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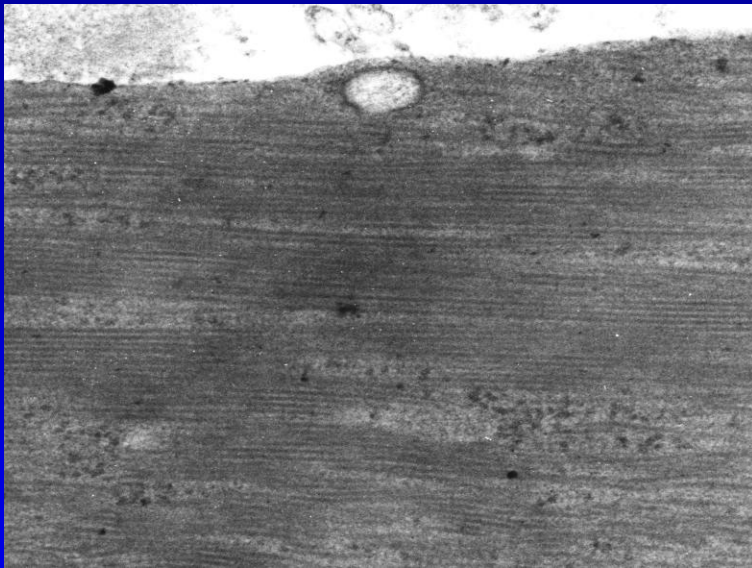
# **Human Globin Gene Regulation and Gene Replacement Therapy for Sickle Cell Disease**

**Tim Townes, Ph.D.**

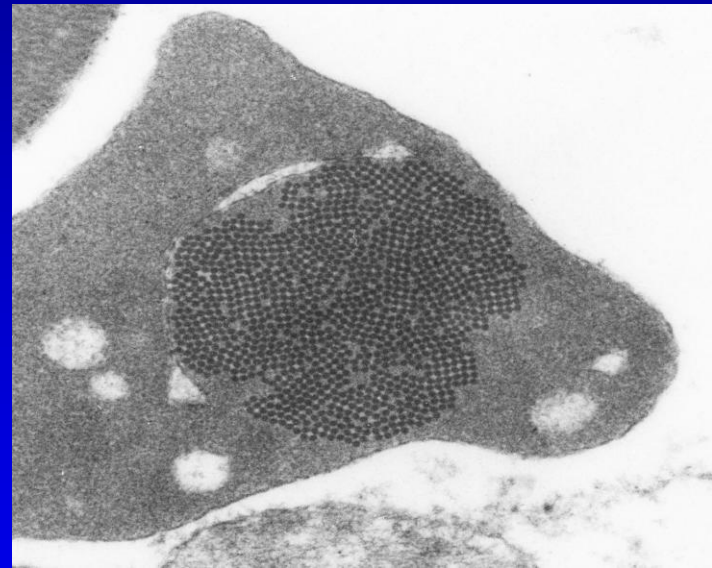
**UAB, Department of Biochemistry and Molecular  
Genetics  
UAB Stem Cell Institute**

