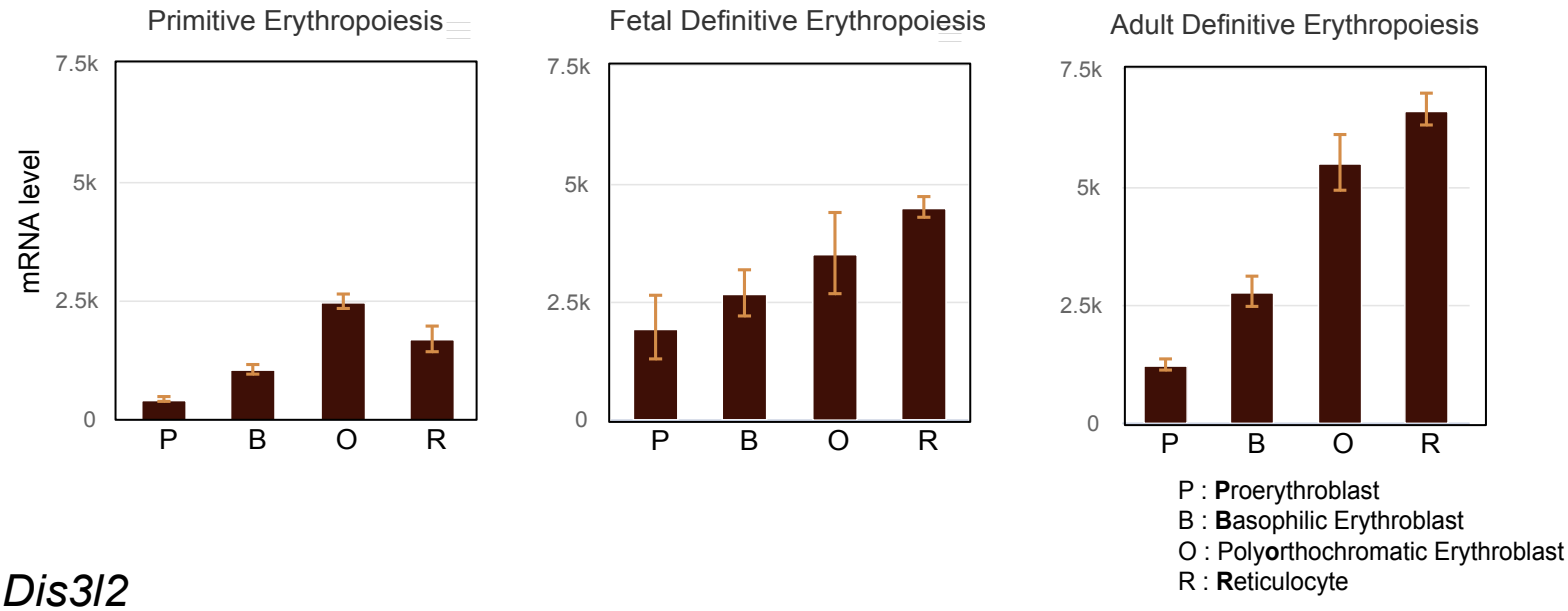
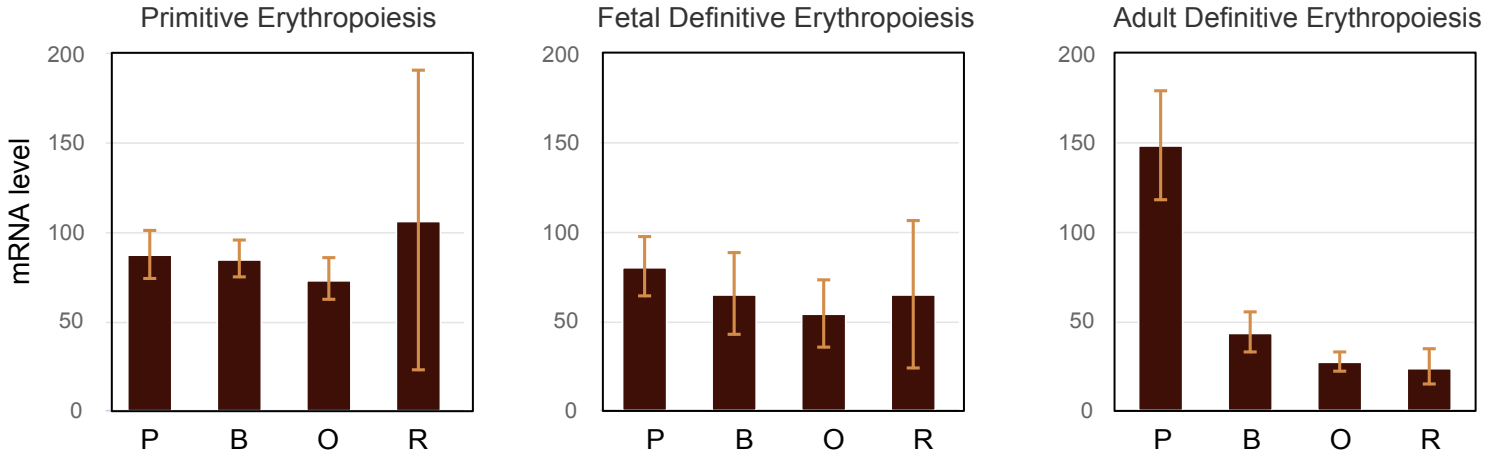
Extended Data Fig. 1

