Systematic Functional Dissection of Common Genetic Variation Affecting Red Blood Cell Traits

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- MPRA : massively parallel reporter assay

  大规模报告基因实验
- MFVs: MPRA functional variants
- ACs : constructs activity
- HSPCs : CD34+ hematopoietic stem and progenitor cells CD34+造血干细胞和祖细胞
- HEPs : human erythroid progenitors/precursors

  人红细胞祖细胞/前体
- MCHC : 红细胞平均血红蛋白浓度

- Designing an MPRA to Screen GWAS Variants.
- Identifies Endogenous Regulatory Elements and MFV.
- Involvement of a GATA1 TF Complex at MFV.
- Isogenic Deletions Identify Multiple Target Genes at Each Locus.
- Altered TF Binding as a Putative Mechanism of Action.
- RBM38 Is a Key Regulator of Alternative Splicing in Terminal Human Erythropoiesis.

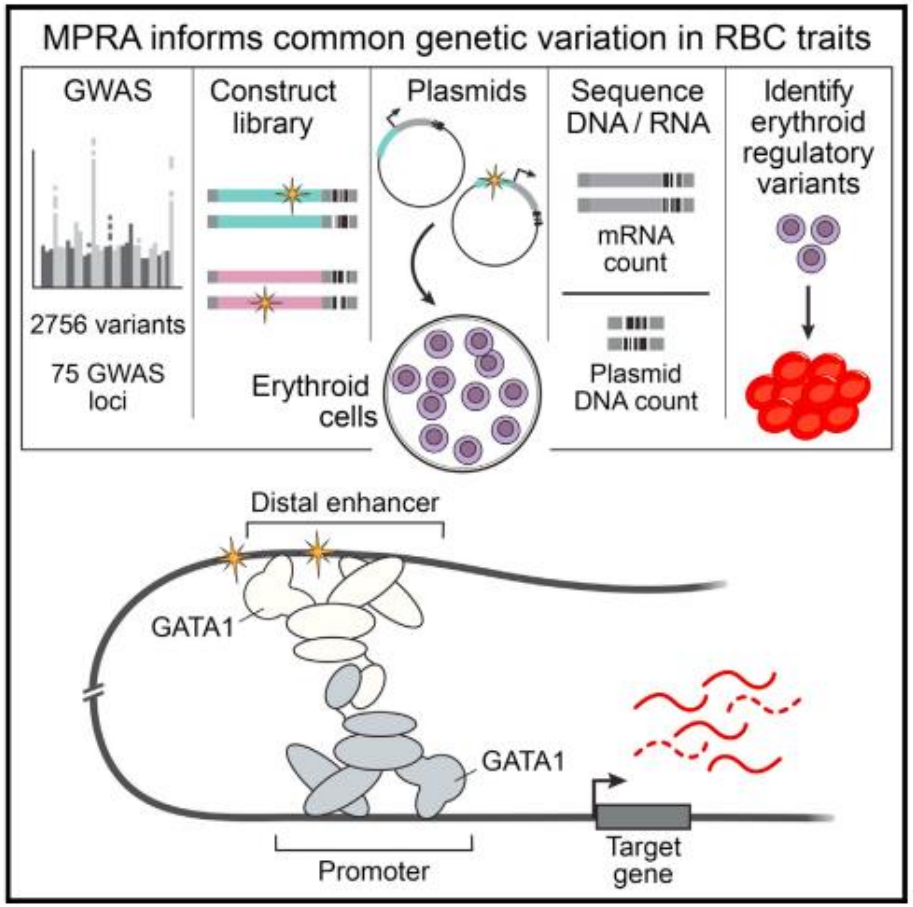
Materials

Our analyses suggested that these variants are enriched for both earlier-stage (CD71+) and later-stage (GlyA+) HEPs.

Upon overexpression of GATA1, we were able to induce a more terminal erythroid gene signature.

