**Supplementary Information**

# Apoptotic Cancer Cell–Primed Cancer-Associated Fibroblasts Suppress Immunosuppressive Macrophages via WISP-1–Integrin α5β3–STAT1 Signaling in Lung Cancer

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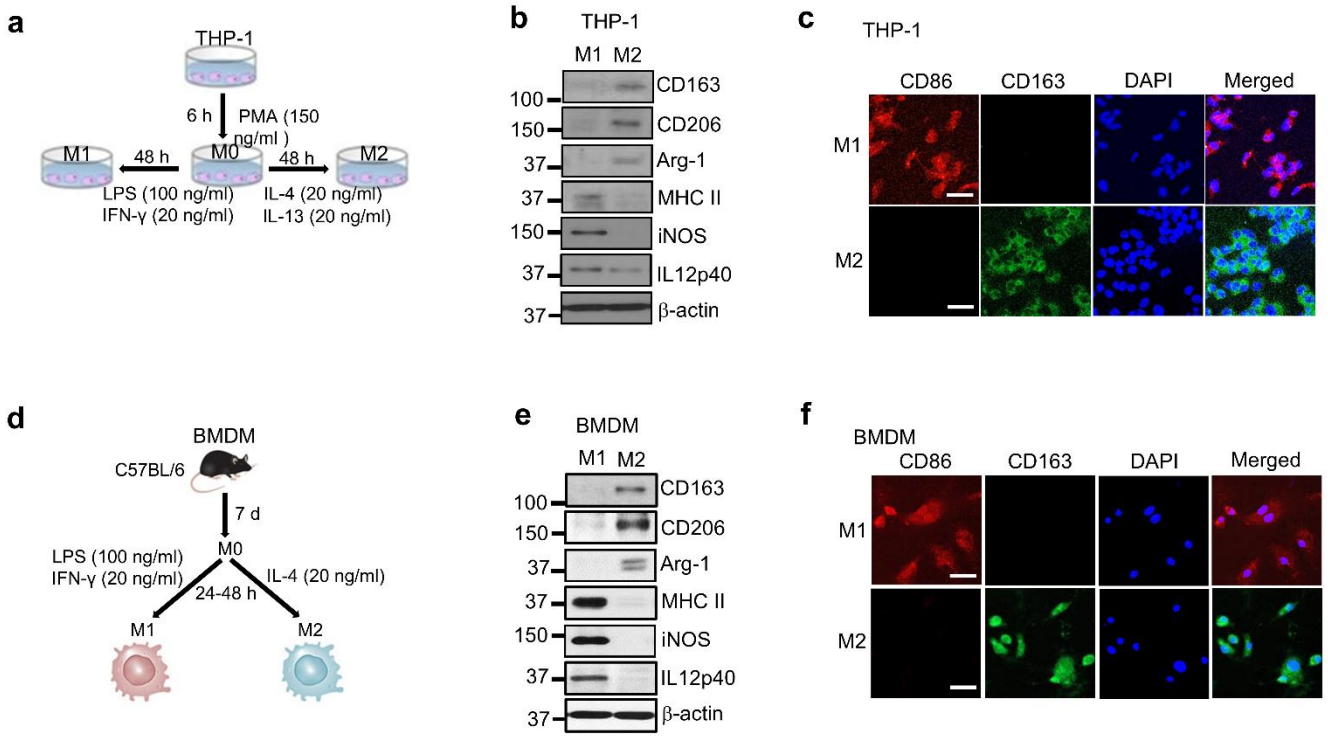
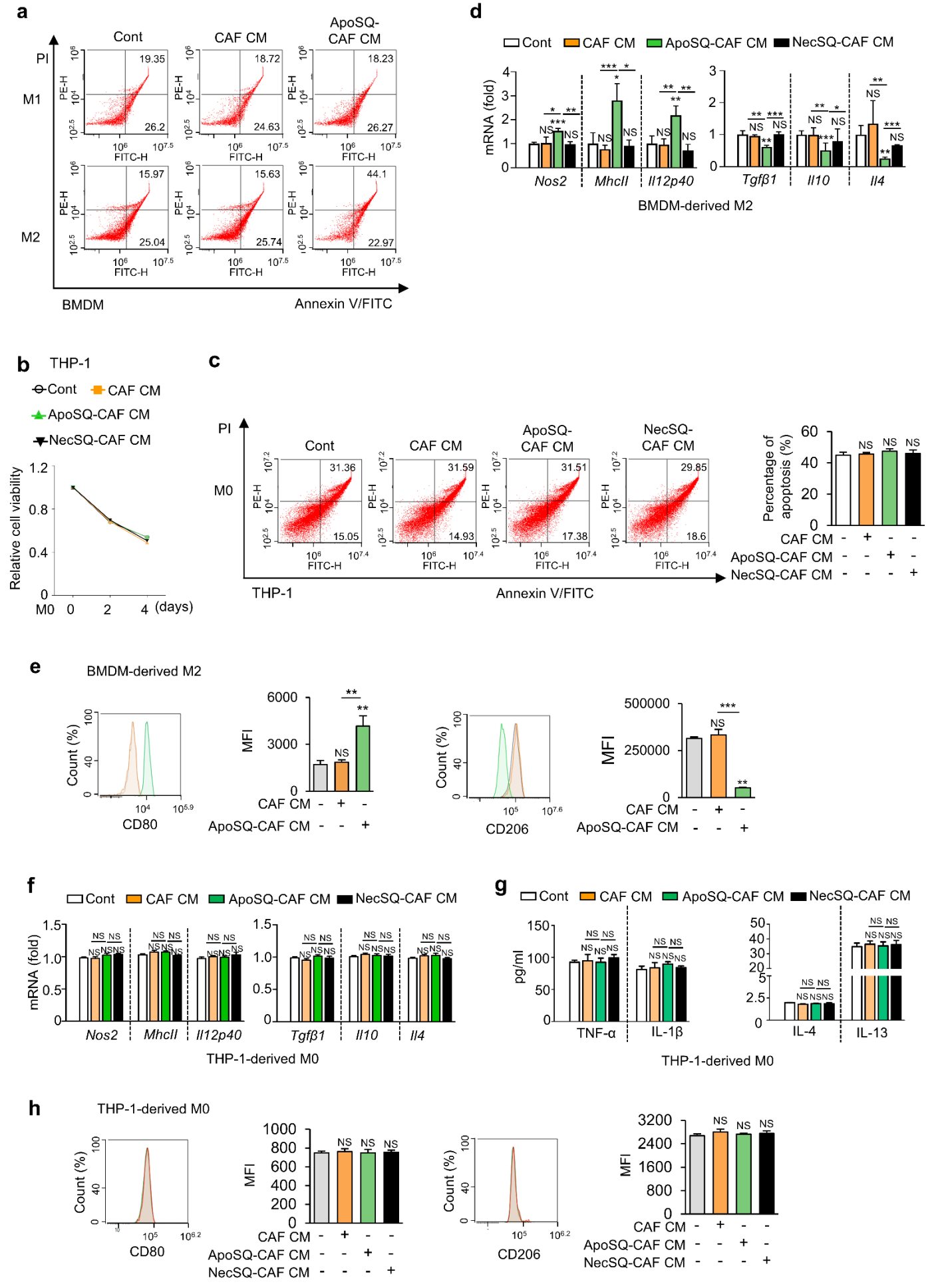
**Supplementary Figure 1**



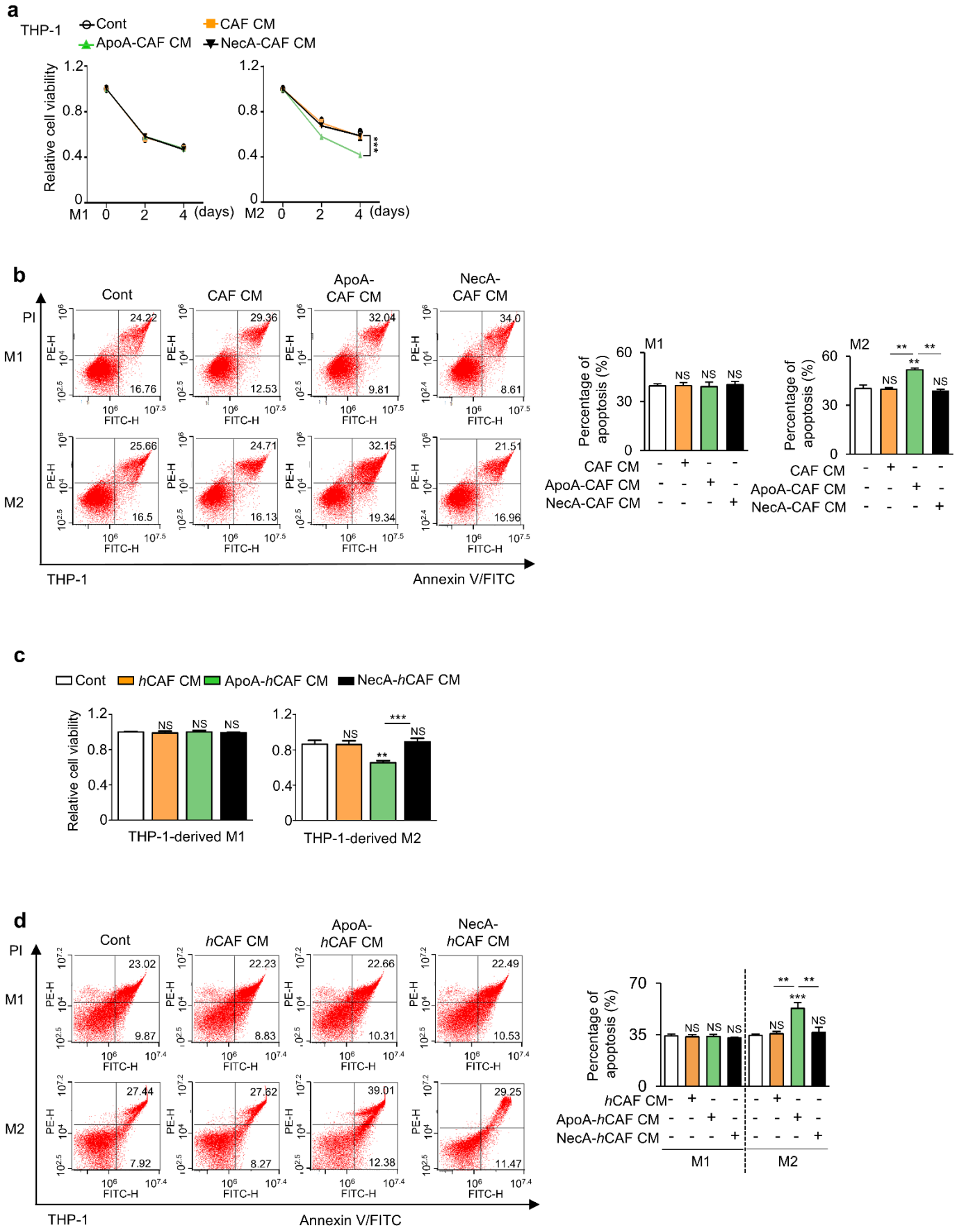

**Supplementary Fig. S1. Assessment of macrophage polarization and effects of ApoSQ-CAF CM on apoptosis.** (**a**, **d**) Schemes of the preparation and polarization of M1- and M2-type macrophages derived from THP-1 cells and mouse bone marrow-derived macrophages (BMDMs). (**b**, **e**) Immunoblot analysis of the M1 and M2 macrophage marker expression in M1 (M1) or M2 macrophages (M2) derived from THP-1 cells and BMDMs. (**c**, **f**) Immunofluorescence staining for the M1 marker CD86 and M2 marker CD163 in M1 and M2 macrophages polarized from THP-1 cells and BMDMs. The imaging medium was VECTASHIELD fluorescence mounting medium containing DAPI. Original magnification: ×400. Scale bars = 20 μm. Data are from one experiment representative of three independent experiments with similar results (**b**, **c**, **e**, **f**).

## Supplementary Figure 2



**Supplementary Fig. S2. Effects of ApoSQ-CAF CM on apoptosis and reprogramming of macrophages.** (**a**, **c**) Apoptosis of THP-1-derived M0 (**c**) and BMDM-derived M1 and M2 macrophages (**a**) was quantified as the sum of the percentages of early and late stages of apoptosis. Flow cytometry analysis after Annexin V−FICT/PI dual staining was employed to evaluate apoptosis. (**b**) Cell viability assay of M0 macrophages (M0) derived from THP-1 cells. (**d**, **f**) qRT-PCR analysis of relative mRNA levels of M1 (*Nos2*, *MhcII*, and *Il12p40*) and M2 (*Tgfβ1*, *Il10*, and *Il4*) markers in THP-1-derived M0 (**f**) and BMDM-derived M2 macrophages (**d**). (**e**, **h**) Flow cytometry analysis of the population of $CD80^+$ and $CD206^+$ macrophages among THP-1-derived M0 (**h**) and BMDM-derived M2 macrophages (**e**). Mean fluorescence intensity (MFI) values (*right*). (**g**) ELISA of TNF-α, IL-1β, IL-4, and IL-13 in culture media of M0 macrophages derived from THP-1 cells. (**a-h**) CAFs were exposed to apoptotic 344SQ cells (ApoSQ) or necrotic cancer cells (NecSQ) for 20 h. Conditioned medium from CAFs only (CAF CM), exposed to ApoSQ (ApoSQ-CAF CM) or NecSQ (NecSQ-CAF CM) was treated to M0, M1 or M2 macrophages for 2 or 3 days. NS, not significant; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, two-tailed Student's *t*-test. Data are from one experiment representative of three independent experiments with similar results (**a**; **c, e**, and **h** ***left***) or from three independent experiments (mean ± standard error: **b**, **d**, **f**, **g**; **c, e**, and **h** ***right***).

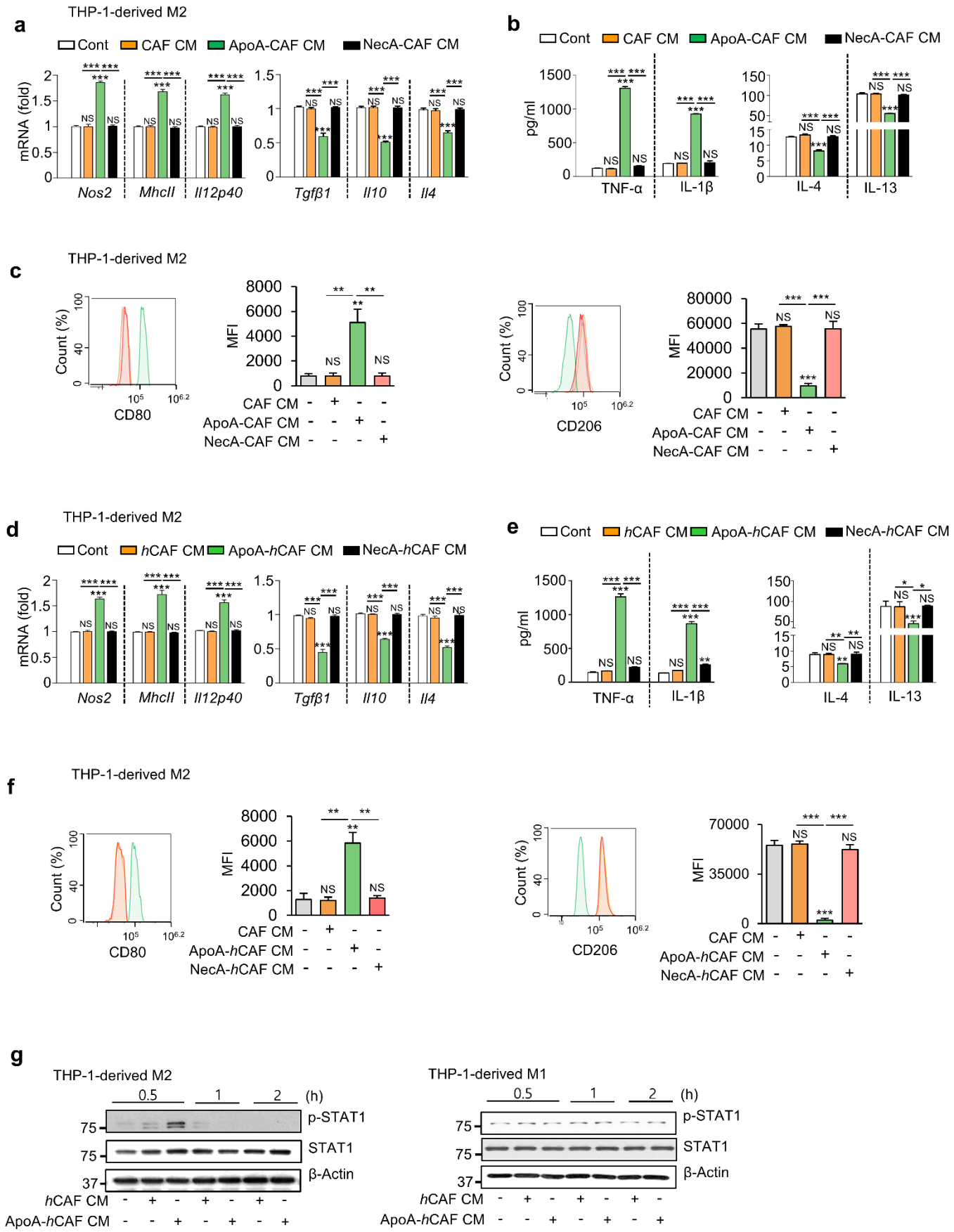
## Supplementary Figure 3




**Supplementary Fig. S3. CM from CAFs exposed to apoptotic A549 cells reduces M2 macrophage survival and induces apoptosis.** (**a**, **c**) Cell viability assay of M1 (M1) and M2 macrophages (M2) derived from THP-1 cells. (**b**, **d**) *Left:* Flow cytometry analysis after Annexin

V−FICT/PI dual staining was employed to evaluate the apoptosis of M1 and M2 macrophages polarized from THP-1 cells. *Right:* Apoptotic cells were quantified as the sum of the percentages of early and late stages of apoptosis. (**a**, **b**) CAFs were exposed to apoptotic A549 cells (ApoA) or necrotic A549 cells (NecA) for 20 h. Conditioned medium from CAFs only (CAF CM), exposed to ApoA (ApoA-CAF CM) or NecA (NecSQ-CAF CM) was treated to M1 or M2 macrophages for the indicated days or 3 days. (**c**, **d**) M1 and M2 macrophages were treated with CM from human CAFs only (*h*CAF CM), ApoA-*h*CAF CM, or NecA-*h*CAF CM for 3 days NS, not significant; $^{**}P < 0.01$, $^{***}P < 0.001$, two-tailed Student's *t*-test. Data are from one experiment representative of three independent experiments with similar results (**b** and **d** ***left***) or from three independent experiments (mean ± standard error: **a**, **c**; **b** and **d** ***right***).

