

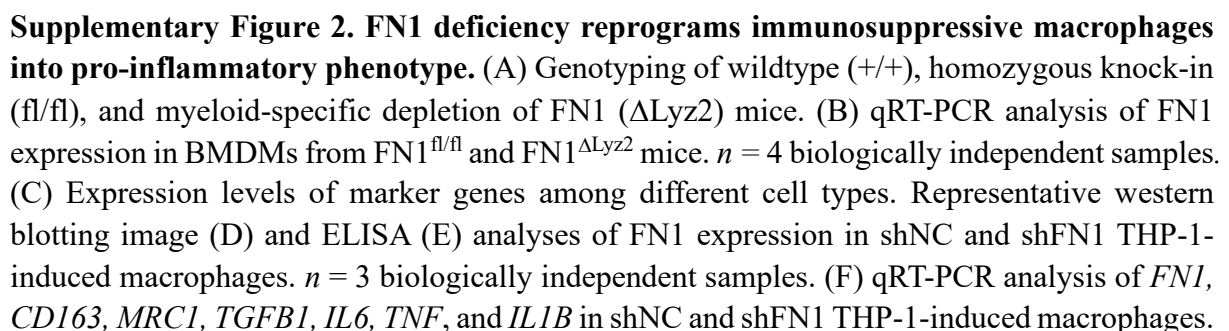
Supplementary Information

**Macrophages-derived fibronectin suppresses anti-tumor immunity via tissue stiffening
and immunosuppression induction**

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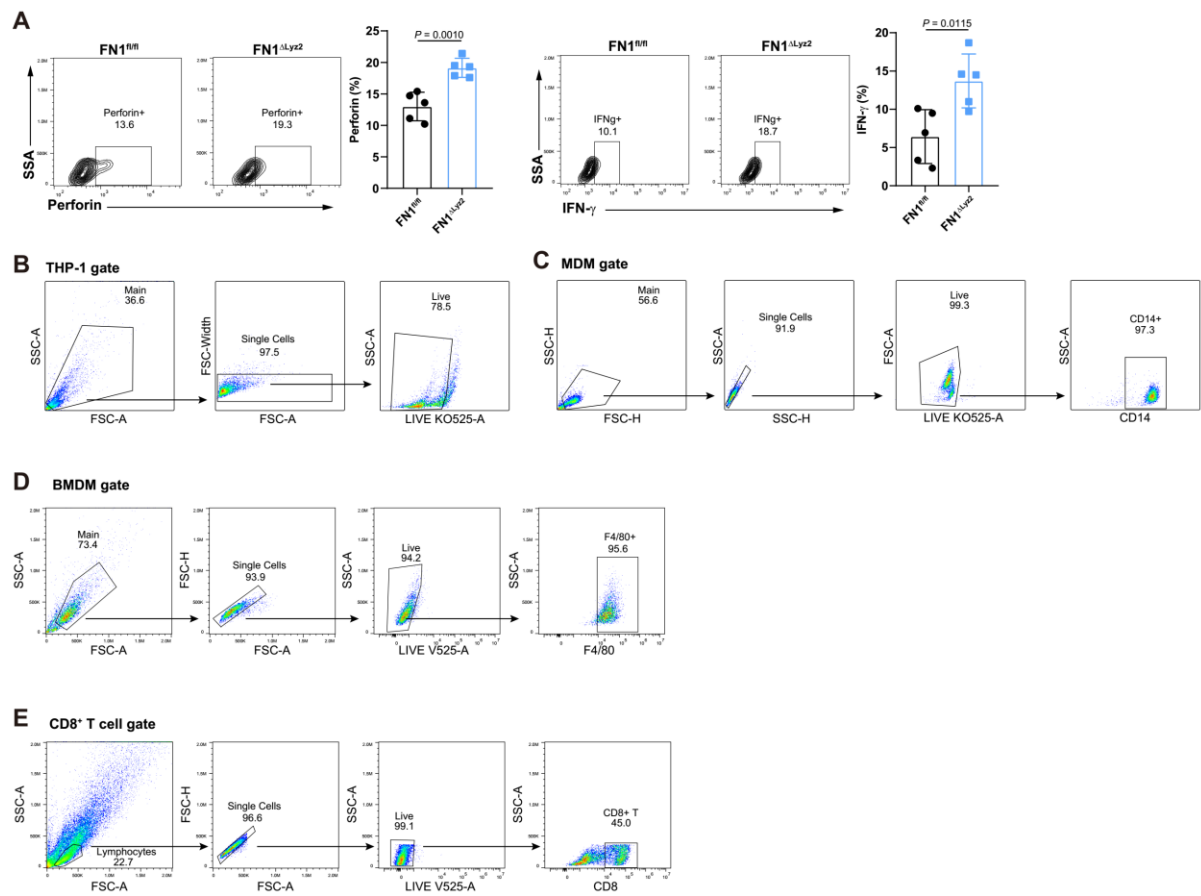
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ELISA analysis of FN1 secretion in the culture medium of M1-like and M2-like MDMs. $n = 3$ biologically independent samples. (G) Imaging flow cytometry analysis of FN1, CD14, CD163, CD206, and HLA-DR in M1-like and M2-like MDMs. Experiment was repeated three times independently with similar results. (H) UMAP plots of cells from public scRNA-seq data GSE148071. (I) Spearman correlation between the expression of FN1 in macrophages or fibroblasts and the proportions of immune cells ($CD4^+$ and $CD8^+$ T cells) from public scRNA-seq data GSE148071. (J) Heatmap of immunosuppressive, lipid metabolism-related, and proinflammatory genes in both $FN1^+$ and $FN1^-$ macrophages from public scRNA-seq data GSE148071. (K) Cell gate protocol for tumor-infiltrating $CD14^+$ macrophages in Fig. 1F, 1G. Data are displayed as the mean (D) and mean $\pm$ SD (E, F), two-tailed unpaired t -test (D, E, F). scRNA-seq, single-cell RNA sequencing; qRT-PCR, quantitative reverse transcription polymerase chain reaction; MRC1, mannose receptor C-type 1; IL, interleukin; TGFB1, transforming growth factor beta 1; TNF, tumor necrosis factor; IFNG, interferon gamma; MDM, monocyte-derived macrophage.

