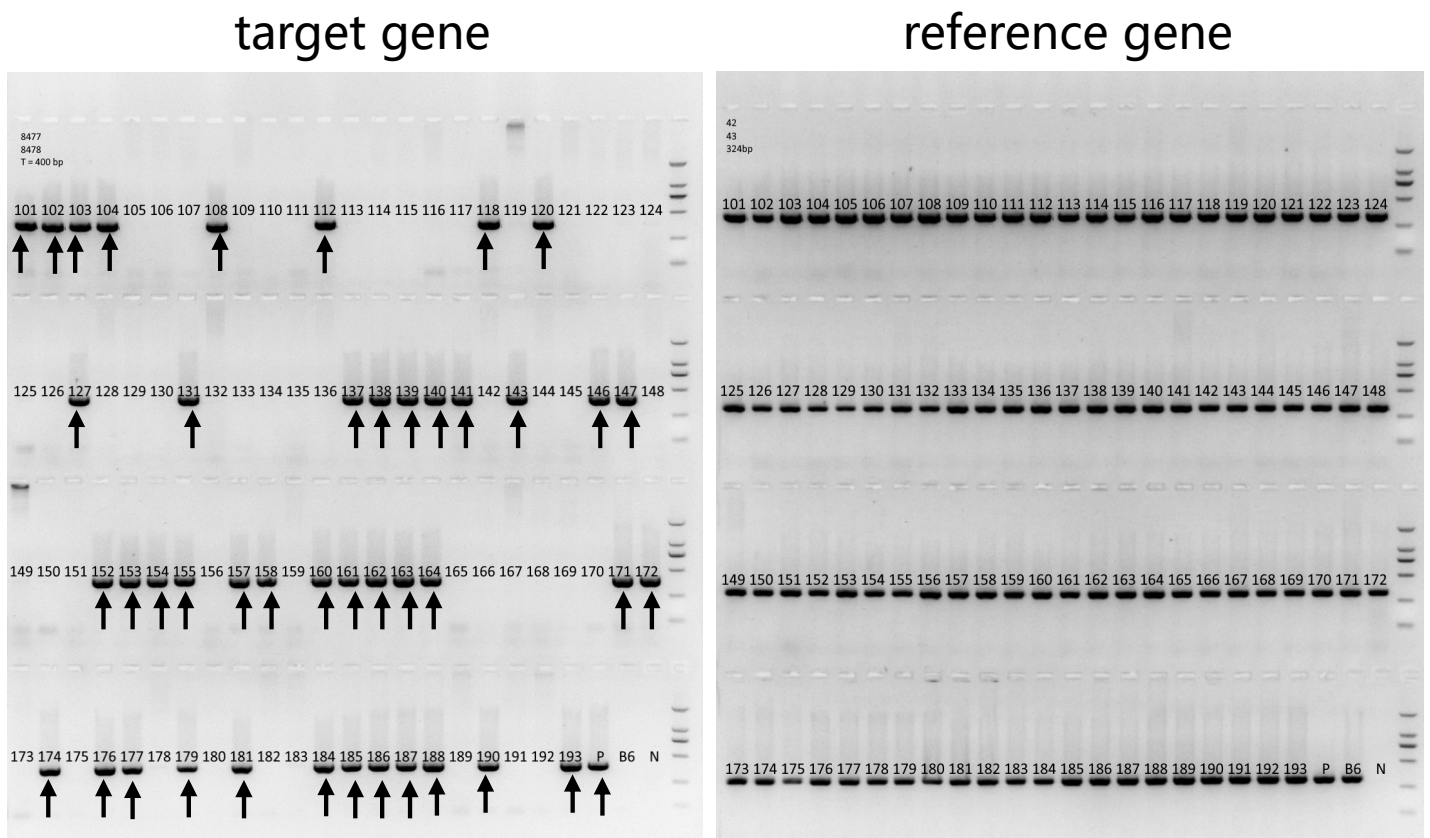


Supplementary Figures and Legends



Supplementary Figure S1. The animal models were identified by agarose gel electrophoresis

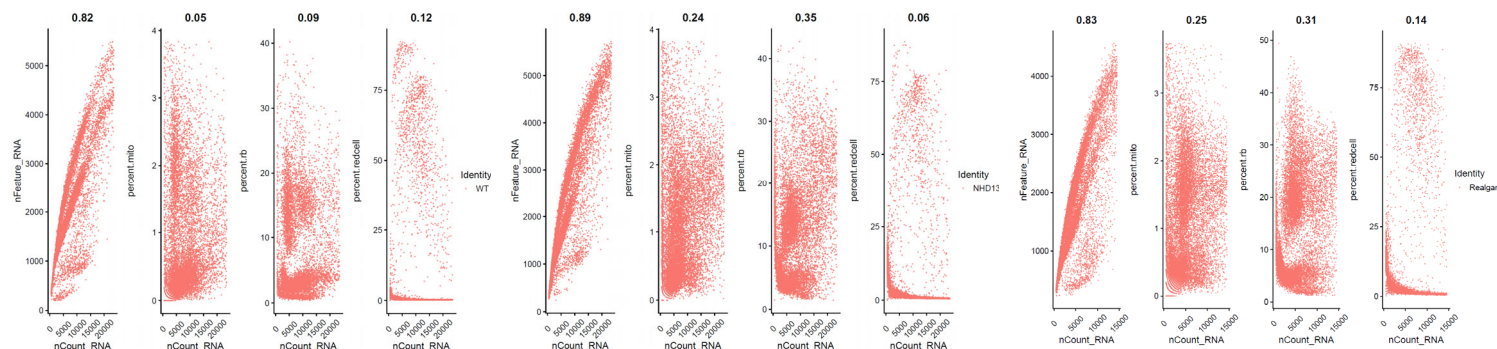
The reference and target genes were expressed in NHD13 transgenic positive mice, Arrows drawn represent transgenic positive mouse numbers