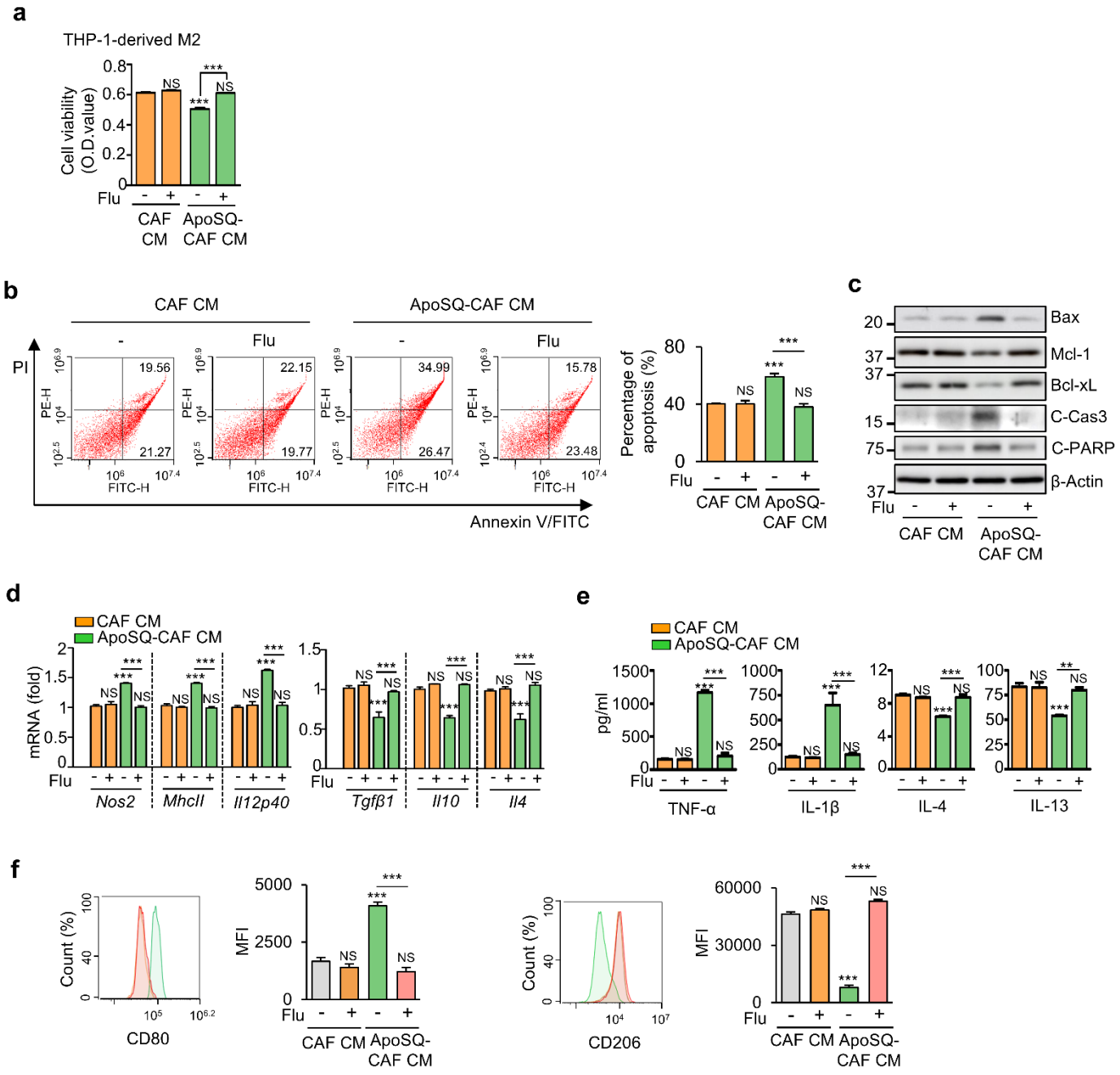
## Supplementary Figure 4




**Supplementary Fig. S4. CM from CAFs exposed to apoptotic A549 cells promotes reprogramming toward an M1-like phenotype and activates STAT1 in M2 macrophages.** (**a**, **d**) qRT-PCR analysis of relative mRNA levels of M1 (*Nos2*, *MhcII*, and *Il12p40*) and M2 (*Tgfβ1*,

*Il10*, and *Il4*) markers in M2 macrophages (M2) derived from THP-1 cells. (**b**, **e**) ELISA of TNF-α, IL-1β, IL-4, and IL-13 in culture media of M2 macrophages derived from THP-1 cells. (**c**, **f**) Flow cytometry analysis of the population of $CD80^+$ and $CD206^+$ macrophages among M2 macrophages derived from THP-1 cells. Mean fluorescence intensity (MFI) values (*right*). (**a-c**) CAF CM, ApoA-CAF CM, or NecA-CAF was treated to M2 macrophages for 2 or 3 days. (**d-f**) *h*CAF CM, ApoA-*h*CAF CM, or NecA-*h*CAF was treated to M2 macrophages for 2 or 3 days. (**g**) Immunoblot analysis of the indicated proteins in THP-1-derived M1 and M2 macrophages treated with *h*CAF CM or ApoSQ-*h*CAF CM for the indicated time. NS, not significant; **P* < 0.05, ***P* < 0.01, ****P* < 0.001, two-tailed Student's *t*-test. Data are from one experiment representative of three independent experiments with similar results (**c** and **f** ***left***; **g**) or from three independent experiments (mean ± standard error: **a**, **b**, **d**, **e**; **c** and **f** ***right***).

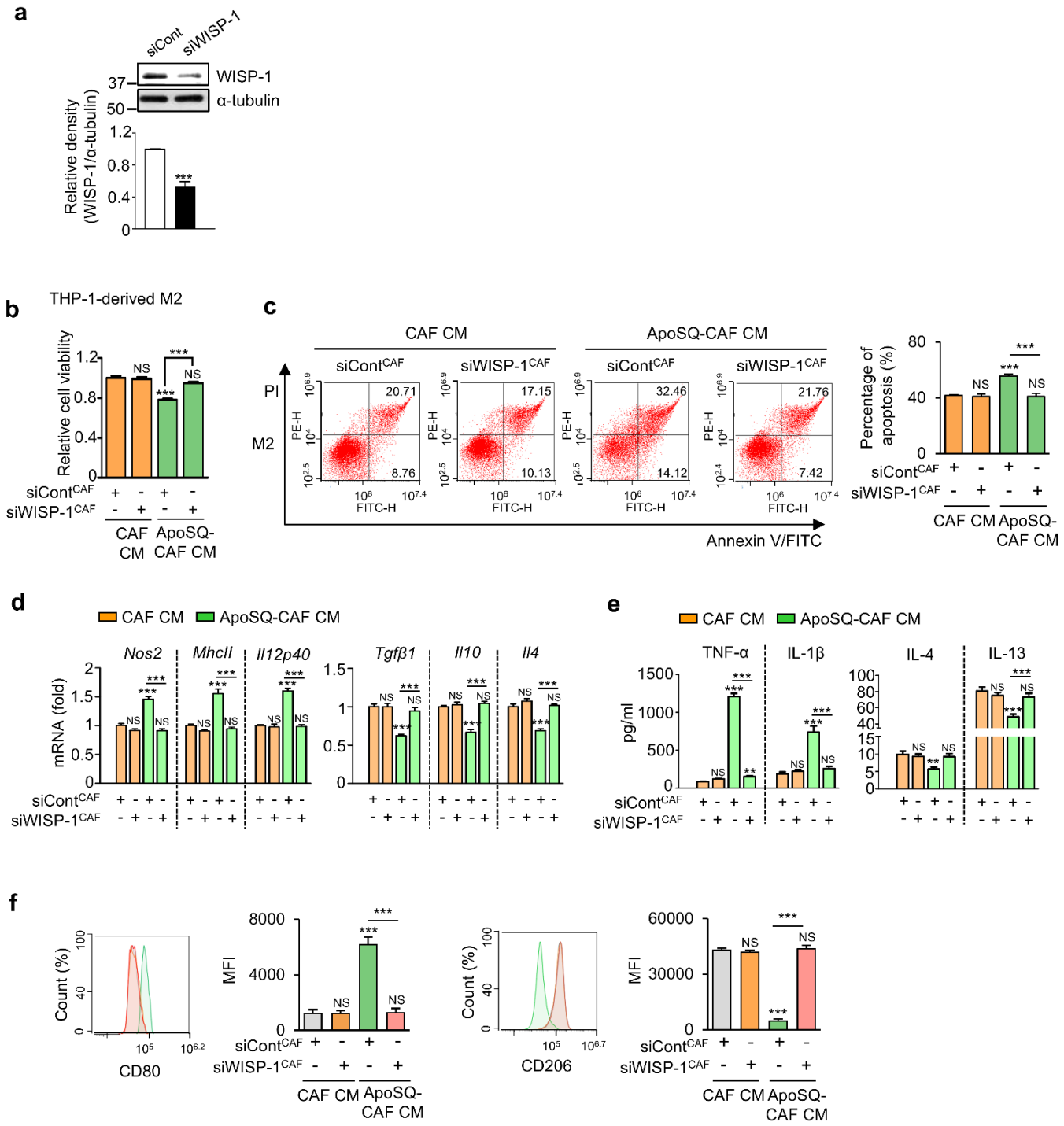
## Supplementary Figure 5




**Supplementary Fig. S5. Fludarabine blocks STAT1-dependent reducing survival, promoting apoptosis and reprogramming of M2 Macrophages.** (**a**) Cell viability assay of THP-1-derived M2 macrophages treated with CM for 3 days. (**b**) *Left:* Flow cytometry analysis after Annexin V−FICT/PI dual staining was employed to evaluate the apoptosis of M2 macrophages. *Right*: Apoptotic cells were quantified as the sum of the percentages of early and late stages of apoptosis 3 days after CM treatment. (**c**) Immunoblot analysis of the indicated proteins in M2 macrophage lysates 3 days after CM treatment. (**d**) qRT-PCR analysis of relative mRNA levels of M1 (*Nos2*, *MhcII*, and *Il12p40*) and M2 (*Tgfβ1*, *Il10*, and *Il4*) markers in M2 macrophages treated with CM for

3 days. (**e**) ELISA of TNF-α, IL-1β, IL-4, and IL-13 in the culture supernatants of THP-1-derived M2 macrophages treated with CM for 3 days. (**f**) Flow cytometry analysis of the population of $CD80^+$ and $CD206^+$ macrophages among M2 macrophages treated with CM for 2 or 3 days. Mean fluorescence intensity (MFI) values (*right*). (**a**-**f**) THP-1-derived M2 macrophages were pretreated with fludarabine (1 μM) 1 h before treatment with CAF CM or ApoSQ-CAF CM. NS: not significant; $^{**}P < 0.01$, $^{***}P < 0.001$, two-tailed Student's *t*-test. The data are from one experiment representative of three independent experiments with similar results (**b** and **f** l***eft***; **c**) or from three independent experiments (mean ± standard error: **a**, **d**, **e**; **b** and **f** ***right***).

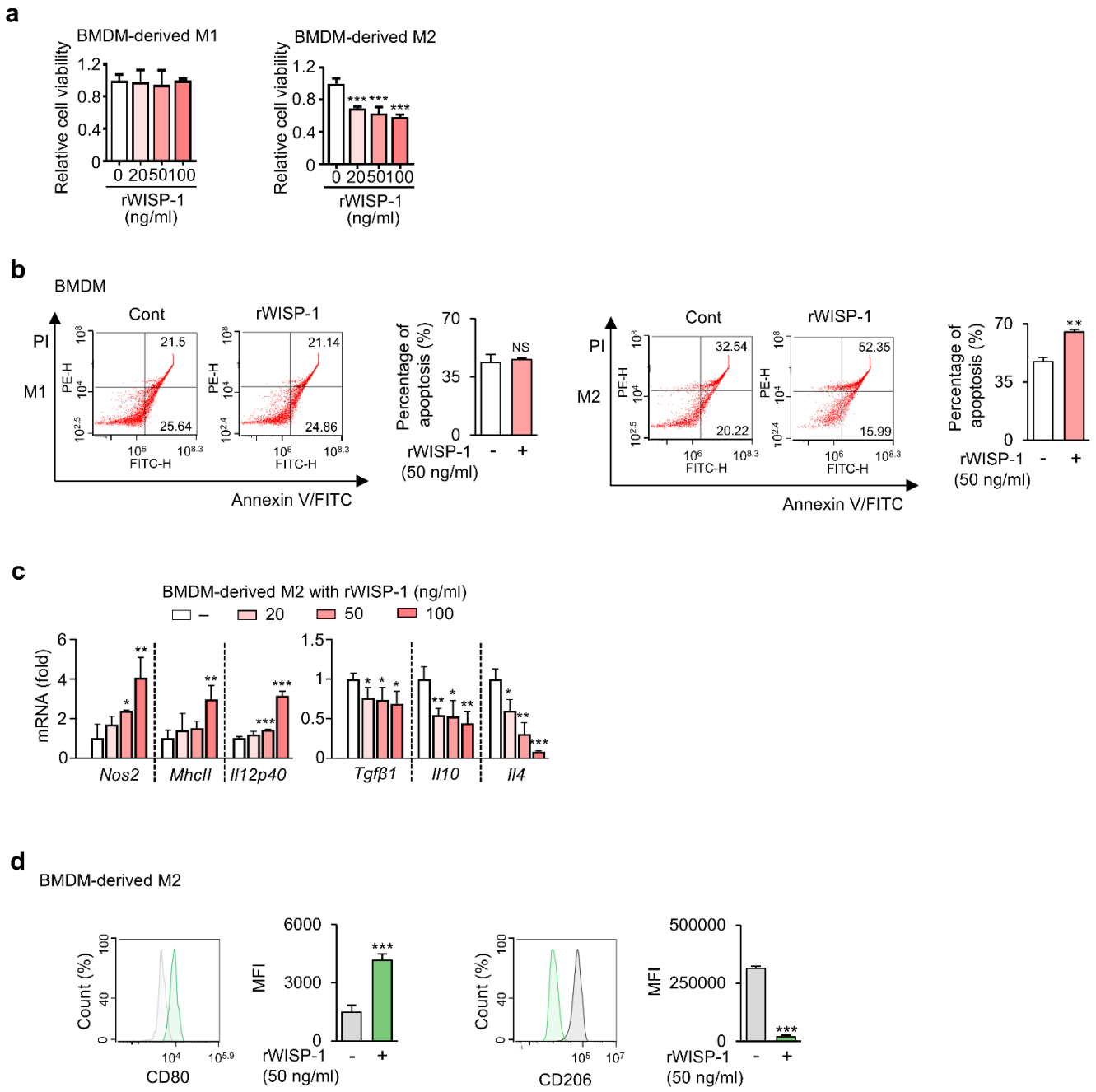
## Supplementary Figure 6




**Supplementary Fig. S6. WISP-1 mediates the anti-survival, pro-apoptosis, and reprogramming effects of ApoSQ-CAF CM on M2 macrophages.** (**a**) Immunoblot analysis of WISP-1 in CAFs transfected with control or WISP-1 siRNA (*upper*). Densitometric analysis of the relative WISP-1 abundance (*lower*). (**b**) Cell viability assay of THP-1-derived M2 macrophages (M2) treated with CM for 3 days. (**c**) *Left:* Flow cytometry analysis after Annexin V−FICT/PI dual staining was employed to evaluate the apoptosis of M2 macrophages treated with CM for 3 days. *Right*: Apoptotic cells were quantified as the sum of the percentages of early and late stages of

