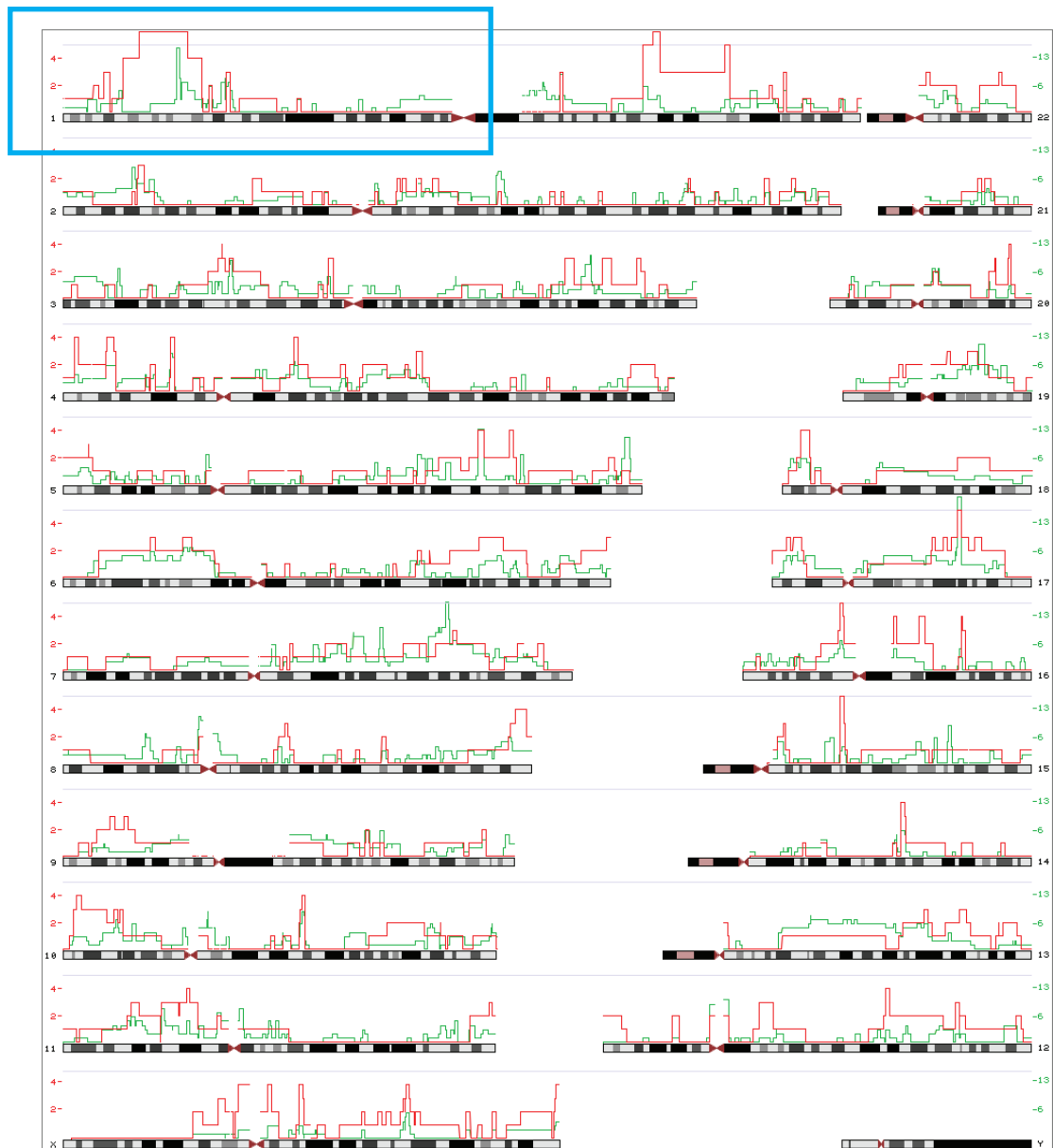
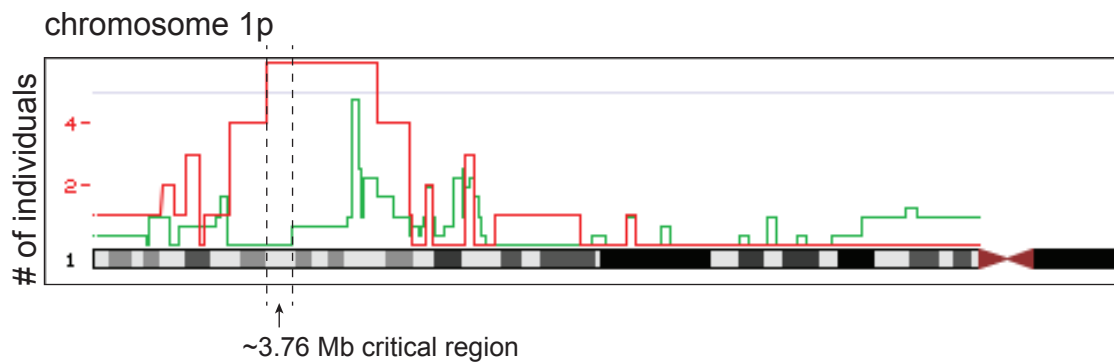