Zcchc6



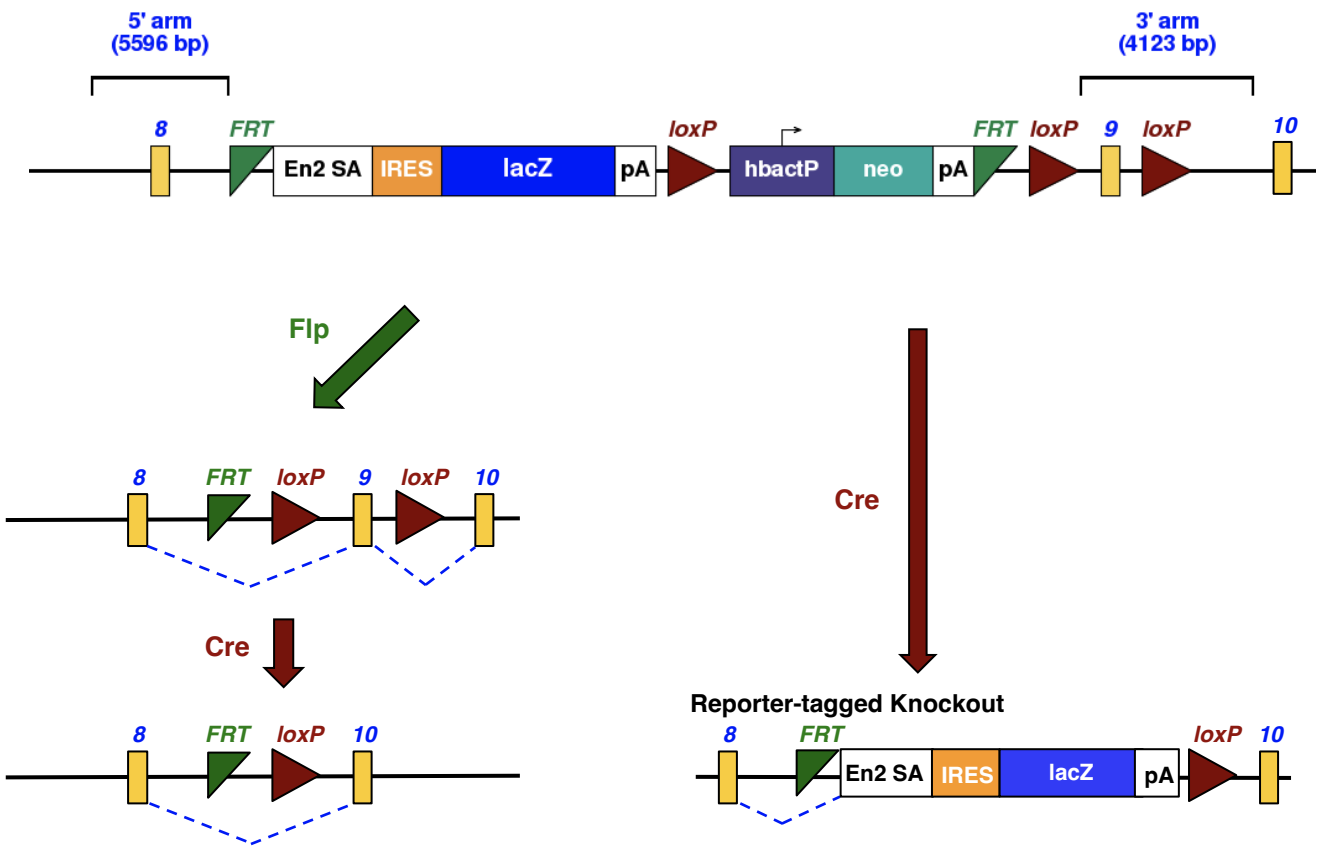
Dis3l2



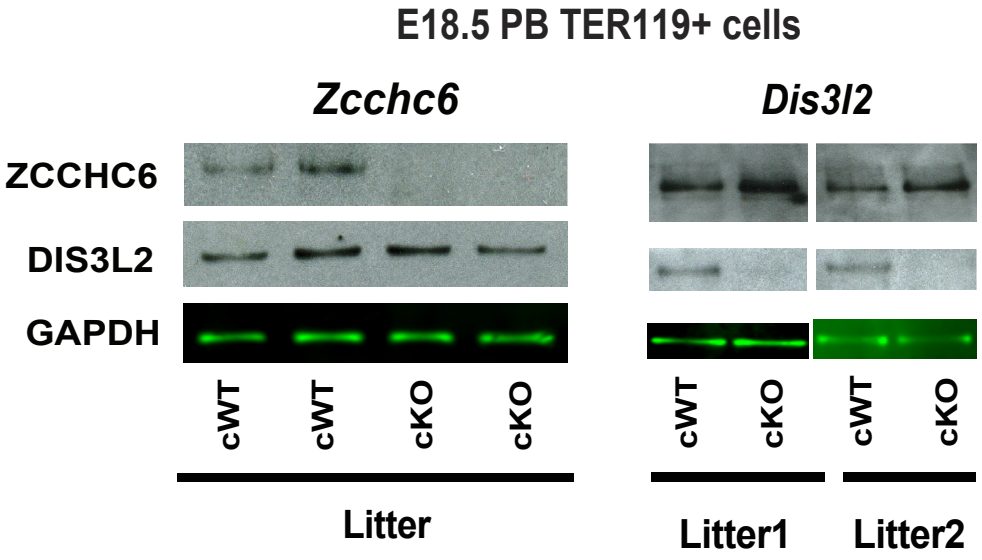
Extended Data Fig. 1.
Expression Profiles of Zcchc6 and Dis3l2 mRNAs During Erythropoiesis (from the Erythron Database).
Expression Levels of Zcchc6 and Dis3l2 mRNAs During Mouse Primitive and Definitive
(fetal liver and adult bone marrow) Erythropoiesis.

Extended Data Fig. 2

a



b



Extended Data Fig. 2. Allele Map of the Dis3l2 Knockout Model and Conditional Knockout Validation.
(a) Allele Map of the *Dis3l2* Knockout Models.
(b) Western Blots of Erythroid Cells from Embryos Carrying only *floxed* (cWT) and both *floxed* and *EpoR-Cre* (cKO) Alleles for Validation.

Extended Data Fig. 3

a

p-values (<0.05 *, <0.01 **, <0.001***)

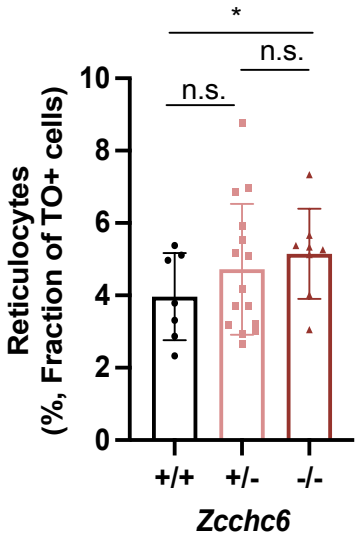
Parameter	Unit	*Zcchc6*			p-value	
		WT	HET	KO	WT vs. KO	HET vs. KO
RBC	M/µl	9.09 ± 0.39	9.03 ± 0.29	9.05 ± 0.39		
HCT	%	43.28 ± 1.52	43.35 ± 1.71	43.99 ± 1.66		
MCH	pg	14.52 ± 0.73	14.66 ± 0.69	15.15 ± 0.76		
MCHC	g/dL	30.48 ± 0.96	30.55 ± 1.17	31.15 ± 1.24		
MCV	fL	47.65 ± 1.50	48.04 ± 1.44	48.61 ± 1.26		
RDW	%	16.49 ± 0.44	16.74 ± 0.60	16.60 ± 0.47		
Hb	g/dL	13.18 ± 0.57	13.23 ± 0.54	13.70 ± 0.79		

b

p-values (<0.05 *, <0.01 **, <0.001***)

Parameter	Unit	*Zcchc6*			p-value	
		WT	HET	KO	WT vs. KO	HET vs.KO
Leukocytes:						
WBC	K/µl	9.65 ± 1.25	9.56 ± 1.57	10.29 ± 1.41		
LY	K/µl	6.15 ± 0.89	6.17 ± 1.29	6.38 ± 1.04		
NE	K/µl	2.86 ± 0.45	2.67 ± 0.48	3.13 ± 0.52		*
MO	K/µl	0.49 ± 0.20	0.64 ± 0.18	0.67 ± 0.15		
EO	K/µl	0.13 ± 0.08	0.07 ± 0.04	0.09 ± 0.05		
BA	K/µl	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.00		
LY	%	63.71 ± 3.99	64.20 ± 5.30	61.88 ± 4.04		
NE	%	29.74 ± 3.29	28.25 ± 4.55	30.54 ± 3.55		*
MO	%	4.96 ± 1.50	6.66 ± 1.50	6.63 ± 1.77		
EO	%	1.40 ± 0.85	0.78 ± 0.50	0.86 ± 0.38		
BA	%	0.19 ± 0.14	0.12 ± 0.10	0.10 ± 0.04		
Thrombocytes:						
PLT	K/µl	870.15 ± 83.11	923.91 ± 120.69	884.38 ± 125.59		
MPV	fL	4.60 ± 0.12	4.63 ± 0.14	4.66 ± 0.15		

c



Extended Data Fig. 3. Analysis of Whole-Body *Zcchc6* Knockout Young Adult Mice (6–7 Weeks).

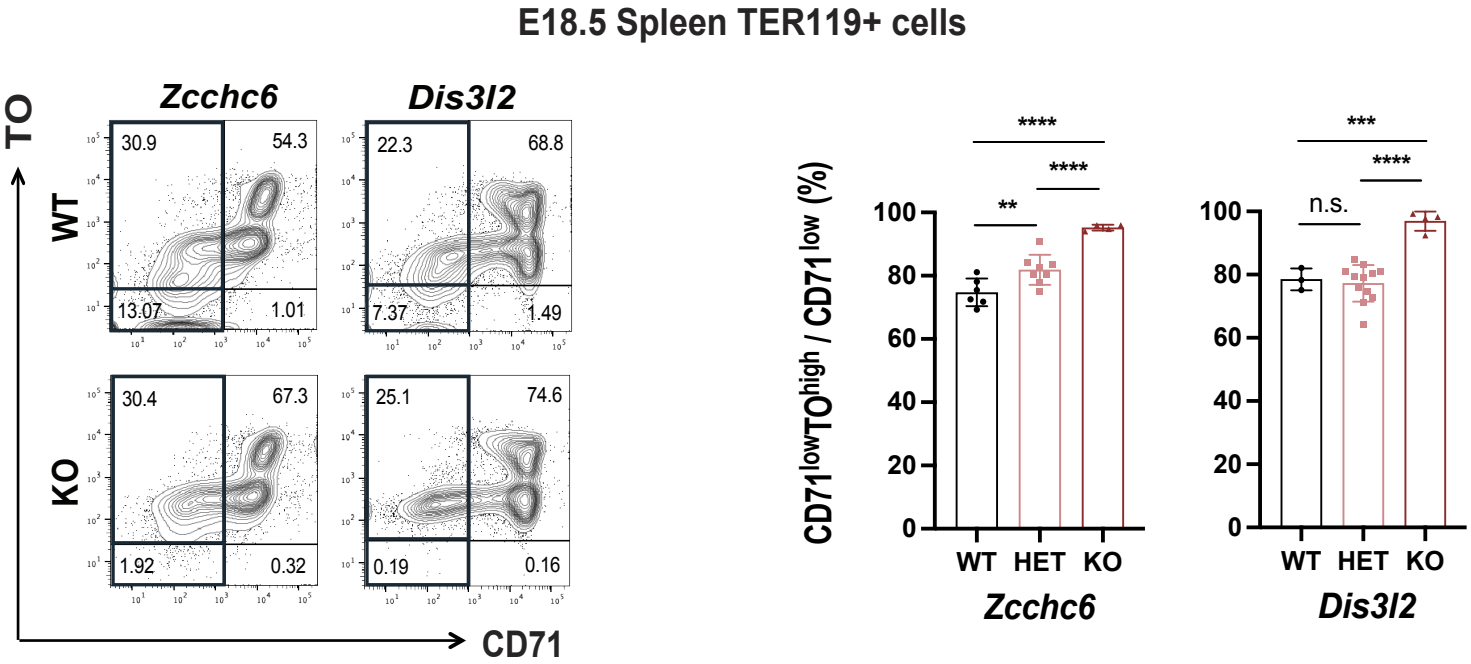
(a) Hematological Profiles of Peripheral Blood from Retro-Orbital Bleeding (mean ± SD).
HCT; Hematocrit, MCH; Mean corpuscular hemoglobin, MCHC; Mean corpuscular hemoglobin concentration, MCV; Mean corpuscular volume, RDW; Red cell Distribution Width. Multiple litters were used.

(b) Leukocytes and Thrombocyte panels.
LY; lymphocytes, NE; neutrophils, MO; monocytes, EO; eosinophils, BA; basophils,
PLT; Platelet or thrombocyte count, MPV; Mean Platelet Volume.