apoptosis. (**d**) qRT-PCR analysis of relative mRNA levels of M1 (*Nos2*, *MhcII*, and *Il12p40*) and M2 (*Tgfβ1*, *Il10*, and *Il4*) markers in THP-1-derived M2 macrophages treated with CM for 3 days. (**e**) ELISA of TNF-α, IL-1β, IL-4, and IL-13 in the culture supernatants of M2 macrophages treated with CM for 3 days. (**f**) Flow cytometry analysis of the population of $CD80^+$ and $CD206^+$ macrophages treated with CM for 2 or 3 days. Mean fluorescence intensity (MFI) values (*right*). (**b-f**) CAFs were transfected with control or WISP-1 siRNA before exposure to apoptotic 344SQ cells (ApoSQ) for 20 h. CAF CM or ApoSQ-CAF CM was treated to THP-1-derived M2 macrophages. NS: not significant; ***$P < 0.001$, two-tailed Student's *t*-test. The data are from one experiment representative of three independent experiments with similar results (**a** ***upper***; **c** and **f** ***left***) or from three independent experiments (mean ± standard error: **a** ***lower***; **b**, **d**, **e**; **c** and **f** ***right***).

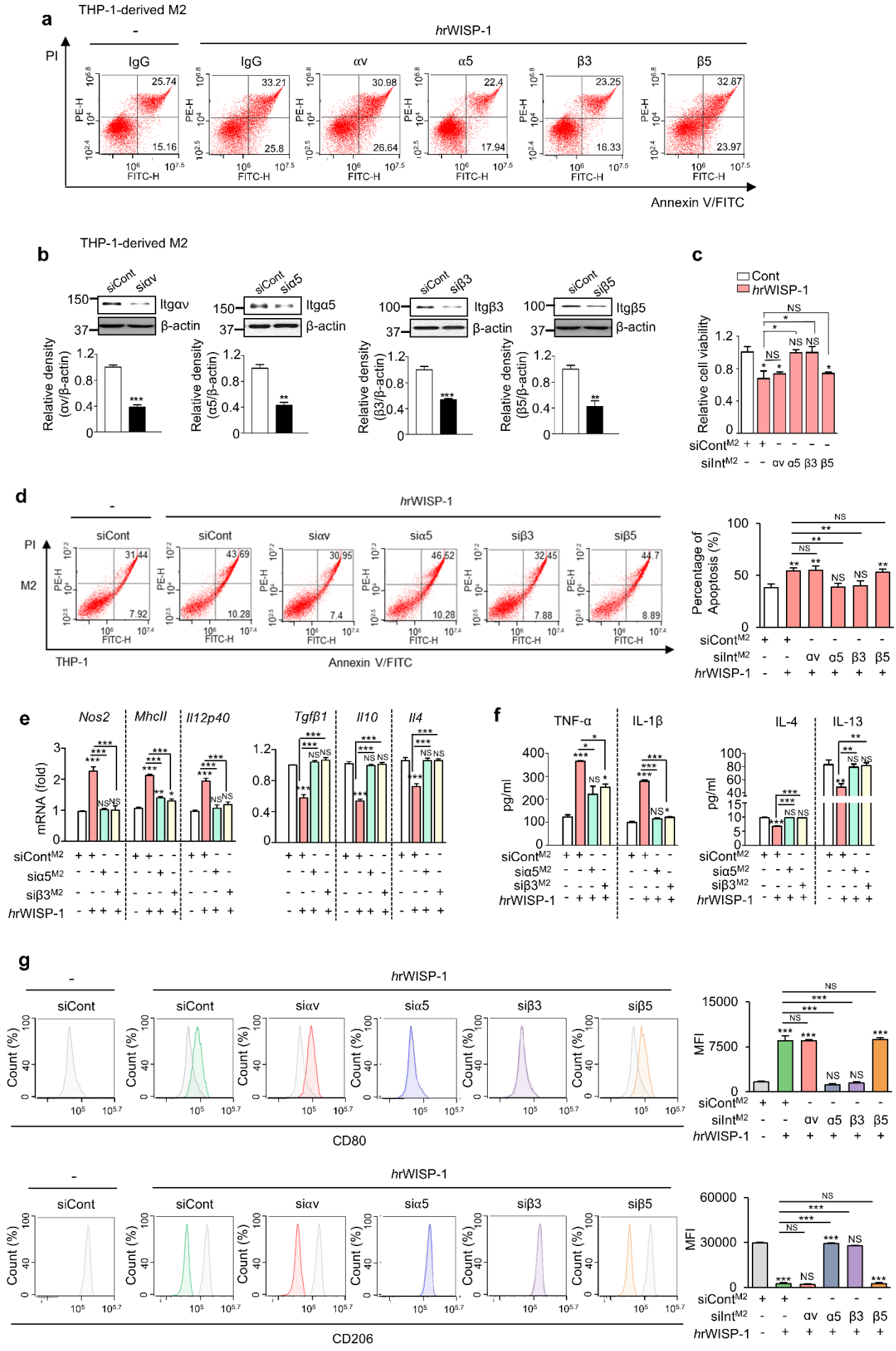
**Supplementary Figure 7**




**Supplementary Fig. S7. Recombinant WISP-1 reduces survival, induces apoptosis, and promotes reprogramming of BMDM-derived M2 macrophages toward an M1-like phenotype.** (**a**) Cell viability assay of BMDM-derived M1 (M1) and M2 macrophages (M2) treated with 20-100 ng/ml mouse rWISP-1 (rWISP-1) for 3 days. (**b**) *Left:* Flow cytometry analysis after Annexin V−FICT/PI dual staining was employed to evaluate the apoptosis of BMDM-derived M1 and M2 macrophages treated with rWISP-1 (50 ng/ml) for 3 days. *Right*: Apoptotic cells were quantified as the sum of the percentages of early and late stages of apoptosis. (**c**) qRT-PCR analysis of relative mRNA levels of M1 (*Nos2*, *MhcII*, and *Il12p40*) and M2 (*Tgfβ1*, *Il10*, and *Il4*) markers in M2

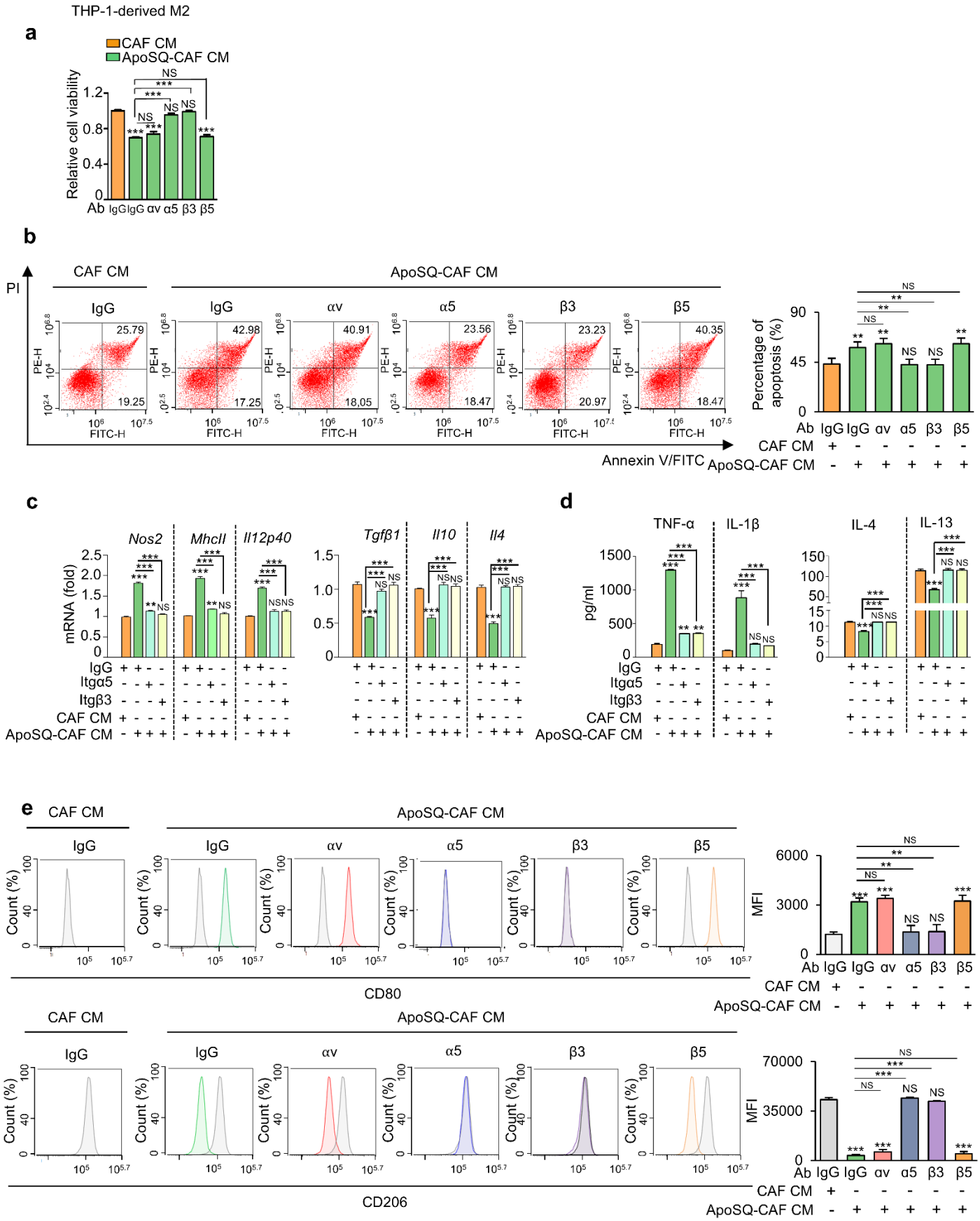
macrophages treated with 20-100 ng/ml rWISP-1 for 3 days. (**d**) Flow cytometry analysis of the population of $CD80^+$ and $CD206^+$ macrophages among M2 macrophages (M2). Mean fluorescence intensity (MFI) values (*right*). BMDM-derived M2 macrophages were treated with 50 ng/ml rWISP-1 for 2 or 3 days. NS: not significant; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, two-tailed Student's *t*-test. The data are from one experiment representative of three independent experiments with similar results (**b** and **d** ***left***) or from three independent experiments (mean ± standard error: **a**, **c**; **b** and **d** ***right***).

# Supplementary Figure 8



**Supplementary Fig. S8. WISP-1-integrin α5β3 signaling mediates the anti-survival, pro-apoptotic, and reprogramming effects of the CM in M2 macrophages.** (**a**) Flow cytometry analysis after Annexin V−FICT/PI dual staining was employed to evaluate the apoptosis of M2 macrophages treated with human rWISP-1 (*h*rWISP-1) for 3 days. THP-1-derived M2 macrophages were pretreated with an anti-integrin blocking antibody (3 μg/ml; anti-integrin αv, α5, β3 or β5) or corresponding IgG isotype control for 30 min prior to *h*rWISP-1 treatment (50 ng/ml) (**b**) Immunoblot analysis of the indicated proteins in THP-1-derived M2 macrophages transfected with control or siRNA of integrin αv, α5, β3, or β5 (*upper*). Densitometric analysis of the relative integrin αv, α5, β3, or β5 abundance (*lower*). (**c**) Cell viability assay of M2 macrophages (M2) treated with *h*rWISP-1 for 3 days. (**d**) *Left:* Flow cytometry analysis after Annexin V−FICT/PI dual staining was employed to evaluate the apoptosis of M2 macrophages treated with *h*rWISP-1 for 3 days. *Right*: Apoptotic cells were quantified as the sum of the percentages of early and late stages of apoptosis. (**e**) qRT-PCR analysis of relative mRNA levels of M1 (*Nos2*, *MhcII*, and *Il12p40*) and M2 (*Tgfβ1*, *Il10*, and *Il4*) markers in M2 macrophages treated with *h*rWISP-1 for 3 days. (**f**) ELISA of TNF-α, IL-1β, IL-4, and IL-13 in the culture supernatants of M2 macrophages treated with *h*rWISP-1 for 3 days. (**g**) Flow cytometry analysis of the population of $CD80^+$ and $CD206^+$ macrophages among M2 macrophages treated with *h*rWISP-1 for 2 or 3 days. Mean fluorescence intensity (MFI) values (*right*). (**c-g**) THP-1-derived M2 macrophages were transfected with control siRNA or siRNA targeting integrin αv, α5, β3, or β5 for 24 h prior to *h*rWISP-1 treatment (50 ng/ml). NS: not significant; **P* < 0.05, ***P* < 0.01, ****P* < 0.001, two-tailed Student's *t*-test. The data are from one experiment representative of three independent experiments with similar results (**a**, **b** ***upper***; **d** and **g** ***left***) or from three independent experiments (mean ± standard error: **b** ***lower***; **c, e**, **f**; **d** and **g** ***right***).

