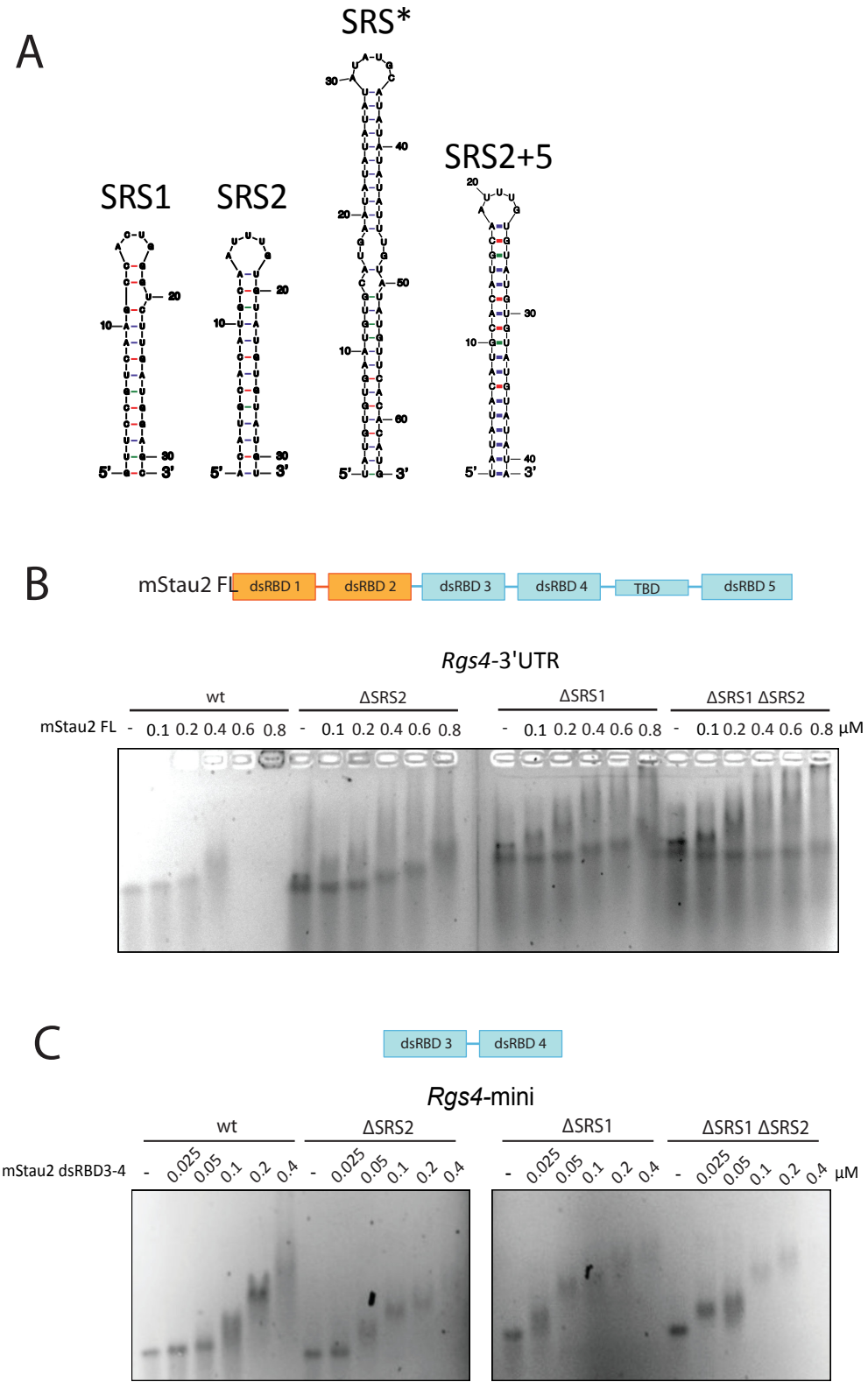

Figure S1: EMSAs with mStau2 and *Rgs4* 3'UTR RNAs. A) mStau2 full-length binds wild type *Rgs4* 3'UTR and SRS deletion mutants with similar apparent affinities in the nanomolar concentration range. **B)** mStau2 dsRBD3-4 binds *Rgs4*-mini wild type and SRS deletion mutants with similar apparent affinities in the nanomolar concentration range. Complexes were resolved in 1.5 % agarose gels and imaged via GelRed staining and UV imaging.

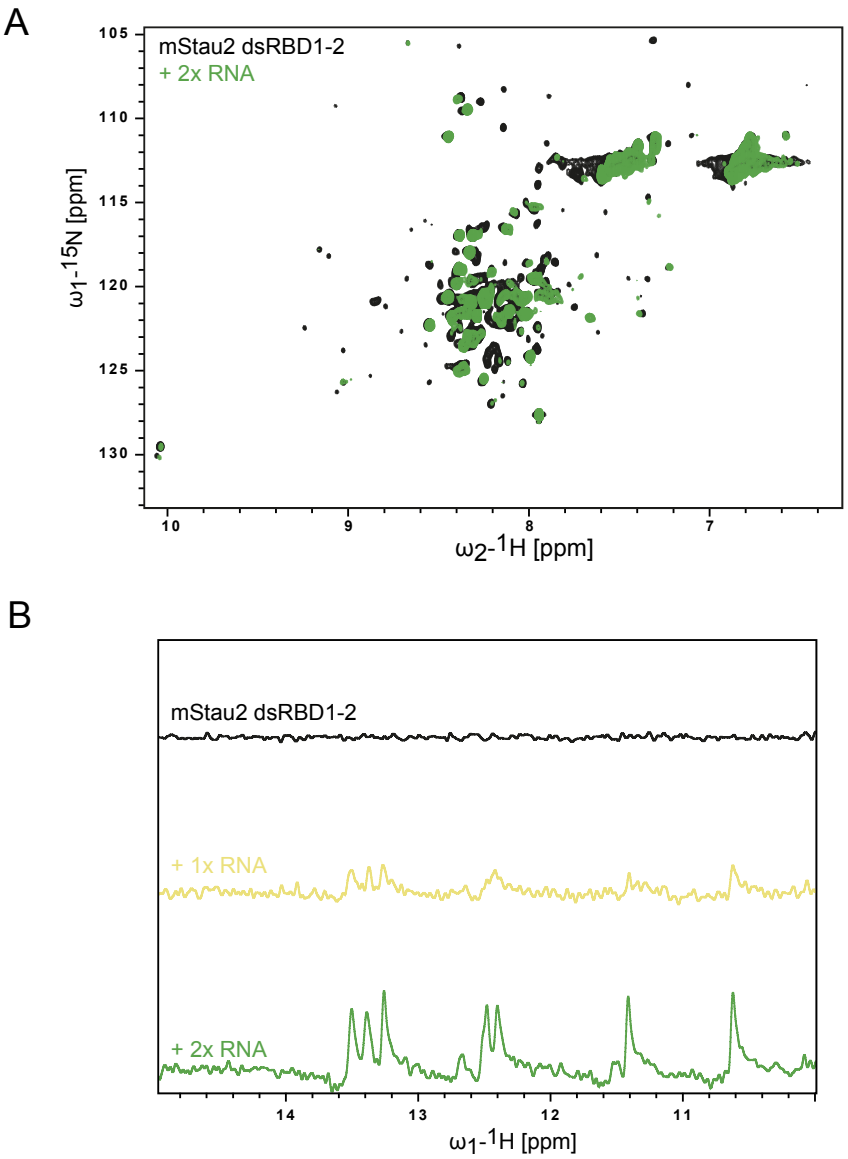

Figure S2: NMR titration experiments of Stau2 dsRBD1-2 with SRS2 RNA. **A)** Overlay of $^{1}H,^{15}N$-HSQC spectra of dsRBD1-2 in absence and presence of 2x excess SRS2 RNA. Resonance shifts and line broadening of several signals are observed. **B)** Comparison of 1D imino traces of SRS2 RNA at different stoichiometric ratios with dsRBD1-2.

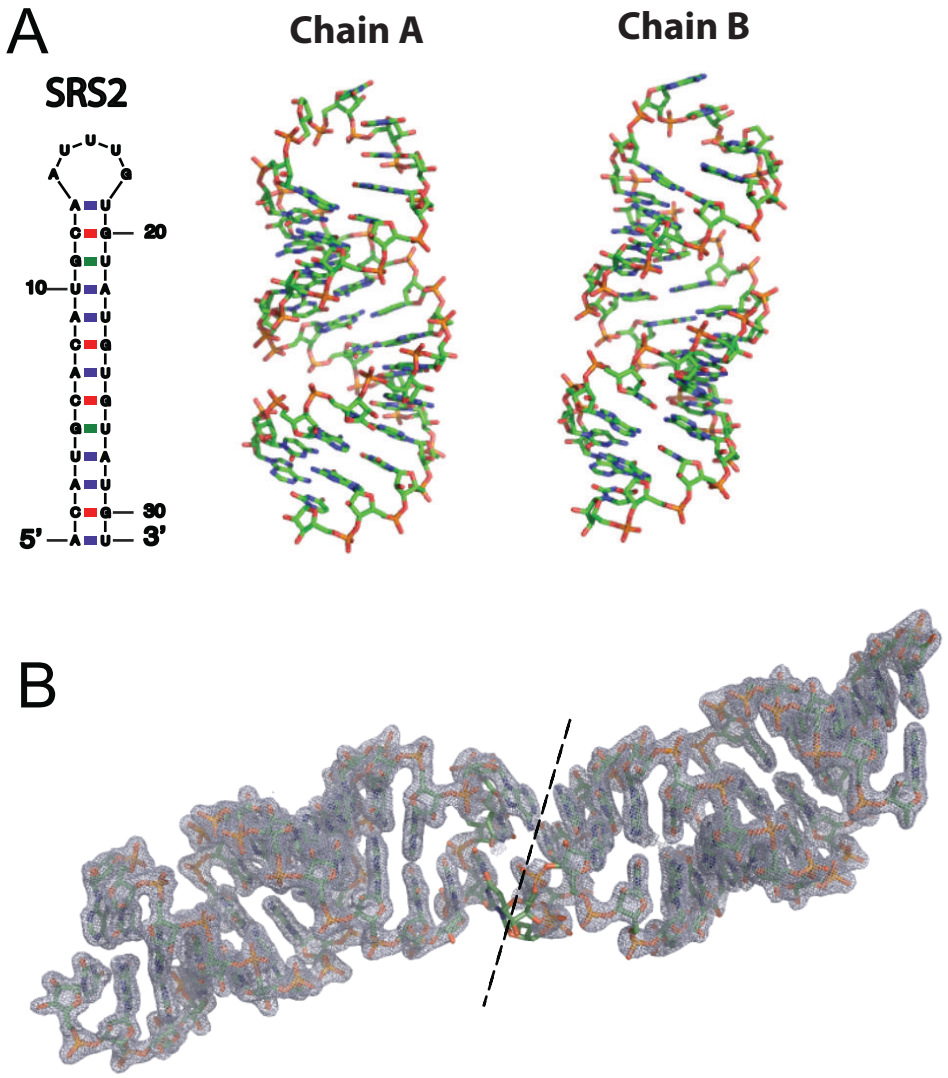

Figure S3: Crystal structure of the isolated *Rgs4* SRS2 RNA stem-loop. A) Schematic drawing of SRS2 RNA and the two molecules chain A and chain B contained in the asymmetric unit of the crystal lattice. Both molecules adopt the typical RNA A-form and form the expected stem-loop. Both chains differ slightly in their loop-regions, which appear to be disordered. **B)** Electron density map of the two RNA molecules in the asymmetric unit. Whereas the density map in the stem region of the RNA is very well defined, it is rather poor in the area of the two loops, indicating disordered loop regions.

