SUPPLEMENTARY FIGURE LEGENDS

Figure S1: Enrichment of AID-Exosome complex.

A) Nomenclature for Eukaryotic RNA exosome subunits. The first vertical column lists from top to bottom the generic names for the 9 RNA exosome core subunits subunits and the two RNA exosome non-core subunits that arose from terminology first used to describe the subunits in yeast. The second column lists the names often used for the mammalian homologues of the yeast proteins. The RNA exosome subunits contain particular domains that confer specific functions. Core subunits: RBD stands for RNA Binding Domain. There can be two different types of RNA binding domains, S1 and K-homology (KH). In addition, some subunits have the primordial RNase PH domain that lacks RNase activity, which presumably was lost during evolution. Non-core Subunits (Rrp44 and Rrp6) have RNase R and RNase D activity, respectively. More details can be found in reviews by Houseley et al., 2006; Lykke-Andersen et al., 2009; Schmid and Jensen, 2008; Shen and Kiledjian, 2006 which are listed in manuscript.

B) Enrichment of AID associated complex generated in a T-7 transcription dependent SHM substrate assay in the presence of Ramos cell extracts. Results here show the transcription and AID-dependent dsDNA deamination activity of the purified protein fractions obtained from incubation of Ramos extract, and SHM substrate followed by elution via a carboxy-methyl sepharose column. Note that a highly active AID complex is eluted from the column when the reaction contains T7 polymerase but not when the reaction lacks T7 polymerase. Load: samples which were loaded on CM column; PT: pass-through of CM column; W1: the first wash fraction of CM column; W2: the second wash fraction of CM column; EI: eluate fraction of CM column. “–T7” and “+T7” indicate substrates with and without of transcription of T7 polymerase

Figure S2: RNA exosome Rrp40-core subunit is required for optimal CSR.

A) Proliferation of Rrp40 knock-down CH12F3 isolates. Growth curves of anti-CD4, IL-4, and TGF-β stimulated Ch12F3 (NS), shRrp40-1 and shRrp40-2 CH12F3 isolates as determined by analyzing live cell population counts over 72 hrs of cell growth. The data in each panel was obtained from represent 3 separate experiments performed simultaneously with each indicated cell type (total of 9 separate experiments). On each curve, points indicate the average and standard deviation from the 3 separate experiments. For main Fig. 3E, the points on the curves shown represent the average and standard from all 9 experiments. The CSR bar graphs shown in Fig. 3C were derived from the 3 experiments used to generate growth curves in panel 1 (shown again on panel 1). The CSR values shown on panels 2 and 3 came from one experiment of the 3 used to calculate growth curves for each panel.

B-F) Rrp40 knock-down experiment with a third independent knock-down line of CH12F3 cells (shRrp40-3) and a separate scrambled non-specific shRNA CH12F3 control (NS-1). Cells were stimulated for CSR to IgA with IL4, anti-CD40 antibodies and TGFβ. **B)** FACS plot of IgA switching from one NS-1 subclone and three independent shRrp40-3 subclones. **C)** Western blotting analysis of Rrp40 and AID expression in the stimulated NS-1 and shRrp40-3 subclones shown in panel B. **D)** Bar graphs show the average and standard deviation from the mean of 4 separate IgA CSR experiments for NS-1 and each shRrp40-3 subclone. **E)** Proliferation of stimulated NS-1 and shRrp40-3 cells. Each point represents the average and standard deviation form the mean for three separate experimental points**. F)** Left panel: The expression of Iα transcripts as analyzed by Northern blotting with Iα and GAPDH probes with RNA prepared from NS-1 and the three separate Rrp40-3 clonal isolates (indicated for panel B). GAPDH is used as a loading control; Right panel: Iμ and Iα transcripts determined in one Rrp40-3 clonal isolate as determined by standard semi-quantitative PCR assay; similar results were obtained for the other clonal isolates.

Figure S3: RNA exosome core subunit Mtr3 is required for efficient CSR.

A) The bar graphs represent the average and standard deviation of the mean for CSR in three separate experiments following anti-CD40, IL-4 and TGF-β stimulation (2 days) of a clonal isolate of CH12F3 cells that expressed either a scrambled shRNA (NS-2) or shRNA directed against Mtr3 (shMtr3-1). **B)** Level of Mtr3 expression in stimulated NS-2 and shMTR3-1 CH12F3 lines as measured by Western blotting with an anti-Mtr3 antibody and with an anti-tubulin as a control. **C)** Level of AID expression in stimulated NS-2 and shMTR3-1 CH12F3 lines as measured by Western blotting with an anti-Mtr3 antibody and with anti-tubulin as a control. **D)** Growth curves of stimulated NS-2 and shMTR3-1 CH12F3 lines. **E)** Levels of Iα and Iμ transcripts in stimulated NS-2 and shMTR3-1 CH12F3 lines as measured by semi-quantitative PCR; **F)** Level of Iα transcripts in stimulated NS-2 and shMTR3-1 CH12F3 as measured by Q-PCR and normalized to GAPDH.

Figure S4: The RNA Exosome associates with S regions during CSR.

This figure shows the primary data used to generate Panels B and D of Fig. 4. **A) Left:** Three independent repeats of ChIP experiments with mock anti-rabbit IgG in CH12F3 cell line are shown. An Sμ sequence was analyzed via PCR with specific primers as indicated in Supplementary Methods. The relative enrichment amounts were calculated by dividing DNA amounts from IP samples to inputs. Numbers indicate fold changes comparing stimulated and un-stimulated samples. **Right**: Three independent repeats of ChIP experiments with anti-Rrp40 antibody in CH12F3 cells. **B)** Three independent repeats of ChIP experiments with anti-Rrp40 antibody in primary splenic B cells are shown. The S regions were amplified with primers indicated in Supplementary Methods.

Figure S5: AID-dependent template strand DNA deamination stimulatory activity co-purifies with RNA exosome.

A) Schematic representation of the assay by which the AID-dependent template strand DNA deamination activity was identified to co-purify with RNA exosome purification fractions. **B)** AID-dependent template strand DNA deamination stimulatory activity was enriched by

fractionation through a S200 gel-filtration column. **C)** Template strand DNA deamination stimulatory activity (top panel) is present in fractions enriched with Rrp40 (western blot, bottom panel) or Flag-Rrp6 (western blot middle panel) in fractions of a 15-40% glycerol gradient fractionation (at low salt conditions). **D)** Template strand DNA deamination stimulatory activity is associated with endogenous RNA exosome. Top panel: Template strand DNA deamination stimulatory activity in S200 gel-filtration fractions. Bottom panel: western blot of fractions corresponding to exosome complex, which is represented by Rrp40 subunit. The activity co-migrates in glycerol gradient centrifugation step as well (data no shown).

Figure S6: Purified 293T Cell RNA and recombinant RNA exosomes stimulate AID template strand deamination activity.

