Supporting Information

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SI Materials and Methods

Cell Lines and Culture Conditions. HeLa cells (ATCC) and HEK293T cells (ATCC) were grown in DMEM (Gibco) supplemented with 10% FBS (Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco) at 37 °C in a 5% CO_2 atmosphere. All experiments were conducted using cells with passage number <30 and free of mycoplasma contamination.

Plasmid Construction and Cell Transfection. Human full-length hnRNPC was cloned from HeLa cDNA into pFLAG-CMV-2 with the following primers: forward primer, 5′-CCC AAG CTT CCA TGG CCA GCA ACG TTA CCA AC-3′ and reverse primer, 5′-GGA ATT CCT AAG AGT CAT CCT CGC CAT TGG-3′. Human full-length MBNL1 was cloned from HeLa cDNA into pEGFP-C1 with the following primers: forward primer, 5′-TAA TCC TCG AGC TAT GGC TGT TAG TGT CAC ACC-3′ and reverse primer, 5′-CGG GAT CCC TAC ATC TGG GTA ACA TAC TTG TG-3′. Human full-length PSMA7 was cloned from HeLa cDNA into p3×FLAG-CMV-10 with the following primers: forward primer, 5′-CCC AAG CTT ATG AGC TAC GAC CGC GCC-3′ and reverse primer, 5′-CGG GAT CCT CAT GAT GCT TTC TTT TGT TTC TTC TTT T-3′. Human full-length VDAC1 was cloned from HeLa cDNA into p3×FLAG-CMV-10 with the following primers: forward primer, 5′-GGG GTA CCA ATG GCT GTG CCA CCC ACG T-3′ and reverse primer, 5′-CGG GAT CCT TAT GCT TGA AAT TCC AGT CCT AGA C-3′. Human full-length NME2 was cloned from HeLa cDNA into pFLAG-CMV-2 with the following primers: forward primer, 5′-CCC AAG CTT ATG GCC AAC CTG GAG CGC AC-3′ and reverse primer, 5′-CGG GGT ACC TTA TTC ATA GAC CCA GTC ATG AGC AC-3′. HeLa and HEK293T cells were transfected with these plasmids using VigoFect (Vigorous Biotechnology) following the manufacturer's instructions.

Antibodies. Antibodies included anti-VDAC1 (ab154856, 1:1,000; Abcam), anti-RAB10 (ab181367, 1:1,000; Abcam), anti-RAP1A (ab181858, 1:2,000; Abcam) (also recognizes RAP1B, which was not identified by CARIC), anti-PSMA6 (ab109377, 1:10,000; Abcam), anti-PSMA2 (ab109525, 1:1,000; Abcam), anti-DDDDK-tag (M185-3, 0.1 μg/mL; MBL), anti-DYKDDDDK-tag (637301, 1:500; BioLegend), anti-GFP (2555, 1:1,000; CST), anti-Nucleolin (ab129200, 1:10,000; Abcam), anti-hnRNPC (sc-32308, 1:400; Santa Cruz) (recognizes both hnRNPC1 and hnRNPC2), anti-FUBP3 (ab181025, 1:2,000; Abcam), anti-PTBP1 (ab133734, 1:2,000; Abcam), anti-mouse IgG (HRP) (ab97023, 1:5,000; Abcam), and anti-rabbit IgG (HRP) (ab6721, 1:5,000; Abcam), and anti-rat IgG (HRP) (ab6734, 1:4,000; Abcam).

Metabolic Incorporation of EU and 4SU in HeLa Cells. EU and 4SU were purchased from Wuhu Huaren Co. and Sigma, respectively. HeLa cells were grown to ∼80% confluence and incubated with 1 mM EU and 0.5 mM 4SU, 1 mM EU alone, or 0.5 mM 4SU alone for 16 h, followed by an additional labeling pulse with 0.5 mM EU and 0.25 mM 4SU, 0.5 mM EU, or 0.25 mM 4SU, respectively, for 2 h. The additional labeling pulse ensured efficient labeling of short-life RNAs. Unless otherwise specified, all of the EU&4SU, EU, and 4SU metabolic labeling experiments were performed using this procedure.

Photocross-Linking and Click Labeling of HeLa Cells. For cells in one 15-cm culture dish that had been metabolically incorporated with EU&4SU, EU, or 4SU, the cells were washed with 5 mL PBS three times, followed by irradiation with 365-nm UV light at 2 J/cm^2 by using a UV cross-linker (CL-1000; UVP). After adding 1 mL buffer [50 mM Tris·HCl, pH 7.5, 100 mM LiCl, 0.02% Nonidet P-40, and EDTA-free protease inhibitor mixture (Thermo)], the cells were harvested by using a cell lifter (Corning). The cell suspension was then adjusted to 6 mL lysis buffer [100 mM Tris·HCl, pH 7.5, 500 mM LiCl, 1% LDS (lithium dodecyl sulfate), 0.003% Nonidet P-40, and EDTA-free protease inhibitor mixture] and homogenized by passing through a narrow needle, followed by incubation at 4 °C with gentle rotation for 1 h. The cell lysate was diluted with 120 mL of 50 mM Tris·HCl (pH 7.5) buffer and divided into 15-mL fractions. Each fraction was concentrated by using an Amicon Ultra-15 centrifugal filter unit (molecular weight cutoff of 10 kDa; Millipore) and washed once with 13 mL Tris·HCl buffer. After combing all of the fractions, the lysate was concentrated to a final volume of 5 mL to remove most LDS. After preclearing with 100 μL streptavidin magnetic beads (Thermo) with gently rotation for 30 min at room temperature (rt), the lysate was reacted with 100 μM azido probe [azide-PEG_3-biotin (Click Chemistry Tools) or azide-Cy5 (Okeanos Tech. Co.)], 500 μM $CuSO_4$, 2 mM THPTA (Sigma), and 5 mM sodium ascorbate (freshly prepared) in a solution with a final volume of 5.5 mL for 2 h at rt with vortex, followed by adding 5 mM EDTA to quench the reaction. When applicable, the reagents used were purchased in the form of RNase-free or dissolved in RNase-free solvents.

On-Bead Flow Cytometry Analysis of the Metabolically Labeled RNAs. Total RNAs HeLa cells treated with EU&4SU, EU, 4SU, or vehicle were extracted using TRIzol (Ambion) following the manufacturer's instructions. A mixed RNA sample was generated by mixing RNAs from EU- and 4SU-treated cells at a 1:1 ratio. Fifty-microgram RNAs from each group were reacted with 100 μM N_3-Cy5 (Okeanos Tech. Co.), 500 μM $CuSO_4$, 2 mM THPTA (Sigma), and 5 mM sodium ascorbate in 300 μL aqueous solution for 2 h at rt, followed by quenching the reaction with 5 mM EDTA. RNAs were precipitated by an equal volume of isopropanol for 10 min at rt. Precipitates were washed with 75% ethanol, air-dried, resuspended in H_2O, and reacted with 0.2 μg/μL HPDP-biotin (1 μg/μL stock solution in dimethylformamide; Thermo) in 10 mM Tris·HCl buffer (pH 7.5) containing 1 mM EDTA for 2 h at rt in the dark. One volume of PCI (acidic phenol: chloroform:isoamyl alcohol, 25:24:1; Sigma) was added to the reaction solution. After centrifugation, the upper aqueous phase containing RNAs was recovered and washed with one volume of chloroform. RNAs were precipitated by 1/10 volume of 3 M NaCl aqueous solution and one volume of isopropanol at rt for 10 min. Precipitates were washed with 75% ethanol, air-dried, resuspended in H_2O, and heat-denatured at 68 °C for 5 min. The solution was incubated with 5 μL streptavidin magnetic beads at rt for 1 h. The beads were washed with high-salt wash buffer (100 mM Tris·HCl, pH 7.5, 10 mM EDTA, 1 M NaCl, and 0.1% Tween-20) three times and analyzed directly by using flow cytometry on a BD Accuri C6 flow cytometer.

