1 **Supplementary Materials for**

2 **G6PD activation in TNBC cells induces macrophage recruitment and M2**
3 **polarization to promote tumor progression**

4 *Cellular and Molecular Life Sciences*

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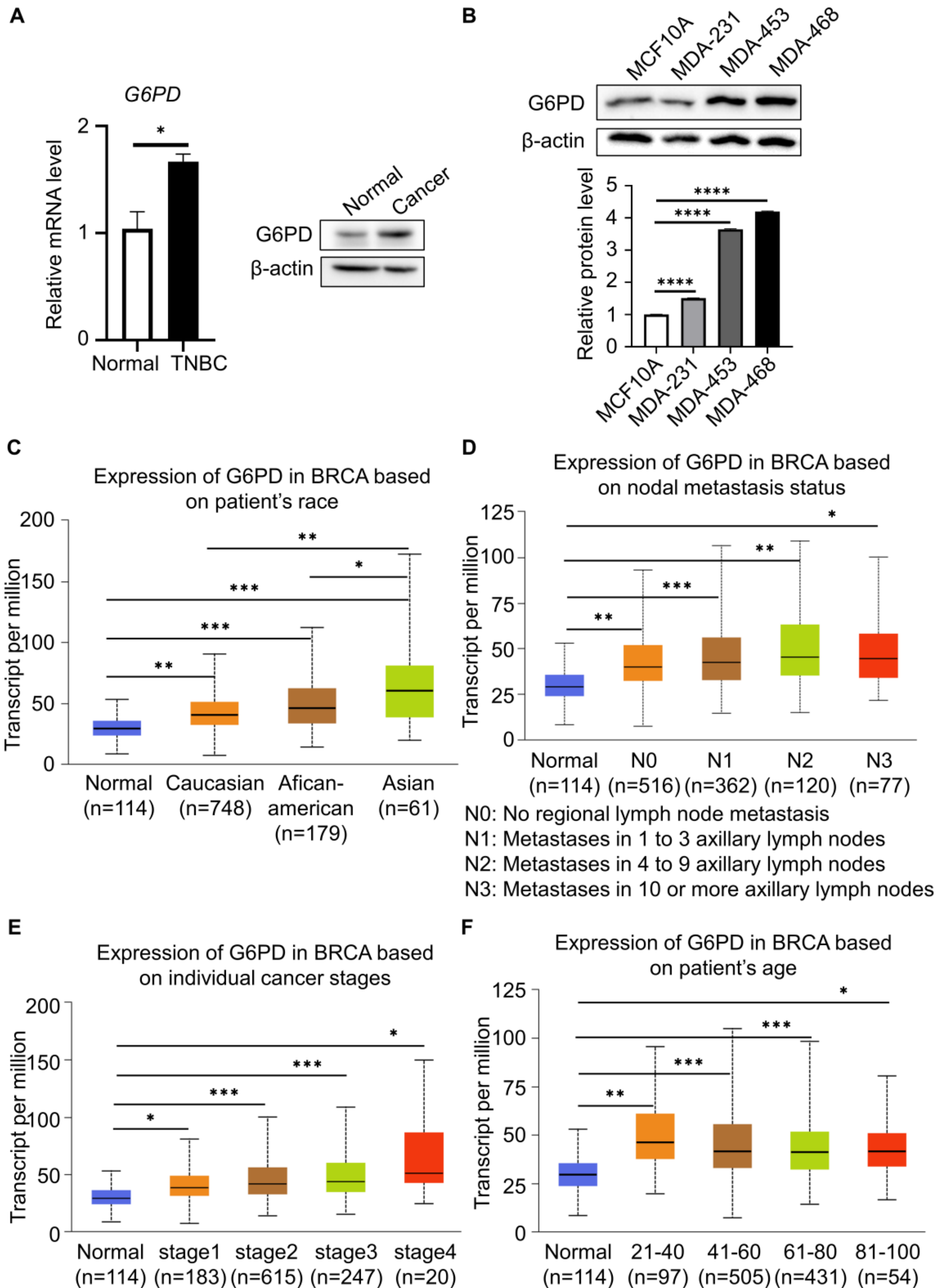
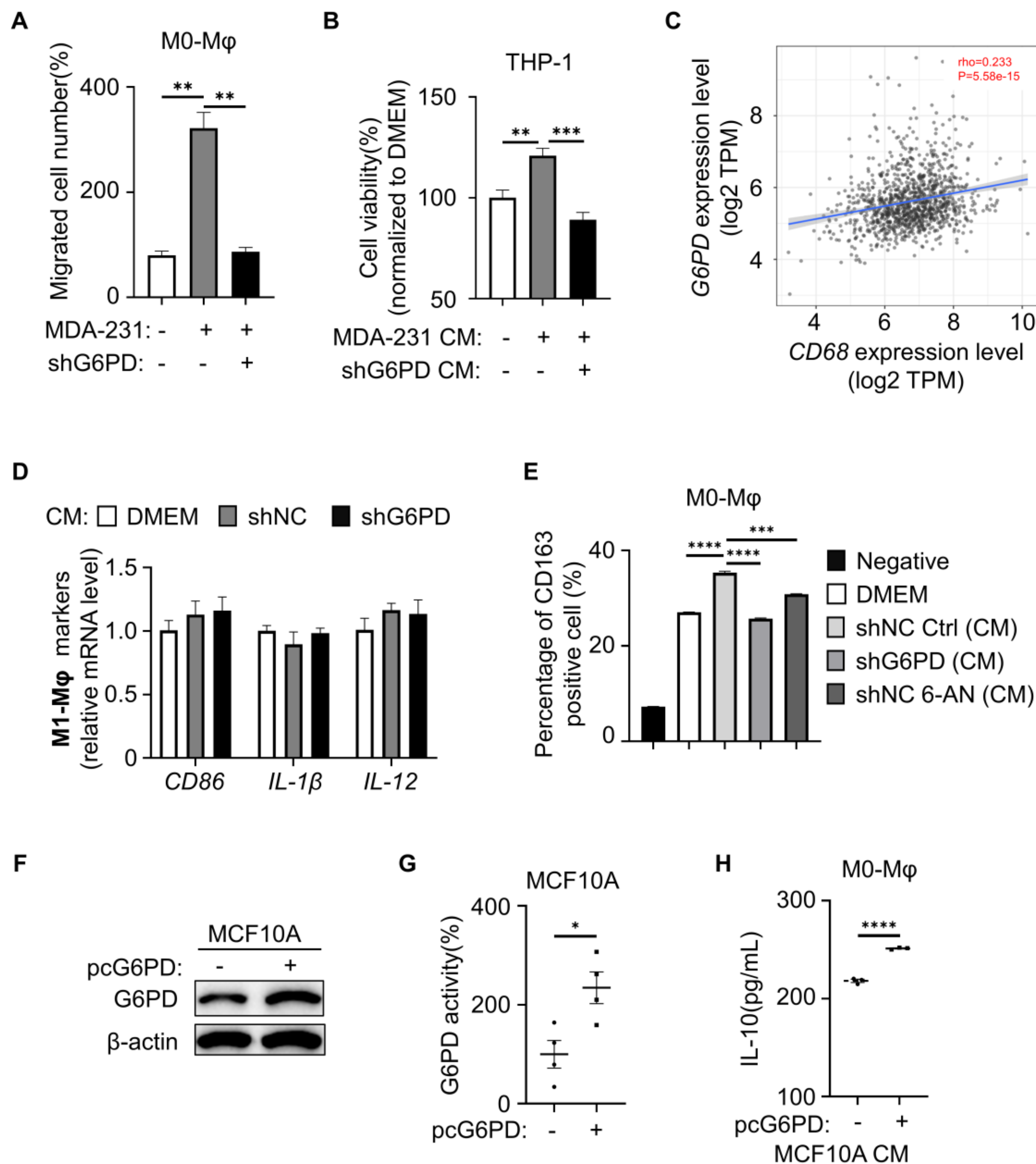
Supplementary 1



