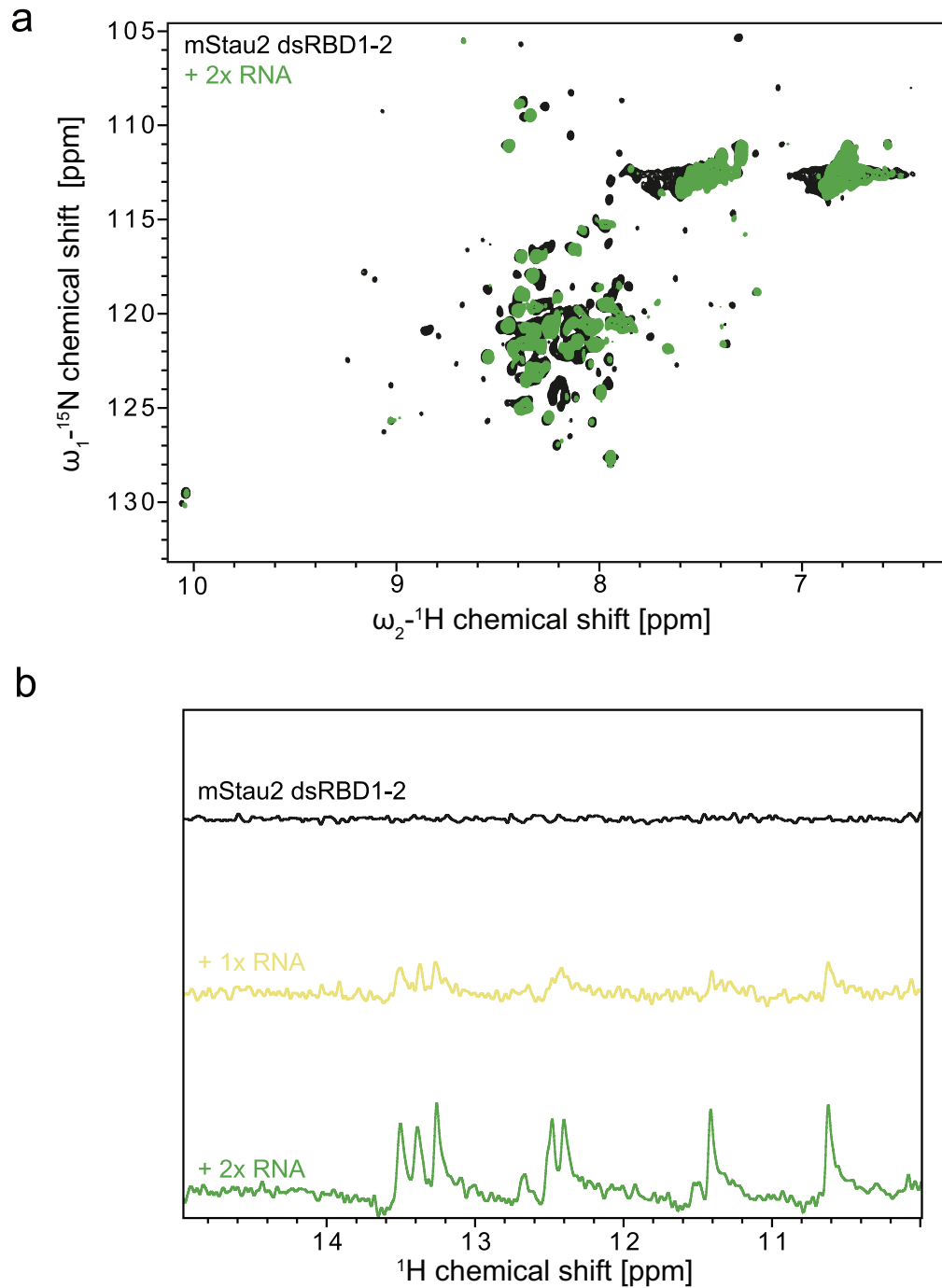


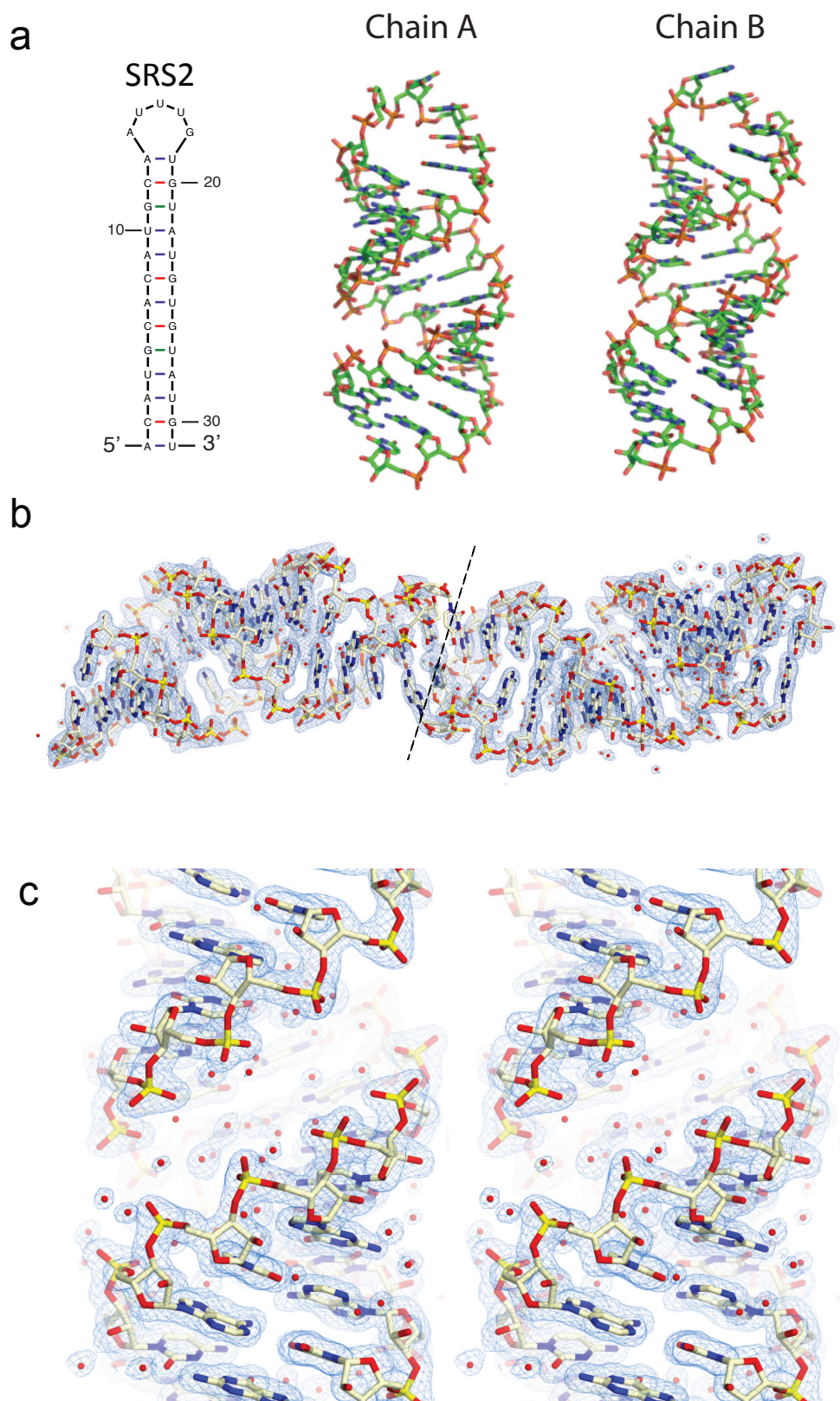
**Supplementary Figure 1: EMSAs with mStau2 and *Rgs4* 3'UTR RNAs.** **a** Schematic representation of the RNAs used in this study. SRS1 and SRS2 are predicted Staufen-recognized structures (SRS) in the *Rgs4* 3'UTR<sup>1</sup>. SRS\* is the most stably predicted secondary structure in the *Rgs4* 3'UTR. SRS2+5 is an elongated version of SRS2. **b** mStau2 full-length binds wild type *Rgs4* 3'UTR and SRS deletion mutants with similar apparent affinities in the nanomolar concentration range. **c** 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. Source data are provided as a Source Data file.

