Human Globin Gene Regulation and Gene Replacement Therapy for Sickle Cell Disease

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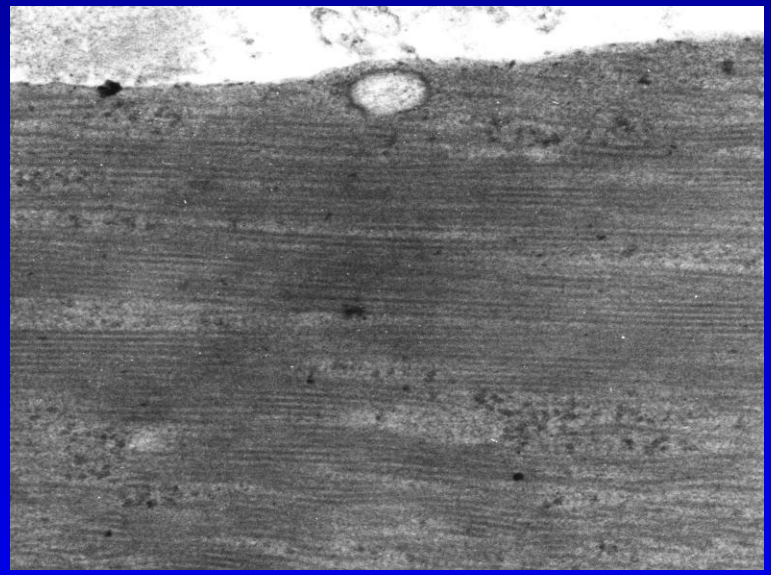
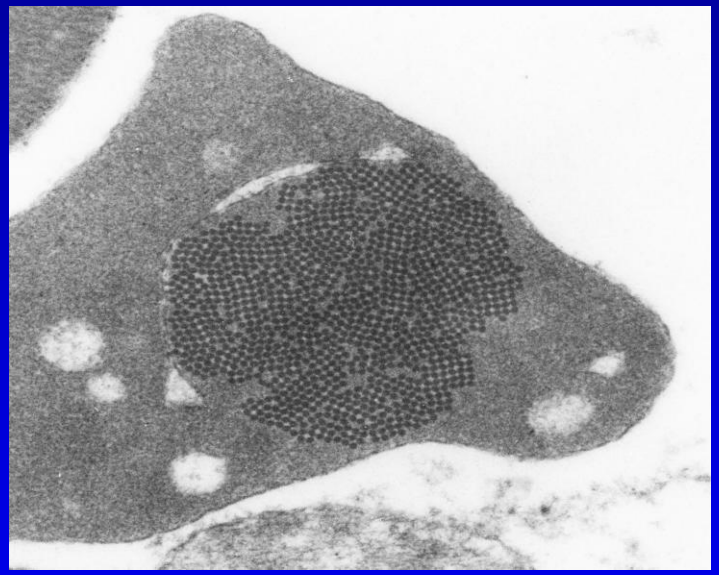
UAB, Department of Biochemistry and Molecular Genetics
UAB Stem Cell Institute

Sickle Hemoglobin Polymers

sagittal



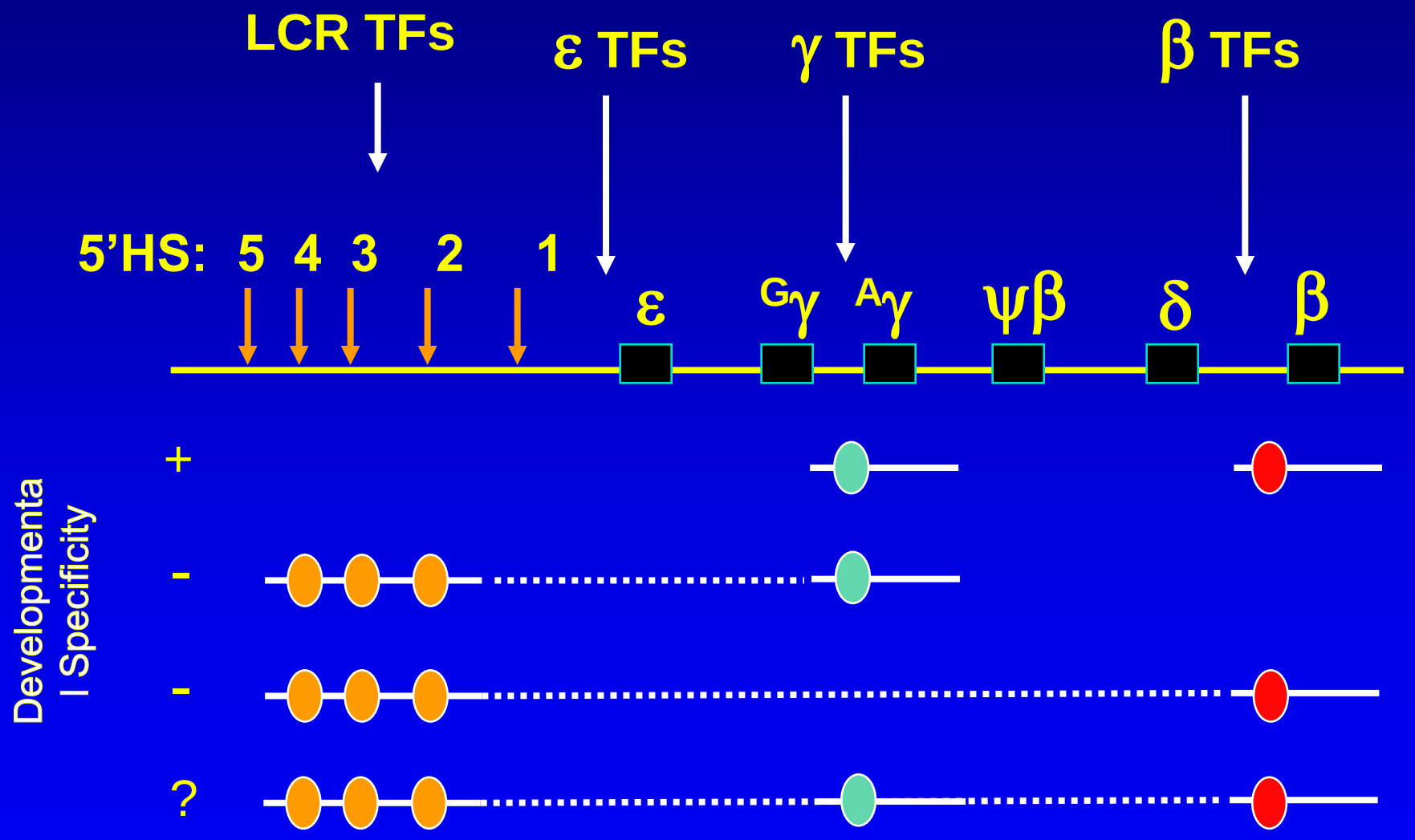
transverse



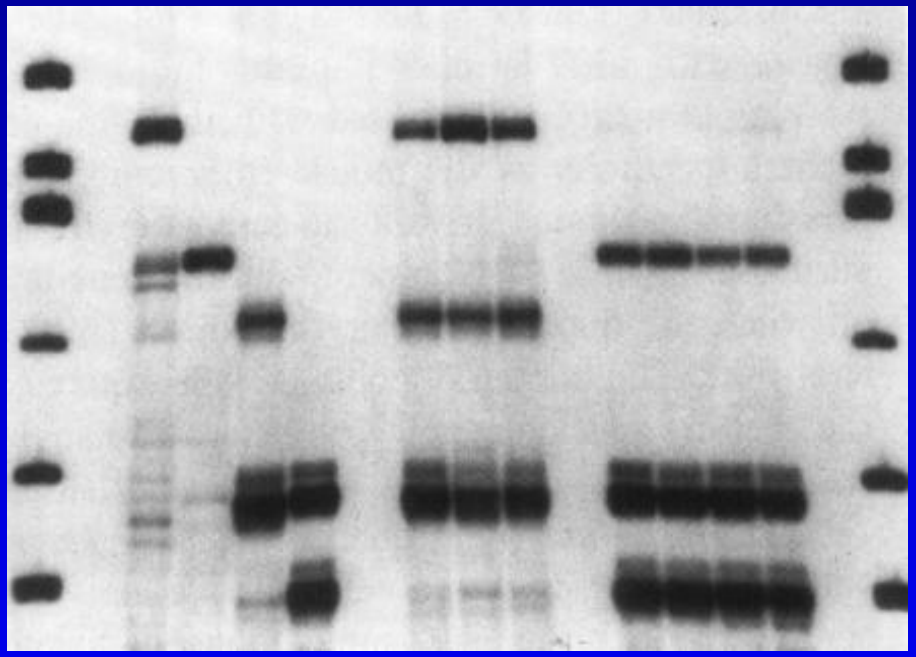
Normal Oxygen tension

Left Middle Cerebral Artery Occlusion

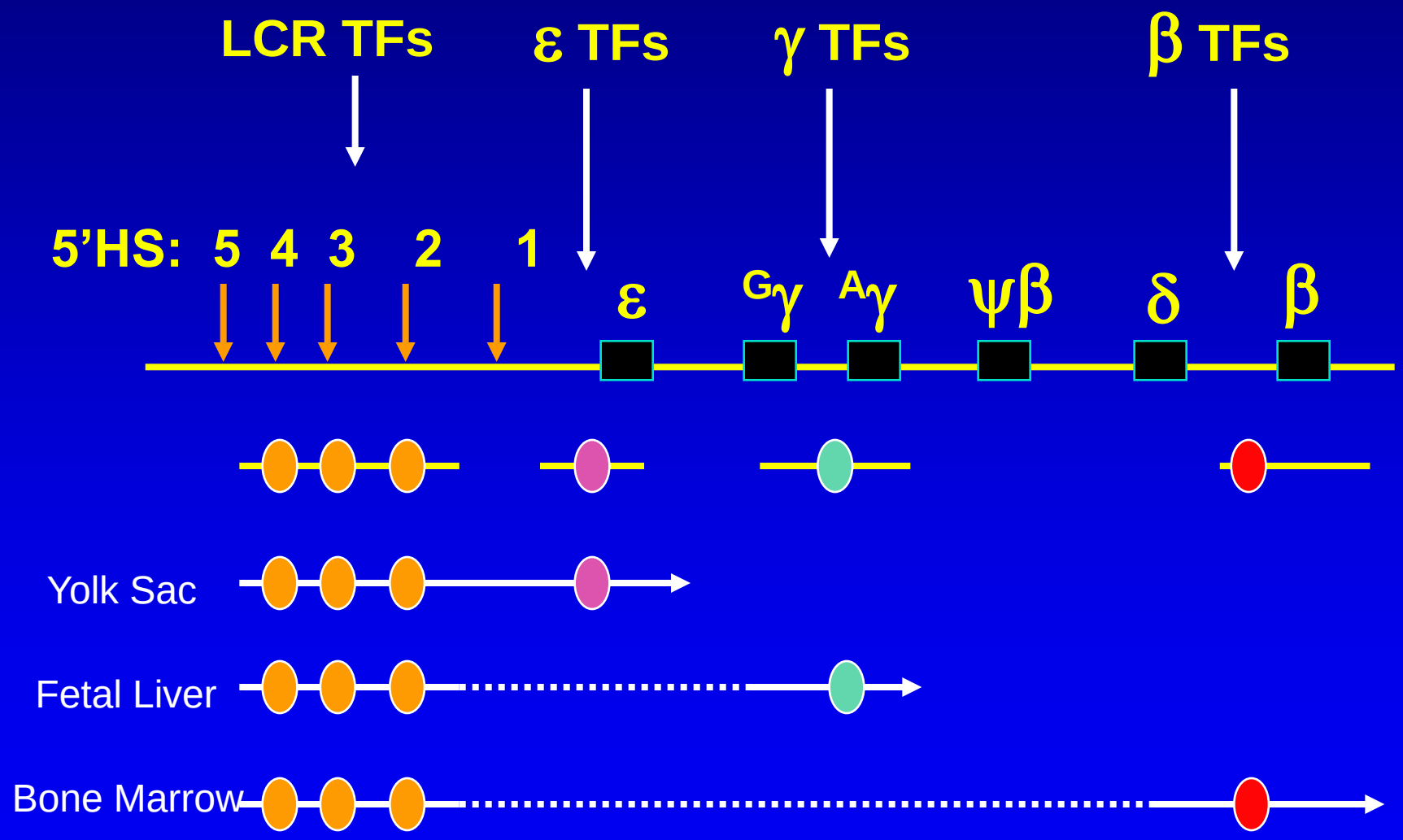
Competition Model for Human Globin Gene Switching



