BACKGROUND/AIMS:
The human β-globin genes are expressed in a developmental stage-specific manner and regulated by many cis- and trans-regulatory components including a locus control region (LCR) and proximal promoter and enhancer elements. The two γ-globin genes, Gγ and Aγ, are expressed in the fetal period. Persistent expression of γ-globin in the adult ameliorates the symptoms associated with mutations of the adult β-globin gene, such as sickle cell disease or β-thalassemia. Thus, understanding the mechanisms by which the fetal globin genes are activated and silenced during development may lead to new avenues for the treatment of hemoglobinopathies.

METHODS:
Bioinformatics, EMSA, Western Blotting, RT-PCR, ChIP

RESULTS & CONCLUSIONS
Using data from the human ENCODE project, we identified a DNase I hypersensitive site located 4 Kbp upstream of the Gγ-globin gene (Gγ -4Kb DHS) in the K562 cell line which expresses high levels of the γ-globin genes. The Gγ -4Kb DHS is characterized by the presence of histone modifications typical for enhancer elements (H3K4 monomethylation and H3K27 acetylation) and binding of hematopoietic (NF-E2) and ubiquitous transcription factors (USFs, E2Fs, YY1, Egr1 etc.).

We expressed and purified a ZF-DBD specifically targeting the Gγ -4Kb DHS. The target site for the ZF-DBD overlaps with a CCCAC Egr1 motif and is close to an E-box sequence, which is predicted to bind USF transcription factors. We analyzed the binding of the ZF-DBD to the target DNA sequence using electrophoretic mobility shift assays (EMSAs). The ZF-DBD showed 3-fold more efficiency in binding affinity (Kd) to the on-target site than to the off-target site. We delivered the ZF-DBD into K562 cells in a temporal manner (6hr, 12hr, 18hr and 24hr). Delivery of the ZF-DBD led to a significant reduction in expression of the γ-globin genes but had no effect on expression of control genes, such as GAPDH, CTCF, GATA-1 and Brg1 etc. Targeting of the ZF-DBD to Gγ -4Kb DHS reduced the enrichment of H3K4 monomethylation and H3K27 acetylation at the Gγ -4Kb DHS and thus changed the epigenetic landscape of the β-globin gene locus in human erythroleukemia K562 cells. Therefore, the Gγ -4Kb DHS contributes to high-level γ-globin gene expression in K562 cells.