Evaluation of EU and 4SU Concentration for Metabolic Incorporation. HeLa cells at ∼80% confluence in 15-cm culture dishes were treated with EU&4SU at varied concentrations (1 mM and 0.2 mM, 1 mM and 0.5 mM, 1 mM and 1 mM, 1 mM and 2 mM, 1 mM and 0 mM, and 0 mM and 0.2 mM) for 16 h, followed by a pulse labeling with EU&4SU at half concentrations. The cells were then photocross-linked and click-labeled with azide-Cy5 as described in *Photocross-Linking and Click Labeling of HeLa*

Cells, except that the CuAAC reaction was performed with no ligand. The lysates were denatured at 55 °C for 5 min in 1× NuPAGE LDS sample buffer (Invitrogen) and then resolved on a NuPAGE 10% bis-Tris gel (Invitrogen), followed by in-gel fluorescence scanning on a Typhoon FLA 9500 laser scanner (GE Healthcare) and stained with Coomassie brilliant blue.

Evaluation of Photocross-Linking and Click-Labeling Conditions. For evaluating the photocross-linking conditions, HeLa cells were treated with EU&4SU as described in *Metabolic Incorporation of EU and 4SU in HeLa Cells*, followed by irradiation with 365-nm UV light at varied energy densities ranging from 0.2 to 8 J/cm^2. The cells were then click-labeled with azide-Cy5 as described in *Photocross-Linking and Click Labeling of HeLa Cells*, except that the CuAAC reaction was performed with no ligand, followed by analysis by in-gel fluorescence scanning. For evaluating the ligands for CuAAC, the cells were treated with EU&4SU as described in *Metabolic Incorporation of EU and 4SU in HeLa Cells*. The cells were then photocross-linked and click-labeled as described in section *Photocross-Linking and Click Labeling of HeLa Cells*, by using various ligands including BTTAA, THPTA, and TBTA, followed by analysis by in-gel fluorescence scanning.

Cell Viability Assays. HeLa cells were seeded on six-well plates and cultured for 24 h. Cells were treated with EU&4SU, EU, 4SU, or vehicle, using the procedure describe in *Metabolic Incorporation of EU and 4SU in HeLa Cells*. The cells were washed twice with 1 mL PBS, followed by incubation with 1 mL PBS containing CCK-8 reagent (1:10 dilution; Beyotime) and 10% FBS at 37 °C for 15 min. The cell viability was then determined by measuring the absorbance at 450 nm of the incubation solution by using a Synergy H4/Hybrid Reader (BioTek).

Analysis of RNP Cross-Linking. HeLa cells were incubated with EU&4SU, EU, or 4SU as described in *Metabolic Incorporation of EU and 4SU in HeLa Cells*. The cells were then photocross-linked and click-labeled with azide-Cy5 as described in *Photocross-Linking and Click Labeling of HeLa Cells*. Each sample was divided into halves. One half of each sample was treated with 0.5 μg/μL RNase A at 37 °C for 1 h, and the other half was not treated. The samples were then analyzed by in-gel fluorescence scanning. To evaluate the AD concentration for complete inhibition of RNA synthesis, HeLa cells were seeded in eight-well chamber slides (Thermo), grown to ∼50% confluence, and treated with AD at varied concentrations ranging from 0.1 to 8 μM for 30 min at 37 °C. To the cells was added 1 mM EU. After incubation for 3 h, the cells were washed three times with 200 μL PBS, followed by fixation and permeabilization by incubation with 200 μL PBS containing 3.7% paraformaldehyde and 0.1% Triton X-100 for 15 min at rt. After washing with PBS, the cells were reacted with 50 μM azide-Cy5, 1 mM $CuSO_4$, and 2.5 mM sodium ascorbate (freshly prepared) in PBS for 30 min at rt. At the last 10 min of the reaction, 2 μg/mL Hoechst 33342 was added to stain cell nuclei. The cells were washed three times with 200 μL PBS containing 0.02% Tween-20 and imaged by a laser scanning confocal microscope (Zeiss LSM 700). For AD inhibition experiments, HeLa cells were treated with or without 8 μM AD for 30 min. The cells were then incubated with EU&4SU, EU, or 4SU for 3 h. The cells were then photocross-linked and click-labeled with azide-Cy5 as described in *Photocross-Linking and Click Labeling of HeLa Cells*, followed by analysis by in-gel fluorescence scanning.

Isolation of RBPs by CARIC. HeLa cells grown in 15-cm dishes were treated with EU&4SU, EU, or 4SU as described *Metabolic Incorporation of EU and 4SU in HeLa Cells*, followed by photocross-linking and click labeling with azide-biotin as described in *Photocross-Linking and Click Labeling of HeLa Cells*, which resulted in 5.5 mL labeled lysate per dish. For UV-omitted experiments, the cells were treated with EU&4SU and kept in the dark for the following experiments until the captured proteins were eluted from the streptavidin beads. The lysate was precipitated with 8 vol of precooled methanol at −30 °C for 30 min. The pellet was washed twice with precooled methanol and dissolved with 5 mL buffer [solution A: 4% SDS and 10 mM EDTA; solution B: 1% Brij-97, 150 mM NaCl and 50 mM triethanolamine, pH 7.4. A:B 1:8 (vol/vol)]. To the solution was added 0.4 mL settled streptavidin agarose beads (Thermo), followed by incubation with gentle rotation overnight at 4 °C. The agarose beads were washed twice with 10 mL PBS containing 2% SDS (pH 7.4) for 10 min, twice with 10 mL of 250 mM NH_4HCO_3 buffer containing 8 M urea for 10 min, and twice with 10 mL PBS containing 2.5 M NaCl (pH 7.4) for 10 min. After a brief wash with 50 mM Tris·HCl (pH 7.5), the agarose beads were resuspended in 400 μL biotin elution buffer (12.5 mM biotin, 75 mM NaCl, 7.5 mM Tris·HCl, pH 7.5, 1.5 mM EDTA, 0.15% SDS, 0.075% sarkosyl, and 0.02% Na-deoxycholate) and incubated on an orbital shaker (1,500 rpm; IKA MS3) at rt for 20 min, followed by heating at 65 °C for 10 min on a thermo shaker. The solution was collected and the beads were eluted again to give 800 μL solution in total, which was diluted to 0.05% SDS by adding 50 mM Tris·HCl (pH 7.5). The solution was concentrated to 40 μL by using an Amicon Ultra-0.5 centrifugal filter unit (molecular weight cutoff of 10 kDa), followed by incubation with 0.5 μg/μL RNase A at 37 °C for 2 h. The resulting sample was then used for analysis on SDS/PAGE with silver staining, Western blotting, and MS-based proteomic analysis.

SDS/PAGE and Western Blotting on Isolated RBPs. The sample of isolated RBPs (one-fifth of that from one 15-cm culture dish) was denatured at 70 °C for 10 min in 1× NuPAGE LDS sample buffer and resolved on a NuPAGE 10% bis-Tris gel, followed by visualization by silver staining. For Western blot analysis of specific proteins enriched by CARIC, the sample of isolated RBPs (one-fifth of that from one 15-cm culture dish) was resolved on a NuPAGE 10% bis-Tris gel, transferred to PVDF membrane (0.2 μm; Millipore), blocked by 5% (wt/vol) skim milk (BD), and incubated with specific primary antibodies for 1.5 h at rt using concentrations suggested by the manufacturers. After washing with TBST three times, HRP-conjugated goat anti-mouse (or rabbit) IgG was applied and incubated at rt for 1 h. HRP substrate peroxide solution (Millipore) was used to visualize proteins on a Tanon-5200 Multi. To obtain the RNase A-treated samples, during the isolation of RBPs as described in *Isolation of RBPs by CARIC*, the sample was treated with 0.2 μg/μL RNase A for 1 h at 37 °C before precipitation of the click-labeled sample with precooled methanol.

