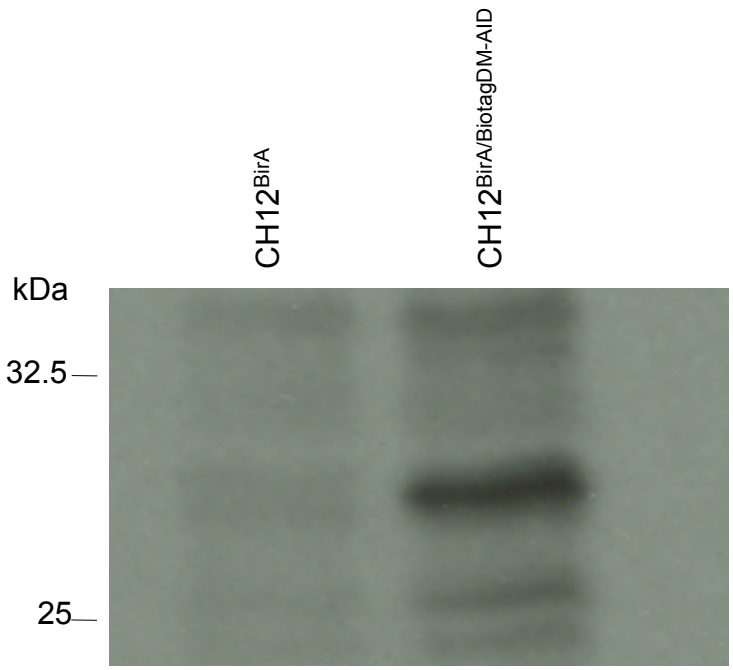
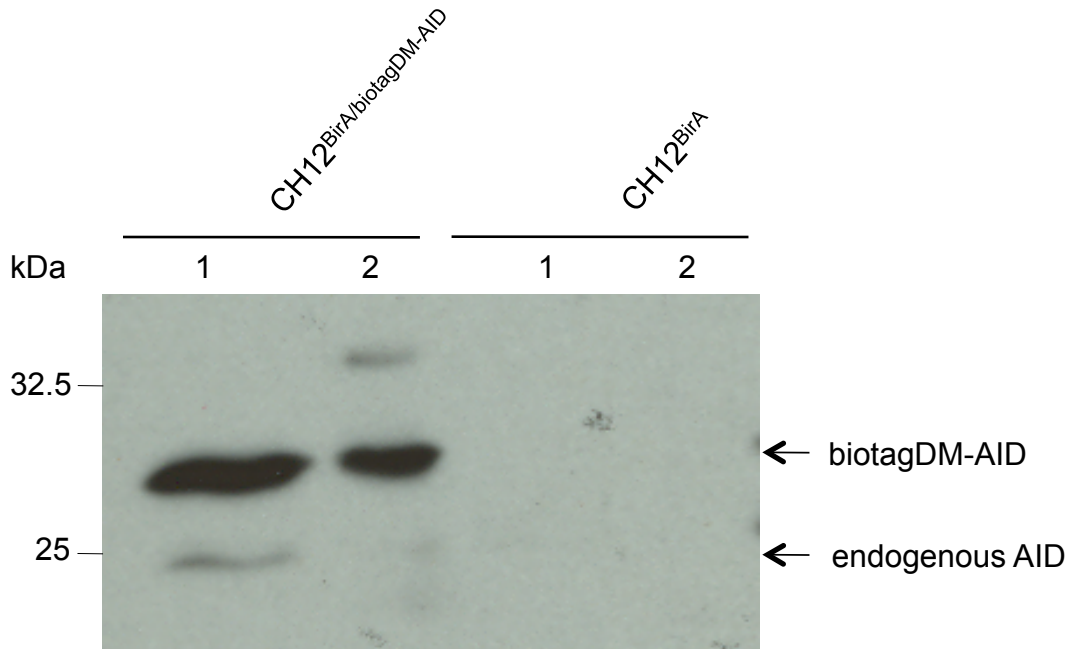
Proteins found in both MS more than once in both sets, never in control	
UPSP:TOP2B_MOUSE	dna topoisomerase 2-beta
UPSP:RAD50_MOUSE	dna repair protein rad50
UPSP:SMCA5_MOUSE	swi/snf-related matrix-associated actin-dependent regulator of chromatin subfamily a member 5
UPTR:Q640Q4_MOUSE	gcn1l1 protein
UPSP:TCPG_MOUSE	t-complex protein 1 subunit gamma
UPSP:TNPO1_MOUSE	transportin-1 (importin beta-2) (karyopherin beta-2)
UPSP:TBB3_MOUSE	tubulin beta-3 chain
UPTR:Q6JIZ1_MOUSE	acetyl-coa carboxylase 1
Proteins found in both MS once in one set and more than once in the other, never in control	
UPTR:Q3U2N8_MOUSE	riken
UPTR:Q3UW53_MOUSE	niban protein
UPSP:SYMPK_MOUSE	symplekin
UPSP:DYHC_MOUSE	dynein heavy chain, cytosolic
UPTR:Q3TML0_MOUSE	thioredoxin domain containing 7
UPSP:MRE11_MOUSE	double-strand break repair protein mre11a (mre11 homolog 1)
UPTR:Q9D2E2_MOUSE	hypothetical zinc finger
UPSP:DDX47_MOUSE	probable atp-dependent rna helicase ddx47
UPSP:GALK1_MOUSE	galactokinase
UPSP:RL4_MOUSE	60s ribosomal protein l4
UPTR:Q8BK60_MOUSE	serpinb1
Proteins found in both MS only once in both sets, never in control	
UPTR:Q3TY88_MOUSE	thyroid receptor interacting protein 12 (trip12)
GP:AK129105_1	riken
UPSP:DNL1_MOUSE	dna ligase 1
UPSP:SMRC2_MOUSE	swi/snf-related matrix-associated actin-dependent regulator of chromatin subfamily c member 2
UPSP:DPOD1_MOUSE	dna polymerase delta catalytic subunit
UPSP:TCPE_MOUSE	t-complex protein 1 subunit epsilon
UPTR:Q3TML0_MOUSE	thioredoxin domain containing 7
UPSP:IRF8_MOUSE	interferon regulatory factor 8
UPTR:Q9CYQ1_MOUSE	cytokine-like nuclear factor n-pac homolog
UPTR:Q2TB02_MOUSE	nf-kappa b inhibitor
UPTR:Q3T9A9_MOUSE	programmed cell death 4
UPSP:TCPW_MOUSE	t-complex protein 1 subunit zeta-2
UPSP:ARP3_MOUSE	actin-like protein 3
UPSP:PTBP2_MOUSE	polypyrimidine tract-binding protein 2, inhibits alternative splicing
UPSP:RBBP4_MOUSE	histone-binding protein rbbp4
UPSP:AMPM1_MOUSE	methionine aminopeptidase 1
UPTR:Q9QZ83_MOUSE	gamma actin-like protein
UPSP:ORC5_MOUSE	origin recognition complex subunit 5
UPSP:PDK3_MOUSE	[pyruvate dehydrogenase [lipoamide]] kinase isozyme 3

