SUPPLEMENTARY FIGURE LEGENDS

Figure S1 The correlation of Rheb1 expression level with AML prognosis. (A) Kaplan-Meier curves of event-free survival of patients with AML, with low expression (≤ 50% quantile, n = 426) and high expression (> 50% quantile, n = 3) based on Verhaak *et al*.'s dataset. (B) Kaplan-Meier curves of overall survival of patients with AML, with or without Rheb1 deletion based on TCGA data in the cBioportal database.

Figure S2 The genotype of established AML mouse model. (A) Western blotting analysis of Rheb1 protein level in WT and AML cells and their densitometry value using β-actin as an internal control (n = 3). (B) Flow cytometric analysis of GFP^+ AML cells using CD3, B220 and CD11b. (C) Rheb1 mRNA expression of $Rheb1^{fl/fl}$ and $Rheb1^{\Delta/\Delta}$ AML cells. (D) PCR confirmation of the genotype of mice. (E) Histopathological analysis of spleen sections in $Rheb1^{fl/fl}$ and $Rheb1^{\Delta/\Delta}$ AML mice. (F) Gating strategy for measuring L-GMP frequencies in the spleens of $Rheb1^{fl/fl}$ and $Rheb1^{\Delta/\Delta}$ AML mice. (G) L-GMP frequency in the spleen (n = 4). (H) The percentages of AML subpopulations using c-Kit and Gr-1 in the spleen (n = 4). The data are presented as the mean numbers ± SEM.

Figure S3 The function and related signaling pathway of AML cells. (A) GFP^+ cells homing to the BM (left panel) and spleen (right panel), n = 5. (B) The differentially expressed genes of $Rheb1^{fl/fl}$ and $Rheb1^{\Delta/\Delta}$ AML cells. (C) Enrichment of M phase mitotic cell cycle-related genes in $Rheb1^{fl/fl}$ vs.

Rheb1$^{\Delta/\Delta}$ GFP^{+} cells. NES (normalized enrichment score), and the P values are indicated in each plot. (D and E) The cell cycle status of K^{+}G^{+} (D) and K^{-}G^{+} cells (E) (n = 4). (F and G) The mRNA expression levels of the indicated genes in GFP^{+} (F) and K^{+}G^{-} cells (G). The GFP^{+} cells were sorted, and the RNA was isolated for RT-PCR using the indicated gene primers. The data are presented as the mean numbers ± SEM. (H and I) The normalized MFI of p-S6 (H) and p-4E-BP1 (I) of K^{+}G^{+} and K^{-}G^{+} cells in the BM of both groups (n = 4). The data are presented as the mean numbers ± SEM.

Figure S4 The signaling pathway in human AMLs and murine AML. (A and B) mTOR (A) and 4E-BP1 (B) mRNA expression in human AML cells with t(8;21)(AMLI_ETO), t(15;17)(APL), inv(16)/t(16;16) and t(11q23)/MLL using the HemaExplorer database. The data were analyzed using the Mann-Whitney U test. Significant differences are indicated with asterisks (*, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001). (C) KEGG analysis of the upregulated pathways in *Rheb1*$^{fl/fl}$ vs. *Rheb1*$^{\Delta/\Delta}$ GFP^{+} cells. (D) KEGG analysis of downregulated pathways in *Rheb1*$^{fl/fl}$ vs. *Rheb1*$^{\Delta/\Delta}$ GFP^{+} cells.

SUPPLEMENTARY METHODS

LSC transplantation

K^+G^- cells (LSCs) were sorted using a BD FACS Aria III flow sorter, and then transplanted into sub-lethally irradiated recipient mice. The survival status was observed and the survival curve was analyzed by Graphpad Prism 6.

Histological and pathological analysis

For pathological analysis, spleen and liver were fixed with 4% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Homing assay

WBMCs were obtained and GFP^+ cells were sorted by Aria III flow cytometer. 1 $\times 10^7$ BM GFP^+ cells were intravenously injected into lethally irradiated (9.5 Gy) recipient mice (CD45.1). The recipient mice were sacrificed to obtain WBMCs and spleen cells after 24 h, and these cells were then labeled for analyzing the number of GFP^+ cells by BD FACS LSRII flow cytometer.