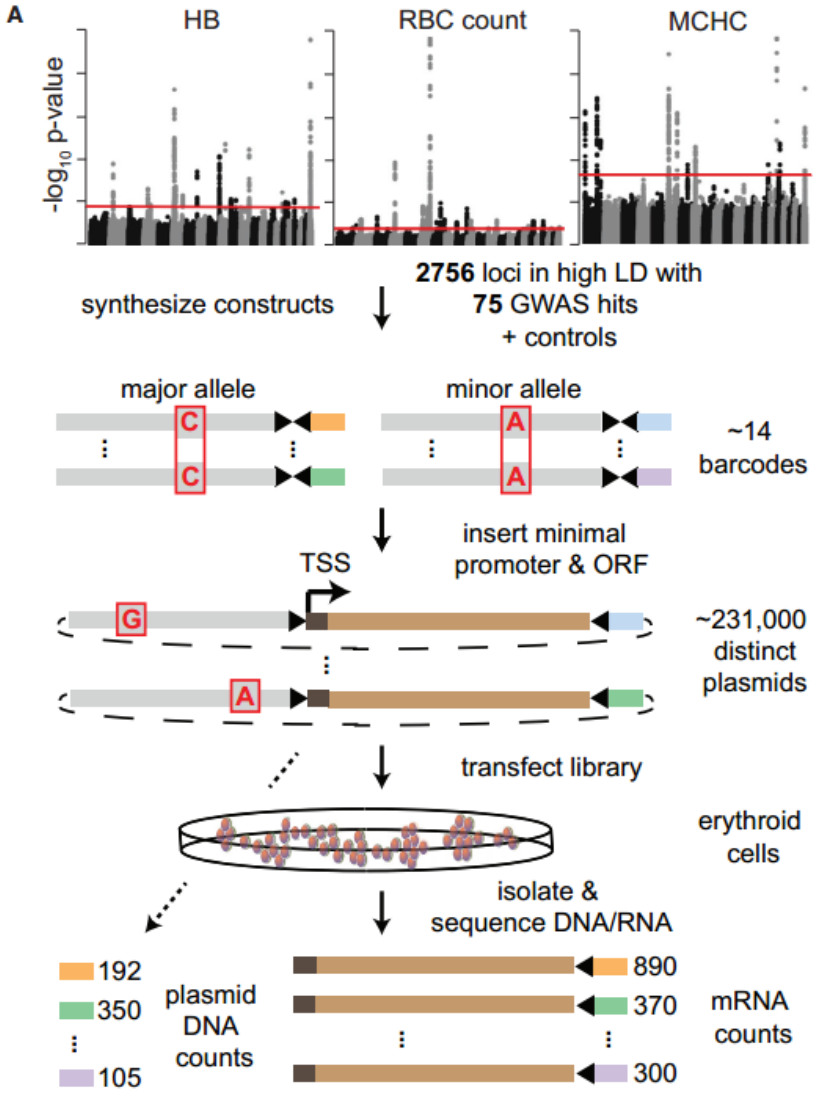
We performed our MPRA in both standard K562 cells, resembling an earlier HEP, and K562+GATA1 cells, resembling a more differentiated HEP.