A) An independent repeat experiment of data shown in Fig. 5. Template and non-template strand DNA deamination by AID in presence of either RPA plus PKA or RNA exosome complex enriched from HEK293 cells via the Flag-tagged Rrp6 sub-unit are shown. The assay is as described in Figure 5C except that a longer substrate was used that yields more focused product bands. An independent experiment of this type that yields similar findings is shown in Figure 5D. Note that the PKA/RPA simulates AID deamination on the transcribed non-template strand where as RNA exosome stimulates AID deamination activity on both template and non-template strands of dsDNA substrates. **B)** Purified AID (AID^{293}) deamination activity on a core Sμ substrate in presence or absence of RNA exosome purified from 293T was measured by strand specific DNA deamination assay by southern blotting using specific probes against the template or the non-template strand. Because of the nature of the substrate (longer and more RGYW motifs) deamination is visualized as a smear. In addition, deamination of the non-template strand is observe with AID alone because of R-loop formation (Chaudhuri et al., 2003). **C)** Purified AID (AID^{293}) template strand DNA deamination activity on a synthetic R-loop forming substrate (Chaudhuri et al., 2003) in the presence or absence of recombinant RNA exosome (rExo) or $RNA\ exosome^{293}$ and transcription (T) was measured using template strand-specific

DNA probe. The strand-specific DNA deamination assay is schematically described in Fig. 5C. **D)** Titration of enriched RNA exosome (Exo^{293}) DNA deamination stimulatory activity measured over different concentrations of partially purified RNA exosome employing ^{3}H-release assay outlined in Fig. 5A. **E)** Titration of different concentration of recombinant RNA exosome as assayed for ability to augment of AID mediated DNA deamination in the ^{3}H release assay outlined in Fig. 5A. **F)** Total DNA deamination measure by ^{3}H-release from radio-labeled templates in presence of AID and either recombinant core RNA exosome (rExo) or cellular RNA exosome enriched form HEK293 cells ($Exo^{293)}$. **G)** Direct comparison of AID template strand DNA deamination activity in presence of optimal amounts (to achieve maximal activities) of the RNA $exosome^{293T}$ preparation (Exo^{293}, 200ng) and recombinant RNA exosome core complex (rExo, 20 pmol). The optimal concentrations for Exo^{293} were obtained from the titration in panel A and that for rExo from the titration in panel B. For further details please see Extended Supplementary Methods.

Figure S7: Analysis of individual subunits of RNA exosome complex for stimulation of AID activity in transcription-dependent SHM substrate assay.

A) Total AID-DNA deamination activity in presence of equimolar amounts of recombinant RNA exosome subunits. The assay was peformed as outlined in Figure 5 **B)** Coomassie stained bands of purified recombinant RNA exosome subunits. **C)** Template strand AID-dependent DNA deamination in presence of recombinant Rrp40, Rrp41, Rrp46 and Mtr3. Assay was performed as outlined for Fig. 5C. **D)** Coomassie stained bands of Rrp40, Rrp41, Rrp46 and Mtr3.

Figure S8: Working Model for RNA exosome-dependent AID activity on transcribed duplex DNA substrates. Please refer to the last paragraph of the “Discussion” section for details of this model.

SUPPLEMENTARY TABLE LEGENDS

Table 1: A list of RNA exosome subunits found in the AID-associated complex as identified by mass spectrometric analysis (MS/MS). The detailed peptide information is available upon request.

SUPPLEMENTARY MATERIALS AND METHODS

Recombinant plasmids, antibodies and proteins.

The RNA exosome recombinant subunits (Csl4, Mtr3, Rrp4, Rrp40, Rrp42, Rrp43, and Rrp46, Rrp6, Rrp41 and Rrp45) were previously published or cloned in pRS-DUET vector for expression of His_6-tagged recombinant proteins. AID was expressed in 293T cells using construct pcDNA-AID as previously described (Chaudhuri et al., 2004; Chaudhuri et al., 2003; Greimann and Lima, 2008). Recombinant RPA was expressed from vectors p11d-RPA plasmid provided by B. Stillman (Stillman and Gluzman, 1985). AID antibodies were generated as previously described (Chaudhuri et al., 2003). Other antibodies were obtained as follows: Rrp40 (Genway); Rrp6 (Genway); Rrp46 (AbCam); Mtr3 (Genway). The recombinant RNA exosome subunits and RNA exosome complex was purified as described (Greimann and Lima, 2008).

AID transcription dependent ^{3}H-release DNA deamination assay.

For the uracil release assay, a [^{3}H]-labeled DNA (1–2 pmol) substrate was transcribed using T7-Maxiscript kit (Ambion) and incubated with 1 μg of AID^{293} protein preparation and indicated additional factors as described (Chaudhuri et al., 2004; Basu et al., 2005; 2008) or indicated in relevant figure legends. The RGYW-rich artificial SHM substrate, R-loop artificial substrate and Sμ substrate were assayed as described previously (Chaudhuri et al., 2003;2004; Basu et al., 2005; 2008). For comparison purposes, the optimal concentration for activity of different enriched cellular or recombinant core exosome protein components used for this assay was determined by titrating varying amounts of the RNA exosome preparations in the ^{3}H-release DNA deamination assay with other conditions remaining fixed (Figure S6). The deaminated DNA products were then deglycosylated with recombinant bacterial UDG (0.2 units, NEB Biolabs) for 1 h at 37 °C in a buffer containing 25 mM Tris-Cl, pH 7.5, 100 mM NaCl, 1 mM DTT and 10 μM $ZnCl_2$. The reaction was terminated by 1%TCA precipitation, centrifuged, and

the radioactivity retained in the supernatant counted via liquid scintillation counter (Wallac 1409). DNA deamination activity was expressed as a percentage of TCA soluble to total [^{3}H] counts in the input DNA substrate. More details on the assay have been described previously (Chaudhuri et al. 2003; 2004).

Strand-specific AID DNA deamination assay

Transcription of the RGYW-rich sequences was performed with a T7-Maxiscript kit (Ambion) using approximately 100 ng of substrate DNA. To determine which strand of the RGYW DNA was deaminated, after incubation with AID plus additional test factors (e.g. RNA exosome/exosome subunits/RPA and PKA) the DNA was de-glycosylated and de-proteinated with proteinase K, treated with hot alkali and then precipitated. Denaturation and Southern analysis of the DNA was done using specific probes designed to recognize the T7 primer binding sequences. Probes used in the Southern analysis hybridize to the 5′ end of non-template strand or the 3′ end of the template strand, respectively. The probes are labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (Chaudhuri et al., 2004; Chaudhuri et al., 2003). The RGYW-rich artificial SHM substrate, R-loop artificial substrate and Sμ substrate assayed as described previously (Chaudhuri et al., 2003;2004; Basu et al., 2005; 2008).

Purification of RNA exosome complex from 293T cells.

A 293T cell line expressing a Flag-epitope was used in these experiments. A total of 4g of cells were used to harvest total cell extracts in buffer A (20 mM Tris (pH7.5), 1mM DTT, 10mM $MgCl_2$ and 10% glycerol) containing 280mM NaCl using a combination of douncing and sonication for promoting lysis of the cells. The extract was dialyzed against Buffer A+280mM NaCl and passed through a 20ml DEAE cellulose column. The elution of proteins was monitored by measuring $O.D._{280}$ absorption and by Bradford assays (Biorad). The total protein (3mg) was concentrated using 0-70% ammonium sulfate precipitation followed by elution through a 35 ml S200-gel filtration column. The protein fraction obtained in the excluded volume was loaded on a 15-40% glycerol gradient (Buffer A+100mM NaCl) and centrifuged at 40,000 rpm for 16hrs. The gradient was fractionated into approximately 500 μl fractions by inserting a needle at the

bottom of the tube and collecting fractions drop wise. The peak fractions containing RNA exosome subunits (400μg) were dialyzed against buffer A+100 mM NaCl. The sample was then immunoprecipitated with 100 μl of FLAG-agarose (sigma) for 12 hrs at 4^{o}C (the FLAG-agarose slurry was pre-washed in PBS and equilibrated). The Immunoprecipitate was washed with buffer A+200mM NaCl and eluted with 500ng of Flag peptide. For elution, the immunoprecipitate was incubated with the Flag peptide overnight at 4'C and then rotated on a nutator for 1 hr. The eluate was obtained by centrifuging the reaction and then isolating the supernatant separated from the beads. The elution step was repeated and the total eluate was dialyzed against Buffer A+50mM NaCl (containing 50% glycerol) and stored in small aliquots.

Purification of AID complex from transcription coupled deamination assays supplemented with Ramos cell extracts.

