

1 Supplemental Tables

2 Supplemental Table 1. Description of clinical trials used in the study

ID	Age	Sex	FAB	Genetic abnormality	Cytogenetics	Treatment	Prognosis
M0-1	9	M	M0	MLL rearrangement	11q23	chemotherapy	NR,death
M2-1	11	F	M2	NPM1 mutation	CN-AML	chemotherapy+transplant	CR
M2-2	6	M	M2	FLT3/ITD	CN-AML	chemotherapy	
M2-3	5	M	M2		t(8;21)	chemotherapy	Death after relapse
M2-4	5	M	M2	CEBPA double mutation	CN-AML	chemotherapy	CR
M2-5	12	M	M2	CEBPA double mutation	CN-AML	chemotherapy	CR
M2a-1	13	F	M2	CEBPA double mutation	CN-AML	chemotherapy	CR
M2a-2	3	F	M2	AML1-ETO+	CN-AML	chemotherapy	death
M2a-3	8	M	M2	AML1-ETO+	CN-AML	chemotherapy	NR,death
M2b-1	11	M	M2	AML1-ETO+	t(8;21)	chemotherapy	CR
M2b-2	8	M	M2	AML1-ETO+	t(8;21)	chemotherapy	Death after relapse
M2b-3	9	M	M2	AML1-ETO+	t(8;21)	chemotherapy	CR
M2b-4	12	F	M2	AML1-ETO+; C-KIT mutation	t(8;21)	chemotherapy	CR
M2b-5	13	F	M2	AML1-ETO+	t(8;21)	chemotherapy	Death
M2b-6	2	M	M2	AML1-ETO+	t(8;21)	chemotherapy	CR
M2b-7	8	F	M2	AML1-ETO+	t(8;21)	chemotherapy	Death after relapse
M2b-8	7	F	M2	AML1-ETO+	t(8;21)	chemotherapy+dasatinib	CR
M4-1	1	M	M4	NPM1、GATA2、KIT	CN-AML	chemotherapy	CR
M4-2	11	F	M4		CN-AML	chemotherapy	Death after relapse
M4b-1	11	F	M4	CBFβ-MYH11+	inv(16)	chemotherapy+transplant	Transplant complications died
M4b-2	8	M	M4	KRAS、FLT3	CN-AML	chemotherapy	
M4eo-1	5	F	M4	CBFB/MYH11+、KIT、NPM1	inv(16)	chemotherapy	
M4eo-2	10	F	M4	CBFB/MYH11+	inv(16)	chemotherapy+dasatinib	CR
M4eo-3	9	M	M4	CBFB/MYH11+	inv(16)	chemotherapy	NR
M4eo-4	7	M	M4	CBFB/MYH11+	inv(16)	chemotherapy	CR
M4eo-5	9	M	M4	CBFB/MYH11+	inv(16)	chemotherapy	CR
M5-1	10	F	M5	KRAS	CN-AML	chemotherapy	CR
M5-2	3	M	M5	RUNX1	CN-AML	chemotherapy	CR
M5-3	1	M	M5	MLL-AF9 +	11q23	chemotherapy	CR
M5-4	5	F	M5	FLT3-ITD	CN-AML	chemotherapy	CR
M5-5	10	M	M5		CN-AML	chemotherapy+transplant	CR
M5-6	5	F	M5	MLL-AF9+; FLT3	11q23	chemotherapy	CR
M5-7	8	M	M5		complex	chemotherapy	Death after relapse
M5-8	12	F	M5		CN-AML	chemotherapy	Death after relapse
M5-9	11	F	M5	KRAS	CN-AML	chemotherapy	NR
M5-10	13	F	M5	FLT3-ITD	CN-AML	chemotherapy	NR
M5-11	6	M	M5	MLL rearrangement	11q23	chemotherapy	PR, death
M5-12	12	M	M5		CN-AML	chemotherapy	NR, death
M5-13	8	M	M5		CN-AML	chemotherapy	NR, death
M5-14	1	F	M5	MLL rearrangement	11q23	chemotherapy	death

M6-1	3	M	M6		complex	chemotherapy	death
M7-1	2	M	M7			chemotherapy	death

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27 **Supplemental Table 2. The antibody panel used in CyTOF**

