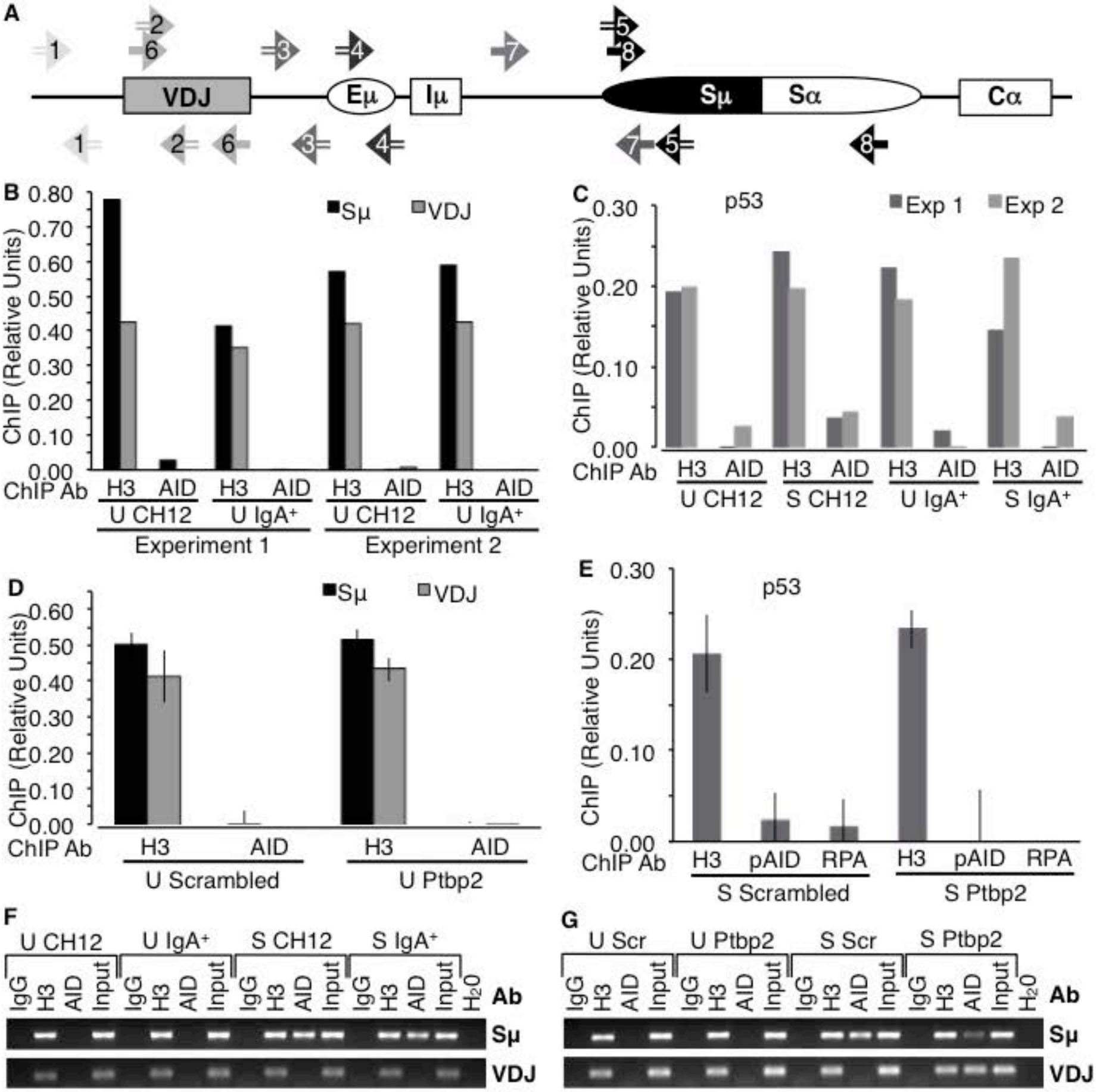
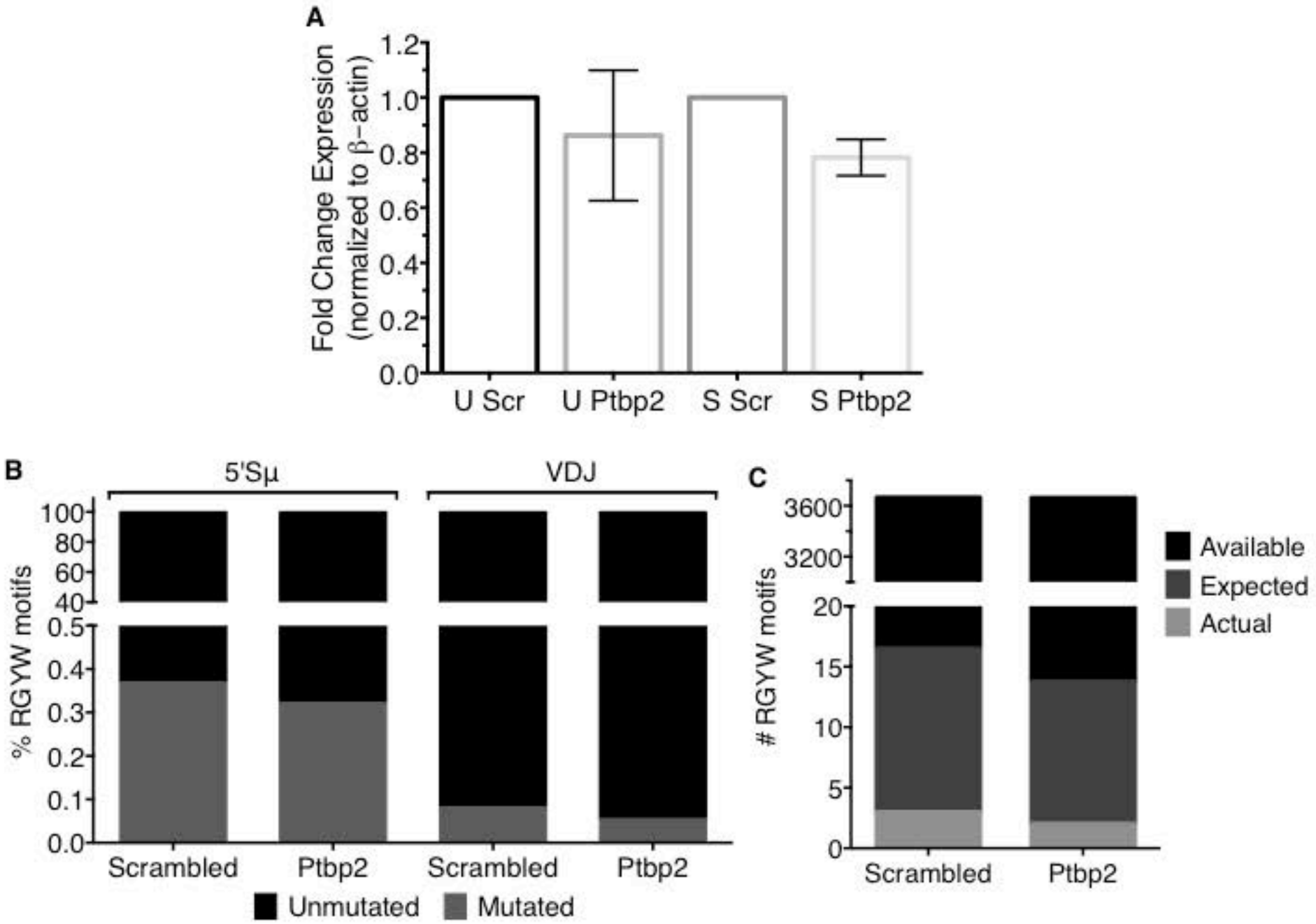