A total of 1.2 liters of Ramos cells were grown in culture to a density of 1x10^{6} cells/ml. These cells were harvested and total protein extract prepared as described Chaudhuri et al., 2004; Chaudhuri et al., 2003). Briefly, we re-suspended the cells in low salt buffer (20 mM Tris (pH 7.5), 10 mM NaCl and 1 mM DTT). The cells were then incubated on ice for 20 minutes and lysed in by douncing. The lysed cells were centrifuged and re-suspended in Buffer A + 280 mM NaCl first by sonication followed by douncing. The extract (approx. 0.15g) was dialyzed against buffer A + 100m M NaCl, centrifuged to remove debris and then incubated with 500μg of T7-RGYW (actively transcribed with 30,000U of T7 polymerase).

The transcription reaction was incubated with cell extract for 30 minutes at 30°C and, thereafter, loaded onto a carboxy-methyl sepharose column (bed volume=5 ml). Eluted fractions were analyzed for ability to deaminate a transcribed dsDNA substrate. The eluted proteins were then passed through a DEAE cellulose column (A+280m M NaCl). The purified fractions were dialyzed against buffer A + 25mM NaCl and passed on a heparin-sepharose column (5 ml bed volume). For the heparin sepharose step, the column was equilibrated with buffer A+20m M NaCl, washed with buffer A+50 mM NaCl and eluted with A+200mM NaCl. The eluted protein fractions were concentrated using 70% ammonium-sulfate precipitation (in cold

with constant stirring and ph adjustment) and loaded on a 35ml S200 gel-filtration column. The protein fraction obtained in the exclusion volume was loaded on a 15-40% glycerol gradient (containing buffer A+100mM NaCl) and fractionated at 40,000 rpm for 16 hrs. The fractions were collected by inserting a needle at the bottom of the tube and collected drop wise in 4'C. The fractions with highest transcription dependent cytidine DNA deamination activity were dialyzed against buffer A+100 mM NaCl. This preparation was immunoprecipitated with anti-AID antibodies for 8 hrs at 4°C. The immunoprecipitated protein complex was eluted with AID specific peptide by incubating at 4ºC overnight. The immunoprecipitate was centrifuged to remove the antibody bound beads and the supernatant collected for further work. The elution was repeated to avoid loss of sample.

The components of the eluate were separated via polyacrylamide gel electrophoresis (15% PAGE) and fragments of the gel were analyzed by mass spectrometry at the Rockefeller University Proteomics Research Center. For identification of the protein samples by mass spectrometry, the protein gel bands were excised from the 1D SDS-PAGE and were subject to in-gel reduction, alkylation, and trypsin-digestion. The digestion was performed for 16 hours at 37 °C. The generated peptides were extracted with 50% acetonitrile and 0.1% TFA twice and dried on Speed-Vac. The dried peptides mixture was subject to LC/MS/MS analysis. For LC-MS/MS analysis, the peptide mixture was separated by a 60 min gradient elution with the Dionex U3000 capillary/nano-HPLC system (Dionex, Sunnyvale, California) at a flow rate of 0.250 mL/min that is directly interfaced with the Thermo-Fisher LTQ-Orbitrap mass spectrometer (Thermo Fisher, San Jose, California) operated in data-dependent scan mode. The analytical column was a home-made fused silica capillary column (75 mm ID, 100 mm length; Upchurch, Oak Harbor, Washington) packed with C-18 resin (300 A, 5 mm, Varian, Palo Alto, California). Mobile phase A consisted of 0.1% formic acid, and mobile phase B consisted of 100% acetonitrile and 0.1% formic acid. The 60 min gradients at 0.250 mL/min flow rate for B solvent went from 0 to 55% in 30 min and then in 10 min to 80%. The experiment consisted of a single full-scan mass spectrum in the Orbitrap (400-1600 m/z, 30,000 resolution) followed by 6

data-dependent MS/MS scans in the ion trap at 35% normalized collision energy. Data was analyzed by MASCOT software and manual inspection.

Immunoprecipitation of AID/RNA exosome complex from primary B cells, CH12F3, Ramos cells and HEK293T cells.

Nuclear extracts from primary B cells and B cell lines were prepared as previously described (Chaudhuri et al., 2004; Chaudhuri et al., 2003). A 500µg aliquot of extract was treated with DEAE cellulose in a buffer containing 280 mM NaCl. The eluates were dialyzed against buffer A+100 mM NaCl and immunoprecipitated with anti-AID antibodies as described previously (Basu et al., 2005; Basu et al., 2008). Immunoprecipitates were washed with buffer A+150 mM NaCl and analyzed by SDS gel electrophoresis followed by western blotting with anti-AID antibodies or specific antibodies against components of the RNA exosome complex. For interaction studies in 293T cells, each individual Flag-tagged exosome subunit, respectively, plus AID were co-expressed from pCMV plasmids following transient transfection into HEK293T cells and cells were harvested 48 hrs later. The immunoprecipitation procedure was same as that for B cells except that immunoprecipitates were washed with buffer A + 400 mM NaCl.

shRNA-mediated knockdown of RNA exosome components in CH12F3 cell lines.

Human fibroblast 293 cells were co-transfected with lentiviral backdone plasmids and plasmids harboring shRNA against subunits of the RNA exosome complex (pL-KO) using a calcium phosphate transfection protocol. Approximately 3 ml of viral supernatant from these transfected cells was overlayed on 3ml of CH12F3 cells in presence of 1ug/ml polybrene. The cells were spin-infected for 2 hours and then incubated at 37°C for 24 hours. Thereafter, the cells were selected in 0.5 ug/ml puromycin for 7 days followed by 1 µg/ml for 3-7 days. During the selection process the CH12F3 cells were grown in culture at dilutions of $1*10^4$ cells/ml media concentration. The sequence of the two shRNAs directed against Rrp40 used are: CCGGGAAAGCTATTAGCTCCAGATTCTCGAGAATCTGGAGCTAATAGCTTTCTTTTTG and CCGGGATCGCTAAATCTGGAGATATCTCGAGATATCTCCAGATTTAGCGATCTTTTTG.

IgA CSR analysis of CH12F3 cells depleted of Rrp40 and Mtr3.

CH12F3 cells were analyzed for expression of surface IgA FITC-conjugated anti-mouse IgA clone (eBiosciences), and APC-conjugated B220 antibodies. The cells were suspended in 2.5% FCS containing PBS, stained and washed with the same buffer before FACS analysis.

Determination of Iμ and Iα germline transcription and cell growth of CH12F3 cells.

Total RNA was extracted from cultured CH12F3-2 cells using Trizol (Invitrogen, Carlsbad CA) according to the manufacturer's instructions, and quantitated by measuring A260 using a Nanodrop spectrophotometer. 2μg of RNA was treated with DNAseI (Invitrogen, Carlsbad CA), immediately primed with oligo-dT and reverse transcribed using Superscript III Reverse Transcriptase (Invitrogen, Carlsbad CA). For absolute quantification, a serial dilution of control sample and experimental sample was prepared and amplified using SYBR Green ROX (Roche Applied Science, Indianapolis IN), using the following primer pairs: GLT IμF 5'-CTCTGGCCCTGCTTATTGTTG-3', GLT CμR 5'-GAAGACATTTGGGAAGGACTGACT-3', GLT IαF 5'- CCTGGCTGTTCCCCTATGAA-3', GLT CαR 5'-GAGCTGGTGGGAGTGTCAGTG-3', GAPDHF 5′-TGTCCCCACTGCCAACGTGTCA-3′, GAPDHR 5′-AGCGTCAAAGGTGGAGGAGTGGGT-3′. Real-Time PCR was performed using the ABI **7500** system (Applied Biosystems). Samples were cycled followed by a dissociation stage. Analysis was performed using the Δ-ΔCT method.

The DNA synthesized was then quantitated using a Roche sybrgreen ROX kit and analyzed in a ABI real time PCR 7500 machine. The synthesized product was also estimated using 0.8% agarose gel electrophoresis. In some experiments, levels of Iα germline transcripts were also determined by Northern blotting analyses as describe (Nakamura, et al. 1996). For analysis of cell growth of CH12F3 and RNA exosome subunit knockdown cell lines, the cells were harvested at respective time points from 15%FCS containing RPMI cultures. The cells were counted after trypan blue staining for estimation of live cell population.

