Supplemental Methods

***In vitro* DNase I foot printing**. *In vitro* DNase I footprinting was performed as described previously [1] [2] using 32P-labeled 211 bp fragment from 3'HS1. Footprinting reaction mixes contained K562 nuclear extract.

Gel Mobility Shift Analysis. Was preformed as previously described[3]. Nuclear extracts from K562 cells and lysed by hypotonic lysis, followed by high salt extraction of nuclei as described by Andrews and Faller [4]. Binding reactions were carried out as described [5] [6]. Competitor oligonucleotides were added at molar excess of 10 fold. Resulting complexes were separated by electrophoresis through 6% polyacrylamide gels at 21°C.

Luciferase Reporter Assay. The 211 bp fragment was inserted either 5' or 3' in either 5' to 3' or 3' to 5' fashion of an *ANK1E* promoter/luciferase or a TK/luciferase reporter gene [7]. 20μg of test plasmid and 0.5μg of the renilla luciferase control plasmid were co-electrophorated into K562 or SH-SY5Y cells using a BioRad Gene Pulser Xcell at 200V at 960 μF. After 48 hours, luciferase and renilla luciferase activity was measured using the Promega Dual-Luciferase Reporter Assay System on a Thermo Fluoroskan Ascent FL [8].

Supplemental Figure Legends

Figure S1. DNase I sensitivity of the erythroid *ANK1* promoter region in erythroid K562 cell chromatin. A. The map indicates the position of ankyrin exon 1E (*ANK1E*) which is specifically expressed in erythroid cells, flanking restriction enzyme sites, locations of 5' and 3' DNase I hypersensitive sites, and locations of probes used in DNase I mapping. B. analysis of the region 5' of the *ANK1E* promoter. Nuclei from K562 cells were treated with increasing amounts of DNase I, digested with SacI, and subjected to Southern Blot analysis using the 5' probe indicated in A. This demonstrated the expected 10kb SacI fragment. C. Mapping the 5.8kb region between the DNase I hypersensitive sites 5' and 3' of the *ANK1E* promoter. Nuclei from K562 cells were treated with increasing amounts of DNase I, digested with SacI, and subjected to Southern blot analysis using the central probe indicated in A. This demonstrated generalized DNase I sensitivity of the 5.8 kb region. D. Mapping the 2kb region 3' of the DNase I hypersensitive site. Nuclei from K562 cells were treated with increasing amounts of DNase I, digested with SacI, and subjected to Southern blot analysis using the 3' probe indicated in A. This demonstrated the expected 2.0kb fragment. E. The final hybridization of the filter was to a probe for the human keratin 14 gene which recognizes 9.0 and 2.1kb bands. Blots shown in B, C, D, and E are the same filter, striped and re-hybridized.

Figure S2. DNase I sensitivity of the erythroid *ANK1* promoter region in nonerythroid SHSY5Y chromatin. A. The map indicates the position of ankyrin

exon 1E (*ANK1E*) which is specifically expressed in erythroid cells, flanking restriction enzyme sites, locations of 5' and 3' DNase I hypersensitive sites, and locations of probes used in DNase I mapping. B. analysis of the region 5' of the *ANK1E* promoter. Nuclei from SHSY5Y cells were treated with increasing amounts of DNase I, digested with SacI, and subjected to Southern Blot analysis using the 5' probe indicated in A. This demonstrated the expected 10kb SacI fragment. C. Mapping the 5.8kb region between the DNase I hypersensitive sites 5' and 3' of the *ANK1E* promoter. Nuclei from SHSY5Y cells were treated with increasing amounts of DNase I, digested with SacI, and subjected to Southern blot analysis using the central probe indicated in A. This demonstrated no DNase I sensitivity in the 5.8 kb region. D. Mapping the 2kb region 3' of the DNase I hypersensitive site. Nuclei from SHSY5Y cells were treated with increasing amounts of DNase I, digested with SacI, and subjected to Southern blot analysis using the 3' probe indicated in A. This demonstrated the expected 2.0kb fragment. E. The final hybridization of the filter was to a probe for the human keratin 14 gene which recognizes 9.0 and 2.1kb bands. Blots shown in B, C, D, and E are the same filter, striped and re-hybridized.

