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Supplementary Materials for

Modulation of pre-mRNA structure by hnRNP proteins regulates alternative splicing of *MALT1*

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Supplementary Figures

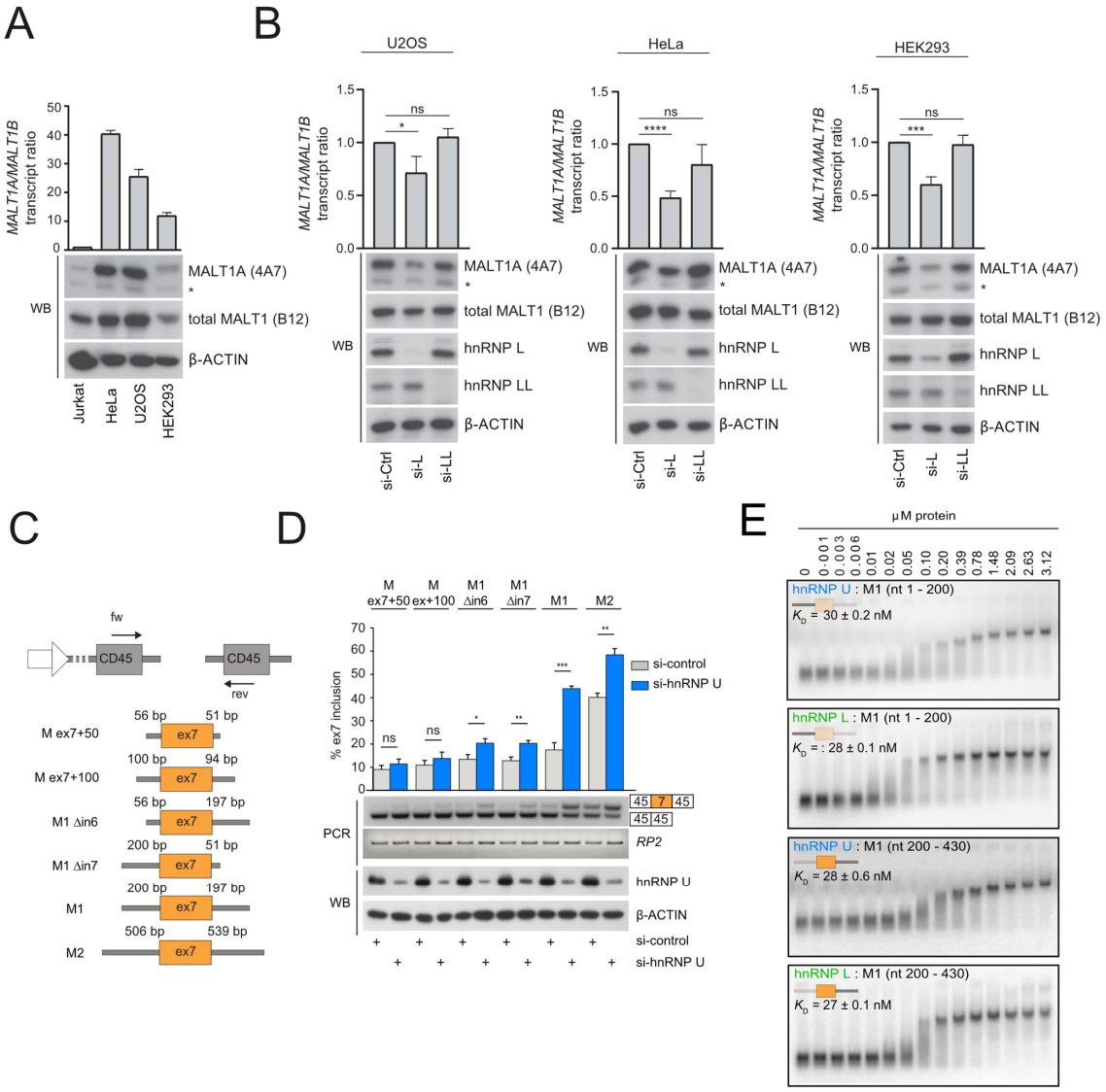

Figure S1. Related to Figure 1. Mapping *MALT1* regions regulated by hnRNP U and L. A,B, Quantification of endogenous *MALT1* transcript and MALT1A protein levels upon knockdown of hnRNP L and LL in different cell lines. Asterisk indicates an unspecific band. The antibody used is in parenthesis. **C,** Minigene constructs used. **D,** Minigene splicing assays to evaluate which regions are required for splicing recapitulation relative to endogenous *MALT1*. Data are representative for three independent experiments. Depicted is the mean ± s.d. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; NS, not significant; unpaired Student's *t*-test. **E,** EMSAs showing that hnRNP U and hnRNP L bind with low nanomolar affinity to sub-fragments of the M1 *MALT1* minigene pre-mRNA (M1 nt 1-200 or M1 nt 200-430).