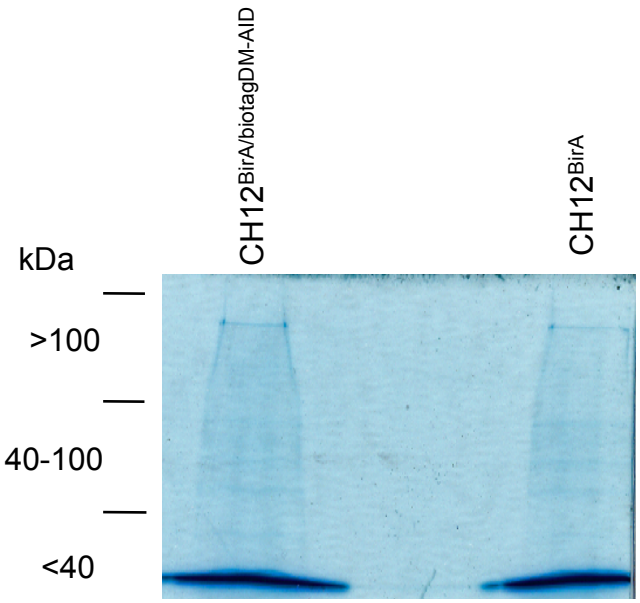
Table S1. List of potential AID interactors common to two independent mass spectrometric analyses.



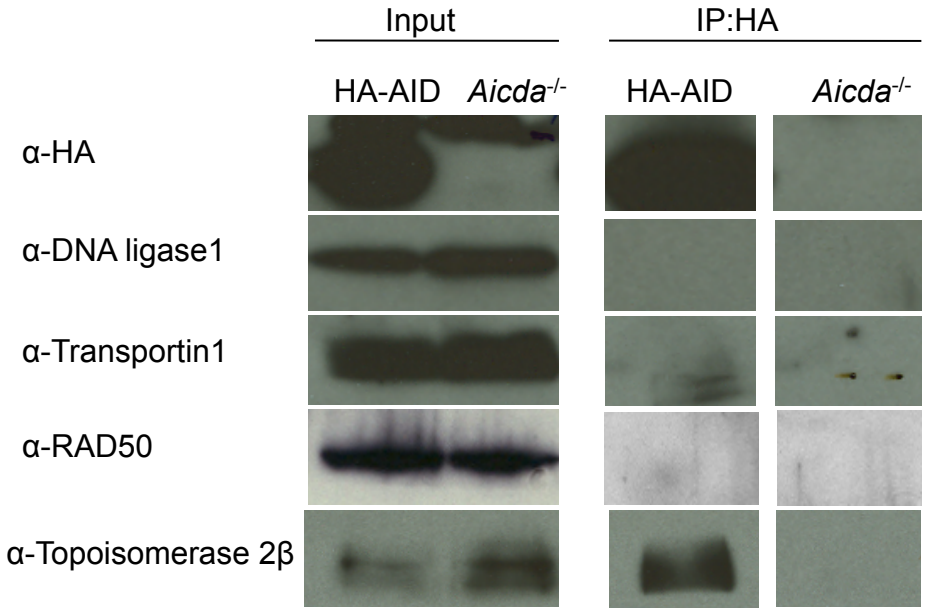
Supplementary Figure 1. Expression of biotagDM-AID in unstimulated CH12$^{BirA/biotagDM-AID}$ cells. Whole cell extracts derived from CH12^{BirA} or CH12$^{BirA/biotagDM-AID}$ cells were probed on an immunoblot with AID antibodies. Immublot shown is representative of three experiments.



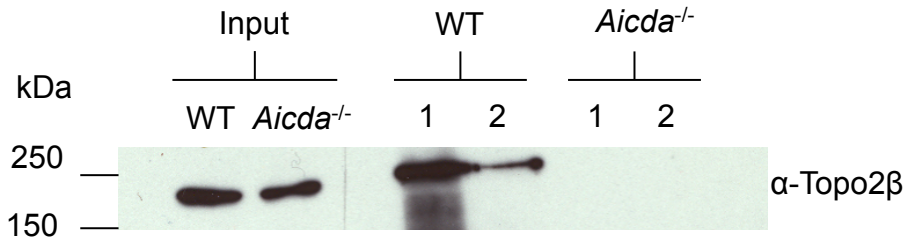
Supplementary Figure 2. Immunoblot for AID in affinity-purified samples analyzed by mass spectrometry. A fraction of nuclear extract from $CH12^{BirA/biotagDM\text{-}AID}$ or $CH12^{BirA}$ affinity purified through streptavidin agarose was analyzed by immunoblot using AID antibodies. Lanes 1 and 2 denote first and second elutions of the affinity-purified samples. Only the first elution was used for mass spectrometric analysis. The immunoblot shown is representative of two independent experiments.