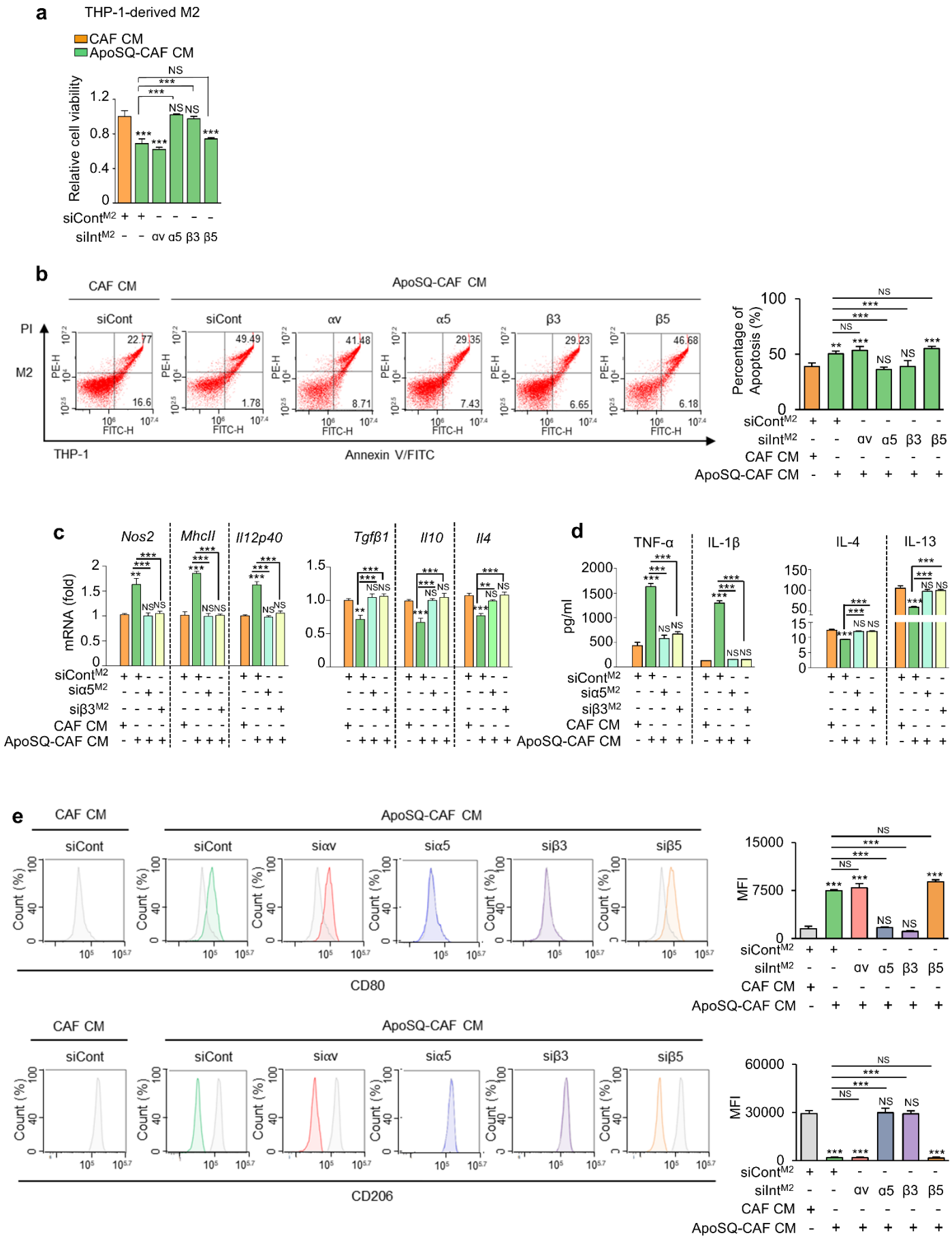
## Supplementary Figure 9




**Supplementary Fig. S9. Neutralizing antibodies against integrin α5 and β3 abrogates the anti-survival, pro-apoptotic, and reprogramming effects of ApoSQ-CAF CM on M2 macrophages.** (**a**) Cell viability assay of M2 macrophages (M2) treated with CM for 3 days. (**b**)

*Left:* Flow cytometry analysis after Annexin V−FICT/PI dual staining was employed to evaluate the apoptosis of M2 macrophages treated with CM for 3 days. *Right*: Apoptotic cells were quantified as the sum of the percentages of early and late stages of apoptosis. (**c**) qRT-PCR analysis of relative mRNA levels of M1 (*Nos2*, *MhcII*, and *Il12p40*) and M2 (*Tgfβ1*, *Il10*, and *Il4*) markers in M2 macrophages treated with CM for 3 days. (**d**) ELISA of TNF-α, IL-1β, IL-4, and IL-13 in the culture supernatants of M2 macrophages treated with CM for 3 days. (**e**) Flow cytometry analysis of the population of $CD80^+$ and $CD206^+$ macrophages among M2 macrophages treated with CM for 2 or 3 days. Mean fluorescence intensity (MFI) values (*right*). (**a-e**) THP-1-derived M2 macrophages were pretreated with an anti-integrin blocking antibody (3 μg/ml; anti-integrin αv, α5, β3, or β5) or corresponding IgG isotype control for 30 min prior to CM treatment. NS: not significant; $^{**}P < 0.01$, $^{***}P < 0.001$, two-tailed Student's *t*-test. The data are from one experiment representative of three independent experiments with similar results (**b** and **e** ***left***) or from three independent experiments (mean ± standard error: **a**, **c**, **d**; **b** and **e** ***right***).

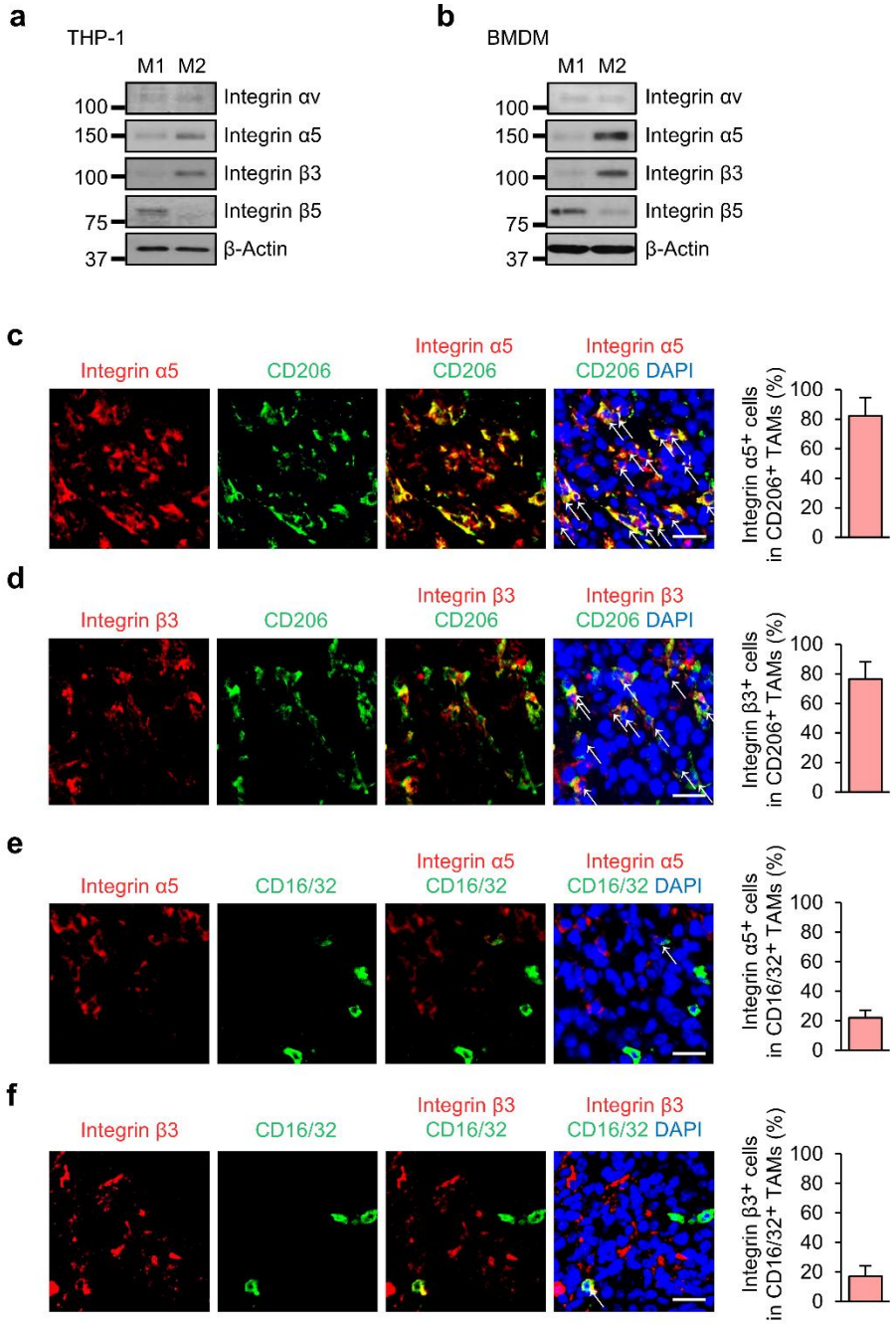
## Supplementary Figure 10




**Supplementary Fig. S10. Knockdown of integrin α5 and β3 abrogates the anti-survival, pro-apoptotic, and reprogramming effects of ApoSQ-CAF CM on M2 macrophages.** (**a**) Cell

viability assay of M2 macrophages (M2) treated with CM for 3 days. (**b**) *Left:* Flow cytometry analysis after Annexin V−FICT/PI dual staining was employed to evaluate the apoptosis of M2 macrophages ) treated with CM for 3 days. *Right*: Apoptotic cells were quantified as the sum of the percentages of early and late stages of apoptosis. (**c**) qRT-PCR analysis of relative mRNA levels of M1 (*Nos2*, *MhcII*, and *Il12p40*) and M2 (*Tgfβ1*, *Il10*, and *Il4*) markers in M2 macrophages) treated with CM for 3 days. (**d**) ELISA of TNF-α, IL-1β, IL-4, and IL-13 in the culture supernatant of M2 macrophages treated with CM for 3 days. (**e**) Flow cytometry analysis of the population of $CD80^+$ and $CD206^+$ macrophages among M2 macrophages treated with CM for 2 or 3 days. Mean fluorescence intensity (MFI) values (*right*). (**a-e**) THP-1-derived M2 macrophages were transfected with control siRNA or siRNA targeting integrin αv, α5, β3, or β5 for 24 h prior to CM treatment. NS: not significant; $^{**}P < 0.01$, $^{***}P < 0.001$, two-tailed Student's *t*-test. The data are from one experiment representative of three independent experiments with similar results (**b** and **e** ***left***) or from three independent experiments (mean ± standard error: **a**, **c**, **d**; **b** and **e** ***right***).

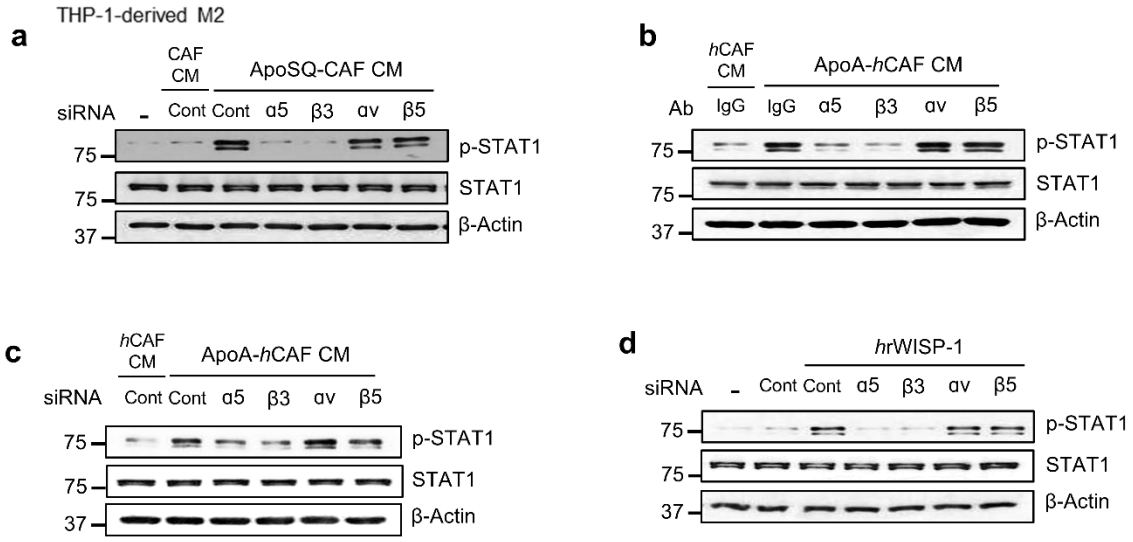
**Supplementary Figure 11**




**Supplementary Fig. S11. Integrin α5β3 is expressed on M2 macrophages and M2 TAMs in primary tumors.** (**a, b**) Immunoblot analysis of Integrin αv, α5, β3, and β5 expression in M1 (M1) or M2 macrophages (M2) derived from THP-1 macrophages and mouse BMDMs. (**c-f**) Immunofluorescent staining of integrin α5 (red) and β3 (red), CD206 (green), and CD16/32 (green) in primary tumor sections. Right: Quantitation of integrin $\alpha5^+$ or $\beta3^+$ cells in $CD206^+$ TAM (M2) and $CD16/32^+$ TAM (M1) in primary tumor. The imaging medium was VECTASHIELD fluorescent mounting medium containing DAPI. Original magnification: ×40. Scale bars = 100 μm. 344SQ cells

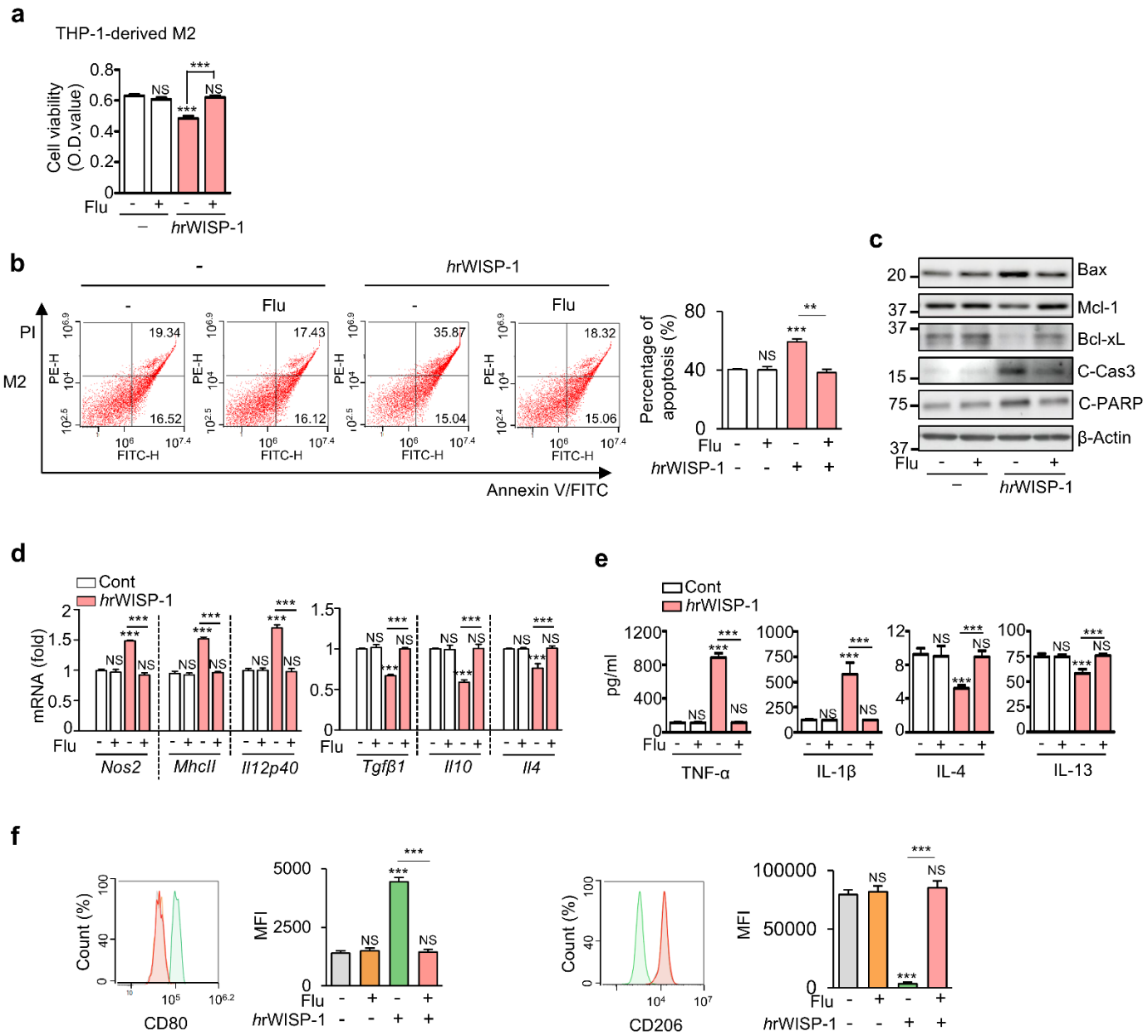
were subcutaneously implanted into syngeneic (129/Sv) mice (n=3 per group). Mice were necropsied 6 weeks after 344SQ cell injection. The data are from one experiment representative of three independent experiments with similar results (**a**, **b**; **c-f** ***left***) or from three independent experiments (mean ± standard error: **c-f** ***right***).

**Supplementary Figure 12**




**Supplementary Fig. S12. CM from CAFs exposed to apoptotic cancer cells and recombinant WISP-1 activates STAT1 through integrin α5β3.** (**a-d**) Immunoblot analysis of phosphorylated STAT1 and total STAT1 in M2 macrophages treated with CM or *hr*WISP-1 for 30 min. (**a**, **c**, **d**) THP-1-derived M2 macrophages were transfected with control siRNA or siRNA targeting integrin αv, α5, β3, or β5 for 24 h prior to treatment with CM or *hr*WISP-1 (50 ng/ml) for 30 min. (**b**) THP-1-derived M2 macrophages were pretreated with an anti-integrin blocking antibody (3 μg/ml; anti-integrin αv, α5, β3, or β5) or corresponding IgG isotype control for 30 min prior to CM treatment. (**a-c**) CM was obtained from mouse CAFs (CAF) exposed to apoptotic 344SQ cells (ApoSQ) or human CAFs (*h*CAF) exposed to apoptotic A549 cells (ApoA). The data are from one experiment representative of three independent experiments with similar results.