Marker Name	Manufacturer	Cat. No.	Conjugate Metal Tag
CD41	Fluidigm	3089004B	89Y
CD45	Biolegend	304045	141Pr
CD47	Biolegend	323102	142Nd
CD117	Biolegend	313223	143Nd
CD38	Biolegend	303535	144Sm
CD163	Biolegend	326502	145Nd
CD64	Biolegend	305029	146Nd
CD11c	Biolegend	301639	147Sm
CD16	Biolegend	302051	148Nd
CD34	Biolegend	343531	149Sm
CD43	Biolegend	343202	150Nd
CD123	Biolegend	306027	151Eu
CD13	Biolegend	301717	152Sm
CD303	Biolegend	354215	153Eu
CD3	Biolegend	300443	154Sm
CD24	Biolegend	311127	155Gd
CD36	Biolegend	366215	156Gd
CD44	Biolegend	338811	158Gd
Tim3	Biolegend	345002	159Tb
CD235a	Biolegend	349102	160Gd
CD244	Biolegend	329502	161Dy
CD18	Biolegend	366302	162Dy
CD35	Biolegend	333402	163Dy
CD19	Biolegend	302247	165Ho
CD7	Biolegend	343111	166Er
CD11b	Biolegend	301337	167Er
CD55	Biolegend	311302	168Er
CD33	Biolegend	303419	169Tm
CD45RA	Biolegend	304143	170Er
CD96	Biolegend	338402	171Yb

CD15	Biolegend	323035	172Yb
HLA-DR	Biolegend	307651	173Yb
CD4	Biolegend	300541	174Yb
CD14	Biolegend	301843	175Lu
CD56	Biolegend	318345	176Yb
CD61	Fluidigm	3209001B	209Bi

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49 **Supplemental Table 3. The antibody used in FCM、WB and IF.**

Antibody	Manufacturer	Cat. No.
Anti-CD7 antibody	Abcam	ab109296
Anti-MRP4 antibody	Abcam	ab313704
Anti-P glycoprotein antibody	Abcam	ab168337
Anti-Bcl-2 antibody	Abcam	ab32124
Anti-XIAP antibody	Abcam	ab229050
Anti- p85 alpha antibody	Abcam	ab191606
Akt (pan) Rabbit mAb	Cell Signaling Technology	4691T
Phospho-Akt (Ser473) Rabbit mAb	Cell Signaling Technology	4060T
NF-κB p65 Rabbit mAb	Cell Signaling Technology	8242S
Phospho-NF-kappaB p65 (Ser536) Rabbit mAb	Cell Signaling Technology	3033S
IKK beta Rabbit Monoclonal Antibody	Cell Signaling Technology	8943T
Src (L4A1) Mouse Monoclonal Antibody	Cell Signaling Technology	2110T
Active Caspase-3 Rabbit mAb	ABclonal	A11021
Caspase-3 Rabbit mAb	ABclonal	A19654
p38 MAPK Rabbit mAb	ABclonal	A4771
Phospho-p38 MAPK Rabbit mAb	ABclonal	AP1311
IκBα Rabbit mAb	ABclonal	A19714
Src Rabbit mAb	ABclonal	A19119
Phospho-IκBα-S32 Rabbit mAb	ABclonal	AP0707
Rabbit IgG isotype control	ABclonal	AC042
Anti-ENT1 antibody	SANTA CRUZ	sc-377283
SECTM1 Mouse Monoclonal Antibody	Proteintech	60281-1
GAPDH Mouse Monoclonal Antibody (HRP Conjugated)	Beyotime	AF2823
α-Tubulin Mouse Monoclonal Antibody (HRP Conjugated)	Beyotime	AF2831
HRP-conjugated Goat Anti-Rabbit IgG	BBI	D110058
HRP-conjugated Goat Anti-Mouse IgG	BBI	D110087
APC-anti-CD11b antibody	Biolegend	301310

Alexa Fluor 568 goat anti-rabbit IgG	Abcam	ab175471
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Supplemental Table 4. Antigen markers of different myeloid cell differentiation stages¹⁻⁴.

Cell type	Characteristics
CD34 ⁺ AML LSC-containing progenitor cells	lin ⁻ CD34 ⁺ CD38 ^{-/low} CD33 ^{low}
AML blast cells	CD34 ⁺ CD33 ⁺ CD14 ⁻ CD117 ⁺
promonocyte	CD34 ^{+/+} CD33 ⁺ CD13 ⁺ CD14 ⁻ CD64 ⁺ CD15 ^{low} CD35 ⁺ CD36 ^{mid}
monocyte	CD34 ⁻ CD33 ⁺ CD13 ⁺ CD14 ⁺ CD64 ⁺ HLA-DR ⁺ CD35 ⁻ CD11b ⁺ CD15 ^{low}
promyelocyte	CD33 ⁺ CD13 ⁺ CD117 ⁺ CD15 ⁺ CD11b ⁻ CD16 ⁻
myelocyte	CD34 ⁻ CD33 ^{low} CD13 ^{low} CD15 ⁺ CD11b ⁺ CD16 ⁻
neutrophil	CD13 ⁺ CD33 ⁻ CD15 ⁺ CD11b ⁺ CD16 ⁺ CD35 ⁺
erythroid	CD235a ⁺ CD36 ⁺
megakaryocytic	CD41 ⁺ CD61 ⁺
CD34 ⁻ cells	CD34 ⁻ CD33 ⁻ CD16 ⁻ CD14 ⁻ CD235 ⁻ CD38 ⁻ CD35 ⁻ CD11b ⁻ CD117 ⁻ CD64 ⁻

A threshold for expression level:

Negative (-): Raw signal values (0–10) were normalized to 0–1.

