Chromatin accessibility mapping of primary erythroid cell populations leads to identification and validation of Nuclear Factor I X (NFIX) as a novel fetal hemoglobin (HbF) repressor

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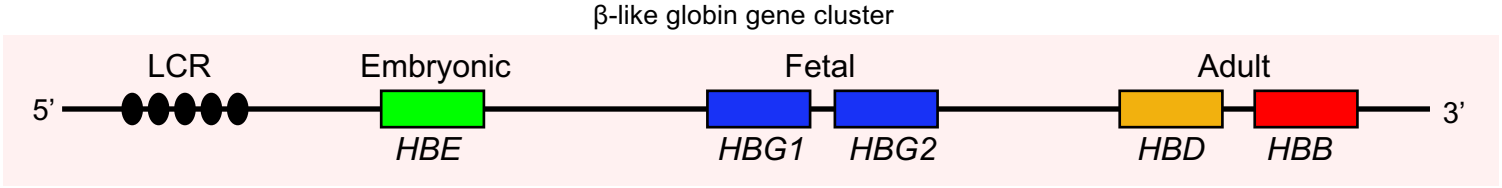
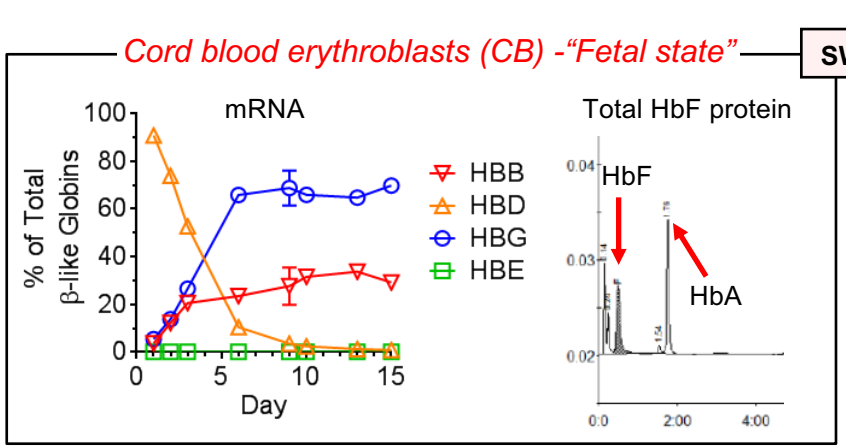
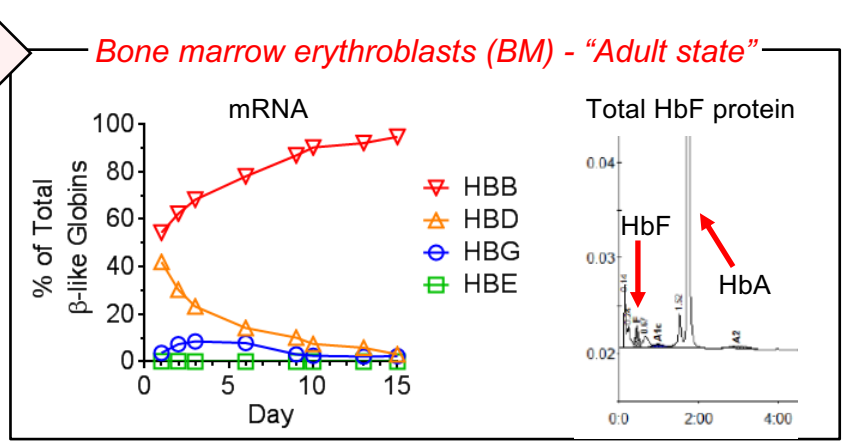
Using fetal and adult state erythroblasts to identify novel HbF repressors



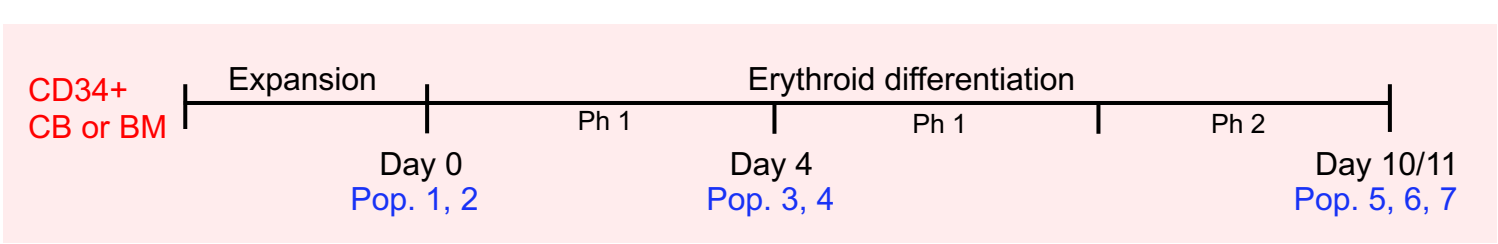


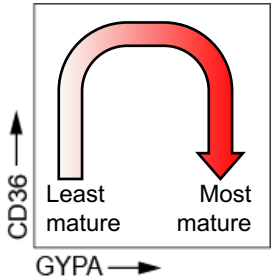
SWITCH



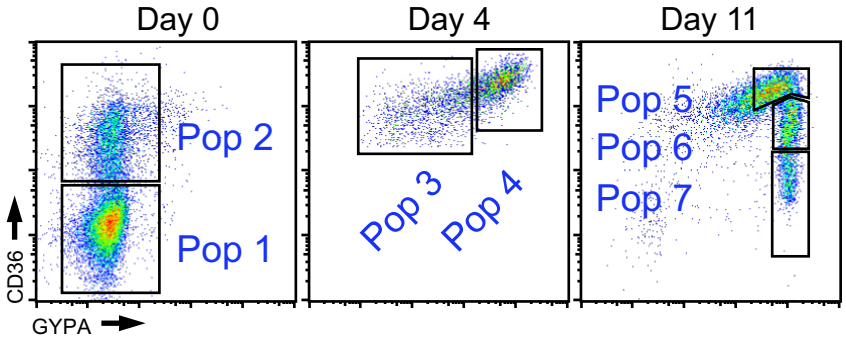
- Human beta-globin genes are developmentally regulated
- RNA-seq and ATAC-seq of BM and CB cells could identify lineage- and stage- specific repressors of HbF

FACS enables isolation of discrete cell populations for ATAC-seq and RNA-seq

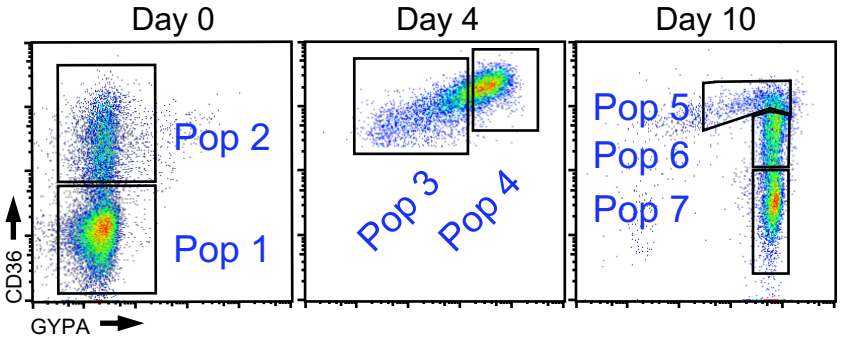




Cord blood erythroblasts (CB) - "Fetal state"