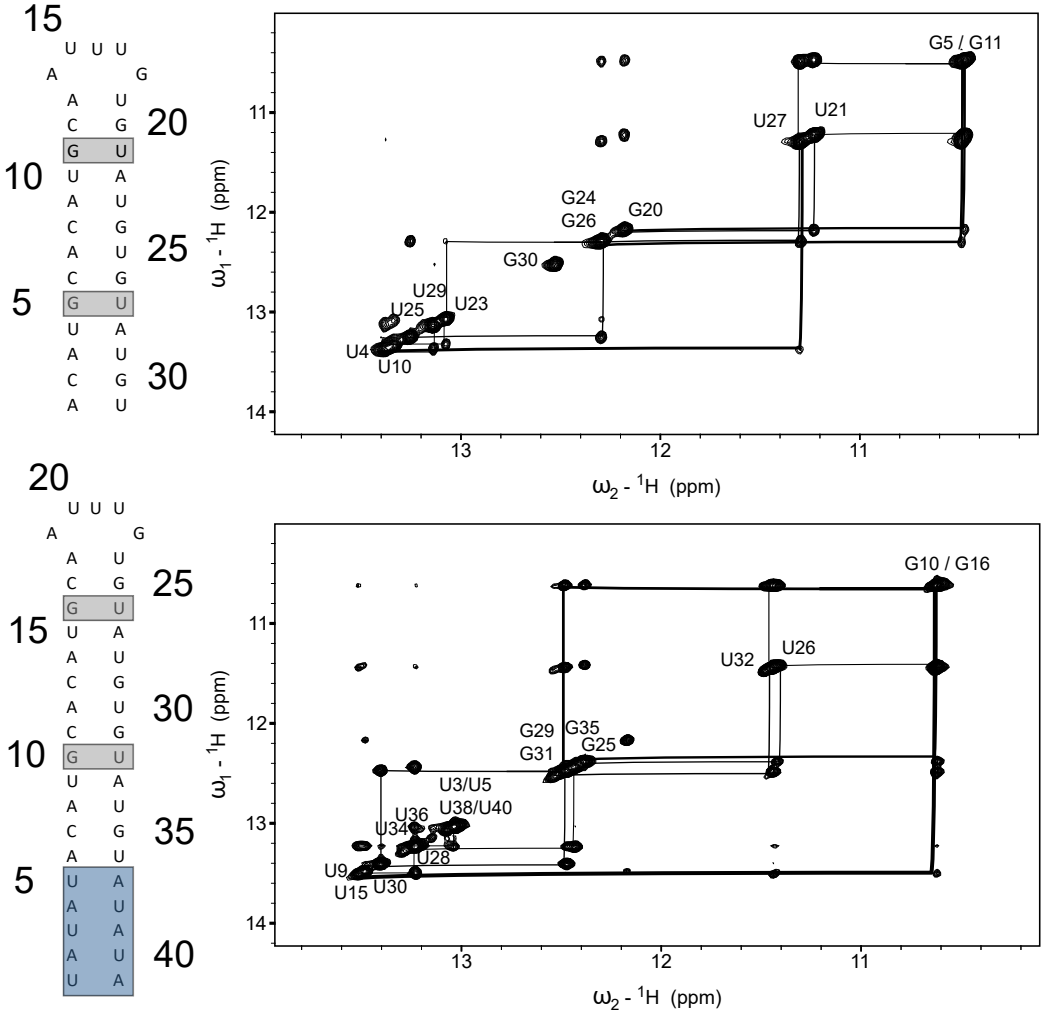

Figure S4: RNA assignment of imino groups based on ^{1}H, ^{1}H- NOESY spectra of SRS2 (upper) and SRS2+5 RNA (lower).

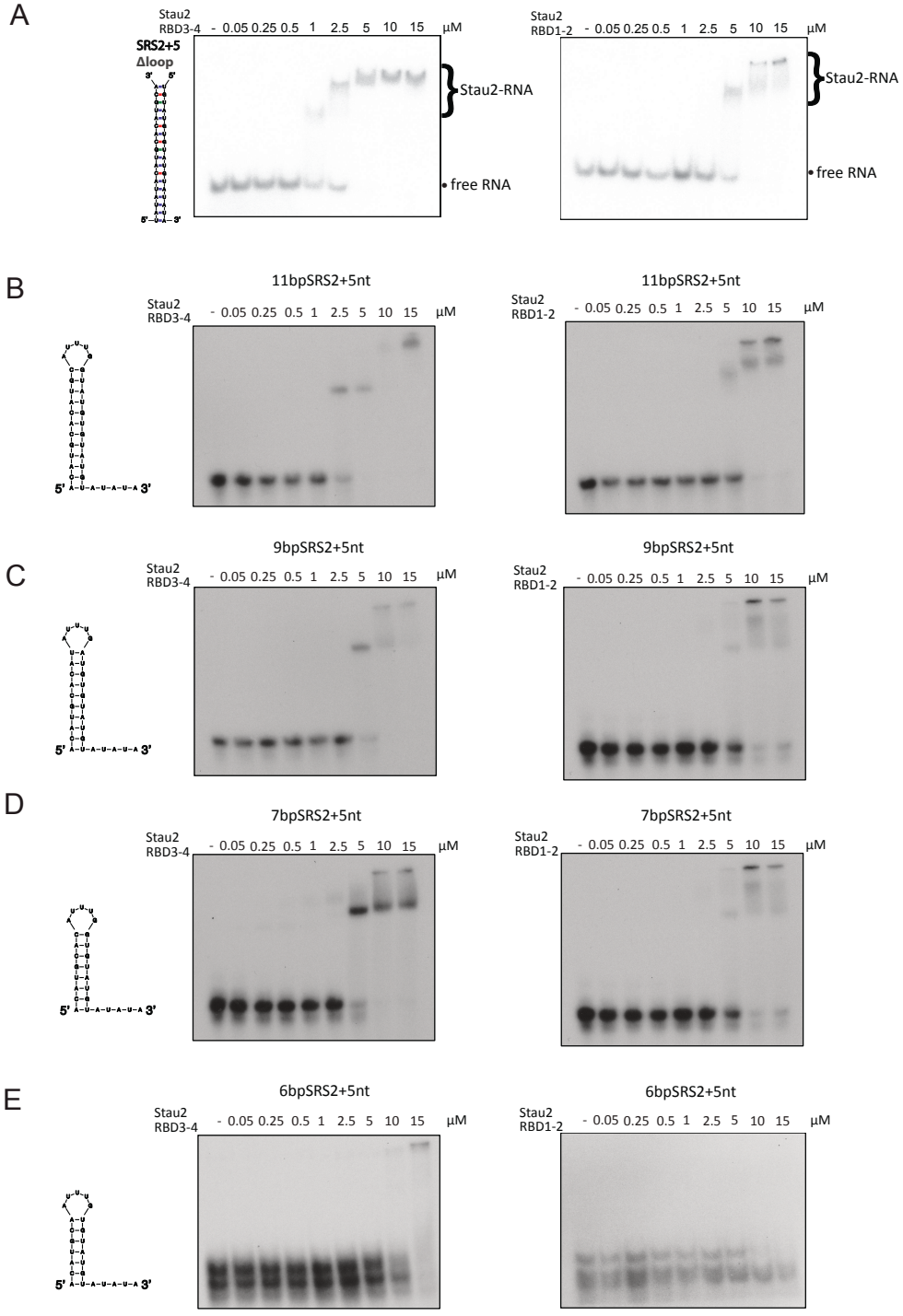

Figure S5: EMSAs with mStau2 tandem domains dsRBD3-4 and dsRBD1-2 and modified SRS2 RNAs. A) mStau2 binding to the elongated stem of SRS2 RNA without the loop region. Binding by both tandem domains to the elongated stem-loop is improved, the stem RNA is bound with similar affinity as SRS2, indicating that total length of the RNA determines binding rather than its specific structure. **B)** dsRNA stem-loops with 11bp, **C)** 9 bp and **D)** 7 bp are bound by both dsRBD3-4 (left) and dsRBD1-2 (right) with similar affinities in the micromolar concentration range. **E)** For a stem-loop with only 6 bp, binding is almost completely abolished. No protein-RNA complex is observed. To increase any effects of shortening the stem, SRS2 RNAs with a single-stranded 3' extension were used. This should improve affinity enough to allow for visualization of a deterioration of binding by mStau2 with shortening of the dsRNA stem. Complexes were resolved by native PAGE and imaged by PhosphorImaging or by exposure of radiograph films.

