RBM25 is a global splicing factor promoting inclusion of alternatively spliced exons

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Supplemental Data

FIGURE S1. RBM25 depletion interferes with cell proliferation in PaCa cells

FIGURE S2. Partial reconstitution of RBM25 depletion restores splicing at RBM25 target exons

FIGURE S3. RBM25 lacking protein interaction domains is unable to promote inclusion of target exons.

FIGURE S4. Known and candidate KMTs do not specifically methylate RBM25 K77

TABLE S1. Gene expression in RBM25-depleted 293T cells relative to cells transduced with control sgRNA.

TABLE S2. Measurement of mRNA splicing events in RBM25-depleted and RBM25 over-expressing cells.

TABLE S3. Splicing events affected by RBM25 depletion in 293T cells relative to cells transduced with control sgRNA.

TABLE S4. Proteins identified in co-immunoprecipitation experiments with RBM25, and relative abundance compared to IgG negative control using SILAC.

TABLE S5. Proteins identified in co-immunoprecipitation with RBM25 domain deletion constructs. Relative abundance was determined using SILAC signal intensities and normalized to the amount of RBM25 determined from peptides shared between each long and short construct.

TABLE S6. Proteins identified in peptide pull-down using RBM25 peptides spanning amino acids 67-85. Methyl vs non-methyl pull-down was measured using SILAC, in duplicate with labels swapped. The entire experiment was conducted twice.

TABLE S7. Primers used for real-time PCR to validate the effects of RBM25 knock-down and reconstitution in 293T and HT-1080 cells.

Figure S1

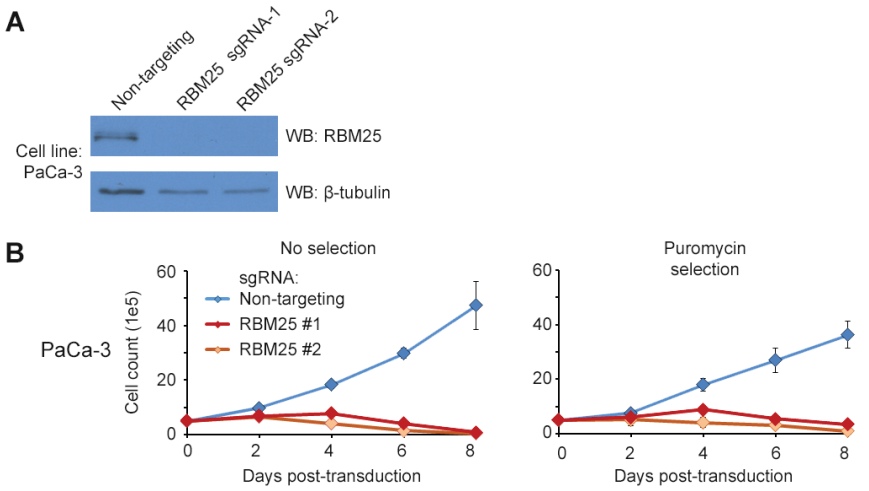

FIGURE S1. RBM25 depletion interferes with cell proliferation in PaCa cells
(**A**) RBM25 levels in PaCa-3 measured by Western blot four days after transduction with the CRISPR/Cas9 system. (**B**) Growth of transduced PaCa-3 cells was tracked for eight days both without selection (left) and with puromycin selection (right).