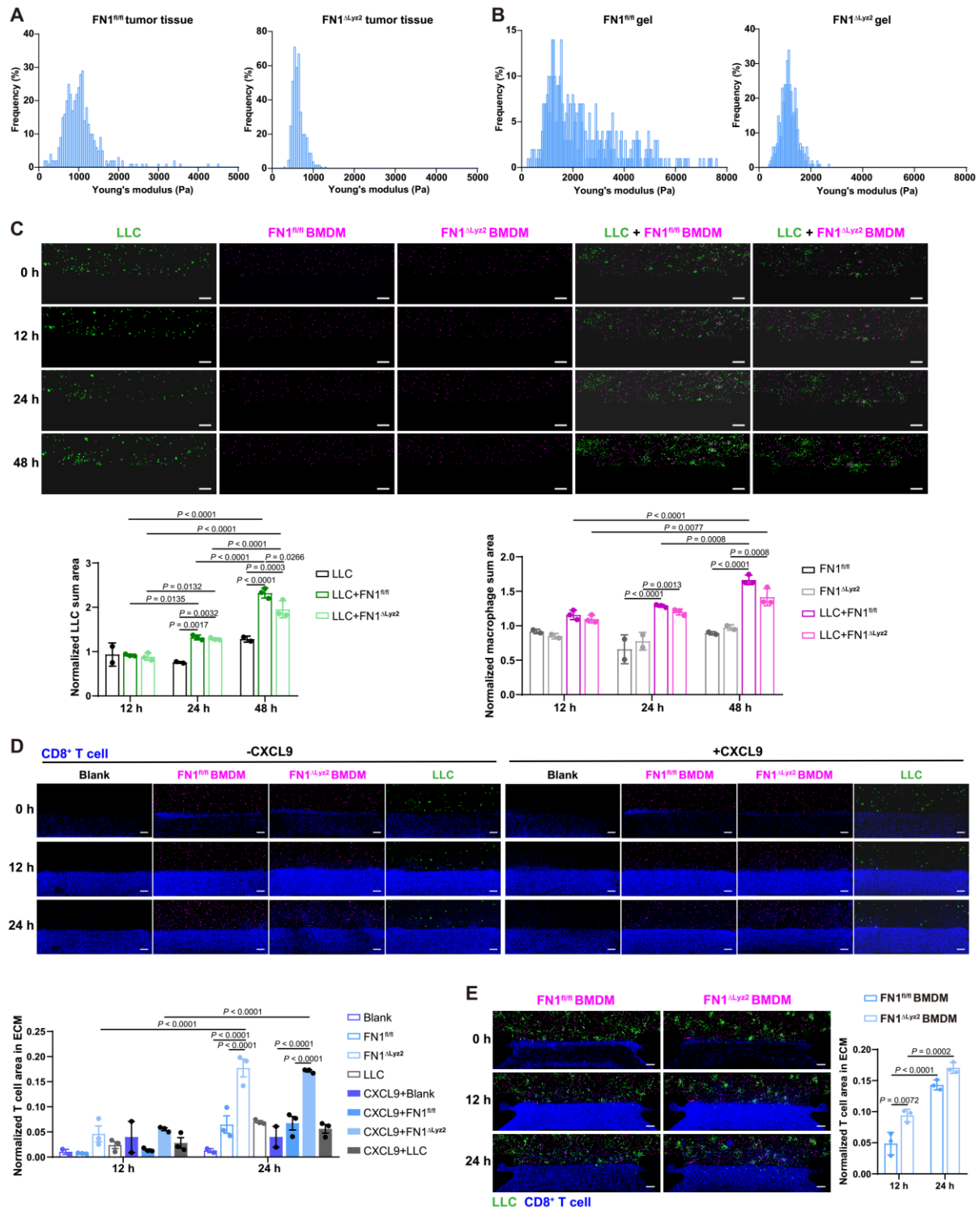


Supplementary Figure 2. FN1 deficiency reprograms immunosuppressive macrophages into pro-inflammatory phenotype. (A) Genotyping of wildtype (+/+), homozygous knock-in (fl/fl), and myeloid-specific depletion of FN1 (Δ Lyz2) mice. (B) qRT-PCR analysis of FN1 expression in BMDMs from FN1^{fl/fl} and FN1 ^{Δ Lyz2} mice. *n* = 4 biologically independent samples. (C) Expression levels of marker genes among different cell types. Representative western blotting image (D) and ELISA (E) analyses of FN1 expression in shNC and shFN1 THP-1-induced macrophages. *n* = 3 biologically independent samples. (F) qRT-PCR analysis of *FN1*, *CD163*, *MRC1*, *TGFB1*, *IL6*, *TNF*, and *IL1B* in shNC and shFN1 THP-1-induced macrophages.

