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AT HOFSTRA/NORTHWELL

Targeting Fetal Hemoglobin Expression in Sickle Cell Disease for Novel Therapies

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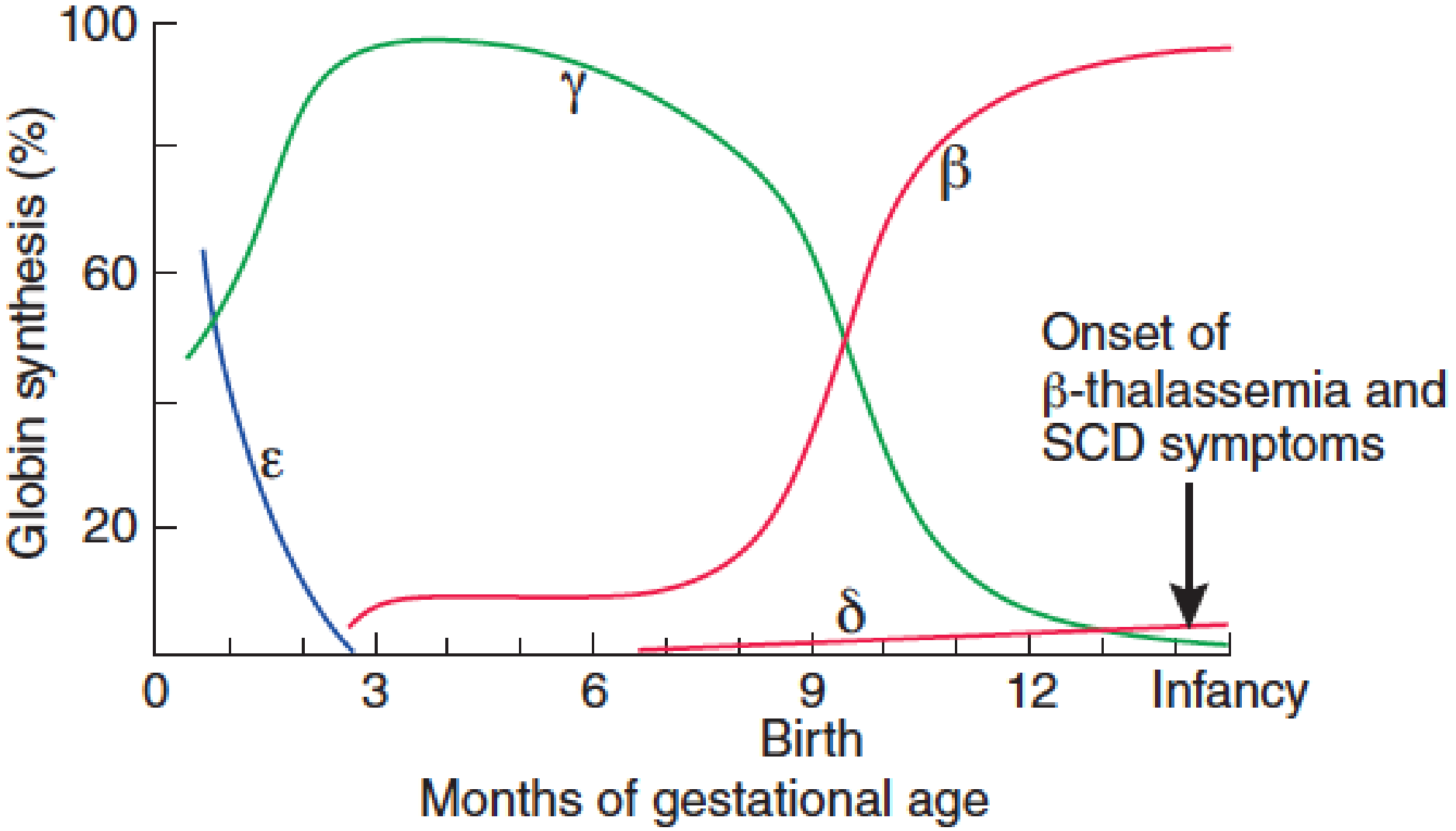
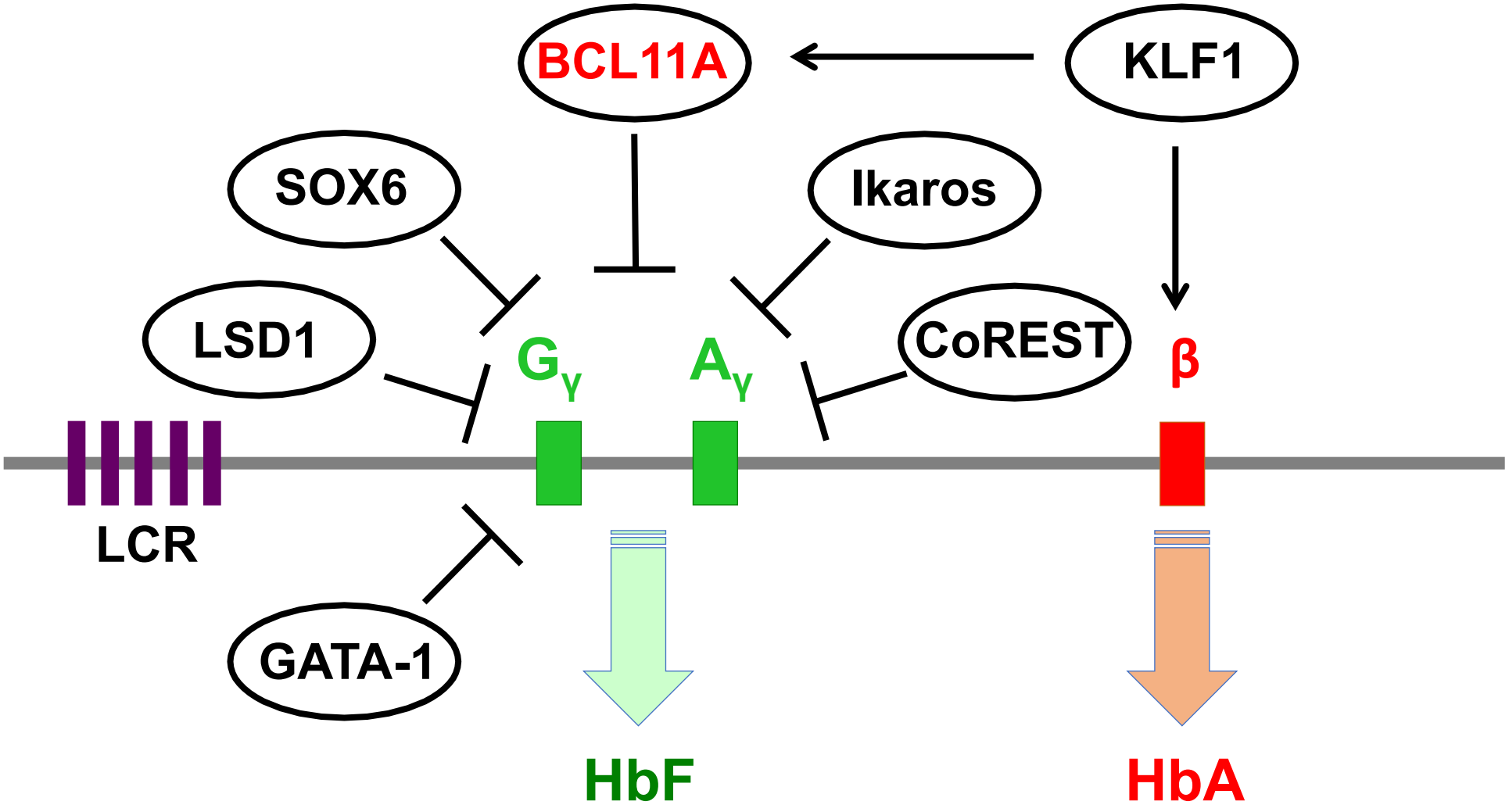
Globin Switch



Sankaran, V.G. and Weiss M.J., *Nat. Medicine*, **2015**

Regulation of the Gamma to Beta Globin Switch



Adapted from Xu, J. *et al.*, The Hematologist **2011**

Number of People with SCD

- Globally: >200,000 births/year
- United States: 70-100,000 persons with SCD
- New York State: 1,911 newborns in 8 years (240/year)
- CCMC: 24 newborns/year, total of >500 children are followed

Dr. Banu Aygun

Strategies for the Treatment of Sickle Cell Disease

. Stem Cell Transplant
. Gene Therapy
. Medication

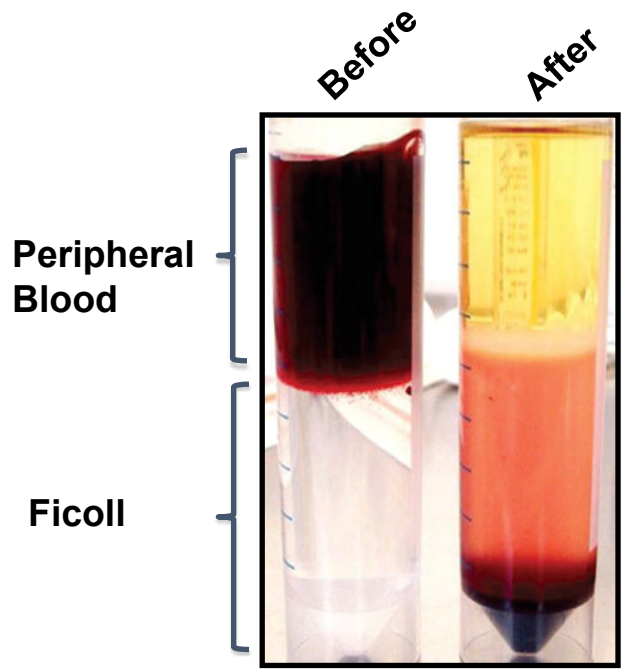
Pomalidomide

- Pomalidomide increases F-cells more than HU *in vitro* by an unknown mechanism of action. Moutouth de Parseval *et al*. JCI 2008

- Pomalidomide is FDA approved for treatment of Multiple Myeloma.

- Our hypothesis is that Pomalidomide acts through the modulation of the transcription networks regulating globin switching.

