Supporting Information for

"Targeted Profiling of Epitranscriptomic Reader, Writer and Eraser Proteins Accompanied with Radioresistance in Breast Cancer Cells"

Tianyu F. Qi[1], Weili Miao[2] and Yinsheng Wang[1,2*]

[1]Environmental Toxicology Graduate Program and [2]Department of Chemistry, University of California, Riverside,

CA, 92521-0403, USA.

[*]Correspondence author: Yinsheng Wang. Telephone: (951)827-2700; E-mail: yinsheng.wang@ucr.edu

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Materials and Methods

Cell Culture and SILAC

The radioresistant clones (C5 and C6) of MDA-MB-231 and MCF-7 cells were generated previously.[1,2] MDA-MB-231/C5 and MCF-7/C5 paired cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen-Gibco) and 1% penicillin/streptomycin. Cells were kept at 37 °C in a humidified atmosphere containing 5% CO_2. For the SILAC labelling experiments, the MDA-MB-231/C5 and MCF-7/C6 paired cells were cultured in the light DMEM media (i.e., normal lysine and arginine), or the heavy DMEM media i.e., [$^{13}C_6$, $^{15}N_2$]-L-lysine and [$^{13}C_6$]-L-arginine, with the addition of 10% dialyzed FBS (Invitrogen) and 1% penicillin/streptomycin, for at least 21 days to complete incorporate isotope-labeled amino acids.

Tryptic digestion of whole cell lysates

After the complete SILAC labelling, MDA-MB-231/C5 and MCF-7/C6 paired cells were lysed on ice for 30-min using CelLytic M cell lysis reagent (Sigma) supplemented with 1% protease inhibitor cocktail, and centrifuged at 9000 g for 30 min at 4°C. The supernatants were collected, and the protein concentrations in the supernatants were quantified using the Bradford assay. In the forward SILAC labelling experiments, light-isotope-labelled C5 and C6 cell lysates were mixed at 1:1 ratio (by mass) with heavy-isotope-labelled MDA-MB-231 and MCF-7 cell lysates, respectively. In the reverse SILAC labelling experiments, light-isotope-labelled MDA-MB-231 and MCF-7 cell lysates were mixed at 1:1 ratio (by mass) with heavy-isotope-labelled C5 and C6 cell lysates, respectively. Two forward and two reverse labelling experiments were carried out for MDA-MB-231/C5 and MCF-7/C6 pairs of cells.

Following the filter-aided sample preparation (FASP) protocol,[3] 50 µg of protein samples (25 µg of heavy- and light-isotope-labelled cell lysate combined) were denatured twice in 8 M urea in 50 mM NH_4HCO_3 in the polyethersulfone (PES) Membrane 30 kDa centrifugal filter unit (VWR) by centrifuging at 11,000g for 25 min. The denatured samples were reduced with dithiothreitol at 37 °C for 1 hr, alkylated with iodoacetamide at room temperature for 30 min, followed by washing twice with 50 mM NH_4HCO_3. The samples were digested with MS-grade trypsin (Pierce) at 1:50 ratio (trypsin: protein, by mass) in 50 mM NH_4HCO_3 at 37 °C overnight. The tryptic peptides were collected by centrifugation, dried in a Speed-vac, desalted using OMIX C18 pipet tips (Agilent Technologies), and redissolved in 0.1% formic acid for LC-PRM analysis.

Establishment of PRM library

A PRM library containing unique tryptic peptides from 152 epitranscriptomic RWE proteins was established in Skyline.[4] Two or three unique peptides exhibiting high intensities in previously published shotgun proteomic data were selected to represent each RWE protein in the PRM library,[5] and the MS/MS of these peptides were deposited into the PRM library.[5] Additionally, iRT of each peptide was derived from the linear regression of RT with iRT of tryptic peptides of BSA (with defined iRT) analyzed under the same chromatographic conditions.