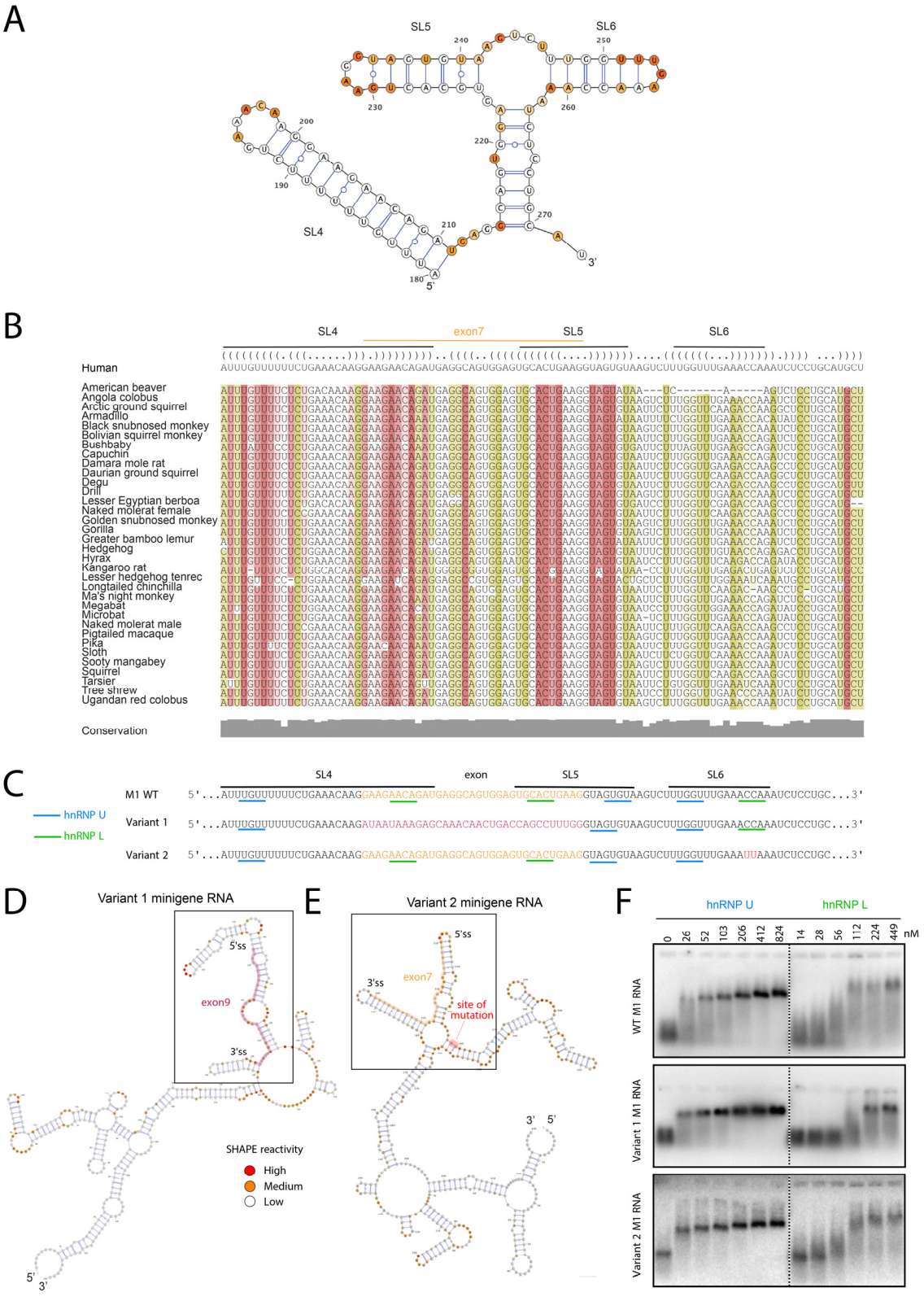

Figure S2. Related to Figure 2. Stem-loop (SL) 4 and the hammerhead, formed by SL5 and SL6, are conserved in mammals. Mutations local to SL4 and the hammerhead *MALT1* minigene pre-mRNA modify secondary structure, but retain binding with hnRNP U and hnRNP L. **A,** SHAPE reactivities for SL4 and the hammerhead, comprised of SL5 and SL6. Non-reactive, semi-reactive and highly reactive

nucleotides are colored white, orange, and red, respectively. **B,** Multiple sequence alignment shows that the RNA sequence and secondary structure for these stem-loops is evolutionary conserved. The alignment is colored using the colorrna.pl of the Vienna package (red marks base pairs with no sequence variation, ochre, green, turquoise, blue, and violet mark base pairs with 2, 3, 4, 5, or 6 different types of base pairs, respectively) (*70*) **C,** Primary sequences of *MALT1* minigene RNA variants, with mutations colored**. D,E,** Secondary structure of variant 1 and 2 minigene RNAs as determined by SHAPE. **F,** Variants 1 and 2 bind to hnRNP U and hnRNP L comparable to wildtype.