Figure S3. *In vitro* NF-E2 binding to 3'HS-1. (A) DNase foot print analysis of the 3'HS1 region. Footprint analysis was preformed as previously described [7]. A 211 base pair fragment of 3'HS-1 was incubated with or without increasing mounts of nuclear extract containing NF-E2 then digested with DNase I. The protected region shown contains the consensus binding sequence for NF-E2 (CTGACTCATATCT). (B) In vitro analysis of NF-E2 binding. Electrophoretic Mobility Shift assay preformed as previously

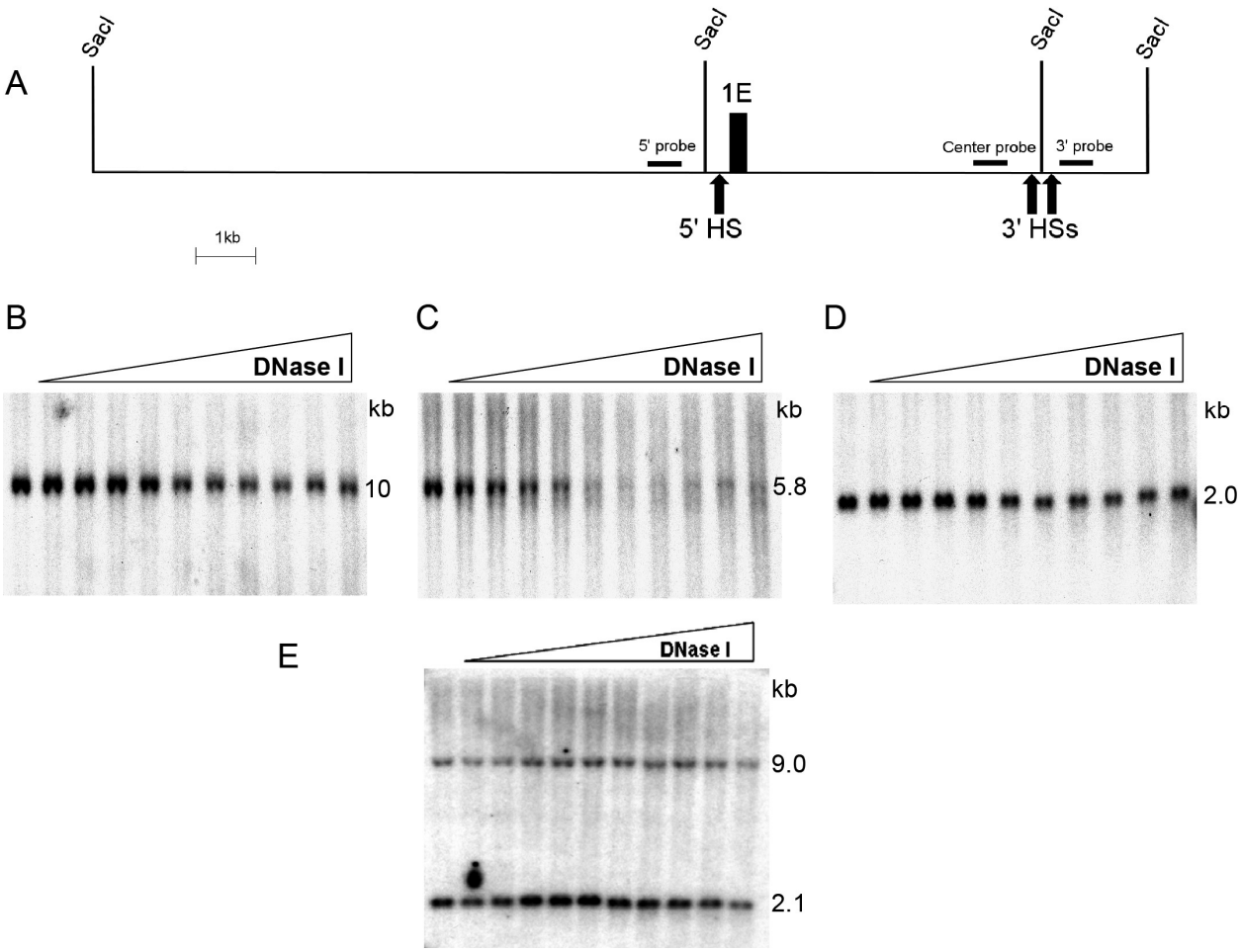
described [7]. Labeled oligonucleotides containing a consensus NF-E2 sequence (Control NF-E2 site; TGGGGAACCTGTGCTGAGTCACTGGAG) or the 3'HS-1 NF-E2 (3' Ankyrin NF-E2 site; CTGAGTTTCTACTCAGTCTACCTCGAC) binding sites were incubated with nuclear extract containing NF-E2 then analyzed on polyacrylamide gels. Both sites bind NF-E2 as evidenced by the slower migrating band, which can be competed off the probe with either unlabeled probes or anti-NF-E2 antibodies.