A

Identity WT

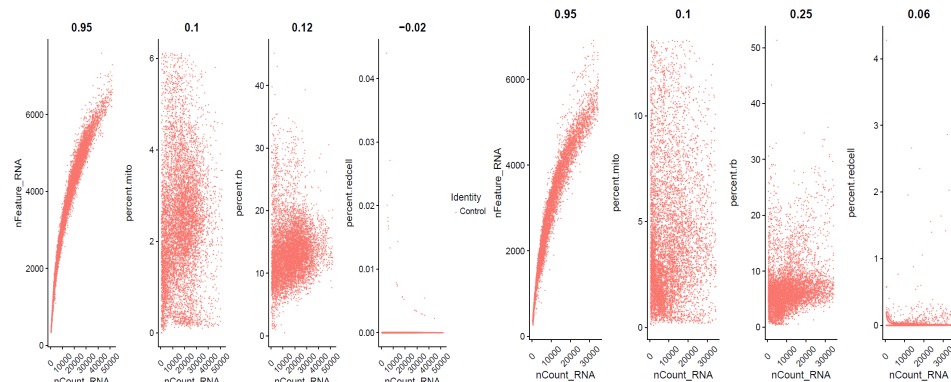
Identity NHD13

Identity Realgar



B

Identity Control

Identity As₂S₂

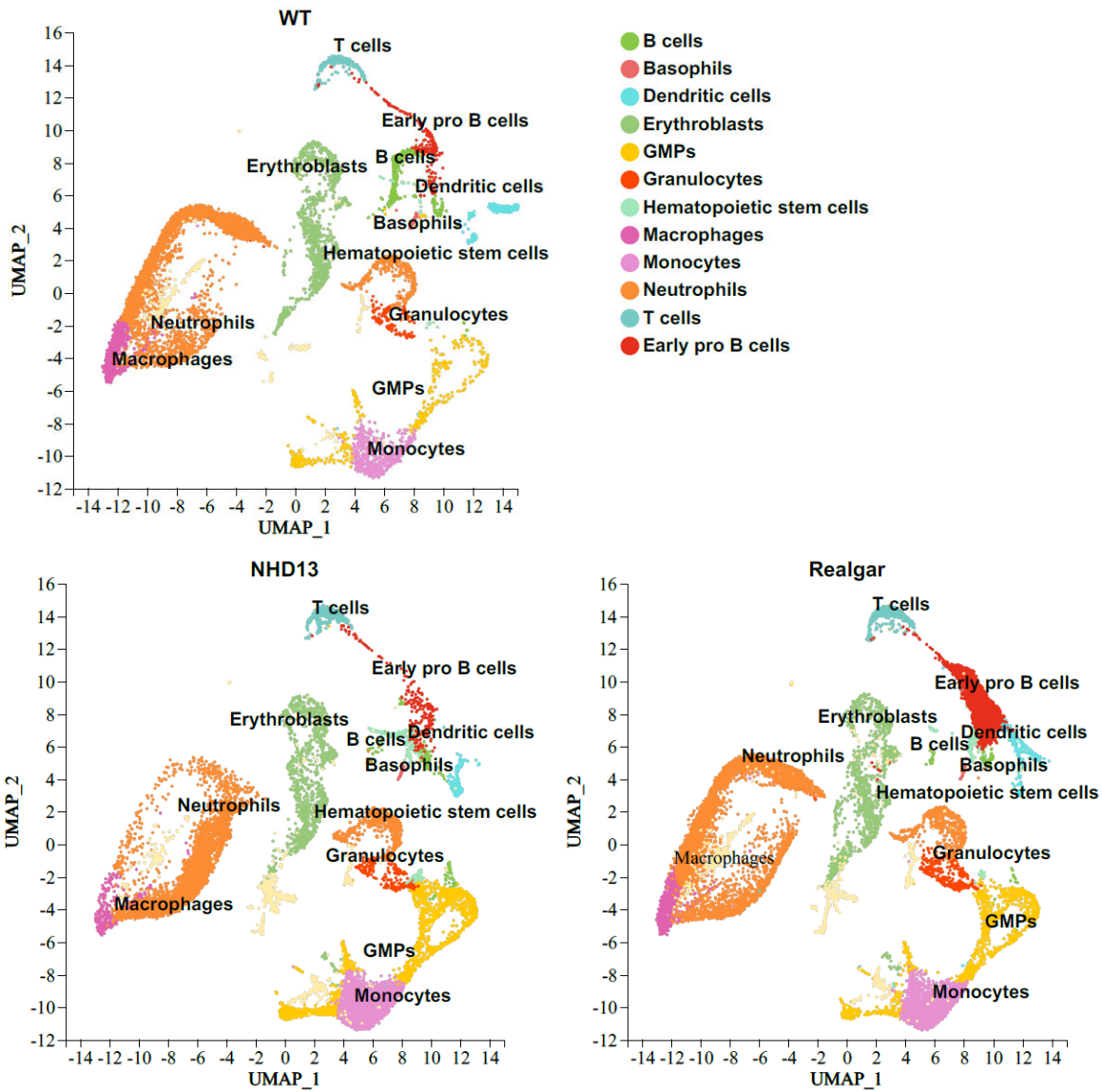
Supplementary Figure S2. Scatter plot for cell quality assessment

A) Scatter plot for single-cell quality assessment of mouse bone marrow cells from in vivo administration, there are a total of 3group, which are WT、NHD13 and Realgar.