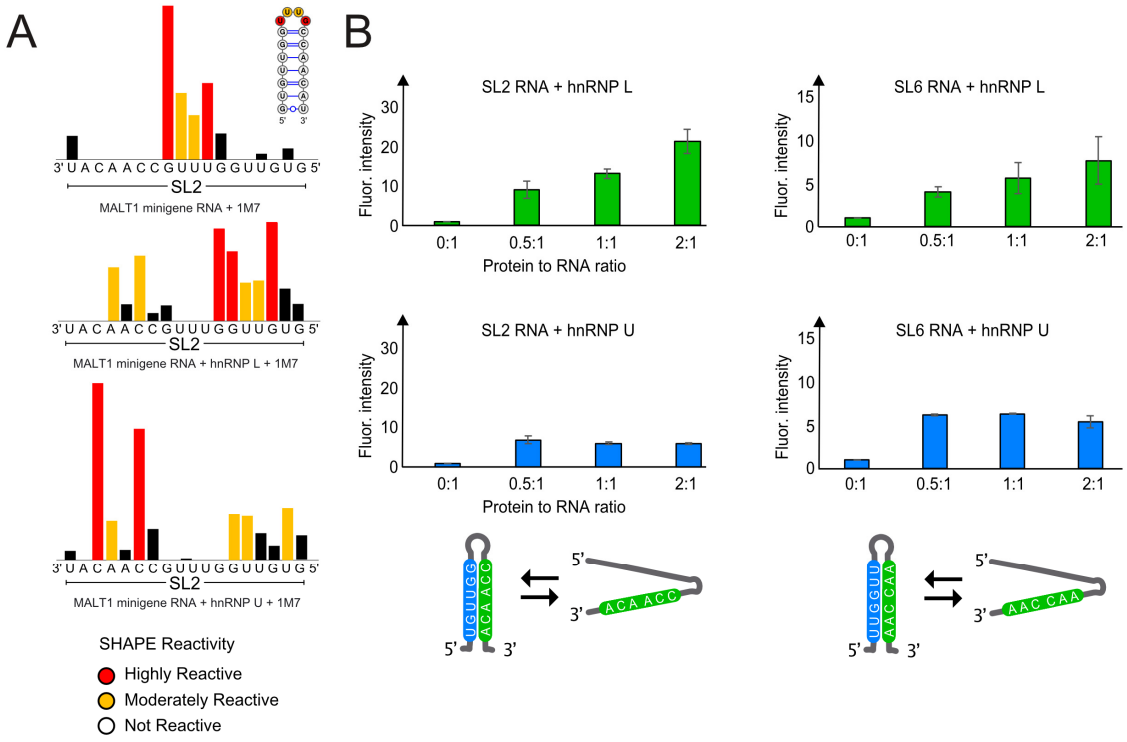

Figure S3. Related to Figure 3. hnRNP U and hnRNP L differentially modulate structured elements of the MALT1 minigene RNA. A, Normalized SHAPE reactivity profiles corresponding to SL2 in the absence and presence of the hnRNP L and hnRNP U proteins. **B,** Fluorescence quenching assays with the SL2 and SL6 RNA hairpins labeled with a fluorescent dye and quencher at the 5' and 3' termini show that hnRNP L unwinds, whereas hnRNP U maintains secondary structures of splice signal-containing stem-loops. Errors refer to 3 biological replicates.