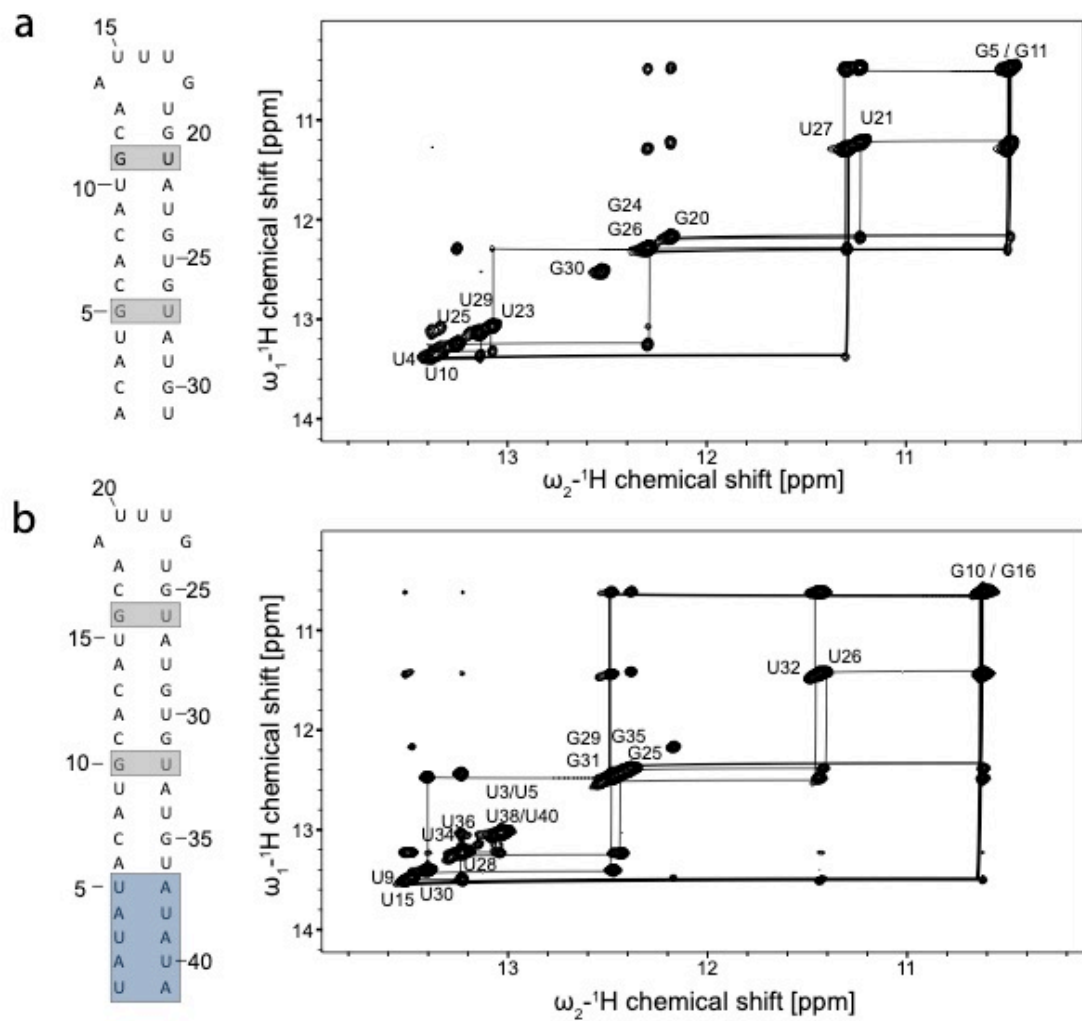


**Supplementary Figure 2: NMR titration experiments of Stau2 dsRBD1-2 with SRS2 RNA.**  
**a** Overlay of  $^1\text{H}$ ,  $^{15}\text{N}$ -HSQC spectra of dsRBD1-2 in absence and presence of 2x excess SRS2 RNA. Resonance shifts and line broadening of several signals are observed. **b** Comparison of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

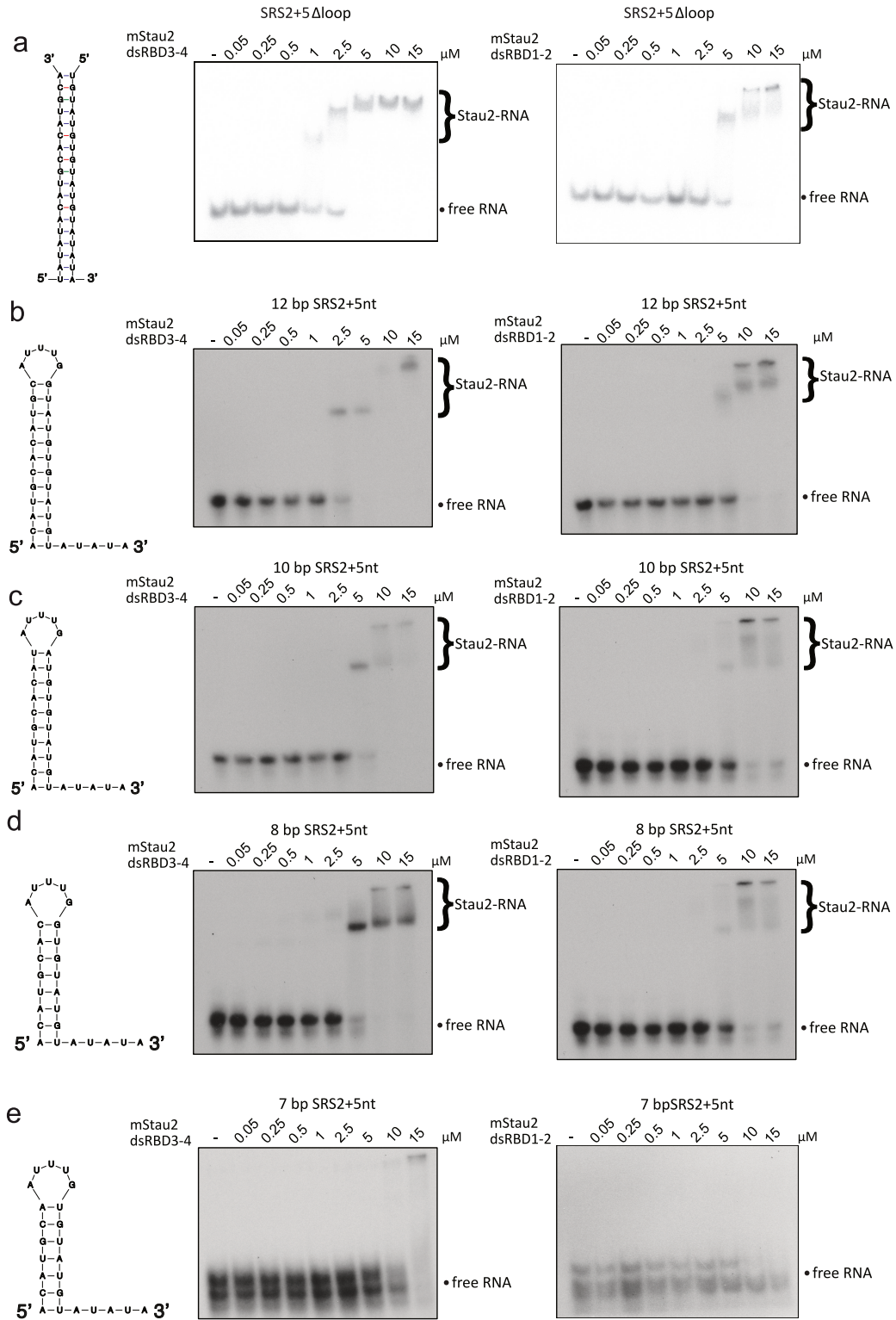




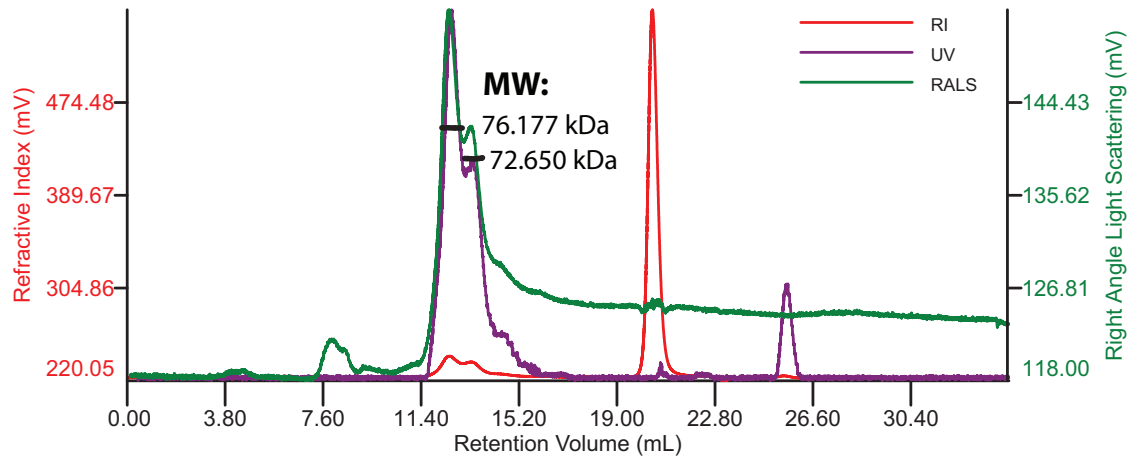
**Supplementary Figure 3: Crystal structure of the isolated *Rgs4* SRS2 RNA stem-loop.** **a** Schematic drawing of SRS2 RNA and the two molecules chain A and chain B contained in the asymmetric unit of the crystal lattice. Both molecules adopt the typical RNA A-form and form the expected stem-loop. Both chains differ slightly in their loop-regions, which appear to be disordered. **b** (2F(o)-F(c)) electron density map of the two RNA molecules in the asymmetric unit at 1 $\sigma$  contour. 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. **c** Stereo image of a fragment of the SRS2 stem showing the (2F(o)-F(c)) electron density map at 1 $\sigma$  contour.



