1010 **Supplemental materials**

1011 **Antibodies and Reagents**

1012 **Antibodies**: Anti-human CD85K-APC (eBioscience, CA, USA, #17-5139-42); Anti-
1013 AnnexinV-APC (BD Pharmingen, CA, USA, #550475); anti-STAT3 (CST, CA, USA,
1014 #30835), anti-phospho-STAT3 (Tyr705) (CST, CA, USA, #9145); anti-IKZF1 (CST, CA,
1015 USA, #5543)；anti–β-actin-HRP (MBL, CA, Japan, PM053-7).

1016 **Reagents:** XF Cell Mito Stress Test Kit (Agilent, China, #103015-100), XF Glycolysis
1017 Stress Test Kit (Agilent, #103020-100), ATP Bioluminescence Assay Kit HS II (Roche,
1018 China, #11699709001).

1019

1020 **Cell lines**

1021 The human MM cell lines MM.1S, OPM2, LP-1, ARP1 and RPMI 8226 (ATCC) were
1022 cultured in RPMI-1640 medium supplemented with 20% fetal bovine serum (FBS) and 1%
1023 penicillin streptomycin. HEK293T cells were cultured in DMEM supplemented with 10%
1024 FBS.

1025

1026 **Luciferase Reporter Assays**

1027 To evaluate the transcriptional regulation of LILRB4 by IKZF1 and PFKFB1 by STAT3, we
1028 performed a luciferase reporter assay in 293T cells. In brief, 293T cells were cotransfected
1029 with PLVX-STAT3-strepII (or empty vector) with the pGL4.27-PFKFB1 promoter or PLVX-
1030 IKZF1-strepII with the pGL4.27-LILRB4 promoter for 24 hrs. Luciferase activities were
1031 measured by using a luciferase reporter system (GloMax®Multi Instrument).
1032 Measurements were calculated as firefly luciferase units vs. Renilla luciferase units.

1033

1034 **Chromatin immunoprecipitation (ChIP) assay**

1035 The ChIP assay was performed using the ChIP Assay Kit (Beyotime). 293T cells were
1036 cotransfected with the PLVX-STAT3-strepII plasmid with the pGL4.27-PFKFB1-promoter
1037 or PLVX-IKZF1-HA plasmid pGL4.27-LILRB4-promoter plasmid and crosslinked with 1%
1038 formaldehyde (Sigma–Aldrich) at 37 °C for 10 min, followed by incubation with anti-strepII
1039 or HA antibodies (Sigma) or control rabbit IgG (Santa Cruz) at 4 °C overnight before
1040 immunoprecipitation. For the sample input, 1% of the sonicated precleared DNA was
1041 purified at the same time as the precipitated immune complex. The ChIP samples were
1042 purified by the Gel and PCR Clean-up Kit (Nucleospin). The STAT3- or LILRB4-binding
1043 sequence was amplified by semiquantitative PCR using primers specific for human
1044 PFKFB1 and LILRB4, as listed in Table S1.

1045

1046 **Immunoblot analysis**

1047 For immunoblot analysis, whole cell lysates were electrophoresed onto 10% sodium
1048 dodecyl sulfate–polyacrylamide gels and transferred to nitrocellulose membranes
1049 (Millipore). After blocking with a 5% solution of nonfat dried milk, the membranes were
1050 incubated with the following primary antibodies at 4 °C overnight: anti-phospho-STAT3
1051 (Tyr705) (CST, CA, USA, #9145), anti-STAT3 (CST, CA, USA, #30835) and anti-β-actin
1052 (MBL). The membranes were further incubated with the appropriate horseradish
1053 peroxidase-conjugated secondary antibody (Sigma) at room temperature for 1 hr, followed

1054 by the detection of the protein level.

1055

1056 **Quantitative RT–PCR**

1057 LILRB4-knockdown MM cells and scrambled MM cells were FACS-purified for RNA
1058 extraction. First-strand cDNA was reverse transcribed using AMV reverse transcriptase
1059 (TaKaRa). PCRs were performed according to the manufacturer's instructions (Roche).
1060 The experiments were conducted in triplicate with the Applied Biosystems 7900HT, and the
1061 mRNA levels were normalized to the level of β-actin RNA transcripts. The sequences of
1062 primers used are listed in Table S1.

