SUPPLEMENTAL METHODS

APOBEC3G purification

Frozen Sf9 cells containing A3G were lysed in 1X hA3G buffer (25 mM Hepes pH 7.4, 500 mM NaCl, 10 mM $MgCl_2$, 5% glycerol, 0.01 M Imidazole, 0.2 mM β-mercaptoethanol and EDTA-free complete protease inhibitor (Roche)). The lysed cells were brought to 1M NaCl, 1% Triton X-100 and 0.1 mM $CaCl_2$, and the samples were digested with 0.025 mg/mL DNase I (Sigma) and 0.03 mg/mL RNase A (Sigma) at 37 °C for 30 min. The lysates were brought to 1 M urea final concentration, incubated at 24 °C for 5 min and centrifuged (10,000 x g for 10 min at 4 °C). Cleared lysates were incubated with 1 mL Ni-NTA agarose (Qiagen, Germany) for 2 h while tumbling at 7 °C. The resin was washed consecutively with 3 column volumes of: 1X hA3G buffer with 0.5 M urea in 1X hA3G buffer containing 0.05 M imidazole, followed by washes with 3 column volumes with the same buffer supplemented to 0.07 M imidazole. Protein was eluted with 1X hA3G buffer with 0.5 M imidazole. Peak A3G containing fractions identified by A280 absorption and analysis of the fractions on Coomassie blue stained SDS PAGE were pooled, snap frozen in liquid nitrogen and stored at -80 °C.

Preparation of A3G for mass spectroscopy.

ssDNA-cross-linked samples were digested with 10 μg of DNase I (Sigma) and 300 units of micrococcal nuclease (Thermo Scientific) and RNA- cross-linked samples were treated with 10 μg RNase A (Sigma) and RNase T1 (Life Technologies/Ambion), both for 4-6 hours at 37 °C. The gel slices were washed three times with 25 mM ammonium bicarbonate (AmBc), dehydrated with solution containing 50% acetonitrile and 25 mM AmBc and dried out in a vacuum-speed centrifuge. The gel slices were rehydrated with 25 mM AmBc and then two more rounds of dehydration-rehydration procedure was performed in order to remove any residual amounts of nucleases or digested nucleic acids followed by treatment with 10 mM DTT at 55 °C for 1 hour and iodoacetimide at room temperature in the dark for 45 min. The samples then were washed with 25 mM AmBc, dehydrated with 50% acetonitrile and 25 mM AmBc solution, vacuum dried and digested overnight with Trypsin Gold (Promega, 0.5 μg/ μl) in 25 mM AmBc at 1:20 ratio.

Mass Spectrometry Analysis Digested fractions were reconstituted with 0.1% formic acid in water and vortexed for 5 minutes, as previously described[1]. Samples were analyzed on two independent mass spectrometers, an LTQ (Thermo Fisher Scientific) and an LTQ Orbitrap XL (Thermo Fisher Scientific), to yield complementary data resulting in high sequence and modification coverage.

For LTQ analysis, each sample was loaded and washed onto a 0.3 x 5mm C_{18} trap column using an 1100 series autosampler (Agilent Technologies) and then eluted onto a home-pulled, home-packed C_{18} analytical column. Columns were pulled with a Sutter Laser puller to a tip width of ~10μm and packed using a pressure bomb to 10cm with C_{18}AQ 5μm 200Å media (Michrom), ending with an internal column diameter of 75μm. Columns were equilibrated to initial run conditions prior to loading the sample on the column. Peptides were separated and eluted with the following chromatographic profile:

3% B for 4 minutes while loading the sample onto the trap column, ramping to 20% B over 1 minutes then to 60% B over 42 minutes, then ramping to 95% B in 1 minute and remaining there for 3 minutes before returning to initial run conditions. Solvent A was LC/MS grade water (Burdick & Jackson) + 0.1% formic acid (Pierce) and Solvent B was LC/MS grade methanol (Burdick & Jackson) + 0.1% formic acid. Flow rate across the column was 100mL/min while loading the sample onto the trap column, and 350nL/min during elution to the analytical column. The mass spectrometer was operated in a data-dependent manner. A survey scan was performed, followed by MS/MS analysis of the top seven most abundant peaks from the survey scan. No dynamic exclusion was employed. Helium was used as collision gas, with an activation Q of 0.25, activation time of 30ms and normalized collision energy of 35%. Data were collected as RAW files.

