Supplemental Data

Chromatin Architecture and Transcription Factor Binding Regulate Expression of Erythrocyte Membrane Protein Genes

Laurie A. Steiner, Yelena Maksimova, Vincent Shultz, Clara Wong, Milind C. Mahajan, Sherman M. Weissman, Patrick G. Gallagher

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Supplemental Table 1. Primers for mRNA Expression Studies.

Gene	Primer Location	Forward Primer	Reverse Primer
ankryin-1	Exons 39-40	GAGGCCAAGAACACCTTCAC	TGCTCCTCTGTCACCTGCT
SPTA1	Exons 50-51	CATGAGCCCAAGTTTGAGAAG	GCAGTATAGTCCTCCAGTGAGACA
Band 3	Exons 19-20	AGGCAACCTTTGATGAGGA	AGGTGGGGATGTGGAATG
actb	Exons1 -2	GCACAGAGCCTCGCCTTT	CGGCGATATCATCATCCAT
ADD2	Exons 4-5	GCAGATGAAGAAGGGGAACA	GGCGTCATCATGGAGACATT
ADD3	Exons 12-13	TCAGTTGCTTGCTGGAATTG	ATGGGTTAGGAGGAGCTGGT
ADD1	Exons 13-14	AACCCCTTCACCACACTCAC	TCTGTTCTCTAGCCTCGTCCA
SPTB	Exons 25-26	CTCCACCTGCTGGGTGTC	ACACCTCCTGCTCCTTGTTC
ICAM4	Exons 1-2	CTCCAGGATCACCGCCTAC	GAATAGATGACCCGGCTTCC
ERMAP	Exons 10-11	GCAAACTCAGGCTGGAGAAG	AGGATGAGTTTGGGATGTGC
EPB41	Exons 15-16	GGTGGGATTTCAGAGACACG	GTCTGGGTGCTGCTCCTTT
EPB42	Exons 12-13	GGGGCTCATTCACAGAGAGA	GAACTGGAACTTGGCACACA
TMOD1	Exons 8-9	CATCCAACGCAATGATGAAC	CACCGCCACACACTAGACAC
TPM3	Exons 2-3	CCTGCAAAAGCTGGAAGAAG	CATCTTTTAAGGCCCGGTTT
EPB49	Exons 10-11	GTCATTGCCGATCCGAAG	TAGACGTTCCCTGGTGCAAG
RPS13	Exon 3-4	CATCTGACGACGTGAAGGAG	CTTGTGCAACACCATGTGAA
RPL27	Exon 2-3	ATGAAACCTGGGAAGGTGGT	GTCAATTCCAGCCACCAGAG
OAZ1	Exon 3-4	AAACGCATTAACTGGCGAAC	GAACTCCAGGAGAACTGCAAA
SRP14	Exon 3-4	GCTACCGATGGGAAGAAGAA	CAGCCCATCCATGTTAGCTC

Supplemental Table 2. Validation Primers for Quantitative-PCR Chromatin Immunoprecipitation Analyses.

Site of NF-E2 Occupancy	Gene	Forward Primer	Reverse Primer
1	Ank1	TCTCATTCACACCAGCAAGG	GCTGGGAGCCAGGAATAAAT
2	SPTA1	CCAGAGCCTCCCAAAACTG	CCATCAGCCATACACAATCG
3	ADD2	TCAACTGGACAGTGGGAGAA	GTGTCTGTTGGGCATAGGTG
4	ADD2	CCAACTGCTTCCCTGACATT	TGCAGAGAGAAATGGGTTTG
5	ADD2	AATGCCTGACCATGACTTGA	TGGTTTTACTAACGTGGCAAGA
6	ADD1	TTCATAGAGCGGGAAGCATT	CCAGCAGGTGTTCCACATATT
7	ERMAP	GGCACACATATTTCACTCAACC	GCATGTATTCTGCCTCCACA
8	ERMAP	CTGCTGCAGTATGTTTTGTGG	CCACTTGCTGAGTTTTGGTG
9	ERMAP	GCTCCATGAGTCAAGCGATTA	TGACAAAATGGGAAGGATGC
10	ERMAP	CTGCTTCAGGGCTGTTTCTT	TTACCTTCCCATTCCCACCT
11	EPB41	TTTGGAGACGGGAGTTCTTG	GCTCACTTAACATGTACTAAATGAAGGAT
12	EPB41	TCTGAAGGGAGCAGGAGAAA	TCAAGAGGCTGAAGGTCACA
13	EPB41	ACAGCTTTCCCGCTTCAGT	GGCACCTGTGTGTGTGTTTC
14	EPB41	GAAGCGTTGATGGAGTGGAC	CCGTAAAAGCGAGTTCTGGA
15	EPB42	GCCGCAGTCTAAGGAATTTG	GAGCACTGACACTGCAGGAA
16	EPB49	GGCAGGGTGTGATTTGAGAG	ATGTGTGAGGGGGAGAGAGA
17	TMOD1	CCATTTCTATCGCCACATCC	AAGAGCAGCATGGAAGAAGG
18	TPM3	AAAAGGATTTCCTGGGGATG	AGGTGAGGGGTGCTATGTGT

Site of GATA-1 Occupancy	Gene	Forward Primer	Reverse Primer
1	Ank1	CTCACTTCAGCCTCACAGCA	GCCTTCTTTCACAAATCAAGTT
2	Ank1	CTTGCAATCCTGTCTAACCTACA	AATGAGATGCAAACTCTCCACA
3	SPTA1	TGAGTGGCTGGCTTATACCC	CAGCTCTCTTCACCCCAAGA
4	ADD1	CTGTATTTCACAGGCCCACA	TACACCATTCCCACCTCTCC
5	ADD3	AGAGAGAGGCTGTGCTTTGTG	CAGAGGCAGTTATTCAGTTTGC
6	ERMAP	TTGGTATTTGTGTTTTGGTATGCT	TGAACGGTGGCTAAACCTTG
7	ERMAP	CTGGAAGACCTGGAGGTTGA	AGGAGGGTAGAGCTGGAAGG
8	ERMAP	AGGGAATGGAGGAGTGGAAA	TCCCAAACCAACTCATCACA
9	EPB41	GAACTCGCTTTTACGGAACG	GGATCTGAATTTCGTCAACCA
10	EPB41	AATCAGTTCTTCGCCAGTTGT	GAATCTGCCATTTATTGAGTGC
11	EPB41	TGGAGTGCACACTGGGTTAG	CACCTCCAAGTTCTGAATACTCC
12	EPB41	CAGGTATCTGGGGAAGAGCA	CCCTAGATCATTTTGACCCAAC
13	EPB41	TGAGAGCTTTCCTGTGTTGG	GCTAAGGATTTCCCATTTTCA
	EPB41	TTGTCTGTTTTGCGGTATTGA	AGAAAGACAGGAAAGGCTGGT
14	EPB41	ATCCCATCAGACCCTTTTGA	TCCTCACAGAGTTATTGCAGGT
15	EPB41	GCCAACAGTAATGTGTTTTGC	TGTGAATTTTCGGCCTCAGT
16	TMOD1	CCATTTCTATCGCCACATCC	AAGAGCAGCATGGAAGAAGG
17	TMOD1	ACCCCAAGAGATGCAAAATG	AACGTCAAAGTTGTCAAGTGGT
18	TMOD1	TTGTGGCTCATAGAGCTTGC	CAGGAGGGGCTCTGTTTGT
19	TPM3	TCACAAACTAGCACAATGTCAGG	CCTGGAAACACCTCCTCAAG
Positive Control	b-globin HS2	AGAACATCTGGGCACACACC	AAGCAAACCTTCTGGCTCAA