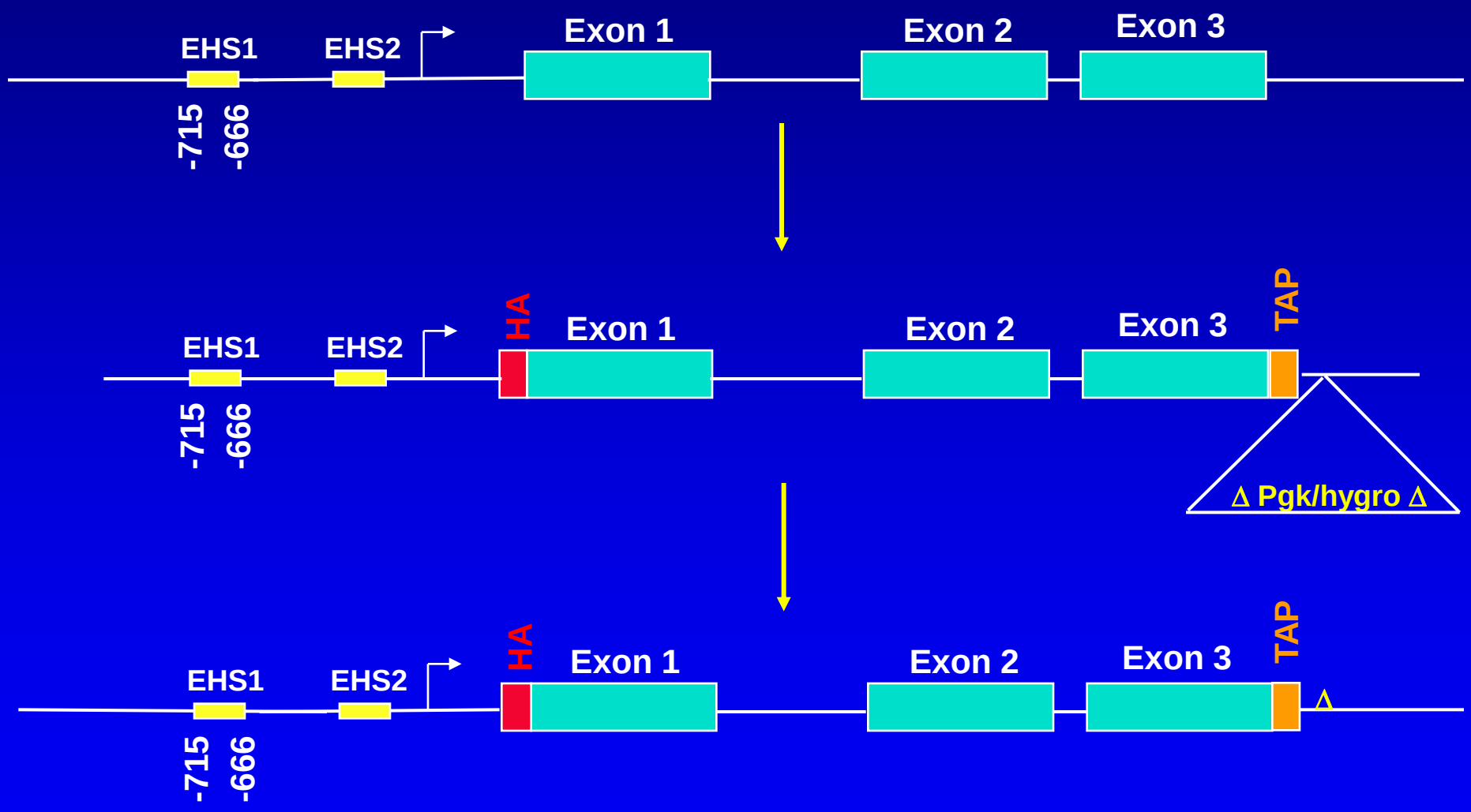
Linkage of γ- and β-Globin Genes Restores Developmental Specificity



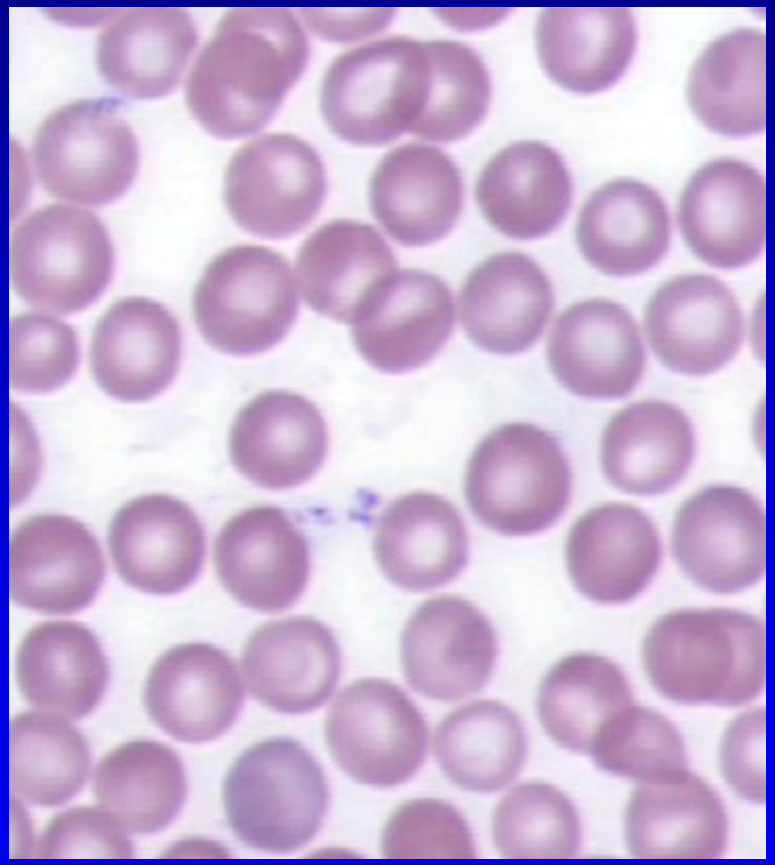
Competition Model for Human Globin Gene Switching



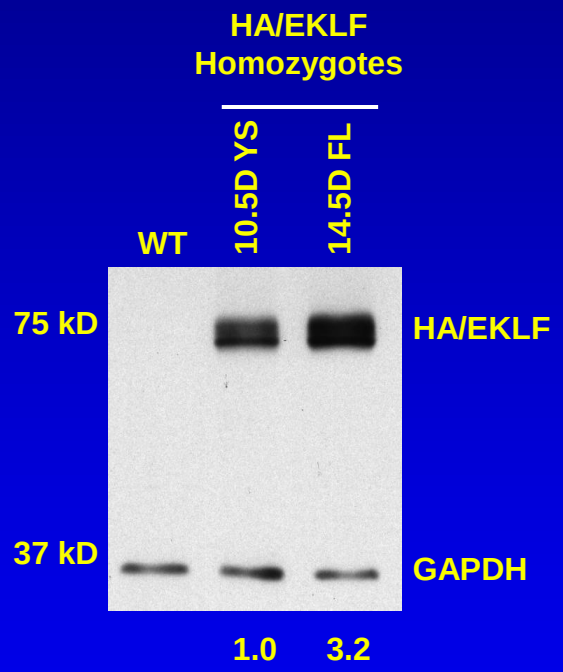
Production of HA/EKLF/TAP Knockin Mice



HA/EKLF Mice

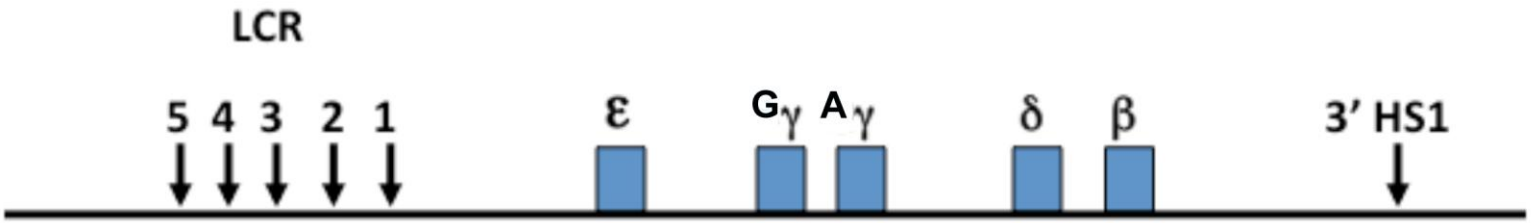


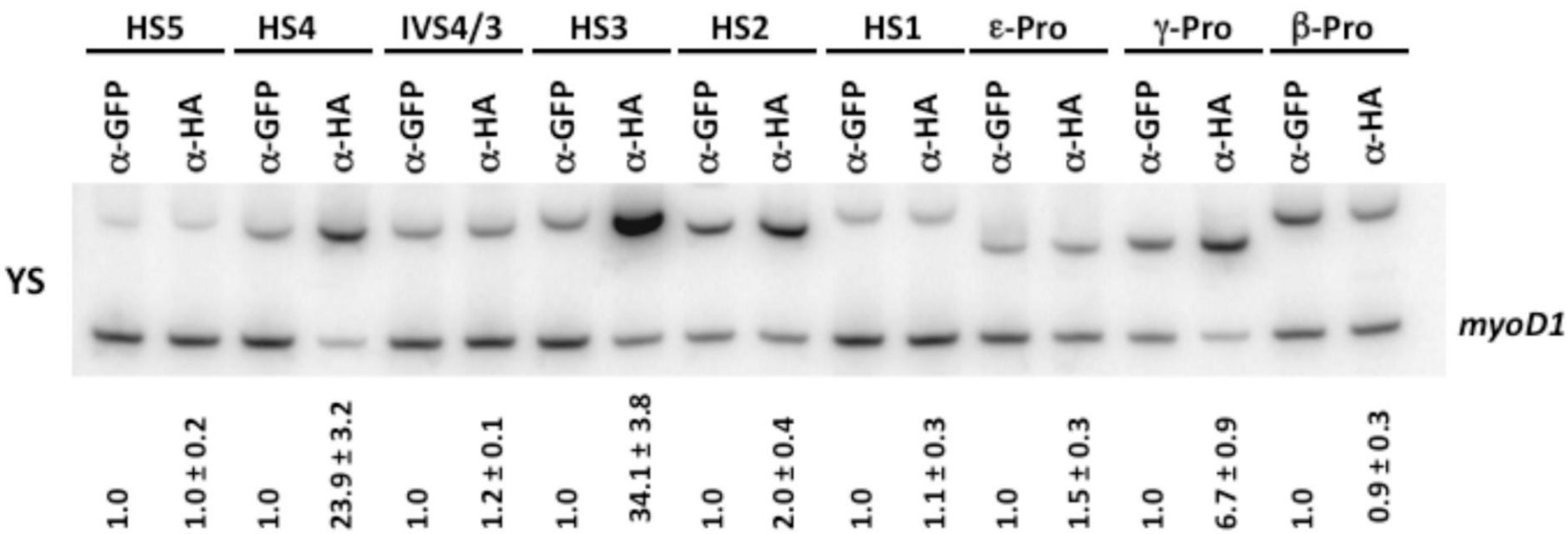
Quantitation of HA/EKLF in Primitive vs Definitive Erythroid Cells



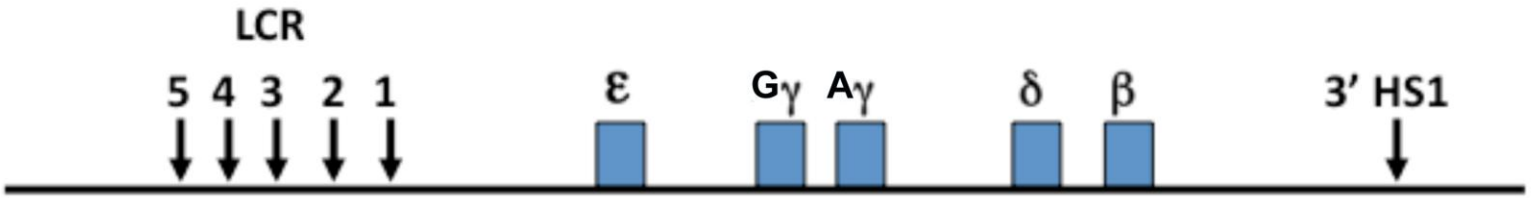
Western Blot with α-HA

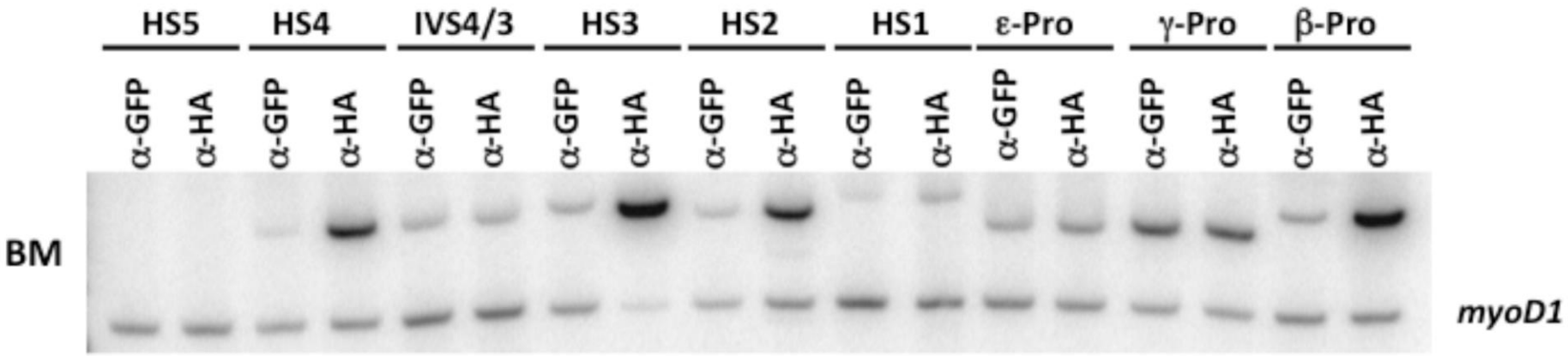
EKLF Binding to the Human β-globin Locus in Murine 10.5D YS