Graphical Abstract

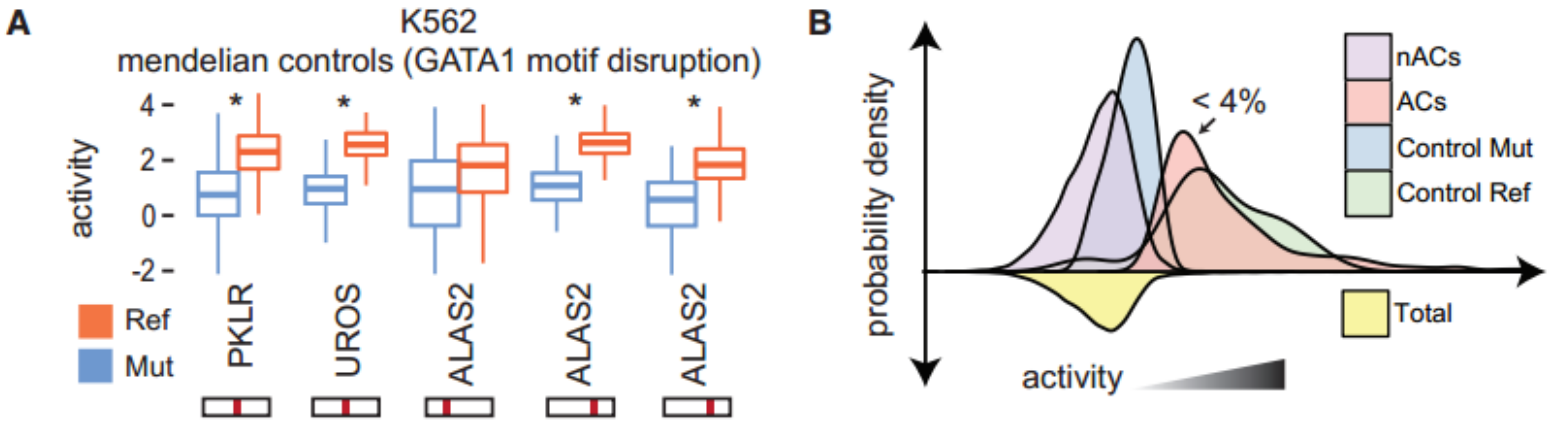


MPRA

- Synthesized constructs
- Constructs were ligated into a plasmid backbone
- The library was transfected into an erythroid cell line
- An mRNA/DNA ratio across each barcode represents the activity for the tagged construct



Identifies Endogenous Regulatory Elements and MPRA Functional Variants



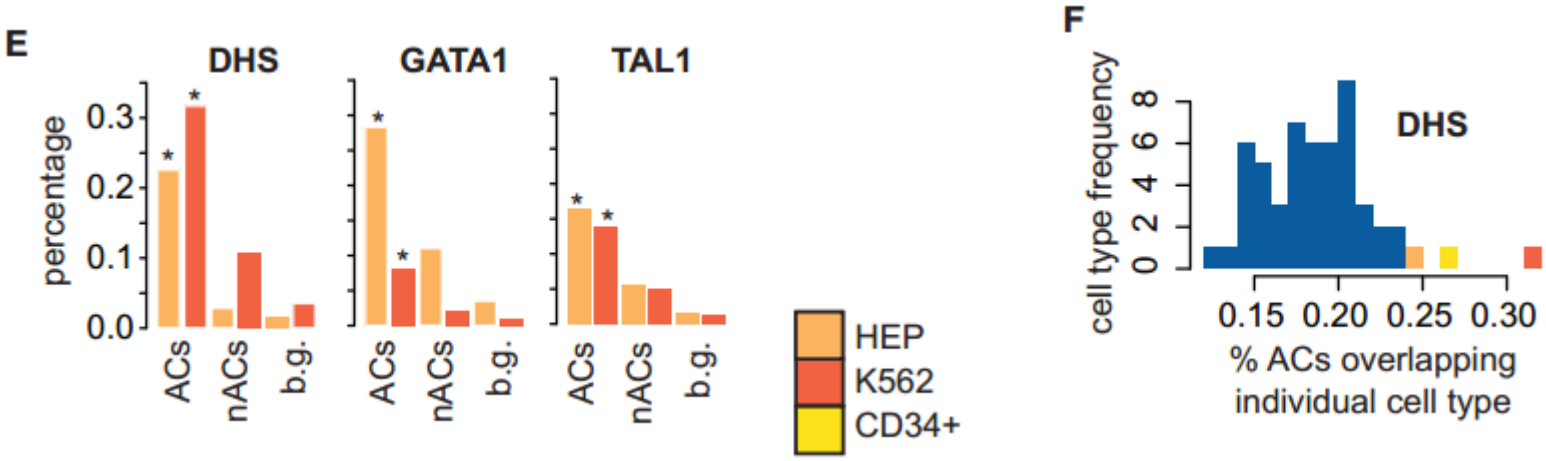
A: activity boxplots of the five unique positive control variants.