Figure S2

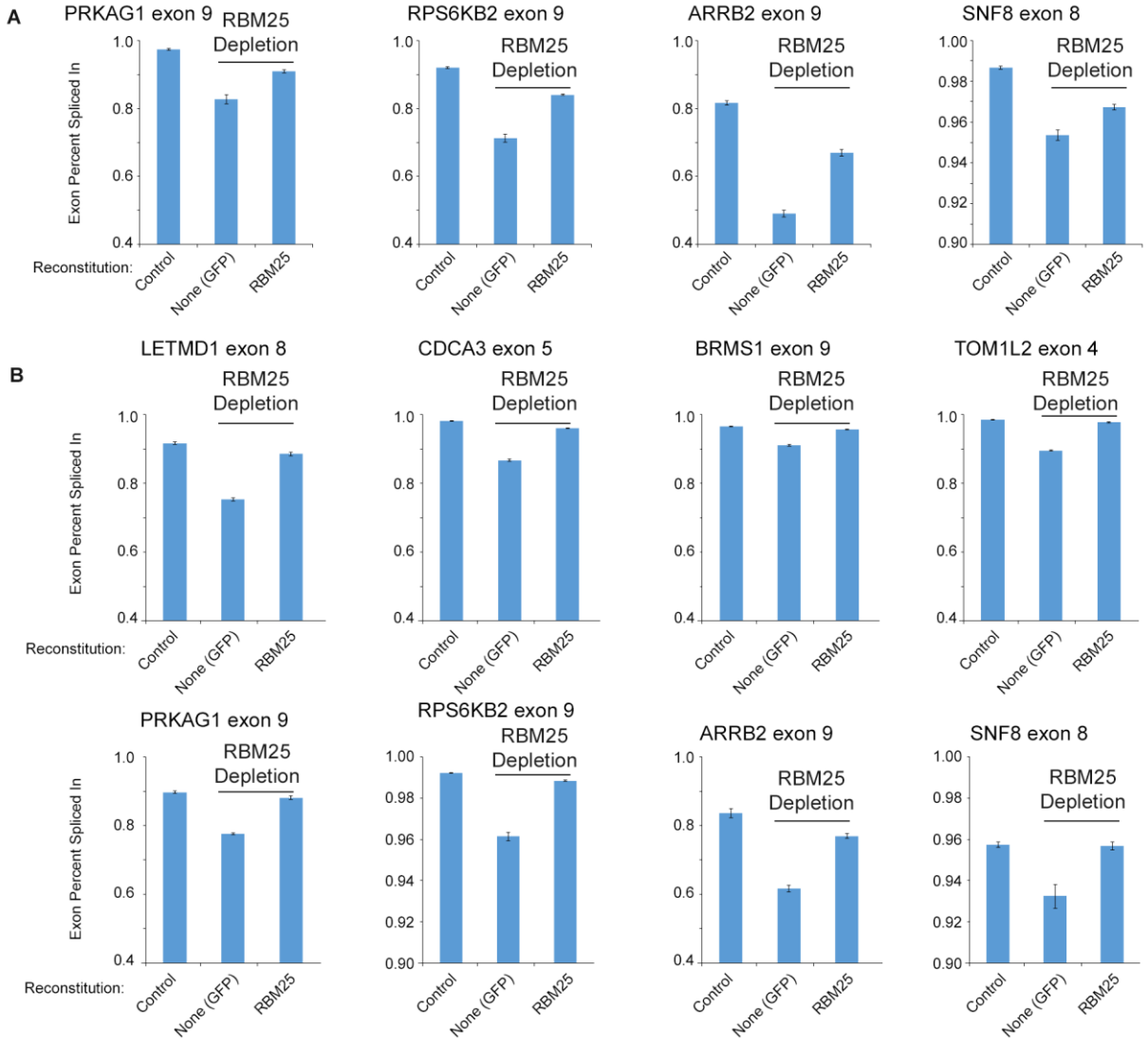

FIGURE S2. Partial reconstitution of RBM25 depletion restores splicing at RBM25 target exons (**A**) Endogenous RBM25 was depleted in 293T cells expressing control vector (GFP) or indicated RBM25. Real-time PCR was used to measure exon inclusion at eight RBM25-target exons identified by RNA-Seq (four shown here, four shown in Fig. 2). Error bars show S.E.M, N=4. (**B**) The same experiment was performed in HT-1080 cells. Error bars show S.E.M., N=3.