(c) Quantification of reticulocyte (TO+) population per genotype (mean ± SD).

Extended Data Fig. 4

a



b

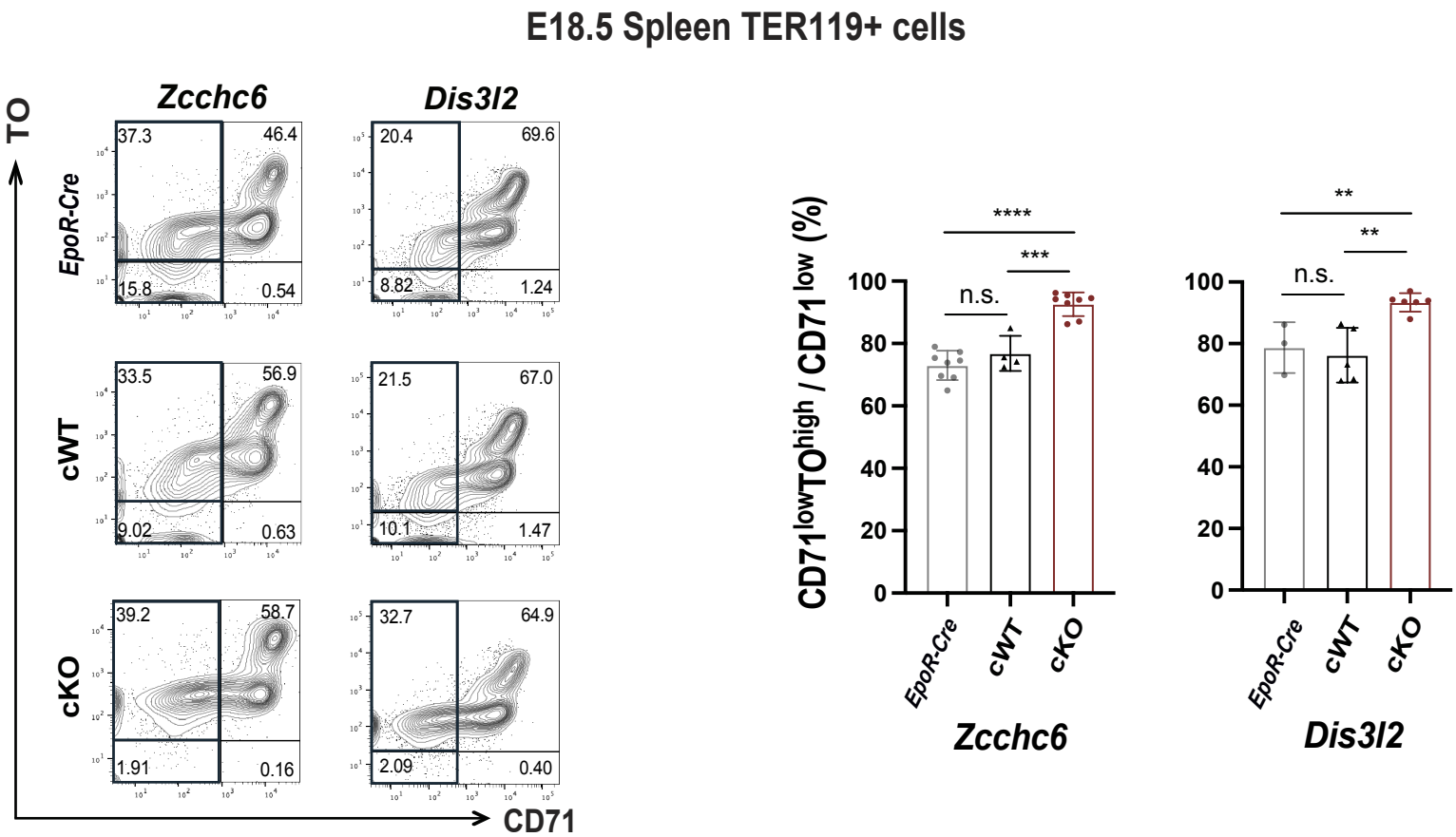
p-values (<0.05 *, <0.01 **, <0.001***)

Parameter	Unit	*Zcchc6*			p-value		*Dis3l2*			p-value	
		WT	HET	KO	WT vs. KO	HET vs.KO	WT	HET	KO	WT vs. KO	HET vs. KO
Leukocytes:											
WBC	K/μl	7.10 ± 1.39	8.96 ± 3.72	21.13 ± 8.52	***	***	3.79 ± 1.22	4.42 ± 1.08	5.53 ± 1.36	*	
LY	K/μl	5.85 ± 1.25	7.60 ± 3.50	18.61 ± 7.52	***	***	1.65 ± 0.62	1.96 ± 0.49	2.77 ± 1.10	*	*
NE	K/μl	0.96 ± 0.14	0.98 ± 0.31	1.66 ± 0.74	**	**	1.58 ± 0.57	1.82 ± 0.57	2.00 ± 0.73		
MO	K/μl	0.17 ± 0.08	0.22 ± 0.07	0.71 ± 0.29	***	***	0.12 ± 0.10	0.12 ± 0.09	0.15 ± 0.09		
EO	K/μl	0.10 ± 0.04	0.11 ± 0.05	0.12 ± 0.04			0.34 ± 0.11	0.43 ± 0.18	0.46 ± 0.10		
BA	K/μl	0.04 ± 0.01	0.04 ± 0.02	0.05 ± 0.03			0.10 ± 0.05	0.10 ± 0.05	0.16 ± 0.09		*
LY	%	82.10 ± 2.33	83.63 ± 5.61	87.87 ± 1.61			43.52 ± 9.67	44.52 ± 7.34	49.51 ± 11.79		
NE	%	13.75 ± 2.22	11.93 ± 4.92	7.92 ± 1.44	*		41.88 ± 9.86	40.86 ± 6.63	36.35 ± 9.34		
MO	%	2.24 ± 0.92	2.60 ± 0.63	3.38 ± 0.63	*		2.73 ± 1.84	2.75 ± 2.05	2.51 ± 1.23		
EO	%	1.37 ± 0.44	1.37 ± 0.61	0.60 ± 0.21		*	9.37 ± 1.14	9.64 ± 3.13	8.58 ± 2.26		
BA	%	0.54 ± 0.06	0.47 ± 0.19	0.22 ± 0.09	**	**	2.51 ± 1.15	2.23 ± 1.16	3.05 ± 1.26		
Thrombocytes:											
PLT	K/μl	496.75 ± 49.31	517.67 ± 35.27	519.13 ± 24.93			425.60 ± 71.20	464.71 ± 86.20	411.64 ± 66.27		
MPV	fL	6.31 ± 0.17	6.42 ± 0.15	6.50 ± 0.11			6.32 ± 0.18	6.10 ± 0.27	6.13 ± 0.22		

Extended Data Fig. 4. Additional Analysis of Whole-Body *Zcchc6/Dis3l2* Knockout Mice.
(a, left figures) Flow cytometry profiles of erythroid cells from spleens.
Representative profiles of each KO mouse and WT littermates were displayed.
(a, right graphs) Quantification of reticulocyte population per genotype.
(b) Hematological profiles in the PB of whole-body KOs (Leukocytes and Thrombocyte panels).
LY; lymphocytes, NE; neutrophils, MO; monocytes, EO; eosinophils, BA; basophils,
PLT; Platelet or thrombocyte count, MPV; Mean Platelet Volume.

