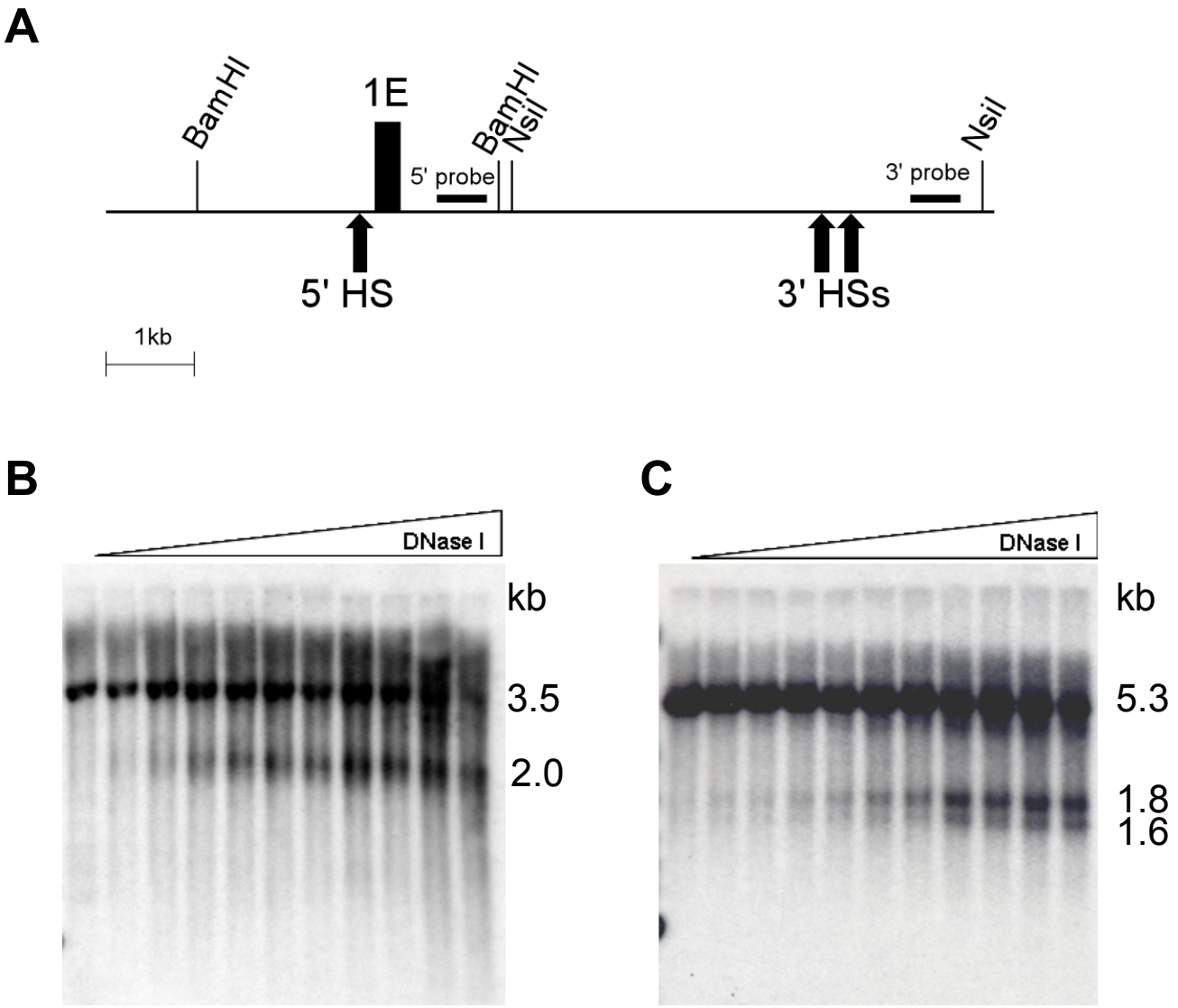