Methods



Isolate CD34+ cells
Miltenyi Column

Cytokines
SCF, IL3,
IL6, Flt3

Expand cells x 4days

Epo
SCF
IL-3
Study Drug

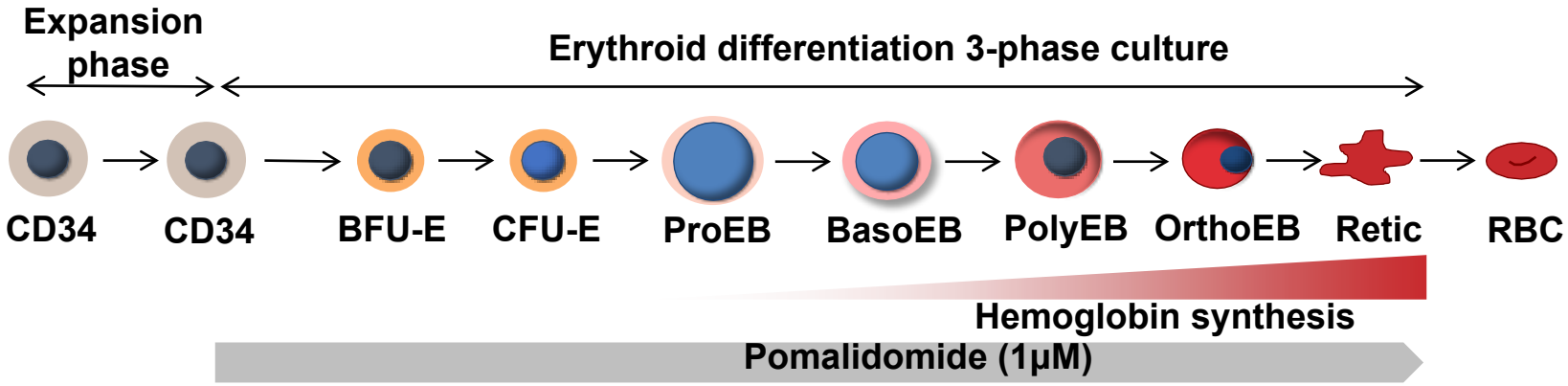
Liquid Culture System

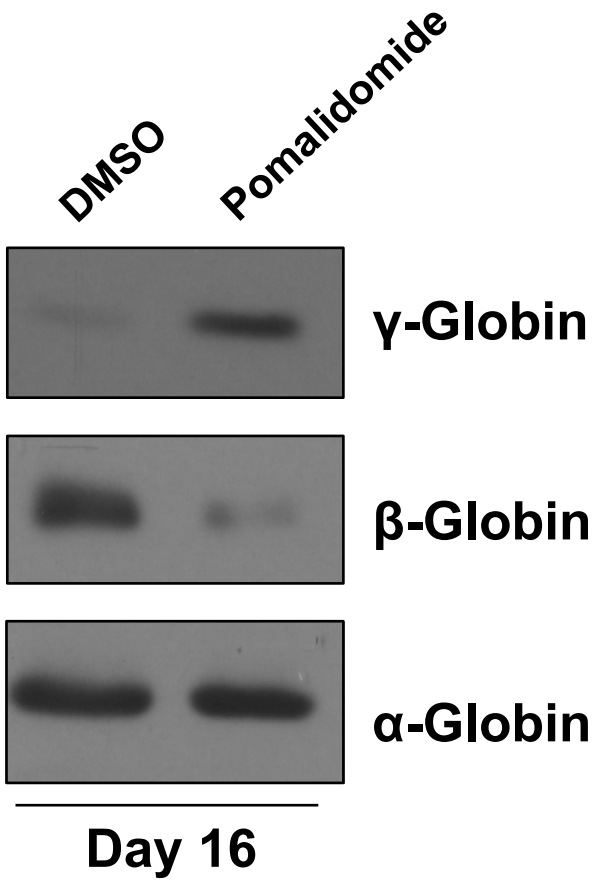
Differentiation for up to 16 days

We use CD34+ cells in vitro culture system

Many different culture systems

Pomalidomide Induces a Reciprocal Globin Switch

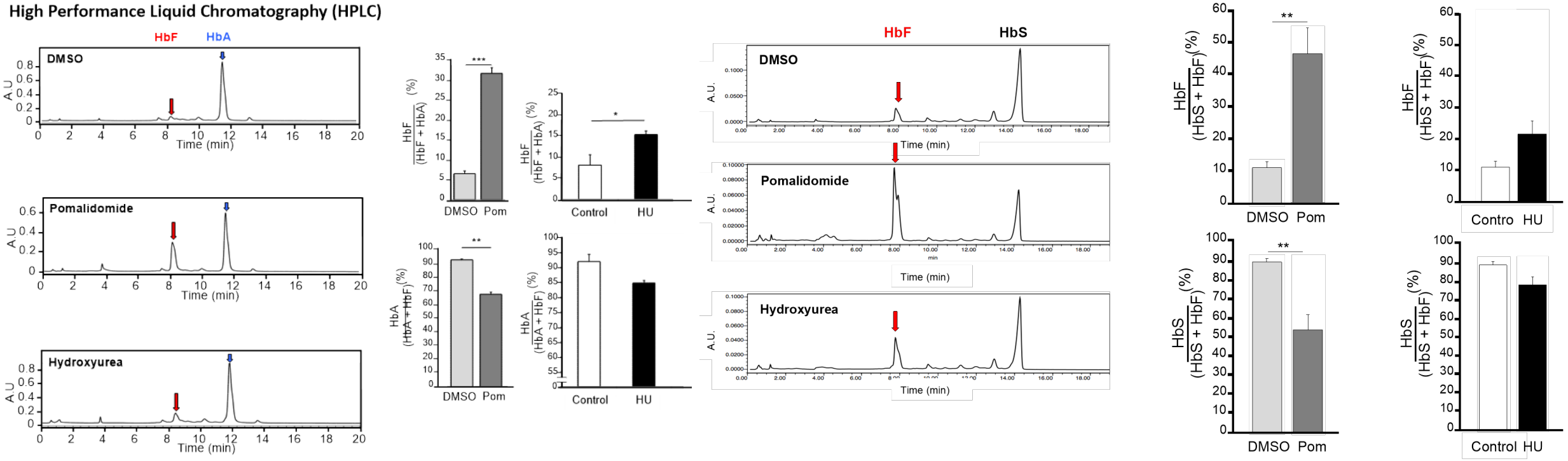




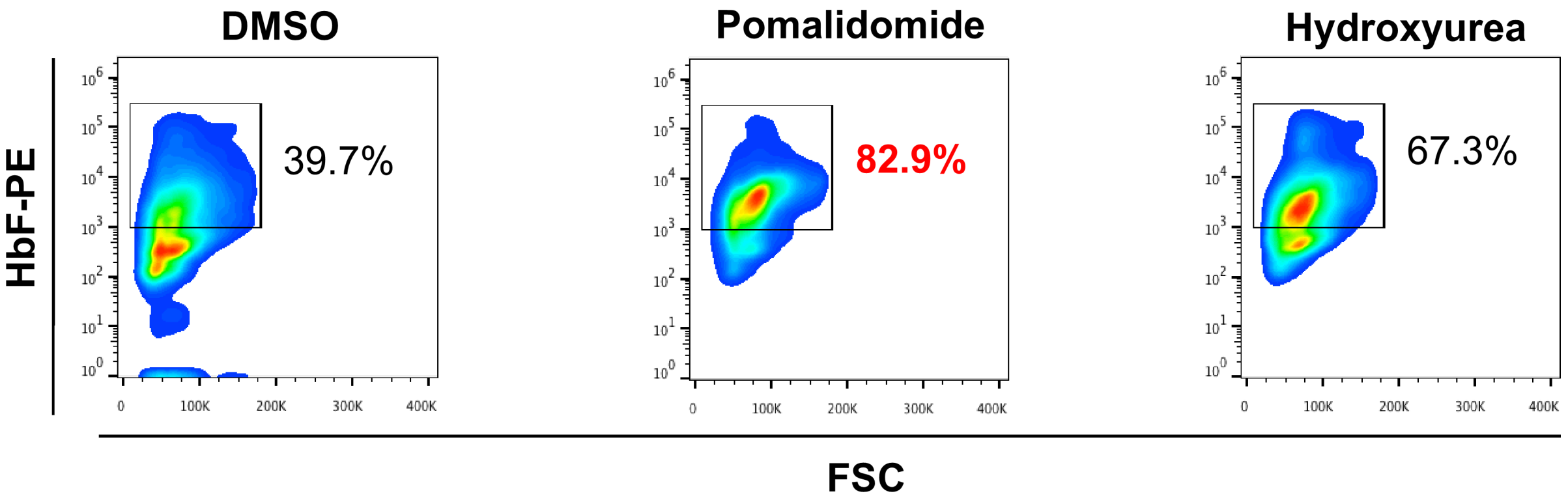
Pomalidomide Induces a Reciprocal Globin Switch

CD34+ cells from control donors

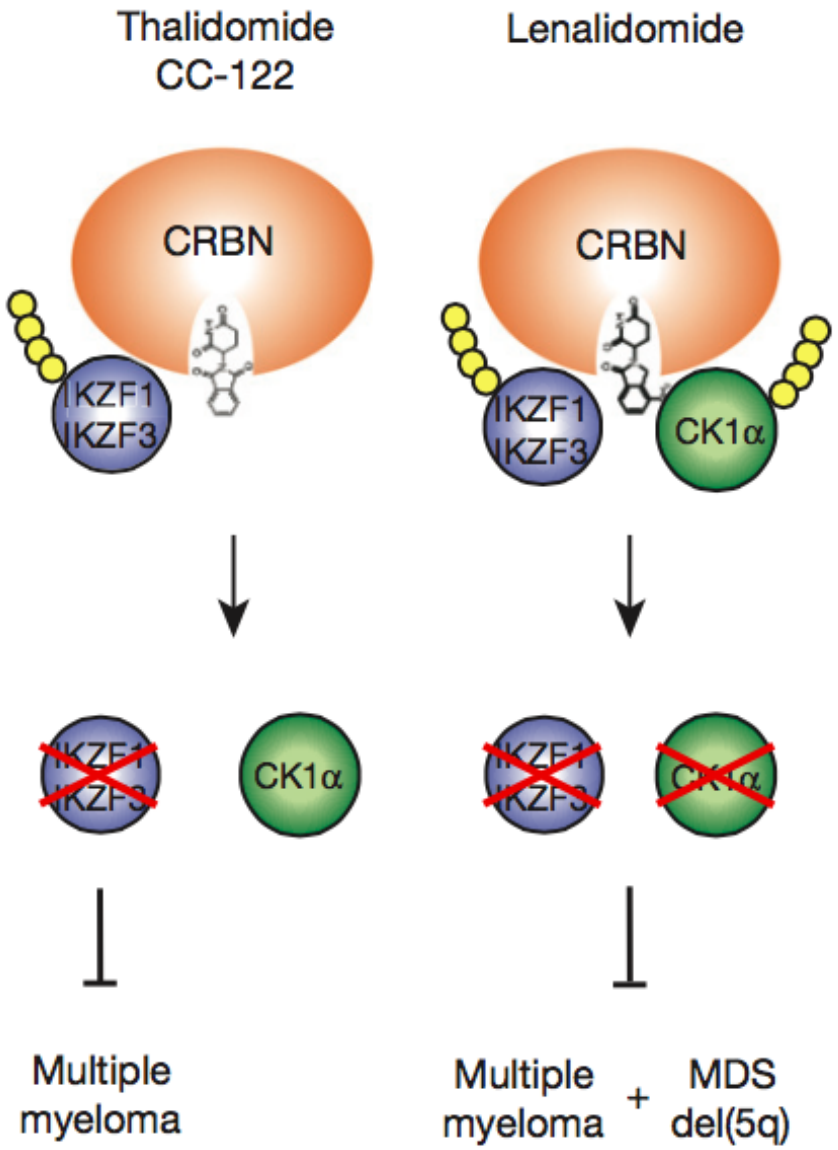
CD34+ cells from sickle cell disease patients



Pancellular Distribution of Fetal Hemoglobin after Pomalidomide Treatment



Pomalidomide and its analogues target Ikaros in the treatment of Multiple Myeloma



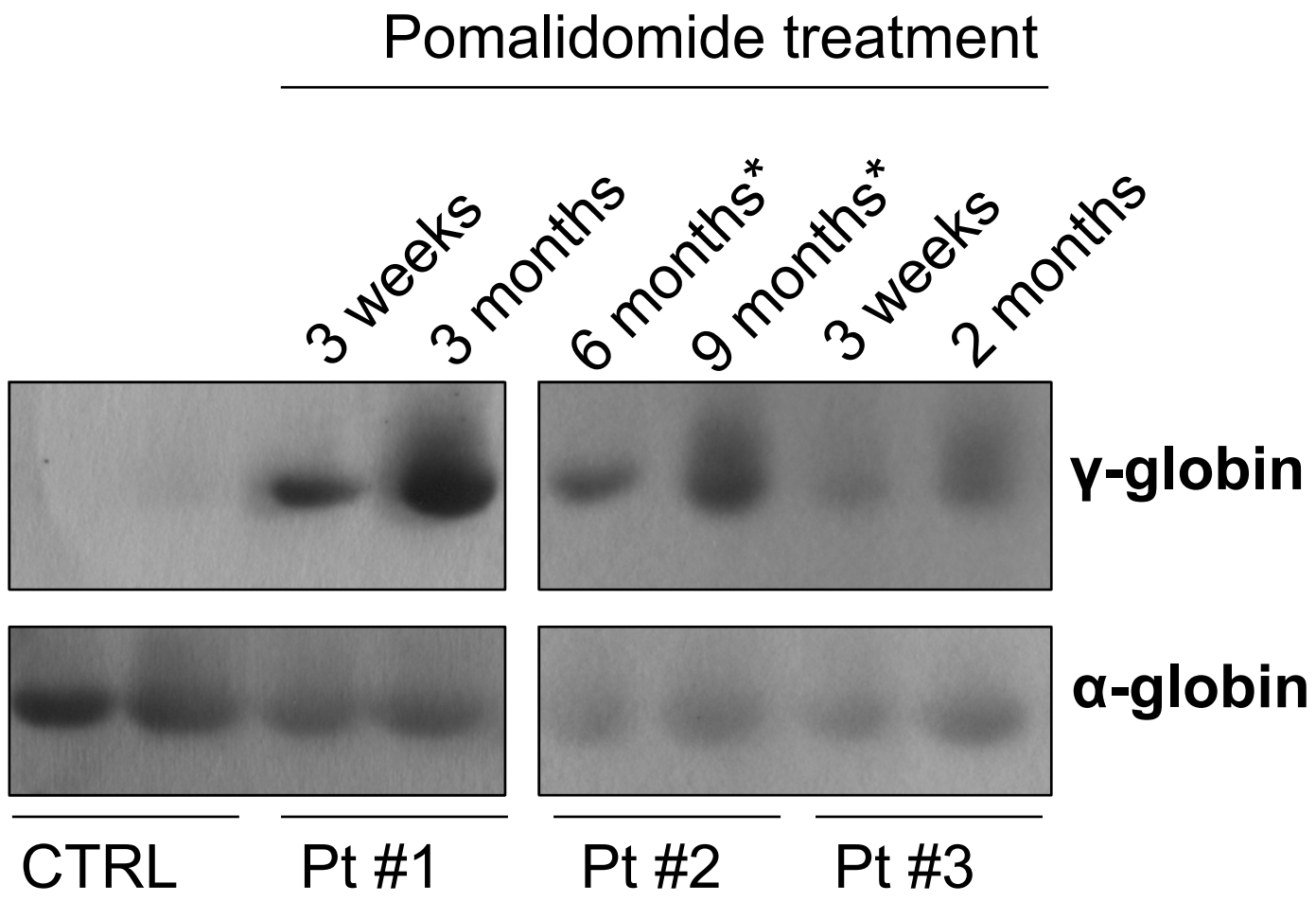
Kronke J et al. *Nature*.2015.