B: ACs show a similar activity distribution to non-mutated controls

Ref : constructs with intact GATA1-binding sites.

Mut : constructs with broken binding sites.

Ref showed strong enhancer-like activity.

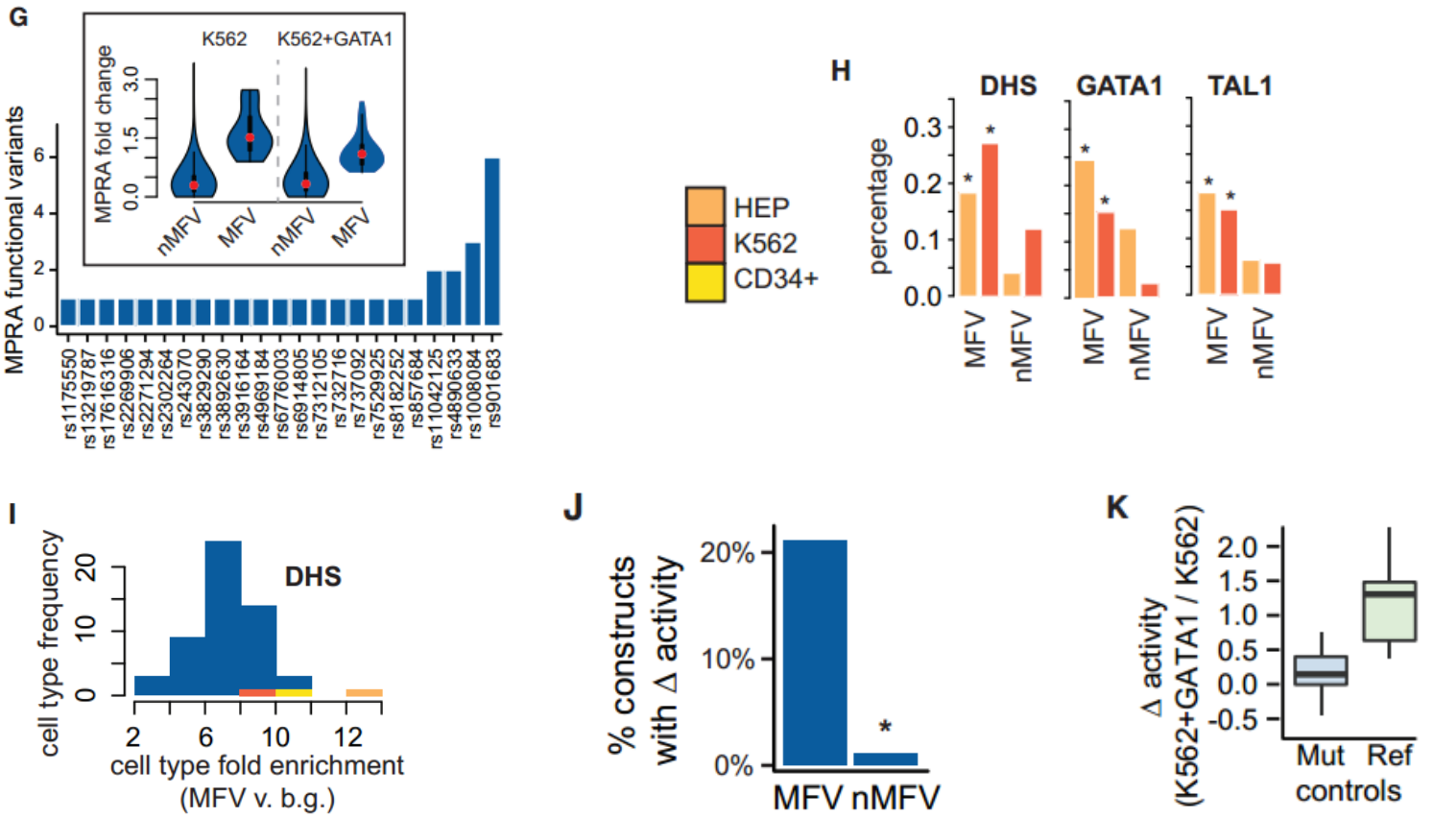


E : ACs are enriched for erythroid DHS and occupancy sites of the erythroid TFs, GATA1 and TAL1.

F : ACs showed the greatest overlap with erythroid cell types.