$n \geq 3$ biologically independent samples. Representative contour plots/histogram and quantification of CD163, CD86 (G), IL-6, IL-1 β , and TNF (H) expression in shNC and shFN1 THP-1-induced macrophages. $n = 4$ biologically independent samples. (I) ELISA analysis of CXLC9 and CXCL10 secretion in the culture medium of shNC/shFN1 THP-1-induced macrophages. $n = 3$ biologically independent samples. Representative dot plots/histogram and quantification of CD163, CD206 (J), IL-6, and IL-1 β (K) expression in FB-treated MDMs. $n = 3$ or 5 biologically independent samples. (L) ELISA analysis of CXLC9 and CXCL10 secretion in the culture medium of FB-treated MDMs. $n = 6$ biologically independent samples. (M) qRT-PCR analysis of *IL6*, *IL1B* and *TNF* expression in FN1^{fl/fl} and FN1 ^{Δ Lyz2} BMDMs. $n = 3$ biologically independent samples. (N) Representative dot plots and quantification of IL-6 and TNF expression in FN1^{fl/fl} and FN1 ^{Δ Lyz2} BMDMs. $n = 8$ biologically independent samples. Data are displayed as the mean $\pm$ SD, two-tailed unpaired (B, E–I, M, N) or paired (J–L) *t*-test. qRT-PCR, quantitative reverse transcription polymerase chain reaction; BMDM, bone marrow-derived macrophage; IL, interleukin; TGFB1, transforming growth factor beta 1; TNF, tumor necrosis factor; FB, fibronectin blocker; MDM, monocyte-derived macrophage.

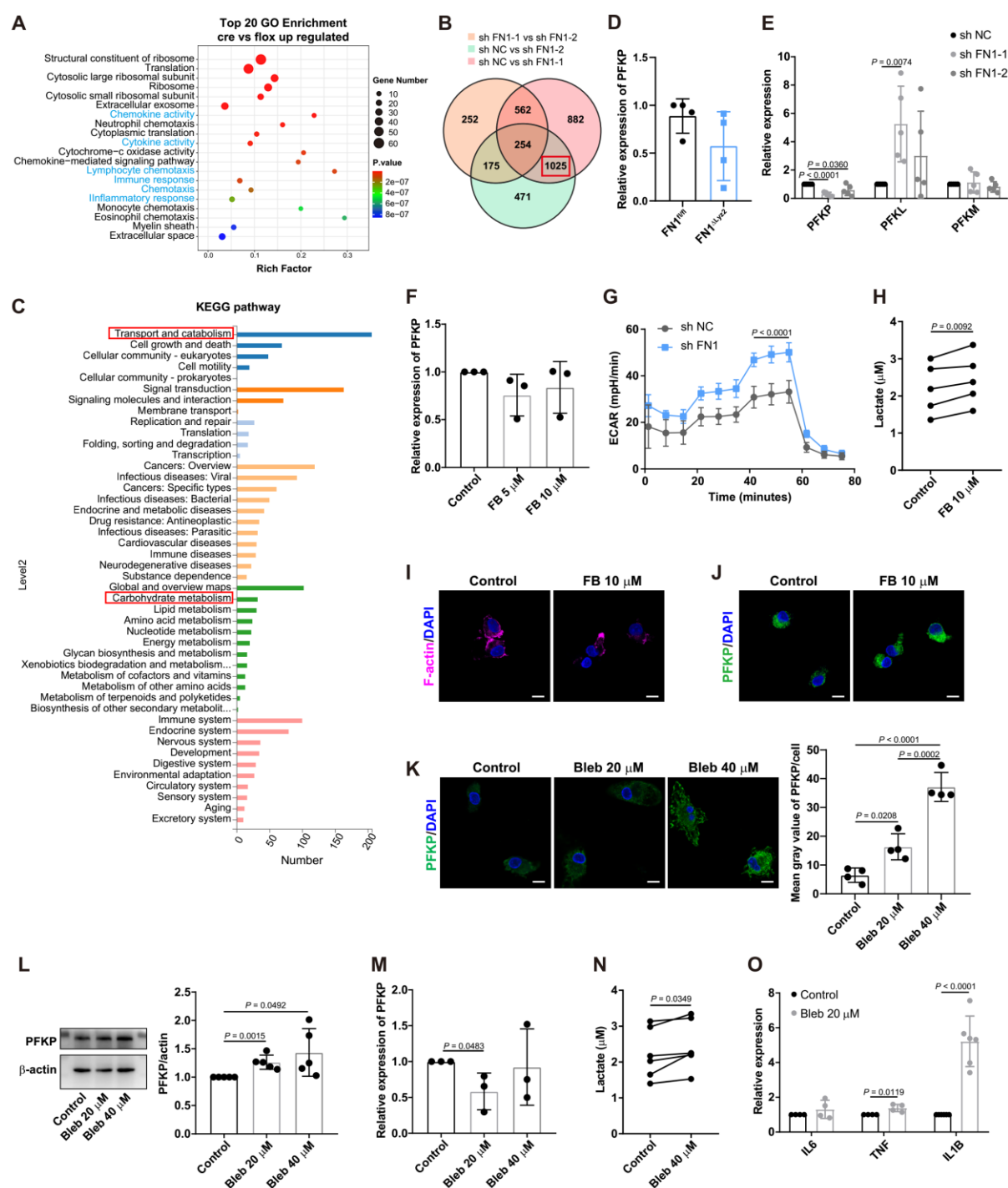


Supplementary Figure 3. FN1 deficiency promotes T-cell cytotoxicity. (A) Representative contour plots and quantification of perforin and IFN- γ expression in CD8⁺ T cells co-cultured with FN1^{fl/fl} and FN1 ^{Δ Lyz2} BMDMs in a ratio of 1:2 for 24h. $n = 5$ biologically independent samples. Cell gate protocol for THP-1-induced macrophages in Sup Fig. 2G, 2H (B), MDMs in Sup Fig. 2J, 2K (C), BMDMs in Sup Fig. 2N (D) and CD8⁺ T-cells in Sup Fig. 3A (E). Data are displayed as the mean $\pm$ SD, two-tailed unpaired t -test. BMDM, bone marrow-derived macrophage; IFN- γ , interferon gamma; MDM, monocyte-derived macrophage.