1063

1064 **Metabolic analyses of the oxygen consumption rate, extracellular acidification rate**
1065 **and ATP level**

1066 The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were
1067 detected in LILRB4-knockdown and scrambled ARP-1 cells with an XF Cell Mito Stress
1068 Test Kit (Seahorse, 103015-100) and an XF Glycolysis Stress Test Kit (Seahorse, 103020-
1069 100) using a Seahorse XF96 analyzer according to the manufacturer's instructions. For
1070 the detection of OCR, 1.5 μM oligomycin, 2 μM FCCP and 0.5 μM rotenone/antimycin were
1071 loaded into injection ports A, B and C, respectively. During sensor calibration, the indicated
1072 cells were incubated in 175 μL of assay medium (XF Base Medium supplemented with 2
1073 mM glutamine, 1 mM pyruvate, and 10 mM glucose, pH 7.4 at 37 °C) in a 37 °C CO_2-free
1074 incubator. For the ECAR test, injection port A on the sensor cartridge was loaded with 10
1075 mM glucose. Then, 2.5 μM oligomycin was loaded into port B, and 100 mM 2-DG was
1076 loaded into port C. During sensor calibration, the cells were incubated in 175 μL of assay
1077 medium (XF Base Medium supplemented with 1 mM glutamine alone, pH 7.4 at 37 °C) in
1078 a 37 °C CO_2-free incubator.

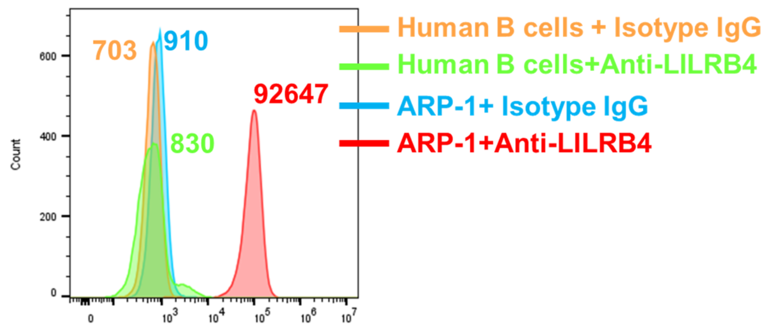
1079

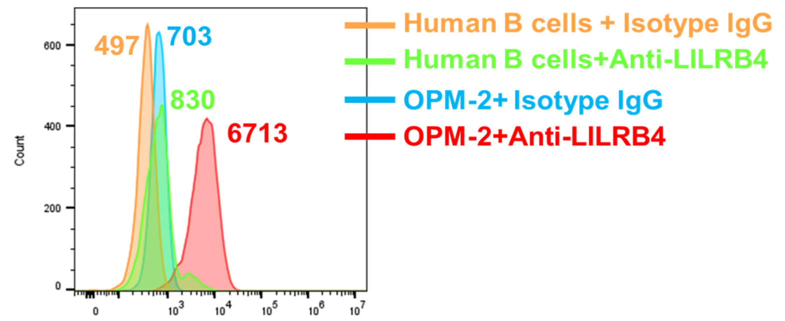
1080 **Lactate measurement**

1081 Lactate levels in the supernatant were measured according to the manufacturer's protocol
1082 (Sigma–Aldrich, MAK064). Briefly, 6 $X10^5$ LILRB4-knockdown and scrambled ARP-1 cells
1083 were plated in RPMI-1640 medium and supplemented with 20% fetal bovine serum (FBS),
1084 followed by incubation at 37 °C for 4 hr. The supernatant was centrifuged at 12,000 g at
1085 4 °C for 5 min to remove cell debris before the analysis of lactate levels. Lactate levels
1086 were determined by a spectrophotometer according to the manufacturer's protocol.

1087

Figure S1





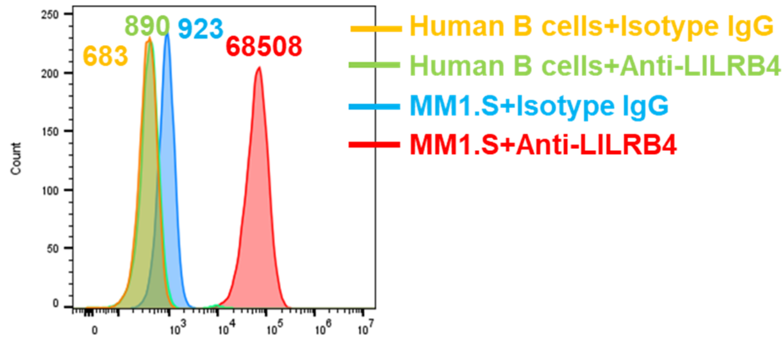


Figure S1, related to Figure 1. LILRB4 is highly expressed on multiple myeloma cells. (A-C) LILRB4 expression levels were quantified by flow cytometric analysis in ARP-1 (A), OPM2 (B), MM1.S (C) and normal B cells.