Supplementary Figure 1. Chromosome 1p haplotypes in family AH. Haplotypes were determined by SNP microarrays. A indicates the variant allele and G the reference allele at genomic position chr1:24125191 (hg19), corresponding to GALE p.R51W (c.C151T). SNP alleles are indicated 1 or 2. The 3.78MB region of homozygosity shared by all affected individuals were bounded by rs4649099 and rs 11247639, based recombination events in relatives III.6, III.16, and III.18.

A

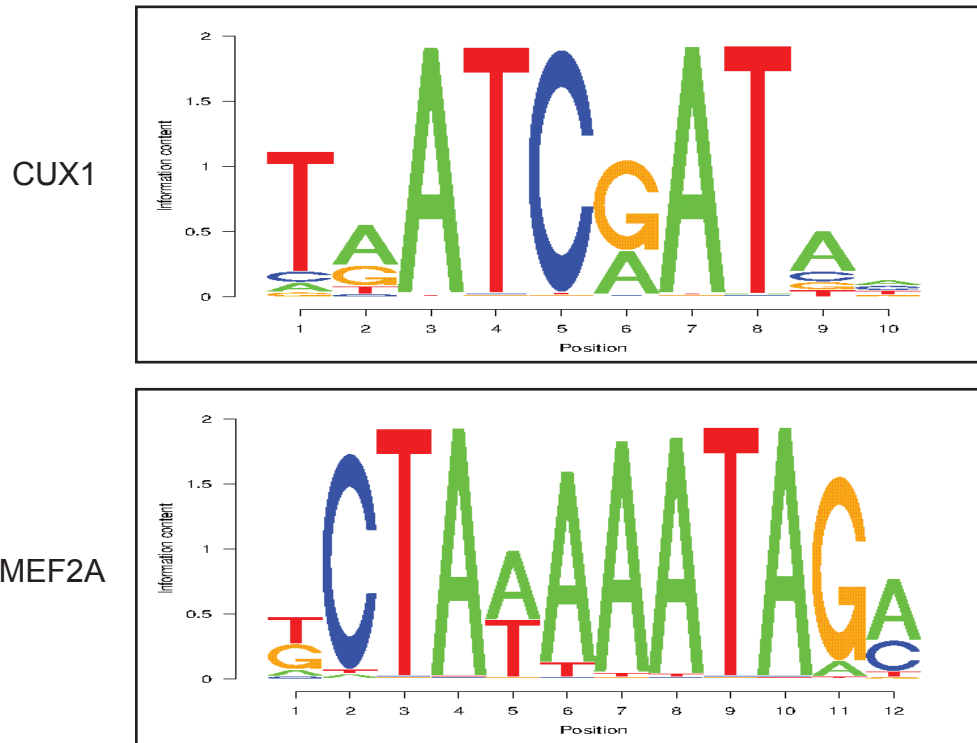
whole genome

**B**

Supplementary Figure 2. Homozygosity mapping. At each chromosomal position, histograms indicate the number of affected (red) and unaffected (green) individuals homozygous for the same allele. A) Whole genome overview. Chromosomes 1-23 are numbered top to bottom (left) then bottom to top (right). Blue box indicates the region enlarged in part B. B) Enlarged view of chromosome 1p. The 3.76 MB region between the dashed lines is the only region genome wide in which all affected individuals, and no unaffected individuals, share homozygosity for the same alleles.