Negative Control	GABRA6	CTGAACCTTTGGAAGCTGAGA	TTATTGGCCTCGGAAGATGA
Negative Control	chr 6	ACTGAAGGCCAGGTGCTATG	GAAATGGGTTTTCATCGTGTT

Site of NF-E2 GATA-1 Co-occupancy	Gene	Forward Primer	Reverse Primer
1	KCC3	GGATACATCTGTGTAGTATGGGAAA	TCCCACCTTAGCCTCTCAAA
2	E2F3	TGGGTTTTCAGTCGCAAGA	GTGCAGGGGTCAAATATGCT
3	E2F3	CTTGAACTCTGCCTGCTCTGT	ATGCCAGGGCAGTTGTATTG
4	E2F3	CCATTGTTTTACTGTGGCCTTT	CATACAAAACTCAACTCCAGACATAC
5	E2F3	TGTGTTCAAATATACCACCTCTCTCT	CGACCGAAACCTGTTGGTAA
6	RPS24	CCATTGTGTTTTCCTGGTATTTT	AGCCATTAGTCATGTAGCGAACT
7	RBM15	GCTTATGGATTGATGGGAAAGA	ACCCCTCAAACTGGCCTAC
8	CFOS	CACAGGTTCGGATGTTAGTGG	AGAGCATCATCCAGGCAAAC
9	CJUN	TCCCATAGTTGTCCCAGTCAG	CCATCTAGGGGCTTCAAAAA
10	p45NFE2	CTGAGCGTTCTGCTGACCTT	CACCGACTCTATGCCCTCTC
11	NRF2	TCACCTTTGGGATTAGGTCTTG	TGTTCCTGAAGTTTCTGTTGG
12	RUNX1	CAGTCATCAAAGACACAGAGGAA	AAAAAGGAACCCCTCTCCAA
13	DLL3	CACCTTGCACAGCCTCTACC	CTGCTTCTCTGTGCGTGTGT

Supplemental Table 3. Oligonucleotide Probes for Electrophoretic Mobility Shift Assays.

Site of GATA-1 Occupancy	Gene	EMSA Oligos
1	ANK1	tgaatgtTTATCAtttttac accctaagctgctatcaaacaccagaactt accagaacttatttcttctatctaactgta cttcctggtctctggtatctattattctat cgtgttgGTATCAttacaag
2	ANK1	gctgtgaAGATAGtatgtgg gggcggtgtgtatctgtgtatgcatgt
3	SPTA1	ttatatgAGATATaaacctt aatgatgGGATATcttcgtg agagctgTGATATtaaagctg cagtgcaGGATAAtttggct cctgcctcTTATCTtcccctg gattatgTTATCAgggtttt tgatgGGATATCTtcgtgtt ttccacaTTATCCagatcaa
4	ADD1	tcgagttGGATATtgagaatg tgtacccAGATAAtttatcc AGATAAtTTATCCgcatatg
5	ADD3	ctttgtgTTATCTatactct ggggaaacacagataagatagatggtt aagacacatgactatcccagcaatgctaa cctctggctttatcactccactaagtt
6	ERMAP	ttttattTTATCAatttata ttcctgaATATCCcaatctt
7	ERMAP	gcttctcCGATAAggagattg tagtcctGGATACtttggtg ggttattTTATCTttaaact
8	ERMAP	aggaagaGGATAAgttagga gaactggAGATACcagtttg
9	EPB41	no GATA-1 sites
10	EPB41	gtgtcttGGATATtattata aaaagcaGGATATatagccc gtacatgCTATCTggcgtac
11	EPB41	attgcatTTATCAtagcatt catagaaAGATAAagaagaa agaacttTGATATtttata
12	EPB41	tgtgcagGTATCTggggaag
13	EPB41	ttgggcaGGATAAtaccttc taggaaaTGATACtaaccta tgtgtatATATCAccatctg atatagaTGATATTATCTaa gaTGATATTATCTaatcata aaatcttGGATAGggattac

14	EPB41	gctttagTGATACTATCAaa agTGATACTATCAaacagtt ctgcactTGATATaactcat cttaccaAGATAAatctgac acatgttTTATCCtcatgtt catactgTGATACcagcctt caaatgtATATCTggtccaa tttccaaAGATACtttaaac atagactTTATCCcatcaga ggtagttTTATCCcaattat ttgatcaATATCAgtaccat accacagTTATCAttctcaa
15	EPB41	tgtgtgcATATCTttagtga gatgtgtAGATAAGATATtt gtAGATAAGATATttttgta cccttttGTATCTtcttttt CTGAAGGagataaTTGGTGT
16	TMOD1	tccatttCTATCGccacatc tcctaagGGATAAagaccaga
17	TMOD1	tgcctgaAGATAAtgcaata
18	TMOD1	cttggtcTTATCTgcagaca cataaggGGATACAGATACt gGGATACAGATACtgcaggg
19	TPM3	ggcatgaAGATAGaagtttt attctgtCTATCTtaatctg

Supplemental Data. Table 4. Evolutionary Conservation at Sites of GATA-1 Occupancy in Major Erythrocyte Membrane Genes[1]

Region of GATA-1 Binding	*Max PhastCons Score*	*Max Regulatory Potential Score*
1	0.320315003	0
2	0.998000026	0.278573006
3	1	0.060624
4	0.614	0.218105
5	1	0.330263
6	1	0.155851
7	0.299213	0.251003
8	0.973	0
9	0.645669	0.21786
10	1	0.281977
11	1	0.337995
12	0.977244	0
13	1	0.211255
14	0.988	0
15	1	0.395043
16	0.224219993	0.468782008
17	1	0.227656007
18	0.424881995	0.347528011
19	0.962	0

[1] Maximum PhasCons and regulatory potential scores were calculated for each region of GATA-1 occupancy. Regions with PhastCons scores >0.8 and Regulatory Potential scores >0 were considered to have conserved regions predicted to contain a *cis*-regulatory module.