Figure S4. Analysis of the cis-acting regulatory activity of 3'HS-1. A 211 bp fragment of 3'HS-1 was tested for orientation, position and promoter independence. The constructs shown at the left were transfected into K562 or neuronal SHSY5Y cells. 3'HS-1 significantly increases expression of *ANK1E* and TK promoters only in erythroid cells and only when adjacent to the promoter regardless of orientation (*$p<0.05$; when compared to promoter alone). Mutation of the NF-E2 (single triangle) or NF-E2/AP1 (double triangle) abolishes the activity of 3'HS-1 (†$p< 0.01$; compared to wild type).

1. Laflamme K, Owen AN, Devlin EE, et al. Functional analysis of a novel cis-acting regulatory region within the human ankyrin gene (ANK-1) promoter. *Molecular and cellular biology*. 2010;30(14):3493-3502.
2. Gallagher PG, Nilson DG, Wong C, et al. A dinucleotide deletion in the ankyrin promoter alters gene expression, transcription initiation and TFIID complex formation in hereditary spherocytosis. *Human molecular genetics*. 2005;14(17):2501-2509.
3. Gallagher PG, Forget BG. An alternate promoter directs expression of a truncated, muscle-specific isoform of the human ankyrin 1 gene. *The Journal of biological chemistry*. 1998;273(3):1339-1348.
4. Andrews NC, Faller DV. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic acids research*. 1991;19(9):2499.
5. Strauss F, Varshavsky A. A protein binds to a satellite DNA repeat at three specific sites that would be brought into mutual proximity by DNA folding in the nucleosome. *Cell*. 1984;37(3):889-901.

6. Carthew RW, Chodosh LA, Sharp PA. An RNA polymerase II transcription factor binds to an upstream element in the adenovirus major late promoter. *Cell*. 1985;43(2 Pt 1):439-448.
7. Gallagher PG, Romana M, Tse WT, Lux SE, Forget BG. The human ankyrin-1 gene is selectively transcribed in erythroid cell lines despite the presence of a housekeeping-like promoter. *Blood*. 2000;96(3):1136-1143.
8. Gallagher PG, Steiner LA, Liem RI, et al. Mutation of a barrier insulator in the human ankyrin-1 gene is associated with hereditary spherocytosis. *J Clin Invest*. 2010;120(12):4453-4465.