# Sickle Hemoglobin Polymers

sagittal



transverse

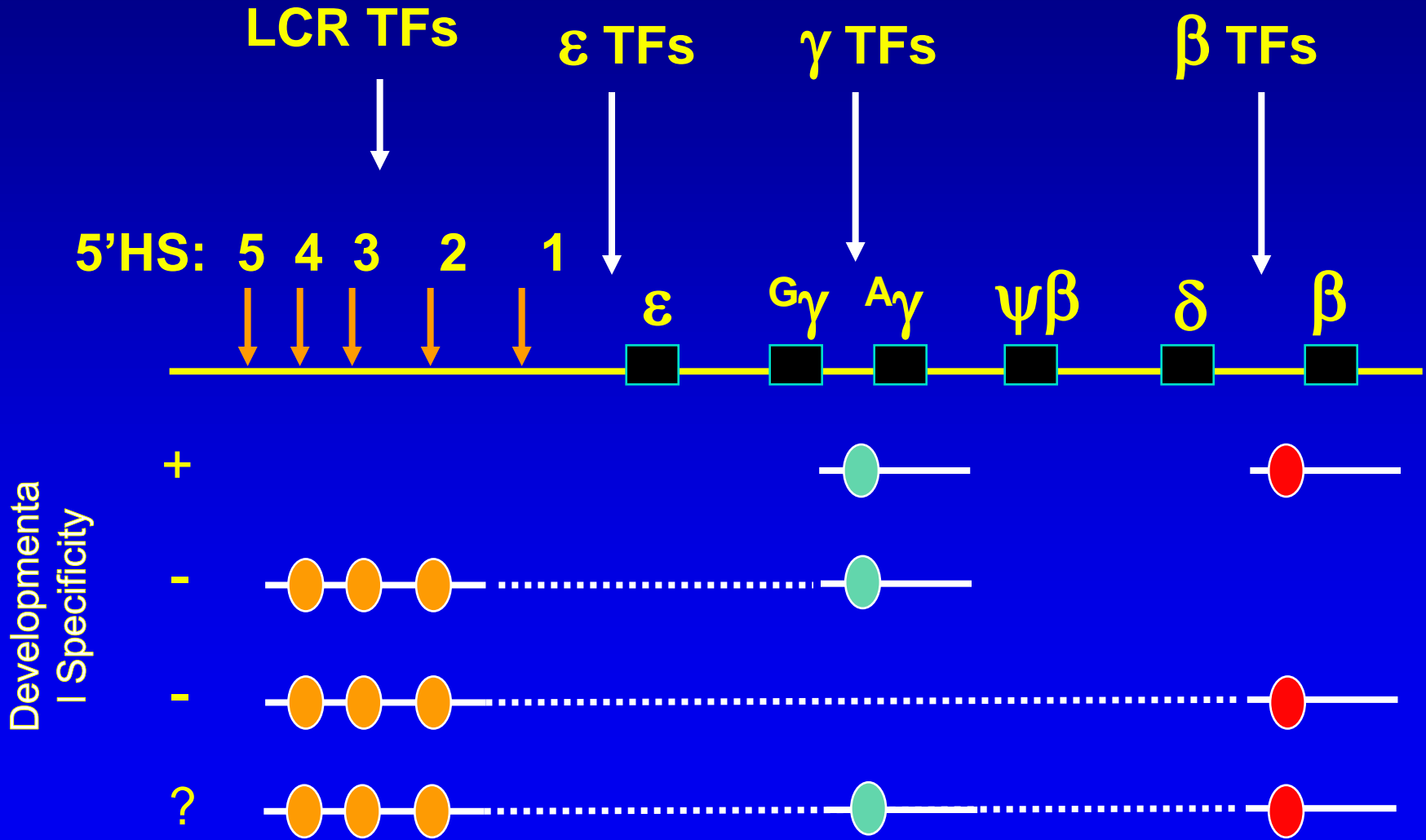


Normal Oxygen tension

# Left Middle Cerebral Artery Occlusion

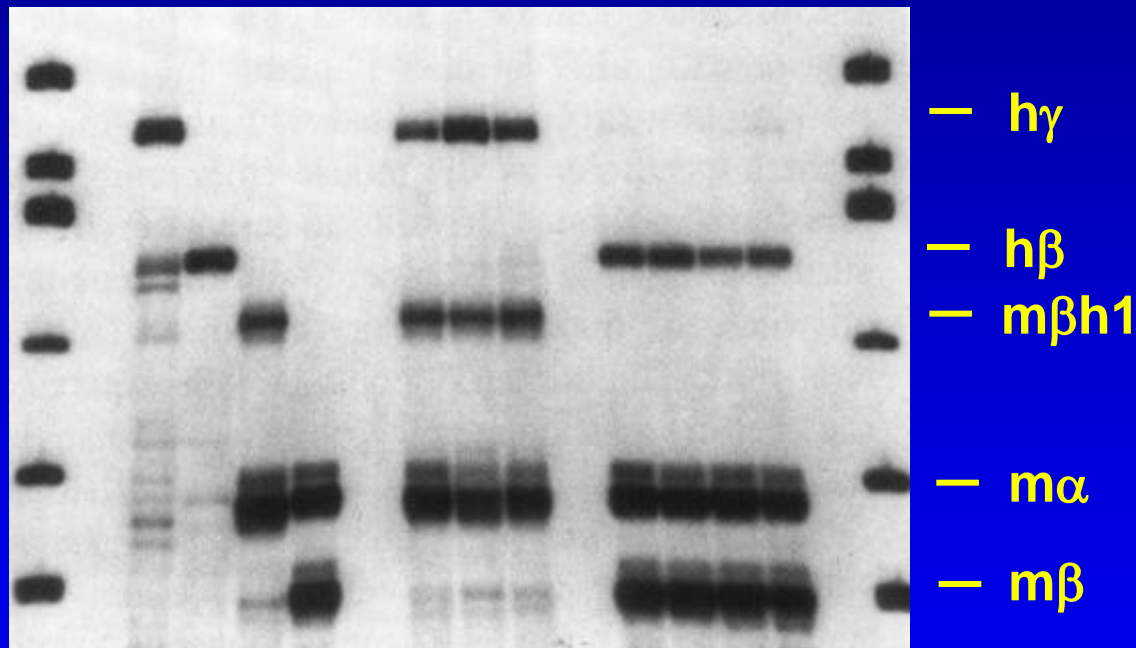


# Competition Model for Human Globin Gene Switching

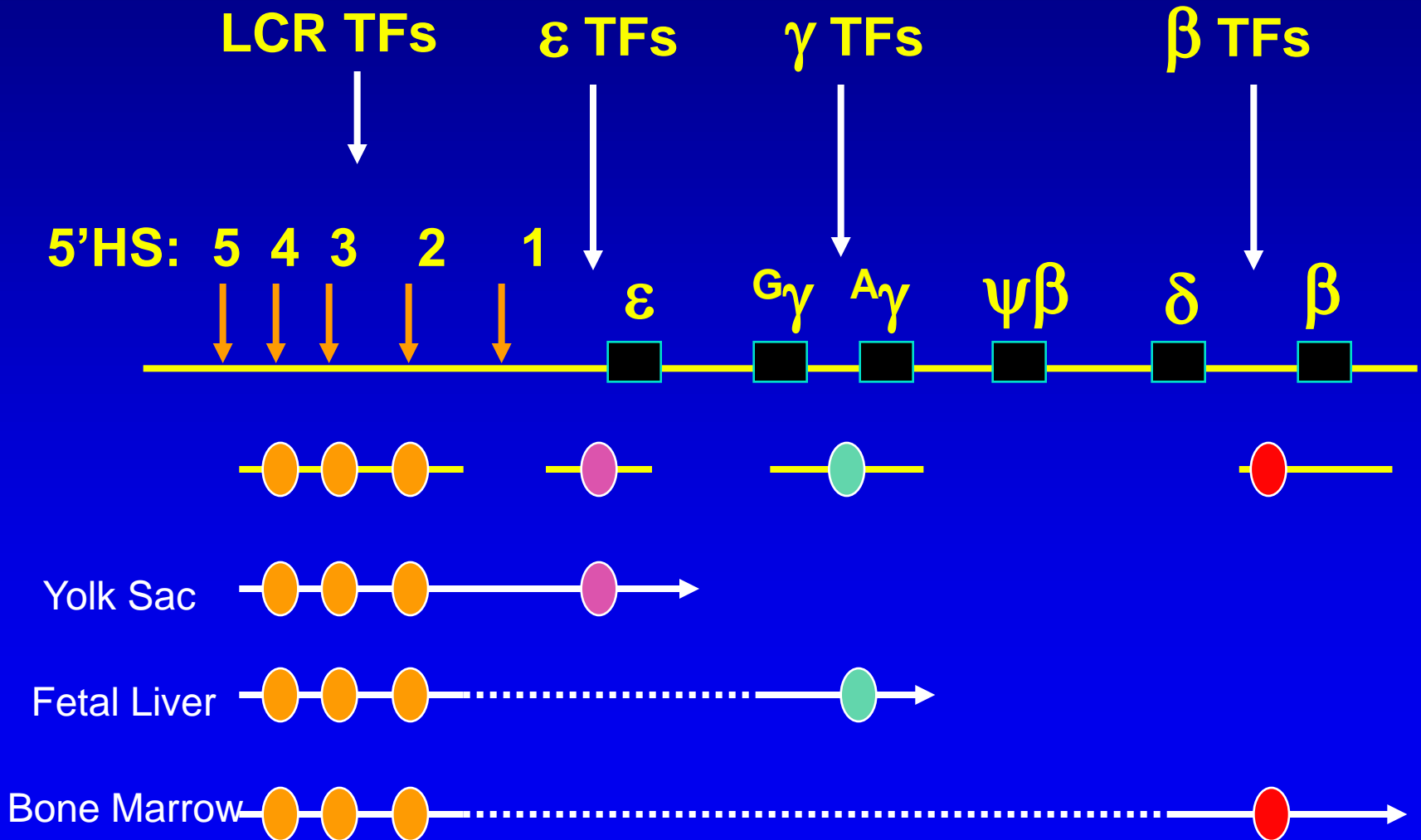


# Linkage of $\gamma$ - and $\beta$ -Globin Genes Restores Developmental Specificity

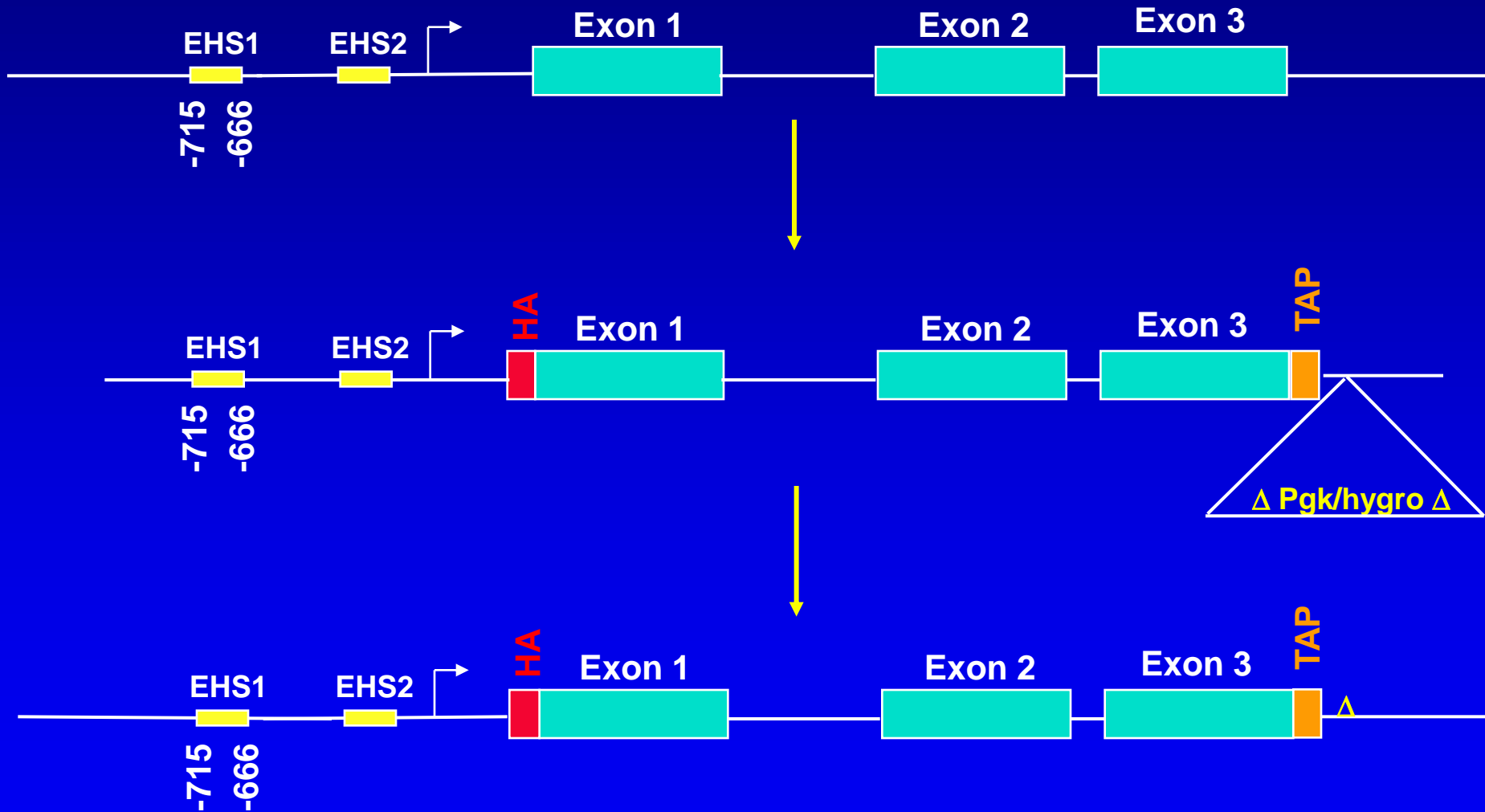
Controls Embryos Adults



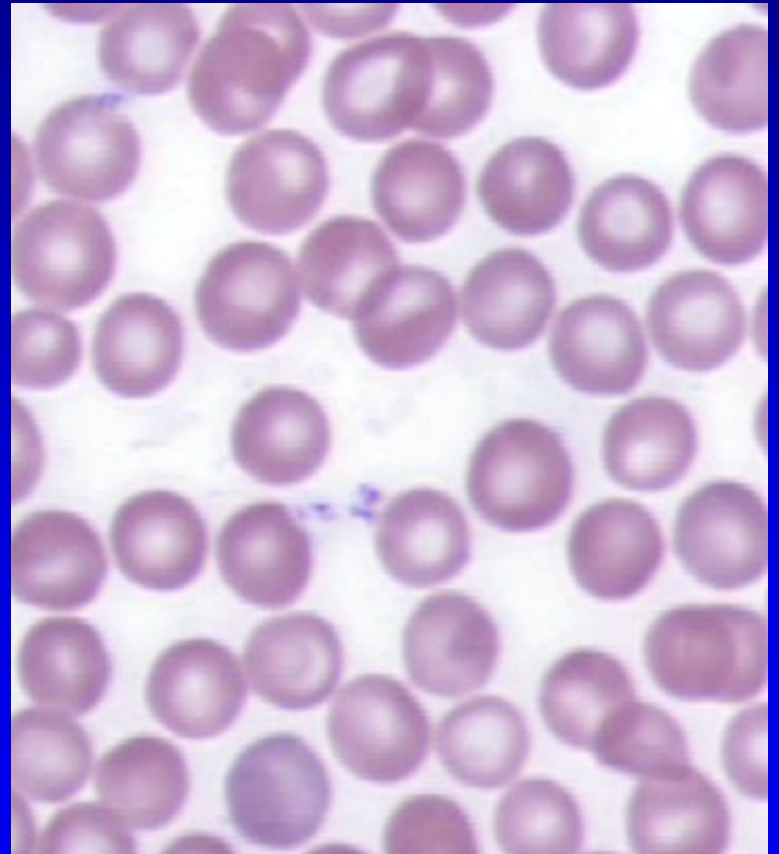
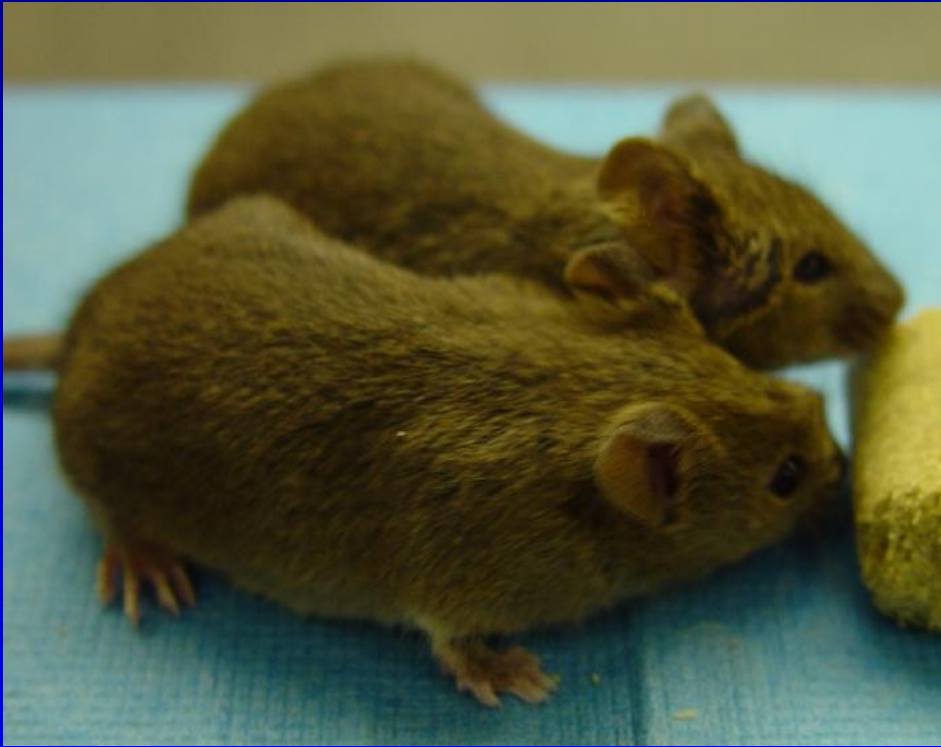
# Competition Model for Human Globin Gene Switching



# Production of HA/EKLF/TAP Knockin Mice

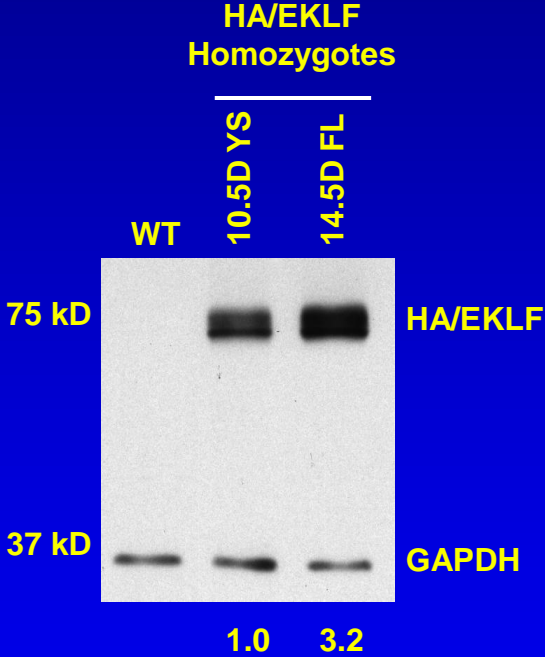


# HA/EKLF Mice



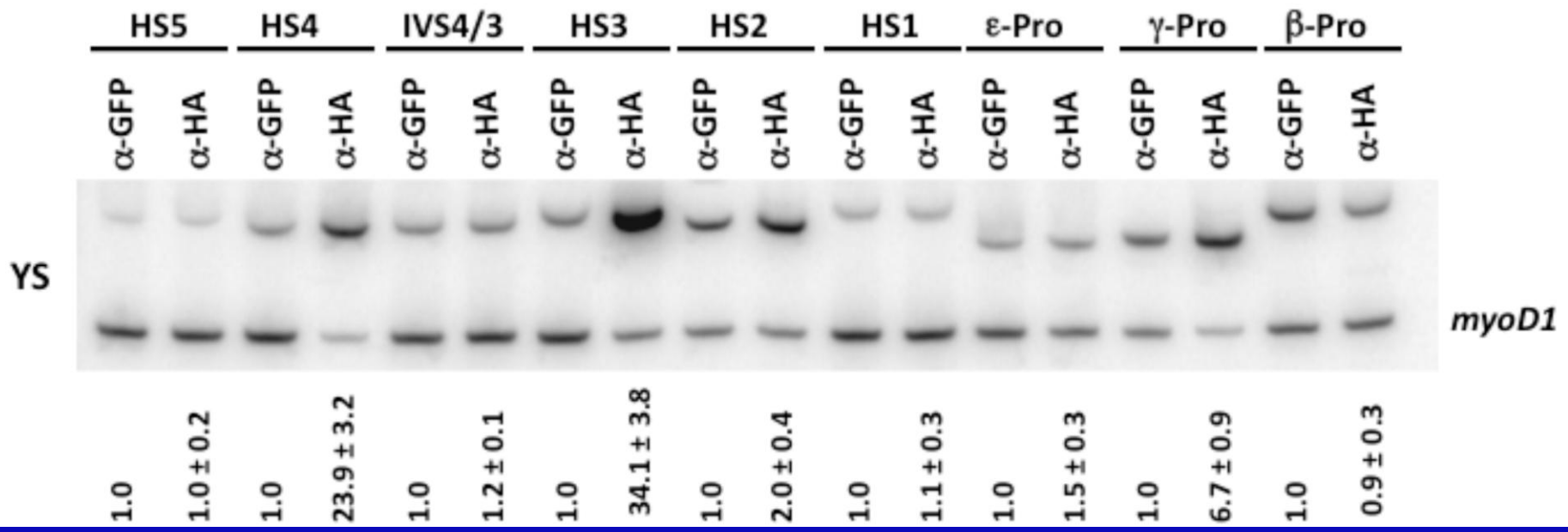
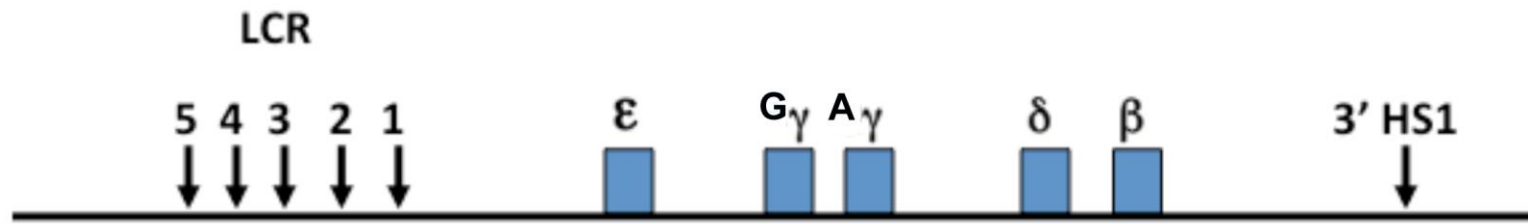