Supplemental Table 5. Results of Electrophoretic Mobility Shift Assays.[1]

Gene	*Site Number*	*Number of Potential GATA Sites*	*Number of EMSA Positive GATA Sites*	*Location*	*Tamalpais Stringency*
Ankyrin	1	5	1	Intragenic	L2-L3
Ankyrin	2	2	0	Intragenic	L1
α-Spectrin	3	8	4	Intragenic	L4
α-Adducin	4	3	0	Intragenic	L4
γ-Adducin	5	4	2	Intragenic	L4
ERMAP	6	2	0	3’ Adjacent	L2-L3
ERMAP	7	3	2	3’ Adjacent	L1
ERMAP	8	2	0	Intragenic	L2-L3
Protein 4.1R	9	0	0	5’Adjacent	L2-L3
Protein 4.1R	10	3	1	Intragenic	L2-L3
Protein 4.1R	11	3	1	Intragenic	L2-L3
Protein 4.1R	12	1	0	Intragenic	L2-L3
Protein 4.1R	13	6	0	Intragenic	L2-L3
Protein 4.1R	14	12	4	Intragenic	L4
Protein 4.1R	15	5	2	Intragenic	L4
Tropomodulin	16	2	0	5’ Intergenic	L1
Tropomodulin	17	1	0	5’ Intergenic	L4
Tropomodulin	18	3	1	Intragenic	L4
Tropomyosin	19	2	1	Intragenic	L2-L3

[1] Intragenic regions of GATA binding are within an exon or intron. Adjacent regions of GATA-1 binding are within 10 kb of the 5’ or 3’ end of the gene. Intergenic regions of GATA-1 binding are greater than 10 kb from the 5’ or 3’ end of the gene.

Supplemental Data Table 6. Evolutionary Conservation at Sites of NF-E2 Occupancy in Major Erythrocyte Membrane Genes[1]

Region of NF-E2 Binding	*Max PhastCons Score*	*Max Regulatory Potential Score*
1	1	0.410688
2	0.645669	0.338894
3	0.977244	0
4	1	0.308142
5	0.932323	0.218286
6	0.948	0
7	1	0.155851
8	0.962	0.282132
9	1	0.323301
10	0.858	0.42025
11	0.327748	0.510371
12	0.991	0.214608
13	0.997	0
14	0.614	0.218105
15	0.653543	0.520551
16	0.998	0.278573
17	0.15874	0.468782
18	1	0.227656

[1] Maximum PhasCons and regulatory potential scores were calculated for each region of NF-E2 occupancy. Regions with PhastCons scores >0.8 and regulatory potential scores >0 were considered to have conserved regions predicted to contain a *cis*-regulatory module.

Supplemental Figure Legends

Supplemental Figure 1. RNA Polymerase II binding at the promoters of the erythrocyte membrane genes. Integrated genome browser view of RNA Polymerase II binding at the promoters of the erythrocyte membrane genes in erythroid (K562) and non-erythroid (HeLa) cells. A. Ankyrin-1 B. Band 3 C. β-spectrin D. β–Adducin E. α-Adducin F. γ-Adducin G. ICAM-4 H. Protein 4.1R I. Protein 4.2 J. Dematin K. β-Actin L. Tropomodulin. M. Tropomyosin

Supplemental Figure 2. H3Me3K4 binding at the promoters of the erythrocyte membrane genes. Integrated genome browser view of H3Me3K4 binding at the promoters of the 15 erythrocyte membrane genes in erythroid (K562) and non-erythroid (HeLa) cells. A. Ankyrin-1 B. α-Spectrin C. Band 3 D. β-Adducin E. α-Adducin F. γ-Adducin G. ICAM-4 H. Protein 4.1R I. Protein 4.2 J. Dematin K. β-Actin L. Tropomodulin. Asterisk denotes the location of “exon 0” which is not listed in the UCSC browser. M. Tropomyosin

Supplemental Figure 3. Integrated genome browser view of regions of GATA-1 occupancy in selected erythrocyte membrane protein genes predicted by Tamalpais in K562 chromatin. A. Ankyrin-1 B. α-Adducin. C. γ-Adducin. D. Protein 4.1R. E. Tropomodulin. F. Tropomyosin.

Supplemental Figure 4. Integrated genome browser view of regions of NF-E2 occupancy predicted by Tamalpais in K562 chromatin. A. Ankyrin-1. B. α-Adducin C. β-adducin. D. Protein 4.1R E. Protein 4.2. F. Dematin G. Tropomodulin H. Tropomyosin.

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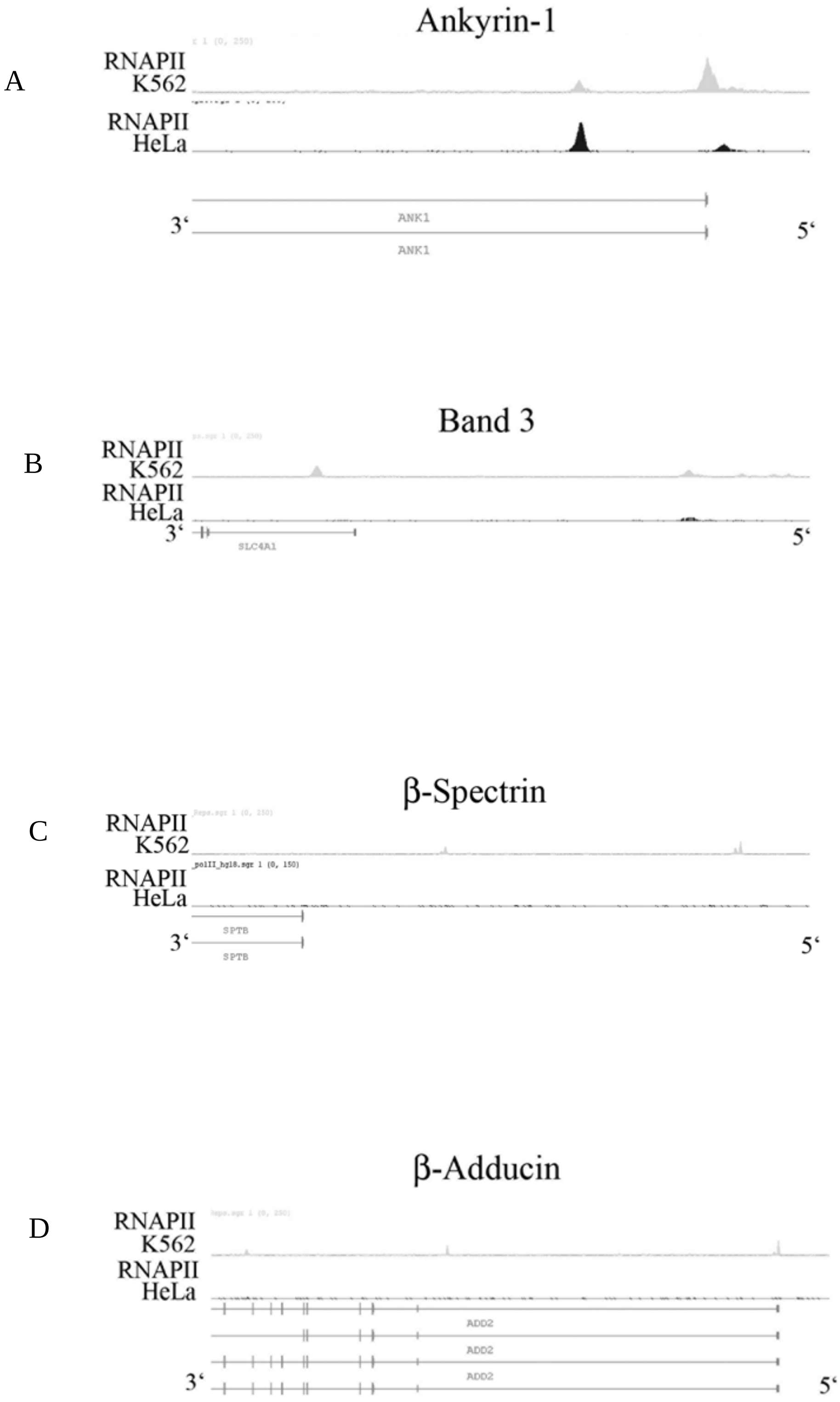

