**Supplemental Information**

## Supplemental Methods

**Gene set enrichment analysis.** Gene set enrichment analysis was performed using Gene Set Enrichment Analysis software (GSEA V2.07) (1, 2). The following running parameters were used: gene set as permutation type, and 1,000 permutations and values of normalized density of classes as metric for ranking genes. Gene sets related to myeloid and lymphoid differentiation were from the MSig database of the Broad Institute, Cambridge, MA.

**Engraftment of human CMML cells in NSGS mice.**
NSGS mice were conditioned with busulfan (Sigma, 30mg/kg, intraperitoneal) 24 hours before intravenous injection of CMML cells. Bone marrow aspirations were performed in order to monitor engraftment. These samples were stained with antibodies against CD45, CD33, and CD19 (BD Biosciences) and human cells were then detected with a FACSCanto flow cytometer (BD Biosciences). Analysis was performed with FloJo software (Tree Star, Inc).

## Supplemental References


1. Mootha, V.K., Lindgren, C.M., Eriksson, K.F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstrale, M., Laurila, E., et al. 2003. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 34:267-273.
2. Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., et al. 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 102:15545-15550.


## Supplemental Figure Legends

**Supplemental Figure 1. Evaluation of CD48$^+$ LSK cells in Nras$^{G12D/G12D}$ mice.**
Control and Nras$^{G12D/G12D}$ mice were treated with pI-pC and sacrificed on Day 12 for analysis of CD48$^+$ LSK cells. We refer the day of the first pI-pC injection as Day 1.

**Supplementary Figure 2. Gene set enrichment analysis (GSEA) of HSC microarray results identifies a gene signature of lymphoid differentiation in Nras$^{G12D/G12D}$ HSCs.**
(A-C) 500 HSCs were purified from control or Nras$^{G12D/G12D}$ mice for microarray analysis. (A) Heat-map analysis of known genes associated with HSC self-renewal. (B) GSEA analysis of HSC self-renewal signature. (C) GSEA analysis of myeloid versus lymphoid differentiation. FDR, false discovery rate; NES, normalized enrichment score. (D, E) Quantification of common myeloid progenitors (CMP), granulocyte-macrophage progenitors (GMP), megakaryocyte-erythroid progenitors (MEP) (D), and common lymphoid progenitors (CLP) (E) in bone marrow (BM) and spleen (SP) from control and Nras$^{G12D/G12D}$ mice. Data are presented as mean $\pm$ s.d.. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

**Supplementary Figure 3. Rapamycin treatment does not rescue the HSC and JMML/CMML phenotypes in Nras$^{G12D/G12D}$ mice.** Rapamycin or vehicle treatment in control or Nras$^{G12D/G12D}$ mice was carried out similarly as AZD6244 treatment as described in Figure 4. (A-C) Absolute numbers of hematopoietic stem cells (HSCs) (A), common myeloid progenitors (CMPs) (B), and common lymphoid progenitors (CLPs) (C) in hind limb (H.L.) bone marrow (BM) and spleen (SP) are presented as mean + s.d. *, $P < 0.05$. (D) Representative flow cytometry analysis of myeloid cells in bone marrow (BM), spleen (SP), and peripheral blood (PB). The percentages of analyzed cells are indicated in each quadrant.

**Supplementary Figure 4. GM-CSF evoked pSTAT5 level is greatly elevated in Nras$^{G12D/G12D}$ MPPs but IL-3 evoked pSTAT5 level is normal in mutant HSCs and MPPs.** Total bone marrow cells from control or Nras$^{G12D/G12D}$ mice were enriched for Sca1$^{+}$ cells. CD150$^{+}$ CD41$^{-}$ cells (enriched for HSCs) and CD150$^{-}$ CD41$^{-}$ cells (enriched for MPPs) were subsequently sorted from Sca1$^{+}$ enriched cells. Sorted cells were serum- and cytokine-starved for 30 minutes at 37°C and stimulated with 10 ng/ml of GM-CSF (A) or IL-3 (B) for 10 minutes at 37°C. Levels of phosphorylated STAT5 were measured using phospho-specific flow cytometry. HSCs (defined as [Lin CD48]$^{-/low}$ cKit$^{+}$ cells from sorted CD150$^{+}$ CD41$^{-}$ cells) and MPPs (defined as [Lin CD48]$^{-/low}$ cKit$^{+}$ cells from sorted CD150$^{-}$ CD41$^{-}$ cells) were gated for data analysis. Results obtained from one representative experiment are shown.

**Supplementary Figure 5. IL-6 evoked Stat3 activation in Nras$^{G12D/G12D}$ HSPCs depends on JAK.** Whole bone marrow cells from control or Nras$^{G12D/G12D}$ mice were serum- and cytokine-starved for 90 minutes at 37°C and incubated with DMSO vehicle or AZD1480 for another 30 minutes at 37°C. Cells were then stimulated with 10 ng/ml of IL-6 for 10 minutes at 37°C. Levels of pSTAT3 were measured using phospho-specific flow cytometry. Nonneutrophil Lin$^{-}$ c-Kit$^{+}$ cells were gated for data analysis. Results obtained from one representative experiment are shown.

**Supplementary Figure 6. Combined inhibition of JAK and MEK is much more efficacious to block ERK1/2 activation than inhibition of JAK or MEK alone.** Whole bone marrow cells from wild-type mice were serum- and cytokine-starved for 90 minutes at 37°C and incubated with DMSO vehicle or various concentrations of drugs for another 30 minutes at 37°C. Cells were then stimulated with 10 ng/ml of GM-CSF for 10 minutes at 37°C. Levels of p-ERK1/2 and pSTAT5 were measured using phospho-specific flow cytometry. Nonneutrophil Lin$^{-}$ c-Kit$^{+}$ cells were gated for data analysis. Results obtained from one representative experiment are shown.

**Supplementary Figure 7. Combined inhibition of JAK and MEK effectively blocks hGM-CSF evoked ERK1/2 and STAT5 activation in human CMML cells.** Primary human CMML cells were serum- and cytokine-starved for 90 minutes at 37°C and incubated with 5 μM DMSO vehicle or drugs for another 30 minutes at 37°C. Cells were then stimulated with 10 ng/ml of hGM-CSF for 10 minutes at 37°C. Levels of p-ERK1/2 and pSTAT5 were measured using phospho-specific flow cytometry. CD38$^{-}$ CD33$^{+}$ cells were gated for data analysis. Results obtained from one representative patient are shown.