# Quantitation of HA/EKLF in Primitive vs Definitive Erythroid Cells

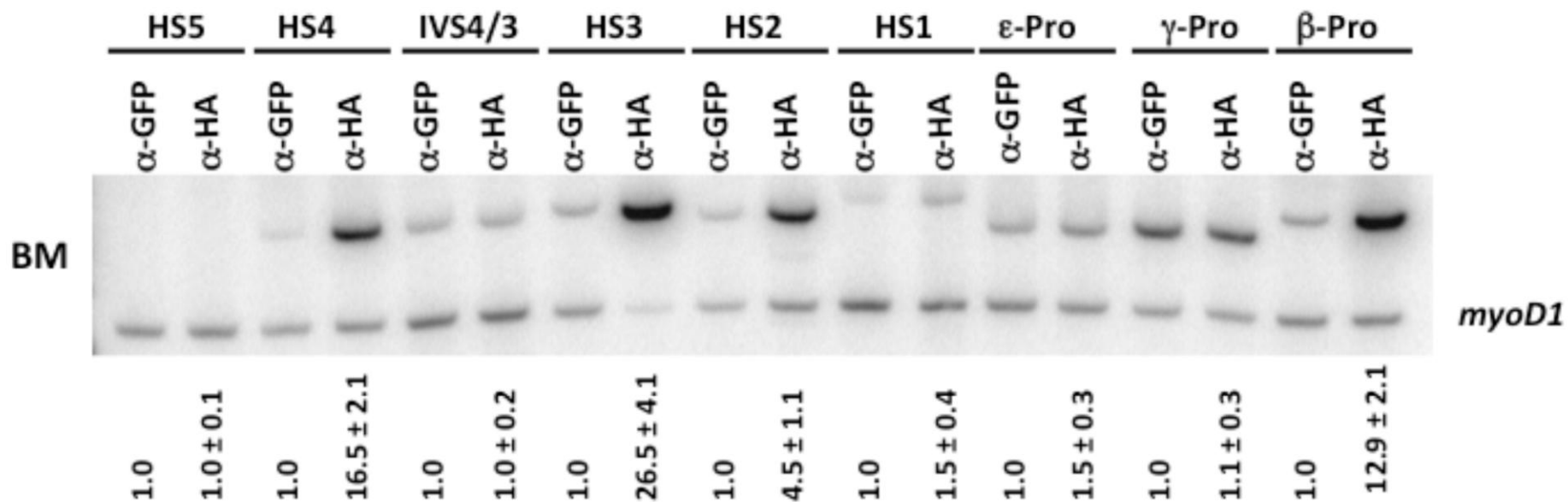
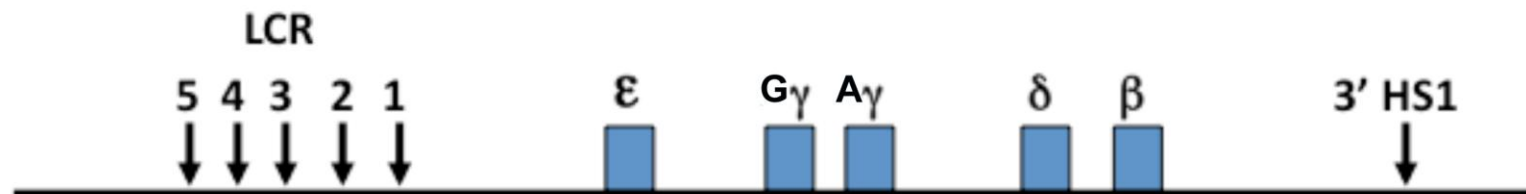


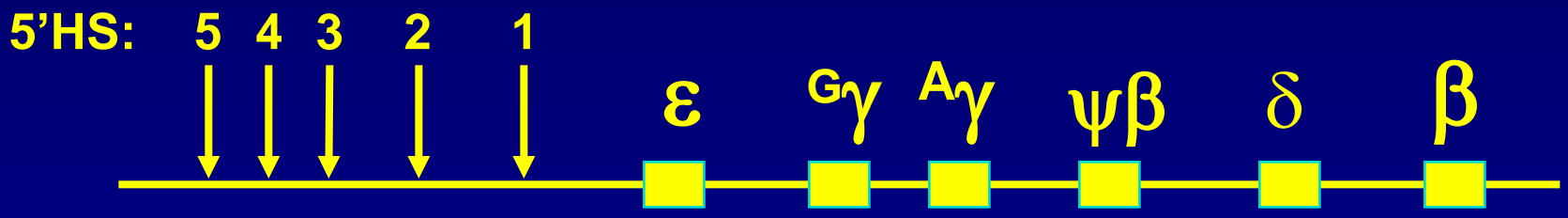
Western Blot with  $\alpha$ -HA

# EKLF Binding to the Human $\beta$ -globin Locus in Murine 10.5D YS

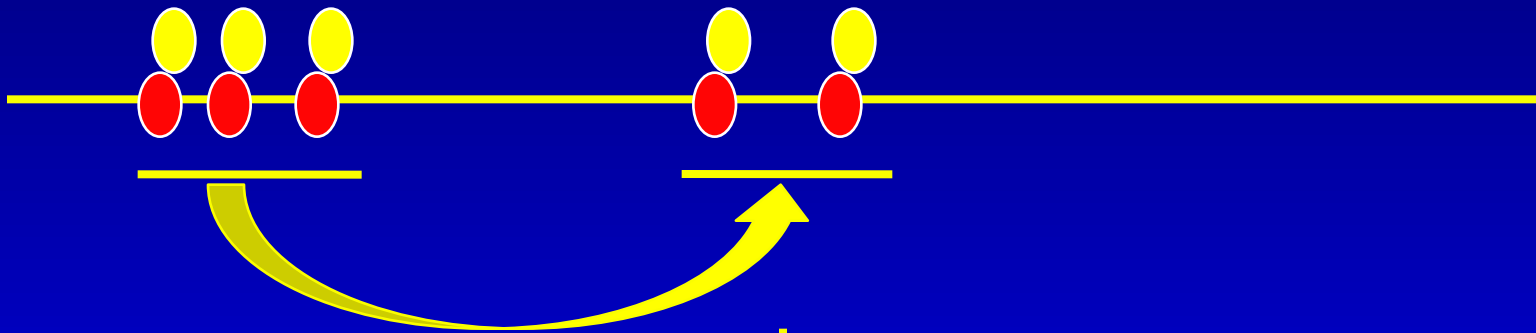


# EKLF Binding to the Human $\beta$ -globin Locus in Murine Adult BM





Fetal Liver

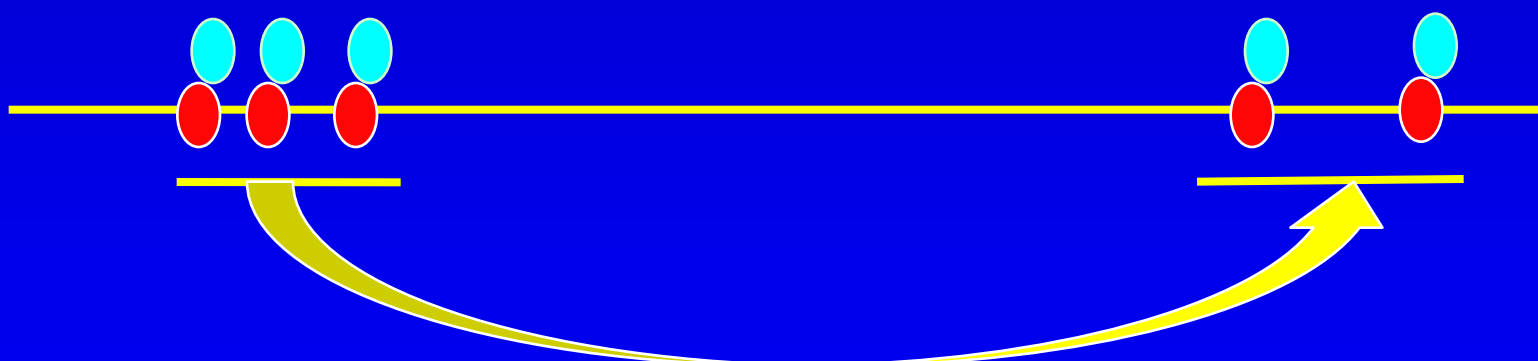


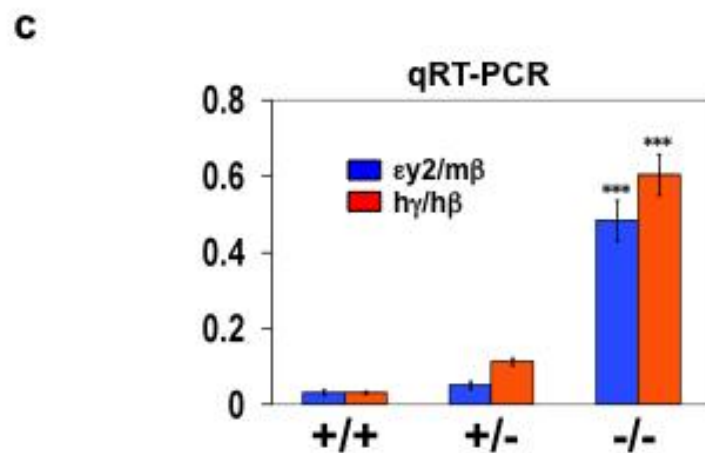
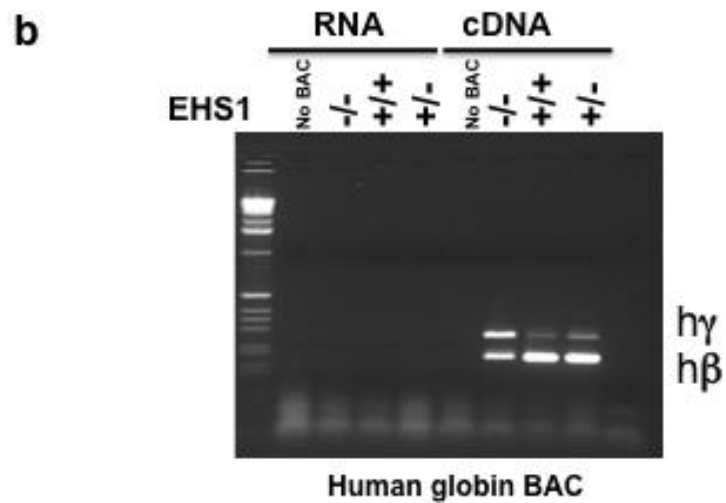
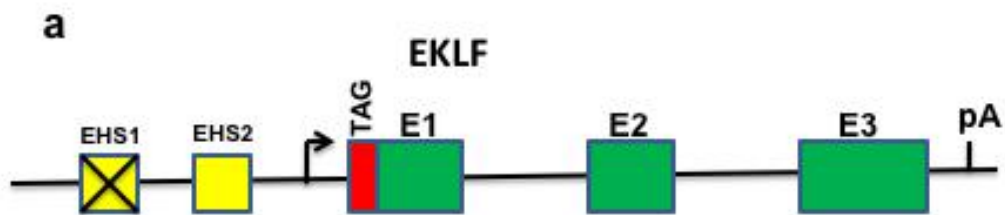
EKLF