Chromatin Immunoprecipitation of Rrp40 from CH12F3 cells and primary splenic B cells.

CH12F3 cells or primary spleen B cells were stimulated for IgA or IgG1 CSR for 48 hours. At this time point, the cells were treated with 1% formaldehyde in culture for 10 minutes

followed by treatment with 0.125 M glycine, to cross link the cellular DNA with bound proteins. The cross-linked cells were then lysed in and cell debris removed by passing the extract through glass wool. The extract was then pre-cleared with ssDNA/Protein A agarose bead slurry in presence of non-specific IgG for an hour. The pre-cleared DNA was immunoprecipitated for Rrp40 using 1ug of anti-Rrp40 antibodies (Genway, rabbit polyclonal) or with control rabbit IgG for 8 hours at 4°C. These immuno-complexes were collected by adding ssDNA/protein A agarose by an incubation of 1hr at 4°C. The beads were isolated by centrifugation and sequentially washed with number of buffers. Each wash is for 5 minutes and repeated 3 times. The buffers used were (a) low salt buffer (b) high salt buffer (c) LiCl buffer and (d) TE. The immuno-complex was eluted in ChiP elution buffer. The eluted DNA was then prepared for further analysis by treatment with 0.3M NaCl and RNase treatment. The DNA was cleaned of associated proteins by proteinase K treatment and extraction with phenol:Chloroform:isoamyl alcohol. The extracted DNA was precipitated in presence of glycogen and washed with 70% ethanol and resuspended in water for further PCR analysis. The composition the buffers are as follows. (a) SDS lysis buffer: 1% SDS, 10 mM EDTA and 50 mM Tris pH 8.1 (b) ChIP dilution buffer: 0.01%SDS, 1.1% TritonX-100, 1.2 mM EDTA, 16 mM Tris pH8.11 and 165 mM NaCl (c) Low salt and high slat wash buffers: 0.1%SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris pH8.1, 150/500mM NaCl (d) LiCl Wash buffer: 0.25M LiCl, 1% deoxycholic acid , 1%IGEPAL-CA630, 1mM EDTA and 10mM Tris8.1 (e) Elution buffer 1%SDS, 0.1M $NaHCO_3$(Chaudhuri et al., 2004; Nambu et al., 2003).

The specific primers used for PCR reactions during analysis of Sμ products in Rrp40 ChIP DNA are: μ-switch region-sense, 5'-TAGTAAGCGAGGCTCTAAAAAGCAT-3'; μ -switch region-antisense, 5'-AGAACAGTCCAGTGTAGGCAGTAGA-3'; γ1-switch region-sense, 5'-TATGATGGAAAGAGGGTAGCATT-3'; γ1-switch region-antisense, 5'-CTGGGCTGGTCTGTCAACTCCTT-3'; α-switch region-sense, 5'-TGAAAAGACTTTGGATGAAATGTGAACCAA-3'; α-switch region-antisense, 5'-GATACTAGGTTGCATGGCTCCATTCACACA-3'. Real-Time PCR was performed using the

ABI **7300** system (Applied Biosystems). Analysis was performed using the Δ-ΔCT method. The relative enrichment amounts were calculated by dividing DNA amounts from IP samples to Inputs (Figure S3). Then, the fold changes were calculated by comparing stimulated versus un-stimulated Ch12F3 cells and wild type versus AID$^{-/-}$ spleen B cells.

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A

Yeast homolog	Human protein	Subunit domains
Csl4	ExoSc1	S1 RBD
Rrp4	ExoSc2	S1/KH RBD
Rrp40	ExoSc3	S1/KH RBD
Rrp41	ExoSc4	RNase PH
Rrp46	ExoSc5	RNase PH
Mtr3	ExoSc6	RNase PH
Rrp42	ExoSc7	RNase PH
Rrp43	ExoSc8	RNase PH
Rrp45	ExoSc9	RNase PH
Rrp6	ExoSc10	RNase D
Rrp44	ExoSc11	RNase R