RNA Sequencing of CARIC Isolated RNAs. RNPs from cells on one 15-cm dish were isolated as described in *Isolation of RBPs by CARIC* until an 800-μL elution solution was obtained, to which an equal volume of 2× proteinase buffer (100 mM Tris·HCl, pH 7.5, 12.5 mM EDTA, 150 mM NaCl, 2% SDS) and 2 mg/mL Proteinase K (Ambion) was added and incubated at 55 °C for 1 h. The RNAs were then isolated by TRIzol following the manufacturer's instructions. For each replicate, 1.5 μg RNA was used to construct the cDNA library using a NEBNext Ultra RNA Library Prep Kit for Illumina (NEB) following the manufacturer's instructions. The cDNAs were purified by AMPure XP magnetic beads (Beckman Coulter, Inc.) and quality-controlled on an Agilent Bioanalyzer 2100 system. The cDNA libraries were sequenced on an Illumina HisEq 4000 platform and 150-bp paired-end reads were generated for each sample. The nucleotide sequences of all transcripts on the human reference genome were downloaded from GENCODE (release 25) with transcript types marked. The sequences of 45S preribosomal 5 were obtained from NCBI and integrated together. The adaptors were removed and low-quality reads were filtered from the raw data using the software Skewer (71). The cleaned data were mapped to the whole transcripts using BWA.

The mapped reads were exported and the number of each type of RNA transcripts was counted.

Sample Preparation for MS-Based Proteomic Analysis. RBPs isolated, as described in *Isolation of RBPs by CARIC*, from cells in two 15-cm dishes were used for one MS run. The isolated RBP samples were resolved by 10% SDS/PAGE and stained by silver staining. Each lane of the gel was sliced into small cubes with the band of RNase A removed. The gel cubes were washed with H_2O, destained with the equivalent volume mixture of 100 mM $Na_2S_2O_3$ and 30 mM $K_3[Fe(CN)_6]$. Then, the gel cubes were washed twice with H_2O and twice with 200 mM ABC (ammonium bicarbonate) till the gel cubes were completely colorless and dehydrated in acetonitrile at rt. The gel cubes were incubated with 200 μL of 10 mM DTT (in 50 mM ABC) at 56 °C for 45 min, blocked by adding an equal volume of 58 mM iodoacetamide (in 50 mM ABC) at rt, and incubated for 45 min in the dark. After washing with H_2O, the gel cubes were dehydrated in acetonitrile and digested with 10 ng/μL trypsin (Promega) dissolved in 50 mM ABC at 37 °C overnight. Digested peptides were extracted from gels by 50% (vol/vol) acetonitrile in H_2O with 5% (vol/vol) formic acid (Sigma) at 37 °C for 30 min. The extraction step was repeated twice. The extracts were combined, SpeedVac-dried (Eppendorf), and redissolved in 200 μL of 100 mM triethyl ammonium bicarbonate (TEAB; Sigma). The stable isotopic dimethyl labeling was conducted as previously described with minor modifications (45). Briefly, the peptide samples (200 μL each) from EU&4SU-treated and UV-irradiated cells (experimental samples) and EU-treated and UV-irradiated cells (no4SU control samples) or EU&4SU-treated and nonirradiated cells (noUV control samples) were added with 8 μL of 4% (vol/vol) CH_2O (Sigma) or $^{13}CD_2O$ (Sigma), respectively, and 8 μL 0.6 M $NaBH_3CN$ (Acros). The solutions were incubated at rt for 1 h, followed by addition of 32 μL of 1% (vol/vol) ammonia solution. After adding 16 μL of formic acid, the light and heavy isotopically labeled samples were mixed. The mixed samples were SpeedVac-dried and redissolved in 50 μL 10 mM ABC (pH 10.0). Then, the peptides were fractionated by high-pH reverse phase StageTip as previously reported (46). Briefly, to prepare the StageTips, 300 μg C18 beads (Agela) suspended in acetonitrile were packed into extended length 10-μL tips (Axygen) inserted with C18 membrane (3M) through centrifugation at 1,400 × *g* for 2 min. The StageTips were washed twice with 50 μL 10 mM ABC (pH 10.0) in 80% acetonitrile, twice with 50 μL 10 mM ABC (pH 10.0) in 50% acetonitrile, and twice with 50 μL 10 mM ABC (pH 10.0). Then the peptides were bound to the StageTips, washed twice with 50 μL 10 mM ABC (pH 10.0) and stepwise-eluted for collection using 12 elution buffers (50 μL 10 mM ABC, pH 10.0, in 6, 9, 12, 15, 18, 21, 25, 30, 35, 40, 80, and 6% acetonitrile, respectively). Every two fractions with equal interval (fractions 1 with 7, 2 with 8, and so on) were combined, SpeedVac-dried, dissolved in 15 μL H_2O containing 0.1% (vol/vol) formic acid, and subjected to analysis by LC-MS/MS.

Proteomic Identification by LC-MS/MS. Samples were loaded onto a 100-μm × 2-cm precolumn and separated on a 75-μm × 15-cm capillary column with laser-pulled sprayer. Both columns were packed in-house with 4 μm C18 bulk material (InnosepBio). An Easy nLC 1000 system (Thermo) was used to deliver the following HPLC gradient: 5–35% B in 40 min, 35–75% B in 4 min, then held at 75% B for 10 min (A is 0.1% formic acid in water and B is 0.1% formic acid in acetonitrile). The eluted peptides were sprayed into a Velos Pro Orbitrap Elite mass spectrometer (Thermo) equipped with a nano-electrospray ionization source. The mass spectrometer was operated in data-dependent mode with a full MS scan (375–1,600 M/z) in FT mode at a resolution of 120,000 followed by collision-induced dissociation MS/MS scans on the 15 most abundant ions in the initial MS scan. Only multiply charged (2+, 3+, and higher) precursor ions were selected to do the MS/MS scan. The dynamic exclusion size was set to 500 entries with a maximum duration time of 25 s.

Mass Spectrometric Data Analysis. The raw data files were analyzed using MaxQuant version 1.5.5.1 (47) and MS/MS spectra were searched using the Andromeda search engine (72) against human proteome downloaded from UniProt (73) on November 4, 2016. The digestion mode was set to trypsin/P with maximum two missed cleavages and the minimal peptide length was set to 6 aa. Carbamidomethyl cysteine was set as fixed modification and methionine oxidation and acetyl N-terminal were set as variable modifications. For first search and main search, the maximal mass tolerance was set to 20 ppm and 6 ppm, respectively. For protein identification, the false discovery rate was set to 0.01 and at least two unique peptides were required. The "requantify" and "match between runs" functions were enabled and only proteins with at least two quantified peptides were reported as quantified proteins. Proteins quantified in at least three replicates (at least one in each control set) were further evaluated for enrichment significance using an empirical Bayes moderated *t* test by the Limma package in R/Bioconductor as described by Kwon et al. (13). The resulting *P* values were adjusted by the Benjamini–Hochberg method. Proteins with adjusted $P < 0.01$ and a fold change of two or greater were reported as CARIC RBPs. CARIC RBPs with a fold change greater than three were classified as class I RBPs and the rest were classified as class II RBPs.