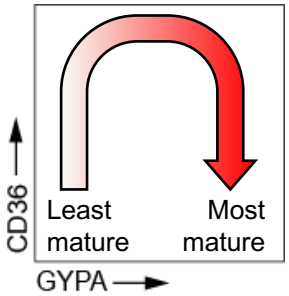


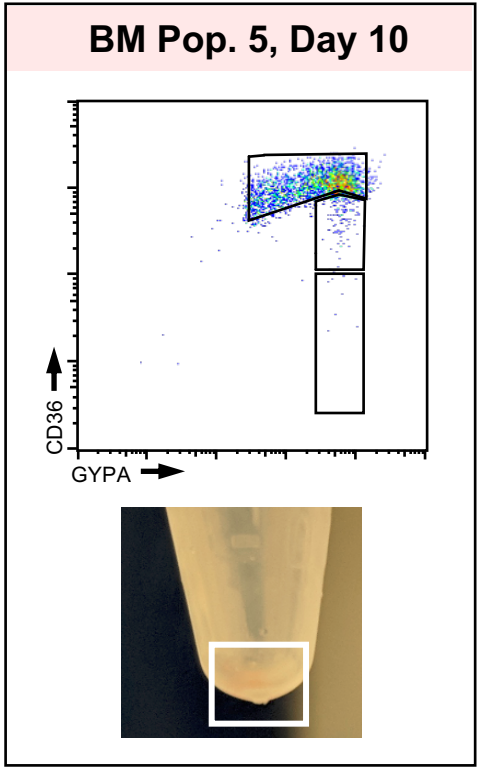
Bone marrow erythroblasts (BM) - "Adult state"

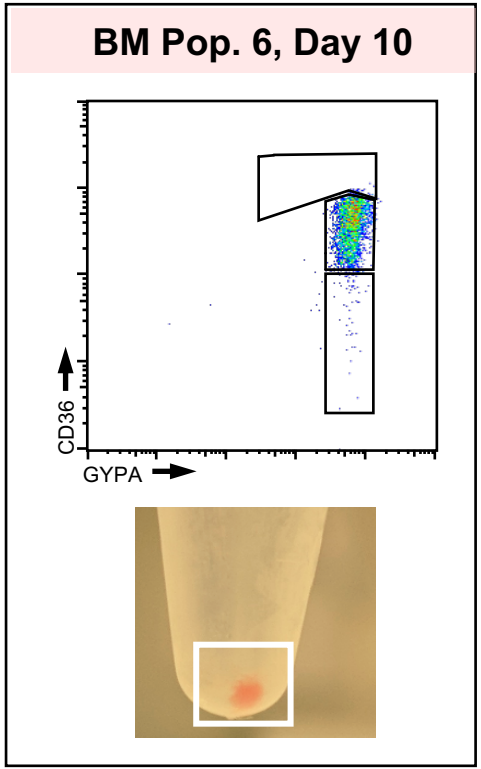


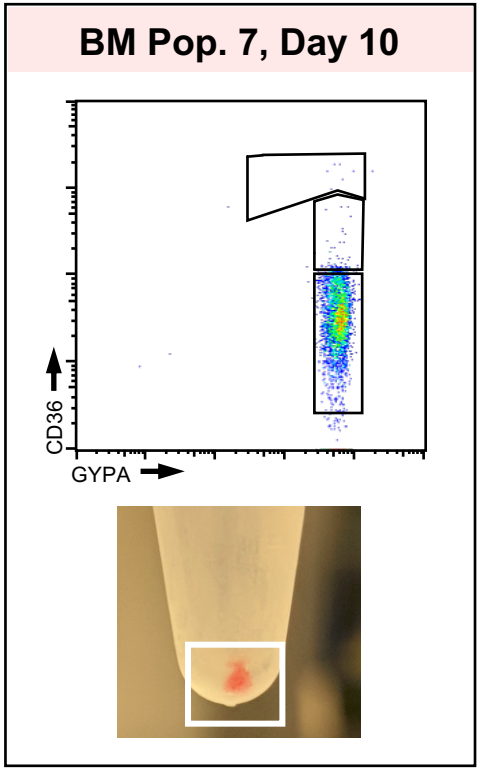
- We isolated 7 increasingly mature, stage-matched cell populations of fetal and adult state erythroblasts based on CD36 and GYPA expression

Sorted cells resolve maturational changes obscured in a pooled approach

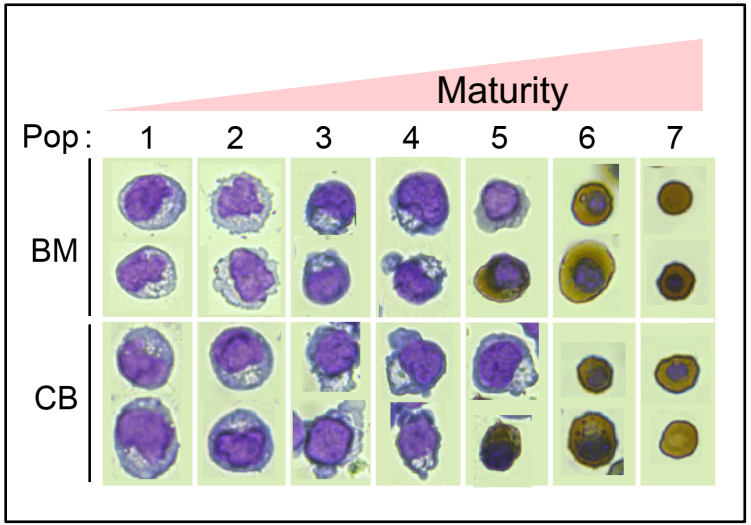


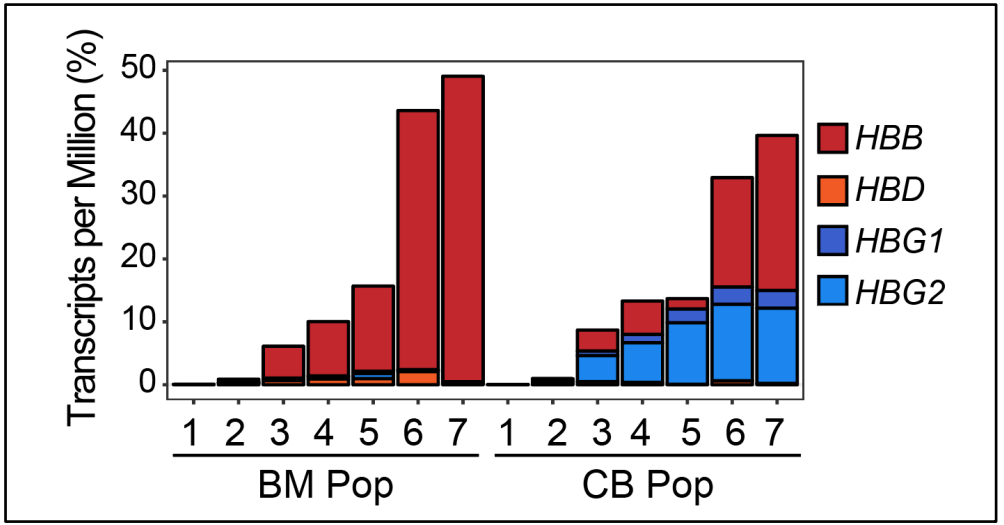






Sorted populations show expected differentiation and globin mRNA profiles





- Cytospin analyses confirmed the purity and differentiation state of each sorted population
- Total beta-like globin mRNA increased during erythroid maturation
  - *HBB* transcripts predominant in BM
  - *HBG* transcripts predominant in CB