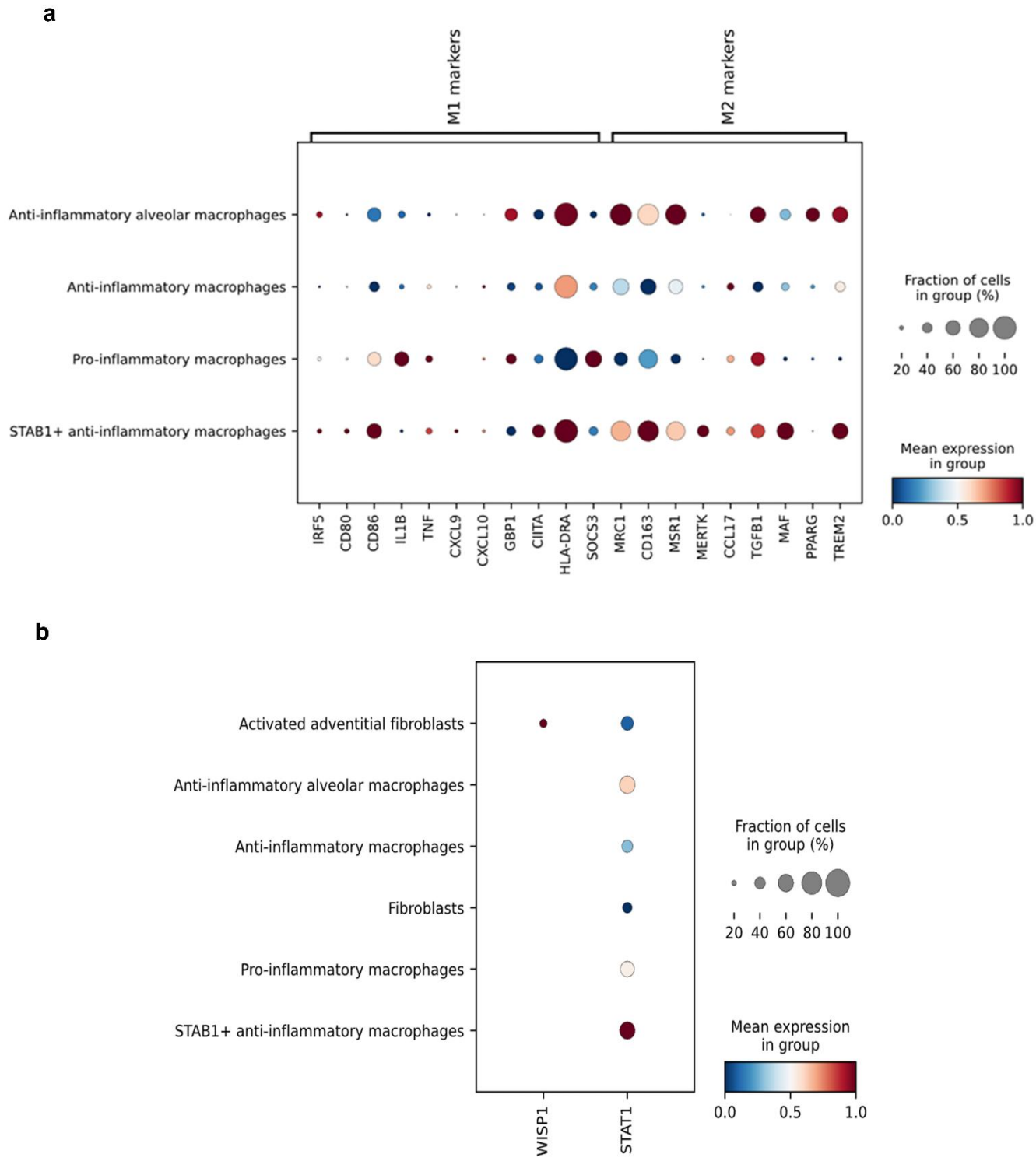
**Supplementary Figure 13**




**Supplementary Fig. S13. Pharmacological inhibition of STAT1 activation abrogates the anti-survival, pro-apoptotic, and reprogramming effects of WISP-1 on M2 macrophages.** (**a**) Cell viability assay of M2 macrophages treated with *hr*WISP-1 (50 ng/ml) for 3 days. (**b**) *Left:* Flow cytometry analysis after Annexin V−FICT/PI dual staining was employed to evaluate the cell apoptosis of M2 macrophages treated with *hr*WISP-1 for 3 days. *Right*: Apoptotic cells were quantified as the sum of the percentages of early and late stages of apoptosis. (**c**) Immunoblot analysis of the indicated proteins in M2 macrophages treated with *hr*WISP-1 for 3 days. (**d**) qRT-PCR analysis of relative mRNA levels of M1 (*Nos2*, *MhcII*, and *Il12p40*) and M2 (*Tgfβ1*, *Il10*, and *Il4*) markers in THP-1-derived M2 macrophages treated with *hr*WISP-1 for 3 days. (e) ELISA of TNF-α, IL-1β, IL-4, and IL-13 in the culture supernatants of THP-1-derived M2 macrophages

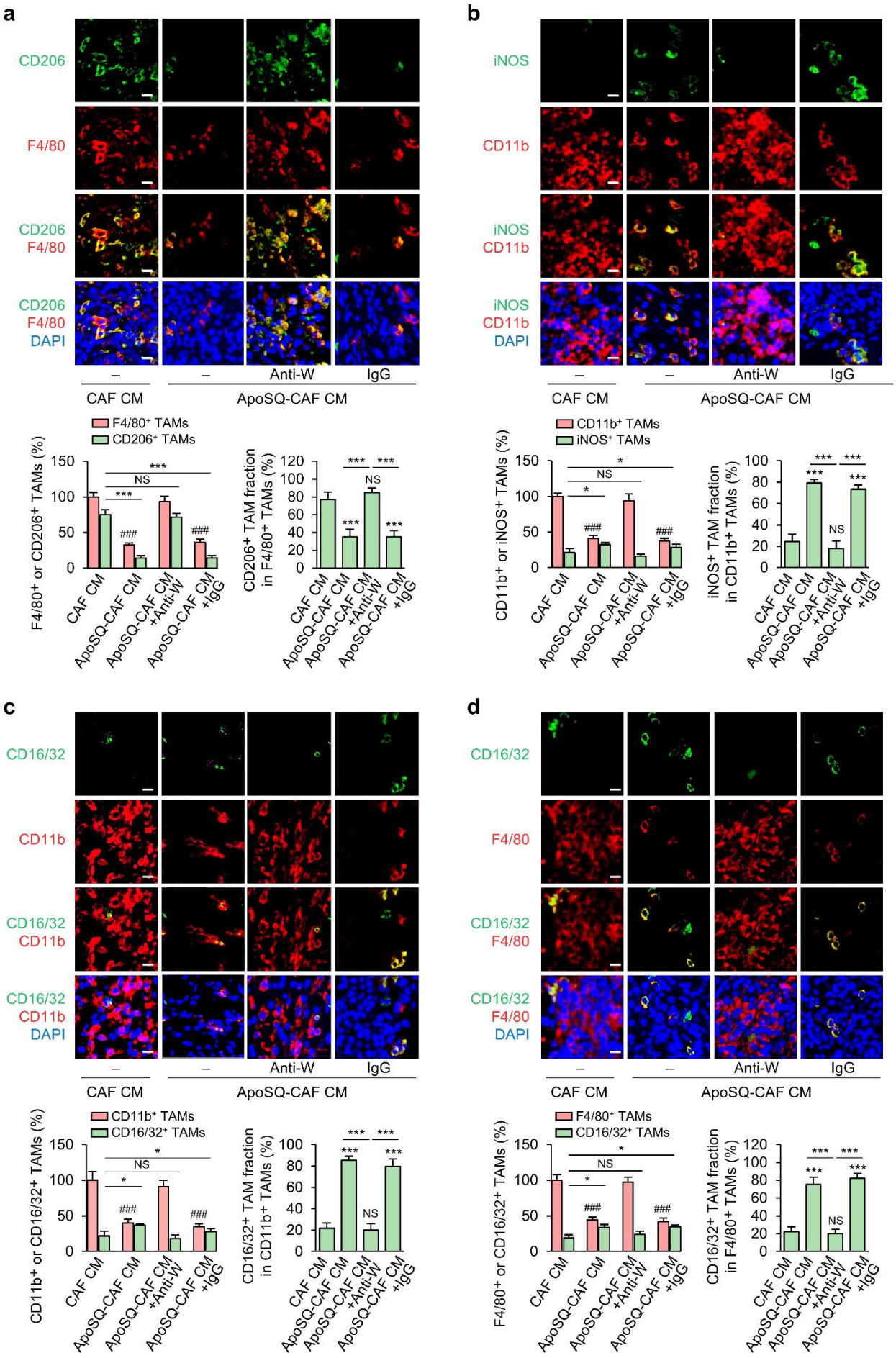
treated with hrWISP-1 for 3 days. (**f**) Flow cytometry analysis of the population of $CD80^+$ and $CD206^+$ macrophages among M2 macrophages treated with *h*rWISP-1 for 2 or 3 days. Mean fluorescence intensity (MFI) values (*right*). (**a-f**) THP-1-derived M2 macrophages were pretreated with fludarabine (1 μM) I h prior to *h*rWISP-1 (50 ng/ml) treatment. NS: not significant; $^{**}P < 0.01$, $^{***}P < 0.001$, two-tailed Student's *t*-test. The data are from one experiment representative of three independent experiments with similar results (**b** and **f *left***; **c)** or from three independent experiments (mean ± standard error: **a**, **d**, **e**; **b** and **f *right***).

**Supplementary Figure 14**



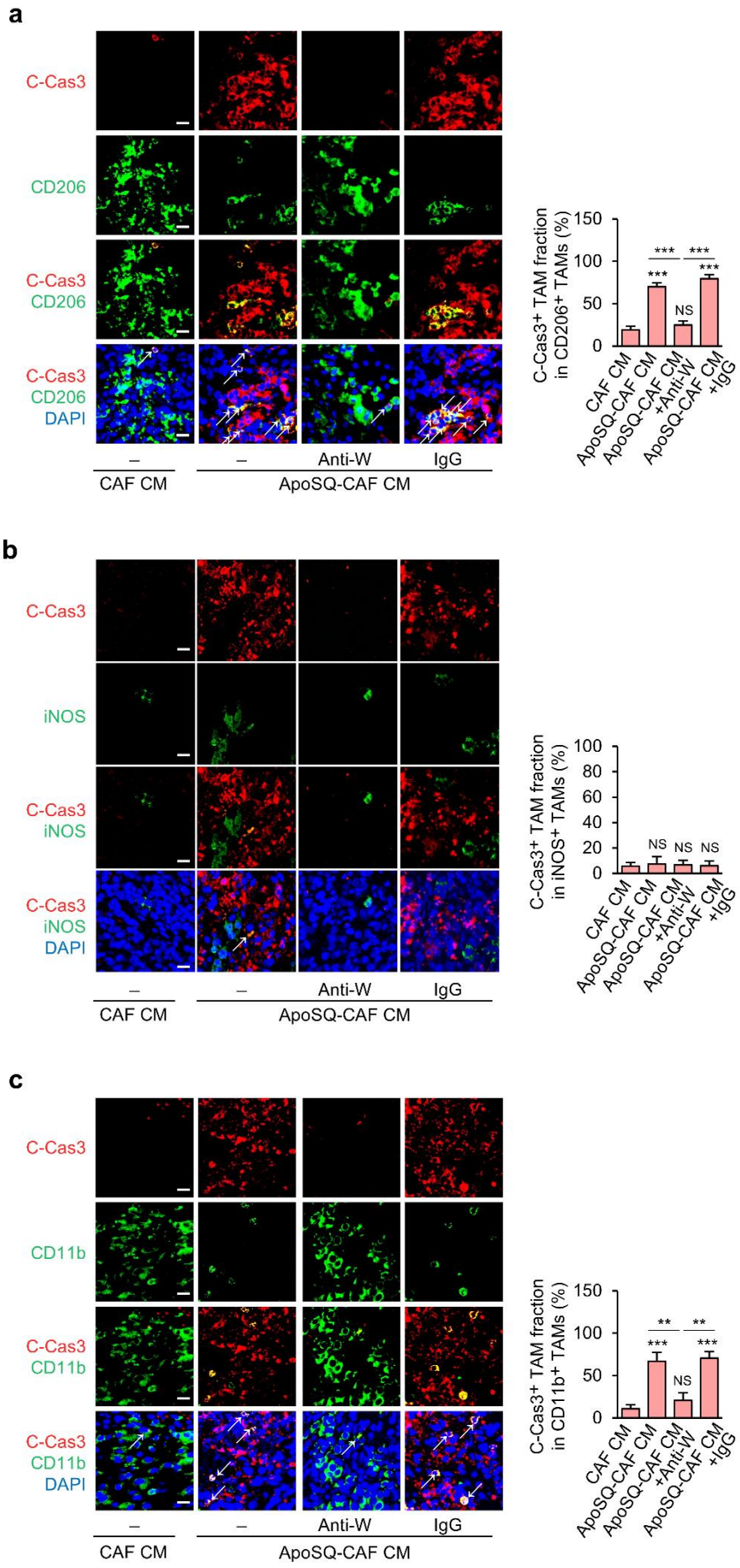

**Supplementary Fig. S14. Expression of macrophage subtype markers and STAT1/CCN4 in non-small cell lung cancer (NSCLC) single-cell datasets.**

## Supplementary Figure 15



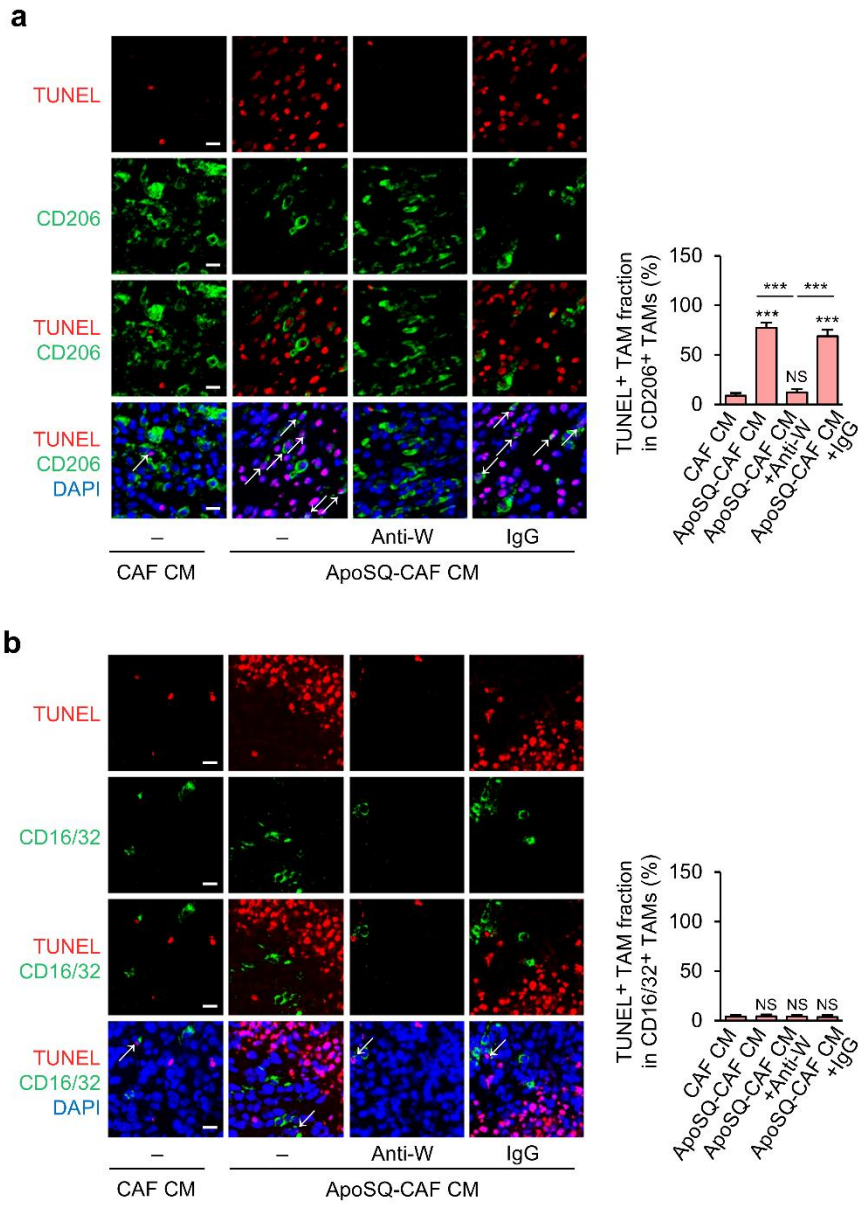
**Supplementary Fig. S15. Administration of ApoSQ-CAF CM reduces M2 fraction and enhances M1 TAM fraction in primary tumors via WISP-1.** The experimental design was described in Fig. 5a. (**a-d**) *Upper*: Immunofluorescent staining of primary tumor sections showing the M2 TAM Marker CD206 (green); M1 markers iNOS (green) and CD16/32 (green); and the pan-macrophage markers CD11b (red) and F4/80 (red). Original magnification: ×40. Scale Bar, 100 μm. (**a**) *Lower*: Quantitation of $CD206^+$ TAM (M2) density and the fraction of M2 TAMs. (**b-d**) *Lower*: Quantitation of $iNOS^+$ and $CD16/32^+$ TAM (M1) density and the fraction of M1 TAMs in primary tumors. The M1 and M2 TAM fraction was determined by the percentage of M1 and M2 TAMs within $CD11b^+$ or $F4/80^+$ TAMs. NS, not significant; $^*P < 0.05$, $^{***}P < 0.001$ compared to CAF CM or as indicated; $^{\#\#\#}P < 0.001$ compared to CAF CM, Analysis of variance with Tukey's post hoc test. The data are from one experiment representative of three independent experiments with similar results (**a-d *upper***). The data are represented as the means ± standard errors from three mice per group (**a-d *lower***).