Positive (+): Raw signal values (>10) were normalized to >1.

Low: Normalized values were 1–2.

Mid: Normalized values were 2–5.

High: Normalized values were more than 5.

Supplemental Figure

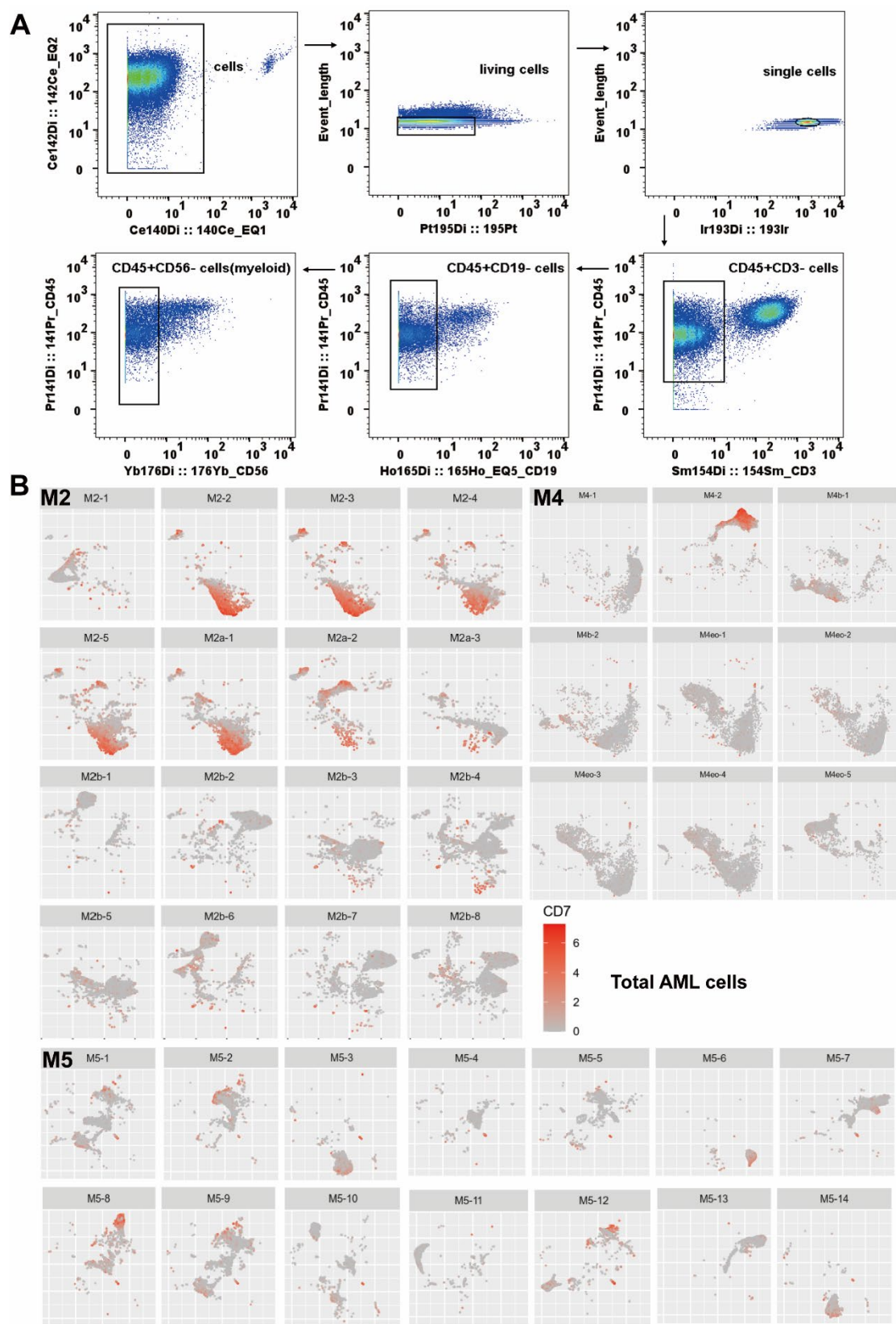
Supplemental Figure 1 (A) Gating myeloid cells in clinical samples using FlowJo. (B) Mass spectrometry single cell CD7 antigen characterization. (C) CD7⁺ AML cell antigen atlas. (D) Differentiation landscape of each positive sample. (E-F) UMAP analysis of CD7⁺ AML cells. (E) Single-cell mass cytometry–based characterization of CD7 expression in AML cells. (F) A UMAP plot of CD7⁺ AML cells. (G-I) Analysis of CD7 expression across FAB classification (G), different molecular mutation (H), cytogenetic (I) derived from 41 pediatric AML samples in protein level. Positive (+): Raw signal values (>10) were normalized to >1.