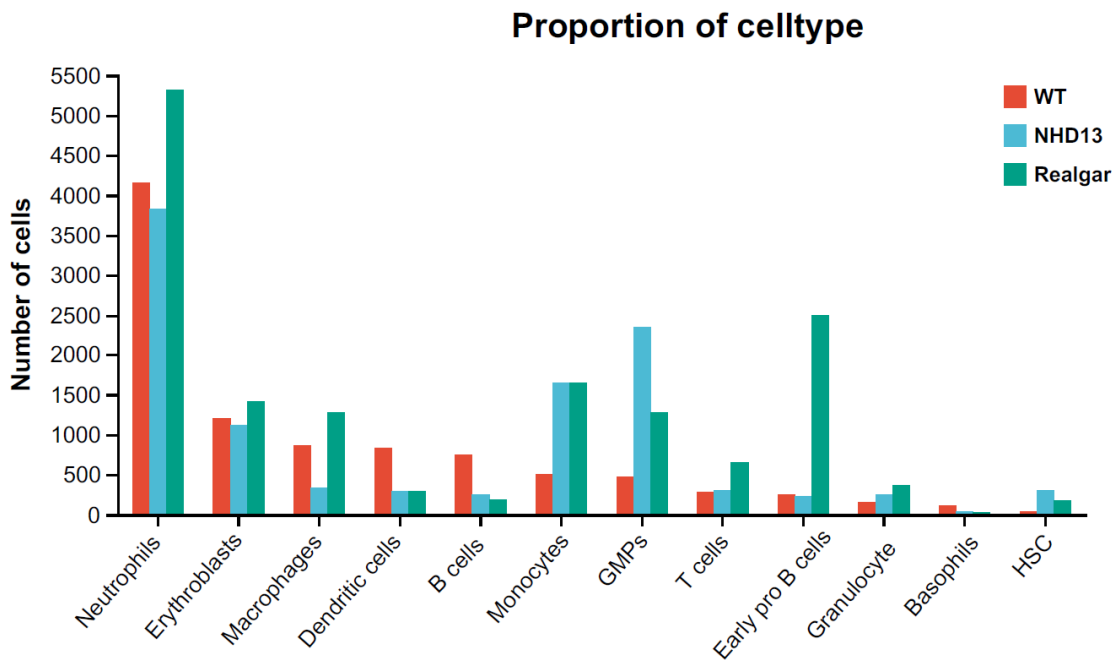
B) Scatter plot of single-cell quality assessment for mouse bone marrow cells from in vitro drug administration,there are a total of 2 groups, which are Control and As₂S₂.

The horizontal axis represents the count within the cells, and the vertical axis represents the number of genes, UMI sequences, and the proportion of UMI sequences from mitochondrial/ribosomal genes in the cells.

A



B

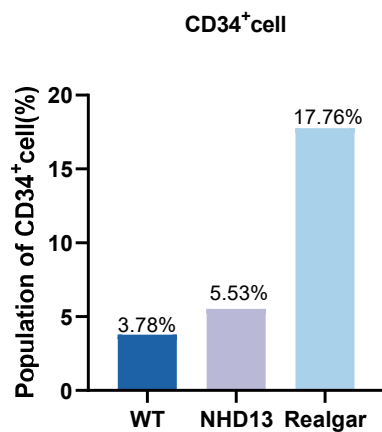


Supplementary Figure S3. Cell type annotation UMAP plot and cell type proportion comparison plot

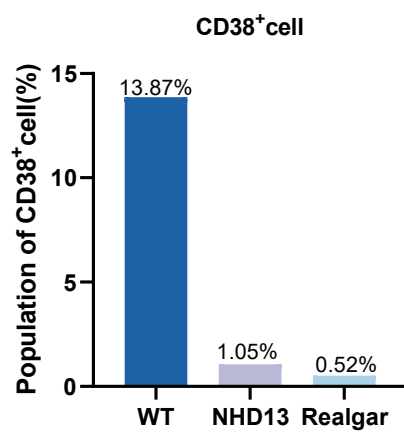
A) UMAP plots of three groups for cell type annotation

B) Proportion comparison plots of cell types for three groups.