29 **Supplementary 1**
30 **(A)** G6PD expression was validated in TNBC patients by q-PCR and Western blot,
31 respectively. **(B)** Immunoblot analyses of G6PD in TNBC compared to MCF10A cells.
32 Densitometry was performed to analyze protein expression, and β-actin served as an
33 internal reference. **(C-F)** The TCGA dataset was used to analyze the association of G6PD
34 expression with clinical parameters from breast cancer patients. Patient's race (C), N stage

35 (D), cancer stages (E) and age (F). There were no significant differences in cancer of
36 different N stages, cancer stages and patient age. The P value between them was greater
37 than 0.5. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.
38

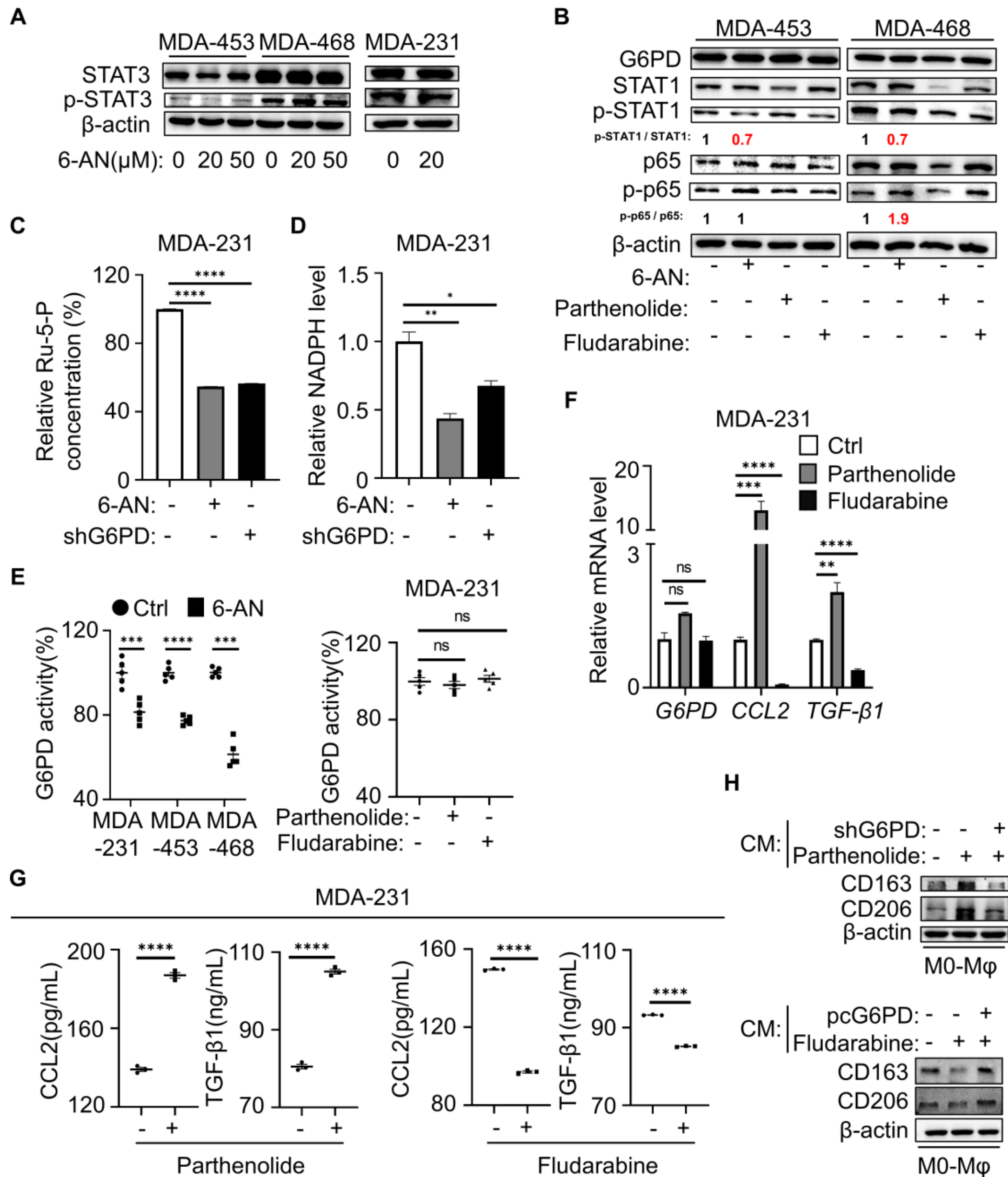
Supplementary 2



39 **Supplementary 2**
40 **(A)** The average numbers of migrated M0-Mφ cells were quantified. **(B)** CCK8 assays are