Patients with mutation in *IKZF1* Do Not Present Elevated Levels of HbF

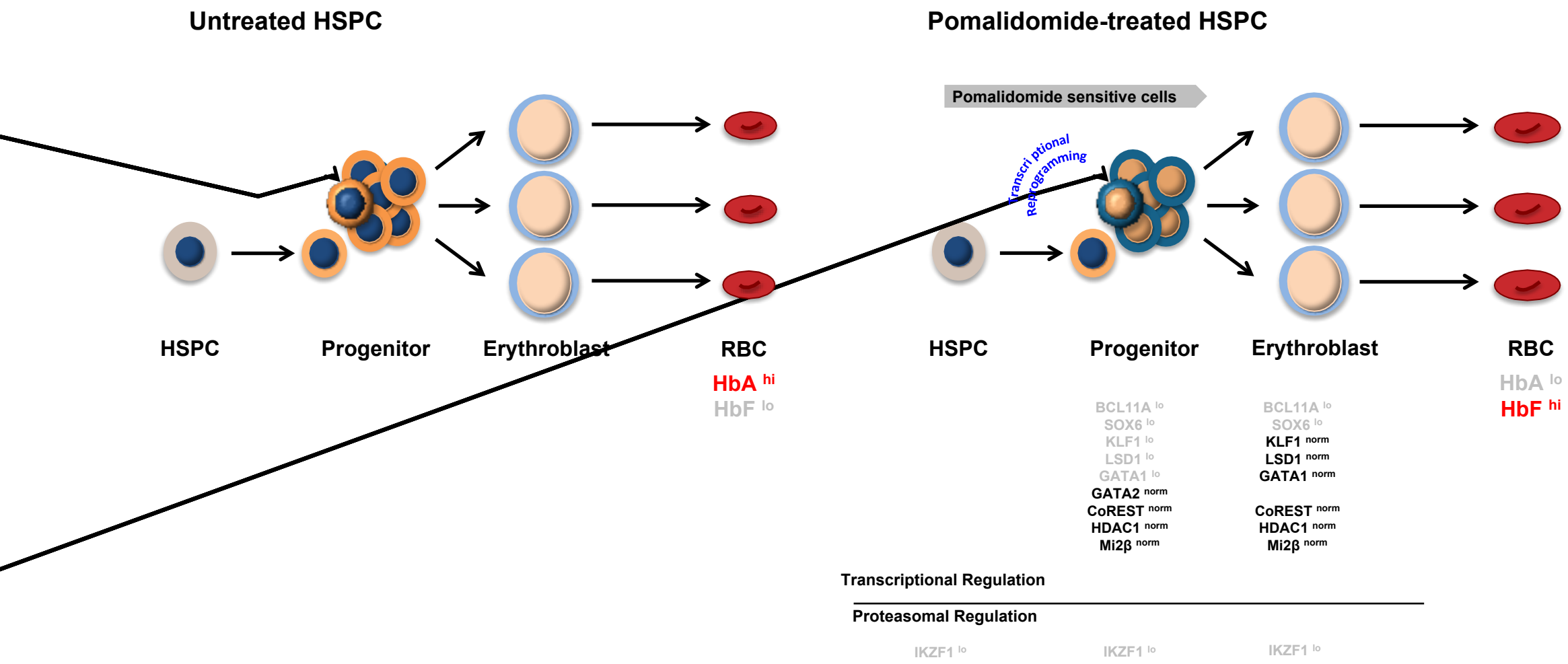
	Family C c.500A→G (p.H167R)					Family F 4.7-Mb deletion on chromosome 7																				Normal Range
Subject	**C1**	**C1A**	**C2**	**C3**	**C1B**	**F1**	**F2**	**F3**	**F4**	**F4A**	**F4B**	**F5**	**F5A**	**F5B**	**F5C**	**F5D**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**	**F14B**	**F14D**	-
Gender	F	M	F	F	F	F	F	M	M	M	F	F	M	M	F	F	M	F	F	M	M	M	M	M	M	M	F	-
Age	49	47	18	16	14	64	71	39	37	10	6	35	11	3	12	6	48	14	10	8	23	21	19	16	44	12	11	-
Affected/ Unaffected (A/U)	A	U	A	A	U	A	A	A	A	U	U	A	U*	U*	U*	U*	A	A	A	A	A	A	A	A	U	U	U	-
WBC, K/uL	9.01	6.34	7.58	9.68	13.08	6.10	3.95	4.70	6.45	7.04	8.36	8.62	8.4	9.84	10.7	7.32	5.40	4.81	5.95	4.74	7.87	8.9	8.68	6.98	10.33	6.32	5.67	3.98-10.04
RBC, M/uL	4.80	5.13	5.08	4.80	4.63	3.40	4.51	4.79	4.65	4.14	3.69	4.31	4.7	3.7	4.71	4.33	5.5	4.98	5.02	4.33	5.19	5.18	4.97	5.13	5.06	4.74	4.35	3.93-5.22
HGB, g/dL	13.9	16.3	13.1	14.6	13.7	10.2	13.9	15.1	14.8	12.2	10.5	13.0	13.6	10.5	13.7	12.5	16.1	14.7	13.9	12.1	16.9	17.4	16.0	16.6	15.2	14.0	13.2	11.0-17.0
HCT, %	41.5	46.9	40.7	42.1	40.3	32.3	43.1	45.8	45.0	37.5	33.2	42.4	42.7	33.2	42.8	39.1	50.6	46.9	43.8	38.8	50.1	52.8	47.9	49.9	49.1	44.6	41.1	34.0-50.0
MCV, fL	86.5	91.4	80.1	87.7	87.0	95.0	95.6	95.6	96.8	90.6	90.0	98.4	90.9	89.7	90.9	90.3	92.0	94.2	87.3	89.6	96.5	101.9	96.4	97.3	97.0	94.1	94.5	79.4-96.0
MCH, pg	29.0	31.8	25.8	30.4	29.6	30.0	30.8	31.5	31.8	29.5	28.5	30.2	28.9	28.4	29.1	28.9	29.3	29.5	27.7	27.9	32.6	33.6	32.2	32.4	30.0	29.5	30.3	25.6-33.0
MCHC, g/dL	33.5	34.8	32.2	34.7	34.0	31.6	32.3	33.0	32.9	32.5	31.6	30.7	31.9	31.6	32.0	32.0	31.8	31.3	31.7	31.2	33.7	33.0	33.4	33.3	31.0	31.4	32.1	32.2-35.5
RDW, %	12.7	12.6	14.1	13.1	12.7	14.1	14.9	13.8	14.1	12.9	13.3	13.4	13.3	14.4	13.2	13.4	14.6	13.5	14.3	13.0	13.5	13.3	14.3	14.0	12.7	13.4	12.3	11.7-14.4
Reticulocyte, %	-	-	-	-	-	1.4	1.3	1.0	1.1	0.9	0.9	1.5	1.2	1.7	1.4	1.4	1.8	0.9	0.8	0.8	2.5	3.3	2.7	2.2	1.5	1.4	1.3	0.5-2.0
Absolute Reticulocytes, K/uL	-	-	-	-	-	46.9	60.0	46.5	49.3	37.7	33.6	64.7	56.6	62.9	64.1	60.6	96.8	44.8	41.2	34.6	126.0	171.5	134.2	113.9	77.4	68.3	56.1	-
HbF, %	1.5	1.1	1.1	0.5	0.8	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2	1.5	0.8	0.3	0.2	0.2	0.4	0.2	0.2	0.7	0.2	0.2	0.3	0.3	0.9	< 2.0
HbA2, %	2.2	2.5	2.1	2.1	2.7	2.8	2.9	2.9	3.1	2.8	3.2	2.7	3.2	3.1	3.0	3.0	3.0	2.8	2.7	3.2	3.0	3.1	2.9	2.9	3.0	2.9	3.1	< 3.5

Adapted from Abdulhay N *et al.Blood*. 2016

Red Cells from Multiple Myeloma Patients on Pomalidomide Express Gamma-Globin

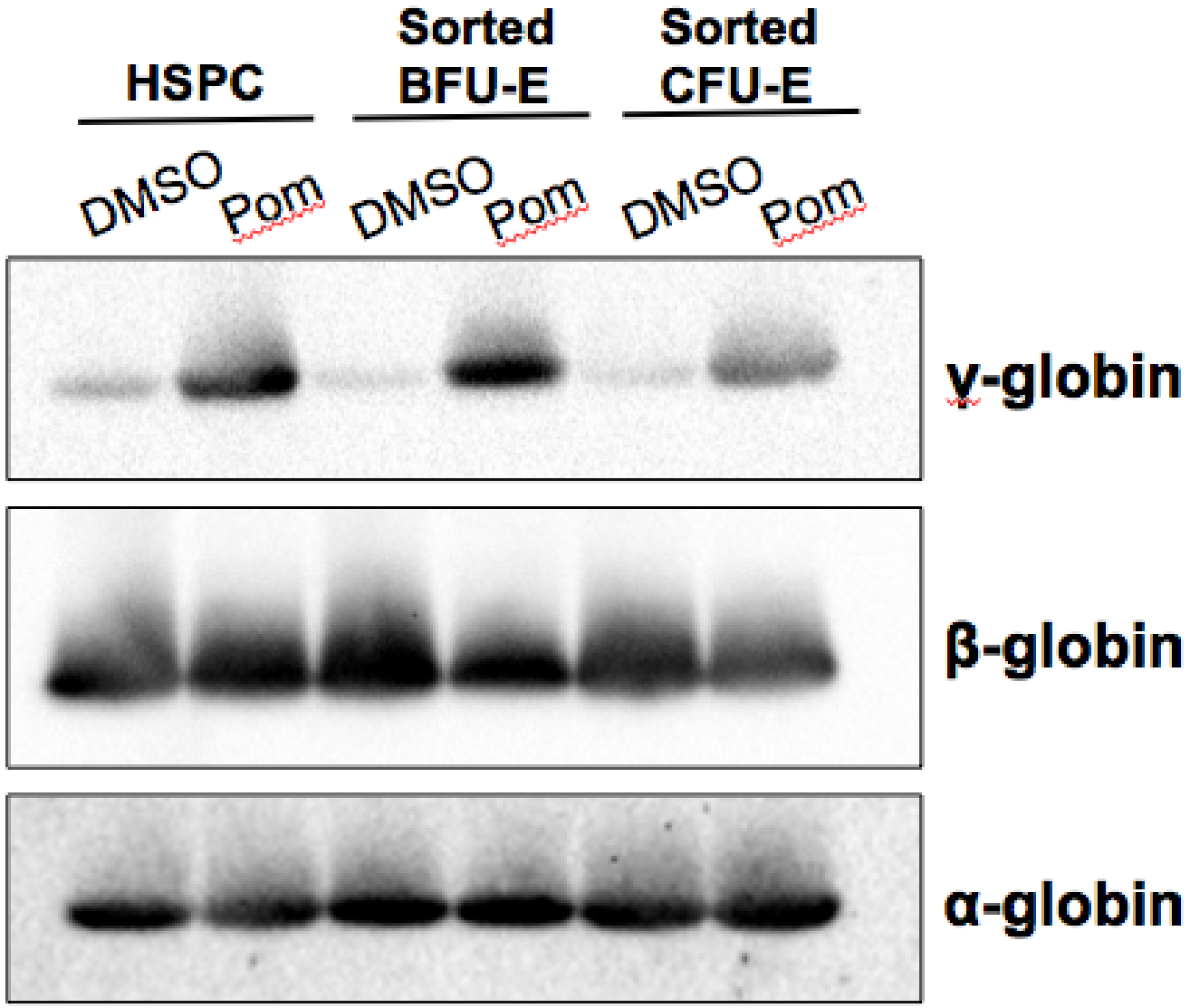


IMiDs in the Reactivation of Fetal Hemoglobin (HbF)



Dulmovits, B.M. *et al. Blood*, 2016

Pom Induces γ-globin Expression in a Stage Specific Manner



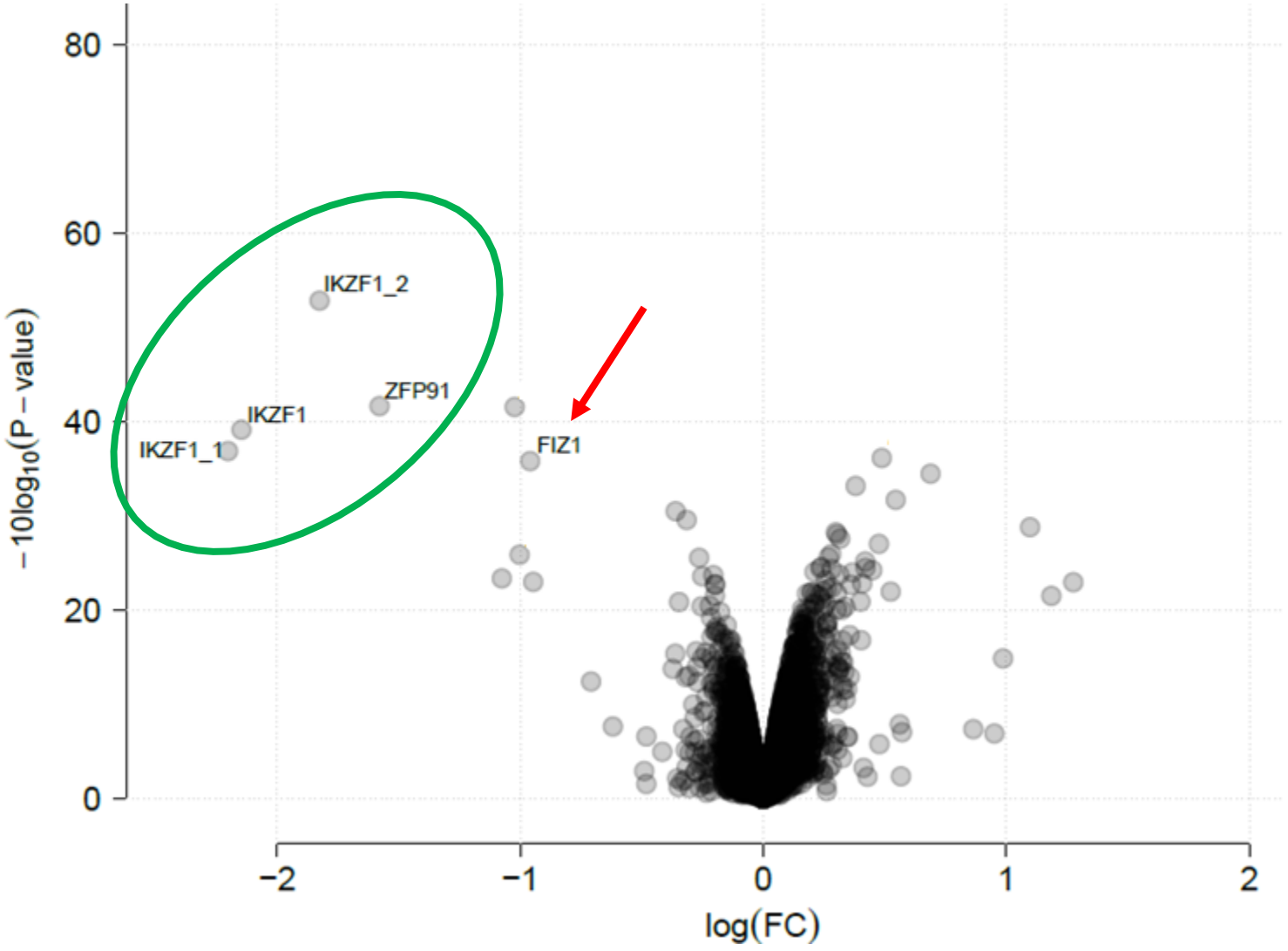
D14 of culture

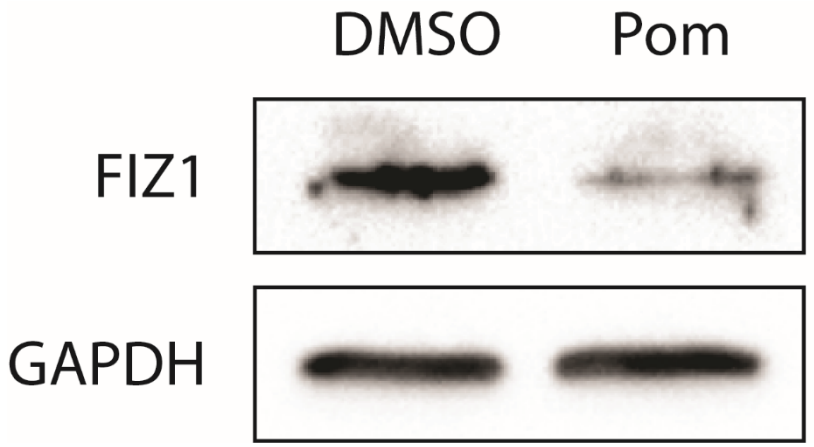
Why Try to Find the Targets of Pom in Erythropoiesis?

