Structural basis of UCUU RNA motif recognition by splicing factor RBM20

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SUPPLEMENTARY MATERIAL

Main Resources

Supplementary Table 1. List of PCR primers.

Supplementary Figure 1. Annotated 2D $^{1}H,^{15}N$-HSQC spectra corresponding to **Figure 1B**.

Supplementary Figure 2. Representative ITC raw data corresponding to **Table 1**.

Supplementary Figure 3. Complete spectra corresponding to the selected regions in **Figure 3C-F**.

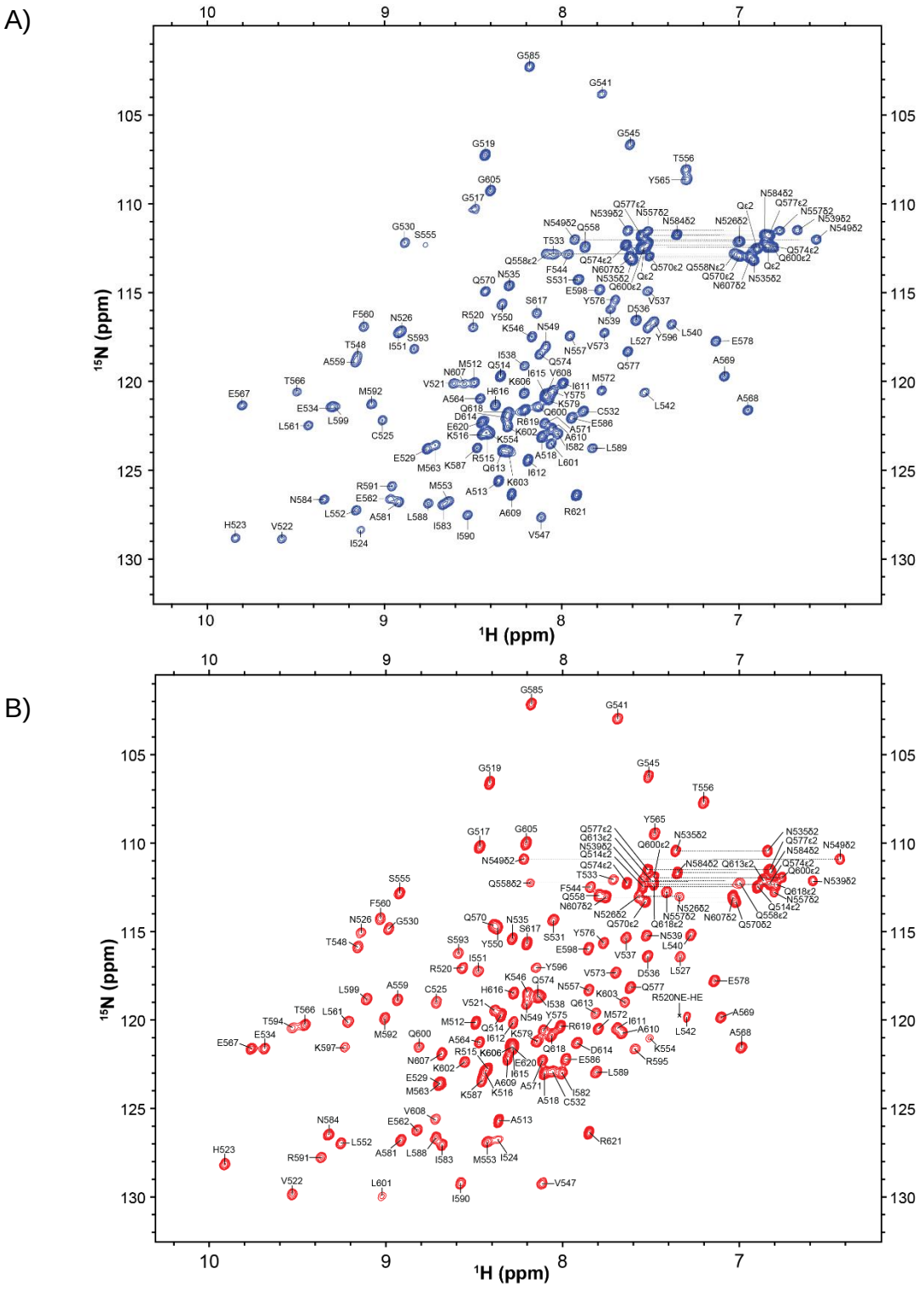
Supplementary Figure 4. Identification of residues in the proximity of Val537.