MPRA can correctly identify regulatory elements that exhibit activity in the endogenous genomic setting and suggest that differences in MPRA activity likely represent changes in binding or activity of cell-type-specific TFs.

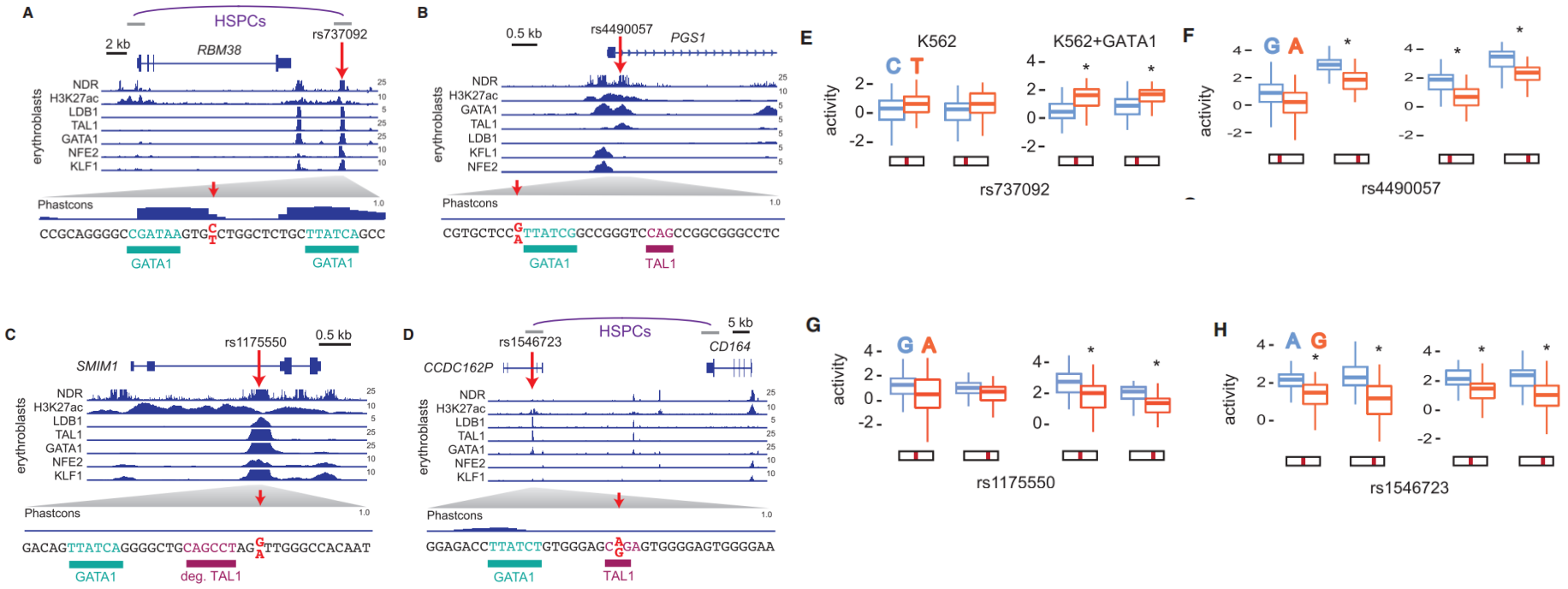
MPRA Functional Variants



J : the group of MFVs was significantly enriched for constructs that exhibit a dosage-dependent response to GATA1