IMiDs may not be used in most patients

Many clinical trials focused on BCL11A

Proteomics of Pom-Treated CD34⁺ Cells Reveal FIZ1 as a Novel Pomalidomide Target

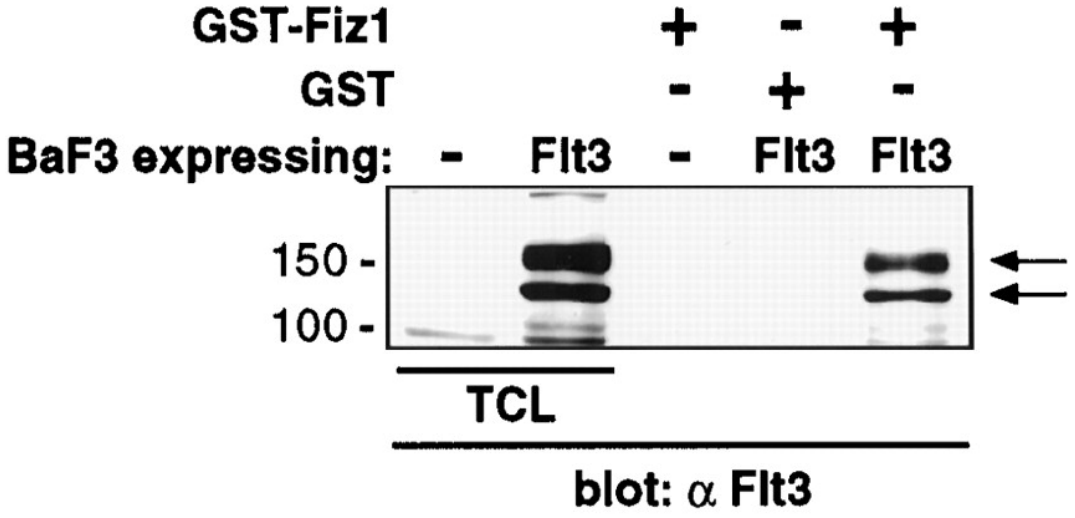




CD34⁺ D4 culture

FIZ1… Some Background

- FIZ1 (FLT3 interacting zinc finger 1)
  - Discovered as a binding partner of FLT3
  - Only significantly studied in retinal biology
  - Contains zinc finger domains similar to those in other IMiD targets: Ikaros, Aiolos and ZFP91



Wolf *et al., J. Biol. Chem.*, 1999

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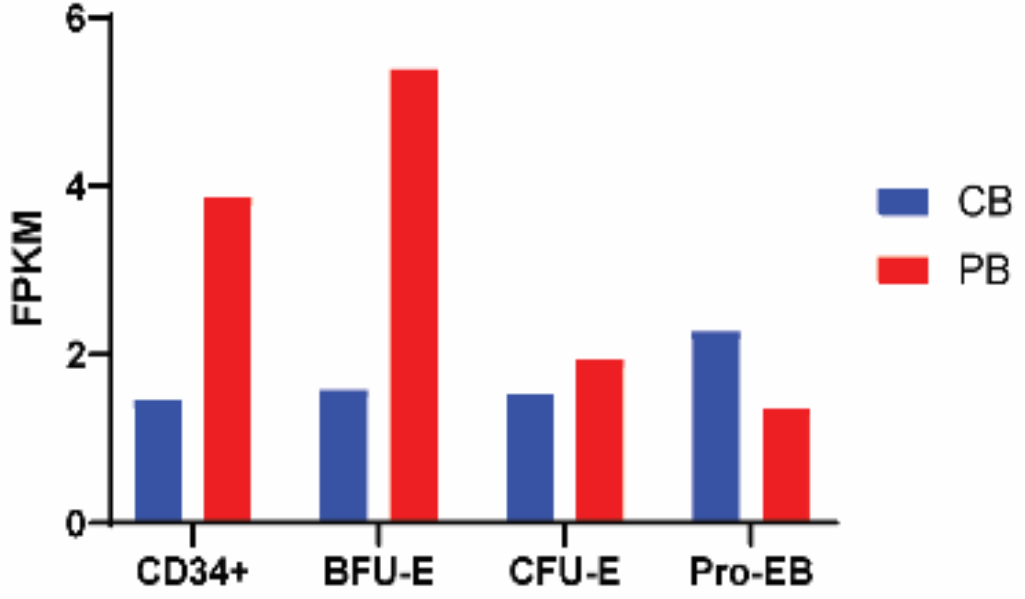
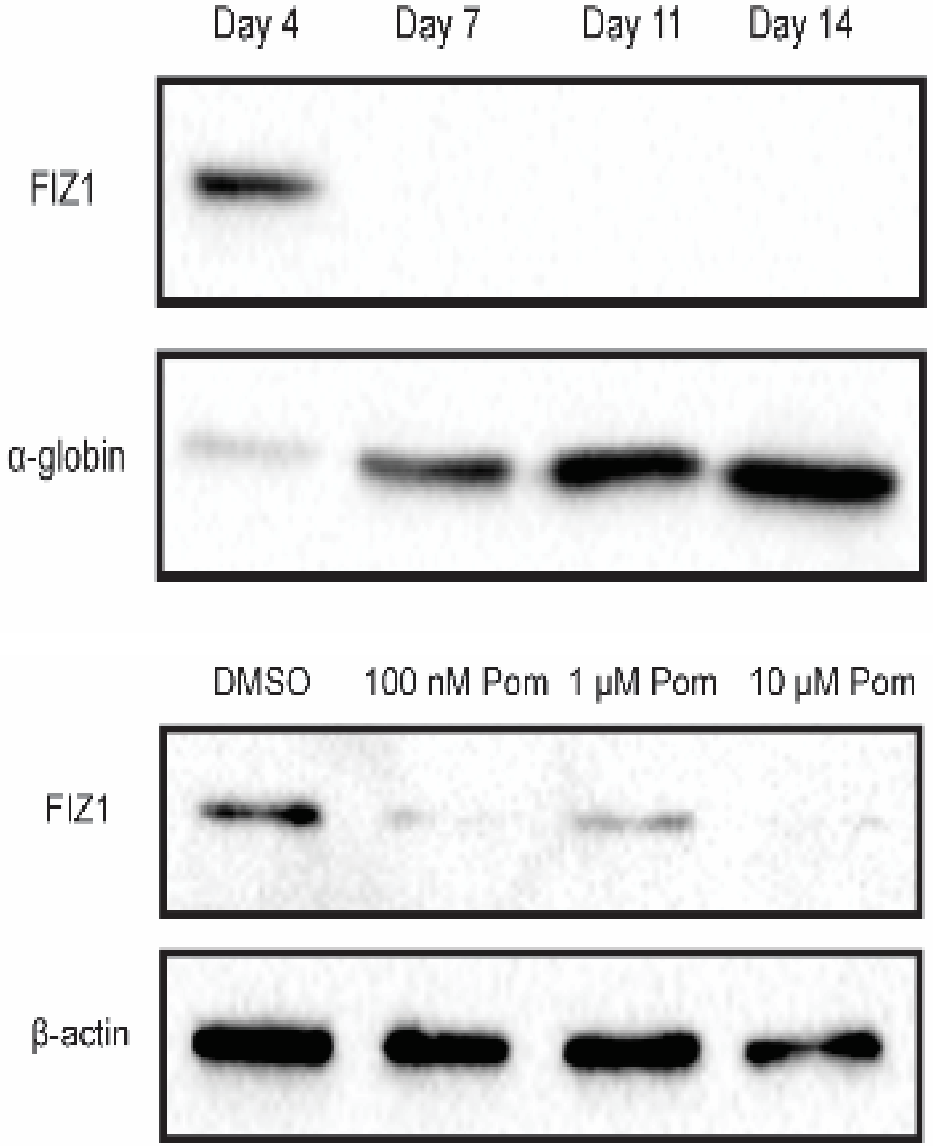
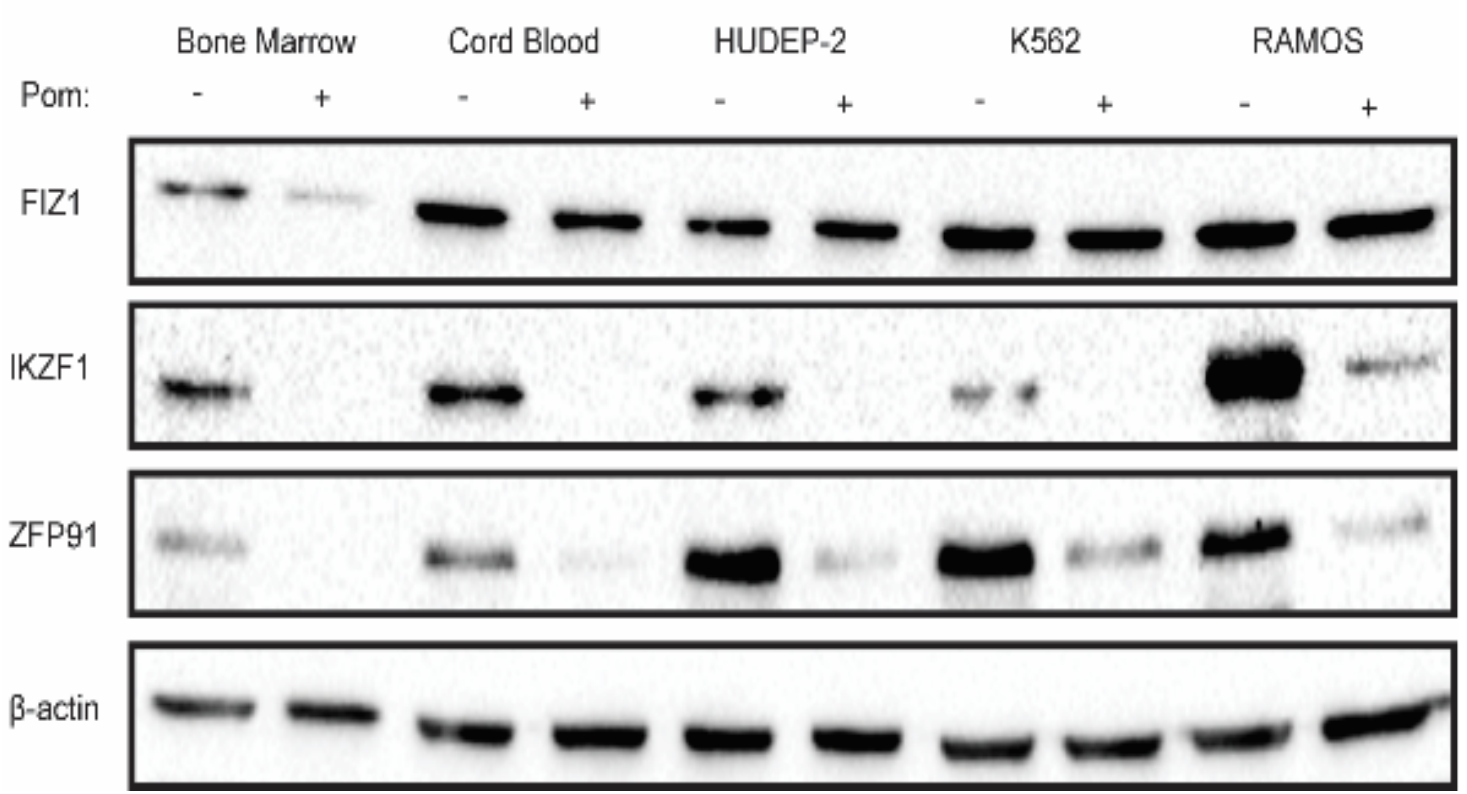