Main Resources

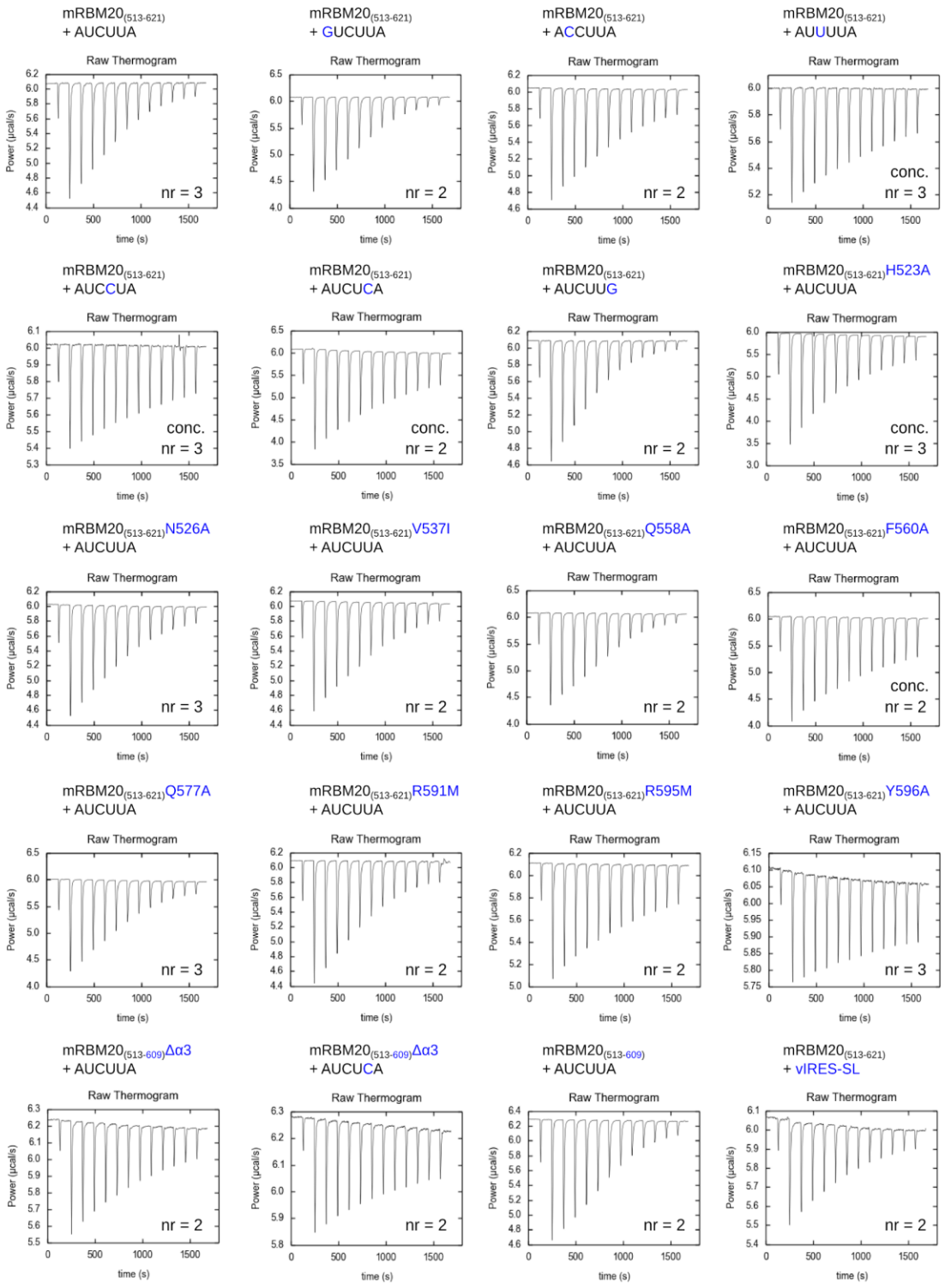
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
5-alpha Competent E. coli	New England Biolabs	Cat# C2987H
T7 Express lysY Competent E. coli	New England Biolabs	Cat# C3010I
Chemicals, Peptides, and Recombinant Proteins		
Nuvia™ IMAC Resin	BioRad	Cat# 7800800
TEV protease	In house produced	N/A
Deposited Data		
RBM20-AUCUUA chemical shifts	BioMagResBank (BMRB)	34428
RBM20-AUCUUA structural ensemble	Protein Data Bank (PDB)	6SO9
unbound RBM20 chemical shifts	BioMagResBank (BMRB)	34429
unbound RBM20 structural ensemble	Protein Data Bank (PDB)	6SOE
Oligonucleotides		
PCR oligos	Sigma	Supplementary Table 1
RNA	In house produced	Table 1
Recombinant DNA		
pET-His1a	EMBL	N/A
mouse RBM20 cDNA	Pamela Lorenzi, University of Verona	N/A
pET-His1a:mRBM20 (513-621)H523A	This paper	N/A
pET-His1a:mRBM20 (513-621)N526A	This paper	N/A
pET-His1a:mRBM20 (513-621)V537I	This paper	N/A
pET-His1a:mRBM20 (513-621)Q558A	This paper	N/A
pET-His1a:mRBM20 (513-621)F560A	This paper	N/A
pET-His1a:mRBM20 (513-621)Q577A	This paper	N/A
pET-His1a:mRBM20 (513-621)R591M	This paper	N/A
pET-His1a:mRBM20 (513-621)R595M	This paper	N/A
pET-His1a:mRBM20 (513-621)Y596A	This paper	N/A
pET-His1a:mRBM20 (513-609)Δα3	This paper	N/A
pET-His1a:mRBM20 (513-649)+RS	This paper	N/A
Software and Algorithms		
Topspin	Bruker BioSpin	Version 4.0
NMRPipe	Delaglio et al, 1995	Version 8.6
Sparky	T.D. Goddard and D.G. Kneller, Univ. of California	Version 3
CNS		Version 1.2
ARIA		Version 2.3