LC-PRM data acquisition

Samples were subjected to LC-PRM analysis on a Q Exactive Plus quadrupole-Orbitrap mass spectrometer coupled with a Dionex UltiMate 3000 RSLCnano UPLC system. The analytical column was packed in-house using 3 µm Reprosil-Pur C18-AQ resin (Dr. Maisch GmbH HPLC)

in a ~ 25-cm long, 75 μm i.d. fused silica column. The trapping column was also prepared in-house using 5 μm Reprosil-Pur C18-AQ resin (Dr. Maisch GmbH HPLC) in a 4-cm long, 150 μm i.d. fused silica column. SILAC samples (500 ng) were separated with a 125-min linear gradient from 6 – 43% mobile phase B (80% acetonitrile in 0.1% formic acid) at a flow rate of 300 nL/min. The spray voltage was 1.8 kV.

Before sample analysis, the tryptic digestion mixture of BSA was analyzed using the LC-PRM method under the same experimental settings, but with *m/z* values of ten tryptic peptides of BSA in the inclusion list. After importing the acquired data for BSA peptides to Skyline, three inclusion lists with *m/z* and a 7-min RT window of each precursor ion were generated and exported from Skyline with the maximum number of concurrent precursor ions being set at 40. Those inclusion lists were imported for LC-PRM analysis on the Q Exactive Plus mass spectrometer, where the precursor ions were distributed in three separate LC-PRM runs. The precursor ions were isolated in the quadrupole at an isolation window of 1.0 *m/z*, fragmented in the HCD collision cell at a normalized collision energy (NCE) of 28. Other settings were: MS/MS resolution, 17,500; automated gain control (AGC) target, 1×10^5; maximum accumulation time: 50 ms.

LC-PRM data processing

The acquired LC-PRM data were imported to Skyline. In Skyline, the acquired MS/MS of each precursor ion was compared with that in the spectral library, where similarity is measured by dot product (dotp) value.[6] A dotp value of > 0.7 is imposed for positive peptide identification. In addition, 4-6 fragment ions in the light and heavy forms should share the same retention time. The potential interfering fragment ions that do not overlay with other fragment ions were manually excluded (i.e., processed data). The SILAC ratios of each precursor ion were calculated automatically in Skyline.

Western blots

MDA-MB-231/C5 and MCF-7/C6 pairs of breast cancer cells were lysed with CelLytic M cell lysis reagent (Sigma) supplemented with 1% protease inhibitor cocktail, and denatured at 95 °C for 5-min with Laemmli loading buffer. The same amount of proteins (10-20 μg) of denatured lysates were separated using SDS-PAGE, and transferred onto a nitrocellulose membrane at 90 V for 60-min at 4 °C. The membrane was blocked with 5% milk in PBS-T (PBS with 0.1% Tween 20) for 45-min, and incubated separately with primary antibodies that recognize human FTO (Abclonal, A1438, 1:1000), TRMT1 (Abclonal, A7116, 1:1000), and GAPDH (Santa Cruz, sc-32233, 1:10,000) at 4 °C overnight. After several thorough washes with PBS-T, the membrane was incubated with donkey anti-rabbit secondary antibody (Sigma, A0545, 1:5,000), or anti-mouse secondary antibody (Santa Cruz, m-IgGκ BP-HRP, 1:5,000), followed by several thorough washes with PBS-T. The protein bands were visualized using Amersham ECL™ Western Blot Detecting Reagent (GE Healthcare).

Bioinformatic analyses

All Kaplan-Meier survival analyses of the TCGA and METABRIC cohorts were carried out in the MedCal software (https://www.medcalc.org/). GSEA enrichment plots were generated in GSEA 4.1.0 software (http://www.broad.mit.edu/gsea) TCGA-BRAC dataset, downloaded from the Xenahubs database (https://gdc.xenahubs.net/download/TCGA-BRCA.htseq_fpkm.tsv.gz), was sorted by mRNA expression level of *TRMT1* from high to low with median value as cutoff. Patients (n = 1,217) were therefore categorized into high- and low-TRMT1-expression group. Gene set

enrichment analysis (GSEA) of the stratified TCGA dataset was carried out against the hallmark gene sets (h.all.v7.4.symbols.gmt) which were downloaded from GSEA Molecular Signatures Database (http://www.gsea-msigdb.org/gsea/msigdb/collections.jsp#H). The number of permutations was set at 1000. A gene set with a false discovery rate less than 0.25 was considered significantly enriched.

Table S1. A list of epitranscriptomic RWE proteins included in the PRM library. ALKHB2, 4, 6, and 7, with unknown functions in RNA modifications, were also listed.