K : the Mendelian constructs with mutated GATA1 motifs do not change in activity with increased GATA1 expression

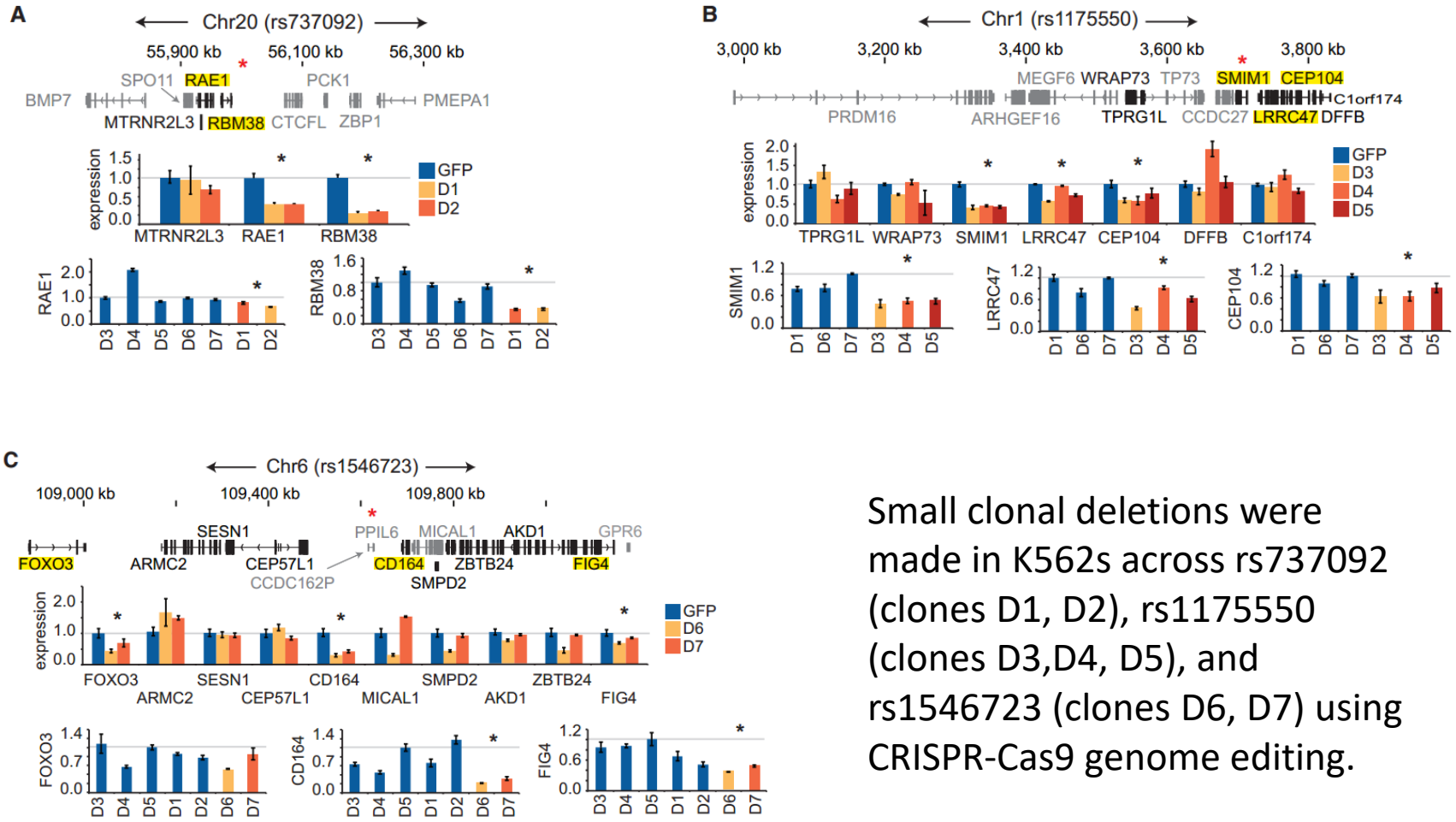
Involvement of a GATA1 Complex at MFV



Predicted TF-binding sites are highlighted proximal to the MFV. (A and D) Interactions between a promoter and HindIII