Supplementary Table 1. List of PCR primers.

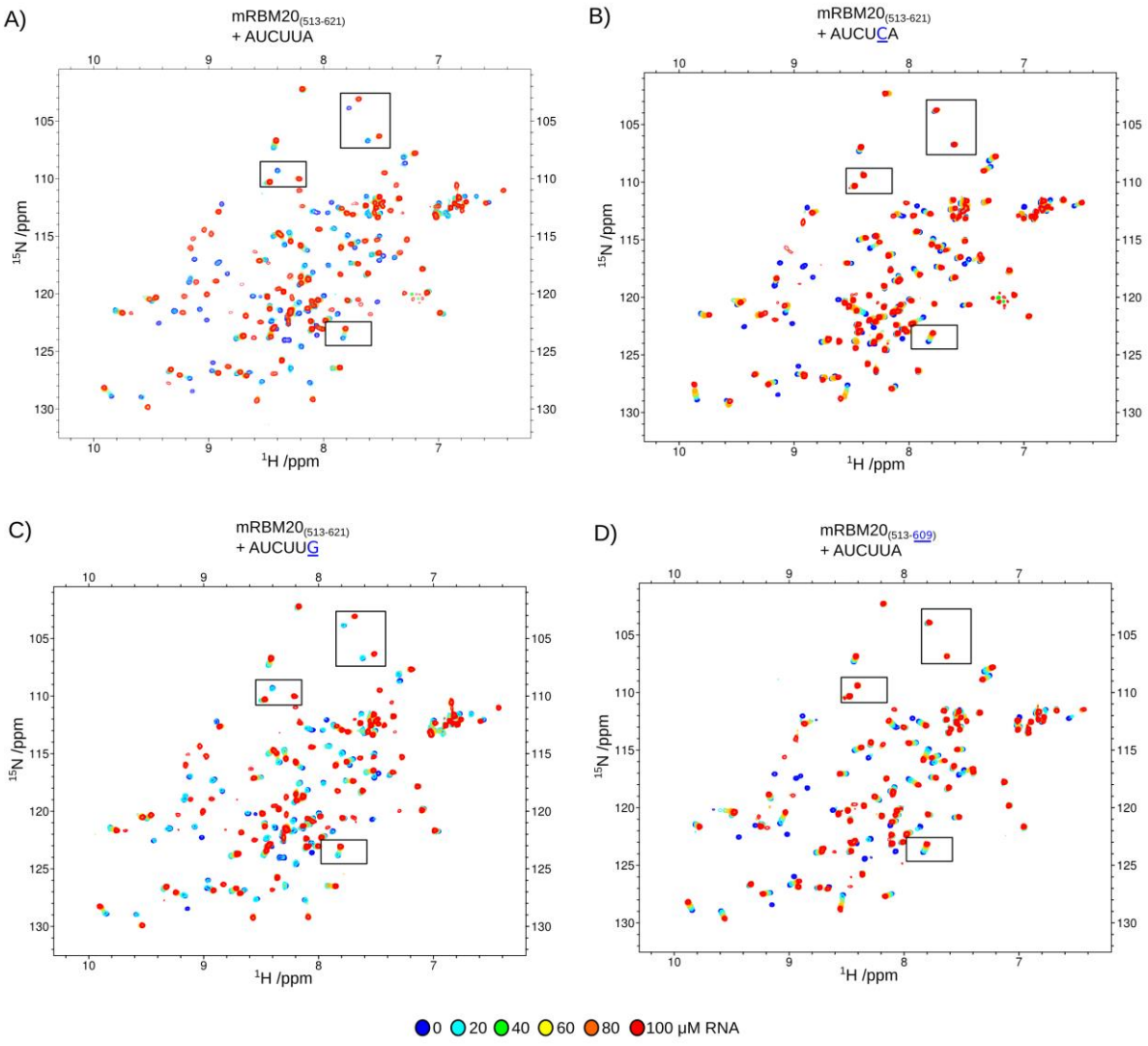
Function	Direction	Primer sequence (5'→3')
Clone mRBM20 (513-621) from cDNA, add stop codon, *Nco*I/*Acc65*I sites	Forward	CAGTAGCCATGGCACAGAGGAAAGGCGCTG
	Reverse	GTGGTACCTTACCTCTCCCTCTGGGAATGG
Remove C-terminal helix (-609), add stop codon, *Acc65*I site	Reverse	GTGGTACCTTAAGCCACATTTTTCCCAGGTTTC
Add RS region (-649), add stop codon, *Acc65I* site	Reverse	GTGGTACCTTAGGATCTTGGGGAGAGTGATC
H523A mutation	Forward	CGGGTAGTGGCGATCTGCAATCTCCCG
	Reverse	GATTGCAGATCGCCACTACCCGTCCAGC
N526A mutation	Forward	CACATCTGCGCGCTCCCGGAGGGCAGC
	Reverse	GCCCTCCGGGAGCGCGCAGATGTGCACTAC
Q558A mutation	Forward	GTCAACTAATGCGGCTTTCTTGGAG