Rapamycin dissolution

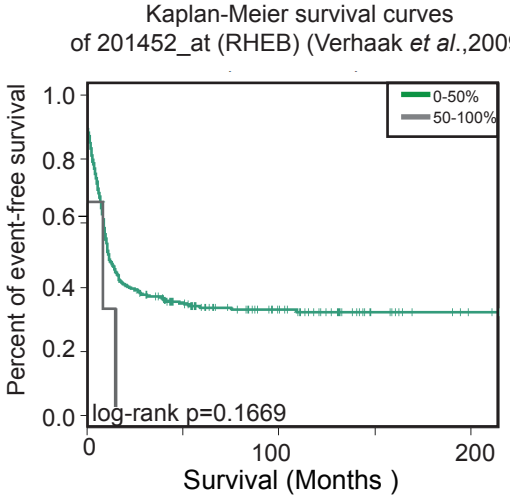
Rapamycin (LC Lab) was dissolved in absolute ethanol at 10 mg/ml and stored at -80°C before use. The stock solution was added to the media at a final concentration of 5.45 nM/ml for all the experiments.

Western blotting analysis

BM PMN cells were lysed and their proteins were extracted, separated and electrophoretically transferred to polyvinylidene difluoride membranes (Millipore). The membrane was blocked with 5% non-fat milk for 1 h, washed

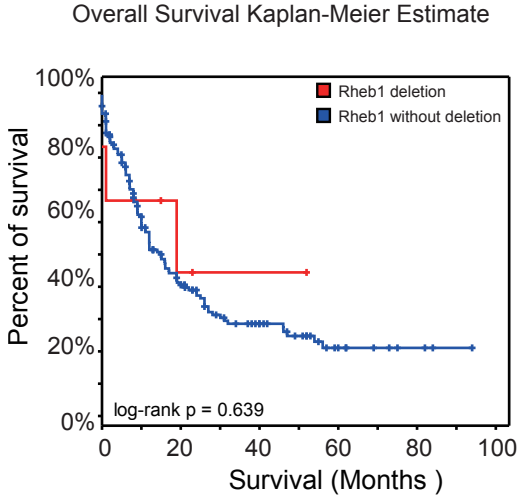
for three times, then incubated with the primary antibodies (β-actin（8H10D10）Mouse mAb: CST; Rheb Rabbit antibody: Thermo Fisher) for 1 h at room temperature, and stay overnight at 4℃. After washing for three times the next day, the membrane was incubated with the relevant horseradish peroxidase-conjugated secondary antibodies (anti-rabbit/mouse IgG HRP-linked antibody: CST) for 1 h, then washed for three times. Finally, the reactive proteins were visualized with ImageQuant LAS4000. The density of each band was analyzed with Image J software and normalized with the control actin band.

A



Rheb1 expression	Median survival time
0-50%	11.47
50-100%	8.41

B



AML (TCGA, Provisional)	#Total cases	#Cases decreased	Median survival (months)
Cases with Rheb1 deletion	6	3	18.96
Cases without Rheb1 deletion	171	111	15.01

