

***Setd2* deficiency in mesenchymal stem cells promotes transformation of myelodysplastic syndrome to leukemia**

Supplementary data

Supplementary Tables

Supplementary Table1. Clinical characteristics of high and low risk MDS patients.....2

Supplementary Table2. The sequence of primers used for real time PCR.....3

Supplementary Table 3. Survival of NHD13 mice and *Lep-Cre/Prx1-Cre*NHD13*Setd2*^{Δ/Δ} mice with MDS or leukemia.....4

Supplementary Figures

Supplementary Figure S1. MSC targeted knockout of *Setd2* inhibited lymphocyte differentiation.....5

Supplementary Figure S2. MSC targeted knockout of *Setd2* prevented bone marrow failure in mice.....6

Supplementary Figure S3. The impact of hematopoietic stem cell transplantation on the bone marrow microenvironment is limited.....7

Supplementary FigureS4. Simplified model of MSC targeted knockout of *Setd2* and F12 mediated control of HSPC self-renewal and differentiation, eventually leading to a trend towards leukemia conversion.....8

Supplementary Figure Legends.....9

Supplementary Table 1. Clinical characteristics of high and low risk MDS patients

Characteristic	High risk MDS (n=6)	Low risk MDS (n=4)	P value
Age, y, median(range)	50(27-85)	67(39-75)	0.51
Male: Female, n (%)	2:4(33.33)	3:1(75.00)	0.52
Diagnosis, n (%)			0.13
MDS-MLD	2	1	
MDS-RS	1	3	
MDS-EB	3	0	
Karyotype			0.64
normal	3	3	
20q-	1	0	
others	1	1	
Complex	1	0	
Blast percentage in marrow, %, mean± SD	7.77±4.64	0.43±0.08	<0.001***
WBC, *10 ⁹ /L, mean±SD	1.84±0.53	5.68±3.62	0.12
Hemoglobin, g/L, mean±SD	84.67±29.00	81.75±19.89	0.86
Platelet, *10 ⁹ /L, mean±SD	59.00±50.57	186.30±64.53	0.02*
IPSS-R, n (%)			0.02*
Low	0	1	
Intermediate	0	3	
High	4	0	
Very high	2	0	
AML transformed, n (%)	2(33.33)	0(0)	NA
Survival, y, median (range)	2(1-11)	2(1-12)	0.35

WBC indicates white blood cells; IPSS-R, Revised International Prognostic Scoring System for Myelodysplastic Syndromes; AML, acute myeloid leukemia; NA, not applicable; *P<0.05, ***P<0.001.

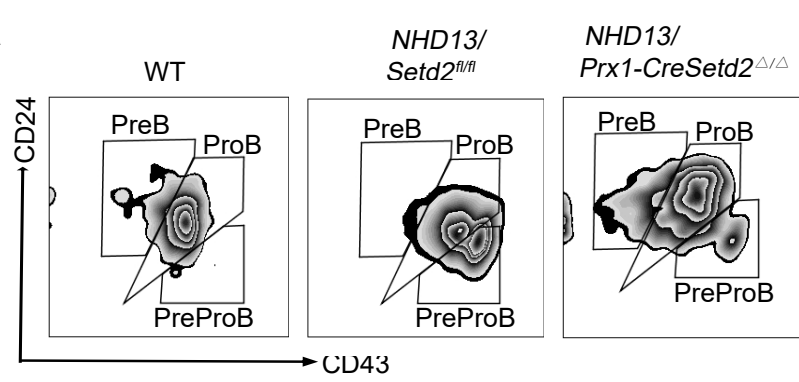
Supplementary Table 2. The sequence of primers used for real time PCR

Gene	Forward primer (5' - 3')	Reverse primer (5' - 3')
GAPDH	GCACCGTCAAGGCTGAGAAC	GTGGTGAAGACGCCAGTGGA
S100A9	TGGCTCCTCGGCTTTGACAGA GT	TGGGTGCCCCAGCTTCACAG A
Prx1-cre	CACCCTGTTACGTATAGCCG	GAGTCATCCTTAGCGCCGTA
Lep-cre	ATGTCCAATTTACTGACCGTAC A	CGCATAACCAGTGAAACAGC ATT
NHD13	TGGAGGGCCTCTTGGTACAGG	GGCTTCTAAGCTGTCTGTGGC C
Setd2	GCCTCGTTTGCATTTGTTTT	TCAGTCAGCTGAAGCAGGAA
Fn1	CCCTATCTCTGATACCGTTGTC C	TGCCGCAACTACTGTGATTCG G