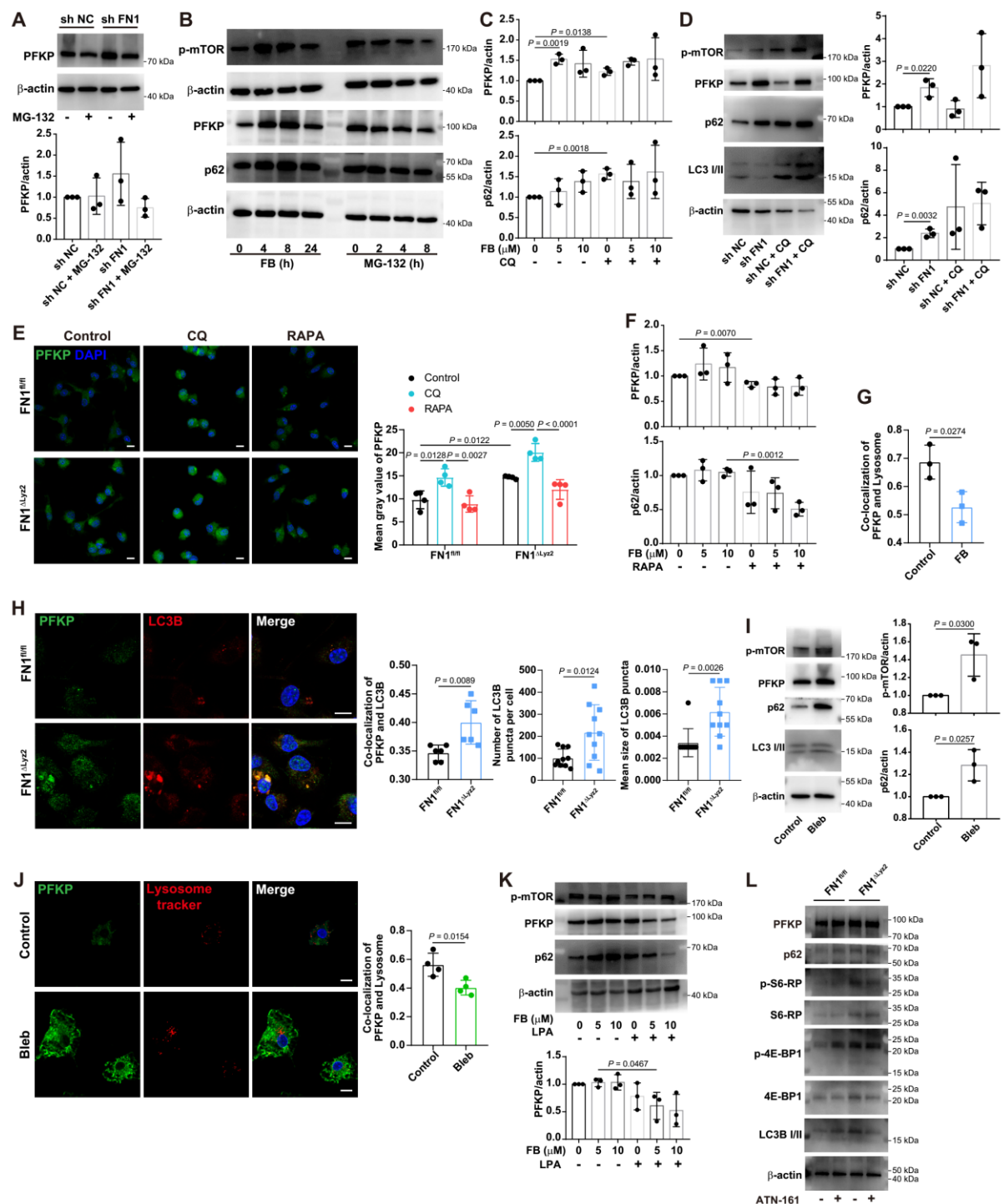
Supplementary Figure 4. FN1 deficiency promotes T-cell infiltration. Stiffness of tumor tissues (A) and BMDM-cultured matrix (B) from FN1^{fl/fl} and FN1^{ΔLyz2} mice. (C) Representative confocal images and analysis of cell proliferation of 3D-cultured LLC and BMDMs from FN1^{fl/fl} and FN1^{ΔLyz2} mice. *n* = 3 biologically independent samples. Scale bar, 100μm. (D) Representative confocal images and analysis of infiltrated Dil⁺CD8⁺ T-cells in hydrogel matrix of BMDMs from FN1^{fl/fl} and FN1^{ΔLyz2} mice or LLC with or without CXCL9 (50 ng/mL). *n* = 3 biologically independent samples. Scale bar, 100μm. (E) Representative confocal images and analysis of infiltrating Dil⁺CD8⁺ T-cells in LLC–FN1^{fl/fl}/FN1^{ΔLyz2} BMDM

co-culture hydrogel matrix. $n = 3$ biologically independent samples. Scale bar, 100 μ m. Data are displayed as the mean $\pm$ SD, two-way ANOVA. BMDM, bone marrow-derived macrophage; LLC, lewis lung carcinom.