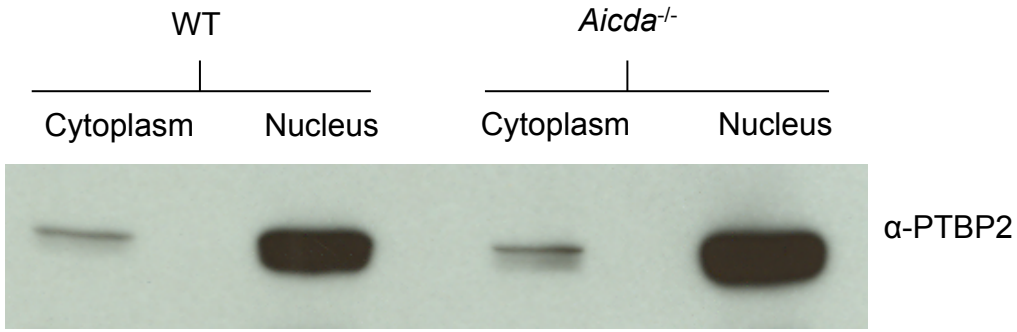
Supplementary Figure 3. SDS-PAGE gel of proteins analyzed by mass spectrometry. Nuclear extract from CH12$^{BirA/biotagDM-AID}$ or CH12^{BirA} was affinity-purified through streptavidin-agarose, bound material eluted, partially resolved on SDS-polyacrylamide gels and stained with Coomassie. Three stacks were excised from each sample on the gel and analyzed by mass spectrometry. The approximate molecular weights of the proteins in each stack is indicated. Gel represents one of two independent purification of AID complex.



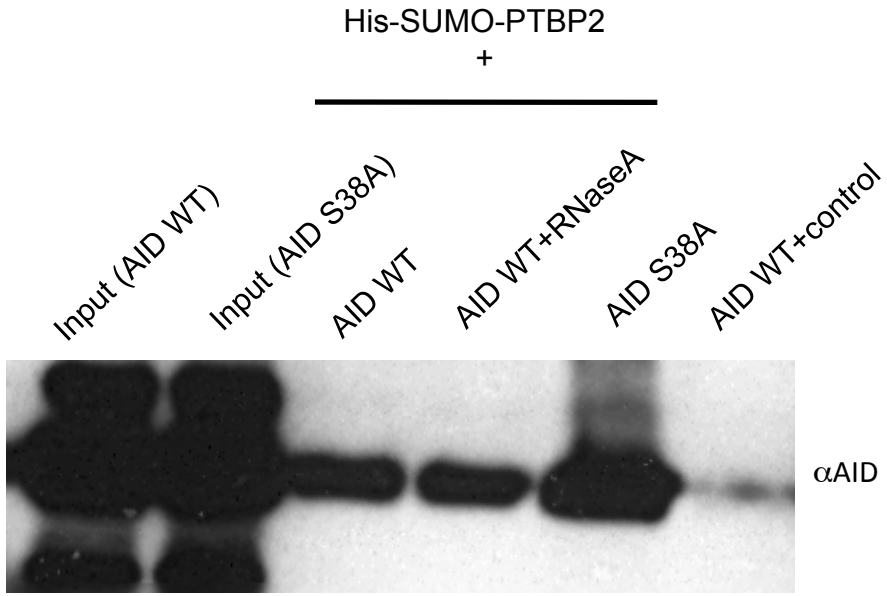
Supplementary Figure 4. Immunoblot for several candidate AID-binding proteins. HA-tagged AID was retrovirally expressed in anti-CD40+IL-4-stimulated AID-deficient mouse splenic B cells. Whole cell extracts were immunoprecipitated with HA antibody and the immunoprecipitate probed with indicated antibodies. The results are representative of two (DNA ligase1, Transportin1 and RAD50) or three (Topoisomerase 2β) independent experiments.



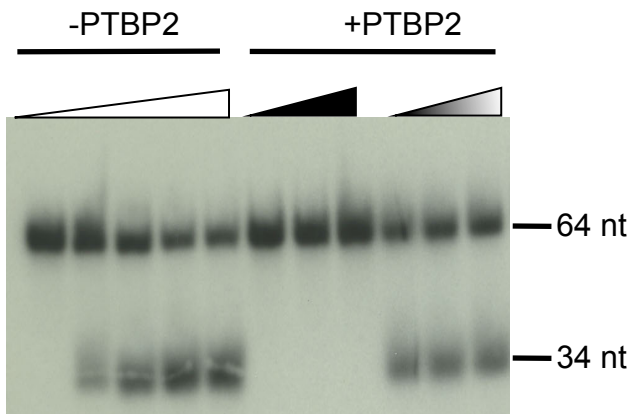
Supplementary Figure 5. Topoisomerase 2β binds to AID in primary B cells. Whole cell extracts from wild-type or *Aicda*$^{-/-}$ mouse splenic B cells stimulated with anti-CD40+IL-4 for 48h were immunoprecipitated with AID antibody and the immunoprecipitate probed on an immunoblot with antibodies against topoisomerase 2β. 1 and 2 denote first and second elutions of the bound proteins during immunoprecipitation. Data are representative of three experiments.



