Supplemental Information

Supplemental Methods

Mice. Mice bearing a conditional floxed *Dnmt3a* allele were crossed to mice carrying *LSL Nras*$^{G12D/+}$ or *Mx1-Cre* to generate *LSL Nras*$^{G12D/+}$*; Dnmt3a*$^{fl/+}$ and *Dnmt3a*$^{fl/+}$*; Mx1-Cre* mice, respectively. These two compound mice were further crossed to generate the experimental mice, including *LSL Nras*$^{G12D/+}$*; Dnmt3a*$^{fl/+}$*; Mx1-Cre*, *LSL Nras*$^{G12D/+}$; *Mx1-Cre* and *Mx1-Cre* mice. These lines were maintained in a pure C57BL/6 genetic background (>N10).

Genotyping of leukemia cells. Genomic DNAs were extracted from bone marrow, spleen, liver, and thymus of diseased recipient mice using Gentra Puregene Cell Kit (QIAGEN). Genotyping of the floxed or recombined *Kras*^{G12D} and *Dnmt3a* alleles was performed as previously described (1, 2).

Colony forming analysis. The methylcellulose based medium M3234 and M3434 were obtained from StemCell Technologies. To detect erythroid burst-forming unit (BFU-E) and other types of colonies (CFU-G, CFU-M, CFU-GM and CFU-GEMM), 2×10^4 bone marrow cells (from all groups of animals) and 2×10^5 (from *Kras*$^{G12D/+}$ and *Kras*$^{G12D/+}$*; Dnmt3a*$^{-/-}$ mice) or 1×10^6 spleen cells (from control and *Dnmt3a*$^{-/-}$ mice) were plated in duplicate in semisolid methylcellulose (M3434) according to the manufacturer's protocol. Colonies were counted after 7 days in culture. For replating analysis, 2×10^4 bone marrow cells isolated from various groups of mice were plated in duplicate in M3234 containing 1 ng/ml of IL-3. Colonies were counted after 7 days in culture and same numbers of cells were re-plated.

To detect CFU-E colonies, 2×10^5 bone marrow and 1×10^6 spleen cells from control mice and 2×10^5 bone marrow and 7.5×10^5 spleen cells from D3a$^{-/-}$ mice were plated in duplicate in M3234 in the absence or presence of various concentrations of EPO (StemCell Technologies) according to the manufacturer's protocol. Colonies were counted after 2 days in culture.

Bone marrow transplantation. Bone marrow cells were isolated from various compound mice. 2.5×10^5 bone marrow cells (CD45.2$^+$) along with same number of competitor cells (CD45.1$^+$) were injected into lethally irradiated CD45.1$^+$ recipients as previously described (3). Four weeks after transplantation, Cre expression was induced as described above. Peripheral blood was collected regularly to monitor the development of hematopoietic malignancies.

Complete blood count (CBC) and histopathology. CBC analysis was performed using a Hemavet 950FS (Drew Scientific). Mouse tissues were fixed in 10% neutral buffered formalin (Sigma-Aldrich) and further processed at the Histology Lab of the University of Wisconsin Carbone Cancer Center.

Supplemental References

1. Tuveson DA, Shaw AT, Willis NA, Silver DP, Jackson EL, Chang S*, et al.* Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. *Cancer Cell* 2004 Apr; **5**(4)**:** 375-387.

2. Nguyen S, Meletis K, Fu D, Jhaveri S, Jaenisch R. Ablation of de novo DNA methyltransferase Dnmt3a in the nervous system leads to neuromuscular defects and shortened lifespan. *Dev Dyn* 2007 Jun; **236**(6)**:** 1663-1676.

3. Zhang J, Wang J, Liu Y, Sidik H, Young KH, Lodish HF*, et al.* Oncogenic Kras-induced leukemogeneis: hematopoietic stem cells as the initial target and lineage-specific progenitors as the potential targets for final leukemic transformation. *Blood* 2009 Feb 5; **113**(6)**:** 1304-1314.

4. Socolovsky M, Nam H, Fleming MD, Haase VH, Brugnara C, Lodish HF. Ineffective erythropoiesis in Stat5a(-/-)5b(-/-) mice due to decreased survival of early erythroblasts. *Blood* 2001; **98**(12)**:** 3261-3273.