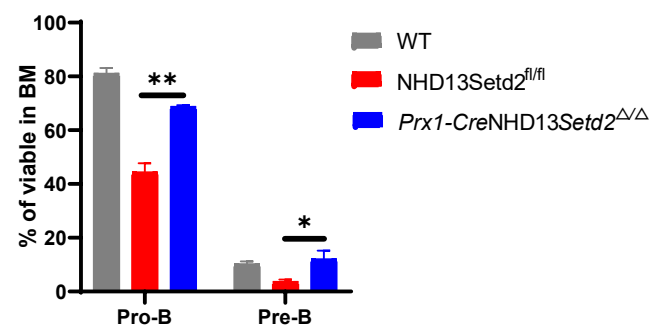
**Supplementary Table 3. Survival of NHD13 mice and
*Lep-Cre/Prx1-Cre*NHD13*Setd2*^{Δ/Δ} mice with MDS or leukemia**

Mice	NHD13	<i>Lep-cre</i> NHD13 <i>Setd2</i> ^{Δ/Δ}	<i>Prx1-cre</i> NHD13 <i>Setd2</i> ^{Δ/Δ}
Median survival, d	301	265	192
Total number (%)			
Died from MDS	10(67)	8(53)	6(40)
Died from AML	5(33)	7(47)	9(60)
Overall	15(100)	15(100)	15(100)