B

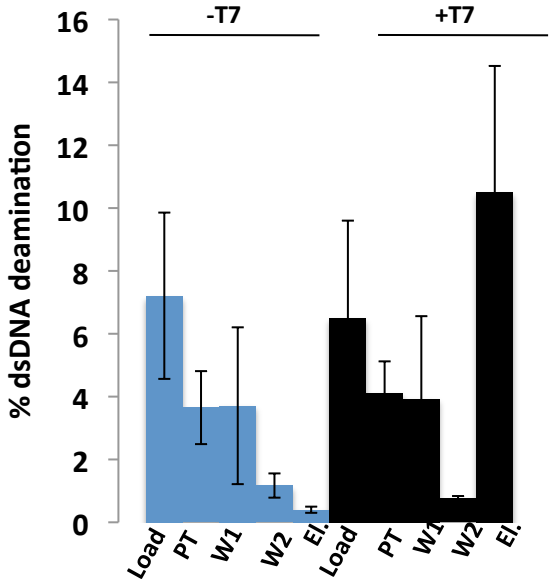


Figure S1

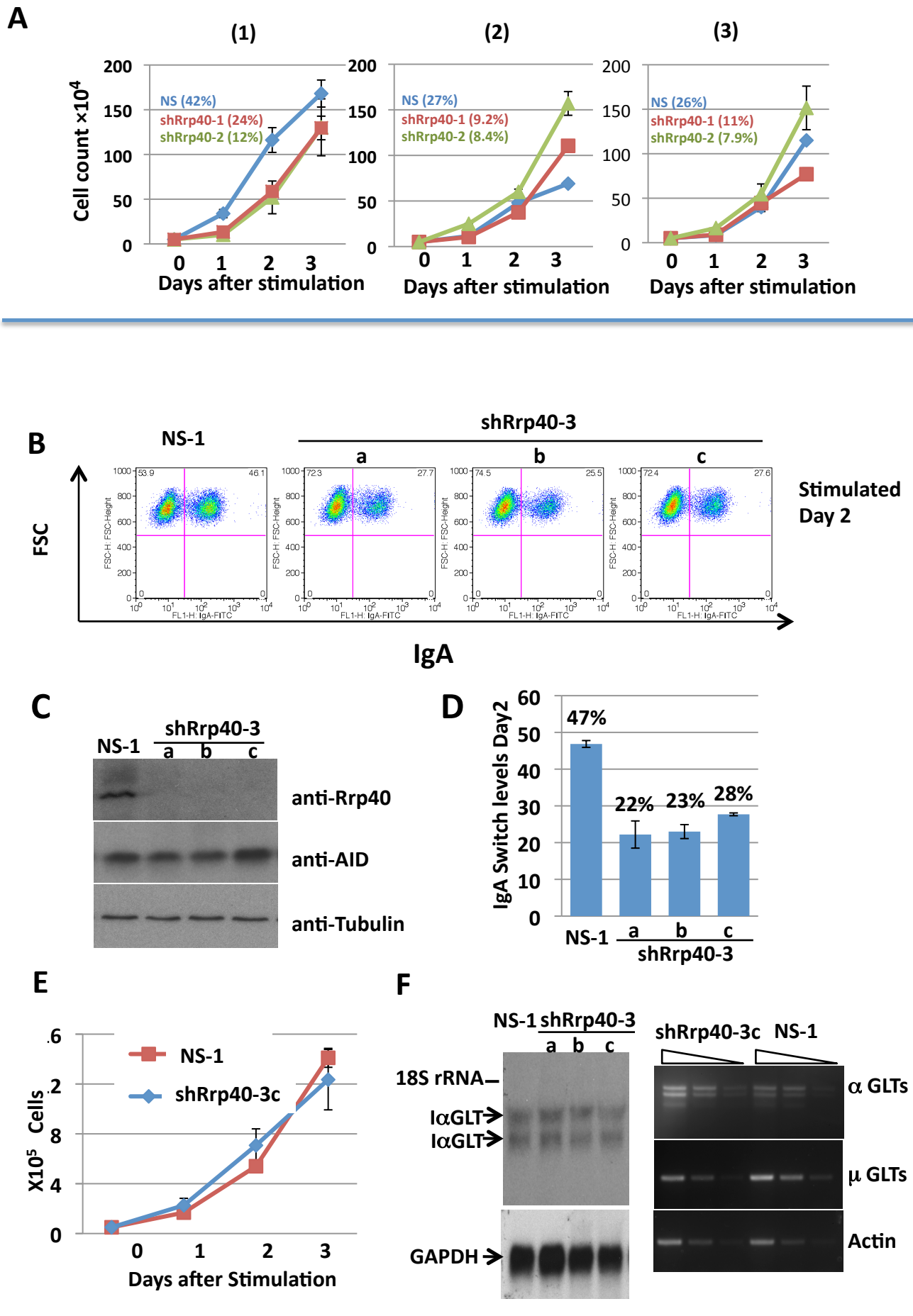

A
(1)
(2)
(3)
Cell count ×10^4
NS (42%)
shRrp40-1 (24%)
shRrp40-2 (12%)
NS (27%)
shRrp40-1 (9.2%)
shRrp40-2 (8.4%)
NS (26%)
shRrp40-1 (11%)
shRrp40-2 (7.9%)
Days after stimulation
B
NS-1
shRrp40-3
a
b
c
FSC
IgA
Stimulated Day 2
C
NS-1
shRrp40-3
a b c
anti-Rrp40
anti-AID
anti-Tubulin
D
IgA Switch levels Day2
47%
22%
23%
28%
NS-1
shRrp40-3
E
NS-1
shRrp40-3c
X10^5 Cells
Days after Stimulation
F
NS-1 shRrp40-3
a b c
18S rRNA
IαGLT
IαGLT
GAPDH
shRrp40-3c NS-1
α GLTs
μ GLTs
Actin

Figure S2

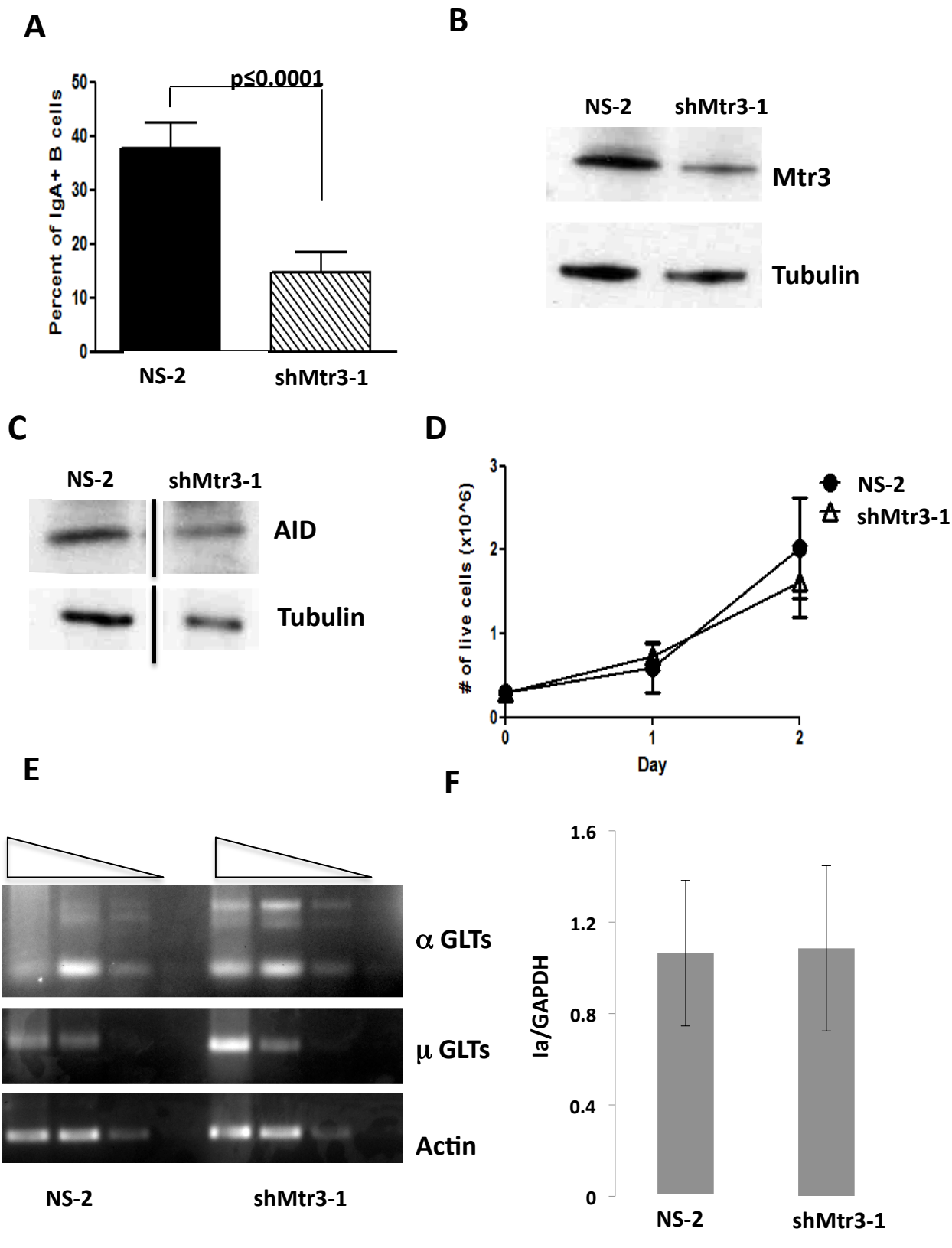
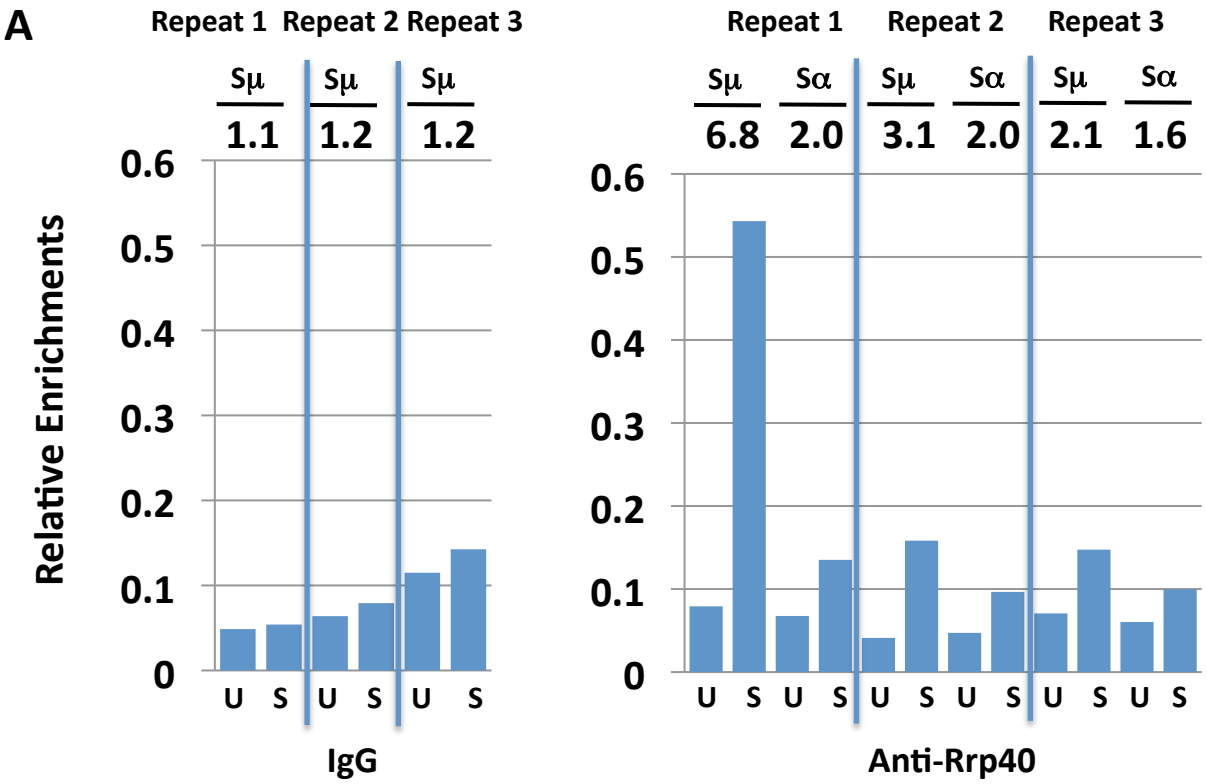