Extended Data Fig. 5

a



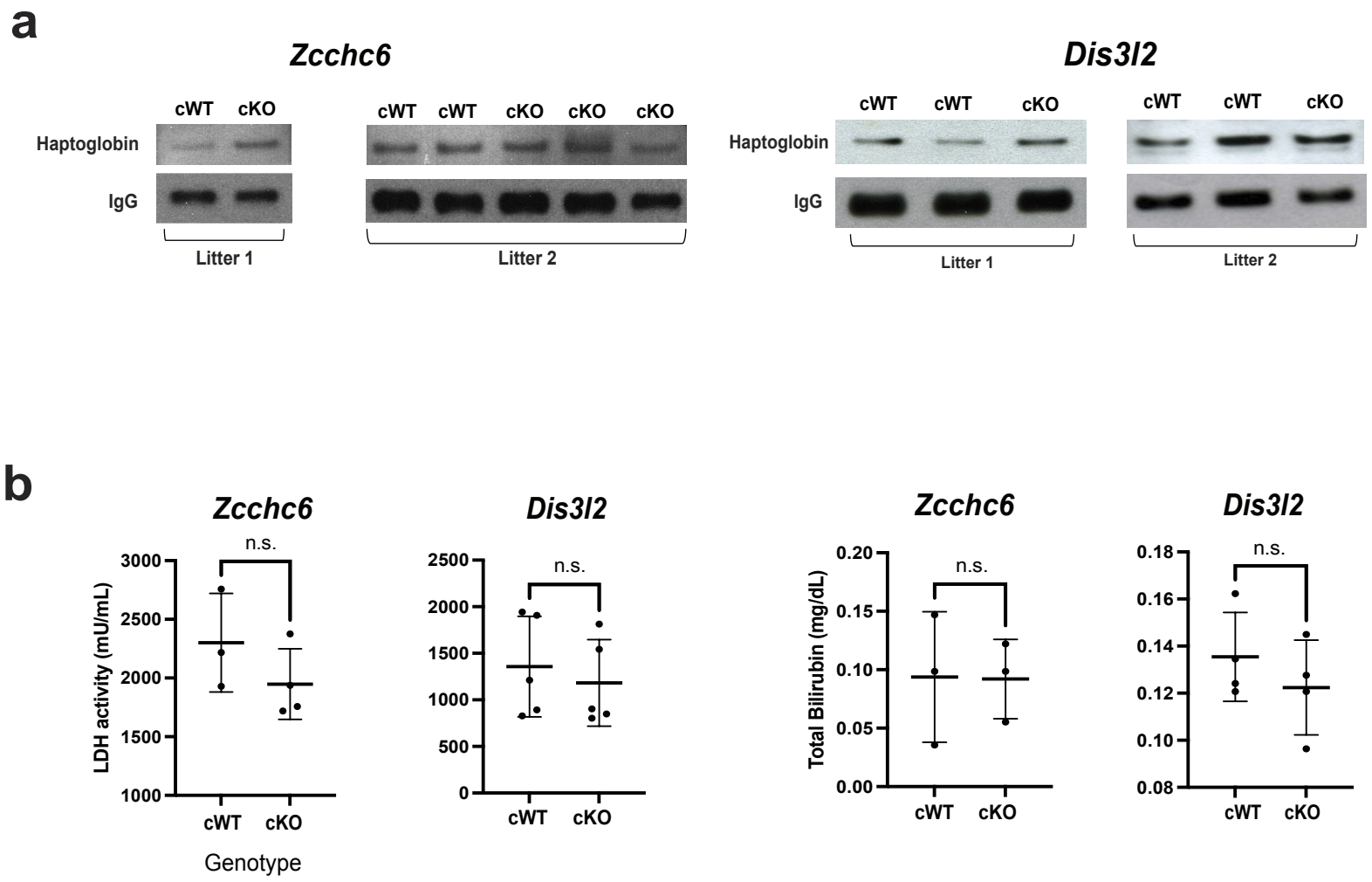
b

p-values (<0.05 *, <0.01 **, <0.001***)

Parameter	Unit	*Zcchc6*			p-value		*Dis3l2*			p-value	
		EpoR-Cre	cWT	cKO	cWT vs. cKO	*EpoR-Cre* vs. cKO	*EpoR-Cre*	cWT	cKO	cWT vs. cKO	*EpoR-Cre* vs. cKO
Leukocytes:											
WBC	K/µl	15.60 ± 5.12	16.54 ± 6.04	30.89 ± 11.89	0.0686	*	17.14 ± 15.10	13.37 ± 7.68	26.49 ± 16.83	***	*
LY	K/µl	14.28 ± 5.13	15.24 ± 5.72	27.93 ± 11.59	0.0913	*	15.49 ± 14.10	12.25 ± 7.40	24.19 ± 15.79	***	*
NE	K/µl	0.92 ± 0.21	0.86 ± 0.20	1.86 ± 0.89			1.18 ± 0.49	0.88 ± 0.25	1.49 ± 0.52	**	
MO	K/µl	0.32 ± 0.13	0.36 ± 0.22	0.91 ± 0.43		*	0.40 ± 0.55	0.17 ± 0.17	0.73 ± 0.66	**	
EO	K/µl	0.06 ± 0.03	0.06 ± 0.03	0.16 ± 0.08			0.06 ± 0.04	0.06 ± 0.03	0.04 ± 0.02		
BA	K/µl	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01			0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01		
LY	%	90.50 ± 4.55	91.88 ± 1.40	89.39 ± 4.33			87.57 ± 7.37	88.59 ± 7.74	90.05 ± 3.11		
NE	%	6.85 ± 4.25	5.58 ± 1.66	7.21± 4.31			10.20 ± 7.96	9.72 ± 7.94	7.41 ± 3.66	*	
MO	%	1.99 ± 0.27	2.02 ± 0.70	2.77 ± 1.08			1.74 ± 0.93	0.94 ± 0.64	2.21 ± 1.09	***	
EO	%	0.47 ± 0.38	0.36 ± 0.20	0.51 ± 0.13			0.38 ± 0.18	0.56 ± 0.34	0.21 ± 0.10	*	
BA	%	0.20 ± 0.15	0.17 ± 0.11	0.11 ± 0.08			0.12 ± 0.06	0.17 ± 0.11	0.11 ± 0.11		
Thrombocytes:											
PLT	K/µl	590.75 ± 38.23	579.50 ± 16.09	609.67 ± 48.13			610.5 ± 133.57	609.11 ± 94.52	569.50 ± 57.43		
MPV	fL	6.00 ± 0.14	6.10 ± 0.18	6.02 ± 0.13			5.98 ± 0.15	6.12 ± 0.16	6.06 ± 0.28		

Extended Data Fig. 5. Additional Analysis of Conditional *Zcchc6/Dis3l2* KO.
(a) Flow cytometry profiles of erythroid cells from the spleen.
Representative profiles and reticulocyte quantification of embryos carrying only the *EpoR-Cre* allele, only the *floxed* allele; cWTs, and both alleles; cKOs.
(b) Hematological profiles in the PB of conditional KOs. (Leukocytes and Thrombocyte panels).
LY; lymphocytes, NE; neutrophils, MO; monocytes, EO; eosinophils, BA; basophils, PLT; Platelet or thrombocyte count, MPV; Mean Platelet Volume.

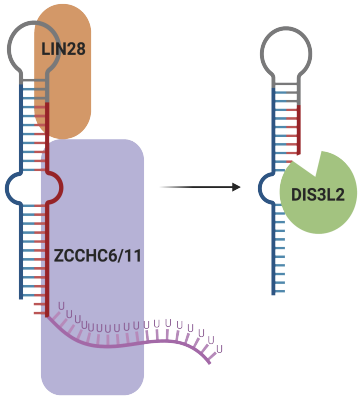
Extended Data Fig. 6



Extended Data Fig. 6. Analysis of Hemolytic Anemia Markers.
(a) Western blots of Haptoglobin in cKOs and cWTs.
(b) LDH and Bilirubin assay results from serum and plasma of cKO mice.

Extended Data Fig. 7

a



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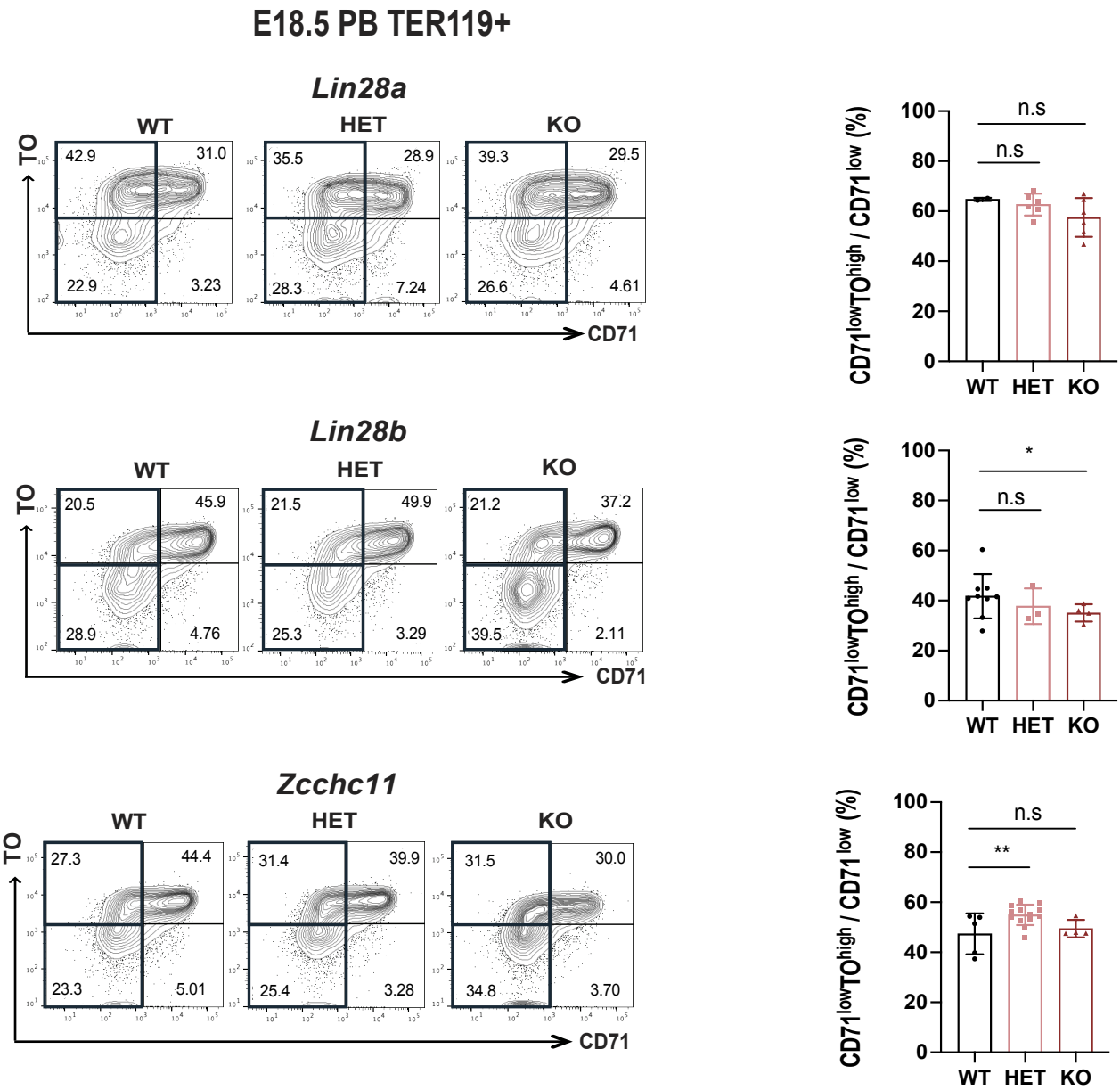
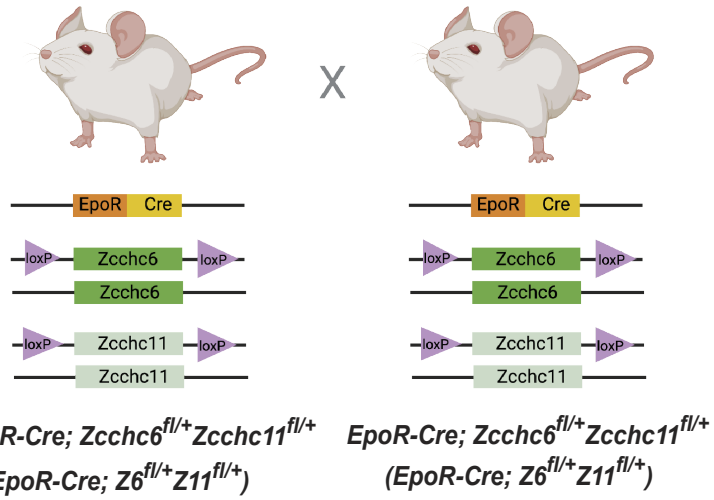