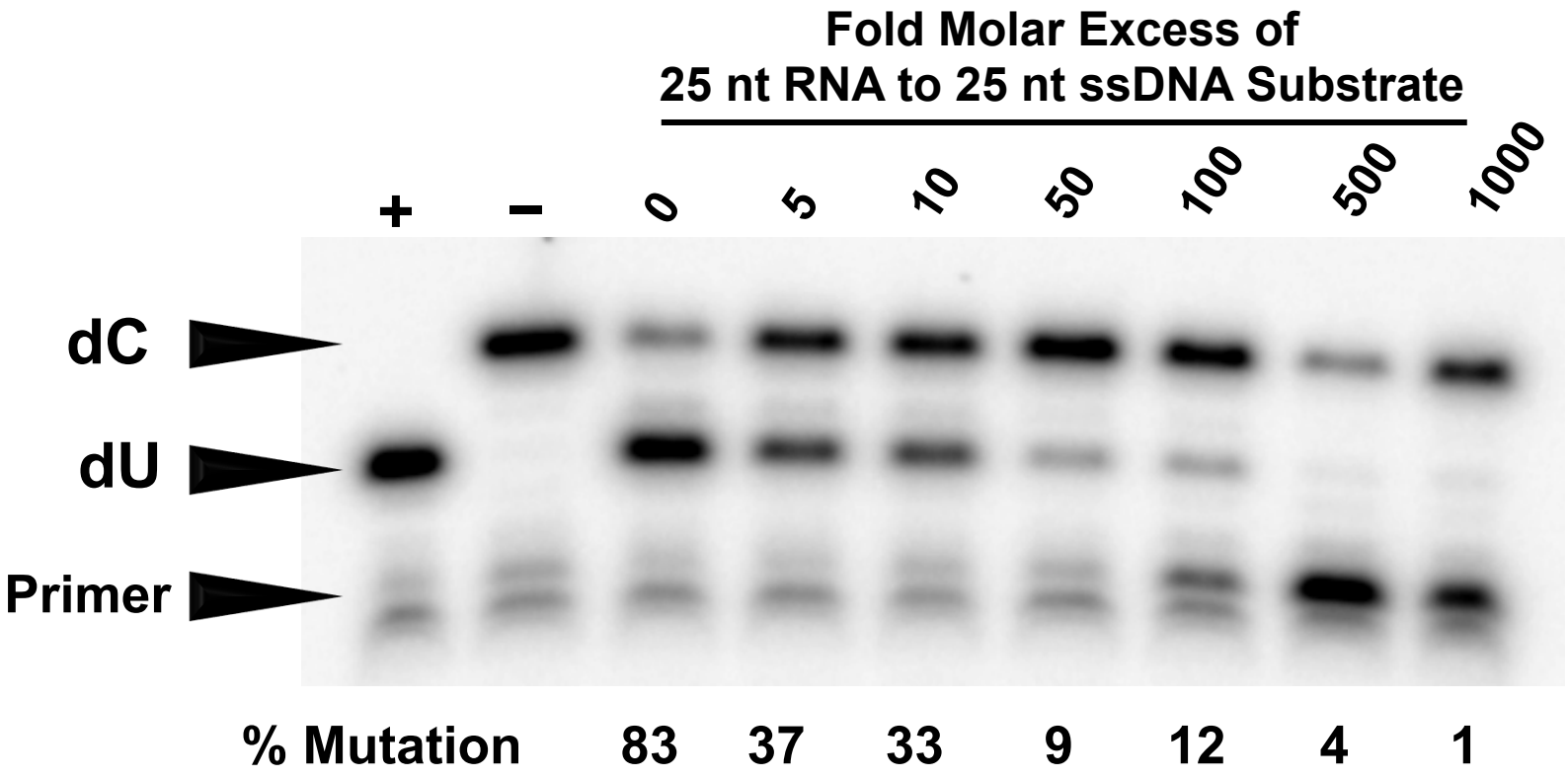
For LTQ Orbitrap XL analysis, a nano-HPLC system (Easy-nLC II, Thermo Fisher Scientific) was coupled to the electrospray ionization source of an LTQ Orbitrap **XL** mass spectrometer (Thermo Fisher Scientific). After digestion, samples were brought up in 30mL of 0.1% formic acid. Each sample was autosampler loaded onto a home-pulled, home-packed C_{18} analytical column. Columns were pulled to a tip of ~10µm with a Sutter Laser puller and packed using a pressure bomb to 10cm with C_{18}AQ 5µm 200Å media (Michrom), ending with an internal column diameter of 75µm. Columns were equilibrated to initial run conditions prior to loading the sample on the column. Solvent A was 0.1% formic acid in water, and Solvent B was 0.1% formic acid in acetonitrile. Peptides were eluted with the following chromatographic profile: 0% B for 2 minutes, ramping to 40% B over 13 minutes then to 70% B over 1 minutes, remaining at 70% B for 3 minutes, and finally returning to initial run conditions. Data were collected as RAW files.