## Supplementary Figure 16



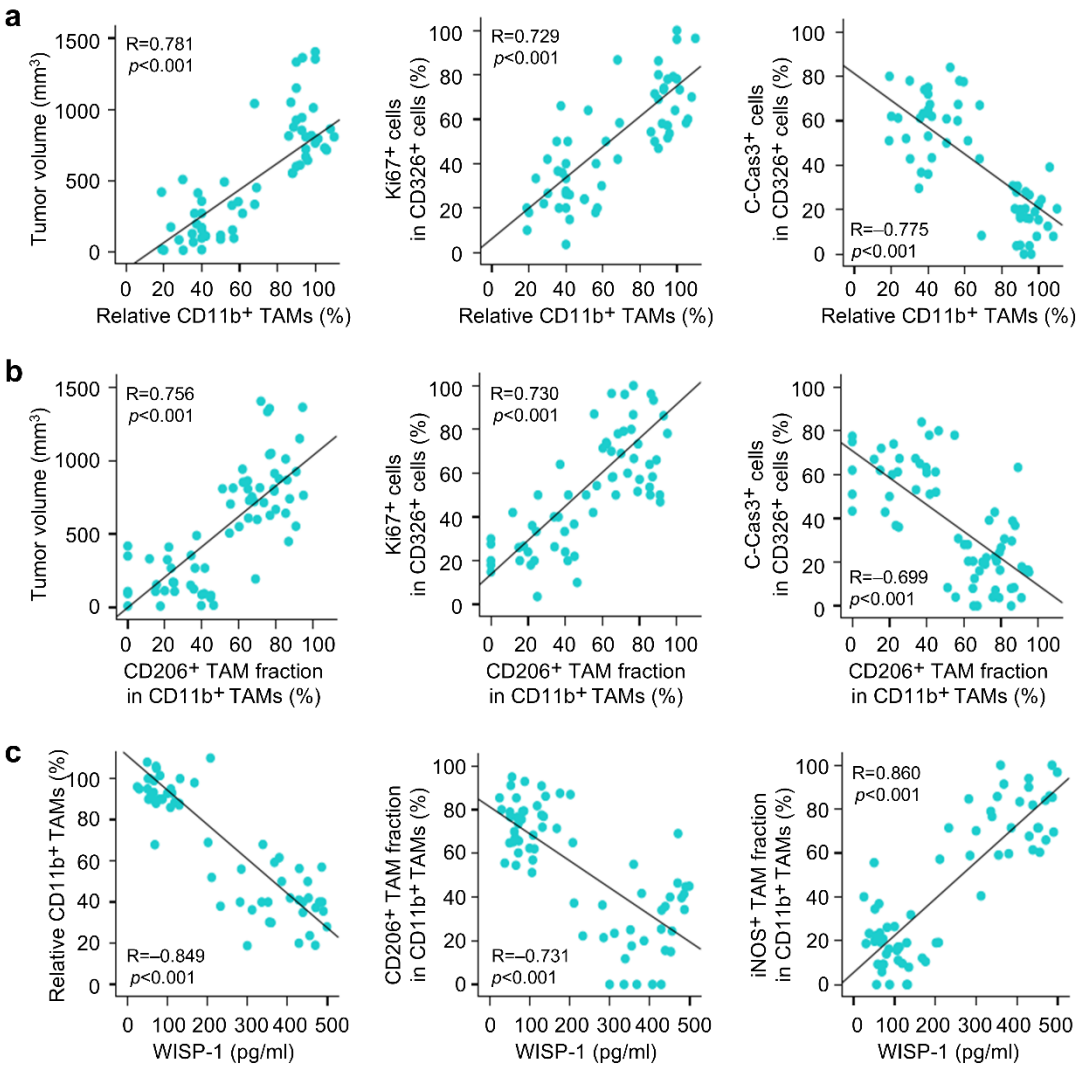
**Supplementary Fig. S16. WISP-1 mediates the pro-apoptotic effect of ApoSQ-CAF CM in M2 TAMs *in vivo*.** The experimental design was described in Fig. 5a. (**a-c**) Immunofluorescent staining of the apoptotic marker cleaved caspase-3 (red), the M2 TAM Marker CD206 (green), the M1 marker CD16/32 (green), the pan-macrophage marker CD11b (red), and DAPI (blue) in primary tumor sections. Original magnification: ×40. Scale Bar, 100 μm. Quantification of cleaved caspase-3$^+$ cells among CD206$^+$, iNOS$^+$, or CD11b$^+$ TAMs (*right*). NS, not significant; $^{**}P < 0.01$, $^{***}P < 0.001$, Analysis of variance with Tukey's post hoc test. The data are from one experiment representative of three independent experiments with similar results (**a-c *left***). The data are represented as the means ± standard errors from three mice per group (**a-c *right***).

**Supplementary Figure 17**




**Supplementary Fig. S17. WISP-1 mediates increased TUNEL positive apoptosis in M2 TAMs by ApoSQ-CAF CM *in vivo*.** The experimental design was described in Fig. 5a. (**a, b**) *Left:* Immunofluorescent staining of the TUNEL (red) the M2 TAM Marker CD206 (green), the M1 marker CD16/32$^{+}$ (green), and DAPI (blue) in primary tumor sections. Original magnification: ×40. Scale Bar = 100 μm. *Right:* Quantification of TUNEL$^{+}$ cells within CD206$^{+}$ (**a**) or CD16/32$^{+}$ TAMs (**b**). NS, not significant; ***$P$ < 0.01, Analysis of variance with Tukey's post hoc test. The data are from one experiment representative of three independent experiments with similar results (**a** and **b** ***left***). The data are represented as the means ± standard errors from three mice per group (**a** and **b** ***right***).

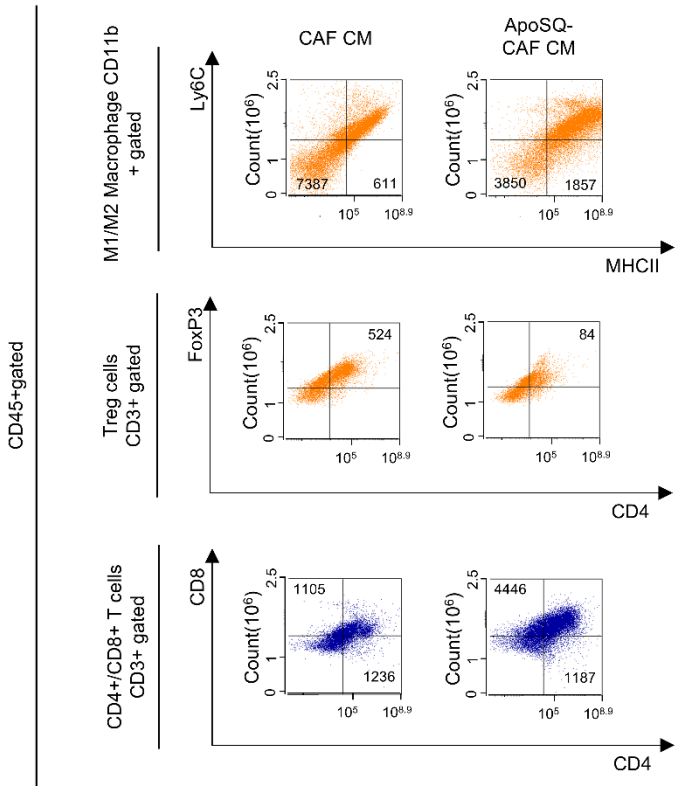
**Supplementary Figure 18**




**Supplementary Fig. S18.** Pearson's correlation analyses of TAM subsets, tumor cell proliferation and apoptosis, and WISP-1 levels in CM. (**a**) Correlation between $CD11b^+$ TAM density in primary tumor tissue and tumor volume, Ki-$67^+$ or cleaved caspase$3^+$ cells among $CD326^+$ tumor cells. (**b**) Correlation between the proportion of $CD206^+$ M2 TAMs ($CD206^+/CD11b^+$) and tumor volume, Ki-$67^+$ or cleaved caspase-$3^+$ cells among $CD326^+$ tumor cells. (**c**) Correlations between WISP-1 levels in the CM evaluated by ELISA and $CD11b^+$ TAM density, the proportion of $CD206^+$ M2 TAMs, or $iNOS^+$ M1 TAMs ($iNOS^+$/ $CD11b^+$). $p<0.001$.

**Supplementary Figure 19**

**a**



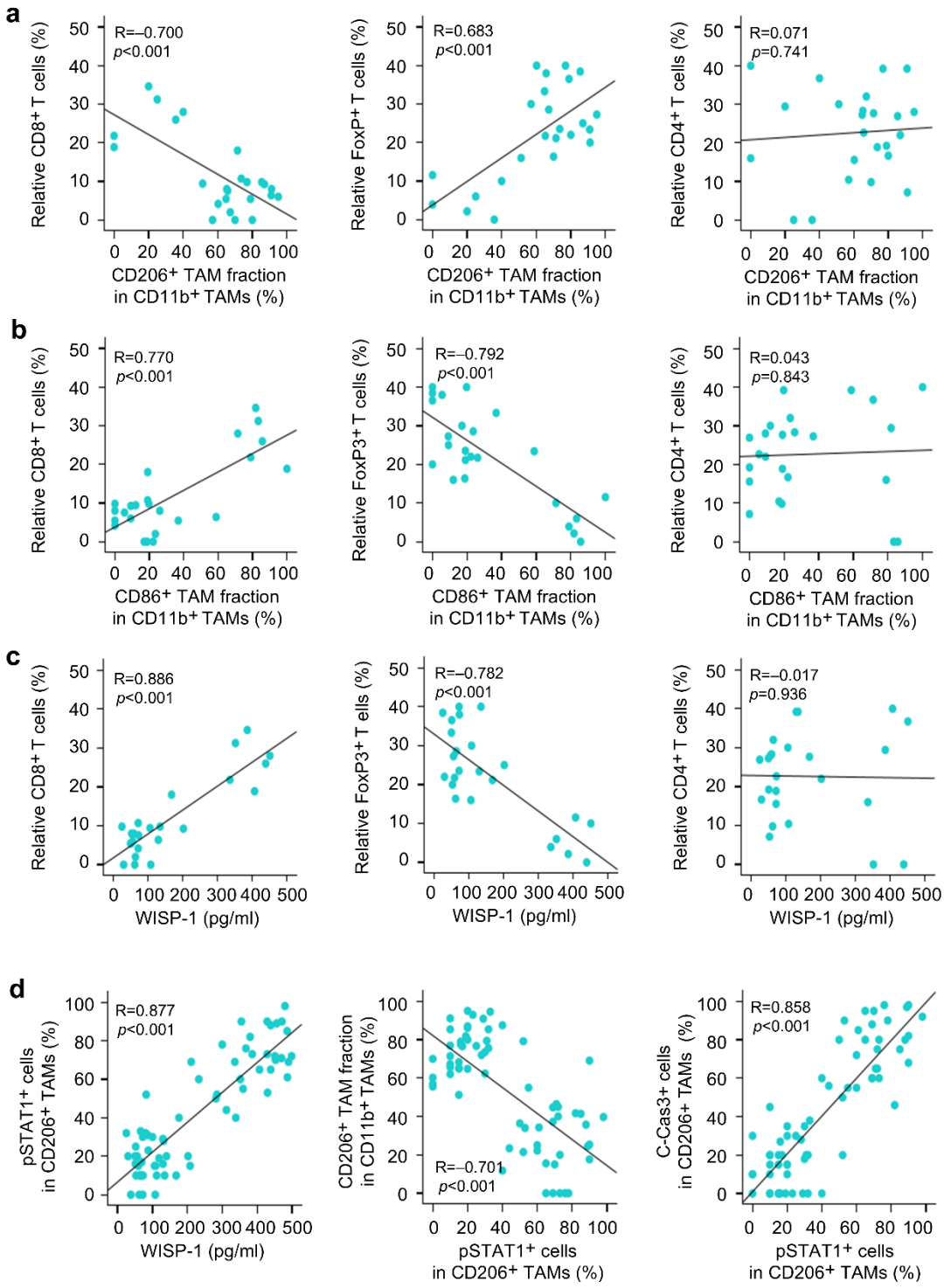

**b**

| Immune population | Gating strategy |
|---|---|
| M2 TAMs | $CD45^+$ $CD11b^+$ $MHCII^-$ $Ly6C^-$ |
| Treg cells | $CD45^+$ $CD3^+$ $FoxP3^+$ $CD4^+$ |
| M1 TAMs | $CD45^+$ $CD11b^+$ $MHCII^+$ $Ly6C^-$ |
| $CD8^+$ T cells | $CD45^+$ $CD3^+$ $CD8^+$ $CD4^-$ |
| $CD4^+$ T cells | $CD45^+$ $CD3^+$ $CD4^+$ $CD8^-$ |

**Supplementary Fig. S19. A representative histogram and gating strategy of immune cell analysis.** (a) Representative histograms illustrating the gating strategy used to identify immune cell populations, including M1 and M2 macrophages, Tregs, $CD8^+$ T cells, and $CD4^+$ T cells, analyzed by flow cytometry. Histograms shown are representative of three independent experiments. Starting two days after subcutaneous injection of 344SQ cells into syngeneic (129/Sv) mice, intratumoral injections of CAF CM or ApoSQ-CAF CM were administered three times per week for six weeks (n = 6 mice per group). Mice were necropsied at the end of the treatment period. (b) Gating strategy for the immune cell population within primary tumors. Single-

cell suspensions were first gated on $CD45^+$ leukocytes, and specific immune cell subsets were subsequently identified based on marker expression.