Extended Data Fig. 7. Characterizing the Impact of Knockouts in Pathway-Related Genes.
(a) Previously published model for the RNA decay machinery (LIN28-ZCCHC6/11-DIS3L2) on the microRNA regulation.
(b) FACS profiles and population quantification for each whole-body KO model (*Lin28a*, *Lin28b*, and *Zcchc11* sequentially).

Extended Data Fig. 8

a



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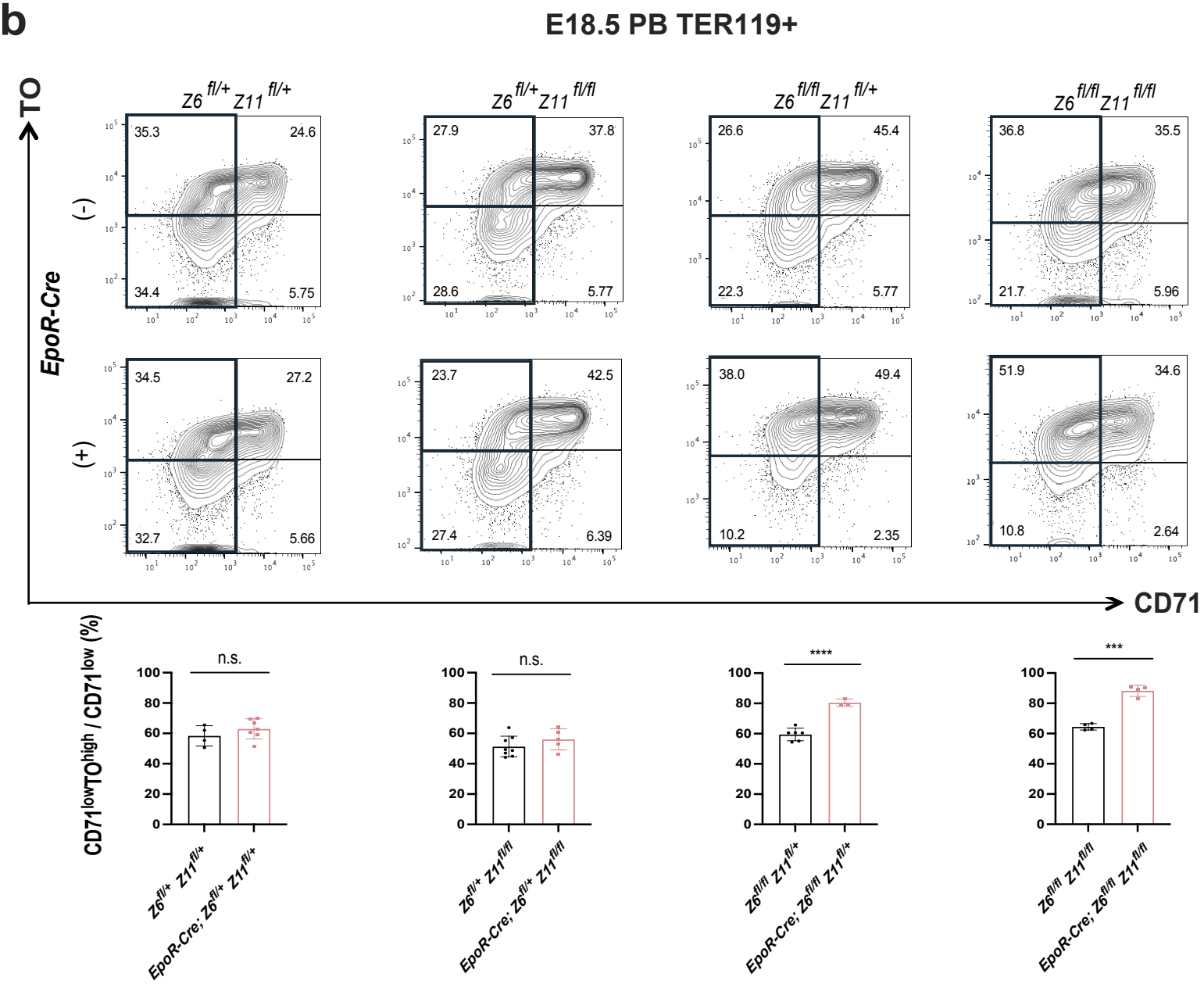
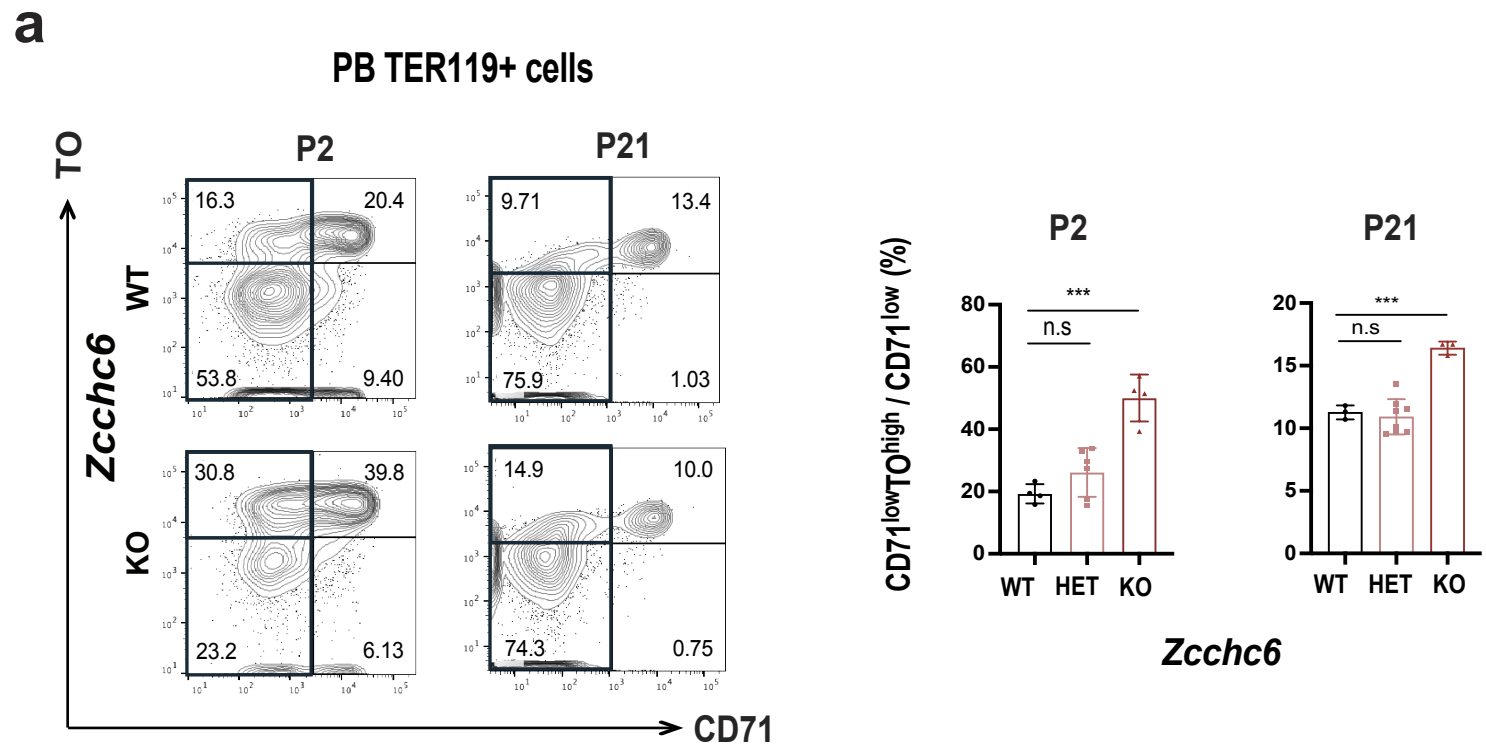
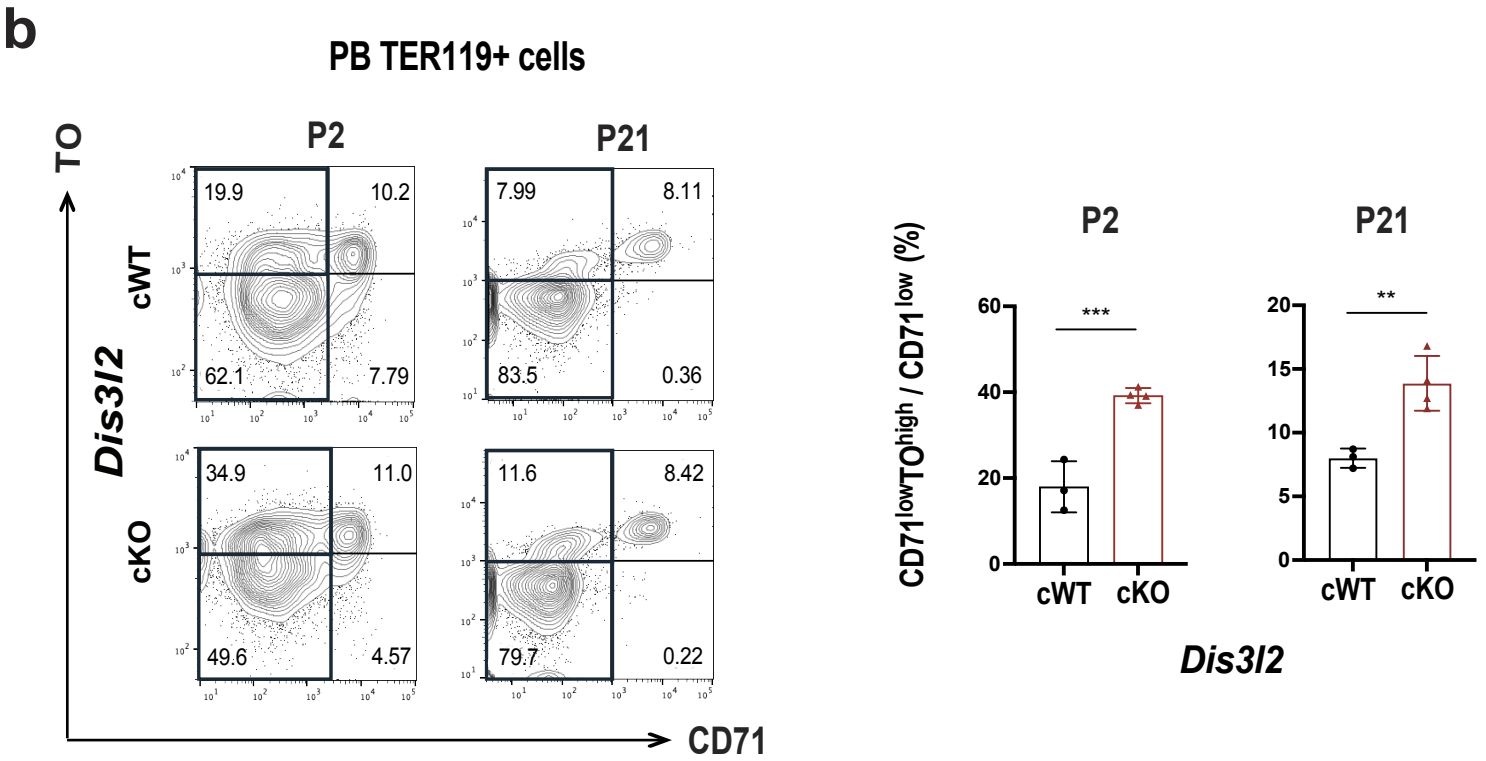