Data Processing and Data Base Searching RAW files from experiments were converted to .mgf files using Bioworks Browser. Resultant .mgf were imported into ProteinScape (Bruker Daltonics) and searched via MASCOT (MatrixScience). Search parameters included: trypsin as an enzyme; 3 missed cleavages; MS tolerance of 1.5Da; MS/MS tolerances of 0.8Da for LTQ data and 0.5Da for MS and MS/MS data from the LTQ Obitrap XL; 1 for #13C, +2; +3 for charge state; decoy search and acceptance criteria of minimum 1 peptide greater than identity score; minimum score of 15; and False Discovery Rate less than 5%. The high number of missed cleavages was chosen using the assumption that trypsin might not cut at a modified residue, as modified residues would not fit in the trypsin active site. The ProteinExtractor function of ProteinScape combined search results and compiled a non-redundant list of identifications. Matched spectra were manually validated using BioTools (Bruker Daltonics). Identification of peptides with a BrdU modification must also show the appropriate ration of $Br^{79/81}$ isotopes in the parent peak.

(1) Lapek, J. D., Jr.; McGrath, J. L.; Ricke, W. A.; Friedman, A. E. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* **2012**, *893-894*, 34.

(2) Shevchenko, A.; Wilm, M.; Vorm, O.; Mann, M. *Anal Chem* **1996**, *68*, 850.