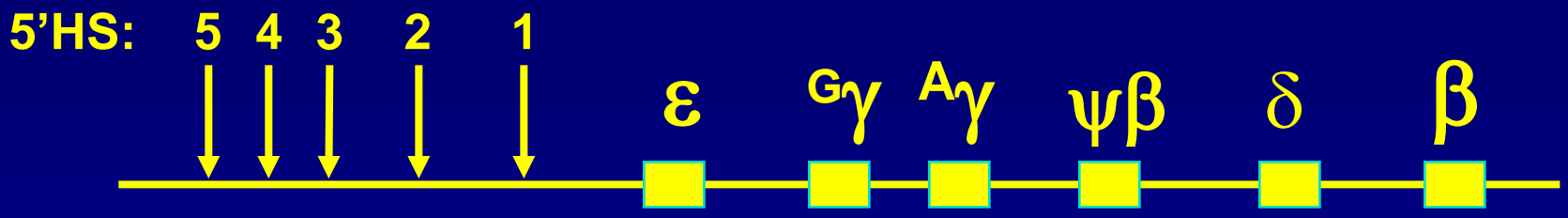
3X EKLF increase



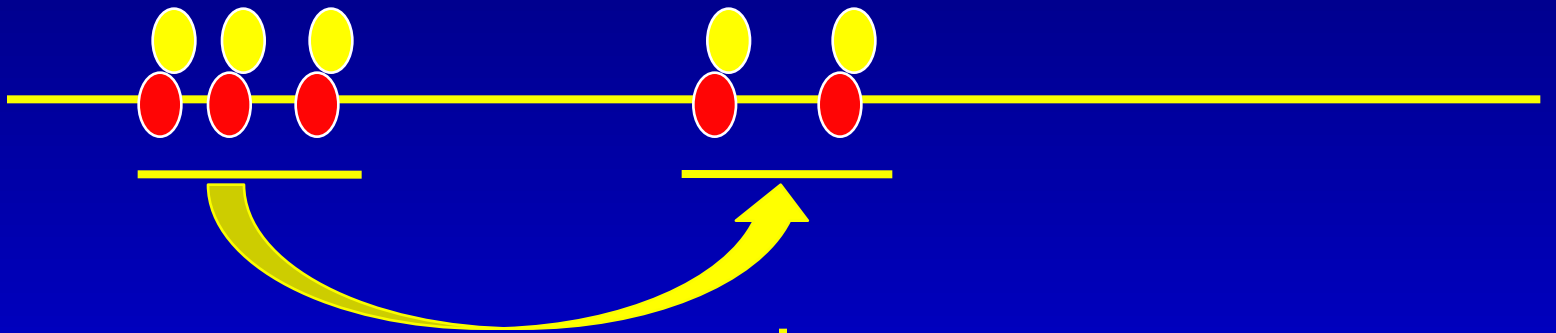
Bone Marrow





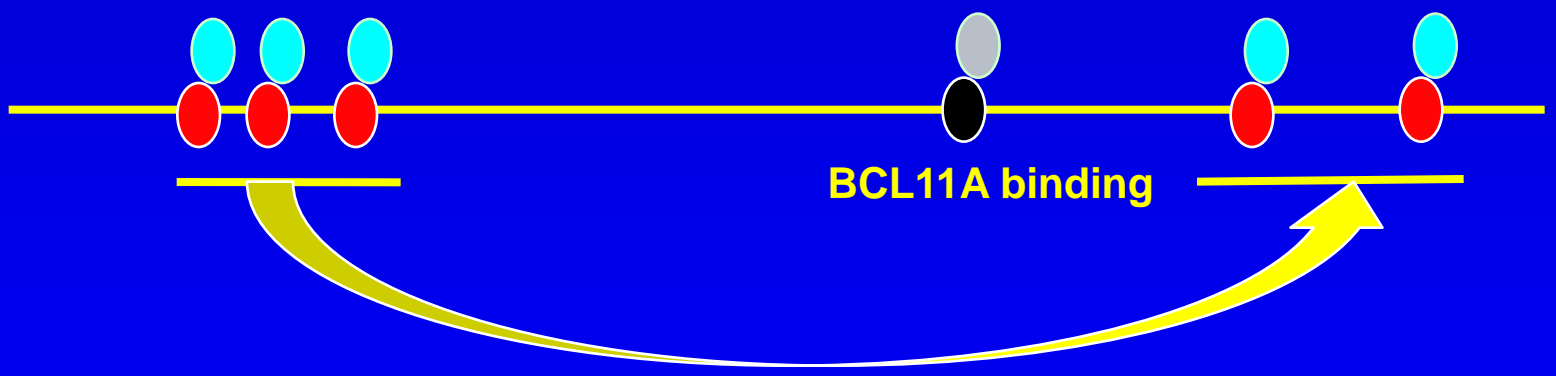


Fetal Liver

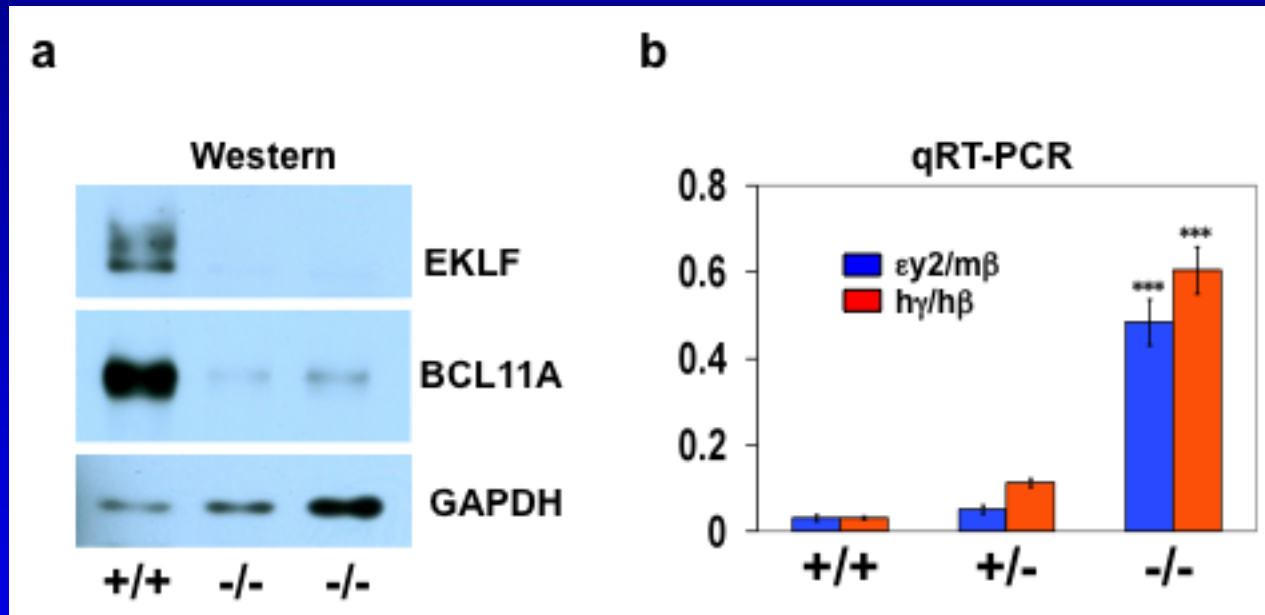


3X EKLf increase

Bone Marrow



# 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

a

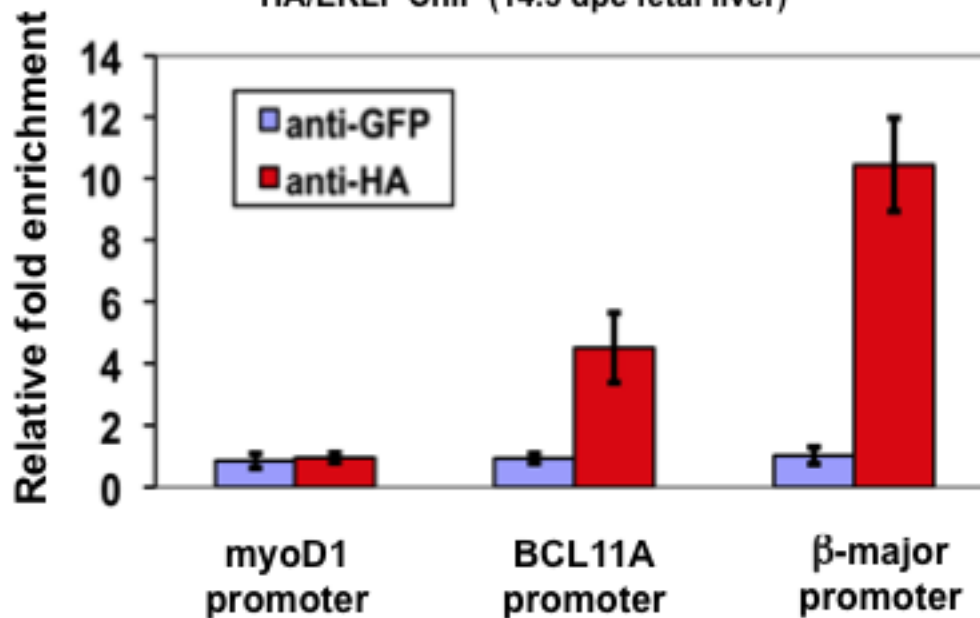
mBCL11A -376 CCTCC**CACCC**-GCC -363

hBCL11A -376 CCTCC**CACCC**CTGCC -362

b

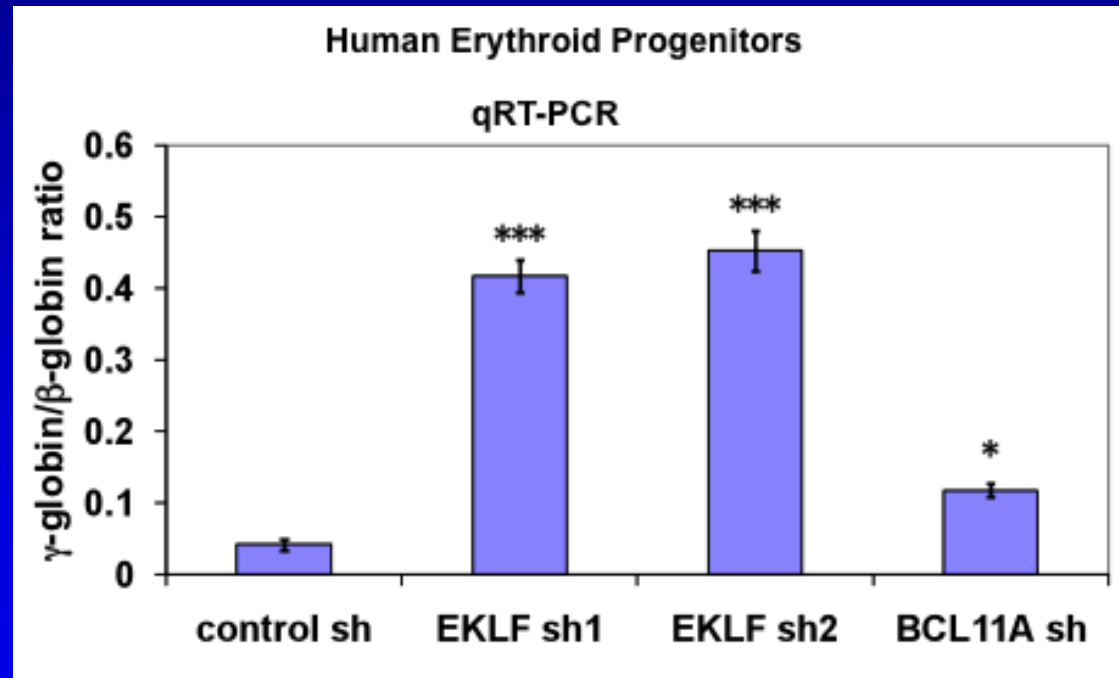
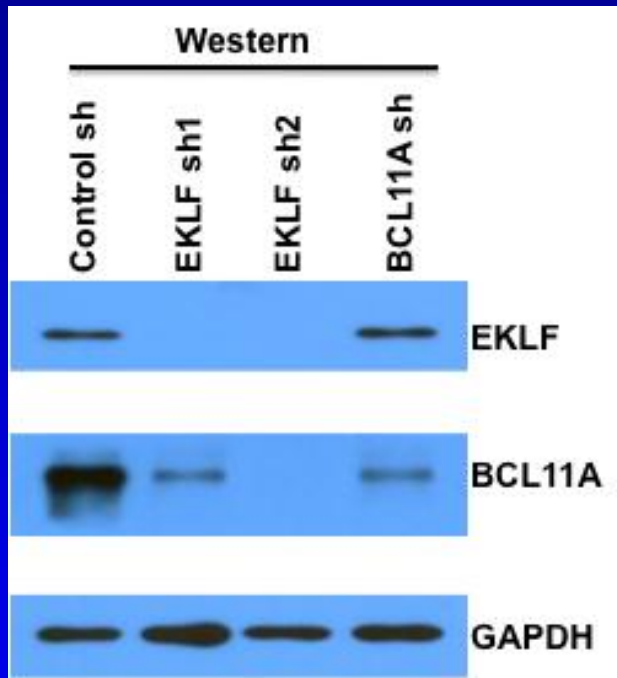
HA/EKLF Knockin Mice

HA/EKLF ChIP (14.5 dpc fetal liver)



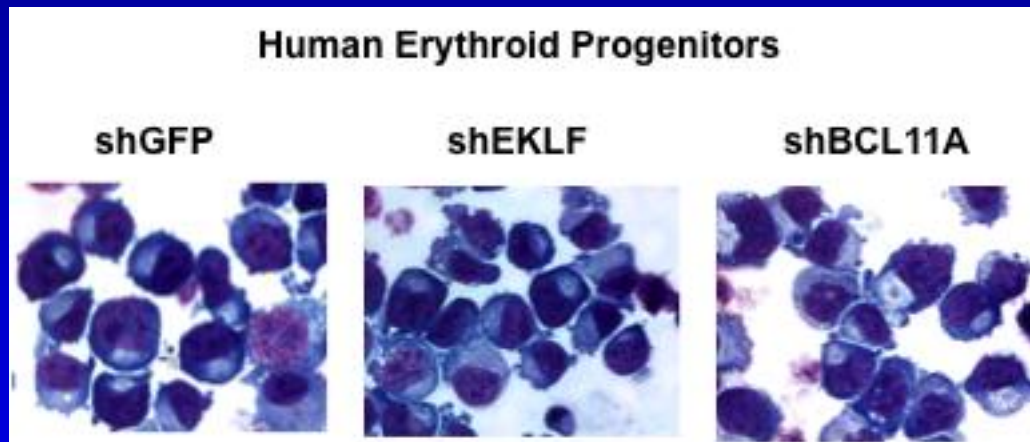


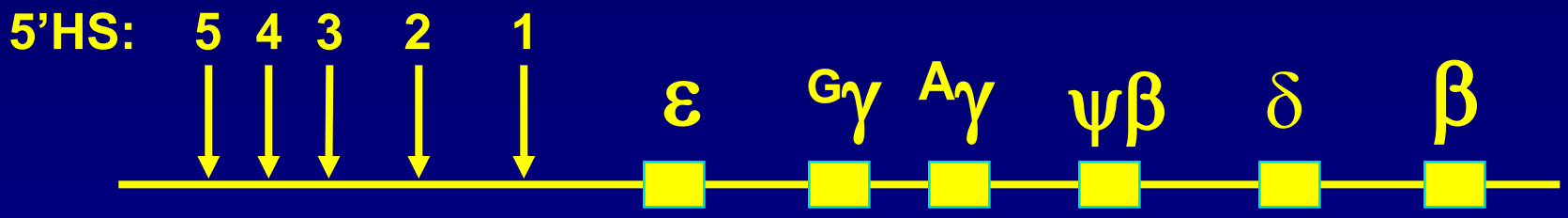
# EKLF Controls Human BCL11A Expression and Globin Gene Switching