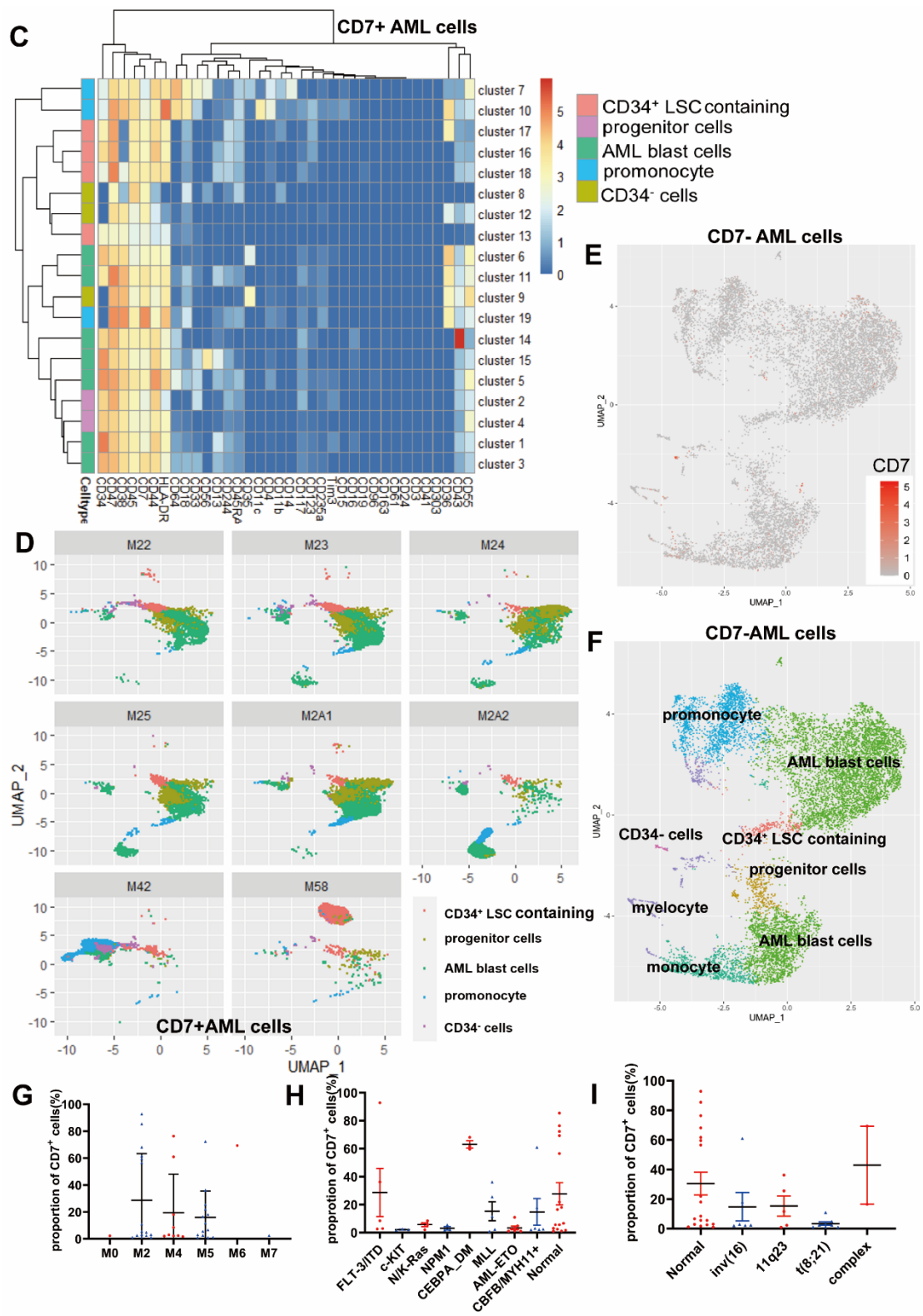
Supplemental Figure 2 (A) Expression of CD7 in Acute Myeloid Leukemia Cell Lines based on GSE59808 data. (B-C) The CD7 protein in the stable cell lines was detected by Western blot. CD7⁺ cell line and knock down cell lines (B), CD7⁺ cell lines and exogenous expression of CD7 cell lines (C). (D-M) Representative histogram blots showing the distribution of cells in different phases of the cell cycle.