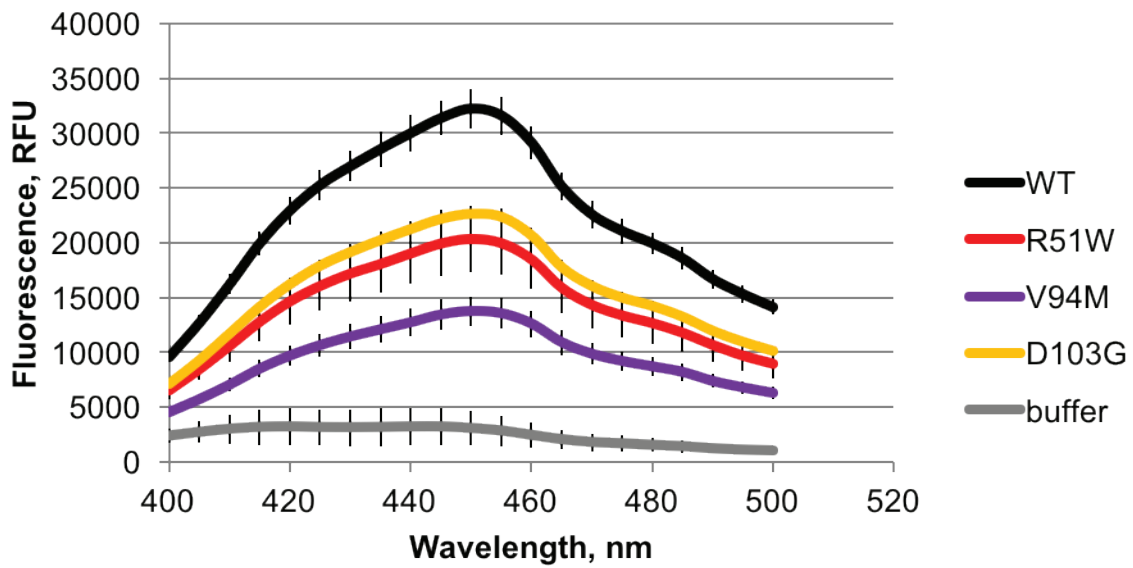
7 bp sequence
flanking intergenic variant

5' GCCACAAATAGCTGT 3'