BM and CB cells exhibit distinct patterns of chromatin accessibility at the β-like globin gene cluster

ATAC-seq

Assay for **T**ransposase-**A**ccessible **C**hromatin

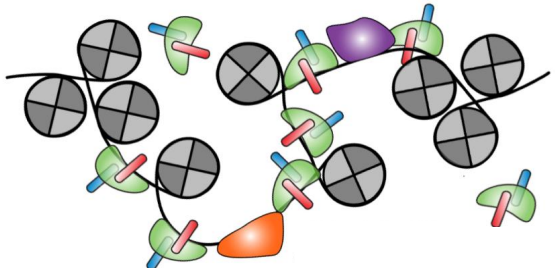
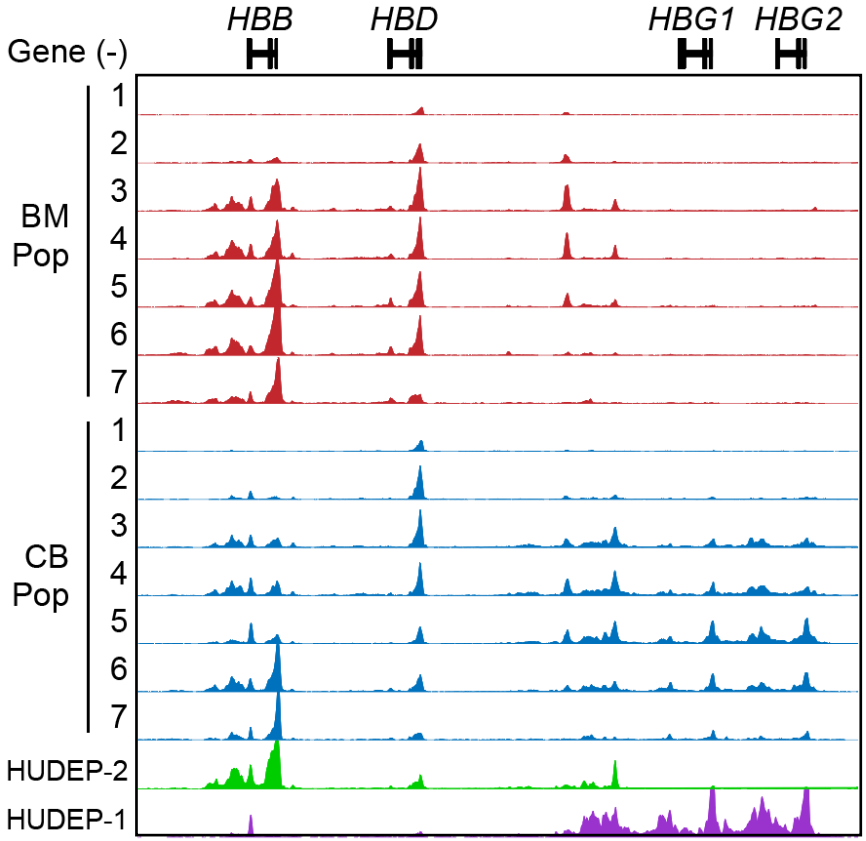


Image: Corces *et al.*, Science 2018



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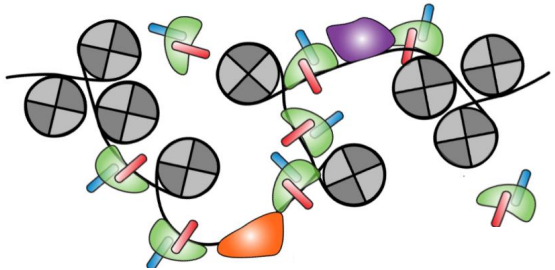

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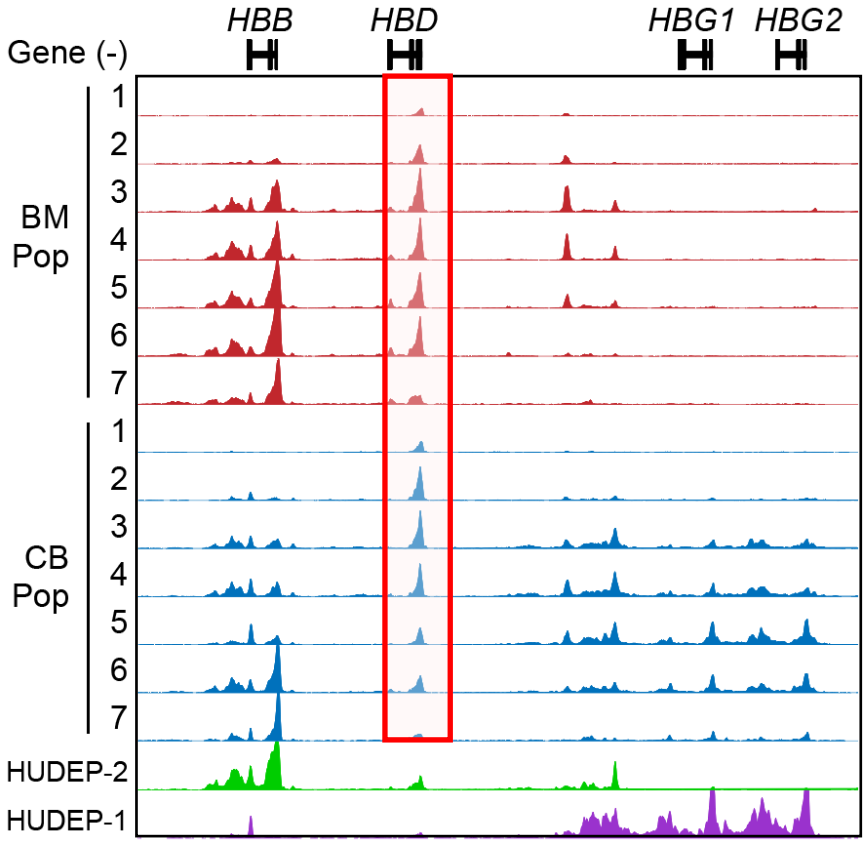
- Accessibility of the chromatin is:
  - initiated at the *HBD* promoter