Figure S2

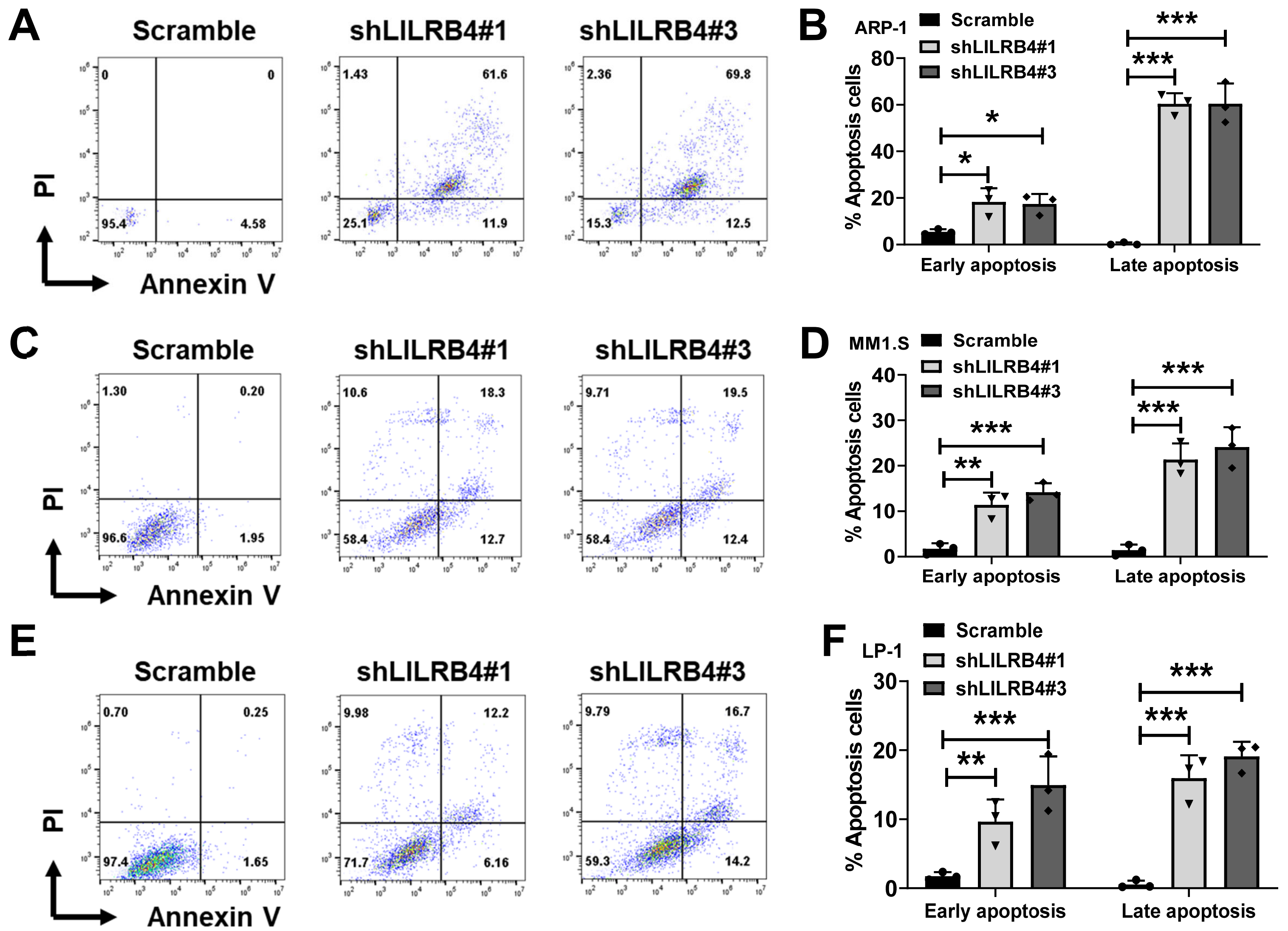


Figure S2, related to Figure 2. LILRB4 supports MM cell proliferation in vitro. (A-B) Representative flow cytometric analyses of percentages of early and late apoptotic cells in scrambled or LILRB4-knockdown (shLILRB4#1 and #3) ARP-1 cells. (C-D) Representative flow cytometric analyses of percentages of early and late apoptotic cells in scrambled or LILRB4-knockdown (shLILRB4#1 and #3) MM1.S cells. (E-F) Representative flow cytometric analyses of percentages of early and late apoptotic cells in scrambled or LILRB4-knockdown (shLILRB4#1 and #3) LP-1 cells. Two-way ANOVA with Sidak's multiple comparison test (B, D and F) was used for the comparison of statistical significance. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Table S1. Primer sequences

Human primers for qRT-PCR	Sequences
STAB1-F	AACCACGTTTGTCACTCATGT
STAB1-R	CGGCAGTCCTGGGTTATCTG
PTGS-F	CTGGCGCTCAGCCATACAG
PTGS -R	CGCACTTATACTGGTCAAATCCC
EGR1-F	GGTCAGTGGCCTAGTGAGC
EGR1-R	GTGCCGCTGAGTAAATGGGA
CDKN1A-F	TGTCCGTCAGAACCCATGC
CDKN1A -R	AAAGTCGAAGTTCCATCGCTC
ALDH7A1-F	CCAGTATGCGTGGCTGAAAGA
ALDH7A1-R	CAGGGCAATAGGTCGTAATAACC
PSPH-F	GAGGACGCGGTGTCAGAAAT
PSPH -R	GGTTGCTCTGCTATGAGTCTCT
GADD45A-F	GAGAGCAGAAGACCGAAAGGA
GADD45A -R	CACAACACCACGTTATCGGG