41 shown for THP-1 cells cultured in complete medium with or without CM from control or
42 G6PD knockdown MDA-231 cells. **(C)** Statistical analysis revealed that the expression
43 intensity of G6PD was positively correlated with CD68. **(D)** Levels of the M1 markers were
44 compared by q-PCR after M0-Mφ coculture with the specified MDA-231-CM for 6 h. **(E)**
45 The percentage of CD163 positive was quantified in macrophages. **(F and G)** G6PD
46 protein expression (F) and enzyme activity (G) in G6PD-overexpressing MCF10A cells for
47 24 h were increased. **(H)** IL-10 contents in the CM of macrophages cells as determined by

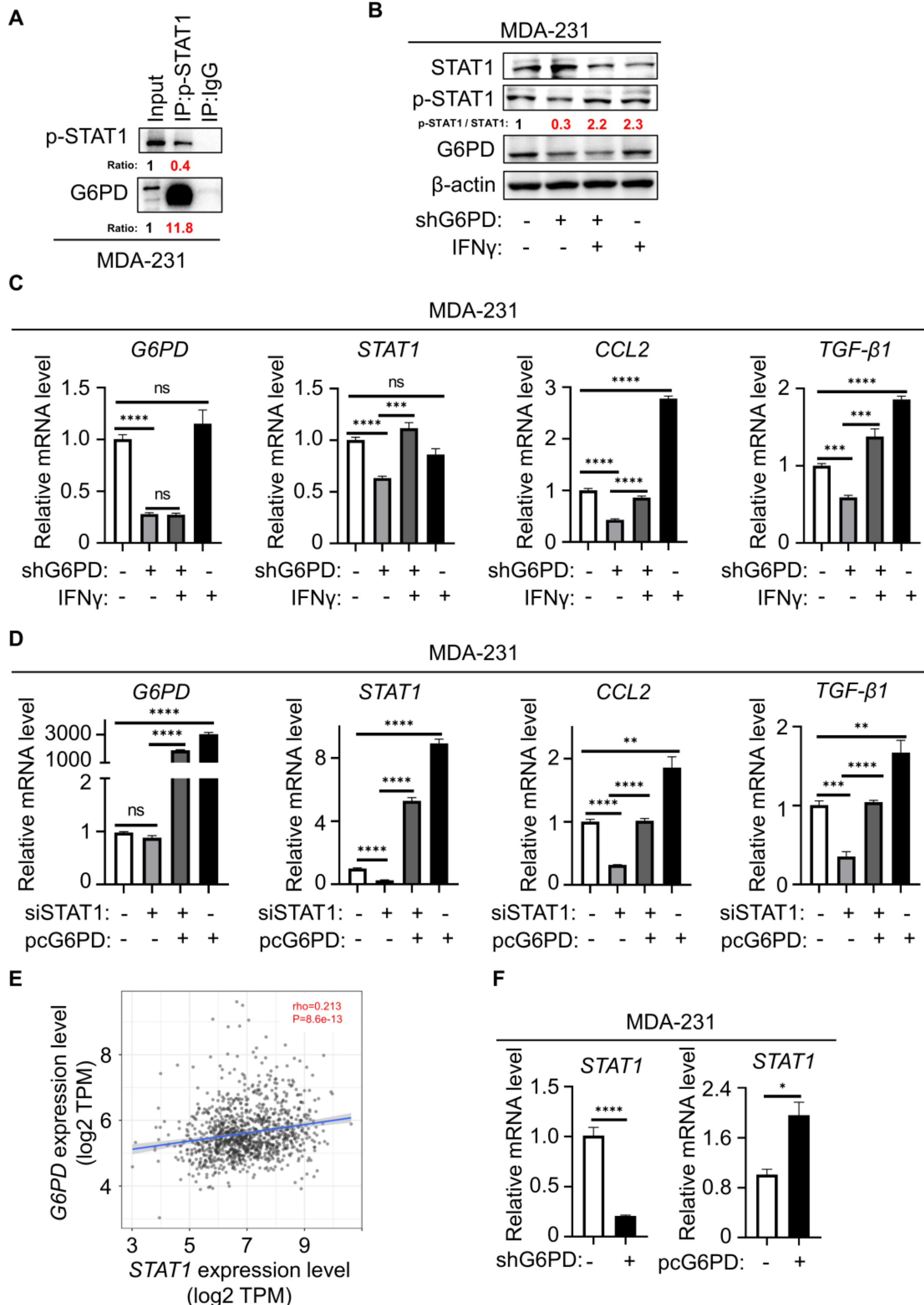
48 ELISA. CM was added to M0-Mφ for 6 h and replaced with serum-free culture for 6 h. The
49 supernatants were recovered and analyzed for IL-10 secretion. *$P < 0.05$, **$P < 0.01$, ***P
50 < 0.001, ****$P < 0.0001$.
51