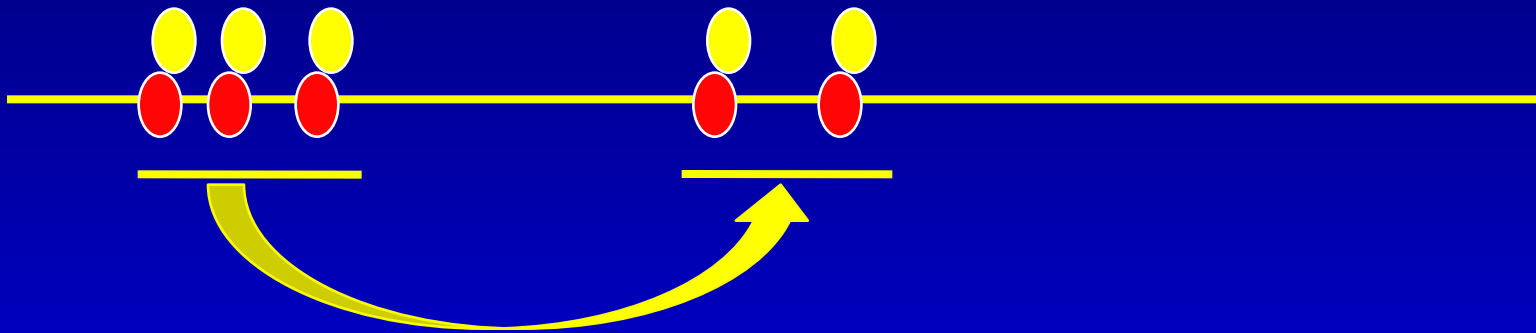
Human Bone Marrow Erythroid Progenitors

# Cytospin of Human Erythroid Progenitors after shRNA Knockdown





Fetal Liver



EKLF decrease  
BCL11A decrease

EKLF increase  
BCL11A increase

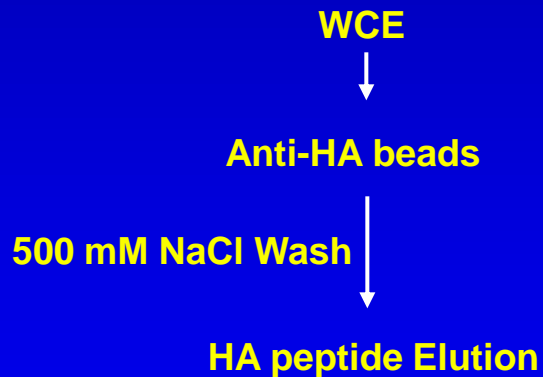


Bone Marrow

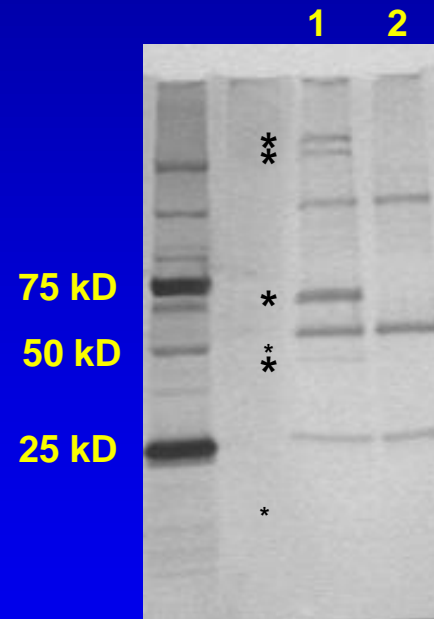


# Purification of Mouse EKLF Protein Complexes from 14.5 dpc Fetal Livers

## HA/EKLF Mice

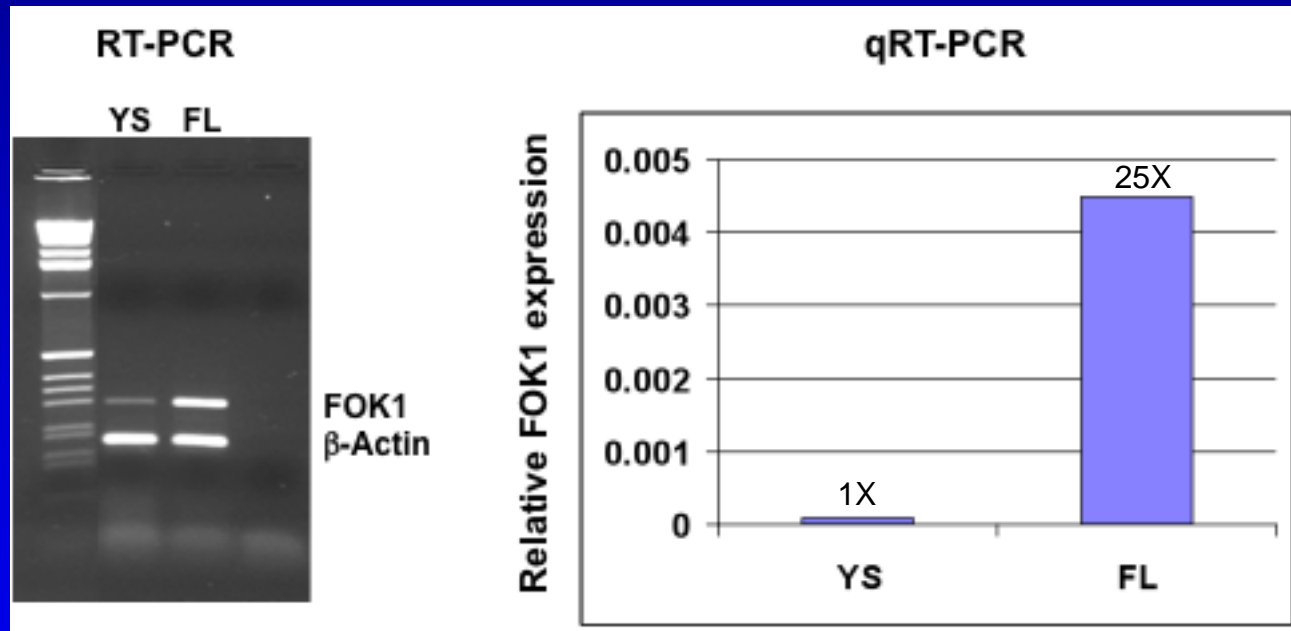


1. HA/EKLF (5 Fetal Livers)      2. Wild Type (5 Fetal Livers)



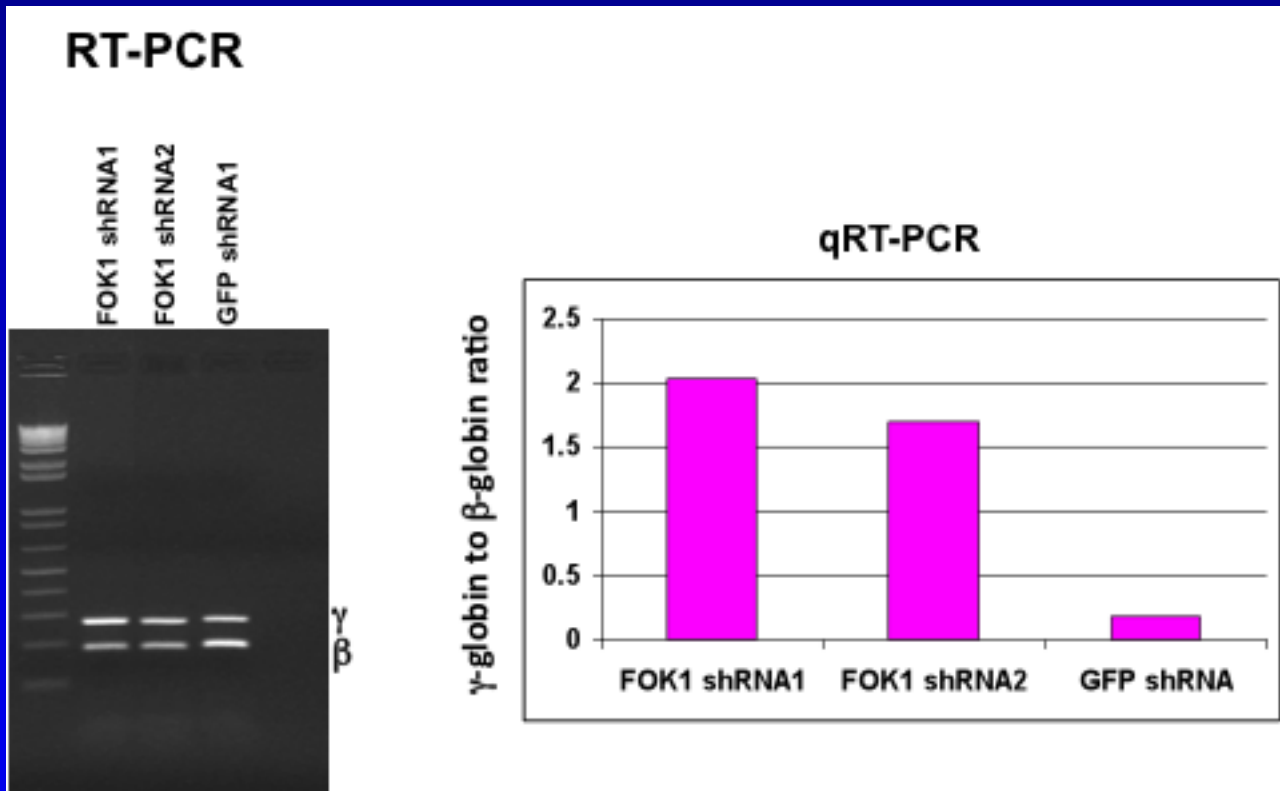
Silver staining

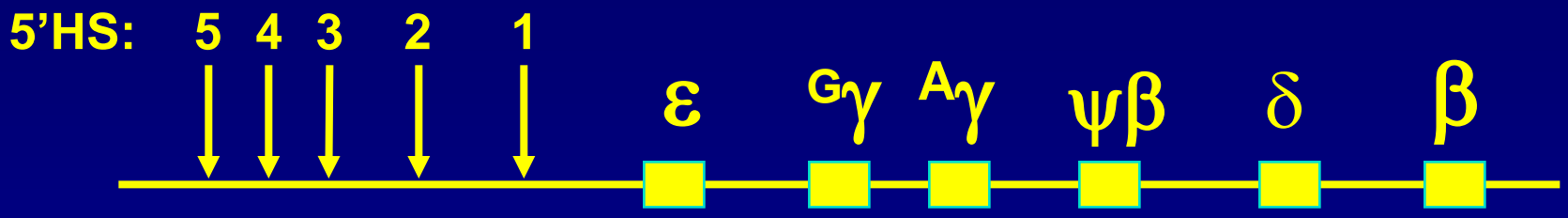
# Friend of KLF1 (FOKLF1)