	Reverse	CAAGAAAGCCGCATTAGTTGACTTCATGAG
V537I mutation	Forward	GAGAATGACATTATTAACCTGGGGCTGCCC
	Reverse	CAGGTTAATAATGTCATTCTCCGTGCAGC
F560A mutation	Forward	CTAATCAGGCTGCGTTGGAGATGGCTTAC
	Reverse	GCCATCTCCAACGCAGCCTGATTAGTTGACTTC
Q577A mutation	Forward	CAGTACTACGCGGAAAAGCCTGCGATTATC
	Reverse	GCAGGCTTTTCCGCGTAGTACTGGACCATAGC
K779M mutation	Forward	CTACCAAGAAATGCCTGCGATTATCAATG
	Reverse	GATAATCGCAGGCATTTCTTGGTAGTACTG
R591M mutation	Forward	GTTACTCATTATGATGTCCACCAGATACAAG
	Reverse	CTGGTGGACATCATAATGAGTAACTTCTCGCC
R595M mutation	Forward	CATGTCCACCATGTACAAGGAATTGCAGC
	Reverse	CCTTGTACATGGTGGACATGCGAATGAG
Y596A mutation	Forward	GTCCACCAGAGCGAAGGAATTGCAGCTG
	Reverse	GCAATTCCTTCGCTCTGGTGGACATGCG