BCL2A1-F	TACAGGCTGGCTCAGGACTAT
BCL2A1-R	CGCAACATTTTGTAGCACTCTG
FLT1-F	TTTGCCTGAAATGGTGAGTAAGG
FLT1-R	TGGTTTGCTTGAGCTGTGTTC
SAGE1-F	TACCAGGGATCTGCATTCTACC
SAGE1-R	CTGTGGGACCAGTTGACAAGA
IKZF1-F	CATCAGCCCGATGTACCAGC
IKZF1-R	CCTCGTTGTTGCTCTCGGT
IKZF3-F	GCTCATACAGACCCGCATGAT
IKZF3-R	AACTGGAACCATCTCCGAGGT
PFKFB1-F	TACCAGAGAACGACGGTCACT
PFKFB1-R	CTGCAATTATGCCAGGGTCATTA
β-ACTIN-F	AGAGCTACGAGCTGCCTGAC
β-ACTIN-R	AGCACTGTGTTGGCGTACAG
Primers for shRNAs	**Target sequences**
Human shLILRB4#1	GCTCATAGTCTCAGGATCCTT
Human shLILRB4#3	CTCGGGAGTACCGTCTGGATA
Primers for vector	**Sequences**
Human PFKFB1-F	TCTATTTCCGGTGAATTCCTCGAGATGTCTCCAG AGATGGGAGA
Human PFKFB1-R	GCACCTCCAGGGATCCGCGGCCGCaGTAGTGGG CTGGGACAGTAT
Human IKZF1-F	TCTATTTCCGGTGAATTCCTCGAGATGGAAGATA TACAAACAAA
Human IKZF1-R	GCACCTCCAGGGATCCGCGGCCGCaCTTCAGCA GGGCTCTGTGTT
Human ALDH7A1-F	TCTATTTCCGGTGAATTCCTCGAGATGTGGCGCC

Human ALDH7A1-R	TTCCTCGCGC GCACCTCCAGGGATCCGCGGCCGCaCTGAAACT TGATTCCTTGGG
Primers for ChIP	**Sequences**
PFKFB1-promoter-ChIP-3-F	CTTGGCATGAGGAACTTTAG
PFKFB1-promoter-ChIP-3-R	TGGGCCCAAGTAGAATGTCA
PFKFB1-promoter-ChIP-4-F	TGACATTCTACTTGGGCCCA
PFKFB1-promoter-ChIP-4-R	CTTAGGAGTCGCACCGAATG
LILRB4-promoter-ChIP-4-F	AGGAGGACACGGCTCTGATA
LILRB4-promoter-ChIP-4-R	GGCGTCTCCTCCCAGGGGCC