Supporting Information for

"Parallel-reaction Monitoring Revealed Altered Expression of a Number of Epitranscriptomic Reader, Writer and Eraser Proteins Accompanied with Colorectal Cancer Metastasis"

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Table of Contents

Materials and Methods

Cell culture

SW480 primary colorectal cancer (CRC) cells (ATCC# CCL-228), derived from a primary human colon adenocarcinoma, and SW620 metastatic CRC cells (ATCC# CCL-227), derived from the lymph node metastasis of the same patient, were purchased from American Type Culture Collection (ATCC) and cultured in Dulbecco's modified Eagle medium (DMEM) (Fisher Scientific) supplemented with 10% fetal bovine serum (Invitrogen-Gibco) and 1% penicillin/streptomycin (Life technologies). For the SILAC experiments (Ong et al., 2002), to the lysine, arginine-depleted SILAC medium were added dialyzed FBS (Invitrogen) and unlabeled lysine/arginine to yield the light DMEM media, or [$^{13}C_6$, $^{15}N_2$]-L-lysine/[$^{13}C_6$]-L-arginine (Cambridge Isotope Laboratories) to give the heavy DMEM media. SW480 or SW620 cells were cultured in the heavy media for at least five cell doublings to enable nearly complete heavy isotope labelling. Cells were kept at 37 °C in a humidified atmosphere containing 5% CO_2.

Western blot

After harvesting, SW480 and SW620 cells were lysed using CelLytic M (Sigma) lysis reagent supplemented with 1% protease inhibitor cocktail (Sigma), and the ensuing proteins denatured in Laemmli loading buffer at 95 °C for 5 min. The same amount of total proteins from the paired CRC cells were resolved on an SDS-PAGE gel, and the proteins in the gel were then transferred onto a nitrocellulose membrane at 60 V for 90 min at 4 °C. The membrane was subsequently blocked using 5% non-fat dry milk in PBS-T (PBS with 0.1% Tween 20) for 45 min, and then incubated with PBS-T containing primary antibodies that recognize human FTO (Abclonal, A1438, 1:1000), hnRNPA2B1 (Santa Cruz, sc-53531, 1:1000), hnRNPC (Santa Cruz, sc-32308,

1:1000), and GAPDH (Santa Cruz, sc-32233, 1:10,000), at 4 °C overnight. After washing using PBS-T for five times, the membranes were incubated with donkey anti-rabbit (Sigma, A0545, 1:10,000) or anti-mouse (Santa Cruz, m-IgGκ BP-HRP, 1:10,000) secondary antibody in PBS-T at room temperature for 1 h. Prior to visualizing the protein bands using Amersham ECL™ Western Blot Detecting Reagent (GE Healthcare), the membranes were washed with PBS-T for five times.

References

Ong, S. E., Blagoev, B., Kratchmarova, I., Kristensen, D. B., Steen, H., Pandey, A., & Mann, M. (2002). Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. *Molecular & Cellular Proteomics*, *1*, 376-386.

Figure S1. A bar graph depicting the relative expression levels of those RWE proteins with expression ratios in SW620 vs. SW480 CRC cells being between 0.67 and 1.5. Error bars represent S.D. of results obtained from two forward and two reverse SILAC experiments.