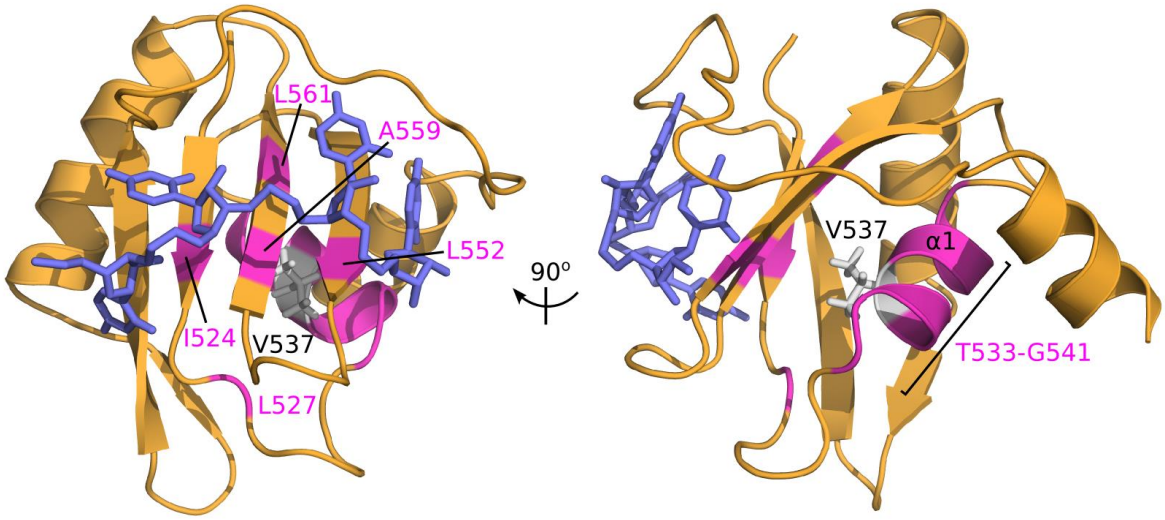
Supplementary Figure 1. Annotated 2D ^{1}H,^{15}N-HSQC spectra corresponding to **Figure 1B**. The backbone and side chain amide cross peaks are labeled with the residue name (single amino acid letter code) and number. The spectra were recorded at 298 K in 20 mM sodium phosphate, 50 mM NaCl (pH 6.5), 2 mM DTT and 10 % (v/v) D_2O. (**A**) NMR sample containing 400 µM [^{13}C,^{15}N]mRBM20$_{(513\text{-}621)}$ and spectrum collected at a field strength of 800 MHz. (**B**) NMR sample containing 400 µM [^{13}C,^{15}N]mRBM20$_{(513\text{-}621)}$ and 480 µM natural abundance AUCUUA RNA, with spectrum collected at a field strength of 700 MHz.



Supplementary Figure 2. Representative ITC raw data corresponding to **Table 1**. Above each thermogram the protein construct in the syringe is listed first, followed by the RNA ligand present in the cell. The number of replicates used to calculate the average and standard deviation in **Table 1** is indicated at the bottom right of each thermogram as 'nr'. In most cases, the target protein concentration was 400 µM, with 40 µM RNA ligand. Those samples which required higher concentrations of 800 µM protein and 80 µM RNA are indicated with 'conc.'.



Supplementary Figure 3. Complete spectra corresponding to the selected regions in **Figure 3C-F**.



Supplementary Figure 4. Identification of residues in the proximity of Val537. Using the structure of the RNA-bound mRBM20$_{(513-621)}$, Val537 is shown as a white stick representation with residues which are within 4 Å coloured in magenta onto the ribbon representation. Although Val537 is distant from the RNA-binding surface, there may be indirect (i.e. allosteric) affects via changes in the hydrophobic core of the protein including to the buried sidechains of Ile524, Leu527, Leu552, Ala559 and Leu561. Since the backbone amide of Leu552 in particular makes a hydrogen bond to the uracil base of U5, this could be one explanation for the slight drop in affinity for the V527I mutant. In addition, the V537I mutant is within helix α1 and thus could alter the folding and stability of helix α3 upon binding the RNA ligand.