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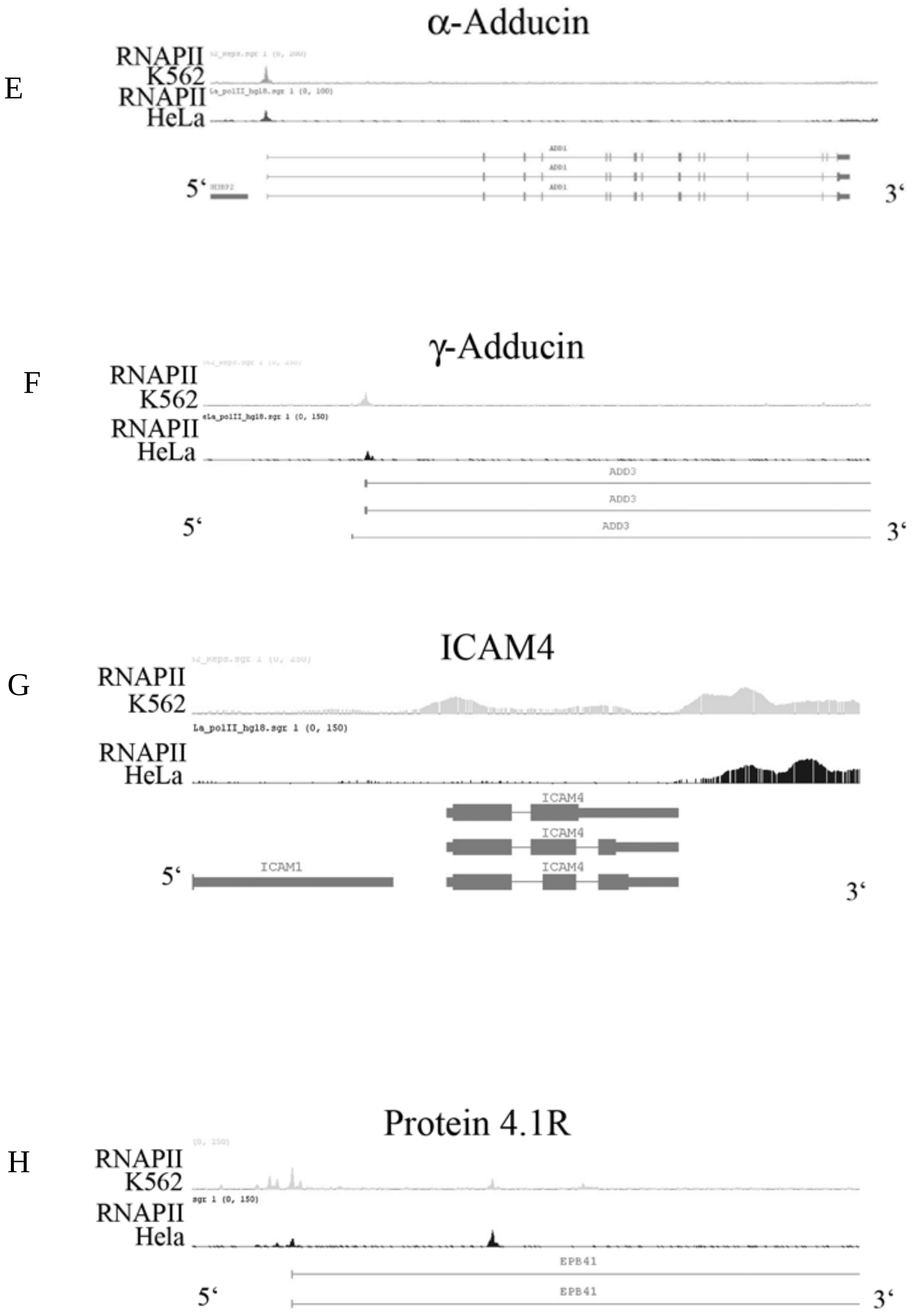