Validation of CARIC RBPs by CLIP. HeLa cells grown on a 10-cm dish per sample were transfected with plasmids expressing FLAG-hnRNPC, FLAG-VDAC1, FLAG-PSMA7, and EGFP-MBNL1, as described in *Plasmid Construction and Cell Transfection*. HEK293T cells grown on a 10-cm dish per sample were transfected with plasmids expressing FLAG-NME2, as described in *Plasmid Construction and Cell Transfection*. Cells were washed with 5 mL PBS three times, cross-linked by 0.15 J/cm^2 254-nm UV light, and lysed in 500 μL CLIP lysis buffer (50 mM Tris·HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, and EDTA-free protease inhibitor mixture). The cell lysate was incubated on ice for 10 min and cleared by centrifugation at 13,000 × *g* for 15 min at 4 °C. Cell lysates were incubated with 1 U/μL RNase T1 (NEB) in 22 °C water bath for 15 min and cooled on ice for 5 min. For IP, 50 μL anti-FLAG-tag mAb-magnetic beads (MBL) or 50 μL anti-GFP mAb-magnetic beads (MBL) per sample were added to incubate with cell lysate for 1 h at 4 °C and washed with 1 mL IP wash buffer [50 mM Hepes-KOH, pH 7.5, 300 mM KCl, 0.05% (vol/vol) Nonidet P-40, and 0.5 mM DTT] twice. The beads were resuspended in 50 μL IP wash buffer, incubated with 100 U/μl RNase T1 in a water bath for 15 min at 22 °C, cooled on ice for 5 min, and washed with 1 mL high-salt wash buffer [50 mM Hepes-KOH, pH 7.5, 500 mM KCl, 0.05% (vol/vol) Nonidet P-40, and 0.5 mM DTT] three times. The beads were resuspended in 50 μL dephosphorylation buffer (5 mM Tris·HCl, pH 7.5, 10 mM NaCl, 1 mM $MgCl_2$, and 0.1 mM DTT), incubated with 0.5 U/μL alkaline phosphatase, calf intestinal (CIP; NEB) in a Thermo-Shaker (Hangzhou Allsheng Instruments Co.) at 37 °C for 10 min, and washed with 1 mL phosphatase wash buffer [50 mM Tris·HCl, pH 7.5, 20 mM EGTA-NaOH, pH 7.5, and 0.5% (vol/vol) Nonidet P-40] followed by washing twice with 1 mL polynucleotide kinase (PNK) buffer without DTT [50 mM Tris·HCl (pH 7.5), 50 mM NaCl and 10 mM $MgCl_2$]. The beads were then resuspended in 50 μl PNK buffer with DTT (50 mM Tris·HCl, pH 7.5, 50 mM NaCl, 10 mM $MgCl_2$, and 5 mM DTT) and incubated with 0.1 μCi/μL [γ-^{32}P]ATP (China Isotope & Radiation Co.) and 1 U/μL T4 PNK (NEB) in a Thermo-Shaker at 37 °C for 30 min. Then, 100 μM nonradioactive ATP was added to fully 5′-phosphorylate all RNAs at 37 °C for 5 min. The beads were then washed by 800 μL PNK buffer without DTT five times, resuspended in 20 μL 1× NuPAGE LDS sample buffer, and incubated in a heat block at 95 °C for 5 min. The supernatant was collected and resolved by SDS/PAGE and transferred to PVDF membrane. The membrane was exposed to a phosphorimaging screen (Fuji) overnight, visualized

on a Typhoon FLA 9500 laser scanner. Western blotting against the FLAG or EGFP tag was used as the protein loading control.

Functional Analysis of Identified Proteins. The theoretical isoelectric point of proteins was calculated by ExPASy (74) and density distribution curves were drawn using MATLAB. The protein domain was manually annotated according to Pfam (75). Metabolism-related proteins were analyzed using Reactome (56). GO and KEGG pathway analyses were conducted on DAVID 6.8 (48, 49) and the 10 most enriched terms against all human proteins were presented. For the GO analysis, proteins annotated in multiple terms were counted multiple times. For the RNA target, metabolic enzyme, and OMIM gene analysis, one protein was counted only once under each term, and proteins annotated with multiple terms were counted multiple times.

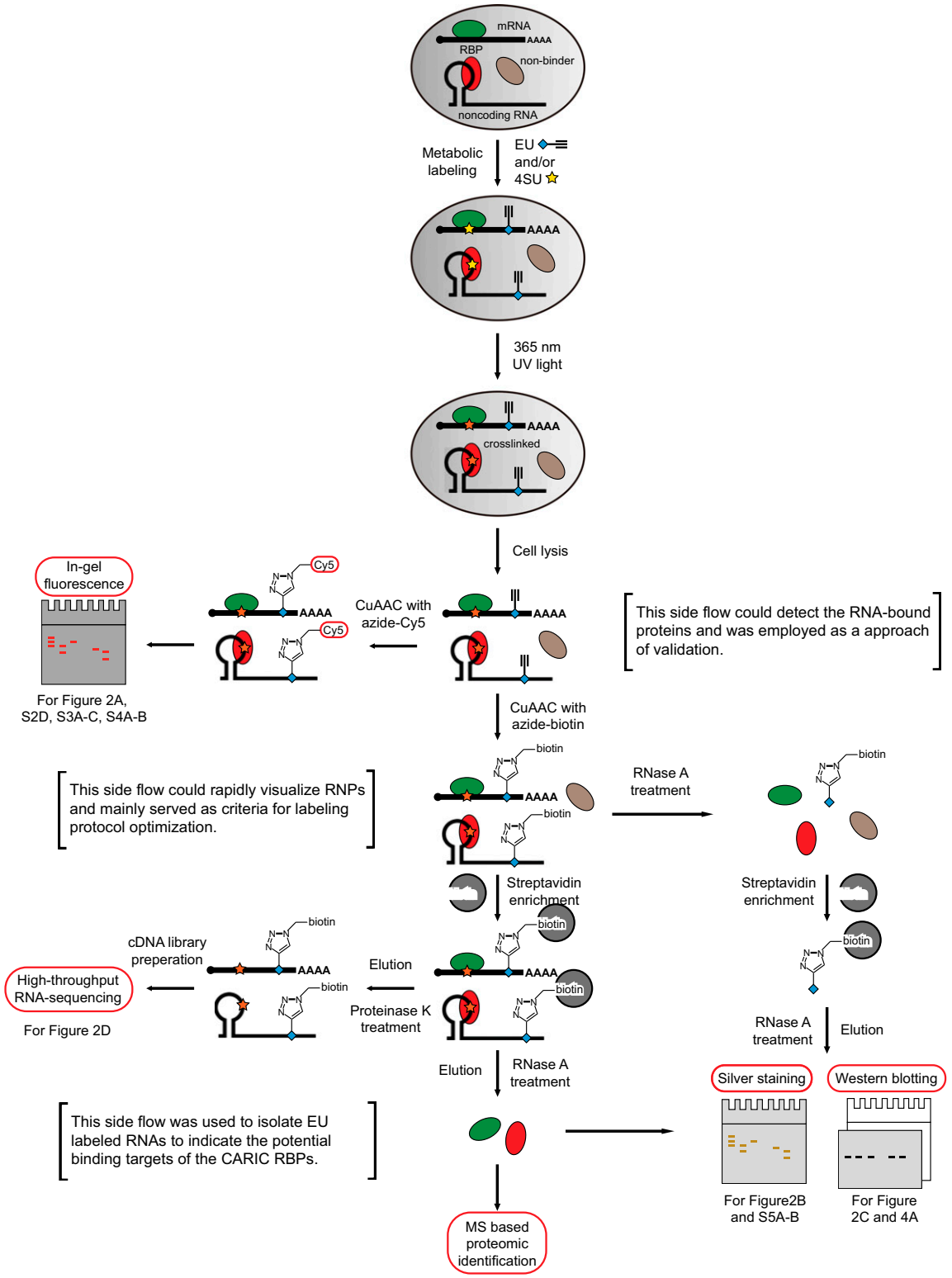

Fig. S1. Detailed workflow of CARIC. The central flow depicts CARIC-enabled enrichment and proteomic identification. The side flows show the procedures for the characterization and validation of CARIC experiments.