Figure S1

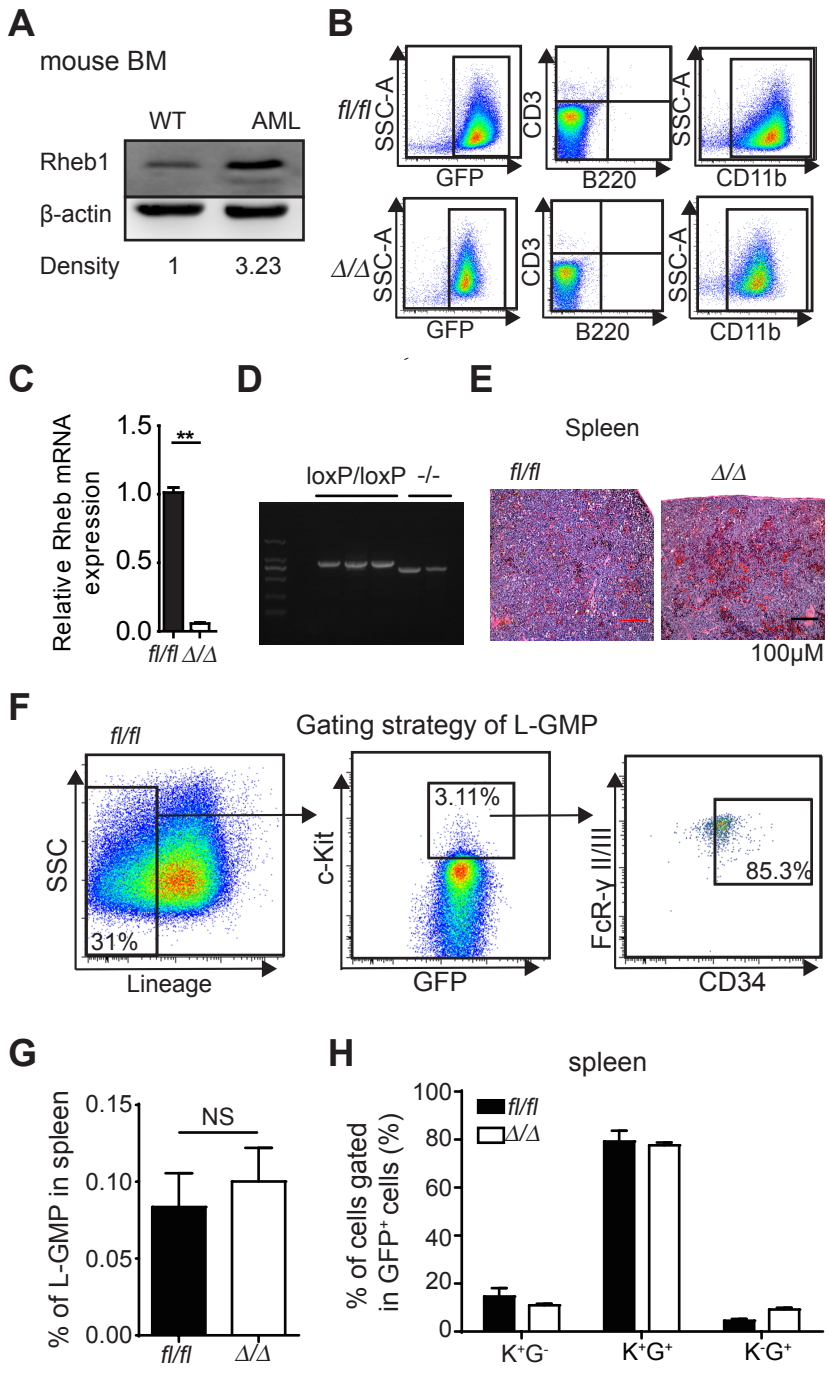

A
mouse BM
WT
AML
Rheb1
β-actin
Density
1
3.23
B
fl/fl
Δ/Δ
SSC-A
GFP
CD3
B220
CD11b
C
Relative Rheb mRNA expression
1.5
1.0
0.5
0.0
**
fl/fl
Δ/Δ
D
loxP/loxP
-/-
E
Spleen
fl/fl
Δ/Δ
100μM
F
Gating strategy of L-GMP
fl/fl
SSC
31%
Lineage
c-Kit
3.11%
GFP
FcR-γ II/III
85.3%
CD34
G
% of L-GMP in spleen
0.15
0.10
0.05
0.00
NS
fl/fl
Δ/Δ
H
spleen
% of cells gated in GFP+ cells (%)
100
80
60
40
20
0
fl/fl
Δ/Δ
K+G-
K+G+
K-G+