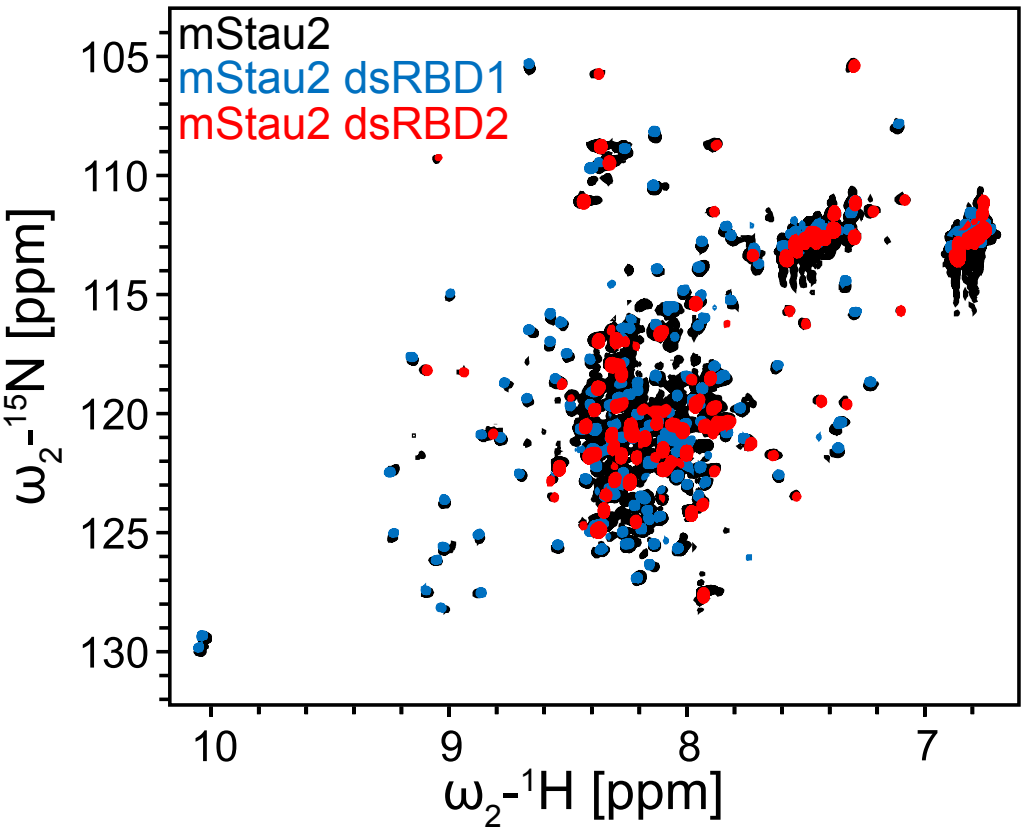

Figure S6: Overlay of the $^{1}H,^{15}N$-HSQC spectra of the tandem domain dsRBD1-2 and the individual dsRBDs 1 and 2. The spectra of dsRBDs 1 and 2 overlap and add up to the spectrum of the tandem domain, indicating that dsRBDs 1 and 2 are separate, partially disordered domains.

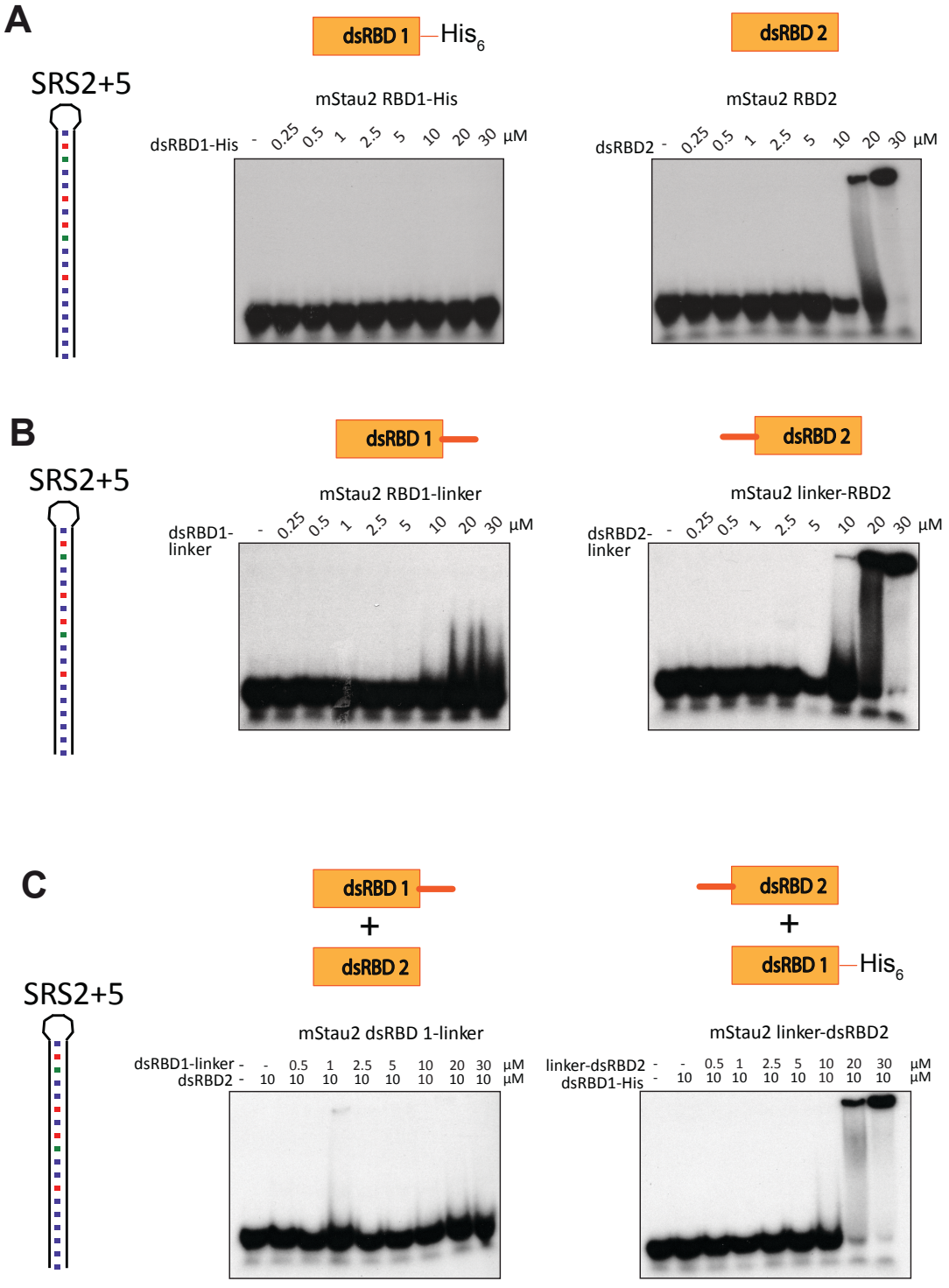

Figure S7: EMSAs with the individual domains dsRBD1 and 2. A) dsSRS2+5 RNA binding of dsRBD1-containing (left) and dsRBD2-containing (right) fragments . **B)** Binding of dsRBD1-linker (left) and linker-dsRBD2 (right) to dsSRS2+5 RNA. **C)** Binding of dsRBD1-linker (left) and linker-dsRBD2 (right) to dsSRS2+5 RNA in presence of the respective other domain at 10 µM.

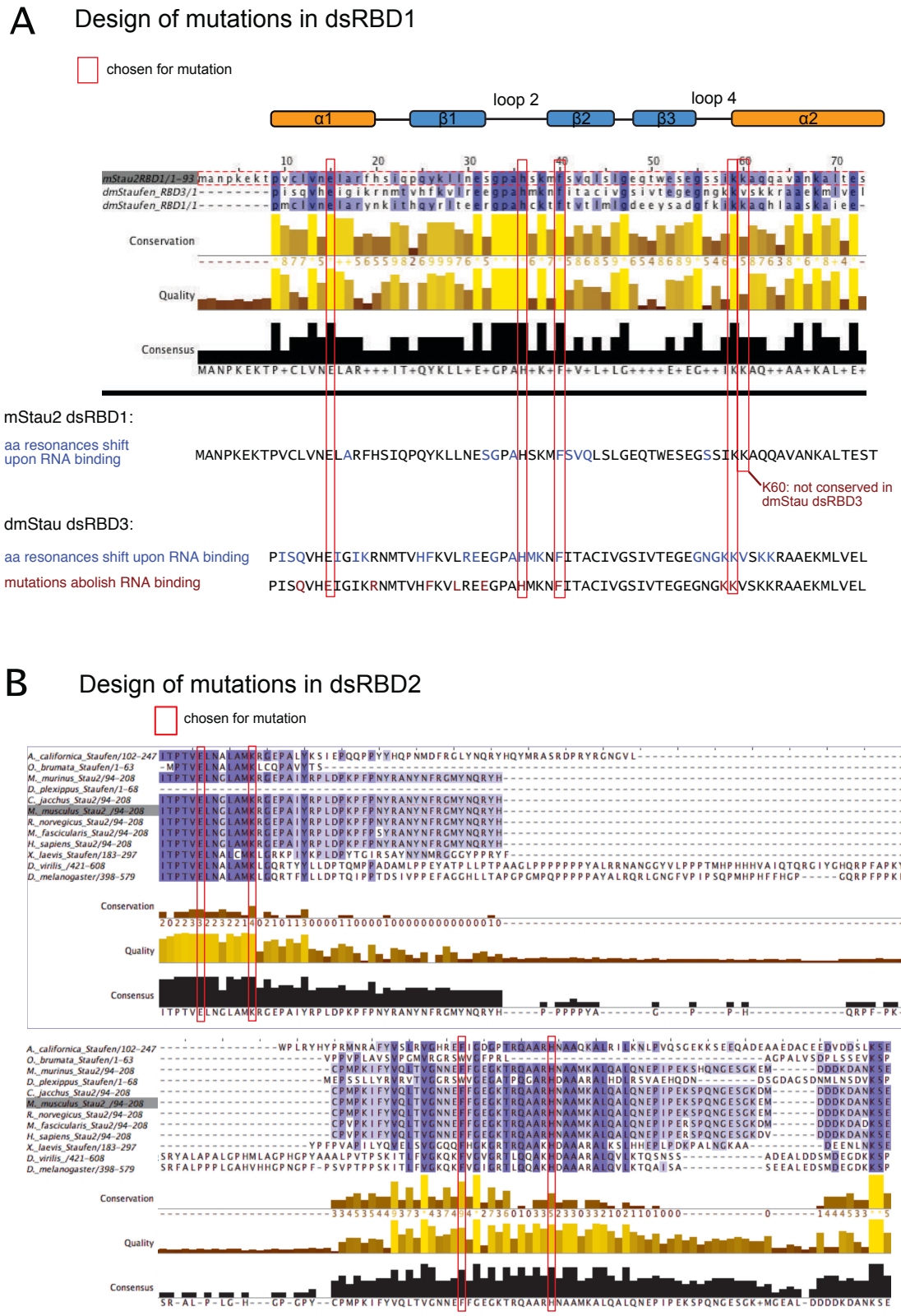

Figure S8: Sequence analysis used for mutant design of dsRBD1 and dsRBD2. A) Multiple sequence alignment of mStau2 dsRBD1, dmStau dsRBD1, and dmStau dsRBD3. Below, assigned residues with NMR chemical shift perturbations upon RNA titration are marked in the sequence of mStau2 dsRBD1 in blue. Residues chosen for mutation are marked by red boxes. Designed mutations map to regions previously identified to be involved in RNA binding in dmStau dsRBD3 (Ramos et al., 2000). **B)** Multiple sequence alignment of mStau2 dsRBD2 to Stau proteins from 11 different species. Residues chosen for mutation are marked by red boxes. Plots were generated with the program JalView.