Supplementary 3



52 **Supplementary 3**
53 **(A)** TNBC cells were treated with 6-AN. The levels of STAT3 and phospho-STAT3 were
54 assessed by immunoblotting. **(B)** MDA-453 and MDA-468 cells were treated with 6-AN,
55 parthenolide or fludarabine. The levels of proteins were assessed by immunoblotting. **(C**
56 **and D)** MDA-231 cells with 6-AN or G6PD stable knockdown were tested for intracellular
57 level of Ru-5-P (C) and NADPH level (D). **(E)** TNBC cells were treated with 6-AN,
58 parthenolide or fludarabine. G6PD activity was studied using a G6PD enzyme activity

59 detection kit. **(F)** MDA-231 cells were treated with parthenolide or fludarabine for 24 h. The
60 mRNA levels of *G6PD*, *CCL2* and *TGF-β1* were detected by q-PCR. **(G)** CCL2 and TGF-
61 β1 contents in the CM of MDA-231 cells as determined by ELISA. Both cell lines were
62 treated with parthenolide or fludarabine. **(H)** Immunoblot analysis of protein expression
63 after M0-Mφ coculture with the specified MDA-231-CM for 6 h. (B-H) Cells were treated
64 with 6-AN (20 μM for MDA-231, 50 μM for MDA-453 and MDA-468), parthenolide (10 μM)
65 or fludarabine (1 μM). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.
66



67 **Supplementary 4**

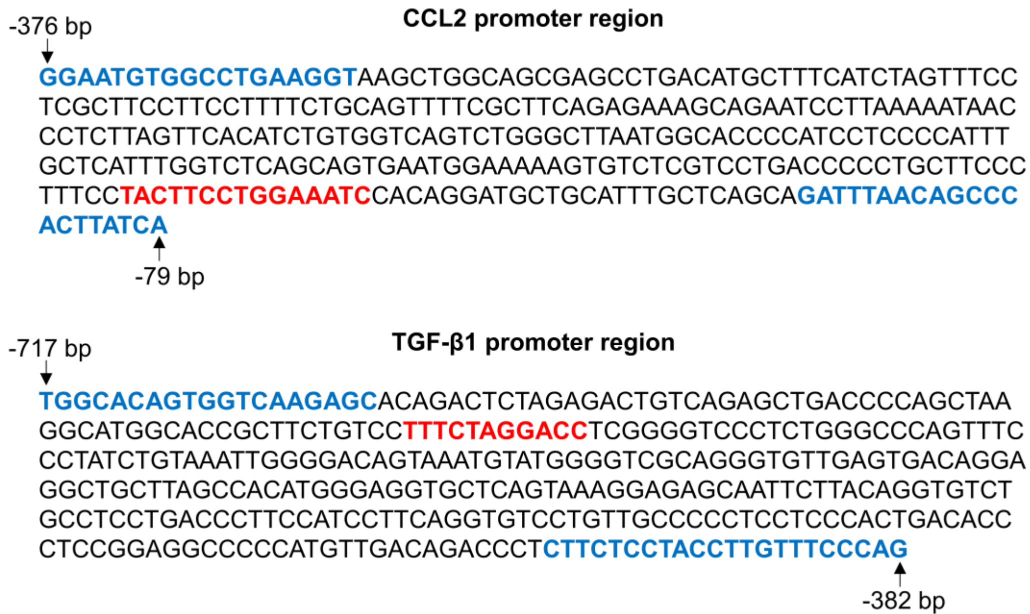
68 **(A)** MDA-231 cells were harvested and subjected to immunoprecipitation with anti-
69 phospho-STAT1, followed by western blot analysis with the indicated antibodies. **(B)**
70 Protein expression in MDA-231 cells were examined using Western blot. **(C and D)** The
71 mRNA levels of *G6PD*, *STAT1*, *CCL2* and *TGF-β1* in MDA-231 cells were examined using
72 q-PCR. **(E)** Statistical analysis revealed that the expression intensity of G6PD was