Figure S2

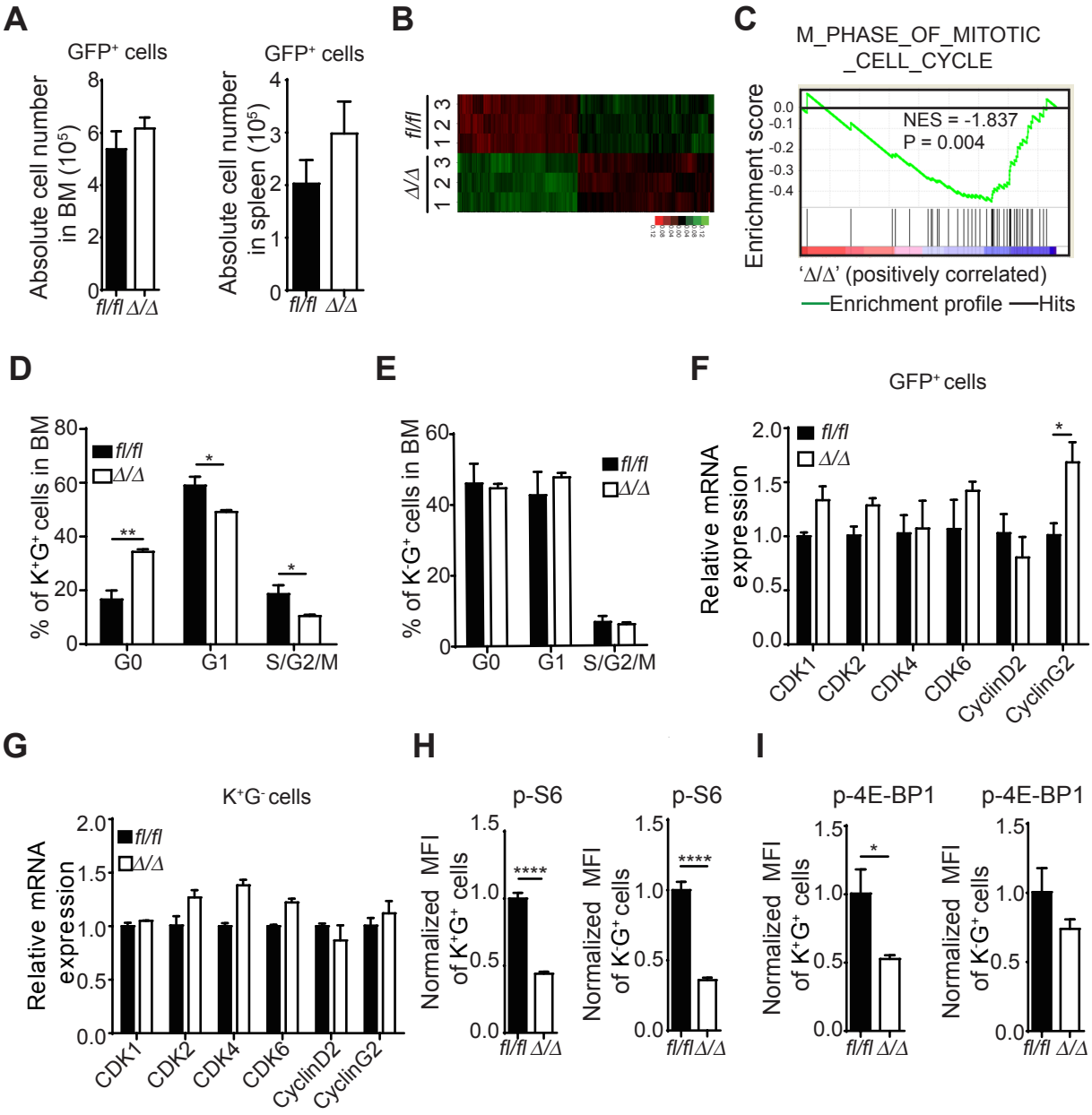

A
GFP+ cells
Absolute cell number in BM (10^5)
fl/fl Δ/Δ
GFP+ cells
Absolute cell number in spleen (10^5)
fl/fl Δ/Δ
B
fl/fl
Δ/Δ
1 2 3
1 2 3
C
M_PHASE_OF_MITOTIC _CELL_CYCLE
Enrichment score
NES = -1.837
P = 0.004
'Δ/Δ' (positively correlated)
Enrichment profile
Hits
D
% of K+G+ cells in BM
fl/fl
Δ/Δ
G0
G1
S/G2/M
E
% of K-G+ cells in BM
fl/fl
Δ/Δ
G0
G1
S/G2/M
F
GFP+ cells
Relative mRNA expression
fl/fl
Δ/Δ
CDK1
CDK2
CDK4
CDK6
CyclinD2
CyclinG2
G
K+G- cells
Relative mRNA expression
fl/fl
Δ/Δ
CDK1
CDK2
CDK4
CDK6
CyclinD2
CyclinG2
H
p-S6
Normalized MFI of K+G+ cells
fl/fl Δ/Δ
p-S6
Normalized MFI of K-G+ cells
fl/fl Δ/Δ
I
p-4E-BP1
Normalized MFI of K+G+ cells
fl/fl Δ/Δ
p-4E-BP1
Normalized MFI of K-G+ cells
fl/fl Δ/Δ