Extended Data Fig. 8. Analysis of *Quad-floxed* Conditional Knockouts.
(a) *Quad-floxed* conditional KO breeding scheme for *Zcchc6* and its paralog *Zcchc11*.
(b) Representative FACS profiles of the *quad-floxed* embryos and quantification of reticulocytes.

Extended Data Fig. 9





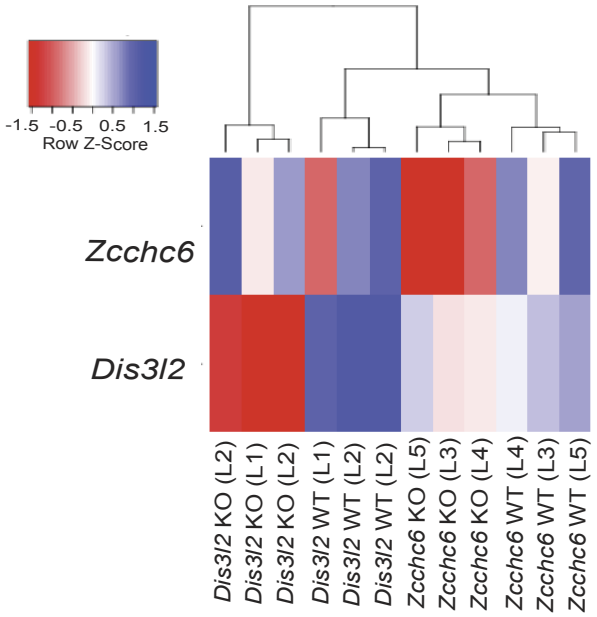
Extended Data Fig. 9. Postnatal Profiling of *Zcchc6* KO and *Dis3l2* cKO.
(a) Postnatal PB FACS profiling and quantification of whole body *Zcchc6* KO model
(b) and *Dis3l2* cKO model.

Extended Data Fig. 10

a

Genotype	Litter	Total Read No.	Mapped Read No.	Mapping Rate
Zcchc6 WT	L3	78,543,026	36,284,397	46.2%
Zcchc6 WT	L4	90,445,954	51,073,608	56.5%
Zcchc6 WT	L5	59,412,646	30,752,512	51.8%
Zcchc6 KO	L3	81,304,604	37,445,722	46.1%
Zcchc6 KO	L4	79,495,636	44,989,077	56.6%
Zcchc6 KO	L5	74,278,610	37,835,979	50.9%
Dis3l2 WT	L1	68,767,710	36,391,345	52.9%
Dis3l2 WT	L2	72,958,170	35,684,609	48.9%
Dis3l2 WT	L2	64,899,346	32,206,562	49.6%
Dis3l2 KO	L1	76,216,868	36,395,571	47.8%
Dis3l2 KO	L2	70,791,404	27,438,581	38.8%
Dis3l2 KO	L2	75,760,250	35,673,350	47.1%