Supplemental Figure Legends

Figure S1. Loss of *Dnmt3a* induces a transient anemia *in vivo*. Control and *Dnmt3a*$^{fl/fl}$; *Mx1-Cre* mice were treated with pI-pC as described in Materials and Methods. (A) Complete blood count was performed regularly after pI-pC treatment. The results are presented as means ± SD. ***P<0.001. (B) Different lineages of cells from bone marrow (BM), spleen (SP), and peripheral blood (PB) of control and *Dnmt3a*$^{-/-}$ mice were analyzed using flow cytometry two months after pI-pC treatment. No significant changes were observed.

Figure S2. Loss of *Dnmt3a* induces moderately increased erythropoiesis in spleen two months after pI-pC treatment. Control and *Dnmt3a*$^{fl/fl}$; *Mx1-Cre* mice were treated with pI-pC and sacrificed two months after pI-pC treatment when *Dnmt3a*$^{-/-}$ (*D3a*$^{-/-}$) mice developed a transient anemia. (A) Representative spleen histologic H&E sections from control and *D3a*$^{-/-}$ mice. (B, C) Erythroid differentiation was analyzed in bone marrow (BM) and spleen (SP) of control and *D3a*$^{-/-}$ mice as previously described (4). (E,F) CFU-E assay was performed in the absence or presence of various concentrations of EPO. Colony numbers cultured in the presence of 3 U/ml of EPO are shown (E) and set as 100% (F). The results are presented as means ± SD. * P<0.05.

Figure S3. Recipients transplanted with *Dnmt3a*$^{-/-}$ bone marrow cells die of hematopoietic malignancies after a prolonged latency. 2.5 X 10^5 control or *Dnmt3a*$^{-/-}$ bone marrow cells along with same number of competitor cells were transplanted into individual lethally irradiated mice. (A) Kaplan-Meier survival curves were plotted against days after transplantation. (B) Quantification of thymus and spleen weights in different groups of recipients. (C) Flow cytometric analysis of lymphoid compartment in different hematopoietic tissues. The results are presented as means ± SD. * P<0.05.

Figure S4. Representative spleen histologic H&E sections from moribund recipient mice transplanted with *Kras*$^{G12D/+}$ (Kras) or *Kras*$^{G12D/+}$*; Dnmt3a*$^{-/-}$ (Kras; D3a$^{-/-}$) cells. MPN, myeloproliferative neoplasm; AML, acute myeloid leukemia.

Figure S5. Genotyping analysis of leukemia cells. Bone marrow cells from moribund recipient mice with myeloid diseases were isolated. Genomic DNAs from various mice were subjected to genotyping analysis as described in Supplemental methods.

Figure S6. Loss of *Dnmt3a* in *Kras*$^{G12D/+}$ cells promotes development of two types of T-ALL. Lethally irradiated mice were transplanted with 2.5×10^5 bone marrow cells from different groups of mice along with same number of competitor cells. Four weeks after transplantation, Cre expression was induced by pI-pC injections. (A) Frequency of donor-derived T cells (Thy1.2$^+$ CD19$^-$) in thymus from different groups of recipients. (B, C) Quantification of CD4$^-$ CD8$^-$ T cells in thymus (B) and thymus weight (C). (D) Analysis of *Notch1* type I deletion in different groups of recipient mice with T-ALL. The results are presented as means ± SD. * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Table S1. Complete blood count (CBC) on Day 5

Parameters	Control (n=14)	D3a$^{-/-}$ (n=4)	Kras (n=12)	Kras; D3a$^{-/-}$ (n=10)
WBC (10^3 cells/μl)	7.1±2.3	7.78±3.77	20.6±11.6***, #	17.3±6.1***, ##
RBC (10^6 cells/μl)	8.9±0.8	8.7±1.89	8.6±1.1	6.3±2.1***; #, ΨΨ
Hemoglobin (g/dL)	13±0.9	11.79±2.88	10.9±1.9**	8.9±2.7***; #, Ψ
Hematocrit (%)	43.1±3.4	41.96±9.85	39.3±3.4**	31.1±9***; #, ΨΨ
RDW (%)	17.9±2.1	17.33±3.14	23.7±2.3***, ###	31.1±4.8***; ###, ΨΨΨ
Platelet (10^3 cells/μl)	882.3±233.9	711.43±170	510.3±138.4***, #	466.2±157.5***, ##