Figure S3

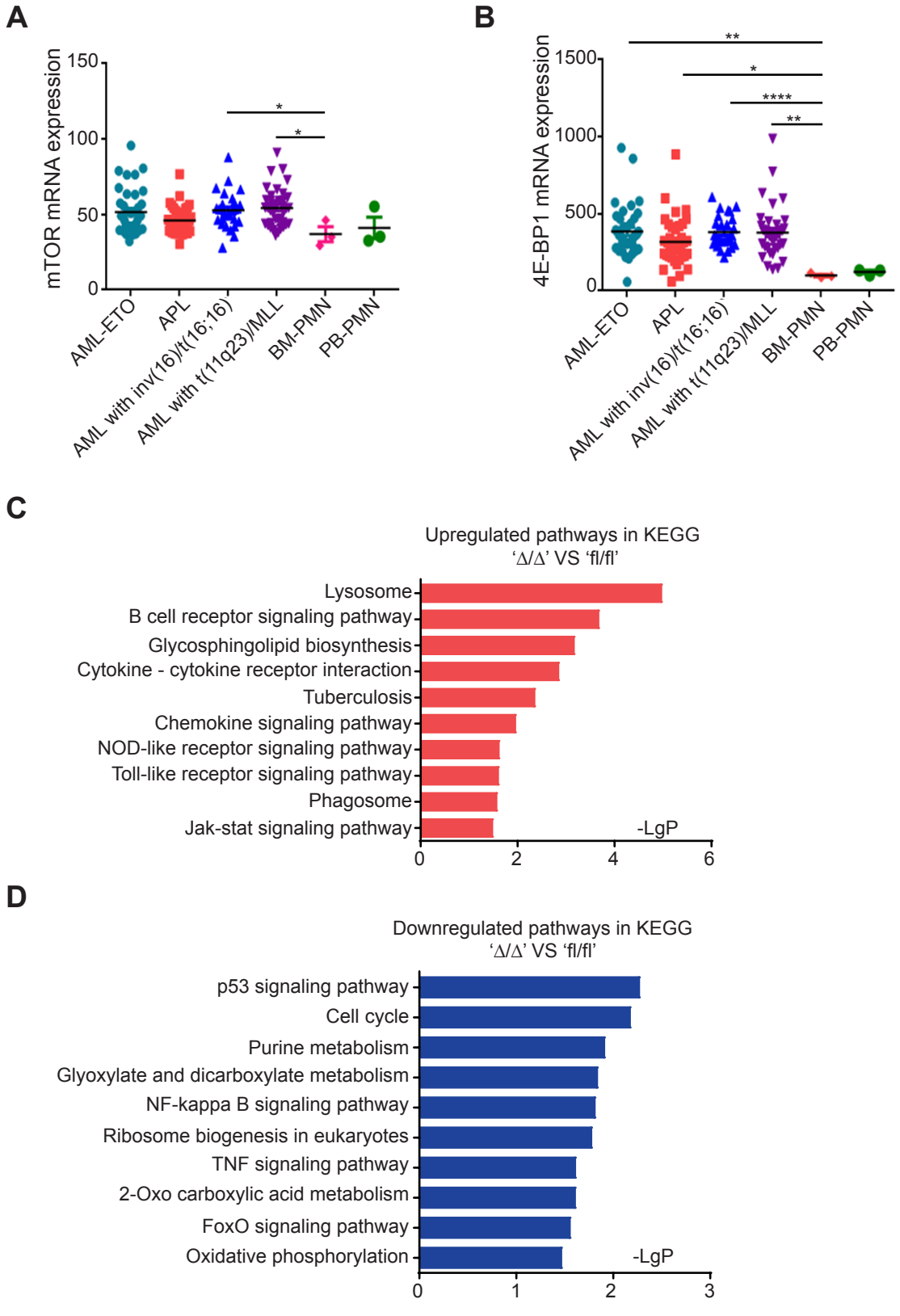

A
mTOR mRNA expression
150
100
50
0
*
*
AML-ETO
APL
AML with inv(16)/t(16;16)
AML with t(11q23)/MLL
BM-PMN
PB-PMN
B
4E-BP1 mRNA expression
1500
1000
500
0
**
*
****
**
AML-ETO
APL
AML with inv(16)/t(16;16)
AML with t(11q23)/MLL
BM-PMN
PB-PMN
C
Upregulated pathways in KEGG
'Δ/Δ' VS 'fl/fl'
Lysosome
B cell receptor signaling pathway
Glycosphingolipid biosynthesis
Cytokine - cytokine receptor interaction
Tuberculosis
Chemokine signaling pathway
NOD-like receptor signaling pathway
Toll-like receptor signaling pathway
Phagosome
Jak-stat signaling pathway
-LgP
0
2
4
6
D
Downregulated pathways in KEGG
'Δ/Δ' VS 'fl/fl'
p53 signaling pathway
Cell cycle
Purine metabolism
Glyoxylate and dicarboxylate metabolism
NF-kappa B signaling pathway
Ribosome biogenesis in eukaryotes
TNF signaling pathway
2-Oxo carboxylic acid metabolism
FoxO signaling pathway
Oxidative phosphorylation
-LgP
0
1
2
3

Figure S4

Table S1　Primer sequence list

Primer	Sequence（5’-3’）	
	mouse	
	Forward	**Reverse**
GAPDH	TGTGTCCGTCGTGGATCTGA	TTGCTGTTGAAGTCGCAGGA
Rheb1	GTTCACCAAGTTGATCACGGT	ATATTCATCCTGCCCCGCTG
BAX	TGGAGCTGCAGAGGATGATTG	AGCTGCCACCCGGAAGA