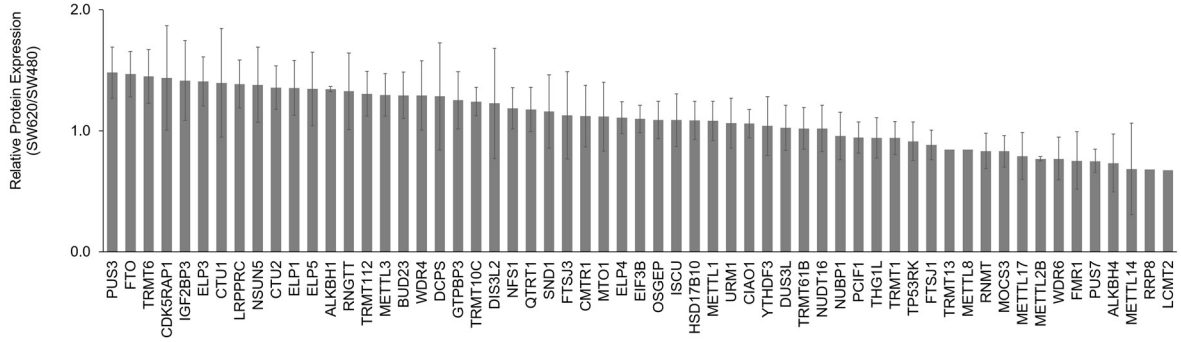


Figure S2. MS/MS of a representative tryptic peptide of hnRNPC (GFAFVQYVNER) in its light- (top panel), and heavy-form (middle panel), and the corresponding MS/MS deposited in the library (bottom panel)

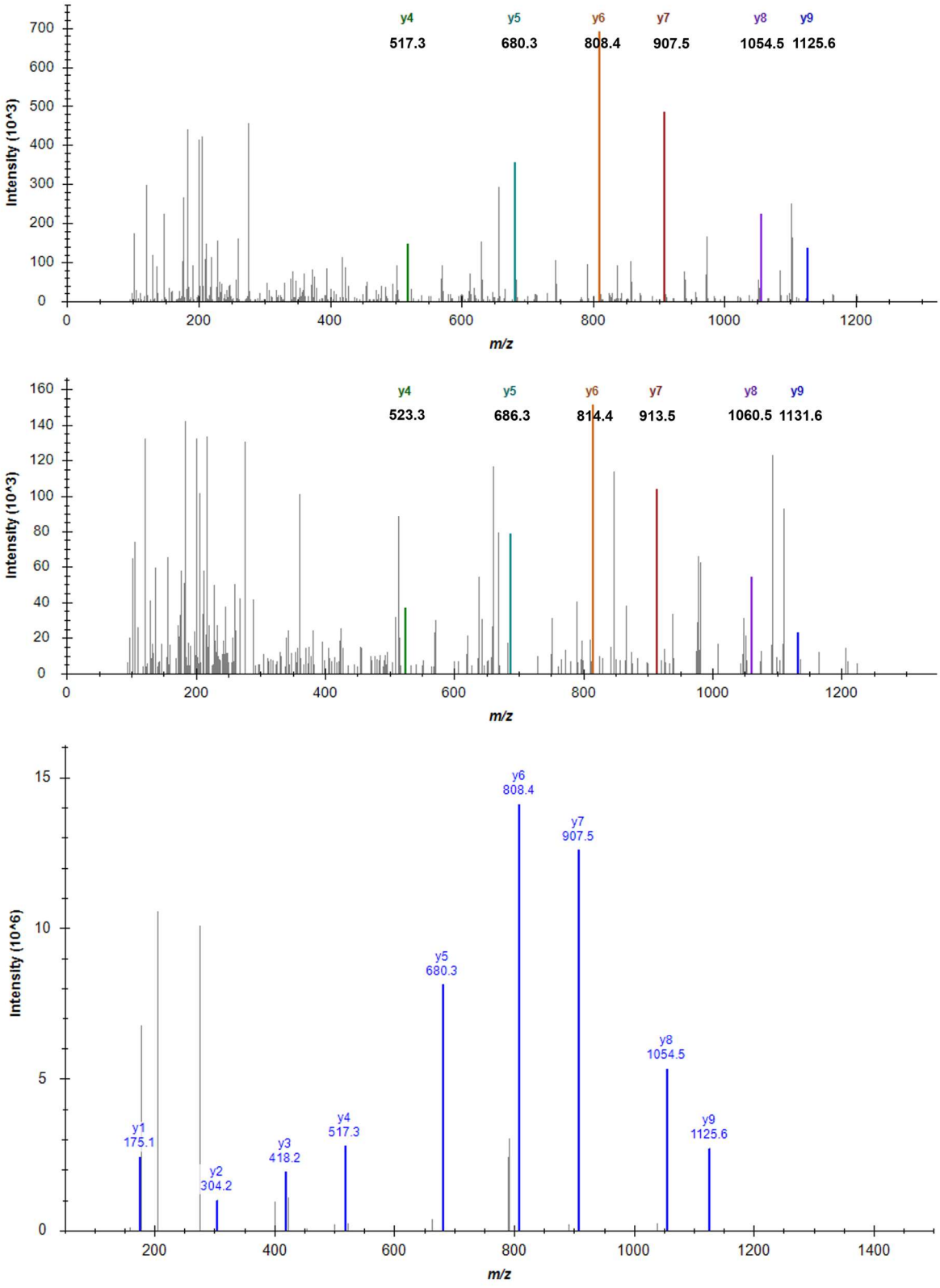
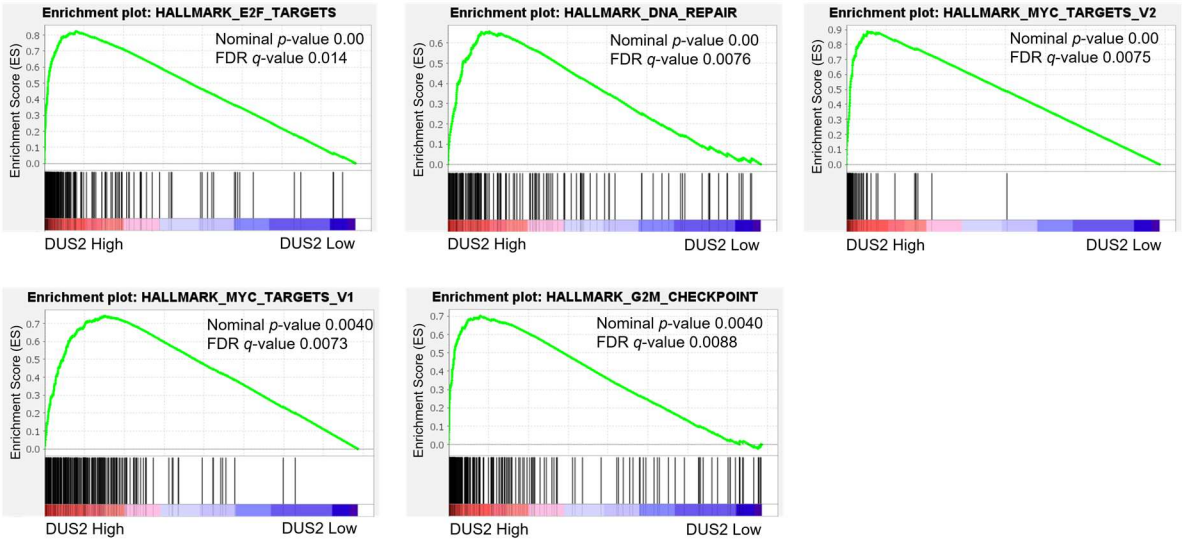