Figure S1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SacI
SacI
SacI
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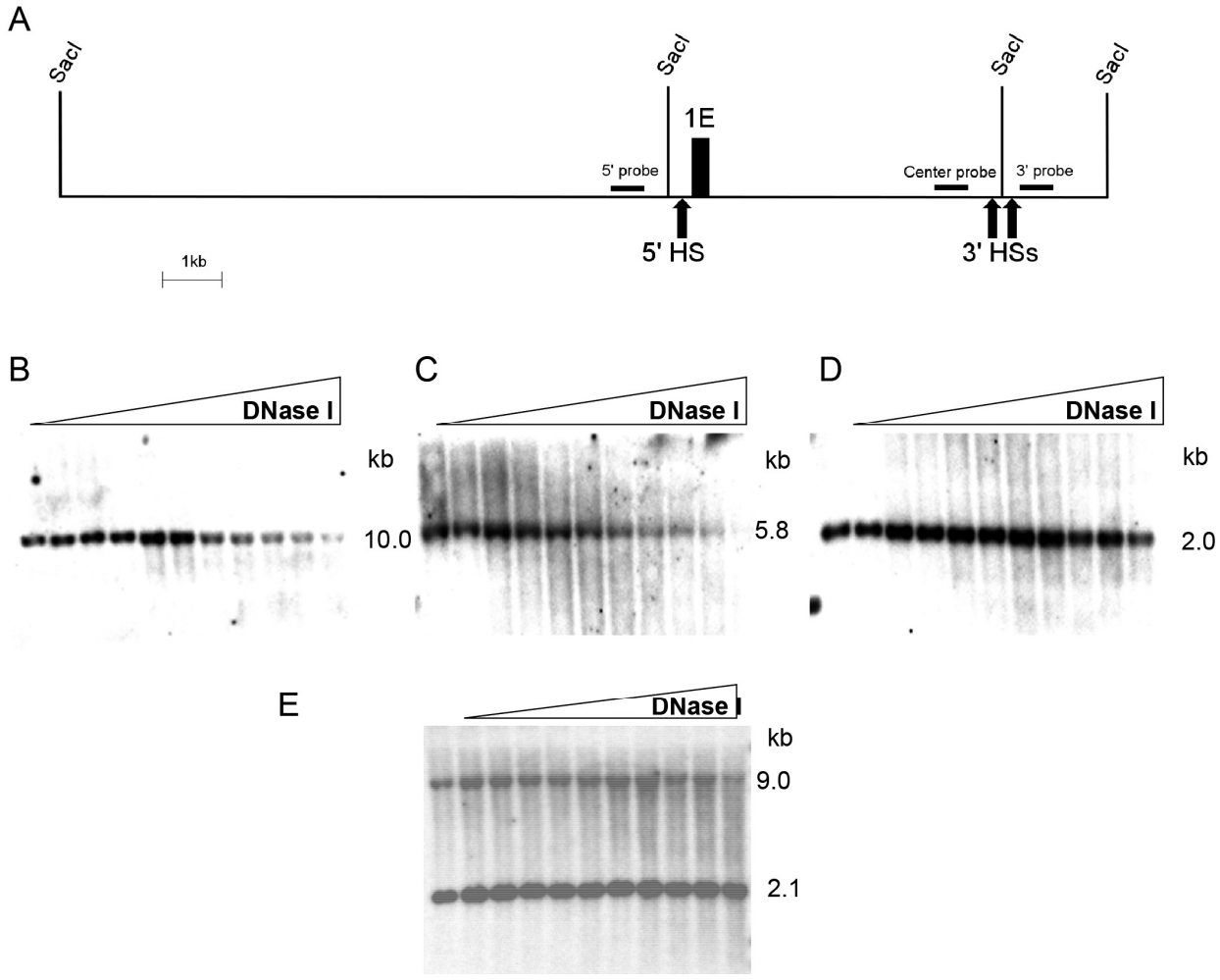
Center probe
3' probe
5' HS
3' HSs
1kb
B
DNase I
kb
10
C
DNase I
kb
5.8
D
DNase I
kb
2.0
E
DNase I
kb
9.0
2.1

Figure S2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SacI
SacI
SacI
1E
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3' HSs
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DNase I
kb
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C
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E
DNase I
kb
9.0
2.1

Figure S3

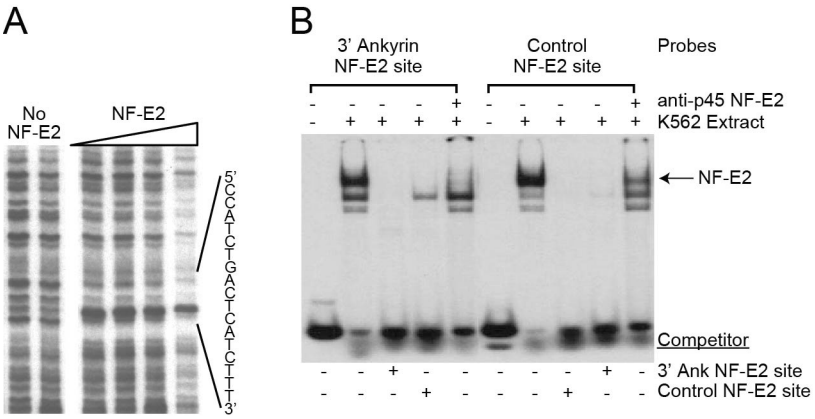
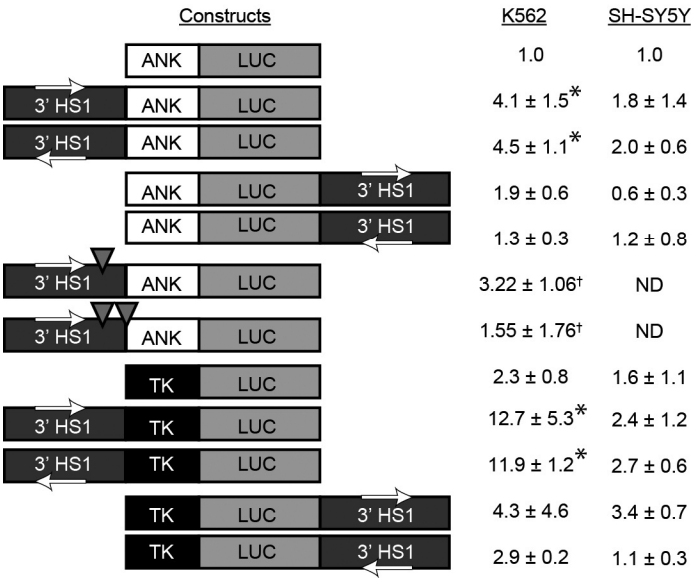