Ensembl Gene ID	Protein Name	Description	Known Functions in RNA modifications	References
ENSG00000160710	ADAR	adenosine deaminase RNA specific	A-to-I writer	[7]
ENSG00000065457	ADAT1	adenosine deaminase tRNA specific 1	A-to-I writer	[7]
ENSG00000189007	ADAT2	adenosine deaminase tRNA specific 2	A-to-I writer	[7]
ENSG00000213638	ADAT3	adenosine deaminase tRNA specific 3	A-to-I writer	[7]
ENSG00000100601	ALKBH1	alkB homolog 1, histone H2A dioxygenase	m^1A, m^5C eraser	[8,9]
ENSG00000189046	ALKBH2	alkB homolog 2, alpha-ketoglutarate dependent dioxygenase	potential eraser	
ENSG00000166199	ALKBH3	alkB homolog 3, alpha-ketoglutarate dependent dioxygenase	m^1A eraser	[10]
ENSG00000160993	ALKBH4	alkB homolog 4, lysine demethylase	potential eraser	
ENSG00000091542	ALKBH5	alkB homolog 5, RNA demethylase	m^6A eraser	[11]
ENSG00000239382	ALKBH6	alkB homolog 6	potential eraser	
ENSG00000125652	ALKBH7	alkB homolog 7	potential eraser	
ENSG00000137760	ALKBH8	alkB homolog 8, tRNA methyltransferase	mcm^5U, mcm^5Um, $mchm^5U$, and mcm^5s^2U writer	[12]
ENSG00000183684	ALYREF	Aly/REF export factor	m^5C reader	[13]
ENSG00000244509	APOBEC3C	apolipoprotein B mRNA editing enzyme catalytic subunit 3C	C-to-U writer	[14]
ENSG00000239713	APOBEC3G	apolipoprotein B mRNA editing enzyme catalytic subunit 3G	C-to-U writer	[15]
ENSG00000186666	BCDIN3D	BCDIN3 domain containing RNA methyltransferase	5′ monophosphate methylation writer	[16]
ENSG00000071462	BUD23	BUD23 rRNA methyltransferase and ribosome maturation factor	m^7G writer	[17]
ENSG00000105879	CBLL1	Cbl proto-oncogene like 1	m^6A writer complex	[18]
ENSG00000101391	CDK5RAP1	CDK5 regulatory subunit associated protein 1	ms^2i^6A writer	[19]
ENSG00000145996	CDKAL1	CDK5 regulatory subunit associated protein 1 like 1	ms^2t^6A writer	[20]
ENSG00000144021	CIAO1	cytosolic iron-sulfur assembly component 1	s^2U, mcm^5s^2U writer	[21]