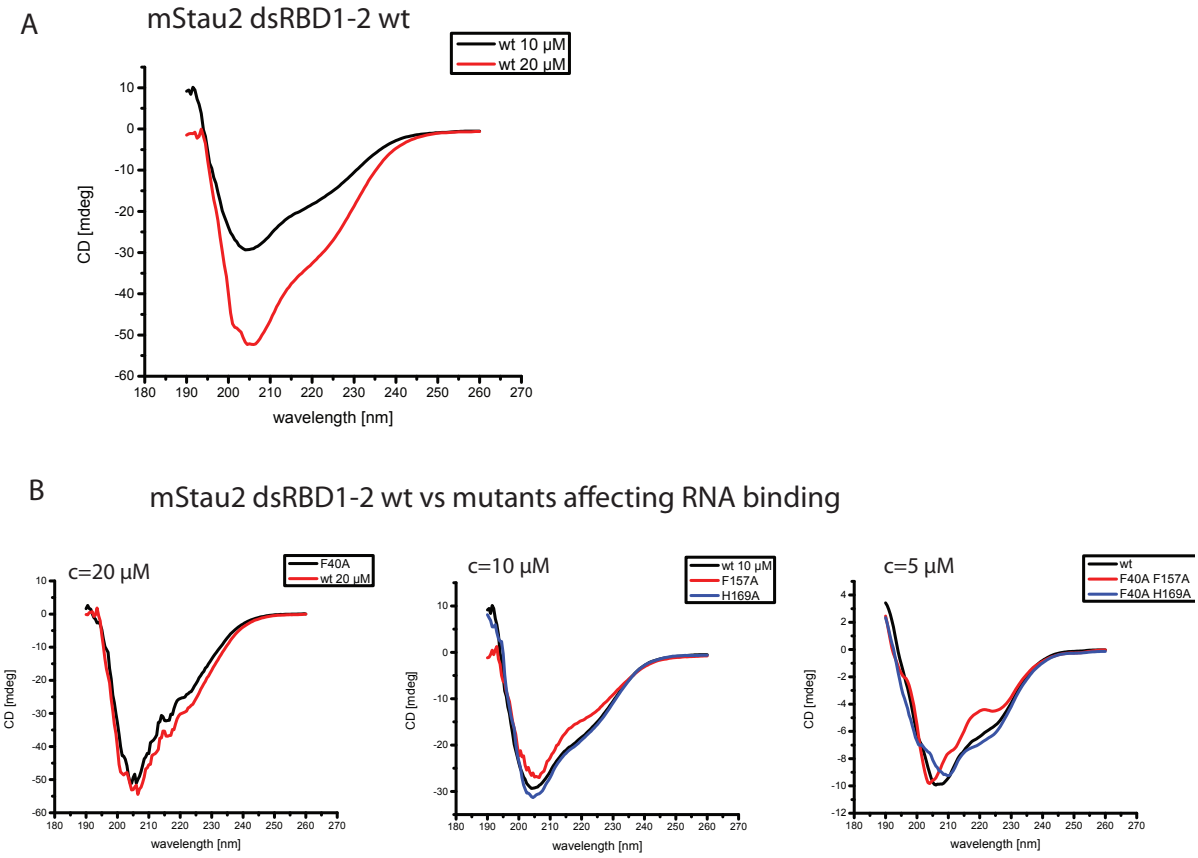

Figure S9: Circular dichroism spectra of mStau2 dsRBD1-2 versions. A) Wild type mStau2 dsRBD1-2 at different concentrations and **B)** RNA-binding mutants of dsRBD1-2 compared to wild type dsRBD1-2. Measurements were performed at the indicated concentrations in buffer containing <50 mM NaCl. All curves show very similar profiles, indicating that mutant proteins adopt the same fold as wild type dsRBD1-2.

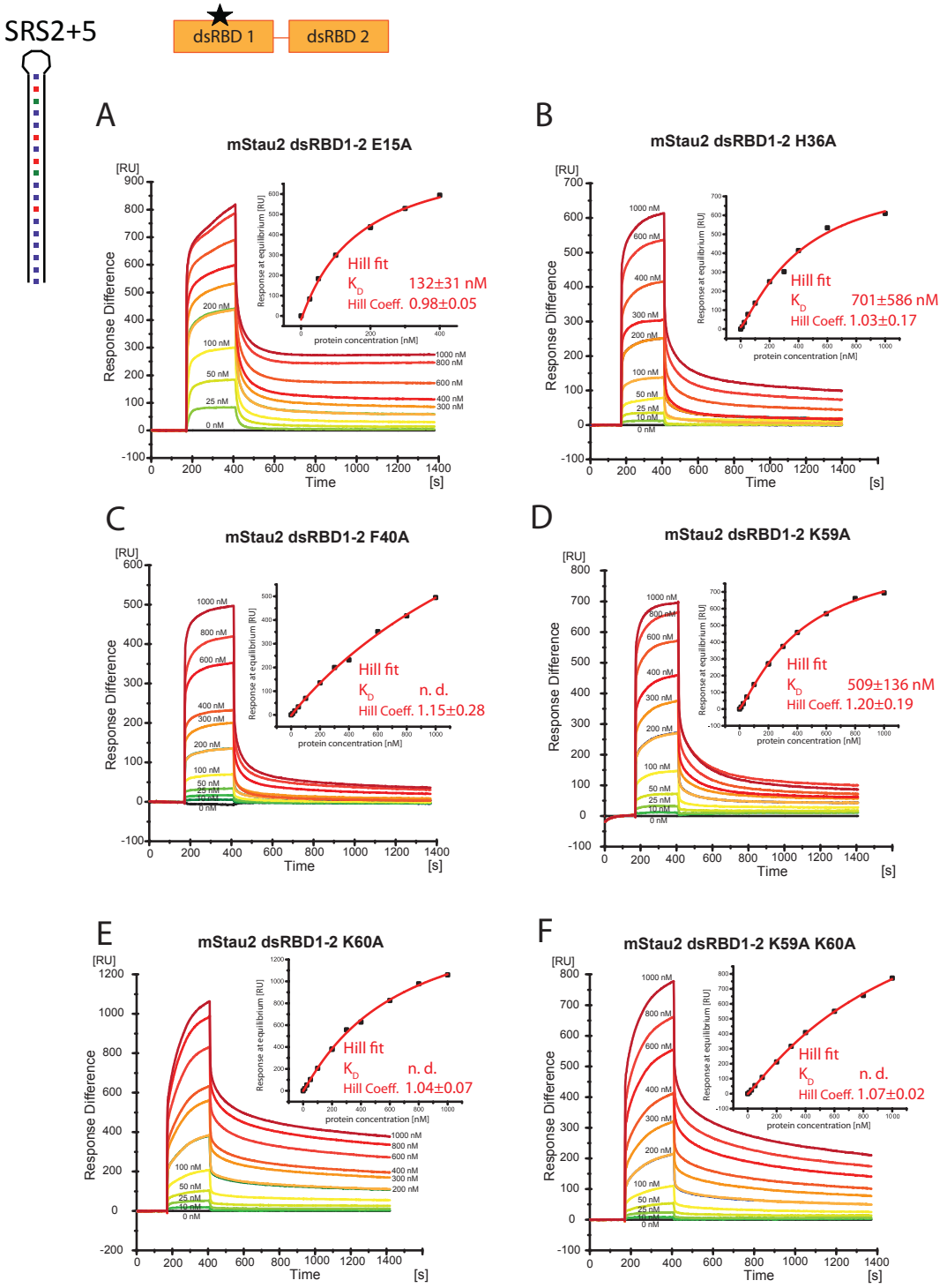

Figure S10: SPR sensorgrams and and curve fitting of SRS2+5 RNA binding by mStau2 dsRBD1-2 with mutations in dsRBD1. A-F) Mutant versions of mStau2 dsRBD1-2. A) E15A, B) K59A, C) H36A, and E) F40A bind SRS2+5 transiently with fast kinetics. The steady-state binding curves are described by Hill-fits with Hill coefficients n ≈ 1, indicating non-cooperative binding.

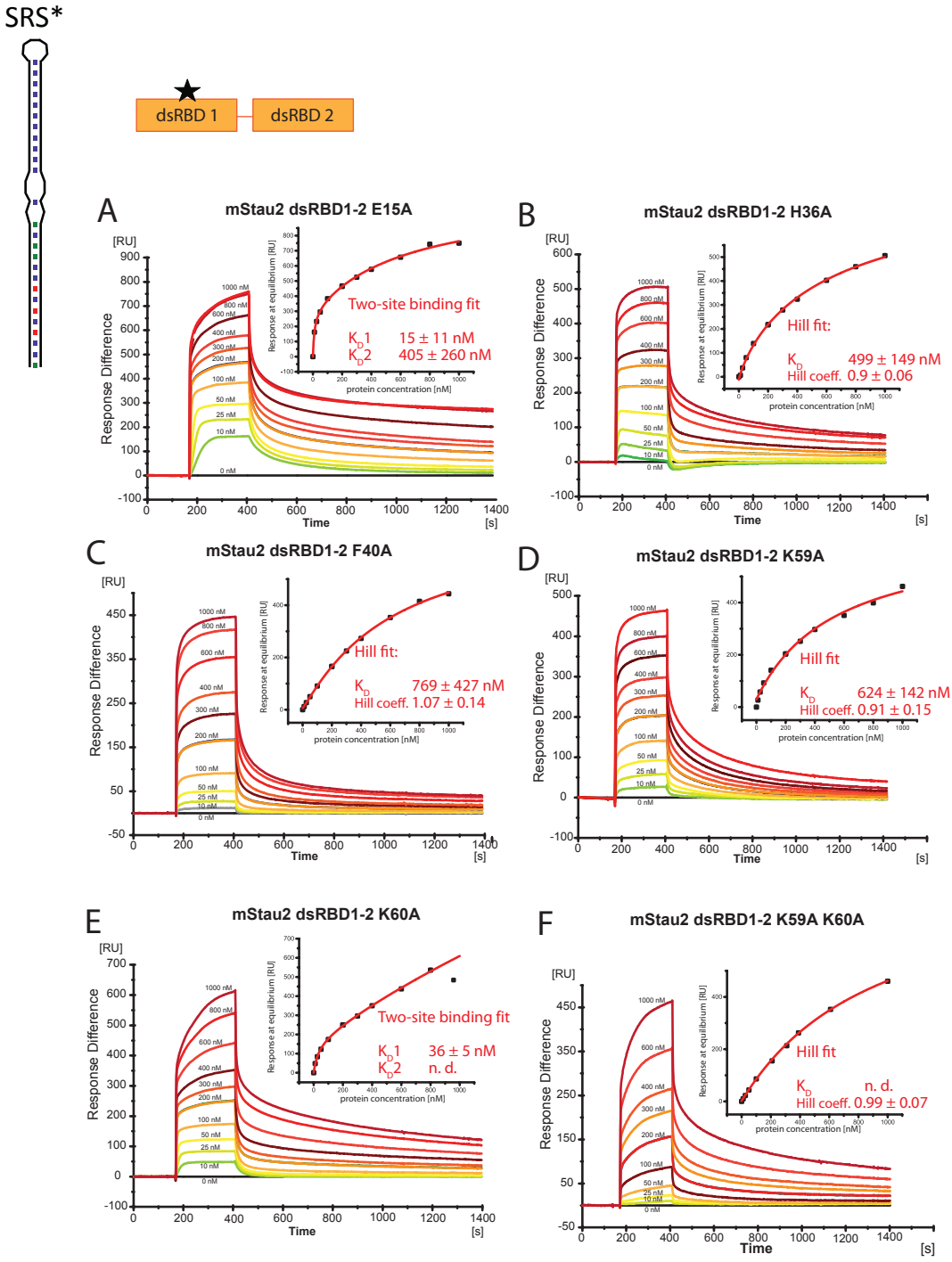

Figure S11: SPR sensorgrams and and curve fitting of SRS* RNA binding by mStau2 dsRBD1-2 with mutations in dsRBD1. A-F) Mutant versions of mStau2 dsRBD1-2. B) K59A, C) H36A, E) F40A and F) K59A K60A bind SRS2+5 transiently with fast kinetics. The steady-state binding curves are described by Hill-fits with Hill coefficients n ≈ 1, indicating non-cooperative binding. Mutants mStau2 dsRBD1-2 A) E15A and D) K60A bind similar to mStau2 dsRBD1-2 wild type.