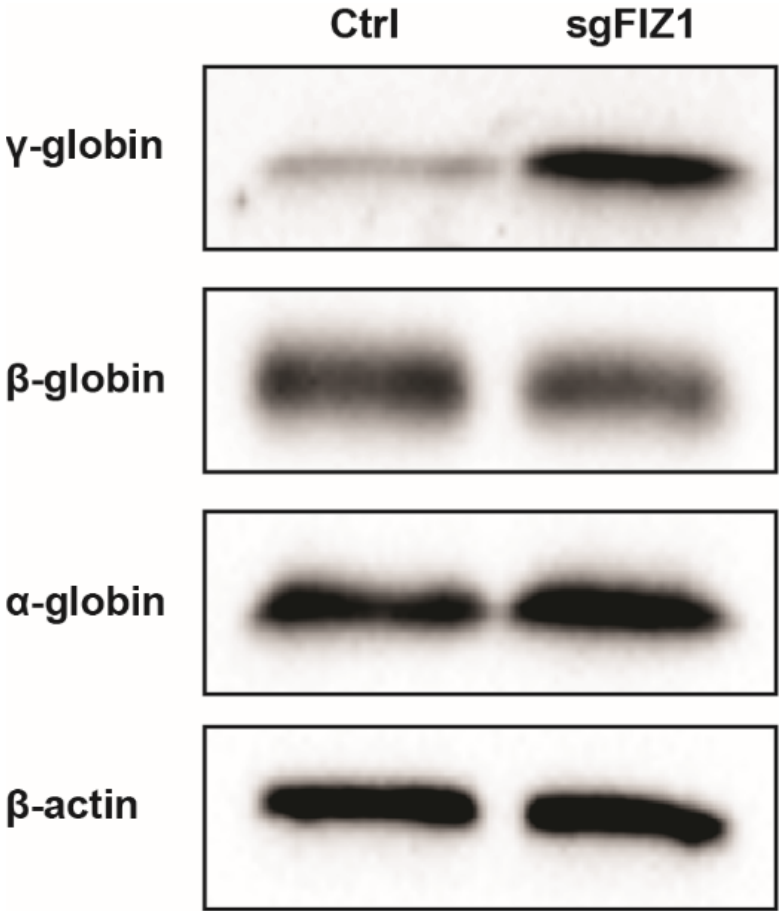
Yan, H. *et al., Am. J. Hematol*, 2018

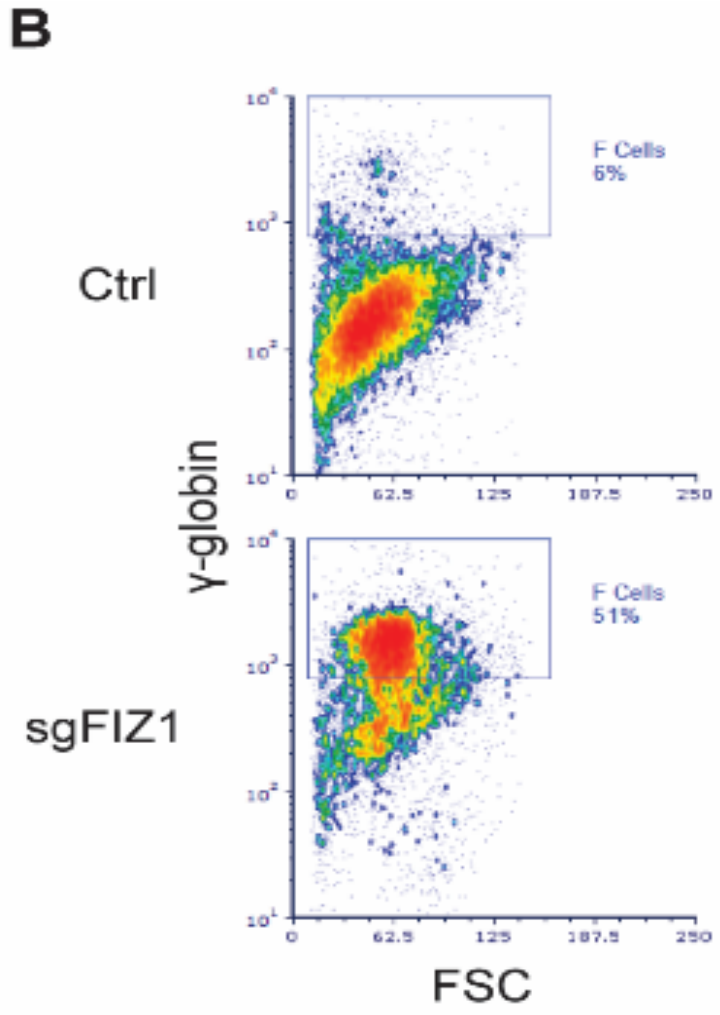
FIZ1 is Expressed Early During Erythropoiesis and its Degradation by Pom is Specific to Bone Marrow Erythroid Cells

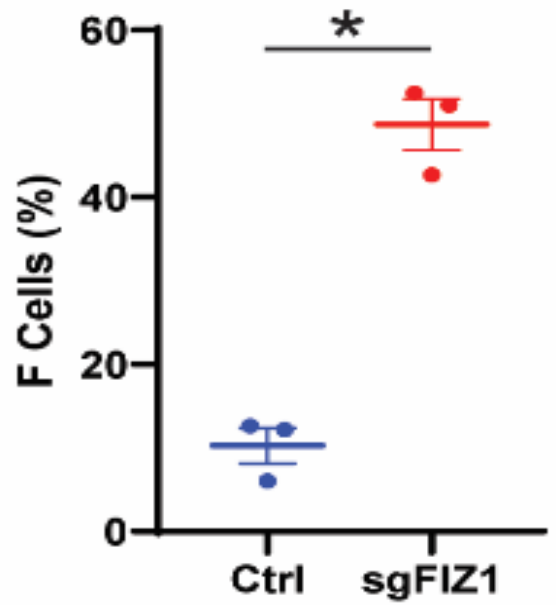




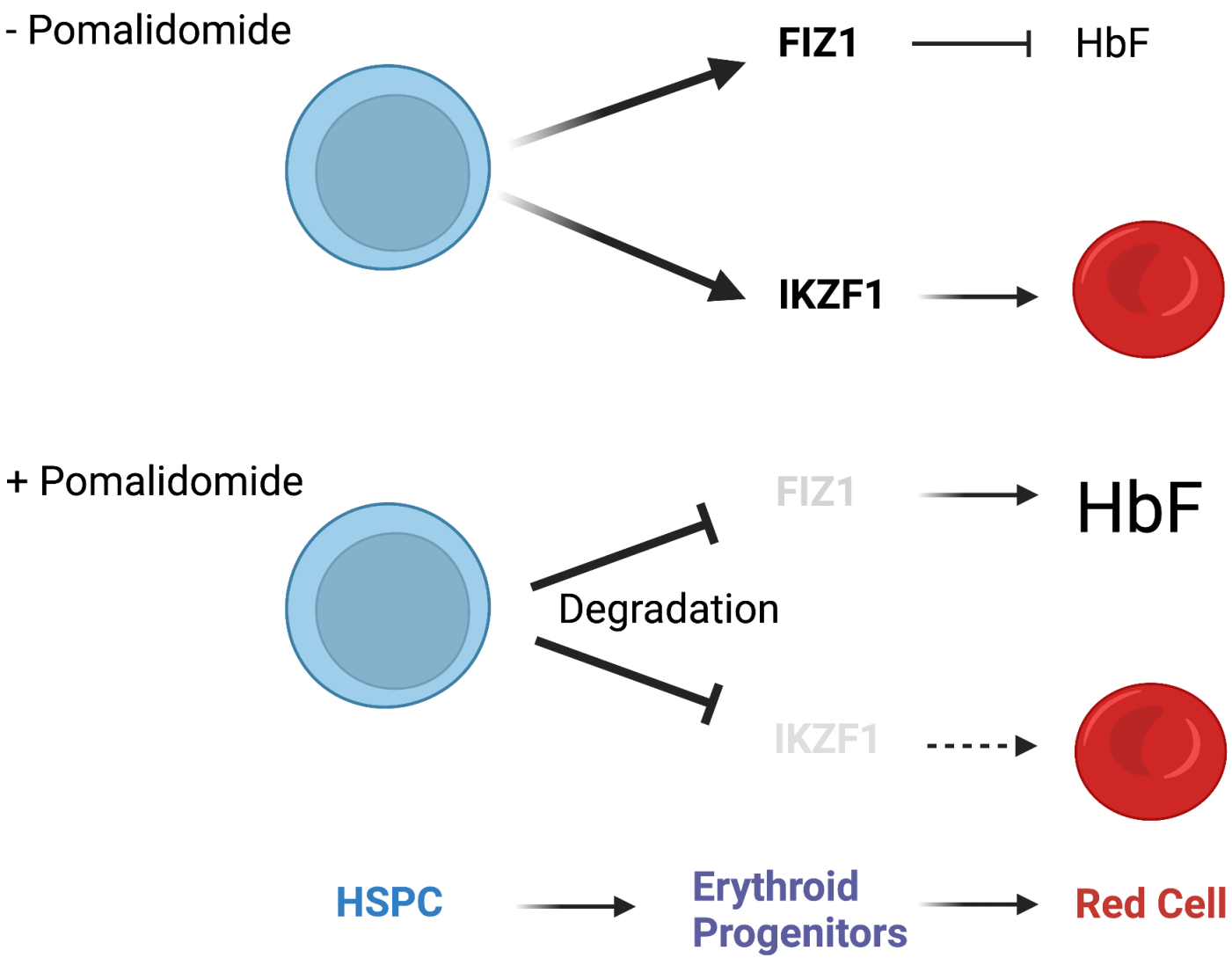
CRISPR/Cas9-Mediated Knockout of *FIZ1* Induces γ-Globin Expression







Summary



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Acknowledgments

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The patients and their families

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R01 HL152099
U01 HL134812

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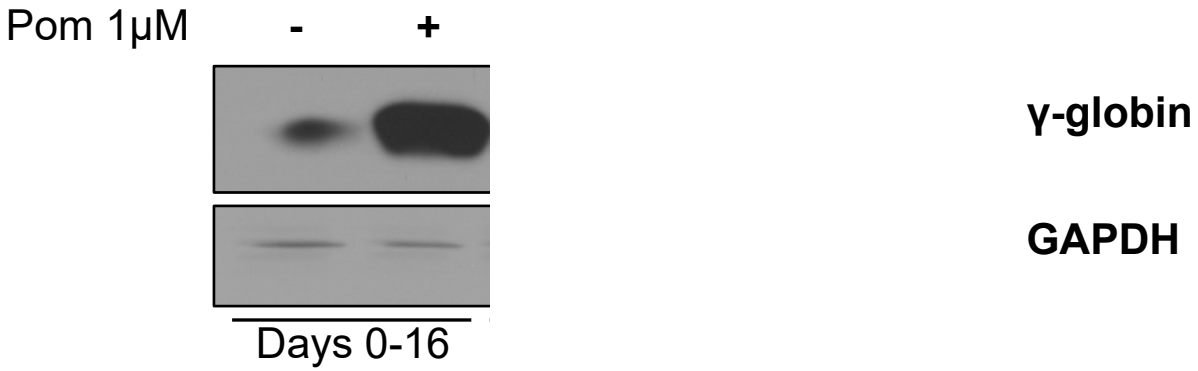
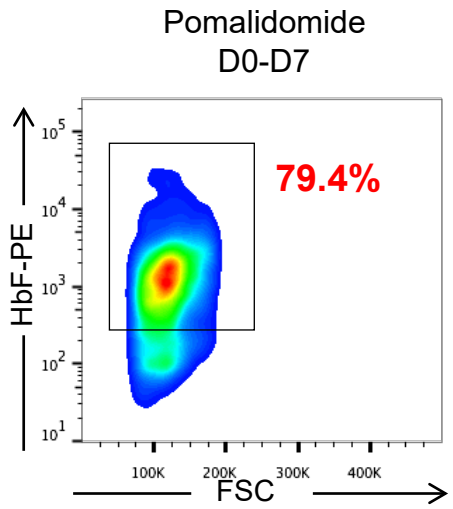
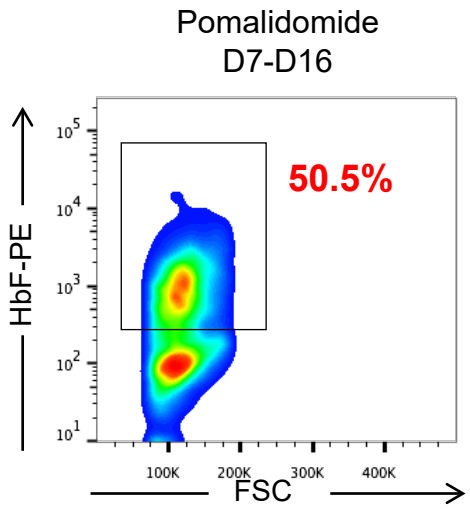
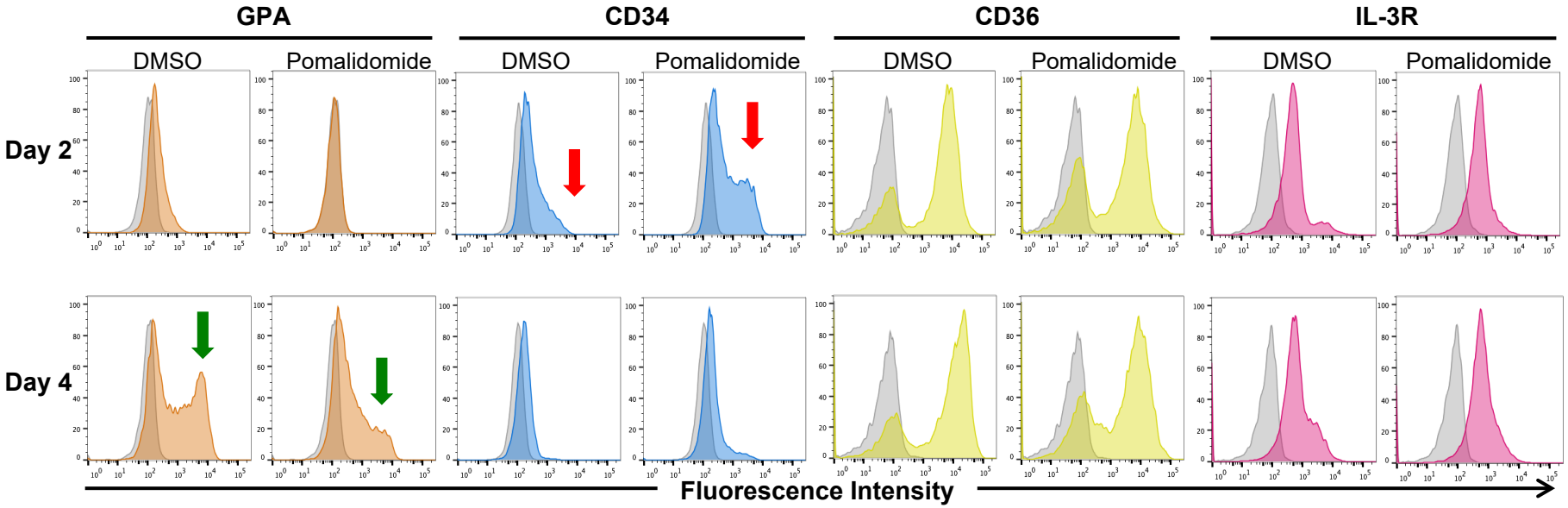
Pomalidomide Acts Early During Erythroid Differentiation