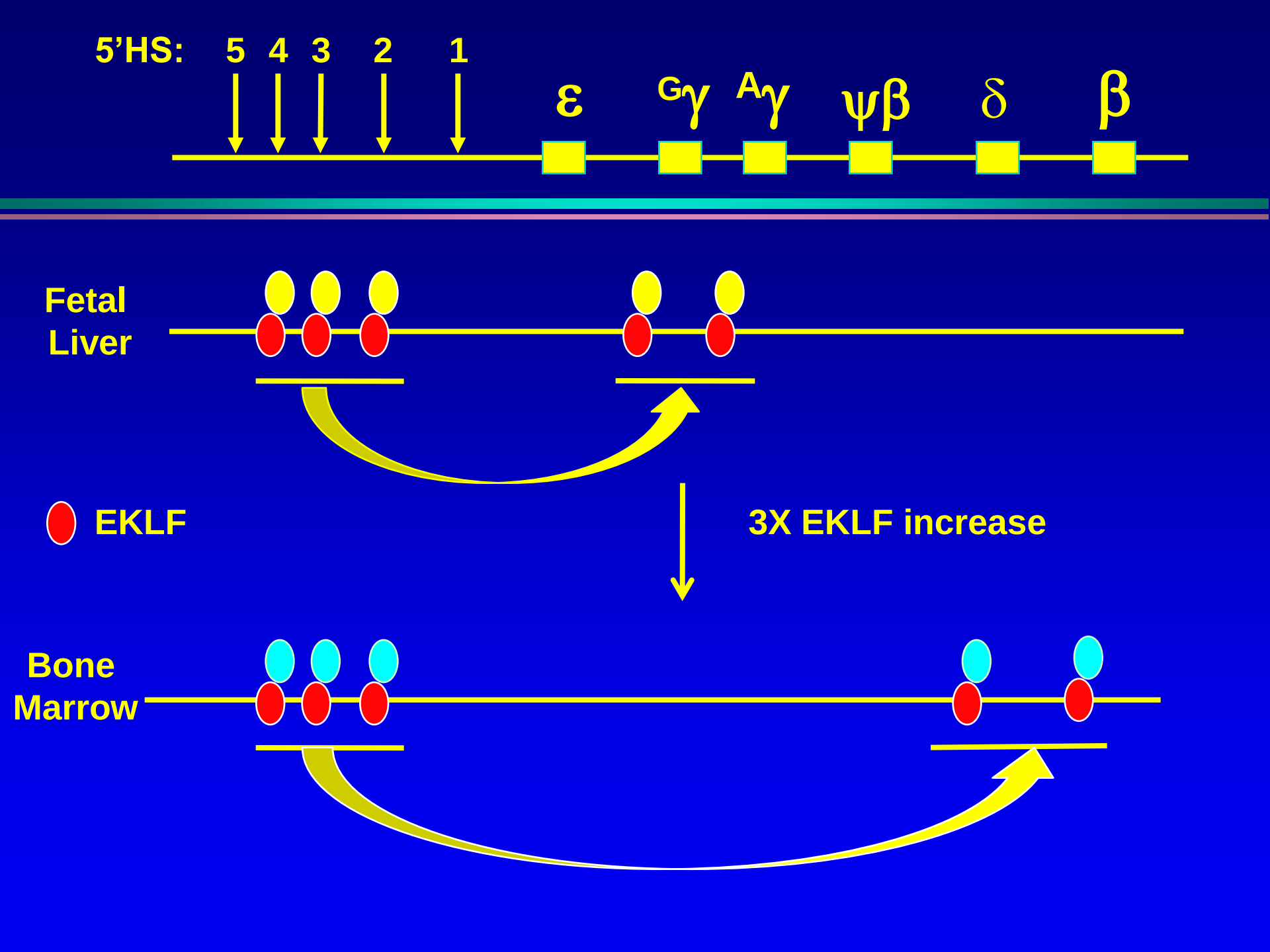


EKLF Binding to the Human β-globin Locus in Murine Adult BM





HS5 α-GFP	HS5 α-HA	HS4 α-GFP	HS4 α-HA	IVS4/3 α-GFP	IVS4/3 α-HA	HS3 α-GFP	HS3 α-HA	HS2 α-GFP	HS2 α-HA	HS1 α-GFP	HS1 α-HA	ε-Pro α-GFP	ε-Pro α-HA	γ-Pro α-GFP	γ-Pro α-HA	β-Pro α-GFP	β-Pro α-HA
1.0	1.0 ± 0.1	1.0	16.5 ± 2.1	1.0	1.0 ± 0.2	1.0	26.5 ± 4.1	1.0	4.5 ± 1.1	1.0	1.5 ± 0.4	1.0	1.5 ± 0.3	1.0	1.1 ± 0.3	1.0	12.9 ± 2.1


5'HS:
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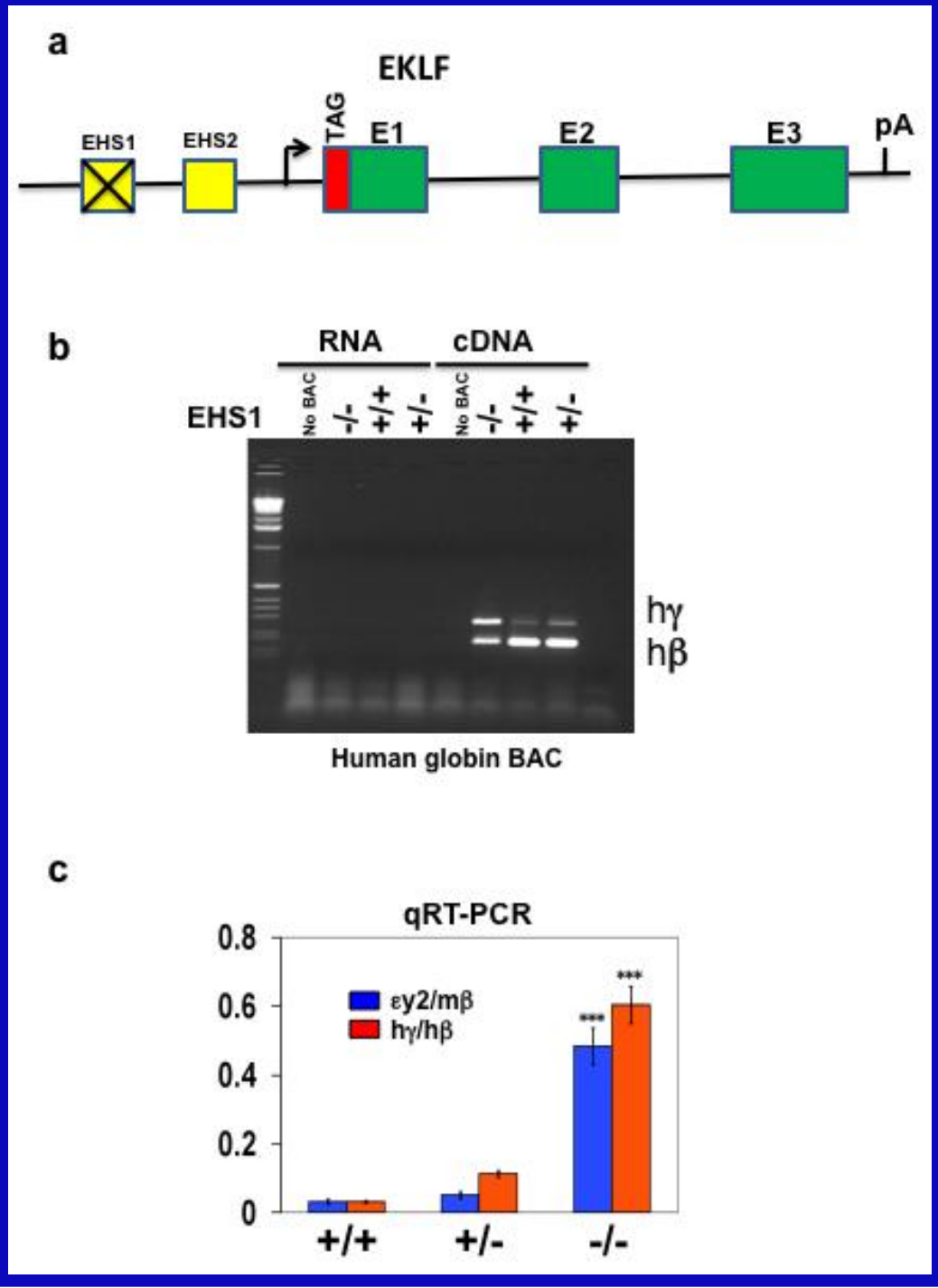
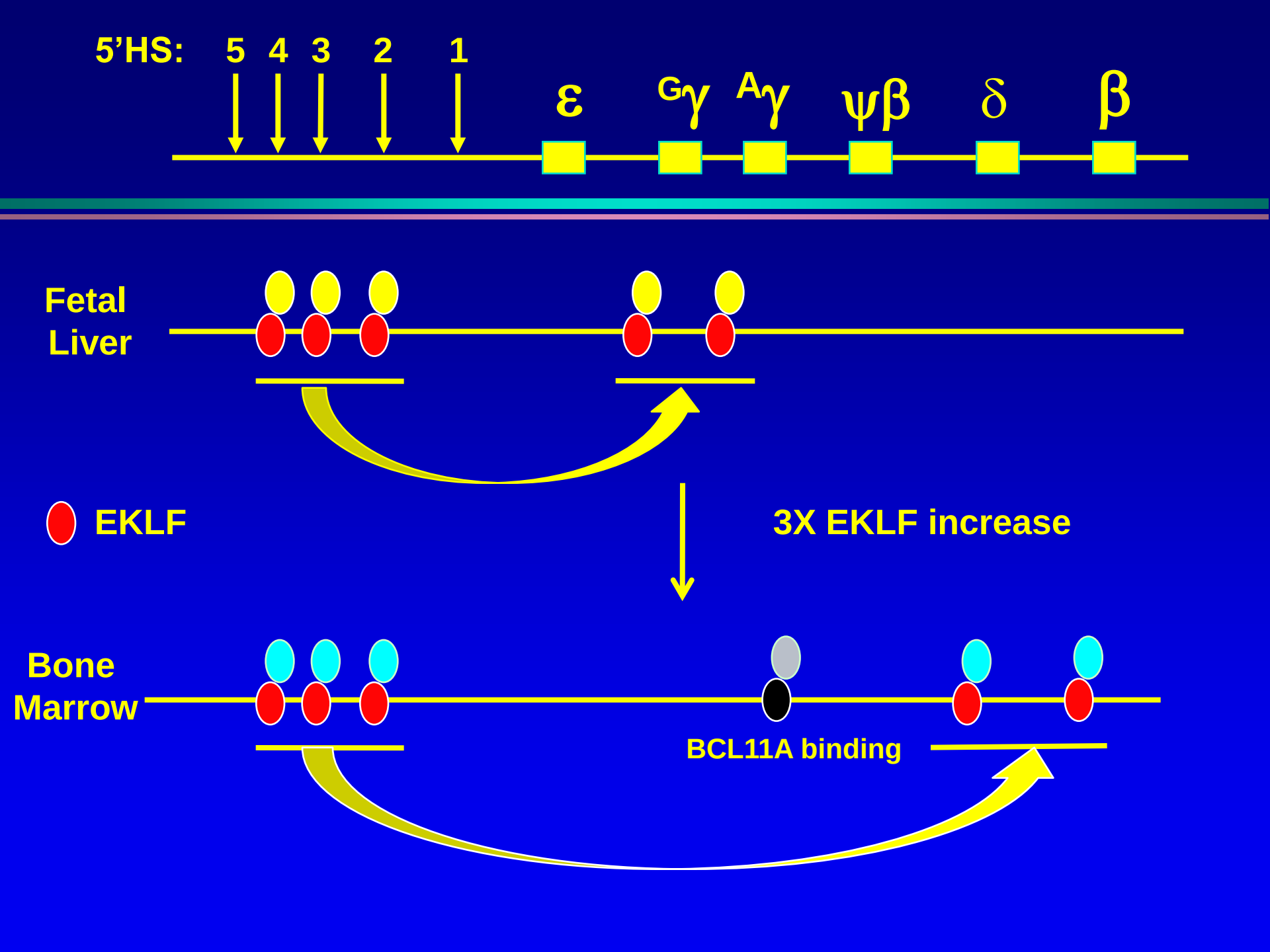
Fetal Liver
EKLF
3X EKLF increase
Bone Marrow


a
EKLF
EHS1
EHS2
TAG
E1
E2
E3
pA
b
RNA
cDNA
EHS1
No BAC
-/-
+/+
+/-
No BAC
-/-
+/+
+/-
hγ
hβ
Human globin BAC
c
qRT-PCR
εy2/mβ
hγ/hβ
0.8
0.6
0.4
0.2
0
***
***
+/+
+/-
-/-