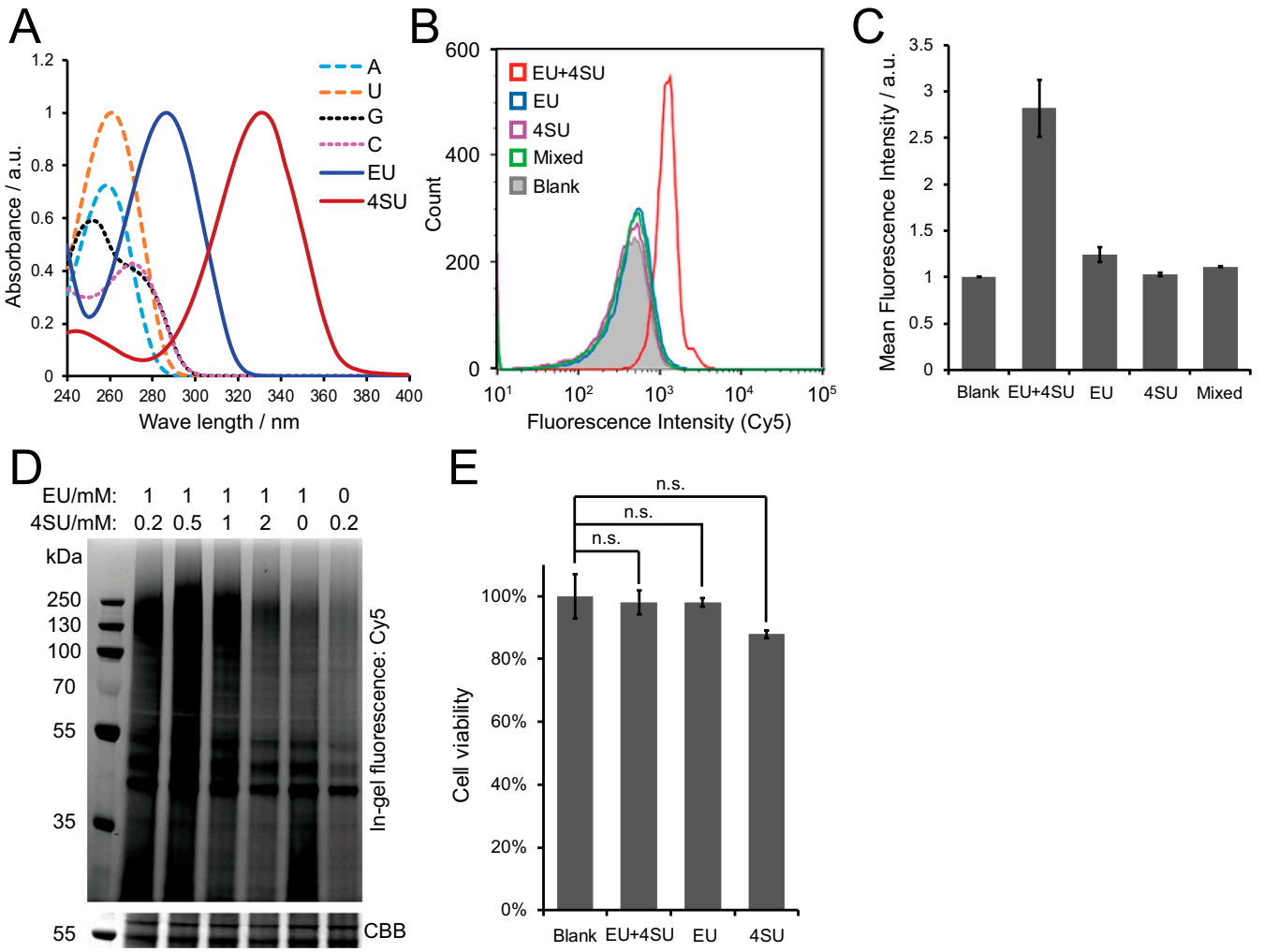

Fig. S2. Optimization of the CARIC procedure: metabolic labeling with EU and 4SU. (*A*) UV-Vis absorption spectra of 4SU, EU, uridine (U), cytidine (C), guanosine (G), and adenosine (A). (*B* and *C*) Incorporation of EU and 4SU into RNAs. HeLa cells were incubated with 1 mM EU and 0.5 mM 4SU, 1 mM EU alone, 0.5 mM 4SU alone, or vehicle for 18 h. The total RNAs were extracted from the cells. A mixed RNA sample was generated by mixing RNAs from EU-incubated and 4SU-incubated cells at a 1:1 ratio. The RNA samples were then reacted with 370 μM HPDP-biotin and 100 μM azide-Cy5 to label 4SU and EU, respectively. The labeled RNAs were incubated with streptavidin beads, followed by detection of Cy5 fluorescence by flow cytometry. The results are shown as a histogram (*B*) and bar graph (*C*). Error bars represent the SD from three replicate experiments. (*D*) Concentrations of EU and 4SU. HeLa cells were incubated with EU together with 4SU at varied concentrations. The cells were irradiated with 365-nm UV light (0.2 J/cm^2). The cell lysates were then reacted with 100 μM azide-Cy5 via CuAAC and analyzed by in-gel fluorescence scanning. The smeared bands at the high molecular weights (>130 kDa) indicate the photocross-linked and click-labeled RNPs. (*E*) HeLa cells were incubated with 1 mM EU together with 0.5 mM 4SU, 1 mM EU alone, 0.5 mM 4SU alone, or vehicle for 18 h. The cell viability was analyzed by the CCK-8 assay. Error bars represent the SD from three replicate experiments. n.s., not significant, $P > 0.05$.