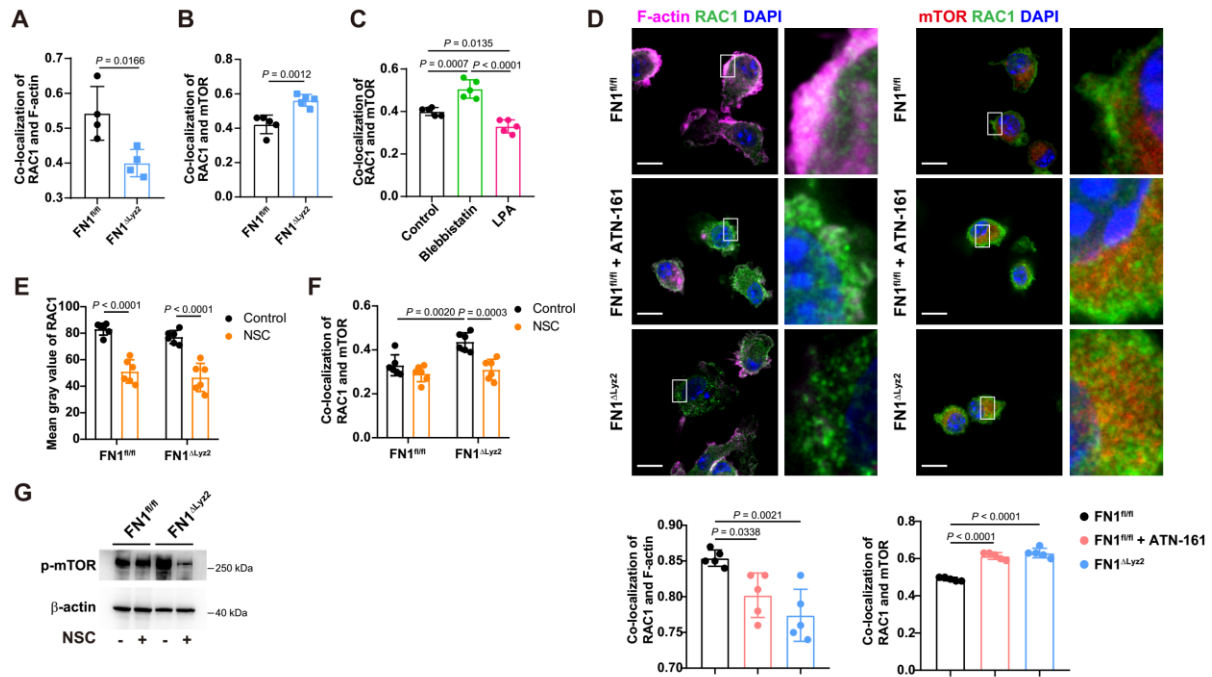
Supplementary Figure 5. FN1 inhibition increases glycolysis by decreasing macrophage actin polymerization *in vitro*. (A) GO enrichment analysis of differentially expressed genes (DEGs) in tumor-infiltrating macrophages from FN1^{fl/fl} and FN1^{ΔLyz2} mice. (B) DEGs in shNC/shFN1 THP-1-induced macrophages. (C) KEGG enrichment analysis of DEGs in shNC/shFN1 THP-1-induced macrophages. (D) qRT-PCR analysis of *PFKP* expression in BMDMs from FN1^{fl/fl} and FN1^{ΔLyz2} mice. *n* = 4 biologically independent samples. (E) qRT-PCR analysis of *PFKP*, *PFKL*, and *PFKM* expression in shNC/shFN1 THP-1-induced macrophages. *n* = 5 biologically independent samples. (F) qRT-PCR analysis of *PFKP*

expression in FB-treated MDMs. $n = 3$ biologically independent samples. (G) ECAR in shNC/shFN1 THP-1-induced macrophages. $n = 5$ biologically independent samples. (H) ELISA analysis of lactate levels in the culture medium of FB-treated MDMs. $n = 5$ biologically independent samples. Representative immunofluorescence image of F-actin (I) and PFKP (J) in FB-treated MDMs. Scale bar, 10 μm . Experiment was repeated three times independently with similar results. (K) Representative immunofluorescence image and quantification of PFKP expression in blebbistatin-treated MDMs. Scale bar, 10 μm . $n = 4$ biologically independent samples. (L) Representative western blotting image and relative analysis of PFKP expression in blebbistatin-treated MDMs. $n = 5$ biologically independent samples. (M) qRT-PCR analysis of PFKP expression in blebbistatin-treated MDMs. $n = 3$ biologically independent samples. (N) ELISA analysis of lactate levels in the culture medium of blebbistatin-treated MDMs. $n = 6$ biologically independent samples. (O) qRT-PCR analysis of *IL6*, *TNF*, and *IL1B* expression in blebbistatin-treated MDMs. $n \geq 4$ biologically independent samples. Data are displayed as the mean $\pm$ SD, hypergeometric test (A), two-tailed unpaired (D–F, L, M and O) or paired (H and N) *t*-test, one-way (K) or two-way ANOVA (G). GO, gene ontology; DEGs, differentially expressed genes; PFKP, phosphofructokinase, platelet; PFKL, phosphofructokinase liver; PFKM, phosphofructokinase muscle; FB, fibronectin blocker; BMDM, bone marrow-derived macrophage; MDM, monocyte-derived macrophage; ECAR, extracellular acidification rate; F-actin, actin filament; IL, interleukin; TNF, tumor necrosis factor.