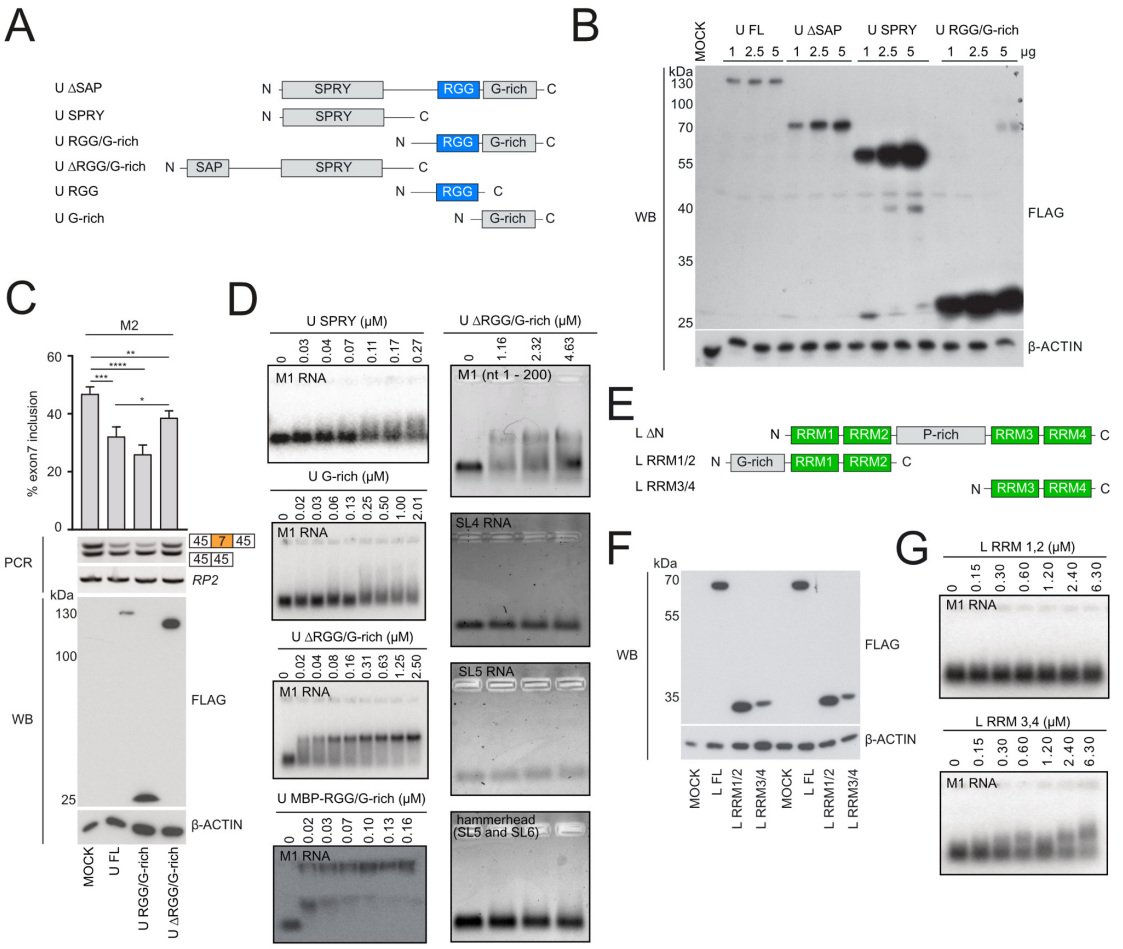

Figure S4. Related to Figure 3. The RGG domain of hnRNP U and the four RRMs of hnRNP L are responsible for binding and regulating splicing of *MALT1* minigene pre-mRNA. A, Mapping regions in hnRNP U and L involved in RNA binding. hnRNP U constructs used to identify region responsible for binding RNA. **B,C,** Minigene splicing assays upon overexpression of various hnRNP U constructs. Data are representative for three (**C**) independent experiments. Depicted is the mean ± s.d. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns, not significant; unpaired Student's *t*-test. **D,** EMSAs with hnRNP U SPRY, ΔRGG/G-rich, G-rich and RGG/G-rich (with MBP solubility tag) domains with *MALT1* minigene pre-mRNAs. **E,** hnRNP L constructs used to identify region responsible for binding RNA. **F,** Minigene splicing assays upon overexpression of various hnRNP L constructs. **G,** Gel shift assays of hnRNP L RRM1,2 and RRM3,4 with *MALT1* minigene pre-mRNA.