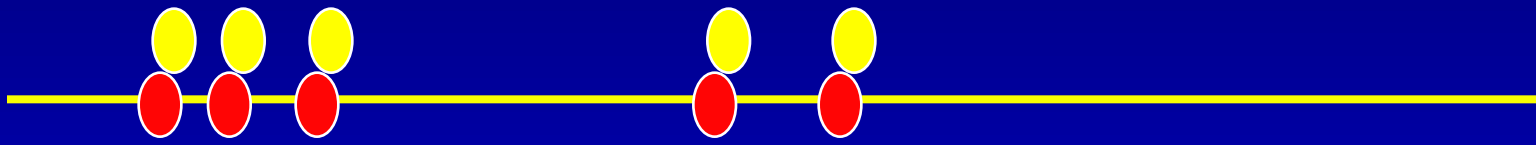
BM Ter119+ FOKLF1 =  
50X

# FOKLF1 Knockdown in Human EP Switches Globin Gene Expression



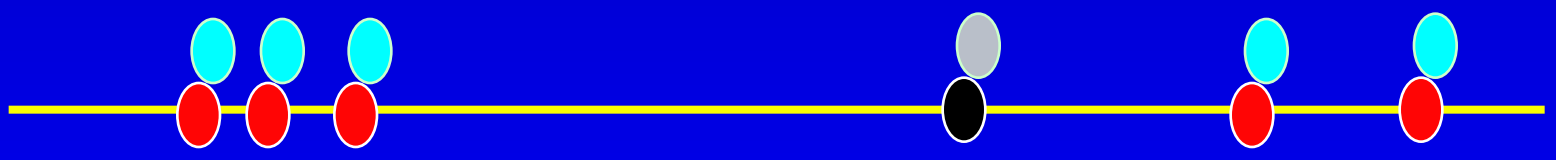


Fetal Liver

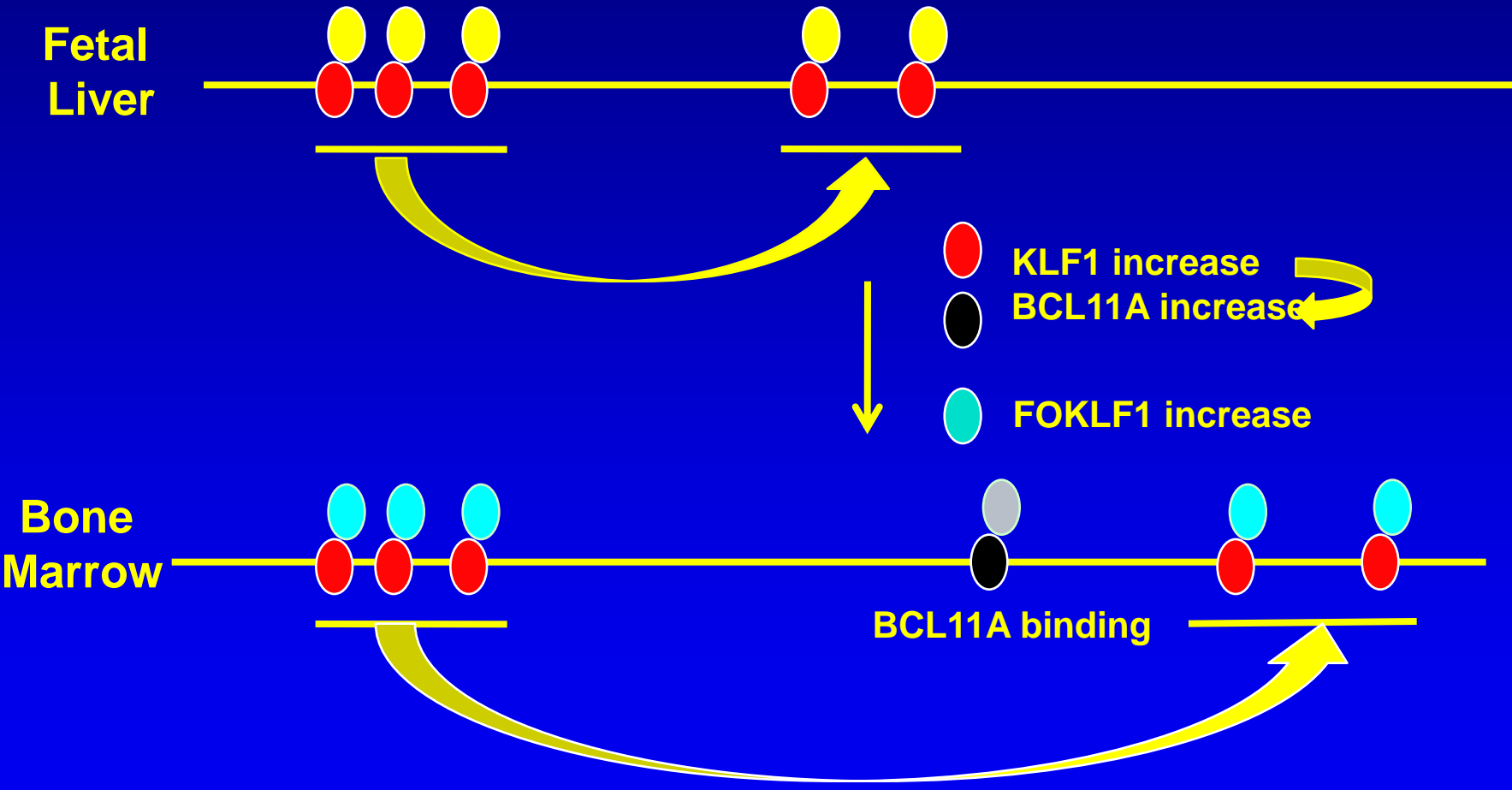


- KLF1 increase
- BCL11A increase
- FOKLF1 increase

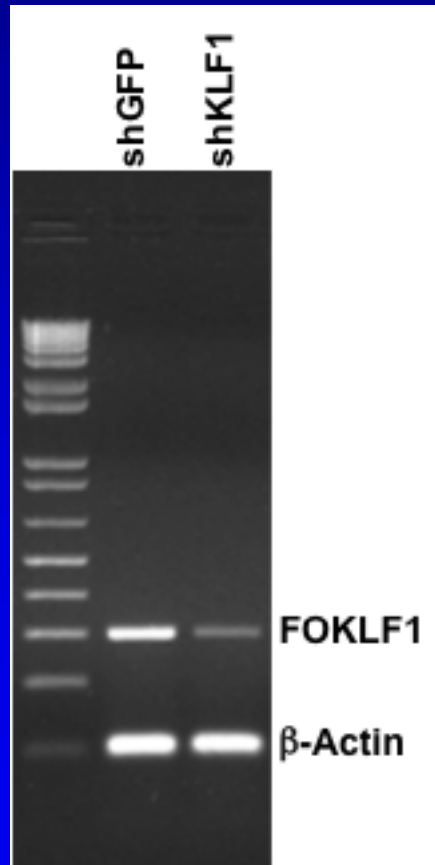
Bone Marrow



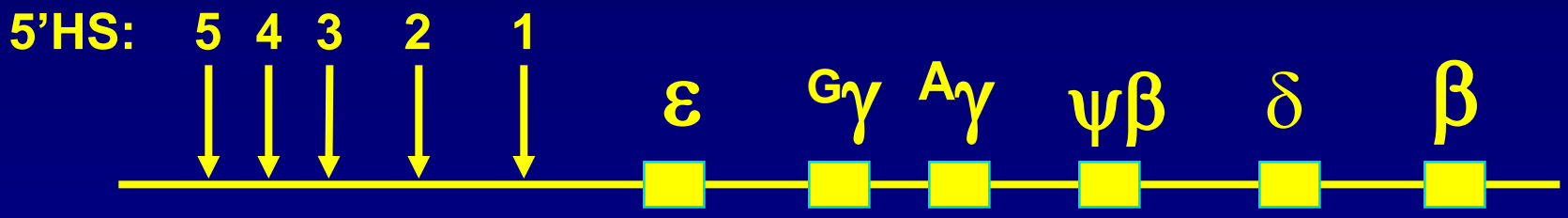
BCL11A binding



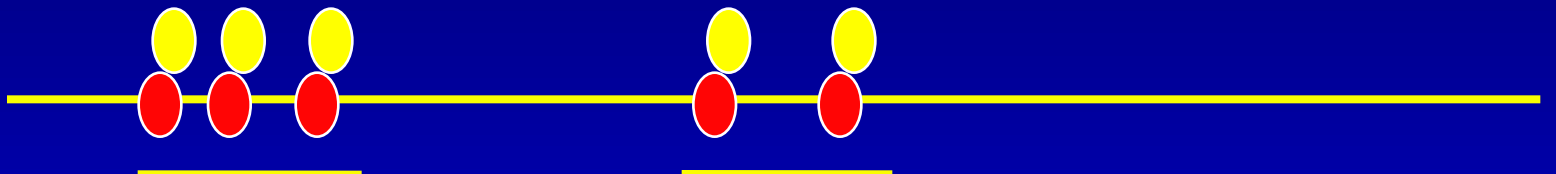
# KLF1 Regulates FOKLF1 in Human Erythroid Progenitors