Bim	TTGGAGCTCTGCGGTCCTT	CAGCGGAGGTGGTGTGAAT
Puma	GCGGCGGAGACAAGAAGA	AGTCCCATGAAGAGATTGTACATG
P53	AAGATCCGCGGGCGTAA	CATCCTTTAACTCTAAGGCCTCATT
Mcl-1	CCCTCCCCCATCCTAATCAG	AGTAACAATGGAAAGCATGCCAAT
P18	TACGAATTCATGGCCGAG	TGGCTCGAGTCACTGCAGGCTT
	CCTTGGGGGA	GTGGCT
P19	AGGCCGGCAAATGATCATAGA	GGTGGATACCGGTGGACTGTG
P21	AATCCTGGTGATGTCCGACC	TTGCAGAAGACCAATCTGCG
P27	TATGGAAGAAGCGAGTCAGC	GCGAAGAAGAATCTTCTGCAG
P57	CAGCGGACGATGGAAGAACT	CTACGCAACCATCTCCGGTT
CDK1	CAAAGCTGGCTGGGTTTCAC	TCTGACCATGCAGAGCACAG
CDK2	AGGTGGAGAAGATTGGAGAGG	CCCGTCAACTTGTTTTTGGCT
CDK4	CCCTCTTCTCACTCTGCGTC	TGCCAGAGATGGAGGAGTCT
CDK6	TGACACTGTGCACACATCAAA	AGACCAGTGAGGAGGGCAT

CyclinG2	AGGGGTTCAGCTTTTCGGATT	AGTGTTATCATTCTCCGGGGTAG
CyclinD2	GAGTGGGAACTGGTAGTGTTG	CGCACAGAGCGATGAAGGT
SLC7A7	CCACACCCATAACGCTGAGT	TGCCCTCTAGCAAACACAGG
CD163	TGCCTCTGCTGTCACTAACG	TTCATTCATGCTCCAGCCGT
TGF β 1	CTGCTGACCCCCACTGATAC	AGCCCTGTATTCCGTCTCCT
MMP8	AGCGCTTCTTCAGCTTAGCA	GTCCACTTGGGACTTCCTGG
LTF	TCCCGAAGCACGAATGACAA	GGGAGGCAAAGAGCTGGAAT
HOXA9	CCCCGACTTCAGTCCTTGC	GATGCACGTAGGGGTGGTG
MEIS1	CATGATAGACCAGTCCAACCGA	ATTGGCTGTCCATCAGGGTTA

Primer	**human**	
	Forward	**Reverse**
GAPDH	GGTCGTATTGGGCGCCTGGTC	TGACGGTGCCATGGAATTTGCCA
Rheb1	AGGGTCATGACGCAGCGAGT	TGCGTCGGGGCGACGTTTTA