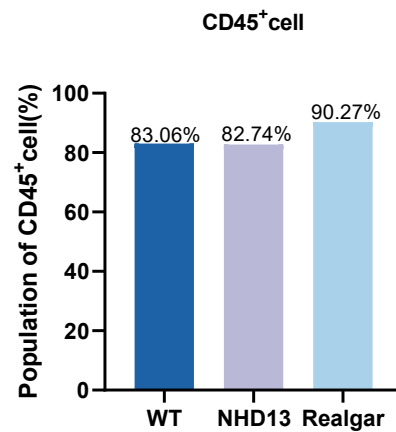
A



B



C



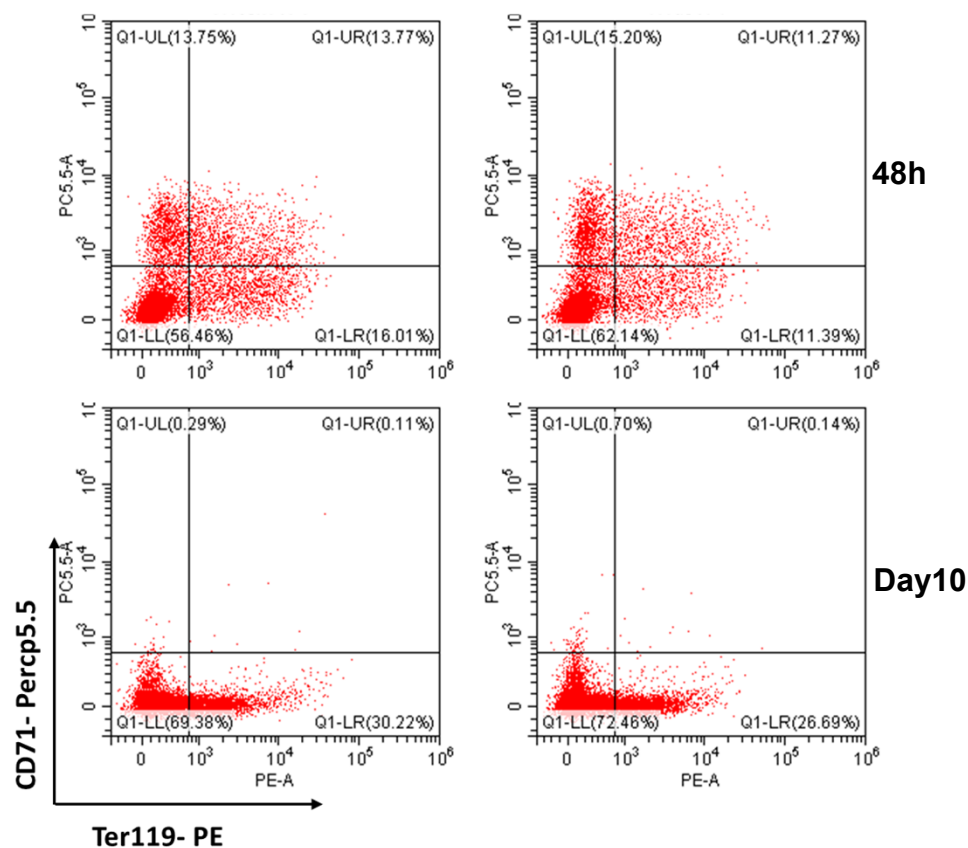
Supplementary Figure S4. Investigating the alterations in the proportions of CD34⁺, CD38⁺, and CD45⁺ cells based on single cell RNA-seq data.

A) Proportion comparison plots of CD34 positive fractions

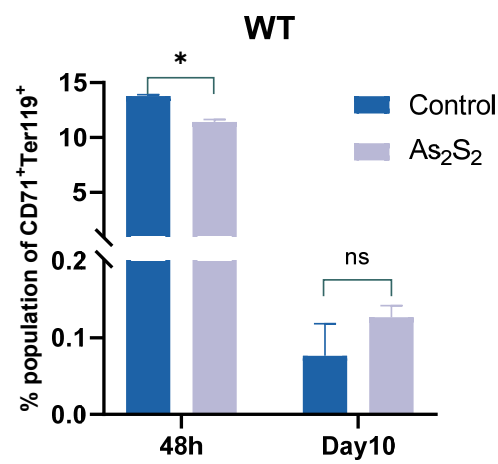
B) Proportion comparison plots of CD38 positive fractions