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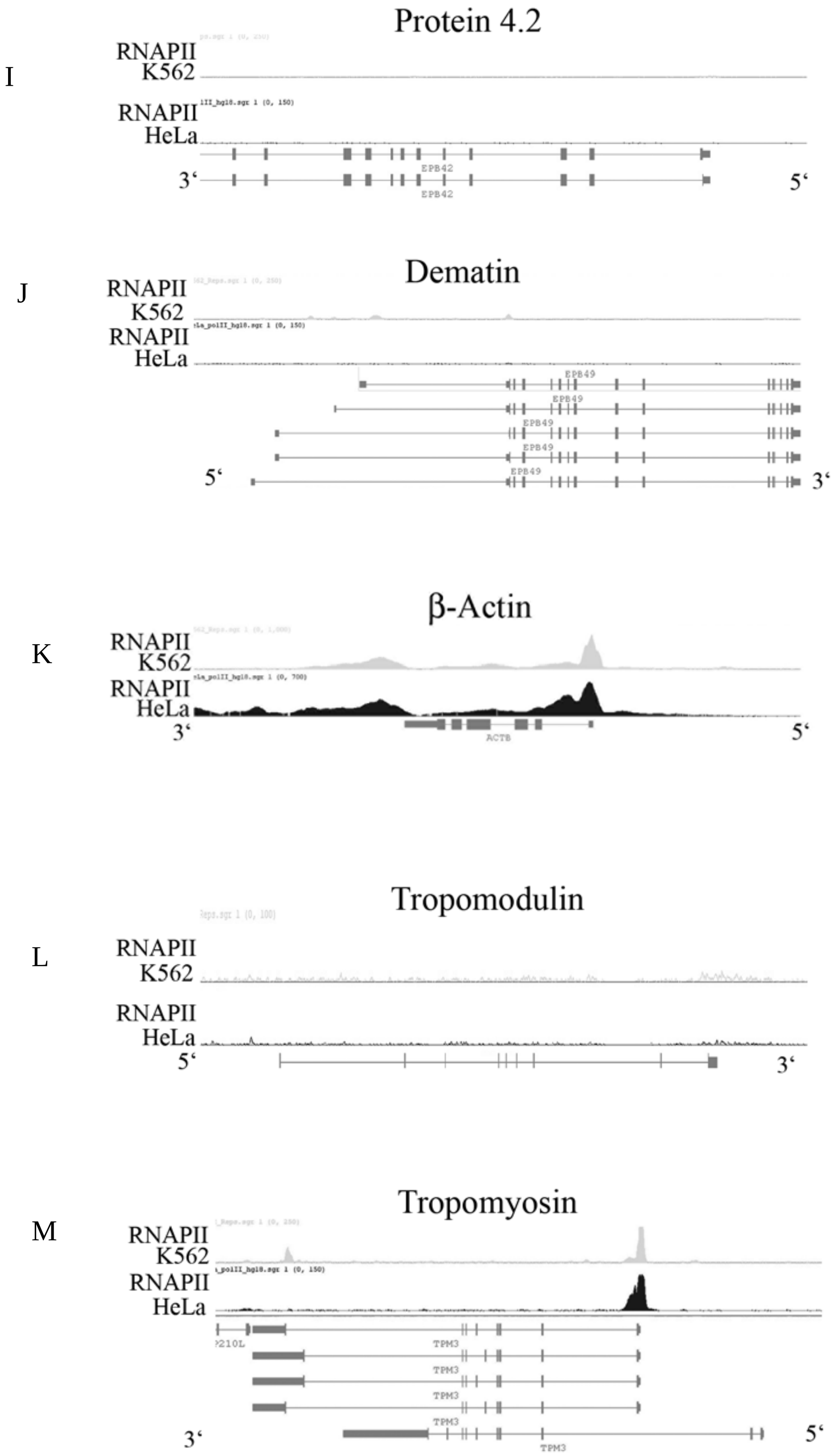

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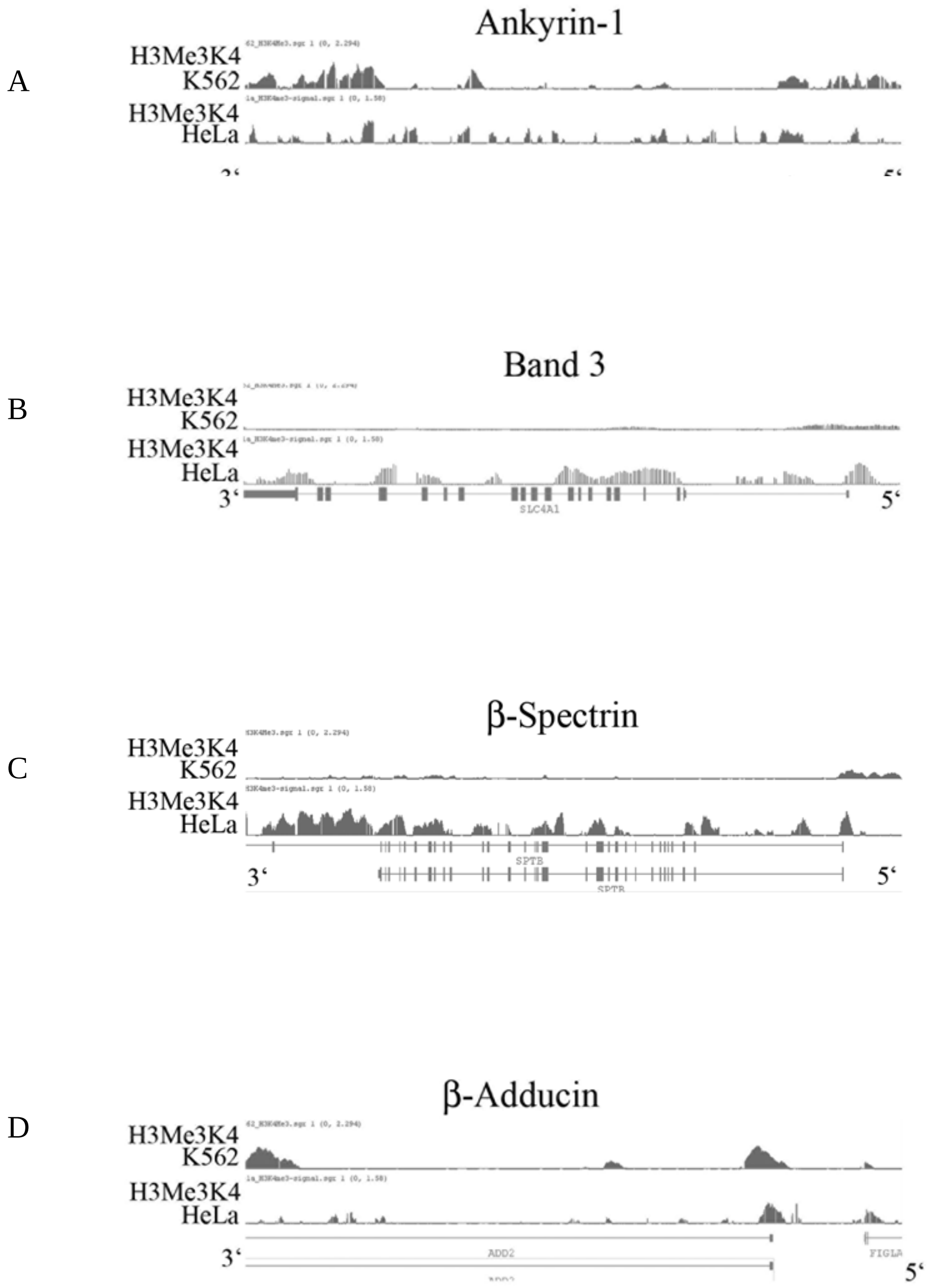