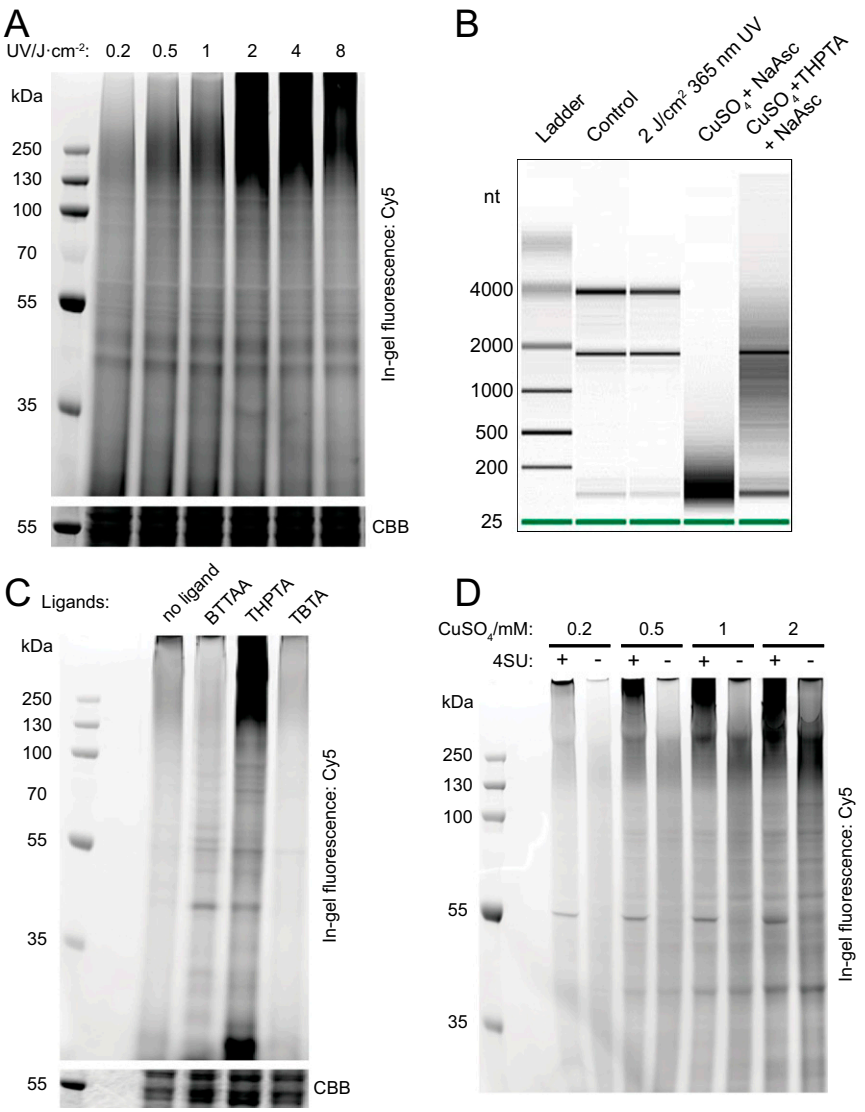

Fig. S3. Optimization of the CARIC procedure: energy density of UV light and CuAAC conditions. (*A*) Energy density of UV light irradiation. The cells treated with 1 mM EU together with 0.5 mM 4SU were irradiated with 365-nm UV light at varied energy densities, followed by reaction with azide-Cy5 and in-gel fluorescence scanning. (*B*) Effects of UV light irradiation and Cu(I) on RNA integrity. The cells were irradiated with 365-nm UV light or incubated with 2 mM $CuSO_4$ or THPTA/$CuSO_4$. The RNAs were extracted and analyzed using BioAnalyzer 2100. (*C*) Effects of Cu(I) ligands on CuAAC. The cells incubated with 1 mM EU together with 0.5 mM 4SU were irradiated with with 365-nm UV light (2 J/cm^2). The cell lysates were reacted with azide-Cy5 via CuAAC with BTTAA, THPTA, TBTA, or no ligand, followed by analysis by in-gel fluorescence scanning. (*D*) Effects of Cu concentrations on CuAAC. The cells incubated with EU together with 4SU were UV light-irradiated. The cell lysates were reacted with azide-Cy5 via THPTA-assisted CuAAC with varied concentrations of $CuSO_4$, followed by in-gel fluorescence scanning.

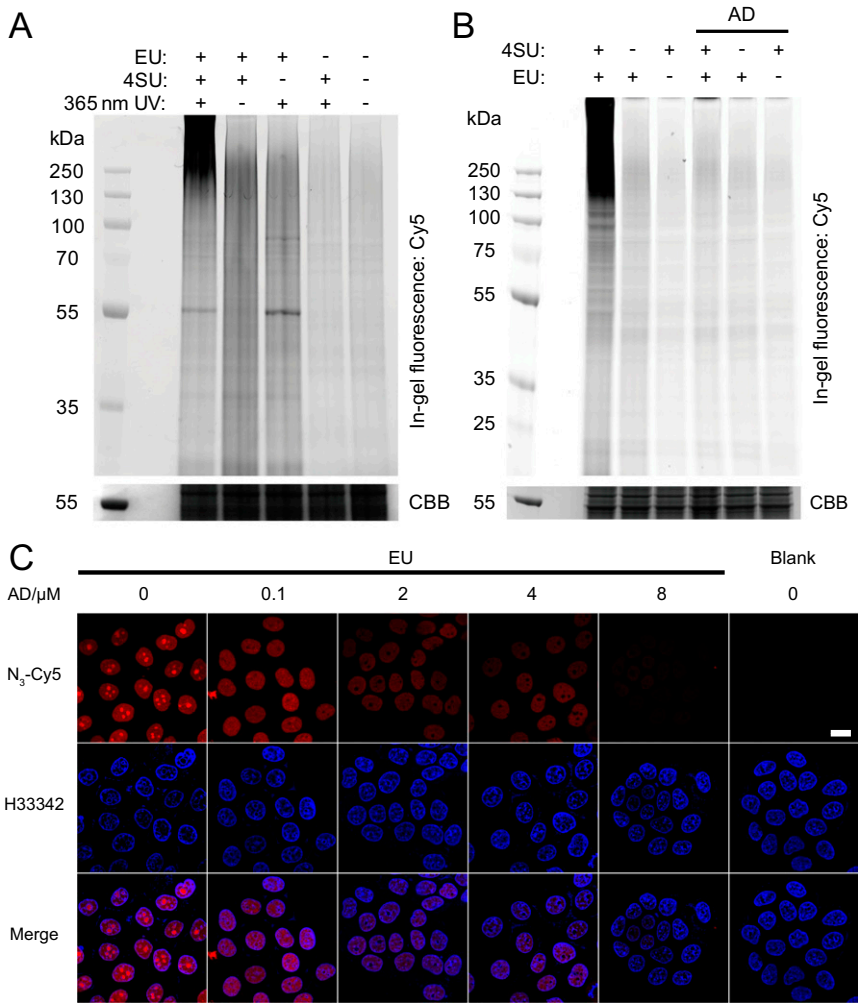

Fig. S4. Validation of CARIC labeling of RNPs. (*A*) In-gel fluorescence analysis of RNPs. The lysates of HeLa cells were incubated with 1 mM EU and 0.5 mM 4SU and irradiated with 365-nm UV light, and control samples which omitted UV or omitted 4SU or omitted EU or vehicle only were reacted with azide-Cy5 via THPTA-assisted CuAAC, resolved by SDS/PAGE, and visualized by in-gel fluorescence scanning. Coomassie brilliant blue (CBB) gel was used as the loading control. (*B*) In-gel fluorescence analysis of RNPs. After treating with or without 8 μM AD for 30 min, HeLa cells were incubated with 1 mM EU and 0.5 mM 4SU, EU, or 4SU alone and irradiated with 365-nm UV light. The lysates were reacted and analyzed as in *A*. (*C*) Inhibition of metabolic incorporation of EU by AD. HeLa cells were treated with AD at varied concentration for 30 min, followed by incubation with 1 mM EU for 3 h. The cells were fixed and permeabilized, followed by click labeling with azide-Cy5. After staining the nuclei with Hoechst 33342, the cells were imaged by confocal fluorescence microscopy. (Scale bar, 10 μm.)

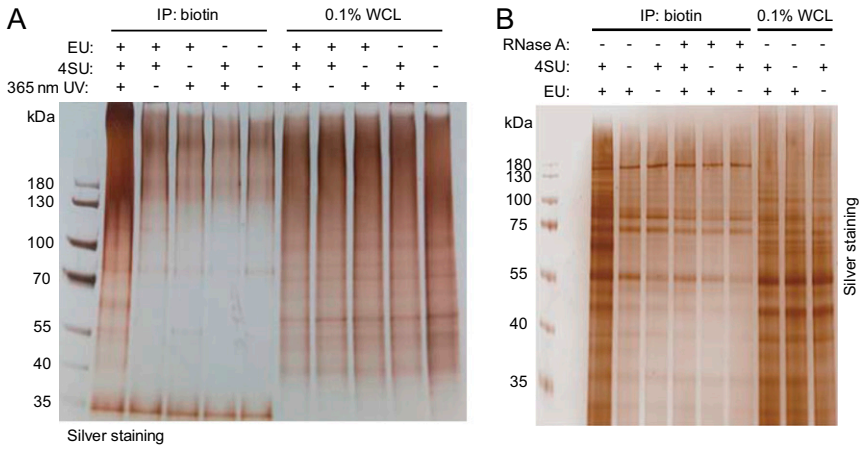

Fig. S5. Validation of CARIC capture of RBPs. (*A*) The lysates of HeLa cells were incubated with 1 mM EU and 0.5 mM 4SU and irradiated with 365-nm UV light, and control samples which omitted UV or omitted 4SU or omitted EU or vehicle only were reacted with azide-biotin via THPTA-assisted CuAAC. The lysates were then enriched with streptavidin beads. After elution, the captured RNPs were digested with RNase A, resolved by SDS/PAGE, and visualized by silver staining. (*B*) After EU&4SU labeling and UV light irradiation, the cell lysates were reacted with azide-biotin. The lysates were then treated with or without RNase A and enriched with streptavidin beads. After elution, the captured RNPs were digested with RNase A, resolved by SDS/PAGE, and visualized by silver staining.