Supplemental Figure 1



Supplemental Figure 2

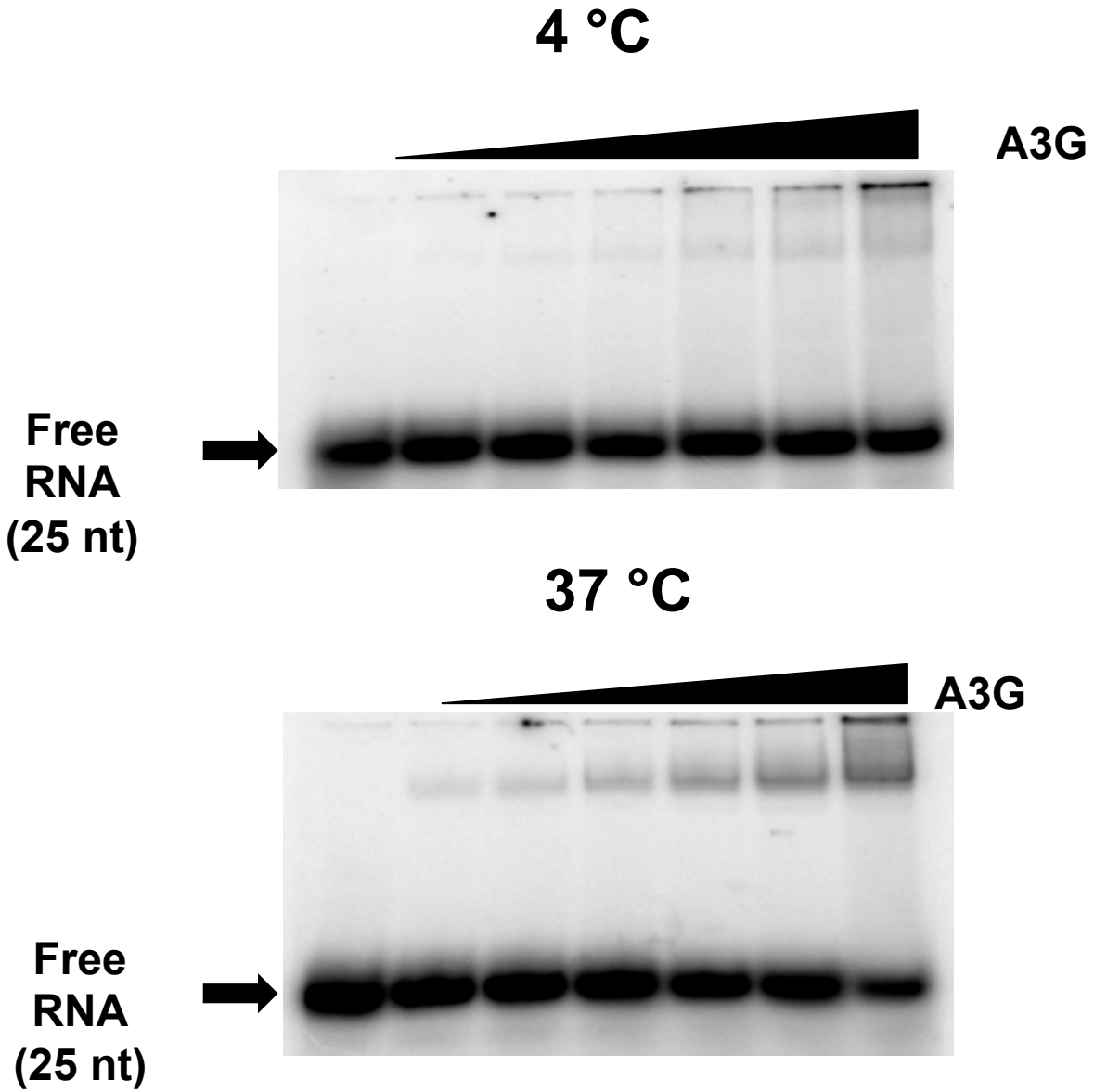


Fig. S3a. A3G tryptic peptide coverage of the control and UV-crosslinked BrdU-modified various length ssDNA or RNA protein samples