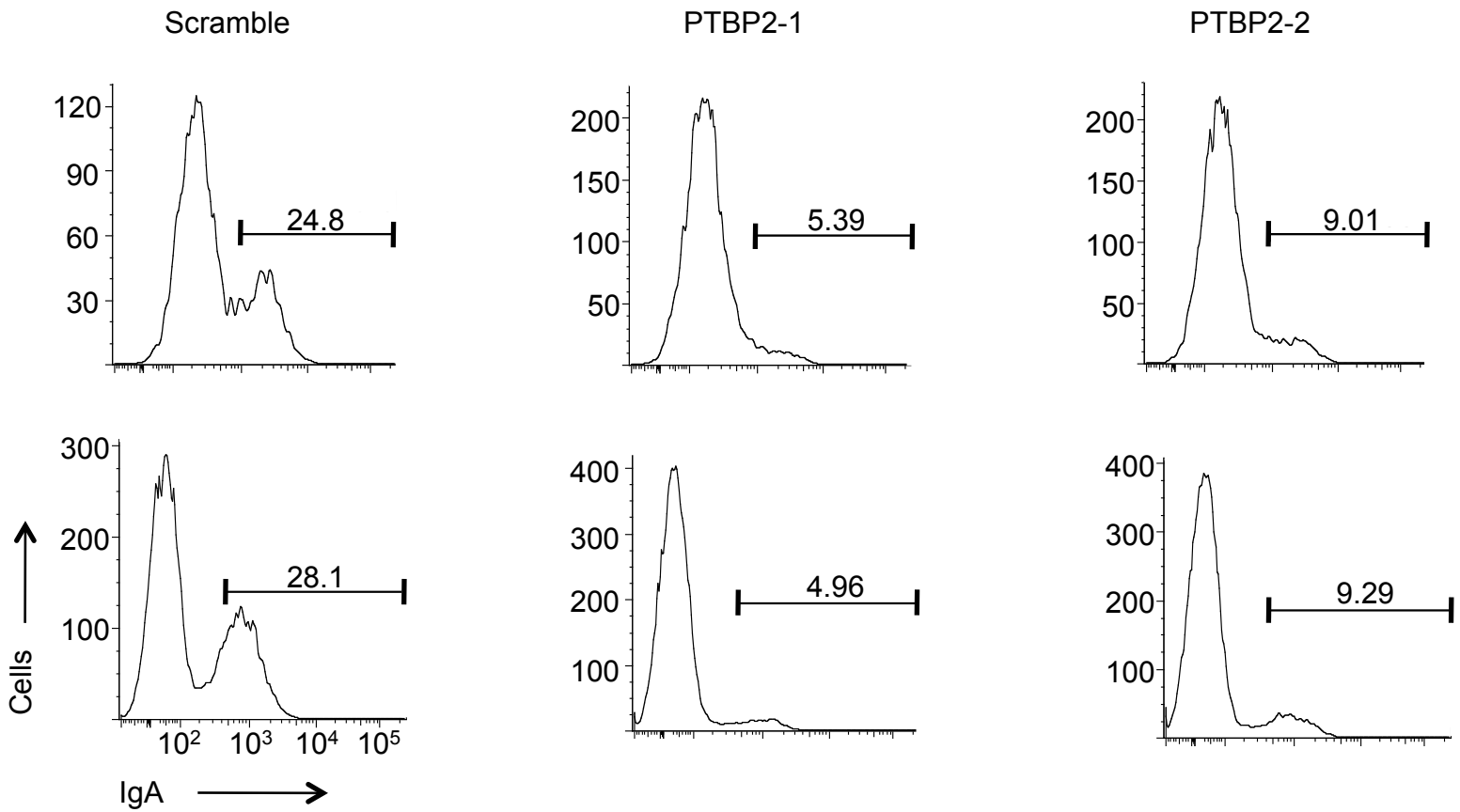
Supplementary Figure 6. PTBP2 is primarily nuclear. Splenic B cells derived from wild type or AID-deficient mice were stimulated with CIT and fractionated into nuclear and cytoplasmic fractions and analyzed by immunoblotting using PTBP2 antibodies. The immunoblot is representative of two experiments.



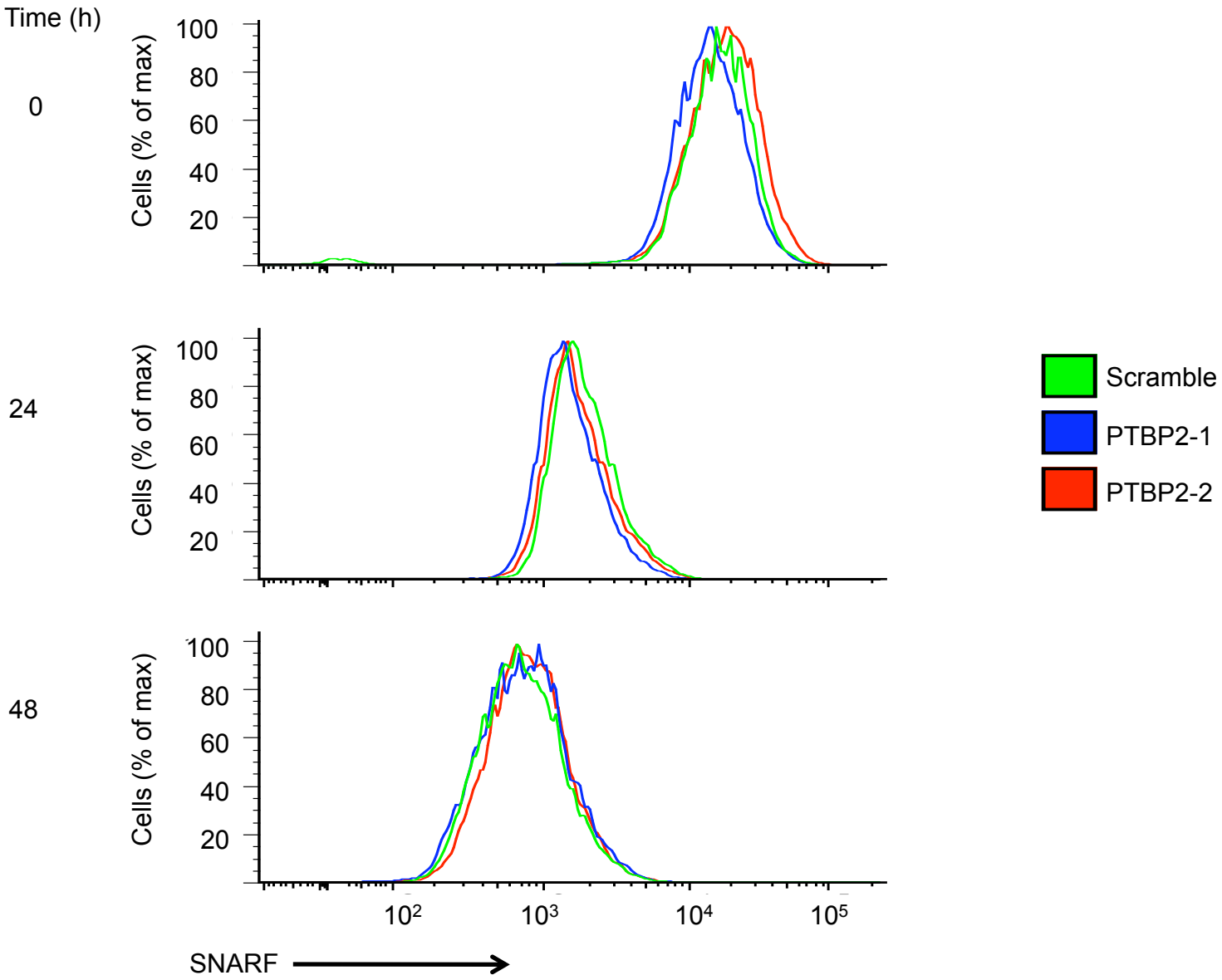
Supplementary Figure 7. Purified recombinant PTBP2 binds to AID partially purified from 293T cells. AID (wild-type or with S38A mutation) was partially purified from 293T cells and incubated with His-SUMO-PTBP2 immobilized on Ni^{2+} agarose beads. Binding of AID to Ni^{2+}-agarose beads alone served as a control. The interaction between PTBP2 and wild type AID was also assayed in the presence of RNaseA. The bound proteins were eluted with 1M NaCl and analyzed on a immunoblot with AID antibodies. The data are representative of two independent experiments.