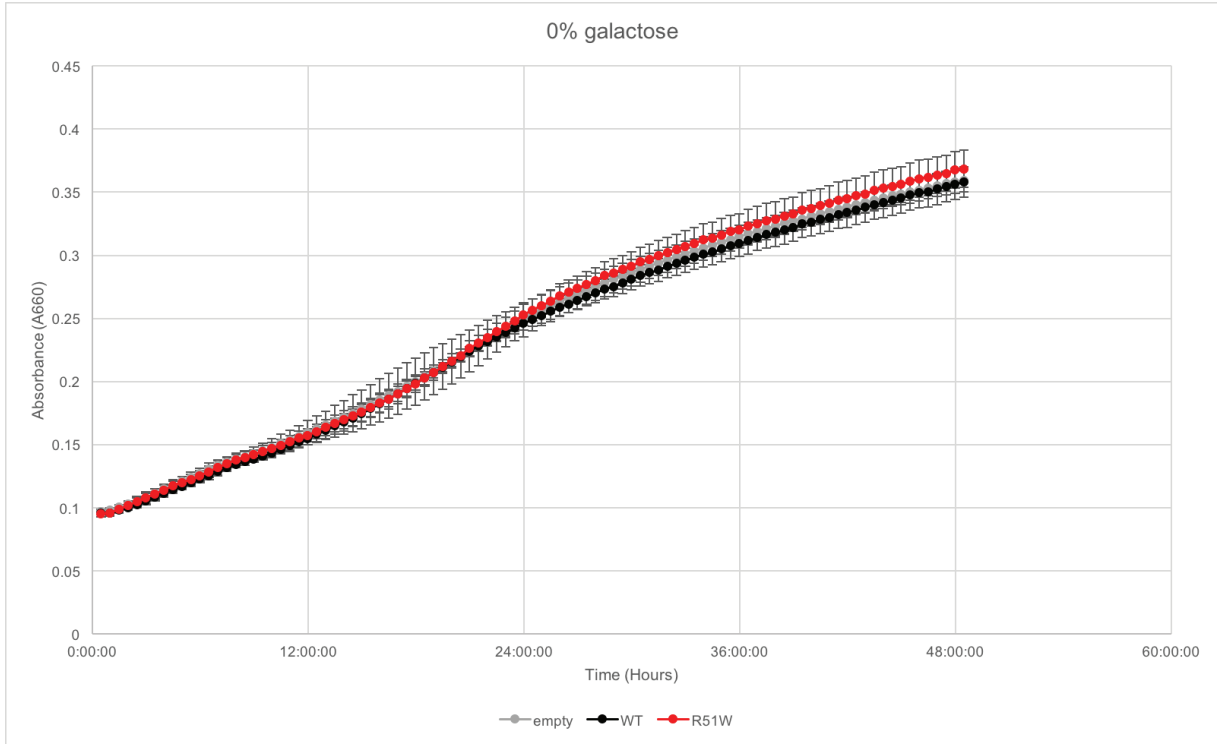
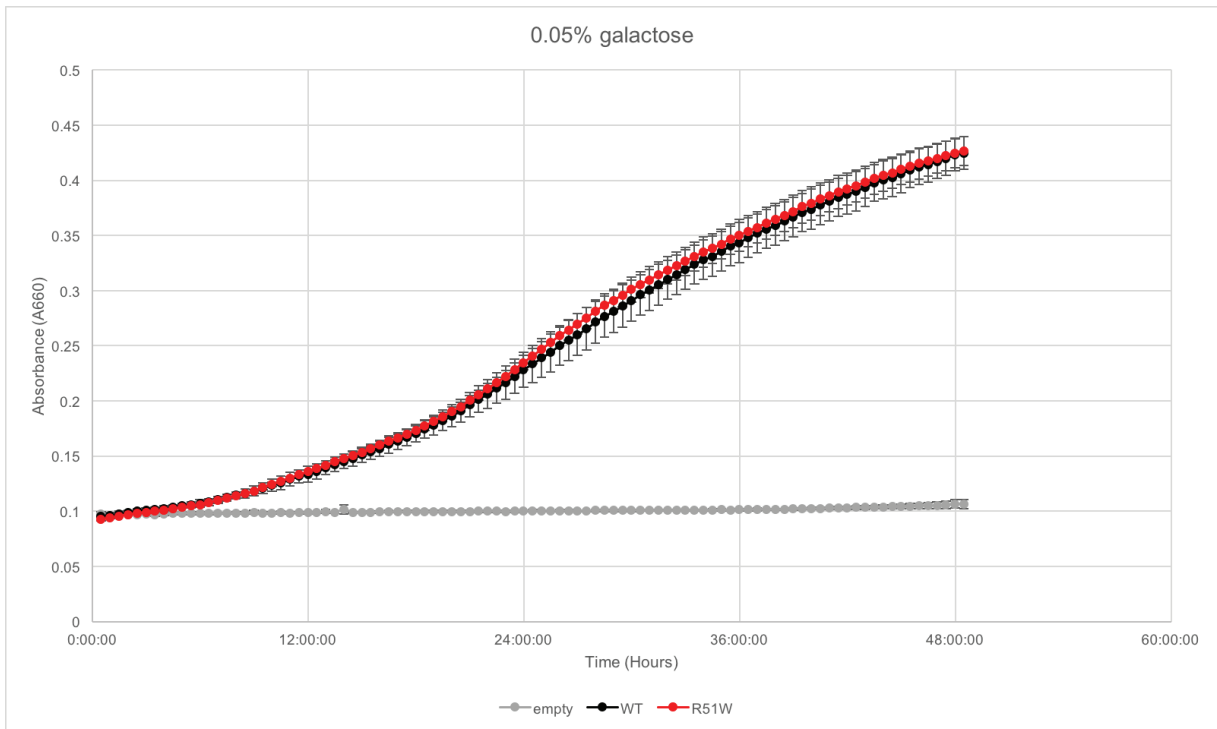
Supplementary Figure 3. Predicted transcription factor binding sites at rs565347299.

Position weight matrices for transcription factors predicted to bind the region surrounding the intergenic variant upstream of *RUNX3*. Matrices based on the JASPAR CORE 2018 database.