5'HS:
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Gγ
Aγ
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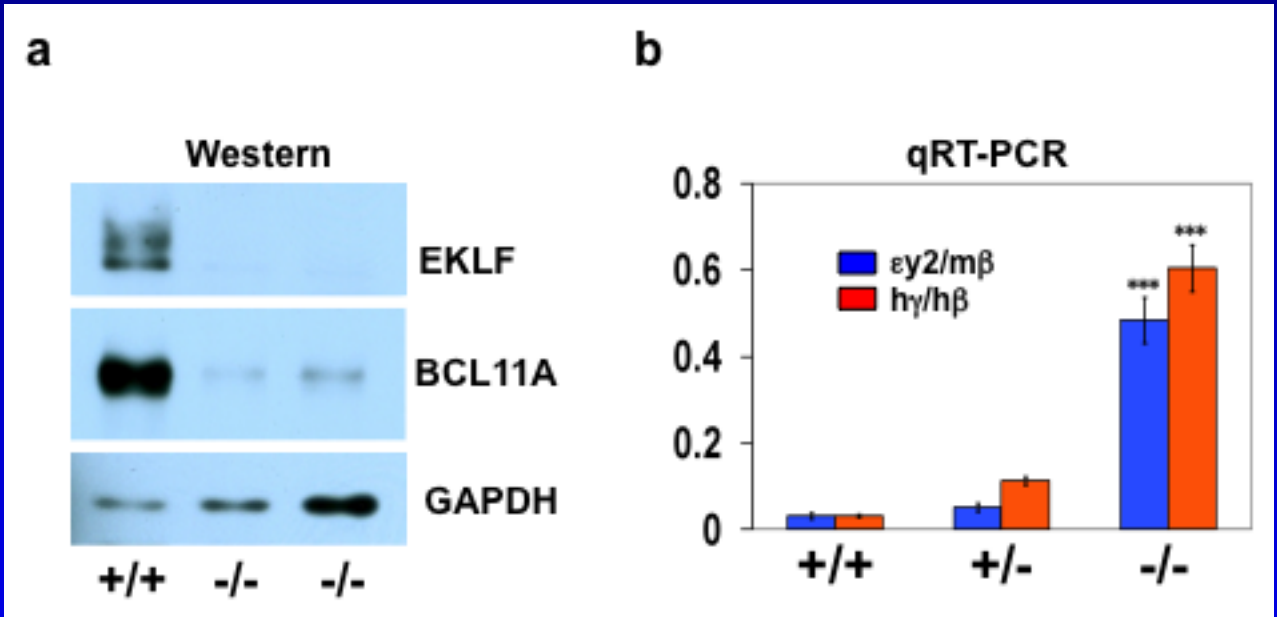
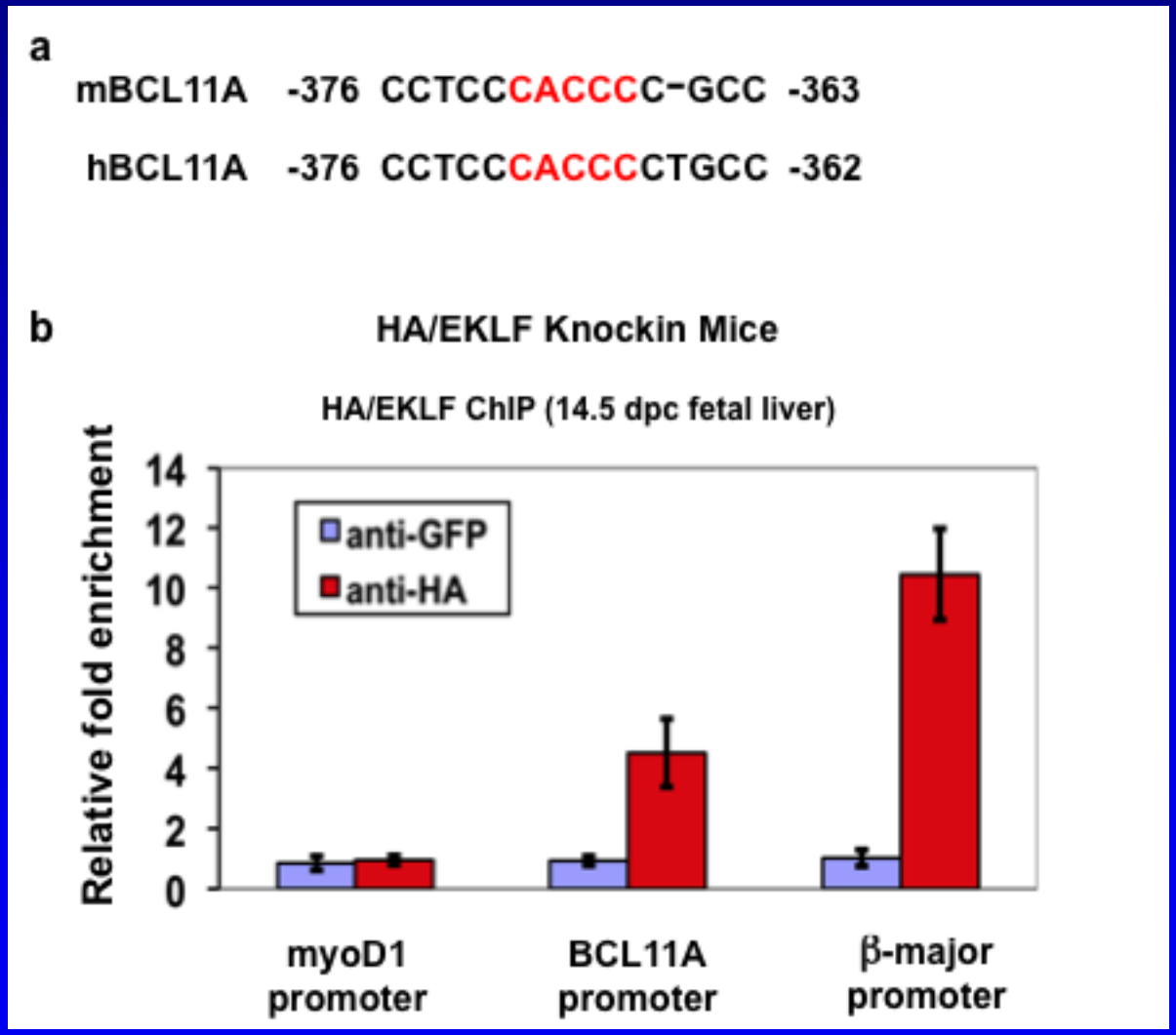
β
Fetal Liver
EKLF
3X EKLF increase
Bone Marrow
BCL11A binding

EKLF Regulates BCL11A Expression and Globin Gene Switching

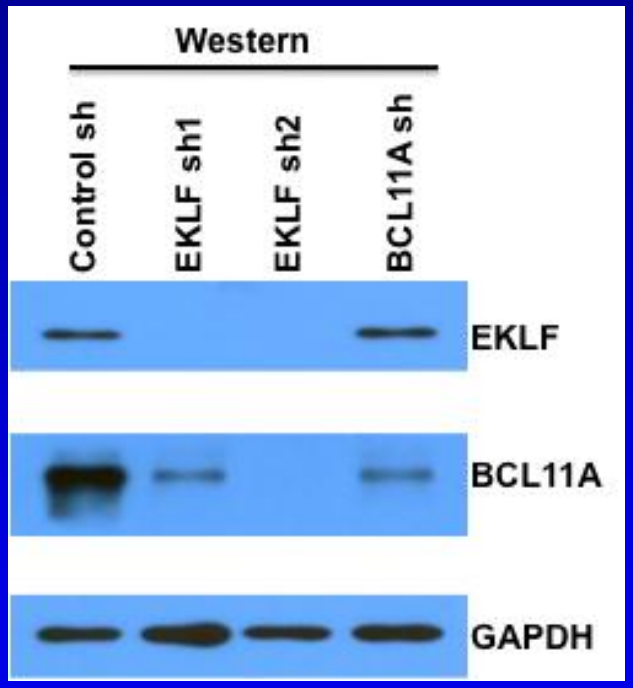


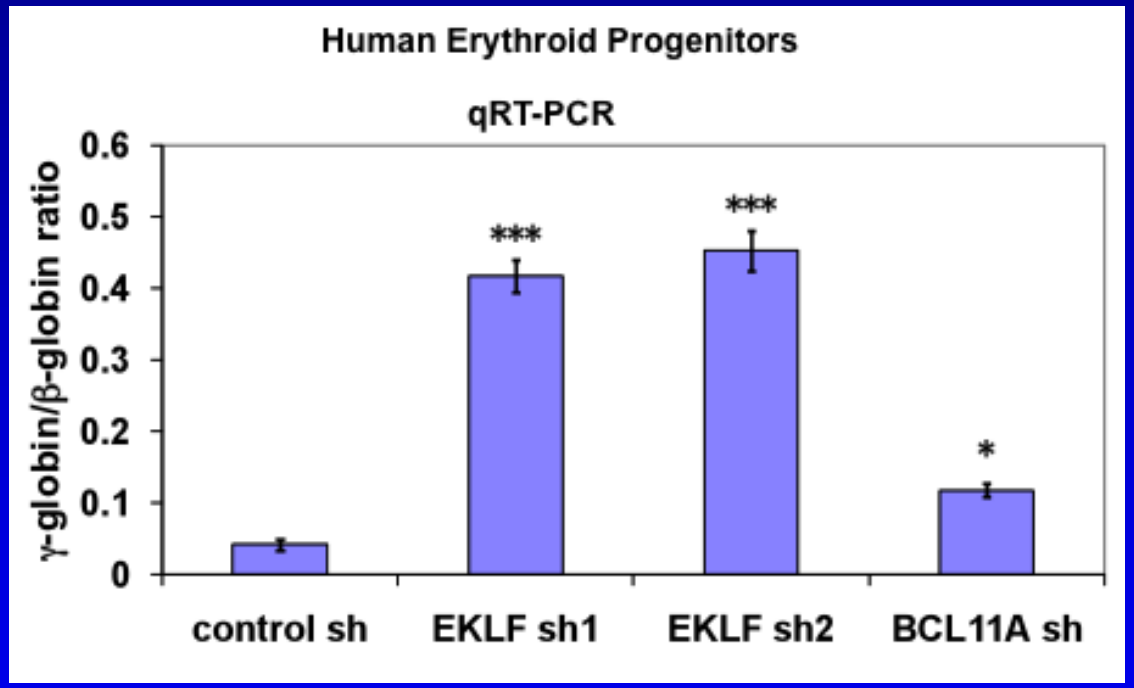
Murine Bone Marrow Erythroid Progenitors
Human Globin BAC Mice