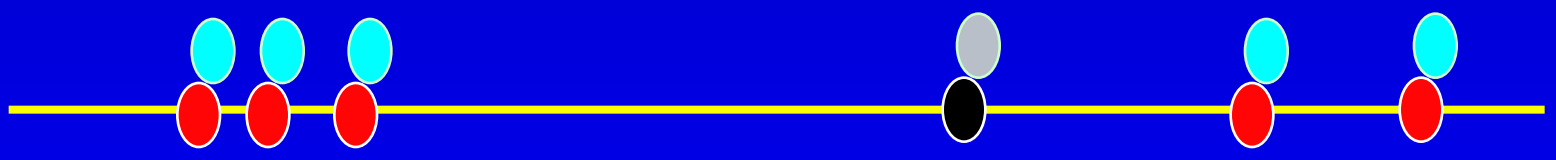


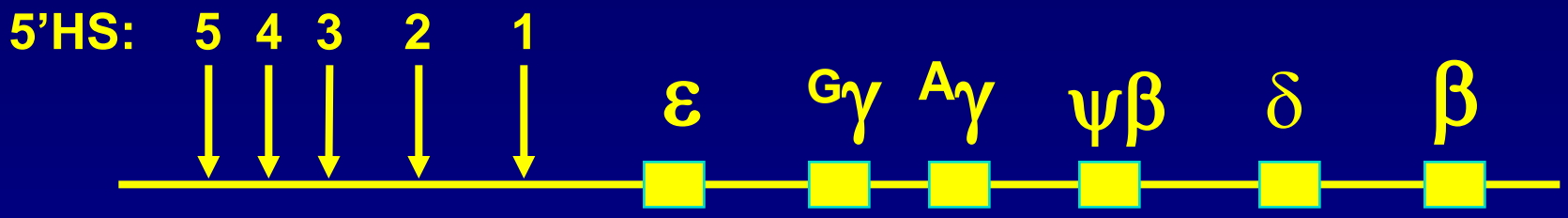


Fetal Liver

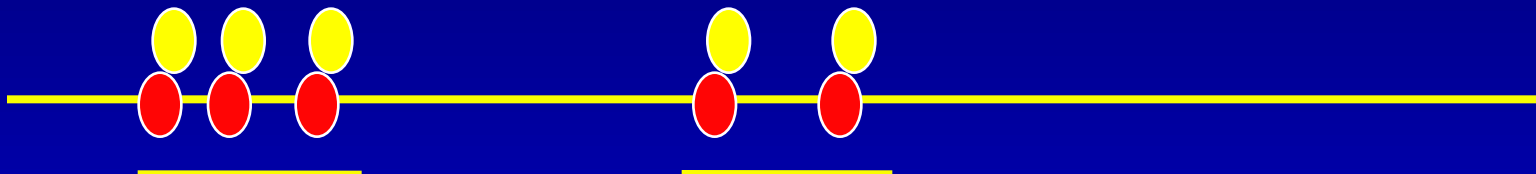


Bone Marrow





Fetal Liver

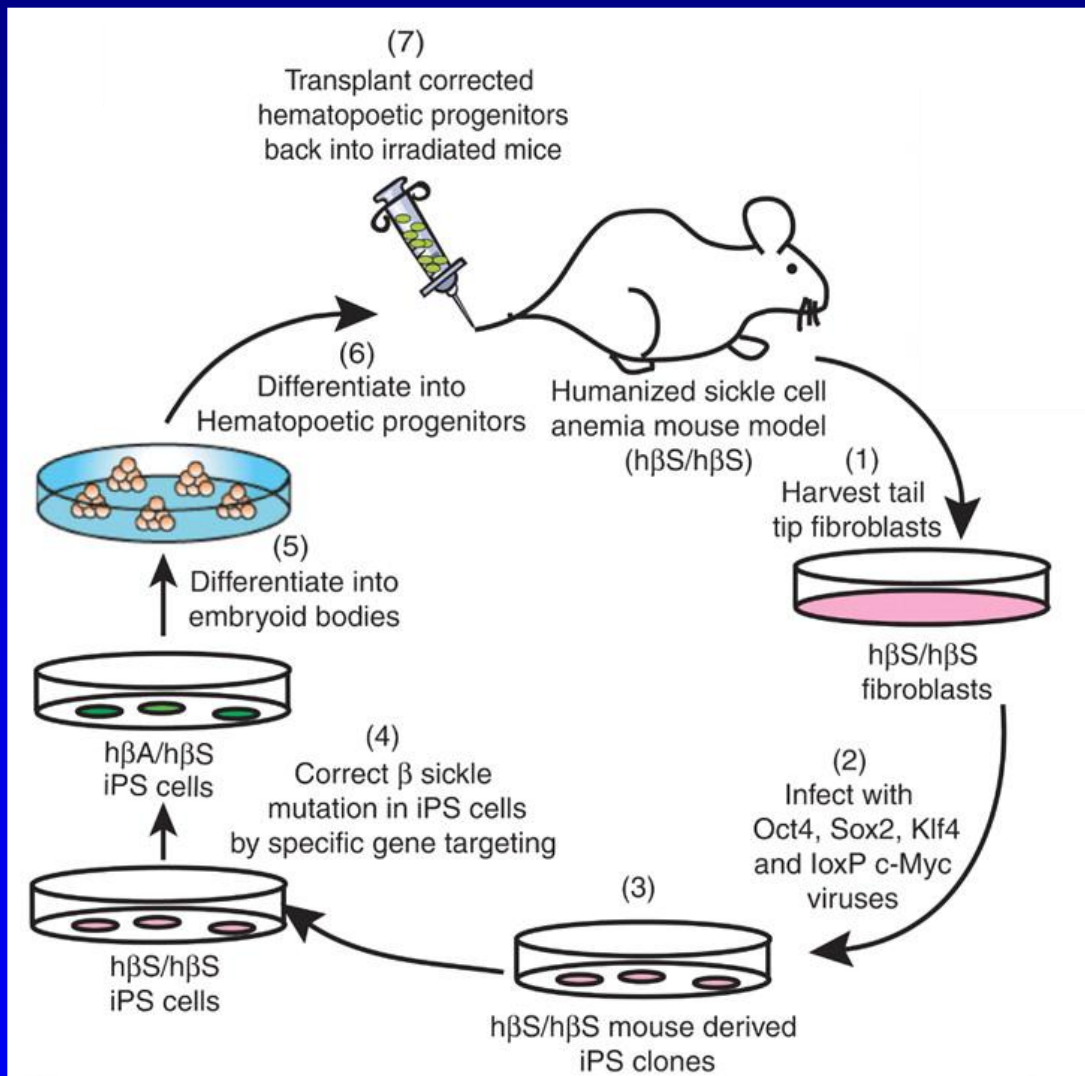


Bone Marrow



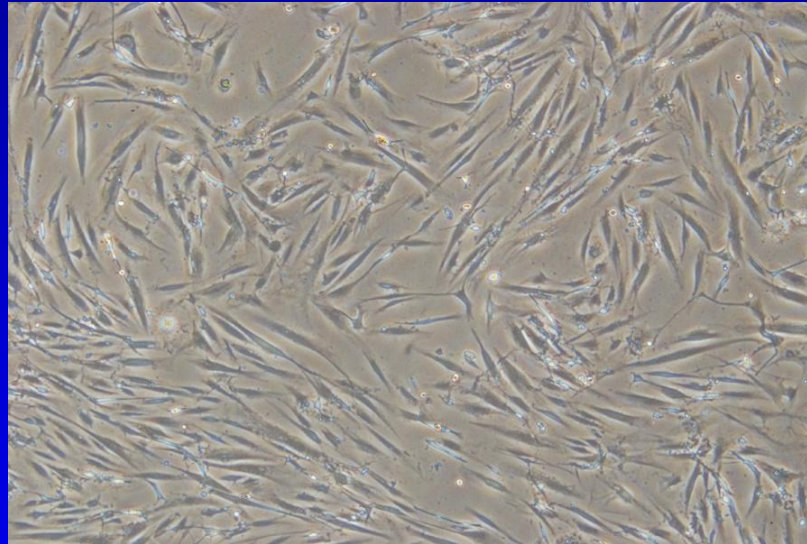
○ Small molecule

# Scheme for Gene and Cell Therapy of Humanized Sickle Mouse



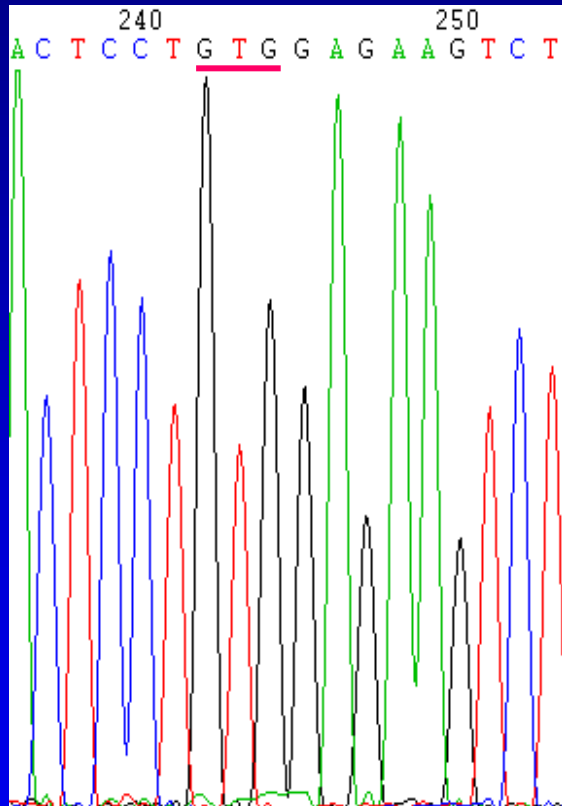
# Gene Replacement in Sickle Skin Fibroblasts?

## Human Sickle Skin Fibroblasts

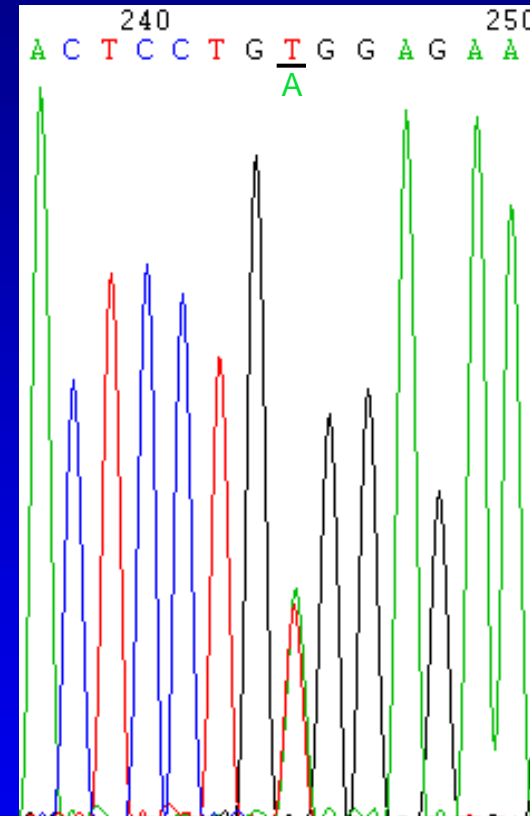


45 year old sickle patient

# Homologous Recombination in Human Sickle Fibroblasts



Sickle Human Fibroblasts



Corrected Sickle Human Fibroblasts

# iPSC Derived From Corrected Human Sickle Fibroblasts

hESC-H1

c-iPSC-1

hESC-H1

c-iPSC-1

anti-TRA-1-60

DAPI

anti-SSEA-4

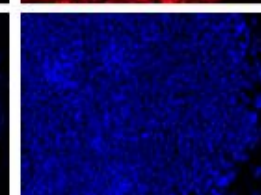
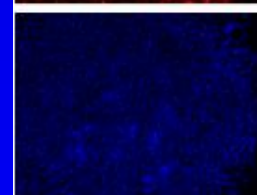
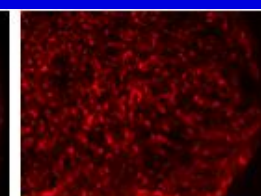
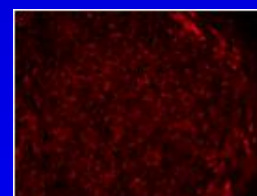
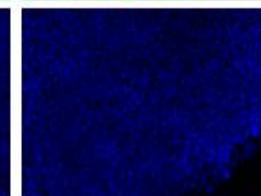
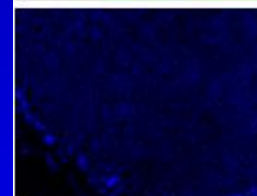
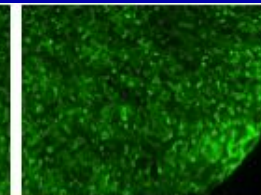
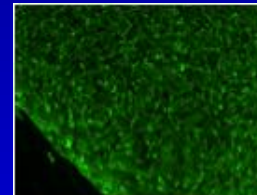
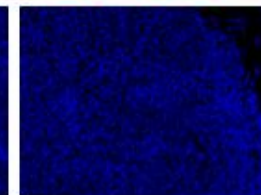
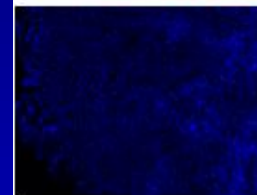
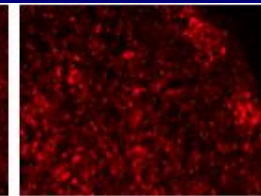
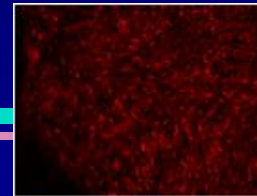
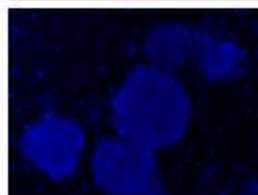
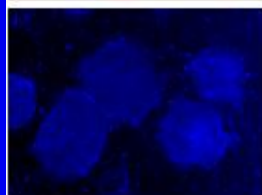
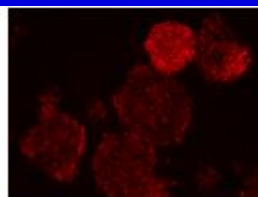
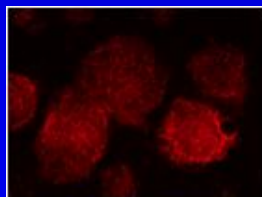
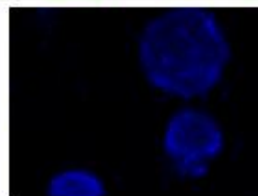
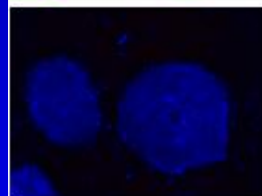
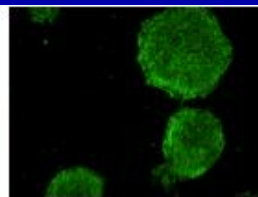
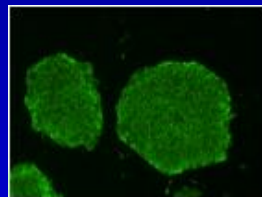
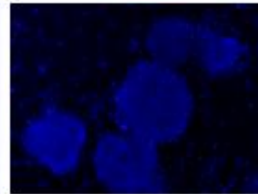
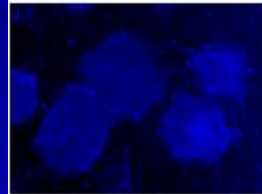
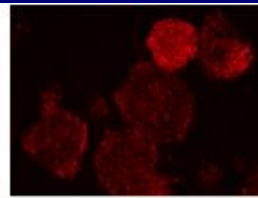
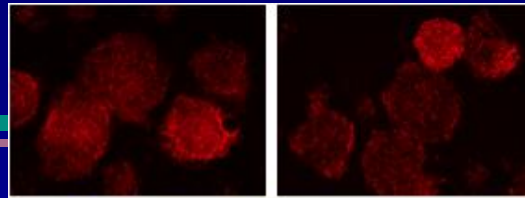
DAPI

anti-TRA-1-81

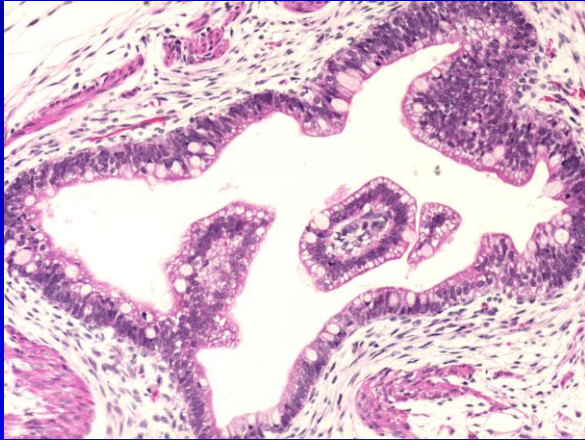
DAPI

2.5 X

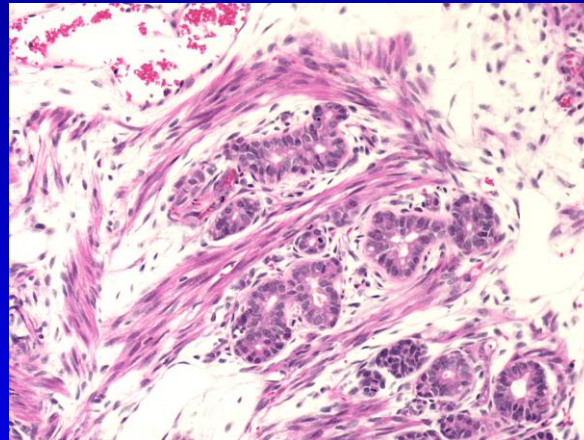
10 X



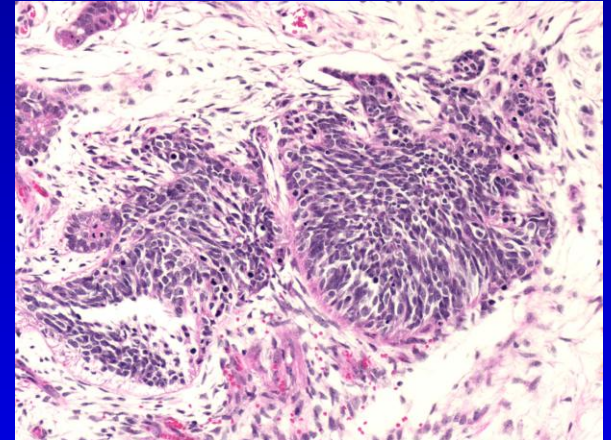
# Corrected Human Sickle iPS Cells Form Tissue Derived From All Three Germ Layers