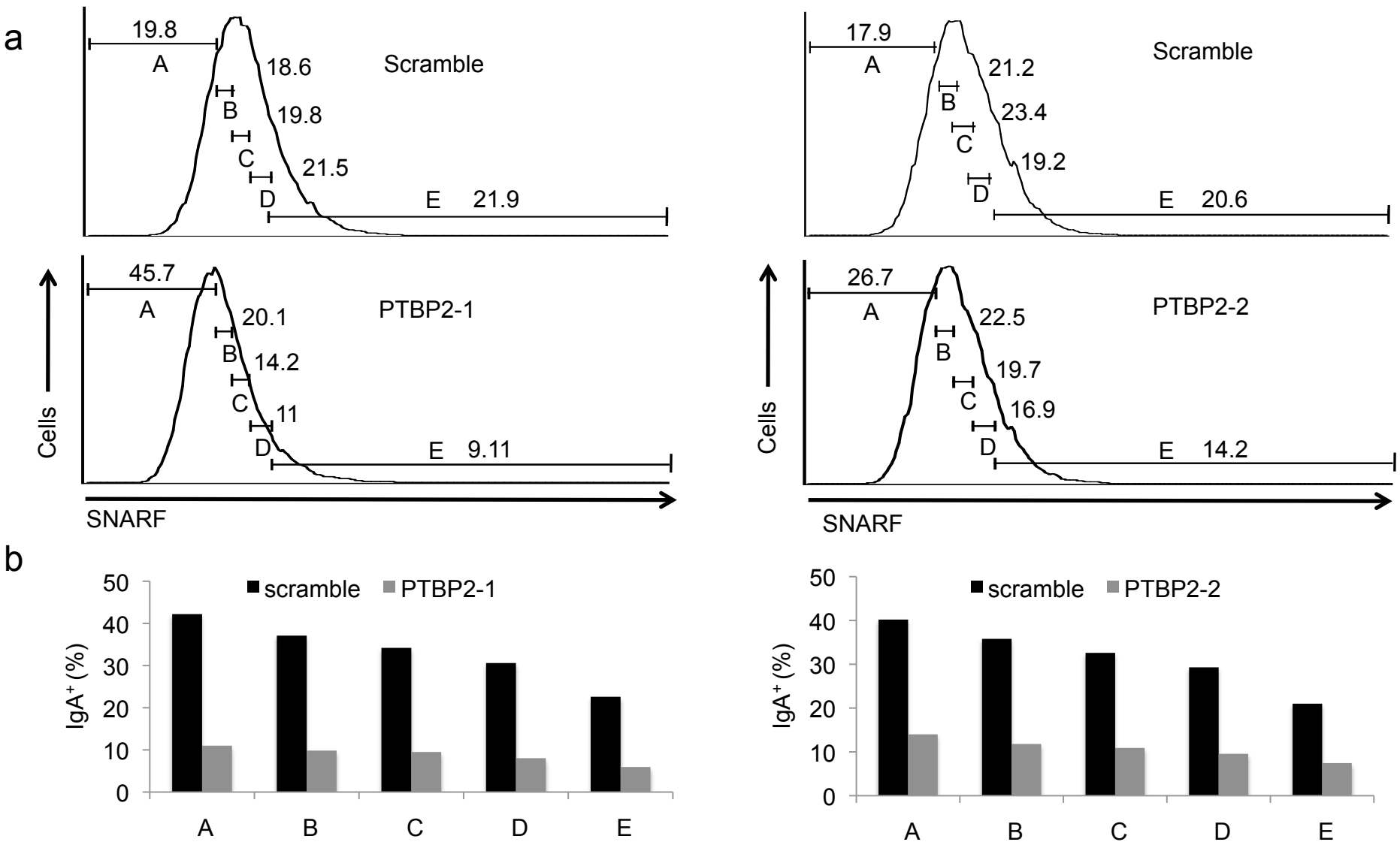
Supplementary Figure 8. PTBP2 does not alter DNA deaminase activity of AID. DNA deamination was determined in a gel-cleavage assay. A 64 base radiolabeled oligonucleotide was incubated with AID alone (open triangle, 0, 20, 50, 100 or 200 μg), PTBP2 alone (filled triangle, 50, 100, 250 μg) or AID (50 μg) and increasing amount of recombinant PTBP2 (shaded triangle, 50, 100, 250 μg). The appearance of a 34 nucleotide cleaved product was monitored by polyacrylamide gel electrophoresis and autoradiography. The assay represents one of two independent experiments.



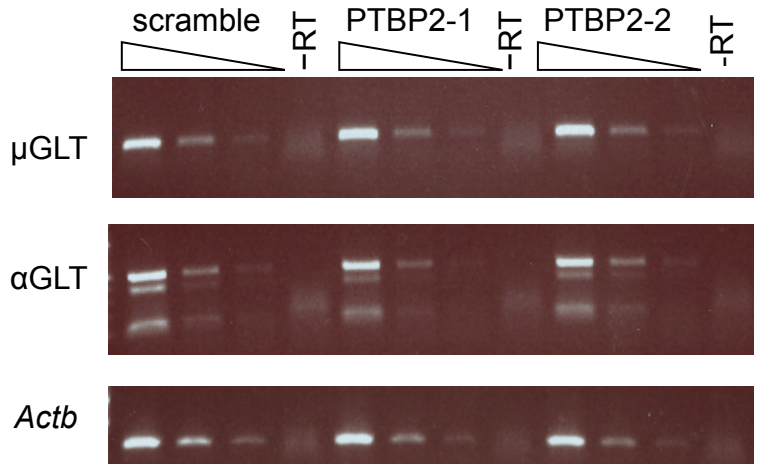
Supplementary Figure 9. PTBP2 knock-down impairs CSR to IgA in CH12 cells. PTBP2 knock-down or scramble CH12 cells were stimulated with CIT for 72 h and switching to IgA was measured by flow cytometry. Numbers indicate percentage of cells that underwent CSR to IgA. Results for two independent experiments are shown.