Figure S3

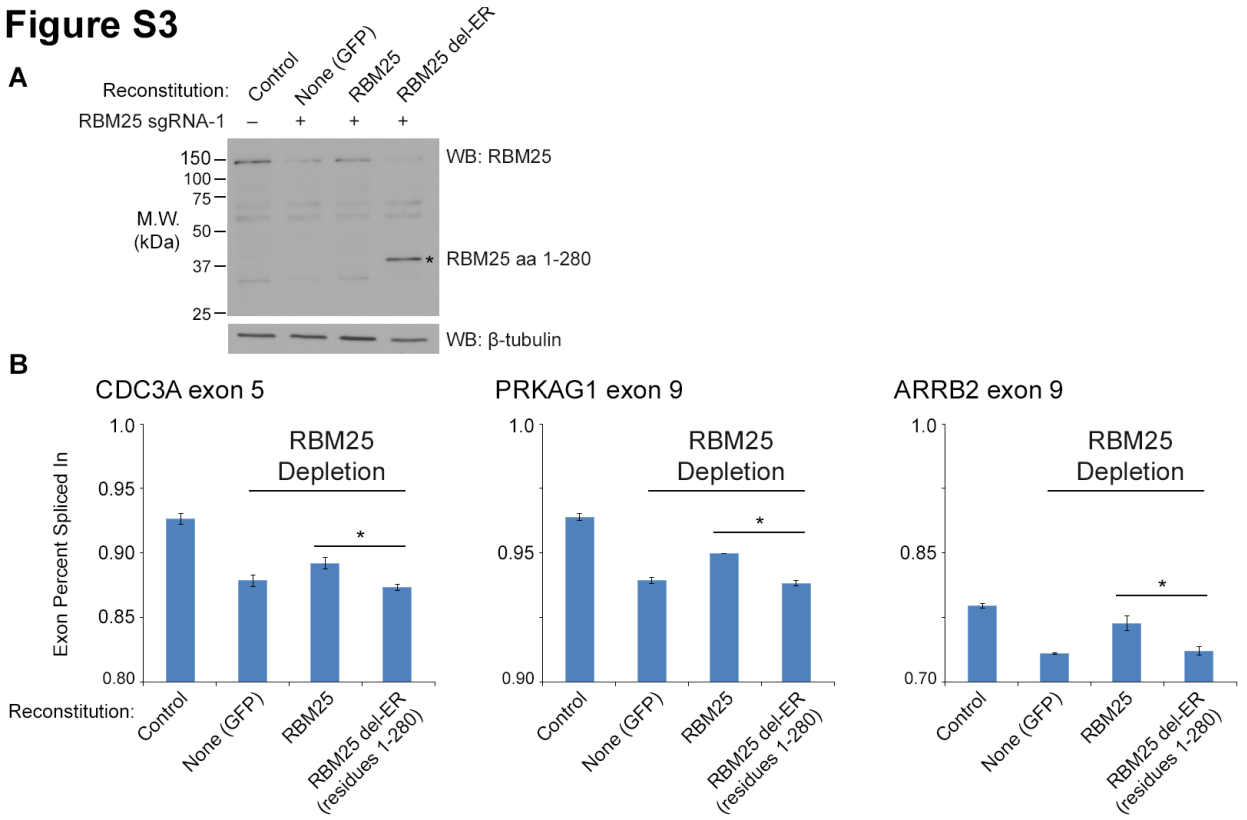

FIGURE S3. RBM25 lacking protein interaction domains is unable to promote inclusion of target exons. (**A**) Endogenous RBM25 was depleted in 293T cells expressing control vector (GFP), full-length RBM25, or RBM25 amino acids 1-280 (the N-terminal portion up to the ER-repeat domain). Western blot used an antibody recognizing an epitope near the N-terminus (Bethyl Labs A301-067A, instead of A301-068A used in all other figures). **(B)** Real-time PCR was used to measure exon inclusion at RBM25-target exons. Error bars show S.E.M, N=3. * $p < 0.05$.