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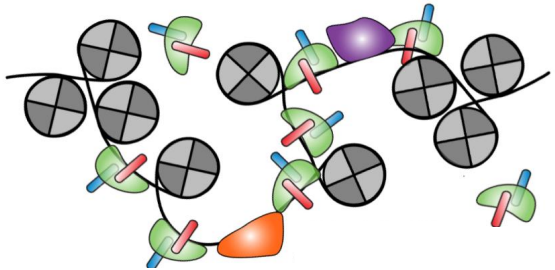
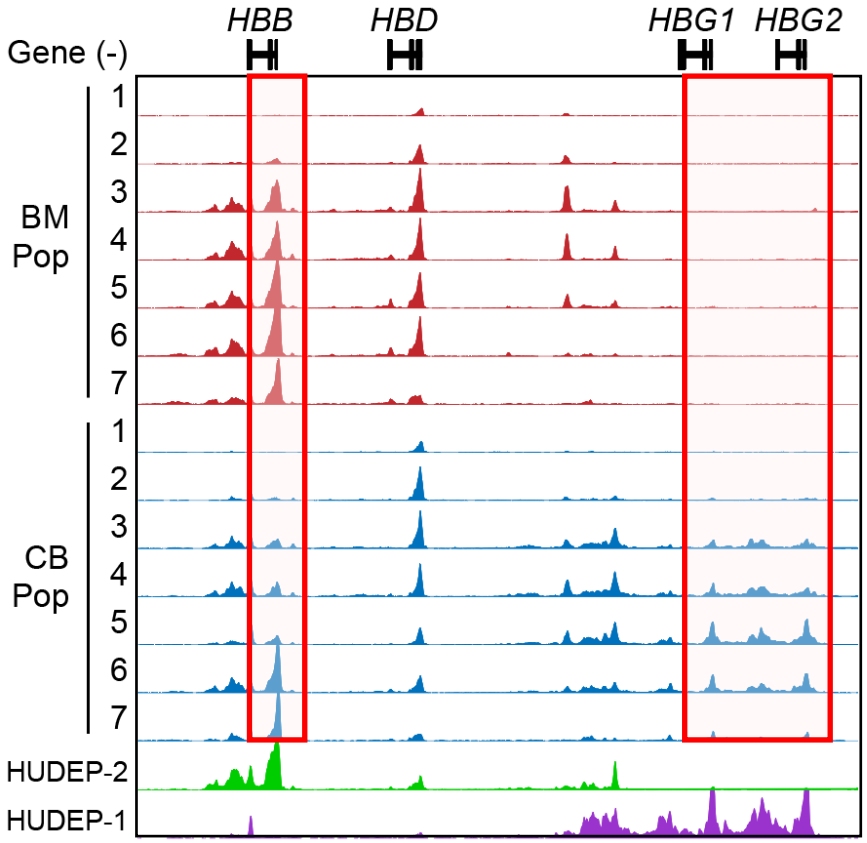


Image: Corces *et al.*, Science 2018

- Accessibility of the chromatin is:
  - initiated at the *HBD* promoter
  - greater at the *HBB* promoter in BM
  - greater at the *HBG* promoter in CB



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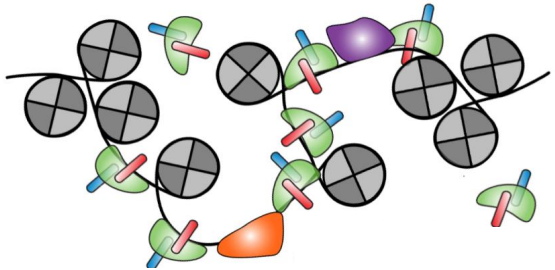

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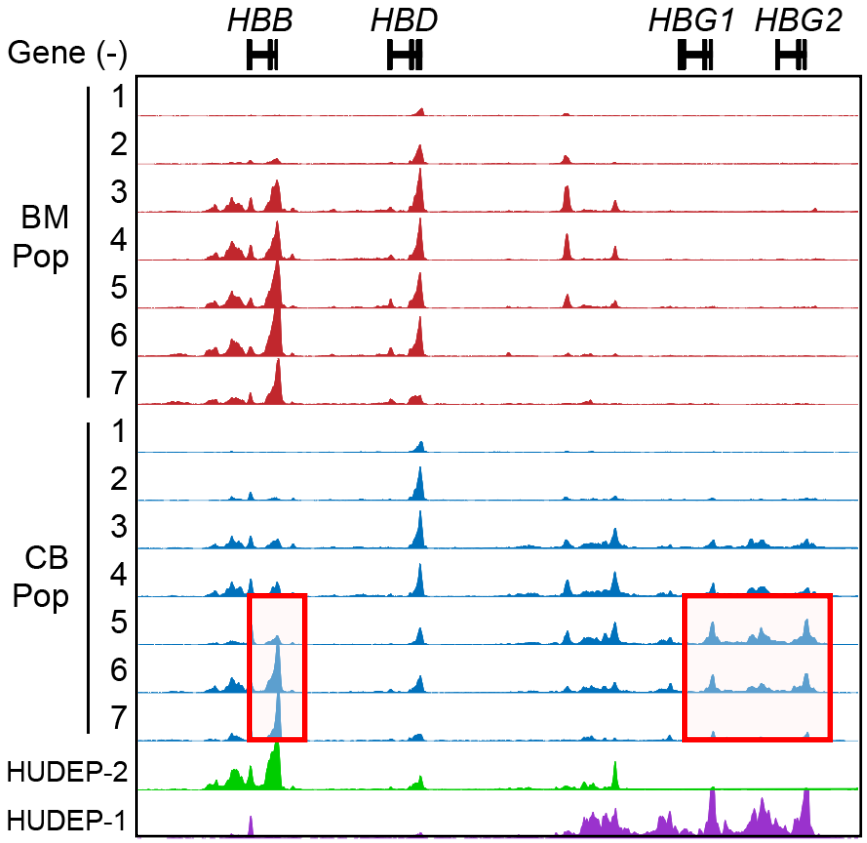
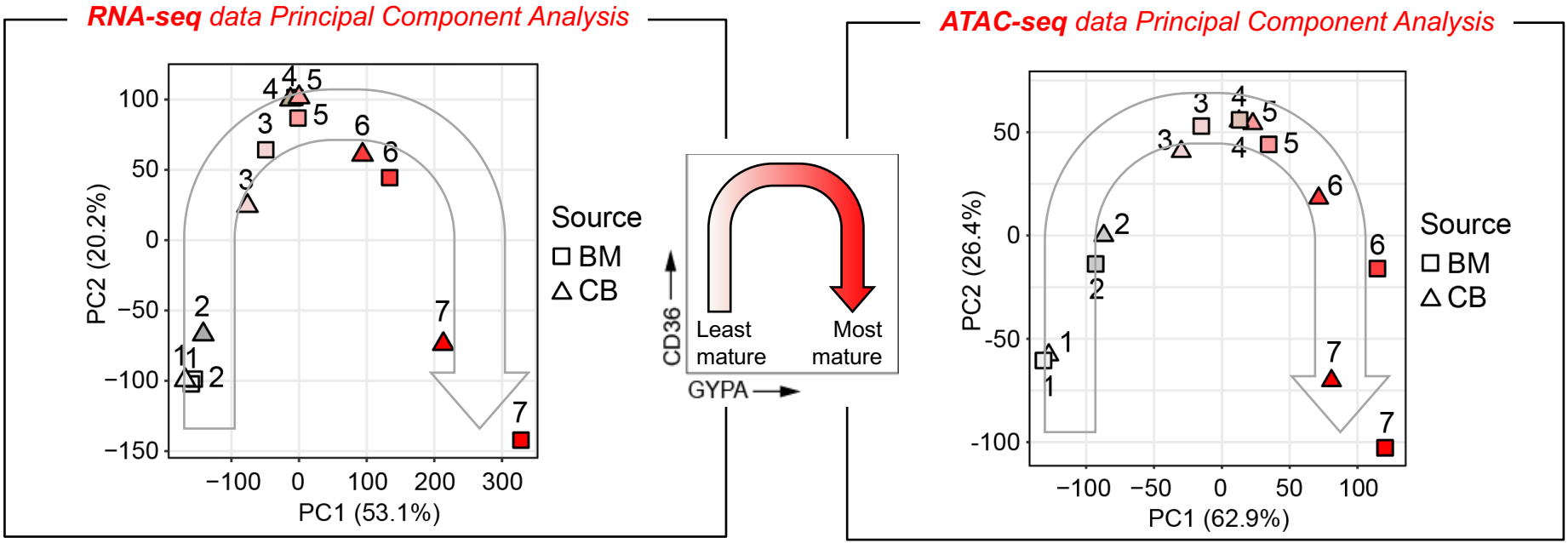
- Accessibility of the chromatin is:
  - initiated at the *HBD* promoter
  - greater at the *HBB* promoter in BM
  - greater at the *HBG* promoter in CB
- *HBG* promoter accessibility appears to be transient in CB