b



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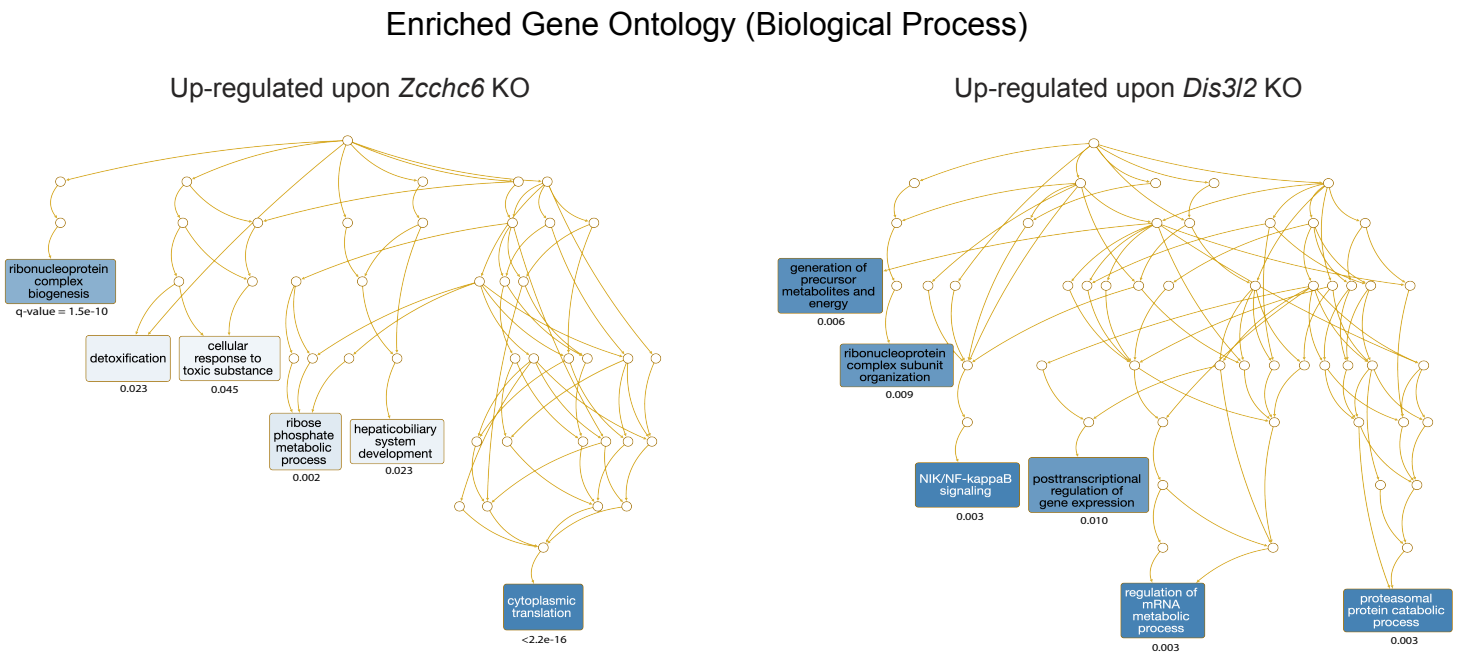
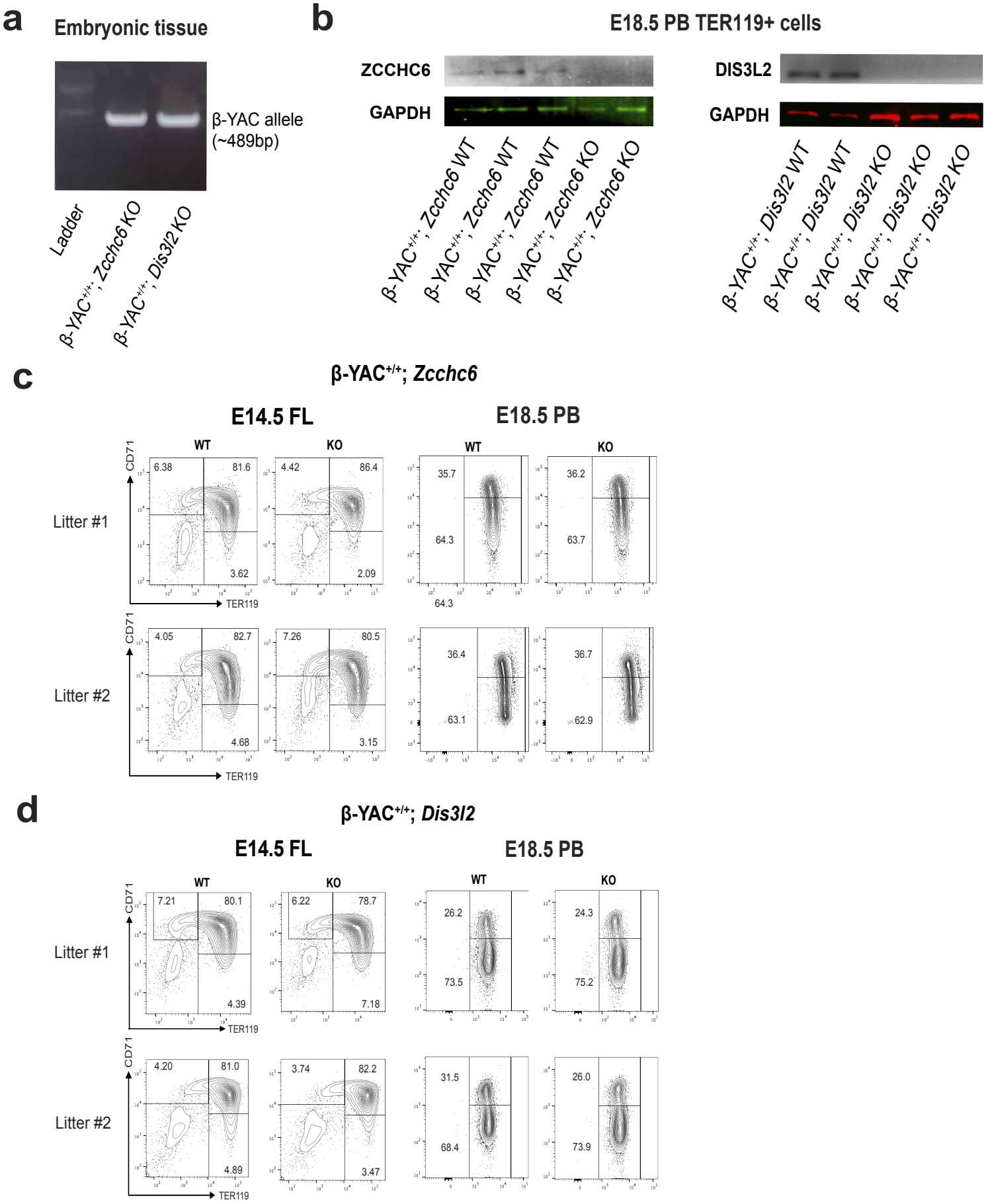


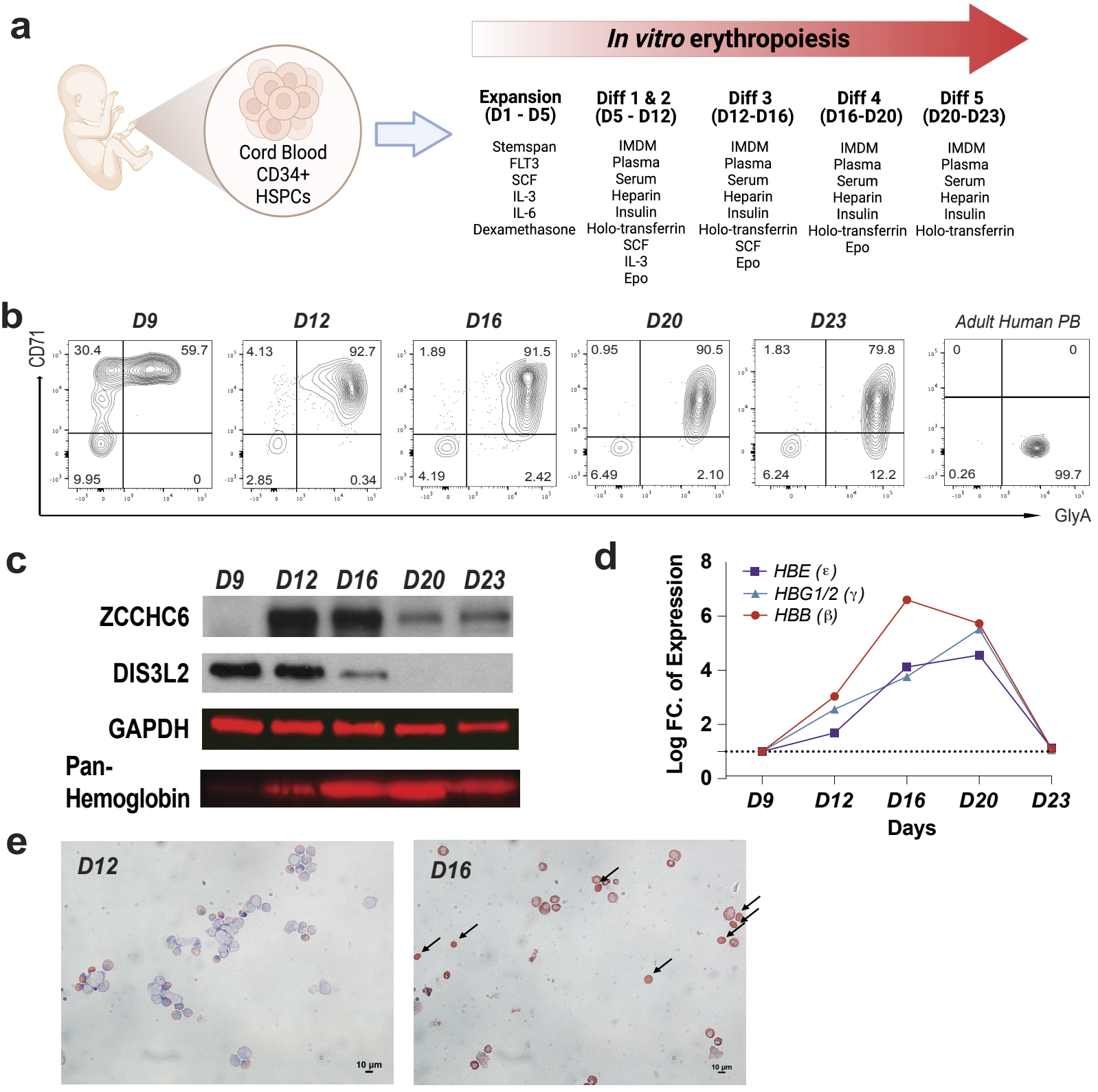
Extended Data Fig. 10. RNA-seq Data Analysis.
(a) Depth and mapping rates of bulk RNA-seq experiments.
(b) Hierarchical clustering of samples based on the expression of *Zcchc6* and *Dis3l2*.
(c) Enriched gene ontology terms and their statistical significances for each KO model.