73 positively correlated with STAT1. **(F)** q-PCR analysis revealed that the expression of G6PD
74 was positively correlated with STAT1. (B and C) Cells were treated with IFNγ (50 ng/mL).
75 *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
76

Supplementary 5

A

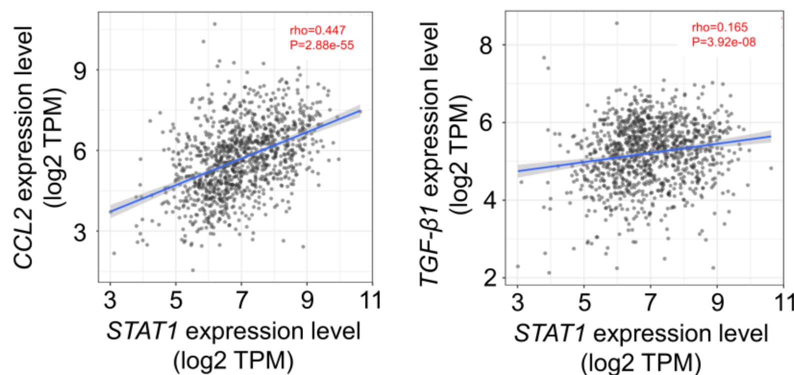
ChIP PCR of CCL2 and TGF-β1 promoter region designed for this study.