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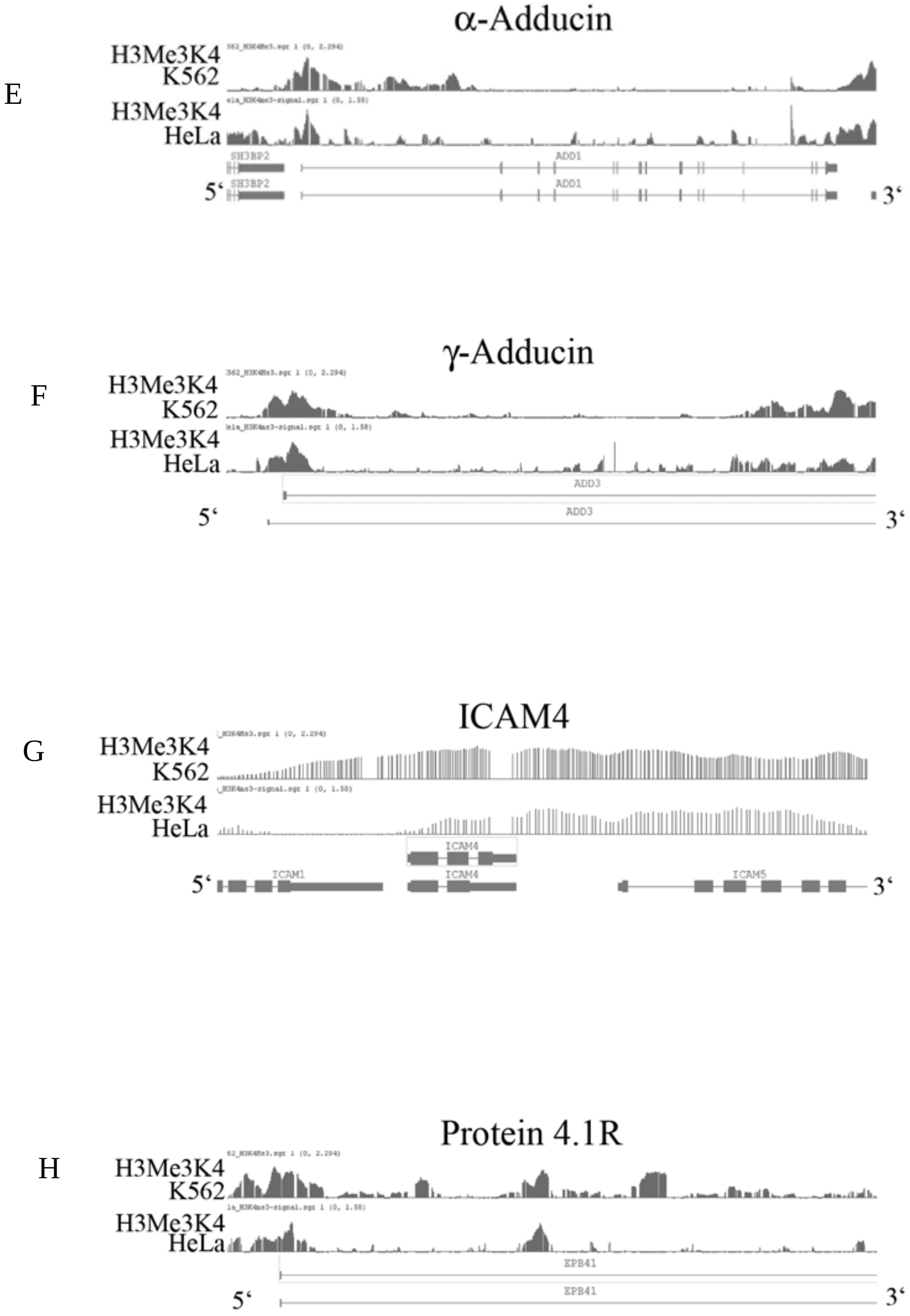

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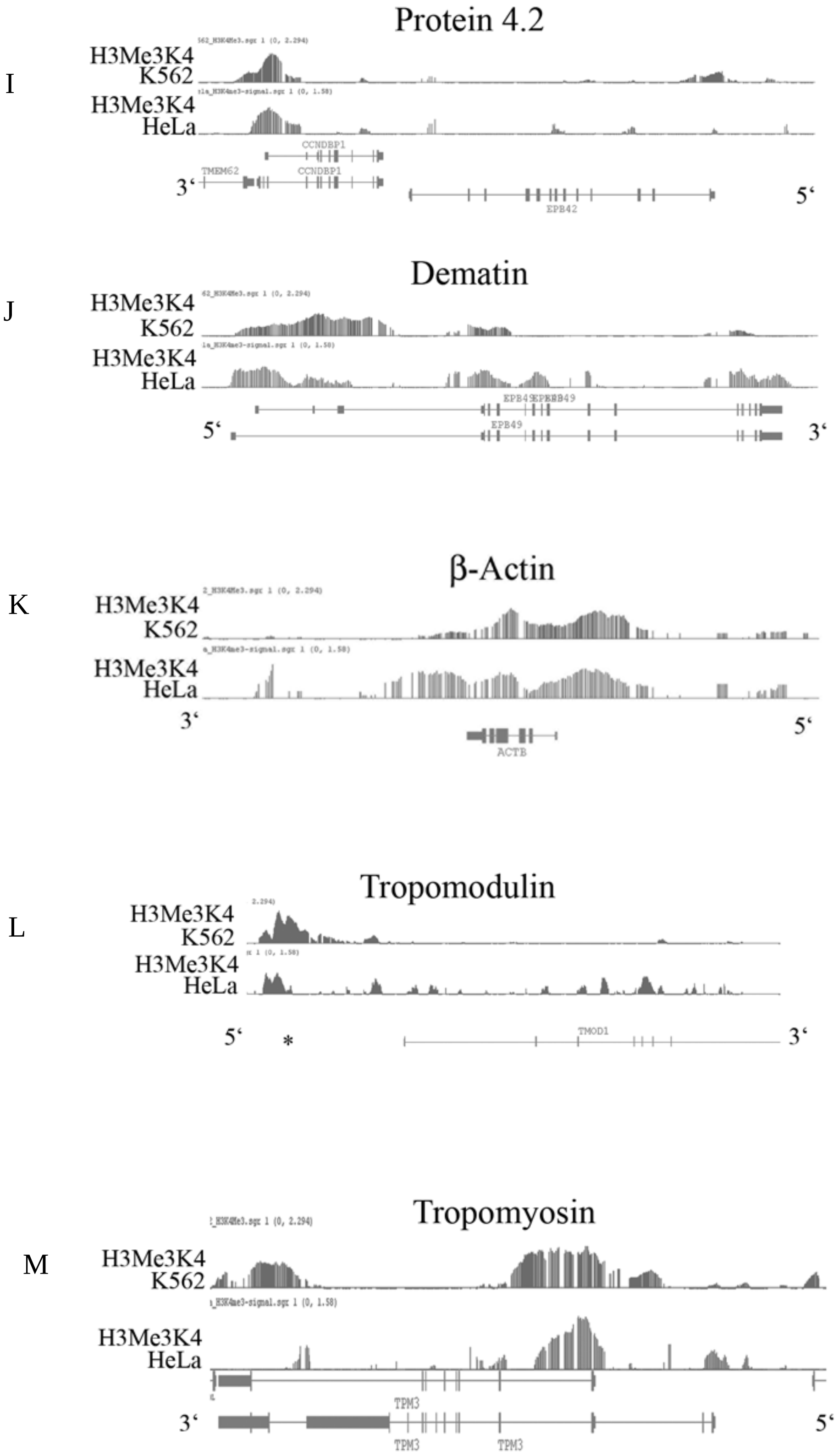