**Supplementary Figure 8. Combined inhibition of JAK and MEK does not lead to significant toxicity in wild-type mice.** C57B/L6 mice (8-12 weeks old) were treated with vehicle or different combined doses of AZD6244 and AZD1480 for 4 weeks. Complete blood count (A), spleen weight and body weight (B) were measured. (C) Representative histologic H&E sections from multiple tissues are shown.

**Supplementary Figure 9. Human grafts from CMML patient samples transiently engraft NSGS mice.** Eight CMML patient samples were engrafted into NSGS mice. (A) Four of the eight samples showed significant human grafts transiently. The mouse engrafted with CMML-20 had to be sacrificed at 10 weeks due to illness. The bone marrow from this mouse was transplanted into secondary mice, none of which showed significant human cell engraftment. (B) Grafts consisted of predominantly myeloid cells. Left panels show bone marrow cells from a mouse engrafted with cord blood cells, containing a mix of human lymphoid and myeloid cells. Panels on the right are from a mouse engrafted with a CMML sample, demonstrating a mix of immature and maturing myelomonocytic cells, with no $CD19^+$ lymphoid cells present.

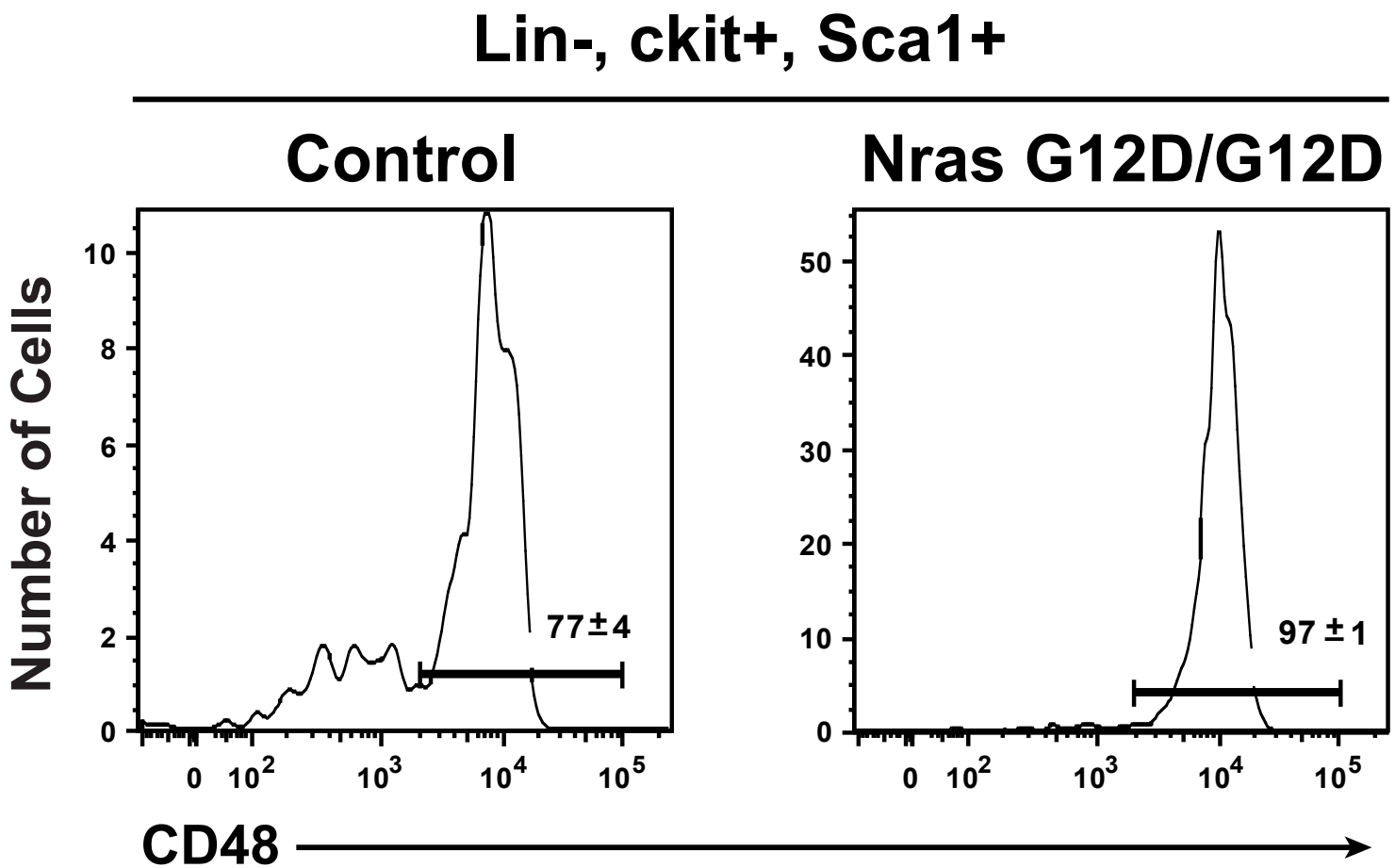
**Supplementary Figure 10. Evaluation of recombination efficiency of the *Nras*$^{G12D/G12D}$ allele in HSCs from *Nras* $^{G12D/G12D}$ mice treated with combination AZD6244 and AZD1480.** $Nras^{G12D/G12D}$ mice with an advanced JMML/CMML were treated with combination AZD6244 and for 20 weeks. Individual HSCs were sorted into 96-well plates and cultured for 14 days. Genomic DNA was extracted from individual colonies and analyzed by PCR. 44 out of 47 HSC colonies show complete recombination.