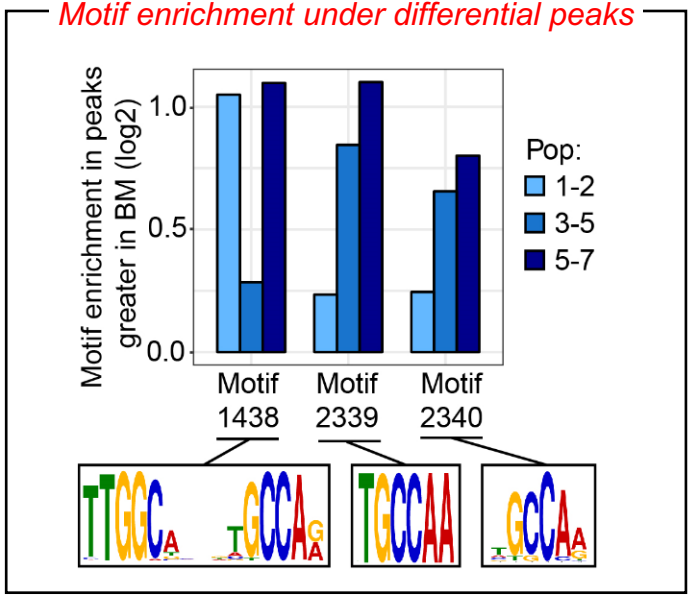
Majority of molecular changes during erythroid maturation are stage-specific

- Sorted populations cluster based on differentiation state rather than by BM or CB lineage, suggesting that most molecular changes are not lineage-specific



NFI factor motifs are enriched in BM erythroblasts

- NFI factor motifs were found to be enriched under ATAC-seq peaks that were larger in BM
  - Lessard *et al.*, Genome Med. 2015, found NFI motifs enriched in regions of differential DNA methylation in adult vs. fetal erythroblasts



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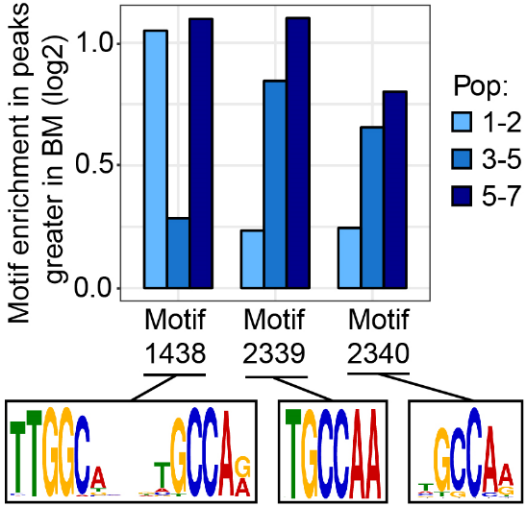
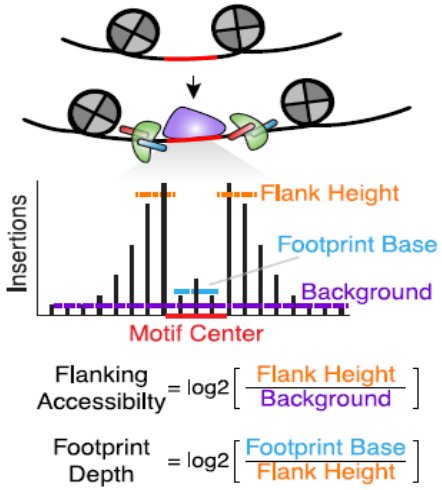
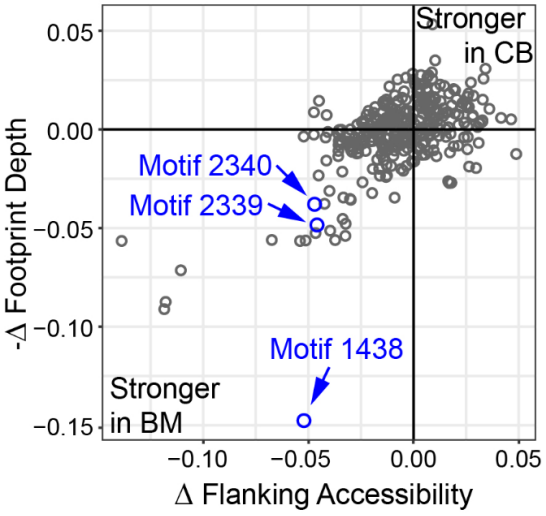
  - Lessard *et al.*, Genome Med. 2015, found NFI motifs enriched in regions of differential DNA methylation in adult vs. fetal erythroblasts
- Flanking accessibility and footprint depth at NFI motifs was also increased in BM