Primers for ChIP PCR, containing STAT1 motif

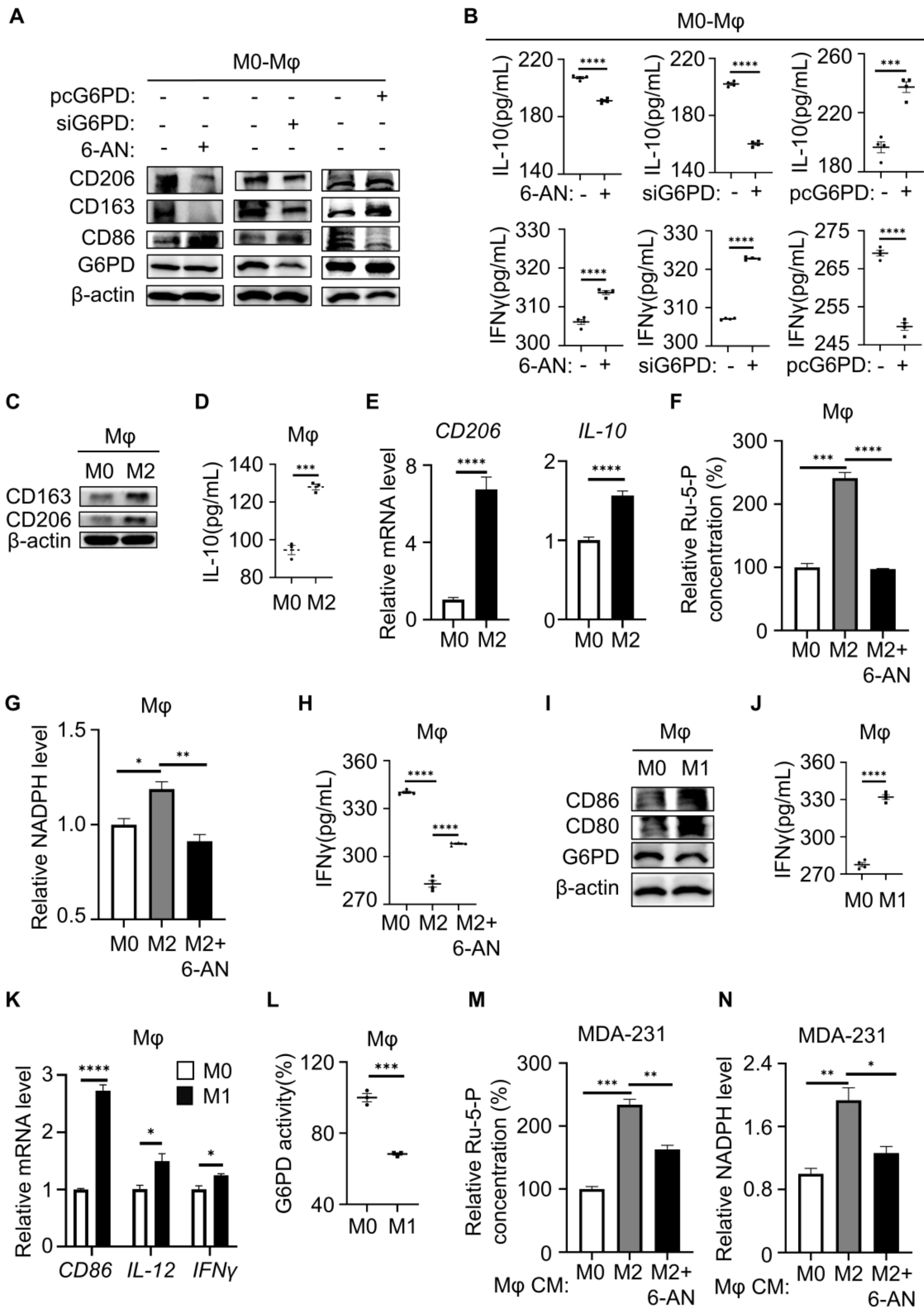
STAT1 predicted binding motif

B



77 **Supplementary 5**
78 **(A)** ChIP PCR of CCL2 or TGF-β1 promoter region designed for this study. The full length
79 of construct contained potential STAT1 predicted binding motif. **(B)** Statistical analysis
80 revealed that the expression intensity of STAT1 was positively correlated with CCL2 or
81 TGF-β1.
82

Supplementary 6



83 **Supplementary 6**

84 **(A)** Altered proteins in M0-Mφ treated with 6-AN/siG6PD/pcG6PD were measured by
85 western blotting. **(B)** ELISA detection of IFNγ after different treatments in macrophages.
86 **(C)** The M2-type markers CD163 and CD206 were determined using western blotting. **(D)**
87 The M2-type marker IL-10 was determined using ELISA. **(E)** The M2-type markers *IL-10*