**Intestine**

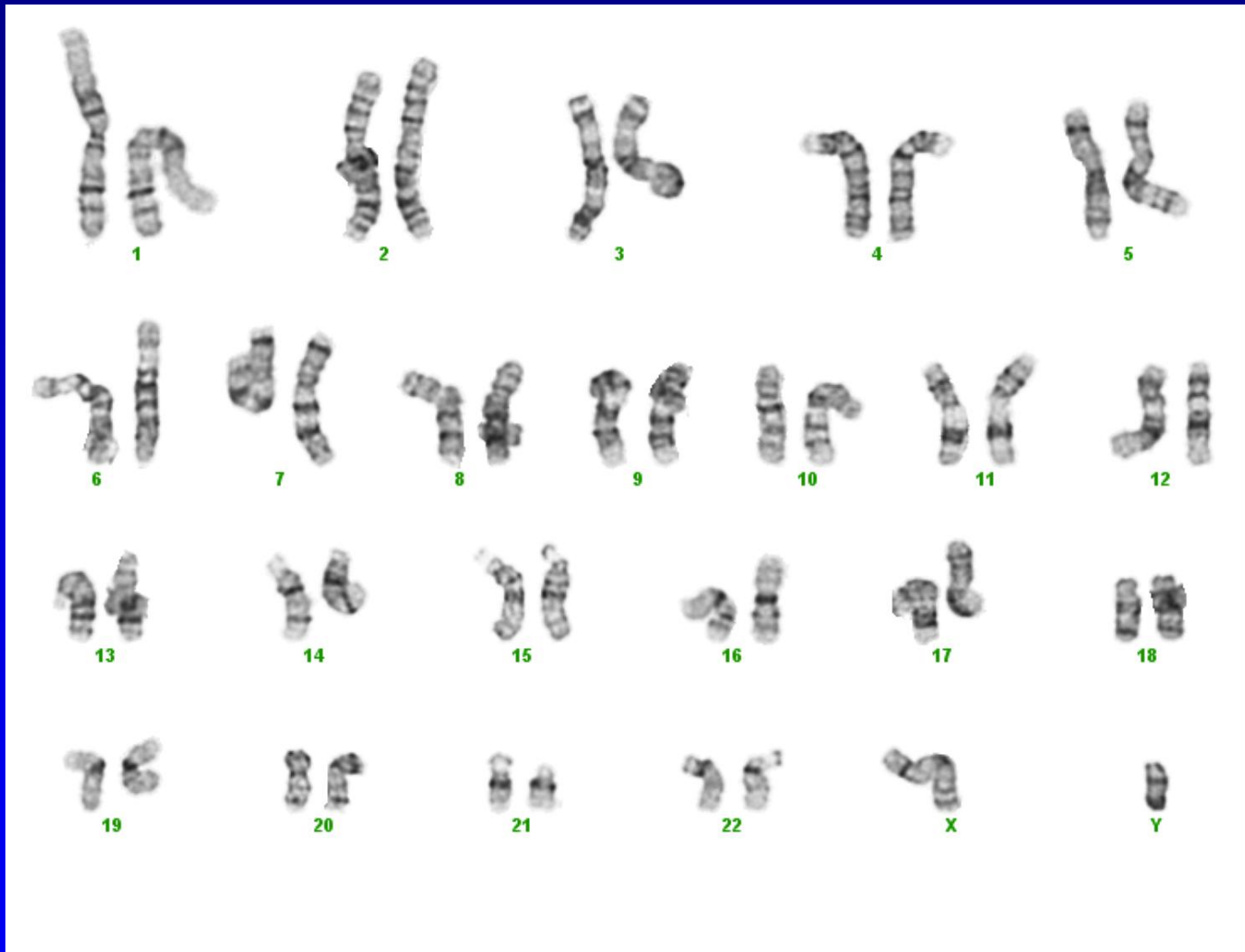


**Smooth Muscle and Gland**



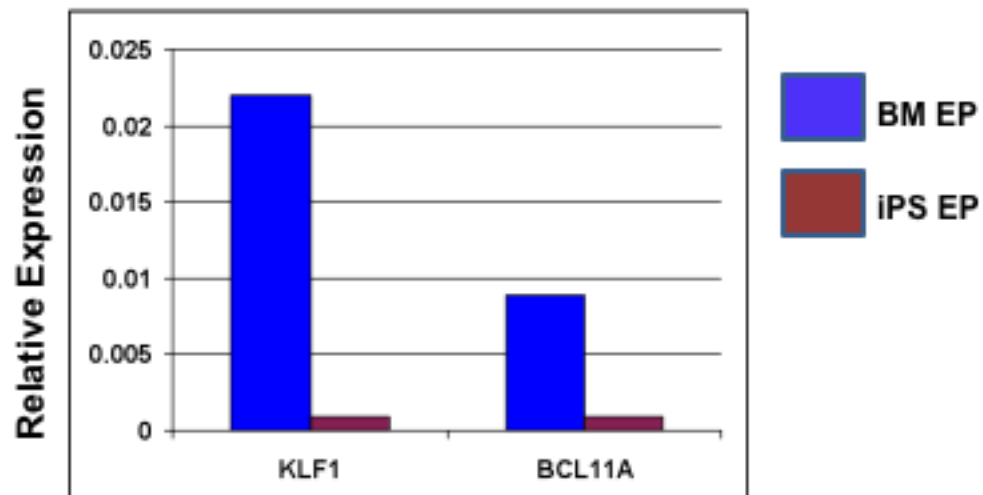
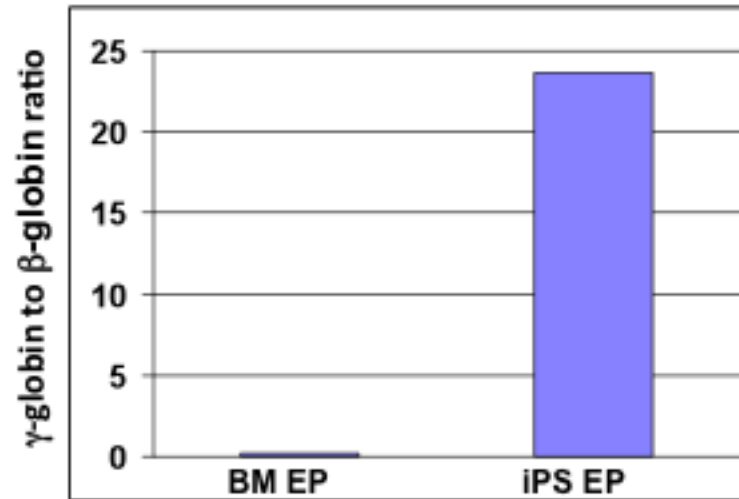
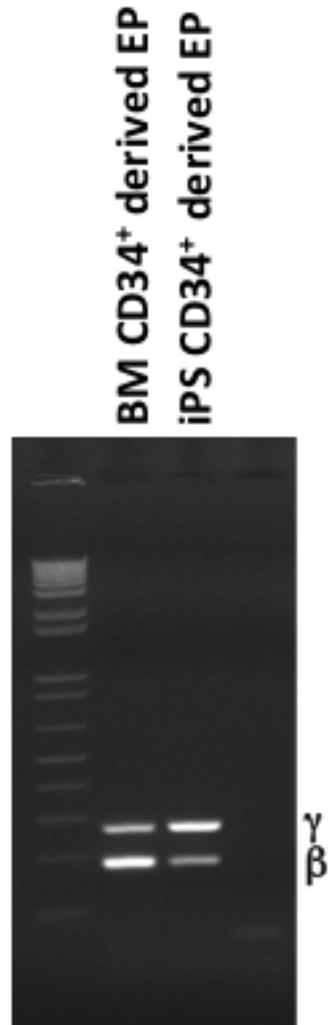
**Neural Ectoderm**

# Normal Karyotype of Corrected Human Sickle iPS Cells

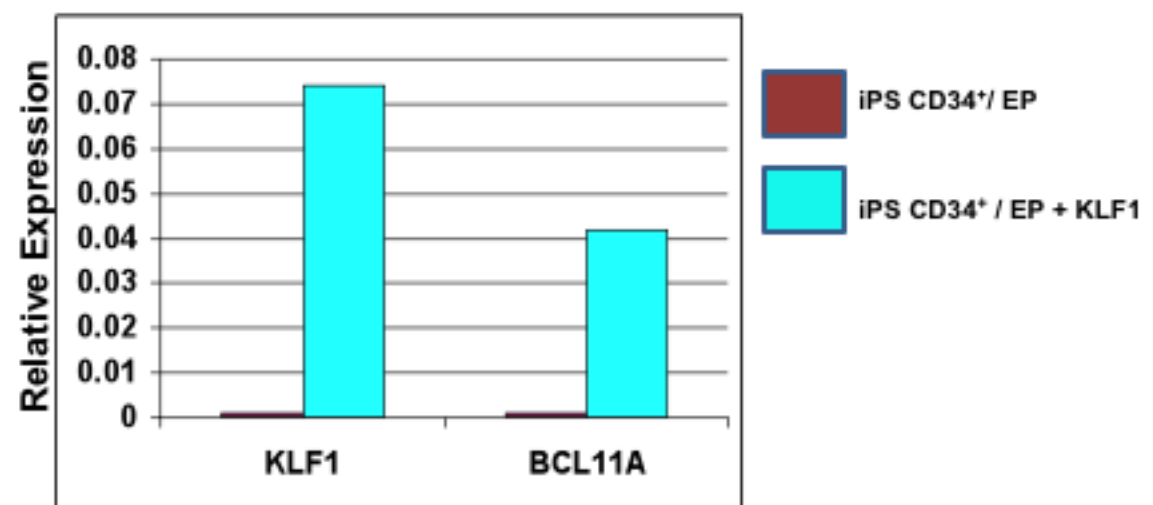
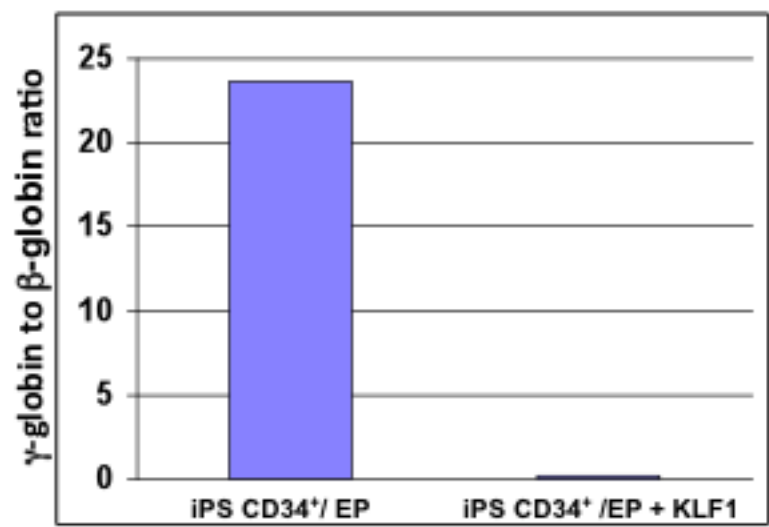
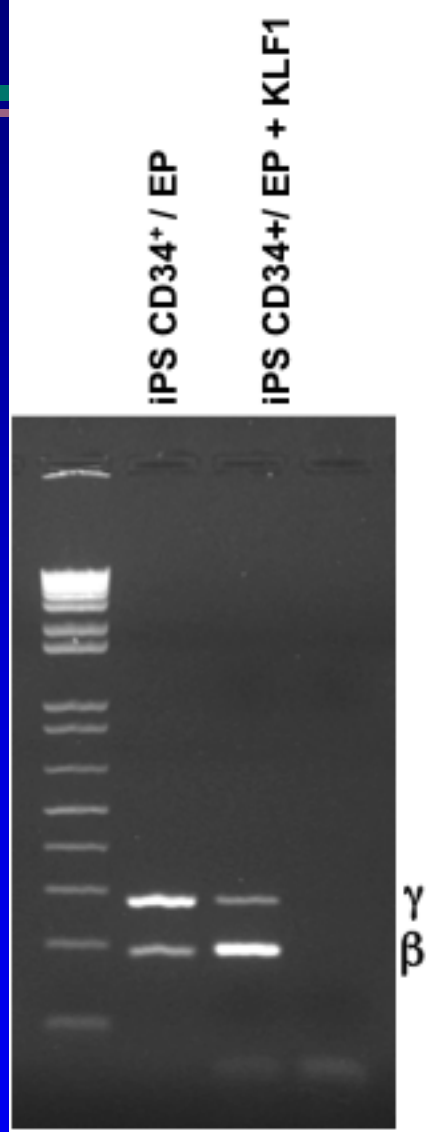




# KLF1 and BCL11A expression are low in human EP derived from iPSC



# KLF1 Expression in IPS Derived EP Switches Globins



# Gene Replacement Therapy for Sickle Cell Disease

**Sickle Patient**

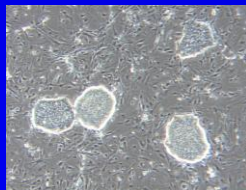
**Transplant corrected blood stem cells into patient**



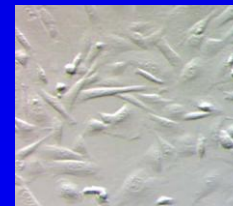
**Skin cell biopsy**



**Culture into blood stem cells**



**Correct Sickle Gene in patient fibroblasts**



**Derive iPS cells**

# 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