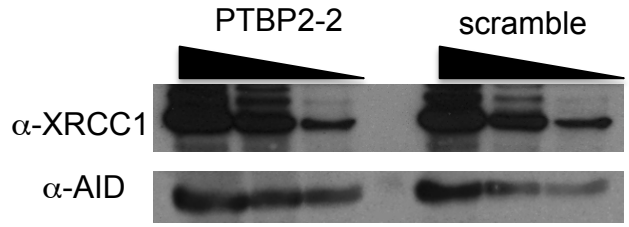
Supplementary Figure 10. Proliferation of control (scramble) or PTBP2 knock-down (PTBP2-1, PTBP2-2) cells. Cells were labeled with the permanent red dye SNARF, stimulated with CIT and analyzed for SNARF dilution by flow cytometry at 0, 24 and 48 h. Results are representative of three experiments.



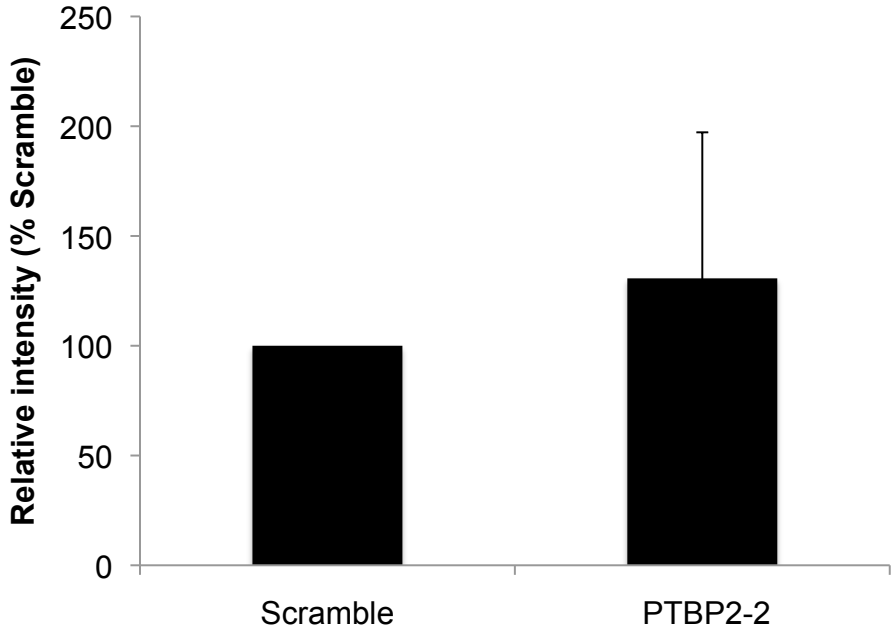
Supplementary Figure 11. PTBP2 knock-down impairs CSR regardless of the extent of proliferation. **(a)** PTBP2 knock-down or control (scramble) CH12 cells were stained with the permanent red dye SNARF (with equal SNARF expression at 0 h). The cells were then stimulated with CIT for 72 h and stained for IgA expression. The extent of proliferation (as indicated by SNARF dilution) was measured by flow cytometry. The control cells were divided into approximate 20-percentile gates based on SNARF expression (labeled A-E, top panels). Identical gates were applied to the PTBP2 KD cells (bottom panels). **(b)** The percentage of cells expressing IgA in each gate for scramble or PTBP2 knock-down is indicated. The results are representative of two experiments.



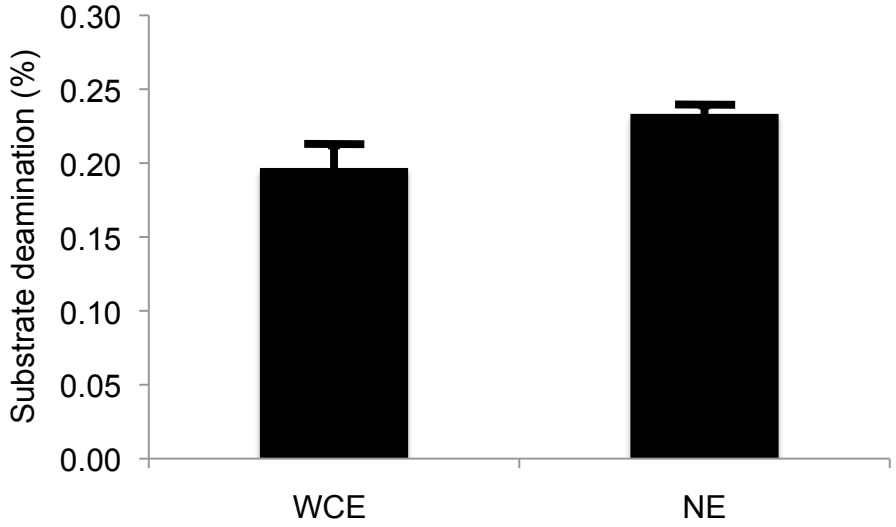
Supplementary Figure 12. Germline transcription is not affected in PTBP2 knock-down cells. RNA from PTBP2 knock-down or control cells was reverse transcribed and 3-fold dilutions of cDNA were analyzed by PCR for the abundance of μ and α germline transcripts (GLT). *Actb* transcript abundance served as a control. –RT represents PCR from template in which reverse-transcriptase was not added. Representative of three experiments.



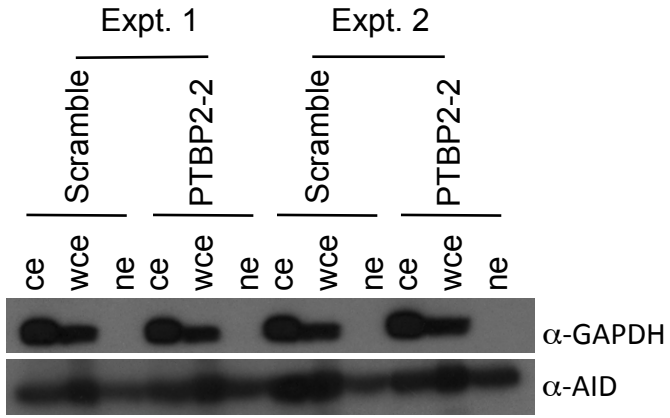
Supplementary Figure 13. Three-fold dilutions of nuclear extracts prepared from control or PTBP2 knock-down cells were analyzed on immunoblots with XRCC1 or AID antibodies. The highest concentration contained 50 μg of protein. Data is representative of two experiments.