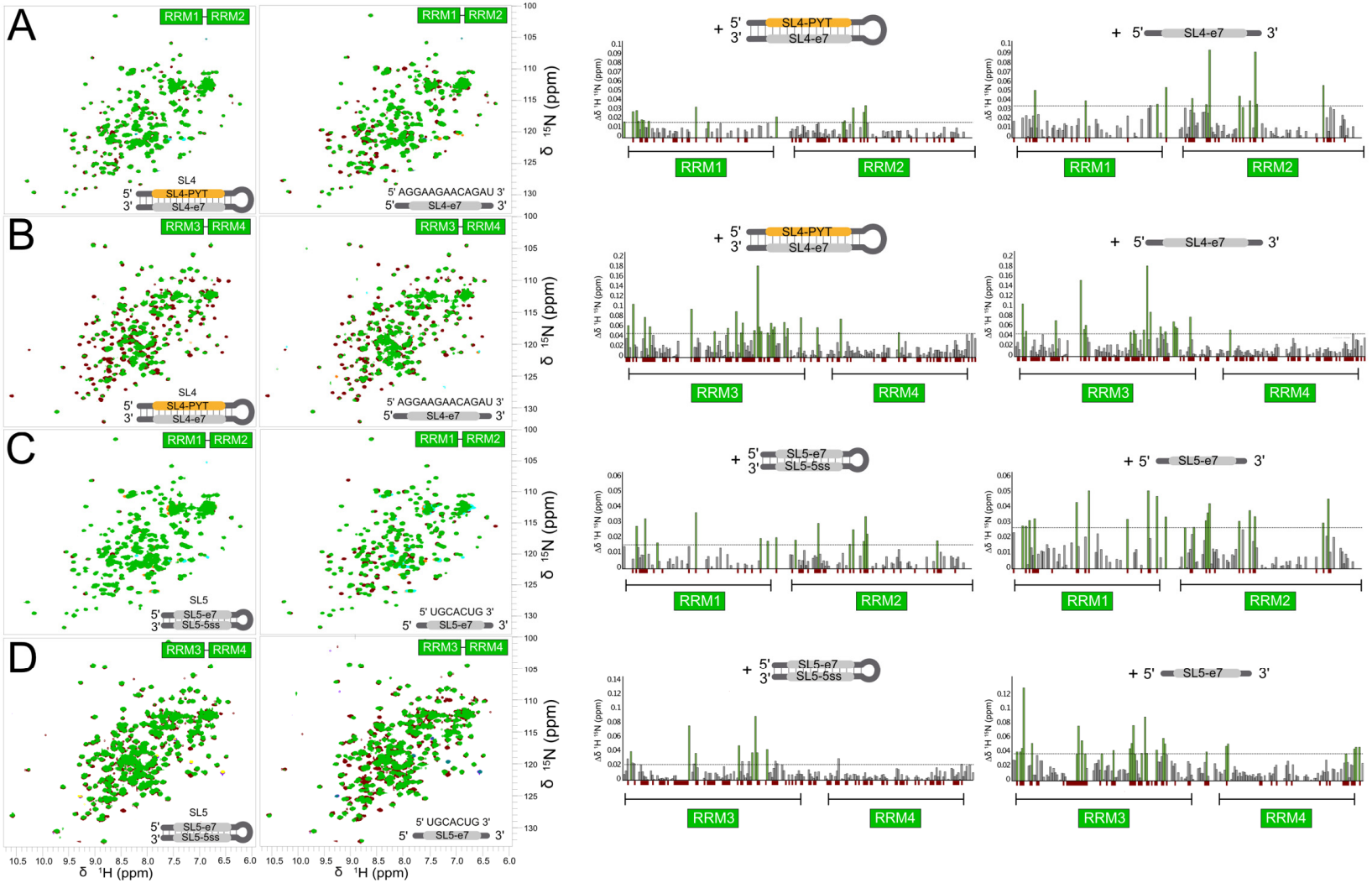

Figure S5. Related to Figure 4. NMR analysis of *MALT1* RNA recognition by hnRNP L. ^{1}H,^{15}N HSQC spectra of free ^{15}N-labeled (**A,C**)

RRM1,2 or (**B,D**) RRM3,4 of hnRNP L (maroon) bound to stem-loops or single-stranded components of (**A,B**) SL4 or (**C,D**) SL5 (green) in the *MALT1* pre-mRNA. Chemical shift perturbation (CSP, green positive bars) and intensity changes (maroon negative bars) for amides in each spectrum are shown on the right. Dotted horizontal lines represent the average CSP plus one standard deviation