C) Proportion comparison plots of CD45 positive fractions

A



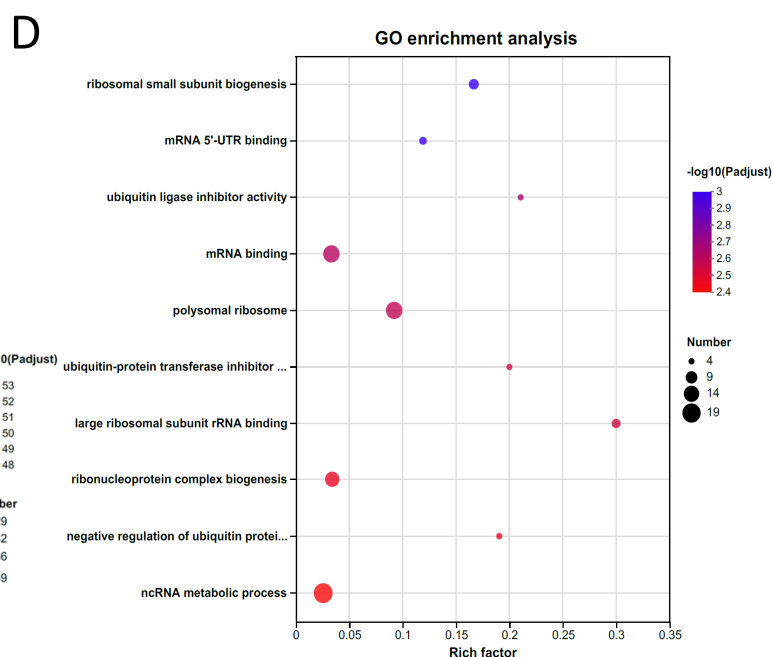
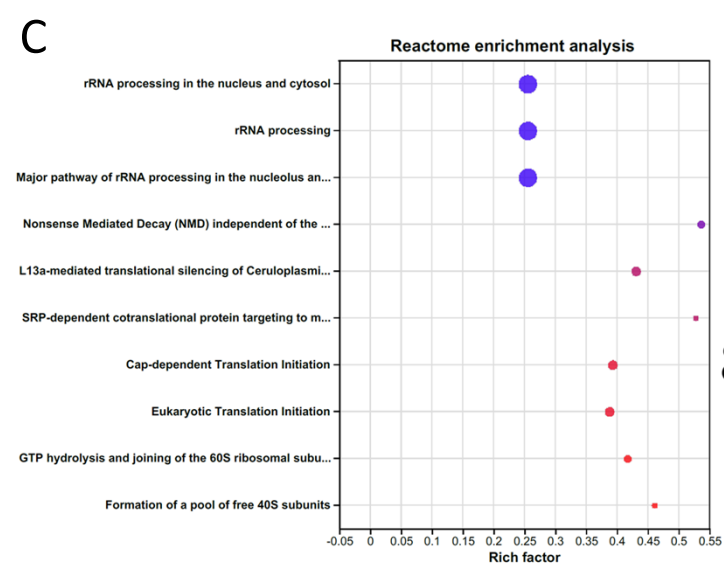
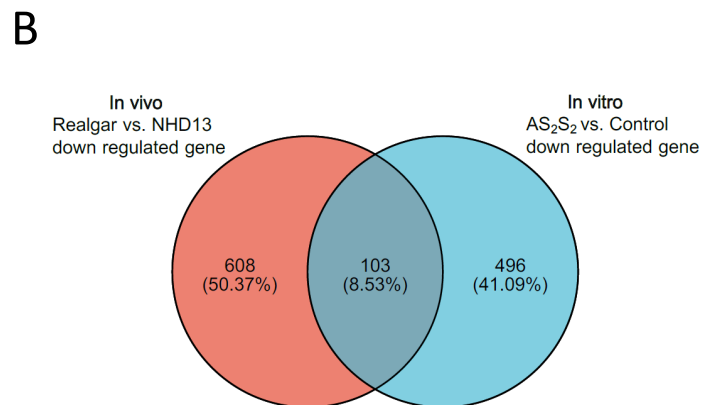
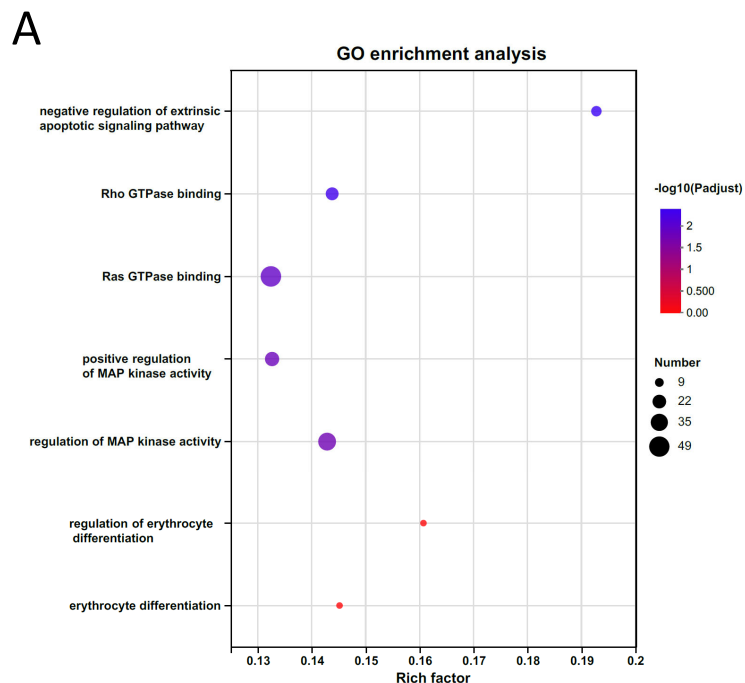
B



Supplementary Figure S5. Flow cytometry for detecting the expression of erythroid markers in wild-type mouse bone marrow cells

A) (up) Flow cytometric analysis of percentages of Ter 119⁺ and CD71⁺ cells in nucleated BM cells from wild-type mice, after 48 hours of intervention with As₂S₂. (Down) Flow cytometric analysis of percentages of Ter 119⁺ and CD71⁺ cells in nucleated BM cells from wild-type mice, after 10 days of intervention with As₂S₂

B) Quantitation of the data in the left panels. Data are the mean $\pm$ SEM (n = 3).



Supplementary Figure S6. Enrichment analysis plot

- A) GO enrichment analysis of AS₂S₂ vs. Control up-regulated differentially expressed genes
- B) Up-regulated differentially expressed genes of Realgar vs. NHD13(in vivo) and down-regulated differentially expressed genes of AS₂S₂ vs. Control(in vitro) shown in Venn Diagram in Erythroblasts. There are a total of 103 overlapping down-regulated genes.
- C) Reactome enrichment analysis of the overlapping down-regulated genes.
- D) GO enrichment analysis of the overlapping down-regulated genes.