KLF1 Binds to the BCL11A Promoter in Adult Erythroid Progenitors



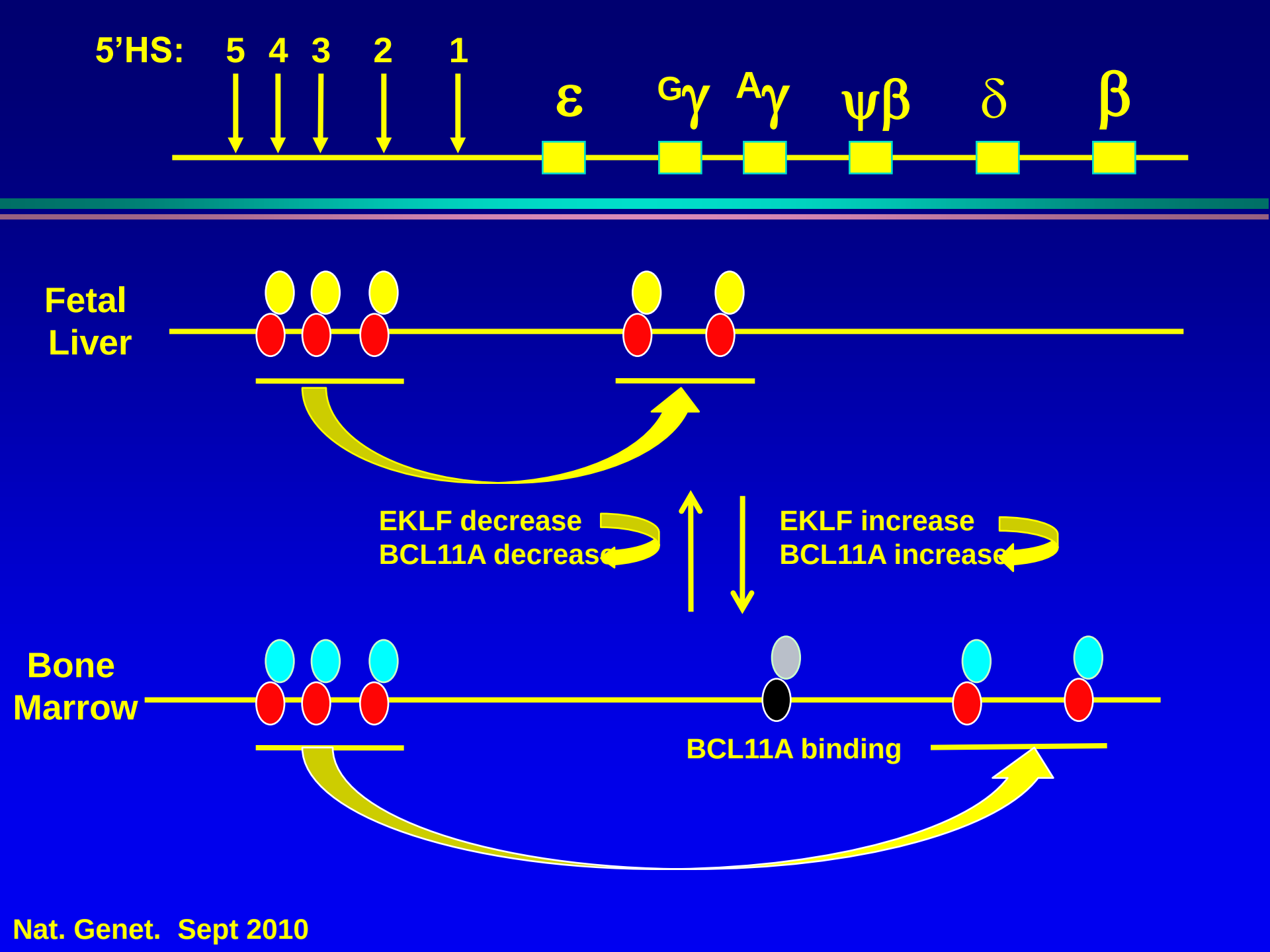
EKLF Controls Human BCL11A Expression and Globin Gene Switching





Human Bone Marrow Erythroid Progenitors

Cytospin of Human Erythroid Progenitors after shRNA Knockdown



Nat. Genet. Sept 2010

Purification of Mouse EKLF Protein Complexes from 14.5 dpc Fetal Livers

HA/EKLF Mice

WCE

↓

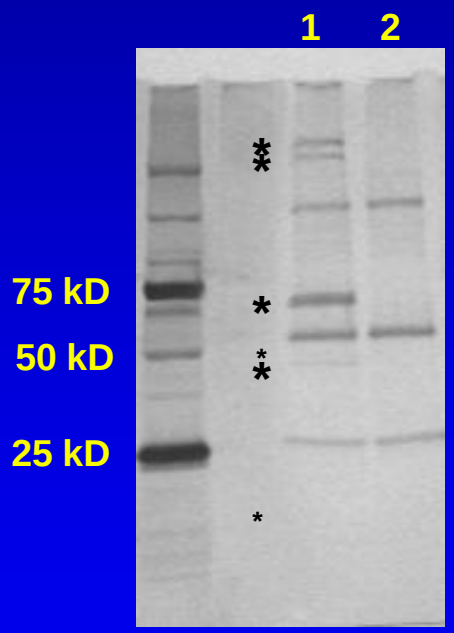
Anti-HA beads

500 mM NaCl Wash ↓

HA peptide Elution

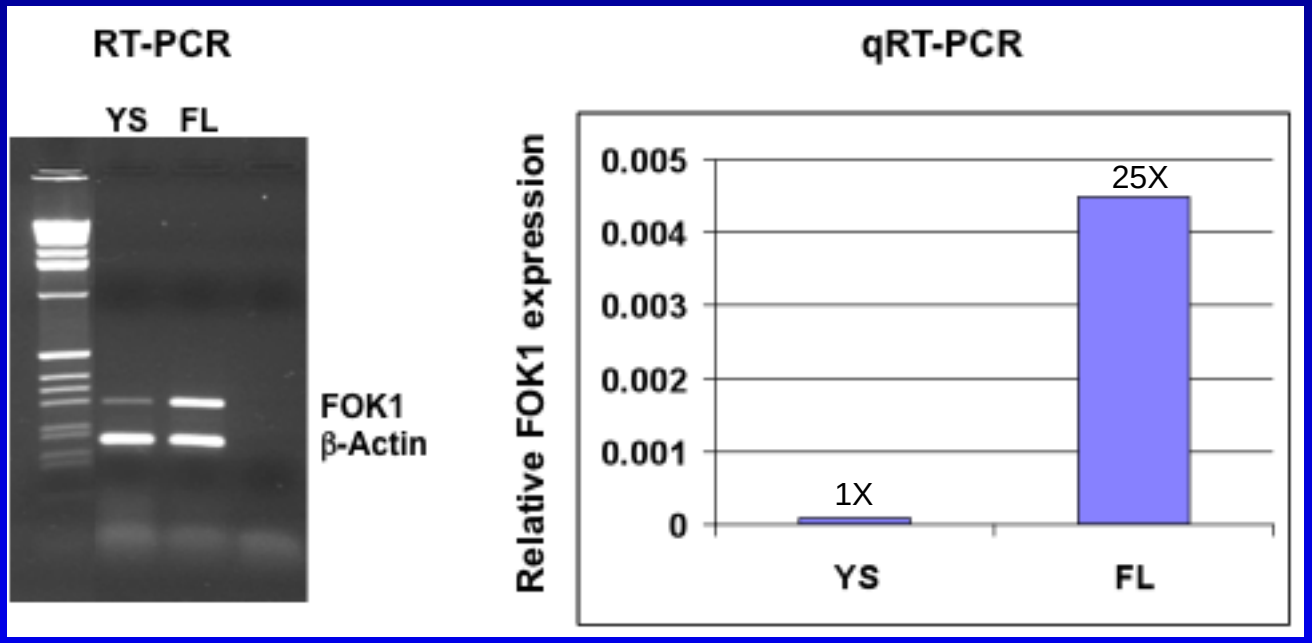
1. HA/EKLF (5 Fetal Livers)

2. Wild Type (5 Fetal Livers)



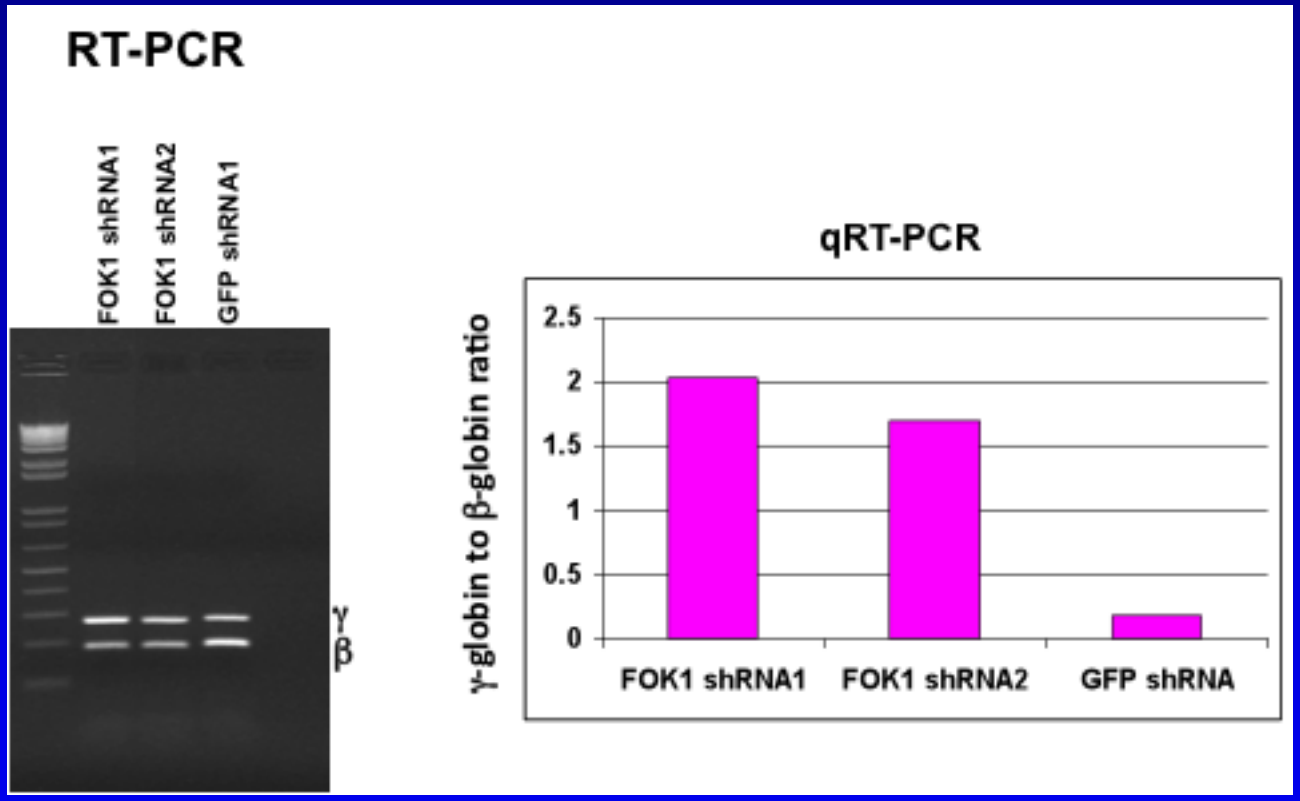
Silver staining

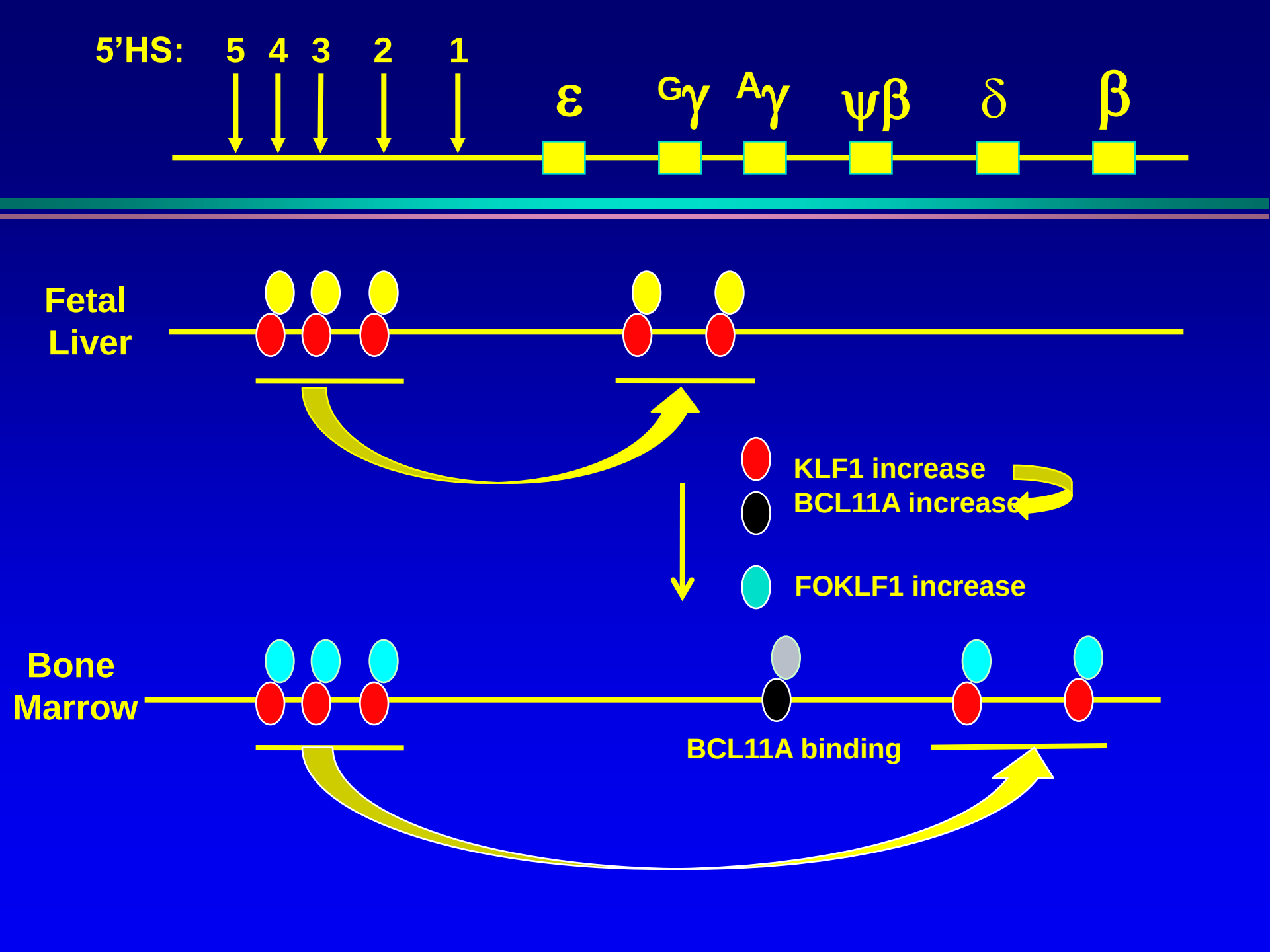
Friend of KLF1 (FOKLF1)