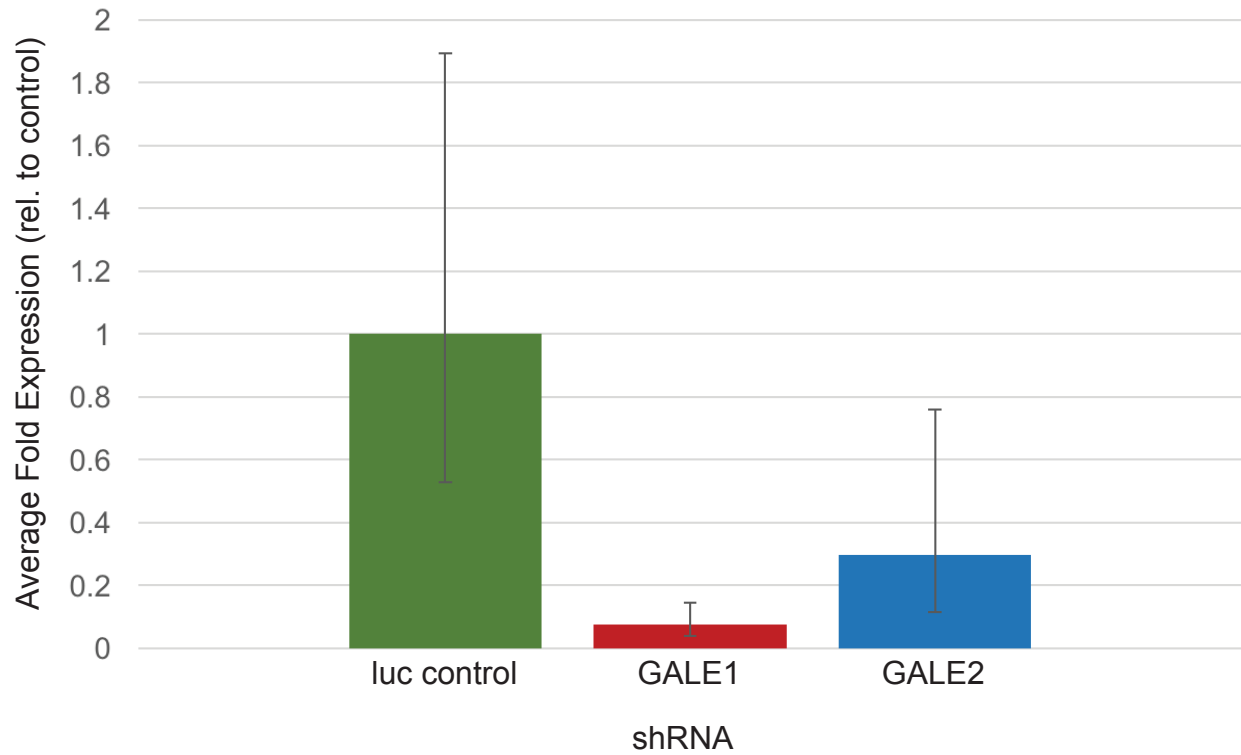


Supplementary Figure 4. NAD⁺ binding to GALE variant proteins.

Protein samples were excited at 350 nm and emission spectra collected at 400-500 nm. Fluorescence +/- SD in RFU is reported for each variant. For each variant protein, each data point is the average of three independent experiments.