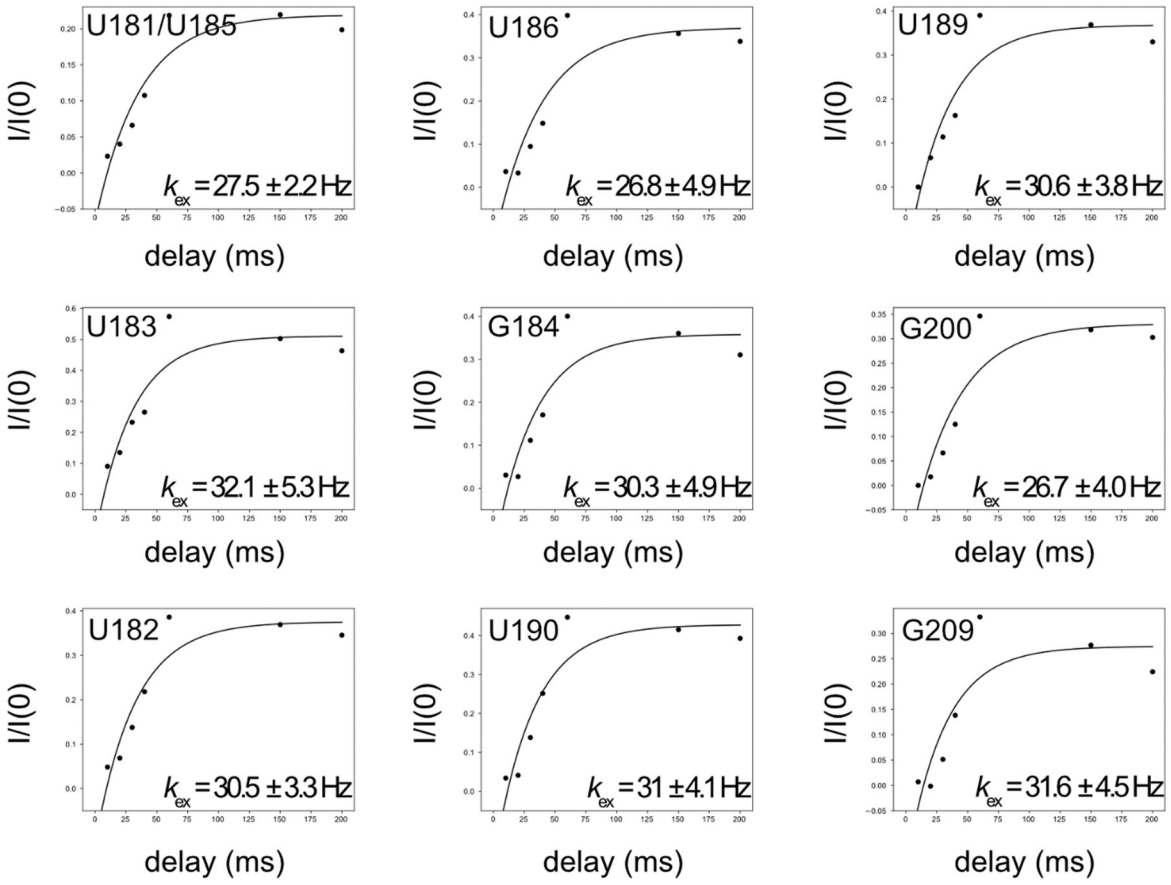

Figure S6. Related to Figure 4. NMR imino proton exchange measurements show reduced stability of *MALT1* SL4 RNA. Fitting of peak intensity as a function of CLEANEX-PM mixing time yields water exchange rates k_{ex} (in Hz). Errors indicate the fitting error.