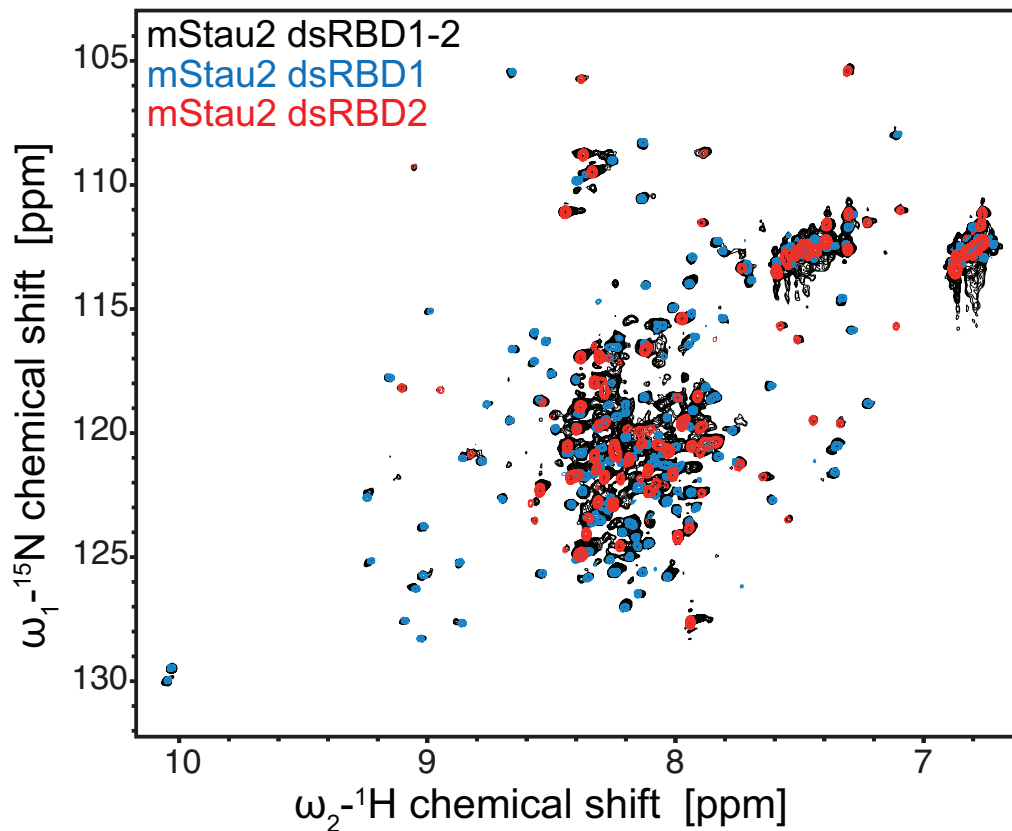
**Supplementary Figure 4: RNA assignment of imino groups based on  $^1\text{H}$ ,  $^1\text{H}$ - NOESY spectra of a SRS2 and b SRS2+5 RNA.**



**Supplementary Figure 5: 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 12bp, **c** 10 bp and **d** 8 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 7 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. Source data are provided as a Source Data file.

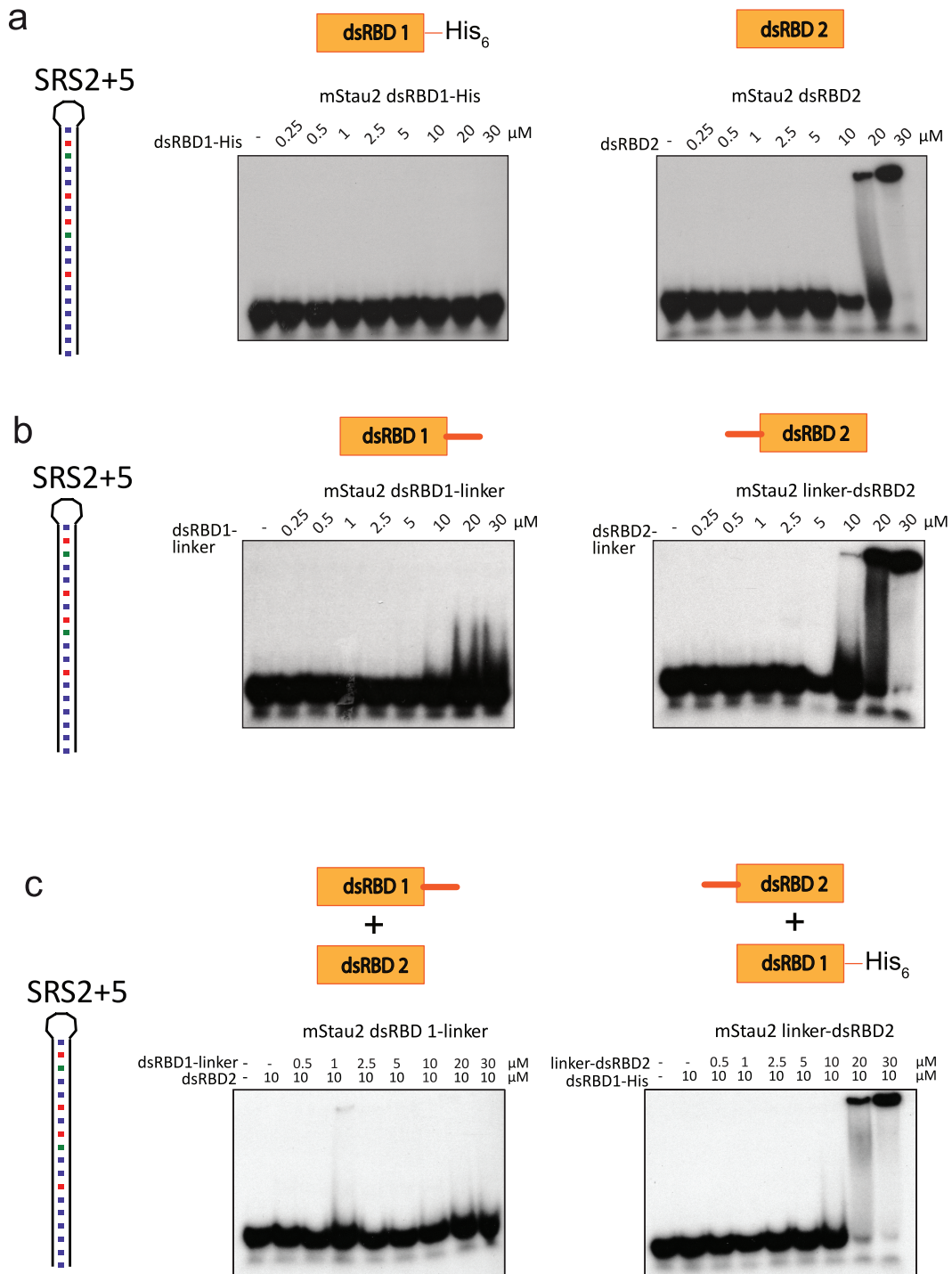


**Supplementary Figure 6: SLS measurements of HisSUMO-Stau2 FL after SEC on 10/300 Superdex200 Increase GL.** SEC chromatogram monitored by UV, refractive index (RI) and right-angle light scattering (RALS). Molecular weight (MW) distribution over peaks 1 and 2 and calculated average MW. The protein elutes in two peaks with molecular weights of 76 kDa and 73 kDa, corresponding to a monomer. The theoretical molecular weight of HisSUMO-tagged Stau2 FL is 75 kDa. The protein co-purified with a slightly smaller degradation product corresponding to peak 2.

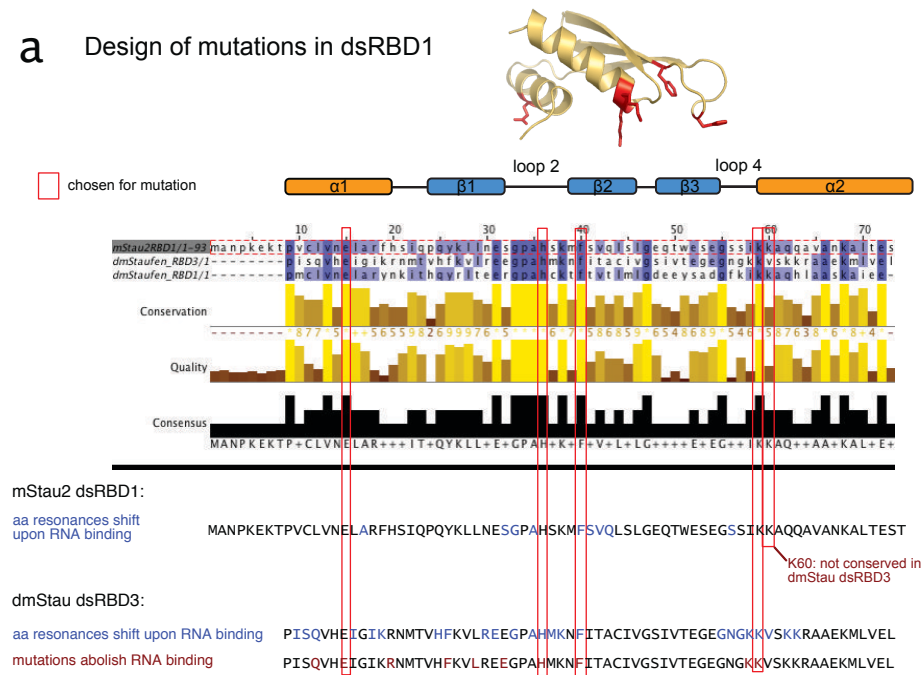
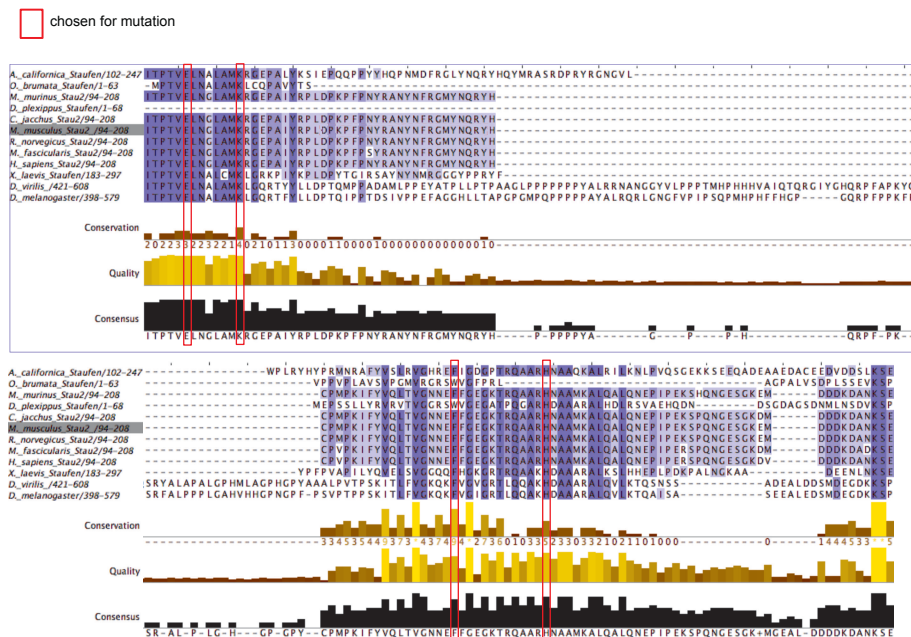


**Supplementary Figure 7: Overlay of the  $^1\text{H}$ ,  $^{15}\text{N}$ -HSQC spectra of the tandem domain dsRBD1-2 and the individual dsRBDs 1 and 2.** The spectra of dsRBDs 1 and 2 overlap and add up to the spectrum of the tandem domain, indicating that dsRBDs 1 and 2 are separate, partially disordered domains.