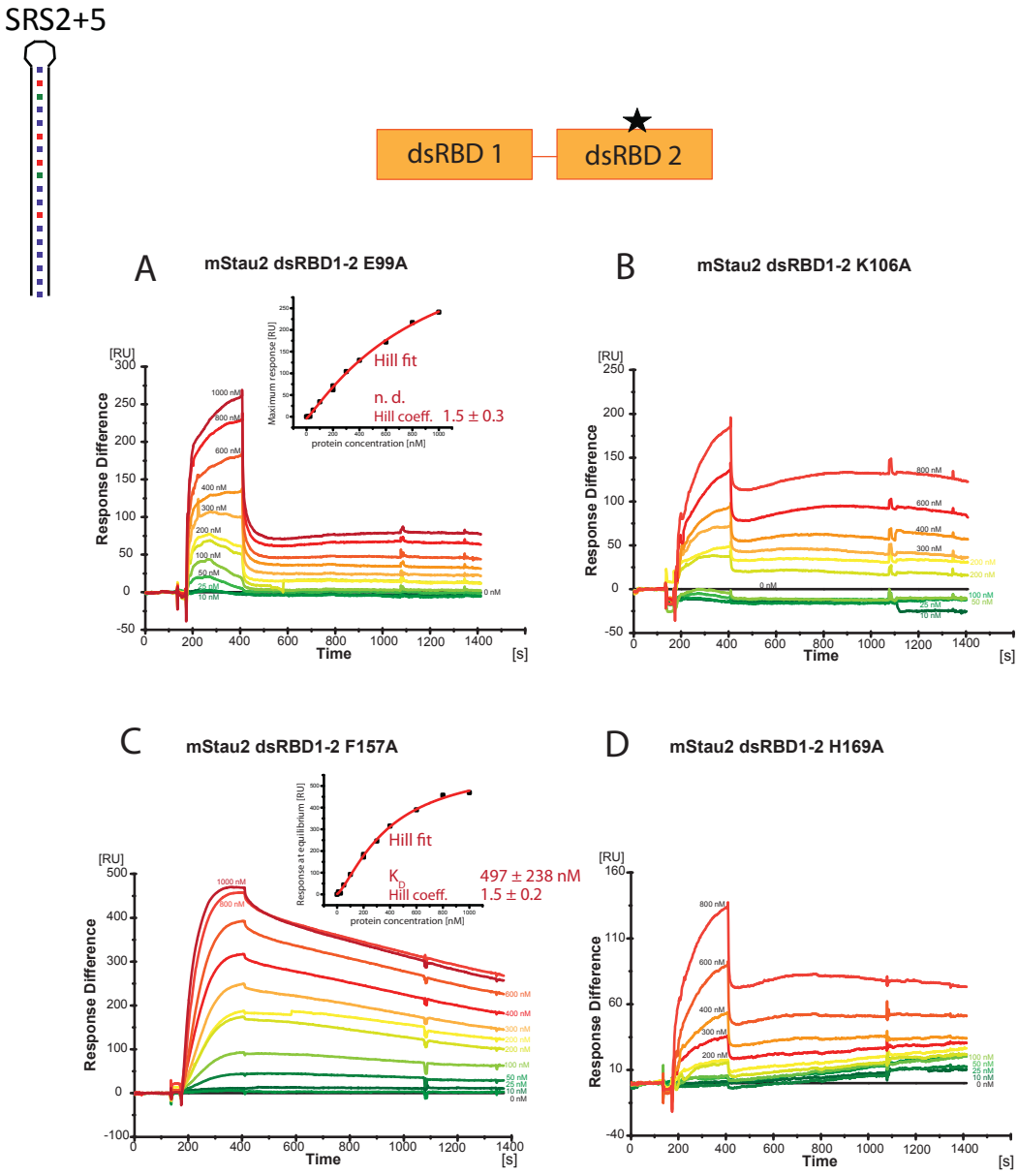

Figure S12: SPR sensorgrams and and curve fitting of SRS2+5 RNA binding by mStau2 dsRBD1-2 with mutations in dsRBD2. mStau2 dsRBD1-2 **A)** E99A, **B)** K106A, **C)** F157A, **D)** H169A show decreased binding to SRS2+5 RNA when compared to wild-type protein. For RBD1-2 E99A (A) and F157A (C), steady-state binding curves are shown. For dsRBD1-2 K106A (B) and H169A (D), steady-state could not be reached.

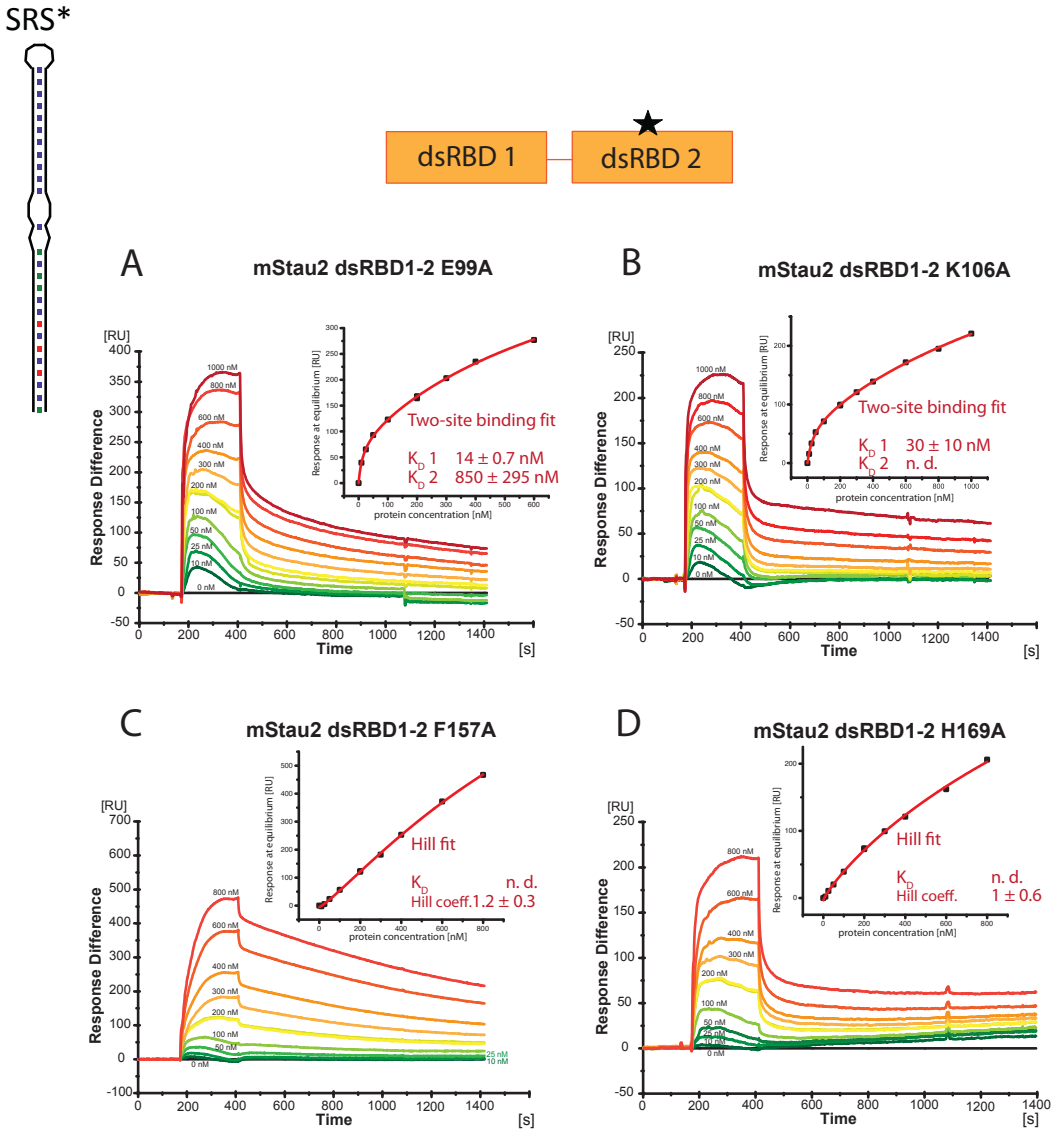

Figure S13: SPR sensorgrams and and curve fitting of SRS* RNA binding by mStau2 dsRBD1-2 with mutations in dsRBD2. mStau2 dsRBD1-2 **A)** E99A, and **B)** K106A bind RNA similar to wild-type dsRBD1-2. In contrast, mStau2 dsRBD1-2 **C)** F157A and **D)** H169A show decreased binding to SRS* RNA.