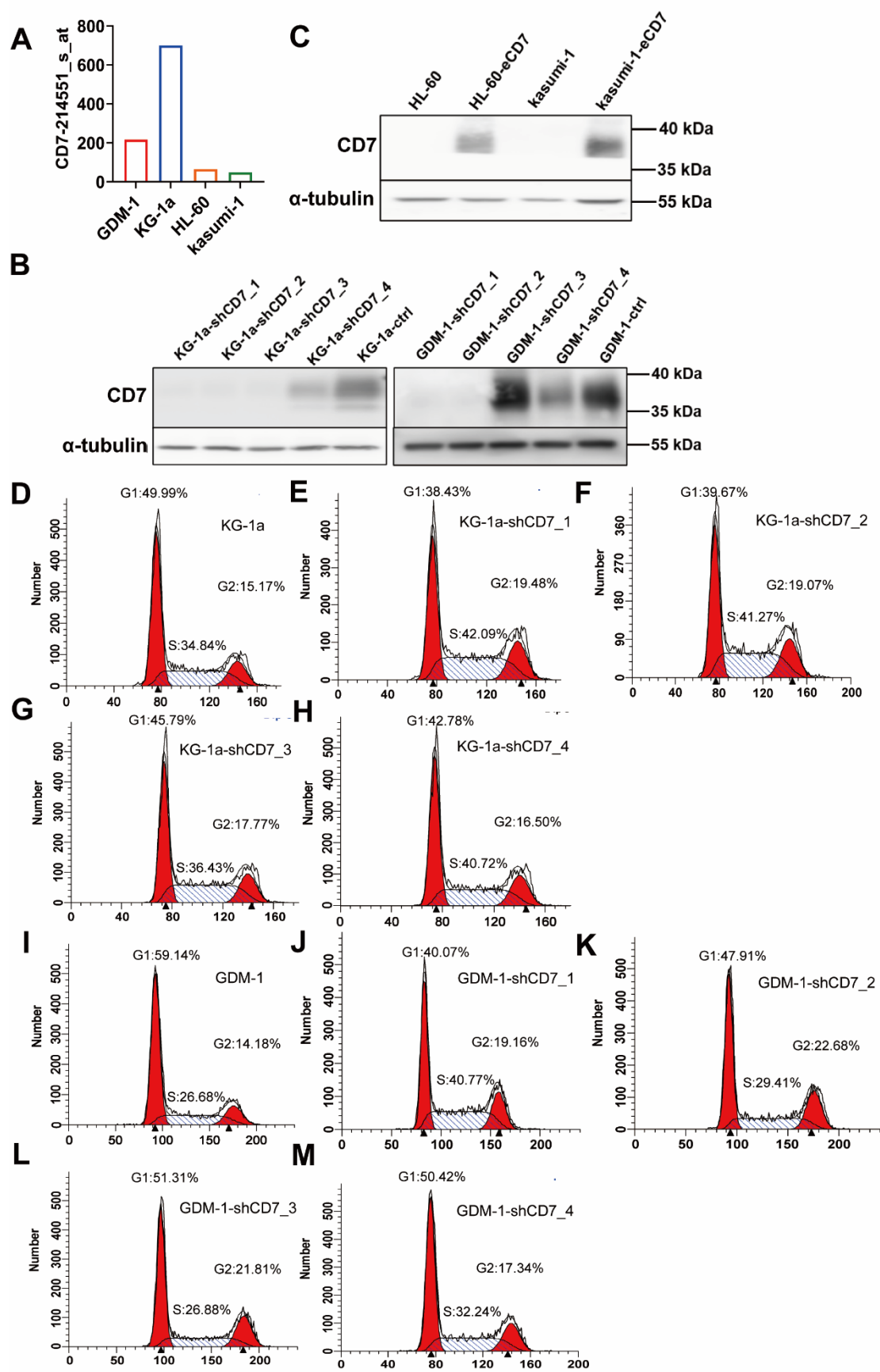
Supplemental Figure 3 (A-B) After treatment with different concentrations of the standard chemotherapeutic drug for 48 hours, cell apoptosis was detected by flow cytometry. Detection of apoptosis after treatment with cytarabine in KG-1a and its CD7 knockdown cell line (A); (B) Apoptosis detection of KG-1a and its CD7 knockdown cell line after daunorubicin treatment (B). (C) Key signaling pathway proteins (NF- κ B, PIK3/AKT and p38), involved in regulating PGP-1 expression, were detected by western blot. (D) After KG-1a cells were treated with JSH-23, the p65 subunit was injected into the nucleus by laser confocal imaging, scale bar=20 μ m. (E) Western blot analysis of XIAP in 6 AML patient samples, including 3 CD7-positive and 3 CD7-negative cases. (F) Detection of BCL-2 after 2 μ M Ara-C or 200 nM DNR treatment of cells. (G) Analysis of XIAP expression following CA-IKK β