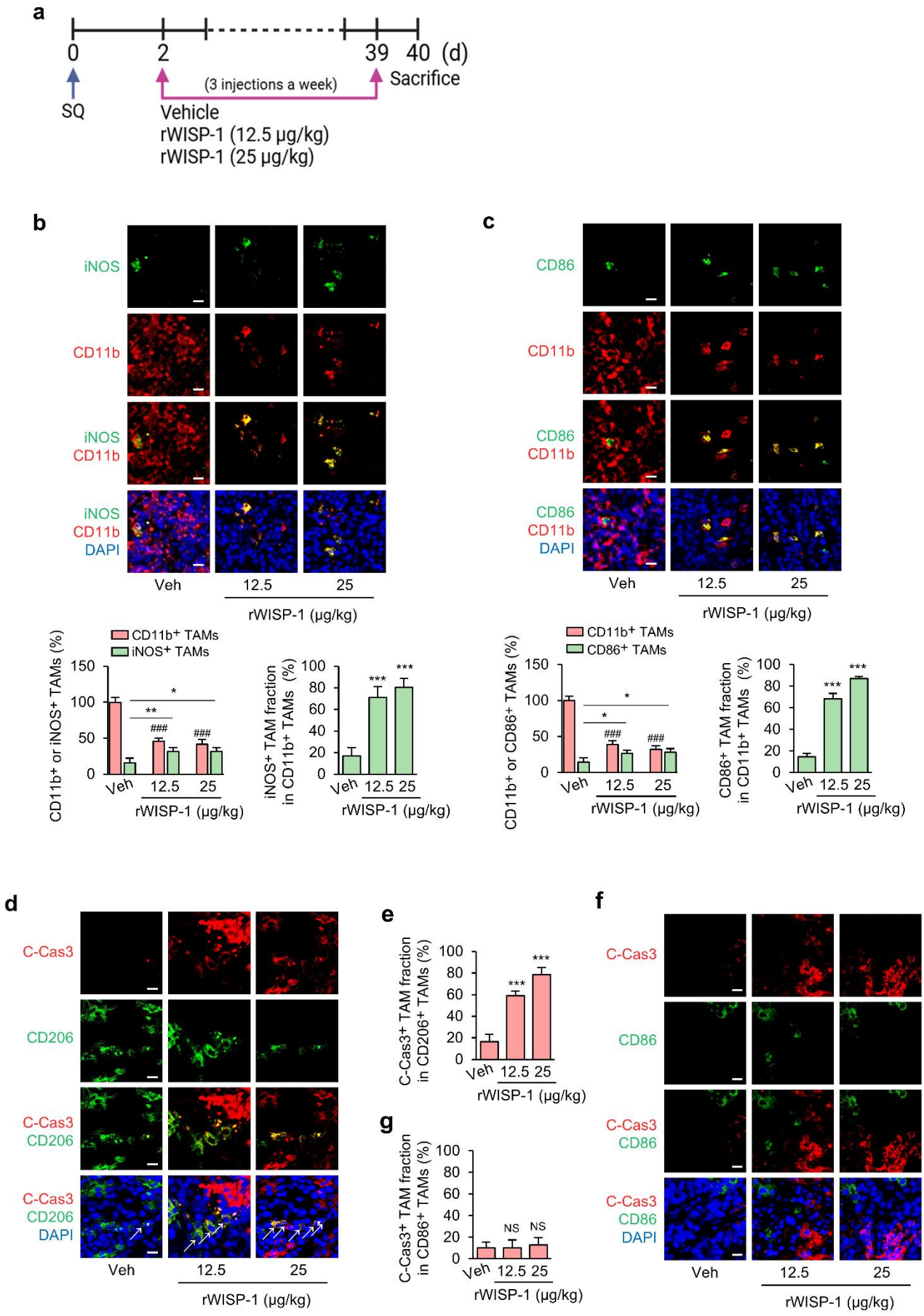
**Supplementary Figure 20**




**Supplementary Fig. S20. Pearson's correlation analyses of TAM subsets, T cell populations in primary tumor sections, and WISP-1 levels in CM.** (**a**) Correlation between the proportion of CD206$^+$ M2 TAMs and the density of CD8$^+$, FoxP3$^+$, or CD4$^+$ T cells in primary tumor tissue. (**b**) Correlation between the proportion of CD86$^+$ M1 TAMs and the density of CD8$^+$, FoxP3$^+$, or CD4$^+$ T cells. (**c**) Correlation between WISP-1 levels in the CM (measured by ELISA) and the density of CD8$^+$, FoxP3$^+$, or CD4$^+$ T cells. (**d**) Correlations between phosphorylated STAT1$^+$ M2 TAMs

(phospho-STAT1$^{+}$/CD206$^{+}$) and WISP-1 the levels in the CM, the proportion of CD206$^{+}$ M2 TAMs, or cleaved caspase-3$^{+}$ cells among CD206$^{+}$ M2 TAMs. $p$<0.001.

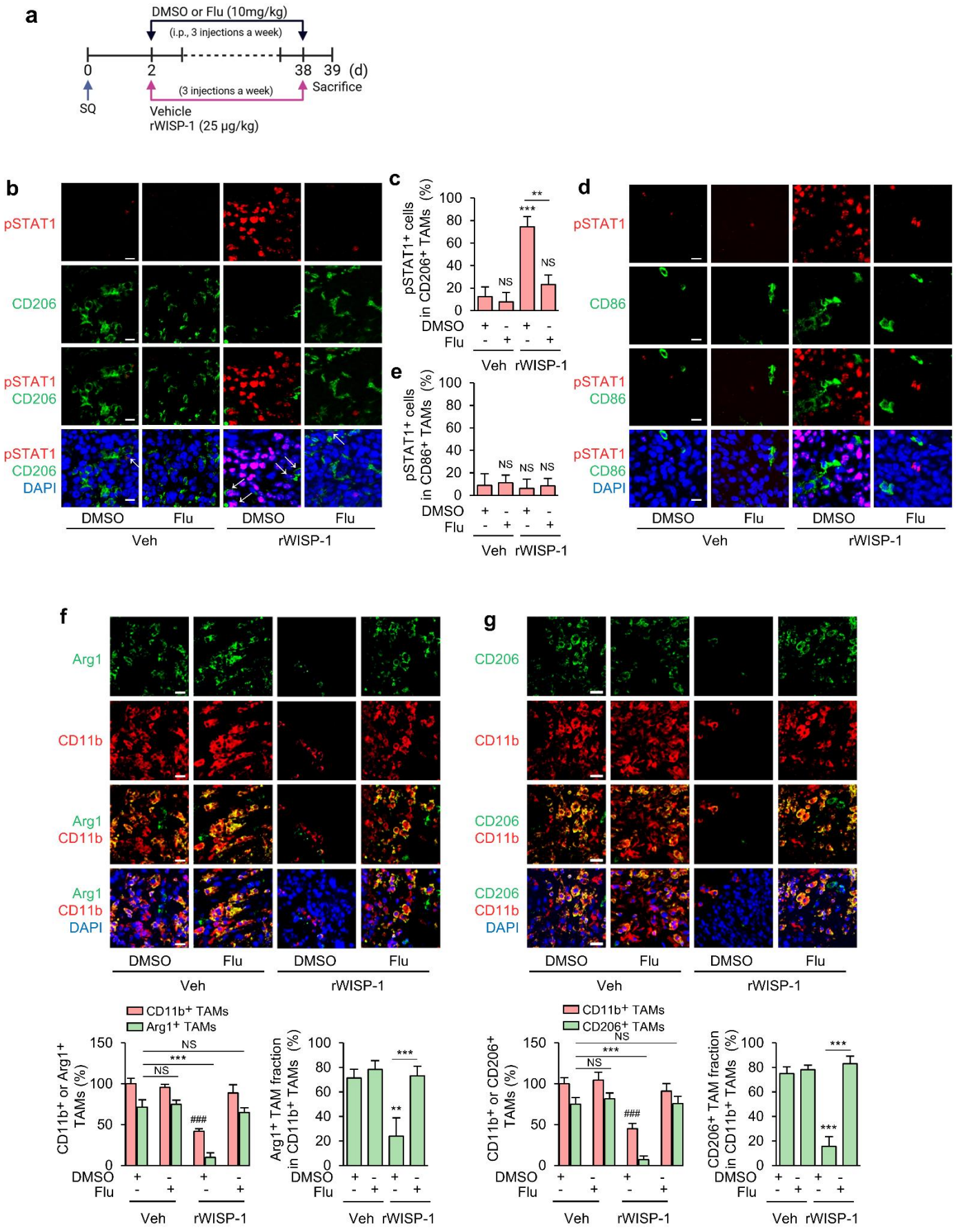
**Supplementary Figure 21**




**Supplementary Fig. S21. Administration of rWISP-1 reduces TAM density and M2 fraction, while promoting M2 apoptosis in primary tumors.** (**a**) Schematic of experimental design and

treatment groups. Where indicated, rWISP-1 (12.5 and 25 μg/kg) was administered intratumorally three times a week for 6 weeks starting 2 days after subcutaneous injection of 344SQ cells into syngeneic (129/Sv) mice (n = 6 mice per group). Mice were necropsied 6 weeks later. (**b**, **c**) *Upper*: Immunofluorescent staining of M2 TAM Markers Arg1 (green) and CD206 (green) and the pan-macrophage marker CD11b (red) in primary tumor sections. Original magnification: ×40. Scale bars = 100 μm. *Lower*: Quantitation of $iNOS^+$ and $CD86^+$ TAM (M1) density (*left*) and the fraction of M1 TAMs (*right*) in primary tumor. The M1 TAM fraction was determined by the percentage of M1 TAMs within $CD11b^+$ TAMs. (**d**, **f**) Immunofluorescent staining of the apoptotic marker cleaved caspase-3 (red), the M2 TAM Marker CD206 (green), the M1 marker CD86 (green), and DAPI (blue) in primary tumor sections. Original magnification: ×40. Scale Bar, 100 μm. (**e**, **g**) Quantification of cleaved caspase-$3^+$ cells in $CD206^+$ or $CD86^+$ TAMs. NS, not significant; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to vehicle or as indicated; $^{\#\#\#}P < 0.001$ compared to vehicle, Analysis of variance with Tukey's post hoc test. The data are from one experiment representative of three independent experiments with similar results (**b** and **c** ***upper***; **d**, **f**). The data are represented as the means ± standard errors from three mice per group (**b** and **c** ***lower***; **e**, **g**).

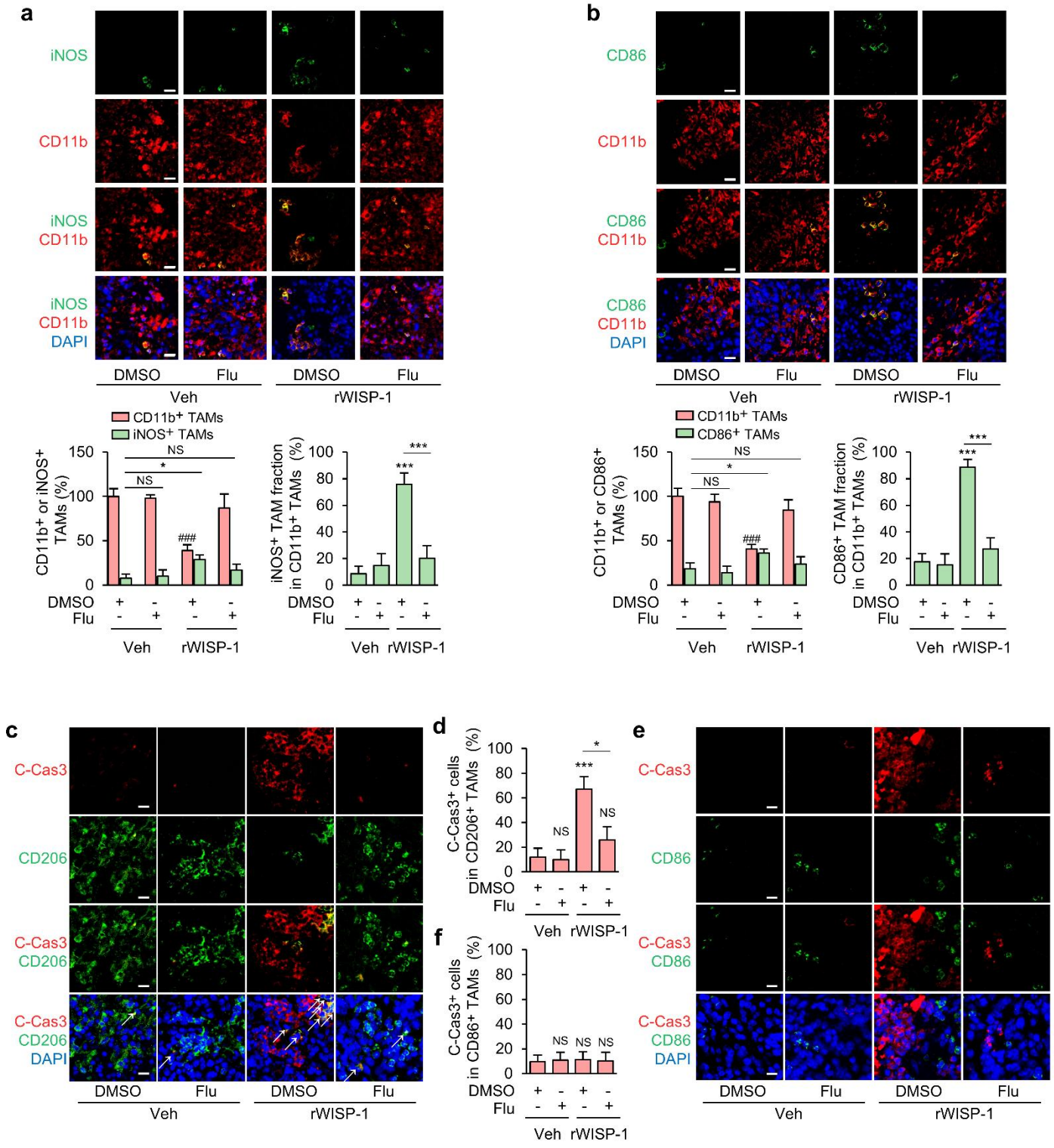
## Supplementary Figure 22




**Supplementary Fig. S22. WISP-1-STAT1 signaling reduces TAM density and lowers the M2 fraction in primary tumors.** (**a**) Schematic of experimental design and treatment groups. Where

indicated, fludarabine (10 mg/kg) or 5% DMSO was administrated intraperitoneally in conjunction with the intratumoral injection of rWISP-1 (25 μg/kg). rWISP-1 was intratumorally injected three times per week for 6 weeks, starting 2 days after the subcutaneous implantation of 344SQ cells into syngeneic (129/Sv) mice (n = 5 mice per group). Mice were necropsied at the end of the 6-week treatment period. (**b**, **d**) Representative confocal images of primary tumor sections stained with an anti-phosphorylated STAT1 (red), anti-CD206 antibody (green), anti-CD86 antibody (green), and DAPI (blue). Original magnification: ×40. Scale bars = 100 μm. (**c**, **e**) Quantification of phosphorylated STAT1$^{+}$ cells among CD206$^{+}$ cells or CD86$^{+}$ TAMs. (**f**, **g**) *Upper*: Immunofluorescent staining of primary tumor sections showing M2 TAM Markers Arg1 (green) and CD206 (green), along with the pan-macrophage marker CD11b (red). Original magnification: ×40. Scale bars = 100 μm. *Lower*: Quantitation of Arg1$^{+}$ and CD206$^{+}$ TAM (M2) density (*left*) and the fraction of M2 TAMs (*right*) in primary tumors. The fraction of M2 TAMs were determined by the percentage of M2 TAMs within CD11b$^{+}$ TAMs. NS, not significant; $^{**}P < 0.01$, $^{***}P < 0.001$ compared to DMSO or as indicated; $^{\#\#\#}P < 0.001$ compared to DMSO, Analysis of variance with Tukey's post hoc test. The data are from one experiment representative of three independent experiments with similar results (**b**, **d**; **f** and **g** ***upper***). The data are represented as the means ± standard errors from three mice per group (**c**, **e**; **f** and **g** ***lower***).

**Supplementary Figure 23**




**Supplementary Fig. S23. WISP-1-STAT1 signaling increases M1 TAM fraction and induces apoptosis of M2 TAMs in primary tumors.** Where indicated, fludarabine (10 mg/kg) or 5% DMSO was administrated intraperitoneally in conjunction with intratumoral injection of rWISP-1 (25 μg/kg). rWISP-1 was injected three times per week for 6 weeks, starting 2 days after the subcutaneous implantation of 344SQ cells into syngeneic (129/Sv) mice (n = 5 mice per group). Mice were necropsied at the end of the 6-week treatment period. (**a**, **b**) *Upper*: Immunofluorescent

staining of primary tumor sections showing M1 TAM Markers iNOS (green) and CD86 (green), along with the pan-macrophage marker CD11b (red). Original magnification: ×40. Scale bars = 100 μm. *Lower*: Quantitation of $iNOS^+$ and $CD86^+$ TAM (M1) density (*left*) and the fraction of M1 TAMs (*right*) in primary tumors. The M1 TAM fraction was determined by the percentage of M1 TAMs within $CD11b^+$ TAMs. (**c**, **e**) Immunofluorescent staining of the apoptotic marker cleaved caspase-3 (red), the M2 TAM Marker CD206 (green), the M1 marker CD86 (green), and DAPI (blue) in primary tumor sections. Original magnification: ×40. Scale Bar, 100 μm. (**d**, **f**) Quantification of cleaved caspase-$3^+$ cells among $CD206^+$ or $CD86^+$ TAMs (*right*). NS: not significant; $^*P < 0.05$, $^{***}P < 0.001$, compared to DMSO or as indicated; $^{\#\#\#}P < 0.001$ compared to DMSO, Analysis of variance with Tukey's post hoc test. The data are from one experiment representative of three independent experiments with similar results (**a** and **b** ***upper***; **c**, **e**). The data are represented as the means ± standard errors from three mice per group (**a** and **b** ***lower***; **d**, **f**).