**Supplementary Figure 11. Evaluation of oncogenic Nras expression in *Nras* $^{G12D/G12D}$ mice treated with AZD6244 and/or AZD1480 at a moribund stage or the end of 20-week treatment.**

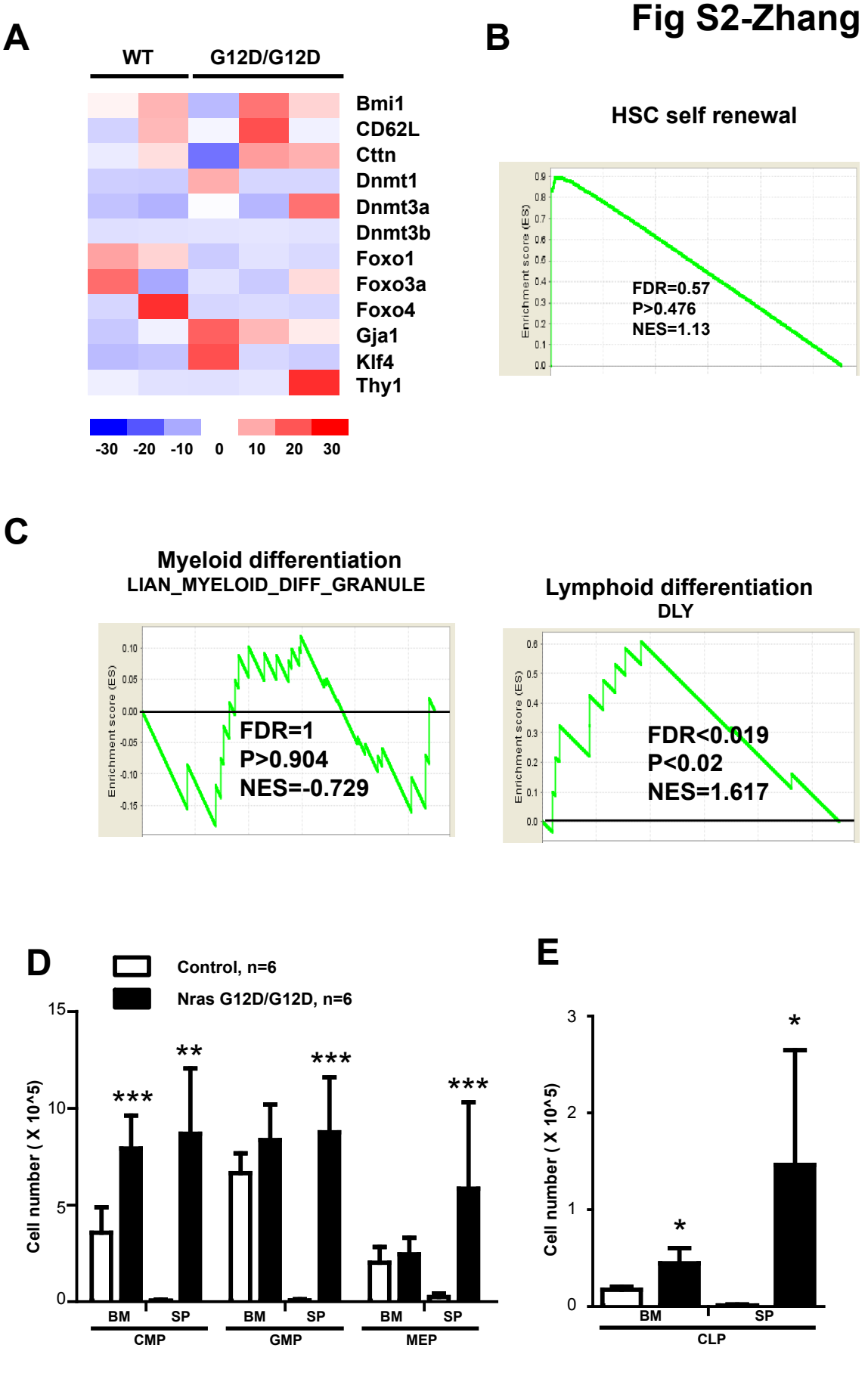
**Supplementary Figure 12. Evaluation of recipient mice transplanted with Nras $^{G12D/G12D}$ cells after various treatments.** $Nras^{G12D/G12D}$ mice with an advanced JMML/CMML treated with vehicle, AZD6244 alone, AZD1480 alone, or combination AZD6244 and AZD1480 until a moribund stage or the treatments were terminated at 20 weeks. $5 \times 10^6$ bone marrow cells isolated from these end-stage mice were transplanted into individual sublethally irradiated mice. Recipients were closely monitored until a moribund stage and analyzed for disease phenotypes. (A) Kaplen-Meier survival curves of recipient mice. P value was determined by the Log-rank test. (B) Dynamic contribution of donor-derived white blood cells ($CD45.2^+$) in peripheral blood of recipient mice at different time points posttransplant. (C) Summary of hematopoietic malignancies developed in recipient mice.

**Supplementary Figure 13. Schematic picture illustrating the consequences of long-term treatment of AZD6244 and/or AZD1480 in Nras$^{G12D/G12D}$ mice.**

# Fig S1-Zhang


Lin-, ckit+, Sca1+
Control
Nras G12D/G12D
Number of Cells
77±4
97±1
CD48
0
10^2
10^3
10^4
10^5

# Fig S2-Zhang


A
WT
G12D/G12D
Bmi1
CD62L
Cttn
Dnmt1
Dnmt3a
Dnmt3b
Foxo1
Foxo3a
Foxo4
Gja1
Klf4
Thy1
-30 -20 -10 0 10 20 30
B
HSC self renewal
Enrichment score (ES)
FDR=0.57
P>0.476
NES=1.13
C
Myeloid differentiation
LIAN_MYELOID_DIFF_GRANULE
Enrichment score (ES)
FDR=1
P>0.904
NES=-0.729
Lymphoid differentiation
DLY
Enrichment score (ES)
FDR<0.019
P<0.02
NES=1.617
D
Control, n=6
Nras G12D/G12D, n=6
Cell number ( X 10^5)
BM
SP
CMP
GMP
MEP
E
Cell number ( X 10^5)
BM
SP
CLP