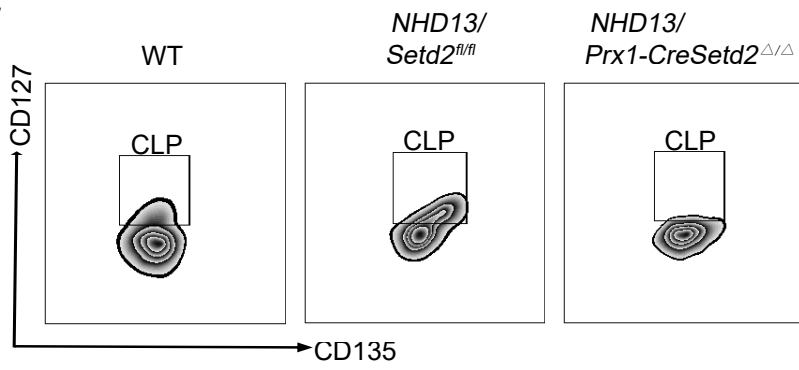
A



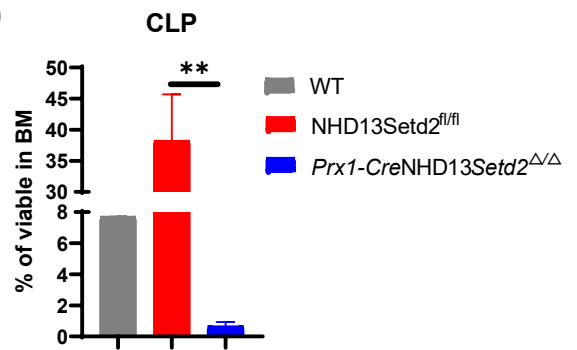
B



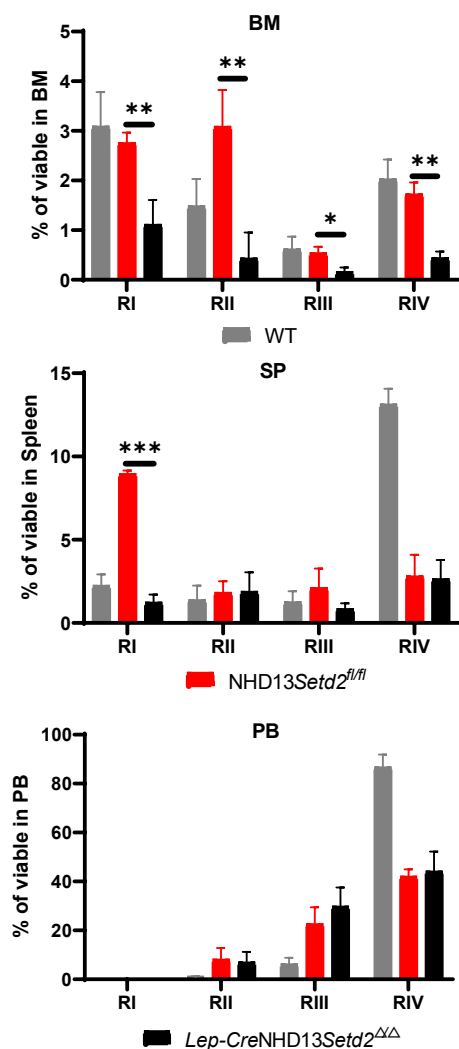
C



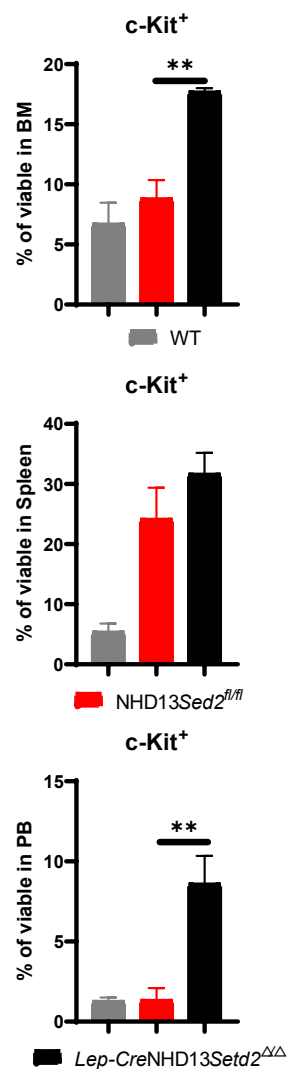
D



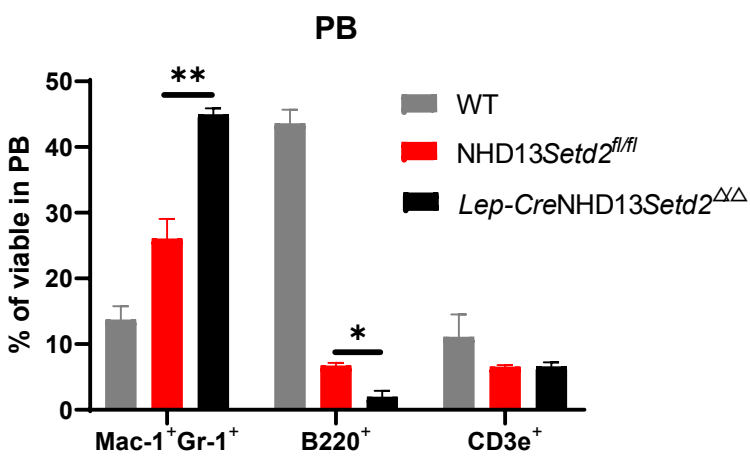
A



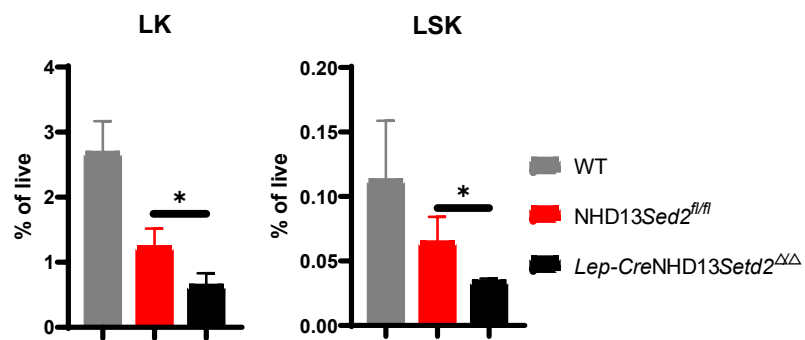
B



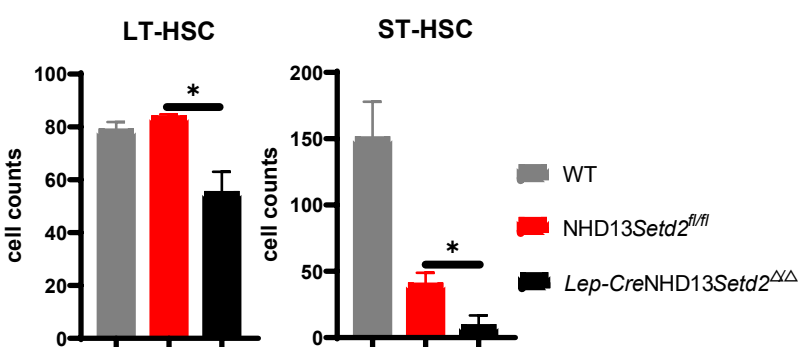
C



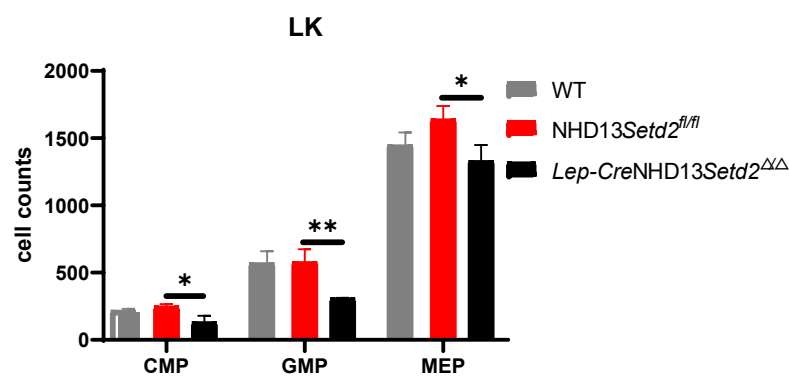
D



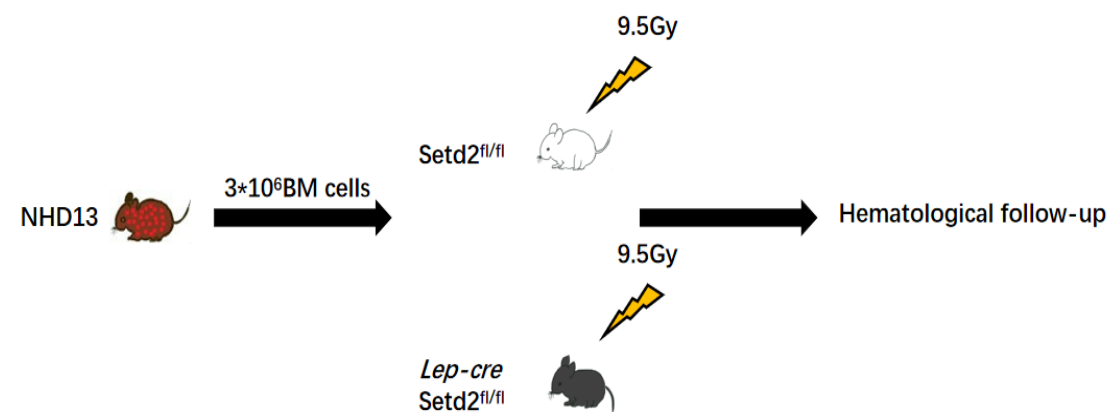
E



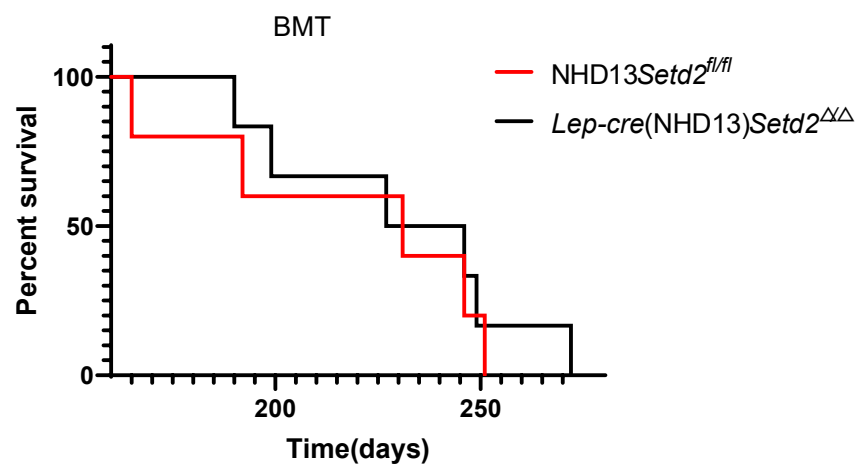