Supplementary Table S1. Key Resources Table

Reagents or Resource	SOURCE	DENTIFIER
Drugs and Reagents		
Realgar	Sanmenxia Yuhuangshan Pharmaceutical Co., Ltd. (Henan, China)	Cat# 180306
AS ₂ S ₂	Sigma Aldrich (St. Louis, Mo., USA)	Cat# 519111
CMC-Na	Sangon Biotech (China, Shanghai)	Cat# 9004-32-4
Stain buffer	BD Pharmingen	Cat#554656
IMDM	Thermofisher Scientific	Cat# 12440053
Hemin	MCE (Shanghai, China)	Cat# HY-19424
ACK-lysis buffer	Beyotime(China, Shanghai)	Cat# A1049201
Other reagents		
Antibodies and Cytokines		
Ter119-PE(mouse)	BD Pharmingen	Cat# 553673
CD71-Percp5.5(mouse)	BD Pharmingen	Cat# 742101
CD235a-FITC (Human)	BD Pharmingen	Cat# 561017
CD71-APC-Cy7 (Human)	BD Pharmingen	Cat# 563671
Purified Rat Anti-Mouse CD16/CD32	BD Pharmingen	Cat# 553141
Anti-S100A8 antibody	Abcam	Cat# 92331
Anti-S100A9 antibody	Abcam	Cat# 242945
β-actin antibody	Abcam	Cat# 115777
Anti-ERK1/2 antibody	Cell Signal Technology	Cat# 9102S
Anti-pERK1/2 antibody	Cell Signal Technology	Cat# 4370S
GAPDH antibody	Cell Signal Technology	Cat# 5174
Hu Recom Flt3/Flk-2 Ligand	Stem Cell Technologies	Cat# 78009.1
Ms Recom SCF	Stem Cell Technologies	Cat# 78064.1
Ms Recom IL-3	Stem Cell Technologies	Cat# 78042.1

Supplementary Table S2. Primer pairs for PCR.

Gene Symbol	Sequences (5'-3')
NHD13	TGGAGGGCCTCTTGGTACAGG GGCTTCTAAGCTGTCTGTGGCC
β -actin	CTAGGCCACAGAATTGAAAGATCT GTAGGTGGAAATTCTAGCATCATCC

Supplementary Table S3. Blood cell counts of mice in each group after treatment

	WBC($\times 10^9/L$)	RBC($\times 10^{12}/L$)	HGB(g/L)	PLT($\times 10^9/L$)
WT	17.39 $\pm$ 2.75	8.66 $\pm$ 0.81	131.4 $\pm$ 14.67	1420.2 $\pm$ 152.32
NHD13	3.25 $\pm$ 0.64	5.30 $\pm$ 1.06	87.6 $\pm$ 9.91	694 $\pm$ 205.09
Realgar	4.96 $\pm$ 1.19	8.79 $\pm$ 1.99	114.4 $\pm$ 14.36	983.2 $\pm$ 160.62

Supplementary Table S4. The weight changes of mice

Time/group	WT(mean $\pm$ SD)	NHD13(mean $\pm$ SD)	Realgar(mean $\pm$ SD)	F value	P value
day1	30.16 $\pm$ 1.49	30.40 $\pm$ 1.80	30.70 $\pm$ 0.93	0.174	0.843
day7	30.12 $\pm$ 1.22	30.44 $\pm$ 1.62	30.78 $\pm$ 0.76	0.348	0.713
day14	30.42 $\pm$ 1.90	30.64 $\pm$ 1.86	31.32 $\pm$ 1.82	0.318	0.734
day21	31.02 $\pm$ 2.03	31.00 $\pm$ 2.03	31.86 $\pm$ 1.84	0.310	0.739
day28	31.58 $\pm$ 1.72	31.62 $\pm$ 1.80	31.80 $\pm$ 1.83	0.022	0.979

Supplementary Table S5. Cell Ranger mapping statistics

	WT	NHD13	Realgar
nCount_RNA	23611	23553	14547
nFeature_RNA	200-5512	200-6258	200-4709
Percent_mito	3.85%	3.83%	3.74%
Percent_rb	30.78%	34.35%	44.39%
Percent_redcell	1.19%	5.16%	7.55%
Percent_double_cell	6.04%	7.77%	6.38%
Num_Orig	11797	14490	18854
Num_filterd	9869	11766	15891

Note: Data from in vivo administration experiment

Supplementary Table S6. Seurat filtration criteria for the exclusion of cells with low qualities

	WT	NHD13	Realgar
Estimated Number of Cells	11862	14490	18854
Mean Reads per Cell	33259	23729	15889
Median Genes per Cell	2100	2166	1666
Valid Barcodes	97%	96.3%	95.9%
Sequencing Saturation	55.3%	37%	39.2%
Total Genes Detected	24229	24227	24163
Median UMI Counts per Cell	7047	6448	4446

Note: Data from in vivo administration experiment

Supplementary Table S7. marker genes for each lineage of blood cells

Cell types	Markers
Basophils	Ccl3; Fcer1a; Mcpt8
Early pro B cells	Dntt; Rag1; Rag2
Erythroblasts	Hbb-bs;Hbb-b2;Bpgm;Tfrc;Mki67;Stmn1
Granulocyte monocyte progenitors	Flt3;Kit;Mpeg1;Itgb2;Ahnak; Pld4; Cd68
Granulocytes	Ltf; Pglyrp1;Lcn2;Camp;Ngp;S100a11
Hematopoietic precursor cells	Kit;Stmn1;Mki67;Adgrg1;Cd34
Macrophages	Ahnak;Mpeg1;Cd68;Ly6d;Irf8;Cd74;Fcer1g Cxcl2;Fpr2;Tlr4;Clec4e;C1qa;C1qb;C1qc;C5ar1
Mature B cells	Cd79a;Cd79b;Cd19;Ms4a1;Cd74
Monocytes	Cd68;Mpeg1;Itgam;Adgre1;Fcgr3;Cd163;Cd14 Fcgr3a
T cells	Ahnak;Thy1;Cd3e
Dendritic cells	Cd1c;Cd209a;Ccr7;Ifitm1;Cd74;H2-Eb1;H2-Aa Mmp25
NK cells	Ncr1;Gzma;Nkg7