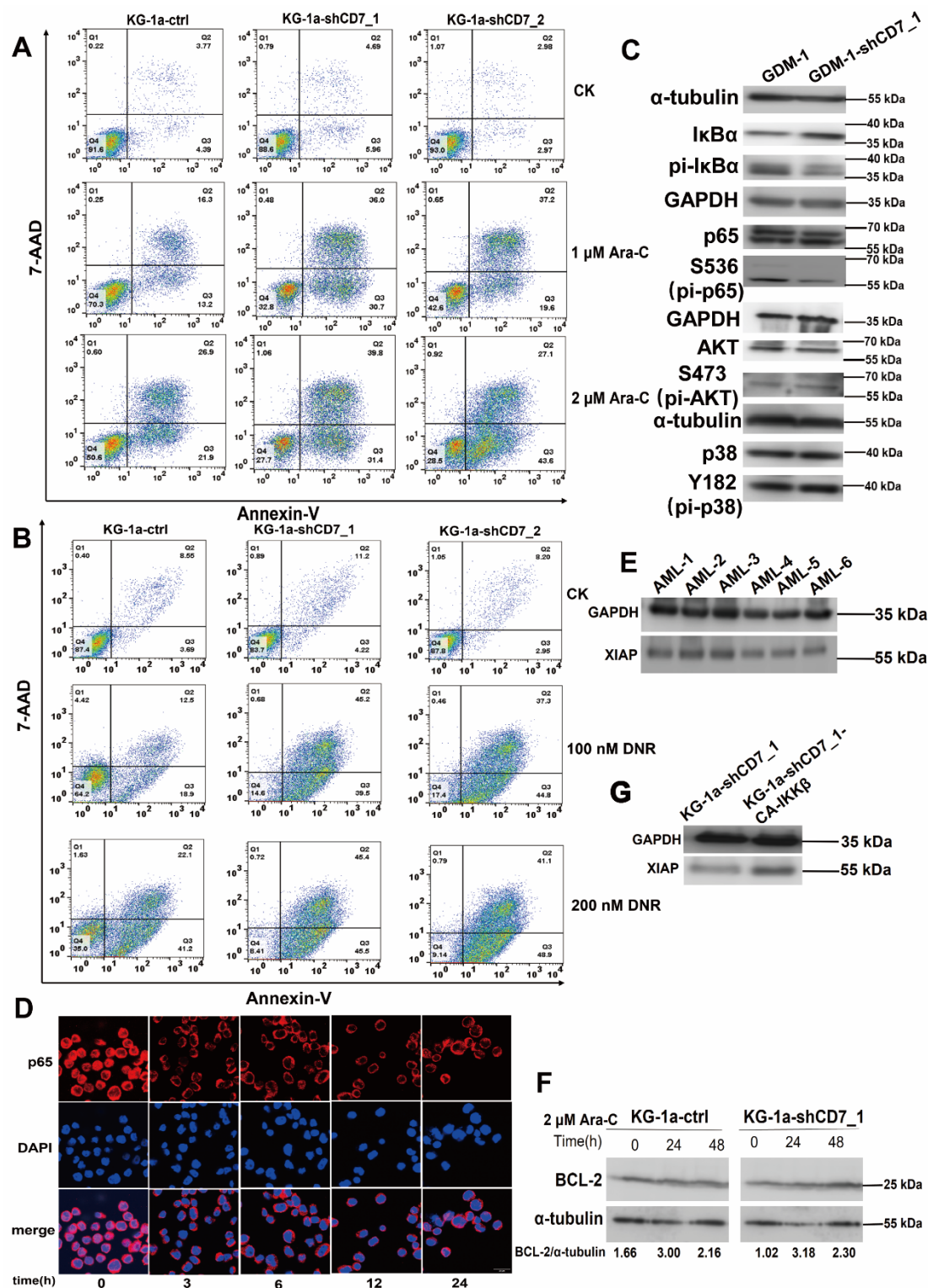
overexpression.