fragment identified from promoter capture Hi-C in CD34+ hematopoietic stem and progenitor cells (HSPCs) are shown.

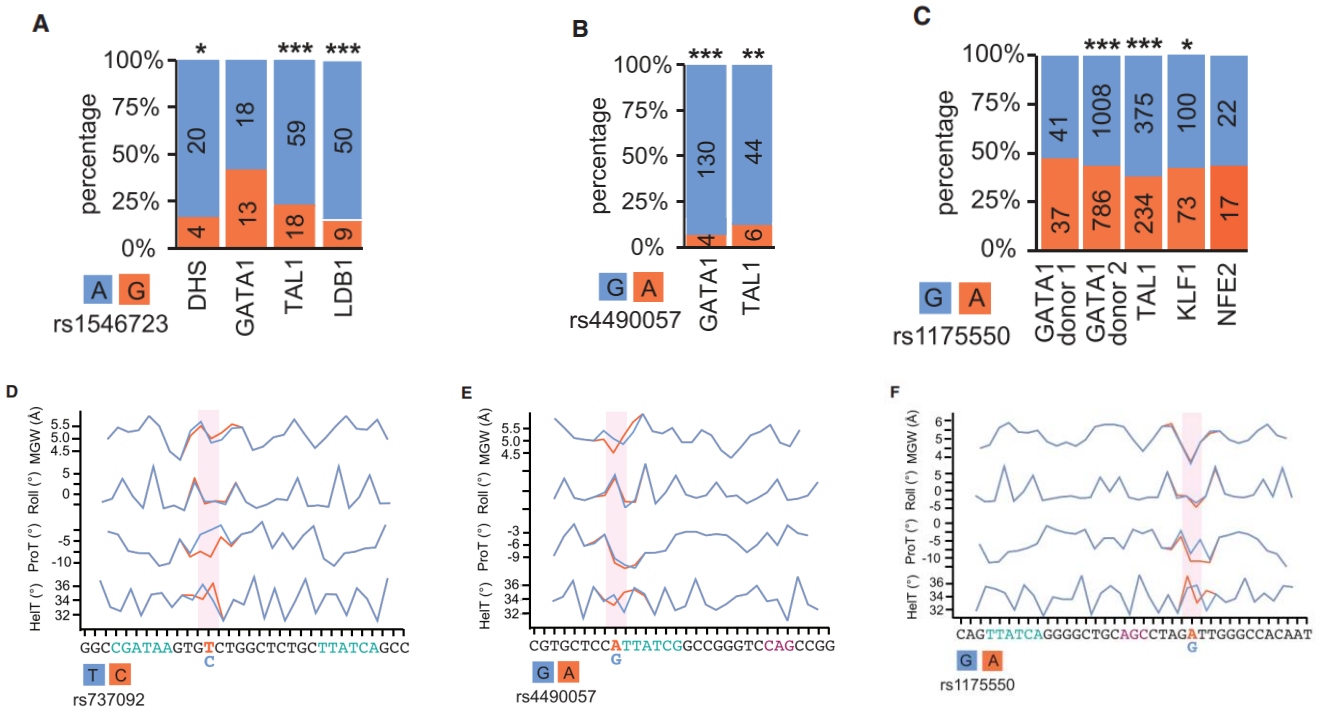
Activity scores for minor and major alleles of 4 variants in the MPRA for the early (K562) and late (K562+GATA1) erythroid progenitor models are shown as boxplots.