All are Mean±SD. Student *t*-test was performed. Compare to control: ** $P<0.01$; *** $P<0.001$. Compare to D3a$^{-/-}$: # $P<0.05$; ## $P<0.01$; ### $P<0.001$. Compare to G12D: Ψ $P<0.05$; ΨΨ $P<0.01$; ΨΨΨ $P<0.001$

Table S2. Complete blood count (CBC) of moribund recipients

Parameters	Control (n=14)	Kras (n=16)	Kras; D3a$^{-/-}$ (n=23)
WBC (10^3 cells/μl)	8.91±2.76	8.53±11.32	14.44±26.97
RBC (10^6 cells/μl)	9.36±0.89	8.48±1.13*	5.68±2***; ###
Hemoglobin (g/dL)	13.55±1.59	12.18±1.99*	8.54±2.62***; ###
Hematocrit (%)	43.06±4	40.45±5.59	28.86±8.49***; ###
RDW (%)	16.25±2.39	19.2±3**	22.72±4.75***; #
Platelet (10^3 cells/μl)	909.57±193.76	622.31±221.28***	285.91±165.82***; ###

All are Mean±SD. Student *t*-test was performed. Compare to control: * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Compare to G12D: # $p<0.05$; ### $p<0.001$.

Fig S1-Zhang

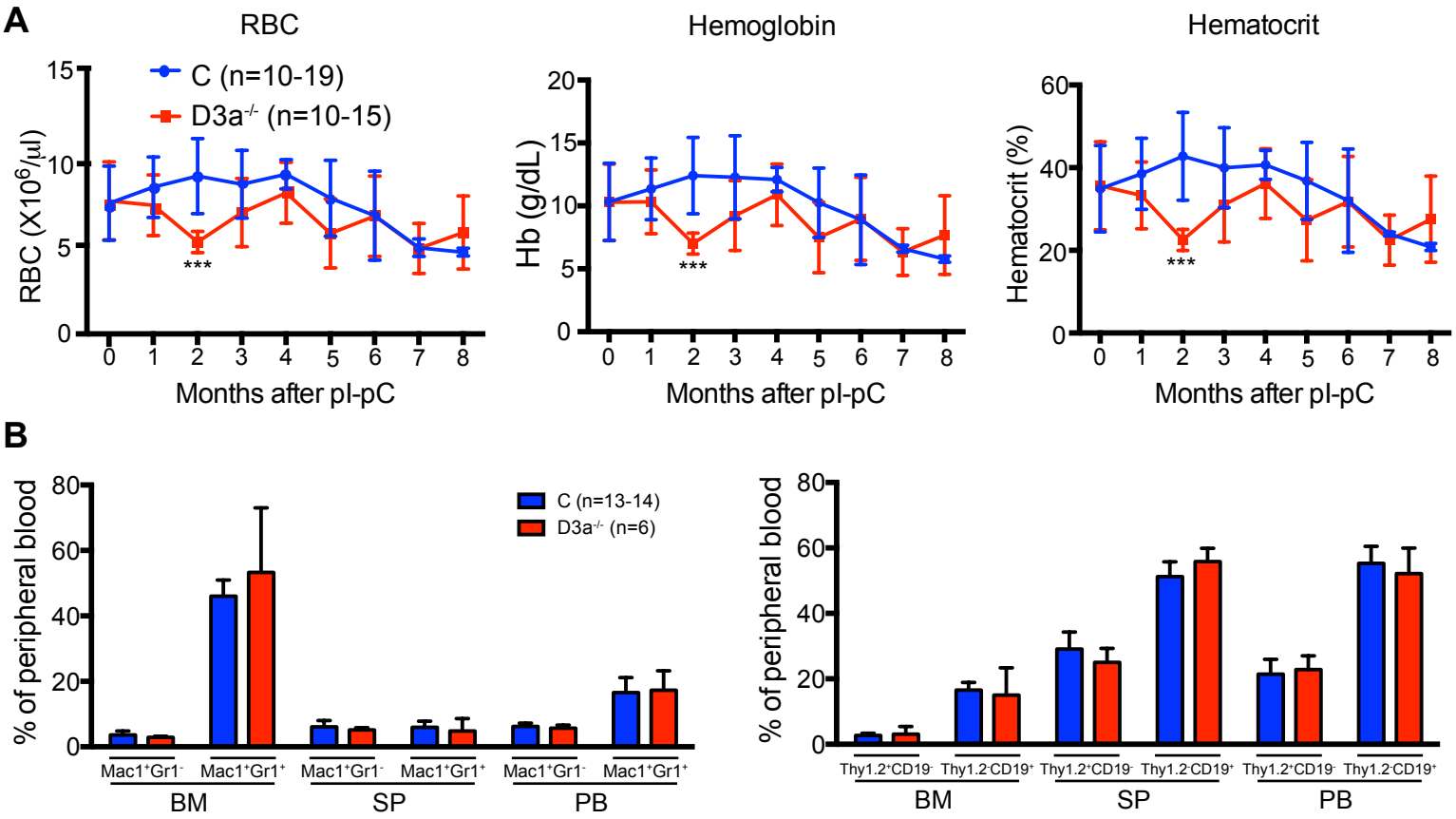

A
RBC
Hemoglobin
Hematocrit
C (n=10-19)
D3a-/- (n=10-15)
RBC (X10^6/μl)
Hb (g/dL)
Hematocrit (%)
***
Months after pI-pC
B
C (n=13-14)
D3a-/- (n=6)
% of peripheral blood
Mac1+Gr1-
Mac1+Gr1+
Thy1.2+CD19-
Thy1.2-CD19+
BM
SP
PB