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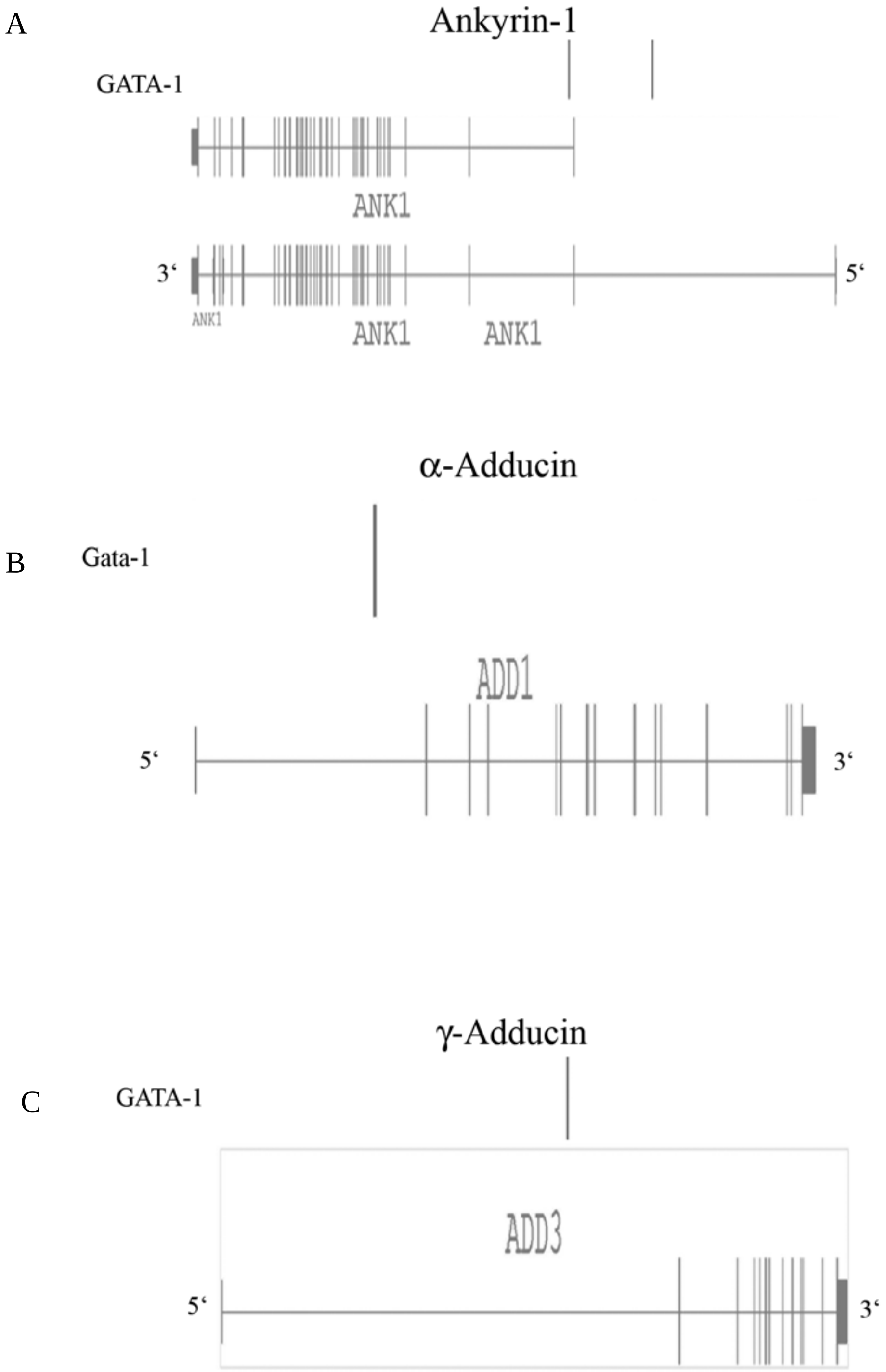

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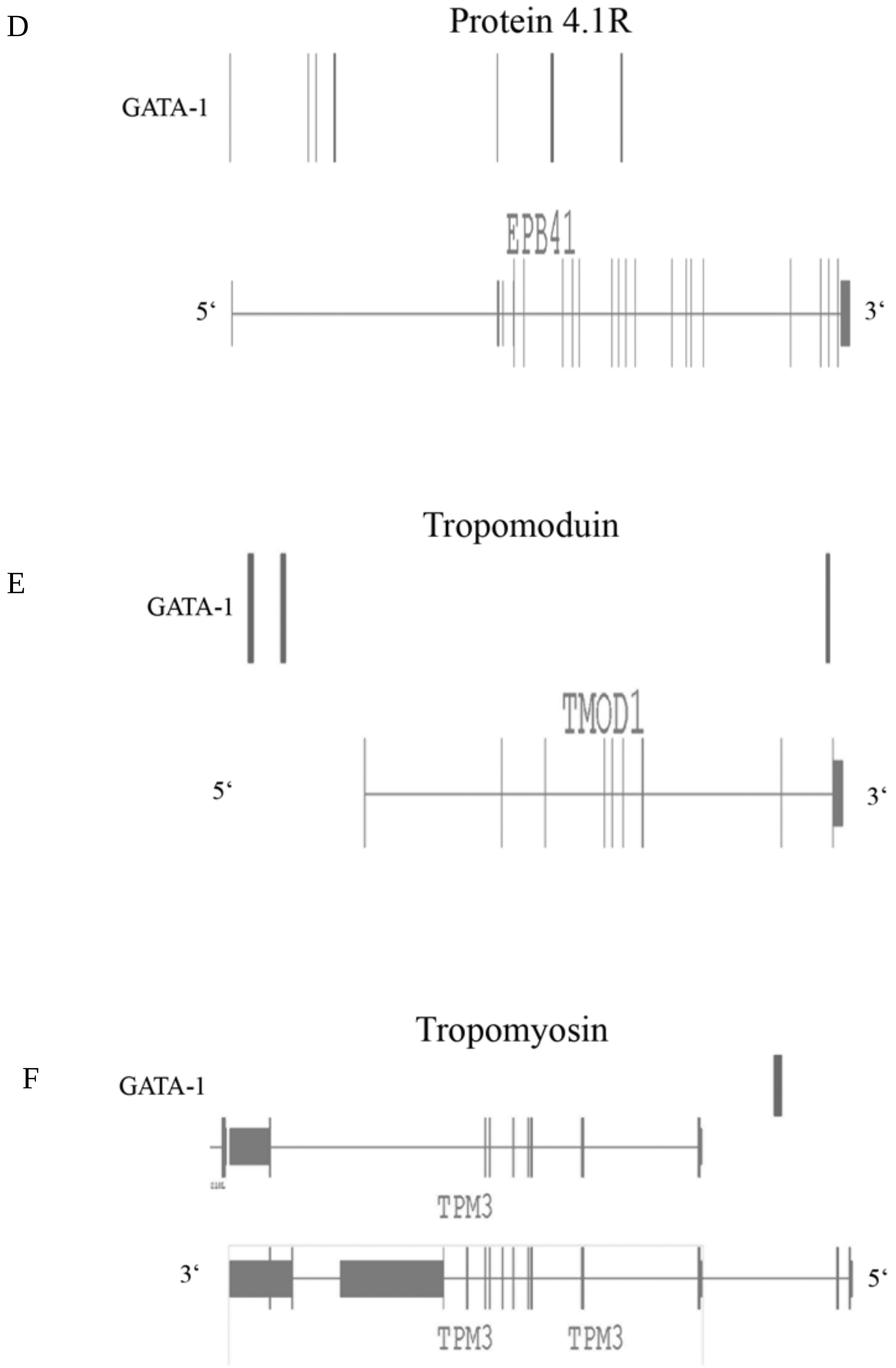