Figure S3

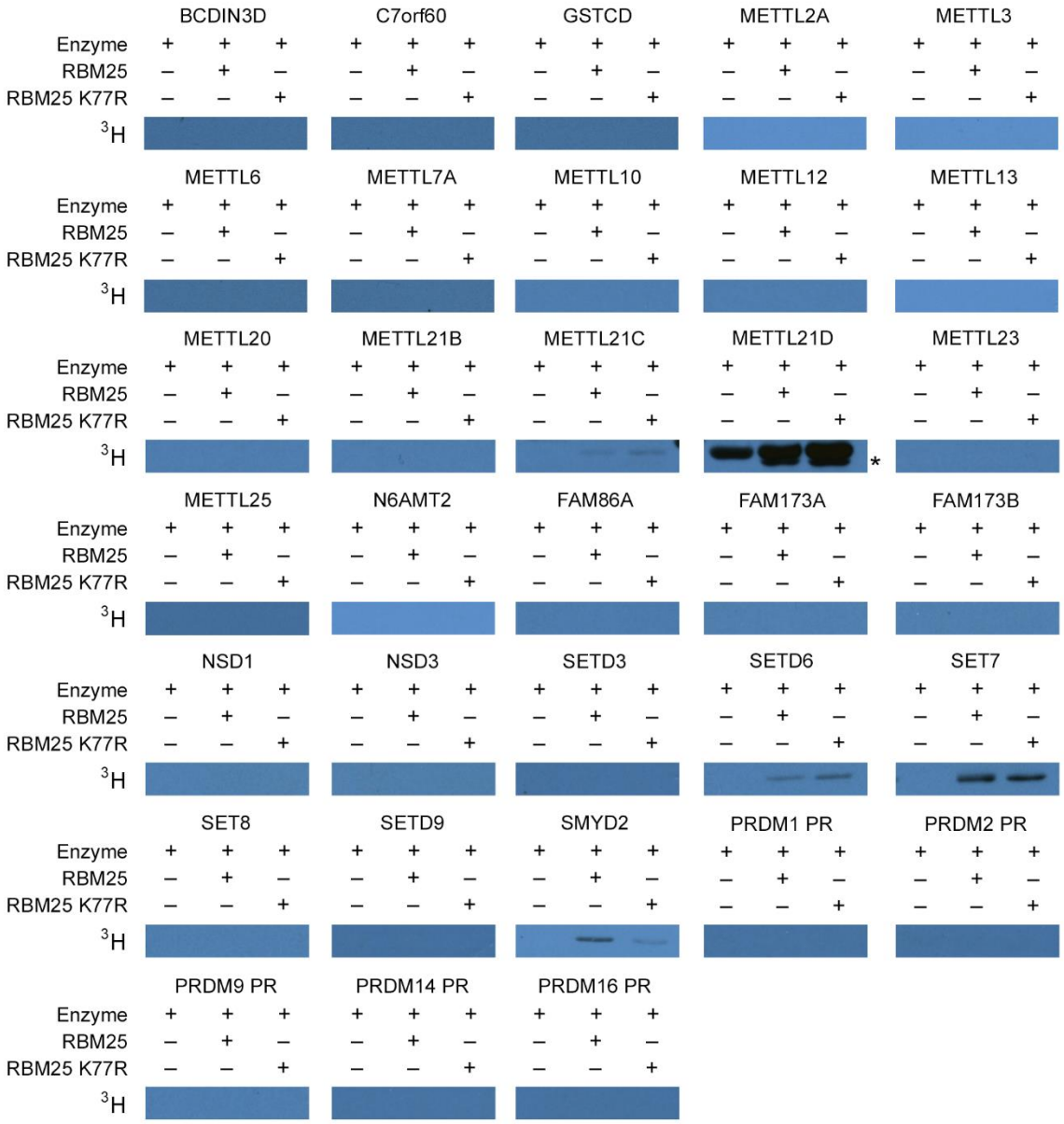

FIGURE S4. Known and candidate KMTs do not specifically methylate RBM25 K77
Each enzyme was expressed in *e.coli* as a fusion with N-terminal GST. Their ability to methylate RBM25 amino acids 1-181 with wild-type or K77R mutation was determined by autoradiography. * Upper and lower bands seen with METTL21D are enzyme and substrate respectively; no difference is seen between wild-type and K77R RBM25.