Fig S2-Zhang

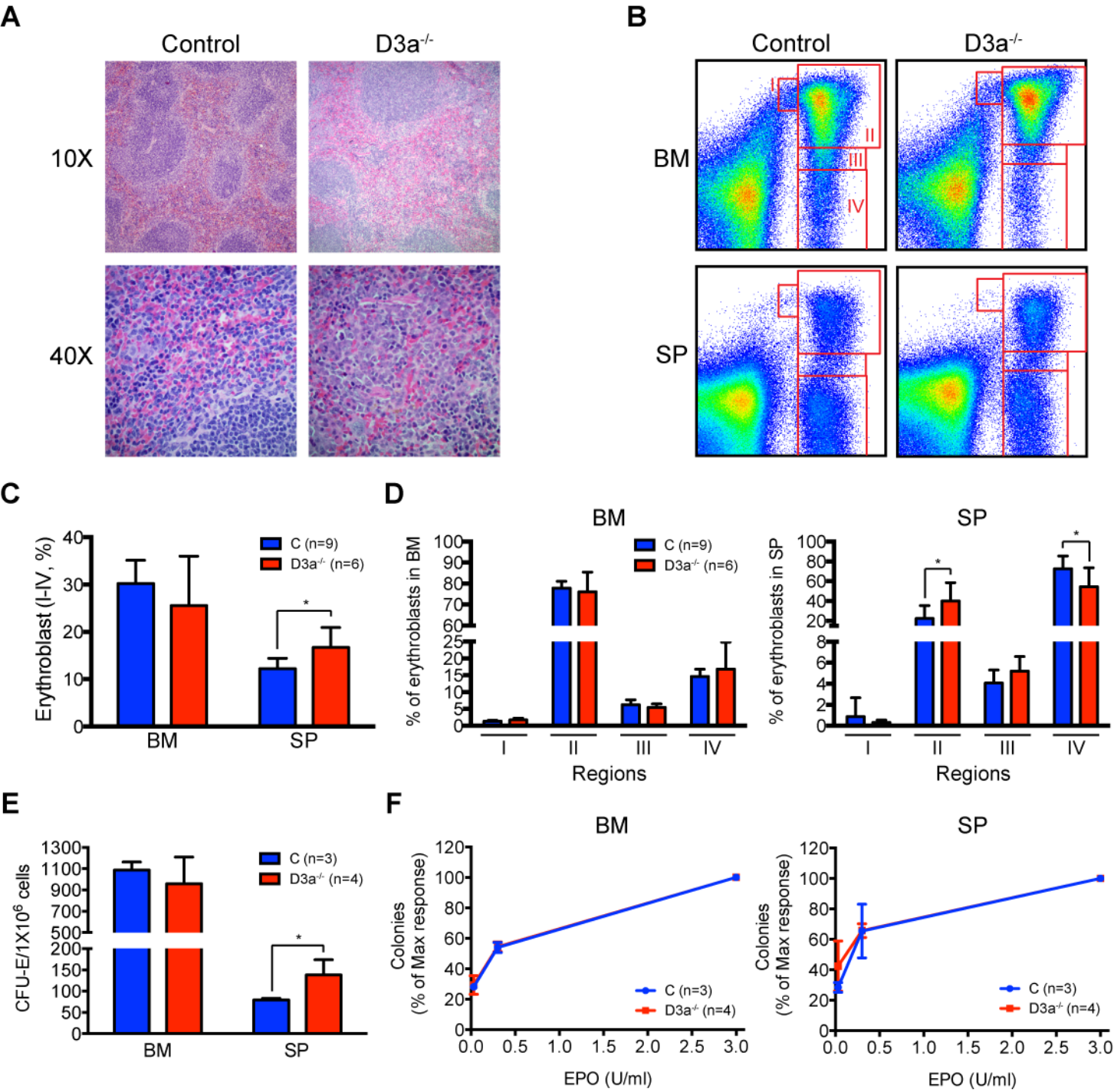

A
Control
D3a-/-
10X
40X
B
Control
D3a-/-
BM
SP
I
II
III
IV
C
Erythroblast (I-IV, %)
C (n=9)
D3a-/- (n=6)
BM
SP
D
BM
SP
% of erythroblasts in BM
% of erythroblasts in SP
C (n=9)
D3a-/- (n=6)
I
II
III
IV
Regions
E
CFU-E/1X10^6 cells
C (n=3)
D3a-/- (n=4)
BM
SP
F
BM
SP
Colonies (% of Max response)
C (n=3)
D3a-/- (n=4)
EPO (U/ml)