Supplementary Table S8. CD34⁺, CD38⁺ and CD45⁺ fractions

Groups	Sum	CD34 ⁺ fractions		CD38 ⁺ fractions		CD45 ⁺ fractions	
		Numbers	Population%	Numbers	Population%	Numbers	Population%
WT	9869	373	3.78	1369	13.87	8197	83.06
NHD13	11766	651	5.53	124	1.05	9735	82.74
Realgar	15891	2822	17.76	82	0.52	14345	90.27

Supplementary Table S9. marker genes for erythroblasts subsets

ProE	BasoE	PolyE	OrthoE
Hbb-bs	Hbb-bs	Hbb-bs	Hbb-bs
Car2	Slc4a1	Slc4a1	Slc4a1
Fth1	Cd24a	Cd24a	Gpx1
Cd24a	Car2	Car2	Alas2
Slc4a1	Gpx1	Gpx1	Car2
Gpx1	Prdx2	Alas2	Tmcc2
Prdx2	Alas2	Prdx2	Cd24a
Rpl8	Fth1	Gypa	Prdx2
Aqp1	Aqp1	Fth1	Slc25a37
Ctse	Gypa	Aqp1	Gypa
Eef1a1	Ctse	Rhd	Pabpc1
Gnb2l1	Slc25a37	Ctse	Hmbs
Rps5	Ube2c	Hemgn	Ube2l6
Tubb5	Rhd	Slc25a37	Fth1
Eef2	Hemgn	Hmbs	Bpgm
Rpl4	Hmbs	Serinc3	Fam46c
Blvrb	Serinc3	Fech	Trim10
Rplp1	Atpif1	Mki67	Fech
Rplp0	Blvrb	Ube2c	Ctse
Car1	Fech	Atpif1	Mktnl
Tmem14c	Mki67	Blvrb	Sncal
Hmbs	Urod	Tspan33	Ppp1r15a
Gypa	Bsg	Urod	Serinc3
Alas2	Grina	Tubb5	Urod
Atpif1	Arf5	Tmcc2	Epb4.1

Supplementary Table S10. Cell Ranger mapping statistics

	Control	As ₂ S ₂
nCount_RNA	52103	34467
nFeature_RNA	200-8906	200-7576
Percent_mito	6.12%	13.61%
Percent_rb	22.62%	13.57%
Percent_redcell	0.00%	0.01%
Percent_double_cell	7.86%	7.92%
Num_Orig	9045	8366
Num_filterd	7687	6735

Note: Data from in vitro experiment

Supplementary Table S11. Seurat filtration criteria for the exclusion of cells with low qualities

	Control	As ₂ S ₂
Estimated Number of Cells	9,057	8,387
Mean Reads per Cell	44,160	40,227
Median Genes per Cell	3,990	2,723
Valid Barcodes	96.80%	97.10%
Sequencing Saturation	43.90%	45.60%
Total Genes Detected	23,065	24,691
Median UMI Counts per Cell	16,501	8,135

Note: Data from in vitro experiment

Supplementary Table S12. Overlapping genes

Gene id	Gene name	Gene description
ENSMUSG00000028063	Lmna	lamin A [Source:MGI Symbol;Acc:MGI:96794]
ENSMUSG00000070056	Mfhas1	malignant fibrous histiocytoma amplified sequence 1 [Source:MGI Symbol;Acc:MGI:1098644]
ENSMUSG00000063856	Gpx1	glutathione peroxidase 1 [Source:MGI Symbol;Acc:MGI:104887]
ENSMUSG00000052187	Hbb-y	hemoglobin Y, beta-like embryonic chain [Source:MGI Symbol;Acc:MGI:96027]
ENSMUSG00000061731	Ext1	exostosin glycosyltransferase 1 [Source:MGI Symbol;Acc:MGI:894663]
ENSMUSG00000027201	Myef2	myelin basic protein expression factor 2, repressor [Source:MGI Symbol;Acc:MGI:104592]
ENSMUSG00000028080	Lrba	LPS-responsive beige-like anchor [Source:MGI Symbol;Acc:MGI:1933162]
ENSMUSG00000058624	Gda	guanine deaminase [Source:MGI Symbol;Acc:MGI:95678]
ENSMUSG00000091694	Apol11b	apolipoprotein L 11b [Source:MGI Symbol;Acc:MGI:3036248]
ENSMUSG00000053279	Aldh1a1	aldehyde dehydrogenase family 1, subfamily A1 [Source:MGI Symbol;Acc:MGI:1353450]
ENSMUSG00000045629	Sh3tc2	SH3 domain and tetratricopeptide repeats 2 [Source:MGI Symbol;Acc:MGI:2444417]
ENSMUSG00000025889	Snca	synuclein, alpha [Source:MGI Symbol;Acc:MGI:1277151]
ENSMUSG00000071226	Cecr2	CECR2, histone acetyl-lysine reader [Source:MGI Symbol;Acc:MGI:1923799]
ENSMUSG00000028716	Pdzk1ip1	PDZK1 interacting protein 1 [Source:MGI Symbol;Acc:MGI:1914432]
ENSMUSG00000035441	Myo1d	myosin ID [Source:MGI Symbol;Acc:MGI:107728]
ENSMUSG00000069919	Hba-a1	hemoglobin alpha, adult chain 1 [Source:MGI Symbol;Acc:MGI:96015]
ENSMUSG00000069917	Hba-a2	hemoglobin alpha, adult chain 2 [Source:MGI Symbol;Acc:MGI:96016]