BM Ter119+ FOKLF1 = 50X

FOKLF1 Knockdown in Human EP Switches Globin Gene Expression




5'HS:
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Gγ
Aγ
ψβ
δ
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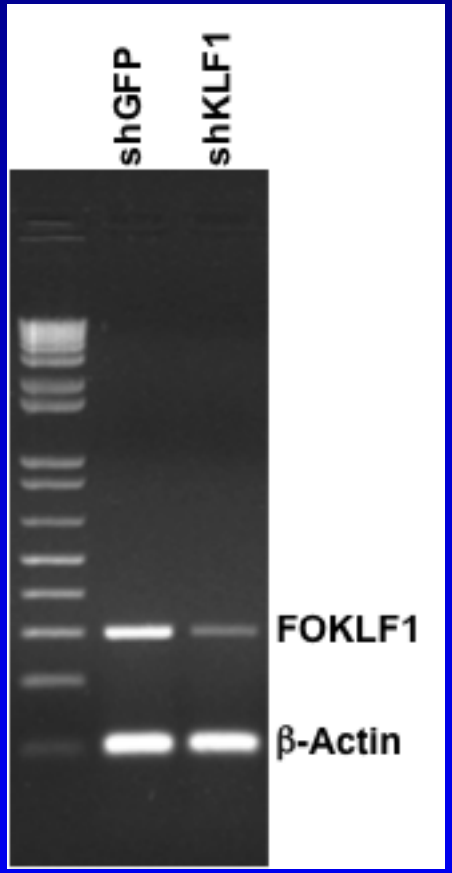
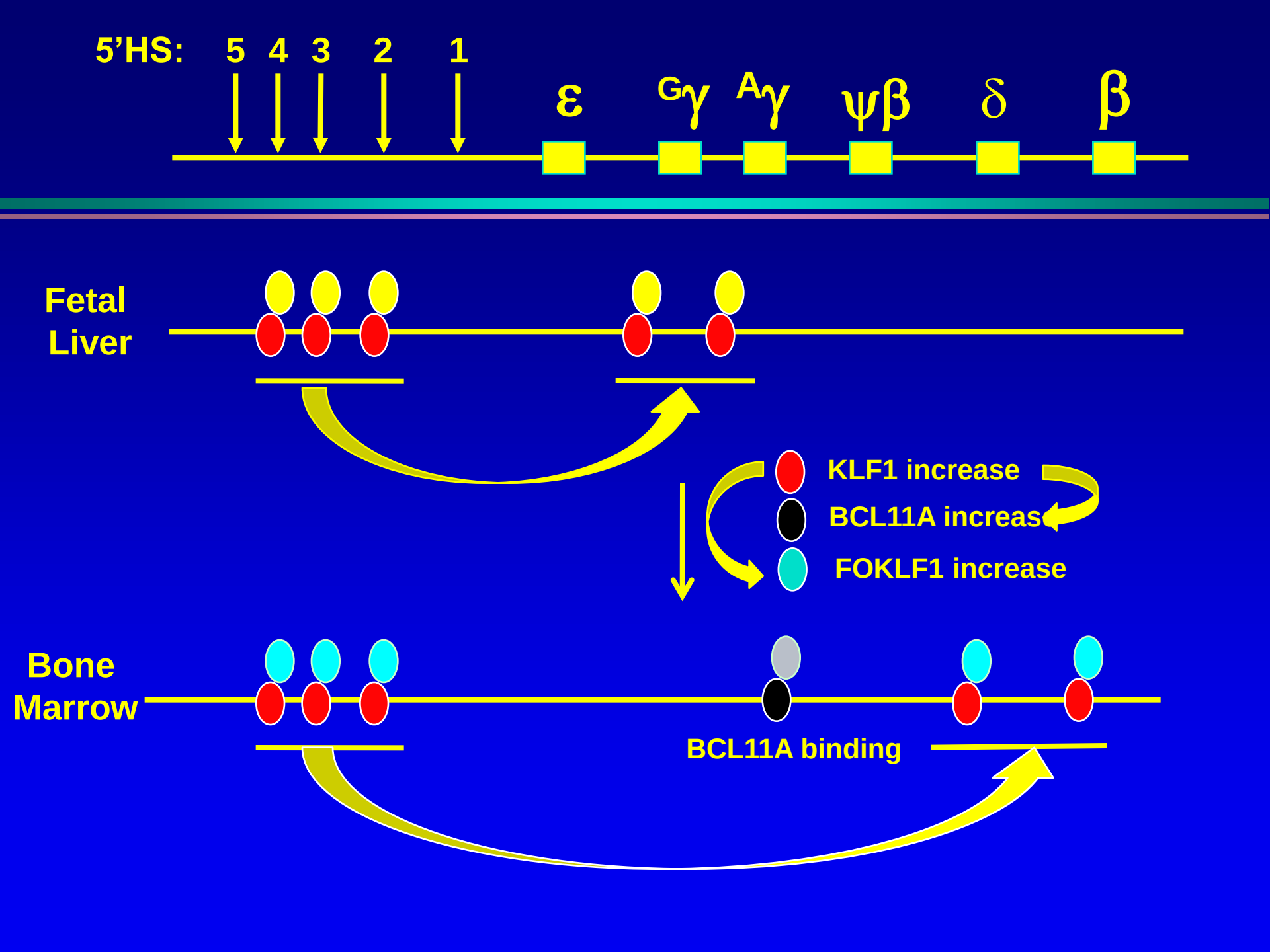
Fetal Liver
KLF1 increase
BCL11A increase
FOKLF1 increase
Bone Marrow
BCL11A binding

KLF1 Regulates FOKLF1 in Human Erythroid Progenitors




5'HS:
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Gγ
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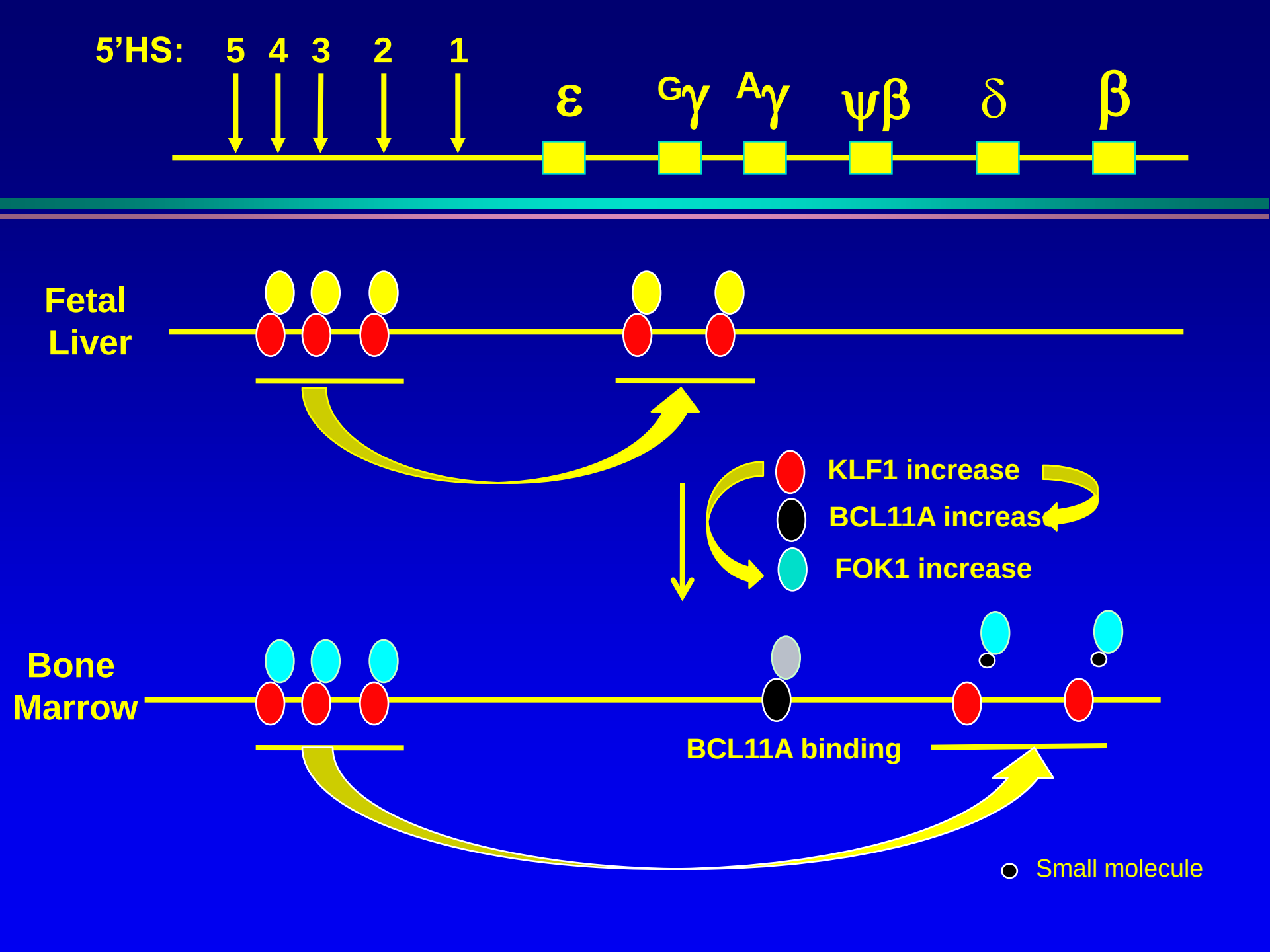
Fetal Liver
KLF1 increase
BCL11A increase
FOKLF1 increase
Bone Marrow
BCL11A binding


5'HS:
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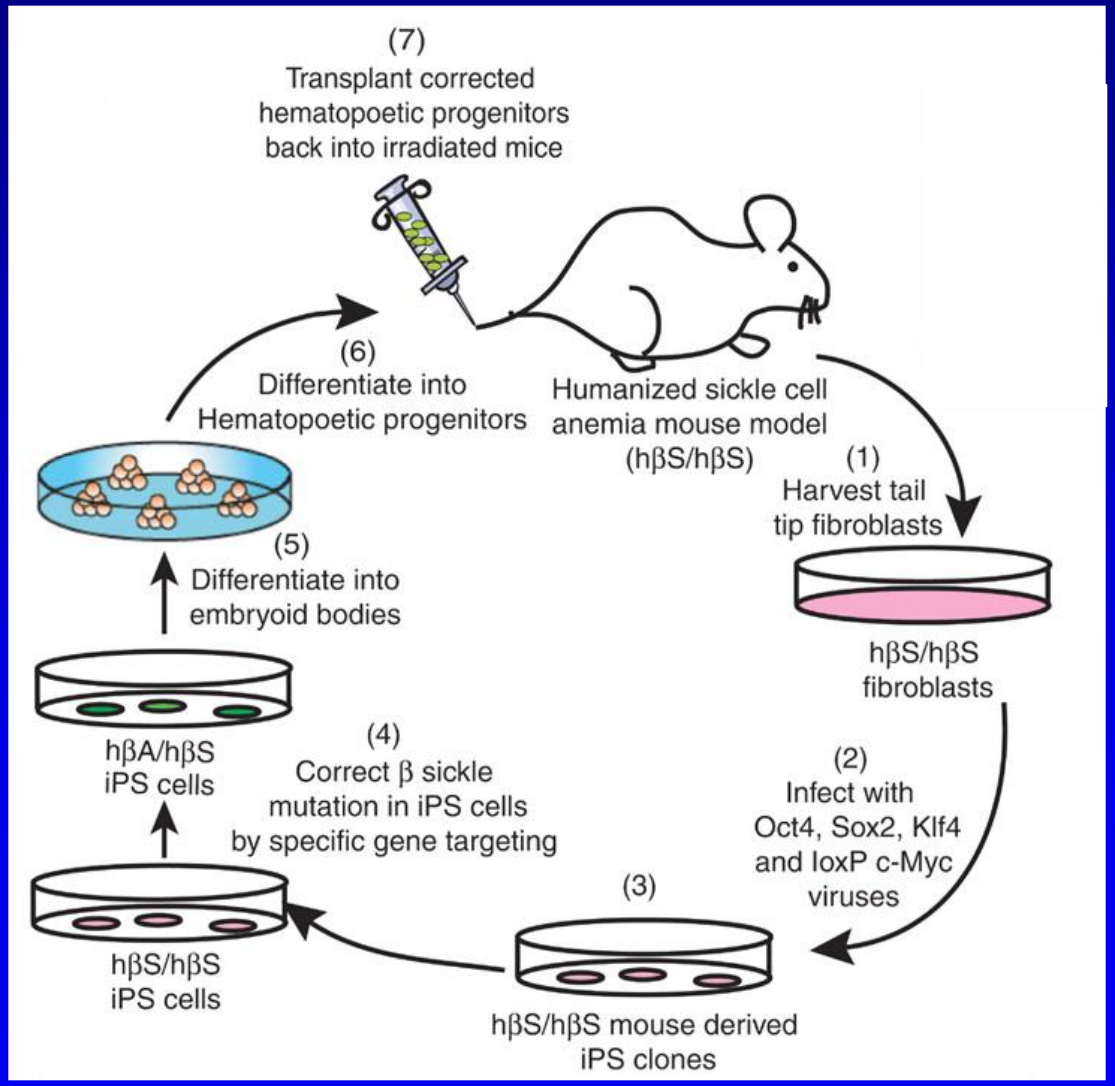
Gγ
Aγ
ψβ
δ
β
Fetal Liver
KLF1 increase
BCL11A increase
FOK1 increase
Bone Marrow
BCL11A binding
Small molecule