A. A3G control (no nucleic acids)

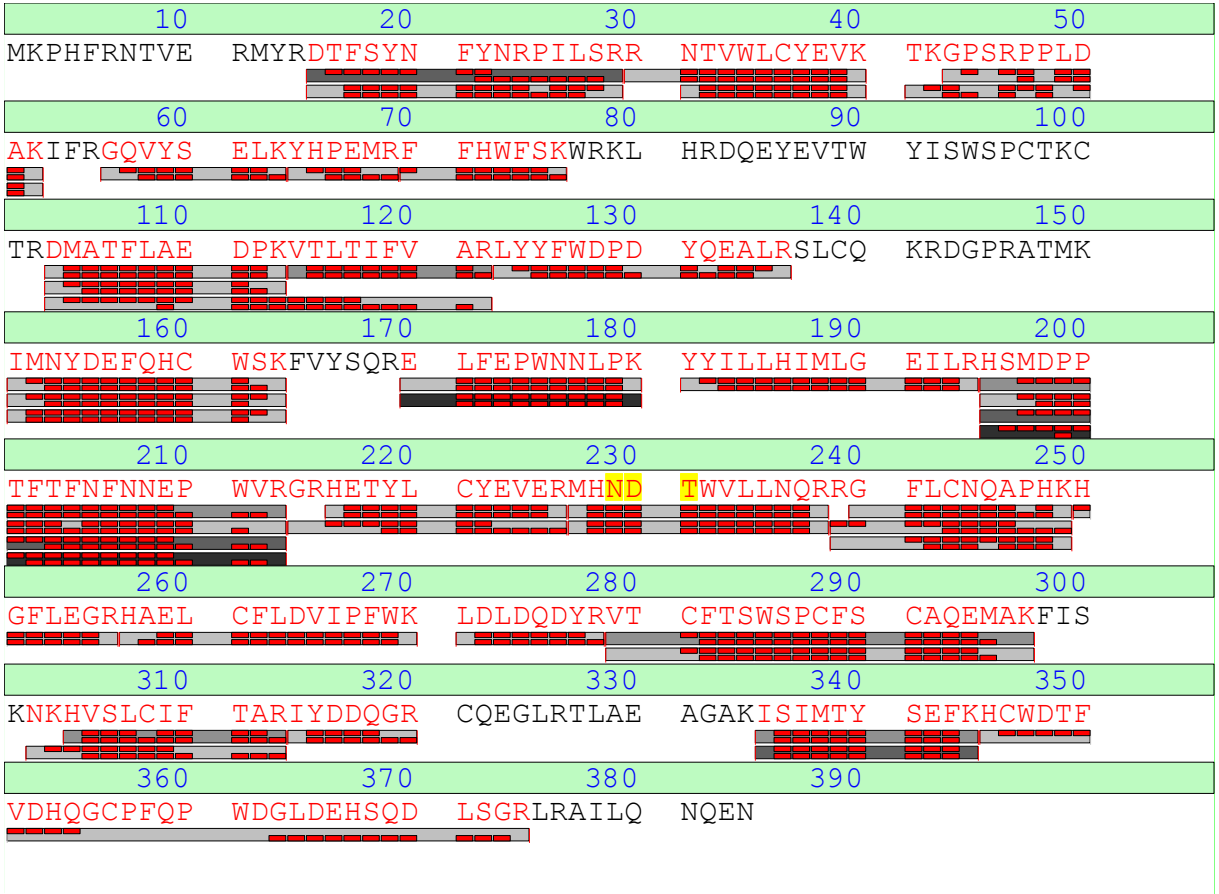


Fig. S3b. A3G tryptic peptide coverage of the control and UV-crosslinked BrdU-modified various length ssDNA or RNA protein samples (continued)

B. 15 nt ssDNA

Fig. S3c. A3G tryptic peptide coverage of the control and UV-crosslinked BrdU-modified various length ssDNA or RNA protein samples (continued)