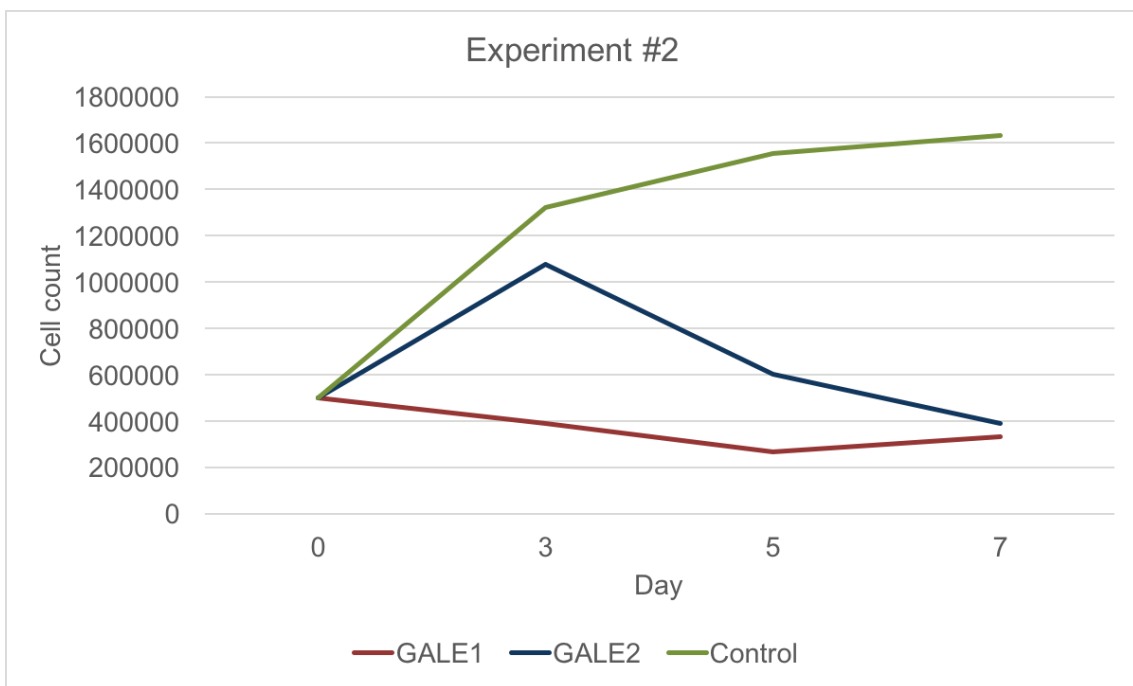
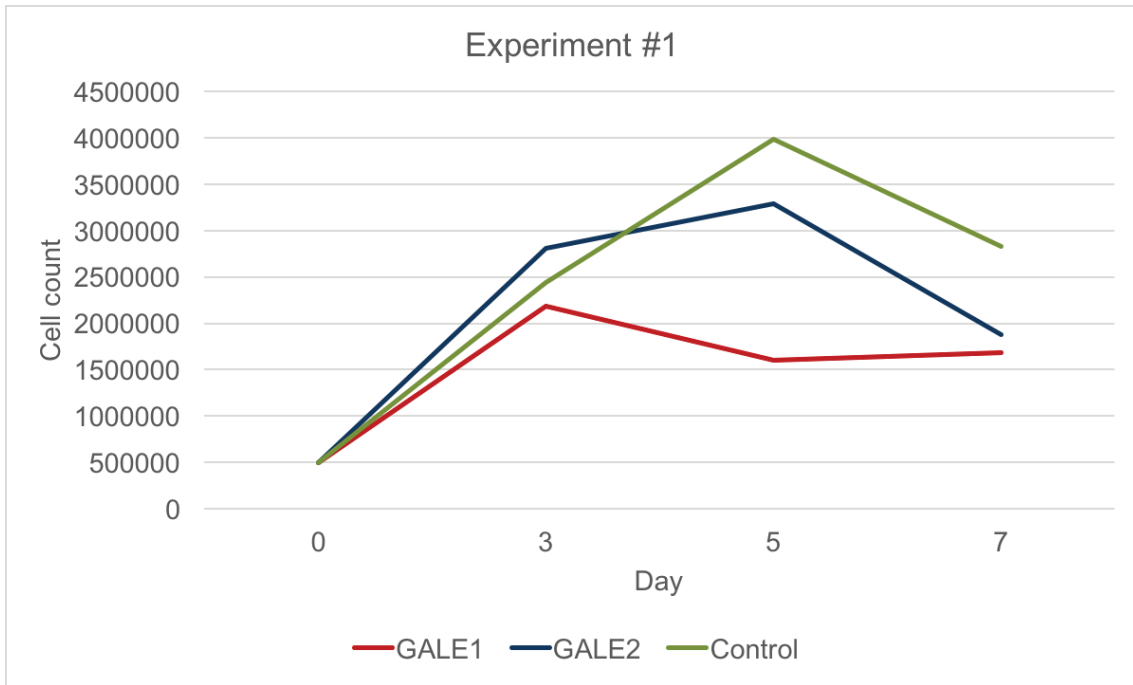
A**B**

Supplementary Figure 5. Complementation by *GALE* alleles in yeast following galactose challenge. *S. cerevisiae* with deletion of endogenous *GALE* was complemented with empty vector, on normal human *GALE* (WT), or human *GALE* R51W. Absorbance at 660 nm was measured every 30 min. Experiment performed in biological triplicate. A) Growth assay with no galactose in media B) Growth assay with 0.05% galactose in media.



Supplementary Figure 6. *GALE* shRNA knockdown efficiency.