Note: The genes are all from the overlapping gene set of WT vs. NHD13 upregulated genes and Realgar vs. NHD13 upregulated genes

Supplementary Table S13. Overlapping upregulated genes of in vivo and in vitro

Gene id	Gene name	Gene description
ENSMUSG00000026202	Tuba4a	tubulin, alpha 4A [Source:MGI Symbol;Acc:MGI:1095410]
ENSMUSG00000032484	Ngp	neutrophilic granule protein [Source:MGI Symbol;Acc:MGI:105983]
ENSMUSG00000073940	Hbb-bt	hemoglobin, beta adult t chain [Source:MGI Symbol;Acc:MGI:5474850]
ENSMUSG00000019820	Utrn	utrophin [Source:MGI Symbol;Acc:MGI:104631]
ENSMUSG00000096054	Syne1	spectrin repeat containing, nuclear envelope 1 [Source:MGI Symbol;Acc:MGI:1927152]
ENSMUSG00000058624	Gda	guanine deaminase [Source:MGI Symbol;Acc:MGI:95678]
ENSMUSG00000056071	S100a9	S100 calcium binding protein A9 (calgranulin B) [Source:MGI Symbol;Acc:MGI:1338947]
ENSMUSG00000056054	S100a8	S100 calcium binding protein A8 (calgranulin A) [Source:MGI Symbol;Acc:MGI:88244]
ENSMUSG00000038116	Phf20	PHD finger protein 20 [Source:MGI Symbol;Acc:MGI:2444148]
ENSMUSG00000025889	Snca	synuclein, alpha [Source:MGI Symbol;Acc:MGI:1277151]
ENSMUSG00000071226	Cecr2	CECR2, histone acetyl-lysine reader [Source:MGI Symbol;Acc:MGI:1923799]
ENSMUSG00000028716	Pdzk1ip1	PDZK1 interacting protein 1 [Source:MGI Symbol;Acc:MGI:1914432]
ENSMUSG00000026822	Lcn2	lipocalin 2 [Source:MGI Symbol;Acc:MGI:96757]
ENSMUSG00000069919	Hba-a1	hemoglobin alpha, adult chain 1 [Source:MGI Symbol;Acc:MGI:96015]
ENSMUSG00000069917	Hba-a2	hemoglobin alpha, adult chain 2 [Source:MGI Symbol;Acc:MGI:96016]
ENSMUSG00000022651	Retnlg	resistin like gamma [Source:MGI Symbol;Acc:MGI:2667763]
ENSMUSG00000038357	Camp	cathelicidin antimicrobial peptide [Source:MGI Symbol;Acc:MGI:108443]

Note: The genes are all from the overlapping gene set of in vivo experiment and in vitro experiment

Supplementary Materials and Methods

AGE(agarose gel electrophoresis)

Mouse tail PCR agarose gel electrophoresis method was used to identify the genotype of mouse pups. DNA: A segment of the mouse tail tip with a length of 0.5 cm was taken using sterile surgical scissors, crushed in liquid nitrogen, and placed in a centrifuge tube for later use. After adding 180 μ L of Buffer GTT and shaking evenly, 20 μ L of Proteinase K was added and vortexed until thoroughly mixed, then incubated at 56 ° C for 6-8 hours in a water bath until the tissue solution became completely clear. After centrifugation at 13,400 $\times$ g for 1 minute, the supernatant was transferred to a new centrifuge tube. Following the addition of GL and shaking evenly, 200 μ L of ethanol was added and mixed well. The resulting solution was added to an adsorption column, centrifuged at 13,400 $\times$ g for 1 minute, discard the waste, add 500 μ L Buffer GW1, centrifuge at 13,400 $\times$ g for 1 minute, discard the waste, add 500 μ L Buffer GW2, centrifuge at 13,400 $\times$ g for 1 minute, discard the waste, then centrifuge at 13,400 $\times$ g for 2 minutes and air-dry the adsorption column at room temperature. The adsorption column was placed in a new centrifuge tube, 50 μ L of Buffer GE was added, left at room temperature for 5 minutes, centrifuged at 13,400 $\times$ g for 1 minute, and the DNA solution was collected. The DNA concentration and total amount were measured using a NanoDrop spectrophotometer and stored at -20° C. Take 2 μ L of the above DNA solution, use NHD13 mutant gene primers (Supplementary Table S2), and perform PCR amplification in a 20 μ L system. Reaction conditions: 95 ° C, 5 min (pre-denaturation); 95 ° C, 30 s (denaturation), 58 ° C, 30 s (annealing), 72 ° C, 30 s (extension), 40 cycles; 72 ° C, 3 min (further extension); 25 ° C, hold. Add 200 μ L Buffer. Finally, perform agarose gel electrophoresis and visualization. Gel preparation: Diluted TAE (1%) with agarose (1.0 g agarose corresponds to 50 mL TAE), heated until clear, cooled, add Safe Green Plus (5 μ L per 50 mL cooled agarose), and pour into the mold. Loading: Directly add PCR products to the wells, 6 μ L per well, and add DL1000 Marker (2 μ L) for molecular weight standards. Electrophoresis: Voltage 120 V, electrophoresis time 30 min, maintain current at 70-80 mA. Imaging: Determine the genotype of mouse pups based on the agarose gel electrophoresis results.