Figure S3. (A) Enrichment plots of gene sets significantly enriched in the high-DUS2-expression group, generated by GSEA 4.1.0. (B) Scatter plots showing the correlation between mRNA expression levels of *MYC* and those of *DKC1* or *NAT10* in TCGA-COAD dataset. The plots were generated using gene expression profiling interactive analysis (GEPIA). Spearman correlation coefficients are displayed.

A



B

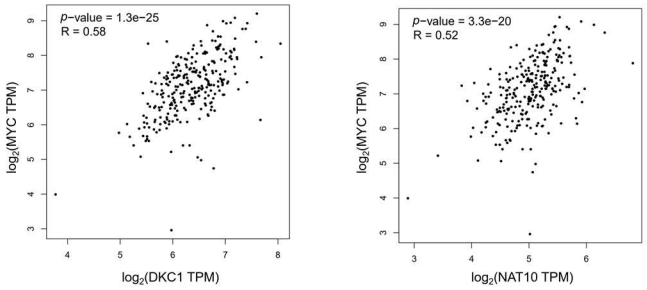


Table S2. A summary of GSEA enrichment results of each of the top 10 up-regulated RWE proteins obtained from LC-PRM analysis. Gene sets with nominal *p*-value (<0.01) and FDR *q*-value (<0.25) were shown.