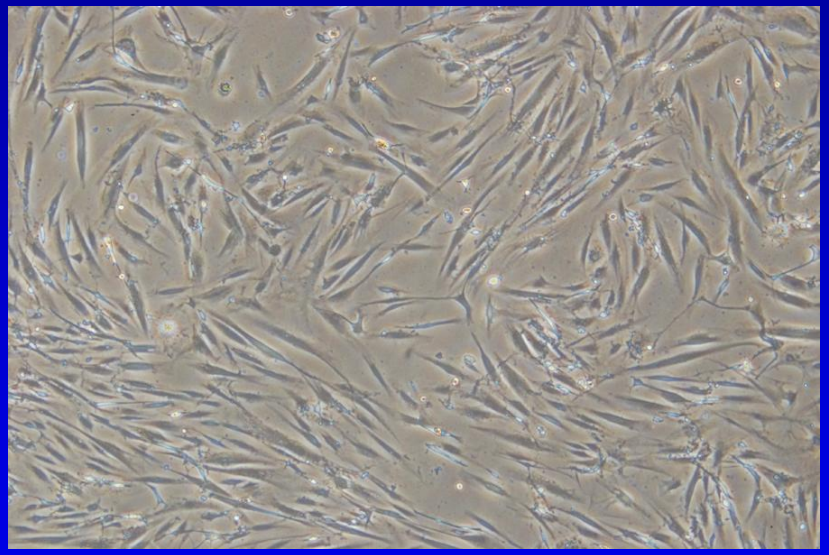
Scheme for Gene and Cell Therapy of Humanized Sickle Mouse



Science 318:1920-1923
Dec 21, 2007

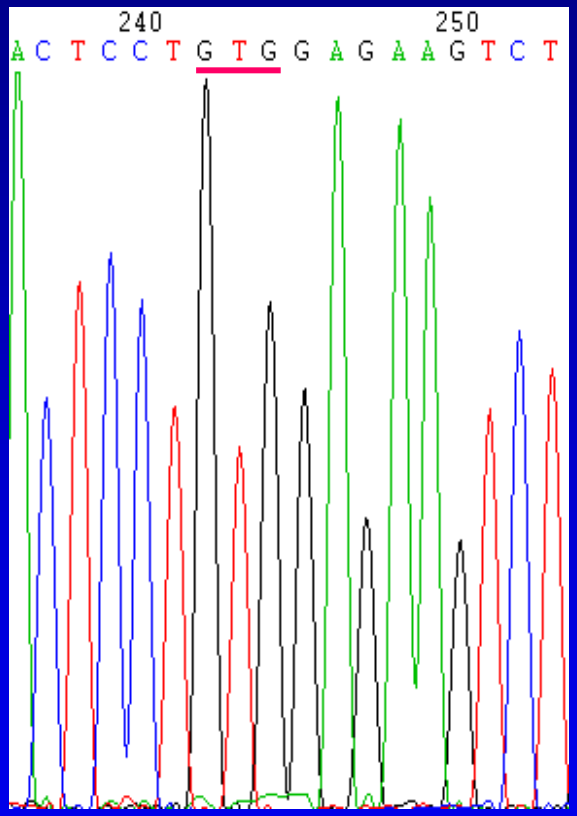
Gene Replacement in Sickle Skin Fibroblasts?

Human Sickle Skin Fibroblasts

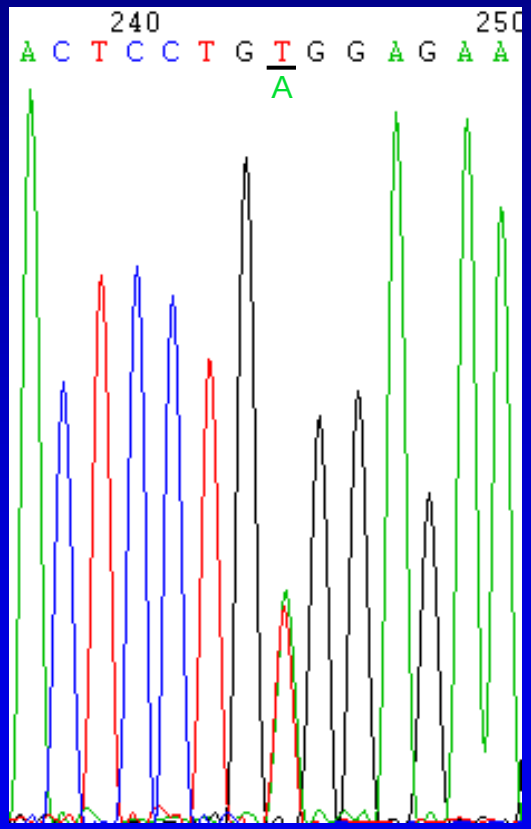


45 year old sickle patient

Homologous Recombination in Human Sickle Fibroblasts

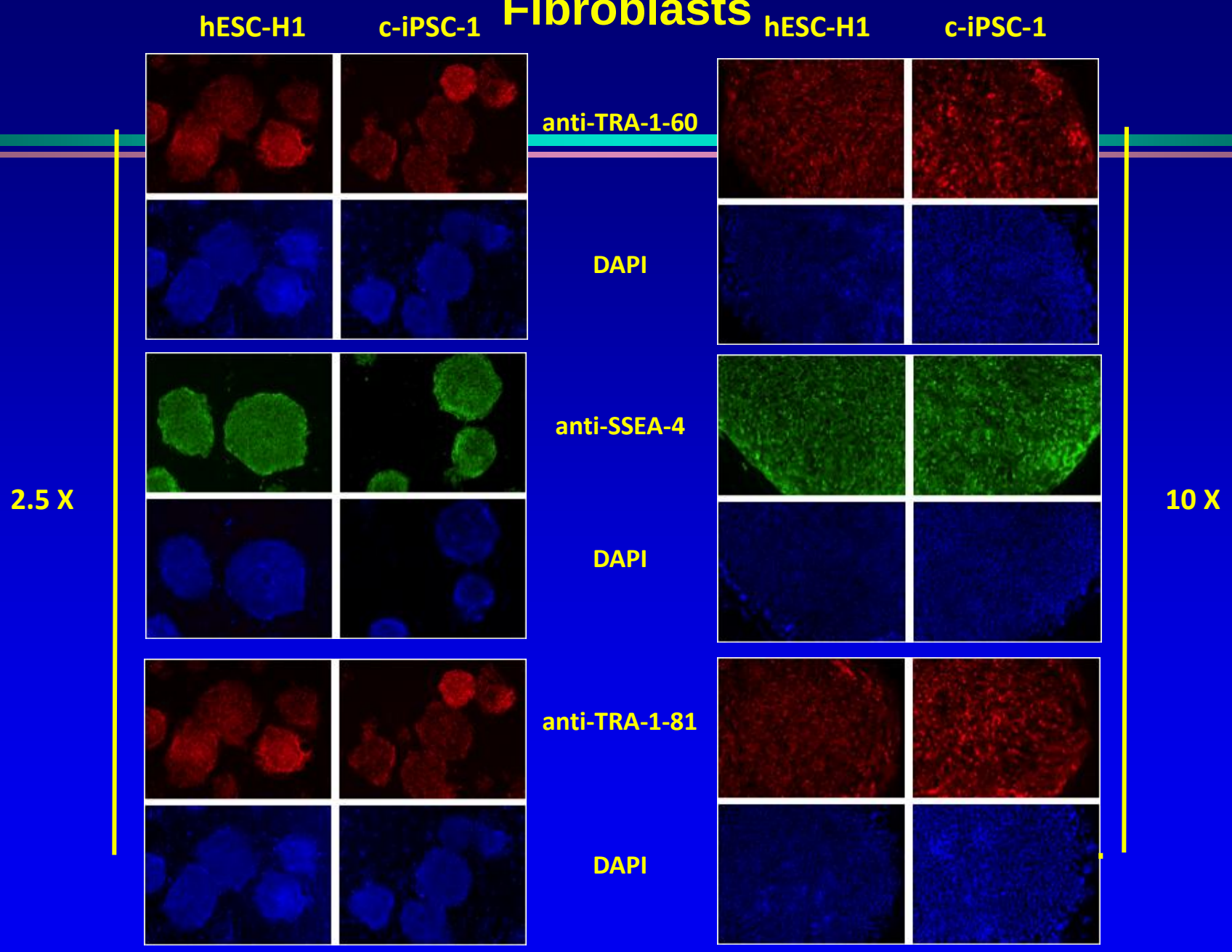


Sickle Human Fibroblasts

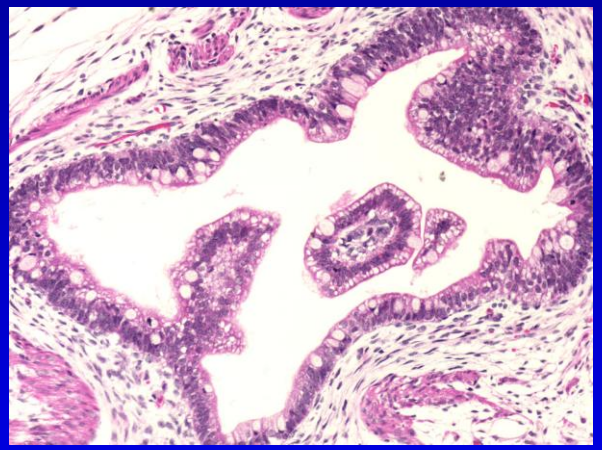


Corrected Sickle Human Fibroblasts

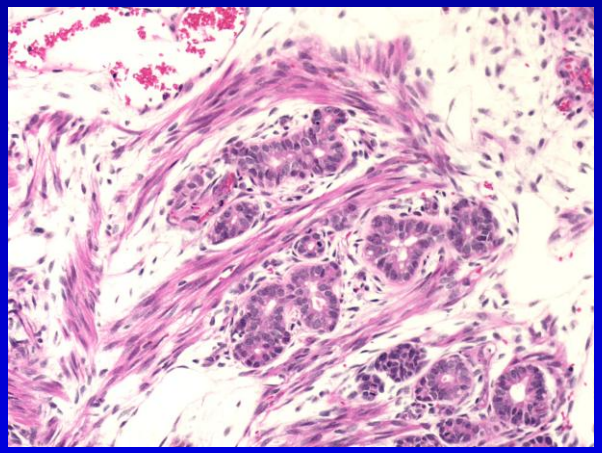
iPSC Derived From Corrected Human Sickle Fibroblasts



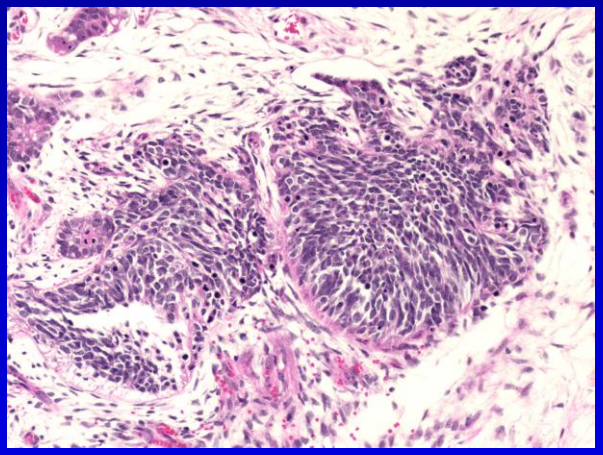
Corrected Human Sickle iPS Cells Form Tissue Derived From All Three Germ Layers