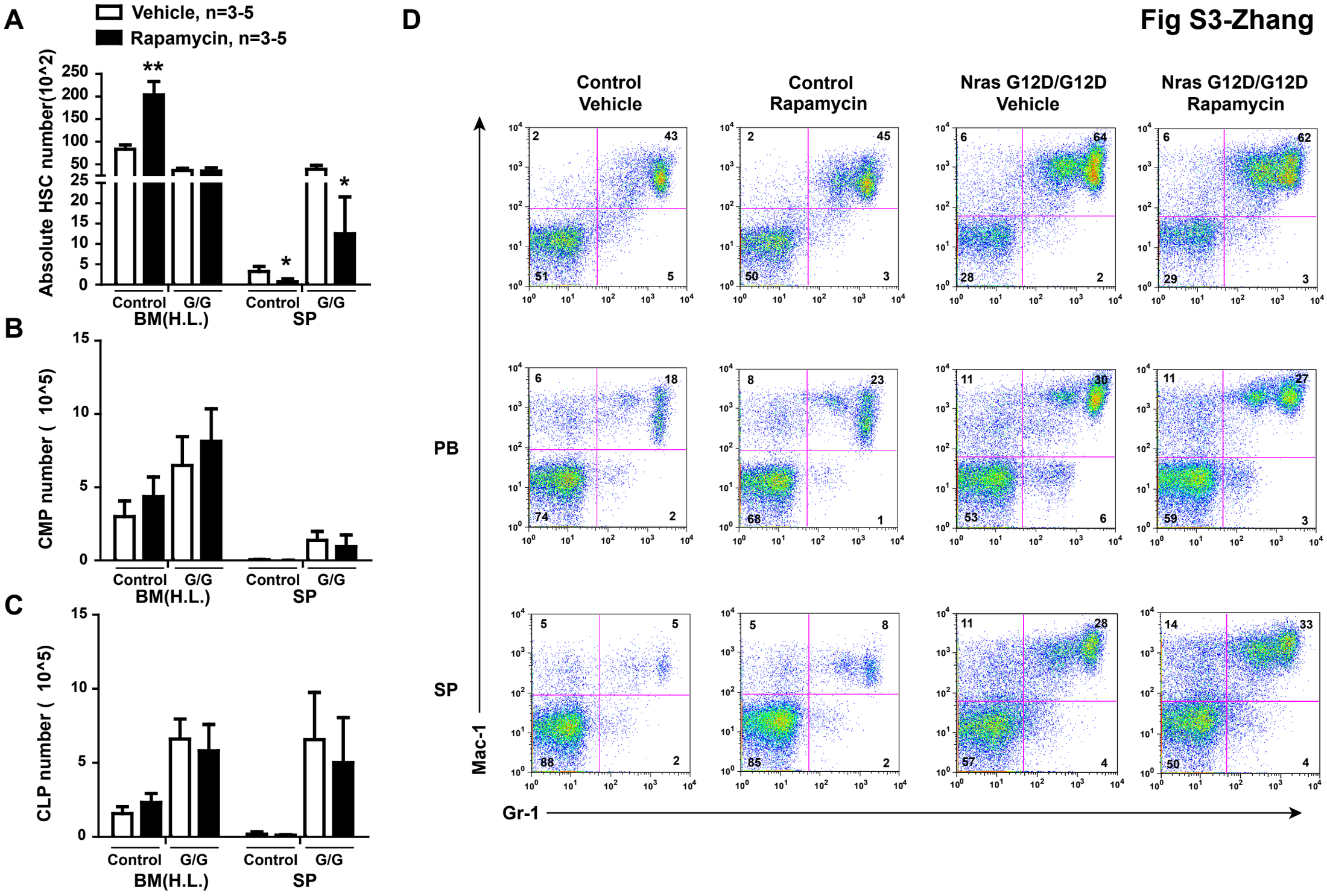
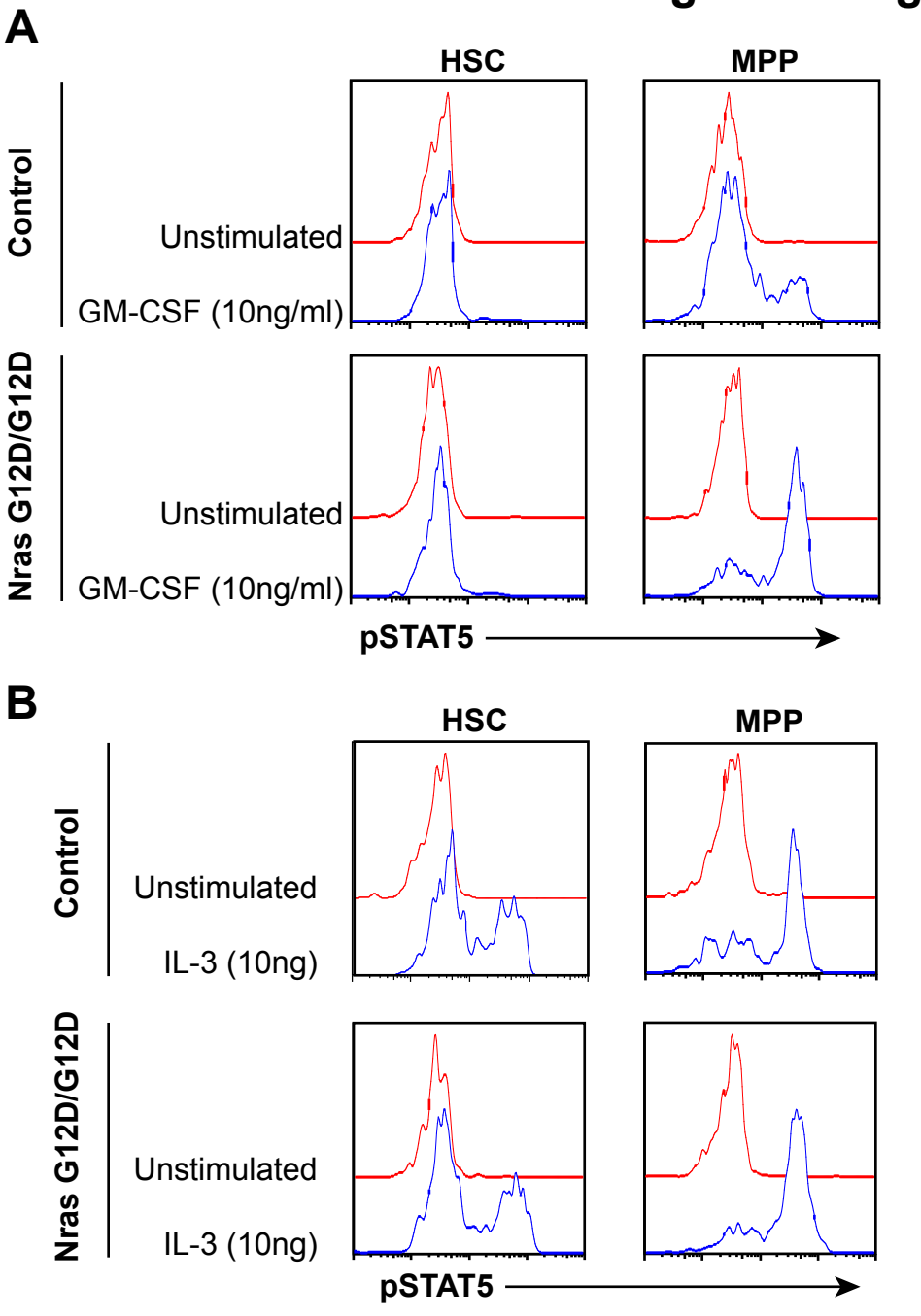
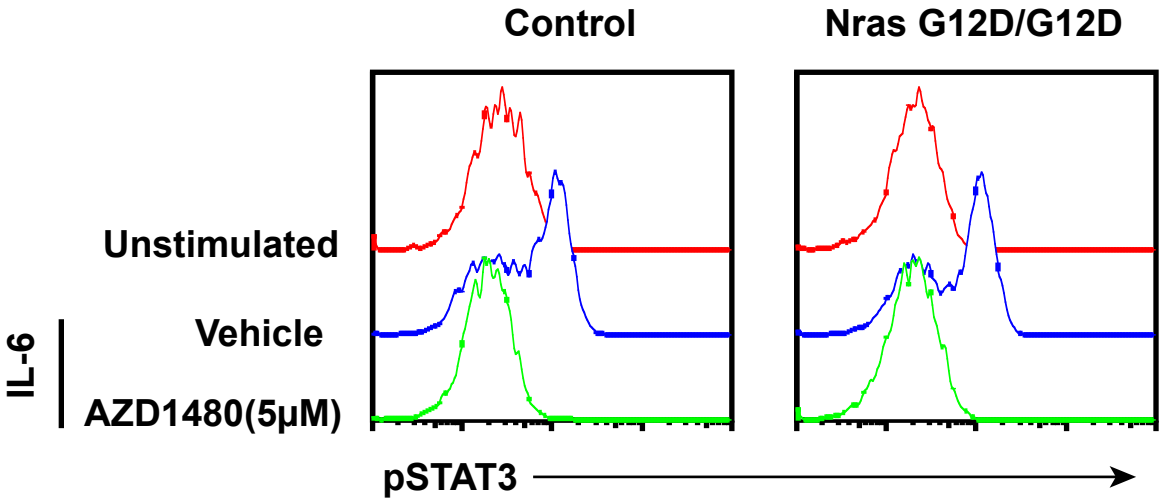