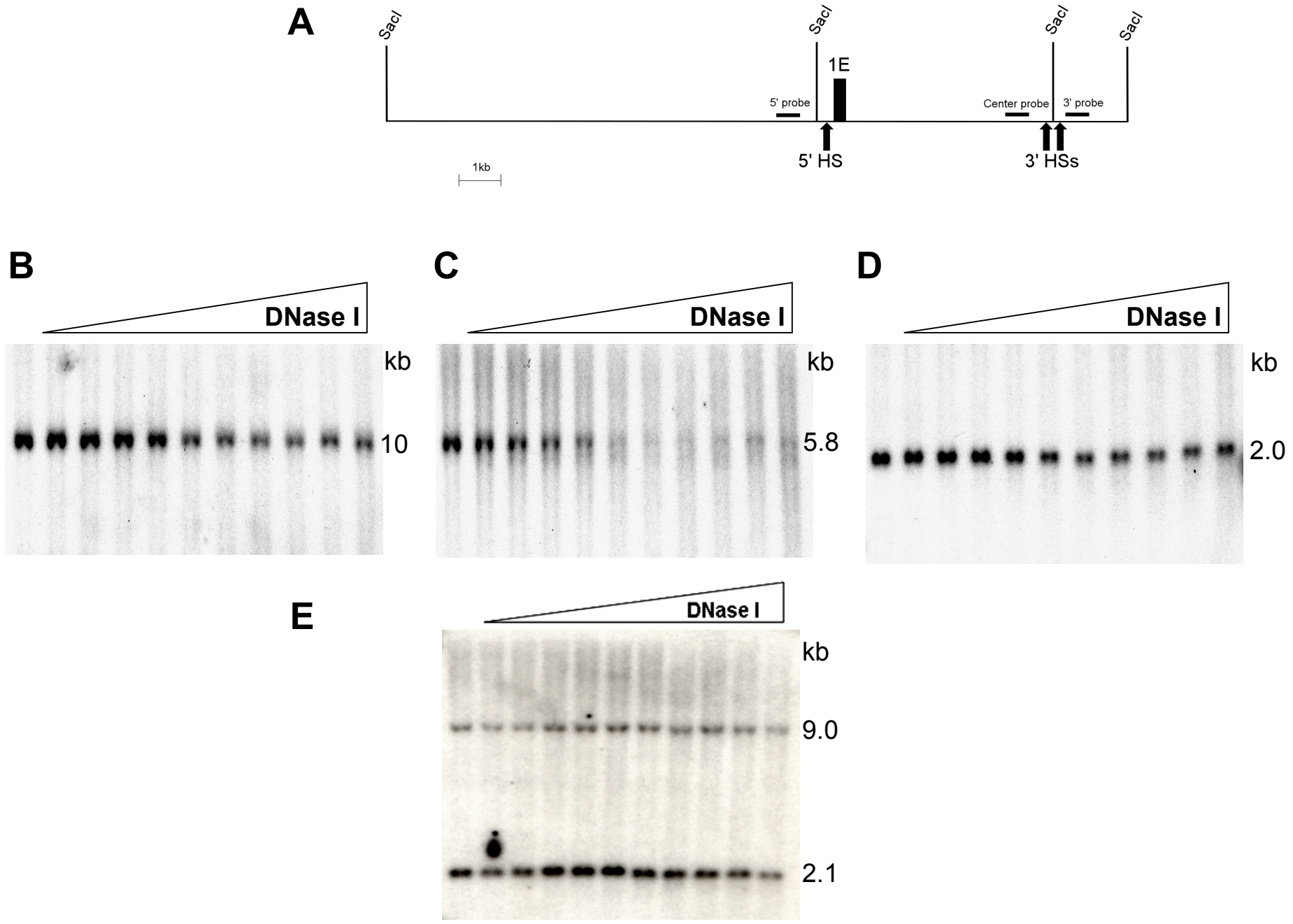