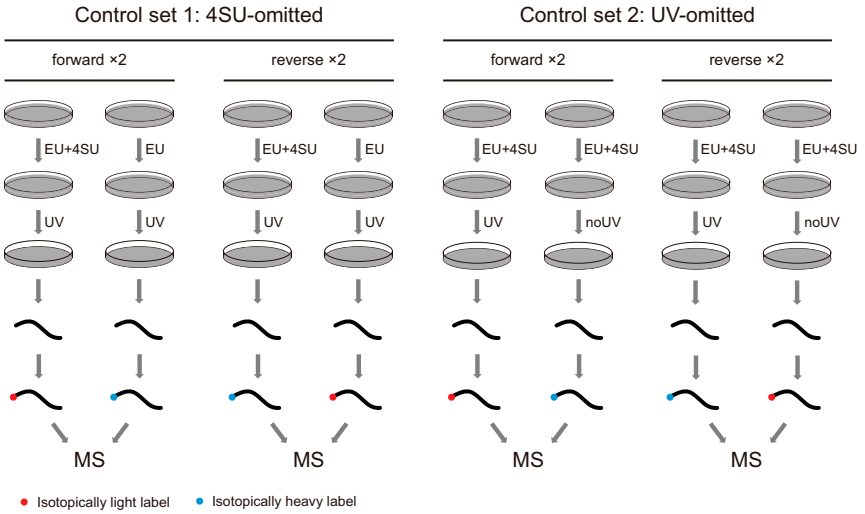

Fig. S6. Workflow for preparing MS samples from a total of eight biological replicates. In control set 1, the 4SU-omitted samples were used as the negative control. In control set 2, the UV-omitted samples were used as the negative control. Both the "forward" and "reverse" dimethyl labeling were formed in duplicate.

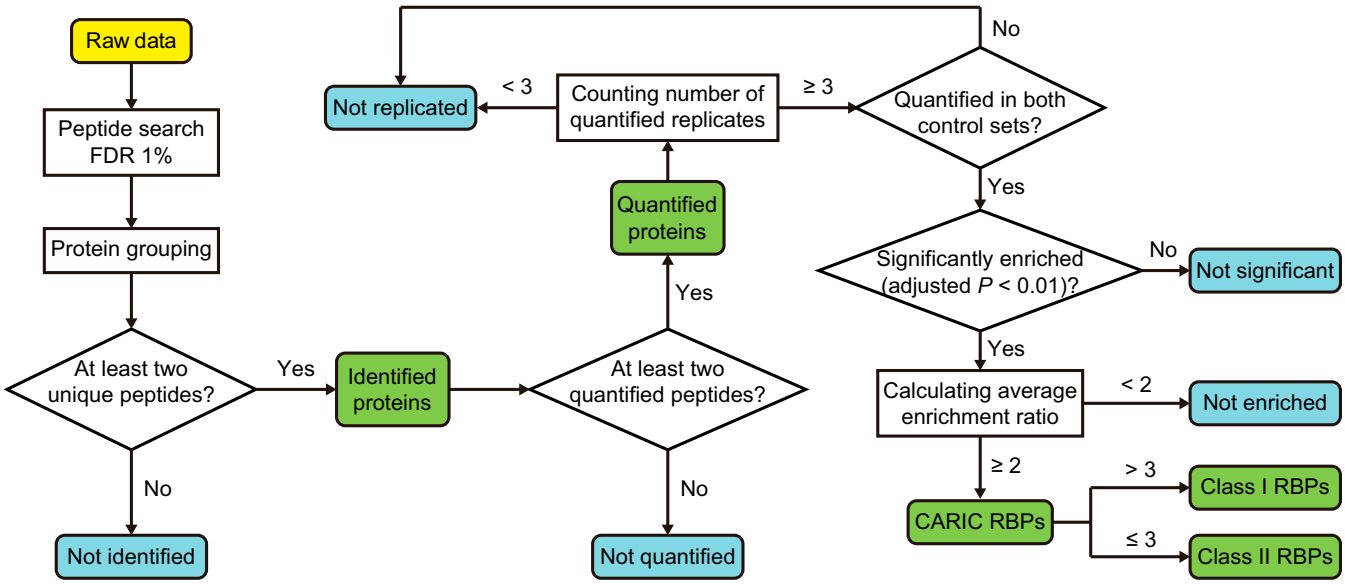

Fig. S7. Flowchart of the streamlined procedure for MS data analysis.

A

Control set	no4SU				noUV				total
Replicate	1	2	3	4	1	2	3	4	
Identified	888	722	793	706	754	733	704	761	1271
Quantified	851	699	769	686	722	707	674	724	1210

B

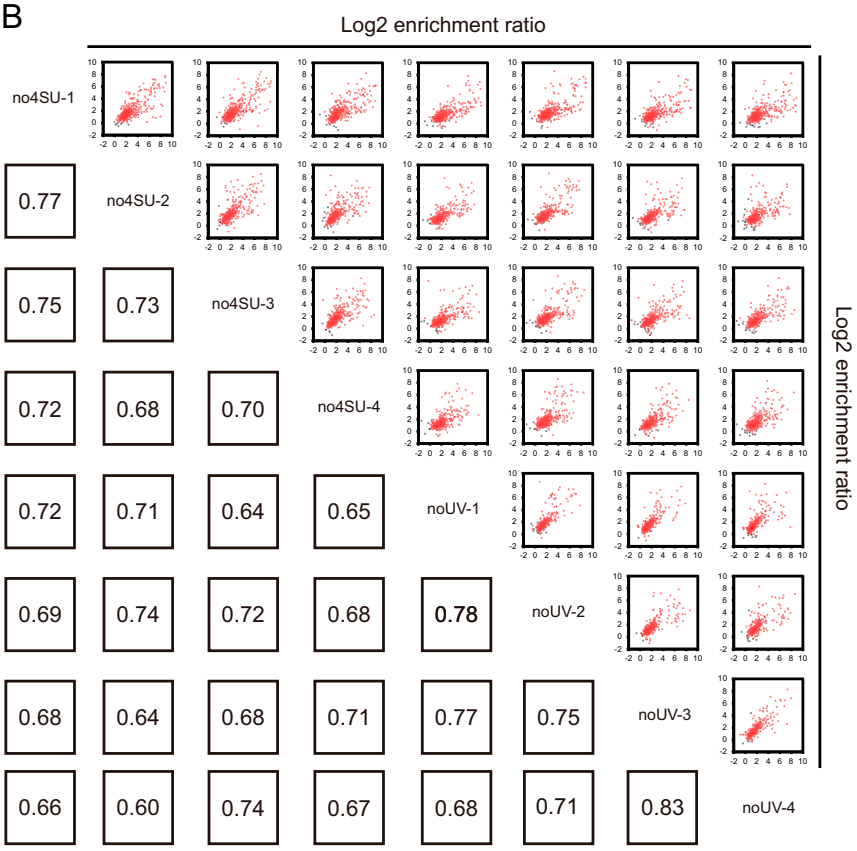

Fig. S8. Proteomic analysis of CARIC RBPs. (*A*) Summary of the proteomic identification and quantification results. (*B*) The scatter plots show the correlation between each biological replicate. Proteins significantly enriched in experimental samples (adjusted $P < 0.01$) are shown in red. The numbers in the boxes below represent the Pearson's correlation coefficient between corresponding replicates.