A
Vehicle, n=3-5
Rapamycin, n=3-5
Absolute HSC number(10^2)
250
200
150
100
50
25
20
15
10
5
0
**
*
*
Control
G/G
Control
G/G
BM(H.L.)
SP
B
CMP number ( 10^5)
15
10
5
0
Control
G/G
Control
G/G
BM(H.L.)
SP
C
CLP number ( 10^5)
15
10
5
0
Control
G/G
Control
G/G
BM(H.L.)
SP
D
Fig S3-Zhang
Control
Vehicle
Control
Rapamycin
Nras G12D/G12D
Vehicle
Nras G12D/G12D
Rapamycin
PB
SP
Mac-1
Gr-1
2
43
51
5
2
45
50
3
6
64
28
2
6
62
29
3
6
18
74
2
8
23
68
1
11
30
53
6
11
27
59
3
5
5
88
2
5
8
85
2
11
28
57
4
14
33
50
4

Fig S4-Zhang


A
HSC
MPP
Control
Unstimulated
GM-CSF (10ng/ml)
Nras G12D/G12D
Unstimulated
GM-CSF (10ng/ml)
pSTAT5
B
HSC
MPP
Control
Unstimulated
IL-3 (10ng)
Nras G12D/G12D
Unstimulated
IL-3 (10ng)
pSTAT5

# Fig S5-Zhang


Control
Nras G12D/G12D
Unstimulated
Vehicle
AZD1480(5μM)
IL-6
pSTAT3

# Fig S6-Zhang

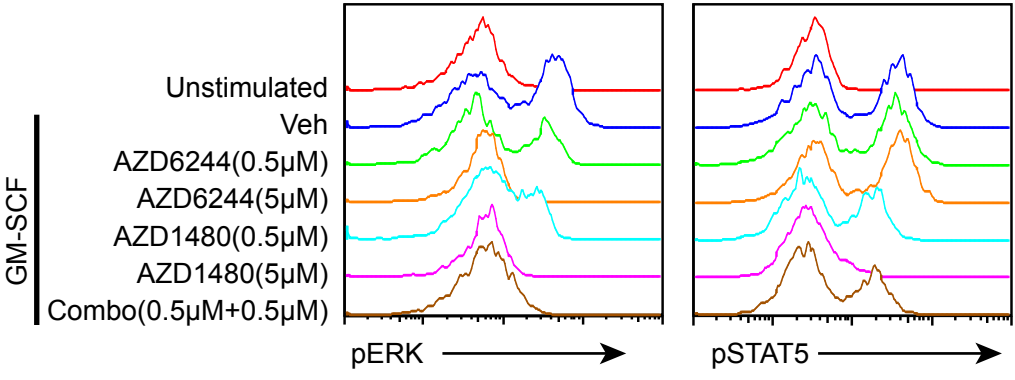
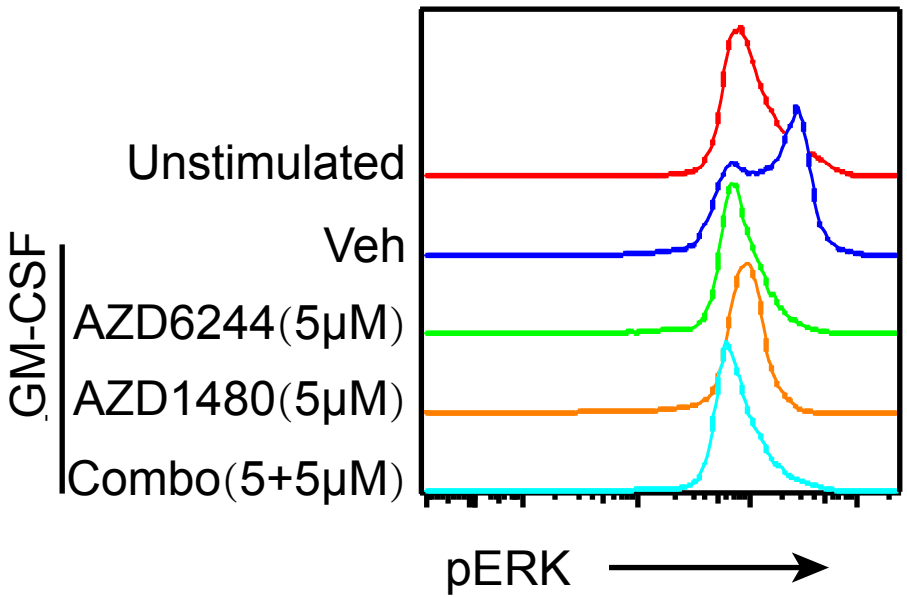
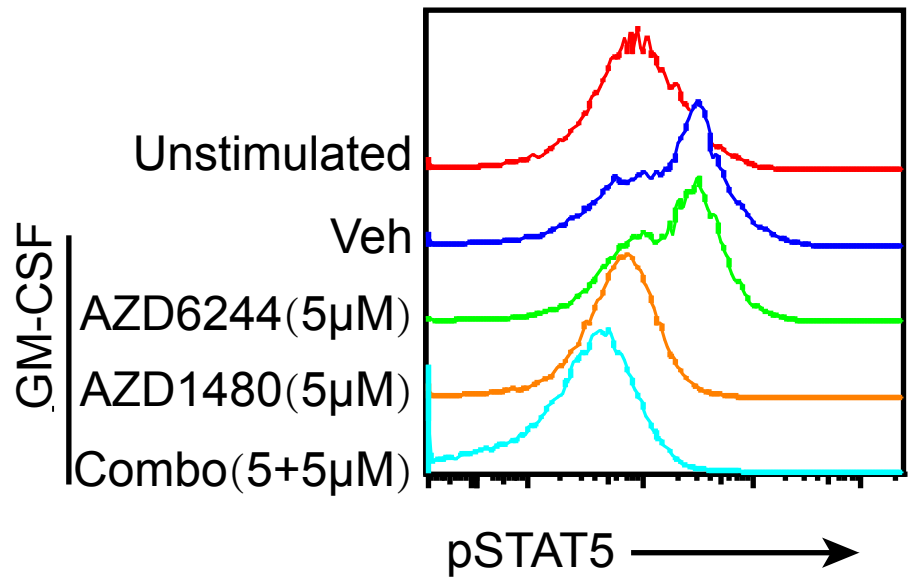