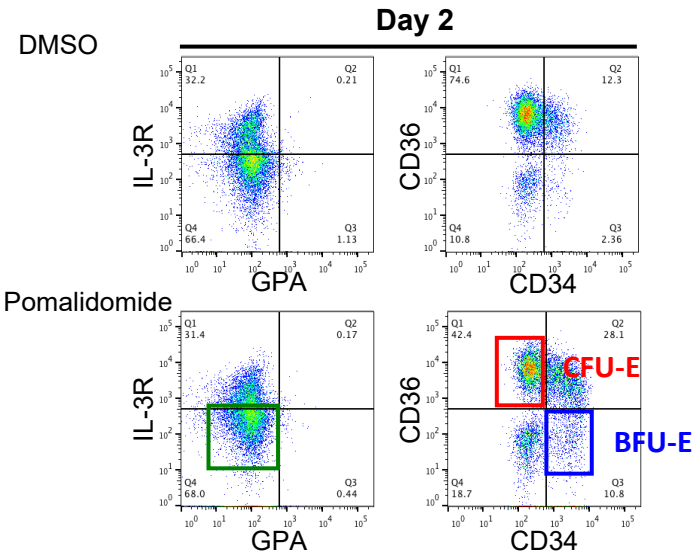


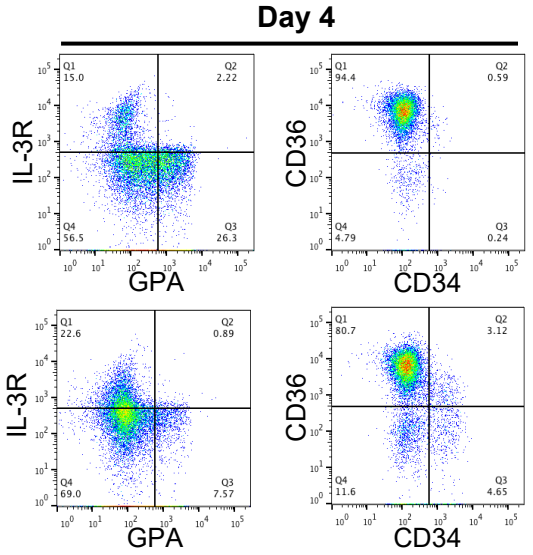




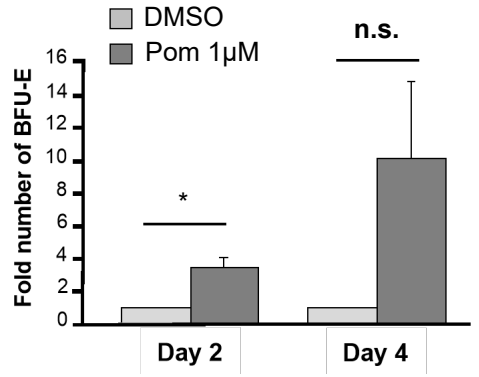
Pomalidomide acts early during erythroid differentiation

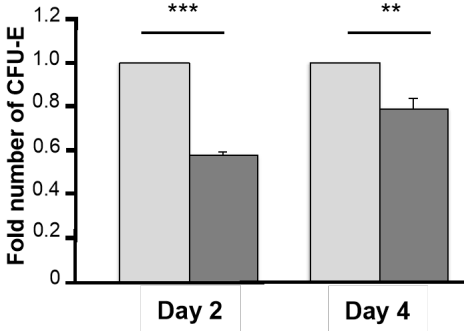




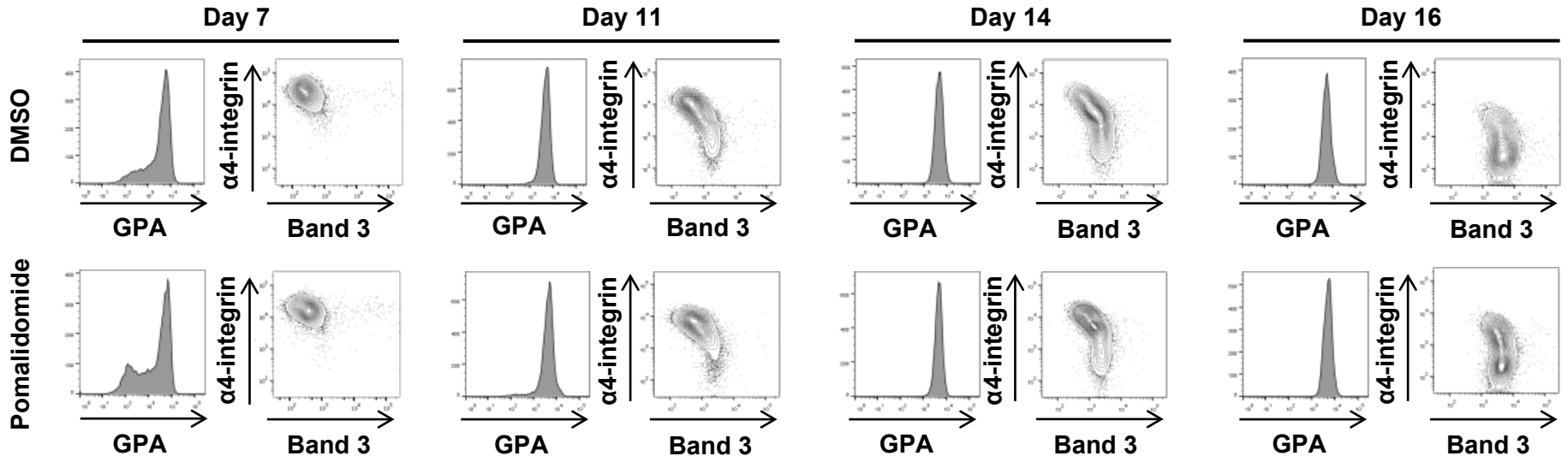


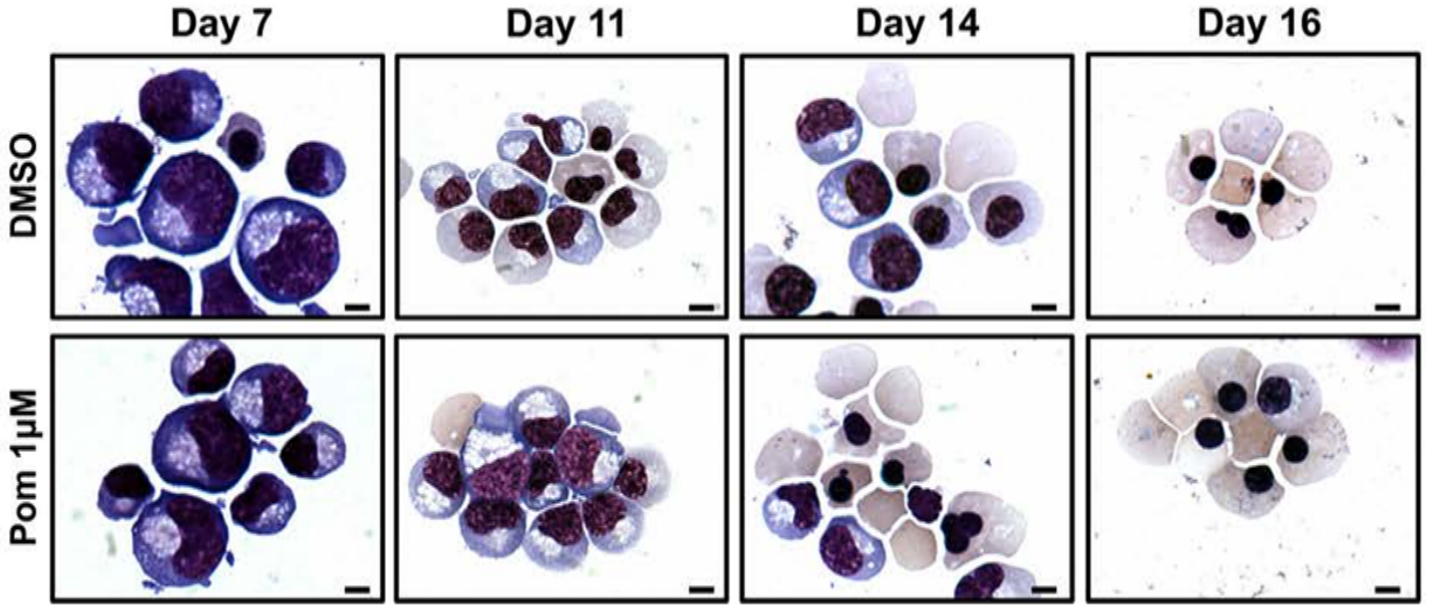
Increased BFU-E
Decreased CFU-E





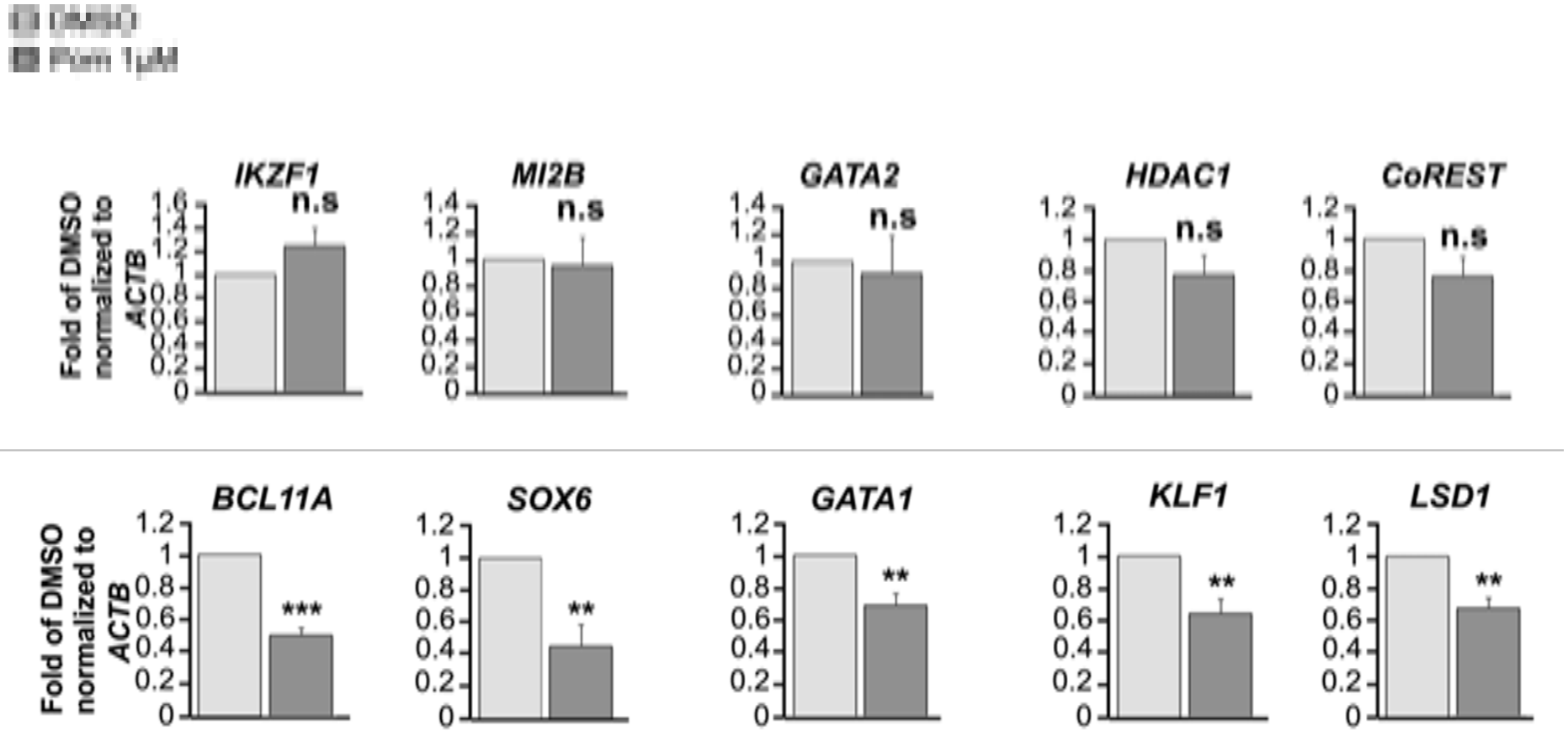
Terminal erythroid differentiation remains unaffected by Pomalidomide treatment





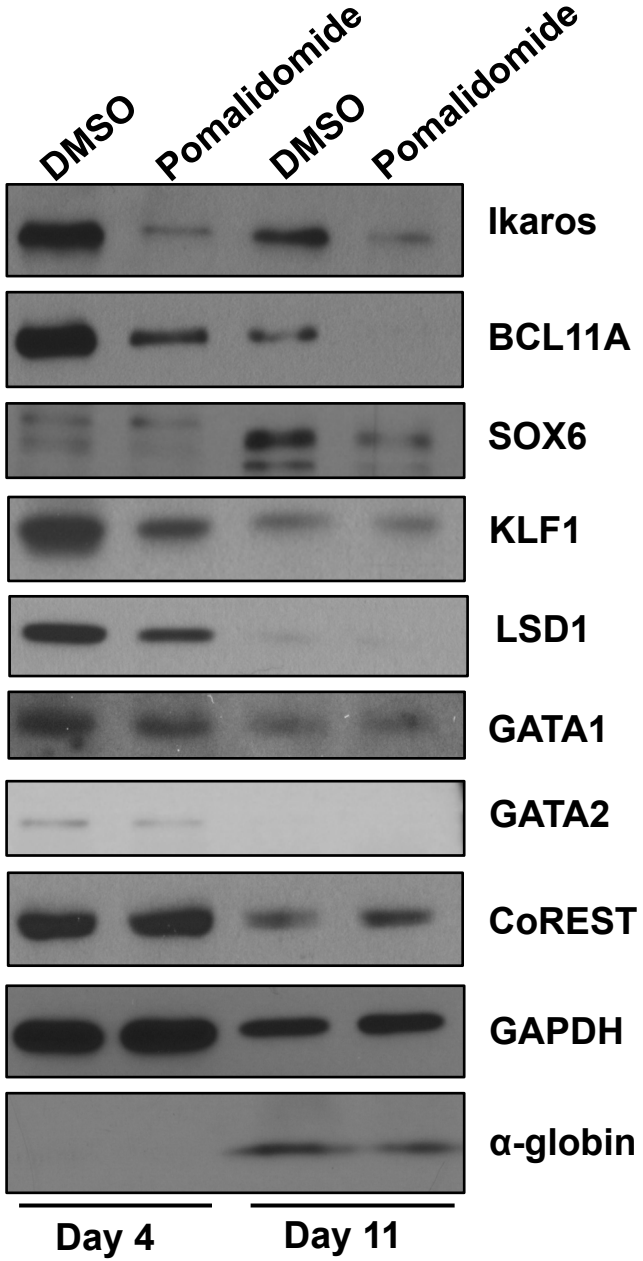
Bar= 5µm

Pomalidomide differentially affects the transcription networks involved in erythropoiesis and globin switching