C. 25 nt ssDNA

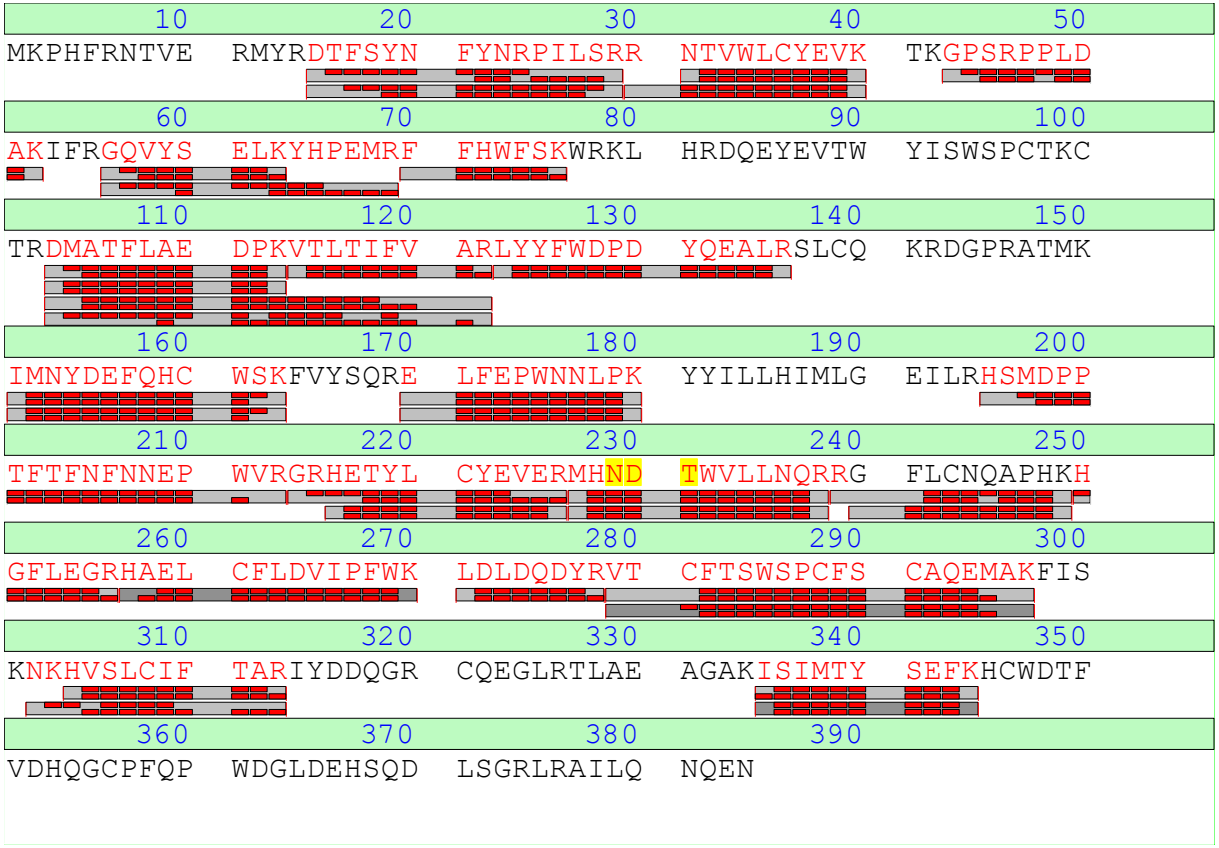


Fig. S3d. A3G tryptic peptide coverage of the control and UV-crosslinked BrdU-modified various length ssDNA or RNA protein samples (continued)

D. 99 nt ssDNA with one BrdU substitution

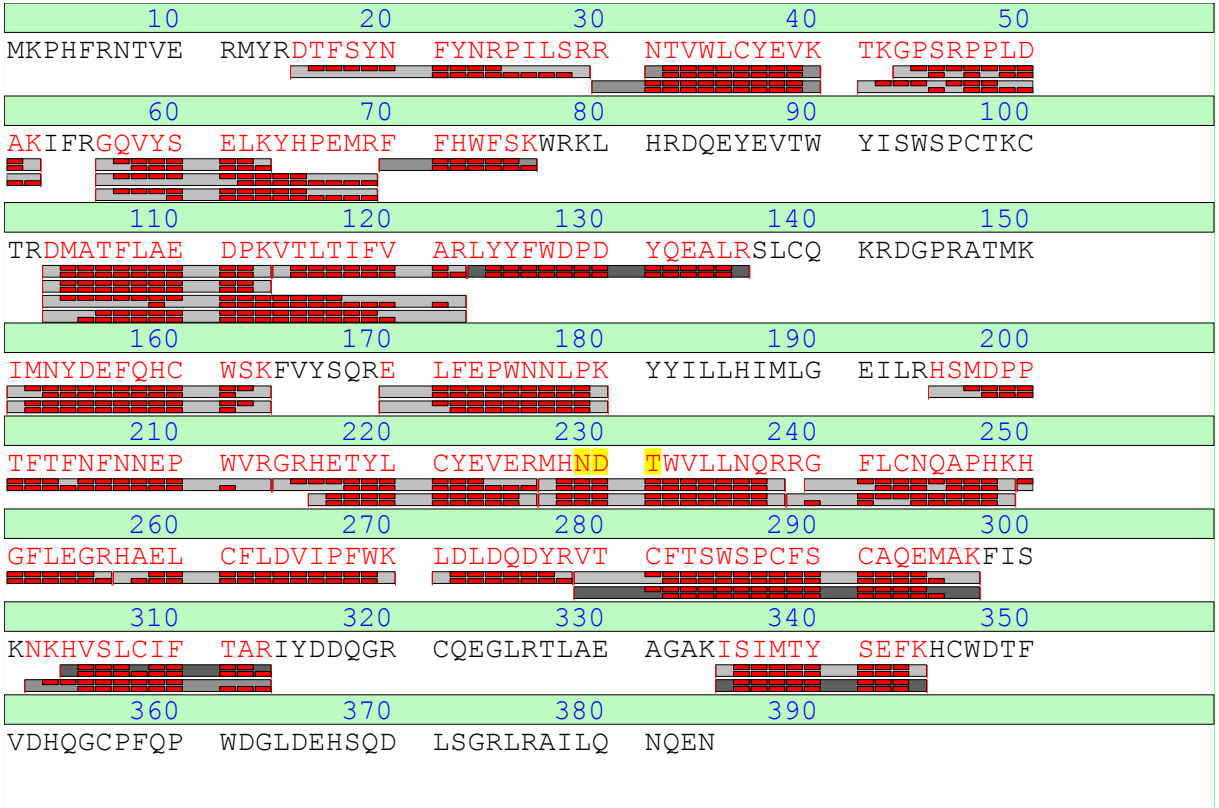


Fig. S3e. A3G tryptic peptide coverage of the control and UV-crosslinked BrdU-modified various length ssDNA or RNA protein samples (continued)