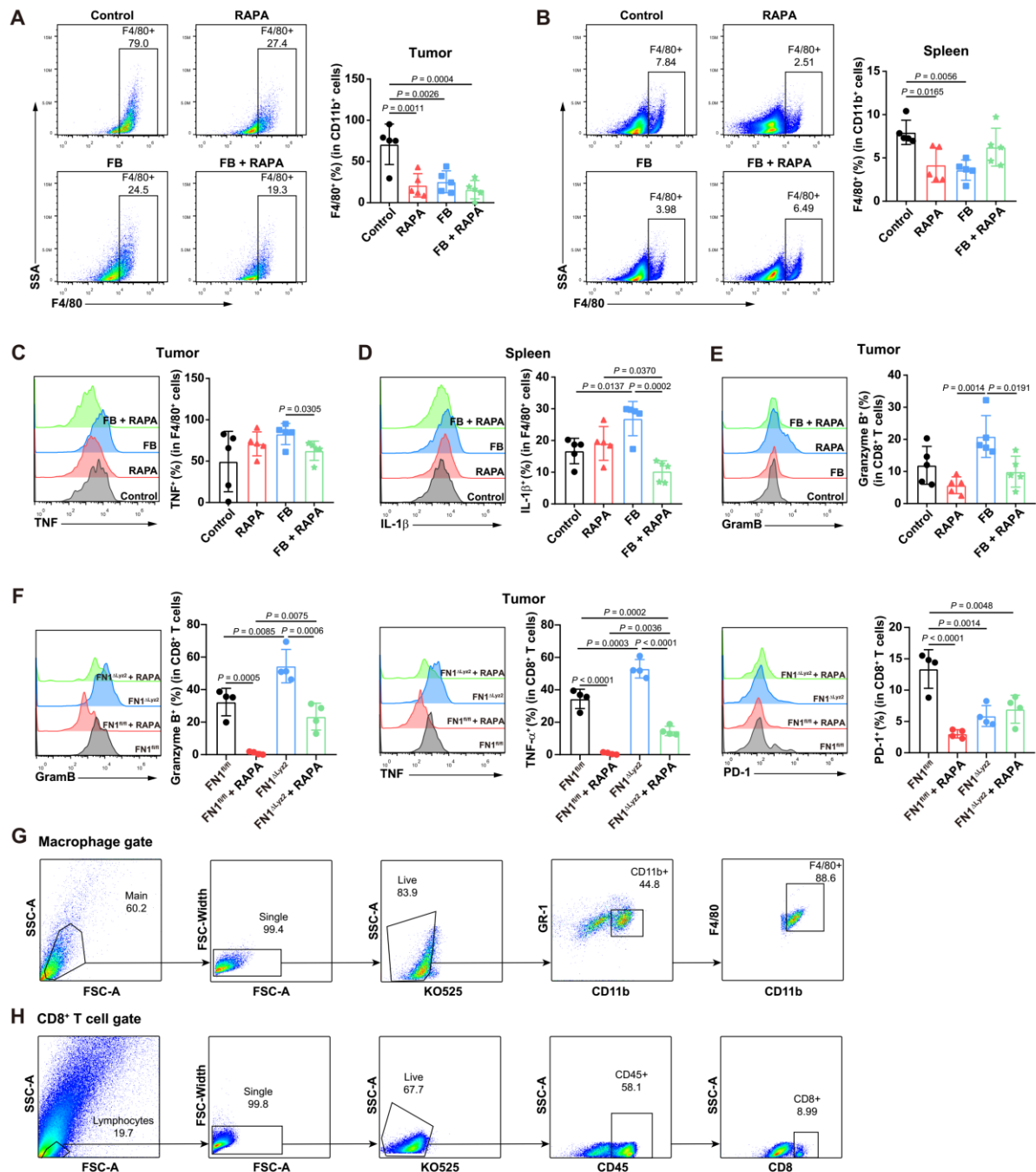


Supplementary Figure 6. Blockade of FN1-integrin interaction increases PFK by inhibiting autophagy through reduced F-actin. (A) Representative western blotting image and relative analysis of PFKP and β -actin expression in shNC and shFN1 THP-1-induced macrophages with MG-132 treatment. $n = 3$ biologically independent samples. (B) Representative western blotting image of p-mTOR, PFKP, p62, and β -actin expression in FB-treated MDMs for 0, 4, 8, and 24 h, as well as after MG-132 usage for 0, 2, 4, and 8 h. Experiment was repeated three times independently with similar results. (C) Quantification of PFKP and p62 expression in Fig. 5C. $n = 3$ biologically independent samples. (D) Representative western blotting image and relative analysis of p-mTOR, PFKP, p62, LC3I/II,

and β -actin expression in shNC and shFN1 THP-1-induced macrophages with CQ treatment. $n = 3$ biologically independent samples. (E) Representative immunofluorescence image and quantification of PFKP expression in FN1^{fl/fl} and FN1 ^{Δ Lyz2} BMDMs treated with CQ or RAPA. Scale bar, 10 μ m. $n = 4$ biologically independent samples. (F) Quantification of PFKP and p62 expression in Fig. 5D. $n = 3$ biologically independent samples. (G) Quantification of co-localization of PFKP and lysosomes within the confocal image in Fig. 5G. $n = 3$ biologically independent samples. (H) Representative immunofluorescence image and co-localization analysis of PFKP and LC3B, and quantification of the number and mean size of LC3B puncta in FN1^{fl/fl} and FN1 ^{Δ Lyz2} BMDMs. Scale bar, 10 μ m. $n = 3$ biologically independent samples. Each dot represents one field. (I) Representative western blotting image and relative analysis of PFKP, p-mTOR, p62, LC3I/II, and β -actin in blebbistatin-treated MDMs. $n = 3$ biologically independent samples. (J) Representative immunofluorescence image and co-localization analysis of PFKP and lysosomes in blebbistatin-treated MDMs. Scale bar, 10 μ m. $n = 4$ biologically independent samples. (K) Representative western blotting image and relative analysis of p-mTOR, PFKP, p62 and β -actin in FB-treated MDMs with LPA usage. $n = 3$ biologically independent samples. (L) Representative western blotting image of PFKP, p62, p-S6-RP, S6-RP, p-4E-BP1, 4E-BP1, LC3BI/II, and β -actin expression in FN1^{fl/fl} and FN1 ^{Δ Lyz2} BMDMs treated with ATN-161 (10 μ M). Experiment was repeated three times independently with similar results. Data are displayed as the mean $\pm$ SD, two-tailed unpaired t -test (A, C, D and F–K) or two-way ANOVA (E). PFKP, phosphofructokinase platelet; FB, fibronectin blocker; MDM, monocyte-derived macrophage; CQ, chloroquine; RAPA, rapamycin; LPA, lysophosphatidic acid; BMDM, bone marrow-derived macrophage.