SUPPLEMENTARY FIGURE 1. Control samples/regions for ChIP analysis and representative gels. (**A**) Location of ChIP and sequencing primers within the *Igh* locus of an IgA+ CH12 cell. Positions of primers used for ChIP (designated by arrows with double lines) and sequencing (designated by arrows with solid lines) analyses are shown. Numbers within arrows correspond to primer sequence listed in Supplementary Table 2. All distances approximate. Presence of specified DNA region was quantitated by qPCR using sequence-specific primers. For n > 2, mean and standard deviation shown. (**B**) AID or H3 binding to Sμ or VDJ in unstimulated (U) CH12 or IgA+ cells. Two independent experiments shown. (**C**) AID or H3 binding to p53 sequence (negative control) in unstimulated (U)- and CIT-stimulated (S) CH12 and IgA+ cells. Two independent experiments shown. (**D**) AID or H3 binding to Sμ or VDJ in unstimulated (U) CH12 cells expressing either scrambled or Ptbp2 shRNA constructs; n = 4. (**E**) Binding of pS38-AID (pAID), RPA or H3 to p53 sequence (negative control) in CIT-stimulated (S) scrambled and Ptbp2-depleted (Ptbp2) CH12 cells; n = 3. (**F-G**) Representative gels of ChIP Sμ and VDJ qPCR reactions from unstimulated (U) and stimulated (S) (**F**) CH12 or IgA+ cells and (**G**) CH12 cells expressing either scrambled (Scr) or Ptbp2 shRNA constructs.