A
p≤0.0001
Percent of IgA+ B cells
50
40
30
20
10
0
NS-2
shMtr3-1
B
NS-2
shMtr3-1
Mtr3
Tubulin
C
NS-2
shMtr3-1
AID
Tubulin
D
# of live cells (x10^6)
3
2
1
0
0
1
2
Day
NS-2
shMtr3-1
E
α GLTs
μ GLTs
Actin
NS-2
shMtr3-1
F
Ia/GAPDH
1.6
1.2
0.8
0.4
0
NS-2
shMtr3-1

Figure S3


A
Repeat 1
Repeat 2
Repeat 3
Sμ
Sμ
Sμ
1.1
1.2
1.2
Relative Enrichments
0.6
0.5
0.4
0.3
0.2
0.1
0
U S U S U S
IgG
Repeat 1
Repeat 2
Repeat 3
Sμ
Sα
Sμ
Sα
Sμ
Sα
6.8
2.0
3.1
2.0
2.1
1.6
U S U S U S U S U S U S
Anti-Rrp40

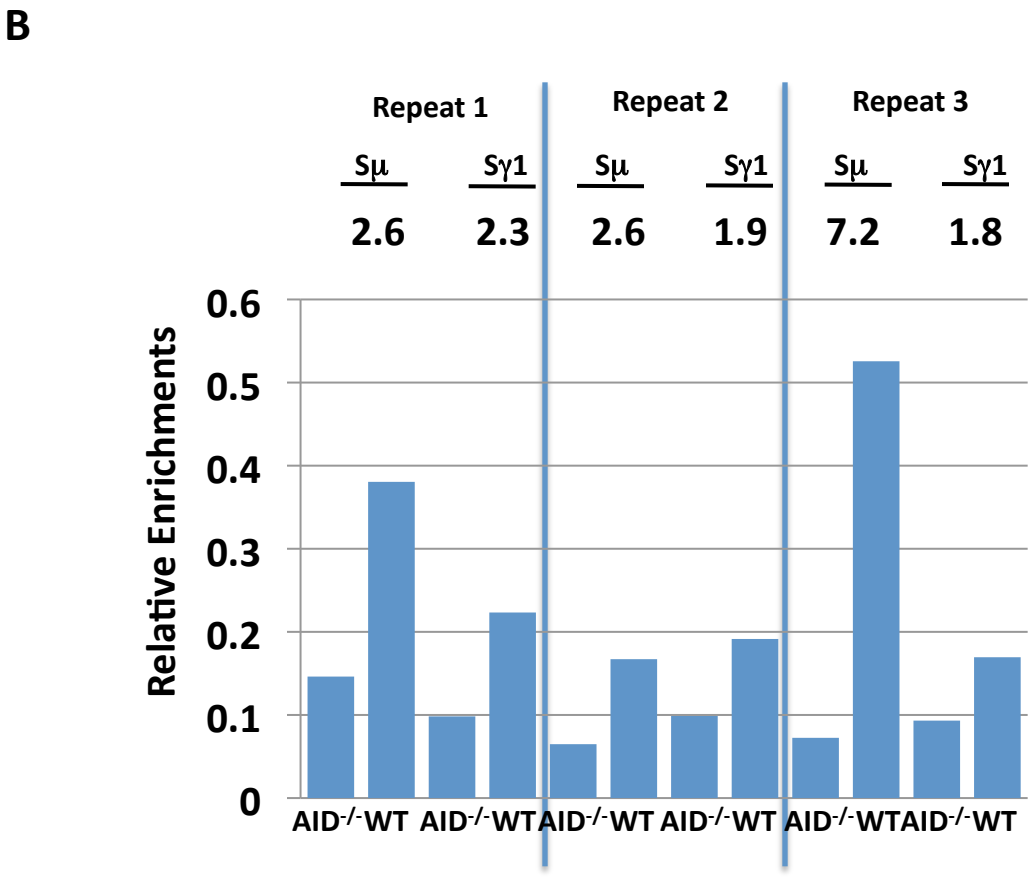

B
Repeat 1
Repeat 2
Repeat 3
Sμ
Sγ1
Sμ
Sγ1
Sμ
Sγ1
2.6
2.3
2.6
1.9
7.2
1.8
Relative Enrichments
0.6
0.5
0.4
0.3
0.2
0.1
0
AID-/- WT AID-/- WT AID-/- WT AID-/- WT AID-/- WT AID-/- WT