Motif enrichment under differential peaks



Transcription factor footprinting analysis



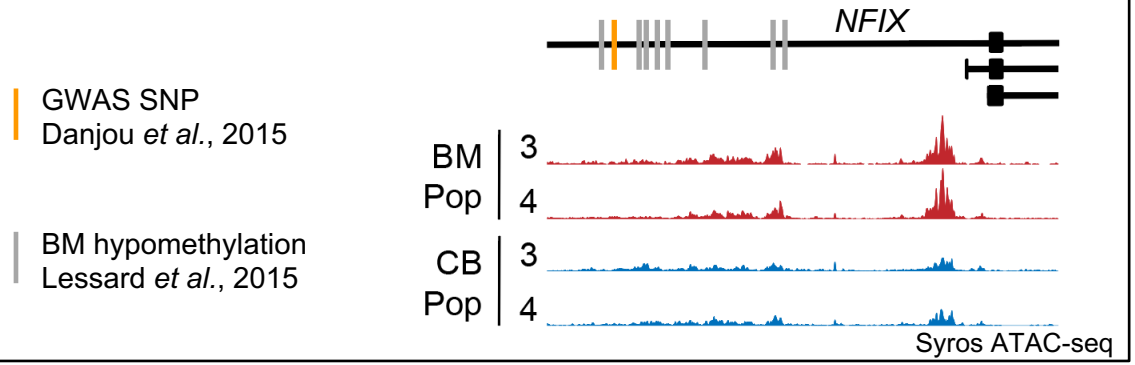


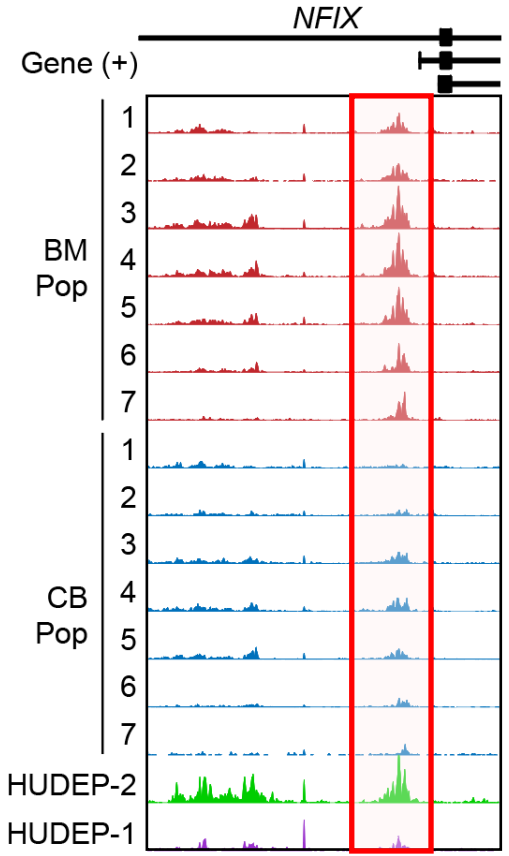
Corces *et al.*, Science 2018

NFIX promoter has increased chromatin accessibility in BM cells

- A region of increased chromatin accessibility at the *NFIX* promoter was observed in BM relative to CB cell population
  - Danjou *et al*., Nat. Genet. 2015 identified a SNP linked to HbF level near this promoter region
    - *p*-value 1.6 x 10^{-8}
  - Lessard *et al*., Genome Med. 2015, showed differentially methylated CpG in adult vs. fetal cells near this promoter region

ATAC-seq peak, GWAS SNP & meCpGs mapped to NFIX promoter

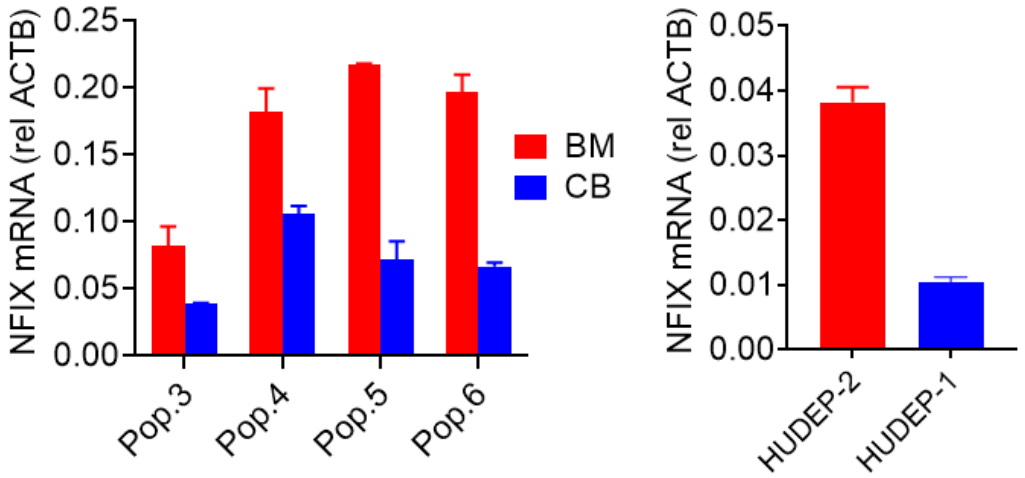




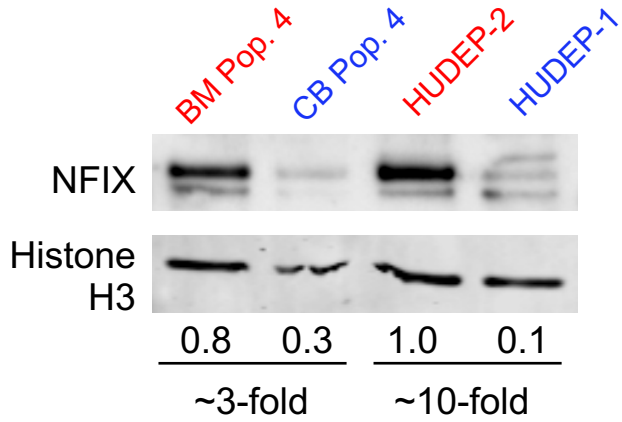
NFIX is differentially expressed in fetal and adult cell types

- Higher levels of *NFIX* transcript and protein in adult globin expressing cells (BM, HUDEP-2) versus fetal globin expressing cells (CB, HUDEP-1)