GM-SCF
Unstimulated
Veh
AZD6244(0.5μM)
AZD6244(5μM)
AZD1480(0.5μM)
AZD1480(5μM)
Combo(0.5μM+0.5μM)
pERK
pSTAT5

**Fig S7-Zhang**

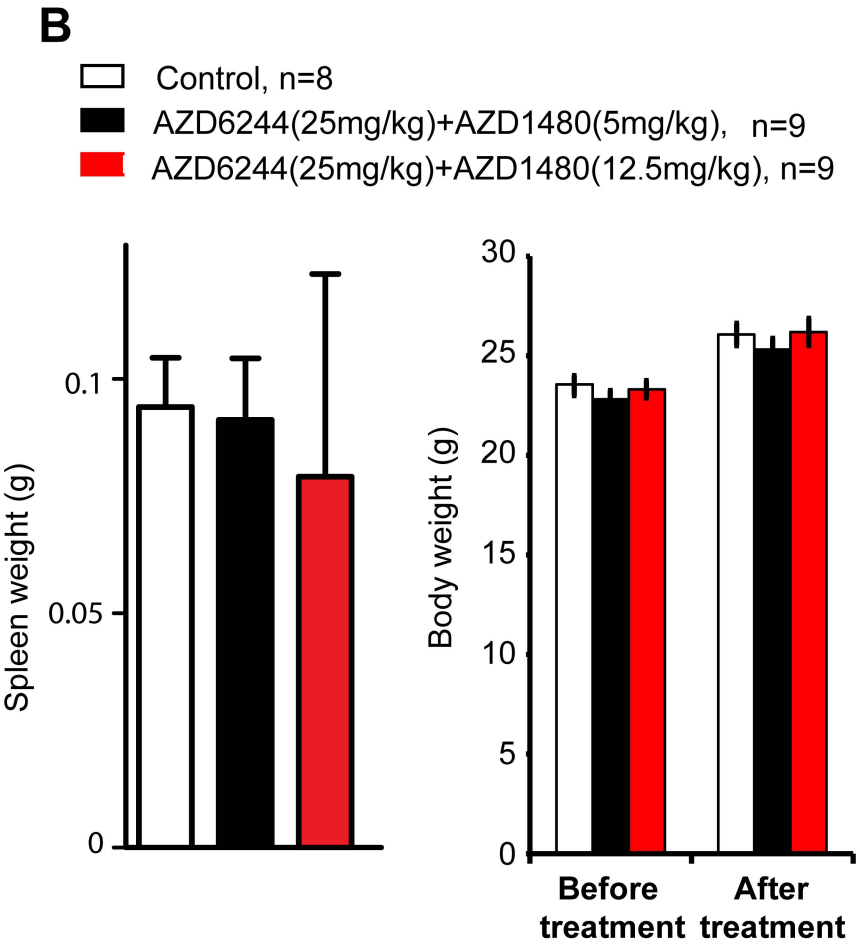

GM-CSF
Unstimulated
Veh
AZD6244(5μM)
AZD1480(5μM)
Combo(5+5μM)
pERK


GM-CSF
Unstimulated
Veh
AZD6244(5μM)
AZD1480(5μM)
Combo(5+5μM)
pSTAT5

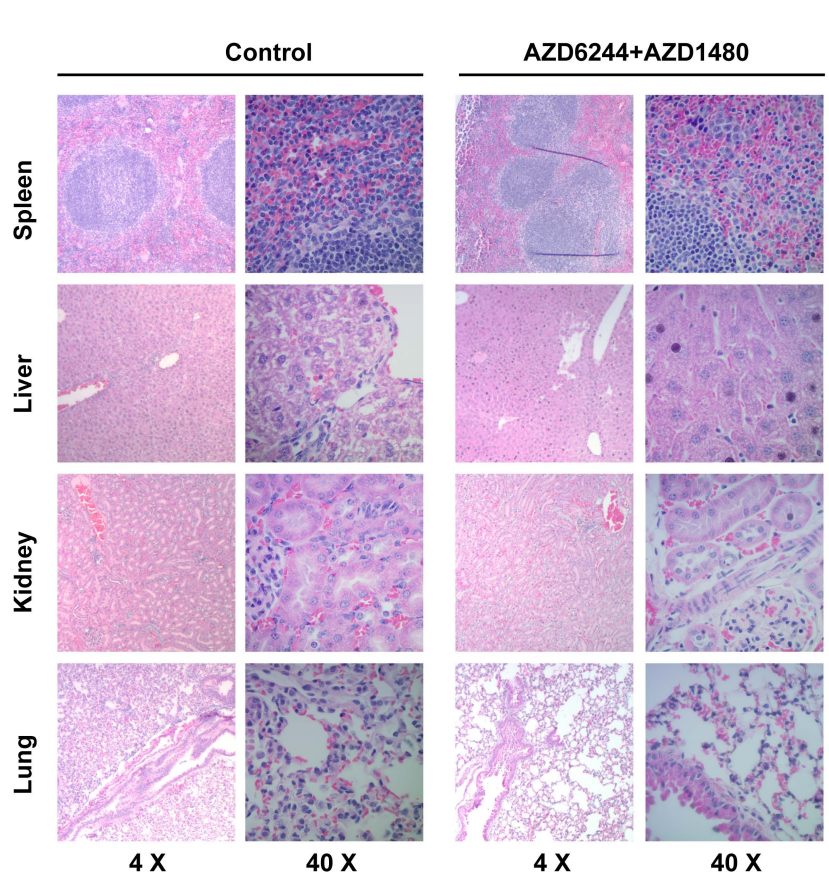
A

| Mice | WBC (× 10^3/μl) Range | Median | Monocytes (× 10^3/μl ) Range | Median | Neutrophils (× 10^3/μl ) Range | Median | Hematocrit (%) Range | Median | RBC×(X 10^6/μl) Range | Median | Platelet (× 10^3/μl) Range | Median |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WT(n=10) | 2.6 -11 | 6 | 0.11-0.72 | 0.43 | 0.25-1.89 | 1.02 | 36-43 | 40.2 | 6.9-9.56 | 8.3 | 804-1230 | 990 |