Fig S3-Zhang

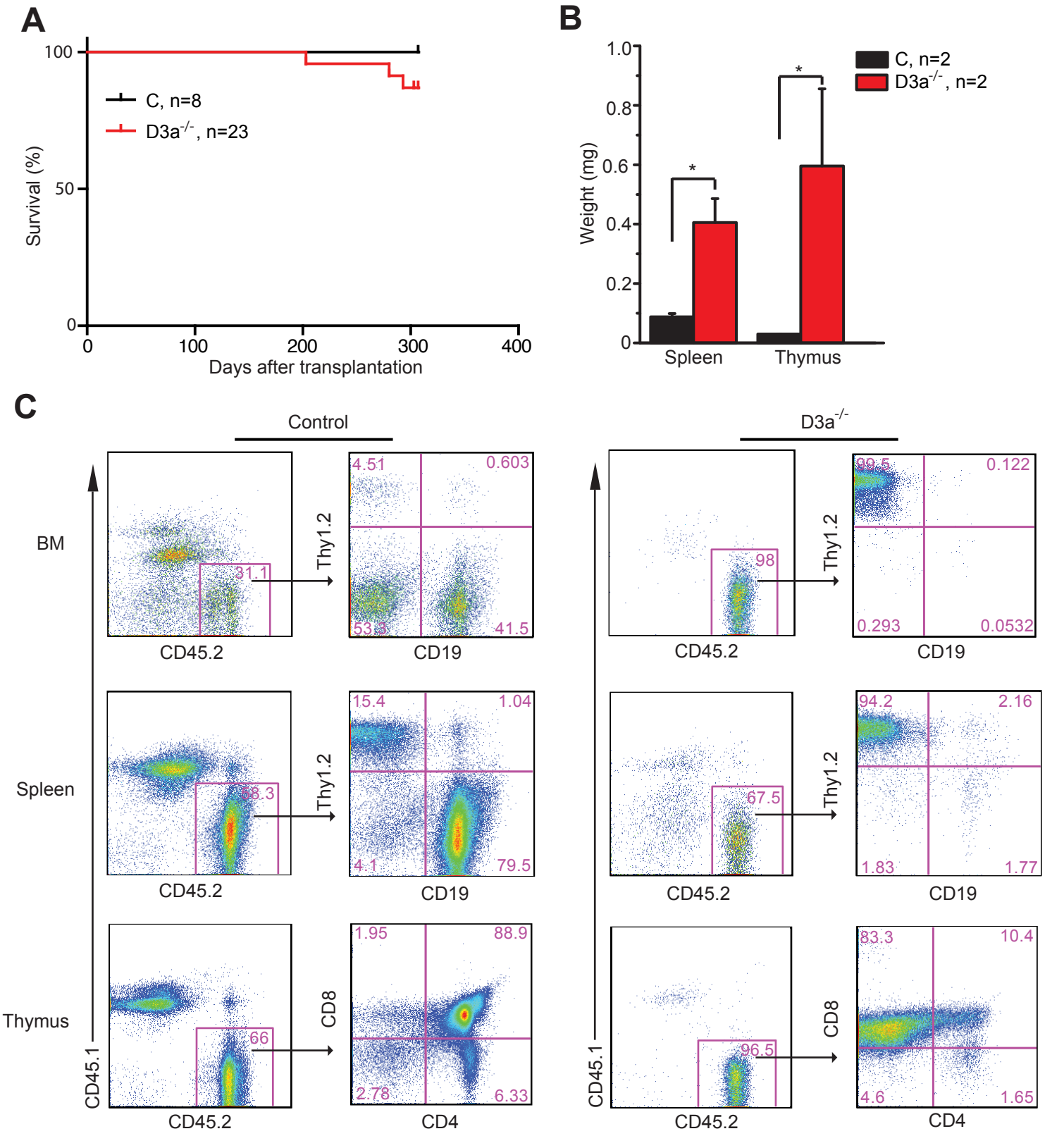

A
100
50
0
Survival (%)
C, n=8
D3a-/-, n=23
0
100
200
300
400
Days after transplantation
B
1.0
0.8
0.6
0.4
0.2
0
Weight (mg)
C, n=2
D3a-/-, n=2
*
*
Spleen
Thymus
C
Control
D3a-/-
BM
Spleen
Thymus
CD45.2
Thy1.2
CD19
CD8
CD4
CD45.1
31.1
4.51
0.603
53.3
41.5
98
99.5
0.122
0.293
0.0532
68.3
15.4
1.04
4.1
79.5
67.5
94.2
2.16
1.83
1.77
66
1.95
88.9
2.78
6.33
96.5
83.3
10.4
4.6
1.65