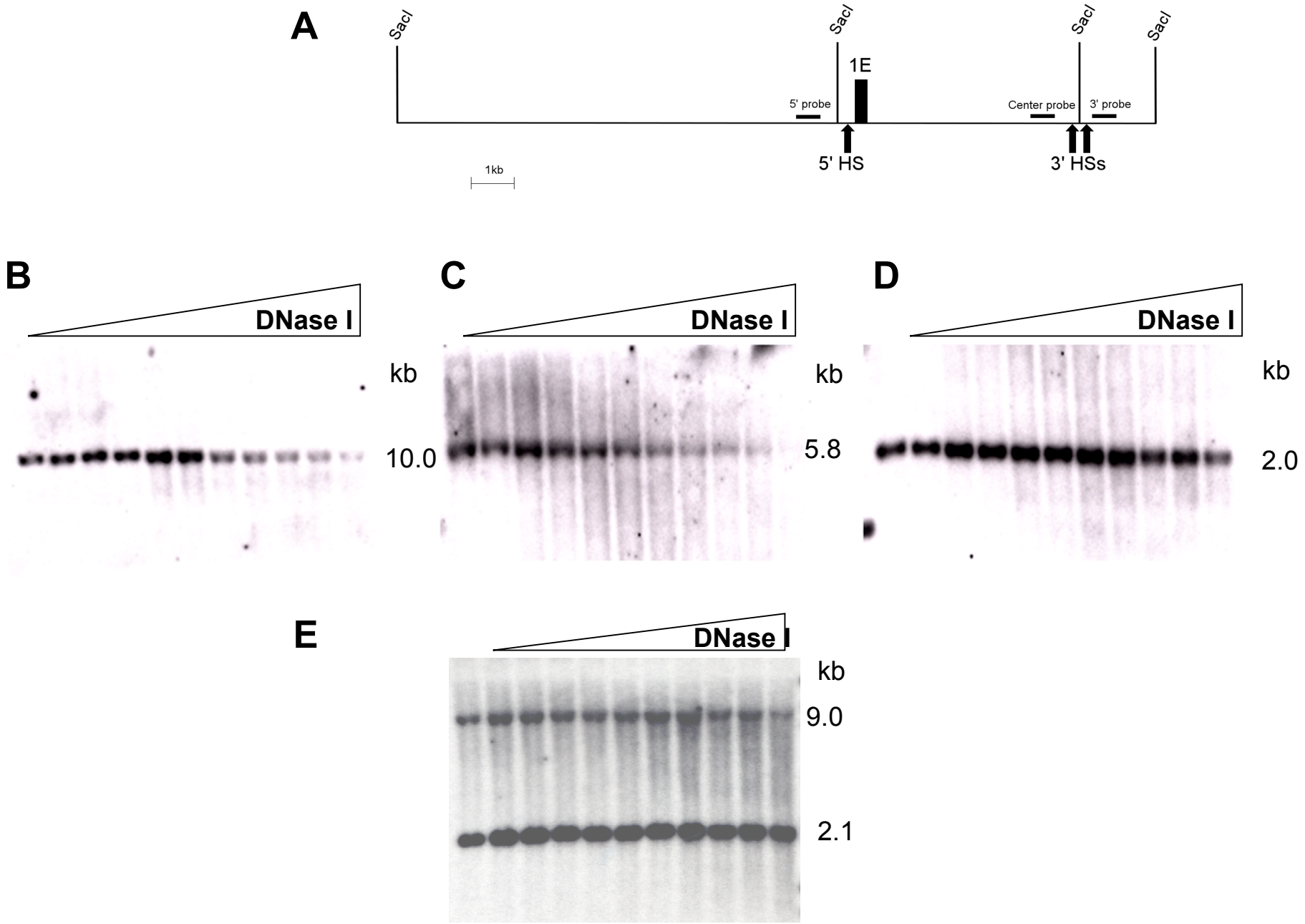
Supplemental Figure S1