| WT+AZD6244(25mg/kg) + AZD1480(5mg/kg), n=5 | 7-11.8 | 7.8 | 0.17-0.50 | 0.30 | 2-5.61 | 3.92* | 42.4-47.6 | 44 | 8.4-9.6 | 9.1 | 1342-1548 | 1451 |
| WT+AZD6244(25mg/kg) + AZD1480(12.5mg/kg), n=5 | 5.8-8.2 | 7.2 | 0.3-0.58 | 0.49 | 1.45-2.05 | 1.8 | 30-42.2 | 38.52 | 6.38-8.6 | 7.8 | 1396-1816 | 1639 |

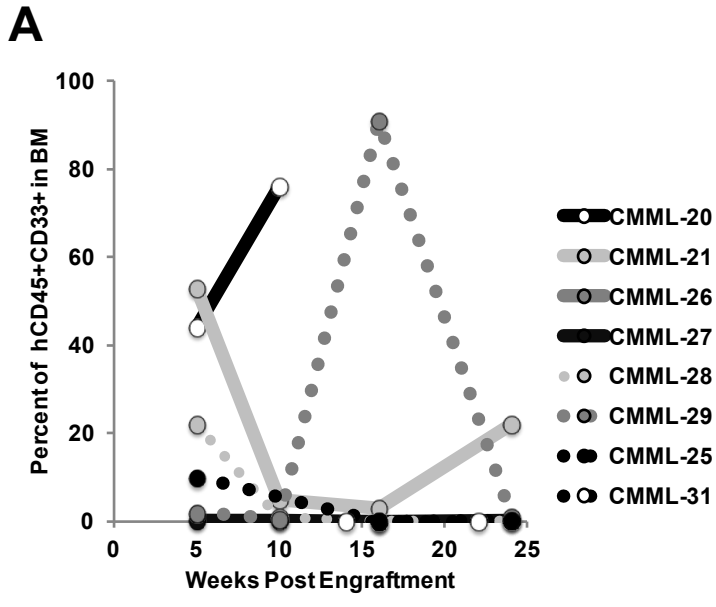
B

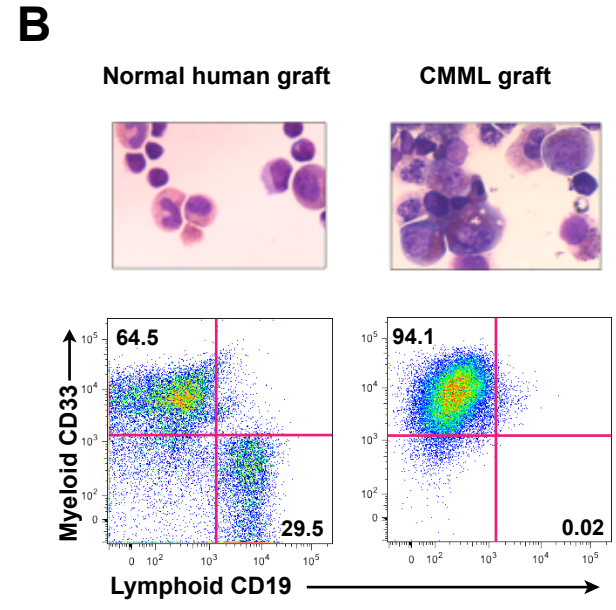



C



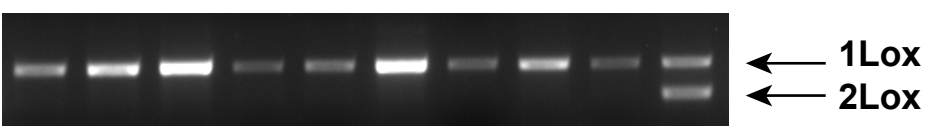
# Fig S9-Zhang


A
100
80
60
40
20
0
Percent of hCD45+CD33+ in BM
0
5
10
15
20
25
Weeks Post Engraftment
CMML-20
CMML-21
CMML-26
CMML-27
CMML-28
CMML-29
CMML-25
CMML-31