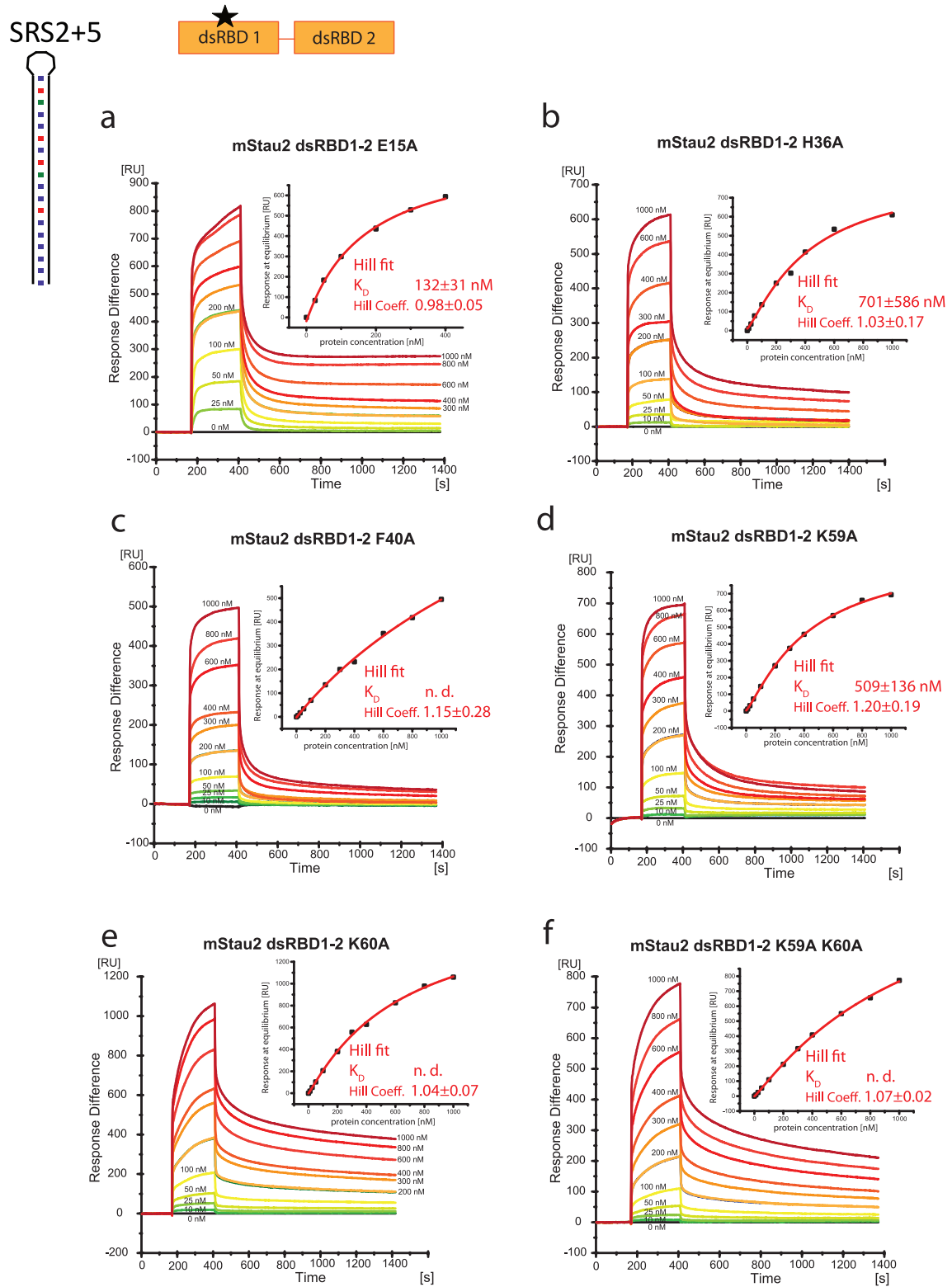




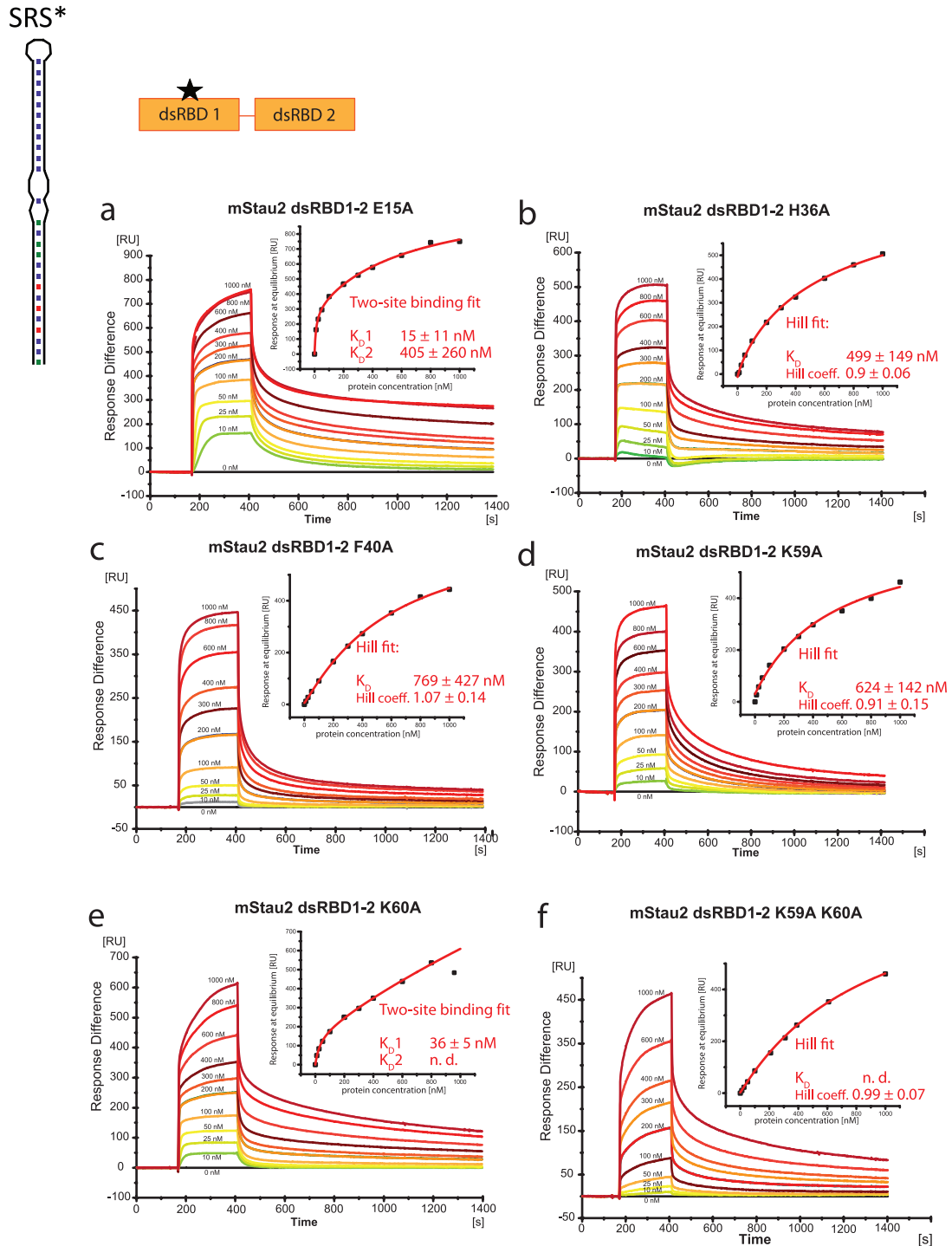
**Supplementary Figure 8: EMSAs with the individual domains dsRBD1 and 2. a** dsSRS2+5 RNA binding of dsRBD1-containing (left) and dsRBD2-containing (right) fragments. **b** Binding of dsRBD1-linker (left) and linker-dsRBD2 (right) to dsSRS2+5 RNA. **c** Binding of dsRBD1-linker (left) and linker-dsRBD2 (right) to dsSRS2+5 RNA in presence of the respective other domain at 10 μM. Source data are provided as a Source Data file.

**a** Design of mutations in dsRBD1**b** Design of mutations in dsRBD2**Supplementary Figure 9: 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 and mapped onto a homology model of dsRBD1. Designed mutations map to regions previously identified to be involved in RNA binding in dmStau dsRBD3<sup>2</sup>. **b** Multiple sequence alignment of mStau2 dsRBD2 to Stau proteins from 11 different species. Residues chosen for mutation are marked by red boxes and mapped onto a homology model of dsRBD2. Plots were generated with the program JalView. Homology models were made using the Phyre2 server<sup>3</sup>.