SUPPLEMENTARY FIGURE 2. *Igh* V gene (VDJ) transcript levels and RGYW mutation spectra in shRNA-transduced CH12 cells. (**A**) Normalized fold change expression of VDJ transcripts quantitated by one-step qRT-PCR with SYBR Green in RNA harvested from unstimulated (U) or CIT-stimulated (S) CH12 cells expressing scrambled (Scr) or Ptbp2 shRNA constructs; n = 4. 'Fold Change Expression' was calculated using crossing threshold (Ct) values and the Pfaffl method for analyzing real-time data assuming 100% efficiency, which sets fold change expression equal to the ratio of [2 to the power of ΔCt target (control – treated)] over [2 to the power of ΔCt reference (control – treated)]; where target is VDJ, reference is β-actin, control is scrambled and treated is Ptbp2. For unstimulated samples U Scr was used as the control, while for stimulated samples S Scr was used as the control. Data represent the mean and error bars show standard deviation from the mean. RNA was isolated from 3 x 10^6 CH12 cells cultured with or without CIT for 96 hours. VDJ primers used for ChIP analysis were used for qRT-PCR analysis. (**B**) Percentage unmutated (black) and mutated (gray) RGYW motifs found in total bases sequenced from the 5′ Sμ region or *Igh* V gene (VDJ) of CIT-stimulated scrambled or Ptbp2-depleted (Ptbp2) CH12 cells. (**C**) Total number of RGYW motifs: available for mutation (black), actually mutated (light gray), or expected to be mutated if mutation frequency was equivalent to that found in 5′ Sμ region (medium gray), of total *Igh* V gene (VDJ) bases sequenced from CIT-stimulated scrambled or Ptbp2-depleted (Ptbp2) CH12 cells. Data represent four independent samples.

A. **VDJ**

	# sequences	# of sequences with n mutations: 0	1	bases sequenced	total mutations	mutation frequency
U IgA$^+$	231	230	1	66,297	1	1.51E-05
S IgA$^+$	239	238	1	68,593	1	1.46E-05

B. **Sμ-Sα**

	# sequences A	# sequences B	total # sequences	# sequences with deletion	# of sequences with n mutations: A 1	A 2	B 1	B 2
U IgA$^+$	43	138	182	1	1	0	1	0
S IgA$^+$	36	132	185	17	13	1	7	0

	total mutations: 0	1	2	bases sequenced	total mutations	mutation frequency
U IgA$^+$	180	2	0	65,515	2	3.05E-05
S IgA$^+$	164	20	1	64,568	20	3.10E-04

C. **VDJ**

	# sequences	# of sequences with n mutations: 0	1	2	bases sequenced	total mutations	mutation frequency
U Scrambled	232	232	0	0	66,584	0	0.00E+00
S Scrambled	228	225	3	0	65,436	3	4.85E-05
U Ptbp2	229	229	0	0	65,723	0	0.00E+00
S Ptbp2	228	226	2	0	65,436	2	3.06E-05
3 wks S Ptbp2	191	190	1	0	54,817	1	1.82E-05

D. **5′ Sμ**

	# sequences	# of sequences with n mutations: 0	1	2	bases sequenced	total mutations	mutation frequency
S Scrambled	111	98	12	1	62,160	14	2.25E-04
S Ptbp2	112	104	7	1	62,720	9	1.43E-04

E. **RGYW**

	5′ Sμ: # seqs	# RGYW motifs	# RGYW muts	% RGYW muts	VDJ: # seqs	# RGYW motifs	# RGYW muts	% RGYW muts	# Expected if equal to 5′ Sμ
Scrambled	111	2163	8	0.37	228	3648	3	0.08	13.49
Ptbp2	112	2173	7	0.32	228	3648	2	0.05	11.75