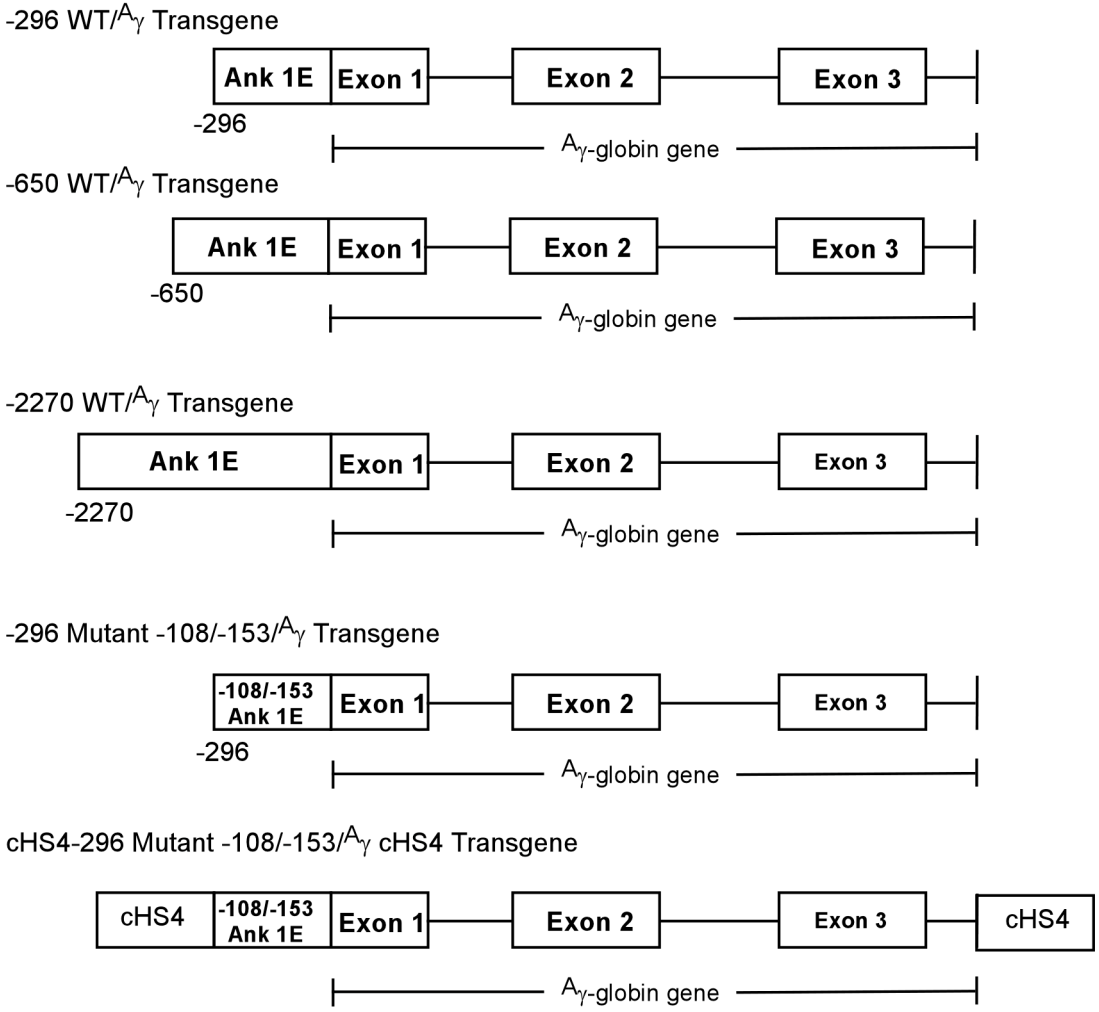
Supplemental Figure S2



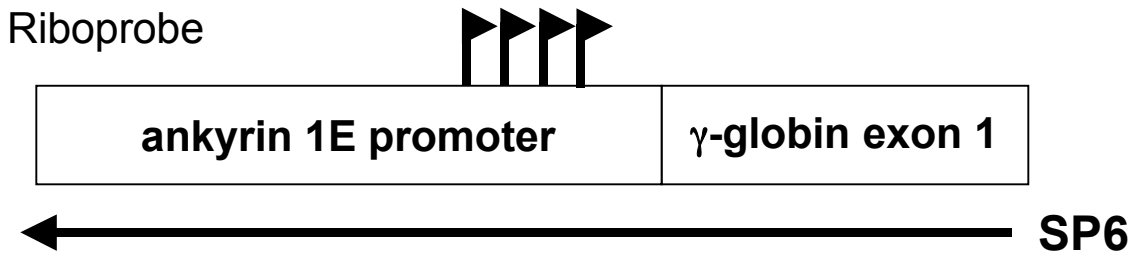
Supplemental Figure S3

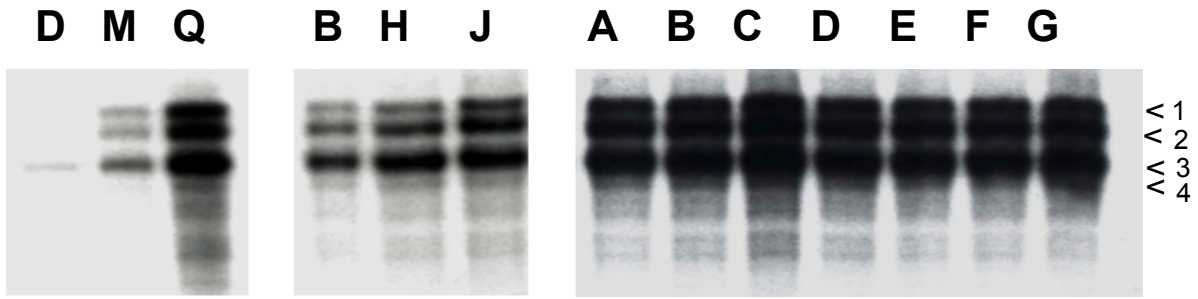


Supplemental Figure S4



Supplemental Figure S5





-296 WT/$^{A}\gamma$ Transgene

-296 Mutant -108/-153/$^{A}\gamma$ Transgene

cHS4 -296 Mutant -108/-153/$^{A}\gamma$ cHS4 Transgene

Supplemental Figure Legends and Tables

Supplemental Figure S1. *Mapping DNase I hypersensitive sites in the erythroid promoter region of the ankyrin-1 locus in neural cells.* A. The map indicates the position of ankyrin exon 1E which is specifically expressed in erythroid cells, flanking restriction enzyme sites, and location of probes used in DNase I mapping. B. Mapping 5' of exon 1E. Nuclei from SH SY5Y cells were treated with increasing amounts of DNase I, digested with BamHI, and subjected to Southern Blot analysis using the 5' probe indicated in A. This demonstrated the expected 3.5 kb BamHI fragment and a smaller 2.0 kb fragment corresponding to a DNase I hypersensitive site. C. Mapping 3' of exon 1E. Nuclei from SH SY5Y cells were treated with increasing amounts of DNase I, digested with NsiI, and subjected to Southern blot analysis using the 3' probe indicated in A. This demonstrated the expected 5.3 kb NsiI fragment and two smaller 1.8kb and 1.6kb fragments corresponding to DNase I hypersensitive sites. Blots shown in B and C are the same chromatin, transferred onto separate filters.

Supplemental Figure S2. *DNase I sensitivity of the ankyrin erythroid promoter region in erythroid chromatin.* A. The map indicates the position of ankyrin exon 1E 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 Mapping 5' of exon 1E. 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 ankyrin exon 1E. 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 between the two 3' DNase I hypersensitive sites. 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.

Supplemental Figure S3. *DNase I sensitivity of the ankyrin erythroid promoter region in nonerythroid chromatin.* A. The map indicates the position of ankyrin exon 1E, flanking restriction enzyme sites, locations of 5' and 3' DNase I hypersensitive sites, and locations of probes used in DNase I mapping. B Mapping 5' of exon 1E. Nuclei from the human neural cell line SH SY5Y 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 ankyrin exon 1E. Nuclei from SH SY5Y 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. D. Mapping the 2kb region between the two 3' DNase I hypersensitive sites. Nuclei from SH SY5Y 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.

Supplemental Figure S4. *Transgenes*. Wild type ankyrin promoter/$^{A}\gamma$-globin reporter transgenes, top three transgenes, are shown. The -296 Mutant -108/-153/$^{A}\gamma$ and the cHS4-296 Mutant -108/-153/$^{A}\gamma$ cHS4 transgenes are shown at the bottom for comparison.