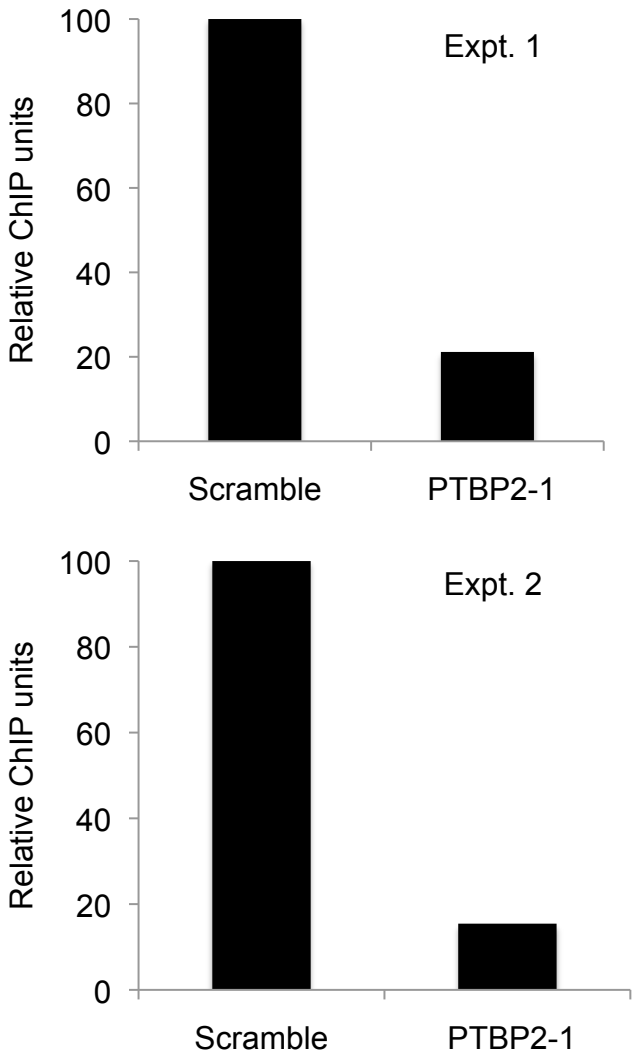
Supplementary Figure 14. Relative abundance of AID on immunoblots of nuclear extracts derived from scramble or PTBP2-2 shRNA expressing CH12 cells. Scanned western blots were densitometrically analyzed by the Image J Program to determine the intensities of AID and XRCC1 (loading control) antibody-reacting polypeptides. Relative abundance of AID was calculated as a ratio of AID:XRCC1 for each of three independent CIT-stimulations. The AID:XRCC1 ratio of scrambled cells was assigned an arbitrary value of 100 and the abundance of AID in PTBP2 knock-down cells depicted as a percentage of scramble shRNA expressing cells. The graph depicts the mean AID expression from three independent CIT-stimulations of cells derived from two independent knock-down experiments. Error-bars represent standard deviation from the mean.



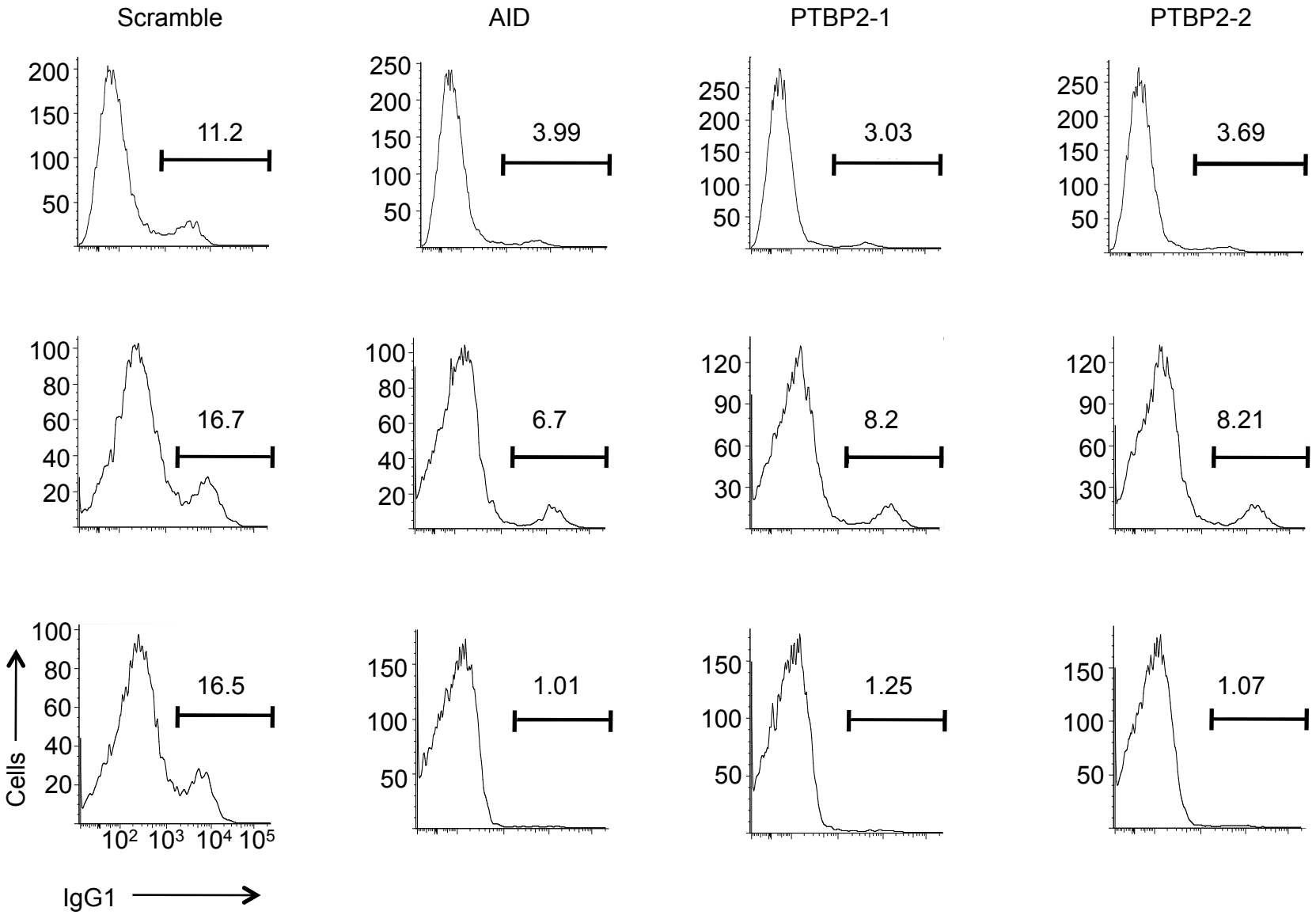
Supplementary Figure 15. Single-strand DNA deamination activity was measured in whole-cell (WCE) or nuclear (NE) extracts derived from unstimulated CH12 cells. Histograms represent mean of three experiments and error bars depict standard deviation from mean.