Fig S4-Zhang

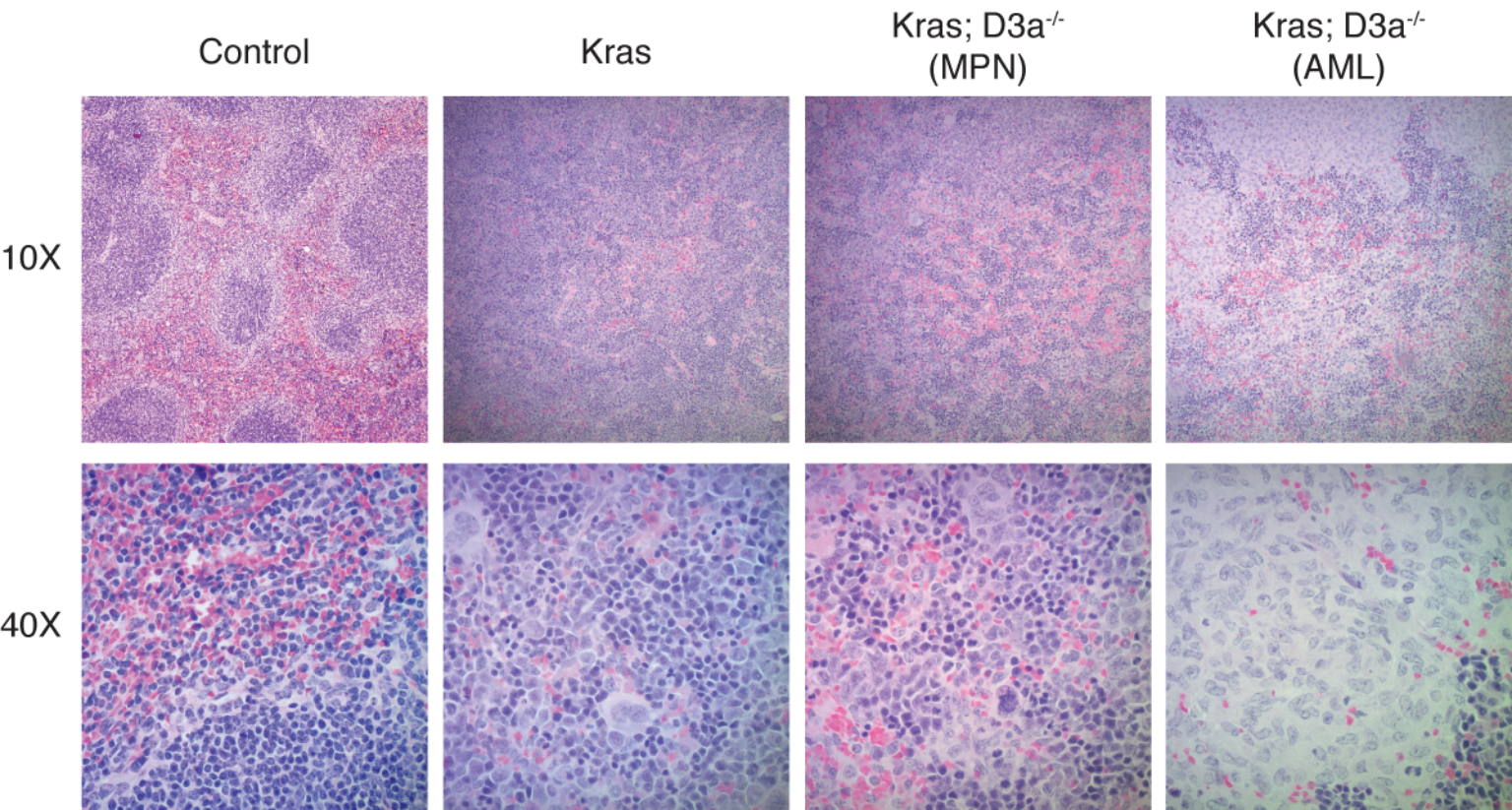


Fig S5-Zhang

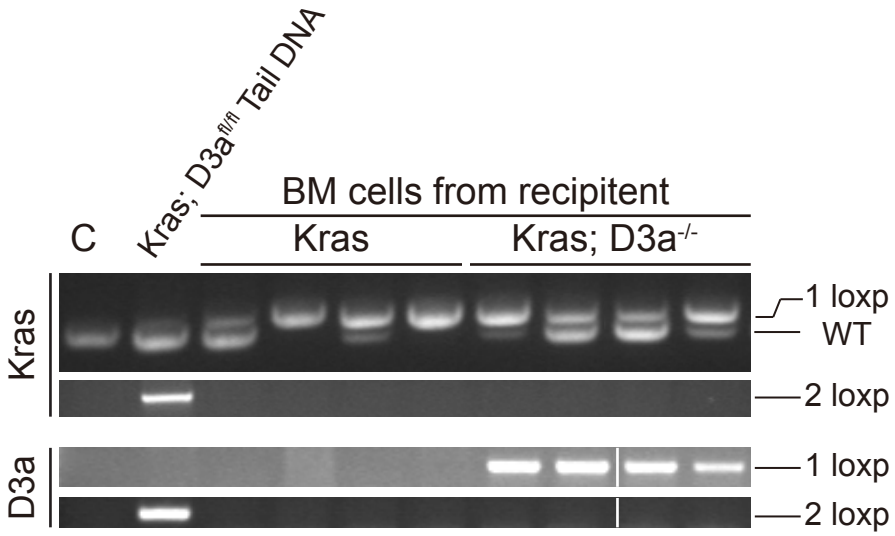
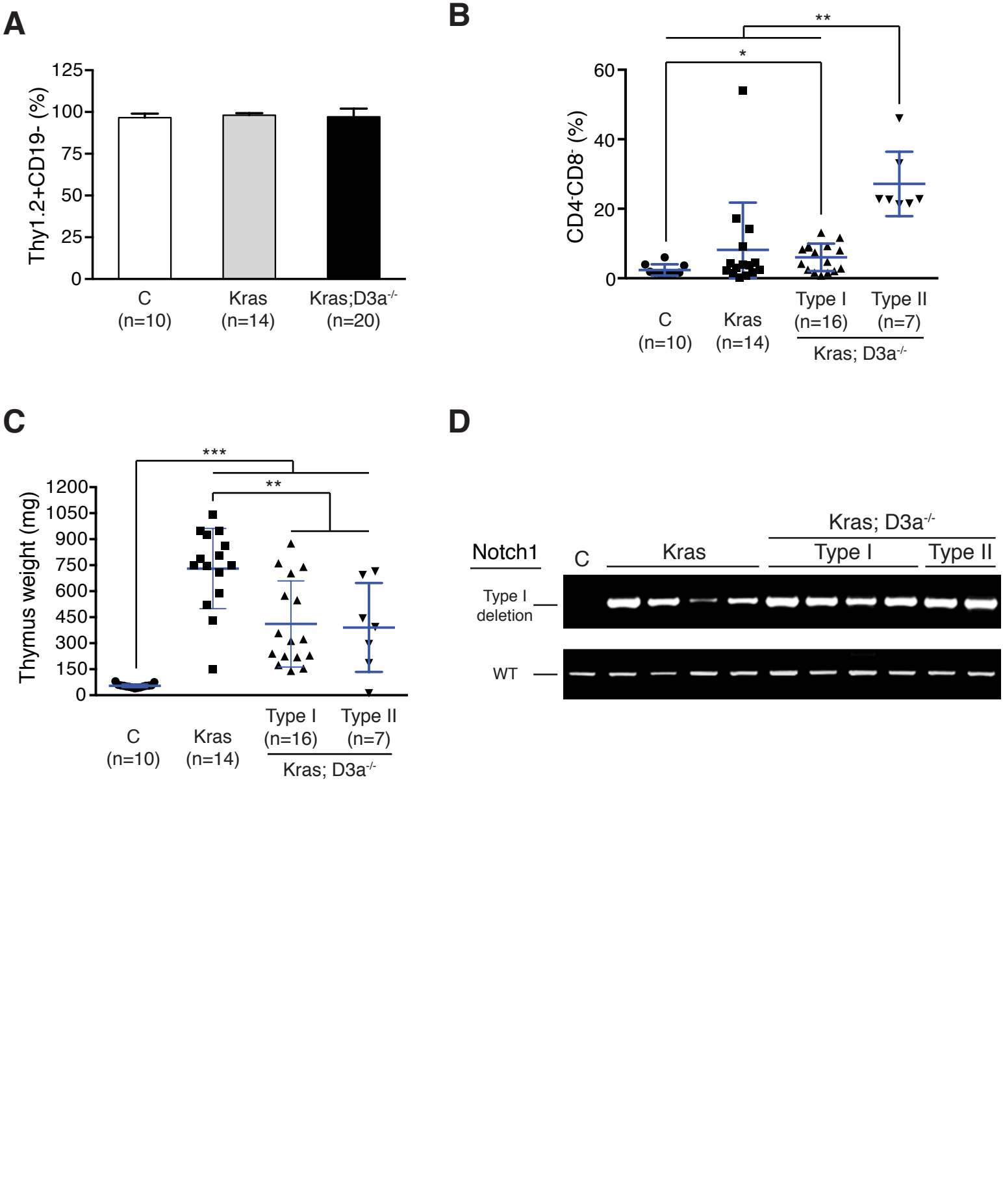

C
Kras; D3a$^{fl/fl}$ Tail DNA
BM cells from recipitent
Kras
Kras; D3a$^{-/-}$
Kras
D3a
1 loxp
WT
2 loxp
1 loxp
2 loxp

Fig S6-Zhang


A
Thy1.2+CD19- (%)
125
100
75
50
25
0
C
(n=10)
Kras
(n=14)
Kras;D3a-/-
(n=20)
B
CD4-CD8- (%)
60
40
20
0
*
**
C
(n=10)
Kras
(n=14)
Type I
(n=16)
Type II
(n=7)
Kras; D3a-/-
C
Thymus weight (mg)
1200
1050
900
750
600
450
300
150
0
***
**
C
(n=10)
Kras
(n=14)
Type I
(n=16)
Type II
(n=7)
Kras; D3a-/-
D
Kras; D3a-/-
Notch1
C
Kras
Type I
Type II
Type I
deletion
WT