F



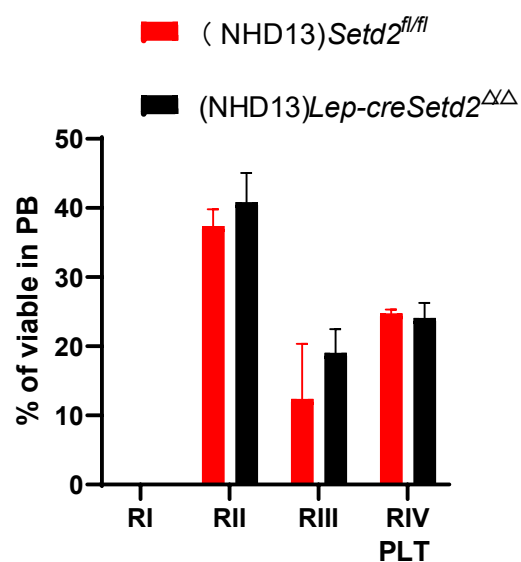
A



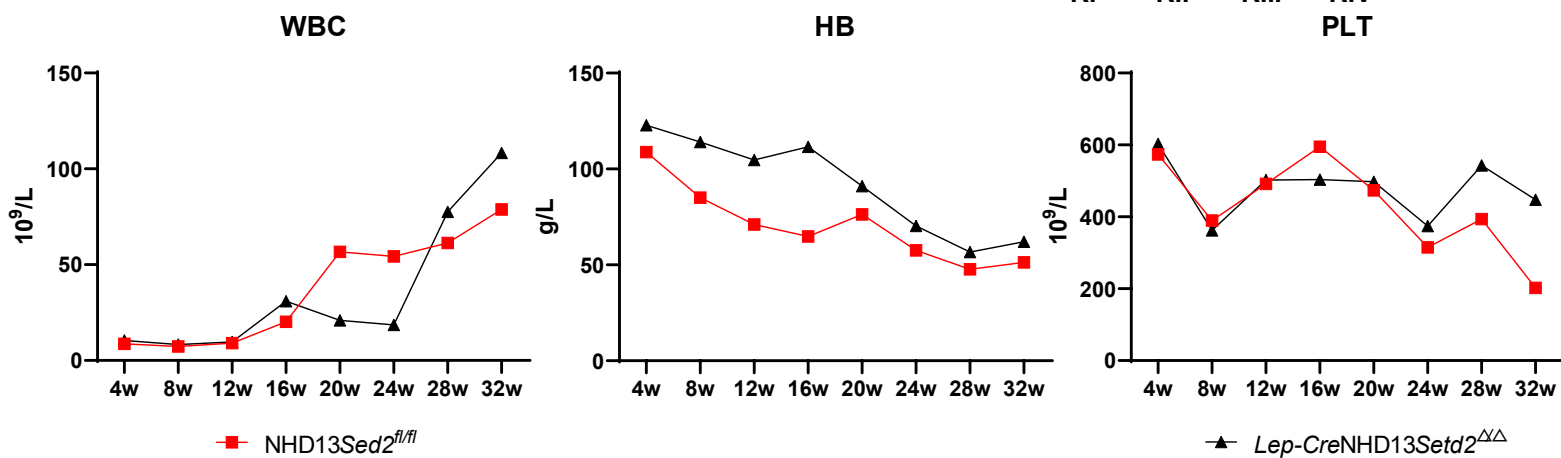
B



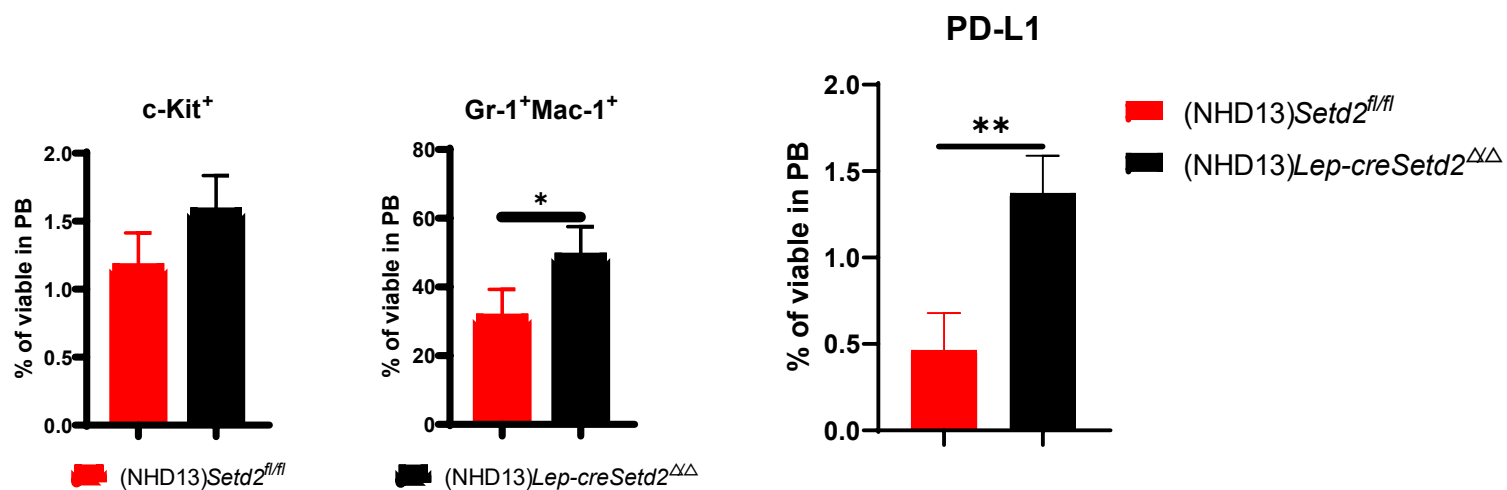
C

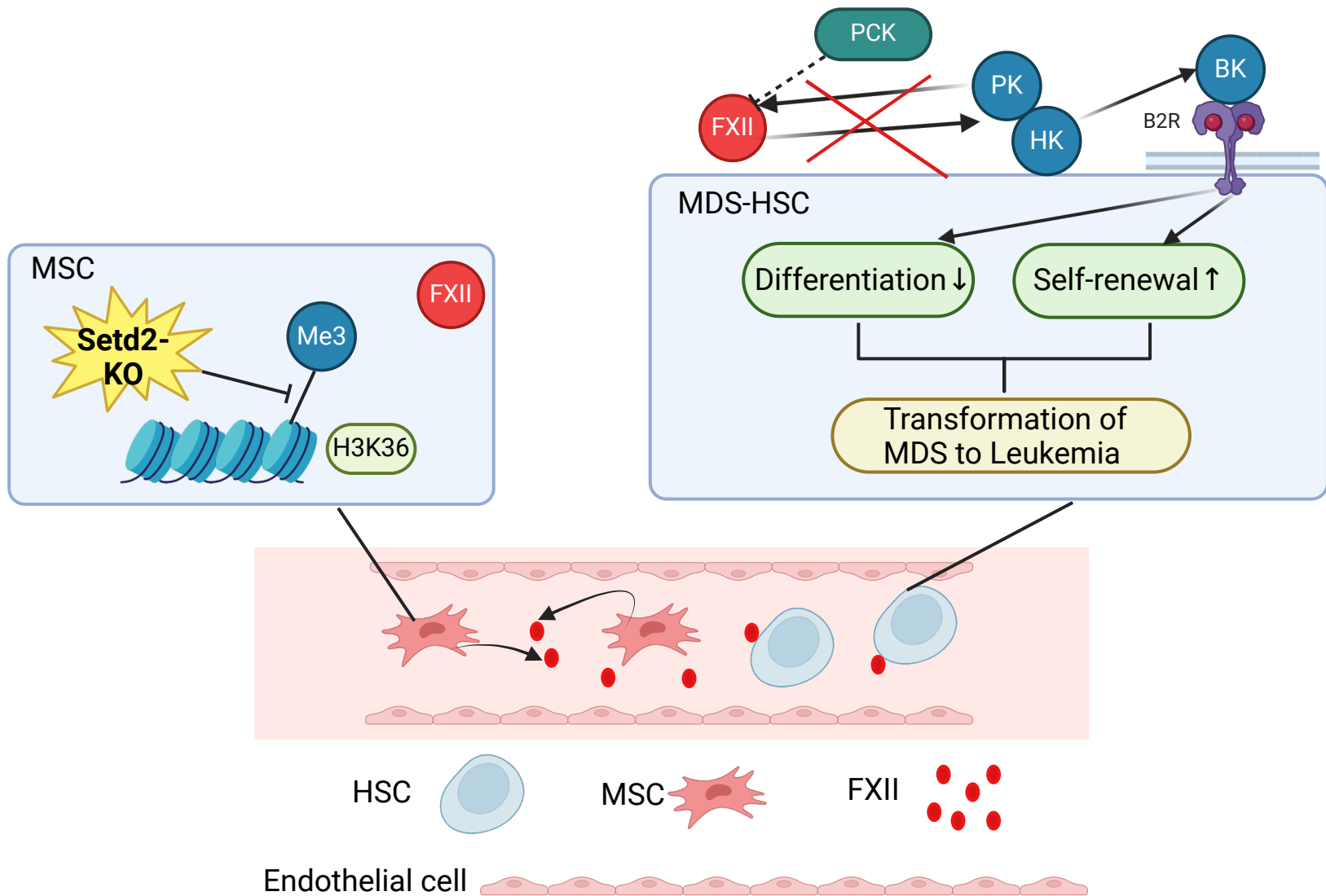


D



E





FigureS1. MSC targeted knockout of *Setd2* inhibited lymphocyte differentiation.

(A, B) The flow cytometry analysis of B cells differentiation in WT, NHD13*Setd2*^{fl/fl} and *Prx1-cre*NHD13*Setd2*^{Δ/Δ} mice. (C, D) The flow cytometry analysis of