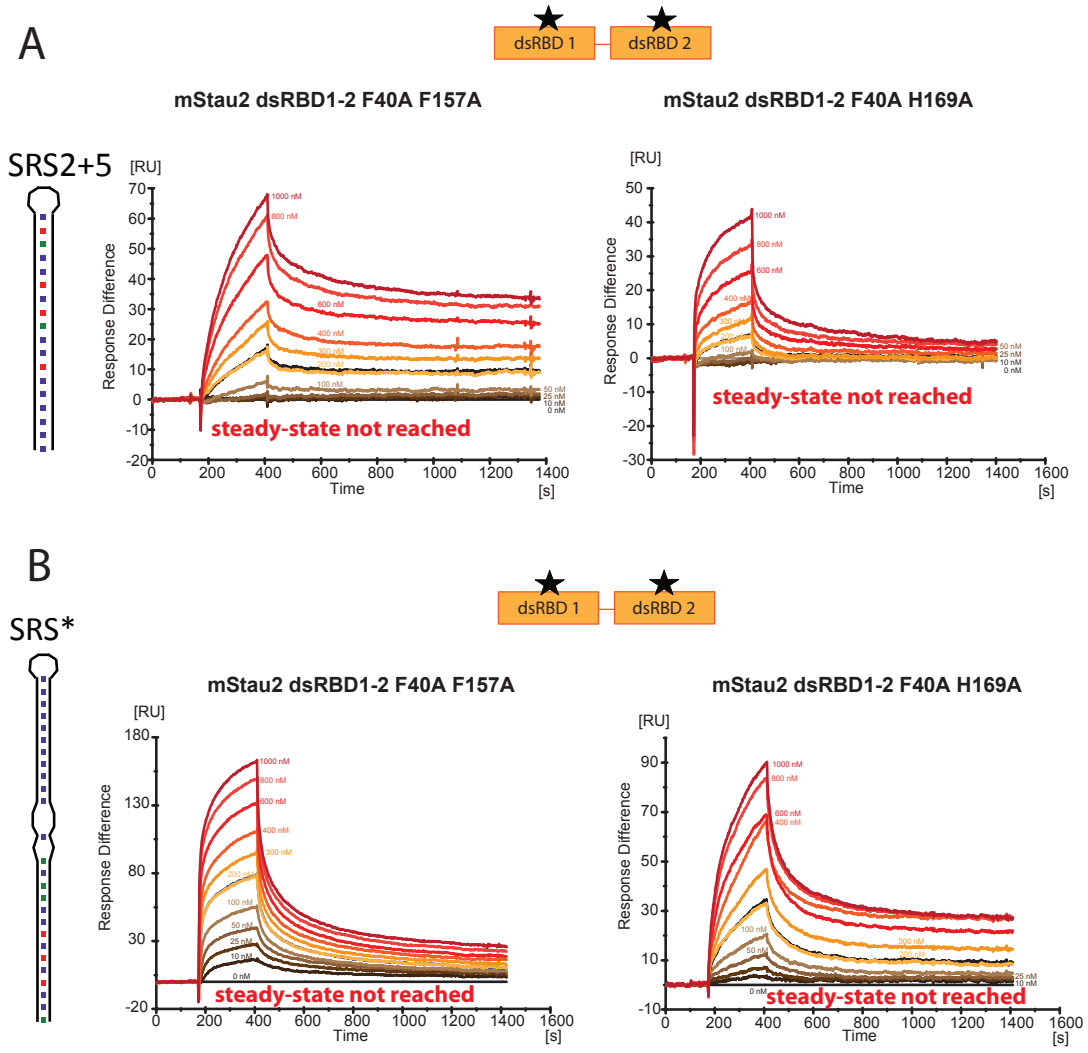

Figure S14: SPR sensorgrams and plots of maximum response against protein concentration of mStau2 dsRBD1-2 double-mutants binding to SRS RNA. Binding to **A)** SRS2+5 and **B)** SRS* is strongly decreased as compared to wild-type mStau2 dsRBD1-2.

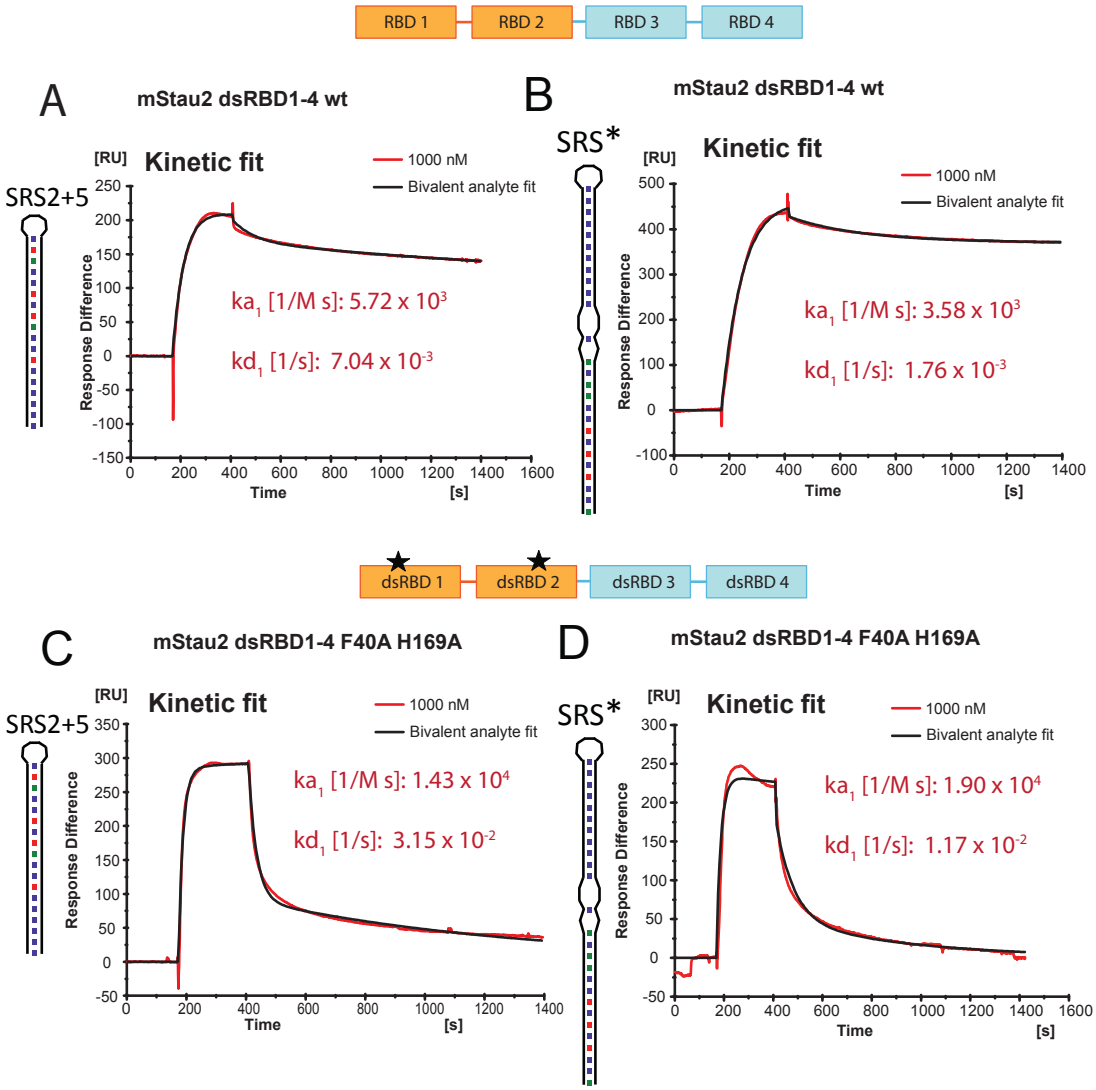

Figure S15: SPR results for mStau2 RBD1-4 and double-mutants binding to RNA. Exemplary kinetic fits (bivalent analyte fit) at 1000 nM protein concentration are shown. **A)** Wild-type mStau2 RBD1-4 binding to SRS2+5 RNA. **B)** mStau2 RBD1-4 F40A H169A binding to SRS2+5. The kinetic fit shows that both ka_1 and kd_1 are ~10-fold increased as compared to wild-type, indicating a transient binding..**C)** Wild-type mStau2 RBD1-4 binding to SRS* RNA. D) mStau2 RBD1-4 F40A H169A binding to SRS*. The kinetic fit shows that both ka_1 and kd_1 are ~5 and ~10-fold increased, respectively, as compared to wild-type.

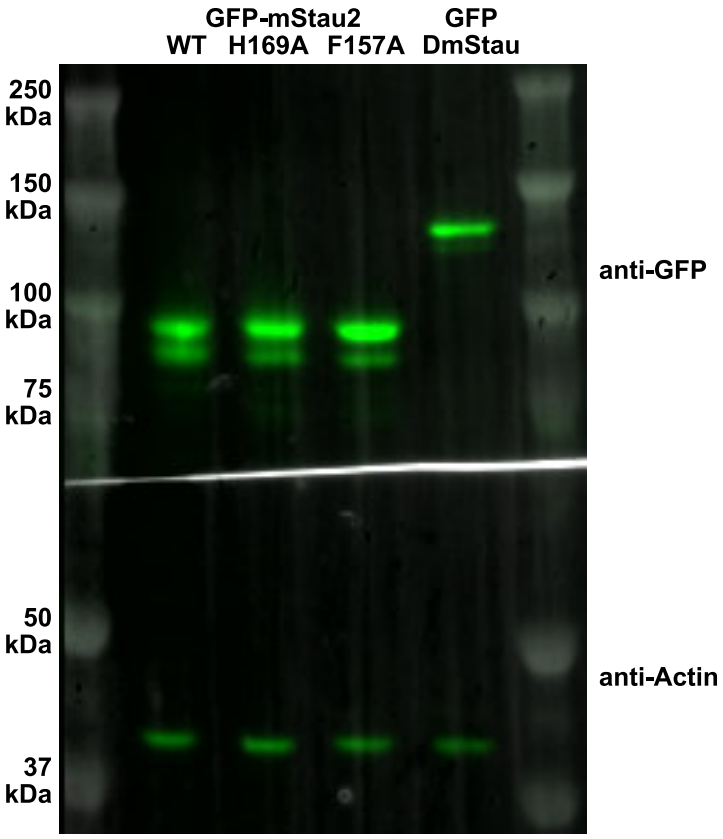

Figure S16: **wt and mutant Stau proteins are equally expressed in the germline of *stau^{R9}/stau^{D3}* mutant flies.** Expression levels were checked by Western blot. The blot was developed with anti-GFP (Torrey Pines lab, #TP401) and anti-Actin (Sigma A-2066) primary antibodies and HRP conjugated anti-rabbit secondary antibodies (JacksonImmunoResearch).