E. 99 nt ssDNA with three BrdU substitution

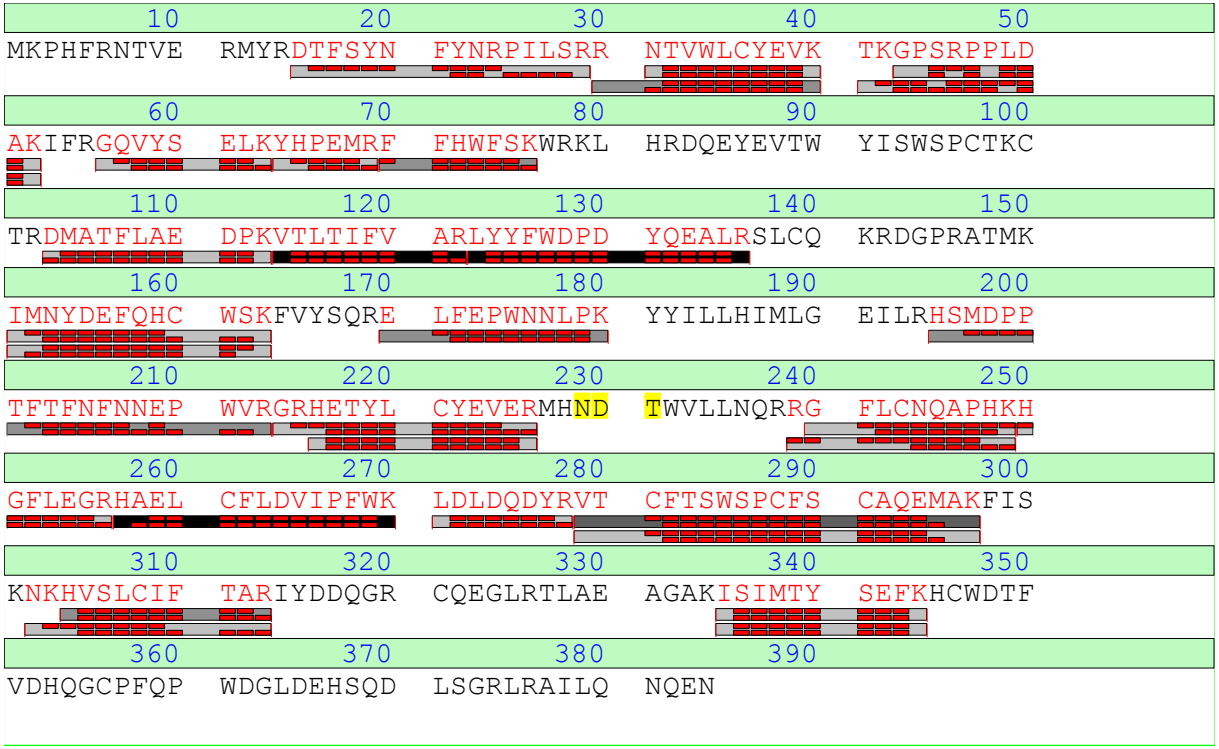
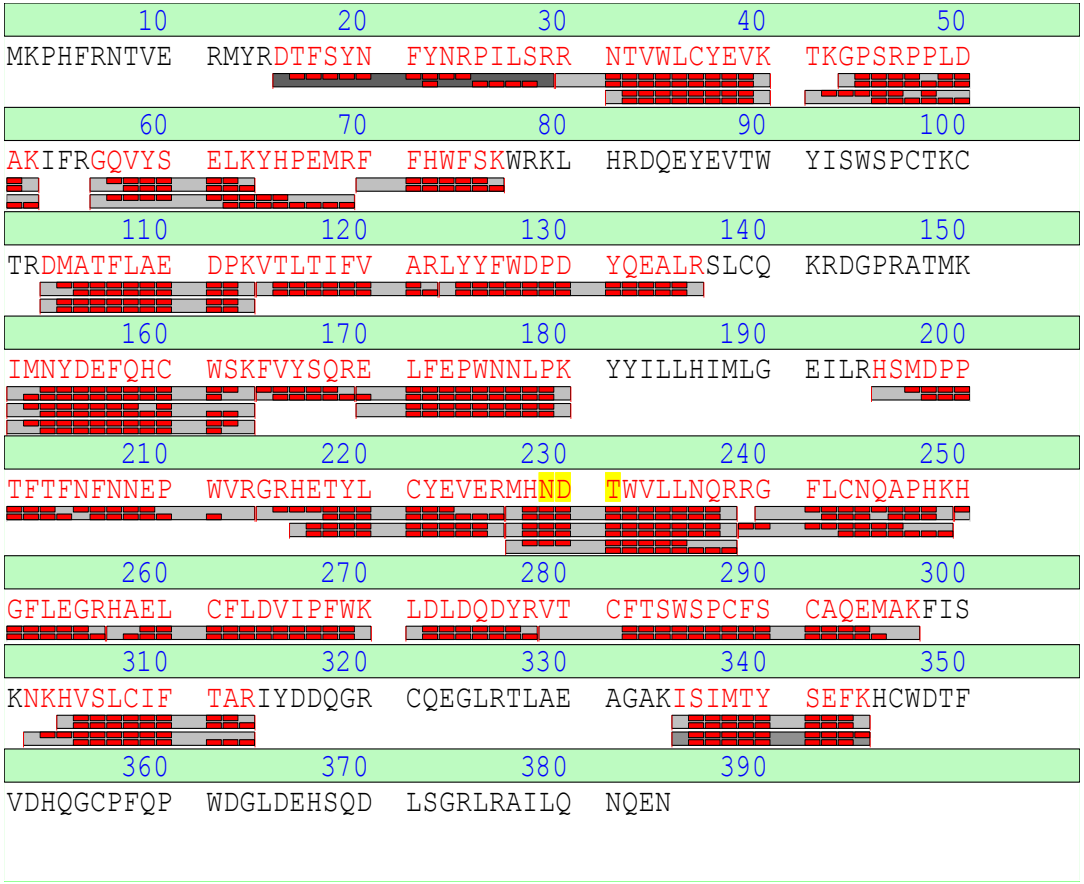