CLP expression in WT, NHD13*Setd2*^{fl/fl} and *Prx1-cre*NHD13*Setd2*^{Δ/Δ} mice. *P≤0.05, **P≤0.01.

FigureS2. MSC targeted knockout of *Setd2* prevented bone marrow failure in mice.

(A, B, C, D, E, F) The flow cytometry analysis of erythroid differentiation, c-Kit⁺ expression, Mac-1⁺Gr-1⁺, B220⁺ and CD3e⁺ expression, LK and LSK expression in WT, NHD13*Setd2*^{fl/fl} and *Lep-cre*NHD13*Setd2*^{Δ/Δ} mice. *P≤0.05, **P≤0.01, ***P≤0.001.

FigureS3. The impact of hematopoietic stem cell transplantation on the bone marrow microenvironment is limited.

(A) *Lep-cre*(NHD13)*Setd2*^{Δ/Δ} and (NHD13)*Setd2*^{fl/fl} transplant mouse models. (B) The survival period of *Lep-cre*(NHD13)*Setd2*^{Δ/Δ} and (NHD13)*Setd2*^{fl/fl} transplant mice. (C, E, F) The flow cytometry analysis of erythroid differentiation, c-Kit⁺ expression, Mac-1⁺Gr-1⁺ and PD-L1⁺ expression in *Lep-cre*(NHD13)*Setd2*^{Δ/Δ} and (NHD13)*Setd2*^{fl/fl} transplant mice. (D) The Complete Blood Count in *Lep-cre*(NHD13)*Setd2*^{Δ/Δ} and (NHD13)*Setd2*^{fl/fl} transplant mice. *P≤0.05; **P≤0.01.

FigureS4. Simplified model of MSC targeted knockout of *Setd2* and F12

mediated control of HSPC self-renewal and differentiation, eventually leading to a trend towards leukemia conversion. HK, high-molecular-weight kininogen; BK, bradykinin; PK, plasma kallikrein; B2R, kinin B2 receptor.