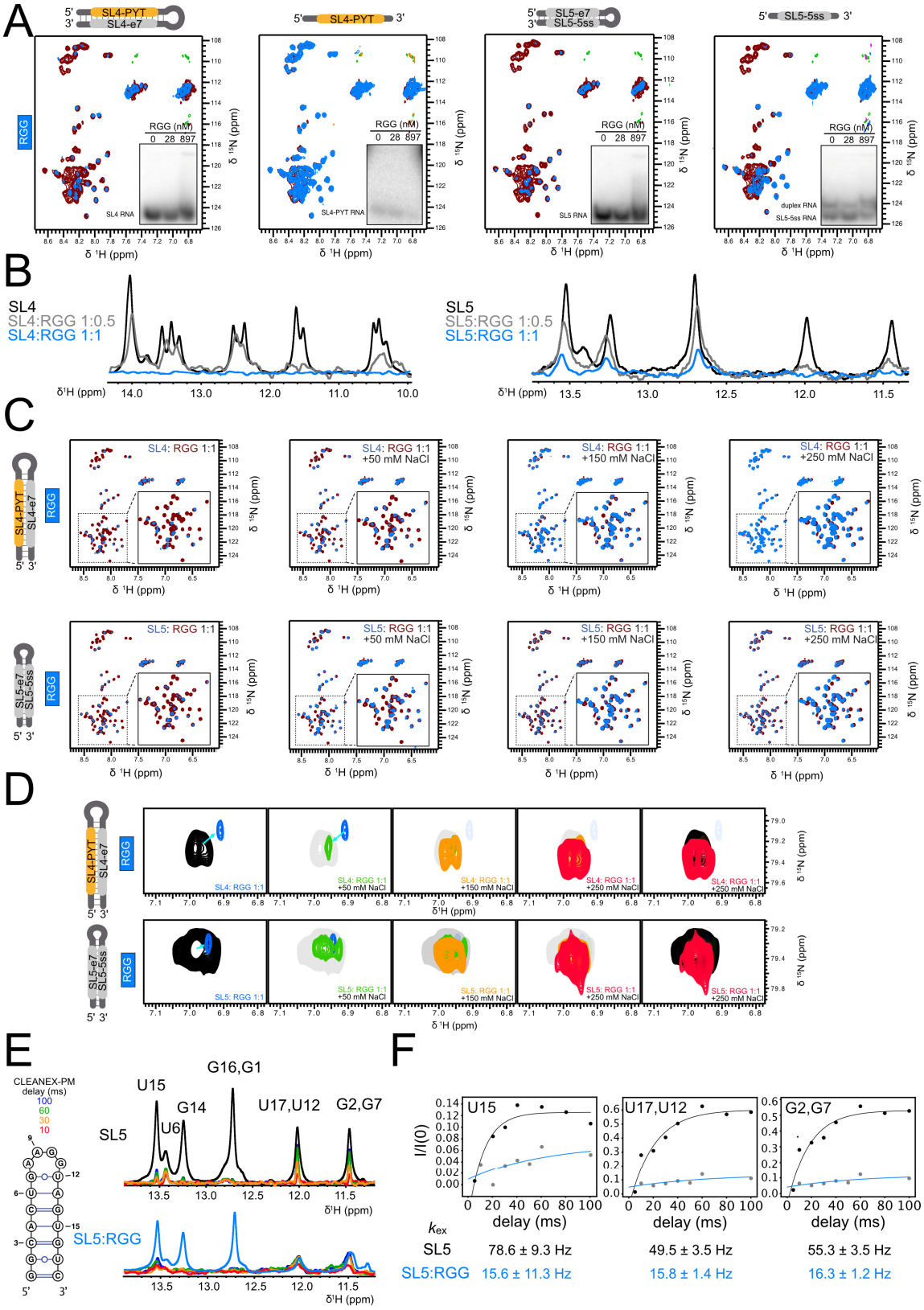

Figure S7. Related to Figure 5. NMR analysis of *MALT1* RNA recognition by hnRNP U RGG. A, ^{1}H,^{15}N HSQC NMR spectra of the ^{15}N-labeled RGG domain of hnRNP U in the presence of *MALT1* SL4

or SL5 RNA hairpins or respective single-stranded regions. Significant spectral changes (line-broadening) are only observed with the structure RNA ligands. While the single-stranded SL4-PYT shows virtually no spectral changes, indicating no binding, the presence of the SL5-5ss RNA induces some line broadening. This is rationalized by the observation that the SL5-5ss is capable of forming an artificial duplex that can bind to the RGG domain as shown in EMSA experiments (EMSA gel, inset). **B,** 1D imino titration spectra of SL4 and SL5 (50 μM) in the presence of 0.5 or 1 molar equivalent of the hnRNP U RGG domain. **C,** ^{1}H ^{15}N HSQC spectra of the ^{15}N labeled RGG domain of hnRNP U (preformed with either SL4, top, or SL5, bottom) in the presence of increasing salt (NaCl) concentration. **D,** ^{1}H ^{15}N HISQC spectra of the arginine side chain H^{ε} protons of the hnRNP U RGG domain (preformed with either SL4, top, or SL5, bottom) in the presence of increasing concentration of NaCl. **E,** Overlay of 1D CLEANEX-PM imino spectra of SL5 in the absence (black) and presence (blue) of the RGG domain of hnRNP U. **F,** Fitting of peak intensity as a function of CLEANEX mixing time to determined imino proton exchange rates k_{ex} (in Hz).