Real-time qPCR was performed in 293T cells with *GALE* TaqMan probe and normalized to GAPDH signal with ddCT analysis. Data are plotted as average fold expression + SD compared to luciferase control. Each experiment was run in quadruplicate.



Supplementary Figure 7. Megakaryocyte proliferation in liquid culture for shRNA knockdown of GALE. Cord-blood-derived CD34+ cells treated with control or *GALE* shRNA viruses were sorted to collect successfully transduced cells. For each condition, 500,000 cells were plated in liquid culture under megakaryocyte growth conditions and cells counted on days 0, 3, 5, and 7. Top and bottom graphs represent counts from two independent experiments.

Supplementary Tables

Table S1. Complete Blood Counts.

Patient	Hemoglobin (g/dL)	MCV (fL)	WBC ($\times 10^9/L$)	ANC	PLT
III-1	8.2	81	4	0.8	8
III-3	8.7	83	4.2	0.6	10
III-11	7.9	61	3	1.8	16
III-16	8.5	85	4.2	1.2	15
III-18	10.5	86	4.3	1	50
IV-1	12.1	81	8.8	1	44

MCV: mean corpuscular volume, WBC: white blood count, ANC: absolute neutrophil count, PLT: platelet

Table S2. Gene expression measured by RNA-Seq for *RPL11* and *RUNX3*.

Samples were prepared in duplicate. For each sample, Z scores were calculated compared to nine control samples. LIN28A expression was not detectable in RNA from lymphoblasts.

Gene	Proband replicate1	Proband replicate2	Mother replicate1	Mother replicate2	Father replicate1	Father replicate2
<i>RPL11</i>	-0.81	-0.56	-0.33	-0.44	-0.41	-0.99
<i>RUNX3</i>	0.83	0.63	-0.11	-1.12	0.19	1.27

Table S3. Rare variants from whole genome sequencing located in the homozygous region shared by all affected individuals in family AH, and either conserved (GERP >2.0) or in proximity to a gene possibly implicated in bone marrow failure.

Chr	Coordinate	Ref	Var	Type	Gene	Gerp	Cons TFS	Encode HMM	Chipseq	DNaseI
1	24,020,197	G	A	intronic	RPL11	-1.3	-	Strong Enhancer	HEY1	5
1	24,125,191	G	A	missense	GALE	+4.8	-	Insulator	CTCF_(SC-5916),CTCF	20
1	25,184,753	A	G	intergenic	RUNX3 (dist=41249)	+0.2	-	Weak Txn	-	-
1	25,186,263	-	CACAC ACACA CACAC ACACA CACAT ATATT	intergenic	RUNX3 (dist=39739)	indel	-	Weak Txn	-	-
1	25,187,920	C	T	intergenic	RUNX3 (dist=38082)	+0.6	-	Weak Txn	-	-
1	25,242,297	G	A	intronic	RUNX3	-3.0	-	Txn Transition	-	13
1	25,250,541	C	T	intronic	RUNX3	+0.7	-	Strong Enhancer	-	-
1	25,252,493	G	A	intronic	RUNX3	-2.9	-	Strong Enhancer	Pol2-4H8	-
1	25,324,183	A	G	intergenic	RUNX3 (dist=32682)	+4.3	CDP CR3_01 (865)	Weak Enhancer	-	7
1	25,905,634	C	T	intergenic	MAN1C1 (dist=38325)	+2.2	-	Heterochrom/lo	-	-
1	26,068,576	G	A	intronic	MAN1C1	+2.1	-	Repressed	-	-
1	26,258,357	T	C	intergenic	PAFAH2 (dist=27901)	-1.0	-	Heterochrom/lo	-	-
1	26,728,707	C	T	intergenic	LIN28A (dist=8562)	-2.4	-	Heterochrom/lo	STAT3	-
1	27,084,517	T	C	intronic	ARID1A	+3.8	-	Txn Elongation	-	-
1	27,320,968	C	T	UTR3	TRNP1	+2.3	-	Poised Promoter	Pol2, GABP, TAF1, ZBTB7A (SC-34508), Pol2-4H8	-
1	27,625,246	C	T	intronic	WDTC1	+3.0	-	Weak Txn	-	5