**Supplementary Figure 24**

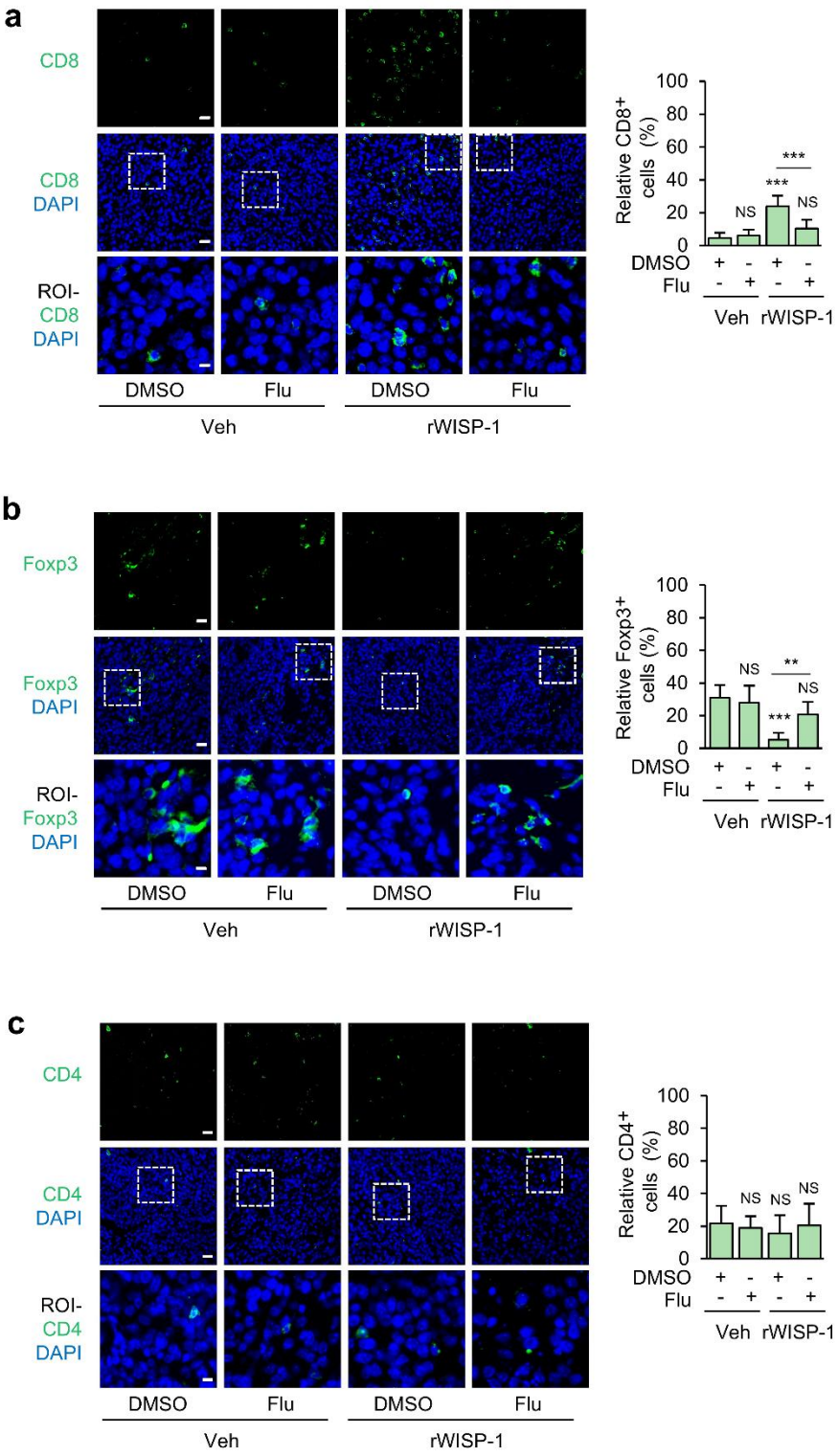



**Figure 24. WISP-1-STAT1 signaling rebalances T cell populations in primary tumors.** Where indicated, fludarabine (10 mg/kg) or 5% DMSO was administrated intraperitoneally in conjunction with the intratumoral injection of rWISP-1 (25 μg/kg). rWISP-1 was intratumorally injected three times per week for 6 weeks, starting 2 days after the subcutaneous implantation of 344SQ cells into syngeneic (129/Sv) mice (n = 5 mice per group). Mice were necropsied at the end of the 6-week treatment period. (**a-c**) *Left*: Immunofluorescent staining of CD8 (green), FoxP3 (green),

CD4 (green), and DAPI (blue) in primary tumor sections. Original magnification: ×40. Scale Bar, 100 μm. *Right*: Quantification of $CD8^+$, $Foxp3^+$, and $CD4^+$ T cell densities. NS, not significant; $^{**}P < 0.01$, $^{***}P < 0.001$ compared to DMSO or as indicated, Analysis of variance with Tukey's post hoc test. The data are from one experiment representative of three independent experiments with similar results (**a-c *left***). The data are represented as the means ± standard errors from three mice per group (**a-c *right***).

**Table S1. List of antibodies used for this study**

| Antigen | Vendor | Cat.No | Source | Species cross-reactivity | Application | Dilution |
|---|---|---|---|---|---|---|
| Arg1 | Novus | NB100-59740 | G | H, M, R | IHC | 1:100 |
| | Cell signaling | 93668 | Rb | H, M, R | IB | 1:1000 |
| Bax | Cell signaling | 2772 | Rb | H, M, R | IB | 1:1000 |
| Bcl-xL | Cell signaling | 2764 | Rb | H, M, R | IB | 1:1000 |
| Bcl-2 | Cell signaling | 3498 | Rb | H, M | IB | 1:1000 |
| C-Cas3 | Cell Signaling | 9661 | Rb | H, M, R, Mk | IB | 1:1000 |
| | | | | | IHC | 1:100 |
| CD11b | NB110-89474 | Novus | Rb | H, M, R, B, C, Pm, RM | IHC | 1:100 |
| | MA1-80091 | Invitrogen | R | H,M,R | IHC | 1:200 |
| CD16/32 | Abcam | ab223200 | Rb | M | IHC | 1:200 |
| | NBP1-27946 | Novus | R | M | IHC | 1:100 |
| CD163 | Abcam | ab182422 | Rb | H, M, R | IB | 1:1000 |
| | Abcam | ab64693 | Rb | H, M, R | IHC, ICC | 1:200 |
| CD206 | Invitrogen | MA5-16871 | R | M | IHC | 1:200 |
| | Cell signaling | 24595 | Rb | H, M, R | IB | 1:1000 |
| CD326 | BD | 552370 | R | M | IHC | 1:100 |
| CD86 | GeneTex | GTX-34569 | M | H, M, R | ICC | 1: 200 |
| C-PARP | Cell signaling | 5625 | Rb | H, M | IB | 1:1000 |
| F4/80 | Cell signaling | 70076 | Rb | M | IHC | 1:100 |
| | Abcam | ab16911 | R | H, M | IHC | 1:100 |
| IL12P40 | abcam | ab106270 | Rb | H, M, R | IB | 1:1000 |
| iNOS | Invitrogen | PA1-036 | Rb | H, M, R | IHC | 1:200 |
| | Invitrogen | PA1-036 | Rb | H, M, R | IB | 1:1000 |
| Integrin αv | Cell signaling | 60869 | Rb | H, M, R | IB | 1:1000 |
| | Invitrogen | 14-0512-85 | Rb | H, M | Neutralizing | 5μg/ml |
| Integrin α5 | Abcam | ab150361 | Rb | H, M, R | IHC | 1:200 |
| | Cell signaling | 98204 | Rb | H, M, R | IB | 1:1000 |
| | Santa cruz | sc-376199 | M | H, M, R | IP | 2μg/ml |
| | Invitrogen | PA5-79529 | Rb | H, M | Neutralizing | 5μg/ml |
| Integrin β3 | Invitrogen | 11-0611-82 | Hm | M, R | IHC | 1:100 |
| | Cell signaling | 13166 | Rb | H, M, R | IB | 1:1000 |
| | Santa cruz | sc-365679 | M | H, M, R | IP | 2μg/ml |
| | Invitrogen | MA1-35264 | M | H | Neutralizing | 5μg/ml |
| Integrin β5 | Cell signaling | 3629 | Rb | H, M, R | IB | 1:1000 |
| | Invitrogen | 14-0497-82 | M | H, M | Neutralizing | 5μg/ml |
| Mcl-1 | Cell signaling | 5453 | Rb | H, M | IB | 1:1000 |
| MHCII | Invitrogen | PA5-116876 | Rb | H, M, R | IB | 1:1000 |
| p-STAT-1 | Cell signaling | 7649 | Rb | H, M, R | IB | 1:1000 |
| | Santa cruz | sc-7988 | G | H, M | ICC | 1:200 |
| | | | | | IHC | 1:200 |
| p21 | Santa cruz | sc-6246 | M | H, M, R | IB | 1:1000 |
| | | | | | ICC | 1:200 |
| p53 | Cell signaling | 2524 | M | H, M, R, Hm, Mk | IB | 1:1000 |
| STAT-1 | Cell signaling | 9172 | Rb | H, M, R | IB | 1:1000 |
| WISP-1 | Abcam | ab260036 | Rb | M, R | IB, IP | 1:1000, 2μg/ml |
| β-actin | Santa cruz | sc-69879 | M | Broad species | IB | 1:1000 |
| Goat IgG (Alexa 488) | Thermo fisher scientific | A11055 | D | Not applicable | ICC | 1:1000 |
| IgG | R&D Systems | MAB005 | R | H, M | Neutralizing | 5μg/ml |
| Mouse IgG (Alexa 568) | Thermo fisher scientific | A11061 | Rb | Not applicable | ICC | 1:1000 |
| Mouse IgG (HRP) | GeneTex | GTX213111 | G | Not applicable | IB | 1:5000 |
| Rabbit IgG (Alexa 488) | Thermo fisher scientific | A11008 | G | Not applicable | ICC | 1:1000 |
| Rabbit IgG (HRP) | GeneTex | GTX213110 | G | Not applicable | IB | 1:5000 |

Abbreviation: H-Human, M-Mouse, R-Rat, Rb-Rabbit, G-Goat, B-Bovine, C- C. Elegans, Pm-Primate, RM-Rhesus Macaque, Mk-Monkey, D-Donkey, Hm-Hamster IB-Immunoblotting, IHC-Immunoprecipitation,IP-Immunoprecipitation, IF-Immunofluorescence

**Table S2. Sequences of qRT-PCR primer.**

| Target gene | Forward (5'→ 3') | Reverse (5'→ 3') |
|---|---|---|
| Human *Il4* | CCGTAACAGACATCTTTGCTGCC | GAGTGTCCTTCTCATGGTGGCT |
| Mouse *Il4* | ATCATCGGCATTTTGAACGAGGTC | ACCTTGGAAGCCCTACAGACGA |
| Mouse *Il4* | CGAGCTCACTCTCTGTGGTG | TGAACGAGGTCACAGGAGAA |
| Human *Tgfβ1* | TACCTGAACCCGTGTTGCTCTC | GTTGCTGAGGTATCGCCAGGAA |
| Mouse *Tgfβ1* | TGGAGCAACATGTGGAACTC | TGCCGTACAACTCCAGTGAC |
| Human *Il10* | TCTCCGAGATGCCTTCAGCAGA | TCAGACAAGGCTTGGCAACCCA |
| Mouse *Il10* | CGGGAAGACAATAACTGCACCC | CGGTTAGCAGTATGTTGTCCAGC |
| Mouse *Il10* | GCTCTTACTGACTGGCATGAG | CGCAGCTCTAGGAGCATGTG |
| Human *Il12p40* | GACATTCTGCGTTCAGGTCCAG | CATTTTTGCGGCAGATGACCGTG |
| Mouse *Il12p40* | TTGAACTGGCGTTGGAAGCACG | CCACCTGTGAGTTCTTCAAAGGC |
| Human *Nos2* | GCTCTACACCTCCAATGTGACC | CTGCCGAGATTTGAGCCTCATG |
| Mouse *Nos2* | GAGACAGGGAAGTCTGAAGCAC | CCAGCAGTAGTTGCTCCTCTTC |
| Mouse *Nos2* | GACATTACGACCCCTCCCAC | GCACATGCAAGGAAGGGAAC |
| Human *MhcII* | GAGCAAGATGCTGAGTGGAGTC | CTGTTGGCTGAAGTCCAGAGTG |
| Mouse *MhcII* | GTGTGCAGACACAACTACGAGG | CTGTCACTGAGCAGACCAGAGT |
| Mouse *Arg1* | GTGGGGAAAGCCAATGAAG | GCTTCCAACTGCCAGACTGT |
| Mouse *Cd206* | CTAACTGGGGTGCTGACGAG | GGCAGTTGAGGAGGTTCAGT |
| Mouse *Cd163* | GGCTAGACGAAGTCATCTGCAC | CTTCGTTGGTCAGCCTCAGAGA |
| Mouse *Tnfα* | CCCCAAAGGGATGAGAAGTT | CACTTGGTGGTTTGCTACGA |
| Mouse *Cd80* | CCTCAAGTTTCCATGTCCAAGGC | GAGGAGAGTTGTAACGGCAAGG |
| Mouse *Ifng* | CAGCAACAGCAAGGCGAAAAAGG | TTTCCGCTTCCTGAGGCTGGAT |
| *Hprt* | CAGACTGAAGAGCTACTGTAATG | CCAGTGTCAATTATATCTTCAAC |

## Table S3. List of siRNA

| Target gene | Sense | Antisense |
|---|---|---|
| Human *Stat1* | 5'-CUGGAUAUAUCAAGACUGA-3' | 5'-UCAGUCUUGAUAUAUCCAG-3' |
| Mouse *Stat1* | 5'-CACAGUUUUAUCCUGAG-3' | 5'-UCUCAGGAUAAAACUGUG-3' |
| *Mouse Wisp-1* | 5′-GGAAUCCUAACGAUAUCUU-3' | 5′- AAGAUAUCGUUAGGAUUCC-3′ |
| Human *integrin αv* | 5′-CCGAAACAAUGAAGCCUUA-3' | 5′-UAAGGCUUCAUUGUUUCGG-3' |
| Human *integrin α5* | 5'-CUGUUGAAGGUACAUCGUU-3' | 5'-AACGAUGUACCUUCAACAG-3' |
| Human *integrin β3* | 5′-UUACUGCCGUGACGAGAUU-3' | 5′-AAUCUCGUCACGGCAGUAA-3' |
| Human *integrin β5* | 5'-CAGCUAAGAACCUGGAACU-3' | 5'-AGUUCCAGGUUCUUAGCUG-3' |

## Table S4. List of antibodies employed for flow cytometry

| Antigen | Vendor | Cat.No | Source | Clone | Dilution |
|---|---|---|---|---|---|
| CD16 | BD Bioscience | 335035 | Mouse | NKP15(IVD) | 1:500 |
| CD206 | eBioscience | 12-2061-82 | Mouse | MR6F3 | 1:100 |
| CD163 | eBioscience | 12-1631-82 | Mouse | TNKUPJ | 1:100 |
| MHCII | eBioscience | 25-5321-82 | Mouse | M5/114.15.2 | 1:100 |
| CD45 | BD Bioscience | 553087 | Rat | RA3-6B2(RUO) | 1:250 |
| CD11b | BD Bioscience | 557397 | Rat | M1/70(RUO) | 1:250 |
| Ly6C | BD Bioscience | 552093 | Rat | RB6-8C5(RUO) | 1:250 |
| FoxP3 | BD Bioscience | 563902 | Rat | R16-715(RUO) | 1:250 |
| CD4 | BD Bioscience | 552775 | Rat | RM4-5(RUO) | 1:250 |
| CD3 | BD Bioscience | 555275 | Rat | 17A2(RUO) | 1:250 |
| CD8 | BD Bioscience | 561092 | Rat | 53-6.7(RUO) | 1:250 |

**Table S5. Macrophage and fibroblast populations in the single-cell transcriptomic dataset**

| Cell types (authors annotation) | Number of cells |
|---|---|
| Anti-inflammatory macrophages | 44,399 |
| Pro-inflammatory macrophages | 14,140 |
| $STAB1^{+}$ anti-inflammatory macrophages | 14,059 |
| Anti-inflammatory alveolar macrophages | 6,574 |
| Activated adventitial fibroblasts | 1,137 |
| Fibroblasts | 1,131 |