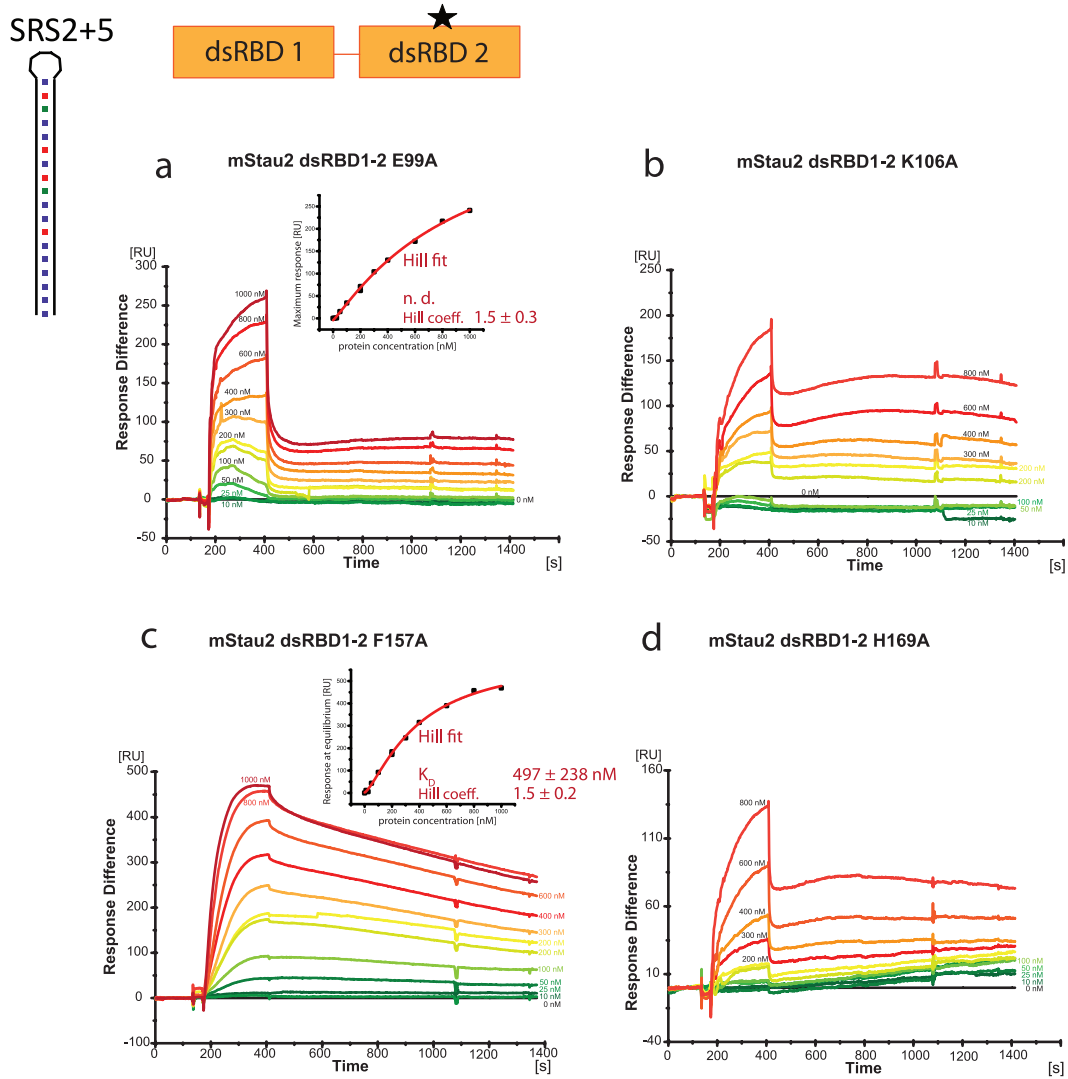


**Supplementary Figure 10: SPR sensorgrams and curve fitting of SRS2+5 RNA binding by mStau2 dsRBD1-2 with mutations in dsRBD1.** a-f Mutant versions of mStau2 dsRBD1-2. a) E15A, b) H36A, c) F40A, d) K59A, e) K60A and f) K29A K60A bind SRS2+5 transiently with fast kinetics. The steady-state binding curves are described by Hill-fits with Hill coefficients  $n \approx 1$ , indicating non-cooperative binding. Source data are provided as a Source Data file.

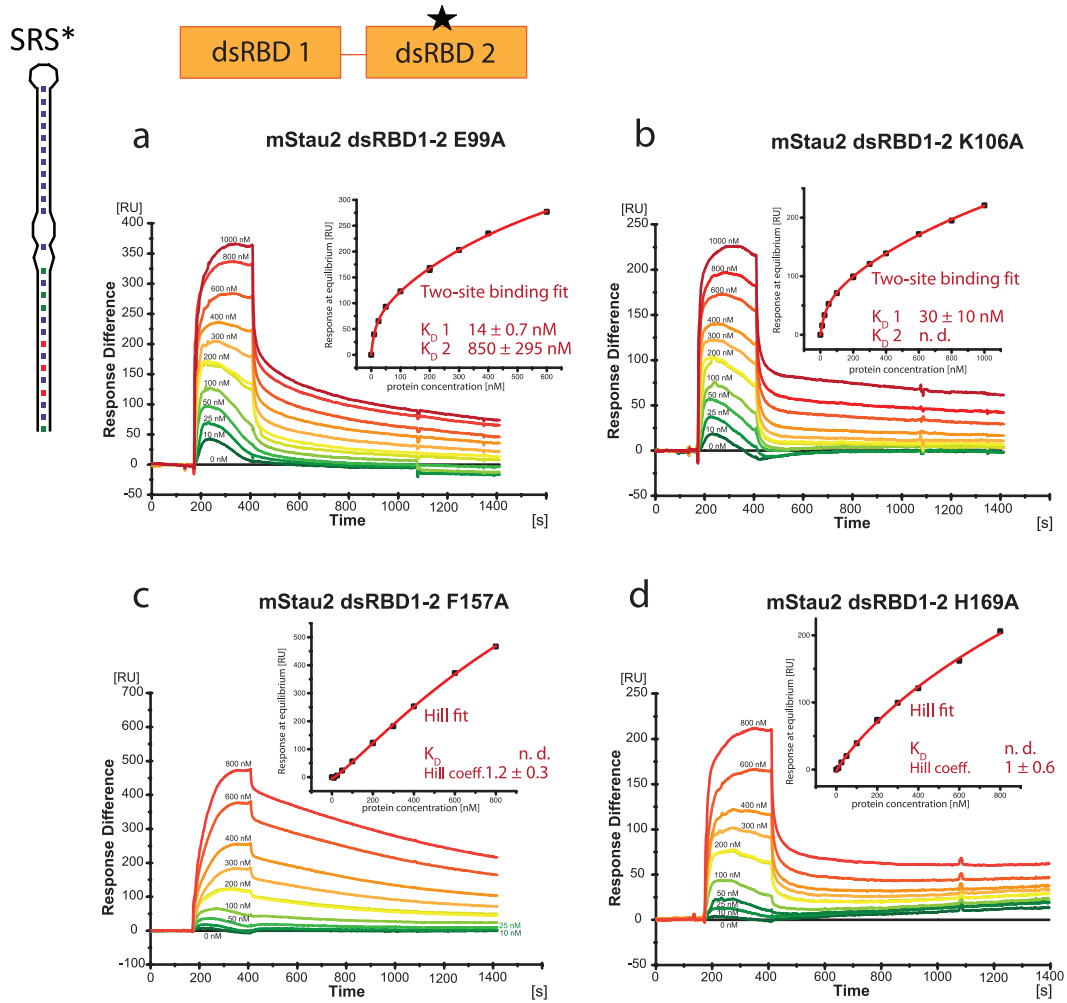


**Supplementary Figure 11: SPR sensorgrams and and curve fitting of SRS\* RNA binding by mStau2 dsRBD1-2 with mutations in dsRBD1. a-f** Mutant versions of mStau2 dsRBD1-2. b) H36A, c) F40A, d) K59A and f) K59A K60A bind SRS2+5 transiently with fast kinetics. The steady-state binding curves are described by Hill-fits with Hill coefficients  $n \approx 1$ , indicating non-cooperative binding. Mutants mStau2 dsRBD1-2 a) E15A and e) K60A bind similar to mStau2 dsRBD1-2 wild-type. Source data are provided as a Source Data file.