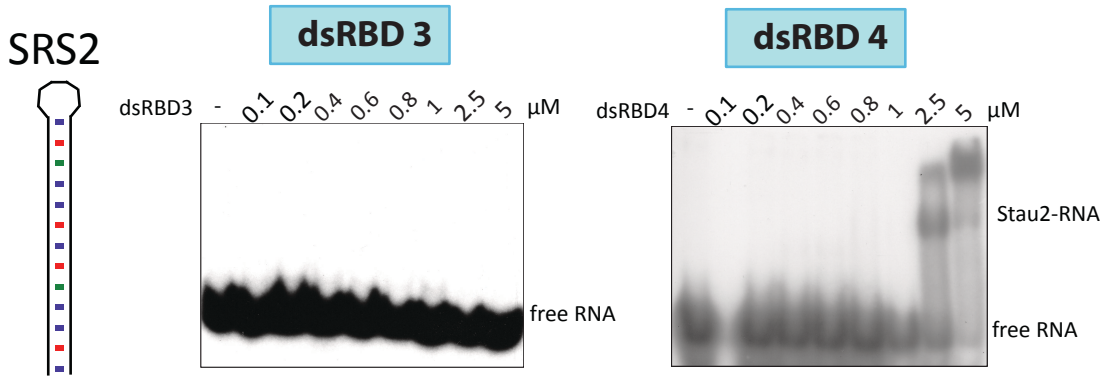

Figure S17: EMSAs with the individual domains dsRBD3 and 4 and SRS2 RNA. While dsRBD4 binds SRS2 RNA at micromolar concentrations, no binding can be observed for dsRBD3 up to 5 µM protein concentration.

Table S 1: Data collection and refinement statistics

	SRS2 RNA
Data collection	
Space group	C 1 2 1
Cell dimensions	
a, b, c (Å)	114.020 32.390 46.370
□, □, □ (°)	90.00 103.47 90.00
Resolution (Å)	55.44 - 1.73
I/□(I)	17.92 (1.88)
$CC_{1/2}$	99.9 (75.7)
Completeness (%)	96.8
(in resolution range)	(39.82 - 1.73)
Redundancy	5.1 (5.4)
Refinement	
Resolution (Å)	39.82 - 1.73
No. of reflections	16965
R_{work}/R_{free}	18.5/23.7
R_{free} test set	819 reflections (4.83 %)
No. of atoms	1421
RNA	1290
Ba ion	13
Mg ion	1
Water	116
Wilson *B* factor ($Å^2$)	30.6
Average B, all atoms ($Å^2$)	45.0
Anisotropy	0.048
Fo,Fc correlation	0.97
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	0.910

Table S2: Plasmids

Short name	Full name/description	Source/Cloning strategy
pRgs4	pExpress1 - Rgs4 ((B) ... IMAGp998E0615376Q)	Kiebler lab (LMU München)
pRgs4 3'UTR ΔSRS2	pEGFP-C2-Rgs4 3'UTR SRS2 deletion 3'UTR	Kiebler lab (LMU München)
pRgs4 3'UTR ΔSRS1	pEGFP-C2-Rgs4 3'UTR SRS1 deletion	Kiebler lab (LMU München)
pRgs4 3'UTR ΔSRS1 ΔSRS2	pEGFP-C2-Rgs4 3'UTR SRS1 and SRS2 deletion 3'UTR	Kiebler lab (LMU München)
pET-dmStaufen	pET3a-Staufen cDNAE10	Ephrussi lab (EMBL Heidelberg)

Short name	Full name/description	Source/Cloning strategy
prsEGFP2		Ephrussi lab (EMBL Heidelberg)
Plasmids created in this study		
SH17	pCR-II-Blunt-TOPO-Rgs4 BR	Blunt end TOPO cloning
SH22	pOPINS3C-mStau2 FL (Staufen homolog 2 isoform 3 [Mus Musculus], NCBI Reference Sequence: NP_079579.2)	InFusion cloning, primers Stau2 FW and Stau2 RV
SH23	pOPINS3C-mStau2 RBD3-4 (mStau2 200-373)	InFusion cloning, primers RBD3 FW and RBD4 RV
SH27	pOPINS3C-Stau2 RBD4 (mStau2 272-373)	InFusion cloning, primers RBD4 FW and RBD4 RV
SH29	pOPINS3C-mStau2 RBD1-4 (mStau2 1-373)	InFusion cloning, primers Stau2 FW and RBD4 RV
SH30	pFastBacDual-HisSUMO-mStau2 FL (Staufen homolog 2 isoform 3 [Mus Musculus], NCBI Reference Sequence: NP_079579.2)	InFusion cloning, primers His-SUMO FW and mStau2 RV-pFBD
SH31	pOPINS3C-mStau2 RBD1-2 (mStau2 1-208)	InFusion cloning, primers Stau2 FW and RBD2 RV
SH41	pOPINS3C-mStau2 RBD1-2 E15A (mStau2 1-208 E15A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 E15A antisense, Stau2 E15A sense+RBD2 RV
SH42	pOPINS3C-mStau2 RBD1-2 K59A K60A (mStau2 1-208 K59A K60A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 K59A K60A antisense, Stau2 K59A K60A sense+RBD2 RV
SH45	pOPINS3C-mStau2 RBD2 (mStau2 94-208)	InFusion cloning, primers RBD2 SM FW and RBD2 RV
SH46	pOPINJ-mStau2 RBD1-6xHis (mStau2 (1-74) - 6xHis)	InFusion cloning, PCR1 with Stau2 FW and RBD1+6xHis RV, PCR2 on product of PCR1 with Stau2 FW and pOPIN-6xHis RV
SH47	pOPINS3C-mStau2 RBD1 linker (mStau2 1-93)	InFusion cloning, primers Stau2 FW and RBD1-linker RV

Short name	Full name/description	Source/Cloning strategy
SH48	pOPINS3C-mStau2 linker-RBD2 (mStau2 75-208)	InFusion cloning, primers linker-RBD2 FW and RBD2 RV
SH49	pOPINS3C-mStau2 RBD1-2 H36A (mStau2 1-208 H36A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 H36A antisense, Stau2 H36A sense+RBD2 RV
SH50	pOPINS3C-mStau2 RBD1-2 F40A (mStau2 1-208 F40A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 F40A antisense, Stau2 F40A sense+RBD2 RV
SH51	pOPINS3C-mStau2 RBD1-2 K59A (mStau2 1-208 K59A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 K59A antisense, Stau2 K59A sense+RBD2 RV
SH52	pOPINS3C-mStau2 RBD1-2 K60A (mStau2 1-208 K60A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 K60A antisense, Stau2 K60A sense+RBD2 RV
SH53	pBlueScript-KS-rsEGFP2-mStau2 FL	InFusion cloning, restriction enzymes BamHI and XbaI, 3-point PCR with primers pBSKS-rsEGFP2 FW + rsEGFP+3C RV, 3C+Stau2 FW+ pBSKS-Stau2 RV
SH55	pOPINS3C-mStau2 RBD1-2 E99A (mStau2 1-208 E99A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 E99A antisense, Stau2 E99A sense+RBD2 RV
SH56	pOPINS3C-mStau2 RBD1-2 F157A (mStau2 1-208 F157A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 F1570A antisense, Stau2 F157A sense+RBD2 RV
SH58	pOPINS3C-mStau2 RBD1-2 H169A (mStau2 1-208 H169A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 H169A antisense, Stau2 H169A sense+RBD2 RV
SH59	pUASp attb-rsEGFP2-mStau2	Infusion cloning with primers pUASp-rsEGFP2 FW and pUASp-Stau2 RV, template SH53

Short name	Full name/description	Source/Cloning strategy
SH60	pOPINS3C-mStau2 RBD1-2 K106A (mStau2 1-208 K106A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 K106A antisense, Stau2 K106A sense+RBD2 RV
SH64	pOPINS3C-mStau2 RBD1-2 F40A H169A (mStau2 1-208 F40A H169A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 F40A antisense, Stau2 F40A sense+RBD2 RV on SH58 as template
SH65	pOPINS3C-mStau2 RBD1-2 F40A H169A (mStau2 1-208 F40A F157A)	InFusion cloning, 3-point PCR with primers Stau2 FW+Stau2 F40A antisense, Stau2 F40A sense+RBD2 RV on SH56 as template
SH66	pBlueScript-KS-rsEGFP2-mStau2 F40A	Site-directed mutagenesis (QuikChange II kit) with Stau2 F40A sense and Stau2 F40A antisense
SH67	pBlueScript-KS-rsEGFP2-mStau2 F40A F157A	Site-directed mutagenesis (QuikChange II kit) on SH66 with Stau2 F157A sense and Stau2 F157A antisense
SH68	pBlueScript-KS-rsEGFP2-mStau2 F40A H169A	Site-directed mutagenesis (QuikChange II kit) on SH66 with Stau2 H169A sense and Stau2 H169A antisense
SH69	pOPINS3C-mStau2 RBD1-4 F40A H169A	InFusion cloning with primers Stau2 FW and RBD4 RV on template SH68
SH70	pOPINS3C-mStau2 RBD1-4 F40A F157A	InFusion cloning with primers Stau2 FW and RBD4 RV on template SH67
SH71	pUASp attB-rsEGFP2-mStau2 F40A F157A	Infusion cloning with primers pUASp-rsEGFP2 FW and pUASp-Stau2 RV, template SH67
SH72	pUASp attB-rsEGFP2-mStau2 F40A H169A	Infusion cloning with primers pUASp-rsEGFP2 FW and pUASp-Stau2 RV, template SH68

Table S3: Cloning primers

Primer name	Primer description	Sequence 5'→ 3'
mStau2 FW	pOPIN- mStau2 FW +PPsite	AAGTTCTGTTTCAGGGCCCGATGG CAAACCCCAAAGAGA
RBD2 FW	pOPIN- mStau2 RBD2 FW +PPsite	AAGTTCTGTTTCAGGGCCCGATGC CCAAGATCTTTTATGTTCAGT
Linker-RBD2 FW	pOPIN- mStau2 RBD2 long FW +PPsite	AAGTTCTGTTTCAGGGCCCGCTTC CCAAACCAGTTCAGAAAC
RBD3 FW	pOPIN- mStau2 RBD3 FW +PPsite	AAGTTCTGTTTCAGGGCCCGATAA GCTTAGTGTTTGAGATTGCGC
RBD1-linker RV	mStau2 RBD1-linker RV-pOPIN	CTGGTCTAGAAAGCTTCTCTAACT ACCTGGGTTATTATTGACATTAC
RBD2 RV	mStau2 RBD2 RV-pOPIN	CTGGTCTAGAAAGCTTCTATTCAG ATTTATTTGCATCTTTATCGTC
RBD4 RV	mStau2 RBD4 RV-pOPIN	CTGGTCTAGAAAGCTTCTAAAGCT GTAACAGCATTGCTT
Stau2 RV	mStau2 FL RV-pOPIN	CTGGTCTAGAAAGCTTCTAGATGG CCGACTTTGAT
pOPIN-6xHis RV	pOPIN-6xHis RV	CTGGTCTAGAAAGCTTCTAgtgatgg tggtgatggtg
RBD1-6xHis RV	RBD1-6xHis RV	gtgatggtggtgatggtgCGTAGATTCGT CAAACGCTT
His-SUMO FW	pFastBacDual-His-SUMO FW	CATCGGGCGCGGATCCATGGCACA CCATCACCAC
Stau2 FL RV-pFBD	Stau2 FL RV-pFastBacDual	ACTTCTCGACAAGCTTCTAGATGG CCGACTTTGAT
pBSKS-rsEGFP2 FW	pBSKS-rsEGFP2 FW	gcggtggcggccgctctagaatggtgagcaag ggcga
rsEGFP+3C RV	rsEGFP+3C RV	cgggccctgaaacagaacttccagcttgtaca gctcgtccatgc
3C+mStau2 FW	3C+mStau2 FW	ctggaagttctgtttcagggcccgATGGCA AACCCCAAAGAGAA
pBSKS-mStau2 RV	pBSKS-mStau2 RV	tcctgcagcccgggggatccCTAGATGGC CGACTTTGATTTCT
pUASp-rsEGFP2 FW	pUASp-rsEGFP2 FW	AGGCCACTAGTGGATCTGGATCCat ggtgagcaagggcga
pUASp-mStau2 RV	pUASp-mStau2 RV	TTAACGTTCGAGGTCGACTCTAGA CTAGATGGCCGACTTTGATT