ENSG0000 0137200	CMTR1	cap methyltransferase 1	N_m writer	[22]
ENSG0000 0180917	CMTR2	cap methyltransferase 2	N_m writer	[22]
ENSG0000 0142544	CTU1	cytosolic thiouridylase subunit 1	s^2U, mcm^5s^2U writer	[23]
ENSG0000 0174177	CTU2	cytosolic thiouridylase subunit 2	s^2U, mcm^5s^2U writer	[23]
ENSG0000 0172795	DCP2	decapping mRNA 2	m^7GpppN eraser	[24]
ENSG0000 0110063	DCPS	decapping enzyme, scavenger	m^7GpppN eraser	[25]
ENSG0000 0086189	DIMT1	DIMT1 rRNA methyltransferase and ribosome maturation factor	$m_2^{6,6}A$ writer	[26]
ENSG0000 0144535	DIS3L2	DIS3 like 3'-5' exoribonuclease 2	uridylation reader	[27]
ENSG0000 0130826	DKC1	dyskerin pseudouridine synthase 1	Ψ writer	[28]
ENSG0000 0169718	DUS1L	dihydrouridine synthase 1 like	D writer	[29]
ENSG0000 0167264	DUS2	dihydrouridine synthase 2	D writer	[29]
ENSG0000 0141994	DUS3L	dihydrouridine synthase 3 like	D writer	[30]
ENSG0000 0107581	EIF3A	eukaryotic translation initiation factor 3 subunit A	m^6A reader	[31]
ENSG0000 0106263	EIF3B	eukaryotic translation initiation factor 3 subunit B	m^6A reader	[31]
ENSG0000 0066044	ELAVL1	ELAV like RNA binding protein 1	m^6A reader	[32]
ENSG0000 0070061	ELP1	elongator acetyltransferase complex subunit 1	cm^5U, ncm^5U, mcm^5U, mcm^5s^2U writer	[21,33]
ENSG0000 0134014	ELP3	elongator acetyltransferase complex subunit 3	cm^5U, ncm^5U, mcm^5U, mcm^5s^2U writer	[21,33]
ENSG0000 0109911	ELP4	elongator acetyltransferase complex subunit 4	cm^5U, ncm^5U, mcm^5U, mcm^5s^2U writer	[21,33]
ENSG0000 0170291	ELP5	elongator acetyltransferase complex subunit 5	cm^5U, ncm^5U, mcm^5U, mcm^5s^2U writer	[21,33]
ENSG0000 0126749	EMG1	EMG1 N1-specific pseudouridine methyltransferase	m^1acp3-Psi writer	[34]
ENSG0000 0105202	FBL	fibrillarin	N_m writer	[35]
ENSG0000 0102081	FMR1	FMRP translational regulator 1	m^6A reader	[36]
ENSG0000 0140718	FTO	FTO alpha-ketoglutarate dependent dioxygenase	m^6A eraser	[37]
ENSG0000 0068438	FTSJ1	FtsJ RNA 2'-O-methyltransferase 1	C_m, U_m, G_m, f^5C_m, hm^5C_m, mcm^5U_m writer	[21,38]

ENSG0000 0108592	FTSJ3	FtsJ RNA 2'-O-methyltransferase 3	C_m, U_m, G_m writer	[39]
ENSG0000 0170270	GON7	GON7 subunit of KEOPS complex	t^6A writer	[40]
ENSG0000 0130299	GTPBP3	GTP binding protein 3, mitochondrial	tm^5U writer	[41]
ENSG0000 0162639	HENMT1	HEN methyltransferase 1	N_m writer	[42]
ENSG0000 0122566	HNRNP A2B1	heterogeneous nuclear ribonucleoprotein A2/B1	m^6A reader	[43]
ENSG0000 0092199	HNRNPC	heterogeneous nuclear ribonucleoprotein C	m^6A reader	[44]
ENSG0000 0072506	HSD17B 10	hydroxysteroid 17-beta dehydrogenase 10	m^1G, m^1A writer subunit	[45]
ENSG0000 0159217	IGF2BP1	insulin like growth factor 2 mRNA binding protein 1	m^6A reader	[46]
ENSG0000 0073792	IGF2BP2	insulin like growth factor 2 mRNA binding protein 2	m^6A reader	[46]
ENSG0000 0136231	IGF2BP3	insulin like growth factor 2 mRNA binding protein 3	m^6A reader	[46]
ENSG0000 0136003	ISCU	iron-sulfur cluster assembly enzyme	s^2U, mcm^5s^2U writer	[21]
ENSG0000 0196976	LAGE3	L antigen family member 3	t^6A writer	[47]
ENSG0000 0168806	LCMT2	leucine carboxyl methyltransferase 2	o2Yw, yW writer	[21]
ENSG0000 0138095	LRPPRC	leucine rich pentatricopeptide repeat containing	m^6A reader	[48]
ENSG0000 0146834	MEPCE	methylphosphate capping enzyme	5′ monophosphate methylation writer	[49]
ENSG0000 0037897	METTL1	methyltransferase like 1	m^7G writer	[50]
ENSG0000 0145388	METTL1 4	methyltransferase like 14	m^6A writer complex	[51]
ENSG0000 0169519	METTL1 5	methyltransferase like 15	m^4C writer	[52]
ENSG0000 0127804	METTL1 6	methyltransferase like 16	m^6A writer	[53]
ENSG0000 0165792	METTL1 7	methyltransferase like 17	m^4C, m^5C writer	[54]
ENSG0000 0165055	METTL2 B	methyltransferase like 2b	m^3C writer	[55]
ENSG0000 0165819	METTL3	methyltransferase like 3	m^6A writer complex	[51]
ENSG0000 0138382	METTL5	methyltransferase like 5	m^6A writer	[56]
ENSG0000 0206562	METTL6	methyltransferase like 6	m^3C writer	[55]