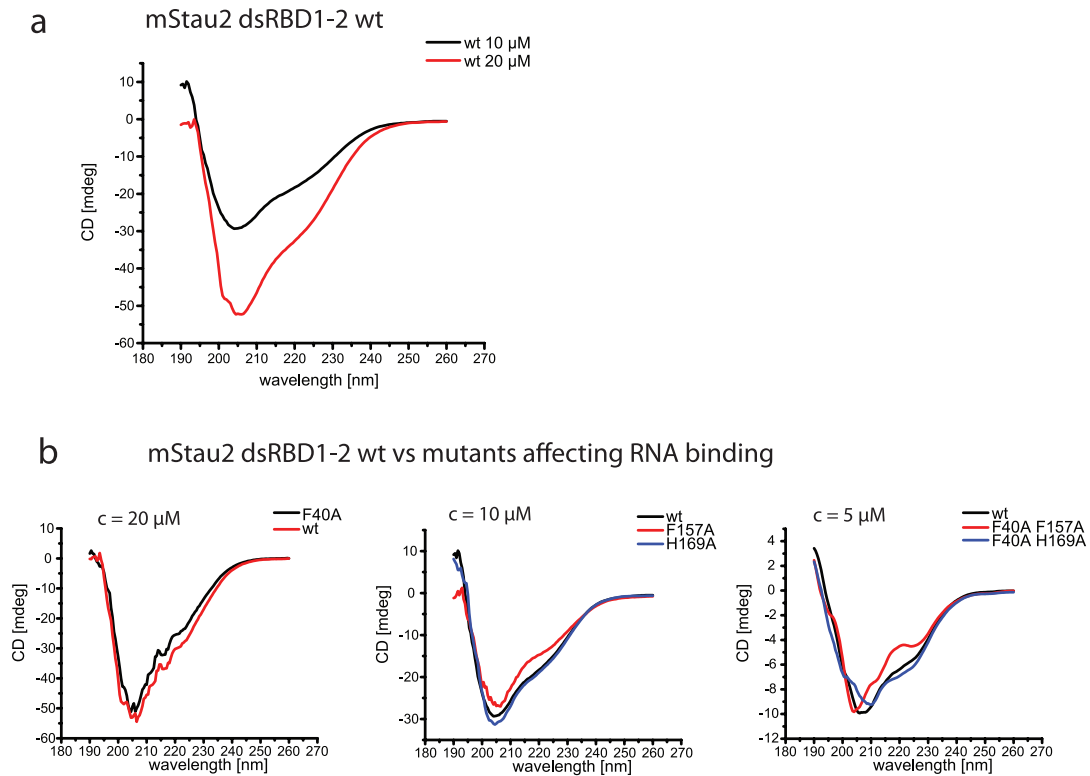




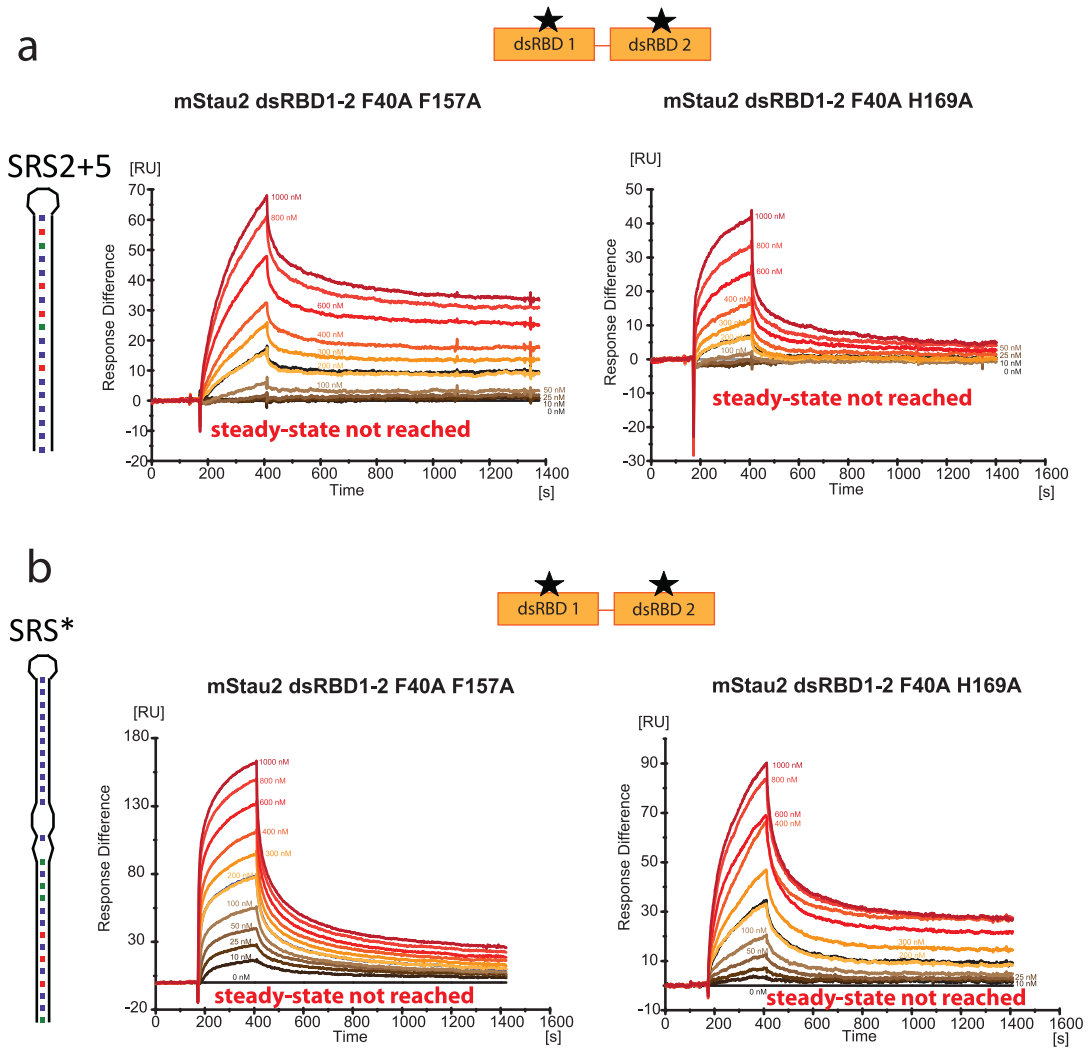
**Supplementary Figure 12: SPR sensorgrams and curve fitting of SRS2+5 RNA binding by mStau2 dsRBD1-2 with mutations in dsRBD2.** mStau2 dsRBD1-2 **a** E99A, **b** K106A, **c** F157A, **d** H169A show decreased binding to SRS2+5 RNA when compared to wild-type protein. For RBD1-2 E99A (**a**) and F157A (**c**), steady-state binding curves are shown. For dsRBD1-2 K106A (**b**) and H169A (**d**), steady-state could not be reached. Source data are provided as a Source Data file.



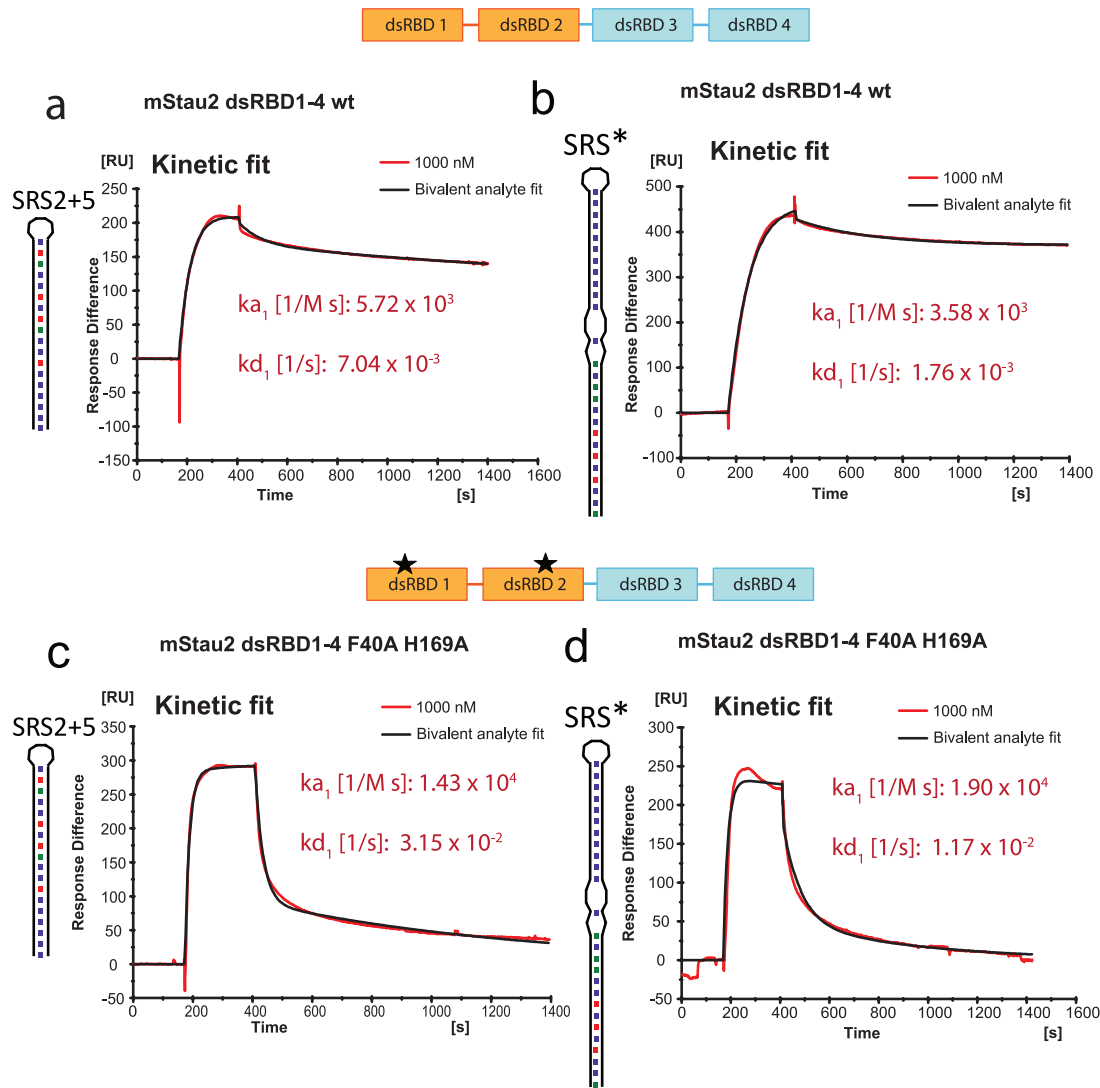
**Supplementary Figure 13: SPR sensorgrams and curve fitting of SRS\* RNA binding by mStau2 dsRBD1-2 with mutations in dsRBD2.** mStau2 dsRBD1-2 **a** E99A, and **b** K106A bind RNA similar to wild-type dsRBD1-2. In contrast, mStau2 dsRBD1-2 **c** F157A and **d** H169A show decreased binding to SRS\* RNA. Source data are provided as a Source Data file.