Table S4. Fraction product (UDP-glc or UDP-glcNAc) from GALE enzyme assays

Substrate	GALE Proteins			
	WT	R51W	V94M	D103G
UDP-gal	0.426	0.235	0.004	0.201
UDP-gal	0.431	0.138	0.003	0.28
UDP-gal	0.434	0.137	0.004	0.179
UDP-galNAc	0.305	0.121	0.017	0.153
UDP-galNAc	0.296	0.114	0.016	0.143
UDP-galNAc	0.287	0.114	0.015	0.121
UDP-galNAc	0.315	0.117	0.012	0.157

Table S5. Cell proliferation under megakaryocyte growth conditions after shRNA knockdown

	Expt	Days after seeding 500,000 cells from each treatment			
		0	3	5	7
GALE1	1	500,000	2,180,000	1,600,000	1,680,000
GALE2	1	500,000	2,810,000	3,289,000	1,880,000
Control	1	500,000	2,440,000	3,989,999	2,826,700
GALE1	2	500,000	388,889	266,667	333,333
GALE2	2	500,000	1,077,778	600,000	388,889
Control	2	500,000	1,322,222	1,555,556	1,633,333

Table S6. Size Distribution of Megakaryocyte Colonies

shRNA	Expt	Rep	total colonies counted	small (3-20 cells)	medium (21-49 cells)	large (>50 cells)	Prop large colonies	P vs control
GALE1	1	A	170	85	56	29	0.171	
GALE1	1	B	137	57	42	38	0.277	
GALE1	2	A	278	124	83	71	0.255	
GALE1	2	B	401	179	127	95	0.237	
GALE1	total		986	445	308	233	0.235	0.008
GALE2	1	A	145	45	53	47	0.324	
GALE2	1	B	125	22	46	57	0.456	
GALE2	2	A	313	89	114	110	0.351	
GALE2	2	B	325	78	101	146	0.449	
GALE2	total		908	234	314	360	0.395	0.0005
Control	1	A	254	172	74	8	0.031	
Control	1	B	246	168	62	16	0.065	
Control	2	A	318	203	76	39	0.123	
Control	2	B	418	257	96	65	0.156	
Control	total		1236	800	308	128	0.094	

Table S7. Chromatographic Pump Gradient parameters

Run time: 9.0 min

Seal wash: 5 min

Pressure limits: low: 0 psi, high: 15,000 psi

Step	Time (min)	Flow (mL/min)	%A	%B	Curve
1	(initial)	0.400	10.0	90.0	(initial)
2	5.00	0.400	20.0	80.0	6
3	5.50	0.400	40.0	60.0	6
4	6.50	0.400	40.0	60.0	6
5	9.00	0.400	10.0	90.0	1

Solvent A = H₂O + 10mM Ammonium Formate + 0.125% (v/v) Formic Acid (FA) (Fisher UPLC-grade)

Solvent B = 95:5 Acetonitrile:H₂O + 10mM Ammonium Formate + 0.125% (v/v) FA

Strong Needle Wash = 95:5 ACN: H₂O

Weak Needle Wash = 90:10 H₂O:ACN

Table S8. Mass Spectrometer Tuning Parameters

Negative ionization mode

Collision energy for different multiple-reaction monitoring (MRM) transitions

Compound	Parent	Daughter		
Name	(m/z)	(m/z)	Cone (V)	Collision (V)
UDP-gal-241	564.90	241.00	35	29
UDP-glc-241	564.90	241.01	35	29
UDP-gal-280	564.90	279.90	35	27
UDP-glc-280	564.90	279.91	35	27
UDP-gal-403	564.90	403.00	35	22
UDP-glc-403	564.90	403.01	35	22
UDP-galNAc-159	606.00	159.00	39	44
UDP-glcNAc-159	606.00	159.01	39	44
UDP-galNAc-273	606.00	272.90	39	32
UDP-glcNAc-273	606.00	272.91	39	32
UDP-galNAc-282	606.00	282.00	39	27
UDP-glcNAc-282	606.00	282.01	39	27
UDP-galNAc-385	606.00	384.90	39	24
UDP-glcNAc-385	606.00	384.91	39	24
UDP-galNAc-403	606.00	403.00	39	23
UDP-glcNAc-403	606.00	403.01	39	23

Table S9. Sugar Retention Times

Sugar	Retention Time (min.)
UDP-glc	4.37
UDP-gal	4.60
DP-glcNAc	4.76
UDP-galNAc	4.98