ENSG0000 0123600	METTL8	methyltransferase like 8	m^3C writer	[55]
ENSG0000 0197006	METTL9	methyltransferase like 9	1-methylhistidine writer	[57]
ENSG0000 0124217	MOCS3	molybdenum cofactor synthesis 3	s^2U, mcm^5s^2U writer	[58]
ENSG0000 0128309	MPST	mercaptopyruvate sulfurtransferase	s^2U, mcm^5s^2U writer	[21]
ENSG0000 0278619	MRM1	mitochondrial rRNA methyltransferase 1	G_m writer	[59]
ENSG0000 0122687	MRM2	mitochondrial rRNA methyltransferase 2	U_m writer	[60]
ENSG0000 0171861	MRM3	mitochondrial rRNA methyltransferase 3	G_m writer	[61]
ENSG0000 0135297	MTO1	mitochondrial tRNA translation optimization 1	tm^5U writer	[21,62]
ENSG0000 0135372	NAT10	N-acetyltransferase 10	ac^4C writer	[63]
ENSG0000 0244005	NFS1	NFS1 cysteine desulfurase	s^2U, mcm^5s^2U writer	[58]
ENSG0000 0111641	NOP2	NOP2 nucleolar protein	m^5C writer	[64]
ENSG0000 0037474	NSUN2	NOP2/Sun RNA methyltransferase 2	m^5C writer	[65]
ENSG0000 0117481	NSUN4	NOP2/Sun RNA methyltransferase 4	m^5C writer	[66]
ENSG0000 0130305	NSUN5	NOP2/Sun RNA methyltransferase 5	m^5C writer	[67]
ENSG0000 0241058	NSUN6	NOP2/Sun RNA methyltransferase 6	m^5C writer	[68]
ENSG0000 0103274	NUBP1	nucleotide binding protein 1	s^2U, mcm^5s^2U writer	[21]
ENSG0000 0198585	NUDT16	nudix hydrolase 16	m^7GpppN eraser	[69]
ENSG0000 0092094	OSGEP	O-sialoglycoprotein endopeptidase	t^6A writer	[70]
ENSG0000 0100982	PCIF1	phosphorylated CTD interacting factor 1	m^6A_m writer	[71]
ENSG0000 0204469	PRRC2A	proline rich coiled-coil 2A	m^6A reader	[72]
ENSG0000 0177192	PUS1	pseudouridine synthase 1	Ψ writer	[73]
ENSG0000 0162927	PUS10	pseudouridine synthase 10	Ψ writer	[74]
ENSG0000 0110060	PUS3	pseudouridine synthase 3	Ψ writer	[75]
ENSG0000 0091127	PUS7	pseudouridine synthase 7	Ψ writer	[76]