B
Normal human graft
CMML graft
64.5
29.5
94.1
0.02
Myeloid CD33
Lymphoid CD19

# Fig S10-Zhang

## Nras G12D/G12D
## (AZD6244+AZD1480)




After 20-week treatment

# Fig S11-Zhang

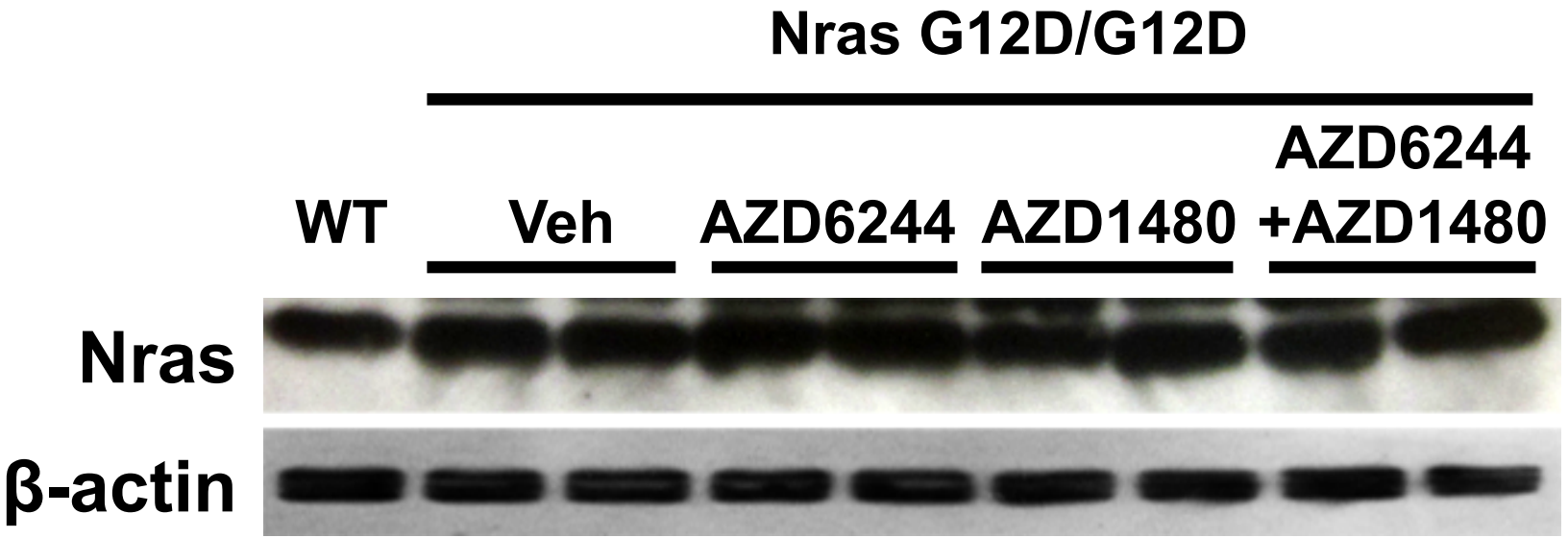


Fig S12-Zhang

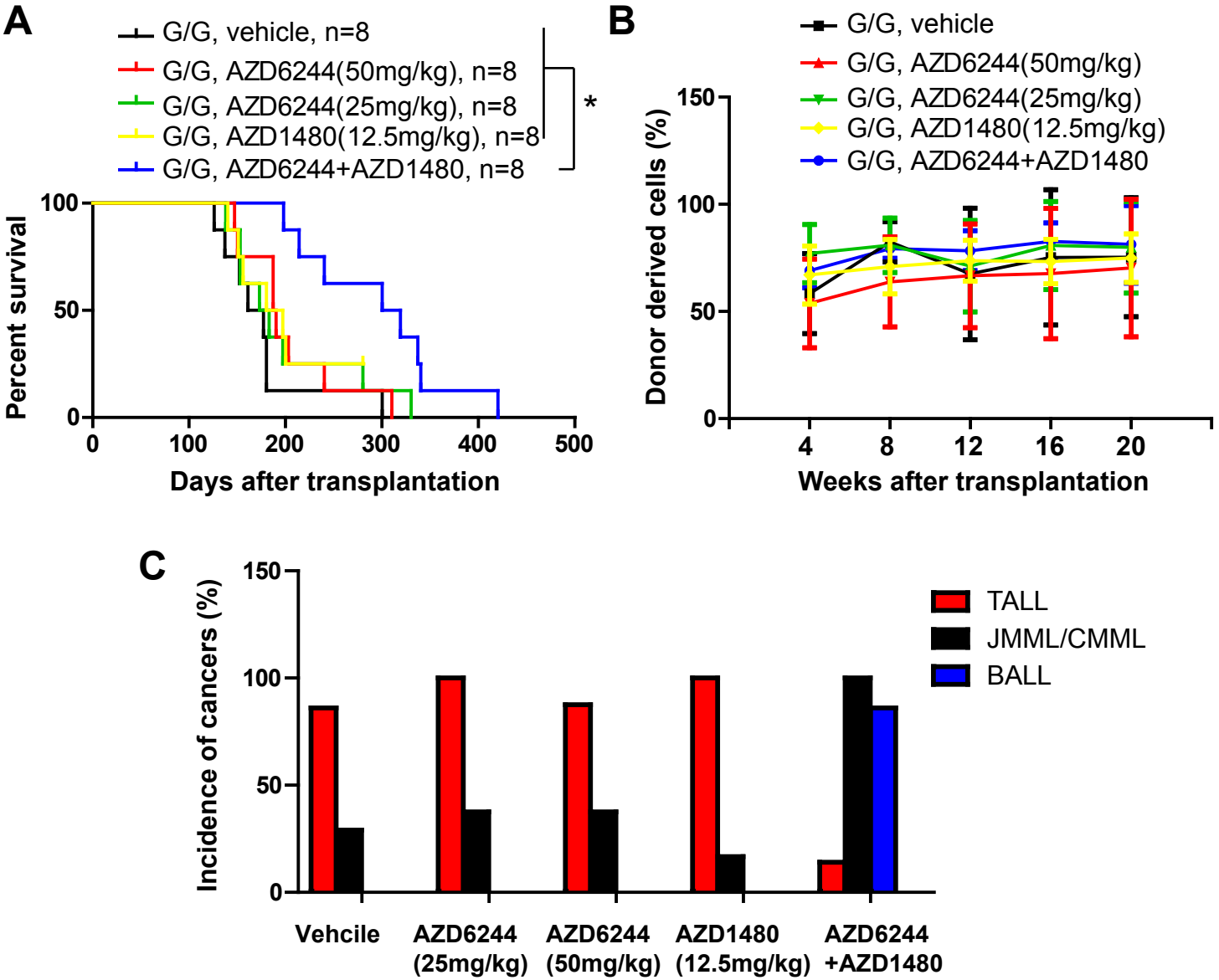


Fig S13-Zhang