Table S4: Mutagenesis primers

Primer name	Primer description	Sequence 5'→ 3'
mStau2 E15A antisense	a44c_antisense	ggaaacgggctaacgcatttaccagacacactggag
Stau2 E15A sense	a44c	ctccagtgtgtctggtaaatgcgttagcccgtttcc
mStau2 H36A antisense	c106g_a107c antisense	caccgaaaacatcttcgaagcagcaggcccgctttcattc
mStau2 H36A sense	c106g_a107c_	gaatgaaagcgggcctgctgcttcgaagatgttttcggtg
mStau2 F40A antisense	t118g_t119c_ antisense	agactcagctgcaccgaagccatcttcgaatgagcagg
mStau2 F40A sense	t118g_t119c	cctgctcattcgaagatggcttcggtgcagctgagtct
mStau2 K59A antisense	a175g_a176c_ antisense	ttgttgggccttcgctatactgctcccttcggattccc
Stau2 K59A sense	a175g_a176c	gggaatccgaagggagcagtatagcgaaggcccaacaa
mStau2 K60A antisense	a178g_a179c_antisense	caacagcttgttgggccgcctttatactgctcccttcgg
Stau2 K60A sense	a178g_a179c_	ccgaagggagcagtataaaggcggcccaacaagctgttg
mStau2 K59A K60A antisense	a175g_a176c_a178g_a179c_antisense	cagcttgttgggccgccgctatactgctcccttcggattcccatgt
mStau2 K59A K60A sense	a175g_a176c_a178g_a179c	acatgggaatccgaagggagcagtatagcggcggccaacaagctg
mStau2 Δ119-135 antisense		ggcaatgatacctctgtggatctagtggcctg
mStau2 Δ119-135 sense		caggccactagatccacagaggtatcattgcc
mStau2 E99A antisense	a296c_antisense	tagcgagcccattcagtgccacagttggagttata
Stau2 E99A sense	a296c_sense	tataactccaactgtggcactgaatgggctcgcta
mStau2 K106A antisense	a316g_a317c antisense	ggcaggctctccccttgccatagcgagcccattc
mStau2 K106A sense	a316g_a317c	gaatgggctcgctatggcaaggggagagcctgcc
mStau2 F157A sense	t469g_t470c	gttcagttaactgtaggaaataatgaagcctttggtgaagggaagactc
mStau2 F157A antisense	t469g_t470c antisense	gagtcttcccttcaccaaaggcttcattatttcctacagttaactgaac
mStau2 H169A antisense	c505g_a506c antisense	ctttcatcgcagcattggctctggcagcttgtcgag
mStau2 H169A sense	c505g_a506c	ctcgacaagctgccagagccaatgctgcgatgaaag

Table S5: Template primers for RNA *in vitro* transcription

Primer name	Primer description	Sequence 5'→ 3'
3'UTR FW	T7prom+*Rgs4* 3'UTR FW	AATTTAATACGACTCACTATAGGttctcacacagaggcagagaacc
3'UTR RV	*Rgs4* 3'UTR 2129 RV	aggcctataaagcacatggcagaaacagacat
BR FW	T7prom+*Rgs4* 3'UTR 257FW	AATTTAATACGACTCACTATAGGtaatggccctgtaggtctgg
BR RV	*Rgs4* 3'UTR 870 RV	acgtgagcaaccaaccac
T7 FW	T7prom FW	AATTTAATACGACTCACTATAGG
SRS1 RV	SRS1-T7prom RV	GCTCCATCAAGACCCAGTGGCTTGACGGAACCCTATAGTGAGTCGTATTAAATT
SRS2 RV	SRS2-T7prom RV	ACATACACATACACAAATTGCATGTGCATGTCCCTATAGTGAGTCGTATTAAATT
SRS3 RV	SRS3-T7prom RV	CatgtgtgaacatatacaaatatatatatgcatatatatatatattcatgcacattcacacataCCTATAGTGAGTCGTATTAAATT
SRS2+5 RV	SRS2+5-T7prom RV	TATATACATACACATACACAAATTGCATGTGCATGTATATACCTATAGTGAGTCGTATTAAATT
11bpSRS2+5 RV	11bpSRS2+5- T7prom RV	TATATACATACACATACAAATCATGTGCATGTCCTATAGTGAGTCGTATTAAATT
9bpSRS2+5 RV	9bpSRS2+5-T7prom RV	TATATACATACACACAAATTGTGCATGTCCTATAGTGAGTCGTATTAAATT
7bpSRS2+5 RV	7bpSRS2+5-T7prom RV	TATATACATACACAAATTGCATGTCCTATAGTGAGTCGTATTAAATT
6bpSRS2+5 RV	6bpSRS2+5-T7prom RV	TATATACATACCAAATGCATGTCCTATAGTGAGTCGTATTAAATT

Table S6: RNA sequences

Short name	Full name/ description (numbering relative to start of 3'UTR)	Production
Rgs4 3'UTR	*Rgs4* 3'UTR FL Rattus norvegicus regulator of G-protein signaling 4 (Rgs4), mRNA NCBI Reference Sequence:	*In vitro* transcription (Ambion MegaScript), primer: 3'UTR FW, 3'UTR RV

Short name	Full name/ description (numbering relative to start of 3'UTR)	Production
	NM_017214.1 (3'UTR: nt 728-2919 of mRNA)	
BR	*Rgs4* 3'UTR (257-890)	*In vitro* transcription (Ambion MegaScript), primer: BR FW, BR RV
BR ΔSRS2	*Rgs4* 3'UTR (257-890) Δ(354-366)(417-429)	*In vitro* transcription (Ambion MegaScript), primer: BR FW, BR RV
BR ΔSRS1	*Rgs4* 3'UTR (257-890)Δ(729-759)	*In vitro* transcription (Ambion MegaScript), primer: BR FW, BR RV
BR ΔSRS1 ΔSRS2	*Rgs4* 3'UTR (257-890)Δ(354-366)(417-429) (729-759)	*In vitro* transcription (Ambion MegaScript), primer: BR FW, BR RV
SRS1	*Rgs4* 3'UTR (729-759)	*In vitro* transcription (Ambion MegaShortScript), Chemical synthesis (IBA)
SRS2	*Rgs4* 3'UTR (354-429)-Δ(367-416)AUUUG	*In vitro* transcription (Ambion MegaShortScript), Chemical synthesis (Dharmacon & IBA)
SRS3	*Rgs4* 3'UTR (492-555)	*In vitro* transcription (Ambion MegaShortScript)
SRS2+5	*Rgs4* 3'UTR (349-434)-Δ(367-416)AUUUG	*In vitro* transcription, primers: T7prom, dsSRS2+5 RV
11bpSRS2+5	*Rgs4* 3'UTR (354-434)-Δ(369-418)AUUUG	*In vitro* transcription (Ambion MegaShortScript), primers: T7prom, 11bpSRS2+5 RV
9bpSRS2+5	*Rgs4* 3'UTR (354-434)-Δ(371-419)AUUUG	*In vitro* transcription (Ambion MegaShortScript), primers: T7prom, 9bpSRS2+5 RV
7bpSRS2+5	*Rgs4* 3'UTR (354-434)-Δ(373-421)AUUUG	*In vitro* transcription (Ambion MegaShortScript), primers: T7prom, 7bpSRS2+5 RV
6bpSRS2+5	*Rgs4* 3'UTR (354-434)-Δ(374-422)AUUUG	*In vitro* transcription (Ambion MegaShortScript), primers: T7prom, 6bpSRS2+5 RV

Table S7. Sequences of ssDNA oligonucleotides specific to *bicoid*

Name	Sequence
bcd_5UTR_1	TGGCAAAGGAGTGTGGAAAC
bcd_CD_1	CTGAAGCTGCGGATGTTGG
bcd_CD_2	TCGAAGGGATTTCGGAATTG
bcd_CD_3	CCATATCTTCACCTGGGCTG
bcd_CD_4	GTCCTTGTGCTGATCCGAT
bcd_CD_5	CTCCACCCAAGCTAAGAGTC
bcd_CD_6	GCGTTGAATGACTCGCTGTAG
bcd_CD_7	TGTGGCCTCCATTGTAGTTG
bcd_CD_8	GGTGATTATGGACCTGCTGC
bcd_CD_9	GCTGGAAGTCAAAGTGATGG
bcd_CD_10	GTAGTACGAGCTGTTGAAGTTG
bcd_CD_11	GTGTTAATGGCTCGTAGACC
bcd_CD_12	CACACAGACTCGGACTTTCG
bcd_CD_13	CTTCTTGCTCGTTCCGTCG
bcd_CD_14	CCCTTCAAAGGCTCCAAGATC
bcd_CD_15	CTAAGGCTCTTATTCCGGTGC
bcd_CD_16	CTCCACGATTTCCGGTTCC
bcd_CD_17	GCTTGCATTATCGTATCCATCG
bcd_CD_18	CATCCAGGCTAATTGAAGCAG
bcd_3'UTR_1	ATGAAACTCTCTAACACGCCTC
bcd_3'UTR_2	GTACAATCAGGAACAACAGTGG
bcd_3'UTR_3	ACACGGATCTTAGGACTAGACC
bcd_3'UTR_4	GAATAGCGTATTGCAGGGAAAG
bcd_3'UTR_5	GCCCAAATGGCCTCAAATG
bcd_3'UTR_6	CCGAAATGTGGGACGATAAC

References

Ramos, A., Grunert, S., Adams, J., Micklem, D.R., Proctor, M.R., Freund, S., Bycroft, M., St Johnston, D., and Varani, G. (2000). RNA recognition by a Staufen double-stranded RNA-binding domain. EMBO J *19*, 997-1009.