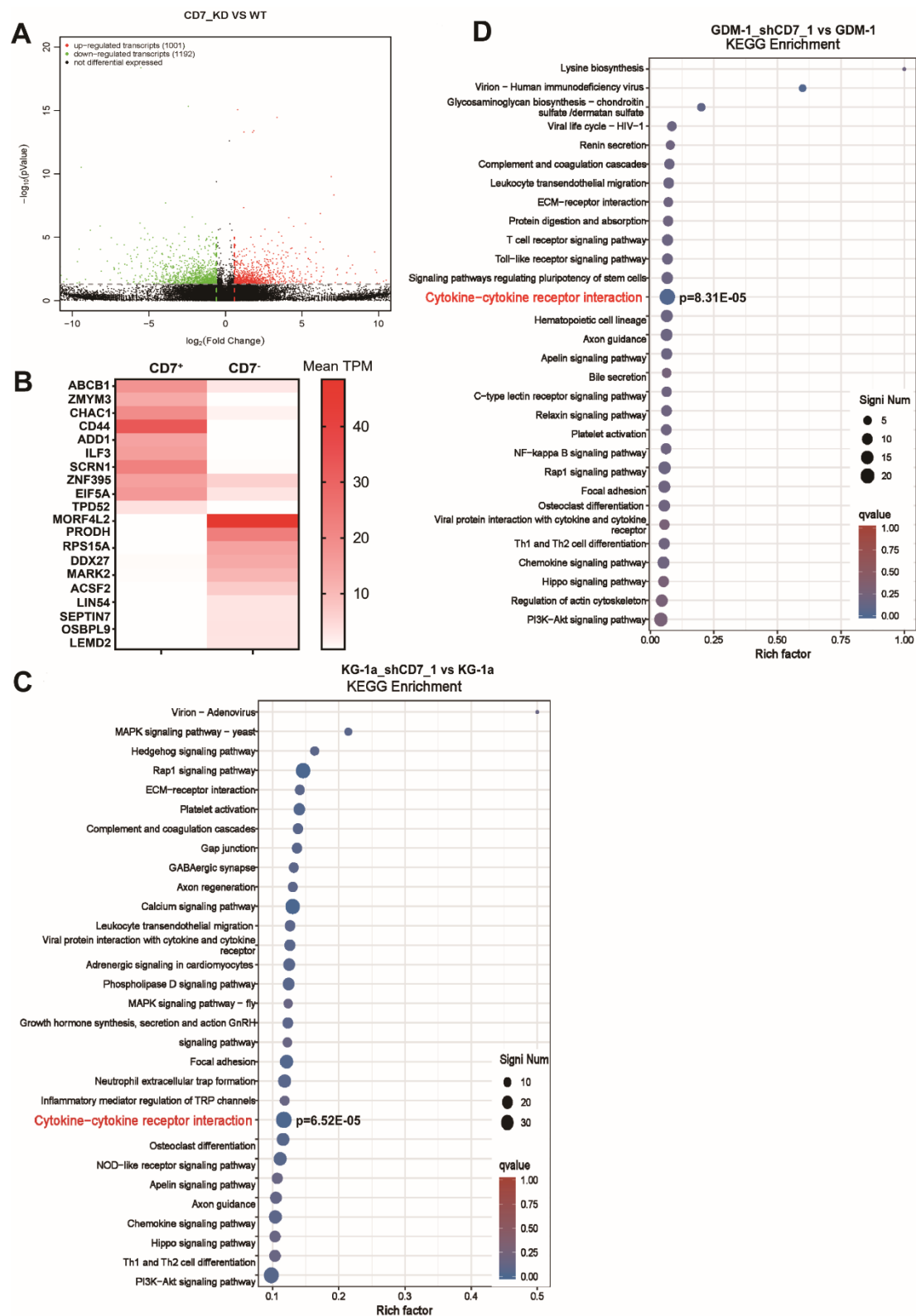
Supplemental Figure 4 (A) Differential gene analysis of CD7⁺ and CD7^{KD}AML cell lines (volcano map); (B) The top ten genes with significant up-regulation and down-regulation (heat map). (C-D) Pathway enrichment analysis of the differentially expressed genes (DEGs), identified from the transcriptome data, was conducted using an R package, and the results were visualized as a bubble plot created with ggplot2. (C) CD7⁺ KG-1a VS shCD7_1 cell; (D) CD7⁺ GDM-1 VS shCD7_1 cell. (E-H) Detection of IL-1 β and TNF- α by Elisa. (E-F) The levels of IL-1 β protein in the supernatant and cytoplasm of CD7⁺ cell lines and their shCD7_1 cell lines were detected by Elisa; (G-H) Elisa was used to detect the level of TNF- α protein in the supernatant and cytoplasm of CD7⁺ cell lines and their shCD7_1 cell lines. (I-L) Nuclear translocation of the NF- κ B p65 subunit in GDM-1 and CD7-knockdown cells following stimulation with 20 ng/mL IL-1 β for 1 h (I-J) or 10 ng/mL TNF- α for 2 h (K-L). scale bar, 20 μ m. The nuclear-to-cytoplasmic ratio was calculated for 60 cells per group.

