

Supplementary Materials

Full Title: Humanized Mouse Models of Cooley's Anemia: Correct Fetal to Adult Hemoglobin Switching, Disease Onset, and Disease Pathology

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## Material and Methods

### Vectors and mice

Gene targeting vectors were cloned as previously described.(1, 2) The -117 G to A mutation were introduced into the  $\gamma$  globin gene promoter by PCR mutagenesis. Correctly targeted human globin gene KI ES cell lines were injected into 8 cell stage embryos to produce mice completely derived from the heterozygous KI ES cells. Heterozygous human  $\alpha$  and  $\beta$ -like globin KI mice were bred together to make homozygous CA mice. Hematology and histology analyses were conducted as previously reported.(1, 2) Transfusion of CA mice was performed as previously described.(1)

### Fetal hemoglobin staining

Mouse blood was collected into PBS with 5mM of EDTA. Two microliters ( $\mu$ l) of blood were washed twice in PBS and fixed in 100  $\mu$ l of 0.05% freshly prepared glutaraldehyde. 100  $\mu$ l of 0.1% Triton X-100 was used to permeabilize the fixed cells. Phycoerythrin conjugated anti-human fetal hemoglobin antibody (BD Pharmingen, NJ) was used to stain fetal hemoglobin according to the manufacture's instruction.

## References

1. Huo, Y., McConnell, S.C., and Ryan, T.M. 2009. Preclinical transfusion-dependent humanized mouse model of beta thalassemia major. *Blood* 113:4763-4770.
2. Huo, Y., McConnell, S.M., Liu, S.-R., Yang, R., Zhang, T.-T., Sun, C.-W., Wu, L.-C., and Ryan, T.M. 2008. Humanized mouse model of Cooley's Anemia. *J. Biol. Chem.*