ENSG0000 0129317	PUS7L	pseudouridine synthase 7 like	Ψ writer	[77]
ENSG0000 0213339	QTRT1	queuine tRNA-ribosyltransferase catalytic subunit 1	Q writer	[78]
ENSG0000 0151576	QTRT2	queuine tRNA-ribosyltransferase accessory subunit 2	Q writer	[79]
ENSG0000 0162775	RBM15	RNA binding motif protein 15	m^6A writer complex	[80]
ENSG0000 0259956	RBM15B	RNA binding motif protein 15B	m^6A writer complex	[80]
ENSG0000 0147274	RBMX	RNA binding motif protein X-linked	m^6A reader	[81]
ENSG0000 0111880	RNGTT	RNA guanylyltransferase and 5'-phosphatase	m^7GpppN writer	[82]
ENSG0000 0101654	RNMT	RNA guanine-7 methyltransferase	m^7GpppN writer	[82]
ENSG0000 0007376	RPUSD1	RNA pseudouridine synthase domain containing 1	ψ (probable) writer	[83]
ENSG0000 0166133	RPUSD2	RNA pseudouridine synthase domain containing 2	ψ (probable) writer	[83]
ENSG0000 0156990	RPUSD3	RNA pseudouridine synthase D3	ψ writer	[83]
ENSG0000 0165526	RPUSD4	RNA pseudouridine synthase D4	ψ writer	[83]
ENSG0000 0132275	RRP8	ribosomal RNA processing 8	m^1A writer	[84]
ENSG0000 0129158	SERGEF	secretion regulating guanine nucleotide exchange factor	s^2U, mcm^5s^2U writer	[21]
ENSG0000 0197157	SND1	staphylococcal nuclease and tudor domain containing 1	m^6A reader	[85]
ENSG0000 0100138	SNU13	small nuclear ribonucleoprotein 13	methylation writer complex	[86]
ENSG0000 0059588	TARBP1	TAR (HIV-1) RNA binding protein 1	G_m writer	[87]
ENSG0000 0029639	TFB1M	transcription factor B1, mitochondrial	$m^6{}_2A$ writer	[88]
ENSG0000 0162851	TFB2M	transcription factor B2, mitochondrial	$m^6{}_2A$ writer	[88]
ENSG0000 0137574	TGS1	trimethylguanosine synthase 1	$m^{2,2,7}G$ writer	[89]
ENSG0000 0113272	THG1L	tRNA-histidine guanylyltransferase 1 like	xG writer	[21]
ENSG0000 0066654	THUMP D1	THUMP domain containing 1	ac^4C writer unit	[90]
ENSG0000 0172315	TP53RK	TP53 regulating kinase	t^6A writer	[21,91]
ENSG0000 0144034	TPRKB	TP53RK binding protein	t^6A writer	[21,91]

ENSG0000 0107614	TRDMT1	tRNA aspartic acid methyltransferase 1	m^5C writer	[92]
ENSG0000 0043514	TRIT1	tRNA isopentenyltransferase 1	i^6A writer	[93]
ENSG0000 0104907	TRMT1	tRNA methyltransferase 1	$m^{2,2}G$ writer	[94]
ENSG0000 0145331	TRMT10 A	tRNA methyltransferase 10A	m^1G writer	[95]
ENSG0000 0174173	TRMT10 C	tRNA methyltransferase 10C, mitochondrial RNase P subunit	m^1A, m^1G writer	[21,96]
ENSG0000 0066651	TRMT11	tRNA methyltransferase 11 homolog	m^2G writer	[97]
ENSG0000 0173113	TRMT11 2	tRNA methyltransferase subunit 11-2	m^7G writer	[98]
ENSG0000 0122435	TRMT13	tRNA methyltransferase 13 homolog	C_m, A_m writer	[99]
ENSG0000 0099899	TRMT2A	tRNA methyltransferase 2 homolog A	m^5U writer	[100]
ENSG0000 0155275	TRMT44	tRNA methyltransferase 44 homolog	U_m writer	[21]
ENSG0000 0126814	TRMT5	tRNA methyltransferase 5	m^1G, m^1I writer	[21,101]
ENSG0000 0089195	TRMT6	tRNA methyltransferase 6	m^1A writer	[96]
ENSG0000 0166166	TRMT61 A	tRNA methyltransferase 61A	m^1A writer	[96]
ENSG0000 0171103	TRMT61 B	tRNA methyltransferase 61B	m^1A writer	[102]
ENSG0000 0100416	TRMU	tRNA mitochondrial 2-thiouridylase	mnm^5s^2U writer	[103]
ENSG0000 0165832	TRUB1	TruB pseudouridine synthase family member 1	Ψ writer	[104]
ENSG0000 0167112	TRUB2	TruB pseudouridine synthase family member 2	Ψ writer	[105]
ENSG0000 0134744	TUT4	terminal uridylyl transferase 4	uridylation writer	[106]
ENSG0000 0083223	TUT7	terminal uridylyl transferase 7	uridylation writer	[106]
ENSG0000 0198874	TYW1	tRNA-yW synthesizing protein 1 homolog	4-demethylwyosine writer	[107]
ENSG0000 0162623	TYW3	tRNA-yW synthesizing protein 3 homolog	7-aminocarboxypropylwyosine writer	[108]
ENSG0000 0167118	URM1	ubiquitin related modifier 1	mcm^5s^2U writer	[108]
ENSG0000 0164944	VIRMA	vir like m6A methyltransferase associated	m^6A writer complex	[109]
ENSG0000 0160193	WDR4	WD repeat domain 4	m^7G writer	[50]