Supplemental Figure S5. *RNase protection analysis of mRNA transcription initiation in ankyrin erythroid promoter/$^{A}\gamma$-globin reporter transgenic mice*. The riboprobe utilized is diagrammed at the top of the figure. Lanes 1-3. RNase protection of reticulocyte RNA from mice carrying 1, 3 or 9 copies of the -296 WT/$^{A}\gamma$ transgene. Lanes 4-12. RNase protection of reticulocyte RNA from -296 Mutant -108/-153/$^{A}\gamma$ transgenic mice. Lanes 13-19. RNase protection of reticulocyte RNA from cHS4-296 Mutant -108/-153/$^{A}\gamma$ cHS4 transgenic mice.

Supplemental Table S1. *Oligonucleotide primers for chromatin immunoprecipitation*

Chromatin immunoprecipitation studies around the ankyrin 5'HS region in K562 cell chromatin

Primer Pair: 6kb 5' of Ankyrin 5'HS

Sense 5'-GGCTAGTCAAGTGAAGCAGTGGGA-3'

Antisense 5'- AGTTTGTCCCTGGCTGGTCTGAGT-3'

Primer Pair: 3kb 5' of Ankyrin 5'HS

Sense 5'-GGGAGGGGAGCAGGGCATGA-3'

Antisense 5'-TGTGCCCATGACCCAGCTAACAA-3'

Primer Pair: 750 5' of Ankyrin 5'HS

Sense 5'-TTATTTTTGGCCCTGGTGTT-3'

Antisense 5'-TCTCCGCGTGCTTTCACTA-3'

Primer Pair: Ankyrin 5'HS

Sense 5'-GCCCAGAGTTGGACATCAGG-3'

Antisense 5'-CGCACCCAGCGACTTTTAGA-3'

Primer Pair: 750 3' of Ankyrin 5'HS Promoter

Sense 5'-GATAATGGCGCGTTAGCAAG-3'

Antisense 5'-ACACTGACACATGGCACAGG-3'

Primer Pair: 3kb 3' of Ankyrin 5'HS

Sense 5'- CTCCTCTCCGGCTACCCCCA-3'

Antisense 5'- GTCCGGGTCGGGTACTCGTT-3'

Primer Pair: 6kb 3' of Ankyrin 5'HS

Sense 5'-CCAAATGCCCCCTAAATAGA-3'

Antisense 5'- GTCACTGAGCCAGCATCAAA-3'

Primer Pair: Beta globin LCR HS2 control

Sense 5'-AGAACATCTGGGCACACACC-3'

Antisense 5'-AAGCAAACCTTCTGGCTCAA-3'

Primer Pair: hsSat2 control

Sense 5'-ATCGAATGGAAATGAAAGGAGTCA-3'

Antisense 5'-GACCATTGGATGATTGCAGTCA-3'

Primer Pair: IL4 promoter control

Sense 5’-CCTGGAAGAGAGGTGCTGA-3’

Antisense 5’-CTGAAACCGAGGGAAAATGA-3’

Primer Pair: CITED2 gene promoter control

Sense 5’-ACACACGGCTGGGACTCTT-3’

Antisense 5’-CGTTGTGGAAGCAAGGTTATT-3’

Primer Pair: Beta actin gene promoter control

Sense 5’-GAGGGGAGAGGGGGTAAAA-3’

Antisense 5’-CCGCTCGAGCCATAAAAG-3

Primer Pair: Hox B7 gene control

Sense 5’-TCTCTTCCTCCCCAAATCTC-3’

Antisense 5’-ATGATCAATAATGAATGGGAAAG-3

Chromatin immunoprecipitation studies around the ankyrin 1E region in stably transfected K562 cells

Ankyrin 1E Region

Sense 5’-ATCTGGGCACACACCCTAAG-3’

Antisense 5’-ACGTCACCGGTTCTAGAGGA-3’

α-Globin Control

Sense 5’-GGCCAGCCTCATCACCC-3’

Antisense 5’-TCACACAAGTACACACAGAGGTGC-3’

Keratin Control

Sense 5’-TACCCATGAGTATAAAGCACTCGC-3’

Antisense 5’-CGGCCAGGACGGAGG-3’