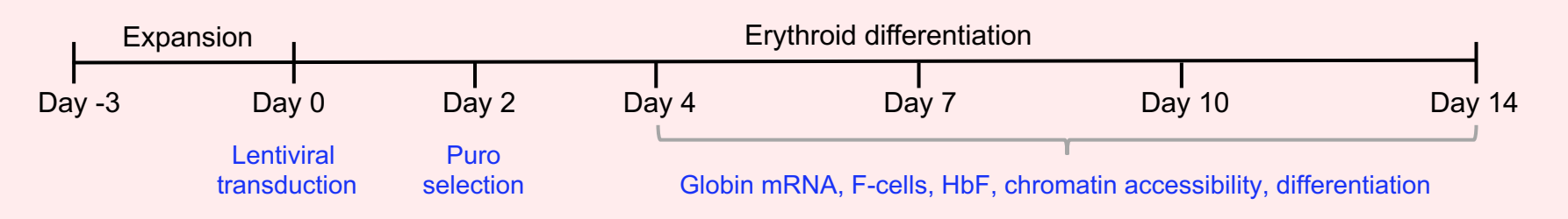
NFIX mRNA – adult and fetal cells

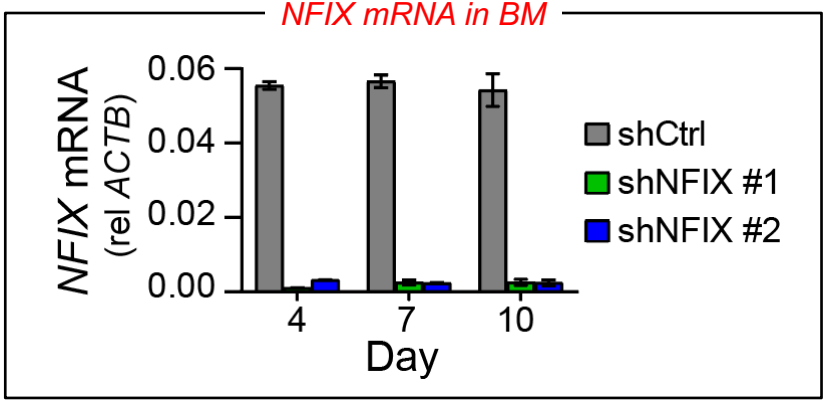


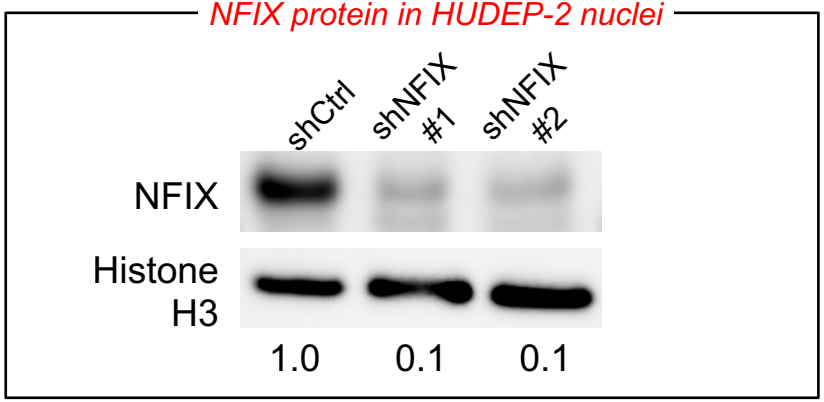
NFIX protein – adult and fetal nuclei



RNAi leads to robust knockdown (KD) of NFIX in BM and HUDEP-2 cells



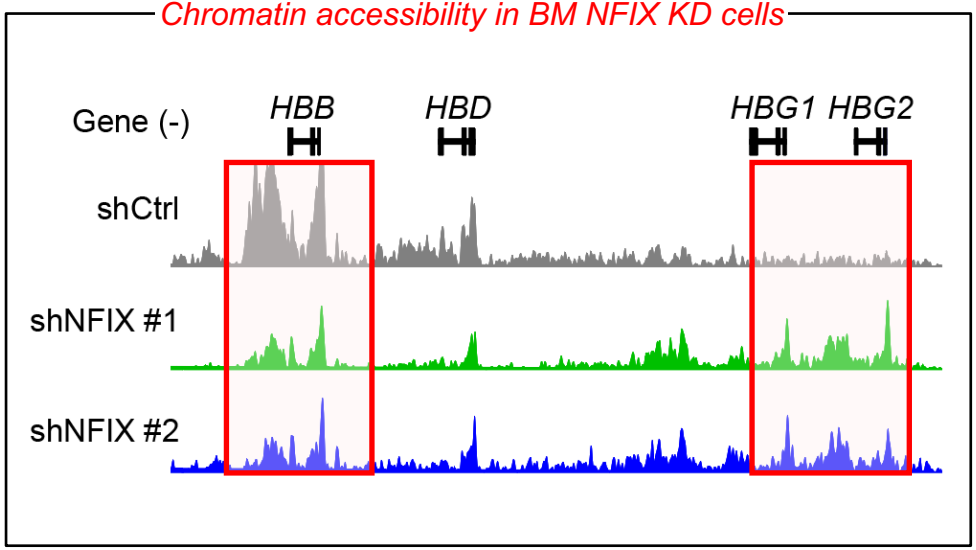




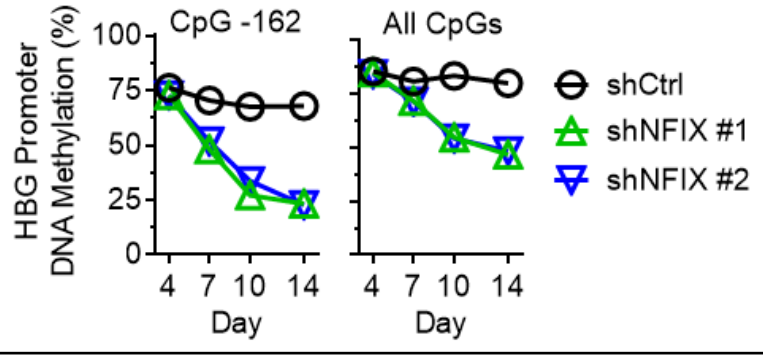
- RNAi knockdown of NFIX leads to >90% reduction in *NFIX* transcripts
- RNAi knockdown of NFIX leads to 90% reduction of NFIX protein

NFIX KD leads to functional changes at the *HBG* promoter

Chromatin accessibility in BM NFIX KD cells



DNA methylation in BM NFIX KD cells



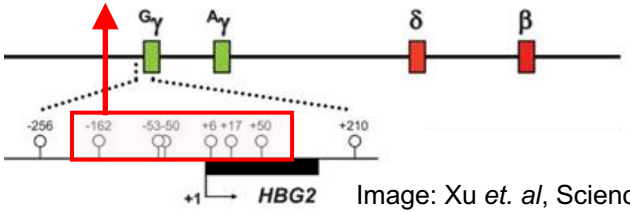
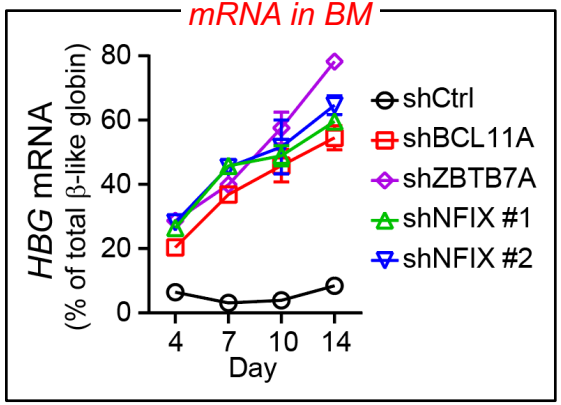
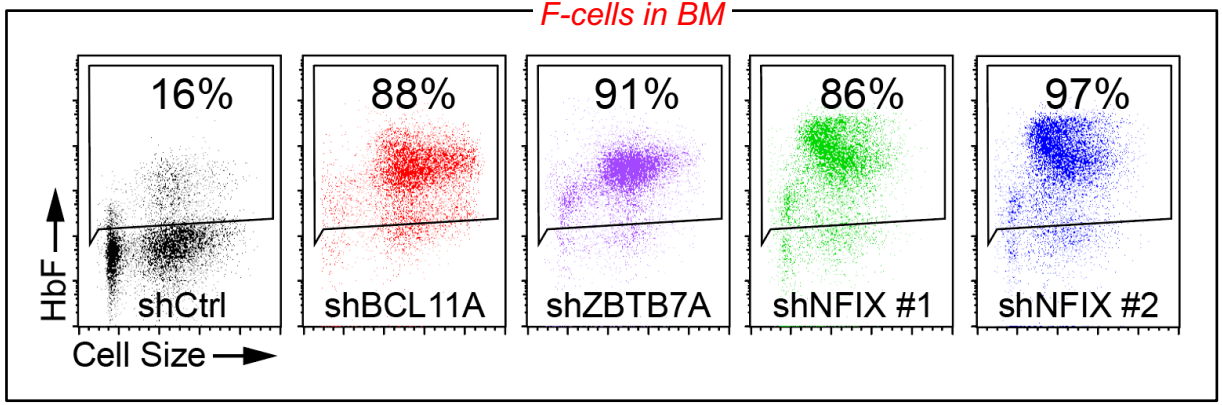