Isogenic Deletions Identify Multiple Target Genes at Each Locus



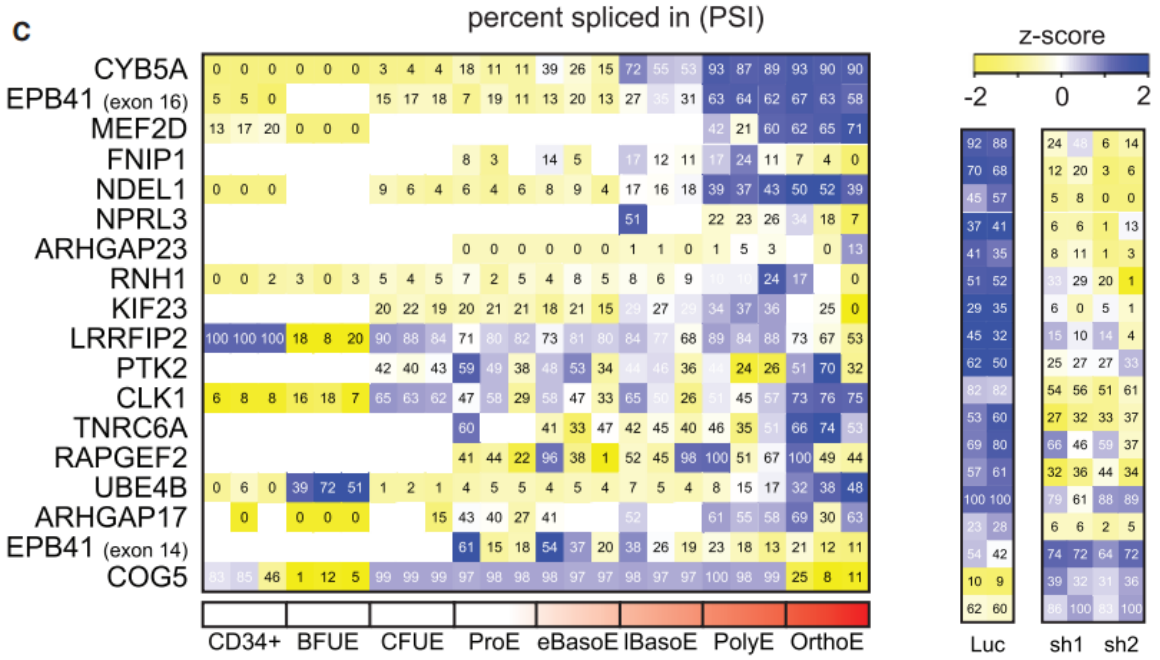
Small clonal deletions were made in K562s across rs737092 (clones D1, D2), rs1175550 (clones D3,D4, D5), and rs1546723 (clones D6, D7) using CRISPR-Cas9 genome editing.

Altered TF Binding as a Putative Mechanism of Action



MFVs can alter the binding and activity of GATA1 and co-factors either directly or by fine-tuning the shape of the DNA adjacent to the core binding motifs

RBM38 Is a Key Regulator of Alternative Splicing in Terminal Human Erythropoiesis



We used two independent short hairpin RNAs (sh1 and sh2) to knockdown *RBM38* along with one control hairpin (shLuc)

A heatmap of exons with a >20% change in PSI shared between RBM38 knockdown and normal erythropoiesis

Highlights

- Identify Multiple Target Genes at Each Locus.
- Functional GWAS variants alter activity of GATA1.
- The target gene *RBM38* regulates mRNA splicing.

总结

优点：

用MPRA对GWAS结果分析，可以鉴定出对基因表达影响最大的变异。

改进 ：

对于MPRA，可以通过加更多barcode来提高灵敏度。

Thanks