ENSG0000 0178252	WDR6	WD repeat domain 6	C_m, G_m, f^5C_m, hm^5C_m writer	21,38
ENSG0000 0146457	WTAP	WT1 associated protein	m^6A writer complex	110
ENSG0000 0065978	YBX1	Y-box binding protein 1	m^5C reader	111
ENSG0000 0196449	YRDC	yrdC N6-threonylcarbamoyltransferase domain containing	t^6A writer	47
ENSG0000 0083896	YTHDC1	YTH domain containing 1	m^6A reader	112
ENSG0000 0047188	YTHDC2	YTH domain containing 2	m^6A reader	113
ENSG0000 0149658	YTHDF1	YTH N6-methyladenosine RNA binding protein 1	m^6A, m^1A reader	112
ENSG0000 0198492	YTHDF2	YTH N6-methyladenosine RNA binding protein 2	m^6A, m^1A, m^5C reader	112,114,115
ENSG0000 0185728	YTHDF3	YTH N6-methyladenosine RNA binding protein 3	m^6A, m^1A reader	112,114
ENSG0000 0123200	ZC3H13	zinc finger CCCH-type containing 13	m^6A writer complex	116
ENSG0000 0168228	ZCCHC4	zinc finger CCHC-type containing 4	m^6A writer	117

RNA modifications and their abbreviations:

I, inosine; m^1A, 1-methyladenosine; m^5C, 5-methylcytidine; m^6A, N^6-methyladenosine; mcm^5U, 5-methoxycarbonylmethyluridine; mcm^5U_m, 5-methoxycarbonylmethyl-2′-*O*-methyluridine; $mchm^5U$, 5-(carboxyhydroxymethyl)uridine methyl ester; mcm^5s^2U, 5-methoxycarbonylmethyl-2-thiouridine; m^7G, 7-methylguanosine; ms^2i^6A, 2-methylthio-N^6-isopentenyladenosine; ms^2t^6A, 2-methylthio-N^6-threonylcarbamoyladenosine; s^2U, 2-thiouridine; N_m, 2′-*O*-methylation; $m_2^{6,6}A$, $N^{6,6}$-dimethyladenosine; Ψ, pseudouridine; D, dihydrouridine; cm^5U, 5-carboxymethyluridine; ncm^5U, 5-carbamoylmethyluridine; m1acp3-Psi, N^1-methyl-N^3-(3-amino-3-carboxypropyl) pseudouridine; C_m, 2′-*O*-methylcytidine; Um, 2′-*O*-methyluridine; Gm, 2'-*O*-methylguanosine; f^5Cm, 5-formyl-2'-*O*-methylcytidine; hm^5Cm, 2′-O-methyl-5-hydroxymethylcytidine; t^6A, N^6-threonylcarbamoyladenosine; tm^5U, 5-taurinomethyluridine; m^1G, 1-methylguanosine; o2Yw, peroxywybutosine; yW, wybutosine; m^4C, N^4-methylcytidine; m^3C, 3-methylcytidine; ac^4C, N^4-acetylcytidine; m^6Am, N^6,2′-*O*-dimethyladenosine; Q, queuosine; $m^6{}_2A$, N^6,N^6-dimethyladenosine; $m^{2,2,7}G$, N^2,N^2,7-trimethylguanosine; xG, unknown modified guanosine; $m^{2,2}G$, N^2,N^2-dimethylguanosine; m^2G, N^2-methylguanosine; m^5U, 5-methyluridine; m^1I, 1-methylinosine; mnm^5s^2U, 5-methylaminomethyl-2-thiouridine

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Figure S1. (a) Gene Ontology (GO) Biological Pathway (BP) analysis of those epitranscriptomic RWE proteins differentially expressed by at least 1.5-fold in either of the two pairs of matched parental/radioresistant breast cancer cell lines. The analysis was carried out using Database for Annotation, Visualization and Integrated Discovery (DAVID). (b) Kaplan-Meier survival analysis of METABRIC cohort who received radiation therapy. Patients were stratified by mRNA expression level of *CTU1* with median value as a cutoff. (c-d) GSEA enrichment plots generated from GSEA 4.1.0 software showing significant enrichment of TRMT1 with Myc_targets_V1 (c) and UV-response_up (d).