was assessed using a commercially available lactate assay kit (Nanjing Jiancheng). 30 μg tissue lysate was used for testing.