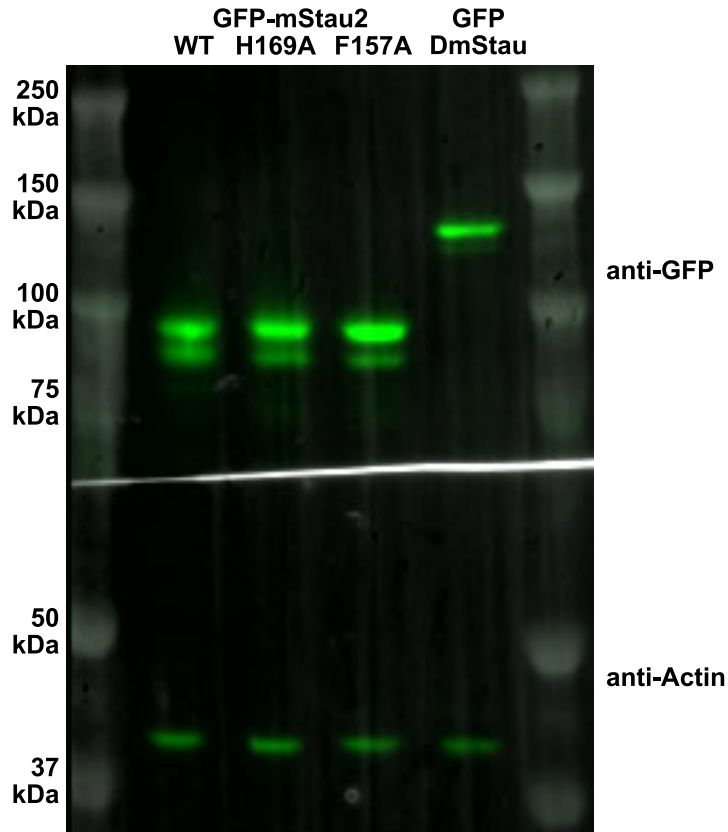
**Supplementary Figure 14: Circular dichroism spectra of mStau2 dsRBD1-2 versions.** **a** Wild-type mStau2 dsRBD1-2 at different concentrations and **b** RNA-binding mutants of dsRBD1-2 compared to wild-type dsRBD1-2. Measurements were performed at the indicated concentrations in buffer containing <50 mM NaCl. All curves show very similar profiles, indicating that mutant proteins adopt the same fold as wild type dsRBD1-2.



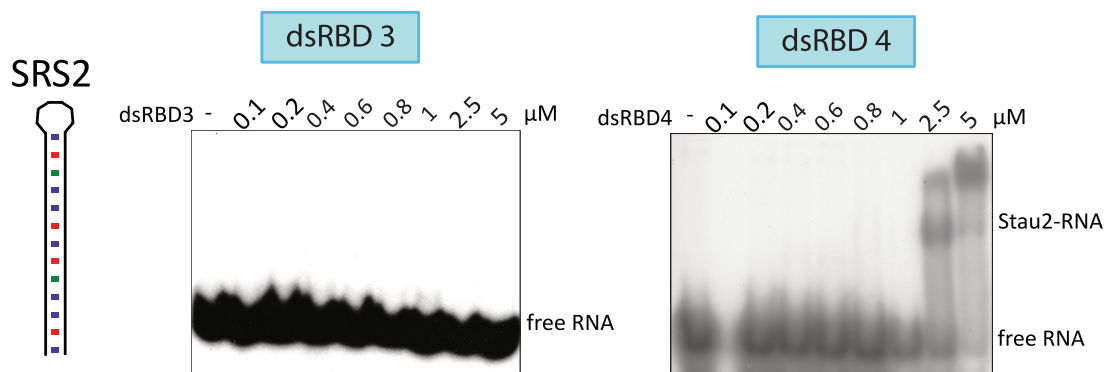
**Supplementary Figure 15: SPR sensorgrams and plots of maximum response against protein concentration of mStau2 dsRBD1-2 double-mutants binding to SRS RNA. Binding to **a** SRS2+5 and **b** SRS\* is strongly decreased as compared to wild-type mStau2 dsRBD1-2. Source data are provided as a Source Data file.**



**Supplementary Figure 16: 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  $k_{a1}$  and  $k_{d1}$  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  $k_{a1}$  and  $k_{d1}$  are ~5 and ~10-fold increased, respectively, as compared to wild-type.



**Supplementary Figure 17: wt and mutant Stau proteins are equally expressed in the germline of *stau<sup>R9</sup>/stau<sup>D3</sup>* 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).



**Supplementary Figure 18: 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  $\mu$ M protein concentration.

**Supplementary Table 1: RNA sequences**

Short name	Full name/ description (numbering relative to start of 3'UTR)	Production
<b>Rgs4 3'UTR</b>	<i>Rgs4</i> 3'UTR FL  Rattus norvegicus regulator of G- protein signaling 4 (Rgs4), mRNA NCBI Reference Sequence: NM_017214.1 (3'UTR: nt 728-2919 of mRNA)	<i>In vitro</i> transcription (Ambion MegaScript), primer: 3'UTR FW, 3'UTR RV
<b>BR</b>	<i>Rgs4</i> 3'UTR (257-890)	<i>In vitro</i> transcription (Ambion MegaScript), primer: BR FW, BR RV
<b>BR ΔSRS2</b>	<i>Rgs4</i> 3'UTR (257-890) Δ(354- 366)(417-429)	<i>In vitro</i> transcription (Ambion MegaScript), primer: BR FW, BR RV
<b>BR ΔSRS1</b>	<i>Rgs4</i> 3'UTR (257-890)Δ(729-759)	<i>In vitro</i> transcription (Ambion MegaScript), primer: BR FW, BR RV
<b>BR ΔSRS1 ΔSRS2</b>	<i>Rgs4</i> 3'UTR (257-890)Δ(354-366)(417- 429) (729-759)	<i>In vitro</i> transcription (Ambion MegaScript), primer: BR FW, BR RV
<b>SRS1</b>	<i>Rgs4</i> 3'UTR (729-759)	<i>In vitro</i> transcription (Ambion MegaShortScript), Chemical synthesis (IBA)
<b>SRS2</b>	<i>Rgs4</i> 3'UTR (354-429)-Δ(367- 416)AUUUG	<i>In vitro</i> transcription (Ambion MegaShortScript), Chemical synthesis (Dharmacon & IBA)
<b>SRS3</b>	<i>Rgs4</i> 3'UTR (492-555)	<i>In vitro</i> transcription (Ambion MegaShortScript)
<b>SRS2+5</b>	<i>Rgs4</i> 3'UTR (349-434)-Δ(367- 416)AUUUG	<i>In vitro</i> transcription, primers: T7prom, dsSRS2+5 RV
<b>11bpSRS2+5</b>	<i>Rgs4</i> 3'UTR (354-434)-Δ(369- 418)AUUUG	<i>In vitro</i> transcription (Ambion MegaShortScript), primers: T7prom, 11bpSRS2+5 RV
<b>9bpSRS2+5</b>	<i>Rgs4</i> 3'UTR (354-434)-Δ(371- 419)AUUUG	<i>In vitro</i> transcription (Ambion MegaShortScript), primers: T7prom, 9bpSRS2+5 RV
<b>7bpSRS2+5</b>	<i>Rgs4</i> 3'UTR (354-434)-Δ(373- 421)AUUUG	<i>In vitro</i> transcription (Ambion MegaShortScript), primers: T7prom, 7bpSRS2+5 RV
<b>6bpSRS2+5</b>	<i>Rgs4</i> 3'UTR (354-434)-Δ(374- 422)AUUUG	<i>In vitro</i> transcription (Ambion MegaShortScript), primers: T7prom, 6bpSRS2+5 RV

**Supplementary Table 2: Plasmids**

Short name	Full name/description	Source/Cloning strategy
<b>pRgs4</b>	pExpress1 - Rgs4 ( (B) ... IMAGp998E0615376Q)	Kiebler lab (LMU München)
<b>pRgs4 3'UTR ΔSRS2</b>	pEGFP-C2-Rgs4 3'UTR SRS2 deletion 3'UTR	Kiebler lab (LMU München)
<b>pRgs4 3'UTR ΔSRS1</b>	pEGFP-C2-Rgs4 3'UTR SRS1 deletion	Kiebler lab (LMU München)