88 and *CD206* were determined using q-PCR. **(F and G)** Intracellular level of Ru-5-P (F) and
89 NADPH level (G) were detected in macrophages. **(H)** ELISA detection of IFNγ after
90 different treatments in macrophages. **(I)** The M1-type markers CD86, CD80 and G6PD
91 were determined using western blotting. **(J)** The M1-type marker IFNγ was determined
92 using ELISA. **(K)** The M1-type markers *CD86*, *IL-12* and *IFNγ* were determined using q-
93 PCR. **(L)** G6PD enzyme activity was detected in macrophages. **(M and N)** MDA-231 cells
94 coincubated with CM from macrophages were tested for intracellular level of Ru-5-P (M)
95 and NADPH level (N). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
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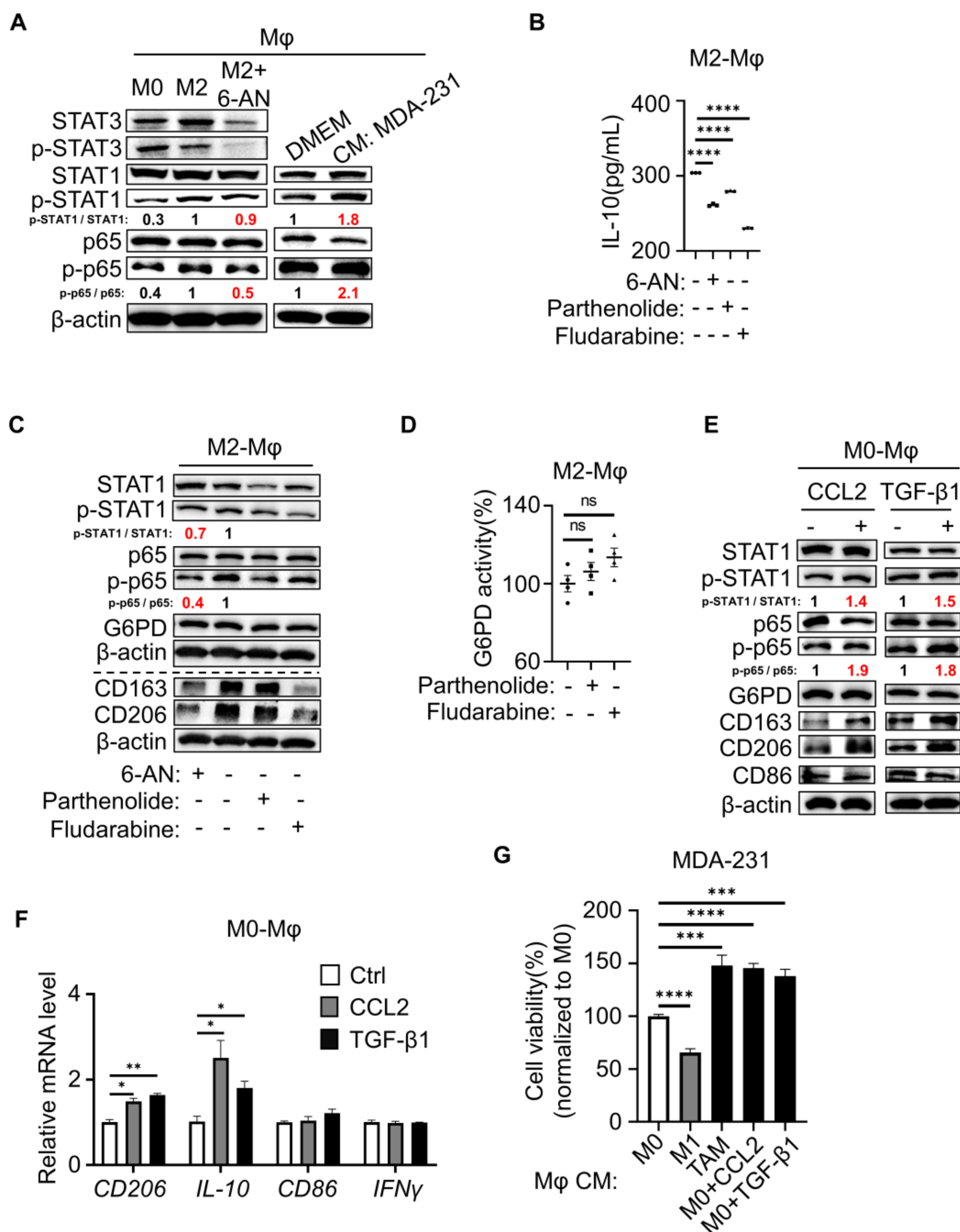
Supplementary 7



97 **Supplementary 7**
98 **(A)** Protein expression in macrophages was examined using immunoblotting. **(B)** ELISA

99 analysis of IL-10 after treating M2-Mφ with 50 μM 6-AN, 10 μM parthenolide and 1 μM
100 fludarabine for 24 h. **(C)** M2-Mφ were treated with 6-AN (20 μM), parthenolide (10 μM) or
101 fludarabine (1 μM). The levels of proteins were assessed by immunoblotting. **(D)** G6PD
102 enzyme activity was detected in macrophages. **(E)** Protein level in macrophages following
103 the addition of 30 ng/mL CCL2 or TGF-β1 as determined by immunoblotting. **(F)** The
104 macrophages markers *CD206*, *IL-10*, *CD86* and *IFNγ* were determined using q-PCR. **(G)**
105 The cell viability of MDA-231 cells cocultured with CM from macrophages detected by MTT
106 assay. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.