Figure S4

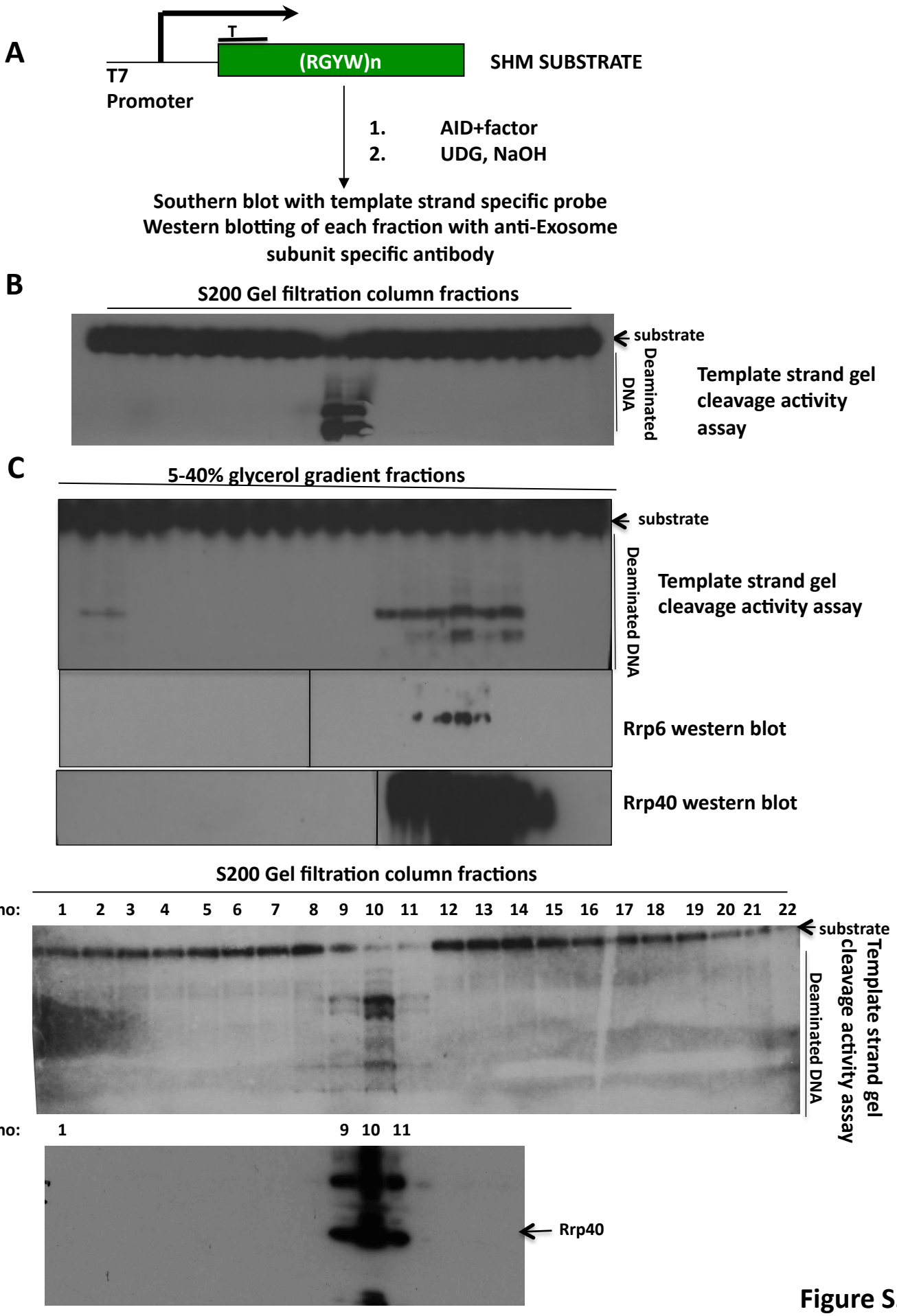

A
T
(RGYW)n
SHM SUBSTRATE
T7
Promoter
1. AID+factor
2. UDG, NaOH
Southern blot with template strand specific probe
Western blotting of each fraction with anti-Exosome subunit specific antibody
B
S200 Gel filtration column fractions
substrate
Deaminated DNA
Template strand gel cleavage activity assay
C
5-40% glycerol gradient fractions
substrate
Deaminated DNA
Template strand gel cleavage activity assay
Rrp6 western blot
Rrp40 western blot
D
S200 Gel filtration column fractions
Frac no: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
substrate
Deaminated DNA
Template strand gel cleavage activity assay
Frac no: 1 9 10 11
Rrp40

Figure S5

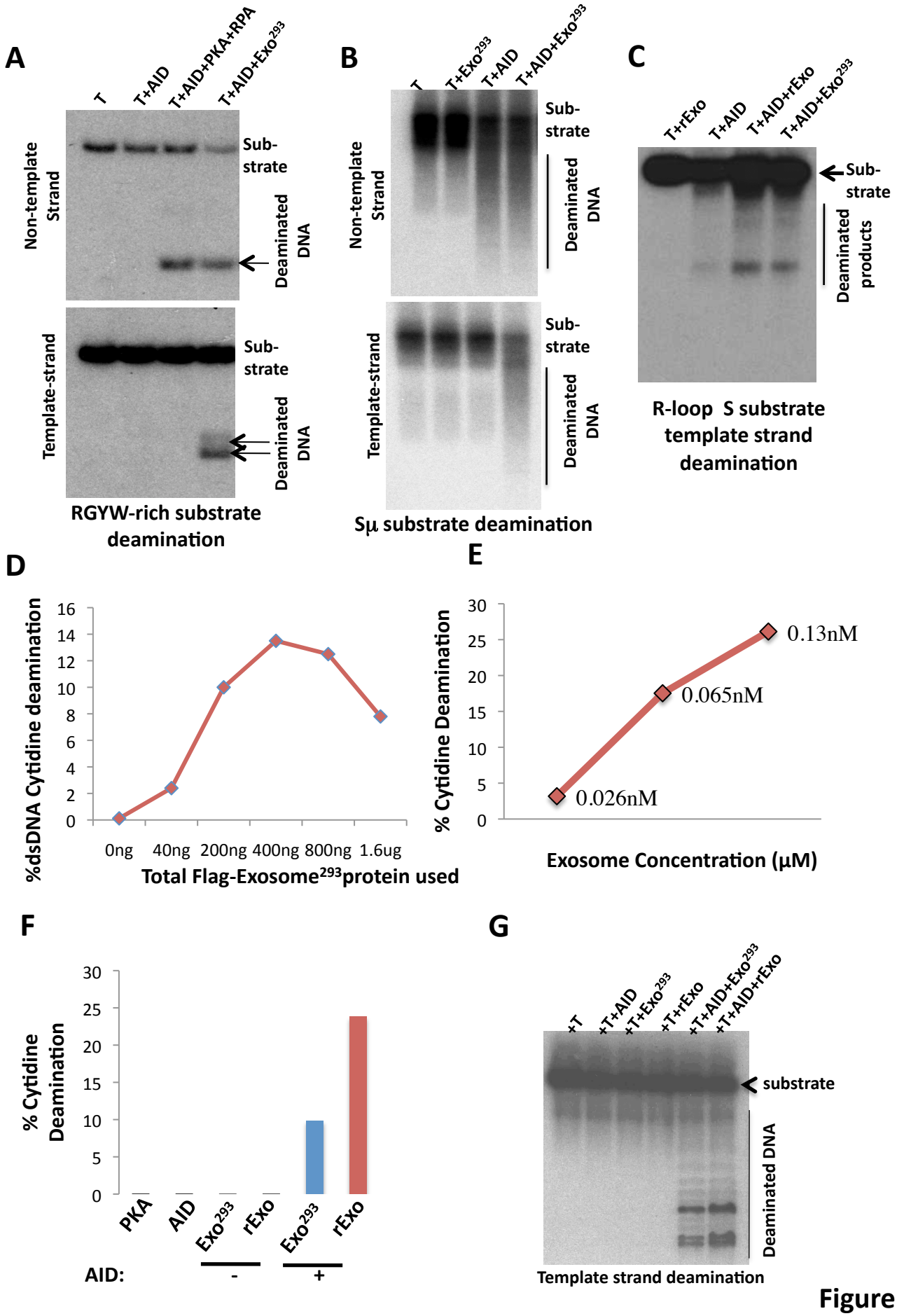

A
T
T+AID
T+AID+PKA+RPA
T+AID+Exo293
Non-template Strand
Sub-strate
Deaminated DNA
Template-strand
Sub-strate
Deaminated DNA
RGYW-rich substrate deamination
B
T
T+Exo293
T+AID
T+AID+Exo293
Non-template Strand
Sub-strate
Deaminated DNA
Template-strand
Sub-strate
Deaminated DNA
Sμ substrate deamination
C
T+rExo
T+AID
T+AID+rExo
T+AID+Exo293
Sub-strate
Deaminated products
R-loop S substrate template strand deamination
D
%dsDNA Cytidine deamination
16
14
12
10
8
6
4
2
0
0ng
40ng
200ng
400ng
800ng
1.6ug
Total Flag-Exosome293protein used
E
% Cytidine Deamination
30
25
20
15
10
5
0
0.13nM
0.065nM
0.026nM
Exosome Concentration (μM)
F
% Cytidine Deamination
30
25
20
15
10
5
0
PKA
AID
Exo293
rExo
Exo293
rExo
AID:
-
+
G
+T
+T+AID
+T+Exo293
+T+rExo
+T+AID+Exo293
+T+AID+rExo
substrate
Deaminated DNA
Template strand deamination