Image: Xu *et. al*, Science, 2011

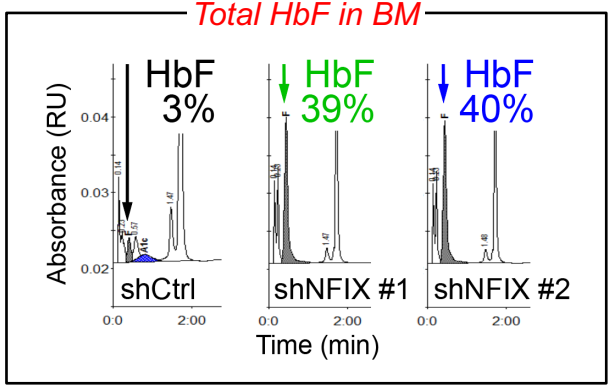
- NFIX knockdown leads to an increase in chromatin accessibility at the *HBG* promoter
- NFIX knockdown leads to a decrease in DNA methylation at the *HBG* promoter

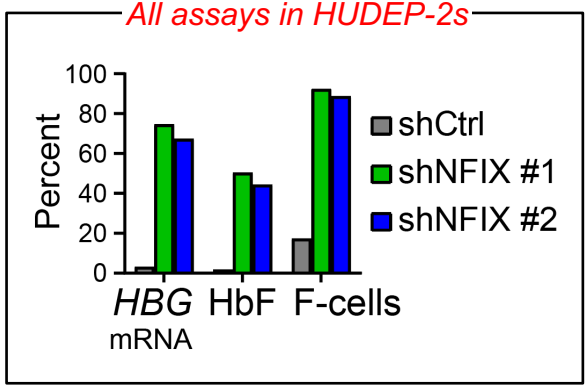
NFIX KD leads to elevated *HBG* mRNA and HbF protein levels



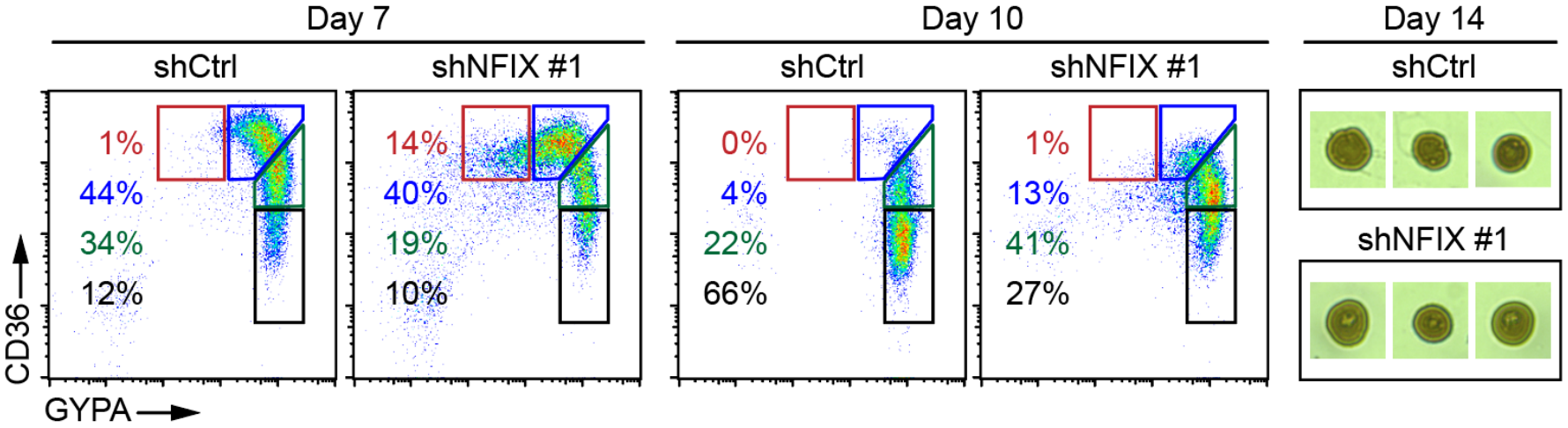


- KD of NFIX in BM or HUDEP-2 cells induces fetal hemoglobin





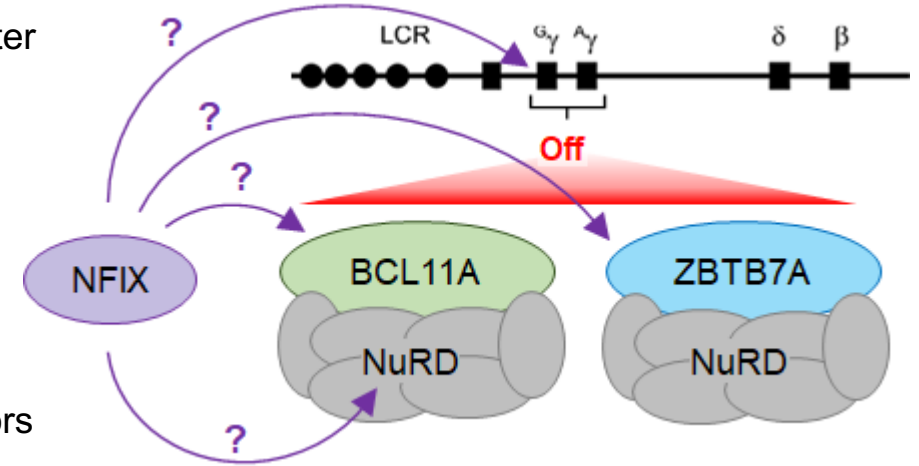
NFIX KD cells are capable of terminal erythroid differentiation



- NFIX KD cells exhibit a delay in erythroid differentiation during early stages of culture
- Despite the maturation delay, NFIX KD cells can differentiate into enucleated RBCs

Summary and next steps

- Performed ATAC-seq on discrete erythroblast populations from adult and fetal state cells
- NFI motifs were enriched under adult versus fetal state ATAC-seq peaks
- NFIX promoter accessibility, mRNA, and protein is increased in adult versus fetal cell types
- NFIX knockdown leads to:
  - Increased chromatin accessibility at *HBG* promoter
  - Elevated *HBG* mRNA and HbF protein
- Future work will investigate if NFIX:
  - Binds directly at the *HBG* promoter
  - Interacts with previously described HbF repressors



Acknowledgements - *Syros SCD Program Team*

NFIX co-authors

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