Extended Data Fig. 11



Extended Data Fig. 11. Analysis of Humanized Mice Upon *Zcchc6/Dis3l2* KO.
(a) Genomic PCR analysis of the human β-YAC allele in offspring.
(b) Validation of preserved *Zcchc6/Dis3l2* KO in the crossed humanized mice using western blots.
(c & d) FACS profiles of erythroid maturation from E14.5 fetal liver and E18.5 PB of subject mice.

Extended Data Fig. 12



Extended Data Fig. 12. Human *In Vitro* Erythropoiesis

(a) Scheme of *in vitro* erythropoiesis from human cord blood-derived hematopoietic stem/progenitor cells (HSPCs; CD34+ cells).

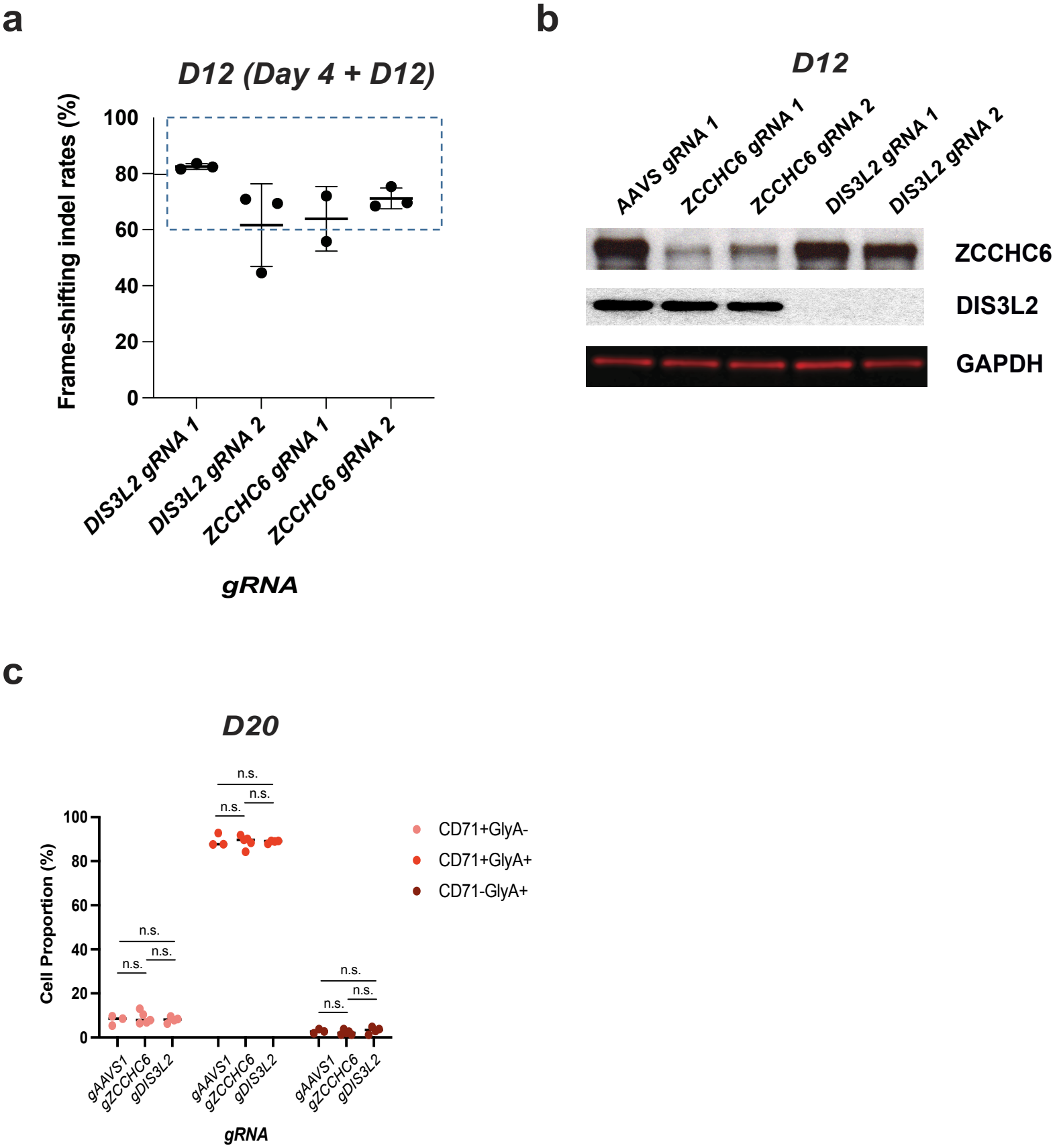
(b) Time-series FACS profiles to follow erythroid maturation status (Adult human PB served as a positive control).

(c) Time-series protein expression profiles of ZCCHC6, DIS3L2, and hemoglobin during the *in vitro* erythropoiesis by western blots.

(d) RNA expression profiles of β-like globins using qPCRs during the in vitro erythropoiesis.

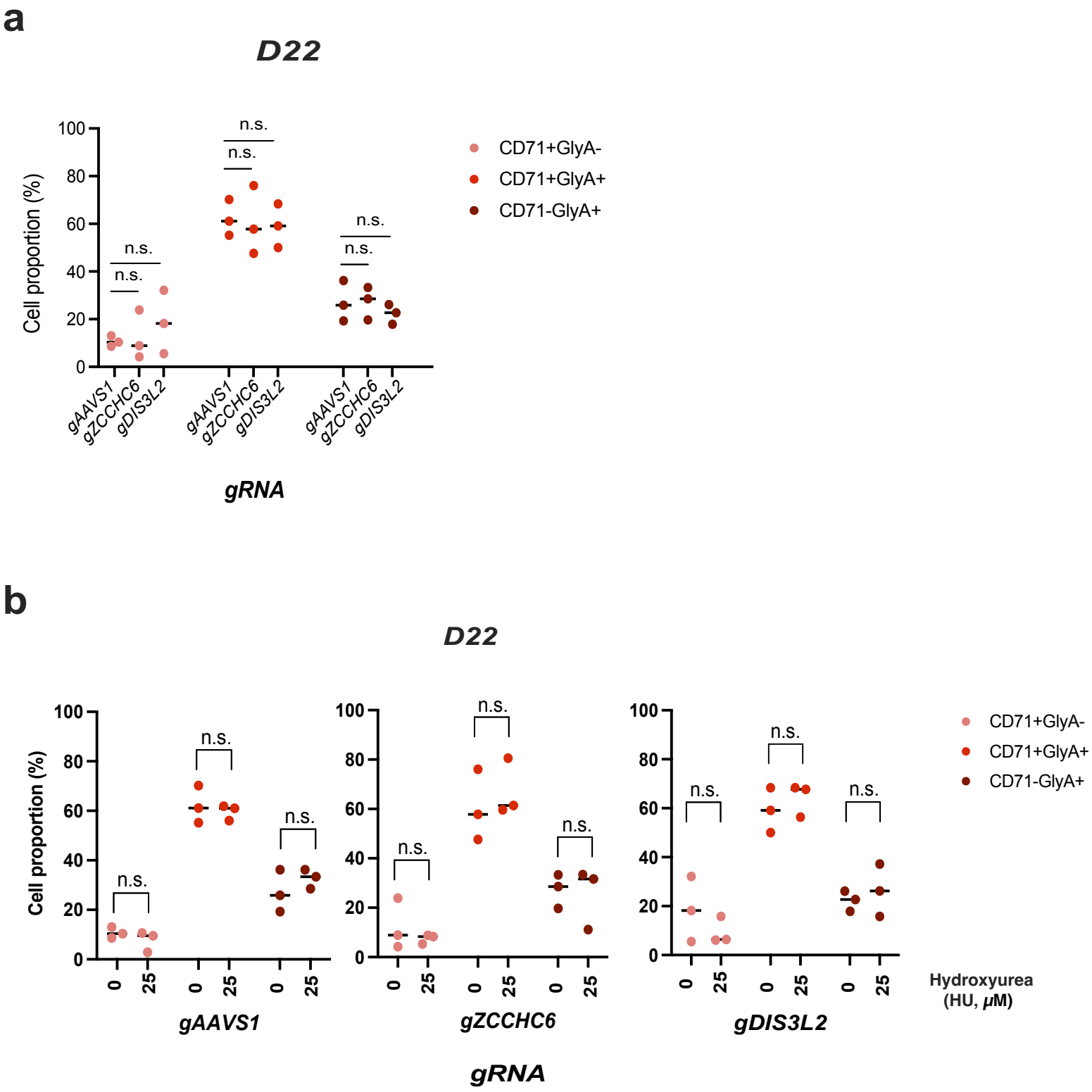
(e) Histology of cytospined cells (stained by May-Grünwald Giemsa method).

Extended Data Fig. 13



Extended Data Fig. 13. Analysis of CRISPR KO in Differentiated Erythroid Cells from CB.
(a) Genomic editing and **(b)** Protein profiles to access efficiency of CRISPR/Cas9-mediated KOs.
(c) Summary graph of erythroid maturation status by FACS (CD71 and GlyA) in CRISPR/Cas9 KOs.

Extended Data Fig. 14



Extended Data Fig. 14.
Analysis of CRISPR KO in Differentiated Erythroid Cells from PB Following HU treatment.
Summary graph of erythroid maturation status by FACS (CD71 and GlyA) in **(a)** CRISPR/Cas9 KOs **(b)** CRISPR/Cas9 KOs & HU treatment