Short name	Full name/description	Source/Cloning strategy
<b>pRgs4 3'UTR</b> <b>αSRS1 αSRS2</b>	pEGFP-C2-Rgs4 3'UTR SRS1 and SRS2 deletion 3'UTR	Kiebler lab (LMU München)
<b>pET-dmStaufen</b> <b>prEGFP2</b>	pET3a-Staufen cDNAE10	Ephrussi lab (EMBL Heidelberg) Ephrussi lab (EMBL Heidelberg)
<b>Plasmids created in this study</b>		
<b>SH17</b>	pCR-II-Blunt-TOPO-Rgs4 BR	Blunt end TOPO cloning
<b>SH22</b>	pOPINS3C-mStau2 FL (Staufen homolog 2 isoform 3 [Mus Musculus], NCBI Reference Sequence: NP_079579.2)	InFusion cloning, primers Stau2 FW and Stau2 RV
<b>SH23</b>	pOPINS3C-mStau2 RBD3-4 (mStau2 200-373)	InFusion cloning, primers RBD3 FW and RBD4 RV
<b>SH27</b>	pOPINS3C-mStau2 RBD4 (mStau2 272-373)	InFusion cloning, primers RBD4 FW and RBD4 RV
<b>SH29</b>	pOPINS3C-mStau2 RBD1-4 (mStau2 1-373)	InFusion cloning, primers Stau2 FW and RBD4 RV
<b>SH30</b>	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
<b>SH31</b>	pOPINS3C-mStau2 RBD1-2 (mStau2 1-208)	InFusion cloning, primers Stau2 FW and RBD2 RV
<b>SH41</b>	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
<b>SH42</b>	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
<b>SH45</b>	pOPINS3C-mStau2 RBD2 (mStau2 94-208)	InFusion cloning, primers RBD2 SM FW and RBD2 RV
<b>SH46</b>	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
<b>SH47</b>	pOPINS3C-mStau2 RBD1 linker (mStau2 1-93)	InFusion cloning, primers Stau2 FW and RBD1-linker RV
<b>SH48</b>	pOPINS3C-mStau2 linker-RBD2 (mStau2 75-208)	InFusion cloning, primers linker-RBD2 FW and RBD2 RV
<b>SH49</b>	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
<b>SH50</b>	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
<b>SH51</b>	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



Short name	Full name/description	Source/Cloning strategy
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
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

**Supplementary Table 3: Cloning primers**

Primer name	Primer description	Sequence 5' → 3'
<b>mStau2 FW</b>	pOPIN- mStau2 FW +PPsite	AAGTTCTGTTTCAGGGCCCGATGGC AAACCCCAAAGAGA
<b>RBD2 FW</b>	pOPIN- mStau2 RBD2 FW +PPsite	AAGTTCTGTTTCAGGGCCCGATGCC CAAGATCTTTTATGTTCACT
<b>Linker-RBD2 FW</b>	pOPIN- mStau2 RBD2 long FW +PPsite	AAGTTCTGTTTCAGGGCCCGCTTCC CAAACCAGTTCAGAAAC
<b>RBD3 FW</b>	pOPIN- mStau2 RBD3 FW +PPsite	AAGTTCTGTTTCAGGGCCCGATAAG CTTAGTGTTTGAGATTGCGC
<b>RBD1-linker RV</b>	mStau2 RBD1-linker RV- pOPIN	CTGGTCTAGAAAGCTTCTCTAACTA CCTGGGTTATTATTGACATTAC
<b>RBD2 RV</b>	mStau2 RBD2 RV-pOPIN	CTGGTCTAGAAAGCTTCTATTGAGA TTTATTTGCATCTTTATCGTC
<b>RBD4 RV</b>	mStau2 RBD4 RV-pOPIN	CTGGTCTAGAAAGCTTCTAAAGCTG TAACAGCATTGCTT
<b>Stau2 RV</b>	mStau2 FL RV-pOPIN	CTGGTCTAGAAAGCTTCTAGATGGC CGACTTTGAT
<b>pOPIN-6xHis RV</b>	pOPIN-6xHis RV	CTGGTCTAGAAAGCTTCTAgtgatggg gtgatggg
<b>RBD1-6xHis RV</b>	RBD1-6xHis RV	gtgatggggtgatgggCGTAGATTCGTCA AACGCTT
<b>His-SUMO FW</b>	pFastBacDual-His-SUMO FW	CATCGGGCGCGGATCCATGGCACA CCATCACCAC
<b>Stau2 FL RV- pFBD</b>	Stau2 FL RV-pFastBacDual	ACTTCTCGACAAGCTTCTAGATGGC CGACTTTGAT
<b>pBSKS-rsEGFP2 FW</b>	pBSKS-rsEGFP2 FW	gcggtggcgccgctctagaatggtgagcaaggg cga
<b>rsEGFP+3C RV</b>	rsEGFP+3C RV	cgggccctgaaacagaactccagctgtacagctc gtccatgc
<b>3C+mStau2 FW</b>	3C+mStau2 FW	ctggaagttctgttcagggcccgATGGCAAAC CCCAAAGAGAA
<b>pBSKS-mStau2 RV</b>	pBSKS-mStau2 RV	tcctgcagcccggggatccCTAGATGGCC GACTTTGATTCT
<b>pUASp-rsEGFP2 FW</b>	pUASp-rsEGFP2 FW	AGGCCACTAGTGGATCTGGATCCatg gtgagcaagggcga
<b>pUASp-mStau2 RV</b>	pUASp-mStau2 RV	TTAACGTTTCGAGGTCTGACTCTAGAC TAGATGGCCGACTTTGATT

**Supplementary Table 4: Mutagenesis primers**

Primer name	Primer description	Sequence 5' → 3'
mStau2 E15A antisense	a44c_antisense	ggaaacgggctaacgcatttaccagacacactggag
Stau2 E15A sense	a44c	ctccagtgtgtctggtaaatgcgttagcccggttcc
mStau2 H36A antisense	c106g_a107c_antisense	caccgaaaacatcttgaagcagcaggcccgcttcattc
mStau2 H36A sense	c106g_a107c_	gaatgaaagcgggcctgctgcttgaagatgtttcggtg
mStau2 F40A antisense	t118g_t119c_antisense	agactcagctgcaccgaagccatcttgaatgagcagg
mStau2 F40A sense	t118g_t119c	cctgctcattcgaagatggcttcggtgcagctgagtct
mStau2 K59A antisense	a175g_a176c_antisense	ttgtgggccttcgctatactgctccctcggtattccc
Stau2 K59A sense	a175g_a176c	gggaatccgaagggagcagtatagcgaaggcccaaca a
mStau2 K60A antisense	a178g_a179c_antisense	caacagcttgtgggcccgcctttatactgctccctcgg
Stau2 K60A sense	a178g_a179c_	ccgaagggagcagtataaaggcggcccaacaagctgtt g
mStau2 K59A K60A antisense	a175g_a176c_a178g_a179c_antisense	cagcttgtgggcccgcgctatactgctccctcggtattccca tgt
mStau2 K59A K60A sense	a175g_a176c_a178g_a179c	acatgggaatccgaagggagcagtatagcggcggccca acaagctg
mStau2 $\alpha$ 119-135 antisense		ggcaatgatacctctgtggatctagtggcctg
mStau2 $\alpha$ 119-135 sense		caggccactagatccacagaggtatcattgcc
mStau2 E99A antisense	a296c_antisense	tagcgagcccattcagtgccacagtggagttata
Stau2 E99A sense	a296c_sense	tataactccaactgtggcactgaatgggctcgcta
mStau2 K106A antisense	a316g_a317c_antisense	ggcaggctctccccttgccatagcgagcccattc
mStau2 K106A sense	a316g_a317c	gaatgggctcgctatggcaaggggagagcctgcc
mStau2 F157A sense	t469g_t470c	gttcagtttaactgtaggaaataatgaagccttgggaagg gaagactc
mStau2 F157A antisense	t469g_t470c_antisense	gagctctccctcaccaaaggcttcattatttctacagttaac tgaac
mStau2 H169A antisense	c505g_a506c_antisense	ctttcatcgagcattggctctggcagctgtcgag
mStau2 H169A sense	c505g_a506c	ctcgacaagctgccagagccaatgctgcgatgaaag

**Supplementary Table 5: Template primers for RNA *in vitro* transcription**

Primer name	Primer description	Sequence 5' → 3'
3'UTR FW	T7prom+ <i>Rgs4</i> 3'UTR FW	AATTTAATACGACTCACTATAGGttctc acacagaggcagagaacc
3'UTR RV	<i>Rgs4</i> 3'UTR 2129 RV	aggcctataaagcacatggcagaaacagacat
BR FW	T7prom+ <i>Rgs4</i> 3'UTR 257FW	AATTTAATACGACTCACTATAGGtaat ggccctgtaggtctgg
BR RV	<i>Rgs4</i> 3'UTR 870 RV	acgtgagcaaccaaccac
T7 FW	T7prom FW	AATTTAATACGACTCACTATAGG
SRS1 RV	SRS1-T7prom RV	GCTCCATCAAGACCCAGTGGCTTGA CGGAACCTATAGTGAGTCGTATTA AATT

Primer name	Primer description	Sequence 5' → 3'
<b>SRS2 RV</b>	SRS2-T7prom RV	ACATACACATACACAAATTGCATGT GCATGTCCCTATAGT GAGTCGTATTAAATT
<b>SRS3 RV</b>	SRS3-T7prom RV	Catgtgtgaacatatacaaatatatatgcatatata tatatatcatgcacattcacacataCCTATAGT GAGTCGTATTAAATT
<b>SRS2+5 RV</b>	SRS2+5-T7prom RV	TATATACATACACATACACAAATTGC ATGTGCATGTATATACCTATAGTGA GTCGTATTAAATT
<b>11bpSRS2+5 RV</b>	11bpSRS2+5- T7prom RV	TATATACATACACATACAAATCATGT GCATGTCCTATAGTGAGTCGTATTA AATT
<b>9bpSRS2+5 RV</b>	9bpSRS2+5-T7prom RV	TATATACATACACACAAATTGTGCAT GTCCTATAGTGAGTCGTATTAAATT
<b>7bpSRS2+5 RV</b>	7bpSRS2+5-T7prom RV	TATATACATACACAAATTGCATGTCC TATAGTGAGTCGTATTAAATT
<b>6bpSRS2+5 RV</b>	6bpSRS2+5-T7prom RV	TATATACATACCAAATGCATGTCCTA TAGTGAGTCGTATTAAATT

**Supplementary Table 6: Sequences of ssDNA oligonucleotides specific to *bicoid***

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

### **Supplementary References**

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