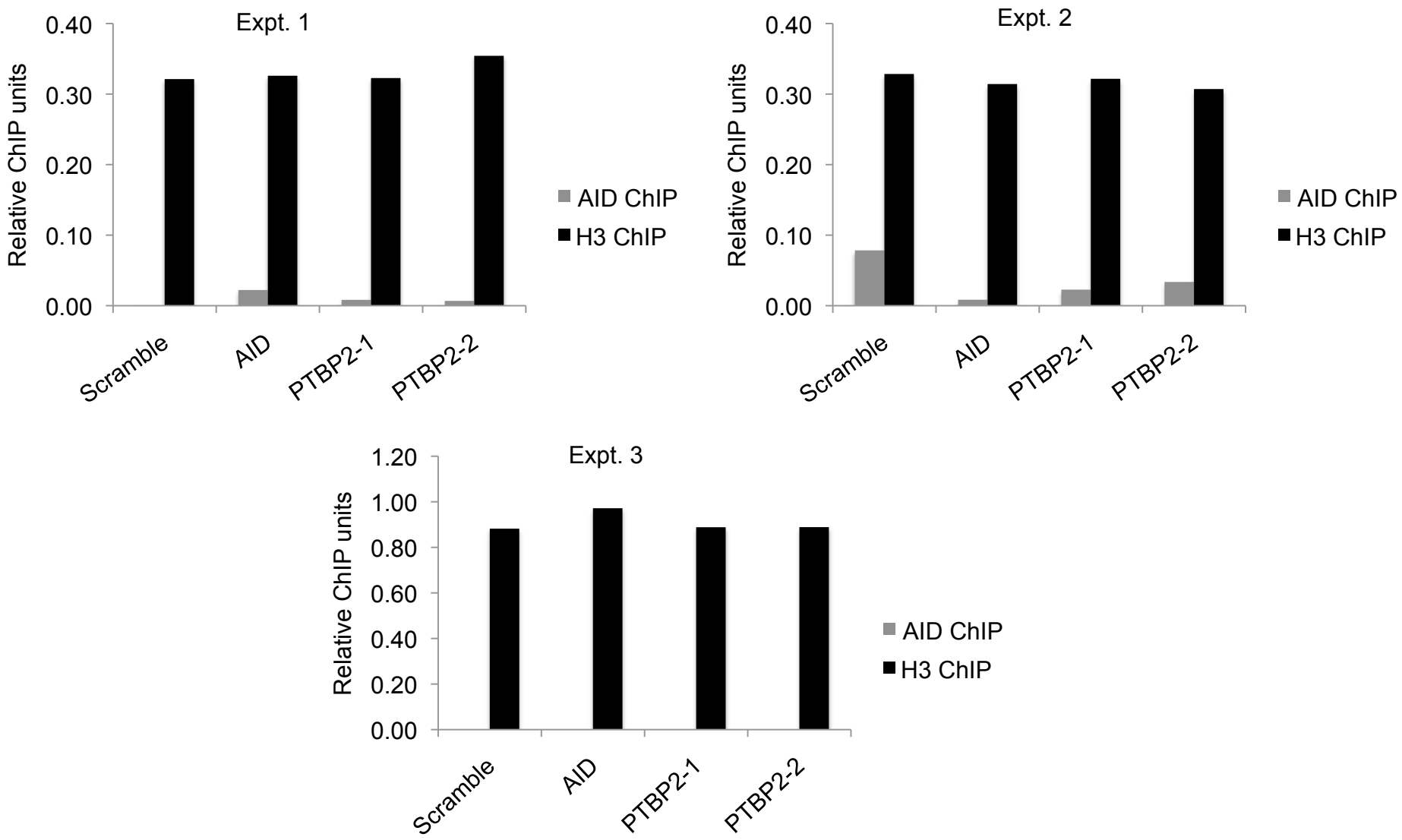
Supplementary Figure 16. Whole cell (wce), cytoplasmic (ce) or nuclear (ne) extracts from scramble or PTBP2 knock-down cells were analyzed by immunoblot using GAPDH or AID antibodies. Approximately 60 μg of each sample was analyzed. Immunoblot analyses of two independent knock-down experiments are shown.



Supplementary Figure 17. The amount of Sμ in anti-AID ChIP samples in CH12 cells expressing scramble or PTBP2-1 shRNA was quantified by real-time quantitative PCR (qPCR). The graph depicts qPCR values expressed as relative ChIP units with scrambled assigned an arbitrary value of 100. ChIP units are derived from normalizing Ct values to input and then subtracting IgG ChIP Ct values as background. Results show the values obtained from each of two independent knock-down experiments.



Supplementary Figure 18. PTBP2 impairs CSR to IgG1 in primary B cells. PTBP2 knock-down (PTBP2-1, PTBP2-2), AID knock-down or control scramble cells were stimulated with anti-CD40+IL-4 for 72 h and switching to IgG1 was measured by flow cytometry. Numbers indicate percentage of cells that have undergone CSR to IgA. Results for three independent experiments are shown.



Supplementary Figure 19. AID does not bind to p53 in primary splenic B cells. Primary splenic B cells expressing indicated shRNAs were stimulated with anti-CD40+IL-4 for 48 h and chromatin immunoprecipitation (ChIP) carried out with non-specific IgG or antibodies against AID or histone H3. Three-fold dilutions of ChIP DNA were analyzed by PCR for the presence of p53. Results for each of three experiments are shown.