Experiments performed at Day 4 of erythroid differentiation

Pomalidomide differentially affects the transcription networks involved in erythropoiesis and globin switching

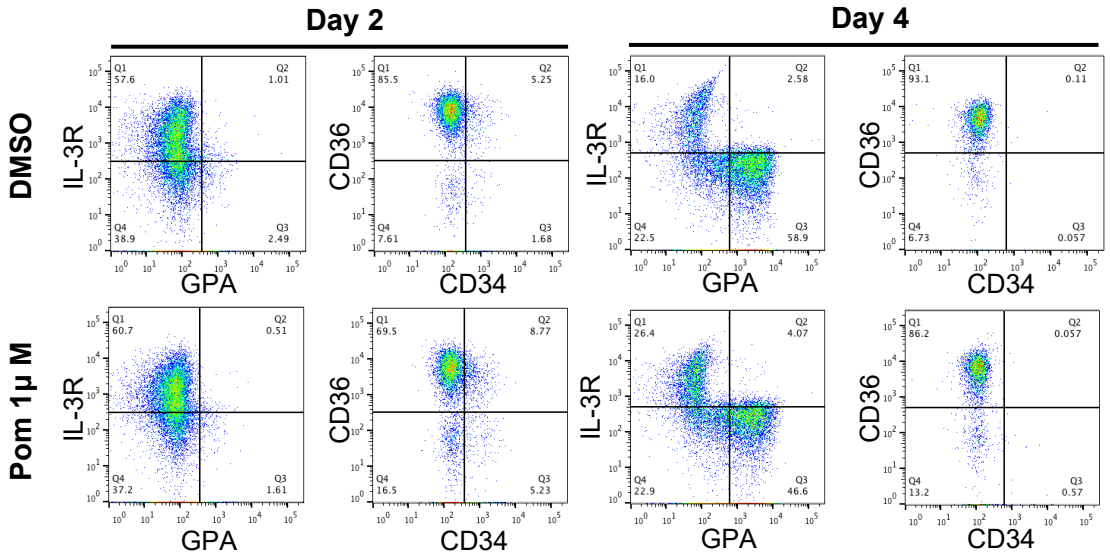


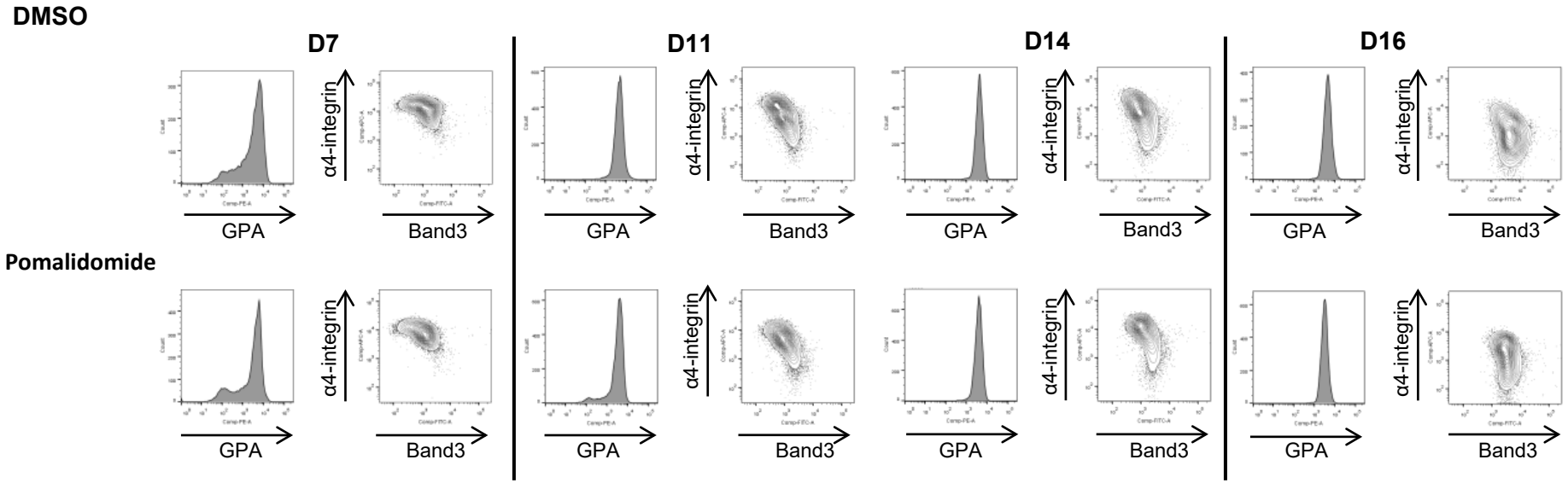
Group I: Expression levels decreased and remained decreased due to pomalidomide treatment

Group II: Expression levels decreased and then normalizing due to pomalidomide treatment

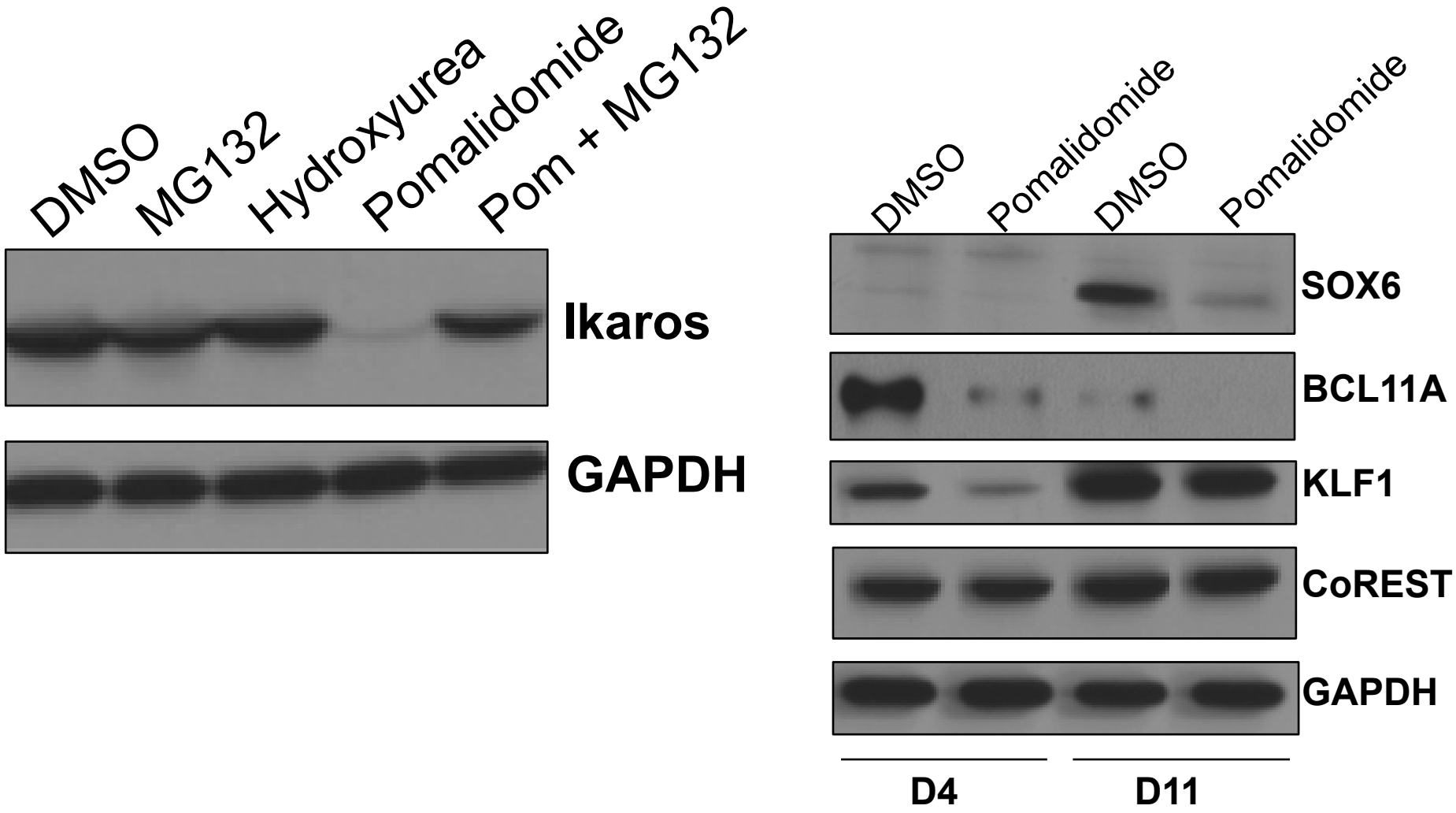
Group III: Expression levels remaining unchanged due to pomalidomide treatment

Pomalidomide Mechanism of Action is Conserved in SCD

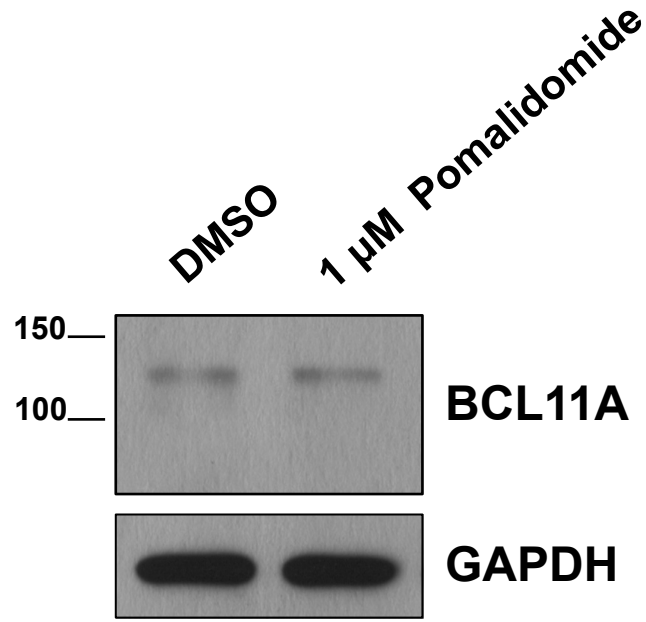




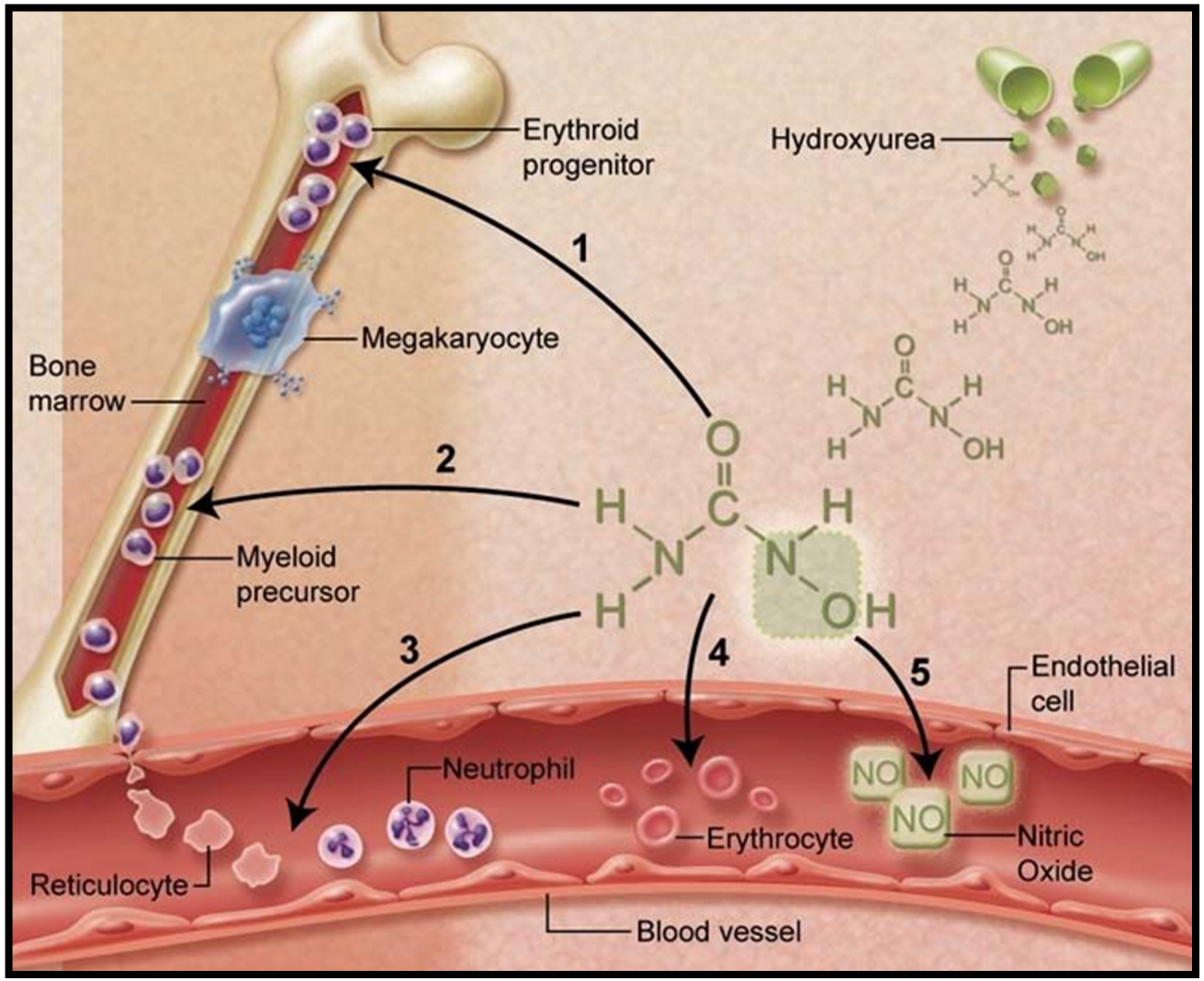
Pomalidomide Mechanism of Action is Conserved in SCD