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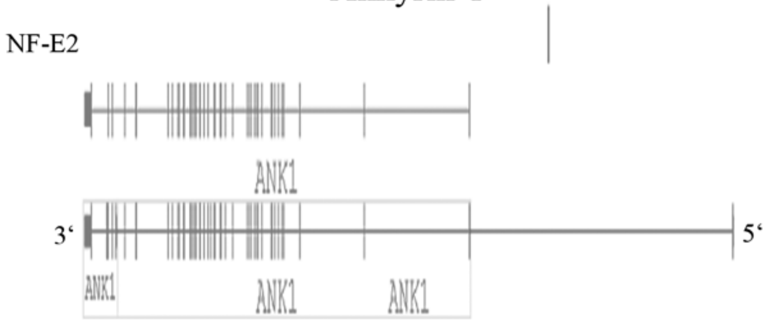
A

Ankyrin-1



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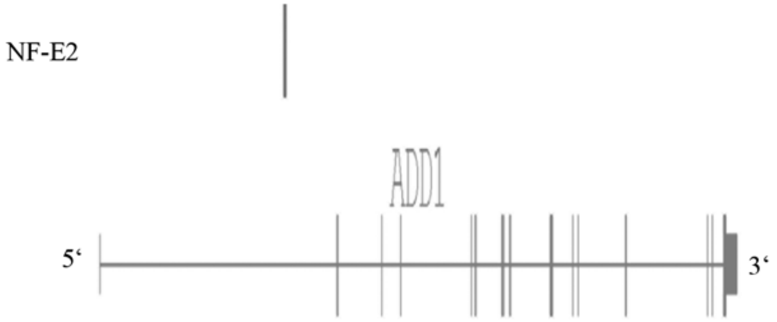

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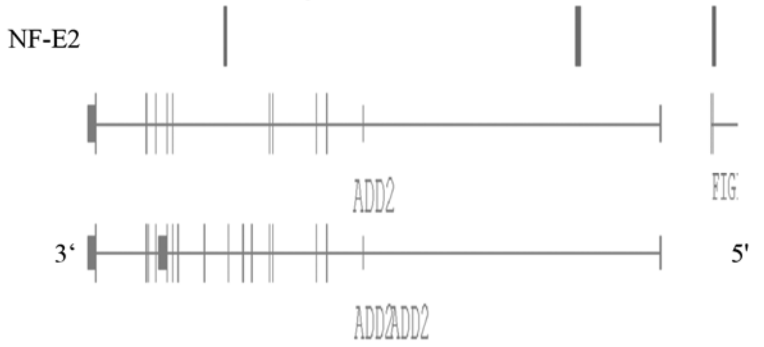
β-Adducin



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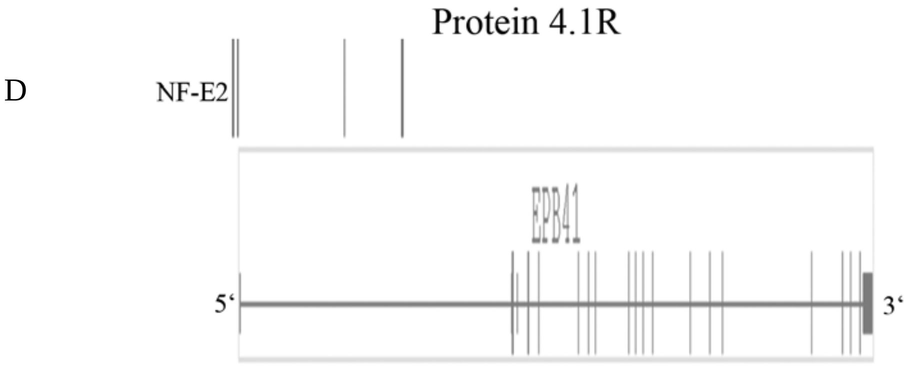

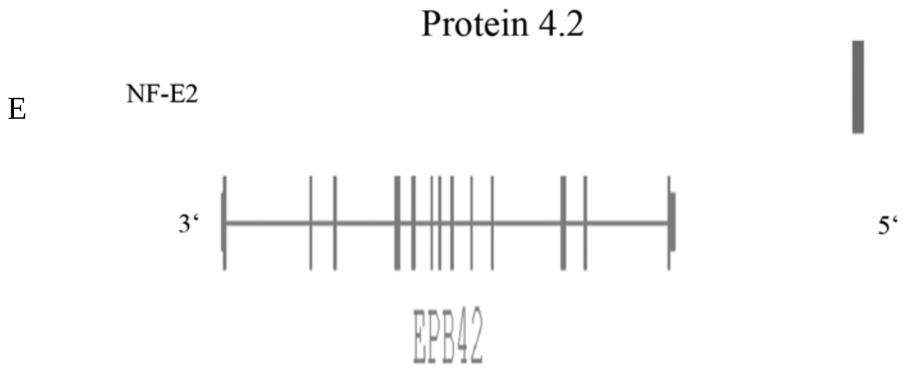

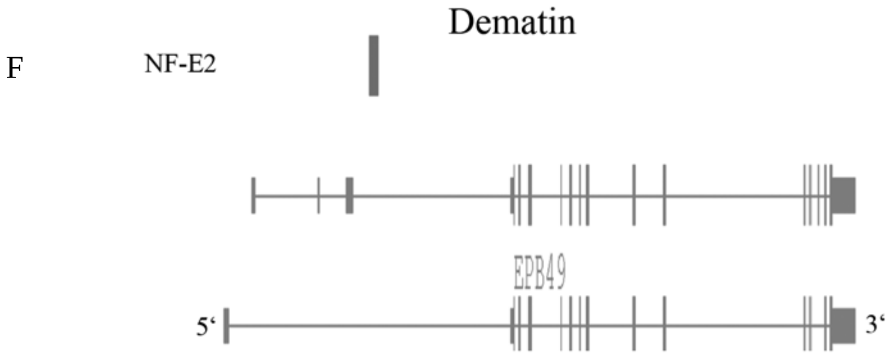

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