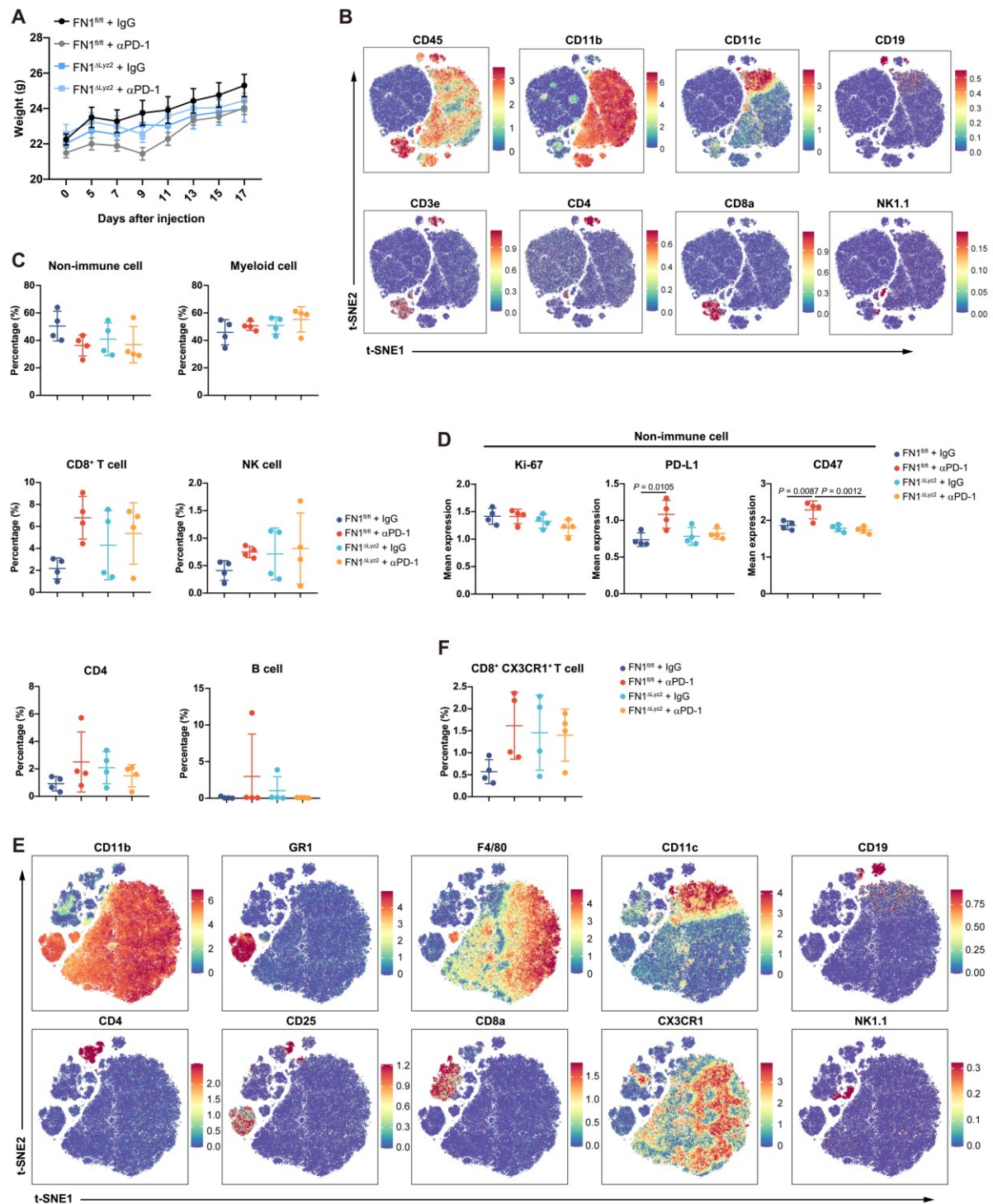


Supplementary Figure 7. Lack of FN1 increases PFK by activating the RAC1-mTOR axis and inhibiting autophagy through reduced F-actin. (A) Quantification of co-localization of RAC1 and F-actin within the confocal image in Fig. 5J. $n = 4$ biologically independent samples. Quantification of co-localization of RAC1 and mTOR within the confocal image in Fig. 5K (B) and Fig. 5M (C). $n = 5$ biologically independent samples. (D) Representative immunofluorescence image and quantification of co-localization of F-actin and RAC1, mTOR and RAC1 in $FN1^{fl/fl}$ and $FN1^{\Delta Lyz2}$ BMDMs treated with ATN-161 (10 μ M). Scale bar, 10 μ m. $n = 5$ biologically independent samples. Quantification of RAC1 expression (E), and the co-localization of RAC1 and mTOR (F) within the confocal image in Fig. 5N. $n = 3$ biologically independent samples. Each dot represents one field. (G) Representative western blotting image of p-mTOR and β -actin in $FN1^{fl/fl}$ and $FN1^{\Delta Lyz2}$ BMDMs treated with NSC-23766. Experiment was repeated three times independently with similar results. Data are displayed as the mean $\pm$ SD, two-tailed unpaired t -test (A and B), one-way (C and D) or two-way (E and F) ANOVA. F-actin, actin filament; BMDM, bone marrow-derived macrophage.