Fig. S3f. A3G tryptic peptide coverage of the control and UV-crosslinked BrdU-modified various length ssDNA or RNA protein samples (continued)

F. 25 nt apoB RNA



SUPPLEMENTAL FIGURE 1. *In vitro* deaminase activity on 25 nt Sub ssDNA is inhibited by 25nt apoB RNA in a concentration-dependent manner. The dC to dU deaminase activity of A3G on 25nt Sub ssDNA was quantified by PhosphorImager scanning densitometry of PAGE upon which poisoned primer extension products had been resolved as described in Methods. The indicated molar ratio of 25nt apoB RNA to 25 nt Sub ssDNA was established before incubating each reactions at 37 ^{o}C. Primer extension control reactions lacked A3G and contained either premutated 25 nt ssDNA (+) or unmutated 25 nt ssDNA (-). Percent mutation was calculated for each reaction as the density of the primer extension band labeled dU divided by the sum of the densities in the primer extension bands labeled as dU and dC, multiplied by 100. The PAGE gel shown is representative of replicate determinations {McDougall, 2011 #362}.

SUPPLEMENTAL FIGURE 2. Temperature dependence of A3G:RNA complex assembly. The efficiency of A3G assembly with 5' [32P] γ-ATP 5' end-labeled apoB 25 nt RNA at 4 °C (**A**) or 37 °C (**B**) was evaluated by EMSA. For both temperatures, the concentration of RNA in each reaction was 2.0 μM, whereas the concentration of A3G was titrated such that reactions contained 0 μM, 0.03 μM, 0.06 μM, 0.11 μM, 0.22 μM, 0.44 μM, 0.86 μM (Lanes 1-7 respectively in each panel). Shifts were visualized by PhosphorImager scanning densitometry. The gels shown are representative of a single independent experiment.

SUPPLEMENTAL FIGURE 3. A3G sequence coverage of the control and UV cross-linked to ssDNA or RNA protein samples. A3G sequences indicated in red are tryptic peptides identified using the LTQ Obitrap XL. Red colored stripes underlying the protein sequences reflect the peptide scores for Mascot searches in each sample that were high score matches for the *b* and *y* ion mass/charge values matches to the predicted MS peptide fragmentation. Gray/black-colored sections indicate low or below threshold peptide fragmentation matches respectively. Representative sequence coverage is shown for the following samples: A, A3G control (no nucleic acids); B, 15 nt ssDNA; C, 25 nt ssDNA; D, 99 nt ssDNA; E, 99 nt ssDNA with three BrdU substitutions; F, 25 nt ApoB