Gene	Number of gene sets are significant at FDR < 25%	Number of gene sets are significant at nominal pvalue < 1%	Enrichment results	Normalized Enrichment Score (NES)	Nominal *p*-value	FDR *q*-value
DUS2	17	5	E2F_TARGETS	2.14	0.0E+00	1.4E-02
			DNA_REPAIR	2.12	0.0E+00	7.6E-03
			MYC_TARGETS_V2	2.09	0.0E+00	7.5E-03
			MYC_TARGETS_V1	2.07	4.0E-03	7.3E-03
			G2M_CHECKPOINT	2.02	4.1E-03	8.8E-03
DCP2	6	2	MITOTIC_SPINDLE	2.11	2.1E-03	2.4E-02
			SPERMATOGENESIS	1.70	4.1E-03	2.0E-01
NAT10	13	7	UNFOLDED_PROTEIN_RESPONSE	2.36	0.0E+00	0.0E+00
			DNA_REPAIR	2.17	2.0E-03	3.3E-03
			G2M_CHECKPOINT	2.16	0.0E+00	3.3E-03
			MYC_TARGETS_V1	2.08	2.0E-03	8.3E-03
			E2F_TARGETS	2.07	0.0E+00	7.7E-03
			MITOTIC_SPINDLE	2.04	1.9E-03	7.8E-03
			MYC_TARGETS_V2	2.01	0.0E+00	8.8E-03
HNRNPC	15	8	MTORC1_SIGNALING	2.38	0.0E+00	0.0E+00
			G2M_CHECKPOINT	2.38	0.0E+00	0.0E+00
			MYC_TARGETS_V1	2.20	0.0E+00	3.0E-03
			PROTEIN_SECRETION	2.19	3.9E-03	2.7E-03
			UNFOLDED_PROTEIN_RESPONSE	2.19	0.0E+00	2.1E-03
			E2F_TARGETS	2.17	0.0E+00	2.4E-03
			DNA_REPAIR	2.01	9.5E-03	1.5E-02
			SPERMATOGENESIS	1.98	0.0E+00	1.5E-02
DKC1	13	5	UNFOLDED_PROTEIN_RESPONSE	2.25	0.0E+00	9.6E-04
			MYC_TARGETS_V1	2.16	2.0E-03	4.2E-03
			E2F_TARGETS	2.12	2.0E-03	6.5E-03
			G2M_CHECKPOINT	2.07	0.0E+00	8.5E-03
			MYC_TARGETS_V2	1.98	0.0E+00	1.3E-02
YRDC	18	9	UNFOLDED_PROTEIN_RESPONSE	2.46	0.0E+00	5.1E-04
			MTORC1_SIGNALING	2.43	0.0E+00	2.6E-04
			MYC_TARGETS_V1	2.30	0.0E+00	6.3E-04
			DNA_REPAIR	2.26	0.0E+00	8.6E-04
			OXIDATIVE_PHOSPHORYLATION	2.21	2.0E-03	1.5E-03
			E2F_TARGETS	2.20	0.0E+00	1.3E-03
			G2M_CHECKPOINT	2.16	0.0E+00	2.1E-03
			GLYCOLYSIS	2.03	8.0E-03	7.3E-03
			MYC_TARGETS_V2	2.02	0.0E+00	6.9E-03
RBMX	11	3	G2M_CHECKPOINT	2.05	2.0E-03	2.6E-02
			E2F_TARGETS	2.02	0.0E+00	1.8E-02
			MYC_TARGETS_V1	1.85	2.0E-03	4.4E-02
DUS1L	5	1	MYC_TARGETS_V2	1.97	1.9E-03	6.9E-02