Supplementary Figure 8. Loss of FN1 attenuates autophagy and the immunosuppressive microenvironment *in vivo*. Representative dot plots and proportion of F4/80⁺ macrophages among myeloid cells from tumors (A) and spleens (B) of C57BL/6 mice. $n = 5$ biologically independent samples. Histogram and quantification of TNF expression in F4/80⁺ macrophages in tumor tissues (C), IL-1 β expression in F4/80⁺ macrophages in spleens (D), and granzyme B expression in CD8⁺ T-cells in tumors (E). $n = 5$ biologically independent samples. (F) Histogram and quantification of granzyme B, TNF and PD-1 expression in CD8⁺ T-cells in tumors from FN1^{fl/fl} and FN1 ^{Δ Ly22} mice with RAPA usage. $n = 4$ biologically independent samples. Cell gate protocol for F4/80⁺ macrophages in Fig. 6D, 6I, Sup Fig. 8A–8D (G) and CD8⁺ T-cells in Fig. 6E, 6J, Sup Fig. 8E, 8F (H). Data are displayed as the mean $\pm$ SD, two-tailed unpaired t -test (C), one-way ANOVA (A, B and D–F). IL, interleukin; TNF, tumor

necrosis factor; PD-1, programmed death-1, RAPA, rapamycin.



Supplementary Figure 9. Inhibition of FN1 improves the efficacy of anti-PD-1 therapy in NSCLC. (A) Mice weight measured every two days. $n = 5$ biologically independent samples for FN1^{fl/fl} + anti-PD-1 group, $n = 6$ biologically independent samples for other groups. (B) tSNE plots showing the overlay with marker genes. (C) Statistical analysis of the proportion of main cell clusters in tumors. $n = 4$ biologically independent samples. (D) Mass cytometry analysis of Ki-67, PD-L1, and CD47 expression in non-immune cells. $n = 4$ biologically independent samples. (E) tSNE plots showing the overlay with marker genes of subsets. (F)

Mass cytometry analysis of the proportion of CD8⁺CX3CR1⁺ T-cell clusters in tumors. $n = 4$ biologically independent samples. Data are displayed as the mean $\pm$ standard error of the mean (SEM) (A) and mean $\pm$ SD (C, D and F), one-way ANOVA. PD-L1, programmed death-ligand 1; PD-1, programmed death-1.

Stratification group		n
Age		42–78
Sex	Male	4
	Female	12
Diagnosis	Lung squamous cell carcinoma	4
	Lung adenocarcinoma	12
AJCC stage	I	11
	II	1
	III	4

Supplementary Table 1. Summary of clinical characteristics of patients with cancer. Samples were obtained from the First Affiliated Hospital of Zhengzhou University (n = 16). AJCC, American Joint Committee on Cancer.

Gene	Forward (5' to 3')	Reverse (5' to 3')	Species
<i>FN1</i>	AGGAAGCCGAGGTTTAACTG	AGGACGCTCATAAGTGTCACC	Human
<i>MRC1</i>	GTGATGGGACCCCTGTAACG	CTGCCCAGTACCCATCCTTG	Human
<i>IL10</i>	TACCACCTCCCGAAAATGTCA	CCCAGTCTGAATGCTCATCTG	Human
<i>PFKP</i>	GTGGATTTCGAGGCCTACCTG	GTTGGACACAGTAGCGGGAA	Human
<i>PFKL</i>	GCTGGGACAGACTGTTTCTTTC	GCCCTCATAGCCCTCGTAGA	Human
<i>PFKM</i>	GTCTCGCCAGTTAGTCAGGA	TGGTGGCTTTGGTATGAGCA	Human
<i>CD163</i>	TGTGGCCTGCATAGAGAGTG	TTCCCCAAAATGAGCAGAAC	Human
<i>IL1B</i>	ATGATGGCTTATTACAGTGCGAA	GTCGGAGATTCGTAGCTGGA	Human
<i>TGFB1</i>	GCCAGAGTGGTTATCTTTTGATG	AGTGTGTTATCCCTGCTGTCAC	Human
<i>IFNG</i>	TCGGTAACTGACTTGAATGTCCA	TCGCTTCCCTGTTTTAGCTGC	Human
<i>TNF</i>	GGCAGTCAGATCATCTTCTCGAA	GAAGGCCTAAGGTCCACTTGTGT	Human
<i>IL6</i>	TACCCCCAGGAGAAGATTCC	TTTTCTGCCAGTGCCTCTTT	Human
<i>GAPDH</i>	GCACCGTCAAGGCTGAGAAC	TGGTGAAGACGCCAGTGGA	Human
<i>FN1</i>	TCTCCTCCCATCCACTCA	TGCCTCTTGCTCTTCCC	Mouse
<i>PFKP</i>	CACCTATAAGCGTCTTGCC	GTGCCCCTACATTGATGAC	Mouse
<i>IL6</i>	AGCCCACCAAGAACGATAG	GGTTGTCACCAGCATCAGT	Mouse
<i>IL1B</i>	AGTTGACGGACCCCAA	TCTTGTGATGTGCTGCTG	Mouse
<i>TNF</i>	CGCTGAGGTCAATCTGC	GGCTGGGTAGAGAATGGA	Mouse
<i>GAPDH</i>	CCTCCTCCAATTCAACCCT	CACCGACCTTCACCATTTT	Mouse

Supplementary Table 2. Primer sequences used for qPCR. qPCR, quantitative polymerase chain reaction; IL, interleukin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase