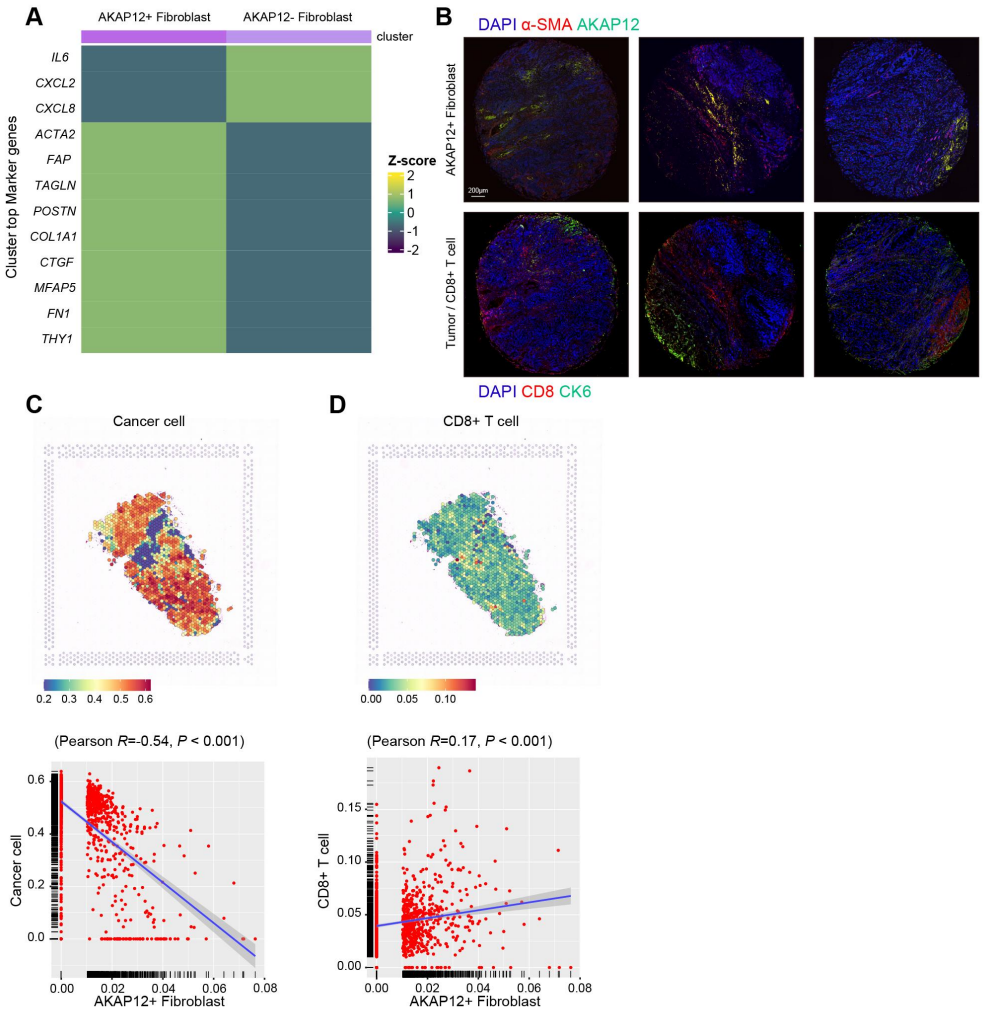
Supplementary Data

AKAP12 positive fibroblast determines immunosuppressive contexture and immunotherapy response in TNBC patients by promoting macrophage M2 polarization

The Supplementary Data consist of:

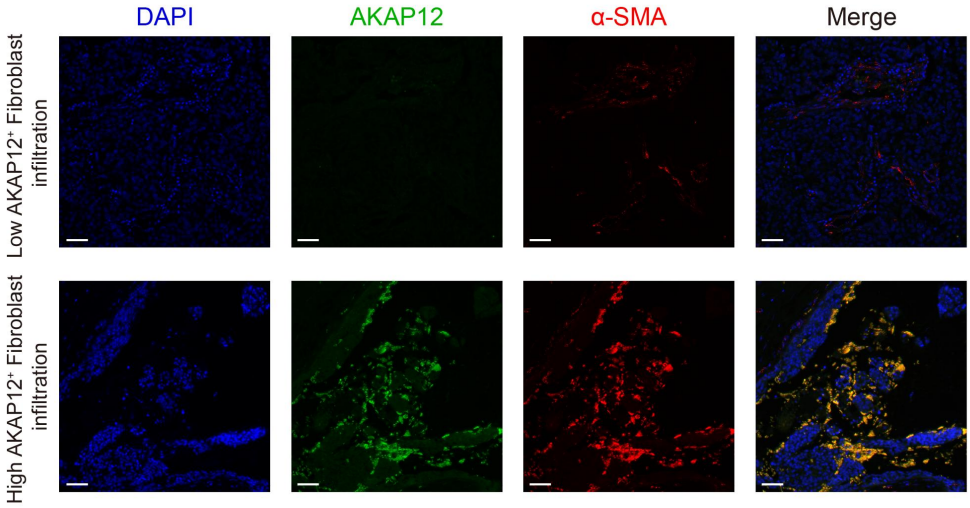
Supplementary Figures 1-5

Supplementary Tables 1-2

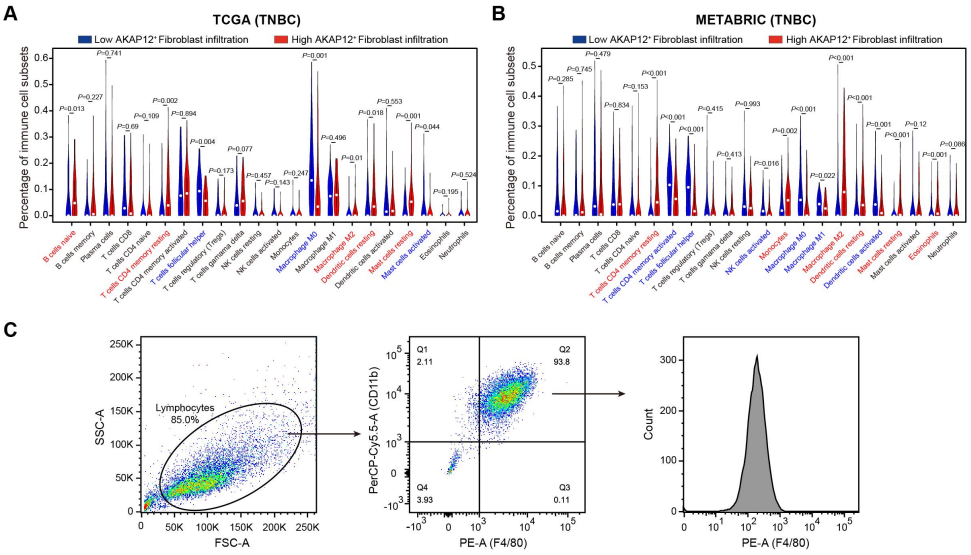


Supplementary Figure 1. Identification of a AKAP12^{+} fibroblast subset in TNBC. (A) Heatmap showing the differential gene expression in AKAP12^{+} fibroblast and AKAP12^{-} fibroblast based on single-cell transcriptomes. (B) Top: Representative images of immunofluorescence co-staining of α-SMA (red) and AKAP12 (green) in TNBC tissues. Bottom: Representative images of immunofluorescence co-staining of CD8 (red) and CK6 (green) in TNBC tissues. Scale bar, 200 μm. (C) Top: Spatial distribution of cancer cell on a representative TNBC tissue section. Bottom: Pearson correlation analysis of proportions of AKAP12^{+} fibroblast and cancer cell in TNBC

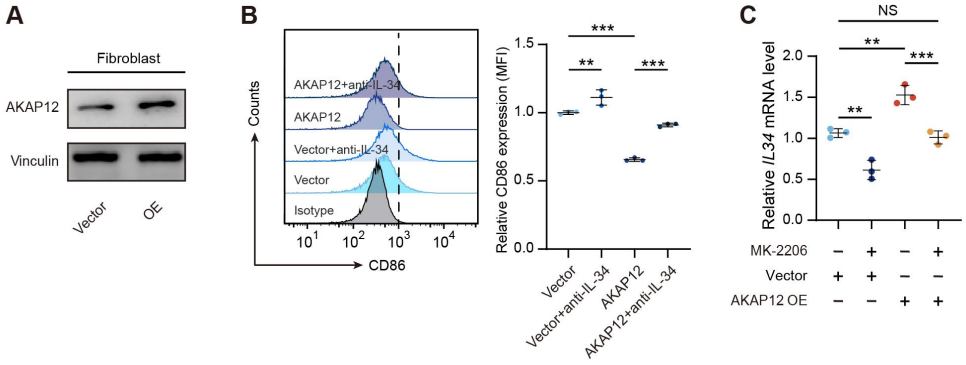
tissues. (D) Top: Spatial distribution of $CD8^+$ T cell on a representative TNBC tissue section. Bottom: Pearson correlation analysis of proportions of $AKAP12^+$ fibroblast and $CD8^+$ T cell in TNBC tissues.



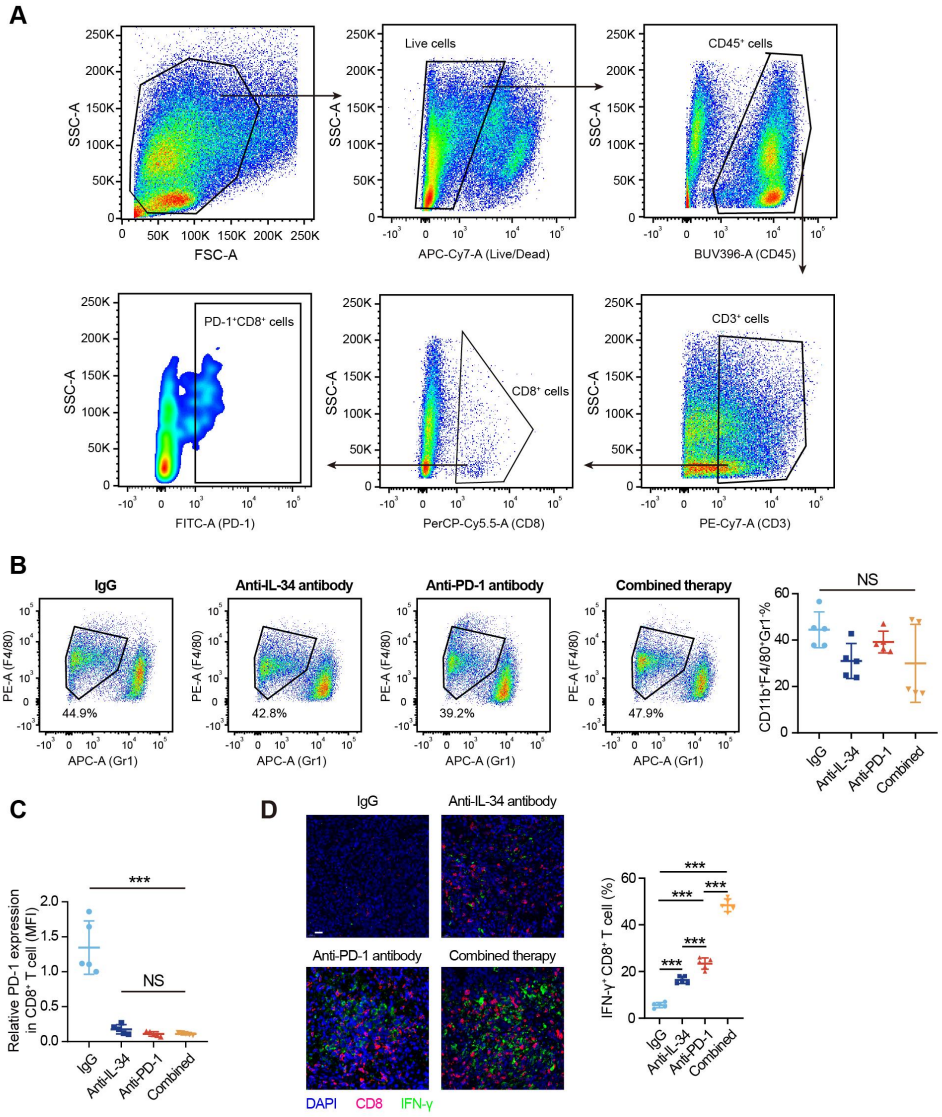
Supplementary Figure 2. Representative images of immunofluorescence co-staining of α-SMA (red) and AKAP12 (green) in mouse orthotopic TNBC tissues from high and low $AKAP12^+$ fibroblast infiltration groups.



Supplementary Figure 3. Intratumoral AKAP12^{+} fibroblasts correlate with immunosuppressive tumor microenvironment in TNBC. (A-B) Violin chart of the infiltration percentage of 22 immune cell types calculated using CIBERSORT algorithm in high and low AKAP12^{+} fibroblast infiltration groups from TCGA (A) and METABRIC (B) cohorts. Increased and decreased cell subsets in the high AKAP12^{+} fibroblast infiltration group compared to the low group are colored by red and blue, respectively. Mann-Whitney U test. (C) Representative example of gating strategy of BMDMs.



Supplementary Figure 4. AKAP12^{+} fibroblasts interact with macrophages via IL-34/CSF1R signaling in TNBC. (A) Western blot of AKAP12 level in vector and AKAP12-overexpressing (OE) fibroblasts. (B) Flow cytometry analyses of CD68 expression on THP-1-differentiated macrophages from the indicated groups. (C) qPCR detecting IL-34 expression levels in vector and AKAP12-overexpressing fibroblasts treated with or without MK-2206. ** P<0.01, *** P<0.001, and NS, not significant; one-way ANOVA test.



Supplementary Figure 5. Pharmacological blockade of the IL-34/CSF1R signaling enhances the efficacy of anti-PD-1 antibody in TNBC. (A) Representative example of the gating strategy of PD-1$^+$ CD8$^+$ T cells. (B) Flow cytometry analyses showing the population proportion of CD11b$^+$F4/80$^+$Gr1$^-$ total macrophages in each group. (C) Flow cytometry analyses of PD-1 expressions in CD8$^+$ T cells in each group. (D) Immunofluorescence analyses showing the IFN-γ$^+$ CD8$^+$ T cell population in each group. *** P<0.001, and NS, not significant; one-way ANOVA test.

Supplementary tables

Supplementary Table 1. The clinicopathologic details of TNBC patients (n=80)

Variables	Number
Age(years)	
≤ 50	40 (50.0%)
> 50	40 (50.0%)
Gender	
Female	80 (100.0%)
Male	0 (0.0%)
TNM stage	
I+II	51 (63.75%)
III+IV	29 (36.25%)
Tumor differentiation	
G1+G2	9 (11.25%)
G3+G4	71 (88.75%)

Supplementary Table 2. Details of 39 antibodies applied in CyTOF assay

Label	Marker	Clone	Manufacturer
89Y	CD45	30-F11	BioLegend
115In	CD3ε	145-2C11	Biolegend
141Pr	Gr-1(Ly-6G/Ly-6C)	RB6-8C5	Biolegend
142Nd	CD11c	N418	Biolegend
143Nd	CD69	H1.2F3	Biolegend
144Nd	CX3CR1	SA011F11	Biolegend
145Nd	CD24	M1/69	Biolegend
146Nd	CD279(PD-1)	29F.1A12	Biolegend
147Sm	CD80	16-10A1	Biolegend
148Nd	Ly-6C	HK1.4	Biolegend
149Sm	CD117(c-kit)	2B8	Biolegend
150Nd	IgD	11-26c.2a	Biolegend
151Eu	CD44	IM7	Biolegend
152Sm	CD19	6D5	Biolegend
153Eu	Siglec-F	E50-2440	BD
154Sm	CD62L	MEL-14	Biolegend
155Gd	CD103	2E7	Biolegend
156Gd	CD317(BST-2)	44E9R	R&D
157Gd	FcεRIα	MAR-1	Biolegend
158Gd	MERTK(Mer)	2B10C42	Biolegend
159Tb	F4/80	Cl:A3-1	BioRAD
160Gd	TCR β chain	H57-597	Biolegend
161Dy	CD49b(pan-NK cells)	DX5	Biolegend
162Dy	CD25(IL-2Rα)	3C7	Biolegend
163Dy	CD45R(B220)	RA3-6B2	Biolegend
164Dy	CD192(CCR2)	475301	RD
165Ho	FOXP3	FJK-16s	eBioscience
166Er	TCR γ/δ	GL3	Biolegend
167Er	CD206(MMR)	C068C2	Biolegend
168Er	CD64(FcγRI)	X54-5/7.1	Biolegend
169Tm	CD38	90	Biolegend
171Yb	CD86	GL-1	Biolegend
172Yb	CD127(IL-7Rα)	A7R34	Biolegend

173Yb	CD172a(SIRPα)	P84	Biolegend
174Yb	CD27	LG.3A10	Biolegend
175Lu	Ki-67	SolA15	eBioscience
197Au	CD4	RM4-5	Biolegend
198Pt	CD8a	53-6.7	Biolegend
209Bi	CD11b	M1/70	Biolegend