Figure S4



Constructs	K562	SH-SY5Y
ANK LUC	1.0	1.0
3' HS1 ANK LUC	4.1 ± 1.5*	1.8 ± 1.4
3' HS1 ANK LUC	4.5 ± 1.1*	2.0 ± 0.6
ANK LUC 3' HS1	1.9 ± 0.6	0.6 ± 0.3
ANK LUC 3' HS1	1.3 ± 0.3	1.2 ± 0.8
3' HS1 ANK LUC	3.22 ± 1.06†	ND
3' HS1 ANK LUC	1.55 ± 1.76†	ND
TK LUC	2.3 ± 0.8	1.6 ± 1.1
3' HS1 TK LUC	12.7 ± 5.3*	2.4 ± 1.2
3' HS1 TK LUC	11.9 ± 1.2*	2.7 ± 0.6
TK LUC 3' HS1	4.3 ± 4.6	3.4 ± 0.7
TK LUC 3' HS1	2.9 ± 0.2	1.1 ± 0.3

Table S1. Primers used for ChIP validation in the *ANK1E* promoter region and for ChIP-loop

Primer	Sequence (5'-3')	Start	End
1F	acagcttttgcggcccc	41657601	41657618
1R	ccccgtgacaaggaactaagg	41657442	41657462
2F	gtgacactctgtgttcatatgctgg	41657050	41657074
2R	cagtaatggcttctggaacgaacc	41656916	41656939
3F	ctgtcaggcactagtccccg	41656550	41656550
3R	cctgacgaggccgcc	41656366	41656381
4F	ctagtgaaagcacgcggac	41655184	41655806
4R	gcttgtctaaaagtcgctgggtg	41655466	41655488
5F	aaggaagggaaagagcgga	41654495	41654514
5R	gtgtggcctgatgtccaactc	41654239	41654259
6F	cggcaagcaattcaggttaag	41654010	41654010
6R	attttacccatttttccatcgg	41653794	41653816
7F	cgtgttggtatcattacaagtcctctc	41653421	41653447
7R	tggtgtttgatagcagcttaggg	41653347	41653369
8F	gggcaccgggaatatagcag	41652146	41652165
8R	ttcccatgacacacgcctg	41651820	41651839
9F	catgagccaccgcactcg	41650354	41650374
9R	ttttcaatcgcaggtttgtgag	41650325	41650346
10F	aggagggaagctcactggag	41650662	41650681
10R	ctcactgtattgcccaggct	41650868	41650887
11F	gatactctgctcttgggattttt	41650268	41650290
11R	gccagggaaaataagaggaa	41650068	41650087
12F	acgaggtttctcagcacagc	41649823	41649842
12R	caggagataaatcagcctctgc	41649639	41649660
13F	gcgtttgtcatcattctccgac	41649766	41649787
13R	ggcccattagcagcagtcag	41649572	41649591
14F	gttagtgcctccccactttg	41649208	41649227

14R	gacttgctctgtctccaggc	41648986	41649005
15F	ggagtcaatggctagaggaccc	41648428	41648449
15R	ttcaaatcacgccgtgtagaca	41648345	41648366
16F	aaaggggttttatatctcaaagtcttc	41648220	41648246
16R	ttccctcctaaccactgcac	41648037	41648056
17F	aggaggcagaggttgcagt	41647701	41647720
17R	atctgggcagttcctgtcac	41647538	41647557

Primer	Sequence (5'-3')	Start	End
F	cgcagtttagcagactcaaaggaaagc	41654660	41654686
1R	cacacctccccgcttattta	41650166	41650185
2R	aaaaggagatgggagccact	41651317	41651336
3R	ctgctgtgaggctgaagtga	41653696	41653715
4R	caagcccccaaggcccttcg	41655081	41655100