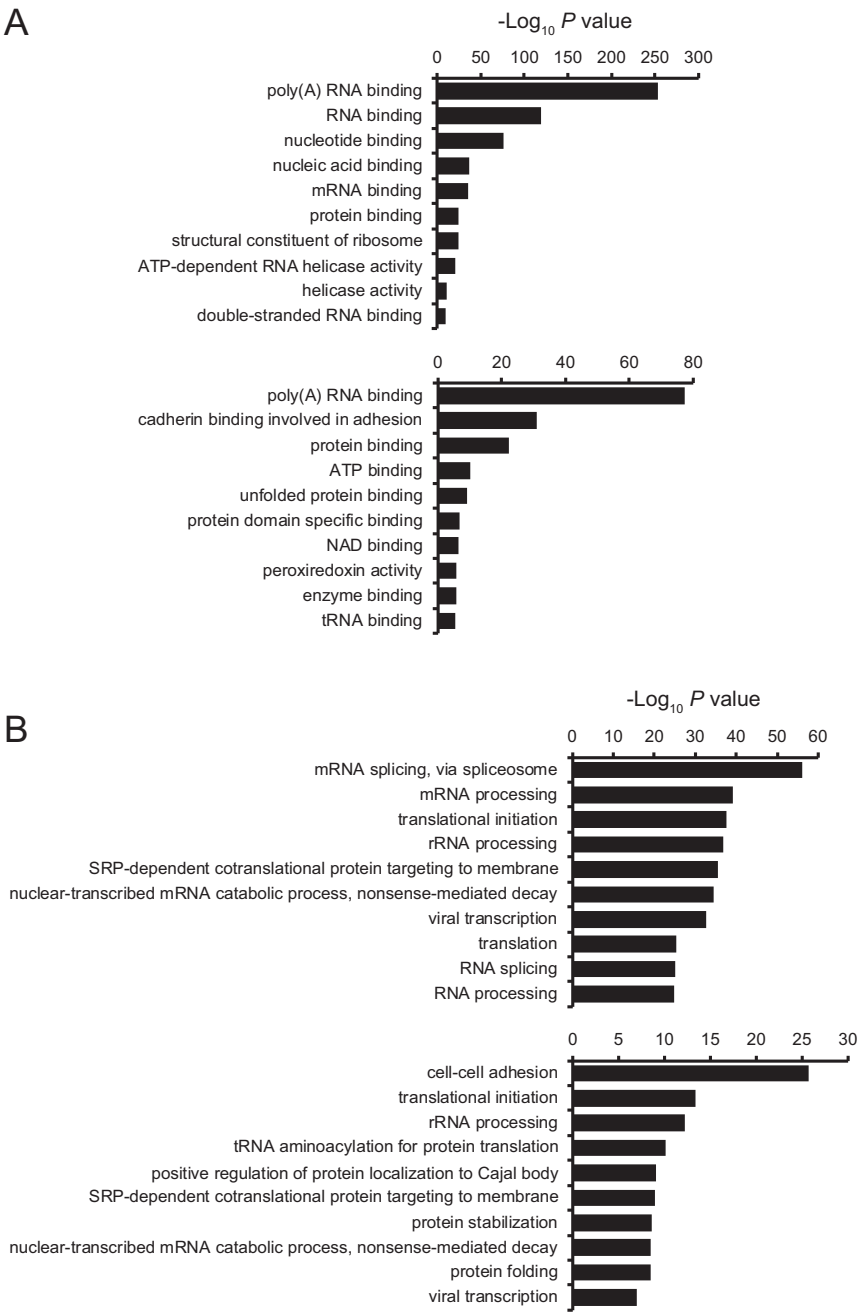

Fig. S9. GO analysis of CARIC RBPs. (*A*) Top 10 enriched terms of molecular function of class I (*Upper*) and II (*Lower*) RBPs. (*B*) Top 10 enriched terms of biological process of class I (*Upper*) and II (*Lower*) RBPs.

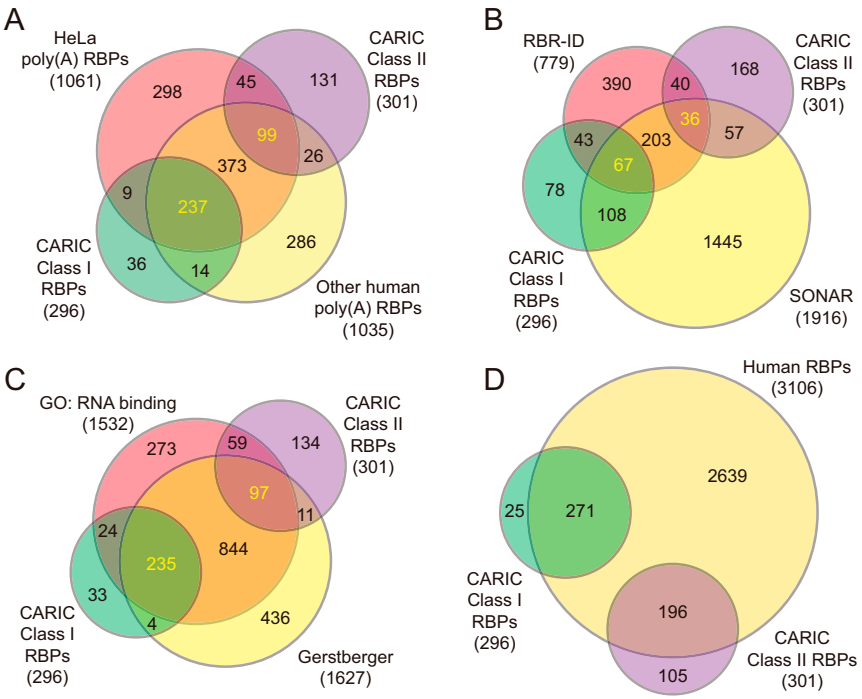

Fig. S10. Venn diagram showing the overlap of CARIC class I and II RBPs with the previously identified human poly(A) RBPs (*A*), the RBPs identified by SONAR and RBR-ID (*B*), the GO-annotated RBP list and the human RBP list manually curated by Gerstberger et al. (3) (*C*), and the human RBP list (*D*).

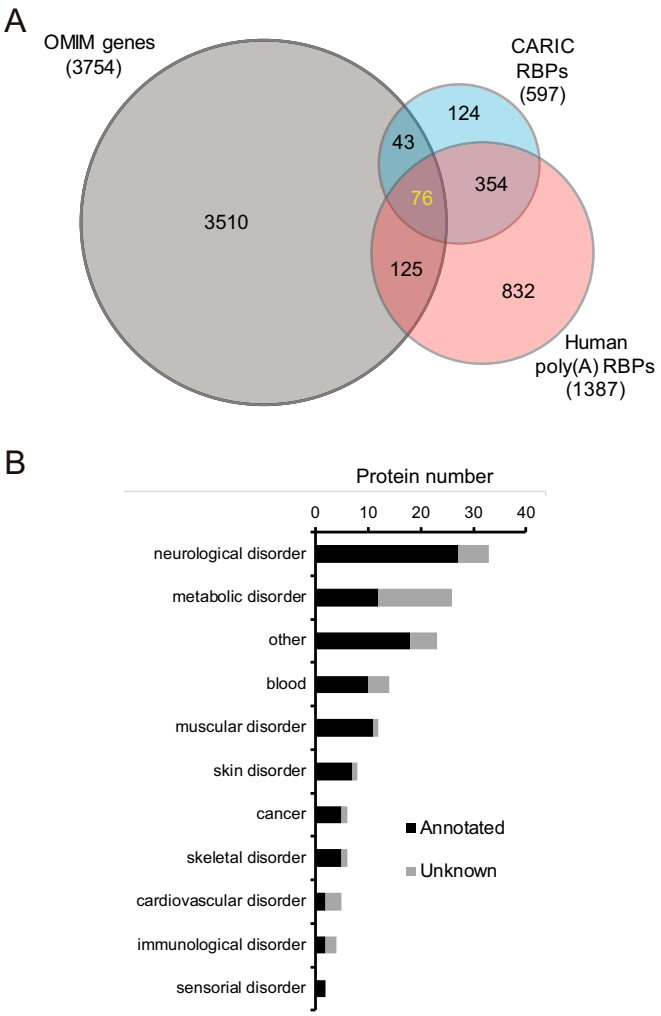

Fig. S11. RBPs in human diseases. (*A*) Venn diagram showing human poly(A) RBPs and CARIC RBPs listed in OMIM. (*B*) Number of CARIC-annotated RBPs and CARIC unknown RBPs related to each type of human disease.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)
Dataset S3 (XLSX)
Dataset S4 (XLSX)
Dataset S5 (XLSX)
Dataset S6 (XLSX)
Dataset S7 (XLSX)
Dataset S8 (XLSX)