Intestine

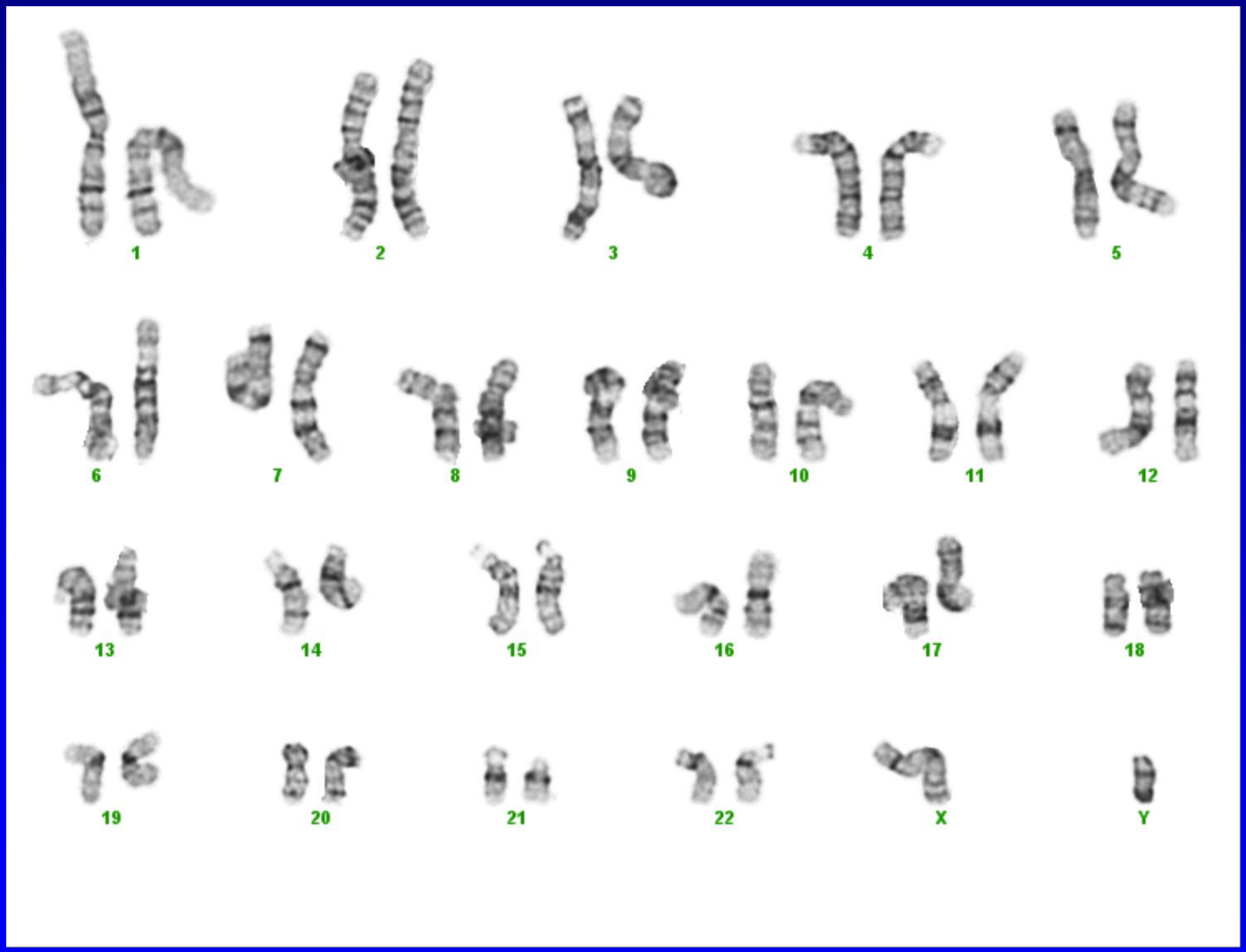


Smooth Muscle and Gland

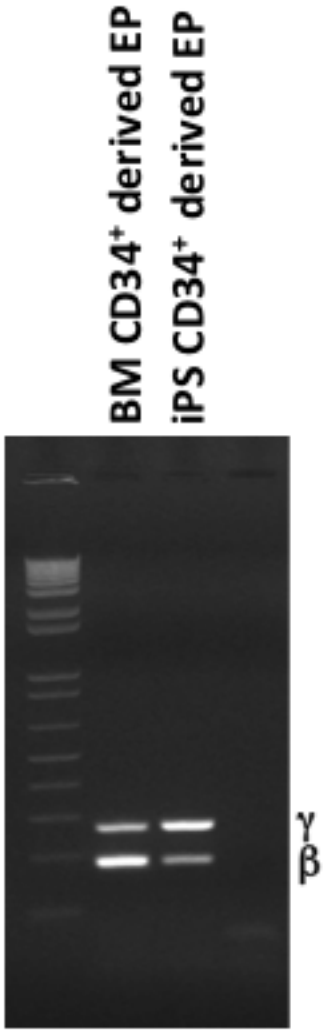


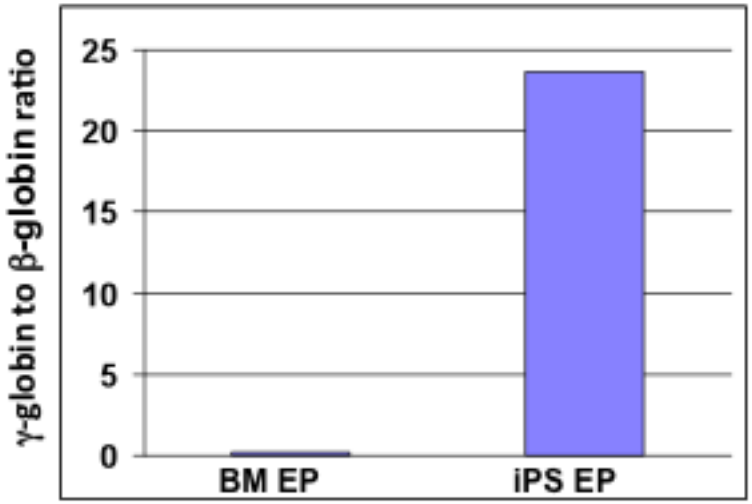
Neural Ectoderm

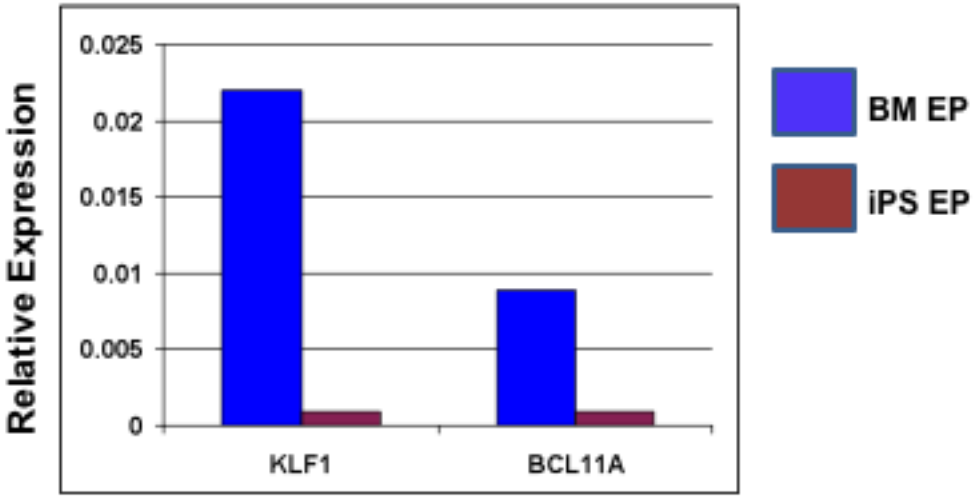
Normal Karyotype of Corrected Human Sickle iPS Cells



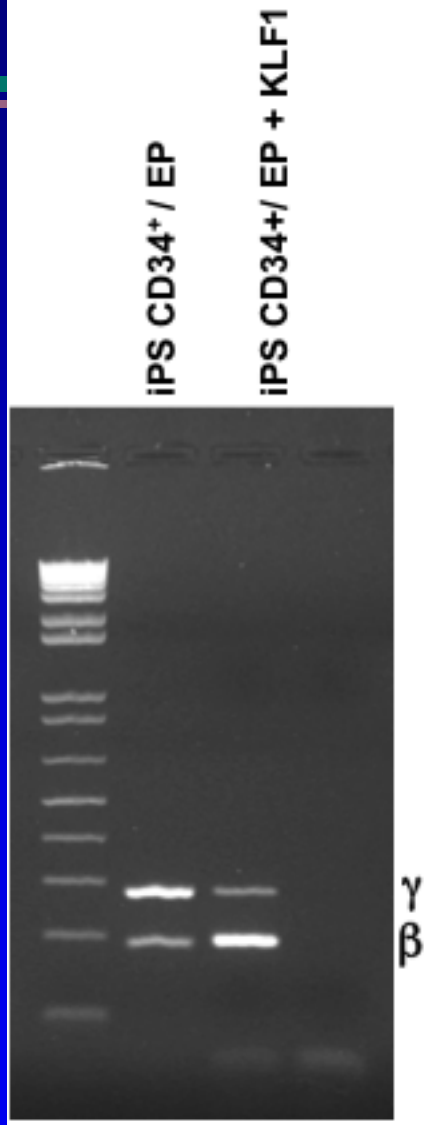
KLF1 and BCL11A expression are low in human EP derived from iPSC

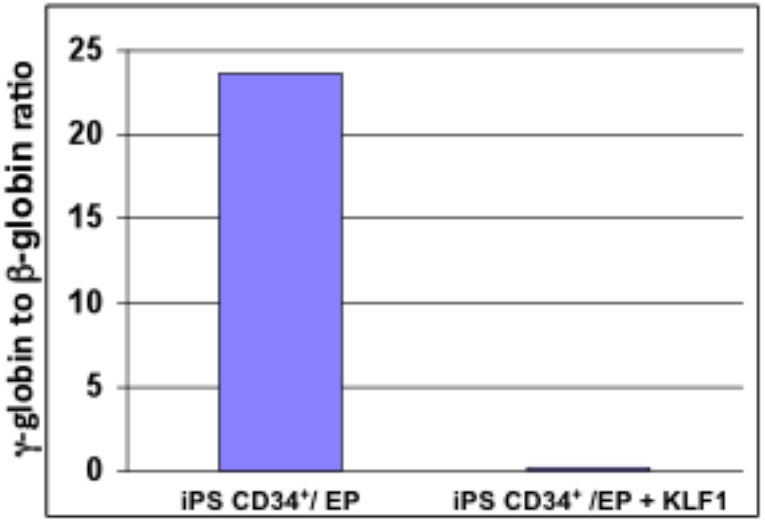


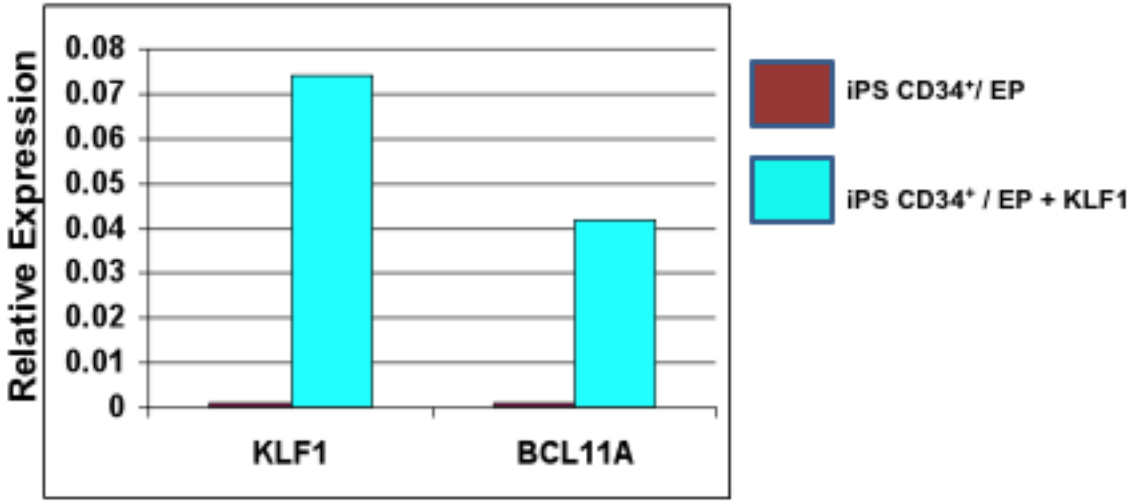




KLF1 Expression in iPS Derived EP Switches Globins





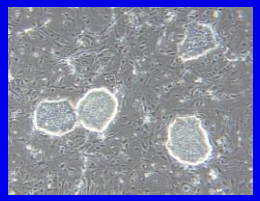


Gene Replacement Therapy for Sickle Cell Disease

Sickle Patient

Transplant corrected blood stem cells into patient

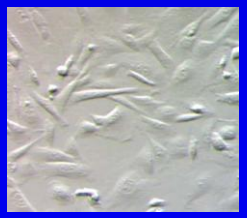
Culture into blood stem cells



Derive iPS cells

Skin cell biopsy

Correct Sickle Gene in patient fibroblasts



Acknowledgements

Chia-Wei Chang
Yi-Shin Lai
Chao Li
Kaimao Liu
Kevin Pawlik
Ivan Popov
Joe Sun
Erik Westin
Jane Wu
Dewang Zhou

Matt Renfrow