Pomalidomide does not appear to affect BCL11A expression levels in cultured human neuronal cell lines



Multiple Effects of Hydroxyurea



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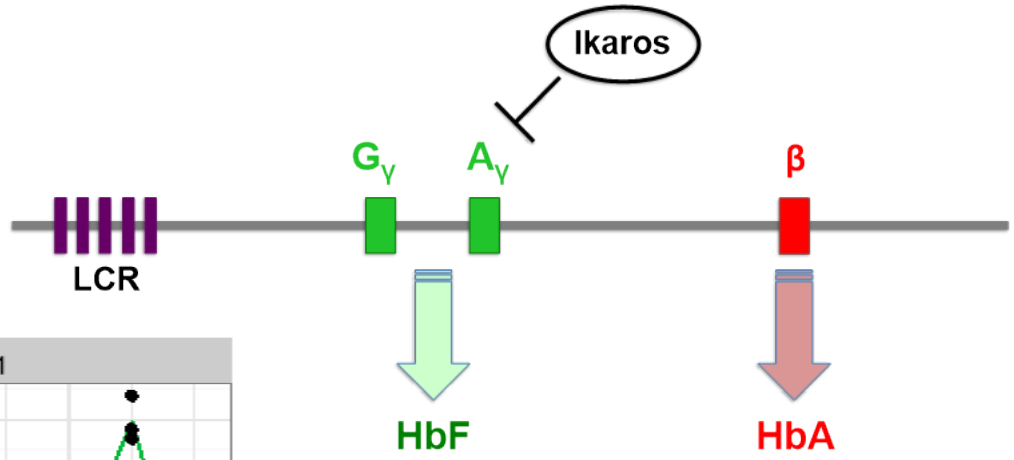
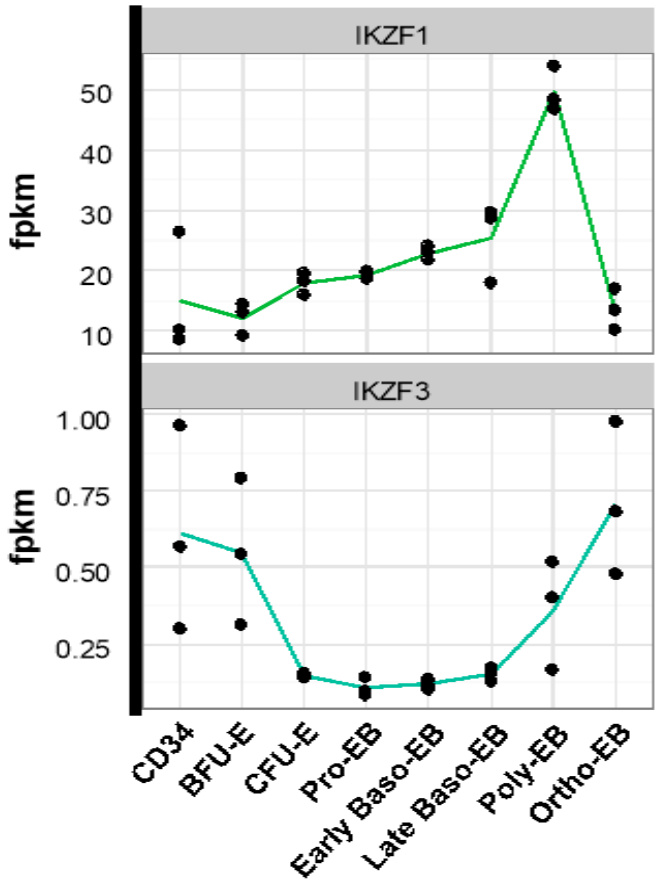
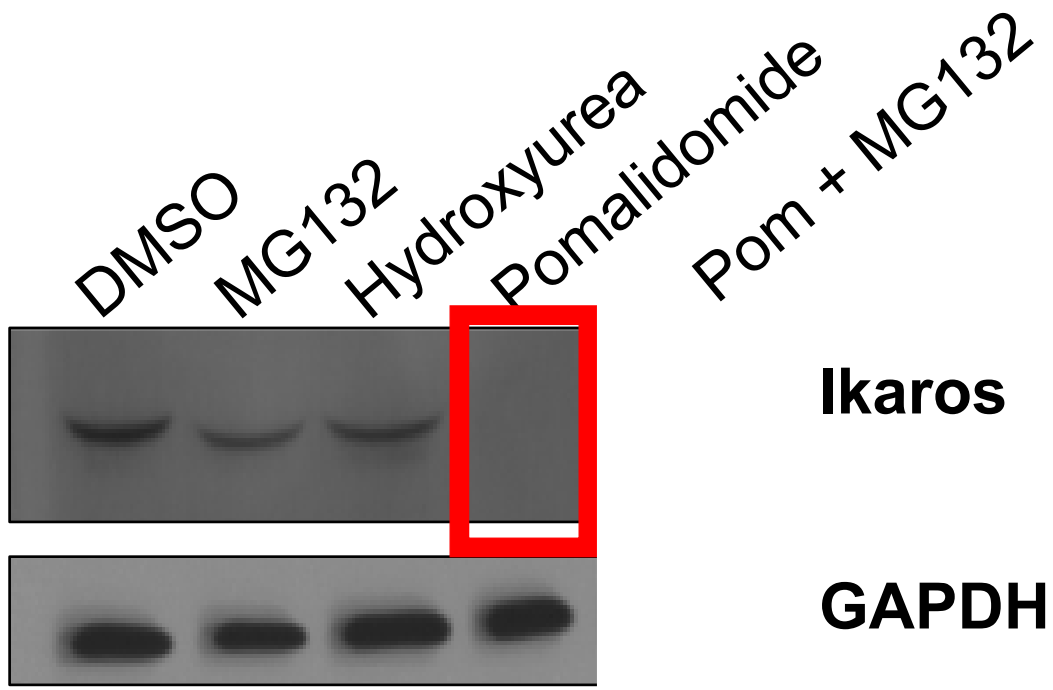
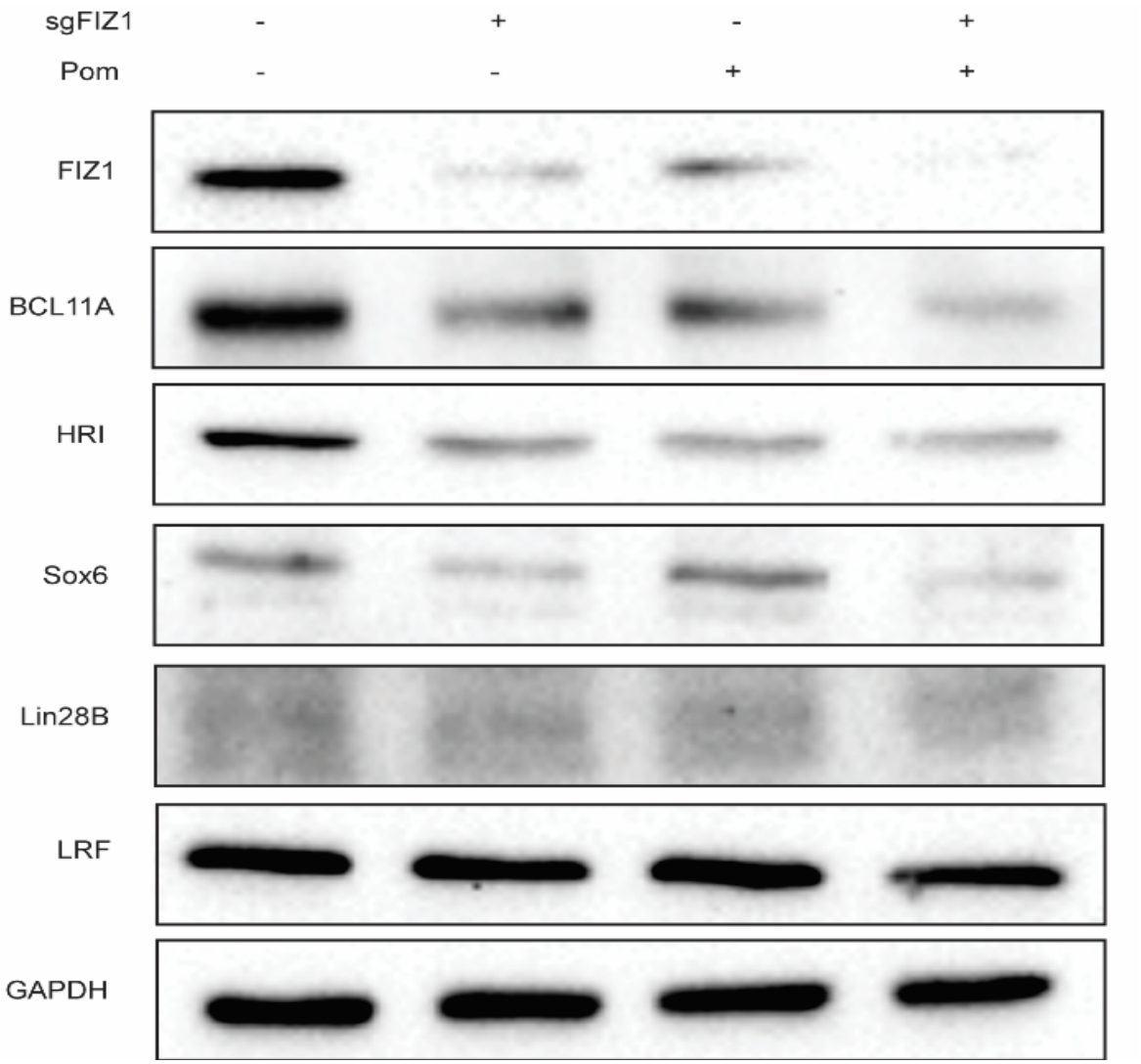
Pomalidomide but not Hydroxyurea targets Ikaros to proteasomal degradation during erythroid differentiation



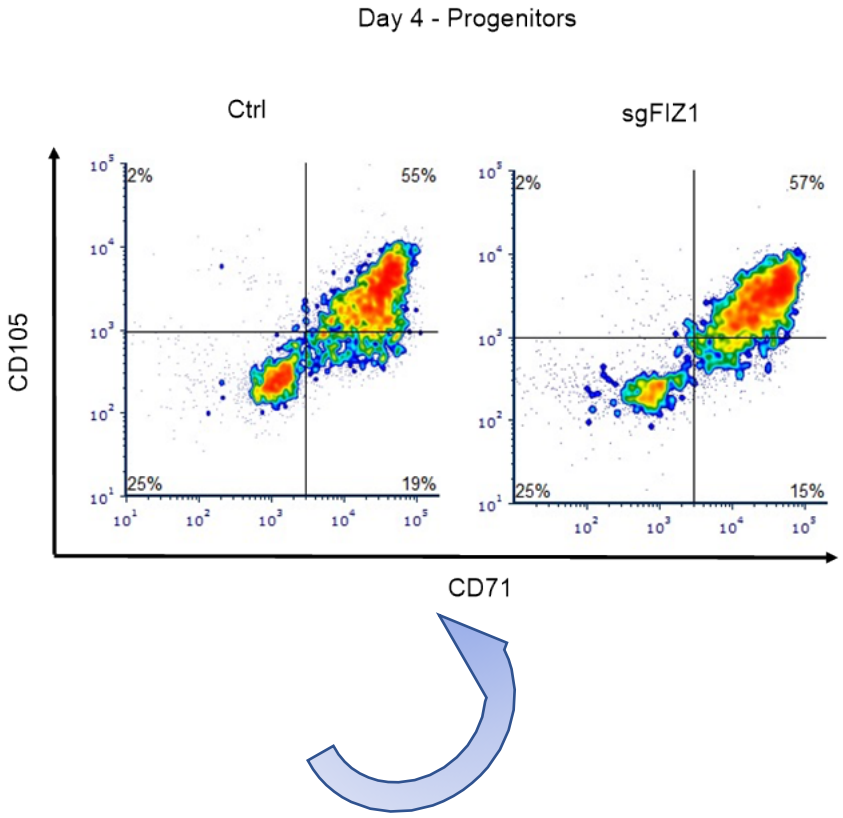


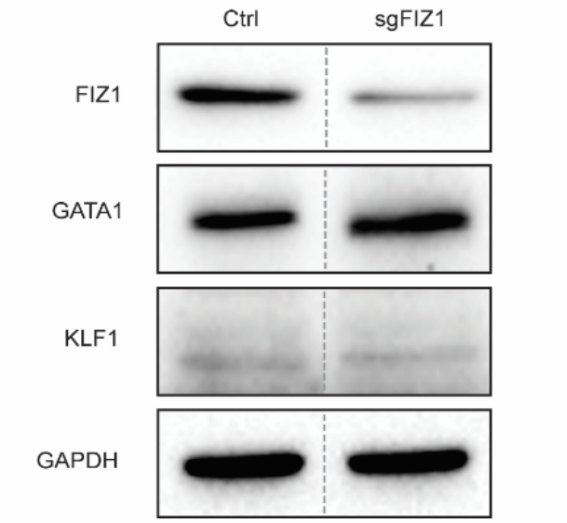


Loss of *FIZ1* Differentially Impacts Regulators of γ-Globin Regulation



FIZ1 Knockout Does Not Delay Erythropoiesis





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