SUPPLEMENTARY TABLE 1. Absolute number of mutations in the *Igh* V gene and S regions of IgA$^+$ or CH12 cells. Mutation frequency expressed as number of unique mutations per total bases sequenced. Mutations present in the (**A**) *Igh* V gene (VDJ) and (**B**) Sμ – Sα junction of unstimulated (U) or CIT-stimulated (S) IgA$^+$ cells. The IgA$^+$ cell line used for mutation analysis consisted of two clonal populations; both with the same *Igh* V gene, but with a different Sμ – Sα junction (indicated as either A or B sequences and mutations). Both A and B populations contained sequences with deletions and mutations (data not shown), indicating that AID was reactivated regardless of the Sμ – Sα junction expressed by the cell. One Sμ – Sα junction sequence contained 2 mutations, but was not included in the mutation frequency calculation since each mutation was also found in 2 separate sequences, and thus were not unique. Mutations present in the (**C**) *Igh* V gene (VDJ) and (**D**) 5′ Sμ region of unstimulated (U) or CIT-stimulated (S) CH12 cells expressing either scrambled or Ptbp2 shRNA constructs. Mutations in VDJ of Ptbp2-depleted CH12 cells continuously CIT-stimulated for 3 weeks (3 wks S Ptbp2) are also listed in (C). (**E**) Mutations (muts) at RGYW motifs in total bases sequenced from the 5′ Sμ region or *Igh* V gene (VDJ) of CIT-stimulated scrambled or Ptbp2-depleted (Ptbp2) CH12 cells. Percent RGYW mutations (% RGYW muts) expressed as number of RGYW motifs mutated (# RGYW muts) per total number of RGYW motifs sequenced (# RGYW motifs = number of sequences multiplied by number of RGYW motifs in sequenced region) multiplied by 100. Number expected if equal to 5′ Sμ region = 5′ Sμ % RGYW mutations multiplied by VDJ number RGYW motifs divided by 100.

#	Name	Forward	Reverse
1	5′Vh1-53	CTAATGGGCAGAGAATGTCTGTACT	CCTCCATTCATGATACATCAACTTA
2	VDJ (ChIP)	AGCCTGGGACTGAACTGGT	AGGTGTAGCCAGAAGCCTTG
3	3′Jh2	TCGGATACTGTATAAATGCTGTCAC	AGGACAGATTATCTCCACATCTTTG
4	Iμ promoter	GCTCAGCCTGGACTTTCGGTTTGGT	GGAGTCAAGATGGCCGATCAGAACC
5	Sμ	TAGTAAGCGAGGCTCTAAAAAGCAT	AGAACAGTCCAGTGTAGGCAGTAGA
6	VDJ	AACTGCAGCAGCCTGGGACTGAACTG	GGTGCCTTGGCCCCAGTAGTCAAAGTAGT
7	5′Sμ	GCGGCCCGGCTCATTCCAGTTCATTACAG	AATGGATACCTCAGTGGTTTTTAATGGTGGGTTTA
8	Sμ – Sα	TTGAGAGCCCTAGTAAGCGAGGCTCTA	GAACTGTGAATAAGTCCAGTCATGCTAAT
	p53	CCCAGAGACTGCTGTTAAAGTAGAACC	CGCCACAGCGTGGTGGTACC
	Cμ	AGCACCATTTCCTTCACCTGGA	TGTCCTCAGTGTTGGGAAGGTT
	VJκ	GTCCTTTCTTTTCCTGCTTAACTCT	GTTTAACAGTTCCATCTGGTTTCTG
	Cdc45l	TGGTCTAGAGCTCGCCAAGAAGC	CCTCCATGAGTGAGCAGTAGAGAAAAG
	Dusp1	CTGAGTTCCACTGAGTTCCTAAGCAG	CAGGCAAGCGAAGAAACTGCCTC
	Fabp5	AGGAGTAGGACTGGCTCTTAGGAAG	GTCGTCTTCACTGTGCTCTCGG
	Melk	GGCAAAGTGACAATGCAGTTTGAACTGG	GGTCACATCTTGCAGCCAGACAAG

SUPPLEMENTARY TABLE 2. Primer sequences. Name and nucleotide sequence of primers used in ChIP and mutational analyses are listed. Number corresponds to numbering depicted in Supplementary Figure 1A.