Figure S6

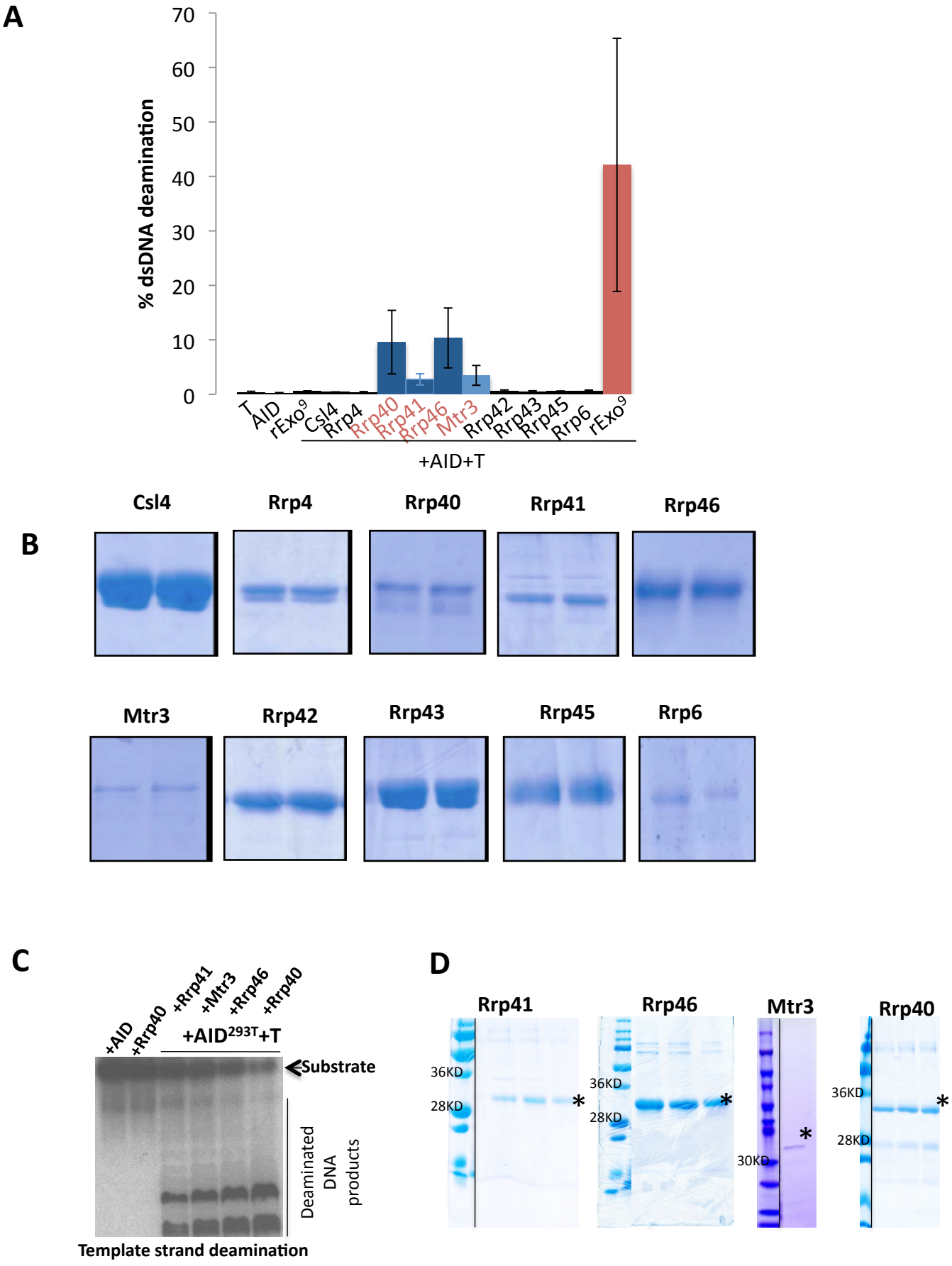
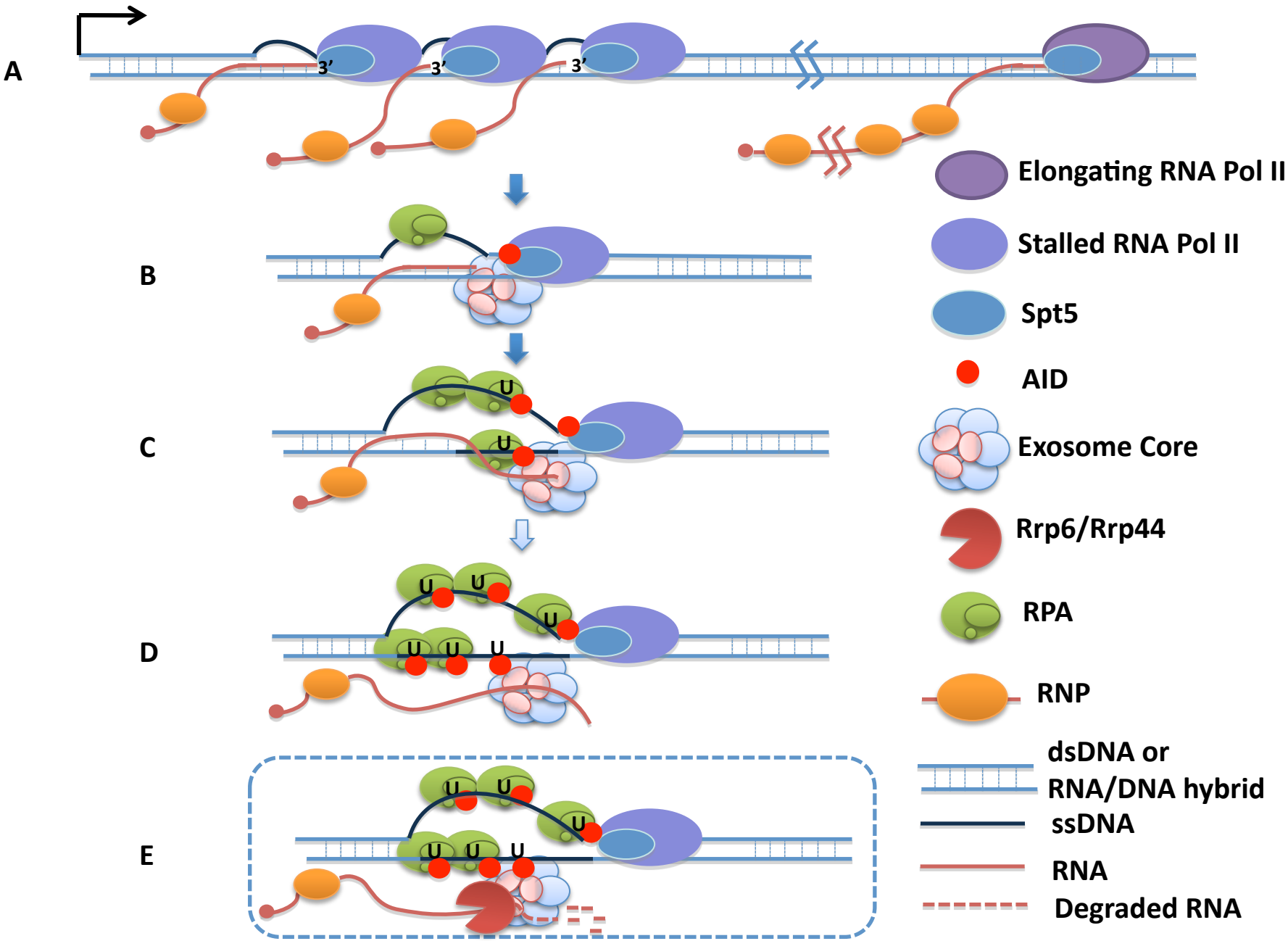

A
% dsDNA deamination
70
60
50
40
30
20
10
0
T
AID
rExo9
Csl4
Rrp4
Rrp40
Rrp41
Rrp46
Mtr3
Rrp42
Rrp43
Rrp45
Rrp6
rExo9
+AID+T
B
Csl4
Rrp4
Rrp40
Rrp41
Rrp46
Mtr3
Rrp42
Rrp43
Rrp45
Rrp6
C
+AID
+Rrp40
+Rrp41
+Mtr3
+Rrp46
+Rrp40
+AID293T+T
Substrate
Deaminated DNA products
Template strand deamination
D
Rrp41
Rrp46
Mtr3
Rrp40
36KD
28KD
36KD
28KD
30KD
36KD
28KD

Figure S7


A
3'
3'
3'
B
C
U
U
D
U
U
U
U
U
E
U
U
U
U
U
Elongating RNA Pol II
Stalled RNA Pol II
Spt5
AID
Exosome Core
Rrp6/Rrp44
RPA
RNP
dsDNA or
RNA/DNA hybrid
ssDNA
RNA
Degraded RNA