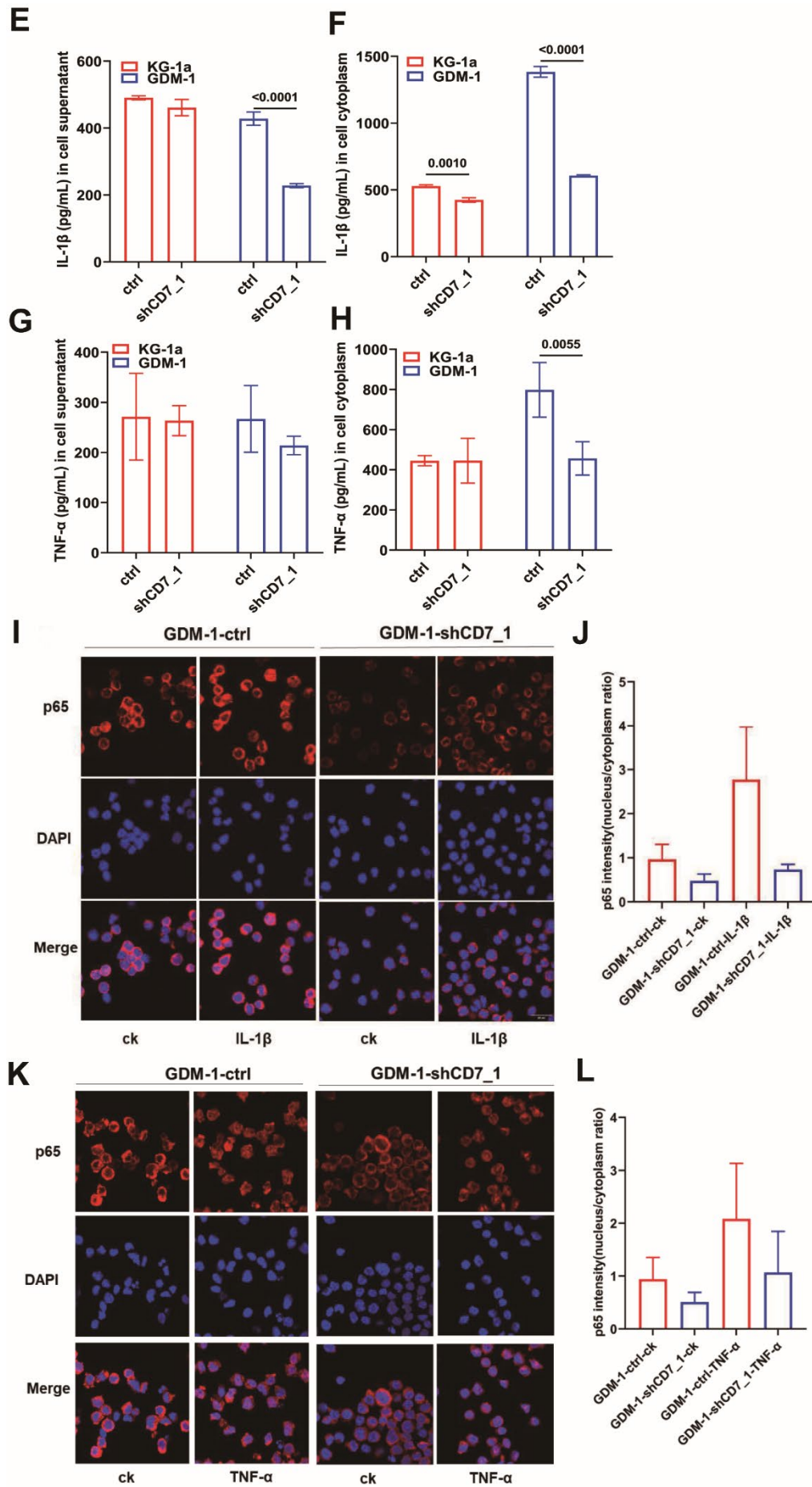












Reference

- 1 Bendall, S. C. *et al.* Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. *Science* **332**, 687–696 (2011). <https://doi.org/10.1126/science.1198704>
- 2 Haubner, S. *et al.* Coexpression profile of leukemic stem cell markers for combinatorial targeted therapy in AML. *Leukemia* **33**, 64–74 (2019). <https://doi.org/10.1038/s41375-018-0180-3>
- 3 van Dongen, J. J. *et al.* EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. *Leukemia* **26**, 1908–1975 (2012). <https://doi.org/10.1038/leu.2012.120>
- 4 van Lochem, E. G. *et al.* Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: Reference patterns for age-related changes and disease-induced shifts. *Cytometry Part B: Clinical Cytometry* **60B**, 1–13 (2004). <https://doi.org/https://doi.org/10.1002/cyto.b.20008>