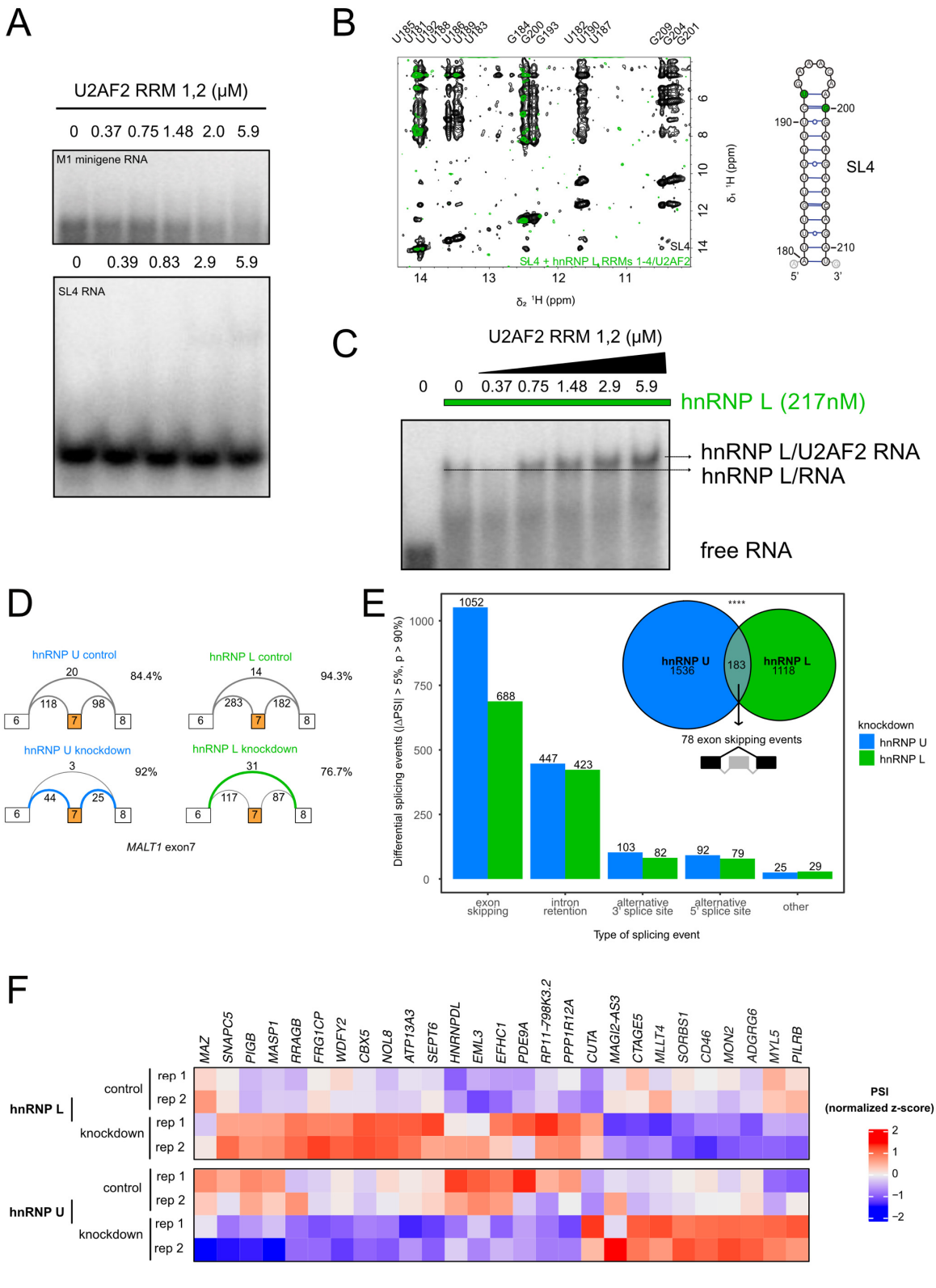

Figure S8. Related to Figure 6. Binding of hnRNP L enables U2AF2 binding to *MALT1* RNA and alongside hnRNP U, regulate splicing events genome-wide. **A,** EMSA of U2AF2 RRM1,2 with *MALT1*

M1 minigene RNA (top) and SL4 (bottom). **B,** Superposition of ^{1}H,^{1}H NOESY spectra of SL4 RNA in the absence (black) or presence (green) of hnRNP L RRM1-4 and U2AF2 RRM1,2. Imino resonances that remain as part of the protein-RNA complex are colored in green on the secondary structure of SL4. **C,** EMSA of U2AF2 RRM1,2 in the presence of hnRNP L RRM1-4 with M1 minigene RNA. **D**, Skipping of exon7 of *MALT1* and its dependence on hnRNP U and hnRNP L is shown from knockdown data available from ENCODE and processed in MAJIQ. Percentage of exon7 inclusion is given for all conditions. **E,** Number of overlapping local splice variations (Venn diagram, number of included exon skipping events given below) and types of splicing events (bar chart) regulated by both hnRNP U and hnRNP L (> 5% change in percent selected index [PSI] in at least one junction of the local splice variation, >90% confidence). **F,** Heatmap showing the comparison of z-score-normalized mean PSI values for 19 antagonistically regulated cassette exons (change in the same junction of the LSV in both comparisons) in hnRNP U and hnRNP L control and knockdown experiments (2 replicates per condition). *MALT1* exon 7 (marked by asterisk) did not reach significance, but is shown for comparison.

Supplementary Tables

Table S1 | Related to Materials and Methods. Primers used for qPCR and minigene assays

Primer	Sequence (5' -> 3')
MALT1A fw	GAAGGTAGAAATCATCATAGGAAG
MALT1A rev	GCTTTGAGCTTGGGGTGCTCC
MALT1B fw	AAGCCCTATTCCTCACTACCAGTGG
MALT1B rev	GGATGACCAAGATTATTTAATTCATCTATG
RP2 fw	GCACCACGTCCAATGACAT
RP2 rev	GTGCGGCTGCTTCCATAA
CD45 fw (minigene)	GGGAGCTTGGTACCACGCGTCGACC
CD45 rev (minigene)	CAGCGCTTCCAGAAGGGCTCAGAGTGG

Table S2 | Related to Materials and Methods. Antibodies used for Western blots

Antibody
anti-hnRNP U (3G6) Abcam
anti-hnRNP L (4D11) Abcam
anti-hnRNP LL (4783S) Cell signaling
anti-MALT1 (B12) Santa Cruz Biotechnology
anti-MALT1A (4A7) HMGU
anti-Flag-M2 Sigma-Aldrich
anti-β-Actin (C4) Santa Cruz Biotechnology
(HRP)-conjugated secondary antibodies (Jackson ImmunoResearch)